ANNOUNCING
Doctoral Dissertation Defense

Samantha L. Gies

“Biophysical Characterization of the Conformational Dynamics and Membrane Interaction of the Bacterial Phospholipase ExoU”

Candidate for Doctor of Philosophy in Biophysics
Graduate School of Biomedical Sciences
Department of Biophysics
Medical College of Wisconsin

Committee in charge:
Jimmy B. Feix, PhD (Advisor and Chair)
Blake Hill, PhD
David Hoogerheide, PhD
Neil Hogg, PhD
Candice Klug, PhD

Wednesday, April 7, 2021, 1:00 pm (CST)
Live Public Viewing (WebEx):

https://mcw.webex.com/mcw/onstage/g.php?MTID=e703c678101e14b6e7dbf9915fe961f86
GRADUATE STUDIES

Biochemistry of the Cell
Techniques in Molecular and Cell
Molecular and Cellular Biology
Mechanism of Cellular Signaling
Classical and Molecular Genetics
Ethics and Integrity in Science
Research Ethics Discussion Series
Intro to Biomedical Research
Advanced Protein Chemistry
Biophysical Techniques in Biochemistry
Biostatistics I
Electron Spin Resonance
Special Topics in Biochemistry
Biophysics Journal Club EPR
Biophysics Reading and Research
Biophysics Seminar
Doctoral Dissertation
Doctoral Dissertation Continued
Pseudomonas aeruginosa is a major cause of nosocomial infections, a type of infection associated with high morbidity and mortality. The morbidity and mortality associated with P. aeruginosa infections appears to primarily be determined by virulence. The expression of a type III secretion system that injects effector proteins directly into the host cell cytosol significantly impacts the virulence associated with P. aeruginosa infection. Four effectors proteins are secreted by the type III secretion system, of which ExoU is the most cytotoxic.

ExoU is a potent bacterial phospholipase that is regulated by ubiquitin. ExoU rapidly destroys host cell membranes following ubiquitin activation, facilitating P. aeruginosa survival and dissemination in the host. P. aeruginosa infection with a positive ExoU genotype strain is a risk factor for acute mortality. Consequently, the development of an ExoU inhibitor could be of significant therapeutic value.

Two crystal structures of ExoU in the presence of its cognate chaperone have been published. ExoU is a patatin-like phospholipase with an α,β-hydrolase catalytic domain fold and a catalytic serine-aspartate dyad. These structures represent an inactive conformation in the absence of ubiquitin cofactor and the catalytic aspartate is not localized in either electron density map. The catalytic serine and aspartate must be in close proximity, with 5-6 Angstroms, to be in a catalytically productive conformation. A key step to developing an ExoU inhibitor is studying the active holoenzyme conformation of ExoU. Additionally, phospholipases act at the water-
membrane interface so understanding the interaction of ExoU with the membrane is also important.

In my dissertation I characterize the conformational dynamics and membrane interaction near the catalytic residues of ExoU. My initial SDSL-EPR studies on the catalytic serine motif suggest a conformational change occurs in which the catalytic serine motif loses tertiary contacts and becomes exposed to the membrane environment upon activation by both ubiquitin cofactor and phospholipid substrate (Chapter 2). Similar studies on the catalytic aspartate indicate the catalytic aspartate region forms a more restrained conformation upon activation but remains exposed to the aqueous environment (Chapter 3). Our neutron reflectometry experiments are the first study to implicate the catalytic domain in membrane interaction and provide evidence that the formation of the holoenzyme state of ExoU does not involve a major reorganization of the protein structure (Chapter 4).
CURRICULUM VITAE

Samantha L. Gies
PhD Candidate
Department of Biophysics
Medical College of Wisconsin

CONTACT INFORMATION
6500 W Howard Ave Apt 210
Milwaukee WI, 53220
(414)688-7197
slgies112190@gmail.com

EDUCATION
07/2014-05/2021
PhD Candidate, Biophysics, Medical College of Wisconsin, Milwaukee, WI

09/2009- B.S., Chemistry, Carroll University, Waukesha, WI

RESEARCH AND PROFESSIONAL EXPERIENCE
6/2015-present
Graduate Research Assistant, Biophysics, Medical College of Wisconsin, Milwaukee, WI

“Biophysical Characterization of the Conformational Dynamics and Membrane Interaction of the Bacterial Phospholipase ExoU”
Primary Advisor: Jimmy B. Feix, PhD

The goals of my dissertation research are to characterize the conformational dynamics and membrane interaction of the bacterial phospholipase ExoU using site-directed spin labeling in conjunction with electron paramagnetic resonance spectroscopy. Specifically, I want to determine if the conserved catalytic serine structural motif and the catalytic aspartate loop change conformation and interact with the membrane upon formation of the active holoenzyme. I found that the catalytic serine structural motif lost tertiary contacts and became exposed to the membrane environment and that the catalytic aspartate loop underwent a conformational change to a more restrained conformation upon formation of the active holoenzyme state. Additionally, the membrane interaction of ExoU was investigated with neutron reflectometry. This study implicates the catalytic domain of ExoU in membrane interaction.
06/2018  Summer School on Methods and Applications of Small Angle Neutron Scattering and Reflectometry, National Institute of Science and Technology (NIST), NIST Center for Neutron Research (NCNR), Gaithersburg, MD

10/2017  Mass Spectrometry Training Workshop for Self-Service Instruments, Medical College of Wisconsin, Center for Biomedical Mass Spectrometry Research, Milwaukee, WI

07/2017  Demystifying the grant process: a grant writing retreat for postdocs and graduate students, Medical College of Wisconsin, Office of Postdoctoral Education and The Graduate School of Biomedical Sciences, Milwaukee, WI

08/2015  EPRWorkshop “EPR Methodologies Enabled by Loop-Gap Resonators”, National Biomedical EPR Center, Milwaukee, WI

07/2015  Principles of EPR Training Workshop, National Biomedical EPR Center, Milwaukee, WI

PUBLICATIONS


CONFERENCE PRESENTATIONS

03/2019  3rd Annual Graduate Student Association Symposium, Harley Davidson Museum Milwaukee, WI.  
Poster: “Membrane composition studies on the phospholipase activity of ExoU”.

03/2019  63rd Annual Biophysical Society Meeting, Baltimore, MD  
Poster: “Site-directed spin labeling EPR studies on the catalytic aspartate loop of ExoU upon interaction with ubiquitin and membranes”.

02/2018  62nd Annual Biophysical Society Meeting, San Francisco, CA  
Poster: “Investigating the Conformational Dynamics and Membrane Interaction Near the Catalytic Serine of ExoU Upon Interaction with Diubiquitin and Membranes by EPR Spectroscopy”.

05/2017  1st Annual Graduate Student Association Symposium, Harley Davidson Museum Milwaukee, WI.  
Poster: “Site-directed spin labeling studies on the bacterial phospholipase ExoU”.
HONORS AND AWARDS
06/2018  NCNR Summer School Travel Award
2009-2012  Carroll University Dean’s List

LEADERSHIP
05/2018-  Graduate Student Assembly, Biophysics Department Representative
05/2020

PROFESSIONAL MEMBERSHIPS
2012-2021 American Chemical Society
2016-2019 Biophysical Society