



FIMB-2023

FRONTIERS IN MODERN BIOLOGY

ORGANIZED BY



**Department of Biological Sciences
IISER Kolkata**

E-ABSTRACT BOOK

**JANUARY
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DIRECTOR'S MESSAGE

I am delighted to note that the Department of Biological Sciences, IISER Kolkata is organizing its flagship conference Frontiers in Modern Biology (FIMB) on January 20th – 22nd, 2023. The Department has been enthusiastically organizing FIMB since 2011 and this would be the 7th edition of this conference. The Department of Biological Sciences integrates cutting edge research with innovative teaching and constantly strives towards academic and scientific excellence. It provides a vibrating and interdisciplinary climate needed for nurturing the next generation of scientists. FIMB is one such initiative to boost the intellectual environment of the Department. Apart from stimulating discussions on core biology areas, I hope that this meeting will also focus on interdisciplinary topics aligned to the vision of the IISER system. I am sure that this conference would provide an opportunity to the young researchers and students to broaden their horizon in various fields and be a great forum for interaction with the eminent scientists and their peers. I am sure FIMB will serve as an excellent platform for initiating collaborations. I am happy to learn that apart from getting an opportunity to listen to the talks of eminent scientists, the students will also have the opportunity to present their works in the form of platform talks and posters. This will be a wonderful opportunity for them to discuss their research work with a knowledgeable audience and receive useful feedback.

On behalf of IISER Kolkata, I thank you for your participation in the conference and welcome you all to our campus for an intellectual stimulating time.

Prof. Prasanta K. Panigrahi

Director

Indian Institute of Science Education and Research (IISER) Kolkata

DBS CHAIRPERSON'S MESSAGE

After a hiatus of a couple of years due to the Covid-19 pandemic, we are back with our flagship conference Frontiers in Modern Biology – 2023 (FIMB-2023) to be held on 20th – 22nd January, 2023.

Our Department has been organizing FIMB since 2011 and this would be the 7th edition of the conference. Celebrating the diversity and interdisciplinarity in biological research has been the focus of FIMB since its inception and I am sure that the same tradition will continue in FIMB-2023. Apart from hosting invited talks from eminent speakers from across the country, FIMB-2023 will also showcase the research of our talented students through oral and poster presentations. It is heartening to see the overwhelming response of students both from within IISER Kolkata as well as outside who are enthusiastic about presenting their work. On behalf of the Department, I welcome you all to be a part of this excitement of enjoying good science in a relaxed setting at our green campus. Lastly, I thank all the speakers, the members of the organizing committee, our institute administration and our sponsors for their active support towards FIMB-2023. Looking forward to three-days of scientific interactions with you all.

Prof. Rupak Datta

Head, Department of Biological Sciences

IISER Kolkata

FIMB 2023

Schedule

DAY 1: 20.01.2023 (Friday)

03:00 – 04:25 PM	Registration (Meghnad Saha Lecture Theatre Lobby, TRC Ground Floor)
Opening Session (Meghnad Saha Lecture Theatre)	
04:30 PM	Welcome remarks by HoD
04:40 PM	Director's address
Technical Session I (Meghnad Saha Lecture Theatre) Chairperson: Syamal Roy	
05:00 – 05:50 PM	Keynote Lecture Speaker: Gagandeep Kang (CMC Vellore) Title: Learning from human biology
07:00 PM onwards	Dinner (Rabindranath Tagore Auditorium)

DAY 2: 21.01.2023 (Saturday)

08:30 – 09:25 AM	Breakfast (Rabindranath Tagore Auditorium)
Technical Session II (Meghnad Saha Lecture Theatre) Chairperson: Mohit Prasad	
09:30 – 10:10 AM	Invited Talk 1 Speaker: Anand Bachhawat (IISER Mohali) Title: Glutathione metabolism revisited: Learnings on the way
10:15 – 10:55 AM	Invited Talk 2 Speaker: Arun Shukla (IIT Kanpur) Title: Structure, function and modulation of G Protein-Coupled Receptors
11:00 – 11:25 AM	Coffee break
11:30 AM – 12:10 PM	Invited Talk 3 Speaker: Gayathri Pananghat (IISER Pune) Title: Cytoskeletal filaments that sculpt a helical bacterial cell
12:15 – 12:30 PM	Oral Presentation 1 Speaker: Firoz Molla (University of Calcutta) Title: Sugar signalling acts as a proxy for cytokinin signalling for de novo meristem formation during nodule organogenesis
12:35 – 12:50 PM	Oral Presentation 2 Speaker: Anirban Roy (IISER Kolkata) Title: Engineering an acetyllysine reader with a photocrosslinking amino acid for interactome profiling



DAY 2: 21.01.2023 (Saturday)	
01:00 – 03:55 PM	Lunch and Poster Session (Rabindranath Tagore Auditorium)
Technical Session III (Meghnad Saha Lecture Theatre) Chairperson: Somdatta Sinha	
04:00 – 04:40 PM	Invited Talk 4 Speaker: Pramod Wangikar (IIT Bombay) Title: Mass Spectrometry based metabolomics and its applications
04:45 – 05:00 PM	Oral Presentation 3 Speaker: Sanjib Das (ICMR-NICED) Title: Establishment of an intra-gastric surgical model in C57BL/6 mice to study the vaccine efficacy against <i>Helicobacter pylori</i>
05:05 – 05:35 PM	Tea break
05:35 – 06:15 PM	Invited Talk 5 Speaker: Manjari Jain (IISER Mohali) Title: Understanding complex communication in avian vocalizations
07:00 PM onwards	Dinner (Rabindranath Tagore Auditorium)
DAY 3: 22.01.2023 (Sunday)	
08:30 – 09:25 AM	Breakfast (Rabindranath Tagore Auditorium)
Technical Session IV (Meghnad Saha Lecture Theatre) Chairperson: Arnab Gupta	
09:30 - 10:10 AM	Invited Talk 6 Speaker: Arnab Mukhopadhyay (NII) Title: Interspecies interactions regulate longevity in <i>Caenorhabditis elegans</i>
10:15 – 10:55 AM	Invited Talk 7 Speaker: Vinita Gowda (IISER Bhopal) Title: What is in a name? Learning from plants on how to create and maintain diversity in nature
11:00 – 11:25 AM	Coffee break
11:30 - 11:45 AM	Oral Presentation 4 Speaker: Apurba Das (IISER Kolkata) Title: Abnormal autophagy in the MPS-VII fly brain is due to reduced expression of Mif, the master regulator of lysosome-autophagy related genes
11:50 AM – 12:30 PM	Invited Talk 8 Speaker: Dipyaman Ganguly (CSIR IICB) Title: Exploring Piezo1 mechanosensing in immune cells
12:35 PM	Prize distribution, valedictory session and vote of thanks
01:30 PM onwards	Lunch (Rabindranath Tagore Auditorium)

Abstracts for Invited Talks

Learning from human biology

Gagandeep Kang

Division of Gastrointestinal Sciences
Christian Medical College, Vellore

Clinical research, particularly related to infectious diseases, is controversial in India, with accusations of treating the vulnerable as guinea pigs. Yet the development of new understanding, diagnostic, preventive or treatment strategies for human disease is impossible without experimenting on humans or learning from individuals and populations. The use of the rotavirus vaccines in different parts of the world has demonstrated that differences in human biology determined in the environment influence the benefit provided by vaccines and the harms experienced by them. An approach to shortening the path to learning about these differences is to consider more human experimental medicine including the use of human infection and challenge studies. Widely accepted in high income countries such studies are now being adopted in low-income countries, with ethical oversight and scientific engagement that should raise global standards for learning from human biology.

Glutathione metabolism revisited: Learnings on the way

Anand K Bachhawat

Department of Biological Sciences
Indian Institute of Science Education and Research Mohali

Glutathione is the most abundant thiol molecule in almost all eukaryotic cells. It plays important functions in the cell, particularly in redox, detoxification and mitochondrial function. Thus, maintaining the levels of glutathione, as well as the equilibrium between the reduced and oxidized forms in the cell is very crucial for health and disease. Glutathione homeostasis can be maintained by its biosynthesis, transport, efflux and its degradation. Discovered in 1888, between 1920 to the 1970s, much of the metabolism of glutathione were unravelled by many of the stalwarts of biochemistry of those years. Thus, when we accidentally began work on glutathione in the late 1990s, it was thought that the biochemistry of glutathione, and its metabolism was completely known. We soon discovered that this was not the case. Some of the reported pathways were wrong, while in other cases they were incomplete, and in some cases, there were enzymes waiting to be discovered. In this overview, I will briefly discuss some of the discoveries that we have made that helped unravel many unknown aspects of glutathione and its metabolism. This eventually led us to propose a new cycle of glutathione metabolism, “The glutathione cycle” which we propose should replace the previously known “ γ -glutamyl cycle”. Some of the new enzymes could be therapeutically important, and we discuss the challenges and attempts in seeking new inhibitors to these enzymes and pathways.

Structure, function and modulation of G Protein-Coupled Receptors

Arun K. Shukla

Department of Biological Sciences and Bioengineering,
Indian Institute of Technology Kanpur

Our research program is focused on understanding the largest class of cell surface proteins in our body which are referred to as G protein-coupled receptors (GPCRs). These receptors are intricately involved in almost every physiological process and approximately half of the currently prescribed medicines exert their therapeutic effects through these receptors. The overarching theme of my research is to understand the structure, function and regulation of GPCRs, and leverage this information to design and discover novel therapeutics with minimal side-effects. Our research has elucidated the details of how clinically prescribed drugs for a range of human disorders interact with and regulate the function of their cognate receptors in human body. We have also discovered previously unappreciated mechanisms that GPCRs utilize to receive the information outside the cells and relay the message across the cell membrane. More recently, we have designed synthetic proteins such as antibody fragments that can be utilized to monitor GPCR activation and trafficking, and to rewire GPCR signaling in cellular context.

Cytoskeletal filaments that sculpt a helical bacterial cell

Gayathri Pananghat

Department of Biology,
Indian Institute of Science Education and Research Pune

Spiroplasma is a helical bacterium devoid of cell wall. It maintains its unique shape in the absence of cell wall, with the help of cytoskeletal filaments forming a ribbon-like organization within the cell. Interestingly, it also undergoes dynamic changes in cell shape during its movement in the viscous environment where it survives. Cytoskeletal proteins, namely MreB, a bacterial actin homolog, and Fibril, a protein of unknown fold, are the components of the cytoskeletal ribbon which confer its shape. I will be describing the structural biology approaches using X-ray crystallography, electron microscopy and electron tomography that we are employing in order to study the cytoskeletal filaments, and thereby understand the molecular basis of motility and shape determination in *Spiroplasma*.

Mass Spectrometry based Metabolomics and its applications

Pramod P. Wangikar

Department of Chemical Engineering,
Indian Institute of Technology Bombay

Metabolomics is the study of metabolites present within an organism, cell, tissue or biofluids. It is a rapidly emerging field, as evident from the surge in publications in the last 10 years. Metabolomics has implications in biomarker discovery, clinical and fundamental research, spent media analysis and bioprocess optimization, metabolic engineering, and many other fields. Mass spectrometry coupled to liquid chromatography (LCMS) or gas chromatography (GCMS) are the instruments of choice for metabolomics studies as these instruments can measure hundreds of compounds in a single run. Our group has developed expertise in mass-spectrometry based metabolomics of various platforms. We apply these techniques for metabolic engineering of cyanobacteria, spent media analysis and biomarker discovery. Cyanobacteria provide an interesting platform for biotechnological applications due to their efficient photoautotrophic growth and amenability to genetic engineering. Developing such strains may involve host and pathway engineering, which can be rationally guided by metabolomics. We have isolated a novel and robust cyanobacterial strain *Synechococcus elongatus* PCC 11801 from Powai Lake which has the potential to be deployed as industrial host strain. For guiding the strain design, we have developed a workflow for isotopic non-stationary ^{13}C -metabolic flux analysis. This in turn provides a high-resolution flux map, which aids the model-based design of strains and efficient channeling of carbon toward the product of interest. Similarly, we have developed LCMS methods to identify and quantify over 500 metabolites in human blood samples. These are being further used in predicting biomarkers and metabolic signatures for various complex disorders. Our group has also developed a GCMS and LCMS based method to analyse spent media from the bioprocess to understand the metabolites consumed or secreted during the process by bacterial or mammalian cells. Better visibility of the media provides insights into batch-to-batch variation in productivity as well as guide efficient feed design. Moreover, analyzing a high-resolution LCMS data is a challenge as the presently available software shows 10-20,000 features due to the noise and redundancy in the data. To overcome the challenge, we have developed a software to analyze LC-MS data using artificial intelligence and machine learning, which has improved the turnaround time and range of data analysis, yielding more accurate and improved results for applications.

Understanding complex communication in avian vocalizations

Manjari Jain

Department of Biological Sciences
Indian Institute of Science Education and Research Mohali

Acoustic signals may have evolved under diverse selection pressures. Two important drivers of signal evolution are sexual selection and sociality. Sexual selection is known to drive vocal complexity in display vocalizations, whereas sociality is likely to drive complexity in contextual calls in social animals. While the role of sexual selection in signal evolution has been studied extensively in birds, the role of sociality in driving vocal complexity has been examined in mammals and has been largely overlooked in birds. Using bioacoustics and behaviour as our research tools we are examining complexity in avian vocalizations, both in terms of structure and function. In this talk I will show how complexity in avian vocalization manifests in variety, function, syntactic rules and even linguistic laws. For this I will focus on our work on two avian species, one in which vocal complexity is likely to have been driven by sexual selection and the other in which the driver is likely to be sociality. Lastly, I will touch upon the value of using common backyard species as model systems in ecological research.

Interspecies interactions regulate longevity in *Caenorhabditis elegans*

Arnab Mukhopadhyay

Molecular Aging Laboratory, National Institute of Immunology

A complex interaction between commensal microbiota and the host regulates organismal aging. Only recently, the molecular details of such interspecies interactions have started to be unravelled. The microbiota is a rich source of vitamin B12 that is required for multiple life history traits, including longevity. *C. elegans* has been instrumental in our understanding of the vitamin B12-driven interspecies relationship that drive cellular signalling and gene expression to influence metabolism and aging. The worms encounter and feed on bacteria of different micronutrient content in their niche but can modulate metabolism to maintain a normal life span in most scenarios. However, in the laboratory, we often encounter mutants that show differential phenotypes on two different bacteria while the wild-type is able to maintain homeostasis. Such diet-gene interactions are critical to our understanding of how nutrition affects longevity. We have been characterizing one such diet-gene pair involving a kinase gene and vitamin B12 supplied by the bacteria. I will discuss our current understanding on this aspect of a micronutrient-driven interspecies relationship.

Learning from plants on how to create and maintain diversity in nature

Vinita Gowda

Department of Biological Sciences,
Tropical Ecology and Evolution Lab,
Indian Institute of Science Education and Research Bhopal

Biodiversity refers to the biological diversity in any region, which is often measured using different ecological metrics such as species diversity or abundance. The critical measuring entity in any of these metric is a “taxa”. The origin, maintenance and extinction of these taxa therefore is key to our understanding of how biodiversity is generated and maintained in any region. And, a taxa is identified by its name. India is known for its two megadiverse regions the Western Ghats and Eastern Himalayas. I will present one case study from each of these regions that will highlight evolutionary and ecological mechanisms that may be used by plants to create as well as maintain taxonomic diversity. The Eastern Himalayan region is represented by taxa from the NE Indian states, which has a continuous distribution with flora from SE Asia, while the Western Ghats is different because it forms an isolated floristic region (from the NE or SE Asia), and shows more resemblance to the African flora. Our studies from both these regions show that most of our flora may be very young (~7-10 mya), and may be rogue when it comes to ‘reproductive isolation boundaries’. Added to this is the presence of hyper diverse niches from the Himalayan uplift, monsoon intensification, and generalist pollinators which has resulted in local hybridisation events that are common in both these regions. While hybridisation can result in taxonomically complex outcomes that seem unresolvable, it also provides us with an opportunity to understand and observe how incipient speciation may look like in many of these taxa, which allows us to witness how species evolve, ultimately creating the floristic biodiversity in these two hyperdiverse regions of India.

Exploring Piezo1 mechanosensing in immune cells

DipyamanGanguly

Division of Cancer Biology and Inflammatory Disorder
CSIR-Indian Institute of Chemical Biology

Piezo1 is a recently discovered professional mechanosensing ion channel that responds to physical cues at the plasma membrane, described widely in CNS and hemodynamic functions. Increase in local membrane tension leads to opening of Piezo1 channels and intracellular influx of bivalent cations like Ca^{2+} , leading to downstream signaling and modulation of cell biology. Our laboratory, primarily focused on human dendritic cell biology, was the first to implicate Piezo1 mechanosensing in human immunocellular functions, which we did while exploring the cell biology of plasmacytoid dendritic cells. We further explored the role of these mechanosensors in human T cells and revealed their essential role in T cell activation and migration, driven by discrete mechanisms. Piezo1 optimizes T cell activation by local bolstering of the actin cytoskeleton at the immunological synapse. On the other hand, Piezo1 plays a critical role in integrin-dependent T cell migration in response to chemokine signaling, downstream of focal adhesion kinase activation. In the present talk I plan to briefly outline the research interests of the laboratory over the past decade, tell the story of how we stumbled upon Piezo1 as a cell membrane-intrinsic sensor for physical cues in immune cells and then briefly discuss the insights we gathered on its function in regulating human immune cells.

Abstracts for Oral Presentations

Sugar signaling acts as a proxy for cytokinin signaling for de novo meristem formation during nodule organogenesis

Firoz Molla, Anindya Kundu, Maitrayee Das Gupta

Department of Biochemistry, University of Calcutta

Symbiosis between plants and diazotrophs require formation of a *de novo* meristem for endocytic accommodation of symbionts, a process that is tightly regulated by plant hormones cytokinin and auxin. Cytokinin signaling through CRE1 receptor causes auxin accumulation by regulating its transport or biosynthesis to initiate cell division for nodule organogenesis. Accordingly, CRE1 mutant (*cre1*) is unable to undertake symbiosis and our objective was to strategize and restore functional symbiosis in *cre1* for understanding the downstream events. First and foremost, we show that sucrose as well as turanose (non-metabolizable sucrose) treatment can recover functional symbiosis in *cre1* indicating the importance of downstream sugar signaling in symbiosis. An auxin conjugate hydrolase MtIAR33 was highly upregulated by sugar signaling leading to *IAA-asp* to *IAA* conversion. Overexpression of MtIAR33 could also restore symbiosis in *cre1* indicating deconjugation of auxin conjugates to be a potential pathway of auxin accumulation during nodule organogenesis. Additionally, sugar signaling significantly upregulated an auxin responsive homeobox transcription factor WOX5 well known for its role in meristem maintenance. Intriguingly overexpression of MtWOX5 from *Medicago* having indeterminate nodule meristem failed to rescue *cre1* but AhWOX5 from *Arachis* having determinate meristem could completely rescue *cre1*. We probed into the mechanism of this differential response and uncovered a striking molecular basis for how these homologous proteins have diverged in these two legumes. We have shown that MtWOX5 function as a repressor whereas AhWOX5 acts as an activator and swapping a single amino acid is sufficient to convert MtWOX5 to AhWOX5 function and vice versa.

Engineering an acetyllysine reader with a photocrosslinking amino acid for interactome profiling

Anirban Roy, Soumen Barman, Jyotirmayee Padhan, Babu Sudhamalla

Department of Biological Sciences
Indian Institute of Science Education and Research Kolkata

Bromodomains are specialized epigenetic readers that bind acetylated lysine residues on histone and non-histone proteins. Docking of bromodomains to acetylated histones provides a platform for other effector proteins to be recruited to the chromatin for downstream cellular functions. Bromodomains bind a vast number of acetylated protein ligands, but traditional methods cannot be employed to identify their entire interactome with spatiotemporal resolution. In contrast, photocrosslinking amino acids are a powerful alternative to study protein-protein interactions that can detect even low-affinity interactions. Photocrosslinkable amino acids carry photoreactive functional groups like diazirines, aryl azides, and benzophenone. Benzophenone, a popular photocrosslinker is activated at 365 nm wavelength of UV light. Upon UV irradiation, it generates a diradical and reacts with nearby C–H bonds. If no functional group is present nearby, it can relax back to its original state. Herein, we engineered the bromodomain of ATAD2B to introduce the photocrosslinking amino acid 4-benzoyl-L-phenylalanine (BzF), which carries a benzophenone moiety for interactome mapping. All possible orientation of the ATAD2B-BzF mutant was predicted by computational modeling and simulation. BzF was site-specifically incorporated into the ATAD2B bromodomain by utilizing amber suppressor mutagenesis using the evolved orthogonal *M. jannaschii* TyrRS-tRNA_{CUA}^{Tyr} pair. Circular dichroism and thermal shift assay were performed to measure the structural and thermal stability of the ATAD2B-BzF mutant protein. In the photo crosslinking experiment, ATAD2B-BzF mutant crosslinked to acetylated histone H4 peptides and full-length histones upon UV irradiation. We show that the ATAD2B-BzF mutant underwent robust crosslinking with hyperacetylated cellular proteome and could capture novel interactome.

Establishment of an intra-gastric surgical model in C57BL/6 mice to study the vaccine efficacy against *Helicobacter pylori*

Sanjib Das, Prolay Halder, Soumalya Banerjee, Shanta Dutta, Asish Kumar Mukhopadhyay, Hemanta Koley

Division of Bacteriology,
ICMR-National Institute of Cholera and Enteric Diseases

Helicobacter pylori (*H. pylori*) cause chronic gastritis including dyspepsia, duodenal ulcers affecting half the population worldwide. A 2012 report estimated around 723,000 deaths (8.8% of all cases) out of a total 14.1 million cancer cases due to gastric cancer only making it the 3rd major cause of deaths globally. Here, we present a newly established intra-gastric surgical model in C57BL/6 mice to check the vaccine efficacy of OMVs based immunogen. To formulate the immunogen, different strains are subjected to biochemical and PCR-based techniques to screen for major virulence factors. Strain positive for most of the parameters, was selected for Outer Membrane Vesicles (OMVs) isolation followed by characterization and immunisation using C57BL/6 mice. Standard protocol was used to isolate the OMVs. A comparative profile was done for protein and LPS (lipopolysaccharide) present on OMVs. Biophysical characterization was performed using DLS, Zeta potential and TEM analysis. For vaccine efficacy study, an intra-gastric surgical model was established through histopathological and immunological analysis. Completion of OMVs based immunization; the intra-gastric challenged mice model reflects the all parameter (histopathological and immunological) of *H. pylori* infection in both placebo group and immunized group; with better cytokine profile, antibody titer and tissue architecture in immunized cohort. This model would be effective in vaccine efficacy study against *H. pylori*.

Abnormal autophagy in the MPS VII fly brain is due to reduced expression of Mitf the master regulator of lysosome-autophagy related genes

Apurba Das, Mohit Prasad, Rupak Datta

Department of Biological Sciences
Indian Institute of Science Education and Research Kolkata

Mucopolysaccharidosis type VII (MPS VII) is a recessively inherited disease that occurs due to deficiency of β -glucuronidase leading to lysosomal accumulation of undegraded glycosaminoglycans. Prominent clinical symptoms include hydrops fetalis, musculoskeletal deformities, neurodegeneration and hepatosplenomegaly leading to premature death. However, the mechanism underlying neurodegeneration in MPS VII is poorly understood. Recently our lab has generated first MPS VII fly model by knocking out the CG2135 gene, the *Drosophila* homolog of β -glucuronidase. The CG2135^{-/-} fly exhibited accumulation of ubiquitinated proteins and mitochondria in the brain indicating defect in cellular clearance pathway. To understand the reason behind such clearance defect, we studied the status of autophagy in the CG2135^{-/-} fly brain. The level of autophagosomal protein Atg8II was found to be significantly low in the brain of the CG2135^{-/-} fly. Expression of the autophagy related protein Atg1 was also found to be downregulated in the CG2135^{-/-} fly brain indicating defective autophagy. TEM images of the CG2135^{-/-} brain showed reduced abundance of autophagic bodies and increased multilamellar bodies confirming the autophagy defect. Next, to understand the reason of autophagy dysregulation we investigated its regulators mTOR and Mitf. Our results showed mTOR is inactive and expression of Mitf is downregulated in the CG2135^{-/-} fly brain. Mitf targets like Vha14, Vha55 and VhaPPA were also found to be downregulated in the CG2135^{-/-} fly brain. Collectively, our results indicate a novel mechanism where Mitf downregulation is affecting lysosomal and autophagy genes that might be responsible for the neuronal pathogenesis in the MPS VII fly.

Abstracts for Poster Presentations

Biochemical characterization and engineering of AfPolX1

Abhijit Behera, Purba Mukherjee

Department of Biological Sciences
Indian Institute of Science Education and Research Kolkata, India

X-family DNA polymerases play a vital role in DNA repair. Although mammalian, especially human, DNA polymerases of this family have been extensively studied, X-family polymerases from other eukaryotes are yet to be well-characterized. We are investigating a novel X-family polymerase from the infectious fungus *Aspergillus fumigatus* Af293 (AfPolX1), annotated as a Terminal deoxynucleotidyl Transferase (TdT) in bioinformatic databases (NCBI Accession ID: XP_755218 & UniProt Accession ID: Q4X1T5). We report that, unlike TdT, AfPolX1 has no template-independent nucleotide incorporation activity. Instead, it has single-nucleotide gap-filling activity, a characteristic feature of repair polymerases such as Pol μ . AfPolX1 has poor ribonucleotide discrimination; however, in the presence of other ribonucleotides, it shows unusually low incorporation activity. We are studying the fidelity of AfPolX1 to examine its role in DNA repair further and to gain insight into AfPolX1's unusual mechanism of nucleotide incorporation. Human Pol μ and TdT share 43% sequence identity with significant structural homology. To shed light on how Pol μ developed template-independent activity and evolved into TdT, we have made an engineered AfPolX1 and are exploring the contribution of the *loop1* region of these polymerases.

Studies towards engineering a trifunctional cellulase

Aditi Konar, ShritamaAich, Supratim Datta

Department of Biological Sciences
Indian Institute of Science Education and Research Kolkata, India

Production of glucose from raw biomass involves the action of 3 cellulolytic enzymes, starting with endoglucanase, followed by cellobiohydrolase and beta glucosidase. This glucose can then be channelised into different pathways like production of ethanol (biofuels), or other metabolic pathways to produce value added products like shikimic acid, levulinic acid, aspartic acid, sorbitol and many more. This work involves making the process of glucose production more economical by replacing the 3 cellulolytic enzymes with just one processive enzyme which shows a tri-functionality fulfilling the roles of all 3 enzymes. Characterisation of this enzyme has shown high stability and catalytic activity along with multi-substrate specificity. Additionally, a study of the substrate breakdown pattern of this enzyme shows immediate production of all the soluble sugars precluding the necessity for a prolonged reaction time. A further study into the mechanistic insights of the aforementioned properties of this enzyme makes it remarkably interesting to find new dimensions from a structural level that can justify this functionality.

Bacterial Outer Membrane Vesicles as an offensive striker for microbes or a defensive custodian of the host?

Afruja Khan¹, Avijit Sardar², Pradip K. Tarafdar², Amirul I. Mallick¹

¹Department of Biological Sciences

²Department of Chemical Sciences

Indian Institute of Science Education and Research Kolkata, India

Naturally secreted outer membrane vesicles (OMVs) from gut microbes carry diverse cargoes, including proteins, nucleic acids, toxins, and other intrinsic secretory components. As a generalized secretion system, bacterial OMVs can shuttle molecules across different cell types facilitating bacterial pathogenicity and self-survival in the gut. Although the harmonized secretion of major virulence factors is a shared mechanism of many mucosal pathogens, *Campylobacter jejuni* (*C. jejuni*) lacks classical virulence-associated secretion systems. Alternatively, *C. jejuni* often employs OMVs to deliver active toxins and secretory proteins into the target cells. Here we studied the “bi-functional attributes” of *C. jejuni* OMVs to venture into their unique functional diversities and explore their potential to influence the immunological correlates of host protection *in vitro*. We performed mass spectrometry of trypsin-digested OMVs protein and identified 237 proteins, including several periplasmic, membrane-associated, and putative cytoplasmic virulence factors, including two key proteins of the bacterial Type VI secretion system, TssM and VgrG. To decipher the mechanisms of cellular uptake of OMVs, combining FRET, R-18 dequenching assay, and *in-vitro* localization study, we further confirmed the involvement of multiple endocytic processes, predominantly via the dynamin-dependent pathway. We found a high degree of positive correlation between OMVs concentration and *C. jejuni* invasion rate of different target cells; on the other hand, we also noticed strong pro-inflammatory responses when cells were incubated with OMVs. Together data presented herein indicates that despite its intrinsic property to deliver a repertoire of virulence factors of parent bacteria (offensive striker), OMVs can serve as “defensive custodians” for the host by eliciting immunoprotective responses.

Handedness in free-ranging Hanuman Langurs (*Semnopithecus entellus*) within an urban landscape

Akash Dutta¹, Dishari Dasgupta², Pritha Bhattacharjee¹, Manabi Paul¹

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Indian Institute of Science Education and Research Kolkata, India

Our study intends to explore the impact of urbanisation on behavioural patterns of non-human primates. From our personal qualitative observations in West Bengal, hanuman langurs (*Semnopithecus entellus*) often come in a large troop, create man-primate conflict while either to scavenge or being provisioned with foods, within urban settlements. Therefore, we chose to study on multiple hanuman langur troops, receiving varying human interferences across different habitats along the urban-rural gradient. Based on our preliminary study, we have already addressed the alteration of food choice in free ranging hanuman langur in urban space as a result of continuous human provisioning (Dasgupta et al., 2021). For further intensive study, we are focusing on the behaviours, that exhibits the trait of individual, as well as group personality of langurs and action reaction interplay with the surrounding factors. We are carrying out field-based experiments that requires them to complete different tasks to explore the trait of handedness of langurs, living under natural social and ecological conditions. Handedness has a variety of positive effects on cognition and social cohesiveness. Till now, we have done total 191 experiments on 32 individuals, from which 156 successful experiments have been considered and more such experiments are still going on. This ongoing study reveals some significant hand preferences at individual level and inter-manual differences between different life stages of langurs. These findings offer us early evidence of handedness in free-ranging hanuman langurs at the individual and population levels.

A study of mechanical heterogeneities during collective cell migration of fish keratocytes

AlekhyaHati, Dipjyoti Das, Bidisha Sinha

Department of Biological Sciences
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Collective cell migration (CCM) is the migration of cells in closely associated cohesive groups, critical to biological processes such as embryogenesis, tissue repair, and cancer metastasis. It is slower than single-cell migration but provides a better net directionality to the cells. To understand underlying mechanisms that govern front coherence yet allow exploration using fingering of the front, we study CCM in fish keratocytes. Unlike systems of study involving a two-dimensional monolayer of cells, in fish keratocytes, a continuous flux of cells occurs from the scale explant during the migration, forming multilayered cell sheets. As cells migrate, the expanding front infrequently develops finger-like multicellular protrusions. Since the leading edge cells are often connected by actomyosin cables, we ask if such cables prevent fingering. We show that mild perturbation of actin polymerization can significantly enhance the propensity for fingering. It also enhances the positive correlation between front curvature and front velocity suggesting that the migrating front may use actin to check fingering instabilities. Our future goals are to understand if heterogeneities in the cable and the front could induce local instabilities for better exploration. In contrast to leaders, follower cells do not directly decide front shape or fingering – but how do they contribute to the overall optimization of CCM? Recently our lab showed that some followers can form "bridges" between substrate-adhered cells. Using YAP1 as an indicator, we uncover that as passive riders, cells are more prone to proliferation, while leaders and substrate-adhered followers contribute to the cell sheet's active migration.

Olaparib induces synthetic lethality in DNA-repair deficient gingivobuccal oral squamous cell carcinoma(OSCC-GB) cells

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Gingivobuccal oral squamous cell carcinoma(OSCC-GB) is the most common form of tobacco-induced oral cancer among Indian men and is associated with high mortality. A previous genomic study on OSCC-GB patients revealed that mutations in DNA repair pathways are associated with lymph node metastasis and poor prognosis. The PARP-inhibitor, Olaparib also known as Lynparza (AstraZeneca) has been already approved for the treatment of ovarian cancer patients with a BRCA mutation and has also shown promising activity in breast and prostate cancer patients. And considered to be the world's first precision medicine for prostate cancer. We investigated the effect of Olaparib on three genomically-characterized OSCC-GB cells, harboring mutations in DNA-repair pathways, of which, OSCC-29B cells additionally possess a BRCA1 mutation in addition to the other mutations. We observed that Olaparib perturbed the viability of OSCC-GB cells in a dose-dependent fashion. The effect was most promising against OSCC-29B cells, having BRCA1 mutation. Interestingly, we observed that Olaparib resulted in genomic instability in OSCC-GB cells, as confirmed by the upregulation and nuclear accumulation of γ H2AX-foci, a prominent marker of double-stranded DNA breaks. Moreover, Olaparib induced the reprogramming of the epithelial-to-mesenchymal transition (EMT) in OSCC-GB cells, by interfering with Akt-signaling. Furthermore, we observed that Olaparib exhibited a promising effect in combination with Cisplatin, in several OSCC-GB cell lines. Interestingly the effects were negligible in the OSCC-GB cell line, without BRAC1 mutation or mutations in repair pathways. Hence it may be concluded that Olaparib induces synthetic lethality in repair-deficient OSCC-GB cells.

LB film decorated with silver nanoparticles SERS biosensor for Pesticide detection in environment

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With the vibrational information available from Surface Enhanced Raman Spectroscopy (SERS), the molecular forms and the orientation of an adsorbed species on the metal surfaces can be delineated from the enhancement of its signal. Fabrication of SERS-active substrates is a thrust area of research in the field of SERS. Here, we report the fabrication of a new SERS-active substrate composed of Ag nanoparticles grown on Langmuir-Blodgett (LB) film. The SERS activity of these SERS-based-biosensor exploited with environmentally important pesticide, and dye namely Rhodamine 6G. Density functional theory (DFT) calculation was performed to study the molecular structure and Raman vibrational modes of the paraquat. A detailed discussion has been devoted to uniformity, reproducibility, and practical utility of the fabricated substrates. The results emanate the ultra-sensitive detection of paraquat, uniform enhanced signals over the substrates, and high reproducibility. The report successfully demonstrated the detection of paraquat in agricultural water, environmental water, and soil extracts. Paraquat, known to be highly toxic to human is widely used as an effective pesticide in many countries. The mortality-rate of paraquat exposure is high because of lack of effective treatments. Many countries regulate traces in drinking water because the solubility is quite high in water. Hence, monitoring the levels of this pesticide is quite significant. We propose that the present sensors hold the potential to be used in pesticide detection and monitoring in-the-field experiments. This method is useful than existing ones as detection can be made at trace-amounts.

Exploring color preference in Indian free-ranging dogs (*Canis lupus familiaris*)

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The lives of Indian free-ranging dogs are intertwined with human habitats and are rich with different kinds of color cues. The dogs, being mainly diurnally active, may have adapted to properly identify these cues and gain an advantage in the urban jungle. Color can provide contrast and context and help the dogs interact with their environment. It is known that dogs are dichromats, being able to see only shades of blue and yellow, with their cone cell activities peaking at 429nm and 555nm. Through a three-choice test among blue, yellow, and neutral gray objects, we found that the dogs prefer yellow as the color to explore first. Such color preferences have been previously found in many species across different phyla, including drosophila, zebrafish, blue-footed booby birds, and lions. A better understanding of the reason for this color preference in dogs can help us explore novel avenues of dog and urban animal ecology.

Understanding cellular mechanoprotection by Ezrin

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Ezrin is an important member of the ERM family of proteins. Its role as an important linker between cell membrane and the underlying actin cortex has been extensively studied but its role in cellular mechanoprotection and sensing is not clear. We first show that at the basal membrane, inhibiting ezrin results in formation of new focal adhesions as well as strengthening of stress fibers by their re-orientation and elongation. We further demonstrate that this is a formin-dependent effect with less or no contribution from the Rock-dependent stress fiber remodeling pathway. Ezrin inactivity is balanced out by enhanced adhesion and reduced motility. However, previous work in the lab reported a less contractile cortex at apical section of cells. In the next part, our study aims at understanding the role of ezrin in caveolae-independent cellular mechanoprotection. We use hypo-osmotic shock on cell pre-loaded with Calcein AM to measure rupturing propensity and rupture time distribution by following their response by epi-fluorescence microscopy for 10 minutes. Under normal conditions volume regulation starts in the first few minutes and a very small percentage of cells rupture. Inhibiting Ezrin's activity doesn't alter rupture propensity or time taken to rupture significantly. Interestingly, when caveolae-mediated membrane homeostasis is perturbed by cholesterol depletion by MBCD or statins, the time taken to rupture is reduced. To check if tension is involved, we study the correlation of cell spread area in rupture time. Our data strongly suggests that ezrin's mechano-protective role may be tension-dependent which we will further establish in future.

Repurposing of approved kinase inhibitors against *Leishmania donovani* targeting cell cycle regulatory kinases

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Leishmaniasis belong to a group of infectious diseases caused by obligate intracellular parasite belonging to the genus *Leishmania*, transmitted by female sand fly vector of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World. *Leishmania donovani* thrives as promastigote in sandfly gut at 22°C at a pH 7.4 and at 37°C at a pH 5.5 in the mammalian macrophages where it transforms into amastigotes. Due to the parasite's intracellular origin and widely dispersed locations, the disease poses a large global concern and presents a significant obstacle in the discovery and delivery of drugs. Drugs recently in use have several restrictions, making the discovery of new drugs a necessity. Moreover, resistance against the approved anti-leishmanials has made it important to look for a few pre-clinical candidates against VL. Enzymes or cell-surface receptors are the major therapeutic targets against *Leishmania*. Among these, modulation of protein kinases might be beneficial for the treatment of leishmaniasis as protein kinases play crucial role in regulation of cell cycle and other aspects of the parasites' biology. This work summarizes the role that protein kinases play in modulating various phenomenon associated with differentiation and infectivity of the parasites and the concept of repurposing the human protein kinase inhibitors against leishmaniasis and their importance in the development of new chemical entities with potential beneficial effects on the diseases caused by different species of *Leishmania*.

Two E3 ubiquitin ligases, RDUFs, are novel regulators of seedling photomorphogenesis in Arabidopsis

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Light is a critical environmental cue that regulates plant growth and development. A group of Really Interesting New Gene (RING) domain-containing E3 ubiquitin ligases, such as COP1, have been shown to play a vital role in regulating seedling photomorphogenesis in *Arabidopsis*. COP1 is a master repressor of photomorphogenesis. Here, we identified and functionally characterized two novel RING domain-containing E3 ubiquitin ligases such as RDUF1 and RDUF2, which have a domain of the unknown function (DUF). Analysis of knock-out mutants of *RDUF1* and *RDUF2* results in significantly longer hypocotyls, while the overexpressed transgenic lines showed shorter hypocotyl and enhanced photomorphogenic response in a wavelength-independent manner. Our genetic analysis revealed that RDUFs function parallelly to HY5 but downstream to COP1. Moreover, our gene expression data showed that HY5 probably promotes *RDUF1/2* expression in a light-dependent manner, suggesting that RDUFs could be novel targets of HY5 for the activation of gene expression. Future work focusing on the molecular interactions of RDUFs with HY5 and COP1 will reveal the underlying mechanism through which RDUFs promote seedling photomorphogenesis in *Arabidopsis*.

Implications of Simple Sequence Repeats in the Viral Genomes of Peribunyaviridae

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Simple Sequences repeats (SSR) as a signature is used to illustrate the evolutionary relation of viruses. Here, Peribunyaviridae has been used, due to its tri-partite (L, M and S) nature, which is assigned based on its fragment size. Peribunyaviridae belongs to Bunyavirales possess 4 Genera. Herbevirus (3), Orthobunyavirus (125) classified, (14) unclassified, Pacuvirus (5), (1) Shangavirus and (3) unclassified Peribunyaviridae. For analysis, several databases and software such as ICTV, NCBI, MISA, MAFFT and MEGA have been utilized. Channelizing them we obtained L (~6.909), M (~4.43) and S (~0.98) genome fragment size in kbp. It is negatively co-related to GC%, as L (33.66%), M (34.47%) and S (40.10%) segments. Three segments consist of 1651, 907 and 229 mono-repeat motifs respectively. Total incidences, of di-repeat (AT/TA) along L (1469), M (1072) and S (115) incidences. The L, M and S consist of 330, 267 and 58 Tri-repeat motifs respectively. Upon further analysis, we observe more than 95% of the SSR where in the CDS region. Mono SSR A+T composition play credential role in evolution as they are in the active site. Phylogenetic tree concluded that segments have been evolved differently; having same host, with most A+T% (100%) mosquitoes.

VEGF-A mediated alteration of GJIC restricts murine- β -Coronavirus infectivity

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At the onset of the COVID-19 pandemic, epidemiologically, the coronavirus pathology in neoplasms has been reported to show a differential effect depending on the etiology of cancer, which remains a critical research question to be studied. This study aims to set up an experimental immunocompetent syngeneic model to observe the effect of murine- β -Coronavirus in ovarian carcinoma pathogenesis and its onset to metastasis, both *in vitro* and in C57BL/6 mice. Epithelial Ovarian Carcinoma Cell Lines derived from C57BL/6 mice, ID8 and ID8 overexpressing VEGF-A (ID8-VEGF) were used in this study to generate a syngeneic Ovarian Cancer Model. ID8 and ID8-VEGF cultures were exposed to MHV-RSA59, a demyelinating strain of MHV (Mouse Hepatitis Virus) to observe its effect on the neoplasticity of Ovarian Cancer Cells *in vitro*. Findings from this study suggest that exogenous overexpression of VEGF-A induced poor DNA damage repair, resulting in the presence of intracellular DNA fragments, which triggered the cGAS-STING pathway and retention of that signal due to poor GJIC formation and ERp29 in ID8-VEGF. Activation of cGAS-STING pathways upregulated the antiviral protein Ifit-2, restricting their replication and subsequent infectivity *in vitro*. However, upon infection, MHV-RSA59 demonstrated an anti-neoplastic effect by suppression of EMT, production of Apoptotic factors and oxidative stress resulting in suppression of metastasis and eventual virus-induced oncolysis *in vitro*. Further studies on the anti-neoplastic nature of MHV-RSA59 with Ifit-2 antagonists are expected to establish non-human-coronaviruses as effective oncolytic agents for peritoneal carcinomas due to their robust immunomodulatory properties that can be redirected toward cancer cells.

Effect of a pesticide on the behaviour of a non-target ant *Diacamma indicum*

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Globally, pesticide consumption has increased which adversely affects the environment, especially non-target species. This is compounded by the use of higher than recommended dose by farmers. *Diacamma indicum*, a non-target, primitively eusocial ant living near human habitat are occasionally exposed to pesticides used in domestic and agricultural fields. We studied the effect of a pyrethroid pesticide, alpha-cypermethrin (widely used in West Bengal), on the behaviour of these ants. We performed three experiments simulating field-like acute exposure of alpha-cypermethrin to ant colonies. First, we conducted a long-term behavioural assay with various doses (4x, 2x, 1x, 0.5x and 0.25x of recommended dose). We identified five pesticide induced behaviours: appendage shaking, staggering, trembling, twitching and paralysis which were absent in non-contaminated colonies. 2x and 4x had significantly greater impact on behaviour. 0.5x and 4x caused significantly higher mortality. Second, long-term effect of recommended dose on colony fitness was tested on 15 control and 25 treatment colonies. No significant effect on colony size, mortality, or status of gamergate was detected. Third, we subjected 16 colonies to relocate in contaminated arena to mimic real-world situation where nests might be destroyed during field pesticide applications. Although 80% colonies successfully relocated, the process was significantly delayed as pesticide impacted their behaviour and task organisation. After 24 hours, colony mortality was 14% while 7% were paralysed. Our results show that although alpha-cypermethrin did not cause severe lethality of ant colonies, it has significant negative effects on *D. indicum* behaviour which might affect their success in the long run.

An efficient UV-C device for decontaminating personal protective equipment (PPE) soiled with human Corona and Influenza virus: Solution for small-scale reuse

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The recent pandemic upsurge and the related substantial rise in the demand and usage of personal protective equipment (PPE) including protective gowns, face masks, face shields, head covers, gloves, etc., have led to severe ecological repercussions and a burden on global waste management systems. The surge of using such non-recyclable items has in turn led to high carbon footprint emissions, and water and soil pollution. Hence, several strategies have been in place to tackle the extreme anomaly in the demand-supply chain and the increased environmental pollution, including repurposing different alternate materials in place of the recommended PPE materials, reducing the overall use of PPE by the maintenance of isolation, sanitation and social distancing and finally, reusing the actual PPE components safely. To this end, an increasing body of studies and evidence, suggest the concept of its reuse after a round of safe and efficient disinfection cycle. Recent studies support the use of Ultra Violet (UV) radiation-based surface disinfection methods and have attracted significant attention and gained marked visibility in the global market. In this study, we have systematically evaluated the effectiveness of an in-house make UV sanitization device (UVSD) in decontaminating PPE and have shown that a 15 min exposure of the virus-contaminated PPE within the UVSD cabinet could effectively inactivate both the human H1N1 Influenza virus (A/PR/8/1934/H1N1) and Human Coronavirus (HCoV) OC43 virus. Our result demonstrates the possible application of UV-C radiation as an effective strategy to reuse PPE in several organizational setups, including healthcare and other occupational settings.

Engineered improved siRNA therapeutics against metastatic breast cancer

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RNA interference has huge potential to treat metastatic breast cancer. However, efficient cytosolic delivery of functional siRNA is a key challenge for developing effective RNAi therapies. We have engineered multifunctional protease-stabilized facial lipopeptide based siRNA transporters which have demonstrated comparable knockdown efficiency like, HiPerFect, a lipid-based widely-used commercial transfection reagent sold by multinational company, Qiagen. Additionally, unlike HiPerFect, our designed siRNA transporters do not show any toxicity and exhibit very high cytosolic delivery in MDA-MB-231 cells (TNBC) and primary cell line HUVEC (hard-to-transfect cell line), better than HiPerFect. Our designed non-immunogenic, protease-resistant siRNA transporters also provide stability to siRNA against RNase. Endosomal escape is an important feature of any efficient siRNA transporter and our *in-vitro* data shows endosomal escape of siRNA transporter by pore formation in endosome mimicking giant unilamellar vesicles. Notch 1 induces metastasis, proliferation and drug resistance in TNBC and our *in vitro* data demonstrates long term and high level of Notch 1 knockdown by our designed siRNA transporter encased Notch 1 siRNA. Downregulation of metastasis-promoting MMP-2 gene, reversion of epithelial-mesenchymal transition and decreased expression of stemness markers were observed in Notch 1 silenced MDA-MB-231 cells, inferring efficient prevention of cancer metastasis in TNBC. Interestingly, nanobridge-mediated interaction between endothelial cells (HUVEC) and epithelial cells (MDA-MB-231) was also inhibited in Notch-1 silenced MDA-MB-231 cells. In *in-vivo* zebrafish model, Notch-1 silenced MDA-MB-231 exhibited prevention of metastasis and cell proliferation. The experimental data demonstrates that our engineered multifunctional siRNA transporters as monotherapy or in combination therapy have potential for performing enhanced RNA interference overcoming cancer metastasis. Such siRNA transporters might be translated for developing improved siRNA based combination therapeutics against metastatic cancer.

Receptor binding analysis predicts potential transmission of SARS-CoV-2 Omicron (B.1.1.529) variant to mammalian hosts

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The currently circulating variant of concern Omicron (B.1.1.529) has higher human infectivity than previously known Severe Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) variants. The extremely high infectivity of this variant makes it possible that it might spread to more intermediate hosts. To establish the SARS-CoV-2 infectivity, the spike (S) protein receptor binding domain (RBD) interacts with the host Angiotensin I Converting Enzyme 2 (hACE2) receptor. In this study, we used protein-protein interaction analysis to evaluate the interaction of Omicron S protein RBD with the ACE2 receptor of 143 mammalian hosts, including humans. The aim of this study is to predict the likelihood of the virus infecting other animal species that live in close proximity to human in urban, rural, agricultural, or zoological settings. The Omicron RBD showed higher binding affinity for the ACE2 receptor of 122 mammalian hosts than human ACE2 (hACE2) and the interactions involve different amino acid residues. The rat (*Rattus rattus*) ACE2 was found to have the strongest interaction with Omicron RBD, with a binding affinity of -1393.6 kcal/mol. The clearly considerable binding affinity between the RBD of Omicron and the host ACE2 implies a greater possibility for infection and transmission to other hosts via intermediary hosts. Though expected, the capacity of the Omicron RBD to bind to the host ACE2 receptor may not be defined by the mammalian species' evolutionary position, indicating the involvement of several variables in the host divergence of the variation.

Targeting Pro-tumorigenic actions of Tankyrase 1 in Colorectal Cancer: Possible Solution through Olive Phenols

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Colorectal cancer (CRC) has already become a healthcare threat worldwide. Tankyrase 1 (TNKS) receives significant attraction as a tumour target as it induces Wnt/ β catenin signalling by destabilising the Axins (negative regulators of Wnt/ β catenin). Tankyrase also promotes telomere elongation by antagonising Telomere repeat factor 1 (TRF1) action, which directly inhibits telomere elongation. Therefore, inhibition of the tankyrase activity can control excessive Wnt- β catenin pathway and blockade telomere elongation in CRC. The Olive tree (*Olea europaea*) is known for its medicinal properties due to the presence of phenolics. This study targeted divulging precise anti-cancer mechanisms of Olive phenolics by revealing the potential interactions between tankyrase 1 and Olive phenolics. Genetic alterations and expression of TNKS and TRF1 were checked by assessing TCGA data via CBioportal and UALCAN databases. A combined genetic alteration frequency of 12% and increased mRNA expression of both genes were observed. The top interacting partners of TNKS were revealed by developing the PPI network via STRING (confidence score 0.7). Functional enrichment analyses (Metascape) revealed TNKS and its interacting partners are involved in telomere maintenance, cell cycle, and Wnt signalling. Seven phenols were selected after applying drug-likeness and toxicity filters to check interactions with Tankyrase 1. Among these eriodictyol, luteolin, and apigenin showed good binding affinities with both the ankyrin-repeat clusters (-6.7, -6.6, and -7.1 Kcal/mol) and PARP catalytic domain (-10.0, -9.8, and -9.6 Kcal/mol). Thus, these three Olive compounds may act as tankyrase 1 inhibitors by resisting its PARP catalytic action and interaction with TERF1.

Deciphering the role of zwitterionic sulfobetaine polymer in actin cytoskeleton dynamics regulation

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The actin cytoskeleton plays an essential role in many cellular processes like cell morphology, cell polarity, cytokinesis, and cell migration. Effective actin dynamics is very crucial for proper cellular functions and is controlled by several actin-binding proteins inside the cell. Many substances, including poly-lysine, polycationic polymer, different polypeptides, and pharmaceuticals have been used to modulate actin dynamics; nevertheless, their effectiveness is often constrained by cells' specific toxic responses. Therefore, synthesizing novel compounds with minimal cellular toxicity that interact with actin are of utmost bio-importance. Herein, we have synthesized a zwitterionic polymer poly sulfobetaine methacrylate (PSBMA) by reversible addition-fragmentation chain transfer (RAFT) polymerization and comprehensively investigated how it influences actin dynamics. Using isothermal titration calorimetry (ITC), we have found that PSBMA interacts with monomeric G-actin. Furthermore, our *in vitro* actin dynamics study employing total internal reflection fluorescence microscopy (TIRFM), pyrene actin polymerization assay, and dynamic light scattering (DLS) indicates that PSBMA polymer acts as a nucleator of F-actin filament assembly and induces actin filament elongation rate. In addition, transmission electron microscopy (TEM) demonstrates that PSBMA has ability to crosslink F-actin. Considering all of our findings, PSBMA has fresh potential as a synthetic compound for the investigation of *in vitro* actin dynamics research. This repeating zwitterion-containing polymer is non-toxic for HeLa cells.

Biochemical and functional characterization of a cytosolic malic enzyme from *Leishmania major*

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Malic enzymes (MEs) are oxidoreductases catalyzing the reversible decarboxylation of malate to pyruvate, thereby generating NADPH. MEs are present ubiquitously across all organisms and are known to be involved in key metabolic pathways namely, fatty acid biosynthesis, redox homeostasis, TCA cycle, gluconeogenesis, etc. However, lower eukaryotic MEs, particularly those of protists, still remain understudied. Here we report the biochemical and functional characterization of a novel malic enzyme from the parasitic protozoa, *Leishmania major*, the causative agent of cutaneous leishmaniasis. Unlike all other *Leishmania sp.* which encodes two MEs, *L. major* is known to code for a single ME isoform (previously reported by our group). We have now identified a novel second ME isoform of *L. major*, the LmME2. We have performed activity assays with purified LmME2 to measure its kinetic parameters. Interestingly, our data revealed oxaloacetate, fumarate, and ATP act as negative regulators of malate decarboxylation activity of LmME2. By immunolocalization studies, we confirmed the cytosolic localization of LmME2. We also successfully generated a LmME2 overexpressing *L. major* strain, which showed increased resistance to oxidative stress than their wild-type counterparts. This data hints towards the potential role of LmME2 in cellular oxidative stress alleviation and provides the first glimpse into activity regulation and the functional role of leishmanial cytosolic ME isoform. We are currently investigating its other functional characteristics and regulatory mechanisms to gain a deeper understanding of the role of LmME2 in parasite metabolism. This may eventually help us in identifying a novel drug target against this pathogen.

Identification of a novel bicarbonate transporter in *Leishmania*

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One of the fascinating properties of the protozoan parasite *Leishmania* is its ability to reside in the acidic phagolysosomes of the host macrophages. However, the survival mechanism of *Leishmania* under acidic condition is still not completely understood. A working model of intracellular pH regulation in *Leishmania* involving two carbonic anhydrases and a putative bicarbonate transporter has been reported by us (Fig 1). The identity of the protein remained elusive so far as no such transporter was predicted in the parasite genome database, but our biochemical data strongly suggested presence of a bicarbonate transporter in *Leishmania*. Upon thorough genome mining of the *Leishmaniamajor*, we could identify an uncharacterized protein-encoding gene with significant sequence similarity to multi-anion transporter family of proteins reported to transport bicarbonate. We verified the presence of this gene in *L. major* genome by genomic PCR. Further, we report its expression at the mRNA and protein level indicating that it is not a pseudogene and also confirmed its localization in the plasma membrane. Bicarbonate transport activity of this putative transporter was confirmed by stably expressing it in HEK-293 cells, followed by intracellular pH measurement, which showed significant alkalization of the cells. Using CRISPR-Cas9 gene editing technology, we generated a heterozygous knockout cell for this putative bicarbonate transporter. The mutant *L. major* strain displayed relatively acidic cytoplasm and reduced cell viability suggesting the importance of this protein in *Leishmania* physiology. This will be the first report of a bicarbonate transporter in the entire Kinetoplastida group of protozoans.

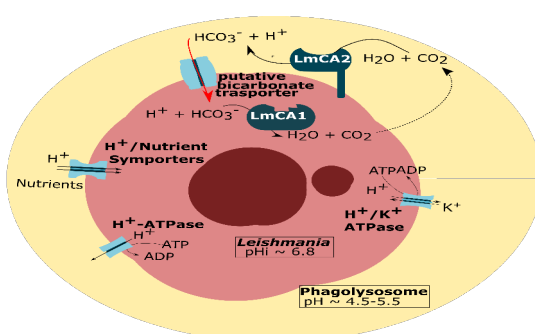


Figure 1: Putative model of pH regulation in *L. major* involving two carbonic anhydrases and a putative bicarbonate transporter. (Adapted from Pal et al., *J Cell Sci.* 2017;130(4):754-766. doi:10.1242/jcs.199422)

Anticancer Effect of *Lantana camara* Leaf Extract on Breast Cancer Cell Lines

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Breast cancer is currently the largest cause of cancer incidence and the fifth leading cause of cancer-related deaths worldwide. Conventional therapies for breast cancer include one or a combination of several procedures such as radiation therapy, surgery, chemotherapy, hormonal therapy and other targeted therapies. Frequent recurrence, chemotherapeutic resistance to common drugs and insensitivity to hormonal therapies are reasons for which novel plant-derived products are constantly being assessed. *Lantana camara*, an invasive weed of the Verbenaceae family, has been utilized in herbal medicines for generations due to its myriad of therapeutic properties. In the present study, *in vitro* anticancer effects of ethanolic extract of *L. camara* leaves (LCLE) were evaluated against two breast cancer cell lines, triple-negative MDA-MB-231 and estrogen receptor-positive MCF-7 cells. Experimental results showed that LCLE could induce cytomorphological changes and significantly reduce cellular proliferation in both cell lines. In MCF-7 cells, exposure to LCLE resulted in increased sub-G1 population whereas G0/G1 phase cell cycle arrest was observed in MDA-MB-231 cells with little increase in sub-G1 population. MDA-MB-231 cells were found to undergo apoptosis in the presence of LCLE. Furthermore, LCLE repressed the wound healing potential of MDA-MB-231 cells to a considerable extent as reflected by its ability to hinder the inherent migratory capability of the cells. Our findings suggest that *L. camara* ethanolic leaf extract has a significant inhibitory effect against both MDA-MB-231 and MCF-7 cell lines, making LCLE a candidate for further exploration to find its therapeutic potential.

Host plant quality Vs. Enemy-Reduced Space: Explaining Oviposition Preference and Larval Performance Patterns in *Plutellaxylostella*

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Oviposition behaviour by herbivorous insects depends on the nutritional quality of host plants and the risk of attack by their natural enemies. We report the oviposition preference of a specialist herbivore and its offspring's performance in a tri-trophic system constituting bottom-up and top-down factors. We observed that oviposition preference and performance are not correlated in *Plutellaxylostella* - plant interactions. The adult preference was more towards secondary metabolite (SM) -rich host plant though the performance of the offspring and the life span was compromised. We hypothesize that plant defensive chemicals provide an enemy reduced space for the herbivore to escape from its natural enemies. Feeding on an SM-rich host provided better larval cellular immunity in terms of higher hemocyte counts and phenoloxidase activity. This elevated immunity also increased larval survival against the entomopathogens and generalist parasitoid wasp. We further observed a positive correlation of the larval immunity with the concentration of SMs. However, the enhanced immunity did not influence the parasitization by a specialist larval parasitoid wasp. We have also characterized close range volatile cues of larval origin that impact parasitoid oviposition. Interestingly, parasitoids host preference guided by these volatile cues correlates with its performance. In summary, our results show that the oviposition of herbivores and parasitoids is highly context-dependent and can be understood better only by studying multiple fitness parameters and interactions with different natural enemies.

Individual personality and water clarity determine mate-association preferences in wild zebrafish (*Danio rerio*)

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Freshwater systems are prone to high levels of turbidity due to rainfall, increasing anthropogenic activities, etc. The fish behavioral traits which are dependent on vision can get affected by changing levels of turbidity, impacting evolutionary and ecological processes shaping fish communities. In this study, we investigated behavioral traits like boldness, aggression and activity and their potential relationship to mate-association preferences in zebrafish in clear and turbid waters. Based on repeated measurements, variations in behavioral traits among individuals maintained in differing water clarity conditions were initially tested. Further tests were conducted to explore whether these traits determine mate-association preferences among tested individuals. Potential differences in their preferences under clear and turbid water conditions were then analyzed. To address these, we conditioned adult zebrafish in clear water and turbid waters, for 30 days. After conditioning, the behavioral tests were carried out to measure their boldness, aggression and activity traits. Following this, mate-association preferences were assessed, based on two-choice tests (in clear and turbid water conditions), comprising different combinations of behavioral traits and sex of the presented fish. We found that of the tested traits, *boldness* was affected in turbid waters while activity and aggression were not. In clear waters, fish showed a preference for reactive males, however, these preferences were not exhibited in turbid waters. Our results show plasticity in boldness and mate choice in response to differing levels of turbidity. As water clarity may vary temporally in natural habitats of zebrafish, the strength of sexual selection on behavioural traits may be diminished under high turbid conditions.

Understanding the Mechanism of Action of Dual specificity tyrosine phosphorylation regulated (DYRK) kinase

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Kinases act as intracellular dynamic protein switches that regulate the function and / or outcome of a signal transduction process by regulating various downstream molecules of the signalling pathway. Dual specificity Tyrosine(Y) phosphorylation Regulated Kinase or DYRK belongs to the CMGC group of kinases that phosphorylates side chain -OH group of both aliphatic (serine/threonine) and aromatic (tyrosine) amino acids of specific protein substrates. The property of co-translational cis-autophosphorylation of the second tyrosine residue of YxY motif of activation loop of DYRK separates these kinases from other members of the CMGC group. DYRKs are present ubiquitously in the eukaryotic kingdom and serves pleiotropic functions including cell division, cell proliferation, cell differentiation, stress response and apoptosis. Aberrant kinase activity often leads to development of various diseases – congenital defects associated with Down's Syndrome, formation of solid tumor, carcinoma, rheumatoid arthritis, diabetes type I and other neurodegenerative disorders. Our goal is to understand the biochemical characteristics of DYRK kinase and to decipher how these kinase functions are translated in the cell to regulate myriad of cellular processes.

Thermal pretreatment - a prerequisite for the reduction of hydrolysis stage during anaerobic digestion of *Lantana camara*

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The present study predominantly focuses on the effect of different thermal pretreatments on *Lantana camara*. Thermal pretreatment reduced the hydrolysis period and at the same time increased biogas production, compared to the untreated *Lantana camara*. The impact of various thermal pretreatment techniques on the anaerobic digestion of *Lantana camara* viz., hot water bath, hot air oven, autoclave and microwave was studied. Among them, autoclave was found to be more efficient succeeded by hot water bath, hot air oven and microwave pretreatment. Autoclave pretreatment enhanced the solubilisation (sSOD) and an increment in volatile fatty acids (VFA) was observed i.e. 45.44% and 56.75% at 110 °C for 80 min respectively, as compared to the control/untreated. Cumulative methane production after autoclave pretreatment had raised to 3656 mL CH₄/g VS in 35 days from 2895 mL CH₄/g VS in 50 days for the untreated sample.

Identification and functional characterization of putative ligand binding domain(s) of JlpA protein of *Campylobacter jejuni*

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A group of proteins, collectively known as Surface Exposed Colonization Proteins (SECPs) of *Campylobacter jejuni* (*C. jejuni*), such as CadF, FlpA, JlpA, and Outer membrane proteins (OMPs), are the critical factors for bacterial adherence to host cells. Among them, JlpA of *C. jejuni* binds to heat shock protein (Hsp)-90 α and triggers a pro-inflammatory signaling cascade via NF- κ B and p38 MAP kinase pathways. However, how the interaction of JlpA with the receptors on the host cell initiates cellular signaling remains unknown. The crystal structure of JlpA indicates that the “hydrophobic concave face” of JlpA protein can form an unusually “large hydrophobic basin” with a “localized acidic pocket”, suggesting the possibility of JlpA binding to multiple ligands. To gain insight into the position and structure of the allosteric binding site of JlpA, molecular docking was performed using several protein-protein docking servers and the top-scored docking model was subjected to molecular dynamics simulation. The findings of the ligand docking studies suggest that human Hsp90 α contacts through the allosteric binding site present in the C-terminal domain of JlpA protein. To determine whether mutations can affect the strength of the interactions, we cloned and expressed JlpA (ligand) protein with a deletion mutation of 18 amino acid sequences from the C-terminal domain of JlpA (Q347 to M367). Together with flow cytometric analysis, the results of ligand-receptor-based ELISA confirm a marked reduction in the receptor binding affinity of JlpA mutant protein when incubated with the N-terminal domain of human Hsp90 α . Further study to define the binding activities of JlpA and identify crucial amino acids can provide an important platform for developing novel therapeutic modalities against Campylobacteriosis.

Methanolic Neem Bark Extract modulates virus induced oxidative stress and inflammation

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The ancient ethnomedicinal plant *Azadirachta indica* A juss or neem has been in use for centuries to cure various diseases and inconveniences to human race. Neem has versatile biological activities including antioxidant, anti-inflammatory, antimicrobial, antiviral and insecticidal activities. Due to presence of different bioactive components such as polyphenols, flavonoids, and limonoids Neem possesses potential antioxidant properties. In this post pandemic era as we are dealing with virus induced oxidative stress, we need a targeted therapeutic approach against oxidation and associated pathogenesis. Furthermore, recent studies explored a β -coronavirus, Mouse Hepatitis Virus or MHV induced oxidative stress, Endoplasmic Reticulum (ER) stress, neuroinflammation in experimental mice model. Parallely, MNE was explored to restrict MHV replication, viral protein translation, spread which leads to neuroinflammation and pathogenesis. This study aims to illuminate the antioxidant and anti-inflammatory activity of Neem. The mechanistic stand point of restricting RSA59 induced oxidative stress, MNBE was able to restrict viral entry, spread, reduced the burden of cellular stress, Reactive Oxygen Species production, modulating Unfolded Protein Response (UPR) pathway and cell signaling. It was observed that in in-vivo mice model, MNBE was able to restrict virus induced oxidative stress by modulating the stress markers like Nrf-2, XBP-1, RelA etc. where as in in-vitro model, both restriction of stress markers Nrf-2, XBP-1, RelA and upregulation of antioxidants like HMOX-1 and Catalase was key to restrict oxidative stress. In conclusion, MNBE might affect the nexus between antioxidant pathway, UPR pathway, and inflammatory pathways.

Deciphering molecular interactions between TCTP and small molecule drugs for a prospective therapeutic strategy in Cancer

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The highly conserved TCTP is essential for early development and cellular processes including regulation of cell cycle progression, apoptosis, genomic stability, DNA repair, and stress response in both animals and plants. Over expression of TCTP is heavily implicated in multiple cancers and so it has potential as an alternative therapeutic target in cancer. Histamine releasing factor, or TCTP, has been demonstrated to bind several anti-histaminic (hydroxyzine) and anti-psychotic (Sertraline, Thioridazine) drugs. These medications may have the potential to destroy cancer cells and prevent the growth of tumours by functional inhibition of TCTP. But the exact mechanism of action of these drugs is still elusive. This study is based on molecular interaction of drug-TCTP reported by *in-silico* docking. We want to understand whether this drug interaction effect cellular TCTP interactome by altering its structure resulting in function regulation. The NMR solution structure of TCTP (PDB ID: 2HR9) and 3D structures of drug ligands from PubChem were energy minimised and ligand binding active site on TCTP were determined to perform docking followed by molecular simulation. Critical residues at ligand binding sites were analysed by sequence conservational analysis using TCTP sequences from various species. Docking has been performed using AutoDock Vina. Binding energy of docking with Buclizine, Sertraline and Thioridazine were -6.4 kcal/mol, -6.3 kcal/mol and -6.0 kcal/mol respectively were obtained using AutoDock Vina, providing buclizine to be the highest affinity ligand. In our lab *in vitro* experiments are being performed to evaluate the anti-cancer effectiveness of these small molecule drugs.

Childhood factors important for predicting COPD and asthma in adulthood

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Childhood exposures and diseases play an important role in determination of lung diseases in adulthood. Thus, it may be important to look into the childhood events in order to predict the possibility of obstructive lung diseases. The aim of the study is to find out specific childhood events that is associated with presence of asthma or COPD selectively. In a detailed questionnaire on history of childhood (age below 8 years) events such as various exposures and diseases were recorded at the point of performing spirometry following presentation to our out-patient department. The diagnosis of obstructive lung diseases was made; COPD: $FEV_1/FVC < 0.7$, and asthma with post-bronchodilator reversibility of $FEV_1 \geq 200$ ml and $\geq 12\%$. The odds ratio of each question was evaluated in both diseases in univariate and multivariate analysis. The odds ratio for asthma is found to be higher with history of childhood asthma ($p < 0.0001$), history of hospitalisation ($p < 0.0001$), history of recurrent antibiotic usage ($p < 0.0001$), eczema ($p < 0.05$), long term wheezing ($p < 0.001$), long term sneezing and running nose ($p < 0.01$), recurrent purulent expectoration ($p < 0.05$), recurrent emergency visit to doctors ($p < 0.05$) in childhood. Chances of COPD were higher if a person had a history of tuberculosis as a child ($p < 0.05$). There was a considerable overlap region between the two diseases in multivariate analysis. The identification of the childhood risk factors associated with COPD and asthma needs to be noted in clinical practice. Further research is needed in this field.

Biophysical characterization of mitochondrial phenylalanyl-tRNA synthetase and its pathogenic variations

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To translate proteins, Mitochondria need to import the component of protein translation machinery encoded by the nuclear gene. During this transportation, these proteins must endure different cellular environment including the pH gradient across the mitochondrial membrane. It was proposed that after entering into mitochondrial matrix the imported proteins are refolded to its native active state. Mitochondrial aminoacyl-tRNA synthetases (mtAARSs) are the critical components for mitochondrial protein translation. Extensive research works suggests that mutations in mtAARSs are responsible for many human neurological disorders. In this study, we focus on human mitochondrial phenylalanyl-tRNA synthetase (HmtFRS) encoded by a nuclear gene (FARS2). *In vitro* studies of these pathogenic mutants demonstrated reduction in enzymatic activity, however, mutational effect on protein stability and unfolding/refolding is not extensively studied. We have selected some reported pathogenic mutations of HmtPheRS (P136H, D142Y, G309S) to evaluate their stability and unfolding/refolding *in vitro*. The wild type protein showed remarkable stability across the pH range 1-8, on the contrary, mutants showed a minor alteration in stability. HmtFRS has propensity to aggregate at physiological temperature that reduced substantially in the presence of the substrates (ATP and L-Phenylalanine, alone or in combination) and under reducing environment. Stabilizing effect of the substrates under identical condition was distinctly different in pathogenic mutants. When aminoacylation assay was performed after incubating the proteins at different pH and refolding to assay condition, stark alteration in activity was observed. Our study indicated that pathogenic mutants may have compromised conformational stability due to improper refolding, though further validations are required.

Linking plant genome to its metabolism

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Understanding the designing principles of several biological processes that define the cellular physiology is a prerequisite to get a high yield plant cultivar. Metabolism is a key cellular process of cellular physiology. Computational analysis of the metabolism of a species starts with a collection of metabolic pathways and their reactions. However, plant specific enzymes are neither fully annotated nor any tool is available for it. Here we aim to develop plant specific enzyme classifier, a tool that can predict the enzyme encoding genes present in a plant from its genome sequence. For prokaryotes there are pre-developed genome annotation tools like PRIAM (Claudel-Renard et al., 2003) or Prokka (Seemann, 2014). Eukaryotic and hence plant genes are different from prokaryotic genes as they contain intron and exon regions. Therefore, different classifier is required to classify enzymes from eukaryotic genome sequences. Here we propose an algorithm which can predict the enzyme classes (EC numbers) present in plants from their genome sequences. This algorithm is based on the hypothesis that for a particular enzyme class the sequences in different plants due to their homology are much more similar among themselves in comparison to other classes. In this work we aim to construct specific and unique descriptor for each enzyme involved in central carbon metabolism. Metabolic pathways of central carbon metabolism of some plants have been reconstructed using this algorithm and their comparative analysis will shed important insights related to plant metabolism and evolution.

Behavioural adaptation of free-ranging langurs and their interaction with free-ranging dogs in urban areas of West Bengal, India

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Habitat degradation due to urbanization has changed the biodiversity pattern worldwide. While some animals tend to exhibit three responses to urbanization – avoid, exploit and adapt, some fail to do so and become extinct. Those species who adapt in urban areas by learning to use anthropomorphic resources for their daily sustenance flourish in urban cities, becoming a part of urban biodiversity. Rhesus macaques and Hanuman langurs are the most common free-ranging non-human primates in urban areas who share human offered food subsidies and shelter with free-ranging dogs. However, unlike non-human primates, free-ranging dogs have been living with humans for centuries, much before other animals began co-existing with us, thereby becoming a ubiquitous part of urban biodiversity. Here, our study focuses on urban adaptation of these free-ranging langurs and their interaction with free-ranging dogs. From our previous field-based experiments in Dakshineswar we saw a shift in feeding preference of these folivorous colobines where they preferred processed food items over unprocessed food items (Dasgupta et.al.,2021). Currently from our long-term field-observations we have found the presence intentional gestural communication in free-ranging langurs where free-ranging langurs ‘beg’ for food from human beings and keep on persisting till they have received the food item, preferably of their choice. Apart from this we are also looking into their interaction with free ranging dogs and how human beings perceive these two urban free-ranging mammals in different locations of West Bengal, India. Our understanding about the behavioural flexibility and survival strategies of these two species is limited, making it difficult for us to come up with sustainable solution to address human-animal conflict in urban ecosystem. We intend to fill up this lacuna with our ongoing study.

RAV1 mediates cytokinin signalling to regulate primary root meristem size in Arabidopsis

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Root system architecture is a complex trait which critically influences plant growth and adaptability. Plant root is characterized by four distinct developmental zones: the meristematic zone (MZ), transition zone (TZ), elongation zone (EZ) and differentiation/maturation zone (DZ). The development of each root zone is a consequence of a specific set of hormonal interplay. Antagonistic interaction of auxin and cytokinin for example, determines the size of root meristem during post-embryonic root development. Here we show that RAV1, a member of the AP2/ERF family of transcription factors, is involved in regulating root meristem size. In Arabidopsis seedlings that lacked functional RAV1, larger root meristem size was observed with increased number of meristem cells and consequently longer primary root. Further experiments showed that absence of RAV1 compromises inhibitory action of cytokinin in root cell proliferation and thus has enlarged root meristem size, compared to wild type. In absence of efficient cytokinin action, the *rav1* mutant line exhibit enhanced auxin response and altered expression of auxin transporter (PIN1) in primary roots. Gene expression analysis further revealed that primary cytokinin-responsive genes, *ARR1* and *ARR12* were significantly downregulated in *rav1* roots that resultantly altered auxin-responsive gene expressions. Interestingly, cytokinin positively regulates *RAV1* gene expression through *ARR1*, as a part of secondary response. RAV1 thereafter augments cytokinin signalling in primary root through suppression of *CRF1* gene expression, which otherwise acts as negative regulator of cytokinin signalling. Taken together, RAV1 is a critical factor that regulates cytokinin signalling in primary roots for efficient control of root meristem size.

Role of Ecdysone signalling in Epithelial Morphogenesis

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Epithelial morphogenesis generates diversity in tissue organization and aids in the formation of various organs in the metazoans. At the cellular level, this process mediates change in the shape and organization of the epithelial cells. Given the wide importance of this process in multicellular development, we still lack clear understanding as to how epithelial morphogenesis is regulated in the metazoans. Employing the *Drosophila* oogenesis model, we have examined the role of Ecdysone (EcR) pathway in mediating the shape transition of epithelial follicle cells. A previtellogenic fly egg is enveloped by a layer of somatic epithelial cells called the follicle cells. A small subset of anterior follicle cells (AFCs) undergo shape transition from cuboidal to squamous fate as the egg enters the vitellogenic phase. We demonstrate that the activity of EcR pathway in the AFCs coincides with the timing of cuboidal-to-squamous shape transition. Depletion of the Ecdysone Receptor function affects cuboidal to squamous transition of anterior follicle cells (AFCs). Employing standard genetic tools, we show that EcR function modulates Notch signaling to facilitate the shape change of the AFCs. Over all, our work provides novel molecular insight as to how Ecdysone signalling mediates shape change in the epithelial follicle cells. Results from the above will be presented.

ERp29 modulates astrocytic gap junction protein Cx43 trafficking and murine β -coronavirus infectivity

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Astrocytes are interconnected by gap junctions (GJ) intercellular communication that is important for the proper maintenance of CNS homeostasis in panglial system. Infection of astrocytes with a neurotropic murine- β -coronavirus (m-CoV), mouse hepatitis virus (MHV-A59) causes intracellular retention of the predominant astrocyte GJ protein, Connexin43 (Cx43), impairing gap junctional intercellular communication (GJIC). Not much is known about the mechanism of how MHV infection reduced Cx43 mediated GJIC. From the mechanistic standpoint, our previous studies highlighted that intracellular retention of Cx43 in MHV-A59 infected primary neonatal mouse astrocytes is associated with increased ER stress marked by upregulation of binding immunoglobulin protein (BiP)/glucose-regulated protein 78(Grp78) and reduced expression of ER-resident chaperone, ERp29. Treatment of MHV-infected primary astrocytes with a well-studied ER stress inhibitor 4-sodium phenylbutyrate (4-PBA), rescued their ability to transport Cx43 to the cell surface and increased GJIC by inducing ERp29 expression and modulating ER stress. Furthermore, studies on mouse astrocytoma-derived DBT cell models stably transfected with exogenous ERp29 showed increased Cx43 trafficking and assembly into GJ plaques. Thus, targeting ERp29 pathway in combination with UPR may be a feasible molecular approach to design therapeutics to protect against m-CoV infection which may also have the potential as pan-CoV antivirals.

Dpb2 – One protein, Various roles

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Dpb2, the second largest non-catalytic subunit of Polymerase Epsilon, the leading strand polymerase in eukaryotes, is indispensable for cell survival. Dpb2 physically tethers polymerase epsilon with the CMG helicase by interacting with Psf1 which along with Psf2, Psf3 and Sld5 make up the GINS complex which itself is a component of the CMG helicase. Dpb2 is also responsible for incorporating polymerase epsilon into the replisome. In our current study, we have been able to pinpoint the conserved amino acid residues of in the N terminal domain of Dpb2 that are involved in this interaction with Psf1. In the future these identified residues could pave the way for developing cancer therapeutics. During replication, cells can and do get exposed to various forms of stresses and in turn, cells themselves employ various mechanisms to overcome such stresses. As previous research has shown, several proteins involved in replication also have important roles to play in these mechanisms. Similarly, Dpb2 has also been shown to participate in such pathways. In the second part of our study, we have used Hydroxyurea (HU) and Methyl Methanesulphonate (MMS) in our laboratory setting, to induce stress during replication. We also show that the absence of the N terminal domain of Dpb2, made yeast cells vulnerable to the cytotoxic effects of HU and MMS. Our present work helps to understand the various roles played by Dpb2 from cellular replication to replication stress response pathways.

Shoals under threat! Use of multimodal sensory cues in predator avoidance by wild-caught zebrafish shoals

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Predation is key driver of shoaling in fishes and here we examine the immediate response of wild caught zebrafish (*Danio rerio*) shoals to cues from a natural predator, the snakehead (*Channa sp.*). Zebrafish shoals (n=15 per treatment) comprising ten fishes were recorded under laboratory condition, for five minutes after exposure to a predator cue (visual or olfactory) or both cues together. In control experiments, shoals received no predator cue. All shoal members were tracked using idTracker following which a detailed analysis of shoal properties was performed. We found that compared to control treatments, shoals receiving either visual or olfactory cues had significantly greater cohesion, polarization and velocity. Surprisingly, when the shoals received both these cues simultaneously, the cohesion, polarization and velocity decreased. On examining the velocity profile of individuals, we found that in addition to acceleration and deceleration phases, a third phase existed in which individuals showed little or no movement (i.e., underwent freezing). Shoals receiving dual cues had significantly greater number of individual freezing events and spent comparable proportion of time freezing as compared to the other treatments. Therefore, zebrafish relied on both visual and olfactory cues to escape predation. When shoals were presented with both the cues together, while freezing frequency increased, other responses were comparable to that of control treatments where no predator cue was provided. While this study clearly shows that multimodal cues elicit a different anti-predator response than the cues singly, more experiments are needed to identify the underlying causes for this behaviour.

Retrospective analysis of blood biochemical parameters and chest CT and their relation to disease severity in COVID 19

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COVID 19 had imposed a global health emergency in past two years. We have undertaken this study to assess the biochemical impairments caused by COVID 19. The retrospective cohort study was conducted for 7 months. The study population comprised of 60-75 years of patients who had been admitted to Medical College and Hospital, Kolkata and have been symptomatic for SARS COV-2 irrespective of RTPCR or RAT outcomes. As evidenced so far, almost all of our patients were diabetic and hypertensive. Low Hb%, TCRBC, red cell volume was constant observation in all cases. This was accompanied by lymphocytopenia and monocytopenia. Multi-organ involvement was evident by elevated serum inflammatory markers like-CK, CKMB, SGPT, SGOT, LDH, lipase, amylase and excess urinary excretion of urea, creatinine, commencing usually in the 2nd week of disease. Hypercoagulability in the form of raised PT, APTT, INR, D-dimer were present in some patients. Alongside biochemical parameters, HRCT chest showed peripherally distributed bilateral ground-glass opacities indicative of pulmonary edema. Classical signs of pulmonary CT of COVID 19 are accompanied with atypical changes, on rare occasions. However, temporal evolution of lung abnormalities in chest CT could not be evaluated at the present moment due to lack of repeat CT chest reports. So, this can be concluded that COVID 19 is a disease with a long course where biochemical alterations are accompanied with parallel deterioration of clinical features. Biochemical parameters aided with HRCT chest might just be a highly valuable tool in our hands in terms of management of the disease.

Exploring novel regulators of innate immune system in health and autoimmunity

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Compartmentalisation of the eukaryotic cell cytosol aids in maintaining distinct local physiological conditions (pH) which are regulated by different protein channels through the exchange of ions across membranes. We explored the role of one such anion exchanging protein from the SLC4A family, SLC4A3, which regulates internal pH through electroneutral exchange i.e influx or efflux of HCO_3^- in exchange for Cl^- across the membrane. Publicly available database BioGPS and gene expression studies revealed that this gene is highly expressed in a unique subset of dendritic cells called plasmacytoid dendritic cells best known for producing large amounts of type-I interferon during viral infection. TLR ligand mediated pDC activation requires endosomal acidification and we hypothesize that being an ion channel SLC4A3 maybe acting as an acid loader in these compartments. To address the functional role of SLC4A3 in pDCs we performed confocal microscopy studies to investigate whether it localises to the acidic vesicle compartments. We also used a $\text{Cl}^-/\text{HCO}_3^-$ exchange inhibitor DIDS and performed siRNA mediated knock down studies to explore whether SLC4A3 regulates IFN- α secretion from pDCs. In-vivo studies revealed DIDS inhibited IFN- α secretion in response to bona fide TLR9 ligands CpGA and CpGB. Type-I interferons have been established to play a pathogenic role in progression of autoimmune diseases and DIDS mediated amelioration of disease symptoms in murine models of SLE and psoriasis showed promising results. These studies will help us gain critical insights on whether SLC4A3 could serve as potential therapeutic targets in autoimmune disease development and progression.

Role of membrane fluctuation tension on TNFR1 clustering

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Interaction of tumour necrosis factor (TNF) and TNF-receptor 1 (TNF-R1) can potentially induce either cell survival or cell death. Localization of TNF-R1 at plasma membrane (PM) leads to survival, involving the NF κ B signaling while its endocytosis leads to cell death via apoptosis. No single mechanism has been identified that controls the choice between the two pathways. Since endocytosis is dependent on cell membrane mechanics, we aim to study if TNF-R1's PM retention vs. endocytosis is mechanically controlled. It is well known that at high PM tension, endocytosis is disfavoured. However, our lab has also shown that for normal functioning of all endocytotic pathways cells retain a lowered PM tension. How does the mechanical axis of TNFR1 signaling function can therefore only be understood by first testing if indeed tension and TNFR1 signaling are intertwined. We demonstrate that blocking Pre-Ligand-Binding Assembly Domain (PLAD) formation - key to formation of the TNFR1 complex in proliferative cells increases tension. This suggests that TNFR1's proliferative signaling arm indeed sustains tension homeostasis at a lowered value. Enhancing tension via cholesterol depletion prior to administration of the inhibitor of PLAD suppresses its effect. To recreate tension dysregulation without directly impacting cholesterol depletion, we perform a long term inhibition of CDC42. This increases tension and suppresses the effect of inhibitor of PLAD. Finally we also show the local coupling between membrane fluctuations and TNF-R1 clustering.

***Leishmania*-induced TLR1 differentially regulates tissue-specific parasite persistence in experimental visceral leishmaniasis**

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Host-tissue preference is a critical aspect of parasitic infection and is directly correlated with species diversity. Even the same species, *Leishmania donovani*, infects the host's bone marrow, spleen, and liver differentially. The tissue-specific persistence of *Leishmania* results from host-pathogen immune conflicts and arguments. The physical conflict in terms of toll-like receptors (TLRs) and the chemical languages in terms of cytokines are the concerns. The host protective or pejorative roles of TLRs during *L. donovani* infection has been well established, but what entirely missing is the influence of TLRs on the parasite persistence in the bone marrow microenvironment as *L. donovani* cleverly induces myelopoiesis and takes complete control of the bone marrow monocytes/macrophages for long-term hidden shelter. This study revealed that TLR1 is differentially expressed throughout the course of infection and distinctively induces either the up-regulation or down-regulation of the Ly6C^{hi} mature inflammatory monocytes in the bone marrow. Interestingly, TLR1 signaling influences the prolonged sustenance of parasites in the bone marrow, but not in the spleen or liver. To the best of our knowledge, this is the first report to show that the TLRs can be instrumental in tissue-specific parasite persistence during *Leishmania* infection.

Multicellular behaviours in bacteria: Implications in therapeutics and bioremediations

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Although the bacteria are unicellular prokaryotes, they are evolved with the ability to have multicellular behaviours in order to cope and survive under harsh or restricted conditions. Swarming and biofilm formation are two major multicellular behaviours which bacteria employ for survival under different conditions. As swarming and biofilm formation are associated with bacterial pathogenesis and bacterial bioremediation processes as well, it is important to study the multicellular behaviours in bacteria in order to identify better therapeutic targets against pathogenic bacteria and to increase the biofilm forming ability of bacteria to have a better bioremediation strategies. In the present study, isolated Gram-negative and Gram-positive bacteria were challenged with different growth conditions to monitor the alterations in their swarming and biofilm formation abilities. An inverse correlation was observed between swarming and biofilm formation abilities in both Gram-negative and Gram-positive bacteria and involvement of quorum sensing was found to be associated as a regulatory factor for both swarming and biofilm formation. Further studies are underway to delineate the molecular mechanism(s) involved in decision making by the bacteria to swarm or to form biofilm under different conditions.

Ifit2 regulates murine- β -coronavirus spread to the spinal cord white matter and its associated myelin pathology

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Ifit2, a protein strongly induced by Interferons, plays an important role in providing protective antiviral immunity. Intracranial inoculation of RSA59 in Ifit2^{-/-} mice caused pronounced morbidity and mortality accompanied by uncontrolled virus replication with significantly impaired microglial activation and reduced recruitment of NK1.1⁺ T cells and CD4⁺ T cells in the brain along with reduced expression of CX3CR1. The spinal cord of RSA59 infected Ifit2^{-/-} mice showed higher viral replication and spread accompanied by reduced recruitment of CD4⁺ T cells. Infected Ifit2^{-/-} mice showed impaired acute microglial activation but developed severe chronic demyelination. Reduced number of CD4⁺ T and effector CD8⁺ T cells were observed in Cervical-lymph-node of these mice in addition to limited permeability of Blood-Brain-Barrier as indicated by ZO-1, a tight junction protein, expression. The reductionist approach showed upregulation of Ifit2 protein in brain derived Primary cells such as astrocytes, neurons, oligodendrocytes precursor cells and meningeal fibroblast upon RSA59 infection. Overall, it can be concluded that Ifit2 deficiency causes uncontrolled viral replication and spread in the grey and white matter of the spinal cord whereas in WT mice RSA59 is restricted to grey matter and grey-white matter junction. Therefore, Ifit2 is not only required for restricting Viral replication in CNS and efficient T cell priming in the CLN but also protects mice from developing severe chronic neuro-inflammatory demyelination.

Early tension lowering is necessary for successful fusion during myoblast differentiation

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Membrane height fluctuations is incessant in nature and governed by cells' mechanical status. Although different molecular players control fusion, mechanical destabilization is key during fusion in invertebrates to vertebrates. High cortical tension has been reported to aid fusion at local fusogenic synapse in *Drosophila* myoblast cells whereas in case of mouse, local pore formation happens by stressing the membrane with a transmembrane small protein myomerger, thus helping in fusion (Kim *et al.*, 2015). To unravel the mechanical aspects, here we study cell fusion during myoblast differentiation and the role of fluctuations and tension during the process. We first measured membrane tension through myoblast differentiation by using basal membrane height fluctuation imaged utilizing Interference reflection microscopy – a non-invasive technique. From population data, single cell tension profiles and by using M β CD-based cholesterol depletion – it was clear that tension lowering is essential for fusion to be initiated in the sample. Interestingly, the local membrane concentration of the fusogenmyomerger showed negative correlation with tension. This was reflected even at the cell level and corroborated by siRNA-based knockdown studies. At later time points – in well-formed myotubes – tension is higher and correlation is not negative. On quantifying the clustering state of myomerger through IF we find that at higher tension or higher intensities it has less cluster-to-background ratio as well as lesser (negative) correlation (Chakraborty, Sivan and Biswas, 2022). We thus postulate clustering-based curvature induction by myomerger to be responsible for early tension reduction.

Unrevealing the molecular basis of VEGFR1 autoinhibition

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The outcome of VEGFR1 and VEGFR2 interplay determines multiple physiological processes like vascular development, glucose metabolism, and immune responses. VEGFR2 attributes the majority of the vascular function, whereas VEGFR1 was proved to be a decoy receptor by negatively regulating VEGFR2 signaling. Even though the functional subunit of VEGFR1 exhibits all the conserved motifs required for the kinase activity, the phosphorylation level was found to be very poor compared to its closest homolog, VEGFR2. In contrast, recent studies show the importance of VEGFR1 signaling in different pathological conditions like Cancer Pain, Diabetic retinopathy, and arthritis but very little is known about mechanisms underlying the regulation of VEGFR1 kinase activity. To understand the molecular basis of poor kinase activity, first, we determined the VEGFR1 activity in different cell lines. We observed that, unlike VEGFR2, the activation pattern of VEGFR1 highly diverges from other classical receptor tyrosine kinases. We speculate that multiple structural elements are involved in stabilizing the autoinhibited conformation of VEGFR1. This study dissects the structural elements that account for this functional difference between these two receptors. We decipher an ionic latch between the kinase domain and the juxta membrane segment that plays an essential role in stabilizing the autoinhibited conformation of VEGFR1. Further analysis shows that cellular phosphatases also play a critical role in regulating VEGFR1 activation. Like other classical RTKs, activation of VEGFR1 can be recovered upon phosphatase inhibition. These results define a unique regulatory mechanism of VEGFR1 and explore the role of phosphatase in modulating VEGFR1 activity, further demonstrating the therapeutic potential of phosphatases in VEGFR1-mediated pathological angiogenesis.

Deciphering the novel role of PIST in *Leishmania major* infection

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PDZ domain protein interacting specifically with Tc-10 (PIST), a possible regulator of intracellular trafficking events, might play an essential role in triggering autophagy against many invaders via the PIST-Beclin1-PI3KC3 pathway. Autophagy is a highly conserved, self-degradative process eventuated via a specialized trafficking pathway ending with lysosomal fusion and the degradation of the cellular constituents. It is also the host microbicidal response against the invaders. Nevertheless, pathogens have evolved strategies to counteract this microbicidal pathway in many ways. The LC3-associated phagocytosis (LAP) and autophagy process in response to the protozoan parasite *Leishmania major* infection remains poorly understood. In this study, we have investigated the role of PIST in the *L. major* infection. We have found that PIST is involved in the process of *L. major* promastigotes internalization. We show that the *trans*-Golgi resident protein PIST migrates from the *trans*-Golgi to the site of infection and colocalizes with the parasite's nucleus. In addition, we show the co-location of Beclin1 and PIST at the *leishmania*-containing parasitophorous vacuoles (LPV). Moreover, we have found that the recruitment of LC3B to the LPV was inhibited, manifesting the sabotage of the autophagy pathway and delay in phagosomal maturation. PIST stays with LPV from the early to the late infection phase and might be responsible for the intracellular growth of the parasite and its pathogenesis by impeding the host autophagy pathway.

Chemical and biological approaches to develop efficient cellulases for biomass degradation towards the biofuel production

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The economic exploitation of lignocellulosic biomass for biofuels requires the efficient use of enzymes. Reusing enzymes across multiple biomass hydrolysis cycles is one approach to reducing the high cost of the enzymes involved in converting cellulose into fermentable sugars. Enzyme immobilization is a well-established method for enhancing enzyme stability and reusability, which are critical in commercial biocatalytic applications. I will report the progress made in our attempt to immobilize enzymes using several supports, such as polymer, nanoparticles, and covalent organic framework (COF), to improve stability and reusability. Another critical requirement for efficient cellulose hydrolysis is the synergistic activity of cellulases. To understand the dynamic basis of synergism and to design an efficient cocktail, we have designed quorum sensing based genetic circuits using the tools of synthetic biology. The progress thus far by this approach will also be discussed.

Metabolic Reprogramming in Cord Blood-Derived NK Cells Promotes Persistence and Enhances Anti-tumor Activity

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Natural killer cell (NK) plays a key role in cancer immunosurveillance. NK cell is an ideal population for allogeneic cell therapy since their activity is regulated by germline-encoded receptors in a non-MHC-restricted manner. Studies have shown that allogeneic NK cells obtained from cord blood are safe and do not cause significant toxicity, like cytokine release syndrome (CRS), neurotoxicity, and graft-versus-host disease (GVHD). Additionally, allogeneic NK cells are effective against liquid tumors such as acute myelogenous leukemia (AML), but they have limited effectivity against solid tumors. Adoptively transferred primary NK cells typically persist in vivo for a short time, limiting their anti-tumor effectiveness. Engineered NK cells could therefore improve their antitumor activity by improving their in vivo persistence. In this study, we found that cord blood-derived NK cells were more metabolically fit when Cytokine-inducible SH2-containing protein (CISH) and membrane glycoprotein CD38, were knocked out through CRISPR-Cas9. By measuring ECAR and OCR with a metabolic flux analyzer (Seahorse Analyzer), we showed that CISH and CD38 individual or double knockout (DKO) produced more ATP along with an increased rate of mitochondrial respiration and glycolysis. Single or DKO cells showed better killing efficiency against K562 cells compared to control cells (EP) as measured by luciferase assay. Single or DKO cells also showed consistent in vitro killing activity in repeated challenge with K562 cells (IncuCyte based tumor challenge assay) compared to the EP cells. These data indicate that knocking out CISH or CD38 improves metabolic fitness and tumor killing efficiency in NK cells.

Assessment of flammability of different plant lifeforms and their contribution towards the maintenance of Terai savanna and grasslands

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Grassland degradation has become a global concern leading to the depletion of floral and faunal diversity. Therefore, it is essential to understand the factors promoting grassland conversion to the forest. We calculated the flammability index and subsequently analyzed the variability in the morphophysiological traits of different life forms and their relationship to flammability using Principal Component Analysis (PCA). The high flammability of grasses plays a critical role in sustaining active fire that helps maintain grassland by impeding shrub encroachment. Our study would also help decide the timings (season) for fire prescription in the grasslands.

Determination of Oligomeric Polydispersity and Oligomers Dependent Holdase Chaperonic Activity of Small Heat Shock Protein (sHSPs) IbpA and IbpB

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Inclusion bodies associated protein IbpA and IbpB are two major small heat shock proteins (sHSPs) from *Escherichia coli* having holdase chaperone activity with molecular weight of 16 KDa per monomer. Report says that the monomeric form of such small heat shock proteins (sHSPs) will oligomerize to make heavy oligomers with molecular range up to 2.0-3.0 MDa made up of 100-150 subunits. During temperature upshift, the 2.0-3.0 MDa small heat shock proteins will dissociate to make relatively smaller size (in the range of KDa) active oligomers of such proteins. The small heat shock proteins IbpA and IbpB have holdase chaperone activity. Holdase chaperone activity of IbpB is more than IbpA and is greater when combining both of them (IbpA and IbpB). The MDa form of IbpA and IbpB are the storage form that will dissociate to make smaller active oligomeric species of KDa range, when they face stresses. The active oligomers will bind with heat denatured, misfolded proteins and will give them the protection against further denaturation-aggregation by making HSP-substrate protein complex in ATP-independent way and transferring them to others ATP-dependent holdase-foldase chaperone system (DnaKJE) to refold the misfolded, aggregated proteins.

Heterologous expression of glyoxylate pathway in a novel fast growing freshwater cyanobacteria for photoautotrophic production of succinate

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Cyanobacteria are prokaryotic organisms that can convert carbon dioxide into valuable chemicals via endergonic reactions driven by light energy. As humans continuously strive to lower the anthropogenic carbon dioxide, photoautotrophic cyanobacteria hold significant promise as hosts for biotechnological applications in creating a sustainable bio-based economy. Succinate is a universal value-added commodity chemical with many applications in the petrochemical and polymer industry. It is present as a natural intermediate in the tricarboxylic acid (TCA) cycle. Our group has isolated a number of robust and fast-growing cyanobacterial strains which have shown higher promise as industrially deployable hosts. In this study, six of those novel *Synechococcus elongatus* isolates have been engineered to express glyoxylate shunt to produce high succinate titers. This pathway is a two-gene variant of the TCA cycle comprising of isocitrate lyase (ICL), which converts isocitrate to succinate & glyoxylate, and malate synthase (MS), which converts glyoxylate & acetyl CoA to malate. We employed synthetic biology tools to introduce this pathway in the cyanobacterial genome along with other auxiliary genes which have been reported to improve the efficiency of the desired pathway. Further, the process was optimized for suitable light and CO₂ conditions by screening the engineered strains for photosynthetic production of succinate via the glyoxylate pathway. Moreover, an advanced gene editing tool, CRISPR-Cpf1 system has been used to knock out competing succinate dehydrogenase enzyme and a glycogen synthase gene for an effective carbon sink. These strategies have shown more than fourfold increase in succinate accumulation in the engineered strains compared to the wild type in five days under optimal light and CO₂. These engineered strains offer tremendous promise to act as chassis for large-scale photoautotrophic succinate production by cyanobacteria.

One-step multiplex PCR assay for the detection of major mosquito vectors causing infectious diseases in India

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Mosquitoes are the major contributors to vector-borne diseases, menacing over 40% of the world's population, by transmitting malaria, dengue, chikungunya, yellow fever, Japanese encephalitis, West Nile virus, and Zika. In spite of organized control efforts, mosquito-borne diseases are expanding throughout the world due to rapid climate change and unplanned urbanization. Historically, vector control is the most effective method for controlling vector-borne diseases. The World Health Organization (WHO) also recommended active surveillance of mosquito vectors in urban and peri-urban areas along with rural areas for better control of vector-borne diseases. The most conventional methods of identification of vectors based on morphology demand enormous man-hours, require specific skills, and are often misleading. An easy, cost-effective, and fast one-step assay is required for the robust identification of pooled samples covering diverse geographical territories. We have developed a multiplex-reverse transcriptase PCR assay for species identification of major mosquito vectors based on internal transcribed spacer 2 (ITS2) of ribosomal DNA. The species-specific abundances can also be identified from the unique band pattern visible on an agarose gel. To the best of our knowledge, this is the first one-step multiplex-PCR assay developed to identify the major mosquito vectors of West Bengal.

Long-term tension regulation of single cells in collective cell migration

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Collective cell migration is central to various physiological processes, such as development, wound healing, and metastasis. When it comes to understanding a collective behavior, it is essential to know how the individual bodies that compose the collective behave and gives rise to the emergent properties of the system we observe. In our case, we are using a wound healing model; cell sheet migration of fish keratocytes (cichlids), to study the mechanical properties of a migrating single cell within the collective, to understand collective cell migration. Previous studies have examined how membrane tension and actin polymerization forces guide single-cell migration. In contrast, the role of membrane tension in collective cell migration remains unexplored, to the point that we still need to know whether the cell maintains a particular tension distribution or changes during migration. To address this question, we followed migrating leader cells in the collective using Interference Reflection Microscopy (IRM) for 40-60minutes, taking 41-second movies in 4 minutes intervals. Intensity-to-height conversion and fitting membrane height fluctuation data into our model yielded the effective membrane fluctuations tension of basal plasma membrane for each time point. The results indicate oscillations in membrane tension distribution and migration speed and interesting correlations between them during migration. These results could imply the presence of negative feedback between membrane tension and actin polymerization forces or the breaking and building up of adhesions synchronously, which will be the next objective of this study.

Catalytic mechanism of protein priming

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DNA polymerases lack the ability to perform de novo DNA synthesis, necessitating a mechanism for ‘priming’. Typically, in prokaryotic and eukaryotic systems, replicative DNA polymerases use RNA primers laid down by primases to initiate DNA replication. Replicons like adenoviruses, bacteriophages, and specific mitochondrial plasmids of plants and fungi evade this “RNA priming” by using a unique protein, referred to as terminal protein (TP). TPs bear a certain hydroxyl residue that substitutes for a 3’ hydroxyl, and specialized protein-priming B-family DNA polymerases utilize this hydroxyl to perform polymerization. Protein-primed replication in the bacteriophage phi29 has been extensively studied; however, a clear understanding of the mechanistic determinants for catalysis using a protein as a primer does not exist. We find that phi29 DNA polymerase (Pol) has the ability to covalently couple non-cognate templates to TP, an observation that provides us new insight into the sequence-independent activity of the Pol. Using electrophoretic mobility shift assays (EMSA), we have been able to determine the conditions for obtaining a stable Pol-TP complex on a replication fork-shaped DNA and are currently undertaking experiments to build the entire phi29 replisome. Long term, these findings are being used to develop an efficient, enzymatic route for synthesizing peptide-oligonucleotide conjugates — molecular hybrids that have numerous applications ranging from hydrogel synthesis to molecular cages for targeted drug therapy.

Autophagy defect results in abnormal clearance of mitochondria and energy depletion in the MPS VII fly brain

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Mucopolysaccharidosis type VII (MPS VII) is an inherited disorder caused due to β -glucuronidase (β -GUS) deficiency resulting in accumulation of undegraded GAGs in the lysosomes. Recently, we developed the first fly model of MPS VII by knocking out the β -GUS gene (CG2135) from the *Drosophila* genome. This MPS VII model successfully mimicked the clinical phenotypes with prominent neuromuscular defects. Further examination of the CG2135^{-/-} fly brain disclosed loss of dopaminergic neurons along with accumulation of engorged lysosomes and ubiquitinated proteins. These initial observations led us to investigate the status of the autophagy, an essential part of the lysosome-mediated clearance system. Interestingly, the level of the autophagosome-associated protein ATG8 II and the expression of autophagy related genes was significantly decreased in the CG2135^{-/-} fly brain indicating an autophagy defect. Moreover, we found increase in the intensity of Mito tracker positive puncta as well as an increased level of mitochondrial protein ATP5A in those brains. ATG8 II protein was also found to be reduced in the isolated mitochondrial fraction from CG2135^{-/-} fly brain signifying that reduced mitophagy could be the cause of mitochondrial accumulation. JC1 staining of the CG2135^{-/-} fly brain revealed that the accumulated mitochondria have reduced membrane potential. Significantly low level of ATP in the CG2135^{-/-} fly brain also suggests reduced mitochondrial functionality in those flies. Our data suggests that energy depletion due to accretion of damaged mitochondria in a high energy demanding tissue like brain could be the reason for increased apoptosis and neuronal death in MPS VII flies.

To Expand’ or ‘To Divide’– A Temperature-Imposed Decision for Cotyledons growth

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Being sessile, plants actively adapt to dynamic environmental cues. Cotyledons are the first aerial organs of a seedling to perceive light and temperature signals. In *Arabidopsis*, the role of PHYTOCHROME-INTERACTING FACTORS (PIFs) in elongating hypocotyl to warm ambient temperature has been well established. However, their role in temperature-mediated regulation of cotyledon growth and architecture remains unknown. Here, we show that PIFs play a vital role in the temperature-mediated regulation of cotyledon growth. Analysis of various natural accessions (wild-type) indicated that elevated ambient temperature reduces cotyledon size by negatively impacting cell expansion but causing a trigger in cell division. Analysis of cotyledon response to temperature revealed that individual *pif* mutants cotyledon showed weak to moderate insensitivity; however, higher order *pif* mutants showed a strong insensitivity to warm temperatures mediated suppression of cotyledon growth. Consistent with this, transgenic lines overexpressing PIFs grow with much smaller cotyledons even under lower ambient temperatures, suggesting that PIFs play a predominant role in warm temperature-mediated suppression of cotyledon growth. Our data also revealed that the upstream regulatory components phyB and COP1, which respectively inhibit and promote PIFs function, play a critical role in regulating cotyledon growth in response to temperature. Collectively, this study highlights the role of the phyB-COP1-PIF module in fine-tuning the regulation of cotyledon growth in response to ambient temperatures. Currently, we are looking into the molecular link between PIFs and the cell cycle to understand how PIFs regulate cell size and division to optimise the growth of cotyledons.

Gut Microbiome – A novel weapon to defeat cancer

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Microbiome studies are rapidly changing the way we understand medicine. The largest reservoir of microorganisms in our body lies in the gut harbouring almost 10^{12} cells/ml. With the advancement of science and technology, the complexity of gut microbiome and its role in the pathophysiology of several diseases never fails to perplex us. The gut-microbiome is a double-edged sword- both health promoter (producing metabolites regulating gut homeostasis) and trouble-maker(toxin production by certain microbial sub-populations might trigger inflammation, and tumorigenesis). Shaped by factors like lifestyle,diet,genetics and age, it is a heterogeneous population of commensals-bacteria, fungi, Archaea,Bacteroides and Firmicutes in the large intestine. Dysbiosis,is a biomarker for pathogenesis. Since gut-microbiota can affect efficacies of immunotherapy/chemotherapy, they are targeted by modern approaches for enhanced cancer treatment. The connection between gut-microbiome and cancer is bidirectional: carcinogenesis alters the microbiome and in turn, changes in normal composition of microbiome might stimulate tumorigenesis. One of the major side effects of radiation therapy is gastrointestinal toxicity that partly depends on gut commensals. Other than metabolic/immunological modulations by gut microbiota inducing anti-metastatic/anti-proliferative state, they also influence metabolism of chemotherapeutic agents determining the outcome of cancer-therapy.Ironically,in recent years, overuse of antibiotics and improper diet has selected for a gut-microbiome that isn't diverse and resilient enough to establish a balanced immune response.Probiotics are ingested to enrich gut microflora and arrest disease progression.What if an optimized cocktail of microorganisms is designed that would increase the efficacy of immune system towards cancer therapy?

Phosphorylation-Induced Conformational Dynamics and Inhibition of Janus Kinase 1 by Suppressors of Cytokine Signaling 1

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The dysfunction of the JAK/STAT (Janus kinase/signal transducers and activators of transcription) pathway consequences several pathophysiological conditions, including autoimmune disorders. The negative feedback regulators of the JAK/STAT signaling pathway, suppressors of cytokine signaling (SOCS), act as a natural inhibitor of JAK and inhibit aberrant activity. SOCS1 is the most potent member of the SOCS family, whose KIR (kinase inhibitory region) targets the substrate-binding groove of JAK with high affinity and blocks the phosphorylation of JAK kinases. Overall, we performed an aggregate of 13 μ s MD simulations on the activation loop's three different phosphorylation (double and single) states. Results from our simulations show that the single Tyr¹⁰³⁴ phosphorylation could stabilize the JAK1/SOCS1 complex as well as the flexible activation segment. The phosphate-binding loop (P-loop) shows conformational variability at dual and single phosphorylated states. The principal component analysis and protein structure network analysis reveal that the different phosphorylation states and SOCS1 binding induce intermediated inactive conformations of JAK1, which could be a better target for future JAK1 selective drug design. The structural protein network analysis suggests that the com-pY1034 system is stabilized due to higher values of network hubs than the other two complex systems. Moreover, the binding free energy calculations suggested that pTyr¹⁰³⁴ states show a higher affinity toward SOCS1 than the dual and pTyr¹⁰³⁵ states. We believe that the mechanistic understanding of JAK1/SOCS1 complexation will aid future studies related to peptide inhibitors based on SOCS1.

Rational engineering of a β -glucosidase (H0HC94) from glycosyl family I (GH1) to improve catalytic performance on cellobiose

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The conversion of lignocellulosic feedstocks by cellulases to glucose is a critical step in biofuel production. β -glucosidases catalyze the final step in cellulose breakdown, producing glucose, and is often the rate-limiting step in biomass hydrolysis, which is unsuitable for industrial use. Rationally engineered previously characterized enzymes may be one strategy to increase catalytic activity and the efficiency of cellulose hydrolysis. The specific activity of most natural and engineered β -glucosidase is higher on the artificial substrate *p*-Nitrophenyl β -D-glucopyranoside (*p*NPGLc) than on the natural substrate, cellobiose. Based on our hypothesis of increasing catalytic activity by reducing the interaction of residues present near the active site tunnel entrance with glucose without disturbing any existing interactions with cellobiose (natural product), we report our engineering of a GH1 family β -glucosidase, H0HC94. The mutant shows a significantly higher specific activity, k_{cat} , and cellobiose specificity over H0HC94. Further, molecular dynamic simulations and protein structure network analysis indicate that the mutant significantly increased the dynamically stable communities and hub residues, leading to a change in enzyme conformation and higher enzymatic activity. This study shows the impact of rational engineering of non-conserved residues to increase β -glucosidase substrate accessibility and enzyme specificity.

Cloning and Characterisation of a thermotolerant GH6 cellobiohydrolase from *Chaetomium thermophilum*

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The consortium of microbial hydrolases is used to degrade the cellulosic biomass for production of biofuels and other valuable bio-products. Glycoside hydrolases like endoglucanase, cellobiohydrolase, and β -glucosidase are major cellulases and their proper proportion used for the efficient degradation of cellulosic biomass. Cellobiohydrolases helps in cleavage of cellulose chain from reducing (CBHI) and non-reducing (CBHII) ends. The biomass conversion processes depend on many enzyme properties including stability, product inhibition. Characterisation and study of thermostable and ionic liquid tolerant cellulase that could make better cellulase cocktail for one pot reaction that is cost effective and efficiently saccharifies biomass. We cloned a cellobiohydrolase from the GH6 family in *Chaetomium thermophilum* and found it to be thermostable and tolerant to 20 % 1-Ethyl-3 Methylimidazolium Acetate (EMIM OAc). This CBH hydrolyses Avicel, CMC, β -Glucan, in a processive manner and produces cellobiose. I will present my initial results of characterizing this enzyme.

Age, Diabetic Status and Vaccine type determine susceptibility to Covid-19

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Equipped with a dramatically high mutation rate, SARS-CoV-2 trampled across the globe infecting individuals of all ages and ethnicities. The condition was further exacerbated by the emergence of different variants of concern, implicated in curtailed vaccine efficacy and risk of reinfection. To test whether age-group, vaccine type, and diabetes have any association with COVID-19 susceptibility, Google Form-based surveys were conducted in order to collect data. 384 responses were received for the studies on age and vaccine type. Sample size for the data on diabetic status was 430. Chi-Square Tests of Independence were conducted for statistical analyses. Age-group and incidence of contracting COVID-19 are not independent of each other ($P < 0.05$). People belonging to the age-group of 41-60 years are most likely, and those below 21 years are least likely to be affected. There is a significant association between the vaccine received and incidence of contracting COVID-19 after being vaccinated ($P < 0.05$). Individuals receiving Covaxin are less likely to contract COVID-19 after being vaccinated than those receiving Covishield. Diabetic status and incidence of contracting COVID-19 are significantly associated ($P < 0.01$). Diabetics have a greater predisposition to COVID-19, and COVID-infected individuals are at a higher risk of developing diabetes. This study statistically proved the association of COVID-19 infection with age. Covaxin (whole virion vaccine) was established to be more potent than Covishield (recombinant vector vaccine). Diabetes and COVID-19 were found to be significantly associated. However, a larger sample needs to be considered for authenticating the findings.

Bioengineering LAB vector expressing reporter protein: a non-invasive tool for real-time tracking of gut microbes

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Lactococcus lactis (*L. lactis*), the first genetically modified probiotic bacteria, is generally regarded as safe (GRAS) and is best studied for its ability to express heterologous proteins. The advantage of *L. lactis* as probiotics is proven unquestionable for their generalized health-promoting effect on maintaining gut homeostasis and immune balance. However, an in-depth understanding of their role in improving gut health requires a selective approach that would allow us to study their bio-distribution, fate, and immunological consequence. The fluorescent labeling techniques are primarily used to chemically conjugate small, non-biological fluorophores and chromophores compounds with the bacterial surface. However, these methods often affect bacterial growth, and more importantly, the functioning of such compounds is impeded by low oxygen availability in the gut and their poor tissue permeability of optical wavelengths. To harness the unique properties of *L. lactis* inexpressing biological macromolecules, we utilized a “live bacterial platform” to express the fluorescence reporter proteins in the bacterial cytosol. Using Nisin Inducible Controlled Expression (NICE) system in pCYT plasmid with the Nisin inducible promoter (P_{nisA}), we bioengineered a stable *L. lactis* vector to express EGFP (Enhanced green fluorescent protein, Ex:488nm; Em:511nm) and mCherry reporter proteins (Ex:587nm; Em:610nm). Following the oral administration of the recombinant *L. lactis* into a mice model, we observed that bacterial localization was more evident in the distal ileum and ileocecal junction at 3 h post-administration using *in vivo* imaging system (IVIS). Moreover, when analyzed for plasmid integrity, we could successfully retrieve the bioengineered bacteria from freshly harvested fecal pellets and retain their ability to express the reporter protein. Together, we describe the optimal non-invasive biomedical applications of bioengineered *L. lactis* as a befitting strategy for active live tracking of the gut microbes.

Investigating importance of endogenous Gyrase inhibitor YacG during cytotoxic stress conditions in *Escherichia coli*

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DNA Gyrase, a Type II topoisomerase is vital for survival as it is responsible for maintenance of negatively supercoiled status of the bacterial genome. DNA supercoiling *in vivo* has been shown to be dynamic and this variability is responsible for differential gene expression under different growth phases, environmental stress conditions. Interestingly, several chromosomally encoded proteinaceous interactors of DNA gyrase have been reported that play an important role in regulating DNA gyrase activity *in vivo*, thereby influencing DNA topology causing differential gene expression pattern based on cellular need. YacG is a specific endogenous inhibitor of DNA gyrase, which interferes with DNA binding and strand passage activity of the enzyme. Although it has been shown to be an inhibitor *in vitro*, we believe, it serves an important role *in vivo*, in safeguarding genome from gyrase poisons and inhibitors. We show that YacG expression protects against the cytotoxic action of novobiocin, ciprofloxacin. YacG also protects from the action of natural proteinaceous toxins targeting DNA gyrase like CcdB and MicrocinB17. Deletion of *yacG* leads to hypersensitivity compared to wild type *E. coli*. However, YacG fails to protect against general DNA damaging agents like Methyl methane sulphonate, suggesting that its protection is restricted to gyrase targeting cytotoxic agents. It also fails to impart any protection from other classes of antibacterial drugs like Nitrofurantoin and Rifampicin. We propose that existence of YacG probably reflects a bacterial strategy to safeguard its genome integrity by protecting essential housekeeping function of DNA gyrase from external agents specifically targeting this vital enzyme.

Bacterial Ghost Cells mediated prophylactic study against *Salmonella* Typhi and *Salmonella* Paratyphi A in mice model

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Typhoid fever is a severe disease caused to ranges from 12 to 21 million cases per year and 1,29,000 to 1,45,000 deaths annually worldwide by *Salmonella* Typhi and in India paratyphoid incidence of 105 (74 to 148) per 1,00,000 person/years. At present, we urgently need a paratyphoid vaccine. Alkaline agent was used to prepare ghost cell of *Salmonella* Typhi and Paratyphi A. After complete immunization, immunoblot and ELISA were performed to assess immunogenicity. We checked CD4+, CD8+ and CD19+ splenic cells changes in immunized animals. Protective efficacies monitored with survival study and histopathological analysis. Electron microscopy image was clearly shown the trans-membrane tunnel in treated bacteria which confirm bacterial ghost formation. Immunogenic bands found against outer membrane protein, lipopolysaccharide in immunoblot and serum IgG, IgA titer also correlates these results. Cell-mediated immunity was observed, especially Th1/Th17 specific cytokine response from splenic cells in ex-vivo experiment. In efficacy study, we observed bivalent Typhoidal ghost cells immunized mice showed better protection than non-immunized control mice in survivality and histopathology. Ghost cells could be a safe (less cytotoxic) immunogen of Typhoidal *Salmonella* in future.

Differential Microbial Signature Associated with Benign Prostatic Hyperplasia and Prostate Cancer

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Apart from other risk factors, chronic inflammation is also associated with the onset of Prostate Cancer (PCa), wherein pathogen infection and tissue microbiome dysbiosis are known to play a major role in both inflammatory response and cancer development. However, except for a few studies, the link between microbes and PCa remained poorly understood. To explore the potential microbiome signature associated with PCa in Indian patients, we investigated differential compositions of commensal bacteria among patients with benign prostatic hyperplasia (BPH) and PCa using 16S rRNA amplicon sequencing followed by qPCR analyses using two distinct primer sets. Using two independent cohorts, we show that *Prevotellacopri*, *Cupriaviduscampinensis*, and *Propionibacterium acnes* represent the three most abundant bacteria in diseased prostate lesions. LEfSe analyses identified that while *Cupriavidustaiwanensis* and *Methylobacteriummorganophilum* are distinctly elevated in PCa samples, *Kocuria palustris* and *Cellvibriomixtus* are significantly enriched in BPH samples. Furthermore, we identify that a number of human tumor viruses, including Epstein-Barr virus (EBV) and hepatitis B virus (HBV), along with two high-risk human papillomaviruses - HPV-16 and HPV-18, are significantly associated with the PCa development and strongly correlated with PCa bacterial signature. The study may thus offer to develop a framework for exploiting this microbial signature for early diagnosis and prognosis of PCa development.

Phosphatase or kinase: Understanding the master regulator of VEGFR-1 signaling

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Reactive Oxygen Species (ROS) is known to portray a significant role as an intracellular messenger in controlling the activation of the Receptor Tyrosine Kinases by inhibiting the Protein Tyrosine Phosphatases. ROS locally inactivates the intracellular negative regulator like Protein Tyrosine Phosphatases (PTPs) and prolongs signal transduction of Receptor Tyrosine Kinases (RTKs). The regulation between RTKS and PTPs is very crucial for the cellular functioning. Vascular Endothelial Growth Factor Receptor 1 (Vegfr1), a well characterized RTK of VEGFR family expressed in endothelial cells and monocytes plays an important pathophysiological role in tumor angiogenesis, Diabetic retinopathy and arthritis although the mechanism underlying its action is not clear. Vegfr1 and vegfr2 have almost similar sequence and binds to VEGFA ligand. However, Vegfr1 has 10-fold high affinity for its ligand VEGFA yet having poor kinase activity compared to Vegfr2 and is known to negatively regulate angiogenesis. The work tries to elucidate the reasons behind autoinhibited kinase activity of Vegfr1, one of which could be its association with PTPs. The results require further demonstration if cellular phosphatase mediated regulation of the Vegfr-1 signaling can become a therapeutic for the treatment of tumor angiogenesis.

Type I Interferon Driven Gut Dysbiosis Contributes to Insulin Resistance and promotes the Development of Metabolic Syndrome

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Insulin resistance in obese individuals has been previously associated with inflammation in both clinical and pre-clinical studies. Type-I interferon plays a crucial role in the initiation of chronic low-grade inflammation or 'metaflammation' in obesity and associated metabolic syndrome. Derangement of the gut microbial consortia is an established signature of pathogenesis in such contexts. We aimed at validating pathogenetic role of type I IFN in loss-of-function (LOF) and gain-of-function (GOF) murine models in wild type C57BL/6 mice by evaluating immunocellular dynamics, investigating the nature of gut dysbiosis and performing serum metabolomic studies. Systemic blockade of type I interferon in the LOF model improved insulin sensitivity and reduced weight gain in high fat diet fed (HFDF) mice. Alternatively, in the GOF model systemic abundance of type I interferon was achieved by administration of adenoviral vector overexpressing murine IFN- α which led to systemic insulin resistance. Fecal transplantation from these mice to wild type C57BL/6 mice depleted of their native gut microbes was sufficient to induce systemic insulin resistance in recipient mice. V3/V4 region sequencing of the 16s-rRNA gene from fecal DNA in the GOF and fecal microbiota transplantation (FMT) model revealed altered microbial signatures in the IFN-AdV and FMT-IFN-AdV as compared to their respective controls. These were associated with systematic changes in serum metabolite abundance and altered immune cell phenotypes. These studies will contribute to gain critical insights into the role of type I IFNs on diet-induced insulin resistance as well as identify possible therapeutic targets in clinical contexts of obesity and metS.

Insights into the antimicrobial prescribing patterns and nosocomial incidence rates across ICU and non-ICU wards of a tertiary care super-speciality hospital of West Bengal

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The study was conducted to gather baseline information to assess the antimicrobial prescribing practices for indoor patients across ten ICU and non-ICU departments in a tertiary care super speciality hospital of West Bengal. Modified version of a patient data collection tool (CRF) proposed by WHO-AMSP scale was adopted for validation using standard methodologies. Data from indoor patients were collected prospectively during March to May, 2022. The use of antimicrobials was categorized as empiric, prophylactic or culture driven. The WHO-AWAKE classification of antimicrobials was used to classify systemic antimicrobials. Study data was archived and analysed using SPSS 20.0 (IBM) and Med Calc 8 (Belgium). Total beds covered during the survey was 1025 and the number of patients on antimicrobials was found to be as high as 99.27 %. Mean age distribution of the patients was found to be 37.62 ± 18.38 years. The patients surveyed were equitably distributed based on gender with female patients accounting for 51.71 %. The mean number of antimicrobials per patient was found to be 2.62 (range of 1.4 to 2.2). Relatively higher number of patients were found to be on 2 or more antimicrobials from WATCH and RESERVE categories. Interestingly, among nosocomials CLABSI and CAUTI rates were 22.05 % and 22.11 % respectively with a relatively lower proportion of SSIs 0.88%. Mortality relatedness to infection was found to be 48.62 %. Compliance rate to hospital antibiotic policy was found to be 83.51% while culture was sent in nearly 59% of the patients surveyed. Double gram negative and Double anaerobic coverage of antimicrobials varied use across departments. Our baseline study was undertaken on a significant cohort of patients in a tertiary care institute of the state and the main take-away included capturing of real time data of antimicrobial consumption, DDD and DOTs and hospital acquired infection rates which may aid policy makers to formulate antimicrobial guidelines in the state and country.

Zooplankton diversity and its relationship to the physico-chemical qualities of water in shallow wetland of Arunachal Pradesh

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Diversity and dynamics of zooplankton being the naturally available fish food organisms seems to have influencing role towards the development of rice field fishery. Plankton samples were randomly collected by filtering 25 L of water and preserved immediately in 4% formalin. The quantification was done by using Lackey's drop count method. Water quality parameters were analyzed with the help of APHA, 2012. Five communities of zooplankton viz. Cladocera, Copepoda, Rotifera, Protozoa, Ostracoda were found. These were comprised of 34 taxa of Cladocera, 7 taxa of Copepoda, 17 taxa of Rotifera and 5 taxa of Protozoa, and only one taxon of Ostracoda. Mean density of zooplankton was highest in May (452 individuals l-1) followed by June (446 individuals l-1) and lowest in September (89 individuals l-1). Cladocera showed maximum Shannon diversity value in June (2.869) and minimum was in September (1.298). Copepod varied from 1.301 (April) to 1.696 (July) whereas Protozoa (1.388) and Rotifera (1.533) showed peak diversity in May while Protozoa (1.132) and Rotifera (1.024) showed least value in July. Physico-chemical qualities of water were under suitable range for better survival and growth of zooplankton. The abundant population of zooplankton show a typical population attribute as well as serves as natural food sources for the fishes during the rice growing season.

How much robust a plant biochemical system is!

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Plants can survive at varied environmental conditions by relying on their robustness even at the cellular level. Although every leaf of a plant and every cell of a leaf contribute towards the overall growth of the whole plant, the biomass are expected to be different in different cells within a leaf. These differences may be related with the availability of light energy and nutrient or biomass requirement and also on the cellular conditions including the enzymatic gene expression and their kinetics etc. We reconstructed Medium-Scale-Metabolic models of C3 (Rice) and C4 (Setaria sp.) plants and analysed them using FBA. The experimentally observed proportion of the biomass components of a plant leaf is, in reality, an average value of multiple leaf cells. Our analysis shows i) to biochemically synthesize a fixed amount of biomass with different components in experimentally observed proportion, the amount of photon needed by a single plant cell is same as the need of a group of cells with a wide metabolic variations, ii) several different metabolic readjustments occur in these cells to maintain the redox balance and to fulfil the energy requirements, iii) these underlying metabolic variations are reflected in different possible modes of TCA cycle and GS-GOGAT pathways, iv) Assimilation Quotient, being dependent on nitrogenous and sulphur sources, is a poor marker for measurement of photorespiration rate. Present analysis confirms that the plant metabolic system is very much robust with large number of potential alternative pathways that may become active to accommodate different kind of stresses.

Exploring the role of Melanoregulin, a novel regulator in Plasmacytoid Dendritic cells

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Plasmacytoid dendritic cells(pDC) are known for their robust production of Type I Interferons in response to viral infection. In our study we described the role of Melanoregulin(MREG), a small highly charged protein with highest expression in plasmacytoid dendritic cells among all major immune subsets, that regulates the production of Type I Interferons in them. Here we showed that MREG localizes in the late endosomal compartment upon stimulation of pDCs with synthetic TLR9 ligands and helps in transportation of cargo from late endosomal to auto-phagosomal compartment. pDCs being a key driver of psoriasis skin pathophysiology, we reanalysed a publicly available single cell RNA sequencing dataset and found out pDCs invading the skin of psoriasis patients expressed more MREG than pDCs infiltrating the skin of control patients. Similar results were also obtained upon analysis of three publicly available RNA transcriptomics datasets for psoriasis and control skin. Collectively, our research reveals that MREG is a novel protein that regulates pDC function.

A glycoconjugate vaccine induces protective immunity against Typhoidal and non-Typhoidal Salmonella

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Vaccines are available against typhi but not against Paratyphi and Typhimurium infection. Our lab earlier reported the protective efficacy of salmonella Typhi/Paratyphi outer membrane conserved protein T2544 and vaccine against Salmonella Typhi and Salmonella Paratyphi infection. Here, we develop a candidate vaccine, containing O-specific polysaccharides (OSP) from S. Typhimurium, conjugated to the S. Typhi/S. paratyphi outer membrane protein T2544 (OSP-T2544). After purification of the respective protein and the polysaccharide, both are allied using ADH linker and the biophysical characterization of the conjugate was performed using several techniques. The protective efficacy of the candidate vaccine was evaluated in different mouse models for Typhoidal and non-Typhoidal infection after subcutaneous immunization. Antibody and avidity titer in serum was measured. The candidate vaccine (OSP-T2544) induces four times higher serum IgG titer compared to OSP alone. The avidity titer was also higher between 120th and 38th-day sera. In the protection study, 75%, 77%, and 80% of OSP-T2544-immunized mice survived the lethal challenge of S. Typhi, S. Paratyphi, and S. Typhimurium respectively. Our proposed candidate vaccine is therefore able to induce protective antibodies against Typhoidal and non-typhoidal salmonella.

Altered cellular prion (PrPC) expression in the murine β -coronavirus (mouse hepatitis virus) and its role in viral infectivity

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The cellular prion protein (PrPC) is a glycosylphosphatidyl inositol (GPI) anchored extracellular cell membrane protein that is expressed in a myriad of tissues and organs, being predominantly expressed by various cell types in the central and peripheral nervous system. Due to its diversified roles and contribution in a plethora of physiological processes like neural homeostasis, neurogenesis, myelin maintenance, signal transduction, copper uptake and many more, a definite role of PrPC has been difficult to be established and so its exact physiological significance continues to remain enigmatic. Previous studies reported that PrPC exerts protective role during neuroinflammation, although its precise role in neuroinflammation demands a thorough investigation. Here we have attempted to envision the function of PrPC in Mouse Hepatitis Virus MHV-A59-induced murine model of neuroinflammation in C57BL/6 mice both in-vivo and in-vitro cell culture system. A significant upregulation of PrPC at both protein and mRNA levels was evident in RSA59 (an isogenic recombinant strain of MHV-A59) infected, inflamed mouse brains which increases during acute viral infection reinforcing its role in neuroinflammation. Furthermore, the upregulation of prion in mice brain samples made it imperative to investigate the effect of RSA59 infection on prion expression in different neural cell lines namely mouse neuroblastoma N2a, microglial N9 and DBT astrocytoma cell lines upon virus infection. The results revealed an upregulation of PrPC in neuroblastoma N2a and microglial N9 cell line while a downregulation was observed in DBT astrocytoma cell line and hence a differential expression of prion during neuroinflammation is clearly evident.

Evaluating the effect of p-Benzoquinone conjointly with NNK in Guinea pig lung- Deciphering the role of Vitamin-C

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Smoking causes various types of chronic and fatal diseases. Chronic obstructive pulmonary disease (COPD), a very common disease related to smoking causes destruction of lung alveolar cells and found to increase the probability of lung cancer by several folds. This observation of association of lung cancer with COPD is surprising considering the totally opposite phenomenon involving redox signalling at the cellular level. So the hypothesis is that the wound healing or repair mechanisms initiated due to inflammation and oxidative stress in COPD patients could also trigger lung cancer development. In this study, we want to decipher the molecular link and common cell signalling events underlying between COPD and Lung cancer and also deciphering the role of Vit-C. We took guinea pigs as model animal because they cannot synthesis Vit-C like human. We used p-BQ alone and also along with NNK, a carcinogen, to observe conjoint effect by systemic injections with Vit-C oral supplementation. The dose of NNK and p-BQ were calculate based on the amount reported in commercial cigarette and also considering long term consumption by a chain smoker. For dosage, the body weight of guinea pigs were adjusted. The lung tissues have collected after particular time points. Histology, western blot, immunohistochemistry and oxyblot analysis were performed to understand the molecular mechanism. Experimental analysis confirmed that the effects of CS exposure in guinea pig lung were mimicked by p-BQ treatment and were prevented by Vit-C. Inflammation and oxidative damage increased gradually, however, Vit-C treatment largely negate the effect.

Scavenging Strategies in Free-ranging dogs

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Foraging and acquiring of food is a delicate balance between managing the costs (both energy and social) and individual preferences. We carried out a multiple-choice test experiment (MCTE) on 68 individual free-ranging dogs (FRD) & 136 groups on urban streets, simulating scavenging from dustbins to see the change in foraging strategies, if any, under the influence of social cost like intra-group competition. We found multiple differences between the strategies of dogs foraging alone versus in groups with competition playing an implicit role in the dogs' decision making when foraging in groups. Dogs continually assessed and evaluated the available resources in a "patch", transitioning from random foraging to systematic foraging with time and more information. Dogs in groups used an, "eat first, sample afterwards" strategy whereas individual dogs sampled thoroughly before eating. Additionally, dogs in groups were quicker and more likely to respond to the experimental set-up and eat from it. The dogs adjusted their behaviour in terms of effort and time allocated according to the quality of the "patch". Foraging in groups also provided benefits of reduced individual vigilance. The various decisions and choices made lend support to the optimal foraging theory wherein the dogs harvested the nutritionally richest patch possible with the least risk and cost involved but were willing to compromise if that was not possible. This underscores the cognitive, quick decision-making abilities and adaptable behaviour of these dogs, which is likely to have influenced the process of dog domestication.

Reciprocal interaction between Copper ATPases of Host and Leishmania regulates infection and pathogenicity

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The study explores the role of copper in host-pathogen interaction using the Leishmania-macrophage model. In case of leishmaniasis, when Leishmania infects human macrophage and harbours in the phagolysosomes as amastigotes, we observed that the human Copper ATPase, ATP7A, traffics from the Golgi to these phagolysosomes and transports copper in these bodies. Copper acts as an antimicrobial agent and is lethal for the amastigotes. Interestingly, we also found that Leishmania reciprocally upregulates its own Copper-ATPase, LmATP7, to export this copper for its survival. We sequenced and characterised Leishmanial Copper ATPase and named it LmATP7. Engineered Leishmania lacking LmATP7 could not infect macrophage cell line and mice successfully but the ones overexpressing the same showed higher infectivity as compared to the wild type parasites. Our study shows host macrophages tries to exert copper stress within Leishmania containing phagolysosomal compartments to kill the pathogen. To counteract this, Leishmania has armoured itself with the copper ATPase, LmATP7.

A multi-tier regulation of the homologous mammalian Copper ATPases by AP1 in polarized epithelia

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ATP7A and ATP7B are P-type ATPases that regulates copper homeostasis in mammalian cells, mutations in which causes Menkes and Wilson disease respectively. Menkes disease is characterized by deficiency of copper, while Wilson disease patients have hepatic copper overload. In a polarised epithelia, both ATP7A/7B localize in the trans-Golgi network (TGN) under basal or Cu-deprived conditions. Under elevated intracellular copper, ATP7A and ATP7B traffic to basolateral and apical membranes, respectively. This differential trafficking can be used to address a fundamental question in cell biology, i.e., how does a cell sort its cargos once asymmetry sets in? In this study, we have used polarised MDCK cell line as they express these highly homologous copper transporters. We have elucidated the differential trafficking itinerary of ATP7A/7B in polarised MDCK cells and their regulatory partners. The trafficking pathways of both ATPases are traced by observing the localization with various endosomal substations like CRE, BSE, ASE, etc. We found that ATP7A/7B localize at distinct domains in TGN, suggesting that the asymmetric trafficking initiates at the TGN itself. They show similar Golgi exit kinetics and copper sensitivity. Using proximity biotinylation technology and mass spectrometry analysis, we found clathrin, AP1 and, AP3 as potential interacting partners, among many others. We also used AP1A and AP1B knockout cells to elucidate its essential role in regulating the differential trafficking at TGN and CRE. We further observed that the combined regulation of AP1(A/B) in the localization of ATP7B, where AP1A gives directionality from TGN to CRE, and AP1B mediates its recycling from CRE to TGN or its retention at CRE. MDCK – Madin-Darby Canine Kidney, TGN – Trans-Golgi Network, CRE – Common Recycling Endosome, BSE – Basolateral Sorting Endosome, ASE – Apical Sorting Endosome, AP1 – Adapter Protein 1, AP3 – Adapter Protein 3

Amelioration of Thioacetamide Induced Liver Fibrosis in *Mus musculus* by Methanolic Leaf Extract of *Amaranthus spinosus* Linn

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The liver interacts with components of circulation, destined for the entire body due to its unique position in the human body. Some of these components may cause harm to the cellular components of the liver, resulting in the activation of the organ's defence or fibrogenesis. Persistent stimulation of the fibrogenic response can result in liver fibrosis which may lead to cirrhosis and hepatocellular cancer. This study focused on the effect of methanolic leaf extract of *Amaranthus spinosus* Linn. in mice with induced liver fibrosis. Fibrosis was induced in *Mus musculus* using intraperitoneal injections of 100 mg/kg body weight of Thioacetamide, 3 times/week for 8 weeks. Animals with established fibrosis were then given an oral dosage of the leaf extract at 50 mg/kg body weight for 10 days. Biochemical parameters like Alanine transaminase (ALT), Aspartate transaminase (AST), Bilirubin (Direct and Total), and Alkaline Phosphatase were assessed in blood. Tissue samples were collected from control, thioacetamide treated and leaf extract-treated groups. Blood biochemical parameters were observed to be significantly reduced in animals with liver fibrosis after receiving plant extract orally, in comparison to thioacetamide-treated animals. Tissue histology also showed improvements in the anatomy of liver tissue after leaf extract dosage. The study is indicative of *Amaranthus spinosus* being potentially anti-fibrotic and may present a prospect for the development of a hepatoprotective herbal medicine.

Non-self ‘carbohydrate oligomer’ protects Ly6C⁺ monocytes and induces infection-incompetent macrophages: A therapeutic adjuvant against *Leishmania donovani* infection

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Leishmania donovani, the obligatory protozoan parasite infection causes visceral leishmaniasis that multiplies inside the macrophages of the bone marrow, spleen, and liver. Macrophages derived from inflammatory monocytes are essential in conferring host protection during this disease by induction of pro-inflammatory Th1 response. However, the host protective mechanisms of these macrophages are hijacked by *Leishmania* inducing an emergency myelopoiesis in bone marrow diverting them to a more benevolent phenotype which can now be utilized by the parasites for their safe shelter to overcome the host's immune clash and aid in parasite sustenance. The sabotage could be overcome by inducing the differentiation of hematopoietic cells towards protective inflammatory monocytes (iMOs) or infection-incompetent macrophages to restrict or deport the parasites from their haven in favour of host protection. In this study, we report a ‘carbohydrate-oligomer’ from the edible mushroom *Pleurotus florida* which was capable of rescuing the innate system from parasitic manipulation and inducing the generation of infection-incompetent macrophages, thereby, promoting host-protective Th1 responses. This effect was also found to induce long-term concomitant immunity and hence, presents itself as a potent adjuvant for therapeutic purposes.

Isolation and Enrichment of Primary Neuroglial cells from Neonatal Mice to study their individual role in the Central Nervous system

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The nexus between neuroglial cells is pivotal for the proper functioning and sustenance of the intricate Central nervous system. The development of a proper *in vitro* system warrants a better understanding of the structural and functional relationship among these cells. Previous studies have established primary neuronal cultures from embryos or neural stem cells attained by sacrificing pregnant mice. As opposed to previously established cultures, our approach utilizes the brain from a single day 0 pup to isolate various neuroglial cells including astrocytes, microglia, oligodendrocytes, and meningeal fibroblasts in addition to the neurons circumventing mice sacrifice, which is comparatively less laborious and cost-effective. The current study aims to develop *In vitro* system of neuroglial cells to highlight the atypical role of glial cells in contributing towards CNS homeostasis maintenance. Further through this protocol, we aim to provide step-by-step instructions for the isolation and culture of primary neuroglial cells along with its characterization using cell-specific markers, which provide an opportunity to carry out various cell-based and biochemical assays to understand their CNS cell-specific behaviour in a reductionist approach.

Copper independent endo-lysosomal localisation of Wilson's disease protein ATP7B in Hepatocytes

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Lysosome is a dynamic organelle that regulates several essential cellular processes including nutrient recycling and cellular clearance. Formation of membrane contact sites with different organelles is one of the modes of lysosomal functioning. At basal cellular copper level, the Wilson disease protein, ATP7B, is a Cu-ATPase that resides on TGN membrane and traffics to endo-lysosomes upon elevated cellular copper to export out excess copper. In our study, we have found that a significant amount of ATP7B is present in the endo-lysosomal compartment of hepatocyte irrespective of cellular copper concentration. This localisation pattern of ATP7B is found to be hepatocyte specific and was not observed for the homologous Cu-ATPase, ATP7A. We discovered the presence of TGN-Lysosome proximity site (TGN-LPS), a novel organelle interface that harbours a fraction of ATP7B. ATP7B that localizes in the TGN in apposition to TGN-LPS occupied a sub-region of the organelle that has a lower pH as compared to rest of the TGN. Localization of ATP7B on TGN-Lysosome hybrid organelle upon Golgi disruption suggested possible exchange of membrane between these two organelles. Interestingly, modulating perinuclear/peripheral lysosomal equilibrium also influences endo-lysosomal pool of ATP7B. On contrary to previous published data, we found that upon copper chelation, ATP7B is not completely retrieved to the TGN; rather it localises to peri-Golgi region of cell. To summarize, we propose the existence of a novel organelle interface (TGN-LPS) that controls copper-independent endo-lysosomal localisation of ATP7B in hepatocyte.

Activation of microglia/macrophages and infiltration of T cell subsets in the CNS together with their priming in the CLN varies in an age-dependent manner in the Mouse Hepatitis Virus (MHV) induced experimental model of Multiple Sclerosis

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Multiple Sclerosis or MS is a multifactorial, chronic demyelinating disease of the Central Nervous System. Understanding the mechanism of T cells regulation is imperial to decipher the immunopathology in CNS neuroinflammation. The Mouse Hepatitis Virus (MHV) induced model of MS helps to establish a virus induced experimental model attempting to explain virus-induced neural-cell damage culminating to demyelination and axonal loss. Here an isogenic recombinant strain of MHV-A59 namely RSA59 was used to induce experimental neuroinflammation in C57BL/6 mice. Previous studies have established that migration of CD4⁺T cells not only assist microglia/macrophages to attain their activated state during the acute phase of neuroinflammation, but are also crucial for maintaining neuronal homeostasis and reducing microglia/macrophage activation during the chronic phase of demyelination. This is in stark contrast to the Experimental Autoimmune Encephalomyelitis model of MS where T cells are myelinolytic. This study focuses on understanding the age-dependent (juvenile, young adult and adult mice) immunological maturity of mice post RSA59 induced acute neuroinflammation and the progression of chronic phase demyelination. The results show that transition from juvenile to adult phase is accompanied by significant microglia/macrophages activation and heightened infiltration of CD4⁺ and CD8⁺T cells in the CNS that significantly reduce viral load in adult mice as compared to juvenile and young adults at the acute phase. This data corroborates with significantly reduced demyelination at chronic phase with age. Additionally, Tregs were significantly increased both in the CNS and CLN of adult mice at acute phase which also resulted in reduced chronic demyelination.

Anticancer therapeutic potential of *Azadirachta indica* (Neem) bark extract and its derived limonoids on cervical cancer *in vitro* and in 3D multicellular tumor spheroids

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Cervical cancer (CC) remains a significant cause of mortality among women worldwide, specifically in Asian countries. To date, treatment includes surgery in combination with chemo- and radiation therapy. Often, cancer acquires chemotherapeutic resistance, and patients suffer from chemotherapy-associated side effects. Hence, the quest to look for novel anticancer drugs and treatment strategies with high therapeutic potential and negligible side effects is still open. Our previous studies have shown the anticancer potential of methanolic neem stem bark extract (MNBE) against CC *in vitro*. In the present study, we investigated the anticancer effect of MNBE on HeLa cell-derived 3D multicellular tumor spheroids mimicking avascular tumors *in vivo*. We found that MNBE significantly decreased the growth and survival of 3D HeLa spheroids in a dose-dependent manner. Further fractionation and characterization of MNBE revealed various compounds of which two limonoids i.e., Nimbolide and Gedunin, were found to have strong anticancer potential. We found that pretreatment with Gedunin, a natural HSP90i, synergistically enhanced the anticancer potential of Nimbolide against CC cell lines, including HeLa, SiHa and ME-180. Protein expression analysis of various ER stress markers (HSF1, HSP90, and HSP70) revealed increased ER stress after Gedunin pretreatment. Additionally, RAD51, a client protein of HSP90 and vital for DNA damage repair, was significantly downregulated which might result in sensitization of cancer cells towards Nimbolide treatment. Moreover, we have generated a CC cell line derived xenograft nude mice model to determine the efficacy of MNBE and its bioactive compounds *in vivo*.

Germline protein, Cup, non-cell autonomously limits migratory cell fate in *Drosophila* oogenesis

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The ability of a stationary cell population to acquire migratory fate is critical both in development as well as in diseased condition like tumor metastasis in the metazoans. Though this transformation in cell fate is physiologically very important, the underlying molecular mechanism as to how migratory cells are delineated from stationary population is not very well understood. The specification of migratory border cells from the follicular epithelium during *Drosophila* oogenesis has emerged as an excellent model system to study cell fate specification. A layer of follicle epithelial cells envelope the germline nurse cells. JAK-STAT activation in a small subset of anterior follicle cells lead to migratory border cell fate. We demonstrate that RNA binding protein Cup modulates border cell fate. Down regulation of Cup function suggests that it function in the germline nurse cell and limits the border cell fate non cell autonomously. Further investigation reveal that Cup affects Delta trafficking in the nurse cells through Rab11GTPase, potentiating Notch activation in the anterior follicle cells. Modulatory effect of Notch activity on JAK-STAT signalling in the anterior follicle cells, impinges on the border cell fate. Overall, we propose that germline Cup regulates the size of the migratory border cell cluster in the anterior follicle cells through Notch and JAK-STAT signalling.

Evaluation of spirometric parameters for identification of concomitant obstruction in patients with diffuse parenchymal lung diseases

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Obstructive airway disease (OAD) and diffuse parenchymal lung diseases (DPLD) often co-exists. Hence, it is important to identify the situation to offer the best possible treatment. The study aims at envisaging the role of FEF25-75 in identifying airflow obstruction in patients with DPLD diagnosed on High Resolution Computed Tomography (HRCT) features. The selected subjects were subjected to spirometry and the GOLD recommendation of FEV1/FVC: <0.7 was used to identify concomitant airflow obstruction. Thus, diagnosed unmixed DPLD and DPLD with OAD overlap were compared based on the other available spirometric variables such as FVC (forced vital capacity), FEV1 (forced expiratory volume at first 1sec), FEV1/FVC and FEF25-75 (forced expiratory flow at 25-75% of vital capacity). ROC curves were drawn using FEF25-75 and the best cut-off value for differentiation between the two groups were identified. The GOLD criteria for OAD could identify 26 (12.93%) [male: female - 19:7] out of 201 subjects keeping pure DPLD as 87.07% (N=175; male: female as 85:90). Notably, the mean %-predicted FEF25-75 was found to be significantly higher in pure DPLD than those with DPLD + OAD overlap (70.36 ± 32.57 vs. 28.35 ± 29.41 , $p < 0.0001$). The ROC curve generated a cut-off value of 45.5 to differentiate the two groups with a sensitivity and specificity of 76.99% and 88.46% respectively. Apart from the conventional markers of airflow obstruction, FEF25-75 can too effectively predict airflow obstruction in a DPLD patient population. Thus, FEF25-75 may also be treated as a marker of airflow limitation.

Ligand-decorated polymeric micelle used for tumor targeting and drug delivery

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The aim of this study is tumor targeted drug delivery for conjured therapeutic benefits using ligand installed polymeric micelle as nanomedicine. This nanomedicine-based approach improves the bioavailability of the drug loaded micelle, exhibit increased dose responses and enhanced targeting efficiency by selectively delivering drugs to the tumor site. Here, we have used folic acid as targeting ligand and doxorubicin as an anticancer drug. We have synthesized folic acid installed and doxorubicin conjugated block co-polymer and subsequently the polymeric micelle, of size 25-50nm. The hydrophilic block of polymer is composed of polyethylene glycol units and the hydrophobic block is made up of aspartic acid. In our preliminary study, we have used 2D cell culture model and have assessed the cellular internalization and further subcellular localization of polymeric micelle. We have observed that the folic acid installed nanomedicine showed higher cellular uptake and co-localization with the nuclei compared to the control micelle. In our future study, we plan to assess the anti-tumor activity of our synthesized polymeric micelle in tumor spheroid model using various cell lines (KB, HeLa etc).

Functional Characterization of Interferon Lambda 4 in Human Primary Cells (PBMCs)

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A dinucleotide variant rs368234815 (Δ G) introduces a new member of the interferon family i.e. *IFNL4*, present upstream of *IFNL3*. It was found that a 179 amino acid (aa) long IFN- λ 4 protein is produced by a frame-shift mutation. Having A Strong antiviral activity, *IFNL4* and its genetic variations were found to be associated with poor clearance of HCV, Asthma, COPD, Prostate Cancer, GI, and Malaria. So, far reports are available that show IFN- λ 4 at the protein level is- Human prostate cancer cell lines with Sendai virus (SeV) induces expression of IFN- λ 4 another is the Expression of *IFNL4* in PHHs at 6 or 24 h post-SeV infection. This leads to our insert to learn more about IFN- λ 4 in human primary cells. What we found is surprising for us that in spite of having any genotype (TT/TT, TT/ Δ G, Δ G/ Δ G) individual produces IFN- λ 4 like protein. A new report generated recently Shows that *IFIIC2* (*IFNL4* pseudogene) which is present upstream of *IFNL2* gene is translated in many mammals giving rise to a unique protein which is 169 aa. We also tested this possibility and reported that *IFIIC2* is not expressed in humans. By using molecular biology approaches we confirmed that TT allele of humans could express an IFN- λ 4 like protein. By doing multiple mutations we confirmed that they use the same start and stop codon like IFN- λ 4 but fail to induce the IFN-stimulated gene expression. We found that this new isoform is glycosylated and secreted like IFN- λ 4. Importantly, we show that the Δ G allele can also potentially express a similarly frameshifted isoform. The functional significance of these novel isoforms remains to be elucidated.

Mapping the intracellular conformational changes of the Cu transporter ATP7B

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ATP7B is a Copper transporter, which, when mutated may lead to copper accumulation in the liver and subsequently, in the brain, resulting in a condition known as Wilson's disease (WD). This P-type ATPase performs a crucial role in maintaining copper homeostasis along with other Cu-binding proteins in hepatocytes, neurons and nephrons, which express ATP7B, predominantly. At basal copper levels, this enzyme resides at the Trans-Golgi network membrane from where it supplies Cu to secretory cuproproteins like ceruloplasmin. At higher copper levels, ATP7B traffics out of TGN into endolysosomal compartments to sequester and eventually extrude out excess Cu from the cell. Structurally, ATP7B consists of a transmembrane region and four major cytosolic domains – an N-terminal domain with six Cu-binding sites, a Nucleotide-binding domain (NBD), a Phosphorylation domain and an Actuator domain. It has been proposed that the intramolecular conformation of ATP7B may play an important role in its trafficking regulation. We are trying to unravel these inter-domain interactions that take place in the protein during its trafficking from the TGN to vesicles by using a bimolecular fluorescence complementation approach. We have generated the recombinant ATP7B construct with the required reporter gene inserts and are in the process of investigating the interactions between the Cu-sensing N-terminal domain and the NBD of the enzyme, which has been postulated to occur at the TGN, based on an in vitro study carried out previously. We plan to throw light on the working and regulation of this remarkable Cu- ATPase as it traffics from one intracellular site to another.

Sound waves can manipulate the way molecules manifest itself in the system

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All creations are energy, and sound is one such manifestations of energy as vibrations. Water that constitutes 60% of adult human body, show structural differences in its crystal when kept under different set of emotions *in vitro*. Therefore, its effect *in vivo* is an area for wide research. Fascinating as it sounds, vibrations within and beyond the sonic range has profound effect on water molecules, DNA and protein structure and organization within system. All matter vibrate at certain unique frequency (resonant frequency). The resonant frequency of cancerous cells, proteins that trigger apoptosis (caspases), oncosis and pyroptosis can be studied and measures to selectively induce, suppress or reprogram their activity based on sound vibrations, without much injury to the surrounding healthy cells. This, in turn, can impel and reprogram immune cells to identify cancer cells. Based on the studies conducted, their resonant frequency cause molecular vibrations to selectively lyse cells, dissipating heat energy. Sonic treatment is an alternative to the crude methods employed in treating diseases that adversely affect the system differentially. HIFU and Histotripsy employed treatment non-invasively and effectively focus ultrasound waves to mechanically destroy targeted cells. Sonosensitive particle enabled drug targeting cause targeted cells to implode by cavitation in real time without excess heat energy. Even after cycles of insonation, cells have tendency to sustain. These challenges can be answered by LIPUS for selective ablation of cancerous cells, providing a hope for a day, when the world can smile and say, "Yes, Cancer has an Answer!".

Distribution of Th2 in OAD

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In the OAD (Obstructive airway diseases) population, a good frequency of Th2 (type II helper T cells) high activity is present. The knowledge of a relative distribution of such Th2 status is important. Consecutive subjects of clinically diagnosed OAD (having any three of wheeze, cough, expectoration and SOB) were subjected to spirometry with measurement of FeNO, absolute eosinophil count (AEC) and IgE. The Th2-high inflammatory pattern was classified as “absolute” or “possible” when all three or any of two of the parameters were high ($AEC \geq 300$ cells/ml, $IgE \geq 375$ IU/ml, $FeNO \geq 25$ ppb). The negativity of all three or any two were regarded as “absolute” or “possible” non-Th2 activity. Asthma was diagnosed as FEV1 reversibility ≥ 200 ml and 12% and COPD as $FEV1/FVC < 0.7$ with reversibility < 200 ml and 12 %. The subjects not falling in “asthma” and “COPD” were denoted as “unclassified”. The distribution of Th2 inflammation was seen statistically in these patients. Of included subjects, (N=246; male:155, female:91) with asthma 70 (28.45 %), COPD 89 (36.17 %) and “unclassified” 87 (35.33 %) were included. The distribution of “absolute” and “possible” Th2-high subjects were 23 (32.85 %) and 20 (28.57 %) for asthma, 15 (16.85 %) and 25 (28.08 %) for COPD and 16 (18.39 %) and 28 (32.18 %) for “unclassified” subjects. Similarly non-Th2-high subjects were distributed as ‘absolute’ and ‘possible’ were 5 (7.14 %) and 22 (31.42 %) for asthma, 20 (22.47 %) and 29 (32.58 %) for COPD and 19 (21.83 %) and 24 (27.58 %) for “unclassified” group. Th2-high activity is variably present (although most frequent in asthma) in OAD. Hence, such classification needs functional correlation and may have therapeutic implications.

Elucidating the effect of temperature on enzyme activity of β -Glucosidase by computational and experimental approach

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β -Glucosidase (EC 3.2.1.21) is a key component of the cellulase cocktail required for the degradation of cellulose in biomass to glucose and its subsequent conversion to chemicals and biofuels. However β -Glucosidases are often inhibited by their reaction product glucose through the accumulation of glucose during saccharification, which in turn inhibits cellobiohydrolase and other cellulolytic enzymes of the cellulase cocktail. Thus, glucose tolerant and active β -Glucosidase is required for the efficient saccharification of biomass. While previous research have implicated the role of the active site pocket in glucose tolerance or inhibition, understanding the precise interactions of the substrate and product with the enzyme is important towards designing glucose tolerant enzymes. We have employed atomistic molecular dynamics and Molecular Mechanics Generalised-Born Surface Area (MMGBSA) approaches to study the protein-ligand (substrate and product) interactions, using GH1 β -Glucosidase from *Paenibacillus polymyxa* (BglB) as model. Through experimental and computational studies we have tried to delineate how enzyme activity is temperature-dependent. The binding free energies derived from MMGBSA studies demonstrated the effect of temperature on enzyme affinity towards ligand. Furthermore, with per-residue energy decomposition and hydrogen-bond occupancy, we identified key residues that may be responsible for substrate and product binding. Thus far, our analyses show that the enzyme glucose interactions at the active site pocket are facilitated by polar interactions.

Repurposing of PDE5 Inhibitor Sildenafil as a Possible Therapeutic Target for Cervical Cancer

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Decreased cyclic nucleotide levels and increased expression of various cAMP/cGMP-dependent phosphodiesterases (PDE) isoforms have been linked to several tumorigenesis including cervical cancer where increased expression of PDE5 have been documented hinting towards the roles of cyclic nucleotide signaling pathways in tumorigenesis. Therefore, modulation of cyclic nucleotide signaling with selective in trial or market available PDE inhibitors might be a cost-effective therapeutic intervention against cervical cancer. Our study has shown that PDE5 inhibitor sildenafil can significantly increase cGMP levels in human cervical cancer cell lines: HeLa and SiHa (HPV⁺ cell lines) but showed no modulation in C33A (HPV⁻ cell line). Cell proliferation study also revealed marked abrogation of cancer cell proliferation upon sildenafil treatment in HPV⁺ cell lines. It was therefore investigated whether such abrogation was the result of altered telomerase expression and activity. Taken together, these findings suggest that inhibition of PDE5 generate anti-proliferative effects in HPV⁺ cell lines but not in cell lines lacking HPV. These results clearly indicate towards the potentiality of sildenafil as anti-cancer targets in HPV⁺ cervical cell lines indicating an association of viral load with cGMP-PDE signaling modulation, while the precise mechanism of the same is yet to be unraveled.

A study on passive protective efficacy in suckling mice model conferred by pentavalent OMVs-based immunized mice sera against infection caused by circulating diarrhoeagenic *Escherichia coli*

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Escherichia coli pathotypes are major causative agents of diarrhoea induced childhood morbidity and mortality in developing countries. Enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC) or Shiga-toxin producing *E. coli* (STEC) and enteroaggregative *E. coli* (EAEC) constitutes the five main pathotypes among seven pathotypes of diarrhoeagenic *Escherichia coli* (DEC). The study will shed light on the potential role of pentavalent outer membrane vesicles (OMV)-based immunogen in prevention of diarrhoea mediated health burden caused by DEC. Transmission electron microscopic (TEM) analysis was done for comparison of sizes of different DEC OMVs. Pyrogenicity and toxicity study were done to standardize immunization dose. Western blot analysis and ELISA against different components of bacterial cell were done for immunogenicity study of pentavalent OMVs. Protective efficacy study conferred by immunized mice sera was assessed in suckling mice. Protective efficacy study revealed that immunized mice sera significantly confer protection in suckling mice against five prevalent strains. This study sheds light on the ability of pentavalent OMV based immunogen of DEC to elicit marked immunogenicity and protective efficacy against different components of DEC when administered intraperitoneally in adult BALB/c mice. Thus, the above-mentioned formulation may act as a novel vaccine candidate to attenuate and subsequently eradicate DEC mediated diarrhoea in the near future.

Molecular Insights into the Recognition of Acetylated Histone Modifications by the BRPF2 Bromodomain

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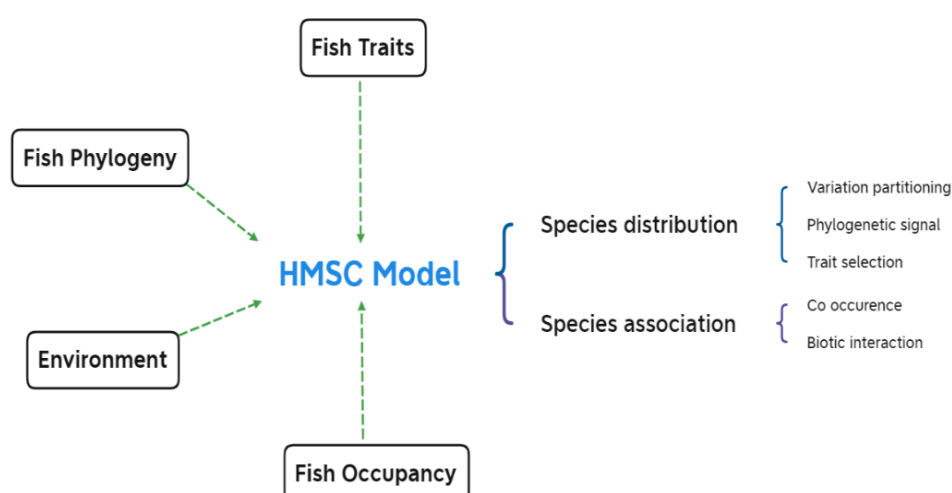
HBO1 [HAT bound to the origin recognition complex (ORC)], a member of the MYST family of histone acetyltransferases (HATs), was initially identified as a binding partner of ORC that acetylates free histone H3, H4, and nucleosomal H3. It functions as a quaternary complex with the BRPF (BRPF1/2/3) scaffolding protein and two accessory proteins, ING4/5 and Eaf6. Interaction of BRPF2 with HBO1 has been shown to be important for regulating H3K14 acetylation during embryonic development. However, how BRPF2 directs the HBO1 HAT complex to chromatin to regulate its HAT activity toward nucleosomal substrates remains unclear. Our findings reveal novel interacting partners of the BRPF2 bromodomain that recognizes different acetyllysine residues on the N-terminus of histone H4, H3, and H2A and preferentially binds to H4K5ac, H4K8ac, and H4K5acK12ac modifications. In addition, mutational analysis of the BRPF2 bromodomain coupled with isothermal titration calorimetry binding and pull-down assays on the histone substrates identified critical residues responsible for acetyllysine binding. Moreover, the BRPF2 bromodomain could enrich H4K5ac mark-bearing mononucleosomes compared to other acetylated H4 marks. Consistent with this, ChIP-seq analysis revealed that BRPF2 strongly co-localizes with HBO1 at histone H4K5ac and H4K8ac marks near the transcription start sites in the genome. Our study provides novel insights into how the histone binding function of the BRPF2 bromodomain directs the recruitment of the HBO1 HAT complex to chromatin to regulate gene expression.

Hierarchical modeling of species communities (HMSC) in freshwater fishes of Terai-Dooars Ecoregion: How likely is it to predict the co-occurrence of fish species?

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Species distribution modeling has advanced over the past two decades. Hierarchical modeling of species communities (HMSC) has recently been found to ease understanding of community structures from various data-driven hypotheses. It has been a pioneering tool to model all species in a given community jointly. Here we attempted to model the presence and absence of 41 fish species as a function of environmental filtering with potential contingencies



on species traits and phylogenetic relationships. This study selected six torrential freshwater rivers of the Terai Dooars ecoregion. The HMSC model was fitted using the *r* package, HMSC (V 3.0-13). Results show higher variation explanations by fixed effects rather than random effects. Stream slope (SL) and annual precipitation (B12) have a higher mean-variance contribution. The presence of *O. barna*, *B. vagra*, and *X. cancila* seems considerably impacted by stream slope. Similarly, *A. botia*, *B. brucei*, *P. balitora*, *P. chola*, and *S. devdevi* seem to be more modulated by annual precipitation. Furthermore, the phylogenetic signal in the beta plot is low, inferring that phylogenetically related species might not have similar responses from the given covariates. Following the biotic assembly rules, species-to-species association matrices have been produced, which indicate the random level co-occurrences of fish species at site and basin levels. No residual association was found at the river level. Such results can infer the probability of species co-occurrences which is not explained by covariates but rather subjected to their dispersal limitations, niche assembly, and facilitative/ competitive associations.

Evaluating the antiviral property of Telmisartan for the management of Chikungunya virus infection

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Chikungunya virus (CHIKV) has become a global pathogen causing significant socio-economic burden and till date no commercial vaccine or drug is available against CHIKV. Drug Re-purposing is an interesting alternative, provided the dose and dosage regimen are patient compatible. The inflammatory pathways of the renin-angiotensin system (RAS) and peroxisome proliferator-activated receptor-gamma (PPAR- γ) are usually involved in viral infections. Thus, Telmisartan (TM), which is known to block the angiotensin 1 (AT1) receptor and activate PPAR- γ , was investigated against CHIKV *in vitro* (Vero, RAW 264.7 cells and hPBMCs) and *in vivo* (C57BL/6 mice). TM was found to abrogate CHIKV infection efficiently (IC₅₀ of 15.34-20.89 μ M in the Vero and RAW 264.7 cells respectively) with remarkable inhibition in the Viral RNA and protein levels. Additionally, TM interfered in the early and late stages of CHIKV life cycle with efficacy during pre-treatment and post-treatment. Moreover, treatment with the agonist of AT1 receptor and antagonist of PPAR- γ increased CHIKV infection which was antagonized by TM treatment, suggesting TM's anti-viral potential by modulating host factors. In addition, reduced activation of all major mitogen-activated protein kinases (MAPKs), NF- κ B (p65) and cytokines by TM occurred through the inflammatory axis. Interestingly, at the human equivalent dose, TM abrogated CHIKV infection and inflammation significantly leading to reduced clinical score and complete survival of C57BL/6 mice. Additionally, TM reduced infection in hPBMC derived monocyte-macrophage populations *in vitro*. Considering its history of long-term safety and anti-viralefficacy against CHIKV, it can be a suitable candidate in future for repurposing against CHIKV.

Network and ecological drivers of scavengers in human-dominated landscapes in India

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Rapid urbanization has led to significant habitat changes, and some species have adapted to the urban ecosystem quite efficiently in recent times. Scavengers are typically well suited to life in human-dominated urban ecosystems. They utilize human-generated wastes as their primary food source and act as the "cleaners" in such habitats. However, scavenging communities have been studied less in urban ecosystems, and we have a limited understanding of their diversity and drivers. We experimented with different sites in West Bengal, India, to identify the scavenging guild within urban habitats in response to human-provided food. A total of 17 other vertebrate species were identified across sites over 498 sessions of observations in our study. We carried out network analysis to understand the system's dynamics, and it came out that the free-ranging dog and common mynah were critical species within the scavenging networks. This study shed light on the complexity of scavenging networks within human-dominated habitats.

Green Medicine for Urolithiasis

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Urolithiasis (*Uro- urinary system; Lithos- stone formation*) is a prevalent urological disorder affecting 12% of global population yearly. There are different types of urolithiasis but out of which calcium oxalate are found to be diagnosed mostly. All the treatment strategies for urolithiasis are invasive i.e. breaking of larger stones into smaller pieces so that those are easily excreted normally. To avoid such invasive treatments, scientists are exploring an alternative option i.e. green medicine. Tulsi was selected for our study because of its versatile therapeutic profile and well availability throughout India. The therapeutic potentials of green extracts are mainly exhibited by the major phytochemical such as eugenol of tulsi leaf extract (TLE). Since, eugenol and other important phytochemicals of TLE are feebly soluble in water, to make them bioavailable, a venture was taken i.e. nanonization. Gelatin-stabilized TLE-nanoparticles (TLE-NPs) and eugenol-nanoparticle (ENPs) were prepared by nanoprecipitation method. Various physico-chemical properties of the nanoparticles were determined by the techniques like FESEM, TEM, DLS, AFM, spectrophotometry and dialysis. Anti-urolithiatic potentials of the nanoparticles were evaluated by measuring a) nucleation time, b) crystallization, c) aggregation, d) dissolution and e) DFT analysis. The cytotoxicity of TLE-NPs and ENPs were assessed on HEK 293T cell line by MTT assay. The anti-urolithiatic potential of TLE, TLE-NPs, eugenol and ENPs were evaluated in a comparative manner and their potentials were found to be as in the following order ENPs > TLE-NPs ≥ Eugenol > TLE. In conclusion, the prepared nanoformulations could be applied as effective drug for calcium oxalate urolithiasis.

Higher Order Interaction in structurally perturbed intransitive cycles

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Given Darwinian selection, maintaining species variety in ecological groups is a difficult task. The current study looks beyond pairwise competitive interactions and offers a fresh viewpoint on how higher-order interactions affect the development of social traits. Our straightforward model provides a positive perspective to show how perturbations affect intransitive competitive higher-order interactions. We demonstrate how, without the aid of a complicated set of ordinary differential equations, the perturbed contact network can swiftly ascertain the coexistence equilibrium of competing species. Our perturbation in the matrix correspond to evolution of species in real world, while one accounts for evolution of weaker species other for evolution of stronger species. Depending on the quantity of disturbances, the system can be divided into a number of viable cluster states. Our study also shows that the ratio of disturbed to unperturbed species is inversely related to the intensity of the perturbation used. Our findings imply that nonlinear dynamical systems and interaction topologies can be used to understand how species might persist under challenging circumstances. Particularly, our results show that when two species compete less, their abundance rises and they outperform others, i.e., positive evolution of weaker species is having a long-term beneficial impact on the dynamics where positive evolution of stronger species lead to reduced density of both the species in ecosystem. Moreover, we also investigated the role of higher order interaction in decreasing the transient time.

Effect of Neem Bark extract on S-palmitoylation, a post-translational modification of viral proteins that modulate viral fusogenicity

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Recent outbreak of SARS-CoV-2 has severely affected human health worldwide driving the urgent need to find effective therapeutic approaches. Murine Hepatitis Virus (MHV) and SARS-CoV-2 belongs to the β -coronavirus family which allows MHV to serve as model system where β -coronavirus induced disease progression can be studied in C57Bl/6 mice. Studies suggest that neem bark extract (NBE) can effectively restrict the infectivity and fusogenicity of murine-coronaviruses (MHV-A59/ RSA59) and human coronavirus (SARS-CoV-2) but underlying mechanism of action is still not understood. One of the most important structural proteins of these viruses is the spike protein which alone is capable of cell to cell fusion. Recent studies have revealed the presence of cysteine residues in the cytoplasmic tail of spike protein that undergo post-translational modification, S-palmitoylation, which is crucial in infectivity and fusogenicity. Deletion of those cysteine residues resulted in lower fusogenic activity of the virus. This study aims to understand the effect of NBE treatment on the spike proteins of different virus strains of MHV – hepatoneurotropic strain RSA59 and neurotropic strain MHV-JHM to give an overall idea of how NBE is affecting the status of palmitoylated regions in the cytoplasmic tail of spike proteins. It can be predicted that there will be reduced palmitoylation of the cysteine residues in the cytoplasmic tail due to NBE which may lead to restricted viral infectivity and fusogenicity. The study sheds light on probable binding of neem phytochemicals to cytoplasmic tail of spike proteins ultimately serving as a novel therapeutic strategy against pan β -Coronaviruses.

PHYTOCHROME INTERACTING FACTOR 3 is an essential component of the thermosensory pathway in Arabidopsis

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Plants, as sessile organisms, adapt to changing environments to survive and optimise their fitness. The warm ambient temperature, i.e. few degrees higher than the optimum temperatures, significantly impacts plant growth and fitness. The average seasonal growth temperatures have been predicted to increase by 2-5°C due to climate change, resulting in severe biodiversity loss and reduced crop yields. Understanding the molecular mechanisms that sense and integrate temperature cues is, therefore crucial. Here, we identified PHYTOCHROME-INTERACTING FACTOR 3 (PIF3) as a novel and essential component of the thermosensory pathway in Arabidopsis. The knockout *pif3* mutant shows significant insensitivity to warm-temperature-induced hypocotyl growth and flowering. In contrast, PIF3 overexpression transgenic lines show constitutive warm temperature responses with long hypocotyls and petioles, bigger rosette architecture and early flowering even under lower ambient temperatures. Further, our pharmacological studies reveal that PIF3 promotes thermosensory growth by inducing auxin biosynthesis and signalling, as the treatment of *pif3* mutants with exogenous auxin could only partially rescue its short hypocotyl phenotype. Our whole genome transcriptomic analysis data reveals that PIF3 activates the expression of crucial auxin biosynthetic and signalling genes and a large number of genes involved in cell-wall remodelling. Currently, we are investigating the actual mechanism through which PIF3 activates thermosensory responses.

A new insight into the reversibility profile of OAD

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Bronchodilator responsiveness is an important assessment issue for obstructive airway disease (OAD) patients. Salbutamol (β_2 -agonist) inhalation is used conventionally which helps to differentiate among the common OADs: asthma, chronic obstructive pulmonary disease (COPD) and Asthma-COPD Overlap Syndrome (ACOS). Of late, another novel bronchodilator, glycopyrronium reversibility (Anti-Muscarinic-Agent) is proposed by us. We have looked for the serial reversibility of salbutamol and glycopyrronium in successive patients who gave us consent. The responsiveness to these agents were assessed in both absolute value and % change for asthma ($FEV_1/FVC \geq 0.7$, reversibility ≥ 200 ml and 12%), COPD ($FEV_1/FVC < 0.7$, reversibility < 200 ml and 12%) and ACOS ($FEV_1/FVC < 0.7$ but reversibility ≥ 200 ml and 12%). Statistical analysis was done using anova in Graphpad prism. Mean age (in years) of the patients was highest in COPD (61.16 ± 11.33) and lowest in asthma patients (40.15 ± 17.21) with ACOS (51.60 ± 14.96) in between. Females were slightly more in asthma (M:F=32:35) while males were dominant in COPD (M:F=126:41) and ACOS (M:F=61:28) patients. COPD group had recorded lowest (40.24 ± 66.49 ml) while asthmatics had the highest (381.6 ± 153.8 ml) salbutamol reversibility on absolute values. However, ACOS has the highest % change (29.00 ± 13.95). On the contrary, glycopyrronium reversibility was highest in COPD (105.6 ± 100.9 ml) followed by ACOS (93.03 ± 138.9 ml) and asthma (73.95 ± 150.5 ml) on absolute values but highest in ACOS (36.82 ± 17.12) followed by asthma (25.61 ± 14.76) and COPD (15.20 ± 17.51) in terms of % change. Glycopyrronium reversibility seems better for COPD and ACOS patients than asthma.

Identifying a New Role of ADAMTS13 in Human Mesenchymal Stem Cells (MSCs) under Serum-Deprivation Stress

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The immunomodulatory potential of mesenchymal stem cells (MSCs) is vital for their application in regenerative therapeutics. MSCs can facilitate angiogenesis and promote neo-vascularization via secretion of trophic factors. However, post-transplantation, MSCs encounter harsh micro-environmental conditions like nutrient deprivation, hypoxia or an inflammatory-milieu at the diseased site, which hamper their efficacy. Our study entails identifying the key molecules regulating the molecular mechanisms of angiogenesis in human umbilical cord-derived Wharton's Jelly MSCs (WJ-MSCs) under serum-deprivation stress. ADAMTS13, a matrix-metalloproteinase responsible for cleaving the Von-Willebrand factor (vWF), is reported to have pro-angiogenic functions. In our study, we found that ADAMTS13 was upregulated in WJ-MSCs under serum-deprivation condition. Correspondingly, potent pro-angiogenic markers like *VEGF*, *PDGF*, *IL-6* and *TNF- α* were also seen to be upregulated. siRNA-mediated knockdown of *ADAMTS13* under serum-deprivation stress led to considerable reversal in the expression of the angiogenesis markers hinting that it might be playing a role in regulating them. Moreover, the p38 and the JNK signalling pathways were identified as negative and positive regulators of ADAMTS13 expression, respectively, under the same condition. Further, our findings indicated that the Notch pathway and p53 could be the other underlying partners modulating the expression of ADAMTS13, and subsequently, the angiogenesis markers under serum-deprivation stress. Additionally, the increased level of ADAMTS13 in serum-deprived WJ-MSCs was functionally demonstrated by efficient disruption of vWF multimers by immunofluorescence studies. Overall, our study highlights ADAMTS13 as a probable key player, regulating the expression of angiogenic markers in WJ-MSCs under serum-deprivation stress condition.

“Ecological suicide” of *C. jejuni* via T6SS dependent functionality: a novel death- pathway of gut pathogens

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Indiscriminate antimicrobial use can alter microbial composition in the gut, destabilizing gut homeostasis and impairing gut barrier function. While most gut pathogens are armed with the intrinsic ability to thrive in the harsh gut environment, selective dysbiosis of gut colonizing pathogens is often challenging. To this end, we demonstrated the unique attributes of *Campylobacter jejuni*, a known gut pathogen that naturally inhabits chicken ceca, serving as the single largest source of human infection. Though the *C. jejuni* armed with T6SS can facilitate bacterial predation, host pathogenesis, how *C. jejuni* harboring functional T6SS coexist with other resident microbes and whether they have any role in the perturbation of gut homeostasis are largely unknown. Here, we demonstrate a novel ecological consequence of *C. jejuni* T6SS as an example of “Ecological suicide” under altered environmental conditions. Using bile salt as a common gut stressor and a non-pathogenic *E. coli* as a T6SS-target (prey), we showed a significant reduction in T6SS-positive cells compared to T6SS-negative. Further, we elucidate the underlying mechanism by tracking fluorophore-conjugated bile salt, which suggests T6SS-mediated stressor influx in *C. jejuni*, leading to enhanced oxidative stress and subsequent cell death. Using chickens as primary hosts of *C. jejuni*, our *in vivo* study also suggests that prey-driven activity of T6SS can perturb *in vivo* intestinal colonization of *C. jejuni* in chickens administered with bile salt solution and prey. Together, we elucidate how T6SS-dependent predation can lead to self-killing under an altered gut environment and highlights the prospect of using this unique feature of T6SS-dependent “predation cost” as an “antibiotic alternative” approach for improving gut health.

Syntaxin-17 is a Novel Inhibitor of Autophagic Lysosomal Reformation in Pancreatic Beta Cells in Diabetes.

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Dysfunction and death of pancreatic islet beta cells develop metabolic disorder type-2 diabetes (T2D). Macroautophagy (autophagy) mitigates pathologic stress and preserves cellular health by catabolising autophagosome-sequestered cytotoxic components within autolysosomes. SNARE/tether (Syntaxin-17/HOPS)-mediated formation of autolysosomes, active digestion by lysosomal hydrolases, and subsequent regeneration of naïve lysosomes from autolysosomes (autophagic lysosomal reformation: ALR) by mTOR/UVRAG perpetuate autophagy. Impaired autophagy is linked to numerous pathologies including T2D that manifest beta cell accumulation of autolysosomes but with unclear mechanisms. In vitro exposure of rat insulinoma beta cells (INS-1) to glucolipotoxic diabetic milieu (palmitate + high glucose) and subsequent fluorescence imaging reveal time dependent accumulation of autolysosomes reminiscent of diabetic beta cells. Biochemical analyses of lysosomal cathepsins and western blot detection of mTOR signalling shows comparable activity to untreated controls indicating possible perturbation in downstream ALR. Interestingly, a depleted level of UVRAG but heightened retention of Syntaxin-17 is obtained in autolysosomes from diabetes-treated cells. Immunoprecipitation of autolysosome UVRAG recruiter HOPS subunits VPS33A and VPS16 shows increased association with Syntaxin-17 but UVRAG. The current data set indicates a novel hitherto unknown function of Syntaxin-17 in ALR, which is mediated by possible perturbation of Syntaxin-17 recycling from post-fusogenic Syntaxin-17/HOPS complex in autolysosomes and rather a stable association that prevents HOPS association/recruitment of UVRAG to inhibit ALR under diabetic milieu. Promoting Syntaxin-17 recycling or dissociation of Syntaxin-17/HOPS complex in beta cell autolysosomes could be an effective therapeutic strategy to activate ALR, and thereby to reinstate autophagy and beta cell health to improve T2D.

Understanding The Role Of Inositol Hexakisphosphate In The Activation Of Bruton's Tyrosine Kinase

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Bruton's tyrosine kinase (BTK) is a non-receptor tyrosine kinase required for regulation of B-lymphocyte development, differentiation and signalling. The activation of BTK is a crucial event in the B-Cell signaling pathway. BTK was known to be activated by its recruitment to the plasma membrane and subsequent phosphorylation by Src family of kinases. Recently, it was found that inositol hexakisphosphate (IP₆) activates BTK, even in the absence of a membrane through its interaction with the PH-TH domain. This interaction is hypothesized to stabilize a transient dimer of PH-TH domain which is yet to be detected in solution or in cellular environment. Interestingly, a point mutation (E41K) in the PH domain constitutively activates BTK, the mechanism of which is yet to be completely understood. In this study, we focus to characterize the IP₆ mediated PH-TH dimer and try to investigate the role of IP₆ in constitutive activation of E41K mutant.

Copper induced regulation of Copper Transporter CTR1 endocytosis

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Copper is an essential micronutrient for all eukaryotic organisms, and it is utilized in several physiological processes. In mammalian system, the high affinity copper transporter, CTR1, resides on the plasma membrane and forms a homotrimer to attain a channel conformation for copper import. At elevated extracellular Cu, as a self-regulatory mechanism, CTR1 are endocytosed via Clathrin mediated. Our study focuses on the Cu dependent regulation of CTR1 endocytosis in relation to conformation of the protein. During membrane targeting, CTR1 remain majorly as 'loose-grip' trimer and localize on membrane as such. High extracellular Cu induces 'tight-grip' trimer formation making it functional for copper uptake. When intracellular Cu concentration outstretch to optimum concentration, CTR1 protein clusters on the plasma membrane, a phenomenon that precedes endocytosis. This CTR1 clustering triggers changes in membrane tension that is favorable for endocytosis initiation. Interestingly, in parallel, CTR1 loses its tight conformation which induces AP-2 bindings to the cytosolic loop of the protein. Following up same cells in IRM-TIRF microscopy along with arresting endocytosis by Dynasore revealed that, upon copper treatment the local clustering of CTR1 increases whereas initial local membrane tension decreases. Similarly, CTR1 puncta analysis showed increase in area fraction in the same ROI of transfected HEK293T cells upon copper treatment; thus, confirming that CTR1 cluster formation is an essential pre-endocytic event.

Translation linked cytoplasmic mRNA decay

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A precise balance among the synthesis, degradation and translation of mRNA is a prerequisite for controlled functioning of a cell. Cells must have an intrinsic signal encoded in its biomolecular sequences, structure and their interactions to regulate and control these three important cellular processes. We hypothesize that codon optimality, internal unstructured segments (IUS), and ribosomal density (Rf) are the potential regulators of both translation and degradation of mRNA. Integrating mRNA sequence, experimentally determined secondary structure, translation rate, ribosomal footprinting and mRNA half-lives data we tried to understand their effects on mRNA half-lives and translation rate. We propose a metric Codon content index (CI) which gives an idea of transcript wide optimal and non-optimal codon content. The developed metric is able to capture the effect of optimal codon on mRNA half-lives and translation rates. We have shown that lower CI denoting abundance of optimal codon elevates translation rate and half-lives of mRNA transcript. We have also shown that CI, along with IUS, destabilizes an mRNA transcript, reducing its half-life. Integration of Rf data indicates that occurrence of ribosomes on mRNA increases the mRNA half-life enhancing its stability, but to a certain extent. Higher Rf probably leads to ribosomal collision, which facilitates transcript's capture by co-translational surveillance directing them to degradation. Further, we integrate the above data to get an insight on how all these features affect the translation rates and half-lives of mRNA transcripts on a genome scale.

A residue-level insight into thermostability

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Thermostable proteins are of immense interests for decades, because of their uses in various industrial and research fields. Proteins from mesophilic origin disintegrate at high temperatures but those from thermophilic and hyperthermophilic organisms are designed differently by nature. Scientist have tried to unravel this designing principle in several theoretical, simulation and experimental studies. So far, we have come to know about few types of inter-residue and residue-solvent interactions that are predominantly present in thermophilic proteins. Among these, in our previous study, we have identified seven features that are nearly universal in thermophilic-mesophilic orthologous pair, regardless of their lengths, three-dimensional (3D) topologies, domains and functions (Hait et al., 2020). Comparing the orthologous sequences, we have showed how the mutations in thermophilic proteins support these thermal adaptation mechanisms. Further, we have found the association of charge-reversal mutations of partially-exposed charged amino acids with increased coulombic interactions in thermophilic proteins (Hait et al., 2021). Recently, we have tried to understand, how the arrangement of interacting (in 3D) amino acids in the primary chain are associated with thermostability. Working on 1560 orthologous protein pairs, we have found out that thermophiles are enriched with long range interactions, they have bigger connected clusters and higher network density compared to their mesophilic orthologs. This is a fascinating outcome for protein engineers as well as structural biologists, because two interacting amino acids in 3D, when distantly placed in the primary chain, would stabilize the tertiary structure and play a key role in maintaining global stability.

***Leishmania* secretory factor GP63 downregulates host Nramp1 to sequester iron in the phagolysosomes**

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Macrophage phagolysosomal iron exporter Natural resistance-associated macrophage protein 1 (Nramp1) is the genetic determinant of resistance against various intracellular pathogens including *Leishmania*. Recently, we reported that *Leishmania major* infection of the macrophages resulted in significant downregulation of the Nramp1 protein with simultaneous increase in phagolysosomal iron content. Here we report that even in uninfected macrophages, Nramp1 was significantly downregulated when the cells were treated with *L. major* conditioned medium (LmCM) resulting in enrichment of phagolysosomal iron content. Using a proteasome inhibitor, we could confirm that LmCM-induced Nramp1 downregulation was caused by ubiquitin-proteasomal degradation. These data indicated a possible involvement of a secretory biomolecules of *Leishmania* in mediating Nramp1 downregulation. The ability of LmCM to downregulate Nramp1 was abolished upon heat treatment or incubation with trypsin, suggesting that the secretory biomolecule which caused downregulation of Nramp1 is protein in nature. Since the metalloprotease GP63 is an abundant secretory protein of *Leishmania*, we pre-incubated the LmCM with metalloprotease inhibitors EDTA and 1,10 Phen. and then used it for treatment of macrophages. Such treatment failed to downregulate Nramp1 indicating that GP63 might be the factor responsible for Nramp1 downregulation. To confirm this, we generated the GP63 knockout *L. major* strain using the CRISPR-Cas9 gene editing tool. LmCM obtained from GP63^{-/-} strain was unable to downregulate Nramp1. Taken together, our results uncovered a novel role of GP63 in targeting host Nramp1 protein to augment the phagolysosomal iron pool.

***Leishmania donovani* preferentially exploits fluid phase endocytosis to supports its intracellular survival**

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Severe hypo-cholesterolemia and reduced lipoprotein are the hall marks of fatal visceral leishmaniasis (VL), caused by intracellular protozoan pathogen *Leishmania donovani* (LD). Lipids/lipoproteins act as a two-edge sword in host-pathogen interaction. While circulating-lipids may contribute in innate-immune defence of host, some pathogens can co-opt and catabolise host lipids/lipoproteins to support their own needs. Although, LDs are auxotrophic to cholesterol, cholesterol is absolute necessity for a productive LD-infection in host. It has been well reported that Intracellular LDs replicating within membrane-bound parasitophorous vacuoles (PV) can accumulate hue of lipid-droplets around PV. However, underlying mechanism how PV-bound LD can acquire, catabolise and utilize these host-lipids for their own benefit (if any) remains elusive. In this study we have tried to answer these intricate details about role of lipids in establishing LD-infection by using an experimental model of murine liver resident macrophages or Kupffer cells which offers the first line of protection against invading LD parasites. Using an array of immunofluorescence combined with label free Raman Spectroscopy and infected host cell transcriptomics we have shown that LD preferentially endorsed fluid-phase-receptor-independent endocytosis to accumulate low density lipoproteins during nascent PV formation. Our data also suggest although *de novo* lipogenesis shoots-off in LD-infected cells, LD-infection deactivates NPC-1, a key host-molecule responsible for supplying lipids to host-membrane. Our data also explains the long-standing observation linked with host-membrane fluidity leading to deactivated T-cell signalling against LD-infected host. Presently we are trying to characterize putative lipoprotein-receptor-like molecules in LD parasite by generating CRISPR-based genetically modified LD lines.

Thioredoxin and dihydrolipoic acid modulate the activity of caspase 9 in HepG2 cells

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Elevated concentrations of Nitric oxide and nitrosative stress may lead to the S-nitrosylation of proteins. The S-nitrosylation mediated redox biochemistry and regulation of proteins have been found to be a regulator of the signaling cascade leading to apoptosis. Previously, we reported the S-nitrosylation mediated regulations of Caspase 3 and 8 activities in HepG2 cells. Thioredoxin system was found to be a modulator to regenerate Caspase activities. Here in, we report that Thioredoxin (Trx) catalyzes the S-denitrosylation of Caspase 9 in HepG2 cells. Moreover, dihydrolipoic acid (a dithiol mimetic of Trx) has been found to regenerate the Caspase 9 activity in Trx reductase-deficient HepG2 cells via S-denitrosylation.

Rainfall has less of an impact on flooding in a Terai grassland than does the upstream river water level

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Manas National Park is a unique mosaic of grasslands, savanna-type vegetation, and woodlands that shelters numerous endangered species. Seasonal floods have been an integral part of the landscape. They are essential for maintaining the balance between grasslands and forests by regularly introducing disturbances and restrictions on the spread of woody vegetation. Characterizing floods for habitat management has always been difficult because the deluge makes affected areas inaccessible, and the cloud cover plagues the freely available satellite data. Synthetic aperture radar (SAR) data from sentinel 1A and 1B, which uses microwave radiation and is unaffected by clouds, has recently been made publicly available by the European Space Agency. We produced the monthly inundation maps by calculating Z-scores and then differentiated waterlogged and non-waterlogged pixels using supervised classification. When we modeled the yearly patterns against rainfall and upstream river water level in one-on-one regression, we found a high correlation with upstream river water level and a weak correlation with rainfall. This result implicates the importance of maintaining the water level of the upstream rivers in Terai and the prevention of artificial alterations of streams.

Mitochondrial citrate transporter SLC25a1 regulate Ulcerative Colitis pathology and it inhibition improves dextran sodium sulfate-induced colitis

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Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract, which includes Ulcerative colitis (UC) and Crohn's disease (CD). Mitochondrial dysfunction, specifically TCA cycle metabolite altered secretion is well documented in IBD without any well-defined cause. IBD is caused due to both genetic and environmental factors which causes alteration in the gut permeability leading to microbiome and their derived products getting access to the intestinal mucosal tissues. Microbial components like LPS, MDP causes TCA cycle break in the immune cells mainly in the macrophages and dendritic cells which leads to accumulation of multiple metabolites including citrate. Mitochondrial citrate subsequently comes out to the cytosol through the citrate transporter (CIC) encoded by SLC25a1 and also acts as an important source for acetyl CoA. In this study, we identified for the first time that SLC25a1 expression is drastically enhanced in the colon biopsy tissues from the ulcerative colitis patients when compared to the control group. Further, blocking the citrate transporter with a specific inhibitor, CTPI-2, led to reduced secretion of pro-inflammatory cytokines from the human primary macrophages. Mechanistically, mitochondrial citrate was found to modulate mitochondrial dynamics and immune function in the UC patients and human primary macrophages. We further validated the inhibitor in an experimental mice model of colitis and observed that disease pathology was ameliorated after CTPI-2 treatment. Overall, we elucidated a previously uncharacterized mode of action of citrate in UC pathogenesis and propose that SLC25a1 can be a promising target in IBD treatment.

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Development of synthetic biology tools for the locally isolated cyanobacteria

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Engineered cyanobacteria are attracting attention as hosts for the photosynthetic conversion of carbon dioxide to chemicals. *Synechococcus elongatus* PCC 11801, a fast-growing and stresstolerant cyanobacteria, has the potential to be developed as a chassis for metabolic engineering. To leverage it as a platform cell factory and to understand its intricate metabolic regulations, stress responses, and for designing a compatible synthetic biology toolbox, a global transcriptome analysis was performed using RNA-Seq under three distinct relative abiotic stress conditions of temperature, carbon and salt. Non-hierarchical clustering of these differentially expressed genes revealed co-regulation of genes and metabolic pathways across these conditions. While we observed significant down-regulation in the expression of bicarbonate transporters under HC and HS, sulfur relay and central carbon metabolism were upregulated under HC and HT. Significant differences in the transcription of ‘hypothetical proteins’ indicated their potential role in metabolic regulations. Overall, this global transcriptomic analysis was further utilized to determine chromosomal integration sites, and characterize the native promoters and ribosome binding sites for this cyanobacterium. Overall, 74 neutral sites were proposed, of which five sites have been experimentally validated for their neutrality, based on their effect on cell growth and photosynthetic efficiency. The promoter library of 48 promoters were built which exhibited a 2- 15-fold dynamic range of strength, and also discovered a switch promoter that would be appropriate to express toxic genes. A small subset of these promoters was also tested with a functional gene tyrosine ammonia lyase (TAL), whose expression was measured by the quantification of p-Coumaric acid in PCC 11801. Comparing the different promoters for the production of this Phenylpropanoids, the effect of growth condition was also investigated. Thus, high cell density growth was tested, which increased the production of p-Coumaric acid to 3- fold. Natural operons of PCC 11801 were mimicked and the intergenic sequences were characterized to build a library of 15 native RBS elements, which had enhanced the fluorescence of the following reporter gene in the range of 2-23fold. In conclusion, this study will expedite the metabolic engineering of this novel system that can be deployed as potential platform cell factory.

Mapping Pharmacological Network for Identification of Molecular Targets of Epigallocatechin-3-gallate against Cervical Cancer

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Epigallocatechin-3-gallate (EGCG), the primary active component of green tea, has a well-established role in cancer therapy and prevention. It has been revealed that EGCG suppresses the initiation, promotion, and progression of carcinogenesis, resulting in a chemo-preventative effect. However, the precise pharmacological target and mechanism of action of EGCG's anticancer activities remain unknown. This study has been designed to identify the major molecular pathways, cellular processes, and potential targets of EGCG in cervical cancer by using systematic network pharmacology and molecular docking approach. The network pharmacology analysis identifies 84 primary assumptive targets of EGCG against cervical cancer, of which 5 core targets have been defined, including AKT Serine/Threonine Kinase 1 (AKT1), tumor protein P53 (TP53), tumor necrosis factor (TNF), Heat shock protein HSP 90-alpha (HSP90AA1), and vascular endothelial growth factor A (VEGFA). Subsequently, the enriched pathways and targets were analyzed using pathway enrichment analyses from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO). Furthermore, bioinformatics analysis was also performed to identify and depict each biological process and signaling pathway influenced by EGCG treatment of cervical cancer. Molecular docking studies reveal the interaction pattern and affinity of EGCG with the predicted cancer targets. This study revealed the primary targets and potential mechanism of action of EGCG against cervical cancer, and these findings may serve as important references for future work on the drug development to treat cervical cancer.

Understanding active regulation of membrane mechanics and its heterogeneity

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At any particular niche, cells maintain their plasma membrane tension at a set-point to enable proper membrane functioning. However, recent work have shown that intracellular variations in tension are possible and can be regulated by the actin cytoskeleton and membrane cholesterol. To understand how cells actively regulate membrane mechanics and its heterogeneity, we study the role of active processes in general and the role of membrane-cytoskeleton linkages via Ezrin and membrane reservoirs – caveolae. We use Interference reflection microscopy (IRM) to map membrane fluctuations and fluctuation-tension of live cells¹. Coupling Total Interference Reflection Fluorescence (TIRF) allows us to measure, in parallel, distribution of the protein players². To test if membrane fluctuations are active, we first tested algorithms based on statistical inference developed³ to detect the presence of non-equilibrium fluctuations. Using ATP-depleted cells we show that membrane fluctuations have an active component that reduces significantly on ATP-depletion. To understand the role of Ezrin, we use Ezrin inhibitor as well as knockdown ezrin using siRNA. We find that Ezrin inhibition enhances fluctuation-tension. Additionally, TIRF-IRM reveals that ezrin can locally reduce as well as enhance fluctuations “actively”. I will also present how ezrin add to tensional heterogeneity or maintenance of local gradients of tension. Finally, to understand the role of caveolae, we first use MbCD, a cholesterol depletion agent and genetically modified cell lines - Cav1 KO, Cavin1 KO and cells with reduced EHD2. We show that caveolae and its most stable configuration is critical for controlling tension heterogeneity in cells.

Analysis of microsatellites in the *Orthopoxvirus*

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Monkeypox is a zoonotic disease caused by monkeypox virus (MPXV), which is an enveloped, double stranded DNA virus belonging to the *Orthopoxvirus* genus and the *Poxviridae* family. Present study is an attempt to extract and analyse the microsatellites from the genomes of 8 species of the genus *OPXV*. Microsatellites or SSR (simple sequence repeat) are usually present as 20-60 repeats of mono- to hexa-nucleotide motif. Distribution of microsatellites is non-random across coding and non-coding regions. The average size of genomes was 205 kb while the GC% was 33% for all but one. A total of 10,584 SSRs and 854 cSSRs were observed. POX2 with the largest genome of 224.499kb had maximum of 1493 SSRs and 121 cSSRs while POX7 with the smallest genome of 185.578kb had minimum incident SSRs and cSSRs at 1181 and 96 respectively. There was significant correlation between genome size and SSR incidence. Di-nucleotide repeats were the most prevalent (57.47%) followed by mono- at 33% and tri- at 8.6%. Mono-nucleotide SSRs were predominantly T (51%) and A (48.4%). A majority of 80.32% SSRs were in the coding region. Ankyrin/Ankyrin like protein and Kelch protein which are associated with host determination and divergence have the highest SSR density in almost all studied viruses. So, the microsatellite signature across the genomes of *Orthopoxvirus* is involved in evolution and other genome functions.

Regulation of membrane fluctuations and effective mechanics during de-adhesion by endocytosis

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Surface area regulation of the plasma membrane (PM) is central for cellular functioning and involves endocytosis. To understand the contribution of early steps of endocytosis, we study membrane height fluctuations and fluctuation-tension in de-adhering HeLa cells. In this work it is shown that de-adhesion initially increases temporal fluctuations, but the membrane gradually smoothens out. The estimated fluctuation-tension is lowered and then starts recovering with the membrane smoothing. That endocytosis is enhanced is clear because area fraction of Rab5-labelled early-endosomes and Rab4-labelled recycling-endosomes are first enhanced then saturate. Blocking pit-scission for dynamin-dependent endocytosis by Dynasore not only fails to stop the tension surge but allows a much higher tension to be reached. Our measurements suggest the tension rise is due to enhanced pit-formation and reduced recycling at lowered tension. This rise persists despite actin depolymerization or ATP depletion. However, for the rise to occur independent of ATP, cholesterol is essential. We finally show using confocal that ATP-independent cholesterol-dependent membrane invaginations help in tension regulation. Therefore, tension regulation starts with pit formation either actively or passively while recycling is important for containing the tension surge – together determining the steady-state tension.

Ezrin along endomembranes - as size and dynamics regulator

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Ezrin, a Ezrin, Radixin and Moesin (ERM) family protein, is involved in pinning the plasma membrane (PM) to the cytoskeletal F-actin. It also anchors specific membrane proteins to the cortical actin cytoskeleton to maintain cell shape. It is potentially activable by PKCs and LOK kinase. Despite being reported to have cytosolic distribution and not having any structural preference for PM, its role at endomembrane for shape/tension regulation remains poorly studied. Another impetus to our work was from the reports that actin helps in endomembrane biogenesis – especially tubulation. Herein we present a comprehensive study on ezrin localization beyond the cortex and its effect on endomembrane morphology and dynamics by colocalization and photobleaching experiments. We report here that PM-derived endomembranes (peripheral early-endosomes) contain Ezrin. The binding propensity decreased as the endosomes matured into recycling endosomes and in organelles located farther away from the cortex (ER, mitochondria). Presence of patchy Ezrin on tubulated Rab4 (18.89%) - rich recycling-endosomes suggest their role in membrane segregation. We also observed a high level of patchy colocalization of p-ezrin on lysosomes (colocalization % - 78.41%) and show that $\sim 1/3^{\text{rd}}$ of the pool use PIP2. We further show that although there is no bias in ezrin's localization to any particular size of lysosomes, once ezrin is inhibited, lysosomes are bigger and fewer. Following their movement and interaction with other lysosomes suggests that Ezrin regulates lysosome fusion. Together, the data shows Ezrin to be specifically enriched in lysosomes among all endomembrane and may affect tension/shape regulation during biogenesis.

P-126

Delineating the genome features for exploring novel Indian isolates of *Synechococcus elongatus* as biochassis for bioproduction

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Cyanobacteria are attractive biochassis for the sustainable conversion of CO₂ to a wide range of chemicals. However, from commercialization perspective, exploration and isolation of fast-growing and stress-tolerant strains become a prerequisite. Therefore, we present a pan-genome analysis of recently isolated *Synechococcus elongatus* strains (IITB3, IITB4, IITB5, IITB6, IITB7 and IITB8) from Powai Lake in Mumbai, India as a photoautotrophic host for metabolic engineering. The Indian isolates shared significant genetic and protein identity, leading to a sub-clade within the *S. elongatus* clade. Comparative genomics of this clade revealed intriguing features about these isolates, such as CRISPR-associated proteins, antibiotic resistance elements for a novel class of phosphonic antibiotics, and also led to the discovery of the gene sequence for an enzyme involved in asparagine biosynthesis, which was earlier unknown for *S. elongatus* strain. The strains presented a notable doubling time of 2.3-3 h under elevated light and CO₂ conditions, which is competitive with heterotrophic hosts. Although the strains had commendable identity at gene and protein levels, their specificity towards light and CO₂ was different. This was explicit from the difference in their doubling time. Further, for ease of genetic amenability, we optimized a transformation protocol through homologous recombination at the genomic neutral site, and the complete segregation and protein expression was confirmed using the eYFP reporter system. To demonstrate the strains as effective hosts for heterologous hydrocarbon synthesis, two-gene pathways for ethanol and mannitol production were introduced in them. In this study, among all the strains, IITB6 exhibited the highest titers for ethanol 60 mg/L and mannitol 450 mg/L. Conclusively, under current investigation, efforts have been made for pan-genome analysis of eight *Synechococcus elongatus* strains, followed by demonstrating their amenability through metabolic engineering for the production of ethanol and mannitol.

P-127

Investigating The Potential of Bacterial Microcompartment Technology in Stabilizing the Enzymes Under Industrial Operating Conditions

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Enzymes are vital biocatalysts for many chemical reactions and industrial processes. However, industrial processes are often employed in harsh conditions such as extreme pH, temperatures, use of organic solvents, and so forth, which drastically affect the stability of the enzymes. Encapsulation has become a popular strategy to stabilize the enzymes. Meantime, bacterial microcompartment (MCP) has been utilized to serve as a great encapsulating platform to optimize several metabolic reactions. MCPs are an ensemble of enzymatic core wrapped within semi-permeable protein shells. They optimize reaction rates by confining toxic or volatile intermediates. Given their attributes, MCPs offer great promise in designing subcellular nanobioreactor for the improved production of industrially important enzymes employing pathway encapsulation. In the current study, MCPs have been shown to maintain enzyme activity for extended periods against various physical challenges such as temperature, pH, and organic solvent. In all the said conditions, the encapsulated enzyme was shown to be more stable than its free counterpart. To establish the significance of an intact shell, the Pdu MCP was mechanically broken, and the enzyme activity was assayed. The broken MCP exhibited lower activity and faster decay in case of higher temperatures, indicating the importance of an intact shell in conferring stability and durability to the lumen enzyme. Bacterial MCP-mediated encapsulation can serve as an effective strategy to protect the enzymes being used under harsh conditions which can open up new tools for stabilizing the enzymes in many bioprocess industries.

P-128

Vitronectin as a key molecular determinant in altered adhesion and migration of human umbilical cord-derived MSCs under stress condition.

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Mesenchymal stem cell (MSC)-based therapy gets compromised as adverse microenvironmental conditions at the target site affect migration, engraftment, and viability of transplanted MSCs. To improve treatment efficacy, it is important to assess the impact of microenvironment and identify key molecular players affecting these characteristics of MSCs. We observed that under serum starvation stress, human umbilical cord-derived MSCs exhibited increase in cell spread area and adhesion, with reduction in cellular migration. The changes in these parameters were accompanied by formation of large number of super mature focal adhesions (FAs) and notable induction in vitronectin (VTN) expression. NF- κ B was found to be a positive regulator of VTN expression while ERK pathway regulated it negatively. Inhibition of these signalling pathways or VTN knockdown under serum starvation established that VTN mediated the changes in adhesion and migration pattern along with formation of mature FAs. Further analysis revealed that phosphorylation of myosin light chain (MLC) was induced under serum starvation and inhibiting MLC kinase led to reversal in the observed changes in adhesion, migration and FA formation. Overall, it was demonstrated that VTN-mediated induction in MLC phosphorylation led to an increase in FA formation and cellular adhesion while compromising migration in WJ-MSCs under serum starvation stress.

P-129

Epstein-Barr virus Nuclear Antigen 2 Regulated Carbonic Anhydrase 9 Expression is Essential for EBV Induced B-Cell Transformation and Survival of transformed B-lymphocytes

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Epstein-Barr virus (EBV) is a B-lymphotropic human gammaherpesvirus that persists indefinitely in latently infected B-cells for the life of the host. In both immunocompetent and immunocompromised EBV infection causes several B-cell lymphomas. *In vitro*, EBV transforms naïve B-lymphocytes into lymphoblastoid cell lines (LCLs), which can be used as a perfect surrogate model to study B-cell transformation process and subsequent B-cell lymphoma development. Whole genome transcriptome profiling reveals that EBV infection in primary B-cells elevates the expression of membrane associated Carbonic Anhydrase 9 (CA9). CAs are one of the major cellular components that regulate overall pH homeostasis to maintain cell proliferation under various cellular stresses including insufficient delivery of oxygen (hypoxia) and accumulation of acidic products of the glycolytic metabolism (acidosis). Our results demonstrate that EBV infection rapidly increases CA activity post 2 to 4 days of infection which further stabilise in the later phase of the infection, while blocking CA activity by CA9 specific inhibitor retards the EBV induced B-Cell transformation process and significantly decreases the viability of EBV transformed cells. We further demonstrate that through inhibiting CA activity and declining intracellular pH, CA9 inhibitor induces apoptosis in LCLs. ChIP-Seq re-analysis of EBV encoded all the latent proteins as well as several B-cell specific transcription factors followed by ChIP-qPCR validation, we demonstrate that EBNA2 complex with RBP-Jk bind to CA9 promoter region and might be responsible for transcriptional activation of CA9 expression. Further, using EBNA2 positive and deleted EBV infected Burkitt's lymphoma cell lines - Jiyoye and P3HR1, respectively, we confirm that presence of EBNA2 is essential for elevated expression of CA9 in EBV positive cells. Overall, our study not only provides fundamental additional clues for EBV induced B-cell transformation, but also offers potential therapeutic options against various EBV positive B-cell lymphomas.

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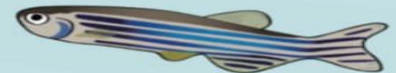
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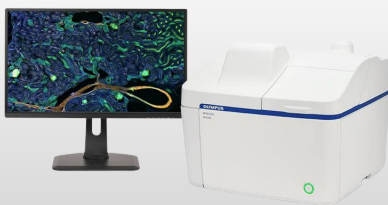
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