BIOGRAPHICAL SKETCH

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NAME: Mark Anthony Herzik Jr.

eRA COMMONS USER NAME: mherzik

POSITION TITLE: Assistant Professor of Chemistry and Biochemistry

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 Houston (Houston, TX)	B.S	05/2007	Biochemistry/Chemistry
University of California, Berkeley (Berkeley, CA)	Ph.D.	05/2014	Molecular and Cell Biology
The Scripps Research Institute (La Jolla, CA)	Post-doc	12/2018	Structural Biology

A. Personal Statement

One of my true passions in science is using complementary structure-function approaches to reverse-engineer the molecular machines that are critical to human physiology. I am particularly interested in the three-dimensional (3D) conformational landscapes endowing proteins with specific functions and how these structures are dynamically related during signal transduction. My research career thus far has entailed using structural methods to obtain mechanistic insights into signal transduction pathways contributing to a variety of disorders, ranging from cardiovascular disease to neurodegeneration and chronic pain.

For the last decade I have been developing targeted biochemical and structural approaches directed towards elucidating transiently populated conformational states of key biomedically relevant protein complexes. As a *Helen Hay Whitney Foundation postdoctoral fellow* in the laboratory of Dr. Gabriel Lander at the Scripps Research Institute, I obtained extensive expertise in the development of novel high-resolution, high-throughput single-particle cryogenic electron microscopy (cryo-EM) methodologies to push the technology closer to the theoretical size and resolution limits and have become a world-recognized leader in the field. These studies have endowed me with substantial expertise in the expression, purification, characterization, and structure determination of various biological specimens of various sizes (~4.5 kDa to ~700 kDa) and complexities (monomer to 28-mer, etc.). From these works, I have established myself as an expert in structural biology and a key member of the global cryo-EM community. My body of work includes a Thermo Fisher Scientific-sponsored webinar for cryo-EM methods developments, an invited book chapter in Methods and Molecular Biology focused on high-throughput and high-resolution single-particle cryo-EM data collection, and have served as an invited speaker at several national workshops and conferences dedicated to cryo-EM. I have also organized/participated in many cryo-EM symposia/workshops emphasizing the latest developments in cryo-EM and am a committee member for the cryo-EM model validation task force.

My extensive background in structural biology, has provided me with the necessary skills to probe important transient pathways in a targeted manner and elucidate the key conformational switches with atomistic precision. My current group uses the latest cutting-edge cryo-EM instrumentation together with novel sample preparation, data collection and processing algorithms to determine the molecular basis for a variety of age-related and neurodegenerative diseases, focusing primarily on mitochondrial biogenesis and molecular transport mechanisms. Building upon my prior research experience, my group develops novel strategies for specimen preparation, imaging, and processing to enable high-resolution structural determination of these critical protein complexes. To further explore the conformational landscape these complexes sample to perform their functions my group also develops novel strategies for quantifying local and global conformational dynamics within cryo-EM data to better understand the molecular-level transitions necessary for protein function. As a result of these

efforts, I was awarded the *Searle Scholars Award* for my innovative approaches to structure determination and its application to mitochondrial protein biogenesis.

My long-term career goal has always been to conduct cutting edge interdisciplinary biomedical research in a stimulating and collaborative environment that prioritizes the education of students from disadvantaged backgrounds, like myself. As a first-generation college student, I take great pride in the education and mentorship of our next generation of scientists and strive to develop engaging and innovative curriculum and learning resources to ensure their success. I specifically chose a position at The University of California, San Diego because of the supportive scientific environment, the breadth of science being performed, the long-term commitment of UCSD to becoming a pioneer in cryo-EM, as well as the extensive supportive training programs and educational resources for training and mentoring students. The goals outlined in Brian's proposal reflect this decision and my lab is ideally suited to develop the innovative cryo-EM technologies necessary for successful structure determination of PINK1. The combined expertise of Dr. Taylor and I, and our dedication to the training young scientists, together with our collaborators Dr. Reck-Peterson, Dr. Villa, will ensure the goals outlined in Brian's proposal come to fruition.

1. Wu M, Lander GC[#], **Herzik MA Jr**[#]. *Sub-2 Å Resolution Structure Determination Using Single-Particle Cryo-EM at 200 keV*. JSB X. 2020 Feb 27; 4(100020). doi: 10.1016/j.yjsbx.2020.100020. eCollection 2020. PMID: 32647824
   [#]corresponding author
2. **Herzik MA Jr.*,** Wu M*, Lander GC. *High-resolution structure determination of sub-100 kDa complexes using conventional cryo-EM.* Nature Communications. 2019 Mar 4;10(1):1032
3. Hirschi M*, **Herzik MA Jr***, Wie J, Suo Y, Borschel WF, Ren D, Lander GC, Lee SY. *Cryo-electron microscopy structure of the lysosomal calcium-permeable channel TRPML3*. Nature. 2017 Oct;550(7676):411-414. Doi: 10.1038/nature24055. PMID: 29019979
4. **Herzik MA Jr**, Fraser JF, Lander GC. Fraser JF, Lander GC. *A multi-model approach to assessing local and global cryo-EM map quality.* Structure. 2019 Feb 5;27(2):344-358.e3. doi: 10.1016/j.str.2018.10.003. Epub 2018 Nov 15. PMID: 30449687

B. Positions and Honors

ACTIVITY/ OCCUPATION	START DATE (mm/yy)	ENDING DATE (mm/yy)	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Undergraduate Researcher	01/06	05/07	Structural Biology	University of Houston	Dr. Glen B. Legge
Research Technician	05/07	08/08	Structural Biology	University of Houston	Dr. Glen B. Legge
Graduate Student	08/08	05/14	Structural Biology/ Biochemistry	University of California, Berkeley	Dr. Michael A. Marletta
Research Associate	05/14	10/14	Chemistry	The Scripps Research Institute	Dr. Michael A. Marletta
Post-doctoral Research Associate	10/14	12/18	Structural Biology	The Scripps Research Institute	Dr. Gabriel C. Lander
Assistant Professor	01/19	Current	Biochemistry/ Biophysics	University of California, San Diego	University of California, San Diego

Academic and Professional Honors

University of California, San Diego:

Lattimer Award — March 2021 – March 2022
Searle Scholar Award — July 2020 – Present
Southern California Society for Microscopy and Microanalysis — April 2019 – Present
Board Member – Executive Council in Biology

The Scripps Research Institute:

The Scripps Research Institute Society of Fellows Research Travel Award — Spring 2017

Helen Hay Whitney Postdoctoral Fellowship April 2016 – January 2019

University of California, Berkeley:
American Heart Association Predoctoral Fellowship (Award# 11PRE7370086) July 2011 – June 2013
NIH Biophysics Training Grant (Award #T32GM008295-24) July 2010 – June 2011
Outstanding Graduate Student Instructor Award for Molecular and Cell Biology Spring 2010

Mentor Experience

INSTITUTION/COMPANY	TRAINEE	OCCUPATION	START (mm/yy)	END (mm/yy)	FIELD
University of California, San Diego	B. Cook, Ph.D.	Postdoc	09/20	Current	Electron Microscopy
University of California, San Diego	K. McGuire, Ph.D.	Postdoc	09/20	Current	Electron Microscopy
University of California, San Diego	H. Nguyen	Graduate Student	02/19	Current	Electron Microscopy
University of California, San Diego	A. Payan	Graduate Student	04/19	Current	Electron Microscopy
University of California, San Diego	K. Nguyen	Undergrad	05/19	Current	Biophysics
University of California, San Diego	N. Mousa	Undergrad	05/19	Current	Biophysics
University of California, San Diego	Y. He	Undergrad	01/20	Current	Biochemistry
The Scripps Research Institute	M. Wu	Graduate Student	08/16	05/19	Electron Microscopy
The Scripps Research Institute	A. Song	Undergrad	03/15	12/18	Biochemistry
University of California, Berkeley	R. Jonnalagadda	Undergrad	09/12	06/14	X-ray crystallography
University of California, Berkeley	Brandyn West	Undergrad	05/10	06/12	Biochemistry

Teaching Experience

University of California, San Diego
Lead Instructor, CHEM265: Introduction to 3D Electron Microscopy Winter 2021
Lead Instructor, CHEM114A: Biochemical Structure and Function Fall 2020
Lead Instructor, CHEM213A: Protein Structure and Function Winter 2020
Lead Instructor, CHEM265: Introduction to 3D Electron Microscopy Winter 2020
Lead Instructor, CHEM114A: Biochemical Structure and Function Fall 2019

The Scripps Research Institute
Guest Instructor, Introduction to Structural Biology Winter 2016
Guest Instructor, Introduction to Structural Biology Winter 2017

University of California, Berkeley
Head Grader, Survey of the Principles of Biochemistry and Molecular Biology Spring 2012 – Spring 2014
Grader, Survey of the Principles of Biochemistry and Molecular Biology Fall 2011 – Spring 2014
Graduate Student Instructor, General Biochemistry & Molecular Biology Laboratory Spring 2011
Graduate Student Instructor, Survey of the Principles of Biochemistry and Molecular Biology Fall 2009

C. Contributions to Science

My contributions to science are organized into three categories: I. Cryogenic Electron Microscopy (cryo-EM) Method Development, II. Protein Dynamics, and III. Structural Studies of Integral Membrane Proteins. I have deposited 40 Protein Data Bank Entries (1 unpublished), 18 Electron Microscopy Data Bank (EMDB) entries (2 unpublished) and authored/co-authored 21 publications/book chapters. A full list of my current publications can be found at the following location: https://www.ncbi.nlm.nih.gov/myncbi/1v1UFrlamy-At/bibliography/public/

I. Cryogenic Electron Microscopy (cryo-EM) Method Development:

From the outset of my introduction to single-particle cryo-EM I have dedicated significant efforts toward furthering the technology beyond the currently perceived limits (1,2,3). Working at the forefront of the field,

I have spearheaded the development of novel cryo-EM sample preparation, data collection, and image processing strategies that have advanced the known lower molecular size limit to obtain high-resolution reconstructions of macromolecular protein complexes that had traditionally been deemed too small to image by cryo-EM (2,3). The smallest of these complexes, ~42 kDa (2), held the world-record for the smallest complex that had been imaged to sub-nanometer resolution at the time of publication. Furthermore, these studies demonstrated not only that such small macromolecular complexes could be imaged by single-particle approaches, but that distinct conformational states of a protein could be resolved for complexes as small as ~64 kDa (2). In addition, I have furthered the attainable resolution limits of single-particle cryo-EM closer to the theoretical limits, obtaining 3D reconstructions of well-behaved mammalian macromolecular complexes to as high as ~1.7 Å resolution (1). These structures are of sufficient quality to resolve ordered water molecules, alternative rotameric conformations, as well as holes in the EM density for aromatic side chains and proline residues (1). Each of these works represent key milestones in the field of EM and have revolutionized the perceived capabilities of current instrumentation. The raw data from each of these works have been uploaded to the Electron Microscopy Publix Image Archive Repository (EMPIAR) and have served as important datasets for the development of new data processing algorithms. Additionally, the methodologies critical to the success of these works is also the focus of a chapter in Methods in Molecular Biology entitled "Setting up parallel illumination on the Talos Arctica for high-resolution data collection", 2020 *in press*, MA Herzik Jr.

Research Papers:

1. Wu M, Lander GC*, **Herzik MA Jr***. *Sub-2 Å Resolution Structure Determination Using Single-Particle Cryo-EM at 200 keV*. JSB X. 2020 Feb 27; 4(100020). https://doi.org/10.1016/j.yjsbx.2020.100020
   **Denotes equal contribution*
2. **Herzik MA Jr.*,** Wu M*, Lander GC. *High-resolution structure determination of sub-100 kDa complexes using conventional cryo-EM.* Nature Communications. 2019 Mar 4;10(1):1032
   **Denotes equal contribution*
   ***Recommended by Faculty of 1000*
3. **Herzik MA Jr***, Wu M*, Lander GC. *Achieving better Than 3 Å resolution by single particle cryo-EM at 200 keV*. Nature Methods. 2017 Nov;14(11):1075-1078. doi: 10.1038/nmeth.4461. PMID: 28991891
   **Denotes equal contribution*

Symposia

4. **Herzik MA Jr** and DeRosier D. 3rd Annual Southern California Cryo-EM Symposium 2018. Organizer.

II. Protein Dynamics:

A longstanding goal of my research has been the development of new strategies directed towards obtaining a molecular-level understanding of the conformational landscape proteins must traverse to perform their functions. During my undergraduate and graduate careers, I developed targeted biochemical and structural methodologies to visualize transiently populated states during signaling cascades (4) and obtain molecular ensembles of these critically important complexes. To glean more information into conformational dynamics present within cryo-EM data I developed a novel, user-independent multi-model pipeline that provided critical insights into the function of the 26S proteasome lid (2) and resulted in the first molecular ensemble derived from cryo-EM data deposited to the PDB (PDB ID: 3JCK). I then applied these methodologies to all high-resolution cryo-EM structures in the EMDB to obtain a molecular ensemble that represented the EM data and provided a quantitative and qualitative means to assess the local and global quality of a cryo-EM reconstruction (1). Using these approaches, in collaboration with the lab of Dr. Seok-Yong Lee (Duke), together with novel sample preparation and data processing methodologies I was able to show for the first time that the transient receptor potential (TRP) channel family of ion channels undergo significant conformational changes during sensitization and activation (3). These studies are evidence of my capable dedication to developing novel approaches to directly visualizing local and global conformational dynamics.

Research Papers:

1. **Herzik MA Jr**, Fraser JF, Lander GC. *A multi-model approach to assessing local and global cryo-EM map quality.* Structure. 2019 Feb 5;27(2):344-358.e3. doi: 10.1016/j.str.2018.10.003. Epub 2018 Nov 15. PMID: 30449687
   ***Recommended by Faculty of 1000*
2. Dambacher CM*, Worden EJ*, **Herzik MA Jr***, Martin A, Lander GC. *Atomic structure of the 26S*

proteasome lid reveals the mechanism of deubiquitinase inhibition. Elife. 2016 Jan 8;5:e13027. (doi: 10.7554/eLife.13027). PMID: 26744777
**Denotes equal contribution*

3. Zubcevic L*, **Herzik MA Jr***, Wu M*, Borschel WF, Hirschi M, Song A, Lander GC, Lee SY. *Conformational ensemble of the human TRPV3 ion channel.* Nature Communications. 2018 Nov 14;9(1):4773. doi: 10.1038/s41467-018-07117-w. PMID: 30429472
**Denotes equal contribution*

4. **Herzik MA Jr,** Jonnalagadda R, Kuriyan J, Marletta MA. *Structural insights into the role of iron-histidine bond cleavage in NO-induced activation of H-NOX proteins*. Proc Natl Acad Sci USA. 2014 Oct 7;111(40):E4156-64. (doi: 10.1073/pnas.1416936111). PMID: 25253889
***Recommended by Faculty of 1000*

III. Structural Studies of Integral Membrane Proteins:

As mentioned above, I have dedicated significant effort to developing novel methodologies to further the size and resolution capabilities of single-particle cryo-EM. Alongside these efforts, I spearheaded the development of high-throughput single-particle cryo-EM approaches that allowed for the rapid screening of numerous conditions (e.g., detergents, lipids, agonists, etc.) directed towards the determination of novel 3D structures of several integral membrane protein complexes (1,2,3,4). In collaboration with the lab of Dr. Seok-Yong Lee at Duke University I was able to determine the first 3D cryo-EM structures of the Transient Receptor Potential Vanilloid-2 (TRPV2) ion channel (1), the Transient Receptor Potential Mucolipin-3 (TRPML3) ion channel (2), the TRPV3 ion channel (3), and served as a key member in determining the first full-length structures of the mitochondrial calcium uniporter (MCU) (4). During these studies, I became an expert in the purification, handling, and manipulation of mammalian integral membrane proteins for structure determination using high-throughput, high-resolution single-particle cryo-EM. These studies are evidence of my capable dedication to developing novel methodologies in order to pursue challenging biological questions and, given my track record as a leader in pushing the molecular envelope of structure determination, I am confident that we will successfully determine the structures of the challenging targets outlined in this proposal.

Research Papers:

1. Zubcevic L*, **Herzik MA Jr***, Chung BC, Liu Z, Lander GC, Lee SY. Cryo-electron microscopy structure of the TRPV2 ion channel. Nature Structural and Molecular Biology. 2016 Feb;23(2):180-6. (doi: 10.1038/nsmb.3159). Epub 2016 Jan 18. PMID: 26779611
**Denotes equal contribution*

2. Hirschi M*, **Herzik MA Jr***, Wie J, Suo Y, Borschel WF, Ren D, Lander GC, Lee SY. *Cryo-electron microscopy structure of the lysosomal calcium-permeable channel TRPML3*. Nature. 2017 Oct;550(7676):411-414. Doi: 10.1038/nature24055. PMID: 29019979
**Denotes equal contribution*

3. Zubcevic L*, **Herzik MA Jr***, Wu M*, Borschel WF, Hirschi M, Song A, Lander GC, Lee SY. *Conformational ensemble of the human TRPV3 ion channel.* Nature Communications. 2018 Nov 14;9(1):4773. doi: 10.1038/s41467-018-07117-w. PMID: 30429472
**Denotes equal contribution*

4. Yoo J, Wu M, Yin Y, **Herzik MA Jr**, Lander GC, Lee SY. *Cryo-EM structure of a mitochondrial calcium uniporter.* Science. 2018 Aug 3;361(6401):506-511. doi: 10.1126/science.aar4056. PubMed PMID: 29954988.

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

NIH R35 GM138206	Herzik (PI)	07/01/20 – 06/31/25
Towards an atomistic understanding of mitochondrial protein biogenesis.		
Searle Scholars Program	Herzik (PI)	07/01/20 – 07/01/23
Developing hybrid methods to understand dynamic mitochondrial protein assemblies		
Exploratory Project Grant – NysnoBio Neurology	Herzik (PI)	04/01/20 – 10/01/20
Molecular understanding of the progression of Parkinson's disease		