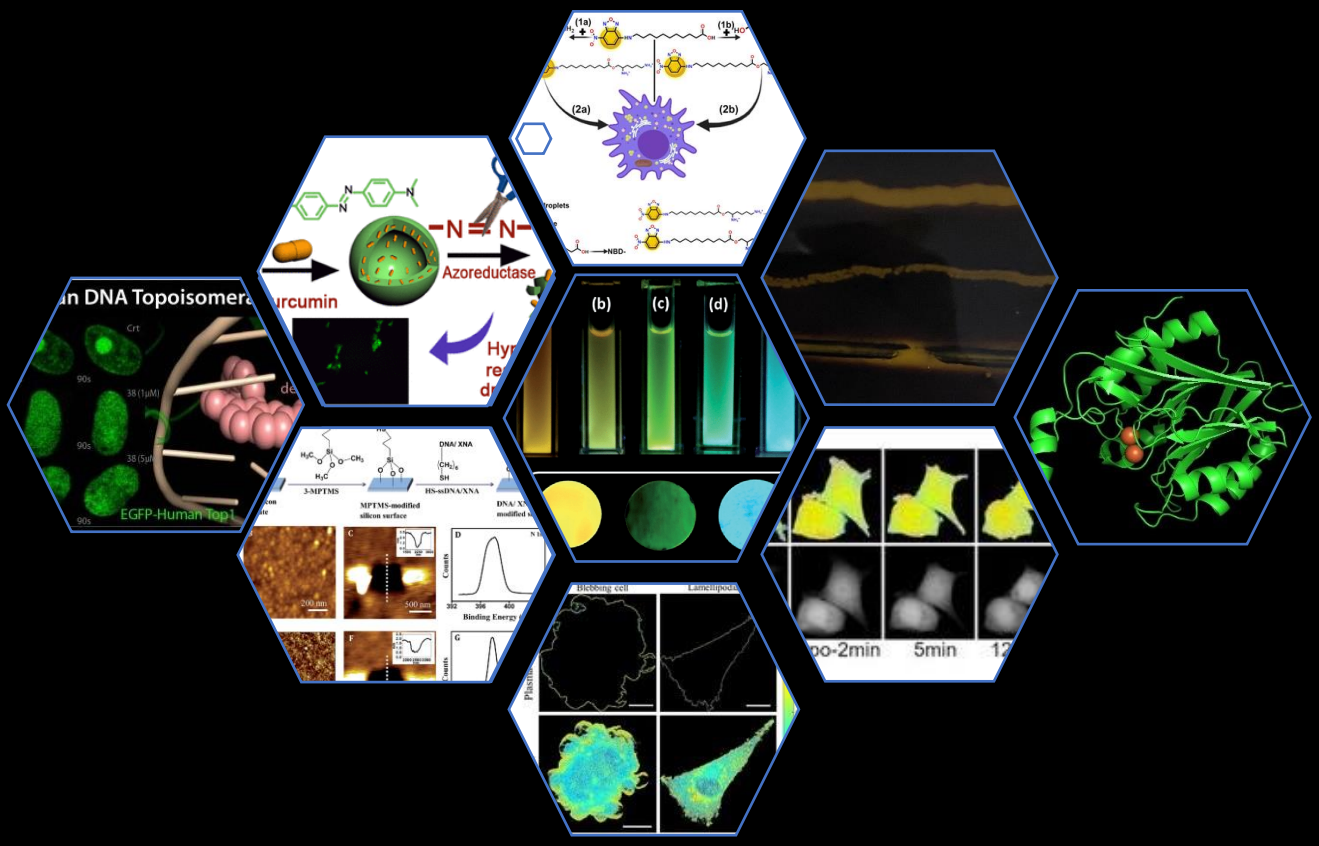
ANNUAL MEETING OF
SCHOOL OF BIOLOGICAL SCIENCES
24th February, 2023



Patron:

Prof. Tapas Chakraborty*, *Director, IACS

Organising Committee:

Prof. Prasanta K Das, Chair-SBS

Prof. Arindam Banerjee

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Prof. Somdatta Ghosh Dey

Prof. Benu Brata Das

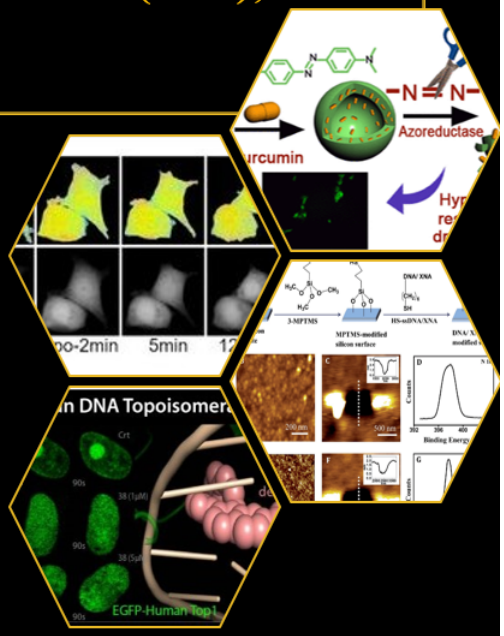
Prof. Deepak K Sinha

Dr. Ritesh R Pal

Annual Meeting of School of Biological Sciences (SBS), IACS

24th February, 2023

CV Raman Hall



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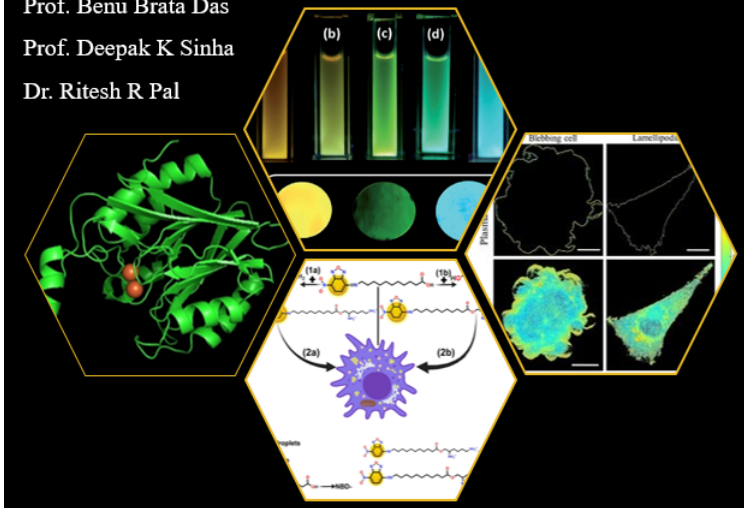
Invited Speakers:

Prof. Amitabha Chattopadhyay, CCMB, Hyderabad- **"Cholesterol and GPCR Signaling: A Molecular Sensor for Cholesterol in the Serotonin$_{1A}$ Receptor"** (Keynote lecture)

Prof. Arun K. Shukla, IIT-Kanpur- *"Structure, function and modulation of* **G Protein-Coupled Receptors"** (Plenary Lecture)

Prof. Deepak Kumar Sinha, IACS, Kolkata- **"Spectral imaging reveals that the composition of the LDs in zebrafish embryos are governed by its size and metabolism"**

Prof. Siddhartha S Jana, IACS, Kolkata- **"Can non-canonical miRNAs regulate gene expression?"**



Student Speakers:

Ditipriya Mallick- "A weak 5'-splice site causes exon skipping of a novel MYH9 isoform, NMII A2"

Subhojit Paul- "Coagulation factor FVIIa enhances PD-L1expression and its stability in breast cancer cells to promote breast cancer immune evasion"

Srijita Paul Chowdhuri- "CDK1 mediated phosphorylation of TDP1 promotes genomic stability by preventing TDP1 trapping on mitotic chromosomes"

Suparno Banerjee- "CpdB – the predicted regulator of nanotube-flagella switch in Pathogenic Escherichia coli"

Sourav Mondal- "Structural consequences of HMGB1 binding to platinum anticancer drug-treated DNA at single molecule level"

Biswanath Hansda- "Amino acid containing amphiphilic hydrogelators with antibacterial and antiparasitic activities"

Monalisa Chowdhury- "Alternative Therapy: An emerging pathway for treating cancer"

Subhamoy Jana- "Study of TNFR1 Cluster Compaction in live cells using Homo-FRET"

Contents

Director's Desk

It gives me immense pleasure to note that the School of Biological Sciences will be organizing its Annual Scientific meeting this year on February 24, 2023. The annual inhouse meeting is a worthy platform for the Ph.D. students, not only to enrich and broaden their knowledge in complementary fields, but also to get reflections of the experts on their own research and knowledge. I sincerely wish success for the endeavour of the school.

Tapas Chakraborty
Director (Additional Charge)

Program Schedule

Welcome Note: Prof. Prasanta K Das, Chair-SBS; 10:00-10:05		
Opening Remark: Prof. Tapas Chakraborty, Director, IACS; 10:05-10:15		
Session 1: Chair: Prof. Arindam Banerjee		
Speaker	**Title**	**Time**
Prof. Amitabha Chattopadhyay **CCMB, Hyderabad** (Keynote Lecture)	Cholesterol and GPCR Signaling: A Molecular Sensor for Cholesterol in the Serotonin$_{1A}$ Receptor	10:15-11:00
Prof. Deepak Kumar Sinha **IACS, Kolkata** (Invited Lecture)	Spectral imaging reveals that the composition of the LDs in zebrafish embryos are governed by its size and metabolism	11:00-11:30
Tea Break: 11:30-12:00		
Session 2: Chair: Prof. Benu Brata Das		
Ditipriya Mallick	A weak 5'-splice site causes exon skipping of a novel MYH9 isoform, NMII A2	12:00-12:15
Subhojit Paul	Coagulation factor FVIIa enhances PD-L1expression and its stability in breast cancer cells to promote breast cancer immune evasion	12:15-12:30
Srijita Paul Chowdhuri	CDK1 mediated phosphorylation of TDP1 promotes genomic stability by preventing TDP1 trapping on mitotic chromosomes	12:30-12:45
Suparno Banerjee	CpdB – the predicted regulator of nanotube-flagella switch in Pathogenic *Escherichia coli*	12:45- 13:00
Lunch: 13:00-14:30		
Session-3: Chair: Prof. Rupa Mukhopadhyay		
In Memory of Prof. Manju Ray		
Prof. Arun K. Shukla **IIT-Kanpur** (Plenary Lecture)	Structure, function and modulation of G Protein-Coupled Receptors	14:30-15:15
Prof. Siddhartha S Jana **IACS, Kolkata** (Invited Lecture)	Can non-canonical miRNAs regulate gene expression?	15:15-15:45
Dr. Alok Ghosh (CU) **Dr. Aparajita Pal (DHWU)**	Commemoration of Prof. Manju Ray by her doctoral students	15:45-15:55
Tea Break: 15:55-16:15		
Session 4: Chair: Prof. Pradyut Ghosh		
Sourav Mondal	Structural consequences of HMGB1 binding to platinum anticancer drug-treated DNA at single molecule level	16:15-16:30
Biswanath Hansda	Amino acid containing amphiphilic hydrogelators with antibacterial and antiparasitic activities	16:30-16:45
Monalisa Chowdhury	Alternative Therapy: An emerging pathway for treating cancer	16:45-17:00
Subhamoy Jana	Study of TNFR1 Cluster Compaction in live cells using Homo-FRET	17:00-17:15
Vote of Thanks: Dr. Ritesh Ranjan Pal: 17:15		

Cholesterol and GPCR Signaling: A Molecular Sensor for Cholesterol in the Serotonin$_{1A}$ Receptor

Amitabha Chattopadhyay

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G protein-coupled receptors (GPCRs) are the largest class of molecules involved in signal transduction across membranes, and represent major drug targets in all clinical areas. The serotonin$_{1A}$ receptor is an important neurotransmitter receptor of the GPCR superfamily and is implicated in the generation and modulation of various cognitive, behavioural and developmental functions. In our earlier work, we demonstrated that membrane cholesterol is necessary for ligand binding, G-protein coupling and signalling of serotonin$_{1A}$ receptors. In the overall context of high-resolution structures of GPCRs showing bound cholesterol molecules, we previously reported the presence of cholesterol recognition/interaction amino acid consensus (CRAC) motifs in the serotonin$_{1A}$ receptor. In our recent work, we explored the molecular basis of cholesterol sensitivity exhibited by the serotonin$_{1A}$ receptor by generating mutants of key residues in CRAC motifs in transmembrane helices (TM) 2 and 5 of the receptor. Our results show that a lysine residue (K101) in one of the CRAC motifs is crucial for sensing altered membrane cholesterol levels. These observations are further supported from all-atom molecular dynamics simulations which reveal a tightly bound cholesterol molecule between TM1 and TM2 by establishing polar contacts with K101 that leads to stabilization of extracellular loop 1 (ECL1). Interestingly, the position of this cholesterol molecule is almost identical to a co-crystallized cholesterol molecule in the recently reported high-resolution cryo-EM structure of the serotonin$_{1A}$ receptor, thereby strongly validating the molecular mechanism for cholesterol sensitivity of the serotonin$_{1A}$ receptor proposed by us. These results constitute one of the first reports comprehensively demonstrating that cholesterol sensitivity could be knocked out by a single point mutation in a specific cholesterol binding site. We envision that progress in deciphering molecular details of the nature of GPCR-cholesterol interaction would lead to better insight into our overall understanding of GPCR function in health and disease.

Structure, function and modulation of G Protein-Coupled Receptors

Arun K. Shukla

Joy-Gill Chair professor
Wellcome Trust-DBT Senior Fellow & EMBO Young Investigator
Department of Biological Sciences and Bioengineering
Indian Institute of Technology, Kanpur

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

Spectral imaging reveals that the composition of the LDs in zebrafish embryos are governed by its size and metabolism

Deepak Kumar Sinha

School of Biological Sciences, IACS, Kolkata

The embryonic body (blastodisc) of zebrafish develops separately from the yolk. The blastodisc stores the lipids as numerous lipid droplets (LDs), whose sizes range two orders of magnitude (0.5). Despite its importance in development, there are fewer studies of lipid metabolism in live biological systems due to a lack of suitable techniques. We utilize the solvatochromism of NR to investigate the lipid metabolism of LDs in live zebrafish embryos. LD's polarity (an indicator of lipidomic composition) decreases during lipolysis and increases during lipogenesis. By measuring the rate of polarity change in the LDs, we validate that the smaller LDs are more efficient in enforcing lipolysis. The biogenesis of LDs in zebrafish embryos depends on the yolk's metabolic activity and occurs through the fission of yolk material near the yolk-blastodisc interface. The yolk interface's local polarity controls the nascent LDs' size and composition. We find a similar correlation between the polarity and the size of the oil droplets synthesized in vitro, suggesting polarity's role in determining the LDs' size. In zebrafish blastodisc, LDs closer to the yolk-blastodisc interface exhibit higher metabolic activity than LDs away, suggesting the uneven metabolic potential of LDs within the cytoplasm. The lipid biochemistry in zebrafish is remarkably similar to that in humans. Therefore, the methodology used in this manuscript is potentially relevant for developing a zebrafish-based model system to investigate human lipid metabolism-related diseases.

Can non-canonical miRNAs regulate gene expression?

Kumarjeet Banerjee, Shekhar Saha, Shaoli Das, Suman Ghosal, Indranil Ghosh and Siddhartha S Jana*

School of Biological Sciences, IACS, Kolkata

Nonmuscle myosin IIC (NM IIC), one of the paralogs of NM II, plays important role in many cellular processes such as cytokinesis, adhesion, migration and mechano-sensitivity. Unlike NM IIA and IIB, NM IIC is relatively less abundant in majority of tissues and cell lines. Whereas expression of NM IIC is inducible in variety of cell lines, its mechanism is yet to be explored. Here, we report a group of micro-RNAs (mmu-miR 200a-5p, mmu-miR 532-3p, mmu-miR 680 and mmu-miR 1901) that can regulate the expression of NM IIC both in-silico and in vitro. All these micro-RNAs have both canonical and non-canonical binding site at 3′UTR and coding region of NMHC IIC mRNA; and each miRNA is able to downregulate NM IIC but at different degree as assessed by dual-luciferase assay and immunoblot analyses. Changing the NM IIC expression by specific mimic miRNAs and anti miRNA oligos in 4T1 cells shows defects in cell migration and growth. This study demonstrates a probable mechanism of the regulation of a key mechanoresponsive element, NM IIC by endogenous miRNAs which have non-canonical binding sites.

A weak 5'-splice site causes exon skipping of a novel MYH9 isoform, NMII A2

Ditipriya Mallick

School of Biological Sciences, IACS, Kolkata

The Non-muscle myosin II paralog, NM IIA, encoded by MYH9 gene, is widely expressed across various tissues. A novel neuron-specific isoform of NM IIA, which contains a 63-nucleotide inserted sequence near the actin-binding region, has recently been reported in brain tissues. This isoform is known as NM IIA2. The tissue-specific alternative splicing of mouse MYH9 pre-mRNA results in exon skipping of this sequence in non-neuronal cells, thus restricting NM IIA2 expression to brain and neuronal tissues. In this study, the 63-nucleotide sequence found in neuronal MYH9 cDNA is determined to be a cassette type-exon, A2, between constitutive exons 15 and 16. We used the minigene construct that contains exon A2 and the flanking introns and exons (15 and 16), to show that sequence elements are critical in A2 exon skipping. Bioinformatic analysis, systemic deletion of flanking intronic sequences and mutation of the 5'splice site and branch-point sequences of the minigene construct established that skipping of the A2 exon might be due to weak 5' splice site of the exon in non-neuronal cells.

Coagulation factor FVIIa enhances PD-L1expression and its stability in breast cancer cells to promote breast cancer immune evasion

Subhojit Paul, Kaushik Das, Arnab Ghosh, Avinandan Bhoumick, Akash Chatterjee, Abhimanyu Basu, Prosenjit Sen*

School of Biological Sciences, IACS, Kolkata

Immunotherapy in breast cancers has not gained significant success. Coagulation factor FVIIa-mediated protease-activated receptor 2 activations are found to promote metastasis and secretion of immune-modulatory cytokines but the role of the coagulation factor in cancer immunology is still not well understood. Here, we aim to investigate whether coagulation factor FVIIa protects breast cancer cells from CD8 T cell-mediated killing. PBMC-derived CD8 T cells are co-cultured with ±FVIIa pre-treated MDA468 cells. The proliferation and activity of CD8 T cells were measured by FACS and ELISA. An allograft model using wild-type or Tissue Factor/PAR2 deleted 4T1 cells was used to determine the effect of coagulation factor FVII on cancer immune evasion *in vivo*. Here, we demonstrate that TF-FVIIa induces PDL1 in breast cancer cells by activating PAR2. PAR2 activation triggers LATS1 kinase inactivation leading to the suppression of YAP/TAZ phosphorylation and ensuing nuclear localization of YAP and TAZ. YAP or TAZ inhibition reduces PD-L1 expression and increases CD8 T cell activity. We further demonstrate that, apart from transcriptional induction of PD-L1, PAR2 activation also increases PD-L1 stability by enhancing its glycosylation through N- glycosyltransferases STT3A and STT3B. In the mouse model of breast cancer tumor cells, specific TF/ PAR2 depletion leads to PD-L1 downregulation and increases Anti PD1 immunotherapy efficacy. In conclusion, we show that coagulation factor FVIIa mediated signaling cascade in cancer cells serves as a tumor intrinsic immunosuppression mechanism to promote cancer immune evasion.

CDK1 mediated phosphorylation of TDP1 promotes genomic stability by preventing TDP1 trapping on mitotic chromosomes

Srijita Paul Chowdhuri

School of Biological Sciences, IACS, Kolkata

Tyrosyl–DNA phosphodiesterase 1 (TDP1) hydrolyzes the phosphodiester linkages at the DNA 3'-ends linked to a tyrosyl moiety of stalled Topoisomerase I–DNA covalent complexes (Top1cc). Top1 activity is not limited to the S-phase of the cell cycle but also promotes chromosomal transactions during mitosis. Defective repair of Camptothecin (CPT)- induced trapped Top1cc triggers DNA double strand breaks (DSBs). Cyclin dependent kinase 1 (CDK1) is the core mitotic regulatory kinase critical for the G2/M phase transition. CDK1- deficiency impedes mitotic progression, underscoring deleterious late mitotic events. Here we show a novel functional coupling between CDK1 and TDP1 which catalyses the phosphorylation of TDP1 at the N terminal domain. We show that TDP1 is primarily phosphorylated by CDK1 in M-phase using site-specific phosphopeptide antibodies. CDK- dependent phosphorylation-defective mutant TDP1 is enriched at the common fragile sites under mild replication stress, which stimulates a marked elevation of mitotic DNA synthesis (MiDAS) and leads to mitotic defects. TDP1 knockout cells complemented with the mitotic phosphomutant TDP1 showed hypersensitivity to CPT and harboured mitotic defects like micronuclei, anaphase bridges, and chromosomal breaks, which culminate into 53BP1 nuclear bodies in the next G1 phase of the cell cycle. This finding shows a novel role for CDK1 mediated phosphorylation of TDP1, which is critical for the release of TDP1 from the Top1cc damage sites and preventing genomic instability.

CpdB – the predicted regulator of nanotube-flagella switch in Pathogenic *Escherichia coli*

Suparno Banerjee

School of Biological Sciences, IACS, Kolkata

Bacteria are the dominant and most abundant organisms on earth, residing in complex multi-species communities. To shape their community and flourish in the niche, bacteria need to maintain intricate molecular crosstalk with proximal prokaryotic and eukaryotic cells. Recent discovery of a novel membranous conduit for bacterial communications has revolutionized concepts of bacterial intra- and inter-kingdom crosstalk. It was found that membranous nanotubes extended from bacterial outer membrane serve to conduct a contact-dependent exchange of cytoplasmic molecules among bacteria belonging to same or different species, as well as between pathogenic bacteria and their human host cells. Further studies showed that a membrane-associated protein, YmdB, could upregulate nanotube formation in Bacillus subtilis.YmdB also acts like a switch between motility and biofilm formation. Thus, using Enteropathogenic Escherichia coli (EPEC) as model bacterium, I am trying to identify a YmdB ortholog and check its effect in the functioning and formation of nanotube. Nanotube formation has been estimated by the ability to exchange amino acid among bacterial cells via nanotubes. Histidine auxotroph of EPEC has been created and the auxotroph was found to grow in amino acid limiting M9 medium only in presence of wild-type EPEC cells by exchanging the amino acid. Furthermore, the probable YmdB ortholog, CpdB, identified through sequence and structural analysis, was deleted. Motiliy of cpdB mutant strains and its ability to form nanotube was estimated against EPEC wild type. cpdB mutant of EPEC also showed growth phase dependent enhancement of motility without any observable growth defect in EPEC.

Structural consequences of HMGB1 binding to platinum anticancer drug-treated DNA at single molecule level

Sourav Mondal

School of Biological Sciences, IACS, Kolkata

High mobility group B1 (HMGB1) is an architectural protein that bends DNA, and binds to DNA damage sites, such as those induced by platinum anticancer agents. Herein, the structural alterations induced by platinum drugs cisplatin or BBR3464, in presence of HMGB1, have been probed by high-resolution atomic force microscopy (AFM) and DNA pulling force-extension analysis. It is observed that drug-induced DNA loop formation enhanced upon HMGB1 binding. This could be because HMGB1 increases DNA conformational flexibility, bringing the drug-binding sites closer to each other, thereby enhancing the propensity of double adduct formation and therefore loop formation via inter-helical cross-linking. Since HMGB1 enhances DNA flexibility, the near-reversible structural transitions as observed in the force-extension curves (for 1h drug treatment), generally occurred at lower forces in presence of HMGB1. Since no reversible transition could be observed after 24h drug treatment, the DNA structural integrity must have been largely lost. The Young's modulus of dsDNA, estimated from the force-extension analysis, increased upon drug treatment due to formation of the drug-induced covalent cross-links and consequent reduction in DNA flexibility. The Young's modulus increased further in presence of HMGB1, possibly as a result of HMGB1-induced enhancement in DNA flexibility that could ease formation of the drug-induced covalent cross-links. Since an increase in DNA rigidity may inhibit basic cellular events like DNA replication and transcription, a cell viability assay was performed using HMGB1-knocked down cells from human breast metastatic cancer cell line that revealed that knocking down HMGB1 makes the cells more insensitive to cisplatin or BBR3464 treatment.

Amino acid containing amphiphilic hydrogelators with antibacterial and antiparasitic activities

Biswanath Hansda

School of Biological Sciences, IACS, Kolkata

Nanoscale self-assembly of peptide constructs represents a promising means to present bioactive motifs to develop new functional materials. Here we introduce a series of peptide amphiphiles C_{14}-Phe-CONH-(CH_2)n-NH_2 (n=6 for P1 and n=2 for P3) and C_{14}-Trp-CONH-(CH_2)n-NH_2 (n=6 for P2 and n=2 for P4) which form hydrogels based on β-sheet nanofibril networks, several of which have promising anti-microbial and anti-parasitic activities, in particular against multiple strains of Leishmania including drug-resistant ones. The amphiphilic gelators (P1 and P3) having L-phenyl alanine residue were active against Gram-positive bacteria B. subtilis and Gram-negative bacteria E. coli and P. aeruginosa. These gelators are highly cytotoxic to promastigotes of Leishmania and trigger apoptotic-like events inside the parasite. The mechanism of killing the parasite is shown, and these gelators are non-cytotoxic to host macrophage cells, indicating a potential use of these gels as therapeutic agents against multiple forms of leishmaniasis in the future.

Alternative Therapy: An emerging pathway for treating cancer

Monalisa Chowdhury

School of Biological Sciences, IACS, Kolkata

Cancer, a multidimensional challenge, has been spreading its clutches globally since many decades. Conventional treatments are being used in the past few decades, yet complete curbing of this deadly disease remains challenging. Conventional cancer treatments have systematic side effects that stand against its desirable therapeutic efficacy. Alternative strategies using biochemical features of cancer cells to promote apoptosis are finding notable significance. One of the ways of unconventional therapy is combination therapy. In recent days, combination therapy is emerging with the hope of achieving minimal side effects and improved therapeutic efficiency. In this context covalently tailored biotinylated blue emitted Fe^{2+} -doped carbon dots (FCD_b) were synthesized. FCD_b can effectively sense H_2O_2 by fluorescence quenching as well as activate H_2O_2 (pro-drug), which oxidatively damage the DNA through the generation of reactive oxygen species (ROS: superoxide ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), etc. Along with this anticancer drug paclitaxel was also loaded to FCD_b. FCD_b-PTX was exhibited ~2.7 to 3.5-fold higher killing of B16F10 cells than normal cells. Another such important biochemical feature of malignant cells is hypoxia, alteration of which can lead to cell death. Hypoxia inducible factor 1α (HIF-1α) has the key role in hypoxia generation. Herein, we synthesized biotinylated Co^{2+} -integrated carbon dot ($CoCD_b$) that specifically diagnose and selectively killed cancer cells with 3-3.1-fold higher efficiency over non-cancer cells by hypoxia induced apoptosis in absence of traditional therapeutic intervention. Immunoblotting assay in $CoCD_b$ treated MDA-MB-231 cells confirmed the increased expression of HIF-1α that was responsible for efficient killing of cancer cells. In 2D cells and 3D tumor spheroid, $CoCD_b$ treated cancer cells showed significant apoptosis that make $CoCD_b$ a potential theranostic agent.

Study of TNFR1 Cluster Compaction in live cells using Homo-FRET

Subhamoy Jana, Priyanka Roy, Nandana Nanda, Deepak Kumar Sinha*

School of Biological Sciences, IACS, Kolkata

Clustering of tumor necrosis factor receptor-1 (TNFR1) is necessary for the activation of the TNFR1-mediated downstream signaling pathway. The ligand (TNFα) enforces the homo-trimeric assembly of TNFR1 whereas the PLAD-PLAD interaction leads to macro clustering of many trimeric TNFR1s. The compaction of the macro TNFR1 cluster is affected by both the ligand as well as the PLAD-PLAD interaction. The role of compaction in determining the TNFR1 signaling is not clear. We investigate the compaction map of the macro TNFR1 clusters using homo-FRET. Our results indicate the differential role of TNFα and PLAD-PLAD interaction in defining the compaction of the cluster. The compaction affects the signaling potential of TNFR1 macro clusters. The spatial map reveals the inhomogeneous and dynamic nature of the compaction of the cluster. In my poster, I will discuss TNFR1 cluster compaction nature, dynamics, and how this compaction changes in live cells exposed to different physiological conditions.