



5<sup>TH</sup>  
**STUDENTS'**  
**SYMPOSIUM**  
**2024**

25<sup>th</sup> – 26<sup>th</sup> July

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**BRIC**

**National Centre for Cell Science**



## Events Sponsor's



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# About the Symposium

Of the Students, By the Students, For the Students:

A Students' Symposium is being organized to provide bachelor's, master's, PhD students and postdocs working in fields related to cell biology / biotechnology to present their research, to exchange ideas and experiences, and to learn from each other, as well as from experienced scientists.

It gives us great pleasure to announce the 2024 NCCS Student Symposium. This is a two-day event to provide a platform for students to showcase their work along with facilitating the exchange of ideas and experiences. Moreover, attendees will also have the unique opportunity to interact with invited guest speakers from renowned institutes of the country along with attending their insightful talks. Join us for an enriching experience at the forefront of academic discourse and innovation.

# 5<sup>th</sup> NCCS STUDENTS' SYMPOSIUM

## Scientific Programme

July 25<sup>th</sup> – July 26<sup>th</sup> 2024

25 JULY 2024

Venue: NCCS Auditorium (GARGI)

Time	Programme
8:00 AM – 8:50 AM	Registration
9:00 AM – 9:25 AM	Inauguration and Welcome address by <b>Director, NCCS, Pune</b>
9:25 AM – 10:05 AM	Plenary Talk 1 (online) <b>Dr Soumya Swaminathan</b> , MSSRF, Chennai <i>Title of the talk: TBA</i>
10:05 AM – 10:25 AM	Oral presentation Session I <b>SS I-1 Lizanne Oliveira</b> , NCCS, Pune <i>Novel Roles of Nucleoporins: Nup358 mediated regulation of ER-Mitochondrial contact sites</i>
10:25 AM – 11:05 AM	Tea break (Outdoor covered canteen area)
11:05 AM – 11:45 AM	Oral presentation Session II <b>SS II-1 Mahak Tiwari</b> , NCCS, Pune <i>CLCs regulate Wnt signaling and actin organization in mESCs</i>  <b>SS II-2 Kundan Kumar</b> , IISER, Pune <i>The Serine hydrolases of Drosophila melanogaster: integrative chemoproteomics and genetics</i>
11:45 AM – 1:00 PM	Poster presentation Session I
1:00 PM – 2:15 PM	Lunch break (Outdoor covered canteen area)
2:15 PM - 2:25 PM	Sponsor's talk (Invitrogen)

2:25 PM - 2:35 PM	<b>Sponsor's talk (DSS Imagetech)</b>
2:35 PM - 3:20 PM	Plenary Talk 2 <b>Dr Abhijit Majumder</b> , IIT Bombay <i>Prizes and Surprises of Interdisciplinary Treasure Hunt: A Personal Anecdote</i>
3:20 PM – 4:10 PM	Oral presentation Session III  <b>SS III-1 Vibhuti Prakash Mahajan, NCCS, Pune</b> <i>Utilizing plasmablasts of infected individuals to understand the B cell repertoire upon SARS-CoV-2 infection</i>  <b>SS III-2 Amruta Jadhav, NCCS, Pune</b> <i>Structural and functional characterization of a novel chimeric transcript RMND5A-ANAPC1</i>  <b>SS III-3 Pradipta Pal, NCCS, Pune</b> <i>Complement deficiency leads to enhanced NK cell activation and regulates tumor growth.</i>
4:10 PM – 5:10 PM	Plenary Talk 3 <b>Dr Vijay Kuchroo</b> , Harvard Medical School, Massachusetts <i>Role of Checkpoint molecules in regulating anti tumor immunity</i>
5:10 PM – 5:20 PM	Concluding remarks Day 1
5:20 PM onwards	Tea break (Outdoor covered canteen area)
<p style="text-align: center;"><b>26 JULY 2024</b>  <b>Venue: NCCS Auditorium (GARGI)</b></p>	
8:00 AM - 9:00 AM	Breakfast (Outdoor covered canteen area)
9:00 AM - 9:35 AM	Oral presentation Session IV  <b>SS IV-1 Jyotsna Singh, NCCS, Pune</b> <i>Nup93 subcomplex and its crosstalk within nuclear pore complex</i>  <b>SS IV-2 Bhagyashree Karmarkar, NCCS, Pune</b> <i>Exploring the human gut microbiome for prolyl peptidases in Celiac disease treatment</i>

9:35 AM - 10:30 AM	Plenary Talk 4 <b>Dr Girish Ratnaparkhi</b> , IISER, Pune <i>The making of a (remarkable) lineage: Primordial germ cells in the early embryo</i>
10:30 AM – 10:55 AM	Tea break (Foyer)
10:55 AM - 11:30 AM	Oral presentation Session V  <b>SS V-1 Asmit Moharana</b> , NISER, Bhubaneswar <i>Characterisation of the lipid environment and interplay of lipids in Gpr161, an orphan GPCR</i>  <b>SS V-2 Manikrao Thakare</b> , NCCS, Pune <i>Animal eat less when on more sugar diet?</i>
11:30 PM - 1:00 PM	<b>Poster presentation Session II</b>
1:00 PM – 2:15 PM	Lunch break (Outdoor covered canteen area)
2:15 PM – 3:00 PM	Plenary Talk 5 <b>Dr Gayathri Pananghat</b> , IISER, Pune <i>Understanding bacterial shape, motility and cell division in cell-wall less bacteria</i>
3:00 PM – 3:50 PM	Oral presentation Session VI  <b>SS VI-1 Praneeta Pradip Bhavsar</b> , NCCS, Pune <i>Proteomic and Phosphoproteomic Profiling of Doxorubicin Mediated Chemoresistance in Breast Cancer</i>  <b>SS VI-2 Gaurav Agarwal</b> , NCCS, Pune <i>Role of PDI in regulation of insulin biosynthesis</i>  <b>SS VI-3 Omika Wadhwa</b> , Max Planck Florida Institute for neuroscience <i>Sensorimotor integration and object tracking in fruit flies</i>
3:50 PM - 4:35 PM	Plenary Talk 6 <b>Dr Vatsala Thirumalai</b> , NCBS, Bangalore <i>Predictive coding in the cerebellum of larval zebrafish</i>

4:35 PM – 4:45 PM

**Sponsor's talk (HiMedia)**

4:45 PM – 5:00 PM

Concluding session and prize distribution ceremony

5:00 PM onwards

High Tea (Foyer)



# **INVITED GUEST SPEAKERS**

## **Prizes and Surprises of Interdisciplinary Treasure Hunt: A Personal Anecdote**

**Abhijit Majumder**

Professor, Department of Chemical Engineering, IIT Bombay

In the past decade of running my independent lab, I have frequently encountered a question from research scholars: "Should I transition to a more interdisciplinary research field?" In this talk, I aim to address this query by drawing examples from my own journey, acknowledging my mistakes, and lessons learned. The central focus of my research group revolves around mechanobiology and microfluidics, merging principles from chemical engineering, physics, material science, and biology. Through various projects—some cantered on science and the others on technology—I will discuss the inherent challenges in interdisciplinary research, discuss strategies to navigate these challenges, and underscore the associated rewards.

## **The making of a (remarkable) lineage: Primordial germ cells in the early embryo biology**

**Girish Ratnaparkhi**

Professor, Department of Biology, IISER Pune

Embryonic development in metazoans is controlled by maternal factors deposited in the egg during oogenesis. In today's talk, I will focus on the specification of primordial germ cells (PGCs), a very distinct cell lineage specified by the end of the maternal-zygotic transition (MZT), in the early embryo. These PGCs, at a later stage, in the mature adult define the stem cells that produce eggs (or sperm) for the next generation, and thus, PGC specification is essential for the continuity of the species.

Using the fruit fly *Drosophila* as an example, I will introduce the significant steps that define the PGCs on the posterior side of the fly embryo. These include the migration of nuclei (2n) to the posterior side, their entry into the germplasm, centrosomal alignment, and the gene regulatory networks (GRNs) that define the PGC identity.

I will highlight recent work from our (*Das et al.*, 2024, *eLife*) and the Lipschitz (*Siddiqui et al.*, 2024, *Sci. Adv.*) laboratories that suggest that the degradation of maternal factors at the end of the MZT plays a significant role in specifying and maintaining the unique PGC lineage. I will focus on the germ cell regulator Smaug (*Tolkein*, 1937), an RNA regulator that needs to be actively degraded in PGCs for proper development.

## **Understanding bacterial shape, motility and cell division in cell-wall less bacteria**

**Gayathri Pananghat**

Associate Professor, Department of biology, IISER Pune

*Spiroplasma* is a helical bacterium devoid of cell wall. It maintains its unique shape in the absence of a 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, while it depends on FtsZ-based mode for cell division.

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, cell division and shape determination in *Spiroplasma*.

## **Predictive coding in the cerebellum of larval zebrafish**

**Vatsala Thirumalai**

Senior Faculty, NCBS, Bangalore

When navigating the world around them, animals encounter a plethora of sensory stimuli, some of which could be stereotyped and/or repetitive. Being able to predict such repetitive or stereotyped stimuli enables animals to respond faster, ignore these stimuli or avoid them. We show that larval zebrafish only a few days old are capable of learning stimulus patterns and using them predictively to respond quickly. These actions are mediated by specific activity in Purkinje neurons of the cerebellum. I will discuss these results and what it means for the evolution of predictive processing.

# **STUDENT TALKS**

### **SS I-1 Lizanne Anasthasius Oliveira, National Centre for Cell Science, Pune**

**Title:** Novel Roles of Nucleoporins: Nup358 mediated regulation of ER-Mitochondrial contact sites

**Abstract:** Certain nucleoporins that localize to and function at the nuclear envelope, are also present as ER sub-domains in the cytoplasm, at structures known as Annulate Lamellae (AL). ER-Mitochondrial Contact Sites (ERMCS) are regions where the two organelles are tethered, for the regulation of their functions. Our lab has shown that AL resident nucleoporin – Nup358 localizes to ERMCS and regulates ERMCS integrity in response to growth factors via the mTORC2 pathway. Whether GSK3 $\beta$  – one of the mediators of mTORC2 pathway – is a player in this signaling cascade remains to be deciphered. Additionally, a shorter fragment of Nup358 – containing Ran binding domains – can also mediate this function. However, whether Nup358 regulates ERMCS integrity and functioning in a RanGTPase dependent manner remains unclear. Our studies attempt to understand these aspects of Nup358 mediated regulation of ERMCS. Moreover, given that both nucleocytoplasmic transport and ERMCS are impaired in neurodegenerative diseases, metabolic disorders, cancers, etc., it would also be interesting to understand if Nup358 at the AL may serve as a link to mediate crosstalk between nucleocytoplasmic transport pathways and ERMCS functions.

### **SS II-1 Mahak Tiwari, National Centre for Cell Science, Pune**

**Title:** CLCs regulate Wnt signaling and actin organization in mESCs

**Abstract:** The development of an organism from a single-celled embryo requires multiple precisely coordinated decisions. These decisions are heavily dependent on cellular processes such as endocytosis and intracellular trafficking. Clathrin light chains are part of Clathrin mediated endocytic pathway, which is involved in the internalization of plasma membrane receptors and macromolecules by forming a clathrin coat. The basic structural unit of the clathrin coat is the triskelion, which comprises of three molecules each of the clathrin heavy chain (CHC), and of the clathrin light chains (CLCs). In mammals, there are two clathrin light chains, CLCa and CLCb which have characteristic tissue specific expression. While CHC plays an important role in the maintenance of mouse embryonic stem cells' pluripotency (Narayana et al; 2019 and Mote et al, 2020), the precise role of clathrin light chains in the context of embryonic stem cells (ESCs) is unexplored. We used CRISPR-Cas9 genome editing to create single and double knock out mESC lines of the clathrin light chains (Clta and Cltb) in order to address gaps in the field. Depletion of CLCa resulted in reduced Wnt secretion which led to reduced output of Wnt signalling pathway.

Furthermore, cells lacking CLCa or both the CLCs showed altered actin organization, compared to WT cells. We also discovered a novel method whereby Wnt signaling and the actin cytoskeleton are interconnected through CLCa. Activation of the Wnt signaling pathway in CLCa knockout cells resulted in a rescue of aberrant actin structures. Our findings reveal new and unique functions for clathrin light chains in the context of mouse embryonic stem cells.

## **SS II-2 Kundan Kumar, Indian Institute of Science Education and Research, Pune**

**Title:** The Serine hydrolases of *Drosophila melanogaster*: integrative chemoproteomics and genetics

**Abstract:** The serine hydrolase (SH) superfamily is one of the largest functional enzyme classes in all life forms and consists of proteases, peptidases, lipases, and carboxylesterases as representative members. We utilize chemical proteomics techniques, activity-based protein profiling (ABPP), to globally interrogate the functions of SHs members in various native, yet complex biological settings. We initially report a bioinformatics analysis toward the identification and categorization of nonredundant SHs in *Drosophila melanogaster*. Following this in silico analysis, leveraging discovery chemo-proteomics, we identify and globally map the full complement of SH activities during various developmental stages and in different adult tissues of *Drosophila*.

Next, we aim to characterize novel SH in *Drosophila*'s systemic wound response (SWR). We identify novel SHs that are enzymatically active during SWR. Mutants of Toll/NFkB pathway reveal novel immune-responsive SHs not previously implicated in these signal transduction pathways. We identify predicted lipase CG17192, as the target for annotating its immune response function. We showed that CG17192 is secretory enzyme and hydrolyzes the Phosphatidylinositol (PI) and phosphatidylserine (PS) as substrate. The CG17192 knockdown results in increase PI and PS in the adult gut. The CG17192 influences DAG signalling upon gut infection. CG17192 is an immune response SH, regulated by NFkB signalling. CG17192 is secretory phospholipase C and regulates DAG, a signalling molecule during the immune response.

## **SS III-1 Vibhuti Prakash Mahajan, National Centre for Cell Science, Pune**

**Title:** Utilizing plasmablasts of infected individuals to understand the B cell repertoire upon SARS-CoV-2 infection

**Abstract:** B cells recognize pathogens and differentiate into memory cells and antibody-secreting plasma cells. Plasmablasts are precursors of plasma cells that secrete highly specific antibodies. In addition, upon secondary immune response memory B cells also convert into plasmablasts initially before terminally differentiating into plasma cells.

Plasmablasts express abundant intracellular immunoglobulin (Ig) RNA and hence can be utilized for the efficient amplification of the Ig gene. Plasmablasts isolated one week after the infection predominantly represent the antigen specific repertoire. We are looking at the B cell receptor (BCR) repertoire that is amplified in response to the SARS CoV-2 infection. We are interested in studying the pathogen specific clonal lineages selected in COVID-19 and unique sequence signatures across individuals. Here we have isolated plasmablasts from infected COVID-19 patients at clonal density. Heavy and light chains of the Ig chain were PCR amplified and cloned into respective vectors. Both heavy and light chains were sequenced to identify the clonal proliferation, BCR repertoire, and heavy and light chain pairing. Moreover, we also expressed paired heavy and light chains to expressed recombinant antibodies in mammalian cells. This allowed us to screen the antibodies against the most immunodominant antigens of the SARS-CoV-2 i.e., nucleocapsid and receptor binding domain (RBD) of the spike protein. Purified antibodies were also assessed for RBD affinity by Surface Plasmon Resonance (SPR). We have used a similar approach to study the memory BCR repertoire and comparison between them shows that the plasmablasts derived repertoire is more diverse. Heavy and light chain sequences obtained from this study are unique and have not been reported elsewhere. This study will help us in understanding the diversity of the plasmablast associated BCR repertoire upon SARS-CoV-2 infection.

### **SS III-2 Amruta Jadhav, National Centre for Cell Science, Pune**

**Title:** Structural and functional characterization of a novel chimeric transcript RMND5A-ANAPC1

**Abstract:** Chimeric transcripts result from the fusion of two transcripts of adjacent or distant genes. Recent studies have provided mounting evidence that chimeric transcripts may also play a functional role in normal physiology and disease. 4437 Chimeric transcripts (CTs) were identified previously in the lab by analyzing RNA-sequencing data of 161 ovarian tumor samples from TCGA. Interestingly, 15 of these candidates had noteworthy matches to known pseudogenes. These chimeric transcripts were further validated by PCR followed by Sanger sequencing. In our study, RMND5A-ANAPC1 was chosen for additional research. The longest read for RMND5A-ANAPC1 matches the reported pseudogene of ANAPC1 with 100% sequence similarity (ANAPC1P2). This is thought to be the genomic level result of a fusion between the two genes, RMND5A (intron2-3) and ANAPC1 (intron 24-25). RMND5A-ANAPC1 was discovered to be expressed in both cancer cell lines and normal cell lines of humans after being screened across numerous distinct cell lines. It was not found in other species. It is also upregulated in response to gamma irradiation stress response. We suspect it might have a role in the response to DNA damage and repair.

### **SS III-3 Pradipta Pal, National Centre for Cell Science, Pune**

**Title:** Complement deficiency leads to enhanced NK cell activation and regulates tumor growth.

**Abstract:** Natural killer (NK) cells play a crucial role in regulating tumor growth, while the complement system is known for its protective function against infections and maintaining cellular balance. Numerous studies have demonstrated the role of the complement system in enhancing tumor growth and promoting metastasis. However, its specific influence on the anti-tumor activity of NK cells remains unclear.

**Methods:** We injected mouse melanoma cells (B16F10) subcutaneously into C57BL/6 wild-type, complement-deficient (C3<sup>-/-</sup>, C5aR1<sup>-/-</sup>, or Factor B<sup>-/-</sup>) mice and monitored tumor growth. Flow cytometry was used to analyze immune cell populations within tumors and secondary lymphoid tissues.

**Results:** Transplanting B16F10 cells into C3<sup>-/-</sup> mice significantly reduced tumor growth and metastasis compared to wild-type mice. Flow cytometric analysis revealed increased infiltration of NK cells secreting cytotoxic proteins Granzyme B and IL-10 into the tumor microenvironment in C3<sup>-/-</sup> mice. Blocking C3aR or C5aR1 receptors enhanced NK cell infiltration into tumors. Under normal conditions, NK cells in the lymph nodes of wild-type mice secreted higher levels of IL-10. Moreover, splenic NK cells exhibited increased cytotoxicity in the absence of complement. The liver, a major source of systemic complement, showed NK cells in C3<sup>-/-</sup> mice with significantly fewer inhibitory NKG2A receptors, resulting in a lower ratio of activating (NKG2D) to inhibitory receptors per NK cells compared to wild-type NK cells. As anticipated, NK cells isolated from tumors in C3<sup>-/-</sup> mice demonstrated robust cytotoxic activity compared to those in C3<sup>+/+</sup> mice with tumors. Depleting NK cells in C3<sup>-/-</sup> mice using anti-NK1.1 monoclonal antibodies prevented the reduction in tumor growth significantly more effectively than using isotype control IgG antibodies.

**Conclusion:** Our findings suggest that deficiency in complement C3 alters NK cells' effector and cytotoxic functions, thereby enhancing anti-tumor immunity and regulating tumor growth. This highlights the potential of disrupting the complement system as a novel therapeutic approach for managing tumor growth.

### **SS IV-1 Jyotsna Singh, National Centre for Cell Science, Pune**

**Title:** Nup93 subcomplex and its crosstalk within nuclear pore complex.

**Abstract:** Nuclear Pore Complexes are megadalton assemblies made up of 34 different proteins that forms large conduits for cargo transport between cytoplasm and nucleus. During transport, the proteins of the NPC undergo numerous rearrangements that allow the NPC to transition from a state of constriction to dilation. To understand this



mechanism of transition, we aimed at studying the crosstalk between two critical proteins of NPC, Nup93 and Nup155 that reside in the inner ring of NPC. Nup 93 is a very versatile linker protein that also interacts with the central transport channel whereas Nup155 has the membrane binding domain that tethers to the nuclear envelope. To delineate the role of these nucleoporins in NPC dynamics, we mapped the interaction interfaces between Nup93-Nup155 and reconstituted the complexes in-vitro. Interestingly, we observed that there are multiple interaction sites present between these proteins that provide scope for vigorous rearrangement of proteins within NPC and help achieve the plasticity required for active transport of cargoes. Furthermore, CryoEM SPA study of purified Nup155-Nup93 complex is ongoing that will also shed light on the conformational dynamics of these proteins.

#### **SS IV-2 Bhagyashree Karmarkar, National Centre for Cell Science, Pune**

**Title:** Exploring the human gut microbiome for prolyl peptidases in Celiac disease treatment

**Abstract:** The incidence of Celiac disease (CeD) in India has shown a notable rise in recent years. While the presence of at least one copy of HLA-DQ2/DQ8 predisposes individuals to CeD, not all carriers develop the disease. This suggests that variations in gut microbiota composition between patients and healthy individuals likely contribute to CeD pathogenesis.

Gut microbes in individuals without CeD may individually or collectively degrade gluten, the environmental trigger for CeD, to a non-immunogenic state. Metagenomic analysis of these challenging-to-culture gut microbes can unveil enzymes potentially capable of breaking down gluten immunogenic epitopes.

My research focuses on identifying such enzymes with the capacity to degrade gluten, employing multiple methodologies to assess their efficacy in this regard. By elucidating the enzymatic capabilities involved, we aim to add to therapeutic strategies utilizing enzymes that target gluten degradation in CeD management.

#### **SS V-1 Asmit Moharana, National Institute Science Education and Research, Bhubaneswar**

**Title:** Characterisation of the lipid environment and interplay of lipids in Gpr161, an orphan GPCR

**Abstract:** The orphan G-protein coupled receptor, Gpr161 is a negative regulator of the Sonic Hedgehog signaling pathway which is a crucial for regulation of developmental processes such as growth and patterning of multicellular embryos (Mukhopadhyay, et al. 2013). Recent Cryo-EM studies have implicated cholesterol and a unique loop dynamics, particularly in the second extracellular loop (ECL2) as being important for its constitutive

activity (Hoppe, et al. 2024). However, the precise molecular mechanism underlying this process remains unclear. In our study we address these questions using coarse-grain molecular dynamics simulations. We have considered the structural model of the Mouse Gpr161 embedded in a model ciliary membrane and have performed a total of 90 $\mu$ s of coarse-grain simulations. We observed high cholesterol density at the previously reported cholesterol binding site at loop seven and six. In addition, we observed the localization of Diarachidoyl (DA) lipids at helices one and seven. These are a class of reactive saturated lipids and functions as secondary messengers in signaling pathways. Further, we have investigated the localisation of other lipids such as cholesterol and PIPs, and explored the unique loop dynamics of the ECL2. Overall, our work will enhance our understanding of Gpr161 functional dynamics and potentially aid in the development of therapeutic strategies for treating cancer and various congenital disorders linked to Gpr161.

### **SS V-2 Manikrao R Thakare, National Centre for Cell Science**

**Title:** Animal eat less when on a more sugar diet?

**Abstract:** In *Drosophila*, direct measurement of food consumption is challenging as the animal is small in size and the amount of food consumed is minute. We have developed a sensitive feeding assay, DIETS (direct intake estimation and longitudinal tracking of solid food consumption). In DIETS, food consumed by a group of flies, from a small cup is directly weighed over hours to days. The dietary choice is measured in the assay by weighing food intake from two cups with distinct diets. Using the DIETS assay we are studying the neuronal regulation of high-sugar diet (HSD) feeding in flies. Our longitudinal HSD feeding data shows that flies show persistently reduced feeding on sucrose-added diets, over days. Our ongoing work suggests that the nutrient value of sugar is necessary for this food-intake suppressing effect. Currently, we are investigating the neural basis of this phenomenon.

### **SS VI-1 Praneeta Pradip Bhavsar, National Centre for Cell Science**

**Title:** Proteomic and Phosphoproteomic Profiling of Doxorubicin Mediated Chemoresistance in Breast Cancer

**Abstract:** Background: Acquired drug resistance during chemotherapy leads to tumor recurrence in breast cancer patients. Identifying chemoresistant specific proteins and their aberrant phosphorylations is indeed important in determining the chemotherapy regimen for patients.

Methods: To identify proteins involved in chemoresistance, a doxorubicin-resistant breast cancer cell line was established and subjected to Label-free quantitative proteomics and phosphoproteomics using an Orbitrap Fusion mass-spectrometer coupled to an EASY-nLC system.

**Results:** The majority of the identified proteins with Fold Change  $\geq 2$  were TRAF2, LACTB, FN1, FDXR, and ABCC1 implicated in various cellular processes, particularly in cell proliferation and drug efflux mechanisms. TRAF2 is involved in various cancer-relevant cellular processes, however, the functional role of TRAF2 in resistance to chemotherapy has not been investigated so far. We observed that TRAF2 deficient cells enhanced sensitivity towards doxorubicin with decreased cell proliferation. Using phosphoproteomic profiling, we identified TNIK (TRAF2 interactor) phosphorylated at the S680 was found unique to the resistant cells with localization probability  $> 90\%$ .

**Conclusions:** Collectively, our proteomics and phosphoproteomic study identified novel protein targets TRAF2 and its interactor TNIK that would provide valuable insights into the mechanisms underlying drug resistance in breast cancer.

#### **SS VI-2 Gaurav Agarwal, National Centre for Cell Science, Pune**

**Title:** Role of PDI in regulation of insulin biosynthesis

**Abstract:** Protein Disulfide Isomerase (PDI) was identified to directly interact with insulin mRNA and regulate its rate of translation in the pancreatic  $\beta$ -cells. PDI lacks any RNA binding motifs and is known to be localized in the ER, where the mRNAs (localized to the cytoplasm) are inaccessible. We are interested in identifying the cause of this interaction and to delineate the mechanism behind the translational regulation governed by this protein.

#### **SS VI-3 Omika Wadhwa, Max Planck Florida Institute for neuroscience**

**Title:** Sensorimotor integration and object tracking in fruit flies

**Abstract:** Flies perform a wide variety of behaviors in nature that require complex sensorimotor transformations. In this study, an attempt has been made to elucidate the neural pathways involved in sensorimotor transformations during courtship behaviors. I present two parallel pathways for angular and forward control of velocity during courtship, in which a visual cue (female fly) must be recognized, and the velocity of the male fly must instantaneously change accordingly.

# **POSTER PRESENTATION**

### **PS I-1 Surabhi Sharma, National Centre for Cell Science, Pune**

**Title:** Understanding the role of Clathrin light chains in regulating mitochondrial function during early development.

**Abstract:** Clathrin mediated endocytosis is one of the key endocytic pathways, and is known to regulate cell fate decisions during early development. The basic unit of clathrin coat is a triskelion structure made up of three heavy and three light chains. Clathrin heavy chain is known to maintain pluripotency in embryonic stem cells, while the role of Clathrin light chains in development is underexplored. Previously our lab has made and characterized Clathrin light chain knockout mouse embryonic stem cells (mESCs) generated using CRISPR-Cas9 technology, which show an altered actin phenotype and expression of specific differentiation markers when compared to their wild type counterparts (ongoing study). During differentiation, pluripotent stem cells undergo a drastic change in mitochondrial morphology, transitioning from small and fragmented to elongated, tubular networks, which is accompanied by a significant increase in the number of mitochondria in mouse and human embryonic stem cells. These changes in mitochondrial shape and number upon differentiation are also accompanied by an increase in oxidative phosphorylation-based metabolism. We therefore characterized whether mitochondrial function was altered in these knockout mESCs. We observed changes in the basal respiration rates and mitochondrial cristae in mESCs upon knocking out specific clathrin light chains. Further, we show that knocking out both Clathrin light chains A and B in mouse embryonic stem cells (mESCs) leads to a change in the overall shape of the mitochondria from tubular to donut, which is also observed upon pyruvate deprivation in these cells. We also observed an increase in the Mitotracker staining intensity and percent empty space in these knockouts. Our results suggest a possible role of Clathrin light chains in regulating mitochondrial function during early development.

### **PS I-3 Bheem Singh Bhandari, National Centre for Cell Science, Pune**

**Title:** Elucidating the structure and function of mammalian Nup88 Complex of Nuclear pore Complex

**Abstract:** Nuclear pore complexes are megadalton assemblies residing in the nuclear envelope of an eukaryotic cell. It directs the import and export of molecules between the cytoplasm and nucleus, which are highly specific, dynamic, and regulated. There are 32 different proteins (nucleoporins) that build this assembly together. The NPC is modular and can be divided into many subcomplexes. One such subcomplex is the Nup88 complex (Nup214•Nup88•Nup62), situated at the cytoplasmic side of the NPC, and their proteins are involved in mRNP export. Although its interactors are known, the domain-wise exact role of their proteins in mammalian NPC is still unknown.

In the absence of a high-resolution structure of these proteins and their complexes, it becomes difficult to delineate and understand the mechanism of mRNP transport by the NPC. In this study, we aim to investigate the critical interacting domains of Nup214 and Nup88. Further, we want to reconstitute the structural domains of Nup214•Nup88•Nup62 with Nup93 (1–150) to obtain high-resolution structures using Cryo-EM. This study will help in understanding the role of the mammalian Nup88 complex and the underlying mechanism by which it drives so many interactions within NPC.

**PS I-4 Kartik Mandal, National Centre for Cell Science, Pune**

**Title:** Deciphering the regulatory mechanisms of SecPD-L1 and its implications in immune checkpoint inhibition

Cancer is the uncontrolled cell proliferation caused by dynamic genome alterations and post-genome events resulting in tumours. Cancer has some common hallmarks, one of the hallmarks is immune evasion. The interaction of PD-L1/PD-1 under physiological conditions maintains peripheral tolerance, preventing T cell hyperactivation and autoimmune disorders. Tumours hijack this inhibitory signal by upregulating PD-L1 expression to evade antitumor immunity. The role of programmed cell death ligand 1 (PD-L1) and immune checkpoint inhibition has garnered significant attention in cancer immunotherapy. Therapeutic antibodies blocking the PD-L1/PD-1 pathway demonstrate notable antitumor efficacy in 15–25% of cancer patients by activating T cell functions. However, most patients either do not respond or develop resistance after an initial short-term response to PD-L1/PD-1 blockade.

We observed that, in addition to being upregulated on the cell surface, PD-L1 is also secreted, and potentially may contribute to the anti-PD-L1 immunotherapy resistance. Our study investigates the molecular pathways behind the secretion of PD-L1 from cancer cells, with a focus on the potential impact of this secretory process on T cell inactivation. This poster presentation aims to elucidate the regulatory mechanisms governing PD-L1 secretion. By unravelling the mechanism of secretory PD-L1 regulation, we strive to uncover novel insights into the modulation of immune checkpoint responses.

**PS I-5 Ritika Gupta, National Centre for Cell Science, Pune**

**Title:** Phenotypic plasticity and molecular heterogeneity in response to tumor niche change is depicted by ID8 syngeneic mouse model

**Abstract:** Research titled "ID8 cells switch phenotypes when presented with a different tissue niche as a response to microenvironmental cues and phenotypic plasticity leading to tumor heterogeneity in HGSC" evaluates tumor heterogeneity in HGSC due to phenotypic plasticity and 'class switching' in immunocompetent ID8 syngeneic mouse

model. ID8 mouse model was stratified throughout disease progression based on histopathology, transcriptomics, proteomics and immune profiles. The study also highlights differences in TME of ascites (secondary tumor) temporally (tap1, tap2, tap3) as the disease progresses for the first time. Earlier efforts have achieved molecular stratification of HGSC into different subtypes that emphasize phenotypic diversity and plasticity (epithelial, mesenchymal, mixed), and their functional/behavioral associations (differentiated, immunoreactive, proliferative). The future of targeted therapies in this disease will rely on a deeper understanding of the underlying molecular differences between these subtypes, validated in a frequently employed immunocompetent ID8 syngeneic mouse model.

#### **PS I-6 Vaishnav Wagh, Intignus Biotech Pvt Ltd, Pune**

**Title:** Antiretroviral Treatment-Induced Galectin-9 Might Impact HIV Viremia in Addition to Contributing to Inflammaging

**Abstract:** Galectin-9 plays a crucial role in both HIV reactivation and inflammaging-related non-AIDS events, prompting a study into its implications for HIV-infected individuals. Plasma galectin-9 levels were analyzed in 152 viremic and 395 aviremic individuals undergoing first-line ART, correlating positively with HIV-1 viral load ( $r = 0.507$ ,  $p < 0.0001$ ) and ART duration ( $r = 0.308$ ,  $p = 0.002$ ), and negatively with CD4 count ( $r = -0.186$ ,  $p < 0.0001$ ). The galectin-9/CD4 count ratio demonstrated strong discriminatory power (AUC = 0.906) with 90.13% sensitivity and 70.05% specificity at a cutoff of 14.47 for detecting viremic status.

Additionally, galectin-9 levels showed correlations with markers of inflammation (cystatin C and IL-18) and cardiovascular health (systolic blood pressure). Individuals on long-term ART exhibited lower galectin-9-induced HIV reactivation compared to those on short-term therapy. These findings suggest galectin-9 as a potential cost-effective biomarker for monitoring HIV viremia. Urgent strategies are recommended to mitigate galectin-9's impact on both HIV replication and the development of non-AIDS-related complications. This research underscores the importance of targeted interventions to optimize clinical outcomes in HIV-infected populations.

#### **PS I-7 Rumpa Mahata, National Centre for Cell Science, Pune**

**Title:** Elucidating the role of lysine-33 linked ubiquitinated protein in cell cycle progression

**Abstract:** Cellular function of proteins are majorly controlled by several post translational modifications through covalent attachment of a functional groups including a sugar moiety, acetyl moiety, phosphate or a small protein like ubiquitin and SUMO. Among these post-translational modifications, ubiquitination plays a central role in cell cycle proliferation, stress response, DNA damage repair, signal transduction through

controlling cellular localization of proteins, protein–protein interaction, stability of proteins. One interesting question would be how the attachment of this small 76 amino acid ubiquitin drives these diverse cellular functions. Here, the concept of ubiquitin linkage comes. Unlike other post-translational modifications, proteins may undergo either mono-ubiquitination or multi-monoubiquitination or polyubiquitination. During polyubiquitination of proteins, ubiquitin-ubiquitin covalent conjugation happens through utilizing the one of the 7 lysine residues of preceding ubiquitin molecule with the C-terminal carboxylic group of next ubiquitin molecule. The cellular fate of a protein is decided by the involvement of specific lysine residue in ubiquitin-ubiquitin linkages. Till date the function of lysine 11 (K11), lysine 48 (K48), lysine 63 (K63) linked ubiquitinated proteins are well established, but the functions of others ubiquitin linkages are poorly understood. In my study, through proteomics approaches we have identified K33-linked polyubiquitination cellular proteins and  $\beta$ -catenin was one of them. The oncogenic function of  $\beta$ -catenin has been widely reported in cancers. However, the role of  $\beta$ -catenin in controlling the cell cycle is still unclear. We observed that APC/CCDH1 ubiquitin ligase (a key player in cell cycle) selectively promotes K33 related ubiquitination of  $\beta$ -catenin in late mitosis and the G1 phase of the cell cycle. Additionally, we have observed that APC/CCDH1 supports the physiological stability of  $\beta$ -catenin. What K33 linkage specific ubiquitination controlling  $\beta$ -catenin function at the late mitosis-G1 phase of the cell cycle is now the subject of discussion.

#### **PS I-8 Harshada Pardeshi, National Centre for Cell Science, Pune**

**Title:** Automated, open-source and one-step command-line pipeline for bacterial whole genome sequencing data assembly and annotation

**Abstract:** Next-generation sequencing (NGS) has developed as an effective technique for decoding millions of DNA fragments. Its high throughput capability and cost-effectiveness have impacted genomic research by providing a better understanding of genome structure, function and dynamics. Advances in NGS have made it feasible to use bacterial whole genome sequencing (WGS) to identify species, strains and genotypes to study genetic mutations, variants, phylogenetic relatedness, disease outbreaks, drug susceptibility, etc.

WGS creates millions of reads, which must be assembled into high-quality draft genomes which can then be annotated and studied for functional properties. Analyzing this huge amount of data requires advanced bioinformatic and computational skills. The prerequisites of advanced computing abilities remain a bottleneck in using these high throughput sequencing techniques which limits its use.

Although the current tools and pipelines are widely available, their use is limited by various factors like complicated installation, size limits, ceased support and so on. Thus, there is



a need to develop an automated pipeline that can assemble and evaluate the sequence reads reducing manual labor and overcome current pipelines' limitations.

Here, we present an automated, open-source and user-friendly command-line pipeline for assembling and annotating bacterial WGS data with minimum manual labor, hassle-free installation and multi-genome analysis in one step.

#### **PS I-9 Manisha Datta, National Centre for Cell Science, Pune**

**Title:** Understanding the effect of mutant forms of clathrin heavy chain on neural development and function

**Abstract:** De novo mutations in the CLTC gene lead to a spectrum of early-onset neurodevelopmental conditions characterized by developmental delay, intellectual disability (ID), epilepsy, and movement disorders. The CLTC gene encodes a widely expressed clathrin heavy chain which plays a crucial role in intracellular trafficking, and synaptic vesicle recycling. In our study, we investigated how pathogenic missense mutations (Trp1108Arg and Leu1047Pro) in the CLTC gene, associated with intellectual disability impact trafficking, neural development, and behaviour using an in vivo *Drosophila melanogaster* model system.

We characterised these flies expressing CLTC mutations and observed that clathrin-coated vesicle movement was compromised in larval haemocytes. Similar disruptions in vesicle movement were also observed in mammalian HEK-293 cell lines. Flies expressing the mutant forms of CLTC demonstrated reduced survival compared to the wild type. Notably, third instar larvae with the Leu1047Pro mutation failed to eclose. Furthermore, significantly reduced locomotion was observed in mutant larvae upon heat stress. Mutant flies also displayed altered mushroom body formation which resulted in impaired learning and memory formation. Together, our results demonstrate that the *Drosophila* model system successfully recapitulates the phenotypes observed in patients carrying CLTC mutations.

#### **PS I-10 Sourav Halder, National Centre for Cell Science, Pune**

**Title:** The role of PIP4K in neuromuscular gene regulation by regulating translation of its target mRNAs.

**Abstract:** Phosphatidyl inositol 5-phosphate 4-kinase (PIP4K) is a key regulator of phosphoinositide metabolism. PIP4K2A can be imported into *Plasmodium* species from the host erythrocytes and interact with UUGU motif containing specific plasmodium transcripts. Since, PIP4k is involved in many neurodevelopmental and neurodegenerative diseases, lead us to study the role of PIP4K in neuronal gene regulation. Transcriptomics

analysis shows that PIP4K2A overexpression leads to downregulation of glutamate receptor's expression and TRIBE-seq analysis shows lots of promising RNA targets of PIP4K including Glutamate receptors RNA. Further, we explored PIP4K can regulate expression of Glutamate receptor (GluRIIA) in drosophila nmj also. Further, we explore dPIP4K can Interact with GluRIIA mRNA and the interaction might be UUGU motif dependent. We also show that neuronal knock down (KD) of PIP4K shows drastic increase of GluRIIA expression in drosophila NMJ. Further, proximity labelling based interatomic analysis shows PABP1 as an interacting partner of PIP4K2A. It has been reported that EIF4G has the affinity for binding to PABP and promotes translation. PIP4K can Interact with its target mRNA and PABP1 together and prevent 80s ribosomal complex formation by competing with the EIF4G for binding to PABP. Thus, PIP4K might be act as a translation repressor & regulates expression of its target genes.

### **PS I-11 Ajay Pradhan, National Centre for Cell Science, Pune**

**Title:** Molecular mechanisms of lysosome-related organelle biogenesis in *Tetrahymena thermophila*

**Abstract:** Secretory granules/organelles are found in a subset of animal tissues and eukaryotic

lineages with few having been analyzed at the molecular level. In ciliates, formation of secretory organelles (mucocyst/ trichocyst) shares striking similarities to insulin granule formation in mammalian pancreatic  $\beta$ -cells. However, recent studies have suggested unexpected similarities between secretory granules and lysosome-related organelles (LROs), making the study of LROs even more important for advancing human health. Although mucocysts have historically been considered as secretory granules, recent studies indicated that mucocyst formation relies on mechanisms that function in lysosome formation. Thus, mucocysts belong to the very broad family of LROs, comparable to secretory LROs like Weibel-Palade bodies in mammalian cells, and detailed studies of mucocyst biogenesis hold great promise to advance our understanding of this class of organelles. Historically, most studies of mucocysts began with biochemical or candidate gene approaches. To complement these approaches, we have explored the use of expression profiling, taking advantage of an online database of *Tetrahymena* gene expression. This approach is based on the discovery that a large set of genes involved in mucocyst biogenesis is coordinately transcribed. Using this technology, we showed that the mucocyst processing enzymes could be identified based on their expression profiles, which differ from >98% of the protease-encoding genes in *Tetrahymena*. We therefore propose to exploit expression profiling to identify other key components of mucocyst biogenesis, starting with the V-ATPases.

**PS I-12 Sanskruti Sanjivan Thakur, MGM School of Biomedical Sciences, MGMIHS, Navi Mumbai**

**Title:** Nanoparticles for zebrafish model development –Aluminium oxide nanoparticles induce cognitive impairment in Zebrafish

**Abstract:** Cognitive studies involving learning and memory require using appropriate model organisms to translate relevant findings to humans. Zebrafish are increasingly being utilized as animal models for neurodegenerative diseases due to their similar neurobehaviors to humans. Al<sub>2</sub>O<sub>3</sub>-NPs, with their unique physical and chemical properties, are widely used in various fields due to their ability to penetrate the blood-brain barrier and distribute and accumulate in different brain areas. The study aimed to biologically synthesize <50nm Al<sub>2</sub>O<sub>3</sub>-NPs, characterize them, and investigate their toxicity and changes in motor behavior in zebrafish larvae. Our study compared chemically(<5nm) and biologically synthesized(<50nm) Al<sub>2</sub>O<sub>3</sub>-NPs through FET and behavior studies on exposure to increasing concentrations. The study revealed that Al<sub>2</sub>O<sub>3</sub>-NPs in zebrafish larvae's embryonic stages can cause impaired neurodevelopmental behaviors, decreased speed, distance travelled, and thigmotaxis, leading to increased mortality which was more prominent in chemically synthesized NPs. The size of the nanoparticles was the primary factor influencing the toxic effects, with dissolved Al<sup>3+</sup> also playing a role. The study suggests that Al<sub>2</sub>O<sub>3</sub>-NPs can be used to model cognitive dysfunction in zebrafish and further examine its effects on latent learning and memory abilities during their development.

**PS I-14 Praneet Wahi, National Centre for Cell Science, Pune**

**Title:** CCR9-CCL25 signaling is involved in maintaining central tolerance and controls acute gut inflammation.

**Abstract:** Background- The thymus is a primary lymphoid organ known as the site for T cell development. Thymic dendritic and thymic epithelial cells are important in developing T cells. It has been reported that thymic DCs are known to express CCR9, and epithelial cells produce its cognate chemokine CCL25. How CCR9-CCL25 signaling alters the phenotype of thymic DCs, which further has an impact on the development of T cells, and how peripheral gut inflammation alters the mature T cell thymic output to the peripheral system, leading to chronic autoimmune disease are not clearly understood. Methods- CCR9<sup>-/-</sup> or CCR9<sup>+/+</sup> mice were given dextran sodium sulfate (DSS, 2% w/v) in drinking water, and disease symptoms were monitored. Mice were sacrificed when their weight was reduced to more than 20% of their initial weight. Tissues and cells from these mice were analyzed using flow cytometry, immunohistological staining, and qRT-PCR. Results- Our data showed that CCR9<sup>-/-</sup> shows severe gut inflammation after exposure to DSS in drinking water compared to wild-type mice. Further, analysis of thymic DCs

showed CD103<sup>high</sup>CD8α<sup>+</sup>CD11c<sup>+</sup> (migratory) DCs and CD103<sup>low</sup>CD11b<sup>+</sup>CCR7<sup>+</sup>CD11c<sup>+</sup> (cDC2) DCs were increased in CCR9<sup>-/-</sup> mice compared to wild-type. To understand the contribution of CCR9<sup>+/+</sup> DCs in gut inflammation, we adoptively transferred CCR9<sup>+/+</sup> or CCR9<sup>-/-</sup> DCs in a CCR9<sup>-/-</sup> host treated with DSS. We observed that CCR9<sup>+/+</sup> DCs could partially rescue colitis symptoms while CCR9<sup>-/-</sup> DCs aggravate the symptoms. Analysis of the thymus showed that adoptively transferred CCR9<sup>-/-</sup> and CCR9<sup>+/+</sup> DCs localized in the specific zone in the thymus. Furthermore, CCR9<sup>-/-</sup> mice showed defective positive and negative selection in the thymus compared to the wild-type mice. In CCR9<sup>-/-</sup> mice treated with DSS showed that the frequency of CCR6<sup>+</sup> T cells was decreased in the thymus whereas the colon showed an increased frequency of CCR6<sup>+</sup> T cells. Conclusion- Our data suggest that deficiency of CCR9 alters the phenotypic profile of DCs in the thymus and shows defective T cell development. This altered cellular phenotype of DCs and T cells shows a disrupted central tolerance mechanism, leading to strong acute gut inflammation. The present mechanism impacts how CCR9-CCL25 signaling can be exploited to establish tolerance and control autoinflammatory diseases in the clinic.

**PS I-15 Aman Soni, Department of Biotechnology, Savitribai Phule Pune University, Ganeshkhind, Pune**

**Title:** Impact of SARS-CoV-2 infection on pancreatic β-cell function: A Mechanistic Study.

**Abstract:** Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-Cov-2), was the causative agent behind COVID-19 (Coronavirus Disease-19) pandemic in 2020. SARS-Cov-2 has a positive single-stranded RNA genome which codes for 5 proteins amongst which the Spike protein (S-protein) plays an important role in viral attachment and fusion in the target cell.

SARS-CoV-2 targets cells via the ACE-2 receptor. The pancreatic β-cells also express the ACE-2 receptor; hence, SARS-CoV-2 can infect pancreatic β-cells via ACE-2-dependent entry and affect their viability and function. A deeper understanding of the underlying mechanism that leads to the development of type 2 diabetes mellitus (T2DM) in COVID-19 patients is required. Here, we infected the human umbilical cord mesenchymal stem cells derived islet-like cellular aggregates (ICAs) with the S-protein. Glucose stimulated insulin secretion assays were performed followed by estimation of insulin and c-peptide levels by ELISA. The results from these experiments will be further elaborated.

**PS I-16 Rutuja Sawant, National Centre for Cell Science, Pune**

**Title:** Consortium on celiac disease: Microbial solution as a potential cure for the disease

**Abstract:** The incidence of celiac disease has increased in the past decade in India. Previously it was thought that celiac disease was prevalent in countries with high wheat consumption. Celiac disease is an autoimmune disease triggered by gluten (found in wheat) and results in gut damage, abdominal discomfort, diarrhea, weight loss etc. Gut bacteria have disease-driving potential as alteration in gut microbiota leads to gut dysbiosis. Some bacteria degrade gluten partially due to which autoimmune peptides are generated. Nevertheless, some bacteria can fully break down the gluten protein, and if their levels in the gut decrease, it may lead to the development of the disease or worsen its symptoms.

At present, the only clinically prescribed treatment is a strict gluten-free diet. This can be difficult to adhere to, is financially restrictive and there may still be accidental exposure to gluten through condiments, pharmaceutical additives, etc. To address this disease, we are isolating anaerobic gut bacteria from the unaffected Indian population with the ability to fully break down gluten without producing immune-triggering peptides. We use Sanger sequencing for bacterial identification and have found promising candidates to degrade gluten.

Bacteria do not operate independently in their natural environment but rather demonstrate complex ecological interactions. Hence, apart from isolates that break down gluten, we aim to create a set of organisms that can alleviate symptoms by fully breaking down gluten.

**PS I-18 Aaradhika Menon, Ankit Chindaliya, Subhanjan Satapathy- Yashraj biotechnology limited, TTC Industrial Area, MIDC, Navi Mumbai**

**Title: Development and characterization of anti NGAL monoclonal antibodies for diagnostic purpose.**

**Abstract:** Neutrophil Gelatinase Associated Lipocalin (NGAL) is a 23 kDa protein belonging to the lipocalin family. Expressed in neutrophils, kidney, prostate, and respiratory epithelia, it is a biomarker for acute kidney injury (AKI) and chronic kidney disease (CKD). During injury to renal tubule, the reabsorption of NGAL is significantly compromised, resulting in high amounts of NGAL in urine. Monoclonal antibodies to NGAL can be useful in detecting this urine NGAL and thereby, helpful in early diagnosis of AKI and CKD. Here, we generated monoclonal antibodies (mAbs) against NGAL using hybridoma technology for diagnostic purpose. We were successfully able to develop two distinct mAbs 3A91E7 and 3F82B11 that identifies different epitopes of NGAL with high affinity, sensitivity and specificity. These mAbs were purified using affinity chromatography and gel filtration chromatography. The purity and aggregation were tested using SEC-HPLC. Furthermore, using mAb 3A91E7 and 3F82B11 as capture and detector, respectively, we developed a quantification sandwich ELISA. We demonstrated

that anti-NGAL mAbs, 3A91E7 and 3F82B11 could be utilized for detection of NGAL in patient urine samples.

**PS II-1 Jyoti Das, National Centre for Cell Science, Pune**

**Title: Clathrin Light chain regulates neural development and function in *Drosophila melanogaster***

**Abstract:** Clathrin is a cytosolic protein involved in the trafficking of a wide range of cargo. The clathrin subunit is composed of three heavy chains and three light chains that polymerizes to form the clathrin coated vesicle. Although the role of clathrin heavy chain in all clathrin dependent physiological functions is well established the role of the light chains remains elusive.

In this study, we use *Drosophila melanogaster* as a model system to understand the role of clathrin light chains in neural development and function. Using CRISPR-CAS9 we show that clathrin light chain Knockout flies show severely hampered locomotion. This phenotype is recapitulated when clathrin is depleted only from the neurons indicating that a neuronal specific function, presumably their role in synaptic vesicle recycling is essential for locomotion behavior. There are also defects in neuronal morphology as seen at the larval neuromuscular junction and deformed mushroom body structures in the adult flies. Other behavioral defects like inability to sense thirst/satiation is also seen in these flies.

**PS II-2 Srinibash Behera, National Centre for Cell Science, Pune**

**Title: Mapping the Genetic Landscape of Brinjal Little Leaf Disease Related to 'Ca. Phytoplasma trifolii' to Identify Key Pathogenicity Factors**

**Abstract:** Brinjal little leaf (BLL) disease, primarily associated with 'Ca. *Phytoplasma trifolii*', significantly impacts eggplant crops (*Solanum melongena* L.), a major vegetable cultivated worldwide, including in India. The disease induces symptoms such as reduced leaf size, excessive shoot proliferation, and abnormal flower development, which lead to sterility and diminished fruit quality. To elucidate the genomic basis of BLL phytoplasma pathogenicity, the BLL strain BR06 was sequenced using the Illumina HiSeq platform. The quality-approved Illumina reads underwent metagenomic binning with MEGAHIT v1.2.9 and MetaBAT 2:2.15, classifying the sequences by taxonomic status. The phytoplasma bin identified was qualitychecked using CheckM v1.1.3. Concurrently, QC-approved raw reads were assembled using existing phytoplasma genomes with Bowtie2 v2.3.5.1. To ensure all reads were accounted for, QC-passed raw reads were mapped back to the MetaBAT-generated phytoplasma bin. The final assembly of the Brinjal Little Leaf (BLL) phytoplasma, comprising 70 scaffolds totaling 529,585 bp, revealed a genomic landscape rich in key components essential for pathogenicity. Among the identified genes

were 466 protein-coding genes, 28 tRNA genes, and three rRNA genes. Analysis with SignalP v.6.0 identified seven putative effector proteins implicated in BLL pathogenicity, all carrying N-terminal secretion signals. Further analysis with cNLS mapper indicated these proteins had low NLS scores, suggesting they are not targeted to the nucleus. These putative effector proteins, confirmed by TMHMM v.2.0 to lack transmembrane domains, are translocated via the Sec translocon pathway. Meanwhile, the inclusion of Protein translocase subunit SecA and SecY in the genome suggests that these putative effectors might utilize this pathway for their extracellular movement, which could be essential for manipulating host plant processes and potentially initiating infection. The presence of Superoxide dismutase [Mn] (sodA) is thought to assist the phytoplasma in combating oxidative stress through unknown mechanism. Single scaffold is essential for enhancing the mapping of effector proteins and other pathogenesis-related proteins in the Brinjal little leaf (BLL) phytoplasma genome using hybrid assembly of long sequencing reads.

### **PS II-3 Snehal Kulkarni, Agharkar Research Institute, Pune**

**Title:** Development of a Nano-Adjuvant for Oral Vaccine Against fish Nodavirus

**Abstract:** Fish Nodavirus (FNV) is a significant aquaculture pathogen that causes viral nervous necrosis. To overcome the economic losses associated with diseases, vaccination is a promising alternative. In this study, inorganic nanomaterials viz., zinc oxide (ZnO), calcium oxide (CaO), hydroxyapatite (HAP), and layered double hydroxide (LDH) were synthesized, characterized. ZnO, CaO, and HAP were non-toxic at 100µg/mL in fish cell lines. The virus was propagated in SISK and SIGE cell lines, purified, and inactivated with formalin (FNVin) and conjugated with nanoparticles. In in-vivo studies following oral routes of administration, ZnO (size 40nm, charge +3.14, stability between pH 7-9, temperature 25 -40C and salinity in range 4-40 PPT) were chosen. 20mg/mL ZnO-FNVin was coated on pelleted fish feed. Under controlled conditions, the whole virus vaccine trials were carried out in 5 susceptible fish species. All groups of the vaccinated animals showed a higher survival percentage (~75%) than the non-vaccinated group, indicating the efficacy of ZnO as an adjuvant for oral vaccination. The study indicates the utility of benign ZnO nanoparticles as a safe alternative and an easy-to-use material for protecting farmed fish against viral infections.

### **PS II-4 Brindaban Das, National Centre for Cell Science, Pune**

**Title:** Elucidating the role of FBXO41 in Breast cancer

**Abstract:** F-box proteins are variable components of SCF (Skp1-Cullin1-F-box) E3 ligases, and they play vital roles in cell cycle progression, apoptosis, DNA damage repair, cell signaling, and other cellular processes by governing the turnover of major proteins.

Human genome encodes for 69 F-box proteins, and the function of most of the proteins remains elusive. From our lab, a tumor suppressor screening of F-box proteins showed that FBXO41 inhibits growth of MCF7 breast cancer cell line through inducing autophagic death. Several unpublished data highlighted that FBXO41 can also target the oncogenic protein CDK5. CDK5, also known as atypical CDK, has a crucial role in post-mitotic neurons. Apart from its physiological role, it is a well-known driver of cancer progression. We have shown that FBXO41 interacts with CDK5. We observed that ectopically expressed FBXO41 downregulates the protein level of CDK5. Further, it is observed that degradation of CDK5 by FBXO41 is blocked with 26S proteasome inhibitor. Lastly, we also discovered that CDK5 may be degraded by forming intact SCF complex. Collectively, our findings suggest that FBXO41 may be functioning as a tumor suppressor in breast cancer by facilitating proteasomal degradation of CDK5.

**PS II-5 Aravindan Narayanan, National Centre for Cell Science, Pune**

**Title:** Inhibiting mitochondrial pathways as a therapeutic strategy to target CSCs in ovarian cancer

**Abstract:** Usage of defined serum-free media is a well-established approach to maintain stem cells and cancer stem cells (CSCs). Here we subjected five different phenotypes of High-Grade Serous Ovarian Carcinoma (HGSC) to serum starved (SS) condition as a surrogate for cancer stem cells' maintenance to identify the CSC-specific features and targeting them therapeutically. A Label Free Quantification (LFQ) proteomics approach revealed that regardless of their phenotypic differences, HGSCs relied on mitochondrial translation and mitochondrial metabolic pathways to survive under SS. This mitochondrial involvement was further validated through the phenotypes' dependency on OXPHOS, enriched mitochondrial biogenesis, elevated transmembrane potential and increased ROS levels after starvation. The necessity of mitochondrial translation and OXPHOS for the maintenance of CSC characteristics were examined both in-vitro and in-vivo using antibiotics and ETC inhibitors respectively. Interestingly, in-vitro spheroid formation was hampered under individual or combinatorial usage of antibiotics and OXPHOS inhibitors. The same result was noticed in-vivo, where the combinatorial drug regimen of Metformin + Erythromycin + Paclitaxel (OXPHOS inhibitor + mito. Translation inhibitor + conventional drug) indicated the maximal tumor inhibitory effect compared to all the other individual or combinatorial drug regimen. This strongly indicate the potential of inhibiting the mitochondrial metabolism and translation along with conventional drugs can improve the treatment and could be an efficient way to address the heterogeneity of the disease.

**PS II-6 Akshay Santosh Lonare, National Centre for Cell Science, Pune**

**Title:** Regulation of calcium homeostasis and autophagy by Nup358



**Abstract:** RanGTPase and transport receptors, through controlled interactions with nucleoporins, regulate the process of nucleocytoplasmic transport (NCT). How NCT is regulated by and/or is coordinated with other cellular events, particularly cytoplasmic processes, is largely unknown. Interestingly, a subset of nucleoporins including a RanGTP-binding nucleoporin, Nup358, is known to be present in the cytoplasm at subdomains of endoplasmic reticulum (ER) called annulate lamellae (AL). We recently found that AL are often found at the ER- mitochondria contact sites (ERMCSs), a cytoplasmic platform for inter-organelle communication including calcium ( $\text{Ca}^{2+}$ ) homeostasis. We find that Nup358 is important for  $\text{Ca}^{2+}$  homeostasis, and depletion of Nup358 decreased levels of mitochondrial  $\text{Ca}^{2+}$  with a concomitant increase in the cytoplasmic  $\text{Ca}^{2+}$  (cyto- $\text{Ca}^{2+}$ ), which in turn stimulates autophagy through activation of the CaMKK2/AMPK pathway. Current studies are focused on addressing the potential regulation of Nup358's function in  $\text{Ca}^{2+}$  homeostasis by RanGTP and NCT receptors. This study is expected to provide new insights into the possible direct regulation of ERMCS functions by the NCT process.

**PS II-7 Shiva Kumar Tomar, National Centre for Cell Science, Pune**

**Title:** Exploring the role of an F box protein, FBXO34, in cancer

**Abstract:** Cancer remains one of the leading causes of mortality worldwide. This high mortality rate is attributed to the dysregulation of various cellular processes, including the turnover of proteins via ubiquitination. The SCF (Skp1-Cullin1-F box proteins) E3 ligase complex, a pivotal unit in ubiquitination, features highly variable F-box proteins. Despite their significance, the role of these proteins in cancer regulation remains underexplored. Our lab screened several F-box proteins and observed significant growth inhibition by FBXO34 in the MCF-7 cell line. Further investigation revealed that FBXO34 functions as a tumor suppressor by inhibiting the proliferation, colony formation ability, and migration of breast cancer cells. Notably, FBXO34 downregulates the protein level of vimentin, a key mesenchymal marker involved in the migration and invasion of cancer cells. Further, we plan to conduct additional key experiments to understand the underlying mechanism of tumor suppression. So far, the data suggest that FBXO34 plays a critical role in cancer regulation and degrades a key oncogenic protein.

These findings highlight the importance of FBXO34 in the regulation of breast cancer progression. Consequently, our study may discover a new molecular player associated with cancer malignancy and potentially lead to novel therapeutic strategies.

**PS II-8 Sushmita Sahoo, National Centre for Cell Science, Pune**

**Title:** Identification and Functional Characterization of candidate chimeric transcripts in ovarian cancer

**Abstract:** Cancer cells rely on genome instability which plays a crucial role in facilitating their survival and fitness. Genome instability can lead to various genetic changes, such as gene mutations, chromosomal rearrangements, and alterations in DNA structure and copy numbers. Due to the consequences of genome instability in cancer cells, two or more mRNAs can be fused to generate chimeric Transcripts (CTs). These CTs may function as novel proteins, long non- coding RNAs or as transcriptional and translational regulators. Earlier, CTs were considered to be exclusive to the cancer cells, but now evidences suggest that, they are also present in the non- cancerous tissues and cells. The study of chimeric transcripts unveils a complex layer of gene regulation and genetic diversity. Our lab has previously validated the breakpoint of various CTs in ovarian cancer cell lines. Therefore, the purpose of this study is to generate full length of validated chimeric transcript and understand the molecular relevance of these candidate Chimeric Transcripts.

**PS II-9 Sananda Kumar Patra, National Centre for Cell Science, Pune,**

**Title:** CD40 expression in stem cells and delineating its functional relevance

**Abstract:** Stem cells can self-renew and differentiate into various cell types, a process controlled by a program called stemness. This regulation is influenced by signaling mechanisms and niche factors. Key pathways involved in embryonic stem cell (ESC) pluripotency include LIF via JAK/STAT, Wnt, Notch, MAPK/ERK, BMP, etc. CD40, a tumor necrosis factor receptor family member, is present in both hematopoietic and non-hematopoietic cells and is presumed to influence hematopoietic stem cell (HSC) fate. However, its precise contribution during stem cell maintenance and differentiation is yet to be discerned. In our study, CD40 expression was notably observed in ESCs and mesenchymal stem cells (MSCs) at both the transcriptional and translational levels. We have also confirmed its activation in bone marrow-derived MSCs (BM-MSCs) through phosphorylation of p38 and Erk. Hence, efforts were directed to gain understanding on the role of CD40 in osteo-, adipo- and chondrogenesis from BM-MSCs. Interestingly, CD40 stimulation demonstrated a dose-dependent effect on MSC osteogenesis, as evidenced by Alizarin Red S (ARS) and Alkaline Phosphatase (ALP) assays. Similarly, during spontaneous differentiation of ESCs, CD40 tilted the differentiation ladder towards ectoderm signifying its plausible role during lineage specification from ESCs. Further work will shed light on its novel role during development.

**PS II-10 Anup Kumar Singh, National Centre for Cell Science, Pune**

**Title:** Role of Interferon inducible GTPase, IIGP1, in macrophage polarization

**Abstract:** Macrophages are innate immune cells vital for inflammation and tissue repair. They are capable of exhibiting a spectrum of polarized phenotypes, from pro-

inflammatory M1 to anti-inflammatory M2. Proteome profiling data of M1 and M2 macrophages from our lab suggests that IFN- $\gamma$  inducible proteins including IIGP1 (Interferon Inducible GTPase) are selectively altered in M1 macrophages compared to M2 macrophages. The aim of the present study was to delineate the potential role of IIGP1 in macrophage polarization. Our results indicate that Knockout of IIGP1 in the macrophage cell line RAW264.7 significantly reduces M1 phenotype markers, NOS2, MHCII, CD86, and CD80, and alters the transcript levels of IL-6, IL-1 $\alpha$ , IL-1 $\beta$ , CCL2 and also the phagocytic ability. These findings underscore IIGP1's crucial role in maintaining the M1 phenotype in macrophages.

#### **PS II-11 Shubham Yadav, National Centre for Cell Science, Pune**

**Title:** Identification and Characterization of Downstream Effector Protein(s) for Gao

**Abstract:** Gao is the most abundant G $\alpha$  protein in the brain and controls both the development and adult physiology of the brain. This study aims to identify and characterize downstream effectors for Gao, which have not yet been fully elucidated. We immunoprecipitated active Gao-GTP or inactive Gao-GDP from protein lysates and performed mass spectrometry to identify proteins that bind specifically to activated Gao. We did this experiment in three ways. First, we generated transgenic strains of *C. elegans* that express epitope-tagged Gao that is either wild-type or constitutively-active (Q205L), and immunoprecipitated Gao. Second, we prepared protein lysates from *C. elegans*, split the lysate in half, activated Gao in one half with GTPS, and immunoprecipitated. The third approach was identical to the second except we used mouse brain lysates instead of *C. elegans* lysates. Mouse brain Gao is >80% identical to worm Gao, and we expect effectors to also be conserved. We identified several known Gao binding proteins, and are working to validate interactions with new ones and to determine the biological functions of any effectors found. Our work is relevant to human health as our studies will focus on defects that are believed to underlie clinical depression.

#### **PS II-12 Puja Ghosh, National Centre for Cell Science, Pune**

**Title:** Screening of psychobiotic strains from stool samples to validate its role in gut brain axis.

**Abstract:** Psychobiotics, a subclass of probiotics with mental health benefits, have garnered increasing attention for their potential role in the gut-brain axis and mental disorders. The role of psychobiotics is thought to have influence on the intestinal barrier, immune processes, and functioning of the nervous system. This study focuses on the isolation and characterization of such psychobiotic organism cultivated under anaerobic conditions. Here, we have employed selective anaerobic culturing techniques to isolate potential psychobiotic strains. The isolates were subjected to a series of phenotypic and

genotypic characterizations, including 16S rRNA gene sequencing, to confirm their identity and taxonomy. One promising strain, designated as *Anaerostipes caccae* is a commensal demonstrated notable resilience and growth under Obligate anaerobic conditions. This strain exhibited key psychobiotic properties, such as the production of GABA and short-chain fatty acids (SCFAs), which are crucial for modulating neural functions. Additionally, in vitro assays indicated that *Anaerostipes caccae* could act as important species in maintaining gut homeostasis and immune modulation thereby, suggesting a potential mechanism for its psychotropic effects. To further substantiate its psychobiotic potential, the strain was tested for its ability to survive gastrointestinal transit. Results from simulated gastric and intestinal fluid tolerance tests showed high survivability rates. The strain's safety profile was also confirmed through antibiotic susceptibility testing and the absence of harmful metabolites. In conclusion, the successful isolation and comprehensive characterization of *Anaerostipes caccae* underline its promise as a psychobiotic candidate. This study lays the groundwork for future in vivo studies to explore its efficacy and mechanisms in promoting mental health through modulation of the gut-brain axis.

**PS II-13 Debolina Sarkar, National Centre for Cell Science, Pune**

**Title:** Understanding the role of CORVET complexes in the delivery of proteins to Lysosome-related organelles in *Tetrahymena thermophila*

**Abstract:** Lysosome-related organelles (LROs), which are diverse intracellular compartments crucial for various physiological functions. Traditionally found in animals, LROs encompass membrane-bound organelles involved in regulated exocytosis, relying on receptors and adaptors for cargo sorting. To explore LRO biogenesis beyond animal models, researchers turned to *Tetrahymena thermophila*, a ciliated protozoan whose mucocysts serve as specialized secretory organelles and belong to the LRO family. The study leverages *Tetrahymena* as a model system because of its comprehensive array of tools and approaches that have been developed over several decades.. This allows for a detailed investigation into the pathways underlying mucocyst formation. Specifically, the research aims to elucidate how proteins are transported to LROs in *Tetrahymena*. To understand the pathways that lead to mucocyst formation in *Tetrahymena*, we will examine protein transport pathways in the endolysosomal network, which connect endocytosis and hydrolytic compartments, and specifically the role of tethers in the CORVET (class C core vacuole/endosome tethering) family. Fundamentally, we will test the hypothesis that distinct CORVET complexes are involved in protein delivery to LROs. This study will advance the frontier area of evolutionary cell biology by determining the roles of CORVET tethers in the endolysosomal network and analysing this network's architecture in a ciliate.

## **PS II-14 Sourabh, National Centre for Cell Science, Pune**

**Title:** Antagonizing muscarinic receptor 3 signaling restricts tumor growth.

**Abstract:** Background:- Neuro-immune communication controls different physiological processes and maintains cellular homeostasis. The neurotransmitter acetylcholine plays a crucial role in the modulation of immune cells. Additionally, acetylcholine is known for stimulating cell division, migration, and survival of cancer cells. But, how acetylcholine interacts with its cognate receptors and regulates immune response to cancer is not entirely understood. Methods:- Orthotropic colon cancer was induced in C57BL/6 or CHRM3-/- mice by subcutaneous injection of a mouse colon cancer cell line MC38. Expression of muscarinic receptors and other molecules in various tissues and immune cells were analyzed using semi-quantitative PCR, Immunohistochemistry (IHC), and multicolor spectral flow cytometry. Results:- Our results showed that the MC38 colon cancer cell line and various immune cells express muscarinic receptors. Intraperitoneal injection of CHRM3 antagonist and CHRM4 antagonist resulted in reduced MC38 tumor growth in C57BL/6 mice. The antagonist treatment reduced tumor growth by enhancing CD8+ T cells and CD4+ T cells. Similarly, mice deficient in CHRM3 molecules showed reduced MC38 tumor growth kinetics and reduction in suppressor cells in the secondary lymphoid tissues. Conclusion:- Our data showed that antagonism of the muscarinic receptor 3 with 4-DAMP or Tropicamide alters the anti-tumor immune response and controls tumor growth. This suggests that the acetylcholine neuro-immune axis through CHRM3 is important in regulating colon tumor growth and can be exploited as a potential therapeutic target to control tumor growth in the clinic.

## **PS II-15 Dipti Ashok Dama, Yashraj Biotech Ltd, TTC Industrial area, MIDC, Navi Mumbai**

**Title:** Optimizing Purification Strategies for His-Tagged Protein X: Insights from IMAC and Biotin-Avidin Affinity Chromatography

**Abstract:** Immobilized Metal Affinity Chromatography (IMAC) is utilized when a biomolecule exhibits a specific affinity for transition metal ions. In recombinant biotechnology, IMAC employs coordination chemistry to facilitate the purification of His-tagged proteins, simplifying their isolation. However, achieving effective and efficient reversible adsorption is influenced by several factors, including the type of ligand on the matrix, the metal ion used for chelation, buffer composition, and the structure of the biomolecule. In our study, we expressed the His-tagged protein coded as IVDCF21 in *E. coli* and purified it using IMAC. IVDCF21 is useful as tumor marker especially for the diagnosis of non-small cell lung cancer (NSCLC). For downstream purification we

conducted experiments using different matrix bases and modified the exposure of the His tag of protein to ligands by incorporating chaotropic and reducing agents. These adjustments aimed to enhance protein binding and yield. Additionally, we utilized chemical methods, specifically sulfo-NHS coupling, for biotin tagging, offering an alternative and versatile approach compared to enzymatic methods. The biotinylated protein was subsequently purified using reliable biotin-avidin affinity chromatography, which is well-suited for applications such as ELISA (for coating, as detector or control) and other detection assays.

#### **PS II-16 Pooja Arya, National Centre for Cell Science, Pune**

**Title:** Understanding the memory B cell immunoglobulin repertoire upon SARS-CoV2 infection.

**Abstract:** B lymphocytes are an integral part of the adaptive immune system which generate immune response against pathogens mainly by secreting antibodies. Antigen binding to the B Cell Receptor (BCR) is a critical step to initiate B cell activation. The activated B cells undergo somatic hypermutation, affinity maturation and class switching in germinal centre leading to differentiation of B cells into memory B cells and plasma cells. Memory B cells are quiescent B cells which either migrate to tissues, secondary lymphoid organs or remain in circulation. Upon a secondary infection with a similar pathogen or booster immunization, memory B cells differentiate into short lived antibody secreting cells (ASC) thereby evoking a quick and enhanced immune response. B cell receptor and its diversification during infection or vaccination can be studied by isolating memory B cells. Alternatively, functional antibodies can also be generated using these memory B cells against the pathogen proteins. Our study focuses on the BCR repertoire of the memory B cells generated during the severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2). The BCR of memory B cells (heavy and light chain) were amplified and sequenced to identify B cells clones proliferated during SARS-CoV2 infection or vaccination. Our data suggests the utilization of selective pairs of VDJ regions across individuals with unique CDR3 which are not reported in any published literature or database. These heavy and light chain pairs also form functional antibodies when expressed in mammalian cells.

#### **PS II-17 Fahima Munavar K, National Centre for Cell Science, Pune**

**Title:** Investigating the role of deubiquitinating enzyme, USP, in mesoderm specification and further differentiation to its derivatives

**Abstract:** Deubiquitinating enzymes (DUBs) are a group of proteases that catalyze the deconjugation of ubiquitin moiety from proteins thereby antagonizing the function of ubiquitin E3 ligases. Growing evidence suggests that the DUBs are involved in processes

like DNA damage response, cell cycle control, transcriptional regulation, regulation of protein turnover rate and localization. Despite several studies, the role of DUBs in specific cellular contexts, especially cell fate specification, is limited. Hence, in the present study, we attempt to address these lacunae by using embryonic stem cells (ESCs) as a model system to assess the modulatory role that the DUBs may play in deciding various cell fate specifications and subsequent maturation. Using the knockdown approach for one of the candidate DUBs (USP) identified via forward genetic screening, we could discern that USP is dispensable for ESC maintenance. However, USP affected the differentiation of cells into three germ layer derivatives; especially significant negative regulation of mesoderm specification was observed. Our findings indicate a differential role of USP in haemato-endothelial differentiation, where USP is required only for haematopoietic specification and not for endothelial differentiation. In contrast, USP inhibits cardiac mesoderm and skeletal muscle specifications. While attempting to deduce the mechanistic role of USP in mesoderm specification, we learned that USP interacts with  $\beta$ -catenin and is regulated by the canonical Wnt signalling pathway. Indeed, our work has highlighted pronounced mediation of Wnt-USP dictating the early cell fate decision.

#### **PS II-18 Vandana Maurya, National Centre for Cell Science, Pune**

**Title:** Identification and characterization of MFS transporter proteins associated with lysosome-related organelles in *Tetrahymena thermophila*.

**Abstract:** All cell types secrete proteins and other molecules, and many cell types have evolved specialized pathways to tailor this secretion to physiological requirements. A remarkable and medically significant example of such specialization is seen in two related compartments called dense core granules (DCGs) and lysosome-related organelles (LROs). The ciliate *Tetrahymena thermophila*, like mammalian cells, has a prominent secretory organelle called a mucocyst, which is LRO. Recently, several studies suggested mechanistic links between LRO and insulin granule formation, making the study of LRO even more important for advancing human health. However, the LRO biogenesis pathway is not well understood in animals. We are proposing to expand analysis of mucocyst/LRO biogenesis in *Tetrahymena*, including its dependence on the major facilitator superfamily (MFS) of transporter proteins, by taking advantage of the many tractable features of this single-cell model organism. MFS is one of the largest and most diverse superfamily of secondary active transporters conserved from bacteria to humans. They are responsible for transporting essential molecules, including drugs and toxins, enabling them to be potential drug targets. However, MFS transporters in humans and other higher eukaryotes have received limited attention due to their biological complexity. In this study, the aims are designed to ask (a) whether a specific MFS is involved in mucocyst biogenesis and (b) what precise role MFS is playing and what proteins it interacts with.

We would then identify the paralog-specific elements that have tailored a particular MFS to function in the pathway of mucocyst biogenesis.

**PS II-18 Sneha Verma, National Centre for Cell Science, Pune**

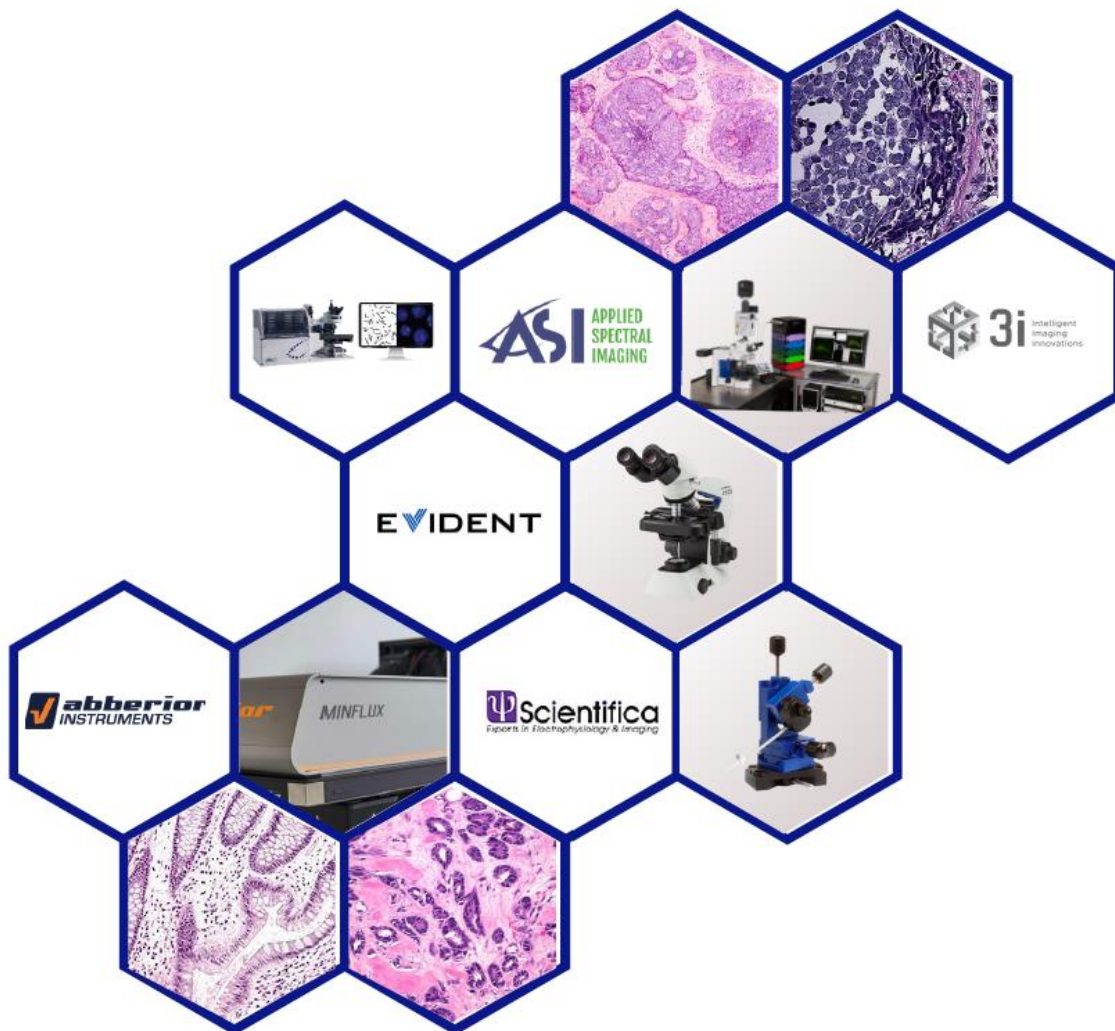
**Title:** Characterization of TENGU Effector Homolog Interactions in 'Ca. Phytoplasma asteris' Implicated in the Pathogenesis of Sandal Spike Disease

**Abstract:** The sandal spike disease (SSD), related to 'Ca. Phytoplasma asteris' (Aster Yellows group), poses a significant threat to Indian sandalwood (*Santalum album* L.), making it the second most expensive wood globally due to declining population density. The association of 'Ca. Phytoplasma asteris' results in chlorosis, leaf yellowing, shortened internodes, and reduced leaf size, leading to a spike-like morphology in sandalwood trees. This progression leads to the gradual decline of twigs and branches, ultimately resulting in dieback and the demise of sandalwood trees. Genome sequencing of 'Ca. Phytoplasma asteris' SW86 isolate from Marayoor reserve, utilizing Illumina and Oxford Nanopore Technology platforms, enabled a targeted hybrid metagenomic assembly resulting in 20 scaffolds totaling 554,025 bp. Further, genome analysis revealed the presence of putative effector genes like homologues of SAP11, SAP05 and Tengu su. TENGU, an effector from 'Ca. P. asteris' Onion Yellow strain (OY), is known to induce dwarfism, witches' broom, and sterility in the host plant. However, the molecular mechanism of TENGU pathogenicity in the host plant remains unexplored. A yeast two-hybrid assay was employed to identify putative interactors of the TENGU homolog from 'Ca. P. asteris' strain SW86, known to be associated with sandal spike disease of sandalwood. The tengu gene, with its signal peptide removed, was cloned into the vector pGBKT7 in fusion with the GAL4 Binding Domain (BD), which was screened against an *Arabidopsis thaliana* cDNA library cloned into pGADT7, which was in fusion with the GAL4 Activation Domain (AD). Blue yeast growth on a medium lacking Adenine, Histidine, Leucine, Tryptophan, and in the presence of aureobasidin, confirms AD and BD construct interaction. 47 potential interactions were identified. Some of the major interactors found were Ubiquitin-Conjugating Enzyme 19 (UBC19), Nuclear Fusion Defective 3 (NFD3), and HY5-homologue (HYH). Based on these findings, we suggest various pathways through which TENGU pathogenicity in the host plant can be explained.





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The advertisement features a ThermoFisher Scientific EVOS M5000 cell imaging system. The microscope is white and black, with a large touchscreen monitor displaying a grid of 96 wells, likely for a microplate. The background is a vibrant, abstract image of cells in shades of purple and blue. The ThermoFisher Scientific logo is in the top right corner. The text 'EVOS Cell Imaging Systems' is prominently displayed, followed by the tagline 'Superb optics, integrated usability, compact, simple yet powerful.' The 'invitrogen' logo is visible in the bottom right corner.

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