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: Herbine, Karl

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

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Temple University	BA	08/2008	12/2016	Biochemistry
University of Pennsylvania	--	02/2017	06/2019	Immunology
Thomas Jefferson University	PhD	07/2019	TBD	Biochemistry & Molecular Pharmacology

A. Personal Statement

My long term research interests focus on the implementation of solving the structures of macromolecules to develop a comprehensive understanding on how protein complexes execute specific biological functions and how defects in molecular machines can contribute to human disease. My academic training and research experience to date have provided me with an excellent background in biochemistry and structural biology. As an undergraduate at Temple University, I had the opportunity to conduct research in two notable labs. I first started research under the mentorship of Dr. Weidong Yang on studying ribosomal subunit processing, mRNA export, and HIV-1 Rev response elements. During my final year of my undergraduate studies, I participated in an Undergraduate Research Program (URP) under the mentorship of Dr. Vincent Voelz on using molecular dynamic simulations to study the folding mechanisms of two protein domains of streptococcal G protein that are similar in sequence but have separate folding and functions. Prior to my graduate training, I joined the lab of Dr. De'Broski Herbert as a research technician where I had many independent research projects in molecular biology and immunology. This research experience resulted in 4 publications, 2 of which I shared co-authorship, and an invitation to present my research at the 22nd Annual Woods Hole Immunoparasitology (WHIP) conference. For my graduate training at Thomas Jefferson University, I have returned to the fields of structural biology and biochemistry by studying the underlying molecular mechanisms of transcription initiation and processive elongation in human mitochondria under the mentorship of Dr. Dmitry Temiakov. Dr. Temiakov specializes in biochemistry and is well known in the field of mtDNA transcription and replication. For my current thesis project, I am currently working on solving the structures of mtDNA transcription initiation complex intermediates using Cryo-electron microscopy (cryoEM) in order to elucidate a step-wise mechanism of transcription initiation in human mitochondria. The field of cryoEM is rapidly growing and requires near perfect sample preparation and immense knowledge of analysis software in order to achieve high-resolution structures. During my graduate studies, I plan on obtaining multiple high-resolution structures of the human mitochondrial transcription complex. Overall, I feel that the resources provided by the National Center for CryoEM Access and Training (NCCAT) will serve as in integral part of my graduate studies and will help me complete 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

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.

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. 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;56(7):791-805. doi: 10.1080/00365521.2021.1917650. Epub 2021 May 3. PMID: 33941035; PMCID: PMC8647134.
    b. 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):eabc6259. doi: 10.1126/sciimmunol.abc6259. PMID: 33188058; PMCID: PMC8257082.
    c. 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. PMID: 31562318; PMCID: PMC6764942.
    d. 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. Epub 2019 Jun 7. PMID: 31175162; PMCID: PMC6615948.

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.

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

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
	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

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: **Temiakov, Dmitry**

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

POSITION TITLE: Associate 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	PhD	02/1996	Molecular Biology
SUNY Downstate Medical Center, New York	Postdoctoral	2001	Molecular Biology
Spring-8, RIKEN, JAPAN	Postdoctoral	2002	Structural Biology

A. Personal Statement

I have 25 years of experience working in the transcription field, with specific training and expertise in studies of function and structure of RNA and DNA polymerases. As a postdoctoral fellow, I carried out biochemical and structural characterization of T7 RNA polymerase, an enzyme related to mitochondrial RNA polymerase. As a PI or co-Investigator on several university and NIH-funded grants, I laid the groundwork for the proposed research by developing methods to isolate and study transcription complexes formed by bacterial, yeast and mitochondrial RNA polymerases. Over the past 5 years two major projects have been developed in my laboratory. The first project focused on structural-functional studies of mitochondrial transcription and replication. We were able to elucidate atomic structures of the several key complexes of mitochondrial polymerase, including the structures of the initiation, elongation and anti-termination complex. In addition, we characterized intermediate complexes along the pathway of transcription initiation and suggested the sequential model of assembly of the initiation complex, which is currently the prevailing model in the field. The second major project undertaken by the lab is based on a novel concept of regulation of replication-transcription switch in human mitochondria. The focal point of the project is the D-loop region of mitochondria, which contains a number of regulatory signals and binding sites for yet unidentified proteins. These studies are centered on regulation of replication and transcription at both molecular and cellular levels.

Ongoing and recently completed projects that I would like to highlight include:

NIH R35 (MIRA) GM131832
Temiakov (PI)
6/1/2019 - 5/31/2024
Molecular mechanisms of mitochondrial transcription and replication

NIH R01 GM104231
Temiakov (PI)
1/14/2013 - 12/31/2019

Molecular mechanisms of mitochondrial transcription initiation

NIH R01 GM118941
Temiakov (PI)
8/16/2017 - 5/31/2019
Replication-transcription switch in mitochondria

Citations:

1. Kang E, Wu J, Gutierrez NM, Amy Koski A, Tippner-Hedges R, Agaronyan K, Luengo AP , Redondo PM , 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, D Lee, Wu D, Huang T, Wolf DP , **Temiakov D**, Izpisua Belmonte JC, Amato P, Mitalipov S. Mitochondrial replacement in human oocytes carrying pathogenic mitochondrial DNA mutations. ***Nature***. 2016 Dec 8;540(7632):270-275
2. Zamudio-Ochoa A, Morozov YI, Sarfallah A, Anikin M, **Temiakov D**. Mechanisms of mitochondrial promoter recognition in humans and other mammalian species. **Nucleic Acids Res**. 2022 Mar 21;50(5):2765-2781. doi: 10.1093/nar/gkac103.
3. 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. doi: 10.15252/embj. 2021107988. Online ahead of print. PMID: 34423452

B. Positions and Honors

1997-2002	Postdoctoral Research Scientist, Department of Microbiology and Immunology, SUNY Downstate Medical Center at Brooklyn, NY
2001, 2002	Visiting scientist, Spring-8, RIKEN, Japan
2002-2004	Research Assistant Professor, Department of Microbiology and Immunology, SUNY Downstate Medical Center at Brooklyn, NY
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 at Stratford, NJ
2018-present	Associate Professor, Department of Biochemistry, Thomas Jefferson University

Awards

1999-2000, 2000-2001	Recipient of SUNY Postdoctoral Fellowship “Dean’s Initiative in Research”
2013	Excellence in Research Award, New Jersey Health Foundation
2015	Faculty Research Achievement Award, Rowan University
2015	Excellence in Research Award, New Jersey Health Foundation

C. Contributions to Science

1. **Regulation of mitochondrial transcription and replication.** These studies provided insights into regulation of transcription by initiation factors TFAM and TFB2M, identified a novel transcription intermediate (pre-initiation complex), and introduced the concept of a molecular switch between replication and transcription in human mitochondria.

a. 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

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

c. Morozov AI, Agaronyan K, Cheung A, Anikin M, Cramer P, **Temiakov D**. A model for transciption initiation in human mitochondria. ***Nucleic Acids Res.*** 2015 Apr 20;43(7):3726-35 PMCID: PMC4402542.

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.

2. **Structural studies of mitochondrial transcription.** The first high resolution crystal structure of mitochondrial RNA polymerase was obtained during these studies. This structure, along with the structures of the initiation, elongation and anti-termination complex are the only source of structural information for mitochondrial RNA polymerases and is being used extensively to guide biochemical and biophysical experiments.

a. 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

b. 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

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.

3. Structural-functional studies of multi-subunit RNA polymerases. These studies were focused on characterization of the elongation complexes of multi-subunit RNA polymerases. I obtained crystals that were used to solve the first atomic structure of the elongation complex of bacterial RNA polymerase, a major breakthrough in the transcription field.

a. **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

b. Kashkina E, Anikin M, Tahirov TH, Kochetkov SN, Vassylyev DG, **Temiakov D.** Elongation complexes of *Thermus thermophilus* RNA polymerase that possess distinct translocation conformations. ***Nucleic Acids Res.*** 2006;34(14):4036-45. Epub 2006 Aug 16 PMCID:PMC1557819.

c. 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):257-66

d. Kashkina E, Anikin M, Brueckner F, Lehmann E., Kochetkov S, McAllister WT, Cramer P, **Temiakov.** Multisubunit RNA polymerases melt only a single DNA base pair downstream of the active cite. ***J. Biol. Chem.*** 2007 Jul 27;282(30):21578-82

4. Studies of T7 RNA polymerase. My earlier studies were focused on mechanisms involved in transition of T7 RNAP from initiation to elongation stage of transcription and fidelity of substrate incorporation. The concept of substrate pre-selection and binding outside of the active site of polymerase ("pre-insertion" site) has been introduced based on these studies. The pre-insertion sites were then confirmed experimentally for multi-subunit RNA polymerases and DNA polymerases. The mechanism of substrate selection in the pre-insertion site is now considered as an integral part of transcription and replication fidelity.

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**, Mentesana PE, Ma K, Mustaev A, Borukhov S. & McAllister WT. The specificity loop of T7 RNA polymerase interacts first with the promoter and then with the elongating transcript, suggesting a mechanism for promoter clearance. ***Proc. Nat. Acad. Sci.*** U.S.A. 2000;97:14109-14 PMCID:PMC18879

d. Pomerantz RT, **Temiakov D**, Anikin M, Vassylyev DG, McAllister WT. A Mechanism of Nucleotide Misincorporation during Transcription due to Template Strand Misalignment. ***Mol Cell***. 2006 24(2): 245-255 PMCID:PMC2810628

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