

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: Brotzman, Shayna

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

POSITION TITLE: PhD 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
University of Michigan, Ann Arbor, MI	BS	08/2019	04/2023	Biochemistry
University of Pennsylvania, Philadelphia, PA	PhD	08/2023	05/2028 (expected)	Biochemistry, Biophysics, and Chemical Biology

A. Personal Statement

My long-term research interests center on understanding the structural and functional mechanisms of proteins that contribute to human disease. My undergraduate education at the University of Michigan gave me a strong foundation in biochemistry and molecular biology. My interest in protein biochemistry led me to join Dr. Markos Koutmos' lab, where I investigated the molecular mechanisms of tRNA-binding proteins involved in cancer and developmental disorders. Through this experience, I gained experience in cloning, protein purification, and kinetic and thermodynamic assays, culminating in a publication and a poster presentation at a regional ACS meeting. Additionally, I developed independence as a scientist and trained incoming undergraduate and graduate students. My passion for mentorship was further reinforced through roles as an Undergraduate Instructor's Aide and as a Peer Mentor for the Women in Science and Engineering Residency Program, further solidified my passion for mentoring future research scientists. I plan to continue mentoring incoming graduate and undergraduate students over the course of my time in graduate school.

My undergraduate research experience solidified my desire to pursue research as a career and propelled me to apply for biochemistry PhD programs that would allow me to gain expertise in protein structural biology. After enrolling in the Biochemistry, Biophysics, and Biological Chemistry program at the University of Pennsylvania, I rotated in Roberto Dominguez's lab, where I was introduced to the actin cytoskeleton and gained hands-on experience with cryo-electron microscopy for the first time. I ultimately chose to join the Dominguez lab because I was drawn to unique integration of structural biology and biochemical techniques to study the molecular mechanisms of actin-binding proteins (ABPs). My thesis project focuses on uncovering the structural and functional mechanisms of three ABPs involved in thin filament length regulation in muscle sarcomeres. I have already obtained a preliminary structure of one of these proteins bound to the pointed end of F-actin, which I anticipate will lead to a first-author publication in the coming year.

Beyond research, my training plan includes opportunities to refine my scientific writing and critical thinking skills, present scientific literature and my own work, and explore potential career paths. With the support of this fellowship, my research advisor, and my department, I aim to achieve my goal of becoming a principal scientist in industry after graduation.

Peer-reviewed articles: Wilhelm, C. A., Mallik, L., Kelly, A. L., **Brotzman, S.**, Mendoza, J., Anders, A. G., Leskaj, S., Castillo, C., Ruotolo, B. T., Cianfrocco, M. A., & Koutmos, M. (2023). Bacterial RNA-free RNase P: Structural and functional characterization of multiple oligomeric forms of a minimal protein-only ribonuclease P. *J Biol Chem.*, 299(11), 105327

I have not published or created research products under another name.

B. Positions, Scientific Appointments and Honors

Positions and Employment

2021 - 2023: Undergraduate Research Assistant at University of Michigan

2021: Undergraduate Instructor's Aide at University of Michigan

2020 - 2021: Peer Mentor for Women in Science and Engineering Residency Program at University of Michigan

Professional Memberships

2023: Member, Phi Beta Kappa Honor Society

Honors

2023: Royal Society of Chemistry Award

2021 – 2022: May-Walt Summer Research Scholarship

2021 – 2022: James B. Angell Scholar

2019 - 2022: University Honors

2019: William J. Branstrom Freshman Prize

C. Contributions to Science

Undergraduate Research: As an undergraduate research assistant in Dr. Markos Koutmos' lab at the University of Michigan, I spent over two years characterizing a newly discovered subclass of RNase P enzymes, known as Homologs of Aquifex RNase Ps (HARPs), that cleave the 5' end of precursor tRNAs (pre-tRNAs) during tRNA maturation. To investigate *H. thermophilus* (*Ht*) HARP substrate specificity and activity, I designed and conducted single-turnover assays with several fluorescently labeled pre-tRNAs to assess the impact of various pre-tRNA structural features on cleavage efficiency. The observed rate constants from those assays indicated *Ht* HARP has the highest activity toward native *H. thermophilus* substrates, with no preference for the length of the 5' pre-tRNA leader sequence length. Additionally, I determined the binding affinity (K_b) of *Ht* HARP for various pre-tRNA substrates using binding electrophoretic mobility shift assays (EMSAs). One key finding from these experiments was that *Ht* HARP appeared to form higher oligomeric states when bound to pre-tRNA. This was later confirmed by cryo-EM and x-ray crystallography, which revealed the existence of dimer, tetramer, dodecamer, and 14-mer states. The cryo-EM structures also revealed a novel tRNA-binding mode that may be conserved in other tRNA-binding proteins. My contributions to the HARP project culminated in several poster presentations as well as a publication in the *Journal of Biological Chemistry* in 2023.

1. Wilhelm, C. A., Mallik, L., Kelly, A. L., **Brotzman, S.**, Mendoza, J., Anders, A. G., Leskaj, S., Castillo, C., Ruotolo, B. T., Cianfrocco, M. A., & Koutmos, M. (2023). Bacterial RNA-free RNase P: Structural and functional characterization of multiple oligomeric forms of a minimal protein-only ribonuclease P. *J Biol Chem.*, 299(11), 105327

Graduate Research: In the lab of Dr. Roberto Dominguez, I study the structural and functional mechanisms by which actin binding proteins (ABPs) regulate actin filament (F-actin) dynamics. During my rotation, I worked to purify and structurally characterize CapG, a gelsolin-related actin binding protein that caps the barbed end of actin filaments. Using cryo-EM, I was able to solve a preliminary structure of CapG bound to the barbed end of F-actin, providing insights into why it lacks the severing activity of other gelsolin protein family. This work culminated in an oral presentation to my department. For my thesis project, I am investigating the molecular mechanisms of ABPs that regulate thin filament length (TFL) in sarcomeres, focusing on leiomodin-2 (Lmod2) and SH3BGR. I have developed methods to purify constructs of both proteins and used pyrene-actin polymerization assays to establish SH3BGR as a novel pointed-end capping protein. Additionally, I have solved a preliminary structure of Lmod2 bound to the pointed end of F-actin in conjunction with tropomyosin, a known thin filament ABP that interacts with Lmod2. My current work focuses on solving the structure of SH3BGR bound to the pointed end, both alone and in conjunction with known pointed-end thin filament ABPs, to elucidate its structural capping mechanism. Combining these structural insights with biochemical experiments will reveal how these proteins contribute to the strict length regulation of actin filaments seen in

sarcomeres in striated muscle. To date, I have presented my research at multiple departmental seminars and plan to showcase my findings in upcoming poster sessions.

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
UNIVERSITY OF MICHIGAN – CUMULATIVE GPA: 3.992		
2019	WISE RP Seminar	A
2019	Writing and Academic Inquiry	A+
2019	Elementary Modern Hebrew I	A
2019	Applied Honors Calculus 2	A
2019	The Land of Israel/Palestine through the Ages	A
2020	STEM Success	A
2020	Introductory Biology – Molecular, Cellular, and Developmental	A
2020	Structure and Reactivity I	A
2020	Investigations in Chemistry	A
2020	Elementary Modern Hebrew II	A
2020	Introductory Biology: Ecology and Evolution	A
2020	Structure and Reactivity II	A
2020	Synthesis and Characterization of Organic Compounds	A+
2020	Mathematical Methods for Physical Scientists	A+
2020	Intermediate Modern Hebrew I	A
2021	Introduction to Buddhism	A+
2021	Chemical Principles	A
2021	Fundamentals of Biochemistry	A
2021	Intermediate Modern Hebrew II	A
2021	Teaching Experience for Undergraduates	A
2021	Introductory Bioinorganic Chemistry: The Role of Metals in Life	A+
2021	Introduction to Biochemical Research Techniques and Scientific Writing	A
2021	Undergraduate Research in Biochemistry	A+
2021	War and Revolution in China	A+
2022	Structural Biology: The Architecture of Life	A
2022	Undergraduate Research in Biochemistry	A+
2022	Organic Chemistry of Drug Design	A-
2022	Advanced Biochemistry: Macromolecular Structure and Function	A+
2022	Genetics	A+
2022	Undergraduate Research in Chemistry	A+
2022	Biophysical Chemistry I: Thermodynamics and Kinetics	A
2022	Russia and the Soviet Union: Reform, Revolution, and the Socialist Experiment	A+
2022	Protein Misfolding Diseases	A+
2023	Undergraduate Research in Chemistry	A+
2023	Advanced Biochemistry: Cellular Processes	A+
2023	Protein Structure and Function	A+
2023	Critical Reasoning	A
UNIVERSITY OF PENNSYLVANIA – CUMULATIVE GPA: 4.0		
2023	Cell Biology	A
2023	Macromolecular Biophysics	A+
2023	Macromolecular Crystallography	A+
2023	Lab Rotation	A+

YEAR	COURSE TITLE	GRADE
2024	Structural and Mechanistic Biochemistry	A
2024	Data Analysis and Scientific Inference	A+
2024	Molecular Basis of Disease	A
2024	Lab Rotation	A
2024	Raiziss Seminar	A
2024	Pre-Dissertation Research	A
2024	Emerging Infectious Diseases	A