SIGNALS AND SENSORS

11,12-DECEMBER, 2019 @ NCBS

General help:

For internet access:

1. Connect to the wifi "NCBS-Hotspot".

2. In your internet browser, you will be directed to a registration page. Fill in your details.

3. A link for activation will be emailed to you. Click on that link to avail internet access for 24 hours.

Accommodation and Transport:

For participants staying in Mandara hostel, transportation has been arranged to and from NCBS. Please assemble at the Mandara main gate at ***7:45am*** on the 11th and a student volunteer will be there to guide you. The meeting takes place in the following address:

NCBS, GKVK Campus, Bellary Road, Bangalore-560065.

NCBS reception (080-2366-6001) can be contacted any time for details.

Food arrangements:

All lunches are provided at the conference venue.

For **out-station participants** breakfasts on the 11th and 12th and for dinner on the 11th, you will receive food coupons (when you collect your name tag) and those coupons can be used at the NCBS Main Canteen. Breakfast times are from 8:15am to 9:30am. Dinner is from 7:15pm to 8:30pm.

Posters:

Poster boards will be available in a designated area in the NCBS SLC building Colonnade. Please contact a student volunteer (Junaid: 6006249437) to find poster boards.

Schedule

Day 1: 11th December, 2019, **Venue:** Malgova (SLC, 2nd floor, NCBS)

8:00 - 9:30	**Registration/Breakfast**
9:30 - 9:45	Welcome
Session 1	**Diverse sensors capture diverse biology** **Chair: Prof. R. Sowdhamini, NCBS.**
9:45 - 10:25	Dr. Ankona Datta, TIFR, Mumbai *"Reversible chemical tools capture life in action"*
10:25 – 11:05	Dr. Akash Gulyani, InStem, Bangalore *"Natural and engineered light sensors: Cellular dynamics and novel photoswitching"*
11:05 - 11:30	**Break**
11:30 - 12:10	Dr. Ashwani Sharma, IISER, Tirupati *"Baby Spinach-based minimal modified sensor (BSMS) for miRNA sensing"*
12:10 - 12:50	Dr. Arati Ramesh, NCBS, Bangalore *"Iron sensing by RNAs: Lessons from the Sensei"*
13:00 - 14:00	**Lunch for all participants @ NCBS main canteen, 1st floor**
14:00 - 16:00	Posters @ SLC Colonnade
Session 2	**Sensing the very basic metabolites** **Chair: Prof. Sandhya S. Visweswariah, IISc**
16:00 - 16:40	Dr. Sunil Laxman, InStem, Bangalore *"Sensing changes in cellular metabolic states"*
16:40 - 17:20	Dr. Harinath Chakrapani, IISER, Pune *"Chemical Biology of Gaseous Signaling Molecules"*
17:20 – 18:00	Dr. Amit Singh, IISc, Bangalore *"Deploying Redox Biosensor to Discover New Drugs Against Tuberculosis"*

Day 2: 12th December, 2019, Venue: Malgova (SLC, 2nd floor, NCBS)

Session 3	**Sensors and sensibilities** **Chair: Dr.Dasaradhi Palakodeti, inStem**
9:45 - 10:25	Dr. Surajit Sinha, IACS, Kolkata *"Delivery of Antisense Reagents and Therapeutic Approach for the Treatment of Duchenne Muscular DystrophyDelivery of Antisense Reagents and Therapeutic Approach for the Treatment of Duchenne Muscular Dystrophy"*
10:25 - 11:05	Dr. Ruchi Anand, IIT, Mumbai *"Design of Selective Aromatic Biosensors for Pollution Monitoring"*
11:05 - 11:30	**Break**
11:30 - 12:10	Dr. Ishu Saraogi, IISER, Bhopal *"Structure based design strategies for the development of novel anti-amyloidogenic and antibacterial agents"*
12:10 - 12:50	Dr. Mahavir Singh, IISc, Bangalore *"Structure specific recognition of telomere DNA and TERRA RNA G-quadruplexes by hnRNPA1"*
13:00 - 14:00	**Lunch for all participants @ NCBS main canteen, 1st floor**
14:00 - 16:00	Panel discussion: ***"Biosensors: how, why, what next... and what not"***
Session 4	**Sharpening the cutting-edge of biosensors** **Chair: Prof. Sudhir Krishna, NCBS**
16:00 - 16:40	Dr. Sarit S Agasti, JNCASR, Bangalore *"Imaging life with programmable supramolecular interaction"*
16:40 - 17:20	Dr. Sandeep Ameta, Campus Fellow, Shashi Thuttupalli's lab, NCBS Bangalore *"Assessing RNA reaction networks using droplet microfluidics and sequencing"*
17:20 - 18:00	Dr. Gaurav Singh, Post-doctoral Fellow, Akash Gulyani's lab InStem, Bangalore *"Linking mitochondrial activity with membrane order using functional probes for high resolution quantitative imaging"*

Abstracts

Wednesday, December 11, 2019

Session 1:

Reversible Chemical Tools Capture Life in Action

Ankona Datta, Department of Chemical Sciences, Tata Institute of Fundamental Research, Mumbai, India

Abstract: Tracking the spatiotemporal dynamics of small molecules and ions which regulate key decision-making processes in living systems can provide critical insights into biological pathways in both normal and disease states. In this context, fluorescent chemical sensors that illuminate molecules of life have emerged as powerful tools. However, major challenges related to distinguishing similar molecules and inability to achieve reversible sensing have restricted live imaging to a chosen few bio-analytes. We have leveraged chemical recognition and secondary non-covalent interactions to develop reversible sensors that can capture a wide range of non-genetically encoded small molecules and ions in action. In this talk, I will highlight our cell-permeable reversible fluorescent sensors for imaging metal ions1 and tracking signaling lipids.2

Akash Gulyani, InStem, Bangalore : Abstract yet to be received

Baby Spinach-based minimal modified sensor (BSMS) for miRNA sensing

Ashwani Sharma, Department of Chemistry and Biology, IISER, Tirupati: Abstract yet to be received

Abstract: Nucleic acid sensing holds great potential in disease diagnosis, gene expression profiling and personalized therapeutic approaches. Several fluorescence based hybridization probes for the detection of nucleic acid has been reported in literature. Most of them, for instance, molecule beacons require chemical labelling followed by purification, and are also not cost effective. Light-up aptamer based sensors have recently shown great potential and has emerged as a promising platform for designing biosensors for small molecule as well as nucleic acid detection. Many of these sensors takes the advantage of a RNA mimic of green fluorescent protein often termed as spinach aptamer that has been shown to activate the fluorescence of otherwise non-fluorescent small- molecule DFHBI (3,5-difluoro-4-hydroxybenzylidene imidazolidinone)[1]. Using a miniature variant of spinach aptamer, termed as baby spinach, we have demonstrated a surprisingly simple, cost effective and label free baby spinach based minimal-modified sensor (BSMS) for fluorescent detection of miRNA. A single BSMS probe can detect either of DNA or RNA analytes including miRNA. The BSMS destabilizes in the absence of analyte by disrupting small molecule dye binding pocket of baby spinach leading to no fluorescence. However, sequence specific binding of target DNA/RNA analyte acts as a turn on switch leading to fluorescence enhancement by several folds. The sensitivity of the BSMS lies in the low nanomolar range for both DNA as well as RNA and also exhibits high specificity towards its target sequence. The sensor design is quite general in nature and can be easily modified to detect varied length nucleic acid sequence. Since the sensor consists of single, short and chemically unmodified ssRNA, it can be genetically encoded, thus holds potential for in vivo applications.

Iron sensing by RNAs: Lessons from the sensei

Arati Ramesh, National Center for Biological Sciences

Abstract: Despite the central role of iron in biology, our understanding of how cells sense iron is limited to only protein dependent mechanisms. Here, we report the discovery of iron-sensing riboswitches (named *Sensei*). These RNAs are present across bacterial phyla, and reside in the 5'untranslated regions or within coding regions of mRNAs encoding iron-related proteins. These riboswitches bind iron with high specificity and selectivity. Iron binding causes conformational changes in the riboswitch that alter the accessibility of conserved nucleotides ultimately enabling a genetic response. Sensei-iron interactions result in increased translation of the riboswitch-associated mRNA *in vivo,* thus positioning these riboswitches as true metalloregulators. Learning from natural *Sensei* RNAs, we engineer metal selectivity, successfully converting a nickel/cobalt sensing riboswitch to exclusively bind iron and a Sensei RNA to now exclusively recognize cobalt. Thus we define the sequence and structural space of iron-sensing RNAs and open avenues for the design of RNA-based biosensors.

Session 2:

Sensing changes in cellular metabolic state.

Sunil Laxman, InStem, Bangalore: Abstract yet to be received

Chemical Biology of Gaseous Signaling Molecules

Harinath Chakrapani, Associate professor, Department of Chemistry, IISER- Pune

Abstract: Maintenance of redox homeostasis is essential for cellular survival and growth. Small gaseous entities which are reactive biological species derived from oxygen, nitrogen as well as sulfur are generated and quenched during these processes. These small gaseous molecules mediate a number of cellular processes and signaling events. The major challenges in this field include reliable detection, controlled generation as well as inhibition of biosynthesis of these species. Our lab works on developing tools to study these complex biological processes in a systematic manner. Using fundamental and mechanistic organic chemistry as the basis, we develop small molecules that can fragment to produce the aforementioned species.

Deploying Redox Biosensor to Discover New Drugs Against Tuberculosis

Amit Singh, Department of Microbiology and Cell Biology, IISc, Banga

Abstract: The capacity of *Mycobacterium tuberculosis* (*Mtb*) to tolerate multiple antibiotics represents a major problem in tuberculosis (TB) management. Heterogeneity in *Mtb* populations is one of the factors that drives antibiotic tolerance during infection. However, the mechanisms underpinning this variation in bacterial populations remain poorly understood. Here, we show that phagosomal acidification alters redox physiology of *Mtb* to generate a subpopulation of replicating bacteria displaying drug tolerance during infection. RNA sequencing of this redox-altered population revealed the involvement of iron-sulfur (Fe-S) cluster biogenesis, hydrogen sulfide (H_2S) gas, and drug efflux pumps in antibiotic tolerance. The fraction of the pH and redox-dependent tolerant population significantly increases when *Mtb* infects macrophages with actively replicating human immunodeficiency virus type-1 (HIV-1), suggesting that it could contribute to high rates of TB therapy failure during HIV-TB co-infection. Pharmacological inhibition of phagosomal acidification by the antimalarial drug chloroquine (CQ) eradicated drug-tolerant *Mtb*, ameliorated lung pathology, and reduced post-chemotherapeutic relapse *in vivo*. The pharmacological profile of CQ (C_{max} and AUC_{last}) exhibited no significant drug-drug interaction when co-administered with first line anti-TB drugs in mice. Our data establish a

link between phagosomal pH, redox metabolism and drug tolerance in replicating *Mtb* and proposed repositioning of CQ to shorten TB therapy and achieve a relapse-free cure.

Thursday, December 12, 2019

Session 3:

Delivery of Antisense Reagents and Therapeutic Approach for the Treatment of Duchenne Muscular DystrophyDelivery of Antisense Reagents and Therapeutic Approach for the Treatment of Duchenne Muscular Dystrophy

Surajit Sinha, School of Applied and Interdisciplinary Sciences, Indian Association for the Cultivation of Science (to be deemed university), Jadavpur, Kolkata

Abstract: Antisense oligonucleotides (AON) are known to inhibit gene expression by blocking translation of mRNA which has brought a new hope in fighting against a variety of genetic disorder diseases. However, the major obstacle is their poor cellular uptake and their delivery is a long standing problem. Duchenne muscular dystrophy (DMD) is a genetic disorder disease, caused by mutations in the dystrophin gene and produced the functionally compromised dystrophin protein. AON-mediated exon skipping strategy is able to correct the mutations in DMD and restore truncated yet functional dystrophins. In 2016 FDA approved the first morpholino oligonucleotide (PMO)-based drug "Exondys 51" (Eteplirsen) for the treatment of DMD. The price of the drug is 300,000$ per patient per year.

Similarly, another 2'-O-Methyl thioRNA-based lead candidate 'Drisapersen' has been designed to skip exon 51 and produced functional dystrophins and completed Phase III trials but has been rejected by FDA largely on the basis of toxicity which limits dosing, and efficacy.

Initially these oligos show some therapeutic benefit, as they can enter diseased "leaky" muscle cells but do not readily enter healthy muscle cells because they require delivery vehicle. Although the transfection reagents are known for their delivery however the correlation between transfection efficiency and cytotoxicity becomes unsatisfactory because reagent capable of having high transfection efficiency often involves undesired cytotoxic effects.

To overcome the delivery problem, we developed a novel internally guanidinium linked nonpeptidic cellular transporter (IGT) composed of heterocyclic six-membered rings which internalizes efficiently into cells and ubiquitously distributed into zebrafish injected embryos. It efficiently transports antisense morpholinos in vitro and in vivo mice model and exhibits antisense effect. Similarly IGT conjugated thioRNA-based AON working well against exon skipping genes of DMD patient to express right dystrophin RNA. Toxicity and immunogenicity tests have been completed which encouraged us to go for clinical trial.

Design of Selective Aromatic Biosensors for Pollution Monitoring

Ruchi Anand, Department of Chemistry, Indian Institute of Technology Bombay, Mumbai

Abstract: Aromatic hydrocarbons like phenol, benzene and its derivatives constitute a class of highly toxic xenobiotics that pollute both river and groundwater due to pollution from tannery, dye and petrochemical industries. In particular due to lack of active functional groups it is difficult to develop selective biosensors for these classes of compounds. Here, we use a highly thermostable enzyme based in vitro bio-sensing scaffold to

develop a universal sensing platform for a battery of aromatics. The sensor system exploited here is a natural phenol sensor that constitutes both sensing as well as the readout domain on a single polypeptide chain. The enzyme system has been adapted to detect phenol down to 10 ppb. Further, the natural phenol sensor has been engineered via structure based targeted design to specifically detect bulkier phenols like xylenols as well as hydrocarbons such as benzene, toluene etc. It has been shown to be capable of direct detection of these aromatics from simulated waste water containing high concentrations of a milieu of pollutants. The setup has been further translated as a chip-based environmental diagnostic device. The sensor strip consists of protein coated on mesoporous silica and this design is competent towards detection of phenol with 95% accuracy in real-time environmental samples collected from local surroundings, making it a viable candidate for commercialization. Overall, this work describes a highly versatile biosensor unit that presents a promising potential for direct detection in a device format for on-site pollutant monitoring.

Structure based design strategies for the development of novel anti-amyloidogenic and antibacterial agents

Ishu Saraogi, Department of Biological Sciences, IISER, Bhopal

Our research is at the interface of chemistry and biology, and currently focuses on the use of structure based design strategies for the development of novel anti-amyloidogenic and antibacterial agents. In my talk, I will summarize our work in these two areas.

Amyloidosis is a well-known, but poorly understood phenomenon caused by the aggregation of proteins, often leading to pathological conditions. The aggregation of insulin, for example, poses significant challenges during the preparation of pharmaceutical insulin formulations commonly used to treat diabetic patients. We have identified a small molecule, which causes a dose dependent reduction in insulin fibril formation. Biophysical analyses and docking results suggested that the inhibitor likely bound to partially unfolded insulin intermediates. Further, molecule-treated insulin had lower cytotoxicity, and remained functionally active in regulating cell proliferation in cultured Drosophila wing epithelium. Thus, our inhibitor is a promising lead for regulating insulin fibrillogenesis.

Bacterial resistance to antibiotics poses an unprecedented challenge to global health. In search of novel antibacterial strategies capable of evading existing resistance mechanisms, we identified the bacterial signal recognition particle (SRP), an essential protein transport machinery, as a potential target. Functional SRP is composed of a protein (Ffh) and a 4.5S RNA component, so we envisioned that antisense peptide nucleic acid (PNA) molecules targeting 4.5S RNA might inhibit the RNA-Ffh interaction, thus compromising bacterial viability. Designed PNA molecules indeed bound specifically to 4.5S RNA, and inhibited the 4.5S RNA-Ffh interaction in a dose dependent manner, leading to inhibition of SRP mediated GTP hydrolysis. The most potent PNA molecule, when tagged with a cell penetrating peptide, was able to effectively inhibit E. coli cell growth. The PNA-mediated inhibition was relieved by overexpression of 4.5S RNA, suggesting that the PNA specifically blocks 4.5S RNA function. Our work validates SRP as an antibacterial target for the first time, and invites research into small molecule inhibitors of bacterial SRP as potential antibacterial agents.

Structure specific recognition of telomere DNA and TERRA RNA G-quadruplexes by hnRNPA1

Mahavir Singh Molecular Biophysics Unit, Indian Institute of Science, Bengaluru NMR Research Centre, Indian Institute of Science, Bengaluru

Abstract: hnRNPA1 is a member of heteronuclear ribonucleoprotein that has been shown to promote the telomere elongation. It is a modular protein with an N-terminal UP1 and a C-terminal region that harbors functional motifs such as RGG-box, a prion like domain, and a nuclear shuttling sequence. UP1 has been known to destabilize

telomeric DNA G-quadruplex structures and participate in telomere remodeling. However, the role of RGG-box in telomere DNA and telomere repeat containing RNA (TERRA) recognition and unfolding remained unexplored. Recently, we have shown that the RGG-box shows structure specific interaction with the telomere DNA and TERRA RNA G-quadruplexes and further it enhances the G-quadruplex unfolding activity of UP1. This work provides new insights into the recognition of higher order RNA/DNA G-quadruplex by the RGG-box.

Session 4:

Imaging life with programmable supramolecular interaction.

Sarit S Agasti, Faculty Fellow, New Chemistry Unit & Chemistry and Physics of Materials Unit, JNCASR, Bangalore

Abstract: Developing strategies to assemble molecular components within the complexities of cells and tissues is of great interest in biology. It drives advancements in various domains of fundamental and medical research, including protein modification, assay development, therapeutic targeting, and cell surface engineering. Importantly, such strategies play a crucial role in applications that require molecular tagging or labeling, such as imaging. Supramolecular non-covalent structural motifs are particularly attractive for this purpose as it allows molecular−level design approach to control properties of the systems in a manner that is life-like (i.e., dynamic behavior, environmental responsiveness, and adaptability). However, the application of synthetic recognition motifs for programming molecular assemblies in living systems remains a challenging task due to the chemical complexities of the living system and lack of selectivity in conventional non-covalent interactions. In my talk, I will describe our recent success of programming molecular assemblies in the living system based on a synthetic host-guest system featuring Cucurbit[7]uril (CB[7]). We demonstrated that highly selective and ultrastable host-guest interaction in CB[7] provides a non-covalent mechanism for assembling imaging agents in cells and tissues. Importantly, we have shown that CB[7]−ADA interaction fulfills the demands of specificity and stability that is required for bioorthogonal assembly in the living cell. We demonstrated this by labeling and imaging the distribution and dynamics of microtubule in HeLa cell. We used the dynamic nature of the supramolecular interaction to develop a new technique for super-resolution imaging with ~20 nm resolution. This technique, which we call SPIN (Supramolecular Probe-based Interaction mediated Nanoscopy), exploits repetitive and transient binding of the fluorescently labeled guest to complementary CB[7] host to obtain stochastic switching between fluorescence ON- and OFF-states. By connecting CB[7] guest to targeting ligands, we demonstrated that this autonomous blinking enables two-dimensional (2D) and 3D super-resolution imaging of biomolecules in cells. We expect that this simple and easy to implement strategies will be easily applicable to address various questions in a wide range of biological and materials research.

Assessing RNA reaction networks using droplet microfluidics and sequencing

Sandeep Ameta, Dr Shashi Thutupalli's lab, National Center for Biological Sciences

Abstract: Reaction networks composed of autocatalytic RNAs (*1, 2*) may have been involved in a rudimentary form of evolution on the Hadean Earth (*3*). However, Darwinian evolution in such reaction networks is not trivial as variation, differential reproduction, heredity, and selection are mediated by the relative proportions of network members rather than a single replicating entity (*4*). To experimentally demonstrate ingredients of Darwinian evolution in reaction networks, we developed an experimental strategy using droplet-based microfluidics and barcoded sequencing. Using this, we screened ~ 20,000 RNA reaction networks corresponding to more than

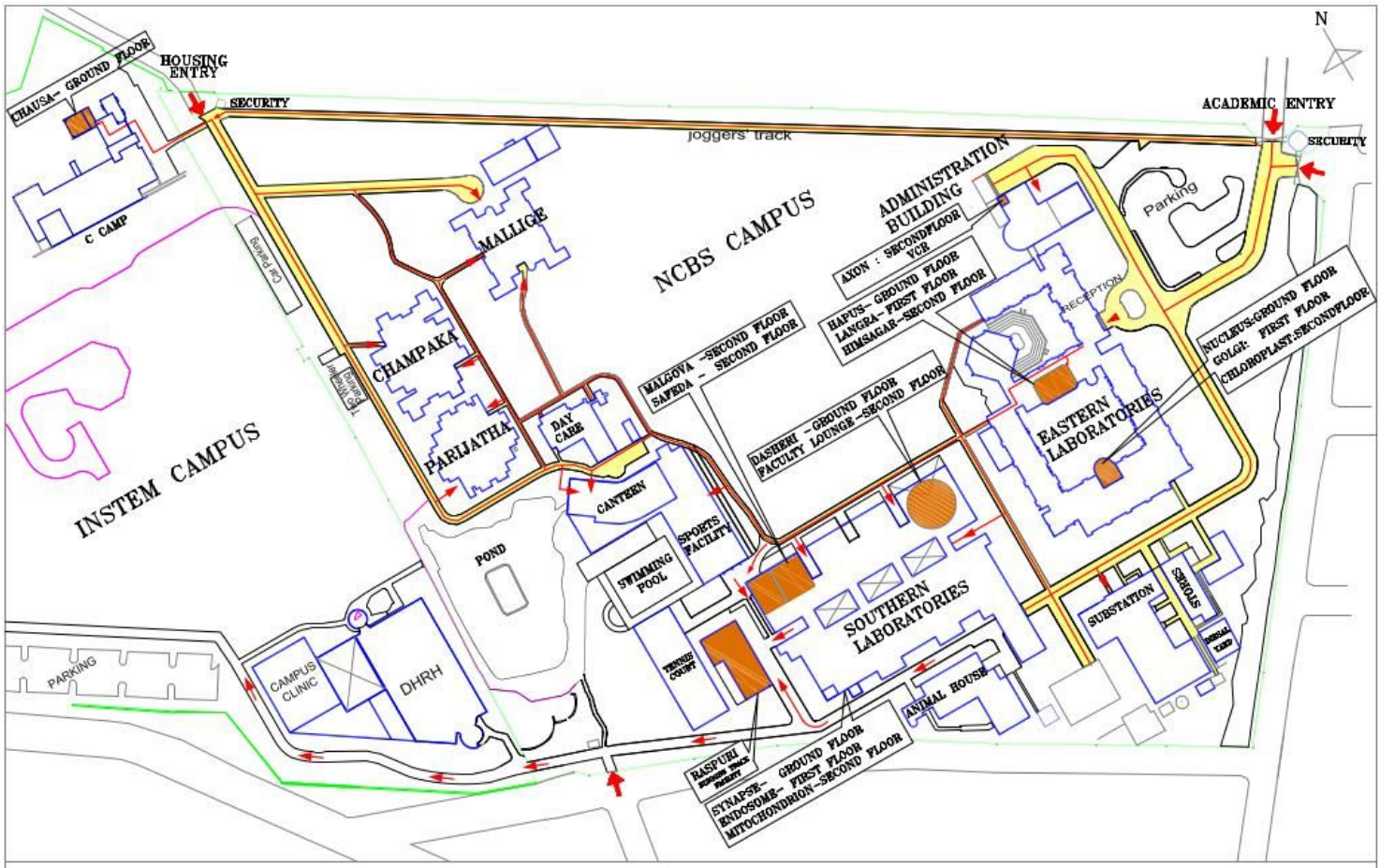
1,800 distinct networks of catalytic RNAs (*5*). In this meeting, I will talk about how to build a barcoded sequencing strategy using microfluidics to quantify different RNAs at high resolution. I will also show how we are using this technology for developing *in vitro* selection cycles (directed evolution) for isolating emerging RNA networks from random sequence pools.

Linking mitochondrial activity with membrane order using functional probes for high resolution quantitative imaging

Gaurav Singh, Dr. Akash Gulyani's Lab, InStem, Bangalore

There is a growing realization that architecture of mitochondrial inner membrane plays a critical role in tuning metabolic output and regulating key mitochondrial functions. The inner membrane is highly protein rich and structured into numerous tubular invaginations called cristae that act like bio-chemical reactors where cristae *shape* modulates organization of mitochondrial respiratory complexes into 'super-complexes'. However, much remains unknown about internal mitochondrial organization and function due to the absence of critical, high-resolution probes and tools. In this talk, I will discuss the development of novel mitochondrial probes, which can uniquely address questions on internal mitochondrial micro-environment, in particular membrane-order. In particular, I will discuss how fluorescence lifetime imaging (FLIM) using our probe allows unprecedented dynamic visualization of mitochondrial membrane order inside living cells and tissues. Further, we show that mitochondrial membrane order is tightly linked to mitochondrial bioenergetics, and find that mitochondrial membrane dynamically restructures to modulate or respond to changes in enzymatic activity of ETC complexes that control ATP production. Our preliminary results also uncover significant mitochondrial heterogeneity, in terms of membrane order, both within a cell as well across cells. What is the origin and functional significance of such microenvironment heterogeneity? Is it linked to cell-state and if so, can we predict cell status or cellular fate on the basis of degree of mitochondrial heterogeneity? I will also briefly discuss development of new class of high-resolution probes that enables us to visualize mitochondrial cristae in live cells using STED microscopy, allowing unprecedented insights into real time dynamics of mitochondrial microstructure.

Campus Map:



ROUTE MAP TO NCBS BUILDINGS AND LECTURE HALLS