OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

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: **Mishghan Zehra Humayun**

eRA COMMONS USER NAME (credential, e.g., agency login): mhpz2

POSITION TITLE: Graduate Student Research Assistant

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 Karachi, Karachi, Pakistan	BSc	12/2015	Biochemistry
University of Karachi, Karachi, Pakistan	MSc	12/2017	Biochemistry
University of Karachi, Karachi, Pakistan	M.Phil	06/2019	Biochemistry
University of Missouri, Columbia, USA.	Ph.D.	Current	Biochemistry

A. Personal Statement

My curiosity about the intricacies of chemical reactions within living organisms on a molecular scale has driven me to delve deeper into the realm of biochemical processes, expanding my understanding of life. Engaging in scientific research has ignited within me a fervent enthusiasm for the sciences and cultivated a keen investigative spirit. The opportunity to study anemia prevalence in pregnant mice models and its association with BMI and dietary diversity during my undergraduate career first piqued my interest in human health-related research. This initial research exposure led me to acquire invaluable skills in basic biochemistry and laboratory procedures. During this early period, I developed evaluative techniques and developed my ability to discuss a wide range of ideas and skills. I excelled at tasks requiring strong organization and attention to detail. To build on these strengths and gain exposure to clinical data, I next worked in an oncology department collecting data from breast cancer patients. I investigated the correlation of vitamin D deficiency with bone turnover markers in diagnosed breast cancer patients and co-authored a paper. As a graduate student, I took more advanced courses in biochemistry, which helped lay the foundation for my research-based mindset, intensifying my interest in the field.

Having decided to pursue a scientific research career at this point, I moved from Pakistan to the USA to explore upcoming opportunities. As a researcher at Marshall University Joan C. Edwards School of Medicine in West Virginia, I participated in a variety of studies investigating the contributions of chronic oxidative stress to kidney disease, non-alcoholic steatohepatitis, obesity, and metabolic syndrome, as well as their associated long-term complications. In addition to my basic science research, I was also extensively involved in clinical research to evaluate some potential biomarkers and circulating miRNAs implicated in chemotherapy-related cardiac

dysfunction and non-alcoholic fatty liver disease (NAFLD) progression. My extensive contributions allowed me to co-author multiple projects. The experience I gained in this lab taught me how to work within deadlines and manage my workload productively, which I believe is an essential skill for studying science. In addition to learning how to communicate and socialize with others, I gained confidence in working cooperatively with others as a team. Training medical students and undergraduates was also part of my responsibilities. My duties required patience and resilience as I handled both teamwork and individual tasks. The experience provided me with a chance to hone my project management and teaching skills. Through my experience, I've demonstrated that I'm hardworking, passionate, and determined, which are qualities that are needed in this work environment.

I gained insight into the nuances of cell signaling in chronic oxidative stress-associated metabolic disorders while participating in research projects studying the role of renin-angiotensin-aldosterone systems (RAAS) in adipose tissue dysfunction, Na/K-ATPase signaling in an experimental model of uremic cardiomyopathy, and adipose tissue transplantation in an experimental model of uremic cardiomyopathy. These studies revealed the importance of individual protein functions. Consequently, I chose a Ph.D. program that provided me with an understanding of protein structure and function as they pertain to human disease. Since then, I have been passionately exploring this topic and intend to continue to do so throughout my career.

My research background offers me a firm grounding in this field. I am well-acquainted with various lab techniques and eager to expand my expertise further. Leveraging my skills and knowledge, I am confident in my ability to excel in this research field and enthusiastic about contributing to advancements in human health.

Ph.D. project: I joined Dr. Clarissa Durie's lab, which specializes in cryo-electron microscopy, a state-of-the-art structural biology tool. I was fascinated by single-particle cryo-electron microscopy, which revolutionized structural biology and was recognized with the Nobel Prize in Chemistry. Further, I had the opportunity to work closely with Dr. Durie and other experienced researchers. The lab project I was assigned aligned with my interests in human diseases. Currently, I am working with a Gram-negative human pathogen, Legionella pneumophila, which causes a potentially fatal form of pneumonia. The pathogen causes infection through the use of a Type IV secretion system, so my primary objective is to characterize this weapon structurally using single-particle cryoEM. In the long run, we hope to understand how *Legionella pneumophila's* Type IV secretion system transports proteins into the host cell and contributes to pathogenicity. Drawing on my past laboratory experiences, I've demonstrated the ability to quickly acquire new techniques. Under the guidance of my principal investigator and senior lab scientist, Wing-Cheung Lai, I've developed a comprehensive understanding of methodologies pertinent to my project in Dr. Durie's lab.

B. Positions and Scientific Appointments:

- Graduate Research Assistant/Ph.D. candidate at the University of Missouri, Columbia, USA (2021-present)
- Graduate Teaching Assistant at the University of Missouri, Columbia, USA (2023)
- Research Associate at the Marshall University Joan C. Edwards School of Medicine, Huntington, WV, USA (2018-2021)
- Clinical Nutritionist at the Jinnah Postgraduate Medical Center, Karachi, Pakistan (2017-2018)
- Graduate Research Assistant at the University of Karachi, Pakistan (2016-2018)
- Clinical Laboratory Assistant at the Darul Sehat Hospital, Karachi, Pakistan (2015)
- Undergraduate Research Assistant at the University of Karachi, Pakistan (2013-2015)

Honors:

- Awarded a gold medal for securing 1st position in the BSc in Biochemistry (2015)
- Awarded a gold medal for securing 1st position in the MSc in Biochemistry (2017)

Poster Presentations:

- Poster Presentation at the Graduate Life Sciences Joint Recruitment Weekend Interdisciplinary Research Poster Session 2024 in Columbia, MO.
- Poster Presentation at the 37th Annual Gibbs Conference 2023 on Biological Thermodynamics in Carbondale, Illinois.
- Poster Presentation at the 21st Annual Great Plains Infectious Disease Conference (GPID 2023) in Columbia, MO.
- Poster Presentation at the Central States Microscopy and Microanalysis Meeting 2023 in Columbia, MO.
- Poster Presentation at the Graduate Life Sciences Joint Recruitment Weekend Interdisciplinary Research Poster Session 2023 in Columbia, MO.
- 3MT presentation at the Department of Biochemistry, University of Missouri, Columbia.
- Poster Presentation at the 36th Annual Gibbs Conference 2022 on Biological Thermodynamics in Carbondale, Illinois.
- Poster Presentation at the 20th Annual Great Plains Infectious Disease Conference (GPID 2022) in Columbia, MO.
- Poster Presentation at the MU Health Science Research Day 2022 in Columbia, MO.
- Poster Presentation at the Research Day 2020 by Marshall University Joan C. Edwards School of Medicine.
- Poster Presentation at the 5th International Conference on Endorsing Health Science Research (ICEHSR-17) in Karachi, Pakistan.

C. Contributions to Science:

1- The review presents a comprehensive analysis of the architectural features of Type IV Secretion Systems (T4SSs) and reveals the diversity of T4SS molecular structures among bacterial species. To better understand the evolution and adaptation of T4SS, the study identifies commonalities as well as differences between structural features. The diverse molecular structures of T4SS among bacterial species have significant implications for bacterial pathogenicity. Variations in T4SS architecture can influence bacteria's ability to deliver virulence factors, such as toxins or effector proteins, to host cells, thereby impacting the severity and outcome of infections. Understanding T4SS diversity and identification of features shared across T4SSs is necessary to exploit these similarities for developing targeted therapies and interventions against pathogenic bacteria. Therefore, Durie Lab aims to establish a method to determine high-resolution structures of T4SSs from different bacterial species so that strategies can be developed to prevent and treat infections.

***Zehra M**, Heo J, Chung JM, Durie CL. Comparative Analysis of T4SS Molecular Architectures. J Microbiol Biotechnol. 2023 Dec 28;33(12):1543-1551. doi: 10.4014/jmb.2307.07006. Epub **2023** Aug 1. PMID: 37528551; PMCID: PMC10772558.*

2- Uremic cardiomyopathy presents as a complex and multifaceted form of pathological cardiac hypertrophy observed in advanced chronic kidney disease. It represents a substantial burden for individuals with end-stage renal disease, carrying a high morbidity and mortality rate. This study showcased the potential significance of adipocyte NKAL in mitigating the systemic effects of uremic cardiomyopathy. The findings in the study provide valuable insights into unraveling the intricate molecular mechanisms underlying the involvement of adipocytes and adipocyte NKAL in the advancement of uremic cardiomyopathy. If these observations are validated in human studies, they may pave the way for the discovery of novel therapeutic targets.

*Sodhi, K., Wang, X., Chaudhry, M.A., Lakhani, H.V., **Zehra, M**., et al. **2023**. Adipocyte Na, K-ATPase Signaling Attenuates Experimental Uremic Cardiomyopathy. Cellular and Molecular Biology, 69 (5), 197-206.*

*Sodhi, K., Pillai, S. S., Lakhani, H. V., **Zehra, M**., Abraham, N. G., & Shapiro, J. I. Therapeutic potential of subcutaneous fat and adipocyte transplantation in experimental uremic cardiomyopathy by antagonism of Na/K-ATPase signaling. Under review at Science Signaling.*

3- Prior investigations have established a positive connection between adipose tissue and brain function. Nevertheless, uncertainty remains regarding whether adipocytes directly impact various neuronal regions of the brain. This study illustrates that stimulating adipocytes' Na, K-ATPase oxidant amplification loop (NKAL) through diet induces a neurodegenerative phenotype, which can be mitigated by inhibiting oxidant production via NaKtide (a signaling antagonist). Peripheral adipocytes are central to brain dysfunction induced by Western diets. Consequently, targeting adipocyte NKAL holds promise for potentially averting or addressing neurodegeneration.

*Sodhi K, Pratt R, Wang X, Lakhani HV, Pillai SS, **Zehra M**, Wang J, Grover L, Henderson B, Denvir J, Liu J, Pierre S, Nelson T, Shapiro JI. Role of adipocyte Na, K-ATPase oxidant amplification loop in cognitive decline and neurodegeneration. iScience. 2021 Oct 12;24(11):103262. doi: 10.1016/j.isci.2021.103262. PMID: 34755095; PMCID: PMC8564125.*

*Lakhani, H. V., **Zehra, M**., Pillai, S. S., Shapiro, J. I., & Sodhi, K. Dysregulation of HO-1-SIRT1 Axis is Associated with AngII-Induced Adipocyte Dysfunction. Under review at the International Journal of Molecular Sciences.*

4- Breast cancer patients undergoing anthracycline treatment commonly experience cardiotoxicity, yet the underlying pathophysiological mechanisms remain unclear. Despite ample evidence supporting the necessity for consensus guidelines to monitor cardiac function both before and after chemotherapy initiation, their absence exacerbates the risk of chemotherapy-related cardiac dysfunction in affected patients. This study aimed to assess the clinical efficacy of a novel panel comprising plasma biomarkers and circulating miRNAs associated with cardiotoxicity in breast cancer patients and to examine their correlation with specific cardiovascular injury markers, troponins I and T. Implementation of this panel could facilitate early detection of chemotherapy-induced cardiac dysfunction, enabling timely intervention to manage disease progression and mitigate irreversible damage.

*Lakhani HV, Pillai SS, **Zehra M**, Dao B, Tirona MT, Thompson E, Sodhi K. Detecting early onset of anthracyclines-induced cardiotoxicity using a novel panel of biomarkers in West-Virginian population with breast cancer. Sci Rep. 2021 Apr 12;11(1):7954. doi: 10.1038/s41598-021-87209-8. PMID: 33846495; PMCID: PMC8041906.*

*Qureshi S.A., Udani S.K., **Zehra M**, Batool T. Ghani F, Azmi M.B. **(2018)**. A Cross-sectional Study: Bone Markers in Different Body Mass Index Groups of Newly Diagnosed Breast Cancer Females in Karachi, Pakistan. International Archives of BioMedical and Clinical Research, 4 (1), pp. 141-145, 21.*

5- In non-alcoholic fatty liver disease (NAFLD), an excess accumulation of fat occurs within the liver, resulting in hepatic inflammation and fibrogenesis, ultimately culminating in nonalcoholic steatohepatitis (NASH) or cirrhosis. Common risk factors associated with the development and progression of NAFLD include diabetes and obesity. Currently, the diagnosis of NAFLD relies on radiological studies and biopsies, procedures that are highly invasive and not cost-effective. Therefore, we have devised a panel comprising circulating biomarkers and miRNA to evaluate individuals at risk of progressing to NASH. This approach will enable the implementation of early intervention strategies.

*Pillai SS, Lakhani HV, **Zehra M**, Wang J, Dilip A, Puri N, O'Hanlon K, Sodhi K. Predicting Nonalcoholic Fatty Liver Disease through a Panel of Plasma Biomarkers and MicroRNAs in Female West Virginia Population. Int J Mol Sci. **2020** Sep 13;21(18):6698. doi: 10.3390/ijms21186698. PMID: 32933141; PMCID: PMC7554851.*

***Zehra M**, Curry JC, Pillai SS, Lakhani HV, Edwards CE, Sodhi K. Elucidating Potential Profibrotic Mechanisms of Emerging Biomarkers for Early Prognosis of Hepatic Fibrosis. Int J Mol Sci. 2020 Jul 3;21(13):4737. doi: 10.3390/ijms21134737. PMID: 32635162; PMCID: PMC7369895*.

*Pratt R, Lakhani HV, **Zehra M**, Desauguste R, Pillai SS, Sodhi K. Mechanistic Insight of Na/K-ATPase Signaling and HO-1 into Models of Obesity and Nonalcoholic Steatohepatitis. Int J Mol Sci. 2019 Dec 21;21(1):87. doi: 10.3390/ijms21010087. PMID: 31877680; PMCID: PMC6982200.*

*Lakhani HV, Pillai SS, **Zehra M**, Sharma I, Sodhi K. Systematic Review of Clinical Insights into Novel Coronavirus (CoVID-19) Pandemic: Persisting Challenges in U.S. Rural Population. Int J Environ Res Public Health. 2020 Jun 15;17(12):4279. doi: 10.3390/ijerph17124279. PMID: 32549334; PMCID: PMC7345039.*

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: Clarissa Durie

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

POSITION TITLE: Assistant 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
College of William and Mary, Williamsburg, VA	BBA	05/ 2003	Business, Marketing
University of Alabama at Birmingham, Birmingham, AL	BS	05/2011	Chemistry
University of Alabama at Birmingham, Birmingham, AL	PhD	08/ 2017	Chemistry
University of Michigan, Ann Arbor, MI	Postdoctoral Researcher	12/2021	Structural Biology & Microbiology

A. Personal Statement

I am a biophysicist pursuing research that combines structural and functional investigations of macromolecular machines to improve our understanding of the fundamental process of protein translocation. My academic training and research experience equip me with a background in multiple disciplines including biochemistry, molecular biology, enzyme kinetics, structural biology, and microbiology. During my PhD research in Aaron Lucius's lab at the University of Alabama at Birmingham, I used transient-state kinetics to investigate how two disaggregases, *Escherichia coli* ClpB and *Saccharomyces cerevisiae* Hsp104, use energy from ATP to disrupt protein aggregates like those implicated in neurodegenerative disease. This work included developing and optimizing fluorescence-based binding and kinetics assays and resulted in six publications.

For my post-doctoral work, I joined the research group of Dr. Melanie Ohi, a leader in cryo-electron microscopy and membrane protein complexes. During this work at the University of Michigan, which was funded by an NIH F32, I initiated a collaboration with Dr. Michele Swanson, a leading expert on the bacterial pathogen *L. pneumophila.* I gained expertise in state-of-the-art cryo-EM, membrane protein purification, bacteriology, and genetics techniques that allowed me to study the structure and function of the *L. pneumophila* Dot/Icm Type IV Secretion System. Throughout my academic career, I have formed and maintained successful collaborations within and beyond my university, as evidenced by my publication record.

I have extensively used negative stain electron microscopy and cryo-electron microscopy to screen and assess numerous samples of across a vast range of sizes, oligomeric states, species of origin and other factors. I have determined structures of everything from large multi-component membrane-bound complexes including the T4SS from *L. pneumophila and H. pylori*, to viruses including Eastern Equine Encephalitis Virus, to enzymes including pyruvate kinase and the AAA+ ATPase ClpA in various ligand bound states. I have contributed images to a recent textbook on single particle cryo-EM as well as co-authored review articles about using cryoEM. During a facility closure at my postdoc institution, I proposed and was awarded time at a national cryoEM center (Stanford SLAC CryoEM Center) for data collection on the Titan Krios, on the strength of preliminary data collected using negative stain EM and cryoEM pilot data on a lower strength cryo electron microscope.

My career path is unconventional, and I think my training and background have uniquely prepared me for success. I will briefly summarize those experiences here. I initially studied business in college and then worked in human resources for a consulting firm for about five years. While I found this work afforded me stability, I thought my work could have greater impact in the biomedical sciences through mentoring and discovery. This led to my career change. The project-, time-, and people management skills I learned in that corporate environment have translated well to my scientific pursuits. Having begun my scientific studies with the goal of leading a research team at a university, I sought opportunities to develop curriculum and deliver content. As a graduate student, while serving as a Teaching Assistant for Organic Chemistry Labs (2011-2014), I implemented a new strategy for teaching scientific writing skills to undergraduate students. This work was selected for a book on teaching undergraduate chemistry to millennial students. In Summer 2017, I co-taught Fundamentals of Biochemistry (CH 460). My current teaching appointment is facilitating small group Patient-Based Learning discussions in the University of Missouri, School of Medicine. As a graduate student and a postdoctoral researcher, I have contributed to the training of high school, undergraduate, and graduate students. As a postdoctoral researcher, I directly mentored a summer undergraduate researcher who was awarded a National Sciences Foundation Graduate Research Fellowship based on her research with me and she is now in graduate school. In my current position as an Assistant Professor, I am supervising, training, and mentoring two graduate students. As a new PI, I have participated in many professional development activities through the Leading Edge, an international program for gender minorities (women, non-binary, and transgender individuals) pursuing and starting faculty positions. I organized and moderated a panel on early career funding, and I have hosted workshops to help post-docs preparing for the academic job market develop and refine the research vision for their independent labs. I also serve the community more broadly by reviewing manuscripts for journals ad-hoc and reviewing applications for electron microscopy instrumentation access at an NIH sponsored national center (NCCAT). Taken together with my scientific pursuits, these experiences have prepared me to succeed as the leader of my independent research group.

Current & Recently Completed Research Support

R35 GM150663 Maximizing Investigators' Research Award (MIRA), National Institute of General Medical Sciences of the National Institutes of Health
07/2023 – 04/2028
Role: PI
Title: Protein Transport Across Membrane by Bacterial Pathogens

F32 I150027 Ruth L. Kirschstein National Research Service Award (NRSA), National Institute of Allergy and Infectious Disease
01/2020 - 12/2021
Role: PI
Title: Structural and functional studies of the *Legionella pneumophila* dot T4SS
Note: I started my faculty position after completing two years of the three-year fellowship.

B. Positions, Scientific Appointments, and Honors

Current Position & Appointments

12/2021 – present Assistant Professor, University of Missouri, Department of Biochemistry

Academic & Professional Achievements & Honors

2021 Protein Society, Diversity Award
2020 University of Michigan, Cell & Developmental Biology, Bradley M. Patten Award for Excellence in Postdoctoral Research
2020 Fellow, HHMI Leading Edge Symposium
2019 University of Michigan, Life Sciences Institute, Outreach Award
2019 University of Michigan, Life Sciences Institute, LSI Cubed Funding Competition Awardee
2017 University of Alabama at Birmingham, Alabama Section ACS Outstanding Graduate Student Fellow
2016 University of Alabama at Birmingham, 3MT ®, People's Choice Award (Three-minute thesis)
2016 FASEB SRC on Protein Folding in the Cell, 4th Place Young Investigator Award
2014 University of Alabama at Birmingham, Structural Biology Symposium, Poster Competition 1st Place
2013 University of Alabama at Birmingham, Outstanding Organic Chemistry Graduate Teaching Assistant Award

C. Contribution to Science

Some research products are published under my previous name, Clarissa L. Weaver.

1. **Understanding pathogenesis through high-resolution cryo-electron microscopy:** As a postdoctoral fellow, I isolated Dot/Icm T4SS core complexes from *L. pneumophila* and determined their structures using cryo-EM. Significant findings include the discovery of three proteins in the core complex not previously known to be part of the system and of unexpected stoichiometry of subunits within the complex. We observed a symmetry mismatch between three sub-regions of the core complex, a feature that we now propose to be important to the function of secretion systems since a symmetry mismatch has been observed in other T4SSs, T2SSs, T3SSs, and T6SSs. We also observed greater similarity between the Dot/Icm T4SS and *H. pylori* Cag T4SS, than between either of these two protein-translocating T4SSs and smaller DNA-translocating T4SSs. Still, remarkable differences between the Dot/Icm and Cag systems exist, including the more compressed outer membrane cap of the Dot/Icm system compared to the Cag system. This work resulted in co-first author publications, as well as contributions to published work on the Cag system. During this time, I also worked with collaborators outside the University of Michigan to characterize antibody-bound Eastern Equine Encephalitis Virus by negative stain and to search for suitable conditions for plunge freezing samples for cryo-EM.

- **Durie, C. L.***; Sheedlo, M. J.*; Chung, J. M.; Byrne, B. G.; Su, M.; Knight, T.; Swanson, M.; Lacy, D. B.; Ohi, M. D. Structural Analysis of the Legionella Pneumophila Dot/Icm Type IV Secretion System Core Complex. *eLife* **2020**, *9*, e59530. (* equal contribution)
- Sheedlo, M. J.*; Chung, J. M.*; Sawhney, N.; **Durie, C. L.**; Cover, T. L.; Ohi, M. D.; Lacy, D. B. Cryo-EM Reveals Species-Specific Components within the Helicobacter Pylori Cag Type IV Secretion System Core Complex. *eLife* **2020**, *9*, e59495. (* equal contribution)
- Williamson, L. E.; Gilliland, T.; Yadav, P.; Binshtein, E.; Bombardi, R.; Kose, N.; Nargi, R. S.; Sutton, R. E.; **Durie, C. L.**; Armstrong, E.; Carnahan, R. H.; Walker, L. M.; Kim, A. S.; Fox, J. M.; Diamond, M. S.; Ohi, M. D.; Klimstra, W. B.; Crowe, J. E. Human Antibodies Protect against Aerosolized Eastern Equine Encephalitis Virus Infection. *Cell* **2020**, *183* (7), 1884-1900.e23.
- Sheedlo, M. J.*; **Durie, C. L.***; Chung, J. M.; Chang, L.; Roberts, J.; Swanson, M.; Lacy, D. B.; Ohi, M. D. Cryo-EM Reveals New Species-Specific Proteins and Symmetry Elements in the Legionella Pneumophila Dot/Icm T4SS. *eLife* **2021**, *10*, e70427. (* equal contribution)

2. **Early contributions to the field from my independent lab** include review articles on the family of type IV secretion systems and the use of AI in cryoEM data analysis.

- Chung, J.M. ; **Durie, C. L.** ; Lee, J.; Artificial Intelligence in Cryo-Electron Microscopy. *Life* 2022, 12(8), 1267. https://doi.org/10.3390/life12081267
- Zehra, M.[†]; Heon, J.[†]; Chung, J. M.*; **Durie, C. L.***; Comparative analysis of T4SS molecular architectures. *Journal of Microbiology and Biotechnology* 2023; 33(12): 1543-1551 ([†]co-first authors; co-corresponding authors)

3. **Kinetic investigation of the mechanisms of protein disaggregating motors**. I used biophysical assays to investigate the molecular mechanisms of protein translocation used by the ATPases *E. coli* ClpB and *S. cerevisiae* Hsp104. These homologues, together with co-chaperones, disrupt protein aggregates in an ATP-dependent fashion, and thus are of interest as potential therapies for neurodegenerative disease, because animals including humans lack homologues. I examined how the ClpB mechanism changes in the presence of its co-chaperone DnaK. I identified a novel role for the co-chaperone DnaK, which acts as a peptide release factor for ClpB. Next, I explored whether Hsp104 translocates substrates processively. At the time, every structure of hexameric Hsp104 previously reported was a symmetric, planar ring. The report of a novel spiral conformation led me to broaden my investigation of Hsp104 to include nucleotide requirements for hexamerization and polypeptide binding. I found that the high and low peptide binding affinity of hexameric Hsp104 structures corresponds to changing peptide affinity as protomers cycle through ATP binding, hydrolysis, and release. Modulation of binding affinity allows Hsp104 to remain engaged with the client protein through multiple rounds of ATP hydrolysis. Finally, I tested a mechanistic model based on a high-resolution cryo-EM structure of Hsp104 bound to a polypeptide substrate. The authors had proposed a model for processive translocation. Remarkably, I found that Hsp104 translocates soluble substrates non-processively. I used the same experimental designs to study a gain-of-function variant, Hsp104A503S, which was developed to disaggregate proteins implicated in human disease. I found that Hsp104A503S remains bound to the polypeptide for a longer time, but takes fewer kinetic steps than Hsp104.

- **Weaver, C. L.**; Duran, E. C.; Mack, K. L.; Lin, J.; Jackrel, M. E.; Sweeny, E. A.; Shorter, J.; Lucius, A. L. Avidity for Polypeptide Binding by Nucleotide-Bound Hsp104 Structures. *Biochemistry* **2017**, *56* (15), 2071–2075.
- Duran, E. C.; **Weaver, C. L.**; Lucius, A. L. Comparative Analysis of the Structure and Function of AAA+ Motors ClpA, ClpB, and Hsp104: Common Threads and Disparate Functions. *Front. Mol. Biosci.* **2017**, *4*, 54.
- **Durie, C. L.**; Duran, E. C.; Lucius, A. L. Escherichia Coli DnaK Allosterically Modulates ClpB between High- and Low-Peptide Affinity States. *Biochemistry* **2018**, *57* (26), 3665–3675.
- **Durie, C. L.**; Lin, J.; Scull, N. W.; Mack, K. L.; Jackrel, M. E.; Sweeny, E. A.; Castellano, L. M.; Shorter, J.; Lucius, A. L. Hsp104 and Potentiated Variants Can Operate as Distinct Nonprocessive Translocases. *Biophys. J.* **2019**, *116* (10), 1856–1872.

4. **Curriculum Development:** While serving as a Graduate Teaching Assistant for Organic Chemistry Labs (2011-2014; CH 234, 235, 236, 238), I worked with another TA and our Coordinator to implement a new strategy for teaching scientific writing skills to undergraduate students in Organic Chemistry I and II labs. This work was later presented at an American Chemical Society and selected to be included in a book on teaching undergraduate chemistry to the millennial student.
- **Weaver CL**, Duran EC, Nikles JA. Addressing the Millennial Student in Undergraduate Chemistry. Potts GE, Dockery CR, editors. Washington, DC: American Chemical Society; 2014. Chapter 8, An Integrated Approach for Development of Scientific Writing Skills in Undergraduate Organic Lab; p.105-123.

Complete list of published works in my bibliography:

https://www.ncbi.nlm.nih.gov/myncbi/clarissa.durie.1/bibliography/public/