Puja Majumder

Curriculum Vitae

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Experience

2024-present **Research Associate**, Memorial Sloan Kettering Cancer Center, New York, USA.
Research area: Cryo-EM structural biology of CRISPR & other antiphage defense systems

2022-2024 **Research Scholar**, Memorial Sloan Kettering Cancer Center, New York, USA.
Research area: Cryo-EM structural biology of CRISPR & other antiphage defense systems

Feb 2021–Oct 2021 **Research Associate**, Molecular Biophysics Unit, Indian Institute of Science, Bengaluru, India.
Research area: cryo-EM of membrane protein

Ph. D Thesis

Title Structural and Functional Investigation of a Multi-drug Efflux Transporter QacA

Supervisor Prof. Aravind Penmatsa

Awards

2019 Best Poster Award, European Molecular Biology Organization (EMBO) Workshop

2018 Best Poster Award, Indian Biophysical Society Meeting, IISER Pune, India

2015 GATE Doctoral Fellowship, Science and Engineering Research Board (SERB), Government of India

2015 DST Inspire Scholarship, Department of Science and Technology (DST), Government of India

2015 First Class First – Gold Medal in Master of Science, Jawaharlal Nehru University (JNU), India

2013 CEEB Fellowship, Combined Entrance Examination for Biotechnology, India

Education

2015–2021 **Doctor of Philosophy**, Indian Institute of Science (IISc), Bengaluru, India.
Membrane protein Structural Biology

2013–2015 **Master of Science**, Jawaharlal Nehru University (JNU), New Delhi, India.
Biotechnology, First Class First

2011–2013 **Bachelor of Science**, St. Xaviers College (Autonomous), Kolkata, India.
First Class Honours in Microbiology

Publications

[8] C.F.Baca[‡], P. Majumder[‡*], J. H. Hickling, D. J. Patel, L. A. Marraffini; Cat1 forms filament networks to degrade NAD^+ during the type III CRISPR-Cas anti-viral response; accepted in Science **([‡]co-first and [*]co-corresponding author)**

[7] C.F.Baca[‡], P. Majumder[‡], J. H. Hickling, L. Ye, S. F. Brady, D. J. Patel, L. A. Marraffini; CRISPR systems convert ATP to ITP by the Cad1 adenosine deaminase to provide antiviral immunity; Cell, 187, 7183–7195 (2024) **([‡]co-first author)**

[6] C. F. Baca, Y. Yu, J. T. Rostøl, P. Majumder, D. J. Patel, L. A. Marraffini; The CRISPR effector Cam1 mediates membrane depolarization to provide phage defense during the Type III CRISPR-Cas immune response; Nature, 625, 797–804 (2024)

[5] P. Majumder, S. Ahmed, P. Ahuja, A. Athreya, R. Ranjan, A. Penmatsa; Cryo-EM structure of antibacterial efflux transporter QacA from *Staphylococcus aureus* reveals a novel extracellular loop with allosteric role; The EMBO Journal, 42:e113418 (2023)

[4] S. Dudey, P. Majumder, A. Penmatsa, A. A. Sardesai; Topological analyses of the L-lysine exporter LysO reveal a critical role for a conserved pair of intramembrane solvent-exposed acidic residues; Journal of Biological Chemistry, 297(4) 101168 (2021)

[3] B. Khatri, P. Majumder, J. Nagesh, A. Penmatsa and J. Chatterjee; Increasing protein stability by engineering the n$\rightarrow \pi^*$ interaction at the β-turn; Chemical Science, 11, 9480–9487 (2020)

[2] P. Majumder, S. Khare, A. Athreya, N. Hussain, A. Gulati and A. Penmatsa; Dissection of protonation sites for antibacterial recognition and transport in QacA, a multi-drug efflux transporter; Journal of Molecular Biology, 431, 2163–2179 (2019)

[1] P. Majumder, A. K. Mallela and A. Penmatsa; Transporters Through the Looking Glass: An Insight into the Mechanisms of Ion-Coupled Transport and Methods That Help Reveal Them; Journal of the Indian Institute of Science, 98, 283–300 (2018)

Selected contributed presentations

Oct 2024 Bacterial novel anti-viral defense strategy by Cat1 (CRISPR associated TIR 1) effector; Memorial Sloan Kettering Cancer Center (MSKCC), Basic Science Retreat 2024, New York. [Talk]

June 2024 Elucidation of a novel anti-viral defense strategy by Cad1 and Cam1; Vagelos education center at Columbia University Medical Center, The 4th Annual NY Area Cryo-EM Meeting 2024, New York. [Talk]

May 2024 Elucidation of a novel anti-viral defense strategy by Cad1, CRISPR-associated adenosine deaminase 1; Memorial Sloan Kettering Cancer Center (MSKCC), Structural Biology Retreat 2024, New York. [Talk]

Sept 2023 Mechanism of Cam1 (Cyclic-oligoadenylate-activated membrane protein 1) mediated phage defense during type III CRISPR-Cas activity; Memorial Sloan Kettering Cancer Center (MSKCC), Basic Science Retreat 2023, New York. [Poster]

Oct 2019 Dissection of protonation sites of antibacterial recognition and transport in QacA, a multidrug efflux transporter; EMBO Workshop, Tools for Structural Biology of Membrane Proteins, EMBL Hamburg, Germany. [Poster]

Skills and Expertise

Cryo-Electron Microscopy Proficient in cryo-EM sample preparation of different biomolecules (*e.g.* soluble and membrane proteins, filament protein, supramolecular protein complex and protein nucleic acid complex) along with grid freezing using Vitrobot setup and grid clipping. Expert in data collection using EPU and Serial-EM, in Thermo Fisher Titan Krios microscopes equipped either with Gatan G^2 or FEI Falcon4i detectors, along with energy filter. Have significant independent usertime at common electron microscopy user facilities, *viz.* **Simons Electron Microscopy Center** facility at MSKCC, also National Centre for Cryo-EM Access and Training **(NCCAT)** and New York Structural Biology Centre **(NYSBC)**.

X-ray crystallography Soluble and membrane protein sample preparation, plate setup techniques such as hanging drop, sitting drop, lipidic cubic phase crystallization using STP Labtech Mosquito Robot, screen preparation using STP Labtech Dragonfly. Experienced in data collection through Synchrotron beamtime (remotely at **Argonne National Laboratory Advanced Photon Source (APS)** and at **Brookhaven National Laboratory NSLS-II beamline** and in-person collection at **European Synchrotron Radiation Facility (ESRF) at EMBL Grenoble, France**.

Biochemistry ITC, UV-Vis spectroscopy, Fluorescence spectroscopy, MST, mass photometry, Western blot, ELISA, EMSA, molecular biology techniques, Transport assays using everted vesicles, proteoliposomes and whole cells.

Biophysics Proficient in biophysical characterization techniques such as SEC-MALS, CD spectroscopy, nano-DSF, FSEC, FPLC, DLS.

Antibody generation Conceptualized and Streamlined Indian Camelid antibody (ICab) or nanobody generation for structural biology. Expert in monoclonal antibody isolation and purification, Fab preparation against target protein from mice.

Software Proficient and experienced in cryo-EM softwares for data collection and processing (data collection using **EPU** and **Serial-EM**, data processing using **CryoSPARC**, **Relion**, **Chimera**, **Coot**, **Phenix**), X-ray crystallography softwares for data processing (**HKL-3000, CCP4i**, **REFMAC**), primary bioinformatics for protein model prediction and search tools (**Foldseek, Dali, AlphaFold3, BLAST**). Data analysis softwares (**GraphPadPrism** and **Origin**) and data illustration softwares (**ChimeraX**, **Pymol**, **Illustrator**).

Summary of scientific contribution

My primary research goals are directed towards exploring novel defense strategies of prokaryotes against foreign toxic elements, starting from antimicrobial chemicals to viruses. This study unravels novel systems employed by bacteria to defeat foreign particles.

Potential applications

- Studying multi-drug efflux systems of bacteria have potential impact in drug-design industries.
- Bacterial anti-viral defense systems can be repurposed as sophisticated biotechnological and biomedical tools like CRISPR-Cas systems.

Using my basic training in biotechnology and microbiology as foundation, I have gained expertise in cryo-EM and X-ray crystallography to solve structure of the aforementioned systems to understand their function. A few notable works are summarized below:

- Structural and functional elucidation of multi-drug efflux transporter QacA:
  In my doctoral dissertation, I worked on bacterial defense against daily usage antibacterial drugs and uncovered the drug efflux mechanism through a transporter QacA from hospital-associated MRSA strains. I performed several biochemical transport experiments to understand the promiscuous drug efflux mechanism of QacA [a]. Additionally, I found a unique role of a structured extracellular loop in QacA and related drug efflux transporters which is involved in the allosteric regulation of the efflux activity, by solving camelid nanobody-bound QacA structure at 3.6 Å resolution through cryo-EM [b]. This work provides a detailed insight about the structure-function correlation of a key drug efflux pump from a pathogen which can contribute to the field of drug-design to combat multi-drug resistance in bacteria.

  Publications from this work

  a P. Majumder, S. Khare, A. Athreya, N. Hussain, A. Gulati and A. Penmatsa; Dissection of protonation sites for antibacterial recognition and transport in QacA, a multi-drug efflux transporter; J Mol Biol. 2019 May 17;431(11):2163-2179. PubMed Central PMCID: PMC7212025

  b P. Majumder, S. Ahmed, P. Ahuja, A. Athreya, R. Ranjan, A. Penmatsa; Cryo-EM structure of antibacterial efflux transporter QacA from *Staphylococcus aureus* reveals a novel extracellular loop with allosteric role; EMBO J. 2023 Aug 15;42(16):e113418. PubMed Central PMCID: PMC10425836

  In my postdoc, I am working towards understanding bacterial antiphage defense systems and their diversification in terms of defense mechanisms. A part of my work is centered on CRISPR-associated CARF-effectors, providing adaptive immunity to bacteria. During phage infection, the type III CRISPR system provides immunity by synthesizing cyclic oligo-adenylates (cOAn) which activate CRISPR-associated effectors fused to CARF domains. As part of a strong collaboration with the Luciano Marraffini lab at Rockefeller University, we targeted the CARF-effectors having diverse effector domains like transmembrane domain (Cam1), adenosine deaminase (Cad1) and TIR (Toll/interleukin-1 receptor) domain (Cat1).
  Our investigations on Cam1 revealed that the effector protein is associated with the abortive infection mechanism and causes bacterial growth arrest by membrane depolarization [a]. To

elucidate the mechanistic insights of membrane depolarization by Cam1, we planned cryo-EM based structural studies which were facilitated by generation of monoclonal antibodies against Cam1. The full-length Cam1-antibody complex structure in the apo form shows that Cam1 tetramer forms a pore by arranging four TM (transmembrane) helices spanning the membrane and the pore lining residues are indicative of cation transport. However, in the apo-Cam1 structure the pore is blocked by a couple of arginine residues positioned within the loop joining the TM domain to the CARF domain. The studies are being extended to cA4 (cyclic tetra-adenylate)-bound Cam1-antibody complex to understand the molecular principles underlying anticipated pore activation mechanism of Cam1.

Publications from this work

a C. F. Baca, Y. Yu, J. T. Rostøl, P. Majumder, D. J. Patel, L. A. Marraffini; The CRISPR effector Cam1 mediates membrane depolarization to provide phage defense during the Type III CRISPR-Cas immune response; Nature. 2024 Jan;625(7996):797-804. PubMed Central PMCID: PMC10808066.

Structure guided mechanistic insights of Cad1 (CRISPR-associated adenosine deaminase 1):
I employed biophysical characterization and biochemical assays to find the signal cOAn molecules that activate Cad1 and its oligomeric states. I performed x-ray crystallography to solve the structure of the CARF domain of Cad1 in the apo, cA4 and cA6 bound forms at 3.6 Å, 2.4 Å and 1.8 Å resolution respectively revealing the mechanism of signal recognition by the dimeric CARF domains during CRISPR antiphage defense. The cryo-EM structure of the full-length Cad1 in the apo state, the ATP-bound state and cA4 bound state in presence of ATP at 3.4 Å, 3.6 Å and 3.2 Å resolution respectively along with collaborative in-vivo functional studies in the Marraffini lab revealed that Cad1 is a hexameric, cA4/cA6 activated, metal dependent, ATP to ITP deaminase. Additionally, the ATP bound apo and cA4-bound Cad1 cryo-EM structures lead to the identification of a unique ATP binding site at the interface of the CARF and deaminase domains which was required for activity. This pocket binds double the number of ATPs in the cA4-bound structure indicating its involvement in ATP sequestration upon signal recognition. Cad1 mediated ATP depletion leads to growth arrest of the phage infected bacteria and stops the spread of viral particles to the rest of the population [a].

Publications from this work

a C.F.Baca,[‡] P. Majumder,[‡] J. H. Hickling, L. Ye, S. F. Brady, D. J. Patel, L. A. Marraffini; CRISPR systems convert ATP to ITP by the Cad1 adenosine deaminase to provide antiviral immunity; Cell. Oct 2024;187, 7183-7195; PubMed PMID: 39471810 **([‡]co-first author)**

- Structure of Cat1 filaments (CRISPR-associated TIR 1) elucidates it's mechanism of defense:
I have utilized cryo-EM microscopy to solve atomic resolution structure of active Cat1 (cA4-Cat1) protein exhibiting unique filament assembly. Our results showed that apo-Cat1 dimers assemble into filaments upon cA4 signal recognition by dimeric CARF domains which form the center of the filament and the TIR domains are radially arranged. Three of these filament-sheets assemble to form a trigonal spiral bundle by inter-filament association with a trigonal pore at the center which further expands to form a pentagonal spiral bundle with a large pentagonal pore in the

middle. The large filament network of active Cat1 effector is required for its potent activity as illustrated by the impact of inter-filament association breaking mutants, both in vivo and in vitro. A pair of TIR domains are required to form a functional composite NADase pocket in active Cat1 protein as displayed by two cryo-EM structures of cA4-Cat1 with NAD analog BAD and with NAD; the finding was further verified by Cat1 active site single-mutants, both in vivo and in vitro. Next, we showed that upon cA4 recognition, Cat1 hydrolyses NAD to ADPR and NAM, thereby depleting NAD in phage infected bacterial cells during CRISPR defense. The depletion of NAD leads to the growth arrest of the host bacteria that prevents phage propagation [a].

Upcoming publication from this work:

a C.F.Baca,[‡] P. Majumder,[‡*] J. H. Hickling, D. J. Patel, L. A. Marraffini; Cat1 forms filament networks to degrade NAD^+ during the type III CRISPR-Cas anti-viral response; Upcoming in Science **([‡]co-first & [*]co-corresponding author)**

References

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Prof. Luciano Marraffini
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Prof. Aravind Penmatsa
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