inStem

INSTITUTE FOR STEM CELL SCIENCE
AND REGENERATIVE MEDICINE

Annual Report
2017-2018

inStem

Image courtesy: Ravi Kumar Boyapati, NCBS

Content

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Director's Note

As predicted last year, we might need sunglasses to watch the bright lights of 2018, and I must say that this has borne out. Our move into our new building is a reality, and several laboratories—in particular the Regulation of Cell Fate **(RCF)** and the Centre for Inflammation and Tissue Homeostasis **(CITH)** themes have transited into this sparkling new space, and have begun to appreciate their spacious new digs. As with any new move, teething problem abound but in balance, the occupation of the new building is a reality and a welcome one.

It's a fitting testament to the construction team and the functional state of the new building, that India's first Cryo-EM was installed under the aegis and made operation as a National Facility, generating very exciting data from laboratories on the campus and across the country. The credit for making this happen should go to the joint efforts of inStem and NCBS investigators, Ramaswamy and Vinothkumar, respectively. This facility was inaugurated in January of 2018 by Bharat Ratna, CNR Rao, in the presence of Nobel Prize-winning Richard Henderson, the man who revolutionized single particle Cryo-EM for structural biology. This facility and its Big Data component reflects one of the outcomes of the Bangalore Life Sciences Cluster growing to foster strong ties between our institutes, and providing the bandwidth for leveraging large scale funding opportunities, necessary for the creating of such facilities.

In the past year, science at inStem has reached new heights and the Annual Report documents this with accounts from most of the laboratories putting out extremely important papers that are building a strong foundation for stem cell science and its translation. Once again, I must congratulate the efforts of our Dean, ***Apurva Sarin,*** and the entire Scientific Advisory Board for their unstinting support and encouragement for helping to build a vigorously collaborative set of themes.

In its formative years, inStem has been driven by a thin sliver of senior scientists and a very large number of younger colleagues. A sign of a maturing institution is the development of a middle-tier of scientists. We are delighted to note that ***Colin Jamora*** provided a great account of his work conducted at the Joint Research Laboratory at inStem at the Centre of Tissue inflammation and Homeostasis (CITH), and successfully navigated a very high power joint review committee with our partners, Institute for Molecular Oncology (IFOM), to be promoted to Investigator at inStem. He has also been commended for adroitly steering his theme. ***Srikala Raghavan,*** also at CITH and ***Dasaradhi Palakodeti*** at the Technology for Advancement of Science (TAS) theme, also successfully negotiated their transitions to Associate Investigators at inStem.

The Centre for Chemical Biology and Therapeutics (CCBT) theme, also underwent a major program review and once again we are delighted to report a very positive and enthusiastic outcome, where the 'undruggable' becoming druggable by the perseverance of the CCBT team was fully recognized. Under the leadership of ***Prof. Ashok Venkitaraman,*** CCBT has shown convincing evidence that new cancer targets and corresponding small molecule drugs are foreseeable in near future form the hitherto 'undruggable' protein-protein interaction inhibitor space. This effort has now opened up a number of new engagements on the campus and brought in many other from beyond to engage with the platforms set up at CCBT, in different protein-protein interaction based disease target screens.

The Accelerator program for Discovery in Brain disorders using Stem cells (ADBS) at the Centre for Brain

Disorders and Repair theme has grown from strength to strength, and now is the site for one of the most deeply clinically phenotyped cohorts for familial neuropsychriatirc disease. Here an inter institutional engagement driven by ***Mahendra Rao,*** Collaborative Science Chair at inStem, with ***Raghu Padinjat*** at NCBS and ***Sanjeev Jain*** and his clinical colleagues NIMHANS, has reached an important juncture. With the publication of the protocol for this cohort study, and the creation of a 300 + and growing target set of 500 individual distributed over several hundred families, this is turning out to be a major resource for research and clinical discovery. The clinical links that the collaborative program Centre for NeuroSynapthopathies (CNS) driven by ***Shona Chattarji*** with the University of Edinburg, has developed neurophysiology and neuronal differentiation protocols which are going to be tremendous opportunities for the ADBS programme too. These two programmes of the DBT in the area of brain disorders, its understanding, and its remediation by the deployment of Stem cell technology, are steering a flagship effort with the DBT in developing a Bio-repository of Stem Cell lines from patients, and this when developed should provide a tremendous resource for the scientific community.

In October we inaugurated The Tata Institute of Genetics and Society Centre at inStem (TIGS-CI) and we are looking forward to the deployment of active genetics in stem cells and any model organism. We also see that this as an extraordinary opportunity for researchers at inStem and elsewhere on the campus to grasp this new technology, while TIGS-CI is dedicated towards clear translational goals in the areas of health and agriculture.

New efforts at engaging in Science communication in a variety of forms have been initiated thanks to ***Mahinn Ali Khan,*** who has joined us as our Head of Communications for the campus. We look forward to the efforts fructifying in the coming years, and warmly welcome Mahinn to the Bangalore Life Science Cluster.

On behalf of all of us at this campus, I would like to offer our sincere appreciation to ***Mr. T T Jagannathan,*** Chairman of TTK Prestige for his generous donation for our international outreach activity, and express our deepest gratitude to ***Kris Gopalakrishnan*** and ***Kiran Mazumdar*** for their constant support and inspiration.

While there is a palpable atmosphere of collaborative inquiry at inStem, each theme has developed a distinct identity and way of functioning. This is an example of how theme based modes of operation create a unique identity of a scientific institute in the 21st century, distinct from the traditional PI driven structure. As we enter our 10th year at inStem, it would be important for us to reflect on future growth, and ensure that the sum of all the parts adds up to more. We need to put in place robust mechanisms for the sustainability of our science, that requires the necessary flexibility to develop new areas and hire the brightest minds and the right staff to secure our future decades. We still have a few more hurdles to cross before we are able to do this, but the seeds of an appropriate culture have been sown and the shoots of these efforts are emerging above ground. We now anticipate the flowering of these saplings in the coming years.

Satyajit Mayor
Director, inStem

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Administration Report

The Institute has completed its ninth year in its pursuit for excellence in stem cell research and allied areas. Following the approval of the Revised Cost Estimate (RCE-II) in February 2017 by the *Department of Biotechnology, Government of India,* the infrastructure development had resumed and the work is in full-swing. Every effort is being made to ensure that these works will be completed by the end of September/December 2018.

The National Centre for Biological Sciences *(NCBS)*/ Tata Institute of Fundamental Research *(TIFR)* and the Centre for Cellular and Molecular Platforms *(C-CAMP)* continued to extend shared services to inStem as participants in the Bangalore Life Science Cluster *(BLiSc).* This has resulted in sharing of resources at optimum level as well as saving in costs, if these services were to be run independently. Based on the positive experience and economies that are being derived a formal system is being proposed through a memorandum of understanding between the participating institutions.

While CSCR's (a centre of the Institute situated in Vellore) accounts are integrated into the accounts of the Institute for the year, an addendum is added giving a bird's eye view of the accounts of C-CAMP (an independent non-profit company registered under the Companies Act) established by and through the initiative of the inStem.

The table below indicates the status of grants received and the manpower count at the end of 31/3/18

DETAILS	2016-17	2017-18
Core grants received	₹ 687.00 million	₹ 853.00 million
EMG grants received	₹ 908.87 million	₹ 457.78 million
No. of active grants	55	56
Manpower	232	308

Within the limited positions that were available to the Institute and with the support of temporary staff checks and balances were introduced wherever feasible to ensure continued systemic improvements as well as to provide services to the scientific and technical activities as smoothly as possible. In achieving this, colleagues in Scientific, Technical *(including in services and construction)* groups worked in unison with the administration, and their support is much appreciated.

With the approval of RCE-II and the sustained efforts of the authorities of the Institute and those in the Department of Biotechnology to obtain pending approval for positions combined with the availability of its own building, the Institute functioning will be much smoother and its contributions will increase considerably in the years to come. The RCE status has closed w.ef. 31/03/2018 and regular budgetary sanctions have been received for 2018-19. Initially a tentative budget of Rs. 835.70 million has been sanctioned.

K. Krishnama Raju *(until September 2017)*

B. S. Nagaraja *(from September 2017), as OSD, holding charge as Head-Administration, inStem*

Image courtesy: Ravi Kumar Boyapati, NCBS

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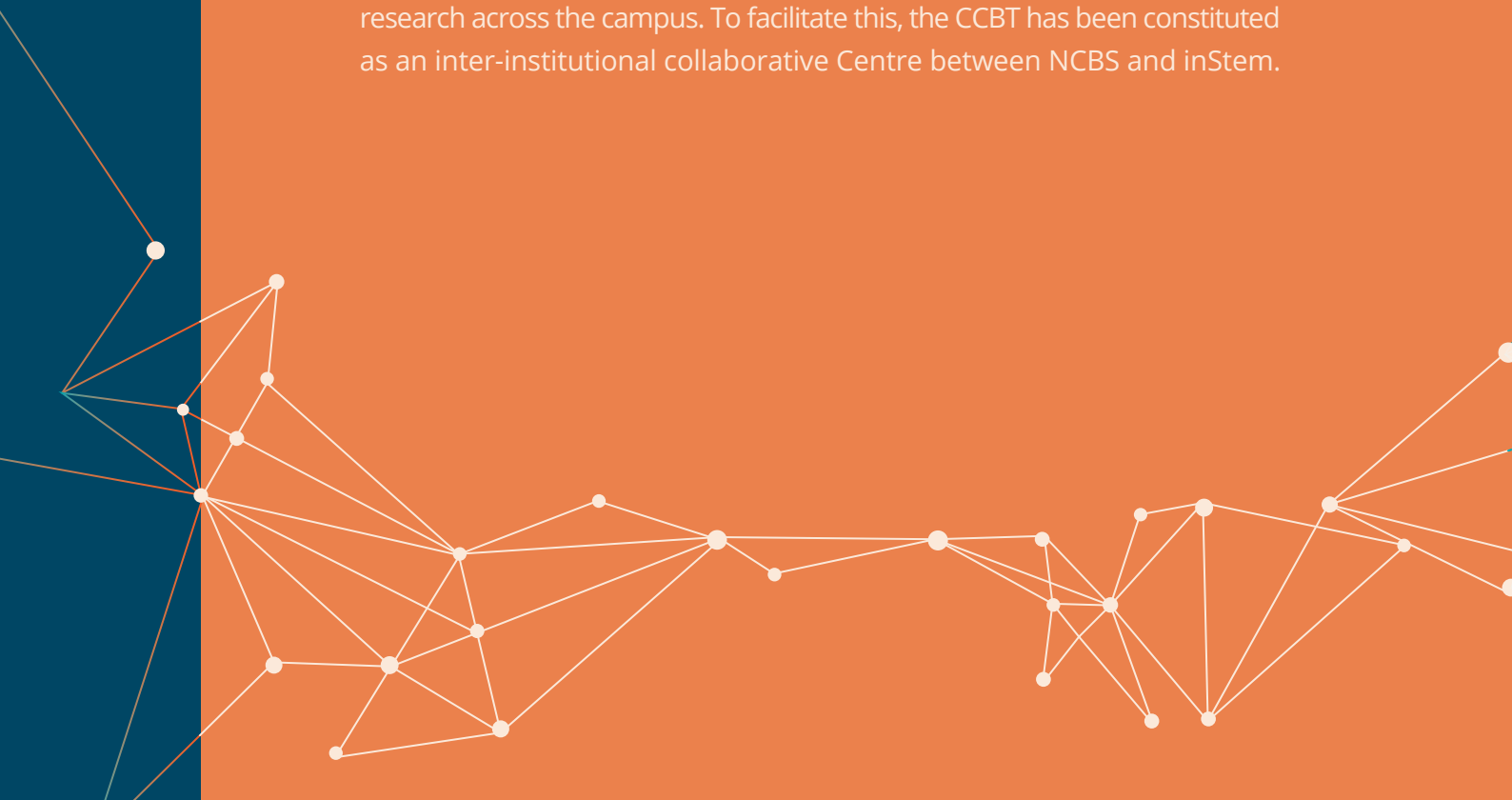
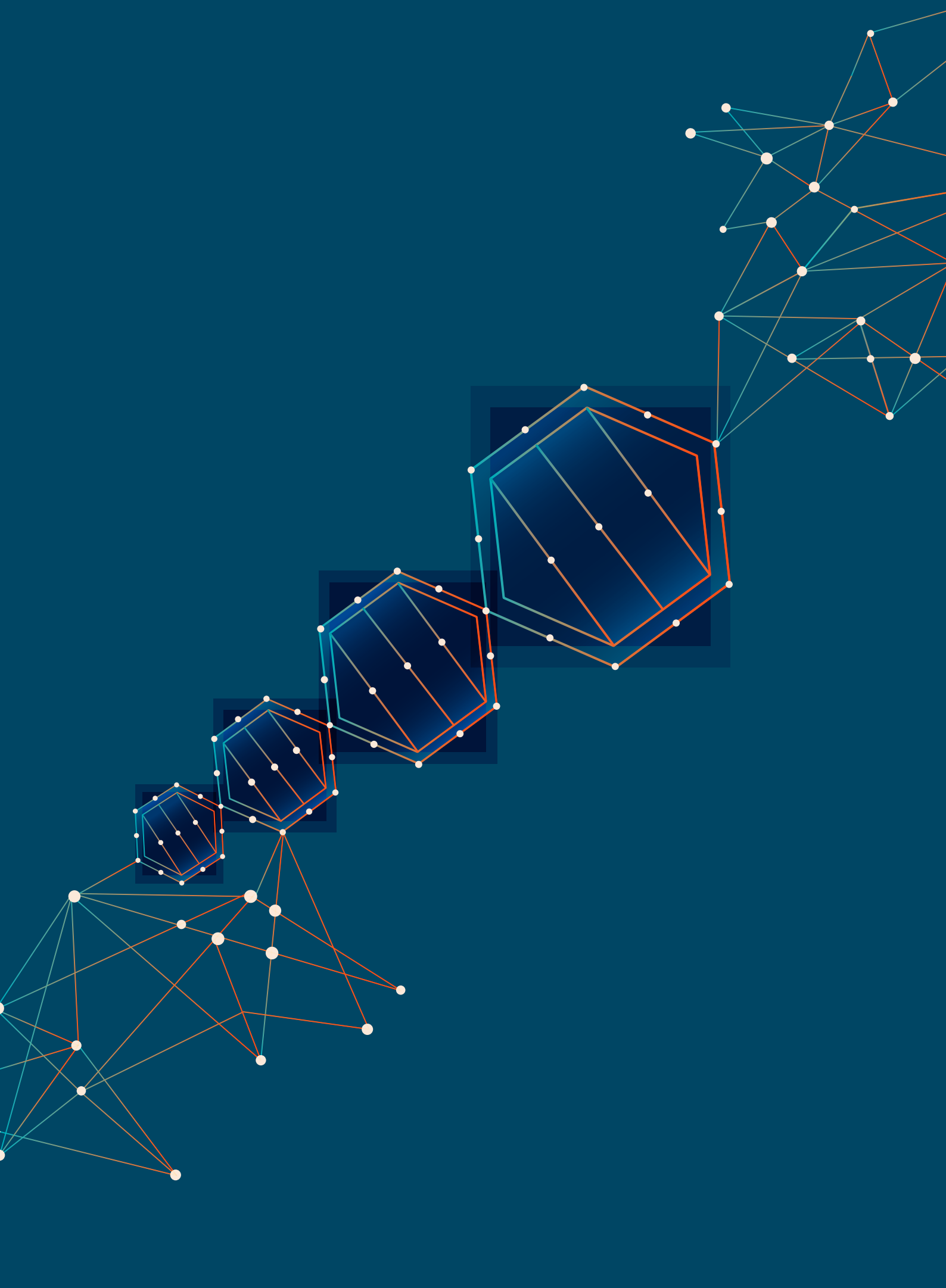
CCBT

Centre for Chemical Biology and Therapeutics

The Centre for Chemical Biology and Therapeutics (CCBT) is an integrated, multidisciplinary programme whose central aim is to develop innovative approaches to create chemical tools that modulate novel classes of targets, in order to explore the fundamental biological mechanisms underlying human diseases like cancer. Our long-term vision is not only to provide novel insights into disease mechanisms, but also to translate this new knowledge into the discovery of novel approaches for therapy. We expect our work to provide a framework for chemical biology and translational research across the campus. To facilitate this, the CCBT has been constituted as an inter-institutional collaborative Centre between NCBS and inStem.





3.1

Interrupting Intracellular Signalling via Phosphopeptide Recognition

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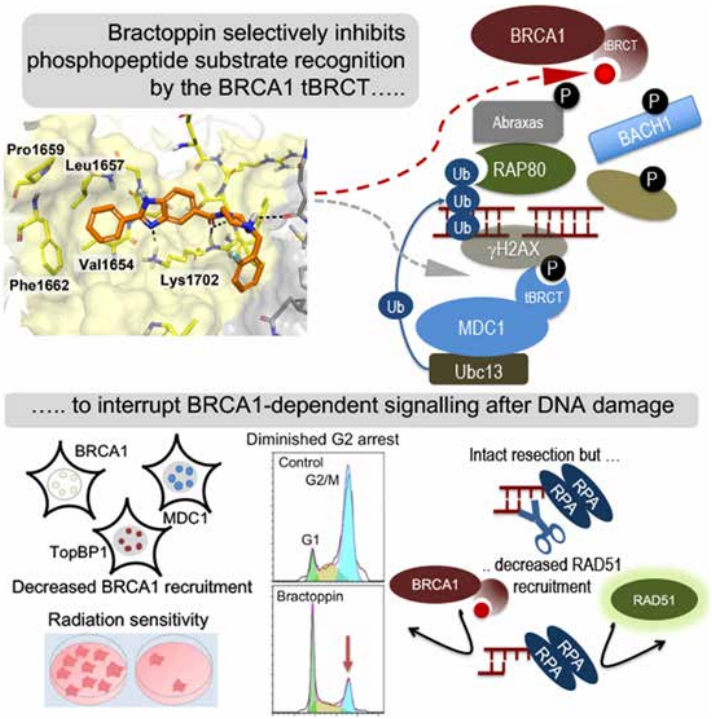
The Centre for Chemical Biology and Therapeutics (CCBT) pioneers innovative approaches to create chemical tools that modulate novel classes of targets, in order to explore the fundamental biological mechanisms underlying human diseases, with the long-term vision to seed the discovery of new therapeutics.

Despite the recent explosion in knowledge concerning the genetic basis for human diseases, there has not yet been a corresponding increase in our ability to develop new medicines for their treatment. One major scientific impediment is that only about 10% of proteins encoded in the human genome has so far been targeted using selective chemical probes or drugs. For instance, our ability to modulate intracellular signalling pathways implicated in disease has thus far been largely restricted to ATP-competitive inhibitors of protein kinase enzymes that initiate these pathways, although this approach is frequently compromised by lack of specificity and the pleiotropy of induced biological effects.

The **CCBT** has embarked over the past three years on a research programme to explore a new approach to modulate intracellular signalling pathways by targeting the molecular recognition of phosphopeptide substrates via specific protein domains. Typically, such molecular recognition events transduce signals downstream of protein kinase activity. Over 10 different domains found in human proteins mediate phosphopeptide recognition during protein kinase signalling. They range from SH2 domains, which recognize pTyr residues embedded in specific motifs, to BRCT domains, which recognize pSer or pThr motifs using structurally distinct mechanisms. Hundreds of proteins – which participate in many different signalling pathways – utilize such domains to transduce intracellular signals.

Whether it is feasible to create drug-like chemical probes that modulate with high selectivity and cellular activity the molecular recognition of phosphopeptide substrates via specific protein domains remains unclear. Our first objective has been to address this question in the context of one important domain family that is critical for intracellular signalling pathways that control genome duplication and repair.

Intracellular signals triggered by DNA breakage in human cells flow through proteins containing BRCT (BRCA1 C-terminal) domains. This family, comprising 23 conserved phosphopeptide-binding modules in man, is inaccessible to small-molecule chemical inhibitors. During 2017-18, we have reported *(Periasamy et al., Cell Chemical Biology)* the development of ***Bractoppin,*** a drug-like inhibitor of phosphopeptide recognition by the human BRCA1 tandem (t)BRCT domain, which selectively inhibits substrate binding with nanomolar potency in vitro. Structure-activity exploration suggests that Bractoppin engages BRCA1 tBRCT residues recog-



nizing pSer in the consensus motif, pSer-Pro-Thr-Phe, plus an abutting hydrophobic pocket that is distinct in structurally related tBRCT domains, conferring selectivity. In cells, Bractoppin inhibits substrate recognition detected by Forster resonance energy transfer, and diminishes BRCA1 recruitment to DNA breaks, in turn suppressing damage-induced G2 arrest and assembly of the recombinase, RAD51. But damage-induced MDC1 recruitment, single-stranded DNA (ssDNA) generation, and TOPBP1 recruitment remain unaffected.

Thus, our findings demonstrate that a drug-like inhibitor of phosphopeptide recognition selectively interrupts BRCA1 tBRCT-dependent signals evoked by DNA damage, and exemplify the potential of such an approach to interrupt intracellular signalling via the modulation of phosphopeptide recognition.

We expect in coming years to report on an ambitious repertoire of ongoing research programmes that aim to build on ourinitial success to deliver, in a stepwise fashion, a palette of novel chemical probes that selectively modulate the molecular recognition of pSer, pThr or pTyr-containing substrates in order to systematically explore how this mechanism contributes to intracellular signalling. These programmes will also establish a powerful inter-disciplinary capability for chemical biology and therapeutics development in the inStem/NCBS campus.

PUBLICATIONS

Targeting Phosphopeptide Recognition by the Human BRCA1 Tandem BRCT Domain to Interrupt BRCA1-Dependent Signalling (Cell Chemical Biology (2018) 25(6):677-690)

Jayaprakash Periasamy, Vadiraj Kurdekar, Subbarao Jasti, Mamatha B. Nijaguna, Sanjana Boggaram, Manjunath A. Hurakadli, Dhruv Raina, Lokavya Meenakshi Kurup, Chetan Chintha, Kavyashree Manjunath, Aneesh Goyal, Gayathri Sadasivam, Kavitha Bharatham, Muralidhara Padigaru, Vijay Potluri, and Ashok R. Venkitaraman

Indian Provisional Patent Application No.: 201741030084

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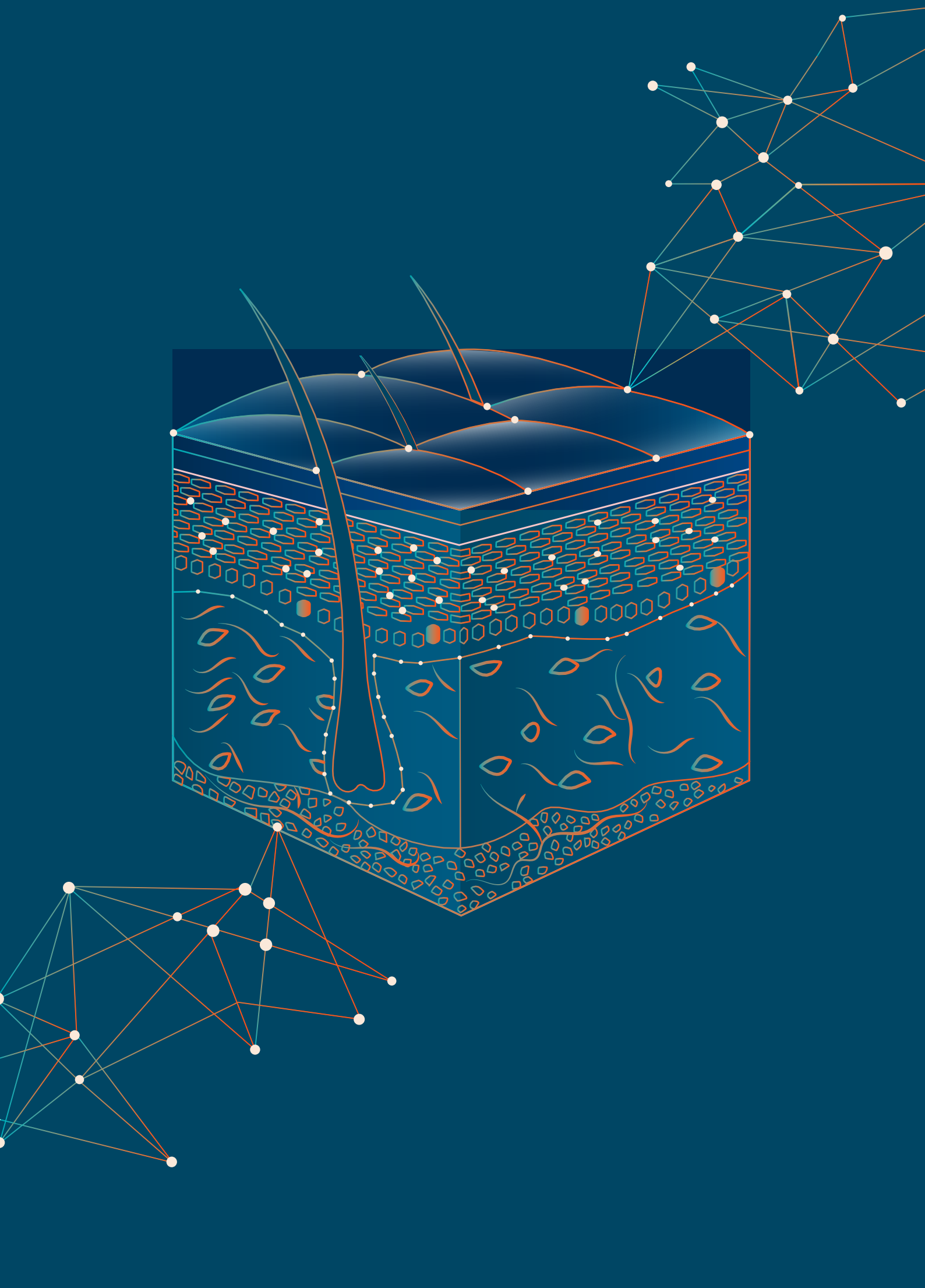
CITH

Centre for Inflammation and Tissue Homeostasis

The Centre for Inflammation and Tissue Homeostasis (CITH) is an inter-disciplinary research hub at inStem for investigating skin regeneration and repair. The mission of the laboratories that comprise CITH is to understand the basic processes of how to heal wounds and regenerate organs lost to age, injury, or disease. It is increasingly being appreciated that a common characteristic of growing older, wound healing, and many diseases is the presence of *tissue inflammation,* which is classically viewed as a physiological response to fight off invading pathogens. Through our scientific activities, we have been advocating the emerging concept that inflammation plays more roles in regulating tissue integrity and repair in addition to merely offering protection from infections. Our aim is to leverage this knowledge to identify and develop new treatments and cures for a host of common diseases such as *diabetes, inflammatory skin diseases,* and *cancer.*



4.1

Understanding Wound Healing Programmes and Allied Diseases

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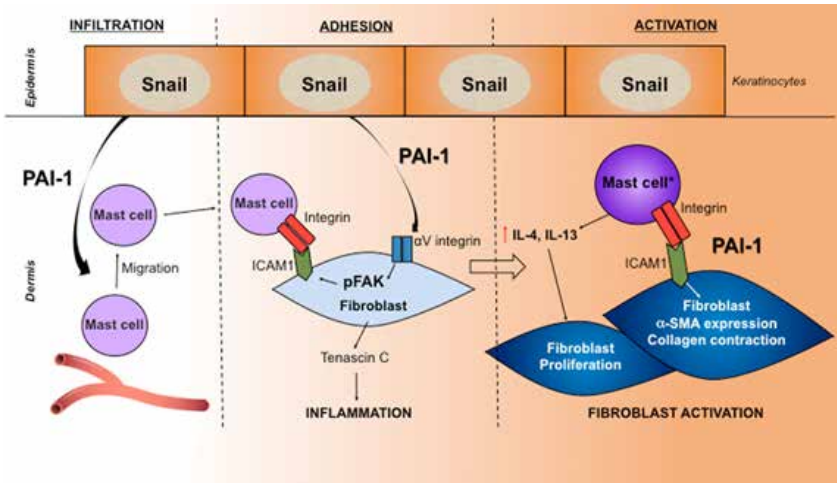
The IFOM-inStem Joint Research Laboratory headed by Colin Jamora works on deciphering the molecular mechanisms underlying wound healing. The goal is to utilize this knowledge to develop therapies for diseases where wound healing is deregulated, such as in diabetes, fibrotic diseases and cancer.

The powerful lure of the field of regenerative medicine is based on the promise of being able to design and repair tissues of the human body to restore parts lost to trauma or disease. Interestingly, regeneration *imitates and adapts* many processes found in embryogenesis and tissue morphogenesis. Therefore, a thorough understanding of these developmental processes beginning with individual stem cells through the formation of functional three dimensional organs is a prerequisite to making this field a reality. Among the organs in the body, the mammalian skin has remarkable regenerative abilities and is thus a prime model for elucidating the fundamental mechanisms regulating tissue regeneration and repair. In particular, the outer layer of the skin *(the epidermis)* is one of the few tissues that constantly regenerates throughout the lifetime of the animal. This capability renders it an outstanding model system for following how stem cells fuel regeneration. Moreover, due to its protective function as a barrier from the external environment, the epidermis is constantly damaged and must mount a wound-healing programme to rapidly restore tissue architecture and function. Both through our work and that of others, important strides have been made in the discovery of the rules of tissue formation, the interactions that occur between different cells within an organ, and the mechanisms underlying the regulation of tissue homeostasis. Importantly, insights gained from these studies will produce novel information on how to combat a plethora of diseases in which one or more of the processes of tissue repair and regeneration go awry.

Over the past year we have made important advances in understanding how cells of the immune system play an important role in mediating tissue repair during the wound-healing programme. Upon injury, the tissue immediately launches a self-limited inflammatory response to provide a defence against pathogens and play an important role in orchestrating activities that expedite recovery. However, the benefits of inflammation recede when these responses fail to resolve in a timely manner. For instance, we found that a type of immune cell that normally resides in the skin, called a γδ T-cell, can activate the stem cells in the hair follicle to stimulate them to assist in the repair of wounded skin *(Lee et al., eLife 2017)*.

Moreover, we recently uncovered how a protein called Plasminogen Activator Inhibitor type 1 (aka PAI-1) can cause another type of immune cell to infiltrate into



▲ ***Figure 1 :*** *Role of PAI-1 in mediating the interaction of mast cells and dermal fibroblasts (From Pincha et al., JCI 2018)*

the skin upon wounding. This immune cell is known as a mast cell and is a well-known mediator of allergic responses. In the context of wound healing, we found that PAI-1 not only can recruit mast cells to the skin but also mediates their interaction with the dermal fibroblasts *(Figure 1)*. These dermal fibroblasts are the major source of collagen, which provides structural support for the skin and is especially important during the rebuilding of the damaged tissue. The binding of the mast cells to the fibroblasts results in their mutual activation – fibroblasts respond by increasing their production of collagen and mast cells become activated and release proteins that further promote the wound healing response *(Pincha et al., JCI 2018)*. Limiting the duration in which these fibroblasts are activated is important as prolonged activation can lead to an over-scarring condition known as *fibrosis*. The danger posed by fibrosis is that the overproduction of collagen and similar proteins can cause the hardening of the tissue and ultimately compromise normal tissue function. As such, PAI-1 may be an attractive target in limiting the activation of fibroblasts so that their normal function doesn't become exaggerated and thereby lead to a pathological condition.

PUBLICATIONS

Interactions Between Epidermal Keratinocytes, Dendritic Epidermal T-Cells, and Hair Follicle Stem Cells. (Methods Mol Biol. 2018 Jun 13. doi: 10.1007/7651_2018_155.)
Badarinath K, Dutta A, Hegde A, Pincha N, Gund R, Jamora C. (2018)

PAI1 Mediates Fibroblast-Mast Cell Interactions in Skin Fibrosis (Journal of Clin. Invest. 2018 May 1; 128(5):1807-1819. doi: 10.1172/JCI99088. Epub 2018 Mar 26.)
Pincha N, Hajam EY, Badarinath K, Batta SPR, Masudi T, Dey R, Andreasen P, Kawakami T, Samuel R, George R, Danda D, Jacob PM, Jamora C. (2018)

Stimulation of Hair Follicle Stem Cell Proliferation Through an IL-1 Dependent Activation of γδT-cells (Elife. 2017 Dec 4;6. pii: e28875. doi: 10.7554/eLife.28875.)
Lee P, Gund R, Dutta A, Pincha N, Rana I, Ghosh S, Witherden D, Kandyba E, MacLeod A, Kobielak K, *Havran WL, Jamora C. (2017)*

4.2

Elucidating Unconventional Mechanisms by which KMTs Influence Cell Fate Decisions

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Broad areas of research in the laboratory lie at the interface of epigenetics and stem cell biology. We are addressing the role of repressive histone methyltransferases in the commitment of cell fate from stem cell through aging.

Lysine methyltransferases (KMTs) that deposit methylation marks on proteins are critical determinants in various facets of development and disease. Deletions mutations of KMTs lead to either embryonic lethality or developmental defects. In addition, misregulation of KMTs is often noticed in multiple cancers. Till date, KMTs have been best studied for their role in methylating histones thereby regulating gene expression. Owing to the scarcity of knowledge on non-histone methylations, it remains elusive how the non-histone methyl proteome impacts development and disease.

My laboratory is investigating the canonical and non-canonical mechanisms by which KMTs regulate cell plasticity in development and how perturbations of these mechanisms lead to disease states. We have been successful in generating human stem cell based tools and mouse models to ask the questions as to how histone (canonical) and non-histone proteins methylations (non-canonical) could converge to ensure desired biological outcomes.

The following are key programmes of study in my laboratory:

Mechanisms Underlying Cellular Plasticity Regulated by KMTs

The relevance of spatial organization of chromatin in ESCs for the maintenance of pluripotency has been extensively studied. In the sequence of events leading to pluripotency during reprogramming of a somatic cell, the genome is reorganized by a number of epigenetic modifiers. In a significant contribution towards defining the role of lysine histone methyltransferase (KMT) EZH2 in reprogramming, work from my laboratory demonstrated that EZH2 drives mesenchymal to epithelial transition during human iPSC generation. Although the requirement of KMTs in cellular reprogramming has been extensively studied in terms of histone modifications, there is a notable lack of understanding of how non-histone protein interactions of KMTs can influence genome reorganization. We identified a key role for Euchromatic histone methyltransferase1 (EHMT1) in organizing heterochromatin via its histone and non-histone lysine methylation. Ongoing work in the laboratory indicates during early stages of reprogramming, EHMT1 is displaced from the

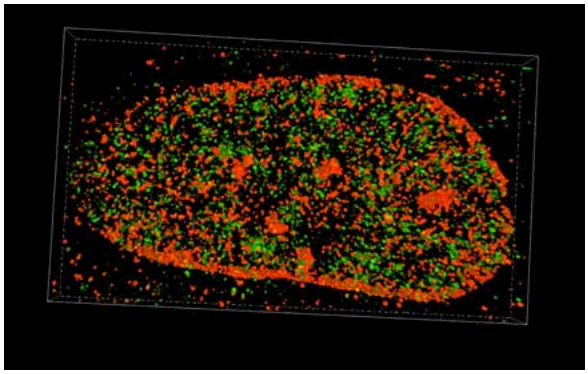
nucleus, which coincides with dismantling chromatin organization. Currently my lab is exploring how EHMT1 is exported out of the nucleus and what role the cytoplasmic pool of EHMT1 might play in reprogramming.

Function of KMTs in Tissue Repair and Homeostasis

The process of tissue repair is highly complex and requires orchestrated and coordinated control on transient activation and repression of genes from cells of different origin. While histone methyltransferases (HMTs) are key players of development and disease, their role in wound healing still remains poorly defined. Studies in our laboratory indicated elevated expression of EZH2 during the process of normal wound healing. Literature demonstrates cell type conversions are triggered by injury as repair response to promote tissue homeostasis. Thus, we are investigating the effects of EZH2 in differentiation/trans differentiating of skin cells during repair and aging.

Contribution of KMTs to Aging and Age Related Diseases

Aging is a pleiotropic and time dependent process governed by genetic and environmental factors resulting in loss of normal cellular plasticity. At the molecular level, aging leads to degenerative epigenomic changes in somatic as well as stem cell components that are responsible for the progressive loss of homeostatic and regenerative potential of a given tissue. In my laboratory we focused our studies on non-histone methylation of HMTs that contributes to loss of cell plasticity thereby resulting in aging. Towards this we have demonstrated that the EHMT mediated H3K9 methylation and non-histone methylation of LaminB1 are critical determinants of heterochromatin organization and its impact on fundamental changes associated with the aging process. We are also investigating the contribution of EHMT1 in age related metabolic disease such as diabetes and obesity. Our data indicates deregulation of EHMT1 expression can serve as an early biomarker for the pathogenesis of obesity.



▲ ***Figure 1:*** *SIM imaging of the fibroblasts nucleus identifies localization of EHMT1 (Green) and LaminB1 (Red) at the nuclear periphery (Width-13.38 µM; height - 23.49 µM; depth- 2.88µM)*

PUBLICATIONS

Setting Up Methylation in Mammalian Cells: Role of Histone Methyl Transferases in Disease and Development (Molecular and cellular therapeutics in human disease. Invited book by Springer Publications 2018)
Abhishek Mohanty and Shravanti Rampalli (2018)

TALKS & OUTREACH

Euchromatic Histone Methyl Transferases Regulate Heterochromatin Organization During Aging – International Congress of Cell Biology CCMB, Hyderabad, India, January 2018.

Euchromatic Histone Methyl Transferases in Aging and Inflammation – Stem Cell Biology and Disease Models. Bangalore, India, February 2018.

Epigenetics in Stem Cells – Epigenetics in Human Health Workshop, Department of Life Sciences, Garden City University, Bangalore, India, March 2018.

Stem Cells and Disease Modelling – Pluripotent Stem Cells Workshop, Centre for Stem Cell Research (CSCR), Vellore, India, April 2018.

Non-histone Interaction of Ehmt1 Regulated Cellular Aging – Transcription Assembly Meeting. Hyderabad, July 2018.

4.3

Epithelial Homeostasis and Inflammation: Integrins, Associated Proteins and Immune Cells

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Research in the Raghavan lab focuses on understanding the role of integrins, its associated proteins and immune cells in maintaining the stem cell niche and extracellular matrix organization, both of which are critical for the maintenance of epithelial homeostasis.

Understanding the Role of Vinculin in Maintaining Stem Cell Homeostasis in Skin

Vinculin is a mechano-coupling, protein that is found both at *cell-cell (adherens)* junctions and *cell-substratum (focal)* adhesions. Vinculin helps to link the actin cytoskeleton to the junctions at the cell membrane. It acts as a docking protein for several focal adhesion partners and alpha-actinin at cell-cell junctions thereby regulating several signaling pathways induced by mechanical forces. In order to study the roles of vinculin in keratiocytes, we generated a skin specific conditional KO. The vinculin knockout mice displayed loss of hair, unusual size and abnormally shaped hair follicles forming cyst like structures and acceleration of the hair follicle cycle, which interestingly did not lead to complete hair loss. Label retaining experiments revealed that the hair follicle stem cells fail to maintain their quiescence in the KO, which may explain their continuous cycling. The bulge cells in the KO show defects in maintaining normal cell-cell junctions, which results in the nuclear translocation of YAP. We also observed that the phenotype of vinculin null cells was similar to the alpha catenin null keratinocytes, highlighted the role of these two proteins in maintaining stable adherens junctions. Our results suggest that the loss of cell junction stability can override the intrinsic quiescence of bulge stem cells.

Understanding the Crosstalk Between Epithelia and the Immune System

Macrophages are highly plastic subsets of leukocytes, which play crucial role in inflammation and tissue homeostasis. Their plasticity and heterogeneity depend on micro environmental cues and organ localization. During initial stages of inflammatory or wound response, they act as potent inflammatory macrophages (M1) and in later stages, where tissue remodeling is crucial, they display a reparative (M2) phenotype. This phenomenon of state switching is termed as Macrophage Polarization and is being extensively studied in adult systems. However, Embryonic inflammatory signature differs from that of the adult mouse. For example, macrophages in embryos originating from erythro-myeloid progenitors seed the tissues as opposed to the bone marrow derived monocytes that are supplied to adult tissues, during inflammation and tissue remodeling. While, the concept of the macrophage polarization states during inflammation

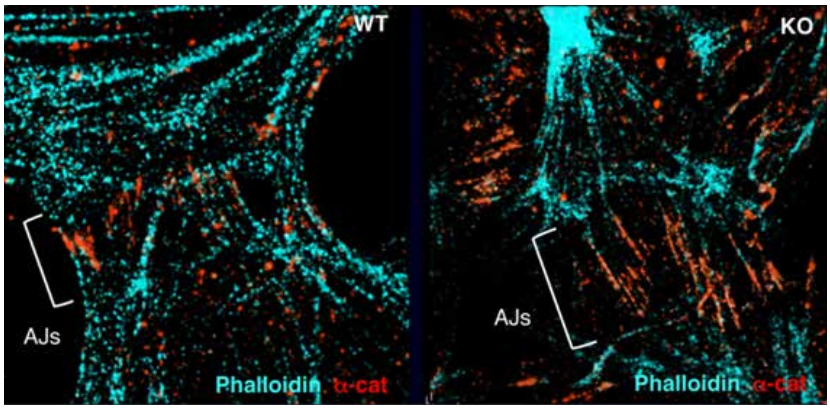

Figure 1: Abnormal cell-cell junctions in vinculin KO cells. Scale bar 5μM.

is still being challenged in adult systems, the role of macrophages in embryonic inflammation is yet to be fully explored. Using the conditional integrin β1 knockout embryos that serve as a very good model for embryonic sterile inflammation, we aim to understand the mechanisms underlying macrophage polarization and its crosstalk with the epithelia in development and disease. Using specific blocking antibodies that block macrophage maturation and recruitment (CSF1R) and NSAIDS like celecoxib we propose to study the crosstalk between epithelia and the tissue resident macrophages during inflammation.

PUBLICATIONS

Cytoplasmic Poly (A)-Binding Protein Critically Regulates Epidermal Maintenance and Turnover in the Planarian Schmidtea Mediterranea (Development. 2017 Sep 1; 144(17):3066-3079.)
Bansal D, Kulkarni J, Nadahalli K, Lakshmanan V, Krishna S, Sasidharan V, Geo J, Dilipkumar S, Pasricha R, Gulyani A, Raghavan S, and Palakodeti D. (2017)

Isolating Immune Cells from Mouse Embryonic Skin (Methods Mol Biol. 2018 May 24. doi: 10.1007/7651_2018_148. [Epub ahead of print])
Kurbet AS, and Raghavan S. (2018)

TALKS & OUTREACH

Breaking Barriers: Role of Integrins in Epithelial Homeostasis and Sterile Inflammation – Indian Society of Developmental Biology, June 2017.

The Integrin Network: Role of Integrins in Epithelial Homeostasis and Sterile Inflammation – RTCMBR Conference, Shiv Nadar University, October 2017.

Role of Mechanotransduction in Maintaining Stem Cell Quiescence in Mouse Skin – EMBO Meeting On Frontiers in Cytoskeleton Research, October 2017.

Role of Vinculin in Maintaining Stem Cell Quiescence in Mouse Skin – Stem Cell Biology and Disease Models Mini-symposium, February 2018.

Role of Mechanotransduction in Maintaining Stem Cell Quiescence – inStem Annual Talks, Bangalore, February 2018.

Breaking Barriers: Role of Integrins in Epithelial Homeostasis and Sterile Inflammation – 5th International Conference on Cellular and Molecular Bioengineering (ICCMB5), Singapore, March 2018.

Role of Mechanotransduction in Maintaining Stem Cell Quiescence – Mechanobiology Institute (MBI), Singapore, March 2018.

The Integrin Network: Role of Integrins in Epithelial Homeostasis and Inflammation – Institute for Medical Biology (IMB), Singapore, March 2018.

5

CBDR

Centre for Brain Development and Research

Neurodevelopmental and ***neurodegenerative*** brain disorders represent a major and growing public health threat. **CBDR** hosts two major programmes, the Centre for Neurodevelopmental Synaptopathies **(CNS)**, an international collaborative centre between *inStem, NCBS and the University of Edinburgh*, and Accelerator Programme for Discovery in Brain Disorders using Stem cells **(ADBS),** a collaboration between *inStem, NCBS and clinicians at the National Institute of Mental Health and Neurological Sciences (NIMHANS)* at Bengaluru. The shared aim of these programmes is to accelerate the discovery and delivery of effective therapeutics for largely untreatable conditions. Although these are a disparate group of currently untreatable conditions that include acquired, developmental and ageing-related diseases, there are common themes and needs. Uniformly, the unmet need is for a human-based approach to investigating the causes, consequences and ultimately treatment of these diseases. To this end, CBDR has generated a range of expertise in several fields of neurobiology including synaptic function and plasticity, human stem cells and cognition behaviour.

5.1

CNS: Centre for Neurodevelopmental Synaptopathies

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Neurodevelopmental and neurodegenerative disorders pose a major and growing public health threat. Our research spans multiple levels of neural organisation and combines animal and human-based model systems to accelerate the discovery and delivery of effective therapeutics for these largely untreatable conditions.

Co-Principal Investigators

Siddharthan Chandran

Peter Kind

David Wyllie

Programme 1: Modelling Human ASDs "in a dish"

Siddharthan Chandran, David Wyllie, Sumantra Chattarji

To reduce ASDs to merely the consequence of genetic and biochemical dysfunction, invariably from a neuron-centric perspective, is to understate the highly evolved, dynamic and cellular connectedness of the human brain. Multiple lines of evidence from pathology, genetics, radiology and experimental systems implicate a central role for glia in: **(i)** not only maintaining *(and tuning)* neuronal function in health but also, dependent on disease context, being **(ii)** injurious or **(iii)** neuroprotective. Yet the role of microglia (astrocytes (AS), oligodendrocytes (OLG)) and microglia (MG) in ASDs is understudied. Dissecting the nature of cellular autonomy and the mechanism of glial-mediated influences on neuronal biology and function in ASDs requires an integration of diverse experimental approaches.

Exploiting human stem cell and gene editing technologies offers an unprecedented opportunity for the creation of both in vitro and, following transplant, in vivo human based systems that model aspects of ASDs. Importantly, human iPSC models of monogenic and even sporadic neurodevelopmental/psychiatric disorders have not only recapitulated disease phenotypes but also informed on disease mechanisms. Although the aetiology of the majority of ASDs remains poorly understood, accumulating genetic and pathological evidence implicate shared mechanisms. Genetics is therefore our starting point and we choose to study the highly penetrant monogenic causes of ASD/ID beginning with fragile X syndrome (FXS) to interrogate the role of cellular autonomy in FXS *(Figure 1).*

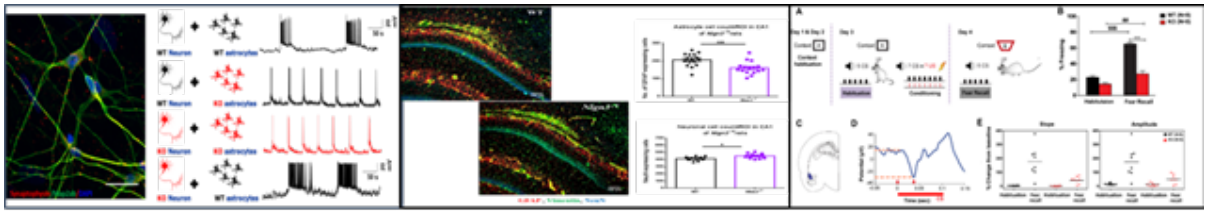

Figure 1 Figure 2 Figure 3

▲ *Figure 1: A illustrates the presence of the synaptic protein –Synaptophysin on human ipsc derived cortical neurons (Map2ab positive) 8 weeks in vitro. B represents the glial modulation of burst firing of cortical neurons.*

Figure 2: Images depict the Vimentin, NeuN and GFAP positive cells in the hippocampal CA1 of both WT and Nlgn3-/Y transgenic animals at the developmental time point of P28. GFAP in red, Vimentin in green and NeuN in blue.Scale bar= 100µm.

*Figure 3: The neural basis of impaired recall of conditioned fear in a rat model of FXS. (A) Experimental design. (B) Significant increase in freezing relative to tone habituation after fear conditioning in both WT. Impaired fear recall in KO rats. (C) Diagram of in vivo electrode placements for recordings. **(D)** Representative raw LFP trace recorded in the LA in response to CS. (E) Changes in auditory evoked potential slope (left) and amplitude (right) in the LA.*

Programme 2: The Autistic Network – From Pathways to Rescue

Sumantra Chattarji and Peter Kind

Using novel transgenic rat models of ASDs, we are addressing whether genetically heterogeneous disorders share common synaptic neuropathology, as well as whether the common synaptic pathophysiology that arises from shared "developmental" mechanisms could be a therapeutic target throughout the lifespan of the animal. For instance, do all forms of ASD have dysregulated protein synthesis or altered network firing as core deficits? This is being pursued in many ways. For instance, we are exploring whether the circuit maturation and development after birth to childhood is uniform. Circuits are not only made of neuronal cells connected with synapses but also require critical cellular interactions with non-neuronal, glial cells including astrocytes and oligodendrocytes. The role of glial cells and their malfunctioning is only being uncovered in ASD. We are characterizing the neuron/glia ratio, astrocyte and microglia shape and number, and finally metabolism. We have found alterations in astrocytic maturation and number in Neuroligin$^{-/Y}$, Neurexin$^{+/-}$ and PTEN$^{+/-}$ models with a concomitant change in populations of mature neurons in the hippocampus and amygdala at post-natal day 10 and 28 animals. Interestingly, no differences were found in the Cntnap2$^{+/-}$ strain. This divergence has important consequences for in vitro electrophysiological signatures and performance in behavioural tasks being characterized by other verticals of CBDR and offer an important avenue of future therapeutic intervention and manipulations *(Figure 2).*

Programme 3: Autistic Function – Rat Behaviour and Imaging

Peter Kind and Sumantra Chattarji

We continue to carry out detailed analyses across biological scales *(behavioural, electrophysiological and biochemical)* in our new rat models of highly penetrant single-gene causes of ASD/ID to better model autistic and cognitive deficits that can accurately reflect autistic features in humans. We are nearing completion of behavioural characterization of most of our ASD lines and several interesting phenomena have been uncovered. For instance, we found the recall of fear memories formed during Pavlovian auditory fear conditioning to be impaired in Fmr1-KO rats. To examine the neural basis of this deficit in the encoding and recall of fear memories formed by auditory fear conditioning, we carried out in vivo recordings of local field potentials (LFP) in the lateral nucleus of the amygdala (LA). This revealed that in the KO rats that showed impaired fear recall, both the slope and amplitude of LFPs evoked by the tone conditioned stimulus (CS) were reduced compared to their wild-type (WT) counterparts. Further, using whole-cell recordings in brain slices, we found a significant impairment in LTP in LA principal neurons in KO rats. This was also accompanied by a reduction in basal synaptic transmission at excitatory glutamatergic inputs to LA neurons of KO rats, as evidenced by decreased pre-synaptic release of transmitters. Taken together, these findings provide the first comprehensive framework, across biological scales from behaviour to synapses, for understanding the neural basis of abnormal amygdalar function in FXS *(Figure 3).*

PUBLICATIONS

Extinction Recall of Fear Memories Formed Before Stress is Not Affected Despite Amygdalar Hyperactivity *(Elife. 2018 Aug 13;7. pii: e35450. doi: 10.7554/eLife.35450)*
Rahman M. M., Shukla A., and Chattarji S (2018)

Repeated Social Stress Leads to Contrasting Patterns of Structural Plasticity in the Amygdala and Hippocampus
Patel D., Anilkumar S., Chattarji S., and Buwalda B (2018)

Hippocampal and Amygdalar Cell-Specific Translation is Similar Soon After Stress but Diverge Over Time (Hippocampus Jun;28(6):441-452)
Madan JS, Gupta K, Chattarji S, and Bhattacharya A (2018)

Activation of the Same Mglur5 Receptors in the Amygdala Causes Divergent Effects on Specific Versus Indiscriminate Fear (Elife. 30;6. pii: e25665)
Rahman MM, Kedia S, Fernandes G, and Chattarji S (2017)

Modeling the C9ORF72 Repeat Expansion Mutation Using Human Induced Pluripotent Stem Cells (Brain Pathology. 27, 4, 518-524)
Thangaraj Selvaraj B, Livesey MR, and Chandran S (2017)

Cell Type-Specific Translation Profiling Reveals a Novel Strategy for Treating Fragile X Syndrome (Neuron. 2; 95(3):550-563.e5)
Thomson SR, Seo SS., Barnes SA, Louros SR. Muscas M, Dando O, Kirby C,Hardingham GE, Wyllie DJA, Kind PC, and Osterweil EK (2017)

Sidekick No More: Neural Translation Control by p70 Ribosomal S6 Kinase 1 (Oxford Handbook on Translation. Online epub.May 2018. Ed. Wayne Sossin doi: 10.1093/oxfordhb/9780190686307.013.10)
Aditi Bhattacharya (2018)

TALKS & OUTREACH

FXS and Fear Learning: Alternative "Facts" from the Amygdala
Gordon Research Conference on Fragile X and Autism-related disorders. IlChiocco, Tuscany, Italy. June 2018.

Closing the Loop on Troubled Translation: Letters from Neuroligin 3 and Neurexin 1 Rat Models
Gordon Research Conference on Fragile X and Autism-related disorders. IlChiocco, Tuscany, Italy. June 2018.

Fragile X and Autism-Related Disorders
Gordon Research Conference 2018, Italy

Stress Neurobiology Workshop (2018)
The Banff Centre, Canada

Simons Foundation Autism Research Initiative (2018)
Science Meeting, New York, USA

PROMEMO Mini-symposium on Memory (2018)
Aarhus University, Denmark

Psychiatry, Genetics & Neuroscience (2018)
Wellcome Trust Genome Campus, UK

Stress: Past, Present and Future Directions (2018)
Princeton University, USA

5.2

ADBS: Accelerator Programme for Discovery in Brain Disorders

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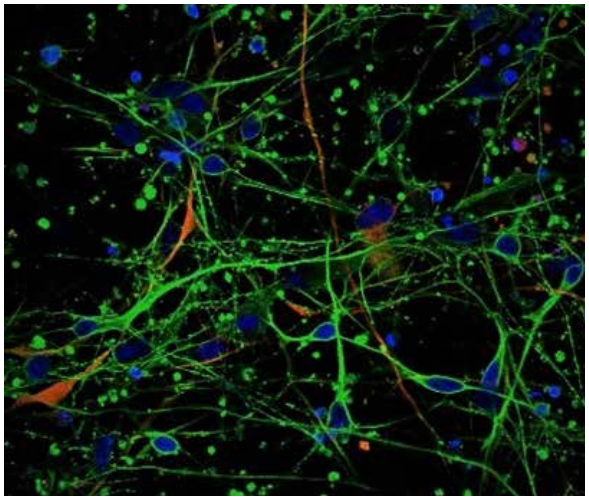
The Accelerator programme for Discovery in Brain disorders using Stem cells (ADBS) is a scientific venture to understand mental illness by harnessing the power of modern human genetics and stem cell technology. The programme is a collaborative initiative of three institutions from Bengaluru, India – the Institute for Stem Cell Science and Regenerative Medicine (inStem), the National Centre for Biological Sciences (NCBS) and the National Institute for Mental Health and Neurosciences (NIMHANS).

The **ADBS programme** is one component of the Accelerating the application of Stem cell technology in Human Diseases (ASHD) programme,which uses modern stem cell technology to create cellular models of the brain derived from human subjects taken from families with a strong history of mental illness. The overall goal is to uncover the genetic, cellular and molecular basis of mental disorders.

Psychiatric disorders are a major source of disability in young adults with about 2–3% of the population at risk for developing these disorders both in India and across the world. There is a pressing need to understand the mechanistic basis of these disorders so that novel diagnostic and therapeutic approaches can be developed.

Mental illnesses are recognized as having an inherited basis. However, despite their high heritability and identification of a large number of 'common' and rare variants, few genetic correlates that could account for their high prevalence have been identified. Many of the genes *(and pathways)* identified suggest aberrant neural development and connectivity in early life as being critical to their pathogenesis. The epigenetic changes that occur due to exposure to environmental influences during windows of sensitivity in the developing brain, give rise to different trajectories of brain development leading to variations in temperament, response to stress and substance abuse.

Given the gene/environment interactions that over time are likely to lead to psychiatric disorders, well-defined, prospective clinical cohorts offer a unique opportunity to understand the pathogenesis of mental disorders. In collaboration with the Department of Psychiatry, NIMHANS, we are assembling a cohort of families with a high density of mental illness. The families will be followed over a period of twenty years in order to observe the development of disease by regular and detailed clinical phenotyping. In addition, we have established immortalized stable cell lines and pluripotent stem cells from affected individuals in these families and unaffected controls. This material will be used to generate cellular models through which the mechanistic aspects of cellular neurobiology that lead to disease can be understood.



▲ *Figure 1: XCL1 Neurons stained with Neuronal marker TUJ1 (Green), Glial Marker GFAP (Red) and Nuclear stain DAPI (Blue).*

Collectively, we aim to understand the relationship between cellular phenotypes and disease progression. This will be accomplished through collaborative research applying genetic analysis and cell-based assays on patient derived cell lines in conjunction with information from detailed clinical analysis. The following are additional activities organized under the aegis of the ADBS programme:

Training programme in generation and maintenance of human iPSC

The CiRA-ADBS training programme is organized annually by the *ASHD programme* and *The Centre for iPS Cell Research and Application (CiRA), Kyoto University, Japan*. Eight Indian researchers *(1 junior faculty, 2 post-doctoral fellows, 3 PhD students and 2 Junior Researchers)* from various Indian institutes, attended the CiRA-ADBS training programme during Nov-Dec 2017.

Workshops:

The first ADBS Human iPSC Workshop 2017 on "Reprogramming Human Somatic Cells to HiPSCs" was held in November 6th -11th, 2017 at inStem, Bangalore. Researchers from ten Indian institutes/companies and one participant from an Institute in Nepal were selected for hands-on training at the workshop. In addition, ADBS organized an internal workshop on iPSC generation and maintenance in May 2018 for researchers in inStem, NCBS, C-CAMP and NIMHANS. 5 researchers were trained in this workshop.

Human Genetics and Disease Biology Meeting

The sequencing of the human genome has raised much excitement in the possibility of understanding the blueprint of life. Further, the development of low-cost high-throughput DNA sequencing has facilitated the analysis of large numbers of human genomes and revealed the diversity in DNA sequence within and between human populations. Upholding the importance of genomics research in human disease biology, the ADBS programme coordinated the *Human Genetics and Disease Biology Meeting* in June, 2018. This meeting brought together eminent leaders in academia and industry to discuss the opportunities and challenges for studies of human genetics and disease biology in India using new advances in DNA sequencing and stem cell technology.

PUBLICATIONS

Discovery Biology of Neuropsychiatric Syndromes (DBNS): A Centre for Integrating Clinical Medicine and Basic Science. (BMC Psychiatry. DOI: https://doi.org/10.1186/s12888-018-1674-2)
Biju Viswanath, Naren P. Rao, Janardhanan C. Narayanaswamy, Palanimuthu T. Sivakumar, Arun Kandasamy, Muralidharan Kesavan, Urvakhsh Meherwan Mehta, Ganesan Venkatasubramanian, John P. John, Odity Mukherjee, Meera Purushottam, Ramakrishnan Kannan, Bhupesh Mehta, Thennarasu Kandavel, B. Binukumar, Jitender Saini, Deepak Jayarajan, A. Shyamsundar, Sydney Moirangthem, K. G. Vijay Kumar, Jagadisha Thirthalli, Prabha S. Chandra, Bangalore N. Gangadhar, Pratima Murthy, Mitradas M. Panicker, Upinder S. Bhalla, Sumantra Chattarji, Vivek Benegal, Mathew Varghese, Janardhan Y. C. Reddy, Padinjat Raghu, Mahendra Rao and Sanjeev Jain (2018)

Developing Two Reference Control Samples for the Indian Population (Stem Cell Res. Jul; 30:38-42. doi: 10.1016/j.scr.2018.05.001. Epub 2018 May 12.)
Iyer S, Bhatia P, Rao M, Mukherjee O (2018)

5.3

Time and Energy Contours of mGluR and NMDAR Mediated Translation

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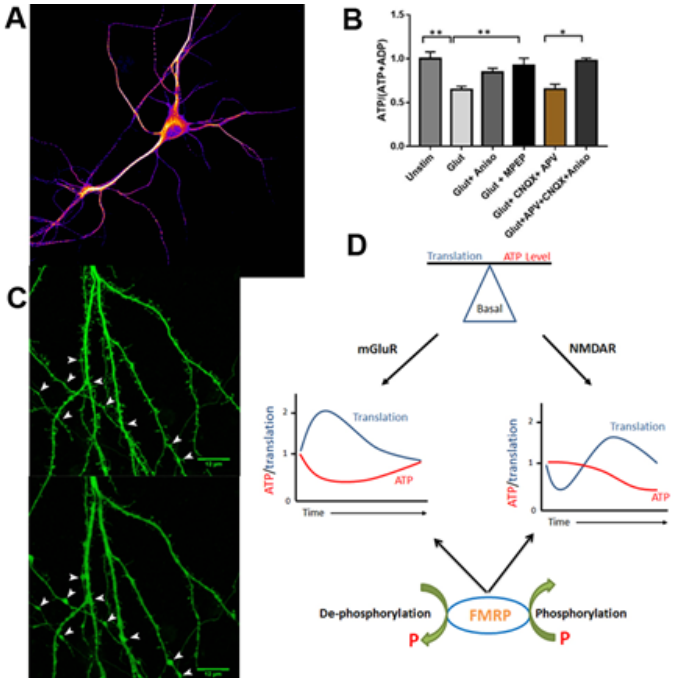
We have identified that activity mediated protein synthesis is the major consumer of energy in the post-synaptic compartment of glutamatergic synapses. Activation of mGluR and NMDAR results in very distinct energy and translation landscape which likely reflect their distinct synaptic response. FMRP, acts as a dual switch for both mGluR and NMDAR signalling through its phosphorylation.

mGluR and NMDAR are two major class of glutamate receptors involved in synaptic plasticity. Activation of both the receptors leads to protein synthesis which plays an important role in synaptic signalling. We have identified that mGluR and NMDAR stimulation leads to translation of very distinct pools of mRNA using ribosome profiling. FMRP, the absence of which leads to Fragile X syndrome *(FXS)*, is integral to translation regulation downstream of both NMDAR and mGluR. We identified that phosphorylation of FMRP is the switch to activate the translation in response to NMDAR while dephosphorylation of FMRP leads to mGluR mediated translation *(Figure 1D).* We went on to demonstrate that FMRP along with AGO2 and MOV10 provides specificity for NMDAR mediated translation. The role of FMRP in NMDAR mediated translation is physiologically important as we show that in the absence of FMRP, NMDAR mediated response such as spine retraction *(Figure 1C)* is defective. This clearly shows the importance of studying NMDAR signalling in FXS and its role in therapeutic strategies.

The brain consumes disproportionally large amount of energy. Synapse is considered to be the primary site of energy drain in nervous system but the contribution of post-synaptic terminal in this is largely unexplored. We have identified that activity mediated protein synthesis is the major consumer of energy in the post-synaptic compartment of glutamatergic synapses. Translation downstream of mGluR and NMDAR forms a very distinct energy and time contour which is likely to be an important part of their synaptic signalling *(Figure 1D)*. On mGluR stimulation, there is a rapid and robust translation leading to reduced ATP levels *(Figure 1B)*. mGluR induced translation and drop in ATP level is sustained for a substantial period of time which in itself results in a signalling conundrum *(see later section).* On the other hand, NMDAR stimulation to begin with leads to stark inhibition of translation at global level. But over time, translation gets activated and there is a concomitant drop in ATP level *(Figure 1D).* mGluR mediated translation is primarily carried out through activation of elongation *(of stalled polysomes?)* while NMDAR mediated translation appears to occur through *de novo* initiation. We are currently studying the temporal and mechanistic difference in mGluR and NMDAR response in generating a distinct translation landscape. This will help us to understand how translation affects synaptic plasticity and the integration of synaptic signalling.



▲ *Figure 1: Time and Energy contours of mGluR and NMDAR mediated translation.* ***A).*** *FUNCAT signal from a cultured cortical neuron* ***B).*** *Change in ATP/ADP ratio from cultured cortical neurons on glutamate stimulation (5') and in combination with antagonists for mGluR (MPEP), NMDAR (APV), AMPAR (CNQX) and protein synthesis inhibitor (anisomycin)* ***C).*** *Frames from cultured cortical neuron live imaging (0 and 5 minutes respectively) after NMDAR stimulation (5') for spine retraction* ***D).*** *Schematic showing the correlation between ATP levels, translation on mGluR and NMDAR stimulation and the role of FMRP in this signalling.*

Neurons *(like other cells)* have a very efficient homeostasis mechanism to control ATP levels primarily through the activity of AMP kinase. AMPK is a sensor of ATP level and regulate protein synthesis and other anabolic processes based on the availability of resources. Interestingly on mGluR stimulation AMPK activity is kept low even with sustained drop in ATP facilitating the activity mediated translation. In contrast on NMDAR stimulation, a sharp increase in AMPK activity is likely to cause global translation inhibition. Our preliminary data indicates that in the absence of FMRP both of these responses are defective which may contribute to the synaptic dysfunction in FXS. Synaptic plasticity is studied extensively through electrophysiological experiments and understanding the molecular basis of many of these synaptic events will complement these electrophysiology read-outs which will significantly enhance our understanding. Studying the mechanism of synaptic translation and understanding this in the context of time and energy landscape is an important aspect of our future studies.

PUBLICATIONS

FMRP interacts with C/D box snoRNA in the nucleus and regulates ribosomal RNA methylation (Online ePub in Cell (iScience - DOI:https://doi.org/10.1016/j.isci.2018.11.007))
Michelle N. D., Naveen K. C. Gowda, Vishal Tiwari, Rosana Babu, Praveen Anand, Sudhriti Ghosh Dastidar, Randhir Singh, Bhuvaneish S., Rakhi Pal, Arati Ramesh, Sumantra Chattarji, Siddharthan Chandran, Akash Gulyani, Dasaradhi Palakodeti, Ravi S. Muddashetty.

TALKS & OUTREACH

A Look into the Brain (Through the Eye) – CNR Rao Hall of Science Programme, JNCSAR, Bangalore, November 2017

Kaliyuvudu Hege matthu Mareyuvudu Eke? (How do we learn and why do we forget) – Talk in Kannada for the JIGYASA Project, NCBS campus, June 2018

How do we learn and why do we forget? – for BLiSc Science Café, Bangalore, July 2018

Image courtesy: Ravi Kumar Boyapati, NCBS

6

CCBD

Centre for Cardiovascular Biology and Disease

The Centre for Cardiovascular Biology and Disease theme at inStem, focuses on the signalling and biomechanical properties of the heart, with an emphasis on genetic hypertrophic and dilated cardiomyopathies, and autosomal dominant myocardial diseases caused by missense mutations in genes encoding the contractile apparatus and the signalling pathways that regulate its contractility. These diseases are common, debilitating and often lead to sudden death. This theme brings together a team of scientists using complementary approaches to a fundamental clinical issue in India and worldwide. Interactions and collaborations across our team members are strong, bringing together biochemistry, biophysics, biology, genetics, structural biology, computational biology and clinical sciences to define how cardiomyopathy mutations affect the power output of the human heart. Our ultimate goal is to understand the underlying molecular mechanisms of hypertrophic and dilated cardiomyopathies in order to develop new therapeutic approaches for these diseases.

James Spudich
Theme Coordinator

Sivaraj Sivaramakrishnan
Theme Coordinator

6.1

Nano-engineering for Discovery and Disease Mechanism Related to Cytoskeleton

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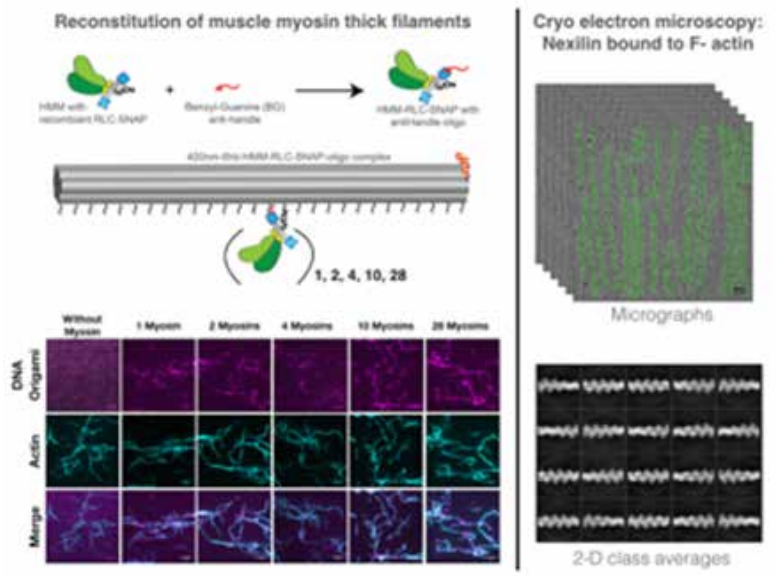
Eukaryotic biological motions across scales and orders of magnitude involve cytoskeleton elements. Mutations in them are frequently associated with human pathology, for e.g., cardiomyopathies, neurological disorders and ciliopathies. Our lab utilizes the power of nano-engineering and in vitro reconstitution to uncover new findings in cytoskeleton biology, thereby bridging the knowledge gap between clinical findings and molecular mechanism.

As a part of the cardiomyopathy team, my research focuses on bridging the knowledge gap between clinical findings and molecular mechanism underlying cardiomyopathy disease causing mutations. Together our theme covers human genetics, animal models, cell biology, biochemistry and structure at the molecular level of cardiomyopathy diseases. Currently our group is focusing on reconstituting a minimal contractile unit and high-resolution structures of sarcomere proteins implicated in cardiomyopathy. Both these project will benefit from the collaborative research environment of CCBD theme and Bangalore Bio-cluster campus.

A major hurdle in achieving reconstitution of minimal contractile unit is generating arrays of myosin motors that approximate the order found in a half-sarcomere *(i.e., one half of a bipolar thick filament).* Engineering a defined number of myosin motors and understanding their cooperativity during force generation represent a fundamental challenge in muscle biology and motor biophysics. In addition to addressing fundamental questions in the muscle field, the reconstituted system will allow us to study cardiomyopathy mutations and their effects in force generation during muscle contraction.

Here we have utilized the self-assembling DNA origami system to achieve a near native structure myosin thick filaments *(dimeric myosin heads displayed in a helical array).* This will enable us to study the collective biophysical properties of contractile unit and compare the cardiomyopathy disease causing mutations. For designing a synthetic hemi-thick filament assembly, we are using computational methods to design a modular assembly to achieve 400 nm DNA structure with precise topology of attachment sites for the dimeric myosin heads. We have successfully folded the designed DNA origami structure and validated it using negative stain electron microscopy. Simultaneously we have developed methods to attach SNAP tagged motors to attach to a linear DNA assembly. On the myosin motor front, we have established methods to attach native myosin heads to the DNA *(Figure 1).*



▲ *Figure 1: Illustration of reconstitution of muscle myosin thick filament using DNA origami (top, left). Total internal reflection microscopy images of reconstituted DNA:Myosin complex bound to filamentous actin (F-actin), shows the proof of concept of designed assay (bottom, left). Cryo Electron microscopy micrographs of Nexilin bound to F-actin, green circles represent the extraction of particles for averaging (top, right). Two-dimensional class averages of extracted particles (bottom, right).*

Several proteins connect the thin and thick filaments and the Z-disc units to each other and mutations in these proteins have also been implicated in cardiomyopathies. There are over a dozen proteins present in the Z-disc structures and mutations in every single component lead to a variety of diseases, including HCM and DCM. Among them, nexilin is the least characterized of the proteins and have been implicated primary cardiomyopathies. So far clinical studies have identified 5 mutations and the mutations that cause HCM and DCM are clustered at the amino- and carboxy-terminal of nexilin respectively.

In order to address what biochemical changes occur upon mutation and understand the difference between the role of nexilin in heart and skeletal sarcomere, we aim to undertake structure-function studies of nexilin. Our work in this direction includes purification of full-length, truncations and mutant nexilin proteins, actin binding assays and cryo electron microscopy of nexilin bound to filamentous actin *(Figure 1).* In addition to the structure-function studies, we are closely collaborating with *Dhandapany Perundurai's* lab to identify new mutations in nexilin and other sarcomere proteins. Dr. Perundurai's lab has already identified a novel mutation in nexilin that is present in Indian population. Future work will involve introducing this mutation in mice to study and model the human patient mutation effects.

PUBLICATIONS

Life as an Early Career Researcher: Interview with Minhaj Sirajuddin (Future Sci OA 2017 Nov 1;4(2): FSO258. doi: 10.4155/fsoa-2017-0106. eCollection 2018 Feb)
Sirajuddin M (2017)

Sequence diversity of tubulin isotypes in regulation of the mitochondrial voltage-dependent anion channel (J Biol Chem. 2018 May 18. pii:jbc.RA117001569).
Rostovtseva T.K., Gurnev P.A., Hoogerheide D.P., Rovini A., Sirajuddin M., and Bezrukov S.M. (2018)

TALKS & OUTREACH

Live Cell Sensors for Tubulin Modifications – Curie Institute, Orsay, France, October 2017

Nanoengineering for Discovery andDisease Mechanisms Related to Cytoskeleton – EMBOCytoskeleton Meeting, Pune, November 2017

Regulation of Molecular Motors by Tubulin Modifications – IISER Trivandrum, March 2018

6.2

Genes, Mechanisms, and Therapies for Cardiomyopathies

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Cardiomyopathies are a group of heart muscle diseases that often lead to progressive heart failure with significant mortality. Every year about 1,000-5,000 new cases are diagnosed with cardiomyopathy, with a large number of the patients being less than 40 years of age. The cause of a significant percentage of cardiomyopathies (~40%) remains unknown with poorly defined mechanisms and no curative therapies.

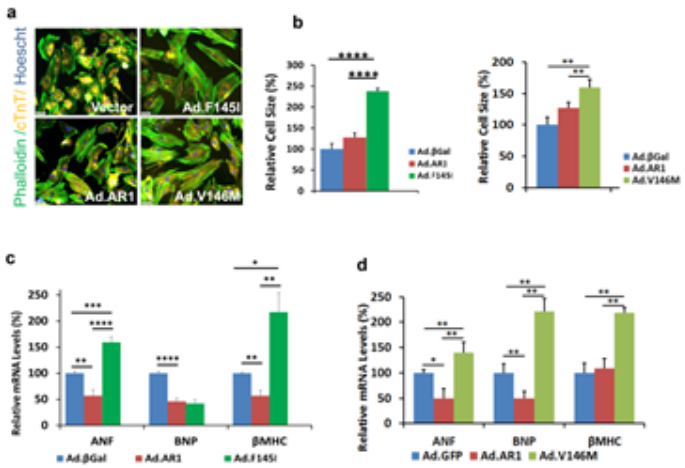
Our lab is interested in exploring *new genes, mechanisms and relevant drugs* that have a significant clinical and curative impact on cardiomyopathies. Our group encompasses a multi-disciplinary approach involving Next Generation Sequencing (NGS) in identifying new genes and various models to understand the mechanistic basis and therapeutic targets for the new cardiomyopathy genes.

Gene discovery for South Asian specific cardiomyopathy patients

We have organized various unrelated Indian cardiomyopathy index patients (~1000) for our genetic studies through a South Asian specific cardiovascular initiate. We performed trio exome sequencing in 200 selected participants. Standard bioinformatics pipelines are used to identify genes associated with cardiomyopathy. Various potential candidate gene variants are identified in the Adiponectin Receptor R1 (ADIPOR1) in four unrelated families. All the respective amino acid changes are evolutionary conserved and predicted to be pathological.

Molecular mechanisms associated with AdipoR1 (AR1) variants

To know the functional consequences of ADIPOR1 variants, we expressed the variants using viral vectors *(Ad.F145I, Ad.V146M and Ad.L157H)* along with the vector control (Ad.βGal) orwild-type (Ad.AR1) in the rat cardiomyocytes (RCM) and assessed the hypertrophic markers including cell surface area and foetal gene expressions. Transient expression of the mutant proteins in RCM showed significant increase in cell sizes, and foetal genes expression including atrial natriuretic factor (ANF), brain natriuretic peptide (BNP) and β-myosin heavy chain (βMHC) compared to Ad.βGal or Ad.AR1 *(Figure 1)*. Collectively these data suggest that constitutive expression of ADIPOR1 mutants can induce cardiac hypertrophy. We next examined the signaling pathways modulated by ADIPOR1 variants by assessing their effects on lysates obtained from cardiomyocytes overexpressing Ad.AR1, Ad.F145I, Ad.V146M or Ad.L157H using immunoblotting for various downstream targets. Surprisingly, the well-known downstream targets of ADIPOR1 including AMPK, liver kinase B1 (LKB1) and acetyl-coA carboxylase



▲ *Figure 1: ADIPOR1 variants induce cardiomyocyte hypertrophy. (a) Representative images and cell surface area measurement (b) in neonatal rat cardiomyocytes (>200 cells) infected with Ad.βGal, Ad.GFP, Ad.AR1, Ad.F145I and Ad.V146M and stained with Phalloidin, cTnT (cardiac troponin T) or Hoechst as indicated. Results presented as relative cell area compared to controls. (c, d) Quantitative RT-PCR analysis of hypertrophic marker genes ANF, BNP and βMHC in adult cardiomyocytes. Expression is normalized to 18s rRNA and presented as a ratio compared with the control (Ad.βGal) (n = 6). *p < 0.05 and **p< 0.01.*

(ACC) were relatively normal in the cardiomyocytes expressing Ad.F145I, Ad.V146M and Ad.L157H compared to Ad.AR1. However, we found evidence that Ad.V146M and Ad.L157H selectively target the p38/mTOR pathway members. The Ad.V146M expressing cardiomyocytes showed increased phosphorylation of p38 kinase, mammalian target of rapamycin (mTOR), 70 kDa ribosomal protein S6 kinase (p70S6K) or eukaryotic elongation factor-2 kinase (eEF2K) with normal or slightly decreased levels of extracellular-signal regulated Kinases (ERKs), cytochrome oxidase subunit 2 (COX2) and eukaryotic elongation factor-2 (eEF2) compared to Ad.AR1, respectively. A similar pattern was observed in the cardiomyocytes expressing Ad.L157H compared to Ad.AR1,respectively. In addition, treatment of known inhibitors of p38/mTOR rescued the cardiomyocyte hypertrophy and restored the normal signalling in a representative mutant (Ad.V146M) expressing cells, respectively. In contrast, Ad.F145I activated the ERK but not p38/mTOR pathway. These data indicate that Ad.V146M and Ad.L157H signal differently compared to Ad.F145I to induce cardiac hypertrophy. These findings demonstrate that the site of amino acid change in the ADIPOR1is crucial for its downstream signalling and the disease pathogenesis. This might partly explain the differences in the patient phenotypes; V146M and L157H genotype-positive patients are associated with HCM with diabetes while F145I genotype-positive patient with HCM alone.

Transgenic mice model of cardiomyopathy and re-purposing FDA drug

To know whether ADIPOR1 variants can independently induce cardiomyopathy, we generated a transgenic mouse that specifically expresses the representative variant protein (V146M) in the heart. At week twelve, the transgenic mice (Cre-V146M) displayed increased heart-to-body weight (HW/BW) ratio, myocytes cross-sectional areas, expression of fetal genes (ANF, BNP and βMHC) and myocardial fibrosis compared to controls (Crenegative mice). Strikingly, p38 and mTOR activation and the associated metabolic alterations elicited by Ad.V146M in the cellular models were also altered in Cre-V146M mice compared to Cre-negative mice. Notably, treatment with rapamycin (an mTOR inhibitor) rescued the cardiomyopathy phenotypes in the Cre-V146M mice by normalizing cardiac function, myocardial fibrosis and the downstream signaling pathways including mTOR levels. Thus, our findings provide evidence that the ADIPOR1 variant can cause cardiomyopathy.

PUBLICATIONS

Melatonin and Human Cardiovascular Disease. Myocardin Ablation in a Cardiac-Renal Rat Model. (Scientific Reports in press)

Anupam M, Santanu R, Rajni S, Akhilesh K, Rishikesh P, Satish K R, Sagartirtha S, Uma Nahar S, Ajay B, Dhandapany P S, and Kullar M (2018)

TALKS & OUTREACH

Novel Genes Associated with Cardiomyopathies in South Asians – IIT, Chennai, May 2017

7

RCF

Regulation of Cell Fate

Metabolic reprogramming is pivotal to cell fate decisions underlying growth, proliferation, differentiation etc. The metabolic state of a cell is dependent upon both, overall nutrient availability, as well as the environmental niches a cell is present in. However, interactions between signal transduction networks and the metabolic state of a cell that culminate in robust cellular behaviours at the scale of tissues and whole organisms, remain poorly understood. Research in the RCF theme, spans from single cells to complex tissues, with participating groups connected by an interest in the control of cell (metabolic) identity and the modulation of these, in contexts of injury, inflammation and hematopoietic stress. The reports that follow, summarize efforts to identify rules governing the emergence of distinct metabolic identities (cell fates); signalling to growth during re-activation of tissue-resident stem cells in response to injury and metabolite control of cell fate decisions in immune cell lineages. The theme also welcomes its newest member, *Arvind Ramanathan,* whose interest in metabolic remodelling as an adaptive response in skeletal muscle, complements and enhances the scope of the theme's activities. Collectively, the theme is poised to investigate how cellular (metabolic) identity is maintained in homeostasis and the response to stress and build an integrated understanding of cell fate decisions in diverse contexts.

7.1

Metabolic Signalling Underlying Cell Fate Decisions in T-cells

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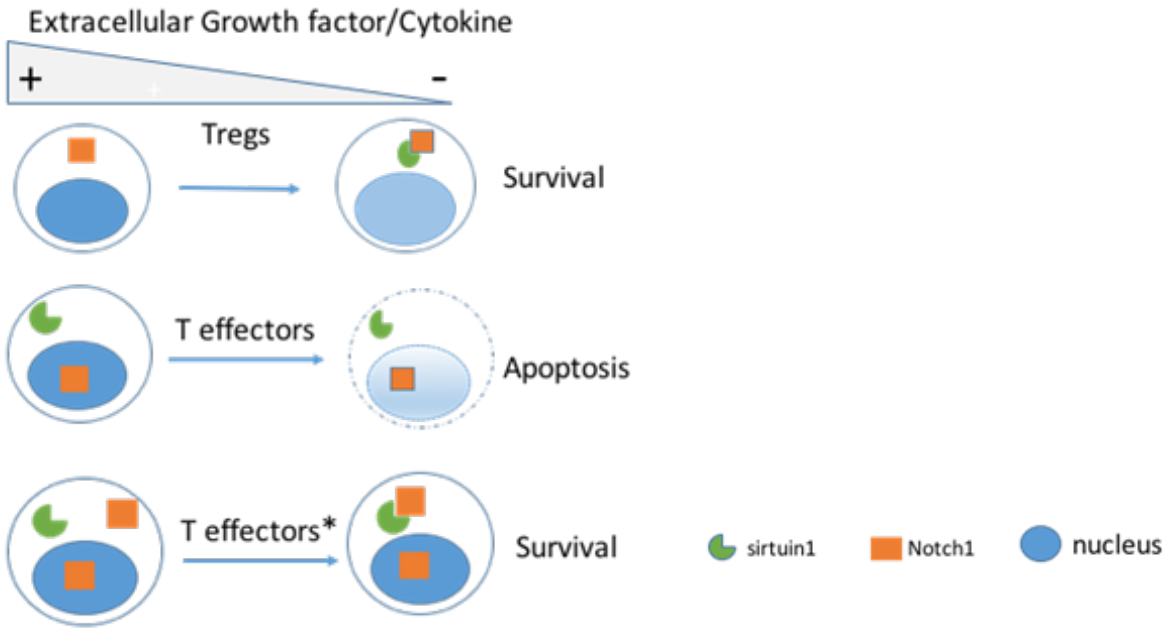
Nutrient uptake is linked to growth factor signalling in T-cell subsets in the immune system. Deviations from this norm, provide insights into cross-talk between signal transduction networks and cell metabolism. We seek to understand the rules governing these interactions and the maintenance of cell (metabolic) identity in the T-cell lineage.

T-regulatory cells (Tregs) suppress adverse immune responses associated with auto-immunity, allergies, and limit inflammation, thereby playing a critical role in balanced immune function. Unlike T-cell subsets that trigger inflammation and are highly dependent on specific extracellular growth factors, Tregs can function in environments where these growth factors are limiting. My laboratory has been interested in understanding how Tregs adapt and survive in these conditions, while maintaining lineage stability, necessary to their function.

T-cell dependence on growth factors for nutrient uptake can be mimicked in culture and has been used to understand intracellular changes and manipulate cellular responses using different approaches. In such an assay, T-cell subset dependencies on growth factor/cytokines are sharply delineated, with the wash-out of growth factors (withdrawal), triggering apoptosis of T-effector subsets, whereas Tregs, survive. Building on this, our earlier work positioned Notch1 signalling, a well characterized and evolutionarily conserved pathway, as a key intermediate in Treg survival; observations that were also confirmed by genetic manipulations in animals *(Perumalsamy et al, 2012).* Since our experiments ruled out Notch1 regulation of growth factor receptor expression, we focused on metabolic characteristics (and possible interactions with the Notch1 pathway), hypothesizing that distinct metabolic features in Tregs would be key to the adaptation to cytokine withdrawal.

In this regard, withdrawal of growth factors was observed to trigger Sirtuin1 deacetylase activity, in Tregs. Sirtuin family proteins are evolutionarily conserved sensors of environmental stimuli. Hence, the activation of Sirtuin1 in itself, was was not unusual in this context. However, our analysis identified Notch1 as a target of Sirtuin1 and showed that this interaction was critical for both spatial orientation and consequently Notch1 activity in Tregs *(Marcel, Perumalsamy, Shukla et al., 2017).* Further, revealing cross-talk that we do not yet fully understand, expressing spatially-restricted Notch1 (N^{SR}) in T-effector cells, ameliorated their dependence on growth factors, as detected in the assay of nutrient stress. This was also linked to the acquisition of sensitivity to Sirtuin1. Hence, the NIC-Sirtuin link was revealed as a key intermediate in the adaptation to nutrient stress in Tregs. Whilst cross talk between signalling networks and metabolism are



▲ *Figure 1: Notch1-Sirtuin interactions in Treg adaptation to nutrient stress. Withdrawal of growth factors triggers the upregulation and interaction between Sirtuin1 and processed Notch1 in the cytoplasm in Tregs. T-effectors, on the other hand do not initiate this interaction, and undergo apoptosis or cell death. Expressing Notch1 adapted to signal from the cytoplasm (N-SR, see text) in T-effectors (*), allows this interaction and, protects T-effectors from apoptosis. Intriguingly, Sirtuin1 is detected in T-effectors, even in the presence of growth factors (+ condition), highlighting differences in the metabolic configurations of these subsets. (Not to scale.)*

known, examples of such regulation in the context of Notch family proteins are few. Since Sirtuin1 dampens transcription by regulating the sub-cellular location of processed Notch1, it is tempting to speculate that this may be a mechanism to transiently silence Notch transcription, while promoting survival in differentiating cells.

With *Sunil Laxman's* laboratory we have initiated attempts to identify metabolites critical for Treg adaptation to nutrient stress. Metabolite profiling and functional outputs of Tregs, revealed an unusual utilization of, and dependence on, specific amino acids for survival. More recent experiments, opened up the exciting possibility that Notch1 may be a player in this process. We are currently focusing on confirming these observations and delineating the mechanism underlying this regulation. These studies suggest a previously unreported role of Notch1 in maintaining intracellular metabolite pools and metabolite homeostasis in cells, with may be more broadly applicable.

PUBLICATIONS

The Lysine Deacetylase Sirtuin 1 Modulates the Localization and Function of the Notch1 Receptor in Regulatory T Cells (Science Signalling. 10(473) aah4679).
Marcel N, Perumalsamy LR, Shukla SK., and Sarin A. (2017)

TALKS & OUTREACH

Notch Signalling in T-Regulatory Cells and Consequences to Immune Homeostasis– Immunology Conference, Nirma University, Ahmedabad, November 2017

Notch Signalling Regulates Autophagy in T-cells– India-EMBO Symposium on Autophagy: Cellular mechanisms and significance in health and disease, December 2017

Notch Signalling and the Control of Cell Fate Through Metabolic Programming – International Congress of Cell Biology, Hyderabad, January 2018

7.2

The Logic of Metabolism, Metabolic Sensing, and Cell Fate Regulation

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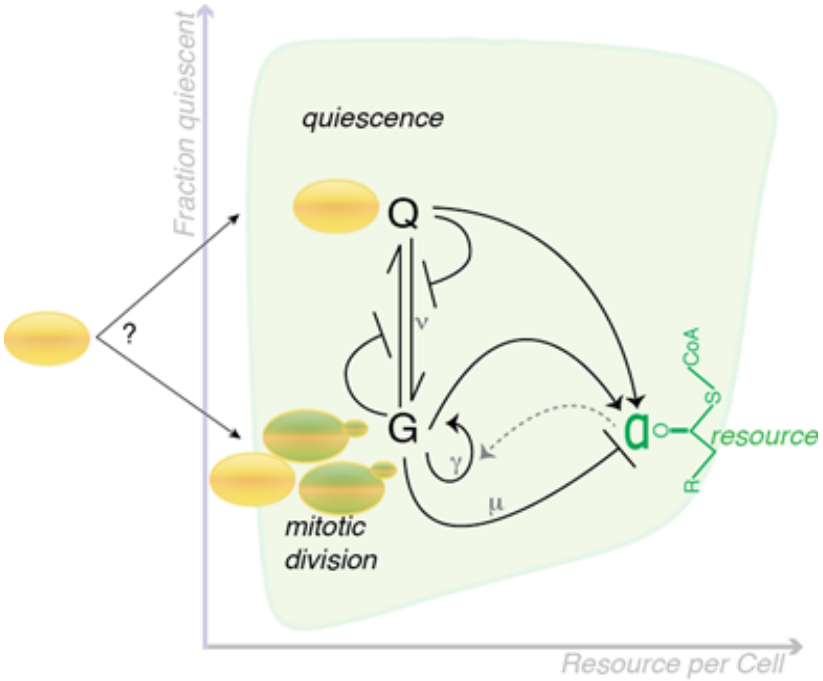
My lab is interested in understanding the logic of metabolic information flow within cells, the organizing principles of metabolic networks, and how that regulates cell fates. Here, we address (i) How metabolites function as signalling molecules; (ii) how cells sense and switch metabolic states; and (iii) how metabolism controls phenotypic heterogeneity.

Metabolism is dismissed as a series of essential but rigid biochemical reactions, of irreducible complexity. However, metabolism is dynamic, and functions with predictable rules and constraints, which can be experimentally or theoretically defined. We now also appreciate that the metabolic state of a cell can causally control multiple cellular outcomes. My lab is interested in deciphering the organizational principles, and logic of metabolic information flow within cells, and how that regulates cell fates. The questions we address include:

1. What makes some metabolites unique in their ability to regulate different cell fates?
2. What molecular machines sense metabolic states, and how do they mediate information transfer to regulate metabolic homeostasis?
3. What is the metabolic basis for the emergence of phenotypic heterogeneity in isogenic cells?

In these contexts, we are especially interested in the versatile functions of amino acids. Amino acids have multiple context dependent functions, ranging from purely metabolic, to signalling roles. However, our understanding how amino acids regulate cell fates *(and the mechanisms by which this is mediated)* remains poor. Given the conserved, fundamental nature of our questions, we utilize a tractable eukaryotic model, *S. cerevisiae,* to uncover universal processes of metabolic sensing and regulation. Appropriate lessons from this model are taken to more complex systems, including mammalian cells. A distinguishing feature of our research is the ability to address questions at both a "systems" level, and a mechanistic level. For systems level studies, we combine quantitative LC/MS/MS based metabolomics, with genomics, estimates of global translation, and build mathematical models where appropriate. To address causal, molecular and mechanistic processes, we use traditional cell biology, biochemistry and genetics. While the questions we ask and address are basic, they have strong implications for synthetic biology and therapy.

The laboratory now has established strengths in quantitative metabolic flux analysis, and genomics/transcriptomics, in addition to our traditional strengths in mechanistic biochemistry and cell biology. A notable expansion in our research programmeis the inclusion of mathematical modelling that complement, and enhances our experimental approaches addressing metabolic determinants of cell fate. This is an ongoing, growing collaboration with *Dr. Sandeep Krishna* and



▲ *Figure 1: A simple model explaining oscillations between quiescent and proliferative states, based on the internal availability of a metabolic resource.*
Adapted from: Krishna S & Laxman S, MBoC 2018

other members at the NCBS-Simons Centre for Living Machines. Additionally, the collaborative effort within the theme, led by *Dr. Apurva Sarin*, addressing how specific metabolic components of cell fate in T-cells, has gathered momentum with jointly mentored trainees.

Over the past year the laboratory, comprised of a team of postdocs, students and research assistants, has made substantial advances in addressing questions in all three areas of research. These include specific studies on how components of the translation machinery regulate overall metabolic homeostasis, how specific amino acids function as metabolic signalling molecules, and how clonal cells spatially organize to create groups of cells with distinct function. Some of these studies are now undergoing peer review, and we anticipate their publication in the coming months.

A recently completed study highlights the potential of combining experiments with modelling, to address core questions in metabolism and cell fate. The quiescent and the rapidly proliferating *(mitotic)* state are two ends of the cell state spectrum. While a plethora of experimental data studying these states exists, there are few universal models that can predict or explain the switch between these two states. Using experimental data from yeast systems, we built a predictive model explaining how threshold amounts of an internal metabolite, as well as a required communication between the quiescent cells, dividing cells and the resource, determine transitions between quiescent and dividing states *(Figure 1).* This is amongst the first models to successfully explain this major cell cycle transition. New directions emerging from this, and our other studies are currently developing in the lab, strengthening the overall research programme in the laboratory.

PUBLICATIONS

A Versatile LC-MS/MS Approach for Comprehensive, Quantitative Analysis of Central Metabolic Pathway
(Wellcome Open Res [2018] 3:122 (doi: 10.12688/wellcomeopenres.14832.1).
Walvekar A, Rashida Z, Maddali H. and Laxman S. (2018)

Methionine Coordinates a Hierarchically Organized Anabolic Program to Enable Proliferation
(Accepted, Molecular Biology of the Cell).
Walvekar A, Srinivasan S, Gupta R, and Laxman S. (2018)

A Minimal "Push-Pull" Bistability Model Explains Oscillations Between Quiescent and Proliferative Cell States (in press, Molecular Biology of the Cell. doi: https://doi.org/10.1091/mbc.E18-01-0017).
Krishna S. and Laxman S. (2018)

Optofluidic Platform to Investigate Cell Community in Microenvironments (Life Sciences Conference (LSC), 2017 IEEE, 51-54).
R. V. R. Choudhury, A. Prabhakar, and Laxman S. (2017)

Thiol Trapping and Metabolic Redistribution of Metabolites Enable Cells to Overcome Cysteine Overload (Microbial Cell, 2017, Vol. 4, No. 4, pp. 112 – 126)
Deshpande A. A., Bhatia M., Laxman S., and Bachhawat A. K. (2017)

Conceptualizing Eukaryotic Metabolic Sensing and Signalling (Review). J Indian Institute of Science, doi: 10.1007/s41745-016-0013-1).
Laxman S. (2017)

TALKS & OUTREACH

Outreach activities from the lab include:

(i) interactions with students from local high schools (grades 11 and 12, Biology and Biotechnology electives), who visited the lab and campus, and got an overview of ongoing science in the lab.

(ii) talks at the JN Planetarium (as part of the REAP programme), and policy/popular science writing in mainstream media in a personal capacity.

7.3

Regulation of Lung Injury-Repair

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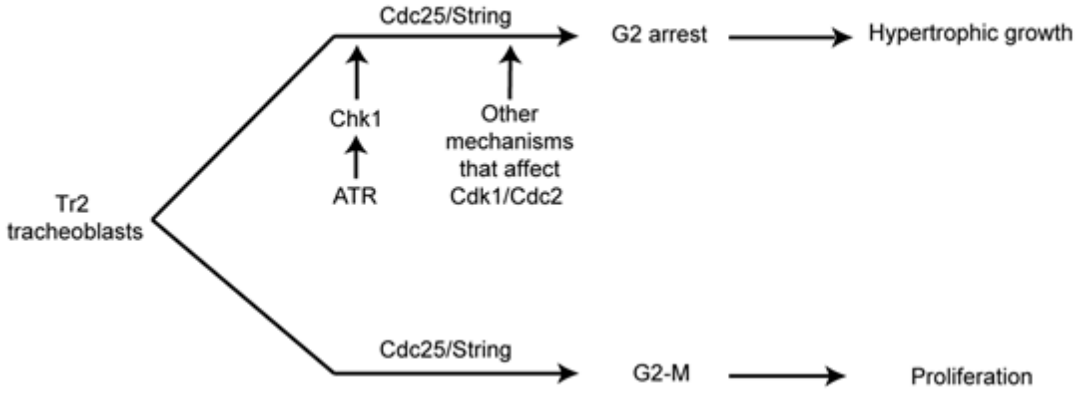
The aim of the research programme is to delineate the mechanisms that protect the lung from chemical injury and repair the lung in its aftermath. Ongoing research in the laboratory probes the mechanisms that regulate cell fate in the respiratory tract during injury-repair.

Epithelial cells line the surfaces of organs throughout the body and serve to protect the organism. The maintenance and post-injury repair of these tissues are consequently of vital importance. Our research focuses on how the epithelial lining of the respiratory tract copes with injury. Some epithelia, such as the skin and the lining of the alimentary canal, have high rates of cell proliferation and turnover during homeostasis. In contrast, others like lining of the respiratory tract in the lung have significantly lower rates but can dramatically upregulate proliferation in response to injury.

As a member of the Regulation of Cell Fate Theme at inStem, our group is pursuing three lines of enquiry. First, we are investigating how in response to chemical injury airway epithelial cells regulate expression of antioxidant enzymes. Second, we are trying to understand how quiescent airway stem/progenitor cells rekindle a mitotic programme. And third, we are probing the role of the Notch signalling pathway in the regulation of airway stem/progenitor cell fate during post injury-repair.



▲ *Figure 1: Model for the regulation of proliferation and growth in thoracic (Tr2) tracheoblasts in Drosophila. We propose that negative regulation of the G2-to-M transition by ATR/Chk1 and by other mechanisms that regulate Cdc2/Cdk1 activity facilitate cellular and hypertrophic organ growth.*

Our efforts to understand the mechanisms of mitotic arrest in stem cells have, in addition to work on mouse models, led us to investigate the regulation of adult progenitors in Drosophila. In this context we have analysed the cell cycle phasing of progenitors of the adult tracheal *(respiratory)* system *(tracheoblasts)*. These progenitors are arrested in the G1 and G2 phases of the cell cycle at different times. Our studies have uncovered a role for the ATR/Chk1 pathway in mediating mitotic arrest in the G2 phase of the cell cycle. Moreover, we find that arrest in G2 is necessary for cellular growth and in turn hypertrophic organ growth *(please see figure).* Ongoing studies aim to elucidate the mechanisms for G1 and G2 arrest in tracheoblasts and apply the knowledge and expertise gained to the characterization of stem/progenitor cells in the lung.

PUBLICATIONS

Negative Regulation of G2-M by ATR (mei-41)/Chk1(Grapes) Facilitates Tracheoblast Growth and Tracheal Hypertrophy in Drosophila (eLife2018;7:e29988)
Amrutha K., Bagul A., and Guha A. (2018)

TALKS & OUTREACH

Mechanisms of Lung Injury-Repair – Departmentof Biochemistry, JSS Medical College, Mysuru, November 2017

Mechanisms of Lung Injury-Repair – Pulmonary, Allergy and Critical Care Unit, JSS Hospital, Mysuru, November 2017

Hosted a student as part of the **Careers Exposure and Higher Education Advisory Programme** conducted by the Mallya Aditi International School, July 2018.

7.4

Systemic and Metabolic Control of Hematopoiesis

Tina Mukherjee
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Metabolic control of diverse biological processes forms the central focus of our theme "Regulation of Cell fate". Allied to this, our research group aims to define metabolic demands underlying hematopoiesis. By employing genetic and genome-wide approaches we seek to identify metabolic regulators of blood progenitor-cell development and function. By uncovering long-range, neuronally-derived systemic metabolites and their physiological implications, we expect to establish the broader relevance of metabolic inputs in blood development.

Olfactory control of blood progenitor competency

The olfactory system is a unique sensory modality that is tuned to promote animal survival by detecting odours to discriminate between favourable and unfavourable conditions. While our understanding of olfaction and its role in survival is limited to initiating behavioural changes, we find that olfaction is necessary for the establishment of a competent immune system where by odour-derived cues establish the development of a blood cell lineage termed *lamellocyte,* which is necessary to combat parasitic wasp-immune infections. Ongoing work reveals an unexpected association of exposure to environmental odours and their capacity to influence cellular immune responses. Specifically, larval odour-detection mediated neuronally-derived, systemic GABA is internalized by blood progenitor-cells. Intracellular blood cell GABA catabolism through the GABA-shunt pathway prevents degradation of HIF-1α (Sima) protein, a potential transcription factor. HIF-1α is necessary and sufficient for lamellocyte induction. Limited GABA availability during larval development restricts blood-progenitor HIF-1α levels and consequently the lamellocyte induction potential. Unexpectedly, preconditioning Drosophila larvae in odour-conditions mimicking parasitoid-threatened environment, raises systemic GABA availability that further elevates blood-progenitor HIFα levels. Subsequently, infection responses in them are rapid and efficient. Overall, this study unravels the adaptive influence of environmental odour-experience on myeloid-progenitor metabolism and immune-potential, the relevance of which may be broadly conserved.

Lipid uptake by circulating myeloid-cells as a global regulator of lipid homeostasis: a role in metabolic homeostasis and animal development

Myeloid functions are dedicated to phagocytosis and immunity. Our ongoing work in Drosophila larval blood system has identified lipid-scavenging functions of circulating blood cells. This allows the circulating blood cells to function as regulators of global metabolic

▲ *Figure 1: Olfaction mediated metabolic priming of immune competency*

equilibrium and proper larval development. We have identified Notch (N) and Croquemort (Crq) as important effectors of this function. Perturbing them drives global changes in larvae and adults, symptomatic of metabolic disorders and enhanced sensitivity to enriched diets. Expressing lipid-scavenging receptors rescues these defects. Unlike canonical scenarios, insulin resistance (IR) and inflammation are not involved, making it a unique process to investigate. We hypothesize a model of nutrient demand by early myeloid-cells as the means to alter nutrient globally that affects overall animal physiology both immediately and in the long-term as well.

PUBLICATIONS

Olfactory Control of Immune-Cell Competency in Drosophila (Manuscript in revision)
Sukanya Madhwal, Minkyu Shin, Manish K. Joshi, Ankita Kapoor, Pirzada Mujeeb Ur Rehman, Kavan Gor, Jiwon Shim, and Tina Mukherjee. (2018)

AWARDS

Innovation Young Biotechnologist Award (2017) – DBT: *I*dentification of regulators of myeloid-cell homeostasis, predisposing animals to metabolic disorders and insulin resistance.

TALKS & OUTREACH

Maintaining Myeloid Cell Fate and Function Through Stress Sensing Pathways – NISER, Bhubaneshwar, 2017

The Impact of Odor Experience on Myeloid Development and Potential – Indian Drosophila Research Conference, Bhopal, 2017

Metabolic Control of Immune-Competency by Odours in Drosophila – Stem Cell Biology and Disease Models, inStem, Bangalore, 2018

Identification of Novel Neuronal Signals in Stem/Progenitor Development and Maintenance – 8th Ramalingaswami Conclave, New Delhi, 2018

7.5

Quiescence and Adult Stem Cell Potency

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Our group is interested in the mechanisms by which the dormant or quiescent state of adult muscle stem cells (MuSC) promotes the acquisition of regenerative function. These temporarily arrested progenitors maintain adult muscle during normal turnover, as well as regenerate damaged tissue following injury. De-regulation of quiescence underlies pathologies at opposite ends of a spectrum- cancer may represent a failure to enter quiescence, while degenerative disease may represent a failure to exit quiescence. Therefore, understanding the acquisition and maintenance of quiescence has broad implications for human disease.

Altered transcription factor partnerships lead to signalling switches in quiescent muscle stem cells

The entry of MuSC into quiescence is characterized by the expression of target genes that are activated by Tcf/Lef1 transcription factors, which mediate the response to Wnt signalling. We found that although Lef1 promotes gene expression by cooperating with β-catenin in proliferating cells, Lef1 changed partners to interact with the TGF-β effector Smad3 to activate target genes in quiescent cells *(Figure 1)*, and switched back to β-catenin during cell cycle re-activation. Thus, crosstalk between Wnt and TGF-β pathways occurs at a transcriptional level to control MuSC quiescence.

A new modulator of RhoA signalling in control of muscle differentiation

Earlier, we showed that mDiaphanous1, an effector of adhesion-dependent RhoA-signalling promotes MyoD expression in myoblasts, linking contractility to lineage determination. Now, we report that mDia1 negatively regulates MyoD function and Myogenin expression in myotubes. We investigated mechanisms that may counteract mDia1 to promote Myogenin expression and timely differentiation. Using yeast two-hybrid and mass-spectrometric analysis, we found that mDia1 has a stage-specific interactome, including Prohibitin2 (Phb2), MyoD, Akt2, and β-Catenin. Phb2 colocalises with mDia1 in cytoplasmic punctae *(Figure2)* and co-expression of Phb2 reverses the anti-myogenic effects of mDia1. Our results suggest that Prohibitin2 sequesters mDia1 to dampen its activity and finetunes RhoA signalling to promote differentiation.

▲ *Figure 1: A single isolated adult muscle fiber with Smad3 (red) expression in quiescent Pax7+ muscle stem cell (green).*

PUBLICATIONS

Lef1 Switches Partners from B-Catenin to Smad3 for Transcriptional Activation of Tcf/Lef1 Target Genes in Quiescent Muscle Stem Cells* (Science Signalling 11, 540, eaan3000. DOI: 10.1126/scisignal.aan3000)
Aloysius A., DasGupta R. and Dhawan J. (2018)

Cycling to Meet Fate: Connecting Pluripotency to the Cell Cycle (Frontiers in Cell Dev Biol. 6: 57. doi: 10.3389/fcell.2018.00057)
Zaveri L. and Dhawan J. (2018)

Induction of Quiescence (G0) in Bone Marrow Stromal Stem Cells Enhances their Stem Cell Characteristics (Stem Cell Res 30: 69-80.)
*Rumman M.**, Majumder A.**, Harkness L.**, Venugopal B., Vinay M. B., Pillai M. S., Kassem# and Dhawan J.# (2018)*

Inter-cellular Force Interaction Overrides Cellular Response to Substrate Stiffness in human Mesenchymal Stem Cells (Biomaterials Science 6(5): 1109–1119)
Venugopal B., Mogha P., Dhawan J., and Majumder A. (2018)

** Highlighted on Science Signalling Website with an Editorial Comment)*
*** Equal contribution*
 # Corresponding authors

TALKS & OUTREACH

Anticipating Change: Paused Genetic Programs in Sleeping Stem Cells – Shiv Nadar University, Delhi, October 2017

Cell Biology of Neuromuscular Disorders: Opportunities for Discovery and Therapeutic Avenues – Indian Society for Human Genetics Annual Meeting, Hyderabad, April 2018

Muscle Stem Cells: Quietly Working to Build and Repair Adult Muscle – Keynote lecture at Indian Zebrafish Investigators Meeting, CCMB, July 2018

Working Quietly Needs Cross Talk-Transcriptional Interactions between Wnt and TGF β Signalling in Muscle Satellite Cells – FASEB Summer Conference on Muscle Satellite Cells and Regeneration Steamboat Springs, USA, July 2018

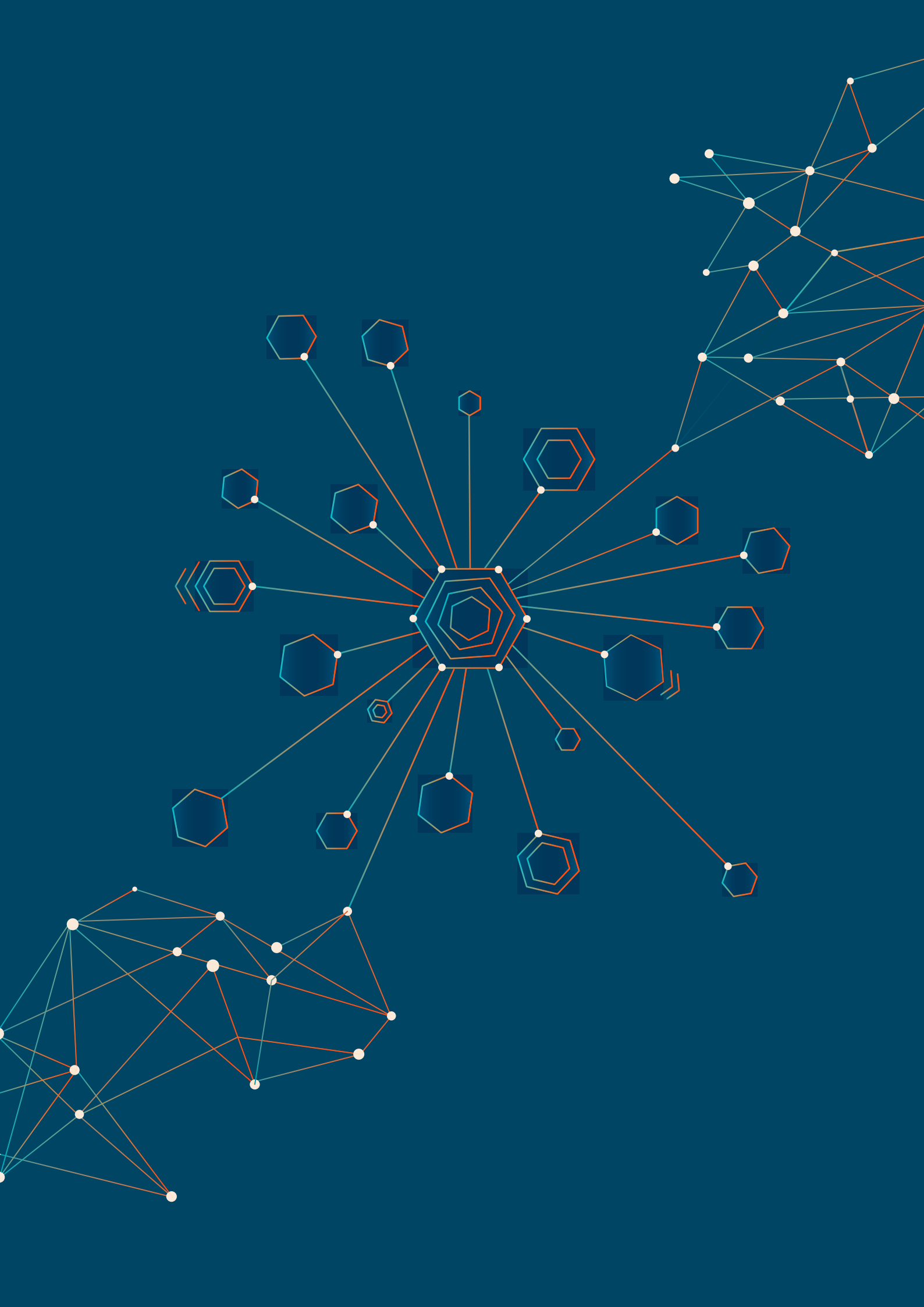
Image courtesy: Ravi Kumar Boyapati, NCBS

8

TAS

Technologies for the Advancement of Science

The TAS theme develops new technologies and approaches to solve difficult problems in biology. Our work is embedded in a culture of collaboration, spans disciplines/scales and has made new advances possible in both fundamental and applied biology. We develop biosensors and probes for functional bio-imaging, advanced materials, for example to alleviate disease and toxicity. We develop new tissue/cell types in the dish using stem cell technology. We have made significant advances in structural biology, biochemistry, developmental biology and study of novel RNA regulation. Our collaborative work on using the planarian model system to mechanisms of regeneration has been transformative, including development of a new way to study functional eye-brain regeneration. While, the work we have done has accumulated over years, this year has seen the burst of publications and patents. Our sphere of influence is visible from productive collaborations within the theme, across the inStem-NCBS campus and with the wider scientific community. The achievements of the TAS theme are a testament to its bold and integrative vision and scientific rigor across disciplines.



8.1

Molecular Form and Function

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Jeff Abramson
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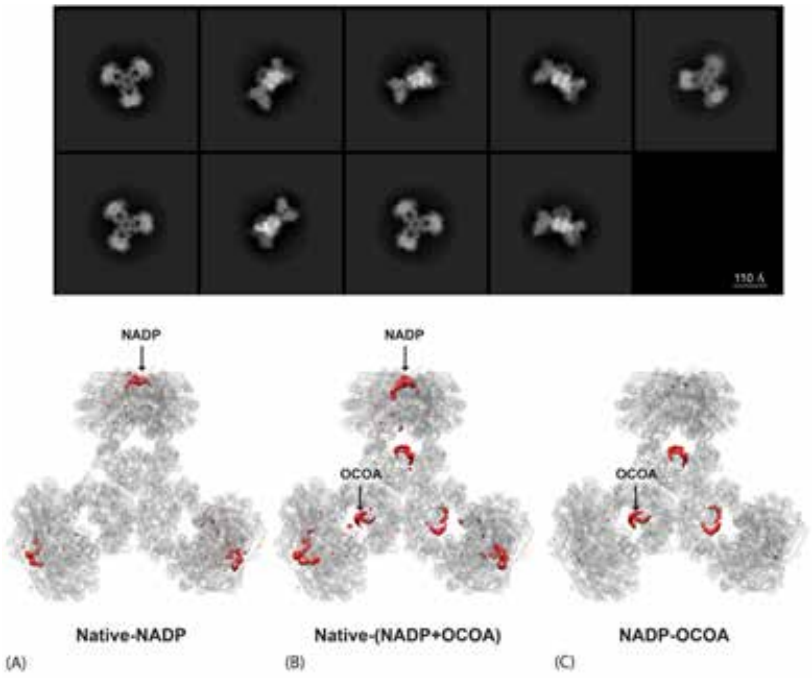
The last year has been an eventful year for the lab. The laboratory moved to new space in the inStem building. Our science has also taken a new direction with the arrival of the cryo-EM and it looks like a lot more action in the lab will be in cryo-EM in the coming years. We are in for some exciting times.

In the last annual report, we had a picture of several of the proteins structures that we had determined involved in the uptake and catabolism of sialic acid by gram negative bacteria. Publications from this project that has been going on for the last 5 years are now coming out. This work has been primarily funded by an Indo-Swedish collaborative grant by DBT *(India)* and Vinnova *(Sweden)*. The structure of the first sialic acid transporter has now been published and the structure reveals a new sodium site. Our work on bacterial sialobiology will continue, while we are busy writing up the basic science work, we have in parallel started work on translational aspects of drug discovery.

It has been suggested that cells marked by high expression of CD44 could be the cancer stem like cells in oral squamous cell carcinoma. To understand the gene expression pattern of these cells, we embarked on a project in collaboration with the *Kidwai Memorial Institute of Oncology*. Transcriptome analysis of CD44+ and CD44- cells from primary tissue was carried out. The study revealed a decreased expression of cell adhesion genes in the CD44+ cells. This work has now been published *(Mishra et.al., 2018).* Further work on understanding the pathways that are regulated will continue as our lab is now part of the Virtual National Oral Cancer Institute of the DBT.

The most exciting development is the arrival and the installation of the new high resolution electron cryo-microscope. The first work from our lab, is a collaboration with *Prof. Sowdhamini* and *Dr. Vinothkumar* at NCBS. We have now determined structures of the bifunctional enzyme PaaZ determined by electron cryo-microscopy without and with bound ligands. PaaZ is an enzyme in the hybrid aerobic-anaerobic hybrid pathway is used by many bacteria to degrade environmental pollutants and catalyses the crucial reaction of ring opening. The structures reveal that three domain-swapped dimers of the bifunctional protein form a hexamer. Combining knowledge of the structures with Small Angle X-ray Scattering and computational studies suggests that the key intermediate is transferred from one active site to another by a mechanism of electrostatic anchoring of the CoA moiety controlled by a set of conserved positively charged residues. A side note of the work is our ability to observe clear ligand density suggesting that this would be a good method to determine structures of protein-ligand complexes – very useful in a drug discovery setting. A second project is on the enzyme dimethyl formamidase an enzyme that is involved in the biodegradation of the common environmental pollutant dimethyl formamide. These studies done in collaboration with *Dr. Vinothkumar (with whose group we now share a joint lab),* has given



▲ *Figure 1: The top part of the figure shows the different 2D class averages, revealing the different views of the molecule. In the bottom the structures of PaaZ in complex with NADP (A), NADP and octonyl CoA bound to the aldehyde dehydrogenase domain (B) and NADP and octonyl CoA bound to the hydratase domain.*

us a new avenue to work on several interesting proteins that were hard to obtain crystals of. We are hoping that next few years the lab will use crystallography routinely, but move more towards using electron microscopy as a primary tool for our structural work.

PUBLICATIONS

Crystal Structures and Kinetic Analyses of N -Acetylmannosamine-6-Phosphate 2-Epimerases from Fusobacterium Nucleatumand Vibrio Cholerae (Acta Crystallogr. Sect. F Struct. Biol. Commun.74, 431–440)
Manjunath L., Guntupalli S. R., Currie M. J., North R. A., Dobson R. C. J., Nayak V., and Subramanian R. (2018)

Decreased Expression of Cell Adhesion Genes in Cancer Stem-Like Cells Isolated from Primary Oral Squamous Cell Carcinomas (Tumour Biol.40, 1010428318780859)
Mishra A., Sriram H., Chandarana P., Tanavde V., Kumar R. V, Gopinath A., Govindarajan R., Ramaswamy S., and Sadasivam S. (2018)

Automation Aided Optimization of Cloning, Expression and Purification of Enzymes of the Bacterial Sialic Acid Catabolic and Sialylation Pathways Enzymes for Structural Studies (Microb. Biotechnol.11, 420–428)
Bairy S., Gopalan L. N., Setty T. G., Srinivasachari S., Manjunath L., Kumar J. P., Guntupalli S. R., Bose S., Nayak V., Ghosh S., Sathyanarayanan N., Caing-Carlsson R., Wahlgren W. Y., Friemann R., Ramaswamy S., and Neerathilingam M. (2018)

"Just a Spoonful of Sugar" : Import of Sialic Acid Across Bacterial Cell Membranes (Biophys. Rev.10, 219–227)
North R. A., Horne C. R., Davies J. S., Remus D. M., Muscroft-Taylor A. C., Goyal P., Wahlgren W. Y., Ramaswamy S., Friemann R., and Dobson R. C. J. (2018)

Substrate-Bound Outward-Open Structure of a Na+-Coupled Sialic Acid Symporter Reveals a New Na+site (Nat. Commun. 10.1038/s41467-018-04045-7)
Wahlgren W. Y., Dunevall E., North R. A., Paz A., Scalise M., Bisignano P., Bengtsson-Palme J., Goyal P., Claesson E., Caing-Carlsson R., Andersson R., Beis K., Nilsson U. J., Farewell A., Pochini L., Indiveri C., Grabe M., Dobson R. C. J., Abramson J., Ramaswamy S., and Friemann R. (2018)

The New Era of Microcrystallography (J. Indian Inst. Sci. 10.1007/s41745-018-0086-0)
Banerjee S., Montaville P., Chavas L. M. G., and Ramaswamy S. (2018)

The Sodium Sialic Acid Symporter from Staphylococcus Aureus Has Altered Substrate Specificity (Front. Chem.6, 233)
North R. A., Wahlgren W. Y., Remus D. M., Scalise M., Kessans S. A., Dunevall E., Claesson E., Soares da Costa T. P., Perugini M. A., Ramaswamy S., Allison J. R., Indiveri C., Friemann R., and Dobson R. C. J. (2018)

Identification of Multiple Isomeric Core Chitobiose–Modified High-Mannose and Paucimannose N-Glycans in the Planarian Schmidtea Mediterranea (J. Biol. Chem.293, 6707–6720).
Subramanian S. P., Babu P., Palakodeti D., and Subramanian R. (2018)

AWARDS

Elected **Fellow of the Electron Microscopy Society of India.**

TALKS & OUTREACH

Science and Scientists – Mallya Aditi International School, December 2017

Why Do Science? – Kensri School, February 2018

What Has Science Done for Society? – Agastya Foundation, February 2018

8.2

Illuminating Cellular Dynamics and Natural Light Sensing

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New advances in science are made through new measurements. We develop tools that make these measurements possible. We have developed biosensors which allow us to 'see' how disease-causing proteins work in live cells and tissues. We have discovered novel light sensing proteins, enabling new technology and understanding of natural light sensing.

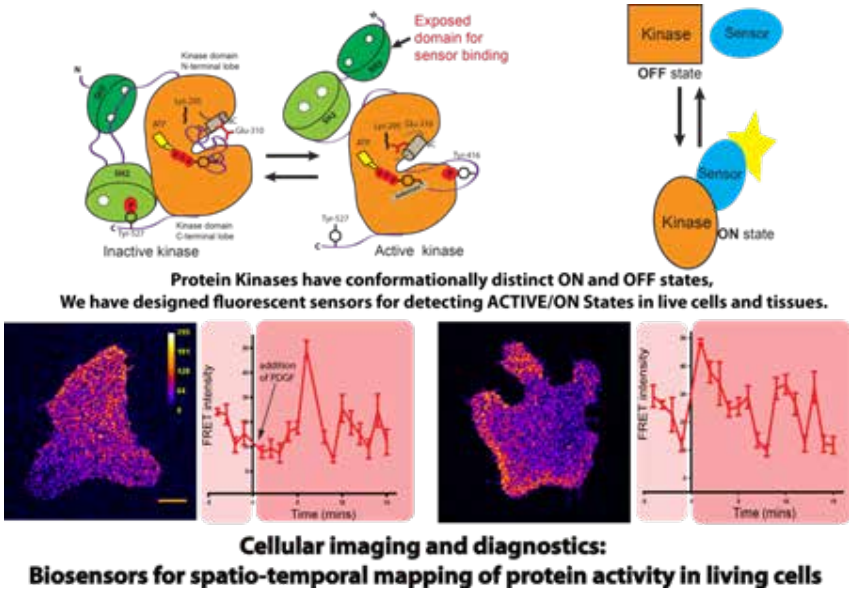
Sensors and Probes for Cellular Imaging

Cell function is controlled by precise activation of signalling protein. These signalling activities are tightly regulated in time and within specific compartments in cells and tissues. We have developed biosensors for directly imaging activities of signalling proteins. For instance, we have built multiple biosensors for visualizing activity of Src family kinases, critical in major diseases like cancer, heart disease and inflammation. Biosensor imaging with a key drug targer, Fyn tyrosine kinase, has revealed for the first time, how this kinase is able to coordinate multiple signalling modules. In the process we have also developed new technology for cellular diagnostics and biosensor generation.

We have also now established a robust programme focused on developing small molecule probes for sensing in cells and complex environments. For eg., we have built probes for mitochondrial imaging, metal ion sensing and for probing biological membranes. We are now using these new technologies for comprehensive imaging of cell function and state.

Shedding 'Light' on Neural Regeneration

Regeneration of organs and tissues is a significant challenge. Employing a highly collaborative and interdisciplinary approach, we have now established a new programme to study 'functional' eye and neural regeneration. Planarian flatworms show dramatic regeneration ability – it is possible to regenerate an entire organism, including its eyes, brain and nervous system, from a small cut piece of an adult worm. While regeneration has been examined, relatively little information is available linking eye and visual network regeneration to functional recovery. We have shown that planarians, despite possessing 'rudimentary' eyes and associated neuronal networks are able to perform complex sensing and processing *(Shettigar et al 2017).* Further, we can 'watch' the recovery of such complex function over regeneration, and ask what elements of the eye and the brain allow such complex sensing and processing to occur. This exciting advance is revealing the molecular and structural requirements for eye regeneration, patterning and function. In addition to this we have also discovered



▲ *Figure 1: Biosensors for imaging protein activity in living cells and tissues. Our group has developed new fluorescent biosensors for directly visualizing activity of Src family kinases, key enzymes critical in controlling cell behaviour and multiple disease states. Our sensors are able to detect and respond to active form of signalling proteins, allowing us to visualize biochemistry in real time and with spatial precision.*

eye-independent sensing in a variety of flatworm species. This work sheds light on how eyes and light sensing evolved and how light-sensing molecules and cells can be used in new applications and technologies.

PUBLICATIONS

Hierarchies in Light Sensing and Dynamic Interactions Between Ocular and Extraocular Sensory Networks in a Flatworm (Science Advances, 3, e160302)
Nishan Shettigar, Asawari Joshi, Rimple Dalmeida, Rohini Gopalakrishna, Anirudh Chakravarthy, Siddarth Patnaik, Manoj Mathew, Dasaradhi Palakodeti, Akash Gulyani (2017)

Facile Synthesis of Highly Sensitive, Red Emitting, Fluorogenic Dye for Microviscosity and Mitochondrial Imaging in Embryonic Stem Cells (Chemistry Select, 2, 4609-4616)
Sufi O. Raja, Gandhi Sivaraman, Ananya Mukherjee, Duraisamy Chellappa, and Akash Gulyani (2017)

Cytoplasmic Poly (A) Binding Protein (PABPC2) Critically Regulates Epidermal Maintenance and Turnover in Planarian Schmidtea Mediterranea (Development, 144 (17), 3066 – 30179
Dhiru Bansal, Jahnavi Kulkarni, Kavana Nadahalli, Vairavan Lakshmanan, Srikar Krishna G., Vidyanand Sasidharan, Jini Geo, Shilpa Dilipkumar, Renu Pasricha, Akash Gulyani, Srikala Raghavan, and Dasaradhi Palakodeti (2017)

Chemically Diverse Small Molecule Fluorescent Chemosensors for Copper Ion (Coordination Chemistry Reviews, 357, 50–104)
Gandhi Sivaraman, Murugan Iniya, Thangaraj Anand, Niranjan G. Kotla, Omprakash Sunnapu, Subramanian Singaravadivel, Akash Gulyani, and Duraisamy Chellappa (2018)

Tunable Emission from Fluorescent Organic Nanoparticles in Water: Insight into Nature of Self-Assembly and Photoswitching (Chemistry – A European Journal, (2018), 24,1–1)
Akash Gulyani, Nilanjan Dey, and Santanu Bhattacharya (2018)

A Unique Self-Assembly Driven Fluorescence Sensing of Lipid Bilayers: Ratiometric Probing of Vesicle to Micelle Transition (Chemical Communications, (2018), 54, 5122-5125; DOI: 10.1039/c8cc01635f)
Akash Gulyani, Nilanjan Dey, and Santanu Bhattacharya (2018)

Highly Responsive Fluorescent Assemblies Allow Unique, Multiparametric Sensing of Phospholipid Membrane Environment (Chemistry – A European Journal, August 2018. Accepted -https://doi.org/10.1002/chem.201803627)
Akash Gulyani, N. Dey, and S. Bhattacharya (2018)

Microenvironment Sensitive Charge-Transfer Dye for Tandem Sensing of Multiple Analytes at Mesoscopic Interfaces (Advanced online article, American Chemical Sciences-ACS Sustainable Chemistry and Engineering 2018. DOI:10.1021/acssuschemeng.8b02065)
Nilanjan Dey, Dipen Biswakarma, Akash Gulyani, and Santanu Bhattacharya (2018)

PATENTS (WITH INSTEM AFFILIATION)

Composition and method for mitochondrial imaging; Patent filed in India, US patent being applied for.
Akash Gulyani, Sufi O. Raja, and Gandhi Sivaraman

AWARDS

Cell Press – TNQ Best Paper in Life Sciences Award to *Nishan Shettigar* for his research paper, "Hierarchies in Light Sensing and Dynamic Interactions between Ocular and Extraocular Sensory Networks in a Flatworm".

TALKS & OUTREACH

Sensing Across Scales: Visualizing Cellular Dynamics and Discoveries in Natural Light Sensing – Advances in Science, Engineering and Technology, ASET colloquium, Tata Institute of Fundamental Research (TIFR) Bombay, July 2018

Multi-layered and Dynamically Interacting Light Sensing Networks in a Fully Regenerating Flatworm – Gordon Research Conference (GRC) on 'Photosensory receptors and signal transduction', Barga, Italy, March 2018

Illuminating Cellular Dynamics and Eye-Brain Regeneration – International Congress on Cell Biology (Joint meeting of Asia-Pacific, Asian and Indian Cell Biology Societies); Hyderabad, January 2018

Sensing and Visualizing Cellular Dynamics and Signalling – Interdisciplinary Approach to Biological Sciences (IABS-2018), Indian Association for the Cultivation of Science, Kolkata, February 2018

Visualizing Signalling Dynamics and Eye-Brain Regeneration – International meeting on Biomaterials at Vellore Institute for Technology, Vellore, TN. June 2018

Sensing Across Scales: Imaging Cellular Dynamics and Eye-Brain Regeneration – Frontiers in Imaging Science, Janelia Research campus, Ashburn, Virginia, USA, March 2017

New Methods for Visualizing Cell Signalling and Neural Regeneration – University of Copenhagen, Denmark, June 2017

Discoveries in Signalling Dynamics, Natural Light Sensing and Eye-Brain Regeneration – Meeting on 'Information transfer across scales- From Molecules to Behaviour, University of Calcutta, Kolkata, March 2017

New Probes and Sensors for Live Cell Imaging of Cell Signalling – Kaleidoscope, a discussion meeting in Chemistry, Goa, July 2017

Visualizing Cellular Dynamics and Discoveries in Natural Light Sensing – Institute of Bioinformatics and Applied Biotechnology (IBAB), Bangalore, India, March 2017

8.3

Laboratory of Self-Assembled Biomaterials: Advanced Materials for Biomedical Applications

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The Vemula group develops a wide range of biomaterials and bioengineering concepts to solve unmet clinical needs. At present his lab is developing prophylactic biomaterials, specially a topical gel to prevent pesticide-induced systemic acetylcholinesterase inhibition thereby preventing neuronal dysfunction and mortality in agriculture farmers.

India is one of the prime countries in pesticide usage in the world, with a vast majority of agricultural workers being repeatedly exposed to pesticides in the field. Organophosphate-based pesticides are acetylcholinesterase (AChE) inhibitors. Inhibition of AChE leads to the accumulation of the neurotransmitter, acetylcholine, resulting in neurological disorders, suffocation, paralysis, and in severe cases death.

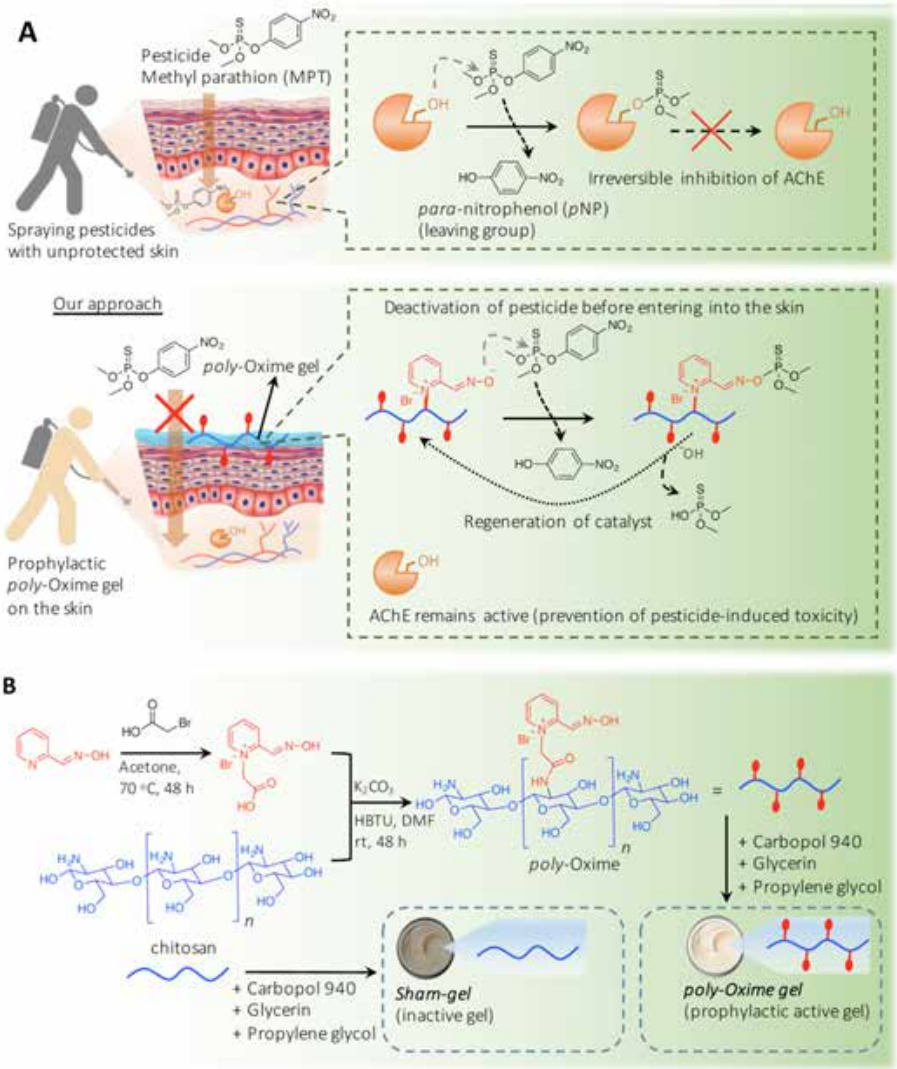
It is clear that systemic exposure to pesticides through dermal route is a health hazard. Preventive strategies to eliminate or minimize dermal exposure to reduce pesticide-induced toxicity is a less attended clinical need. Although, the personal protective equipment (PPE) such as suits, gloves, face masks, headgear, and boots are available; they are scarcely used, mainly due to high cost and discomfort under tropical conditions. An ideal user compliant solution would be a low-cost, easy-to-use, non-obstructive biocompatible material which could inactivate pesticides on the skin and prevent their penetration. A topical gel with a catalytic activity to cleave/hydrolyse pesticides before entering into the skin may serve as a prophylactic strategy to prevent the chronic exposure *(Figure 1A)*.

Herein, we present a nucleophilic poly-Oxime *(pyridine-2-aldoxime conjugated chitosan)* topical gel *(Figure 2B)* capable of catalytically deactivating organophosphates efficiently on the skin, thereby preventing inhibition of AChE quantitatively, and preventing pesticide-induced toxicity and mortality. Direct dermal exposure of widely used pesticide, methyl parathion (MPT) in rats significantly inhibited the activity of AChE in blood, brain, lung, liver and heart. A single dose dermal exposure of MPT (150 mg/kg) in rats dampened the locomotor coordination function, altered neuromuscular signalling and lead to death with a median survival time of four days. The same dose of MPT in the presence of poly-Oxime topical gel did not induce any adverse effects, and a 100% survival was observed. The poly-Oxime gel could hydrolytically cleave a wide range of commercial organophosphate formulations.

To demonstrate the efficiency of poly-Oxime gel to prevent pesticide-induced mortality, we used two sets

of experiments. In the first set, rats were repeatedly exposed to a pesticide while every day a thin layer of poly-Oxime gel was applied prior to pesticide exposure. While rats have died in the absence of poly-Oxime gel (MST = 4 days), the presence of poly-Oxime gel prevented mortality and a 100% survival has been observed *(Figure 1C)*. In the second set, the poly-Oxime gel was applied only once on day-0, and repeatedly exposed to pesticide for four days. Even a single application of poly-Oxime gel completely prevented mortality with a 100% survival. Daily or single application of sham-gel led to 100% mortality with MST of 7 and 6 days, respectively. This demonstrates the stability, robustness and true catalytic nature of poly-Oxime gel.

Farming in developing countries is labour-intensive and demands strenuous physical activity from agriculture workers. As pesticide exposure is known to affect endurance and neuromuscular coordination, farmers lose their ability to work with full capacity. Our data demonstrate that the presence of poly-Oxime gel completely prevented pesticide-induced loss of endurance and neuromuscular coordination. Additionally, pesticide exposure can severely damage nerves such as a sciatic nerve. Through Gait analysis we have demonstrated that direct exposure to pesticide significantly damaged the sciatic nerve while the presence of poly-Oxime gel prevented such damage.



▲ *Figure 1: (A) Schematic of dermal penetration of pesticides which induce toxicity and mortality through AChE inhibition. (B) Synthesis of poly-Oxime polymer and a dermal gel preparation including sham gels.*

PUBLICATIONS

Prevention of Pesticide-Induced Neuronal Dysfunction and Mortality with Nucleophilic Poly-Oxime Gel (Sci. Adv.2018, in press)
Thorat K., Pandey S., Chandrashekharappa S., Vavilthota N., Hiwale AA., Shah P., Sreekumar S., Upadhyay S., Phuntsok T., Mudnakudu-Nagaraju K. K., Mahato M., Sunnapu O., and Vemula P. K. (2018)

Exploring Membrane Permeability of Tomatidine to Enhance Lipid Mediated Nucleic Acid Transfections (BBA Biomembranes 2018, in press)
Rangasami V. K., Lochania B., Voshavar C., Rachamalla H. R., Banerjee R., Dhayani A., Thangavel S., Vemula P. K., and Marepally S. (2018)

Bioresponsive Drug Delivery Systems in Intestinal Inflammation: State-of-the-Art and Future Perspectives (Adv Drug Deliv. Rev.2018, doi.org/10.1016/j.addr.2018.06.021)
Kotla N. G., Rana S., Sivaraman S., Sunnapu O., Vemula P. K., Pandit A., and Rochev Y. (2018)

Local Injections of Tacrolimus-Loaded Hydrogel Reduce Systemic Immunesuppression-Related Toxicity in Vascularized Composite Allotransplantation (Transplantation2018, in press)
Dzhonova D., Olariu R., Leckenby J., Banz Y., Prost J-C., Dhayani A., Vemula P. K., Voegelin E., Taddeo A., and Rieben R. (2018)

Towards an Arthritis Flare-Responsive Drug Delivery System (Nat. Commun. 2018, 9, 1275)
Joshi N., Yan J., Levy S., Bhagchandani S., Slaughter K., Sherman NE., Amirault J., He X., Rui TS., Valic M., Vemula P. K., Miranda O. R., Levy O., Aliprantis A., Ermann J., and Karp J. M. (2018)

The Mir-124 Family of Micrornas Critical for Regeneration of the Brain and Visual System in the Planarian Schmidtea Mediterranea (Development 2017, 144, 3211-3223)
Sasidharan V.,Marepally S., Eliott S. A., Baid S., Lakshmanan V., Nayyar N., Bansal D., Sanchez-Alvarado A., Vemula P. K., and Palakodeti D. (2017)

Metal Sequestering Dermal Cream Exhibits Beneficial Effects in Patients with Dyshidrotic Eczema Associated with Nickel Induced Allergic Contact Dermatitis - A Pilot Study (Allergy2017, 72, 310)
Gergovska M., Briand E., Karp J. M., Jacob S. E., Vemula P. K., Gospodinov D., and Kazandjieva J. (2017)

Targeted Delivery of Microbial Metabolite, Urolithin A Protects from Chemically (DSS or TNBS) Induced Colitis in Pre-Clinical Models (J. Immunol2017, 198 (1 Supplement), 65-6)
Singh R., Hegde B., Baby B. V., Sandeep C., Kotla N., Chandrasekar B., Marepally S., Bodduluri H., Vemula P. K., and Jala V. R. (2017)

Scaling the Effect of Hydrophobic Chain Length on Gene Transfer Properties of Di-Alkyl, Di-Hydroxy Ethylammonium Chloride Based Cationic Amphiphiles (RSC Adv.2017, 7, 25398-405)
Hiwale A. A., Voshavar C., Dharmalingam P., Dhayani A., Muktavaram R., Nadella R., Sunnapu O., Gandhi S., Naidu V. G. M., Chaudhuri A., Marepally S., and Vemula P. K. (2017)

Morphology Transition in the Helical Tubules of Supramolecular Gels Driven by Metal Ions (Chem. Commun. 2017,53, 1538-41)
Lalitha K., Sridharan V., Maheswari C. U., Vemula P. K.,and Nagarajan S. (2017)

Structural Studies of 1,2-O-cyclohexylidene-myo-inositol: Insights of Hydrogen Bonding Interactions (Acta Crysts. C2017, C73, 20-27)
Puroshothaman G., Juvale K., Vemula P. K., Kirubakaran S., and Thiruvenkatam V. (2017)

BOOK CHAPTERS

Prevention of Metal Exposure: Chelating Agents and Barrier Creams in: Metal Allergy (Springer)
Mahato M., Sherman N. E., Mudnakudu K. K. M., Joshi N., Briand E., Karp J. M., and Vemula P. K. (2017)

Viral and Non-Viral-Based Hybrid Vectors for Gene Therapy in: Gene and Cell Therapy: Biology and Applications (Springer Nature)
Mahato M., Rao J. G., and Vemula P. K. (2018)

TALKS & OUTREACH

Proactive Acceleration of Biomedical Translational Research – National workshop on Nanomedicine: Challenges and Barriers in Translation, New Delhi, April 2018

Disease-responsive Nanomaterials: their Biomedical Applications – Science Academies Lecture Workshop, Mody University, Sikar, Rajasthan, April 2018

Key Steps in Science-Entrepreneurship: A Tale of Three (Ad)Ventures Based on Nanomaterials – Science Academies Lecture Workshop, Mody University, Sikar, Rajasthan, April 2018

Disease-Responsive Biomaterials: An Emerging Concept for the Treatment of Inflammatory Diseases – School of Regenerative Medicine, MAHE, Bangalore, April 2018

Disease-Responsive Biomaterials: An Emerging Concept for the Treatment of Inflammatory Diseases – Amrita Institute of Medical Sciences, Kochi, April 2018

Disease-Responsive Biomaterials: An Emerging Concept for the Treatment of Inflammatory Diseases – Special Interest Group Series, JSS University, Mysore, February 2018

Translational Science: A Tale of Three (Ad)Venturesin Science Entrepreneurship! – National Seminar at Sir. M. Visvesvaraya Institute of Technology, Bangalore, January 2018

Research and Entrepreneurship: A Roadmap to Science Entrepreneurship! – Govt. Degree College, Nizamabad, Telangana, October 2017

Translational Science: A Tale of Three (Ad) Venturesin Science Entrepreneurship! – Workshop for undergraduate students, National Institute of Technology, Warangal, October 2017

Nanomaterials for Biomedical Applications – Science Academies Lecture Workshop, SJR College for Women, Bangalore, August 2017

Disease-controlled Pharmacokinetics: Its Applications in Biomedical Sciences – Research to Industry: Pharmacological Insights – Workshop, Acharya & B.M. Reddy College of Pharmacy, Bangalore, March 2017

PATENTS

Conjugate, a Composition, an Article, Process of Preparation and Application Thereof – (Indian Patent Application Number: 201841006678)
Vemula P. K., Thorat K. V., Chandrashekharappa S., and Pandey S.

Synthetic Analogs of Gut Microbial Metabolites for Protection of Endothelial and Epithelial Barriers and Applications Thereof – (USPTO #62/671,737)
Jala V. R., Bodduluri H., Singh R., Vemula P. K., Chandrashekharappa S., and Hiwale A. A.

8.4

Polyadenylation and poly A Binding Protein Regulate Neoblast Function in Planarians

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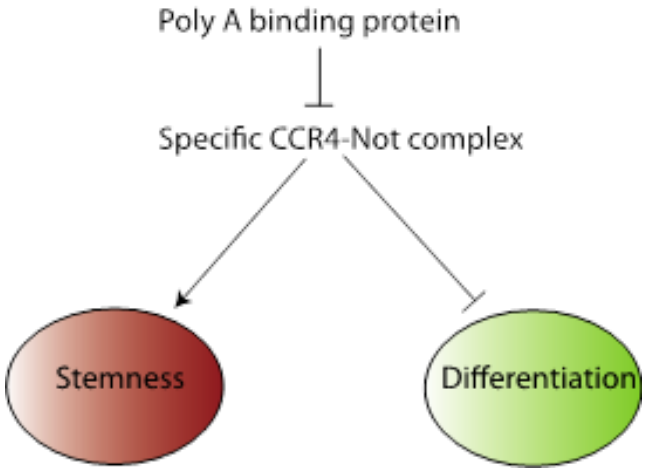
Initial events critical for stem cell state transition involve change in the protein repertoire. Translation regulation is one of the key mechanisms by which the protein levels could be modulated during stem cell state transitions. Using mouse embryonic stem cells and regenerative model, planaria Schmidtea mediterranea, we identified several cis and trans regulators of translation critical for stem cell state transitions.

Eukaryotes are typically characterized by the presence of poly A tail, which are bound by a specific set of proteins known as Poly A binding proteins. Poly A binding proteins are known to regulate mRNA stability and translation. However, the interacting partners of poly A binding proteins and the mechanism by which they regulate poly A length are not well understood. My laboratory has identified several classes of RNA binding proteins expressed in the stem cell population *(neoblast)* of planarians. Knockdown studies identified poly A binding proteins such as Poly A binding proteins Cytoplasmic *(PABPC2)* and Poly A binding proteins *(PABP1)* as critical regulators of neoblast function. We have recently shown that PABPC2 is essential for the epidermal turnover and knockdown lead to dramatic defect in the planarian wound healing and epidermal organization *(Bansal et al. 2017).* The other poly A binding protein, PABP1 is expressed in a specific subset of neoblast population and its knock down lead to increased neoblast proliferation and defect in differentiation. Transcriptome analysis identified specific class of CCR4-NOT proteins that were up-regulated in the neoblast progeny. Currently, we are testing the hypothesis that the PABP is essential for controlling the levels of of specific deadenylase complex in neoblast progeny. Upon knockdown of PABP, the deadenylase complex destabilizes the transcripts critical for differentiation and thus promote the stemness. Together, our study will unravel the key role of poly A binding proteins in regulating de-adenylases complexes critical for stem cell function.

Ribosome Heterogeneity Regulate Stem Cell Function

Translation regulation is majorly regulated by the binding of the RNA binding proteins or various classes of small RNAs to the UTR regions of the mRNA. However, recent studies have implicated changes in the ribosomal composition as a key regulator of translation. We want to dissect the role of ribosomal proteins and their post-translational modification in stem cell function. Using planaria as a model system, we have identified several ribosomal proteins en-



▲ *Figure 1: Schematic showing the Poly A binding protein in regulating specific CCR4-Not complex protein essential for stem cell function.*

riched in the neoblast population. Knockdown of some of those protein lead to varying defects in the neoblast function. Currently, we are investigating the transcripts that could be affected by the knockdown of these ribosomal proteins and their potential role in neoblast function. The other aspect of ribosomal heterogeneity involves differential post-translation modification of the ribosomal proteins that could influence the ribosomal loading and its elongation during translation. Using embryonic stem cells, we are investigating the post-translation modification on the ribosomes that could affect translation during stem cell proliferation and differentiation.

PUBLICATIONS

Quantification of Neurotransmitters from Intact and Regenerating Planarians Using UHPLC-MS/SRM Method (cMol Biol. 2018;1774:555-570. doi: 10.1007/978-1-4939-7802-1_25)
Rangiah K., and Palakodeti D. (2018)

Post-Transcriptional Regulation in Planarian Stem Cells (Semin Cell Dev Biol. 2018 Jun 8. pii: S1084-9521(17) 30204-5.Review)
Krishna S., Palakodeti D., and Solana J. (2018)

Identification of Multiple Isomeric Core Chitobiose-Modified High-Mannose and Paucimannose N-Glycans in the Planarian Schmidtea Mediterranea (J Biol Chem. 2018 May 4;293(18):6707-6720)
Subramanian SP., Babu P., Palakodeti D., and Subramanian R. (2018)

Developmentally Regulated Higher-order Chromatin Interactions OrchestrateB Cell Fate Commitment (Nucleic Acids Res. 2017 Nov 2;45(19):11070-11087).30204-5.Review)
Boya R., Yadavalli AD., Nikhat S., Kurukuti S., Palakodeti D., and Pongubala JMR. (2017)

Cytoplasmic Poly (A)-binding Protein Critically Regulates Epidermal Maintenance and Turnover in the Planarian Schmidtea Mediterranea (Development. 2017 Sep 1;144(17):3066-3079)
Bansal D., Kulkarni J., Nadahalli K., Lakshmanan V., Krishna S., Sasidharan V., Geo J., Dilipkumar S., Pasricha R., Gulyani A., Raghavan S., and Palakodeti D. (2017)

The miR-124 Family of microRNAs is Crucial for Regeneration of the Brain and Visual System in the Planarian Schmidtea Mediterranea (Development. 2017 Sep 15;144(18):3211-3223. doi: 10.1242/dev.144758. Epub 2017 Aug 14)
Sasidharan V., Marepally S., Elliott SA., Baid S., Lakshmanan V., Nayyar N., Bansal D., Sánchez Alvarado A., Vemula PK., and Palakodeti D. (2017)

Hierarchies in Light Sensing and Dynamic Interactions Between Ocular and Extraocular Sensory Networks in a Flatworm (Sci Adv. 2017 Jul 28;3(7):e1603025)

Shettigar N., Joshi A., Dalmeida R., Gopalkrishna R., Chakravarthy A., Patnaik S., Mathew M., Palakodeti D., and Gulyani A. (2017)

TALKS & OUTREACH

Expected Role of Serotonin in Planarian Eye Regeneration – EVODECE workshop, Observatoire Oceanologique de Banyuls sur Mer, France, January 2018

Role of tsRNA in Cell State Transitions – ICCB meeting, Hyderabad, January, 2018

RNA Mediated Regulation of Stem Cell Function and Regeneration – Genomics workshop, IBAB, December 2017

Planaria as a Model System to Study Regeneration – Centre for Human Genetics, Bangalore, May 2018

8.5

Gene Regulatory Mechanisms Governing Vertebrate Development

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Our research programme aims to uncover mechanisms of cell fate commitment during early vertebrate development. We then leverage this mechanistic insight to tackle human disease.

Intense efforts are on to investigate the developmental mechanisms driving the differentiation of specific cell types from pluripotent stem cells. This knowledge is the basis for rational approaches to guide stem cell differentiation into organoids or regenerative cell types. These applications will impact disease modelling, drug screening and regenerative medicine. Our interest is to study mechanisms governing the cell fate commitment during vertebrate development, with a specific focus on mesoderm. We study two key stem cell types in vertebrate embryos, **1)** cardiopharyngeal mesoderm (CPM), which gives rise to heart and a specific group of skeletal muscles in the head and **2)** neuromesoderm (NM), which contributes to spinal cord and musculoskeletal system *(see Graphic)*. In addition, we study cranial neural crest (NC), which make the cranial ganglia as well as craniofacial skeleton. Our work has revealed the developmental mechanisms governing these clinically important cell types.

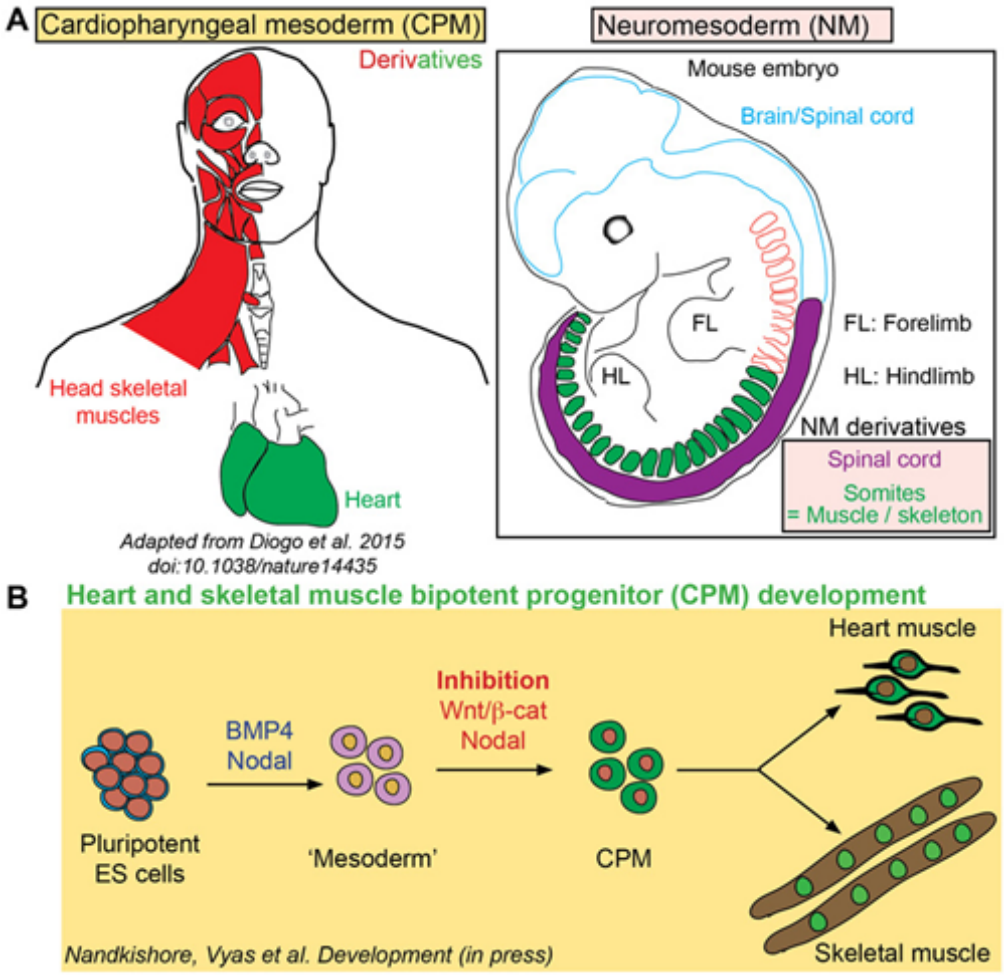
Building on our work, we have directed the differentiation of pluripotent cells to skeletal muscle via CPM developmental route *(see Graphic; Nandkishore, Vyas et al., under revision).* This is a significant breakthrough since a specific subtype of skeletal muscle has never been generated in vitro previously. Generating subtypes of skeletal muscle in the dish will enable research and therapies directed at the muscle-wasting diseases preferentially involving these specific muscle groups. Our current research aims to build on the foundation of this success and develop strategies for efficient skeletal muscle derivation from pluripotent cells.

In vertebrate embryos, a pool of 'axial progenitors' ensures the generation of the body below the level of forelimbs. A subset of these progenitors, known as neuromesoderm (NM), contributes to both the spinal cord and the mesoderm, which gives rise to the musculoskeletal tissue below neck. The mechanism regulating the maintenance of such bipotent progenitors and governing the binary fate choice remains ill defined. Our work has revealed a key function of Tbx6, a developmental T-box transcription factor in the NM-lineage *(Javali, Misra et al. 2017)*. Using mouse genetics as well as molecular studies to identify transcriptional targets, our work has begun revealing the function of Tbx6 in balancing the neural fate in order to maintain the bipotent progenitor state and subsequently, in inducing mesoderm differentiation during fate choice.

In vertebrates, a migratory population of cells known as the cranial neural crest (NC) originates from the border of developing brain. It gives rise to a vast array of cell types including neurons and glia of cranial nerves as well as cartilage and bone of the craniofacial skeleton. Perturbed NC development causes craniofacial abnormalities, the major group of birth defects

in humans. In spite of this clinical significance, NC development in mammals is poorly understood. Our work has identified novel components of the regulatory network controlling NC development. We have identified NC-enriched microRNAs (miRs) by comparative small RNA-sequencing. Our work indicates that these miRs control the migratory behaviour of NC via modulation of Wnt / β-catenin signalling pathway. The fate adopted by NC depends on the destination and our study is unravelling the machinery controlling NC differentiation by regulating its migration. Furthermore, using ChIP-sequencing, we have dissected the function of Twist1, a key transcription factor, in NC. Twist1 is mutated in Saethre-Chotzen Syndrome characterized by craniofacial defects. We have dissected the neural crest regulatory network controlled by Twist1 and find that it controls craniofacial skeleton development globally. This study has provided the framework to address the molecular basis of craniofacial defects in the syndrome.

In summary, our work has yielded important insights into the mechanisms governing early cell fate during vertebrate development. The knowledge as well as resources generated by our studies are creating unique possibilities to expand the scope of our research to study human disease.



▲ *Figure 1: (A) A common pool of progenitors, the cardiopharyngeal mesoderm, generates heart and head musculature of vertebrates. Neuromesoderm progenitors contribute to spinal cord as well as somite-forming paraxial mesoderm below forelimb.*
(B) Leveraging our findings on CPM development, we have guided the differentiation of stem cells into heart as well as skeletal muscle cells. The differentiation regimen, a recapitulation of developmental sequence, is shown.

PUBLICATIONS

Co-expression of Tbx6 and Sox2 Identifies a Novel Transient Neuromesoderm Progenitor Cell State (Development. 2017 Dec 15;144(24):4522-4529. Doi: 10.1242/dev.153262)
Javali A., Misra A., Leonavicius K., Acharyya D., Vyas B., and Sambasivan R. (2017)

Early Mesoderm Specification Underlies Divergent Head and Trunk Muscle Development in Vertebrates (Development - in press)
Nandkishore N., Vyas B., Javali A., and Sambasivan R. (2018)

Infectivity of Adeno-Associated Virus Serotypes in Mouse Testis (Under revision in BMC Biotech)
Rajasekaran S., Thatte J., Periasamy J., Javali A., Jayaram M., Sen D., Krishnagopal A., Jayandharan GR., and Sambasivan R. (2018)

TALKS & OUTREACH

International Society of Developmental Biology, Singapore, June 2017

Indian Society of Developmental Biology – Biennial meeting, Pune, June 2017

Advancing biology to therapy: From Stem Cells to Organs – Seminar in a symposium organized by Bharatidasan University, Tiruchirapalli, March 2018

9

TIGS-CI

Tata Institute for Genetics and Society – Centre at inStem

The goal of **TIGS-CI** is to advance global science and technology research in a socially-conscious and ethical manner to address some of the world's most pressing issues, ranging from public health to agriculture. **TIGS-CI** is also committed to capacity building for science and science policy in India.

TIGS-CI functions in a close collaborative partnership between two institutes - one at the University of California, San Diego (TIGS-UCSD) and the other in India. TIGS-CI, a public charitable Trust, was launched in India in 2017, and operates as a not- for-profit, basic research centre within the Institute for Stem Cell Science and Regenerative Medicine (inStem) in Bangalore, Karnataka. TIGS at UCSD and India will work collaboratively to train personnel, advance research and facilitate the broad applications of the latest genetic technologies to ultimately improve human health and agriculture in India.

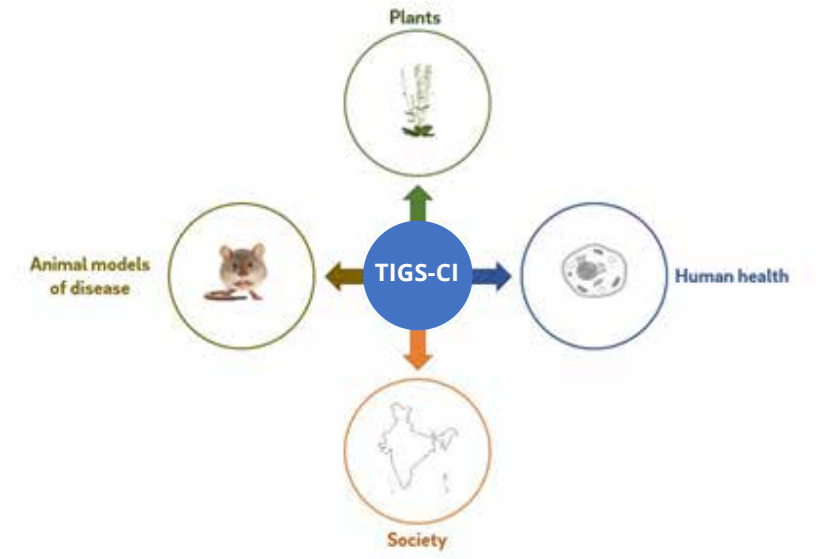
The emphasis is on capacity building to ensure that solutions to real-life problems will be created by trained scientists working in India. The scientific efforts are focused in three main areas:

1) Understanding the behaviour, population genetics and ecology of insect vectors that cause diseases in humans and plants.

2) Reducing antimicrobial resistance and the exploration of applications of the latest technologies using stem cells.

3) Using stem cell models to simulate and correct diseases in mammalian cells.

In order to further the commitment to advance research in a socially conscious and ethical manner, **TIGS-CI** will engage with governmental and educational institutions, communities, as well as other stakeholders to provide education about the technological advances. Such engagement will allow **TIGS-CI**, in partnership with the stakeholders, to strike the right balance between the benefits versus the risks of implementing the new technologies, thus, addressing any ethical issues that may exist, all within the regulatory framework established by the appropriate Government agencies.



10

CSCR

Centre for Stem Cell Research

(A translational unit of inStem, Bengaluru at Christian Medical College Campus, Bagayam, Vellore)

The Centre for Stem Cell Research continues to focus on translational research in cell and gene therapy towards regenerative medicine to bring stem cell science and other novel therapies to the management of patients with unmet needs. The concept of teams working on specific themes through multidisciplinary collaborations is being further enhanced to move closer to this goal. Four thematic research programmes in multi-individual, multi-disciplinary and multi-institutional programmes are described as follows. More details can be found on the CSCR website.


Alok Srivastava


R. V. Shaji


Saravanabhavan T.


Mohankumar M.


Srujan Marepally


Asha M. Abraham


Kuryan George


Shantidani Minz


Dolly Daniel


Vrisha Madhuri

1. Musculoskeletal Regeneration

This programme is coordinated by *Vrisha Madhuri* with a large team of clinical and basic scientists including several external collaborators. This group aims to develop novel therapies to address unmet needs of patients with bone, cartilage and muscle disorders. The major current focus is on clinical translation related to physis, articular cartilage, and bone regeneration. A novel pilot study of scaffold-based autologous mesenchymal stromal cells for correction of large segment bone loss in children has been completed; two phase I/II clinical trials have been initiated for the treatment of physeal bars and osteogenesis imperfecta. Two preclinical studies are in progress for osteochondral and segmental bone repair using functionalized scaffolds.

For articular cartilage regeneration small and large animal studies have been completed with differentiated MSCs on indigenous scaffolds with a successful outcome. Osteoarthritis prevention is another area that is being explored. There is a new focus on using biomolecules on the scaffold for regeneration with in vitro studies completed and ongoing large animal studies. The continued follow up for pilot human physeal regeneration with culture-expanded autologous chondrocytes has shown success at five years, and a Phase 1 clinical trial has been initiated with DHR funding. The group also achieved success in physeal regeneration using hydrogel scaffolds in the large animal model. A first of its kind pilot study on human bone defect regeneration study has been completed with a follow up of 1.5 - 3 years and further preclinical work is ongoing in the area of bone regeneration using biomolecules. A new phase I/II clinical trial has been initiated in collaboration with the *Karolinska Institute*, Sweden for treatment of osteogenesis imperfecta using foetal liver mesenchymal stem cells. Under international collaboration, the work on non-invasive manipulation of physeal cartilage and muscle derived stem cell for sphincter repair continues. This group has also successfully completed two preclinical studies for the treatment of a ventral hernia using muscle derived stem cells and tested the effect of novel tissue engineered construct using Wharton's Jelly MSC in a rat burn model.

▲ *Figure: Outcome of tissue engineered bone transplant in proximal humerus at three years follow up. **(A)** Preoperative radiographs of a 12-year-old with chronic osteomyelitis and gap non-union **(B)** Postoperative radiograph after 3 years shows union and well incorporated scaffold. **(C)** Shows the SEM analysis of cell seeded scaffold showing good cell to scaffold and cell to cell interaction at 8 days.*

2. Gene Therapy

Our goal is to capitalize on the recent advances in the world towards gene therapy of monogenic haematological disorders and make them possible for patients in India. Several scientists and physicians are involved with this work, coordinated by *Alok Srivastava (AS)*, and includes *R V Shaji (RVS), Saravanabhavan Thangavel (ST), Mohankumar Murugesan (MM)* and *Srujan Marepally (SrM)* at CSCR, and other faculty members from CMC, Vellore, in addition to several external collaborators.

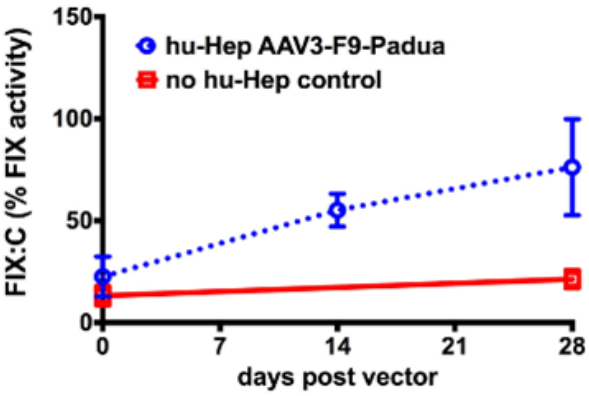
2.1 Haemophilia

The development of AAV vector-based gene therapy clinical trial for haemophilia B is the major initial thrust of this program led by AS. A clinical trial for gene therapy of haemophilia B is being planned. A unique vector (AAV3) and FIX transgene have been designed for this clinical trial, and its functionality has been confirmed in vitro and in vivo *(Figure 1).* The results are very encouraging in both of these models which sets the stage for clinical studies. However, there have been unanticipated challenges in the plans for GMP production of AAV3, which are being addressed with our collaborators in the USA. We are hopeful of completing this in the next 6-9 months and propose a clinical trial.

A lentiviral vector-based gene therapy for haemophilia A has also been developed over the last two years in collaboration with the Emory University, Atlanta, USA (AS). A proposal for a Phase 1 clinical trial has been submitted to the CDSCO three months ago. This approach is also important to explore for haemophilia as >50% of the patients may be ineligible for current AAV-based gene therapy due to pre-existing anti-AAV antibodies. The concept here is to transplant autologous haematpoietic stem cells (HSCs) ex-vivo transduced with a lenti viral vector carrying the FVIII transgene. Though this approach is well established for several diseases including the major haemoglobin disorders, this is the first such proposal for haemophilia in the world. It is also the first proposal for a clinical trial of gene therapy in India.

To explore other options for gene-transfer technologies for haemophilia, work is ongoing for developing a novel ex-vivo gene therapy by targeted integration of FVIII transgene in hematopoietic stem cells through CRISPR-Cas9 technology (MM). Given the expertise for lipid-based gene transfer at CSCR, we are also working on applying this approach to haemophilia through liver-targeted liposomal formulations (SrM). Both cellular and transgenic haemophilia animal models will be used to test these approaches.

An industry collaboration has been established with Intas Pharmaceuticals for the development of rAAV8-hFIX-Padua based gene therapy for Haemophilia B. This work is coordinated at CSCR by *Sanjay Kumar*. In-vivo efficiency of expression in being evaluated in the transgenic haemophilia mouse models at CSCR.



▲ *Figure: TK-NOG mice with humanized liver & AAV3-F9 Padua – hFIX activity 14- and 28-days post 5e11vg vector infusion.*

2.2 Haemoglobin Disorders

Another major thrust of the gene therapy is on the major haemoglobin disorders such as thalassemia and sickle cell disease which are major public health problems in India. Two approaches are currently under development – lentiviral vector-based gene transfer approach which is already being evaluated in animal models *(RVS/AS - details under the NAHD section)* and a novel gene-editing approach using the CRISPR-Cas9 technology for correction of the phenotype of β-thalassemia major and sickle cell disease by altering the expression γ-globin chains through transcriptional modifications (ST/MM) in collaboration with two groups at the University of California, USA through a INDO-US exchange programme.

2.3 Other Diseases

Using CRISPR/Cas9 gene editing tools preclinical studies are also ongoing to develop gene correction in Wiskott-Aldrich syndrome (WAS). Gene editing tools and strategies are being tested for the targeted integration of the WAS transgene in the hematopoietic stem cells.

2.4 Technology Development

I. A novel nucleofection reagent to deliver ribonucleo protein complex (Cas9-sgRNA) into hematopoietic cells, including HSCs (SrM/ST/MM).

II. A novel lipid nano-carrier system to deliver nucleic acids specifically to the liver. This technology is being explored in hemophilia in collaboration with the University of Florida, USA (SrM/AS).

3. Cellular Reprogramming and its Applications - Disease Modeling and Haplobanking

The area of cellular reprogramming technology is coordinated by *R. V. Shaji* at CSCR. This technology is now being applied to two areas of translational research, disease modeling,andhaplobanking. iPSC lines generated from a patient with Fanconi anemia are being used for understanding the molecular basis of bone marrow failure phenotype in this disease. As Fanconi anaemia pathway is required for reprogramming, we generated iPSCs using a doxycycline inducible lentiviral vector for complementation of the gene expression. The generated iPSCs showed disease specific phenotypes in the absence of doxycycline in the culture medium, and this helped us to have isogenic wild type and mutant iPSC lines for Fanconi anaemia. RNA sequencing carried out for the iPSCs cultured in the presence and absence of doxycycline identified previous pathways associated with Fanconi anaemia, and we also have identified several other pathways, which are currently under investigation. We also identified the small molecules that could be used for restoring the normal phenotype in the mutant iPSCs. Future experiments will be focussed on validating these small molecules to test whether they are potential drugs for this disease. Other diseases for which iPSCs is being developed include Diamond Blackfan anaemia and congenital dyserythropoietic anaemia. We are also developing isogenic mutant and wild type iPSC lines for these diseases by creating mutations using CRISPR/Cas9. Please see NAHD section for more details.

A major translational effort has also been initiated towards establishing a, *"haplobank"* for generating iPSCs from individuals homozygous for HLA haplotypes. This area is coordinated by *Dolly Daniel* from Department of Transfusion Medicine and Immunohaematology, CMC, Vellore and *R. V. Shaji* from CSCR. Liaising with the stem cell registry DATRI, and also including patients/donors HLA typed in CMC, to date 224 donors representing the top 18 haplotypes have consented and samples were drawn for iPSC production. Processes are in place for collection of donor samples and protocols for the derivation of iPSCs in a GMP facility have been standardized. HIPAA and HITEC compliant biobanking system has been installed and is in use. It is intended that during the next year, samples from individuals with the top 30-40 haplotypes will be collected. The MoU signed with Histogenetics will allow installation of an NGS system for confirmatory HLA typing of iPSCs made in the lab. We have established protocols for derivation of clinical-grade iPSCs. For this, using xeno-free reagents and episomal plasmids to deliver the reprogramming factors, we generated iPSC cells from cultured erythroid cells from donors. The iPSCs generated using this protocol showed high level expression of pluripotency markers, trilineage differentiation potential and normal karyotypes *(Figure1)*.

4. Novel Approaches to Haematological Disesaes (NAHD) Programme:

A major enhancement of some of the existing efforts at CSCR has come through the programme on Novel Applications in Haematological Diseases (NAHD) under the multi-institutional Indo-Japan programme on Accelerating Applications of Stem Cells to Human Diseases (ASHD). The NAHD segment of this programme is carried out at CSCR / CMC and has the following components:

4.1 Clinical Trial for Gene Therapy of Hemophilia B

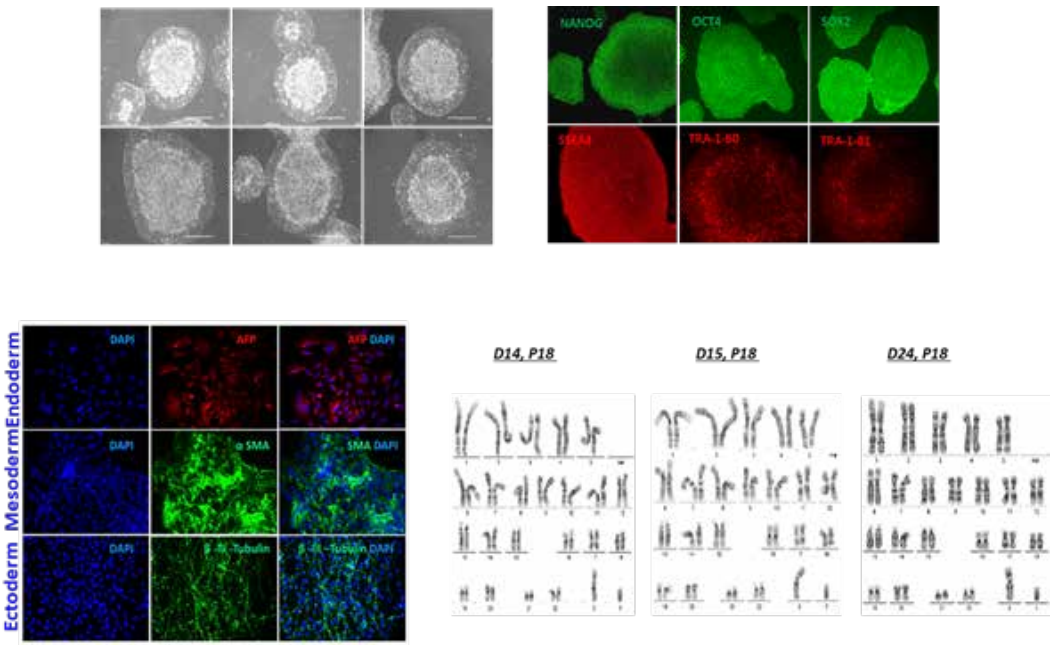
Details of this project are provided under the "Gene Therapy" section of this report.

4.2 Standardization of Anti-AAV Antibody Assays

The goal is to standardize assessment of anti-AAV antibody through different assays to allow appropriate selection of patients for gene therapy. This work is coordinated by *Asha M Abraham*, along with *Hubert Daniel* and *Rajesh Kannangai* from Department of Clinical Virology, CMC, Vellore, and *Sanjay Kumar* and *Alok Srivastava* from CSCR. It is being done in collaboration with the University of Florida, USA. Both binding and neutralizing antibodies are being assessed through the whole capsid and serotype specific peptide ELISAs and transduction inhibitions assays (TIA), respectively. The whole capsid and peptide ELISAs have been standardized for AAV 3, 5 and 8. TIA by mCherry based flow-cytometry had been standardized for AAV 3 & 5. Fifty hemophilia A or B and 50 healthy individual sera were tested for whole capsid and specific peptide antibodies. While about 50% of patients tested so far are positive for AAV3 antibodies, 18% of individuals are negative for antibodies to AAV 3, 5, and 8 *(Figure 2).*

4.3 Late Pre-Clinical Research - Lentiviral and Genome Editing Approach for Thalassemia and Sickle Cell Disease

This project aims to evaluate lentiviral vectors for developing gene therapy for the major haemoglobin disorders. This is coordinated by *R V Shaji* and *Alok Srivastava.* Four lentiviral vectors have been developed in collaboration Emory University. The lentiviral globin vectors showed significant expression in the ex-vivo erythropoiesis model and consistent expression 17 weeks after transplantation in sickle cell mice. New vectors for higher transgene expression are being generated. In parallel, immortalized erythroid progenitor cells were generated from CD34+ cells, and, in



▲ *Figure: iPSCs generated by episomal expression of reprogramming factors and using xeno-free cell culture media. **(A)** morphology **(B)** immunofluorescence analysis of pluripotency markers **(C)** trilineage differentiation of iPSCs and **(D)** karyotype analysis of the iPSCs generated using these conditions.*

future, these immortalized cells will be used for testing the lentiviral vectors for their transgene expression before testing them in mouse models.

Another important component of this programme is the gene editing approach to reactivation of fetal haemoglobin production. This work is being carried out by *Saravanabhavan Thangavel* and *Mohankumar Murugesan* using the CRISPR-Cas9 technology in collaboration with the University of California. They have screened and identified BCL11A enhancer, BCL11A binding site, and Sicilian HPFH deletion as the targets. The gene editing of these targets resulted in the fetal haemoglobin level of at least 10% in erythroid progenitor cell lines.

4.4 Early Pre-Clinical Research for Hemoglobin/ Erythroid Disorders

We aim to create disease models for two monogenic erythroid disorders, Diamond Blackfan Anemia (DBA) and Congenital Dyserythropoietic Anemia (CDA), by creating mutations in the associated genes by CRISPR/ Cas9. We have successfully disrupted the target genes by CRISPR/Cas9. Currently, we are establishing a novel approach to introduce biallelic mutations along with selection markers to screen colonies for target mutations. Methods to differentiate iPSCs to haematopoietic progenitor to erythroid cells have also been developed. An iPSC line that expresses Cas 9 from AAVS1 site in doxycycline individual manner has been developed.

4.5 HAPLOBANKING – Bank of iPS Cells from Individuals with Homozygous HLA Haplotypes

This project is aimed at creating a bank of iPSCs derived from individuals homozygous for the most common HLA haplotypes in the Indian population. Details of this project are provided under the, "Cellular Reprogramming and Its Applications" section of this report.

4.6 Control of Thalassemia and Sickle Cell Disease – Creating a Model for India

This programme is led by *Kuryan George* and *Shantidani Minz* along with several other colleagues from the departments of Community Health, Haematology and Transfusion Medicine and Immunohaematology at CMC, Vellore. A collaboration was established with the Department of Health and Family Welfare and the National Health Mission of the Govt. of Odisha. This is a unique programme in terms of scale and complexity in this field in the world. Four districts have been identified to implement the first phase of this programme. Towards increasing capacity and capability for treatment of major haemoglobin disorders in Odisha, workshops are being arranged at different levels *(State/Regional/District levels)* for doctors/other healthcare workers of Odisha.

PUBLICATIONS

Physeal Regeneration: From Bench to Bedside (Mukhopadhyay A. (eds) Regenerative Medicine: Laboratory to Clinic. Springer, Singapore)
Madhuri V., Rajagopal K., and Ramesh S. (2017)

The Effect of Low Intensity Shockwave Treatment (Li-SWT) on Human Myoblasts and Mouse Skeletal Muscle (BMC Musculoskeletal Disorders BMC series 2017.18:557)
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Isolation, In-Vitro Expansion, and Characterization of Human Muscle Satellite Cells from the Rectus Abdominis Muscle (Paediatricorthopaedics and related sciences. Volume 3)
David Livingstone, Albert A Kota, Sanjay K Chilbule, Karthikeyan Rajagopal, Sukria Nayak, and Vrisha Madhuri (2017)

Tomatidine, a Steroidal Alkaloid Improves Liposomal Transfections (BBA Biomembranes 2018 – Accepted)
Vignesh K. Rangasami, Brijesh Lochania, Chandrashekhar Voshavar,Harikrishna R. Rachamalla, Rajkumar Banerjee, Ashish Dayani, Saravanabhavan Thangavel, Praveen K. Vemula, and Srujan Marepally(2018)

Tocopherol-Ascorbic Acid Hybrid Antioxidant Based Cationic Amphiphile for Gene Delivery: Design, Synthesis, and Transfection (Accepted - Bioorganic Chemistry 2018).
Venkanna M. V. N., Brijesh Lohchania, Harikrishnareddy Rachamalla, Rajkumar Banerjee, Srujan Marepally, and Srilakshmi P. V. (2018)

Targeted α-Tocopherol Based pH Sensitive Galactosylated Lipid: Design, Synthesis, and Transfection Studies (MedChemComm, 2018, 9, 264-274)
Venkanna M. V. N. , Brijesh Lohchania, Srujan Marepally, and Srilakshmi P. V. (2018)

Green Transfection: Cationic Lipid Pool Derived from Vegetable Fat Palm Stearin Enhances Nucleic Acid Transfections (ACS Omega, 2017, 2 (11), 7892–7903)
Priya Dharmalingam, Hari Krishna Reddy R., Bhanuprakash B., Saravanabhavan Thangavel, Mohan Kumar K. M., Rajkumar Banerjee, Arabinda Chaudhuri, Chandrashekhar Voshavar, and Srujan Marepally (2017)

Rhodamine-Based Fluorescent Turn-On Probe for Facile Sensing and Imaging of ATP in Mitochondria (ChemistrySelect, 2017, 2 (25) 7654-7658)
Omprakash Sunnapu, Niranjan G. Kotla, Balaji Maddiboyina, Duraisamy Chellappa, Srujan Marepally, Jayabalan Shanmugapriya Subramanian Singaravadivel, and Gandhi Sivaraman (2017)

Generation of an Integration-Free iPSC line (CSCRi005-A) from Erythroid Progenitor Cells of a Healthy Indian Male Individual (Stem Cell Research 29,148–151)
Manian K. V., Bharathan S. P., Maddali M., Srivastava V. M., Srivastava A., and Velayudhan S. R. (2018)

TALKS & OUTREACH

Lentiviral Vector-Based Gene Therapy for Major Haemoglobin Disorders – (Poster presentation;Accepted) – American Society of Gene & Cell Therapy, May 2018
Abhirup Bagchi, Shaji R. Velayudhan, David Archer, Jordan Shields, David Mc Carty, H. Trent Spencer, and Alok Srivastava

An Optimized Approach for the Generation of Induced Pluripotent Stem Cells from Peripheral Blood Derived Erythroid Progenitors – Poster presentation (Accepted) – International Society for Stem Cell Research (ISSCR) Annual Conference, Melbourne, Australia, June 2018
Kannan V. Manian, Alok Srivastava, and Shaji R. Velayudhan

Human induced Pluripotent Stem Cells (iPSC) Workshop – Centre forStem Cell Research (CSCR) conducted its first Human induced Pluripotent Stem Cells (iPSC) workshop in April 2018. The workshop was designed to provide better iPSC culture practice and train the researchers to translate the techniques in their own laboratories, and involved hands-on-training and guest lectures by invited experts in the field.

Annual Cell and Gene Therapy Symposium – CSCR has been organizing an annual symposium on Cell and Gene Therapy for the last twoyears. The aim of this meeting is to provide a platform for scientists and physicians to come together and discuss the advances in the field. The 3rd Annual Cell and Gene Therapy Symposium was held in September 2018. Over 120 scientists/physicians from 46 institutions attended this meeting including those from three industry groups and which had nine international speakers.

11

Research Development Office

Research at the ***Bangalore Life Science Cluster*** which includes *NCBS, inStem* and *CCAMP,* spans a diverse range of questions and approaches in the broad area of life sciences. The Research Development Office (RDO) was created to facilitate research and training at the Cluster, via research funding.

Over the course of the last eight years, the RDO has supported the diverse needs of the campus in fund-raising, grants management and contract negotiation for research funding from funding agencies, corporate sources and charitable organizations.

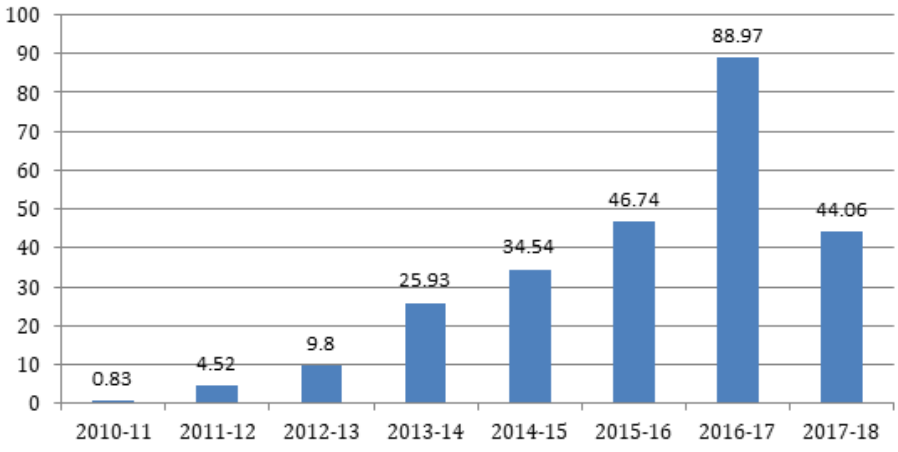
Generous funding from the Government has been invaluable in establishing large institutional programmes on campus such as the *Centre for Chemical Biology and Therapeutics* (CCBT), *Bangalore Life Sciences Cluster for Multiscale Basic and Applied Research in the Biological Sciences* (B-LIFE), the *Programme on Chemical Ecology*, the *National Mouse Research Resource* (NaMoR) and the *Macromolecular Crystallography and Scattering Facility*. The RDO manages all these large programmes.

The campus has also invested considerable effort into developing a mixed funding portfolio including charitable funding to complement funding from Government and international grants on campus. Some successful examples of programmes benefiting from such mixed funding include the *Centre for Brain Development and Repair (CBDR)* at inStem supported by the Shanta Wadhwani Foundation and the Department of Biotechnology (DBT). More recently, the Pratiksha Trust and DBT have supported a major programme on *"Accelerator Programme for Discovery in Brain disorders using Stem cells"* (ADBS) at inStem and NCBS, with institutional collaborations with NIMHANS.

In 2017, TTK Prestige Group awarded a generous grant for supporting our vision of *"Scientists beyond boundaries"* which has given a significant boost to our Campus Fellows Programme and enabled support to International researchers at BLiSc. In addition, this grant also enables students and postdoctoral fellows to attend international conferences and workshops through the *"TT Narasimhan travel awards"*.

Through the generosity of our philanthropic partners we have initiated the *Endowment Fund* in 2016 for research, training, innovation and outreach.

Work at the RDO is made possible by a dynamic and professional team who are committed to offering several key services to the campus at the boundaries of science, management and outreach. We look forward to a rewarding journey further ahead for the RDO, supporting campus research funding and the Endowment Fund.



▲ *Figure: Extramural funds at inStem (in Crores INR)*

12

Communications Office @ inStem

The BLiSc Communications Office aims to bring about greater awareness of, and interaction with the Bangalore Life Sciences cluster via communication and promotion of the research, discoveries, and, events of NCBS, inStem, and C-CAMP—thereby reinforcing its reputation for innovation and scientific excellence.

The office supports in Stem by developing collateral material, providing communications counsel and services to augment its presence through various channels, outreach, and events. We develop and implement strategies to enhance awareness and understanding of inStem and the value it brings to the scientific community.

PR and Media

The Communications Office has maintained an up-to-date, online repository of *publications, popular science articles,* and *news reports* on inStem's research. We also work closely with the institution in the creation, planning, and management of inStem events – providing communication support. We have continually nurtured publicity and growing reach via our social media engine. Some examples of our work include:

- Feature on *Prof. Ramaswamy's* Tedx talk on "Science and Scientists".
- Coverage of the EMBO Size and Shape event hosted by inStem.
- Write up on collaborative paper with the Simons Centre co-authored by *Dr Sunil Laxman* of inStem received encouraging feedback on social media.
- Coverage of inStem's presence at the India International Science Festival (IISF), in Lucknow.

Outreach Activities 2018

In the past year, the Communications Office has been involved in several outreach initiatives that have included inStem participation. In June, we partnered with *Mandram* – a platform to promote Tamil language and literature – for the Jigyasa Project to present an event comprising science talks in Kannada and Tamil.

In June 2018, we launched ***Science Café***– a monthly session of informal, relaxed exchange of scientific knowledge and innovative ideas that brings science to the public at an accessible venue in the city. *Dr Ravi Muddashetty* of inStem was part of both initiatives, and we are slated to have more inStem scientists participate in future iterations. June also marked our ***'Out of the Lab'*** outreach where citizens can invite scientists for popular talks and Q&A sessions in their neighbourhoods.

The Comms Office also actively manages ***school outreach programmes*** and has facilitated several schools and colleges for facility visits, where students are introduced to different laboratories within the cluster and can interact with the scientists. This is in addition to the various school outreach initiatives inStem PIs undertake independently.

What's Next

In November 2018, we are reviving the Jigyasa Project to deliver popular science talks in Kannada, Tamil and Hindi languages.

Science Café will travel to India's North East region and present talks from Cluster PIs in concert with local scientists delivering talks in local Khasi and Assamese.

The Comms Office will also endeavour to redesign the inStem website and give it a facelift that reflects its dynamic charter.

We also have lined up a series tentatively named, ***"inLab – covering a lab a week"***, that provides an insight

into a week in the life of a lab at inStem, covering the themes, areas of research, and interactions with scientists and students alike. The purpose behind this initiative will be to create a bridge of understanding and awareness of the institute and make it more accessible to the public.

Upcoming events, such as the TIGS symposium, will be undertaken with complete support from the Office, both as pre-event organisers as well as offering media and publicity support.

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inStem Collaborations


IFOM,
Italy
IBDM,
Marseille
University of
Cambridge, UK
RIKEN, Japan
Kyoto
University,
Japan
Transdisciplinary University
(TDU), Bangalore
NIMHANS, Bangalore
Manipal Academy of
Higher Education, Manipal
Rajiv Gandhi
University of Health
Sciences, Bangalore
Jawaharlal Nehru Centre for
Advanced Scientific Research
(JNCASR), Bangalore
Indian Institute of
Science (IISc), Bangalore
University of Agricultural
Sciences (UAS), Bangalore
Christian Medical
College, Vellore
St. John's Medical College
Hospital, Bangalore

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inStem Investigators

Apurva Sarin, Senior Professor & Dean
S. Ramaswamy, Senior Professor
Colin Jamora, Investigator[1]
Dasaradhi Palakodeti, Associate Investigator
Srikala Raghavan, Associate Investigator
Shravanti Rampalli Deshpande, Assistant Investigator
Akash Gulyani, Assistant Investigator
Praveen Kumar Vemula, Research Investigator
Ravi S. Muddashetty, Research Investigator
Ramkumar Sambasivan, Research Investigator
Tina Mukherjee, Assistant Investigator
Minhaj Sirajuddin, Assistant Investigator
Sunil Laxman, Assistant Investigator
Arjun Guha, Research Investigator
Dhandapany Perundurai, Assistant Investigator

VISITING FACULTY

James Spudich ***(Stanford),*** Collaborative Science Chair
Ashok Venkitaraman ***(Cambridge),*** Collaborative Science Chair
Jeff Abramson ***(UCLA),*** Collaborative Science Chair (until 31/12/17)
Siddharthan Chandran ***(U. Edinburgh),*** Collaborative Science Chair
Peter Kind ***(U. Edinburgh),*** Collaborative Science Chair
Mahendra S. Rao ***(NYIRM, NewYork),*** Collaborative Science Chair
Sivaraj Sivaramakrishnan ***(U.of Minnesota),*** Visiting Faculty
Maneesha Inamdar ***(JNCASR),*** Adjunct Faculty
Anil Prabhakar ***(IIT,Madras),*** inStem Associate
Sanjeev Jain ***(NIMHANS, Bangalore),*** Adjunct Faculty
Jyotsna Dhawan ***(CCMB, Hyderabad),*** Visiting Senior Professor

1 Joint Appointment with IFOM (Milan, Italy).

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inStem Leadership Committees

A. SOCIETY

Dr. Renu Swarup, Secretary to the Government of India, DBT, New Delhi (from March 2018);
Prof. K. Vijay Raghavan, Secretary to the Government of India, DBT, New Delhi (up to February 2018)
Prof. Satyajit Mayor, Director, NCBS & inStem, Bengaluru
Dr. Alka Sharma, Advisor & Scientist G, DBT, New Delhi
Ms. Gargi Kaul, JS & FA, DBT, New Delhi (until February 2018)
Mr. B. Anand, AS & FA, DBT, New Delhi (from March 2018)
Mr. Chandra Prakash Goyal, Joint Secretary (Admin), DBT, New Delhi
Dr. Satyajit Rath, Scientist, NII, New Delhi
Dr. Kiran Mazumdar Shaw, CMD, Biocon India Ltd., Bengaluru
Dr. Sunil Thomas Chandy *(until September 2017);* **Dr. J. V. Peter** *(from September 2017)*, Director(s) CMC, Vellore
Prof. H. Sharat Chandra, Hon. Director, Centre for Human Genetics
Prof. Alok Srivastava, Head, CSCR & Professor of Medicine, CMC, Vellore
Prof. K. Muniyappa, Department of Biochemistry, IISc, Bengaluru
Prof. Goverdhan Mehta, Former Director, IISc & CSIR Bhatnagar Fellow, Bengaluru
Prof. P. Balaram, Molecular Biophysics Unit, IISc, Bengaluru
Dr. Chittaranjan Yajnik, Director, KEM Hospital, Pune
Dr. Chandrima Shaha, Professor of Eminence, NII, New Delhi
Prof. Jyotsna Dhawan, Visiting Senior Professor, inStem & Chief Scientist, CCMB, Hyderabad
Prof. Apurva Sarin, Dean, inStem, Bengaluru
Prof. S. Ramaswamy, inStem, Bengaluru
Prof. Upinder S. Bhalla, Dean, NCBS, Bengaluru
Mr. B. S. Nagaraja, Officer on Special Duty (OSD), holding charge of Head - Administration, inStem, Bengaluru

B. GOVERNING COUNCIL

Dr. Renu Swarup, Secretary to the Government of India, DBT, New Delhi (from March 2018);
Prof. K. Vijay Raghavan, Secretary to the Government of India, DBT, New Delhi *(up to February 2018)*
Prof. Satyajit Mayor, Director, NCBS & inStem, Bengaluru
Dr. Alka Sharma, Advisor & Scientist G, DBT, New Delhi
Ms. Gargi Kaul, JS & FA, DBT, New Delhi *(until February 2018);*
Mr. B. Anand, AS & FA, DBT, New Delhi *(from March 2018)*
Mr. Chandra Prakash Goyal, Joint Secretary *(Admin),* DBT, New Delhi
Dr. Satyajith Rath, Scientist, NII, New Delhi
Dr. Chandrima Shaha, Professor of Eminence, NII, New Delhi
Dr. Chittaranjan Yajnik, Director, KEM Hospital, Pune
Dr. Sunil Thomas Chandy *(until September 2017)*; **Dr. J. V. Peter,** *(from September 2017)*, Director(s) CMC, Vellore
Prof. K. Muniyappa, Department of Biochemistry, IISc, Bengaluru
Prof. Alok Srivastava, Head- CSCR & CMC, Vellore
Prof. Jyotsna Dhawan, Visiting Senior Professor, inStem & Chief Scientist, CCMB, Hyderabad
Prof. S. Ramaswamy, inStem, Bengaluru
Prof. Upinder S Bhalla, Dean, NCBS, Bengaluru

Prof. Apurva Sarin, Dean, inStem, Bengaluru
Mr. B. S. Nagaraja, Officer on Special Duty (OSD), holding charge of Head-Administration, inStem, Bengaluru

C. SCIENTIFIC ADVISORY COMMITTEE

Prof. Azim Surani, Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK
Prof. Alejandro Sanchez Alvarado, Howard Hughes Medical Institute, USA
Prof. Utpal Banerji, University of California, Los Angeles, USA
Prof. Francesco Blasi, IFOM *(FIRC Institute of Molecular Oncology, Milan),* Italy
Prof. Marco Foiani, IFOM *(FIRC Institute of Molecular Oncology, Milan),* Italy
Dr. Satyajit Rath, National Institute of Immunology, New Delhi, India
Prof. Mriganka Sur, Picower Institute for Learning and Memory, Massachusetts Institute of Technology, USA
Prof. Helen Skaer, Emeritus Professor, University of Cambridge
Dr. Mahendra Rao, Senior Scientific Advisory, NYSCF *(New York Stem Cell Foundation*)
Prof. Satyajit Mayor, Director, NCBS & inStem
Prof. S. Ramaswamy, Senior Professor, inStem
Prof. Jyotsna Dhawan, Visiting Senior Professor, inStem & Chief Scientist, CCMB, Hyderabad
Prof. Upinder S. Bhalla, Dean, NCBS
Prof. Apurva Sarin, Dean, inStem

D. FINANCE COMMITTEE

Prof. Satyajit Mayor, Director, NCBS & inStem, Bengaluru
Mr. B. Anand, AS & FA, DBT, New Delhi *(from March 2018)*
Dr. Alka Sharma, Advisor & Scientist G, DBT, New Delhi
Prof. S. Ramaswamy, inStem, Bengaluru
Prof. Apurva Sarin, Dean, inStem, Bengaluru
Prof. Upinder S. Bhalla, Dean, NCBS, Bengaluru
Mr. B. S. Nagaraja, Officer on Special Duty (OSD), holding charge of Head-Administration, inStem, Bengaluru

NON ACADEMIC STAFF

E. ADMINISTRATIVE STAFF

B. S. Nagaraja, Officer on Special Duty (OSD), holding charge of Head – Administration, inStem, Bengaluru
K. P. Pandian, Head – Strategy, Bangalore Life Sciences Cluster
K. M. Basavarajappa, Project Officer
Sreenath B. A., Purchase Officer
Shrikant Bhat, Assistant Accounts Officer
Valsala Neyyan, Administrative Assistant
Shobha R., Assistant Administrative Officer
Sunitha R., Project Assistant (Admin)
Shobha B. N., Project Secretary
Supriya N., Project Assistant

F. SCIENTIFIC & TECHNICAL STAFF

Sai Sudha, Scientist D
Rajesh R., Engineer C *(Systems Administrator)*
Anand Kumar V., Engineer C *(Electrical)*
Chakrapani N., Junior System Administrator

Design: Sumita Nanda

Image courtesy: Ravi Kumar Boyapati, NCBS

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