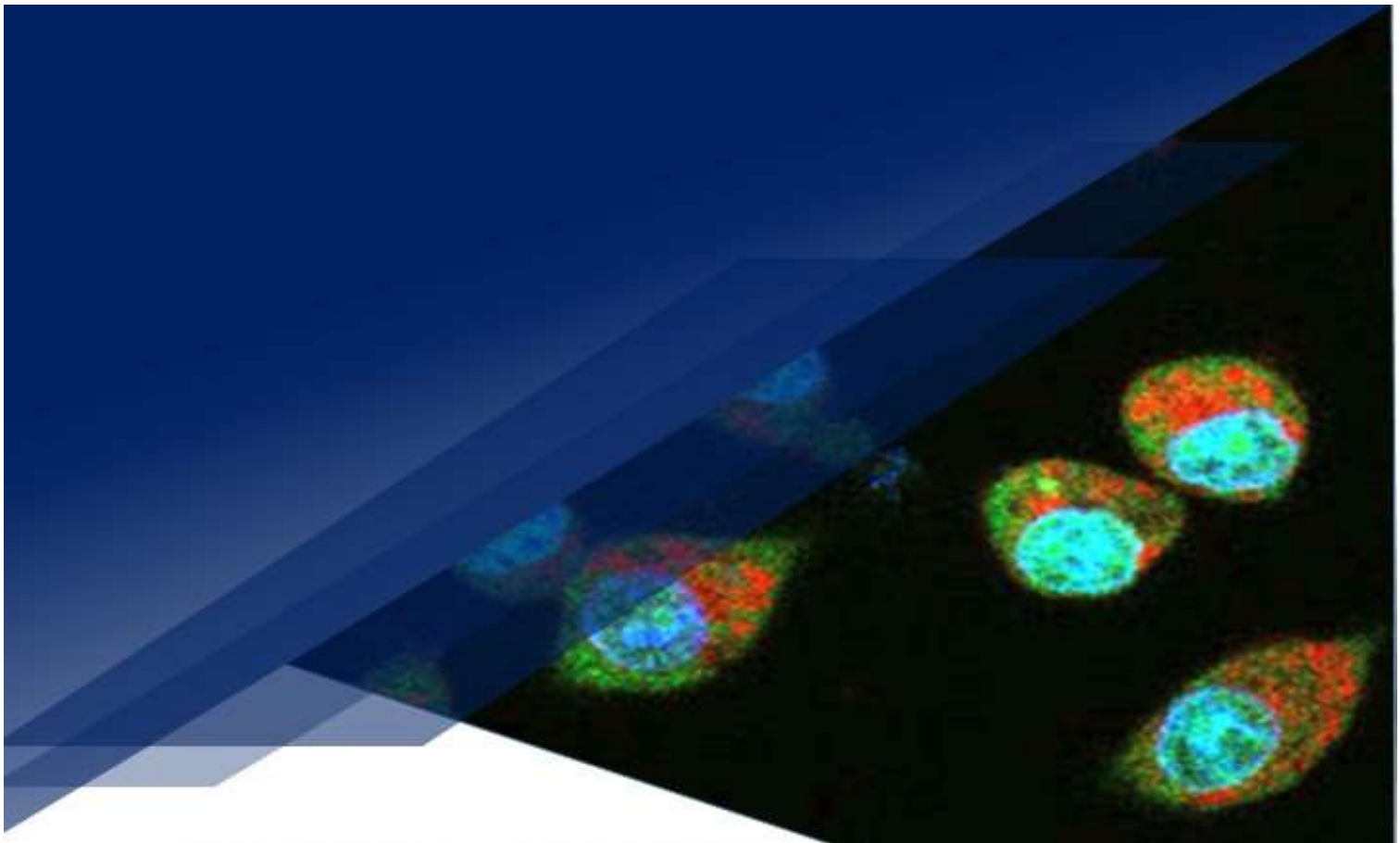


CSIR-IICB
Annual Report
2021-22

CSIR-INDIA
सीएसआईआर
CSIR
भारत का नवाचार इंजन
The Innovation Engine of India

CSIR-Indian Institute of Chemical Biology
4, Raja S. C. Mullick Road, Kolkata 700032

1

CSIR-IICB Annual Report 2021-22

Cover picture:

1. Live mouse monocyte/macrophage cell that has been stained by Dapi (nuclear stain) and Mito Tracker red (mitochondrial stain) to visualize mitochondria health and activity
2. Molecular structure of Mahanine

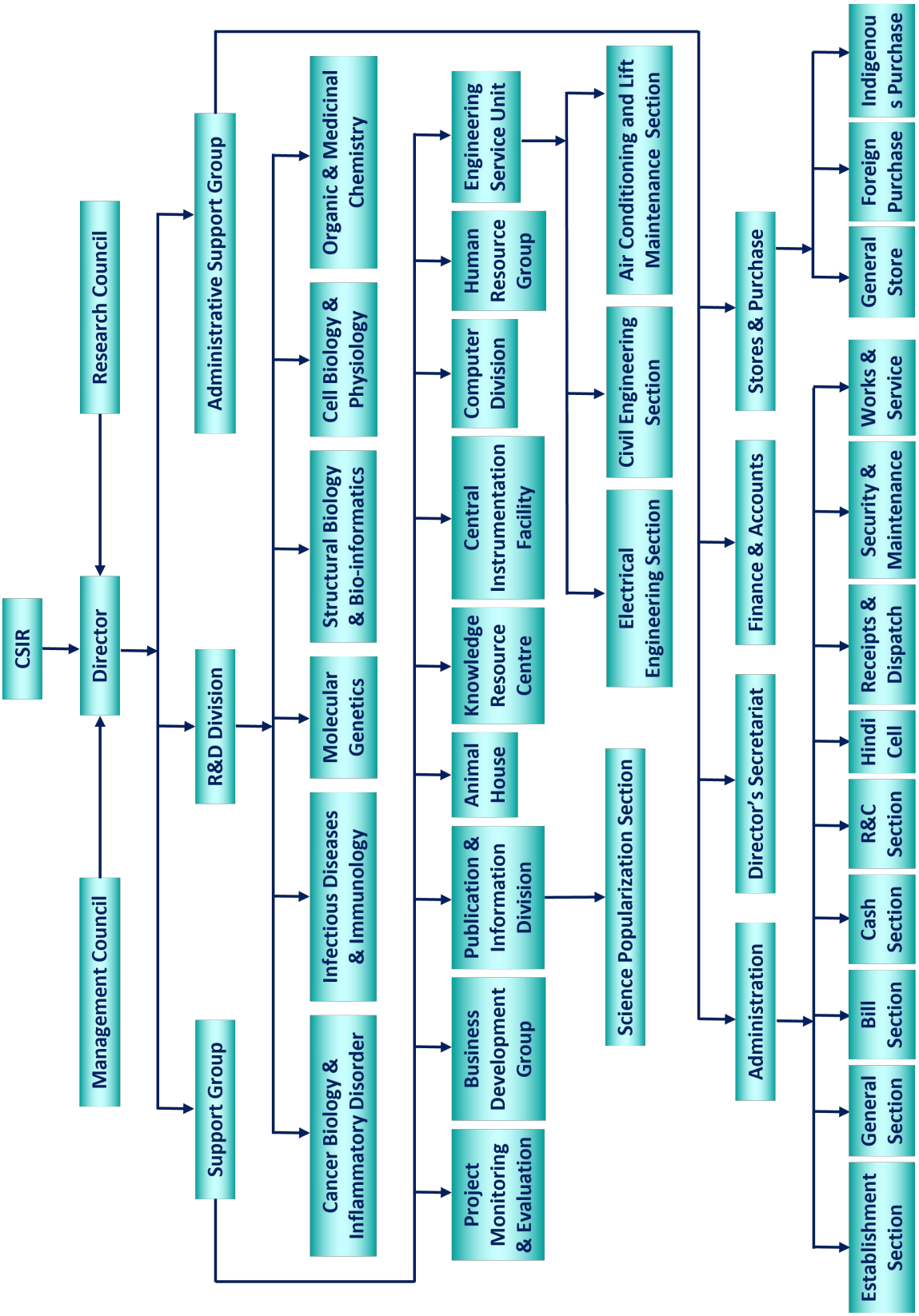
CSIR-Indian Institute of Chemical Biology
4, Raja S. C. Mullick Road, Kolkata 700032

2

वार्षिक प्रतिवेदन
Annual Report
2021-22

सी.एस.आई.आर.-भारतीय रासायनिक जीवविज्ञान संस्थान
4, राजा एस. सी. मल्लिक रोड, यादवपुर, कोलकाता - 700032, भारत
CSIR-Indian Institute of Chemical Biology
4, Raja S. C. Mullick Road, Jadavpur, Kolkata - 700032, India

CSIR-IICB Organisation Chart



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From the Desk of the Director

I am pleased to announce the publication of the Annual Report of CSIR-IICB for the year 2021-22. It carries glimpses of the achievements of the Institute during the year - particularly the efforts undertaken to deliver the benefits of science and technology to all corners of the society.

The Institute was established in 1935 as the Indian Institute of Medical Research. Presently it is one of the constituent laboratories of the CSIR and is involved in development of therapies and diagnostic strategies of human diseases. Besides the main campus at Jadavpur, Kolkata, it has a Translational Research Unit of Excellence (TRUE) at Salt Lake. Six research divisions manage the research and development activities of CSIR-IICB.

This institute significantly contributes to the knowledge generation in the fields of Chemical Biology, Biochemistry, Cell Biology, Genomics, Epigenomics, Structural Biology, Bioinformatics, and Medicinal Chemistry. Cutting edge research carried out at the Institute addresses the fundamental as well as applied biological and biomedical research challenges associated with communicable and noncommunicable diseases that are most relevant for the country.

Intervention by our scientists in areas like understanding structural and mechanistic features of various proteins, macromolecular complexes and cellular pathways, RNA-targeted small molecules, cost-effective process chemistry of life-saving drugs and agrochemicals, identification of pure herbal and synthetic compounds/herbal extracts having *in vitro* anti-proliferative activities against different cancer cells has resulted in significant contribution to the healthcare sector.

The excellent scientific infrastructure available in the Institute includes X-ray crystallography, Nuclear Magnetic Resonance (NMR), Cryo-EM, single molecule fluorescence measurements and Fluorescence Correlation Spectroscopy, Raman spectroscopy, mass spectrometry and Nano-separation technology. In addition to probing the frontiers of research, scientists of the Institute are also involved in guiding young students to ensure continuation of a steady stream of researchers dedicated to the progress of the nation.

I take this opportunity to thank our collaborators, sister laboratories of CSIR and the CSIR-Headquarters for their timely help, support and cooperation. I deeply appreciate each individual of the CSIR-IICB family for his/her contribution in making this Institute one of the premier research Institutes in the country.

Dr. Arun Bandyopadhyay

CSIR-IICB Research Council (2020-2023)

Dr. Debashis Mitra JC Bose National Fellow, National Centre for Cell Science, Pune	Chairman
Dr. Vidita Vaidya Hutment Area, Department of Biological Sciences Tata Institute of Fundamental Research, Mumbai	Member
Dr. Saman Habib Chief Scientist, Molecular & Structural Biology CSIR-Central Drug Research Institute, Lucknow	Member
Dr. Uday Saxena Chief Scientific Officer, Dr. Reddy's Laboratories Ltd., Hyderabad	Member
Prof. Anand Bachhawat Department of Biological Sciences, IISER Campus, Knowledge city, SAS Nagar (Mohali)	Member
Dr. Meenakshi Sharma Division of Non-Communicable Diseases Indian Council of Medical Research, New Delhi	Member
Dr. Vinay Kumar Nandicoori Director CSIR-Centre for Cellular and Molecular Biology, Hyderabad	Member
Dr. Mitali Mukherjee Chief Scientist and Head Genomics & Molecular Medicine CSIR-Institute of Genomics and Integrative Biology, New Delhi	Member
Dr. Geetha Vani Rayasam Council of Scientific & Industrial Research, New Delhi	Member
Dr. H S Subramanya Director, Institute of Bioinformatics and Applied Biotechnology (IBAB), Bengaluru	Member
Prof. Partha Sarathi Mukherjee IISc, Bangalore	Special invitee
Dr. Arun Bandyopadhyay Director, CSIR- IICB	Director, CSIR- IICB
Dr. Suvendra Bhattacharyya Senior Principal Scientist, CSIR-IICB	Secretary

CSIR-IICB Management Council

(01.01.2020 – 31.12.2021)

Dr. Arun Bandyopadhyay, Director, CSIR- IICB	Chairman
Dr. Tapas Kundu, Director, CSIR-CDRI, Lucknow	Director of sister laboratory
Dr. P. Jaisankar, Chief Scientist	Scientist of the laboratory representing the staff of various age groups
Dr. K.N. Chattopadhyay, Senior Principal Scientist	
Dr. S.N. Bhattacharyya, Senior Principal Scientist	
Dr. Ranjan Jana, Principal Scientist	
Dr. Arindam Talukdar, Principal Scientist	Head, RPBD/PME of the Laboratory
Dr. Aparna Laskar, Principal Technical officer	Representative of the technical personnel
Shri Asim Kumar Jha, Finance & Accounts Officer	CoFA/FAO of the laboratory
Shri Suprokash Halder, Administrative Officer	Member Secretary

(01.01.2022 – 31.12.2023)

Dr. Arun Bandyopadhyay, Director, CSIR- IICB	Chairman
Dr. Suman Kumari Mishra, Director, CSIR-CGCRI, Kolkata	Director of sister laboratory
Dr. Jayati Sengupta, Senior Principal Scientist	Scientist of the laboratory representing the staff of various age groups
Dr. Debabrata Biswas, Senior Principal Scientist	
Dr. Siddhartha Roy, Principal Scientist	
Dr. Smrutisanjita Behera, Scientist	
Dr. S.C. Biswas, Senior Principal Scientist	Head, RPBD/PME of the Laboratory
Dr. Binayak Pal, Principal Technical officer	Representative of the technical personnel
Finance & Accounts Officer, CSIR-IICB	CoFA/FAO of the laboratory
Sr. Controller of Administration / Administrative Officer, CSIR-IICB	Member Secretary

Research & Development @ CSIR-IICB

Cancer Biology and Inflammatory Disorder Division

Diverse arrays of aberrations are required before a normal cell will defy 'social behavior' to fulfill its only purpose – to proliferate – at the expense of life. By then, deep down at the molecular level crucial signalling pathways would have been extensively rewired. The long-term goals of this division are to focus on the comprehensive understanding of cancer at many levels ranging from the investigation of molecular and genetic basis of cancer, the elucidation of cellular processes altered during development of cancers, immune response and inflammation. The faculties conduct both basic and translational research on a range of topics that include lung, brain, oral, breast, prostate, colon, pancreatic, cervical cancers and leukemia.

Comprehensive insights into the molecular interactions and signaling crosstalks that contributes towards cancer progression and a quest for therapeutic avenues

Dr. Mrinal Kanti Ghosh, mrinal.res@gmail.com

Group Members

Dr. Sibani Sarkar (WoS, DHR, ICMR)
Sourav Dey (Lab Manager)
Gouranga Saha, Ex-SRF
Satadeepa Kal, Ex-SRF
Bhaskar Basu, Ex-SRF
Shaheda Tabassum, Ex-SRF
Rajni Shaw, SRF
Shrabastee Chakraborty, SRF
Sunny Kumar, SRF
Subhojit Karmakar, SRF
Srija Roy, SRF
Sayanyi Ghosh, JRF
Mouli Chatterjee, JRF
Sabana Begam, JRF

Collaborators

Dr. Sandeep Chatterjee, Park Clinic, Kolkata
Dr. Suresh Bajoria, RTIICS (NH), Kolkata
Dr. Uttara Chatterjee, IPGMER & Park Clinic, Kolkata

Background

Cancer is characterized by the undue proliferation of cells and the overhauling of homeostatic cellular conditions. The cells manage to efficiently evade the control mechanisms and give rise to a proliferative mass of cells that transform into tumors. The different types of cancers are characterized based on the organ or tissue of origin, along with the molecular characteristics of the respective cancer cells. Oncogenesis is defined as the process of transformation of normal cells into cancer cells through characteristic molecular reprogramming of a cell to undergo uninhibited cell division, allowing the formation of a malignant mass. According to the World Health Organization, one of the leading global causes of death was attributed to cancer in the year 2022, accounting for nearly 10 million deaths. The most common cancers are breast, lung, colon, glioma, and prostate cancers. At the molecular level, there exist numerous types of oncogenes that are dysfunctional genes owing to specific mutations or genetic expressions, that trigger deregulated cellular signaling pathways and suppress apoptosis in driving cancer. Contrarily, tumor suppressors are specific molecular players that help in decelerating the pace of carcinogenic aggravation in a cell by promoting anti-tumorigenic profiles and signaling networks. Due to rapid technological advances, it has become possible to gain insights into the molecular makeup of different cancer types, the development, and progression of cancer, and utilize the knowledge in designing effective diagnostics and cancer therapies to combat the disease and provide alleviation of complications in cancer patients and heed towards the pathway to curb oncogenesis.

Furthermore, it has been observed that despite the advancements in the field of cancer therapeutics, the management and treatment of disease is far from being under control. This necessitates the need for higher comprehensive ideas about the cellular machinery, molecular networks, and signaling mechanisms that together contribute to the oncogenic scenario. Additionally, there is a need to speed up translational research for unraveling revolutionary roles of different targeted drug delivery approaches, big-data analysis, and other bioinformatics tools to boost our understanding of cancer. Consequently, our lab aims to conjointly utilize the advancements in the scientific world and transcend into the arena of basic and translational research to gain a mechanistic understanding of the factors and signaling pathways leading to oncogenesis and seek effective therapeutic avenues of interventions through novel research ideas and scientific findings.

Aims and Objectives

- To unravel a detailed understanding of the role and regulation of key molecular players such as DDX5, RelA, CHIP, HAUSP, MGMT, FOXM1,

and PTEN in curbing oncogenesis.
- To study the transcriptional and post-translational modifications such as ubiquitination and deubiquitination in cancer.
- To identify novel molecular targets and gain insights into their tumorigenic propensity.
- To seek out novel therapeutic delivery techniques such as the use of nanoparticles and liposome-mediated methods for targeted drug delivery and cancer treatment.

Work Achieved

USP7 targets XIAP for cancer progression: Establishment of a p53-independent therapeutic avenue for glioma

Glioma is characterized by the malignancy of the central nervous system that often gives rise to the deadly Glioblastoma multiforme (GBM: grade IV glioma), a primary malignant brain tumor with minimal survival of three months without any proper medication. Despite advancements in diagnosis and therapeutics, the statistics of survival of glioma patients show dismal profiles. This necessitates a detailed understanding of the molecular players catering to glioma progression and therapeutically combating the scenario. Ubiquitin-specific peptidase 7 (USP7/HAUSP) is a deubiquitinating enzyme (DUB) that performs the function of the removal of characteristic ubiquitin tags from specific target protein substrates and alters their degradation rate, sub-cellular localization, interaction, and activity. Generally, in cancers characterized by the expression of wild-type p53, the induction of apoptosis by USP7 inhibition is well established and mediated by the 'USP7-Mdm2-p53' axis. However, in cancers without functional p53, USP7-dependent apoptosis follows several alternative pathways. The Inhibitor of apoptosis proteins (IAPs) are negative regulators of caspases found to be frequently overexpressed in various human cancers and widely targeted in many anticancer therapies. XIAP is one of the well-characterized members of the IAP group with its overexpression being linked to chemoresistance and overall poor prognosis. However, there exists a paucity of sufficient research interconnecting these molecular players and deciphering their interactive roles in cancer progression. Thus, we sought to broadly understand the role of USP7 in cancer progression and therapy.

In our lab, we have successfully identified the anti-apoptotic protein XIAP as a possible candidate for controlling p53-independent apoptosis under the influence of USP7's deubiquitinase activity. XIAP was identified as a potential target of USP7-dependent deubiquitylation through proteomics analysis. Thereafter, GSEA analysis revealed elevated regulation of apoptosis signaling upon USP7 inhibition associated with XIAP down-regulation. Modulation of USP7 expression and activity in multiple cancer cell lines showed that USP7 deubiquitinates XIAP to inhibit apoptosis in a caspase-dependent pathway. Additionally, we employed the USP7 inhibitor P5091 alone or combined with XIAP inhibitor Embelin and found their induction of cellular apoptosis at a higher magnitude than individual inhibition of USP7 in vitro and in vivo. Immunohistochemical staining revealed a sequential accumulation of USP7 grade-wise and was found to be correlated with an elevated level of XIAP in glioma tissue. Therefore, to the best of our knowledge, this is the first report on the identification and validation of XIAP as a novel substrate of USP7 and their conjoint roles in empowering the tumorigenic propensity of cancer cells by inhibiting apoptosis through an alternate route of tumor progression involving XIAP through the USP7-XIAP axis.

Future Research Plans

- To elucidate the transcriptional and post-translational regulation of important molecular players involved in oncogenesis such as HAUSP, CHIP, DDX5, PML, and PTEN.
- To develop an omics-based study (proteomics, transcriptomics) and employ the use of big data analysis techniques for successfully identifying novel factors involved in oncogenesis.
- To test the therapeutic efficacy of natural compounds and delve into the roles of small molecules in the avenue of cancer therapeutics and focus upon the synergistic mode-of-action of anti-cancer agents.
- To develop novel targeted drug delivery-based systems to assuage tumorigenesis.

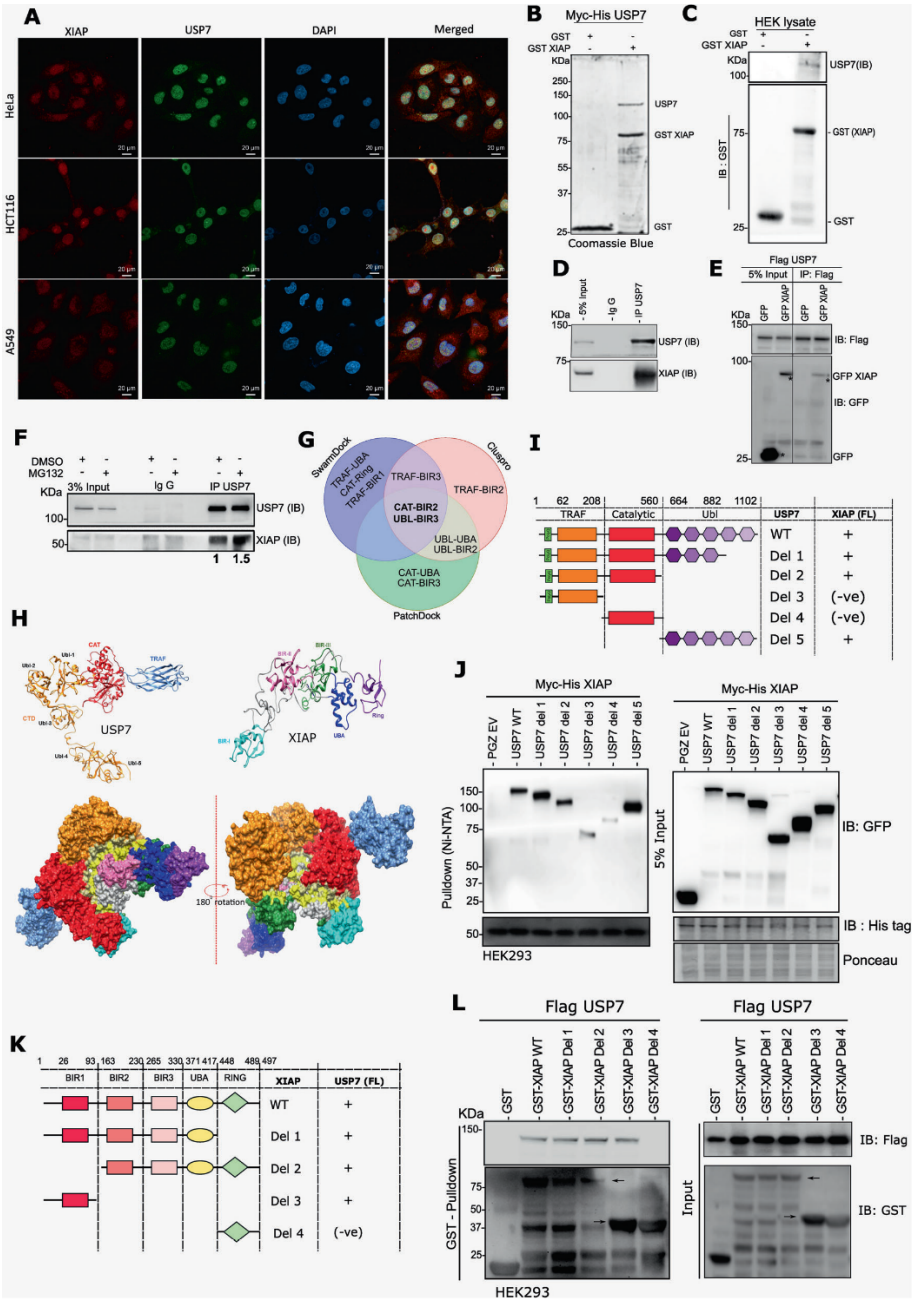

Figure 1: XIAP and USP7 colocalize and physically interact. A Representative image of immunofluorescence staining of USP7 (green), XIAP (red) in HeLa, HCT116, and A549 cells. B Purified Myc-His-USP7incubated separately with GST and GST-XIAP in the presence of GSH- beads for 4 h. SDS-PAGE separated samples were stained with Coomassie blue. C HEK293 cell lysate was incubated separately with purified GST and GST-XIAP in the presence of GSH-beads followed by IB using indicated antibodies. D HEK293 cell lysate was immunoprecipitated with USP7 antibody followed by IB using indicated antibodies. E Interaction in overexpressed conditions: HEK293 cells were transiently transfected with FLAG-USP7 and GFP-XIAP or GFP as the control, followed by immunoprecipitation and IB. F HEK293 cells were treated with either DMSO or MG132 (25 μM) for 8 h followed by IB with indicated antibodies. G Venn diagram shows a comparison of the top-six interacting domain pairs of USP7 and XIAP obtained using three different docking software. H Upper panels showing full-length USP7 and XIAP proteins generated by stitching the available USP7 and XIAP domains in PDB. Lower panels were showing the USP7-XIAP complex, where the interface region was highlighted in yellow. I Schematic representation of USP7 deletion mutants interacting with full-length XIAP. J HEK293 cells were co-transfected with indicated plasmids; cell lysates were pull-down with Ni-NTA beads for 1 hr at room temperature. Pulled-downed and input were analyzed by IB with the indicated antibodies. K Schematic representation of XIAP deletion mutants interacting with full-length USP7. L HEK293 cells were transiently transfected with Flag-USP7. The lysates were incubated separately with GST or GST-XIAP deletion mutants overnight at 4 °C. After pull-down with GSH-bead for 4 h at RT, pulled-down proteins were checked by IB.

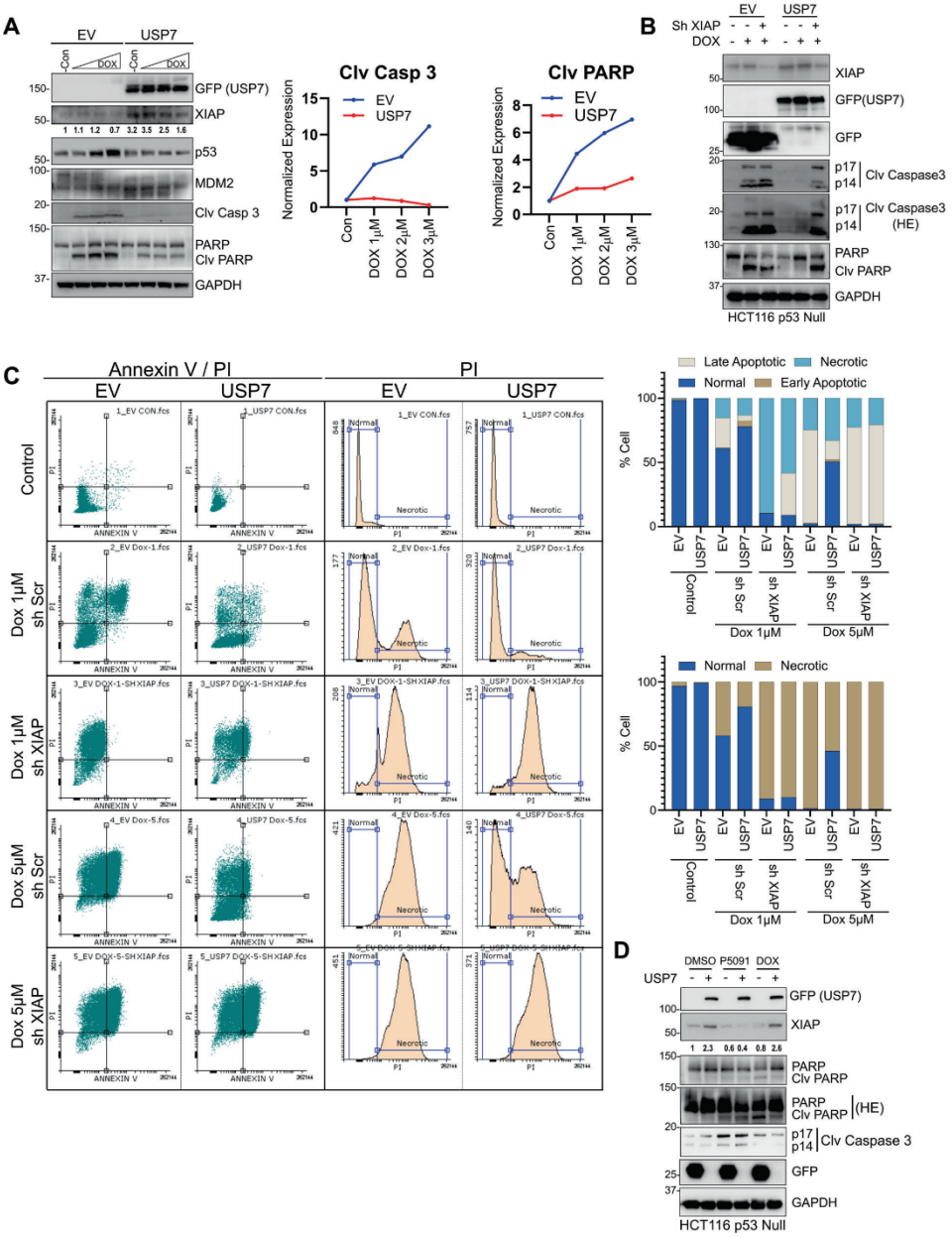

Figure 2: USP7 regulates cellular fate through the involvement of XIAP. A HEK 293 cells were transfected as indicated, for 24 h followed by Dox treatment (0, 1, 2, 3 μM) for another 6 h, and the expression of the indicated proteins was analyzed by IB. B HCT116 (p53Null) cells were transfected with indicated plasmids for 24 h, followed by Dox (1 μM) treatment for another 6 h. The expression of indicated proteins was analyzed by IB to detect apoptosis intensity. C Annexin V/PI apoptosis of HCT116 p53Null cells transfected as indicated. Before analysis, transfected cells were treated with 1 μM and 5 μM of DOX for another 12 h as indicated; per cent of cells at different phases as indicated in the bar graph. The normal and necrotic cell was detected in HCT116 p53Null cells in a similar USP7 overexpression and Dox concentration but for 24 h. D HCT116 (p53Null) cells were transfected with Flag-USP7, as mentioned. After 24 h, cells were treated with P5091 (10 μM for 24 h) or Doxorubicin (1 μM for 6 h). Cell lysates were analyzed by IB using the indicated antibodies.

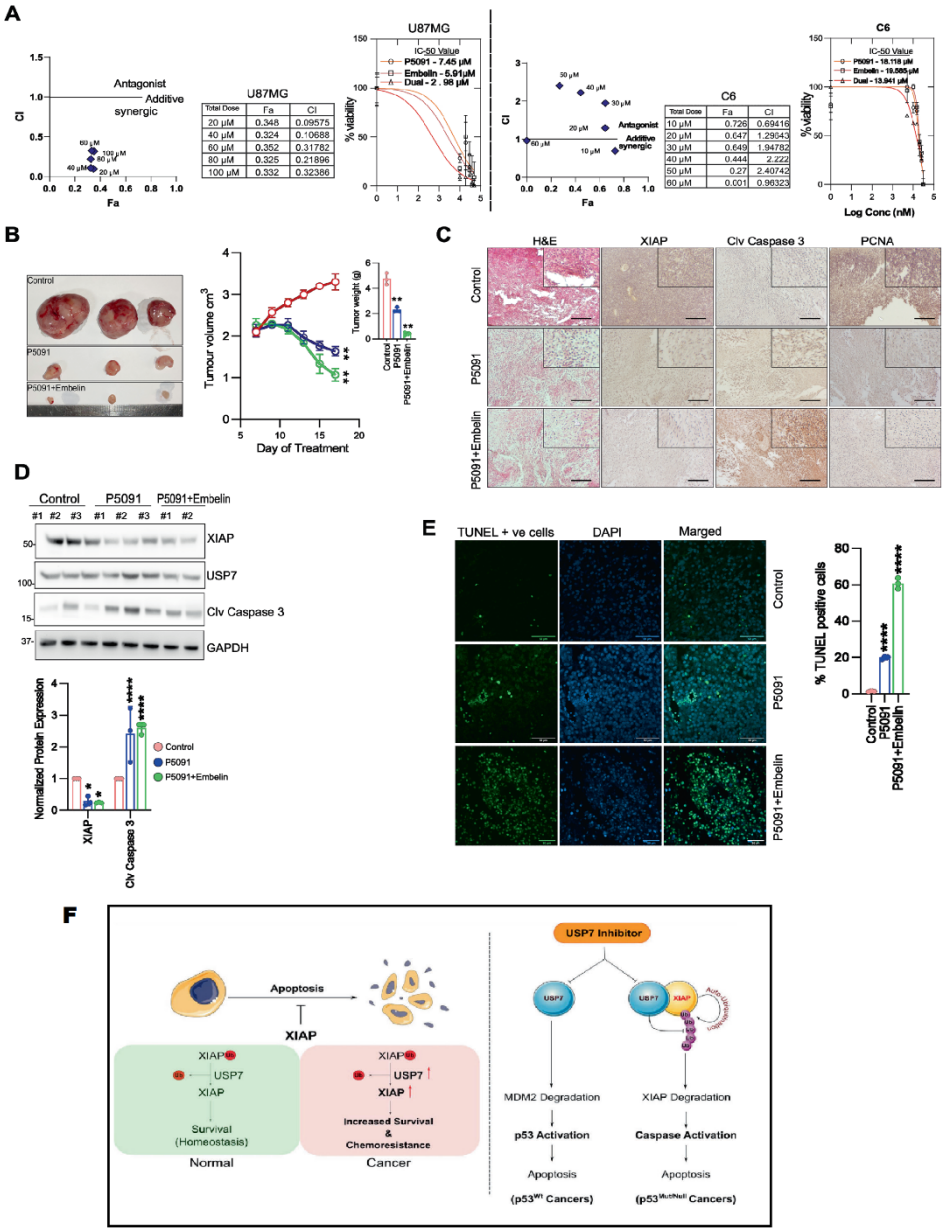

Figure 3: USP7—XIAP axis is important in tumor progression and a schematic representation of USP7-XIAP in promoting cancer cell survival. A Isobologram analysis showed the synergistic cytotoxic effect of the dual drug combination in U87MG and C6 cells. B Female SD rats (200–250 g; 3–4 weeks) were subcutaneously injected with 1 × 106 C6 cells (rat glioma) as a syngeneic system to develop a tumor. Then, 14 days post-injection, rats were treated with vehicle control, P5091 (5 mg/Kg body weight), or in combination with Embelin (5 mg/Kg body weight) every alternate day. Tumor volume was measured every alternate day. C Paraffin-embedded sections from tumor tissues were analyzed by H&E staining. D Expression of indicated proteins in treated tumor tissue samples was analyzed by WB. E Apoptosis of tumor cells was analyzed by TUNEL assay. F Proposed model of USP7-mediated deubiquitination and stabilization of the anti-apoptotic protein XIAP and promotion of cancer cell survival.

Publications

- Saha, G., Sarkar, S., Mohanta, P. S., Kumar, K., Chakrabarti, S., Basu, M., & Ghosh, M. K. (2022). USP7 targets XIAP for cancer progression: Establishment of a p53-independent therapeutic avenue for glioma. Oncogene, 41(47), 5061–5075. https://doi.org/10.1038/s41388-022-02486-5
- Basu, B., & Ghosh, M. K. (2022). Ubiquitination and deubiquitination in the regulation of epithelial-mesenchymal transition in cancer: Shifting gears at the molecular level. Biochimica et biophysica acta.-Molecular cell research, 1869(7), 119261. https://doi.org/10.1016/j.bbamcr.2022.119261
- Tabassum, S. & Ghosh, M. K. DEAD-box RNA helicases with special reference to p68: Unwinding their biology, versatility, and therapeutic opportunity in cancer. Genes Dis. (2022) ISSN 2352-3042. doi: https://doi.org/10.1016/j.gendis.2022.02.008.
- Kal, S., Chakraborty, S., Karmakar, S., & Ghosh, M. K. (2022). Wnt/β-catenin signaling and p68 conjointly regulate CHIP in colorectal carcinoma. Biochimica et biophysica acta.-Molecular cell research, 1869(3), 119185. https://doi.org/10.1016/j.bbamcr.2021.119185
- Kumar, S., Basu, M., & Ghosh, M. K. (2021). Chaperone-assisted E3 ligase CHIP: A double agent in cancer. Genes & Dis, 9(6), 1521–1555. https://doi.org/10.1016/j.gendis.2021.08.003

Book Chapters / Invited Reviews

Sarkar S, Basu M and **Ghosh MK*** (2022). ROS induced cancers with special interest in glioma: targeting ROS in a combinatorial approach for successful chemotherapy. *Handbook of Oxidative Stress in Cancer: Therapeutic Aspects.* Published by Springer (*Invited*). https://doi.org/10.1007/978-981-16-1247-3_244-1.

Extramural Funding

- Restoration of p53 and Rb through targeting their post-translational modifiers (PTMs) HAUSP and MDM2 in Glioma. FBR (Focused Basic Research), CSIR
- PARP inhibitor (PARPi) treatment for effective Breast Cancer therapy: Pre-Clinical Validation & Combination Therapy with PTEN-CT Peptide. PAN-CSIR Cancer Research, CSIR

Invited Talk

Frontiers of Biological Research: Challenges and Opportunities. Amity University, Kolkata, August 18. Online faculty development programme during August 13-18, 2021.

Acute myeloid leukemia hematopoiesis and epigenetic dependencies

Dr. Amitava Sengupta, amitava.sengupta@iicb.res.in; amitava.iicb@gmail.com

Group Members

Subham K. Bandyopadhyay, DBT-JRF (enrolled at AcSIR)
Subhadeep Ghosh, CSIR-JRF (enrolled at AcSIR)
Satyaki Bhowmik, CSIR-JRF (enrolled at AcSIR)
Anwesha Bose, CSIR-JRF (enrolled at AcSIR)
Wasim Shaikh, CSIR-JRF (enrolled at AcSIR)
Sayan Chakraborty, PhD (soon to join pdf at OUMC, USA)
Liberalis Debraj Boila, PhD (currently pdf at Fred Hutch Cancer Center, USA)
Sayantani Sinha, PhD (currently pdf at Fred Hutch Cancer Center, USA)

Background

Physiological aging is associated with the onset of chronic and lifestyle diseases including cancer. Hematopoietic aging is characterized by clonal expansion of myeloid-biased hematopoietic stem cells/ progenitors and by increased risk of development of myeloid leukemia. Although the majority of patients with acute myeloid leukemia (AML) initially respond to chemotherapy, many of them subsequently relapse, and the mechanistic basis for AML persistence following chemotherapy remains poorly understood. We are trying to test the hypothesis that epigenetic dysregulation within hematopoietic compartment is involved in hematopoietic aging and causes AML pathogenesis. We are particularly interested at understanding the cell-autonomous and non-cell-autonomous molecular determinants that regulate HSC self-renewal, differentiation and interaction with hematopoietic microenvironment or niche. Our overreaching aim is identification of altered and unique epigenetic fingerprints in human myeloid leukemia, and characterization of epigenetic vulnerabilities in AML.

Aims and Objectives

- *Epigenetic regulation of AML hematopoiesis and targeted therapy*
- *Evolution of AML immune microenvironment contexture and immunomodulation*

Work Achieved

Acute myeloid leukemia (AML) is a heterogeneous, aggressive malignancy with dismal prognosis and with limited availability of targeted therapies. Epigenetic deregulation contributes to AML pathogenesis. KDM6 proteins are histone-3-lysine-27-demethylases that play context-dependent roles in AML. Recently we have identified that KDM6-demethylase function critically regulates DNA-damage-repair-(DDR) gene expression in AML. Mechanistically, KDM6 expression is regulated by genotoxic stress, with deficiency of KDM6A-(UTX) and KDM6B-(JMJD3) impairing DDR transcriptional activation and compromising repair potential. Acquired KDM6A loss-of-function mutations are implicated in chemoresistance, although a significant percentage of relapsed-AML has upregulated KDM6A. Olaparib treatment reduced engraftment of KDM6A-mutant-AML-patient-derived xenografts, highlighting synthetic lethality using Poly-(ADP-ribose)-polymerase-(PARP)-inhibition. Crucially, a higher KDM6A expression is correlated with venetoclax tolerance. Loss of KDM6A increased mitochondrial activity, BCL2 expression, and sensitized AML cells to venetoclax. Additionally, BCL2A1 associates with venetoclax resistance, and KDM6A loss was accompanied with a downregulated BCL2A1. Corroborating these results, dual targeting of PARP and BCL2 was superior to PARP or BCL2 inhibitor monotherapy in inducing AML apoptosis, and primary AML cells carrying KDM6A-domain mutations were even more sensitive to the combination. Together, our study illustrates a mechanistic rationale in support of a novel combination therapy for AML based on subtype-heterogeneity, and establishes KDM6A as a molecular regulator for determining therapeutic efficacy.

Bone morphogenic protein (BMP/TGF-β) signaling determines mesenchymal-stromal-cell (MSC) osteolineage commitment and tissue identity. However, molecular integration of developmental signaling with MSC-intrinsic chromatin regulation remains incompletely understood. SWI/SNF-(BAF)

is an ATP-dependent chromatin remodeler implicated in multi-cellular development. We identified that BMPs and long-term osteogenic signals in MSCs selectively induce expression of polybromo BAF (PBAF) components Pbrm1, Arid2, and Brd7. Loss of Pbrm1/Arid2/Brd7 profoundly impairs osteolineage gene expression and osteogenesis without compromising adipogenesis. Pbrm1 loss attenuates MSC in vivo ossification. Mechanistically, Pbrm1/PBAF deficiency impairs Smad1/5/8 activation through locus-specific epi-genomic remodeling, involving Pbrm1 bromodomains, along with transcriptional downregulation of Bmpr/TgfβrII affecting BMP-early-responsive gene expression. *Gain of function* of *Bmprlβ, TgfβrII* in PBAF-deficient MSCs partly restores Smad1/5/8 activation and osteogenesis. Pbrm1 loss further affects hematopoietic stem/progenitor activity through non-cell-autonomous regulation of microenvironment and niche-factor expression. Together, these findings reveal a link illustrating epi-genomic feedforward control of BMP/TGF-β signaling to transcriptional and cellular plasticity in the mesenchymal microenvironment and account for stromal-SWI/SNF in hematopoiesis.

Future Research Plans

Functionally dissect contributions of chromatin remodelers and epigenetic modifying-enzymes in HSPC transformation in myeloid leukemia.

Publications

- Boila LD, Ghosh S, Bandyopadhyay SK. Dick JE, **Sengupta A.** KDM6 demethylases integrate DNA repair gene regulation and loss of KDM6A sensitizes human acute myeloid leukemia to PARP and BCL2 inhibition. ***Leukemia*** 2023: 37, 751-764.
- Boila LD, **Sengupta A**. Evolving insights on histone methylome regulation in human acute myeloid leukemia pathogenesis and targeted therapy. ***Experimental Hematology*** 2020: S0301-472X(20)30554-3.
- Sinha S, Biswas M, Chatterjee SS, Kumar S, **Sengupta A**. Pbrm1 steers mesenchymal stromal cell osteolineage differentiation by integrating PBAF-dependent chromatin remodeling and BMP/TGF-β signaling. ***Cell Reports*** 2020: 31, 107570.
- Chakraborty S, Sinha S, **Sengupta A.** Emerging trends in chromatin remodeler plasticity in mesenchymal stromal cell function. ***FASEB J*** 2021: 35, e21234.

Awards/Honours

- Scholarship, Keystone Symposia: Hematopoiesis, USA *(Invited Talk),* 2021
- ICMR-National Inst of Immunohaematology (NIIH) Diamond Jubilee Oration Award, 2021
- Prof. Saroj Chandrasekhar Memorial Primary Award, Sastra University, *(lab),* 2021

Extramural Funding

Deciphering epigenetic dysregulation in hematopoietic stem cell transformation in human myelogenous leukemia, DBT

Invited Lecture

- 2022: *Harnessing epigenetic and transcriptional dependencies for combination targeted therapy in acute myeloid leukemia.* Society of Biological Chemists Annual Meeting, Kolkata.
- 2022: *Epigenetic regulation of hematopoietic stem cell function in myeloid leukemia*. National Symposium: *'Stem Cell and Cellular Therapy in Human Diseases'*, Stem Cell Research Center, Sanjay Gandhi Post Graduate Institute of Medical Science, Lucknow.
- 2022: *Epigenetic regulation of leukemia pathogenesis & targeted therapy.* IISER-Thiruvananthapuram, Kerala.
- 2022: *From leukemia epigenetics to targeted therapy.* BAW 2022, Ooty, Organized by IISER-Kolkata.
- 2022: *Discovery hematology research in a non-clinical academic setup in India: promises & challenges.* Prof. Sandhya Ghose Memorial Oration, Bengal Society of Haematology (BeSHCON), Kolkata.
- 2021: *Polybromo SWI/SNF integrates chromatin remodeling & BMP/TGF-β signaling to determine mesenchymal stromal osteolineage commitment and hematopoiesis.* Keystone Symposia: Hematopoiesis (EK37-2021), Colorado, USA, sponsored by American Association for Cancer Research (AACR).
- 2021: *Epigenetic insights of mesenchymal stromal cell lineage commitment & hematopoiesis.* 2nd International Conference on Cell and Experimental Biology, Houston, Texas, USA.
- 2021: *Molecular genetic determinants for human AML targeted therapy.* ICMR-National Institute of Immunohaematology (NIIH) Diamond Jubilee Oration.
- 2021: *Targeted therapy & next-gen precision medicine for human leukemia.* CSIR Young Scientists Conclave.
- 2021: *Harnessing epigenetics for human AML targeted therapy.* International eSymposium frontiers in Blood, cancer & development (fBcd21): systems to medicine, Kolkata, India

Dendritic cell biology at the crossroads of autoimmunity, cancer and protective immunity

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Group Members

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Background

The basic premise for immune algorithm is distinguishing self from nonself. This is achieved by different modules of host immune system. The 'innate' immune system recognizes the nonself based on predominantly nonself-associated molecular patterns (PAMPs), while the 'adaptive' immune axis adapts to the nonself molecular determinants. These two works together toward an effective immune response. An effective immune response to an invading pathogen (nonself) leads to protective immunity and a defective response leads to overt infection. On the other hand, an unintended response to the self-entities leads to autoimmune disorders, while a misjudged tolerance to the altered self contributes to tumorigenesis. Our research broadly concentrates on role of innate immune axis in the crossroads of infection, autoimmunity and cancer. Dendritic cells (DCs) are the innate cells with most of the decision-making responsibilities for an ensuing immune response or tolerance. We try to decipher the governing principles of self-nonself discrimination by the germline-encoded invariant pattern recognition receptors (PRRs) expressed by DCs and how they work in a given clinical context.

Aims and Objectives

There are three major aspects that we explore:

- Role of innate immune deregulation in autoreactive inflammation
- Role of mechanical cues in immune cells

Work Achieved

- Development of novel small molecule antagonists for toll-like receptor 9 and 7, promising therapeutic targets in systemic autoimmune diseases as they inhibit activation of plasmacytoid dendritic cell activation and type I IFN induction. [*Das N et al., J Med Chem, 2022; Pal S A et al., Molecules, 2022.*]
- Exploration of host immune response in COVID-19 and clinical trial on convalescent plasma therapy in severe COVID-19. [*Raychaudhuri D et al., Mayo Clin Proc IQ&O, 2022; Ray Y et al., Nat Commun, 2022, Park H et al., JAMA Netw Open, 2022*].

Future Research Plans

We plan to further investigate the role of plasmacytoid dendritic cell in contexts of sterile inflammation, perform preclinical validation of the novel TLR9 antagonists in diabetes and systemic lupus. A program on molecular characterization of innate immune events in terms of mechanotransduction and endocannabinoids signaling are also ongoing.

Figures

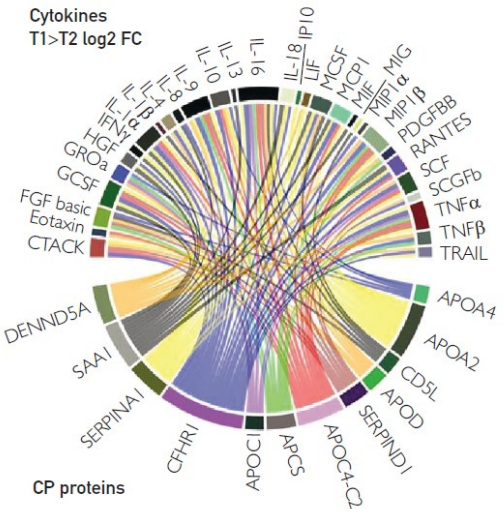

Figure 1: Correlation of plasma proteins in convalescent plasma with plasma abundance of cytokines in severe COVID-19 recipients (from Raychaudhuri D et al., Mayo Clin Proc IQ&O, 2022)

Publications

- Raychaudhuri D, Bandopadhyay P, D'Rozario R, Sarif J, Ray Y, Paul SR, Singh P, Chaudhury K, Bhaduri R, Pandey R, Bhattacharya P, Sengupta S, Chatterjee S, **Ganguly D***. Clinical trial sub-group analyses to investigate clinical and immunological outcomes of convalescent plasma therapy in severe COVID-19. **Mayo Clinic Proceedings: Innovation, Quality & Outcome.** 2022 Dec;6(6):511-524.
- Singh P et al. and CSIR Phenome India Cohort Investigators. A machine learning-based approach to determine infection status in recipients of BBV152 (Covaxin) whole-virion inactivated SARS-CoV-2 vaccine for serological surveys. **Computers in Biology and Medicine**. 2022 Apr 25;146:105419.
- Das N#, Bandopadhyay P#, Roy S#, Sinha BP, Ghosh Dastidar U, Rahaman O, Pal S, **Ganguly D***, Talukdar A*. Development, Optimization and In-vivo Validation of New Imidazopyridine Chemotype as Dual TLR7/TLR9 Antagonists Through Activity Directed Sequential Incorporation of Relevant Structural Sub-units. **Journal of Medicinal Chemistry**. 2022 Sep 8;65(17):11607-11632.
- Pal S*, Ghosh Dastidar U, Ghosh T, **Ganguly D**, Talukdar A*. QSAR Assisted Exploration of Ligand-Based Insights for the Design of Small-Molecule TLR7 Antagonists. **Molecules**, 2022 Jun 23;27(13):4026.
- Ray Y*, Paul SR, Bandopadhyay P, D'Rozario R, Sarif J, Lahiri A, Bhowmik D, Vasudevan JS, Maurya R, Kanakan A, Sharma S, Kumar M, Singh P, Roy R, Chaudhury K, Maiti R, Bagchi S, Maiti A, Perwez MM, Mondal A, Tewari A, Mandal S, Roy A, Saha M, Biswas D, Maiti C, Chakraborty S, Sharma Sarkar B, Haldar A, Saha B, Sengupta S, Pandey R, Chatterjee S, Bhattacharya P, Paul S, **Ganguly D***. A Phase 2 Single Center Open Label Randomised Control Trial for Convalescent Plasma Therapy in Patients with Severe COVID-19. **Nature Communications**, 2022, 13(1):383.
- Park H, Tarpey T, Liu M, Goldfeld K, Wu Y, Wu D, Li Y, Zhang J, **Ganguly D**, Ray Y, Paul SR, Bhattacharya P, Belov A, Huang Y, Villa C, Forshee R, Verdun NC, Yoon HA, Agarwal A, Simonoivich VA, Scibona P, Pratx LB, Belloso W, Avendaño-Sola C, Bar KJ, Duarte RF, Hsue PY, Luetkemeyer AF, Meyfroidt G, Nicola AM, Mukherjee A, Ortigoza MB, Pirofski LA, Rijnders BJA, Troxel A, Antman EM, Petkova E*. Development and Validation of a Treatment Benefit Index to Identify Who May Benefit from Convalescent Plasma among Patients Hospitalized With COVID-19. **JAMA Network Open**. 2022, in press.

Conferences/Events Organized by CSIR-IICB

Indo-French Symposium on Metaflammation, March 2022, co-organizer, with Vanja Sisirak (L'universite de Bordeaux, France)

Invited Talks

- March, 2022: CNRS ImmunoConcept University of Bordeaux, Bordeaux, France.
- April, 2022: Department of General Medicine, University of Crete, Heraklion, Greece.
- April, 2022: IMBB FoRTH, University of Crete, Heraklion, Greece.
- March, 2022: Lupus Initiative Kolkata, Annual Meeting, Kolkata, India.

Extramural Funding

No.	Title	Funded by	PI/Co-PI	Amount (Lakh INR)	Brief description
1	Swarnajayanti Fellowship grant	DST	PI	200.0 (5 years, 2018-2023)	Mechanistic exploration of the pathogenetic role of type I interferons in metabolic syndrome and preclinical validation of therapeutic targeting
2	National Bioscience Award	DBT	PI	15.0 (3 years, 2018-2021	
3	Indo-French Collaborative Project	CEFIPRA	PI	80.0 (3 years, 2019-2022)	Role of DNAse1L3 in metabolic syndrome
4	CSIR Focussed Basic Research	CSIR	PI	70.0 (3 years, 2020-2023)	Exploring the role of mechanical cues in human immune cells.

Delineating the role of CD38 in promoting the terminal exhaustion and immunotherapy unresponsiveness in CD8+ T cells in cancer

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Research activities

1. **CD38-RyR2 mediated chronic Ca^{2+} signaling promotes the differentiation of $TCF1^{lo}$ terminal exhausted $CD8^+$ T cells in tumor.** Preponderance of terminally exhausted $CD8^+$ T cells (Tex^{term}) at the tumor site is the major impediment for successful immunotherapy of cancer. Despite the recent understanding in epigenetic, transcriptomic and metabolic features of Tex^{term}, the cellular pathway underpinning the differentaion of Tex^{term} remains elusive. Upon assessing intratumoral $CD8^+$ T cells from both clinical samples and pre-clinical mouse models, we observed that $CD8^+$ T cells co-expressing CD38 and PD1 displayed the characteristic features of Tex^{term}. Single cell RNA sequencing analysis of both murine tumor infiltrating $CD8^+$ T cells and *in vitro* chronically exhausted human $CD8^+$ T cells revealed that clusters expressing CD38 diplayed the transcriptomic signature of Tex^{term}, and importantly, were mutually exclusive to the cluster expressing *TCF7*. Pharmacological and genetic knockdown of CD38 maintained TCF1 expression in chronically exhausted $CD8^+$ T cells and significantly improved their ability to control tumor growth upon adoptive transfer in B16-F10 melanoma bearing mice. Mechanistic studies revealed that CD38 expression on Tex^{term} led to chronic elevation of intracellular Ca^{2+} levels through c-ADPR-RyR2 axis, which activated Akt signalling. Activation of Akt restrained the nuclear localization of Ezh2, which found to be crucial in maintaining TCF1 expression in Tex^{term} and inhibition of which in $CD8^+$ T cells resulted in poor anti-tumor response in mice. Thus our data suggests that CD38 could be a dugrable target to improve the immunotherapeutic efficacy of $CD8^+$ T cells in cancer. **(Figure 1).**

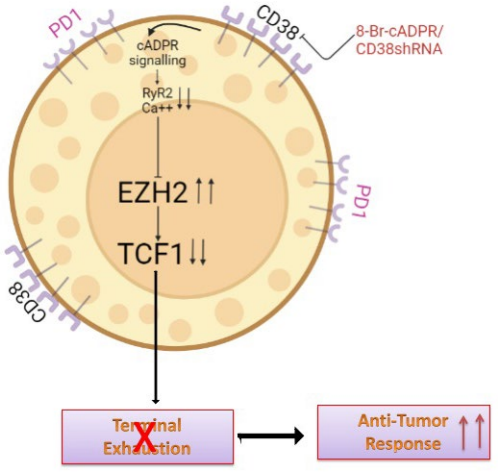

Figure 1: Schematic diagram representing the role of CD38 in regulating the effector function of $CD8^+$ T cells in tumor

2. **Intratumoral $PD1^+CD38^+Tim3^+$ $CD8^+$ T cells in pre-BCG tumor tissues are associated with poor responsiveness to BCG immunotherapy in patients with non-muscle invasive bladder cancer.** Intravesical immunotherapy with Bacillus Calmette-Guerin (BCG) has been a standard of care therapy for non-muscle invasive bladder cancer (NMIBC) which accounts for about 75% of newly diagnosed urothelial cancer. However, given the frequent recurrence and progression,

identification of a pre-treatment biomarker capable of predicting responsiveness to BCG in NMIBC is of utmost importance. Herein, using multiparametric flow cytometry, we comprehensively characterized $CD8^+$ T cells from peripheral blood and tumor tissues from 27 pre-BCG patients bearing NMIBC to obtain immune correlates of bladder cancer prognosis and responsiveness to BCG therapy. We observed that intratumoral $CD8^+$ T cell subsets were highly heterogenous in terms of their differentiation state and exist at different proportions in tumor tissues. Remarkably, among the different $CD8^+$ T cell subsets present in the tumor tissues, the frequency of terminally exhausted-like $CD8^+$ T cell subset marked as $PD1^+CD38^+Tim3^+$ $CD8^+$ T cells were inversely correlated with the favourable outcome of the patients and responsiveness to BCG therapy. Moreover, we also noted that the intratumoral abundance of progenitor exhausted-like $PD1^+CD8^+$ T cell subset in pre-BCG NMIBC tumor tissues were indicative of better recurrence-free survival after BCG. Collectively, our study led to the identification of biomarkers that can predict the therapeutic responsiveness of BCG in NMIBC. **(Figure 2)**.

Future directions

Our lab would be predominantly involved in deciphering the mechanisms of how metabolic rewiring instill T cells with a distinct transcriptional program that shape up the functional outcome of T cell in tumor. We would also explore whether the combinatorial approach of targeting metabolic feature of T cells along with immune checkpoint blockade could improve the therapeutic efficacy of T cells against cancer.

Extramural funding

- Role of endoplasmic reticulum (ER) stress induced UPR signaling in regulating the metabolic fitness and functionality of CD8+ T cells in cancer.
  Start year: 2020,
  Duration: 5 years
  Agency: India Alliance DBT Wellcome.
- Mechanistic exploration of the role of shingosine-1-phosphate receptor 1 (S1PR1) in regulating the functional fate of tumor infiltrating CD8 T cells.
  Start year: 2020;
  Duration: 3 years
  Agency: SERB, India

Publication from the lab

- Chowdhury S, Kar A, Bhowmik D, Gautam A, Basak D, Sarkar I, Ghosh P, Sarkar D, Deka A, Chakraborty P, Mukhopadhyay A, Mehrotra S, Basak S, Paul S, **Chatterjee S.** *Intracellular Acetyl CoA Potentiates the Therapeutic Efficacy of Antitumor CD8+ T Cells.* **Cancer Research.** 2022, 82(14):2640
- Ray Y, Paul SR, Bandopadhyay P, D'Rozario R, Sarif J, Raychaudhuri D, Bhowmik D, Lahiri A, Vasudevan JS, Maurya R, Kanakan A, Sharma S, Kumar M, Singh P, Roy R, Chaudhury K, Maiti R, Bagchi S, Maiti A, Perwez MM, Mondal A, Tewari A, Mandal S, Roy A, Saha M, Biswas D, Maiti C, Bhaduri R, Chakraborty S, Sarkar BS, Haldar A, Saha B, Sengupta S, Pandey R, **Chatterjee S**, Bhattacharya P, Paul S, Ganguly D. *A phase 2 single center open label randomised control trial for convalescent plasma therapy in patients with severe COVID-19.* **Nat Commun**. 2022, 13(1):383
- Sarif J, Raychaudhuri D, D'Rozario R, Bandopadhyay P, Singh P, Mehta P, Hoque MA, Sinha BP, Kushwaha M, Sahni S, Devi P, Chattopadhyay P, Paul SR, Ray Y, Chaudhuri K, Banerjee S, Majumdar D, Saha B, Sarkar BS, Bhattacharya P, **Chatterjee S**, Paul S, Ghosh P, Pandey R, Sengupta S, Ganguly D. *Plasma Gradient of Soluble Urokinase-Type Plasminogen Activator Receptor Is Linked to Pathogenic Plasma Proteome and Immune Transcriptome and Stratifies Outcomes in Severe COVID-19.* **Front Immunol.** 2021, 12:738093.
- Bandopadhyay P, D'Rozario R, Lahiri A, Sarif J, Ray Y, Paul SR, Roy R, Maiti R, Chaudhuri K, Bagchi S, Maiti A, Perwez MM, Sarkar BS, Roy D, Chakraborty R, Vasudevan JS, Sharma S, Biswas D, Maiti C, Saha B, Bhattacharya P, Pandey R, **Chatterjee S**, Paul S, Ganguly D. *Nature and Dimensions of Systemic Hyperinflammation and its Attenuation by Convalescent Plasma in Severe COVID-19.* **J Infect Dis**. 2021, 224(4):565
- Biswas D, Maiti C, Talukder B, Azharuddin M, Saha S, Pandey S, Das A, Adhikari SD, Ray Y, Sarkar BS, Paul SR, Saha B, Paul S, **Chatterjee S**, Ganguly D, Bhattacharya P. *A prospective study on COVID-19 convalescent plasma donor (CCP) recruitment strategies in a resource constrained blood centre.* **ISBT Sci Ser**. 2021, 16(4):276

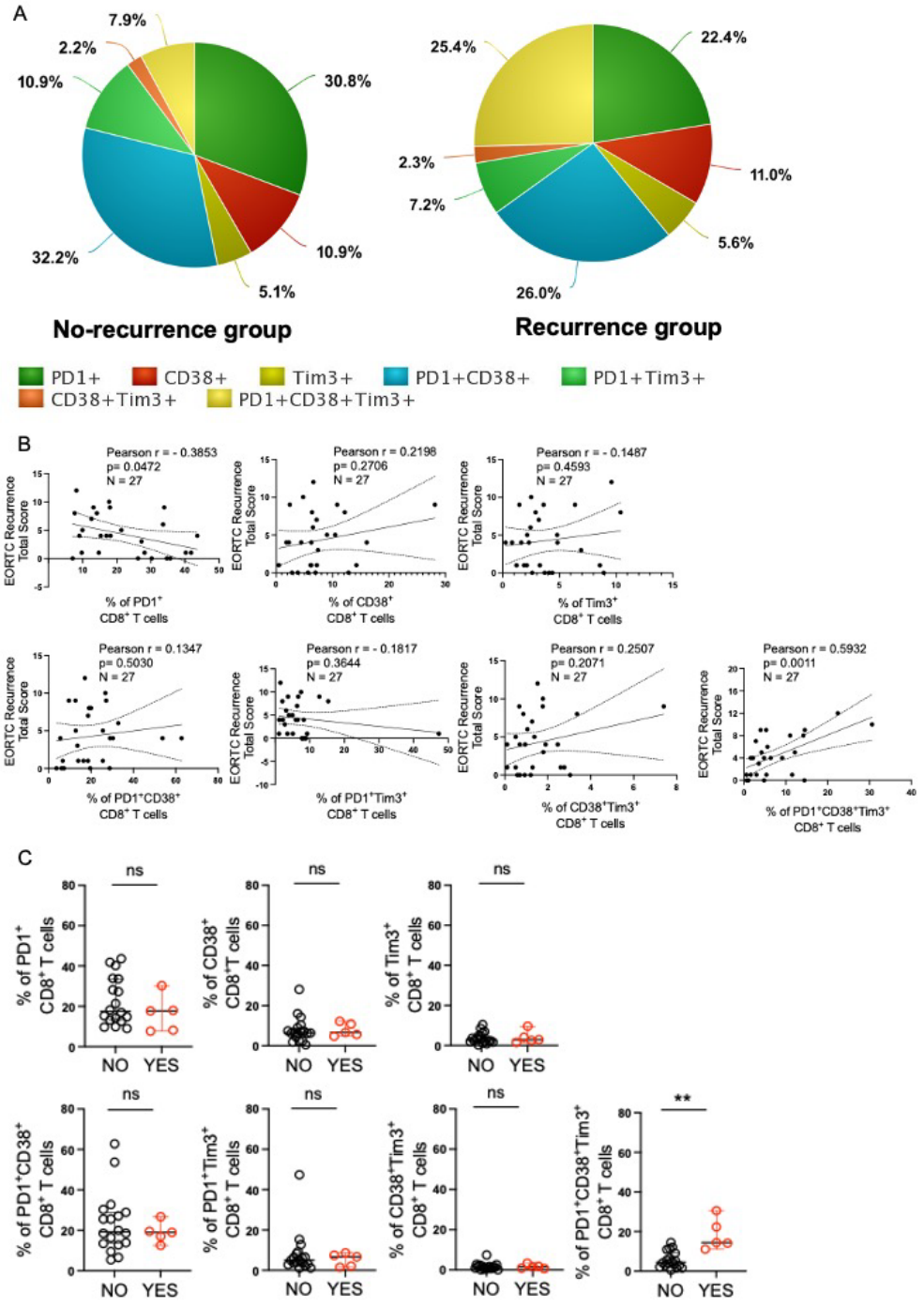

Figure 2: Intratumoral PD1+CD38+Tim3+ CD8+ T cells in pre-BCG tumor tissues predict the post-BCG recurrence in NMIBC. (A) Pie chart representation of frequency of 7 different subsets of intratumoral CD8+ T cells with their relation to tumor recurrence. (B) Pearson correlation between EORTC recurrence-risk and frequency of different intratumoral CD8+ T cell subsets. (C) Graphical representation of various intratumoral CD8+ T cell subsets at the pre-BCG tumor tissues of non-responders as compared to those who exhibited durable response to BCG. Data from 27 NMIBC patients are presented.

Chimeric antigen receptor T (CAR-T) cell targeting immune checkpoint and cancer marker/ antigen in breast and ovarian cancer

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Research Activities

Ovarian cancer is associated with the highest mortality rate of all gynecologic malignancies and high grade serous ovarian cancer (HGSOC) is the predominant histotypes. The standard-of-care for HGSOC patients includes cytoreductive surgery followed by chemotherapy with platinum-based drugs (e.g., cisplatin or carboplatin) [Malekshah, Sarkar *et al.* 2019]. Although HGSOC showed initial pathological response with platinum-based drugs, majority of the patients' experience relapse within 18–24 months. Moreover, in the multi-centric retrospective study of ovarian cancer patients treated with carboplatin or cisplatin, the neuro-toxicities, thrombocytopenia, anemia was noticed [Pignata, De Placido *et al.* 2006].

Cell based therapies in recent past have emerged as a potent strategy for targeted therapy. We have previously successfully used bioengineered mesenchymal stem cells as a carrier to deliver secretory suicidal gene therapy particularly in tumor microenvironment against chemo-resistant ovarian cancer [Malekshah, Sarkar 2019]. Chimeric antigen receptor T (CAR-T) cell utilizing cancer antigens or markers is another potent option for cell based targeted therapy, perhaps, the most powerful one among them. *Here we propose to use CAR-T cell based therapy to treat HGSOC in an anticipation to improve overall survival of HGSOC*. We will use our bi-specific CAR-T cells that expresses nanobody or single variable domain (VHH)) raised against mesothelin in tandem with anti-PDL1 followed by transmembrane and intracellular domains fused with costimulatory signals (Fig. 1). These CAR-T will specifically target immunosuppressive PD-1/PDL1 signaling pathway as well as concomitantly activate chimeric T cell receptor signalling resulting in activation of T cell and killing of cancer cells through cytotoxic or effector T cells [Yan, Hu *et al.* 2019].

Work achieved

We have successfully designed our CAR construct and validated our CAR construct through PCR, q-RT-PCR, FACS and confocal microscopy. We have designed specific primers for CAR construct spanning the junction region between α-PDL1 and α-MSLN and performed PCR amplification using those primers. After PCR amplification, agarose gel electrophoresis result showed our desired band in the gel.

Similarly, we have designed q-RT-PCR primer for CAR construct. First, we have transfected the HEK-293T cells with CAR construct and 48 hours post transfection, we have isolate the RNA. cDNA was prepared from the isolated RNA using oligo-dT primers and q-RT-PCR was performed using specific primer spanning the anti-PD-L1 nanobody and anti-mesothelin (MSLN) nanobody sequence. We have found significant mRNA level in transfected one. So, CAR is expressed at mRNA level.

We have also validated CAR expression at protein level. HEK-293T cells were transfected with CAR construct and 48 hours post transfection, cells were stained with fluorochrome conjugated PD-L1 and mesothelin and performed flow cytometry analysis. FACS analysis reveals that transfected cells were PD-L1 and mesothelin positive. The dual targeting CAR-T also retains its binding conformation with both PD-L1 and mesothelin at the same time. The

co-localization study was performed using confocal laser scanning microscopy. It shows that fluorochrome conjugated PD-L1 and mesothelin binds simultaneously on the cell surface and appears as a ring around the cell surface. Hence, epitope or antigen recognizing domain is found in the extracellular region of the chimeric antigen receptor (Fig.2).

Of-target toxicity is one of the major problem with CAR-T cell therapy, to monitor CAR-T cell activation in real time we have designed reporter construct which will signal bioluminescence upon CAR-T cell activation. We have also validated our reporter construct through bioluminescence signal. We have transfected Jurkat (immortalised human T cell) with reporter construct and activate Jurkat with cell stimulation cocktail and measure bioluminescence after addition of D-luciferin. We have noticed significant bioluminescence signal in the activated Jurkat compared to non-activated transfected one. So, reporter signals only upon Jurkat activation and this reporter can be used for real time monitoring of CAR-T cell in-vivo settings.

Future research plans

Testing the efficacy of bispecific CAR-T cell in-vitro and in-vivo xenograft models in NOD-SKID mice.

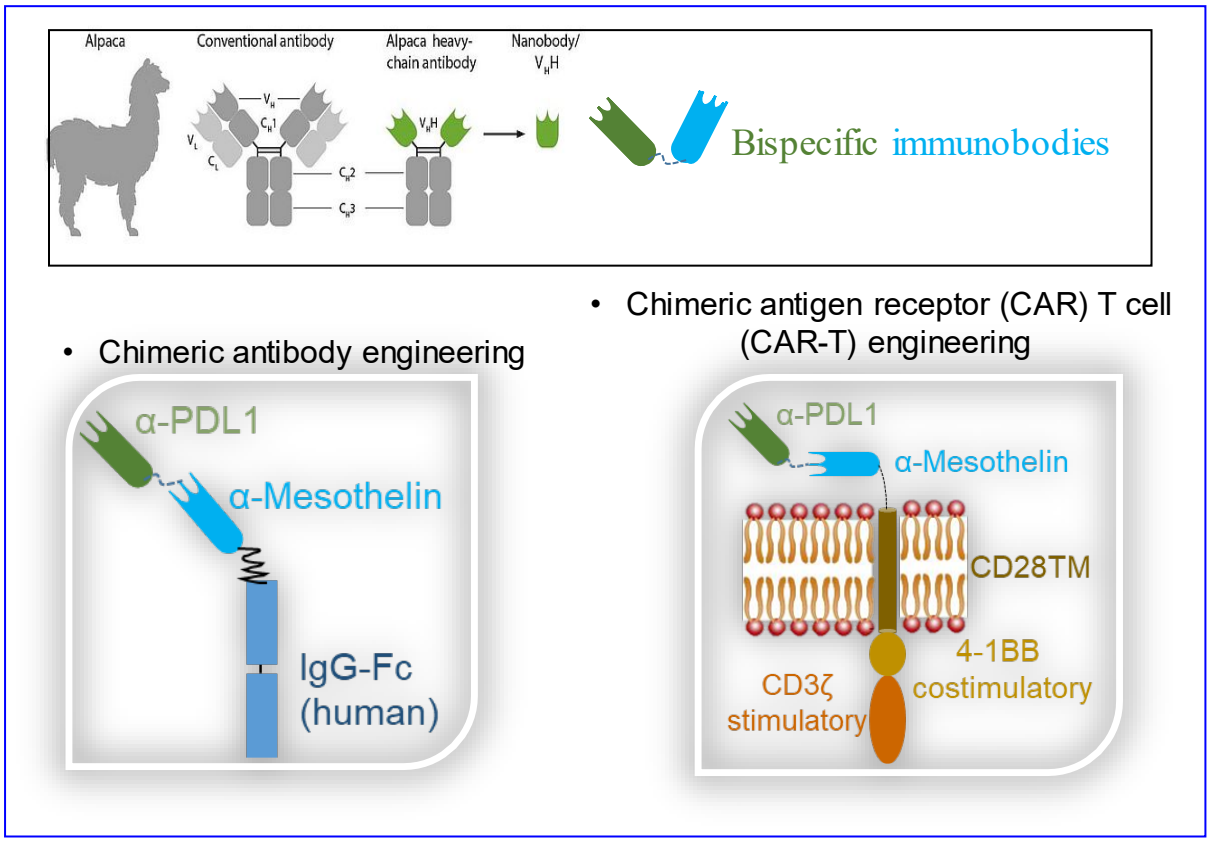

Figure 1: Bispecific CAR-T targeting cancer cells expressing cancer antigen mesothelin and PDL1 (iimune checkpoint). Nanobodies are comprised of only single variable domain (VHH) as opposed to conventional antibody; A. The two nanobody sequences (Nbs) will be inserted in tandem with human IgG-Fc region to generate chimeric antibodies. The similar strategy will be used to generate CAR-T construct by inserting these nanobody sequences upstream of transmembrane domain of CAR-T construct. This construct upon transduction will produce CAR-T expressed on the cell membrane with extracellular antigen recognition domain that upon binding with cancer markers (mesothelin) will produce plethora of antitumpr cytokines resulting in cancer cell death.

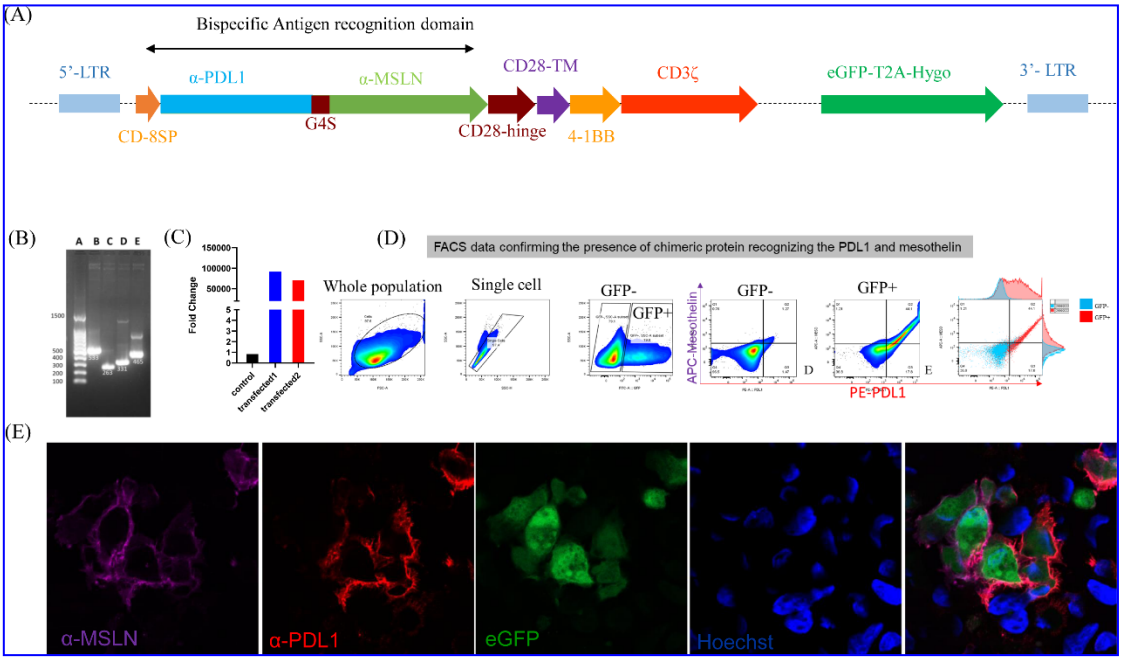

Figure 2: Bispecific Chimeric antigen receptor CAR-T targeting PDL1 and mesothelin. *A*; Construct design in pLenti vector. *B*; Insertion of α-PDL1 and α-MSLN Nanobody sequence is validated by Agarose gel electrophoresis. Transfection infection by Lentivirus showing expression as shown by qRT-PCR (*C*) and flow cytometry data (*D*). The co-localization of CAR-T showed membrane localization of CAR-T expressed protein as shown by laser scanning confocal microscope; *E*.

Extramural funding

- Genomic Surveillance program for SARS-CoV-2: Consortium of India and Sri Lanka (Ref: 223547/Z/21/Z Dated: 24.01.2022). Funding Agency: The Wellcome Trust, UK. Role: PI. Tenure: 2022-2023
- Suicidal gene therapy using engineered secretory carboxylesterse-2/ Camptothecin-11 targeting chemoresistant ovarian serous adenocarcinoma. Funding Agency: Science and Engineering Research Board (SERB): File. SRG/2019/001880) Role: PI. Tenure: 2019-2022
- Sentinel site for Indian SARS-CoV-2 Genomics Consortium (INSACOG): Genomics & Molecular Surveillance of SARS-COV-2: Funding Agency: Jointly supported by DBT, CSIR, National Center for Disease Control and Public Health (Head quarter: Georgia, US).

List of Publications (journal and conference)

- Sarkar P, Banerjee S, Saha SA, Mitra P, **Sarkar S**. Genome surveillance of SARS-CoV-2 variants and their role in pathogenesis focusing on second wave of COVID-19 in India. Scientific Reports. 2023;13:4692. doi:10.1038/s41598-023-30815-5
- Sarkar P, Banerjee S, Chakrabarti S, Chakrabarti P, Bandyopadhyay A, Ghosh AM, Saha S, Roy A, **Sarkar S**. Genome characterization, phylogenomic assessment and spatio-temporal dynamics study of highly mutated BA variants from India. Indian J Med Microbiol. (Elsevier). 2022; S0255-0857(22)00232-8. PMID: 36400646. doi: 10.1016/j.ijmmb.2022.10.006
- Chandra Jena B, **Sarkar S**, Rout L, Mandal M. The transformation of cancer-associated fibroblasts: Current perspectives on the role of TGF-β in CAF mediated tumor progression and therapeutic resistance. Cancer Lett. 2021 Nov 1; 520:222-232. doi: 10.1016/j.canlet.2021.08.002. Epub 2021 Aug 5. PMID: 34363903.

Understanding and targeting chemoresistance mechanisms in cancer

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Devendra Shukla (CSIR-SRF)
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Bilash Chatterjee (UGC-SRF)
Subhankar Bose (CSIR-SRF)
Md. Tanjim Alam (Project-JRF)

Research Activities

Targeting DNA polymerase eta-mediated mutagenic translesion DNA synthesis by small molecules

Development of chemoresistance and tumor relapse is the major challenges associated with successful chemotherapy. Accumulated pieces of evidence including data from our laboratory suggest that targeting Pol η mediated-mutagenic translesion synthesis (TLS) is a potential strategy for improving chemotherapy. Therefore, targeting Pol η with small-molecule inhibitors is a promising strategy for combating chemoresistance in certain types of cancer. However, the identification of small molecules which specifically target Pol η -mediated TLS with high *in vivo* efficacy has been challenging. Using *in silico* screening approach, we have identified a small-molecule inhibitor, chrysin that can bind with Pol η more efficiently out of active 230 compounds screened (Saha et al., 2020). A few of the reported interacting residues (Arg 93, Arg 111) are the ones that impart stability to the palm domain of Pol η. We have also performed molecular dynamics studies of this small molecule-target complex. Further, we demonstrated that chrysin treatment sensitizes the ovarian cancer stem-like cells to cisplatin treatment via inhibiting η-mediated TLS. Remarkably, chrysin treatment inhibits Pol η expression and enhances the cisplatin-induced cell death in ovarian CSLCs both *in vitro* and *in vivo*. Furthermore, our data demonstrated a reduction in cisplatin-induced mutagenesis following chrysin treatment. Combinatorial treatment of chrysin with cisplatin significantly reduces the tumor growth in ovarian cancer human xenografts (Figure 1), establishing a framework for developing chrysin as a novel class of TLS inhibitors that may be used as chemotherapy adjuvants.

Deciphering the role of miRNAs in ovarian cancer progression and chemoresistance

Cancer stem cells (CSCs) are considered to play a central role in the process of tumor initiation, progression, invasion, metastasis and therapy resistance. MicroRNAs (miRNAs) have important roles in regulating CSC properties like self-renewal, differentiation and stemness, and are considered to be potential pharmacological targets. Altered expressions of various miRNAs have been reported in ovarian cancer cells. However, only few miRNAs have been reported to link with stemness, chemoresistance and progression of ovarian cancer. Tumor suppressive role of miR-379-5p have been reported in various malignancies such as breast, gastric, non-small cell lung carcinoma and hepatocellular carcinoma. However, we still lack evidence for the inhibitory function of miR-379-5p in ovarian cancer metastasis and chemoresistance. Herein, we propose to investigate the role of miR-379-5p in regulation of characteristics of CSCs in ovarian cancer. Our preliminary investigations have shown lower levels of miR-379-5p in ovarian CSCs as compared to corresponding bulk cells. We demonstrated that enforced expression of miR-379-5p in ovarian cancer cells reduces the CSC population and sensitizes ovarian CSCs to cisplatin treatment. Our studies will lead to a fundamental understanding of whether miR-379-5p deficiency promotes ovarian cancer progression and chemoresistance, and define a novel function of miR-379-5p in regulating the CSC populations in ovarian cancer.

Future research plans

In next few years, the major focus of our lab would be unravelling the mechanisms lead to development of acquired drug resistance in ovarian cancer. Our long-term goal is to develop small molecules/small molecule-anti-cancer drug conjugate for targeting molecular signalling pathways leading to chemoresistance.

List of Publications (journal and conference)

- Ganju R, Mishra S, Ahirwar DK, **Srivastava AK**, Tripathi PP. Inflammation and Myeloid Cells in Cancer Progression and Metastasis. **Frontiers Cell and Developmental Biology, 2022 IF: 6.6**
- Pandit A, Begum Y, Saha P, **Srivastava AK** and Swarnakar S. Approaches Toward Targeting Matrix Metalloproteases for Prognosis and Therapies in Gynecological Cancer: MicroRNAs as a Molecular Driver **Frontiers in Oncology, 2022, IF-6.24**
- Saha P, Mandal T, Talukdar AD, Kumar D, Kumar S, Tripathi PP, Wang QE, **Srivastava AK**. DNA polymerase eta: a potential pharmacological target for cancer therapy. **Journal of Cellular Physiology**. 2021 Jun; 236 (6):4106-20. **IF: 6.384,** PMID:33184862
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List of IPRs

Title of invention: Trypsin/trypsin-like protease (e.g., Trypsin and TMPRSS2) based SARS-CoV-2 spike protein cleavage assay system. (Application number: 202111049482). Role**:** Co-inventor

Achievements

Guest Editor: Frontiers in Cell and developmental Biology Topic: "Inflammation and Myeloid Cells in Cancer Progression and Metastasis during the reporting period. (Individual achievement)

Extramural funding

Sl.No	Title of Project	Participating Agencies	Role
1	DBT-Ramalingaswami Fellowship	**DBT**, New Delhi, Govt. of India	PI
2	Transcriptomic analysis of µTumor spheroid derived from single cancer stem cell to identify novel therapeutic targets in breast cancer	**SERB,** Govt. of India	PI
3	Understanding the role of translesion DNA synthesis in chemoresistance of lung adenocarcinoma.	**ICMR**, New Delhi	PI
4	Bio-assay guided isolation of anti-cancer compounds from *Pterocarpus santalinus* and assessment of cytotoxicity, pharmacokinetics and detailed molecular mechanisms.	**National Biodiversity Authority** (NBA) of India	PI
5	Anticancer potential and molecular mechanism of Rasa sindhoor on Ovarian Cancer Cells	CCRAS, **Ministry of Ayush**	PI
6	Evaluation of cytotoxicity, pharmacokinetics, anti-cancer activity, detailed molecular mechanisms of Bhallatakadi Modka in in vitro and in vivo tumor xenograft models	CCRAS, **Ministry of Ayush**	PI
7	Development of Genetically Engineered "off the Shelf" Inducible CAR-T Cells for the Cancer Therapeutics	**DBT**, New Delhi, Govt. of India	Co-PI
8	Functional dissection of Jmjd3 during embryonic and adult hippocampal neurogenesis.	**SERB,** Govt. of India	Co-PI
9	Utilizing the Principles of Coordination Chemistry to Develop Combination Prodrugs and Nanotherapeutics with a Synthetic Lethality-Like Concept	**SERB,** Govt. of India	Co-PI

Cell Biology and Physiology Division

Cell Biology and Physiology division deals with different research domains of modern biology with an aim towards understanding both basic fundamental as well as clinical biology. The investigators associated with this division are particularly interested in systems and cell & molecular biology, investigating the functions of subcellular organelles and intracellular signaling events in normal as well pathophysiological condition. The research endeavours are uptaken with a strong and persistent outlook towards development of novel therapeutic targets, diagnostics and therapeutic leads.

Understanding the molecular mechanism and pathophysiology of Cancer

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Background

Over the last decade, there has been a massive surge in reports highlighlting the role of metabolic reprograming in tumorigenesis. Reportedly, mutations harboured in tumor suppressor genes/oncogenes serve as a key driving phenomenon in altering several signaling pathways, eventually contributing to metabolic reprograming in cancer. One such important oncogenic transcription factor is Ets1 protooncoprotein, belonging to Ets family. Ets1 contributes to oncogenesis through regulation of a myriad facades of cancer, including angiogenesis, metastasis, chemoresistance, etc. Recent reports have also highlighted its role in acquisition of favourable metabolic alterations in cancer. Mitochondria serve as the major site for bioenergetic regulation in the cellular system and the significance of its structural and functional alterations in driving cancer is yet another aspect that is dynamic and fairly elusive. In this view, we were intrigued to understand the implications of Ets1 proto-oncoprotein in regulating mitochondrial structure, through regulation of mitochondrial fission-fusion proteins; followed by understanding the resultant connotations on mitochondrial bioenergetics. Sirtuins, a class of epigenetic modifiers are actively associated with metabolic modulations in cancer cells. In this direction, our group was particularly keen on finding the role of different sirtuin 6 (Sirt6) in modulating bio-energetic profile of malignant cells, through mitochondrial regulation.

The tumor microenvironment (TME) is the cellular environment in which the tumor exists, including surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, lymphocytes, signalling molecules and the extracellular matrix (ECM). TME derived factors like hypoxia, growth factors etc. play a significant role in oncogenesis by providing a suitable niche for cancer propagation. This necessitates identifying the regulatory role exhibited by these TME derived factors in understanding cancer progression through regulating the functional dynamics of transcription factors. Thus, our subsequent study is focused at understanding the role of Ets1 in regulating glycolytic genes, and its resultant implications on the metabolic status of the cancer cells, in response to an important TME derived cue, i.e. Epidermal Growth Factor.

Protein-protein interaction (PPI) is an emerging approach of regulating the functionalities of myriad transcription factors. DAXX (Death Domain Associated Protein 6)/EAP (Ets1 Associated Protein) is reported to repress Ets1's transcriptional activity through PPI and has emerged as an important oncogenic player across different cancer types and as a potent therapeutic target. Recent reports have established DAXX as an important candidate in ovarian cancer, where-in DAXX has been shown to regulate different aspects of cancer progression namely, proliferation, migration and chemoresistance. Considering the importance of DAXX and Ets1 as individual driver players of ovarian cancer progression, this study was performed with a view to further unravel how this interaction might be involved in regulating the different aspects of cancer progression.

Furthermore, several reports highlight the role of Monocarboxylate transporters (MCTs) in metabolically active solid tumors in regulation of concentration dependent transport of different important monocarboxylates including pyruvate, lactate etc. and are encoded by the SLC16A family of genes. Ultimately, our studies were also directed towards extensively studying the immense role of these MCTs in ovarian cancer metastasis/EMT with an intent to substantially mitigate the same through pharmacological intervention.

Aims and Objectives

- To understand the mechanism of oncogene-mediated mitochondrial fission-fusion imbalance in cancer cell.
- To elucidate the underlying mechanism of EGF)-mediated metabolic reprograming through oncogenic transcription factor, Ets-1, in cancer.
- To understand the functional regulation of ETS1 oncogenic transcription factor through protein-protein interactions.
- To study the role of monocarboxylate transporter (MCT1/2/3/4) as oncogenic drivers in ovarian cancer.
- To study the role of Sirt6 in mitochondrial function to promote invasiveness in ovarian cancer.

Work Achieved

- ETS1 oncogenic transcription factor, established to be associated with glycolytic reliance of cancer cells, has been found to be facilitating mitochondrial fragmentation (**Figure 1. A**) in ovarian cancer cells through Drp1 augmentation. Furthermore, ETS1 mediated Drp1 increment is shown to be a repercussion of ETS1 binding at DNM1L (Drp1) promoter sequence, resulting in an increase in its expression at both transcript and protein levels. The mitochondrial fragmentation resulting from Drp1 augmentation results in mitochondrial load reduction and a decreased expression of mitochondrial OXPHOS protein complexes, primarily complex 5 (ATP synthase). The decrease in Complex 5 expression further results in a compromised OXPHOS level of the cancer cells, rendering them more reliant on glycolysis. This phenomenon of the classical Warburg effect has been known to be a driver of EMT/metastasis in cancer, Interestingly, our findings suggest that molecular (siRNA mediated) knockdown or pharmacological inhibition (MDiVi-1) of Drp1 significantly mitigates Ets1 associated EMT/Metastasis in ovarian cancer (**Figure 1. B**).
- ETS1, an onco-protein vastly related to metastasis in different cancers, shows an increase upon Epidermal Growth Factor (EGF) treatment *in vitro.* ETS1 increases the transcript and protein level of different glycolysis associated genes to achieve the elevated glycolysis and compromised OXPHOS in ovarian cancer cells. DAXX, an interacting partner of ETS1, represses the transcriptional efficiency of ETS1 to reduce metastasis.
- Extensive literature reports suggest that Ets1 undergoes a host of protein-protein interactions (PPI), often exerting regulatory implications on its functioning. One such significant interaction partner for Ets1 is DAXX (Death Domain Associated Protein 6), reported to repress Ets1's transcriptional activity through PPI. In this study, we have validated a strong PPI between Ets1 and DAXX proteins in both OC cell lines and patient tissue samples. Further, in silico approaches identified the N-terminal of Ets1 DAXX interacting domain (DID) and C-terminal of DAXX Ets1 binding domain to be involved at the interaction interface, subsequently validated through in vitro pull-down assays and microscopy based colocalization studies of deletion constructs (DEL) with respective wildtype (WT) interaction partners. Ets1-DAXX PPI resulted in an EMT suppression by repressing Ets1 transcriptional activity on DAXX WT overexpression, subsequently rescued on DAXX silencing in OC cell lines. Furthermore, this repression by WT DAXX was abrogated on DAXX DEL overexpression. Cumulatively, our studies put forth ETS1-DAXX PPI as an important conjuncture of therapeutic intervention in ovarian cancer progression.
- Given the vast array of functions that the transporters play in oncogenesis, our objective was to look into the association of MCT1 (SLC16A1), MCT2 (SLC16A7), MCT3 (SLC16A8), and MCT4 (SLC16A3) with Epithelial ovarian cancer (EOC) pathophysiology by exploiting various publicly available databases and web resources. MCT1 and MCT4 were found to be upregulated at mRNA level in OC tissues compared to normal, however only higher levels of MCT4 mRNA was found to be associated with poor patient survival. Gene association study revealed that MCT4 was positively related to the genes

responsible for biological adhesion, migration, immune modification, and other oncogenic attributes proving its potential to serve as a therapeutic target (**Figure 2**). We compared the effects of MCT1/2 blocker SR13800 and a broad-spectrum MCT blocker α-Cynao Hydroxy Cinnamic Acid (α-CHCA) to MCT4 silencing *in vitro* to find that α-CHCA has a greater effect on the invasive behaviour of the cancer cells than MCT1/2 blocker SR13800. From our study, MCT4 has emerged as a prospective marker for predicting poor patient outcomes and a potential therapeutic target.

- SIRT6 is found in higher levels in ovarian cancer cells. SIRT6 being a metabolic sensor increases cellular glycolysis as well as mitochondrial oxidative phosphorylation. SIRT6 promotes cancer cell migration and invasion by facilitating DRP1-induced mitochondrial fragmentation (**Figure 3**) and their localization towards the leading edge of invading cells. Thus, we indicate an oncogenic role of SIRT6, wherein it regulates mitochondrial function induced cancer cell invasion and metastasis.

Future Research Plans

Exploring alternative treatment options in context of ovarian cancer is an urgent need given the very high incidence and mortality rate associated with the cancer. Metabolic rewiring of the cancer cells resulting in aerobic glycolysis and lactate production, has been identified as one of the major targets for therapeutic intervention. Dual blocking of Glycolysis and lactate transport using minimal concentrations of the respective blockers was found to be effective in mitigating growth factor induced migration and invasion of the Ovarian cancer cells. Adopting such combinatorial approaches using metabolic inhibitors not only addresses the issues of dosage reduction but also helps to prevent associated cytotoxicity. Similarly, blockers that can mediate changes in the mitochondrial dynamics was also found effective at mitigating EMT/Invasion of the cancer cells. In future, different combinatorial approaches could be developed by blocking mitochondrial and glycolytic feeder pathways in mitigating oncogenesis. These could be tested in animal models to standardize the effective dosages for cancer remission without causing much toxicities. More specific inhibitors to address the off-target effects could also be explored.

Publications

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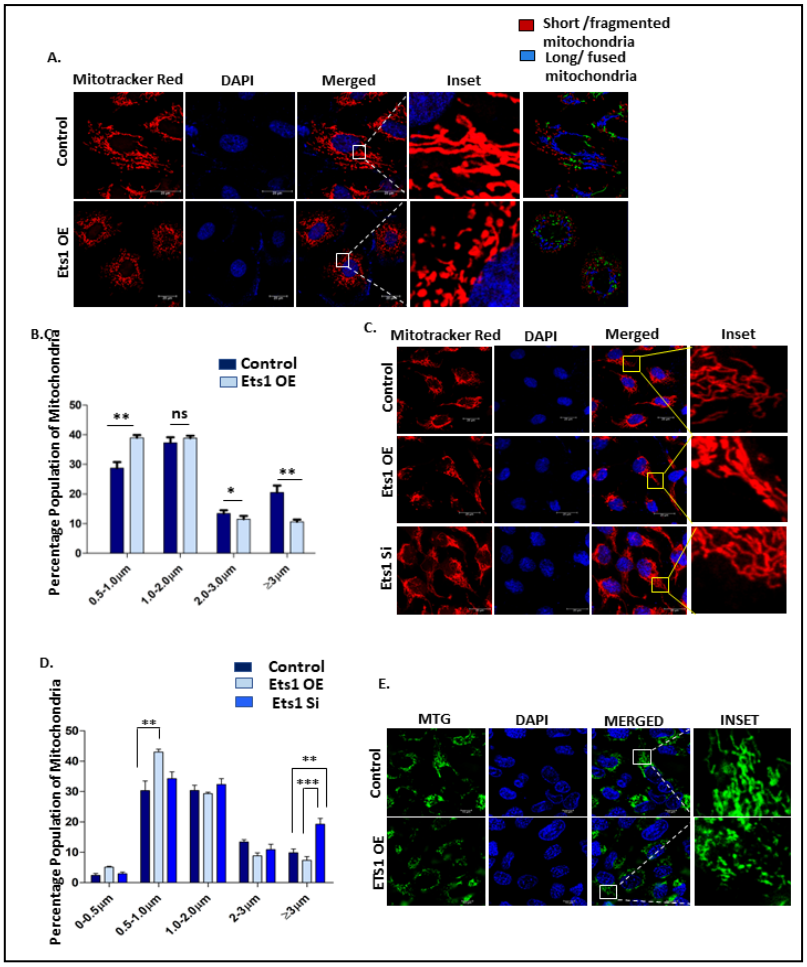
- Bandopadhyay S, Prasad P, Ray U, Das Ghosh D, **Roy SS***. SIRT6 promotes mitochondrial fission and subsequent cellular invasion in ovarian cancer. **FEBS Open bio.** 2022 Sep;12(9):1657-76.

Extramural Funding

Sib Sankar Roy (PI) and Prof Satinath Mukhopadhyay, SSKM Hospital (Co-PI)
"To evaluate the effect of black tea intake on chronic anovulation and insulin resistance in polycystic ovarian syndrome (PCOS)". Code NTRF:207/2018; Sanctioned Fund Rs. 34.45 lakhs, 24/04/2019 to 23/4/2022 (National Tea Research Foundation, NTRF).

a.



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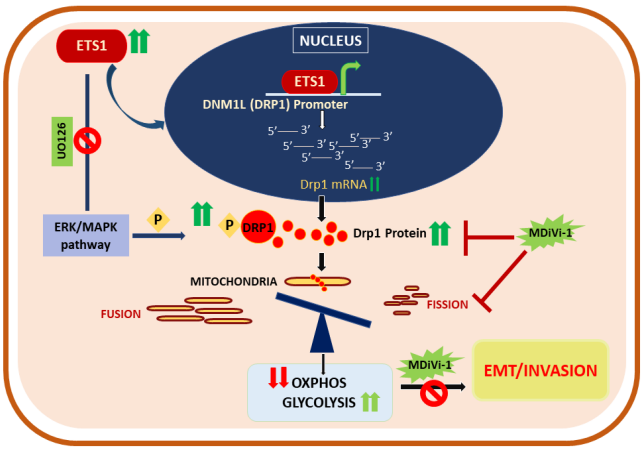

Figure 1: Ets1 oncoprotein causes fragmentation of mitochondria (a.) A. Confocal microscopy-based mitochondrial imaging using Mitotracker Red (MTR) is performed in control (untransfected) and Ets1 overexpressing OAW42 cells (*n=3*). Short/fragmented mitochondrion have been colour coded with RED and long/fused mitochondria have been coded with BLUE colour in the representative images. **B.** Mitochondrial length estimation is performed using LASX software and represented as histogram. **C-D.** MTR based mitochondrial length estimation in Control, Ets1 overexpressing and Ets1 knockdown SKOV3 cells (*n=2*). Mitochondrial length estimation is performed on LASX software. **E.** Mitotracker Green (MTG) based live-cell imaging is performed for control and Ets1 overexpressing SKOV3 cells. Scale bars 20µm (A, C), 10µm (E).
(**b.**) Schematic representation of Ets1 mediated Drp1 augmentation through direct binding at DNM1L promoter. Increased Drp1 undergoes activating phosphorylation at ser616 residue and enhanced mitochondrial recruitment to facilitate fission. Fragmented mitochondria due to pronounced fission leads to glycolytic bias over OXPHOS, resulting in EMT/invasion. MDiVi-1 administration blocks Drp1 activity ultimately to mitigate mitochondrial fission, glycolytic bias and EMT/Invasion associated with Ets1.

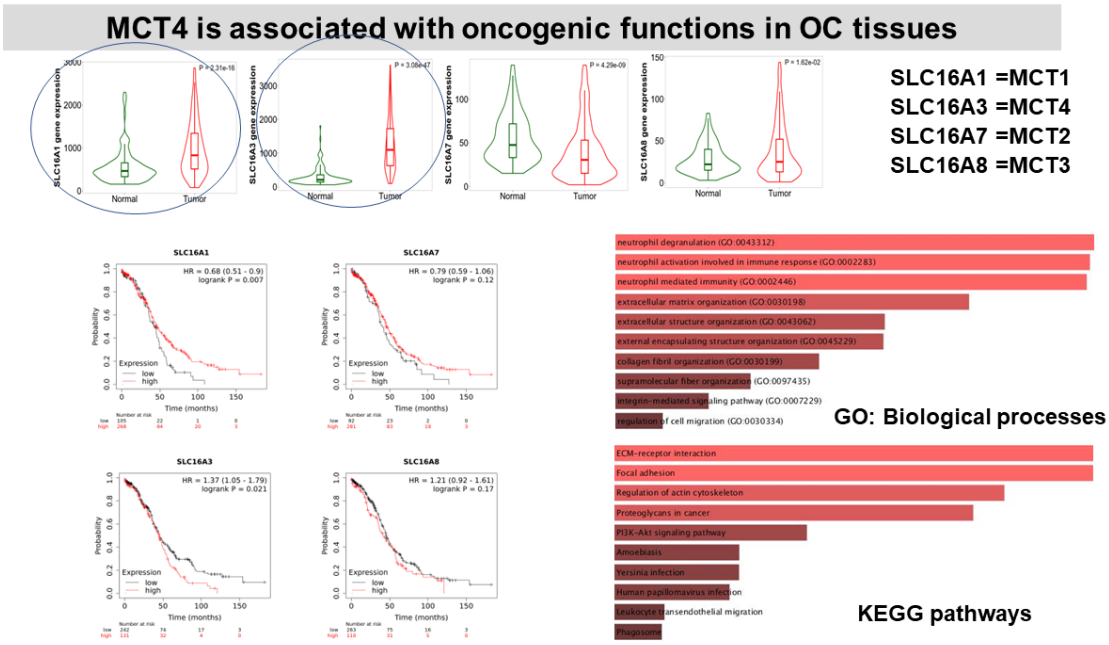

Figure 2: Violin plot for gene expression (RNA-Seq) profile for SLC16A1/3/7/8 genes from TNMplot comparing normal vs ovarian cancer tissues. Overall survival of Ovarian cancer patients associated with mRNA levels of the SLCA16 genes (TCGA-OV). Pathway enrichment analysis for the SLC16A3 genes in TCGA-OV. Gene Ontology (Biological pathway) enrichment and KEGG pathway analysis showing fold change (length of the bars) of all the pathways that are associated with SLC16A3 gene in TCGA-OV. (Darker the colour- lower the FDR value)

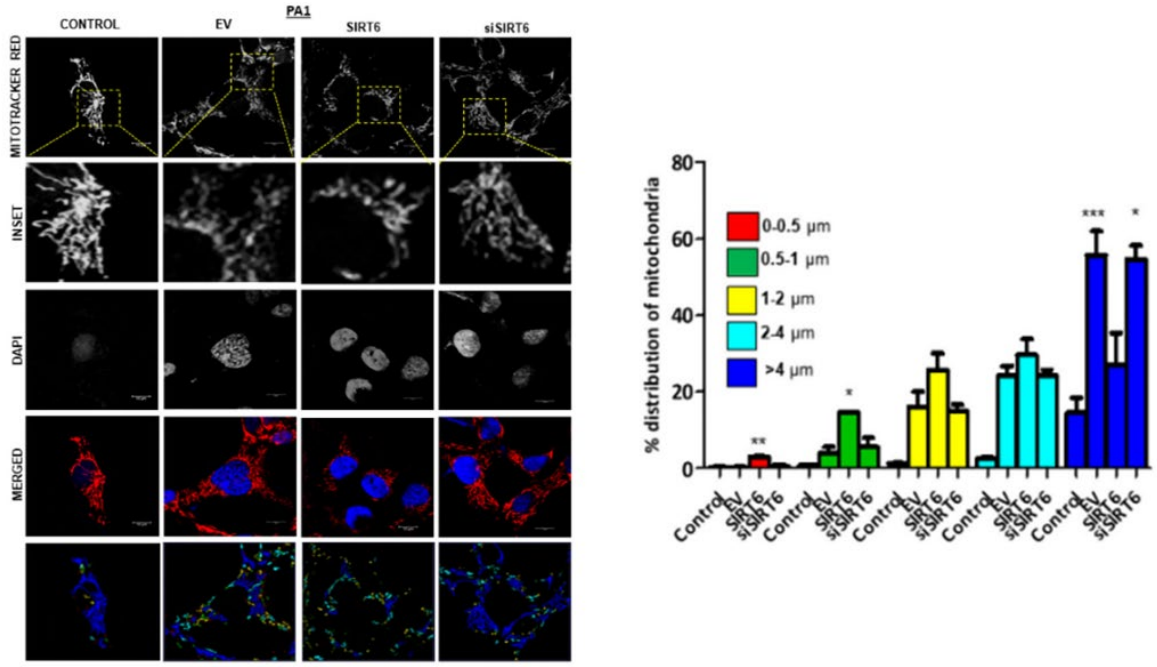

Figure 3: Mitotracker Red staining showing mitochondrial structure is depicted in PA1 cell with EV, SIRT6 overexpression and SIRT6 siRNA transfection and corresponding mitochondrial length distribution calculations were done in Leica mitochondrial structural analysis software. Nucleus is stained with DAPI. EV= Empty vector. (Bandopadhyay S. et. al., FEBS Open bio, 2022.)

Vitronectin is associated with aortic inflammation and Atherosclerotic plaque progression in ApoE-Knockout mice

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Background

Atherosclerosis is a chronic inflammatory disease that primarily affects the intimal layer of large and medium-sized arteries, making it the leading cause of cardiovascular complications. Recent research has emphasized the role of inflammation in aortic atherosclerosis, where the balance between pro-inflammatory and anti-inflammatory mechanisms determines the fate of the plaque. Therefore, understanding the factors that govern athero-inflammation is essential for effectively managing patients with cardiovascular diseases (CVDs).

The accumulation of LDL-cholesterol has long been recognized as a genetic and environmental promoter of atherosclerotic disease. The initial steps of atherosclerosis development involve the sequestration of LDL-cholesterol in the subendothelial space, where it becomes oxidized, giving rise to ox-LDL. This oxidation triggers inflammatory responses in the aorta, particularly in areas of non-laminar blood flow. Endothelial cell adhesion molecules (CAMs) play a crucial role in the early stages of plaque formation by facilitating the infiltration of cells into the subendothelium and the subsequent formation of foam cells. Over time, a necrotic core forms, and if not timely cleared, it can lead to atherothrombotic vascular diseases such as stroke or myocardial infarction. Several adhesion molecules have been studied in the context of atherosclerosis, as they are present in both the vascular endothelium and leukocytes. The coordinated interactions between these molecules are necessary for the recruitment of inflammatory cells from the blood to the site of injury, i.e., the vessel wall, in atherosclerosis. If the underlying triggers are removed, the inflammation phase is replaced by a resolution phase. However, if these triggers persist, repeated cycles of inflammation and resolution lead to tissue remodeling. Certain adhesion molecules not only orchestrate inflammation but also contribute to tissue remodeling. VTN, a multifunctional protein primarily found in plasma and the extracellular matrix, is one such adhesion molecule. Although VTN is known to play a role in cell spreading and wound healing, its functional involvement in pathological atherosclerosis remains largely unexplored. In other inflammatory airway diseases like asthma and COPD, where tissue remodeling is a significant phenomenon, VTN levels have been found to be reduced. However, there are currently no reports on the specific role of VTN in pathological atherosclerosis in the existing literature.

Aims and Objectives

- Effects of Dietary cholesterol on the expression of Vitronectin in Liver and level in Circulation
- Whether VTN is associated with inflammation and plaque progression
- Clinical correlation of plasma level VTN in human subjects

Major findings

Our investigation involved the analysis of VTN expression in the livers of Apolipoprotein E (ApoE) Knockout mice, which are commonly used as a model for studying atherosclerosis. The mice were fed a high cholesterol diet (HCD) to induce

atherosclerotic progression. Our results revealed a significant downregulation of VTN mRNA and protein levels after 60 days of HCD feeding. To explore the underlying mechanisms, we conducted a promoter assay, which confirmed that cholesterol influences the expression of VTN by modulating its promoter.

To further understand the impact of VTN reduction, we employed small interfering RNA (siRNA) to mimic VTN deficiency in HCD-fed ApoE Knockout mice. The reduction of VTN accelerated athero-inflammation, as evidenced by an increase in the expression of NF-kB, ICAM-1, and VCAM-1 at the plaque site. Additionally, inflammatory proteins such as MCP-1 and IL-1β were upregulated in the plasma. We also observed increased expression of MMP-9 and MMP-12, along with a decrease in collagen content within the plaque, under conditions of VTN deficiency. These changes may pose a challenge to plaque integrity.

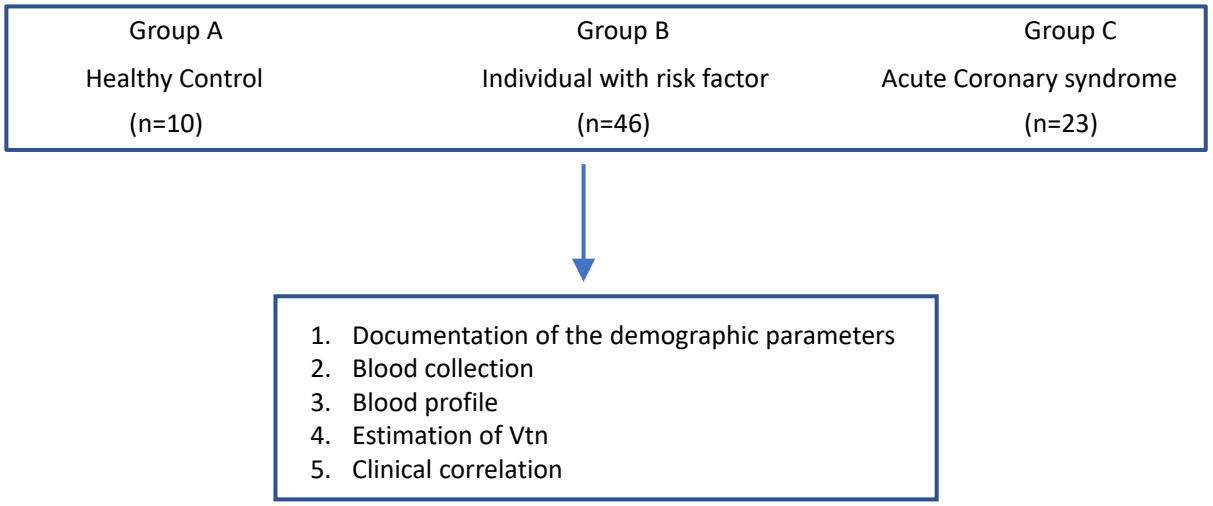
In a clinical context, our findings hold significance. Human subjects with acute coronary syndrome or those at risk for atherosclerosis demonstrated lower levels of VTN in their plasma compared to healthy controls. This suggests that plasma VTN has clinical relevance in the pathophysiology of coronary artery disease. Taken together, our study establishes the pivotal role of VTN in cholesterol-driven atherosclerosis and aortic inflammation. Furthermore, it suggests that VTN could serve as a useful indicator for assessing atherosclerotic plaque burden and stability

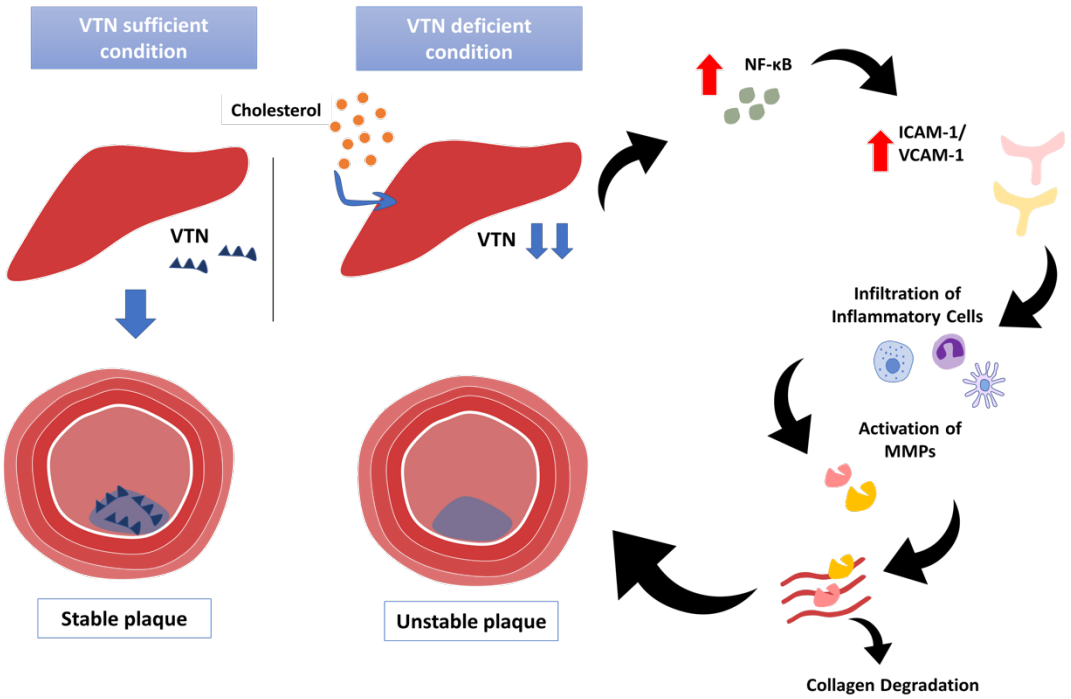
In this study, we present compelling evidence demonstrating that systemic deficiency of VTN is associated with inflammation and plaque progression in ApoE Knockout mice, a widely used animal model for atherosclerosis. We establish a reciprocal relationship between VTN and cholesterol load, indicating that VTN downregulation promotes unresolved inflammation and the formation of rupture-prone plaques by accumulating cells and overexpressing adhesion molecules. Furthermore, we establish an inverse correlation between VTN levels and the severity of coronary artery disease (CAD) in human subjects. Collectively, our findings highlight the critical association of VTN with the ongoing inflammatory process in the aorta, suggesting a novel role for VTN in CAD.

Publications

Chakravarty D, Guha Ray A, Chander, V, Mabalirajan U, Mondal PC, Siddiqui KN, Sinha BP, Konar, A and ***Bandyopadhyay A***. Systemic deficiency of vitronectin is associated with aortic inflammation and plaque progression in ApoE-Knockout mice. ***FASEB BioAdvances***. 2021;00:1–17

Flow chart of observational study in human subjects





Schematic representation depicting the role of VTN in atherosclerotic plaque

The supply of sufficient VTN in the atherosclerotic plaque maintains stable conditions in the plaque, while chronic cholesterol load causing VTN downregulation induces a series of events which initiates inflammation leading to upregulation of NF-κB, which in turn enhances ICAM-1 and VCAM-1 secretion. This leads to inflammatory cells build up and a shifting towards unresolved and chronic inflammation in the plaque mileu. In addition, MMPs are activated which leads to severe collagen degradation, resulting in an unstable plaque, indicating vulnerability towards acute events. NF-κB (nuclear factor kappa light chain enhancer of activated B cells), ICAM-1 (Intracellular Cell Adhesion Molecule-1), VCAM-1 (Vascular Cell Adhesion Molecule-1), MMPs (Matrix metalloproteases), VSMC (Vascular smooth muscle cells). Up arrow indicates upregulation and down arrow indicates down regulation.

Cellular and molecular rendezvous at the maternal-fetal interface: Implication in placental pathophysiology

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Research Activities

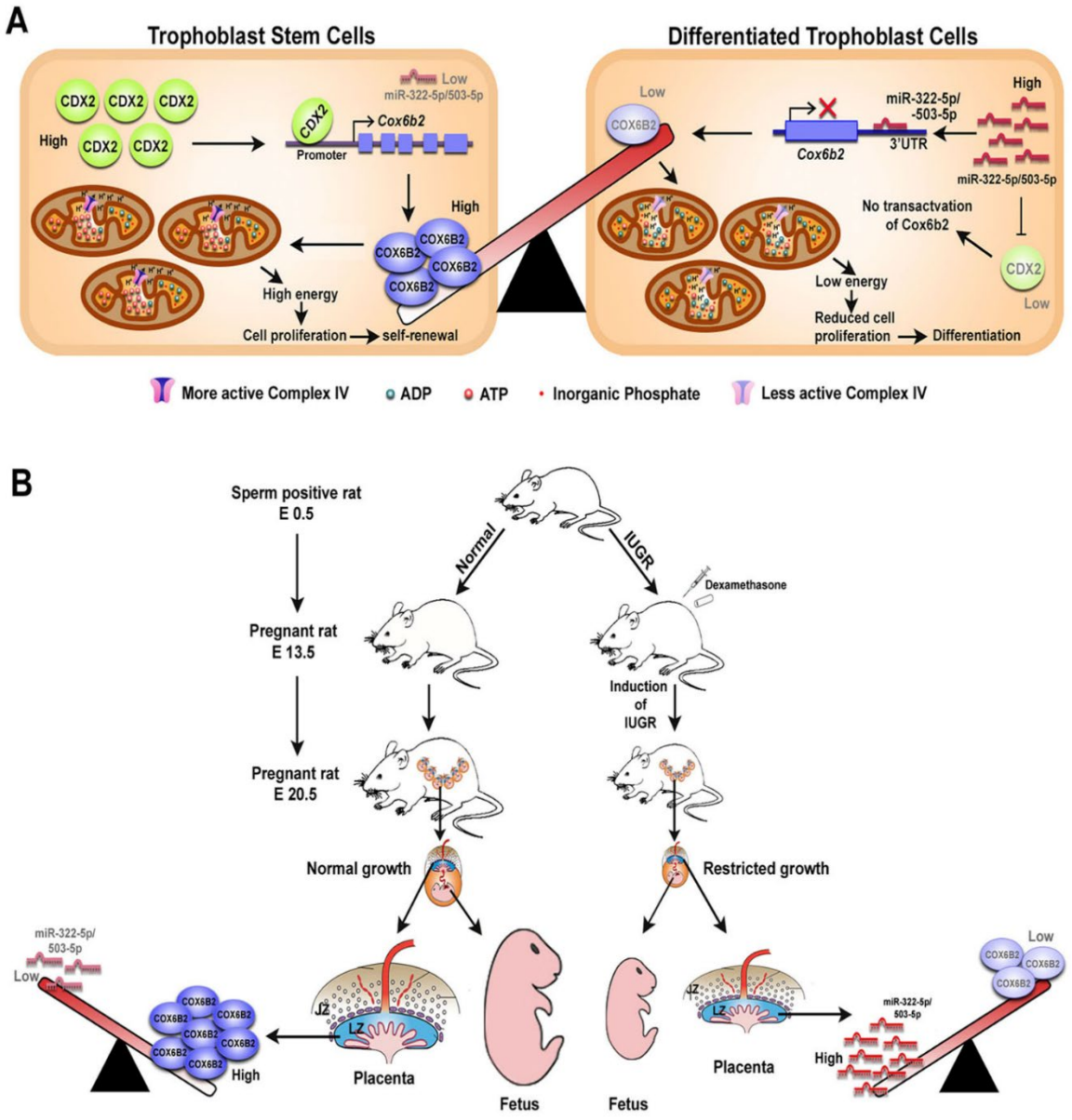
We are interested in how the early trophoblast niche is regulated by miRNAs, transcription factors and specific cellular signalling events to ensure normal development. We also investigate how trophoblast derived factors modulate vascular smooth muscle cells (VSMC) to impart phenotypic changes required for normal progression of pregnancy. We use the tools and concepts of molecular biology, cell and developmental biology, genetics, microscopy to further our understanding of trophoblast stem cell differentiation, angiogenesis and placental development.

1) Energy metabolism during trophoblast stem cell self-renewal, differentiation and intra-uterine growth restriction

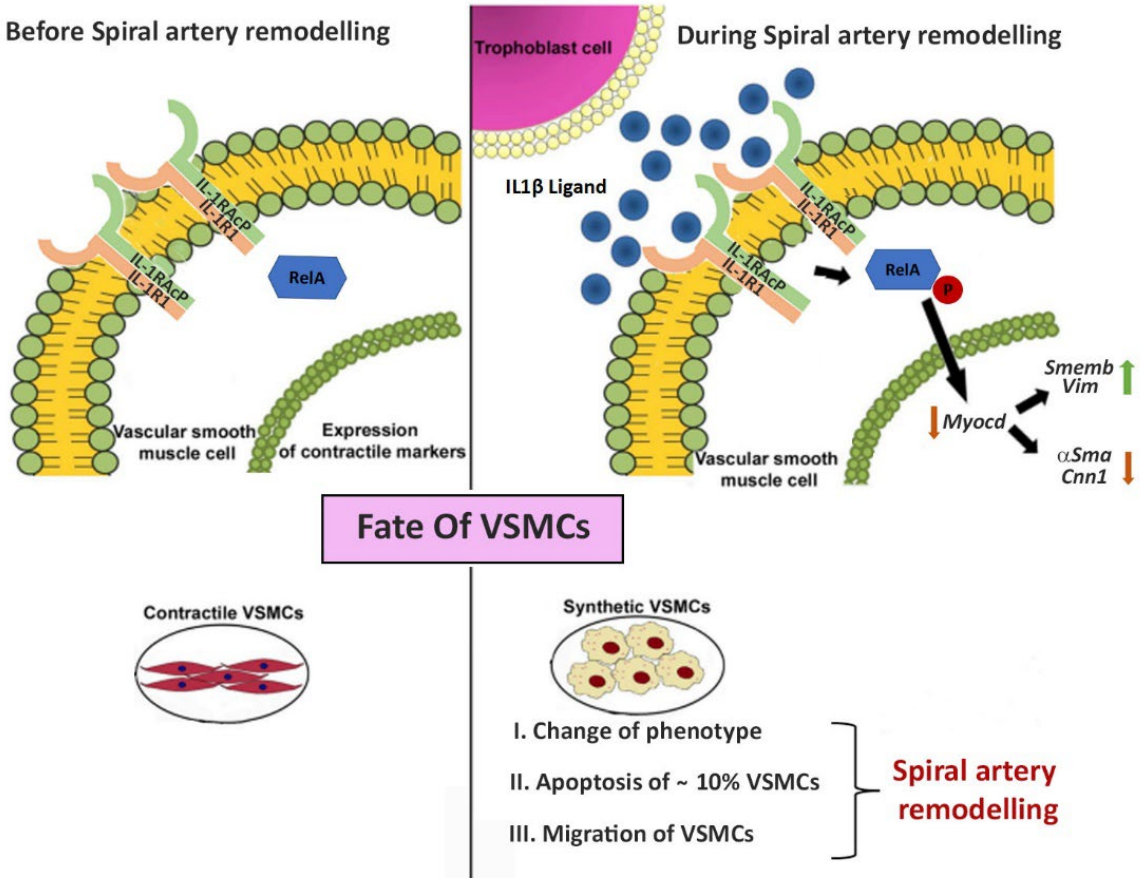
Metabolic effector(s) driving cell fate is an emerging concept in stem cell biology. Here we showed that Cytochrome C Oxidase Subunit 6B2 (Cox6B2) is essential to maintain the stemness of trophoblast stem (TS) cells. RNA interference of Cox6b2 resulted in decreased mitochondrial Complex IV activity, ATP production, and oxygen consumption rate in TS cells. Furthermore, depletion of Cox6b2 in TS cells led to decreased self-renewal capacity indicated by compromised BrdU incorporation, Ki67 staining, and decreased expression of TS cell genetic markers. As expected, the consequence of Cox6b2 knockdown was the induction of differentiation. TS cell stemness factor CDX2 transactivates Cox6b2 promoter in TS cells. In differentiated cells, Cox6b2 is post-transcriptionally regulated by two microRNAs, miR-322-5p and miR-503-5p, leading to its downregulation as demonstrated by the gain-in or loss of function of these miRNAs. Cox6b2 transcripts gradually rise in placental trophoblast gestation progresses in both mice and rats with predominant expression in labyrinthine trophoblast. Cox6b2 expression is compromised in the growth-restricted placenta of rats with reciprocal up-regulation of miR-322-5p and miR-503-5p. These data highlight the importance of Cox6B2 in the regulation of TS cell state and uncompromised placental growth.

2) Trophoblast-directed signalling affects Myocardin-driven vascular smooth muscle cell fate

In this project, we demonstrated that a) disappearance of α-SMA, a VSMC contractile marker, is associated with trophoblast invasion during rat gestation; b) Trophoblast cells (TC) direct VSMC phenotype switching *in vitro* when both cell types are co-cultured; c) VSMC phenotype switching is associated with Myocardin downregulation at the maternal-fetal interface; d) Gain in or loss of function of Myocardin enhances and reduces α-SMA expression, respectively indicating Myocardin mediates VSMC phenotype switching, e) Myocardin down regulation is caused by IL-1β – relA signaling axis.



Summary of the influence of COX6B2 and trophoblast stem cell self-renewal, differentiation, and placental pathophysiology. In TS cells CDX2 transactivates Cox6b2. COX6B2 potentiates complex IV activity and mitochondrial respiration yielding more ATP and aids in TS cell self-renewal. Induction of differentiation is associated with an increase in miR322-5p and miR503-5p, that target Cox6b2 leading to its downregulation and subsequent decrease in its activities (A). With the progression of gestation, COX6B2 is expressed in the labyrinthine zone of the placenta. Low levels of COX6B2 and reciprocally high levels of its regulatory miRNAs in the placenta mark IUGR in the rat (B).



Our studies proved that that the co-activator Myocardin is a key regulator for spiral artery remodelling. It will be interesting to explore the expression as well as role of Myocardin in placental pathophysiology wherein spiral artery remodelling is compromised, such as, IUGR cases. Furthermore, Myocardin regulatory molecules, which will be explored later might be used as targets in preclinical models to combat IUGR.

Future research plans

Cell fate determination and differentiation accompany an exquisite molecular orchestra that is still an active area of research. Trophoblast cells, recognized as parenchymal cells of the placenta, execute most placental functions, indispensable for successful pregnancy. They differentiate from multipotent trophoblast stem (TS) cells during development. Despite being recognized as the developmental counterpart of embryonic stem (ES) cells in the context of placental development, many facets of regulation of trophoblast development remained elusive. In rodents and primates, specialized populations of trophoblast cells of the placenta invade the uterine stroma and establish relationships with uterine blood vessels supplying the placenta. Two populations of invading trophoblast cells can be identified: (i) interstitial and (ii) endovascular. Interstitial trophoblast cells penetrate through the uterine stroma and are often situated in perivascular locations, whereas endovascular trophoblast cells enter uterine blood vessels, where they replace endothelial cells. It has been proposed that the "trophoblastic vascular colonization" is an effective mechanism for removing maternal vasomotor control and thus dramatically augmenting the delivery of maternal resources to the placenta. This hallmark developmental event in effect creates flaccid, low-resistance blood vessels, known as spiral artery remodelling, and is fundamental for optimal delivery of nutrients to the fetus. Our future goal is to dissect the mechanism of spiral artery remodelling by cellular rendezvous involving TS cells, VSMCs and endothelial cells of the spiral arteries.

Extramural funding

Ongoing

- Regulation of trophoblast development and placental pathophysiology by long non-coding RNA (2021-2024) **SERB-POWER (PI).**
- Hippo dynamics is a critical regulator of trophoblast stem cell self-renewal and differentiation (2020-2023) **ICMR, (PI).**
- Molecular regulation of spiral artery remodeling (2019-2023), **DBT, (PI).**

Completed

- Cellular Prion: a novel regulator of decidual cell function at the maternal-fetal interface (2019-2022), **ICMR, (PI) .**

List of Publications (journal and conference)

In SCI Journal

- Basak T and **Ain R** (2021) Sequestration of eIF4A by Angiomotin: a novel mechanism to restrict global protein synthesis in trophoblast cells. ***Stem Cells*** 39(2):210-226. doi: 10.1002/stem.3305.

In conference proceedings

- Basak T and **Ain R** (2021) Hippo components YAP and LATS1 regulate trophoblast stem cell self-renewal and giant cell differentiation. In the proceedings of the International Society for Stem cell Research, P-460.
- Paul M, Gope TK and **Ain R** (2021) Novel Role of Nitric-Oxide Synthase Trafficking Inducer (NOSTRIN) in Combating Colon Cancer Aggressiveness. *FASEB J.*, Vol 35, issue S1.
- Saha S, Das S, Chakraborty S and **Ain R** (2021) Molecular Orchestration of Epithelial Mesenchymal Transition During Trophoblast and Placental Development. *FASEB J.*, Vol 35, issue S1.
- Basak T and **Ain R** (2021) Polarity switches governing murine trophoblast stem cell differentiation. In the proceedings of International Conference on Challenges and Strategies in Reproductive and Environmental Health, P-216, Page-397.
- Ghosh M, Saha S, Basak T and **Ain R** (2021) Long Non-Coding RNA MALAT1 in Placental Development, Trophoblast Differentiation. In the proceedings of International Conference on Challenges and Strategies in Reproductive and Environmental Health, P-96, Page-215.
- Das P, Basak T, Saha S and **Ain R** (2021) FoxO4: A Pivotal Regulator of Trophoblast Development and Placental Pathophysiology. In the proceedings of International Conference on Challenges and Strategies in Reproductive and Environmental Health, P-144, Page-290.

Invited lecture

COX6B2-directed mitochondrial function is pivotal in equipoising trophoblast stem cell self-renewal and differentiation. 90th Annual meeting of Society of Biological Chemists, Manesar, India, December 2021.

Alzheimer's disease: regulation of autophagic neuron death

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Background

Alzheimer's disease (AD) is characterized by accumulation of senile amyloid-β (Aβ) plaques and hyperphosphorylated tau tangles causing progressive loss of synapse and neuronal death. Out of the various neuron death modalities, autophagy and apoptosis are reported to be the major death paradigms in AD. However, how these two processes lead to neuronal loss is still inconspicuous.

Autophagy is a major intracellular catabolic pathway which eliminates the damaged cellular contents like proteins, lipids and other organelles, with the help of lysosomal enzymes. Neurons are highly dependent on autophagy for their survival due to their extreme polarized and post mitotic nature. Autophagy maintains the health of neurons by preventing the accumulation of aggregated cytosolic wastes of proteins or membranes. Accumulation of cellular wastes in neurons may incur great burden in the cells which are not diluted due to their non-dividing nature. The role of autophagy in AD is quite controversial. Accumulation of autophagy vacuoles (AVs) is reported in AD patients' brains as compared to normal brain biopsies. While few reports suggest autophagy being crucial in degradation of Aβ plaques, APP and tau tangles, there are many reports suggesting presence of aberrant AVs containing Aβaggregates in AD brains. Since there are varied reports suggesting the beneficial and deteriorating effects of autophagy in AD, its exact role in AD pathogenesis still remains uncertain.

In AD, it is reported that the Aβ plaque may generate oxidative stress or trigger expression/activation of a number of pro-apoptotic proteins in neurons leading to cell death by apoptosis. Reports of increased DNA fragmentation, granulated chromatin, changes in cell shape and size, caspase activity, changes in levels of the apoptosis related proteins of Bcl-2 family have been cited in the AD patients' brains bearing senile plaque depositions. Bcl-2 protein family plays important roles in the interplay of autophagy and apoptosis. Reports suggest that BH3-domain only pro-apoptotic protein, Bim, sequesters Beclin1 or ATG6, which is a vital autophagic protein and inhibits autophagic initiation in starved HeLa cells. Puma (p53 upregulated modulator of apoptosis) is a potent pro-apoptotic protein like Bim; however, its role in autophagy is not yet revealed. The C-terminal fragment of Beclin1 is reported to translocate to the mitochondria thus releasing apoptotic signals. There are many levels of regulations and interactions between the autophagic and apoptotic machineries which are not yet clear. In this lab we concentrate primarily on the activation of both autophagy and apoptosis in neurons under the influence of Aβ and find out the role of autophagy in determining the neuronal fate. We specifically look into the effect of BH3-only proteins, Puma and Beclin1 in regulating autophagic death of neurons under Aβ toxicity.

Aims and Objectives

- To study if Aβ induces both autophagy and apoptosis.
- To determine if Beclin1 promotes neuronal cell death upon Aβ treatment.
- To study if Puma evokes autophagy in neuronal cells upon Aβ insult.
- To investigate whether autophagy and apoptosis is induced simultaneously in brains of 5xFAD mice
- To determine if down regulation of Puma and Beclin1 leads to inhibition of both autophagy and apoptosis *in vivo.*
- To study wheather autophagic BH3-only protein Beclin1 interacts with pro-apoptotic BH3-only protein Puma.

Work Achieved

Aβ induces both autophagy and apoptosis

We began our quest using neuronally differentiated PC12 cells being treated with oligomeric Aβ at a concentration of 5μM for overnight. We checked the onset and progression of autophagy and apoptosis by checking the levels of their markers by immunocytochemistry. We found an increase in the expression of autophagosome markers such as LC3, p62, a lysosomal marker, Lamp1 and a marker of DNA double strand breaks, pH2AX in differentiated PC12 cells under Aβ toxicity over a period of 0-24h. Expression of these proteins gradually increased over time. We also checked for apoptosis by TUNEL assay under same condition and obtained similar results. This upregulation of autophagic and apoptotic proteins were further checked by western blotting and an increase of LC3B, p62, Lamp1 and pH2AX levels was found as early as 8h with a peak at 16h (LC3B and p62) or 24h (Lamp1 and pH2AX) post Aβ treatment. Having observed induction of both autophagy and apoptosis after Aβ treatment, we were intrigued to investigate the specific cellular population inwhich both these phenomena occurred simultaneously. Our co-immunocytochemistry studies revealed that following Aβ insult, there was a gradual increase of cells exhibiting both LC3 and pH2AX, and the number of cells showing co-staining increased significantlyin a time dependent manner from 8h to 24h of treatment.

Since PC12 cells is an immortal cell line, we checked the status of autophagicand apoptotic proteins in primary cortical neurons. We found a significant and gradual increase in the expressions of autophagic initiation marker, LC3B under Aβ insult in a time dependent manner as checked by both immunocytochemistry and western blotting. Protein levels of another important autophagic protein, Beclin1 and autophagy flux marker, p62 were also elevated gradually and significantlyunder Aβ toxicity in cortical neurons from 8h to 24h.The apoptotic effector, cleaved caspase-3 staining was also shown to be increased under the influence of Aβ from 8h to 24h (Fig 2I-J).Collectively, these data indicate that both apoptosis and autophagy are induced upon Aβ insult in neuronal cells and both the phenomena occur simultaneously in a particular cell.

Induction of autophagy does not protect cells from Aβ toxicity

Since we found that both autophagy and apoptosis are induced in response to Aβ treatment in neuronal cells, we therefore intended to check if autophagy is beneficial on cell viability in presence of Aβ. We treated neuronally differentiated PC12 cells with Aβ for overnight in the presence of pan caspase inhibitor (zVAD-FMK) to inhibit apoptosis, rapamycin to induce autophagy or both zVAD-FMK (to inhibit apoptosis) and rapamycin (to simultaneously induce autophagy). Results showed that the death caused by Aβ was rescued when apoptosis was inhibited by zVAD-FMK. However, there was no rescue seen when autophagy was induced using rapamycin. Further, simultaneous induction of autophagy and inhibition of apoptosis by rapamycin and zVAD-FMK respectively did not provide any more significant protection as compared to whenonly apoptosis was blocked, suggesting that induction of autophagy does not play a major role in cell survival under Aβ insult, at least in cultured neurons.

Beclin1 promotes neuronal cell death upon Aβ treatment

Beclin1 is a major autophagy protein which has been reported to be induced under neurodegenerative conditions and plays an essential role in autophagy-apoptosis crosstalk. Interestingly, recent evidences have also shown that proteolytic cleavage of Beclin1 causes apoptotic neuronal loss in two different models of neurodegeneration. Since our previous experiments showed that autophagy induction does not protect neurons from Aβ toxicity, we next checked whether inhibition of autophagy by downregulating Beclin1 could provide protection to neurons. Neuronally differentiated PC12 cells were transfected with either shBeclin1 or shRand (control), and then were subjected to Aβ treatment for 24h and 48h. Results showed significant protection, where Beclin1 was downregulated compared to cells transfected with shRand. Similar protection was obtained in cortical neurons treated with 1.5μM Aupto 48h. Knockdown of Beclin1 not only protects neuronal cells from death but also retains the overall neuronal morphology of neurons even after 48h of Aβ treatment. We further observed that number of cells in which

Beclin1 was downregulated, levels of cytochrome-c was significantly low as opposed to control plasmid transfected cells upon treatment with Aβ. Taken together, these results indicate that Beclin1 plays an essential role in neuron death evoked by Aβ by inducing both autophagy and apoptosis.

Puma and FoxO3a evoke autophagy initiation but hinder autophagy flux in neuronal cells upon Aβ insult

We have previously shown the activation of Puma in response to Aβ in neurons leads to neuronal death and it has been also shown that Puma plays an essential role in regulation of mitophagy in cancerous cells. Taking cue from these studies, we checked the effect of downregulation of Puma, on the regulation of autophagy initiation and flux under Aβ toxicity. We observed a significant drop in the levels of LC3B and p62 in the differentiated PC12 cells in which Puma had been downregulated as compared to the control cells, suggesting that downregulation of Puma inhibited the autophagy initiation but helped in maintaining the autophagic flux, as indicated by the reduced levels of p62, which is otherwise seen to be accumulated and upregulated under the influence of Aβ. Since Puma is directly activated by a transcription factor FoxO3a and FoxO3a is known to play a major role in autophagy induction in cardiomyocytes for normal cardiac functioning, we checked if this transcription factor plays anyrole in autophagy initiation and maintaining the autophagy. Performing an immunocytochemistry analysis in FoxO3a downregulated PC12 cells, we found a significant decrease in autophagic initiation and p62 accumulation as compared to the control cells under Aβ toxicity. We also determined these protein levels by western blotting and found a decrease in the levels of LC3B and p62 in both shPuma and shFoxO3a transfected PC12 cells as compared to control transfected cells under Aβ treatment, indicating a dual role of both Puma and FoxO3a in the regulation of autophagy and apoptosis.

Autophagy and Apoptosis is induced simultaneously in brains of transgenic AD mice

To validate our *in vitro* studies on the occurrence of autophagy and apoptosis under Aβ toxicity, we first checked the autophagy levels *in vivo* in 12 months APP/PS1 transgenic mice brain and found an increase in the levels of both LC3 and p62 as compared to the wild type control mice brain. Next, we checked for the advent of autophagy and apoptosis in 6 months old 5xFAD mice brain as compared to the age-matched wild type mice brain. Our immunohistochemistry studies show that there is an increase in the expression of autophagy marker proteins LC3 and Lamp1, in the 5xFAD mice brain compared to wild type. Also, we found an increase in the staining of Pumain 5xFAD brain as compared to wild type brains. Further, protein levels of autophagy markers were checked by western blot and we found significantly elevated levels of both LC3B and Lamp1 in 6 months old 5xFAD mice brain tissue lysates from cortex and hippocampus as compared to the age-matched wild type mice cortex and hippocampus lysates respectively.In order to check if both the processes, viz, autophagy and apoptosis occurred simultaneously in the same cells, we performed the immunohistochemistry and TUNEL assays together in the same brain sections of 5xFAD mice and in corresponding wild type sections. We found an increase in the levels of LC3 and TUNEL positive cells in 5xFAD mice as compared to the wild type set. Interestingly we also observed that many cells underwent both autophagy and apoptosis simultaneously in 5xFAD. We thereby confirm that both autophagy and apoptosis simultaneously occur in brain of AD mice. The level of crosstalk between both these processes may finally determine the survival or death of neurons.

Down regulation of Puma and Beclin1 inhibit both autophagy and apoptosis *in vivo*

Since both autophagy and apoptosis are triggered simultaneously in both cellular and animal models of AD, we next checked the role of two key BH3-only proteins, Beclin1 (essential for autophagosome formation) and Puma (a pro-apoptotic protein) in cell death in an *in vivo* model. We created an animal model of AD by injecting oligomeric Aβ in the cortex of rat brains by stereotaxy. Puma and Beclin1were downregulated in Aβ-infused rats by injecting specific shRNAs against them, exclusively. We infused reverse Aβ peptide (rAβ) in rat brains, to create negative controls and we infused PBS in the rat brains for control, in similar locations. We took the tissue lysates of the cortex and hippocampus of the brains of these rats and performed western blotting where we found a significant reduction in the levels of autophagy proteins LC3B and p62 in the cortex (Fig 7A-D) and hippocampus (Fig 7E-H), in both Puma or Beclin1 knockdown rat brains as compared to the Aβ-infused rat brains. We also found a decrease in the cleaved PARP levels in the brains of rats where we downregulated Puma or Beclin1 as compared to that of the Aβ-infused rat brains (Fig 7A-B, 7E-F), suggesting that both Puma and Beclin1 play an essential role in the regulation of both autophagy and apoptosis, in determining the fate of neurons in AD.

Autophagic BH3-only protein Beclin1 interacts with pro-apoptotic BH3-only protein Puma

Structural studies indicate that Beclin1 is a BH3-only protein with a BH3 binding motif through which it

interacts with Bcl-2/Bcl-xL. Reports have prompted towards a close interaction between BH3-only proteins Bim and Beclin1 which holds the essence of regulation of autophagy in starved conditions. Taking cue from such reports, we investigated whether Puma, due to the presence of BH3 motif, interacted with Beclin1at any level in our model. Since we found that downregulation of Pumaor Beclin1 leads to a substantial amount of inhibition of autophagy as well as apoptosis, it was imperative to have checked whether these proteins show any kind of interaction between them or not. We performed immunoprecipitation studies on tissue lysates of cortical neurons. Our study suggested a potential interaction between Puma and Beclin1. Furthermore, we see an increase in the interaction of the two BH3-only proteins under Aβ treated conditions as compared to the control (Fig 1A-D). Collectively, these findings hint towards the fact that pro-apoptotic protein Puma regulates autophagy by its close association with Beclin1 which is an important autophagy protein (Fig 1E). Downregulation of any of these proteins blocks initiation of autophagy and accumulation of cargo leading to survival of neurons.

Thus, our study reveals a direct interaction between Puma and Beclin1 upon Aβ treatment. However, final outcome differs as Bim inhibits autophagy, whereas Puma induces autophagy but results in an impaired autophagic flux which causes neuron death. Further investigation is required to reveal this disparity between two similar molecules and to understand how Puma leads to impaired autophagic flux in AD.

Future Research Plans

- To study the mechanisms of glia origin cytokines that promotes neuronal survival and ameliorates cognititive behaviours in animal models of Alzheimer's disease.
- Studying glia subtypes, their secretory profiles and role in disease pathogenesis.
- Determing the role of autophagy regulators (small molecule or siRNA) in neurodegeneration and amelioration of behaviour in AD mice.
- Role of miRNA in disease pathogenesis and its implication in diagnosis and therapy.
- Detection of disease specific signatures for Alzheimer's disease in clinical samples.

Publications

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Extramural Funding

Alzheimer's disease: is age-related subtle DNA damage the trigger of the sporadic Alzheimer's disease? 2019 – 2022. (DST, India)

Conferences / Workshops

- Number of Abstracts in National/International Conference: 3
- Number of Abstracts in International (Overseas) Conference: 2

Conferences/Events Organized by CSIR-IICB

- NeuroUpdate; Nov 27-28, 2021
- 39th Annual meeting of Indian Academy of Neurosciences; Dec 16-19, Virtual

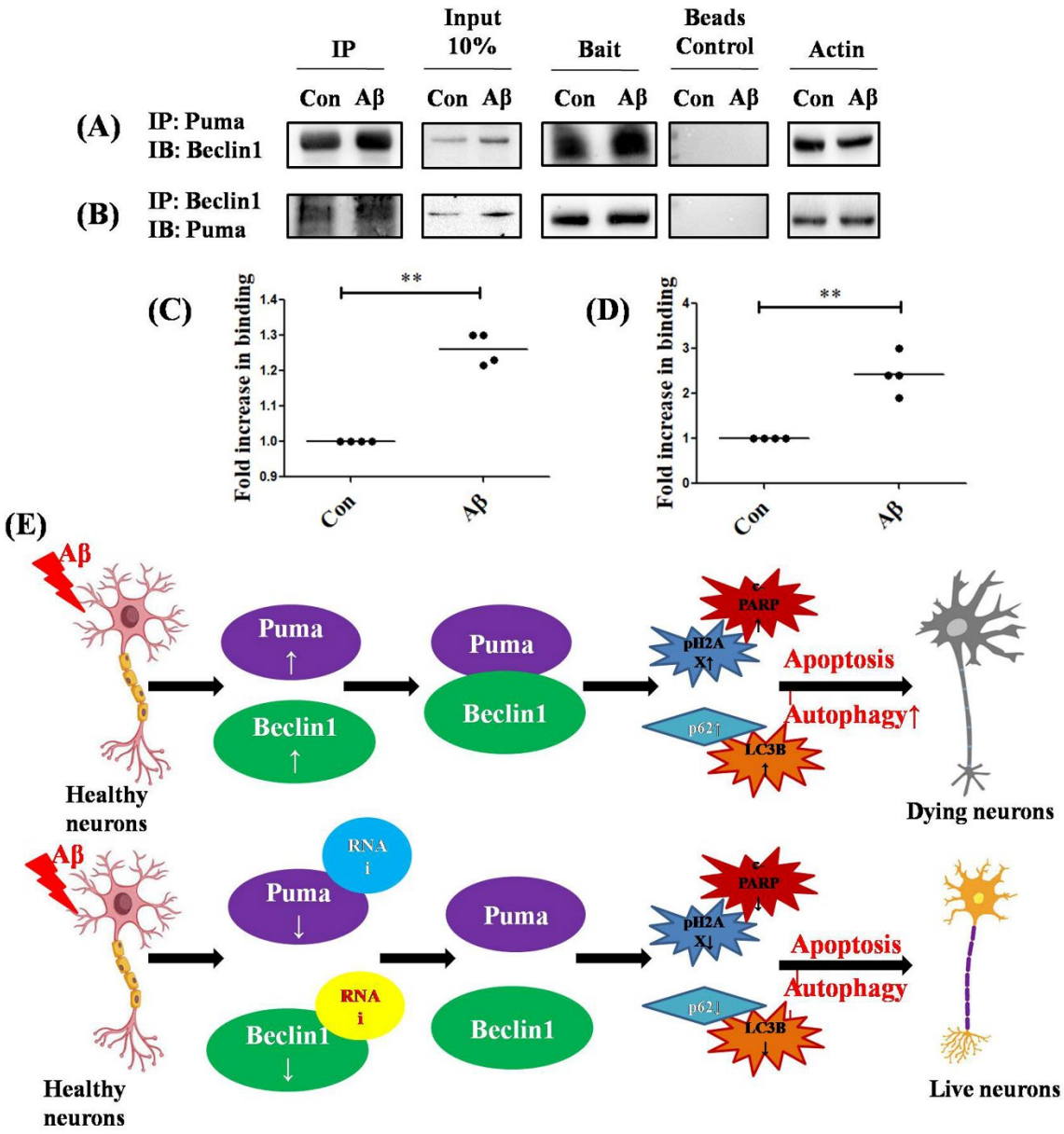

Figure 1: Autophagic BH3-only protein Beclin1 interacts with pro-apoptotic BH3-only protein Puma in cortical neurons. (A) Primary rat cortical neurons were cultured and treated with and without 1.5μM Aβ for overnight and the cell lysates were immunoprecipitated (IP) with Puma antibody and immunoblotted (IB) for Beclin1. (B) Reverse immunoprecipitation was also performed to check interaction of Beclin1 with Puma; cell lysates were immunoprecipitated with Beclin1 antibody and immunoblotted for Puma. The inputs and baits for the respective immunoprecipitation studies are shown in the second and the third column of (A) and (B). The beads controls are shown in the fourth column and the loading controls (Actin) for each immunoprecipitation study are represented in the fifth column of (A) and (B). (C) and (D) Graphical representation of the fold change in the interaction between Beclin1 and Puma under Aβ treatment as compared to control. Data represented were collected from 4 independent experiments. The asterisks denote statistically significant differences between corresponding conditions; **p<0.001. (E) Schematic representation depicting the role of BH3-only proteins Puma and Beclin1 in regulation of autophagic death in neurons exposed to Aβ.

Unraveling the role of Ubiquitin Proteasome System in Nonalcoholic fatty liver disease (NAFLD)

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Background

Ubiquitination is a post translational modification wherein ubiquitin is added to a target protein. The process is mediated in three consecutive steps by three different enzymes. The first step consists of ATP-dependent activation of ubiquitin by E1-ubiquitin activating enzyme followed by transfer of ubiquitin from E1 to the active site cysteine of E2 via trans thioesterification reaction. The final step is carried out by E3 ubiquitin ligases which catalyses the formation of an isopeptide bond between lysine of the target protein and C-terminal glycine of ubiquitin. Multiple ubiquitin residues can thereafter be added to the first residue yielding a polyubiquitin chain. Once a protein is ubiquitinated, it is usually targeted for degradation by the 26S proteasome.

Regulation of ubiquitylation of proteins is achieved by deubiquitinases or deubiquitinating enzymes (DUBs) which de-conjugate ubiquitin from ubiquitylated substrates to regulate their activity and stability, thereby maintaining homeostasis. Humans harbor roughly around 100 DUBs and they are divided into five different families, four of which (UCH, USP, OTU, MJD) are cysteine proteases and the fifth family (JAMM) constitutes zinc-dependent metalloproteases. The role of DUBs in NAFLD remains largely unexplored. Recently, CYLD (Yan-Xiao Ji, Nature Medicine 2018), TNFAIP3 (Peng Zhang, Nature Medicine 2017) have come in the forefront as potential players in NASH.

Based on our preliminary data, we intend to investigate the roles of DUB and ubiquitin-proteasome pathway in the pathogensis of NAFLD.

Ongoing research questions

- Implications of ubiquitin proteasomal system (UPS) and autophagy triad in the pathogenesis of NAFLD.
- Identification of specific deubiquitinase in the pathology of NAFLD
- Therapeutic and intervention for NAFLD - Identifying novel small molecules and agents for already identified pathways in fatty liver disease including E3 ubiquitin ligase COP1.

Summary of progress made

- We have identified a COP1 as the bona fide E3 ligase for the rate-liming lipolytic enzyme ATGL in liver and their interaction modulates fat content in liver. Further, we have shown that a hepatokine PEDF helps transport of ATGL into nucleus where COP1 mediated degradation of ATGL. We in collaboration with Dr Arindam Talukdar have developed a series of small molecules that targets this pathway and helps in reduction of lipid in hepatocytes (IP protection done).
- We uncovered proteasomal dysfunction as one key pathological feature in diet-induced murine model and human NASH patients and ASK1 and PPARγ serve as molecular complementation in proteasome-mediated liver injury. Moreover, we established a pharmacologically synergistic drug regimen of ASK1 inhibitor and PPARγ agonist that ameliorated liver pathology in these two different preclinical settings.
- We have identified a small group of deubiquitinases (DUB) that could modulate hepatic steatosis and liver injury by specific targeting of cognate substrate.

Future directions

- New knowledge generation through deciphering novel molecular and cellular mechanisms of NAFLD pathogenesis including proteotoxicity

due to dysfunctional ubiquitin proteasomal system
- (UPS)-mitochondrial dysfunction-autophagy triad.
- Identify lead compounds targeting ubiquitin proteasomal pathway, mTOR pathway and dysfunctional mitochondria for further development based on pharmacokinetics, proof of concept, efficacy and toxicity profile.

Selected relevant publications

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To explore the molecular mechanisms of adaptive responses of airway epithelial cells against various exogenous and/or endogenous insults, for a fuller understanding of respiratory diseases

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Background

Our lab is focusing on challenging lung diseases like steroid resistant asthma, COPD and lung fibrosis. We found crucial molecular clues towards understanding of these diseases. Using these clues we are in the process of translational research.

Ongoing research questions

- To understand the basis of steroid resistance in asthma and chronic obstructive pulmonary disease.
- To find the novel targets in COPD
- To understand the basis of idiopathic lung fibrosis.

Summary of progress made

- Our lab had earlier demonstrated the dysfunctional mitochondria in airway epithelium of the asthmatic mice lungs. We show now that Ku70 modulation by the administration of Ku70 plasmid attenuates asthma features and reduces mitochondrial dysfunction in the lungs of allergen exposed mice. Ku70 is a key DNA repair protein with diverse roles including VDJ recombination, telomere maintenance, and maintenance of cell homeostasis. Recently, we found a reduction in Ku70 expression in asthmatic airway epithelium, and this was associated with mitochondrial dysfunction in asthmatic condition. We have shown that Ku70 over-expression in asthmatic mice attenuated airway hyperresponsiveness, airway inflammation, sub-epithelial fibrosis along with reduction in TGF-β with no effect in IL-13 levels and goblet cell metaplasia. Ku70 over-expression in asthmatic mice reduced 8-isoprostane, a marker of oxidative stress, and restored the mitochondrial function in asthmatic mice. We further found these roles of Ku70 to be independent of DNA damage as Ku70 overexpressed mice did not show any reduction in DNA tail, an index of DNA damage. Thus, our findings indicate that Ku70 can attenuate crucial features of asthma along with the restoration of mitochondrial function. This implies that Ku70 could be a therapeutic target for asthma without affecting DNA repair function.
- Our lab found that DHA, RXR ligand, helped in improving steroid sensitivity in five steroid insensitive asthmatic patients. Demonstrated the beneficial role of retinoid X receptor (RXR) in steroid resistant asthma.
- Found the moonlighting role of Rad50 in maintaining airway epithelial barrier using singlecell transcriptomics strategy followed in depth investigations.
- Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease with a very poor prognosis as it has a 2.5 to 5 years mean survival after proper diagnosis. Even nintedanib and pirfenidone cannot halt the progression, though they slow the progression of IPF. Hence, there is a need to understand the novel pathophysiology. Phospholipase A2 (PLA2) could be the ideal candidate to study in IPF, as they have a role in both inflammation and fibrosis. We have shown the expression profile of various secretory

Phospholipase A2 (PLA2) isoforms by analyzing publicly available transcriptome data of single cells from the lungs of healthy individuals and IPF patients. Among 11 members of sPLA2, PLA2G2A is found to be increased in the fibroblasts and mesothelial cells while PLA2G5 is found to be increased in the fibroblasts of IPF patients. We identified a subset of fibroblasts expressing high PLA2G2A with moderate expression of PLA2G5 and which are specific to IPF only; we named it as PLA2G2A+ IPF fibroblast. Pathway analysis revealed that these PLA2G2A+ IPF fibroblast have upregulation of both inflammatory and fibrosis-related pathways like the TGF-β signaling pathway, IL-17 signaling, the arachidonic acid metabolism pathway and ECM-receptor interaction. In addition to this, we found elevated levels of sPLA2-IIA in plasma samples of IPF patients in our cohort. PLA2G3, PLA2G10 and PLA2G12B are found in to be increased in certain epithelial cells of IPF patients. Thus, these findings indicate that these five isoforms have a disease-dominant role along with innate immune roles as these isoforms are found predominantly in structural cells of IPF patients. Further, we have targeted sPLA2 in mice model of bleomycin-induced lung fibrosis by pBPB, a known sPLA2 inhibitor. pBPB treatment attenuated lung fibrosis induced by bleomycin along with a reduction in TGF-β and deposition of extracellular matrix in lung. Thus, these findings indicate that these sPLA2 isoforms especially PLA2G2A may serve as a therapeutic target in lung fibrosis. This work has been communicated to AJP-Lung journal.

- Found the protective role of Smar-1 (a nuclear matrix associated protein) in steroid resistant asthma. We also found a reduction in the expression of Smar-1 (a nuclear matrix associated protein) in human COPD lungs and smoke exposed mice emphysematous lungs. We are in the process of understanding the possible mechanims underlying the beneficial protective effects of Smar-1 in COPD conditions.
- Our lab had found that beneficial role of retinoid X receptor (RXR) in COPD. We are in the process of determining the effects of RXR ligand, DHA for anti-COPD effects.

Future directions

- We want to determine how RXRgamma is involved in COPD pathogenesis.
- We want to determine how SMAR-1 is involved in COPD pathogenesis.
- We want to prepare the formulation of Standardised *Sonneratia apetala with Dr. Jaisankar and Dr, Arun Bandyopadhyay*
- We want to prepare the water soluble form novel benzoxazinone derivative, PD-05, potent human neutrophil elastase inhibitor to test its activity in neutrophil elastase dominant lung diseses (*Dr. Jaisankar and Dr, Arun bandyopadhyay)*
- Using the clues obtained from our lab, we will be heading towards identification of epithelial protective agents for incorporation in existing therapeutic regimens in asthma and COPD.

Publications

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Characterization mitochondrial form-function relationship during neurodegeneration

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Background

Mitochondria play the central role in neuronal maintenance and survival. Biogenesis and degradation of mitochondria are precisely balanced during aging and any instability results in cell death. Changes in mitochondrial function during this imbalance are reflected on its morphological features and thus affect the clearance as well as strategic positioning for optimal functioning. Hindrances in mitochondrial clearance could be due to the deviations in protein population on outer mitochondria surface. The precise research areas of our laboratory are:

- Pathways leading to mitochondrial autophagy (mitophagy) and their cross talk during aging and neurodegeneration (especially in Parkinson's disease and Huntington's disease).
- Role of mitochondrial outer membrane protein complexes to segregate and tag mitochondria for clearance.
- Pharmacological and genetic modulation of mitophagy: therapeutic aspects in neurodegenerative diseases.

Research questions

Our group studies the relevance of maintaining healthy mitochondrial population for the neuronal survival from three different perspectives:

- We are deciphering the basic mechanisms of sorting mitochondria for efficient degradation after depolarization.
- We are currently addressing the effect of available neurotransmitter supplementation therapy on mitochondrial health.
- In collaboration, we are developing supportive molecules which can synergistically improve the efficacy of currently available therapies against neurodegenerative disorder.

Research Progress

- We found that substrate proteins of Parkin and Prohibitin 2 (PHB2) oligomerization levels accompany mitochondrial depolarization.
- We discovered that though mitophagy receptor PHB2 does not change with aging or PD related neurotoxic insults in different parts of rat brain, proteasome inhibitory deubiquitinase USP14 increases with age or neurotoxic insults.
- It was found that Monoamine oxidase mediated degradation of its substrates can lead to apoptotic mitochondrial fragmentation and thus magnify the progressive nature of age-related neurodegeneration.
- We have screened around 100 compounds, isolated from natural resources and identified the ones which are potent Monoamine oxidase inhibitor. The screening, characterization and in vivo validation is a continuous on-going process. We have identified one formulation which has huge therapeutic implication. Patent has been filed for composition of the formulation (Indian patent file no: 202311006898, date: 02-02-2023).

Future Research Plans

- Identifications of core components of PHB2-Parkin substrate protein and characterization of the interaction during aging and progression of familial Parkinson's disease.
- We will validate whether inhibition of Monoamine oxidase mediated apoptotic mitochondrial fragmentation can offer

therapeutic implication.

- Development of phytopharmaceutical formulations to regulate Monoamine oxidase activity in vivo.

Extramural funding

- Department of Biotechnology (DBT), Government of India. Project title: “Impact of Voltage-dependent anion-selective channel 1 (VDAC1) protein on mitochondrial shaping and dynamics”.
- Science and Engineering Research Board (SERB)-DST, Government of India. Project title: “Mitochondrial clearance in aging brain: therapeutic approach against differential neurodegeneration in Parkinson’s disease.”
- Indian Council of Medical Research (ICMR), Government of India. Project title: “Managing mitochondria from dopamine: halting Parkinson's disease progression”.

Publications

- Myricitrin – a flavonoid isolated from Indian olive tree (Elaeocarpus floribundus) inhibits Monoamine oxidase in brain and elevates striatal dopamine levels: therapeutic implications against Parkinson’s disease. Banerjee C, Nandy S, Chakraborty J*, Kumar D*. Food & Function journal - The Royal Society of Chemistry. 2022. (*co-corresponding author). Doi: 10.1039/D2FO00734G

Role of endogenous neuronal progenitors in neurogenesis after acute neuronal loss and electrochemical detection of Alzheimer's disease biomarker, β-secretase enzyme (BACE1), with one-step synthesized reduced graphene oxide

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Research Activities

A) Role of endogenous neuronal progenitors in neurogenesis after acute neuronal loss

Kainic Acid (KA) causes hippocampal neuron cell death: We have examined neuronal cell death in the KA 30 mg/kg treated mice using TUNEL and immunofluorescence staining. In the KA-treated mice, TUNEL positive cells were observed in the CA1 and CA3 region of the hippocampus, 3 days after KA treatment. TUNEL positive cells were also seen in the CA1 and CA3 region of the hippocampus 5 days after KA treatment. No TUNEL-positive cells were seen in the hippocampus of control mice.

Increased proliferation following KA induced cell death: In order to check the effect of KA on adult neurogenesis, we have injected KA 25-30 mg/kg in P28-P35 mice to induce neurogenesis and behavioral seizure score was observed (Racine et al. 1972). New-born neurons were marked by acute intraperitoneal injection of BrdU. We have observed an approximate increase in neurogenesis marked by BrdU+ cells in SGZ of hippocampus. Quantitative analysis of BrdU+ cell in the SGZ of dentate gyrus of the hippocampus was performed. Every 10th section of hippocampus was analyzed from rostral to caudal. Quantification data confirmed that BrdU+ cells were increase four fold in KA treated mice in comparison to control animals.

Increased neurogenesis and migration of newly born cells to the site of cell death: To further confirm if Brdu+ cells are increased and they also migrate to the injury site, we have injected KA, allowed the degeneration to happens for three days followed by BrdU for injections for seven consecutive days to follow the migration of newly generated neuronal progenitors. We have found that BrdU+ neuronal progenitors are increased at the DG and there are significantly higher numbers of Brdu+ newborn cells to the CA1 and CA3 region of the hippocampus which was prone to KA induced injury/cell death. This highlights the increased neurogenesis at DG regions of the hippocampus and their migration to the site of injury that may be further differentiated to integrate into the circuitry.

Increased adult neural stem cells and their migration to the site of cell death: To further understand if newly generated Brdu+ neuronal progenitors also express the neural stem cell markers, we have done double immunofluorescence of Brdu and Sox2. We have found that Brdu+Sox2+ newly born neuronal progenitors are increased in the DG region of the hippocampus. We have also confirmed that there are significantly higher numbers of Brdu+Sox2+ newly born progenitors in the DG and they are migrated to the site of injury that is CA3 and CA1 region of the hippocampus.

Increased adult neural stem cells and their migration to the site of cell death:
To further understand if newly generated Brdu+ neuronal progenitors also express the neuronal markers, we have done double immunofluorescence of Brdu and NeuN. Our recent experiments suggest that these higher number of stem progenitors at injury site correlated with higher number of Brdu+NeuN+ at injurt site suggesting the new born neuron.

Future Plans

- **Whether basal progenitors get activated and generate new neuron following KA inducedneurodegeneration:** For this purpose, we will characterize the proliferation, mitotic

activity, and differentiation status of progenitor cells and neuroblasts localized in neurogenic regions in SVZ, SGZ and pPV; at 7, 14, 28 and 42 days after KA induced cell death by labeling the new neurons with BrdU.

- **Whether newly generated neuroblasts get recruited at damaged site after KA induced neurodegeneration and participate in the brain circuitry:** Integration of regenerated neurons into the existing neural circuitry is a prerequisite for restoration of brain functions so in the last phase, we will examine the maturation and connectivity of regenerated neurons. To test the hypothesis that newly generated neuron actually participate in the brain circuitry, we will perform triple staining with BrdU, MAP2, and synaptophysin to suggest that newly generated neurons received synaptic inputs. In addition BrdU, Tbr1, and synaptophysin triple immunofluorescence will also be performed to proof that new cells integrate into the brain circuitry

B) Electrochemical Detection of Alzheimer's Disease Biomarker, β-Secretase Enzyme (BACE1), With One-Step Synthesized Reduced Graphene Oxide

The schematic and working principle of the fabricated electrode proposed in this research work is based on electrochemical detection of current variation upon interaction of antibody and antigen, as shown in Figure 1.

Synthesis, Conjugation, and Characterization of rGO (reduced graphene oxide) and BACE1 Ab Along with Ag Capture. The morphological studies of the fabrication and working of the electrode were analyzed using SEM. In the corresponding scanning electron micrographs, bare rGO appeared as flakes (Figure 2Di), immobilized BACE1 Ab was observed as white globular structures on the rGO surface (Figure 2Dii), and BACE1 Ag layer captured by the specific Ab was observed.

Characterization of Optimized FTO/rGO/BACE1Ab Sensor- The electrode performance was checked within a range of rate of scan from 0.01 to 0.1 V/s (Figure 3C) and reduced current output was seen with reduction in the rate of scan, which was also depicted by a linear regression of peak current vs. scan rate (Figure 3D). As the peak current decreased with scan rate, 0.1 V/s was optimized as the most efficient rate of scan for electrode testing.

Analytical Output of Modified FTO/rGO/BACE1Ab Sensor for BACE1 Ag Detection- differential pulse voltammograms of the electrodes tested with increasing concentration of BACE1 Ag in buffer samples from 1 fM to 1 µM. The peak current of the electrodes was observed to decrease as a function of BACE1 Ag concentration. he LOD in the case of buffer samples was calculated to be 0.64 fM (Figure 5Aii) and 1 fM (Figure 5Bii) in spiked serum. This slight decrease in sensitivity in serum samples is due to the matrix effect caused by other components such as different proteins present in serum, in addition to the target antigen.

Extramural funding

- "Functional dissection of Jmjd3 during embryonic and adult hippocampal neurogenesis" Funding agency: SERB (2021-2024) Grant amount. 44.22 lakhs (PI)
- "Anticancer Potential and Molecular Mechanism of Rasa sindhoor and Guggulu tiktaka kashaya on ovarian cancer cells" AYUSH (2022), Amount: 34 Lakhs

List of Publications (journal and conference)

- Dey J, Roberts A, Mahari S, Gandhi S, **Tripathi PP**. Electrochemical Detection of Alzheimer's Disease Biomarker, β-Secretase Enzyme (BACE1), With One-Step Synthesized Reduced Graphene Oxide Front Bioeng Biotechnol. 2022 Mar 24;10:873811. doi: 10.3389/fbioe.2022.873811
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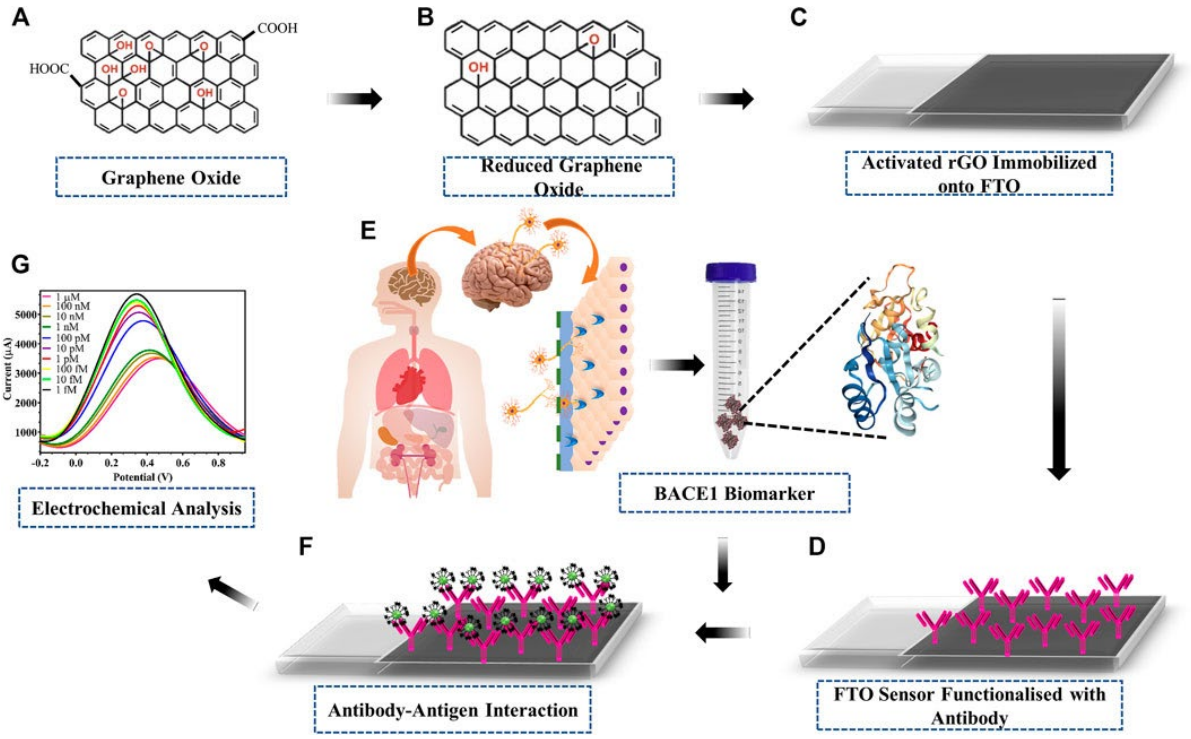

Figure 1: Schematic of fabrication and working of developed electrode: (A) GO; (B) one-step synthesised rGO; (C) activation of rGO via EDC-NHS carbodiimide coupling chemistry coated on the surface of FTO; (D) immobilization of BACE1 Ab onto activated rGO; (E) Alzheimer's disease BACE1 biomarker protein sample; (F) capture of target BACE1 Ag upon interaction with immobilized BACE1 Ab; (G) electrochemical detection of the current output upon Ab–Ag interaction.

Infectious Diseases and Immunology Division

Faculties, fellows and technical staffs of Infectious Diseases and Immunology Division of CSIR-IICB are involved in various research works including basic science, application science related to the prevention and control of cholera, leishmaniasis, malaria, gastric ulcer and viral diseases like dengue, chikungunya, herpes and hepatitis B. Work is also being carried out on endometriosis, a disease of reproductive womens. The other interest lies on wound healing pharmacophores (nucleoside analogues and drug loaded nanoparticles) to be used as potential radiopharmaceuticals for scintigraphic diagnosis and therapy.

Role of Matrix Metalloproteases (MMPs) in Disease Prognosis

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Background

We worked on the pathogenesis of different diseases including gastric ulcer, gastric cancer, ovarian cancer, endometriosis and neurodegenerative disorders. The main focus of my laboratory is to understand the role of matrix metalloproteinases (MMPs), a group of zinc containing calcium dependent endopeptidases, which are involved in the degradation of different extra-cellular matrix proteins during disease progression. Previous studies from my laboratory have identified altered expression of few MMPs in gastric ulcer. MMP-9 in particular, was found to be very important protein as it is involvement in ECM degradation and development of ulcer as well as neurodegeneration.

Our findings suggest that targeting MMP-9 activity can offer a new therapeutic avenue for multiregulated pathways of gastric ulcers. From the ancient ages it was known that medicinal plants are rich sources of diverse small molecules with various therapeutic properties. One such medicinal plant is Neem which is well-known source of several bioactive compounds that used extensively in traditional medicine in the Indian subcontinent. The investigation on gastroprotective and therapeutic potential of the GQDs (molecule synthesized from neem bark extract) found to be very promising.

Another compound rotenone has widespread beneficial effects in agriculture, fisheries and animal husbandries. Although prolonged exposure causes a detrimental health hazard. Rotenone during its extraction, formulation or usage may cross the blood brain barrier leading to neurodegeneration and the development of Parkinson's disease like symptoms. It is a known inhibitor of the mitochondrial ETC complex I. The studies executed in my laboratory showed that rotenone exposure results in inflammation and programmed necroptotic death.

Aims and Objectives

- To investigate the molecular process that may be involved in rotenone toxicity induced degradation of neuronal cells that mimics PD pathogenesis.
- To explore the possibility of using GQDs derived from neem bark for antioxidant therapy.

Work achieved

Rotenone alters cell viability, intracellular ROS production, ATP level and mitochondrial membrane potential along with the activity and expression of MMP-9 and TIMP-1

Mouse Neuroblastoma (N2A) cells were grown in DMEM medium (Gibco, Invitrogen, USA) containing 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin (Gibco, Invitrogen, USA) in a humidified settings having 5% CO_2 and 95% air at 37°C. Cell survival was assessed thereafter using CellTiter-Glo reagent solution. Luminescent signal thus generated was measured in a microplate luminometer (Glomax, 20/20 Luminometer, USA). Control and rotenone treated cells were next processed for live cell imaging using Mitosox Red (5 µM) for 30 min in Krebs–Ringer's buffer (130 mM NaCl, 5 mM KCl, 10 mM glucose, 2 mM MgCl2, 1 mM CaCl2, 20 mM HEPES) maintaining dark condition. The dye laden cells were viewed under a high resolution Stimulated Emission Depletion Microscopy (STED) (Leica TCS SP8). To observe the changes in mitochondrial transmembrane potential upon rotenone exposure we used JC-1 dye. Mitochondrial membrane potential within intact cells was calculated as the ratio of fluorescence intensities at 590 nm and 535 nm. Further, the intracellular ATP level was evaluated in non-treated and rotenone exposed N2A cells using a commercial

kit relied on luciferin–luciferase reaction. The luminescence thus generated was monitored immediately in a microplate luminometer (Glomax, 20/20 Luminometer, USA) according to the manufacturer's protocol. An ATP-standard curve of known concentration was used to calculate intracellular ATP content and the values were represented as nmol of ATP/mg of protein. To monitor the gelatinase activity in control and rotenone treated N2A cells we performed gelatin zymography. The gelatinolytic activity has shown as negative staining. The band intensities were quantified by densitometry using ImageJ software. The activity of TIMP-1, at the same time was analysed by reverse zymography using MDA-MB-231 cells as MMP source. The regions of TIMP-1 activity have shown as coloured bands and the band intensities were quantified densitometrically by ImageJ software. SDS-PAGE was then performed using 50 µg of total protein followed by immunoblotting to evaluate the protein expression patterns.

We observed a significant loss of cell viability upon rotenone exposure. Nearly 50% cells died after 24 hours of 100 µM rotenone exposure (**Fig. 1A**). Again, while performing live cell imaging probing with mitochondrial ROS specific dye (MitoSOX Red) we observed high fluorescence intensity in the rotenone exposed cells under confocal microscope (**Fig. 1B**). The calculated amount of intracellular ATP in rotenone exposed cells is far less than the control cells. We observed ~6 fold decrease in in ATP content of rotenone exposed cells compared to that of control cells (**Fig. 1C**).

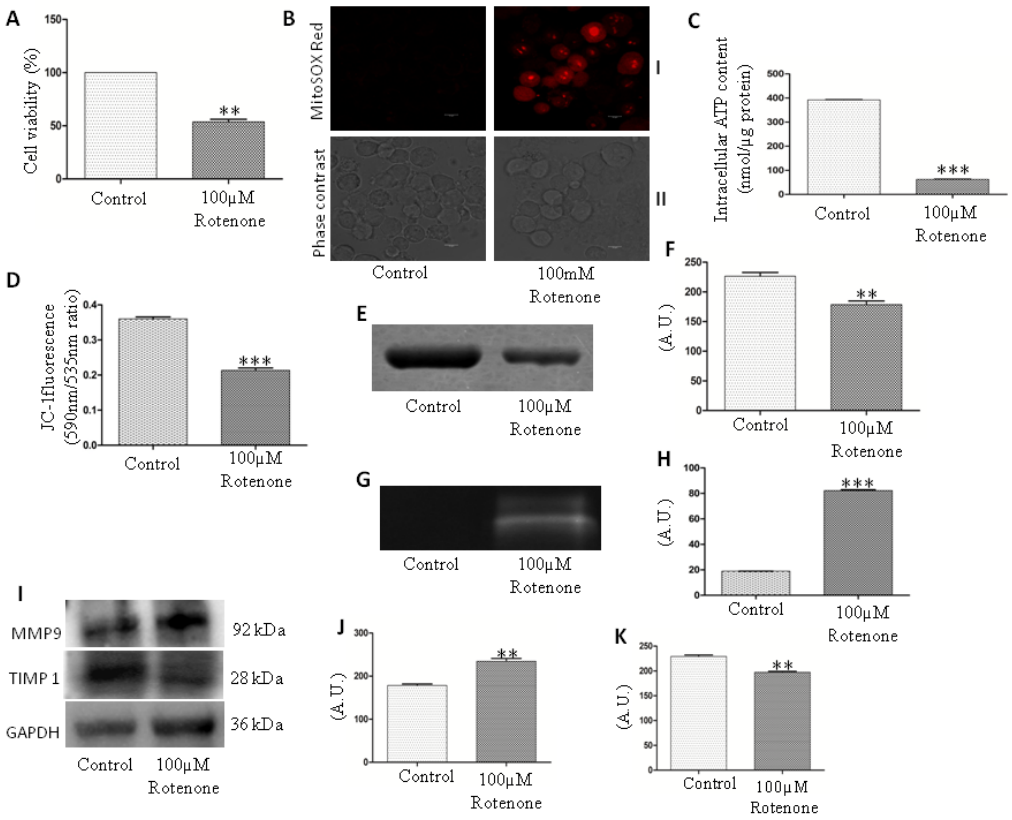

Figure 1: Effects of rotenone exposure on N2A cell **(A)** Effect of Rotenone on cell viability. N2A cells were treated without (control) or with rotenone (100 µM) for 24 hours. At the end of the incubation period, the degree of viability was assayed by CytoTox-Glo cytotoxicity assay (**P<0.005 vs control) **(B)** ROS generation in N2A cells after rotenone exposure was monitored using MitoSOX Red **(C)** Alteration in intracellular ATP content in N2A cells treated without (control) or with rotenone (100 µM) for 24 hrs. (***P<0.0005 vs control) **(D)** Alteration in Mitochondrial trans-membrane potential in intact N2A cells after Rotenone exposure was measured by JC-1 (***P<0.0005 vs control). **(E)** Reverse zymography showing the activity of TIMP-1 after rotenone exposure. **(F)** Densitometric analysis of TIMP-1 activity (**P<0.01 vs control). **(G)** Activity of MMP9 was observed by Gelatin zymography. (H) Densitometric analysis of MMP9 activity (***P<0.0001 vs control). **(I)** Western immunoblots for MMP9 and TIMP-1. (J) Densitometric analysis of MMP9 expression (**P<0.01 vs control). **(K)** Densitometric analysis of TIMP-1 expression (**P<0.01 vs control). Student's t-test, paired, two-tailed.

Another important observation was a striking loss of mitochondrial membrane potential, evident from a significant (~58%) decline in the ratio of JC-1 fluorescence emitted at 590nm and at 527nm respectively (**Fig. 1D**). Further, Rotenone exposure alters the activity and expression of MMP-9 and TIMP-1. We observed ~1.3 fold decrease in the activity of TIMP-1 in rotenone exposed cells in comparison to that of control cells (Fig. 1E & F); whereas, the expression of TIMP-1 after rotenone exposure decreased by ~1.2 times as observed by western immunoblotting (**Fig. 1I & K**). We also found that the active MMP-9 level increased by ~4.1 fold upon rotenone exposure (**Fig. 1G & H**), while the expression of MMP-9 increased by ~1.2 fold as compared to untreated cells (**Fig. 1I & J**).

Rotenone alters the expression patterns of various proteins, destabilizes cell cytoskeleton and induces necroptosis in N2A cells

N2A cells were treated with or without rotenone for 24 hours. Afterwards, cells were lysed and SDS-PAGE was performed followed by western immunoblotting. The changes in expression patterns were observed thereafter. The changes in expression of alpha-synuclein and the formation of aggregates were visualized by immunocytochemistry using Texas-red conjugated secondary antibody. FITC Annexin V apoptosis detection kit I was next used to analyze the cell death, following the manufacturer's instructions. The stained cells were then analysed by BD LSRFortessa™ Cell Analyzer-Flow Cytometers and data was processed using Flowing Software 2. Cytoskeleton degradation was studied using TRITC-Phalloidin stain and nucleus was stained following incubation with DAPI. The changes were observed by a high-resolution Stimulated Emission Depletion Microscopy (STED) (Leica TCS SP8).

We observed a marked increase in alpha-synuclein expression in rotenone treated cells. The expression of UCHL1, a deubiquitinating enzyme, diminished upon rotenone exposure. Furthermore, cofilin - the actin binding protein showed significant increase in its expression upon rotenone exposure (**Fig. 2A**). We next observed an upregulation of TNF-α., RIPK1 upon rotenone exposure; whereas, no detectable change in caspase 8 expression was observed during the study, in addition, caspase 3 expression increased upon rotenone exposure (**Fig. 2B**). the expression and aggregation were increased after rotenone exposure and the aggregated regions were marked with arrowheads (**Fig. 2C**). The results of flow cytometry revealed that rotenone has no effect on early apoptosis and has nominal effect on late apoptosis after 24 hours of exposure. Hence, it could be concluded that rotenone induced cell death was not dependent on apoptosis. (**Fig. 2D-G**). This observation along with the modification in the RIPK pathway confirmed the involvement of programmed necroptosis involves in this process. As rotenone alters the expression levels of cofilin, the G actin binding protein, we also investigated the organization of actin filaments upon rotenone exposure. The disruption and destabilization of the actin cytoskeleton of N2A cells were visualized and the changed structures have been shown by the arrowheads (**Fig. 2H**).

Graphene Quantum Dots (GQDs) protect the gastric mucosa against forced swim-stress-induced gastric ulcer in murine model

In this work, we examined the intracellular ROS scavenging and anti-inflammatory effects of Graphene Quantum Dots (GQDs), synthesized from neem bark extract, in a model of stress-induced gastric ulcer. The ulcer was induced in Balb/c male mice by stressor using forced swim stress for 4 h. Carbon dots at different doses (2 and 10 mg/kg b. w.) were administered orally 1 h prior to swim stress. Next, the mice were forced to swim inside a vertical cylinder (height 40 cm and diameter 20 cm) containing water up to about 20 cm height. The height of the water column is so in tune that the mice were not able to jump out of the cylinder. Following forced swim stress, animals were sacrificed after 4 h, and the stomachs of the mice were isolated for scoring the ulcer index.

An ulcer index of 70 was induced in the gastric tissues of mice by swim stress for 4 h. This time point was chosen for the rest of the experiments unless mentioned otherwise. Interestingly, in this murine model of gastric ulcer, GQDs drastically reduced swim-stress-induced gastric lesions dose dependently and showed nearly 95% inhibitions at 10 mg/kg (**Fig. 3A & B**). GQDs used at low (2 mg/kg b.w.) and high (10 mg/kg b.w.) doses were chosen for the rest of the experiments for the comparative analysis between low versus high dose. Microscopic examination revealed that forced swim stress caused extreme hemorrhagic wounds all through the epithelium, whereas those wounds were not found in GQDs-pre-treated group, particularly at 10 mg/kg b.w. (**Fig. 3A**). Moreover, the investigation from histology of tissue indicates that the stress causes shedding of the gastric epithelial cells, besides interruption of the epithelial-mucosal barrier (red bolt) of the stomach in contrast with that of control,

and the ulcer formation is prevented in tissues that are treated with varying dosages of GQDs (**Fig. 3A**; middle and lower panel). In addition, the results of the TUNEL assay (**Fig. 3C**) indicate that stress-induced gastric cell death while pre-treatment with GQDs completely reduces that damage at a dose of 10 mg/kg b.w. Dark brown dots (**Fig. 3C**) (black arrow) are used to indicate the end-labelling that is restricted inside the nucleus and increased DNA disintegration inside the nuclei of gastric cells after generation of forced swim stress.

GQDs exert gastroprotective effect by dampening inflammation and apoptosis as well as by down-regulating MMP-9 activity

Matrix metalloproteinase (MMP)-9 is well known for its inflammatory action in gastric tissues. Upon inhibition of MMP-9, pathogenesis is ameliorated; therefore, the tissues from GQDs-treated and untreated groups were processed for in vivo analysis of MMP-9 expression using immunofluorescence staining. Red fluorescence represents MMP-9, which is immune stained with anti-MMP-9 antibody and Texas Red conjugated secondary antibody (**Fig. 4A**). Blue fluorescence represents DAPI-stained nuclei and documented equal fluorescence in control, stress ulcerated, and GQDs-pre-treated stressed gastric tissue sections (**Fig. 4A**). The increased expression of MMP-9 is observed in the epithelial layer and glandular regions of stress ulcerated stomach compared with control. Both low (2 mg/kg b.w.) and high (10 mg/kg b.w.) doses of GQDs-pre-treated sections exhibit inhibited MMP-9 signals as evident from merging the images with corresponding DAPI-stained nuclei.

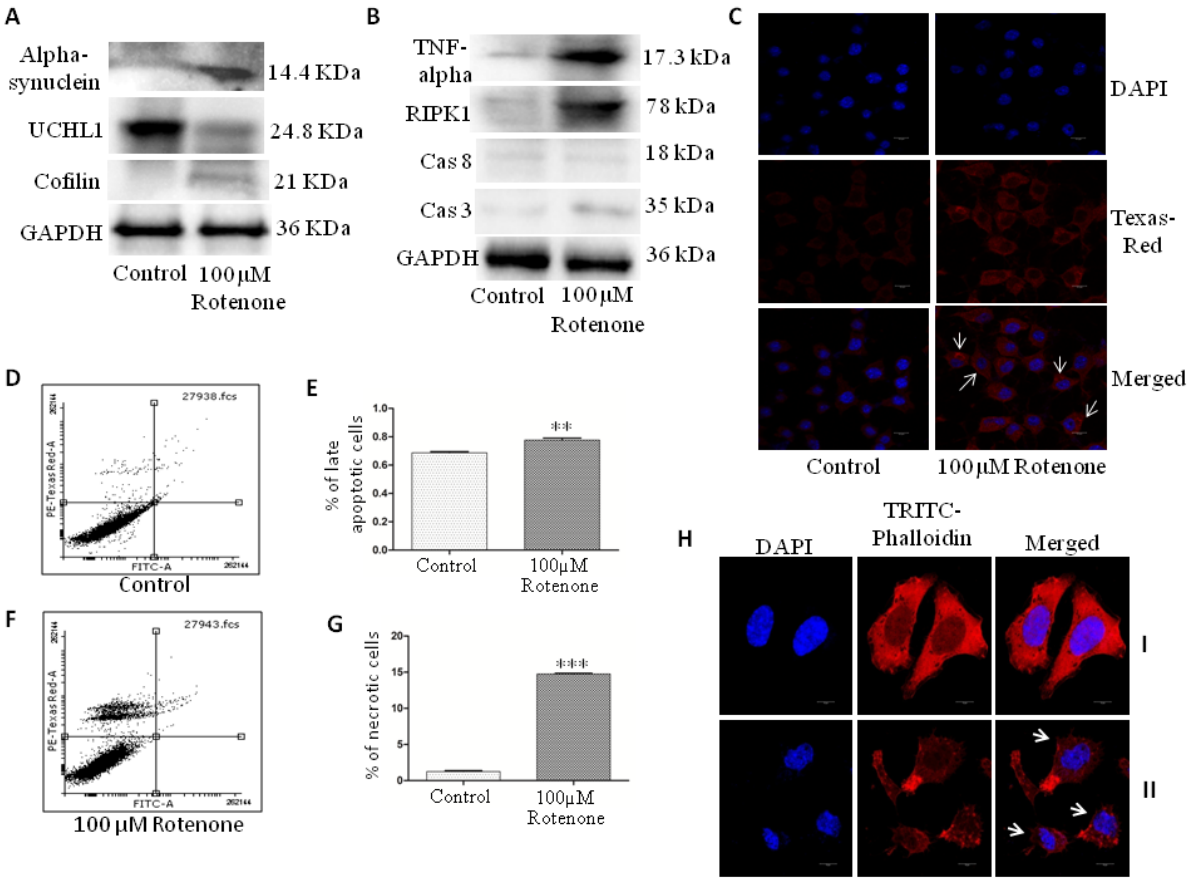

Figure 2: Effect of Rotenone on protein expressions and cell death mechanism in N2A cells **(A)** Expression patterns of proteins involved in PD pathogenesis by Western immunoblotting **(B)** Expression patterns of proteins involved in necroptosis by Western immunoblotting **(C)** Confocal microscopic images showing eexpression pattern of alpha-synuclein and its aggregate formation **(D, E)** Annexin V-FITC apoptosis detection method was used to evaluate apoptotic and necroptotic cells respectively. **(F)** Densitometric analysis of percentage of apoptotic cells (**P<0.01 vs control Student's t-test, paired, two-tailed). **(G)** Densitometric analysis of percentage of necroptotic cells (**P<0.01 vs control). Student's t-test, paired, two-tailed. **(H)** Degradation of actin cytoskeleton in cultured N2A cells by Rotenone. Condensed and fragmented nuclei and discontinuous and distorted staining for actin cytoskeleton are indicated by arrows.

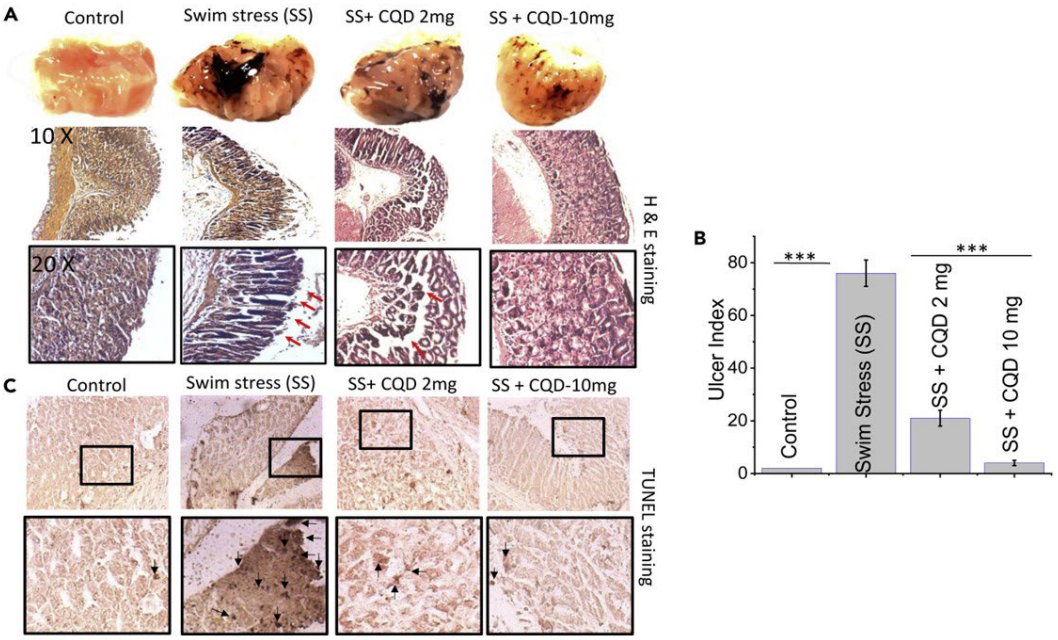

Figure 3: Protection by graphene quantum dots (GQDs) against forced swim-stress-induced gastric ulcer in mice. **(A)** Macroscopic observation of control, forced swim-stressed and GQDs-treated mouse stomach (upper panel). Histological representation of gastric tissue sections from different groups. The red arrow indicates disruption of the tissue layer of the stomachs from different groups (lower panel). **(B)** Ulcer indices for each stomach and plotted for GQDs-treated and untreated groups (data were indicated as the mean ± SEM). Evaluation between groups was executed using one-way analysis of variance (ANOVA) after that Student–Newman–Keuls t test. **(C)** TUNEL staining of gastric tissue sections from different groups. Black arrow indicates that the incidence of apoptosis was majorly concentrated in ulcerated sections.

Furthermore, in gelatin zymographic analysis, we found an increase in each pro and active MMP-9 activity in acute ulcerated stomach tissue extract, whereas GQDs treated at doses 2 and 10 mg/kg b.w. effectively reverse that upregulation during gastroprotection (**Fig. 4B**). The densitometric evaluation of zymographic bands (**Fig. 4C**) shows that stress increases the pro and active MMP-9 activity by 60% and 50%, respectively, in comparison with control, whereas GQDs prevent this activity and bring it almost near to control.

Furthermore, to delineate the function of GQDs in expressing secreted MMP-9, western blotting was performed using PBS extracts of control, ulcerated, and GQDs-treated tissues. Stress causes significant enhancement of MMP-9 expression in secreted form, whereas GQDs pre-treatment, even at a dose of 2 mg/kg b.w, significantly reduces MMP-9 secretion during the prevention of ulceration (**Fig. 4D & E**). In addition, the effect of GQDs in stress-responsive chaperone heat shock protein 70 (Hsp70) was also observed. Hsp70 expression was significantly reduced in ulcerated gastric tissue and was upregulated by GQDs (**Fig. 4D & E**). The HSPs preserve cellular functional stability caused by sublethal injury and are known to induce through oxyradical, thermal, and inflammatory stress. The chaperone-denatured intracellular proteins and signal transduction proteins modulate signaling events in repetitive stress. Herein, we hypothesize GQDs-induced overexpression of anti-apoptotic Bcl-2 along with a sustained increased level of Hsp70 is sufficient to clear gastric damage during gastroprotection as evident from **Fig. 4D & E**. However, excess and sustained accumulation of apoptotic BAX ultimately triggers BAX-dependent apoptosis and consequent caspase-3 expression (**Fig. 4D & E**). Caspase-3 is one of the main initiator caspases and is linked to the mitochondrial death pathway, whereas Caspae-3 is an effector caspase. Oxidative stress damages mitochondria, which in turn results in the associated functional failure and programmed cell death. Cumulatively, GQDs significantly prevent inflammation and subsequent apoptosis in the gastric milieu even after stress by inhibiting the expression of MMP-9, BAX, and Caspase-3 and upregulating the BCL2 as evident from densitometry analysis (**Fig. 4D & E**).

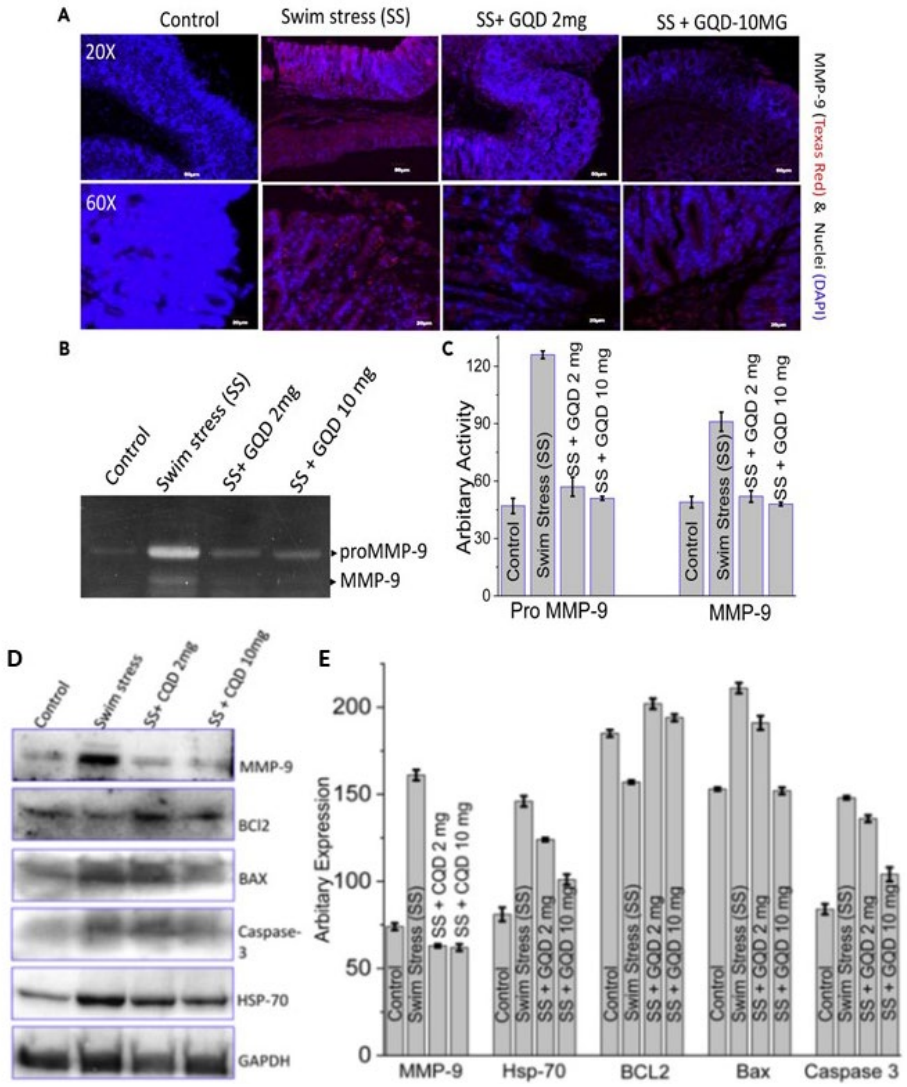

Figure 4: Gastroprotective mechanism of GQDs. **(A)** GQDs downregulate in vivo MMP-9 expression and activity in forced swim-stress-induced gastric ulcer in mice: here MMP-9 was immune-stained with anti-MMP-9 antibody and Texas Red (red) conjugated secondary antibody. The nuclei were stained with 4', 6-diamidino-2-phenylindole or DAPI (blue). Both low (2 mg/kg b.w.) and high (10 mg/kg b.w.) doses of GQDs treated tissues exhibited significant inhibition of MMP-9 signals (red) compared with stress as evident from merged images with DAPI. **(B)** Gelatin zymographic representation of both pro and active MMP-9 activity in acute ulcerated stomach-tissue-extract-treated, as well as GQDs-treated, groups. **(C)** Histographic representation of pro- and active-MMP-9 activity in control, stress ulcerated, and GQDs-treated gastric mucosa. Graph shows fold change versus both form of MMP-9 under stress (data were indicated as the mean ± SEM). Statistical evaluation was done using ANOVA after that Student–Newman–Keuls t test. **(D)** Effect of GQDs pre-treatment (2 and 10 mg/kg b.w.) on expressions of MMP-9, BCL2, Bax, Caspase-3, Hsp70, and GAPDH (loading control) in gastric tissues after 4 h of stress as presented in respective western blots for these molecules. Each blot is representative of three independent experiments. **(E)** Histographic representation shows the significance of fold changes versus expressions of MMP-9, Hsp70, BCL2, Bax, and Caspase-3 under stress and GQDs-treated conditions compared with control (data were indicated as the mean ± SEM). Statistical evaluation was performed using ANOVA after that Student–Newman–Keuls t test.

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- Choudhary P, Biswas S, Kandoth N, Tayde D, Chatterjee A, Chattopadhyay S, **Swarnakar S****, Das A and Pramanik SK **(2022)** Graphene quantum dots to control ROS-mediated gastric damage. ***iScience*** **25(4):** 104062. **IF=5.74**; doi: 10.1016/j.isci.2022.104062.
- Manna A, Roy T, Das T, Bandyopadhyay S and **Swarnakar S**** **(2022)** Potency of

Phytocompounds as Hydroxychavicol and Plumbagin in Combination to Fight Against Leukemia via Activation of MAPK-Mediated Apoptotic Pathway: An *In-vitro* Approach. ***Int J Pharm Sci Drug Res***. **14(1):** 1-8. **IF=1.2**; doi: 10.25004/IJPSDR.2022.140116.

- Deb N, Jha T, **Swarnakar S** and Besra SE **(2021)** Apoptosis Inducing Activity of *Diospyros melanoxylon* Bark Extract on Molt-3 Cell Line via Activation of Mitochondrial Membrane Caspase Cascade and Cell Cycle Arrest with its Antioxidant Potential. ***Int J Pharm Sci Rev Res.*** **71(1):** 148-155. **IF=0.131**; doi: 10.47583/ijpsrr.2021.v71i01.018.
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- Pandit A, Begum Y, Saha P, Srivastava AK and **Swarnakar S** (2022)** Approaches towards targeting matrix metalloproteases for prognosis and therapies in gynecological cancer: microRNAs as a molecular driver. ***Front Oncol.*** **11:** 720622. **IF=6.24**; **Citation=01**; doi: 10.3389/fonc.2021.720622.
- Gupta R, Chakravarti R, Ghosh A, Singh R, Ravichandiran V, **Swarnakar S****, Ghosh D **(2022)** Cas13d: Anew molecular scissor for transcriptome engineering. ***Front Cell Dev Biol.*** **10:** 866800. **IF=5.69**; **Citation=03**; doi: 10.3389/fcell.2022.866800.
- Begum Y, Pandit A **and Swarnakar S** (2022)** Insights into the regulation of gynecological inflammation-mediated malignancy by metalloproteases. ***Front Cell Dev Biol.*** **9:** 780510. **IF=5.69**; doi: 10.3389/fcell.2021.780510.

Book Chapters

- Pandit A, Begum Y, Saha P and **Swarnakar S (2022)** Nanotechnology based gene delivery strategies towards diseases therapy; Advances and applications. **Book:** ***Advances in Nanotechnology-based Drug Delivery Systems.*** Edited by Drs. Anupam Das Talukdar, Satyajit Dey Sarker and Jayanta Kumar Patra. *Elsevier Publisher (Netherlands),* pp. 197-226. **ISBN No.** 978-0-323-88450-1.
- Chatterjee A, Mishra VK, Saha S and **Swarnakar S (2022)** An Insight into Targeted Therapy for Ovarian Cancer. **Book:** ***Handbook of Oxidative Stress in Cancer: Therapeutic Aspects.*** Edited by Prof. Sajal Chakraborti. *Springer Nature, Singapore*. **ISBN No.** 9789811654213; 9811654212.
- Choudhary P, Mishra VK and **Swarnakar S (2022)** Zymography and reverse zymography for testing proteases and their inhibitors. **Book:** ***Cancer Biomarkers: Methods and Protocols.*** Edited by Dr. Gagan Deep. *Springer Nature,* pp. 107-120, **ISBN No.** 978-1-0716-1896-7.
- Roy T, Mishra VK, Mallick S and **Swarnakar S (2022) Chapter:** Functional regulation between matrix metalloproteases and cell junction proteins in gastric cancer. **Book:** ***Handbook of Oxidative Stress in Cancer: Mechanistic Aspects***. Edited by Drs. Sajal Chakraborti, Bimal K. Ray, Susanta Roychoudhury. *Springer Nature Singapore Pte Ltd.,* pp. 153-164, **ISBN No.** 978-981-15-9410-6, **eBook ISBN No.** 978-981-15-9411-3 & **Print and electronic bundle ISBN No.** 978-981-15-9412-0.

Invited Lectures

Sl. No.	Title of the talk	Name of the Meeting, Venue & Year	Remarks
01.	**Association of matrix metalloprotease 7 and metastasis in gastric cancer**	Adamas University, Kolkata, 13th August, 2021	Colloquium speaker
02.	**Empowering Girls for a brighter tomorrow**	Seminar series in NIPER-Kolkata on 7th September, 2021	Guest lecture
03.	**Heavy metal toxicity and impact on fishes**	Seacom University, West Bengal 18th November, 2021	Guest lecture
04.	**The experience of scientific processes and the joy of discovery**	Techno University, Kolkata on 28th February, 2022	Guest lecture

Investigating the molecular details of virus infection and pathogenesis, particularly virus replication; viral protein translation and trafficking, with the aim of developing novel strategies of diagnosis and intervention (antivirals)

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Group Members

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Abinash Mallick, SRF
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Background

We are looking at the existence, prevalence & aetio-pathogenesis of various viral infections in India with special reference to-

- RNA viruses like dengue virus (DENV) and SARS-CoV-2. We are also working on faster diagnostic methods for these endemic viruses.
- DNA viruses like human herpesviruses and hepatitis B virus.
- We are also investigating whether the elsewhere-reported Leishmaniaviruses (LRVs) are also present in Indian visceral leishmaniasis (VL)/kala-azar cases.
- We are also tracking dengue from pre-pandemic to post COVID-19 pandemic times and analyzing the implications of the observed serological cross-reactivity between these two viruses.

Aims and Objectives

- Molecular analysis of DENV serotypes in cell culture and characterization of NS1 protein.
- Characterization of herpesviruses in clinical samples from Kolkata in terms of their growth properties and sensitivity to available antivirals.
- Our discovery that the human host is exposed to an RNA virus (Lepsey NLV 1) in *Leptomonas seymouri* (LS), another co-infecting protozoan parasite with *Leishmania donovani* (LD), i.e. the "LD-LS-Lepsey NLV1 triple pathogen" phenomenon, unveiled a new paradigm of research towards revisiting the mysteries of kala-azar/Indian leishmaniasis pathogenesis and management.
- Diagnosis of COVID-19 (by CRISPR-Cas9-based, FELUDA technology) and understanding the globally observed "dengue and COVID-19" conundrum.

Work Achieved

1. Dengue virus RNA-based diagnosis without PCR amplification

We optimized the protocol for rapid pan-dengue detection in DENV-infected serum samples with pan-dengue probes, immobilized on magnetic beads and applied for patent (Fig.1.). International application was published under the PCT on 11th November 2021 and republished on 20th January, 2022 (1).

2. DENV NS1 ELISA-escape mutations identified

Dengue diagnosis primarily relies on NS1 ELISA and serological (IgG/IgM) tests. There are reports of low and variable sensitivity of the widely-used NS1 ELISA tests. We investigated whether NS1 mutations directly have any impact on NS1 ELISA-based dengue virus (DENV) detection in clinical samples.

Fifty-eight serum samples were collected from dengue-endemic area during 2015-17 and tested with three commonly-used NS1 ELISA kits. The samples were subjected to diagnostic RT-PCR and sequencing of structural gene(s). Sequencing of NS1 gene revealed amino acid changes which were transferred to respective wild type NS1 backbone to determine their effects on NS1 production and secretion in Huh-7, Vero and A549 cells.

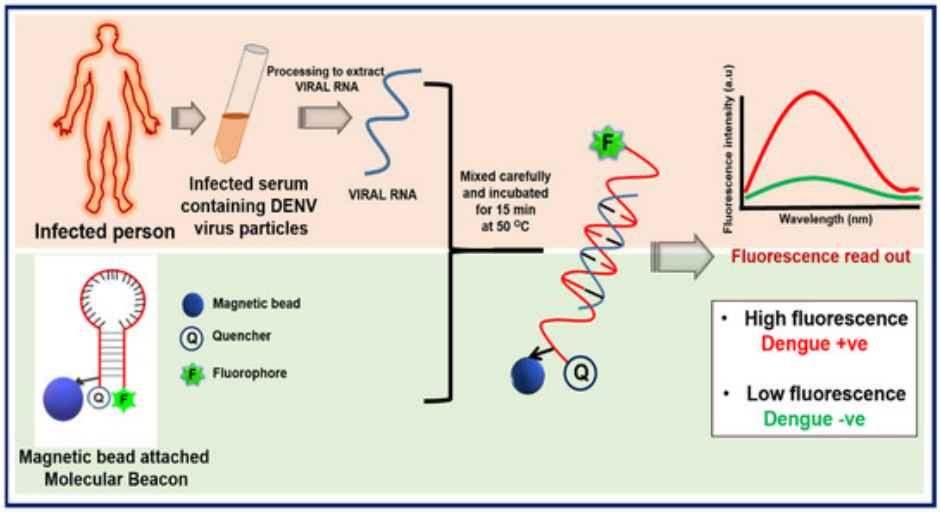

Figure 1: Schematic representation of the functional workflow of the fluorescence quencher system

Eighty-seven percent samples were virus RNA-positive but 65% of these were NS1 ELISA-positive. NS1-gene mutations like *Val236 to Ala* (DENV2) or *Trp68 to stop codon* in DENV3 were associated with decreased NS1 production and secretion. These mutations were originally identified in NS1 ELISA-negative clinical isolates. All DENV1 and > 80% DENV2 were NS1 ELISA-positive. The three NS1 ELISAs could not detect circulating (2015-17) DENV3 single infections, despite being RNA-positive. Among serotypes 1-3, wild type NS1 production was highest for DENV1 and lowest for DENV3 in all cell lines tested (Fig. 2).

Further studies to define more NS1 mutations in clinical samples, is needed to optimize ELISA kits for more sensitive dengue diagnosis (2).

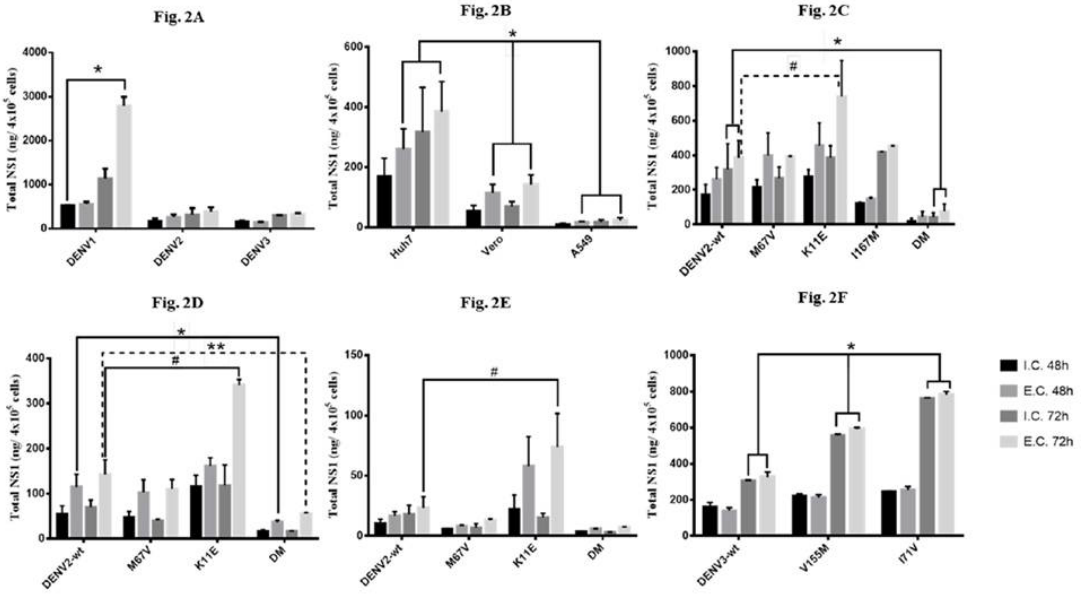

Figure 2: Variable DENV NS1 production and secretion pattern based on serotypes, cells and mutations
(A, C, F) In Huh7 cells ;**(D)** In Vero cells; **(E)** In A549 cells. The Y-axis represents the total NS1 quantity (ng/ 4x10^5 cells) measured from the transfected well i.e. intracellular NS1 in total cell lysate (left column) and extracellular NS1 in total supernatant (right column), at a given time point, i.e. 48 or 72hwt: wild-type; DM: double mutant; I.C.: Intracellular; E.C.: Extracellular. Error bars indicate SD.

3. Discovery of non-cytopathic herpes simplex virus (HSV) from clinical samples of recurrent herpes disease

HSV usually produces cytopathic effect (CPE) within 24-72hrs post-infection (P.I.). Clinical isolates from recurrent HSV infections in patients on Acyclovir therapy were collected between 2016 & 2019 and tested in cell cultures for cytopathic effects and further in-depth characterization.

Fourteen such isolates did not show any CPE in A549 or Vero cell lines even at 120hrs P.I. However, these cultures remained positive for HSV-DNA after several passages. Sequence analysis revealed that the non-CPE isolates were all HSV-1. Analysis of the thymidine kinase gene from the isolates revealed several previously reported and two novel ACV-resistant mutations. Immunofluorescence and Western blot data revealed a low-level expression of the immediate early protein, ICP4. Late proteins like ICP5 or capsid protein, VP16 were almost undetectable in these isolates. AFM imaging revealed that the non-CPE viruses had structural deformities compared to wild-type HSV-1 (Fig. 3).

Our findings suggest that these strains are manifesting an unusual phenomenon of being non-CPE herpesviruses with low level of virus protein expressions over several passages. Probably these HSV-1 isolates are evolving towards a more "cryptic" form to establish chronic infection in the host thereby unraveling yet another strategy of herpesviruses to evade the host immune system (3).

Future Research Plans

- Few DENV-infected sera samples with pre-determined viral load (by qRT-PCR) had been tested using the pan-DENV probe and it was found that the virus RNA load corroborated with the level of fluorescence-based detection (1). Larger numbers of samples are needed to be tested for further clinical validation.
- We are currently pursuing questions such as how Lepsey NLV 1, a protozoan virus persists in the human system for so long, and in considerable numbers, making its recovery possible from even archived human serum samples.
- Devising strategies of differential serological diagnosis of dengue and COVID-19 in the post-pandemic era (4).
- Screening for antivirals against coronavirus, dengue and herpes simplex virus.

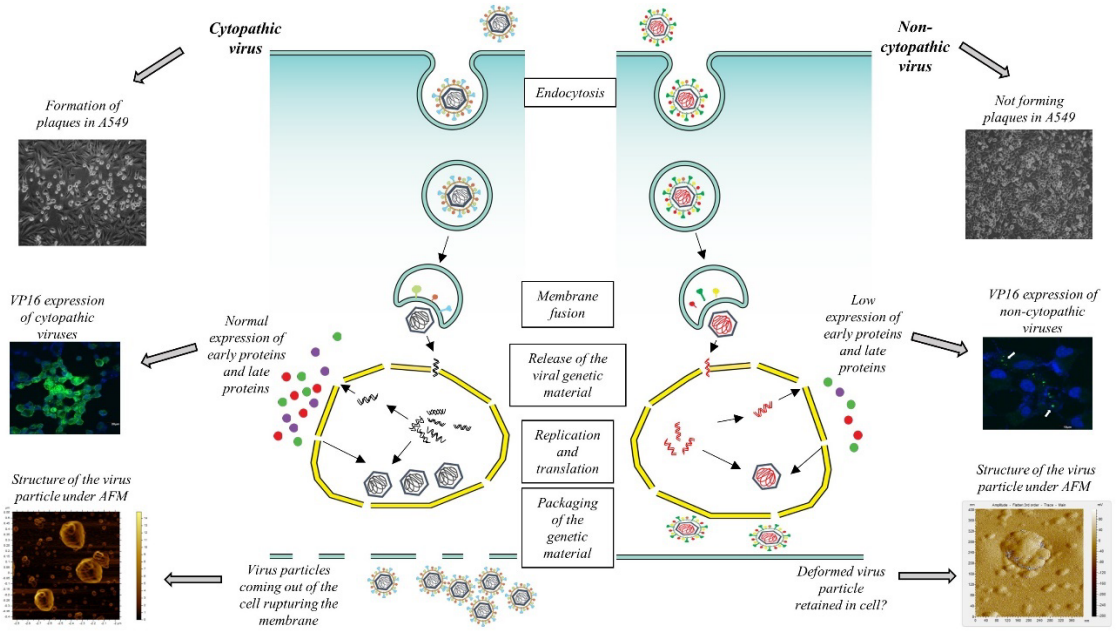

Figure 3. Schematic representation of the difference in pattern of cell infection between cytopathic (left panel) and non-cytopathic HSV-1 isolates (right panel). Normally, HSV-1 enters into the cell through endocytosis and releases nucleic acid into the nucleus where multiple copies of DNA are generated. Viral DNA is transported to cytoplasm for expression of viral proteins, which helps to form capsid particles in the nucleus. After formation of the complete enveloped virus particle, it ruptures the cell membrane and egresses. On the other hand, non-cytopathic virus replication produces lower number of DNA copies resulting in low expression of the virus proteins. Thus, faulty/low level virus replication or packaging hinders proper release of the progeny virions

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- Sukla, S., Mondal. P., Biswas, S. and Ghosh, S. (2021) A Rapid and Easy-to-Perform Method of Nucleic-Acid Based Dengue Virus Diagnosis Using Fluorescence-Based Molecular Beacons. *Biosensors (Basel)* **11**, 479. doi: 10.3390/bios11120479.
- Ghosh, A., Sukla, S., Nath, H., Saha, R., De, A. and Biswas S. (2022) Non-structural protein 1 (NS1) variants from dengue virus clinical samples revealed mutations that influence NS1 production and secretion. *Eur J Clin Microbiol Infect Dis* **41**, 803-814.
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- Nath, H., Mallick, A., Roy, S., Sukla, S., Basu, K., De, A. and Biswas, S. (2021) Archived dengue serum samples produced false-positive results in SARS-CoV-2 lateral flow-based rapid antibody tests. *J Med Microbiol*. **70**, 001369. doi: 10.1099/jmm.0.001369.

Extramural Funding

Characterization of herpesviruses in clinical samples from Kolkata in terms of their growth kinetics and sensitivity to available antivirals. 2018-2021, with no cost extension to 2022 (**Higher Education, S&T & Bio-Technology,** Government of West Bengal).

Invited Talks

- Invited speaker to deliver a **popular science lecture** as **Resource Person in Virology** on 22nd February, 2022 at 3:00 p.m. The event was organized by the **Tetulia Sarrang N.N. High School (Higher Secondary), Egra –I Block of Purba Medinipur, West Bengal** on occasion of their Diamond Jubilee Celebration. This was part of outreach program by the **CSIR-IICB JIGYASA** team.
- Delivered **online lecture in Bengali** on 21st February, 2022 at 3:00 p.m. The title of the talk was "**The importance of covid safety rules in today's world**". The lecture was organized by CSIR-IICB on the occasion of **International Mother Language Day**.
- Invited speaker to deliver a **virtual lecture as Resource Person in Virology** on 11th June, 2021 at 3:00 p.m. The event was organized by the **Jhargram Raj College, West Bengal, pursuing DBT Star College Scheme under the Ministry of Science and Technology, Govt. of India**. Title of the talk: **Viral diseases: our constant struggle to be one step ahead**.

Nanotechnology approach to prevent bacterial infection

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Background

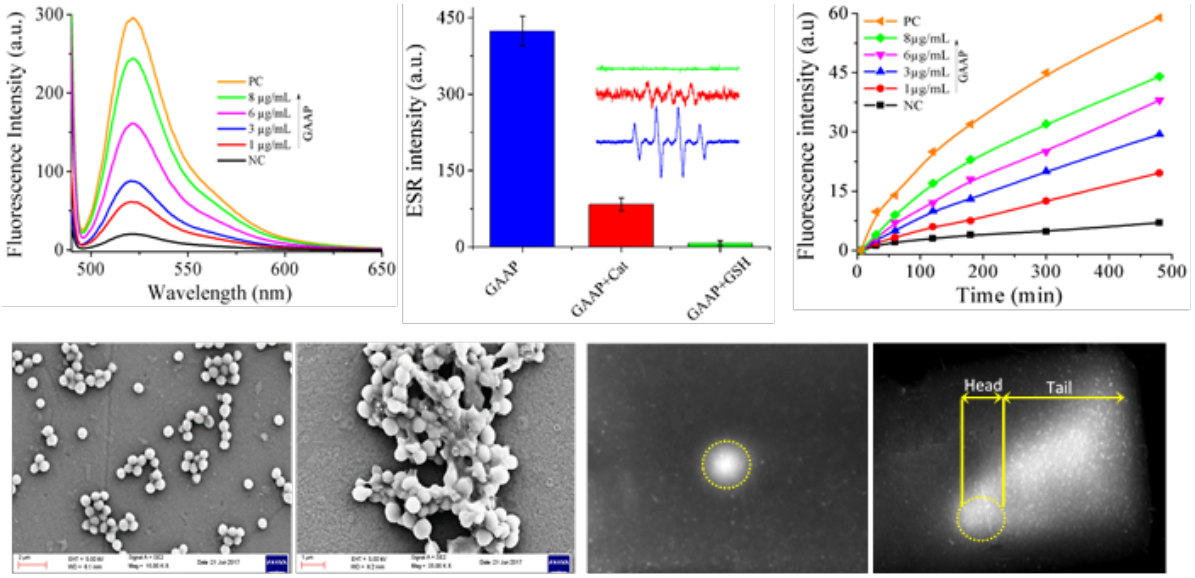
Bacterial infections caused by ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas. aeruginosa, Enterobacter* spp.*)* is one of the growing global concerns for health care sectors, causing wound infections, septicemia, pneumonia, bone and joint infections. As per the reports of WHO, *S. aureus* infections lead to 2 million illnesses and 23,000 deaths per year. Antibiotic associated adverse effects and emergence of bacterial resistant strains necessitates the development of an alternative yet effective approach. The nanotechnology based therapy has emerged as a potential therapeutic strategy to combat the bacterial infection. The innovative nanomaterials could enable interventions at the molecular length scale and are, therefore, an important cornerstone in building solutions. In spite of significant advances, it remains difficult to construct nanomaterials with precisely defined therapeutic effects, release of therapeutic molecule at targeting site and minimize any adverse effect to human health. The primary research focus of our group is to develop biomimetic and surface-functionalized nanomaterials for targeted drug delivery for infection control, tissue engineering application and medical device development

Aims and Objectives

- Designing of bacterial cell wall targeting nanoconstruct and bacterial infection control
- Understanding biochemical mechanisms of nanomaterial based antimicrobial toxicity and biofilm disruption property
- Nanobiomaterial based transdermal scaffold for tissue engineering application and medical device development

Bacterial cell wall targeting nanoconstruct

The cationic antimicrobial peptides, which occur naturally as part of host defense systems, can act both as antibiotics and immunomodulatory agent and potential to target the bacterial cell wall. We constracted antimicrobial peptide conjugated graphene–silver nanocomposite (GAAP) as cell wall targeting nanoconstract for the treatment of bacterial infection. The cell wall targeting nanoconstract effectively killed the planktonic cells and biofilms of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* depending upon the concentration and duration of the interaction. The complete eradication of preformed biofilm was achieved when treated with 10 µg/ mL of GAAP for 5 h. To understand the interaction of GAAP with bacterial cells, the intracellular ROS production was measured. The ESR and fluorescence spectroscopic studies showed generation of singlet oxygen ($O_2^{-\bullet}$) and hydroxyl radicals ($^{\bullet}OH$) upon treatment with GAAP nanocomposite, while ζ value revealed electrostatic interaction of GAAP with bacterial cell wall, leading to depolarization of the cell membrane potential (ψ). This further caused pore formation on the cell membrane and disrupted the cell wall integrity. The single-cell gel electrophoresis study also revealed a comet tail suggested that the internalization of GAAP into the cytoplasm through the damaged cell membrane caused considerable DNA damage upon treatment of GAAP. The antimicrobial peptide functionalization enhanced the biofilm inhibition and disruption activities of GAAP, and minimizing its toxicological impact by preventing dissolution of Ag^{+} ions. Understanding the bacterial killing mechanism opens up new strategy for devising nanomaterial-based antibacterial strategies.



This work has been published in *ACS Biomater. Sci. Eng.* **2021,** 7, 5899−5917.

Future Research Plans

Stimuli responsive delivery of therapeutic agent

In the area of drug delivery, we focus on designing of polymersomes for controlled delivery of therapeutic agent to create translatable targeted drug delivery technology, where delivery of the therapeutic agent is facilitated by reduction in pH as observed in the bacterial biofilm, tumor interstitium or in the endosome. This approach has the potential for effective delivery of the therapeutic agent at the targeted site and to reduce side effects associated with systemic exposure.

Cell-targeted photodynamic and phtothermal therapy

Photodynamic and phtothermal therapy emerges as a promising treatment strategy to reduce the potential risks of systemic toxicity, collateral healthy tissue damage and surgical trauma. We are designing formulation of light activated photosensitizer, which results in energy transfer cascades to yield cytotoxic reactive oxygen species, rendering apoptotic and necrotic cell death.

Publications

- Antibacterial and antibiofouling activities of antimicrobial peptide-functionalized graphene–silver nanocomposites for the inhibition and disruption of *Staphylococcus aureus* biofilms. Thanusu Parandhaman; Priyadarshani Choudhary; Baskaran Ramalingam; Michael Schmidt; Sridevi Janardhanam; and **Sujoy K. Das**.* *ACS Biomater. Sci. Eng.* **2021,** 7, 5899−5917.
- Inhibitory Potential of iRGD Peptide-Conjugated Garcinol-Loaded Biodegradable Nanoparticles in Rat Colorectal Carcinoma. Brahamacharry Paul, Raghuvir H. Gaonkar, Debasmita Dutta, Rajesh Dasi, Biswajit Mukherjee, Shantanu Ganguly, and **Sujoy K. Das**. *Biomaterials Adv.* **2022**, *134*, 112714.

Awards/Honours/Memberships

- Member of Royal Society of Chemistry (MRSC)
- Member of Royal Society of Biology (MRSB)
- Member of American Chemical Society

Extramural Funding

Principle Investigator: Sujoy K Das
Funding Agency: SERB, Govt. of India

Discovery of plant based molecules/extracts as therapeutics for colon cancer

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Group Members

Purnachandra Pal, NIPER-JRF
Deethi Jyothi, CSIR-JRF
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Collaborators

Dr. Snehasikta Swarnakar, CSIR-IICB
Dr. Krishna Das Saha, CSIR-IICB

Background

Swietenia macrophylla King in Hook (SM) is known to have several medicinal properties. The crude ethyl acetate extract of Mahogany seeds (SMEA) has earlier been reported to be active with IC_{50} of 35 µg/mL against the human colorectal cancer (CRC) cell line HCT116 (Goh and Kadir 2011; Goh et al. 2014) but the active principles, responsible for its anti-CRC activity, have not yet been reported. Chloroform extracts of SM seeds (SMCE) as well as two isolated limonoids IICB-UPS-SM-1 and IICB-UPS-PC-SM-2 showed significant *in vitro* anti-CRC activity in human colon carcinoma (HCT116) cell line. IICB-UPS-SM-2 (IC_{50} = 5.6 µM) was found to be two times more potent than IICB-UPS-SM-1 (IC_{50} = 10µM). Both compounds showed anti-CRC activity through inhibition of the Mouse Double Minute 2 homolog (MDM2) of the MDM2-p53 pathway. The Selectivity Index (S.I.) of isolated compounds IICB-UPS-SM-1 and IICB-UPS-SM-2 for cancer cells were about 6.6 and 12.8 fold respectively which was significantly better that S.I. of the extract (S.I. ~1.5).

Objectives

- To prepare extracts of *Swietenia macrophylla* King in Hook (SM) seeds
- To test the activity of the different extracts
- To identify different molecules, present in the extracts
- To isolate different components of the active extracts
- To identify the pure compound(s) responsible for the activity
- To further develop the active component as therapeutic agents against colorectal cancer

Expected output and outcome of the work

- The identification of the component(s) responsible for therapeutic action from identified plant
- Plant based theraputic agents (pure substances) as therapeutics for colon cancer.

Work Achieved

The chloroform extract was prepared for SM (SMCE). Its different components were characterized with HPLC (Fig1) and LC-MS analysis.

SMCE was tested against three different cancer cell lines HCT116 (colon cancer cell line), HepG-2 (Human hepatocellular carcinoma), and A549 (Human lung carcinoma). SMCE showed its highest activity with IC_{50} value of 50.2 ± 8.5 µg/mL against HCT116 cell line as compared to IC_{50} value of 72.1 ± 7.5 µg/mL and 80.9 ± 7.9 µg/mL against HEPG2 and A549 cell lines respectively (Figure2). Similarly, IC_{50} of IICB-UPS-SM-1 and IICB-UPS-SM-2 were 10.5 ± 2.7 µM and 5.6 ± 1.4 µM (against HCT116); 10.5 ± 2.7 µM and 5.6 ± 1.4 µM (against HepG2); and 44.5 ± 4.3 µM and 37.5 ± 3.6 µM (against A549) respectively. Both IICB-UPS-SM-1 and IICB-UPS-SM-2 were significantly more active than SMCE and IICB-UPS-SM-2 showed higher activity than IICB-UPS-SM-1 in all the cell lines tested. SMCE as well as IICB-UPS-SM-1 and IICB-UPS-SM-2 showed better activity against HCT116 cell lines compared to other cancer cell lines.

The ability of IICB-UPS-SM-1 and IICB-UPS-SM-2 to specificially target cancer cells than normal cells were also studied. It was found that in the normal human epithelial kidney (HEK293T) cell line 50% of cells dead (CC_{50}) happens at 73.5 ± 5.8 µg/mL with SMCE, 69.2 ± 3.1 µM with IICB-UPS-SM-1 and 71.9 ± 7.5 µM with IICB-UPS-SM-2 (Figure-2). Thus, for HCT116 cell lines IICB-UPS-SM-1 and IICB-UPS-SM-2 offer a selectivity index [SI = (CC_{50}) / IC_{50}] of

about 6.6 fold and 12.8 fold respectively compared to S.I. of only about 1.5 for SMCE. Hence both IICB-UPS-SM-1 and IICB-UPS-SM-2 may be more useful agents against CRC than SMCE due their much higher selectivity indices than that of SMCE.

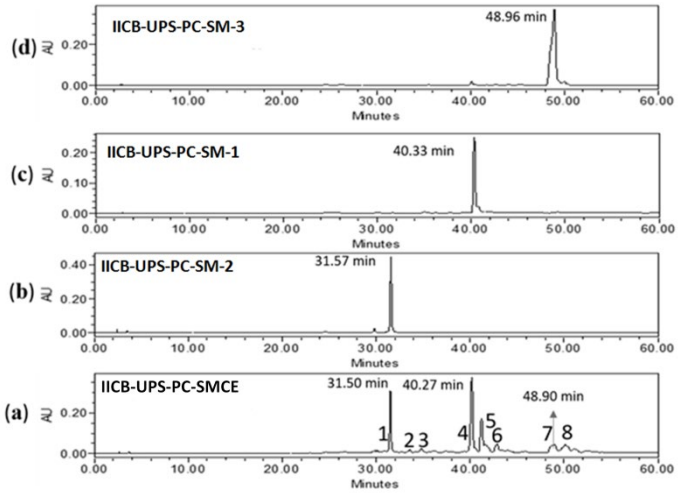

Figure 1: High-performance liquid chromatography (HPLC) of (a) the SMCE extract, (b) IICB-UPS-SM-2, rt. 31.57(±9) min, (c) IICB-UPS-SM-1, rt. 40.33(±9) min and (d) IICB-UPS-SM-3, rt 48.96(±9) min, obtained by relay synthesis from IICB-UPS-SM-1. The rt. of 48.96(±9) min for IICB-UPS-SM-3 matched well with the rt. of peak number-7 of SMCE extract observed at 48.90(±9) min. The reverse-phase C_{18} column was used for chromatographic separation and the peaks were observed at 230 nm wavelength.

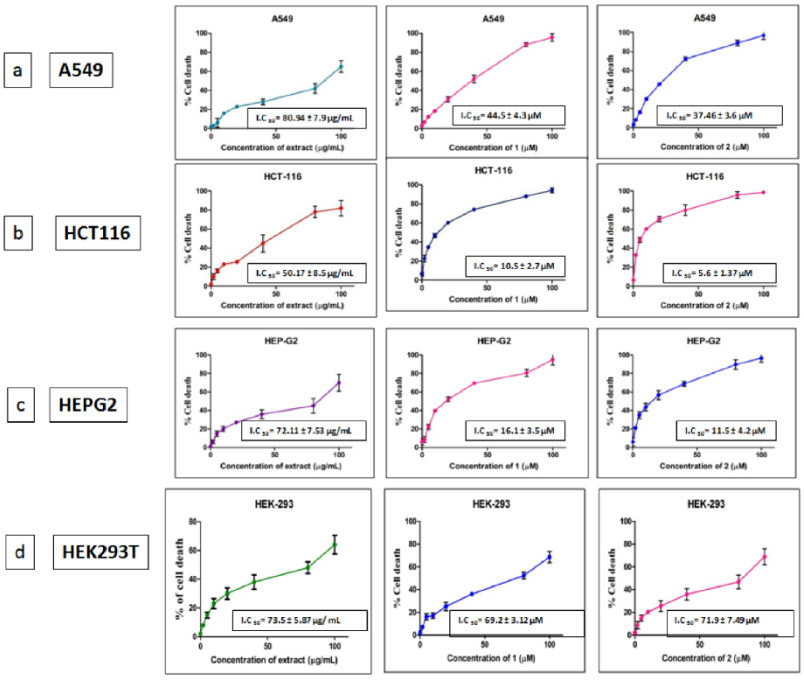

Figure 2: Concentration dependent growth inhibitory effect of the extract SMCE, IICB-UPS-SM-1 and IICB-UPS-SM-2 on different cell lines (a) A549; (b) HCT116; (c) HEPG2 and (d) HEK293. Cells were treated with different concentrations of SMCE (0-100 µg/mL) or IICB-UPS-SM-1 and IICB-UPS-SM-2 ranging in concentration from (0-100µM) for 24 h and viability was measured using an MTT assay. The IC_{50} of each of SMCE, IICB-UPS-SM-1 and IICB-UPS-SM-2 are shown in the corresponding figures. The data are represented as mean ± SD from two independent experiments performed in triplicate.

The mechanism of cell death (Figure3) was studied using IICB-UPS-SM-1 and IICB-UPS-SM-2 on HCT116 cell line with 5-fluorouracil (5-FU) (a marketed colon cancer drug, +ve control), at an IC_{50} value of 5µM (Shakibaei et al. 2013) for 24h. With escalating doses, IICB-UPS-SM-1 and IICB-UPS-SM-2 showed a concomitant significant dose-dependent increase of p53 and p21expression levels compared to the untreated cancer cells. The effect was more marked for IICB-UPS-SM-1 than IICB-UPS-SM-2 with latter showing an almost flat dose-response. IICB-UPS-SM-2 also shows slight reduction in MDM2 expression level, which is significantly more than that shown by IICB-UPS-SM-1. This coupled with the inhibition of MDM2 by both IICB-UPS-SM-1 and IICB-UPS-SM-2, suggests probable saturation of MDM2 activity of IICB-UPS-SM-2 at the given concentrations but not of IICB-UPS-SM-1, which shows dose dependency for its activity.

Future Research Plans

Apart from working on the above anti-cancer project, we also have ongoing anti-viral program going on in the lab as reported earlier. We want to discover anti-virals (inhibitors) against dengue and SARS-Cov-2 virus from synthetic and natural sources. In this direction, we have already prepared different serotypes of dengue NS5-methyltransferase (NS5-MTase), NS5-helicase, NS3/NS2B-protease set up activity assays for the above proteins. Similarly, we have also prepared SARS-Cov-2 proteases viz. Mpro and PL-pro and RNA dependent RNA Polymrase (RdRp) enzymes. The activity assays of these proteins is also ready. Hence, we plant to discover target specific small molecule/peptide based inhibitors against these viruses by design of synthetic molecules (in collaboration with chemists at CSIR-IICB/other CSIR labs) as well as screening of *in-house* (CSIR) compound library containing both syntheic as well as natural product based molecules.

References

- Goh BH, Chan CK, Kamarudin MNA, Abdul Kadir H. 2014. Swietenia macrophylla King induces mitochondrial-mediated apoptosis through p53 upregulation in HCT116 colorectal carcinoma cells. J Ethnopharmacol. 153(2):375–385. https://doi.org/10.1016/j.jep.2014.02.036
- Goh BH, Kadir HA. 2011. In vitro cytotoxic potential of Swietenia macrophylla King seeds against human carcinoma cell lines. J Med Plants Res. 5(8):1395–1404.
- Shakibaei M, Mobasheri A, Lueders C, Busch F, Shayan P, Goel A. 2013. Curcumin Enhances the Effect of Chemotherapy against Colorectal Cancer Cells by Inhibition of NF-κB and Src Protein Kinase Signaling Pathways. PLoS One. 8(2):1–13. https://doi.org/10.1371/journal.pone.0057218

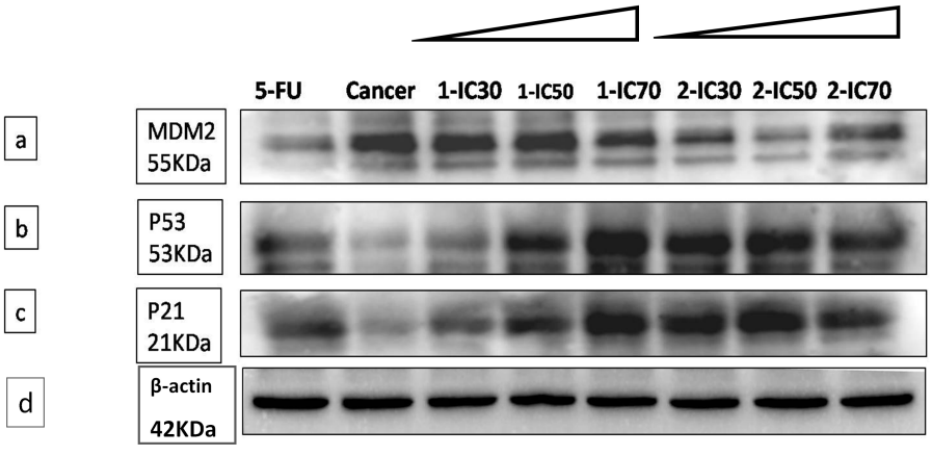

Figure 3: Cells were treated with IC_{50} dose of the standard anti-cancer drug 5-fluorouracil (5-FU, 5µM), which is the positive control, and IC_{30}=3.6 µM, IC_{50}=10.5 µM and IC_{70}=30.8 µM dose of **IICB-UPS-SM-1** (**1**) (lane 3-5) and IC_{30}=1.7 µM, IC_{50}=5.6 µM and IC_{70}=18.7 µM dose of **IICB-UPS-SM-2** (**2**) (lane 6-8) respectively for 24 h. The untreated cancer cells (lane 2) is the –ve control group. Cells were lysed and cell lysate was used for: (a-d) western blot analysis to check expression levels of MDM2, p53, p21, and β-actin respectively. β-actin was used as the loading control.

Publications

Publications in international journals

- Design and synthesis of novel spirocyclic carboxylic acids as potent and orally bioavailable DGAT1 inhibitors and their biological evaluation, *Summon Koul, Suresh Kurhade, Sandeep Bhosale, Keshav Naik, Videsh Salunkhe, Sudhir Ravula, Prasad Punde, Ravikumar Velayutham, Atul Tiwari, Daniela Ahl, Srividya Malkapuram, Vamsi Mudagala, Amol Raje, Dhananjay Umrani, Suhas Tambe, Poonam Patil,* ***Umesh Singh****, Debnath Bhuniya, Narayanan Hariharan, Kasim Mookhtiar,* Bioorg. Med. Chem. Lett. (2022); 62: 128632 [IF=2.823], https://doi.org/10.1016/j.bmcl.2022.128632
- Conformation of Achiral α/β Hybrid Peptides Containing Glycine and 1-Aminocyclohexaneacetic Acid, *Sudha Shankar, Deeti Jyothi, Junaid ur Rahim, Purna Chandra Pal,* ***Umesh Prasad Singh**** *and Rajkishor Rai**, ChemistrySelect (2022), 7(10), e202104453 [IF=2.109], https://doi.org/10.1002/slct.202104453

Publications in Conferences

- 91st Annual Meeting of Society of Biological Chemists (India) on "Life at the Confluence of Biology and Chemistry" (SBC2022), Biswa Bangla Convention Centre, Kolkata, Dec 2022 **(P-274)**, Antiviral activity of amarogentin from *swertia chirata* against dengue virus serotype-2, Purna Chandra Pal, Deeti Jyothi, SudesnaDas, Ayesha Noor, AlagarasuKalichamy and **Umesh Prasad Singh***
- 91st Annual Meeting of Society of Biological Chemists (India) on "Life at the Confluence of Biology and Chemistry" (SBC2022), Biswa Bangla Convention Centre, Kolkata, Dec 2022 **(P-275)**, Biochemical and computational studies for discovery of inhibitors against sars-cov2 main protease, Sudesna Das, Purna Chandra Pal, Mohammad Rafi, Sunnapu Prasad, Sudip Dey, P. Jaisankar and **Umesh Prasad Singh***
- 91st Annual Meeting of Society of Biological Chemists (India) on "Life at the Confluence of Biology and Chemistry" (SBC2022), Biswa Bangla Convention Centre, Kolkata, Dec 2022 **(P-112)**, Melatonin reverses hyperglycemia induced gastric cancer cell proliferation, Abhishek Chatterjee**,** Tapasi Roy, Vineet Kumar Mishra, Deeti Jyothi, **Umesh Prasad Singh** and Snehasikta Swarnakar *

Memberships

- Life member of the Chemical Research Society of India (CRSI) (LM-1200)
- Life member Indian Crystallographic Association (ICA) (LM196)
- Life member of Society of biological chemists, India (SBC) (LM-4558)

Conferences/Workshops

91st Annual Meeting of Society of Biological Chemists (India) on "Life at the Confluence of Biology and Chemistry" (SBC2022), Biswa Bangla Convention Centre, Kolkata, 8-11 Dec 2022

Conferences/Events Organized

14th Annual Meeting of the Proteomics Society, India and International Conference on Proteins & Proteomics (PSI-ICPP 2022), 3-5 Nov 2022, CSIR-IICB, Kolkata

Trypsin/Trypsin-like protease based SARS-COV-2 spike protein cleavage assay system

Dr. Upasana Ray, upasana.ray@iicb.res.in

Group Members

Debica Mukherjee, CSIR-JRF
Feroza Begum, CSIR-JRF
Sandeepan Das, CSIR-JRF
Dluya Samuel Thagriki, CSIR-TWAS JRF

Aims and Objectives

Designing and establishment of reporter based in vitro trypsin/trypsin-like protease-based SARS-CoV-2 spike protein cleavage assay system.

Work Achieved

We designed a substrate that is the main component of the assay system. The substrate contains a reporter protein followed by spike protein cleavage sequence and a matrix binding domain, all as fusion protein. Upon addition of the trypsin like protease, the substrate should get cleaved at the cleavage site and reporter protein dislodged. The reporter protein will be available from the reaction supernatant for reporter assay. Positive reporter signal will indicate cleavage by protease. The proof of concept of the working model of the assay system has been established and a provisional patent filed [Provisional patent filed: Trypsin / Trypsin-like protease based SARS-CoV-2 spike protein cleavage assay system for drug screening {202111049482 (dated: 28/10/2021)}]

Utility of the assay system

This assay system can be used to screen drugs against trypsin like proteases which cleave SARS-CoV-2 spike protein.

Future Research Plans

In future we plan to work with alternate reporter systems and upgradation of the assay system to highthroughput form.

Awards/Honours/Memberships

- Selected as member of the **Global Young Academy** (GYA) for a term of 5 years {hosted by the German National Academy of Sciences Leopoldina in Halle (Saale), Germany}. GYA members engage in various working groups, strategic projects and collaborate with international partner organisations.
- Selected for getting showcased in the book **She Is: 75 Women in STEAM. Announced by Principal Scientific Advisor (PSA) to GoI and British High Commissioner** on the occasion on India's 75th anniversary of independence-**Azadi ka Amrit Mahotsav**. This book is to showcase women role models in Science, Technology, Engineering, Arts and Mathematics (STEAM) for youth.
- 'Valuable Member of the Year' award by Indian National Young Academy of Science (INYAS) for outreach activities.
- Selected for inclusion in the compendium, "WE" The Scientists" where 32 short biographies of Women from INYAS (& Alumni) were highlighted who have made great strides during their academic and research career besides contributing directly to the society through various outreach programs. This was released on the occasion of International Women' Day 2022.
- Included as a part of STEM Women ASIA (an initiative by Australian Academy of Science, AASSA and IAP, InterAcademy Partnership)
- Invited to be a part of the official launch of STEM Women Asia as an inaugural member. The event was hosted by Australian Academy of Science. (September, 2021)

Extramural Funding

- Studying SARS-CoV-2 spike protein and ACE2 interaction to identify amino acid positions and residues of spike protein that would enhance ACE2 attachment and thus entry. SERB POWER fellowship project
- Scanning through the molecular interactome of Dengue virus NS1 protein and host cell factors (miRNA, mRNA and proteins) to identify potential targets for anti-Dengue antivirals. SERB Women Excellence award project

- Understanding the role of Dengue NS1 protein in endothelium leakage and therapeutic intervention of NS1 pathogenesis to design anti-Dengue antiviral candidate. Department of Science & Technology and Biotechnology, Govt. of West Bengal

Invited Talks (2021-2022)

- COVID-19 vaccine landscape for DST, Haryana (2020)
- Viral structural proteins, self-assembly and VLP vaccines. Webinar on viral diseases organized by Mahatma Gandhi University, Kerala (29.10.2021)
- Cosmic Rays. Delivered talk at Science from India's Past and Present (SIPP) series to highlight work of Dr. Vikram Sarabhai. (7.8.2021)
- What are vaccines? Invited talk as a part of popular talk series of DST Rajasthan and INYAS (1.7.2021)
- COVID-19 Vaccination. Organized by CSIR-CSIO Chandigarh as a part of Jigyasa (5.5.2021)
- Vaccine platforms based on repetitive multivalent display of antigens. At Immunology symposium organized by IIT, Roorkee (29. 4.2021)
- Virus like particle based vaccine against JC polyomavirus and progressive multifocal leukoencephalopathy. Invited by AIIMS, New Delhi (17.4. 2021)
- Vaccine strategies for COVID-19: Indian perspective. Invited by MEDEX Malaysia medical expo MYMEDEX 2021 (8.4.2021)

Role of ribosomal P-proteins in the asymmetric nuclear division of Plasmodium falciparum

Dr. Sudipta Das, sudipta.das@iicb.res.in

Introduction

Plasmodium falciparum is a unicellular eukaryotic protozoan parasite which causes malaria, a catastrophic disease in many developing countries. Malaria alone is responsible for millions of deaths annually across the globe. In India, the cases of malaria are alarmingly increasing and the hope of prevention and cure is extremely challenging due to the emergence of drug resistant parasites. Drug resistance in malaria parasites is a grave concern, so in this situation the battle against malaria must go on by emphasizing on discovering new drug targets and inventing novel small molecules which can target multiple pathways together ensuring reduced chances of resistance. Thus, understanding the basic biology of malarial parasite is extremely important in devising novel drugs and drug targets. The peculiar proliferative cycles of apicomplexan parasites differ substantially from the hosts they inhabit and could offer novel molecular targets. To progress in this direction, we need a good understanding of the unique structural and molecular features of parasite proliferation. Apicomplexan parasites and many of their relatives undergo a complex developmental process in the cells of their hosts, which includes genome replication, cell division and the assembly of new invasive daughter cells. *Plasmodium* cells in particular, exhibit a peculiar cycle of cell division (schizogony), which is unique. In the vertebrate hosts, schizogony occurs in the liver and red blood cells. The growth of the parasite is mostly confined to the trophozoite stage during which nuclear and other organellar genomic division occurs followed by the formation of cell bodies. The mechanism and the regulation of schizogony are poorly understood.

Proposed work with Plasmodium falciparum P2 protein

Understanding the function of P2 on red cell surface

Localization of P2 on red cell surface appears to be an absolute requirement for parasite growth. Blocking red cell surface P2 using monoclonal antibody, and by inhibiting P2 using conditional degradation by allelic exchange using ribozyme construct (Fig.1) both showed nuclear division arrest. Pixel density quantification of ribozyme mediated P2 degradation showed 90-95% degradation and remarkably over 90% parasites were arrested at nuclear division stage, (Fig. 1). Despite this remarkable indispensability of P2 on red cell surface, it is an open question as to what might be the role of this protein in this 6-8h of window on red cell surface. During intraerythrocytic development and subsequent cell cycle progression, import of serum factors such as lipids into the parasite has been shown to be an absolute necessity. In particular, palmitic and oleic acids have been shown to be indispensable for cell cycle progression. Palmitic and oleic acids depleted serum did not support parasite growth and that resulted into the arrest of cell division. P2 on red cell surface appears to play a role as a sensor through the binding to some components in serum, hence, to check this possibility dot blot was performed and that came out astonishing. When recombinant tetrameric P2, but not the monomer, were checked for its binding ability to a set of lipid molecules, such as Phosphatidic acid (PA), Phosphatidyl ethanolamine (PE), Phosphatidylserine (PS), Sphingomyelin (SM), Sphingosine (Sph), Phosphatidyl inositol (PI), Phosphatidyl choline (PC), remarkably, P2 bound only to three lipids, PA, PS, and PC (Fig. 2 Disha et al., unpublished data). To explore the possibility of P2-lipid interaction on red cell surface, several biochemical and biophysical experiments are needed to be performed using wild type and transgenic parasites.

To understand the function of P2 on red cell surface, it is unquestionable to understand whether P2 tetramer alone is acting as a channel or as a pump or P2 is complexed with other parasite proteins to sense and/or transport lipid molecules into the parasites. To address this question, I propose to identify proteins interacting with and proximal to red cell surface P2 by applying proximity biotinylation

approach developed by Roux et al.. Using this method, biotinylating enzyme of *E. coli* can attach biotin to a subunit of acetyl CoA carboxylase but due to a mutation at R118G, it is capable of promiscuously biotinylating proteins in its proximity. By fusing the mutated enzyme (BirA*) to a protein of interest it is possible to preferentially label other proteins either in complex with or proximal to the protein of study, which then is followed by affinity capture of the labeled proteins and identification by LC MS/MS (Fig. 3). This approach was originally developed for mammalian cells but now is being used for other organisms including Trypanosoma and Toxoplasma. Using this method, a transgenic *P. falciparum* line which will express P2-BirA* will be generated. With this fused protein, I will assess whether the pattern of proximity biotinylation of P2-BirA* changes during different stages of intraerythrocytic development. A catalogue of proteins identified through this study could then form the basis for further experimentation using a variety of biochemical and genetic tools. Overall, I believe that this proximity-biotinylation assay would reveal the identity of different molecular players which might be associated with P2 for its novel function on red cell surface and it would also provide inklings of the regulation of time dependent appearance and disappearance of tetrameric P2 on red cell surface.

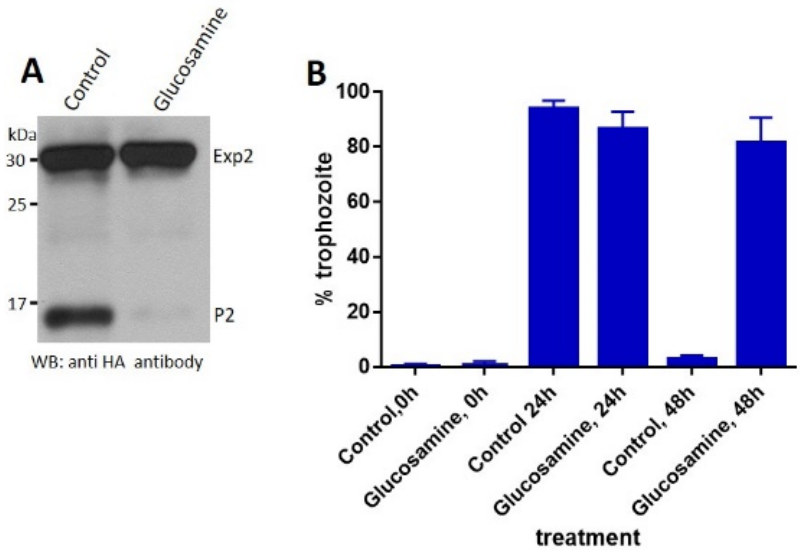

Figure 1: (A) P2-HA-Ribozyme transfected transgenic parasite was treated with 5mM glucosamine in cRPMI for 24h (ring to trophozoite). Parasites were harvested by gentle saponin lysis. SDS-PAGE and Immunoblot was probed with HA antibody. Exp2 was used as a loading control. (B) Quantitation of arrested trophozoite morphology at 0h, 24h and 48h after glucosamine treatment post merozoite invasion (n=5).

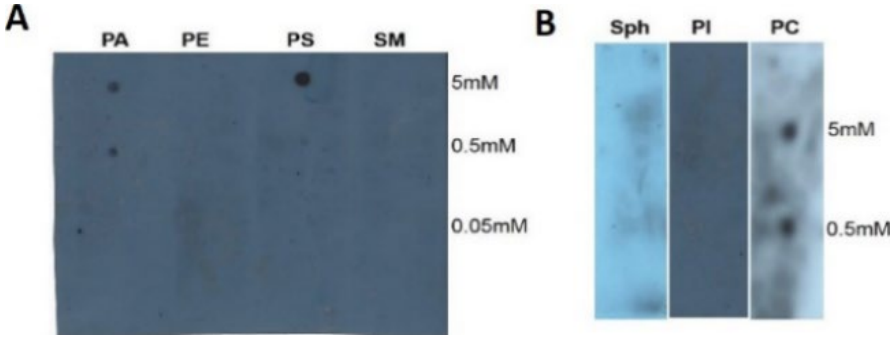

Figure 2: (A&B) Three different concentration of Phosphatidic acid (PA), Phosphatidyl ethanolamine (PE), Phosphatidyl serine (PS), Sphingomyelin (SM), Sphingosine (Sph), Phosphatidyl inositol (PI), Phosphatidyl choline (PC) were blotted and incubated with recombinant P2 protein. Subsequently, P2 protein was detected using anti-P2 monoclonal antibody E2G12) (Disha et al., unpublished data)

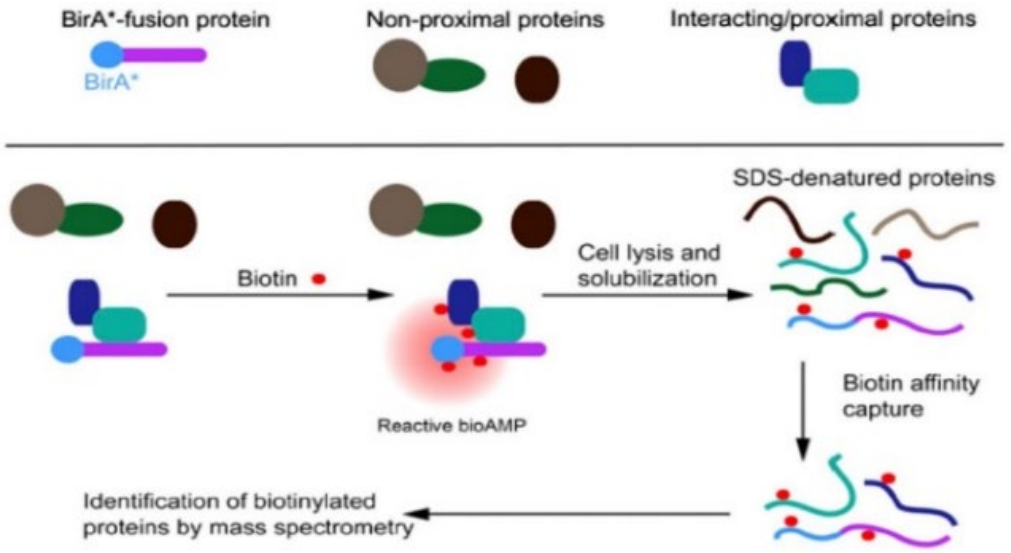

Figure 3: Schematic of proximity-biotinylation method. In a cell expressing a protein fused with BirA*, biotin addition results in its activation and biotinylation of interacting and proximal proteins while non-proximal proteins are not labeled. Affinity capture of biotinylated proteins can be followed by their mass spectrometric identification. From Roux et al. 2013.

Awards / Honour

- Ramalingaswami Fellowship from the Department of Biotechnology (DBT), India.
- Core Research Grant, Department of Science and Technology (DST), SERB, India.
- Medical Biotechnology Grant, DBT
- https://twitter.com/DBTIndia/status/1340315698485084161/photo/1

Publication

- **Sudipta Das#**, Anwesa Manna, Oindrila Majumder, Leena Dhara, Kaustav Majumder, Anwesha Bhattacharjya. Denaturation resistant P2 tetramer is required to imports fatty acids from human serum into intraerythrocytic Plasmodium falciparum. bioRxiv 2022.06.11.495734; doi: https://doi.org/10.1101/2022.06.11.495734 (# corresponding author).
- **Das S#**, Roy B, Chakrabarty S. Non-ribosomal insights into ribosomal P2 protein in Plasmodium falciparum-infected erythrocytes. Microbiologyopen. 2021 Aug;10(4):e1188. doi: 10.1002/mbo3.1188. PMID: 34459544; PMCID: PMC8380560. (# corresponding author)
- **Sudipta Das#** Evolution of nuclear asymmetry across unicellular primitive eukaryotes. (Under revision) (# corresponding author)

Host-pathogen interactions: protozoal evasion of host defense and implications in disease pathogenesis

Dr. Chandrima Shaha, cshaha04@gmail.com

Research Activities

Understanding host-pathogen interactions is a complex issue and therefore, it is important to constantly study and analyse the evolution of these processes to understand diseases. The evasion strategies used by protozoal parasites like the trypanosomatids involve the modulation of host components that make up the large arsenal of host defense mechanisms. This ever-evolving area of study needs to be constantly worked upon and analyzed for a holistic understanding of a set of neglected tropical diseases.

Our studies show a novel role of the cytosolic tryparedoxin peroxidase (cTXNPx) of *Leishmania donovani* in the manipulation of host-pathogen interactions. cTXNPx is a defensive enzyme in a non-secretory form. Apart from that, the cTXNPx is released in the spent media of *Leishmania* cultures and also in the host cell cytosol by the way of secretion. This secretory form of the enzyme interacts with multiple proteins in the host cell cytosol, one of the prominent one being the apoptosis-inducing factor (AIF). Immunoprecipitation with relevant antibodies suggests a strong interaction between AIF and cTXNPx. The migration of AIF to the nucleus to precipitate apoptosis after infection is inhibited in the presence of recombinant cTXNPx expressed in the host cell suggesting a functional role of the enzyme. This inhibition of AIF movement results in lesser host cell death, giving an advantage to the parasite for continued survival. This model of interference with host apoptosis-inducing proteins by parasitic protozoa presents an opportunity to identify potential molecules that could be a part of an important array of proteins amenable for manipulation to inhibit parasite growth.

Future research plans

Despite a definitive pattern of apoptosis, main proteins involved in apoptosis are absent in the *Leishmania* parasite. Recent studies have helped to develop a better understanding of parasite cell death, identifying new proteins or even new apoptotic pathways. However, there are very few comprehensive collection of thoughts about the apoptotic pathways that could serve as a model for similar organisms or could be considered as targets for drug development. Proteins like endonuclease G, metacaspase, aquaporin Li-BH3AQP, calpains, cysteine proteinases, and tryparedoxin peroxidases could be considered as primary targets and an analysis of the involvement of these molecules could form the basis of a review that provides a cogent proposal for further research. Further, the analyses of protozoa-host interactions using current knowledge about Trypanosoma proteins as a pivot will provide much relevant information.

Extramural funding

National Academy of Sciences funding for JC Bose Distinguished Chair Professorship.

Publications (journal and conference)

- Shaha C*, An exciting journey in science: from stars to cells. Science Reporter, 59:3, 14-17 (2022).
- Shaha C*, Misinformation through predatory practices. Proceedings of the Indian National Academy of Sciences, 88:829-831 (2022).
- Das S, Saha T, Yadav S, and Shaha C*, A novel role of secretory cytosolic tryparedoxin peroxidase in delaying apoptosis of *Leishmania* infected macrophages. Molecular Cellular Biology 42: e0008122. doi: 10.1128/mcb.00081-22 (2022).

Achievements

Awards

- Life time Achievement Award, Informa, Mumbai (September, 2021) https://www.biopharmahonours.com/winner-2021
- Dr. SP Ray-Chaudhuri Endowment Award 2020 by Society of Cell Biology. 2021.

Professional engagements

- President, Indian National Science Academy, New Delhi
- Chair, SSB selections, Biological Sciences
- Chair, GD Birla Awards
- Member, Selection Committee for TWAS Awards in Biological Sciences
- Member, NASI-Reliance Award Committee
- Member, Fellows selections for Biological Sciences, NASI
- Member, Promotions and Assessment Committee, Indian Institute of Science, Bangalore
- Member, Selection Committee for Scientists, Office of PSA
- Member, Governing Council, National Institute of Plant Genome Research
- Member, KVPY selection committee, DST
- Member, Science and Engineering Research Board- Empowered Committee
- Member, Jury, Sun Pharma Awards
- Member, Scientific Advisory Committee, Rajiv Gandhi Centre for Biotechnology, Trivandrum
- Member, Distinguished Scientist Selections, Department of Biotechnology
- Member, Director Selections, Indian Association for Cultivation of Science

Outreach activities during the reporting period

- **80th CSIR Foundation Day Lecture**, September 26, 2021. Title: *Looking into life, the beauty of cellular struggle*.
- **ICAR distinguished lecture,** for Azadi Ka Amrit Mahotsav. November 23, 2022. Title: *Emerging diseases and our immunity*
- **Distinguished Lecture**, UGC-Human Resource Development Centre, Panjab University, Chandigarh November 26, 2021. Title: *The current challenges faced by human immunity.*
- **INSA Presidential Lecture,** Dec 15, 2021. *Science Communication in current times: Challenges and Opportunities.*
- **Inspirational Lecture.** Kendriya Vidyalaya, Khanapara, Guwahati, January 20, 2022 *Fun Journey of a scientist.*
- **Keynote Address.** International symposium on "Emerging Materials for Biomedical Engineering " January 30th, 2022. *The relevance of Science Academies in Indian Science.*
- **Distinguished Lecture in Life Sciences. USBT Distinguished Lecture Series in Life Sciences. Guru Govind Singh Indraprastha University.** February 1, 2022. *The beauty of cellular struggle: of invaders and host.*
- **Panel lecture, Indian National Science Academy,** Misinformation in science, March 3, 2022. *Predatory Journals and its effect on scientists.*
- **Keynote Lecture**, **National Academy of Sciences** symposium March 8, 2022. *Celebrating women Scientists as leaders.*
- **Keynote lecture**, **National Institute of Animal Biotechnology**, Hyderabad on Women in STEMM: facts and inspirations. March 8, 2022. *Celebrating the success of scientists who happens to be women.*

Addressing key components of macrophage defense signaling targeted by *Leishmania* parasites for successful survival towards developing robust anti-leishmanials with drug delivery systems

Dr. Pijush K. Das, pijushdas@iicb.res.in

Brief Summary of the Scientific Achievements

The work in my laboratory is centered on studying macrophage biology using visceral leishmaniasis as a model macrophage disease. The key question we try to address is how macrophage signaling pathways that lead to the production of robust defense molecules are hijacked by *Leishmania* parasites and therefore have tremendous potential for the development of therapeutic targets in general for macrophage-associated diseases. Earlier we identified key regulatory molecules along with their underlying mechanisms exploited by *Leishmania,* which include A20, a host deubiquitinase, and UCP2 (uncoupling protein 2), an inner mitochondrial membrane protein.

Earlier we exploited UCP2 to develop anti-leishmanial by using genepin, which is a specific inhibitor of UCP2. Now, we would like to exploit A20 as it is up-regulated by *Leishmania* and is instrumental in suppressing NF-κB and inflammasome complex formation. The work is being carried out and is on the verge of completion.

Another aspect of our study is the relationship of the programmed cell death 1 receptor (PD-1) with the onset of leishmaniasis. Earlier we showed that *Leishmania* induces differential regulation of PD-1 in the early and late phases of infection, which exploits anti-inflammatory responses to suppress host-protective activities. Now we have unraveled the complete cascade of signaling events how the PD-1 pathway fine-tunes the pro-inflammatory responses in macrophages to prevent exacerbating inflammation and how *Leishmania* exploits it to foster their intracellular survival. This study has been communicated for publication.

In addition, we published comprehensive literature to highlight the challenges of the current anti-leishmanial therapies, coupled with the unraveling of the new therapeutic modalities and their mechanisms of action which potentiates them as better anti-leishmanial agents, thereby overcoming the problems of present-day therapeutics. Furthermore, it also sheds light on the importance of various immunomodulators and investigational drugs that might come up as effective remedial therapeutics against leishmaniasis as future drugs.

We have also published a comprehensive review to summarize our current understanding of the mechanisms of inflammasome activation in macrophages and cAMP homeostasis of the parasite, leading to parasite viability within the macrophages and the establishment of infection. We have also published Protocols for inflammasome study for the benefit of relevant researchers. Furthermore, we took into account recent progress in translating these research areas into therapeutic strategies aimed at combating macrophage-associated diseases.

Publications

- Roy, S., Gupta, A.K., Ukil, A. and **Das, P.K.** (2022) PD-1 negatively regulates macrophage immune activation by turning off JNK and STAT1 signaling: Exploited by *Leishmania* for its intra-macrophage survival. (Communicated).
- Saha, S., **Das, P.K.** and Ukil, A. (2022) Heme Oxygenase 1: A potential drug target having multiple functions. (2022). Invited Book Chapter, Nova Science Publishers, New York, USA.
- Biswas, A., Bhattacharjee, A. and **Das, P.K.** (2022) Modulation and determination of the status of inflammasomes in *Leishmania*-infected macrophages in Immune Homeostasis for Lab Protocol Series Methods in Molecular Biology, Springer publications (In press).
- Bhattacharjee, A., Biswas, A. and **Das, P.K.** (2022) The tale of mastering macrophage environment through the control of

inflammasome-mediated macrophage activation and cAMP homeostasis by the protozoan parasite *Leishmania.* doi: 10.5772/intechopen.102671, InTech Open Limited. UK.

- Roy, S., Ukil, A. and **Das, P.K. (**2021) Anti-infectives to Combat Leishmaniasis, in: Frontiers in Clinical Drug Research-Anti-Infectives, Vol. 8, p 174-208. Bentham Publishers.

Scientific activities in which involved

Member of Sectional Committee VI (General Biology) of INSA for 2021-2024, Member of Fellowship Securitizing Committee of NASI, Chairman and Member of CSIR SRF/RA committee, Expert in the Assessment Committee of CSIR scientists, Member of Life Sciences discipline of CSIR-NET, Expert for assessment of Professor at (NIT) Rourkela and JNU, New Delhi, Member of Award Selection committee of SBC, Reviewer of projects from National and International funding agencies, Reviewer of papers submitted to National and International journals. Ph.D. thesis Examiner of JNU, Delhi University, Kalyani University, ACSiR, CCMB, etc., and actively involved in delivering Science Popular Lectures to various schools and colleges as part of the Science Awareness Programme of INSA.

Future Plan

- Complete understanding of the underlying mechanism of GRA as a suppressor of A20 function and genipin as a suppressor towards NLRP3 inflammasome mediated activation of pro-inflammatory cytokine production.
- Deciphering the intricate signaling events involving the exploitation of anti-inflammatory PD-1 pathway to suppress host-protective responses towards the development of potential anti-parasitic compounds.
- Comprehensive review of literature of my career-long extensive work on how macrophage signaling pathways that lead to the production of robust defense molecules are hijacked by macrophage-associated pathogens like *Leishmania* which might lead to the development of potential therapeutic leads.

Multifaceted roles in the management of Leishmaniasis

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Group Members

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Anirban Bhattacharyya, SRF (ICMR)
Sohitri Mukherjee, Project Associate-I (GCRF)
Suman Midya, Project Assistant Level-I (JCB)

Research Activities

Visceral Leishmaniasis (VL) is a deadly parasitic disease occurring worldwide. As a comprehensive approach towards its control, my lab has been working on its diagnosis, therapeutics, vaccine strategies, host-pathogen interaction and variation in immunological parameters for the last three decades.

For robust diagnostic tests and effective treatment against VL, we have identified, cloned, and purified a noble antigen, Repressor of Differentiation Kinase 2 (RDK2), and checked its immunodiagnostic potential. RDK2 has an important role in life cycle progression in various parasites. However, its character in leishmaniasis remains elusive. Herein, we used RDK2 for the diagnosis of humans in VL using serum and urine samples. In this study, we found that urine samples exhibit better sensitivity and specificity, at 93.8% and 85.7%, respectively, compared to serum at 78% and 87%, respectively. Thus, the rRDK2 antigen can be applied for non-invasive diagnosis with urine as a sample over serum with a possibility of also acting as a test of cure.We also found a novel recombinant antigen from *Leishmania donovani* called Otubain Cysteine Peptidase (Ld-OCP) and tested its diagnostic and prognostic potential using well characterised patients of human VL. Our results in serological assay showed that Ld-OCP had comparable sensitivity with Indian patients and improved response for diagnosing Brazilian patients when compared with the commercially available test rK39. Moreover, our rapid diagnostictest could also monitor the treatment response of Indian VL patients which was not possible with rK39.

The immunochromatography-based lateral flow assay (LFA) has attracted attention for a long time due to its ability to give results within a few minutes, mainly in resource-poor settings. In our study, we optimized and developed the LFA to detect anti-*Leishmania* antibodies for VL diagnosis. The performance of the developed test was evaluated with serum and urine samples of Indian VL patients and Brazilian sera. The new test exploits well-studied and highly-sensitive purified antigens, LAg isolated from *L. donovani* promastigotes and protein G conjugated colloidal-gold as a signal reporter. The intensity of the bands depicting the antigen-antibody complex was optimized under different experimental conditions and quantitatively analyzed by the ImageJ software. For the diagnosis of human VL in India, LFA was found to be 96.49% sensitive and 95% specific with serum, and 95.12% sensitive and 96.36% specific with urine samples, respectively. The sensitivity and specificity of LFA were 88.57% and 94.73%, respectively, for the diagnosis of Brazilian VL using patients' sera infected with *Leishmania infantum*. LFA is rapid and simple to apply, suitable for field usage where results can be interpreted visually and particularly sensitive and specific in the diagnosis of human VL. Serum and urine LFA may improve diagnostic outcomes and could be an alternative for VL diagnosis in settings where tissue aspiration is difficult to perform.

Various T cell subsets such as Tregs are involved in pathologies in suppressive host immune response by modulation Th17/Th1 effector response. However, in VL the role of immunoregulatory cytokines and then correlation with Th17 cell is poorly understood. Our results in progressive VL model of BALB/c mice indicate that during the initial phase of infection. Th17 response is heightened and during late phase of the disease this response is suppressed. This suppresses response of Th17 is actually happening because of the elevation of

regulatory T cell during onset of the disease which actively suppressed the proliferation of Th17 cells. Mechanistically, regulatory cytokines secreted from Treg cell such as IL-35 and TGF-β synergistically downregulates Th17 during chronic infection. This modulation of Th17-Treg module during VL could be harnessed for host derived therapeutic intervention.

Acute toxicities of available chemotherapeutic regimes customarily available for leishmaniasis along with lack of vaccines currently necessitate the development of new drugs. In this regard, we have evaluated a liposomal formulation of HO-3867 (a curcumin analogue) for the first time against *Leishmania*. This nanoformulation of HO-3867 (PC-SA/HO-3867) was observed to be a strong apoptosis inducer in *L. donovani*, being non-toxic towards healthy cells. PC-SA/HO-3867 treatment induced *L. donovani*metacaspase and PARP1 activation along with downregulation of the Sir2 gene in promastigotes. PC-SA/HO-3867 arrested intracellular *L. donovani* amastigote burden *in vitro*, with reactive oxygen species (ROS) and nitric oxide (NO)-mediated parasite killing. Thus, liposomal HO-3867 can be a highly promising and non-toxic nanoparticle-based therapeutic alternative against leishmaniasis, warranting preclinical studies in future.

Future Plans

- Investigating the effects of novel cationic liposome mediated immunotherapy on checkpoint blockade in cancer.
- Development of GMP/GLP grade liposomal amphotericin vaccine against VL.

Extramural funding

Indian Funding

Awarded a Sir J. C. Bose Fellowship under DST research grant, of Rs. 89,75,000/- project entitled "Exploration of recognized potent antigen and liposomal tools to design a defined subunit T cell vaccine and chemotherapy against VL" from 2016-2022.

International Collaborators

- Awarded a MRC-Global Challenge Research Fund (GCRF) Capacity of British Pound 7.8M along with Prof Graham Sandford, Dr Steven Cobb, Prof Patrick Steel and Dr Ehmke Pohl of Durham University, U.K. Project entitled "A Global Network for Neglected Tropical Diseases" from 2017-2022.
- Awarded a Engineering and Physical Sciences Research Council (EPSRC), of British Pound 62,256.46 along with Prof Patrick Steel as Principal Investigator, Durham University, and Prof. Nahid Ali as Co-Investigator, CSIR-Indian Institute of Chemical Biology, U.K. Project entitled "Patient-centric supramolecular formulations of new anti-leishmanial drugs for Indian Communities" from 2020-2022.

Publications (journal and conference)

- Kamran, M., Ejazi, SA., Choudhury, ST., Bhattacharyya, A., Tanishka, K., Pandey, K., Das, VNR., Das, P., da Silva, FO., Costa, DL., Costa, CHN., Rahaman, M., Goswami, RP., **Ali, N**. 2021. A novel antigen, otubain cysteine peptidase of *Leishmania donovani* improves serodiagnosis of visceral leishmaniasis and monitors treatment response. *Clinical Infectious Diseases*.73(7):1281-1283.
- Ejazi, SA., Choudhury, ST., Bhattacharyya, A., Kamran, M., Pandey, K., Das, VNR., Das, P., da, Silva, FO., Costa, DL., Costa, CHN., Rahaman, M., Goswami, RP., **Ali, N**. 2021. Development and Clinical Evaluation of Serum and Urine-Based Lateral Flow Tests for Diagnosis of Human Visceral Leishmaniasis. *Microorganisms*. 2021;9(7):1369.
- Asad, M., Sabur, A., Kamran, M., Shadab, M., Das, S., **Ali, N**. 2021. Effector functions of Th17 cells are regulated by IL-35 and TGF-β in visceral leishmaniasis. *FASEB J*. 35(9):e21755. doi:10.1096/fj.202002356RR.
- Das, A., **Ali, N**. 2021. Nanovaccine: an emerging strategy. *Expert Review of Vaccines*. 2021;20(10):1273-1290. doi:10.1080/14760584.2021.1984890.
- Chakraborty, A., Banerjee, S., Mukherjee, B., Poddar, MK., **Ali, N**. 2021. Calorie restriction modulates Neuro-immune system differently in young and aged rats. *International Immunopharmacology*. 100:108141. doi:10.1016/j.intimp.2021.108141
- Das, A., Kamran, M., and **Ali, N**. 2021. HO-3867 induces ROS-dependent stress response and apoptotic cell death in *Leishmania donovani*.*Frontiers in Cellular and Infection Microbiology*.Dec 3;11:774899.
- Bhattacharyya A, Kamran M, Ejazi SA, Das S, Didwania N, Bhattacharjee R, Rahaman M, Goswami RP, Pandey K, Das VNR, Das P, Gayen S, **Ali N**. 2022. Revealing a Novel Antigen Repressor of Differentiation Kinase 2 for Diagnosis of Human Visceral Leishmaniasis in India. Pathogens. Jan 20;11(2):120.

DNA topoisomerases of *Leishmania* Parasites

Dr. Hemanta K. Majumder, hemantamajum@gmail.com

Research Activities

The Scientist has made outstanding contributions in the area of basic biochemistry of kDNA metabolism and DNA topoisomerases of Leishmania donovani. He has worked on the development of DNA topoisomerases targeted therapeutics. He has also made significant contribution in the area of cell death induced by DNA topoisomerases inhibitors.

Dr. Majumder is the convener of INSA Local Chapter and the President of NASI local Chapter, Kolkata.

Future Research Plan

To understand the unique features of type IA topoisomerase of L. donovani and to know how the enzyme differs from bacterial and yeast homologues.

Extramural funding

INSA Senior Scientist contingency fund.

Publications

- Chowdhury S.R, Das S.K, Banerjee B. Chowdhury S.P., Majumder H.K. and Das B.B. TdP1 knock out L. donovani accumulate topoisomerase 1-linked DNA damage and are hyper Annual sensitive to clinically used anti leishmanial drugs. FASEB Journal, Vol. 36(4) e22265 (2022)
- Chowdhury S.R., Bhoumik, A, Gupta, V.K. and Majumder H.K. Type II topoisomerases in trypanosomatids and apicomplexan parasites. ACTA Tropica 234, 1066B (2022). (submitted in March 2022 and accepted in July 2022)

Achievements (Awards, honours and membership)

K.S. Krishnan Memorial Lecture Award of INSA dated December 23,2021. The Award comprises a citation and an honorarium of Rs. 25000/-.

Lectures delivered

- Parasites are smarter than humans. Delivered as a Science lecture at German Consulate. Kolkata on October 15,2020.
- A Tale of Cell's Magicians: DNA topoisomerases and an old enemy Leishmania. Delivered at Royal Society of Biology, Northern Region, RSB, Durham, UK on May 26,2022.
- Introduction to Molecular Cloning and Recombinant DNA Technology at Sister Nivedita University, on December 21,2021.
- Leishmania research: Four decades of journey. When Science meets life at SBCI Kolkata Chapter Conference at Sister Nivedita University, Kolkata on April 09, 2022.

Spike protein induced altered membrane fluidity and phase transition in antigen presenting cells and effect on T-cell stimulating ability

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Research Activities

SARS-CoV-2 spike protein (S) is associated with the entry of virus inside the host cell by recruiting its loop dominant receptor binding domain (RBD). RBD plays a crucial role in the attachment of the virus particles on the surface of host cells through strong interaction with the ACE2 receptor. It is known that cholesterol, the master regulator of infection modulates both the early (receptor binding, S1-S2 segmentation through proteolysis) and late events (the viral membrane-host cell membrane fusion by S2 region and entry of viral RNA inside the host cell accompanying the immune deactivation process). Thus the presence of cholesterol-rich motif in spike RBD could potentially influence the interaction with the host ACE2 receptor. Here, we employed molecular docking of RBD with cholesterol using iGemdock (http://gemdock.life.nctu.edu.tw) software to identify the cholesterol binding pocket and to evaluate the binding energies as well as the most stable cholesterol bound structure. We found that the N-terminal motif (^{21}ERFPNITN^{28}, red) and C-terminal motif (^{221}PKKSTNL^{227}, blue) create the cholesterol binding pocket (Figure 1). The N-terminal binding motif resides near the CRAC domain (^{29}LCPFGEVFNATR^{40}) which is present in RBD. Since the pocket is formed in the vicinity of the CRAC domain of RBD, we believe that cholesterol binding presumably effects the interaction property of RBD with host cell receptor by stabilizing the RBD-ACE2 complex. Also altering this cholesterol level from the lipid bilayer in the host cell might affect the interaction between the RBD and ACE2 receptor. We also showed that pre-pulsing of Antigen presenting cells with spike protein caused upregulation of class II MHC protein coupled with upregulation of TNF-alpha and IL-6 production suggesting immunostimulatory role of spike protein.

Publications

- K Roy, S Roy and S Roy (2021) A mimotope attached to an ITIM–SHP-1 interaction inhibitory peptide boosts immune response and efficacy. **RAC Med Chem**. *DOI: 10.1039/d1md00099c*
- K Mondal, S Das, K Naskar and S. Roy (2021) Modulation of Splenic B Cell Subsets during Experimental *Leishmania donovani* Infection in BALB/c Mice. *Pathogens* **2021**, *10*(7),814; **https://doi.org/10.3390/pathogens10070814**
- B Mukherjee , K Mukherjee , P Nanda , R Mukhopadhayay , V Ravichandiran , S N Bhattacharyya , S Roy (2021)Probing the molecular mechanism of aggressive infection by antimony resistant Leishmania donovani. Cytokine. Sep;145:155245.doi: 10.1016/j.cyto.2020.155245.
- A Ghosh, P K. Kar, A Gautam, R Gupta, R Singh, R Chakravarti, V Ravichandiran, S Ghosh Dastidar, D Ghosh and S Roy (2022) An insight into SARS-CoV-2 structure, pathogenesis, target hunting for drug development and vaccine initiatives. RSC Med Chem 13 (6), 641

Stringent response regulator DksA, signaling and regulation of virulence in *Vibrio cholerae*

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Group Member

Dr. Priyanka Dhar

Collaborator

Dr. Bhabatosh Das, Associate Professor, Translational Health Science and Technology Institute (DBT), Faridabad, India.

Research Activities

Nutritional deficiency mediated stringent response regulatory circuit involving *relA*, *spoT*, *relV*, *gppA* and *dksA* genes of the cholera pathogen *Vibrio cholerae* has been successfully established from our group and it has been observed that the deletion of the *dksA* gene encoding the stringent response regulatory protein DksA leads to decrease in motility, haemagglutinin protease (HAP) and cholera toxin production, which are critical virulence traits of *V. cholerae*. The phenotypes exhibited by the Δ*dksA* mutant are similar to that of increased levels of intracellular signaling molecule 3', 5' cyclic diguanylic acid (c-di-GMP). Recently, it has been shown that c-di-GMP controls motility of *V. cholerae* via the positive regulatory protein TfoY and also through the LonA protease (encoded by the *lonA* gene), which degrades TfoY. Thus, it seems that the LonA protease serves as a negative regulator of motility in *V. cholerae*. Previously it has been shown that the *V. cholerae* Δ*dksA*Δ*lonA* double mutant CΔdksAΔlonA is hypermotile compared to the control strains used, namely, C6709 (wild-type), CΔdksA (Δ*dksA*), and CΔlonA (Δ*lonA*). In the Δ*dksA* mutant probably due to high intracellular c-di-GMP level there is rapid degradation of TfoY leading to hypomotility ofΔ*dksA* cells. Thus, it has been concluded that deletion of the *lonA* gene in *V. cholerae* under Δ*dksA* genetic background probably leads to significant accumulation of TfoY protein in the double mutant CΔdksAΔlonA, which ultimately rescued Δ*dksA* cells from hypomotility to hypermotility phenotype. We have shown that expression of the *vieA* gene encoding c-di-GMP degrading phosphodiesterase (PDE) enzyme of *V. cholerae* cloned under the arabinose inducible promoter P_{BAD} of the expression vector pBAD24 in Δ*dksA* mutant led to rectification of its motility defect. It has also been found that the decreased production of HAP in Δ*dksA* mutant could be rescued by deletion of the *lonA* gene, i.e., in CΔdksAΔlonA double mutant exhibited similar HAP production like wild-type strain C6709. Deletion of *dksA* probably led to increase in intracellular levels of c-di-GMP which modulates *hapR* gene expression and hence there is a decrease in HAP production. Thus, deletion of the *lonA* gene under Δ*dksA* background can restore normal HAP production probably by down-regulating c-di-GMP production, which needs further investigation.

Future research plans

- In this phase of work the recombinant plasmid pClVieA (insert DNA codes for a PDE) will be introduced in different *V. cholerae* Δ*lonA* mutant strains followed by controlled expression of the cloned gene by induction with defined L-arabinose concentration and examination of the motility and HAP phenotypes. Similarly, cloning of a gene encoding c-di-GMP synthesizing enzyme (Accession No. *vca0956*) will be attempted followed by examination of its overexpression effects in wild-type and in different mutant strains.
- Experiments will be done to know the intracellular levels of c-di-GMP in wild-type and different *V. cholerae* mutants including Δ*dksA* cells by thin layer chromatography.
- Writing of a manuscript has been initiated. A relevant review article entitled 'c-di-GMP and stringent response mediated gene regulation in human diarrheal pathogens' has been compiled and will be submitted for publication.

Extramural funding

Funded by CSIR Emeritus Scheme (No. 21(1100)/20/EMR-II, CSIR, New Delhi.

Publications (journal and conference)

- Biswas Q, Purohit A, Kumar A, Rakshit D, Maiti D, Das B, **Bhadra RK.** Genetic and mutational analysis of virulence traits and their modulation in an environmental toxigenic *Vibrio cholerae* non-O1/non-O139 strain, VCE232. *Microbiology.* 2022 Feb; 168(2). doi: 10.1099/mic.0.001135. PMID: 35113781.
- Dhar P, Rakshit D, **Bhadra RK**.. DksA-LonA mediated regulation of motility and haemagglutinin protease (HAP) production in *Vibrio cholerae.* (Presented in the 16th Asian Conference on Diarrhoeal Disease and Nutrition held during 11-13 November 2022, Kolkata, India).
- Attended and Chaired a Session in the 91st Annual meeting of Society of Biological Chemists (India) held during 8-11 December 2022, Kolkata.

Molecular Genetics Division

This department has the mandates to identify the importance of small RNA level -modulation in the mammalian cell physiology; to determine the molecular basis of gene delivery to mitochondria, and to understand the eukaryotic transcriptional regulatory mechanisms and their role in human diseases. One objective of this department is poised to indentify the mechanisms that regulate activity and transport of different microRNAs in mammalian cancer and immune cells and tissues to relate these to disease onset and progression. Mechanistic understanding and regulation transcription process at molecular level is also under investigation. Using a combination of basic and applied approaches we will study the molecular basis of genetic disease and its probable therapy targeting diverse steps both in pre- and post-transcriptional events.

Regulation of miRNA Activity in Mammalian Cells: Importance of Extracellular Vesicles

Dr. Suvendra Nath Bhattacharyya, suvendra@iicb.res.in

Group Members

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Background

The understanding of post-transcriptional regulation of mRNAs added an additional layer after the discovery of microRNAs (miRNAs). miRNAs, short tiny regulators of mRNA activity binds to the 3' UTR of mRNA with partial complementarities and represses its' expression either by target mRNA degradation or translational repression. miRNAs are 20-22 nucleotide long short RNAs contains 5' conserved seed sequence complementary to 3' UTR of target mRNA. miRNA biogenesis is a sequential modification process where miRNAs firstly transcribed from its' specific gene in the form of primary miRNA transcripts (pri-miRNAs) with the help of RNA polymerase II. Pri-miRNAs then processed into precursor miRNAs (pre-miRNAs) with the endonucleolytic activity of micro processor complex (DROSHA/DGCR8) to form a ~70nt. long cleaved pre-cursor miRNA (pre-miRNA) in the nucleus. Pre-miRNA then exported out from the nucleus with the anchorage of Exportin 5 protein complex. Pre-miRNA then further cleaved to form miRNA/miRNA* with the catalytic activity of DICER1. miRNAs then incorporated into RNA induced silencing complex (miRISC) consists of AGO and TRBP proteins for further miRNA activity on target mRNA.

Aims and Objectives

Research at RNA Biology Research Laboratory is concentrated on identification of novel gene regulatory mechanisms operating in mammalian cells that are primarily executed by small regulatory RNA- the microRNA. We explore the mechanisms that regulate microRNAs levels and activities in mammalian immune, neuronal and cancer cells. The major goal of our research is to identify the machineries that either by regulating the intra and extracellular transport of microRNAs or by modifying microRNA interacting proteins affects the microRNA function in mammalian cells. Contributions of subcelluar structures and organelles in compartmentalization of miRNAs biogenesis, function and turnover in mammalian cells is also explored under this research aim. Alteration in miRNA-machinery upon exposure of mammalian cells to pathogens and its implications in the disease process is the other major thrust area of our research.

Work Achieved

"Intercellular communication via extracellular Vesicles is an extraordinary concept that received momentum when the epigenetic information in forms of miRNAs was found to be exchangeable via EVs between mammalian cells. This have revolutionized our concept of gene regulation in higher eukaryotes. Among several key questions that remained unanswered in the filed of intercellular miRNA transport between mammalian cells, the specificity and mechanism of miRNA export via EVs is one of the most fascinating questions. How, selectively miRNAs are getting picked up and sorted into EVs is the question that Dr Suvendra Bhattacharyya's laboratory has addressed. With an expertise in

miRNA research field, he discovered the mechanism of selective miRNA export from stressed hepatic cells; identified key factors controlling miRNA export process from macrophage and non-macrophage cells; identified specific role of mitochondria in intra and extracellular miRNA transport; and identified the role of RNA processing bodies and GW182 bodies in controlling miRNA transport in mammalian cells. We have recently published a paper to show how the pathogen invasion control the miRNA export and shuttling machineries of the infected and neighbouring cells to create the infection niches via miRNAs associated with extracellular vesicles secreted by infected cells (Fig.1).

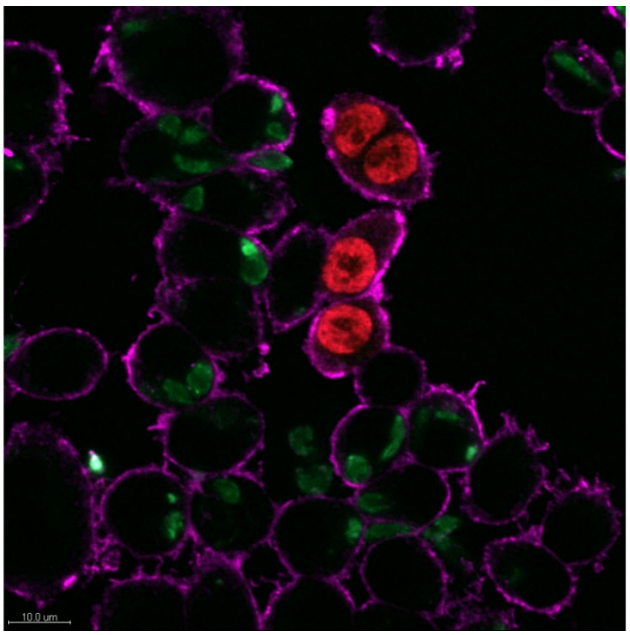

Figure 1: miRNA trafficking controls the pathogen infection process. Extracellular vesicle from infected cells carry miR-146a to get transferred to naive macrophage to polarize them to M2 stage.

Future Research Plans

How the EV-containing miRNAs are taken to neighbouring cells through the extracellular matrix is a fascinating question and we have identified a matrix metaloproteases that by digesting the extracellular matrix can allow the movement of EVs across the cell boundaries. We are currently exploring the mechanistic detail of this process.

Publications

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- Ganguly, S. Ghoshal, B., Banerji, I., Bhattacharjee, S. Chakraborty, S, Goswami, A., Mukherjee, K. and **Bhattacharyya, S.N.** (2022) Leishmania survives by exporting miR-146a from infected to resident cells to subjugate inflammation. **Life Sci Alliance.** 5(6)pii: e202101229.
- Das, A., Basu, S., Bandyopadhyay, D., Mukherjee, K., Dutta, D., Chakrabarti, S., Adak, M., Jana, S., Bose, S., Swarnakar, S., Chakrabarti, P. and **Bhattacharyya, S. N.** (2021) Inhibition of Extracellular Vesicle-Associated MMP2 Abrogates Intercellular Transfer of Hepatic miR-122 to Tissue Macrophages and Curtails Liver Inflammation. **iScience** 12(24) 103428.

Awards/Honours/Memberships

- Dr Suvendra N Bhattacharyya featured in in the book '75 under 50 Scientists Shaping Today's India' by Dept. of Science and Technology, Govt. of India and VIGYAN PRASAR
- Dr Suvendra N Bhattacharyya selected for Sun Pharma Research Award in Medical Sciences in Basic Research

Understanding mechanisms of eukaryotic transcriptional regulation and leukemia development by MLL and MLL fusion partner proteins

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Collaborators

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Background

Human MLL protein is a histone H3-K4 methyl transferase that is associated with transcriptional activation. Balanced chromosomal translocations between MLL and variety of MLL fusion partners (>80) give rise to both acute myeloid and lymphoid leukemia with two-year survival rate of <50%. Recently, attempts have been made towards understanding molecular mechanisms of action of MLL fusion partners and corresponding MLL fusion proteins in transcriptional regulation and leukemia development. These studies have suggested a unified mechanism of action of common MLL fusion partners in regulating transcription through their presence in a large multi-subunit Super Elongation Complex (SEC). However, mouse models of MLL fusion proteins suggest distinct mechanisms of action of individual MLL fusion proteins and corresponding leukemia development.

In support of this hypothesis, our earlier studies and few recent studies have shown that, in contrast to a large megadalton static complex, the MLL fusion partners form various sub-complexes with overlapping subunits for dynamic regulation of different steps of transcription. Further, few recent studies have also shown different requirement of MLL fusion partners (outside the context of SECs) for transcriptional regulation and leukemia development. Therefore, for better understanding of overall mechanisms of functional regulation and disease pathogenesis, more detailed analyses are required.

In our lab, we are currently exploring detailed mechanisms of action of few MLL fusion partners in transcriptional regulation that is both dependent and independent of SECs. Further, we would extend our studies towards exploring importance of these novel mechanistic understanding in MLL fusion-mediated leukemogenesis.

Aims and Objectives

- Detailed studies on role of human ZMYND8 protein in positive regulation of transcription
- Understanding of role of human TFIID complex in regulation of elongation control through promoter proximal pausing
- Mechanistic understanding of role of human DBC1 protein in regulation of ELL functions within mammalian cells
- Temporal regulation of functions of SEC through post-translational modification of AFF1
- Understanding of mechanisms of transcriptional regulation by human FKBP5 through regulation of P-TEFb complex functions within mammalian cells.

Work Achieved

Negative feedback loop mechanism between EAF1/2 and DBC1 in regulating ELL stability and functions

Although ELL-associated factors 1 and 2 (EAF1/2) have been shown to enhance RNA Polymerase II-mediated transcription *in vitro*, their functional roles *in vivo* are poorly known. In this report, we show functions of these proteins in regulating ELL stability through their competitive binding with HDAC3 at the N-terminus of ELL. Reduced HDAC3 binding to ELL causes increased acetylation leading to reduced ubiquitylation-mediated degradation. Similar functional roles played by DBC1 in regulating ELL stability further prompted in-depth analyses that

demonstrated presence of negative feedback loop mechanisms between DBC1 and EAF1/2 in maintaining overall ELL level. Mechanistically, increased DBC1 reduces EAF1/2 level through increased ubiquitylation involving E3 ubiquitin ligase TRIM28, whereas, increased EAF1/2 reduces DBC1 level through reduced transcription. Physiologically, after few passages, ELL levels in either DBC1 or EAF1 knockdown cells are restored through enhanced expression of EAF1 and DBC1 respectively. Interestingly, for maintenance of ELL level, mammalian cells prefer EAF1-depndent pathway during exposure to genotoxic stress, and DBC1-dependent pathway during exposure to growth factors. Thus, we describe coordinated functions of multiple factors including EAF1/2, HDAC3, DBC1, and TRIM28 in regulating ELL protein level for optimal target gene expression in a context-dependent manner within mammalian cells (Fig. 1).

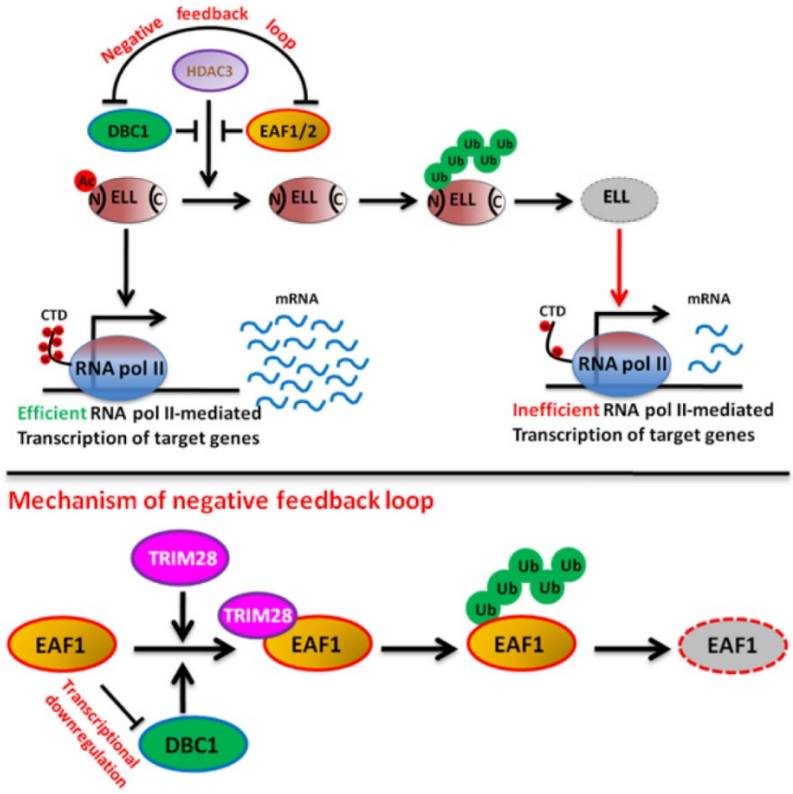

Figure 1: Model depicting the overall regulatory mechanisms in play for maintaining ELL levels within cells. ELL acetylation at its N-terminus mediates its stability and thus causes efficient Pol II-mediated transcription of target genes. Upon deacetylation by HDAC3, ELL is polyubiquitylated and subsequently degraded by the ubiquitin proteasome system. EAF1 and EAF2, by virtue of their ability to competitively bind with HDAC3 at the N-terminus of ELL, cause stability and target gene expression by ELL. Interestingly, DBC1 which also stabilizes ELL in a similar manner, cross-talks with EAF1 through a negative feedback loop mechanism. DBC1 regulates expression of EAF1 through enhancing its interaction with ubiquitin E3 ligase TRIM28,whereas, EAF1 on the other hand regulates expression of DBC1 by regulating its mRNA expression through transcription. Implications of these mechanisms of regulation of ELL stability in physiological response to external cues are also deciphered in this study as described.

ATM-mediated ELL phosphorylation enhances its self-association through increased EAF1 interaction and inhibits global transcription during genotoxic stress

Mammalian cells immediately inhibit transcription upon exposure to genotoxic stress to avoid fatal collision between ongoing transcription and newly recruited DNA repair machineries to protect genomic integrity. However, mechanisms of this early transcriptional inhibition are poorly understood. In this study, we decipher a novel role of human EAF1, a positive regulator of ELL-dependent RNA Polymerase II-mediated transcription *in vitro*, in regulation of temporal inhibition of transcription during genotoxic stress. Our results show that, besides Super Elongation Complex (SEC) and Little Elongation Complex (LEC), human ELL (aka ELL1) also forms a complex with EAF1 alone. Interestingly,

contrary to the *in vitro* studies, EAF1 inhibits ELL-dependent RNA polymerase II-mediated transcription of diverse target genes. Mechanistically, we show that intrinsic self-association property of ELL leads to its reduced interaction with other SEC components. EAF1 enhances ELL self-association and thus reduces its interaction with other SEC components leading to transcriptional inhibition. Physiologically, we show that upon exposure to genotoxic stress, ATM-mediated ELL phosphorylation-dependent enhanced EAF1 association results in reduced ELL interaction with other SEC components that lead to global transcriptional inhibition. Thus, we describe an important mechanism of dynamic transcriptional regulation during genotoxic stress involving post-translational modification of a key elongation factor (Fig. 2).

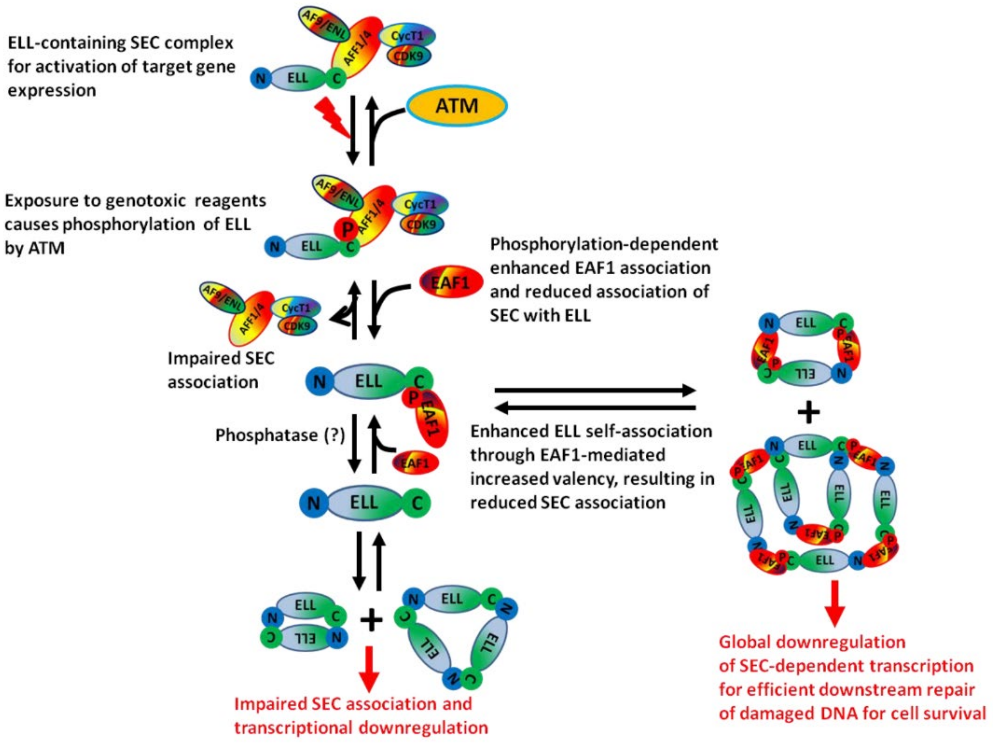

Figure 2: This model of transcriptional regulation is based on our observation of the absence of EAF1 in ELL-containing SEC. ELL protein, by virtue of its tendency to self-associate that requires both N and C terminus, the C-terminal end becomes inaccessible for its interaction with other SEC components. EAF1, by virtue of its ability to interact at both N and C terminus of ELL, provides additional valency for the ELL protein to interact with and further self-associate, thus enhancing overall self-association of ELL upon its binding and therefore causing transcriptional downregulation. Interestingly, genotoxic stress-dependent ATM-mediated ELL phosphorylation increases ELL-EAF1 interaction and thus leads to enhanced ELL self-association. This enhanced self-association further leads to reduced ELL binding with other SEC components and ultimately causes global transcriptional downregulation in response to genotoxic stress. This transcriptional downregulation is key for efficient downstream repair of damaged DNA, for overall cell survival upon exposure to genotoxic stress. ELL mutants with impaired ATM-mediated phosphorylation and EAF1 interaction, fail to efficiently downregulate global transcription upon exposure to genotoxic stress and leads to accumulation of DNA damage, causing reduced cell survival.

Future Research Plans

In future, majority of our research efforts would be directed towards addressing dynamic regulation of functional activity of different SEC factors during transcription-coupled DNA repair process. Especially we would like to focus more on regulation of SEC functional activity by various post-translational modification.

Publications

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- Dipika Yadav, Koushik Ghosh, Subham Basu, Robert G. Roeder, and Debabrata Biswas: Multivalent role of human TFIID complex in recruiting elongation components at the promoter proximal region for transcriptional control. ***Cell Reports.*** 2019 Jan 29;26(5):1303-1317.e7. doi: 10.1016/j.celrep.2019.01.012.
- Nidhi Kumari, Md. Abul Hassan, Xiangdong Lu, Robert G. Roeder, and Debabrata Biswas: AFF1 acetylation by p300 temporally inhibits transcription during genotoxic stress response. ***Proc Natl Acad Sci U S A.*** 2019 Oct 29;116(44):22140-22151. doi: 10.1073/pnas.1907097116. Epub 2019 Oct 14.
- Subham Basu, Mahesh K. Barad, Dipika Yadav, Arijit Nandy, Bidisha Mukherjee, Jit Sarkar, Partha Chakrabarti, Satinath Mukhopadhyay, and Debabrata Biswas: **DBC1, p300, HDAC3 and Siah1 coordinately regulate ELL stability and function for expression of Type 2 diabetes-linked genes**. ***Proc Natl Acad Sci U S A.*** 2020 Mar 24;117(12):6509-6520. doi: 10.1073/pnas.1912375117. Epub 2020 Mar 9.
- Dheerendra Pratap Mall, Subham Basu, Koushik Ghosh, Nidhi Kumari, Abhishake Lahiri, Sandip Paul, and Debabrata Biswas: Human FKBP5 negatively regulates transcription through inhibition of P-TEFb complex formation. ***Mol Cell Biol.*** 2022 Jan 20;42(1):e0034421. doi: 10.1128/MCB.00344-21. Epub 2021 Nov 15.
- Subham Basu, Arijit Nandy, Mahesh K. Barad, Sujay Pal, and Debabrata Biswas: Negative Feedback Loop Mechanism between EAF1/2 and DBC1 in Regulating ELL Stability and Functions. ***Mol Cell Biol.*** 2022 Oct 20;42(10):e0015122. doi: 10.1128/mcb.00151-22. Epub 2022 Aug 29.
- Sujay Pal, Dipika Yadav, and Debabrata Biswas. ATM-mediated ELL phosphorylation enhances its self-association through increased EAF1 interaction and inhibits global transcription during genotoxic stress. ***Nucleic Acids Res***. 2022 Oct 28;50(19):10995-11012. doi: 10.1093/nar/gkac943.

Extramural Funding

- Wellcome-Trust DBT India Alliance Intermediate Fellowship
- Dept. of Science and Technology (DST)

Investigation of the early immune signal perception and regulation of plant immunity by sphingolipids during Xanthomonas oryzae pv oryzae (Xoo) infection in in rice

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Background

Rice is the staple food of more than half of the world's population and rice production in India is an important part of the national economy. Numerous pathogenic microorganisms cause rice diseases that lead to huge yield loss worldwide. For a reliable and affordable use of the resistant cultivars it is crucial to understand the molecular basis of plant host-pathogen interaction. Plants have two types of innate immunity (Dodds and Rathjen, 2010). One is mediated by surface-localized pattern recognition receptors (PRRs). PPRs recognize pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) and activate PRR-triggered immunity (PTI). The other one uses intracellular immune receptors that recognize virulence effectors secreted by pathogens and induces effector-triggered immunity (ETI) (Boller and Felix, 2009). PRRs recognize a wide range of ligands including lipid, protein, nucleic acid, carbohydrate etc. PAMPs such as bacterial flagellin, peptidoglycan, lipopolysaccharide, and fungal chitin are reported to be sensed by rice cells and trigger innate immunity (Kaku et al. 2006, Chen et al. 2011, Liu et al. 2012).

Sphingolipids are important constituents of plasma membrane and endo-membranes in all eukaryotes. They have a sphingoid long chain carbon base (LCB), an amide group and an N-acylated fatty acid (FA), which is bonded to a polar head group. There are more than 168 different sphingolipids in *Arabidopsis*. Sphingolipids are involved in many aspects of plant development and plant's response to biotic and abiotic stress stimuli. Numerous studies in animal cells establish important role of sphingolipids in processes like cell development, cell proliferation and cell death. Emerging evidences suggest their role in (PCD). Studies in plant also report the involvement of sphingolipids in growth, endo-membrane differentiation, programmed cell death, plants response towards chilling stress, dehydration stress, pathogen attack and defence responses cell. In plants, the plasma membrane forms the 1st line of defence against pathogens. Several proteins related to plant defence assemble in plasma membrane micro-domains. Work done by Nagano et al. 2016, elucidated the relationship between micro-domain and innate immunity in Rice. Importantly, this work demonstrates that in rice micro-domains are essential for conferring resistance to rice blast fungus infection and 2-hydroxy sphingolipids contribute to the ordered structure of plasma membrane micro-domain in rice (Nagano et al. 2016).

My current research also establishes that sphingolipids induce cell death and affect the growth and development of Arabidopsis seedlings. Rice and *Xanthomonas oryzae pv oryzae* (Xoo) are well known host pathogen system. It would be interesting to find out if LCBs are involved in perception of Xoo at the membrane or if sphingolipids regulate downstream response reactions during Xoo infection.

Future Research Plans

- Study PAMP-induced cytosolic Ca^{2+} dynamics in rice plants and understand how specificity is achieved during perception of PAMP secreted by Xoo.
- Investigation of sphingolipids profile of rice leaves after *Xanthomonas oryzae pv oryzae* (Xoo) infection.
- Isolation of membrane rafts after Xoo infection, lipid profiling and protein profiling of the isolated membrane raft.
- Investigation of crosstalk between sphingolipids signal and hormonal signal during biotic stress response.

Organic and Medicinal Chemistry Division

The Organic and Medicinal Chemistry (OMC) Division at CSIR-Indian Institute of Chemical Biology is comprised of dedicated scientists with a diverse array of expertise in synthetic organic chemistry, catalysis, chemical biology, medicinal chemistry, natural product chemistry and supramolecular chemistry. The strength of the OMC division is in its heterogeneity with scientists from diverse field of researchers are present under one roof, which helps IICB to address any scientific problems from multiple angles. Scientists from this division play a crucial role for the genesis of several research activities to address biological, environmental and other chemical problems which are burning issues of the nation. The department is actively engaged for the development of green chemical processes and technologies to boost up Indian pharma, agro and chemical industries for self-sustainability. Simultaneously, several biomedical challenges are tackled through a symbiotic collaboration between chemists and biologists to make Swasth Bharat.

Development of lead molecules of natural and synthetic origins with specific targets against various diseases

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Dr. Arun Bandyopadhyay, CSIR-IICB
Dr. Snehasikta Swarnakar, CSIR-IICB
Dr. Saikat Chakrabarti, CSIR-IICB

Background

The main aspect of our work involves the development of lead molecules having anti-cancer, anti-leishmanial, anti-bacterial anti-ulcer, and anti-viral properties. Our group is also engaged in the development of fluorescent probes for plausible application to live cell imaging. Our investigations towards the biological applications of our various synthesized compounds revealed that they have potent anti-ulcer property by inhibiting MMP-9. We are also engaged in making use of enzymes from edible sources as catalysts in organic reactions to synthesize specific epigenetic enzyme inhibitors by understanding the molecular targets as well as in the synthesis of precursors to pharmaceutically active compounds. We also investigate asymmetric organic transformations by using chiral ligands/catalysts to synthesize bioactive scaffolds. Introduction of stable atropisomerismis another field that our group explores. Atropisomerism which gives optically rich compounds is responsible for target selectivity of a promiscuous scaffold.

Aims and Objectives

- To identify lead molecules by virtual screening of natural products inspired in-house library to discover potential lead molecules against the SARS-CoV-2 main protease
- To develop fluorescent probes with applications for live cell imaging.
- To perform asymmetric reactions by using chiral catalysts.
- To develop lead molecules of natural origins with specific targets against various diseases.

Work Achieved

Virtual screening of natural products inspired in-house library to discover potential lead molecules against the SARS-CoV-2 main protease

SARS-CoV-2, a new coronavirus emerged in 2019, causing a global healthcare epidemic. Although a variety of drug targets have been identified as potential antiviral therapies, and effective candidate against SARS-CoV-2 remains elusive. One of the most promising targets for combating COVID-19 is SARS-CoV-2 Main protease (M^{pro}, a protein responsible for viral replication. In this work, an in-house curated library was thoroughly evaluated for druggability against M^{pro}. We identified four ligands (FG, Q5, P5, and PJ4) as potential inhibitors based on docking scores, predicted binding energies (MMGBSA), in silico ADME, and RMSD trajectory analysis. Among the selected ligands, FG, a natural product from Andrographis nallamalayana, exhibited the highest binding energy of −10.31 kcal/mol close to the docking score of clinical candidates Boceprevir and GC376. Other ligands (P5, natural product from cardiospermum halicacabum and two synthetic molecules Q5 and PJ4) have shown comparable docking scores ranging −7.65 kcal/mol to −7.18 kcal/mol. Interestingly, we found all four top ligands had Pi bond interaction with the main amino acid residues HIS41 and CYS145 (catalytic dyad), H-bonding interactions with GLU166, ARG188, and GLN189, and hydrophobic interactions with MET49 and MET165 in the binding site of M^{pro}. According to the ADME analysis, Q5 and P5 are within the acceptable range of drug likeliness, compared to FG and PJ4. The interaction stability of the lead molecules with viral protease was verified using

replicated MD simulations. Thus, the present study opens up the opportunity of developing drug candidates targeting SARS-CoV-2 main protease (M^{pro}) to mitigate the disease. Figure 1.

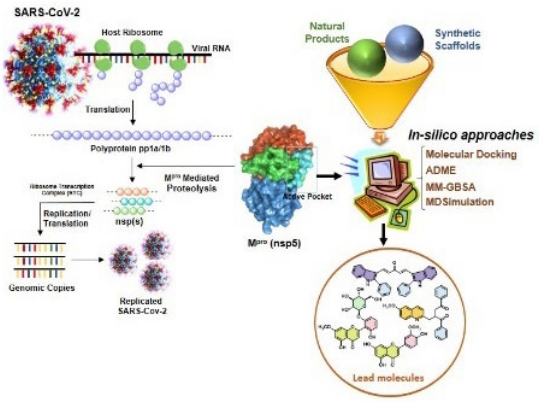

Figure 1: Strategy for drug candidates targeting SARS-CoV-2 main protease (M^{pro}) to mitigate the disease

Molecular docking studies for lead identification

Boceprevir (Boc) and GC376, FDA approved clinical candidates inhibiting viral proteases, were considered as a standard reference molecule in present study (Ma, Sacco et al. 2020) (Sacco, Ma et al. 2020) . Crystal structure of SARS-CoV-2 M^{pro} was adopted as an origin point for computer-aided drug design due to its functional relevance in the survival of CoV. Our docking protocol was validated by re-docking of Boceprevir into active pocket of prepared protein (PDB ID: 7BRP), has shown null deviation (0.00 Å RMSD) from the initial co-crystalize pose reported in literature (Fu, Ye et al. 2020) (Jain 2008) (Mandal, Garg et al. 2016). Along with the in-house library (a set of 150 molecules), four clinical antiviral candidates (Boceprevir, GC376, Lopinavir and Ritonavir) were then docked into the validated active pocket of M^{pro}. Furthermore, the interactions of top scorer ligands (5 selected ligands, FG, Q5, P5, PJRJ_37A and PJ4, see Fig. 2) with the amino acid residues of Mpro were carefully investigated using the Maestro (Schrödinger, LLC, New York, NY, 2021) and Discovery studio visualizer (BIOVIA DS Visualizer 4.5, San Diego: Dassault Systèmes, 2020).

Conclusion: The new findings indicate that computational approaches can be used to find inhibitors of SARS-CoV-2, which is still causing a global pandemic. In this work, 150 natural and synthesized molecules were checked for the target of the Mpro of SARS-CoV-2. These compounds were screened against SARS-CoV-2 M^{pro} with four potential repurposed drugs (Boceprevir, GC376,

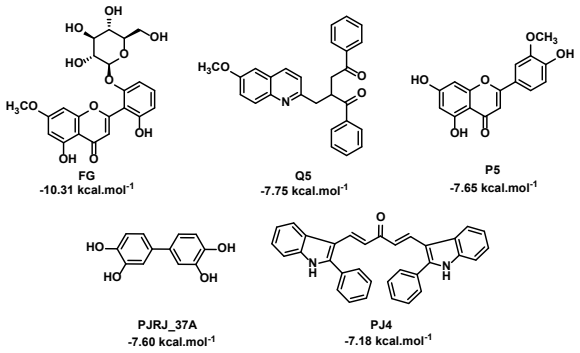

Figure 2: Structure of top five ligands (with respect to docking score)

Ritonavir, and Lopinavir). Our study identified four compounds from an in-house chemical library of natural and synthetic compounds based on docking score, predicted binding energies, Predicted ADME score and averaged RMSD trajectories. SARS-CoV-2 main protease inhibitors are anticipated to be ligands FG, P5 (natural flavone class of products) and Q5, PJ4 (synthetic ligands). Even though the scores of molecular docking are not so high when compared to Standard reference molecules understudy except for the ligand FG which is comparable with the reference, but the interaction profiles of the top ligands attracted the interest of authors to take the ligands for further study as leads. We presume that logical structural modifications of the leads selected may give the potent catalytic inhibitors for the M^{pro} to abort the readily mutating and replicating SARS-CoV-2 virus. To enhance the ADME profile of molecules FG and better binding to the site of M^{pro} further derivatization in structure is suggested and *in vitro* investigations are required for further confirmation of the findings.

Future Research Plans

- Development of high-affinity BET Bromodomain Ligands as anticancer agents and as SynTEFs for activation of FXN and other genetic diseases.
- Development of Synthetic and Natural Product based inhibitors against histone methyltransferases (G9a/GLP).
- Target oriented development of small molecules against COVID-19, *Leishmania Donovani* and Chagas diseases.
- Elucidating the mechanism of the small molecular fluorescence probes for Lipid droplets (LD) and exploring their qualitative and quantitative estimation of LDs in various diseases models for a wide range of biomedical applications.

- The development of stable atropisomers for biological applications is an ongoing effort.
- Synthesis of new lead compounds of natural product origin and their analogues having various biological activities, with special focus on anticancer, anti-microbial, anti-leishmanial and anti-inflammatory compounds.
- Extraction, isolation and purification of compounds and evaluation of their biological activities from medicinal plants are also on-going.

Publications

- Novel Spirooxindole Derivatives as Potential Non-Detergent Type Spermicides, Shrabanti Kumar, Chiranjit Acharya, Nipun Abhinav, **Parasuraman Jaisankar***, *ChemistrySelect*, **2022**, 7 (35), e202202210.
- Design and Development of Benzothiazole-Based Fluorescent Probes for Selective Detection of Aβ Aggregates in Alzheimer's Disease, Rathnam Mallesh, Juhee Khan, Krishnangsu Pradhan, Rajsekhar Roy, Nihar Ranjan Jana, **Parasuraman Jaisankar**, Surajit Ghosh, *ACS Chemical Neuroscience*, (**2022**), 13 (16), 2503-2516.
- Amino acid containing amphiphilic hydrogelators with antibacterial and antiparasitic activities, Biplab Mondal, Vivek Kumar Gupta, Biswanath Hansda, Arpita Bhoumik, Tanushree Mondal, Hemanta K Majumder, Charlotte JC Edwards-Gayle, Ian W Hamley, **Parasuraman Jaisankar*,** Arindam Banerjee*, *Soft Matter* (**2022**), *18 (37)*, 7201-7216.
- Virtual screening of natural products inspired in-house library to discover potential lead molecules against the SARS-CoV-2 main protease, Aakriti Garg, Narender Goel, Nipun Abhinav, Tanmay Varma, Anushree Achari, Pinaki Bhattacharjee, Izaz Monir Kamal, Saikat Chakrabarti, Velayutham Ravichandiran, Araveeti Madhusudhana Reddy, Sreya Gupta & **Parasuraman Jaisankar*** *Journal of Biomolecular Structure and Dynamics,* (**2022**), 2033-2045

Awards/Honours

- Awarded Fulbright – Nehru Academic and Professional Excellence Fellowship for 2021 by USIEF.
- Appointed by NGCMA-DST as one of the DST-GLP Lead Inspectors for carrying out inspection of Test Facilities for OECD-GLP compliance of NGCMA, DST, Govt. of India.
- Appointed as Associate Editor of Frontiers in Chemistry (Chemical Biology) in 2021.
- Awarded Gold Medal by the Society "Chirantan Rasayan Sanstha®" (CRS) for the year 2021, India.

Conferences/Workshops

Webinar on "Post Covid-19: Resurgence of Indian Industry and R & D," organised by SRM University-AP, 15th May 2020.

Conferences/Events Organized

Skill Development programs of CSIR-IICB, 2021-2022 organized by CSIR-IICB, Kolkata, India.

Invited Talks

- Delivered Fulbright Outreach Lecture Series online at University Minnesota, Minneapolis, MN 55455-0431, USA funded by Fulbright Foundation, USA on "Target Based Novel Molecules of Synthetic and Natural Product Origins for Drug Discovery in Neglected Infectious Diseases - Leishmaniasis and Chagas Diseases", August 13, 2021.
- Delivered Fulbright Outreach Lecture Series online at NIAAA/NIH, USA funded by Fulbright Foundation, USA on "Small molecular Fluorescence Probes for Live Cell Imaging applications and anti-inflammatory agents targeting Matrix Metalloproteinase-9 (MMP-9)" October 20, 2021.
- Delivered Fulbright Outreach Lecture Series online at Department of Chemistry and Biochemistry, The University of Texas at Arlington, USA funded by Fulbright Foundation, USA on "Unravelling the Complexity of Chirality, Development of Small Molecular Probes for Live Cell Imaging Applications, and Anti-Inflammatory Agents Targeting Matrix Metalloproteinase-9 (MMP-9), November 19, 2021.
- Delivered a special lecture at Chemical Biology and Therapeutic Department (CBT), St. Jude Children's Research Hospital, Memphis, Tennessee, USA on "Tuning of Small Molecules of Synthetic and Natural Products Origins with Specific Targets in Drug Discovery", June 3, 2021.
- Delivered Gold Medal award lecture on "Tuning of Small Molecules of Synthetic and Natural Product Origins as Potential Bioactive Agents with Specific Targets" in Drug Discovery in the "One-Day Webinar on Science Beyond Boundary: Invention, Discovery, Innovation and Society "Rasayan 11", on October 20, 2021.

Synthesis of Novel Heterocycles of Biological Interests

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Background

Carbazoles are considered as privileged scaffolds because they form the core structure of numerous bioactive alkaloids, serve as pharmacophore in medicinal chemistry and constitute an important building block in organic synthesis. Besides, these are potential electroluminescent materials because of their photorefractive, photoconductive and light emitting properties. Attempts to synthesize them began in the late nineteenth century[3a-b] but the field continues to be an active area of research.

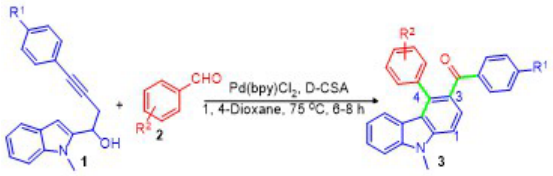
Importantly, the metal catalyzed cascade reactions of 1-(indol-3/2)-yl)-3-alkyn-1-ols proved to be an efficient strategy to deliver carbazoles in high yields. Typically, iodine mediated and gold(I)-catalyzed cyclizations (*5-endo-dig*) of 1-(indol-3-yl)-3-alkyn-1-ols are reported to form carbazoles through a *Wagner-Meerwein* type 1,2-alkyl shift. On the other hand, 1-(indol-2-yl)-3-alkyn-1-ols are used as potential substrates for benzannulations by employing costly Au(I), Au(III), or Pt(IV) catalyst. Surprisingly, palladium-catalyzed reactions do not appear to have been explored for this purpose, calling for straightforward and practical methods for their general synthesis.

Aims and Objectives

- An atom economic direct synthesis of 4-aryl-3-arylcarbonyl-carbazoles through palladium-catalyzed alkyne-carbonyl metathesis strategy employing 1-(indol-2-yl)-3-butyn-1-ols and aromatic aldehydes as substrates.
- A palladium catalyzed synthesis of 4-aryl-carbazoles via intramolecular cyclizations of 1-(indol-2-yl)-3-butyn-1-ols.
- Palladium(0)-catalysed stereoselective synthesis of 3-ylidene-[1,4]benzo- diazepin-5-ones/benzo[f][1,2,5]thiadiazepine-1,1-dioxides
- Screening of natural products based compounds for anti-cancer activities and preclinical studies on a nanoencapsulated betulinic acid analogue (BC-2c) to improve the efficacy against colorectal carcinoma.

Work Achieved

We developed a facile method for the general synthesis of 4-aryl-3-arylcarbonyl-carbazoles **3** via palladium(II)-catalyzed alkyne-carbonyl metathesis strategy employing substrates such as 1-(indol-2-yl)-3-butyn-1-ol **1** and aldehydes **2** (**Scheme 1**) The optimized reaction conditions were found after screening different reaction parameter (i.e., catalyst, ligand, solvent etc.). We then expanded the scope of this reaction by using various 1-(indol-2-yl)-3-butyn-1-ols **1** and aryl aldehydes **2**. A strong electron-withdrawing group (EWG, viz. $R^2 = NO_2/CF_3$) at the para position of the phenyl ring of aldehyde **2** facilitated the reaction with **1a** (R^1=H) resulting in the formations of products **3** (viz., **3a**/**3b**) within 6-8 h with very good yields (68-72%). On the other hand, an electron-donating group (EDG; viz., R^2 = OMe/Me) at the same position of aldehyde **2** afforded the product **3** in reduced yield (59-64%) requiring longer reaction time.



Scheme 1: Synthesis of 4-aryl-3-arylcarbonyl-carbazoles **3**

Next, we studied the reactivity of the acetylene **1b** having an EDG (R^1 = OMe) in the phenyl ring attached to the acetylenic carbon with aldehydes **2a** ($R^2 = NO_2$) and **2b** ($R^2 = CF_3$) having an EWG at the para position; gratifyingly, these reactions afforded products **3** within 6-7 h with very good yields (68-71%). While substrate **1c** carrying an EWG (R^1 = F) also underwent reactions with aldehydes **2** containing an EWG resulting in products **3** within 7 h, albeit in somewhat lower yields (60-66%).

Interestingly, omission of the aldehyde **2** from the aforesaid reaction led to the development of a general synthesis of carbazoles **4** (Scheme 2). We also explored the scope of this reaction as before. This showed that incorporation of an EWG (viz., R = Cl) at C5 position of the benzene ring in the indole moiety of substrate of **1** reduced the yield of the product **4** compared to the substrate having an EDG (viz. R = OMe). Furthermore, substrates **1** containing either strong (R^1 = COOMe/NO_2) or moderate (R^1 = CF_3/F/Cl/Br) EWG hindered the reaction to some extent, delivering the products **4** in moderate to good yields (58-70%). The reaction was amenable to the synthesis of bis-carbazole derivatives. In addition, a plausible reaction mechanism has been established using various control experiments to explain the product formations.

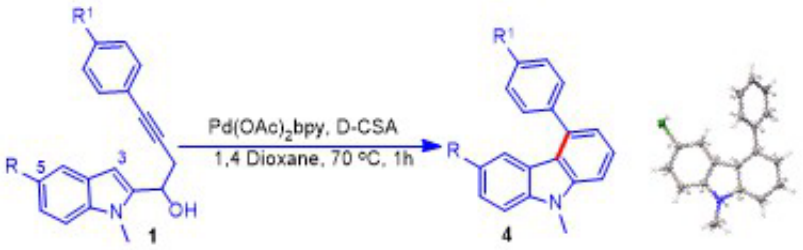
Besides, we evaluated the anti-proliferative and cytotoxic and apoptogenic activity of methanolic extract of *Oroxylon indicum* Bark (MEOIB) on U937, K562 cells and normal lymphocytes. The cell viability was studied by MTT assay. Cell morphology was determined by fluorescence, confocal microscope and DNA fragmentation by agarose gel electrophoresis study. The MEOIB inhibited the viability of cells in a time & concentration dependent manner. Morphologically, it was found that externalization of PS from inner leaflet to outer leaflet of the cell membrane and condensed chromatin, membrane blabbing, fragmentation of nuclei in all the treated cells than control and DNA bands appeared in the form of ladder after treatment with MEOIB. The present study reveals that the MEOIB possesses potent anti-leukemic activity against U937 and K562 cell lines but there no toxicity in normal lymphocytes.

Future Research Plans

- Development of efficient palladium catalysed methods for the synthesis δ-carbolines and benzofuro[3,2-*b*]pyridines via metal-catalyzed heteroannulations of allenamides.
- Iron(III)-catalyzed straight forward methods for the synthesis of spiro-indenyl 1,4-benzoxazines and 2-(2,2-diarylvinyl)quinoxalines.
- Nanoparticulate targeted delivery of Betulinic acid derivative (BAD) to enhance therapeutic efficacy towards colorectal cancer.

Publications

- Pramanik, S., Chatterjee, S., Banerjee, R., and Chowdhury, C. (2022) Palladium-catalyzed Benzannulations of 1-(Indol-2-yl)-3-butyn-1-ols: Easy Access to Functionalized Carbazoles. *Organic Lett*. **24**, 1895−1900.
- Besra, S. E., Ray, M., Roy, S., and Chowdhury, C. (2022) Cytotoxic Activity of Bark of *Oroxylon indicum* Benth against U937and K562*. Int. J. Pharm. Sci. Rev. Res*. **75**(1), 73-78.
- Mondal, D., Pal, G., and Chowdhury, C. (2021) Palladium(0)-catalysed regioselective cyclisation of 2-amino(tosyl) benzamides/sulphonamides: Stereoselective synthesis of 3-ylidene-[1,4]benzodiazepin-5-ones/benzo[f][1,2,5]thiadiazepine-1,1-dioxides. Chem. Commun. 57, 5462-5465



Scheme 2: Synthesis of 9-Methyl-4-aryl-9H-carbazoles **4**.

Synthesis of Novel Poly-N-Fused Heterocyclic Scaffolds by C-H Activation Strategies and Their Efficacy Studies

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Background

Our lab is engaged in variety of different projects covering highly diversified areas. Transition metal catalyzed C-H activation reactions to synthesize novel poly-N-fused heterocycles to targeting SARS-COV2 spike protein allosteric site in search of new Inhibitors and b-Turn induced helical self assembly formation by a small dipeptide mainly to HSp70 Inhibitor design and synthesis are only few of these projects. Strategically we design our projects to utilise both my own chemistry and also our inhouse biology collaborations.

Aims and Objectives

- To develop novel methodologies to synthesize *N*-fused poly-heterocyclic scaffolds and study their bio-medical applications.
- To Study the photophysical properties of these highly conjugated aromatic poly-N-heterocycles by UV-fluorescence spectroscopy and their applications
- Synthesis & Biophysical propertiy (structure elucidation) studies of Small synthetic peptide based nano structures and their applications
- New Anti-viral therapeutic regimen design, synthesis and validation against Dengu and Covid-19.
- Synthesis of Novel molecules as Molecular Rotor

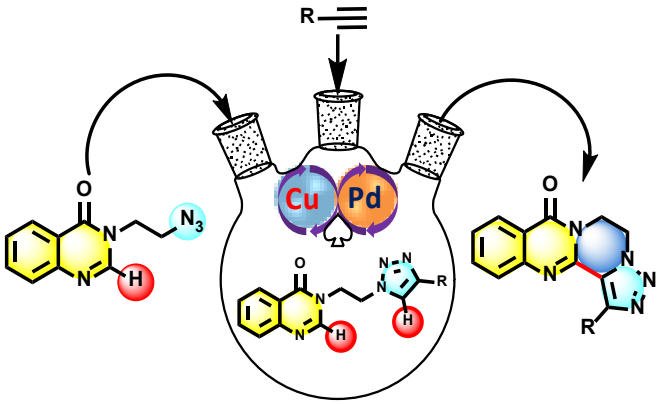
Work Achieved (Some Representative works)

N-Fused Triazolo-Piperazino-Quinazolinone Poly-heterocycles Synthesis via One Pot Tandem Click Reaction and Cross Dehydrogenative Coupling

A simple, one pot method to synthesize tetracyclic quinazolinone-piperazine-triazole fused poly-N-heterocyclic scaffolds was developed. In this strategy, a tandem approach of two highly efficient synthetic reactions, Click and Cross dehydrogentive coupling (CDC), with high atom economy was employed to get the target N-fused scaffolds. Here the choice of three different heterocycles (triazole, piperazine, quinazolinone) were dictated by their known & established individual bioactivities. Some of these derivatives showed bright fluorescence in solid and solution states.

Rh^{III}-Catalyzed Redox Neutral Cascade of Carbenoid Functionalization and Dephosphonylative Annulation

A Rh(III)-catalyzed regioselective redox-neutral cascade process of carbenoid functionalization followed by dephosphonylative annulation of benzoic acids with □-Diazo-□-keto phosphonatehas been realized, which led to the direct synthesis of privileged 3-substituted isocoumarin scaffold. According to the best of our knowledge, this is the first report of a complete redox neutral method to synthesize isocoumarins using C□H functionalization strategy. In the catalytic cycle of this reaction, there are two possible pathways for the C–C coupling between ortho-positioned carbon atom of benzoic acid and the diazo carbon atom: (i) concerted 1,2-aryl shift (ii) stepwise metal-carbene formation followed by migratory insertion. DFT study has predicted that the concerted pathway has lower activation energy as compared to the stepwise pathway by 1.5 kcal/mol.



- One pot protocol
- 36 examples were synthesized yield upto 89%
- Novel quinazolinone fused scaffolds
- Bright white solid state emission

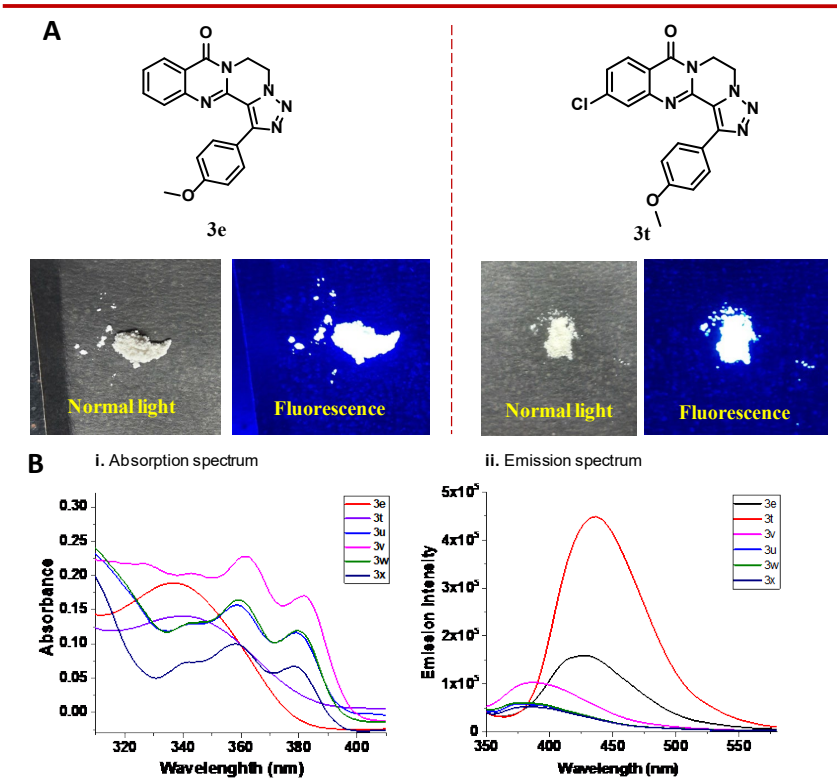


Figure 2. (A) Normal light picture (left) and solid state fluorescence of **3e** and **3t** captured by irradiation at 365 nm with dark background (right) **. (B)** (i) UV absorption of six compounds (5 μM in CH_2Cl_2, λ_{Ex} = 330-390 nm); (ii) Emission intensities of six compounds (5 μM in CH_2Cl_2, λ_{Em} = 360-450nm)

Figure: Quinazolino-piperazino-triazoles synthesis and their photophysical properties (Ref: 1)

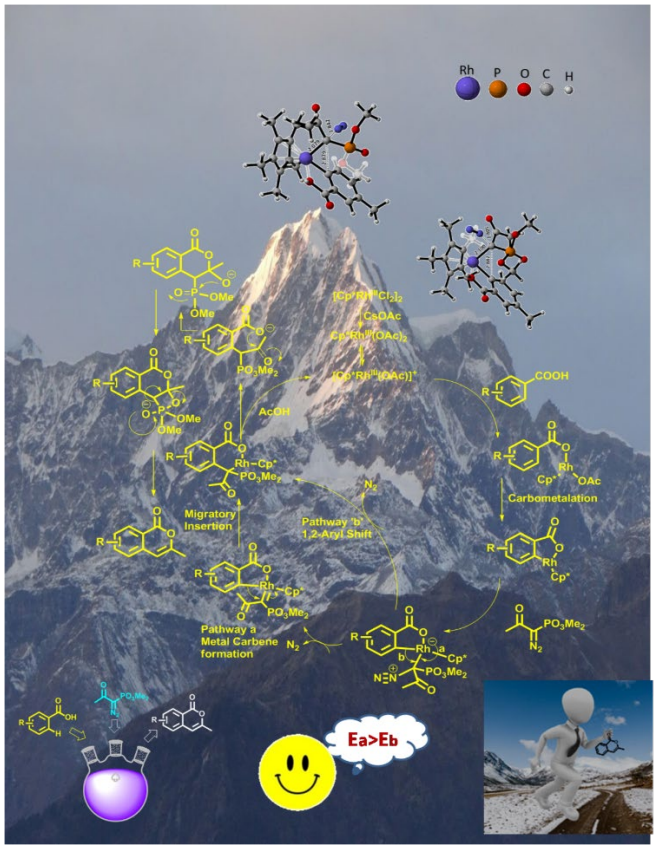


Figure: Accepted Front Cover art of Ref: 3

Publications (Ref):

1. Synthesis of N-Fused Triazole–Piperazine–Quinazolinones via One-Pot Tandem Click Reaction and Cross-Dehydrogenative Coupling; Ravuri Srinath, Arindam Manna, Subhankar Shee, Vijay Babu Pathi, Saswati Ghosh, Krishnendu Khamaru, Nakul Chandra Maiti and **Biswadip Banerji***; *Org. Lett.* 2021, 23, 24, 9365–9370.
2. *S* -Benzyl cysteine based cyclic dipeptide super hydrogelator: Enhancing efficacy of an anticancer drug via sustainable release; Saswati Ghosh, Sayoni Nag, Krishna Das Saha and **Biswadip Banerji***; Journal of Peptide Science, https://doi.org/10.1002/psc.3403
3. Synthetic and Computational Studies on RhIII-Catalyzed Redox Neutral Cascade of Carbenoid Functionalization and Dephosphonylative Annulation; Suvankar Bera, Partha Mondal, Debabrata Sarkar, Vijay Babu Pathi, Sourav Pakrashy, Ayan Datta*, **Biswadip Banerji***; Journal of Organic Chemistry, 2021, 68, 10, 7069-7077

Molecular Diversity through Cascade C-H Activation

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Background

Development of previledged medicinal scaffolds is the key step in drug discovery program. We have initiated a cutting-edge C-H activation technology for the synthesis of heterocycles and other medicinally relevant molecules. Furthemore, this technology is particularly important for the late-stage diversification of functional molecules. C-H activation in organic synthesis (CHAOS) not only accelerate the synthesis but also allow us to achieve molecules which was unimaginable before. Furthermore, multiple C-H activation in a cascade manner will enable us to achieve molecular diversity as well as complexity from simple, readily available, inexpensive starting materials. This approach will generate a library of multifunctional molecules for Alzheimer's Disease, breast cancer etc. Furthermore, cost effective processes for the off-patent drug and agrochemicals will be developed. Recently, we have initiated a research program for the utilization of CO_2, SO_2 etc. which are feedstock chemicals for the production of value-added products (Fig. 1).

Aims and Objectives

- Development of synthetic methodology for the synthesis of basic pharmacophores through cascade C-H activation.
- Late-stage diversification via site-selective C-H activations will be performed for the synthesis of unnatural amino acids.
- Chemical methodology and library development (CMLD) will be performed.
- Initial phenotypic screen of this library of compounds against MCF-7 primary breast cancer cell line and the corresponding MCF-10A normal cell line in 3D cell culture for hit optimization
- Affinity-based protein profiling for target identification, validation and hit to lead optimization through structure modifications
- Multi-functional molecular probe will be developed against Alzheimer's Disease

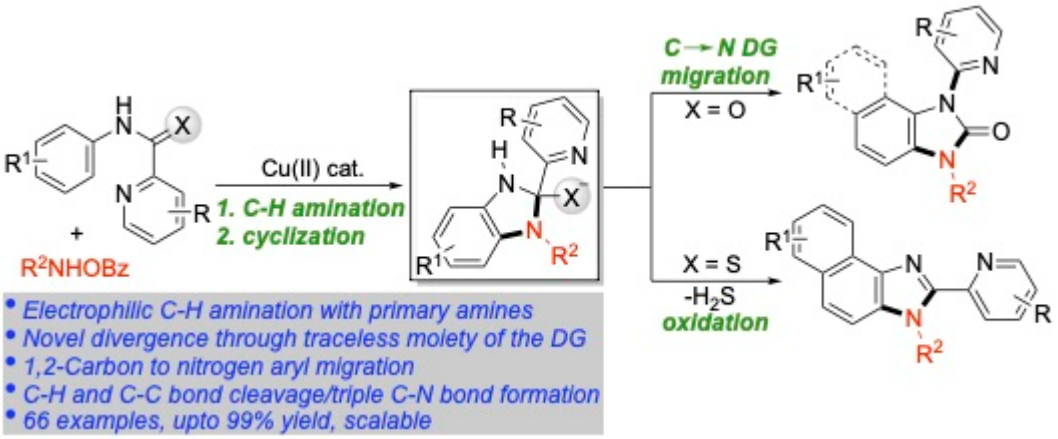

Figure 1

Work Achieved

We have initiated and optimized the synthesis of basic scaffolds through C-H activation. We have accomplished a palladium-catalyzed synthesis of 2-arylindoles, and indolines from readily available and inexpensive aryl ureas and vinyl arenes merging C-H activation and alkene difunctionalization at room temperature. This strategy has been extended to the other pharmacophores such as 2-methyl indole and indolines, 2-quinazolinones etc. The 2-arylindole moiety has been extended to the dibenzofused carbazole system through multiple C-H activations. We have developed inexpensive copper-catalyzed C-H hydroxylation reaction to achieve a multifunctional molecular scaffold which is exhibiting neuroprotection in Alzheimer's Disease model. We have also identified a lead molecule against breast cancer cell lines. We have synthesized a library of carbazole alkaloids present in the *Murayya koinigii* to evaluate their anticancer efficacy (Fig.2).

Future Research Plans

We like to apply this cutting-edge technology for the late-stage modification of amino acids to generate non-protinogenic amino acids for chemical biology and receptor specific drug delivery applications.

Publications

- Nandi, S.; Jana, R.* (2022) Toward Sustainable Photo-/Electrocatalytic Carboxylation of Organic Substrates with CO_2, Asian, J. Org. Chem. 11, e202200356 (selected as cover page).
- Nandi, S.; Mondal, S.; Jana, R.* (2022) Protocol for chemo- and regioselective C(sp3)-H activation using a heterogeneous copper powder-catalyzed reaction, Star Protoc, 26;3(4):101781.
- Nandi, S.; Mondal, S.; Jana, R.* (2022) Chemo- and Regioselective Benzylic C(sp3)–H Oxidation Bridging the Gap Between Hetero- and Homogeneous Copper Catalysis, , iScience, accepted. doi.org/10.1016/j.isci.2022.104341.
- Begam, H. M.; Nandi, S.; Jana, R.* (2022) A Directing Group Switch in Copper-catalyzed Electrophilic C–H Amination/Migratory Annulation Cascade: Divergent Access to Benzimidazolone/Benzimidazole, Chem, Sci., 13, 5726-5733. doi.org/10.1039/D2SC01420C.
- Das, P.; Das, S.; Jana, R.* (2022) Aryldiazonium Salts and DABSO: A Versatile Combination for Three-Component Sulfonylative Cross-Coupling Reactions, Chem. - Asian J., Ahead of Print. doi.org/10.1002/asia.202200085.
- Manna, K.; Ganguly, T.; Baitalik, S.*; Jana, R.* (2021) Visible-Light- and PPh3-Mediated Direct C–N Coupling of Nitroarenes and Boronic Acids at Ambient Temperature, Org. Lett., 23, 8634–8639.
- Jana, R.*; Begam, H. M.; Dinda, E. (2021) The emergence of the C–H functionalization strategy in medicinal chemistry and drug discovery, Chem. Commun., Feature article, 57, 10842-10866.

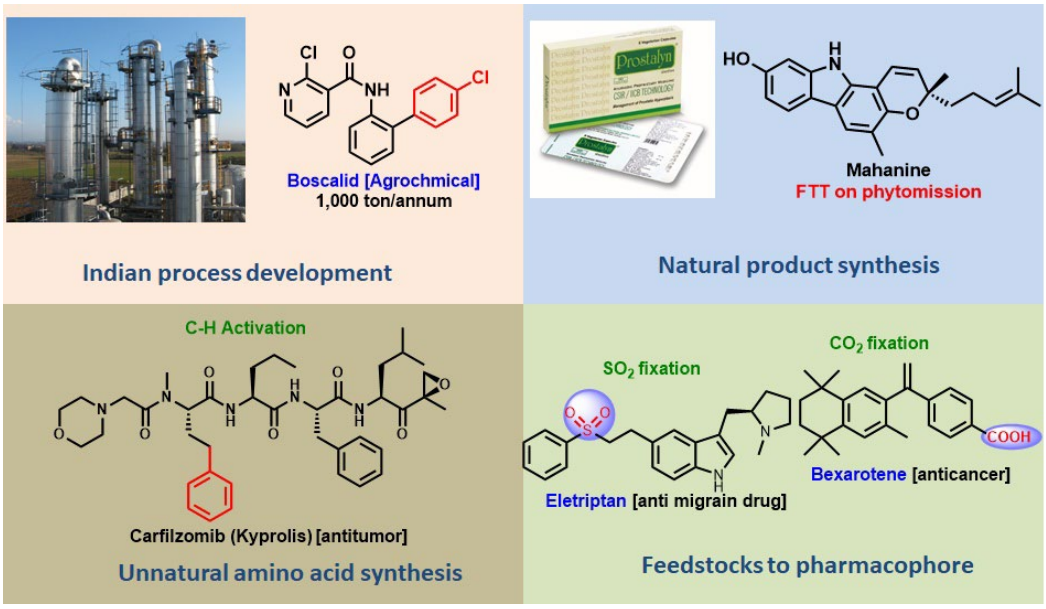

Figure 2

Extramural Funding

- Molecular Diversity through Cascade C-H Activations, 2015-2018, DST, SERB, Govt. of India, Project # EMR/2014/00469
- Phytomission II (HCP-38), CSIR
- Antiviral mission mode (HCP-41), CSIR
- Pan CSIR Anticancer mission mode (HCP-40), CSIR

Conferences/Workshops

- Invited lecture at University of Calcutta, teacher's orientation program.
- Invited lecture at NITM.
- Invited lecture at JIS university.
- Invited lecture at Surendranath College.
- Invited lecture at BITS-Hyderabad.
- Popular Science Lecture for Gigyasa program on Green Chemistry and Engineering from Societal Perspective
- Coordinating skill development program on LCMS training

Development of novel antivirals targeting Hepatitis C Virus RNA: The liver specific delivery of these antivirals and DNA damage and Cancer: Development of Novel Theranostics Targeting Nucleic acid

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Background

Hepatitis C Virus (HCV) is a worldwide threat. Currently, about 175 million people are the infected with Hepatitis C Virus (HCV). HCV mainly affects the liver, and leads to liver cirrhosis and hepatocellular carcinoma. The major problems associated with the virus are detection of the viral load in acute conditions and the absence of effective vaccination against the virus. The available therapy for HCV is immunostimulatory pegylated interferon alfa (IFN-α) and in combination with guanosine analogue ribavirin which suffered from low efficacy and serious side effects. Rapid mutations in protein envelop and drug resistances are great concerns and targeting viral conserved region is more imperative strategy to deal with this issue.

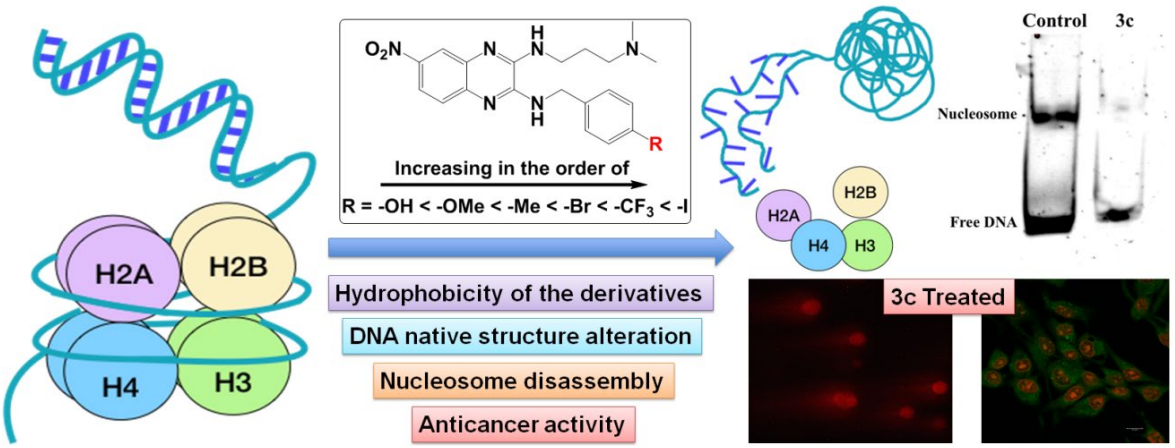
Work done

Recent studies have shown that the Internal Ribosome Entry Site (IRES) RNA of 5'-untranslated region (UTR) is a target for antiviral development due to the highly conserved region of HCV with unique structural features. We have synthesized a series of novel quinoxaline small molecules based on the crystal structure of 2-amino-benzimidazole bound to HCV IRES RNA. The quinoxaline derivatives inhibit the viral protein translation in a Dual Luciferase reporter based assay containing the HCV IRES in low micromolar range. Mutagenesis studies of HCV IRES show that the compounds can target the domain IIa of HCV IRES RNA. Circular dichroism, molecular dynamics and docking study further confirm that the quinoxaline derivatives target the domain IIa. Our studies provide potential leads for the development of antiviral therapy against HCV.
(Currently the project is funded by CSIR under a NCP project for 5 years, "Targeting RNA driven processes: Novel Chemical Biology Approaches to Identify New Classes of RNA Modulators").

1. Substituent effect of benzyl moiety in nitroquinoxaline small molecules upon DNA binding: Cumulative destacking of DNA nucleobases leading to histone eviction

Cooperative disruption of Watson-Crick hydrogen bonds, as well as base-destacking, is shown to be triggered by a quinoxaline-based small molecule consisting of an *N,N*-dimethylaminopropyl tether, and a para-substituted benzyl moiety. These events lead to superstructure formation and DNA condensation as evident from biophysical experiments and classical molecular dynamics simulations. The DNA superstructure formation by mono-quinoxaline derivatives is highly entropically favored and predominantly driven by hydrophobic interactions. Furthermore, oversupercoiling of DNA and base-destacking cumulatively induces histone eviction from *in-vitro* assembled nucleosomes at lower micromolar concentrations implicating biological relevance. The DNA structural modulation and histone eviction capacity of the benzyl para-substituents are in the order:

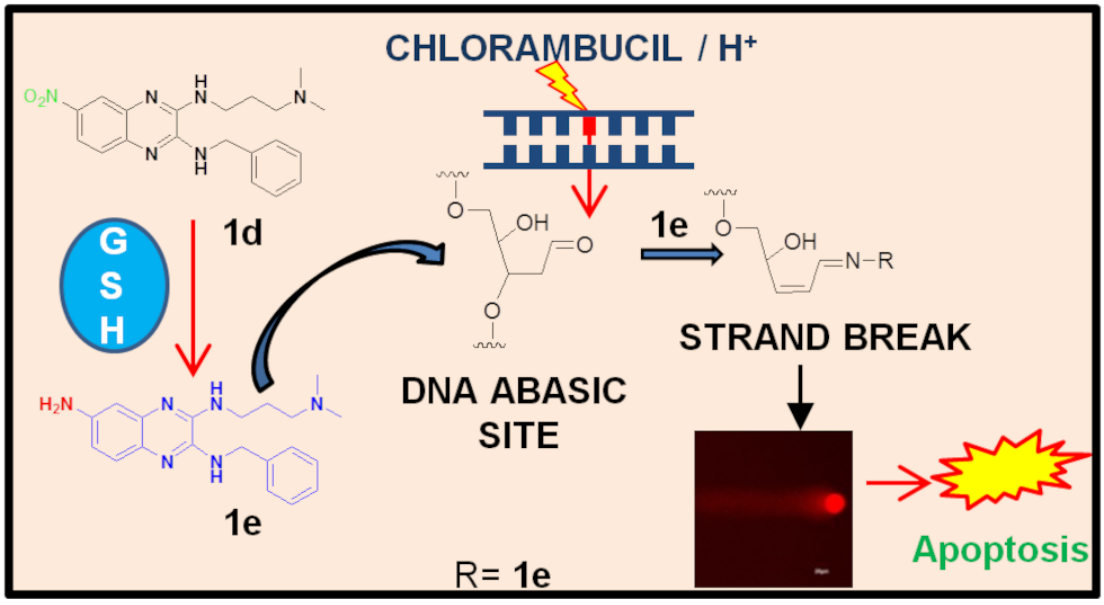
-I > -CF3> -Br > -Me > -OMe > -OH, which is largely guided by the polarity of benzyl para-substituent and the resulting molecular topology.



The most hydrophobic derivative **3c** with para-iodo benzyl moiety causes maximal disruption of base pairing and generation of superstructures. Both these events gradually diminish as the polarity of the benzyl parasubstituent increases. On the other hand, quinoxaline derivatives having heterocyclic ring instead of benzyl ring, or in the absence of *N,N*-dimethylamino head-group, is incapable of inducing any DNA structural change and histone eviction. Further, the quinoxaline compounds displayed potent anticancer activities against different cancer cell lines which directly correlates with the hydrophobic effects of the benzyl para-substituents. Overall, the present study provides new insights into the mechanistic approach of DNA structural modulation driven histone eviction guided by the hydrophobicity of synthesized compounds leading to cellular cytotoxicity towards cancer cells. (Published in ***European Journal of Medicinal Chemistry Volume 229, 5 February 2022, 113995, https://doi.org/10.1016/j.ejmech.2021.11399. IF 6.514***)

2. Cleavage of Abasic sites in DNA by an aminoquinoxaline compound: Augmented cytotoxicity and DNA damage in combination with an anticancer drug Chlorambucil in human colorectal carcinoma cells

The elevated level of endogenous oxidative DNA damage and spontaneous deamination of DNA bases in cancer cells substantially increase the abasic sites in DNA via base excision repairs (BERs). Thus, the predominant BER pathway is a favorable target for cancer therapy. Interestingly, elevated levels of glutathione (GSH) in certain cancer cells, such as colon cancer, are associated with acquired resistance to several chemotherapeutic agents, which increase the difficulty for the treatment of cancer. Here, we have reported an ideal nitro group-containing monoquinoxaline DNA intercalator (**1d**), which is reduced into a fluorescent quinoxaline amine (**1e**) in the presence of GSH; concurrently, **1e** (~100 nM concentration) selectively causes the *in vitro* cleavage of abasic sites in DNA. **1e** also binds to the tetrahydrofuran analogue of the abasic site in the nanomolar to low micromolar range depending on the nucleotide sequence opposite to the abasic site and also induces a structural change in abasic DNA. Furthermore, the amine compound (**1e**) augments the response of the specific bifunctional alkylating drug chlorambucil at a much lower concentration in the human colorectal carcinoma cell (HCT-116), and their combination shows a potential strategy for targeted therapy. Alone or in combination, **1d** and **1e** lead to a cascade of cellular events such as induction of DNA double-stranded breaks and cell arrest at G0/G1 and G2/M phases, eventually leading to apoptotic cell death in HCT-116 cells. Hence, the outcome of this study provides a definitive approach that will help optimize the therapeutic applications for targeting the abasic site in cancer cells. *(Published in* ***ACS Omega*** **2022**, 7, 8, 6488-6501 DOI 10.1021/acsomega.1c04962. IF 3.512)



3. Interaction of a triantennary quinoline glycoconjugate with Asialoglycoprotein receptor

Targeted intracellular delivery is an efficient strategy for developing therapeutics against cancer and other intracellular infections. Non-specific drug delivery shows limited clinical applications owing to their high dosage, cytotoxicity, non-specific action, high cost, etc. So, targeted delivery of less cytotoxic drug candidates to hepatocytes through ASGPR mediated endocytosis could be an efficient strategy to surmount the prevailing shortcomings. In the present work, the gene encoding ASGPR-H1-CRD has been amplified from Huh7 cells, cloned into pET 11a vector and the ASGPR-H1-CRD protein was expressed and purified from *E. coli*. A novel triantennary galactose conjugated quinoline derivative **4** has been synthesized that demonstrates a 17 fold higher binding affinity to isolated ASGPR-H1-CRD protein receptor (K_d ~ 54 µM) in comparison to D-Galactose (Kd ~ 900 µM). Moreover, micro-calorimetric studies for the interaction of glycoconjugate **4** with ASGPR protein on live hepatocytes showed notable thermal response in case of ASGPR containing Huh7 cells, in comparison to non-ASGPR Chang cells. Our research outcome might serve as an approach towards targeted delivery of small glycoconjugates to hepatocytes. (***ChemMedChem* 2021**, 16, 2211–2216 https://doi.org/10.1002/cmdc.202100158. IF 3.466)

4. Binding Studies of Aloe-Active Compounds with G-Quadruplex Sequences

G-quadruplex, a unique DNA quartet motif with a pivotal role in regulation of the gene expression, has been established as a potent therapeutic target for the treatment of cancer. Small-molecule-mediated stabilization of the G-quadruplex and thus inhibition of the expression from the oncogene promoter and telomere region may be a promising anticancer strategy. Aloe vera-derived natural compounds like aloe emodin, aloe emodin-8- glucoside, and aloin have significant anticancer activity. Comparative binding studies of these three molecules with varieties of G-quadruplex sequences were carried out using different biophysical techniques like absorption spectral titration, fluorescence spectral titration, dye displacement, ferrocyanide quenching assay, and CD and DSC thermogram studies. Overall, this study revealed aloe emodin and aloe emodin-8-glucoside as potent quadruplex binding molecules mostly in the case of c-KIT and c-MYC sequences with a binding affinity value of 10^5 order that is higher than their duplex DNA binding ability. This observation may be correlated to the anticancer activity of these aloe-active compounds and also be helpful in the potential therapeutic application of natural compound-based molecules. (***ACS Omega*, 2021**, 6, 18344−18351, https://doi.org/10.1021/acsomega.1c02207. IF 3.512)

Future Research Plans

Development of an effective small molecule targeting HCV IRES RNA which can work in nanomolar range and decrease viral translation. Design of small molecules which can sequence specifically target DNA and have anticancer properties which can be developed as therapeutics. We are also working on a glycomimetic-quinoline analogue which will be effective in reducing hepatic fat content and

improved metabolic outcome. Can be later translated into a compound to decrease hepatic fat content.

Extramural Funding

- "Synthesis of novel imipramine derivatives targeting Leishmania Donovani" (West Bengal DBT).
- "Development of Novel Theranostics Targeting Nucleic acids" P.I: Dr. Sanjay Dutta (DST-SERB)

Publications

- Chandra Sova Mandi, Tridib Mahata, Dipendu Patra, Jeet Chakraborty, Achyut Bora, Ritesh Pal and **Sanjay Dutta*** "Cleavage of Abasic sites in DNA by an aminoquinoxaline compound: Augmented cytotoxicity and DNA damage in combination with an anticancer drug Chlorambucil in human colorectal carcinoma cells" ***ACS Omega* 2022**, 7, 8, 6488-6501 DOI 10.1021/acsomega.1c04962. IF 3.512
- Ritesh Pal,# Jeet Chakraborty,# Titas Kumar Mukhopadhyay,# Ajay Kanungo, Rimita Saha, Amit Chakraborty, Dipendu Patra, Ayan Datta* and **Sanjay Dutta*** "Substituent effect of benzyl moiety in nitroquinoxaline small molecules upon DNA binding: Cumulative destacking of DNA nucleobases leading to histone eviction" ***European Journal of Medicinal Chemistry* Volume 229, 5 February 2022, 113995**, https://doi.org/10.1016/j.ejmech.2021.113995. IF 6.514 (#These authors contributed equally.)
- Subhadeep Palit,# Sayanika Banerjee,# Tridib Mahata,# Sougata Niyogi, Tanusree Das, Chandra Sova Mandi, Partha Chakrabarti, **Sanjay Dutta*** "Interaction of a triantennary quinoline glycoconjugate with Asialoglycoprotein receptor" ***ChemMedChem* 2021**, 16, 2211–2216 https://doi.org/10.1002/cmdc.202100158. IF 3.466, (#These authors contributed equally.)
- Abhi Das* and **Sanjay Dutta*** "Binding Studies of Aloe-Active Compounds with G-Quadruplex Sequences" ***ACS Omega*, 2021**, 6, 18344−18351, https://doi.org/10.1021/acsomega.1c02207. IF 3.512

Target Based Design, Synthesis, Development and Validation of Novel Small Molecules Inhibitors/ Modulators against Auto Immune Disorders, Anti-Cancer Therapeutics and Neglected Tropical Diseases

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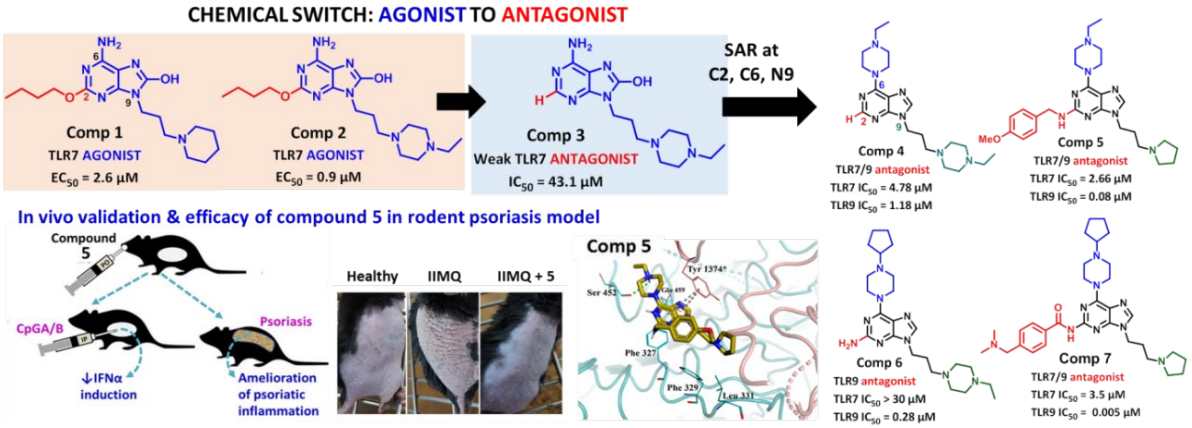
Prof. Malcolm McConville, University of Melbourne, Australia
Structural Genomics Consortium, Canada

Background

Probing Endosomal Toll-like Receptors (TLRs)

TLRs are the members of the larger family of evolutionary conserved pattern recognition receptors (PRRs) which plays critical and important role in first line of defence for self-nonself discrimination by the host immune response. Aberrant endosomal TLR activation is implicated in auto-reactive inflammation in different autoimmune diseases. Herein, have reported SAR strategies for the systematic development of potent dual TLR7/9 antagonists (4-7) starting from TLR7 agonist (1 and 2). The SAR analysis revealed that there exist concerted relationships between the nature of substituents at C2, C6, and N9 positions. Interestingly, the authors identified a singular 'chemical switch' at C2 that could make a potent purine scaffold TLR7 agonist (1 and 2) to lose agonism and acquire antagonist activity (in 3), which could further be potentiated by the introduction of an additional basic center at C6 (in 4). At the C2 position, suitable substitutions such as –NH2, substituted benzylamines and benzamides are well-tolerated for TLR9 inhibition (in 5 &7). On the other hand, when –NH2 substitution is present at C2 position suitable N9 substituents can modulate TLR7 inhibition (in 6). At the C6 position, piperazine with hydrophobic ethyl and cyclopentyl groups is favorable as compared to electron-withdrawing amides. At N9 both ethyl piperazine and pyrrolidine groups can manifest promising TLR9 antagonism with high selectivity against TLR7. Among the set of hit compounds, the lead compound 5 was selected based on reporter assays and in vitro pharmacokinetic profile. The in vivo therapeutic efficacy of 5 was established in a preclinical murine model of psoriasis. Histomorphological evaluation of organs did not show any major morphologic changes following oral administration of 5. These results provide compelling evidence which advocates 5 as a potential candidate for future development into a clinically relevant therapeutic molecule. The highlight of the work was a small structural modification in TLR7 ligand could lead to a reversal in their functional activity.



Designing of Development of Novel Small molecules ameliorating Non alcoholic Steatohepatitis (NASH)

Nonalcoholic fatty liver disease (NAFLD) is the most chronic liver disease in which >5% steatosis has been occurred in absence of significant alcohol consumption, monogenic hereditary disorders, and steatogenic medications. Also it is strongly associated with type 2 diabetis and obesity. NAFLD is subdivided into two classes based on histological outcome. Nonalcoholic steatohepatitis (NASH) is the invasive progression of NAFLD where extracellular matrix proteins, notably collagen fibres, accumulate in the liver encircling hepatocytes and forming scar tissue resulting in irreversible hepatocellular damage (ballooning) and inflammation occurs. Nonalcoholic fatty liver (NAFL) is the pre-sign of NASH, usually considered as benign (accumulation of fat). NASH progression leads to fibrosis, cirrhosis, liver failure and finally hepatocellular carcinoma (HCC). There is no current therapeutics to prevent NASH. So our lab is mainly focusing on novel small molecules curing NASH in a rational way starting with Quinazolinediones and Quinazolinones cores. We have filled two patents in on the molecules from these cores.

Development of new drugs for Leishmaniasis

Leishmaniasis is a disease caused by parasites of the Leishmania type which is spread by the bite of certain types of sandflies. Most prominently it can be observed on underdeveloped areas specially in Africa, Brazil, Nepal, Bangladesh. In India Bihar, Jharkhand, UP, West-Bengal are the prevalent area for this disease. Skin ulcers, fever, low red blood cells, enlarged liver are the prominent indications occurred due to this. So our lab is focused for curing this traumatic disease by designing the small molecules in a rationalized way. I've set up a bilateral collaboration between IICB, Monash University and University of Melbourne, Australia to develop new drugs for Leishmaniasis. A grant was approved under Australia-India Strategic Research Fund (AISRF) (Indo-Australian Biotechnology Fund stream to the Department of Biotechnology, Government of India.

Aims and Objectives

Our lab aims to answer fundamental questions that lie at the interface of chemistry and biology by integrating the concept of organic chemistry, biochemistry and molecular modeling to perform Structure-Based Design and Synthesis of novel chemical entity to unravel the molecular mechanism and develop affordable drugs for potential treatment for human diseases.

Work Achieved

- We have successful developed potent and dual/selective TLR9 and TLR7 antagonists and filled two patents to protect the IP. Two patents are now in pipieline in filing in US. We also published three articles in various reputed journals (Journal of Medicinal Chemistry, ChemMedChem and Molecules) to establish our understanding in the autoimmune diseases and viability of the developed molecules.
- We have reported design, synthesis, and validation of "non-camptothecin" Topoisomerase 1 (Top1) poisons as anticancer agents. We filed provisional as well as PCT/WO to protect our IP. We published three articles; one in Journal of Medicinal Chemistry (ACS), European Journal of Medicinal Chemistry (Elsevier), and another in Computational and Structural Biotechnology Journal.
- The novel class of Top1 poisons is based on the quinoline core developed through an understanding of the structural features of ligands essential for binding in the active site. We provided compelling evidences that advocate for our novel Top1 poisons as a

potential anticancer agent.
- We recently filed provisional patent in NASH. Application no: 0186NF2019
- We've set up a bilateral collaboration between IICB, Monash University and University of Melbourne, Australia to develop new drugs for Leishmaniasis. A grant was approved under Australia-India Strategic Research Fund (AISRF) (Indo-Australian Biotechnology Fund stream to the Department of Biotechnology, Government of India.

Future Research Plans

- Preclinical development of rationally designed inhibitors for the nucleic acid-recognizing Toll-like receptors (TLRs) for which two patent has been filed. In vivo validation of our novel Topoisomerase 1 (Top1) poisons as anticancer agents.
- Establish industrial partner to take forward our IP protected knowledge to commercialization.
- To seek ways to extend a long term scientific collaboration on mutual strength between India and Australia (Monash University and University of Melbourne) in neglected disease drug discovery.

Publications

- ICBS 2021: Looking Toward the Next Decade of Chemical Biology. Clinton G. L. Veale, Arindam Talukdar, Boris Vauzeilles. *ACS Chemical Biology 2022*, 17, 728–743. DOI: 10.1021/acschembio.2c00209
- Fascinating Transformation of SAM-Competitive Protein Methyltransferase Inhibitors from Nucleoside Analogues to Non-Nucleoside Analogues. Arindam Talukdar, Ayan Mukherjee, Debomita Bhattacharya. *Journal of Medicinal Chemistry,* 2022, 65, 3, 1662-1684. DOI: 10.1021/acs.jmedchem.1c01208
- Systematic Optimization of Potent and Orally Bioavailable Purine Scaffold as a Dual Inhibitor of Toll-Like Receptors 7 and 9. Biswajit Kundu, Deblina Raychaudhuri, Ayan Mukherjee, Bishnu Prasad Sinha, Dipika Sarkar, Purbita Bandopadhyay, Sourav Pal, Nirmal Das, Debdeep Dey, Kantubhukta Ramarao, Kasireddy Nagireddy, Dipyaman Ganguly, Arindam Talukdar. *Journal of Medicinal Chemistry*, 2021, 64, 13, 9279-92301. DOI: 10.1021/acs.jmedchem.1c00532
- Structural Evolution and Translational Potential for Agonists and Antagonists of Endosomal Toll-like Receptors. Arindam Talukdar, Dipyaman Ganguly, Swarnali Roy, Nirmal Das, Dipika Sarkar. *Journal of Medicinal Chemistry*, 2021, 64, 12, 8010-8041. DOI: 10.1021/acs.jmedchem.1c00300
- Identification of small molecule allosteric modulators of 5,10-methylenetetrahydrofolate reductase (MTHFR) by targeting its unique regulatory domain. Gustavo A. Bezerra, Alexander Holenstein, William R. Foster, Bing Xie, Kevin G. Hicks, Céline Bürer, Seraina Lutz, Ayan Mukherjee, Dipika Sarkar, Debomita Bhattacharya, Jared Rutter, Arindam Talukdar, Peter J. Brown, Minkui Luo, Lei Shi, D. Sean Froese, Wyatt W. Yue. *Biochimie*, 2021. 183, 100-107. DOI: 10.1016/j.biochi.2021.01.007

Memberships

Life member of Chemical Biology Society

Extramural Funding

- Design and Development of Selective inhibitors of protein arginine methyltransferase 1 involved in epigenetic modifications. Core Research Grant. SERB. February 2020. CRG/2019/000853.
- Development of new drugs for leishmania- an australia-indian partnershipAustralia-India Strategic Research Fund (AISRF): Start Year: June 2017. End Year: June 2020. Agency: DBT, Gov. of India.
- Probing endosomal toll-like receptor 9 biology using novel small molecule antagonists. Start Year: September 2016. End Year: March 2019. Agency: EMR/2015/000117. Agency: DST-SERB, Gov. of India.
- Exploring Therapeutic Efficacy of Novel Toll like Receptor 9 Antagonist in Type II Diabetes. Start Year: March 2017 End Year: March 2020. Agency: EMR/2016/003021, DST-SERB, Gov. of India.

Patent

Sl No.	Title	Country	Filed on (Date)	Names of the inventors
1	Preparation of quinazolinediones and use thereof	World	PCT Application No.: PCT/IN2022/051099 Dated 25.12.2022	Arindam Talukdar, P Chakrabarti, D Sarkar, S Chowdhury, S Goon, A Sen, U Ghosh Dastidar, B Patra, I Hoque
2	Quinazolinones derivatives for treatment of Non-Alcoholic Fatty Liver disease, preparation and use thereof.	World and US	WO2022003712 (A1). US patent filing done. Dated 25.06.2021 US application no. : 18013681 Dated 29.12. 2022	Arindam Talukdar, P Chakrabarti, D Sarkar, S Chowdhury, S Goon, S Das, N Das, D Sarkar

Development of synthetic supramolecular receptors and crystal engineering of functional molecules

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Background

Design and synthesize of synthetic supramolecular receptors capable of binding desired and targeted analytes or molecules is an emerging area to develop functional materials. Polycyclic aromatic hydrocarbons, carbohydrates and anions are three diverse target molecules. Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic in nature, persistent in environment and their removal is crucial. Synthetic receptors capable of binding PAHs are highly sought-after, but hard to realize due to the size, solubility and other physical properties. Carbohydrates are challenging class of molecules to design synthetic receptors, due its polar nature and diverse isomers. Mimicking the natural lectins to recognize carbohydrates with synthetic receptors provides excellent opportunity in different biological functions ranging from managing diabetes to early-stage cancer detection. Synthetic molecules capable of binding anions, especially chloride anion, and transporting it across bilayer membrane can play therapeutic role in diverse physiological conditions. Despite with the tremendous progress made in synthetic molecules capable of binding anions, only a few are capable of transporting it across lipid membranes, and they are hard to access. We aim to develop synthetic receptors against these challenging class of targets in a readily accessible manner. We have earlier developed a cage-like macrocycle with ease of synthesis in high yield to bind PAH molecules. We have further developed chiral-cage molecule built with steroids, although it did not bind the carbohydrates, as indented. We are working on the development of chloride anion transporting receptor with ease of synthesis.

We also focus on understanding of molecular recognition and crystal engineering, especially with the tool of single-crystal x-ray diffraction techniques. Crystal engineering has become a powerful tool in developing formulation as cocrystals. Further crystal engineering is a reliable tool to modulate the emission properties of flurorescent molecules. Many fluorescent molecules which are emissive in solution state are not emissive in the solid state. However, cocrystallization with designer molecules can enable them emissive at solid state with desired features.

Aims and Objectives

- Development of novel synthetic receptors for the recognition of biologically relevant molecules of PAHs, carbohydrates and anions through specific intermolecular interactions.
- Crystal engineering of organic molecules to develop functional materials and modulating the properties of drugs.

Work Achieved

We have earlier demonstrated the synthesis of cofacial cage molecules capable of binding with PAH guests in competitive solution. Subsequently, we have expanded the scope of the synthesis which resulted in diverse organic cage molecules. We have utilized coordination driven self-assembly to form synthetic receptors of metal-organic cage molecules. We have applied the strategy to form hydrogen bonding appended chiral organic linker to form metal-organic cage molecules, recently. Further work in progress in examining the binding with carbohydrates.

While working on developing organic and metal-organic cage molecules, we come across certain intermediate molecules which serve excellent platform for crystal engineering. Earlier we have reported cocrystal engineering with triazine-triphenol with di-pyridyl molecules. Likewise, we have come across tetra-hydroxybiphenylethylene while developing a certain organic cage molecule.

Herein, we report the cocrystals of AIEgenic

tetraphenol, namely tetrakis(4-hydroxybiphenyl)ethylene (THBPE), with a few bispyridyl azadonors, built with phenol···pyridine hydrogen bonding. We employed two different crystallization conditions: diffusion of hexane into THF and slow evaporation from ethyl acetate. The former condition readily afforded cocrystal in all instances except one, whereas the latter afforded only one. Attempted cocrystallization with 4,4' -bipyridine (BP) did not result in any cocrystals but instead afforded THF solvates of the tetraphenol. The rigid coformers, 4,4' -azopyridine (AP) and 1,2-bis(4-pyridyl)ethylene (BPE), resulted in non-emissive close-packed cocrystal solvates. In both cases, the THF solvent competes with pyridyl moiety for hydrogen bonding at two of the four possible sites in the tetraphenol, which is uncommon for phenol···pyridine supramolecular synthon. However, the synthon wins out in the crystals from ethyl acetate solution with one coformer (AP) to form a fully hydrogen bonded square lattice (***sql***) network. Moderately flexible 1,2-bis(4-pyridyl)ethane (BPA) led to a close-packed hydrated cocrystal solvate. Highly flexible 1,2-bis(4-pyridyl)propane (BPP) afforded emissive cocrystals, with nanotubular pores of 10 Å diameter. The later cocrystal adopts ***qtz*** topology with eight-fold interpenetration. The latter two cocrystals are emissive in the solid-state, which is unusual for phenol···pyridine hydrogen bonded complexes. The quantum yields of the solid-state emission exhibited by the latter two cocrystals are 16% and 25%, which are higher than that of the tetraphenol with 9%. The observed emission property can be correlated with how unique and efficient the AIEgens are packed in the solid with the assistance of coformers. The cocrystallization experiments with rigid tetraphenol and diverse bispyridyl coformers also provide insight into the formation of cocrystals along with solvates and hydrated solvates. Notably, four, among five, cocrystals reported herein, have Z'=2.

The results which we have demonstrated are remarkable in that this is the rare example of modulating the solid-state emission of fluorescent molecules by hydrogen bond mediated cocrystallization.

Future Research Plans

Development of organic cage molecule with the above emissive fluorescent molecule soluble in organic solvent and water for the recognition of biologically relevant analytes.

Publications

- Samanta, K.; Maji, S.; Samanta, J.; Natarajan, R. (2021) Crystal Engineering of a Tetraphenol with Bispyridines toward Hydrogen-Bonded Emissive Cocrystals. *Cryst. Grwoth Des 21*, 6485-6496.

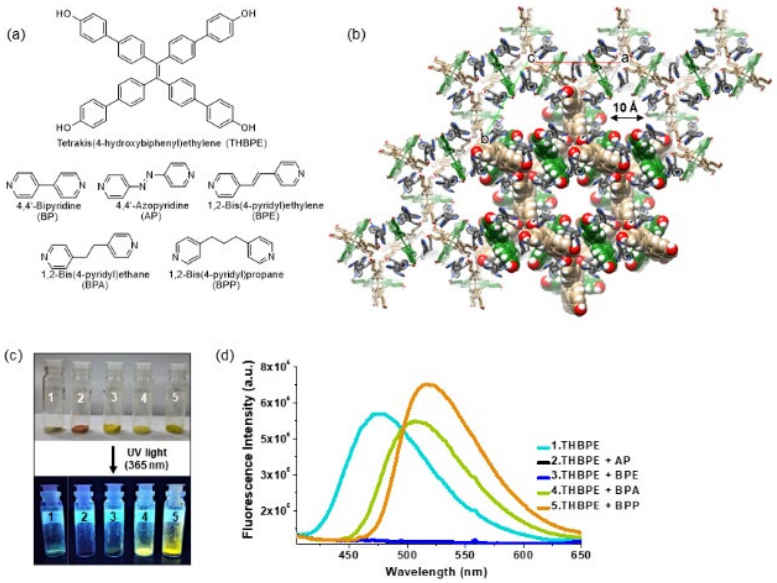

Figure 1: (a) Molecular structure of THBPE and di-pyridtyl coformers; (b) Crystal structure of THPBE-BPP which is eight-fold interpenetrated and in qtz topology; (c) Photomicrographs of the cocrystals, under UV (365 nm) irradiation, and (d) Solid-state emission profile of THBPE and its cocrystals

Development of Catalytic and Electrochemical Approaches for Late-Stage Functionalization and Synthesis of Potential Bioactive Molecules

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Background

Our research group has substantially contributed in the field of **catalysis** in the organic chemistry area (C-H bond activation/functionalization) to develop affordable, efficient, and innovative as well as the industry-friendly synthetic processes for the synthesis of functionalized potential bio-active molecules such as Anthrone, Benoxazine, Benzosultam, Oxaziridine, acridine, as well as Benzofuranone, employing transition metal(free)-catalysis employing the concept of **electrocatalyzed** and **transition metal-catalyzed** (Pd, Fe, Co, Ni, Ru, Rh & Ir) C–H/C–X bond activation and metal-free C–H/C–X bond functionalizations.

Aims and Objectives

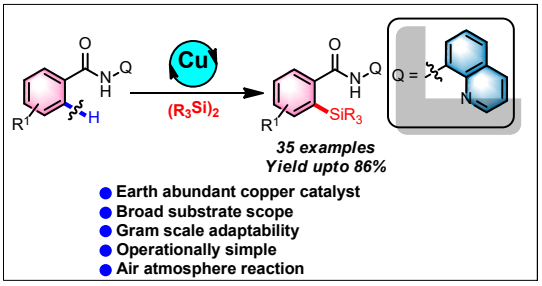
- Development of electro-catalyzed, Metal-free and transition metal-catalyzed cost-effective, affordable and industry-friendly C-H activation/functionalization process for the late-stage functionalization of pharmacophores such as benzoxazines, benzosultams, anthrone, benzofuranone and quinoline.
- Development of a process for the synthesis of the generic version of FDA approved drug against COVID-19 and other infectious diseases.

Work Achieved

Employing catalysis, and electrochemical synthesis we have developed the methodologies to synthesize functionalized spirocycles and heterocycles. We have published **three publications Organic Letters, 2021, 18, 4521–4526 (IF: 6.005), Chemical Communication, 2022, 58, 2902-2906 (IF: 6.222).and Book Chapter (Wiley) and Two Indian Patents.** We have developed the process for APIs such as Tilorone (anti-viral) and Tropisetron.

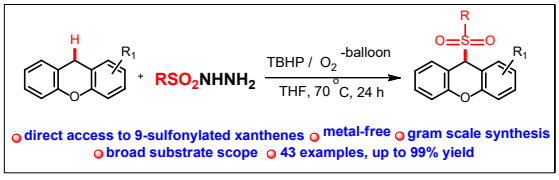
Copper-Catalyzed Direct sp2 C-H Silylation of Arylamides using Disilanes (May 2021):

A copper-catalyzed method for direct intermolecular ortho-silylation of benzamides has been developed that affords organosilane products in moderate to high yields. The key features include: (i) use of commercially available disilanes as silicon source with 8-aminoquinoline as a bidentate directing group, (ii) use of earth-abundant first-row transition metal, (iii) operationally simple conditions without the need of an inert atmosphere, and (iv) tolerance of a wide range of functional groups. The practicality and effectiveness of this method has been demonstrated by a gram-scale experiment. This strategy therefore, constitutes a convenient way of constructing C–Si bonds useful for synthetic organic chemistry. **The manuscript is published in Organic Letters, 2021, 18, 4521–4526 (IF: 6.005), This work has been selected by ACS for cover page**.



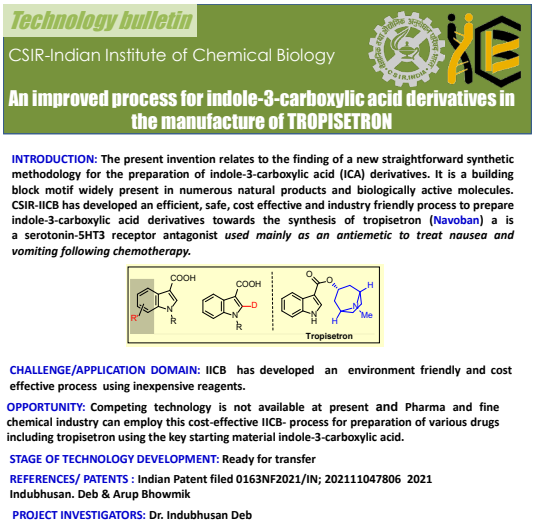
A Metal-free Radical/Radical Cross-Coupling Reaction of Xanthene with Sulfonyl Hydrazides: Facile Access to Xanthen-9-sulfone Derivatives (Feb 2022):

A straightforward strategy for direct incorporation of sulfonyl units into xanthene moiety for accessing xanthen-9-sulfone derivatives in good to excellent yields has been established via metal-free oxidative cross-coupling reaction of xanthenes and sulfonyl hydrazides. Using easily accessible starting materials this methodology proceeds efficiently with a high degree of functional group compatibility and with a wide scope of both xanthenes and sulfonyl hydrazides under operationally simple reaction conditions. Mechanistic investigations revealed that sulfonyl radicals could be generated from sulfonyl hydrazides in the presence of TBHP under oxygen atmosphere. **The manuscript is published in Chemical Communication, 2022, 58, 2902-2906 (IF: 6.222).**



Provisional Indian Patent:

Indole-3-carboxylic acid derivatives represent a vital building block stipulates an immense recognition owing to their wide range of application as a key starting material to synthesize several artificial drugs such as Arbidol, Tropisetron, Dolasetron, etc. or it's derivative broadly used as anticancer agents (CPI-1205), serotonin 5-HT4 and 5-HT6 antagonists, EphB3 receptor tyrosine kinase inhibitors, potential therapeutic agents for Alzheimer's disease, mast cell tryptase inhibitors and widely present in numerous natural products. We have demonstrated the use of suitable reagents towards the synthesis of diverse, indole-3-carboxylic acid derivatives with high functional group tolerance, thereby generating a broad substrate scope with excellent yields. This protocol is applicable for the synthesis of indole 3-carboxylic acids. The making of desired ICA and AA derivatives is most important as these have been used as a key intermediate to synthesize several biologically active molecules including tropisetron. We have extended our methodology for the synthesis of tropisetron, a commercially available drug as serotonin 5-HT4 and 5-HT6 antagonists. Which has been **filed for patent PATENT 0163NF2021/IN; 202111047806 2021 and given for expression of Interest to attract Industry partner.** By slightly tuning the reaction conditions, a divergent synthesis of the library of anthranilic acids has also been established.



We are actively involved in **COVID-API mission.** To support urgent research to combat the ongoing outbreak of COVID-19, caused by the novel coronavirus SARS-CoV-2, we have to develop a cost-effective and industry-friendly process for the synthesis of Tilorone dihydrochloride from commercially available cheap starting materials. Tilorone has a broad-spectrum of antiviral activity through an IFN-related innate immunity pathway and orally bioavailable interferon inducer. In vivo efficacy studies from the literature showed possible uses of tilorone against a broad array of infections including Middle East Respiratory Syndrome

(MERS), Chikungunya, Ebola and Marburg and more recently against human coronaviruses including MERS-CoV which highlights that this old drug may be repurposed against the new viruses that are responsible for the pandemic situation right now. **The process has been filed for a patent, 0211NF2020,** 202111047809, Dated 20th Oct, 2021

Future Research Plans

- Development of the process for the synthesis of the broad spectrum of pharmaceutically relevant (chiral) molecules employing **Electrocatalysis, photocatalysis** and **Metal (free)-catalysis**. Bioactivity study of newly synthesized molecules will be pursued.
- Development of the affordable process for the generic version of FDA approved API (anti-viral Mission and anti-Cancer mission) and agro chemicals.

Publications

- Writhabrata Sarkar, Aniket Mishra, Arup Bhowmik and **Indubhusan Deb*** "Copper-Catalyzed Direct sp2 C–H Silylation of Arylamides Using Disilanes" ***Organic Letters, 2021, 18, 4521–4526*** **(IF: 6.005), This work has been selected by ACS for cover page**.
- Sumit Das; Arup Bhowmik; Writhabrata Sarkar; Imtiaj Mondal, Aniket Mishra, Subhra J. Saha, Sudip Karmakar and **Indubhusan Deb* "**A radical–radical cross-coupling reaction of xanthene with sulfonyl hydrazides: facile access to xanthen-9-sulfone derivatives" ***Chemical Communication,*** **2022, 58, 2902-2906 (IF: 6.222).**
- Arup Bhowmik; **Indubhusan Deb*** An improved synthetic method for the preparation of indole-3-carboxylic acid derivatives: useful key intermediate for the synthesis of several biologically active molecules including Tropisetron **Provisional Patent**: **0163NF2021/IN; 202111047806 Dated, 20th Oct 2021**
- Parasuraman Jaisankar, **Indubhusan Deb,** Pinaki Bhatacharaya**,** An improved synthetic method for 2,7-dihydroxyfluorenone in the manufacture of tilorone and its salts **0211NF2020,** 202111047809, Dated 20th Oct, 2021

Awards/Honours/Memberships

- Bristol Mayer Squibb Research Fellowship-USA
- Life member of Chemical Biology society
- Membership of ACS Chemical Society

Extramural Funding

- SERB, DST, 12/2020-12/2023 INR 41,7 Lakh
- Bristol Mayer Squibb Research Grant. $ 10,000 (BMS-USA), 2020

Integrating Regioselective *E* → *Z* Isomerization of Trienones with Cascade Sequences under Photosensitizer-Free Direct Irradiation at 390 nm

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Background

The activation of chemical reactions using light as an external stimulus is an extremely powerful tool for synthetic organic chemistry. However, a photosensitizer is often required for harvesting light energy and transferring it to the target substrate or reagent via energy transfer. There is a persistent challenge to find a suitable photosensitizer. While direct irradiation of reactant(s) has provided an alternative solution in this endeavour. Not only is this direct irradiation strategy operationally simple and cost-effective, it also removes the need for photosensitizers and additional reagents that would mitigate competing side reactions and subsequently would impact overall efficiency. The past few years have witnessed a renaissance in visible-light-mediated contra-thermodynamic *E* → *Z* alkene isomerization employing selective energy transfer catalyst. The fundamental design principle of such isomerization is deconjugation of the *Z*-isomer and thereby raising the triplet energy relative to the substrate, subsequently inhibiting re-excitation of the *Z*-isomer. The *E* → *Z* directionality has been mainly controlled by using noncovalent destabilizing 1,3-allylic strain or stabilizing through-space interactions such as $n_O \rightarrow p$ (boron p-orbital), $n_S \rightarrow Zr$ (zirconium), and $n_O \rightarrow \pi_{C=O}^*$, or through the in-situ formation of a transient chromophore species. Although the emphasis remains mostly on the selective manipulation of isolated alkene units in chromophore, the regioselective *E* → *Z* isomerization of target alkene units embedded in conjugated dienes or polyenes remains mostly underexplored. This is presumably due to the additional complexities arise in selective chromophore deconjugation of the extended π-system and thereby inhibiting the process with an intrinsic directionality. It is pertinent to note that such a photosensitized alkene isomerization under UV-light irradiation has been well-established for decades, however, their potential applications have been restricted due to the problems associated with general applicability, temperature regulation, and functional group compatibility issues.

Aims and Objectives

The regioselective *E* → *Z* isomerization of a target olefin unit embedded in a conjugated polyene is challenging. Examples are limited only with retinal and its derivatives. The problem is further amplified when such an isomerization is integrated into cascade sequences, among which the regioselectivity and subsequent directionality are the major bottlenecks. Indeed, there are no reports till date for such a transformation. Herein, we report that such a controlled isomerization and subsequent cyclization cascade can be enabled by photosensitizer-free direct irradiation of linearly conjugated acyclic polyenes in dichloromethane solvent using a 390 nm LED. The directionality results from deconjugation of the extended π-system in the transient *Z*-isomer due to stabilizing n → π* interactions between 1,4–dicarbonyls (C=O → C=O) or 1,4–carbonyl/–aryl (C=O → aryl) groups. The involvement of such noncovalent interactions has been supported by X-ray crystallography and control experiments. Thus, conjugated trienones can be converted into the oxabicyclo[3.2.1]octadienes stereoselectively in an atom- and step-economic manner, including the first example via regioselective isomerization of a tetrasubstituted alkene. The reaction conditions are very general (>46 examples) and can be conducted in an open air at ambient temperature. Such a cascade cyclization can also be realized in the solid state.

Work Achieved

We have developed an unprecedented atom- and step-economical route to oxabicyclo[3.2.1]octadienes stereoselectively from linearly conjugated acyclic trienones mediated by direct excitation at 390 nm LED in CH_2Cl_2 solvent. The reaction proceeds under mild conditions in open air at ambient temperature and shows high functional group tolerance. This strategy further underscores the importance of sensitizer-free and operationally simple direct irradiation to generate molecular complexity under green and sustainable conditions. Such a cascade cyclization can also be done in the solid state, albeit in moderate yields. Crucial to the success of this endeavour is the highly regioselective contra-thermodynamic alkene isomerization of the terminal double bond bearing 1,4–dicarbonyls or 1,4–carbonyl and aryl groups. The directionality in the cascade has been ensured by stabilizing n→π* interactions which deconjugate the intermediary *Z*-isomer. After isomerization, highly-strained *Z*-configured double bond would undergo subsequent reactions cascade under the same photochemical conditions to deliver the product. The isomerization and subsequent cyclization of a tetrasubstituted alkene embedded in trienone serves as a rare example in this cascade sequences. We hope our findings on regioselective isomerization of polyenes using stabilizing noncovalent interactions will find wide applications.

Future Research Plans

Following our success in C3-thioester/-ester substituted dienones as pluripotent molecular platform in diverse organic transformations, we are interested in further developing methodologies that harness the power of visible-light to mediate reactions that are useful in the synthesis of biologically active molecules and pharmaceuticals.

Publications

- S. Naskar, R. Moi, I. Das, K. Biradha, *Angew. Chem. Int. Ed.* **2022**, *61*, e202204141; (2) R. Maity, S. Ghosh, I. Das, *Chem. Eur. J.* **2023**, e202300421.

Organosulfide rich ferula oil loaded o/w delivery system: Effect of lipid phase on fabrication, stability, release, bio-accessibility and anti-cancer potential

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Research Activities

- Isolation and characterization of compounds from *Ferula assa-foetida* oil
- Development of analytical methods for isolation, purification of organosulfides
- Studies as per phytopharmaceutical guidelines – Stability studies, pre-formulation studies, etc.

Purification and identification of anticancer organosulfides from Ferula assa-foetida gum: integrative analysis employing GC/GC-MS/RP-HPLC/NMR

Ferula assa-foetida, containing organosulfides is widely used in Indian cuisine and traditionally claimed to have several medicinal properties including anticancer properties. Ferula oil enriched with organosulfides displayed significant inhibition of the cell growth in-vitro against SKOV3 and A549 cancer cells in a dose-dependent manner. This prompted us to investigate and delineate the compounds responsible for the activity. In this endeavor, the employed GC/GC-MS analysis resulted in the indecisive outcome. This led to the development of an expedient isocratic RP-HPLC method for the separation and purification of four major compounds which were further unambiguously characterised as (-)-E-2-butyl propenyl disulfide, (-)-Z-2-butyl propenyl disulfide, (-)-1-(methylthio)propyl(E)-1-propenyl disulfide, and (-)-1-(methylthio)propyl(Z)-1-propenyl disulfide employing ^{1}H, ^{13}C, and 2 D NMR (Figure 1). The isolated compounds were further evaluated for their potential against SKOV3 and A549 cell lines where a trisulfide has displayed better activity.

Effect of lipid phase on fabrication, stability, release, and bio-accessibility

Organosulfides-enriched ferula oil (FO) have several proven pharmacological effects besides its culinary usage. However, its volatile nature and water insolubility limit its application as a therapeutic and nutraceutical agent. The present study, focusing fabrication of ferula oil nano-emulsion with tween 80 using ultrasonication, revealed that the ferula oil itself cannot serve as an oil phase. Therefore, different lipid phases were selected, wherein fatty acid/ester containing lipid phases (isopropyl myristate/IPM, ethyl oleate/EO, and caprylic acid/CA) resulted in clear and transparent nano-emulsions with average globule size of 26.77 ± 8.94, 27.11 ± 4.29, and 25.80 ± 0.49 nm, respectively. Further, stability studies at three different temperatures and Turbiscan analysis revealed that the carbon length influences the stability as well as ferula oil retention capacity and bioaccessibility. Findings, provide a basis for the development of ferula oil-loaded nano-emulsions as functional food and therapeutic agent.

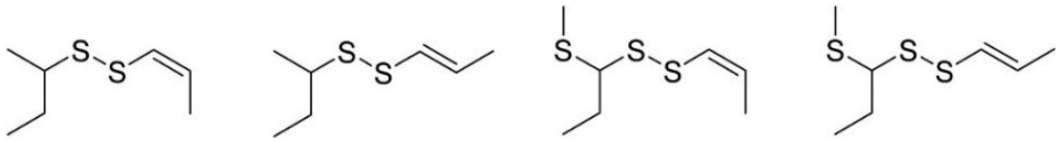

Figure 1: Structures of the organosulfides

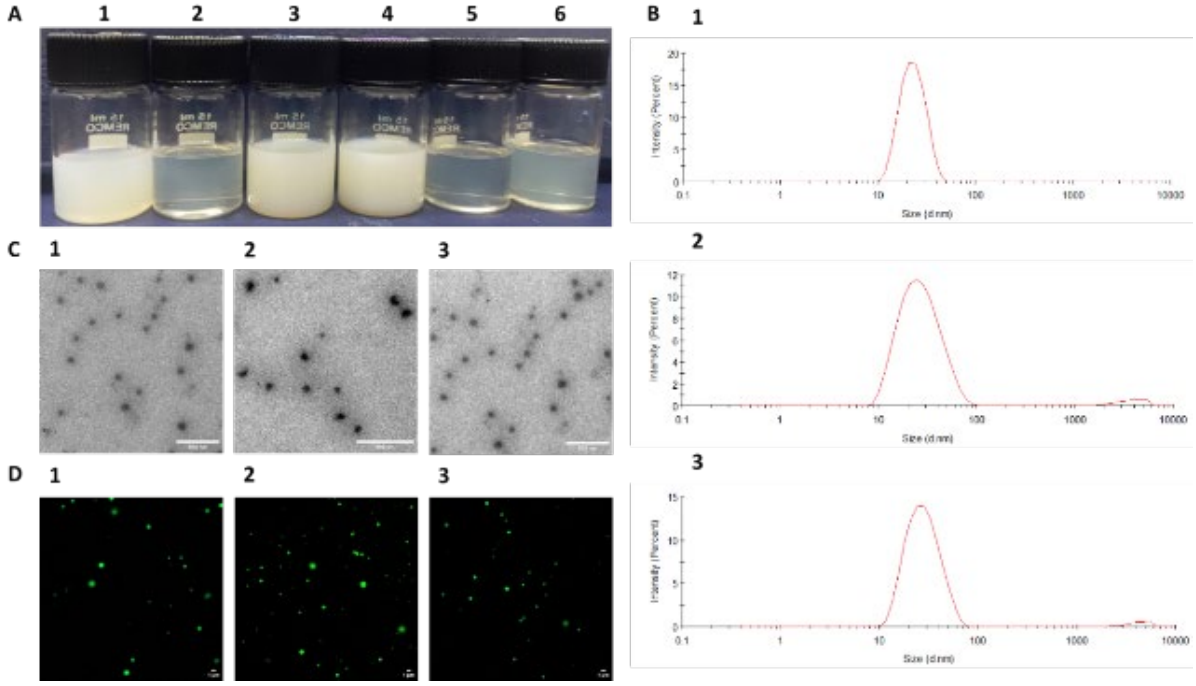

Figure 2: 2A - Oil-in-water (o/w) nano-emulsions prepared with the ferula oil (1A1), FOEO (1A2), FOCO (1A3), FOOO (1A4), FOCA (1A5), FOIPM (1A6). **2B -** Globule size distribution of o/w nano-emulsion fabricated with FOIMP (1B1), FOEO (1B2), FOCA (1B3). **2C -** TEM images of o/w nano-emulsion fabricated with FOIPM (1C1), FOEO (1C2), FOCA (1C3); scale bar 500 nm. **2D** - CLSM images of o/w nano-emulsion fabricated with FOIPM (1D1), FOEO (1D2), FOCA (1D3); scale bar 1 µm.

Future Research Plans

In future, majority of our research efforts would be directed towards the development of stable formulation and its exploration in animal models as per the phytopharmaceutical drug guidelines.

Publications

- Myricitrin – a flavonoid isolated from Indian olive tree (*Elaeocarpus floribundus*) inhibits Monoamine oxidase in brain and elevates striatal dopamine levels: therapeutic implications against Parkinson's disease. Banerjee C, Nandy S, Chakraborty J, **Kumar D**. Food Funct. 2022, https://doi.org/10.1039/D2FO00734G
- Seedpod extracts of Wrightia tinctoria shows significant anti-inflammatory effects in HepG2 and RAW-264.7cell lines. Karmakar E, Das P, Yatham P, **Kumar D**, Mukhopadhyay S, Roy SS. Nat Prod Res. 2022. https://doi.org/10.1080/14786419.2022.2146688
- Two new cycloartane type triterpenes from *Dysoxyllum binectariferum*. Singh CP, Sharma A, Sharma K, Darshani P, Katare A, **Kumar D**, Bharitkar YP. Nat Prod Res. 2022. https://doi.org/10.1080/14786419.2023.2172008

Patent

- A composition for treating parkinson's disease. Kumar D, Chakraborty J, Yatham P, Banerjee C, Sarma SS. 2023. 202311006898

Deciphering the functional role of GSH in plant defense with special reference to the identification of GSH-regulated stress responsive microRNA/s in *Arabidopsis thaliana*

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Research Activities

Arabidopsis thaliana plants, both wild type Col-0 cultivar as well as transgenic lines viz. *AtECS*, overexpressing LeɣECS gene, previously developed in our lab, were grown. Earlier studies noted the enhanced GSH content in transgenic *AtECS* lines as well as demonstrated its potentially significant stress tolerance aptitude as compared to wild type Col-0. Interestingly, mechanistic understanding on the *AtECS* lines displayed the interplay of ET biosynthesis besides SA signaling with GSH in addition to the induction of several other stress modulators viz. HSPs, MAPKs etc. to mitigate environmental stress conditions. To obtain an in depth understanding, various other experiments were carried out. In this context, one of the key objectives was to identify the GSH-responsive microRNA in *A. thaliana* under both control and stressed conditions. For stress conditions, both abiotic and biotic stress conditions were implemented. Stress analysis of Col-0 and *AtECS1* plant for abiotic assay was performed with salinity stress using NaCl treatment. Whereas biotic stress analysis was performed with bacterial strain *Pseudomonus syringae* and fungal strains of *Botrytis cinerea* and another necrotrophic fungal pathogen *Alternaria brassicicola*. Analyses were executed to decipher how GSH imparts stress tolerance via miRNA-mediated gene regulation in both wild-type Col-0 and transgenic *AtECS1* under stressed condition. Construction of miRNA library under both control and stressed conditions were performed following the identification of GSH-responsive miRNA. Further validation of GSH-regulated stress responsive microRNA was also performed to show the accuracy of the dataset. Downstream analysis revealed miRNAs targeting defense-related genes like LRRK, MYB transcription factors, TCP8, etc. - down regulated in *AtECS1* line, which further relieved repression of their target gene expression, leading to stress resistance. Together, findings suggest that GSH plays a functional role in modulating miRNA-mediated regulation of defense-related genes to mitigate stress conditions *in planta*.

Future Research Plans

- Overexpression of GSH responsive miRNA in *Arabidopsis thaliana* and its validation.
- Confirmation of target gene cleavage using the degradome database of *Arabidopsis.*
- Development of stress tolerant crop plants useful for the agricultural sector.

Extramural Funding

CSIR, New Delhi

Publications

- Aparupa Bose Mazumdar Ghosh, Anindita Banerjee, **Sharmila Chattopadhyay*** (2022). An insight into the potent medicinal plant *Phyllanthus amarus* Schum. and Thonn. **The Nucleus 65, 437–472.** https://doi.org/10.1007/s13237-022-00409-z
- Riddhi Datta, Kajal Mandal, Priyanka Boro, Asma Sultana, **Sharmila Chattopadhyay*** (2022). Glutathione imparts stress tolerance against *Alternaria brassicicola* infection via miRNA mediated gene regulation. **Plant Signal Behav** 17(1):2047352. doi: 10.1080/15592324.2022.2047352.
- Priyanka Boro, Asma Sultana, Kajal Mandal, **Sharmila Chattopadhyay*** (2022). Interplay between glutathione and mitogen-activated protein kinase 3 via transcription factor WRKY40 under combined osmotic and cold

stress in *Arabidopsis*. **J. Plant Physiol.** 271. https://doi.org/10.1016/j.jplph.2022.153664

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- Soumi Biswas, Saptarshi Hazra, and **Sharmila Chattopadhyay*** (2021). Deep sequencing unravels methyl jasmonate responsive novel miRNAs in *Podophyllum hexandrum*. **J. Plant Biochem. Biotechnol.**, https://doi.org/10.1007/sl3562-021-0069

Structural biology & Bioinformatics Division

With a view to understand cellular function and dysfunction in human health and disease, researchers at the Structural Biology & Bioinformatics Division studies attempt to probe into the structural and mechanistic features of various proteins, macromolecular complexes and cellular pathways, using integrative, trans-disciplinary approaches. Basic as well as translational research are being carried out on protein structure, functions, protein-protein interactions, protein-nucleic acid interactions, applying state-of-the-art technologies like X-ray crystallography, Nuclear Magnetic Resonance (NMR), Cryo-EM, single molecule fluorescence measurements and Fluorescence Correlation Spectroscopy, Raman spectroscopy, mass spectrometry, Nano-separation technology etc. Bioinformatic studies involving big data analysis, genome/proteome data mining, molecular dynamic simulations, molecular docking and pathway analysis are also being pursued. Special emphasis is given on macromolecules and small molecules of therapeutic interest against diseases like leishmaniasis, tuberculosis, malaria, multiple amyloid-related neurodegenerative diseases, systemic diseases like cancer and diabetes and microbial infections.

Using the spectroscopy at ensemble and single molecule resolution to study protein folding/aggregation and its implications in Neurodegenerations and Infections

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Background

We have been studying the early and unexplored events of protein aggregation and its implications in neurodegenerations and infections. We first showed using spectroscopy at single molecule resolution that a protein at the early microsec fluctuates between conformers of different radii and forms oligomers (**JBC 2010, JBC 2012, JBC 2015**). We then combined FRET and FCS to develop methodology to detect the formation of early oligomers, which contribute maximally to the cellular toxicity of neurodegeneration (**Langmuir 2014**). We then followed up using cryo-EM to report for the first time the structural understanding of toxicity induced by alpha synuclein oligomers in Parkinson's Disease, in which targeting a crucial histidine residue of alpha synuclein arrests fibril propagation (**Communications Biology 2021**).Our applications of chemical biology tools on spectroscopy in vitro and in live cells show that, the nature of fluctuations of a protein and its ability to form early oligomers can have profound implications on how this protein would form amyloid at the late stage (**ACS Chem Neurosci 2014; Sci. Rep 2018**), Recently, using more than 140 mutants of superoxide dismutase (SOD1) we have developed a cofactor based membrane association model of ALS, and provided experimental validations (**eLife 2021**).

Protein conformational changes during protein-lipid interactions play ubiquitous roles in governing a number of cellular events. Using two proteins of unknown functions, we investigated the role of protein-lipid interactions to define the mechanistic pathway regarding infectious disease progression. Our findings suggested that KMP-11-membrane interaction is modulated by the cholesterol content of lipid bilayer which is directly linked with the mechanism of parasite survival strategy (**J. Phys. Chem. B 2017**) in leishmaniasis. On the other hand, environment-dependent binding events between immunogenic KMP-11 and membrane ergosterol resolved the missing link between ergosterol biogenesis and immune suppression. In another context, we established that MPT63, a protein of M.Tb with immunoglobulin like fold, loses its immunogenic responses through surface binding and this happens through the environment-sensitive conformational switching from native beta sheet to helical conformation (**Langmuir 2018**). Nevertheless, this environment-dependent switch event of MPT63 is also responsible for host cell death through membrane pore formation. Our investigation has revealed that the helix conformer of MPT63 creates toxic oligomers in order to perforate host membranes (**ACS Chemical Biology, 2019**). Using the understanding of the conformational switch of a protein towards the productive infections as a dark side of nature, we investigated the unexplored binary combination of the sequences of SARS COV2 spike protein and the similarity with diverse pathogen derived proteins, which may provide novel molecular insights into the process of infection (**Communications Biology 2021a**).

Ongoing Research Questions

- Developing the molecular understanding of the roles of aggregation intermediates in neurodegeneration
- Studying the role of protein-membrane interactions in infections

Summary of progress made in last one year

Using a combination of CryoEM and biophysics, we report the first time three dimensional structure of a non-toxic early oligomers in the aggregation pathway of alpha synuclein, a protein whose aggregates are found in PD brain. Using chemical biology as a tool, we show that an interface histidine could be targeted by metallo prophyrins and allied proteins to block the toxic pathway.

Using detailed mutational analyses and spectroscopy, we developed a 'co-factor derived membrane association model' of SOD aggregation wherein mutational stress closer to the Zn (but not to the Cu) pocket is responsible for membrane association-mediated toxic aggregation and survival time scale after ALS diagnosis.

Using extensive use of Fluorescence Correlation Spectroscopy at single molecule resolution and biological quantification exercise, we developed detailed protein-membrane interaction landscapes in representative neurodegenerative and infectious diseases and showed the properties of different modulators, like cholesterol.

Future Direction

Our lab would continue to use a combination of chemical biology, biophysics and mathematical biology to work towards developing a molecular grammar, which applies to key aspects of complicated human diseases. While we have made some progress in our in vitro studies, we are now applying our experimental methodologies in live cells measurements.

Publications

- Goswami, Shubhasish; Manna, Bharat; **Chattopadhyay, Krishnananda**; Ghosh, Amit; Datta, Supratim (2021) Role of conformational change and glucose binding sites in the enhanced glucose tolerance of Agrobacterium tumefaciens 5A GH1 β-glucosidase mutants, Journal of Physical Chemistry **125**; 9402-9416
- Mahapatra, Anindita; Mandal, Narattam; **Chattopadhyay, Krishnananda** (2021) Cholesterol in Synaptic Vesicle Membranes Regulates the Vesicle-Binding, Function, and Aggregation of α-Synuclein, Journal of Physical Chemistry **125**; 11099-11111
- Sannigrahi, Achinta; **Chattopadhyay, Krishnananda** (2022) Pore formation by pore forming membrane proteins towards infections, Advances in Protein Chemistry and Structural Biology **128**, 79-111
- Mandal, Narattam; **Chattopadhyay, Krishnananda**; Sannigrahi, Achinta (2022) Studying protein-folding dynamics using single-molecule fluorescence methods 225-236.
- Sannigrahi, A; Chowdhury, S; Das, B; Banerjee, A; Halder, A; Saleem, M; Naganathan, A N; Karmakar, S; **Chattopadhyay, K* (2021)** The metal cofactor zinc and interacting membranes modulate SOD1 conformation-aggregation landscape in an in vitro ALS Model, eLife, 10, E61453 DOI: 10.7554/eLife.61453
- Chakraborty, R.; Dey, S.; Sil, P., Paul, S. S.; Bhattacharya, D.; Bhunia, A.; Sengupta, J.; **Chattopadhyay, K***. (2021) Conformational distortion in a fibril-forming oligomer arrests alpha-Synuclein fibrillation and minimizes its toxic effects; Communications Biology 4, 518 DOI: 10.1038/s42003-021-02026-z

Discovery of parasite derived factors those modulate the pathogenicity of *Leishmania*

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Research Activities

Leishmania infection results into severe, life-threatening disease and is a growing public health concern in many countries including India. With no approved vaccines in sight, the situation is becoming alarming because current containment strategies are not entirely successful and *Leishmania* infections are on the rise. With this perspective, scientists are actively looking at various drug-targeting sites. Our laboratory has had a long-standing interest in *Leishmania* biology for identifying new five genes, specific for the parasite that can be potential target sites for drug development. These new parasite specific proteins are heme containing adenylate cyclase, ascorbate peroxidase, pseudoperoxidase, NAD(P)H cytochrome b_5 oxidoreductase and PAS domain containing phosphoglycerate kinase.

Aims and Objectives

- Deciphering the function of PAS domain containing phosphoglycerate kinase in pathogenesis of *Leishmania*.
- Exploring the non-glycolytic role of leishmanial glyceraldehyde 3 phosphate dehydrogenase in immunoregulation of macrophage.
- Screening of known adenylate cyclase inhibitors for their ability to restrict *Leishmania* growth.

Work Achieved

Recently, we described the PAS domain-containing phosphoglycerate kinase (PGK) from *Leishmania major* (LmPAS-PGK) that shows acidic pH (5.5)-dependent optimum catalytic activity. The PAS domain of LmPAS-PGK is expected to regulate PGK activity during catalysis, but the mechanism of regulation by PAS domain at the molecular level is uncharacterized. We have utilized the full-length, PAS domain-deleted, and mutant enzymes to measure the enzymatic activity in the presence of divalent cation at various pH values. Catalytic activity measurement indicates that Mg^{2+} binding through PAS domain inhibits the PGK activity at pH 7.5, and this inhibition is withdrawn at pH 5.5. To identify the Mg^{2+} binding residues of the PAS domain, we exploited a systematic mutational analysis of all (four) His residues in the PAS domain for potential divalent cation binding. Replacement of His-57 with alanine resulted in depression in the presence of Mg^{2+} at pH 7.5, but H71A, H89A, and H111A showed similar characteristics with respect to the wild-type protein. Fluorescence and isothermal titration calorimetry studies revealed that H57 is responsible for Mg^{2+} binding in the absence of substrates. Thus, the protonated form of His57 at acidic pH 5.5 destabilizes the Mg^{2+} binding in the PAS domain, which is an essential requirement in the wild-type LmPAS-PGK for a conformational alteration in the sensor domain that, sequentially, activates the PGK domain, resulting in the synthesis of higher amounts of ATP.

Extracellular vesicles (EVs) secreted from *Leishmania*, like entire parasites, were found to have a significant impact on macrophage cell signaling and function, indicating cross talk with the host immune system. GAPDH from *Leishmania major* (LmGAPDH) is highly enriched within the EVs secreted during infection. To understand the function of LmGAPDH in EVs, we generated control, overexpressed, half-knockout (HKO), and complement cell lines. HKO cells displayed lower virulence compared with control cells when macrophages and BALB/c mice were infected with them, implying a crucial role for LmGAPDH in *Leishmania* infection and disease progression. Furthermore, upon infection of macrophages with HKO mutant *Leishmania* and its EVs, despite no differences in TNFA mRNA expression, there was a considerable increase in TNF-α protein expression

compared with control, overexpressed, and complement parasites as determined by ELISA, RT-PCR, and immunoblot data. *In vitro* protein translation studies suggest that LmGAPDH-mediated TNF-α suppression occurs in a concentration-dependent manner. Moreover, mRNA binding assays also verified that LmGAPDH binds to the AU-rich 3'-UTR region of TNFA mRNA, limiting its production. Together, these findings confirmed that the LmGAPDH contained in EVs inhibits TNF-α expression in macrophages during infection via posttranscriptional repression.

As parasite specific proteins like, globin-coupled heme containing adenylate cyclase are more reliable to choose as drug target and this strategy could avoid the side effects in host, our future goal is rational designing specific inhibitor against this enzyme. The background informations from the literature, ribose components have earlier shown to have very low Ki values of adenylate cyclase indicating that not only the greater the binding affinity but also smaller quantity of compound may be needed to inhibit the activity of that enzyme. Cordycepin (3'-deoxyadenosine) and MDL-12,330A (cis-N-(2-Phenylcyclopentyl) azacyclotridec-1-en-2-amine, HCl) had shown the inhibition of 100% the growth of *Leishmania* promastigotes at 5 micro molar concentration in vitro. Our future objective is pre-clinical validation and regulatory studies of these Hit/lead molecule.

Future Research Plans

In the long term, we would like to emphasize on targeting parasite specific essential enzyme through small molecules in collaboration with other scientists at CSIR-IICB. In future, if drugs can be designed against potential targets (if identified) can be used as new therapeutics for treatment of leishmaniasis.

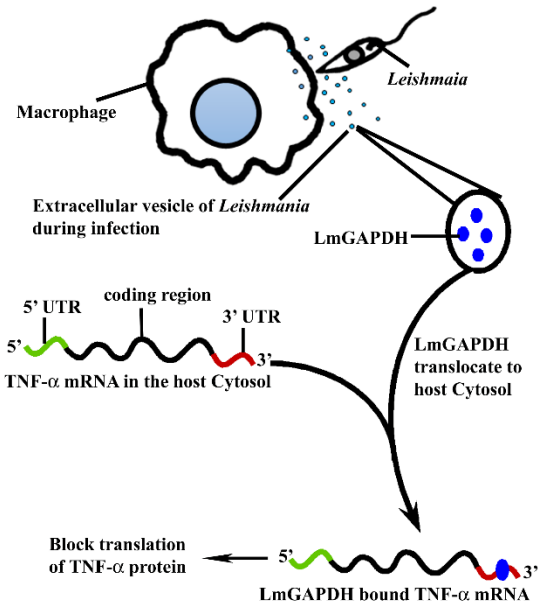

Figure 1: A possible schematic diagram for a novel virulence function of LmGAPDH contained in extracellular vesicle.

Extramural Funding

Translocation and regulation of an unusual novel PAS domain containing phosphoglycerate kinase in *Leishmania*. 2021-2024 (DST, India)

Recent publications

- Glyceraldehyde-3-phosphate dehydrogenase present in extracellular vesicles from *Leishmania major* suppresses host TNF-alpha expression. Das P, Mukherjee A, **Adak S**. *J Biol Chem.* 2021 Oct; 297 (4): 101198. doi: 10.1016/j.jbc.2021.101198.

Bacterial ATPase of Type Three Secretion System (T3SS) from Yersinia enterocolitica

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Background

The type three secretion system (T3SS) has been regarded as major virulence determining factor in numerous plants and animal pathogenic bacteria. A broad spectrum of diseases is caused by pathogenic bacteria such as enteric infections caused by enteropathogenic E. coli, Shigella, Salmonella, and Y. enterocolitica containing T3SS. These pathogens use T3SS to inject effector toxins directly inside the host cell to manipulate host cellular processes. Mutations, deletions, or blocking of the T3SS apparatus components result in reduced virulence and disease manifestations in mouse model experiments. T3SS has been predicted to be evolved from flagellar T3SS (fT3SS) through horizontal gene transfer and both of them share a common ancestor. Both T3SS and fT3SS share many structurally similar components at their core. Bacterial T3SS is a complex structure composed of approximately 20 different proteins and broadly can be divided into three basic parts namely the basal body, needle complex, and a large cytosolic component known as the sorting platform or the C- ring complex. The basal body consists of two co-axial homomeric protein complex rings across the inner and outer plasma membrane including the peptidoglycan layer. The needle is present in association with the outer ring projecting away from the bacterial membrane providing approximately 1.5–2.5 nm conduit for translocation of unfolded effectors. Tip complex protein associated with the needle helps the bacteria to integrate with the host plasma membrane. Once the association with the host membrane is established, the export apparatus helps in the translocation of effectors through the needle to the host cell cytoplasm. Previous structural studies involving Cryo-Electron microscopy have revealed the global structure of T3SS Injectisome. However, the precise details of the cytoplasmic components are largely undetermined because of their dynamic nature of association with the injectisome.

Energy for unfolding and translocation of effectors is provided by both, Proton Motive Force (PMF) and ATP hydrolysis by a highly conserved ATPase ring complex which remains in close association with the active needle complex at the sorting platform. These ATPases also have structural and functional similarities with the F0- F1 ATPase—an AAA+ ATPase (ATPase associated with various cellular activities). Structural and functional similarity between T3SS ATPases and the AAA + enzymes suggest the possibility of a universal model of mechanism among such molecular motors.

Aims and Objectives

T3SSs are complex nano-syringe-like structures used by many pathogenic bacteria to inject effector toxins directly into the host cell cytoplasm. Efficient unfolding and translocation of an effector require active interaction of the effector-chaperone complex with the highly conserved T3SS ATPases inside bacterial cytoplasm. The oligomeric form of these ATPases is the most active form present at the export gate. In previous studies, hexamer and dodecamer have been reported as functional oligomeric forms of these ATPases. In Y. enterocolitica, YscN (a putative energizer of yscyop T3SS for Yop secretion) has been suggested to be activated through oligomerization and work as a hexamer. However, the true nature of the activation and formation of the different oligomers is still poorly understood. Our main objective is to understand the various oligomeric nature ofYscN and to correlate with its catalytic efficieny.

Another focus of this study is the characterization of the formation of different functionally relevant higher-

order oligomers based on its N- terminal region i.e., partially active hexamer and the most active higher-order oligomer forms of YsaN.

Work Achieved

Y. enterocolitica uses contact-dependent T3SS for the delivery of anti-host effector proteins directly into the eukaryotic host cell. In addition to fT3SS, Y. enterocolitica maintains two distinct, independently regulated T3SSs. The first one is Ysa-Ysp T3SS encoded by Ysa Pathogenicity Islands located on the chromosome and another one is Ysc- Yop T3SS which is encoded by ≈70 Kb virulent plasmid pYV/pCD1. The Ysa- Ysp T3SS is required for the gastrointestinal phase of infection and intracellular survival of Y. enterocolitica within macrophages and is important in virulence. In our previous study, it was shown that YsaN, a T3SS ATPase in Y. enterocolitica encoded by Ysa- Ysp T3SS is a magnesium-dependent ATPase and the probable dodecamer state is the most active form of YsaN. Moreover, it was also shown that the N- terminal 1–20 amino acid residues of YsaN is crucial for YsaL binding (a negative regulator of YsaN ATPase).

Here, we report the existence of a higher-order oligomer (probable dodecamer) as well as a hexamer, as the functional oligomeric form of ATPase YsaN from Y. enterocolitica ysa-ysp T3SS. In our previous in-vitro study, the possible dodecamer has been identified as themost active form of YsaN over the weakly active monomeric form. Based on different biochemical and biophysical methods, the present study analyzes the mechanism of formation of different functional oligomers of YsaN and its correlation with the N-terminal region.

We have performed the in-vitro characterization of untagged YsaN and various deletion constructs. Another focus of this study is the characterization of the formation of different functionally relevant higher-order oligomers based on its N- terminal region i.e., partially active hexamer and the most active higher-order oligomer forms of YsaN. The present study indicates the involvement of distinct regions on the N- terminal domain of YsaN in the formation of hexamer and its higher-order oligomer. It has also been shown that the transition to a higher oligomer state of YsaN is an ATP concentration-dependent event following two-step kinetics. The existence of the hexamer as well as the higher-order oligomer complex has also been observed in negative TEM **(Figure 1)** which suggest that the formation of higher-order oligomer occurs by stacking of two homo- hexamers through their N- terminal faces which are also supported by perfect docking between two hexamer rings during in-silico studies **(Figure 2)**.

Future Research Plans

My laboratory is working on several T3SS ATPase and its regulators. Structural characterization of T3SS ATPase with its regulator using TEM and CryoEM is our laboratory's future research plan.

Publications

- Kumar R, Roy C, **Datta S**. Delineating specific regions of N- terminal domain of T3SS ATPase YsaN of *Yersinia enterocolitica* governing its different oligomerization states. Front Mol Biosci. 2022 Sep 8;9:967974. doi: 10.3389/fmolb.2022.967974.
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Extramural Funding

CSIR-NCP: Genome mining, cloning and characterization of the gene clusters and the secondary metabolites coded by them for biological activity in indigenous extremophilic Cyanobacteria. (PI: Sucheta Tripathy, Co-PI: Saumen Datta, Co-PI: Smrutisanjita Behera

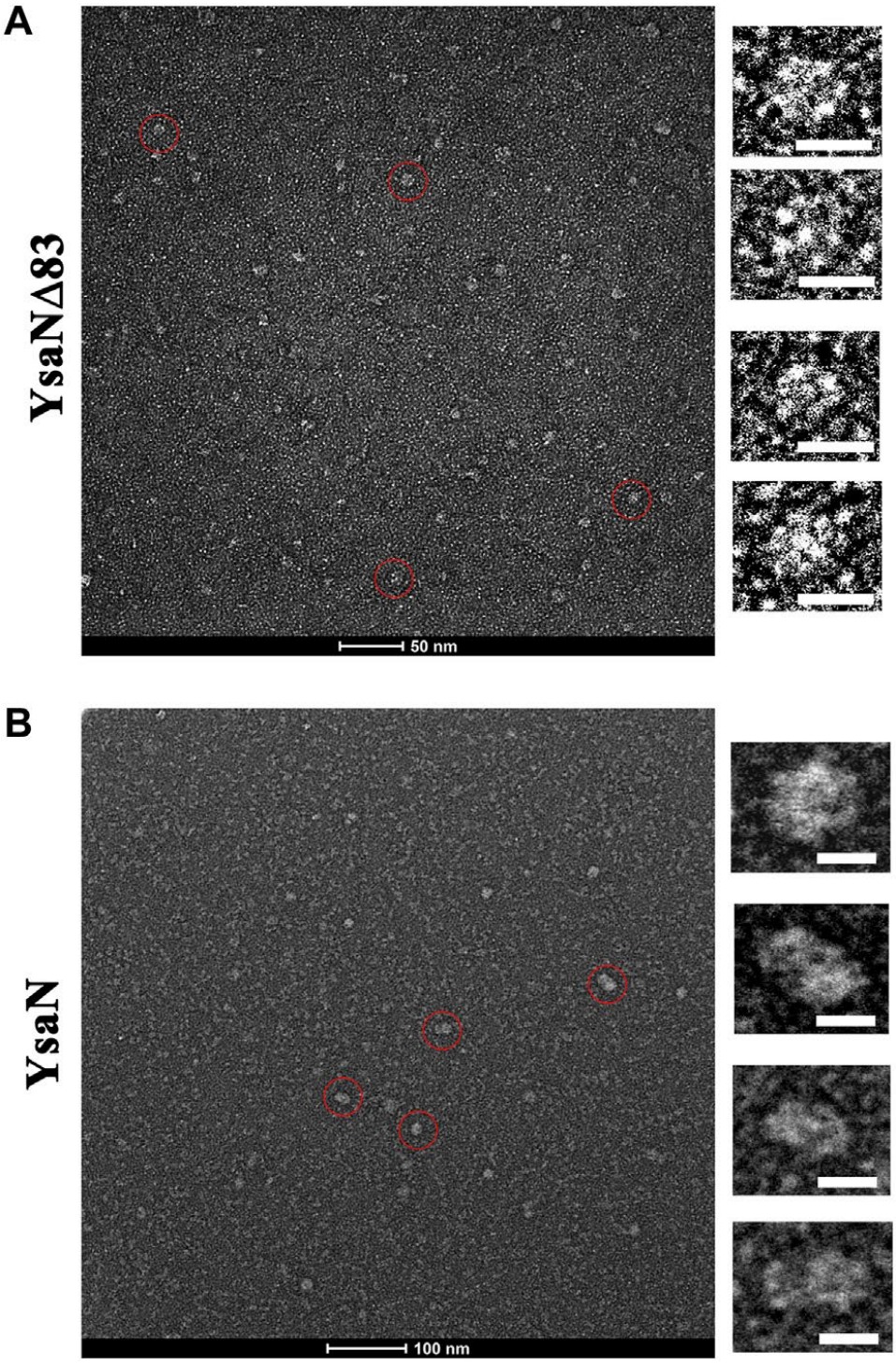

Figure 1: Negative TEM image of YsaN and YsaNΔ83 oligomer in presence of ADP. AlFX. (A) YsaNΔ83 oligomer. The Zoomed image represents an approximately 10 nm hexamer ring assembly of particles marked by the red circle in a different orientation. (B) The YsaN higher-order oligomer complex. The zoomed image represents particles marked by the red circle in a different orientation having a size of approximately 15–20 nm in different orientations representing probable dodecamer. The prepared sample grids were stained with 1% uranyl acetate. The white color scale bar represents 10 nm in all cases.

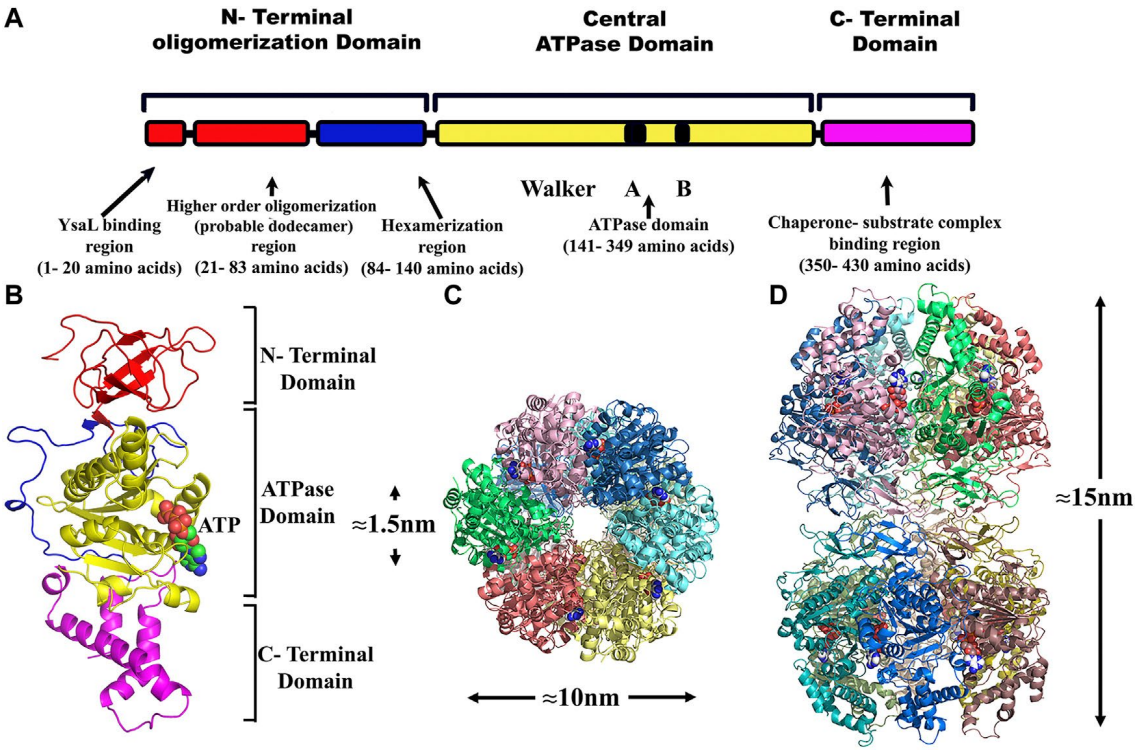

Figure 2: Structure modeling of YsaN and YsaN dodecamer complex- (A)- Linear representation of different predicted domains in YsaN. (B)- The model structure of YsaN is constructed on basis of FliI- FliH crystal structure (flagella T3SS ATPase structure PDB ID 5B0O). (C,D)- Model of probable dodecamer build by stacking two homo-hexamers through their N terminal faces following docking study. C- top view, (D)- side view.

Understanding the molecular mechanisms underlying systemic diseases and host-pathogen interactions using systems biology approaches

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Background

We are actively involved in identification and subsequent analysis of important bio-molecular interactions involving proteins, DNA and other macro-molecules in systemic diseases like cancer and infectious diseases, like malaria, leishmaniasis, etc. Primary objectives and/or contributions of our research group are to develop robust and efficient approaches to understand the hidden properties of protein-protein interaction (PPI) systems leading to infection/systemic disease by a) integrating large scale "multi-omics" data through network biology/graph theoretical algorithms, mathematical modelling and machine learning techniques, b) in-depth analysis of molecular interaction patterns using state-of-the-art molecular modeling, docking and dynamics strategies, and c) experimental characterization/verification of the identified bio-markers and/or therapeutic targets.

We utilize large-scale genomics, transcriptomics, proteomics, and metabolomics data to construct disease or context specific bio-molecular interaction networks and further study them to understand and decipher their biological significance using meta-interactome analysis. Our group also develops various computational tools, techniques and web servers, which are freely available for users and are beneficial to the scientific community throughout the world.

Recently, we have also ventured into developing image processing and machine learning / deep learning based software packages to process and analyze brain MRI and CT images for detection of early features of Alzheimer's disease and stroke, respectively.

Please visit our lab website (**http://www.hpppi.iicb.res.in/saikatlab/**) for more detailed overview and description of the previous and ongoing projects and to use various tools (servers and databases) that we have developed over the years.

Aims and Objectives

- Computational systems biology analysis of cancer interactome network.
- Detection of Alzheimer's disease using brain magnetic resonance image (MRI) processing and machine learning techniques.
- Systems biology analyses in delineating spatial interconnectivity of ER stress mediated cellular responses.
- Target-dependent coordinated biogenesis (TDCB) of secondary MicroRNAs.

Work Achieved

Computational systems biology analysis of cancer interactome network

Identification of cancer signature markers from patient-derived multi-omics data using machine learning techniques:

We are developing a computational pipeline to integrate and analyze multiple 'omics' data (multi-omics) retrieved from cancer patients and subsequently implementing machine learning (ML) and deep learning (DL) based protocol to identify multi-layer signature markers which could be utilized as prognostic and diagnostic bio-markers. One of the

approach aims to identify best features derived from single omics data types and further integrating the best single omics features into a multi-omics data type to distinguish cancer cohort from the healthy cohort via implementation of supervised machine learning techniques like support vector machine (SVM), random forest (RF), and XGBoost classifiers, respectively. The second approach intends to implement convoluted neural network (CNN) based deep learning protocol to identify best performing features to distinguish cancer cohorts from the healthy ones.

Detection of Alzheimer's disease (AD) using brain magnetic resonance image (MRI) processing and machine learning techniques

Early and accurate identification of the underlying causes of dementia, such as Alzheimer's disease (AD) is of utmost importance. A large body of research has shown that imaging techniques are most promising technologies to improve subclinical and early diagnosis of dementia. Morphological changes, especially atrophy in various structures like cingulate gyri, caudate nucleus, hippocampus, frontotemporal lobe, etc., have been established as markers for AD. Being the largest white matter structure with a high demand of blood supply from several main arterial systems, anatomical alterations of the corpus callosum (CC) may serve as potential indication neurodegenerative disease.

Hence, we have performed automatic detection and segmentation of the CC and calculated its morphological features to feed into a multivariate pattern analysis using support vector machine (SVM) learning techniques. Our results using large patients' cohort show CC atrophy-based features are capable of distinguishing healthy and mild/moderate AD patients. Our classifiers obtain more than 90% sensitivity and specificity in differentiating demented patients from healthy cohorts and importantly, achieved more than 90% sensitivity and 80% specificity in detecting mild AD patients (1). Comparison of the length (horizontal span) and thickness of the segmented CC between healthy and demented (mild and moderate) shows marked reduction in thickness and length (Figure 1).

Results from this analysis are encouraging and advocate development of an image analysis software package to detect dementia from brain MRI using morphological alterations of the CC.

Integrative network and pathway analyses of deregulated miRNAs and their target genes upon endoplasmic reticulum (ER) stress

miRNAs are shown to play substantial role in ER stress mediated pathophysiology. Thus, it is important to correlate miRNAs expression with the corresponding mRNA targets in order to understand the functional aspect of miRNA regulome in the context of ER stress mediated cellular response. We identified and compared deregulated miRNAs and their target mRNAs across three different chemically (tunicamycin, DTT, and thapsigargin, respectively) induced ER stress scenarios. Over-representation analysis of target gene sets indicates involvement of some pathways that are common among the three ER stress conditions while many that are specific to each drug treatment. Further, we retrieved gene/mRNA expression data from the gene expression omnibus (GEO) datasets that indicate usage of similar drugs to induce ER stress in human cell lines. Comparison of the deregulated genes under similar conditions revealed lesser amount of overlap between cell types and species type. We also generated the interactome of miRNA-target genes and subsequently isolated GEO dataset specific protein interaction networks. Figure 2A contains deregulated miRNAs (obtained through miRNA sequencing) and their validated target genes retrieved from miRTarBase and Tarbase databases whereas Figure 2B shows the 1st level protein interactors of the thapsigargin and tunicamycin treated deregulated genes obtained from the afore-mentioned GSE datasets (GSE19519 and GSE24500). Again, we observed presence of many unique molecular players both at the level of miRNA targets (~29%) as well as PPIs of GSE dataset mRNAs (~57%), color coded differently in panels A and B, respectively. These findings in turn advocate the complexity and probable involvement of a wider variety of uncharacterized genes and pathways in triggering and maintaining ER stress (2).

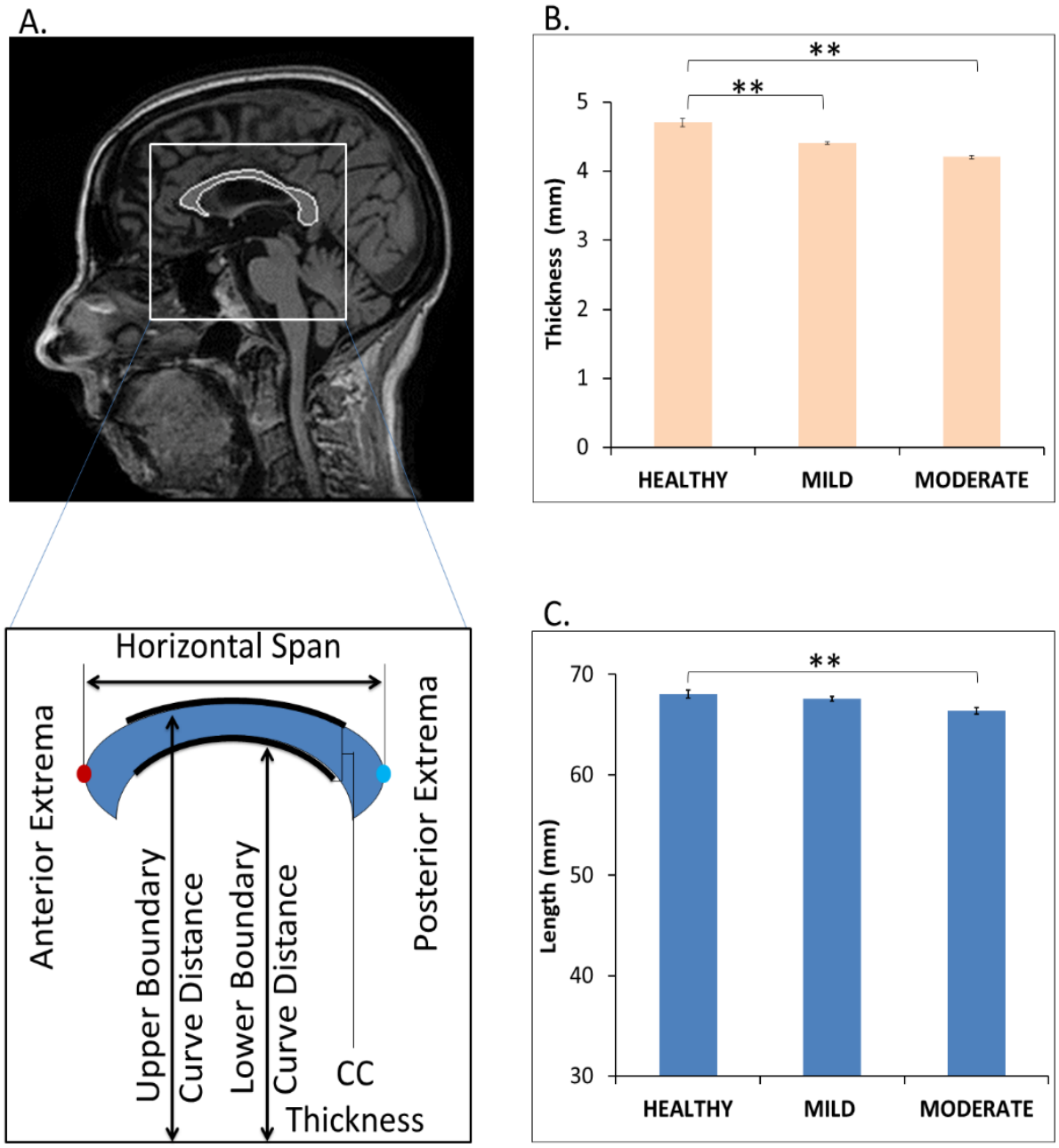

Figure 1: Decrease in corpus callosum (CC) length and thickness in mild and moderate dementia patients. Panel A shows a sample mid sagittal view of the segmented CC from healthy person MRI. It also provides a pictorial depiction of the measurement of CC length (horizontal span) and thickness. Panel B and C compare the average CC thickness and length among healthy, mild, and moderately demented samples. $p < 0.05$ is signified by ** symbol

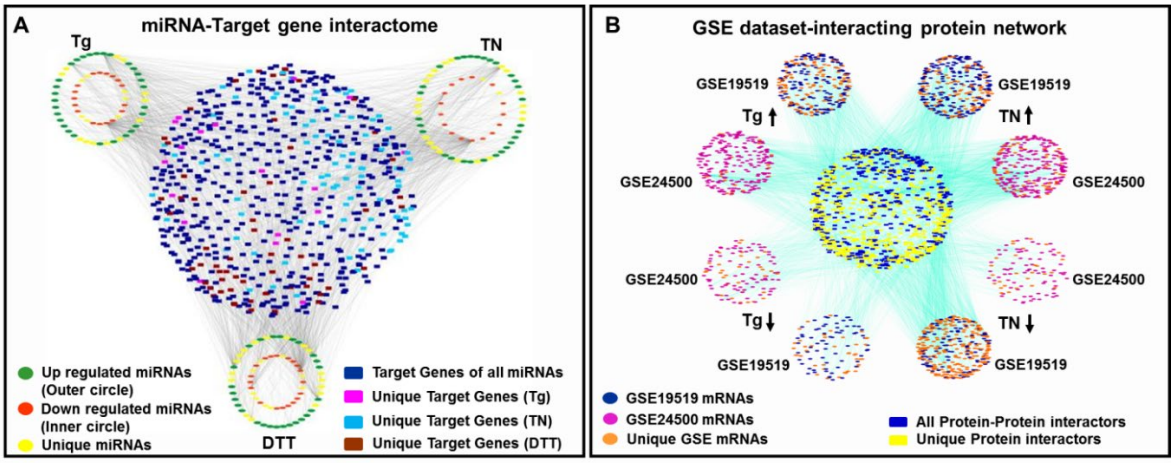

Figure 2: Representation of miRNA-Target gene and GSE dataset specific protein-protein interactomes. Tg, TN, DTT specific miRNA-Target gene interactions retrieved from miRTarBase and Tarbase databases shown in Panel A. Three outward projecting clusters having drug specific deregulated miRNAs (inner and outer circles made with down and up regulated miRNAs, respectively, with unique miRNAs marked in yellow). All their targets are placed at the center, with different color-coded unique players obtained from each drug treated transcriptomics profile. miRNAs and their targets are in oval and rectangular shapes, respectively. GSE19519 and GSE24500 dataset specific targets and their corresponding protein interactors (retrieved from the STRING database) were used to generate the network shown in panel B. Tg induced deregulated targets of GSE datasets are on the left (upregulated in top left, downregulated in bottom left); TN induced GSE dataset targets are on the right (upregulated in top right, downregulated in bottom right). All the interacting protein partners are placed at the center. GSE mRNAs and their interactors are shown in oval and rectangular shapes, respectively. Unique GSE players and their unique targets are colored separately. Up and down arrows indicate up and downregulated status of the genes, respectively. Tg and TN specify the drug treatments

Target-Dependent Coordinated Biogenesis of Secondary MicroRNAs

We report a target-dependent coordinated biogenesis (TDCB) mechanism of miRNAs, where target mRNAs increase the biogenesis of a family of secondary miRNAs that share binding sites in the 3' UTR of common target mRNAs having binding sites for a primary miRNA. We identified and unraveled how the TDCB operates in a physiological context to regulate the miRNA network and fine-tune the inflammatory responses. We have determined the likely conditions under which this coordinated biogenesis (CB) can occur and developed a computational module to predict regulatory miRNAs and their network under these contexts. Analysis of a few CB regulatory networks suggests that multiple miRNAs may potentially exhibit this phenomenon in a condition-specific, cell type-specific, and species-specific manner. Coordinate biogenesis data for each CB regulator (miRNA) can be obtained from the COORD-BIO repository developed by us. Herein, the data mined during this work to study the coordinate biogenesis phenomenon are queried by considering a particular CB regulator and expression profiles (miRNA and mRNA) in a scenario of interest. The front end of the server is HTML, PHP, and Java based, while back end scripts determine all possible CB regulator-target relationships for a particular CB regulator (miRNA) based on the conditions proposed for the coordinate biogenesis phenomenon to occur. Further, context-specific miRNA and mRNA expression profiling data may be utilized to determine the most likely sets of CB relationships. The web server for determining probable CB miRNA pairs and associated secondary effector mRNA is freely available for use (http://www.hpppi.iicb.res.in/coordB2/index.html) (3).

Future Research Plans

- To identify multi-layer cancer signature markers which could be utilized as prognostic and diagnostic bio-markers.
- Deep learning (DL) based algorithm development in order to utilize both whole brain structural and functional magnetic resonance imaging (MRI) data to aid early detection of dementia.
- To identify novel ER-mitochondria cross-talk proteins under ER stress condition. Investigating the probable roles of novel ER stress and UPR pathway genes in progression of female specific cancer.

Publications

- Das S, Panigrahi P, **Chakrabarti S*.** Corpus Callosum Atrophy in Detection of Mild and Moderate Alzheimer's Disease Using Brain Magnetic Resonance Image Processing and Machine Learning Techniques. ***Journal of Alzheimer's Disease Reports* 2021.**
- Mallick P, Maity S, Chakrabarti O, **Chakrabarti S***. Role of systems biology and multi-omics analyses in delineating spatial interconnectivity and temporal dynamicity of ER stress mediated cellular responses. ***Biochim Biophys Acta Mol Cell Res.* 2022** Apr;1869(4):119210.
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- Saha G, Sarkar S, Mohanta PS, Kumar K, **Chakrabarti S**, Basu M, Ghosh MK. USP7 targets XIAP for cancer progression: Establishment of a p53-independent therapeutic avenue for glioma. ***Oncogene.* 2022** Nov;41(47):5061-5075.
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- Garg A, Goel N, Abhinav N, Varma T, Achari A, Bhattacharjee P, Kamal IM, **Chakrabarti S**, Ravichandiran V, Reddy AM, Gupta S, Jaisankar P. Virtual screening of natural products inspired in-house library to discover potential lead molecules against the SARS-CoV-2 main protease. ***J Biomol Struct Dyn.* 2022** Jan 19:1-13.
- Das R, Kamal IM, Das S, **Chakrabarti S***, Chakrabarti O. MITOL-mediated DRP1 ubiquitylation and degradation promotes mitochondrial hyperfusion in a CMT2A-linked MFN2 mutant. ***Journal of Cell Science* 2022.**
- Khatun M, Kumar K, Baidya A, Mondal RK, Baszczyňski O, Kalčic F, Banerjee S, Dhali GK, Das K, Chowdhury A, Janeba Z **Chakrabarti S***, Datta S. Variability in the response of HBV D-subgenotypes to antiviral therapy: designing pan D-subgenotypic reverse transcriptase inhibitors. *Journal of Virology* 2021.
- Das A, Basu S, Bandyopadhyay D, Mukherjee K, Datta D, Chakraborty S, Jana S, Adak M, **Bose S**, **Chakrabarti S**, Swarnakar S, Chakrabarti P, Bhattacharyya SN. Inhibition of extracellular vesicle-associated MMP2 abrogates intercellular hepatic miR-122 transfer to liver macrophages and curtails inflammation. *iScience* 2021.

Extramural Funding

Platform integration for high Through-put multi omics data analysis and text processing. 2020 - 2025. (Department of Biotechnology, India). Saikat Chakrabarti (Co-PI)

Cryo-EM structural analyses to understand the functional mechanisms of biological macromolecules

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Krishnamoorthi Srinivasan, JRF
Priya Baid, SRF
Sandip Dey (Ph.D. degree awarded, Project Associate)

Work achieved

Structural understanding of the mechanism of ligand-dependent functional selectivity of 5-HT_{2A} G protein-coupled receptor:

Our lab investigates the structure and dynamics of various biological macromolecules and assemblies that are implicated in serious human diseases. Thus, all these systems could be potential drug targets.

This project aims to understand the mechanism of the function of serotonin 2A receptor (5-HT_{2A}R) subtype of the G-protein coupled receptor (GPCR) family, one of a key players involved in various neuromodulatory functions. Pharmacologically distinct classes of ligands either activate or inactivate the 5-HT_{2A}R receptor. Although high-resolution structures of 5-HT_{2A}R as well as several other 5-HT GPCRs provided snapshots of both active and inactive conformational states, these structures, representing truncated form of the receptor, cannot fullt describe the mechanism of conformational transitions between the inactive and active states. The importance of the intrinsically disordered intracellular regions, e.g. ICL2 and ICL3, has been proposed but not yet explored.

In the study mentioned here, we investigated the conformational dynamics of ligand-free and five distinct ligand-bound full-length 5-HT_{2A}R using unbiased atomistic molecular dynamics (MD) simulations. Selected ligands belong to either of the full, partial, or inverse agonist classes. We have observed remarkable differences in structural, dynamical, as well as thermodynamic parameters within ligand-bound complexes. Our results revealed, for the first time, that either activation or inactivation of the receptor upon specific ligand binding is primarily achieved through conformational transitions of its second and third intracellular loops (ICL2 and ICL3). Moreover, we found a remarkable allosteric crosstalk between the drug-binding site and distal intracellular parts of the receptor where binding of a specific ligand thermodynamically controls (either stabilizes or destabilizes) the intracellular region, consisting of crucial conformationally dynamic ICL2 and ICL3 loops, that leads to functional selectivity. Our findings would open up possibilities of designing novel ligands to specifically regulate the intracellular cavity of the receptor to adopt a particular conformational state of the receptor for a certain signalling.

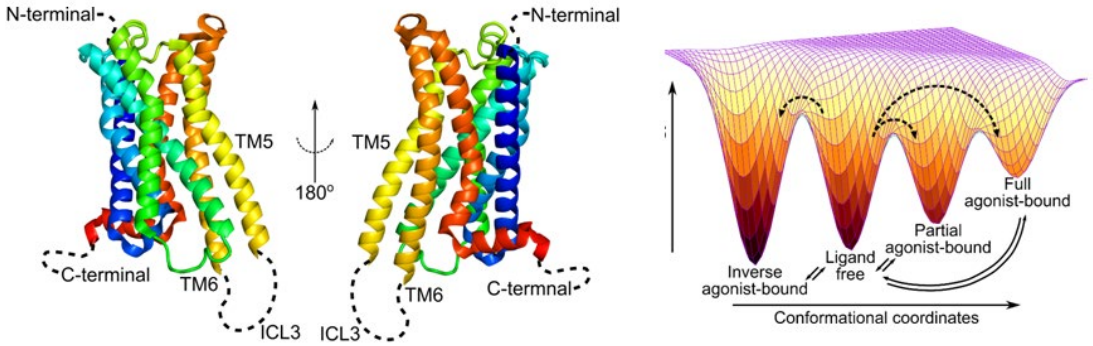

Figure 1: Structural study on 5-HT_{2A}R. Experimentally determined structure of 5-HT_{2A}R is shown (PDB ID – 6A93; left panel). Energy landscape for the activation and inactivation of 5-HT_{2A}R upon specific ligand binding is depicted schematically. Arrows between energy wells show transition from one conformation to another (right panel)

Future Research Plans

Our lab is one of the first few labs in India to start 3D cryo-electron microscopy (cryo-EM) of biological molecules. Our research particularly focuses on the translational machinery of pathogenic bacteria. We aim at identifying hitherto unknown ribosome-associated factors instrumental in various stress-related mechanisms of pathogenic bacteria and characterizing the ribosome-factor interactions using high-resolution3D cryo-EM.

We have been successfully elucidating ribosome-associated mechanisms in pathogenic bacteria using primarily high-resolution cryo-EM, with the aim to identify species-specific potential drug targets.

Awards

Recipient of SERB-Power Fellowship (2022)

Publications

- Mozumder S, Bej A, **Sengupta J.** (2022) Ligand-Dependent Modulation of the Dynamics of Intracellular Loops Dictates Functional Selectivity of 5-HT_{2A}R., J Chem Inf Model. (ACS) 62(10): 2522-2537. (**Cover Art selected**)
- Bhattacharjee S, **Sengupta J.** (2021) Hidden electrostatic energy contributions define dynamic allosteric communications within p53 during molecular recognition. Biophys J. (Cell Press) 120(20): 4512-4524.

Book Chapters

- Srinivasan K, Banerjee A, Baid P, Dhur A, **Sengupta J. (2022)** Ribosome-membrane crosstalk: Co-translational targeting pathways of proteins across membranes in prokaryotes and eukaryotes. Advances in Protein Chemistry and Structural Biology (APCSB) (Membrane proteins), Chapter 5**,** Vol 128, Academic Press (Elsevier), Editor: Prof. Rossen Donev
- Pathak BK, Dey S, Mozumder S, **Sengupta J. (2022),** The role of membranes in function and dysfunction of intrinsically disordered amyloidogenic proteins. Advances in Protein Chemistry and Structural Biology (APCSB) (Membrane proteins), Chapter 11, Vol 128, Academic Press (Elsevier), Editor: Prof. Rossen Donev

Extramural Funding

Ongoing SERB (DST) funded project (CRG/2019/001788) entitled 'High-resolution structural descriptions of HflX's interactions with 70S ribosome and ribosomal subunits to decipher its functional role in bacteria under stress'; Sanctioned in February, 2020.

Piecing together genomes of microbes for exploring the biological treasure trove

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Dr. Swasthi Tiwari, PGI, Lucknow, UP.
Prof. Brett Tyler, Oregon State University, USA.
Dr. Takao Kasuga, University of California Davies, USA.
Dr. Ramesh Ventukuri, Sweedish University of Agricultural Sciences, Sweden.

Background

India is a major mega diverse nation with most of its microbial populations lying under explored. We work on a plethora of organisms belonging to different phylogenetic clades towards solving the biological riddles encoded in their genomes and exploiting them for beneficial purposes. We use existing and in house softwares and some custom made softwares in joining the shorter reads generated by the nextgen sequencing methods into larger contiguous segments. We use these contigs in predicting genes and assigning biological functions into them. We have already sequenced the genomes and transcriptomes of prokaryotic and eukaryotic organisms in discovering major genes including anti-freezing genes in endophytes that helps them sustain in sub-zero temperatures. These genes have huge economic significance. We have been able to over produce cell wall degrading enzymes in some fungal species that can have major implications in paper industry. We have predicted novel effectors that lie in the repeat rich regions of the genomes that evolve faster than other regions of the genomes - re-iterating the two-speed genome evolution concept in pathogens. We have created computational resources for genomic data analysis in forms of lightweight genome analyzers. Our interest in prokaryotes centers on photosynthetic Cyanobacteria that grow in extreme environment. These organisms are shown to be extremely rich in signalling molecules that help them adapt quickly to changing environments. They also produce a plethora of secondary metabolites that has huge commercial significance. In future, we would like to use this information for commercial level production of bio-enzymes and metabolites and bio-remediation agents.

Aims and Objectives

- Analyzing complex genomes in understanding the genomic re-arrangements for adaptations.
- Harvesting genes for metabolic re-engineering for commercial exploitation.
- Developing biological softwares for data analysis.

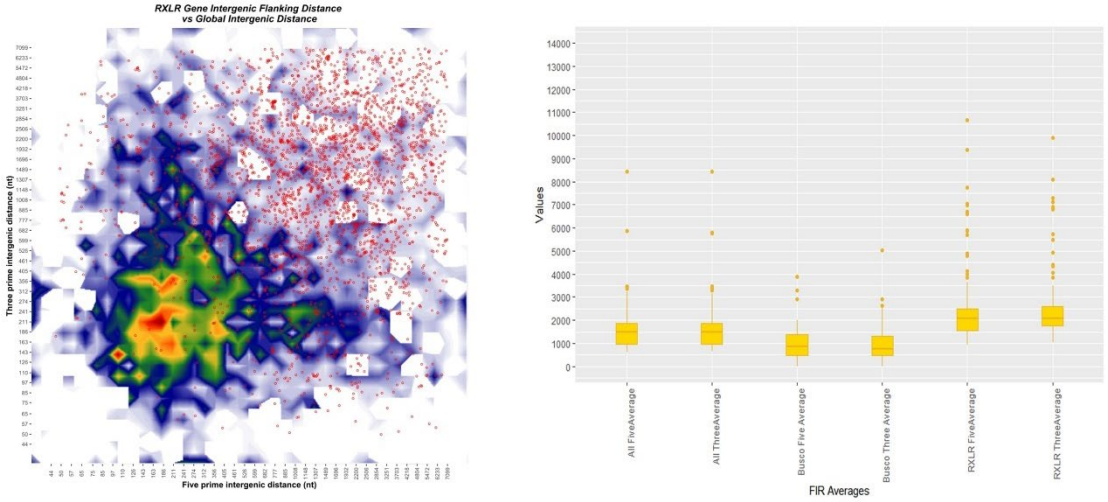
Work Achieved

Our lab focuses on analyzing genomes and transcriptomes of extremophile and pathogenic microbes that are exclusively isolated from India. Our main goal is to understand their adaptation and localized evolution. In this context, we have already sequenced several genomes of Cyanobacteria to unearth their genetic components.

The oomycetes pathogens are fungi like organisms but have different arsenals for causing infection than the fungi and are therefore, are difficult to control. One such pathogen *Phytophthora capsici* is the causal agent for large scale damage of vegetables crops. We have isolated 2 strains from West Bengal that are pathogens to solanaceous crops. In order to understand the effector biology, we carried out a mammoth task of comparing 128 genomes of Phytophthora. We have re-established that there is a two speed genome prevalent as in case of many pathogens [Fig 1]. We also show that the genomes

undergo evolution in a selective fashion mostly guided by the geographical regions driven by the selection pressure exerted by the host [Fig 2].

Apart from that, we have been working on machine learning approaches in analysis and assembly of simple and complex genomes. We are now characterizing the genes of interest that are involved in production of metabolites.



A B

Figure 1

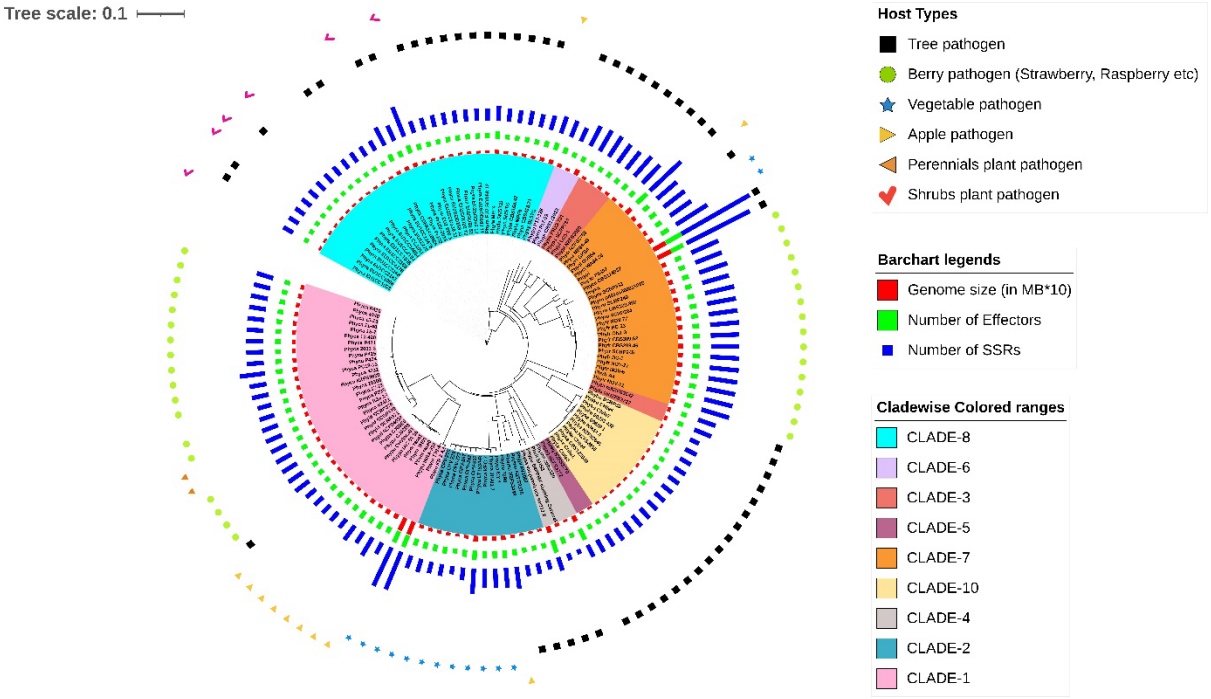


Figure 2

Future Research Plans

We are now working towards over production of metabolites as well as other biologically active components from the Cyanobacterial cells. With the fungal dried cell wall, we wish to produce bio-materials that can be directly used for bio-remediation purposes.

Publications

- Comparative Genome Analysis Across 128 Phytophthora Isolates Reveal Species-Specific Microsatellite Distribution and Localized Evolution of Compartmentalized Genomes. K Mandal, S Dutta, A Upadhyay, A Panda, S Tripathy*.Frontiers in Microbiology 13. [IF: 6.067]
- Factors governing the sinterability, In vitro dissolution, apatite formation and antibacterial properties in B2O3 incorporated S53P4 based glass powders. Sakthi Prasad, Margit Fábián, Anal Tarafder, Shashi Kant, Prasanta Kumar Sinha, Sucheta Tripathy, Annapurna Kalyandurg, Amarnath Reddy Allu, Kaushik Biswas. Ceramics International. https://doi.org/10.1016/j.ceramint.2021.10.237 [IF: 4.6]
- Editorial: Genomics and Effectomics of Filamentous Plant Pathogens.Ye W*, Wang Q*, Tripathy S*, Zhang M*, Vetukuri RR*. Front Genet. 2021 Feb 3;12:648690. doi: 10.3389/fgene.2021.648690. eCollection 2021. PMID: 33613652 [IF: 4.5]

Awards/Honours/Memberships

- Research Advisory board of National Tea Research Foundation (2019-2022)
- Associate Editor of Frontiers in Genetics and Microbiology (2020)
- Senior Editor for Molecular Plant Microbial Interaction (An American Phytopathological Society Journal)

Extramural Funding

- Platform integration for high Throughput multi omics data analysis and text processing. (DBT-Bioinformatics Center)
- Development of Genome Consortium Databank from plants and microbial population emerging from India. DBT (National Networking Program)

Conferences/Workshops

International conference on cyanobacteria research-May 26,27-Tokyo (Virtual conference)

Invited Talk

"Tenable solutions for tantalizing data – A brief overview" Invited talk in Health and Informatics Summit in IIIT Delhi, held between 1st -6th October 2021.

Protein Conformation Linked Human Diseases and Nano-Formulation for Therapy

Dr. Nakul Chandra Maiti, ncmaiti@iicb.res.in

Group Members

Krishnendu Khamaru, SRF
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Dr. Sauman Saha, Research Associates

Collaborators

Dr. Achinta Kumar Saha, University of Calcutta, Kolkata
Dr. Anirban Bhunia, Bose Institute, Kolkata
Dr. Biswadip Banerji, CSIR-IICB
Dr. Snehasikta Swarnakar, CSIR-IICB

Background

Several proteins and their aggregates such as amyloids are found to be cause of several human diseases, like Huntington's, Alzheimer's, Parkinson's, etc. Studies also suggested formation of functional amyloids and membrane less organelles due to formation of assembly structures of proteins particularly those contain significant amount of disordered regions. Some of these provide crucial beneficial role to our cellular systems. Therefore, major research initiatives are carried out on proteins structure, stability and formation of several type of protein aggregates. To realize the details of the events and the linked entities we utilized Raman spectroscopy as a major technique in our investigation.

We utilize Raman spectroscopy to investigate protein aggregation and fibrillation of several proteins and peptides. Particularly we analyzed the protein secondary structure of different assembly structure captured in the fibril formation pathway with particular focus on oligomeric intermediate which is believed now to be most cytotoxic. This intermediate structure attains characteristic morphological features while the constituent protein may/may not differ much in their secondary and tertiary structure in the native physiological conditions. Conformation states of proteins in the oligomeric state obtained by Raman spectroscopic analysis particularly aid to comprehend the structure of the oligomer and overall mechanisms of fibrillation and amyloid formation. It has been established that the backbone amide band and side-chain vibrations of amino acid residues present in the protein molecules largely affected in the fibril formation pathway and it follow a concerted reaction pathway, i.e. the protein molecule transformed into β- sheet rich amyloid fibril via formation of an oligomeric intermediate. However, the intriguing and interesting fact was that the proteins maintained/attained some helical pattern in the oligomeric step. Raman analyses established the distribution of residues in secondary structural conformation i.e. both helical and β-domain, possesses similarity with molten globule like structure. However, in the fibrillar state, the protein backbone attains anti-parallel β-sheet structure and several side-chain residues may be exposed to the surface of the protein and it was evidenced in the Raman spectra of the fibrils.

Aims and Objectives

The research in my laboratory, using modern techniques of NMR and Raman spectroscopy and other biological methods, mainly relate to (i) understanding the structural intricacy of amyloid proteins and their aggregates which could be neurotoxic or pathogenic, (ii) synthesis of small molecules and their spectroscopic characterization and (iii) synthesis of different types of bio-nanomaterials and their bio-chemical applications. (iv) Recently also we oriented our research to realize the structural aspects of main enzyme SARS-Cov-2 and Dengue.

Work Achieved

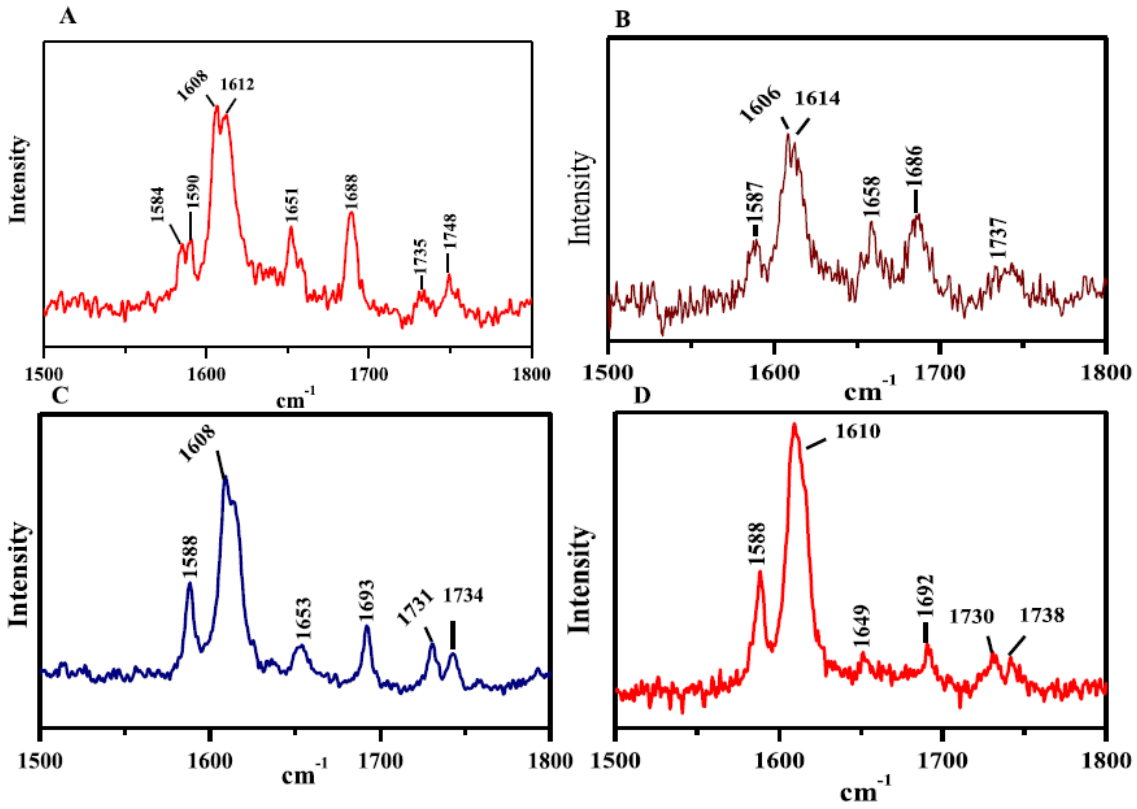


Figure 1: Experimental Raman spectra of P1 and P2 in two different sample conditions. (A) Raman spectra of P1 in solid state. (B) Raman spectra of P1 in methanol. (C) Raman spectra of P2 in solid state. (D) Raman spectra of P2 in methanol

Short peptides are known to form self-organized nano-architectures useful for advanced biomedical applications. Both hydrogen-bonding and hydrophobic interactions play a pivotal role in molecular assembly, including self-assembly of proteins and peptides. In the current investigation, we reported the synthesis, self-assembly pattern, and physicochemical properties of two de novo dipeptides: N-Boc-(O-benzyl)-tyro- (dimethyl)-glutamate and N-Boc-(O-benzyl)-glu-(O-benzyl)-tyrosine-methyl ester, abbreviated as P1 and P2, respectively. In methanol, P1 gave rise to a unique fibrillar network whereas P2 self-assembled into oligomers of 20-30 nm diameters. The UV-Vis, FT-IR and Raman spectra were recorded using experimental methods and compared with the computational spectra obtained by density functional method (B3LYP/6-31G(d)). The electronic absorption spectra of both P1 and P2 measured in methanol showed broad absorption peaks in the UV region with an absorption maximum at ~ 273 nm. FT-IR and Raman spectra of both the dipeptides in solid state showed amide I vibration bands at ~1650 cm^{-1} indicating a similar conformation state of the peptide bonds. However, H/D exchange ^1H NMR measurements confirmed that the amide NH of P1 was strongly involved in H-bond formation compared to P2 amide NH and rapid H/D exchange occurred upon addition of D_2O in the solution. Thioflavin T (ThT) fluorescence assay measurement suggested that incubation of P1 in methanol at room temperature produced fibrillar aggregates enriched with compact β-sheet conformation.

Dengue virus (DENV) encodes a unique protease (NS3/NS2B) essential for its maturation and infectivity and, it has become a key target for anti-viral drug design to treat dengue and other flavivirus related infections. Present investigation established that some of the drug molecules currently used mainly in cancer treatment are susceptible to bind non-active site (allosteric site/ cavity) of the NS3 protease enzyme of dengue virus. Computational screening and molecular docking analysis found that dabrafenib, idelalisib and nintedanib can bind at the allosteric site of the enzyme. The binding of the molecules to the allosteric site found to be stabilized via pi-cation and hydrophobic interactions, hydrogen-bond formation and π-stacking interaction with the molecules.

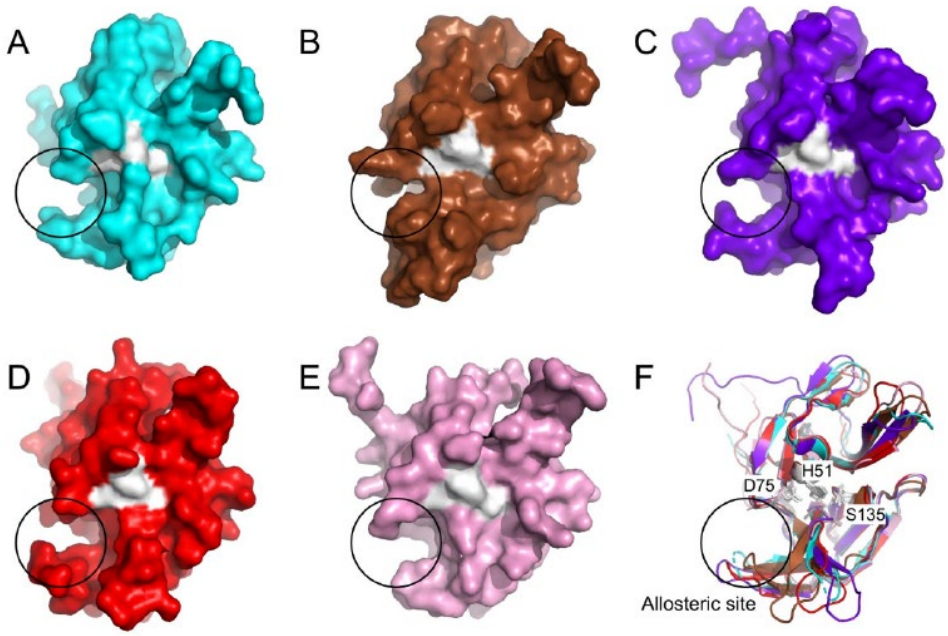


Figure 2: Comparison of allosteric sites in DENV protease of different serotypes of dengue in surface representation

Molecular dynamics (MD) analysis further revealed that the molecules on binding to NS3 protease caused significant changes in structural fluctuation and gained enhanced stability. Most importantly, the binding of the molecules effectively perturbed the protein conformation. These changes in the protein conformation and dynamics could generate allosteric modulation and thus may attenuate/alter the NS3 protease functionality and mobility at the active site. Experimental studies may strengthen the notion whether the binding reduce/enhance the catalytic activity of the enzyme, however, it is beyond the scope of this study.

Future Research Plans

- Drug discovery for SARS-CoV-2 targeting the 3CLpro
- Drug discovery for Dengue targeting NS3 protease
- Understanding amyloid formation mechanism and its implication in amyloid diseases
- Nanocomposites for theragnostic applications

Publications

- Rudra, D.S.; Pal, U.; Chowdhury, N.; **Maiti, N.C.**; Bagchi, A.; Swarnakar, S., (2022) Omeprazole prevents stress induced gastric ulcer by direct inhibition of MMP-2/TIMP-3 interactions, *Free Radical Biology and Medicine*, 181, 221. Impact factor – 7.376
- Uday, R.V.S; Misra, R.; Harika, A.; Dolui, S.; Saha, A.; Pal, U.; Ravichandiran, V.; **Maiti, N.C.**, (2021) Dabrafenib, idelalisib and nintedanib act as significant allosteric modulator for dengue NS3 protease. PLoS ONE, **16:9**, e0257206. Impact factor –3.24
- Dolui, S.; Bera, K.; Mondal, A; Khamaru, K.; **Maiti, N.C**. (2021), Application of Raman Spectroscopy in Amyloid Research, *Surface Enhanced Raman Spectroscopy: Biosensing and Diagnostic Technique for Healthcare Applications*. **1**, 91.
- Srinath, R.; Manna, A.; Shee, S.; Pathi, V.B.; Ghosh, S.; Khamaru, K.; **Maiti, N.C.**; Banerji, B., (2021), Synthesis of N-Fused Triazole−Piperazine−Quinazolinones via One-Pot Tandem Click Reaction and Cross-Dehydrogenative Coupling, *Org. Lett.*, **23**, 9365, Impact factor – 6.0
- Chakraborty, I.; Kar, R.K.; Sarkar, D; Kumar, S; **Maiti, N.C.**; Mandal, A.K.; Bhunia, A. (2021) Solvent Relaxation NMR: A Tool for Real-Time Monitoring Water Dynamics in Protein Aggregation Landscape, *ACS Chem. Neurosci.*, **12**, 2903. Impact factor – 4.4

Book Chapters / Invited Reviews

Book: Surface Enhanced Raman Spectroscopy: Biosensing and Diagnostic Technique for Healthcare Applications. Editors: Swati Jain, Sruti Chattopadhyay, Bentham Science Publishers pvt Ltd, Singapore 2021

Chapter: **Application of Raman Spectroscopy in Amyloid Research**

Extramural Funding

Translocation and regulation of an unusual novel PAS domain containing phosphoglycerate kinase in Leishmania, SERB, DST, Co-PI

Conferences/Workshops

Participant, Neuro-Update, November 28, 2021

Conferences/Events Organized

- Proteomics Day Symposium, 'Proteomics for Future Diagnostics' March 21, 2022, CSIR-IICB, Organizing Secretary
- 4th Annual Meeting of the Chemical Biology Society, August 11-12, 2021, CSIR-IICB, Organizing committee member

Understanding the structure and mechanism of action of human epigenetic regulators

Dr. Siddhartha Roy, roysiddhartha@iicb.res.in

Group Members

Sinjini Dhang, SRF
Avradeep Karmakar, JRF
Asmit Banerjee, JRF
Deep Basu, JRF
Sayan Gupta, JRF

Collaborator

Dr. Chandrima Das, Saha Institute of Nuclear Physics, Kolkata

Background

The eukaryotic chromatin is composed of DNA and histones, which come together to form functional repeating units called Nucleosomes. Chromatin regulation is a dynamic and complex process, which occurs on various levels in association with different epigenetic regulatory factors and is of utmost importance in gene expression, development and disease. Chromatin structure is subjected to various alterations, which includes the likes of histone modifications, chaperone mediated assembly and disassembly of the histones and ATP dependent remodeling enzymes. Our lab seeks to find out how these different enzymatically driven processes come together to ensure efficient regulation of gene expression. For this, we employ a broad range of molecular, biochemical and biophysical research tools to gain insights into protein structure, function and their corresponding mechanisms.

Research questions

- Exploring the mechanism of ubiquitin transfer mediated by human H2BK120 monoubiquitin ligase UBR7.
- Molecular characterization of H3-H4 directed chaperone activity by NAP-family member TSPYL5.

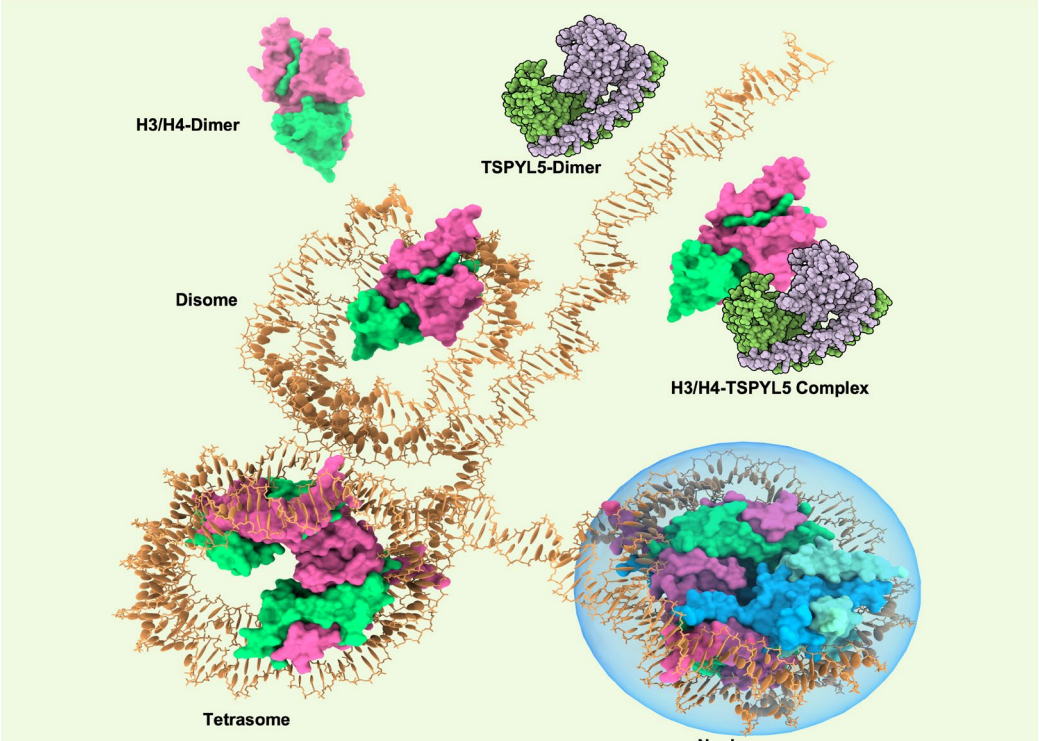
Progress made

Substrate H2B causes a conformational change within UBR7 catalytic domain and induces ubiquitin discharge from UbcH6.

We have found that the plant homeodomain (PHD) finger of UBR7 is essential for its association with E2 UbcH6 and consequent ubiquitin transfer to its substrate histone H2B. We have since identified the critical region of UbcH6 involved in this function. Our work demonstrates that H2B C-terminal tail is sufficient for UBR7/UbcH6- mediated ubiquitin transfer. We were able to confirm UBR7-mediated ubiquitination of the H2B C-terminal tail through a mass spectrometric approach. The PHD finger of UBR7 forms a dimer and this dimerization is essential for ubiquitination of histone H2B. The critical residues involved in the dimerization were identified and upon mutation of these residues, E3 ligase activity of UBR7 was drastically reduced. The dimer deficient mutations that we have identified also corelates with previous reports of cancer mutations in UBR7. Furthermore, we compared the mode of ubiquitin discharge from UbcH6 mediated by UBR7 and RING finger protein 20 (RNF20). While binding of substrate H2B to UBR7 was essential to effectively discharge ubiquitin from the E2-Ub thioester, no such constraint was found in case of RNF20. Conformational change within the UBR7 PHD finger was also detected upon H2B binding, indicating the activation of E3 for its ubiquitin ligase function.

TSPYL5 specifically targets histone H3/H4 and promotes nucleosome assembly.

We have characterized the chromatin association of TSPYL5 and found that it preferentially binds histone H3/H4 via its C-terminal NAP like domain in vitro and ex vivo. The critical residues involved in TSPYL5-H3/H4 interaction were identified and their importance was explored through the binding studies of TSPYL5 towards H3/H4. We successfully purified a stable TSPYL5-H3/H4 complex and further determined the binding stoichiometry of TSPYL5-H3/H4 complex in solution using chemical cross-linking assay and SEC-MALS. Our results indicate that a TSPYL5 dimer binds to two histone H3/H4 dimers or a tetramer. We also found TSPYL5 to have a specific affinity towards longer DNA fragments and



Human TSPYL5 is a H3/H4 specific Histone Chaperon

uses the same histone binding residues for its DNA binding. Furthermore, we established that TSPYL5 is a histone chaperone that is responsible for histone H3/H4 deposition and nucleosome assembly using histone deposition and supercoiling assays.

Future Research Plans

In future, we would like to focus on the roles of non-catalytic domains of UBR7 on histone binding and catalysis and their biological significance. We would also like to explore the basis and mechanism of transcriptional regulation by the NuRD complex.

Recent publications

- Dasgupta A, Mondal P, Dalui S, Das C and **Roy S**. Molecular characterization of substrate-induced ubiquitin transfer by UBR7-PHD finger, a newly identified histone H2BK120 ubiquitin ligase. ***FEBS J.*** 2022 Apr;289(7):1842-1857. *** Editor's Choice Article**
- Sengupta I, Mondal P, Sengupta A, Mondal A, Adhikari S, Dhang S, **Roy S** and Das C. Epigenetic regulation of Fructose-1,6-Bisphosphatase 1 by host transcription factor Speckled 110 kDa during Hepatitis B Virus infection. **FEBS J.** 2022 (in press)
- Dalui S, Dasgupta A, Adhikari S, Das C , **Roy S.** Human testis-specific Y-encoded like protein 5 is a histone H3/H4 specific chaperone which facilitates histone deposition in vitro. (2022) ***JBC*** (Re-submitted after minor revision).
- Adhikari S, Bhattacharya A, Adhikary S, Singh V, Gadad SS, **Roy S**, Das C. The paradigm of drug resistance in cancer: an epigenetic perspective. **Biosci Rep.** 2022 Apr 29; 42(4)
- Srivastava DK, Gunjan S, Das C, Seshadri V, **Roy S**. Structural insights into histone chaperone Asf1 and its characterization from Plasmodium falciparum. ***Biochem J.*** 2021 Mar 12;478(5):1117-1136. *** Cover Page Article**
- Adhikary S, **Roy S**, Chacon J, Gadad SS, Das C. Implications of Enhancer Transcription and eRNAs in Cancer. **Cancer Res.** 2021 Aug 15;81(16):4174-4182.
- Mukherjee S, Adhikary S, Gadad SS, Mondal P, Sen S, Choudhari R, Singh V, Adhikari S, Mandal P, Chaudhuri S, Sengupta A, Lakshmanaswamy R, Chakrabarti P, **Roy S**, Das C. Suppression of poised oncogenes by ZMYND8 promotes chemo-sensitization. ***Cell Death Dis.*** 2020 Dec 15;11(12):1073.

Investigating the Role of Secreted Protein, Acidic and Rich in Cysteine (SPARC) in early diabetic retinopathy

Dr. G. Senthil Kumar, skumar@iicb.res.in

Group Members

Ms. Nidhi Kumari, CSIR-SRF
Ms. Aditi Karmakar, UGC-SRF
Ms. Chirasmita Das, DBT-JRF
Mr. Shubhrajit Barman, DBT-JRF
Mr. Maqsood Ahamad Khan, ICMR- Project Assistant

Collaborators

Dr. Krishna Pada Baidya, Professor, Department of Ophthalmology, Nil Ratan Sircar Medical College & Hospital (NRSMC&H), Kolkata
Dr. Sujoy K Das, Principal Scientist, CSIR-IICB

Background

Diabetic retinopathy is a leading cause of blindness and is characterized by inflammation and oxidative stress in the retina. Retinal glial cells, including Müller cells and astrocytes, play an important role in maintaining retinal homeostasis and have been implicated in the pathogenesis of diabetic retinopathy. Secreted Protein, Acidic and Rich in Cysteine (SPARC) is a matricellular protein that regulates cellular proliferation and migration, and has been shown to be upregulated in diabetic retinopathy. SPARC is involved in the regulation of extracellular matrix (ECM) homeostasis and interacts with various ECM proteins to modulate cell behaviour. In diabetic retinopathy, SPARC has been shown to promote inflammation and inhibit angiogenesis. However, the role of SPARC in early diabetic retinopathy is not well understood.

Aims and Objectives

- To investigate the retinal cell type-specific expression and localization of SPARC in diabetic retinopathy.
- To understand the effects of SPARC on the neuroinflammation process during early diabetic retinopathy.
- To study the mechanisms by which SPARC regulates the inflammatory response and proliferation of retinal glial cells in early diabetic retinopathy.

Work Achieved

We have analyzed the single-cell dataset of various DR models and found the SPARC gene expression significantly dysregulated in retinal Gila cells. Experimental validations have shown that SPARC is strongly associated with the activation of Gila cells during hyperglycemic conditions. We have also found Inflammatory markers and Reactive oxygen species (ROS) were influenced by the SPARC expression on Gila cells.

Future Research Plans

We are in the process of doing functional validation of the SPARC gene by knockdown study. We also plan to do the proteomics of SPARC $^{-/-}$ retinal Gila cells to elucidate the molecular pathway through which it activates the Gila cells during the hyperglycemic condition.

Publications

- Karmakar A, Ahamad Khan MM, Kumari N, Devarajan N, Ganesan SK. Identification of Epigenetically Modified Hub Genes and Altered Pathways Associated with Retinoblastoma. Front Cell Dev Biol. 2022 Mar 10;10:743224. doi: 10.3389/fcell.2022.743224. PMID: 35359459; PMCID: PMC8960645.
- Kumari N, Karmakar A, Ahamad Khan MM, Ganesan SK. The potential role of m6A RNA methylation in diabetic retinopathy. Exp Eye Res. 2021 Jul;208:108616. doi: 10.1016/j.exer.2021.108616. Epub 2021 May 9. PMID: 33979630.

Extramural Funding

- Evaluation of Insulin-Like Growth Factor 2 (IGF2) as a potential epigenetic biomarker and therapeutic target to abolish the metabolic memory in diabetic retinopathy funded by ICMR, Govt of India

- Evaluating the epigenetic adaptation of COL4A1, COL4A2 as a potential therapeutic target for early pathological events in diabetic retinopathy funded by ICMR, Govt of India

Conferences/Workshops

28th Annual Meeting of the Indian Eye Research Group (ARVO- IC – IERG 2022)

Collaborative Projects

PAN CSIR Cancer Research Program

Making Cancer Care Affordable

Empowering Women Health: Focusing on Breast and Gynaecological Cancers of Indian Relevance

Project Summary

Scientists of IICB along with other CSIR laboratories have decided to focus their concerted effort on gynecological cancers of Indian relevance. We are actively focused to find the solution of breast and ovarian cancers that are on the rise among Indian women. There are serious clinical challenges on breast and ovarian cancers; such as, a) affordability of costly targeted cancer drugs b) lack of targeted therapy c) tremendous disease heterogeneity leading to therapy resistance, metastasis and relapse, these are very important parameters that dictate high mortality rates among the patients of BC and OC. Considering all these, the researchers of different CSIR Institutes urge to make a concerted effort to address these very pertinent issues, which will be useful for the management of these deadly diseases.

CSIR-CDRI as a nodal lab and six major CSIR labs (IICB, IGIB, IIIM, IICT, IMTECH, and NCL) have strong scientific strength in the area of Cancer Biology with adequate facilities for preclinical and clinical research. Approximately 80 scientists are working in Cancer Biology area in the CSIR family. This is a ongoing mission mode project (HCP-40) .

Global Objectives

Our overall aim is to provide affordable cancer care to Indian patients. Our proposed objectives are to:

- Develop generic targeted cancer drugs at affordable cost
- Develop new targeted therapy against women cancer of Indian relevance
- Develop generic anticancer MAbs at affordable costs
- Develop new herbal and phytopharmaceuticals as effective cancer therapy

Antiviral Mission CSIR: Discovery & Pre-clinical Development of Antivirals for COVID-19 & other diseases

Pan CSIR antiviral network and platform will be created for preparedness and to effectively combat future epidemics. The mission proposes to identify new molecular entity towards IND filling stage.

Specific protein targets

- Fusion or Entry Inhibitors (Spike-ACE2 interactions)
- Endosomal modulators or Uncoating Inhibitors
- Nucleic acid synthesis Inhibitors (RdRp)
- Viral Protease Inhibitors (Mpro and Plpro)
- Virion Release (Neuraminidase)
- Other Non-structural proteins (Methyltransferases)

The selection of the protein target for drug design will be done keeping in mind the significance of the target protein, drugability and evolutionarily conserved proteins across these viruses. Also, considering the scientific knowledge-based strength within the CSIR. Scientists at different CSIR labs has a broad range of expertise in different field such as Organic Chemistry, Medicinal Chemistry, Bioinformatics, Structural Biologists, Virologists, Disease Biology, Molecular Biology, Pharmacokinetics etc., breadth not available within the boundary of any academic organization in India. Apart from the depth of expertise, CSIR labs have the infrastructure and capabilities required foster drug discovery efforts in focused areas.

Role of CSIR-IICB in the Antiviral Mission

- Setting up of PAN CSIR in-vitro host-dependent assay facility for the antiviral screening program.
- Various viral protein-based assays, interactions between the Spike-RBD domain of the virus and the ACE2 receptor on the human cell will be developed.
- Identification and validation of active compounds from the FDA library and IICB library using in silico structure-based methods
- Generation of leads-optimized candidates using focused Medicinal Chemistry approaches from privilege scaffolds ready for in-vivo validation
- Generation of antiviral hits from natural product and their derivatives and their optimization
- Generation of IP
- Identification of preclinical antiviral lead ready for IND-enabling studies

Central Instrumentation Facility

Central Instrumentation Division (CIF)

Members:

Dr. Sib Sankar Roy (Head), Dr. Indu Bhusan Deb (Deputy Head), Dr. Ramdhan Majhi, Dr. Ardhendu Kumar Mandal, Dr. E. Padmanaban, Mr. Sandip Chowdhury, Mr. Sandip Chakraborty, Mr. Jishu Mandal, Mr. Binayak Pal, Mr. Sounak Bhattacharya, Mr. Soumik Laha, Mr. Sandip Kundu, Mr. Santu Paul, Mr. M. Vigneshwaran, Mr. Tapas Chowdhury, Mr. Tarak Prasad Nandi, Mr. Hari Shankar Beni, Mr. Nimai Charan Pradhan, Mrs. Arpita Maji, Mr. Nilaksha Swarnakar.

The Central Instrumentation Facilities (CIF) Division provides facilities to the researchers at CSIR-IICB along with different academic and R&D organizations, including Universities and Colleges located at different parts of the country. The objective is to provide accurate experimental data to the researchers of our Institute and also to the external users. We have about thirty high end and sophisticated instruments in our facility. Most of them have well-trained operators, who runs the instruments efficiently to get reliable data. This is indeed a pleasure for us to share that a few new types of equipment, like Fluorescence Microscope and CD spectrometer has been inducted to the facilities this year and the latest model of Atomic Force Microscope and the Raman Spectroscope have been ordered. We plan to procure more high-end instruments in the coming years to enrich our facility.

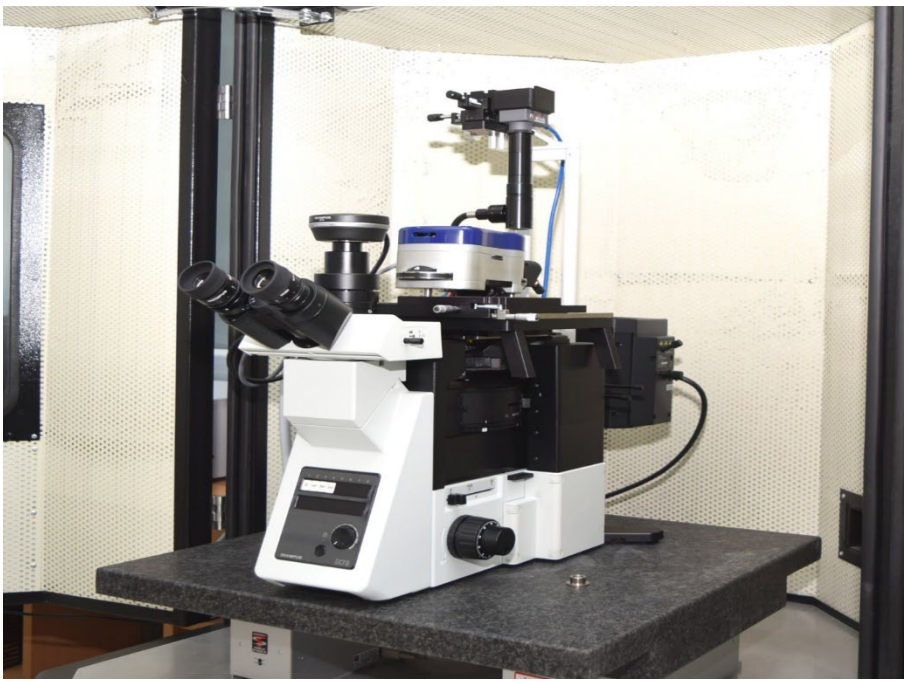
Central Instrumentation Facilities organizes, special hands-on training sessions in a regular fashion for students and staffs to train them about the principle and application and the division also actively participate in **Skill Development Programme (SDP**). CIF at IICB has also demonstrated and trained college students about different instruments. For the mandatory course work of PhD students Instrumentation and techniques happen to be a compulsory paper, where theoretical and practical aspects of relevant Instruments for Biology and Chemistry students are imparted by the experienced faculty and the technical operators. During past few years, the 'Skill Development Program' of CSIR has been introduced. As a part of this program, our technical officers of CIF train the candidates coming from all over the country to take the benefit of this program. This division is taking care of extending all sorts of support to the recipient students and faculties under the science dissemination and popularization scheme of CSIR-IICB. The instrument facility was showcased to many students came from colleges and Universities on several occasions and these instruments include NMR, LCMS, AFM, XRD, FACS, etc. In the 'Jigyasa' program, the instruments of CIF are shown to the school students. In addition, during the open day program, the school students visit our facility throughout the day.

The list of the instruments of CIF is available on the IICB-webpage with their physical location. As per CSIR guideline booking for the instruments with AnalytiCSIR portal has been initiated. CSIR's objective is to share all major R&D facilities to research community all over India. "AnalytiCSIR" is a web-based portal and a gateway for common users to locate the nearest CSIR facility which is required for their R&D activities. The CIF Division has the compulsion of providing service to each and every user of our Institute as well as external users throughout the country. The process of booking instruments and data collection have been simplified, which allows easy access to these instruments for all. We feel that still there is a lot of scopes to improve the service of CIF and all the staffs of this division are always working for improvement.

It is really satisfying that our instrument-generated data are included in many papers published by scientists at IICB and other Institutes. The number of such papers in each year is very high and increasing day by day. Although our objective is to provide satisfactory and accurate service to the users, in parallel our division is earning a considerable amount of revenue. Finally, with the help of all stakeholders, CIF@IICB is capable and determined towards providing instrumental service and facilities towards the research fraternity of our country.

Some Major High End Instruments Facilities in CIF Division

ATOMIC FORCE MICROSCOPE



NEW AFM

FLOW CYTOMETER

HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

FAB MASS SPECTROMETRY

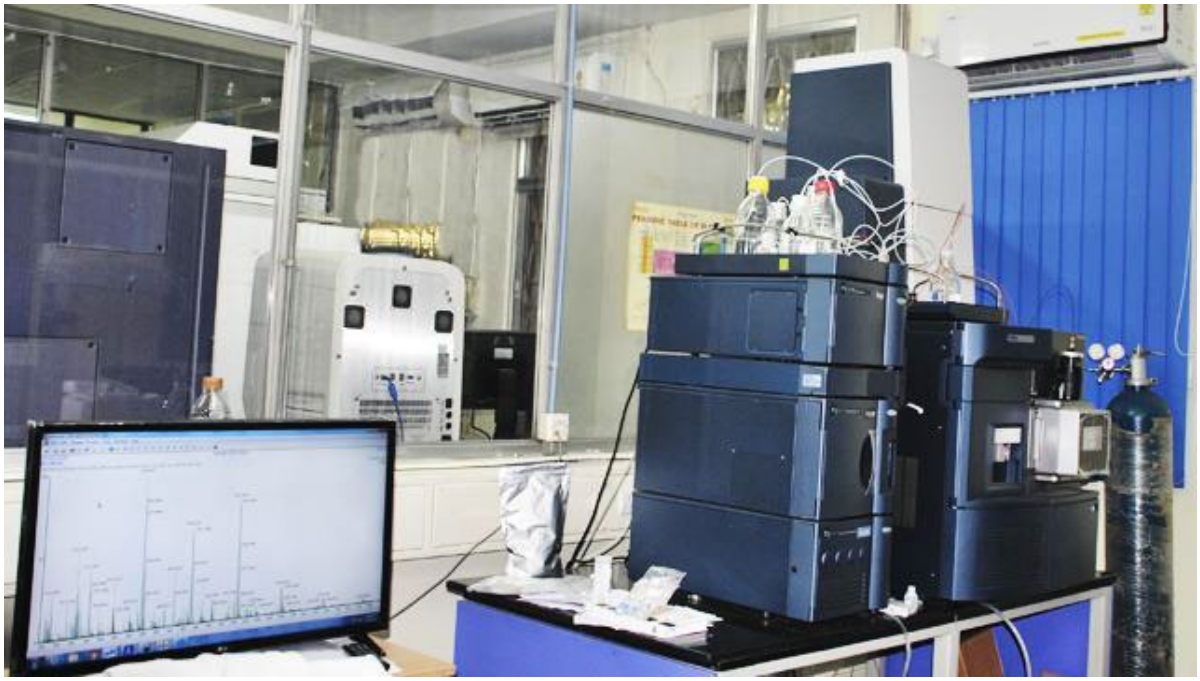
MALDI TOF/TOF

NMR SPECTROMETER 400 MHz

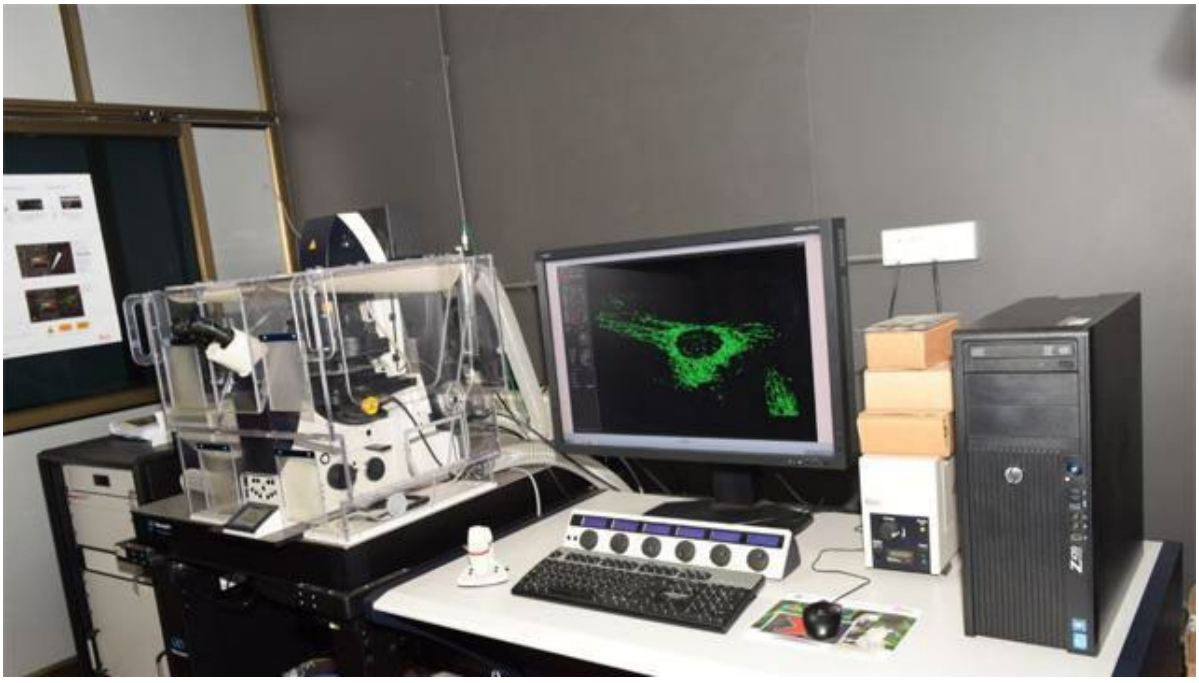
SEA HORSE EXTRACELLULAR FLUX ANALYZER

ORBITRAP MASS SPETROMETRY

NMR SPECTROMETER 600 MHz



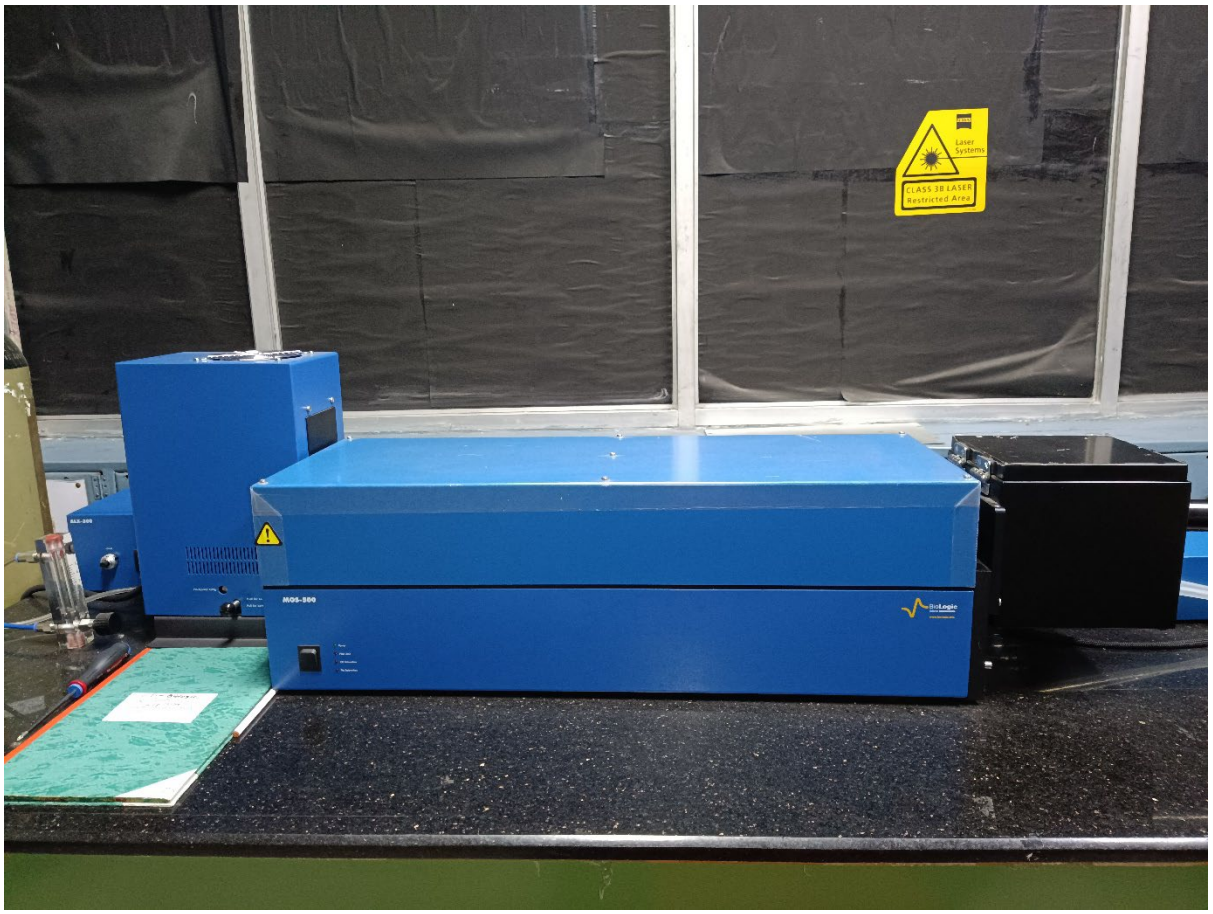
Q TOF-ESI MASS



SUPER RESOLUTION CONFOCAL MICROSCOPE

New facility creation

A circular dichroism spectrometer was installed at CSIR-IICB in February 2022. This facility provide important features of biomolecules and the analysis of chiral substances.



CIRCULAR DICHROISM SPECTROMETER

Group members of CIF

Support Divisions

Animal House

Members:
Dr. A. Konar (Head), Mr. S.S. Verma, Mr. R Sarkar, Mr. J. Midya, Mr. P. Midya, Mr. Lalu Sardar, Mr. G. C. Sardar, Mr. S. K. Midya

The animal Facility in CSIR-IICB is a CPCSEA registered (Regisgration No. 147/GO/REBI/S/99/CPCSEA) key source for supply of authentic animals used in biomedical programme of the Institute. All (annimals) but a few special strain of mouse, are bred and supplied from the in-house breeding colony.

The animals are produced and kept in a scientifically maintained environment (Room Temp. 24±2°C; relative humidity 55-60 %; light and dark schedule 12:12 hrs; illumination 400 lux at 1 mt above the floor). They are raised under strict health and genetic monitoring. The house keeping of the facility acclaimed high appreciation not only from the associated scientists but also the representative of the CPCSEA, Representative of different NGOs and private entrepreneurs, visiting guests and scientists.

Animals (specially mouse) were purchased from other registered breeders only when the required strain was not available in the colony or when animals of same specification was required in a bulk. However, proper unitization of the animals was strictly monitored and animals were produced in a such a number, that the number of utilized animals be minimum but the scientists get their animals as and when required.

The species and strains of animals, routinely maintained in the facility are as follows:
Mice - Balb/C, C57BL/6J, Swiss Albino and transgenic strain.
Rat - Sprague Dwaley
Hamster - Syrian Golden
Rabbit - New Zealand (white)

Species	Stock on 1st April 2021	No. of animals		Total (A)	No. of Animal issuded		No. of Animal		Total (B)	Stock on 31st March 2022
		Produced	Purchased		Produced	Purchased	Died in-stock	Supplied to other R&D organization		
Mouse	1338	2892	83	4313	2259	0	0	0	2342	1971
Rat	309	376	0	585	289	0	0	0	289	296
Hamster	80	33	0	113	17	0	0	0	17	96
Rabbit	59	05	0	64	0	0	0	0	0	64

Business Development Group

Members:
Dr. Arindam Talukdar (Head), Mr. Sandeep Aggarwal, Dr. Aparna Laskar, Mr. Arupesh Majumder, Mr. Saibal Giri

CSIR-Indian Institute of Chemical Biology is engaged primarily in research on diseases and certain biological problems of global interest and is continuously developing its knowledge base through world-class scientific research and innovation. The Institute is conducting basic research related to human health care with an intention to translate basic knowledge into technologies for the benefit of mankind.

In its constant endeavor to translate the research carried out at the Institute into meaningful products, the Business Development and Intellectual Property Management Group performs the dual functions of protecting the various aspects of the research as well as taking steps for early translation of the developed products and processes.

All innovations of the Institute after an assessment of the potential for commercialization, are protected through the filing of patents or copyrights by its Intellectual Property Management (IPM) cell. The IPM cell of CSIR-IICB, in close co-ordination with the Unit For Research & Development of Information Products (URDIP) and Innovation Protection Unit (IPU) of CSIR, is engaged in protecting the technologies developed with an objective to put forward these technologies towards the benefit of mankind. With the help of a new Comprehensive Patent Database prepared by this cell, now brief information about a patent filed by CSIR-IICB, since 1990 is just a click away.

This cell maintains liaison with Scientists of CSIR-IICB and IPU, CSIR to protect the Intellectual Properties of CSIR-IICB/CSIR. This cell functions with advice from the Head, BDG & IPM Cell, and Patent Advisory Committee whenever required. The IPM Cell always extends co-operation to the inventors, CSIR-IICB in writing and filing patent applications and prosecution of filed applications. This cell provides necessary information on technologies developed, patents filed and granted whenever required; provides information on patents and technology to IPU, CSIR regarding Audit and Parliamentary Question; prepares year-wise documents on total Patents of CSIR-IICB filed and granted.

Group members of BDG

List of MoU Signed and agreements

Sl no.	Name of the party	MoU/Agreements	Signed
1	'Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Dhanvantri Nagar, Gorimedu, Puducherry 605006 'Cadila Healthcare Limited', Zydus Corporate Park, Scheme No. 63, Survey No. 536, Khoraj (Gandhinagar), Ahmedabad – 382481 'Sanjay Gandhi Postgraduate Institute of Medical Science', Raebareli Road, Lucknow – 226014 'Shiv Nadar University', NH 91, Tehsil Dadri, Greater Noida, Uttar Pradesh – 20131	Memorandum of Understanding	16.04.2021
2	'Netaji Subhas Chandra Bose Cancer Hospital (NSCB Cancer Hospital)', 3081, Nayabad, Kolkata – 700 094	Memorandum of Understanding	07.09.2021
3	National Informatics Centre (NIC), Department of Information Technology, Ministry of Communication and Information Technology, Government of India, Block A, CGO Complex, Lodi Road, New Delhi – 110 003	Renewal of Agreement	08.11.2021
4	Central Ayurveda Research Institute, Central Council for Research in Ayervedic Sciences, Kolkata	Memorandum of Understanding	11.11.2021
5	Jadavpur University, 188, Raja S. C. Mullick Road, Jadavpur, Kolkata – 700 032	Memorandum of Understanding	23.12.2021
6	Bose Institute (BI), DST, G.O.I., 93/1, Acharya Prafulla Chandra Road, Kolkata – 700 009	Memorandum of Understanding	19.01.2022
7	Dr. Reddy's Institute of Life Sciences (DRILS), University of Hyderabad campus, Gachibowli, Hyderabad-500046	Memorandum of Understanding	21.01.2022
8	Gufic Biosciences Limited, 37, Kamala Bhavan II, S. Nityanand Road, Andheri (East), Mumbai – 400 069 and Bose Institute, Kolkata	Confidentiality Agreement	21.01.2022
9	St. Jude Children's Research Hospital, 262, Danny Thomas Place, Memphis, TN 38105, USA	Memorandum of Understanding (International)	18.02.2022
10	Kalinga Institute of Medical Sciences - KIMS Hospital, Kalinga Institute of Industrial Technology (KIMS Hospital)	Memorandum of Understanding	28.02.2022
11	Manasha Biotech Pvt. Ltd. 79, Ananda Avhasan, Garia Station Road, P.S. Sonarpur, South 24 Parganas, Kolkata – 700 084	Memorandum of Understanding	21.03.2022

Patents Filed/Granted 2021-22

- ### Granted in India

S. No.	Title	Inventors	Prov. Filing Date	Comp. Filing Date	Application No.	Grant Date	Patent No.
1	A LDV LIPOSOMAL FORMULATION OF PHOTO SYSTEM - i FOR TREATMENT OF CANCER	SURAJIT GHOSH, ABHIJIT SAHA, SUBHAJIT GHOSH, SASWAT	05/Oct/2016	06/Sep/2017	201611034058	31/May/2021	368113

		MOHAPATRA, BATAKRISHNA JANA, DEBMALYA BHUNIA					
2	3-INDOLYL FURANOIDS AS INHIBITORS OF MATRIX METALLOPROTEINASE-9 FOR PREVENTION OF GASTRIC ULCER AND OTHER INFLAMMATORY DISEASES	PARASURAMAN JAISANKAR, SNEHASIKTA SWARNAKAR, SOURAV CHATTERJEE, SUGREEV VERMA, MADHUMITA MANDAL, SUSRI RAY CHAUDHURI	15/Feb/2017	15/Feb/2018	201711005308	24/Aug/2021	375121

- **Granted Abroad**

S No.	Country	Title	Inventors	Grant Date	Patent No.
1	AUSTRALIA	BLOCKING TOLL-LIKE RECEPTOR 9 SIGNALING WITH SMALL MOLECULE ANTAGONIST	ARINDAM TALUKDAR, DIPYAMAN GANGULY, BARNALI PAUL, AYAN MUKHERJEE, SHOUNAK ROY, SWARNALI ROY, AMRIT RAJ GHOSH, ROOPKATHA BHATTACHARYA, OINDRILA RAHAMAN, BISWAJIT KUNDU	06/May/2021	2017238758
2	BRAZIL	BIOMARKER FOR VALVULAR HEART DISEASE	ARUN BANDYOPADHYAY, TANIMA BANERJEE, SOMADITYA MUKHERJEE, SANTANU DUTTA	03/Aug/2021	BR 112013008701-3
3	NEW ZEALAND	ANTI-CANCER ACTIVITY OF STEARYLAMINE BEARING CATIONIC LIPOSOMAL DRUG FORMULATIONS	NAHID ALI, MANJARIKA DE, TRIPARNA SEN	31/Aug/2021	717409
4	EUROPE	PURINE BASED COMPOUNDS AS TOLL-LIKE RECEPTOR 9 ANTAGONIST	TALUKDAR ARINDAM, GANGULY DIPYAMAN, MUKHERJEE AYAN, PAUL BARNALI, RAHAMAN OINDRILA, KUNDU BISWAJIT, ROY SWARNALI, DEBLINA RAYCHAUDHURI	13/Oct/2021	3707140
5	GREAT BRITAIN	PURINE BASED COMPOUNDS AS TOLL-LIKE RECEPTOR 9 ANTAGONIST	TALUKDAR ARINDAM, GANGULY DIPYAMAN, MUKHERJEE AYAN, PAUL BARNALI, RAHAMAN OINDRILA, KUNDU BISWAJIT, ROY	13/Oct/2021	EP3707140

			SWARNALI, DEBLINA RAYCHAUDHURI		
6	FRANCE	PURINE BASED COMPOUNDS AS TOLL-LIKE RECEPTOR 9 ANTAGONIST	TALUKDAR ARINDAM, GANGULY DIPYAMAN, MUKHERJEE AYAN, PAUL BARNALI, RAHAMAN OINDRILA, KUNDU BISWAJIT, ROY SWARNALI, DEBLINA RAYCHAUDHURI	13/Oct/2021	EP3707140
7	GERMANY	PURINE BASED COMPOUNDS AS TOLL-LIKE RECEPTOR 9 ANTAGONIST	TALUKDAR ARINDAM, GANGULY DIPYAMAN, MUKHERJEE AYAN, PAUL BARNALI, RAHAMAN OINDRILA, KUNDU BISWAJIT, ROY SWARNALI, DEBLINA RAYCHAUDHURI	13/Oct/2021	EP3707140
8	SWITZERLAND	PURINE BASED COMPOUNDS AS TOLL-LIKE RECEPTOR 9 ANTAGONIST	TALUKDAR ARINDAM, GANGULY DIPYAMAN, MUKHERJEE AYAN, PAUL BARNALI, RAHAMAN OINDRILA, KUNDU BISWAJIT, ROY SWARNALI, DEBLINA RAYCHAUDHURI	13/Oct/2021	EP3707140
9	USA	PURINE BASED COMPOUNDS AS TOLL-LIKE RECEPTOR 9 ANTAGONIST	TALUKDAR ARINDAM, GANGULY DIPYAMAN, MUKHERJEE AYAN, PAUL BARNALI, RAHAMAN OINDRILA, KUNDU BISWAJIT, ROY SWARNALI, DEBLINA RAYCHAUDHURI	09/Nov/2021	11168084

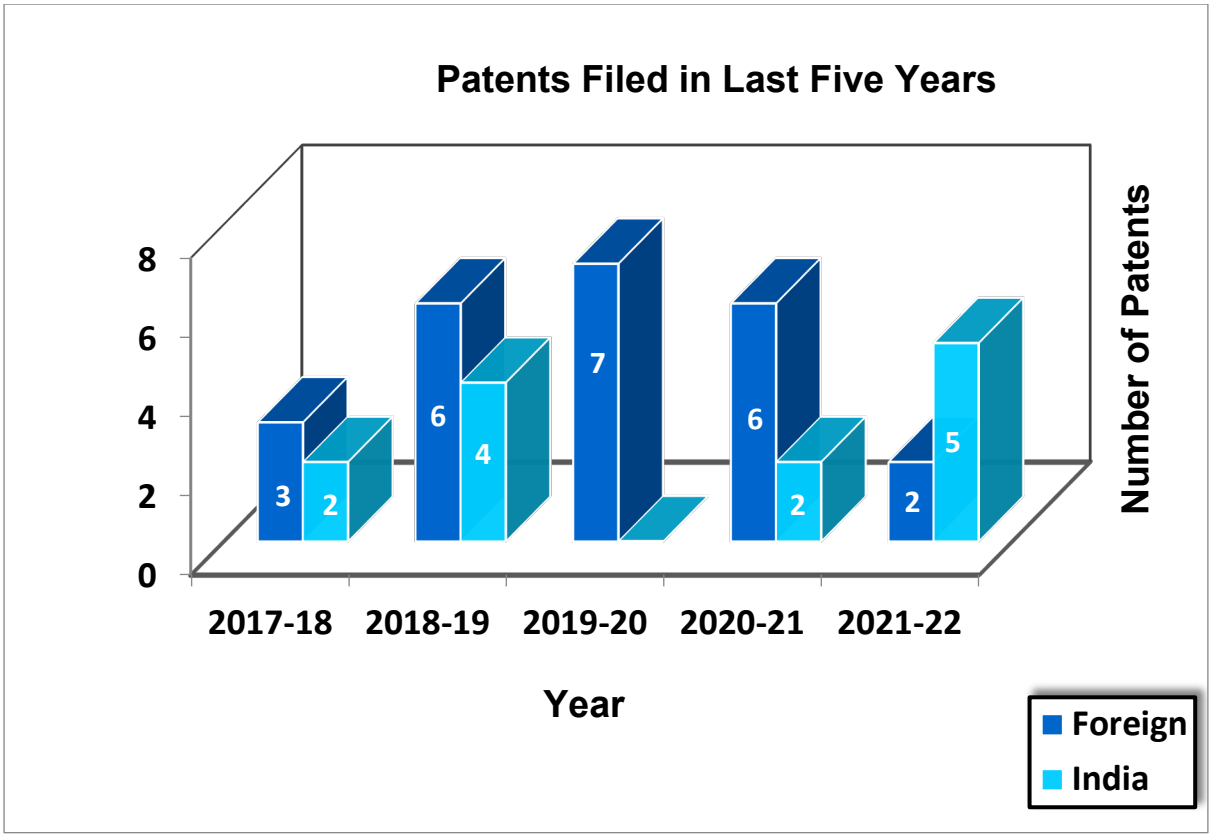
- **Filed in India**

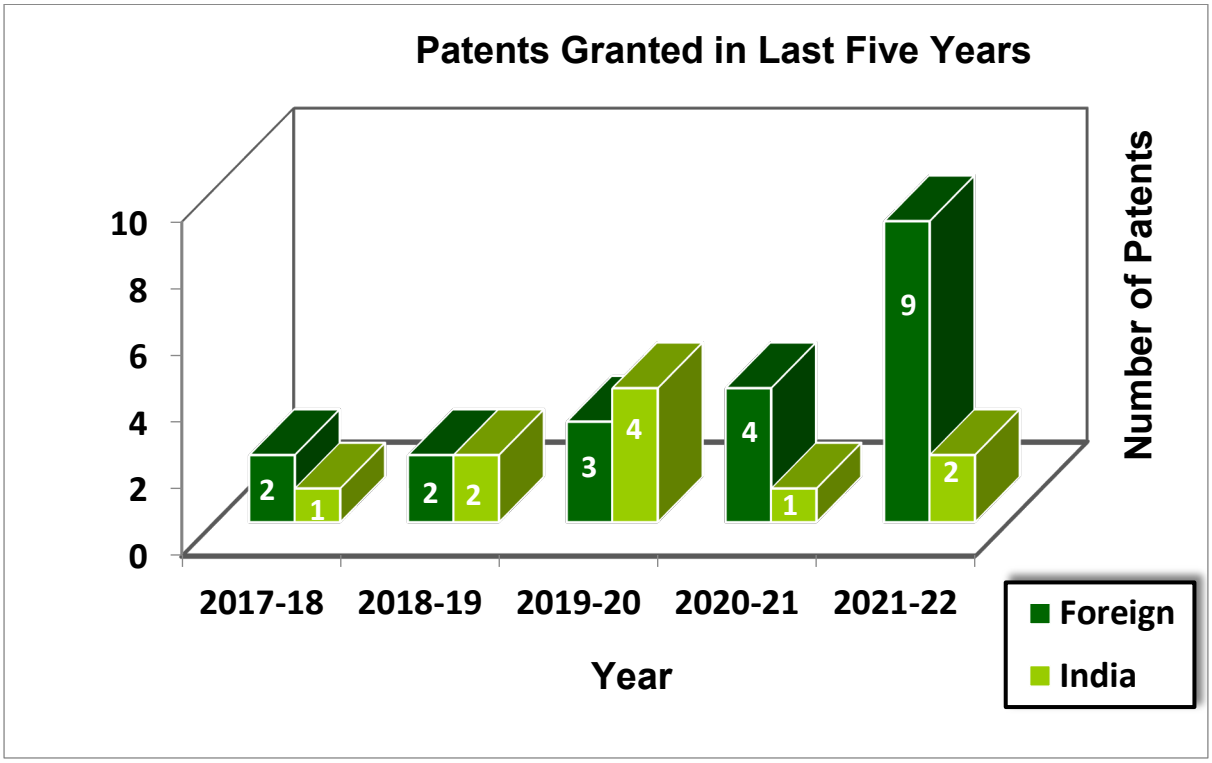
S. No.	Title	Inventors	Prov. Filing Date	Comp. Filing Date	Application No.
1	A NOVEL COMPOSITION OF CATIONIC METAL NANOPARTICLE CONJUGATED NANOEMULSION AND A PROCESS FOR THE PREPARATION THEREOF	SUJOY KUMAR DAS, DEEPAK KUMAR, SNEHASIKTA SWARNAKAR, SOMASHREE BOSE, YOGITA DAHAT	05/Oct/2021	---	202111045225
2	AN IMPROVED SYNTHETIC METHOD FOR THE PREPARATION OF INDOLE-3-CARBOXYLIC ACID DERIVATIVES: USEFUL KEY INTERMEDIATE FOR THE SYNTHESIS OF SEVERAL BIOLOGICALLY ACTIVE MOLECULES INCLUDING TROPISETRON	INDUBHUSAN DEB, ARUP BHOWMIK	20/Oct/2021	---	202111047806

3	A PROCESS FOR THE PREPARATION OF 2,7-DIHYDROXYFLUORENONE USEFUL FOR THE SYNTHESIS OF TILORONE AND ITS SALTS	PARASURAMAN JAISANKAR, INDUBHUSAN DEB, PINAKI BHATTACHARJEE	---	20/Oct/2021	202111047809
4	IN VITRO PROTEASE-BASED SARS-COV-2 SPIKE PROTEIN CLEAVAGE ASSAY SYSTEM	UPASANA RAY, PREM PRAKASH TRIPATHI, FEROZA BEGUM, AMIT KUMAR SRIVASTAVA	28/Oct/2021	---	202111049482
5	PREPARATION OF QUINAZOLINEDIONES AND USE THEREOF FOR TREATMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE	ARINDAM TALUKDAR, PARTHA CHAKRABARTI, DIPAYAN SARKAR, SAHELI CHOWDHURY, SUNNY GOON, ABHISHEK SEN, UDDIPTA GHOSH DASTIDAR, BINITA PATRA, ISRAFUL HOQUE	---	25/Dec/2021	202111061087

- **Filed Abroad**

S. No.	Country	Title	Inventors	Comp. Filing Date	Application No.
1	WORLD	AN EASY-TO-USE DIAGNOSTIC SYSTEM FOR RAPID DENGUE VIRUS DETECTION USING FLUORESCENCE-BASED MOLECULAR PROBES	BISWAS SUBHAJIT, GHOSH SURAJIT, SUKLA SOUMI, MONDAL PRASENJIT	04/May/2021	PCT/IN2021/050432
2	WORLD	QUINAZOLINONES DERIVATIVES FOR TREATMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE, PREPARATION AND USE THEREOF	TALUKDAR ARINDAM, CHAKRABARTI PARTHA, SARKAR DIPAYAN, CHOWDHURY SAHELI, GOON SUNNY, DAS SUBRATA, DAS NIRMAL, SARKAR DIPIKA	25/Jun/2021	PCT/IN2021/050621


Patents Filed in Last Five Years
Number of Patents
8
6
4
2
0
3
2
6
4
7
6
2
2
5
2017-18
2018-19
2019-20
2020-21
2021-22
Year
Foreign
India


Patents Granted in Last Five Years
Number of Patents
10
8
6
4
2
0
2
1
2
2
3
4
4
1
9
2
2017-18
2018-19
2019-20
2020-21
2021-22
Year
Foreign
India

Computer Division

Members:

Dr. Sucheta Tripathy (Head), Dr. Saikat Chakrabarti, (In-charge, TRUE campus), Mr. Pradeep Sypureddi, Mr. Shiv Kumar Gupta

Introduction

Computer Division provides essential IT services to the institute including scientists, staff membersand students. The IT group works towards maintaining uninterrupted IT services to both campuses of the Institute. It has been at the forefront of deploying information technologies towards modernizing the IT infrastructure and facilities besides providing technical support services to the ongoing R&D projects. The Division has also extended its services to CSIR-IICB TRUE, Salt Lake campus through Point-to-Point connectivity.

Major Achievements in 2021-2022

Next-Generation Firewall Procurement and Deployment for the Institute Gateway Services and Cyber Security: Next Generation Firewall was Procured successfully and Deployed for the institute to defend itself from external threats including installation of Routing policies, Security policies, Application Control, Intrusion Protection System, WAN Fail Over, Botnet, Bandwidth Control, etc.

CSIR-IICB Salt Lake Campus Network Backbone System and Infrastructure Upgradation: Salt Lake Campus Network Backbone System and Infrastructure was upgraded successfully with New Layer 3 and Layer 2 Smart Network Managed Switches including Guest House Block for better Connectivity and Scalability. New IP Schema and Local DHCP Services were Implemented for Research Block and Guest house Block with VLAN configuration. Upgraded the Wi-Fi system at Salt Lake Campus and introduced a controller-based system to control connected clients, monitor logs, implement MAC Authentication, etc.

Emergency Backup Internet Connection with 100 Mbps Bandwidth Capacity at CSIR-IICB Jadavpur Campus: Introduced Backup Internet Connection for CSIR-IICB for continuous Internet services with 100 Mbps connection to the Users. The Backup connection also keeps high availability for all internally hosted servers. Link Failover implemented for high availability.

Upgradation of Point-to-Point Connection between CSIR-IICB Jadavpur Campus and CSIR-IICB Salt Lake Campus to 100 Mbps Bandwidth: Upgraded the Salt Lake Campus Point to Point Connection with 100 Mbps Bandwidth for better internet speed and services at CSIR-IICB Salt Lake Campus Research Block and Guest House Block including ring topology for high availability of Internet and Internal Services.

Emergency Backup Internet Connection at CSIR-IICB Salt Lake Campus Guest House Block: Introduced Backup Internet Connection at Salt Lake Campus Guest House Block, Director Conference Room, and Mini Auditorium for High availability of Internet services to conduct very important programs like Research Council Meetings, Performance Appraisal Board Meetings, Scientist and Staff Interviews, CSIR Programs and hosting Conferences and Events, etc.

Deployed Enterprise Client Server Architecture Antivirus System: Introduced New Enterprise Client Server Architecture Antivirus System and End Point Software for the institute's Desktops and Laptops which includes Upgraded Malware defending system including browser protection and other end-user protection systems.

Technical Services of the Computer Division, CSIR-IICB

- IT and Network Infrastructure Management Services for both campuses of the institute.
- Gateway and Firewall Security Services.
- VPN Services for the Scientists to work from home facility.
- Emergency IT Services during COVID-19 outbreak & Natural disasters.
- Email Services for Staff & Students.

- Group Email Services for the Staff and Students.
- NIC VPN Services for the Creating, Managing, and Updating Staff Emails.
- Hosting Websites and Providing Hosting Infrastructure.
- SSL Certificates for the websites and Content Management Services.
- Maintenance of the Institute Website and Intranet Portal.
- Design, Development, and Content Management Services for Skill Development Programs and Conference / Events Web portals from time to time.
- Administration and Maintenance of Computer Division Servers and Sophisticated Core and Distribution Network Appliances.
- Biometric Attendance System for Staff and Students.
- Video Conferencing Services for the institute's Virtual Meetings.
- Display System Services for Quick Information and announcement to the inside users.
- IT Facilities Arrangements for the Conferences, Events, and Institute Annual Programs like Foundation Day Celebrations, etc. including DAC Meetings, Internal Meetings, Student Classes, Administration Meetings, Student Thesis defense Meetings, Colloquium lectures, Viva Voce Exam meetings, Summer Trainee Programs, Skill Development Programs, Jigyasa Programs, etc.
- Technical Support, Call Assignment, and Monitoring Services to the users to provide support services for Desktops, Servers, Work Stations, Laptops, Printers, Scanners, Software, etc.
- Technical Documents and Other Special Services.

Group members of Computer Division

Engineering Services Unit (ESU)

The Engineering Services Unit (ESU) is comprised of Civil Engineering, Electrical Engineering and Air-conditioning & Refrigeration sections.

Civil Engineering Section

Member:

Dr. N. C. Maiti (Head), Mr. Sandip Saha, Mr. Susanta Ray, Mrs. N. Bage, Mr. D. Banik, Mr. A. Paul, Mr. S.R. Tudu, Mr. S.K. Ghosal, Mr. Ptradip Kumar Mondal.
In Works Section under ESU: Mr. Sujit Majumder and Md. Muktar Ahmed.

The Civil Engineering Division under Engineering Services Unit of CSIR-IICB takes major role to render services in board areas of infrastructural development, new construction, renovation and up-gradation of laboratories for Scientific Research Activities and common facilities, maintenance of campus, water supply, sewerage and drainage systems, cleaning and house-keeping work at both the campuses at Jadavpur as well as Salt Lake.

In addition to the above works, ESU Civil Section have been looking after the Gardening and Horticulture work, Audio-Visual works at Auditorium and Seminar as well as Conference Rooms in coordination with Electrical and Computer sections, Housekeeping and Conservancy works, Guest House management Services etc.

The major repair & renovation works taken up in the Financial year 2021-22 are as follows:

- External repair and renovation of CSIR-IICB building (Phase-B) at Jadavpur campus.
- Repair and Renovation of building, sanitary and water supply services.
- Repairing and painting works of room nos. 112,222,315,322,344 and all toilets of guest house at CSIR-IICB, Saltlake campus
- Repair and renovation of staff quarter at west side of IICB Jadavpur campus
- Repair and renovation of erstwhile incinerator room for using it as laboratory room at CSIR-IICB Jadavpur campus.
- Repair & renovation of Different laboratories and rooms (Phase-1) at IICB, Jadavpur campus.
- Providing shed over DG set at Salt Lake campus of CSIR-IICB
- Repair and renovation of HT Panel room adjacent to electrical substation room at CSIR-IICB Jadavpur campus, Kolkata.
- Repairing of boundary wall, fencing and cleaning of garbage at Scientist apartment.
- Repairing & renovation of NMR Room (Room no. 33A) at IICB, Jadavpur, Kolkata.
- Repair and maintenance of various civil works at CSIR-Indian Institute of Chemical Biology, Jadavpur and Saltlake campus, Kolkata.
- Repairing & renovation of committee at Ground Floor at IICB, Jadavpur Kolkata.
- Repair and renovation of damage drain cover near electrical generator room at CSIR-IICB, Jadavpur Campus, Kolkata-32.
- Providing and fixing of iron grid pathway for maintenance of central AC chillar at CSIR-IICB, Saltlake campus, kolkata-700091.
- Repair and Renovation of Electrical Generator Room at CSIR-IICB, Jadavpur campus. Maintenance of central AC chillar at CSIR-IICB.

Electrical Engineering Section

Member:
Dr. N.C. Maiti (Head), Mr. Susanta Roy, Mr. Ujjal Roy, Mr. Sourin Ghosh, Mr. Abhijit Paul, Mr. Samir Majumder, Mr. Arup Karmakar, Mr. Tanmoy Biswas

Electrical Engineering Section under ESU has various activities and involvements towards overall management of Electrical Power Distribution systems installed at the Campuses of CSIR-IICB at Jadavpur and Salt Lake. In addition to this, ESU Electrical Section having regular activities for Estimation, Planning, Execution and Monitoring of all types of works related to Electrical Power and associated system including major modernization works. Different works like Provision of LED Lighting Fittings, Renovation and upgradation of Electrical systems of different Rooms and Working areas, renovation of CCTV Systems etc. were taken up during last financial year.

Air Conditioning section

Member:
Dr. N. C. Maiti (Head), Mr. Prosenjit Gangopadhyay, Mr. Shubhendu Ghosh, Mr. Sanjib Biswas and Mr. Monoranjan Adhikari

Air Conditioning Section is one of the important functioning groups under ESU. This section has various activities at CSIR-IICB, Jadavpur and Salt Lake Campuses.

The important activities performed by this section are as follows

- Operation service and maintenance of Central Air Conditioning Plants of CSIR-IICB Jadavpur campus to ensure normal activities of the Auditorium and Library.
- More than 600 Split ACs are maintained round the clock by this section for Scientific and Research purpose.
- Lifts total 6 nos. are maintained round the clock at both the campuses of CSIR-IICB
- Cold Rooms two nos. at different floors of CSIR-IICB Jadavpur Campus are maintained properly throughout the year for different scientific purposes.

Group members of ESU

Human Resource Group (HRG)

Academic Affairs Division

Members:

Dr. Sanjay Dutta (Head), Ms. Arti Grover, Ms. Debasree Das, Md. Ayub Shah

Human Resource Group (HRG) of CSIR-IICB promotes professional Human Resources Management in this institute by evolving and implementing HR development plan. The HRD group is involved in a wide range of HRD related activities in various areas of PhD student affairs, academic affairs and fellowship related activities. The major area where HR group contributes: Activities related to Academic–Administration concerning PhD program, student affairs, post-graduate training programme, and different other training program. HRG provides assistance in the preparation of teaching and research materials and co-ordination of various programs critical to the mission of the Institute

The functions include: Guidance and co-ordination of different HR development program & talent-management activities. HRG section function as a coordinating centre for the CSIR-IICB PhD course work for the PhD students. Head, HRG serves as the co-ordinator of the Academic Affairs committee.

Student Affairs & Academic Affairs

- PhD course work and PhD program: Management of Course work schedule, course administration and curriculum planning, attendance and class schedule, coordination with the teachers, management of semester examinations, evaluation, seminar, and publication of result, issuance of certificates and statement of marks.
- Scrutinization of applications for PhD registration and documents of RFs/RAs related to academic affairs and maintenance of PhD registration related information.
- Scrutinization of academic records, selection and placement of PG summer/winter trainees and co-ordination of this program.
- Coordination of Academic Affairs committee meeting and content development in this regard.
- NET JRF entrance interview: Information, web notification, list of number of NET JRFs intake. Maintenance of RFs record for the individual PhD supervisor and associated jobs.
- Content development for Research fellow's handbook, publication of course catalogue, Teacher's guideline, academic Calendar and different guidelines related to IICB PhD program and PhD course work.
- Organization of Orientation programme for PhD students.
- Interacting and coordinating with CSIR HRDC

Human Resources: PhD students (as on March 2022)

At a Glance:

Number of existing Research Fellows: 262 (approx.)
(CSIR/UGC/DST/DBT/ICMR)

Number of students awarded PhD degree during 2021-22: 35
(Doctorate degree received from AcSIR/University of Calcutta/ Jadavpur University

Summer Training / Project Work / Dissertation Work

HRG coordinates Summer Training Programme for the eligible Post Graduate students of different Universities, Institutions and Colleges for partial fulfilment of their degrees. The aim is to let young minds feel the thrill and excitement of science by working on a project requiring application and critical appreciation of scientific principles. It also aims at active participation in the learning process through experimentation and putting into practice the knowledge acquired in the classrooms.

The summer program is primarily designed to provide the opportunity to do basic research in top-notch research areas, in a supportive learning environment with plenty of interaction with PhD research fellows and faculty members. Detailed guidelines are made available in CSIR-IICB website

Number of Summer Trainee/Project Trainee (2021-22): 52

Learning and instructional support: Academic Affairs

To conduct and coordinate the IICB PhD course work is the major focus of this Division which includes activities related to CSIR-IICB PhD Course Work program and academic-administrative guidance to the AcSIR activities in this institute.

The CSIR-IICB "**Academic Affairs Committee (AAC)"** acts as an Advisory Committee to the Academic Affairs Division/HRG in connection with CSIR-IICB PhD program and the AcSIR programme.

Members of AAC are as follows

Dr. Suvendra Nath Bhattacharyya, Chairperson
Dr. Sib Sankar Roy, Member
Dr. P. Jaisankar, Member
Dr. K. Chattopadhyay, Member
Dr. Mrinal K Ghosh, Member
Dr. Chinmay Chowdhury, Member
Dr. Jayati Sengupta, Vice- Chairperson
Dr. Subhas Ch. Biswas, Member
Dr. Saikat Chakrabarti, Member
Dr. Debabrata Biswas, Member
Dr. Partha Chakrabarti, Member
Dr. Sanjay Dutta, Member - Convener

CSIR-IICB PhD Course Work (CW)

CSIR-IICB offers a mandatory PhD course work for the Research Fellows in their first year, taught by faculty members of in-house as well as from other Institutes/Universities. The framing of the course content & guidelines is designed in the line of AcSIR courses as well as per UGC requirement. The curriculum of Course work plays a pivotal role for rejuvenating the creative nature in the scientific area of research.

The existing CSIR-IICB PhD Course Work programme constitutes basic and advanced level courses. The basic course is for bridging the gap between M.Sc. and PhD. The advanced level course comprises of frontline areas of research and covers research methodology and review of current literature.

CSIR-IICB PhD CW comprises of Basic & Advanced level courses [total 14 credits taken by any student]:

Basic courses

Research Methodology (Safety and Behavior at Workspace, Laboratory and Institutional Campus, Research Problem Identification and Research Design/Plan, Good Experimental, Observational and Data Analysis including Computer Applications, Intellectual Property, Patent Database Search and Patent Writing, Writing & Communication of Research Results and Inferences, Analytical Tools and Techniques in Research - A General Cross Disciplinary Exposure).

Research and Publication Ethics (Philosophy and Ethics, Scientific Conduct, Publication Ethics, Open Access Publishing, Publication Misconduct, Databases and Research Metrics)

Basic Biology & Basic Chemistry

Inter-disciplinary Learning

Cell and Tissue Engineering, Chemical Biology & Supramolecular chemistry and green chemistry

Advanced Courses

Biology of Macromolecules, Cancer Biology, Cell Biology and Cell Signaling, Eukaryotic Gene Regulatory Mechanisms, Molecular and Cellular Immunology, Protein Science and Proteomics, Advanced Analytical Chemistry, Advanced Organic Chemistry, Natural Products and Drug Discovery & Total Synthesis

'Societal program: Problem Understanding and Analysis'

HRG functions as overall coordinating centre of **CSIR-IICB PhD course work** for PhD students. The PhD course work is carried out with the advice of Chairperson & members of Academic Affairs Committee and also the Examination committee constituted for this purpose.

Total number of Course work students for 2021-22: 55
(Chemistry - 08, Biology - 47)

AcSIR Academy of Scientific & Innovative Research
Set up by an Act of Parliament, An Institute of National Importance

Academy of Scientific and Innovative Research (AcSIR) is established by an Act of Indian Parliament as an Institute of National Importance, provides opportunity to the CSIR students to work in areas of integrative and interdisciplinary areas of Science and Engineering.

AcSIR at IICB started enrolling students from 2012 session in the area of biological and chemical sciences.

Number of students enrolled in 2021-22: 22
Number of students awarded PhD degree during 2021-22: 10

AcSIR@IICB enrolls students twice in a year in January and August session. Students after enrolling with AcSIR had to take coursework in first two semesters. Also a Doctoral Advisory committee is formed for each research student to review their research progress and recommends thesis submission. The maximum duration of the PhD program for the AcSIR students in 6 years and minimum is 3 years which is followed in all CSIR institutes across India.

AcSIR Co-ordinator – Dr. Sanjay Dutta, Principal Scientist CSIR-IICB
Executive Assistant – Ms. Rohini Majumdar

Group members of HRG

List of Doctorates from CSIR-IICB (during April 2021 – March 2022)

Recipient's Name	Supervisor's name	University	Dt. of Award	Title of Thesis
Dr. Liberalis Debraj Boila	Dr. Amitava Sengupta	Jadavpur University	25th August 2021	Histone demethylases in myeloid leukemia
Dr. Sayantani Sinha	Dr. Amitava Sengupta	Jadavpur University	06th September 2021	Chromatin Regulators in Stromal Microenvironment and Hematopoiesis
Dr. Dishari Ghatak	Dr. Amitava Sengupta & Dr. Susanta Roychowdhury (Asssociate Supervisor)	Calcutta University	19th August 2021	Studies on the gain-of –function mutant p53 mediated cancer cell stemness
Dr. Sourav Pal	Dr. Arindam Talukdar	AcSIR	15th December 2021	Design and exploration of toll-like receptor 7 antagonists and topoisomerase I Inhibitors"
Dr. Dibyanti Mukherjee	Dr. Arun Bandyopadhyay	Calcutta University	17th September 2021	Understanding The Role Of Mitophagy In Hypertrophied Cardiomyocytes
Dr. Chinky Shiu Chen Liu	Dr. Dipyaman Ganguly	Jadavpur University	21st January 2022	Exploring the role of mechanical cues in T cell function
Dr. Sandip Naskar	Dr. Indrajit Das	Calcutta University	11th February 2021	Visible-Light-Catalysis For Selective Organic Transformations
Dr. Sukanya Mozumder	Dr. Jayati Sengupta	AcSIR	1st June 2021	Investigation on ligand-based structure and dynamic properties of serotonin (5-HT) G-protein coupled receptor (GPCR)
Dr. Rajanya Bhattacharyya	Dr. Jayati Sengupta	Calcutta University	07th October 2021	Structural And Functional Insight Into Amyloidogenic Protein Aggregation Inhibition
Dr. Shirin Akbar	Dr. Jayati Sengupta	Calcutta University	27th December 2021	Understanding The Mechanism Of Nascent Polypeptide Chain Processing On Bacterial Ribosome By A New Genearation Drug Target Protein Peptide Deformylase
Dr. Snehasish Mishra	Dr. Krishna Das Saha	Calcutta University	22nd February 2022	Therapeutic approach of selected nanocomposites with mechanisms of action in colon cancer
Dr. Anindita Mahapatra	Dr. Krishnananda Chattopadhyay	AcSIR	30th November 2021	Biophysical investigations of the aggregation pathway of alpha-synuclein and its modulations by novel and repurposed molecules
Dr. Indrani Nandi	Dr. Krishnananda Chattopadhyay	AcSIR	9th February 2022	Biophysical investigation of protein-Metal nanocluster interactions

Dr. Suborno Jati	Dr. Malini Sen	Calcutta University	07th October 2021	Role Of WNT Signalling In Bacterial Infection
Dr. Neerajana Datta	Dr. Mrinal Kanti Ghosh	Calcutta University	16th November 2021	Mechanistic Study Of PML Regulation, Towards Curbing Oncogenesis
Dr. Veenita Khare	Dr. Mrinal Kanti Ghosh	Calcutta University	21st June 2021	Involvement Of p68 In Oncogenic Signaling Pathways
Dr. Animesh Mondal	Dr. Nakul Ch Maiti	Jadavpur University	20th August 2021	Structural Variation of Disorder Proteins and It's Alteration in the Processes of Aggresgation & Fibrillation
Dr. Gajbhiye Rahul Laxman	Dr. P. Jaisankar	Jadavpur University	07th December 2021	Exploring Antidiabetic Antileishmanial and Anticancer Lead Molecules from Indian Medicinal Plants
Dr. Anushree Achari	Dr. P. Jaisankar	Calcutta University	20th December 2021	Synthetic studies on natural products and their analogues using bio and organocatalysts an d study of their bioactivity
Dr. Jit Sarkar	Dr. Partha Chakrabarti	AcSIR	14th June 2021	Pathophysiological characterization of non-obese type 2 diabetes mellitus
Dr. Anand Kumar Gupta	Dr. Pijush K Das	Calcutta University	05th July 2021	Evasion Of The Host Macrophage Defence Machinery-Inflammasomes, By Intracellular Parasite, Leishmania donovani
Dr. Krishanu Samanta	Dr. R Natrajan	AcSIR	10th November 2021	Molecular self-assembly: Fluorescent cage, cocrystals and peptide aggregation
Dipayan Rakshit Dr.	Dr. Rupak K Bhadra	Calcutta University	06th December 2021	Molecular Studies On The Stringent Response Related Guanosine Pentaphosphate Phosphohydrolase Coding Gene *gppA* Of *Vibrio cholerae*
Dr. Dipendu Patra	Dr. Sanjay Dutta	AcSIR	21st October 2021	Design and synthesis of fluorescent quinoxaline molecules targeting nucleic acid
Dr. Soumi Biswas	Dr. Sharmila Chattopadhyay	AcSIR	17th May 2021	An investigation on Podophyllum hexandrum – a potential source of the anticancer drug podophyllotoxin
Dr. Santanu Adhikary	Dr. Siddhartha Roy	Calcutta University	07th October 2021	The Role of Putative Chromatin Readers UBR7 And ZMYND8 In Regulating Cellular Oncogenesis And Differentiation.
Dr. Deep Sankar Rudra	Dr. Snehasikta Swarnakar	Calcutta University	07th March 2022	Role of matrix metalloproteinase in restraint cold stress-induced gastric ulcer under diabetic and non-diabetic condition.

Dr. Pijush Das	Dr. Sucheta Tripathy and Dr. Susanta Roychowdhury (Asssociate Supervisor)	Calcutta University	27th July 2021	Development Of A Novel Computational Algorithm To Identify Aggressive Subset Of Head And Neck Cancer
Dr. Samrat Ghosh	Dr. Sucheta Tripathy	AcSIR	3rd August 2021	Analyzing big data with novel combinatorial approach
Dr. Deeksha Singh	Dr. Sucheta Tripathy	AcSIR	24th September 2021	In-depth analysis of a newly discovered marine cyanobacteria with bioremediation and bio-metabolite production potential
Dr. Kumar Singha Roy	Dr. Susanta Roychowdhury & Dr. Sib Sankar Roy (Joint supervisor)	Calcutta University	19th August 2021	Regulation of mitosis by wild type and gain-of-function mutant p53
Dr. Bartika Ghoshal	Dr. Suvendra Nath Bhattacharyya	Calcutta University	11th March 2022	Mechanism of intercellular transfer of microRNAs between mammalian cells.
Dr. Parash Prasad	Dr. Sib Sankar Roy	Calcutta University	11th March 2022	Understanding the role of growth factors and metabolites in metabolic reprogramming of tumor cells and subsequent oncogenesis
Dr. Arnab Bandyopadhyay	Dr.Krishnananda Chattopadhyay	Calcutta University	27th August 2021	Unfolding and amyloidogenesis studies of yeast prion protein In-vitro and inside live cells.
Dr. Ishita Mukherjee	Dr. Saikat Chakrabarti	Calcutta University	20th December 2021	Understanding the intra and inter-cellular interaction complexities and flexibilities using systems and sequence analysis approach.

Knowledge Resource Centre (KRC)

Members:
Dr. Krishnananda Chattopadhyay (Head), Dr. Sankar Maitra, Mr. Manas Samanta, Mr. Tapan Das, Mr. S. Nath and Ms. Mahua Bhattacharyya

Knowledge Resource Centre (Library & Documentation Division) is actively engaged in giving access to the information sources online and off-line. The KRC has been continuing its efforts in expanding services to the users' community through its information base. The Division is playing a pro-active role in establishing itself as one of the important infrastructure divisions by providing literature supports to the scientific community.

Collection strength

Books (including Hindi)	14430
Journals (online only)	109
Bound volumes	33860
Science-Direct (Back files) (http://www.iicb.res.in/bkfiles_library.html)	202 journals full text up to 1994
Newspapers (English, Bengali & Hindi)	3

National Knowledge Resource Consortium (NKRC)

NKRC is a strong network of all CSIR & DST Institutions for pooling and sharing of Knowledge Resources for catering best possible services to their users. The CSIR-NISCAIR is the nodal agency for implementing and monitoring the activities of the NKRC and venturing the project successfully. Presently, about 5000 scholarly journals are accessible in full-text through NKRC across the CSIR & DST Institutions.

Services

- The KRC serves as an important interface between users and the literature by ensuring uninterrupted access to the subscribed content besides regular services like **circulation, reference** and **referral**, **photocopying** and **printouts** services including the following others.
- The Online Public Access Catalogue (OPAC) is available at **http://14.139.223.107:8080/webopac/html/SearchForm** which has been utilized as a very useful search interface for the library holdings.
- Open Access Repository (IR) maintaining in E-prints for archiving peer-reviewed journals articles, Conference papers, Theses and other research documents produced by IICB researchers. The URL for accessing the repository is **http://www.eprints.iicb.res.in**. So far 2006 documents in full-text have been uploaded to the repository.
- **Resource sharing** among CSIR & DST Libraries based on the demand placed by the users.
- **Similarity Index Report** generation for all the theses and research papers from IICB before submitting and communicating accordingly. For reviewing the manuscripts, **iThenticate** – plagiarism detection database service is available in KRC.
- The KRC provides personal information services using Science Citation Index Expanded. IICB-KRC has been subscribing the **'Web of Science'** for such services.
- **CSIR Virtual Union Catalogue** is available with the URL http://14.139.223.115/ for resource sharing among CSIR KRCs.
- Several'User Awareness cum Training Programmes" organized by the KRC to maximize its utilization among the researchers.
- A collection on **Hindi books** has been developed by KRC as advised by Official Language Implementation Committee (**OLIC**).

Group members of KRC

Project Monitoring & Evaluation Group (PME)

Members:

Dr. Subhas Chandra Biswas (Head), Mr. Soumalya Sinha and Mr. Samir Thami

The planning, monitoring and evaluation (PME) division manages the Institute's plan and non-plan projects, grant-in aid projects (GAP), sponsored and collaborative R&D projects, consultancy and technical service projects. This division maintains liaison with Scientists and Technical officers who are Investigators of these projects and liaison with the Finance section and Purchase section and the funding agencies. PME provides proper logistic support for the management, monitoring and implementation of CSIR funded in house projects (Mission Mode, Fast Track Translational) and other externally funded projects that include those obtained from sponsored international agencies. PME's role is in an effective and successful implementation of the institute's commitments to all R&D endeavours. PME is also entrusted with appropriate dissemination of information regarding ongoing and completed projects to all statutory agencies like CSIR audit party, CAG audit etc. PME of CSIR-IICB, like other CSIR laboratories, is actively involved in the timely preparation and maintenance of databases for all intramural and extramural research projects, monitoring of project expenditure of projects, preparation of responses to Parliamentary queries in relation to the activities of the Institute, dissemination of information on all relevant National and International research program requests. PME from time to time provides information to scientists regarding terms and conditions of funding agencies, timely requirement of progress report and completion reports, respectively, of ongoing and completed projects. PME division participates in the preparation of the Institute's annual plan and the budget and maintains the expenditure data, monitors and accounts the receipts of cheques as well as online transfer of fund by the sponsors against the project sanctioned, and request for sanctioned fund, and maintains proper record keeping of all aspects of projects. It does regularly interaction with finance division regarding the expenditure carried out against the projects and prepares the data on this a monthly basis. PME also processes all the relevant requirements for collaborative projects, approvals from competent authorities like Research Council, Management Council, and Director enabling smooth and quick submission of new projects to external funding agencies. Details of CSIR and other extramural projects sanctioned during the reporting period (sanctioned, ongoing and completed) are provided as separate lists.

Group members of PME Group

Completed Extramural/Sponsored/Consultancy Projects (2021-2022)

Sl. No.	Name of the project	Project code	Name of PI	Project Cost (Rs. In Lakhs)	Funding Agency	Start Date	Completion Date
1	Multi-dimensional Research to Enable Systems Medicine : Acceleration using a ClusterApproach	GAP-357	Dr. Samit Chattapadhyay	1,818.00	DBT, Govt.of India	07.03.2017	06.03.2021
2	Role of endogenous intermediate progenitors in neurogenesis following neurodegeneration	GAP-383	Dr. Prem Prakash Tripathi	33.966	SERB	24.05.2018	23.05.2021
3	Research and development work for value addition of the product Parkers Medha Plus	SSP-384	Dr. Subhas Chandra Biswas	9.003	Parker Robinso n	02.07.2018	01.07.2021
4	Histone chaperon Asf1 in Plasmodium falciparum: Novel anti-malarial targets	GAP-385	Dr. Siddhartha Roy	52.500	DBT	04.07.2018	03.07.2021
5	Development of a combinatorial nano-vehicales assisted therapeutic system for the effiecient treatment of Glioma	GAP-390	Dr. Mrinal K Ghosh	41.982	DST, Govt. Of India	23.08.2018	22.08.2021
6	Role of Wnt5A Signaling in Bacterial Infection	GAP-391	Dr. Malini Sen	67.060	DBT, Govt. of India	15.09.2018	14.09.2021
7	Characterization of herpesvirus in clinical samples from Eastern India in terms of their growth kinetics and sensitivity to available antivirals together with screening for novel herpes	GAP-392	Dr. Subhajit Biswas	26.466	DBT, Govt. Of West Bengal	14.09.2018	13.09.2022

8	Glucocorticoid Receptor-Assisted Drug Sensitization (GRADS) in colorectal cancer therapy: Nano-therapeutic strategy towards repurposing of anti-cancer drugs	GAP-393	Dr. Mrinal K Ghosh	12.420	SERB, Govt. Of India	18.09.2018	17.09.2021
9	Development of Novel Theranostics Targeting Nucleic acids	GAP-394	Dr. Sanjay Dutta	25.300	SERB, Govt. Of India	06.11.2018	05.11.2021
10	A Novel Nanotechnology based Approach of Glioma Therapy by Targeting HAUSP-MDM2 axis in combination with Temozolomide	GAP-398	Dr. Mrinal K Ghosh	40.138	SERB, GOVT. Of India	15.01.2019	30.06.2022
11	To evaluate the effect of black tea intake on chronic anovulation and insulin resistance in polycystic ovarian syndrome (PCOS)	GAP-399	Dr. Sib Sankar Roy	34.454	NTRF, Govt. Of India	19.12.2018	23.10.2022
12	Alzheimer's disease: is age related subtle DNA damage the trigger of the sporadic Alzheimer's disease?	GAP-400	Dr. Subhas Chandra Biswas	30.390	SERB. Govt. Of India	04.03.2019	03.03.2022
13	Exploring the mechanistic link between type I interferons, gut dysbiosis and regulatory T cell abundance in obesity associated metaflammation and insulin resistance	GAP-402	Dr. Dipyaman Ganguly	15.000	DBT, Govt. Od India	18.03.2019	17.03.2022
14	Understanding the role of Dengue NS1 protein in endothelium leakage and	GAP-403	Dr. Upasana Ray	23.750	DBT, Govt. Of West Bengal	30.03.2019	29.03.2022

	therapeutic intervention of NS1 pathogens						
15	Understanding entry of Chikungunya virus and engineering self-assembly system based anti-Chikungunya vaccine	GAP-404	Dr. Upasana Ray	35.881	SERB. Govt. Of India	30.03.2019	29.03.2022

Sanctioned and Implemented Extramural/Sponsored/Consultancy Projects (2021-2022)

Sl. No.	Name of the project	Project code	Name of PI	Project Cost (Rs. In Lakhs)	Funding Agency	Start Date	Completion Date
1	Regulation of trophoblast development anda placental pathophysiology by long non-coding RNA	GAP-437	Dr. Rupasri Ain	51.87	SERB, Govt. Of India	12.07.2021	11.07.2024
2	Evaluation of cytotoxicity, pharmacokinectics, anti-cancer activity, detailed molecular mechanisms of Bhallatakadi Modaka	GAP-438	Dr. Amit Kumar Srivastava	23.92	CCRAS, Govt. Of India	12.11.2021	11.11.2023
3	Development of visible-light photoredox and transition metal dual catalytic alkene difunctionalization reactions	GAP-439	Dr. Ranjan Jana	25.06	SERB, Govt. Of India	20.12.2021	19.12.2024
4	Translocation and regulation of an unusual novel PAS domain containing phosphoglycerate kinase in Leishmania	GAP-440	Dr. Subrata Adak	35.75	SERB, Govt. Of India	30.12.21	29.12.2024

5	Understanding the regulatory role of co-activator binding protein PIMT in the pancreatic Beta cells of diabetic animals and T3c diabetic (chronic pancreatisis) humans	GAP-441	Dr. Partha Chakrabarti	12.96	SERB, Govt. Of India	31.12.2021	30.12.2024
6	Evaluation of insulin-like growth factor 2 (IGF2) as a potential epigenetic biomarker and therapeutic target to abolish the metabolic memory in diabetic retinopathy	GAP-442	Dr. G Senthil Kumar	38.00	ICMR, Govt. of India	26.12.2021	25.12.2024
7	Clinical role of a pair of novel mutations in BCR-ABL1 towards therapy switch in imatinib-resistant chronic myeloid leukemia	GAP-443	Dr. Arindam Talukdar	79.93	LADY TATA Memorial Trust	04.02.2022	03.02.2025
8	Functional dissection of Jmjd3 during embryonic and adult hippocampal neurogenesis	GAP-444	Dr. Prem Prakash Tripathi	42.22	SERB, Govt. Of India	23.02.22	22.02.2025
9	Phase II/III clinical trials of an indigenously-developed hepatitis E vaccine and evaluating its immune correlates	GAP-445	Dr. Dipyaman Ganguly	57.80	BIRAC	09.02.2022	08.08.2024
10	In quest of novel drug targets : Investivation on structural dynamics of the	GAP-446	Dr. Jayati Sengupta	38.10	SERB	28.03.2022	27.03.2025

	mycobacterium ribosome using high-resolution cryo-electron microscopy						
11	Platform integration for high through-put multi omics data analysis and text processing	GAP-447	Dr. Sucheta Tripathy	227.21	DBT	07.03.2022	06.03.2027
12	Identification and characterization specific genes/metabolites linked with Rancidity and their Bioavailability Patterns in landraces and elite cultivars of pearl millet for the development of Nutri-Rich products	GAP-451	Dr. U. Mabalirajan	20.98	ICAR	26.08.2022	25.08.2025
13	Investigation of the regulation of plant immunity by sphingolipids in rice	GAP-452	Dr. S. Behera	27.77	SERB	13.12.2022	12.12.2024
14	Exploring the heterogeneity of systemic lupus patients based on antibodies distribution and multi-omics approaches compared in two ethnic cohorts	GAP-453	Dr. Dipyaman Ganguly	138.81	DST	20.10.2022	19.10.2025
15	Hemostat: A lifesaving bandage for faster Blood Arrest	GAP-454	Dr. Sujoy Das	46.65	BIRAC	12.01.2023	12.01.2023
16	Anticancer potential and Molecular Mechanism of Rasa sindhoor on Ovarian Cancer Cells	GAP455	Dr. Amit Kumar Srivastava	40.65			

17	Genomic Surveillance program for SARS-CoV-2: Consortium of India and Sri Lanka	GAP-456	Dr. Siddik Sarkar	50.00	Wellcome Trust, UK	07.11.2022	06.11.2023
18	Evaluation of Anti-Cancerous potential in silico pharmacological analysis and molecular mechanism of Kanchanar guggulu in ovarian cancer µ Tumor spheroids, and in In-vivo model	GAP-457	Dr. Amit Srivastava	44.65	CARI	10.03.2023	09.03.2025
19	Determination and analysis of biological activities of Identified Molecules from Mistletoe	SSP-458	Dr. P. Jaisankar	21.24	Swasthani ketan Integrated Healthcar e & Research Foundatio n	01.02.2023	31.01.2025
20	Single molecule studies of transcription-translation coupling in bacteria	GAP-459	Dr. Abhishek Mazumder	357.28	DBT India Alliance, Wellcome	01.01.2023	31.12.2027

Ongoing In-House Projects (2021-2022)

Sl. No.	Name of the project	Project code	Name of PI	Project Cost (Rs. In Lakhs)	Start Date	Completio n Date
1	CSIR Integrated Skill Initiative Program	NWP 100	Dr. P. Jaisankar	343.000	16.10.2020	31.03.2025
2	PAN CSIR CANCER RESEARCH PROGRAM making Cancer care affordable empowering women's health: Focusing on breast and gynaelogical cancers of Indian Relevance	HCP 40	Dr. Sib Sankar Roy	1008.100	29.06.2021	28.06.2026
3	CSIR Phyto pharmaceuticals Mission Phase-II	HCP 38	Dr. Ranjan Jana	71.150	06.10.2021	31.03.2023

4	Discovery & Pre-clinical Development of Antivirals for COVID-19 & other Diseases.	HCP 41	Dr. Arindam Talukdar	247.000	08.11.2021	31.03.2023
5	Indian Breast Cancer Genome Atlas	HCP 43	Dr. Shilpak Chatterjee	175.500	02.11.2021	31.03.2026
6	Classification and characterization of Phytophthora capsici effectors in understanding the early, late onset of pathogenesis and developing inhibition strategies	MLP134	Dr. Sucheta Tripathy	191.18	28.01.2021	31.03.2023
7	Deriving a pan-omics diagnostic pipeline for systems level immune health and therapeutic targeting in systemic autoimmunity	MLP135	Dr. Dipyaman Ganguly	370.00	03.03.2021	31.03.2025
8	Leishmaniasis: Target specific approaches to affect host-pathogen interaction and disease process	MLP136	Dr. Subrata Adak	300.00	03.03.2021	31.03.2025
9	Modern innovative solutions for Environmental/ Occupational Lung Health challenges using clinical and pre-clinical strategies	MLP137	Dr. U Mabalirajan	406.00	03.03.2021	31.03.2025
10	Non-alcoholic Fatty Liver Disease (NAFLD): Novel Pathogenetic mechanism and therapeutic development	MLP138	Dr. Partha Chakrabarti	499.49	03.03.2021	31.03.2025
11	Targeting RNA driven processes: Novel Chemical Biology Approaches to Identify New Classes of RNA Modulators	MLP139	Dr. Suvendra N Bhattacharya	410.00	03.03.2021	31.03.2025
12	Exploring role of Mechanical cues in Immunocellular Regulation	MLP140	Dr. Dipyaman Ganguly	100.00	03.03.2021	31.03.2023
13	Mechanistic understanding of role of human ZMYND8 in tumor microenvironment-dependent transcriptional reprogramming leading to metastasis in colorectal cancer	MLP141	Dr. Debabrata Biswas	120.00	03.03.2021	31.03.2023
14	Restoration of p53 and Rb through targeting their post-translational modifiers (PTMs) HAISP and MDM2 in glioma and stepping towards development of novel inhibitory peptide designing from interacting interface	MLP142	Dr. Mrinal K Ghosh	113.00	03.03.2021	31.03.2023
15	Theranostic Approach in Glioblastoma Multiforme (GBM) using Engineered	MLP143	Dr. Upasana Ray	125.00	03.03.2021	31.03.2023

	Oncolytic Virus Derived Particles (VDPs)					
16	Development of proof of concept vaccine strategies for COVID-19	MLP144	Dr. Upasana Ray	35.00	14.12.2020	30.09.2021
17	Development of Drug-target based Assay platforms and screening against COVID-19	MLP2037	Dr. Arindam Talukdar	51.01	14.12.2020	31.03.2021

Publication and Information Division

Science Popularization Section (SPS)

Members:

Dr. Snehasikta Swarnakar (Head), Dr. Neeta V.M. Khalkho (Head, SPS), Dr. Sarita Ghosh, Mrs. Arti Grover, Mr. Saibal Giri, Mr. Paresh Sarkar, Mr. P.C. Dehuri

This department is responsible for showcasing the institute activivies to other laboratories and the common public through publishing the annual report, participating in exhibitions, conducting laboratory visitis by different academic institutions, etc. The Jigyasa proramme of CSIR, aimed at popularizing science among school students, is also organised by the Publication and Information Division.

CSIR Jigyasa Programme

The SPS undertakes and deals with a wide range of informational activities and the publication of reports related to these outreach activities such as JIGYASA, participation in technology fairs (both at the local and national levels). Such information is endeavoured to be disseminated both in electronic and printed forms for extensive publicity of CSIR-IICB technologies, products and innovations. The major contribution of SPS lies in assisting scientists to organize outreach regarding above-mentioned technologies, products and innovations**.** The section was involved in the following programmes during the reporting year:

Preparation of documents released during events such as JIGYASA, and updating the English and Hindi Technology Standees to be made available at periodic intervals. SPS also undertook dissemination of information to the scientific milieu on relevant subjects. SPS also maintains record for testing and calibration and updates SPS events and ancillary information on the CSIR-IICB website. The SPS is also entrusted with the responsibility of science news dissemination and display achievements of the institution through exhibitions

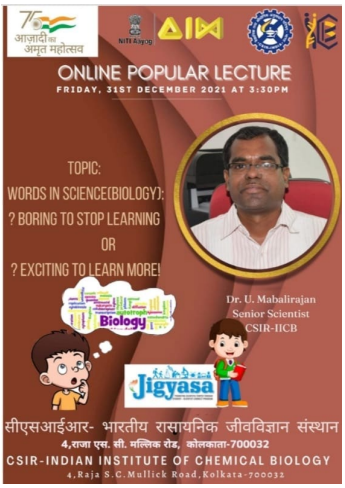
The section was further involved in various outreach activity throughout the year under **'JIGYASA'.** The Year started with staging of **Science Fun Show** on 7th April 2021 in collaboration with Birla Industrial & Technological Museum (BITM) to celebrate 65th CSIR-IICB Foundation Day.The program was on hybrid mode and attended by about 435 students.

We have organized **popular science talks in Bengali, Hindi and** English at different occasions **for Atal Tinker Labs**, where CSIR-IICB Scientists were actively involved through Virtual platform.

The First talk in the series was delivered by **Dr. Dipyaman Ganguly** in Bengali 'বিজ্ঞান পড়া ও বিজ্ঞান করা পেশা বনাম যাপন' which was aired on 22nd July 2021 and was attended by 335 students

Team CSIR-IICB participated in 24th National Exhibition held during 28th October -31st October 2021 on Science city **ground The CSIR-IICB Pavilion was attended by 300 students.**

In the series of events to mark आजादी का अमृत महोत्सव Puppet show in Hindi was staged by Kolkata dolls theature "कोरोना की कहानी बच्चों की जुबानी" which highlited CSIR-IICB contribution in fight against Corona and Testing facility. This was attended by 422 students in offline and online mode.



A webinar on MS team was done on 31st Dec 2021 where our Scientist **Dr. U. Mabalirajan** talked on '*Words in Science - Boring : To stop learning ,Exciting :To learn* more' attended by 107 ATL students.

CSIR-IICB Participated in **CSIR-NPL's Foundation Day & Platinum Jubilee Exhibition** held during 4th-6th January 2022 where CSIR-IICB Scientist **Mr. Sandeep Aggarwal** demonstrated activities related to Business Development Group and Science Popularization Section (JIGYASA). The event witnessed participation from various industries, MSMEs, faculty members and approximately 500 students visited CSIR-IICB counter

The next event was organized in Hindi on 10th January 2022 Vishwa Hindi Diwas jointly by Hindi Cell and CSIR-IICB -JIGYASA Team week on MS team where **Dr. Rakesh Sharma,** Hindi officer CSIR-NIO **talked about** "*कंप्यूटर में हिंदी में प्रयोग में आने वाली टूल्स एवं अप्प्स* "for school students and teacher The event was organized on MS team and there were 895 attendees**.**

CSIR-IICB participated in Jigyasa Vigyan Mahotsav 2022 organised by CSIR HQ during Feb 2022. Dr Dipyaman Ganguly contributed in this event as an online expert speaker.

The First offline outreach program was organized on 22nd February 2022 CSIR-IICB JIGYASA Team at Village Tetulia Sarrang on the special invitation for popular talk in Science to mark the Diamond Jubilee celebration of Tetulia Sarrang High School. Two Scientist from CSIR-IICB **Dr. Ranjan Jana and Dr. Subhajit Biswas** delivered lecture in Bangla "*বিজ্ঞানের জনপ্রিয় বক্তৃতা*". The program was attended by 300 Students and Faculty.

Group members of P&I Division

Administrative Support Divisions

Administrative Division

- The role of Administration is to provide support system to rum the Institute smoothly by ensuring adherence and compliance with Govt. of India /CSIR guidelines issued from time to time.
- Besides processing LTC, Leave, Advance, Salary, all the issues/queries received from CSIR-HQ/CSIR-RAB i.r.o administrative matters/Parliaments Questions/RTI, data for assessment promotion of scientists, etc. were replied with in time manner.
- All efforts are being taken to digitize the records of staff members in line with the CSIR directives.
- The guest House in the CSIR-IICB TRUE Campus is Fully operational now. All efforts to run this were taken by the Administration. Various in house meetings, CSIR HRDC/RAB led meetings were also organised in this campus.

Members of Director's Secretariat

Members of AO's Secretariat

Members of Establishment Section

Members of General Section

Members of Bill Section

Members of R&C Section

Members of Canteen

Security officer

Finance & Accounts Division

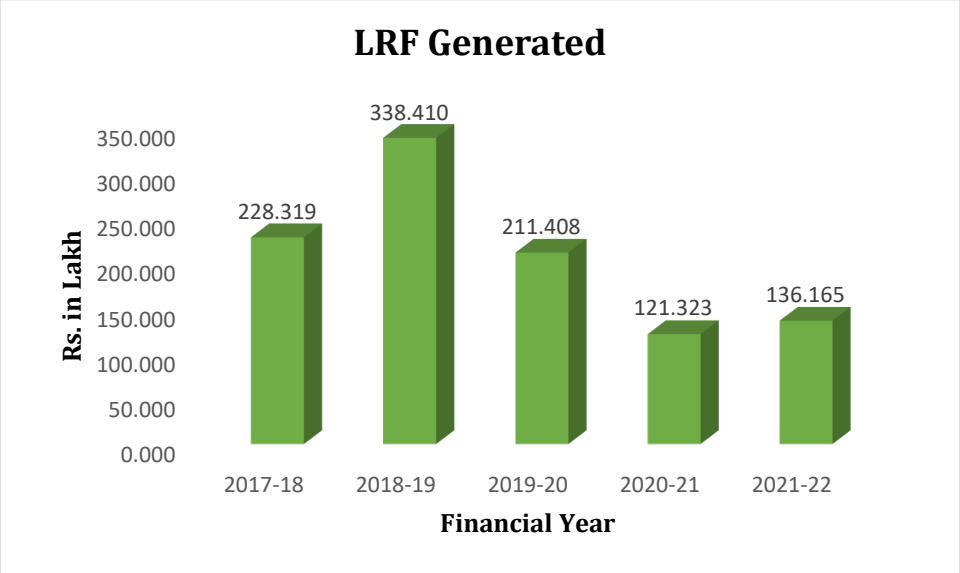
Core Area of Activity

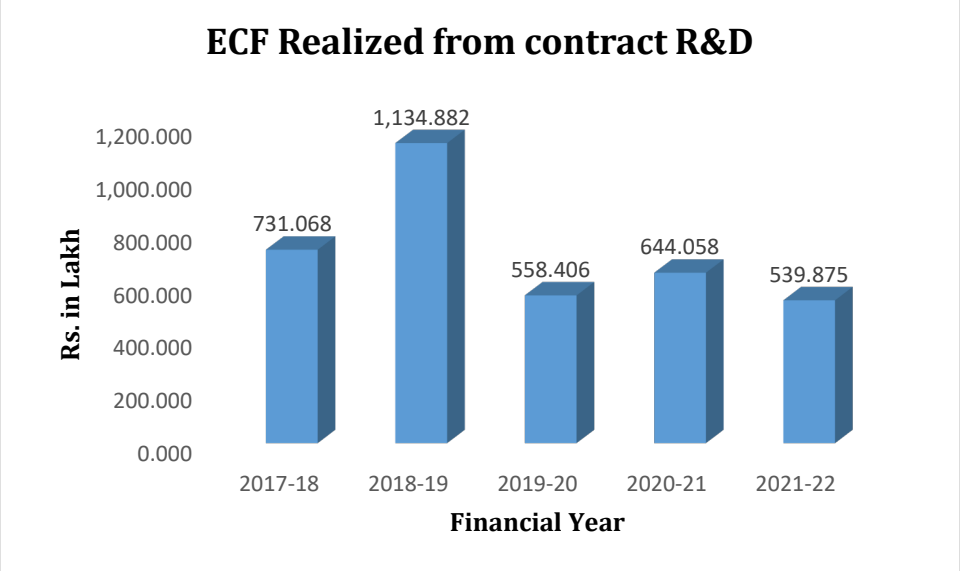
CSIR-IICB Finance & Accounts Division is the pivotal Management wing of the Organisation dealing with Financial Management and Accounting of the Funds. IICB being a constituent Laboratory of CSIR, is mainly run with Government Grants along with External Cash flows through Projects, Consultancy, Testing & Analysis and Technology Transfer fees, etc.

The Finance & Accounts Division ensures optimum utilisation of Public Money and Institutional funds as per the canons of financial propriety. Reflection of the Financial Transactions and dealings are transmitted Internally to CSIR Head Quarters through Monthly Reporting and Annual Accounts. External monitoring of the Institute's Financial aspects is also being handled by IICB Finance through coordination with the Government Audit Departments.

Overall Finance wing Advises and Assists the Director in Financial matters and envisioning the road map for sustainable and self-supporting Financial Infrastructure for IICB.

Group Members of Finance & Accounts Division


LRF Generated
Rs. in Lakh
350.000
300.000
250.000
200.000
150.000
100.000
50.000
0.000
228.319
338.410
211.408
121.323
136.165
2017-18
2018-19
2019-20
2020-21
2021-22
Financial Year


ECF Realized from contract R&D
Rs. in Lakh
1,200.000
1,000.000
800.000
600.000
400.000
200.000
0.000
731.068
1,134.882
558.406
644.058
539.875
2017-18
2018-19
2019-20
2020-21
2021-22
Financial Year

Store & Purchase Department

Mandate

Procurement of Scientific Equipment, Office Equipment, Chemicals, Glassware and other Consumables and also Receipt, Storage and Safe Custody of Material, Issue and Disposal of the same by following laid down CSIR Purchase Rules 2019 and in consonance with GFR 2017 provisions so as to support the R&D activities of the Institute.

Ensuring complete compliance with all laid down procedures, rules and instructions issued by CSIR/Government of India from time to time.

Basic activities

- Prioritization of equipment to be procured and to utilize the CSIR Funds received during the financial year
- Processing of Indents for procurement of items / equipment by floating of Limited Tender Enquiry, Open Tender etc. till the placement of Purchase Orders
- Procurement of indented items through Government e Market Place (GeM) & Central Public Procurement Portal (CPPP) of Govt. of India.
- Processing of Indents for AMC, Chemicals, Gases, Consumables, etc.
- Opening of Letter of Credits/ Wire transfers through bankers in r/o import purchase orders.
- Import of item/equipment after obtaining necessary approval of competent authority.
- Receipt of material, DRR Entries, Opening of the consignments, Preparation of SRV, Stock Entry & Issue of Material, PIR entries
- Return of serviceable/unserviceable material and Disposal of material.
- Reconciliation of Asset Abstract Register with Accounts.
- Disposal of obsolete, unserviceable and surplus items which are recommended for disposal.
- Replies to audit queries and preparation of audit replies.

(The above list of activities is only illustrative)

Group members of Store & Purchase Department

Hindi Activities

Official Language in the Institute is being implemented with regular meetings, everyday Hindi words and phrases displayed in the electronic boards, Hindi workshops, Hindi meetings, observance of Hindi week etc. The year 2021 saw many activities of the Official Language with organising of Hindi workshop every quarter (three months). Official Language meetings were held regularly with Director being the Chairman of all the OLIC meetings.

In this year, due to COVID-19 and social distancing etc., Hindi Pakhwara (14 to 29 September 2021) was celebrated. Only Hindi poetry recitation and Hindi essay writing competition were being arranged maintaining the Covid protocols of the office.

Hindi Pakhwada started on 14th September, a one-day Hindi workshop through MS Team was organized for the technical staff members of the Institute. The day began with the welcome address of Dr. Arun Bandyopadhyay, Director of the Institute. Dr. Swati R. Chadha, Hindi Officer, CSIR-NCL Pune conducted the workshop, which was very useful for the staff members where they were taught to use 'voice typing' to do official work in the official language in an easy manner. Overall the presentation was very interesting.
Considering social distancing under Hindi Pakhwada, Hindi essay writing competition was organized on 24th September, 2021 in which the employees and research scholars of the institute participated. The result was declared after checking the written Hindi essays by Shri L. K. Singh, Hindi Teacher, Hindi Teaching Scheme, Nizam Palace, Kolkata.

On 27th September, 2021, Hindi recitation competition was organized in which the staff and researchers of the institute participated. Shri Priyankar Paliwal, Senior Hindi Officer, CSIR-Central Glass and Ceramic Research Institute, Kolkata was the judge of this competition.

The closing ceremony of the Hindi Pakhwara Celebration 2021 was held on 29th September 2021 in the auditorium of the Institute. Dr. Shubhra Upadhyay, Head, Hindi Department, Khudiram Bose Central College, Kolkata, Chief Guest of the programme delivered the Hindi Pakhwara address. Dr. Arun Bandyopadhyay, Director, CSIR-IICB address the members of the Institute. The prize distribution program was held on the same day and the successful participants in the competition organized during Hindi Pakhwada were awarded by the chief guest Dr. Shubhra Upadhyay and Dr. Arun Bandyopadhyay, Director, CSIR-IICB. The program ended with a vote of thanks to all by Smt. Ambalika Nag, Hindi officer of the institute.

A one day quarterly Hindi workshop was organised on 20th December 2021 for Administrative staff members. The workshop was conducted by Mr. Priyankar Paliwal, Senior Hindi Officer, CSIR - Central Glass and Ceramic Research Institute, Jadavpur, Kolkata on **Official language policy of the Government of India and our responsibility**.

A quarterly Hindi workshop was held on 31st March 2022 for Scientific staff members. The speaker of the workshop was Mr. Anoop Kumar, Assistant Director, Hindi Teaching Scheme, Ministry of Home Affairs, Kolkata on **Unicode & Google Voice Typing**. It was an informative and interesting session.

Hindi officer

हिंदी दिवस
पर सबको हार्दिक शुभकामनाएं
दिनांक : 14 सितंबर, 2021
सीएसआईआर - भारतीय रासायनिक जीवविज्ञान संस्थान
4, राजा एस. सी. मल्लिक रोड, यादवपुर, कोलकाता – 700 032

हिंदी कार्यशाला
14 सितंबर, 2021

हिन्दी कविता पाठ
प्रतियोगिता
27 सितंबर, 2021

हिंदी पखवाड़ा
समापन समारोह
29 सितंबर, 2021
भारतीय रासायनिक जीवविज्ञान संस्थान

29 सितंबर, 2021

हिन्दी कार्यशाला
20 दिसंबर, 2021

हिंदी कार्यशाला

Skill Development Programme

Skill Development Programme

On 24th Sept 2016 honorable Prime Minister inaugurated "CSIR Platinum Jubilee" celebration. On this occasion "CSIR Integrated Skill Initiative" programme was launched on PAN India basis in CSIR laboratories. The aim is to train people in different skill areas, to generate quality human resource at various levels by providing and upgrading skills in all the possible fields.

CSIR-IICB is contributing in this programe with a view to accelerate the aptitude towards research in students and to provide extensive practical exposure in latest technologies which are linked to industry/academia requirement thus would invariably contribute to subsequent employment generation. Institute had offered 26 certificate courses under CSIR-IICB Skill Development Programme during 2021-2022. These courses had provided an opportunity to our youth for hands-on-training, scientific knowledge, analytical perspective & technical skill for understanding the latest technologies used in a research field. The trainees had received 2-weeks exposure in different skilling programs in various advanced areas of IICB expertise through lectures and practical sessions.

During 2021-22, CSIR-IICB had conducted its various skilling courses under the CSIR-Integrated Skill Initiative. Two sessions of Skill Development Program were conducted during this period in which-

- 14 skill courses were run parallel in the Session-I (held during September-October 2021)
- 12 skill courses were run parallel in the session-II (held during March 2022)

The Courses conducted and Number of Candidates trained during the 2021-22 are tabulated below:

Course	Session-I	Session-II	Grand Total
Cryo EM:Sample optimization and 3D structure reconstruction	06	03	09
X-ray Crystallography(small molecules)	06	-	06
High end equipments for clinical applications -Flow cytometry	10	09	19
Liquid Chromatography-Mass Spectrometry	05	03	08
Clinical Biochemistry,Microbiology and Pathology techniques for biomedical applications	07	06	13
Nuclear Magnetic Resonance Spectroscopy	03	03	06
High end equipments for clinical applications-Optical Microscopy	07	06	13
Real time PCR (Duration:1 week)	05	02	07
Protein X-ray crystallography	08	02	10
Gas Chromatography Mass Spectrometry	02	-	02
Molecular Cloning,Protein expression and structural characterization	09	06	15
Separation Techniques for organic molecules	05	05	10
Advanced Bioinformatics	08	06	14
High Performance Liquid Chromatography	02	03	05
Total	**83**	**54**	**137**

Glimpses of Practical sessions during CSIR-IICB Skill Development Programme

Glimpses of Valedictory Sessions

Visits of Dignitaries and Other Events

हिंदी कार्यशाला, 31 मार्च, 2022

CSIR-IICB and MONOSHA, a serpentarium venom bank and research centre, signed a Memorandum of Understanding on March 24, 2022

Dr Didier Raboisson, Attaché for Scientific Cooperation at the Embassy of France in India, Delhi visited CSIR-IICB on March 22, 2022

Proteomics Day Symposium on 'Proteomics for Future Diagnostics' was organized at CSIR-IICB on March 21, 2022

Dr. Pradip Maity, Senior Scientist, CSIR-NCL delivered an invited lecture on 'Organophosphites as Nucleophile Catalysis for Asymmetric Rearrangements and Radical Reactions' on March 11, 2022.

Celebration of International Women's Day 2022 on March 08, 2022.

National Science Day celebration on February 28, 2022. Prof. Krishanu Ray, Chairperson, Department of Biological Science, Tata Institute of Fundamental Research, Mumbai delivered a lecture on 'Going to places inside a cell riding a motor protein'.

An outreach program at Tetulia Sarran N.N. High School on February 22, 2022.

PopularTalk on 'Corona' in Bengali by Dr Subhajit Biswas on International mother language day on February 21, 2022

MoU signed with St. Jude Children Research Hospital, Memphis, TN, USA on February 18, 2022 at CSIR-HQ in the presence of Dr. Shekhar C. Mande, DGCIR and Secretary, DSIR, Govt. of India

विश्व हिंदी दिवस के अवसर पर 10 जनवरी को संस्थान के तरफ से हिंदी संगोष्ठी का आयोजन किया गया।

National Seminar on "Ethnopharmacology for wellness : Tradition to Translation" on December 10, 2021 in association with Society for Ethnopharmacology, (SFE-India)

IISF Curtain Raiser Event on November 26, 2021. Dr Asima Mukhopadhyay Consultant Gynaecological Oncologist and Clinician Scientist, Chittaranjan National Cancer Institute, Kolkata delivered popular lecture on Women's Cancer Care in India.

CSIR Foundation Day Celebration on September 26, 2021

हिंदी पखवाड़ा, 14 सितंबर से 29 सितंबर 2021.

Performance Parameter

Awards/Honours

1	Dr. Suvendra Bhattacharyya received the Sun Pharma Prize in Medical Sciences (Basic Research) in the year 2021-2022 given by the Sun Pharma Science Foundation.
2	Acharya Jagadish Chandra Bose Memorial Award 2022 has been conferred on Dr. Arun Bandyopadhyay, Director, CSIR-IICB for his outstanding contribution on promotion and popularization of science & technology in society by The Science Association of Bengal in collaboration with Vijaygarh Jyotish Ray College on March 02, 2022 at The Ramakrishna Mission Institute of Culture, Golpark, Kolkata.
3	Dr. Suvendra Bhattacharyya was featured in the 75 UNDER 50 SCIENTISTS SHAPING TODAY'S INDIA.
4	Dr. Jayati Sengupta bagged the SERB Power fellowship.
5	Dr. Upasana Ray was selected in the final list of 75 women to be honoured in the book, 'She is: 75 Women in Steam'. The book is a part of the celebration of 75th anniversary of independence. The partners are PSA Office, Red Dot Foundation and Beyond Black.
6	Dr. Amitava Sengupta received the ICMR-National Institute of Immunohaematology (NIIH, Mumbai) Diamond Jubilee Oration Award
7	Dr. Amitava Sengupta delivered the Bengal Society of Haematology, Prof. Sandhya Ghose Memorial Oration
8	Dr. Amitava Sengupta received the Keystone Symposia-Hematopoiesis Scholarship & delivered a Invited Talk
9	Dr. Santanu Adhikary, students of Dr. Siddhartha Roy received NASI Platinum Jubilee Young Scientist Award, in Biomedical Sciences
10	Dipika Sarkar, PhD student of Dr. Arindam Talukdar received 'Best Poster Award-ICBS 2021' At the 'International Chemical Biology Society (ICBS)' Annual Conference: held at Emory University, USA in November 2021.
11	Pallabi Bhattacharyya, student of Dr. Subhas C Biswas awarded the First Prize for an oral presentation under the 'Young Talent Forum' at the NeuroUpdate conference, November 28 2021.
12	Pallabi Bhattacharyya, student of Dr. Subhas C Biswas received EMBL Corporate Partnership Programme Registration Fee Waiver for the virtual EMBL Course: Genome Engineering: CRISPR/Cas from Cells to Mice, 27 September - 4 October 2021.
13	Sukanya Sarkar, Senior Research Fellow (DST-INSPIRE) of Dr. Subhas C Biswas received Best Poster Presentation at the IBRO-APRC Georgian Associate School of Neuroscience held virtually, 21-25 August in Tbilisi, 2021, Georgia.
14	Sukanya Sarkar, Senior Research Fellow (DST-INSPIRE) of Dr. Subhas C Biswas received Travel fellowship by International Brain Research Organization for attending Alzheimer's Association International Conference (AAIC), 26th-30th July 2021, Denver, USA.
15	Sukanya Sarkar, Senior Research Fellow (DST-INSPIRE) of Dr. Subhas C Biswas received the first prize in Oral Presentation in IBRO-APRC Associate School of Neuroscience "Biophysical to Molecular Techniques: An interface in Neurobiology Research" (August 23-27, 2021) by Indira Gandhi National Tribal University Amarkantak (MP), India
16	Sayantani Sinha, former PhD student of Amitava Sengupta received SASTRA-Prof. Saroj Chandrasekhar Memorial Award, NJ

Journal Publications in the year 2021

Sl No.	Paper
1	Das, M; Mukherjee, S; Brandao, P; Seth, SK; Giri, S; Mati, SS; Samanta, BC; Laha, S; Maity, T, Active Bromoaniline-Aldehyde Conjugate Systems and Their Complexes as Versatile Sensors of Multiple Cations with Logic Formulation and Efficient DNA/HSA-Binding Efficacy: Combined Experimental and Theoretical Approach, ACS OMEGA, 2021, 6(5), pp. 3659-3674
2	Biswas, N; Kumar, K; Mallick, P; Das, S; Kamal, IM; Bose, S; Choudhury, A; Chakrabarti, S, Structural and Drug Screening Analysis of the Non-structural Proteins of Severe Acute Respiratory Syndrome Coronavirus 2 Virus Extracted From Indian Coronavirus Disease 2019 Patients, FRONTIERS IN GENETICS, 2021, 12, article no. 626642
3	Bera, S; Mondal, P; Sarkar, D; Pathi, VB; Pakrashy, S; Datta, A; Banerji, B, Synthetic and Computational Studies on Rh-III-Catalyzed Redox-Neutral Cascade of Carbenoid Functionalization and Dephosphonylative Annulation, JOURNAL OF ORGANIC CHEMISTRY, 2021, 86(10), pp. 7069-7077
4	Biswas, N; Mallick, P; Maity, SK; Bhowmik, D; Mitra, AG; Saha, S; Roy, A; Chakrabarti, P; Paul, S; Chakrabarti, S, Genomic Surveillance and Phylodynamic Analyses Reveal the Emergence of Novel Mutations and Co-mutation Patterns Within SARS-CoV-2 Variants Prevalent in India, FRONTIERS IN MICROBIOLOGY, 2021, 12, article no. 703933
5	Yadav, VK; Kumar, A; Tripathi, PP; Gupta, J, Long noncoding RNAs in intestinal homeostasis, regeneration, and cancer, JOURNAL OF CELLULAR PHYSIOLOGY, 2021, 236(11), pp. 7801-7813
6	Ramalingam, B; Venkatachalam, SS; Kiran, MS; Das, SK, Rationally designed Shewanella oneidensis Biofilm Toilored Graphene-Magnetite Hybrid Nanobiocomposite as Reusable Living Functional Nanomaterial for Effective Removal of Trivalent Chromium, ENVIRONMENTAL POLLUTION, 2021, 278, article no. 116847
7	Parandhaman, T; Choudhary, P; Ramalingam, B; Schmidt, M; Janardhanam, S; Das, SK, Antibacterial and Antibiofouling Activities of Antimicrobial Peptide-Functionalized Graphene-Silver Nanocomposites for the Inhibition and Disruption of Staphylococcus aureus Biofilms, ACS BIOMATERIALS SCIENCE & ENGINEERING, 2021, 7(12), pp. 5899-5917
8	Gupta, AK; Roy, S; Das, PK, Antileishmanial effect of the natural immunomodulator genipin through suppression of host negative regulatory protein UCP2, JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY, 2021, 76(1), pp. 135-145
9	Das, A; Dutta, S, Binding Studies of Aloe-Active Compounds with G-Quadruplex Sequences, ACS OMEGA, 2021, 6(28), pp. 18344-18351
10	Rahaman, O; Ganguly, D, Endocannabinoids in immune regulation and immunopathologies, IMMUNOLOGY, 2021, 164(2), pp. 242-252 article no.
11	Ghosh, S; Mukherjee, K; Chakrabarty, Y; Chatterjee, S; Ghoshal, B; Bhattacharyya, SN, GW182 Proteins Restrict Extracellular Vesicle-Mediated Export of MicroRNAs in Mammalian Cancer Cells, MOLECULAR AND CELLULAR BIOLOGY, 2021, 41(5), article no. e00483-20
12	Begum, Y; Pandit, A; Swarnakar, S, Insights Into the Regulation of Gynecological Inflammation-Mediated Malignancy by Metalloproteinases, FRONTIERS IN CELL AND DEVELOPMENTAL BIOLOGY, 2021, 9, article no. 780510
13	Das, S; Mukherjee, S; Ali, N, Super enhancer-mediated transcription of miR146a-5p drives M2 polarization during Leishmania donovani infection, PLOS PATHOGENS, 2021, 17(2), article no. e1009343
14	Chakravarti, R; Singh, R; Ghosh, A; Dey, D; Sharma, P; Velayutham, R; Roy, S; Ghosh, D, A review on potential of natural products in the management of COVID-19, RSC ADVANCES, 2021, 11(27), pp. 16711-16735
15	Sarif, J; Raychaudhuri, D; D'Rozario, R; Bandopadhyay, P; Singh, P; Mehta, P; Hoque, MA; Sinha, BP; Kushwaha, M; Sahni, S; Devi, P; Chattopadhyay, P; Paul, SR; Ray, Y; Chaudhuri, K; Banerjee, S; Majumdar, D; Saha, B; Sarkar, BS; Bhattacharya, P; Chatterjee, S; Paul, S; Ghosh, P; Pandey, R; Sengupta, S; Ganguly, D, Plasma Gradient of Soluble Urokinase-Type Plasminogen Activator

	Receptor Is Linked to Pathogenic Plasma Proteome and Immune Transcriptome and Stratifies Outcomes in Severe COVID-19, FRONTIERS IN IMMUNOLOGY, 2021, 12, article no. 738093
16	Kumari, N; Karmakar, A; Khan, MMA; Ganesan, SK, The potential role of m6A RNA methylation in diabetic retinopathy, EXPERIMENTAL EYE RESEARCH, 2021, 208, article no. 108616
17	Das, P; Mukherjee, A; Adak, S, Glyceraldehyde-3-phosphate dehydrogenase present in extracellular vesicles from Leishmania major suppresses host, JOURNAL OF BIOLOGICAL CHEMISTRY, 2021, 297(4), article no. 101198
18	Das, D; Paul, A; Lahiri, A; Adak, M; Maity, SK; Sarkar, A; Paul, S; Chakrabarti, P, Proteasome dysfunction under compromised redox metabolism dictates liver injury in NASH through ASK1/PPAR gamma binodal complementary modules, REDOX BIOLOGY, 2021, 45, article no. 102043
19	De, K, Decapeptide Modified Doxorubicin Loaded Solid Lipid Nanoparticles as Targeted Drug Delivery System against Prostate Cancer, LANGMUIR, 2021, 37(45), pp. 13194-13207
20	Mukherjee, D; Chander, V; Bandyopadhyay, A, PARIS-DJ-1 Interaction Regulates Mitochondrial Functions in Cardiomyocytes, Which Is Critically Important in Cardiac Hypertrophy, MOLECULAR AND CELLULAR BIOLOGY, 2021, 41(1), article no. e00106-20
21	Mondal, D; Pal, G; Chowdhury, C, Palladium(0)-catalysed regioselective cyclisations of 2-amino(tosyl) benzamides/sulphonamides: the stereoselective synthesis of 3-ylidene-[1,4]benzodiazepin-5-ones/benzo[f][1,2,5]thiadiazepine-1,1-dioxides, CHEMICAL COMMUNICATIONS, 2021, 57(44), pp. 5462-5465
22	Das, S; Chakrabarti, S, Classification and prediction of protein-protein interaction interface using machine learning algorithm, SCIENTIFIC REPORTS, 2021, 11(1), article no. 1761
23	Karmakar, J; Mandal, C, Interplay Between Sialic Acids, Siglec-E, and Neu1 Regulates MyD88- and TRIF-Dependent Pathways for TLR4-Activation During Leishmania donovani Infection, FRONTIERS IN IMMUNOLOGY, 2021, 12, article no. 626110
24	Jati, S; Sengupta, S; Sen, M, Wnt5A-Mediated Actin Organization Regulates Host Response to Bacterial Pathogens and Non-Pathogens, FRONTIERS IN IMMUNOLOGY, 2021, 11, article no. 628191
25	Sarkar, W; Mishra, A; Bhowmik, A; Deb, I, Copper-Catalyzed Direct sp(2) C-H Silylation of Arylamides Using Disilanes, ORGANIC LETTERS, 2021, 23(12), pp. 4521-4526
26	Sengupta, A; Padhan, DK; Ganguly, A; Sen, M, Ccn6 Is Required for Mitochondrial Integrity and Skeletal Muscle Function in Zebrafish, FRONTIERS IN CELL AND DEVELOPMENTAL BIOLOGY, 2021, 9, article no. 627409
27	De, DP; Mukherjee, I; Guha, S; Paidi, RK; Chakrabarti, S; Biswas, SC; Bhattacharyya, SN, Rheb-mTOR activation rescues A beta-induced cognitive impairment and memory function by restoring miR-146 activity in glial cells, MOLECULAR THERAPY-NUCLEIC ACIDS, 2021, 24(), pp. 868-887
28	Bhattacharjee, S; Bhattacharyya, R; Sengupta, J, Dynamics and electrostatics define an allosteric druggable site within the receptor-binding domain of SARS-CoV-2 spike protein, FEBS LETTERS, 2021, 595(4), pp. 442-451
29	Talukdar, A; Ganguly, D; Roy, S; Das, N; Sarkar, D, Structural Evolution and Translational Potential for Agonists and Antagonists of Endosomal Toll-like Receptors, JOURNAL OF MEDICINAL CHEMISTRY, 2021, 64(12), pp. 8010-8041
30	Dahat, Y; Saha, P; Mathew, JT; Chaudhary, SK; Srivastava, AK; Kumar, D, Traditional uses, phytochemistry and pharmacological attributes of Pterocarpus santalinus and future directions: A review, JOURNAL OF ETHNOPHARMACOLOGY, 2021, 276, article no. 114127
31	Mukherjee, B; Mukherjee, K; Nanda, P; Mukhopadhayay, R; Ravichandiran, V; Bhattacharyya, SN; Roy, S, Probing the molecular mechanism of aggressive infection by antimony resistant Leishmania donovani, CYTOKINE, 2021, 145(), pp. - article no. 155245
32	Karmakar, J; Mukherjee, K; Mandal, C, Siglecs Modulate Activities of Immune Cells Through Positive and Negative Regulation of ROS Generation, FRONTIERS IN IMMUNOLOGY, 2021, 12, article no. 758588
33	Mondal, A; Chowdhury, C, Palladium-Catalyzed Synthesis of 1-Vinyltetrahydro-beta-carbolines and Aza-spiroindolenines: Access to the Syntheses of 1-Vinyl-beta-carbolines and Eudistomins Y1 and Y2, JOURNAL OF ORGANIC CHEMISTRY, 2021, 86(5), pp. 3810-3825

34	Kundu, B; Raychaudhuri, D; Mukherjee, A; Sinha, BP; Sarkar, D; Bandopadhyay, P; Pal, S; Das, N; Dey, D; Ramarao, K; Nagireddy, K; Ganguly, D; Talukdar, A, Systematic Optimization of Potent and Orally Bioavailable Purine Scaffold as a Dual Inhibitor of Toll-Like Receptors 7 and 9, JOURNAL OF MEDICINAL CHEMISTRY, 2021, 64(13), pp. 9279-9301
35	Basak, T; Dey, AK; Banerjee, R; Paul, S; Maiti, TK; Ain, R, Sequestration of eIF4A by Angiomotin: A novel mechanism to restrict global protein synthesis in trophoblast cells, STEM CELLS, 2021, 39(2), pp. 210-226
36	Samanta, K; Maji, S; Samanta, J; Natarajan, R, Crystal Engineering of a Tetraphenol with Bispyridines toward Hydrogen-Bonded Emissive Cocrystals, CRYSTAL GROWTH & DESIGN, 2021, 21(11), pp. 6485-6496
37	Kumar, K; Chakraborty, A; Chakrabarti, S, PresRAT: a server for identification of bacterial small-RNA sequences and their targets with probable binding region, RNA BIOLOGY, 2021, 18(8), pp. 1152-1159
38	Ejazi, SA; Choudhury, ST; Bhattacharyya, A; Kamran, M; Pandey, K; Das, VNR; Das, P; da Silva, FO; Costa, DL; Costa, CHN; Rahaman, M; Goswami, RP; Ali, N, Development and Clinical Evaluation of Serum and Urine-Based Lateral Flow Tests for Diagnosis of Human Visceral Leishmaniasis, MICROORGANISMS, 2021, 9(7), article no. 1369
39	Mandal, AK, Dendrimers in targeted drug delivery applications: a review of diseases and cancer, INTERNATIONAL JOURNAL OF POLYMERIC MATERIALS AND POLYMERIC BIOMATERIALS, 2021, 70(4), pp. 287-297
40	Srinath, R; Manna, A; Shee, S; Pathi, VB; Ghosh, S; Khamaru, K; Maiti, NC; Banerji, B, Synthesis of N-Fused Triazole-Piperazine-Quinazolinones via One-Pot Tandem Click Reaction and Cross-Dehydrogenative Coupling, ORGANIC LETTERS, 2021, 23(24), pp. 9365-9370
41	Sarraf, NR; Mukhopadhyay, S; Banerjee, A; Das, AK; Roy, S; Chakrabarti, S; Manna, M; Saha, P, Genome wide comparison of Leishmania donovani strains from Indian visceral leishmaniasis and para-kala-azar dermal leishmaniasis patients, ACTA TROPICA, 2021, 223, article no. 106086
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- Novel Process for Manufacturing TROPISETRON
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