BIOGRAPHICAL SKETCH

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NAME: Moitrayee Bhattacharyya

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Jadavpur University, Kolkata	BSc	2002-2005	Chemistry
Jadavpur University, Kolkata	MSc	2005-2007	Chemistry
Molecular Biophysics Unit, Indian Institute of Science, Bangalore	PhD	2007-2012	Computational Biophysics
Molecular and Cell Biology (MCB), University of California, Berkeley	Postdoctoral researcher	2013-2019	Structural Biophysics and biochemistry

A. Personal Statement

My research goals center around understanding the molecular mechanism of signal transduction in neuronal cells that are pivotal to cognitive development and maintenance. I plan to take a reductionist slant in approaching this problem. Starting with biophysical/biochemical characterization of individual proteins that are critical to neuronal signal transduction, I aim to get a global view of their functioning in the context of complex signaling pathways in cells.

My graduate studies were focused on understanding the physical principles underlying allosteric communication in proteins and its complexes with RNA/DNA. I developed and implemented methods, combining molecular dynamics simulations and network theory, to understand how subtle reorientations at the level of side-chain interactions can mediate long-distance communication in proteins. I have also developed a widely used open-source software, for the easy implementation of this method for characterization of protein structure and dynamics (*reviewed in*[7]).

My postdoctoral work, as a *Human Frontier Science Program* Long Term Fellow (2013-2016) in the Kuriyan lab, focused on understanding the molecular mechanism for the regulation of activation in CaMKII, an oligomeric Ser/Thr kinase that is critical for learning and memory. I was part of the discovery of activation-triggered subunit exchange in CaMKII, a phenomenon that may have implications in the spread of CaMKII activity in cells. My next goal was to uncover the molecular mechanism of activation-triggered subunit exchange in CaMKII. Using a combination of experimental and computational techniques, I have uncovered that the calmodulin-binding element in CaMKII binds to the hub, weakens the integrity of the hub to release dimers and mediates subunit exchange between activated and unactivated holoenzymes. Additionally, the phosphorylation sequesters the calmodulin-binding element from the kinase and calmodulin and biases its interaction with the hub. These findings were reported in two *eLife* papers[1,2].

The activity of CaMKII holoenzymes is regulated by autophosphorylation at an activating (Thr 286) and an inhibitory site (Thr 305/306) on a regulatory segment. During the *mentored phase of my K99/R00 award*, I have also shown that the balance between activating and inhibitory phosphorylation is controlled by a flexible linker that connects the CaMKII kinase domain to the hub (kinase-hub linker). Using a single-molecule assay that I developed, I showed that the two brain-specific isoforms, CaMKII-α and CaMKII-β, which principally differ in this flexible linker, show opposing tendencies of autophosphorylation[3].

Drawing on my expertise in molecular biophysics and biochemistry, structural biology, microscopic techniques, native mass spectrometry (native-MS) and computational biology, the long-term goal of my lab will be to uncover the regulation of signaling proteins and pathways that are implicated in learning and memory and how they get perturbed in Down Syndrome (DS). Specifically, the immediate goals of this project are the

following: (1) To understand how cells can tune the response of CaMKII to Ca^{2+} pulses in the brain by mixing the brain-specific isoforms (α and β) in different ratios within the CaMKII heterooligomers. The goal here is to map the CaMKII-isoform ratios along the spatiotemporal axis of signaling in the brain, using single-molecule microscopy and native mass spectrometry. This will provide an explanation for the role of CaMKII linker-variants in tuning its activity in response to activation stimulus. (2) Dyrk1a is a constitutively active kinase that is overexpressed in trisomy 21 and is a key therapeutic target for treating cognitive impairments associated with DS. The goal of this project is to identify the direct substrates of Dyrk1a and to uncover the molecular basis of its regulation. A molecular understanding of how Dyrk1a is regulated and the pathways directly affected by this kinase will help guide the design of therapeutic strategies targeting Dyrk1a. (3) Dyrk-family kinases are emerging as key regulators of cellular phase-separation. I plan to interrogate what sequence-structure features of these kinases (using Dyrk3 as an example) impact this regulation and what is the role of kinase activity in this process. A molecular understanding of how Dyrk3 regulates cellular-phase separation will help inform future studies aimed at correlating aberrant phase-separation with neuropathologies involving cognitive impairments.

B. Positions and Honors

Positions and employments:

2007-2012: **Graduate Research**, Indian Institute of Science, Bangalore
(*Advisor: Professor Saraswathi Vishveshwara, PhD*)

2013-2019: **Postdoctoral Research Fellow,** University of California Berkeley
(*Advisor: Professor John Kuriyan, PhD*)

July 2020: **Assistant Professor**, Yale University

Honors and awards:

2005-2007: Indira Gandhi Post graduate fellowship, UGC, Govt. of India
2007: Tushar Mahalanabis Memorial Silver Medal in Organic Chemistry, Jadavpur University
2007-2009: Council of Scientific and Industrial Research, Govt. of India, Junior Research Fellowship
2009-2012: Council of Scientific and Industrial Research, Govt. of India, Senior Research Fellowship
2010: Department of Science and Technology, Govt. of India, Young scientist travel award
2010: Indian Council of Medical Research, International Travel by Non-ICMR Scientists award
2013: Eli Lilly and Company Asia Outstanding thesis award
2013-2016: Human Frontier Science Program (HFSP) Long Term Fellowship
2017-2019: NIH Pathway to Independence Award (K99/R00), K99-phase, NIGMS
2020-2023: NIH Pathway to Independence Award (K99/R00), R00-phase, NIGMS

Professional Societies and Public Advisory Committees:

2014, 2016: Reviewer – Current Opinion in Structural Biology
2014, 2016: Reviewer – Molecular BioSystems
2014-2016: Reviewer – PLoS ONE
2016: Reviewer - RSC Advances
2017: Reviewer – The Journal of Physical Chemistry, Journal of Chemical Information and Modeling
2018: Reviewer – Cellular Signaling, Nucleic Acids Research
2020: Reviewer - PNAS
2016-present: Member - Alumni association, Human Frontier Science Program (HFSP)
2019-present: Member – The Protein Society

C. Contributions to Science

Postdoctoral Research:

Molecular mechanism of activation-triggered subunit exchange in Ca^{2+}/CaM-dependent kinase II (CaMKII)

As a postdoctoral scholar in the Kuriyan lab, I have outlined the molecular mechanism of subunit exchange in Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)[1], whereby activation triggers the exchange of subunits in

this oligomeric enzyme. My studies have shown that the human CaMKII holoenzyme exists in dodecameric and tetradecameric forms and that the calmodulin-binding element of CaMKII can bind to the hub of the holoenzyme and destabilize it to release dimers. I have also solved the structures of CaMKII from two divergent organisms, which suggest that the CaM-binding element of activated CaMKII acts as a wedge by docking at intersubunit interfaces in the hub. This converts the hub into a spiral form that can release or gain CaMKII dimers. My work reveals a three-way competition for the CaM-binding element, whereby phosphorylation biases it towards the hub interface, away from the kinase domain and calmodulin, thus unlocking the ability of only activated CaMKII holoenzymes to exchange subunits[2]. Following up on this observation, I have shown that addition of a regulatory segment-derived peptide to the CaMKII hub leads to hub disassembly by native mass spectrometry and long-scale MD simulations that are run on ANTON[3]. Using a single-molecule microscopy assay, I have also established that activation leads to disassembly of CaMKII holoenzymes[3].

The activity of CaMKII holoenzymes is regulated by autophosphorylation at an activating (Thr 286) and an inhibitory site (Thr 305/306) on a regulatory segment. During the mentored phase of my K99/R00 award, I have also shown that the balance between activating and inhibitory phosphorylation is controlled by a flexible linker that connects the CaMKII kinase domain to the hub (kinase-hub linker). Using a single-molecule assay that I developed, I showed that the two brain-specific isoforms, CaMKII-α and CaMKII-β, which principally differ in this flexible linker, show opposing tendencies of autophosphorylation[4]. This result suggests that varying the proportions of α and β isoforms in CaMKII heterooligomers may allow the kinase to tune its response to variations in the amplitude and frequency of calcium spike trains in the brain.

1. Margaret Stratton[#], Il-Hyung Lee[#], ***Moitrayee Bhattacharyya***, Sune M Christensen, Luke H Chao, Howard Schulman, Jay T Groves, John Kuriyan, Activation-triggered subunit exchange between CamKII holoenzyme facilitates the spread of kinase activity, ***eLife*****,** 3**,** e01610 (2014).

2. ***Moitrayee Bhattacharyya***[#], Margaret M. Stratton[#], Catherine C. Going[#], Ethan McSpadden, Yongjian Huang, Anna C. Susa, Anna Elleman, Yumeng Melody Cao, Nishant Pappireddi, Pawel Burkhardt, Christine Gee, Tiago Barros, Howard Schulman, Evan R. Williams, John Kuriyan, Molecular mechanism of activation-triggered subunit exchange in Ca2+/calmodulin-dependent protein kinase II, ***eLife*****,** 5, e13405 (2016).

3. Deepti Karandur[#], ***Moitrayee Bhattacharyya***[#], Zijie Xia, Young Kwang Lee, Serena Muratcioglu, Darren McAffee, Ethan McSpadden, Baiyu Qiu, Jay T Groves, Evan R Williams, John Kuriyan, 2020. Breakage of the oligomeric camkii hub by the regulatory segment of the kinase. ***BioRxiv****,* doi.org/10.1101/2020.04.15.043067 (2020).

4. ***Moitrayee Bhattacharyya***[#], Young Kwang Lee[#], Serena Muratcioglu, Baiyu Qiu, Priya Nyayapati, Howard Schulman, Jay T. Groves, John Kuriyan, Flexible linkers in CaMKII control the balance between activating and inhibitory autophosphorylation. ***eLife***, 9, e53670 (2020).

Graduate Research:

Probing ligand induced perturbations in protein structure networks

The fidelity of biological processes/reactions, in spite of widespread diversity, is programmed by highly specific physico-chemical principles. A key question is whether these diverse biological processes have a unifying theme when probed taking into account even subtle re-orchestrations of the interactions and energetics at the protein/nucleic acid side-chain level (using network theory) from a dynamical perspective (from Molecular Dynamics simulations).

The focus was to elucidate the general principles underlying biological phenomena, such as allosteric communication, ligand induced modulation of rigidity/flexibility and half-sites-reactivity, in protein complexes showing subtle structural variations upon ligand binding. Several proteins and their complexes have been investigated to formulate general methodologies to address these issues, with special emphasis on the mechanism and fidelity of the aminoacylation reaction[5,6]. I have also examined ligand-induced percolations in protein structure network from a global perspective to understand the effect of ligand binding from a global perspective[7]. I have been the first author on a review article summarizing the application of network theory to protein structure networks[8]. While general applications of network theory to analyze molecular dynamics simulations data are discussed in this review, challenging problems that have demanded the attention of the scientific community, such as allosteric communication and protein folding, are considered in greater detail. My aim has been to explore these important problems using concepts of network theory[8].

5. ***Bhattacharyya M*** and Vishveshwara S, *Probing the allosteric mechanism in pyrrolysyl-tRNA synthetase using energy-weighted network formalism*, **Biochemistry,** 50, 6225 (2011).

6. ***Bhattacharyya M***, Ghosh A, Hansia P and Vishveshwara S, *Allostery and conformational free energy changes in human tryptophanyl-tRNA synthetase from essential dynamics and structure networks,* **Proteins: Struct Funct Bioinfo,** 78, 506 (2010).

7. Sukhwal A, ***Bhattacharyya M*** and Vishveshwara S, *Network approach for capturing the ligand-induced subtle global changes in protein structures***, Acta Cryst D**, D67, 429 (2011).

8. ***Bhattacharyya M***, Ghosh S, and Vishveshwara S, Protein Structure and Function: Looking through the Network of Side-Chain Interactions, **Current Protein and Peptide Science**, 17, 4 (2016)

Methodology development and design of open source software

I have developed an open source software package, PSN-Ensemble, for network theory-based analyses of an ensemble of protein structures[9]. PSN-Ensemble can handle structural ensembles generated through molecular dynamics (MD) simulation/NMR studies or from multiple X-ray structures. The novelty in network construction lies in the explicit consideration of side-chain interactions among amino acids. The program evaluates various network parameters related to the topological organization of the protein structure network and long-range allosteric communication. Also, the results are mapped on a graphical display of the structure, allowing an easy access of network analysis to a general biological community.

Disulfide crosslinks are ubiquitous in natural peptides and proteins, providing rigidity to polypeptide scaffolds. The assignment of disulfide connectivity in multiple crosslinked systems is often difficult to achieve. I have developed a methodology for the rapid determination of disulfide connectivity using a direct mass spectrometric fragmentation of the native disulfide bonded polypeptides and subsequent analysis using a newly developed software, DisConnect[10]. The method provides an unambiguous, straightforward approach, especially useful for the rapid screening of the disulfide crosslink fidelity in recombinant proteins, determination of disulfide linkages in natural peptide toxins and characterization of folding intermediates encountered in oxidative folding pathways.

9. ***Bhattacharyya M***, Bhat CR, and Vishveshwara S, *An automated approach to network features of protein structure ensembles*, **Protein Science,** 22**,** 1399 (2013).

10. ***Bhattacharyya M*[#]**, Gupta K[#], Gowd KH, and Balaram P, *Rapid mass spectrometric determination of disulfide connectivity in peptides and proteins*, **Mol BioSystems,** 9**,** 1340 (2013).

[#]**=**co-first authors

A comprehensive list of published work is in **MyBibliography:**

https://www.ncbi.nlm.nih.gov/myncbi/12U7ai889uuQ8/bibliography/public/

D. Additional Information: Research Support

Research support:

2020 – 2023
NIH Pathway to Independence (R00) Award (NIGMS)