BIOGRAPHICAL SKETCH

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

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

POSITION TITLE: Postdoctoral Researcher / Biochemist

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
Rutgers University, New Brunswick, NJ	B.A.	05/2017	Molecular Biology & Biochemistry
Rutgers University, New Brunswick, NJ	Ph.D.	01/2025	Molecular Biosciences
Rutgers University, New Brunswick, NJ	Postdoc		Biochemistry

A. Personal Statement

I am a molecular biologist and biochemist with a strong foundation in biochemical and biophysical assay development, particularly in protein-ligand interactions and innate immune signaling. My doctoral research at Rutgers University has focused on the molecular mechanisms of the innate immune receptor RIG-I, including loss- and gain-of-function variants relevant to COVID-19 disease manifestation. Using a combination of techniques including protein expression and purification, RNA transcription, fluorescence-based binding, enzymatic characterization, functional screening, and structural protein analysis, I have contributed to advancing our understanding of viral RNA recognition and immune activation mechanisms.
Beyond my research expertise, I have leadership experience in interdisciplinary collaborations and scientific communication. My goal is to continue driving translational research at the intersection of biophysics, drug discovery, and immunology, ultimately contributing to therapeutic advancements.

- **Solotchi, M.,** & Patel, S. S. (2024). Proofreading mechanisms of the innate immune receptor RIG-I: distinguishing self and viral RNA. Biochemical Society transactions, 52(3), 1131–1148. https://doi.org/10.1042/BST20230724
- Schweibenz, B. D.*, **Solotchi, M.*,** Hanpude, P., Devarkar, S. C., & Patel, S. S. (2023). RIG-I recognizes metabolite-capped RNAs as signaling ligands. Nucleic acids research, 51(15), 8102–8114. https://doi.org/10.1093/nar/gkad518
- Schweibenz, B. D., Devarkar, S. C., **Solotchi, M.,** Craig, C., Zheng, J., Pascal, B. D., Gokhale, S., Xie, P., Griffin, P. R., & Patel, S. S. (2022). The intrinsically disordered CARDs-Helicase linker in RIG-I is a molecular gate for RNA proofreading. The EMBO journal, 41(10), e109782. https://doi.org/10.15252/embj.2021109782

B. Positions, Scientific Appointments, and Honors

Positions and appointments:

2025 – Postdoctoral Researcher, Rutgers University, New Brunswick, NJ
2018 – 2025 Graduate Researcher, Rutgers University, New Brunswick, NJ
2017 – 2018 Research Associate, QuantumSi, Guilford, CT
2014 – 2027 Undergraduate Researcher, Rutgers University, New Brunswick, NJ

Honors and Awards:

2023 Certificate of Recognition for Excellence in Research, RNA Society
2023 Poster Prize Certificate for an Exceptional Poster Presentation, FASEB SRC

C. Contributions to Science

1. Mechanistic Characterization of RIG-I Loss- and Gain-of-Function Variants
I characterized RIG-I mutations associated with severe COVID-19 outcomes, uncovering how single amino acid substitutions impact innate immune signaling. Using hydrogen-deuterium exchange mass spectrometry (HDX-MS), biochemical ATPase assays, and cellular interferon reporter assays, I demonstrated how mutations disrupt RIG-I's autoregulatory mechanisms, either locking it in an inactive RNA-bound state (loss-of-function) or compromising RNA discrimination (gain-of-function) leading to dysregulated immune responses. These bodies of work are in preparation for publication, and I am proceeding with ongoing efforts to structurally resolve the unique RNA-bound conformation of the loss-of-function variant by cryo-EM.

- **Solotchi, M.,** Gebauer, E., Novick, J. S., Pascal, D. B., Tung, W., Jing, H., Hanpude, P., Su, C. H., Marcotrigiano, J., Griffin P. R., Patel, S. S. A COVID-19-related Loss-of-Function RIG-I Variant is Trapped in an RNA-bound Signaling Inactive State. (*in preparation*)
- **Solotchi, M.,** Jing, H., Tung, W., Gebauer, E., Novick, J. S., Pascal, D. B., Madura, T., Griffin P. R., Marcotrigiano, J., Su, C. H., Patel, S. S. Novel RIG-I variants reveal a disrupted autoinhibitory system as the molecular basis of their hyperactive phenotypes. (*in preparation*)

2. Development of Novel Assays for Protein-Protein Interactions
I designed and validated a bioluminescent NanoBiT protein complementation assay to study RIG-I CARD-CARD interactions *in cellulo*, complemented by a single-molecule FRET system to analyze protein interactions *in vitro*. These tools provide quantitative, real-time measurements of protein activation dynamics, aiding drug discovery efforts targeting antiviral pathways.

- **Solotchi, M.,** Lee, K. Y., Hanpude, P., Balakrishnan, A., Singh, A., Patel, S. S. RIG-I signal transduction is mediated by conditional CARD interactions. (*in preparation*)

3. High-Throughput Screening for Small-Molecule Modulators of RIG-I
My work applied functional high-throughput screening (HTS) to identify small-molecule activators and inhibitors of RIG-I, which could serve as potential antivirals and immune modulators. Using a combination of structure-based drug design, computational docking, and biochemical assays, I validated multiple candidates with therapeutic potential. This ongoing project has reached the hit-to-lead (H2L) optimization stage, and future plans are to patent our bottom-up process of discovering a novel class of small-molecule RIG-I modulators.

- **Solotchi, M.,** Forli, S., Marcotrigiano, J., Patel, S. S. *Regulation of RIG-I Activity by Small-Molecule Modulators: A Bottom-Up Approach.* Provisional Patent Filing, USPTO. (*in preparation*)

4. Structural and Functional Studies of Influenza and Viral RNA Recognition

I contributed to a study demonstrating how influenza B virus NS1 protein binds to triphosphorylated dsRNA, effectively suppressing RIG-I activation. This work provided insights into viral immune evasion strategies and identified novel antiviral targets.

- Woltz, R., Schweibenz, B., Tsutakawa, S. E., Zhao, C., Ma, L., Shurina, B., Hura, G. L., John, R., Vorobiev, S., Swapna, G., **Solotchi, M.,** Tainer, J. A., Krug, R. M., Patel, S. S., & Montelione, G. T. (2024). The NS1 protein of influenza B virus binds 5'-triphosphorylated dsRNA to suppress RIG-I activation and the host antiviral response. bioRxiv : the preprint server for biology, 2023.09.25.559316. https://doi.org/10.1101/2023.09.25.559316

5. Transcriptional Fidelity of Mitochondrial RNA Polymerases
As an undergraduate researcher, I investigated the transcriptional fidelity of mitochondrial RNA polymerases, using radiometric sequencing assays and high-resolution gel electrophoresis to compare RNA polymerase error rates across species.

- Sultana, S., **Solotchi, M.,** Ramachandran, A., & Patel, S. S. (2017). Transcriptional fidelities of human mitochondrial POLRMT, yeast mitochondrial Rpo41, and phage T7 single-subunit RNA polymerases. The Journal of biological chemistry, 292(44), 18145–18160. https://doi.org/10.1074/jbc.M117.797480

BIOGRAPHICAL SKETCH

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

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

POSITION TITLE: Graduate Student

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
Worcester Polytechnic Institute, Worcester, MA	BS	05/2021	Biochemistry
Rutgers University, New Brunswick, NJ	PhD		Biochemistry

A. Personal Statement

What has constantly motivated me through my career as a student and scientist during my undergraduate and postgraduate years was a constant curiosity in how the world works and an eagerness to learn more. An education in biochemistry has helped me learn about the mechanisms that drive life and make living organisms possible. By enrolling in Worcester Polytechnic Institute's biochemistry program as an undergraduate I was able to envision myself as a capable scientist. As a graduate student at Rutgers University, I will learn how to lead meaningful research projects through scientific inquiry.

I am a graduate student working in Dr. Smita Patel's lab researching the function and structure of the RIG-I Like Receptor family which includes RIG-I, MDA5, and LGP2. These immune receptors are essential in innate immunity activation in response to viral infections and understanding the mechanism behind this process can lead to development of therapeutics or agonists that can activate the immune response to fight off infections or stabilize the immune response to prevent autoimmune disease. I am currently exploring the dominant loss of function RIGI-I variant G731R and determining the role of LGP2 in activating MDA5's viral RNA recognition and antiviral response. I use protein purification techniques, protein activity assays, cell culture, molecular biology assays, and Cryo Electron Microscopy techniques as part of the skills needed to conduct this research.

After my PhD, I plan on working in industry focusing on drug development. As a scientist in industry, I plan on helping validate candidate molecules for their ability to target certain diseases. I hope to create therapeutics capable of targeting certain cancers providing more useful alternatives to conventional treatments.

B. Positions, Scientific Appointments, and Honors

2021 Provost's MQP Award, Worcester Polytechnic Institute

C. Contributions to Science

1. I participated in a Research Experience for Undergraduates(REU) at Penn State University where I partnered with a graduate student to design a drug delivery platform that took advantage of the high loading properties of graphene oxide nanoparticles to transport therapeutics directly to cell organelles such as the mitochondria. My role in the project was to test the impact of lipid-reduced graphene oxide(rGO) on the cell viability of MDA MB 231 breast cancer cells. To construct the lipid-rGO assembly, I first made lipid vesicles using a lipid extruder, and then sonicated a mixture of lipid vesicles with rGO to form the final lipid rGO assembly. The MDA-MB 231 breast cancer cells had to be

maintained daily to keep them at an optimal confluence. Experiments were conducted by treating the cells with various concentrations of the lipid-rGO assemblies. Fluorescence microscopy enabled me to differentiate between dead and live cells which I used to calculate the cell viability. From these experiments, I concluded that lipids play a vital role in reducing the toxicity normally observed with reduced graphene oxide, possibly by preventing the reduced graphene oxide from interacting with certain metabolic pathways that would cause these cells to undergo apoptosis. My research contributed to the following research publication from the lab.

   a. Farell M, Self A, Guza C, Song H, Apollon L, Gomez EW, Kumar M. Lipid-Functionalized Graphene Loaded with hMnSOD for Selective Inhibition of Cancer Cells. ACS Appl Mater Interfaces. 2020 Mar 18;12(11):12407-12416. doi: 10.1021/acsami.9b20070. Epub 2020 Mar 6. PMID: 32077682.

2. My undergraduate education at Worcester Polytechnic Institute culminated with my senior thesis project (Major Qualifying project). This was a project that my group and I designed, carried out, and presented over the course of the academic year. My senior thesis, titled 'Selegiline: The Effects of Monoamine Oxidase Inhibitors on the Behavior and Physiology of Caenorhabditis Elegans', I explored how the antidepressant selegiline affected the lipid metabolism in C. elegans. Selegiline is a monoamine oxidase inhibitor that increases dopamine levels for patients suffering from Parkinson's or other neurological disorders. Throughout this project I developed cell culture skills, learned purification techniques that applied column chromatography, and utilized Gas-Chromatography Mass Spectrometry (GCMS) to analyze lipid metabolites. One challenge I faced was that the glycolipids and phospholipids investigated were not identifiable using our GCMS techniques, which prevented me from extrapolating trends from our data. I instead focused our investigation on saturated and unsaturated fatty acids and the pathways where these biomolecules are synthesized and consumed. I observed an increase in monounsaturated fatty acids in C. elegans when exposed to selegiline which I proposed was caused by decreased enzymatic activity of the enzymes FAT-5, FAT-6, and FAT-7. Although my research showed increases in saturated fatty acid content in C. elegans exposed to selegiline, addition studies will have to be conducted to determine if the changes in fatty acid composition occurred organism wide or was isolated to neurons affected by the antidepressant. This project won the 2021 Provost's MQP Award.

3. I spent two years after completing my bachelors degree working at Curia Global as a research scientist. My research group utilized bioreactors for cell research. We maintained and grew transfected CHO cells until those cells reached a targeted production length. This would be done from 30ml up to 50L scales. From there the cells were harvested and the media was run through various purification columns. We also conducted mRNA research in which we optimized an in vitro mRNA transcription process. We designed multiple runs of experiments varying specific reagents to optimize mRNA production. A downstream purification step using oligo-dT columns was optimized to purify the in vitro transcribed mRNA reaction products. I also researched and carried out experiments to create a process flow for synthesizing template DNA that can be used for our in vitro transcribed mRNA process. I used in vitro technologies such as rolling circle DNA amplification to create template DNA.

BIOGRAPHICAL SKETCH

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NAME: Hou, CF David

eRA COMMONS USER NAME (credential, e.g., agency login): CXH401

POSITION TITLE: Adjunct Faculty - Lead Microscopist

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)	END DATE MM/YYYY	FIELD OF STUDY
National Chung Hsing University, Taichung City	BS	06/2005	Entomology
The Australian National University, Canberra, ACT	PHD	05/2014	Chemistry

A. Personal Statement

David is a highly motivated and creative researcher specializing in protein chemistry, crystallography, and cryo-electron microscopy. With a strong focus on developing and researching protein targets with crucial implications for the environment and biomedical applications, his goal is to design next-generation therapeutics. David's expertise is evident in his work as a Research Scientist and Cryo-EM Manager, where he skillfully oversees cryo-EM facility operations and conducts groundbreaking virus/phage-related model reconstructions. His innovative contributions extend to protein purification, structural analysis, and drug screening, showcasing a commitment to advancing the frontiers of science for the betterment of our world.

1. Iglesias SM, Hou CD, Reid J, Schauer E, Geier R, Soriaga A, Sim L, Gao L, Whitelegge J, Kyme P, Birx D, Lemire S, Cingolani G. Cryo-EM analysis of Pseudomonas phage Pa193 structural components. Commun Biol. 2024 Oct 6;7(1):1275. PubMed Central PMCID: PMC11456595.
2. Lokareddy RK, Hou CD, Forti F, Iglesias SM, Li F, Pavlenok M, Horner DS, Niederweis M, Briani F, Cingolani G. Integrative structural analysis of Pseudomonas phage DEV reveals a genome ejection motor. Nat Commun. 2024 Oct 1;15(1):8482. PubMed Central PMCID: PMC11445570.
3. Hou CD, Li F, Iglesias S, Cingolani G. Use of Localized Reconstruction to Visualize the Shigella Phage Sf6 Tail Apparatus. Methods Mol Biol. 2024;2738:215-228. PubMed Central PMCID: PMC10655839.
4. Li F, Hou CD, Lokareddy RK, Yang R, Forti F, Briani F, Cingolani G. High-resolution cryo-EM structure of the Pseudomonas bacteriophage E217. Nat Commun. 2023 Jul 8;14(1):4052. PubMed Central PMCID: PMC10329688.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2024 -	Adjunct Faculty - Lead Microscopist, RUTGERS THE STATE UNIVERSITY OF NJ
2020 - 2023	Research Scientist and Facility Manager, THOMAS JEFFERSON UNIVERSITY
2018 - 2020	Postdoctoral Associate, TEMPLE UNIV OF THE COMMONWEALTH
2014 - 2017	Postdoctoral Associate, Structural Genomics Consortium, Toronto

C. Contribution to Science

1. PHAGE ASEMBLIY OF PODOVIRIDAE. These publications provide a detailed structural characterization of the Shigella phage Sf6 tail apparatus and describe the conformational changes involved in genome delivery. Furthermore, the methods paper shares the workflow and strategies for using localized reconstruction to solve such structures, offering valuable insights for the cryoEM community. I contributed to cryoEM sample preparation, screening, imaging, and data processing for these studies. Additionally, I drafted sections of the manuscript, participated in revisions, and defended the work during the review process.

a. Hou CD, Li F, Iglesias S, Cingolani G. Use of Localized Reconstruction to Visualize the Shigella Phage Sf6 Tail Apparatus. Methods Mol Biol. 2024;2738:215-228. PubMed Central PMCID: PMC10655839.

b. Li F, Hou CD, Yang R, Whitehead R 3rd, Teschke CM, Cingolani G. High-resolution cryo-EM structure of the Shigella virus Sf6 genome delivery tail machine. Sci Adv. 2022 Dec 9;8(49):eadc9641. PubMed Central PMCID: PMC9728967.

2. PHAGE ASEMBILY OF MYOVIRIDAE. These publications provide high-resolution structural insights into the Pseudomonas phage E217, including its genome packaging machinery. The work advances our understanding of phage assembly and function, while also demonstrating the application of cryoEM to study large viral complexes at near-atomic resolution. I was responsible for cryoEM sample preparation, data collection, and image processing for these studies. Additionally, I contributed to manuscript drafting, revisions, and the review process.
   a. Li F, Hou CD, Lokareddy RK, Yang R, Forti F, Briani F, Cingolani G. High-resolution cryo-EM structure of the Pseudomonas bacteriophage E217. Nat Commun. 2023 Jul 8;14(1):4052. PubMed Central PMCID: PMC10329688.

   b. Lokareddy RK, Hou CD, Doll SG, Li F, Gillilan RE, Forti F, Horner DS, Briani F, Cingolani G. Terminase Subunits from the Pseudomonas-Phage E217. J Mol Biol. 2022 Oct 30;434(20):167799. PubMed Central PMCID: PMC10026623.

3. GENOME EJECTION SYSTEM IN PHAGES. These publications provide high-resolution structural insights into viral genome ejection mechanisms, including the architecture of the Pseudomonas phage DEV ejection motor and the periplasmic tunnel of the T7 DNA-ejectosome. Furthermore, our review on viral ejection proteins explores their conserved structural features and conformational flexibility. Collectively, these studies enhance our understanding of phage infection mechanisms and the molecular basis of viral genome delivery. I contributed to cryoEM sample preparation, data collection, image processing, and structural analysis for these studies. Additionally, I participated in manuscript drafting, revisions, and the review process.
   a. Lokareddy RK, Hou CD, Forti F, Iglesias SM, Li F, Pavlenok M, Horner DS, Niederweis M, Briani F, Cingolani G. Integrative structural analysis of Pseudomonas phage DEV reveals a genome ejection motor. Nat Commun. 2024 Oct 1;15(1):8482. PubMed Central PMCID: PMC11445570.

   b. Swanson NA, Hou CD, Cingolani G. Viral Ejection Proteins: Mosaically Conserved, Conformational Gymnasts. Microorganisms. 2022 Feb 24;10(3) PubMed Central PMCID: PMC8954989.

   c. Swanson NA, Lokareddy RK, Li F, Hou CD, Leptihn S, Pavlenok M, Niederweis M, Pumroy RA, Moiseenkova-Bell VY, Cingolani G. Cryo-EM structure of the periplasmic tunnel of T7 DNA-ejectosome at 2.7 Å resolution. Mol Cell. 2021 Aug 5;81(15):3145-3159.e7. PubMed Central PMCID: PMC8349896.

4. DIRECTED EVOLUTION OF ANTIBIOTICS RESISTANCE. The study provides key insights into the evolutionary strategies bacteria employ to develop antibiotic resistance, highlighting the molecular mechanisms underlying this process. Our findings emphasize the growing threat of superbugs and the urgent need for new approaches to combat antibiotic resistance. This publication is a condensed report from my PhD thesis, where I led the project from inception to completion. My contributions included DNA cloning, gene editing, protein expression, experimental design, protein crystallography, structural analysis, manuscript drafting, and defense.
   a. Hou CD, Liu JW, Collyer C, Mitić N, Pedroso MM, Schenk G, Ollis DL. Insights into an evolutionary strategy leading to antibiotic resistance. Sci Rep. 2017 Jan 11;7:40357. PubMed Central PMCID: PMC5225480.

BIOGRAPHICAL SKETCH

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NAME: PATEL, SMITA S

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

POSITION TITLE: Distinguished 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)	END DATE MM/YYYY	FIELD OF STUDY
Bombay University, Bombay	BS	05/1981	Physics and Chemistry
Indian Institute of Techonology (IIT), Bombay	MS	06/1983	Chemistry
Tufts University, Boston, MA	PHD	12/1988	Chemistry
Pennsylvania State University, State College, PA	Post-doc	12/1991	Fidelity of replicative DNA polymerase using transient state kinetics

A. Personal Statement

My lab studies the mechanisms of biological motor proteins such as helicases and polymerases in fundamentally important cellular processes such as viral RNA recognition by human innate immune receptors (RIG-I like helicases) and the replication and transcription of human mitochondrial DNA. We reconstitute the multicomponent complexes in vitro from pure proteins and assemble them on appropriate nucleic acid scaffolds to study their functions employing a variety of biochemical and biophysical approaches, including structural (cryo-EM, HDX), and single-molecule kinetics. Cellular assays in parallel enable validation of the biochemical mechanisms. Through these structure-function studies, we quantitatively dissect the multistep pathways of RNA recognition by the viral RNA sensors and transcription initiation and DNA replication mechanisms of mitochondrial enzymes. Mechanistic studies of naturally occurring disease mutants of viral RNA sensors as well mitochondrial enzymes offer critical insights into key catalytic and regulatory steps. These approaches lead to a deep understanding of the biological processes and the development of novel assays that can be used to screen inhibitors, activators, antagonists, or agonists. Ultimately, our basic science research holds promise for the development of therapies targeting mitochondrial disorders, viral infections, and autoimmune diseases.

I am committed to nurturing the next generation of biomedical researchers. Over the years, I have mentored ~20 undergraduates, 26 graduate students (10 female), and 20 post-docs (8 female) of various nationalities, socioeconomic backgrounds, and experiences – guiding them towards impactful careers across sectors from academia to industry and beyond. I am motivated to participate in the training of graduate students from underrepresented groups. Currently, my lab consists of three postdoctoral fellows (one female), five graduate students (one female and one IMSD trainee), and one undergraduate. We foster a collaborative and supportive environment for students to learn research methods in structural biology, biophysics, biochemistry, and cellular biology while addressing cutting-edge biological questions. I enjoy working with my students in the lab as needed and offering one-on-one mentorship. I meet with each student for two hours weekly, in addition to our regular lab meetings, to review their goals, experimental design, methods, data analysis, and interpretation and facilitating collaborations. In my mentoring approach, I emphasize the importance of scientific rigor, reproducibility, encouraging my students in independent thinking. I support their professional development by encouraging participation in skill-building programs such as the Rutgers boot camps run by the Proteomics Institute, as well as the IMSD and Rutgers iJOBS programs, which introduce them to diverse career opportunities.

I teach enzymology to first-year Medical, Master’s, and graduate students, and have taught biophysics, and served as a co-director of our core course for first-year graduate students at Rutgers University's

multidisciplinary Molecular Biosciences Program for eleven years. I contribute to the larger scientific community by regularly participating in peer review of research papers and proposals including NIH study sections. I have organized scientific conferences and currently serving on the Biophysical Journal editorial board.

1. Lee KY, Craig C, Patel SS* (2024). Unraveling blunt-end RNA binding and ATPase-driven translocation activities of the RIG-I family helicase LGP2. Nucleic Acids Res. 52(1):355-369. PMC10783506.
2. Singh A, Patel G, Patel SS* (2023). Twinkle-Catalyzed Toehold-Mediated DNA Strand Displacement Reaction. J Am Chem Soc. Nov 2; PMC11063129.
3. Goovaerts Q, Shen J, De Wijngaert B, Basu U, Patel SS*, Das K* (2023). Structures illustrate step-by-step mitochondrial transcription initiation. Nature. 622(7984):872-879. PMC10600007.
4. Schweibenz BD, Devarkar SC, Solotchi M, Craig C, Zheng J, Pascal BD, Gokhale S, Xie P, Griffin PR, Patel SS* (2022). The intrinsically disordered CARDs-Helicase linker in RIG-I is a molecular gate for RNA proofreading. EMBO J. 41(10):e109782. PMC9108607.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2022 –	Distinguished Professor, Rutgers - RWJ Medical School, Piscataway, NJ
2013-2022	Professor, Rutgers - RWJ Medical School, Piscataway, NJ
2002-2013	Professor, UMDNJ-RWJMS, Piscataway, NJ
1999-2002	Associate Professor, UMDNJ-RWJMS, Piscataway, NJ
1996-1999	Associate Professor, The Ohio State University, Columbus, OH
1992-1996	Assistant Professor, The Ohio State University, Columbus, OH
2024	MRAA NIH study section standing member
2019-	Editorial Board, Biophysical Journal
2020 and 2002	Organized FASEB meetings on Helicases: Structure, Function and Roles in Human Disease
2011 - 2015	Editorial Board, JBC
2005 - 2007	Gender Equity Program Mentor, CUNY
2002 -	Ad hoc Reviewer, NSF, ACS, NIH MGA, Eureka, Pathway to Independence, MSFE, PCMB, Special Emphasis panel,
1997 - 2002	Member, NIH Biochemistry Study Section

Honors

2017	Biomedical Research Exemplar, P.I. Program at Washington University School of Medicine
2015	Board of Trustees Award for Excellence in Research, Rutgers University
2014	Excellence in Research Award, New Jersey Health Foundation
2013	Outstanding Medical Research Scientist Award for Basic Biomedical Research , Edward J. Ill Excellence in Medicine Awards
2010	Research Award for Basic Sciences, UMDNJ Foundation
2009	Master Educator Guild, UMDNJ-RWJMS
2007	MERIT Award, NIH
2007	Antoine Saugrain Award and Lecture, Chemistry and Biochemistry at Hunter College
2005	Frontiers in Biology, Stanford University Biochemistry Graduate Students invite
1995	Junior Faculty Research Award, American Cancer Society
1989	NIH Postdoctoral Fellowship, NIH
1985	DuPont Fellowship for Academic Excellence, Tufts University
1983	Silver Medalist, IIT Bombay, India

C. Contribution to Science

1. RIG-I FAMILY OF VIRAL RNA RECEPTORS. Our research into helicases led us to the RIG-I family of innate immune receptors—RIG-I, MDA5, and LGP2—which serve as critical viral RNA sensors. RIG-I and MDA5 detect a broad range of RNA viruses, activating a powerful interferon-mediated antiviral response, while LGP2 modulates this response. What intrigued us was that these receptors belong to the helicase family of proteins. In collaboration, we determined the first high-resolution structure of RIG-I bound to double-stranded RNA (dsRNA) and ADP.BeF_3 (ref d) and m7g capped RNA (ref c). We later demonstrated that RIG-I's helicase/ATPase activity does not unwind RNA but facilitates its unidirectional translocation along dsRNA (ref b). This activity plays a dual role: proofreading by dissociating RIG-I from self-RNAs and promoting signaling-competent oligomerization on viral RNAs. RIG-I is tightly regulated, both intrinsically and by external factors. We discovered that an acidic, intrinsically disordered linker within RIG-I plays a key role in blocking self-RNA binding, thereby preventing inappropriate interferon responses (ref a). By studying RIG-I mutations associated with autoimmune disorders and COVID-19, we are uncovering mechanisms leading to both loss- and gain-of-functions. Our assays developed to quantitatively assess each step in RIG-I's RNA binding and activation processes are useful in screening RIG-I agonists and antagonists as potential treatments for viral infections, autoimmune dysfunction, and as vaccine adjuvants. Current focus is to understand LGP2's role in regulating the activities of RIG-I and MDA5.

   a. Schweibenz BD, Devarkar SC, Solotchi M, Craig C, Zheng J, Pascal BD, Gokhale S, Xie P, Griffin PR, Patel SS*. The intrinsically disordered CARDs-Helicase linker in RIG-I is a molecular gate for RNA proofreading. EMBO J. 2022 May 16;41(10):e109782. PubMed Central PMCID: PMC9108607.
   b. Devarkar SC, Schweibenz B, Wang C, Marcotrigiano J*, Patel SS*. RIG-I Uses an ATPase-Powered Translocation-Throttling Mechanism for Kinetic Proofreading of RNAs and Oligomerization. Mol Cell. 2018 Oct 18;72(2):355-368.e4. PubMed Central PMCID: PMC6434538.
   c. Devarkar SC, Wang C, Miller MT, Ramanathan A, Jiang F, Khan AG, Patel SS*, Marcotrigiano J*. (2016) Structural basis for m7G recognition and 2'-O-methyl discrimination in capped RNAs by the innate immune receptor RIG-I. Proc Natl Acad Sci U S A. 113(3):596-601. PMC4725518.
   d. Jiang F, Ramanathan A, Miller MT, Tang GQ, Gale M Jr, Patel SS*, Marcotrigiano J* (2011). Structural basis of RNA recognition and activation by innate immune receptor RIG-I. Nature 479(7373):423-7. PMC3430514.

2. MECHANISM OF MITCHONDRIAL DNA TRANSCRIPTION. We have a long-standing interest in studying single-subunit RNA polymerases that phages and mitochondria use to transcribe their genomes. We study bacteriophage T7 RNA polymerase (T7 RNAP) and the homologous mitochondrial RNA polymerases from yeast (y-mtRNAP) and humans (h-mtRNAP). T7 RNAP is an essential tool in biotechnology, widely used in RNA research and RNA vaccine development, while the mechanisms of mitochondrial RNA polymerases remain understudied. To investigate these systems, we have reconstituted the transcription initiation complexes of the mtRNAPs (ref c,d) and employing both ensemble and single-molecule kinetics to quantitatively measure each step in transcription initiation (ref b). These steps include promoter DNA binding, DNA melting, DNA bending, RNA extension rates, initial bubble collapse, promoter release, transition to elongation, and fidelity of nucleotide addition during elongation. The biochemical analyses provided a strong foundation for capturing the structures of eight initiation intermediates of y-mtRNAP using cryo-electron microscopy (cryo-EM) (ref a). Current focus is on understanding the transcription initiation mechanism of human mtRNAP and how it is regulated at the three human mitochondrial promoters.

   a. Goovaerts Q, Shen J, De Wijngaert B, Basu U, Patel SS*, Das K* (2023). Structures illustrate step-by-step mitochondrial transcription initiation. Nature. 622(7984):872-879. PMC10600007.
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3. MITOCHONDRIAL DNA REPLICATION. My interest in human mitochondrial DNA replication began when Hans Spelbrink identified Twinkle as the human mitochondrial helicase and showed that it is homologous to the bacteriophage T7 helicase we had been studying for two decades. I was fascinated to learn that many of the point mutations in Twinkle linked to mitochondrial diseases were the same mutations we had identified as helicase-deficient in our genetic screen with T7 helicase. We devoted significant effort to expressing soluble and active Twinkle from bacterial systems and reconstituting the mitochondrial replisome—comprising DNA polymerase gamma, Twinkle, and mitochondrial single-stranded binding protein for structural and mechanistic studies (refs a,c,d). We discovered a new DNA binding activity in the N-terminal domain of Twinkle whose function remains unknown (ref a). We found that Twinkle has DNA annealing, strand displacement activities (ref b-d), and more recently, we uncovered a novel RNA chaperone activity in Twinkle, which we are particularly excited to explore.

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   d. Sen D, Nandakumar D, Tang GQ, Patel SS (2012). Human mitochondrial DNA helicase TWINKLE is both an unwinding and annealing helicase. J Biol Chem. 2012 Apr 27;287(18):14545-56. PMC3340288.

4. MECHANISM AND STRUCTURE OF REPLICATIVE RING-SHAPED HELICASES. We discovered the ring-shaped hexameric structure of the T7 helicase and were the first to demonstrate that the replicative helicase ring binds DNA in the central channel (ref a). This mode of DNA binding is now recognized as a general feature of ring-shaped helicases. The structure raised many immediate questions, and the one that intrigued me the most was the motor function and the mechanistic basis for directional translocation on ssDNA and the order of nucleotide hydrolysis around the ring. Using mutant poisoning, transient state kinetics, and computational kinetic modeling with two different ring helicases, T7 and Rho, we showed that helicases employ a sequential mechanism of catalysis to efficiently move on ssDNA and unwind dsDNA by a wedge mechanism (refs b-d). Using similar approaches, we are now investigating the mechanism of human mitochondrial DNA helicase, Twinkle.

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5. FUNCTIONAL COORDINATION BETWEEN HELICASE, POLYMERASE, AND PRIMASE We discovered the mechanistic basis for coupling helicase and polymerase at the fork junction. The helicase is a poor unwindase; we showed that an actively synthesizing T7 DNA polymerase stimulates the helicase. When coupled, the helicase and polymerase create an efficient combined motor that moves in steps of one ATP/bp to unwind and copy the DNA strand (ref b-d). We demonstrated that cooperativity for leading strand synthesis requires physical proximity of helicase and polymerase at the leading strand fork junction. On the other hand, we found little cooperativity between primase and helicase during lagging strand

synthesis. Our studies showed that helicase does not pause during primer synthesis, contrary to published results, and single molecule FRET kinetics demonstrated a priming loop between helicase and primase. We will use similar approaches to study the coordination between helicase and polymerase of the human mitochondrial replisome.

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d. Pandey M, Patel SS* (2014). Helicase and polymerase move together close to the fork junction and copy DNA in one-nucleotide steps. Cell Rep. 6(6):1129-1138. PMC4010093.

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