BIOGRAPHICAL SKETCH

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NAME: Herbine, Karl

eRA COMMONS USER NAME (credential, e.g., agency login): KRH021

POSITION TITLE: Post-Doctoral Fellow

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
Temple University	BS	08/2008	12/2016	Biochemistry
University of Pennsylvania	--	02/2017	06/2019	Immunology
Thomas Jefferson University	PhD	07/2019	12/2024	Biochemistry, Structural, and Molecular Biology

A. Personal Statement

My long-term research interests lie in solving macromolecular structures to develop a comprehensive understanding of how protein complexes execute specific biological functions and how defects in molecular machines contribute to human disease. My academic training and research experience have provided me with a strong background in biochemistry and structural biology. As an undergraduate at Temple University, I conducted research in two notable labs. Under the mentorship of Dr. Weidong Yang, I studied ribosomal subunit processing, mRNA export, and HIV-1 Rev response elements. In my final year, I participated in an Undergraduate Research Program (URP) with Dr. Vincent Voelz, using molecular dynamics simulations to investigate the folding mechanisms of two streptococcal G protein domains with similar sequences but distinct folding and functional properties. Before beginning my graduate training, I worked as a research technician in Dr. De'Broski Herbert's lab, where I led independent projects in molecular biology and immunology. This work resulted in four publications, including two co-authored papers, and an invitation to present at the 22nd Annual Woods Hole Immunoparasitology (WHIP) conference. For my Ph.D. at Thomas Jefferson University, I returned to structural biology and biochemistry, studying the molecular mechanisms of transcription initiation and processive elongation in human mitochondria under the mentorship of Dr. Dmitry Temiakov, a leading expert in the mtDNA transcription and replication field. My thesis project focused on solving the structures of mtDNA transcription initiation and elongation complex intermediates using cryo-electron microscopy (cryo-EM) to elucidate stepwise mechanisms of transcription initiation, promoter recognition, transcription elongation, and substrate selection by human mitochondrial RNA polymerase. Cryo-EM is a rapidly evolving field that demands precise sample preparation and deep expertise in computational analysis to achieve high-resolution structures. Throughout my graduate studies, I aim to obtain multiple high-resolution structures of the human mitochondrial transcription complex. The resources provided by the National Center for Cryo-EM Access and Training (NCCAT) will be invaluable in advancing my research and helping me achieve my long-term goals.

B. Positions, Scientific Appointments and Honors

Positions and Employment

2015 – 2016 Lab Assistant, Temple University
2016 – 2017 Undergraduate Research Assistant, Temple University
2017 – 2019 Research Technician, University of Pennsylvania
2019 – 2024 Graduate Student, Thomas Jefferson University
2025 – Current Postdoctoral Fellow, Thomas Jefferson University

Other Experience and Professional Memberships

2012 Member, Temple Ambler Health Organization
2016 Participant, Undergraduate Research Program

Honors

2015 Dean's list
2016 Dean's list, Distinction in Major

C. Contribution to Science

1. **Undergraduate Research (1):** In the laboratory of Dr. Weidong Yang, I Studied the effects of CRISPR/Cas9 mediated gene knockout of critical genes that chaperone the export of mRNA and pre-ribosomal RNA through the Nuclear Pore Complex in HeLa cell lines. The project involved FRET and SPEED microscopy to analyze single molecule trajectory data to resolve export pathways and efficiencies. My contributions to this work were included in a submitted manuscript to PNAS and most recently the Journal of Molecular Biology.
    a. Junod SL, Tingey M, Kelich JM, Goryaynov A, **Herbine K**, Yang W. Dynamics of nuclear export of pre-ribosomal subunits revealed by high-speed single-molecule microscopy in live cells. iScience. 2023 Jul 21;26(8):107445. doi: 10.1016/j.isci.2023.107445.

2. **Undergraduate Research (2):** I was part of an undergraduate research project in the laboratory of Dr. Vincent Voelz at Temple University. Dr. Voelz's laboratory specializes in applying statistical mechanical models in MDS to better understand the fine microscopic details underlying the mechanisms of protein structure, function, and folding. My research done in the Voelz lab consisted of analyzing large trajectory data of Protein G from Streptococcal bacteria using Python and the UNIX Command Line to see the effects of amino acid substitutions on secondary protein structure and free energy contributions of residues to the overall structure.

3. **Post-Undergraduate Research**: my most recent and comprehensive research experience was as a research technician in the Herbert Lab of Mucosal Immunology at the University of Pennsylvania. I was involved in many independent and collaborative projects, but my main project was on studying the immunological consequences of mice that lacked IL-33 specifically in conventional Dendritic Cells (cDCs) that were subjected to gastrointestinal parasites. Unexpectedly, our data showed that loss of cDC-derivedIL-33 augmented worm clearance and Type 2 cytokine production, indispensable for host immunity, despite IL-33 being a potent inducer of Type 2 cytokine production. The revealing of this unexpected role for IL-33 in dendritic cells in developing an immune response.
    a. Hung LY, Johnson JL, Ji Y, Christian DA, **Herbine KR**, Pastore CF, Herbert DR. Cell-Intrinsic Wnt4 Influences Conventional Dendritic Cell Fate Determination to Suppress Type 2 Immunity. J Immunol. 2019 Jul 15;203(2):511–519. doi:10.4049/jimmunol.1900363
    b. Belle NM, Ji Y, **Herbine K**, Wei Y, Park J, Zullo K, Hung LY, Srivatsa S, Young T, Oniskey T, Pastore C, Nieves W, Somsouk M, Herbert DR. TFF3 interacts with LINGO2 to regulate EGFR activation for protection against colitis and gastrointestinal helminths. Nat Commun. 2019 Sep 27;10(1):4408. doi:10.1038/s41467-019-12315-1
    c. Hung LY, Tanaka Y, **Herbine K**, Pastore C, Singh B, Ferguson A, Vora N, Douglas B, Zullo K, Behrens EM, Li Hui Tan T, Kohanski MA, Bryce P, Lin C, Kambayashi T, Reed DR, Brown BL, Cohen NA, Herbert DR. Cellular context of IL-33 expression dictates impact on anti-helminth immunity. Sci Immunol. 2020 Nov 13;5(53):1–14. doi:10.1126/sciimmunol.abc6259

d. Zullo KM, Douglas B, Maloney NM, Ji Y, Wei Y, **Herbine K**, Cohen R, Pastore C, Cramer Z, Wang X, Wei W, Somsouk M, Hung LY, Lengner C, Kohanski MH, Cohen NA, Herbert DR. LINGO3 regulates mucosal tissue regeneration and promotes TFF2 dependent recovery from colitis. Scand J Gastroenterol. 2021 Jul 3;56(7):791–805. doi:10.1080/00365521.2021.1917650

4. **Graduate Research**: My ongoing PhD thesis research is focused on studying the underlying molecular mechanisms of transcription initiation and processive elongation in human mitochondria through structural and biochemical techniques. The results from my research will help us understand how defects in mitochondrial transcription machinery can lead to human diseases. I am currently utilizing cryo-electron microscopy to determine the complex intermediates of the transcription complex as it progresses from imitation to elongation.
    a. Buchel G, Nayak AR, **Herbine K**, Sarfallah A, Sokolova VO, Zamudio-Ochoa A, Temiakov D. Structural basis for DNA proofreading. Nat Commun. 2023 Dec 27;14(1):8501. doi:10.1038/s41467-023-44198-8
    b. **Herbine K**, Nayak AR, Temiakov D. Structural basis for substrate binding and selection by human mitochondrial RNA polymerase. Nat Commun. 2024 Aug 20;15(1):7134. doi:10.1038/s41467-024-50817-9
    c. Nayak AR, Sokolova V, Sillamaa S, **Herbine K**, Sedman J, Temiakov D. Structural basis for intrinsic strand displacement activity of mitochondrial DNA polymerase. Nat Commun. 2025 Mar 11;16(1):2417. doi:10.1038/s41467-025-57594-z
    d. **Herbine K**, Nayak AR, Temiakov D. Structural Basis for Promoter Recognition and Transcription Factor Binding and Release in Human Mitochondria. Submitted to Molecular Cell, In review, 2025.

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	TEMPLE UNIVERSITY	
2012	General Chemistry	B
2012	General Chemistry II	B
2013	Introduction to Biology	B
2013	Introduction to Biology II	B
2013	Organic Chemistry	B
2014	Organic Chemistry II	B
2014	Inorganic Chemistry	A
2014	Biochemistry I	B
2014	Precalculus	A
2015	Genetics	B
2015	Biochemistry II	A
2015	Calculus I	A
2015	Classical Physics I	A
2015	Calculus II	A
2015	General Physics II	A
2015	Cell Structure and Function	B
2015	Molecular Biology	A
2015	Analytical Chemistry	A
2015	Calculus III	B
2016	Research Techniques/Senior Project	B
2016	Virology	B
2016	Cell & Molecular Neuroscience	A
2016	Physical Chemistry of Biomolecules	A
2016	Structural Bioinformatics	A
2016	Thermodynamics & Kinetic Theory	A

YEAR	COURSE TITLE	GRADE
	THOMAS JEFFERSON UNIVERSITY	
2019 - Current	Seminar in Biochemistry	S
2019	Foundation in Biomedical Sciences	A
2020	Genetic Information Transfer	B
2020	Seminar in Biochemistry	S
2020	Macromolecular Structure	A
2020	Macromolecular Function	A
2020	Applied Statistics in Neuroscience	A
2020	Research Ethics	S
2020	Cell Signaling	A
2021	Planning & Writing Research Grants	S
2021	General Pharmacology	A

BIOGRAPHICAL SKETCH

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NAME: **Temiakov, Dmitry**

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

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Mendeleev University of Chemical Technology, Moscow, Russia	MS	02/1993	Microbiology
Institute of Genetics, Moscow, Russia	Ph.D.	02/1996	Molecular Biology
SUNY Downstate Medical Center, New York	Postdoctoral	2001	Molecular Biology
Spring-8, RIKEN, Japan	Postdoctoral	2003	Structural Biology

A. Personal Statement

My research program is driven by a passion for understanding the intricate interplay between structure and function in mitochondrial DNA (mtDNA) replication and transcription. By integrating diverse experimental approaches, we aim to provide groundbreaking insights into these fundamental processes, with potential implications for understanding and treating human diseases associated with mitochondrial dysfunction.

Over the years, my laboratory has achieved significant milestones, particularly in elucidating key mechanisms involved in mitochondrial DNA maintenance, replication, and transcription. Our findings have contributed to shaping the prevailing model of transcription in the mitochondrial field and identified essential elements of transcription and replication fidelity. Our current aspiration is to unravel mechanisms underpinning the replication-transcription switch, a critical system in human mitochondria preventing collisions between the replisome and transcription machinery while regulating mtDNA copy numbers in cells. Of particular interest is the regulation of replication and transcription in the mitochondria of human gametes and the molecular mechanisms governing the dogma of uniparental inheritance of mtDNA. Our studies seek to determine the consequences of eliminating paternal mitochondrial DNA, exploring the transfer of paternal proteins into an oocyte, and their impact on fertility mechanisms. My research program focuses on elucidating the molecular mechanisms of mitochondrial DNA expression through a systemic approach that integrates biochemistry, structural biology, genetics, bioinformatics, and cell biology.

As the Director of the Integrated Structural Biology Shared Resources at Thomas Jefferson University, I am dedicated to supporting investigators in their research involving cryo-electron microscopy (cryo-EM) and other structural biology techniques. My role involves facilitating access to state-of-the-art instrumentation, providing technical guidance, and assisting researchers with experimental design, data acquisition, and structural analysis. The facility serves a broad scientific community, including investigators from Jefferson, Temple University, the

Wistar Institute, the University of Pennsylvania (UPenn), Rutgers University, and Rowan University. By fostering collaborations across institutions, I aim to advance structural biology research, enhance accessibility to cryo-EM methodologies, and contribute to groundbreaking discoveries in molecular and cellular biology.

B. Positions, Scientific Appointments, and Honors

2023-present	**Director**, Integrated Structural Biology Core Facility, Sidney Kimmel Cancer Center,Thomas Jefferson University
2018-present	**Associate Professor**, **Professor**, Department of Biochemistry and Molecular Biology, Thomas Jefferson University in Philadelphia, PA
2005-2018	**Assistant Professor**, **Associate Professor, tenured**, Department of Cell Biology, University of Medicine and Dentistry of New Jersey - Rowan University School of Osteopathic Medicine in Stratford, NJ
2002-2004	**Research Assistant Professor**, Department of Microbiology and Immunology, SUNY Downstate Medical Center in Brooklyn, NY
2002, 2023	**Visiting scientist**, Spring-8, RIKEN, Japan
1997-2002	**Postdoctoral Research Scientist**, Department of Microbiology and Immunology, SUNY Downstate Medical Center in Brooklyn, NY

Awards

2023	SKMC Michael and Melina Pellini Award for Innovation in the Biomedical Sciences
2015	Excellence in Research Award, New Jersey Health Foundation
2015	Faculty Research Achievement Award, Rowan University
2013	Excellence in Research Award, New Jersey Health Foundation
1999-2000, 2000-2001	SUNY Postdoctoral Fellowship “Dean’s Initiative in Research.”

C. Contributions to Science

1. **Structural-functional studies of mitochondrial transcription.** My laboratory is the leading group in structural and functional studies of human mitochondrial transcription. Over the past decade, we have successfully determined the atomic structures of the RNA polymerase and its principal transcription factors, leading to the elucidation of the mechanisms underlying transcription initiation, elongation, and anti-termination within human mitochondria. Our findings constitute a pivotal source of structural information for mitochondrial RNA polymerases and have been extensively employed to guide a multitude of biochemical and biophysical experimentation.

a. Hillen H, Parshin A, Agaronyan K, Morozov YI, Graber J, Chernev A, Schwinghammer K, Urlaub H, Anikin M, Cramer P, **Temiakov D**. Mechanism of transcription anti-termination in human mitochondria. ***Cell***. 2017 Nov 16; 171(5):1082-1093 PMCID: PMC5798601

b. Hillen H, Morozov MY, Sarfallah A., **Temiakov D**., Cramer P. Structural basis of mitochondrial transcription initiation. **Cell** 2017 Nov 16;171(5):1072-1081 PMCID: PMC6590061

c. Schwinghammer K, Cheung A, Morozov AI, Agaronyan K, **Temiakov D**, Cramer P. Structure of mitochondrial RNA polymerase elongation complex. ***Nat Struct Mol Biol.*** 2013 Nov;20(11):1298-303. doi: 10.1038/nsmb.2683 PMCID: PMC4321815.

d. Ringel R, Sologub M, Morozov Y, Litonin D, Cramer P, **Temiakov D**. Structure of human mitochondrial RNA polymerase. ***Nature***. 2011 Sep 25; 478(7368):269-73.

2. **The Dogma of uniparental inheritance of mtDNA and regulation of mitochondrial transcription and replication.** Uniparental inheritance of mitochondrial DNA is an evolutionary trait found in nearly all eukaryotes; however, the molecular mechanism behind it has remained unknown. We found that re-localization of human mitochondrial transcription factor A, TFAM, from mitochondria to the nucleus of spermatozoa results in elimination of mitochondrial DNA, explaining its maternal inheritance in our species. These studies have implications for mechanisms of fertility as well as for evolution of mitochondria. We also introduced the concept of a molecular switch between replication and transcription in human mitochondria. The switch prevents head-on collision inevitable during simultaneous transcription and replication of the circular mitochondrial genome. This research brings us closer to understanding the mysteries of human inheritance and the vital role of mitochondria in health and disease.

a. Lee W., Zamudio-Ochoa A., Buchel G., Podlesniy P, Marti Gutierrez N., Puigros M., Calderon A., Amy Koski A., Tang H.Y., Li L., Trullas R., Mitalipov S, **Temiakov D**. Molecular Basis for Maternal Inheritance of Human Mitochondrial DNA. **Nature Genetics**. 2023 Sep;(55)10

b. Kang E, Wu J, Gutierrez NM, Koski A, Tippner-Hedges R, Agaronyan K, Platero-Luengo A, Martinez-Redondo P, Ma H, Lee Y, Hayama T, Van Dyken C, Wang X, Luo S, Ahmed R, Li Y, Ji D, Kayali R, Cinnioglu C, Olson S, Jensen J, Battaglia D, Lee D, Wu D, Huang T, Wolf DP, **Temiakov D**, Belmonte JC, Amato P, Mitalipov S. Mitochondrial replacement in human oocytes carrying pathogenic mitochondrial DNA mutations. ***Nature.*** 2016 Dec 8; 540 (7632):270-275

c. Agaronyan K, Morozov AI, Anikin M, **Temiakov D.** Replication-transcription switch in human mitochondria. ***Science.*** 2015 Jan 30. 347 (6221): 548-551 PMCID: PMC4677687

d. Sologub M, Litonin D, Anikin M, Mustaev A, **Temiakov D.** TFB2 is a transient component of the catalytic site of the human mitochondrial transcription initiation complex. ***Cell.*** 2009 Nov 25; 139(5):934-44 PMCID: PMC2806307.

3. Mechanisms of replication and repair of human mitochondrial DNA. These studies are focused on structural studies of mitochondrial DNA replication and repair. We determined the structural basis for the proofreading mechanism for pol A family of DNA polymerases that includes DNA polymerase I (Klenow fragment), T7 DNA polymerase, and mitochondrial DNA polymerase Gamma. The proofreading mechanism remained elusive since the discovery of exonuclease activity in DNAP I. Besides the importance of proofreading mechanisms for PCR applications, our findings reveal novel structural intermediates of DNA polymerase Gamma, providing a path for the development of novel treatments for mitochondrial diseases.

a. Nayak A, Sokolova V, Herbine K, **Temiakov D**. Structural basis for intrinsic strand displacement activity by mitochondrial RNA polymerase. **Nat Commun** 2025 In Press
b. Herbine K, Nayak A, **Temiakov D**. Structural Basis for Substrate Binding and Selection by Human Mitochondrial RNA Polymerase. **Nat Commun** 2024, Aug 20;15(1):7134. doi: 10.1038/s41467-024-50817-9.
c. Buchel G, Nayak A, Herbine K, Sarfallah A, Sokolova V, Zamudio-Ochoa A, Temiakov D. Structural Basis for DNA Proofreading. **Nat Commun** 2023 Dec 27;14(1):8501. doi: 10.1038/s41467-023-44198-8. doi: 10.1038/s41467-023-44198-8. PMID: 38151585
d. Sarfallah A, Zamudio-Ochoa A, Anikin M, **Temiakov D**. Mechanism of transcription initiation and primer generation at the mitochondrial replication origin OriL. **EMBO J.** 2021 Aug 23:e107988. PMID: 34423452

4. **Studies of phage and bacterial RNA polymerases.**
During my earlier research, I investigated transcription mechanisms involving T7 and bacterial RNA polymerase. Through these studies, I introduced the concept of substrate pre-selection and its binding

outside the polymerase's active site, known as the "pre-insertion" site. Subsequent experimental confirmation revealed the existence of the pre-insertion site not only in T7 but also in multi-subunit RNA polymerases and DNA polymerases. The mechanism of substrate selection within this pre-insertion site is now recognized as a crucial aspect of ensuring transcription and replication fidelity. Subsequent studies focused on characterizing the elongation complexes of multi-subunit RNA polymerases and examining the fidelity of transcription.

a. **Temiakov D**, Patlan V, Anikin M, McAllister WT, Yokoyama S, Vassylyev DG. (2004) Structural basis for substrate selection by T7 RNA polymerase. ***Cell***. 2004;116(3):381-91

b. Tahirov TH[1], **Temiakov D**[1], Anikin M, Patlan V, McAllister WT, Vassylyev DG & Yokoyama S. Structure of a T7 RNA polymerase elongation complex at 2.9 A resolution. ***Nature.*** 2002; 420:43-50

c. **Temiakov D**[1], Zenkin N[1], Vassylyeva M, Perederina A, Tahirov T, Kashkina E, Savkina M., Zorov S., Nikiforov V., Igarashi N, Matsugaki N, Wakatsuki S, Severinov K., Vassylyev D.G. Structural basis of transcription inhibition by antibiotic streptolydigin. ***Mol. Cell*** 2005. 19 (5):655-666

d. Kashkina E, Anikin M, Brueckner F, Pomerantz RT, McAllister WT, Cramer P, **Temiakov D.** Template misalignment in multisubunit RNA polymerases and transcription fidelity. ***Mol. Cell.*** 2006 Oct 20;24(2):257- 6

Complete List of Published Work in My Bibliography:

https://www.ncbi.nlm.nih.gov/sites/myncbi/dmitry.temiakov.1/bibliography/44958885/public/?sort=date&direction=descending

BIOGRAPHICAL SKETCH

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NAME: Ashok Ranjan Nayak**,** PhD.

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

POSITION TITLE: Senior Research Investigator, CryoEM Manager

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
Tamil Nadu Agricultural University, Coimbatore, India	M.Sc	2010	Biotechnology
Jawaharlal Nehru University, New Delhi, India	Ph.D.	2016	X-ray crystallography
Virginia Commonwealth University, Richmond, VA, USA	Post-doctoral	2021	Cryo-electron microscopy
Thomas Jefferson University, Philadelphia, PA, USA	Post-doctoral	2023	Cryo-electron microscopy

A. Personal Statement

I am a cryo-electron microscopist/ structural biologist with seven years of experience in single-particle cryo-EM, and over 10 years in protein 3D structure determination. I obtained my initial training in protein crystallography during my PhD, where I solved the structure of a coronin non-canonical coiled-coil from a parasitic protist (Leishmania) by Selenium phasing. This antiparallel tetrameric structure was the first asymmetric coiled-coil structure in the left-handed four-helix bundle fold which implicated a different actin architecture in parasitic protists compared to their mammalian isoforms (from the antiparallel tetrameric arrangement vs parallel trimeric mammalian coronins) consistent with the functional studies.

I trained in single particle cryo-EM during my post-doctoral tenure, Virginia Commonwealth University, where I solved several Calcium-inactivated and magnesium-inhibited structures of Ryanodine receptors which were instrumental in answering the longstanding questions in the field - how the intracellular Calcium channel gets activated at low uM [Ca2+] and inactivated at high mM [Ca2+] and how Magnesium inhibits the RyRs? We discovered a common molecular mechanism underlying the dual Calcium modulation of RyR1 and demonstrated shared inhibitory sites for Calcium and Magnesium. Under a collaborative project, I solved the structure of a bacterial pili-extension motor that powers the pilus-forming machinery, which showed bacteria use different quaternary arrangements of this AAA-ATPase to power pilus extension, a process critical for pili formation.

More recently during my post-doctoral tenure in Thomas Jefferson University, we uncovered the DNA proofreading mechanism from an ensemble of structures of human mitochondrial (mt)DNA polymerase and discovered how exactly a wrong nucleotide inserted in a nascent formed DNA strand is sensed and eventually gets repaired by this family (PolA) of DNA polymerases. Our work clearly supported the intramolecular model of DNA proofreading, proposed a bolt-action working model of DNA proofreading, and discovered backtracking in DNA polymerases for the first time. Currently, I am involved in several projects that aim to understand – 1/ structural basis of strand displacement activity by mitochondrial DNA polymerase Pol-Gamma 2/ substrate trafficking to mitochondrial RNA polymerase, nucleotide pre/selection, entry, and insertion during the nucleotide incorporation cycle during mitochondrial transcription, 3/ mitochondrial DNA

replication through UV lesions 4/ off-target effects of drugs on mitochondrial RNA polymerase during transcription 5/ structural characterization of mitochondrial replisome, etc.

B. Positions, Scientific Appointments, and Honors

Positions

2023, October-Present	**CryoEM Manager, Senior Research Investigator**
2021-2023	**Postdoctoral Research Scientist,** Thomas Jefferson University, Philadelphia, PA, USA
2016-2021	**Postdoctoral Research Scientist**, Virginia Commonwealth University, Richmond, VA, USA

Honors

- Early-career reviewer in the journal eLIfe.
- Early-career reviewer in the Journal of Biological Chemistry (JBC)
- National Talent Fellowship by Indian Council of Agricultural Research, Pusa, New Delhi during undergraduate studies.
- National Talent Fellowship at Jawaharlal University, New Delhi, India during MSc Biotechnology.
- Junior Research Fellowship by Council of Scientific & Industrial Research (CSIR), Government of India during Ph.D.
- Junior Research Fellowship by the Department of Biotechnology Government of India for Ph.D.
- Graduate Engineering Aptitude Test for admission to MTech and Ph.D.
- Member of the biophysical society during post-doctoral tenure (2017-2021).
- Member of the American society of Biochemistry and Molecular Biology.

C. Contributions to Science

1. Molecular Mechanism of DNA proofreading

DNA polymerase (DNAP) can correct errors in DNA during replication by proofreading, a process critical for cell viability. The proofreading is based on the exonucleolytic activity of DNAP, however, the mechanism by which an erroneously incorporated base trans locates from the polymerase (pol) to the exonuclease (exo) site, the mismatched primer is separated from the template strand and diverted into the exonuclease channel, and the corrected DNA terminus returns back remains elusive. In this work, we presented an ensemble of high-resolution structures representing human mitochondrial DNA polymerase Gamma, Polg, captured during consecutive proofreading steps. The structures revealed: the states with mismatched base recognition and its uncoupling from the pol site, the consequent forward translocation of DNAP and changes in DNA trajectory, repositioning and refolding of the structural elements involved in primer separation, the consequent backtracking of DNAP, and displacement of the mismatched base from the template strand and its positioning in the exo site. Altogether, our findings suggested a ‘bolt-action’ mechanism of proofreading based on iterative cycles of DNAP translocation without dissociation from the DNA, which transfers the primer between two catalytic sites. This mechanism was corroborated by functional assays and mutagenesis, linking known pathogenic mutations with key structural elements essential for different steps of proofreading.

Buchel, G*., **Nayak, A.R***., Herbine, K. *et al.* Structural basis for DNA proofreading. ***Nat Commun*** **14**, 8501 (2023). https://doi.org/10.1038/s41467-023-44198-8 (Equal Contribution)

2. Calcium and Magnesium inhibition in Ryanodine Receptors

My postdoctoral work at Virginia Commonwealth University, Richmond provided insights into structural changes leading to calcium-dependent inactivation (CDI) and Mg inhibition in type 1 ryanodine receptors (RyR1), a large intracellular Ca^{2+} channel present in the skeletal muscle. The results rationalized how some disease-causing mutations in RyR1 eliminate CDI of the channel and will be of interest to ion channel structural biologists and physiologists studying skeletal muscle pathologies. The RyR1 channel is regulated by Ca^{2+} and Mg^{2+}. The channel undergoes activation at a micromolar cytosolic Ca^{2+}, while elevated cytosolic Ca^{2+} inhibits it in a negative feedback mechanism. Mg2+ is a constitutive inhibitor of the channel which keeps the channel closed in the resting state. Cryogenic electron microscopy of rabbit RyR1 embedded in nanodiscs under inhibiting Mg^{2+} and Ca^{2+} conditions revealed shared and exclusive sites for both the divalent ions. The open and closed Ca^{2+} inactivated structures demonstrate a centralized mechanism underlying the activation of the RyR1 at micromolar Ca^{2+} and inactivation at millimolar $Ca^{2+.}$ During activation, Ca^{2+} binds to the high-affinity site and engages the transmembrane domain into a rigid block, thus relaying the conformational to the inner transmembrane helices, S6 to open. Further, a rise in Ca^{2+} twists the rigid entity more, closing the inner pore helices, thus (inactivating) the channel. However, Mg causes inhibition by binding at the high-affinity Ca^{2+} site and plugging the channel pore at a site shared by several toxins that cause partially open sub-conductance state of the channel. Most significantly, we found that the EF-hand domain and the S2–S3 cytoplasmic loop located at the intersubunit space of the inactivated structure, acts as a divalent sensor, which relays the conformational changes to neighboring subunits through two pairs of salt bridges. The site is mutated in some muscle diseases that eliminate CDI. The structural insights from these two pieces of work demonstrated that prior Ca^{2+} activation is a prerequisite for Ca^{2+} inactivation and provides for a seamless transition from resting to activated and inactivated to the Mg^{2+} bound conformations of RyR1.

Nayak AR and Samsó M. Ca^{2+} inactivation of the mammalian ryanodine receptor type 1 in a lipidic environment revealed by cryo-EM. 2022. ***eLife*** 11:e75568.https://doi.org/10.7554/eLife.75568

Nayak AR, Rangubit W, Will AH, Hu Y, Castro-Hartmann P, Lobo JJ, Dryden K, Lamb GD, Sompornpisut P and Samsó M . Interplay between Mg^{2+} and Ca^{2+} at multiple sites of the ryanodine receptor***. (accepted, in press Nat Commun.***

3. Structure of Type IV Pilus Extension ATPase from Enteropathogenic Escherichia coli

Type 4 pili (T4P) are retractable surface appendages found on numerous bacteria and archaea that play essential roles in various microbial functions, including host colonization by pathogens. An ATPase is required for T4P extension, but the mechanism by which chemical energy is transduced to mechanical energy for pilus extension has not been elucidated. We reported the cryo-electron microscopy (cryo-EM) structure of the BfpD ATPase from enteropathogenic Escherichia coli (EPEC) in the presence of either ADP or a mixture of ADP and AMP-PNP. Both structures, solved at 3 Å resolution, reveal the typical toroid shape of AAA+ ATPases and unambiguous 6-fold symmetry. This 6-fold symmetry contrasts with the 2-fold symmetry previously reported for other T4P extension ATPase structures, all of which were from thermophiles and solved by crystallography. In the presence of the nucleotide mixture, BfpD bound exclusively AMP-PNP, and this binding resulted in a modest outward expansion in comparison to the structure in the presence of ADP, suggesting a concerted model for hydrolysis. *De novo* molecular models reveal a partially open configuration of all subunits where the nucleotide binding site may not be optimally positioned for catalysis. ATPase functional studies reveal modest activity similar to that of other extension ATPases, while calculations indicate that this activity is insufficient to power pilus extension. Our results revealed that, despite similarities in primary sequence and tertiary structure, T4P extension ATPases exhibit divergent quaternary configurations. Our data also raised new possibilities regarding the mechanism by which T4P extension ATPases power pilus formation.

Nayak AR, Singh PK, Zhao J, Samsó M, Donnenberg MS. Cryo-EM Structure of the Type IV Pilus Extension ATPase from Enteropathogenic Escherichia coli. 2022. **mBio**. Nov 3:e0227022. doi: 10.1128/mbio.02270-22. Epub ahead of print. PMID: 36326250

4. Structure of Leishmania Coronin coiled coil

As a part of my doctorate work, I elucidated the crystal structure of coronin oligomerization domain from *Leishmania donovani* implicated in actin crosslinking of parasitic protists by single-wavelength anomalous dispersion method. Coiled

coils are ubiquitous structural motifs that serve as a platform for protein–protein interactions and play a central role in myriad physiological processes. Though the formation of a coiled-coil requires only the presence of suitably spaced hydrophobic residues, sequence specificities have also been associated with specific oligomeric states. RhXXhE is one such sequence motif, associated with parallel trimeric coronins, found in mammals. Coronin, present in all eukaryotes, is an actin-associated protein involved in regulating actin turnover. Most eukaryotic coronins possess the RhXXhE trimerization motif. However, a unique feature of parasitic kinetoplastid coronin is that the positions of R and E are swapped within their coiled-coil domain. Leishmania donovani coronin coiled-coil domain crystal structure at 2.2 Å, revealed a surprising anti-parallel tetramer assembly, compared to a trimeric parallel structure in most of the eukaryotic counterparts. Structural analyses revealed that LdCoroCC possesses an inherent asymmetry, wherein one of the dimers had a helix shifted by a heptad or one and a half alpha-helical turns. Such a large shift in the alpha-helical register makes leishmania coronin to be the sole member in the left-handed four-helix bundle family to have asymmetry, only observed in another right-handed helical bundle in the Mnt repressor coiled coil structure. The structure also possesses a unique polar core compared to other four-helix bundles with apolar core.

Nayak AR, Karade SS, Srivastava VK, Rana AK, Gupta CM, Sahasrabuddhe AA, Pratap JV. Structure of Leishmania donovani coronin coiled coil domain reveals an antiparallel 4 helix bundle with inherent asymmetry. **J Struct Biol**. 2016 Jul;195(1):129-38. doi: 10.1016/j.jsb.2016.02.020. Epub 2016 Mar 2. PMID: 26940672.

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