BIOGRAPHICAL SKETCH

NAME: PARIJAT SARKAR

eRA COMMONS USER NAME: SARKAR.PARIJAT

POSITION TITLE: Postdoctoral Scholar

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Start Date	Completion Date	FIELD OF STUDY
Indian Institute of Science Education and Research, Kolkata (IISER-K), India	Integrated Bachelor's and Master's of Science	08/2008	05/2013	Biological Sciences
CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India	Ph.D.	01/2014	04/2021	Membrane Biology and Biophysics
Stanford University, Stanford, CA	Postdoctoral Scholar	06/2022	Present	Biochemistry

A. Personal Statement

My long-term research goal is to understand how lipids regulate cellular signaling events in healthy and disease states. My upbringing in the society has always enabled me to come across people deeply involved in science, which has always been one of my major motivating factors. It is worth mentioning that I did my undergraduate from Indian Institute of Science Education and Research (IISER Kolkata, India), which follows an interdisciplinary approach toward teaching science with an emphasis on experiments. As a result, I was exposed to many fields of science besides majoring in Biological Sciences. I had undertaken a number of courses in Physics, Chemistry and Mathematics that inspired me to pursue graduate research in biophysics. My interest in lipids began during my graduate studies in Prof. Amitabha Chattopadhyay laboratory that focuses on lipid-protein interactions using spectroscopy-based techniques. While in Prof. Chattopadhyay's lab, I grew fascinated with how membrane cholesterol affects the biochemical activity of GPCRs, and I contributed to multiple projects investigating the neurotransmitter GPCR serotonin$_{1A}$ receptor as a model system for studying how cholesterol and the cytoskeleton can fine tune receptor function using cell biological and confocal imaging-based approaches. (*Journal of Lipid Research 2022*, *Science Advances 2021, Biophysical Journal 2022, Journal of Membrane Biology 2022*). This work has expanded our understanding of cholesterol in the field of GPCR biology by setting a precedent for how other proteins may sense cholesterol levels in the membrane.

My interest in lipids led me to choose Dr. Rajat Rohatgi in the Biochemistry Department at Stanford University, an expert in receptor signaling, as my postdoctoral mentor. His laboratory has pioneered in elucidating signaling pathways that play important roles in both development and disease, with a particular focus on cilia-dependent mechanisms. Prof. Rohatgi's laboratory recently made the exciting and unprecedented discovery that the Hedgehog signaling pathway is triggered by an acute change in the organization of cholesterol in the ciliary membrane (*Elife* 2019, 2021). Consequently, this is an ideal lab to carry out my research on delineating the

molecular pathways that regulate the lipid composition of the ciliary membrane. This effort will benefit from the synergistic combination of my background in membrane biology with my mentor's expertise in primary cilia, CRISPR-based genetic screens and specific lipid probes. Finally, I would like to highlight Dr. Rohatgi's exceptional record of mentorship, which has directly led to his trainees receiving highly competitive career development grants (such as the K08 and K99 awards from the NIH) and group leader positions at top academic institutions (Yale, University of Washington, NCI/NIH).

Additionally, I joined Dr. Rohatgi's lab because I believe that this fundamental problem can be solved by the application of cutting-edge techniques that are very distinct from the biophysical tools I used in graduate school: genome editing and genome-wide CRISPR screens combined with innovative probes for the characterization of membrane domains and physiologically-relevant signaling assays. While membrane biology has been historically studied using biophysical methods, my mentor's lab will enable me to apply the completely different lens of CRISPR-enabled somatic cell genetics and genomics to the study of the unique membrane microdomain that envelops primary cilia.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

06/2022-present	Postdoctoral Scholar, Stanford University, CA
05/2021-05/2022	Senior Project Associate, Prof. Amitabha Chattopadhyay's Laboratory, CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India
09/2020-present	Student Member, Biophysical Society, USA
05/2016-06/2016	Visiting Researcher in Prof. Trevor Smith's Laboratory, University of Melbourne, Australia
01/2015-present	Member, Fluorescence Society, India
01/2015-present	Member, Society of Biological Chemists, India
01/2015-present	Member, Indian Biophysical Society, India
01/2015-present	Member, Indian Society of Cell Biology, India
01/2014-04/2021	Graduate Researcher in Prof. Amitabha Chattopadhyay's Laboratory, CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India
05/2013-12/2013	Project Assistant, Prof. Amitabha Chattopadhyay's Laboratory, CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India
05/2011-07/2011	DAAD Working Internships in Science and Engineering (WISE) Scholar in Prof. Jasmin Joshi's Laboratory, University of Potsdam, Germany
05/2010-07/2010	Summer Research Fellow (Indian Academy of Sciences) Dr. B V Rajarama Bhat's Laboratory, Indian Statistical Institute (ISI), Bangalore, India
08/2012-present	Member, Electron Microscope Society of India, India
08/2012-04/2013	Undergraduate Researcher in Dr. Partha Pratim Datta's Laboratory, Indian Institute of Science Education and Research, Kolkata (IISER-K), India
08/2008-04/2013	Undergraduate Research, Indian Institute of Science Education and Research, Kolkata (IISER-K), India

Honors and Awards

2021 The National Academy of Sciences, India (NASI) Young Scientist Platinum Jubilee Award 2021 in Bio-Medical, Molecular Biology and Biotechnology
2019 Outstanding poster presentation award in Hy-Sci 2019, CCMB, Hyderabad, India
2018 Sun Pharma Science Scholar Award-2018 in Biomedical Sciences for doctoral research by Sun Pharma Science Foundation, Sun Pharmaceutical Industries Ltd., India
2017 Grant awarded by Avanti Polar Lipids and Full foreign travel grant awarded by Council of Scientific & Industrial Research (CSIR), India to participate in Joint EMBO-FEBS Advanced Lecture Course on 'Biomembranes: Molecular Architecture, Dynamics and Function', Corsica, at France
2016 FEBS Youth Travel Fund for participating in FEBS advanced course on 'Lipid-protein interactions and organelle function' at Spetses Island, Greece
2015 Anindya Kumar Ghosh Memorial Award for the Best Student Seminar (2014-2015), CSIR-CCMB, Hyderabad, India
2014 Dr. Shyama Prasad Mukherjee Fellowship by CSIR, Human Resource Development Group, for Graduate Research Fellowship India
2013 Director's Gold Medal for 1st rank in Department of Biological Sciences after 5 years Integrated BS-MS course at IISER, Kolkata, India
2013 CNR Rao Foundation Prize Certificate for securing the highest marks in the first semester of Integrated BS-MS course at IISER, Kolkata
2012 Visiting Students' Research Programme at Tata Institute of fundamental Research, Mumbai, India
2012 Indian Academy of Sciences Scholarship SRF (Summer Research Fellowship)
2011 DAAD Working Internships in Science and Engineering (WISE) Scholarship
2011 Indian Academy of Sciences Scholarship SRF (Summer Research Fellowship)
2010 Indian Academy of Sciences Scholarship SRF (Summer Research Fellowship)
2008 INSPIRE, Department of Science and Technology, India, for Undergraduate Research Fellowship

C. Contributions to Science

1. Intimate association between the actin cytoskeleton and membrane cholesterol

Depletion of membrane cholesterol is a widely used approach in cell membrane biology to explore cholesterol dependence of the function and dynamics of membrane proteins and receptors. Although cholesterol manipulation by agents such as statins could lead to changes in the actin cytoskeleton polymerization status (in terms of relative contents of G- and F-actin), interpretations of these experiments often does not include this aspect. This is due to lack of a proper perspective at a conceptual level and lack of a convenient assay to provide a readout of actin polymerization status during such processes. The central theme of my PhD thesis was to explore the interplay between plasma membrane and the underlying actin cytoskeleton network that supports the membrane. By building all the requisite tools and establishing new assays, I comprehensively solved the mechanism by which the actin cytoskeleton polymerizes in response to cholesterol modulations in membranes. In addition, using fluorescence recovery after photobleaching (FRAP), Single Particle Tracking (SPT, in collaboration with Dr. Laurence Salomé, CNRS, Université de Toulouse, France) and fluorescence loss in photobleaching (FLIP) approaches, I showed that such reorganization of actin cytoskeleton affects lateral diffusion of both membrane lipids and GPCRs by formation of confinement zones in the membrane. In

summary, my work demonstrated that the dynamic reorganization of the actin cytoskeleton could represent an important determinant in membrane protein dynamics and signaling in diseases that are due to defects in cholesterol biosynthesis pathways such as Smith-Lemli-Opitz syndrome.

1. **Sarkar, P.,** Kumar, G.A., Shrivastava, S., and Chattopadhyay, A. (2022) Chronic Cholesterol Depletion Increases F-actin Levels and Induces Cytoskeletal Reorganization *via* a Dual Mechanism. ***Journal of Lipid Research*** 63: 100206. *(Featured on the cover)*
2. **Sarkar, P.,** and Chattopadhyay, A. (2022) Statin-induced Increase in Actin Polymerization Modulates GPCR Dynamics and Compartmentalization. ***Biophysical Journal*** (DOI: 10.1016/j.bpj.2022.08.039)
3. Shrivastava, S.[†], **Sarkar, P.[†]**, Preira, P., Salomé, L., and Chattopadhyay, A. (2022) Cholesterol-dependent Dynamics of the Serotonin$_{1A}$ Receptor utilizing Single Particle Tracking: Analysis of Diffusion Modes. ***Journal of Physical Chemistry B*** ([†]equal contribution; *Featured on the cover*) (DOI: 10.1021/acs.jpcb.2c03941)
4. Shrivastava, S.,[†] **Sarkar, P.**,[†] Preira, P., Salomé, L., Chattopadhyay, A. (2020) Role of Actin Cytoskeleton in Dynamics and Function of the Serotonin$_{1A}$ Receptor. ***Biophysical Journal*** 118: 944-956. ([†]equal contribution)

2. A molecular sensor for cholesterol in the serotonin$_{1A}$ receptor

The G protein-coupled receptors (GPCRs) represent the largest group of integral membrane proteins in the human proteome accounts for ~40% of current drug targets. The sensitivity of GPCRs toward their immediate membrane lipid microenvironment constitutes an exciting yet poorly resolved area of contemporary GPCR biology. In particular, cholesterol sensitivity of GPCRs is attributed to specific sequence and structural features, such as the cholesterol recognition/interaction amino acid consensus (CRAC) motif, that facilitate their cholesterol-receptor interaction. I focused on the serotonin$_{1A}$ receptor as a representative neurotransmitter GPCR for cholesterol sensitivity and examined the molecular basis of cholesterol sensitivity of the serotonin$_{1A}$ receptor function by mutating various key residues of the receptor and monitoring corresponding functional readouts. My work showed that the functional sensitivity of the serotonin$_{1A}$ receptor to membrane cholesterol is lost when the residue K101 in CRAC motif I is mutated (in both single and double mutants), indicating the role of K101 as a molecular sensor of membrane cholesterol. I believe this work represents the first comprehensive mechanistic study on cholesterol sensitivity of GPCR function using a combination of experimental and computational approaches (in collaboration with Dr. Jana Selent, University Pompeu Fabra, Spain).

1. Kumar, G.A.,[†] **Sarkar, P.,[†]** Stepniewski, T.M.,[†] Jafurulla, M., Singh, S.P., Selent, J., Chattopadhyay, A. (2021) A Molecular Sensor for Cholesterol in the Human Serotonin$_{1A}$ Receptor. *Science Advances* 7, eabh2922 ([†]equal contribution; *Recommended in F1000 prime (Faculty Opinions)*).
2. **Sarkar, P.,** Bhat, A., and Chattopadhyay, A. (2022) Lysine 101 in the CRAC Motif in Transmembrane Helix 2 Confers Cholesterol-induced Thermal Stability to the Serotonin$_{1A}$ Receptor. *The Journal of Membrane Biology* (DOI: 10.1007/s00232-022-00262-w)
3. **Sarkar, P**., Jafurulla M., Bhowmick, S., Chattopadhyay, A. (2020) Structural Stringency and Optimal Nature of Cholesterol Requirement in the Function of the Serotonin$_{1A}$ Receptor. *The Journal of Membrane Biology* 253: 445-457.

3. Membrane dipole potential: a unique approach to explore membrane organization and function

Dipole potential is a relatively less known membrane potential and is generated due to nonrandom organization of dipoles within the membrane. Alteration in membrane dipole potential has been shown to modulate the activity of membrane proteins such as Na^+/K^+-ATPase, ion channels and membrane-active proteins. During my graduate research, I carried out a number of projects on dipole potential and this has resulted in a number of interesting and novel publications. An important aspect of my work was the innovation that allows to image dipole potential using commercial confocal microscopy set-up commonly available in many labs. This would increase the reach of dipole potential measurements to cellular systems, wherein a number of exciting and relevant biological questions can be probed using dipole potential imaging approach. For example, using this approach, I showed that the membrane dipole potential varies with cell cycle progression which correlate with differential membrane composition and GPCR function.

1. **Sarkar, P**., Rao, B.D., Chattopadhyay, A. (2020) Cell Cycle Dependent Modulation of Membrane Dipole Potential and Neurotransmitter Receptor Activity: Role of Membrane Cholesterol. *ACS Chemical Neuroscience* 11: 2890-2899.
2. **Sarkar, P.,** Chakraborty, H., Chattopadhyay, A. (2017) Differential Membrane Dipolar Orientation Induced by Acute and Chronic Cholesterol Depletion. *Scientific Reports* 7: 4484.
3. **Sarkar, P.**, and Chattopadhyay, A. (2022) Membrane Dipole Potential: An Emerging Approach to Explore Membrane Organization and Function. *Journal of Physical Chemistry B* 126: 4415-4430 *(Invited Feature Article; Featured on the cover).*

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/parijat.sarkar.2/bibliography/public/

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	CSIR-Centre for Cellular and Molecular Biology, India	
2014	Biostatistics	A+
2014	Computation/Bioinformatics	A
2014	Basic Chemistry	A+
2014	Research Methodology/Ethics/Safety	A+
2014	Scientific Writing	A+
2014	Biological Macromolecules	A+
2014	Biology of Inheritance	A+
2014	Biology of Infection	A
2014	Stem cells, Regeneration, and ageing	A+
2014	Self Organization in Biology	A+
2014	Cell Biology	A+
2014	NMR micro-imaging and Spectroscopy	A+
2014	Mass Spectroscopy in Biology	A+
2014	Immunology	A+
2015	Seminar	A+
2015	Grant Proposal	A-

CSIR-Centre for Cellular and Molecular Biology graduate courses are graded A+ (91-100%), A (81-90%), A- (71-80%), B+ (61-70%), B (51-60%), B- (41-50%) and F (<40%). Passing is B- or better.