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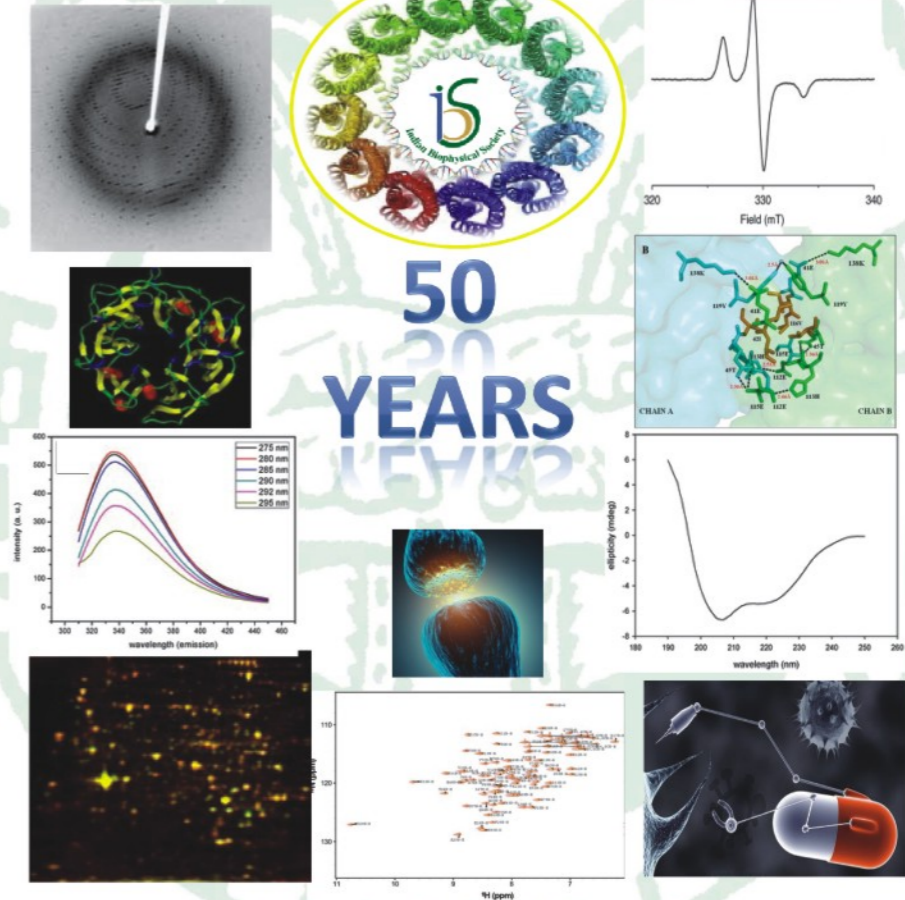
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Special Issue on : BIOPHYSICS IN INDIA



Conference Proceedings
National Symposium on Biophysics &
Golden Jubilee Meeting of Indian Biophysical Society



Organized by: Centre for Interdisciplinary Research in Basic Sciences
Jamia Millia Islamia, New Delhi, India

International Science Press

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Cover page : The Issue Celebrates 50th Year of IBS.

The cover page figure was designed by Pushpanjali Dasauni, Sanjay Kumar Dey, Mamta and Gagandeep from the group of Dr Suman Kundu. The former two are Ph.D students, while the latter two are M.Sc. students of the Department of Biochemistry, University of Delhi

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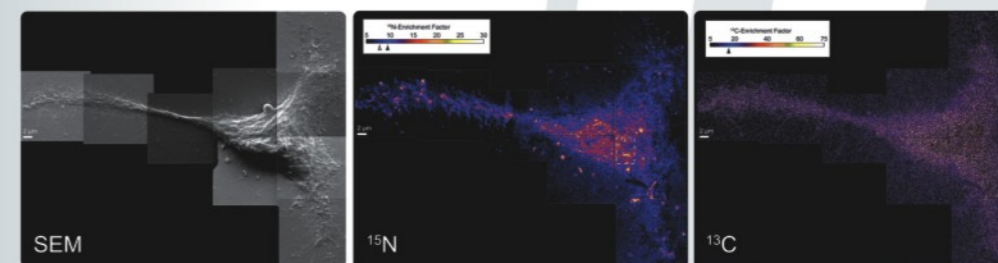


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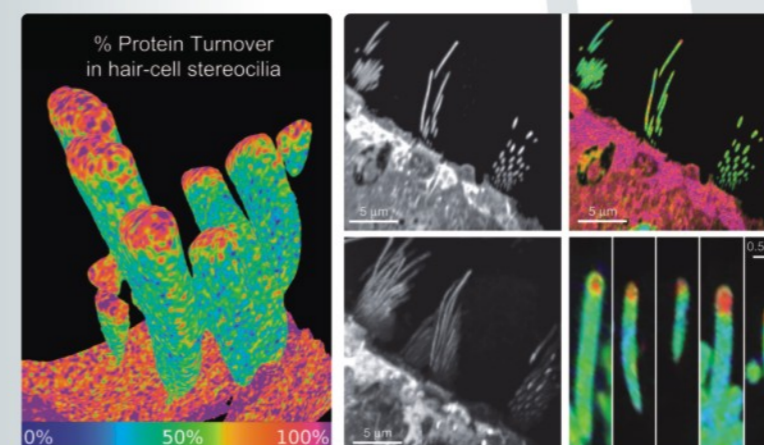


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Mapping the distribution of ¹⁵N-enriched ions from metabolically labeled ¹⁵N-sphingolipids in the plasma membrane, revealing sphingolipid-enriched plasma membrane domains distinct from lipid rafts.



Protein turnover quantification by feeding animals with a ¹⁵N-labelled precursor amino acid to measure appearance of new protein. 2D & 3D images.

From: Multi-isotope imaging mass spectrometry reveals slow protein turnover in hair-cell stereocilia. Duan-Sun Zhang et al. NATURE, vol 481, 26 Jan 2012.

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SPECIAL ISSUE ON**BIOPHYSICS IN INDIA****GUEST EDITORS****Dr. Md. Imtiyaz Hassan**Centre for Interdisciplinary Research in Basic Sciences
Jamia Millia Islamia, Jamia Nagar
New Delhi-110025, India**CONTENTS**

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GENERAL INFORMATION

The Journal: Journal of Proteins and Proteomics (JPP), with its genesis in India, is a peer reviewed journal envisaged to serve the world wide community of researchers and teachers dealing with the challenges of proteins and proteomics resulting in an improved understanding of protein science in general. Published bi-annually, the aim is also to supplement the regular issues with special issues in selected, relevant topics of protein science. The journal has an online presence at <http://www.jpp.org.in>.

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WELCOME TO THE FOURTH SPECIAL ISSUE (“BIOPHYSICS IN INDIA”) OF JOURNAL OF PROTEINS AND PROTEOMICS

After the exciting and memorable first 5 years, JPP walks into the 6th year of journey and is met with an eventful beginning right away. Unlike the last three years, JPP begins the current year with a special issue. Special issue it is – and what a special issue – to *commemorate 50 golden years of Indian Biophysical Society*. Biophysics, the application of physical principles to biology, plays an essential and integral role in the understanding of proteins and proteomics, deciphering their structure-function relationship, mechanistic roles, interplay with other biological molecules and translation into medical and biotechnological uses. The association of JPP and Indian Biophysical Society (IBS) is thus a natural extension of common goals and objectives. JPP is honored to present a *special issue on “Biophysics in India”* and wishes the society everlasting success and glory. JPP wishes to walk alongside IBS for years to come.

Biophysical research in India, through luminaries like G.N. Ramachandran, has excelled in India and is respected worldwide. IBS has shepherded biophysical research in India since its inception. It has encouraged and motivated biophysicists nationwide. Through its annual meetings it has laid down an enviable platform for biophysicists across the country and abroad to update their knowledge, share their findings, discover talents, share experiences, connect and build networks. IBS has been instrumental in inspiring generation of biophysicists in India. It has rewarded valuable research through memorial lectures, travel grants, awards and honors. The 50 year journey has been remarkable and extraordinary and deserves special accolades. JPP congratulates IBS on this landmark achievement.

Jamia Millia Islamia, with its rich heritage of education and research, and proven ability to disseminate knowledge efficiently through conferences and symposiums was chosen to host the annual meeting of IBS in its 50th year. Centre for Interdisciplinary Research in Basic Sciences (CIRBSc), bestowed with the honor, happily accepted the challenge and shouldered the responsibility with great success. JPP congratulates the team from CIRBSc.

JPP is elated to present the conference proceedings of the National Symposium in Biophysics and Golden Jubilee Meeting of Indian Biophysical Society through this special issue. The commemoration of 50 years begins with two G.N. Ramachandran Memorial Lectures (unlike the usual norm of one each year) to be delivered by two most pioneering biophysicists of our times – Dr. Girjesh Govil and Dr. M. Vijayan – whose contributions to biophysics in India is a common knowledge and needs no mention. To make the special year even more special, all past and present Presidents of IBS, who have served IBS selflessly and led the society from forefront, will be felicitated as well. This indeed will be a poignant moment worthy of memories to cherish forever.

The meeting will witness high quality scientific presentations from distinguished invited speakers, many of whom are still in their mid-career or formative years. The highlight of the meeting is the number of young scientists who have been provided platform to showcase their research to eager and supportive audience. No less significant is the fact that an unprecedented number of students will also present their work orally. It is an honor for them and a boost to their confidence. This special issue brings together all the abstracts of the above talks as well as those which are slated to present their findings through posters. The array of abstracts is a testimony to the depth, diversity, advancement and success of biophysical research in India. It is truly awe-inspiring and a great victory for IBS. It presents to the world the current trends in Biophysical research in India.

In addition, the issue also carries a short article that chronicles the IBS in the last 50 years. This is an asset for us as well. We hope that all delegates and participants will enjoy the issue as well as the deliberations of the meeting, the wonderful weather of the great city of Delhi and the remarkable ambience of Jamia Millia Islamia. We take the opportunity to appreciate the support from IBS and CIRBSc extended to JPP and allowing us the honor to present this very special “special issue”.

Editor in Chief
Suman Kundu

INSTRUCTIONS TO AUTHORS

Scope of the Journal: Authors are invited to submit original research articles, in-depth critical reviews, mini-reviews, commentaries, surveys, essays, opinions, insights and introspections in the area of Proteins and Proteomics for consideration and possible publication. The journal will focus on "proteins" and scope includes all facets of proteins and proteomics, like but not limited to, protein structure conformation and dynamics, protein-ligand or protein-protein interactions, protein folding, enzyme mechanisms and kinetics, protein models and predictions, physical properties and function, protein based diagnostics and therapeutics, protein engineering, protein expression and purification, whole proteome analysis, protein expression profiling, proteomics related to disease, proteomic analysis of cellular systems and organelles, methods and technologies related to protein investigations. Authors are requested to consult editor-in-chief, if in doubt about any aspect of scope, format or content of a proposed paper.

Submission and Peer Review: Authors interested in submitting articles are requested to send their manuscript as per guidelines of this journal for review directly to the editor-in-chief at jppindia@gmail.com. Alternately, manuscripts can be submitted online at <http://www.jpp.org.in>. The submission of a paper implies the author's assurance that the paper has not been published and that it has not been submitted simultaneously for publication elsewhere. An expert panel of reviewer will independently review all papers and suggest the Editor-in-Chief about its standard and suitability for acceptance. When submitting a paper authors are requested to suggest 6 potential referees across the globe with full name and address in each case with e-mail address. The final choice of referee's selection will remain entirely with the Editor-in-Chief. Authors of manuscripts can expect a decision normally within 4-6 weeks of receipt. Once a paper is accepted, authors are required to transfer the copyrights of the paper over to the publisher and should submit the copyright form. The submission should include the email and regular address of the corresponding author and all co-authors.

Style for Manuscript: All papers must be written in clear, concise English and should follow the guidelines as outlined below:

- (a) **Format:** The original typescript should be submitted electronically on Letter size paper, as a comprehensive MS Word file, with 2.5 cm margin at the top, bottom, left and right in 1.5 line spacing in Times New Roman font with font size of 12. The flow of article should be: title page, abstract and keywords, text including acknowledgement, abbreviations, references and notations, tables, figure legends and figures.
- (b) **Title Page:** The title should describe the contents fully and shall be as short as possible without the use of abbreviations. Maximum character in title should not exceed 150. Below the title include full names of author (s), department or division and name of institution with complete postal address. This should be followed by name of corresponding author including telephone number, fax and e-mail. Finally, the title page should also include a brief running title. The title page should be on a separate page.
- (c) **Abstract and Keywords:** An abstract of not more than 250 words describing the novelty and outcome of research findings of the paper should be provided. The abstract should be followed by a maximum of 5 keywords separated by semi colon. The abstract and keywords should be on a separate page, usually the second page of the manuscript.
- (d) **Main text of manuscript:** The text should be brief and crisp without repetitions. Figures and tables may not be described in details in the text. The text can be sub-divided into sections like Introduction, Materials and methods, Results and Discussion and Conclusion or Summary. Each section and corresponding sub-section in the text of review articles must be numbered appropriately (viz 1, 2, 2.1, 3, etc). The text should end in Acknowledgement section, which should be brief, mainly to acknowledge funding agencies and individuals who have helped with the manuscript.
- (e) **Abbreviations:** The abbreviations used throughout the text should be listed after the acknowledgement section. The acronym should be written first followed by comma and the corresponding expansion. Multiple acronyms should be separated by semi colon.
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 - Reference to a journal publication: Chakravarti, D.N., Fiske, M.J., Fletcher, L.D. and Zagursky, R.J. (2000). Application of genomics and proteomics for identification of bacterial gene products as potential vaccine candidates. *Vaccine* 19, 601-612.
 - Reference in Book Chapter : Chakravarti, B. and Chakravarti, D.N. (1998). Onchocerciasis. In *Encyclopedia of Immunology*, 2nd ed. (eds. I.M. Roitt and P.J. Delves), Academic Press, San Diego, pp 1872-1975.
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- (i) **Figures:** Figures should be high quality in black and white (colored when absolutely necessary and subject to reviewers' discretion) and numbered sequentially, appropriate to their appearance in text. They should be placed at the end of the manuscript.

One comprehensive file, preferably in x.doc format containing all the sections listed above must be submitted. In addition, authors are encouraged to submit the same as a PDF file as well. High quality figures may be submitted separately in a suitable format as well.

*National Symposium on Biophysics and
Golden Jubilee Meeting of the Indian Biophysical Society*

February 14-17, 2015

Organized by

**Centre for Interdisciplinary Research in Basic Sciences
Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India**

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JAMIA MILLIA ISLAMIA

Jamia Millia Islamia came into existence in 1920 through the tireless efforts of its founders, such as Shaikhul Hind Maulana Mahmud Hasan, Maulana Muhammad Ali Jauhar, Hakim Ajmal Khan, Dr. Mukhtar Ahmad Ansari, Jenab Abdul Majeed Khwaja and Dr. Zakir Husain. It symbolizes the unflinching and resolute commitment of these great visionaries in bringing about socio-economic transformation of common masses, in general, and Muslims, in particular through the vehicle of education. The distinct identity of Jamia Millia Islamia has been eloquently explicated by Dr. Zakir Husain.

The mission of the founding fathers of this great institution should not only serve as a beacon of light for all the stakeholders, but should also inspire us in making this university as one of the premier institutions of higher learning in the world. It should attain distinction in providing cutting edge learning experience, internationally benchmarked education, intellectual freedom and critical research opportunities in frontier areas of contemporary concern.

While the University has come a long way since its inception, it has to keep pace with the changing needs and expectations of the society. It has to successfully perform the multiple roles of creating new knowledge, acquiring new capabilities and producing an intelligent human resource pool for the promotion of economic growth, cultural development, social cohesion, equity and justice.

In view of special status of Jamia Millia Islamia in our country, we need to deliberate upon reconstituting the academic programmes through introduction of Choice-based Credit System, discuss suitable examination and administrative reforms and consider use of international benchmarks, such as Citation index, Journal Impact factor etc. for evaluating the quality of our research. We also exhort all the faculty members to formulate research project proposals either singly or in collaboration with other researchers of this or other institutions for financial support by the national and international funding agencies.

Apart from strengthening the teaching-learning process, research and extension programmes, special focus on extracurricular, sports and other similar activities to foster global competencies among the students is our priority.

CENTRE FOR INTERDISCIPLINARY RESEARCH IN BASIC SCIENCES

The Centre for Interdisciplinary Research in Basic Sciences was established in the year 2006. The reason for its establishment was a realization of the fact that each modern development in biology has its roots in basic sciences. In order to achieve its goal the Centre has both basic scientists and life scientists as faculty members who are doing research in modern areas of life sciences. There are 13 faculty members; a few of them have received significant professional recognitions. Being an interdisciplinary research centre our faculty members are divided into self-selected research programmes in thrust areas such as Protein Folding Problem, Structure Biology, Systems Biology, Bioinformatics, Nano-biotechnology, Bioimaging using SPM, Medicinal Chemistry, Molecular Biology, Virology, and Molecular Reproduction Biology. The size of our research programme allows the Centre to provide challenging research experiences to our students and researchers. The Centre offers two semesters extensive pre-Ph D course of 24 credits (16 credits of theory courses and 8 credits of practical courses). The purpose of this course is to prepare basic scientists (M.Sc. in chemistry/ physics/ mathematics/ computer science), and life scientists (M.Sc. in all branches of biology) to pursue interdisciplinary research in the novel areas of biological sciences. This is achieved by introducing courses from life sciences to basic scientists and courses from basic sciences to life scientists during their pre-Ph.D. course work.

This is a unique feature of the Centre, which is available only in a few Indian universities. We make every effort to integrate our educational programmes into research programmes for both graduate and post-graduate students. The research experience of our faculty members is brought to the classroom, giving students a sense of the excitement and the cutting edge nature of the discipline. In addition, the Centre is committed to provide short term (3 months) and long term (6 months) research trainings to national and international students and research facilities to researchers who are deprived of such facilities for extended periods. The research grants brought by our faculty members are important in supporting research and training programmes of the Centre. As a whole, the faculty members are pursuing major research projects worth 5 crores funded by various national and international funding agencies and have published more than 250 papers in peer reviewed journals in the last five years.

The objectives of the Centre are:

1. To promote interdisciplinary scientific research, advanced teaching and training in chosen areas of interdisciplinary basic sciences leading to M. Phil and PhD degrees;
2. To provide a forum for interaction among scientists, research workers, teachers and students with national and international experts;
3. To provide research facilities to individual workers or research groups, especially to those who are deprived of such facilities for extended periods;
4. To create tenure-based short term and long term chairs and visiting positions for experts in identified areas for interaction with the Centers' faculty, carrying out research and exchange of ideas; and
5. To conduct seminars, workshops, conferences and extension lectures to promote interdisciplinary research in basic sciences.

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Professor Talat Ahmad

FNA, FASC., FNASC., J.C. BOSE Fellow
Vice Chancellor



Vice-Chancellor's Message

I am happy to learn that Centre for Interdisciplinary Research in Basic Sciences is organizing the National Symposium on Biophysics and the Golden Jubilee Meeting of Indian Biophysical Society during February 14-17, 2015. I extended a warm welcome to all the delegates, participants and other distinguished guest to Jamia Millia Islamia, New Delhi.

Biophysics has emerged as an interdisciplinary science as it utilizes of physical sciences to design and develop of tools for unveiling the molecular of biomolecules like DNA. The tool have become so precise detailed that the structure of proteins like haemoglobin is studied by resolving the position of each atom. Biophysics has a tremendous impact now with the nanotechnology, stem cells and of modern imaging techniques. Recent researches in Biophysics are now heading towards the personalized medicines to solve the current and future problems with the help of other science disciplines.

I am sure this symposium will provide an opportunity to young students and researcher's to listen and interact with scientist working on various aspects of Biophysics. I am happy to note that young researchers would also get a chance to present their own work. I hope that this symposium will provide a platform to exchange research ideas and sharing of experience on interdisciplinary research themes. I wish a great success and a fruitful interaction for a deliverable research outcome.

(Prof. Talat Ahmad)
Vice-Chancellor

MESSAGE FROM ORGANIZING COMMITTEE

It is our privilege and great pleasure to organize the **National Symposium on Biophysics and Golden Jubilee meeting of the Indian Biophysical Society** during February 14-17, 2015 in Jamia Millia Islamia, New Delhi, India. Meeting of Indian Biophysical Society (IBS), held annually, is a world premier technical event covering all aspects of Biophysics and its diverse applications. This is a special year because the IBS is successfully completing 50 years of a memorable journey. In this meeting more than 50 invited talks, 20 Young investigators' talks, 64 oral presentations and more than 200 posters will be presented to more than 400 attendees across the country. This year, the program spans four days and includes one day inaugural session followed by three days of the main conference. Distinguished scientists, Professor G. Govil and Professor M. Vijayan are delivering the prestigious G. N. Ramachandran (GNR) Memorial Lectures in this symposium. This meeting will provide ample opportunities for discussions, debate, and exchange of ideas and information among conference participants.

The theme of this meeting is Biophysics, an interdisciplinary science that uses the methods of, and theories from physics to study biological systems. This area is attractive to scientists because it is fairly new, and draws from many other disciplines. Biophysics helps to carry out significant original research that reveals the molecular mechanisms underlying life of all varieties. Many of today's major biological challenges, such as heart disease, crop protection and age-related macular degeneration demand an approach that crosses the boundaries between biology and the physical sciences. For young scientists who have an interest in the fundamental aspects of science, but at the same time feel that their work should (when possible) contribute to a better understanding of biology and perhaps even improve life quality, biophysical chemistry ought to be an attractive research area.

Over the past 50 years, IBS has provided a cross-disciplinary venue for researchers and practitioners not only from Physics, Chemistry and Biology, but also from other related areas such as Biotechnology, Bioinformatics and Medicine. As a vision for the future, we believe in keeping up with the trend of expanding the society and vouching for knowledge sharing in research community. We hope that the entire society works as a team towards newer dimensions in bringing together ideas, innovations and developments from various enthusiastic researchers, and bring them to a common platform to rise together. This meeting will be very special in the sense that all past presidents of IBS will be felicitated by the Organizer.

We are grateful to all authors who trusted us with their work; without them there would be no conference. Our ambition is to realize a successful and memorable event for all participants, delegates, sponsors and exhibitors. We look forward to an exciting week of insightful presentations, discussions, and sharing of technical ideas with colleagues from around the county. We thank you for attending the conference, and we hope that you enjoy your visit to the Delhi.

(Organizing Committee)

PRESIDENTS OF INDIAN BIOPHYSICAL SOCIETY

B. B. Singh (1991-1993)



Dr. B.B.Singh holds a first class Master's degree in Physics and Ph. D. in the field of radiation biology from the University of London. He has worked in IAEA as Head Radiation Biology & Health Related Environmental Research. He retired from BARC in 1938 as Head, Radiation Biology & Biochemistry Divisions. His basic research work relates to free radical biology and oxidative stress. His cancer related research has added hyperthermia as an additional arm in radiotherapy of cancer. In recognition of his research work, the Indian Association for Hyperthermic Oncology and Medicine has instituted "**Dr. B. B. Singh Oration Award**" for foreign scientists to attend its biennial Meetings in India

After retirement from science Dr. Singh started practicing law and holds LL.M. degree from Mumbai University specialising in *Laws Relating to Intellectual Property Rights*. He has played an important role in formulation of laws relating to Patent Act 2005 and Biological Diversity Act 2002. He is a Scientific Advisor *appointed under The Patent Act 2005*. He is a frequent writer in daily Newspapers, weeklies and monthly magazines on India's nuclear laws. He is associated with several NGOs and plays a major role in defining nuclear policies.

He is elected Fellow of the Indian Academy of Sciences and National Academy of Sciences of India.

He is past President of Indian Biophysical Society; Indian Society for Radiation Biology and Indian Association for Hyperthermic Oncology & Medicine.

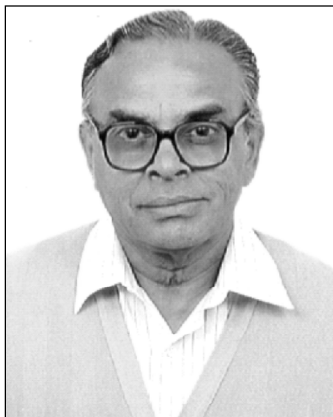
He has been on the Editorial Board of *International Journal of Radiation Biology; International Journal of Hyperthermia; Radiation Physics & Chemistry- An International Journal- Regional Editor, International Journal of Nuclear Law and Atoms For Peace-An international Journal*.

He is recipient of numerous awards most significantly the Veer Savarkar Award, Jack Goldberg Award - Oregon Health Science University, Portland, USA; Society for Cancer Research & Communication Award; 25 years of Service to Freemasonry and Best Rotarian Award.

He has been an active member of Ethics Committees of several medical research institutions in Mumbai including BARC Hospital; Raheja Hospital, Tata Memorial Hospital and Kaivalyadham Institute of Yoga & Ayurvedic Research, Lonavla.

He has given numerous Workshops on IPR and other laws for the corporate world at various places in India and continues to teach IPR subject to M.Sc. students of biotechnology and UGC Faculty improvement courses at Mumbai University.

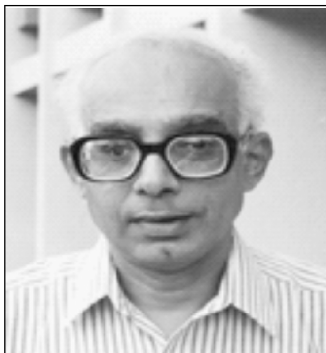
M. Vijayan (1993-1995)



Prof. M. Vijayan is a distinguished biophysicist and structural biologist. He obtained his Ph.D. from the Indian Institute of Science, Bangalore (1967). His postdoctoral stint in Professor Dorothy Hodgkin's laboratory at Oxford facilitated his transition from physics to biology. After his return in 1971, he has all along been at the Indian Institute of Science where he is currently the INSA Albert Einstein Research Professor. He pioneered the development of biological macromolecular crystallography in the country and played an important role in nurturing structural biology in India and abroad. His personal research has been on structural studies on lectins, TB and other mycobacterial proteins, protein hydration and mobility and supramolecular association with emphasis on its relevance to chemical evolution and origin of life. He has guided more than 40

Ph.D. students and 25 postdoctoral fellows and published 290 research papers. The honours received by him include: Bhatnagar Prize; GN Ramachandran Medal, Nehru Birth Centenary Fellowship and KS Krishnan Memorial Lecture of Indian National Science Academy (INSA); FICCI Award; Bhasin Award; Ranbaxy Award; Jawaharlal Nehru Birth Centenary Award, Indian Science Congress Association; CSIR/Science Congress G.N. Ramachandran Award; Goel Prize; Distinguished Alumni Award of Indian Institute of Science; Honorary Fellowship of the Indian Association for the Cultivation of Science; Lakshmi Pat Singhania-IIM Lucknow National Leadership Award for Science and Technology; Sir Devaprasad Sarvadhikari Medal of University of Calcutta; Champion of CSIR Award, Professor Krishnaji Memorial Lecture Award of the National Academy of Sciences, India; Kerala Science Award, 2012; SASTRA-G. N. Ramachandran Award, 2014 and Padma Shri. Prof. Vijayan is a fellow of all three science Academies of India and the TWAS. He has been active in the International Unions of Crystallography (IUCr) and Pure & Applied Biophysics (IUPAB). He was President of the Asian Crystallographic Association (2004-2007). He is a past President of the Indian National Science Academy (2008-2010)

D. Balasubramanian (1995-1997)



Balasubramanian did his BSc (1957) from Presidency College, Madras and MSc (1959) from Birla College, Pilani. He obtained his PhD degree (1965) in Chemistry from Columbia University, New York, USA, working in the area of polypeptide synthesis and conformations, using structural spectroscopy. After 18 months of post-doctoral research at the University of Minnesota Medical School in the area of protein chemistry (particularly the study of hydrophobic interactions), he returned to India in 1967 and joined IIT, Kanpur. In 1977, he moved to the University of Hyderabad and in 1982 to the Centre for Cellular and Molecular Biology (CCMB), where he was Deputy Director and later became Director. He took early retirement

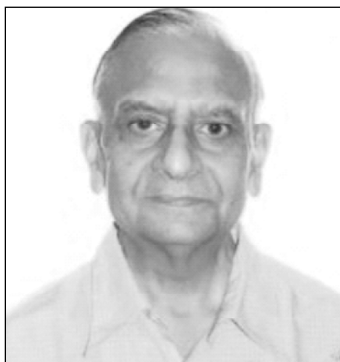
from CCMB in 1998 to join the LV Prasad Eye Institute, Hyderabad, as its Director of Research, a position he continues to hold.

On return to India, Balasubramanian joined the Department of Chemistry, Indian Institute of Technology, Kanpur, where he taught chemistry to undergraduates and spectroscopic methods and frontier topics in biological sciences to postgraduate students. His research work was focused on biophysical chemistry, particularly, on peptides and proteins, and membrane structures. Since 1985, he has focused attention on the biochemistry and cell biology of the eye, particularly, on cataract and glaucoma. He has over 160 publications and has mentored 16 PhD students.

Balasubramanian has been a promoter and activist in the area of the public understanding of science, and writes a regular fortnightly science column in the newspaper *The Hindu*, Member of UNESCO's International Basic Sciences Panel, Member of UNESCO's Bioethics Group, and Chairman of the Indian Department of Biotechnology's (DBT) Task Force on Stem Cell Research. He has been Chief of the Andhra Pradesh Government's Advisory Committee on Biotechnology (1997-2006). He is on the Editorial Board of several international and national professional journals.

Balasubramanian has received the Padma Shri (2002) and l'Ordre de National du Merite from France (2002). He has received the INSA's Indira Gandhi Prize for Popularization of Science and JC Bose Medal (1998), DST's National Prize for Popularization of Science, UNESCO's Kalinga Prize for the Popularization of Science, TWAS Prize in Basic Medical Sciences and Iran's Khwarizmi Prize in Basic Medical Sciences. He is also the recipient of SS Bhatnagar Prize, Goyal Award, Bhasin Award, FICCI Award and Sarma Award of India. He is also a Fellow of all three Science Academies of India and served as the Editor of INSA Publications (1955-97). Currently, he is the President of the Indian Academy of Sciences, Bangalore (2007-10), Secretary General of the Academy of Sciences for the Developing World (TWAS; 2007-10), President of Society of Biological Chemists India (2007-09).

Girjesh Govil (1997-1999)



Girjesh Govil (born 25 Sep 1940), obtained Masters from Allahabad University, and spent one year with BARC Training School. He secured 1st rank in both courses. Obtained Ph.D. degree (1964) from Tata Institute of Fundamental Research (TIFR) Mumbai, in the emerging field of Nuclear Magnetic Resonance (NMR), and initiated Chemical Applications of NMR in India. He served TIFR in various capacities, including as Dean of Physics Faculty. He has co-authored five books and has published over 300 papers in International Journals. His book entitled "*NMR in Biological Systems, From Molecules to Humans*" (Springer, 2008) is used as text book in several American Universities. He has held visiting positions at the National Physical Laboratory, UK, National Research Council, Canada,

National Institutes of Health, USA and Kobe University, Japan. He has served on important positions on International Bodies. These include: Vice-President of International Union for Pure and Applied Biophysics (IUPAB); Chairman of the Governing Council for the International Conferences on Magnetic Resonance in Biological Systems (ICMRBS); UNESCO Expert Committee on Biophysics; Executive Committee of the UNESCO Global Network for Molecular and Cell Biology; Editorial Board, Journal of Bio-molecular NMR and IUPAB Task Force on NMR. He has served on the Organizing Committees of several International Conferences, besides being a plenary/invited speaker.

Govil is a Fellow of Third World Academy of Sciences; Indian National Science Academy; Indian Academy of Sciences; National Academy of Sciences, India and the Royal Society of Chemistry (UK). He has handled Indian interest of the Royal Society. He has served as: Secretary for the Council of Indian National Science Academy; President of Indian Biophysical Society and President, of National Magnetic Resonance Society. He has also served on a number of National Committees dealing with Chemistry, Biophysics, Non-Conventional Energy Sources and Education. He is responsible for the creation of the National Facility for High Field NMR at TIFR, which has made valuable contributions towards research and education in the field of magnetic resonance.

Awards and honors received include: The first G. N. Ramachandran award of the International Union for Pure and Applied Sciences; Shanti Swarup Bhatnagar Award for Chemical Sciences; Federation of Industry and Chamber of Commerce of India Award for Biological Sciences, R.K. Asundi Memorial Lecture Award for Spectroscopy, Goyal Award, Acharya P.C. Ray Memorial Award, Vikram Sarabhai Gold Medal, Homi Bhabha Medal and the Fourth Jawaharlal Nehru Birth Centenary Visiting Fellowship. He has been conferred life-time achievement awards of the Indian Chemical Society and the Indian Science Congress Association.

Anil Saran (1999-2001)

Anil Saran earned his BSc and MSc from Patna University, after which he joined Indian Association for the Cultivation of Science, Kolkata. He worked under the guidance of Professor BN Srivastava on the 'transport properties of gases' and earned DPhil (1967) from Calcutta University. He then went to Ottawa, Canada on NRC Postdoctoral Fellowship. He returned to India in 1969 and joined Tata Institute of Fundamental Research (TIFR), Bombay as CSIR Pool Officer and along with Professor G Govil started work on 'conformation of bio-molecules by quantum mechanical methods'. On Invitation from Professor Bernard Pullman (1971), he joined the Institute de Biologie Physico Chimique, Paris, France as a CNRS Postdoctoral Fellow. On returning to India (1975), he joined TIFR, Bombay as a Fellow and worked there till 2000, when he retired as Professor 'H'. He worked as CSIR Emeritus Scientist (2000-05), and continued as INSA Senior Scientist at the Bombay College of Pharmacy.



He was Visiting Scientist at the German Cancer Research Centre, Heidelberg (1994, 1996); and University of Frankfurt, Germany (2000) under INSA-DFG Exchange Program.

While at TIFR, he along with Professor G Govil initiated the molecular biophysics programme and contributed significantly to set up the National Facility for High Field NMR. His work on the conformation of nucleic acids exhibited the importance of backbone on the helical structure of DNA, the base-pairing hydrogen bonds being additional factor in stabilizing the helical structure. He worked on several other bio-molecules by theoretical and experimental NMR techniques and his results on nucleoside antibiotics, anticancer drugs, hypertensive and AIDS drugs underline the basic feature of their drug action. His review article with Professor Pullman on the conformation of nucleic acids was published in *Progress in Nucleic Acid Research and Molecular Biology* in 1976 and considered a classic. He also authored more than 110 research articles in journals of national and international repute.

Professor Saran served as Scientific Secretary of International Conference on Theoretical Biochemistry and Biophysics at Goa in 1980 and IUPAB XIII International Biophysics Congress at New Delhi in 1999. He served the Indian Biophysics Society (IBS) as the Secretary (1991-95), Vice President (1996-99) and President (1999-2001). He served as Symposium Leader and Chairman, Sanibel International Symposium on Quantum Biology and Quantum Pharmacology, Palm Coast, Florida, USA (1982). He was on the Editorial Board of *Indian Journal of Chemistry, Section B*; Member of the CSIR Chemical Science and Technology Research Committee (1992-95) and IUPAB Commission of Biophysical Physics (1996-2002). He co-edited the Annual reports of National NMR Facility and also *Magnetic Resonance in Biology and Medicine* (Tata-McGraw-Hill, 1985).

Professor Saran was conferred UDCT Golden Jubilee Fellowship (1991) and Professor MN Saha Memorial Lectureship (1997). He was President, Section of Biochemistry, Biophysics and Molecular Biology, 87th Session of Indian Science Congress (1999-2000). He was also elected Fellow of the National Academy of Sciences (India), Allahabad (1984).

Rabi Majumdar (2001-2003)



Dr. Rabi Majumdar is a theoretical physicist and received his Ph.D. degree in 1966 for his work in the area of Nuclear and Elementary Particle Physics at the University of Delhi. He continued his postdoctoral work in the same area at various laboratories in Europe and USA including CERN, Geneva. In later years, his research contributions were mainly confined to the interdisciplinary areas of science involving the molecules of life. These include the nature of structural transitions in long chain molecules like DNA and proteins as well as the mechanism of action of some chemical carcinogens. The investigations were carried out by using the methods of computational biophysics and the results were published in reputed

journals.

Dr. Rabi Majumdar was in the faculty of the University of Delhi for 15 years and later joined Saha Institute of Nuclear Physics, Kolkata in 1983. He served the Institute as Professor and Head of the Biophysics Division during 1993-2000. After retirement in 2000, he was appointed CSIR Emeritus Scientist in the Department of Biophysics, Molecular Biology and Bioinformatics at the University of Calcutta for 5 years. Later he became an Advisor to the West Bengal University of Technology and is now an Emeritus Professor in the same University. He was appointed Visiting Professor at the University of Illinois at Chicago several times from 1997 to 2012.

Dr. Rabi Majumdar was President of the Indian Biophysical Society during 2001-2003. He is also a Fellow of the National Academy of Sciences, India.

Ramakrishna V. Hosur (2003-2005)



BASIC INFORMATION: Born, 1953; B. Sc from Karnatak University; M. Sc (Chemistry) from IIT-Bombay; Ph. D from TIFR (Mumbai university); Post-doc at ETH Zurich (Prof. Kurt Wuthrich, NL); Employed at TIFR since 1978, currently, Senior Professor; Director, UM-DAE Centre for Excellence in Basic Sciences, since Dec 2009.

RESEARCH : Pioneered research in biomolecular NMR, molecular biophysics, biophysical chemistry. Published over 200 research articles; Written and Edited books.; Taught courses at TIFR, IIT-Bombay, IIT-Roorkee on NMR, mathematics, biophysics; Guided 22 Ph. D students.

KEY CONTRIBUTIONS : Discovered new structural motifs in quadruplex DNA structures: A-tetrad, T-tetrad, C-tetrad. • Developed new multidimensional NMR pulse sequences. • Elucidated the folding hierarchy in HIV-1 protease. • Developed 'scaling techniques' for sensitivity and resolution enhancement. • Suggested a pH switch mechanism for cargo trafficking by DLC8 protein inside living cells. • Elucidated the native state energy landscape, and structural and dynamic characteristics of SUMO-1 protein along the equilibrium folding pathways; demonstrated that the native contacts do not progress monotonously. • Elucidated by NMR the self-association pathway of GED domain of dynamin into a large >5 MDa assembly; unraveled the hierarchy of folding and self association of Malarial protein PfP2.

RECOGNITIONS : Convener, National Facility for high field NMR; Member of sectional committees of INSA, Indian Academy of Sciences and National Academy of Sciences; DST, CSIR and DBT expert committees; Chair, IUPAB national committee of INSA (2008-2011); council member, INSA (2008-2010), NASI (2011-12, 2014-); RC member, IICB, Kolkata; President, Indian Biophysical Society (2003-05); President, new biology section of Indian Science Congress, 2005; President, National Magnetic Resonance Society (NMRS) (2009-11); Council Member, Centre for Biomedical Research, Lucknow; Member, Council of International Conference on Magnetic Resonance in Biological Systems (2004-2014); Council of International Society of Magnetic Resonance (2014-2018).

ACADEMY FELLOWSHIPS : Indian Academy of Sciences (1994); National Academy of Sciences (1997); Indian National Science Academy (1995); The World Academy of Sciences (2008).

AWARDS: B. M. Birla award (1992), INSA young scientist (1984), BRUKER young scientist (1983), B. D. Tilak lecture (1989), R. K. Asundi lecture (1998), Jagdish Shankar lecture (2003), 3rd annual award of society for cancer research (1998), JC Bose Fellowship (2007-2017); GN Ramachandran Gold medal (2009); P. C. Ray Memorial Lecture (2011); Padma Shri (2014).

K. P. Mishra (2005-2007)



Dr Mishra was **President** of Indian Biophysical Society, 2005-2008. Prior to this honor, he served on the **Executive Council** of IBS. He served as **Member** of National Committee of Biophysics of INSA for IUPAB. He was a **Member** of INSA delegation to IUPAB Congress in Hungary and a **Member** of Asian Biophysics Committee during his Presidency of IBS.

Dr Mishra obtained his **M.Sc.** from Allahabad University and **Ph.D.** from Gujarat University. He has a bright educational career throughout and is a recipient of National Scholarship of Govt of India.

After his post graduation, he joined Bhabha Atomic Research Center through prestigious Training School in Nuclear Science and Technology in all India competition. He acquired experience and expertise of close to 40 years as **Scientific Officer, Research management and educational administration and teaching at BARC, Mumbai(1968-2006)**. He superannuated as **Head**, Radiation Biology and Health Science Division and **Senior Scientific Officer** of Bhabha Atomic Research Center, Department of Atomic Energy, Government of India. He was **Member**, Standing Selection Committee Trombay Scientific Council, BARC, Health, Safety & Biomedical Group Board, **Member**, Committee of Atomic Energy Commission on Health and Environment Aspects of Uranium Mining at Jaduguda, **Member, Chairman**, Standing Selection and Promotion Committee of Staff, served as **Member**, Selection Committee of recruitment and promotion of scientific staff of BARC **Core Expert Member** on Chemical Disaster and Nuclear Disaster Management of National Disaster Management Authority of India (NDMA). **Member**, Committee on Disaster Management of Planning Commission, Govt of India, served as **Member**, Life Science Board of DRDO, Ministry of Defence, Govt of India. He served as **Expert** in Selection Committee of AssociateProf./Professor in many universities and IITs in India.

He is elected of **Fellow** of National Academy of Sciences, Allahabad, **Fellow** of Maharashtra Academy of Science and **Senior Vice President and Fellow** of Microscope Academy of India. He is a **Fellow** of International Academy of Physical Sciences and Hon. Hon.**Professor/Fellow** of Semipalanisk Academy of Medical Sciences, Kazakhstan.

Prof Mishra has held many official positions in academic and professional bodies; **President**, Indian Society for Radiation Biology 2004-06, **President**, Indian Biophysical Society 2005-2007, **President**, New Biology Section, 88th Session of Indian Science Congress; 2000-2001. **Chairman**, Executive Council, Nehru Gram Bharati University, Allahabad (2010-present), **Chairman**, Research Council, NGBU, **Chairman**, Academic Council, NGBU, **Vice President**, World Council of Nuclear Workers (WONUC) India, 2006-2009, **Convener**, Mumbai Chapter, Indian Science Congress Association, 2006-2009 and **Vice President**, Asian Association of Radiation Research, 2005-2013, **Vice President**, Society of Free Radical Research of India 2003-2005, **Vice President** Bioelectrochemical Society of India, **Member**, National Committee of IUPAB of Indian National Science Academy, **Member**, Executive Council, National Magnetic Resonance Society of India, **Adjunct Professor**, Institute of Technology, Manipal Academy of Higher Education, Manipal, **Adjunct Professor**, Department of Life Science, Mumbai University, 2006-2008, **Member**, Governing Council, Kamala Nehru Memorial Hospital and Research Center, Allahabad, **Member**, Governing Council, United College of Engineering and Research, Allahabad (2004-2009) and **Member**, Governing Council, Padmashri Dr. D.Y. Patil Deemed University, Navi Mumbai (2003-2006), **Councillor**, International Association of Radiation Research (2007-2011).

Life Member, Indian Science Congress Association, **Life Member**, Indian Society for Radiation Biology, **Member** of Society for Cancer Research and Communication, **Life Member**, Society of Biological Chemists, Life Member of IANCAS; Mumbai, **Life Member** of Mutagen Society of India.

He is **Editor-in-Chief** of Indian Journal of Radiation Research and **Co-Editor**, International Journal of Low Radiation, **Editor-in-Chief**, Journal of Nehru Gram Bharati University, **Member**, Editorial Board, Korean Journal of Environmental Biology, **Member**, Editorial Board, Iranian Journal of Radiation Research, **Member**, International Journal of Radiation Biology, **Member**, Editorial Board, World Journal of Radiology, **Editor-in-Chief**, J.Nehru Gram Bharati Univ., **Chief Editor**, Frontiers in Research and is actively involved as a Member of Editorial Board of many of National & International Journals.

Dr Mishra was invited as **Visiting Scientist** to Kyoto University, **Japan**; Institute of Biochemical Genetics, Tolouse, CNRS; **France**, Univ of Bielefeld; **Germany**, Institute of Biological Science; **Croatia**, Himeji Institute of Technology; Japan, Osaka University; **Japan**, Kobe University, **Japan**; Okinawa Institute of Science & Technology, Okinawa, **Japan**; Hiroshima University; **Japan**, Montpellier and Curie Institute, Paris, **France**; McMaster University, Hamilton, **Canada**; Sydney University, Sydney, **Australia**; Budapest University; **Hungary**, The University of Queensland, Brisbane, **Australia**; New York State University; New York State, **USA**. University of Nagoya, **Japan**, Tokyo Institute of Technology, **Japan**, University of Bremen, Germany, Korea Atomic Energy Research Institute, **South Korea**, Beijing University, **P.R.China**, National University of Singapore, **Singapore** and many other countries.

He has guided **51 Ph.D. students** and has published more than **275 research papers and proceeding papers** in National and International Journals. He has delivered more than 350 invited talks in Symposia and Conferences in India and abroad. He has edited books 05 books and has written about 40 Chapters in Books.

Recipient of **Life Time Achievement Award** of Indian Society for Radiation Biology (2006), received **Distinguished Science Award** of Asia- Pacific ESR Society; 2004, ESR Spectroscopy Research Promotion, **Diploma Award** from International EPR Society; 2004, **Distinguished Professor Fellowship** Award from Sydney University; Australia; 2005. Dr Mishra received **CSIR visiting Fellowship** to Nepal under Indo-Nepal Academy Exchange Program of Nepal Nuclear Society and National Academy of Science and Technology, Nepal, Heramb Mishra **Gaurav** award of Allahabad, **Award** of Allahabad Chapter of Indian Medical Association, **Felicitatation Award** of Society of Cancer research and communication, Mumbai, **Education Excellence Award** of CCI, 2014.

He served as **Expert** to **WHO** for Public Health response to Nuclear events for South East Asia Region and **Expert** of Radiation Biology for **IAEA**, Vienna, Austria, served **Advisor**, **WONUC**.

N. R. Jagannathan (2007-2009)



N. R. Jagannathan is a Professor & Head of the Department of NMR & MRI Facility at the All India Institute of Medical Sciences, New Delhi. His research interests are in clinical and pre-clinical cancer research using molecular imaging methods like magnetic resonance imaging (MRI) and NMR Spectroscopy. He is author or co-author of over 300 publications and 5 edited volumes.

Prof. Jagannathan is a Fellow of the Indian Academy of Sciences (FASc, Bangalore); Fellow of the National Science Academy (FNASc, Allahabad); Fellow of the National Academy of Medical Sciences India (FAMS), Fellow of the Indian National Science Academy (FNA; INSA) and Fellow of the International Society for Magnetic Resonance in Medicine (ISMRM).

Currently, he serves as a member of the Editorial Boards of: (i) NMR Biomedicine (John Wiley); (ii) MAGMA (Springer); (iii) Magnetic Resonance Imaging (Elsevier); (iv) Magnetic Resonance Insights (Libertas Academica); (v) Biophysical Reviews (Springer); and (vi) Biomedical Spectroscopy & Imaging (IOS Press).

Prof. Jagannathan is a recipient of the prestigious Drs. Kunti & Om Prakash Oration Award for significant contribution in the field of biomedical sciences, Indian Council of Medical Research (ICMR), Government of India in September 2013. He delivered the Achanta Lakshmipathi Oration for outstanding contributions in the field of MRI of Cancer from the National Academy of Medical Sciences (NAMS), India in October 2012.

Dr. Jagannathan is currently the President of the Indian Chapter of the International Society for Magnetic Resonance in Medicine (ISMRM), USA; Vice-President of the Molecular Imaging Society of India (MISI); member of the Young Investigators Committee of the International Society for Magnetic Resonance in Medicine (ISMRM), USA. He was a Member of the Executive Council of the International Union of Pure & Applied Biophysics (IUPAB - 2009-14); Member of Council of ICMRBS; member of the Program Committee of 2014 World Molecular Imaging Society (2014 WMIC). Prof. Jagannathan also served as a Member of the Annual Program Committee (AMPC; 2008-10), Education Committee (2007-09) and Chapters Committee (2010-11) of the International Society for Magnetic Resonance in Medicine (ISMRM), USA. He was President of the Asian Biophysics Association (2011-13) and President of the Indian Biophysical Society (2007-09).

Ch. Mohan Rao (2009-2011)



Dr. Ch. Mohan Rao combines biophysical, molecular biological and cell biological approaches to address problems of biomedical importance. His research interests include protein folding, molecular chaperones and heat shock proteins, molecular basis of eye diseases such as, cataract and keratitis and retinopathy, photodynamic therapy for eye diseases and cancer, DNA based diagnostics, Microfluidics and Nanobiology. His recent research addresses role of small heat shock proteins in gene expression, cell division, differentiation and apoptosis.

He is the President of the Andhra Pradesh Akademi of Sciences; Fellow of The World Academy of Sciences, Trieste, Italy; Fellow of Indian National Science Academy, Fellow of National Academy of Sciences, India; Fellow of Indian Academy of Science and Andhra Pradesh Akademi of Sciences. He is elected as a member of the Council of International Union of Pure and Applied Biophysics (IUPAB). He has been also a member of the Council of Federation of Asian and Oceanian Biochemists and Molecular Biologists (FAOBMB). He is recipient of several awards including Shanthi Swarup Bhatnagar Prize (1999) and the Ranbaxy Award for Basic Medical Sciences (2000). He has been elected as "J C Bose National Fellow". He was awarded Honorary Doctor of Science by Kakatiya University. He is a member of editorial boards of scientific journals and Section Editor for BBA-Proteins and Proteomics.

He has obtained his PhD from the University of Hyderabad in Chemistry. Later he joined CCMB and initiated intact lens spectroscopic investigation. During 1990-92, he visited National Eye Institute of the National Institutes of Health, Bethesda, USA. He was a visiting Professor at the Tokyo Science University, Japan during 1996, Visiting Scientist at the University of Texas Medical Branch, Galveston, USA during 2000, Visiting Professor at the Institute for Protein Research, Osaka, Japan during 2002, JSPS invitation fellow at the University of Tokyo, 2004.

In addition to his research activity, he has been playing a significant role in popularizing science among the school children, science teachers and uninitiated public in general. He is Honorary President of Jana Vignana Vedika (JVV), a "science for the people" movement. Government of India recognized the efforts of JVV with a National Award for outstanding efforts in science and technology communication (2005), presented by Dr Abdul Kalam, the then president of India.

He is presently the Director of Centre for Cellular and Molecular Biology, Hyderabad.

K. V. R. Chary (2011-2013)



Prof. Chary is a Senior Professor at Tata Institute of Fundamental Research Mumbai/Hyderabad. He has made significant contributions in the areas of Molecular Biophysics, Biological Chemistry, Structural Biology and Nuclear Magnetic Resonance Spectroscopy. His research is primarily focused on the investigation of the 3D structures and properties of biological molecules in atomic detail and their correlation with biological activity. His work on biologically rare DNA forms unveiled for the first time, structural insights on duplex and triplex mismatches, hair-pin bends and strand disproportionation in adopting triplex structures. Most intriguingly, he showed that triplexes are subject to strand slippages in one setting, while in another the same act as a rigid hindrance for incoming DNA replication (An un-anticipated DNA dynamics). He demonstrated the capability of solving complete 3D solution structures of proteins by NMR in India for the first time and continues to lead this pursuit. He decoded the implications of calcium-binding proteins from *Entamoeba* and archaea in pathogenicity. The archeal protein was shown to be an ancient, single domain α -crystallin, with its uncanny similarity to mammalian lens crystallins, a result of divergent evolution. He demonstrated myristoylation-dependent plasticity and signaling of a neuronal calcium sensor, a protein implicated in neurophysiology. He identified a protein from a halophile as intrinsically disordered, which acquires a typical α -crystallin fold upon Ca^{2+} -binding. He demonstrated conformational heterogeneity and dynamics in this protein when it is natively unfolded. In these endeavours, several NMR methodologies were developed, which speed up both acquisition and analysis of multidimensional NMR data by several orders of magnitude and push the boundary of NMR capability. To sum up, Chary's research work elegantly combines high-resolution 3D structural and dynamics information with that of judicious mix of other supporting biophysical tools to achieve a wholesome understanding of the system on hand. Several of these approaches have yielded first time milestones.

Chary has co-authored a book entitled "NMR in Biological Systems: From Molecules to Humans" (Springer). In addition to his contributions to fundamental research, he continues to take very keen interest in science education and induction of young members into mainstream fundamental research. He has guided several Ph.D students and Post-doctoral Fellows. He has been regularly invited to speak at various national and international conferences and work-shops. Chary has been involved in many international activities, such as organizing International Conferences, workshops and training scientists. He has taught in India, Europe, China, Japan and Taiwan. He played major roles in international bodies such as ICMRBS (International Council on Magnetic resonance in Biological Systems), IUPAB (International Union of Pure and Applied Biophysics), ABA (Asian Biophysics Association), Royal Society of Chemistry (West India Section) and Marie Curie International Research Staff Exchange Scheme of European Union. Chary is an elected Fellow of The World Academy of Sciences, Indian National Science Academy, New Delhi; Andhra Pradesh Academy of Sciences and National Academy of Sciences, Allahabad. He is also J.C. Bose National Fellow of Department of Science and Technology, Government of India, New Delhi.

T. P. Singh (2013- till date)



Dr. T. P. Singh works in the Department of Biophysics at All India Institute of Medical Sciences, New Delhi as a DBT-Distinguished Biotechnology Research Professor. He obtained his M.Sc. degree from University of Allahabad in 1971 and Ph.D. from Indian Institute of Science, Bangalore in 1975 on Structure -Function Studies of Analgesics/anti-inflammatory Agents. He worked as an Alexander von Humboldt Foundation and Max-Planck post-doctoral Fellow in the Max-Planck Institute for Biochemistry in Martinsried, Germany during 1978-80. He has been Professor and Head of the Department of Biophysics at All India Institute of Medical sciences, New Delhi during 1984-2006. He was conferred the degree of Doctor of Science, D.Sc. (*h.c.*) by the Karnataka State Open University, Mysore in 2010. He is the Life-Long Distinguished Professor of the University of Mysore since 2014.

He is a Fellow of the TWAS, the Academy of Sciences for the Developing World (FTWAS), the Indian National Science Academy New Delhi (FNA), the Academy of Sciences, Bangalore (FASc), the National Academy of Sciences, Allahabad (FNASc) and the Biotec Research Society of India (FBRs).

His recognitions include Professor D.M. Bose (129th Birthday) Memorial Lecture of Bose Institute, Kolkata-2013, the 52nd Foundation Day Dr.S.P. Agharkar Oration of the Agharkar Research Institute (DST), Pune - 2012, Bhramara's Y.T. Thathachari Award in Life Sciences, Mysore -2012, Jawaharlal Nehru Birth Centenary Lecture Award of INSA - 2011, Annual Award of the Instrumentation Society of India-2011, CSIR Foundation day Lecture Award of IMTECH, Chandigarh-2010, Goyal Prize in Life Sciences, 2007, Professor G.N. Ramachandran CSIR Gold Medal for Excellence in Biological Sciences and Technology-2006, Professor G.N. Ramachandran 60th Birthday Commemoration INSA Medal-2006, Sir J.C. Bose Memorial Award of the Indian Science Congress-2006, K.K. Foundation award for Science and Technology, 2001, etc. He obtained the First Rank in M.Sc. in the University of Allahabad in 1971.

He was the Editor-in-Chief of INSA journal 'Proceedings of the Indian National science Academy', New Delhi (2011-13). He was the President of the Indian Crystallographic Association (2010-13). He was a Vice President of the Indian National Science Academy, New Delhi (2007-09). He is currently the President of the Indian Biophysical Society.

He has guided a large number of Ph.D. students and published extensively in the field of structural biology and ligand design. His fields of research interests include X-ray Crystallography, Protein structure determination, ligand and Peptide Design and Drug Discovery.

INDIAN BIOPHYSICAL SOCIETY (IBS) – ITS GROWTH IN 50 YEARS

In the early days of Indian science, the great and visionary scientist, J. C. Bose realized the importance of physics in biology. Later scientists like N. N. Dasgupta, R. K. Poddar and A. R. Gopala Ayengar played a key role in various activities of biophysics research and teaching. In early 1950s, G. N. Ramachandran at the University of Madras developed a laboratory for studying structures of biologically important molecules using of x-ray crystallography. From his laboratory, the pioneering work of the triple helical structure of collagen and the famous Ramachandran plot (used by all protein crystallographers for predicting the protein secondary structures) emerged, which put India on the world map on Biophysics. By this time, in several other parts of the country, physicists and biologists working in different areas found it relevant to apply their expertise in diverse problems of biophysical research.

In 1965, the Indian Biophysical Society (IBS) was formed and registered under Act XXVI of 1961 at Kolkata with its office at Saha Institute of Nuclear Physics (SINP). The memorandum of association drafted by the founder members included various rules and regulations of the IBS. The founder-members of IBS comprised of Prof. D. M. Bose as the President, Prof. N. N. Dasgupta and Prof. B. Mukherjee as Vice-Presidents, Prof. N. N. Saha as the Secretary, Prof. B. D. Nagchaudhuri as the Treasurer while the members of the executive council were Prof. A. K. Saha, Prof. M. N. Rao and Prof. S. N. Chatterjee. The council decided that IBS would hold its National Symposium every year in collaboration with different host Institutions in the country and Prof. N. N. Saha hosted the first three annual IBS symposia at SINP in Kolkata since 1965. Thereafter, the IBS symposia were hosted by other institutions like University of Gorakhpur,

Lucknow University, Tata Memorial Centre, Mumbai and Panjab University, Chandigarh etc. Thus IBS, slowly and steadily gained a true national character.

However, there was a temporary setback for few years from 1985 to 88. However, the society was revived again in 1989 due to the efforts of senior members like Prof. Girjesh Govil, and Prof. Anil Saran with a National Biophysics Symposium held at BHU, Varanasi in 1989. At this an adhoc executive council was formed with Prof R. Srinivasan as the President and Dr. B. B. Singh as the Secretary. The next meeting was organised at Kolkata (then Calcutta) by Prof. S. N. Chatterjee, a founder member of the Society at SNIP in 1990. It was during 1991-1993, the Society has a duly elected executive council with the keen interest taken collectively by Prof. G. Govil, Prof. M. Vijayan, Dr. B. B. Singh and Prof. Anil Saran. Since then, the IBS meetings are hosted annually by various Universities and research Institutes all over the country. Since then, the Society became very active and grew in strength year by year and presently IBS is now one of the healthiest and most active scientific societies of India and is a platform where youngsters are given ample opportunity to present their work and discuss freely with their senior colleagues.

Over the years, the members of IBS have also developed the quality to respect each other while deliberating on diverse views. IBS functions in such a way that most of the important decisions at the Executive Council (EC) and the Annual General Body (AGB) meetings are arrived at by consensus in a friendly environment. While deliberating on the venues of the IBS Symposia and the possible Executive Council (EC) Members, special care is taken to see that all parts of India as well as all disciplines under biophysics research are represented. At present, the Society

has expanded considerably and there are over 900 Life Members from all over the country. The IBS President's has a two-year term in accordance with the society norms. The IBS Presidents elected sine 1989 were: Late Prof. R. Srinivasan (University of Madras; 1989-91), Dr. B. B. Singh (BARC; 1991-1993), Prof. M. Vijayan (IISc; 1993-1995), Prof. D. Balasubramanian (CCMB; 1995-97), Prof. Girjesh Govil (TIFR; 1997-99), Prof. Anil Saran (TIFR; 1999-2001), Prof. Rabi Majumdar (SINP; 2001-2003), Prof. R. V. Hosur (TIFR; 2003-2005), Dr. K. P. Mishra (BARC; 2005-2007), Prof. N. R. Jagannathan (AIIMS; 2007-2009), Dr. Ch. Mohan Rao (CCMB; 2009-2011), Prof. K. V. R. Chary (TIFR, 2011-13) and presently Prof T. P. Singh (AIIMS; 2014- 15). One may visit the IBS website at 'indianbiophysicalsociety.org' for more information on the current council members, list of life members, upcoming symposia and other academic programs of the society.

The biophysics research in India during the last two decades has spread to all parts of the country and many laboratories have facilities that are of international standard for carrying out advanced biophysical research. Efforts have been made to host the annual meeting of the IBS at smaller cities like Pune, Ananatapur, Roorkee, etc to take Biophysics far and wide across India. The IBS has instituted many awards for youngsters by giving cash awards with certificates for poster presentations in the National Symposium every year. These awards are named after great biophysicists of the country namely, D. M. Bose, N. N. Dasgupta, N. N. Saha, A. R. Gopala Ayengar, K. S. Korgaonkar, S. N. Chatterjee, V. S. R. Rao and R. Srinivasan. All these awards are of equal value. Further, IBS had established an award for a Young Scientist since 2006 to commemorate the memory of Prof. Ratna Phadke, a distinguished scientist in the field of Molecular Biophysics and is given to a young scientist below 35 years, during the IBS Annual Symposium. The IBS also gives two travel awards to cover partial expenses for attending the IUPAB International Biophysics Congress held abroad once every three years and the Asian Biophysics Association (ABA) meeting, which is held once in two years. These awards are: Prof. J. C. Bose award for senior scientists above 35 years age and Prof. G. N. Ramachandran award for younger scientists below 35 years age.

In the year 2002, IBS also instituted a prestigious Prof. G. N. Ramachandran (GNR) Memorial Lecture to be given by a renowned biophysicist at each annual IBS meeting. The first GNR Memorial Lecture was held at the IBS-2002 National Symposium in January, 2002 at the University of Madras, where GNR started his pioneering work. This lecture was delivered by Prof. V. Ramakrishnan from UK on *Crystal Structure of the 30S Ribosomal Subunit*. It is pertinent to point out here that Prof. Ramakrishnan eventually shared the Nobel Prize with Prof. Thomas A. Steitz and Prof. Ada E. Yonath in 2009. The others who gave subsequent GNR Memorial Lectures so far include the Nobel Laureates Prof. Hartmut Michel (2004), Prof. Kurt Wuthrich (2005), Prof. Richard R. Ernst (2006), Prof. Martin Karplus (2009) and Prof. Ada E. Yonath (2011), and others like Prof. Wim G J Hol (2003), Prof. Seji Ogawa (2007), Prof. Tom Blundell (2008), Prof. Robert H. Austin (2010), Prof. Chris Dobson (2012), Prof. Nenad Ben (2013) and Prof. S. Mayor (2014). All the GNR awardees are made Honorary Fellows of the Indian Biophysical Society. Prof. G. Govil and Prof. M. Vijayan would be delivering the GNR lecture at the IBS 2015 at New Delhi.

IBS fosters active cordial relation with the Indian National Science Academy (INSA) and the International Union of Pure & Applied Physics (IUPAB) and Prof. G. Govil and Prof. M. Vijayan have been instrumental in forging this link. The President of IBS is a member of the INSA National Committee for IUPAB, which helps the process of continuing cooperation between these three important bodies. As a result, *the XIII International Biophysics Congress* was successfully organized by INSA, IBS, and IUPAB at New Delhi in September, 1999 with Prof. Govil as Convener and Prof. Anil Saran as the Scientific Secretary. This was followed by three satellite symposia in Mumbai, Kolkata and Hyderabad. The proceedings of the Kolkata satellite symposium on *Structural Biology and Molecular Recognition* were later published in the Indian Journal of Biochemistry and Biophysics (IJBB). This activity put the IBS on a stronger footing and the number of Life Members of the Society shot up from about 300 to nearly 500 around that time. In 2003, the IBS meeting was held at IIT Roorkee that

contained a two-day exclusive IUPAB International Workshop on *Education and Capacity Building in Biophysics: Needs of the Asian African Region*, which was attended by many participants from Asian and African countries. In 2009, an International Workshop on NMR in Biological Systems held at TIFR Mumbai, under the aegis of IUPAB.

India is represented well in various committees and Task Force of IUPAB, namely, task force on Application of Biophysics (Prof. N. R. Jagannathan), Bio-informatics (Prof. M. Vijayan), Biomedical Spectroscopy (Prof. G. Govil), and NMR in Biological Systems (Prof. K. V. R. Chary). Working in these Task Forces, our colleagues have made significant contributions in the formulation and steering of Task Force programmes. We take pride that Prof. G. Govil served as member of the IUPAB council and also as Vice-President while Profs. M. Vijayan, T. P. Singh and N. R. Jagannathan served as elected members of the Council of IUPAB for a period of 6 years term, each. Presently India is represented by Dr. Ch. Mohan Rao in the Council for IUPAB. Recently, IUPAB along with the publisher M/s. Springer has launched a new journal "*Biophysical Reviews*" with Prof. Jean Garnier as Editor-in-Chief with Prof. N. R. Jagannathan from India as one of the members of the Editorial Board.

IBS also has very close association with the Biophysics Societies/Associations of Asian countries thanks to the efforts of Prof. Girjesh Govil, who was instrumental in establishing a platform for cooperation between the scientists belonging to different countries in the Asian

region. This gave birth to Asian Biophysics Association (ABA), with its principal members from Australia, China, Hong Kong, India, Japan, Korea and Taiwan. ABA provides a greater scope for cooperation between scientists in the Asian region. The most important outcome of this effort is in winning the bid to hold the ABA Conference in New Delhi during January/February 2011, which was organized jointly by IBS, INSA and AIIMS by Prof. N.R. Jagannathan. In 2011, Prof. N. R. Jagannathan was elected as President of ABA and he served ABA till 2013. At the steering committee meeting at Jeju Island, Korea held in May 2013, Prof. K. V. R. Chary was elected as Treasurer of ABA and India has been assigned to have the ABA office. Accordingly due to the efforts of Prof. K. V. R. Chary, very recently the ABA office has been started at Hyderabad. Presently the ABA steering committee members from India includes the IBS President (Ex-officio), Prof. K.V.R. Chary and Prof. N. R. Jagannathan (Honorary Member).

Not though exhaustive, in this write up, we have given some glimpses of the growth of IBS and very briefly the biophysics research activities in India in the last 50 years. We sincerely hope that the younger generation of excellent scientists that we have in India, the IBS and the biophysics research, will reach greater heights.

February, 2015

Anil Saran
(President; 1999-2001)

N. R. Jagannathan
(President; 2007-09)

National Symposium on Biophysics and Golden Jubilee Meeting of the Indian Biophysical Society

February 14-17, 2015

Programme Details

Day 1: February 14, 2015 (Saturday)

14:00 to 15:00

Registration

15:00-16:00

Inaugural Session

Professor G.N. Ramachandran Memorial Lecture I

16:00-16:45

Prof. M. Vijayan, Indian Institute of Science, Bangalore

Research with an Indian Flavour: Structural Biology of Mycobacterial Proteins

16:45-17:15

HIGH TEA

Academic and Industrial Scientific Session

17:15-17:45

K. P. Mishra, BARC, Mumbai

Electroporation mediated anticancer drug delivery: biophysical basis and application in cancer treatment

17:45-18:15

Francois Horréard, CAMECA, Gennevilliers, France

The NanoSIMS 50L: chemical and isotopic imaging & analysis down to intracellular level

18:15-18:30

Waters (India) Private Limited
TA Instruments Division

18:30-19:00

EC Meeting of IBS

19:00-20:00

General Body Meeting of IBS

20:00-21:00

DINNER

Day 2: February 15, 2015 (Sunday)

8:30 to 9:30

BREAKFAST

Session I

Protein Structure and Dynamics

Chair: KVR Chary, TIFR, Mumbai

R. V. Hosur, CEBS, Mumbai

Nano-Biotechnology

Chair: D. N. Rao, AIIMS, New Delhi

Arun K. Attri, JNU, New Delhi

9:30 to 10:00

B. Gopal, IISc Bangalore

A structural enzymology approach to understand cell wall synthesis in gram positive bacteria

D. K. Dhawan, Panjab University, Chandigarh

Radio-labeled Resveratrol loaded Gold Nanoparticles as a Cancer Targeting Probe

10:00 to 10:30	Karthe Punnoraj, Madras University, Chennai <i>Crystal structure of the keratin 4 binding domain of serine rich repeat protein-1 (Srr-1) from Streptococcus agalactiae reveals an unexpected dimeric assembly through β-sheet complementation and provides hints on multi-ligand binding mode of Srr-1</i>	Mohd. Owais, AMU, Aligarh <i>Multifunctional nanosystems: growing sanguinity in development of particulate antigen delivery vehicle based vaccines</i>
10:30 to 11:00	Monica Sundd, NII, New Delhi <i>The structure of the holo-acyl carrier protein of Leishmania major displays a remarkably different phosphopantetheinyl transferase (PPTase) binding interface</i>	Avinash Bajaj, RCB, Gurgaon <i>Bile Acid Amphiphiles Targeting Cellular Membranes for Next Generation Therapeutics</i>
11:00 to 11:30	TEA	
Session II	Nucleic Acid Structure and Dynamics <i>Chair: M.V. Hosur, ACTREC, Mumbai S. Kukreti, Delhi University</i>	Computational Biology and Genome Informatics <i>Chair: Rabi Majumdar, Kolkata B. Gopal, IISC Bangalore</i>
11:30 to 12:00	Deepak T. Nair, RCB, Gurgaon <i>DNA polymerase IV and Oxidative stress</i>	Debashis Mohanty, NII, New Delhi <i>In silico approach for deciphering substrate specificity of PDZ domains</i>
12:00 to 12:30	Souvik Maiti, IGIB, New Delhi <i>Combinatorial role of two G-quadruplexes in 5' Untranslated region of Transforming Growth Factor β2 (TGFβ2)</i>	Shailza Singh, NCCS, Pune <i>Engineering signal transduction pathways through synthetic modular systems: Dissecting the puzzle in infectious disease</i>
12:30 to 13:00	AS Ethayathulla, AIIMS, New Delhi <i>DNA binding specificity of p73 DNA binding domain with different p53 response elements</i>	Ranjit P. Bahadur, IIT Kharagpur <i>Probing binding hot spots at protein-RNA recognition sites</i>
13:00 to 13:30	Sobhan Sen, JNU, New Delhi <i>Monitoring Ligand Association and Dissociation Kinetics with G-quadruplex-DNA in Solution at Single Molecule Level: Role of Water on Ligand Binding to DNA</i>	C. Gopi Mohan, Amrita Vishwa Vidyapeetham University, Kochi <i>Understanding the Structure-Function Relationship of Lysozyme Resistance in Staphylococcus aureus Using Molecular Docking, Dynamics and Lysis Assay</i>
13:30 to 14:30	LUNCH	
Session III	Recent Advances in Biophysics <i>Chair: R. P. Roy, NII, New Delhi Anil Saran, TIFR, Mumbai</i>	Recent Advances in Biophysics <i>Chair: N. R. Jagannathan, AIIMS, New Delhi K. P. Mishra, BARC, Mumbai</i>
14:30 to 15:00	Lipi Thukral, IGIB, New Delhi <i>Understanding "split personality" of lipidated protein using molecular dynamics simulations</i>	P. K. Madhu, TIFR, Mumbai <i>NMR investigations of fibrils and transient oligomers of Aβ peptide</i>
15:00 to 15:30	Lipika Adhaya, B. P. Poddar Institute of Management and Technology, Kolkata <i>Bio-electrostatics of the voltage gated potassium ion channel in neuron</i>	Suneel Kateriya, Delhi University, New Delhi <i>Biophysical Characterization of Natural and Engineered Optogenetic Tools</i>
15:30 to 16:00	G. Hariprasad, AIIMS, New Delhi <i>Comparative proteomic analysis of advanced ovarian cancer tissue to identify potential biomarkers of responders and non-responders to first line chemotherapy of carboplatin and paclitaxel</i>	Gopala Krishna, IIT Madras, Chennai <i>Non-canonical EF-hand Ca²⁺ binding sites act as structural switches in Calumenin</i>

Satish Kumar, IVRI, Bareilly
Peptide and PNA Activated AuNPs Plasmon Changes in Visual Detection and Quantification of Virus and Virus Genes

16:00 to 16:30	S. Aravamudhan, NEHU, Shillong <i>Estimating Ring-current Contributions by Quantum Chemical shift Calculations</i> Praveen Singh, IVRI, Bareilly <i>Gold Nanoparticles-Plasmonic Biosensor For Real-Time Detection Of Peste Des Petits Ruminants Antigen</i>	Ashish Arora, CSIR-CDRI, Lucknow <i>NMR Structures of Proteins with Actin Depolymerization Factor Homology, Domain</i> K.D. Sonawane, Shivaji University, Kolhapur <i>Molecular modeling techniques to understand structural significance of biomolecules</i>
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16:30 to 17:00 **TEA**

Session IV	Students Oral Presentation <i>Chair: Anuman Lahiri, Calcutta University</i> <i>G. Hairsh Kumar, DST, New Delhi</i>	Students Oral Presentation <i>Chair: Yogendra Sharma, CCMB, Hyderabad</i> <i>S N Sanyal, Punjab University, Chandigarh</i>
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17:00 to 19:00 **OP1 to OP16** **OP17 to OP32**

19:30 to 21:30 **DINNER**

Day 3: February 16, 2015 (Monday)

8:30 to 9:30 **BREAKFAST**

Session V	Macromolecular Assembly <i>Chair: C. S. Dey, IIT Delhi</i> <i>M. V. Jagannadham, BHU</i>	Biophysics of Cell Signaling <i>Chair: Pawan Malhotra, ICGEB, New Delhi</i> <i>J. K. Batra, NII, New Delhi</i>
9:30 to 10:00	Manidipa Banerjee, IIT Delhi <i>Characterization of Membrane-Active Peptide Components of Hepatitis A Virus (Hav)</i>	B. Anand, IIT Guwahati, Guwahati <i>Functional Insights into the Mechanism of Prokaryotic Specific RNA Guided Gene Targeting CRISPR-Cas system</i>
10:00 to 10:30	Asish Ganguly, IMTECH, Chandigarh <i>Watching the acrobatics of actin de-/polymerizing protein, Gelsolin by SAXS/SANS</i>	Sneha Sudha Komath, JNU, New Delhi <i>Ras dynamics and its dependence on sterol in fungal membranes-a hypothesis on Ras hyperactivation in GPI biosynthesis mutants</i>
10:30 to 11:00	Jeetender Chugh, IISER Pune <i>Visualizing Transient Structures in A-site RNA of the Ribosome: New Structures of Known Molecules for Drug Target</i>	Rajanish Giri, IIT Mandi <i>Proteins without structure and deviations from structure function paradigm</i>

11:00 to 11:30 **INDUSTRIAL TALK**

Session VI	Protein Folding and Diseases <i>Chair: M.N. Gupta, IIT Delhi, New Delhi</i> <i>Rajiv Bhat, JNU, New Delhi</i>	Nucleic Acid Biophysics <i>Chair: N. Chakraborty, NIPGR, New Delhi</i> <i>Rakesh Bhatnagar, JNU, New Delhi</i>
11:30 to 12:00	Kaushik Sengupta, SINP, Kolkata <i>Role of LMNA mutations in higher order protein assembly: A possible mechanism in the pathogenesis of Dilated Cardiomyopathy (DCM)</i>	Pradeep Kumar, IIT Bombay, Mumbai <i>Specific Detection and Stabilization of G-Quadruplex DNAs by Designer Molecules</i>

12:00 to 12:30	Samir K. Maji, IIT Mumbai <i>Understanding the mechanism of α-synuclein aggregation associated with Parkinson's disease</i>	Ranjan Sen, CDFD, Hyderabad <i>Mechanism of transformation of a transcription elongation/termination factor, NusA, into a transcription antiterminator by the bacteriophage protein N</i>
12:30 to 13:00	Kausik Chakraborty, IGIB, New Delhi <i>Chemical chaperones and their mechanism of action</i>	Md. Faiz Ahmad, Case Western, Research University, Cleveland, USA <i>The Structural Basis for the allosteric regulation of one of life's most essential enzymes, the ribonucleotide reductase</i>
13:00 to 13:30	Sashank Deep, IIT Delhi, New Delhi <i>Curcumin binds to the amyloid intermediates of Cu/Zn Superoxide Dismutase (SOD1) converting them into less toxic species</i>	M. Rajeshwari, AIIMS, New Delhi <i>Role of DNA Triple Helical Structures In Neurodegenerative Disorders</i>

13:30 to 14:30 LUNCH

Session VII POSTER SESSION

14:30 to 16:30 Poster Presentation

16:30 to 17:00 TEA

Session VIII	Cutting-Edge Methods in Biophysics Chair: <i>Purnananda Guptasarma, IISER Mohali</i> <i>Karthe Ponnuraj, University of Madras, Chennai</i>	Young Investigators & Industrial Talk Chair: <i>P. C. Sharma, Indraprastha University</i> <i>Harpreet Singh, ICMR, New Delhi</i> <i>(10 minutes Each)</i>
17:00 to 17:30	Yogendra Sharma, CSIR-CCMB Hyderabad <i>Archaeal $\beta\gamma$-crystallins: may be twins but one of a kind</i>	Amresh Prakash, Jamia Millia Islamia, New Delhi Molecular Dynamics Simulation study of β -Lactoglobulin with co-solvent to reveal structural transition of equilibrium intermediate
17:30 to 18:00	Tapan K. Chaudhuri, IIT Delhi <i>Irreversible unfolding of a large Escherichia coli protein Maltodextrin Glucosidase</i>	D. Zareena, JNTUA Anantpur Structural and functional analysis of novel antimicrobial peptides
18:00 to 18:30	S. N. Sanyal, Panjab University <i>Telomerase as a chemopreventive molecular target in colon cancer</i>	Himanshu Ojha, INMAS, N. Delhi Development of 3D-pharmacophore model followed by successive virtual screening, molecular docking and ADME studies for the design of novel HDAC inhibitors.
18:30 to 18:45	N.C. Maiti, IICB, Kolkata <i>Physicochemical Aspects of Amyloidogenic and Non-amyloidogenic Intrinsically Disordered and Structured Human Proteins</i>	Sujoy Mukherjee, CSIR - IICB, Kolkata Correlation of conformational dynamics with the pathogenicity in an amyloid forming protein
18:45 to 19:00	V. Krishnan, RCB, Gurgaon <i>X-ray diffraction analysis of two crystal forms of backbone pilin SpaA fragment from Lactobacillus rhamnosus GG</i>	Indrakant Singh, Deshbandhu College, N. Delhi Identification of probable lead compounds for inhibition of CRY protein in Pisum sativum by structure modeling and pharmacophore designing Nitesh K. Poddar, Invertis University, Bareilly Unfolding of HIV TAR"DNA Hairpin: a Single Molecule FRET studies Rajan Patel, Jamia Millia Islamia, New Delhi An Insight in to the Binding of Pyrrolidinium Based Ionic Liquid with Human Serum Albumin

Rajendra V Joshi SM College, Surat

Are Frequent Exposures to Electromagnetic Field Radiation Emitted by Cellular Phone Base Station Affecting Autonomic Function in Human Being?

Sudip Majumder, ASAS, AMITY Manesar

Structural & Functional aspects of Trypsin-Gold Nanoparticle Bioconjugates: Stoichiometry determines Efficiency

Timir Tripathi, NEHU, Shillong

Dynamics of thiol-based redox switches in the parasitic flatworm Fasciola gigantica

V. Tiwari, Central University of Rajasthan

Alternate drug for carba penem resistant strain of Acinetobacter baumannii

19:30 to 21:30**DINNER****Day 4: February 17, 2015 (Tuesday)****8:30 to 9:30****BREAKFAST****Session IX****Advances in Structural Biology**

*Chair: Ashish Arora, CDRI, Lucknow
Faizan Ahmad, JMI, New Delhi*

Methods in Biophysics and Bioinformatics

*Chair: Ranjan Sen, CDFD, Hyderabad
D. Bhattacharyya, SINP, Kolkata*

9:30 to 10:00

Sri Rama Koti, TIFR Mumbai

Quantification of Protein Dynamics in terms of Flexibility/Rigidity by Single-Molecule Force Spectroscopy Experiments

B. Jayaram, IIT Delhi

Genomes to Hit Molecules In Silico: A country path today, a highway tomorrow- An update

10:00 to 10:30

Neeraj Sinha, Centre of Biomedical Research, Lucknow

Structural and interaction study of collagen protein in native state by solid state NMR spectroscopy

Saachi Gosawi, NCBS, Bangalore

Understanding conformational changes using structure-based models of proteins

10:30 to 11:00

Krishna Mohan Poluri, IIT Roorkee

Differential Glycosaminoglycan Interactions of Murine and Human CXCL1 Proteins

Surat Kumar, Dayalbagh Educational Institute, Agra

Computation Complimenting Experimentation: Vinca Alkaloid Binding to DNA

11:00 to 11:30**TEA****Session X****Students Oral Presentation**

*Chair: P M Dongre, University of Mumbai, Mumbai
M. Aman Jairajpuri, JMI, New Delhi*

Students Oral Presentation

*Chair: Lallan Mishra, BHU
P. K. Madhu, TIFR, Mumbai*

11:30 to 13:30**OP33 to OP48****OP49 to OP64****13:30 to 14:30****LUNCH**

Session XI	Neuro-Biophysics Chair: <i>Subhendu Ghosh, Delhi University</i> <i>D. K. Dhawan, Panjab University, Chandigarh</i>	Molecular Structure and Dynamics Chair: <i>Sher Ali, NII, New Delhi</i> <i>Punit Kaur, AIIMS, N. Delhi</i>
14:30 to 15:00	Anant Bahadur Patel, CCMB, Hyderabad <i>¹³C NMR Studies of Brain Energy Metabolism in Parkinson's Disease</i>	R. Swaminathan, IIT Guwhati <i>Crowding by Specific size of Dextran switches the Substrate Specificity of Acetylcholinesterase enzyme</i>
15:00 to 15:30	M. Venkata Satish Kumar, Tejpur University <i>Inhibition of amyloid fibrils formation as well as disassembly of amyloid fibrils in Alzheimer's disease using the poly-ion, single stranded nucleotide sequence: an in silico Study</i>	Bechan Sharma, University of Allahabad, Allahabad <i>Mechanistic aspect of drug resistance in HIV-1 and new targets for drug design and development</i>
15:30 to 16:00	B. Padmanabhan, NIMHANS, Bangalore <i>Structure Biology of the Epigenetic Marker, BRD2</i>	Avinash Kale, CEBS, Mumbai <i>Structural elucidation of the resolvase activity by RecU from B. subtilis</i>
16:00 to 16:15	J. K. Yadav, Central University of Rajasthan, Ajmer <i>Effect of N-terminal modifications on Aβ amyloid formation</i>	Vibha Tandon, JNU, New Delhi <i>Novel Smart C2-Symmetrical NDI Derivatives as G-Quadruplex Stabilizing Ligand with a Potential to Differentiate between Topological Structures</i>
16:15 to 16:30	Manisha Chug, NBRC, Manesar <i>Somatosensory function is affected by stroke-like injury in motor cortex</i>	Arun Shukla, IIT Kanpur <i>Structural basis of β-arrestin interaction with G Protein-Coupled Receptors</i>

16:30 to 17:00

TEA

Session XII

CONCLUDING SESSION

Professor G.N. Ramachandran Memorial Lecture II

17:00-17:45 **Prof. Grijesh Govil, TIFR Mumbai**
Nuclear Magnetic Resonance in Biology: Application to Study Cell Biology

17:45 to 18:15 **Concluding Remarks and Prize Distribution**

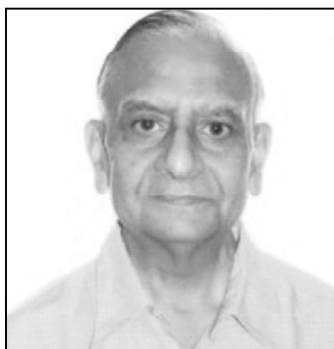
18:15 to 19:00 **High Tea**
Certificate Distribution

G. N. Ramachandran (GNR) Memorial Lecture I

Nuclear Magnetic Resonance in Biology: Application to Study Cell Biology

Girjesh Govil

INSA Honorary Scientist, Tata Institute of Fundamental Research,
Mumbai 400 005



The talk is divided into three parts. First, we will talk about the history and the previous speakers of GNR Lectures organized by IBS and IUPAB. This will be followed by introduction to NMR and its applications in biological systems. Finally, we will focus on applications in cell-

biology, in particular spermatozoa.

NMR is based on the fact that some nuclei have spins and act as magnets. When placed in an external magnetic field (B_n), the spins can orient either parallel or anti-parallel to B_n . Interaction of the nuclear magnetic moment, with magnetic field leads to an energy level diagram, with different energies for the two states. One can change the magnetic orientation by a suitable RF field, leading to a transition from one state to the other. The transition can be detected through a detecting system and is called resonance signal.

Biological molecules are composed of six major elements C, H, N, O, P and S. Of these, the isotopes which are useful from NMR view point are ^1H , ^{13}C , ^{15}N , and ^{31}P . Unfortunately, O and S do not have isotopes suitable

for NMR. The resonance frequencies of these isotopes are widely different. At an applied magnetic field of 10 T, ^1H resonates at 426 MHz, ^{13}C at 107 MHz, ^{15}N at 43 MHz and ^{31}P at 172 MHz. One can study biology using NMR at several levels: Study of the structures of individual biological molecules such as proteins and nucleic acids; Study of cells and cell metabolism; and study of whole organs or living systems such as plants, animals and human (MRI and MRS).

After a brief introduction to NMR in biological systems, I shall concentrate on cellular NMR. Cells are the smallest unit of life. While uni-cellular systems are known, it is more important to study mammalian cells and changes in their metabolism that take place following stress, during medical treatment or during cell-development. My focus will be on the work done by my colleagues on spermatozoa. These cells derive energy through glycolysis. In the male reproductive track, the cells undergo maturation at three different levels. The cells are born in the caput region and mature as they pass through corpus. The cells are fully matured cells in the cauda region. There are significant changes in the levels of different metabolites during cell maturation. The changes can be detected using ^1H , ^{13}C , ^{15}N , and ^{31}P NMR. ^{31}P NMR from cells from the three regions shows cell-maturation. This is reflected in the level of ATP. ^1H NMR provides information on all the metabolites. The spectra are complex and require the use of 2D NMR spectroscopy. Analysis of the metabolic profile, show some unexpected molecules. Such studies are important in animal husbandry.

I shall conclude by giving major developments in NMR of biological systems and the Nobel Prizes in the field of NMR.

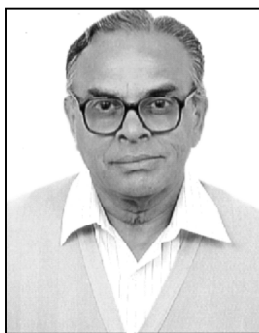
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G. N. Ramachandran (GNR) Memorial Lecture II

Research with an Indian Flavour. Structural Biology of Mycobacterial Proteins

M. Vijayan

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The outstanding contributions of G. N. Ramachandran and his colleagues in the fields of computational biology, modelling and crystallography gave a head start to research in structural biology in the country. One field in which he wished to initiate research, but could not, was biological macromolecular crystallography. Fittingly, one of the two schools he established,

namely, the Molecular Biophysics Unit at the Indian

Institute of Science, Bangalore, subsequently turned out to be the national nucleus for the development of this field in the country. By the turn of the century macromolecular crystallography in India had come of age and was poised to address specific issues with national relevance. One such issue was concerned with mycobacterial proteins with special emphasis on TB proteins. Structural biology of mycobacterial proteins is now being pursued in about ten centres in the country. India accounts for more than ten percent of the total number of TB proteins analysed in the world. The work on mycobacterial proteins being pursued in the author's laboratory encompass recombination and repair, translation, stringent response and specific pathways such as that for CoA biosynthesis. Work on mycobacterial lectins have also been recently initiated. In addition to illuminating several areas of mycobacterial biology, the structural biology efforts at different centres in India on mycobacterial proteins now provide a platform for structure based inhibitor design with the eventual aim of developing drugs.

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Industry Talk

The NanoSIMS 50L: chemical and isotopic imaging & analysis down to intracellular level

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The NanoSIMS is an imaging instrument delivering *Trace Element* and *Isotopic ratio* distribution maps down to 50nm lateral resolution. In life sciences these distribution maps are most of the time coupled with a labelling strategy: giving a plant or an animal nutrients, drugs or any molecules labelled with *one or several stable isotope(s) or/and chemical elements*. The fate of the labelled molecules is then imaged and measured through the local isotopic or elemental enrichment.

Such in-situ mappings and measurements down to sub-cellular level give a unique insight on localization, accumulation, trafficking, internalization of molecules in cells or tissues, turnover and fluxes of molecules. In addition to the greatly improved lateral resolution the stable isotope strategy avoids problems or limitations associated with fluorophores or radioactive labels.

In environmental microbiology coupling NanoSIMS with isotopic labelling gives access to *single cell metabolic activity*, permits unravelling complex symbioses and is applicable to *rare and uncultivable species*. Mapping of individual cell metabolic activity is generally *coupled* with in-situ HISH or external FISH (fluorescence in-situ hybridization) giving the *identity* of individual cells.

Method [1]: A beam of reactive Cs⁺ or O⁻ primary ions of down to 50nm size (= lateral resolution) is rastered on the surface of a sample. In order to increase sensitivity all molecules are broken and (much more numerous) atomic ions are detected instead. The sputtered secondary ions are collected and mass filtered by a magnetic sector mass analyzer allowing *parallel detection* of seven elements or isotopes, ensuring reliable isotopic ratio and perfect

image registration.

Each 2D image is obtained through the consumption of typically a few nanometers of the sample. The scanning is repeated and a 3D image can be reconstructed from the stack of images.

The biological samples must be prepared using transmission electron microscopy (TEM) methods: cells or tissues can be analyzed after dehydration or after fixation, resin embedding and microtoming. Harder materials (corals, bones) can be polished in order to obtain a flat surface.

Applications: We will show many applications from literature in life science from trace element mapping in plants and animal tissues to single cell level studies in environmental microbiology, including element fixation, exchanges, symbioses and metabolic pathways. Industrial application will be illustrated through cosmetic development. Cell biology applications will be illustrated by results in various fields including measurement of protein turnover at small scale [2], replication of cells after injury in tissues, lipid distribution on cell membrane and neuron protein synthesis study. Perspective in extending immunohistochemistry capabilities will be explained based on parallel labelling of antibodies on tissue sections. [3]

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Invited Speakers

¹³C NMR Studies of Brain Energy Metabolism in Parkinson's Disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide, affecting 1-2% of population above 60 years of age. The loss of dopamine in the basal ganglia due to the death of dopaminergic neurons in substantia nigra pars compacta is the central axis of PD. Glutamate and GABA, the major excitatory and inhibitory neurotransmitters in the matured central nervous system, are energetically supported by oxidative glucose metabolism [1, 2]. We are investigating neurotransmitter metabolism in chemical (manganese & MPTP) and genetic (Pitx3^{-/-}) models of PD by ¹H-¹³C-NMR spectroscopy together with an infusion of ¹³C labeled glucose or acetate. In addition, efficacy of different interventions (caffeine, levodopa and dietary restriction) for the improvement of brain energy metabolism in PD is being evaluated.

Metabolic analysis in mice have indicated that the glutamatergic and GABAergic TCA cycle and neurotransmitter cycle fluxes are very distinct across different brain regions [3]. The PD mice showed impaired motor function, a hall mark of the disease. The levels of striatal and olfactory bulb GABA were found to be elevated in these mice. The metabolic analysis revealed a widespread reduction in the excitatory and inhibitory activity in PD mice. The acute levodopa intervention in MPTP treated mice could recover the motor and neuronal activities completely but not the altered metabolite homeostasis [4]. Most interestingly, the altered neurometabolite homeostasis and impaired neuronal metabolism could be restored to normal level following dietary restriction in Pitx3^{-/-} mice. Our findings indicate that the elevated GABA level together with decreased neurometabolic activity could be used as biomarker for diagnosis of PD. In this presentation, I will be discussing these findings in detail.

Acknowledgement: This study was supported by funding from CSIR (BSC0115), Govt of India.

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Watching the acrobatics of actin de-/polymerizing protein, Gelsolin by SAXS/SANS

Ashish

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Gelsolin is a six-domain protein which can polymerize monomeric form of actin (G-actin) into its filamentous state (F-actin), and *vice versa*. Its seemingly contradictory functions are regulated by Ca²⁺ ions and low pH. Insight into how these functions are achieved by gelsolin is a challenge for biophysical chemists. In my talk, I will address how small angle X-ray and neutron scattering (SAXS/SANS) could be applied to understand large degree of shape changes, mainly repositioning of domains of gelsolin to achieve its activated shape and how that shape binds actin molecules. Overall, audience will get a preview of application of scattering methods to address biomolecular systems.

Bile Acid Amphiphiles Targeting Cellular Membranes for Next Generation Therapeutics

Avinash Bajaj

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Cellular membranes are attractive targets for engineering of new antibacterial, antiviral, anti-mycobacterial, and anticancer drugs as it is difficult for the bacterial and mammalian cells to generate resistance against these membrane targeting drugs. Mammalian, gram-positive bacteria, gram-negative bacteria, and mycobacteria present interest membrane characteristics that differ from each other in lipid composition, surface charge, and hydrophobicity. Amphiphilic molecules possess charged surfaces on hydrophobic scaffold that can disrupt cellular membranes through their binding via electrostatic and hydrophobic interactions. Bile Acid present interesting amphiphilic skeleton for engineering of membrane targeted bioactive molecules due to their diverse chemistry, concave structure, and interesting stereochemistry. In my talk, I would present how differential amphiphile-membrane interactions between bile acid based amphiphiles and mammalian/bacterial/mycobacterial membranes can be explored for generating specific anticancer and antibacterial activities. My talk would also highlight how the balance of electrostatic and hydrophobic interactions is critical for membrane specific bioactive molecules.

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Functional Insights into the Mechanism of Prokaryotic Specific RNA Guided Gene Targeting CRISPR-Cas system

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CRISPR-Cas system represents an adaptive and heritable immune system that defends the bacteria and archaea against the invasion of mobile genetic elements. Though this prokaryotic specific immune system is functionally analogous to the RNA interference (RNAi) in the higher organisms, it is mechanistically distinct. The interference mechanism proceeds via three distinct stages: acquisition of protospacer from the invading genetic elements, maturation of CRISPR RNA and RNA guided target recognition and cleavage. Using a combination of biophysical, biochemical and molecular genetics approaches, we have embarked to decipher the underlying mechanism by which the RNA guided targeting occurs. In this talk, I will discuss about the new insights obtained on the functioning of the ribonucleoprotein surveillance complex involved in this defense system.

A structural enzymology approach to understand cell wall synthesis in gram positive bacteria

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The peptidoglycan layer is a major component of the cell wall in gram-positive bacteria. This layer comprises of glycan chains and peptide stems consisting of both L and D-amino acids. The influence of the cell wall constituents on cell shape, motility, division, virulence and pathogenicity have been examined in several bacterial species. These studies reveal a remarkable complexity that facilitates bacterial adaptation to different micro-environments. The synthesis of the peptidoglycan layer is an elaborate process involving the choreographed action of two classes of enzymes – those that synthesize peptidoglycan precursors and enzymes that participate in cross-linking. This talk would describe the structural and functional characteristics of some of these enzymes.

Probing binding hot spots at protein-RNA recognition sites

Amita Barik^a, NithinC^a, Naga BhushanaRao K^b and Ranjit Prasad Bahadur^{a,b,1}

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Prediction of protein-RNA binding energy hot spots using sequence and structural attributes is still elusive. In this study, we use evolutionary conservation derived from the structural alignment of amino acid sequences along with the structural and physicochemical attributes of the protein-RNA interfaces to probe the binding hot spots at the protein surfaces that recognize RNA molecules. As expected, irrespective of the structural class of the complexes, residues at the RNA binding sites are evolutionary better conserved than those at the solvent exposed surface. However, we find some proteins in contact with RNA evolve rapidly compared to others. Complexes where the recognition involves duplex RNA, residues that interact with the major groove of the RNA are better conserved than those interact with the minor groove. We identified multi-interface residues, which participate simultaneously in protein-protein and protein-RNA interfaces in complexes where more than one polypeptide is involved in RNA recognition. These multi-interface residues are the most conserved compared to any other residue; and an interesting study would be to probe their contribution to the stability of the complex. Additionally, we find that the residues at the water preservation site are the most conserved followed by the residues at the hydrated and dehydrated sites. Finally, we developed a Random Forests model using the structural and physicochemical attributes to predict the binding hotspots. The model successfully predicts the –^oG values obtained from the alanine scanning experiments, and provide a stepping-stone towards the engineering of protein-RNA recognition sites with desired affinity.

Mechanistic aspect of drug resistance in HIV-1 and new targets for drug design and development

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Recently, WHO have reported that 35.3 million people were living with HIV/AIDS (PLWH) globally at the end of 2012 which included about 0.8% of adults aged 15-49 years. National AIDS Control Organization (NACO) of India has shown the prevalence of AIDS in India in 2013 to be 0.27 million. In the low and middle income countries

with resource limited settings, more than 8 million PLWH were receiving antiretroviral therapy (ART) at the end of 2011. Application of highly active antiretroviral therapy (HAART) worldwide has been able to significantly reduce the mortality and morbidity of human immunodeficiency virus type 1 (HIV-1) infected individuals. However, the appearance of clinical drug resistance in AIDS patients due to various factors including nonadherence to medication (intake of antiretroviral) has been found to be associated to chemotherapeutic and virologic failure. In addition, high rate of viral replication, appearance of heterogenous circulating viral quasispecies, infidelity in proviral cDNA synthesis as well as immunological and pharmacological factors are also associated to drug resistance. The present research paper describes our understanding on the mechanistic aspect of drug resistance in HIV-1 against the application of the second generation antiretrovirals and possible strategies to encounter the issue. The recent approaches to explore new targets for actions for specific actions of the antiretrovirals and to develop target-structure based new small molecules with least toxicity to the host would be discussed.

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Understanding the Structure-Function Relationship of Lysozyme Resistance in *Staphylococcus aureus* Using Molecular Docking, Dynamics and Lysis Assay

C. Gopi Mohan

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Lysozyme is an important component of the host innate defense system. It cleaves the α -1,4 glycosidic bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine of bacterial peptidoglycan and induce bacterial lysis. *Staphylococcus aureus* (*S. aureus*), an opportunistic commensal pathogen, is highly resistant to lysozyme, because of the *O*-acetylation of peptidoglycan by *O*-acetyl transferase (*OatA*). To understand the structure-function relationship of lysozyme resistance in *S. aureus* by peptidoglycan *O*-acetylation, we adapted an integrated computational and *in vitro* technique.

Our results indicated for the first time that the active site cleft of lysozyme binding with *O*-acetylated peptidoglycan in *S. aureus* was sterically hindered and the structural stability was higher for the lysozyme in complex with normal peptidoglycan. This could have conferred reduced survival of *S. aureus oatA* mutant in different human biological fluids. Consistent with this computational analysis, the experimental data confirmed decrease in the- growth, lysozyme induced lysis and

lysozyme resistance, due to peptidoglycan *O*-acetylation in *S. aureus*.

In silico approach for deciphering substrate specificity of PDZ domains

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PDZ domains are peptide recognition modules which mediate specific protein-protein interactions. Recent high throughput studies have revealed that, PDZ domains have a complex specificity landscape. Hence, prediction of their interaction partners has been a challenging task. We have developed a novel structure based multi-scale approach which identifies crucial specificity determining residues (SDRs) of PDZ domains from explicit solvent all atom molecular dynamics (MD) simulations on representative PDZ-peptide complexes and uses these SDRs in combination with knowledge-based scoring functions for developing genome scale approach for predicting interaction partners of PDZ domains. Multiple explicit solvent all atom simulations ranging from 5ns to 50ns duration have been carried out on 28 PDZ-peptide complexes with known binding affinities. MM/PBSA binding energy values calculated from these simulations show good correlation with the experimental binding affinities with a correlation coefficient of 0.719. Our analysis also shows that the SDRs of PDZ domains identified by MD simulations can be used to develop residue level statistical pair potential based approaches for predicting interaction partners of PDZ domains belonging to diverse families (1). We have benchmarked this multi-scale approach on mouse and human PDZ data sets available from peptide array and phage display experiments. In addition we have also attempted to extend such structure based approach for PDZ domains which recognize internal peptides.

We have also attempted to understand the structural basis of the small molecule-PDZ interactions by *in silico* analysis of the binding modes and binding affinities of a set of 38 small molecules with known K_i or K_d values for PDZ2 and PDZ3 domains of PSD-95 protein. Optimum binding modes for these ligands for PDZ2 and PDZ3 domains have been identified by using a novel combination of semi-flexible docking and explicit solvent molecular dynamics (MD) simulations. MM/PBSA binding free energy values show good agreement with experimental binding free energies with a correlation coefficient of approximately 0.6. Thus our study demonstrates that combined use of docking and MD simulations can help in identification of potent inhibitors of PDZ-peptide complexes (2).

DNA polymerase IV and Oxidative stress

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Reactive oxygen species arise due a variety of reasons and damage genomic DNA and the cellular nucleotide pool. The action of ROS leads to the appearance of 8oxodeoxyguanine (8oxodG) in DNA and replication across this lesion is generally mutagenic. DNA polymerase IV (PolIV) from *E. coli* is a translesion bypass enzyme. Although PolIV can accurately bypass the 8oxodG lesion, the same enzyme is also responsible for incorporation of oxidized dGTP (8oxodGTP) opposite template dA in newly synthesized DNA. The accurate bypass of 8oxodG prevents the appearance of deleterious transversion mutations in the cell, but the presence of many closely spaced 8oxodG lesions in newly synthesized DNA leads to multiple double strand breaks that are lethal for the cell. I will present data that provides deep mechanistic insight into these different activities of PolIV.

Radio-labeled Resveratrol loaded Gold Nanoparticles as a Cancer Targeting Probe

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Cancer targeting efficacy of polyphenolic compounds such as resveratrol could be increased by using nanoparticle-mediated drug delivery system. This could increase the amount of polyphenol finally available for uptake by cancer cells *in vivo* in a number of ways. Synthesized resveratrol loaded gold nano-particles (Res-AuNps) and unloaded gold nano-particles (AuNPs) were radio-labeled with ^{99m}Tc by using direct radio-labeling technique. Greater than 95 % radiolabeling efficiency was obtained for both ^{99m}Tc-AuNPs and ^{99m}Tc-Res-AuNPs within 15 min of incubation at room temperature. Rf = 0.8 ± 0.1 and 0.65 ± 0.2 were obtained for ^{99m}Tc-AuNPs and ^{99m}Tc-Res-AuNPs respectively using pyridine: water: acetic acid in the ratio 3:5:1.5 as mobile phase and silica coated ITLC strips as stationary phase. The radio-complexes were stable upto 6 hours in aqueous medium at room temperature and in serum at 37°C. The percentage of protein binding for ^{99m}Tc-AuNPs and ^{99m}Tc-Res-AuNPs was assessed in rat blood plasma and was found to be 25.5 ± 4.43 % and 23.3 ± 2.18 % respectively. Log Po/w value for ^{99m}Tc-AuNPs and ^{99m}Tc-Res-AuNPs were found to be -1.63 ± 0.41 and -1.2 ± 0.24 respectively, indicating that both ^{99m}Tc-AuNPs and ^{99m}Tc-Res-AuNPs were highly soluble in water. Cytotoxicity results on HT 29 cells after incubation with ^{99m}Tc-AuNPs and ^{99m}Tc-Res-

AuNPs for 24 hours with increasing concentrations (5-160 µg/ml) revealed >70% cell viability upto a concentration of 40 µg/ml for both, ^{99m}Tc-AuNPs and ^{99m}Tc-Res-AuNPs. Haemolysis observed after 24 hours of incubation for ^{99m}Tc-AuNPs and ^{99m}Tc-Res-AuNPs with whole blood at different concentrations, revealed nontoxic behaviour of both ^{99m}Tc-AuNPs and ^{99m}Tc-Res-AuNPs on RBCs even at concentration as high as 100 µg/ml. The percentage of total amount of ^{99m}Tc-AuNPs/^{99m}Tc-Res-AuNPs that was internalized by HT 29 cells after 2 hours of incubation ranged between 8-9 % for ^{99m}Tc-Res-AuNPs and 4-4.5% for ^{99m}Tc-AuNPs. Whereas, cell internalization of ^{99m}Tc-AuNPs was primarily by passive uptake but in case of ^{99m}Tc-Res-AuNPs both active and passive internalization processes contributed towards total internalization at all concentrations. After 4 hours, ^{99m}Tc-Res-AuNPs showed maximum binding to fraction containing soluble cytosolic proteins and free membranes whereas, ^{99m}Tc-AuNPs showed maximum binding to nuclear pellets. The results from *in vitro* investigations on ^{99m}Tc-Res-AuNPs revealed that the cancer targeting efficacy of gold nanoparticles was improved on loading resveratrol on the surface of these nanoparticles. Therefore, radiolabeled resveratrol loaded nanoparticles could be used as cancer imaging probe although further validation is required to conclude its efficacy in clinical diagnosis.

DNA binding specificity of p73 DNA binding domain with different p53 response elements

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p73 is a transcription factor belong to p53 family tumor suppressors and play crucial role in triggering different pathways involved in cell development, cell arrest and apoptotic pathways. p53 family proteins bind to 20-bp response element formed by two 10-bp half-sites where each 5-bp quarter-site is an inverted repeat of the adjacent one. All the p73 response elements match a loose consensus sequence and how the response element sequence variability influences the rate of transcription of genes regulated by p53 family proteins is long standing question. In order to understand how the variations in the response element change the affinity of the p73 DNA-binding domain. Binding studies were carried out by varying each position in the 5-bp quarter-site using fluorescence polarization and sedimentation velocity and also crystallize different response elements with the p73 DNA binding domain. For each quarter-site response element, the fourth and the fifth nucleotides are the most important where change in nucleotide decrease DNA

binding affinity of p73. The spacing between two 10-bp half sites also influences the transcription activity of p73. The structure analysis showed that the zero and one base-pair spacers show compact p73 DNA-binding domain tetramers with large tetramerization interfaces; a two base-pair spacer results in DNA unwinding and a smaller tetramerization interface, whereas a four base-pair spacer hinders tetramerization. Functionally, p73 is more sensitive to spacer length than p53, with one base-pair spacer reducing 90% of transactivation activity. The results establish the quaternary structure of the p73 DNA-binding domain required as a scaffold to promote transactivation.

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The Structural Basis for the allosteric regulation of one of life's most essential enzymes, the ribonucleotide reductase

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Ribonucleotide reductase (RNR) is an enzyme essential to all forms of life, as it is responsible for catalyzing the first step in the production of the building blocks of DNA. Specifically, RNR actively facilitates the conversion of ribonucleoside diphosphates (NDP) to deoxyribonucleoside diphosphates (dNDPs). RNR regulation is essential for maintaining a balanced nucleotide pool. Tight control of the dNDP pool is essential for genomic stability, as nucleotide imbalances lead to severe effects on cell growth and survival. Thus, RNR is an attractive target for cancer and antiviral therapy. Recent work, from our group and others, have provided a molecular basis for understanding how RNR recognizes its diverse set of substrates and, particularly, how ATP and dATP behave as allosteric regulators of RNR. Additionally, our recent structural and biochemical/biophysical studies have been pivotal in describing how subunit oligomerization regulates RNR activity. Armed with an improved understanding of RNR function, we have been able to design and characterize chemotherapeutic agents that interact with RNR to regulate its functions.

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Non-canonical EF-hand Ca²⁺-binding sites act as structural switches in Calumenin

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Calumenin is an ER resident, multiple EF-hand Ca²⁺-binding protein involved in maintaining Ca²⁺ homeostasis by interacting with the SERCA pump (Sahoo *et al.*, 2009) and the Ryanodine receptor (Jung *et al.*, 2006). Calumenin also interacts with a plethora of other proteins, mostly in a Ca²⁺-dependent manner, problems in which are causal to pathological conditions (Teng *et al.*, 2012). Neither the structure nor the functions of calumenin are understood yet. Using a variety of spectroscopic methods and intelligently designed fragments, we have studied the ion-binding properties of calumenin in order to understand its structure-function relationship. We demonstrate that calumenin is a natively unfolded protein and Ca²⁺ induces structural transition (coil to α -helix) with structure formation "nucleation" site being EF-4 and 5, a region known to bind SERCA. Calumenin possesses five canonical and two non-canonical EF-hands. In the non-canonical sites the highly conserved glycine at sixth position in the EF-hand loop is substituted by Alanine and Leucine in first and fourth EF-hands, respectively. Reverting these residues to glycine leads calumenin to adopt α -helix conformation even in absence of Ca²⁺. Based on our results, we hypothesize that calumenin has evolved to adopt a Leucine mediated structural switch that plays an important physiological role for regulating its functions in signaling.

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Genomes to Hit Molecules *In Silico*: A country path today, a highway tomorrow- An update

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The advent of information rich era grants us the opportunity to sketch a pathway from Genome → Gene → Protein → Drug to develop personalized medicine almost in an automated way. Currently however, without the help of any database, an inspection of a DNA sequence does not tell us whether it is likely to be a gene and if it is a gene for mRNA, what the likely three dimensional structure of its protein product is. Also drug design softwares fall short of expectations even if the structures of drug targets are known.

Addressing these issues from a *physico-chemical perspective*, we have developed all atom energy based methodologies for whole genome analysis (1) (*ChemGenome*), tertiary structure prediction of proteins (2) (*Bhageerath* and *Bhageerath-H*) and protein/DNA targeted lead molecule design (3) (*Sanjeevini*). During the process, we discovered that physico-chemical properties such as hydrogen bonding, stacking and solvation energies convey the functional destiny of DNA sequences. *Bhageerath-H* is rated among the leading servers for predicting tertiary structures of soluble proteins to medium resolution. *Sanjeevini* in collaboration with experimental groups delivered a micromolar compound against breast cancer and a sub-micromolar compound against malaria. The presentation will highlight as to how these can be configured into (*Dhanvantari*) an assembly line to deliver hit molecules from genomic information.

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Visualizing Transient Structures in A-site RNA of the Ribosome: New Structures of Known Molecules for Drug Target¹

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Dynamic changes in RNA structure drive many essential processes in living cells. Studies of RNA dynamics have focused on fluctuations about the dominant ground state at sub-microsecond timescales or large-scale transformations in secondary structure occurring at timescales slower than seconds. By using NMR relaxation dispersion and mutagenesis, we show that non-canonical regions of A-site Ribosomal RNA undergo transient excursions away from the ground state towards short-lived (μ s lifetimes) and low populated (2%) excited states that feature local rearrangements in secondary structure and base-pair alignment in regions rich in non-canonical residues. A-site ribosomal RNA contains two highly conserved internal loop adenines A1492 and A1493, which serve to decode the mRNA message by looping out and stabilizing a codon-anticodon mini-helix when it is formed between mRNA and its cognate aa-tRNA. A-site is also known to bind to many antibiotics where drug binds the internal loop, flips the two adenines out and the adenines are forced to bind the codon-anticodon minihelix irrespective of correctness of tRNA. The excited state conformation we proposed is highly conserved and defines a new type of RNA switching that can be integrated into biological circuits. The A-site ES sequesters the A92 and A93 into base-pairs, such that they are no longer available for interacting with incoming tRNAs. Indeed, the C1407U mutation, which stabilizes the A-site ES has previously been shown to significantly increase the stop-codon readthrough and frame shifting, suggesting that the mutation weakens codon-anticodon interactions in the A-site and decreases the fidelity of elongating ribosomes.

Note

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Crystal structure of the keratin 4 binding domain of serine rich repeat protein-1 (Srr-1) from *Streptococcus agalactiae* reveals an unexpected dimeric assembly through α -sheet complementation and provides hints on multi-ligand binding mode of Srr-1

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The serine rich repeat protein-1 (Srr-1) is an adhesive protein of *Streptococcus agalactiae* which has been identified as the first bacterial binding partner for human Keratin 4 (K4). The minimal binding region for K4 within Srr-1 has been localized to the residues 485-642 (termed Srr-1-K4BD) which is the C-terminal part of the non-repeat region of Srr-1 (Srr1-BR; comprising of residues 311-641). Interestingly, Srr1-BR has recently been identified to bind to fibrinogen. Srr1-BR is made up of two domains, namely N2 and N3 as observed in the Clf-Sdr family of fibrinogen binding adhesins. Srr-1-K4BD corresponds to the N3 domain of Srr1-BR and we recently solved the structure of Srr-1-K4BD. The overall structure reveals two molecules in the asymmetric unit, exhibiting a novel parallel α -sheet complementation where the C-terminal strand of one monomer is positioned anti-parallel to the N-terminal strand of the adjacent monomer. In the Clf-Sdr family proteins, including Srr1-BR, both N2 and N3 domains are essential for binding to fibrinogen. Contrastingly only the N3 domain (Srr-1-K4BD) was found sufficient in K4 binding with Srr-1. The dimerization of Srr-1-K4BD observed both in the crystal structure and in solution suggests that the similar molecular association could also be possible *in vivo* and this association would likely generate a new binding site that would recognize another host molecule. It is likely that the adhesin can recognize multiple ligands through its binding region based on the presence of number of ligand binding sub-domains (combined N2-N3 domains, individual N2 and N3 domains) and their association with one another.

Role of LMNA mutations in higher order protein assembly: A possible mechanism in the pathogenesis of Dilated Cardiomyopathy (DCM)

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Lamin proteins are type V intermediate filament proteins which form a scaffolding network underlying the inner nuclear membrane in metazoan cells excepting plants. The nuclear lamins, are primarily of two types, A and B.

Lamin A is encoded by the *LMNA* gene, of which lamin C is a major isoform, whereas, lamin B1 and B2 are encoded by the *LMNB1* and *LMNB2* genes respectively. Expression of lamin A is generally triggered by the onset of differentiation while lamin B1 and B2 are expressed in both embryonic and differentiated tissues. Nuclear lamins have a tripartite structure, characteristic of intermediate filaments, comprising of a central α -helical rod-domain flanked by a short unstructured N-terminal head and a longer unstructured C-terminal tail domain. A stretch of 115 amino acid residues in the tail domain form a β -barrelled structure akin to that of immunoglobulin, hence, known as the Ig-fold domain. The rod is yet again divided into four regions (1A, 1B, 2A and 2B) by certain breaks in the helicity known as linkers (L1, L12 and L2). The α -helical domain is characterized by heptad repeat in the amino acid residues which is responsible for the formation of a left-handed coiled-coil structure, the first step in the homotypic interaction of lamin, finally leading to the formation of the nuclear lamina by further homotypic and heterotypic interactions.

The nuclear lamins have gained increasing importance owing to the fact that over 450 mutations in *LMNA* have been found to be associated with at least 11 of human diseases known as laminopathies including Dilated Cardiomyopathy (DCM), Hutchinson-Gilford progeria syndrome (HGPS), autosomal dominant Emery-Dreifuss muscular dystrophy (AD-EDMD), atypical Werner's syndrome (WS), restricted dermopathy (RD) and mandibuloacral dysplasia (MAD). Dilated Cardiomyopathy (DCM) is a condition characterized by left ventricular dilation leading to an increase in systolic and diastolic volume, cardiac arrhythmia and myocardial infarction related sudden death. A huge number of point mutations in lamin A have been found associated with DCM patients, most of them mapping in the rod region. Several *in vivo* and *ex vivo* studies have been reported trying to focus upon the effect of DCM associated mutants, but a detailed mechanism at the molecular level of this disorder is lacking. Thus, we have focused on some mutations in the (K97E, E161K, L183P, R190W, K219T, K260N and Y267C) reported to be found among 27 Italian families whose proband was diagnosed with DCM, and their effect on the structure of the protein and homotypic and heterotypic interaction of Lamin A and their probable manifestations in the disease.

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Chemical chaperones and their mechanism of action

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Protein folding in the cell is associated with problems of aggregation and misfolding. This is taken care of by

cellular quality controls that detects and upregulates proteins that aid in protein folding and clearance. Although molecular chaperones are primarily upregulated during misfolding stress, it is also known that many small-molecules known as osmolytes or chemical chaperones, accumulate during similar stresses. These are known to stabilize proteins *in vitro* and *in vivo*. *De novo* design of chemical chaperones with higher activity than currently known examples, is desirable to ameliorate protein misfolding and aggregation in multiple contexts. However, this development has been hindered by limited knowledge of activity of these molecules. It is thought that chemical chaperones are typically poor solvents for protein backbone and hence facilitate native structure formation. However, it is unknown if different chemical chaperones can act differently to modulate the landscape in distinct ways. Using a model slow folding protein, double-mutant Maltose-binding protein (DM-MBP), we show that a canonical chemical chaperone, Trimethylamine-N-oxide (TMAO) accelerates refolding by decreasing the flexibility of the refolding intermediate (RI). To compare effects of different chemical chaperones on the same protein, we screened and identified a number of small-molecules that chaperone DM-MBP folding. Interestingly, while Proline and Serine, both having amino acid backbones, assist folding by stabilizing the transition state (TS) enthalpically, Trehalose behaves like TMAO and increases the rate of barrier crossing through non-enthalpic processes. We propose a two-group classification of chemical chaperones, based upon their thermodynamic effect on intermediate and transition states. Single molecule Förster Resonance Energy Transfer studies on the refolding intermediate formed in presence of the chemical chaperones, also support this classification. Interestingly, for a different test protein, although the different groups affect protein folding in a contrasting manner, their molecular mechanism is not conserved. Chaperones that acted to decrease enthalpic barrier for one test protein was found to increase the barrier for the other. This provides a glimpse into the complexity of chemical chaperoning activity of osmolytes. Future work would allow us to engineer synergism between the two classes to design more efficient chemical chaperones to ameliorate protein misfolding and aggregation problems.

Quantification of Protein Dynamics in terms of Flexibility/Rigidity by Single-Molecule Force Spectroscopy Experiments

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Flexibility is critical for the ability of proteins to fold and function. Conformational flexibility of a protein might

also dictate its dynamics as well as ligand binding efficiency. Here, we used single-molecule force spectroscopy to quantify protein dynamics in the native state in terms of stiffness of its unfolding energy landscape, using a harmonic approximation. Protein stiffness is estimated as a spring constant or curvature of its unfolding potential by measuring the barrier to the unfolding transition state and the width of the unfolding potential.

From the stiffness, measured as a spring constant for the deformation along the N-C termini pulling direction, of a ubiquitin-like protein, SUMO1, it is found that SUMO1 is six times more flexible than ubiquitin. The stiffness of SUMO1 increased upon binding to small peptides (SUMO binding motifs) from ~ 1 N/m to ~ 3.5 N/m. The relatively higher flexibility of ligand-free SUMO1 might play a role in accessing various conformations before binding to a target. The conformational flexibility of SUMO1 reduces once the ligand is bound which is measured as increase in the stiffness in our experiments. The flexibility and the associated conformational dynamics of SUMO might help it in interacting with its target proteins at various binding sites, and once the target is bound, the complexed SUMO becomes rigid for the subsequent sequence of events that occur during the SUMOylation. Measuring the stiffness of overall protein or protein-ligand complexes could serve as a measure of their conformational entropy.

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Electroporation Mediated Anticancer Drug Delivery: Biophysical Basis and Application in Cancer Treatment

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Exposure of living cells to high intensity short electrical pulses (a few kV/cm, ms duration) has become an active area of biophysical research with applications in biotechnology, gene delivery and cancer treatment. Plasma membrane of cells become transiently permeable on application of external pulsed electric field above a characteristic threshold value permitting exchange of molecules between cell interior and the surrounding. This phenomenon, popularly called electroporation, has

opened many new possibilities of bacteria, plant and mammalian cell manipulation. Electroporation method allows gaining direct access into the cell cytoplasm, to transfer gene into a variety of cells giving efficient transformation, to enhance drug toxicity to tumor cells and achieve cell fusion for hybridoma technology. Our research group has extensively contributed to understanding of basic biophysical mechanisms involved in rendering the plasma membrane of cell permeable by implanting micropores thereby permitting entry of otherwise impermeant drugs and a host of other agents. This method offers to introduce therapeutic drugs into target disease cells. Studies on Ehrlich ascites cells have shown that at non-toxic concentrations of doxorubicin, an antitumor drug, produced substantial cytotoxic effects when drug treated cells were subjected to electroporation due to enhanced intracellular incorporation of the drug. More recently, results from our laboratory have shown that tumor cells can be sensitized to α radiation when combined with electroporation indicating a possibility of treating radioresistant tumors. A brief highlight of the developing new biomedical applications based on membrane electroporation will be outlined with demonstration of powerful new protocol for effective cancer treatment together with ionizing radiation.

A Summary of talk at IBS Annual meeting, Jamia Millia University, Delhi, Feb 14-17, 2015

Differential Glycosaminoglycan Interactions of Murine and Human CXCL1 Proteins

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Chemokines constitute the largest subfamily of cytokines that provide important regulatory cues in trafficking leukocytes. Glycosaminoglycans (GAGs) such as heparan sulfate are highly negatively charged linear polysaccharides. They are ubiquitously found on cell surfaces, and mediate a wide variety of biological functions. Though it is now well established that chemokine-heparan sulfate interactions regulate leukocyte recruitment, the structural basis and molecular mechanisms underlying these interactions and their evolutionary origins are not well understood. GRO proteins are proinflammatory neutrophil activating chemokines, and exist in equilibrium between monomers and dimers. Analysis on ~ 12 species across mouse to humans suggested that the hierarchies of gene duplication events are not coherent throughout the evolution. Protein sequence and structural analysis of these GRO chemokines using sequence alignment and molecular modelling studies revealed that the electrostatic surfaces of these chemokines are markedly different due to evolutionary mechanisms although their tertiary/

quaternary structures are very similar. Moreover, our structural analysis on murine and human CXCL1/GRO α proteins using various biophysical techniques and NMR spectroscopy indicated that both of them remains stable in the native state up to 90 °C. Heparin binding studies with murine CXCL1 established that the GAGs bind orthogonal to the helical axis spanning the dimer interface. Residue level dynamics and stability measurements of apo- and GAG-bound murine dCXCL1 demonstrated GAGs enhanced chemokine's structural integrity. Comparative analysis of GAG binding studies on murine and human GRO α proteins unraveled that the glycosaminoglycan binding interactions with the engineered disulfide trapped CXCL1 dimer (dCXCL1) vary significantly pointing their differential gradient forming capabilities during neutrophil recruitment. These data together indicate GAG-bound dimers regulate in-vivo neutrophil trafficking by multiple mechanisms including, defining the gradient formation and increasing the life time of 'active' chemokines on the endothelial cell surface for sustained recruitment.

Bio-electrostatics of the voltage gated potassium ion channel in neuron

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Ion Channel protein, involved in transmission of nerve impulses, undergoes reversible conformational changes under the influence of transmembrane electric field. There are experimental evidences of the structure of the voltage sensor domain at zero potential, but the basis of the conformation is not yet clear. Using our innovative theoretical method, utilizing the electrostatic theory, an indigenous Fortran based software has been developed to compute the interaction energy to understand the electrostatics of the ion channel. We have studied the tetramer KvAP channel protein and have postulated that the pole charges, charged residues of the constituent α -helices and the dielectric media contribute significantly to the relative positioning of the helices in a stable conformation. Here we will illustrate how the differential dielectric environment affects the conformation of the α -helix pairs, leading to the structural resemblance of the experimental results.

Understanding "split personality" of lipidated protein using molecular dynamics simulations

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Lipid modification of cytoplasmic proteins initiates membrane recruitment that is vital for organelle and vesicular dynamics. Initiation of cellular autophagy is regulated through covalent conjugation of microtubule-associated protein light chain 3 (LC3) protein with phosphatidylethanolamine (PE) lipid chain. While experimental approaches provide insights into membrane-protein association, unbiased dynamical characterization of this process is challenging. We perform extensive molecular dynamics simulations to reveal components underlying this key process i.e., membrane recruitment of cytosolic proteins. We show that partitioning of the lipid anchor chain commences in a concerted process, with the two acyl-chains inserting one after the other. Concomitant conformational rearrangement of specific region of the LC3 protein, especially the three basic amino acids LYS65, ARG68 and ARG69 ensures a stable and efficient delivery of PE chain into membrane. Comparison of the energetic profiles between non-lipidated and lipidated protein show distinct affinity for LC3 to be present at lipid-water interface. Our study captures the trajectory of a water-favored LC3 protein to be recruited to the membrane and thus open new avenues to explore cellular dynamics underlying vesicular trafficking.

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Characterization of Membrane-active Peptide Components of Hepatitis A Virus (Hav)

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Hydrophobic or amphipathic peptide components, which can interact with host cell membranes, are utilized by viruses during several phases of their life cycle. During entry into host cells, enveloped and non-enveloped viruses utilize membrane active peptides for orchestrating membrane fusion or disruption, respectively. During the latter phases of the viral life cycle, membrane-active proteins called viroporins are utilized for remodeling of internal cellular membranes, altering ionic concentrations and promoting virus replication and assembly. We are characterizing membrane active components in the structural and non-structural proteins of the human

picornavirus, Hepatitis A Virus, through utilization of biophysical and computational methods. We have identified a small structural peptide, called VP4, which is probably utilized by HAV to disrupt endosomal membranes during entry into host cells. We have also identified a 60 amino acid region at the C-terminal of HAV non-structural protein 2B, which interacts with the membranes of endoplasmic reticulum and golgi bodies during HAV replication. The manner of interaction of these components with cellular membranes will be discussed.

Multifunctional nanosystems: growing sanguinity in development of particulate antigen delivery vehicle based vaccines

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Development of protective immunity against many pathogens, particularly viruses, requires fine orchestration of both humoral- and cell mediated-immunity. The immunization of animals with soluble antigens usually leads to the induction of humoral immune responses. In contrast, the activation of a cell-mediated immune response against exogenous antigens has always been a challenge, requiring special strategies to expose them to the proteasome, a multifunctional protease complex in the cytosol of the target cells. The degradation of the protein by the cytosolic proteolytic system forms a cardinal step for the induction of cytotoxic T lymphocytes (CTLs).

In the present study we describe strategies to develop nano-particle based antigen delivery vehicle that have potential to deliver encapsulated antigen to cytosol of the target cells. To develop safe and effective methods for generating CD8⁺ T lymphocytes, we exploited the fusogenic character of lipids derived from lower organisms, that is baker's yeast (*Saccharomyces cerevisiae*) and some other important bacteria. The degree of fusion with model membrane systems of microbial lipid based nano-particles varied from 40-70%, as opposed to 1-8% observed with egg PtdCho liposomes, depending on the assay system used. The fusion of microbial lipid nanoparticles with macrophages resulted in effective delivery of the entrapped solutes into the cytoplasmic compartment. This was further supported by the inhibition of cellular protein synthesis in J774 A1 cells by ricin A, encapsulated in the microbial lipid based nanoparticles.

Our data support that novel fusogenic nano-particles made up of lipid from lower microbes have strong tendency to fuse with the plasma membrane of target cells and thereby delivering the entrapped contents into their cytosol. The delivery of entrapped antigen in cytosol of the target cells ensues its processing and presentation along with MHC class I pathway that eventually elicit

antigen specific cytotoxic T cells. The protocol involving immunization of BALB/c mice with nano-particle-encapsulated antigens resulted in the augmentation of antigen specific cytotoxic T cell lymphocyte as well as IgG responses.

Further, immunization with nano-particle encapsulated antigen resulted in significant enhancement in the release of IFN-gamma and IgG2a in the experimental animals. Finally, we also established that immunization of animals with nano-particle encapsulated relevant antigen protected them against virulent forms of various intracellular infections. This was evident by increased survival, and reduced bacterial burden in vital organs of the immunized animals. The data of the present study suggest that in-house developed lipid based nano-particles can emerge as an effective vehicle for intracellular delivery of antigen and thus hold promise in development of nanosized vaccine against *Mycobacterium sps* and other intracellular pathogens.

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The structure of the holo-acyl carrier protein of *Leishmania major* displays a remarkably different phosphopantetheinyl transferase (PPTase) binding interface

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The genome of *L. major* encodes a type II pathway for which no biochemical or structural information is available. Here, for the first time, we have characterized the central player of the pathway, the acyl carrier protein (LACP) using NMR. Structurally, the ACP molecule is similar to other type II ACPs, comprising of a four helix bundle, enclosing a hydrophobic core. Striking dissimilarities in sequence however exist in the helix II (recognition helix) of LACP, at highly conserved positions, known to interact with PPTases. The conserved DSL motif of the type II ACPs is substituted with an NSL motif in LACP. Furthermore, the conserved methionine at position 45 is substituted with a phenylalanine in LACP. In order to understand the basis for the selection of these residues in LACP, we have studied its interaction with group I and group II PPTases (4'-phosphopantetheinyl transferases) using a range of biochemical, biophysical and bioinformatics approaches. The enzymatic activity of various PPTases towards LACP, and other type II ACPs has also been compared. Our studies shed light on the unique interaction surface of *Leishmania* ACP that has evolved to interact with its cognate PPTase.

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Structural and interaction study of collagen protein in native state by solid state NMR spectroscopy

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Collagen is the most abundant protein of animal system. The collagen protein in bone and cartilage provide structural integrity and flexibility. Structural changes in the collagen protein due to disease such as osteoporosis, give rise to bones with weaker mechanical properties. We have developed methods to look into high - resolution structural details of collagen protein in its absolute native state inside bone matrix. The method is based on ^1H detected solid state NMR experiments. Our experiments map three - dimensional collagen fiber packing to the two - dimensional NMR spectrum and capture subtle structural changes. The sensitivity of the experiments is enhanced by Dynamic Nuclear Polarization (DNP) coupled with solid - state NMR measurements. The method also captures rare aromatic - imino acid interactions in native collagen protein.

Structure Biology of the Epigenetic Marker, BRD2

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Epigenetics, through the modulation of genetic information, plays essential roles in fundamental life process, such as cell-proliferation, cell development and decision between cell survival and cell death. Aberrations in epigenetic mechanisms results in abnormal cellular functions, and can lead to the development of cancer, neurodegeneration, autoimmune/inflammatory diseases, metabolic diseases and viral infections. Hence, the epigenetic targets are of great importance to discover new drug molecules for various major diseases. While drugs targeting histone deacetylase (HDAC) and DNA methyltransferase (DNMT) are clinically approved recently, drug discovery targeting other epigenetic markers are in the pipeline. One of the epigenetic 'reader' proteins, bromodomain containing proteins recognizes acetylated-lysine histones (H3 and H4) and non-histones, such as the tumor suppressor, p 53.

The BET family nuclear proteins have a unique architecture with two tandem bromodomains (BD1 and BD2) and a conserved extra-terminal domain. The BRD2 protein, which belongs to the BET family, recognizes mono-acetylated histone H4 at Lys12 (H4K12ac) and di-acetylated histone H4 at Lys5 and Lys12 (H4K5ac/H4K12ac) through N- and C-terminal bromodomains,

respectively. The BRD2 protein are reported to possess potential role in the pathogenesis of cancer, defects in embryonic stem cell differentiation, seizures and neurodegenerative disorders (NDD) such as Parkinson's disease.

The structure of BRD2 bromodomains complexes with acetylated histone peptides, and few potential inhibitors obtained by virtual screening will be discussed.

NMR investigations of fibrils and transient oligomers of Ab peptide

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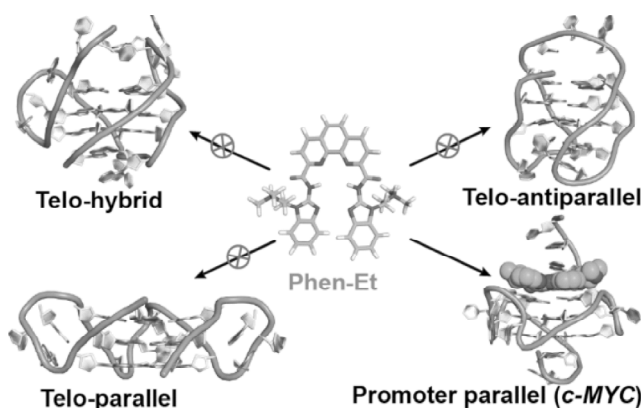
Ab peptides are interesting models for investigating different aspects of amyloid aggregation. On the basis of amyloid cascade hypothesis, which has dominated amyloid disease research for the past two decades, the main therapeutic strategies have aimed either to prevent the aggregation of Ab, or to remove toxic oligomeric and fibrillar species of Ab. Since Ab is ordinarily produced in the brain, and there is no proof that Ab overproduction underlies sporadic Alzheimer's disease (AD), the pursuit of former strategy demands a thorough understanding of all the neurochemical factors that initiate A β deposition in brain. One such factor is the presence of metal ions, especially Zn^{2+} . Observations like high Zn^{2+} concentrations in senile plaques found in the brains of Alzheimer's patients and evidences emphasising the role of Zn^{2+} in Ab-induced toxicity have triggered interest in understanding the nature of Zn^{2+} -Ab interaction. The second strategy generally involves usage of external agents/drugs which can make Ab follow such aggregation pathways which yield non-pathological species of Ab. In this regard, curcumin, a small phenolic compound and a common Asian spice, has been found to ameliorate the effects of Ab induced neurodegeneration in AD models. A structural understanding of how curcumin interacts with Ab can provide a significant impetus to such efforts. Here, we present our results from studies of interaction of both $\text{A}\beta_{40}$ and $\text{A}\beta_{42}$ fibrils with Zn^{2+} ions and curcumin. We also will highlight a method to study the small oligomers of the Ab peptide that are suspected to initiate AD. The smallest of such oligomers that demonstrate a potential sign of toxicity, namely, an enhanced affinity for cell membranes, was studied with fluorescence and solid-state NMR methods. This study may provide clues for AD therapeutics and also suggest a general method to studying transient protein structures.

Specific Detection and Stabilization of G-Quadruplex DNAs by Designer Molecules

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G-quadruplexes are four stranded nucleic acid structures formed by stacking of two or more G-quartets assembled via Hoogsteen base pairing. Potential G-quadruplex forming sequences are present in the telomeres, promoters and introns of the genome; also in the UTRs of mRNAs. Depending upon the sequence and environment, G-quadruplexes can adopt different topologies such as parallel, antiparallel and hybrid structures. Stabilization of quadruplex structures by small molecules offers a way to halt the function of telomerase enzyme or gene expression at the transcriptional or at the translational levels. Our lab focuses on developing chemical tools to stabilize and sense the quadruplex structures. In this direction, recently we reported the use of bis-quinolinium based ligands, which can selectively discriminate quadruplex from duplex DNAs, as a SERS sensor for quadruplexes. We have also demonstrated the use of Thioflavin T as a selective fluorescent light-up probe for quadruplex structures. To specifically recognize and stabilize *c-MYC* and *c-KIT* promoter quadruplexes, which adopt parallel topology over telomeric quadruplexes, that form antiparallel or hybrid topology, we have designed and synthesized a new class of scaffolds based on benzimidazole moiety. Various biophysical and biochemical studies unambiguously provided evidence for the specific stabilization of promoter quadruplexes. Structural basis for this topology specific recognition was unraveled by molecular modeling and dynamics studies.



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Role of DNA Triple Helical Structures in Neurodegenerative Disorders

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It is now established that a small fraction of genomic DNA does adopt non-canonical B-DNA structure or "unusual" DNA structure. The Unusual DNA structures are represented as hotspots of chromosomal breaks, homologous recombination and gross chromosomal rearrangements since they are prone to the structural alterations. These structures, for example, DNA-hairpin, cruciform, Z-DNA, H-DNA (Triplex), tetraplex etc show different biophysical and biochemical properties. At least 30 human hereditary disorders are known to arise as a result of expansions of simple DNA repeats. Most of these disorders are caused by the triplet repeat expansion (TRE) like (CGG) n •(CCG) n , (CAG) n •(CTG) n , (GAA) n •(TTC) n and (GCN) n •(NGC) n .

Friedreich's ataxia, first autosomal recessive degenerative disorder of nervous and muscle tissue caused by the massive expansion of GAA repeats. GAA repeats occurs in the first intron of Frataxin gene X25 on chromosome 9q13-q21.1 and affects both male and female children which manifests itself before puberty and successively there is loss of voluntary muscle coordination. The GAA repeats form a H-DNA by folding back of the purine strand in a parallel fashion leading to parallel Pyrimidine * Purine ?Pyrimidine (pY*R?Y) type of "DNA Triple Helix". The (RfÜR, \Y) triplex further reduces the frataxin gene expression. Till date, FRDA is the only disease known so far associated with DNA triplex. Biophysical studies on the DNA triplexes (containing GAA repeats) using UV melting, UV absorption, Fluorescence, Circular dichroic spectroscopy and EMSA will be presented. Genetic confirmation, circulating plasma DNA and MALDI-TOF proteomic data of FRDA patients (with (GAA)900 repeats will be also discussed.

Mechanism of transformation of a transcription elongation/termination factor, NusA, into a transcription antiterminator by the bacteriophage protein N.

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The bacterial transcription elongation/termination factor, NusA, functions as an antiterminator when it is bound

to the lambdaoid phage derived antiterminator protein, N (1). The mode of N-NusA interaction *vis a vis* the conversion of the latter into an antiterminator is unknown. We asked two questions; i) What is the functional interaction surface on NusA for N and ii) how N modulates NusA-RNA polymerase (RNAP) in the transcription elongation complex (EC)?

In the first part of the study, we identified mutations in NusA-N-terminus (NTD), specifically defective for N-mediated antitermination. These are located at a convex surface of the NusA-NTD, situated opposite to its concave RNAP binding surface. We also showed that when bound to the EC, N makes specific contacts with this convex surface of NusA. We concluded that the N- interaction surface of NusA shifts from the AR1 domain to its NTD domain in the presence of the EC. We propose that the close proximity of this altered N-interaction site of NusA to its RNAP-binding surface, enables N to influence the NusA-RNAP interaction during transcription antitermination that in turn facilitates the conversion of NusA into an antiterminator (2). In the second part of the study, we now show that the aforementioned N-NusA interaction allosterically modulate the NusA-RNAP b-subunit flap domain interactions. This modulation most likely changes the orientation of the flap domain over the RNA exit channel, and this re-orientation of the flap domain makes the exit channel more constricted, which in turn prevents the terminator hairpins to form inside the channel.

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Understanding the mechanism of a-synuclein aggregation associated with Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative movement disorder mostly characterized by the presence of proteinaceous inclusions called Lewy body (LB) and Lewy neuritis (LN) in the brain. The main component of these inclusions is special form of a protein (α -synuclein) aggregate, called amyloid. The *in vitro* as well as various cellular and animal studies have suggested that α -synuclein protein aggregation is indeed a pathogenic event causing cell death occur in PD. Recent studies suggest that soluble oligomeric assemblies of α -synuclein that appear in the early stages of fibrillar assembly, are

the most cytotoxic species. Therefore, understanding the a-synuclein aggregation mechanism and targeting the cytotoxic oligomeric assemblies are crucial steps for the drug development against PD. Our study suggest that a-synuclein aggregation is a complex protein aggregation process, which possess multiple conformational intermediates with various sized oligomers. This understanding of a-synuclein aggregation mechanism(s) might help to design small molecules modulator for protein aggregation associated with Parkinson's disease.

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Telomerase as a chemopreventive molecular target in colon cancer

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The limitless cell proliferation is the hallmark of cancer, and cancer cells have typically acquired damage to genes that directly regulate their cell cycles. Telomerase confers uncontrolled proliferative potential to most cells through its ability to elongate telomeres, the natural ends of chromosomes, which otherwise would undergo progressive attrition and eventually compromise cell viability. The universal presence of telomerase in tumor suggests that telomerase activity is required for cell immortality *in vitro* and *in vivo*, and targeting telomerase may represent a prevalent marker to specifically block tumor cell growth with minor effects on normal cells. We studied the regulation of telomerase and telomerase reverse transcriptase catalytic subunit (TERT) by Diclofenac and Curcumin, alone and also in combination, in 1,2-dimethylhydrazine dihydrochloride (DMH) induced colorectal cancer in S.D male rats. The relationship of telomerase activity with pro-inflammatory molecules, tumor suppressor proteins, cell cycle machinery and also apoptosis were studied. Telomerase is highly expressed in DMH group and its high activity is associated with increased TERT expression. However, telomerase is either absent or present at lower levels in the normal tissue. Cox-2, NF- κ B, IKK α , I κ B α , TNF- α , IL-1B, and IL-2 were highly expressed in DMH as assessed by RT-PCR, qRT-PCR, western blot and immunofluorescence analysis. Diclofenac and Curcumin overcome these carcinogenic effects by down regulating telomerase activity and preventing inflammatory responses raised by NF- κ B to induce apoptosis. Diclofenac and Curcumin also up regulated the expression of tumor suppressor proteins (p51, Rb and p21) and subsequently inhibiting the expression of cell cycle regulatory proteins (CDK4, CDK2, Cyclin D1 and Cyclin E). The anti-carcinogenic effects shown after the inhibition of telomerase activity by Diclofenac and Curcumin may be associated with inhibition of inflammation, up regulation of tumor suppressor proteins p51, Rb and p21,

whose activation induces the cells cycle arrest and apoptosis.

Curcumin binds to the amyloid intermediates of Cu/Zn Superoxide Dismutase (SOD1) converting them into less toxic species.

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that affects motor neurons. Several *in vivo* studies suggest that almost 20% of familial ALS (fALS) is associated with pathological deposition of Superoxide Dismutase (SOD1). SOD1-containing pathological inclusions in ALS exhibit amyloid like properties. In the search of a compound which can inhibit amyloid formation, we observed the fibrillation of SOD1 with reduced disulfide in the presence of curcumin using ThT binding assay, AFM, TEM images and FTIR. Further, there is significant increase in SOD1 mediated toxicity in the regime of pre-fibrillar and fibrillar aggregates which is not evident in curcumin containing samples. Curcumin inhibits DTT-induced fibrillation of SOD1 by binding to oligomers and pre-fibrillar aggregates of SOD1. Docking studies indicate that putative binding site of curcumin may be the amyloidogenic regions of SOD1.

Inhibition of amyloid fibrils formation as well as disassembly of amyloidfibrils in Alzheimer's disease using the poly-ion, single stranded nucleotide sequence: an *in silico* Study

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The plaque formed by aggregation of amyloid $A\beta_{1-42}$ peptide fibril is a major pathological hallmark of Alzheimer's disease. It is the second most prevailing form of dementia. At present there is no cure or treatment for this disease. So significant research studies are focused now on to find drugs to cope with this disease. One of the promising approaches is to prevent the amyloid fibril formation at an early stage. In this study, we used the poly-ion, single stranded nucleotide sequence(ss-DNA) (5'-AAAGAGAGAGAG-3') to prevent the $A\beta$ peptide fibril formation. The interaction between the ss-DNA and the $A\beta_{1-42}$ peptide was studied using all atom molecular dynamics simulation. The initial structure of the complex system containing $A\beta_{1-42}$ peptide and ss-DNA was built using LeaP module of AMBER package. Then the resultant complex system was solvated using TIP3P water

model, made neutral by adding counter ions and then subjected to minimization, equilibration and finally the MD production run for 30 ns. In this simulation study we used ff99SB Amber force field. From the analysis of the MD simulation trajectory, we observe that the poly-ion, ss-DNA (because of the presence of negative charges of the phosphate groups along the backbone) showed electrostatic attraction towards the $A\beta_{1-42}$ peptide which has significant number of positively charged amino acids in it. As a result the $A\beta_{1-42}$ peptide was wrapped (encapsulated) by the ss-DNA and formed a micellar type complex. Therefore it would be very difficult for another unit of $A\beta_{1-42}$ peptide to approach the encapsulated $A\beta_{1-42}$ peptide. From our findings we see this method to be significant method to inhibit the $A\beta$ peptide fibril formation at an early stage and also to disassemble the amyloid fibrils and thereby reduce the risk of Alzheimer's disease.

Understanding conformational changes using structure-based models of proteins

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Ligand binding in several proteins is accompanied by conformational changes. The order of ligand binding and whether it precedes or succeeds the conformational change determines the mechanism of ligand recognition. In conformational selection (CS), the substrate exists in a dynamic equilibrium between an open conformation, and, a closed conformation which is competent to bind ligand. The ligand then binds the closed conformation and increases its population. In induced fit (IF), the substrate populates mainly the open state and the presence of the ligand induces it to adopt the closed conformation. In this talk, I will introduce structure-based models and explain their in the calculation of the conformational dynamics of a protein. These dynamics can they be used to understand ligand recognition.

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Engineering signal transduction pathways through synthetic modular systems: Dissecting the puzzle in infectious disease

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Cellular phenotypes arise due to complexspatio-temporal interplay of biomolecules. Characterizing these biomolecules through genomics and other highthroughputtechniques has made comprehensive

computational modelling of biological systems possible. Such models structured within a mathematical framework can be powerful tools to predict cellular phenotypes aiding construction of therapeutic designer circuits. One such area of application would be predicting the cellular phenotypes of immune cell in infectious disease such as malaria, leishmaniasis, tuberculosis, trypanosomiasis etc. This is of particular interest and importance as the causative agents of these diseases reside within the macrophages; the sentinels of the immune system and modulate their phenotypic behaviour for safe intracellular survival. Experimental studies have revealed an abundance of information about the changing cellular composition with regard to signalling molecules, transcription factor activation-repression and corresponding gene expression patterns in diseased condition leading to a specific immune effector state. It is interesting to note that from developmental standpoint, some immune effector states or cell fates are 'hard-wired' and are acquired in the absence of a pathogen. Nevertheless to say, under both scenarios, cellular fate is the interplay of the complex genomic circuitry or the gene regulatory network (GRN) integrated through signal activated TFs and their corresponding target genes leading to heterogeneous ensembles. Such ensembles establish diverse functional division of the immune effector cells. It is the reprogramming ability of the GRN long side the TFs that helps in the transition of a diseased immune state to a healthy state i.e. the network has a highly evolvable yet robust architecture. Through evolutionary developments often selected traits enhance robustness of the organism and therefore robustness is ubiquitous in living organisms that have evolved. But, a robust system is often fragile facing performance setback as an inherent trade-off. So, understanding the associated trade-offs is essential for identifying their faults that may help design countermeasures for effective synthetic circuit therapeutics. TFs while acting on their target genes modulate the fundamental molecular functions of a cell by specifying promoter regulatory elements, modulating gene output, tuning molecular noise, recruiting coactivator/repressor complexes and cooperating with other TFs to regulate a gene, giving rise to complex behaviour of transcriptional networks. A framework of transcription regulation would be valuable to synthetic biology efforts, which construct synthetic regulatory elements based on the design principles of a GRN. Such bottom-up circuit construction, enable us to improve our understanding of the natural cell signaling and gene networks in diseased condition, but also help modify the phenotypic fate at a cellular level, by engineering them to perform new customized tasks, like specifically invading cancer cells or resolution of an intracellular pathogenic microorganism.

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Ras dynamics and its dependence on sterol in fungal membranes-a hypothesis on Ras hyperactivation in GPI biosynthesis mutants

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My lab has been working to understand the molecular details of glycosylphosphatidylinositol (GPI) anchor biosynthesis in the human pathogen, *C. albicans* pathway. Building on previous work from my lab showing an intimate link between the first step of GPI biosynthesis and two other very important pathways, ergosterol biosynthesis and hyphal morphogenesis via Ras signaling, in *C. albicans* this presentation will attempt to explain the unusual Ras dynamics that is observed in hyperfilamentous GPI anchor mutants and its relationship with ergosterol.

Conventionally, ergosterol deficiency and higher membrane dynamics are associated with poor ability to form true hyphae that is essential for infection and virulence of pathogenic fungi. Yet ergosterol depletion via downregulation of lanosterol 14- α -demethylase (*ERG11*) in certain GPI biosynthetic mutants (*CaGPI19* mutants) of *C. albicans* is accompanied by hyperfilamentation resulting from an upregulation of Ras signaling. Using a combination of steady state anisotropy, fluorescence lifetimes and fluorescence correlation spectroscopy (FCS), we show that membrane-specific probes experience faster dynamics in the membranes of *CaGPI19* mutants, and accumulation of sterol intermediates do not compensate for packing defects due to ergosterol depletion. Yet, Ras dynamics are significantly slower in the membranes of these cells. Using a range of other *C. albicans* mutants including those that overexpress Ras1 as well as those expressing hyperactive mutants of Ras1, we attempt to present a viable model to explain the hyperfilamentous phenotypes observed.

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Combinatorial role of two G-quadruplexes in 5' Untranslated region of Transforming Growth Factor β 2 (TGF β 2)

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RNA G-quadruplexes in untranslated regions (UTR) have been shown to play an important role in translational regulation. Although studies indicated a consistent inhibitory role of G-quadruplex sequence per se in gene expression, role of G-quadruplex in context of entire 5' UTR is found to be inconsistent. However, recently we

demonstrated its completely contrasting activating role in 5' UTR of Transforming growth factor β 2 (TGF β 2). We showed that though 23-mer G-quadruplex sequence (PG4) inhibited the gene expression, G-quadruplex in context of entire 1368 nts long 5' UTR of TGF β 2 augments gene expression. Our *in vitro* experiments confirmed the ability of this sequence to form G-quadruplex and *in cellulo* studies further indicated its activating role in modulating TGF β 2 gene expression. Following the characterisation of each of the two G-quadruplexes in isolation, we examined whether two G-quadruplexes-mediated gene regulation in 5' UTR of TGF β 2 mRNAs are mutually dependent or exclusive events. Our works suggested that these two 5' UTR G-quadruplexes operate synergistically to substantially increase gene expression of TGF β 2. None of G-quadruplex alone is sufficient enough to drastically augment protein production. Both G-quadruplexes are essential for increasing protein output. To our knowledge, our study is the first report showcasing the combinatorial role of two G-quadruplexes in the 5' UTR of an mRNA.

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Biophysical Characterization of Natural and Engineered Optogenetic Tools

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cAMP mediated signalling is present in all domain of life and plays a key role in controlling biological processes. Adenylate cyclase works as a central relay station, which can receive and amplify primary signal such as presence of hormones, alternations in ionic components or change in pH in the environment of the cell. In turn, cAMP activates target proteins such as protein kinases, ion channels, and transcription factors and finally results in cellular response to primary signal(s). In very few organisms, adenylyl cyclase (AC) constitutes of modular architecture where cyclase homology domain (CHD) is fused with regulatory domains like histidine kinase (HK), GAF domain, BLUF domain (sensor of blue light using FAD). In *Euglena gracilis* regulatory domain is BLUF, which fuses with AC and is responsible for photobehavioral responses. These light-gated adenylyl cyclases were applied as optogenetic tools for manipulating cAMP level simply by illumination in many cellular systems and provided opportunity to reversibly manipulate cAMP mediated signaling in living cells with very high spatial-temporal precision, which are not achievable using traditional pharmacological or genetic approaches. Recently, *Naegleria gruberi* genome was sequenced and published. It is a free living amoeboid flagellate. *Naegleria* genome is crucial for studies of cell biology, evolution, environmental and medical microbiology. It has a closely related pathogenic relative

N. fowleri (also known as Brain Eating Amoeba) that causes severe amoebic meningitis. Mining of *N. gruberi* genome and bioinformatics analysis suggest the presence of light-gated adenylyl cyclases. These light-gated adenylyl cyclases (named-NgPACs) consist of two conserved domains BLUF and cyclase homology domain (CHD), respectively. Biophysical characterizations of photoactivated adenylate cyclase (NgPACs) and engineered photoactivated gunylate cyclase (PGC) will be presented in detail. Relative low molecular mass, solubility, light regulated cyclase activities of natural NgPACs and engineered PGCs provides optogenetic tools for non-invasive light regulated control of cAMP in Cell Biology.

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Computation Complimenting Experimentation: Vinca Alkaloid Binding to DNA

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NMR spectroscopy and X-ray crystallography have been effectively employed to provide structural information about the ligand-receptor complexes. Molecular docking approaches are efficiently applied to generate ligand-receptor complexes in order to understand binding interactions between the two entities. However, we have shown in this study that fluorescence spectroscopy, circular dichroism (CD) spectroscopy, electrochemical methods and gel electrophoresis along with molecular docking can successfully explain the phenomenon of the ligand-DNA binding with good correlation between experimental and computational results. The binding affinity data was obtained from fluorescence spectroscopy and electrochemical methods. Structural rigidity of DNA upon ligand binding was identified by CD spectroscopy. Docking was carried out using DNA-Dock program which gave binding affinity data along with structural information like inter-atomic distances and H-bonding, etc. A new tool to identify the weak base sequence specificity viz. pur-pyr specificity was identified.

Crowding by Specific size of Dextran switches the Substrate Specificity of Acetylcholinesterase enzyme

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The cell cytoplasm, the red blood cell interior or the mitochondrial matrix are examples of crowded

environments. The consequences of crowding by proteins and nucleic acids on physiological functions like enzyme catalysis, is still far from being understood. Our interest here was to mimic such macromolecular crowding *in vitro* to understand how crowding by inert high molecular weight dextrans affect the catalytic activity of acetylcholinesterase (AChE) enzyme. We monitored the catalytic rate of AChE in a medium crowded with dextrans of different sizes (15 to 2000 kDa) at different dextran concentrations (0–20 % w/w) employing two different substrates. Investigations revealed enhanced activity (> 2 fold increase) with 2-naphthyl acetate as substrate in comparison to 3-indoxyl acetate (IA), for all dextran sizes at low concentrations (0–10% w/w). Interestingly while AChE activity with 2-naphthyl acetate was specifically enhanced in presence of 200 kDa dextran, its activity with 3-indoxyl acetate in presence of same 200 kDa dextran, was drastically diminished in comparison to all other dextran sizes employed. Fluorescence arising from ANS bound to AChE in presence of dextrans indicated a subtle change in AChE structure coinciding with same dextran concentration and size. Our results suggest that crowding by dextrans of molecular size similar to enzyme itself, can critically influence the enzyme specificity towards a substrate.

Irreversible unfolding of a large *Escherichia coli* protein Maltodextrin Glucosidase

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Investigation of the unfolding processes of proteins relates to the requirement of understanding the mechanism, determining thermodynamic stabilities, and working out parameters related to stability of the proteins and formation of various non-native species across the pathway. In order to deduce various thermodynamic parameters from the unfolding process of the proteins, it has to be ensured that the process is reversible. However, the reversibility of the unfolding process is an intrinsic property of some of the proteins. There are several instances available in the protein folding / unfolding literature that unfolding processes of the proteins are not reversible. In majority of the cases the irreversible unfolding of the proteins end up with the formation of aggregates. It is therefore challenging to deduce stability parameters of those proteins which unfold irreversibly.

In this presentation, the irreversible thermal denaturation of a large monomeric protein from *E.coli* cytosol, Maltodextrin glucosidase has been reported. The denaturation process has been monitored through differential scanning calorimetry, circular dichroism, and

UV-absorption measurements. The denaturation was irreversible, and the thermal transition was significantly scan-rate dependent, which indicated that the thermal denaturation was kinetically controlled. The thermal transition was insensitive towards protein concentration effect; this indicated that the rate limiting step in the transition involved monomolecular species. Based on the outcome of the analysis of data from calorimetrically monitored thermal transition, the thermal denaturation of MalZ protein can be represented by a one-step irreversible model. The thermal transition obtained from calorimetric study exhibited very good coincidence with the turbidity transitions, as well as with the unfolding transitions monitored by circular dichroism. Hence, it has been elucidated that the thermal denaturation of the MalZ protein was rate-limited by conformational unfolding, which preceded by formation of aggregates irreversibly, and thus produced the turbidity in solution. Hence it may be noted that the absence of protein concentration effect on irreversible thermal denaturation does not exclude protein aggregation. Thus the differential scanning calorimetry along with turbidity measurement can be a very effective method for understanding the mechanism of irreversible denaturation.

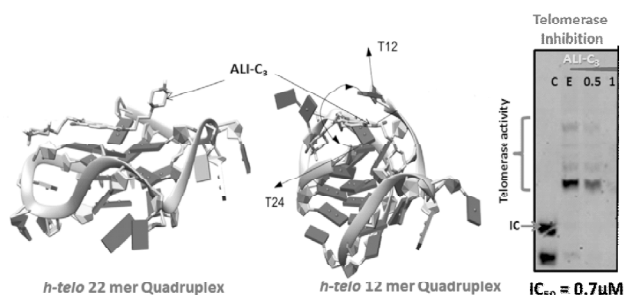
Novel Smart C₂-Symmetrical NDI Derivatives as G-Quadruplex Stabilizing Ligand with a Potential to Differentiate between Topological Structures

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Recent evidences of the formation of DNA G-quadruplexes in cells have provided solid support for these structures to be considered as valuable targets against cancer therapy. Three novel symmetric bisubstituted naphthalenediimide G-quadruplex stabilizing ligands; **ALI-C₃**, **BBZ-ARO** and **BBZ-AROCH₂** were designed and evaluated as telomerase inhibitors. The unfused aromatic rings containing “benzimidazole” in ligands allowed a flexible and adaptive conformation to ligand recognition with G-quadruplexes. ΔG_{-free} obtained from MMPBSA calculations and hydrogen bond occupancy after MD Simulations were decisive, as we found that electrostatic interactions (hydrogen bond occupancy) between ligand side chains and DNA grooves played main role not only in the amount of G-quadruplex formed, but also in selecting its topology, as **ALI-C₃** stabilized preferentially an antiparallel intrastrand 22 mer over **BBZ-ARO**, stabilizing a parallel interstrand 12 mer G-quadruplex more. Their interactions with telomeric G-quadruplex DNA were studied with competitive FRET melting, SPR and CD spectroscopy suggested that the

disubstituted NDI derivatives could strongly bind and effectively stabilize the telomeric G-quadruplex structure, and had significant selectivity for G-quadruplex over duplex DNA. Moreover, ligands showed also a different ability to inhibit telomerase. The correlation of these findings suggests the intriguing possibility that different G-quadruplex structures could differently inhibit the enzyme. **ALI-C₃** was found to be most potent telomerase inhibitor ($IC_{50} = 0.7 \mu\text{M}$) among three. Hence, easy synthetic access combined with selective recognition of different G-quadruplex conformations, stand out **ALI-C₃** as lead to be attenuated as anticancer agent.



Archaeal $\beta\gamma$ -crystallins: may be twins but one of a kind

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$\beta\gamma$ -Crystallins are the ancestrally related, prevalent protein domains with all- β structure organized in specific Greek key pattern, forming the $\beta\gamma$ -crystallin superfamily. Diversification of this domain in the primary structure, during its recruitment in different proteins from evolutionarily distantly placed organisms, therefore altering the properties (or functions), has been an important subject of investigation. Among the >170 archaeal genome sequences currently available in database, only two methanoarchaea viz. *Methanosarcina acetivorans* and *Methanosaeta thermophila* have $\beta\gamma$ -crystallins (M-crystallin and Methallin respectively), while widely prevalent in bacteria and eukaryotes. Both structurally similar methanoarchaeal $\beta\gamma$ -crystallins are different from each other: M-crystallin binds Ca^{2+} similar to many bacterial homologue, whereas Methallin does not. Methallin which does not have bacterial type Ca^{2+} -binding motif, exclusively binds trace metals, viz., Fe, Ni, Co and Zn at the site formed by six histidine residues in the core of the trimeric arrangement assisted by two histidines from one molecule. Methallin binds nickel with a high affinity (dissociation constant (K_d) of 44.8 nM at 30°C). Such binding geometry is unprecedented not only in $\beta\gamma$ -crystallins but also in other proteins and is a classic example of diverse evolutionary inventions and

adaptations manifested in this ancient methanogen, i.e., *Methanosaeta thermophila*. Incorporating two histidine residues at equivalent position does not lead to the formation of such binding site in other $\beta\gamma$ -crystallins, thus metal coordination by histidine and trimeric organization is specific to Methallin. Nickel is an essential trace metal required by methanoarchaea for the methanogenesis and their growth. The study, therefore, suggests the adaptability of the $\beta\gamma$ -crystallin fold to the presence of excess nickel in the environment and also indicate its potential early origin. Methallin, which represents a distinct $\beta\gamma$ -crystallin with a strong possibility for a role in combining trace metal biology with the geological record, enlightens us for exploring the ancient life and evolution of these molecules. Thus, it is quite possible that *Methanosaeta thermophila* is one of those organisms, which existed covering the transition periods of anaerobic to aerobic life forms overlapping to the Great Oxidation Event.

Structural elucidation of the resolvase activity by RecU from *B. subtilis*

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We have determined the crystal structure of the enzyme RecU from *Bacillus subtilis* in complex with a 12 base palindromic DNA fragment. The structure reconfirms the presence of active sites formed around clusters of acidic residues that are known to bind divalent cations. Our structure also shows the metal mediated binding of the DNA to the enzyme. Our complex structure shows the previously missing stalk and the N-terminal residues regions reported in our apo structure. We also present the the extensive molecular dynamic (MD) studies that we carried out on RecU studied using the Param Yuva II facility (C-DAC, Pune). The MD provides more insights to the N-terminal region (first 33 residues) which was always missing in the apo crystal structure.

Young Scientist

Molecular Dynamics Simulation study of β -Lactoglobulin with co-solvent to reveal structural transition of equilibrium intermediate

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The conformation transitions in the secondary/tertiary structures of proteins may lead to misfolding, self-association and aggregation in cells, causing various severe diseases in humans. We have selected β -lactoglobulin as a model protein and carried molecular dynamics (MD) simulation in presence of co-solvent urea and guanidium chloride (GdmCl) to understand the molecular mechanism of α/β conformational transition. Normal mode analysis carried to capture the propensity of residues involve in conformational transition. Results from radius of gyration shows a significant increase in total and hydrophobic solvent accessible area. The highest transition of 7 Å is recorded in trajectory of urea induced simulation and transition of 5 Å is seen at 8 and 22 ns of simulation. RMSD trajectory recorded for simulation in presence of gdmcl shows a continuous transition of 5 Å up to first 5 ns and attain the stable equilibrium after 12 ns, which is continued up to 40 ns of simulation. Result suggests that intermediate frames are stable in comparisons to native β -lactoglobulin (simulation carried in aqueous environment). Secondary structure composition analysis clearly depicted the transition in content of β -helix and β -sheet during the simulation in comparison to native protein. The results shown here may provide the structural evidence on the possible mechanism of structural conformational transition and amyloid formation in cells.

Keywords: β -lactoglobulin; MD simulation, Urea; guanidium chloride; amyloid formation

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Structural and functional analysis of novel antimicrobial peptides

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Antimicrobial peptides (AMPs) are small peptides with microbicidal properties and their length varies from 6 to 50 amino acids. Skin secretions of Chinese odorous frogs contain many such AMPs. These peptides are majorly

cationic and have affinity to the negatively charged bacterial membrane which results in cell death by lysis. The growing problem of resistance to conventional antibiotics and the unique mode of action of AMPs have made such peptides promising candidates for the development of a new class of antibiotics. Peptidomic analysis of purified AMPs reveals that the post-translational modification rarely happens in odorous frogs and thus helps to characterize AMPs by cloning techniques. While a number of peptides have been isolated till date, their mechanistic aspects remain unclear. High-resolution three-dimensional structures of these antimicrobial peptides provide insights into the mechanism of action, enabling thus to develop peptides with enhanced anti-microbial activity. This project is focused at chemical synthesis of short length peptides, cloning a few selected genes of frog peptides in pET32a+ expression vector, *E.coli* BL21 (DE3) expression and purification, structural characterization by solution NMR, antimicrobial activity assays and mechanistic studies.

Comparative proteomic analysis of advanced ovarian cancer tissue to identify potential biomarkers of responders and non-responders to first line chemotherapy of carboplatin and paclitaxel

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Ovarian cancer is the fifth most common cause of cancer in women and is the leading cause of death among cases of gynecologic cancer. In addition to the burden of the cancer, the complexity and uniqueness of the ovarian cancer is that it is diagnosed only in the advanced stages. The conventional treatment for advanced ovarian cancer is an initial debulking surgery followed by chemotherapy combination of carboplatin and paclitaxel. Despite initial high response, many women to the tune of 70% experience disease recurrence with a dismal prognosis. In this study, we have done a comparative gel based

proteomic analysis between the responder and non-responder-advanced ovarian cancer tissues obtained from six patients during debulking surgery. The fluorescent based *Differential In Gel Experiments* showed 312 spots out of which eight differentially expressed proteins with significant statistical significance were picked, trypsin digested and identified by mass spectrometric analysis. While aldehyde reductase, hnRNP, cyclophilin A, β -globin were up-regulated in the responder state, the proteins prohibitin, mitochondrial enoyl coA hydratase, peroxiredoxin, fibrin- β were up-regulated in the non-responder state. These proteins are seen to be functionally and clinically relevant in the respective responsive and non-responsive states to chemotherapy. These 'inherent' proteins, either individually or as a panel, are potential biomarkers to identify advanced ovarian cancer patients with inherent resistance to chemotherapy. This prior knowledge of the chemotherapy response will help clinicians and patients in the therapeutics of advanced ovarian cancer.

Development of 3D-pharmacophore model followed by successive virtual screening, molecular docking and ADME studies for the design of novel HDAC inhibitors.

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Histone deacetylases (HDAC) have emerged as an important therapeutics target for cancer, a neurodegenerative disorder, a neurological disease, traumatic brain injury, stroke, malaria, an autoimmune disease, autism, and inflammation. There are various side effects like myocardium damage and bone marrow depression have been observed with the treatment involving HDAC inhibitors. Therefore, discovery and development of new improved specific inhibitors is of both research and clinical interest.

A dataset of 23 compounds-podophyllotoxin based derivatives with well defined inhibitory activity against HDACs was used for pharmacophore modelling and 3D QSAR analysis. Vorinostat (SAHA) was used as standard compound in the data set. Thereafter virtual screening was performed with flexible docking (XP mode) and ADME filters.

109 five features pharmacophore was developed as hypothesis. Thereafter best 5 hypothesis (AADRR.67, AAADR.120, AAADD.870, AAADD.789, AAADD.425) were selected based upon the active and inactive scores for 3D QSAR validation. Thereafter, the generated pharmacophore model AAADD.870 was used as a 3D query to fetch potent molecules having structures and desired features of a potent HDAC inhibitor using the

virtual screening workflow (containing Asinex, TOSLab, Maybride, and Binding database) which has approximately one million compounds. We obtained 1000 molecules matching our model. Further the virtual screening search retrieved 65 most potent candidates which were further filtered using suite (ADME analysis). The proposed model has been validated by docking the screened compounds into the binding pocket of Human HDAC3 (PDB ID:4A69, RCSB Protein Data Bank). Our discovery will help in the identification of more specific HDAC inhibitors.

Identification of probable lead compounds for inhibition of CRY protein in *Pisumsativum* by structure modeling and pharmacophore designing

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DELLA proteins are growth-repressing factors and play central role in Gibberlic Acid signal transduction. CRY and LA are two main DELLA-encoding genes in pea that are responsible for inhibition of seed germination. Thus, it is mandatory to inhibit the activity of these proteins in order to increase percentage seed germination. In this study, we hunted for inhibitors of CRY protein by using pharmacophore search technology. To begin with, the primary sequence of CRY protein of *Pisumsativum* (Accession number: ABI34432) was retrieved from NCBI and its secondary and 3D structure was predicted by homology modeling, threading and ab-initio modeling. The model generated by I-TASSER server is analyzed to be the best modeled structure, on the basis of Z-score comparison and analysis of secondary structure. Further, the active site analysis of the modeled protein indicated that the third largest pocket is the best site for inhibiting its action as it has a stable conformation and it possesses the active domain. Moreover, the composition of this site is also favorable for interaction with other molecules. This selected site was targeted against the zinc database to find probable ligand molecules that can inhibit its action. The hit against a collection of purchasable drug category consisting of 215,407,096 conformations of 22,723,923 compounds in the database generated a total of 2854 ligand hits. Finally ten molecules were obtained by the analysis of the group of ligands obtained.

Effect of N-terminal modifications on A β amyloid formation

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Background: Alzheimer's disease (AD) is characterized by extracellular deposition of A β amyloid structures in the brain tissues. Previous studies suggest that the amino acid residues in N-terminal sequence play crucial role in determining the toxic potential of A β amyloid structures. In addition, the AD brain plaques consist of many N-terminally modified A β peptides (such as, deletion of first two amino acid, residues and pyroglutamate formation of third glutamine residue). Previous studies suggest that such modifications result in formation of highly toxic A β species. However, the effect of such modifications in A β on its assembly, structure of aggregates and toxicity are poorly defined.

Methodology: In order to address these issues and to probe the structural consequences of N-terminal modification, initially we focused on a series of three A β species: (i) unmodified full length peptide, termed A β (1-40), (ii) A β lacking the two N-terminal residues, termed A β (3-40), and (iii) A β lacking the two N-terminal residues and carrying a pyroglutamate ring at the third N-terminal glutamate residue, which is referred as pEAB β (3-40). We subjected modified and unmodified A β peptide to a series of biophysical analyses such as, Thioflavin T (ThT) kinetic assay, Congo red absorption and birefringence assay, FT-IR, Circular dichroism and fluorescence spectroscopy, X-ray diffraction and Transmission electron microscopy.

Results: Through our study, we have revealed that the N-terminal modifications results increased fibrillation potential, formation of smaller aggregates and fragmentation of longer fibrils. These smaller aggregates of modified A β show higher seeding activity than the longer fibrils of unmodified A β . Therefore, the high toxic potential of these modified A β can be explained by formation of smaller aggregates and an increased fragmentation of longer fibrils.

Somatosensory function is affected by stroke-like injury in motor cortex

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Focal brain damage produces loss of function of the body controlled by the injured area mainly because of

destruction of neuronal tissue at the site of damage. Sometimes behavioural deficits are observed in patients that cannot be attributed to the site of injury in brain. These deficits may arise due to changes occurring in regions distant to the site of primary brain damage. Therefore the present study was taken to investigate changes occurring in the function of anatomically connected intact regions following focal brain damage. The somatosensory and motor cortices in the brain have reciprocal anatomical connections and are functionally interdependent. Therefore stroke-like lesions were produced in motor cortex and its influence on function of somatosensory cortex was determined. Lesions were produced in adult Long-Evans rats by photochemical activation of rose bengal (20mg/kg) with green laser (532 nm). Sham controls were operated in a similar manner but they did not receive laser exposure. Seven days following the lesion, electrophysiological data was acquired from layers 2/3, layer 4 and layer 5 of somatosensory cortex of anesthetized animals. The multiunit data recorded on seventh day showed an increase in excitability in layers 2/3 and layer 5 of the lesioned animals compared to controls. In addition to changes in the neuronal activity, focal motor cortex damage also affected the sensory behaviour of rats in gap-cross task.

Physicochemical Aspects of Amyloidogenic and Non-amyloidogenic Intrinsically Disordered and Structured Human Proteins

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Formation of amyloid fibrils from proteins which is soluble in their native state involves several intermediates and the mechanistic detail of the process is still very obscure. It has been, however, observed that to nucleate the amyloid fibril formation partial unfolding of globular proteins or some structural order for disordered protein is desirable. In addition amyloidogenic region (AR) in protein sequence plays a significant role in protein aggregation and amyloid formation. These make overall physicochemical properties, such as hydrophobicity, PI, charge and other, of a protein very important factors, apart from amyloidogenic region which is a stretch of amino acid residues in a protein sequences prone to form amyloid fiber for a protein's solubility, stability and energetic of amyloid fiber. Based on sequence analysis we presented statistical comparison and distribution pattern of isoelectric point (pI), hydrophobicity, aliphatic index (AI) and instability index (II) of amyloidogenic and non-amyloidogenic proteins present in human proteome. The analysis was performed for both the structured and

intrinsically disordered proteins (IDPs). pI followed a symmetric bimodal normal distribution for structured proteins whereas an asymmetric bimodal distribution was a signature for the disordered proteins. Mean pI of amyloidogenic proteins in the basic range was significantly lower than that of the non-amyloidogenic protein. Further, we analyzed sequence complexity of ARs which are present in intrinsically disordered human proteins. More than 80% human proteins from the disordered protein databases (DisProt + IDEAL) contained one or more amyloidogenic regions (ARs). A significant increase in AR content was observed as the disorderiness of protein sequences decreased. A probability density distribution analysis and discrete analysis of AR sequences indicated that ~8% protein sequences were in the AR and average length of the ARs was 8 residues. Another important observation was that the residues in the ARs were high in sequence complexity and the ARs seldom overlapped with low complexity regions (LCRs). In addition, the AR sequences showed mixed conformational adaptability towards helix, β -sheet/strand and coil conformations.

Unfolding of HIV TAR—DNA Hairpin: a Single Molecule FRET studies

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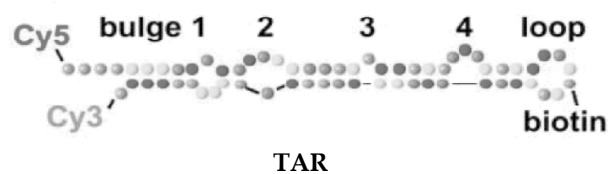
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Current estimates suggest that at least 34 million people in the world are infected with the human immunodeficiency virus (HIV), the cause of the acquired immunodeficiency syndrome. Knowledge of the life cycle of HIV has provided the fundamental information necessary to initiate programs that will identify drugs to treat the infection. Various drugs targeted to the different steps of viral replication have been selected but have shown little or no clinical benefit. Reverse transcription (retroviral life cycle) involves multiple steps, including several nucleic acid rearrangements catalyzed by the HIV- nucleocapsid protein (NC). NC protein helps in the opening of HIV trans-activation response (TAR) – DNA hairpin (Figure) to facilitate the binding of TAR-RNA which helps in the viral replication and infection.

We directly measure the dynamics of the HIV TAR – DNA hairpin with multiple loops in the absence of NC

protein using single-molecule Forster resonance energy transfer (smFRET) methods. We introduced two different strategies to slow down the open/close dynamics of the hairpin. Multiple FRET states are identified that correspond to intermediate melting states of the hairpin. The FRET efficiency of each intermediate state is calculated from the smFRET data. The results indicate that hairpin unfolding obeys a “fraying and peeling” mechanism, and evidence for the collapse of the ends of the hairpin during folding is observed. These results suggest a possible biological function for hairpin loops serving as additional fraying centers to increase unfolding rates in otherwise stable systems.



Proteins without structure and deviations from Structure-Function-Paradigm

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One of critical component of central dogma of molecular biology states for Structure-Function-Paradigm. The recent discovery of proteins which are unstructured in physiological buffer conditions but still functional in native cell environment, posed a challenge to classical understanding of protein structure and function. These proteins are called Intrinsically Disordered Proteins (IDPs). The emerging concept of Disorder-Function-Paradigm is one of the most debated area in modern protein sciences. There are reports that nearly 40% of human proteome is disordered in full or part. On the basis of biological functions and regulation in protein interaction networks, IDPs are implicated in various human diseases. Examples include Oncoprotein p53, Myc and E1A. These IDP are involved in regulating large numbers of genes important in key cellular processes such as growth, differentiation, metabolism and apoptosis. During last decade, some progress has been made in structural determination of disordered proteins complex with their partners. Our focus of this project is to decipher molecular determinants of interaction between a disordered protein and its ordered partner. Such type of molecular detailing in biophysics is not only important for basic research but it has a tremendous importance in drug discovery and cellular metabolic control. Due to elusive nature of these IDPs there are no drugs developed so far, so this study could be informative for new understanding for drug development in an IDP.

Molecular detailing of the disordered system will be discussed in reference to folding and dynamics.

Gold Nanoparticles-plasmonic Biosensor for Real-time Detection of *Peste Des Petits Ruminants* Antigen

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The PPR is a contagious viral infection of both wild and domestic cloven-hoofed small ruminants. The morbidity and mortality rates from PPR can be up to 100% and 90% in severe outbreaks. Due to high economic loss by PPR, it became necessary to develop assay which will be applicable under field conditions outside laboratory without handling of toxic reagents. Chromatographic strip test also known as lateral flow assay is a very simple diagnostic assay which can be used for diverse types of analytes. Application includes, tests on drugs, hormones and metabolites in biomedical, phytosanitary, veterinary, feed, food and environmental contaminants. This assay can be used for qualitative as well as for quantitative evaluation of analytes and easy visual judging of the antigen-antibody reaction using gold nanoparticle. The lateral flow assay strips were prepared using nitrocellulose membrane, sample pad, conjugate pad and absorption pad. All these pad and membrane were fixed on solid laminate for support. 4G6 Mab (reactive against nucleoprotein of PPR virus) was used for conjugation with gold nanoparticle (20nm), purified PPR virus (100x) for test line and goat antimouse antibody (1mg/ml) as control line. The analysis of standard solution of PPR virus, serial dilution was done from stock solution of 100x prepared in milliQ water. From each diluted solution 4 μ l was mixed with 4 μ l of antibody gold conjugate. This solution was kept for incubation of 5 minutes. After incubation, above solution was applied over conjugate pad. Then running buffer (PBS with pH of 7.5) was poured on sample pad drop by drop to carry the whole complex of gold-antibody and analyte (PPR virus) through nitrocellulose membrane. The complex was allowed to run up to the top of nitrocellulose membrane and strips were kept for drying. The color signal was produced on control line of strips immediately. Before this analysis the gold-antibody probe was tested for development of control and test line without addition of standard solutions of PPRV producing the color signal on both control as well as test line. There are routinely used procedures based on enzyme-linked immunosorbent assay (ELISA) for PPRV detection. The procedures based on ELISA, however, are time consuming, complicated, and costly but our test is a rapid, very simple, cost effective and sensitive immune-chromatographic assay using gold nanoparticles. The assay developed is rapid and simple, the results of this qualitative one-step test

can interpret visually. Our sensitive immune-chromatographic assay is a promising candidate for diagnostic application at field level even for non-expert users.

An Insight in to the Binding of Pyrrolidinium Based Ionic Liquid with Human Serum Albumin

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The interaction between synthesized pyrrolidinium based ionic liquid, N-butyl-N-methyl-2-oxopyrrolidinium bromide (BMOP) and human serum albumin (HSA) have been investigated through fluorescence, UV-visible, FT-IR, and molecular docking methods. The BMOP was synthesized and characterized by using $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, FTIR techniques. The steady state fluorescence spectra revealed that BMOP quenched the fluorescence of HSA through combined quenching mechanism. Corresponding thermodynamic parameters viz. Gibbs free energy change (ΔG), entropy change (ΔS) and enthalpy change (ΔH) suggested that the binding process was spontaneous and entropy driven. It was also suggested that hydrophobic forces play a key role in the binding of BMOP to HSA. In addition, the pyrene probe analysis again suggests the involvement of hydrophobic interaction in HSA-BMOP complex formation. Surface tension profile showed that the CMC value of BMOP in the presence of HSA is higher than the CMC value of pure BMOP. The FT-IR results show a conformational change in the secondary structure of HSA upon the addition of BMOP. The molecular docking result indicated that BMOP binds with HSA at hydrophobic pocket domain IIA with hydrophobic and hydrogen bond interactions in which hydrophobic interaction are dominating.

Key words: Ionic liquid, human serum albumin, quenching, hydrophobic interactions, molecular docking.

“Are Frequent Exposures to Electromagnetic Field Radiation Emitted by Cellular Phone Base Station Affecting Autonomic Function in Human Being?”

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The development of wireless communication systems has given rise to concerns about the potential human health hazards of increased and chronic exposure to electromagnetic field (EMF) and radio frequency (RF) radiation. The aim of this study is to evaluate whether

the frequent exposures to electromagnetic field radiation emitted by cellular phone base station affecting autonomic function in human being.

The present study covers urban as well as rural areas of Surat districts. It was under taken in 66 subjects, including control group. The study includes anthropometric parameters (like Height, Weight, BMI), Clinical examination (like Pulse rate, base-line blood pressure etc.) and standard cardiovascular autonomic function assessments test for evaluating Sympathetic and Parasympathetic activity were performed in both groups. Ethical approval and other aspects were taken into consideration. The autonomic function tests were performed in all the 66 apparently healthy subjects. The data obtained was tabulated with respect to various parameters and was statistically treated and analysed.

In our study resting blood pressure i.e. systolic and diastolic blood pressure values were observed to be significantly increased in base station resident group as compared to the control group, it may be due to more pronounced vasoconstriction. Our observations reveal autonomic function changes do not alter in all the subjects but if we compare the study group with the controls than we find the values are significantly increased. We found a significant alteration of the value and these were evident from the change in heart rate and blood pressure.

Estimating Ring-current Contributions by Quantum Chemical shift Calculations

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NMR Chemical shifts, Aromatic ring current effect, Induced fields due to ring currents

There has been significant data available on the ring current induced field values at distant protons and thus contribution to chemical shift by this ring current mechanism. Most of such results have been obtained (1) invoking the classical model for the dipoles placed at the center of ring current circulating regions. The question as to whether it would be possible make such ring current estimates by using the soft-wares used for Quantum Chemical Calculation of chemical shifts would be discussed in this contribution in such a way that it would be possible to the discern how exactly such QM calculations of ring current shifts would help in the context of biological macromolecules.

Peptide and PNA Activated AuNPs Plasmon Changes in Visual Detection and Quantification of Virus and Virus Genes

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Gold nanoparticles (AuNPs) are uniquely nontoxic and have characteristic plasmon making them ideal theranostics. Among different nanomaterials developed so far gold nanoparticles (AuNPs) are unique to their nontoxic and optical characteristics, particularly their potential as theranostics in cancer research as photo-thermal therapeutics and contrast imaging agents. Activation of AuNPs using various ligands may help them as targeted vehicle for controlled drug release and visual *in-vitro* and *in-vivo* diagnostic reagent as a result of plasmon changes. Characteristic plasmon colour of AuNPs changes as a result of variation in their size and charge due to interaction among molecules at the interface of AuNPs from solution, thus provides vital information on specific molecules presence in the sample, when AuNPs are specifically tuned and activated. AuNPs were activated with peptides and peptide-nucleic acids (PNA) and respectively allowed their reaction with specific virus and virus genes, that led to change in plasmon color of AuNPs helping in the detection of the virus in solution. Plasmonic shift in visual spectra of AuNPs solution as function of additive concentration and time led to the quantification.

Specific peptides were identified and used for activation of AuNPs that created nanoparticles networks due to interactions with virus leading to visual assay for their detection and quantification. Specific AuNPs network generated due to interactions of antigen- antibody not only led to color changes but may also be used for other biosensor platforms (Total Internal Reflection, TIR,). Peptide nucleic acids (PNAs) reaction with viral genes at AuNPs interface accompanied specific color changes leading to the visual detection of the virus. Further estimation color in visual spectroscopy helped in the quantification of viral genome (RNA). Strong hybridization characteristics of PNAs and their use in AuNP activation provided not only detection but also antiviral intervention in *in-vitro* and *in-vivo* systems. Thus, another likely theranostic applications of activated AuNPs in virus research.

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Structural & Functional aspects of Trypsin-Gold Nanoparticle Bioconjugates: Stoichiometry determines Efficiency

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Trypsin is arguably the most important member of the Serine Proteases. It has wide implication in different biochemical techniques like limited proteolysis, residual enzymatic activity & proteolytic assays. Interaction of nanoparticles (NPs) with proteins nowadays has become immensely important considering the biological importance of proteins in living system. To understand the mode of interaction of Nano-Protein Complex, considerable impetus has to be given to the initial adsorption of the NPs on the protein surface & the conformational & morphological changes of the protein and NP respectively. Bioconjugates of Gold Nanoparticles (GNP) with trypsin has been known to exhibit increased efficiency & stability in various biophysical & biochemical experiments. However, the structural aspects of this high efficiency & the true mode of interaction of trypsin & GNP's are yet to be discovered largely. Formations of bioconjugates also require tedious chemical conversions which is not easily assessable always. Here we report simple trypsin GNP mixtures in different stoichiometry which also exhibit higher efficiencies in biochemical assay, cell trypsinization & higher resistance to autolysis. Furthermore we have done extensive studies to understand the structural consequence of this trypsin GNP interaction by using AFM, TEM, FTIR & Circular Dichroism techniques. Our findings suggest that upon interaction, the trypsin forms an adherent layer with gold nanoparticles that effectively changes the morphology & dimensions of the nano constructs. However, the stoichiometry of Trypsin & GNP is also extremely important, as higher concentration of GNP might damage the conformation of protein. Stability studies related to GdMCI denaturation shows that 1:1 Trypsin-GNP construct exhibit maximum stability & high efficiency in all the assays performed.

Correlation of conformational dynamics with the pathogenicity in an amyloid forming protein

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The conversion of native protein into insoluble fibres and their deposition in tissues is the hallmark of various types of amyloidosis. While detailed understanding of the process is not clear, conformational dynamics is believed to play a key role in this conversion of native proteins via transiently formed non-native intermediates.¹ In recent work,² we have probed the role of backbone dynamics in the amyloid forming protein, transthyretin (TTR), that is implicated in neuropathies, cardiomyopathy, ocular, leptomenigeal and other forms of amyloidosis.³ We have investigated motions from fast (picoseconds-nanosecond) to slower timescales (~milliseconds) using NMR and molecular dynamics simulations. Residue specific backbone ¹⁵N spin relaxation rates on wild type and mutant TTRs were used to obtain order parameters (S^2), which suggest that most of the residues exhibiting fast motions lie in the unstructured regions of TTR's periphery while the hydrophobic core is virtually rigid. In contrast, results of ¹⁵N C.P.M.G. relaxation dispersion NMR probing slower (~ms) motions show that the dynamic residues are predominantly located in the α -strands of TTR's hydrophobic core that also forms the tetramer's interface. We found that approximately 10% of wild type TTR exists as a minor conformer in exchange with the native state. Similar experiments on many widespread pathogenic mutants (V122I, V30M and L55P) reveal that the proportion of the non-native conformer is higher in pathogenic mutants than wild type and their formation is thermodynamically favoured in case of pathogenic mutants as well, which hints to a role of these intermediates in protein aggregation.

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Dynamics of thiol-based redox switches in the parasitic flatworm *Fasciola gigantica*

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Fascioliasis, a food-borne trematodiasis is caused by two species of *Fasciola*. WHO estimates that at least 2.4 million people are infected in more than 70 countries worldwide, with several million at risk, particularly in developing countries in South-East Asia. Farm animals usually perpetuate transmission of the infection in the environment, though reports suggest that in some areas transmission to humans is constant and intense. Triclabendazole, the only drug recommended by WHO against fascioliasis, is active against both immature and adult parasites, and is therefore employed during the acute and chronic phases. Recent reports suggest increasing resistance to triclabendazole recommending the necessity to develop new chemotherapeutic strategies.

Maintaining redox homeostasis is a major adaptive problem faced by parasites and its disruption can shift the biochemical balance toward the host. Thus redox-active protein system can be a potential drug target. The redox based thiol/disulfide system is the key antioxidant system in defense against oxidative stress among many cellular roles. In recent years it has become evident that reactive oxygen and nitrogen species, despite their traditional reputation as components of radical chains and harbingers of damage, act as physiologically essential messengers in signal transduction. The signaling properties of particular oxidants are primarily sensed and mediated by 'protein thiol switches', which are protein thiols that are specifically and reversibly modified by oxidation, thereby switching the protein between different conformational and functional states.

We have performed biochemical and functional experiments on the thiol based antioxidant system of *Fasciola gigantica*. The proteins of thioredoxin and glutathione systems have been studied *in vitro* and *in vivo*. We will discuss the results in detail.

X-ray diffraction analysis of two crystal forms of backbone pilin SpaA fragment from *Lactobacillus rhamnosus* GG

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Lactobacillus rhamnosus GG is a Gram-positive probiotic strain, which has been extensively studied for its various

health benefits that includes stabilization of gut microbiota, exclusion of pathogens colonization, and modulation of protective innate immunity. Hair-like surface appendages known as pili have been recently identified in *L. rhamnosus* GG. These pili (called SpaCBA) play a key role in host interactions by mediating adherence, stimulating biofilm growth, and modulating immune activity. The SpaCBA pilus consists backbone pilin SpaA, and two ancillary pilins (SpaB and SpaC). Here, we report purification, crystallization, and X-ray diffraction analysis of two crystal forms of SpaA fragment obtained by hanging-drop vapor diffusion method. Crystal form I belong to space group $P2_12_12$ with unit-cell parameters $a = 57.08\text{\AA}$, $b = 74.36\text{\AA}$, and $c = 116.8\text{\AA}$. Whereas, the crystal form II exhibits space group $I422$ with unit-cell parameters $a = 100.35\text{\AA}$, $b = 100.35\text{\AA}$, and $c = 57.34\text{\AA}$. Both the crystal forms diffract to 2\AA resolution at the home-source. According to Matthews coefficient (V_M) calculation, the asymmetric unit contains four molecules in crystal form I, and one molecule in crystal form II. The progress of structural investigation will be discussed.

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Alternate drug for carbapenem resistant strain of *Acinetobacter baumannii*

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Acinetobacter baumannii has been identified by Infectious Diseases Society of America as one of the six pathogens that cause majority of hospital infections. Carbapenems are the most effective β -lactam antibiotics that are routinely prescribed by clinicians to patients infected with *A. baumannii*. Emergence of resistance against carbapenem makes it difficult to treat *A. baumannii* and is therefore a threat to public health. Proteomics, biophysical and bioinformatics have been used to understand the different resistant mechanisms of *Acinetobacter*. The result showed that *Acinetobacter* develop carbapenem resistance due to the over-expression of beta-lactamase, altered Omps, altered PBPs, elevated metabolism. Therefore it is very important to find a suitable alternative to the carbapenem. We have tried different alternative to find suitable drug to carbapenem resistant strain of *Acinetobacter baumannii*. Silver nanoparticles has been prepared using ethylene glycol mono methyl ether as solvent and PVP as capping agent. Transmission electron microscopic study revealed silver nanoparticles in spherical shape with narrow size distribution. The average size of silver nanoparticles has varied by changing the metal precursor to capping agent ratio, and corresponding characteristic size dependent plasmonic properties were observed using UV-Vis

spectroscopy. It was found that novel silver nanoparticles having particle diameter <10 nm exhibited a tremendous potential antibacterial activity against the carbapenem resistant strain of *Acinetobacter baumannii*. Therefore, PVP-capped silver nanoparticle can be developed as an alternative to carbapenem, the most prescribed antibiotics for the *Acinetobacter baumannii* infections.

Keywords: PVP-capped Silver nanoparticles; Carbapenem resistance; *Acinetobacter baumannii*

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NMR Structures of proteins with Actin Depolymerization Factor Homology Domain

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The proteins of the ADF/cofilin family are essential and key regulators of actin filament dynamics. They regulate actin filament dynamics through G-actin binding, F-actin binding and depolymerization, F-actin severing, G-actin monomer sequestering activity, and controlling the rate of nucleotide exchange from actin monomers.

We are working on characterization of non-vertebrate ADF/cofilins and their structural homologs. So far, we have characterized the solution structures and dynamics of these proteins from *Leishmania donovani* (LdCof), *Toxoplasma gondii* (TgADF), *Caenorhabditis elegans* (UNC-60A and UNC-60B) and *Drosophila melanogaster* (Twinstar) using NMR spectroscopy [1-5]. Comparison of the structures and dynamics of these proteins with each other and with other ADF/cofilin suggests that almost all ADF/cofilins have conserved ADF/cofilin fold with structurally conserved G/F-site and F-site for interaction with G-actin and F-actin. But, finer structural and dynamics differences are observed for the F-site of strong and weak F-actin severing proteins. The F-loop of proteins displaying strong severing, viz. UNC-60B, yeast cofilin, actophorin, human cofilin and chick cofilin, is relatively less inclined towards their C-terminals and is also more flexible. While the F-loop of proteins displaying weak severing, viz. UNC-60A and LdCof, is relatively more inclined towards C-terminal and is also less flexible. This small change in conformation and dynamics is sufficient to explain both the F-actin severing activities and co-sedimentation properties of ADF/cofilins. For related *C. elegans* glia maturation factor (CeGMF), which

structurally shares the ADF-homology fold, the dynamics of F-loop are significantly altered and biochemical factors seem to restrict its binding to G-actin. We have also characterized human plasma gelsolin fragment 28 - 161, which shares structural features with ADF/cofilin family proteins.

Molecular modeling techniques to understand structural significance of biomolecules

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Hypermodified nucleosides are found at various positions in transfer RNA molecule. The role of these modified nucleosides is to optimize codon-anticodon interactions to enable smooth and in phase protein biosynthesis process as well as in tertiary folding of transfer RNA. The frequency of such modified bases is more at first 'Wobble' (34th) position and 3'-adjacent (37th) position in anticodon loop of tRNA. Wybutosine (yW) and its derivatives such hydroxywybutine (OHyW), peroxywybutosine are most complex modified nucleosides found in the anticodon loop at 37th position of eukaryotic tRNA^{Phe}. It has been reported that lack of tRNA modification (wybutosine) leads to translational defects which have been associated with certain human diseases. Similarly, the modified nucleosides taurine, lysidine, m²G, and m²₂G are known to provide structural stability to tRNA molecule. Semi-empirical quantum chemical, molecular mechanics methods along with MD simulations have been used to investigate structural significance of such modified nucleosides.

Other techniques such as sequence analysis, homology modeling, molecular docking and molecular dynamics simulation have also been used to investigate structure-function relationship of enzymes responsible to cleave amyloid beta peptides a causative agent of Alzheimer's disease. The RMSD and RMSF results obtained using MD simulation revealed the stability of enzymes-wild type amyloid beta peptide complexes. The molecular basis of amyloid beta peptide degradation at specific sites by ACE, hECE-1, cathepsin B, aminopeptidase and gelatinase enzymes might be helpful to design new approaches to clear A β peptide.

Oral Presentations

NMR Characterization of Molten Globule and Pre-Molten Globule States of L94G mutant of horse Cytochrome-c

OP 1

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Proteins fold *via* a number of different intermediates such as MG and pre-MG states. Understanding structure of these intermediates at atomic level is often a challenge, as these states not only are observed mostly under extreme conditions of pH, temperature and chemical denaturants but these are also extremely dynamic in nature. Several other processes such as chemical modification, site directed mutagenesis (or point mutation) and cleavage of covalent bond of natural proteins often lead to MG and pre-MG states under physiological conditions (near neutral pH and room temperature). The dynamic nature of the protein in these folding intermediate states makes them unsuitable for 3D structure determination. Most of the intermediate states studied so far have been characterized using techniques such as circular dichroism, fluorescence, viscosity, dynamic light scattering measurements, dye binding, infrared techniques, etc. There is limited amount of structural data available on these intermediate states by nuclear magnetic resonance (NMR), if any, and hence the need to characterize these states at the atomic level. We have previously identified a mutant L94G of horse cytochrome-c, which shows characteristics of MG state near room temperature and pH 6.0. We are in the process of characterizing this MG state at atomic level resolution by NMR using a ¹⁵N/¹³C labeled protein. Our preliminary studies with number of NMR experiments suggest that the molten globule state is remarkably different from the wild type horse cytochrome-c.

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Sanjeevini: A Freely Accessible Web-Server for Target Directed Lead Molecule Discovery

OP 2

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Computational methods utilizing the structural and functional information help to understand specific molecular recognition events between the target biomolecule and candidate hits and make it possible to design improved lead molecules for the target.

Sanjeevini represents a massive on-going scientific endeavour to provide to the user, a freely accessible state of the art software suite for protein and DNA targeted lead molecule discovery. It builds in several features, including automated detection of active sites, scanning against a million compound library for identifying hit molecules, finding a target for synthesized molecules, all atom based docking and scoring and various other utilities to design molecules with desired affinity and specificity against biomolecular targets. Each of the modules is thoroughly validated on a large dataset of protein/DNA drug targets.

Sanjeevini is a freely accessible user friendly web-server, to aid in drug discovery. It is implemented on a teraflop cluster and made accessible via a user friendly web-interface at www.scfbio-iitd.res.in/sanjeevini/sanjeevini.jsp. A brief description of various modules, their scientific basis, validation, and how to use the server to develop *in silico* suggestions of lead molecules is presented.

Microenvironment based functional preferences of Cystine residues in high resolution protein crystal structures

OP 3

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Microenvironment is the three dimensional spatial arrangement of atoms around any functional group of

amino acids embedded in protein structure. It has been shown earlier that the micro-environments around conserved amino acids are conserved throughout the protein families (Bandyopadhyay and Mehler, 2008). In this work, we have hypothesized that amino acids perform dissimilar functions when embedded in dissimilar microenvironments. To test this hypothesis disulfide-bridged Cystine residues were chosen from high resolution protein crystal structures; different micro-environments around these Cystine residues and were clustered using agglomerative clustering; functions of Cystines in individual clusters were extracted from published literature.

Three dissimilar microenvironment clusters were identified, i) buried-hydrophobic, ii) exposed-hydrophilic and iii) buried-hydrophilic. The literature survey and functional annotation of Cystine residues have shown that buried hydrophobic cluster caters redox-active Cystine residues in alpha helical conformation. All these redox-active Cystines are part of -C-x-x-C- motif mainly from thioredoxin fold. The local sequence alignment and structural alignment of these folds have shown that microenvironments around these redox-active Cystines are conserved. All these Cystines have relatively high strain energies suggesting that slight perturbation in the microenvironment would perturb the redox state of the disulfide-bridged Cystine.

Secretagogin: Not just a Secretagogue "Identification of SCGN as a Cytosolic Insulin Binding Protein"

OP 4

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Secretagogin (SCGN) is a sparsely explored hexa EF-hand Ca^{2+} binding protein abundantly expressed in $\hat{\alpha}$ -pancreatic cells. It is implicated in insulin and corticotropin-releasing hormone (CRH) release and is shown to favour cell survival. This led us to investigate the role of Ca^{2+} signalling via secretagogin. In our *in-silico* analysis, we observed a striking subsistence of secretagogin at all possible locations of insulin existence *i.e.*, at the site of insulin synthesis ($\hat{\alpha}$ -pancreatic cells cytosol), on the path of maturation (secretory vesicles), at the extracellular environment and also on the cellular targets (including specific neuronal population). This observation prompted us to explore the functionality of the coexistence of two critical proteins. We report here an extremely important feature of secretagogin, that is, it binds insulin. Our data evoke an essential role of Ca^{2+} on the complex formation of insulin and secretagogin. Weaker affinity (albeit strong enough to make a stable complex in the presence of Ca^{2+}) may also suggest an

insulin transporter like function of secretagogin, which binds/releases insulin at specific Ca^{2+} signals. We elucidate that secretagogin protects insulin from reducing agent induced aggregation in a Ca^{2+} dependent manner which suggests an anti-aggregation type function of secretagogin for insulin. This observation suggests that secretagogin might be assisting insulin to fold correctly *in-vivo*. The decline in insulin level/effectiveness (*i.e.*, diabetes), with the ever increasing victims, is among the most rampant disease world-wide. Our study implies that secretagogin might become a novel target for developing future therapeutics for diabetes and other insulin-related pathologies.

New challenges of nanoscience on society and environment

OP 5

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Potential impact of nanoscience and nanotechnology can be widely seen on society with certain issues of concern. Nowadays nanomaterials are progressively entering in the everyday life, including the use of nanoscale materials in biomedical research, catalysis and electronics, thus attracting an increased importance in many fields of technology. It is making considerable improvements to protect the environment by the incorporation of nanoscale devices for treating and remediating environmental contaminants and enhanced sensing. It has found application in the detection and tracking of pathogens, contaminants, heavy metals, nutrients, particulates, environmental characteristics and allergens. Defined approaches to the coherent design of nanobio interfaces for binding of protein and enzyme to nanomaterials are vital for advanced engineering and functional nanobiomaterials for sensing, biocatalysis and biomedical applications. In view of their phenomenal expansion and growth, the historically unparalleled developments of nanoscience and nanotechnology brazen out our society and natural environment with new challenges. Some interior materials of the nanoparticles can be contaminated to the environment. Environment itself affects the toxicity and fate of nanoparticles. Particles in the air, soil or water can interact with nanoparticles and affect what these particles become and where they eventually end up. Technological revolutions have shown that frontiers of science have the prospective to overlay the way for integrated and radically innovative approaches, providing new solutions for some of the most burning problems. Thus, the societal propositions of the newly promising fields need to be known and understood in a better way in order to enable the researchers to respond to what is best for society and environment at large.

Preferential Recognition of DNA G-Quadruplex Topologies

OP 6

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Nucleic acids containing successive runs of guanines (G) tend to self-associate into a four stranded G-quadruplex (G4) structure stabilized by Hoogsteen hydrogen – bonding under some monovalent cations. In recent years, these structures have attracted intense interest because of their role in potential biological functions, such as gene regulation, gene expression and antitumor potential. G-rich sequences are unevenly distributed in some regions of the human genome, including telomeric ends, immunoglobulin switch regions and regulatory elements in some gene promoters, such as *c-myc*, *c-kit*, and so on. G-quadruplexes are considered to be promising targets for antitumor drug design. Several classes of small molecules that are able to selectively bind to G-quadruplexes have been known. The cationic meso-tetrakis (4-(N-methylpyridiumyl) porphyrin (TMPyP4), as a potential inhibitor of telomerase activity, has been the subject of extensive investigations.

We report here on the interaction of G-quadruplex binding ligand (TMPyP4), with G-rich sequences of various genomic locations. The sequences primarily adopt parallel as well as antiparallel quadruplex structures in solution containing monovalent cation (K⁺). Circular dichroism (CD), Ultra-Violet Absorption (UV-Vis), and Fluorescence spectroscopy were used to investigate the effect of TMPyP4 on the topology of various G-quadruplexes. CD spectra of TMPyP4 complexes with all the G-rich sequences suggest a preferential destabilization of quadruplex with parallel topology while the TMPyP4 induced formation of antiparallel G-quadruplexes. To the best of our knowledge this is the first report on preferential destabilization and stabilization (induction) of DNA G-quadruplex topologies by a quadruplex specific synthetic ligand TMPyP4.

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Misfolding of Gelsolin a large multidomain protein involves transitional pathways of morphologically disordered aggregates and structured amyloid like aggregates

OP 7

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Frustration from strong interdomain interactions result in complex folding and misfolding pathways in large multi-domain proteins. Although several pathogenic and non-pathogenic proteins from diverse sources have been implicated to form amyloids, there is still a dearth of reports on amyloid formation by large, multidomain proteins. Here, we report the misfolding and association properties of wild type and mutant forms of human plasma gelsolin, a large (~82kDa), six domain protein. While the proteins differed in their stabilities, both showed three-state unfolding with clearly distinguishable intermediate population. Under reduced acidic conditions at 37 °C, both the proteins progress into amyloid fibrillation. The kinetics as well as dye-binding affinities differed for the two types of misfolding assemblies and their differences in conformational and morphological features was confirmed by AFM/EM and FTIR analysis. Both the proteins involved the formation of amorphous and morphologically disordered aggregates that result in their differential proteinase K stabilities. The chemical stability assessment using urea and acid denaturation, mechanical strength assessment via peak-force QNM analysis showed higher stability of wild-type amyloid fibrils. Thus, we show for the first time that overcoming the energy barrier during misfolding for a large, multidomain protein involves transitional pathways involving both amorphous as well as structured amyloid like aggregates.

Keywords: Gelsolin, Amyloid, Electron microscopy, Atomic force microscopy, FTIR, Peak-force QNM.

Investigating the specificity determinants of different basic-region leucine zippers(bZIP)

OP 8

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Basic-region leucine zipper (bZIP) proteins are a class of eukaryotic transcription factors that contain an N-terminal DNA binding basic region followed by a leucine rich dimerization region, collectively known as the bZIP domain. The C-terminal dimerization domain interacts

with other bZIPs to form homo- or heterodimeric coiled-coil structures, whereas the N-terminal basic region makes sequence specific contacts with the respective cognate DNA sequences. It has been reported that some bZIPs preferentially interact with the CREB/ATF site (ATGACGTCAT), some prefer the AP1 site (ATGACTCAT) while others interact with both. We have employed a high-throughput free energy calculation method to identify the specificity determinants for these interactions.

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A genomics approach for identification of proteases as major potential virulence factors in the pathogenic fungus, *T. rubrum*

OP 9

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Trichophyton rubrum are a monophyletic group of fungi best known for affecting the skin of animals and humans. These fungi cause a variety of skin diseases, including athlete's foot (tinea pedis), jock itch (tinea cruris), and ringworm (tinea capitis or tinea corporis, depending on area of the body infected). During disease, these exclusively infect and multiply within keratinized host structures-the epidermal stratum corneum, nails or hair. During *in vitro* cultivation with protein as sole nitrogen and carbon source, dermatophytes were proven to secrete multiple proteases, some of which have been identified and are thus putative virulence factors. Clustering of secreted proteins of *Trichophyton rubrum* revealed several protease and keratinase that might play major role in establishment of infection by degrading proteins present in skin of host. Comparative secretome analysis along with other dermatophytes and non dermatophytes revealed enrichment of serine protease family in *Trichophyton rubrum* thereby further supporting their functional importance in *Trichophyton rubrum*. In order to gain insight in the same, whole genome sequencing was carried out of *Trichophyton rubrum*. Preliminary annotation was done using Augustus web server

followed by manual curations. Analysis of secretome revealed protease families to be one of the largest secreted family comprising of members that work effectively in acidic, neutral as well as alkaline pH. Further characterization of these proteases will bring insight into the mechanism of action of *Trichophyton rubrum*.

Gold-mining from junk: a computational tool to short list repeat elements that may influence gene expressions

OP 10

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Repeat sequences form a significant portion (30-45%) of the mammalian genomes. Recent findings indicate that repeat elements enriched within genomic neighborhood of co-expressed genes may be crucial in regulating gene expression by harboring functional transcription factor binding sites (TFBS). Already existing computational tools can only identify and/or classify the repeat elements. We have developed a PERL-CGI based web tool 'Genomic Repeat Element Analyzer for Mammals' (GREAM) to short-list repeat elements of potential functional importance, within the neighborhood of genes or other regions of interest.

GREAM uses repeat annotation of reference genome to obtain frequency distribution of all the repeat elements within the neighborhood of input genes. Then a binomial test is employed to short list the repeat elements that are significantly enriched compared to the expected population frequency. By providing a list of putative TFBSs within each of the enriched repeat element, the tool also facilitates *in silico* analysis of its regulatory role. In addition, the tool has assists in two other types of analysis: a) identification of evolutionarily enriched repeat element; b) differential repeat element distribution within specific chromosomal location(s). All of the above mentioned features work for 17 mammalian species.

The functionality of GREAM was tested by analyzing neighborhood of endometrial expressed genes. The tool detected enrichment of a DNA transposon "MER20", which is known to rewire their regulatory network.

Structural studies of cyclophilins and its interactome network analysis

OP 11

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Cyclophilins (Cyps) are highly conserved, ubiquitous in nature and catalyze the interconversion of peptidyl prolyl imide bonds in peptide and protein substrates. In eukaryotic cells, Cyps are found in all cellular compartments with a variety of functions being ascribed to them including cell division, transcriptional regulation, protein trafficking, cell signaling, pre-mRNA splicing, molecular chaperone mechanism and stress tolerance. We have studied structural dynamic property of a RNA binding protein named as Cyclophilin (PiCypA) from a plant-root-colonizing Basidiomycete fungus, discovered in the Indian Thar desert that has shown to provide strong growth-promoting activity during its symbiosis with a broad spectrum of plants. Crystal structure of PiCypA was solved at 1.97 Å resolutions by molecular replacement method. Crystal arrangement has shown three molecules per asymmetric unit arranged in a side-by-side orientation. PiCypA is a monomeric single domain protein and forms a canonical cyclophilin fold comprising two α -helices and eight β -strands. To investigate specificity of RNA binding, PiCypA was titrated against different RNA constructs and we obtained quantitative thermodynamic parameters of binding. RNA molecule was titrated against U -¹⁵N labeled PiCypA at pH 6.5 and shifts in the backbone ¹⁵N and ¹H^N resonances in 2D [¹⁵N,¹H] HSQC spectra were monitored. A gradual shift in the resonances was observed for all the residues that showed changes indicating a fast exchange ($\tau_{ex} < \mu s$) between free and RNA bound PiCypA. Recently, we have also studied the dynamicity of CPR3 (cyclophilin protein from yeast) and its interacting putative protein partners. It was to be found that Ubiquitin and proteins involved into glucose metabolism interacts with CPR3. Surface Plasmon Resonance analysis showed CPR3 interaction with ubiquitin. So, overall structural dynamic study of multiple cyclophilins revealed its involvement in wide range of cellular pathways.

Synthesis, characterization and antibiofilm efficacy of Chitosan–silver nanocomposite

OP 12

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Nano-biotechnology is being envisioned as a burgeoning field with immense application in various allied disciplines of science. An interesting avenue for their exploration is their use as antimicrobials to target various deadly and drug resistant microbes. Over the decades, there occurs alarming number of cases wherein bacteria develop resistance to most commercially available antibiotics. One of the modes by which bacteria exert resistance is their ability to develop biofilms. In the recent scenario, the formation of biofilms is a major cause of implant failure and often limits the timeline of many indwelling medical devices. This raises an urgent need to develop strategies that can inhibit these sessile bacterial communities developing in the form of biofilms. It is in general consensus that nanoparticle based approaches would open new horizons for preventing biofilm based infections. Reckoning with this fact, we endeavoured development of Cht-Agnps, wherein the chitosan moiety itself acts as a versatile agent, acting both as reducing agent as well as capping agent. The as synthesized nanoformulation were characterized employing various physiochemical techniques including UV-Vis spectroscopy, XRD, FT-IR, EDX spectroscopy and TEM, and thereafter their antimicrobial potentialities against *Staphylococcus aureus* were investigated. Basically, a few sets of experiments were carried out, wherein the antibiofilm efficacy of the as synthesized AgNPs against *Staphylococcus aureus* were investigated through Congo Red agar plate method, crystal violet and SEM analysis. Intriguingly, the as synthesized Cht-AgNPs nanocomposite bestowed an assemblage with synergistically emerged attributes which was not only effective against *S. aureus* planktonic cells but also obliterates biofilms formed by these spp. Collectively, the data of the present study instigates that these adjunctive Cht-AgNPs nanocomposite could leads towards newer arsenal of effective therapeutics to combat medical repercussions.

Physicochemical and Immunochemical characterization of HNE-modified HSA: Anti-HNE-HSA antibodies as an immunochemical probe for detection of HNE-induced damage to serum albumin in SLE

OP 13

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Lipid peroxidation of cellular membranes occurs during periods of sustained oxidative stress. 4-hydroxynonenal (HNE), which is considered to be the most reactive lipid peroxidation product, is capable of modifying and/or cross-linking proteins thus impairing their physiological functions. The formation of adducts on proteins produce structural modifications generating neoantigens, which consequently may elicit auto-antibodies response. Enhanced oxidative stress and formation of HNE-modified proteins are reported in Systemic lupus erythematosus (SLE) and other autoimmune diseases. The aim of this study was to elucidate the role of lipid peroxidation derived aldehyde in SLE. We report the structural perturbations in human serum albumin (HSA) upon modification with HNE. Induced antibody, although highly specific for the immunogen, exhibited cross-reactivity with other HNE-modified proteins, amino acids and nucleic acid, suggesting that HNE-derived epitopes share common antigenic determinants. The experimentally induced antibodies against HNE-modified HSA were used as an immunochemical probe to detect the HNE induced damage in SLE patients. Since albumin is the most abundant protein in the circulatory system, it is susceptible to damage. HSA was isolated from SLE patients and probed for HNE induced modification by the experimentally induced antibodies against HNE-HSA by competition ELISA. HSA from healthy individuals was used as control. Significantly high recognition of HSA from SLE patients by experimentally induced antibodies against HNE-modified HSA is a clear indicator of epitope sharing between the HSA modified *in vitro* by HNE and the HSA isolated from SLE patients.

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Polyols affect the stability and amyloid fibril formation of recombinant human lysozyme

OP 14

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'Systemic amyloidosis' is a protein aggregation disorder in which mutations in the human lysozyme gene lead to the production of toxic aggregates in the body.

Considerable work is being carried out towards better understanding and modulation of the amyloid formation pathways and to design effective therapeutic strategies against these disorders. Polyols have been extensively used for protein stabilization and prevention of aggregation during refolding. In this work we have attempted to understand their effect on amyloid aggregation using recombinant human lysozyme as a model protein. ThT assay, TEM and light scattering studies revealed inhibition of lysozyme fibril formation and DSC measurements revealed thermodynamic stabilization of human lysozyme with increasing concentration of polyols with sorbitol being the most effective towards both stabilizing the protein and inhibiting its fibrillation. DLS and ANS binding studies, soluble protein estimation and activity assay showed smaller and less hydrophobic species, increased soluble protein content and increased activity in samples in the presence of polyols compared to the control samples under fibrillation conditions. Addition of polyols at different stages during fibrillation pathway indicated maximum inhibition when polyols were added at the beginning of fibrillation. Prevention of protofibril conversion into fibrils was observed even when polyols were added after the formation of protofibrils and no effect was observed when they were added after the formation of fibrils. These results signify stabilization of the native state as well as of the early intermediates in the fibrillation pathway upon addition of polyols, leading to prevention of their progression into fibrils. This study may prove helpful in better understanding of the fibril formation pathway in terms of modulation by inhibitors and also to analyze the efficacy of polyols as inhibitors of amyloid aggregation.

Sequence Analysis and Function Prediction of Hypothetical proteins from *Streptococcus pyogenes*

OP 15

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Streptococcus pyogenes is a Gram positive, non-motile facultative anaerobe, one of the most frequent pathogen of humans. *S. pyogenes* can infect when defenses are compromised or when organisms are able to penetrate the constitutive defenses and can cause variety of suppurative infections. In last century, infections by *S. pyogenes* claimed many lives as the organism cause puerperal fever, Scarlet fever and Pharyngitis which prompted to name it "flesh eating bacteria". As some strains of pathogen has been reported resistance against commonly used drugs such as Erythromycin, Penicillin,

Clindamycin and Vancomycin. A comprehensive study of *S. pyogenes* become necessary for better treatment and eradication of the diseases. Here, we studied M1 GAS strain of *S. pyogenes*. After analysis of the proteome data of *S. pyogenes*, we found that ~ 36% proteome are listed as conserved hypothetical protein (HP) for which there is no biochemical evidences have been reported so far. Study of these HPs offers presentation of new structures and novel functions of HPs and also new cascade of biochemical pathways. Furthermore, new HPs may be serving as markers and pharmacological targets. We have identified HPs from *S. pyogenes* genome and subsequently annotated their corresponding functions using various tools for sequence alignments, domain and motif search. Also, functions may be predicted on the basis of properties that are associated with a specific functional class and family of the proteins. We also studied their sub-cellular localization and signal peptide if present. Moreover, sequence analysis was also performed to find out the Physiochemical properties including amino acid composition, aliphatic index and instability index of HPs from *S. pyogenes*, which may lead to better understanding of its virulence mechanism, adaptability in host system, tolerance for host immune system and emergence of drug resistance strain and finally drug/vaccine discovery.

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Hinge bending action concomitant with sharp curvature governs substrate recognition of RNaseH

OP 16

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RNaseH hydrolyses RNA in a DNA.RNA duplex formed during diverse biological processes crucial in maintaining genomic integrity. RNaseHI requires minimum of 4 ribonucleotides for cleavage, while RNaseHIII processes even a single rNMP, RNA in a RNA-DNA.DNA chimera junction. **Mutations in RNaseHIII are linked to neurodegenerative disease.** Existence of RNaseH fold in retroviruses renders it to be drug target. Mechanistic insights of substrate recognition crucial for devising better inhibitors and therapeutics remain elusive. We have examined the conformational flexibility of *E.coli* RNaseHI & *Mj.* RNaseHIII and their substrate complexes by MD simulation. Significant bending at the hinge formed between the basic protrusion (BPR) and N terminal RNaseH fold forming region (in *E.coli* RNaseHI) and between the N-terminal (RNaseH fold forming domain) and C-terminal domain (in *Mj.* RNaseHIII) is required for the substrate to bind snugly and interact with residues identified to be decisive for hydrolysis. Several conserved residues (W81, W85, R88, T92, and K96) from the BPR in *E.coli* RNase HI and Tyr 184, Ser 186, Arg209,

Lys 213, Lys216 Arg217 of the C-terminal domain in *Mj.* RNaseHIII provide additional sites for plausible mutations. Hybrid DNA.RNA duplex exhibits strong curvature upon binding to RNaseHI and RNaseHIII and is decisive for recognition. The curvature is independent of base sequence while it is sequence dependent in the enzyme-free situation, implying an induced-fit conformational change. Non-sequence dependent hydrolysis can be accounted as nearly all the interactions involve phosphates. This also offers clues for lack of antisense activity for S- phosphate modified antisense agents.

Binding studies of Curcumin to the Human Calcium-calmodulin Dependent Protein Kinase IV: A Protein Involved in Neurodegenerative Diseases and Cancer

OP 17

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Calcium-calmodulin dependent protein kinase IV (CAMK4) is a multifunctional enzyme belongs to the Ser/ Thr kinase family and present in the nucleus of a cell. This protein has wide range of tissue expression especially in the brain thymus (Jang et al. 2001), neuronal subpopulations, spleen and testis. CAMK4 playing critically important role in the cell proliferation, gene expression, apoptosis, muscle contraction and neurotransmitter release. Curcumin is a naturally occurring yellow bioactive component of turmeric, possess wide spectrum of biological actions and widely used to cure atherosclerosis, diabetes, cancer, and inflammation and is also an antioxidant. Here, we studied the interaction of curcumin, with human CAMK4 at physiological pH 7.2 by using molecular docking, molecular dynamics (MD) simulations and fluorescence methods. We performed MD simulations for both neutral and anionic forms of curcumin-CAMK4 complexes for a reasonably long time (150 ns) to see the overall stability of the ligand-protein complex. Molecular docking studies revealed that the curcumin binds in the large hydrophobic cavity of kinase domain of CAMK4 with several hydrophobic and hydrogen bonded interactions. Additionally, the MD simulations studies contributed in understanding the stability of protein-ligand complex system in the aqueous solution and the conformational changes in the CAMK4 upon binding of curcumin. The experimental results show that the intrinsic fluorescence

of CAMK4 was significantly quenched by addition of curcumin. A remarkable blue shift in the fluorescence spectra was also observed which is in agreement with the MD simulation study, suggest that binding of curcumin on causes a significant conformational changes in the active site region of CAMK4. This study will provide insights into designing of the new inspired curcumin derivatives as therapeutic agents against many life threatening diseases.

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Protein Stabilization in Context of Excluded Volume Effect

OP 18

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Living organisms accumulate small organic molecules i.e. osmolytes, during various stress conditions. These osmolytes stabilizes the cellular proteins and create a molecular crowding environment inside the cell. Excluded volume effect is an important non-specific interaction which is always presents in crowded environment while other non-specific interactions may or may not present. Excluded volume effect is an important parameter which determines the thermodynamic stability of proteins. Shape and size of protein and cosolute (osmolytes) are the two important parameters which affect the excluded volume effect. To determine the effect of size of osmolytes on the excluded volume effect, we have carried out thermal denaturations of apo alpha-lactalbumin (α -LA) and lysozyme in the absence and presence of different sizes of sugar osmolytes (mono-, di-, tri- and tetra- saccharides) at pH 7.0. Entire ($y(T)$, T) data of each denaturation curve was modelled via non-linear least square equation to obtained best fit values of T_m^0 (mid-point of thermal denaturation in the absence of sugar), $\Delta H_{T_m^0}$ (change in enthalpy at T_m^0), ΔC_p (heat capacity change at constant pressure), a_1 - a_6 (temperature-independent coefficients) and α (excluded volume). α is stabilization parameter which depends upon protein-sugar pair in terms of relative size and shape of each. We modelled a combination of data set obtained with different sugar concentrations and model provides an extremely parsimonious description of the data. For both the proteins, α increases with increase in degree of oligomerization of sugars. Therefore, extent of stabilization of proteins will be more in tetrasaccharides than trisaccharides and then disaccharides; and increasing order of stabilization of protein by sugars will be mono- < di- < tri- < tetra- saccharides.

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Methylglyoxal modified humanserum albumin playing role in diabetes Mellitus complication

OP 19

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Methylglyoxal is a highly reactive dicarbonyl compound that act as a potent glycation agent under physiological conditions. MG can modify circulatory proteins like albumin. Modification of albumin by MG was studied by various biophysical and biochemical techniques and later analyzed for their variation of immunogenicity upon modification. We report hyperchromicity in UV region, loss in intrinsic tyrosine fluorescence intensity, aggregation in SDS PAGE, and increased alpha helix in CD studies. The carbonyl content increased on glycation and generation of novel peaks FTIR spectra was observed. The antiserum against modified protein shows higher titre in comparison to that of native protein. The antiserum against modified protein showed higher binding to its immunogen showing generation of neoepitopes in the modified protein. The study suggests a significant damage caused to serum albumin by MG.

Role of enhanced cationic charge on Staphylocidal activity and cell selectivity of novel analogues of α -Melanocyte Stimulating Hormone

OP 20

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α -Melanocyte Stimulating Hormone (α -MSH) is an endogenous neuropeptide with potent anti-inflammatory and antibacterial activity. Towards optimization of its antibacterial activity while maintaining cell selectivity here we report novel analogues of α -MSH with systematically increased cationic charge (from +1 to +5). Increase in net cationic charge improved killing efficacy of α -MSH analogues (+4 and +5 charge) against *Staphylococcus aureus* (ATCC 29213) as compared to α -MSH. Moreover, flow cytometric analysis on intact *S. aureus* cells showed increased membrane permeabilization of α -MSH analogues with highest charge (+5) indicating more membrane damaging effect as compared to α -MSH. All the designed analogues exhibited minimal toxicity on mouse RBC with less than 25% hemolysis at concentrations much higher than the dose required for their antibacterial effect. Insertion depth of α -MSH and designed analogues in negatively charged artificial bacterial mimic vesicles [DMPG:DMPC, 7:3, w/w] and neutral zwitterionic vesicles [DMPC] indicated a clear preference for all the peptides to insert better in bacterial mimic membranes as compared to mammalian mimic membranes. Although unstructured in buffer, α -MSH and the designed analogues showed a clear

tendency to acquire helical conformation in helix inducing solvent 2,2,2- trifluoroethanol. Overall the increase in cationic charge of α -MSH improved Staphylocidal activity and membrane damaging efficacy of the peptides without compromising their cell selectivity. It also indicated that electrostatic interactions and ability to acquire ordered conformation may be crucial for α -MSH and its analogues to selectively disrupt microbial membrane leading to bacterial cell death without harming host cell membrane.

Structure based Function Prediction of Hypothetical Proteins from *Neisseria gonorrhoeae*

OP 21

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Neisseria gonorrhoeae is a Gram-negative bacteria predominantly causes a sexually transmitted infection known as gonorrhoea. The frequent emergence of new multiple drug resistant strain need the extensive study of its genome and development of new drug/vaccine against these pathogen. After analysis of the proteome data of *N. gonorrhoeae* we found that a large fraction proteome are listed as conserved hypothetical protein (HP) for which function are still not known. Study of these HPs offers presentation of new structures and novel functions of HPs and also new cascade of biochemical pathways. Furthermore, new HPs may be serving as markers and pharmacological targets. We have identified 10 HPs showing lyases activity from *N. gonorrhoeae* from sequence analysis and performed homology modeling to reveal the structural basis of the function, structural motifs and amino acids involved in catalytic sites. Along with this, function may be inferred on the basis of properties that are associated with a specific functional class and family of the proteins. We also studied the sub-cellular localization and presence of signal peptide in the HPs. Moreover, sequence analysis was also performed to find out the physicochemical properties including amino acid composition, aliphatic index and instability index of HPs. Finally, function annotation and identification of functionally important regions in HPs from *N. gonorrhoeae* may leads to better understanding of its virulence mechanism, adaptability in host system, tolerance for host immune system and emergence of drugs resistance strain and finally drug/vaccine discovery.

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Study of dynamics of Protein Nanoparticle complex in aqueous medium using Impedance spectroscopy

OP 22

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A rapid development of nanosciences and nanotechnology offers nanoparticles great applications in various fields, like drug development, materials for drug delivery and biosensors etc. Nanoparticles possesses a unique physico-chemical properties such as optical, magnetic, large surface to volume ratio etc. Nanoparticles also confer properties of enhanced bioavailability and improved solubility. When nanoparticles enters in biological fluids, it leads undesired effects. The effects may or may not favour to the systems. Hence it is prime important to understand interaction between nanoparticles and biological materials like proteins, enzymes and nucleic acids etc.

In the present investigation we studied the nanoparticle-protein complex using dielectric spectroscopy. Dielectric spectroscopy reveals typical signature of relaxation process of aqueous media namely dielectric constant, dielectric loss which commonly known as α , β , γ and δ relaxation.

The Chemical route synthesized Silver Nanoparticles were characterized using Dynamic Light Scattering (DLS), Transmission Electron Microscope (TEM) and UV visible spectroscopy. The protein nanoparticles complex was confirmed by fluorescence and UV visible spectroscopy. The Frequency dependent Dielectric dispersion of Silver NP's and Silver NP-protein complex was investigated in the temperature range of 263K - 373K and in a frequency range of 20Hz - 2MHz by impedance spectroscopy (Agilent LCR component analyzer). Variation in dielectric constant (ϵ') and dielectric loss (ϵ'') was observed nanoparticle and protein and Protein-nanoparticle complex. Bode plot (impedance vs frequency) showed increased in impedance (Z) of protein-npts complex. Average relaxation time (τ) for α -region (20HZ-100KHZ) and β -region (> 200 KHZ) was tabulate in control and test condition as a function of reciprocal temperature. In addition, the electrical conductivity was monitored. The several relaxation processes were attributed to motions that range from side groups of the proteins in the aqueous medium.

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Role of K-mer in Optimising Genome Reconstruction using Big Data from Next Generation Sequencing

OP 23

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Next generation sequencing has recently emerged as the most preferred technology to investigate the connection between unique phenotypes of a species to the respective genotypes. Denovo assembly of hundreds of millions of highly redundant short sequencing reads into longer meaningful contigs for genome reconstruction remains a computational challenge because of high RAM requirement on the system. The complexity of the process increases with the complexity and size of the genome sequenced. Hence, the assembly of a eukaryotic genome requires optimisation of assembly parameters to minimize the computational costs. In denovo assembly tools, this is achieved by using both deBruin graph theory and optimal K-mer size. While lower K-mers require lesser memory, they compromise on the quality and useful length of the assembled contigs. Hence, there is a need to maximize Kmer size to improve quality and minimize the RAM requirement to reduce cost in genome assembly. In this study, we discuss the effect of different kmer lengths on the quality of denovo assembly of the recently published genome of *Amaranthus hypochondriacus* that was sequenced inhouse on Illumina platform. We have reported contigs useful for biological interpretation from 869 million reads using 31mers with SOAPDenovo on a 96 GB RAM system in the publication. Here, we further present our efforts to compare the relative merits of assemblies using SOAPDenovo2 with varying k-mer lengths and identify the optimum k-mer length that provides the best assembly in terms of quality of the contigs assessed by their biological significance.

Comparative insight into the anionic and cationic surfactants induced aggregation in hen egg white lysozyme

OP 24

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Electrostatic and hydrophobic interactions have an important role in the protein aggregation. In this study, we have investigated the effect of charge and hydrophobicity of oppositely charged surfactants i.e., anionic (AOT and SDS) and cationic (CTAB and DTAB) on hen egg white lysozyme at pH 9.0 and 13.0

respectively. We have employed various methods such as Rayleigh scattering, turbidity measurements, ThT and Congo Red dye binding assays, far-UV CD, transmission electron and fluorescence microscopy. At sub-micellar concentrations of both anionic and cationic surfactants promote amyloid fibril formation in lysozyme at pH 9.0 and 13.0 respectively. The aggregation was proportionally increased with respect to lysozyme concentrations. The morphology of aggregates at both the pH was fibrillar in structure, as visualized by ThT dye binding, transmission electron microscopy and fluorescence microscopy. Initially the interaction between surfactants and lysozyme was found electrostatic and then hydrophobic investigated by ITC. This study demonstrates the crucial role of charge and hydrophobicity during amyloid fibril formation. The mechanistic model of protein aggregation proposed here will help in designing molecules that can prevent or reverse the amyloid fibril formation or the aggregation.

In silico study on the conformational behavior of N-terminal domain of PABPN1 protein with varying poly-Ala Stretch

OP 25

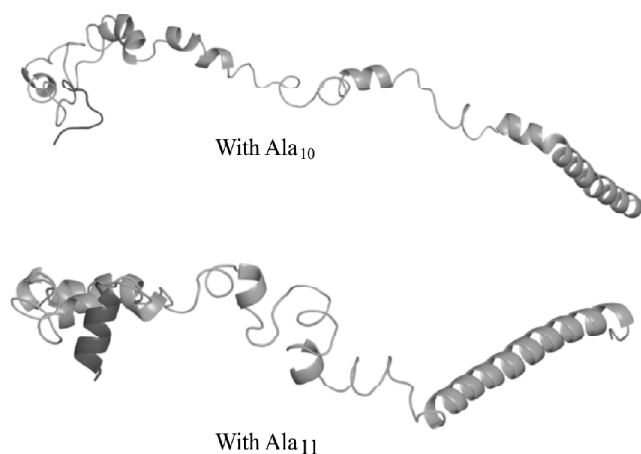
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Expansion in polyalanine stretch (Ala₁₀ to Ala₁₁₋₁₇) of protein polyadenylating binding protein nuclear 1 (PABPN1) causes Oculopharyngeal muscular dystrophy (OPMD)-a late onset, autosomal dominant, slowly progressing disease. The polyalanine stretch (poly-Ala₁₀) is present at N-terminal domain of PABPN1, immediately after the starting amino acid methionine. Poly-Ala expansion results in aggregation of PABPN1 protein. Thus, the study of conformational behavior of polyalanine stretch in normal Poly-Ala₁₀ and expanded poly-Ala₁₁₋₁₇ PABPN1 may throw light on the misfolding and affliction to form stable aggregates by the protein. In addition, the effect of poly-Ala expansion on the conformation of N-terminal domain of PABPN1 may also be helpful for designing of inhibitors to combat OPMD complications.

Molecular dynamics approaches proved to be very useful to study the conformational behavior of bio macromolecules as function of time, especially for proteins. Therefore, simulation studies of poly-Ala peptides with and without protecting groups of chain length 10-17 have been carried out in water by taking three different starting geometries. Simulation study on the conformation of N-terminal domain of PABPN1 protein with Ala₁₀ and expanded Ala residues >Ala₁₀ shows different conformations adopted by poly-Ala Stretch. Simulation studies on the N-terminal domain of PABPN1 protein give interesting results i.e., Ala₁₀ behaves similar manner as in isolated poly-Ala₁₀ peptides, but

simulations of N-terminal domain with poly-Ala₁₀₊₁ forms a stable helix. Expanded alanine stretch (with Ala e"11) also found to adopt α -helix at N-terminal domain of PABPN1 protein with interesting features.



Classification and analyses of putative virulence factors from *Rickettsia rickettsii*

OP 26

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Rickettsia rickettsii is an obligatory, Gram-negative and pleomorphic coccobacillus belongs to the family *Rickettsiaceae*, which is the primary causative agent of Rocky Mountain spotted fever in human beings. The sequenced genome of this pathogen (str. 'Sheila Smith') contains 1,343 protein-coding genes, 3 rRNA genes and 33 transfer RNA genes and 680 uncharacterized hypothetical proteins (HPs). The functions of these proteins were annotated by using the information derived from the *in silico* methods such as sequence similarity, protein clustering, protein-protein interactions etc. The 214 HPs showed positive results in majority of predicted tools; therefore their functional annotations were considered more reliable. These HPs were further classified into 88 enzymes, 59 transport and membrane proteins, 35 binding proteins, 12 structural motifs etc. The sequences of 214 HPs were further used for the prediction of their virulence characteristics. 15 HPs were found to be virulent and two proteins with highest virulence scores were selected for further analyses. The Molecular Dynamics (MD) simulations were performed on these selected virulent proteins using GROMACS package to observe their nature and stability profile. These analyses can be useful in the identification of new drug targets and help in the development of better therapeutic agents to fight against the infection caused by *R. rickettsii*.

HDI: Human Disease Insight

OP 27

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Integration of human diseases, genes, drugs and computational tools at a common platform will enable researchers, physicians and non-professionals to access related information that will exhibit awareness and thereby reducing the chances of suffering due to ignorance. Bioinformatics tools have been integrated in the database that enable researchers to perform comparison among the disease specific genes, and perform protein analysis, search for biomarkers and identification of potential vaccine candidates. Eventually the tools would be of great help to analyze facts about the diseases. Eventually the tools would be of great help to analyze facts about the diseases. The HDI is a knowledge based resource for human disease information to both scientists as well as general public. Here, our mission is to provide a comprehensive human disease database containing most of the useful information with extensive cross-referencing. HDI is a knowledge management system that acts as a central hub to access information about human diseases, drugs and genes involved in various diseases. In addition, the HDI contains well classified bioinformatics tool with description. HDI provides two types of search capabilities, and has provision for downloading, uploading and searching disease/gene/drug related information. Logistics designed for HDI allow regular updation of the database. The database is designed to work best with Mozilla Firefox and Google Chrome. The HDI is freely accessible at <http://humandiseaseinsight.com>, with user-friendly web interface, and is highly useful to the physicians, researchers, patient and general public.

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A...A mismatch instigates B- to Z-DNA transition in a DNA duplex comprising of CAG & GAC trinucleotide repeat expansions

OP 28

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Tandem repeats or microsatellites are commonly observed in eukaryotic genome. Abnormal expansion of such tandem repeats with repeating unit of 3 nucleotides form unusual nucleic acid structures and cause many

incurable genetic diseases in human irrespective of the repeating unit and its location in the genome, known as trinucleotide repeat expansion disorders (TREDs). For instance, hairpin DNA structures that are formed due to overexpansion of CAG trinucleotide repeat leads to Huntington's disorder (coding region) and several spinocerebellar ataxias (5' UTR). Nonetheless, DNA hairpin stem structure that generally embrace B-form with canonical base pairs is poorly understood in the context of periodic noncanonical A...A mismatch as found in CAG/GAC repeat overexpansion. In this context, molecular dynamics simulations with explicit solvent system is performed for CAG/GAC trinucleotide repeats containing DNA duplex with noncanonical A...A mismatches. Results reveal conformational transition from right to lefthanded form, namely, B-to- Z-DNA transition regardless of the initial *glycosyl* conformation.

Transition from B-to-Z is due to the mechanistic effect that originates from the pronounced nonisostericity of A...A mismatch with the flanking canonical base pairs facilitated by base extrusion, backbone and/or base flipping. Thus, we have shown for the first time that A...A base pairing in a CAG/GAC repeat expansion provokes the formation of left-handed Z-DNA, leading to periodic B-Z junction. These results offer a new dimension about CAG/GAC repeat expansion containing DNA duplexes and thus, would facilitate the design of drugs to treat the disorders caused by CAG/GAC expansion at the DNA level.

Impact of glucose modification on the structure of Amadori HSA: Characterized by different biophysical and biochemical techniques OP 29

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Protein modification in diabetes mellitus may lead to early glycation products (EGPs) or amadori product as well as advanced glycation end products (AGEs). Millard reaction or nonenzymatic glycation reaction accelerate in diabetes due to hyperglycation and alter serum protein's structure, their normal functions that lead micro and macro vascular complications in diabetic patients.

In this study, Human serum albumin (HSA) was incubated with different concentrations of glucose at 37°C for a week. At 4th day, Amadori product was formed that was confirmed by colorimetric method NBT assay which is authenticate early glycation product. Conformational changes in native as well as Amadori modified albumin with different concentrations of glucose were investigated by various biophysical and biochemical techniques. Main biophysical techniques hyperchromacity, quenching of fluorescence intensity, CD, FTIR and SDS-PAGE were

used. Further conformational changes were observed by biochemical assays mainly fructoseamine, reduction of fructoseamine with NaBH₄, carbonyl content estimation. This study find structural and biochemical changes in Amadori modified HSA with normal to hyperchronic range of glucose with respect to native HSA. When glucose concentration was increased from normal to chronic range biochemical and structural changes also increased. Highest alteration in secondary and tertiary structure and conformation in glycated HSA was observed at the highest concentration (75mM) of glucose. Although it has been found that Amadori modified proteins is also involved in secondary complications of diabetes as AGEs but very few studies have been done to analyze the conformational changes in Amadori modified proteins due to early glycation. Most of the studies were found on the structural changes in Amadori protein at a particular glucose concentration but no study was found to compare the biophysical and biochemical changes in HSA due to early glycation within a range of glucose concentrations. So this study provide the information about the biochemical and biophysical changes occur in Amadori modified albumin at a range of glucose normal to chronic in diabetes.

Role of a non-catalytic residue in the stimulated GMP formation of Human Guanylate Binding Protein-1 OP 30

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Apart from regulating various cellular processes, IFN-induced GTPases are also key components of cellular immunity. Human Guanylate Binding Protein-1 (hGBP1) is a member of large GTPases, which is induced by IFN-gamma and is important mainly for invading the viral pathogens. hGBP1 has unique ability to hydrolyze GTP to both GDP and GMP, with GMP being the major product of the reaction. The protein has a characteristic of substrate induced higher order structure formation. However, the mechanism of oligomerization and its role in the regulation of activity in hGBP1 are not clearly understood. Oligomerization is associated with conformational changes of the protein. These changes can be probed by intrinsic tryptophan fluorescence as it is very sensitive to its local environment. hGBP1 has four tryptophans which are present in the GTPase (globular) domain. To understand the conformational changes, tryptophans were individually mutated to alanine. Fluorescence studies showed that all mutants except W79A get solvent exposed to some extent, indicating that W79 undergoes a conformational change during tetramerization of the protein. The activity assay using radiolabeled [α -³²P]GTP showed that all other mutants

are catalytically active, but W79A was inactive for the second catalysis at lower protein concentration and showed ~60 fold lower catalytic efficiency compared to its wild type. However, W79F rescued the second catalysis to some extent but could not restore completely. This suggests that the hydrophobicity of W79 is critical in positioning the second catalytic machinery for efficient cleavage of GDP. Thus, this study explains how hydrophobicity of a tryptophan near the active site is critical for enhanced GMP formation.

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Computational prediction of miRNAs and their targets in *Phaseolus vulgaris* using simple sequence repeat signatures

OP 31

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MicroRNAs (miRNAs) are endogenous, noncoding, short RNAs directly involved in regulating gene expression at the post-transcriptional level. In spite of immense importance, limited information about the miRNAs of *P. vulgaris* and their expression patterns prompted us to identify new miRNAs of *P. vulgaris* by computational methods. Besides conventional approaches, we have used the conserved simple sequence repeat (SSR) signatures as one of the prediction parameter. The presence of SSRs in pre-miRs is already established, although their role in pre-miRs is unknown. We could not find any universal SSR that is conserved among all the miRNA families of Viridiplantae. However, we find conserved SSR signature(s) when we consider a particular miRNA family and is used in our prediction. Moreover, for all other parameters including normalized Shannon-entropy, normalized base-pairing index and normalized base-pair distance, instead of a fixed cut-off value, 99% probability range derived from the available data was used. We have identified 208 mature miRNAs in *P. vulgaris* of which 201 are novel. Distribution of these miRNAs in 118 families varies and the most populated ones are MIR1533, MIR1527, MIR5021 and MIR848 with 15, 10, 10 and 7 members, respectively. A total of 1305 targets were identified for 130 miRNAs. Using 80% of sequence identity cut-off, proteins coded by 563 targets were found. Our findings will contribute to the present knowledge of miRNAs and their targets in *P. vulgaris*. The computational methods developed in this study is not only restricted to *P. vulgaris* but can be applied to any species of Viridiplantae.

A Mechanistic Model for Amorphous Protein Aggregation and its Prevention

OP 32

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Study of protein aggregation has become a leading theme in research with relevant interest in molecular medicine and biotechnology. We are investigating the mechanism of amorphous aggregation of Hen Egg White Lysozyme (HEWL) in the presence of a reducing agent (TCEP) which disrupts the disulphide bonds present in the native structure. The amorphous aggregation of proteins is the process where unstructured protein aggregates are formed and this process is associated with many phenomena like formation of protein wine haze, development of cataract in the eye lens and the precipitation of recombinant proteins during their expression and purification (Stranks et al., 2009). While aggregation process resulting in structured aggregates like amyloid fibrillation process have been attempted to be explained by many models and grabbed large attention, amorphous aggregation have been scarcely studied. We have proposed a new comprehensive model to explain amorphous aggregation which hitherto remains vaguely understood.

90° light scattering technique complemented with end point analyses were used to study aggregation kinetics. Dye binding studies and CD spectroscopy were used for characterizing the type of aggregate. It was found that TCEP reduces only the surface exposed disulphide bond and caused conformational alteration in the native protein. Partially folded monomers then associate to form dimers and amorphous aggregates are then formed by dimer addition. We found that the Aggregation involves both Nucleation and isodesmic mechanisms at the same time. We also established that light scattering can be used to probe amorphous aggregating protein systems and the results are consistent with that from other techniques like end point analyses.

Effects of Zinc Oxide Nanoparticles on the Hepatic Tissue of Chicken Embryo

OP 33

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In the past few decades, nanoparticles have attracted worldwide attention due their particular properties like nanoscale size and enhanced surface areas. These properties make nanoparticles quite different from their bulk materials. Due to rapid growth of Nanoscience and

Nanotechnology, the major applications are being developed in the medicine and biology. Studies have revealed that the same properties that make nanoparticles so unique could also be responsible for their potential toxicity. In the present study, we report the effects of Zinc Oxide (ZnO) nanoparticles on hepatic tissue of chick embryo using histopathological techniques. The ZnO NPs were synthesized in thermal plasma reactor by gas phase homogeneous condensation process and characterized using Transmission Electron Microscopy (TEM) and X-ray diffraction method. The average size of the ZnO NPs was found to be 25 nm. The histochemical study of hepatic tissues at the doses of ZnO NPs (50 and 150 µg/g of egg weight) exhibited concentration dependent disorganization, loss of cellular integrity and physical nature of the tissues. The observations indicate the hepatotoxic nature of ZnO NPs in the present model studied. The possible mechanisms of interaction of ZnO NPs with hepatic tissue will be explored.

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Biophysical and Molecular Docking Insight into the Interaction of Cytosine β-D Arabinofuranoside with Human Serum Albumin

OP 34

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Interaction of pharmacologically important anticancer drug Cytosine β-D Arabinofuranoside with Human serum albumin (HSA) at physiological pH 7.4 has been studied by utilizing various spectroscopic and molecular docking strategies. Fluorescence results revealed that Cytosine β-D Arabinofuranoside interacts with HSA through static quenching mechanism with binding affinity of $2.4 \times 10^3 \text{ M}^{-1}$. The average binding distance between drug and Trp²¹⁴ of HSA was found to be 2.23 nm on the basis of the theory of Förster's energy transfer. Synchronous fluorescence data indicated that interaction of drug with HSA changed the microenvironment around the tryptophan residue. UV-visible spectroscopy and circular dichroism results deciphered the complex formation and conformational alterations in the HSA respectively. Differential light scattering was utilized to understand the topology of protein in absence and presence of drug. Thermodynamic parameters obtained from Isothermal titration calorimetry ($\Delta H = -26.01 \text{ KJmol}^{-1}$ and $T\Delta S = -6.5 \text{ KJmol}^{-1}$) suggested the involvement of van der Waal interaction and hydrogen bonding. Molecular docking and displacement study with site specific markers suggested that Cytosine β-D Arabinofuranoside binds to subdomain IB of HSA which is also known as the heme binding site. This study will

be helpful to understand the binding mechanism of CBDA with HSA and associated alterations.

Structural and Functional Alteration in the Y250X Mutant of Transferrin Receptor 2 in Iron Homeostasis

OP 35

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Iron an essential constituent of our body plays an important role in different pathways like cofactor for enzymes, oxygen transport etc. It is an indispensable nutrient as its deficiency causes anemia whereas excess of it causes hereditary hemochromatosis (HH). Iron overloading problems are insidious, causing progressive as well as irreversible end-organ injuries. Regulation of iron and its fine tuning according to the body requirement takes place with the help of a network of proteins named transferrin receptor 1 (TfR1), TfR2, hereditary hemochromatosis factor E (HFE), ferroportin, BMP and hepcidin. These proteins along with ferritin helps in uptake, distribution and storage of iron. In our body there is no excretory mechanism for iron, so it should be critically regulated at intestinal absorption level. TfR2 is a new member of TfR family known to play a role in iron-homeostasis. A nonsense mutation Y250X was reported in TfR2 gene in patients of HH type-III. Here we cloned and expressed mutant TfR2 in different CHO cell line variants-TRVb, TRVb-1 and TRVb-2. We observed that Y250X mutation deleted the ectodomain of TfR2 and it failed to exit the ER and Golgi body. In this study, we monitored iron uptake in different TfR mutant cell lines. Association between TfR1 and TfR2 were also studied. Y250XTfR2 does not affect transferrin dependent iron uptake, although Tf binding was reduced upto 40 %. Further studies are going on to investigate the role of TfR2 in Tf-independent iron-uptake.

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NMR Investigation of Neuronal and Astroglial Metabolic Activity in Depression

OP 36

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Major depressive disorder is a complex neuropsychiatric syndrome and often very severe and life threatening¹. The

objective of the current study was to evaluate the excitatory and inhibitory metabolic fluxes in chronic unpredictable mild stress (CUMS) model of depression. Two month old male C57BL6 mice were subjected to CUMS paradigm for 21 days. Depression like phenotype was assessed using sucrose preference and forced swim test (FST). Cerebral metabolism in the prefrontal cortex was measured by ^1H - ^{13}C -NMR spectroscopy² together with infusion of $[1,6\text{-}^{13}\text{C}_2]\text{glucose}$ or $[2\text{-}^{13}\text{C}]\text{acetate}$ ³. The ^{13}C turnover of amino acids were analyzed to derive the TCA cycle and neurotransmitter cycling fluxes between neurons and astrocytes⁴.

Mice subjected to CUMS paradigm exhibited reduced sucrose preference and increased immobility in FST. The reduced ^{13}C labeling of Gln_{C_4} , GABA_{C_2} and Glu_{C_4} from $[2\text{-}^{13}\text{C}]\text{acetate}$ indicates decreased astroglial metabolic activity in CUMS mice. Glutamatergic TCA cycle (CUMS $0.54 \pm 0.06 \mu\text{mol/g/min}$; Control $0.70 \pm 0.06 \mu\text{mol/g/min}$) and neurotransmitter cycling (CUMS $0.23 \pm 0.03 \mu\text{mol/g/min}$; Control $0.33 \pm 0.03 \mu\text{mol/g/min}$) were found to be reduced significantly in CUMS mice. These data suggest that excitatory and inhibitory neurotransmission is decreased in the prefrontal cortex in depression.

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Identification of single mutations in Recombinant Hemoglobin Variants using modified digestion method and separation power of LC coupled with MALDI MS/MS

OP 37

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Around 7% of the global population carries an abnormal hemoglobin gene. Over 330,000 infants are born annually with hemoglobinopathies and it is the major cause of morbidity and mortality in early childhood. The treatments rely heavily on the diagnosis of hemoglobin variants. The routine/conventional techniques used for the identification of mutation in hemoglobin variants have their own limitations like co-migration of variants in electrophoresis and co-elution in HPLC. The WHO (2002) report on Genomics and Health has emphasized on the development of simple and precise molecular techniques for screening of hemoglobin disorders. A sensitive, robust and reproducible method was thus developed to attempt to identify/characterize single substitution mutations in the hemoglobin disorders which

require very small amount of hemoglobin sample. The method was MS compatible and also dealt with certain limitations like difficulty in getting good sequence coverage and identification of mutation. The method was tested with recombinant hemoglobin mutants as a proof of concept. In addition to conventional in solution trypsin digestion method, the digestion mixture was treated with 10% acetonitrile prior to incubation. The separation power of LC was coupled with MALDI MS/MS for peptide identification. In this method, the results from two proteases were combined to obtain 100% sequence coverage in the α chains. A hemoglobin variant database was created to specify the search and reduce the search time. All the target mutations were identified by this method in recombinant haemoglobin variants. This method could be used in future for regular screening/diagnosis of any single mutation in hemoglobin variants.

Mining BIG DATA in biology using cloud computing: A case study to decipher Prostate Cancer specific Coding and Non-coding RNA profile

OP 38

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As the DNA sequencing cost (NGS) has kept plummeting in the last decade, the sequencing throughput kept defying Moore's law. As a result, the data analysis cost kept spiraling upwards leading to the growing public repositories with largely underutilized data. The volume of sequence data in the repositories, the speed with which new sequences keep pouring into the repositories and the vast number of applications spawned by the lowered sequencing cost, are similar to the big data challenges faced by other areas such as media, medicine and engineering. The challenge here is to translate solutions developed for handling big data in other fields to managing and analyzing data in life sciences. In the last five years cloud computing has emerged as the preferred choice for handling and analyzing big data in biology. The extraction of minable information from the raw/highly-redundant nature of sequence data is the unique challenge facing data driven biology. Specialized bioinformatics tools along with cloud computing platform is becoming a melting pot for the integration of datomes for a given biological context derived from diverse 'omics' technologies. Towards this end, as a case study, we have deciphered prostate tumor-specific, coding and non-coding gene signatures from publically available datasets using AWS Cloud. Here, we will be sharing the challenges in data handling and discuss our findings.

Bhageerath-H: A homology/ *ab initio* hybrid server for predicting tertiary structures of monomeric soluble proteins

OP 39

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Experimental structural data of proteins exhilarated computational approaches to predict tertiary structures. Diminishing similarities of protein sequences to available experimental structures has derived some advanced homology/ *ab initio* hybrid approaches to solve protein tertiary structure prediction problems. Here, we describe *Bhageerath-H*¹, a homology/ *ab initio* hybrid method for predicting tertiary structures of proteins. *Bhageerath-H* software suite incorporates six modules. Starting with protein amino acid sequence as input, it implements an exhaustive conformational sampling using *Bhageerath-H* Strgen² algorithm and NCL based decoy generation, the generated decoys are k-mean clustered and submitted to physico-chemical scoring metric (pcSM)³ for decoy selection, selected decoys are validated using ProTSAV⁴ meta-server and the final five selections are processed by quantum mechanics (PM6) based method for bond angle optimization and REMD refinement. The final five native-like candidate structures are ranked using distance to native algorithm (D2N)⁵ and ProTSAV metaserver. *Bhageerath-H* algorithm has extensive applications in the fields of protein function prediction, active-site directed drug design, studying protein-protein interactions, protein structure design and engineering. *Bhageerath-H* web-server was validated on 75 CASP10 targets and showed TM-scores of > 0.5 in 91% of the cases and C α RMSDs of < 5Å from the native in 58% of the targets. The methodology has performed reasonably well in CASP11 experiment. *Bhageerath-H* is ranked at 7th position in terms of predicting structures under 5 Å in CASP11.

This tool is freely available at http://www.scfbio-iitd.res.in/bhageerath/bhageerath_h.jsp.

Adaptability and versatility of a bacterial immunoglobulin-like (Big) domain containing protein

OP 40

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Bacterial immunoglobulin-like (Big) domains are present mainly in bacterial proteins of 74–95 amino acids in

tandem repeats. Big domain-containing surface proteins have been identified in a number of pathogenic species and provide an evolutionarily tunable protein-binding functionality for mimicking natural host interactions. Although the sequence identity among Big domains is quite low, the structures of Big domain containing proteins are expectedly similar and representative of the stably folded α -sheets. The Big domain α -sandwich is typically stabilized by a conserved hydrophobic core and a Greek key topology. We are intrigued by the lack of disulphide bonds in Big domain containing proteins. Since insertion of disulphide bridge changes the compactness of the protein and it is believed that disulphide bond may have more of a functional than structural role. The absence of this structural constraint in many Big domains containing proteins may allow adaptation to specific biological functions or to particular structural features. However, in a Big domain containing protein Arig (*Arthrobacter aureescence immunoglobulin-like* domain; strain isolated from a highly oxidative stress condition), a pair of Cys is present in the linker between the two Big₂ domains. Here various dynamic properties including that of Ca²⁺ binding and the role of disulphide bond are presented. Arig and its Big domain show different hydrodynamic radius under reducing conditions. Cys to Ser mutation in Arig shows altered hydrodynamic radius and stability. Our results support the notion that in order to build the stable protein nature has introduced both disulphide bridge and Leu in place of Phe (to build a Hydrophobic core) in Arig to tackle oxidative conditions.

Synthesis, Characterization and Assessment of Genotoxicity of Chromium Oxide Nanoparticles

OP 41

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Synthesis, characterization and toxicity evaluation of nanoscale materials have become a significant part of the scientific investigation due to their importance for obtaining new and improved products for all fields of human activity. However, as the use of nanomaterials have increased, so does the concern about their short-and long-term effects on health and the environment. Nanoscale chromium oxide (Cr₂O₃) particles have recently attracted much consideration because of their numerous specific applied applications, which necessitates a thorough evaluation of its toxicity. In this study, the chromium oxide (Cr₂O₃) nanoparticles were synthesized, characterized and assessed for genotoxicity on male

albino rats (*Rattus norvegicus*) of wistar strain. Cr₂O₃ nanoparticles (NPs) were synthesized by sol-gel method and characterized by standard tools like X-ray diffraction (XRD), fourier transform infrared spectroscopy (FTIR), scanning/transmission electron microscopy (SEM/TEM), and UV-Visible spectroscopy which demonstrated their successful synthesis. Dose and time dependent genotoxic effects were investigated by employing assays to detect micronuclei and chromosomal aberrations in bone marrow as well as abnormalities in sperms after exposure to Cr₂O₃ nanoparticles (NPs) via oral gavage.

Functional Insights into a unique 4' Phosphopantetheinyl transferase from *Leishmania major*

OP 42

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Fatty acid biosynthesis of *Leishmania* has gained widespread attention recently due to the alteration of the composition of fatty acids in the drug resistant strains, associating fatty acid synthesis with virulence. An important step that initiates this pathway involves the priming of the acyl carrier protein (ACP) with the Coenzyme-A derived 42'-phosphopantetheine moiety, the reaction catalyzed by a 4' phosphopantetheinyl transferase (PPTase). Genome sequence analysis suggests the presence of a single PPTase in *Leishmania*, which is a monomeric Sfp (*Bacillus subtilis*)-type enzyme.

In vitro characterization of this PPTase with its cognate ACP (LACP, TypeII) and non-cognate substrates (Mtb, pf *E.coli* and Human) reveals some interesting and unusual properties. *In vivo* data sheds light on its sub-cellular localization in the kinetoplast. PPTase exhibits high catalytic efficiency with Mtb ACP as compared to other Type II ACPs. Our biochemical studies provide interesting insights into its possible biological function and might have implications in therapeutic design against the deadly disease leishmaniasis.

Consequences of hyperoxaluria with and without acidic urination on Unfolded Protein Response (UPR)

OP 43

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Chaperones are known to play a crucial role in the proper folding and quality control of proteins. Calnexin being

an ER-resident chaperone assist in the folding process of glycoproteins. Any insult incurred to ER environment leads to a cascade or initiation of unfolded protein response (UPR). Unfolded protein response can be attributed to the unwanted accumulation of misfolded proteins in the ER lumen as a result of compromised protein folding capability mediated by chaperones. UPR regulates a cascade of events resulting in the activation of ER stress pathway. Our present study aims at investigating the role of UPR involved in the hyperoxaluric conditions in renal tissue. For this study, animals were divided into three groups viz., Group 1: served as control which received normal drinking water *ad libitum*, Group 2: were given chronic dose of 0.75% ethylene glycol alone in drinking water for 28 days and Group 3 animals were administered with sub-chronic dose of 0.4% EG with 1% ammonium chloride in drinking water for 9 days. Followed by confirmation of crystalluria in the urine of treated rats and damage to renal functioning, expression of GRP78 a marker of unfolded protein load in ER was estimated. Further UPR pathway was elucidated by estimating ATF4, XBP1, and CHOP mRNA expression. It was found that GRP78 increased group 3 but decreased in group 2 animals. On assessing mRNA expression of UPR pathway ATF4 and XBP1 increased in both group 2 and group 3 but expression of CHOP decreased in group 2 and increased in group 3 animals. These results suggest that EG alone is not effectively initiating UPR whereas with ammonium chloride its effect is more pronounced.

Study of aggregation behavior of amyloid beta peptide (25-35 fragment) in presence of metal ions at different pH

OP 44

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Amyloid beta (A β) is a peptide of 36-42 amino acids that is processed from a transmembrane glycoprotein called Amyloid Precursor Protein (APP). The aggregation and fibrillization of Amyloid-beta (A β) peptide is a major pathological hallmark of Alzheimer's Disease (AD). The full length A β peptide has widely been studied for the structural alterations, aggregation and fibrillization in connection to their perilous role in AD. However, a smaller fragment of Ab peptide, consisting of the 25-35 amino acid stretch of Ab42 is also highly neurotoxic and has emerged as an interesting model to study the aggregation behaviour. Although the smaller fragment of the peptide lacks Histidine residue as the potential metal binding site, but significant effect of metal ions like Copper(II) and Zinc(II) has been observed in the aggregation kinetics of the peptide. Studies with Cu(II) and Zn(II) have been carried out at acidic pH and also at

physiological pH to understand the possible role of pH in the metal ion induced aggregation. Using UV-absorption spectroscopy, Turbidity assays, Circular Dichroism and FTIR spectroscopy the alterations in the secondary structure and the aggregation profile of the peptide have been studied in vitro. The results imply predominant aggregation at acidic pH and in presence of Copper ions whereas at physiological pH more aggregation was induced by Zinc than Copper. The results might be correlated with the enhanced toxicity and precipitation of the amyloid peptide in presence of copper at acidic pH in the physiological acidosis condition in AD.

Reoptimization of the glycosidic torsion and Lennard-Jones parameters leads to improved description of conformational distributions of modified uridines

OP 45

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Nucleoside modifications in tRNA are known to influence conformational preferences of the modified and neighbouring residues, modulate anticodon domain structure, control codon recognition, affect ribosomal binding and influence reading frame maintenance. Accurate force field parameters for the modified residues are therefore necessary for reliable molecular modeling of biophysical and biochemical processes involving tRNA. Over the last decade, force field developers are mainly focusing on the torsional terms and Lennard-Jones parameters of the standard nucleic acid residues for both the CHARMM and AMBER molecular modeling suites for obtaining better agreement with experimentally observed conformational and structural characteristics and thermodynamic properties. Recently, we examined the transferability of currently revised torsion parameters for standard nucleic acid systems by combining them with the only available comprehensive set of parameters for modified ribonucleosides/nucleotides for AMBER. Extensive molecular dynamics (MD) simulations and Replica Exchange MD (REMD) simulations for a set of modified uridines with AMBER FF99 \ddagger , FF99TOR and FF10 force fields showed significant deviation from available experimental observations for most of the modifications. We report here a general approach for the development and fine tuning of AMBER force field parameters for a set of modified uridines (2-thiouridine, 4-thiouridine and pseudouridine) with diverse conformational preferences. We observed that the previously suggested reoptimization of the glycosidic torsion potential led to improvement but was still

insufficient in describing the sugar pucker preferences. We shall also report the effect of revising other torsion parameters and/or the Lennard-Jones sigma (5σ) parameters in achieving further improvement in the description of conformational preferences.

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Role of denatured state in protein stabilization by osmolytes

OP 46

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It is widely known that heat and GdmCl induce structurally and energetically different denatured states of most proteins. Heat-induced denatured state contains many unmelted residual structures that has significant amount of secondary and tertiary interactions, while GdmCl-induced denatured state behaves as random coil with very little stable local structures, if any. Here we have investigated whether the residual structures present in the thermally denatured state plays any significant role in protein stabilization by osmolytes (low molecular weight organic compounds accumulated in stress conditions) as compared to the osmolytes effect on random coil structure. Using three disulfide-free proteins we performed measurements of ΔG_D° (Gibbs energy change at 25 °C) of proteins associated with heat-induced and GdmCl-induced denaturations in the presence and absence of osmolytes, and systematically compared the measured ΔG_D° value for both modes of denaturation. We discovered that structural characteristics of the denatured state of a protein determine the magnitude of protein stabilization in terms of ΔG_D° by an osmolyte, i.e. the more is backbone exposure the more the stabilization. Another important finding is that, among proteins, there exists a good correlation between the $\Delta\Delta G_D^\circ$ and number of peptide bonds in the case of GdmCl-induced denaturation but the correlation breaks down if the $\Delta\Delta G_D^\circ$ values were derived from heat-induced denaturation.

Stacking Interactions in DNA and RNA

OP 47

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Sequence directed structural studies of nucleic acids observed from experimental data suffers limitations due to statistically insufficient available structural data in

many cases. Therefore we have attempted to understand sequence dependent stacking energy along twist, roll and slide degrees of freedom for all possible dinucleotide sequence structures using quantum chemical methods. Stacking energy analysis in these dinucleotide parameter hyper-space was initially done for RNA with AU/AU dinucleotide sequence, which showed that the DFT-D method by ω B97X-D/6-31G(2d,2p) appears to satisfactorily explain the conformational preferences. This methodology was applied to ten unique dinucleotide steps in RNA and DNA. We found that values of roll, slide and twist of most of the dinucleotide sequences in crystal structures fall in the low energy region in the energy contour diagrams. The minimum energy regions with higher twist values are associated with the roll and slide values of B-DNA, whereas, smaller twist values resemble to higher stability to RNA and A-DNA like conformations. Incorporation of solvent is more significant for stability of B-DNA and it explains the preference shown by some sequences to appear in B-DNA or A-DNA conformation. Conformational preference of BII sub-state in B-DNA duplex is exhibited by pyrimidine-purine steps and partly by purine-purine steps in accordance with the experimental studies. The purine-pyrimidine steps shows largest effect of 5-methyl group of Thymine in stacking energy and the introduction of solvent reduces this effect significantly. In case of non-canonical base pair containing dinucleotides where the roll, slide and twist parameters are varied in accordance to RNA structures illuminates for the first time the structural preference of G:U containing 11 possible dinucleotide steps along with corresponding stacking energy. The results so far indicate that G:U wobble base pair can stack well with the canonical base pair giving rise to stable interaction in terms of energy.

Potential Peptide Vaccine Candidates Against Mrsa (Methicilin Resistant Staphylococcus Aureus): A Reverse Vaccinology Approach

OP 48

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The mayhem caused by MRSA is widespread causing a large spectrum of infections and affecting a significant population worldwide. A potential Vaccine candidate against MRSA should be effective against large variety of strains worldwide and also should show strong effectiveness against key materials (e.g. Cell Wall Proteins) for the survival and virulence of the organism. Here, we have developed a novel *in silico* way of identifying potential candidate vaccines against MRSA.

In the past few decades, genome-based approaches have contributed significantly to vaccine development. Our

aim was to identify the most conserved and immunogenic antigens of MRSA, which can be potential vaccine candidates in the future.

In this study, 49 surface proteins from various literatures were screened initially. Similarity of these 49 proteins was searched against all the strains in NCBI through BLASTp. In this step total no. of screening were 833. PSORTb 3.0.2 was used to predict the subcellular localization of the proteins. B cell epitope prediction was done for the immunogenicity testing with the help of IEDB bepiped tools. Antigenicity was tested using VAXIJEN tool. Finally, BLASTp was done for verifying the extent of similarity to human proteome to exclude the possibility of autoimmunity. Proteins failing to comply with the set parameters were filtered at each step. Based on the above criteria, out of the initial 49 proteins selected for screening three were found to be the most promising candidate proteins, namely **Cell surface anchored protein, atl nag (Bifunctional autolysin), ebpS (Elastin-binding protein)**

Bidirectional hydraulic conductivity of *E. coli* outer membrane lectin (Wzi) is regulated by surface aromatic residues and luminal hydrophobic plug

OP 49

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Capsular polysaccharides (CPS) are one of the major virulence determinants of Gram-negative bacteria. Group 1 CPS of *E. coli* is synthesized by a Wzy-dependent mechanism and is non-covalently anchored to the surface by the outer membrane lectin Wzi. We have uncovered a unique, bidirectional, voltage-independent hydraulic conducting property of Wzi by molecular dynamic simulation technique. Conserved residues, Y380 and W39 regulate the water flux at the extracellular and periplasmic ends respectively in concert with a hydrophobic plug. The plug narrows the lumen, making Wzi highly specific for water conduction. Intriguingly, Y380 is part of a conserved "YQF" triad, which is also seen in the sugar-binding site of vSGLT (Sodium Galactose cotransporter), implicating that it might be the CPS surface anchoring site. A potassium ion-binding pocket coinciding with the point of water entry is also observed at the extracellular side. Thus, we hypothesize that Wzi acts an osmotic pressure regulator, aiding in non-covalent attachment of capsular antigen onto the bacterial surface facilitated by the ion pocket. Despite being a lectin, Wzi also functions as a porin, justifying its beta-barrelled structure. Further, modeled extracellular loop 5 (absent in the crystal structure) is found to play a major role in anchoring the protein onto the membrane. Thus,

the outcome of this study may facilitate the design of antibacterial drugs that can specifically alter the water conductance of Wzi. This may further perturb CPS binding to the bacterial surface and thus, lead to decreased virulence and higher antibiotic susceptibility.

Biophysical and Biochemical validation of new inhibitors identified through rational structure based design against Dopamine- β -hydroxylase to combat cardiovascular diseases

OP 50

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Human dopamine- β -hydroxylase (hDBH), expressed in nerve terminals and adrenal medulla, is a key constituent of catecholamine biosynthetic pathway. hDBH is inhibited to treat hypertension and cardiac heart failure-major causes of mortality worldwide. Existing hDBH inhibitors are too few; often result in side-effects and frequently non-responsive to specific population. Since no 3D structure existed for full-length hDBH, structure based rational drug design has been elusive till date. We solved the issue lately by building an experimentally validated *in silico* model for hDBH (Kapoor et al., 2011). The model was used for structure based virtual-screening against small molecule databases. Top hits were tested *in vitro* against human serum DBH and its homologue-bovine DBH, with known inhibitors nepicastat and disulfiram as positive controls. Three potent inhibitors of DBH were discovered with IC₅₀s in low-micro-molar range. Binding of the lead compounds to the enzyme were validated using fluorescence and CD spectroscopy as well as ITC, revealing K_d values in the range of 100-1000nM. *In silico* ADMET profiling indicates the molecules to be latest generation of DBH-antagonists with high cell-permeability and inability to cross the BBB. High doses ($\leq 50\mu\text{M}$) of the lead-compounds showed acceptable cellular tolerance against HEK293 cell-line and insignificant hemo-toxicities against human RBCs. Hence, *in vivo* evaluation of these compounds has been done in model organisms, like- *C. elegans* and *D. melanogaster*, reconfirming their nontoxic properties up to 15 μM doses. These compounds are now being tested in Rat model of CVDs. Lead compounds have also been optimized computationally for their pharmacokinetic improvement.

DNA Sequence specificity & cytotoxicity of harmalol

OP 51

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Binding data obtained from absorbance according to neighbor exclusion model indicated that the binding constant decreased in the order poly(dG-dC).poly(dG-dC) > poly(dA-dT).poly(dA-dT) > poly(dA).poly(dT) > poly(dG).poly(dC). The same trend was shown by the competition dialysis, change in fluorescence steady state intensity, stabilization against thermal denaturation, increase in the specific viscosity and perturbations in circular dichroism spectra. Among the polynucleotides, poly(dA).poly(dT) and poly(dG).poly(dC) showed positive cooperativity where as poly(dG-dC).poly(dG-dC) and poly(dA-dT).poly(dA-dT) showed non cooperative binding. Isothermal calorimetric data on the other hand showed enthalpy driven exothermic binding with a hydrophobic contribution to the binding Gibbs energy with poly(dG-dC).poly(dG-dC), and poly(dA-dT).poly(dA-dT) where as harmalol with poly (dA).poly(dT) showed entropy driven endothermic binding and with poly(dG).poly(dC) it was reported to be entropy driven exothermic binding. The study also tested the *in vitro* chemotherapeutic potential of harmalol in HeLa, MDA-MB-231, A549, and HepG2 cell line by MTT assay. DNA fragmentation study by gel-electrophoresis, ROS generation by FACS and identification of nuclear changes by DAPI staining, internucleosomal DNA damage by comet assay in HepG2 cell line were also studied.

Studies unequivocally established that harmalol binds strongly with hetero GC polymer by mechanism of intercalation and showed maximum cytotoxicity on HepG2 with IC₅₀ value of 14 mM, indicating anticancer potential of harmalol through its ability to induce apoptosis. The results contribute to the understanding of binding, specificity, energetic and cytotoxicity that will guide synthetic efforts of medicinal physiologists and chemists for developing better therapeutic agents.

Catalytically inactive sperm lysozyme like protein 1 possesses intact binding pocket for lysozyme substrates and inhibitors: Possible role in spermovum binding

OP 52

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Lysozymes are well-recognized bacteriolytic proteins widely distributed in the animal kingdom and play a protective role in host defense. Existence of various

isoforms of lysozyme in different tissues led to convergent and adaptive evolution. Gene duplication and mutations resulted into evolution of lysozyme like genes (*Lyzl2*, *Spaca3/Lyzl3*, *Lyzl4/Lyzl5/Spaca5* and *Lyzl6*) in the testis. In the present study, cloning and sequence analyses of buffalo *Spaca3* gene was carried out. The *Spaca3* gene encoding sperm lysozyme like protein 1 (SLLP1) was expressed in *Pichia pastoris* and purified in biologically active form. The signature of c-type lysozyme family and four disulfide bonds were conserved, whereas the catalytic residues (Glu35 & Asp52) were replaced by Ala35 and Asn52. The recSLLP1 showed catalytic inactivity towards bacteriolysis. CD spectra suggested secondary structure of recSLLP1 similar to that of c-type lysozymes. The thermal stability of SLLP1 was lower than HEWL in pH range of 2-10. Homology model of SLLP1 showed overall structure and substrate binding pocket similar to catalytically active c-type lysozymes. Native-PAGE suggested binding of SLLP1 with a lysozyme inhibitor, however, fluorescence spectroscopy and competitive-binding assay suggested weaker binding of the inhibitor with SLLP1 as compared to HEWL. Docking of inhibitor with SLLP1 also suggested weaker binding as compared to binding with lysozyme. Docking studies also suggested binding of NAG, diNAG and triNAG with SLLP1. The study suggest that SLLP1 although catalytically inactive retained the lysozyme like scaffold and binding pocket for possible interaction with NAG glycan moieties on the ovum surface and might have evolved for facilitating sperm-egg binding.

Fructosylated human serum albumin plays a crucial role in diabetic pathophysiology

OP 53

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Diabetes mellitus is amongst the most fast spreading pandemic of metabolic disorders. The defining feature of diabetes mellitus is hyperglycemia. The persistently high levels of reducing sugars cause glycation of various biological macromolecules. Human serum albumin (HSA), being the most abundant serum protein, is prone to glycation and consequent alteration in its properties. This study aimed to assess the role of fructose-modified human serum albumin as a marker of diabetic pathophysiology. In the present study, we carried out modification of HSA with fructose. The changes induced were studied by UV and fluorescence spectroscopy, NBT assay, SDS-PAGE, carbonyl content estimation, CD spectroscopy and FT-IR spectroscopy. Type I and II

diabetes patients were followed up for one year to study the antibody level in their sera against native and fructose-modified HSA. Also, the oxidative stress in these patients and the follow-up counterparts was studied by carbonyl content estimation. Fructose modified-HSA showed hyperchromicity in UV spectrum and increased AGE-specific fluorescence as well as quenching of tryptophan fluorescence. On SDS-PAGE protein aggregation was seen. Early glycation end-products were detected in fructose-modified HSA. The fructose-modified HSA had more content of protein bound carbonyls compared to native HSA. Perturbations in the secondary structure of HSA were evident on far-UV and FT-IR spectroscopy. Patient studies revealed that fructose-modified HSA acts as a potent immunogen compared to its native form and the levels of antibodies against fructose-modified HSA served as a good parameter for tracking the glycemic control, disease status as well as oxidative stress (carbonyl content) in diabetic patients.

Dissecting macromolecular recognition sites in ribosome

OP 54

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We analyzed the protein-protein (PP), protein-RNA (PR) and RNA-RNA (RR) interfaces in a dataset of 13 ribosomal assemblies curated from the Protein Data Bank with resolution better than 4 Å. They belong to six different organisms. We find a total of 846 pairwise interfaces: 421 PP interfaces, 403 PR interfaces and 22 RR interfaces. The average size of the interface in each category is 752Å², 3899Å² and 2296Å², respectively. In both prokaryotes as well as in eukaryotes, the PR interfaces are significantly larger than RR and PP interfaces. On average PR interfaces and RR interfaces have nearly the similar number of H-bonds, whereas PP interfaces have much lower H-bond density. In PR interfaces on the protein side the main chain N and Lys/Arg account for nearly half of all H-bonds. On the RNA side of PR interfaces phosphate group (45%) is dominant compared to base and ribose. The role of ribose sugar is important in RR and PR interfaces. In RR interfaces ribose is heavily involved in H-bonds and the 2' OH is the largest single contributor. The atoms are less compact at the interfaces between large and small subunits of ribosome, which may be justified by the fact that during translation the two subunits periodically associate and dissociate. In case of chemical composition of PR interfaces, on the RNA side the negatively charged phosphates are abundant and on the protein side the positively charged residues (Lys/Arg) are abundant. Thus it may be due to the charge neutralization of negative charges of RNA by positive charges of protein to stabilize the PR complexes.

Different denaturants induces different denatured states in yeast iso-1-cytochrome c and its N-terminal deletants.

OP 55

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It has been observed that different denaturants may give different denatured states of the same protein. We denatured yeast iso-1-cytochrome c (y-cyt-c) and its deletants with heat, guanidinium chloride (GdmCl) and urea. The denatured states thus obtained were compared to see whether they are thermodynamically and structurally are same. Heat-induced denaturation of all proteins was monitored by spectroscopic methods as well as differential scanning calorimetry (DSC). The thermodynamic parameters thus obtained show that proteins follow two-state denaturation. Isothermal denaturation of y-cyt-c and its deletants by urea and GdmCl were also carried out. Coincidence of normalised denaturation curves of different optical properties ($\Delta\epsilon_{405}$, $[\theta]_{222}$, $[\theta]_{405}$ and $[\theta]_{416}$) suggested that the urea-induced denaturation follows a two-state mechanism. On the other hand, GdmCl-induced denaturation of these proteins showed non-coincidence of normalised transition curves suggesting that denaturation does not show a two-state behaviour. A comparison of thermodynamic and structural parameters of denatured state induced by heat with those of GdmCl-induced denatured state showed the presence of residual structure in the denatured state induced by heat. All these experimental observations have also been verified with molecular dynamics (MD) simulations. Thus, it may be concluded that though denaturation of y-cyt-c and its deletants induced by different denaturants may follow a two-state mechanism, they produce different denatured states.

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Tetramer of hGBP1 acts as an allosteric switch for stimulated GMP formation

OP 56

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The interferon- γ induced human guanylate binding proteins (hGBPs) belong to dynamin family of large GTPases. Among them, hGBP1 and hGBP2 share almost 80% sequence identity. These proteins show a unique

feature of hydrolysing GTP into a mixture of GDP and GMP in a sequential manner with unequal proportions. Previous studies from our laboratory with mutant proteins showed that the mutants defective in tetramer formation with the transition state analog did not show stimulated GMP formation, suggesting the role of a tetramer in the stimulated GMP production. To understand how inter-domain interactions could play a critical role in tetramer formation leading to stimulated GMP formation, we prepared chimeric proteins in which the Globular (catalytic) domain of hGBP1 was intact, but the other two domains were systematically swapped with that of hGBP2. Unlike wt-hGBP1, these chimeras showed basal GMP formation with loss of allosteric interactions and no tetramer with the transition state analog. Taken together, these data indicate that a tetramer is critical for stimulated GMP formation through allosteric interaction. To further validate this, we prepared a double mutant of wt-hGBP1 at a site of the interaction between the Globular domain and the helical domain. Interestingly, this mutant showed a higher amount of GMP formation with increase in the allosteric interaction compared to wt-hGBP1. Additionally, this mutant shows a tetramer even with the substrate analog, suggesting that disruption of the inter-domain interaction favours tetramer formation. These data collectively indicate that a tetramer of wt-hGBP1 is indeed important for the stimulated GMP formation through allosteric interaction.

Mechanistic insight into the anti-amyloidogenic behavior of taurine against thermally aggregated human serum albumin

OP 57

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Interaction of small molecule inhibitors with protein aggregates has been studied extensively, but how these inhibitors modulate aggregation kinetic parameters is little understood. In this work, we investigated the inhibitory ability of Taurine on the thermally induced aggregation of Human Serum Albumin (HSA) and also examined how it modulates the kinetic parameters of the aggregation process. We demonstrate the structural changes in the HSA by exploiting various biophysical techniques after binding to the Taurine at 65°C. UV/VIS spectroscopy was applied to check the turbidometric changes in the protein. Thioflavin T fluorescence kinetics was subjected to explore kinetic parameters comparing the amyloid formation in the presence of varying concentration of taurine. Further Congo red binding confirmed the inhibitory effect of taurine on HSA fibrillation process. ANS Binding assay was done to determine the surface hydrophobicity changes occurring before and after the addition of taurine with protein. Far

UV CD and Dynamic Light Scattering (DLS) was done to measure the changes in protein secondary structure and hydrodynamic radii in the presence of taurine and it was seen that Taurine stabilized the protein α -helical structure. Microscopic imaging techniques were also done to analyse the morphology and the amount of aggregation formed. Taurine is also capable of altering the cytotoxicity of the proteinaceous aggregates. Molecular docking study also deciphered the possible residues involved in protein and drug interaction. This work would provide insight into the mechanism of aggregation inhibition by small drug molecules.

Crowder shape: A probable cause for the enhanced thermodynamic stability of the protein. OP 58

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For years, thermodynamic and structural properties of isolated proteins have been measured in dilute buffer solutions. It has been assumed that this *in vitro* condition mimics the *in vivo* picture. On the contrary, the cellular environment contains large biomolecules (proteins, nucleic acids, ribosomes and carbohydrates) whose total concentration ranges from 80 to 400 mg/ml. Such a cellular condition has been termed as macromolecular crowding. This implies that in the cell proteins' physico-chemical properties may be influenced by steric repulsions of macromolecules (macromolecular crowding). That is, this crowding could affect the thermodynamic stability and kinetics of protein folding, and proteins structure, function and dynamics. To mimic cellular environments, inert synthetic crowding agents are used in *in vitro* studies. In the present study effects of different concentrations of ficoll 70 and dextran 70 on the thermodynamic stability of HEW lysozyme were measured at different pH values by following changes in molar absorption coefficient at 300 nm ($\Delta\epsilon_{300}$) as a function of temperature. It has been observed that T_m (transition temperature) and ΔG_D° (Gibbs free energy change at 25 °C) of lysozyme increased with increasing concentration of both the crowders at a given pH in the range 7.0 - 2.0. These observations led us to conclude that, owing to excluded volume effect, both the crowding agents stabilized lysozyme. The percent stabilization (% - ΔG_D°) showed that dextran 70 provides more stabilization than ficoll 70. This observation may be explained by the fact that the rod shaped dextran is expected to exclude more volume than the spherical shaped ficoll, although they have same molecular mass of 70 kD.

Application of next generation sequencing and analysis towards understanding repeat elements in Caryophyllales: *Amaranthus hypochondriacus*

OP 59

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Genomes of the order Caryophyllales are underrepresented in the copious plant genome sequencing efforts. As a result, in the publicly available databases of known repeats such as RepBase and mips-RE-dat, Caryophyllales-specific repeats are also underrepresented. In the work presented here, we have made use of the draft genome sequence of *Amaranthus hypochondriacus*, sequenced and assembled in-house, and the genome of *Beta vulgaris* to characterize Caryophyllales-specific repeats. Homology-based and *de novo* prediction tools were used for discovery of repeats in both these species and RepeatMasker was used to mask the genomes to estimate the repeat content in their respective genomes. When the genomes were masked using the repeats predicted from the respective plant genomes, it revealed the presence of significantly lower percentage of repeats in *A. hypochondriacus* genome compared to other plant species. A more thorough prediction of repeats using RepeatModeler masked only 13.76% of *A. hypochondriacus* genome, still far below the 63% reported for *B. vulgaris*. The lower percentage of repeats in *A. hypochondriacus* genome may suggest that either amaranth specific repeats have diverged from those of other species represented in Repbase or that *B. vulgaris* has been under the influence of active evolution after speciation owing to its history of domestication. Also, the lower percentage of retrotransposons seen in *A. hypochondriacus* compared to other species in Repbase may be because of its lower susceptibility to environmental conditions and viruses. The lower repeat content in *A. hypochondriacus* is further validated using C_0t analysis of the genome.

Available Methods for Estimating Change in Solvent-Accessible Surface Areas of Soluble Proteins during Folding. OP 60

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Solvent accessible surface area (SASA) of soluble proteins has always been considered as a decisive factor in protein

folding studies. It is defined as the surface characterized around a protein by a hypothetical centre of a water molecule with its van der Waals contact surface. Based on SASA values, amino acid residues of a protein can be classified as buried or exposed. There are various types of SASAs starting from relative solvent accessibility to absolute surface areas. SASA of amino acid residues of a native protein can be computed from its atomic coordinates given in Protein Data Bank (PDB). There are various methods but they all give almost same value of SASA of a residue. On the contrary, such estimation is not possible for the unfolded proteins. In efforts to estimate changes in SASA related to the protein folding, a number of the unfolded state models have been developed. We have summarized different algorithms and computational tools for SASA estimations (Ali *et al.*, 2014). Here we show its applications by calculating different stability parameters like m -value, ΔC_p , ΔG_D° , etc. for a number of proteins.

Reference

Ali SA; Hassan I; Islam A; Ahmad F. (2014) A Review of Methods Available to Estimate Solvent-Accessible Surface Areas of Soluble Proteins in the Folded and Unfolded States. *Curr Protein Pept Sci.* 15,456-476.

A compound heterozygous (g.2603T>C, g.13362_13363insA) condition in an Indian family with type II Antithrombin III deficiency leads to *in vivo* polymerization

OP 61

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The serpins (Serine proteinase inhibitors) are a superfamily of inhibitors that mostly inhibit proteinases of the chymotrypsin family. Antithrombin III (AT) is an important endogenous anticoagulant and its deficiency has helped in understanding the molecular mechanisms underlying serpinopathies. AT deficiency can be of either type I where both AT activity and antigen levels are reduced or type II where normal antigen levels are associated with low activity levels. Type II AT deficiency is normally associated with single base pair substitutions whereas type I AT deficiency is commonly associated with insertions and deletions. In the present study an Indian family with type II AT deficiency in four members spanning two generations has been studied. In addition to a previously known defect in heparin binding site g.2603T>C (p.R47C), a novel single base insertion

g.13362_13363insA was identified as the cause of deep vein thrombosis in this family. The single base insertion resides near s4B and s5B of AT. The insertion causes frameshift of amino acids from 416 to 432 (p.P416SfsX16). Elution profile of variant AT at lower ionic strength from Hi-trap heparin column indicated a shift in the inhibitory activity and appearance of larger peak at much lower ionic strength. Oligomeric bands were observed in the Western blot of the proband but not the mother who carried only p.R47C variant. *In silico* studies showed expulsion of s3B and s4B which may result in the insertion of RCL of another intact molecule into the sheet B resulting in loop sheet type of polymer formation.

Modeling the dynamics of cytokine signaling pathways in Rheumatoid Arthritis using systems biology approach

OP 62

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Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects the synovial joints.

In RA, the imbalance between pro- and anti-inflammatory cytokines induces synthesis of enzymes such as inducible nitric oxide synthase (iNOS) and matrix metalloproteinases (MMP-1 and MMP-3). In RA patients, iNOS catalyzes the production of nitric oxide (NO) in much higher quantities (micro Molar) in affected tissues compared to healthy individuals (nano Molar). It is necessary to understand and mathematically model the dynamics of signaling network that affects NO production from a system biology approach. We have constructed a signaling network of pro- and anti-inflammatory cytokines regulating the production of NO during RA based on an extensive literature survey. TNF is one of the important pro-inflammatory cytokines included in this network and we report here the modeling of pathways involving TNF induced NO production. Based on published literature, we have also created a metabolic pathway of nitrate, nitrite and NO across seven body compartments. A systems biology approach using Ordinary Differential Equations has been adapted to build a mathematical model to describe the dynamics of NO and its metabolites by using the experimentally obtained kinetic rate parameters. The steady state values of the NO metabolites generated in the model match well with their respective values in the published experiments. Clinical experiments have been performed on healthy volunteers to measure the time profiles of NO metabolites in various compartments on administration of oral and intravenous nitrate doses. These results have been successfully simulated in our model.

Structural stability of Nucleobindin1/Calnuc: Temperature and pH dependent studies

OP 63

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Nucleobindin1 (Nucb1) or Calnuc is a multi-domain, Ca^{2+} - and DNA-binding protein predominantly localized in the Golgi but also seen in nuclear, cytosolic and extracellular regions. Nucb1 is known to play multiple roles, e.g., in stress response, apoptosis and inflammation. We have observed that the protein degrades easily and stabilizes at a molecular weight of ~ 25 kDa. In order to understand the physiological relevance of this phenomena, we studied the thermal stability and pH based conformational changes of Nucb1. Compared to other calcium binding proteins, Nucb1 is an unstable protein (with a melting temperature (T_m) of 49.8 °C). To identify the regions causing its instability, fragment based approach was followed. Various domains were deleted and corresponding T_m was measured. pH induced structural and conformation changes in *apo*- and *holo*-Nucb1 were monitored by far- & near-UV Circular Dichroism (CD), Tryptophan fluorescence, 8-anilino-1-naphthalenesulfonic acid (ANS) binding and acrylamide quenching. Deletion studies indicated that the poly-glutamine region in the C-terminal end of Nucb1 could be responsible for its instability. Fluorescence and CD studies indicated Nucb1 retained most of its structural elements at pH 1-12. Precipitation was observed at pH 5 in *apo*-Nucb1. Calcium binding rescued Nucb1 from being precipitated at pH 5. We hypothesize that an alteration in surface charge distribution of *holo*-Nucb1 could be protecting it from precipitating. Near-UV CD studies at pH 2, 8 and 11 revealed Nucb1 retained its tertiary structure with minor changes.

Hypothetical Proteins from *Borrelia burgdorferi*

OP 64

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Borrelia burgdorferi belongs to the spirochete family, which is a causative agent of Lyme disease. Species of *Borrelia* are considered as double-membrane bacteria rather than gram positive or negative. Small mammals are the most common reservoir of *Borrelia burgdorferi*. Infection of these natural hosts does not cause any disease, but infection of humans may result in Lyme disease, as a result of the human pathological response to *B. burgdorferi*. While analyzing the proteome data of *B. burgdorferi*, we found that 241 proteins are listed as conserved hypothetical protein (HP) for which any biochemical evidences have not been reported so far and the function of these proteins are still unknown. Study of these HPs offers a new cascade of biochemical pathways. Additionally, HPs may serve as biomarker and pharmacological targets. We have identified HPs from the *B. burgdorferi* genome and annotated their corresponding functions using different tools for sequence alignment, motifs and domain search. Along with this function may be concluded on the basis of properties that are associated with a specific functional class and family of the proteins. We also studied the presence of signal peptide and sub-cellular localization in the HPs. Additionally sequence analysis was also performed to find out the physicochemical properties containing amino acid composition, aliphatic index and instability index of HPs. Finally, function annotation and identification of functionally important regions in HPs from *B. burgdorferi* may lead to suitable understanding of its virulence mechanism, adaptability in host system, tolerance for host immune system and emergence of drug resistance strain and finally drug/vaccine discovery.

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Poster Abstracts

Surface functionalized Superparamagnetic iron oxide Nanoparticles: Design, synthesis and hyperthermia therapy application

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Magnetic nanoparticles (MNPs) with appropriate surface chemistry have been widely used extensively for numerous in vivo applications such as magnetic resonance imaging contrast enhancement, immunoassay, detoxification of biological fluids, hyperthermia, drug delivery and in cell separation, etc. All these biomedical and bioengineering applications require that these NPs which have high magnetization values and size smaller than 100nm with overall narrow particle size distribution, so that the particles have uniform physical and chemical properties. The size and different properties of MNPs are strongly influenced by synthesis method adopted.

In this context in the present investigation, we adopted a new approach to synthesis of Fe_3O_4 MNPs. We chose alkaline media (DIPA) as a co-precipitating agent. Surface of Fe_3O_4 NPs were successfully modified with Pluronic F127. The particle size of the synthesized NPs was ~10 nm. The Pluronic F127coated NPs exhibit narrow size distribution, good dispersibility and very good suspension stability as compared to uncoated Fe_3O_4 NPs. It was found that coating with Pluronic F127 enhances the hydrophilicity caused the nanoparticles to form stable suspension in water. Pluronic F127 coated NPs show superparamagnetism at room temperature with blocking temperature $T_b \sim 200\text{K}$. Induction heating studies of Pluronic F127coated NPs were investigated for their possible application in magnetic fluid hyperthermia (MFH). The coated NPs exhibited self-heating temperature rise characteristics when subjected to external magnetic field at different particle concentration and field amplitudes and from results found that the synthesized Nanoparticles show highest specific absorption rate ~600 Watt/gm. Magnetic properties of synthesized MNPs were measured by a superconducting quantum interference device (SQUID) and crystallographic and chemical properties by TGA, XRD, FTIR and TEM etc.

H2AX a Novel Biomarker for DNA Double-strand Breaks: Purification and Characterization from Mammalian Kidney

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H2AX is a variant of the H2A protein family, which is a component of the histone octamer in nucleosomes, and is a key factor in the repair process of damaged DNA. DNA is normally wrapped around a core histone molecule forming the nucleosome complex. Histone cores are made up of individual histone proteins called H2A, H2B, H3 and H4. The H2A protein family has the greatest number of variants including H2A1, H2A2, H2AX and H2AZ plus many others. The H2A variants only differ by a few residues, but the H2AX protein is unique in eukaryotes due to its carboxy tail and has molecular weight of 15 kDa. When DNA gets damaged, either by endogenous or exogenous or forms double stranded breaks (DSBs), it is always followed by the phosphorylation of the histone H2AX, which is the first step in recruiting and localizing DNA repair proteins. H2AX also has applications in the detection of genomic damage caused by cytotoxic chemical agents and environmental and physical damage, especially in the context of cancer treatment and therapy. Here, we are reporting a simple procedure for the purification of H2AX protein from mammalian kidney. To isolate H2AX protein, sheep kidney was crushed and kept for 12 hours in the extraction buffer. The lysate was centrifuged, and supernatant was precipitated by ammonium sulphate. The precipitate of 100% ammonium sulphate was extensively dialyzed and loaded on Mono Q and Superdex 200 chromatography columns. The purity of H2AX was confirmed by SDS-PAGE. Finally, the protein was identified by matrix-assisted laser desorption/ionization time of flight. Our purification procedure is fast and straightforward with high yield.

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Screening of bioactive molecules from cyanobacteria: a traditional as well as modern approach

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Increasing drug resistance in pathogenic bacteria along with fungi is causing concern to drug developers. Health hazard is increasing with cancer patients. Genome sequencing of cyanobacterial NRPS and PKS genes revealed the potentiality of cyanobacteria to provide array of lead molecules for synthetic biochemists for drug development. Cyanobacteria are little explored in this regard. The sequenced genomes of many cyanobacteria opened the new vistas of such investigations. Generally, active biomolecules are secondary metabolites associated with polyketide synthase (PKS) and non ribosomal peptide synthetase (NRPS) and hybrid thereof. We have PCR amplified these genes from locally isolated genera of cyanobacteria (*Phormidium* CCC727, *Geitlerinema* CCC728, *Arthrospira* CCC729, *Leptolyngbya* CCC732, *Phormidium* CCC730, *Phormidium* CCC731) and amplified PKS as well as hybrid genes. These amplified sequence were sequenced and putative protein structures predicted using bioinformatic approach. In addition to the above, we have also bioassayed the crude extracts of target organisms against pathogenic bacteria as well as fungal strains. Calcein assay, multiplex analysis and flow cytometric analysis were performed using HT29 and A498 cell lines for anticancer activity. The data on bioassay approaches were validated *via* modern approaches using molecular and bioinformatics.

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Screening of bioactive molecules from cyanobacteria: a traditional as well as modern approach

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Increasing drug resistance in pathogenic bacteria along with fungi is causing concern to drug developers. Health hazard is increasing with cancer patients. Genome sequencing of cyanobacterial NRPS and PKS genes revealed the potentiality of cyanobacteria to provide array of lead molecules for synthetic biochemists for drug development. Cyanobacteria are little explored in this regard. The sequenced genomes of many cyanobacteria opened the new vistas of such investigations. Generally, active biomolecules are secondary metabolites associated with polyketide synthase (PKS) and non ribosomal peptide synthetase (NRPS) and hybrid thereof. We have

PCR amplified these genes from locally isolated genera of cyanobacteria (*Phormidium* CCC727, *Geitlerinema* CCC728, *Arthrospira* CCC729, *Leptolyngbya* CCC732, *Phormidium* CCC730, *Phormidium* CCC731) and amplified PKS as well as hybrid genes. These amplified sequence were sequenced and putative protein structures predicted using bioinformatic approach. In addition to the above, we have also bioassayed the crude extracts of target organisms against pathogenic bacteria as well as fungal strains. Calcein assay, multiplex analysis and flow cytometric analysis were performed using HT29 and A498 cell lines for anticancer activity. The data on bioassay approaches were validated *via* modern approaches using molecular and bioinformatics.

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Antiproliferative activity of novel chalcones: Insights into in silico binding interactions with multiple kinases

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Previous work done in our laboratory on design and synthesis of Epidermal Growth Factor Receptor (EGFR) inhibitors gave us 3-Phenyl-1-(pyridin-3-yl)prop-2-en-1-one chalcone as a lead after database screening. We modified the lead and synthesized some novel chalcone analogues with various different substitutions. The synthesized compounds were tested for in-vitro cytotoxic activity by sulforhodamine B assay against human cancer cell lines of different origin, viz. MCF-7 (breast), K562 (leukaemia), A549 (lung), HT29 (colon) and U373MG (glioma). Among the chalcone derivatives tested in the growth inhibitions against several human cancer cell lines, nitro and halide group containing analogues showed high antiproliferative effects. To preliminarily investigate the potential molecular target(s) and to confirm the experimental activity testing for these synthesized compounds, the compounds were docked into various kinases associated with cell cycle and cell growth viz. EGFR, Vascular endothelial growth factor receptor (VEGFR), Cyclin dependant kinases (CDKs), Aurora kinases. Docking was performed using GLIDE module of Schrodinger molecular modeling suite. The analysis of the best docked ligands permitted us to know the binding mode of compounds and confirm the role as anticancer agent. Binding energies of the drug-enzyme (receptor) interactions are important to describe how fit the drug binds to the target macromolecule. The obtained results are useful to understand the structural features required to enhance the inhibitory activities.

Non – canonical mode of RNA recognition by human TBP associated factor 2 N, a key player in amyotrophic lateral sclerosis (ALS)

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Human TATA binding protein associated factor 2 N (TAF15) is a nucleic acid binding protein belonging to the conserved FET class of proteins. It is involved in processes such as pre-mRNA splicing, mRNA transport and DNA binding. Nucleic acid binding is mediated by an RNA recognition motif (RRM) at the C- terminus. Mutations within this RRM region have been implicated as probable causes of amyotrophic lateral sclerosis (ALS). A disordered amino – terminal transactivation domain, a RanBP2 – type zinc finger and a pair of interspersed RGG repeats complete the domain architecture of TAF15. Our study provides a molecular basis of RNA recognition by the RRM – RanBP2 pair using a combination of nuclear magnetic resonance spectroscopy, calorimetry and docking studies. An investigation of the RRM solution structure in complex with its cognate RNA (*SON* hairpin) has yielded conclusive evidence of a non-canonical mode of RNA recognition. Rather than the classical stacking interactions that occur across nitrogen bases and aromatic amino acids on ribonucleoprotein sites, TAF15-RRM – RNA interaction is primarily mediated by a moderate - affinity hydrogen bonding network between nitrogen bases and a concave face on the RRM surface. We further determined that RNA binding depended upon secondary structure rather than sequence by comparing binding affinities across a set of ssRNA oligonucleotides. These findings provide a platform for subsequent investigations of nucleic acid binding by other members of the FET family to further elucidate the molecular basis of ALS progression.

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A Raman spectroscopy and DFT study of the effect of mutation in vkorc1 on anticoagulant activity

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In humans, vitamin K epoxide reductase complex, subunit 1 named as vkorc1 by HUGO gene nomenclature committee (HGNC). vkorc1 is present on short arm of homo sapiens chromosome number 16 at position 11.2

from centromere. The nucleotide length is 4,102 from 31102175 to 31106276 on negative strand (1). We have taken the bacterial homologue of this gene because its structure is solved (2). The product of this gene encodes an enzyme which reduce vitamin K 2,3-epoxide to active form which is essential for blood clotting. Warfarin (3) inhibits the vitamin K dependent synthesis of biologically active forms of the calcium dependent clotting factors II, VII, IX and X, as well as the regulatory factors protein C, protein S, and protein Z. We want to do the theoretical investigation of interaction of warfarin with VKORC1 protein. For this first we predicted the 3-D structure of VKORC1 using Ab Initio approach and then docked the warfarin with predicted VKORC1 protein structure using Auto Dock software. After taking the help of docking results, we have done the density functional quantum chemical calculation for ligand (warfarin) with amino acids present at the active sites of receptor (VKORC1) and analyzed the effect of mutation in vkorc1 on warfarin action of anticoagulation. Raman spectra were acquired in order to see the interaction of amino acids present at the active sites of receptor (VKORC1) with warfarin and the changes after mutation.

Isolation, purification and characterization of cathepsin B from the sheep kidney

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Cathepsin B is a lysosomal cysteine protease of the papain family with a molecular weight of 38-kDa. It has two lobes with its catalytic site located at the interface between the two lobes. The amino acids histidine, cysteine and aspartic acid found at the catalytic site of the enzyme, with histidine on the right lobe interacting with cysteine on the left lobe to catalyze peptide bond cleavage. Cathepsin B, exhibits both endopeptidase and exopeptidase activity in contrast to other cysteine protease of the papain-like family. Its main function is the degradation of proteins that have entered the lysosomal system from outside the cell via endocytosis or phagocytosis or from other compartments within the cell. In biological system this enzyme, have significant role in various pathological and oncogenic processes. Here, we are reporting a simple procedure for the purification of cathepsin B from sheep kidney. To isolate cathepsin B, sheep kidney was crushed and kept for 12 hours in the extraction buffer. The lysate was centrifuged, and supernatant was precipitated by ammonium sulphate. The precipitate of 60% ammonium sulphate was extensively dialyzed and loaded on Mono S and Superdex 200 chromatography columns. The purity of cathepsin B was confirmed by SDS-PAGE. Finally, the protein was identified by matrix-assisted laser desorption/ ionization

time of flight. Our purification procedure is fast and straightforward with high yield.

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Hydrogen and CO gas sensor based on bimetallic oxide composite nanomaterials

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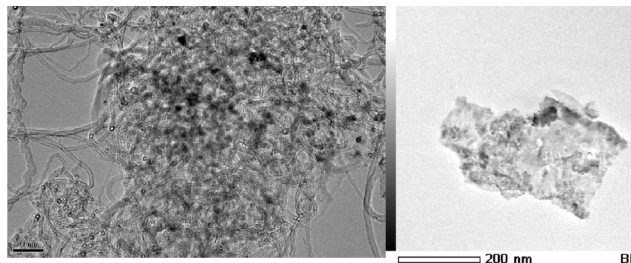
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A dwindling fossil fuel scenario has created a surge in development of hydrogen based economy. Hydrogen finds widespread application in many fields like in development of fuel cells, fuel for rockets and missile systems, as reducing agent in chemical reactions etc. Monitoring hydrogen gas concentration is important in chemical synthesis of ammonia, methanol, hydration of hydrocarbons, in desulphurization of petroleum products, in metallurgical processes and production of rocket fuels [1,2]. Therefore, demand for hydrogen sensors has arisen for safety monitoring of hydrogen gas concentration from production, storage and transportation of this gas. The utmost importance in development of hydrogen sensor is due to its high permeability across materials, high combustibility and low ignition energy which makes it important to help prevent the risk of an explosion in case of leakage at any stage. Similarly, CO being highly toxic with concentrations as low as 667 ppm might cause death [3]. Therefore, the study of materials that may be used in the fabrication of low-cost gas sensors for detection both H₂ as CO is important for avoiding accidents. Palladium is a transition metal widely studied for its high capability of hydrogen storage and in sensors for hydrogen detection [4]. However, Pd is expensive for fabricating devices based only on Pd as active material. Therefore there is a growing need to search alternative materials with potential properties both for storage and detection.

Various hydrogen detection methods use gas chromatographs, mass spectrometers or specific ionisation gas pressure sensors which cost high having high maintenance cost and slow in sensing [5]. However these instruments are huge and need to be made portable and easy to use with similar efficiency or more. Looking into this aspect nanomaterial based sensors is being developed which are transducer devices.

In this work, an attempt is made to detect H₂ and CO using a composite bimetallic oxide nanoparticles of copper and tin. Effect of calcination is also studied. In addition to bimetallic nature, CNT were also mixed with the synthesized powder for improved conduction and adsorption capacities. These materials were synthesized by hydrothermal technique and characterized by UV-

Visible and FTIR spectroscopy along with XRD and SEM analysis. These nanomaterials along with 5% glass powder were printed on alumina substrate using doctor's blade method and baked at 850p C. These materials will be studied in detecting hydrogen and CO gas molecules (10 ppb to 100 ppm) using static and dynamic test system. Figures below shows the TEM images of CNT mixed in CuO and Copper-Tin oxide composite.



Conformation, Stability and Free Energy Calculation of Parallel Modified Quadruplexes: MD Study

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Locked nucleic acid (LNA) is the term for oligonucleotides that contain one or more nucleotide building blocks in which an extra methylene bridge fixes the ribose moiety either in the C3'-endo (beta-D-LNA) or C2'-endo (alpha-L-LNA) conformation. This study explains the relative stability of five quadruplex models and helps to the understanding of interactions between the strands with the help of MM-PBSA calculations. MD simulation were performed for an unmodified structure with telomeric DNA sequence, a completely modified structure for which the experimental data is also available and three hybrid modeled quadruplex structures. LNA was taking for modification in modified structures. The MD results show that the structure of quadruplexes in solution maintain their integrity. The binding free energy calculation suggest that each quadruplex, unmodified, modified and the hybrid structure of modified and unmodified oligonucleotide follow the rule of dimeric interaction in the formation of quadruplex structure. The hybrid complex also form very stable quadruplex structure. There stability may be confirmed with the experimental result. The experimental results for the hybrid duplex structure of the LNA is known to be very stable. But the experimental results for the hybrid quadruplex structure is not available in the literature. Since such modification can form stable hybrid structure, therefore, such modified oligonucleotide may be helpful to completely inhibit the telomerase activity in cancerous cell. Such study may be helpful for the researchers in the

field of biochemistry, molecular biology, medicinal chemistry etc.

Biophysical characterization of dietary flavonoid with Serum albumin using computational approaches

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Flavonoids are polyphenolic compounds which are ever-present in plants and used as therapeutic agents in human health disparities [1]. The 3-D structures of dietary flavonoid were built using the CHEMSKETCH 2.1. AutoDock4.0 [2] was employed to gain an insight into the dietary flavonoid binding with serum albumin (SA). A 3-D atomic coordinates of SA were obtained from the Brookhaven PDB (i.e. Protein Data Bank) and further prepared for docking. All hetero atoms were deleted and non-polar hydrogens were merged. The Kollman united-atom charge model applied to SA. A special attention was given to the set of parameterization for SA. After that, partial atomic charges in SA were loaded according to the Gasteiger-Marsili method. The atomic solvation parameters were also added to SA. Grid maps used by the empirical free-energy scoring function in AutoDock were generated. A grid box of $100 \times 100 \times 100$ grid points in size with a grid-point spacing of 0.375 \AA was used for docking [3]. The map was centered such that it covered the entire protein including all probable binding sites. The rotatable bonds were assigned for the ligand (i.e. a dietary flavonoid) and partial atomic charges were calculated using the Gasteiger-Marsili method after merging non-polar hydrogens.

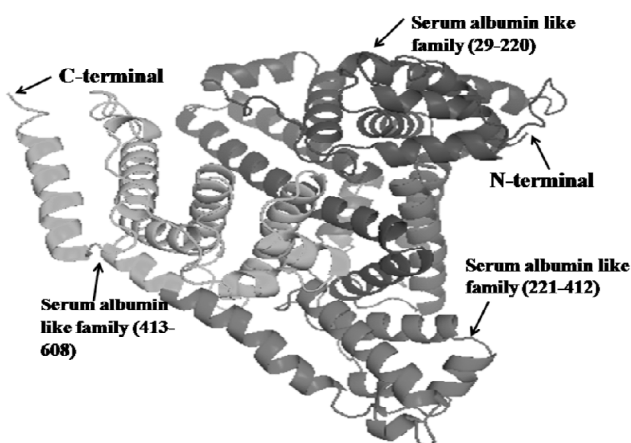


Figure 1: A cartoon diagram showing the overall model of rat serum albumin from *Rattus norvegicus* with the ordered N-terminal arm shown in blue color and C-terminal extension in green color. It shows total 27 α -helices, 2 3_{10} α -helices and rest are loop regions and produced using PyMol v0.99.

Probing hot spots at protein-RNA recognition sites

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Prediction of protein-RNA binding energy hot spots using sequence and structural attributes is still elusive. In this study, we use evolutionary conservation derived from the structural alignment of amino acid sequences along with the structural and physicochemical attributes of the protein-RNA interfaces to probe the binding hot spots at the protein surfaces that recognize RNA molecules. As expected, irrespective of the structural class of the complexes, residues at the RNA binding sites are evolutionary better conserved than those at the solvent exposed surface. However, we find some proteins in contact with RNA evolve rapidly compared to others. Complexes where the recognition involves duplex RNA, residues that interact with the major groove of the RNA are better conserved than those interact with the minor groove. We identified multi-interface residues, which participate simultaneously in protein-protein and protein-RNA interfaces in complexes where more than one polypeptide is involved in RNA recognition. These multi-interface residues are the most conserved compared to any other residue; and an interesting study would be to probe their contribution to the stability of the complex. Additionally, we find that the residues at the water preservation site are the most conserved followed by the residues at the hydrated and dehydrated sites. Finally, we developed a Random Forests model using the structural and physicochemical attributes to predict the binding hot spots. The model successfully predicts the $\Delta\Delta G$ values obtained from the alanine scanning experiments, and provide a stepping-stone towards the engineering of protein-RNA recognition sites with desired affinity.

Base pair dynamics of non-Watson & Crick U•U pair: Implications in Myotonic Dystrophy

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Abundant expansion of trinucleotide repeats is implicated in broad range of neurological diseases termed Trinucleotide Repeat Expansion Disorders (TREDs). The human Myotonic Dystrophy type 1 (DM1), a multi-systemic disorder, is associated with the occurrence of large copies of trinucleotide CUG repeats in the

dystrophia myotonica-protein kinase (DMPK) transcript. These assemble into RNA duplexes comprising the canonical Watson & Crick (WC) base pairs interspersed with non-Watson & Crick (nWC) U·U base pairs. Repeats comprising U·U base pairs sequester MBNL-1 protein and modulates its intended function of mRNA splicing leading to DM1. Although much is known about the etiology of DM1, not much is understood about the underlying structural role. We examine the mechanistic effects of U·U base pairs on RNA duplex using molecular dynamics simulations. Results indicate a dynamic behaviour of U·U base pairs with different hydrogen bonding schemes, that not only dictate base pair stability but also influence global RNA structure with implications on protein recognition and binding. U·U base pairs, during the course of simulations, are observed with two and one hydrogen bonds with varying levels of stability. The U·U hydrogen bonding schemes are not only non-isosteric with respect to WC and nWC pairs but are also non-self-isosteric. Using the non-isosteric metric, residual twist (Dt°) and radial difference ($Dr \text{ \AA}$), we have analysed the effects of different hydrogen-bonded base pairing schemes of U·U on the immediate helical neighbourhood. Simulations also indicate that CUG repeats impart a kink in the RNA duplex that may be essential for protein binding.

Molecular Dynamics Simulation of Human Telomeric Quadruplex DNA

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G-quadruplex DNA (qDNA) has emerged as a three-dimensional structure of special interest due to its functional role in biological processes like transcriptional regulation, DNA replication and genome stability. These bead-like structures adopted by human telomeric DNA are now attractive anticancer drug targets. The four-repeat human telomeric sequence, $d[AGCG(TTAGGG)]_3$, gives rise to three G-quartets stacked in the form of four-stranded helix connected by loops. The single stranded qDNA can attain four different topologies depending on the environment: parallel, anti-parallel, Form1-mixed-(3+1) and Form2-mixed-(3+1). Characterization of these topologies is necessary to understand the structure of biologically relevant telomeres and assist the drug development process. We have performed all-atom Molecular Dynamics Simulation considering two types of counter-ions (Na^+ and K^+) along with Steered Molecular Dynamics Simulation of the four NMR-reported G-quadruplex DNA topology types. We have also simulated a non-telomeric sequence and two X-ray reported structures. A total of 1.7 is MD simulation has been formed for the seven systems.

The ions (Na^+/K^+) inside the G-tetrad core form an integral part of the quadruplex. The analysis of coordinate bonds formed inside the G-tetrad core, i.e., $O6-K^+-O6$ and $O6-Na^+-O6$ angles, indicates that the G-tetrad core may have a distorted cubic or square antiprismatic geometry. The exclusion of ions (Na^+/K^+) from the G-tetrad core during the course of simulation induces non-planarity in the cyclic arrangement of Hoogsteen (G:G H:W) basepairs, supporting the fact that presence of ions in the G-tetrad core is necessary for the stability of a quadruplex. The analysis of MD simulation trajectory corresponding to non-telomeric G-rich sequence solved as quadruplex-DNA structure, gives high RMSD for the Guanine bases and high standard deviations of the basepair parameters, indicating that the structure adopted by a non-telomeric G-rich sequence may not necessarily have the same stability as that of telomeric qDNA. The SMD simulations along with equilibrium MD studies indicate that the parallel and non-telomeric forms are comparatively less stable. We could come to the conclusion that the mixed-(3+1)-form1 is the most stable topology although its structural alike mixed-(3+1)-form2 is also stable.

Molecular Mechanism of Signaling by Human APJ Receptor

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Human APJ Receptor (APJR) is 380 amino acids long cardiac G-protein coupled receptor (GPCR) activated by apelin ligand isoforms such as apelin-13, apelin-17 and apelin-36 (O'Dowd *et al.*, 1993, Tatemoto *et al.*, 1998). An insight into its ligand-binding and downstream signaling is of paramount importance as it is involved in various physiological processes like angiogenesis, cardiovascular development and homeostasis (Chen *et al.*, 2003, Zhou *et al.*, 2003) and acts as co-receptor for HIV pathogenesis (Sorli *et al.*, 2007). Like other GPCRs, APJR also exhibits signaling bias - G-protein dependent and β -arrestin dependent pathways (O'Carroll *et al.*, 2013). This signaling diversity not only establishes receptor plasticity but also promises specific novel and effective therapeutics. In the current study conserved amino acids in the extracellular loop domains (ECL) and transmembrane segments (TM) across species have been mutated and functionally analyzed by a time-dependent *in vivo* pull down assay upon activation with apelin-13 for both β -arrestin1 & 2. The mutant receptors demonstrated β -arrestin2 activation to varying extents, confirming existence of the receptor in different conformational states, aiding activation of different downstream effectors. ¹⁸³MDYS¹⁸⁶-AAAA mutation led to activation of β -arrestin2 like wild type but failed to show

G-protein mediated activity. On the contrary, V¹⁹⁹S²⁷⁵-HH in ECL3 and ²⁶⁸KTL²⁷⁰-AAA in ECL3 lacked noteworthy β -arrestin2 activation. In case of β -arrestin1, activation was not exhibited in any mutant. Our results therefore demonstrate biased signaling by APJR.

Identification and Structural Investigation of Novel Immunogenic Epitope on the Chikungunya Envelope Glycoprotein 2

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Chikungunya virus (CHIKV), a mosquito-borne pathogen that causes Chikungunya fever (CHIKF), has been spreading throughout Asia, Africa, and parts of Europe in recent times. There are no effective vaccines, drugs and diagnostics for early detection available for this virus. Envelope glycoprotein E2 is the major immunodominant structural protein for CHIKV with crucial role in virus attachment and entry and can prove to be potential diagnostic, vaccine and drug target. Based on informatics a putative Epitope of E2 glycoprotein with high antigenic score, flexibility and surface accessibility was selected for stereo dynamic investigation. Immune response to unconjugated peptide (E2.4) was evaluated in BALB/c mice and immune response titers were measured with enzyme linked immunosorbent assay (ELISA) using anti mouse IgG. Results show that the synthetic peptide (E2.4) is immunogenic. To determine uniqueness of the peptide segment identified, three-dimensional structure of E2.4 peptide Epitope (RVPKARNPTVYTGKNQVTML-NH2) was studied in SDS micelles by two-dimensional proton nuclear magnetic resonance spectroscopy and distance geometry calculations. Micelle bound E2.4 peptide shows characteristic extended or coiled coil for N-terminus of the peptide followed by type II reverse turn for residues Tyr 12- Gly 13- Lys 14-Asn 15 connecting well folded C-terminus. As a preliminary data, orientation of E2.4 peptide immunogen in MHCII peptide binding groove can be predicted based on its three dimensional conformation and hence forth correlated to its immune response. The immunogenic Epitope identified and its structure in membrane mimetic SDS micelles provides platform to initiate further preclinical studies to evaluate the immunological potential.

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Examining the mechanical strength of copper ligand bonds in Azurin using Single-Molecule Force Spectroscopy

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The mechanics of protein are important in many processes in living organism. But mechanical processes are also important for our understanding of protein activity and metal ligand bond interactions in metallo-proteins. Depending on the nature of these interactions different chemical bonds display different strength and stability. Recently Li et al showed that Ferric-Thiolate bonds in spite of being highly stable exhibits surprisingly low mechanical stability in rubredoxin. In biological systems, these Fe-Thiolate bonds are mechanically labile and fully distinguish themselves from typical covalent bonds. It is important to study other metal binding proteins to infer insights into the relation between covalency and mechanical stability of metal-ligand bonds.

In this regard, we want to get further insights into this problem by studying Azurin, which is a copper binding protein involved in electron transfer reactions. We are interested in investigating interaction of copper with various ligands such as cysteine, histidine and methionine using single molecule force spectroscopy. The nature of metal-ligand bonds is of debate whether they are of covalent or non-covalent in terms of their strength and stability, and whether the protein environment would have any effect on their bond strength.

A comparative study of stereotactic and systemic animal model of Parkinson's disease

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Dysfunction in ubiquitin proteasome system (UPS) has been implicated in Parkinson's disease as manifested by intracellular protein aggregates known as Lewy bodies which is a hallmark of PD. It is associated with degeneration of dopaminergic neurons in substantia nigra a mid brain region. In this study, PD models were established by systemic administration of rotenone (2mg/kg body weight for 5 consecutive weeks) and a single intranigral stereotactic injection of proteasome inhibitor MG-132 (0.01 mg in 2 μ l) in male Sprague Dawley rats (200-250g). Behavioral analysis along with evaluation of neurotransmitters, apoptosis, oxidative stress and

histopathological analysis were performed. Administration of rotenone results into significant loss of behavioral motor movements, dopaminergic neurons and around 50% loss of dopamine was observed when compared to control group. However MG-132 administration results into approx. 45% loss of dopamine, 28% decline in tyrosine hydroxylase and 57% loss of dopaminergic neurons was observed as compared to the control group. Collectively, these findings demonstrate that both rotenone and MG-132 plays important role in inducing the dopamine depletion and nigral dopaminergic degeneration in systemic and stereotactic animal model. However, rotenone model showed progressive alterations in the motor functions, neurotransmitter levels and oxidative stress whereas stereotactic model (surgical treated animals) showed the delayed symptoms of motor abnormalities.

The parasitic flatworm *fasciola gigantica* encodes a bifunctional thioredoxin that can protect DNA from free radical damage

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Fascioliasis is caused by two species of the genus *Fasciola*. WHO estimates that at least 2.4 million people are infected in more than 70 countries worldwide, with several million at risk, particularly, in developing countries like India. Triclabendazole, the only drug recommended by WHO against fascioliasis, is active against both immature and adult parasites. Recent reports suggest increasing resistance to triclabendazole recommending the necessity to develop new chemotherapeutic strategies. Maintaining redox homeostasis is a major adaptive problem faced by parasites and its disruption can shift the biochemical balance toward the host. Thus redox-active protein system can be a potential drug target. The thioredoxin system is a key antioxidant system in defense against oxidative stress by regulating protein dithiol/disulfide balance. We have performed biochemical experiments on *Fasciola gigantica* Thioredoxin1. The recombinant FgTrx1 exists as a monomer under native conditions and catalyzes the reduction of insulin. FgTrx1 is bifunctional and shows dual activity of both thioredoxin and glutaredoxin, indicating that it is regenerated not only by thioredoxin reductase but also with glutathione system. DNA nicking assay and hydroperoxide assay suggests that it protects the DNA from oxidative damage and thus is essential for survival under oxidative stress. We also propose that the glutathione reductase and thioredoxin reductase activity of FgTrx1 is not only restricted to -CXXC- motif as suggested recently, but may

also be regulated by other residues present in close proximity to the -CXXC- motif through manipulation the pKa of The active site cysteines.

Biochemical studies and intrinsic tryptophan fluorescence assays reveal striking differences in the active site architecture of *H.pylori* arginase compared to mammalian homolog

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Arginase is a binuclear Mn^{2+} -metalloenzyme that hydrolyzes arginine to urea and ornithine. *Helicobacter pylori* arginase (rocF) exhibits different features compared to other homologs like higher activity with Co^{2+} than Mn^{2+} , dimer instead of a trimer, optimum activity at pH 6.5, presence of a SSEHA motif instead of GGDHS and an insertion of 13 residues non-conserved motif. These suggest that the active site architecture of the *H.pylori* arginase could be altered than its mammalian counterpart. To explore this, we used known inhibitors of human arginase for the *H.pylori* enzyme. Potent inhibitors of human arginase, ABH and BEC ($K_i \sim \mu M$) did not inhibit the *H.pylori* enzyme upto 1mM concentration. Also, NOHA and nor-NOHA showed higher K_i values and different nature of inhibition with Co^{2+} - (competitive) and Mn^{2+} -enzymes (noncompetitive). This implies that the metal ions could modulate the active site architecture in a manner that the inhibitors bind differently with different metalloenzymes. Time dependent progress curves of Mn^{2+} -enzyme revealed slow tight binding inhibition with both NOHA and nor-NOHA. The pH dependent experiments with NOHA and nor-NOHA showed that pH could alter the interaction of the inhibitor at the active site of the enzyme. At pH 7.4, one step binding was observed in which reversible EI complex was formed. But at pH 6.5, two steps binding was observed where the EI complex isomerizes to an irreversible EI* complex. Our intrinsic tryptophan fluorescence data is in agreement with the two steps binding mechanism with a red shift of approximately 5nm. These results suggest that the active site architecture of the *H.pylori* enzyme vary from its mammalian counterpart.

Biochemical and spectroscopic characterization of a peroxidase from a medicinal Plant *Euphorbia tirucalli*

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A Peroxidase was purified to homogeneity from the latex of *Euphorbia tirucalli* (Pencil cactus) by cation exchange chromatography. The purified enzyme was found to be monomeric with a molecular mass around 35kDa as determined by SDS-PAGE and MALDI-TOF. Peroxidase activity of enzyme was confirmed by zymogram. The purified enzyme is a glycoprotein with 10-12% glycosylation. The enzyme is stable in the pH range of 5.5-8.5, temperature range of 40-60°C and the optimal activity was observed at pH 6.0 and temperature 45°C. The protein molecule consists of 17 tryptophan, 29 tyrosine, and 10 cysteine residues. The purified enzyme has a broad specificity towards some phenolic substrates in the order of 4-aminoantipyrine >O-phenylenediamine > pyrogallol > guaiacol. The enzyme is also very stable at very high concentration of chemical denaturants like GuHCl, Urea, and SDS and metal ions and organic solvents thus it is applicable in various industrial applications. The activity of enzyme is strongly inhibited by β -mercaptoethanol, Glutathione, L-cysteine, Hydroxylamine, Sodium azide, Sodium sulphite. The enzyme was found to be very stable at room temperature and retained more than 80% activity even after a period of 2 months and was stable for more than 6 month at 4°C without any additive or preservative. Adequate amount of latex, easy purification method, broad substrate specificity, and high stability against pH, temperature, chaotrophs and organic solvents makes this enzyme a potential candidate in biotechnological and industrial applications.

Elucidating the role of protein-lipid interactions underlying membrane curvature – a computational approach

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The protein-lipid interactions play a central role in several cellular biological processes which involve major physical changes in the lipid bilayer such as membrane curvature, bilayer thickness, density etc. Since these parameters represent dynamic supra-molecular biological events and occur at fast-timescale, it is extremely difficult to

characterize experimentally. Therefore, molecular insights driving these physical changes remain largely unknown. A cellular process of degradation known as autophagy that results in a double membrane vesicle, autophagosome involves several such membrane remodelling events, such as curvature, vesiculation and invagination. In particular, membrane curvature has been proposed to be associated with gamut of factors, including protein dynamics, lipid heterogeneity and oligomerization of scaffolding proteins. Here, we use explicit solvent atomistic simulations to determine the membrane modulating effects of one such protein, Atg17. Recently, the structure of this protein was experimentally determined, in complex with other autophagic proteins Atg29 and Atg31. The complex has been proposed to drive pre-autophagosomal structure formation, however molecular mechanism is not known. Preliminary analyses of our simulations reveal gradual increase in membrane curvature during the course of multiple trajectories. Concurrent conformational changes also occur in the protein structure while its associated with the membrane. Our work therefore suggests there exists a fine interplay between protein and lipid mediated factors driving membrane curvature.

A Synergistic Solution for Wastewater Management : A Decentralised Approach

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This paper illustrates that the use of decentralized wastewater treatment system (DWWTs) approach to wastewater treatment have had more success and there is a need to make wastewater treatment people-centric and effective through the “waste to resource” - approach. It provides a state-of-the-art-technology at affordable prices because all of the materials used for construction are locally available. This approach is an effective, efficient and affordable wastewater treatment solution for not only small and medium sized enterprises (SME) but also for the un-served (rural and urban) households in developing countries. Alternative technologies such as DWWT is making water treatment ‘fashionable’ to minimize waste going out of the habitats at micro-level. This can go a long way in changing people’s mindsets towards waste-minimization and up-gradation of the environment. Such an approach permits for flexibility in management as well as complex technologies are available. The decentralized system is not only a long-term solution for small communities but is more dependable. This paper presents the decentralized technical approaches to wastewater treatment and management. A discussion as to their applicability in developing countries, primarily in rural areas, and challenges faced is emphasized all through the paper

.Centralized management of the DWWTs is essential to terminate the havoc of untreated water.

Effect of Oxicam Non Steroidal Anti-Inflammatory drugs on membranes and their consequences on Membrane Fusion

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Non Steroidal anti-inflammatory drugs (NSAIDs) constitute the most common class of analgesic, anti-pyretic and anti-inflammatory agents. Besides these principle functions, they are known to exhibit several alternate functions including anti-cancer and neuro-protective properties. A new and alternate function of NSAIDs has been shown by our group viz. they can induce membrane fusion at physiologically relevant concentrations. This is not a general property, but is specifically shown by NSAIDs belonging to the oxicam chemical group. The ability of these drugs to induce membrane fusion in a protein-free environment has been attributed to their ability to cause adequate perturbations in the membrane structure, so as to bring down the energy barriers associated with each step of the fusion process. In our effort to understand how the different isosteric substitutions on the drugs would affect their interaction with the membranes, which in turn would affect the kinetics of the various steps of fusion, we have presented here a detailed biophysical characterization of drug-membrane interaction for the five oxicam NSAIDs viz. Meloxicam, Piroxicam, Tenoxicam, Lornoxicam and Isoxicam, using various spectroscopic techniques. Partition-coefficients of the drugs between vesicles and buffer have been calculated using 2nd derivative-absorbance spectrophotometry. Drug location has been probed using steady-state and time-resolved fluorescence quenching of membrane-probe DPH. Drug induced membrane fluidization has been monitored using steady-state fluorescence anisotropy of DPH. Membrane fusion kinetics has been probed using lipid-mixing, content-mixing and leakage assays. The differential interaction of the drugs with the membranes could be correlated to the differences in their fusogenic ability.

Urea-induced denaturation studies and MD simulation of yeast iso-1-cytochrome c and its deletants at pH 6.0 and 25 °C

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Sequence alignment of horse cytochrome *c* and Yeast iso-1-cytochrome *c* (y-cyt-*c*) shows that y-cyt-*c* has five extra residues at N-terminus. These residues are numbered as -5 to -1. Here, these extra residues are sequentially removed from y-cyt-*c* to establish their role in folding and stability of the protein. We performed urea-induced denaturation of wild type (WT) y-cyt-*c* and its deletants. Denaturation was followed by observing change in $\Delta\epsilon_{405}$ (probe for measuring change in the heme environment within the protein), $[\theta]_{405}$ (probe for measuring the change in Phe82 and Met80 axial bonding), $[\theta]_{222}$ (probe for measuring change in secondary structure), and $[\theta]_{416}$ (probe for measuring change in the heme-methionine environment). The urea-induced reversible denaturation curves were used to estimate ΔG_D^0 , the value of Gibbs free energy change (ΔG_D) in the absence of urea; C_m , the midpoint of the denaturation curve, i.e., molar urea concentration ([urea]) at which $\Delta G_D = 0$; and m , the slope ($=\partial\Delta G_D/\partial[\text{urea}]$). Our *in vitro* results clearly show that except $\Delta(-5/-4)$, all deletants are less stable than WT protein. Coincidence of normalized transition curves of all probes suggests that unfolding/refolding of WT protein and its deletants is a two-state process. To confirm our *in vitro* observations, we performed 40 ns MD simulation of WT y-cyt-*c* and its deletants. MD simulation results clearly show that extra N-terminal residues play a role in stability but not in folding of the protein.

Structural and thermodynamic characterizations of pre-molten globule state occurring on the folding/unfolding pathway of yeast iso-1-cytochrome c and its deletants at pH 6.0 and 25 °C

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Cytochromes *c* (cyts-*c*) has served as a paradigm for the study of protein stability, folding, and evolution.

Sequence alignment of the yeast iso-1-cyt-c with mammalian cyts-c suggests that the yeast has five extra N-terminal residues. To understand the role of five extra N-terminal residues in folding and stability, we prepared wild type (WT) yeast iso-1-cytochrome *c* (*y-cyt-c*) and its deletants ($\Delta(-5/-1)$, $\Delta(-5/-2)$, $\Delta(-5/-3)$, $\Delta(-5/-4)$ and $\Delta(-5/-5)$) by subsequently deleting these residues. LiCl induced denaturation was followed by observing changes in molar absorption coefficient at 405 nm ($\Delta\epsilon_{405}$), the mean residue ellipticity at 222 nm ($[\theta]_{222}$), and the difference mean residue ellipticity at 409 nm ($\Delta[\theta]_{409}$) near physiological pH and temperature (pH 6.0 and 25 °C). Biphasic transition, N (native) state \leftrightarrow X (intermediate) state \leftrightarrow D (denatured) state, observed in each case. The intermediate (X) was characterized by the far-UV, near-UV and Soret circular dichroism, ANS (8-anilino-1-naphthalenesulfonic acid) binding and dynamic light scattering measurements. These measurements led us to conclude that X state of each protein has structural characteristics of PMG (pre-molten globule) state. Thermodynamic stability of all proteins was also determined. It was observed that the N-terminal extension stabilizes the native WT protein but it has no effect on the stability of PMG state. Another state was observed for each protein, in the presence of 0.33 M Na_2SO_4 at pH 2.1, which when characterized showed all structural characteristics of MG (molten globule) state.

Studies on prevalence and dissemination of *bla*_{CTX-M} β -lactamases among microbial inhabitants of natural environment

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Bacterial resistance to handful of antibiotics available has revolutionized the field of drug therapy. β -lactam, a major class of antibiotics has become ineffective by the evolution of β -lactamases. CTX-M class A type β -lactamases employ a reactive serine (Ser70), catalytic water molecule and the activator residue Glu166 to hydrolyze the β -lactam ring. By conferring resistance to Oxyimino-cephalosporins, they pose a serious threat to human health worldwide. CTX-M-14 and -15 belonging to CTX-M group 1 are the most prevalent variants found in India. Substitutions are believed to amend their catalytic profile, where anthropogenic sources contribute significantly in recruitment of resistance genes into clinically relevant pathogens. Compared to isolates that are prevalent in clinical settings, little information is available about the prevalence and dissemination of resistant genes among isolates that are inhabitant of natural environment. In this regard, bacterial isolates from Delhi stretch of river Yamuna after screening for ESBLs production were assessed for prevalence and diversity of *bla*_{CTX-M}

gene. Among these, a novel variant of CTX-M with 98.6% nucleotide sequence homology to CTX-M-25-group member (i.e. CTX-M-78) was identified in *Kluyverageorgiana* isolate, that differs from CTX-M-25 by the substitutions M10I, C27Y, G92D, F122L and D149G. The substitution of histidine in place of glutamine at position 29 believed to have a crucial role in enzymatic catalysis. The isolate was resistant to 13 out of 17 different antibiotics tested, and able to withstand high concentrations of heavy metals particularly mercury and cadmium. The studies on various aspects may eventually help in getting a better understanding of antibiotic resistance in non-clinical environment in order to counteract the emergence and evolution of resistance.

Determining conformational dynamics of unbound- and cholesterol-bound Apolipoprotein-A1: *in silico* approach

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Cholesterol and its relevance as major modulator for cardiovascular disease is a crucial theme in current biology and serves as a key cellular constituent for most of the biological functions. Cholesterol efflux is maintained by transporting cholesterol from extra hepatic tissues to liver by the reverse cholesterol pathway. In this regard, two models have been proposed to describe the cholesterol efflux, namely Diffusion model that describes the spontaneous movement of cholesterol outside the cells in to interstitial space in the form of vesicles, emulsions of phospholipids or as a complex with albumin and phospholipid. The other model is through the highly efficient transporter such as ABCA1 and SR-EB1 on the plasma membrane, which interacts with Apolipoprotein-A1 (ApoA1) protein. However, molecular insights underlying structural correlations upon cholesterol binding are largely unknown. ApoA1 is secreted as 249-aa pro-protein and is cleaved by circulating protease to form functional 243 aa polypeptide. Here we embarked on modeling studies to obtain a relatively accurate structure of ApoA1 protein, including the crucial C-terminal. We also perform extensive molecular dynamics simulations in explicit solvent for native and cholesterol-bound protein to dissect the structural basis for cholesterol efflux mediated through ApoA1. Our preliminary findings reveal that there exists distinct structural dynamics upon cholesterol binding. Further, our trajectories also reveal multiple conformational states (or highly fluctuating) for C-terminal region. Overall, our work directly addresses initiation mechanism of reverse-cholesterol pathway and hence provides structural

framework for designing apolipoprotein modulators in disease biology.

Structural basis of β -arrestin interaction with G Protein-Coupled Receptors

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G Protein-Coupled Receptors (GPCRs) represent the largest family of cell surface receptors in the human genome and they are involved in almost every physiological and pathophysiological processes in the human body. Currently, about half of the marketed medicines target this class of receptors including several blockbuster drugs such as opiates, α - and β -blockers, antihistamines and angiotensin receptor blockers. However, the structural basis of activation and regulation of these receptors has just started to emerge and still remains in its infancy. The functions of G-protein coupled receptors (GPCRs) are primarily mediated and modulated by the heterotrimeric G proteins, the G-protein coupled receptor kinases (GRKs), and the β -arrestins. G proteins mediate activation of second messenger generating enzymes and other effectors, GRKs phosphorylate activated receptors, and β -arrestins subsequently bind phosphorylated receptors and cause receptor desensitization. However, β -arrestins activated by their interaction with phosphorylated receptors can also mediate receptor endocytosis and G protein independent signaling. Despite their central role in regulation and signaling of GPCRs, a structural understanding of β -arrestin activation and interaction with GPCRs is still lacking.

My talk will essentially focus on our efforts to understand the biophysical and structural basis of β -arrestin mediated regulation and non-canonical signaling of GPCRs. I'll present our research findings pertaining to the conformational switching in β -arrestins that underlies non-canonical GPCR signaling, activation mechanism of β -arrestin upon interaction with GPCRs as revealed by X-ray crystallography and the first snapshot of a GPCR- β -arrestin complex as visualized by Electron Microscopy. I'll also discuss how these findings not only provide novel insights into GPCR regulation and signaling but also offer unique translational opportunities.

Prolactin Inducible Protein Interacts with Semenogelin I Fragments in Human Seminal Plasma: Plausible Role In Sperm Motility

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Protein-protein interactions are very crucial as they are known to play key roles in structural and functional organization of the cells. This study presents detection of semenogelin I fragments as new interacting partners of PIP in human seminal plasma (SP) by co-immunoprecipitation followed by MALDI-TOF/MS analysis. Co-immunoprecipitated PIP interacting proteins were identified as HSA (~66kDa), ZAG (~40kDa), semenogelin I (SEG1) (~20kDa fragment), PIP (~18kDa) and SEG1 fragment (~14kDa). Thus, three major PIP interacting proteins have been identified- SEG1 fragments, ZAG and HSA; of which ZAG and HSA been also previously known. For the first time, SEG1 fragments are reported to interact with PIP in human SP or elsewhere with a possible role in reproductive physiology which might be helpful for spermatozoa to acquire their motility. Structural analysis reveals that there is a strong interaction between PIP and SEG1, as inferred by twenty intermolecular hydrogen bonds. β -sheet 5 is main interaction site of PIP with the OPIP at the same time due to common interaction regions. Semenogelins (I & II), along with fibronectin form gel-like coagulum of freshly ejaculated semen. Prostate specific antigen (PSA) dissolves the coagulum by proteolytic degradation of semenogelins which helps in the release of sperms and thus to induce their motility. It is possible that soon after degradation by PSA, low molecular weight SEG1 fragments bind to free PIP and inhibit its interaction with ZAG (an inducer of forward sperm motility) thus representing a biophysical process for sperms to acquire motility without any interruption.

Biophysical Characterization of DNA and HSA Glycated by Fructose

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The nonenzymatic reaction between reducing sugars and amino groups of long-lived macromolecules (Nucleic Acids and Proteins) results in an array of chemical modifications that may account for several physiological

complications. *In vitro* studies suggest that fructose compared to glucose is a much more potent initiator of the Maillard reaction. In the present study, DNA and HSA were modified with fructose and the structural changes were analysed or visualized by various biophysical techniques. Fructose mediated generation of DNA-AGEs and HSA-AGEs was quantified with UV, fluorescence and FTIR. The modified DNA showed hyperchromicity, enhanced emission in fluorescence intensity, change in the peak position in FTIR profile. Compared to native DNA, the electrophoretic migration of modified samples (0.8% agarose gel) indicates structural alteration/fragmentation. Similarly, modified HSA showed hyperchromicity and loss in fluorescence intensity and shift in the peak positions. Significant changes in the electrophoretic migration pattern of HSA glycosylated by fructose were also observed on 10% SDS-PAGE. This study carried out so far suggests that the modifications of DNA and HSA with fructose leads to alteration in the native structure and hence, it might play an important role in the pathogenesis of liver diseases.

Drug binding interactions of Neuraminidase proteins from Indian strains of Avian Influenza A/H5N1 viruses

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Outbreaks of Avian Influenza A/H5N1 in India since 2006 is a cause of concern as these resulted in considerable socio-economic burden with impact on the poultry farming. Stockpiling drugs is a part of preparedness for emerging outbreaks. This necessitates research in determining effectiveness of the existing drugs on emerging strains. The Neuraminidase (NA) protein of the Avian Influenza virus is the main target for antiviral drugs. Mutations in NA protein may result in resistance to these drugs. The motivation of the study is to check reactivity of antiviral drugs Oseltamivir (OSL), Zanamivir (ZMR) and Laninamivir (LAN) with the Avian Influenza A/H5N1 strains from Indian outbreaks.

While screening was carried out for many strains, data for three strains from the Indian outbreaks is presented here: Ck/India/NIV33487/06 (IND06), Ck/India/WB-NIV2654/08 (IWB08) and Ck/India/WB-NIV16915/08 (IRW08). 3D structures for the NA proteins were predicted by homology modeling and evaluated from PROCHECK analyses. Docking of the NA proteins with the said antiviral drugs were obtained using HEX. Docked NA-drug complexes were subjected to MD simulations under physiological conditions to check the stability of binding (YASARA MD protocol).

It was observed that the NA from IND06 and IWB08 bind to all three drugs. The strain IRW08 having mutations I117V, E119A, H126Y (near the drug binding pocket) does not bind to Oseltamivir and Zanamivir but binds to Laninamivir. Stable binding of NA from IRW08 to Laninamivir were also confirmed by GOLD docking. Thus, Bioinformatics study helped in identification of strains with altered drug binding ability.

Functional annotation and characterization of Rv2345, a TPM domain containing protein from *Mycobacterium tuberculosis*.

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Mycobacterium tuberculosis is the causal agent of tuberculosis, a second largest infectious disease which alone eliminates 1.5 millions of life in a year, as per a recent WHO report. The multi-drug resistant strains of *M. tuberculosis* pose a serious challenge and there is a constant need to find new drug targets in the pathogen. Genome of *M.tb* has been sequenced completely; however a significant percentage of it still remains to be annotated (1), where various genes could be identified as potential targets for new drugs. Proteins participating in cell division in *Mycobacterium* are important targets for developing new drugs. The existing literature documents FtsZ, a tubulin like molecule to initiate formation of Z-ring complex, the primary location for septum formation. ZipA and FtsA are suggested to stabilize Z-ring structure (2, 3) and thus have a crucial part to play in cell division and growth of the bacterium and are reported to be absent in the mycobacterium cell division complex. In this study, putative function is assigned to a gene from *M. tuberculosis* through functional annotation using bioinformatics tools followed by *in vitro* study. Rv2345 is a hypothetical protein and is reported to be a ZipA ortholog (2). It is a membranous protein (70kDa). On its sequence analysis, we found Rv2345 to have TPM domain at its N-terminal (cytoplasmic) region. By sequence based functional annotation, we predicted the domain to have phosphatase activity, where similarity between putative active site residues of Rv2345 with that of earlier known and characterized proteins with TPM domain was analyzed. Further, experimental validation of the proposed functional annotation of Rv2345 was carried out by cloning and purification of TPM domain of the gene, followed by *in vitro* assay for phosphatase activity of the purified TPM domain. Our results confirmed the domain

to have phosphatase activity. Purification and characterization of the whole Rv2345 and identification of its interacting partners is required to substantiate its role in cell division of *M. tuberculosis* and to study its potential as an effective drug target.

Targeting of a potent polyamine analog, 1-naphthyl acetyl spermine, to DNA and RNA polynucleotides

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An understanding of the polyamine function and metabolism is essential for anticancer and antiproliferative treatment. A recent approach has been to utilize the self-regulatory nature of polyamine metabolism by using polyamine analogs. Since the polyamine analog does not substitute for the natural polyamines in the growth accelerating function it exhibits selective anti-cancer cytotoxicity. 1-naphthyl acetyl spermine (NASPM) is a synthetic analog of the biogenic polyamine spermine. Nucleic acid binding efficacy of this analog is not yet revealed. We have been interested in developing fundamental knowledge in the area of polyamine analog-nucleic acids interaction by studying the binding of NASPM with DNA and RNA polynucleotide structures. Therefore, biophysical, calorimetric and imaging studies were conducted to understand the structural, conformational aspects, and the energetics of the interaction. The interactions were favored by positive entropy changes and total enthalpy-entropy compensation. Circular dichroism spectroscopy, thermal melting and ethidium bromide displacement assay were also used to characterize the binding. The binding affinity of NASPM with the double stranded DNA polynucleotides was of the order of 10^6 - 10^5 M⁻¹ and also revealed AT specificity where poly(dA-dT).poly(dA-dT) showed preference over poly(dA).poly(dT) followed by poly(dG-dC).poly(dG-dC) and poly(dG).poly(dC). Interaction of NASPM with three double stranded RNA polynucleotides, revealed binding affinity to be strongest for poly(I).poly(C) followed by poly(C).poly(G) and then poly(A).poly(U).

Computational analysis of macromolecular interactions in proteasome assembly

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The proteasome is an intracellular molecular machine that maintains protein homeostasis by regulating the

degradation of misfolded as well as misassembled proteins. This multi-protein complex comprises of a 20S core particle (CP) which has the proteolytic activity and two 19S regulatory particle (RPs). The core is a barrel shaped structure where two heptameric beta rings are sandwiched between two heptameric alpha rings. The exact mechanism of proteasome assembly remains elusive, although it has been reported that some chaperones assist in their formation. Since the crystal structure of entire proteasome assembly is not available, we have tried to understand the interactions between different subunits of the proteasome assembly in the core particle. In this study, we studied the interactions between the different subunits of the proteasome, in terms of their structural, geometric and physicochemical properties and then compared them with the homodimeric protein-protein interfaces. In total, there were 93 pair-wise interactions between the subunits of the 20S proteasome of *S. cerevisiae*. Since, the structure has two-fold symmetry, similar values have been obtained between the two alpha, two beta and two alpha-beta rings suggesting the involvement of similar subunits within the respective rings. The amino acids Arginine and Tyrosine are the major contributors at the interface. The implications of this study could be used to understand the assembly of the core particle of the proteasome.

Binding of the phenazinium dye janus green blue to DNA : Microcalorimetry and spectroscopic approach

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Thermal melting and isothermal titration calorimetry studies have been carried out to investigate the thermodynamics of the interaction of the phenazinium dye janus green blue (JGB) with deoxyribonucleic acid. The calorimetric data was supplemented by spectroscopic studies. Calorimetry results suggested the binding affinity of the dye to DNA to be of the order of 10^5 M⁻¹. The binding was predominantly entropy driven with small negative favourable enthalpy contribution to the standard molar Gibbs energy change. Binding affinity became weaker as the temperature was raised. The temperature dependence of the standard molar enthalpy changes yielded negative values of standard molar heat capacity changes for the complexation revealing substantial hydrophobic contribution in the DNA binding of JGB. An enthalpy-entropy compensation behavior was also seen in the system. Higher salt concentration decreased the binding of JGB to DNA. The salt dependence of the binding yielded the release of 0.69 number of cations on binding of each dye molecule. The non-polyelectrolytic

contribution was found to be the predominant force in the binding interaction. Thermal melting studies revealed that the the dye stabilized DNA helix against denaturation. The binding was also characterized by absorbance and resonance light scattering spectral measurements. The binding constants from these results were close to those obtained from the calorimetric data. The energetics aspects of the interaction of the dye JGB to DNA is supported by strong binding revealed from the spectral data.

Mutational Analysis of the Highly Conserved core Tryptophan Residues in the Fibronectin Type-II Domains of the Major Protein of Bovine Seminal Plasma, PDC-109.

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The Major protein of bovine seminal plasma PDC-109 is mixture of BSP- A1 and - A2, which have same amino acid sequence, but differ in the degree of glycosylation. PDC-109 has two Fn type II domains, each Fn II domain binds to choline phospholipids with high specificity, which plays an important role in lipid efflux at the time of capacitation, so it is important for successful fertilization. Also, it has been shown in our previous study that PDC-109 act as molecular chaperone by protecting target proteins from various denaturing condition. Multiple sequence alignment of Fn II domain of different proteins shows that a core tryptophan is highly conserved. Crystal and solution structures of PDC-109 suggested this core tryptophan is important for ligand binding. However no previous study has been done to understand significance of these conserved tryptophan residues in any Fn II domain. In the present work two important aspect of PDC-109 have been studied. (1) the role of glycosylation (2) importance of the conserved tryptophan residue, on chaperone like activity (CLA) and its lipid binding ability. The results obtained shows glycosylation increases the CLA and decreases lipid binding ability of PDC-109. From mutational analysis of PDC-109 it is clear that the conserved tryptophan is crucial for its function. Single mutant in 1Fn2 (W47A) resulted in a drastic decrease in its ligand binding ability, and single mutant in 2FN2 with W93A and W106A resulted in complete loss of its ability in ligand binding. And only mutant W47A retains its CLA, but it is decreased to half and W93A and W106A do not show CLA.

Spectroscopic, isothermal titration calorimetry and molecular docking studies on binding nature of interaction of melatonin with bovine serum albumin

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Melatonin (N-Acetyl-5-methoxytryptamine) is an endogenous molecule produced primarily in pineal gland and it is also available in many edible plants and fruits. Melatonin is reported to possess ability to scavenge ROS, stimulating the antioxidant enzymes and reduces lipid peroxidation etc. Therefore, the remedial properties of melatonin were tested in the prevention and treatment of various ailments induced by oxidative stress.

The plasma proteins-ligand binding is reported to influence significantly the pharmacokinetics of ligands and so far no study has ever been reported for protein-melatonin binding mechanism. Therefore, an attempt was made to study binding mechanism of melatonin with BSA (as standard plasma protein) using biophysical techniques under physiological conditions.

UV Vis absorption data indicated binding and associated parameters (K , n) were $2.9 \pm 0.21 \times 10^6 M^{-1}$ and $n = 1.24 \pm 0.04$ suggestive of non-covalent interaction with the single class of binding sites. CD data confirmed after binding of melatonin causes alteration in secondary structure of BSA. ITC data fitted with two site model provided binding parameters values at two sites $K_1 = (6.7 \pm 0.34) \times 10^6 M^{-1}$ and $K_2 = (3.5 \pm 0.5) \times 10^4 M^{-1}$ with respective stoichiometry values 2.7 ± 0.2 and 0.9 ± 0.01 . The enthalpy change and entropy change confirmed the role of electrostatic and hydrophobic interactions in binding. Docking data suggested melatonin binds in two domains, fragment I (residues 198-582) and fragment II (residues 307-582, P-A) and the binding is dominated by both hydrogen bonding and hydrophobic interactions.

Non-additive effects of Mutations on the Thermostability of *Bacillus subtilis* Lipase: An in-silico mutagenesis and Molecular dynamics simulation investigation

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Though many empirical rules have been proposed for predicting the role of point mutations in protein thermostability and activity, it remains one of the important and challenging problems in protein science and engineering. The fact that mutations that alter enzymatic activity may also destabilize the protein complicates matters further. Such mutations may not be useful from a protein engineering point of view unless accompanied by compensatory stabilizing mutations. We have used molecular dynamics simulations at high temperature to probe into the stabilizing effects of potentially useful point mutations by studying the variation in conformational stabilities, of a set of active thermostable mutants of *Bacillus subtilis* Lipase. In the process, we also studied mutants, having different combinations of point mutations, generated through in-silico mutagenesis. Our studies show that some combinations of mutations show their synergistic effect on stabilization or destabilization of lipase mutants and highlight the importance of non-additivity of successive point mutations in determining the conformational stabilities of the mutants.

Heme binds to malarial Glyceraldehyde-3-phosphate Dehydrogenase and inhibits its catalytic activity

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Heme metabolism in malaria parasite has been studied extensively and targeted for drug development. The intraerythrocytic malaria parasite depends heavily on hemoglobin as the main source of protein. In the acidic digestive vacuole, the parasite degrades hemoglobin, producing amino acids and free heme (iron protoporphyrin IX). Free heme is toxic to the parasite and is detoxified by forming hemozoin. However, a portion of this heme gets released in the cytosol of the parasite and how parasite counters this "free" heme is not very well understood. Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) is a glycolytic enzyme and has

been identified to bind heme *in vitro*. However, the details of heme binding to the *Plasmodium falciparum* GAPDH (PfGAPDH) have not been characterized in detail.

Therefore, I studied the interactions of heme and PfGAPDH. Malarial enzyme was cloned and overexpressed using lactose and purified in enzymatically active form. Purified enzyme was used to calculate the binding constants between heme and PfGAPDH using electronic absorption spectroscopy as well as Isothermal Titration Calorimetry (ITC). This enzyme binds heme with micromolar affinity and heme binding results in inhibition of the enzymatic activity. To the best of my knowledge, there is no other report available in the literature about the titration of heme in the presence of DMSO, a non-aqueous solvent used to prevent heme dimer formation, with any protein. Further, I used fluorescence quenching to confirm binding and monitor structural dynamics. Finally, docking studies were carried out to propose the mechanism of inhibition of the enzymatic activity of PfGAPDH by heme. Docking studies indicate that heme binds near the NAD binding pocket of PfGAPDH and possibly interferes with it resulting in the loss of activity.

Probing the binding of two alkaloids sanguinarine and chelerythrine to lysozyme: A spectroscopic, calorimetric and structural investigation

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Sanguinarine (SGR) and Chelerythrine (CHE) are quaternary benzophenanthridien alkaloids that can exist in charged iminium (SGRI/CHEI) and neutral alkanolamine (SGRA/CHEA) forms. The binding of these two forms to the protein lysozyme (Lyz) was studied by several spectroscopic and calorimetric techniques. A higher binding affinity was confirmed for the alkanolamine form of these alkaloids. Among SGR and CHE the highest binding affinity was observed for CHEA interaction. Calorimetry data revealed that SGRI/CHEI binding is endothermic and predominantly involves electrostatic and hydrophobic interactions, whereas SGRA/CHEA binding is exothermic and dominated by hydrogen-bonding interactions. These data suggested that both forms were bound near the Trp-62/63 residues of Lyz. Molecular docking results are in accordance with the spectroscopic and thermodynamic data, further validating the stronger binding of alkanolamine over iminium form. The binding site of both alkaloids is situated near the deep crevice (catalytic site) on the protein surface and is close to several crucial amino acid residues. Docking study could also predict the actual

reason for the higher interaction of CHEA to the protein wherein the classical H-bond formation with Glu-35, Trp-63, Arg-45 and nonclassical H-bond formation with Gln-57, Ala-107 and Trp-108 residues of protein stabilized the CHEA-Lyz complex more. This study advances our knowledge of the structural and thermodynamic aspects of binding between the putative anticancer alkaloid sanguinarine/chelerythrine and lysozyme.

Biophysical characterisation of a new variant of Dihydrofolate reductase

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Dihydrofolate Reductase (DHFR, EC 1.5.1.3) is an important enzyme which converts dihydrofolate into tetrahydrofolate (THF) using NADPH as electron donor, a methyl group shuttle, required for the de novo synthesis of purines, thymidylic acid & certain amino acids which are required for synthesizing DNA in rapidly growing cells. In-vivo folding studies of recombinant proteins provide the understanding on their ability to fold by itself inside the cell, which always dictates the downstream processing for the purification process. Furthermore, in-vitro protein folding studies provide information on the conformational transition during the events.

Being an important enzyme involved in the synthesis of precursors of DNA, it is useful to carry out folding and unfolding study of a new variant of DHFR, which may provide some insight into the various conformational states of the enzyme those are important for designing of selective inhibitors etc. Cloning & over-expression of Zebrafish (*Danio rerio*) DHFR has already been published. So, there are enough scopes for further biophysical characterisation of zDHFR which can give information about thermodynamic parameters and conformational stability profile of the enzyme. In the present study, we have attempted to perform unfolding and refolding studies of zDHFR using various biochemical and biophysical tools. It has been observed that the conformational states of zDHFR are different in presence of various denaturants like urea, acid, temperature etc. Similarly, the yield of spontaneously refolded protein from various denatured states also differs substantially. Since there is irreversibility in the unfolding process of zDHFR under specific denaturant conditions, chaperone assisted refolding was attempted and it has been observed that chaperonin GroEL and GroES enhanced the extent of refolding from acid denatured state. Further, we are trying to carry out experiments on chaperone assisted folding of urea denatured zDHFR and also to understand the mechanism of chaperone assisted folding of the protein.

Structural and functional studies on an archaeal tyrosine decarboxylase (MfnA) from *Methanocaldococcus jannaschii*

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Group II, fold type I, PLP dependent amino acid decarboxylases (AADC) is a family of enzymes that are functionally distinct but structurally conserved, with homologs present across the tripartite kingdoms. They catalyze the decarboxylation of diverse amino acids resulting in the formation of biogenic amines involved in regulating important functions such as neurotransmission and hence are targets of various pathological conditions. Despite low sequence identity amongst these ubiquitous proteins, they share extensive similarities in their subunit structure and kinetic properties. While the structure and function of the bacterial and eukaryotic AADCs have been extensively characterised, little is known about their archaeal orthologs. In this project, we characterize the structure and function of a tyrosine decarboxylase (MfnA) belonging to the above mentioned group, from *Methanocaldococcus jannaschii*, an archaeal thermophilic methanogen. The spectral properties of recombinant MfnA (rMfnA) purified from *E. coli*, were deduced using UV-visible, fluorescence and CD spectroscopy. The kinetic parameters at optimum temperature and pH for tyrosine substrate were determined. rMfnA was crystallised in 2 different conformations with PLP. In one conformation where the cofactor is not bound to the protein, the catalytic loop of the protein remains ordered while in a PLP bound, internal aldimine form, that loop gets disordered. Interestingly, this catalytic loop is disordered in crystals of all the other active amino acid decarboxylases. We expect comparative analysis of the properties of MfnA with the bacterial and eukaryotic orthologs to assist in deducing the structural basis of discrimination between different amino acid substrates in a highly conserved active site pocket.

Selectively and potent inhibitors of human carbonic anhydrase IX: A potential biomarker for hypoxia induced tumours

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Carbonic Anhydrase IX (CAIX) is membrane-associated member of the α -CA enzyme family and over-expressed

in many tumors such as colon, cervical, breast, renal carcinomas and brain tumors and show very restricted expression in normal tissues. Therefore, CAIX is an attractive target for anticancer therapy. Various CAIX inhibitors (sulfonamides/sulfaumates and coumarins) are reported as promising anti-cancer agents, showed appreciable affinity and selectivity. Novel chemical scaffolds with improved pharmacological properties are essential for the development of safe and potent CAIX inhibitors. We have generated CAIX containing catalytic domain only and sub-cloned CAIX in pET21c, respectively. CAIX was expressed in *E. coli* BL21(DE3) as soluble protein and are subsequently purified using Ni²⁺-NTA affinity chromatography. Further CAIX was purified by gel filtration chromatography. The truncated CA IX is 1,131 bp long and encodes a 377-amino acid polypeptide with the molecular mass of 41kDa. The purity of protein was checked by SDS-PAGE in which single band of 41 kDa was visualized, and it was further confirmed by Western blot. The peptide sequence of purified protein was confirmed by MALDI-TOF-MS. Novel phenyl-piperazine compounds are designed and synthesised. Molecular docking result show that compounds: CBMT-01 CBMT-05 and CBMT-17 have potential binding affinity with CAIX. Isothermal titration calorimetry (ITC) and fluorescence spectroscopy based binding study carried to identify the inhibition constant (K_i) and dissociation constant (K_d), respectively of selected compounds.

Dielectric Relaxation Process in Human Blood

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The AC relaxation in human blood is very important to understand dielectric behavior. The dielectric behaviors of human blood have been studied by measuring dielectric constant, dielectric loss and impedance. We measure the dielectric parameter of blood by using a commercial capacitance bridge (LCR HiTESTER meter, Hioki 3532-50). The dielectric spectra were recorded in the frequency range from 42 Hz to 5 MHz. We have recorded the dielectric properties of "A" positive healthy human fresh blood. The temperature dependent properties are observed in present study. We did not observe any evidence for a low-frequency relaxation (α -relaxation) caused by counterion diffusion effects as reported for some types of biological matter. The analysis of a strong Maxwell-Wagner relaxation is due to polarization of the blood cell in the 1-5 MHz region (β -relaxation) allows for the test of model predictions and the determination of various intrinsic blood cell properties. The reorientation motion of water molecules in the blood plasma leads to another relaxation feature (β -relaxation). The temperature

dependent β - and γ -relaxation process is responsible for dielectric properties of human blood.

Hemin interactions with erythroid spectrin and phospholipid bilayers

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Free heme exerts toxic effects like lipid peroxidation, DNA damage and protein aggregation. In case of severe hemolysis, occurring during pathological states like sickle cell disease, ischemia reperfusion and malaria, levels of free heme increase inside erythrocyte. In this study our aim was to investigate whether spectrin, being the major erythroid cytoskeleton protein has any role as acceptor of free heme. We have compared the interactions of three heme derivatives, hemin chloride, hematoporphyrin and protoporphyrin-IX, with dimeric and tetrameric spectrin. The values of the binding dissociation constants (K_d) for binding to spectrin dimer and tetramer were found to be 0.57 μ M and 1.16 μ M respectively. The thermodynamic parameters associated with such binding revealed the binding to be favored by positive change in entropy. Experimental results indicated a binding stoichiometry of 1 heme attached to both dimeric and the tetrameric spectrin indicating the common self associating domain to be the potential binding site. We've also noticed heme-induced structural changes in the membrane skeletal protein. In addition to these we have also studied partitioning of heme derivatives into model membranes and investigated their fusogenic properties in phospholipid bilayer.

Molecular docking of laccases from pathogenic gut bacteria suggests efficient transformation of non-steroidal anti-inflammatory drugs (NSAIDs)

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Laccases (EC1.10.3.2) are multicopper oxidases (MCO), recently reported from different serotypes of *Yersinia enterocolitica*. Laccase plays important role in survival of

pathogenic basidiomycetes yeast, *Cryptococcus neoformans*, in macrophages as well as cerebrospinal fluid, with roles in increased prostaglandin production and decreased IFN- γ levels. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit prostaglandins synthesis and increase IFN- γ . Clinically, NSAIDs are used in the treatment of pain, inflammation and diseases like arthritis. In the present work, the transformation of NSAIDs by laccase from gut pathogenic bacteria was studied *in silico* and validated *in vivo*. Genomic DNA from fifteen strains of seven different serotypes of *Y. enterocolitica* was isolated and PCR was done with primers designed for MCO (*Yak*). Thereafter, the amplicons were cloned in a TA cloning vector (pTZ57R/T) for sequencing. The phylogenetic study of all the cloned *yak* genes showed serotype specific clades. The internal short branches showed discrete clades of pathogenic strains (*Y. enterocolitica* 8081 and *Y. enterocolitica* W22703), emerging pathogenic strains (*Y. enterocolitica* 33, *Y. enterocolitica* 53 and *Y. enterocolitica* E15) and non-pathogenic strains of *Y. enterocolitica*. Laccases from *Escherichia coli* and *Shigella dysenteriae* shared a clade with *Salmonella enterica*. The docking of laccase specific substrate, ABTS, gave maximum GoldScore of 72.64 with *Y. enterocolitica* strain 43. Molecular docking with NSAIDs, indomethacin, gave maximum GoldScore of 55.81 with *Y. enterocolitica* strain 8081. The GoldScore of diclofenac (53.62) and etodolac (52.30) was highest with *S. enterica*. The docking results validated with molsoft ICM Software, were further simulated by Gromacs 4.6.

Effect of Gluten free Diet on Metabolic Profile of Blood Plasma in Celiac Disease Studied using NMR spectroscopy

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Celiac disease (CeD) is an autoimmune enteropathy caused by ingestion of gluten in genetically predisposed individuals. The diagnosis of CeD is challenging and only treatment is exclusion of gluten from diet. Present study investigated the metabolic profile of blood plasma of treatment naïve CeD patients (n=50), CeD patients after six months of gluten free diet (GFD) (n=11) and healthy controls (n=35) using ¹H NMR spectroscopy at 700 MHz to determine the biomarker for diagnosis and follow up of CeD patients after GFD. An informed consent was taken and the Institute Ethics Committee approved the study. The concentration of various metabolites such as leucine, valine, isoleucine, choline, phosphocholine, glycerophosphocholine, creatine, phosphocreatine and

creatinine was significantly lower in blood plasma of treatment naïve CeD patients compared to healthy controls. While a significant increase in the concentration of leucine, valine, isoleucine creatine, phosphocreatine and creatinine was observed following GFD for 6 months compared to the baseline. Partial least squares-discriminant analysis showed distinct clusters of CeD patients and healthy control. Further, separate clusters were observed for the samples examined from CeD patients at baseline and after GFD. Lower level of metabolites in the blood plasma of treatment naïve CeD may be attributed to the malabsorption of metabolites due to villous abnormality as seen on histopathological evaluation. The increase in the concentration of these metabolites after GFD is indicative of mucosal recovery. The present study demonstrates the potential role of metabonomics in identification of biomarker for the diagnosis and for follow up of CeD.

Studies on electrical hysteresis in buffalo horns

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The aim of this paper is to investigate the electrical behaviour of buffalo horn samples when voltages are applied. We measured the current (NADC) as a function of voltage in the interval of the 10 volts to 300 volts, both in increasing and decreasing order. We found that the characteristic Voltage-Current curve shows a hysteresis loop and voltage measurements should be considered perturbative with respect to the electrical response of the samples. The energy stored in fresh and oven dried buffalo horns calculated by electrical hysteresis loops (V-I characteristics) is in the range 3.93 ± 2.1 to $8.9 \pm 3.7 \mu\text{J cm}^{-3}\text{cycle}^{-1}$ and 1.341 ± 0.14 to $3.412 \pm 0.91 \mu\text{J cm}^{-3}\text{cycle}^{-1}$. Significant variation was observed. This variation may be attributed to water content and chemical composition of horn. This paper constitutes a step towards the application of electrical hysteresis for *in vivo* horn diagnosis and to develop new low cost horn disease diagnosis techniques.

Key Words: Electrical hysteresis, buffalo horns, voltage and current.

Structural studies of cyclophilins and its interactome network analysis

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Cyclophilins (Cyps) are highly conserved, ubiquitous in nature and catalyze the interconversion of peptidyl prolyl imide bonds in peptide and protein substrates. In eukaryotic cells, Cyps are found in all cellular compartments with a variety of functions being ascribed to them including cell division, transcriptional regulation, protein trafficking, cell signaling, pre-mRNA splicing, molecular chaperone mechanism and stress tolerance. We studied structure and dynamics of a RNA binding protein named as Cyclophilin (PiCypA) from a plant-root-colonizing Basidiomycetes fungus *Piriformospora indica*, discovered in the Indian Thar desert. The fungus provides strong growth-promoting activity during its symbiosis with a broad spectrum of plants. Crystal structure of PiCypA was solved at 1.97 Å resolutions by molecular replacement method. Crystal arrangement has shown three molecules per asymmetric unit arranged in a side-by-side orientation. PiCypA is a monomeric single domain protein and forms a canonical cyclophilin fold comprising two α -helices and eight β -strands. To investigate specificity of RNA binding, PiCypA was titrated against different RNA constructs and we obtained quantitative thermodynamic parameters of binding. RNA molecule was titrated against U -¹⁵N labeled PiCypA at pH 6.5 and shifts in the backbone ¹⁵N and ¹HN resonances in 2D [¹⁵N, ¹H] HSQC spectra were monitored. A gradual shift in the resonances was observed for all the residues that showed changes indicating a fast exchange ($\tau_{ex} < \mu s$) between free and RNA bound PiCypA. For further comparative study regarding interactome specificity and dynamics we have selected another cyclophilin named as CPR3 from *Saccharomyces cerevisiae*. It shows very well folded conformation as we reported through HSQC and other 3D spectra. The crystals were observed in PEG4000 buffer condition and diffracted at 1.87 Å. It was reported that CPR3 interacts with various putative proteins such as Ubiquitin, Glycogene debranching enzyme and DNA/RNA helicase. Surface Plasmon Resonance analysis showed CPR3 interaction with ubiquitin. Further work regarding detailed structural analysis and dynamics are going on for elucidation of mechanism of enzymatic property and interactome signaling cascade. In conclusion, we are

studying detailed structural insights into different cyclophilins.

Role of α -helical structure in amyloid aggregation of β_2m_{59-71}

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Human β_2 -microglobulin (β_2m) is a 99-residue serum protein that constitutes the light chain of MHC class I molecules. The protein gets deposited as amyloid fibrils in the osteoarticular sites in the patients undergoing hemodialysis. The peptide corresponding to residues 59-71 (DWSFYLLYYTEFT) of intact β_2m is a highly hydrophobic stretch and has been reported to form fibrils in isolation(1). Presence of long stretches of α -helix/ α -strand discordance in proteins is one of the properties associated with the amyloid fibril formation. Helix stabilization may facilitate as well as inhibit fibril formation depending on its strength(2). β_2m_{59-71} has shown to form α -helical conformation when it is dissolved in hexafluoroisopropanol (HFIP). When the peptide dissolved in HFIP is diluted into aqueous buffer, it rapidly self-assembles into amyloid fibrils(3).

β_2m_{59-71} and its four analogs were chemically synthesized using Fmoc chemistry. Analogs were designed so as to introduce the cation- π interaction in the α -helical conformation of the peptide. Two such analogs are β_2m_{59-71} (L64R) and β_2m_{59-71} (Y66R). The control peptides having citrulline instead of arginine were also synthesized. The peptide stocks were prepared in HFIP and diluted to different concentrations in deionized water. FTIR and CD spectra were recorded to check the secondary structural elements and ThT fluorescence to ascertain the amyloid formation. All the analogs caused lesser enhancement in ThT fluorescence compared to the wild-type peptide. CD spectra of all of the peptides indicate predominantly α -helical conformation in 100%, 50%, and 20% HFIP. When dried on the ZnSe crystal from 50% and 20% HFIP, all the peptides except β_2m_{59-71} (Y66R) resulted in β -conformation. β_2m_{59-71} (Y66R) retained substantial α -helical conformation suggesting that cation- π interaction could stabilize the initial α -helical conformation of the peptide. This implies that weak interactions such as cation- π interactions could also be looked at for designing the amyloid-inhibiting molecules.

Dimeric L-asparaginase of hyperthermophile *Pyrococcus furiosus* shows sequential folding of domains with concomitant association of subunits

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Most of the protein folding studies are performed on monomeric small globular proteins. Folding studies of multidomain multimeric proteins is often complicated not only because of domain-domain and domain-linker interactions, but also by interaction between subunits. Here we present studies on the factors contributing to the folding and stability of a dimeric, 2-domain protein L-asparaginase (PfA) from *Pyrococcus furiosus*. PfA is a homodimer in which each monomer consists of two distinct N- and C-terminal domains (NPfA and CPfA respectively). Each domain contains one tryptophan. By studying single Trp mutants, we studied the domains folding with respect to full protein. Domains cloned, expressed and studied in isolation, provided the folding behaviour of each domain in isolation. Equilibrium denaturation experiment on WT PfA, its isolated domains and Trp mutants, indicated that the NPfA is thermodynamically more stable, unfolds at higher guanidine concentration compared to CPfA. Fluorescence quenching experiment along with size exclusion chromatography showed sequential folding of domains, where NPfA folds first extending complementary hydrophobic surface which provides assistance for CPfA to fold. The folding of NPfA also resulted in the association of subunits. Overall, subunit folding and association were found to be highly cooperative in PfA. While each domains exhibit highly cooperative folding behaviour in full protein, isolated domains displayed poor cooperativity in their folding. *In silico* analysis revealed the presence of strong network of hydrophobic interactions between the domains, rendering stability to the dimeric structure against chemical denaturation. To our knowledge, this is the first study showing domainwise folding behaviour of a multidomain multimeric protein together (in Trp mutants) and in isolation (isolated domains).

Determination of Mass Attenuation Coefficient of Wood Biomaterials

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In the present work, mass attenuation coefficient of ten types of wood materials were measured and compared

with XCOM theoretical values. The effective atomic number and effective electron density of the wood compounds were obtained using the measured values of their mass attenuation coefficients in the photon energy range 511 to 1332 keV, analysis of the data measured by NaI(Tl) scintillation detector. The study of effective atomic numbers of biologically important compounds is very useful for many technological applications. The mass attenuation coefficient, effective atomic number and effective electron density are the basic quantities required for determining the penetration of photons in matter. In the present study on the basis of the results, Mass Attenuation Coefficient decreases with increasing gamma energy. Mass Attenuation Coefficient increases with increasing density, decreases with increasing thickness of the wood materials.

Experimental and computational studies of thermostable chitinases isolated from *Thermomyces lanuginosus*

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Chitin, the second most abundant natural biopolymer, is composed of repeating units of N-acetyl-β-D-glucosamine and primarily forms the structural component of protective biological matrices such as fungal cell walls and exoskeletons of insects. Chitinases are a ubiquitous class of extracellular enzymes that have gained attention in the past few years due to their wide range of biotechnological applications, especially in the field of agriculture for bio-control of fungal phytopathogens. They play an important role in the defense of organisms against chitin-containing parasites by hydrolyzing the α-1,4-linkages in chitin and hence act as anti-fungal as well as anti-biofouling agents. In recent years, thermostable enzymes isolated from thermophilic microorganisms have gained widespread attention in industrial, medical, environmental and biotechnological applications due to their inherent stability at high temperatures and a wide range of pH optima. The main focus of the present study was to gain a better understanding of the structural features of chitinases obtained from this thermostable fungus *Thermomyces lanuginosus* using both experimental and computational techniques and their relationship with their activity profiles. The genes encoding thermostable chitinase I and chitinase II from *T. lanuginosus* were isolated and cloned in both *E. coli* as well as the *Pichia pastoris* expression system. Analysis of the nucleotide sequences revealed that the chitinase I gene (1538 bp) encodes a 400 amino acid protein of molecular weight

41.14 kDa whereas the chitinase II gene (1196 bp) encodes a 343 amino acid protein of molecular weight 36.65 kDa. *In silico* protein modeling was helpful in predicting the 3D models of the novel chitinases enzyme, followed by the prediction of its active sites. The presence of Glu176 was found to be essential for the activity of chitinase II. Similarly, analysis of chitinase I revealed several active sites in its structural framework. A 10 ns Molecular dynamics (MD) simulations were implemented to assess the conformational preferences of chitinases. The MD trajectories at different temperatures clearly revealed that the stability of the enzymes were maintained at higher temperatures. A constant pH molecular dynamics simulations at a wide range of pH establish the optimum activity and stability profiles of chitinase I and chitinase II at pH 5.0 (323 K) and pH 4.0 (313 K), respectively. The results suggest a strong conformational pH dependence of chitinases. These enzymes retained their characteristic TIM Barrel fold at the respective protonated conditions, thus validated the experimental outcomes.

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Aggregation of Microtubule Affinity-Regulating Kinase 4 (MARK4) can be prevented at extreme of pH

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MAP/Microtubule affinity-regulating kinase 4 (MARK4) belongs to the adenosine monophosphate-activated protein kinases (AMPK)/sucrose non-fermenting 1 (Snf1) subfamily of the Ca²⁺ calmodulin-dependent protein kinases (CaMK) group of kinases in the human kinome which phosphorylates the microtubule associated proteins (MAP), causing their detachment from the microtubules, and thus increasing microtubule dynamics. MARK4 is comprised of 752 amino acids (82,520 Da), which divided into three distinct domains, protein Kinase domain (59-314), ubiquitin associated domain (322-369), and kinase association domain (649-752). The residues 65-73 are considered for ATP-binding domain. The Lys88 is considered as ATP-binding site. MARK4 plays central roles in the cellular signaling pathways and its abnormal phosphorylation to various substrates is inseparably linked with many human diseases including cancer, diabetes and neurodegenerative disorders. We have successfully cloned, expressed and purified the kinase domain of MARK4 protein. The purity of protein was checked on SDS-PAGE and identified by using mass spectrometry and immunoblotting. Refolding of the recombinant protein was validated by ATPase assay. To detect and to characterize the conformational transitions of MARK4 as a function of pH, we took MARK4 protein

in different pH conditions at 25 °C and found out that at the extreme of pH (below pH 3.5 and above pH 9) MARK4 is fully stable. Along with it, we noticed that at intermediate pH values (between pH 3.5 and 9) MARK4 showed aggregation.

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Conformation of Feglymycin: A Unique Antibacterial and Antiviral Peptide

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Feglymycin is an amphoteric 13 member linear peptide, synthesized by the bacterium *Streptomyces sp.* exhibits remarkable antiviral and antibacterial activities.

It contains unusual (non-proteinogenic) amino acids 4-hydroxyphenylglycine and 3,5-dihydroxyphenylglycines. Except the first hydroxyphenylglycine all other three hydroxyphenylglycines are in S form and all the dihydroxyphenylglycines are in the R form. Thus the residues 2-11 are in alternate R and S form and neither the N terminal nor the C terminal is protected. Therefore, we have carried out conformational study of this molecule, feglymycin to address the following; (i) What is the role of alternate R and S form ii) Why the first two residues are in R form, (iii) Likewise the last three residues are in S form (iv) What is the role of free N and C terminal

To address these points, the conformations of the following peptides containing phenylglycine-PG, parahydroxyphenylglycine-HPG and 3, 5-dihydroxyphenylglycine-DPG in both R and S form, has been carried out by quantum mechanical approach; Ac-X-NHMe with X= PG, HPG, DPG.

Ac-S/R-HPG-R-DPG-NHMe, Ac-S-Val-R-DPG-NHMe, R-HPG-RDPG-S-Val-RDPG-S-HPG-NHMe, Ac-S-HPG-R-DPG-S-HPG-R-DPG-NHMe, Ac-S-HPG-R-DPG-S-Val-R-DPG-S-HPG-S-Phe-S-Asp.

Quantum mechanical-QM calculations clearly show that the first two residues must be in the R form. The QM also predicts that the more stable states of these peptides are stabilized by the non covalent interactions like *carbonyl – carbonyl interactions, carbonyl-lp – π (aromatic ring) interactions, OH/NH – π interactions, CH – π , and Stacking interactions* depending upon the length of the peptide.

Most of these interactions disappear or becomes weaker in simulation studies in water due to interaction of water molecules with the carbonyl groups of backbone and with the aromatic ring through O_w-H- π or O_w-lp- δ interactions. Interestingly, the interaction of water molecule with the aromatic rings through O_wH- π and O_wlp- π leads to non-planarity of the aromatic rings and this is the first study of its nature. These results have been supported by interaction of water with benzene. The Φ ,

Ψ values after 24ns simulation in water under NVT conditions for full length feglymycin are found to lie in the second and fourth quadrant of the Ramachandran map for the residues 2-11. It may be mentioned that the Φ , Ψ values for the last three values, which are in the S form they corresponds to the beta strand region. The driving force to attain this structure is provided by the interaction of the water molecules with carbonyl groups of the backbone. Mutation of phenylalanine by phenylglycine at position 12 altogether changes the structure of the molecule. Esterification of the carboxylic group at the C terminal decreases of the charge and leads elongation of the molecule. Results are in conformity with the experimental observation that Ala scan of this peptide show the important role of L-Asp at position 13 for its anti HIV and inhibition of MurA and MurC activity.

The Conformational Dynamics and Binding Energy Profile of NS5B Polymerase of HCV with 2 Monocyclic Dihydro-Pyridinone Inhibitor : MD Simulations and Free Energy Study

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Hepatitis C Virus (HCV) is a small (~55 nm), spherical, enveloped, hepatropic RNA virus that causes chronic hepatitis in humans. Hepatitis C Virus belongs to the family of Flaviviridae, which also includes viruses like Dengue Virus, West Nile Virus and Yellow Fever. The structural and nonstructural parts of HCV virion are separated by a short membrane peptide p7. The nonstructural proteins NS2 to NS5B are involved in polyprotein processing and viral replication. NS5B is a 65KDa protein that acts as RNA dependent RNA polymerase, and has a remarkable role in the fusion of the new RNA genome. NS5B is being targeted to develop novel antiviral drugs against HCV. To gain deeper insight and to know the molecular mechanism we employed M.D. Simulation at nano second scale. Studies show that, the structure of NS5B polymerase can be viewed as a right hand, where the palm domain contains the active site. The present work contains RMS Deviations which tells about the conformational changes in protein NS5B in presence of 2 MONOCYCLIC DIHYDRO-PYRIDINONE inhibitor. The Binding energy was calculated by free energy methods using Molecular Mechanics Poisson Boltzmann surface area (MM-PB/SA) method. Hydrogen Bond formation was also observed for several residues. MD Simulation and QM/MM methods were used to understand the behavior and binding of ligand with the receptor. We also inspected the role of salt bridge in the complex which effects the stability of protein.

Multifaceted Personality of an Enigmatic Enzyme: Aldose Reductase as a case study

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Aldose reductase (AR) is an NADPH-dependent reductase, which acts on a variety of hydrophilic as well as hydrophobic aldehydes. It catalyzes the first step in the polyol pathway, in which glucose is transformed into sorbitol by AR and then to fructose by an NAD⁺-dependent dehydrogenase. An excessive flux of glucose through the polyol pathway (as can occur in hyperglycaemic state) with the subsequent accumulation of sorbitol, was initially proposed as the basic event in the aetiology of secondary diabetic complications. This led effort for decades to target the enzyme for a specific and strong inhibition. However, surprisingly the ability of AR to reduce toxic alkenals and alkanals, which are products of oxidative stress, presented a new dimension of enzyme, and raised a serious issue if AR shall be classified as a detoxifying enzyme. Recently it has been established that AR acts as a key mediator of certain oxidative and inflammatory signaling pathways that are involved in the development of different human pathologies, such as cardiovascular disorders, sepsis, and cancer. This new information raises serious question to develop strong and specific inhibitors for the enzyme. We propose that new class of inhibitors need to be designed or discovered which may selectively inhibit reduction of either hydrophilic or hydrophobic substrates.

Illuminating Chaperonin-Assisted Protein Folding Demonstrated at Single-Molecule Level

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Many newly synthesized unfolded polypeptides require assistance by molecular chaperones in order to reach their active folded states. The GroEL/GroES chaperonin system is one of the important bacterial chaperonin system which fulfills an essential function in assisting the folding of cytosolic proteins. GroEL is a homo-tetradecameric double-ring assembly of 60 kDa subunits with two opposing central cavities stacked back to back. Upon binding of ATP and the co chaperone GroES, GroEL transiently encapsulates newly synthesized or misfolded substrate proteins, which then can fold to the native state while being shielded from the crowded cytosolic environment. It remains controversial whether GroEL

acts as a passive cage, merely providing an aggregation-preventing microcompartment, or whether GroEL actively accelerates the refolding of a subset of substrate proteins by modulation of the folding energy landscape.

Variety of single molecule fluorescence techniques has been used to address this controversy. A novel and sensitive dual color fluorescence cross-correlation spectroscopy assay shows the absence of any transient aggregates at single molecular concentration. Using a single molecule FRET based assay, we show that the acceleration of substrate protein folding by GroEL is preserved under single molecule conditions, where aggregation is excluded. We applied photoinduced electron transfer with fluorescence correlation spectroscopy to demonstrate that the acceleration of substrate protein folding is achieved by restricting the polypeptide chain dynamics of the encapsulated protein inside the GroEL cavity. Furthermore, we provide evidence that modulation of the folding energy landscape is a result not only of steric confinement during encapsulation, but also of the net negative charge of the GroEL cage wall. Taken together, our findings suggest that GroEL, by an active chaperonin mechanism, promotes substrate protein folding by entropic destabilization of folding intermediates.

Alteration in salt concentration leads in to fibrillar aggregate formation by Mycobacterium Isocitrate Lyase

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Several diseases are linked with protein aggregation but aggregation is also an intrinsic property of polypeptides as the formation of catalytic globular proteins comes at the expense of an inherent propensity of proteins to aggregate. Several globular proteins can access aggregation-prone states from native like conformations by crossing the energy barrier for unfolding. Isocitrate lyase is one of the crucial enzyme of mycobacterium tuberculosis which is involved in catalysing glyoxylate shunt and provides help in persistent infection of mycobacterium under stressed condition. At physiological pH and salt deprived condition, the enzyme undergoes partial unfolding and further self-assembles into a fibrillar structure in vitro. Interestingly, the mature fibrillar structure of MtbICL is so dynamic in nature that upon treatment with the small amount of salt concentration it undergo structural changes and retain its original conformation of tetramer, that's why it remain active, as activity requires at least 5 mM of $MgCl_2$. The formation of fibrillar like structure has been observed for

the first time among the Isocitrate lyases. The interaction of thioflavinT (ThT) with MtbICL has been investigated by CD and fluorescence spectroscopy. The K_{sv} for MtbICL native and MtbICL in fibril form was found to be $2.22 \pm 0.03 \times 10^4 M^{-1}$ and $2.18 \pm 0.02 \times 10^4 M^{-1}$ respectively. The data described here will help to increase our understanding about the interaction of ThT with native proteins. This results also indicate that care must be taken while using ThT as a probe for detecting amyloid fibrils.

Structural and functional characterization of a DnaB helicase from *Pseudomonas aeruginosa*

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Helicases are motor proteins capable of unwinding DNA or RNA duplex substrates and are essential in the cellular processes of DNA replication and repair. DnaB family of helicases is the major replicative helicases in most Eubacteria. They function as hexamers, unwinding DNA in the 5' to 3' direction, by Mg-dependent hydrolysis of nucleotide tri-phosphates (NTPs). DNA unwinding is a dynamic process with major conformational changes, involving entire subunits.

The domain architecture of DnaB consists of an N-terminal regulatory domain (NTD) separated by a flexible linker or "hinge" region from the catalytic C-terminal domain (CTD). The NTD is involved in protein-protein interactions and affects helicase activity by influencing the conformation of DnaB. The conserved CTD consists of a core α - β RecA-like fold and comprises sites for binding NTP and DNA.

In this study, we focus on characterisation of a "putative" DnaB from *Pseudomonas aeruginosa* which will help understand the structure-function relationship of DnaB helicases. Far-UV Circular Dichroism (CD) and thermal melting, intrinsic Tryptophan fluorescence and ATP hydrolysis assays were performed to characterise the protein. Oligomeric state of PaDnaB was determined to be hexamer and the NTD as dimer in solution. The crystal structure of PaNTD was solved to a resolution of 2.4 Å. The dimer formed by crystal contacts is identical to the dimer in the functional hexamer of homologous DnaB helicases.

Pharmacophore Modeling for VEGFR-2 Inhibitors

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Vascular endothelial growth factor (VEGF) is a key stimulant of angiogenesis, which is the process of generating new capillary blood vessels. Inhibition of the Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) kinase is known to result in blockage of angiogenesis. The induction of angiogenesis, or the "angiogenic switch," is a critical step in tumor progression, and inhibitors of VEGFR-2 have been demonstrated both to induce tumor regression and reduce metastatic potential. The ATP site of kinases such as VEGFR-2 displays remarkable conformational flexibility when accommodating chemically diverse small molecule inhibitors. The so-called activation segment, whose conformation controls catalytic activity and access to the substrate binding pocket, can undergo a large conformational change with the active state assuming a 'DFG-in' and an inactive state assuming a 'DFG-out' conformation. The inhibitors are thus classified into Type-I (DFG-in state) and Type-II (DFG-out state) inhibitors. Pharmacophore models were developed for VEGFR-2 inhibitors using the PHASE module of Schrodinger Suite. The Common Pharmacophore Hypotheses (CPH) generated were validated by 3D-QSAR model development. For Type-I inhibitors, the CPH AADRR was selected as it had an $R^2=0.98$ and $Q^2=0.92$. AADRR ($R^2=0.92$, $Q^2=0.41$), ADRRR ($R^2=0.92$, $Q^2=0.30$), AADHR ($R^2=0.92$, $Q^2=0.42$), ADHRR ($R^2=0.93$, $Q^2=0.42$), ADDRR ($R^2=0.93$, $Q^2=0.47$) and ADDHR ($R^2=0.93$, $Q^2=0.44$) were the best CPH generated for Type-II inhibitors.

Rational drug design and development of neuraminidase inhibitors against H1N1 using molecular docking approach

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Structure based drug design such as Molecular docking involves the construction of ligands that fit into the active site of a receptor, forming favorable interactions with residues in the active site. The highly pathogenic influenza virus has caused an increasing pandemic threat. Neuraminidase inhibitors such as oseltamivir and zanamivir have gained remarkable success but the

concern for drug resistance still remains a question. The highly conserved active site of NA and its role in influenza virus replication has made it an interesting target for the development of newer NA inhibitors. Recently, in depth analysis of the crystal structure of the enzyme newly proposed spots within the 150 and 430-loop regions in N1 makes it distinguishable among the subtypes. Thus efforts should be directed to be accessible towards 150-loop open conformation, as it is energetically favorable. But once it binds tightly to this binding pocket, conformational change should be such that it can attain similar structure as that of the closed conformation. This complexity in the design of newer NA must be considered as it is evident from the findings of 2009 pandemic structure that it does not have 150-cavity. In this view we have developed series of compounds with scaffolds like chalcones, flavones and quinolones which are mainly present in naturally occurring molecules that are known for their various pharmacological activity. We have used molecular docking approach to explore the active site of H1N1-NA in both standard (contains 150-loop) and pandemic (lacks 150-loop) virus which are showing interesting and promising results.

NMR structural characterization of a putative UV inducible protein (UVI31+) from *Chlamydomonas reinhardtii* that exhibits DNA endonuclease activity

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Chlamydomonas reinhardtii is a single celled alga, undergoes apoptosis in response to UV-irradiation. UVI31+ in *C. reinhardtii* exhibits DNA endonuclease activity, induced upon UV-stress. UVI31+ that normally localizes to cell-wall and pyrenoid regions gets redistributed into punctate foci within the whole chloroplast, away from the pyrenoid, upon UV-stress. Here we present NMR characterization of the structure, dynamics and the putative function of UVI31+. Calculated structure of UVI31+ has $\alpha_1\beta_1\beta_2\alpha_2\alpha_3$ fold very similar to BOLA family of proteins. Moreover, BOLA domain is similar to the well-described K-Homology class-II (KH) domain that contains RNA and DNA binding motif. KH domains bind RNA or ssDNA, and are found in proteins associated with transcriptional and translational regulation, along with other cellular processes. Three α -helices of UVI31+ form one side of the protein interface while other side is formed by three β -strands and are glued by a strong hydrophobic core, providing a compact 3D protein structure. Twenty-three residues long polypeptide stretch connecting β_1 and β_2

strands is found to be highly flexible. Further, UVI31+ is found to recognize DNA primarily by its β -sheet domain with a dissociation constant of 52 nM. Mutation at β -sheet as S114A reduced endonuclease activity drastically. S114A mutation showed DNA binding 10 fold weaker than wild type-UVI31+. It also possesses a weak β -lactamase activity. The specific motif along with the long flexible loop holds the key to its multiple functions.

Dynamical characteristics of FFL network motif in Biological Networks

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Networks are the representation of interaction of molecules in various aspects such as protein-protein interaction, metabolic network, transcriptional regulatory network, neural network etc. Networks exist in microscopic to macroscopic level. There are various local and global patterns occurring in networks. Network motif is a characteristic that networks have small connected sub networks whose occur significantly higher frequencies than the expected in random network. By comprehensively studying their dynamics, we show that some network motifs are fundamentally more versatile—capable of executing a variety of tasks—than others.

In this study we have found Network Motifs or subgraph of node and there different properties and analysed them on the basis of their original frequencies and Z-Scores individually. This is a local characteristic which will impact globally are found in the various transcription regulatory network. Contrary, this characteristic is found absence in neural network. After analysing we got a vital characteristics feed forward loop (FFL) in all transcriptional regulatory networks with high significance. On the bases of this result, we are seeing dynamics behaviour and how they are depend on the other in loop. Dynamical behaviour of whole network is highly depends on these network motifs.

Extraction and phytochemical screening of *Curculigo Orchioides* Gaertn. Root tubers

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Curculigo orchioides Gaertn. (Family: Hypoxidaceae), commonly known as Kali-Musli, is found to be growing

in tropical and subtropical Asia. Rootstocks are highly valued as a tonic, bitter, restorative, aphrodisiac and sustainable solutions to drug discovery and development in modern society. Many uses of these plants in the traditional medicines have been validated by pharmacological investigation. The fresh rhizomes were purchased from the market and were thoroughly washed with distilled water, air-dried at room temperature under shade. It was then pulverized into uniform powder manually. The sample was then sieved (40 mesh sizes), weighed, bottled, sealed, labeled and kept for analysis. The pulverized rhizomes were extracted with Soxhlet apparatus and extracted with methanol and then fractionated sequentially with hexane, di ethyl ether, chloroform, ethylacetate, methanol and water.

Resulting solution was then concentrated under reduced pressure 35°C using a rotary evaporator and evaporated to dryness under vacuum. The yield of the extract was calculated. The phytochemical screening revealed the presence of carbohydrate, glycosides, saponins, alkaloids, phenolics and tannins.

Three dimensional homology modeling of lanosterol 14- α demethylase of *Candida albicans* and docking studies with new triazole derivatives

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The lanosterol 14 α -demethylase (CYP51) is a Cytochrome P450 enzyme and plays an important role in the biosynthesis of fungal ergosterol, an integral part of fungal plasma membrane. It converts lanosterol into 4,4'-dimethyl cholesta-8,14,24-triene-3-beta-ol which finally converts into ergosterol. CYP51 serves as target protein for most potent antifungal class of azoles. Although the advance in bioinformatics tools may help to develop new bioactive molecules, the absence of crystal structure of CYP51 from *Candida albicans* is a major obstacle in the drug design effort. In this study, three dimensional structure of lanosterol 14 α -demethylase of *Candida albicans* was modeled and used for docking with eighteen newly synthesized triazole derivatives. The model was generated by multiple threading alignment and iterative structure assembly simulation by I-TASSER. The server generated five models with different C-score. Model 1 (C-score=1.30) was selected as best model which was formed on the template of 4LXJA structural analogue. The predicted model was further validated by Ramachandran plot using PROCHECK and MolProbity servers. It showed 89.2% residues in most favoured region and 7.1% residues in additional allowed regions. The docking studies of triazole derivatives with model were done using AutoDock Vina 4.2 software. All the compounds showed appreciating binding energy in the range of -7.4

to -9.8 kcal/mol. The study suggest that the predicted 3D model of lanosterol 14 α -demethylase can used in drug designing process in the search of more potent antifungal compounds.

Synthesis of a novel non-heparin anticoagulant agent with potent *in vitro* and *in vivo* anticoagulant activity

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The counteracting coagulation and fibrinolytic systems act in coordination to maintain the physiological hemostatic balance. Aberrant clot formation inside a blood vessel due to an imbalance of procoagulant and anticoagulant factors may lead to serious pathological conditions presented clinically as life threatening thrombosis and thromboembolism. Currently, anticoagulant therapy is the first line of treatment for these conditions with heparin being most widely used. Heparin exerts its anticoagulant activity through endogenous coagulation inhibitor, antithrombin. However, heparin therapy is associated with various drawbacks due to its polyanionic nature, thus giving rise for the development of safer agents. Here we report the synthesis of a novel saccharide based compound; trehalose octasulfate and the evaluation of its *in vitro* anticoagulant and *in vivo* antithrombotic potential. *In silico* docking of trehalose and trehalose octasulfate with antithrombin showed a differential interaction pattern, where trehalose octasulfate interacts with critical residues of AT that are either directly involved in heparin binding or in the conformational rearrangement of AT on heparin binding. The activity of trehalose octasulfate showed prolongation of clotting time by influencing the extrinsic as well as intrinsic coagulation pathways *in vitro*. Further, when intravenously infused in occlusion induced thrombotic rats trehalose octasulfate exhibited significant reduction in the size and weight of the clot at a low dose. Also coagulation was delayed *in vivo* as was observed by analysing clotting time in the blood plasma isolated from rats preinfused with trehalose octasulfate. In addition, antiplatelet effect of trehalose octasulfate was also observed by the decrease in Adenosine 5'-Diphosphate induced platelet aggregation in its presence, thus indicating its dual; anticoagulant and antiplatelet mechanism of action. Overall, the study reports trehalose octasulfate as a novel, dual acting antithrombotic agent.

Structure and mode of interaction of PfSUMO with its E2 enzyme PfUbc9

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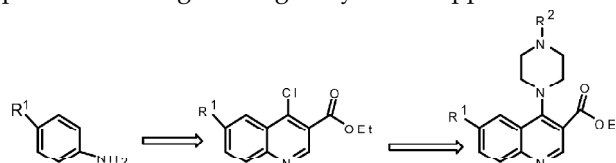
Post-translation modification of proteins is a regulatory mechanism in cell signaling cascade. Sumoylation is an important post-translational modification that contributes to subcellular localization, activity and stability of the target protein. SUMOs are about 100 amino acid residue proteins, where covalent modification (sumoylation) is mediated by a cascade of enzymes known as activating enzyme (E1), conjugating enzyme (E2) and ligating enzyme (E3). Sumoylation is an important regulator of oxidative stress response during the intra-erythrocyte developmental cycle, and disease progression. Since the structure and interacting residues of PfSUMO and PfUbc9 are unknown, we tried to elucidate the structure of PfSUMO and non-covalent mode of interaction between PfUbc9 and PfSUMO using NMR spectroscopy. For structure determination of PfSUMO, we recorded a series of 2D and 3D experiments such as HSQC, CBCANH, CBCA(CO)NH, HNCO, HNCOCA, HCCCONH, TOCSY-HSQC, NOESY-HSQC and different MUSIC experiments. We have assigned all backbone residues and their side chain resonances of PfSUMO and identified the active residues involved in interaction with PfUbc9. Structure elucidation of PfSUMO and its interaction mode with PfUbc9 and changes in related recognition dynamics upon complex formation is under progress.

Design and Synthesis of Quinoline-Piperazine as antineurodegenerative agents

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Quinoline and Piperazine are important constituents of many important drugs. In our study we have designed a series of antineurodegenerative agents having quinoline-piperazine hybrids as key scaffold. Here we have used docking and molecular Dynamics techniques to design these inhibitors. Syntheses of these molecules have performed using convergent synthetic approach.



These hybrids have β -Amyloid disaggregation property.

High Yield Purification and Backbone Dynamics of p53 DNA Binding Domain

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p53 is a 393 residue transcription factor consisting of an N-terminal transactivation domain, a DNA binding domain (p53DBD) and a C-terminal tetramerization domain.⁽¹⁾ While it is a major tumour suppressor protein, mutations, mostly in p53DBD, are believed to inactivate tumour suppression (loss-of-function) or confer new tumour propagation (gain-of-function) capabilities (**Figure 1A**). Consequently, p53 is involved in ca. 50% of all cancers. Given the plasticity of p53 protein, conformational flexibility is believed to play an important role in its function although its detailed mechanism is still unknown.⁽¹⁾ To probe the conformational dynamics of p53DBD, the wild type gene was cloned, expressed in *E. coli* BL21 (DE3) Rosetta and grown in (¹⁵N/¹³C) enriched minimal media. To avoid its accumulation in inclusion bodies and subsequent refolding, the expression conditions were optimized which lead to higher expression of soluble protein. Furthermore, to ensure high recovery of pure protein, the protocol was optimized to enable purification in a single step ion exchange chromatography. The buffer (50 mM phosphate pH=7.1, 150 mM KCl, 5 mM DTT) for NMR studies were fine tuned to avoid sample precipitation and ensure stability.⁽¹⁾

The backbone amide resonances were assigned using triple resonance experiments (HNCA and HN(CA)CB) followed by measurement of ¹⁵N spin relaxation rates (R_1 , R_2) and heteronuclear ¹H-¹⁵N NOE. The relaxation rates were used to obtain quantitative order parameters (S^2), which identified three regions as dynamic hotspots in p53DBD (**Figure 1B**).

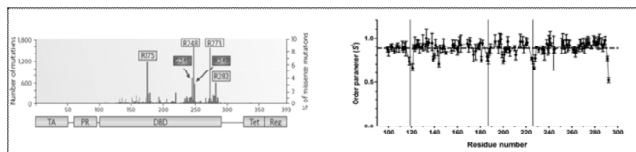


Figure 1: (A). The missense mutations distributed along the p53 sequence. The most common hot-spot mutations are highlighted (2). (B) Plots of backbone amide order parameter (S^2) of wild type p53DBD at pH=7.1. Red dashed lines in the plots show dynamic regions of p53DBD.

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Development of Random Positioning Machine to study microgravity effects on biological systems

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With a great success in previous space missions, India is now in a position to send biological samples in space. Microgravity, observed in spacecraft, is the condition where gravity value is below earth's gravity (1 g). It can be simulated on earth using a laboratory instrument, called Random Positioning Machine (RPM). We have successfully developed this instrument in our laboratory. Essentially, Random positioning machine comprises of two-axis rotating frame, rotating independently of each other by two separate motors with speed ranging from 1-10 rpm. The object under study is placed at the centre of the inner frame of RPM. The instrument randomly changes direction as well as speed of rotating object relative to earth's gravity vector, so that effective magnitude of gravity-vector is averaged to zero over time. The level of simulation within RPM depends on the speed of rotation and the distance of the sample from the centre of rotation. The level of gravity achieved using this instrument varies between 10^{-1} (hypo) g to 10^{-5} g (micro) as per the calibration curve. The whole instrumental set-up is controlled through the software. The experiments using RPM can be performed under controlled environmental conditions of temperature, humidity, CO₂ and light. RPM can be used to study the effects of microgravity condition on many biological samples such as bacteria, blood cells, cell cultures, insects, plants etc. Preliminary experiments on plants are being carried out and obtained results will be discussed.

Modelling cell electroporation II : Effects of variation in membrane cholesterol content on small molecule delivery

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Electroporation is a physical process involving enhanced permeability of biological cell membrane due to application of high voltage electric pulses of very short duration [1, 2]. The enhanced permeability results from formation of micro-pores in the cell membrane. The technique has found wide range of applications in biomedical research including cancer therapy and vaccine delivery [3, 4]. Though considerable efforts have been

made to understand the physical process, differences exist between experimental observations and theoretical models due to various approximations such as uniform composition of lipid bilayer, uniform poration, etc.

Present work is a theoretical formulation and numerical implementation of small molecule uptake during electroporation of a mammalian cell with cholesterol containing membrane. The anticancer drug Doxorubicin (DOX) is considered as the small molecule. Diffusion [6] of DOX through micro-pores on the cell membrane is evaluated in PS cells. Cell geometry for the cell lines were determined experimentally and parameters used as inputs in computer simulations. Numerical solutions (simulations) of the equations carried out in MATLAB. Effect of variation in membrane cholesterol content (15-29% mole-fraction) on drug diffusion vs time was evaluated. It was observed that with the increase in membrane cholesterol content, the pore dimension decreases subsequently affecting DOX uptake by cells. This model can be a useful for prediction of electroporation mediated drug delivery in mammalian cells.

Studies on Interactions of HIV-1 Vif Protein and its Interacting Partners

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HIV-1 virion infectivity factor (Vif) is an accessory protein that is essential for viral replication of HIV. In the absence of Vif, a host cytidine deaminase, APOBEC3G, is incorporated into virions and delivered to target cells where it mutates viral cDNA. Vif recruits a cullin 5-based ubiquitin ligase that targets APOBEC3G for proteosomal degradation. The goal of this research is to determine the mechanisms by which HIV accessory protein Vif recruit their cellular binding partners, pinpoint the molecular interactions that stabilize the virus-host protein complexes, and develop molecules that inhibit complex formation. Vif, ElonginB, ElonginC, Cul5 and APOBEC3G sequences were downloaded from the NCBI Genpept sequence database and were subjected to the Secondary Structural analyses, Comparative Modelling and Simulation. For the study of protein-protein interaction rigid body docking calculations were used using a background Fast Fourier transform (FFT) approach. Here for each molecule encoding of both surface shape and electrostatic charge and potential distributions was modelled using 3D expansions of real orthogonal spherical polar basis functions; which enables the representation of each property by a coefficient vector. The DIMPLOT program was used to plot the interactions

across the protein-protein interface. The interactions plotted were hydrogen bonds and non-bonded contacts. They were extracted from the usual HBPLUS output by the program DIMER; and the plot was generated by LIGPLOT. *In silico* mutagenesis was performed using SPDB viewer and separate models were generated for each interacting residue.

Isolation, Identification and Characterization of novel bioactive compound(s) from *Callistemon viminalis*: In vitro evaluation as potential anti-cancer agents

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To find the novel antitumor compounds with low side effects could be interesting field for the propose of research. The isolation of compounds from plant extracts can possibly be explored for the development of new anticancer drugs. Secondary metabolites like polyphenols, terpenes and alkaloids are extensively distributed in plants and these are reported as free radical scavengers, anti-mutagenic and anticancer activities. To evaluate the biological activity of *Callistemon viminalis* plant we have designed to check the cytotoxic potential of the different solvent extracts and their compounds isolation from different extracts of plant. *Callistemon viminalis* is belongs to Myrtaceae family and is an ornamental plant distributed throughout India. In this study, different solvent extracts (Hexane, Chloroform, Ethylacetate, Acetone, Methanol, and water) of leaves of *C. viminalis* was evaluated for its phytochemical composition by *invitro* methods. First the extracts was screened for the presence of carbohydrates, phenolic compounds, saponins, alkaloids, flavonoids, phytosterols, tannins, and glycosides and will check for their anti cancer activity on Hela, HepG2, and MCF-7 cancer cell-line by using MTT assay. The cell line will treat from concentrations range 0.1-100 µg/ml of plant extract and observe for the affect on the growth of cancer cell line. From these will select the best extracts and compounds contents isolate by RP-HPLC. All the isolated compounds will be screened for their effect on the growth of different cancer cell line. Since natural products occur widely in living organisms and many possess a broad range of activities. Keeping the importance of natural products in mind and vastness of this field believed that this research work would be helpful to find new natural products with better anti cancer activity to overcome the side effects of presently using antitumor drugs and this would be leading steps towards the development of new anti cancer drugs.

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An experimental study of passive diffusion of silver nanoparticles and amino acids through model biological membrane

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This study reports the passive diffusion of silver nanoparticles, tryptophan, phenylalanine and tyrosine through biological membrane model. The experiments were carried out under physiological environment at pH 7.4. The chicken egg shell outer membrane model was used to study the passive diffusion. The passive diffusion was performed against and towards gravitation for 48 hrs. Fick's first law of diffusion under steady state was adopted to evaluate the diffusion rate (J), diffusion coefficient (D), change in concentration with thickness of

membrane (dc/dx) by equation 1 $J(x,t) = -\frac{Ddc(x,t)}{d(x)}$ (1)

Diffusion coefficient of solute calculated by eqn. (2),

$D = \frac{KT}{6\pi rN_0}$ (2) Where K is the Boltzmann constant ($1.38 \times$

$10^{-23} \text{m}^2 \text{kg s}^{-2} \text{K}^{-1}$), N_0 is the Avogadro Number ($6.023 \times 10^{23} \text{mol}^{-1}$), T is the temperature, r is the radius of the molecules, n is the viscosity of the solution. Viscosity was estimated using Ostwald viscometer. The permeability coefficient (P) was estimated according to the Fick's law of diffusion based on the steady state flux and the concentration of the donor compartment eqn.(3).

$$P = \frac{J}{c_{\text{donor}}} \quad (3)$$

Viscosity of the solvent was determined of each solution i.e silver nanoparticles, amino acids using Ostwald viscometer and calculated using following eqn.(4).

$$n_2 = \frac{n_1 \times \rho_1 \times t_2}{\rho_2 \times t_1} \quad (4) \quad n_1 \text{ is the viscosity, } \rho_1 \text{ is the density}$$

of the distilled water, t_1 is the time taken by the to flow between the region A and B marked on the viscometer. ρ_2 is the density of the test sample and t_2 is time taken by the test sample to flow between the region A and B marked on the viscometer.

The egg shell membrane was characterised using scanning electron microscopy. The silver nanoparticles were synthesized chemical degradation route and characterised by UV visible and dynamic light scattering. An average size of nanoparticles was obtained to be 62nm. The diffusion rate of amino acids was higher than

silver nanoparticles; however it was enhanced in combination of silver nanoparticles. Permeability and diffusion coefficient were higher in amino acids than silver nanoparticles. The possible mechanisms have been explained on the basis of molecular property

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Role of weak salts in folding and stability of cyanobacterial phycoerythrin and its truncated variant

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The crystal structure of truncated form of α -subunit of cyanobacterial phycoerythrin (α C-PE) from cyanobacteria *Phormidium tenue* offered a new approach in defining the structure-function relationship of α C-PE. To compare the folding and stability of the full length α C-PE (FL- α C-PE) and its biologically functional truncated form (Tr- α C-PE), after GdmCl and urea we further explored lithium salts (LiCl & LiClO₄)-induced denaturation. This was done by observing changes in visible absorbance at 565 nm, fluorescence at 350 and 573 nm, and circular dichroism at 222 and 560 nm. The denaturation profile of each protein was analyzed for ΔG_D^0 , the value of Gibbs free energy change on denaturation (ΔG_D) in the absence of denaturant; m , the slope ($=\partial\Delta G_D/\partial[\text{Lithium salt}]$), and C_m , the midpoint of the transition curve i.e., [Lithium salt] at which $\Delta G_D=0$. A difference of about 10% in ΔG_D^0 was observed between FL- α C-PE and Tr- α C-PE, suggests that the two proteins are almost equally stable, and natural deletion of N-terminal 31 residues does not alter its stability. Thus, 31 N-terminal residues may provide structural rigidity but no significant stability. Furthermore, it is the 32-164 residues which determine the functional structure of the α C-PE. Since both proteins are reversible to lithium salt-induced denaturation, hence the 31 amino terminal residues are not essentially required for folding. Moreover, the concurrence of the normalized denaturation profiles of different optical properties conclude that denaturation of both the C-PEs is a two-state mechanism with single transition phase, unlike its hexameric phycobilisome structure which consists of the intermediate states.

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A Comparative Study on the Effect of pH on Cyanobacterial α -phycoerythrin and its Naturally Truncated Variant

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Crystal structures of phycobiliproteins enables us to investigate their structural and functional relationships with respect to change in their environmental conditions. In this work, we have exploited the optical properties of chromophore in relationship to bilin proteins and explored the pH-induced conformational changes and relationships, and functional dynamics of cyanobacterial phycoerythrin (C-PE) and its truncated variant in comparative manner, using visible absorbance, fluorescence and circular dichroism (CD), together with analysis of their crystal structures. The α -protein of C-PE from *Phormidium tenue* consists of full length 164 residues (FL- α C-PE) while its naturally truncated form constitutes 133 residues lacking 31 residues (Tr- α C-PE) from the N-terminal side and both belong to all α -protein class. Both of the variants show strong and substantial structural and functional stability in the pH range of 4.75 - 9.25 as compared to their structural stability. Exceeding this range, results into noticeable and pronounced structural and functional changes. Both proteins also lose their characteristic red-pink color beyond this range. Refolding experiments suggest that the two forms of α C-PE are reversible in limited pH range of 3.0-10.5. Beyond this range proteins lose their refolding ability and lost their characteristic color permanently. Extreme change in pH-environments significantly changes the protein-chromophore relationship. Presence of isosbestic and isodichroic points by visible absorbance and far-UV CD spectra, respectively indicate that the two natural versions of α C-PE unfold in two state mechanism comprising native and denatured states, when undergo pH-induced denaturation.

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Structure aided drug design of newer neuraminidase inhibitors and probing their molecular mechanism in treatment of swine flu

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The highly pathogenic swine flu H1N1 virus remains a constant threat due to its high fatality. High rate of mutation in influenza virus, its virulence and resistance to currently available neuraminidase inhibitors viz. oseltamivir and zanamivir necessitates development of new drugs using rapid, robust and automated methods to test a large number of newly synthesized drugs in less time. Chalcones are an important class of naturally occurring molecules and are known for their pharmacological activities. The activity is associated with the ability of chalcones to influence membrane-dependent processes. As a part of our ongoing program of developing novel influenza virus inhibitors, we have developed chalcone derivatives by molecular docking approach to explore the active site of both standard and pandemic H1N1-NA and then synthesized the promising ones. The candidates were further evaluated using *in-vitro* cell based and enzyme based study on standard influenza virus (H1N1). We have then selected few of them for multinuclear NMR and DSC studies in order to probe the molecular mechanism of their antiviral action by using DPPC as model membrane. Results obtained explained the effect of our synthesized candidate molecules on membrane stabilization, fluidity and mobility which further strengthen molecular basis of their antiviral action. Overall results illustrate that these compounds possess remarkable antiviral activity against H1N1 virus using oseltamivir as standard. All the candidates under study showed different mode of binding and mostly all showed good antiviral activity. The most buoyant candidate can be explored further by evaluating its interaction with pandemic neuraminidase.

Diffusion-weighted imaging (DWI) as a tool to differentiate Breast lesions

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Dynamic contrast enhanced magnetic resonance imaging (DCEMRI) has evolved as an adjunct modality for diagnosis of breast cancer with a sensitivity of 89–100%. However, significant overlap between MR characteristics of benign and malignant tumors has been reported leading to a low specificity ranging from 50–90% for DCEMRI. Present study investigated the potential of diffusion weighted imaging (DWI) in the differentiation of malignant breast lesions from benign tumors. 86 women with histopathologically confirmed breast lesions including infiltrating ductal carcinoma (IDC) (n=40) and four categories of benign lesions comprising of 9 intermediate (phyllodes), 22 fibroadenoma, 10 cysts and 6 benign ductal epithelial cells were investigated using DWI at 1.5 T MR scanner. Written informed consent was obtained from each subject and study approved by Institutional ethical committee. Apparent diffusion coefficient (ADC) was calculated by drawing small circular ROIs of 5 pixel on ADC map. Significantly decreased ADC of malignant ($1.01 \pm 0.14 \times 10^{-3} \text{ mm}^2/\text{s}$) compared to the benign cases (fibroadenoma: $1.50 \pm 0.19 \times 10^{-3} \text{ mm}^2/\text{s}$; benign ductal epithelial cells: $1.41 \pm 0.19 \times 10^{-3} \text{ mm}^2/\text{s}$; phyllodes: $1.51 \pm 0.21 \times 10^{-3} \text{ mm}^2/\text{s}$; cysts: $1.52 \pm 0.34 \times 10^{-3} \text{ mm}^2/\text{s}$) was observed showing increased cellularity during malignancy. While, ADC of different benign lesions did not show any significant change. Therefore, present study suggests the potential ability of non-invasive techniques like DWI in increasing specificity of breast tumor diagnosis as it displays distinct tumor features which may reduce false-positive findings and consequentially may reduce unnecessary biopsies.

Folding study of a novel plant peroxidase from *Artocarpus lakoocha* (Biochemical, biophysical and crystallographic aspects)

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A highly glycosylated heme-peroxidase is isolated from the latex of a medicinally important plant *Artocarpus lakoocha*. Purification using anion exchange chromatography isolated a peroxidase of 53 kDa molecular mass with 10.8U/mg specific activity,

extinction coefficient ($\epsilon_{280}^{1\%}$) of 16.3 and an isoelectric point of 7.4 pH. It consists of 17 tryptophan, 14 tyrosine, and 8 cysteine residues per molecule. Spectroscopic studies by using K2D2 software reveal that the enzyme has α/β type secondary structure. From various techniques like, Activity measurements, far UV CD, fluorescence spectroscopy total of six different folding states of peroxidase are predictable: (a) The native POD (N) at neutral pH (b) The pre-MG state at pH 2.0 (c) The molten globule state (MG) at pH 2.0 with 1 M GuHCl (d) The acid-unfolded state (UA) at pH 8.0-4.0 (e) The alkaline denatured state (UB state) at pH 8.5-12.0 (f) The complete denatured state (D) onward 5M GuHCl. For better understanding of our studies on structure-function relationship of this peroxidase, X-ray diffraction studies were done. Crystals were prepared which results in anisotropic diffraction about 6-7Å. With two space groups orthorhombic P121 & P2221 space groups, and unit cells parameters $a=101.71$, $b=57.62$, $c=116.9$ & $a=59.14$, $b=102.69$, $c=122.56$ Å respectively was observed. In conclusion, this peroxidase retain its structural and functional integrity over a wide range of pH (3.0-10.0), temperature (65°C), GuHCl (5M) and urea (8M), and catalyzes oxidative polymerization of active free radicals, which suggest its potential to be used in inflammation prevention, wound healing, waste water bioremediation and other applications.

Serine racemase inhibitor: Mass spectrometric identification of ligand

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D-Serine is present at high levels in the mammalian brain. It is an endogenous ligand of the “glycine site” of N-methyl D-aspartate (NMDA) receptor that plays key roles in excitatory synaptic transmission, plasticity and learning and memory. Overactivation of the NMDA receptor and the resultant influx of calcium into cells is a major culprit in the cell death that occurs following stroke and neurodegenerative diseases. Blockers of the “glycine site” of the receptor have demonstrated neuroprotection in animal models of stroke and AD.

Serine racemase (SR) reversibly isomerizes L-serine to D-serine. It is expressed in glial cells and neurons and constitutes the sole endogenous source for D-serine in mammals. It has been shown that the blockers of the D-serine binding site of the NMDA receptor are neuroprotective in animal models of stroke. Other studies suggest that D-serine and NMDA receptor dysfunction plays a role in the pathophysiology of schizophrenia and may play a role in the pathophysiology of Alzheimer’s disease. The involvement of D-serine in this breadth of

pathophysiological processes makes serine racemase an excellent drug target.

It is imperative to look for specific SR inhibitors. We have Purified SR inhibitor from methanolic extract of *Withania somnifera* by affinity method. Ultra high accurate mass spectrometer was used to determine the molecular identity of the ligand. In “in vitro” set up we have successfully shown that these newly identified small molecule inhibit the recombinant human serine racemase. We will present our recent data on isolation and identification of new SR inhibitors.

Biochemical, biophysical and cellular characterization of algal rhodopsins and their optogenetic applications.

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All organisms including animals, plants and microbes interact with light using photoreceptors. These photoreceptors captures light as a signal and further process it for different purposes like vision, photoperiodism, photomovement and photomorphogenesis. These photoreceptors are mainly classified into six families: Phytochromes, Cryptochromes, Phototropins, BLUF (Blue Light using FAD), Rhodopsins and UVR8. Rhodopsin belongs to G-protein coupled receptor family consisting retinal as chromophore. It switches between all-*trans* and *cis* forms in the retinal binding pocket of the opsin. Rhodopsins can be classified into two types on the basis of presence of a form in ground state: microbial type rhodopsins (MTR, all *trans*) and animal type rhodopsins (ATR, 11 *cis*). Microbial type rhodopsin has been reported in archaeobacteria, bacteria and in green algae (*Chlamydomonas reinhardtii*, *Volvox*, *Chlorella* etc). Optogenetics is a method that uses light to modulate living tissues or signaling events in a targeted manner. Two of the eight rhodopsins, identified in *Chlamydomonas reinhardtii*, (named as chlamyopsins) are light-gated ion channels known as channelrhodopsins. These are widely used as optogenetic tools as they have ability to depolarize membrane non-invasively upon illumination. We are studying different chlamyopsins from *Chlamydomonas reinhardtii*. Chlamyopsin 3 and chlamyopsins 4 responds in blue light region whereas chlamyopsin 5 has both UV and blue light absorbing phases. Chlamyopsin 5 consists of modular domains like histidine kinase, response regulator and adenylyl/guanylyl cyclase that together form a signal transducing complex. These modules can be used in engineering photoreceptors. Their light intensity dependent and wavelength specific expression has been studied. So, here is an attempt to characterize these photoreceptors and

identify their interaction with other photoreceptors in nature.

Significance of Phosphorylation on R120G α B Crystallin, a disease causing mutant in Desmin related myopathy

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Desmin-related Myopathy (DRM) is a condition that results from certain mutations in the desmin gene or because of defects in chaperones that bind to desmin in order to prevent its aggregation. Earlier, a familial form of DRM was reported to be associated with a mutant form of α B-crystallin (R120G α B-crystallin). The disease causing mutant is known to be hyperphosphorylated *in vivo*. We have studied the effect of phosphorylation on R120G α B-crystallin using its phosphorylation mimic (R120G-3D) to study the changes in its structure and stability. The structural change, oligomeric status and the aggregation tendency of phosphorylated R120G were studied using circular dichroism, fluorescence, turbidometry, dynamic light scattering and analytical ultracentrifugation. Our results show that the phosphorylated mimic of R120G α B-crystallin has significantly altered secondary and quaternary structure. Further investigation revealed that R120G-3D has a high propensity to undergo aggregation at physiologically relevant temperatures (37-42°C) suggesting that the self aggregation of the mutant upon phosphorylation and loss of chaperone activity are involved in the molecular mechanism of pathology in desmin-related myopathy.

Aggregation analysis of Concanavalin A binding proteins of human seminal plasma

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Concanavalin A (Con A) binding are known to have decapacitating activity; thus, are very critical for fertility related processes. In present study, we have isolated Con A binding proteins by lectin affinity chromatography and analysed their aggregation behavior by dynamic light scattering (DLS). These proteins form high molecular weight aggregates at near physiological pH (7.0). DLS analysis was also performed at different pH values and

in presence of various additives including NaCl, EDTA, cholesterol and sugars, such as d-glucose, d-fructose and d-mannose. The degree of aggregation was observed reduced in presence of d-fructose, EDTA and at lower and higher pH values as depicted by lowering of hydrodynamic radii. Here, we hypothesize that this aggregation behaviour of Con A binding proteins might be decisive for fertility related events with a suggestive role towards inhibition of premature capacitation.

Structural and functional study of Mycobacterial GTPase Obg

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GTPase -Obg have many roles in cellular processes like cell cycle control, ribosome biogenesis, protein translation, DNA replication and sporulation. In present study we have cloned, over expressed and purified mycobacterial GTPase Obg. CD spectroscopy of GTPase-Obg was carried out at various concentrations of GTP. Results inferred a significant change in secondary structure in presence of GTP. Differential GTPase Obg mRNA expression under different stress conditions was done. Further, an in-silico study was performed to find the binding sites of GTP on GTPase Obg using molecular docking study. Autodock 4 software was utilized to predict the GTP binding sites in the target protein. Our result suggest that GTP potentially interact with ASP (212) and LEU (262) residues of target protein by hydrogen bonds.

EKDB: An *E. coli* K antigen Database

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Multidrug resistant Gram-negative bacterial strains such as *Salmonella*, *Pseudomonas*, *Escherichia*, *Klebsiella* etc of Enterobacteriaceae family cause worldwide mortality every year. One of the major virulence determinants of these bacteria is surface antigens like capsular polysaccharides (CPS), exopolysaccharides (EPS) & lipopolysaccharides (LPS). A detailed understanding of the structure and function of these surface antigens is essential to develop drugs against these bacterial infections. Due to the structural diversity of surface antigens, the existing databases provide only limited

information. Nonetheless, an organized repository of the structures, functions and biochemical information about the various Gram-negative surface antigens is highly essential. Thus, in the present study, we focus on developing a database of *E. coli* capsular polysaccharides (K antigens), as the capsular polysaccharides of *E. coli* have been well characterized. The database is named as *E. coli* K antigen Database (EKDB) and provides information about the structure of *E. coli* capsular antigens as well as the proteins involved in its biosynthesis. The database will also enable the generation of polymeric CPS structures of varying lengths that can be used for modeling studies. Thus, EKDB will provide comprehensive information about the K antigens of *E. coli* and be a valuable resource for researchers all around the globe.

Identification of an allosteric binding site for the inhibition of the type II human Ribonuclease H (hRNase HII)

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Enzymatic cleavage of most ribonucleic acid molecules is necessary for producing their mature functional forms or conversely, for their degradation. These cleavage reactions are performed by cellular ribonucleases (RNases). RNase H is one such ribonuclease that specifically degrades RNA strand in a RNA/DNA hybrid and is involved in various biological processes such as removal of RNA primers from Okazaki fragments, processing R-loops to modulate replication and to restore DNA topology. The type II RNase H (RNase HII) is ubiquitously expressed and constitutes the major source of cellular ribonuclease activity in eukaryotes. Reduced RNase HII function is thought to trigger inflammation through the abnormal accumulation of immunogenic intracellular nucleic acids. RNase HII has also been suggested as a putative anticancer drug target, based on its overexpression in cancer cell lines and findings of a genome-wide siRNA screen. Significantly, it has recently been established that RNase HII is required for maintaining genome stability in mammals. Therefore, small-molecule modulators of RNase HII activity may have utility in therapeutics and as tools to investigate its cellular functions. A recent high-throughput screening study by White *et al.*, identified several small-molecule inhibitors against hRNase HII, but the exact inhibitory mechanism of the compounds remains elusive. In this regard, *in silico* studies have been taken up to (i) identify

the putative binding site of the inhibitors in hRNase HII, to (ii) explain the inhibitory mechanism of these inhibitors and to (iii) derive some rationale for the design of high-affinity inhibitors with high selectivity for hRNase HII.

Human Dopamine Receptors Interaction Network (DRIN): A systems biology perspective on schizophrenia disease biology

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Dopamine receptors (DR) are members of GPCR family involved in the control of locomotion, cognition, affect and neuroendocrine secretion. Malfunctioning of these receptors is well established to trigger many neurological and psychiatric disorders. In this context, a network based approach is more suited to capture the combined effect of multiple genes, accompanied by their interactions with external effectors (such as drug molecules) to enable a global understanding of the disease. To capture comprehensive interactions of candidate proteins associated with human dopamine receptors into the human interactome, we performed a protein-protein interaction network (PPIN) analysis and constructed a human Dopamine Receptors Interaction Network (DRIN) [1]. Furthermore, based upon the co-expression statistics of the disease genes, we constructed a common interacting co-expression network of disease genes in schizophrenia. We examined fundamental network topologies to sequester essential common candidates for schizophrenia. In a comprehensive search against all the available drugs for schizophrenia, we appreciated that our topmost probable candidates can serve as novel drug targets for the disease [2]. Besides, we explored the topology of dopamine receptors as molecular network, revealing their characteristics and the role of central network elements. More to the point, a sub-network analysis was done to determine major functional cluster in human DRIN that governs key neurological pathways. Conclusively, our study pinpointed distinctive topological and functional properties of human dopamine receptors that have helped in identifying potential therapeutic drug targets in schizophrenia disease network.

Biophysical Characterization of Armadillo Repeat Domain of FANCI

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Fanconi anemia (FA) is a genomic instability and cancer predisposition disorder. FANCI protein has different functional domain including armadillo (ARM) repeat and DNA binding region. Looking at the functional diversity and importance of ARM repeat, we have characterized human- FANCI Armadillo repeat region from 985-1207 amino acids. FANCI (985-1207) was cloned, expressed and purified in bacterial system and further purified by size exclusion chromatography using FPLC. Circular Dichroism (CD) & Fluorimetric analysis have revealed that purified protein has correctly folded secondary & tertiary structures with predominantly alpha helices. We have observed the melting temperature (T_m) using Thermal denaturation by CD and Fluorimetric analysis. Further to explore homogeneity we have performed Dynamic Light Scattering (DLS), and found the protein is homogenous. The biophysical analysis may help to understand the folding behaviour and stability of ARM repeat of FANCI.

Duplex to Cruciform transition in a Quasipalindrome present in Human Neuronal Growth Regulator 1 (NEGR1) gene, associated with Cancer

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The role of structural polymorphs of duplex DNA in various cellular processes like replication, recombination, nucleosome structure and regulation of gene expression is well documented. It has also been demonstrated recently that the structures other than canonical duplex DNA are present *in vivo* as transient species. The earlier work from our laboratory on HS4 site of the human β -globin gene LCR has established an equilibrium between hairpin, duplex and cruciform structures in a quasi-palindromic sequence [d-TGGGG(A/G)CCCCA] and its extended 21-mer version,^(1,2) while its RNA counterpart [UGGGG(A/G)CCCCA] is shown to exist only in duplex form.⁽³⁾

In view of these studies, the positional isomorphs of the studied sequences were designed. The bioinformatics study revealed that these sequences [d-TCCCC(A/G)GGGGA] are a part of neuronal growth factor 1 (NEGR1) gene, which has recently shown its association

with cancer.⁽⁴⁾ Biophysical and biochemical studies have been performed for investigating the effect of positional changes of G- and C-stretches within the DNA sequence. These isomorphous quasi-palindromic sequences show quite distinct behaviors. The DNA sequences with A/T and G/C at the centre, exhibited a structural switch from hairpin→cruciform, and duplex→cruciform, respectively. The observed structural changes in the positional isomorphs with just single nucleotide difference, makes this study interesting and might add to our understanding and insights for hypothesizing specific models of the polymorphic structures adapted by DNA sequences, in regulatory and architectural protein binding and other possible biological roles.

The effects of interfacial potential on antimicrobial propensity of ZnO nanoparticle

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The work investigates role of electrostatic interaction in defining antimicrobial propensity of ZnO nanoparticle (ZnONP) against different Gram positive and negative bacteria. Positively and negatively charged nanoparticles are tested against a range of Gram positive and Gram negative bacteria with varied bacterial surface potentials, ranging from -14.7 to -23.6 mV. Chemically synthesized positively charged ZnONP shows a very high antimicrobial propensity with MIC, 50 and 100 µg/mL for Gram negative and Gram positive bacterium, respectively. On the other hand, ZnONP of same size but with negative surface potential shows insignificant antimicrobial propensity against the studied bacteria. Unlike the positively charged nanoparticle, neither Zn²⁺ ion nor negatively charged ZnONPs shows any significant inhibition in the growth or morphology of the bacterium. Charge neutralization and CFU studies together proved the adverse effect of the resultant nano-bacterial interfacial potential on the bacterial viability. Thus, ZnONP with positive surface potential on interaction with bacterial membrane negative surface potential enhances production of the reactive oxygen species and exerts mechanical stress to the membrane, resulting in depolarization of the membrane. From the observations, antimicrobial propensity of metal oxide nanoparticle mainly depends upon the interfacial potential, the potential resulting upon interaction of nanoparticle surface with bacterial membrane.

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Chemical Chaperones Mediated Strategy for Prevention of Antithrombin Polymerization Based Thrombosis

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Serpins (**serine** proteinase **inhibitors**) are the largest super family of protease inhibitors that share common secondary folds but functionally diverse proteins that fold into a conserved structure and follow a common unique suicide substrate-like inhibitory mechanism. Members of serpin play an important role in regulation of enzyme involved in proteolytic cascades. Antithrombin is one of the important family member of serine protease inhibitors, which is synthesized in the liver and circulates in plasma (approx 150 µg/ml). Antithrombin is the main inhibitor of enzymes involved in blood coagulation pathway, mainly inactivates thrombin (factor IIa) and factor Xa (FXa) and to a lesser extent, factors XIIIa, XIa, IXa, tissue plasminogen activator (tPA), urokinase, kallikrein, trypsin, and plasmin. Mutations in antithrombin leads directly to functional defects and polymer formation that compromises the specific function and reduced its inhibitory activity. Our work focused on structural defects of antithrombin that result in its insoluble oligomers and polymers/aggregates that exert toxic effects which lead to antithrombin deficiency mediated thrombosis. We have screened a number of chemical chaperones that retard antithrombin polymerization on native gel. Decrease in the high molecular weight polymer bands were also followed by increased inhibition of thrombin in presence of chemical chaperones but not in its absence. The mixed chaperones based strategy between possible double combinations of lead chemical chaperones showed a reduction in concentration of chemical chaperones at which it hinder polymerization. These specific double combinations of lead chemical chaperones also maintain the inhibitory activity and bis-ANS fluorescence changes during antithrombin polymer formation.

Studies on the interactions of SAP-1 (an N-terminal truncated form of cystatin S) with its binding partners by CD-spectroscopic and molecular docking methods

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SAP-1 is a 113 amino acid long single-chain protein which belongs to the type 2 cystatin gene family. In our previous study, we have purified SAP-1 from human seminal plasma and observed its cross-class inhibitory property. At this time, we report the interaction of SAP-1 with diverse proteases and its binding partners by CD-spectroscopic and molecular docking methods. The circular dichroism (CD) spectroscopic studies demonstrate that the conformation of SAP-1 is changed after its complexation with proteases, and the alterations in protein secondary structure are quantitatively calculated with increase of α -helices and reduction of β -strand content. To get insight into the interactions between SAP-1 and proteases, we make an effort to model the three-dimensional structure of SAP-1 by molecular modeling and verify its stability and viability through molecular dynamics simulations and finally complexed with different proteases using ClusPro 2.0 Server. A high degree of shape complementarity is examined within the complexes, stabilized by a number of hydrogen bonds (HBs) and hydrophobic interactions. Using HB analyses in different protein complexes, we have identified a series of key residues that may be involved in the interactions between SAP-1 and proteases. These findings will assist to understand the mechanism of inhibition of SAP-1 for different proteases and provide intimation for further research.

An insight into the binding between synthesized triazole-tryptophan conjugate and bovine serum albumin: A spectroscopic approach

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Novel triazole-tryptophan conjugate (TTC) was synthesized by copper-catalyzed cycloaddition approach

and characterized by various spectroscopic analyses. The interaction of TTC with bovine serum albumin (BSA) was studied using fluorescence, time resolved fluorescence and absorption spectroscopy. Various binding parameters like binding constant (K_b), binding number (n), and binding forces were calculated. The results showed that TTC quenches the intrinsic fluorophore of BSA through static quenching mechanism. It was observed that compound strongly binds with BSA, however, the strength of complex decreases with increase in temperature. The thermodynamic parameters viz. Gibbs free energy change (ΔG), enthalpy change (ΔH), and entropy change (ΔS) for BSA-TTC interaction indicated that the binding process was spontaneous, enthalpy driven and van der Waals interactions are the major driving forces.

Biophysical analysis on structure and stability of T7 bacteriophage Zinc amidase

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T7 bacteriophage is a DNA virus that has a lytic life cycle and infects most strains of Escherichia coli. It is a 17 kDa novel bifunctional Zinc amidase protein (also known as N-acetylmuramyl-L-alanine amidase or T7 Lysozyme, T7-Lys). It also interacts and inhibits T7 RNA polymerase transcription cycle in order to regulate T7 phage propagation and maturation. In contrast to the hydrolysis action of traditionally known lysozymes (such as T4-Lys, and hen egg lysozyme-HEL), T7-Lys hydrolyzes the link between the N-acetylmuramoyl residues and the L-alanine of the glycopeptides on the cell wall to infect the bacteria. The protein folds into an alpha/beta-sheet structure with a prominent cleft, in which a zinc atom is located. The 3D-structural fold and the catalytic residues involved in hydrolysis are entirely unique compared to HEL/T4L. In order to understand the structure-function relationships and the stability features of T7-Lys, we have cloned, expressed and purified the protein. The pH dependent secondary, tertiary structural features and the conformational stability of T7-Lys have been investigated using NMR spectroscopy and optical spectroscopy techniques. Secondary structural analysis was done using the Far-UV circular dichroism and the tertiary structural analysis was followed using tryptophan and ANS fluorescence spectroscopy. Further, the pH dependent conformational changes were established by using the NMR spectroscopy. Our results suggested that, in contrast to the HEL, the pH dependent structural and stability features of T7-Lys are substantially different. In summary, this study presents a comprehensive analysis of pH dependent structure-stability relationship of this zinc amidase.

Extended C-terminus and conserved residues off lavinbinding pocket modulates the photophysical properties and structural integrity of the BLUF photoreceptor

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Living organisms possess variety of photoreceptors to sense and respond to the light signals. BLUF (Blue lights ensorsusing FAD) domaincontainingproteinsareflavin-basedbluelightphotoreceptorswhich receive blue light signal and are involved in sensory transduction and forphotoadaptive responses in manyprokaryotesand forfeweukaryotes. Thisphotoreceptorregulates the phototaxisincyanobacteria, photosyn the ticgene regulation, biofilm form ationin phototrophic organism and virulencein pathogenicbacteria. BLUF domain proteins exhibit a unique light induced spectral red shift, which is reversible upon darkincubation. Based on domain structure, BLUF domain proteinsaredivided into twocategories: multidomainprotein (complexprotein) linkedtoeffector domain and “shortprotein” thatiscomposedofanN-terminus BLUF domain followed by a 40-50additional stretchofaminoacidresiduesat theC-terminus. In this study, we have report a short BLUF domain protein (SmB) from *Strenotophomonas maltophilia*, which is composed of an N-terminal BLUF domain and an extended C-terminus of 50 amino acid residues. The extended C-terminus residues (outside of the canonical BLUF domain) governthe spectralproperties, thermal recovery kinetics and structural integrityof SmB BLUF protein. Conserved residues within and outside flavin binding pocket maintain the integrity of the Bluf domain fold. Mutagenesis studies also suggest that these conserved residues within and outside flavin binding pocket also regulates the dark recovery kinetics of the SmB BLUF protein. Biophysical characterization of the BLUF domain protein (SmB) will also be presented in detail.

Porphobilinogen Deaminase: Conserved Features and Structural Dynamics Across Species

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Porphobilinogen deaminase (PBGD) catalyzes the formation of 1-hydroxymethylbilane (HMB), in

tetrapyrrole biosynthesis, through a step-wise polymerization of four molecules of porphobilinogen, using dipyrromethane cofactor. Residues of catalytic importance have been suggested by Structural and biochemical studies, but their specific role in the mechanism and the dynamic behavior of the protein remains unknown. To understand the sequence-structure-function relationship during the step-wise polymerization reaction, PBGD homologs from *E. coli*, *Human*, *A. thaliana* and *P. falciparum*, were studied. Multiple sequence alignment shows the residues lining the active site of PBGD across species are conserved. One of the conserved lysines varies to aspartate in AtPBGD and to leucine in PfPBGD. Detailed analysis, including RMSD, RMSF, SASA, volume, Essential dynamics, Protein contact networks and Free energy landscapes were performed on the MD trajectories of the protein through four stages of chain elongation. Domain motions and active-site loop movement seem to aid the accommodation of polypyrrole in the active site of PBGD. It is observed that the functionally important and conserved residues R11, Q19 and D84, in *E.coli* and equivalent residues in other organism, interact with the elongating pyrrole chain in each stage and may participate in the catalysis as indicated in biochemical studies. Exit of the product, HMB, is through the interface between domain 1, domain 2 and active site loop. Limited active-site loop movement restricts the access of the incoming substrate to the catalytic site in PfPBGD. This might explain its low catalytic efficiency compared to other PBGD homologs.

Diketo acids and their peptidic analogues as promising scaffolds for the development of bacterial methionine aminopeptidase inhibitors

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Using diketo esters as the template, various derivatives were designed and the selected compounds were synthesised as bacterial methionine aminopeptidase (MetAP) inhibitors based on virtual screening and molecular dynamics simulation studies. The results of *in vitro* antibacterial screening revealed 15 compounds (**1a-**

c, 1e-h, 1j, 1l, 2a-c, 3d, 5c and 5e) as most potent against different bacterial strains. By using MTT assay on human hepatic cancer cell line (HepG2), the viability of cells was evaluated and all the tested compounds showed no cytotoxic effect at the concentration range of 50-450µg/ml. In the biochemical evaluation against MetAPs from *Streptococcus pneumoniae* (SpMetAP), *Mycobacterium tuberculosis* (MtMetAP), *Enterococcus faecalis* (EfMetAP) and human (HsMetAP), 14 compounds displayed differential behavior against these four enzymes. Moreover, compounds **1g, 3d** and **5e** showed about >80% inhibition of various MetAPs at 100µM inhibitor conc. with little or no inhibition of HsMetAP. The interactions of these inhibitors at the active site of MetAPs provide the structural basis for further development of these diketo acid based inhibitors for improved potency and selectivity.

Importance of helix F in neuroserpin structure and inhibitory mechanism

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Neuroserpin, member of serpin superfamily is known to inhibit tissue type plasminogen activator (tPA) in nervous tissues. The natural variants of neuroserpin cause familial encephalopathy with neuroserpin inclusion bodies (FENIB) that leads to dementia and epilepsy. Native state of neuroserpin is metastable which converts to a more stable state due to conformational changes related with its inhibition mechanism. However these changes also make it vulnerable to polymer formation. Neuroserpin possess canonical serpin three dimensional structure consisting of 3 β -sheets, 9 α -helices and a reactive centre loop (RCL). RCL upon binding to protease inserts in β -sheet A as strand 4 A. Helix F which stays on α -sheet A is predicted to undergo structural changes during loop insertion. We have mutated tryptophan at position 154 to alanine (W154A) and proline (W154P) in helix F, and subsequently studied changes in neuroserpin structure and inhibition mechanism. Far-UV CD spectrum showed minor differences at 222 nm and 216 nm wavelength upon mutations. T_m of W154A and W154P as calculated by studying changes in CD spectra was found to be 54.7±.4°C and 51.1 ±.5 °C respectively as compared to native neuroserpin 56.4±.2 °C, which indicated increased instability. Emission spectra upon excitation at 280 nm revealed that W154P mutation caused higher exposure of residues as compared to W154A mutation. To assess the extent of exposure of hydrophobic patches we undertook Bis-ANS binding study. Bis-ANS binding to hydrophobic patches on the surface of protein enhances its emission spectrum on excitation at 390 nm. W154A

mutation significantly diminished inhibitory activity of neuroserpin whereas W154P showed complete loss of inhibitory activity. The study indicates significant role of helix F in neuroserpin inhibitory mechanism and in maintaining its stability.

Homology modelling of UDP-galactopyranose mutase (UGM) from *Leishmania major* and molecular docking for investigation of new antileishmanial drugs

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The important component of cell wall of *Leishmania major* (Lm) is β -Galactofuranose (β -GalF) which is attached to the cell surface and play critical role in the pathogenesis of parasites. The enzyme UDP-galactopyranose mutase (UGM) present in the β -GalF biosynthetic pathway of Lm converts UDP-galactopyranose (UDP-Galp) into UDP-Galactofuranose (UDP-Galf) which acts as precursor of $\hat{\alpha}$ -GalF thereby having central role in its production. Because of its unique chemical nature and absence in humans it is selected as potential drug target in search of new antileishmanial drugs. Due to absence of crystal structure the 3D structure of UGM from Lm (LmUGM) was generated by using homology modelling. The stereochemical quality was validated. Effective drugs altering cell wall biosynthesis were selected that target active site residues of LmUGM for investigating better chemotherapy regime. The fidarestat, glyburide and 320KAW73 were found to be potent inhibitor of LmUGM as they occupy the UDP binding active sites of the enzyme. Phylogenetic analysis revealed UGM are conserved among *Leishmania* species suggesting that same drugs can be used in other species of the parasites. In this study we show new insight towards understanding the 3D structure of LmUGM thereby providing rational drug discovery process for antileishmanial treatment.

Spectroscopic investigation of interaction between CT-DNA and New Methylene Blue

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Interaction studies of DNA with various drugs play a very crucial role in laying down a concrete foundation for developing the understanding of the rational designing of ligands for diseases like cancer. Methylene blue (MB) has already shown quite promising results for the

photodynamic therapy. Keeping that in mind, a structure analogue, New methylene blue (NMB) has been selected for certain biophysical and biochemical studies using Calf-thymus (CT) DNA. Interaction studies of CT-DNA with NMB were performed using UV-Visible, UV-thermal denaturation, fluorescence, circular dichroism (CD) spectroscopy etc. The change in UV-Visible spectrum demonstrated the intercalation binding mode between NMB and DNA. Fluorescence emission data showed significant quenching in fluorescence spectra of CT-DNA with various concentrations of NMB, indicating the formation of DNA-Drug complex. The binding constants were calculated at different temperatures by UV-Vis and fluorescence spectroscopy and were found to be nearly identical. The thermodynamic parameters, enthalpy change (ΔH°) and entropy change (ΔS°) were computed to be $-6.11 \times 10^4 \text{ J mol}^{-1}$ and $-128.96 \text{ JK}^{-1} \text{ mol}^{-1}$ at 290 K respectively. CD studied have shown that the B-form of DNA changes to a more compact form on interaction with NMB. Comparative thermal melting profiles of CT-DNA alone and in presence of NMB showed DNA stabilization by the ligand. These results are also in accordance with the molecular docking study of NMB dye binding to DNA. The overall results suggest that intercalation mode; hydrogen bonding and Van der Waals forces might be responsible for interaction of NMB dye with ctDNA.

Thermal study of Biocompatible Rosin Modified Cationic Surfactant QRMAE on the Conformation of Human Serum Albumin to Induce Amorphous Aggregation

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The present study is based on the thermal aggregation of human serum albumin (HSA) induced by novel biocompatible rosin surfactants. The aggregation process causes conformational alteration in secondary and tertiary structures of protein. In this study the conversion of globular protein to amorphous aggregates was followed by spectroscopic and microscopic techniques to investigate factors which are responsible for the structural and conformational change and morphology of the protein. Our results show that the aggregation of the HSA was dependent on hydrophobicity, charge and temperature, because the formation of amorphous aggregates occurs in the presence of cationic novel rosin compound quaternary amine of rosin diethylaminoethyl ester (QRMAE) at 40 °C and pH 7.4 (at 25 °C there was no evidence of the formation of aggregates). Beside this its parent compound, abietic acid, and nonionic rosin compounds [ester of rosin acid with polyethylene glycol

monomethyl ether (RMPEG-750) and ester of rosin maleic anhydride with polyethylene glycol monomethyl ether (RMA-MPEG-750)] doesn't show this property. This work provides precise and necessary information that helpful to understand the effects of rosin surfactants. This study also provides very important information for the point of view of athletics, health, pharmaceutical companies, industries, soft drink processing companies.

Sucrose Stabilizes Ribonuclease-A more in the Presence of Macromolecular Crowding Agent

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Proteins are known to perform their specific tasks in the cellular environment made of high concentration of macromolecules. Levels of macromolecules are very high *in vivo* as compared to the very dilute solutions commonly used *in vitro*. The estimated concentration of macromolecules present in the cytoplasm is in the range of 80–400 mg/ml. This means a large fraction of the interior space of a cell is not available to other macromolecular species. Excluded volume theory suggests that any reaction that increases the available volume will be stimulated by macromolecular crowding. Compact folded states of proteins are indirectly stabilized due to destabilization of extended unfolded conformations. In this study, we examined the thermal stability ribonuclease-A (RNase-A) in mixed macromolecular crowded environment. We have mimicked crowding conditions experimentally by adding inert macromolecules, dextran 70 and sucrose (a sugar osmolyte). The concentration of dextran 70 was fixed to 300 g/l which happens to be *in vivo* concentration (300–400 g/l) of macromolecules in the cell and the concentration of sucrose was varied from 0.00 to 0.25M, 0.50M and 0.75M. To study the effect of macromolecular crowding on the stability of the protein at pH values, namely, 2.0 and 7.0, RNase-A was heated at 287 nm in the presence of dextran-70 and sucrose. Results show that T_m is increased to 4.2 °C on addition of 300 g/l dextran 70 in the absence of sucrose while 0.75 M sucrose it increased the T_m to 11.7 °C in the presence of 300 g/l dextran 70 at pH 2.0. At pH 7.0, T_m is increased to 3.4 pC on addition of 300 g/l of dextran 70 in the absence of sucrose while it is increased to 10.1 °C in the presence of 0.75 M sucrose and 300 g/l dextran 70. Our *in vitro* results are in good agreement with excluded-volume theory which demonstrates that the macromolecular crowding acts on unfolded state conformations. The native state is indirectly stabilized via making denatured state more compact and thereby less energetically favourable.

Neuroprotective efficacy of Apocynin against transient global cerebral ischemia in rats

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Background and purpose: Reactive Oxygen Species (ROS) is the key mediator following transient ischemia and is majorly derived from NADPH oxidase. The present study evaluates the potentials of Apocynin, a specific inhibitor of NADPH oxidase in attenuating the neuronal injury following transient global ischemia in rat model.

Experimental approach: Male Wistar rats were subjected to ischemia by occlusion of both common carotid arteries for 15 min followed by reperfusion. Apocynin (5 mg/kg bwt; i.p) was administered 1 hr before surgery and then daily up to 7 day post-surgery. Different behavioural, biochemical, histopathological changes and infarct volume were estimated.

Key results: Apocynin treatment significantly reduces infarct volume, ROS levels, Nitric oxide (NO) levels and lipid peroxidation. Also significant improvement in the reduced glutathione (GSH) levels and the antioxidant enzymes, superoxide dismutase (SOD) and catalase was seen. All these biochemical changes correlate well with the behavioural and histopathological alterations.

Conclusion and implication: Apocynin successfully attenuate ROS levels which results in protection against ischemic insult which is also seen in structural and behavioural changes.

Molecular modeling and dynamics of GPR87 and docking with some anti-cancer drugs

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Orphan G-protein coupled receptor 87 (GPR87) in human is a very recently discovered orphan GPCR means that the search for their endogenous ligands has been a challenge. GPR87 was shown to be over expressed in squamous cell carcinoma (SCCs) or adenocarcinoma in the lung and bladder carcinomas. We have predicted the comparative account on 3D structures of GPR87 on the basis of PDB ID: 3ODU|A. The model was further validated by comparison with structural features of the template proteins by using Verify-3D, ProSA and ERRAT servers were used for determining the stereo-chemical parameters of 3D structure of GPR87 predicted by

Ramachandran plot and good 3-D structure compatibility as assessed by DOPE score. Molecular dynamics (MD) simulation of models is studied of protein by conjugate gradient method. The DRY-motif (Asp- Arg-Tyr sequence) at the end of helix 3 is highlighted, where the G-protein binds and thus the activation signals are transduced. Protein-ligand interactions shows highest dock score with doxorubicin is 96.654, and involved binding site residues of GPR87 are Phe67, Lys247, Lys249, Asn330 and Asp357. In search for a better inhibitor for GPR87, insilico modification of some anti-cancer ligands shows doxorubicin has shown the highest binding affinity with GPR87. So our study provides an early insight into the structure of major drug target GPR87, thus facilitating the inhibitor design.

Unravelling the Structural Features Governing Ligand Recognition in HisJ and MBP

Nahren Manuel Mascarenhas and Shachi Gosavi

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Protein function in most cases is linked with a conformational change. Investigation of such conformational transitions would provide new insights to understand the structure-function paradigm and the different mechanism by which proteins recognize their binding partners. Ligand recognition by proteins are classified into two mechanisms, induced-fit (IF) and conformational selection (CS). In IF, the protein samples the ligand-bound state only upon ligand binding. In case of CS, the protein exists in a dynamic equilibrium between the two states (ligand-bound and -unbound) and the ligand selects the conformation (the ligandbound state, which is) most optimal for its binding. In this work, ligand-binding mechanisms for two structurally similar two-domain proteins, maltose-binding protein (MBP) and histidine-binding protein (HisJ), which operate by IF and CS, respectively, are investigated using coarse-grained structure-based models (SBMs). We establish the structural features inherent to these proteins that govern their ability to exhibit different ligand binding mechanisms. We find that a helix connecting the two domains and an additional interface at the back of the binding pocket serves specifically to stabilize the open-state in MBP. A structurally equivalent feature is absent in HisJ enabling it to access the ligand-bound state even in the absence of its ligand. We propose a model for MBP which could potentially switch its ligand binding mechanism from IF to CS.

Global network, sequence and structural features of LC3 family of proteins-*in silico* approach

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Autophagy is a cellular process by which the cellular materials are degraded and recycled. The process of autophagy proceeds in multiple steps, namely, initiation, elongation, and degradation. The first step initiation involves the formation of a double membrane shaped organelle called autophagosome and during this formation. LC3 protein plays a major role by expanding the isolated membrane and is commonly used as autophagy marker. It is also an essential protein and achieves this initiation of autophagy via association with multiple proteins complexes. Here, we are investigating the LC3-family of proteins to understand their underlying functional diversity. We obtain a global protein-protein network of proteins acting as hub or central residues in this family and further decipher their sequence and structural role in detail. To evaluate this, we perform extensive explicit solvent molecular dynamics simulation of LC3 protein and its orthologues. Overall, our approach addresses a longstanding question that, whether there exists any structural basis for functional diversity amongst protein and its orthologs. Our work thus reveals atomic level resolution information on LC3 protein and its complex orthologs partners and open future aspects for understanding subtle functional differences within same class of proteins.

Investigating Structural and Functional Conservation of Protease Activated Receptors.

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Protease-activated receptors (PARs) are G-protein-coupled receptors (GPCRs) that mediate cellular responses to specific proteases. Four Protease Activated Receptors are known in mouse and human. Human PAR1, PAR3, and PAR4 can be activated by thrombin whereas PAR2 is activated by trypsin and tryptase as well as by coagulation factors VIIa and Xa, but not by thrombin. Interestingly, PAR1 and PAR3 works at low thrombin concentration while PAR4 works at high concentration. In adult mammals, these four members of the PAR family link tissue injury and local generation of active coagulation proteases to cellular responses that help to orchestrate haemostasis, thrombosis, inflammation and most likely tissue repair. Protease activated receptor 1 (PAR1) is the prototypical member

of a family of G-protein-coupled receptors which plays an important role in platelet activation, vascular development and myocardial infarctions. In this study, we have studied the evolutionary conservation of PARs across the species in terms of sequence and structure. Analysis of protein interaction partners of different PARs have led us to identify common and unique interaction partners of these four PARs. Our study provides clues to functional specificity of these PARs.

Effect of Vitamin D supplementation on markers of oxidative stress and endothelial dysfunction in Type 2 diabetes

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Diabetes Mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Type 2 diabetes is accompanied by many complications most of which occur due to oxidative stress, which is defined as an imbalance between oxidant-antioxidant status. Oxidative stress occurs mainly due to the generation of reactive oxygen species (ROS) which lead to cell injury. Increased oxidative stress and endothelial dysfunction have important role in the development of insulin resistance and late complications of T2DM. Vitamin D supplementation increases pancreatic insulin release and improves insulin resistance in type 2 diabetic patients. In the present study, the effect of Vitamin D supplementation on oxidative stress markers such as lipid peroxidation product, malondialdehyde (MDA) and endothelial dysfunction marker like nitric oxide (NO) as well as the level of reduced glutathione (GSH) in patients with T2DM. Results of this study show that Vitamin D supplementation significantly decreases the levels of oxidative stress markers and favourably improves the endothelial dysfunction markers.

Application of Pr-doped Zinc Oxide synthesized by hydrothermal method for organophosphate sensing

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There is an enduring need for improved sensors or techniques to detect organophosphate (OP) and

carbamate (CM) pesticides in agricultural products/food as well as air, soil, and water. Chlorpyrifos is a broad-spectrum, chlorinated organophosphate (OP) insecticide, acaricide and nematicide. The common name for this is (0,0-diethyl 0-3,5,6-trichloropyridin-2-yl phosphorothioate). Chlorpyrifos is used on agricultural food and feed crops, cattle ear tags, golf course turf, industrial plants and vehicles, non-structural wood treatments including processed wood products, fence posts and utility poles, and to control public health pests such as mosquitoes and fire ants. Chlorpyrifos is registered for indoor residential use only in the form of containerized baits. Uses for individual products containing chlorpyrifos vary widely. Chlorpyrifos is a non-systemic insecticide designed to be effective by direct contact, ingestion, and inhalation. Signal words for products containing chlorpyrifos may range from Caution to Danger. Most organophosphorus pesticide, chlorpyrifos is oxidized to its oxon form, chlorpyrifos oxon which is generally regarded as the principal toxic metabolites and responsible for the inhibition of the enzyme AChE (acetyl cholinesterase). Chlorpyrifos-oxon, hydrolysed to form the diethyl phosphate and 3,5,6-trichloro-2-pyridinol (TCPy). metabolite of chlorpyrifos excreted in urine. Detection of organophosphorus using traditional methods are time consuming, costly, and requires highly trained technician to monitor the instrument. The development of a novel device that efficiently, reliably and accurately detects toxic OP and CM pesticides in real time is of substantial use for many applications in the environmental, agricultural and pharmaceutical industries. Although traditional analytical methods have been widely used for detection and estimation of residues of these pesticides, but these methods are time consuming, costly, complex, requiring skilled technicians and lot of organic chemicals (solvents) which are harmful to human beings as well as the whole environment. Research has shown direct electrochemical methods are

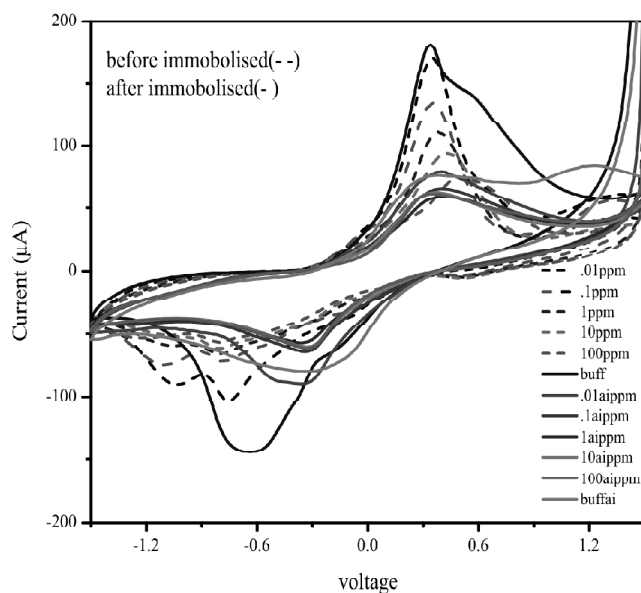
emerging as an alternative method for the detection of OP and CM pesticides. Sensors based on electrochemical technique reduce the possibility of false positives along with versatile detection of analyte. Therefore, the innovation of miniaturized, rapid, portable and cost-effective sensor system is vital towards progression in electrical sensor technology.

For this work, we used Pr-ZnO which was synthesized using Zinc nitrate (1 mM) with 10 mM of praseodymium nitrate and resultant product (Pr-ZnO) is used as sensing material. The synthesis was done by hydrothermal method at 165°C for 5 hours. These powders were then dried and used for sensor fabrication. Powder was characterized by XRD, FESEM, UV-Vis spectroscopy in terms of morphological and elemental analysis. Films of these were screen printed on screen printed gold electrodes (SPE) as working electrode while counter and reference electrodes were of gold. The electrochemical characterization was carried out at a fixed scan rate and variable scan rate for understanding charge transfer characteristics. The pesticide concentration was varied from 0 to 1000 ppb. CV analysis was performed for both with and without enzyme AChE on the working electrode of SPE. The change in the CV curves can be seen as reduction in anodic current with increasing concentration in both cases (as shown here). However, initial studies confirm the possibility of using Pr-doped ZnO as a promising material for enzyme less sensing of organophosphate. This can reduce the cost of sensor fabrication significantly. The revival of AChE enzyme immobilized sensor was also performed with atropine treatment. Further experiments are going on to validate our results.

Dose and time dependent induction of MT by Zinc supplementation in rat liver

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Metallothioneins (MTs) are low molecular weight proteins characterized by high cysteine content and give rise to metal-thiolate clusters. The liver is important for the regulation of zinc homeostasis, while zinc is necessary for proper liver function. The intracellular concentration of Zn is tightly regulated, MT plays crucial role in its regulation. The Objective of this experiment is to determine the relationship between the Zn status in the rat liver and the occurrence of Hepatic MT. In this study we investigated the MT expression trend in rat liver, when Zn is given in dose and time dependent manner. Dose dependent study of MT by the subcutaneous injection of different amount of Zn 10mg, 50mg, 100mg Zn²⁺/kg body weight and for the time dependent behavior of MT 50mg/kg (body weight) dose was given at different time

intervals i.e. 6,18,48 hours. Isolation of MT was done by using combination of Gel filtration and ion exchange chromatography while Characterization of MT fraction was carried in the wavelength range 200-400 nm. Expression of MT was studied by Western blot. The metal stoichiometry is being studied by using ESI-MS and PIXE. The study makes us understand that rapid incorporation and prolonged occurrence of metals with MT plays important role in the detoxification of heavy metals.

Methods of Crop Improvement in Plant Tissue Culture

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Plant tissue culture comprises a set of *in vitro* techniques and strategies by which plant can adapt for specific needs or opportunities. Tissue culture has been exploited to create genetic variability from which crop plants can be improved to provide sustainable food and healthful nutrition, protection of the environment and increasing the number of desirable germplasm available to the plant breeder. Tissue-culture protocols are available for most crop species although continued optimization is still required for better improvement of crops. The use of molecular assisted-technology in Tissue culture has been successfully carried out to incorporate specific traits into plants where they are needed. Techniques involved the culture of protoplast, ovules, microspores and embryos have been used to create new genetic variation in the breeding lines by means of haploid production. Furthermore, tissue culture has also been used to create new varieties of crops through Somaclonal and Gametoclonal technology. To improve the yield of established cultivars, the culture of single cells and meristems can be effectively used to eradicate pathogens from planting materials. The Micropropagation technology fulfilled the needs of millions of plants for the commercial and agricultural markets. With the help of Plant tissue culture, the new selected trait can be available and reach the commercial market in a short span of time as compared to traditional breeding technique. This technology can be expected to have an ever increasing impact on crop improvement as we approach a new decade.

Keywords: Plant tissue culture, Crop, Improvement, Technique, Plant, Trait

Interaction of Adriamycin with a regulatory element of *hmgb1*: Spectroscopic and Calorimetric approach

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HMGB1 is a non histone nuclear protein which plays important role in transcription, VDJ recombination, chromatin remodeling, DNA repair etc and its overexpression is directly correlated with various human malignancies and inflammatory diseases. Because of the clear association between HMGB1 and cancer, we studied the binding of adriamycin (ADM), a well known anti-cancer drug with the promoter region (-165 to -183) of *hmgb1* by using a variety of spectroscopic, calorimetric techniques and in-silico molecular modeling. Changes in UV and CD spectral characteristics (intensity and wavelength) of ADM and DNA associated with an induced peak (300nm) in CD spectrum of DNA and a high binding constant of $2.0 \times 10^5 \text{M}^{-1}$ suggest a strong and stable complex formation between DNA and ADM. Scatchard analysis of spectroscopic data indicate that ADM binds to DNA in a non cooperative nature. Further the quenching of fluorescence emission of ADM and isothermal titration calorimetry of ADM in presence of DNA points out to the intercalative mode of ADM binding to DNA which is enthalpically driven with additional small entropic contribution. Results from molecular modeling, ITC and Fourier transform infrared spectroscopy (FTIR), reveal that ADM has no marked preference between A-T vs G-C base pair in binding to DNA. Therefore, *hmgb1* can be considered as a novel potential chemotherapeutic target in treating cancers associated with HMGB1 upregulation.

Probing the interaction between aceclofenac and bovine serum albumin: A spectroscopic Approach

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The interaction between aceclofenac and bovine serum albumin (BSA) was investigated by using fluorescence, UV-visible and time resolved spectroscopy. The results of fluorescence and time resolved spectroscopy shows that aceclofenac quench the intrinsic fluorescence of BSA through dynamic quenching mechanism. UV-vis

absorption results support for the complex formation between aceclofenac and BSA. The calculated thermodynamic parameters viz. enthalpy change (ΔH), and entropy change (ΔS) for BSA and aceclofenac interaction indicated that the major driving forces are hydrogen bonding and van der Waals interaction. In addition, the negative value of Gibbs free energy change (ΔG) indicates that the binding process was spontaneous. The donor-to-acceptor distance, which was calculated through fluorescence resonance energy transfer (FRET), was found to be less than 8 nm which indicated that the energy transfer from BSA to aceclofenac occurred with high probability.

Biophysical characterization of CNL-26 protein from mature *Lageneria siceraria* seed and its plausible role in urinary bladder carcinoma

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NBS-LRR (nucleotide binding leucine rich repeat) proteins are highly conserved family of immune receptor found in both plants and animals which is type of R proteins, belongs to the STAND family (signal transduction ATPase with numerous domains), classified as NBS-LRR protein (plants), APAF (apoptotic-protease-activating factor 1) (animals) and NLRs (NOD like receptor). On the basis of N-terminal of plant NBS-LRR proteins are classified into three groups are TIR-NBS-LRR (TNL) (Toll interleukin like receptor), CC-NBS-LRR (CNL) (coiled coil) and XNL. We have purified CNL-26 protein from mature *Lageneria siceraria* seeds in two steps by chromatography. CNL-26 protein was identified by MALDI TOF/MS; it shows 82% sequence identity with N-terminal of NBS-LRR protein from *Oryza sativa japonica*. Sequence alignment score of CNL-26 protein with previously reported CNLs protein from other plant source like MLA1 (*Hordeum vulgare* (Barley)) and RPS5 (*Arabidopsis thaliana* (thale cress)) is 21. Higher order oligomerization of CNL-26 protein was observed by gradient native-PAGE and BS3 cross linkers. Far-UV Circular dichroism (CD) spectroscopy of CNL-26 protein concludes that it is most stable with 0.5mM ATP and 1mM MgCl₂. 8-Anilino-naphthalene-1-sulfonic acid (ANS) increased the fluorescence intensity in presence of 0.5mM ATP and 1mM MgCl₂ which relates its oligomerization. ATP and MgCl₂ assist decline in molecular volume of

CNL-26 protein demonstrated by atomic force microscopy (AFM) and transmission electron microscopy (TEM) which infer its structural stability in its presence. Polyclonal antibody was developed against immunoreceptor CNL-26 protein in New Zealand rabbit and Ig G purified by protein agarose A from rabbit sera. Polyclonal antibody titer was calculated by indirect ELISA and western blot analysis as 1:2000 and 1:1500 respectively, shows its high immunogenicity. CNL-26 protein was immunolocalized in plasma membrane, conclude that it carry immune function through receiving chemical signals from outside the cell and translating chemical signals into intracellular action. Therapeutic importance of CNL-26 protein evaluated for anticancer activity on urinary bladder carcinoma (HT1376) with IC50 value of 280 µg/ml and 210 µg/ml in absence and presence of (ATP+MgCl₂) respectively. Cell cycle arrest at G2/M phase of Urinary bladder carcinoma (HT1376) and apoptosis at G0/G1 phase.

Neuroprotective Effects of Apocyanin, a NADPH Oxidase Inhibitor in Lipopolysaccharide Induced Parkinson's Disease Model

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Recent studies have revealed an essential role for neuroinflammation that is initiated by microglial and infiltrated peripheral immune cells and their toxic products (cytokines, chemokines etc) in pathogenesis of PD. Lipopolysaccharide, a bacterial endotoxin is the most extensively utilized glial activator for the induction of inflammatory DA-nergic neurodegeneration. Therefore, for the present study LPS was utilized and injected stereotaxically into the SN of rats at a dose of (5µg/5µl PBS) for the establishment of PD model. This in turn leads to microglial activation subsequently leading to increased level of pro-inflammatory cytokines and excessive superoxide anion as a result of NADPH oxidase activation. Further, LPS caused DA-nergic neuronal death as revealed by IHC of Tyrosine Hydroxylase (TH) and neurotransmitter Dopamine loss resulting in behavioural impairment in rats. Apocyanin prevented the loss of DA-nergic neurons as depicted from IHC of TH and glial cell activation as depicted from IHC of GFAP and Iba-1 in the SN. Apocyanin treatment ameliorated LPS induced alterations in neurochemical (Dopamine and its metabolites DOPAC and HVA), behavioural (Actophotometer, Rotarod, Bar test), biochemical oxidative stress markers (GSH, O₂⁻, SOD, Catalase, LPO) and inflammatory markers. Hence, Apocyanin exhibits profound neuroprotective effect in LPS induced PD model by attenuating PHOX mediated oxidative damage as well as neuroinflammatory response. Thus based on

the findings the neuroprotective role of apocyanin in treatment of PD should be evaluated further.

Key words: Parkinson's disease, NADPH oxidase, motor dysfunction, Immunohistochemistry, Tyrosine Hydroxylase

Micro RNAs in Therapeutics

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Micro RNAs are short (20 – 25 nucleotides), non-coding RNAs that have been known to exist in various organisms including human. Micro RNA are considered to down regulate the gene expression by unswervingly slicing the mRNA, mRNA decay by deadenylation or by translational repression. miRNA direct Argonaute proteins to bind and negatively regulate the complimentary mRNA. miRNA is responsible for a new therapeutics target to countless diseases as in let-7 miRNA inhibit propagation of cancer stem cell, miRNA have been found to play an important role in metabolic diseases like obesity, miR 143 is responsible for differentiation of adipocytes and diabetes, miR 375 regulate insulin secretion in pancreatic islet cells. Tissue-specific miRNAs may perhaps be intricate in the pathogenesis of Cardiovascular, muscular and neurodegenerative diseases. MiRNAs have several significant advantages in that they are small and comprise of a known sequence that is often completely conserved among species, which are very attractive features from a drug development standpoint. Antisense technologies, potent oligonucleotide chemistries to target miRNAs, known as anti-miRs, are currently being generated. One way to therapeutically mimic or re express a miRNA is by using synthetic RNA duplexes designed to mimic the endogenous functions of the miRNA of interest, with modifications for stability and cellular uptake. A hepatic-specific miRNA, miR-122, was found to be of critical importance to the replication of hepatitis C virus (HCV). Seizure of miR-122 with antisense constructs targeting the miRNA significantly reduced the replication of HCV RNA, proposing that miR-122 may present a target for antiviral intervention.

Future Perspective of Immunotherapy In Glioblastoma Multiforme

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Glioblastoma Multiforme (GBM) is the most common malignant form of primary brain tumor. It represents one and a half year of the deadliest human cancers, with

an average survival of one year following diagnosis. Despite the availability of various therapeutics modalities, prognosis is poor, and warrants the need for development of suitable therapeutic strategies. To this end targeted therapy achieves most overwhelming attention. In this regard the role of polyclonal antibody has been well recognised, considering this in view, we envisage a study to evaluate the development of antibodies against GBM. The active targeting of nanoparticle decorated with polyclonal antibody could enhance the efficacy of nanoparticles for delivering several agents specifically into the tumor milieu along with significantly reducing the related toxicity issues.

Glioblastoma Multiforme tissue was provided by the Department of Neurosurgery, J.N medical college, Aligarh in a sterile condition. Membrane protein were isolated from tissue sample and polyclonal raised against this targeted membrane protein. In order to examine whether the polyclonal antibody diminish the level of signalling pathway we will follow the planning to develop Monoclonal antibody against surface protein of GBM.

Dynamical Characterization of Dopamine Receptor D4 using Molecular Dynamics Simulations

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G-protein coupled receptors (GPCRs) constitute a large and diverse family of membrane receptors of high pharmacological interest. Regardless of the high prevalence of GPCRs, structure-function relationship of receptor activation is poorly understood. Here, we employ a range of computational methods to interpret the ligand activation of human Dopamine Receptor D4 (DRD4). We report the modeled structure of DRD4 alone and in complex with dopamine and spiperone, its natural agonist and antagonist, respectively. In order to assess the conformational dynamics induced upon ligand binding, all-atom explicit solvent molecular dynamics (MD) simulations in membrane environment were performed [1]. Detailed analyses of simulations indicate conformational changes in transmembrane region upon agonist binding, characteristic of transmission and tyrosine toggle molecular switches. Moreover, native trajectories reveal the dynamic nature of the third intracellular loop (ICL3) which also represents an open conformation ideal for G protein binding. Further, in addition to the wild type, MD simulations on two variant

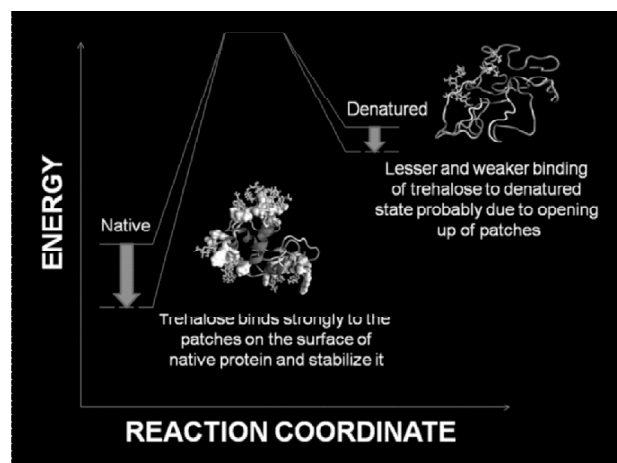
forms of DRD4, V194G and R237L along with its complexes with agonist and antagonist were also performed. The mutation in transmembrane domain was observed to disrupt the ligand binding of DRD4 while the other, R237L interferes with G-protein binding in intracellular loop 3. In comparison with the native receptor, the variants greatly affect the overall stability of the structure. Together, these studies open future avenues to agonist/antagonist that could be employed in the treatment of DRD4 implicated neurological disorders.

Revisiting the conundrum of Trehalose stabilization

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Protein aggregation and loss of protein's biological functionality are manifestations of protein instability. Cosolvents, in particular trehalose, are widely accepted antidote against such destabilization. Although numerous theories have been promulgated in literature with regard to its mechanism of stabilization, the present scenario is still elusive in view of the discrepancies existing in them. To this end, we have revisited the conundrum and attempted to rationalize the mechanism by conducting thorough investigation of the effect of trehalose on the native, partially unfolded and denatured state of protein "Lysozyme" by means of molecular dynamic (MD) simulations under different temperature and concentration regime. Two-dimensional contour plots along with principal component analysis suggests that trehalose molecules offer on-pathway stabilization unaltered principal direction of protein's motion, although it slows down protein dynamics so that the protein gets trapped in homogeneous ensemble of conformations closer to the native state. Free energy landscape reveals higher population of native compared to intermediate and denatured states. Delphi results and calculation of preferential interaction parameter demonstrates that this relative stabilization of native state



can be ascribed to be the consequence of favourable interactions of trehalose with side chains of certain loci on protein surface encompassing polar flexible residues. We propose trehalose-protein interactions to be of primary importance in light of observed demarcations in binding affinity with native and denatured state of protein. Our findings are at variance with the common conception of relative destabilization of denatured state. Rather, we provide evidence for relative stabilization of native state.

Identification of fertility markers in seminal plasma of crossbred bulls

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Problem of sub-fertility in crossbred bulls leads to disintegration of breeding systems and huge economic loss. In this study, we have performed proteomic analysis of seminal plasma (SP) from bulls with varying fertility to identify the fertility-associated proteins by 2D-PAGE and differentially expressed proteins were identified through MALDI-TOF mass spectrometry. Out of the 18 differentially expressed proteins, 9 were found overexpressed and 9 underexpressed in SP of high-fertile bulls in comparison to that of low-fertile bulls. The differential expressions ranged from 1.5- to 5.5-fold between the two groups, where protection of telomeres-1 protein (POT1) was highly overexpressed (2.9-fold) in high-fertile group and prostaglandin E2 receptor EP3 (PTGER3) was highly abundant (5.5-fold) in low-fertile group. The protein interaction network was elucidated using STRING software tool, and the functional bioinformatics analysis was done using Blast2Go software. Most of the differentially expressed proteins were found to be involved in cellular processes and biological regulation with binding and catalytic function.

Expression Profiling of Genes Involved in Lysine Biosynthesis in Edible Plants Including *Amaranthus hypochondriacus* using High Throughput Transcriptomic Data

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Protein energy malnutrition among the underprivileged part of society, especially in children under five years of age, is a major global concern. It accounts for almost half

the child deaths worldwide and leads to poor IQ in those that survive. The condition actually boils down to lysine malnutrition, since lysine is most limiting among the essential amino acids which have to be supplemented through diet. Unfortunately, the major cereals consumed, especially rice and wheat that are staple to India, lack lysine. Efforts to engineer high-lysine rice and wheat have failed because of an inherent inverse correlation between high-lysine content and yield. It is therefore, important to know how *Amaranthus hypochondriacus*, a pseudo-cereal, produces a high yield of lysine-rich grains. To this end, we have utilized next generation RNA sequencing data and compared the transcriptomes of many plants, including that of *A. hypochondriacus*, that have varying percentages of seed lysine content, to see whether the genes involved in lysine biosynthesis contribute to the high lysine phenotype. The expression levels of eight enzymes involved in the lysine biosynthetic pathway in the seed and leaves of *Zea mays*, *Vitis vinifera*, *Glycine max*, *Beta vulgaris* and *A. hypochondriacus* have been compared, for which RNA-Seq data were available in the public repositories. One of the challenges encountered in this study was that the publicly available data were generated under different conditions using different sequencers which could cause variability and bias. We present the strategy used to normalize data from diverse sources and discuss the results here.

Impact of Composite Dielectric Media on S4 α -helix Of KvAP

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The Voltage-gated K⁺ ion channels of Aeropyrum pernix (KvAP) is a transmembrane tetramer protein with two distinct domain: (i) Voltage Sensor Domain (VSD), which comprises of four α -helices (S1, S2, S3, S4) and senses the voltage to control the flow of ions and (ii) Pore Domain (PD), which consists of two α -helices (S5, S6) and helps passage of ions through the channel. These α -helices are antiparallel to each other which behave like a macrodipole with negative C terminal and positive N-terminal. Using basic electrostatic principles, we have developed an innovative software with which we have computed the interaction energy between the residues of the pair of α -helices. The S4 α -helix show various conformational orientation with respect to S3b α -helix when their charged residues (positive Arginine and Histidine and negative Glutamic acid) are exposed to differential dielectric media, which is surrounding the α -helix pair. The theoretical results showing evolution of minimum potential energy, explain the different mutual conformational orientation of S3b-S4 α -helix which closely relates to the structure of KvAP (PDB-2KYH).

Extraction and purification of a therapeutically important protein from a traditional medicinal plant of Kashmir Himalayas

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Majority of the extraction studies on medicinal plants have been largely focusing on metabolite analysis even if a number of therapeutically active proteins have been found to be present in various medicinal plants. It has been argued and accepted that proteome analysis offers a new approach for identification of wide spectrum of genes that are expressed in living system. Keeping this in view, this study was devised to isolate and purify a pharmacologically active glycoprotein from *Withania*. Most of its pharmacological activities like physiologic and metabolic restoration, antiaging, nerve tonic, impairment of cognitive function and prevention of various central nervous system and neurodegenerative disorders (Alzheimer's, Parkinson's diseases etc.) have been associated with its various alkaloid and steroidal compounds. We have been so far able to locate this protein in 90% ammonium sulphate precipitated fraction by SDS-PAGE but not yet successful in resolving the peaks by SDS-PAGE but not yet successful in resolving the peaks for the same protein in anion exchange chromatography as the elution time for the two obtained peaks is quite close. Gel filtration chromatography was used to remove secondary metabolites as they lead to oxidation of proteins.

Biophysical Properties of Hereditary Hemochromatosis Factor-E Protein Expressed in the *Escherichia-coli*

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Hereditary hemochromatosis factor-E (HFE) is a MHC class-I like protein, plays an important role in iron regulation of our body. Mutational studies of HFE suggested its importance in the iron-uptake and regulation. Patients of hereditary hemochromatosis (HH) carries sever iron-overload and a nonsense mutation C282Y in HFE protein was reported. HFE along with other proteins like ferritin and TfR help in iron uptake, storage and regulation. This protein also a part of body's iron level sensory system. Besides these widely important roles of HFE, we don't have any idea about the structural

stability and folding of this protein. Here we have first time cloned and expressed the HFE protein in the *E. coli* strain BL21 (DE3) as soluble protein, and is purified using Ni⁺⁺ Sepharose resin. After affinity chromatography, further purity was achieved by performing weak-anion chromatography on DEAE-Hi Trap column. Purity of protein was checked by SDS-PAGE and confirmation of purified protein was done by immunoblotting. Presence of secondary structure was verified by CD-spectra, which confirms that our protein contains 42 % α -sheet and 18% α -helix. Thermal and pH based structural studies are going on to evaluate the stability of HFE protein. Purified HFE was also used for binding studies with Tf by isothermal titration calorimetry. Binding studies helps us to evaluate whether HFE binds to Tf or not. These studies helps us to establish a relationship between working and stability of iron regulatory proteins.

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Drug Dosage Activity Studies on Fluoroquinolone Antibiotics Through Optical and Related Properties

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Fluoroquinolones are a family of synthetic broad spectrum antibiotics with bactericidal drugs, interfering with DNA replication by the process of Topoisomerase-I. The Drug DNA interaction gives an idea of understanding the various molecular reactions. With the help of physical parameters such as refractivity, susceptibility, Electron ionization cross sections and dosages, the drug activity correlated. The other medicinal important parameters like Bioavailability, Half Life, Protein Binding and log P values are taken from the literature. The correlation between physical parameters and medicinal values gives new approach for drug activity and drug toxicity. The molecular electron ionization cross section obtained from Diamagnetic susceptibilities which are derived from mean molecular polarizabilities. The dosages of the each Fluoroquinolone drug (Narfloxacin, Ciprofloxacin, Ofloxacin, Sparfloxacin, Moxifloxacin and Levofloxacin) calculated by using simple algebraic formulae. Mean molecular polarizabilities have been evaluated from FITR spectra. By utilizing above data drug activity studied without any kind of expensive medical techniques.

Engineering heme retention stability in hemoglobins by mimicking the natural strategy employed in *Synechocystis* Hemoglobin – Promise for Hemoglobin Based Blood Substitute

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In recent years, acellular hemoglobin was being exploited for use as substitute for whole blood under medical emergencies due to its ability to efficiently transport oxygen and thus save lives. However, recombinantly expressed hemoglobin targeted for such use suffers from stability issues and side effects arising out of them. The major stability problem that hemoglobin faces is the loss of the heme prosthetic group that is required for function and stability. Dissociated heme also results in cytotoxicity and efficient use of haemoglobin based blood substitute depends on our ability to prevent heme dissociation. One of the ways to engineer stability is to mimic the strategy of nature since other protein engineering strategies were not successful. We observed that truncated Hb from the *Synechocystis* sp. (SynHb) has such enhanced stability. Our studies demonstrated that the non-axial His 117 in Syn Hb render the protein stable and prevent the loss of heme. Similar strategy was utilized successfully in sperm whale myoglobin (Mb) by mutating topologically equivalent position (Ile107His), which is in close proximity to vinyl group of myoglobin. Reversed phase chromatography, ESI-MS and MALDI TOF analyses confirmed the presence of covalent linkage in Mb Ile107His. The Mb mutant with the engineered covalent linkage was stable to denaturants and exhibited ligand binding and autooxidation rates similar to the wild type protein. This novel finding indicates that a similar strategy in human haemoglobin can help it retain heme stably, which will pave the way for production of stable hemoglobin based blood substitute. We have laid down strategies for such stability enhancement and identified the strategic residues that can prove to be useful towards this goal.

Modelling cell electroporation I : Effects of variation in membrane cholesterol content on pore dynamics

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Electroporation is a physical process involving enhanced permeability of biological cell membrane due to

application of high voltage electric pulses of very short duration [1-2]. The enhanced permeability results from formation of micropores in the cell membrane. Since its inception in the 1970s, the technique has found wide range of applications in biotechnology including cancer therapy and anti-viral vaccine delivery [3,4]. Though considerable efforts have been made to understand the physical process, differences exist between experimental observations and theoretical models [5] due to various approximations such as uniform composition of lipid bilayer, uniform poration, etc. Here we present a theoretical formulation and numerical implementation of electroporation of a mammalian cell with cholesterol containing membrane. The cell geometry of PS cells (porcine kidney) have been determined experimentally and parameters used as inputs in the simulations. Numerical solutions (simulations) of the equations carried out in MATLAB. Effective electroporation was evaluated in terms of transmembrane potential, average pore radius, pore count and distribution vs time. Effect of variation in membrane cholesterol content (15-29% mole-fraction) was evaluated in terms of changes in these parameters. It was observed that variation of cholesterol-content resulted in significant alteration of pore radius, number and distribution of pores on the cell surface. This model can be a useful tool for prediction of electroporation phenomenon in mammalian cells (of various descriptions) at higher magnitude of applied electric fields.

Structural preference and base pair specificity of the DNA binding of the alkaloid chelerythrine: Spectroscopic and calorimetric investigations

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The DNA structural preference and base pair specificity of the plant alkaloid and putative anticancer agent chelerythrine was investigated by biophysical techniques. Interaction with double stranded (ds), heat denatured (hd) and single stranded (ss) DNAs clearly suggested its preference to the ds geometry. Thermodynamics of the interaction revealed exothermic binding of chelerythrine to all three DNA forms, but with highest affinity towards the ds DNA. DNA base-pair specificity was evaluated from study with four synthetic DNA polynucleotides. Hypochromic and bathochromic effects in the absorbance spectrum of the alkaloid was evident in each case; the fluorescence intensity enhanced on complexation with both homo and hetero AT sequences and the homo GC polynucleotide, while it quenched with the hetero-GC sequences. The intercalative mode of binding of the alkaloid was evaluated by anisotropy, quenching and

viscosity study. The binding enhanced the thermal stability and induced moderate conformational changes in these ds polynucleotides. High binding affinity values of the order of $>10^6$ M⁻¹ was observed from both spectroscopic and calorimetric data. The binding was characterized to be exothermic and favored by negative standard molar enthalpy and positive standard molar entropic contributions except for the homo-AT polynucleotide, where it was endothermic and entropy driven. The results revealed the specificity of chelerythrine to vary as homo-GC > hetero-GC > hetero-AT = homo-AT. Detailed spectroscopic and thermodynamic results on the structural aspects and thermodynamics of chelerythrine-DNA interaction will be discussed.

In silico* and *in vivo* study of phenoxy derivatives of sulfonamides as potential Juvenile Hormone Analogs against *Galleria mellonella

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In silico study of chemical databases using protein-ligand interactions by docking techniques is becoming very important and popular nowadays. There are a wide range of comparable and contrasting methodological protocols available in screening databases for the lead compounds. Molecular modeling study gives the direction of what to synthesize and how before synthetic experimental work being carried out. Presently, classical insecticide has been used world-wide to counter insect problem. Though in some developed countries insecticides like DDT etc. are completely prohibited but in some developing countries they are still in use because of its cheaper price and quick action. The idea of using Juvenile hormone mimics represents a promising approach for insect control¹. Insects have the fast multiplication rate, which are responsible for the transmission of various diseases. The insect juvenile hormone analogues are class of compounds which control post-embryonic development of insects from egg to the adult stage and therefore, have the potential to be much safer and more effective insect control agent². *Galleria mellonella* (Honey comb pest) is a major threat to honey bee industries. Pest causes a heavy economic loss all over the world including Asian countries. Juvenile Hormone Binding Proteins (JHBP) of *G. mellonella* which are believed to be the binding site of JH has been crystallized by Kolodziejczyk group³. They have found two binding cavity (W and E); proposed W cavity as binding site to Juvenile Hormone (JH). Studying the nature of W cavity; we have designed sulfonamides bearing phenoxy derivatives. Molecular modeling study has been performed using AutoDock 4.2 software⁴⁻⁵. Best analogs on the basis of binding energy profile have been selected for the further study.

Thus, main objective of the present research work is to design, synthesize and *in vivo* analysis of JH like substances with varied functionalities with a view to obtain some JH analogues which exhibit universal action on unrelated species.

Virtual screening of peptidomimetic compounds as potential activators of glucokinase for treatment of Type 2 diabetes

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Owing to the unique function in regulating glucose metabolism, glucokinase has drawn special attention as a drug target for type-2 diabetes. Glucokinase (GK), an enzyme that phosphorylates glucose to glucose 6-phosphate, serves as the glucose sensor that regulates insulin secretion in pancreatic β cells. GK activators (GKAs) activate GK via binding to an allosteric site of the enzyme. GKAs increase glucose stimulated insulin secretion and regulate blood glucose levels. In the present study, a library of 78 peptidomimetic GKAs was designed based on the known carbon centered GKAs, using Glide docking and e-pharmacophore techniques. Results of docking studies depicted that the designed peptidomimetic GKAs have better relative binding affinity than the known compounds. It also showed additional pi-pi interactions of some of the designed ligands with the receptor. Enrichment of the peptidomimetic library showed ROC at 0.90, RIE 4.61 when area under curve (AUC) was 0.89 while BEDROC varied between 0.39 and 0.534, with varied alpha values. 70% active compounds were recovered at 5.34 enrichment factor. It was further validated by the outcome of their e-pharmacophore rank and pharmacophoric features that the designed peptide based GKAs could be potential glucokinase activators.

Spectroscopic studies on the interaction of Arsenic (III) with Glutathione

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Arsenic contamination in groundwater has reached alarming levels worldwide including India. According to a recent report published in "The Hindu" nearly 70 million people in 86 districts across 10 States in India are affected by arsenicosis from drinking water. In several organisms including humans, Glutathione (γ -L-Glutamyl-L-cysteinylglycine, GSH) plays a crucial role in the detoxification of heavy elements including Arsenic from

the body. GSH has eight dense binding sites i.e. Glu- α COO⁻, Glu- α NH₃⁺, Glu- γ CO, Cys-NH, Cys-CO, Cys-SH, Gly-NH and Gly-COO⁻, to which electrophiles coordinate depending upon the pH of the system and the steric constraints. As-GSH conjugates, mainly in form of As(GS)₃ (Arsenic triglutathione, ATG) and CH₃As(GS)₂ (Monomethylarsenicdiglutathione, MADG) are excreted from the liver into bile via MRP2 transporters. However, the third form, (CH₃)₂AsGS (Dimethyl arsenic mono glutathione, DMAG) has been found to be unstable and gets converted into a volatile compound i.e. C₂H₇As (Dimethylarsine) that diffuse into blood and binds to hemoglobin. Half-life of these complexes have been found to be between 5 to 40 minutes. Various methods such as Nuclear magnetic Resonance spectroscopy, ESI-MS, Liquid chromatography-mass spectrometry (LC-MS), Inductively coupled plasma mass spectrometry (ICP-MS), Hydride Generation Atomic Absorption Spectroscopy (HGAAS) and Electrochemical scanning tunnel microscopy (ECSTM) have been used to investigate these complexes both *in vitro* and in biological fluids. However, due to their instability and the harsh conditions of the commonly employed methods, these complexes are difficult to be detected in biological samples. The present study was designed to exploit spectroscopic methods to give direct evidence about the stoichiometry and stability of Arsenic-Glutathione complex *in-vitro*. Moreover, determination of the most stable conditions for these complexes would help in devising appropriate extraction procedures for their detection and quantification in biological samples.

Determination of Mass Attenuation Coefficient of Wood Biomaterials

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In the present work, mass attenuation coefficient of ten types of wood materials were measured and compared with XCOM theoretical values. The effective atomic number and effective electron density of the wood compounds were obtained using the measured values of their mass attenuation coefficients in the photon energy range 511 to 1332 keV, analysis of the data measured by NaI(Tl) scintillation detector. The study of effective atomic numbers of biologically important compounds is very useful for many technological applications. The mass attenuation coefficient, effective atomic number and effective electron density are the basic quantities required for determining the penetration of photons in matter. In the present study on the basis of the results, Mass Attenuation Coefficient decreases with increasing gamma energy. Mass Attenuation Coefficient increases with

increasing density, decreases with increasing thickness of the wood materials.

Crystal structure of a reconstituted archaeal L-asparaginase obtained by linker-less association of domains: isolated N-domain acts as non-specific chaperone

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Here we report that the isolated N- and C-terminal domains of a dimeric L-asparaginase from *Pyrococcus furiosus* (PfA) can assemble into a functional conjoined entity even in the absence of covalent linker. The conjoined PfA was found structurally similar and functionally superior to the parent protein as investigated using a series of spectroscopic probes, SAXS experiments and X-ray crystallography. Domain interfacial interaction analysis revealed the presence of strong network of non-covalent interactions holding the domains together in the conjoined molecule. Moreover, while the C-domain (CPfA) did not fold individually, the N-domain (NPfA) folds spontaneously forming stable, large, polydispersed oligomers that displayed chaperoning properties. Like other small molecular chaperones, the NPfA protected a variety of substrate proteins (carbonic anhydrase, α -amylase, malate synthase G) from thermal and refolding-mediated aggregation. In addition, NPfA inhibited polyQ-mediated amyloid formation and also facilitated disintegration of preformed amyloid fibrils of A β (1-42). Altogether, our study presents the following novel findings; 1) without any sequence homology to α -crystallin or any known molecular chaperone, NPfA emerged as a novel small molecular chaperone. 2) The linker region (19 residues stretch) in PfA was found dispensable. 3) Structural insights from substrate-bound complex also provided a novel active site composition and mechanism of action of this enzyme.

Cooperative assembly of isolated domains results in reconstitution of an active archaeal L-asparaginase

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L-asparaginase which hydrolyzes L-asparagine into L-aspartic acid and ammonia is being used as chemotherapeutic agent in the treatment of acute

lymphoblastic leukemia (ALL). Earlier our lab reported a L-asparaginase from the hyperthermophile *Pyrococcus furiosus* (PfA) and proved it as a better alternative to commercially available enzymes. From homology modeled structure of PfA we found that it consists of 2 distinct α/β domains; a large N-terminal domain (NPfA) and a small C-terminal domain (CPfA) connected by a 19 residues linker region.

To understand the effect of domain stabilities and inter-domain interactions in overall protein folding and functioning, the constituent domains were studied in isolation as well as in combination. Differential stabilities were observed at the domain level as independently expressed NPfA folded spontaneously, while the CPfA did not fold and formed insoluble aggregates. However, in a refolding mixture where both the domains were present in equimolar ratios, the NPfA was found to nucleating the folding of the CPfA. Thus, the former possibly acted as an internal chaperone. This co-refolded mixture of the domains assembled into a 'conjoined' protein, which matched well in size, hydrodynamic radii and solution scattering profiles with the wild type protein. SAXS and crystal data (2.3Å) of the conjoined protein indicated tetrameric complex of the constituent domains having similar molecular alignment and structure to the parent protein. Interfacial interaction analysis revealed the presence of a strong network of non-covalent interactions holding the domains together in the conjoined molecule. Interestingly, the conjoined enzyme not only regained functional active sites but also displayed higher activity than the wild type protein at all experimental temperatures. Structural insights from substrate-bound complex provided a novel active site composition and mechanism of action for this class of enzyme. Thus, this report proves that for the functioning of a multi-domain protein, inter-domain interactions are sufficient to reconstitute a functional active-site even in the absence of a linker. Moreover, the stability of the protein relied heavily on molecular recognition between the domains.

Identification of serum sirtuins as novel noninvasive protein markers for frailty

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Frailty has emerged as a major health issue among older patients. A consensus on definition and diagnosis is yet to be achieved. Various biochemical abnormalities have been reported in frailty. Activation of sirtuins, a conserved

family of NAD-dependent proteins, is one of the many mimics of calorie restriction which improves lifespan and health in experimental animals. In this cross-sectional study, we assessed the circulating sirtuin levels in 119 (59.5%) nonfrail and 81 (40.5%) frail individuals, diagnosed by Fried's criteria. Serum SIRT1, SIRT2, and SIRT3 were estimated by surface plasmon resonance (SPR) and Western blot. Serum sirtuins level in mean+SD; SIRT1 (nonfrail -4.67 ± 0.48 ng/ μ L; frail -3.72 ± 0.48 ng/ μ L; $P < 0.0001$), SIRT2 (nonfrail -15.18 ± 2.94 ng/ μ L; frail -14.19 ± 2.66 ng/ μ L; $P = 0.016$), and SIRT3 (nonfrail -7.72 ± 1.84 ng/ μ L; frail -6.12 ± 0.97 ng/ μ L; $P < 0.0001$) levels were significantly lower among frail patients compared with the nonfrail. In multivariable regression analysis, lower sirtuins level were significantly associated with frailty after adjusting age, gender, diabetes mellitus, hypertension, cognitive status (Mini Mental State Examination scores) and number of comorbidities. For detecting the optimum diagnostic cutoff value a ROC analysis was carried out. The area under curve for SIRT1 was 0.9037 (cutoff -4.29 ng/ μ L; sensitivity -81.48% ; specificity -79.83%) and SIRT3 was 0.7988 (cutoff -6.61 ng/ μ L; sensitivity -70.37% ; specificity -70.59%). This study shows that lower circulating SIRT1 and SIRT3 levels can be distinctive marker of frailty.

Biophysical Characterization of functional domain of BARD1 and mutants

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BARD1 (BRCA1-associated RING domain protein 1) plays a central role in DNA -damage repair mechanism which forms a heterodimeric complex with BRCA1. Few cancer predisposing mutations such as C645R, V695L, and S761N have been discovered within the C-terminal domain (BRCT) of BARD1. To dissect the structural alterations occurred due to mutations in BARD1BRCT (563-777) domain, we have cloned, expressed and purified different variants in bacterial system. FPLC, Chemical crosslinking assay and mass spectrometric analysis has confirmed the identity and monomeric behavior of the purified proteins. We performed Far-UV and Near-UV range CD spectroscopy to characterize the secondary and tertiary structure of proteins. Thermal denaturation study reveals that wild-type and BARD1 C645R mutant proteins unfold with similar T_m , however significant decrease in T_m were observed in BARD1 V695L, S761N mutants. Limited Proteolysis, DLS and Fluorescence spectroscopy were performed to characterized folding pattern of proteins. Comparative study of wild- type and mutants will thus be helpful to understand the folding pattern of BARD1 wild- type and mutant.

Optimization and Development of Force Field for Some Thiadizole Derivative

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Thiadiazole derivatives possess interesting biological activity probably conferred to them by the strong aromaticity of this ring system. Which lead to great *in vivo* stability and generally a lack toxicity for higher vertebrates including humans when diverse functional groups that interact with biological receptor are attached to this ring and possessing outstanding properties are obtained (1). The biological importance of thiadiazole derivative has promoted us to optimize some members of this family using Density function theory for various biological activities.

All the Molecule's are optimized by choosing Gaussian 6-31g(d) basis set using Gaussian-09 code. The comparative study of physical properties of the derivatives is shown. The force-field parameters of these compound is calculated for MD simulation using leap and antichamber under AMBER 14 code.

1. Thiadiazole – a Promising Structure in Medicinal Chemistry, Yijing Li, Jingkun Geng, Yang Liu, Shenghui Yu, and Guisen Zhao, Chemmedchem 2013,8, 27-41

Production and purification of Amyloid-beta peptide for in vitro studies

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Amyloid- β peptide ($A\beta$) mediates several neurodegenerative diseases. The 42 amino acid $A\beta_{1-42}$ is the predominant form found in neuritic plaques, and has been shown to be neurotoxic *in vivo* is the predominant form found in neurotoxic *in vivo* and *in vitro*. A synthetic oligonucleotides encoding $A\beta_{1-42}$ were constructed and amplified through assembly PCR followed by amplification PCR. A gene was cloned in to pET 41 a(+) vector. Addition of ethanol and GST tag present in the vector resulted in the production of large amounts of soluble $A\beta$ fusion protein. A fusion protein was subjected to a Ni-NTA affinity chromatography. $A\beta$ was cleaved from GST using Entriokinase and purified using glutathione shepharose affinity chromatography. The peptide yield was more than 15 mg/L culture, indicating the utility of method for high yield production of soluble $A\beta$ peptides. SDS-PAGE analysis confirmed the

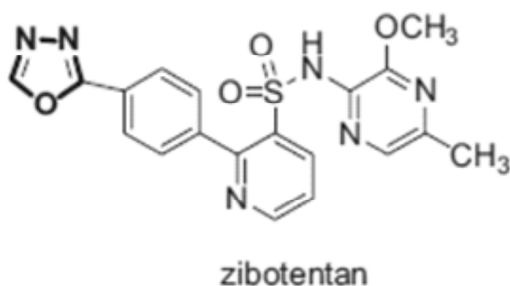
identity of purified A β fusion protein and A α peptides. This system provides the advantages over the chemical synthesis, avoiding human samples ethical issue and other conventional methods for Abeta peptide production. The availability of large quantities of A β peptides will help in several biochemical and biophysical studies which may lead to investigate the mechanism of aggregation and toxicity of these aggregated proteins or peptides.

Synthesis, Characterization and Biological Screening of Novel Heterocyclic Compounds With Biological Significance

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Medicinal chemistry is emphasizing on the search for new agents that could be effective against cancer. Cancer occurs due to abnormalities in cell cycle because of which cells proliferate and divide uncontrollably resulting in formation of malignant tumor. It is one of the major health issue in world. Various heterocyclic compounds have been recognized through molecular biology, empirical screening and rational drug development for assessment of anticancer agents during the past decades. Within drug discovery and development, a number of compounds containing an oxadiazole moiety are extensively used in late-stage clinical trials such as ataluren for the treatment of cystic fibrosis, raltegravir for the treatment of HIV infection, zibotentan as an anticancer agent.



Oxadiazoles group also display a broad range of biological profile such as antimicrobial, antineoplastic, fungicidal, inhibition of tyrosinase and cathepsin K. Also in organic synthesis they act as useful intermediates and widely employed as electron-transporting as well as hole-blocking materials. Further, they are excellent bioisosteres of amides and esters, which can enhance pharmacological activity by participating in hydrogen bonding interactions with the receptors. Keeping in view the significance of this moiety and in continuation to scope of medicinal chemistry we have designed to synthesize Oxadiazoles with free mercapto group and substituted mercapto group. Oxadiazole and its derivatives have been

synthesized according to the multisteps schematic chemical route. The reaction progress was monitored continuously using silica gel coated TLC plates under UV cabinet using hexane: ethylacetate (9: 1) solvent system. Various physical parameters were taken which involves determination of R_f values, LogP values, melting point and purification of compounds was performed using Column chromatography. All synthesized compounds were then characterized using different spectroscopic techniques such as Elemental analysis, FTIR (using ATR mode), ^1H NMR, ^{13}C NMR, UV-visible and ESI-MS spectral data. All synthesized compounds were shown to exhibit the characteristic peaks in their respective spectral data.

Azadirachtin inhibits the toxic aggregation of Human Islet Amyloid Polypeptide (hIAPP)

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Protein misfolding and aggregation plays an important role in many human diseases including Alzheimer's disease, Parkinson's disease and Type 2 diabetes mellitus (T2DM). Type 2 Diabetes mellitus, is a metabolic disorder that is characterized by the loss of insulin producing β cells. Human Islet Amyloid Polypeptide (hIAPP), a glucose metabolism associated polypeptide hormone is co-secreted with insulin in the pancreatic β cells. In pathological condition hIAPP plays a crucial role in amyloid deposition followed by deterioration of pancreatic islet function which further leads to β cell apoptosis. Since hIAPP is one of the most amyloidogenic polypeptide, therefore the development of effective inhibitors against formation of amyloid has been extremely challenging. Using computational approach, we obtained a series of drugs candidates that could be potential therapeutic agents against Type 2 diabetes. On the basis of Autodock results, we narrowed down our investigation towards Azadirachtin and its interaction with hIAPP. Azadirachtin is a tetraterpenoid polyphenolic compound, extracted from traditional plant *Azadirachta indica* (neem), which has been used as a medicinal extract against many diseases. We demonstrated that Azadirachtin may inhibit the amyloid fibril formation of hIAPP by various biophysical techniques. Circular dichroism (CD) and Fourier transformation infra red (FTIR) spectroscopy revealed the structural transition of Human IAPP in the presence of Azadirachtin. Fluorescence spectroscopy showed inhibition of Amyloid formation by Azadirachtin. ANS binding assay also showed the amyloid inhibition by decreasing the hydrophobic surface area in the presence of Azadirachtin. The morphological changes of hIAPP aggregation by Azadirachtin are also confirmed by Transmission Electron Microscopy (TEM). Our results suggest that Azadirachtin may act as good therapeutic agents against Type 2 diabetes.

Study of the role of arginine in stabilizing insulin fold using Biased-Exchange-Metadynamics Simulations

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Arginine is known to assist in folding of proteins. Present work is focused on study of the role of arginine in stabilizing the insulin fold. Bias-Exchange-Metadynamics (BEMD) simulations were carried out for insulin monomer in an aqueous solution at conditions determined by an experimental DOE on temperature, pH and arginine concentration. These molecular dynamics simulations accelerated by BEMD allowed us to estimate effect of DOE variables on equilibrium free energy of insulin fold. We were also able to determine relative stability of other metastable structures vis-à-vis the insulin fold. Finally a detailed structural analysis was carried out to determine secondary structure content, radius of gyration, hydrophobic surface area and arginine distribution around stable and metastable configurations of insulin monomer. To assess the accuracy of simulation protocol, effect of DOE variables on insulin stability and structure as obtained in our simulations was compared against corresponding experiments. Further, role of arginine in stabilizing the insulin fold was quantified by monitoring its interaction with the insulin backbone. Finally, aggregation prone segments of insulin molecule were identified by analyzing aggregation propensity of various metastable structures. In future we will use this information to either mutate or shield the aggregation prone segments to slow down fibrillation of insulin.

First comprehensive *in silico* identification of deleterious nsSNPs of human peroxiredoxin6 and their structural and functional characterization

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Peroxiredoxin 6 (Prdx6), a unique 1-cys mammalian peroxiredoxin having pH-dependent dual function of phospholipase A2 (maximal activity at acidic pH) and glutathione peroxidase (maximal activity at cytosolic pH), is ubiquitously expressed in most biological tissues and cell types. Despite the reported association of Prdx6 with various respiratory, neurological, ophthalmic, metabolic, cardiovascular diseases, and cancers, the comprehensive computational analysis of coding, noncoding and regulatory SNPs, and their functional impacts on protein

level, still remains unknown. Therefore, sequence- and structure-based computational tools were employed to screen the entire listed coding SNPs of *Prdx6* gene in order to identify and characterize them. We have identified 6 deleterious nsSNPs on the basis of concordant *in silico* analysis of four consensus classifiers for prediction of disease related mutants, namely, PredictSNP, PON-P, CONDEL, and Meta-SNP, and further their influence on protein's stability, aggregation propensity and ligand binding interactions has been studied via SDM, Met-Amyl and Discovery studio respectively. These genetic variants may help to develop novel therapeutic elements for associated diseases.

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Structural and functional characterization of *ribR* and *ribT* from *B.subtilis*

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Riboflavin biosynthesis is an obligatory pathway for many microorganisms as riboflavin is sole precursor of flavin coenzymes which are involved in several cellular processes. Bacteria and plants have ability to synthesize riboflavin *de novo*, but higher organisms depend on exogenous supply of riboflavin to meet their requirement. As riboflavin biosynthesis pathway is absent in humans, the enzymes involved in this pathway are considered as potential target for drug discovery. Riboflavin biosynthesis has been extensively studied in *Bacillus subtilis* and some other flavinogenic bacteria, yet not much is known about its regulatory mechanism. In *B.subtilis*, two genes *ribR* and *ribC* have been reported to play an important role in regulation of riboflavin biosynthesis. *ribC* has been studied extensively but not much is known about *ribR*. N-terminal domain of *ribR* is known to have flavokinase activity, whereas the function of C-terminal domain is yet unknown and is reported to bind riboflavin (RFN) element. In *B.subtilis*, an additional gene *ribT* with unknown function was also found to be part of *rib* operon and BLAST search have shown it to have an N-acetyltransferase domain. Since this protein is a part of *rib* operon it might play a role in riboflavin biosynthesis. Thus, using various biochemical, biophysical, structural and computational methods current study is intended to understand the molecular mechanism of both *ribR* and *ribT*. Accordingly, both *ribR* and *ribT* has been cloned, expressed and purified to near homogeneity. Further structural and functional characterization studies are under progress.

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Structural & Biophysical characterization of the Acyl CoA binding proteins (ACBPs) of *Leishmania major*

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Acyl-CoA-binding proteins (ACBPs) function as intracellular carriers of acyl-CoA esters, binding medium- and long-chain acyl-CoAs with high affinity. ACBPs have also been regarded as a model system for understanding the folding of all alpha helical proteins. In *Leishmania major*, we have identified 2 ACBPs whose function remains largely unknown. Given the fact that the amastigote stage (blood stage) of *Leishmania* depends largely on fatty acids and amino acids as the energy source, of which a large part is derived from its host, these proteins might have an important role in its survival. Here we report structural studies on two isoforms of ACBP, their localization in the cell and affinity for various acyl CoA's.

Dose and time dependent induction of MT by Zinc supplementation in rat liver

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Metallothioneins (MTs) are low molecular weight proteins characterized by high cysteine content and give rise to metal-thiolate clusters. The liver is important for the regulation of zinc homeostasis, while zinc is necessary for proper liver function. The intracellular concentration of Zn is tightly regulated, MT plays crucial role in its regulation. The Objective of this experiment is to determine the relationship between the Zn status in the rat liver and the occurrence of Hepatic MT. In this study we investigated the MT expression trend in rat liver, when Zn is given in dose and time dependent manner. Dose dependent study of MT by the subcutaneous injection of different amount of Zn 10mg, 50mg, 100mg Zn²⁺/kg body weight and for the time dependent behavior of MT 50mg/kg (body weight) dose was given at different time intervals i.e. 6,18,48 hours. Isolation of MT was done by using combination of Gel filtration and ion exchange chromatography while Characterization of MT fraction was carried in the wavelength range 200-400 nm. Expression of MT was studied by Western blot. The metal stoichiometry is being studied by using ESI-MS and PIXE. The study makes us understand that rapid incorporation and prolonged occurrence of metals with MT plays important role in the detoxification of heavy metals.

In silico Functional Annotation of Putative Conserved Protein from *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae is an obligate human specific pathogenic bacteria cause sexually transmitted diseases gonorrhoeae and its infection has adverse consequences on reproductive health and facilitates the transmission of human immunodeficiency virus. The emergence of multiple drug resistant strains, have led to new challenges for scientists to develop drug/vaccine for it. Analysis of proteomic data of *N. gonorrhoeae* showed that ~43% of the proteome are listed as hypothetical protein (HP), for which functions are not known. Study of these HPs leads identification of new biochemical pathways which appear to be involved in showing the resistance to the drug/vaccine. Furthermore, these HPs may serves as markers and pharmacological targets. Here, we have used various tools for sequence alignment, domain, motif search and search of possible family to annotate the functions of these HPs. Further, sub-cellular localization of these HPs gives an insight for possibility of being these HPs, a drug or vaccine target. Moreover, sequence analysis was performed to find out the amino acid composition and other physiological parameters such as aliphatic index, instability index and theoretical pI. Finally, functional annotation and identification of functionally conserved regions in HPs from *N. gonorrhoeae* may lead to better understanding of mechanism of its pathogenesis, adaptability and survival strategies to host cell, tolerance to host immune system, emergence of resistant strain and hence development of new drug/ vaccine.

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Conformational selection and ligand binding in uracil-DNA glycosylase from *Mycobacterium tuberculosis*

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Uracil, a promutagenic base, can arise in DNA either from deamination of cytosine or incorporation of dUMP during replication. Uracil-DNA glycosylase excises uracil from a single or double stranded DNA by cleaving the N-glycosidic bond. We report here seventeen independent crystal structures of family I uracil-DNA glycosylase from *M. tuberculosis* (MtUng) and their complexes with uracil

and its derivatives, distributed among five distinct crystal forms. The two domain protein is known to exist in a closed conformation when bound to DNA and in an open conformation when not. The existence of open and closed conformations of the enzyme in the present structures, suggests that the closure of the domain on DNA binding might involve conformational selection. The segmental mobility in the protein is confined to a 32-residue stretch in second domain, which plays a major role in DNA binding. Uracil and its derivatives can bind to the protein in two possible orientations. The positions of the uracil moiety in the two cases are related by a two-fold symmetry passing through N3 and C6. The thermodynamic parameters of binding of these ligands to the enzyme have also been determined using isothermal titration calorimetry. A reasonable rationale for the observed thermodynamic parameters is provided by the crystal structures of the complexes. Apart from the new insights into the structure, plasticity and interactions of the protein molecule, the results of the present analysis provide a platform for structure-based inhibitor design.

Molecular Mechanisms of Microbial Stress Response Regulators

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Microbes cope with unfavourable changes in the environment which include extreme physical conditions, presence of toxic chemical substances, lack of essential nutrition, etc. Survival of a species essentially depends on its ability to sense and adapt to such stress. Here, I present the recent results of the studies of a few regulatory proteins that confer resistance to a) arsenic toxicity or b) redox stress. a) ArsR belong to the SmtB/ArsR family of metalloregulatory proteins that sense the presence of toxic metalloids such as arsenic, antimony etc and trigger pathways to extrude them from the cell. To complement the wealth of biochemical information available on these molecules, we have undertaken structural investigations using X-ray crystallography. ArsR from a few species with high innate resistance to arsenic have been identified expressed and purified using recombinant techniques. We have successfully crystallized ArsR proteins from *Corynebacterium glutamicum* and *Acidithiobacillus ferrooxidans* in complex with arsenic and have made preliminary X-ray analysis. The crystals diffracted to 2.8 Å resolution. Experiments are on to grow bigger crystals for further X-ray crystallographic studies. b) Rex is a redox sensing protein that continually monitors the levels of oxygen in the cellular environment by sensing the ratio of cytosolic concentrations of NADH to NAD⁺ and triggers appropriate respiration pathways. Crystal

structures of Rex in complex with DNA/NAD⁺ or NADH are available. Rex is a homo dimer with each monomer consisting of two domains 1) DNA binding domain with HTH fold and 2) a nucleotide binding domain with Rossman fold. Binding of NAD⁺ or NADH at the nucleotide binding domain induces or inhibits DNA binding respectively and the mechanism has been proposed to be allosteric. We have undertaken targeted dynamics simulation studies and principal component analyses on Rex from *S. agalactiae*. Results indicate that binding of NADH causes 40.4° overall rotation of the DNA binding domain through a cascade of interactions originating from the nucleotide binding domain which results in bringing a few positively charged residues closer to the DNA binding site, causing instability resulting in dissociation from bound DNA and hence derepression.

Modulating drug release kinetics from gelatin nanoparticles by tailoring the secondary structure content of gelatin

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Drug delivery through nanoparticles offers several advantages over conventional methods. Gelatin owing to its biocompatible and biodegradable nature has been a polymer of choice for nanoparticle based applications. "Double desolvation", the preferred protocol for gelatin nanoparticle synthesis, involves harsh reaction conditions such as high temperature and extreme pH values. Any alteration in these parameters is known to affect the size of the particles synthesised. While some of these size variations are obvious, others are intriguing and require an understanding of the changes in the triple helical structure of gelatin molecules. Here we provide information regarding the gelatin structure as studied by different biophysical techniques under various conditions (temperature and pH) of particle synthesis, giving an insight into the structural basis of size variation in gelatin nanoparticles. Moreover, by screening various conditions for particle synthesis we have synthesised nanoparticles with native (triple helices intact) and denatured (random coils) gelatin. The degradation of gelatin by proteases is dependent on its structure, with the triple helices being less susceptible to protease action than random coils. Thus, by controlling the triple helical structure content in gelatin nanoparticles, we provide a novel method for modulating gelatin nanoparticle degradation and hence drug release. Through the present study we have made an attempt to address a basic issue of drug release modulation in gelatin based nanoparticles and provide a method of tailoring the structure content in gelatin nanoparticles to achieve desired drug release rates.

Structural status of a quasipalindromic segment present in the Human SCAI gene

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The binding of small molecules to DNA has been of great interest in order to design new efficient drugs targeted to DNA. Methylene Blue (MB), a phenothiazinyl dye, a kind of photosensitizer molecule showing promising applications in the photodynamic therapy (PDT) for anticancer treatment. It is known to interact with DNA due to its planar structure (1). We present here some results of our study related interaction of MB with a 24-mer DNA sequence (SG24) whose SNP (a G→T single base substituent) lies in the intronic region of Suppressor of Cancer Cell Invasion (SCAI) gene. SCAI is a highly conserved protein that regulates invasion cell migration. Decreased levels of SCAI are tightly correlated with increased invasive cell migration i.e., it is downregulated in several human tumours (2). Using biophysical and biochemical techniques Circular Dichroism spectroscopy, UV-Thermal-melting studies and Fluorescence spectroscopy, and Gel Electrophoresis, we show here that this 24-nt long DNA segment may possibly exist two polymorphic states most possibly the hairpin and bulge duplex forms. The results of the interaction studies between Methylene Blue (MB) and oligomer SG24, conclude that the sequences under study forms structures and interact strongly with methylene blue via intercalation mode mainly. The study may add to the knowledge of drug-DNA binding modes.

Biophysical Studies on the Binding of Janus Green Blue to Hemoglobin and Myoglobin

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Hemoglobin and myoglobin are heme proteins whose main physiological function is to bind molecular oxygen. Janus green blue (JGB) is a positively charged phenazinium dye useful in many biological applications. The binding of JGB to these proteins was studied by various spectroscopic and microcalorimetry techniques. The binding induced quenching of the protein fluorescence was characterized to be due to the formation of specific complexes at the ground state. The affinity of JGB to myoglobin was higher than that to hemoglobin as revealed from the spectral data. The dye induced conformational changes in the proteins and this was revealed from circular dichroism, synchronous fluorescence and 3D fluorescence spectra. The binding was characterized in calorimetry by endothermic heats.

The negative Gibbs energy, the positive enthalpy, and positive entropy changes characterized the binding of JGB to both proteins. The higher affinity of the dye to myoglobin ($K = 8.21 \times 10^4 \text{ M}^{-1}$) over hemoglobin ($K = 6.60 \times 10^4 \text{ M}^{-1}$) was confirmed from ITC results. Parsing of the Gibbs energy revealed that both polyelectrolytic and non-polyelectrolytic forces were involved in the binding interaction. The involvement of ionic interactions in the complexation was also confirmed from pH dependent studies. Detailed structural and energetics aspects of the interaction will be presented.

Investigating the Role of Fractional Surface Area ($\Delta\alpha$) in Counteracting Urea's Effect on Protein Stability

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Urea is found to accumulate in large concentrations in many marine organisms and mammalian kidneys. Urea is a potent denaturant and has been observed to perturb enzyme catalysis and protein-protein interactions. In order to counteract the deleterious effects of urea, organisms build up a class of osmolytes called methylamines. *In vitro* studies have shown that methylamines such as TMAO, sarcosine and betaine stabilize proteins and also have the ability to counteract the denaturing effects of urea. This counteraction happens at a concentration ratio of 2:1 (urea: methylamine). Earlier studies on the urea-methylamine counteraction on protein stability at this ratio resulted in partial counteractions, in fact, counteraction phenomenon is protein specific. It was argued that the most probable reason behind partial compensation or protein specific counteraction might be the difference in the fractional exposure ($\Delta\alpha_i$) of protein groups in different proteins. In order to examine this possibility, we have chosen three disulfide free protein having same GdmCl-induced denatured states and different heat-induced denatured states. We estimated ΔG_D° of proteins by GdmCl-induced denaturation and heat-induced denaturations. We observed that urea-methylamine compensatory ratio is different in case of thermally denatured Mb, barstar and b-cyt-c, but it is similar in the case of GdmCl-induced denatured proteins indicating that the structural characteristics of the denatured state of proteins plays a role in the urea-methylamine counteraction. We also observed a perfect urea-methylamine counteraction of proteins at 2.0:1.2 molar ratios in case of GdmCl-induced equilibrium of all proteins. The most probable reason for this is the difference in the structural characteristics of the denatured states of these proteins. Taken together, our data for urea-methylamine counteraction ratio clearly indicate that the structural differences of the denatured

states of proteins determine the perfect counteraction between urea and methylamine on protein stability.

Structural and computational study of RecU-Holliday Junction complex

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We have determined the X-ray structure of RecU from *Bacillus subtilis* in complex with DNA (Holliday Junction) at resolution of 3.2 Å. RecU is a general Holliday junction resolvase in gram-positive bacteria and catalysase in vitro DNA strand annealing. Two arms of Holliday junction are modelled into the electron density. It has provided structural insights into the binding of RecU to Holliday junction. Additionally a flexible stretch of 33 N-terminal residues is modelled into the density with the knowledge of probable binding to the 4-fold symmetric Holliday junction. Previous study of N-terminal deletion mutants report an important role of N-terminal region in binding of RecU to Holliday junction. Ab-initio modelling was performed based on SAXS data of Apo form of RecU. Resulting envelope is observed to reasonably fit the X-ray structure and gives an indication of high flexibility of N-terminal region in solution. Extended arm conformation was further confirmed with ensemble optimized modelling of SAXS data. This N-terminal region consist of 3 proline residues of which Proline 5 is conserved in the entire RecU family while Proline 12 is conserved in close family as seen from phylogeny analysis. To confirm the role of these prolines in the conformation of N-terminal arm, we performed MD simulations with single, double and triple P to A mutants. MD simulations of Holliday junction in complex with RecU were performed to get further insight to the binding mechanism.

Temperature Dependent Molecular Dynamics Simulation of Ubiquitin

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Since the temperature is a crucial sensitive factor for the behavior of the biomolecules, the study of controlled temperature is an important insight for the molecular dynamics simulation of proteins. In the present study,

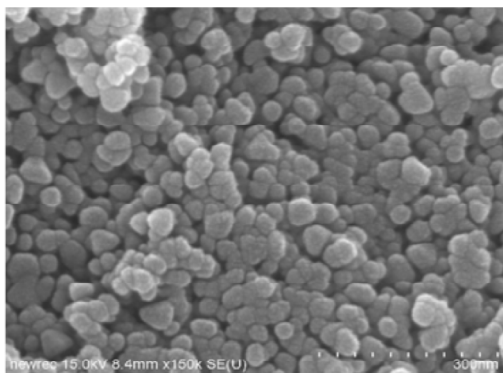
we have tried to estimate the role of temperatures on the atomic change in properties of the Ubiquitin by CHARMM forcefield. We have simulated our system with the solvated protein to bring a comparison with the normal one. Firstly, we have calculated the energies of both the normal and solvated Ubiquitin with water and K⁺ and Cl⁻ ions at room temperature. Then, we analyzed the effect of temperature on the protein by controlled variation in temperatures. Additionally, at varying temperatures we have calculated the binding interactions between Ubiquitin and lipids to study the real phenomenon in the human body. In this study, we have worked with POPG (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol) lipid. From our investigation, it is concluded that there is a significant change in energies during the conformational trajectory change of the protein with varying temperatures. The standard analysis shows a comparison of crucial energy drift between non-equilibrium and equilibrated molecular dynamics. By the apparent detailed comparative method, it will be potentially useful in the future study of the temperature effect on other proteins.

Effect of surface charges on bacterial cytotoxicity of Zinc Oxide nanoparticles

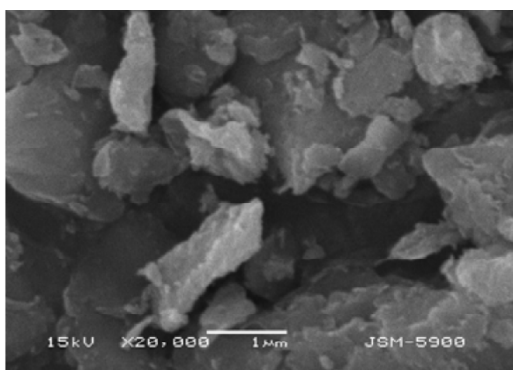
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With the rapid developments in the field of nanotechnology, our exposure to nanomaterials in daily life in cosmetics, medicines, electronics, etc., is continuously increasing. Hence, in recent times there has been a growing concern over the potential toxic effects of nanomaterials. Earlier studies investigating the effect of parameters such as particle size and surface charge of nanoparticles on living systems have reported that smaller sized particles are more toxic and negatively and positively charged particles have different effects. In particular, studies report that positively charged nanoparticles are more toxic than negatively charged ones, but this is still under speculation. In our study we have synthesized ZnO nanoparticles having positive (using IPA) and negative charges (using Zinc acetate dihydrate) on their surface and have used them for toxicological studies on *E. coli*. These particles were characterized using SEM for morphology and size estimation while surface charges were estimated using Zeta potential measurement. The nanoparticles were added to the LB medium at a fixed concentration of 2mM and the media was inoculated with different sizes of inoculums ranging from 0.005% to 1%. Growth kinetics were studied by plotting absorbance at 600nm versus time. Fluorescence emission study was also performed at time points of 2 hrs. Our results show that anionic ZnO



Cationic ZnO



Anionic ZnO

nanoparticles with are more toxic than cationic ZnO nanoparticles. Further, the effect of the differently charged nanoparticles becomes clearer at higher inoculum concentrations.

Surface functionalized Superparamagnetic iron oxide Nanoparticles: Design, synthesis and hyperthermia therapy application

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Magnetic nanoparticles (MNPs) with appropriate surface chemistry have been widely used extensively for numerous in vivo applications such as magnetic resonance imaging contrast enhancement, immunoassay, detoxification of biological fluids, hyperthermia, drug delivery and in cell separation, etc. All these biomedical and bioengineering applications require that these Nanoparticles which have high magnetization values and size smaller than 100nm with overall narrow particle size distribution, so that the particles have uniform physical and chemical properties. The size and different properties of magnetic nanoparticles are strongly influenced by synthesis method adopted.

In this context in the present investigation, we adopted a new approach to synthesis of Fe_3O_4 MNPs. We chose alkaline media (DIPA) as a co-precipitating agent. Surface of Fe_3O_4 nanoparticles were successfully modified with Pluronic F127. The particle size of the synthesized nanoparticles was ~ 10 nm. The Pluronic F127coated nanoparticles exhibit narrow size distribution, good dispersibility and very good suspension stability as compared to uncoated Fe_3O_4 nanoparticles. It was found that coating with Pluronic F127 enhances the hydrophilicity caused the nanoparticles to form stable suspension in water. Pluronic F127 coated nanoparticles show superparamagnetism at room temperature with blocking temperature $T_b \sim 200\text{K}$. Induction heating studies of Pluronic F127coated nanoparticles were investigated for their possible application in magnetic fluid hyperthermia (MFH). The coated nanoparticles exhibited self-heating temperature rise characteristics when subjected to external magnetic field at different particle concentration and field amplitudes and from results found that the synthesized Nanoparticles show highest specific absorption rate ~ 600 Watt/gm. All syntheses were carried out in a well defined and controlled reaction setup. Magnetic properties of synthesized MNPs were measured by a superconducting quantum interference device (SQUID) and crystallographic and chemical properties by TGA, XRD, FTIR and TEM etc.

Signature of functional ligand binding to proteins from conformational thermodynamics

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Functional ligand binding to a protein is often specific to the conformational state of the protein. We show that the thermodynamically destabilized and disordered residues in different conformational states of a protein can serve as binding sites for ligands. This is illustrated in case of alpha-lactalbumin (aLA), a divalent metal ion binding protein. We use the histograms of dihedral angles of the protein generated from all-atom molecular dynamics simulations to calculate the conformational thermodynamics. The ligand binding site is successfully tested for β -1,4-galactosyltransferase ($\beta 4\text{GalT}$) binding to the Ca^{2+} -aLA complex through the destabilized and disordered residues. However, in the Mg^{2+} -aLA complex no such thermodynamically favourable binding residues can be identified. Applying similar analysis we predict that Ca^{2+} -aLA can bind to oleic acid through the basic histidine (H) 32 and nearby hydrophobic residues of the interfacial cleft, where, the resulting complex is known to exhibit cyto-toxic activity against tumor cells.

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Probe-dependent study of protein aggregation in crowded environments

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Protein aggregation or misassembly is a grave problem in human beings which ultimately leads to various neurodegenerative diseases like Alzheimer's (β -amyloid protein), Parkinson's (α -synuclein protein), Type-II diabetes (amylin protein), Prion disease (prion protein), etc. To design a proper and effective clinical treatment for these debilitating diseases, it is imperative to know the underlying mechanism of this process and also devise possible counter-measures.

Study of protein aggregation in crowded media apart from studying in buffer is of much relevance because inside the cell the aggregation or misassembly problem is even more acute.¹ It is well known that crowding enhances association reactions by one to three orders of magnitude.² To simulate the actual interior environment of the cells some inert macromolecular crowders such as Dextran (M.W 6/40/70 kDa) and Ficoll (M.W 70 kDa) have been used in this study. One of the prime concerns of this study is to establish curcumin as a novel probe for monitoring the process of protein aggregation.

Curcumin (diferuloylmethane), a naturally occurring polyphenol has been proposed here as a molecular probe alternate to thioflaving T (ThT), the latter being one of the most commonly used assays for protein aggregation. To overcome the insolubility of this polyphenol in aqueous medium, the well-known drug carrier molecule β -Cyclodextrin (β -CD) has also been used in our study.³

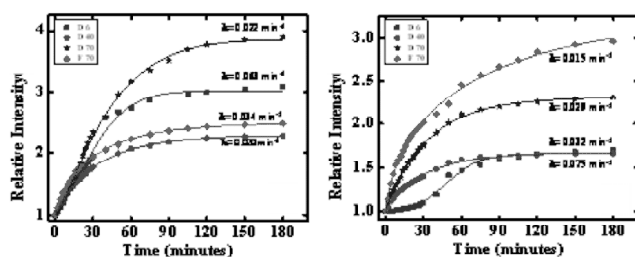


Figure 1: Comparison of relative fluorescence intensity of curcumin in absence of CD (left figure) & presence of CD (right figure) at 520 nm. The aggregation was carried out at 200 g/L of crowder concentration.

Using bovine serum albumin (BSA) as the model protein for the aggregation study, our data show that curcumin is not only responsive, i.e., senses protein aggregation but also provides a different insight into the heterogeneity of BSA aggregation. The obtained results have been explained on the basis of curcumin binding to different sites on the aggregates as that compared to ThT thereby

providing an alternate means of investigating and approaching the problem of protein aggregation. The presence of β -CD and macromolecular crowders also adds to the importance of these findings, especially with β -CD having often been used as a pseudo-chaperone.

In-silico docking studies of curcumin and its analogues in search of potent inhibitors of α Synuclein aggregation

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Parkinson's disease (PD) is associated with oligomerization and amyloid formation of α -synuclein (AS). The polyphenolic Asian culinary ingredient curcumin has proven to be effective against a wide range of human diseases including cancers and neurological disorders. Using docking experiments we have studied the effects of 44 natural and synthetic analogues of curcumin on AS. This *in silico* study investigates the inhibitory effects of curcumin analogues against AS self-aggregation. Collectively our results suggest that curcumin analogues can be pursued as candidate drug targets for treatment of PD and other neurological diseases.

Do macromolecular crowding agents only exert excluded volume effect? A protein solvation study

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Crowding at the physiological level stands well-appreciated in the scientific community. This has resulted in a recent surge in studies of biomolecular conformations and dynamics in presence of both synthetic and protein based macromolecular crowding agents (1). A general consensus is that the crowders influence the protein conformations by the well-known 'excluded volume effect' wherein these reduce the available conformational space to the biomolecules. Usage of BSA and Lysozyme as crowding agents have however revealed enough evidence toward the presence of non-specific electrostatic associations with the protein molecule of interest. This is indeed expected as the proteins have an appreciable distribution of surface charged residues that can potentially show significant interaction with the probe molecule (2) which itself has its own share of charged amino acid side-chains. Though the same has been hinted at to some extent for the Ficoll, PEG and Dextran based

synthetic crowders that are uncharged and are often used to mimic the intracellular milieu, strong evidence of the same is still hard to come across. The latter arises in part because majority of studies dealing with modulations in protein conformations in presence of these synthetic crowders are performed at quite high concentrations of these crowding agents. Under such conditions the excluded volume phenomenon predominates and masks the other crowder-induced effects that can also influence the proteins. In this study, we have probed how ANS bound to the serum albumin proteins, BSA and HSA, is solvated by the proteins in response to the synthetic crowders, Dextran (6, 40 and 70) and PEG (200, 8000). Our study reveals that the crowders at very low concentrations (2 - 10 g/L), wherein the effect of excluded volume is minimum, can have significant influence on how the protein solvates the bound ANS molecules. Negligible changes at the equilibrium level but dramatic modulations in dynamics imply that the crowders show appreciable transient/weak non-specific interactions with the proteins thereby opening up a new direction that will significantly contribute to the overall importance and understanding of the phenomenon of macromolecular crowding.

Novel peptides binding to glucokinase and its therapeutic implications in type 2 diabetes: a computational study

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Glucokinase (GK) is a type -IV hexokinase, a glucose phosphorylating enzyme that is known to regulate secretion of insulin and hepatic metabolism. Its loss of function is implicated in diabetes type 2. Recently it has been shown that phosphomimetic of BCL-2 homology 3 (BH3) α -helix derived from human BAD, a GK-binding partner protein, can stimulate GK. The interaction between GK and BAD -BH3 is known to enhance insulin secretion. This BAD - BH3 phosphomimetic peptide binds to a new site apart from the allosteric site in GK. In the present study, 29 novel peptide sequences based on the phosphomimetic BAD-BH3 have been designed. A state of the art protein -protein docking program, PIPER (Schrodinger 2014 v9.3) was used to dock all the 30 molecules with closed crystal structure of GK. 26 peptides docked at a new site different from the allosteric site. The binding site of the phosphomimetic BAD-BH3 and computationally designed dehydrophenylalanine (\ddot{A} F) containing peptides, in GK has been compared. This cluster of peptides has been analyzed with respect to points, distance and energy of interaction between the designed peptides and the protein.

Optical Tweezer a tool to study Bacteria – Nanoparticle interaction

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Optical tweezer system is a versatile high resolution force measurement tool. The principle of optical trapping is based on light particle interaction and to measure forces on these particles in picoNewton range. In the present work, modular optical tweezer consisting of mainly laser diode ($\lambda=975\text{nm}$), high numerical aperture objective, Quadrant photodetector system and image acquisition system is used to trap *E-coli* bacteria. Optical trapping has been used to study bacterial dynamic, sorting of bacteria and antibacterial activity. Rotation of *E-Coli* bacteria within the optical trap is measured by varying the laser power. Effect of ZnO nanoparticles on *E-Coli* bacterial rotation is observed. However, Optical tweezer is used to measure the forces between *E-Coli* and ZnO nanoparticles.

Synthesis and Characterisation of Apocynin loaded PLGA nanoparticles – An *in vitro* study.

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Apocynin is widely used as a neurodegenerative cure along with known anti-inflammatory properties. Due to poor solubility of this drug there are certain apprehensions about its bio-availability. Since PLGA (Poly Lactic-co-Glycolic Acid) encapsulated drug nanoparticles have capability to attain a sustained release and cause minimal drug loss, they enhance the efficiency and bio-availability of drugs. Therefore our present study is aimed at envisaging a novel approach of synthesising and characterisation of apocynin loaded PLGA nanoparticles. Nanoparticles both unloaded and loaded were prepared using solvent extraction method followed by its analysis in terms of size and stability. Characterisation of nanoparticles was performed by TEM (transmission electron microscopy), measuring zeta potential and Dynamic Light Scattering (DLS) to check the average size of prepared nanoparticles. Furthermore, drug release and encapsulation efficiency of the drug was calculated *in vitro* by forming a standard curve. Our results suggested a stable drug loaded nano-suspension with desired size ranging from 217-259 nm and controlled percentage release of 0.3-0.5%. These new nanoparticles may have a better ability to counteract pathogenic conditions believed to be treated by apocynin in its natural state.

Role of Lysine residues on the Origin of Novel Absorption Spectra in the Near Ultraviolet region among Proteins devoid of aromatic residues

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Absorption in the UV region has been dominated by aromatic amino acids in peptides and proteins owing to the presence of a conjugated structure in their side chains. However, L-Lysine hydrochloride was reported to display a unique absorption and luminescence feature at high concentrations (~0.5 M) in aqueous medium. Blue luminescence (~435 nm) was visible on excitation at 355 nm. The above features which were concentration dependent are attributed to likely aggregates of L-lysine. [Homchaudhuri & Swaminthan, Chem. Lett. 2001]. Similar observations arising from interactions between two or more lysine residues present in close spatial vicinity in lysine rich proteins like Human Serum Albumin have also been reported. [Homchaudhuri & Swaminthan, Bull. Chem. Soc. Jpn. 2004]. It is intriguing to know the origin of these novel spectra in the absence of any aromatic moiety. In an attempt to further understand the nature and origin of these unusual spectra we present here the investigations on the interactions among lysine residues in Alpha 3C, a 68 residue protein containing 17 Lysine residues while being devoid of aromatic amino acids. Some work on absorption spectra of amine containing non-aromatic compounds and lysine containing short peptides devoid of aromatic amino acids in aqueous medium will also be presented.

Sub-stoichiometric concentrations of polyphenols in combination with β -cyclodextrin can inhibit as well as disaggregate α -synuclein amyloids under cell mimicking conditions

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Protein aggregation results in pathogenesis of a number of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Type 2 diabetes, prion disease, Huntington's disease etc.¹ Human α -synuclein is known to be present as inclusion bodies known as Lewy bodies and Lewy neuritis in the brain tissues during the

course of Parkinson's disease.² In our earlier studies, we have shown that an optimized cocktail of curcumin and β -cyclodextrin (β -CD) can not only inhibit the aggregation of α -synuclein but also disaggregate the preformed aggregates *in vitro*.³ In the current work we have shown that the combination of curcumin and β -cyclodextrin also works under conditions mimicking the intracellular environment i.e. in the presence of macromolecular crowding agents. This work has been further extended to include polyphenols like resveratrol (from red grapes), baicalein (from *Scutellaria baicalensis*) and epigallocatechin gallate (EGCG) (from green tea) to test the efficacy of these in presence of β -CD in blocking aggregation and breaking up pre-formed aggregates. Our data reveal that these compounds work via a synergistic mechanism thereby bringing down the actual effective concentrations of these agents had these been used alone.

Fas-activated serine/threonine kinase: A Multidisciplinary Protein

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Human fas-activated serine/threonine kinase (FASTK) plays a significant role to protect the cells under stress conditions. It is a mitochondria-associated protein that promotes the survival of cells exposed to adverse environmental conditions. The polypeptide structure of human FASTK comprised 465-amino acid residues. The total number of Ser and Thr residue and aromatic amino acid constitutes 10.1% and 8.38%, respectively. The theoretical pI, molecular mass and molar extinction coefficient was calculated which was found as 9.82, 51504.29 Da and 29870 M⁻¹cm⁻¹ respectively. The sequence alignment of FASTK was evaluated with other mammal which shows 90, 91, 98, 87, 98, 88% similarity with the *Bos Taurus*, *Sus scrofa*, *Pan troglodytes*, *Mus musculus*, *Pan paniscus*, *Elephantulus edwardii* FASTK counterpart respectively which further supported by phylogenetic tree. Interestingly, this protein contained 14 cysteine residues that form seven disulfide linkages. The number of disulfide bond may be decrease because out of seven, three Cys residues (Cys21, Cys61, and Cys62) has been predicted as metal binding site. The conserved motifs have been identified by using motif scan online tool. This protein has a putative myristoylation site, casein kinase II phosphorylation site and protein kinase-C phosphorylation site. These motifs may be responsible for phosphorylation property and may help for its role in signaling pathways. The association of FASTK with various interacting protein was analyzed by using online server STRING. In the confidence view of SRING server, strong association of FASTK was found with T cell intracellular Ag-1 (TIA1)

highest score is 0.946. Furthermore, the antigenicity plot has shown that the highly antigenic amino acid residues has constituted. In the best of our knowledge, there is no report on the biophysical analysis of FASTK and its structural interaction with TIA-1. This work is an effort to provide an idea to understand and explore the role of FASTK in the aspect of cell biology.

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Binding Studies of cysteine protease inhibitor SAP-1 with Heparin

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SAP-1, low molecular weight cysteine protease inhibitor (CPI), belongs to type-2 cystatins family. SAP-1 protein purified from human seminal plasma (HuSP) has been shown to inhibit cysteine and serine proteases and exhibit interesting biological properties, including high temperature and pH stability. Heparin is a naturally occurring glycosaminoglycan (with varied chain length) which interacts with a number of proteins and regulates multiple steps in different biological processes. In this study, we have employed surface plasmon resonance (SPR) to improve our understanding of the binding interaction between heparin and SAP-1 (protease inhibitor). SPR data suggests that SAP-1 binds to heparin with a significant affinity ($K_D = 158$ nm). SPR solution competition studies using heparin oligosaccharides showed that the binding of SAP-1 to heparin is dependent on chain length. Large oligosaccharides show strong binding affinity for SAP-1. Further to get insight into the structural aspect of interactions between SAP-1 and heparin, we performed *in silico* docking studies of the SAP-1 with heparin and heparin-derived polysaccharides. The results suggest that a positively charged residue lysine plays important role in these interactions. We believe that this information will improve our understanding about how heparin, present in the reproductive tract, regulates activity of cystatins.

An iterative method for error correction in PacBio reads using multiple next generation sequencing read sets: a case study with big genome data

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Next generation sequencing technologies have revolutionised the field of genomics by providing an

economical, high throughput alternative to first generation sequencing. However, they produce shorter reads, of lengths ~ 30700 base pairs. In addition, their reads can contain context specific errors for example, a base composition bias in Illumina reads, introduced during an amplification step required before sequencing. A key third generation technology, that of Pacific Biosciences (PacBio), addresses these issues by examining single molecules in real time using their SMRT technology, thereby eliminating the need for amplification and generating very long reads (up to 20000 base pairs). This should ease the task of assembling reads into sequences, but for one major limitation: the base call error rate, which is $\sim 11\%$ insertion deletions and $\sim 3\%$ mismatches, uniformly dispersed across the read length. Often these errors are removed using less error prone Illumina sequences, but existing implementations are demanding from the standpoint of computational resources.

Here we present a method for correcting PacBio reads, which iteratively targets errors using short reads, and can be adapted to suit existing data and computational resources. The method is implemented in a case study using genomic data from *Amaranthus hypochondriacus*, a dicot of high nutritional and research value, in which ~ 2.4 million reads sequenced at 25X coverage on PacBio's RSII platform with C5 chemistry, are corrected in 4 iterations for substitutions followed by insertion deletions, first using Illumina mate paired reads sequenced at 31X coverage, then using Illumina pairedend reads sequenced at 74X coverage. The effectiveness of correction is studied by mapping the short reads back to the corrected long reads.

Thermal and chemical denaturation studies of horse cytochrome c and its mutants.

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Cytochromes *c* (cyts-*c*), a small globular protein (12.5 kDa) in which heme is covalently bound to the polypeptide chain via thioether bonds. Cyt-*c* has been widely used as a model protein to gain information about the mechanism of protein folding and unfolding processes. More than 287 complete sequences of cyts-*c* are reported from various sources (www.uniprot.org), and they show many evolutionary conserved residues. It is evident from structure and function studies of cyts-*c* that only few residues are important for protein folding and stability. Apart from heme-binding residues such as Cys14, Cys17, His18, there are four positions in cyts-*c*, which are conserved in all subfamilies. These positions are Gly/

Ala6, Phe/Tyr10, Leu/Val/Ile94 and Tyr/Trp/Phe97. Crystal structure studies on all cyts-c yielded identical 3-D structure and showed that these four residues form many significantly strong contacts. From these studies, it was concluded that these are quite important for the protein folding and stability. It is noteworthy that these residues at position 6, 10, 94 and 97 of horse cytochrome *c* (h-cyt *c*) offer 23 interatomic contacts between the N and C-terminal helices alone. To determine the importance of conservative substitution at position 94 of h-cyt *c* in the structure, folding and stability of the protein, we made L94V/L94I/L94F mutants by site directed mutagenesis. Structure of these mutants were characterised by the far-UV, near-UV, and Soret circular dichroism, absorbance, intrinsic and 1-Anilino-8-naphthalene sulfonate fluorescence, and dynamic light scattering measurements at pH 6.0 and 25 ± 0.1 °C. Furthermore, thermal and chemical stabilities of all mutants were compared with those of the wild type protein.

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Cloning, Expression, Purification and Biophysical Characterization of Mammalian cell entry (Mce4A) Protein

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Mammalian cell entry (Mce4A) is an α/β type of protein, belongs to the MCE-family of *Mycobacterium tuberculosis*. The gene *mce4A* is homologous to *mce1A* of *M. tuberculosis*, which plays a central role in host cell invasion. Mce4A also helps in the long term survival by cholesterol utilization and leads to persistent infection of *M. tuberculosis*. Host cholesterol utilization mechanism by Mce4A is not clearly understood. Here, we performed cloning, expression and purification of Mce4A. The gene of *mce4A* was cloned in *pET28a* with hexahistidine tags under control of inducible promoter and expressed in the *E. coli* BL-21(DE3) strain. The recombinant protein was purified under denaturing condition by affinity chromatography on Ni-sepharose. The purified protein was checked by SDS-PAGE and Western blot which shows that Mce4A protein is ~43kDa. We further performed biophysical studies to know the structural features of this protein under different conditions of temperature, pH and salt.

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Analyzing Node Interference in Wnt activated p53 repair network

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The availability of high-throughput data from experiments increases the availability of the large network datasets. It requires the detection of quantitative and qualitative changes in the topological properties in the biological networks. In order to quantify the properties and analyze the functionality of nodes, the measure of interference between the nodes (gene, protein, metabolites) is important. Within this time, many different methods have been developed (barbasai et al 2004 etc.) which focuses on the global network properties and on their global modifications. Recent fundamental studies by liu et al 2011, shows that identifying driving nodes in a network is fruitful in establishing the functionalities of the networks and also getting the deeper understanding of the large biological network. We analysed the theoretical significance of centraliy based node interference on wnt activated p53 repair pathway using equation ($Int_{Btw}(i,n,G)=relBtw(G,n) - relBtw(G_{|i}n)$). Since, Node interference allows us to measure or topologically determine the functional influence of the node (i.e specific) on the whole network. Notably we found interesting results that are in agreement with the experimental findings.

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Effect of Natural molecular crowding on the structure and stability of DNA Duplex

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We systematically and quantitatively investigated the effect of glucose as a model of natural molecular crowding agent on the structure and thermodynamics of Watson-Crick base paired three duplexes (named as D1, D2 and D3) of different base compositions and lengths. Structural analyses demonstrated that duplexes (D1 and D2) folded into B-form with different cations in the absence and presence of glucose while duplex (D3) folded into mixed A and B-form. Moreover, we demonstrate that the duplex is more stable without glucose under all cationic conditions tested (Na^+ , K^+ , $Na^+ + Mg^{++}$ and $K^+ + Mg^{++}$)

[free energy change at 25°C (ΔG_{25}°) of for duplex (D1) were -13.56, -13.76, -12.46, and -12.36 kcal/mol, for duplex (D2) were -13.57, -12.93, -12.86, and -12.30 kcal/mol, for duplex (D3) were -10.05, -11.76, -9.91, -9.70 kcal/mol] respectively. Moreover, these duplexes are marginally destabilized at high concentration of glucose (DNA: glucose ratio was 1:10000), [ΔG_{25}° for duplex (D1) -12.47, -12.37, -11.96, -11.55 kcal/mol, for duplex (D2) -12.37, -11.47, -11.98, -11.01 kcal/mol and for duplex (D3) -8.47, -9.17, -9.16, -8.66 kcal/mol]. These results suggest that glucose molecules strongly interact with water and act as a weak structure breaker on the tetrahedral network of water.

In silico Function Prediction of Conserved Telomerase Reverse Transcriptase (Tert) Therapy in Delaying Ageing

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Telomeres are the tandem repeats of the TTAGGG sequence and these are bounded by a complex of protein called Shelterin. Telomere protection targets six proteins, TRF1, TRF2, TIN2, RAP1, POT1, and TPP1, the shelterin complex protects the ends of telomeres from genomic rearrangement and binds to telomeres, and protects telomeres by repressing DNA damage response (DDR) at telomeres and preventing chromosomes fusions. Loss of shelterin proteins derepresses DDR and allow non homologous end joining (NHEJ) of chromosomes ends, resulting in chromosome end-to-end fusions and genomic instability. Telomerase confirms limitless proliferation potential to most human cells. Dysfunction of telomerase can lead to either cancer or ageing pathogenesis. Anti-ageing activity of telomerase has been demonstrated in mice and it is found that over expression of TERT genetically engineered causes enhanced expression of the p53, p16, p19ARF tumor suppressors. This enhance the fitness of various physiological barriers accompanied by systemic delays in ageing.

Archaeal $\beta\gamma$ -crystallins: may be twins but one of a kind

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$\beta\gamma$ -Crystallins are the ancestrally related, prevalent protein domains with all- β structure organized in specific Greek key pattern, forming the $\beta\gamma$ -crystallin superfamily. Diversification of this domain in the primary structure,

during its recruitment in different proteins from evolutionarily distantly placed organisms, therefore altering the properties (or functions), has been an important subject of investigation. Among the >170 archaeal genome sequences currently available in database, only two methanoarchaea viz. *Methanosarcina acetivorans* and *Methanosaeta thermophila* have $\beta\gamma$ -crystallins (M-crystallin and Methallin respectively), while widely prevalent in bacteria and eukaryotes. Both structurally similar methanoarchaeal $\beta\gamma$ -crystallins are different from each other: M-crystallin binds Ca^{2+} similar to many bacterial homologue, whereas Methallin does not. Methallin which does not have bacterial type Ca^{2+} -binding motif, exclusively binds trace metals, viz., Fe, Ni, Co and Zn at the site formed by six histidine residues in the core of the trimeric arrangement assisted by two histidines from one molecule. Methallin binds nickel with a high affinity (dissociation constant (K_d) of 44.8 nM at 30°C). Such binding geometry is unprecedented not only in $\beta\gamma$ -crystallins but also in other proteins and is a classic example of diverse evolutionary inventions and adaptations manifested in this ancient methanogen, i.e., *Methanosaeta thermophila*. Incorporating two histidine residues at equivalent position does not lead to the formation of such binding site in other $\beta\gamma$ -crystallins, thus metal coordination by histidine and trimeric organization is specific to Methallin. Nickel is an essential trace metal required by methanoarchaea for the methanogenesis and their growth. The study, therefore, suggests the adaptability of the $\beta\gamma$ -crystallin fold to the presence of excess nickel in the environment and also indicate its potential early origin. Methallin, which represents a distinct $\beta\gamma$ -crystallin with a strong possibility for a role in combining trace metal biology with the geological record, enlightens us for exploring the ancient life and evolution of these molecules. Thus, it is quite possible that *Methanosaeta thermophila* is one of those organisms, which existed covering the transition periods of anaerobic to aerobic life forms overlapping to the Great Oxidation Event.

Combined Effect of Hypergravity and Fertilizer on Growth Rice Seedlings

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Plants have evolved under the constant force of gravity and its presence strongly influences growth and development of plants. Gravity provides a directional stimulus which plays an important role in basic life process in the cell, like biosynthesis, membrane exchange, and cell growth and development. Earlier studies carried

out in our laboratory showed decrease in growth and chlorophyll content in rice seeds under hypergravity. Studies related to seeds soaked in different fertilizer solutions before planting showed significant enhancement in growth and chlorophyll content. In the present work, experiments were carried out to study the combined effect of hypergravity and N:P:K (50:30:30 kg/ha) fertilizer on rice (*Oryza sativa* var. Ratanagiri) seeds. Seeds were soaked in fertilizer solution of concentration N=0.02601g/l, P=0.00660g/l, K=0.01257g/l for 12 hr, and exposed to Hypergravity value 2500g using a centrifuge (Super spin-R-V/FM, manufactured by Plasto Crafts Industries (P) Ltd, Mumbai, India) for 10min. Seeds were then grown on 0.8% agar for 10 days in controlled environmental conditions. Physiological response of 10 days old rice seedlings was studied by comparing it with control. Root length, shoot length were measured on 10th day. Chlorophyll was extracted by using DMF as a solvent and its absorption spectra were recorded. The protein concentration from seeds of rice seedlings was estimated by using Bradford method on 10th day of their growth. The results obtained showed that root length, shoot length increases in seedlings raised from seeds exposed combinely to hypergravity and fertilizer than the control one. Similar results were obtained in chlorophyll content, alpha amylase activity, and total amylase activity. The results obtained showed that protein concentration increases in seedlings raised from seeds exposed to only with fertilizer solution than control and other treatments.

Inhibition of long term memory and induction of biochemical deficits in rats following protofibrillar A β 1-42 injection.

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Amyloid-beta(A β) peptide deposition into insoluble plaques is a pathological hallmark of Alzheimer's disease (AD) but the soluble oligomeric A β has been hypothesized to directly impair the learning and memory in AD. Evidences from some clinical studies indicated that A β protofibril formation is induced by the Arctic mutation (E22G) and is the major cause for early AD onset. However, the biochemical mechanism involved in the protofibril-induced toxicity is not very well addressed. So, the present study was undertaken to study the effects of single intracerebroventricular (i.c.v.) injection of protofibrillar A β 1-42 on the long term memory. Rats were divided into two groups (n = 5 per group): (1) sham control group and (2) A β 1-42 injected group. A single dose of protofibrillar A β 1-42 (5 ul) through i.c.v. injection was bilaterally administered to 2nd group animals while sham control animals were administered with 5 ul of vehicle. The results demonstrated that protofibrillar A β

significantly induced reactive oxygen species (ROS) production, acetylcholinesterase activity, nitrite levels and lipid peroxidation in hippocampus, cortex and striatum regions after six weeks. Also, the behavioral studies have shown an increase in the anxiety levels and inhibition of long term memory after protofibrillar A β injection. The activity of antioxidant enzymes was also significantly reduced after protofibrillar A β injection in hippocampus, cortex and striatum regions. Thioflavin-T staining confirmed the presence of amyloid deposits and Nissl's staining have shown the neuronal loss after six weeks of protofibrillar A β injection. So, the present study indicated that protofibrillar A β 1-42 injection inhibits the long term memory and also leads to various biochemical alterations.

Exploring the anti-angiogenic role of traditional and Cox-2 selective NSAIDs in experimental colorectal cancer

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The present study explores the chemopreventive role of traditional and Cox-2 selective NSAIDs in 1,2 dimethylhydrazine (DMH) - induced colon carcinogenesis in the rat model. The animals were divided into nine groups: Group 1 served as control and Group 2 served as vehicle treated control. Group 3 received a weekly injection of 30mg/kg body weight of freshly prepared DMH in 1mM EDTA saline. Group 4 received DMH along with daily oral administration of celecoxib (6 mg/kg body weight), Group 5 received DMH along with daily oral administration of etoricoxib (0.6 mg/kg body weight), Group 6 received DMH along with daily oral administration of diclofenac (0.6 mg/kg body weight), Group 7 received celecoxib daily orally, Group 8 received etoricoxib daily orally and Group 9 received diclofenac daily orally. Gross morphological analysis revealed the occurrence of raised mucosal lesions called MPL or multiple plaque lesions, which were maximum in the DMH group and their number was regressed with the co-administration of imatinib. Further, aberrant crypts foci (ACFs), the regions of abnormal cell growth, were found to be decreased in imatinib co-administration groups. Histological analysis was also performed and abnormal histo-architecture like hyperplasia and dysplasia were evident in the carcinogenic group, which were found to be reduced with imatinib co-administration. The signal transduction pathways for angiogenesis will be explored which may reveal that the NSAIDs could be a potential target for the chemoprevention of colorectal cancer.

Switch in the Aggregation Pathway of Bovine Serum Albumin Mediated by Electrostatic Interactions

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A strong denaturant, guanidinium hydrochloride (GdnHCl), is shown to delay and alter the inherent aggregation pathway of bovine serum albumin (BSA) from a downhill polymerization to a nucleated polymerization. We hypothesize that such an alteration is closely connected to the conformational population of the protein, and ion-binding to such an ensemble. Hindered molecular collisions due to electrostatic repulsions in an ion-bound denatured ensemble increase the activation barrier for aggregation to such an extent that the growth, which was spontaneous in the absence of any cosolute, goes through an unfavorable nucleation phase. Our study shows that the behavior in GdnHCl is not unique to it, but occurs in a certain class of cosolutes—those which are charged and bind to BSA. Variation in pH of the medium, which gives rise to extra charges on the protein backbone, also showed such repulsive effects, further confirming the involvement of electrostatic interactions. We have further shown that the coexistence of both an appropriate population and an appropriate cosolute is necessary. An absence of either of these prevents a switch in the pathway.

Functional studies on *Staphylococcus aureus* surface protein FnBPA and its interaction with nanoparticle

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Pathogenic bacteria specifically recognize ECM molecules of the host (e.g. collagen, fibrinogen and fibronectin) through their surface proteins known as MSCRAMMs and initiate colonization. The Fibronectin binding protein A (FnBPA) is a MSCRAMM of *Staphylococcus aureus*. The N-terminal A-region of FnBPA (332 amino acids) binds to fibrinogen (Fg) by a unique ligand binding mechanism called 'Dock, lock and latch' which is a common binding theme for MSCRAMMs of some Gram-positive bacteria. To understand the interaction of FnBPA with nanoparticle and to analyze the effect of this interaction with the ligand binding mechanism of FnBPA with Fg, we cloned, expressed and purified the Fg binding region of FnBPA. Later, FnBPA-AgNP protein corona was analysed by gel shift assay, blotting assays, ELISA and circular dichroism

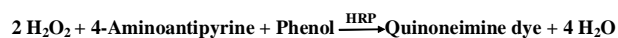
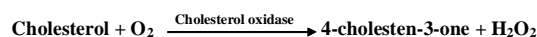
spectroscopy. The results suggest that the protein-corona formation at a particular ratio of FnBPA-AgNP hinders the ligand binding mechanism, the 'Dock, lock and latch'. On increasing the concentration of AgNP, FnBPA loses its secondary structure conformation. This inhibition mechanism can be useful in the development of anti adhesion drugs and antimicrobial materials.

Immobilization of Horseradish Peroxidase on magnetic Chitosan/Graphene Oxide beads for Cholesterol Oxidase estimation

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Horseradish Peroxidase (HRP) is widely used in the Peroxidase based assay methods for the estimation of H₂O₂. In the Cholesterol Oxidase assay, H₂O₂ is liberated from the oxidation of cholesterol in the presence of oxygen which is assayed using HRP.



The use of HRP makes this assay method expensive for each time use where the HRP is present in the soluble form and the recovery of HRP is not possible and hence it cannot be reused. The aim of this study was to prepare the HRP immobilized beads having magnetic properties for the ease of separation and increasing the reusability of HRP for cost effective assay conditions. In the present work, we prepared polymeric support (chitosan)-Ferric oxide beads using the blend of chitosan and Fe₂O₃ nanoparticles, adsorbed with the Graphene oxide and subsequently activated with 2.5% glutaraldehyde. The immobilization of HRP was confirmed by FTIR analysis and SEM. The optimal enzyme concentration was 0.16 mg/ml, binding efficiency was 80% and optimal pH and temperature for the binding of enzyme were 8.0, 25°C respectively. The immobilized HRP showed 85% and 60% of its initial activity up to 10 and 13 cycles of reuse respectively. The pH and temperature optima were shifted from 6.5 and 50°C for soluble HRP to 7.0 and 55°C for the immobilized HRP respectively. On the basis of present study, the magnetic chitosan/graphene oxide HRP due to its reusability and improved stability could be useful for biotechnological applications and bioassay.

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Intrinsically Disordered Monomeric Chorismate Mutase: Purification, Mutagenesis and Structural Investigations

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A topologically redesigned chorismate mutase enzyme has challenged the paradigm that structural disorder in general reduces enzyme efficiency [Hilvert et al., PNAS, 2004]. Monomeric chorismate mutase (mCM) is an intrinsically disordered protein with properties of a molten globule. The ability of this highly dynamic ensemble to efficiently catalyse enzyme reaction is under scrutiny. This enzyme undergoes binding induced folding in presence of its substrate yet much of its disordered nature is conserved. In this work, we have expressed and purified mCM from a recombinant bacterial host. Site directed mutagenesis at selected sites was carried out to insert single tryptophan residues along the length of the protein. We intend to utilize the fluorescence from tryptophan or extrinsic probes attached to sole cysteine residue to monitor the local structural dynamics of mCM in presence of suitable ligands and substrate. This work will provide insights on how the local and global rotational dynamics of the protein is coupled with its function.

Biophysical and Biochemical Characterisation of Mg-Ros Modified Human IgG

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IgG is a major serum protein found in all body fluids. It is rich in arginine and lysine residues making it a good target for post translational modifications (PTM). Among these PTMs "glycation" and "oxidation" are of special interest in diseases like Diabetes, Rheumatoid Arthritis, Aging etc. The synergistic interaction between glycation and oxidation also known as glycooxidation is highly relevant due to its involvement in the production of deleterious changes at the molecular level. Methylglyoxal (MG) is a potent glycating agent and an important precursor of advanced glycation end products. MG is formed by non enzymatic sugar fragmentation reactions and spontaneous decomposition of triose phosphate intermediates of glycolysis. Reactive Oxygen Species (ROS) are formed in the living organisms during normal metabolic reactions as well as environmental stress. Among various ROS, $\text{OH}\cdot$ is extremely reactive with a short half life. As oxidative stress and AGE formation are inextricably interlinked, we studied the same in vitro. In this study glycation followed by hydroxyl radical

modification of IgG was assessed by various physicochemical techniques like UV absorption spectroscopy, Tryptophan/Tyrosine fluorescence, AGE specific fluorescence, SDS page, Carbonyl group estimations, Thioflavin T and Congo Red binding assay. The results revealed that oxidation followed by glycation caused extensive damage to IgG leading to structural perturbations. The possible role of damaged IgG in Rheumatoid arthritis and Diabetes is suggested.

Effect on thermodynamic and spectroscopic properties of yeast iso-1-cytochrome c on the deletion of extra N-terminal residues

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Cytochrome-c (cyt-c) is a thoroughly studied small globular protein that plays an indispensable role in the electron transport chain. Vast data set of varied sequences and high-resolution crystal structures of cyt-c, made this protein as a model system to study the evolution of protein sequence and structure. Sequence analysis suggests that yeast iso-1-cytochrome c (y-cyt-c) has five extra N-terminal residues in comparison to the mammalian cyts-c. *In silico* analysis proposes the existence of several van der Waals interactions between these five N-terminal residues and the protein atoms. In order to ascertain the role of these extra N-terminal residues in the conformation and stability y-cyt-c, we sequentially deleted all five N-terminal residues. All the five deletants and wild type protein were expressed in BL21 (ϕE3). All the variants were biophysically characterized by UV-Vis absorbance, SDS-PAGE and CD. Thermal stability of these variants was studied by heating the proteins in the temperature range 20 – 80 °C. The data was fitted to two state-denaturation model and T_m (melting temperature) and DH_m (change in enthalpy) were determined.

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Neuromodulatory Potential of Ginkgo Biloba Leaf Extract against Aluminium Induced Neurotoxicity in Female Rat Brain

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Purpose: Aluminium, a known neurotoxin, has long been implicated in the pathogenesis of Alzheimer's disease. Al

induced pro-oxidant activity and the protective role of exogenous Ginkgo Biloba leaf extract (GBE) as well as various behavior studies and oxidative stress parameters were determined in the Hippocampus and cortex of rats.

Methods: SD female rats were intraperitoneally injected with Al (as Al lactate) at doses of 10 mg/kg body wt/day for a period of 6 weeks. GBE was administered in conjunction with Al at a dose of 100 mg/kg of body wt by oral gavage for a period of 6 weeks.

Results: The study indicates significant increase in ROS levels, lipid peroxidation and NO levels and reduced the GSH levels in the two regions of rat brain. These alterations were also depicted in the histology which shows signs of hypoxia, vacuolation and paucity of neurons in the hippocampus and cortical region. Co-treatment with GBE has improved the acetylcholinesterase activity and hence has improved spatial memory as assessed by behavior tests.

Conclusion: The biochemical changes observed in these tissues indicate that Al acts as pro-oxidant agent, Co-treatment with GBE was able to normalize significantly the oxidative stress parameters as observed in the histology and hence has improved the anxiety as assessed by elevated plus maze and the locomotor activity of the rats which were altered following Al treatment. So, GBE might be administered as a potential supplement in the treatment of neurological disorders in which oxidative stress is involved.

The Prophage encoded Streptococcal Hyaluronate Lyase has broad substrate specificity and function

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Streptococcus equi (group C streptococcus) and *Streptococcus pyogenes* are associated with a wide variety of mucosal and invasive infections that claim human life. Spreading of infection in host tissues is thought to be facilitated by the bacterial gene encoded extracellular hyaluronate lyase (HL) which degrades hyaluronan (HA), chondroitin-6-sulfate (CS-C) and dermatan sulfate (DS) of the extracellular matrix (ECM). The clinical strain *S. equi* 4047 however, lacks a functional extracellular HL. The prophages of *S. equi* and *S. pyogenes* encodes intracellular HLs which are reported to partially degrade HA and do not cleave any other glycosaminoglycans (GAGs). The phage HLs are thus thought to play a role limited to the penetration of streptococcal HA capsules, facilitating bacterial lysogenization and not in the bacterial pathogenesis. We have systematically looked into the structure-function relationship of phage HLs and

observe that, while HA is the preferred substrate, these phage HLs have weak activity towards CS-C and DS and can completely degrade all of them to disaccharides. Even though the catalytic triple-stranded β -helix (TS β H) domain of phage HLs are functionally independent, its catalytic efficiency and specificity is influenced by the N-terminal domain. The phage HLs also interacts with human transmembrane glycoprotein CD44. The CD44 seems instrumental in optimum HA degradation by phage HLs *in vivo*. The above results suggest that the streptococci can use phage HLs to degrade GAGs of ECM for spreading virulence factors and toxins, while utilizing the disaccharides as a nutrient source for proliferation at the site of infection.

Structural and functional characterization of Hylp-type bacteriophage encoded hyaluronate lyases

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Streptococcus sps is associated with a wide variety of mucosal and invasive human infections that claim human life. Spreading of infection in host tissues is thought to be facilitated by the bacterial gene encoded extracellular hyaluronate lyase (HL) which degrades glycosaminoglycans (GAGs) of the extracellular matrix (ECM). The role of phage HLs were postulated to be limited to bacterial lysogenization only and not in the bacterial pathogenesis, due to their strict substrate specificity to hyaluronic acid (HA). Phage HLs are substantiated upon the presence of Gly-X-Y (collagen rich) motif at the N-terminal region of Hylp-type and absence in Hylp2-type. We systematically looked into the structure-function relationship of Hylp-type phage HL. The study envisages that apart from HA as a preferred substrate, phage HL also has weak activity towards chondroitins and dermatans and can completely degrade all of them. Even though the catalytic triple-stranded β -helix (TS β H) domain of phage HL is functionally independent, its catalytic efficiency and specificity is influenced by the N-terminal domain. The phage HL enhances the HA degradation while interacting with human transmembrane glycoprotein CD44 and catalytically active in physiological buffer. The above results are suggestive of the role of phage HLs in pathogenesis and streptococci can use phage HLs to degrade GAGs of ECM for spreading virulence factors and toxins. Thus, the study re-evaluated the role of phage HLs as a potential virulence factor. The collagenous GlyX-Y motif present at the N-terminal of the enzyme influences stability and interact with calcium ions

suggesting its role in the enzyme regulation. The study also unravelled the localization of active site pocket by site-directed mutagenesis of amino acid residues involved in the catalysis. In addition, the construct of phage HL without the head domain can be used as an alternative for suppressing hyaluronan-mediated tumor progression and specific determination of HA.

Quantum mechanical calculations and molecular docking study on diallyl disulphide derivatives

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Diallyl disulphide (DADS) is an oil soluble organosulphur compound derived from garlic possessing various remedial applications such as antimicrobial, antiinflammatory, anticancer, immune modulatory, antioxidant and antihistaminic. However, its use is limited due to its extreme volatility. In order to overcome this problem 7 new DADS analogs were synthesized and characterized by Rai et al., 2008¹. In the present study, we have theoretically characterized the molecular structure of these DADS derivatives conformers using density functional theory (DFT/B3LYP) with 6-311++G(d,p) basis set by Gaussian 09W suite. Subsequently, we have explored the Histone deacetylase (HDAC) inhibition potential of these derivatives using docking study (Schrodinger, 2012). Docking study was carried out with HDAC 8 (1T67, Protein Data Bank) protein considering allyl mercaptan as the reference HDAC inhibitor. Density functional calculations involving NMR, IR, UV - Visible, HOMO- LUMO analysis were carried on the most stable conformer and NMR and IR theoretical data were compared with the experimental values to verify the correct geometries of DADS derivatives.

DADS analogs in the binding pocket majorly show hydrophobic interactions with important amino acid residues which play an important role in our docking study. It was also observed sulphur atom plays an important in binding with Zn atom of the binding site. Based upon the glide score values and S-Zn bond length within the active site three derivatives namely, DADS 5, DADS 10 and DADS 11 were found to be more effective HDAC 8 inhibitors than allyl mercaptan.

Signature networks underlying unfolded intermediates of an obligate GroEL substrate

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Maintaining protein homeostasis is essential for cell survival. Many topologically complex proteins require an elaborate machinery of molecular chaperones, to assist efficient folding into their native form. GroEL chaperonin binds with a large subset of these protein substrates lacking ordered tertiary structure, to facilitate folding thereby mitigating the risk of aggregation in *E.coli*. The overall chaperone complex has been well studied however structural scaffolds of the non-native substrates that dictate chaperone-mediated folding remain poorly understood. Here we employ an integrated framework of *in silico* and *in vitro* techniques to investigate non-native ensemble of an obligate GroEL substrate, DapA. Long is unfolding simulations reveal compact intermediates with distinct structural characteristics. ANS binding experiments and computational analysis display significant increase in surface exposed hydrophobic patches of the unfolding intermediates. Based on atomistic-resolution data, we obtain that these patches are primarily contributed from native and non-native ²-sheet elements. Further, we constructed dynamic network graphs of the trajectories to elucidate long-range intra-protein connectivity of native and intermediate topologies, highlighting residue clusters that serve as central hubs. Our findings underscore the key structural properties of an unfolded chaperone-substrate and suggest that there is an extant underlying coherent pattern within the disordered nature of the protein that serves as molecular signal, guiding them to chaperone binding.

Challenges in Preventing Aggregation in Multidomain Proteins

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Human serum albumin (HSA) is a multidomain protein and is known to exist as N and F isomeric forms at pH 7.4 and 3.4, respectively. During N-F transition, domain I undergoes a structural rearrangement with minor changes in secondary structure, whereas domain II transforms to a molten globule like state and domain III is unfolded and separated from rest of the molecule without significantly affecting rest of the protein molecule. In this work, we studied the aggregation behaviour of F isomers in the absence and presence of *curcumin*, a polyphenol compound found in turmeric. The

aggregation process was monitored using 90° light scattering and ThT-fluorescence, congo red binding and ANS fluorescence. 90° Light scattering and ANS monitor total aggregation while ThT and CR binding monitor fibrils formation. We found that F isomers of HSA forms amorphous aggregates without any added ligand. Surprisingly, curcumin, commonly used as an inhibitor of aggregation, induced fibrillation of the protein. We found that curcumin-induced aggregate showed all tinctorial properties of amyloid fibrils. Binding and conformational studies indicated that curcumin binds strongly to both N and F isomers and cause major structural alteration in the protein. We conclude that curcumin binding induce conformation changes in F isomers, generates amyloid precursor state which at higher concentration collapses unspecifically and then rearrange in structured aggregates. This study poses challenges in preventing aggregation by small molecule inhibitors in multidomain proteins where each domain is capable of unfolding/refolding independently and inter-domain and intra-domain interactions can affect the overall aggregation reaction.

Synthesis of Praseodymium doped Zinc oxide nanoparticles by hydrothermal method for possible use in glucose sensing

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Glucose biosensors are by far the most widely studied type of biosensors and numerous designs have been proposed. Three generations of glucose biosensors based on glucose oxidase can be identified using (i) natural oxygen as co-substrate and generation and detection of hydrogen peroxide, (ii) synthetic electron mediator and (iii) direct electron transfer between glucose oxidase and the electrode. Zinc oxide is known for its biocompatibility, stability and environmental safety, with wide range of applications, in photocatalysis, dye degradation, solar cells, optical devices etc. which led to direct applications of this material in the preparation of biosensors. Praseodymium a well-known activator dopant is chosen for the current study. For this work, we used different ratios of Zinc nitrate and praseodymium nitrate (1:1, 1:10, 1:50) and resultant product Pr-ZnO is used as sensing material, synthesized by hydrothermal method at 165°C for 5 hours. The synthesized powder is also characterized by Fourier transform infrared spectroscopy (FTIR) and UV-Visible spectroscopy. Films of the doped powder were screen printed on a screen printed gold electrodes (SPE). The electrochemical characterization was carried at a fixed scan rate and variable scan rate for understanding charge transfer characteristics. Films were characterized for glucose sensing in the concentration

range of 0 to 200 mg/dL. Further studies are in progress to determine the sensitivity with and without glucose oxidase immobilization.

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Tracking interaction induced conformational changes in protein-RNA recognition

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Protein-RNA interactions are essential for gene expression and its regulation. During the interactions both protein and RNA undergo conformational rearrangements for a structurally and functionally favorable binding. Here, we have studied the changes adapted by protein and RNA during the conformational transitions in terms of accessible surface area and torsional angles obtained from the unbound and the bound structures of the interacting components. A non-redundant dataset of 112 protein-RNA complexes was curated from the Protein Data Bank for this study. Of these, the unbound structures of protein and RNA are available for 25 complexes; only unbound structures of proteins are available for 77 complexes; and only unbound structures of RNA are available for 10 complexes. The unbound structures helps us to understand the pre-association conformation and the subsequent changes that occur during interaction. In this study, we find that positively charged residues change significant conformation and gets exposed to interact with the negatively charged counterpart of the RNA backbone. Aromatic residues also change significant conformations while interact with the nucleotide bases. The protein surface that recognizes RNA undergoes disordered to ordered transitions during complexation compared to other part of the protein surface. The extent of side-chain deviation of amino acid residues increased with the length of the side-chain. The flexibility of side-chains present in the protein interior are more constrained than those present in the interface followed by the surface, which can be attributed to the packing density of these regions.

Antimicrobial and antiadhesive properties of biosurfactant produced from probiotic bacteria

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Probiotic bacteria or 'Lactic acid bacteria' (LAB) have significant impact on human health and immune system.

Among LAB, *Lactobacilli* species show antimicrobial activity against various pathogens from intestinal tract and female urinogenital tract. Lactobacilli species are known to produce various components namely lactic acid, bacteriocins, H₂O₂ and biosurfactants. Adhesion of pathogens to the epithelial cells is prevented through the production of biosurfactant. We have screened lactobacilli species for biosurfactant production. Work on antimicrobial, antiadhesive properties of biosurfactant is in process. We have selected SPPU1 for biosurfactant production for further studies along with the media optimization, purification and characterization of biosurfactant. SPPU1 produced biosurfactant in the fermentation medium supplemented with lactose and sucrose (1% w/v) with the reduction of surface tension up to 26 mN/m with good foaming property. Further physical properties like interfacial tension, wetting property, contact angle, emulsification, Lactobacilli strains obtained from nationalized culture banks are also processed for the current studies.

Structural and Biophysical Characterisation of Rv1828 of *M. tuberculosis*

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Mycobacterium tuberculosis (*Mtb*), the principal etiological agent of tuberculosis in humans, is reported to infect more than two million people every year. Although reasonable number of drugs are available in market for treatment of tuberculosis, emergence of multidrug resistant *Mtb* against these drugs necessitates an urgent need for development of new drugs. In order to develop novel drugs new targets should to be identified. Rv1828 one of the essential protein for *Mtb* is known to be indirectly involved in providing resistance to bacterium against drugs currently available in market. Structural and functional elucidation of this protein will help in understanding its role in *Mtb* which can be explored for designing new drugs against multidrug resistant *Mtb*. According to tuberculist database Rv1828 is a protein of unknown function. The conserved domain database in NCBI predicts it in the family of HTH MerR like transcriptional regulator and therefore anticipated that it may be involved in regulating one of the essential process of pathogen. Various biophysical techniques will help us to identify function of Rv1828 and three dimensional structure will give its molecular details. So far we have cloned, expressed and purified the protein Rv1828. Preliminary biophysical characterisation has been done using circular dichroism and dynamic light scattering experiments. Crystallisation trials are under progress for structural characterisation of Rv1828.

Molecular plasticity of β -catenin: insights from molecular dynamics simulations

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The β -catenin, member of armadillo family, is general regulator of Wnt-Signalling pathway. In cytosol it is under strict regulation of various kinases. Structurally β -catenin has three domains; the N-terminal domain is primarily for regulation of β -catenin's activity, a large middle domain consists of twelve armadillo repeats that provide platform for assembly of various transcription factors of Wnt regulated genes in nucleus and the C terminal domain. Phosphorylation of β -catenin at N terminal results in its ubiquitination and ultimately proteasomal degradations. The Wnt signals or mutations in β -catenin stabilizes it by preventing phosphorylations, the stable β -catenin ultimately reaches in nucleus and interact with transcription factors such as TCF/LEF that result in various types of cancers. Here we explore the nature of interactions between β -catenin and transcription factors such as hLEF1 and hTCF4. Moreover, we also present virtual screening against β -catenin in search of potential therapeutics against β -catenin. Validation of binding interactions is performed by Molecular Mechanics-Poisson Boltzmann Surface Area (MM-PBSA) studies.

In vitro study of protein aggregation and polyphenol release efficiency of human serum albumin nanoparticles

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Polyphenols are ubiquitous in our diet and have a multitude of beneficial activities that are investigated in our laboratory. Human γ B-crystallin undergoes UV induced photo damage that results in modification of the Trp residues. We have monitored the effect of epigallocatechin gallate (EGCG) on the irradiation of human γ B-crystallin, from recombinant *Escherichia coli*, with UV in presence of a modified Fenton's reagent to assess the damage to Trp residues. It was found that EGCG is able to effectively protect human γ B-crystallin from oxidative stress induced photo-damage. Fisetin loaded human serum albumin (HSA) nanoparticles were prepared and characterized and the anticancer activity using MCF-7 breast cancer cell lines investigated. The encapsulation efficiency was ~84% and the *in vitro* release

profile showed an initial burst release followed by a slow and controlled release of fisetin. Cytotoxicity assays showed that fisetin loaded HSA nanoparticles kill the breast cancer cells more efficiently compared to fisetin alone. Prior to investigating polyphenols in case of fibrillation the effect of surfactants has been studied with amyloid beta peptide fragment ($A\beta_{25-35}$) and hen egg white lysozyme (HEWL). Interactions of sodium dodecyl sulfate (SDS, anionic), dodecyl trimethyl ammonium bromide (DTAB, cationic) and Triton X-100 (TX, neutral) with preformed $A\beta_{25-35}$ fibrils showed that TX could promote fibrillation whereas DTAB breaks down the $A\beta_{25-35}$ fibrils. We have also studied the effects of benzoic acid and gallic acid on HEWL fibrillation and preliminary results indicate that gallic acid is able to inhibit the process.

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Biophysical characterization of novel low molecular weight anticancer protein from wheat seeds

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Plants defend themselves from pathogens and pests by different pathways of which producing antimicrobial peptides are most important. Thionins are cationic, antimicrobial, and generally basic, plant peptides with a molecular weight of 5000 Da, which contain 6 or 8 conserved cysteine residues; abundant in wheat and barley. Their in vitro toxicity against plant pathogenic bacteria and fungi indicates a role in the resistance of plants. In the current study, we report successful and easy purification of purithionin (a type of thionins) like low molecular proteins from wheat seeds. Several biophysical studies showed the presence of an intrinsic fluorophore, tyrosine. The ground state absorption of protein shows classical spectra of tyrosine, even though it showed unusual fluorescence behavior. Usually tyrosine fluoresces maximum at 310nm but in the present study, the tyrosine fluorescence was observed at 342nm which is unusual. The structural studies showed higher helical content in the protein which was reestablished by computational analysis using Psipred. The protein showed structural changes in circular dichroism spectra in the presence of SDS. In addition, the protein happens to be a potent anticancer agent, effective against Human hepatocellular liver carcinoma cell line (HepG2). Molecular dynamics study of the protein showed stability in both water and membrane, however no pore formation was observed in the membrane. Dynamic stability of purothionin dimer in the membrane also suggests that the purothionin can get inserted into the membrane thermodynamically favourably.

Plausible G-Quadruplex Elements in the Human GRIN1 Promoter Region

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It is well known that G-rich sequences can fold into four-stranded secondary structures called quadruplexes. These structures are potentially useful therapeutic targets. N-methyl-D-aspartate (NMDA) receptor has been associated with many diseases that include Neurodegenerative disorders, Alzheimer's disease, Huntington's disease, but mainly the involved in Schizophrenia syndrome [1]. In the present study, we have focussed on the GRIN1 gene, which encodes NR1 subunit of NMDA receptor. Numerous single nucleotide polymorphisms (SNPs) have been identified in different regions of GRIN1 gene among schizophrenic patients of different parts of the world. While analyzing the promoter region of GRIN1, it was found out that G1001C polymorphism is associated with schizophrenia in the Italian population [2]. So, we have selected a 27-mer sequence of GRIN1 gene whose SNP has been associated with schizophrenia. With the help of different techniques such as Gel Electrophoresis, Circular Dichroism spectroscopy and Thermal-melting studies, we have indicated that this 27-nt sequence may possibly have Watson-Crick hydrogen bonding in addition to typical Hoogsteen hydrogen bonding of G-quadruplex. A truncated sequence GRIN15 from which bases that are involved in Watson-crick H-bonding are excluded is studied to further ensure the presence of tetramolecular G-quadruplex. Another sequence GRIN15M (a mutated version of GRIN15) has been employed to prove the non-existence of homo-duplex structure formation by GRIN15 sequence. Experimental results conclude that the sequence under study forms tetramolecular G-quadruplex structure coexisted with Watson-Crick H-bonding under physiological salt and pH conditions.

Characterizing the Meropenem and Human Serum Albumin Interaction by Spectroscopic and Computational Approaches

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Meropenem (carbapenem drug) is referred as the drug of last resort to check the outbreak of resistant bacteria in hospital and community acquired infections. The overall effectiveness of meropenem shall be determined by its interaction with plasma proteins, principally human serum albumin (HSA). In the present work, we invigilated

the mode of interaction between meropenem and HSA using various techniques. We found that the meropenem quenches the fluorescence of HSA and the rate of quenching decreases with increasing temperature. It clearly shows that fluorescence quenching is due to the formation of a complex between HSA and meropenem i.e. static quenching. The interaction between HSA and meropenem is spontaneous in nature ($\Delta G < 0$) having a binding constant of the order of 10^4 M^{-1} and only one type of binding site. HSA-meropenem complex is stabilized primarily by electrostatic interactions along with some contribution from hydrophobic interactions and hydrogen bonding. FRET analysis shows that the distance between meropenem and Trp-214 of HSA is optimum for quenching to take place. CD data shows a minor conformational change in the overall structure of HSA upon meropenem binding. Molecular docking studies predict that meropenem binds at Sudlow's site II located in subdomain IIIA of HSA with a few residues of subdomain IIA also contributing favourably to HSA-meropenem complex stability. The amino acid residues that interact with meropenem are: Lys-195, Trp-214, Arg-218, Lys-436, Cys-437, His-440, Lys-440, Pro-447, Cys-448, Asp-451, Tyr-452 and Val-455. This study provides an insight into the molecular basis of interaction between meropenem and HSA.

The impact of urea and guanidinium chloride on the secondary structure of D-phosphoglycerate dehydrogenase from *Entamoeba histolytica*

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A phosphoglycerate dehydrogenase (PGDH) is a protein that catalyzes the oxidation of D-phosphoglycerate to 3-phosphohydroxypyruvate in the phosphorylated serine metabolic pathway. The *E. histolytica* is an enteric protozoan parasite that possesses both phosphorylated and non phosphorylated serine metabolic pathways. The *E. histolytica* PGDH gene (*EhPGDH*) encodes a protein of 299 amino acids with a molecular mass of 33.5 kDa. It has an isoelectric point of 8.11. *EhPGDH* catalyzed reduction of phosphohydroxypyruvate to phosphoglycerate utilizing NADH and NADPH. The *EhPGDH* protein is present as a homodimer, which is demonstrated by gel filtration chromatography. The PGDH from *Entamoeba histolytica* has been over-expressed and purified using various chromatographic techniques. The purified protein was homogenous as indicated by a single protein band on SDS-PAGE and a single peak in ESI-MS. The urea and guanidinium chloride induced non co-operative unfolding of D-phosphoglycerate dehydrogenase was studied by means of Circular Dichroism. Since *EhPGDH* showed high homology to

PGDH from bacteroides and other enteric protozoan ciliate, *E. caudatum* and it has no /very little homology with human protein, which might be important to study the usefulness of this protein. Thus this protein is considered as a new drug target against amoebiasis or amoebic dysentery.

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Interaction of phospholipids with Human Protein Z

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Human Protein Z is a 62 kDa, multi-domain vitamin K dependent anticoagulant that acts as a cofactor of ZPI (protein Z dependent protease inhibitor) by increasing the rate of fXa inhibition by ~1000 fold in presence of procoagulant Ca^{2+} and phospholipid. PZ binds to lipid bilayer through its γ -carboxy glutamic acid rich (GLA) domain and forms stabilizing interactions with fXa bound to the same membrane surface, bringing the bound ZPI into close proximity to fXa. Cofactor activity of PZ thus originates from its anchoring to the phospholipid bilayer. Phosphatidylserine (PS), and possibly phosphatidylethanolamine (PE) of platelet membrane are the key lipids regulating many stages of coagulation and anticoagulation. In the present study, we are interested to examine if both PS and PE regulate the cofactor activity of PZ. A six carbon chain soluble form of PS and PE was used as tools for this purpose. Preliminary data demonstrated that both PS and PE bind to PZ with similar affinity ($k_d \sim 49 \mu\text{M}$). Synergy between binding of PS and PE was also observed. Other phospholipids such as phosphatidic acid, phosphatidyl choline and phosphatidyl glycerol were also shown to bind PZ but with much less affinity. Similar effect of PZ on antithrombin inhibition of fXa was observed in presence of both PS and PE. Binding stoichiometry of both the lipids with PZ was also detected to be two. More experiments are underway to conclusively show both PS and PE upregulate cofactor activity of PZ in a manner very similar to each other.

Decoding the Moonlighting trait of Lipoyl Domain in Dihydrolipoamide dehydrogenase

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Our current report highlight the Lipoyl domain in Dihydrolipoamide dehydrogenase (DLDH) and exposing its cardinal role in catalyzing transfer of acetyl group to

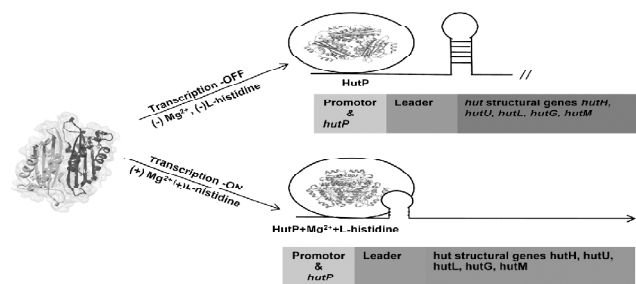
some receptor proteins through structural biology studies. Although, the presence of lipoyl domain is a characteristic of E2 component of the pyruvate dehydrogenase complex, but certain organism are reported to have lipoyl domain in Dihydrolipoamide dehydrogenase (DLDH) eg. *Streptococcus pneumonia*, *Mycoplasma capricolum*, *Neisseria meningitis*. *Streptococcus pneumonia* rDLDH, being efficient in mediating protein acetylation is suggestive by harboring lipoyl domain. Besides of the re-oxidation of Dihydrolipoamide, DLDH is well known to possess several other metabolic activities. Such property of DLDH is described as the moonlighting function. These enzymes have developed a second function during a long course of evolution. The moonlighting protein enables the cell to synthesize a few proteins and synthesize less DNA to replicate, thereby preserving a great deal of energy for proliferation of cell. moonlighting include several different kinds of proteins and since a past decade a list of growing numbers of diverse proteins were being recognized as a moonlighting. Dihydrolipoamide dehydrogenase (DLDH) represents an excellent example of a 'moonlighting' protein that overcomes the one gene-one structure-one function concept to justify the characteristic of harboring the lipoyl domain in DLDH of *streptococcus pneumonia*.

Structural and Functional Analysis of RNA Binding Antitermination Protein

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Expression of bacterial operons is controlled at transcriptional level. RNA binding proteins involve in controlling the gene expression either as an activator or a repressor. The positive gene expression through transcription regulation in operons is referred as transcriptional antitermination. HutP is an RNA binding antitermination protein and the activated form of HutP binds with its mRNA. The downstream genes of hut operons are regulated with the activated HutP.



The activated form of HutP complex from *Geobacillus thermodenitrificans* and RNA binding analysis are to be presented in detail. The antitermination mechanism of HutP is shown above.

Crystal structure of dihydrodipicolinate synthase (DHDPS) of *Aquifex aeolicus*

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The essential amino acid Lysine plays a vital role in the survival and growth of many micro-organisms. Dihydrodipicolinate synthase (DHDPS, E.C.4.2.1.52) catalyzes the first committed step in the lysine biosynthetic pathway: the condensation of (S)-aspartate semialdehyde and pyruvate to form (4S)-4-hydroxy-2,3,4,5-tetrahydro-(2S)-dipicolinic acid. Since (S)-lysine biosynthesis does not occur in animals, DHDPS is an attractive target for rational antibiotic and herbicide design. Here, we report the crystal structure of DHDPS from a hyperthermophilic bacterium *Aquifex aeolicus* (AqDHDPS). L-Lysine is used as an important animal feed additive where the production is at the level of 1.5 million tons per year. The biotechnological manufacture of lysine has been going for more than 50 years which includes over synthesis and reverse engineering of DHDPS. AqDHDPS revealed a unique disulfide linkage which is not conserved in the homologues of AqDHDPS. *In silico* mutation of C139A and intermolecular ion-pair residues and the subsequent molecular dynamics simulation of the mutants showed that these residues are critical for the stability of AqDHDPS tetramer. MD simulations of AqDHDPS at three different temperatures (303, 363 and 393 K) revealed that the molecule is stable at 363 K. Thus, this structural and *In silico* study of AqDHDPS likely provides additional details towards the rational and structure-based design of hyper-L-lysine producing bacterial strains.

Secondary metabolites from plants: An overview on its role as a potential source for drug development and design

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Plants have evolved themselves to produce bioactive secondary metabolites that have allowed them to produce a range of chemicals which are not directly responsible for its metabolism and survival but are produced in case of extreme environmental factors such as predation and nutrient deprivation. These metabolites are now being regarded as successful leads to future drug development. Morphine from *Papaver somniferum* (pain control), digitoxin from *Digitalis purpurea* (for congestive heart failure), taxol from *Taxus braviifolia* (anticancer) are a few examples of secondary metabolites based drugs available.

Traditional use is an important approach to discovering newer bioactive compounds, as validation of traditional medicine is of great significance in enabling human access to affordable medicines. Most plant secondary metabolites are derived from only a few building blocks but, after the formation of the basic skeletons, each plant species has different, highly specific enzymes that modify these compounds. Their use can be broadly classified into three ways: (a) natural compound is used intact (vincristine), (b) semi synthetic derivative of a natural product is used (podophyllotoxin), (c) basic skeleton of the compound is derived from plants (diosgenin).

There are several ways of increasing production of a particular bioactive compound using metabolic bioengineering. Each major class of secondary metabolites (polyketides, isoprenoids, alkaloids, phenylpropanoids and flavonoids) is further subdivided into several subclasses. Because of the activity of enzymes with different substrate- and stereo-specificity, the chemical diversity and biological activity of the molecules belonging to the same subclass can be enormous.

Biochemical characterization of Lipoate protein ligase/octanoyltransferase of *Leishmania major*

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Lipoic acid is an essential cofactor, indispensable for the functioning of α -ketoacid dehydrogenase complexes (α -KADHs) and the glycine cleavage complex. The enzymes involved in lipoylation have gained increasing attention

lately, owing to their association with pathogenicity. A *Listeria monocytogenes* mutant strain lacking lipoate protein ligase A (LplA) is unable to grow in the host cytosol and is less virulent. Likewise, the expression of lipoate protein ligase B (LipB) is strongly up-regulated in the lungs of patients with pulmonary multiple-drug-resistant tuberculosis. Leishmaniasis is a similar infectious disease where multidrug resistant strains have raised heightened concern. No information is available regarding its lipoic acid metabolism. In our quest for the identification of new targets for drug intervention to combat leishmaniasis, we have cloned, purified and biochemically characterized its putative lipoate protein ligase (LipB) and followed the transfer of the octanoyl-moiety from C₈-ACP to the lipoyl domain of Glycine cleavage system H protein. Our studies provide interesting insights into its function.

Allosteric and Druggability Prediction by Molecular Docking

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Allosteric is a direct and efficient way of regulating biomacromolecule function. The allosteric modulators can fine-tune protein mechanics. Therefore, targeting of allosteric sites is gaining attention as a strategy in drug design. However, the experimental approaches provide a limited degree of characterization of new allosteric sites. Computational approaches are useful in to analyze and select potential allosteric sites for drug discovery. In this report we have discussed the use of molecular docking, which has become an integral part of the drug discovery process, to predict the druggability of novel allosteric sites on target proteins. We have used genetic algorithm in docking and placed the whole protein in the search space. Fragment library was used as drug probes. Multiple run of the genetic algorithm populated all the druggable sites in the target protein, which was translated to two/three dimensional patterns. Such, novel pattern based analysis of the clusters gives druggability scores of allosteric pockets in terms of weighted probabilities. This structure based analysis method will help researchers develop allosteric modulators and to identify novel target sites on drug resistant proteins as well.

Cryptdin-2: An Insight Into the Structural and Conformational Behaviour Using MD Simulations

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Simulation studies have been carried out for Cryptdin-2 and its analogues with one/two/all disulphide linkage(s) cleaved. Analogues with one disulphide linkage cleaved and the analogue with two disulphide linkage cleaved AA3 are found to adopt better amphipathic structure than cryptdin-2 and thus may prove out to be better antimicrobial peptides. These analogues are predicted to be more stable than the other similar analogues in simulations under NVT conditions. Carbonyl-carbonyl interactions, CH–O interactions, OH- interactions, NH - interactions and the interactions of water molecules generally with the carbonyl oxygen of the backbone contribute to the stability of these molecules. Deviations in amide bond geometries were observed within +/- 20. This has been explained in terms of the interactions which involve backbone peptide bond moieties i.e. carbonyl-carbonyl and the interactions of water molecules with the carbonyl oxygen of the backbone. The side chains of five residues Arg, Tyr, His, Glu and Asn in cryptdin-2 and its analogues are thought to contain planar moieties. Due to the carbonyl- carbonyl interactions, hydrogen bonding CH – / NH – / OH – , -CH–O interactions and stacking interactions, the side chain of these residues does not remain planar. By MD simulations of cryptdin-2 in POPC bilayers reveal that this is inclined w.r.t. the lipid molecules whereas in bacterial membrane it is almost parallel to the lipid molecules. This implies that the hydrophobic moment of cryptdin-2 is less than the POPC bilayer and almost compatible to the hydrophobic moment of bacterial membrane.

Epigallocatechin gallate (EGCG) binds to alpha-synuclein and modulates its fibrillation: Spectroscopic and Calorimetric Studies

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Alpha-synuclein (α -syn) is a 14 kD intrinsically disordered protein, associated with Parkinson's disease (PD). There have been many reports linking aggregation of alpha-synuclein with neurodegeneration in PD. Many plant products e.g. polyphenols have been shown to have high potential to counter this aggregation. We have investigated the effect of one such polyphenol from green tea, epigallocatechin-3-gallate (EGCG) on α -syn amyloid fibril formation and characterized its binding by

isothermal titration calorimetry (ITC). There is no noticeable shift in ANS binding fluorescence intensity of α -syn on incubation with EGCG. Addition of EGCG to α -syn leads to static quenching of tyrosine fluorescence indicating interaction between EGCG and α -syn. ITC data show weak to moderate sequential binding of EGCG to α -syn at 25°C, 37°C and 45°C. Thioflavin T (ThT) assay to study the effect of EGCG on the fibrillation kinetics of α -syn reveals increase in the lag time of α -syn aggregation and reduction in the fibrillation of α -syn by EGCG. Transmission electron microscope (TEM) images of the samples show marked suppression of fibrillation of α -syn at high EGCG concentration and correlate well with ThT studies. Direct binding interaction of EGCG with α -syn protein at multiple sites with varying affinities are, therefore, responsible for modulating and suppressing fibrillation of the protein.

Effect of mechanical vibrations in the range of earthquake frequencies on hemoglobin protein

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Mechanical vibrations in the range of earthquake frequencies up to 50Hz are more detrimental for human system. Hemoglobin plays a very important role in red blood cell for blood purification. Hemoglobin carries oxygen from the respiratory organs to the rest of the body. Electrodynamic shaker from M/s Spectral Dynamics, USA, was used for generation of sinusoidal mechanical vibration in the frequency range of 10 - 50 Hz, and displacement of 10 mm. Vibration Test System consists of Shaker, Power Amplifier, Control System including Data analysis Software, accelerometers, charge amplifier and cooling blower. In the present study, the effect of mechanical vibrations on hemoglobin proteins were studied on the basis of spectroscopy i.e. ultra violet and visible spectral range 200 - 800 nm absorption spectra and Fourier Transform Infra Red (FTIR) spectroscopy with spectral range 400 - 4000 cm⁻¹. Surface tension of protein solution was measured by using pendant drop method. Mechanically vibrated hemoglobin samples were compared to non vibrated hemoglobin sample. UV-Visible absorption spectroscopy shows that hemoglobin exhibits strong absorption peak in the Soret band region. Hemoglobin protein was mechanically vibrated at different frequencies ranging from 10 Hz to 50 Hz with amplitude of 10 mm for exposure 10 minute time. Vibrated and untreated hemoglobin proteins were compared on the basis of ultra violet -visible spectroscopy, FTIR spectroscopy and surface tension.

Keywords: Vibration, Frequency, Earthquake, hemoglobin, proteins, Spectroscopy

Conformation of DNA Duplex in Presence of LNA as a Third Strand

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The conformation of DNA triplex formed by mixed sequences has biotechnological importance. LNA is a modified oligonucleotide with locked structure of furanose ring of sugar in C3'-endo conformation by forming a 2'-O, 4'-O methylene linkage. It is used as a modified TFO to form DNA triple helix. The parameters of modified nucleotides with LNA's are prepared using Gaussian 09, using 6-31G(d) basic set. ff99ps0 force field parameters are used for nucleic acid under AMBER12 software. The initial structure of complex is generated for a DNA duplex sequence d(AGGCCGGACCCGGCG)-d(CGCCGGGTCGGCCT) with corresponding LNA modified strand d(AGGCCGGACCCGGCG) in major groove of duplex keeping LNA strand parallel to first strand of duplex by using LEAP and NAB program under AMBER12 software. 10 angstrom buffer of TIP3P water molecules are placed around the complex in each direction to form a cubic periodic system. The complex is neutralized by adding Na⁺ ions. The system is equilibrated at 300K. To see the behavior of complex in water solution molecular dynamics with 100 ns time period has been performed by using Particle Ewald Sum Method with 10Å cut-off. The free energy and rmsd calculation shows that LNA can form stable triplex of DNA with R-type hydrogen bonding pattern. The DNA parameters are calculated by using PTRAJ and X3DNA.

Expression studies of important calcium signaling genes in *Neurospora crassa*

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The filamentous fungi *Neurospora crassa* has unique calcium (Ca²⁺) signaling machinery. The *N. crassa cmd* gene encodes a highly conserved Ca²⁺ signaling protein calmodulin (CaM). Only one out of the 48 Ca²⁺ signaling genes in *N. crassa* encodes for a CaM that appears to be an essential gene for viability. Moreover, we studied two other Ca²⁺ signaling genes *trm-9* and *nca-2* using the knockout mutants. We found that the *N. crassa* homologues of *cmd*, *trm-9* and *nca-2* gene play a role in

growth, Ca²⁺ sensitivity, UV survival, and in acquisition of thermotolerance induced by heat shock temperature. We further did expression analysis of *cmd*, *trm-9*, *nca-2* and some important calcium signaling genes through real-time PCR in various conditions. We found that fold change expression of these three genes were highest in $\Delta ncs-1$ knockout mutant strain. Fold change expression of *nca-2* and *trm-9* gene was high in $\Delta trm-9$ and $\Delta nca-2$ knockout mutant strain respectively. We further checked the expression profile of these three genes in double mutant background, and we found that fold change expression of *cmd* gene was low in both Ca²⁺/CaM-dependent protein kinase ($\Delta camk-2\Delta camk-3$, $\Delta camk-2\Delta camk-1$) and Ca²⁺/cation ATPases ($\Delta trm-9\Delta nca-2$) double mutant strains. Also fold change expression of *nca-2* gene was high in $\Delta camk-2\Delta camk-1$ double mutant background but fold change expression of *trm-9* gene was high in $\Delta camk-2\Delta camk-3$ double mutant strain. Additionally when we checked expression profile of these three genes in response to Ca²⁺ and UV stress, it indicated a complex regulatory mechanism of expression.

Characterization of tryptophan containing mutants of SUMO proteins using fluorescence spectroscopy

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SUMOs are members of the ubiquitin-like protein family that act as reversible post-translational modifiers of proteins in eukaryotes. The mechanism of SUMO ligation to target proteins, which is called SUMOylation, is similar to that of ubiquitination. In spite of their high structural similarity, SUMOs and ubiquitin are functionally divergent. Ubiquitin's function is well-defined in targeting proteins for degradation by tagging them with ubiquitin chains. Unlike ubiquitin, the function of SUMOs is to modulate the function of the target protein. SUMO targets are involved in various cellular processes such as regulating mitochondrial function, chromatin structure and signal transduction. In all these processes, SUMO proteins bind to other proteins and modify their function. The reasons for the functional differences between SUMOs and ubiquitin remain to be understood. Hence, it would be important to study the biophysical properties of SUMOs and compare them with ubiquitin to gain further understanding on the functional diversity and the possible role of SUMO polymers *in vivo*. In this work, site-direction mutagenesis has been used to construct two mutants of SUMO1 and SUMO2, each containing single tryptophan at the core. Equilibrium studies of chemical denaturation using urea and thermal denaturation were probed using steady-state and time-resolved fluorescence spectroscopy. It was found that the protein stability of mutant proteins remains unchanged after mutagenesis.

These tryptophan mutants of SUMO proteins will serve as model proteins to understand unfolding and folding kinetics, and protein dynamics using fluorescence spectroscopy.

In silico structural insight of mutation in PDGFR- β tyrosine kinase domain by molecular dynamics and docking approach

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Platelet derived growth factor receptor beta (PDGFR β) belongs to the receptor tyrosine kinase family, and controls a wide range of biological functions by regulating the cellular proliferation, survival, angiogenesis, migration and differentiation. A major challenge in drug designing efforts is due to development of drug resistance, initiated by the missense mutations in the protein kinase catalytic domain. This gain or loss of function due to mutations in PDGFR β tyrosine kinase genes associated with the development various diseases: cancers, atherosclerosis, fibrosis and nephritis. However, the structural evidence of impair in bio-molecular functions, due to mutation are yet to determine. Therefore, the aim of the present work is to map the modeled structure of wild-type (WT) and mutant and their effect on drug binding mechanisms. Docking analysis is carried with the potential inhibitor: sunitinib. Furthermore, molecular dynamics simulations are commenced to understand the mechanism of cancer associated mutations in altering the PDGFR β kinase structure, dynamics, and stability. Our result shows that the turnover rate of the wild type (WT) and mutated PDGFR β receptor simulation analysis are similar in absence of ligand. Specifically, the mutation at residue 634 (K634A), and is present in the ATP binding pocket, which slightly may affect the protein stability and confers resistance to the drug sunitinib. Present findings may provide the structural insights to understand the molecular mechanism of PDGFR β mutation and development of effective drugs.

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The Inter Helical Interactions for trimeric gp41 of HIV-1 envelope protein: MD Simulations and Free Energy calculations

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Entry of the viral genetic material of HIV-1 into host cell is mediated by the fusion of viral and cellular membranes. The envelope protein of HIV-1 plays the fundamental role in attachment of virion to the cell surface and promotes membrane fusion. The noncovalently associated gp-41 and gp-120 defines the structure of HIV-1 envelope protein. The inter- and intra- protein-protein interactions play important role in the conformational changes needed for the fusion process. In the present study, we have performed 100 ns MD simulations of trimeric form of gp-41 to explore the inter helical protein-protein interactions which provide the stability of trimeric complex. We have also performed the MMPBSA free energy method to study the stability of complex and residue-wise interactions during dynamics. Using residue decomposition analysis we have highlighted the key residues which participate into protein-protein interactions into trimeric gp-41. We have also highlighted to those protein residues which are most likely prone to conformational changes during fusion process. We found that the fusion peptides in each chain show the largest conformational changes during the MD simulations which is in accordance with the previous predictions where the conformational changes in these area have been predicted during the entry of virion into the host cell.

Purification of Triose Phosphate Isomerase and its Complex with Globin Like β -Subunit from Sheep Kidney

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Triose phosphate isomerase (TIM) is a ubiquitous dimeric enzyme with a molecular weight of 27-kDa. This enzyme catalyzes the reversible inter-conversion of the triose phosphate isomers dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde-3-phosphate (GAP), which is an essential process in the glycolytic pathway. This enzyme is composed of two identical subunits and each consisting of a roughly 250 amino acid residues. TIM belongs to the class of the α/β barrel enzymes. We have first time observed that TIM is forming a stable complex with the

α -subunit of hemoglobin. To successfully purify this naturally occurring complex, sheep kidney was crushed and isolated protein was subjected to ammonium sulphate precipitation. Fraction obtained at 90% ammonium sulphate was extensively dialyzed and applied to the anion-exchange chromatography on Hi-trap DEAE-FF in 10 mM tris buffer (pH 8.0). The eluted fractions were concentrated and loaded to the gel filtration column. We observed a band of trios phosphate isomerase (TIM) at 27-kDa along with a 16-kDa band of α -subunit on SDS-PAGE. Both proteins exist in the form of a complex, was further confirmed by passing onto the superdex column, showing a single peak which was further confirmed by native PAGE. This is first report on the isolation, purification and characterization of a naturally occurring complex of TIM with the hemoglobin α -subunit.

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Low molecular weight vicilin-like glycoprotein from the seeds of *Citrullus lanatus*: Purification and biochemical characterization

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The watermelon (*Citrullus lanatus*) seeds are highly nutritive and its extracts are traditionally used to cure cancer, cardiovascular diseases, hypertension, and blood pressure. These extracts are also used as home remedy for edema and urinary tract problems. In this study, we have isolated protein fraction of *C. lanatus* seeds and successfully purified a low molecular weight vicilin-like glycoprotein using chromatographic methods. Purification was analyzed by SDS-PAGE and purified protein was identified by MALDI-TOF/MS. To show its presence at gene level, we extracted mRNA from immature seeds and reverse transcribed it using suitable forward and reverse primers for purified glycoprotein. The PCR product was analyzed on 1% agarose gel and was subsequently sequenced by Dideoxy DNA sequencing method. An amino acid translation of the gene is in agreement with amino acid sequences of the identified peptides. This is the first report of purification of a vicilin like polypeptide from *C. lanatus* seeds.

Molecular characterization of modular microbial LOV domain based photoreceptors and their optogenetic potential

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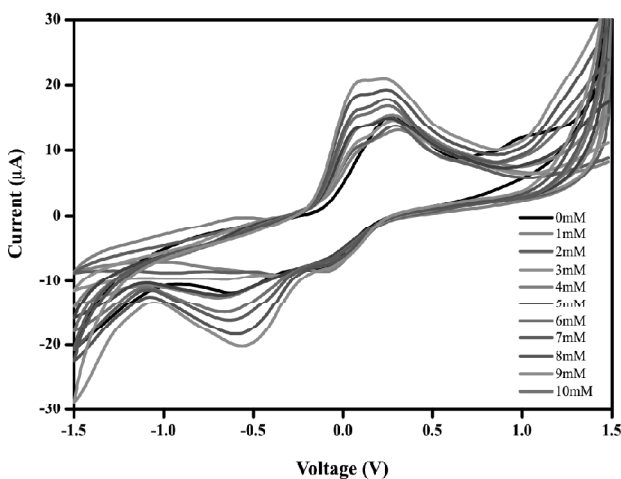
Photoreceptor proteins are light sensing proteins which comprise of a protein moiety and a chromophore group. They are divided into six classes based on their chromophore structure, among which are UV-A/blue light sensing modules, LOV (Light, Oxygen and Voltage), BLUF (Blue Light Utilizing FAD) proteins and cryptochromes. Upon UV-A/blue light illumination, these proteins get activated while they return to their ground state with dark incubation. LOV domain containing proteins are modular wherein; at one terminus is a light sensor which is coupled to an effector domain like, Ser/Thr kinases, histidine kinases, DNA binding domain, phosphodiesterases, etc. BLUF domains also offer versatility by being naturally coupled to cyclases, DNA binding motifs and oxygen sensing motifs. Cryptochromes are structurally similar to DNA photolyases that repair UV damage induced DNA breaks but lack photolyase activity. An emerging field of science, optogenetics combines optics and genetics to control and regulate cellular events with spatio-temporal precision by illumination. LOV and BLUF proteins make an attractive component of the optogenetic toolkit by virtue of having small size (~ 100-140 amino acids), higher solubility, diverse photophysical properties, and ubiquitous flavin cofactor and by being light switchable. Their modularity only adds to this repertoire for biotechnological and biomedical applications. Moreover, engineering of photosensors with altered photocycle lifetimes and coupling to novel modular domains increases targeted studying of cellular and biological events. Here, we study and analyze some of these blue light sensing proteins from a variety of organisms using bioinformatic, biophysical, biochemical approaches and discuss their optogenetic potential.

Hydroquinoneelectrochemical sensing Properties of Ni-SnO₂ Powder synthesized by soft chemical route

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Hydroquinone (HQ) is an important organic compound that is widely used in many fields such as pharmaceutical, antioxidant, dye, photography and cosmetic industries. In human medicine, hydroquinone (HQ) is used as a topical application for skin whitening for reducing the colour of the skin. Also HQ is considered as an environmental pollutant by the US Environmental Protection Agency (EPA) and the European Union (EU) for the high toxicity and low degradability in the ecological environment. Therefore it is necessary to develop simple and rapid analytical methods for the detection of micro-amount of HQ. Many determination methods such as high performance liquid chromatography and spectro photometry chemiluminescence and electrochemical methods and have been used for the detection of HQ and its isomers. Electrochemical techniques offer the opportunity for portable, cheap and rapid methodologies. Nanomaterial based sensors are being developed for its detection. In this work, an attempt is made to detect using a composite metal oxide. A soft chemical reaction was used for synthesis of NiSnO₃ nanopowder. The synthesized nanomaterial was characterized by XRD, SEM, TEM, thermal analysis and FT-IR. The X-ray diffraction patterns of bimetallic oxide after annealing indicate the formation of composite material of NiSnO₃. These powders were screen printed over a pre-printed gold electrode on glass epoxy substrate (SPE). Electrochemical characteristics of the sensors were studied at various hydroquinone concentrations (1mM to 10m M). Cv curves were obtained as a function of



concentration and scan rate. The charge transfer characteristics were also studied to understand the diffusion or surface controlled process. It is realized that anodic peak current increases linearly as a function of hydroquinone concentration, as shown in the CV curve. The results of various analysis techniques and sensor characteristics are carried out and correlated.

Investigating the structure of C-Myc PEST region after Pyrophosphorylation

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The C-Myc oncoprotein is a transcription factor which plays an important role in various processes like cell growth, proliferation, differentiation and programmed cell death. Deregulation of C-Myc expression causes many types of cancer in human beings. It's centrally located intrinsically disordered PEST region (amino acid 201 to 268), is rich in Proline (P), Glutamic acid (E), Serine (S) and Threonine (T). This PEST region lacks a stable tertiary structure which is responsible for rapid degradation of the C-Myc oncoprotein. The C-Myc oncoprotein undergoes various post-translational modifications like phosphorylation, glycosylation and acetylation at different sites which is responsible for its stability and proper functions. Pyrophosphorylation of a protein is a post-translational modification event which plays an important role in protein signaling. The aim of our present study is to investigate the consequences of both phosphorylation and pyrophosphorylation on the structure of C-Myc PEST region. For this purpose we have cloned and expressed the PEST fragment in bacteria. We present here the results of cloning and expression of C-Myc PEST region for the wild type as well as its Tryptophan and Serine mutants. Tryptophan fluorescence data from purified PEST fragments shall shed light on how post-translational modifications in Intrinsically Disordered Regions (IDRs) cause structural changes such as disorder to order transition which control protein function.

Impact of p53-MDM2 Molecular Network on Wnt Signaling

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p53 is the most studied protein in terms of cancer and also signaling pathways leading to it. In normal cells, p53 activity is inhibited while on the other hand it is activated under stress conditions. P53 decides the fate of the cell either to go for apoptosis or to be arrested for repair. Since nutlin3a and nutlin 3b are the two enantiomerisms that up-regulates p53 and competes for the binding sites of p53 on MDM2. We designed an integrated network of p53 and wnt oscillators describing the effect of nutlin and wnt in the stress cell on the basis of regulation of p53 signaling. Thus the communication between two oscillators can be described deterministically by translating the interaction of different molecules into a set of ordinary differential equations (ODE) using the chemical kinetics law. These independent oscillators are reported to interact with each other via Gsk3, under the stress of regulating the interaction process which is revealed by high concentration of β -Catenin, which is being monitored by the axin expression in a feedback loop mechanism by aggregation to form the destruction complex. Our numerical simulation results of the model, pointed out very important and interesting phenomenon. Presently we studied this phenomenon in the deterministic model. Further, stochastic modelling could provide the realistic behavior of this model.

Surface functionalized Superparamagnetic iron oxide Nanoparticles: Design, synthesis and hyperthermia therapy application

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Magnetic nanoparticles (MNPs) with appropriate surface chemistry have been widely used extensively for numerous in vivo applications such as magnetic resonance imaging contrast enhancement, immunoassay, detoxification of biological fluids, hyperthermia, drug delivery and in cell separation, etc. All these biomedical and bioengineering applications require that these NPs which have high magnetization values and size smaller than 100nm with overall narrow particle size distribution, so that the particles have uniform physical and chemical properties. The size and different properties of MNPs are

strongly influenced by synthesis method adopted. In this context in the present investigation, we adopted a new approach to synthesis of Fe₃O₄ MNPs. We chose alkaline media (DIPA) as a co-precipitating agent. Surface of Fe₃O₄ NPs were successfully modified with Pluronic F127. The particle size of the synthesized NPs was ~10 nm. The Pluronic F127coated NPs exhibit narrow size distribution, good dispersibility and very good suspension stability as compared to uncoated Fe₃O₄ NPs. It was found that coating with Pluronic F127 enhances the hydrophilicity caused the nanoparticles to form stable suspension in water. Pluronic F127 coated NPs show superparamagnetism at room temperature with blocking temperature T_b ~ 200K. Induction heating studies of Pluronic F127coated NPs were investigated for their possible application in magnetic fluid hyperthermia (MFH). The coated NPs exhibited self-heating temperature rise characteristics when subjected to external magnetic field at different particle concentration and field amplitudes and from results found that the synthesized Nanoparticles show highest specific absorption rate ~600 Watt/gm. Magnetic properties of synthesized MNPs were measured by a superconducting quantum interference device (SQUID) and crystallographic and chemical properties by TGA, XRD, FTIR and TEM etc.

Role of a single tryptophan residue in maintaining structural integrity of *Helicobacter pylori* arginase.

Ginto George and Apurba Kumar Sau

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Helicobacter pylori arginase plays a critical role in providing acid resistance and thus important for colonization of pathogen in the gastric epithelial cells. It helps in evading the host immune response by down-regulating nitric oxide (NO) production from L-arginine by the inducible isoform of NO synthase (iNOS), as L-arginine is a common substrate for both iNOS and arginase. Our previous studies reported that Trp159-Asp126 interaction of this single tryptophan protein plays an important role in maintaining the bimetallic cluster and thus for the function of the enzyme. We also highlighted the hydrophobic and / or π - π interactions of tryptophan with the surrounding aromatic amino acids Tyr125 and His122 by molecular dynamic simulations. In the present study, we are elucidating the role of Asp126, Tyr125 and His122 for retaining the tryptophan residue in the close proximity of the active site, which may be essential for maintaining structural integrity and function. By steady-state and time-resolved fluorescence analyses, tryptophan accessibility and steady-state anisotropy measurements, our data suggest that Trp is more solvent exposed in the metal reconstituted double mutants, Tyr125Ala, Asp126Ala, His122Ala, Asp126Ala as well as in the single

mutant Asp126Ala compared with the wild type. This indicates that His122 and Tyr125 individually play important role in positioning the Trp at the active site by hydrophobic/ or π - π interactions. The increasing order of ANS binding to apo as well as holo mutant proteins compared with the wild type shows more hydrophobic patches are exposed in case of the mutants indicating the difference in the tertiary structure. The overall studies suggest that the interactions of the tryptophan residue with the histidine, tyrosine and aspartic acid residues are indispensable for maintaining the structural stability in *H. pylori* arginase.

Molecular insights into L-asparaginase I of *Leishmania donovani*: Computational analysis and structure based design of inhibitors.

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Leishmaniasis caused by *Leishmania sp.*, a protozoan parasitic disease is endemic in Asia, South America, Middle East, Africa and some European countries. With diverse and complex epidemiology, the pathogen employs strategic evasion from destruction by host cellular immune system for growth and survival. Forward line chemotherapy for Leishmaniasis includes, Pentavalent antimonials, Anti-fungal antibiotics, Aromatic dimidines. Rapidly developing multi-drug resistance poses a serious threat to current line therapies, demanding structured development of alternate therapeutic strategies. Here, with the aid of comparative genome and proteome analysis of various pathogenic protozoans, we report L-asparaginase I of *Leishmania donovani* (LdAI) as a new bimolecular target against Leishmaniasis. Comparative modelling and MD simulations were employed to obtain a reliable model of LdAI. Virtual screening of compounds from entire ZINC library (zincdocking.org) based of physicochemical properties of the substrate, L-asparagine/ L-glutamine. Docking based affinity prediction of top 100 screened compounds was further analysed for their toxicity profiles. Subsequently, structure based redocking approach was employed to screen five potential inhibitors against Leishmanial L-asparaginase.

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Development of Random Positioning Machine to study microgravity effects on biological systems

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With a great success in previous space missions, India is now in a position to send biological samples in space. Microgravity, observed in spacecraft, is the condition where gravity value is below earth's gravity (1 g). It can be simulated on earth using a laboratory instrument, called Random Positioning Machine (RPM). We have successfully developed this instrument in our laboratory. Essentially, Random positioning machine comprises of two-axis rotating frame, rotating independently of each other by two separate motors with speed ranging from 1-10 rpm. The object under study is placed at the centre of the inner frame of RPM. The instrument randomly changes direction as well as speed of rotating object relative to earth's gravity vector, so that effective magnitude of gravity-vector is averaged to zero over time. The level of simulation within RPM depends on the speed of rotation and the distance of the sample from the centre of rotation. The level of gravity achieved using this instrument varies between 10^{-1} (hypo) g to 10^{-5} g (micro) as per the calibration curve. The whole instrumental set-up is controlled through the software. The experiments using RPM can be performed under controlled environmental conditions of temperature, humidity, CO₂ and light. RPM can be used to study the effects of microgravity condition on many biological samples such as bacteria, blood cells, cell cultures, insects, plants etc. Preliminary experiments on plants are being carried out and obtained results will be discussed.

The structural basis for the prevention of NSAID-induced gastrointestinal tract damage by the C-lobe of bovine lactoferrin

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most well recognized drugs worldwide for the treatment of pain, inflammation, and fever. NSAIDs are commonly administered for treatment against inflammatory diseases, rheumatoid arthritis, osteoarthritis, dysmenorrhea, and ischemic cerebrovascular disorders. Though NSAIDs are highly effective in their function, their chronic administration causes damage to the gastro duodenal mucosa causing gastropathy, which is a broad

term that includes gastric and intestinal injuries, peptic ulcers and formation of strictures within small and large intestines. The C-lobe was prepared proteolytically using serine proteases. The eight NSAIDs from three different classes, were selected for solution studies, these NSAIDs bind to C-lobe with binding constants ranging from 10^{-4} M to 10^{-5} M, thus having reasonable affinities for sequestration of these compounds. In order to establish the mode of binding and sequestration properties, the complexes of C-lobe with all these eight compounds were prepared and the structures of their complexes with C-lobe were determined using X-ray crystallographic method. The structures showed that all the eight NSAIDs molecules not only bound to C-lobe at the well established ligand binding site in C-lobe which is formed involving two α -helices $\alpha 10$ and $\alpha 11$ and also on the different side opposite to the NSAIDs binding site. The ligand binding site is separated from the well known iron binding site by the longest and the most stable β -strand βj in the structure. This established binding site along with new binding site in C-lobe of lactoferrin shows a good complementarity for the acidic and lipophilic compounds such as NSAIDs. This indicates that C-lobe of lactoferrin can be exploited for the prevention of NSAID-induced gastropathy.

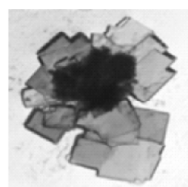
Crystallization and Structure Determination of Lactoperoxidase in Complex with Acetylsalicylic acid, Salicylhydroxamic acid and Benzylhydroxamic acid

¹A. K. Singh, ²Rashmi P. Singh, ²M. Sinha, ²P. Kaur, ²S. Sharma and ²T. P. Singh

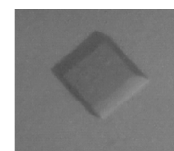
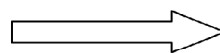
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Lactoperoxidase (LPO), (EC. 1.11.1.7) is a member of the family of glycosylated mammalian heme-containing peroxidase enzymes which also includes myeloperoxidase (MPO), eosinophil peroxidase (EPO) and thyroid peroxidase (TPO). The binding studies of bovine lactoperoxidase with three aromatic ligands, acetyl salicylic acid (ASA), salicylhydroxamic acid (SHA) and benzylhydroxamic acid (BHA) have indicated that these three compounds bind to lactoperoxidase. The freshly purified and lyophilized samples of LPO were dissolved in 0.01 M phosphate buffer to a concentration of 25 mg/ml. The ligands (ASA, SHA and BHA) were dissolved in the above buffer containing 30% (v/v) methanol. Both solutions were mixed in equal volume giving protein to ligand molar ratios of approximately 1:10. A reservoir solution containing 0.2M ammonium iodide and varying concentrations of PEG-3350 from 10% to 30% (w/v) were prepared. 6ml of protein-ligand solution was mixed with 6ml of reservoir solution to

prepare 12ml of drops for hanging drop vapour diffusion method. This experiment was repeated in the three set ups of crystallization for LPO with ASA, SHA and BHA. The rectangular and dark greenish colored crystals measuring up to $0.3 \times 0.2 \times 0.2 \text{ mm}^3$ were obtained after five days in 0.2M ammonium iodide and 20% (w/v) PEG-3350. The crystals obtained in that condition appeared in clusters and found to diffract poorly. Since LPO is a calcium binding protein therefore in order to improve the crystallization process and crystal quality, 2mM CaCl_2 was added in the above crystallization condition. This addition resulted in the growth of single crystals measuring up to $0.4 \times 0.3 \times 0.2 \text{ mm}^3$. These crystals were tested in the X-ray beam and were diffracted nicely.



Initial results



After optimization



LABELLED BIOMOLECULAR NMR COMPOUNDS AND CELL GROWTH MEDIA

Nucleoside Triphosphates and Phosphoramidites:

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- **dNTPs** (¹⁵N)(¹³C,¹⁵N)(D)
- **Deoxyphosphoramidites** (¹⁵N)(¹³C,¹⁵N)

Protein Standards:

- **Solution NMR**
- **Solid State NMR**

Cell Growth Media:

- **Rich *E. coli* Media**
- **Yeast Media**
- **Celtone**

Amino Acids:

- **Protected Amino Acids**
- **Free Amino Acids**

Minimal Media Reagents:

- **D-Glucose** (¹³C₆)(¹³C₆,D₇)(D₇)
- **Glycerol** (¹³C₃)(¹³C₃,D₈)
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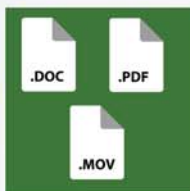
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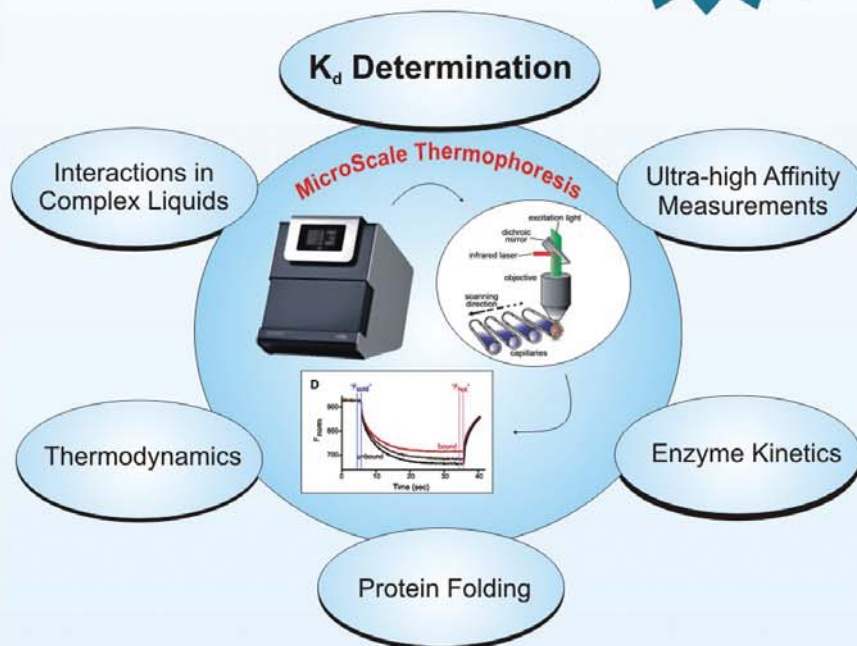
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Microscale Thermophoresis

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Installations
&
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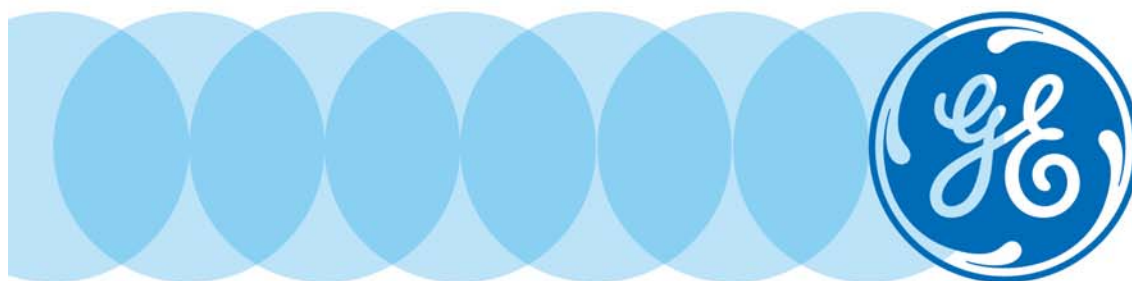


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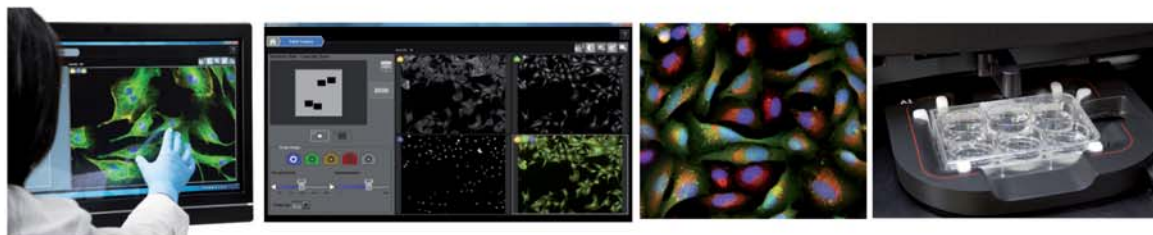
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