Doctoral Dissertation Defense Announcement

Samuel A. DeCero II

“Characterization of the allosteric activation mechanism of the bacterial toxin ExoU mediated by ubiquitin”

Candidate for Doctor of Philosophy in Microbiology & Immunology
Graduate School of Biomedical Sciences
Medical College of Wisconsin

Committee in Charge
Dara W. Frank, PhD (Advisor)
   Jimmy B. Feix, PhD
   Joseph T Barbieri, PhD
   Amy W. Hudson, PhD
   Brian C. Smith, PhD

Tuesday, July 26th, 2022, at 10 am (CST)

Hybrid defense
Room: H1400 Bolger Auditorium
Live Public Viewing: https://mcw-edu.zoom.us/j/91691169093?pwd=Q0JjbVArTnpCM1NOK2w0YmszeWh5dz09
Graduate Studies:

Biochemistry of the Cell
Techniques in Molecular and Cellular Biology
Molecular and Cellular Biology
Mechanisms of Cellular Signaling
Classical and Molecular Genetics
Ethics and Integrity in Science
Advanced Protein Chemistry
Research Ethics Discussion Series
Cellular Microbiology
Bacterial Toxin Mucosal Cell Interactions
Seminar Series
Biophysical Techniques in Biochemistry
Advanced Bacterial Physiology
Abstract:

ExoU is the most potent of the four type III secretion system (T3SS) effectors encoded by *Pseudomonas aeruginosa* (Pa) and *exoU*+ infections are associated with poor clinical outcome and increased patient mortality. ExoU is characterized as a 74 kDa patatin-like phospholipase with a broad substrate specificity. ExoU is oriented to the membrane by an interaction with phosphatidylinositol 4,5-bisphosphate (PIP2) and synergistically activated by interactions with lipids and host ubiquitin (Ub). After transitioning to an active holoenzyme state, ExoU acts on the host displaying phospholipase A2 activity cleaving phospholipids within the inner leaflet of the plasma membrane producing water, lyso-phospholipids, and free fatty acids as byproducts. As a result of intoxication and phospholipase activity, ExoU induces acute cellular necrosis. Studying how ExoU is activated is necessary to develop a specific inhibitor that selectively impedes ExoU activity without impacting host phospholipases.

Studying ExoU with conventional structural approaches such as x-ray crystallography or nuclear magnetic resonance (NMR) are difficult since ExoU is a large, flexible toxin. Using an approach without size and flexibility limitations allows electron paramagnetic resonance (EPR) to be invaluable in understanding the transition of ExoU conformational states. Like crystallography and NMR, EPR has its own limitations. Almost all forms of EPR require the mutagenesis of one or more residues to introduce cysteine residue(s) that could be subsequently labeled using a nitroxide spin label (MTSL, R1 or bromo-MTSL, R5) which is called site-directed spin labeling (SDSL). Together SDSL coupled with EPR can provide insights into structural dynamics, conformational changes, and binding interactions. The two most common EPR techniques to study ExoU have been continuous wave EPR (CW-EPR) and double electron-electron resonance (DEER). CW-EPR provides insight into the tertiary and quaternary structural changes local to the spin label. DEER provides distance distribution measurements between two different spin labels. When coupled with *in silico* modeling, these approaches are capable of deducing conformational changes and or modeling protein-protein interactions.

In this dissertation, I have used a variety of biochemical, biophysical and *in silico* techniques to provide insight into the allosteric activation of ExoU by Ub. Site-directed mutagenesis revealed that a key Ub residue, L8, specifically drives ExoU activity in both residue biochemical properties and specific location in relation to ExoU. CW-EPR and DEER have added to our understanding of the structural dynamics of ExoU in the inactive apoenzyme state and the active holoenzyme state. The development of a microscale thermophoresis (MST) lipid-binding assay allowed for the determination that Ub doesn’t significantly impact ExoU affinity for bicelles. A variety of *in silico* techniques have allowed for the allosteric pathway induced by Ub to be predicted and suggests that Ub L8 is an important residue involved in hydrophobic interactions between ExoU and Ub which are correlated to ExoU activity. Collectively, the data in this dissertation supports that Ub L8 is a specific residue that participates in hydrophobic interactions with ExoU and likely propagates an allosteric pathway that induces ExoU activity.
Samuel A. DeCero II

Education

Doctor of Philosophy, PhD
Department of Microbiology and Immunology
2017-2022 (5 years)
Anticipated, August 31st, 2022

B.A., Biology; Minor, Studio Art
Carthage College
Department of Natural Science
2013-2017 (4 years)

Experience

2017-2022 Graduate Research Assistant/PhD Candidate
Dr. Dara W. Frank, PhD
My dissertation work investigated the potent bacterial toxin ExoU. ExoU is a patatin-like phospholipase encoded by Pseudomonas aeruginosa and delivered to the eukaryotic cytosol via the type 3 secretion system (T3SS). Upon delivery to the host cytosol, ExoU binds the inner leaflet of the phospholipid bilayer, interacts with lipid substrate, and binds ubiquitin. ExoU then displays broad substrate recognition subsequently breaking down substrate, inducing membrane stress, and ultimately acute cell necrosis.

Primary Foci
- Expanded previous studies supporting that a single amino acid of ubiquitin specifically drives ExoU activation using in vivo and in vitro phospholipase activity assays.
- Characterized the impact of ubiquitin on allosteric changes to ExoU structure induced by protein-protein interactions using electron paramagnetic techniques.

Secondary Foci
- Developed a solution-phase lipid-binding assay to determine if ubiquitin influences ExoU lipid affinity using microscale thermophoresis.
- Generated and utilized existing in silico pipelines to model the interaction of ExoU and ubiquitin as well as the potential allosteric pathway of ExoU activation.
- Predicted and generated intramolecular non-native disulfide bonds to study ExoU/ubiquitin structure, ExoU phospholipase activity, and ExoU conformational change using combined SDS-PAGE, in vitro activity assays, and electron paramagnetic techniques.

2013-2017 Research Mentee
Dr. Deborah Tobiason, PhD
- Isolated and characterized environmental acquired bacteria producing antimicrobial compounds.
- Adapted the protocol of Gordonia terrae bacteriophage isolation based on framework and funding by the Howard Hughes Medical Institute SEA-PHAGES program.

2016 Summer Undergraduate Research Experience Mentee
Dr. Andrea Henle, PhD
- Characterized the effects of GNAQ mutations in uveal melanoma oncogenesis using a zebrafish model.
- Extracted protein from zebrafish tissue for use in pull-down assays to detect putative protein-protein interactions with novel binding partner.
Bibliography


Conferences & Presentations

**Conferences**
- 2021 31st Annual Graduate School Research Symposium (Virtual)
- 2020 Lakeside Conference for Protein Toxins and Effectors (Virtual)
- 2019 Midwest Microbial Pathogenesis Conference, Toledo, OH
- 2019 29th Annual Graduate School Research Symposium

**Presentations**
- 2022 The role of ubiquitin L8 in ExoU activity.
- 2021 The contribution of ubiquitin to ExoU’s activity.
- 2020 The impact of ubiquitin on ExoU’s activation.
- 2019 L8R inactivation of ExoU.
- 2019 Visualizing U21 golgi exit using RUSH fluorescent microscopy.
- 2019 The dependence of diubiquitin orientation when activating ExoU.

Leadership and Service

**Leadership**
- 2020-2022 Graduate School Association (GSA) Representative
- 2017-2022 Enhancing Scholarly Culture Committee Chair/Member
- 2019-2020 Spotlight on Science Planning Committee Member

**Appointments**
- 2021-2022 GSA Research Affairs Committee Student Representative
- 2021-2022 GSA Awards Committee Student Representative
- 2020-2021 GSA Fundraising Committee Member
- 2020-2021 GSA Travel Award Policy Committee Member
- 2020-2021 GSA Academic Standing & Welfare Committee Student Representative
- 2020-2021 GSA Peer Mentorship (Big/Little Sib.) Committee Co-chair

Public Service
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<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td>2022</td>
<td>5th Annual Graduate School Association Symposium</td>
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<td>2021</td>
<td>4th Annual Graduate School Association Symposium</td>
<td>Presentation Judge</td>
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<tr>
<td>2018-2020</td>
<td>Milwaukee Public School Science Fair</td>
<td>Science Fair Judge</td>
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### Certifications and Honors

#### Certifications

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<tr>
<td>2024 (Exp.)</td>
<td>Good Clinical Practices (NIDA)</td>
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<td>2017</td>
<td>National Coalition Building Institute (NCBI) Training</td>
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#### Honors

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<td>2019</td>
<td>Graduate School Research Symposium Poster Awardee</td>
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<td>2019</td>
<td>The American Association for the Advancement of Science</td>
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<tr>
<td>2017 &amp; 2016</td>
<td>Earl Lambert Achievement Award for Academic Excellence and Service</td>
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