BIOGRAPHICAL SKETCH

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NAME: Dinshaw J. PATEL

eRA COMMONS USER NAME (credential, e.g., agency login): PATELDJ

POSITION TITLE: Member and Abby Rockefeller Mauze Chair in Experimental Therapeutics

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
University of Mumbai, Mumbai, India. Chemistry	BS	1961	Chemistry
California Institute of Technology, Pasadena, CA.	MS	1963	Chemistry
New York University, New York, NY.	PhD	1968	Chemistry
New York Univ. Medical School New York, NY.	Postdoc	1967	Biochemistry
AT&T Bell Laboratories, Murray Hill, NJ.	Postdoc	1968-1969	Biophysics

A. PERSONAL STATEMENT

I received my PhD in Chemistry from New York University (NYU) in 1968 for research in the photochemistry. I decided next to shift the emphasis of my research to the life sciences and hence completed postdoctoral training (one year) in Biochemistry at NYU School of Medicine followed by postdoctoral training (two years) in Biophysics at AT&T Bell Laboratories. I was next promoted to permanent Member of Technical Staff at Bell Labs and spent the next 15 years undertaking NMR-based studies of the structure and dynamics of cyclic peptides, proteins and nucleic acids. I moved to Columbia University Medical School in 2004 as a tenured Professor of Biochemistry and Molecular Biophysics where my group spent the next 8 years doing NMR-based research on DNA mismatches, bulges and junctions, on DNA triplexes and G-quadruplexes, and drug-DNA complexes. I was recruited in 1992 as a tenured Member to the Cellular Biochemistry and Biophysics Program at the Memorial Sloan-Kettering Cancer Center to set up a Structural Biology component to the program. My group's research during the 1990s focused on NMR-based studies of covalent chiral carcinogen-DNA adducts, and complexes of antibiotics and peptides with natural and *in vitro* selected RNA targets.

My laboratory began to increasingly use x-ray crystallography starting around 2000 with the emphasis initially on RNA-mediated gene regulation, with subsequent extension to histone-mark and DNA-mark mediated epigenetic regulation, to lipid transfer proteins, and more recently to nucleic acid pattern recognition receptors and CRISPR-Cas surveillance complexes. We have complemented our structural efforts with functional studies undertaken by collaborators to deduce mechanistic insights into the biological systems of interest.

Starting in 2019, my group has increasingly used cryo-EM to study macromolecular structure, recognition and regulation.

Dr. Patel has published 550 papers and reviews. His h-index (Google Scholar) is 144.

B. Positions and Honors Appointments

1970 - 1984 Member of Technical Staff, Polymer Chemistry Department,
AT&T Bell Laboratories, Murray Hill, NJ
1984 - 1992 Professor of Biochemistry & Molecular Biophysics,

College of Physicians & Surgeons, Columbia University, New York, NY
1992 - Member, Structural Biology Program
Memorial Sloan-Kettering Cancer Center (MSKCC), New York, New York
1994 - Professor, Graduate Program in Biochemistry & Structural Biology,
Weill School of Medical Sciences, Cornell University, New York, NY

Honors and Awards

1961 - 1963 Jamshetjee N. Tata Fellow
1983 AT&T Bell Laboratories Distinguished Technical Staff Award
1992 - Abby Rockefeller Mauzé Chair in Experimental Therapeutics (MSKCC)
1997 Distinguished Alumnus Award, New York University
1997 - 1999 Harvey Society (Vice-President 97-98; President 98-99)
2013 NIH Directors Transformative R01 Award (with Thomas Tuschl and Uwe Ohler)
2014 2014 FEZANA Jamshed and Shirin Guzdar Excellence in Profession Award
2015 Einstein Professorship of Chinese Academy of Sciences, China
2019 Lifetime Achievement Award, American Association of Indian Scientisits in Cancer Research
2019 Inaugural Tan Jiazhen International Life Science Collaboration Award
2023 The Shizhang Bei International Award

Academy Memberships

2009 Member, National Academy of Sciences, USA
2014 Member, American Academy of Arts and Sciences, USA

External Review Committees

1984 - 2005 National Institutes of Health, Bethesda, MD
• Member, Molecular and Cellular Biophysics Study Section (84-88)
• National Cancer Institute, Board of Scientific Counselors-B (00-05)
1989 - 1996 Howard Hughes Medical Institute, Chevy Chase, MD
• Member, Scientific Review Board - Structural Biology (89-92)
• Member, Medical Advisory Board (93-96)
2015 - Joint Center for Life Sciences, Tsinghua-Beijing Universities, Beijing, China
2017 - 2018 Watson-Cheerland Precision Medicine Institute, Shenzhen, China

Scientific Advisory Boards

2009 - 2018 European Institute of Chemistry & Biology, Bordeaux, France
2010 - 2011 Epinova, GlaxoSmithKline, Stevenage, United Kingdom
2011 - 2022 Institute for Research in Biomedicine, Barcelona, Spain
2016 - Beijing Advanced Innovation Center for Structural Biology, Beijing, China
2016 - Center for Life Sciences, Harbin Institute of Technology, Harbin, China
2019 - 2020 Shenzhen Bay Area Committee, Shenzhen, China
2019 - Biology Department, Southern University of Science and Technology, Shenzhen, China

Consultancies

2020 - 2022 Ventus Pharmaceuticals, Boston, MA, USA

C. CONTRIBUTIONS TO SCIENCE

The publication list below is restricted to the Patel labs studies on class 1 and 2 CRISPR-Cas surveillance pathways, as well as prokaryotic and eukaryotic Argonaute silencing compolexes and the cGAS-STING pathway.

1. Single-subunit CRISPR-Cas Surveillance Systems and their Anti-CRISPR Complexes

Efficient and site-specific genome engineering can be achieved based on programmable dsDNA cleavage using CRISPR-Cas systems. Our structural studies on single-component Cas complexes are shedding light on the principles underlying cleavage chemistry of dsDNA and RNA targets. Future challenges include an

understanding of the diverse mechanisms adopted by distinct CRISPR-Cas systems in efforts to broaden and enhance their applicability as genome editing tools. Efforts are also underway to provide a structural understanding of recognition principles involving evolved bacteriophage suppressor proteins that inhibit the CRISPR-Cas pathway, thereby regulating the genome engineering activities of CRISPR-Cas systems.

Gao, P., et al. & Patel, D. J. (2016). Type V CRISPR-Cas Cpf1 endonuclease employs a unique mechanism for crRNA-mediated target recognition. ***Cell Research*** 26, 901-913. PMCID: PMC4973337.

Yang, H., et al., & Patel, D. J. (2017). PAM-dependent target DNA recognition and cleavage by C2c1 CRISPR-Cas endonuclease. ***Cell*** 167,1814-1828. PMCID: PMC5278635.

Yang, H. & Patel, D. J. (2017). Inhibition mechanism of an anti-CRISPR suppressor targeting SpyCas9. ***Mol. Cell*** 67, 117-127. PMCID: PMC5595222.

Meeske, A. J., Jia, N., et al., Patel, D. J. & Marraffini, L. A. (2020). Phage-encoded anti-CRISPR enables full escape from type VIA CRISPR-Cas immunity. ***Science*** 369, 54-59.

2. Multi-subunit CRISPR-Cas Surveillance Systmes and their Anti-CRISPR Complexes

The structural studies are also being extended to multi-component CRISPR-Cas systems and their anti-CRISPR complexes with an emphasis on type III systems given their ssRNA cleavage, ssDNA cleavage and cyclic-oligoadenylate (cOA) formation multiple activities. We are also interested in how cOA second messengers activate and regulate CARF domain containing RNases and DNases [collaborators: Sriram Subramanian (NCI) and Luciano Marraffinin (Rockefeller)].

Guo, T. W., et al., Patel, D. J. & Subramaniam, S. (2017). Cryo-EM structures reveal mechanism and inhibition of DNA targeting by a CRISPR-Cas surveillance complex. ***Cell*** 171, 414-426. PMCID: PMC5683424.

Jia, N., et al., Marraffini, L. A. & Patel, D. J. (2019). Type III-A CRISPR Csm complexes: Assembly, target RNA recognition, periodic cleavage and autoimmunity. ***Mol. Cell*** 73, 264-267. PMCID: PMC6355164.

Jia, N., et al., & Patel, D. J. (2019). Second messenger cA_4 formation within the composite Csm1 Palm pocket of type III-A CRISPR-Cas Csm complex and its release path. ***Mol. Cell*** 75, 933-943. PMCID: PMC6731140.

Jia, N., et al., & Patel, D. J. (2019). CRISPR-Cas III-A Csm6 CARF domain is a ring nuclease triggering stepwise cA_4 cleavage with ApA>p formation terminating RNase activity. ***Mol. Cell*** 75, 944-956. PMCID: PMC6731128.

3. Prokaryotic Argonaute Silencing Complexes

In the RNA silencing area, my group has made fundamental discoveries related to the structural biology of prokaryotic Argonaute proteins and their complexes with guide and target strands, thereby providing mechanistic insights into the nucleation, propagation and cleavage steps of Ago-mediated cleavage of mRNA [collaborator: Thomas Tuschl (Rockefeller) and David Bartel (MIT)].

Wang, Y., et al., Tuschl, T. & Patel, D. J. (2008). Structure of the guide-strand-containing argonaute silencing complex. ***Nature*** 456, 209-213. PMCID: PMC4689319.

Wang, Y., et al., Tuschl, T. & Patel, D. J. (2008). Structure of an argonaute silencing complex with a seed-containing guide DNA and target RNA duplex. ***Nature*** 456, 921-926. PMCID: PMC2765400.

Wang, Y., et al., Tuschl, T. & Patel, D. J. (2009). Nucleation, propagation and cleavage of target RNAs in Ago silencing complexes. ***Nature*** 461, 754-761. PMCID: PMC2880917.

Swarts, D. C., et al., Patel, D. J., Berenguer, J., Brouns, S. J. & van der Oost, J. (2014). DNA-guided DNA interference by prokaryotic Argonaute. ***Nature*** 507, 258-261. PMCID: PMC4697943.

4. Eukaryotic Argonaute Silencing Complexes

The research on prokaryote Argonautes has been extended to their eukaryotic counterparts, thereby identifying a catalytic tetrad responsible for modulation of cleavage activity [collaborator: David Bartel (MIT), Nam-Hai Chua (Rockefeller) and Mien-Chie Hung (M. D. Anderson Cancer Center)]

Zhang, X., et al. Tuschl, T., Patel, D. J. & Chua, N-H. (2006). Cucumber mosaic virus-encoded 2b suppressor inhibits *Arabidopsis* AGO1 cleavage activity to counter plant defense. ***Genes Dev.*** 20, 3255-3268. PMCID: PMC1686603.

Nakanishi, K., Weinberg, D. E., Bartel, D. P. & Patel, D. J. (2012). Structure of yeast Argonaute with guide RNA. ***Nature*** 486, 368-374. PMCID: PMC3853139.

Shen, J., et al., Patel, D. J. & Hung, M. C. (2013). EGFR modulates miRNA maturation in response to hypoxia through phosphorylation of Ago2. ***Nature*** 497, 383-387. PMCID: PMC3717558.

Nakanishi, K., et al., Tuschl, T. and Patel, D. J. (2013). Eukaryote-specific insertion elements control human ARGONAUTE slicer activity. ***Cell Reports*** 3, 1893-1900. PMCID: PMC3757560.

5. cGAS-STING Pathway

My group has recently turned its attention to the field of pattern recognition receptors that sense double-stranded nucleic acids in the cytosol, thereby triggering a cascade of events that activate the innate immune response. Our efforts have focused on cGAS, the metazoan sensor of cytosolic dsDNA, the second messenger cGAMP and the adaptor STING [collaborators: Thomas Tuschl (Rockefeller), Winfried Barchet (University Hospital-Bonn) and Roger Jones (Rutgers)]. Our structural studies identified cGAMP, produced by DNA-activated cGAS from GTP and ATP, to be c[G(2',5')pA(3',5')p], that contained an unanticipated 2',5' linkage at the GpA step. Our research was next extended to STING activation by cGAMP and targeting by the anti-viral agent DMXAA.

Gao, P., et al., Tuschl, T. & Patel, D. J. (2013). Cyclic [G(2',5')pA(3',5')p] is the metazoan second messenger produced by DNA-activated cyclic GMP-AMP synthase. ***Cell*** 153, 1094-1107. PMCID: PMC4382009.

Gao, P., et al., Tuschl, T. & Patel, D.J. (2013). Structure-function studies of STING activation by c[G(2',5')pA(3',5')p], its linkage isomers and DMXAA. ***Cell*** 154, 748-762. PMCD: PMC4386733.

Gao, P., et al., Hartmann, G., Tuschl, T., Deng, L., Barchet, W. & Patel, D. J. (2014). Binding pocket and lid region substituents render human STING sensitive to mouse-selective drug DMXAA. ***Cell Reports*** 8, 1668-1676. PMCID: PMC4381552.

Xie, W., et al., Tuschl, T. & Patel, D. J. (2018). Human cGAS catlytic domain has an additional DNA-binding interface that enhances enzymatic activity and liquid phase condensation. ***Proc. Natl. Acad. Scis. USA*** 116,11046-11955. PMCID: PMC6575157.

D. Additional Information: Research Support

ONGOING SUPPORT

NIH R01 GM129430 (Patel, PI) 04-08-19 to 03-31-24 NCE 2.4 cal. mths
Class I and III multi-subunit CRISPR-Cas surveillance complexes: recognition, cleavage, autoimmunity and inhibition.
Determine cryo-EM structures of Csy and Csm complexes to deduce mechanistic insights into target cleavage and its regulation, as well as principles underlying anti-CRISPR recognition and inhibition.
Role: PI

NIH R01 AI141507 (Tuschl, PI) 06-10-19 to 05-31-23 NCE 1.2 cal. mths
Development of small molecule cGAS inhibitors for repression of dsDNA-triggered interferon expression.
Design and structural characterization of small molecule inhibitors of cytoplasmic dsDNA sensor cGAS and their optimization.
Role: co-PI

Leukemia Lymphoma SCOR 7021020 (Licht, PI) 10-01-19 to 09-30-24 1.2 cal mths
Consortium for the study of epigenetic targeting in hematological malignancy.
Apply cryo-EM approaches to structurally characterize large complexes involved in writing, reading and erasing epigenetic marks.
Role: co-PI

MSKCC BRIA Grant (Zhao, PI) 10-01-22 to 09-30-24 0.6 cal mths
Mechanisms of Smc5/6 engagement and manipulation of DNA

A combined cryo-EM structural study and yeast genetics to investigate Smc5/6 binding to duplex and junctional DNAs to investigate the role of Smc5/6 in genome integrity.
Role: Co-PI

NIH 1U19 AI1711401 (Perlin, PI) 05-10-22 to 05-09-25 2.4 cal mths
Metropolitan Antiviral Drug Accelerator
A collaborative public-private partnership to deliver orally available small molecule antiviral drugs to treat infections caused by coronaviruses including SARS-CoV-2 and a variety of additional viruses of pandemic potential.
Role: co-Director: Structural Biology Core

NIH R01 GM145888 (Patel, PI) 08-15-22 to 08-14-26 1.2 cal mths
Structure-activity based mechanistic insights into cleavage chemistry by self-cleaving nucleolytic ribozymes.
This project will address structure-function studies of the hovlinc and related nucleolytic ribozymes.
Role: PI

NIH R01 HD110120 (Keeney, PI) 07-01-22 to 06-20-27 1.2 cal mths
Structural and functional principles undergoing germline genome transmission
This project will address fundamental questions about the mechanism and control of meiotic homologous recombination that is directly relevant to reproductive health and disease.
Role: co-PI

BIOGRAPHICAL SKETCH

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NAME: You, Yu

eRA : COMMONS USER NAME (credential, e.g., agency login): YUYOU3

POSITION TITLE: Senior Research Scientist

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Jilin University, Changchun, China	BS	09/2006	07/2010	Life Science
Tsinghua University, Beijing, China	PHD	09/2012	01/2017	Molecular Biology
Memorial Sloan Kettering Cancer Center	Researh Fellow	06/2017	05/2021	Structural Biology
Memorial Sloan Kettering Cancer Center	Research Associate	05/2021	06/2022	Structural Biology
Memorial Sloan Kettering Cancer Center	Senior Research Scientist	06/2022	Current	Structural Biology

A. PERSONAL STATEMENT

After completing my B.Sc and M.Sc in interdisciplinary backgrounds from New Delhi, India, I decided to pursue my PhD in Biochemistry from the University of Illinois at Urbana-Champaign. My interest in defensive strategies utilized by eukaryotes and prokaryotes throughout evolution enabled me to take up multiple projects revolving around antiviral and antibacterial proteins including the virus-induced protein, Viperin, the antibacterial protein of the Toxin-antitoxin system, RtcB and the much more recently characterized proteins of the antiphage CBASS systems. With the aim of deciphering more of such defensive strategies employed by bacteria and eukaryotes, I decided to pursue my post doctorate from Memorial Sloan Kettering Cancer Center in Structural Biology, which has provided me with a platform to undertake structure-guided approaches towards uncovering various mechanisms along with an excellent collaboration with leading scientists in the field. These mechanisms are part of a recently characterized universe of novel and elusive antiphage protective operons present in the dynamic pan-genomic defense islands of bacteria. By employing innovative structural and functional studies both at MSKCC and with its collaborators, I seek to add to my repertoire of uncovering mechanisms of phage-induced robust measures employed by bacteria which might have evolutionary links with the eukaryotic immune system.

During my early period Ph.D. training in Tsinghua University, I conducted studies to investigate the structure of Nickel/Cobalt ion specific ECF transporter, an ECF transporter consist of membrane protein NikM, CbiT and

soluble ATPase protein CbiO, which is essential for Nickel/Cobalt ion uptake. During my late period of Ph.D. I gained rich experience in studying membrane protein-drug molecular complex structures and functions. NADH-ubiquinone oxidoreductase of Plasmodium falciparum (PfNDH2) represents a viable target for antimalarial drug development. Structural and functional studies revealed the RRL-552 exhibits excellent potency against both drug-resistant strains in vitro and parasite-infected mice in vivo via a potential allosteric mechanism. I also worked on structural and functional study on acid-sensing ion channel (ASIC) with mambalgin1, which is toxin isolated from black mamba snake. I reported a cryo-EM structure of chicken ASIC in complex with mambalgin1 at 5.4 Å resolution. The structure provided the first experimental evidence that mambalgin-1 interacts directly with the extracellular thumb domain of cASIC1a, rather than inserting into the acid-sensing pocket, as previously reported. Binding of mambalgin-1 leads to relocation of the thumb domain that could disrupt the acidic pocket of cASIC, illustrating an unusual inhibition mechanism of toxins on ASIC channels through an allosteric effect.

In June 2017 I joined in Dr. Dinshaw Patel's laboratory at Memorial Sloan Kettering Cancer Center, I was trained to apply electron microscopy techniques to examine SMC complexes with DNA for structural study. I learned the Smc5/6 complex, MRN complex and Spo11 complex with DNA. These works suggested SMCs' DNA-clamp mechanisms and allowed us to understand the detailed interactions among the subunits and DNA.

I also investigated the role of cyclic-oligoadenylate-activated membrane protein 1 (Cam1) during the type III CRISPR immunity. Structural and biochemical analysis reveals that the CARF domain of Cam1 binds cyclic tetra-adenylate second messengers. *In vivo*, Cam1 localizes to the membrane, is predicted to form a tetrameric transmembrane pore, and provides defense against viral infection through the induction of membrane depolarization and growth arrest.

B. Positions, Scientific Appointments and Honors

2013 National Scholarship for Graduate Students, Ministry of Education of PRC.

2014 National Scholarship for Graduate Students, Ministry of Education of PRC.

2016 Innovation Award of Beijing Advanced Innovation Center for Structural Biology, 2016, Tsinghua University

2018 Outstanding Contribution in Reviewing, Elsevier Publishing Company.

C. Contributions to Science (* denotes co-first authors)

1) 2010-2014 (Graduate Research Assistant), College of Life Science, Tsinghua University
Project: Nickel/Cobalt specific ECF transporter.

- We purified Ecf-S (NikM, membrane protein) and Ecf-A (NikO, soluble protein) components from Nickel/Cobalt specific ECF transporter. We crystallized NikM with Nickel/Cobalt ion and NikO in complex with DDM detergent. X-ray data collection and data procession results showed the first time structures of NikM at 1.83 Å resolution and NikA+DDM at 2.30 Å resolution.

•We identified the ion types in NikM through X-ray fluorescence spectrum and the anomalous scattering signal screening. Structural analysis and comparison indicated that NkiM unique N terminal loop is required for ion binding.

•Nickel ion uptake activity analysis and quantum chemical analysis identified the substrate selection of NikM and key residues for nickel ion binding of NikM.

•Structural analysis of NikO indicated that NikO forms a C terminal-mediated homodimer, with unexpected DDM binding in the N terminal canonical nucleotide-binding domain (NBD)

a. **Yu, Y**., Zhou, M., Kirsch, F., Xu, C., Zhang, L., Wang, Y., Jiang, Z., Wang, N., Li, J., Eitinger, T., and Yang, M. (2014) Planar substrate-binding site dictates the specificity of ECF-type nickel/cobalt transporters. ***Cell research*** 24, 267-277
b. Chai, C.*, **Yu, Y.***, Zhuo, W.*, Zhao, H., Li, X., Wang, N., Chai, J., and Yang, M. (2013) Structural basis for a homodimeric ATPase subunit of an ECF transporter. ***Protein & cell*** 4, 793-801.

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2) 2014-2016 (Graduate Research Assistant), College of Life Science, Tsinghua University
Project: Protein complex between Pathogenic bacteria/HIV protein and human host

•We cooperated with the Yongqiang Jiang lab to purify, crystallize and determine the structure of the complex structure of *Streptococcus suis* adhesin factor H-binding protein (Fhb) with Gb2 (host cellular receptor glycolipid GbO3 analog), which provided structural insight into Fhb-mediated host-pathogen interactions of *S. suis*.

•Cooperated with the Zhiwei Huang lab to solve the complex structure of the Vif–CBF-β–CUL5–ELOB–ELOC complex from HIV and human host, which revealed the structural basis for Vif(HIV) hijacking of the CBF-β(human host) and CUL5 E3 ligase complex (human host).

a. Guo, Y. Y.*, Dong, L. Y.*, Qiu, X. L.*, Wang, Y. S., Zhang, B. L., Liu, H. N., **Yu, Y.**, Zang, Y., Yang, M. J., and Huang, Z. W. (2014) Structural basis for hijacking CBF-beta and CUL5 E3 ligase complex by HIV-1 Vif. ***Nature*** 505, 229-233
b. Zhang, C., **Yu, Y.**, Yang, M., and Jiang, Y. (2015) Expression, purification, crystallization and structure determination of the N terminal domain of Fhb, a factor H binding protein from Streptococcus suis. ***Biochemical and biophysical research communications*** 466, 413-417
c. Zhang, C., Hao, H., Zhao, j., **Yu, Y.**, Kong, D., Chen, S., Jiang, H., Yuan, Y., Zheng, Y., Yang, M., Jiang, Y. (2016) Structural basis of the interaction between the meningitis pathogen Streptococcus suis adhesin Fhb and its human receptor. ***FEBS Lett.*** 590,1384-1392

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3). 2016-2017 (Graduate Research Assistant), College of Life Science, Tsinghua University
Project: Drug target (Membrane protein) complex with anti-malarial chemicals and molecular mechanisms of pain release

•Structural and functional studies of type-II NADH dehydrogenases (NDH2) from plasmodium falciparum (*Pf*NDH2). X-ray crystallography to determine the high resolution (higher than 3.0 Å) structures of *Pf*NDH2 with different substrates and three inhibitors, respectively. Our study revealed a novel mechanism for inhibition of *Pf*NDH2 with allosteric inhibitors and affirms *Pf*NDH2 as a valid drug target for anti-malarial treatment.

•Acid-sensing ion channels (ASICs) are neuronal voltage-independent Na^+ channels, which are new potential therapeutic targets in the management of psychiatric disorders, neurodegenerative diseases and pain. Mambalgin-1 (isolated from black mamba venom) specifically inhibits ASICs to exert strong analgesic effects and are thought to have therapeutic potential against pain. We purified the complex and solved the cryo-EM structure of chicken ASIC1a (cASIC1a) in complex with mambalgin-1, established a structural basis for its channel inhibition mechanism and provided crucial insights for the development of new optimized inhibitors of ASICs.

a. Yang, Y*., **Yu, Y***., Li, X*., Li, J., Wu, Y., Yu, J., Ge, J., Huang, Z., Jiang, L., Rao, Y. and Yang, M., (2017) Target Elucidation by Cocrystal Structures of NADH-Ubiquinone Oxidoreductase of Plasmodium falciparum (Pf NDH2) with Small Molecule To Eliminate Drug-Resistant Malaria. ***Journal of medicinal chemistry***, *60*(5), pp.1994-2005.
b. Sun, D.*, **Yu, Y*.,** Xue, X*., Pan, M*., Wen, M., Li, S., Qu, Q., Li, X., Zhang, L., Li, X., Liu, L., Yang, M., Tian, C., (2018) Cryo-EM structure of the ASIC1a–mambalgin-1 complex reveals that the peptide toxin mambalgin-1 inhibits acid-sensing ion channels through an unusual allosteric effect**.** ***Cell Discovery*** 4:27. *

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4) 2017-current (Senior Research Scientist), Structural Biology Program, Memorial Sloan Kettering Cancer Center

Project: Structural and functional study on SMC5/6 complex and MRN complex.

•The Smc5/6 complex plays multiple roles in DNA replication and repair. Its genome-protecting functions rely on its interaction with DNA. Human Smc5/6 is uniquely emerging amongst SMC complexes as a critical viral restriction factor. Further, Smc5/6 inhibits the transcription and/or replication of several viruses including hepatitis B, herpes simplex (HSV-1), human papillomavirus (HPV), Epstein-Barr virus (EBV), and unintegrated human immunodeficiency virus (HIV). We first solved the cryo-EM structure of Nse5-6 subcomplex at 3.2 Å resolution, which is a unique feature of the SMC5/6 complex distinct from other SMC complexes. We purified SMC5/6 core complex (hexamer: Smc5-Smc6-Nse2-Nse3-Nse4) and solved the cryo-EM structue of SMC5/6 core complex with ATP and dsDNA. Finally, we got the cryo-EM structure of SMC5/6-ATP-DNA complex at the resolution 3.8 Å resolution. Our studies revealed the structures of all SMC5/6 subunits at atomic-level resolution for the first time and claimed that the SMC5/6 subunits form a clamp to encircle a double helical DNA. We identified subunit transformations upon DNA capture and functional impact of multiple DNA contact sites. These studies laid the foundation for an in-depth under-standing of how Smc5/6 fulfill unique roles in genome protection and anti-virus process.

systems including viperin, CdnG, Cap5 and others and contributed to the discovery of novel small molecule termed AIPP, the generation of which is predicted to be the mechanism through which viperin executes its antiviral functions. Viperin is known to be induced in a variety of viral infections including Dengue, HIV and Influenza and the discovery of AIPP by fungal, archaeal and human viperin potentially added to the list of ways in which viperin inhibits viruses. Additionally, my work on the CdnG-Cap5 cyclic-oligonucleotide driven CBASS system led to the discovery of a novel small molecule with a unique linkage specificity termed as 3',2'-cGAMP which was 10,000-fold more potent in activating the operonically linked Cap5 nuclease effector as compared to other similarly reported cyclic-dinucleotide ligands. This led us to publish our manuscript in a top journal where it was selected as a featured article. Furthermore, I have also worked on cyclic mononucleotide based antiphage systems which are different from CBASS.

a. Li, S.*, Yu, Y.*, Zheng, J., Miller-Browne, Victoria., Zheng, S., Kuang, H., Patel, D.J., and Zhao, X.(Submitted). Molecular basis for Nse5-6 mediated regulation of Smc5/6 functions. ***Proceedings of the National Academy of Sciences***.
b. **Yu, Y**. *, Li, S. *, Ser, Z., Kuang, H.H., Than, Thane., Guan, D.Y., Zhao, X and Patel, D.J., (2022). Cryo-EM structure of DNA-bound Smc5/6 reveals DNA 5 clamping enabled by multi-subunit conformational changes. ***Proceedings of the National Academy of Sciences***, 119(23).
c. **Yu, Y**. *, Li, S. *, Ser, Z. *, Sanyal, T. *, Choi, K., Wan, B., Kuang, H., Sali, A., Kentsis, A., Patel, D.J. and Zhao, X., (2021). Integrative analysis reveals unique structural and functional features of the Smc5/6 complex. ***Proceedings of the National Academy of Sciences***, 118(19).

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5) 2020-current (Senior Research Scientist), Structural Biology Program, Memorial Sloan Kettering Cancer Center

Project: Type III CRISPR-Cas immune response by Cam1 mediates membrane depolarization.

Prokaryotic type III CRISPR-Cas systems provide immunity against viruses and plasmids using CARF protein effectors. Recognition of specific sequences of these invaders by crRNA guides leads to the production of cyclic oligoadenylate second messengers, which bind CARF domains and trigger the activity of an effector domain. While most effectors degrade host and invader nucleic acids, some are predicted to contain transmembrane helices (TMH) without an enzymatic function. Whether and how these CARF-TMH fusion proteins facilitate the type III CRISPR-Cas immune response has not been studied. Here we investigate the role of cyclic-oligoadenylate-activated membrane protein 1 (Cam1) during the type III CRISPR immunity. Structural and biochemical analysis reveals that the CARF domain of Cam1 binds cyclic tetra-adenylate second messengers. In vivo, Cam1 localizes to the membrane, is predicted to form a tetrameric transmembrane pore, and provides defense against viral infection through the induction of membrane depolarization and growth arrest. These results reveal that CRISPR immunity do not always operate through the degradation of nucleic acids, as initially thought, but through a wider range of cellular responses.

a. Baca, C.F. *, **Yu, Y. *,** Rostol, J.T.*, Majumder, P., Patel, D.J. and Marraffini, L.A., (2023). Cam1 mediates membrane depolarization to provide phage defense during the Type III CRISPR-Cas immune response. ***Nature, (in revised)***.
b. Patel, D.J., **Yu, Y.**, Xie, W., (2023) cGAMP-activated cGAS-STING signaling: its bacterial origins and evolutionary adaptation by metazoans. ***Nature Structural & Molecular Biology***, 352(2), 1-16.
c. Patel, D.J., **Yu, Y.**, Jia, N., (2022) Bacterial origins of cyclic nucleotide-activated antiviral immune signaling. ***Molecular Cell***, 82(24) 4591-4610.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: ARPITA CHAKRAVARTI

eRA : COMMONS USER NAME (credential, e.g., agency login): ARPITA_CHAKRAVARTI

POSITION TITLE: RESEARCH SCHOLAR

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Bhaskaracharya College of Applied Sciences, New Delhi	B.Sc (HON)	06/2009	06/2012	Biomedical Sciences
Jawaharlal Nehru University, New Delhi	M.Sc	07/2012	07/2014	Life Sciences
University of Illinois at Urbana-Champaign	Ph.D	05/2015	05/2022	Biochemistry
Memorial Sloan Kettering Cancer Center	Post Doc	08/2022	-	Structural Biology

A. PERSONAL STATEMENT

After completing my B.Sc and M.Sc in interdisciplinary backgrounds from New Delhi, India, I decided to pursue my PhD in Biochemistry from the University of Illinois at Urbana-Champaign. My interest in defensive strategies utilized by eukaryotes and prokaryotes throughout evolution enabled me to take up multiple projects revolving around antiviral and antibacterial proteins including the virus-induced protein, Viperin, the antibacterial protein of the Toxin-antitoxin system, RtcB and the much more recently characterized proteins of the antiphage CBASS

systems. With the aim of deciphering more of such defensive strategies employed by bacteria and eukaryotes, I decided to pursue my post doctorate from Memorial Sloan Kettering Cancer Center in Structural Biology, which has provided me with a platform to undertake structure-guided approaches towards uncovering various mechanisms along with an excellent collaboration with leading scientists in the field. These mechanisms are part of a recently characterized universe of novel and elusive antiphage protective operons present in the dynamic pan-genomic defense islands of bacteria. By employing innovative structural and functional studies both at MSKCC and with its collaborators, I seek to add to my repertoire of uncovering mechanisms of phage-induced robust measures employed by bacteria which might have evolutionary links with the eukaryotic immune system.

B. Positions, Scientific Appointments and Honors

2023 Awarded the Anne. A Johnson Outstanding PhD Student Award

2022 Robert L. Switzer award in Excellence in teaching, Department of Biochemistry, University of Illinois, Urbana-Champaign

2021 Nature Communications Editor's Highlights, Manuscript on antiphage defense systems

2021 Teachers ranked as excellent Department of Biochemistry, University of Illinois, Urbana-Champaign

2013 All India Rank of 36, National Entrance Test (Junior Research Fellowship)), India, 2013

2012 DBT Builder Scholarship, Department of Life Sciences, Jawaharlal Nehru University

2012 Delhi State rank of 6, Delhi University, Genetics Examination.

C. Contributions to Science

Throughout my career, I have worked with proteins and reaction mechanisms of a variety of antiphage defense systems including viperin, CdnG, Cap5 and others and contributed to the discovery of novel small molecule termed AIPP, the generation of which is predicted to be the mechanism through which viperin executes its antiviral functions. Viperin is known to be induced in a variety of viral infections including Dengue, HIV and Influenza and the discovery of AIPP by fungal, archaeal and human viperin potentially added to the list of ways in which viperin inhibits viruses. Additionally, my work on the CdnG-Cap5 cyclic-oligonucleotide driven CBASS system led to the discovery of a novel small molecule with a unique linkage specificity termed as 3',2'-cGAMP which was 10,000-fold more potent in activating the operonically linked Cap5 nuclease effector as compared to other similarly reported cyclic-dinucleotide ligands. This led us to publish our manuscript in a top journal where it was selected as a featured article. Furthermore, I have also worked on cyclic mononucleotide based antiphage systems which are different from CBASS.

1. Fatma, S*., Chakravarti, A*, Zeng, X. et al. (2021). Molecular mechanisms of the CdnG-Cap5 antiphage defense system employing 3′,2′-cGAMP as the second messenger. ***Nat Commun*** 12, 6381.

2. Chakravarti, A., Selvadurai, K., Shahoei, R., Lee, H., Fatma, S., Tajkhorshid, E., & Huang, R. H. (2018). Reconstitution and substrate specificity for isopentenyl pyrophosphate of the antiviral radical SAM enzyme viperin. ***The Journal of biological chemistry***, *293*(36), 14122–14133.

- These authors contributed equally.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: ABHISHEK SUMAN

eRA COMMONS USER NAME (credential, e.g., agency login): SUMAN_A

POSITION TITLE: Postdoctoral Research Scholar

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Lovely Professional University, Punjab, India	BTech	08/2009	05/2013	Biotechnology
ICMR-Rajendra Memorial Research Institute of Medical Sciences, Bihar, India	Research Intern	05/2013	07/2014	Microbiology
Lovely Professional University, Punjab, India	MTech	08/2014	05/2016	Biotechnology
Indian Institute of Technology Hyderabad, India	Ph.D.	07/2016	07/2022	Structural Biology
Memorial Sloan Kettering Cancer Center, New York, NY	Postdoctoral Research Scholar	02/2023	Present	Structural Biology

A. Personal Statement

I received my PhD in Structural Biology from the Indian Institute of Technology Hyderabad (IITH), India, where I worked with Prof. Eerappa Rajakumara to explore the mechanistic insights into the functioning of DNA binding proteins involved in epigenetic gene regulation. In my doctoral studies, I focused on exploring the binding preferences of epigenetic regulator proteins for different epigenetic marks on DNA and histone variants, using biochemical, biophysical, and computational approaches. I employed X-ray crystallography and computational biology approaches to explore the structural insights into epigenetic regulation. I also substantially contributed to the collaborative projects related to drug design and understanding their mechanisms of action, where we developed potential drug candidates for amyotrophic lateral sclerosis (ALS), which was featured as a 'Research highlight' in Nature India (Nature publishing group), and in vitro fertilization (IVF) which got national media coverage. I also started and established the computational biology facility in Prof. Rajakumara's lab. With substantial contribution, I co-authored 18 articles (15 published, 3 under review). One of my research publications was featured as the cover page article in Biochemistry (American Chemical Society publication). I also serve as a reviewer for scientific journals, including PLoS One, Biophysical Chemistry and Journal of Biomolecular Structure and Dynamics. Apart from research, I had also been involved in mentoring students, classroom teaching and live demonstrations, organizing workshops/seminars and was invited for a guest lecture.

Currently, I am a postdoctoral trainee in Prof. Dinshaw Patel group at Memorial Sloan Kettering Cancer Center for my postdoctoral training. Prof. Patel is an internationally recognized leader in Structural Biology, with a h-index (Google Scholar) of 144 and has an extensive record of training postdoctoral fellows. In the group, my research focuses on exploring structural and mechanistic insights into the bacterial antiphage defense systems for which I am employing multi-pronged approaches including cryogenic electron microscopy (Cryo-EM), macromolecular X-ray crystallography, and other biophysical and biochemical approaches. In addition, the postdoctoral training outlines a set of career development activities including grant writing, public speaking, and lab management to enhance my ability to become an independent investigator. This postdoctoral will be helpful in building a strong foundation for achieving my long-term research goal of exploring structural and mechanistic

insights into diverse biological phenomena, including epigenetic regulation, antiviral surveillance, and drug designing for disease treatment.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2023 – Present **Postdoctoral Research Scholar, Memorial Sloan Kettering Cancer Center, New York, NY**

2013 – 2014 **Research Intern, Indian Council of Medical Research (ICMR) – Rajendra Memorial Research Institute of Medical Sciences, Patna, Bihar, India**

Honors

2022 **'Excellence in Research' award, Indian Institute of Technology Hyderabad, India**

2018 – 2022 **Senior Research Fellowship, Ministry of Education, Govt. of India**

2016 – 2018 **Junior Research Fellowship, Ministry of Education, Govt. of India**

2016 **All India Rank 9 in General Aptitude Test in Engineering (GATE)**

2016 **M. Tech awarded with distinction, Lovely Professional University, India**

C. Contributions to Science

1. **Epigenetic regulation:**
DNA and histone modifications, also known as 'epigenetic marks', play an important role in regulating gene expression. These modifications are recognized by specialized domains in regulator proteins that further relays a signal for downstream processing. The dual domain cassette containing Tandem Tudor domain (TTD) and Plant Homeodomain (PHD) of UHRF1, a eukaryotic protein, is one such 'reader' domain that has been known to recognize tri-methylated lysine 9 on histone H3 (K3K9me3) and unmodified arginine 2 (H3R2) through TTD and PHD domains, respectively. Our studies showed that TTD domain has a higher preference for di-methylated H3K9. PHD finger of another protein VIM1, which has similar sequence feature as PHD finger of UHRF1, doesn't recognize H3R2 mark. Our biophysical, structural, and computational studies revealed that though the sequence features are conserved, there is the conformational changes in the peptide recognition motifs lead to abrogation of H3R2 readout. Similarly, SRA domain recognizes methylated cytosine in DNA. SRA domain of UHRF1 (SRA_{UHRF1}) is. Known to be a bona fide reader of hemi-methylated cytosine in CpG DNA context (hemi-mCpG). Our biophysical and structural studies revealed that SRA_{UHRF1} also recognizes fully-methylated cytosine not only in CpG DNA (fully-mCpG) context, but also in CpHpG DNA (fully-mCpHpG), however the stoichiometry of protein:DNA is 1:1 in prior and 2:1 in the later contexts. Another SRA domain containing protein, SUVH5 from plant origin, recognizes methyl cytosine is all the DNA contexts (CpG, CpHpG and CpHpH) through SRA domain. In addition, SUVH5 also performs H3K9 methylation through SET domain. Our studies revealed that there is an allosteric crosstalk between the two domains. We also employed quantum-mechanical (QM) calculations to determine why the catalytic activity of the SET domain of SUVH5 is limited to di-methylation of H3K9 and not extended to tri-methylation.

    a. **Abhishek S**, et al. (2018). Biochemical and dynamic basis for combinatorial recognition of H3R2K9me2 by dual domains of UHRF1. *Biochimie*, 149, 105–114.

    b. **Abhishek S**, et al. (2021). Mechanistic insights into recognition of symmetric methylated cytosines in CpG and non-CpG DNA by UHRF1 SRA. *Int J Biol Macromol*, 170, 514-522.

    c. **Abhishek S**, Deeksha W & Rajakumara E. (2021) Helical and β-turn conformations in the peptide recognition regions of the VIM1 PHD finger abrogate H3K4 peptide recognition. *Biochem*, 60, 2652-62.

    d. **Abhishek S**, Deeksha W & Rajakumara E (2023) Mechanistic insights into allosteric regulation of methylated DNA and histone H3 recognition by SRA and SET domains of SUVH5 and the basis for di-methylation of lysine residue. *FEBS J*, 290 (4), 1060-1077.

e. Satish M, Nivya MA, **Abhishek S**, et al. (2018). Computational characterization of substrate and product specificities, and functionality of S-adenosylmethionine binding pocket in histone lysine methyltransferases from Arabidopsis, rice and maize. *Proteins*, 86(1), 21–34.

2. DNA repair:

ADP-ribosylation is an important epigenetic mark which is catalyzed by PARP1, the genome guardian protein. PARP1 has been known to sense DNA breaks – double strand break (DSB) and single strand break (SSB). We used biochemical and biophysical approaches to unravel the novel feedback allosteric regulation of PARP1 by its catalytic product poly(ADP-ribose) (PAR). We also showed that PARP1 not only senses DNA breaks, but also single stranded DNA (ssDNA), which further suggests the yet unexplored role of PARP1 in ssDNA regulation. We also unraveled the regulation and mechanism of PARP1 in apoptosis.

a. Deeksha W, **Abhishek S** & Rajakumara E. (2022) PAR recognition by PARP1 regulates DNA-dependent activities and independently stimulates catalytic activity of PARP1. BioRxiv, 2021.12.21.473685. *(Under review)*

b. Deeksha W, **Abhishek S**, Giri J & Rajakumara E. (2023) Regulation of PARP1 and its apoptotic variant activity by single-stranded DNA. *FEBS J*. 2023 May 28.

3. Drug/vaccine design:

In a collaboration with Dr. Guruprasad Kalthur, Manipal Academy of Higher Education, India, we design and optimize an inhibitor of sperm phosphodiesterase variants (PDEs) to enhance sperm functions for effective the treatment of infertility in males, using computational approaches. We further confirmed it by in vitro and in ex vivo experiments. In collaboration with Dr. Basant K Patel, IIT Hyderabad, we developed and tested a small molecule inhibitor, named as AIM4, against TDP-43, a key protein responsible for ALS. We also employed computational approaches to propose the mechanism of action of Auranofin, a known inhibitor of thioredoxin/glutathione reductases (TR/GRs) in different pathogens, including bacteria and protozoa. We showed that Auranofin has different target sites in bacterial and protozoal TRs/GRs. Using computational approaches, we also designed a multi-epitope fused ferritin nano-cage based vaccine candidate against SARS-CoV-2.

a. Satish M, Kumari S, Deeksha W, **Abhishek S**, et al. (2021) Structure based redesigning of pentoxifylline against selective phosphodiesterases to modulate sperm functional competence for assisted reproductive technologies. *Sci Rep*, 11, 12293.

b. Raj G, **Abhishek S**, Nitin K, Sreenath D, Rajakumara E. Computational, in vitro binding and competition binding studies of theophylline against phosphodiesterases functioning in sperm. *(Under review)*

c. Girdhar A, Bharathi V, Tiwari VR, **Abhishek S**, et al. (2020). Computational insights into mechanism of AIM4-mediated inhibition of aggregation of TDP-43 protein implicated in ALS and evidence for in vitro inhibition of liquid-liquid phase separation (LLPS) of TDP-432C-A315T by AIM4. *Int J Biol Macromol*, 147, 117–130.

d. **Abhishek S**, et al. (2019). Dynamic Basis for Auranofin Drug Recognition by Thiol-Reductases of Human Pathogens and Intermediate Coordinated Adduct Formation with Catalytic Cysteine Residues. *ACS Omega*, 4(5), 9593–9602.

e. Pratibha M, **Abhishek S** & Rajakumara E. (2022) Designing ferritin nanocage based vaccine candidates for SARS-CoV-2 by in silico engineering of its MHC I and MHC II epitope peptides. *J Biomol Struct Dyn*, Jul 22.

4. Other articles (published / under review)

Apart from above mentioned areas, I have a key contribution in other projects related to mechanistic studies on DNA bending by high mobility group (HMG) proteins, elucidating structure-function relationship of enzyme, mechanistic studies on toxin-induced stress in zebrafish, formulation of culture medium for

Leishmania species. I have also co-authored review articles related to enzyme engineering, minichromosome maintenance, and allosteric regulation of proteins.

a. Rajakumara E, Satish M, & **Abhishek S**. (2020). In vitro studies on non-canonical DNA binding specificities of KAP6 and HMO1 and mechanistic insights into DNA bound and unbinding dynamics of KAP6. *Int J Biol Macromol*, 160, 925–933.

b. Uma Mahesh MVN, **Abhishek S**, Faidh MA, Rajakumara E & Chadha A. Structure and mechanism of an Ornithine cyclodeaminase/μ-crystallin homolog from the yeast Candida parapsilosis ATCC 7330. *(Under review)*

c. Pullaguri N, Grover P, **Abhishek S**, et al. (2021). Triclosan affects motor function in zebrafish larva by inhibiting ache and syn2a genes. *Chemosphere*, 266, 128930.

d. **Abhishek S**, et al. (2021) A simple monophasic LGPY medium for routine maintenance of Leishmania donovani promastigotes. *Parasitol Res*, 120, 1269-71.

e. Rajakumara E, **Abhishek S**, et al. (2022) Structure and Cooperativity in Substrate-Enzyme Interactions: Perspectives on Enzyme Engineering and Inhibitor Design. *ACS Chem Biol*, 17, 266-280.

f. Mehta G, Sanyal K, **Abhishek S**, et al. (2022) Minichromosome maintenance proteins in eukaryotic chromosome segregation. *Bioessays*, 44, e2100218.