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

Dinshaw J. PATEL

Member and Abby Rockefeller Mauzé Chair in Experimental Therapeutics
Structural Biology Program
Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, NY, 10065, USA

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Naturalized US citizen: 1990.

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 540 papers and reviews. His h-index (Google Scholar) is 126.

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

Awards and Honors

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

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 -	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 -	Shenzhen Bay Area Committee, Shenzhen, China
2019 -	Biology Department, Southern University of Science and Technology, Shenzhen, China

C. CONTRIBUTIONS TO SCIENCE

Pertinent to this application, Dr. Patel has used structural biology and biochemistry to fundamentally advance our understanding of molecular mechanisms underlying cGAS-STING and CRISPR-Cas surveillance pathways, as well as piRNA-mediated protection of genome integrity.

The publication list below is restricted to these topics.

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.

Inhibitors Targeting cGAS

We are interested in identifying activators and inhibitors of human cGAS and STING given the importance of the cGAS-STING patway in innate immunity. To date, we have made progress in identifying inhibitors of cGAS that exhibit distinct specificities for mouse versus human cGAS [collaborators: Fraser Glickman and Thomas Tuschl (Rockefeller) and Roger Jones (Rutgers)].

Vincent, J., et al., Tuschl, T., Patel, D. J., Glickman, J. F. & Ascano, M. (2017). Small molecule inhibition of cGAS reduces interferon expression in primary macrophages from autoimmune mice. ***Nat. Commun.*** 8:750. PMCID: PMC5622107.

Lama, L., et al., Glickman, J. F., Patel, D. J. & Tuschl, T. (2019). Development of human cGAS-specific small molecule inhibitors with biochemical and cell-based activity for repression of dsDNA-triggered interferon expression. ***Nat. Commun.*** 10: 2261. PMCID: PMC6529454.

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.

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.

piRNA-mediated Protection of Genome Integrity against Transposons

The PIWI-intercating RNA (piRNA) pathway protects genome integrity in part through establishing heterochromatin at transposon loci. The goal of the research is to decipher molecular events associated with silencing and the requirement of piRNA-guided targeting of nuclear PIWI proteins to nascent transposon transcripts [collaborator: Alexei Aravin, Caltech). To this end, we recently contributed to the determination that nascent RNA binding complex SFiNX licenses piRNA-guided heterochromatin formation [collaborators: Julius Brennecke, IMP, Austria}

Le Thomas, A., et al., Patel, D. J. & Aravin, A.A. (2014). Trans-generationally inherited piRNAs trigger piRNA biogenesis by changing the chromatin of piRNA clusters and inducing precursor processing. ***Genes Dev***. 28, 1667-1680. PMCID: PMC4980073.

Webster, A., et al., Patel, D. J. & Aravin, A. A. (2015). Aub and Ago3 are recruited to nuage through two mechanisms to form a ping-pong complex assembled by Krimper. ***Mol. Cell*** 59, 564-575. PMCID: PMC4545750.

Chen, A., et al., Patel, D. J., Smibert, C. A., Lipshitz, H. D., Toth, K. F. & Aravin, A. A. (2016). Cutoff suppresses RNA polymerase II termination to ensure expression of piRNA precursors. ***Mol. Cell*** 63, 97-109. PMCID: PMC4980073.

Batki, J., et al., Patel, D. J. and Brennecke, J. (2019). The SFINX complex licenses piRNA-guided heterochromatin formation. ***Nat. Struct. Mol. Biol.*** 26,720-731. PMCID: PMC6828549.

D. RESEARCH SUPPORT

Ongoing Support

NIGMS-NIH (Patel) 04-08-19 to 03-31-23 1.2 cal. mths
1 R01 GM129430 (SKI-16547) $200,000 dc/yr
Title: 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

NIAID-NIH (Tuschl) 06-10-19 to 05-31-23 1.2 cal. mths
1 R01 AI141507 (SKI-17128) $120,075 dc/yr
Title: 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 (Licht) 10-01-19 to 09-30-24 1.2 cal mths
(SKI-xxxxx) $136,699 dc/yr
Title: 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

Geoffrey Beane-MSKCC (Zhao) 08-01-19 to 07-31-21 0.6 cal mths
(SKI-17037) $86,957 dc/yr
Title: Structural and functional illumination of a tumor suppressing SMC genome maintenance complex
Cryo-EM structural studies of sub-complexes and the intact Smc5/6 genome maintenance complex and the functional impact of disrupting the identified intersubunit interfaces.
Role: co-PI

BRIA-MSKCC (Keeney) 09-01-19 to 08-31-21 0.6 cal mths
(SKI-17002) $95,000 dc/yr
Title: Elucidating the structural and functional principles of germline genome transmission.
Apply x-ray and cryo-EM studies to determine structures of complexes contributing to meiosis
Role: co-PI

ETC-MSKCC (Patel) 01-01-20 to 12-31-21 0.6 cal mths
(SKI-17308) $130,435 dc/yr
Title: Development of small molecule inhibitors of METTL3-METTL14 m6A RNA merthyltransferase as drugs against acute myeloid leukemia.
Identify small molecules from high-throughput screens for targeting human METTL3-METTL14 and then optimize them through a combination of structure- and computational-guidance of medicinal chemists.
Role: PI; co-PI (Kharas)

Ludwig-MSKCC (Maciejowski) 09-01-19 to 08-31-21 0.6 cal mths
(SKI-17099) $130,435 dc/yr
Title: Innate immune responses to homologous recombination deficiency
Development of a sensor that monitors cGAMP at the cellular level
Role: co-PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: JIA, Ning

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

POSITION TITLE: Postdoctoral Associate

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
Ocean University of China	B.S.	06/2010	Biological Sciences
University of Science and Technology of China	Ph.D.	04/2016	Structural Biology
Memorial Sloan Kettering Cancer Center	Postdoctoral	To date	Structural Biology

A. Personal Statement

I have the training, expertise and motivation necessary to successfully carry out the proposed research project. I have a broad background in biological sciences, with specific training and expertise in structural biology. My research mainly focuses on molecular understanding of protein-nucleic acid interactions in the area of CRISPR/Cas systems. As a postdoctoral associate, I am skilled in X-ray crystallography and Cryo-EM for structural studies, and also biochemical assays including biomolecular interactions (ITC, SPR, ELISA, pull-down assay), immunological assays (Immunofluorescence, western blot), nanodisc technology, liposomes and lipid monolayer preparation, liposome dye leakage assay, chemical cross-linking, ultraviolet-visible spectrophotometry, capillary electrophoresis (CE), high performance liquid chromatography (HPLC), circular dichroism (CD), dynamic light scattering (DLS), RNA transcription in vitro, baculovirus expression system, sf9 insect cell culture, HEK 293F cell culture and luciferase reporter assay. Based on these experiences, I successfully performed the research projects, collaborated with other researchers, and successfully produced several peer-reviewed publications from each project.

Publications

(# co-first author, * corresponding author)

Postdoctoral Training

1. **Jia, N.***, Xie, W., De La Cruz, J.M., Eng, E.T., Patel, D.J.* (2020) Structure-function insights into the initial step of DNA integration by a CRISPR-Cas-Transposon complex. *Cell Research*. 30, 182-184. .

2. **Jia, N.#,** Unciuleac, M.#, Xue C., Greene E.C., Patel, D.J., Shuman, S. (2019) Structures and single-molecule analysis of bacterial motor nuclease AdnAB illuminate the mechanism of DNA double-strand break resection. *Proc. Natl. Acad. Scis. USA*. 2019 Nov 18. pii: 201913546. doi: 10.1073/pnas.1913546116.

3. **Jia, N.***, Jones, R., Sukenick, G., & Patel, D.J.* (2019) Second Messenger cA4 Formation within the Composite Csm1 Palm Pocket of Type III-A CRISPR-Cas Csm Complex and Its Release Path. *Molecular Cell* 75(5):933-943 e936.

4. **Jia, N.***, Jones, R., Yang, G., Ouerfelli, O., & Patel, D.J.* (2019). CRISPR-Cas III-A Csm6 CARF Domain Is a Ring Nuclease Triggering Stepwise cA4 Cleavage with ApA>p Formation Terminating RNase Activity. *Molecular Cell* 75(5):944-956 e946.

5. **Jia, N.***, Mo, C.Y., Wang, C., Eng, E.T., Marraffini, L.A., and Patel, D.J.* (2019). Type III-A CRISPR-Cas Csm Complexes: Assembly, Periodic RNA Cleavage, DNase Activity Regulation, and Autoimmunity. *Molecular Cell 73*, 264-277 e265.

Graduate Training

6. **Jia, N.**, Liu, N., Cheng, W., Jiang, Y.L., Sun, H., Chen, L.L., Peng, J., Zhang, Y., Ding, Y.H., Zhang, Z.H., et al. (2016). Structural basis for receptor recognition and pore formation of a zebrafish aerolysin-like protein. *EMBO reports* 17, 235-248.

7. Zhou, K., **Jia, N.**, Hu, C., Jiang, Y.L., Yang, J.P., Chen, Y., Li, S., Li, W.F., and Zhou, C.Z. (2014). Crystal structure of juvenile hormone epoxide hydrolase from the silkworm Bombyx mori. *Proteins* 82, 3224-3229.

8. Song, N., **Jia, N.**, Yanagimoto, T., Lin, L., and Gao, T. (2013). Genetic differentiation of Trachurus japonicus from the Northwestern Pacific based on the mitochondrial DNA control region. *Mitochondrial DNA* 24, 705-712.

9. He, Y.X., Zhang, N.N., Li, W.F., **Jia, N.**, Chen, B.Y., Zhou, K., Zhang, J., Chen, Y., and Zhou, C.Z. (2012). N-Terminal domain of Bombyx mori fibroin mediates the assembly of silk in response to pH decrease. *Journal of molecular biology* 418, 197-207.

B. Positions and Honors

Positions and Employment

2017.6-2019.5	Research Fellow, Memorial Sloan Kettering Cancer Center
2019.6-2019.10	Research Scholar, Memorial Sloan Kettering Cancer Center
2019.11-present	Research Associate, Memorial Sloan Kettering Cancer Center

Honors

2007	China National Scholarship, China
2009	China National Scholarship, China
2010	Scholarship for outstanding students, China
2015	Caisi Scholarship for outstanding students, China

C. Contribution to Science

1. My graduate work focused on exploiting the receptor recognition and pore formation of a zebrafish aerolysin-like protein Dln1, which may function as a defense molecule in zebrafish. We present the first crystal and electron microscopy structures of a vertebrate aerolysin-like protein from *Danio rerio*, termed Dln1, before and after pore formation. Structural analyses combined with computational simulations and biochemical assays suggest a pore-forming process with an activation mechanism distinct from the previously characterized bacterial members. Moreover, Dln1 and its homologs are ubiquitously distributed in bony fishes and lamprey, suggesting a novel fish-specific defense molecule. As the first author, this work was published on *EMBO reports* (2016).

    a. **Jia, N.**, Liu, N., Cheng, W., Jiang, Y.L., Sun, H., Chen, L.L., Peng, J., Zhang, Y., Ding, Y.H., Zhang, Z.H., et al. (2016). Structural basis for receptor recognition and pore formation of a zebrafish aerolysin-like protein. *EMBO reports* 17, 235-248.

2. My postdoctoral work firstly focused on structure-function studies of type III CRISPR-Cas surveillance complexes. Our higher-resolution cryo-EM Csm structures allowed structural characterization in greater detail of bound protein and RNA and precisely defined alignments, intermolecular contacts, and catalytic pocket architectures necessary for a detailed, mechanistic understanding of function. Our work on type III-A CRISPR-Cas Csm systems provide mechanistic insights into Csm complex assembly, RNA targeting, RNA binding-activated DNA cleavage and cA_n synthesis. Besides, we provide the molecular basis for how Csm6 binds to the produced second messenger cA_4, which activates Csm6 to cleave ssRNA, leading to the degradation of host and invasive RNA. Importantly we demonstrated that the cA_4-activated Csm6 also cleaves the cA_4, in turn terminates the Csm6 RNase activity, which could potentially lead to the unconstrained RNase activity and cell death. As the first author and corresponding author, these work were published on three Molecular Cell papers in 2019.

    a. **Jia, N.***, Mo, C.Y., Wang, C., Eng, E.T., Marraffini, L.A., and Patel, D.J.* (2019). Type III-A CRISPR-Cas Csm Complexes: Assembly, Periodic RNA Cleavage, DNase Activity Regulation, and Autoimmunity. *Molecular Cell 73*, 264-277 e265.
    b. **Jia, N.***, Jones, R., Sukenick, G., & Patel, D.J.* (2019) Second Messenger cA4 Formation within the Composite Csm1 Palm Pocket of Type III-A CRISPR-Cas Csm Complex and Its Release Path. *Molecular Cell* 75(5):933-943 e936.
    c. **Jia, N.***, Jones, R., Yang, G., Ouerfelli, O., & Patel, D.J.* (2019). CRISPR-Cas III-A Csm6 CARF Domain Is a Ring Nuclease Triggering Stepwise cA4 Cleavage with ApA>p Formation Terminating RNase Activity. *Molecular Cell* 75(5):944-956 e946.

3. Recently, bioinformatics analyses have revealed the presence of CRISPR-Cas loci in bacterial Tn7-like transposons, thereby implicating a functional relationship between RNA-guided DNA targeting and transposition, with the latter representing a new role unrelated to host defense. Support for this concept has emerged from recent functional studies on type I-F and type V-K effectors involved in sequence-specific DNA transposition, thereby significantly broadening the potential biological applications of

CRISPR-Cas technology. To complement the available functional studies, our efforts have focused on structural studies of the *Vibrio cholerae Tn6677* multi-subunit type I-F Cascade^{crRNA}-TniQ complex, whereby transposition subunit TniQ initiates DNA transposition with the eventual help of other transposition-associated proteins TnsA, TnsB and TnsC in the gene cluster. Our studies provide insights into DNA-targeting by the Cascade^{crRNA}-TniQ complex, which represents an essential initial step in crRNA-guided DNA integration, providing structural insights into the potential use of RNA-guided Tn7-like transposons for genome editing. As the first author and corresponding author, this contributed was accepted by Cell Research in 2020.

a. **Jia, N.***, Xie, W., De La Cruz, J.M., Eng, E.T., Patel, D.J.* (2019) Structure-function insights into the initial step of DNA integration by a CRISPR-Cas-Transposon complex. Cell Research, in press (2020).

4. Besides studying on how DNA/RNA nucleases function in CRISPR-Cas systems, we are also interested in multi-subunit nucleases in other systems. Multi-subunit motor nucleases recognize, unwind, and resect DSB ends during homologous recombination in bacteria. RecBCD, AddAB, and AdnAB exemplify 3 distinct clades of end-resection machines in different bacterial taxa. Mycobacterial AdnAB is thought to represent an ancestral state of the machine. By collaborating with Dr. Stewart Shuman's group at MSKCC, we determined cryo-EM structures of mycobacterial AdnAB, in the absence of DNA and bound to a forked duplex DNA, that illuminate its domain architecture, the structural basis for 5' strand cleavage by the AdnA nuclease, and DNA interactions of the AdnB motor that ensure unidirectional translocation. Our findings add to an emerging appreciation of the evolutionary path and functional diversity of bacterial DSB resection systems. As the first author, these work were published in *Proceedings of the National Academy of Sciences of the United States of America* in 2019.

a. **Jia, N.[#],** Unciuleac, M.[#], Xue C., Greene E.C., Patel, D.J., Shuman, S. (2019) Structures and single-molecule analysis of bacterial motor nuclease AdnAB illuminate the mechanism of DNA double-strand break resection. *Proceedings of the National Academy of Sciences of the United States of America*. 2019 Nov 18. pii: 201913546. doi: 10.1073/pnas.1913546116.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/10qMigym9pxQq/collections/59193535/public

D. Additional Information: Research Support and/or Scholastic Performance

Research Support

NIGMS-NIH 1 R01 GM129430 (Patel) 04-08-19 to 03-31-23 1.2 cal. mths
Title: 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: Research Associate