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: Lacy, D. Borden

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

POSITION TITLE: 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
University of North Carolina, Chapel Hill, NC	B.S.	1990-1994	Chemistry
University of California, Berkeley, CA	Ph.D.	1994-1999	Chemistry
Harvard Medical School, Boston, MA	Postdoctoral	1999-2005	Microbiology

Over the past 20 years I have made contributions to our structural and mechanistic understanding of (i) botulinum neurotoxin, (ii) anthrax toxin (iii) *Helicobacter pylori* VacA, (iv) the large glucosylating toxins of *Clostridioides difficile* and *C. sordellii,* and (v) the *Staphylococcal aureus* leukocidins. My laboratory relies on a diverse array of experimental tools (structural biology, targeted gene disruption, chemical biology, and cell/tissue-based functional assays) to address questions related to how the toxins bind, enter, and modify host cell function. The goal is to create a structural and mechanistic framework for understanding the roles these toxins play in pathogenesis and for pursuing novel therapeutic strategies.

One area of recent focus has been the toxins produced by *C. difficile*, TcdA and TcdB. These toxins are the major virulence factors in *C. difficile* infection, but relatively little is known about the molecular structures of these toxins, their receptors, and the respective roles these toxins play in disease. My laboratory has made several key contributions to this field of study, as highlighted in sections 4 and 5 below.

From a technical perspective, my background was initially in X-ray crystallography. Like many, however, the technical advances in cryo-EM over the last few years have led me to realize that cryo-EM may serve as a better technique for some of our projects. With this, I have been actively attending meetings and workshops that have allowed me to get up-to-speed on the technical aspects of sample preparation, data collection, and data processing. In addition, I now have several trainees who are or are becoming proficient in these methods. I highlight two recent papers where all of the cryo-EM sample preparation, data collection, computation, and model building was done by members of my laboratory.

1. M.J. Sheedlo, D.M. Anderson, A.K. Thomas, **D.B. Lacy.** (2020) Structural elucidation of the *Clostridioides difficile* transferase toxin reveals a single-site binding mode for the enzyme. *Proc Natl Acad Sci U S A.* 117(11):6139-6144. doi: 10.1073/pnas.1920555117. Epub 2020 Mar 2. PMID: 32123082
2. D. M. Anderson[1], M.J, Sheedlo[1], J.L. Jensen, **D.B. Lacy.** (2020) Structural insights into the transition of *Clostridioides difficile* binary toxin from prepore to pore. *Nat Microbiol.* 5(1):102-107. doi: 10.1038/s41564-019-0601-8. Epub 2019 Nov 11.PMID: 31712627. [1]Co-first authors

B. Positions and Honors.

Positions and Employment

2006 - 2011	Assistant Professor, Departments of Microbiology and Immunology and Biochemistry, Vanderbilt University Medical School
2012 - 2016	Associate Professor, Departments of Pathology, Microbiology and Immunology and Biochemistry, Vanderbilt University Medical School
2015 - present	Principal Investigator, Veterans Affairs Tennessee Valley Healthcare System
2017 - present	Professor, Departments of Pathology, Microbiology and Immunology and Biochemistry, Vanderbilt University Medical School

Other Experience and Professional Memberships

2013-present	Member, American Association for the Advancement of Science
2014-present	Member, American Society for Microbiology
2014-2017	Member, International Scientific Program Committee, Clostpath: International Meeting on the Molecular Biology and Pathogenesis of the Clostridia
2014-2018	Member, Host Interaction with Bacterial Pathogens (HIBP) NIH Study Section
2017-2021	NIH, Steering Committee for the NIAID Systems Biology for Antibacterial Resistance program
2017-2022	Member and Chair-elect, Clostpath: International Meeting on the Molecular Biology and Pathogenesis of the Clostridia Steering Committee
2017-2022	Member, Target Selection Board for the NIAID Structural Genomics Centers for Infectious Diseases
2019-2023	Member, Bacterial Pathogenesis (BACP) NIH Study Section

Honors

1994	Phi Beta Kappa
1995-1998	NSF Graduate Student Fellowship
1999-2000	NIH National Research Service Award
2000-2003	Helen Hay Whitney Postdoctoral Fellow
2004-2005	Charles A. King Trust Postdoctoral Fellow
2008-2012	Burroughs Wellcome Investigator in the Pathogenesis of Infectious Disease
2014	Margaret C. Etter Early Career Award, American Crystallographic Association
2015	Ernest W. Goodpasture Research Award, Vanderbilt University
2015-2017	Chancellor Faculty Fellow, Vanderbilt University
2017-2024	Edward and Nancy Fody Chair in Pathology, Vanderbilt University
2017	Elected Fellow of the American Association for the Advancement of Science
2018	Elected Fellow of the American Academy of Microbiology

C. Contribution to Science

1. BoNT. I determined the first X-ray crystal structure of botulinum neurotoxin (BoNT) as part of my graduate work with Ray Stevens at UC Berkeley. The structure (a) and accompanying analysis (b) has served as a platform for novel inhibitor design, the optimization of neutralizing antibodies, and new hypothesis-driven investigations into the mechanism of BoNT entry into cells**.** In starting an independent laboratory, I returned to the study of BoNT, in particular addressing the mode in which different serotypes recognize different host receptors (c) and the structure that allows BoNT to survive passage through the low pH environment of the stomach and the protease-rich environment of the small intestine (d).

a. **D.B. Lacy**, W. Tepp, A.C. Cohen, B.R. DasGupta, and R.C. Stevens (1998) The crystal structure of botulinum neurotoxin type A and implications for toxicity. *Nature Structural Biology* 5**,** 898-902. PMID: 9783750

b. **D.B. Lacy** and R.C. Stevens (1999) Sequence homology and structural analysis of the clostridial neurotoxins. *Journal of Molecular Biology* 291, 1091-104. PMID: 10518945

c. J. Schmitt, A. Karalewitz, D.A. Benefield, D.J. Mushrush, R.N. Pruitt, B.W. Spiller, J.T. Barbieri, **D.B. Lacy** (2010) A structural analysis of botulinum neurotoxin type G receptor binding. *Biochemistry*, 49(25), 5200-5. PMCID: PMC2894633.

d. D.A. Benefield, S.K. Dessain, N. Shine, M.D. Ohi, **D.B. Lacy**. (2013) Molecular assembly of botulinum neurotoxin progenitor complexes. *Proc Natl Acad Sci U S A*. 110(14):5630-5. PMCID: PMC3619295.

2. Anthrax Toxin. During a postdoctoral fellowship with John Collier, I was able to make several contributions to the understanding of anthrax toxin mechanism. Anthrax toxin is formed from three proteins: the cell binding protective antigen (PA) and the enzymes edema factor (EF) and lethal factor (LF). I helped analyze the first crystal structure of LF (a) and mapped the binding site for PA on EF and LF using a cell-based binding assay (b). I determined the crystal structure of the newly discovered anthrax toxin receptor (CMG2) both alone (c) and in complex with the PA heptamer (d) which led to the insight of how the receptor creates a barrier for PA insertion into membranes which can be overcome through exposure to the low pH of the endosome.

a. A.D. Pannifer, T.Y. Wong, R. Schwarzenbacher, M. Renatus, C. Petosa, J. Bienkowska, **D.B. Lacy**, R.J. Collier, S. Park, S.H. Leppla, P. Hanna, and R.C. Liddington (2001) Crystal structure of the anthrax lethal factor. *Nature* 414, 229-33. PMID: 11700563.
b. **D.B. Lacy**, M. Mourez, A. Fouassier, and R.J. Collier (2002) Mapping the anthrax protective antigen binding site on the lethal and edema factors. *J Biol Chem.* 277, 3006-10. PMID: 11714723.
c. **D.B. Lacy**, D.J. Wigelsworth, H.M. Scobie, J.A.T. Young, and R.J. Collier (2004) Crystal structure of the von Willebrand factor A domain of human capillary morphogenesis protein 2: an anthrax toxin receptor. *Proc Natl Acad Sci U S A* 101, 6367-72.
d**. D.B. Lacy,** D.J. Wigelsworth, R.A. Melnyk, S.C. Harrison, and R.J. Collier (2004) Structure of heptameric protective antigen bound to an anthrax toxin receptor: A role for receptor in pH-dependent pore formation. *Proc Natl Acad Sci U S A* 101, 13147-51. PMCID: PMC516539.

3. *Helicobacter pylori*. During my first week at Vanderbilt, my new colleague, Tim Cover, invited me to collaborate on the structural analysis of VacA, a key virulence factor made by the pathogen *H. pylori*. My first graduate student, Kelly Gangwer, began work on this project, and together we determined the first crystal structure for this toxin (a), a 55 kDa domain involved in host cell binding. We continue to enjoy a very productive collaboration, which now includes Melanie Ohi, to understand how the toxin oligomerizes and forms pores in the host cell (b-c). We are also working to understand the structure and function of the Cag Type IV Secretion System (d).

a. K.A. Gangwer, D.J. Mushrush, D.L. Stauff, B. Spiller, M.S. McClain, T.L. Cover, **D.B. Lacy** (2007) Crystal Structure of the Helicobacter pylori Vacuolating Toxin p55 Domain. *Proc Natl Acad Sci U S A*, 104, 16293-8. PMCID:PMC2042200
b. C. González-Rivera, AM Campbell, SA Rutherford, TM Pyburn, NJ Foegeding, TL Barke, BW Spiller, MS McClain, MD Ohi, **D.B. Lacy[1]**, T.L. Cover TL[1]. (2016) A non-oligomerizing mutant form of *Helicobacter pylori* VacA allows structural analysis of the p33 domain. *Infect Immun* 84(9):2662-2670. [1]Co-corresponding authors. PMCID: PMC4995914
c. M. Su, A.L. Erwin, A.M Campbell, T.M. Pyburn, L.E. Salay, J. L. Hanks, **D.B. Lacy**, D. L. Akey, T. L. Cover, M.D. Ohi. (2019) Cryo-EM Analysis Reveals Structural Basis of *Helicobacter pylori* VacA Toxin Oligomerization. *J Mol Biol.* 431(10):1956-1965. PMCID:PMC6625667
d. JM Chung[1], MJ Sheedlo[1], AM Campbell, N Sawhney, AE Frick-Cheng, **D.B. Lacy[2]**, TL Cover[2], MD Ohi[2]. (2019) Structure of the *Helicobacter pylori* Cag type IV secretion system. *Elife.* 2019 Jun 18;8 pii: e47644. [1]Co-first authors, [2]Co-corresponding authors. PMCID:PMC6620104

4. *C. difficile* Toxin Structure. Like anthrax toxin and BoNT, the TcdA and TcdB toxins made by *C. difficile* are AB toxins that enter host cells by endocytosis and use the low pH of the endosome to form a pore and deliver an enzymatic moiety across the membrane. TcdA and TcdB are unlike these toxins in size and sequence however. The opportunity to contribute to a mechanistic understanding of how these medically important toxins function became an area of focus for me and my second graduate student, Rory Pruitt. Since TcdA is a large protein (300 kDa), our approach was to combine high-resolution structures of individual domains with an electron microscopy structure of the holotoxin (a). The collective work has provided insight into how autoprocessing is activated by inositol hexakisphosphate and how TcdA and TcdB can glucosylate different substrates within host cells. In 2016, my laboratory succeeded in elucidating the first TcdA crystal structure that includes the pore forming delivery domain (b). This structure indicates that the mechanism for TcdA/TcdB pore formation is entirely novel. In two of our more recent publications we have used the toxin structural framework to define mechanism of action for antibodies that neutralize TcdA (c) and TcdB (d).

a. R.N. Pruitt, M.G. Chambers, K. Ng, M.D. Ohi, and **D.B. Lacy** (2010) Structural organization of the functional domains of *Clostridium difficile* toxins A and B. *Proc Natl Acad Sci U S A*. 107(30) 13467-72. PMCID: PMC2922184

b. N.M. Chumbler, S.A. Rutherford, Z. Zhang, M.A. Farrow, J.P. Lisher, E. Farquhar, D.P. Giedroc, B.W. Spiller, R.A. Melnyk, **D.B. Lacy**. (2016) Crystal structure of *Clostridium difficile* Toxin A. *Nature Microbiology.* 1:15002. PMCID: PMC4976693
c. Kroh HK, Chandrasekaran R, Rosenthal K, Woods R, Jin X, Ohi MD, Nyborg AC, Rainey GJ, Warrener P, Spiller BW, **D.B. Lacy**. (2017) Use of a neutralizing antibody helps identify structural features critical for binding of toxin TcdA to the host cell surface. *J Biol Chem.* 292(35) 14401-14412. PMCID: PMC5582835
d. Kroh HK, Chandrasekaran R, Zhang Z, Rosenthal K, Woods R, Jin X, Nyborg AC, Rainey GJ, Warrener P, Melnyk RA, Spiller BW, **D.B. Lacy**. (2018) A neutralizing antibody that blocks delivery of the enzymatic cargo of toxin TcdB into host cells. *J Biol Chem*. 293(3) 941-952. PMCID: PMC5777265

5. *C. difficile* Toxin Function. The last four papers that I am highlighting are important in that they challenge dogma that prevailed at the time about TcdB function (a-c), or describe a novel mechanism of TcdA endocytosis (d). My graduate student, Nicole Chumbler, learned that the potent cytotoxicity associated with TcdB was due to necrosis (rather than the accepted mechanism of apoptosis). Further, she discovered that the necrosis was independent of the TcdB autoprocessing and glucosyltransferase activities (a). The finding suggested that simple mutation of these activities would not be enough to ensure vaccine antigen safety, an idea that has now been echoed in independent reports. My post-doctoral trainee, Melissa Farrow, went on to discover the mechanism of the necrosis (b). Specifically, TcdB induces an aberrant activation and assembly of the epithelial cell NADPH-oxidase complex that results in the formation of redox-active endosomes and the production of reactive oxygen species (ROS). More recently, my graduate student, Mitch LaFrance, discovered that PVRL3 (or NECTIN3) can serve as a cellular receptor for TcdB (c). The prevalence of NECTIN3 in human colonic tissue and its colocalization with TcdB suggest the relevance of this interaction in human disease. Finally, my graduate student, Ramya Chandrasekaran discovered a novel mechanism of endocytosis for TcdA (d) which is distinct from the clathrin-mediated entry of TcdB.

a. N.M. Chumbler, M.A.Farrow, L.A.Lapierre, J.L. Franklin, D. Haslam, J.R.Goldenring **D.B. Lacy**. (2012) *Clostridium difficile* Toxin B causes epithelial cell necrosis through an autoprocessing-independent mechanism. *PLoS Pathog.* 8(12):e1003072. PMCID: PMC3516567
b. M.A. Farrow, N.M. Chumbler, L.A. Lapierre, J.L. Franklin, S.A. Rutherford, J.R. Goldenring, **D.B. Lacy** (2013) *Clostridium difficile* toxin B-induced necrosis is mediated by the host epithelial cell NADPH oxidase complex. *Proc Natl Acad Sci U S A.* 110(46):18674-9. PMCID: PMC3831945
c. M.E. LaFrance, M.A. Farrow, R. Chandrasekaran, J. Sheng, D.H. Rubin, **D.B. Lacy**. (2015) Identification of an Epithelial Cell Receptor Responsible for *Clostridium difficile* TcdB-Induced Cytotoxicity. *Proc Natl Acad Sci U S A.* 112(22):7073-8. PMCID: PMC4460460
d. R. Chandrasekaran, A. Kenworthy, **D. B. Lacy**. (2016) *Clostridium difficile* Toxin A Undergoes Clathrin-Independent, PACSIN2-Dependent Endocytosis. *PLoS Pathogens*. 12(12):e1006070. PMCID: PMC5152916

Complete List of Published Work in MyBibliography: http://www.ncbi.nlm.nih.gov/sites/myncbi/1bS-2BjDgr-QO/bibliograpahy/42611860/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

R01 AI095755-09 Lacy (PI) 4/1/2016-3/31/2021
NIH/NIAID
Structural mechanisms of *Clostridium difficile* pathogenesis
The goal of the proposed project is to identify the structures and molecular mechanisms of action for TcdA, a key virulence factor in *C. difficile* pathogenesis.
Role: PI

I01-BX002943-05 Lacy (PI) 1/1/2016-12/31/2023
Veterans Administration
The role of toxins in *Clostridium difficile* infection pathogenesis

The goal of the proposed project is to identify the structures and mechanisms of the *C. difficile* transferase (CDT) binary toxin.
Role: PI

Merck Foundation Lacy (PI) 11/1/2016-10/31/2020
A high-resolution structure of bezlotoxumab bound to *Clostridium difficile* TcdB
This proposal is designed to provide structural insight into the mechanism by which the monoclonal antibody bezlotoxumab provides protection against *Clostridium difficile* toxin TcdB.
Role: PI

Pfizer Lacy (PI) 1/1/2019-12/31/2021
This project involves defining the mechanism of action for a panel of antibodies that neutralize TcdA or TcdB function.
Role: PI

R01 AI039657-20 Cover (PI) 5/01/2012-4/30/2022
NIH/NIAID
Structure Function Analysis of *Helicobacter pylori* VacA
To 1) investigate VacA structural features that are required for intracellular toxin activity and membrane channel formation, 2) analyze differences in functional properties of VacA proteins encoded by different *H. pylori* strains, and 3) identify and analyze host cell components that are required for VacA cytotoxicity.
Role: Co-investigator

R01 AI118932-04 Cover (PI) 1/01/2016-1/31/2021
NIH/NIAID
Type IV Protein Secretion in *Helicobacter pylori*
Dr. Lacy assists Dr. Cover with structural analysis of proteins in the *H. pylori* type IV secretion system.
Role: Co-investigator

R01 AI069233-14 (Skaar, PI) 5/1/2016-4/30/2021
NIH/NIAID
Mechanism and function of heme-iron utilization in staphylococcal pathogenesis
Dr. Lacy assists Dr. Skaar with structural analysis of proteins in the *S. aureus* heme biosynthesis pathway and their complexes with allosteric activators.
Role: Co-investigator

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: Jensen, Jaime Lea

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

POSITION TITLE: Postdoctoral Research Trainee

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
North Dakota State University	B.Sc.	05/2009	Biochemistry/Molecular Biology and Philosophy
North Dakota State University	Ph.D.	07/2017	Biochemistry
Vanderbilt University Medical Center	Postdoctoral	In progress	Microbiology

A. Personal Statement

Throughout my graduate career and into my postdoctoral research, I have made contributions to the structural and mechanistic understanding of (i) cell-surface signaling regulation of iron transport in Gram-negative bacteria, (ii) the interaction of S100B with the receptor for advanced glycation endproducts (RAGE), (iii) colonic epithelial receptor interactions with large clostridial toxins, and (iv) the Type III secretion system. I have incorporated several biochemical and biophysical techniques into my research, including molecular biological methods, chromatography, circular dichroism spectroscopy, isothermal titration calorimetry, microscale thermophoresis, bio-layer interferometry, X-ray crystallography, small-angle X-ray scattering, electron microscopy, and various cellular assays.

In the Lacy lab at VUMC, my primary projects have focused on the large clostridial toxins, namely, *C. difficile* toxins TcdA and TcdB, and the *C. perfringens* toxin TpeL. As the main virulence factors of *Clostridioides* infections, understanding their interactions with host cell receptors and the roles the toxins play in pathogenesis is of paramount importance for developing novel therapeutics.

B. Positions and Honors

Positions and Employment

2017 – Present Postdoctoral Research Fellow/Trainee, Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center
2011 – 2017 Graduate Research/Teaching Assistant, Department of Chemistry and Biochemistry, North Dakota State University
2009 – 2010 Quality Control Scientist, Aldevron, LLC
2007 – 2009 Research Specialist Apprentice/Undergraduate Research Assistant, Center for Nanoscale Science and Engineering

Teaching Experience

Spring 2020 Teaching/Communications Assistant, Bioregulation II, Vanderbilt University
Fall 2012 – 2014 Teaching Assistant, Methods of Biochemical Research, North Dakota State University
Spring 2014 Teaching Assistant, General Chemistry Lab, North Dakota State University
2010 ESL Instructor, Oasis English Language Institute, Mysore, India
Fall 2008 Teaching Assistant, Fundamentals of Biochemistry Lab, North Dakota State University

Honors

2017	NDSU College of Science and Math Graduate Student Research Award
2015 – 2017	NSF ND EPSCoR IIP Doctoral Dissertation Assistantship
2016	American Society for Biochemistry and Molecular Biology Travel Award
2016	NDSU College of Science and Mathematics Travel Award
2014	Frontiers in Biomedical Research Symposium, student poster award
2014	Bruker Travel Sponsorship, travel award to national ACA meeting
2014	NDSU College of Science and Mathematics Travel Award
2014	American Crystallographic Association Young Scientist Travel Grant
2012	James D. Geerdes Memorial Scholarship for Biochemistry Excellence
2012	NIH National IDeA Symposium of Biomed Research Excellence Student Travel Award

Specialized Training

June 2014	Neutrons in Structural Biology Workshop, Oak Ridge National Laboratory
June 2013	CCP4 School in Macromolecular Crystallography, Argonne National Laboratory
June 2012	NMRviewJ Training Course, University of Maryland Baltimore County

Professional Memberships

2017 – Present	American Society for Microbiology
2017 – Present	The Biophysical Society
2016 – Present	American Association for the Advancement of Science
2013 – Present	American Crystallographic Association
2007 – Present	American Society for Biochemistry and Molecular Biology

C. Contributions to Science

Peer-Reviewed Publications

Jensen, J. L. Yamini, S., Rietsch, A., and Spiller, B. W. "Structural characterization of the T3SS export gate platform with the central stalk protein." *PLoS Pathogens* (In preparation)

Mileto, S. J., Jarde, T., Childress, K. O., **Jensen, J. L.**, Rogers, A. P., Kerr, G., Hutton, M. L., Sheedlo, M. J., Bloch, S. C., Shupe, J. A., Horvay K., Flores, T., Engel, R., Wilkins, S., McMurrick, P. J., Lacy, D. B., Abud, H. E., and Lyras, D. (2020) "*Clostridioides difficile* infection damages colonic stem cells via TcdB, impairing epithelial repair and recovery from disease." *Proceedings of the National Academy of Sciences.* (In press)

Jensen, J. L., Jernberg, B., Sinha, S. C., and Colbert, C. L. (2020) "Structural basis for cell-surface signaling by a conserved sigma regulator in Gram-negative bacteria." *Journal of Biological Chemistry.* (In press)

Indurthi, V. S. K.*, **Jensen, J. L.***, Leclerc, E., Sinha, S., Colbert, C. L., and Vetter, S. W. (2020) "The Trp triad within the V-domain of the receptor for advanced glycation endproducts (RAGE) modulates folding, stability, and ligand binding." *Bioscience Reports.* https://doi.org/10.1042/BSR20193360 *Co-first authors.

Anderson, D. M., Sheedlo, M. J., Jensen, J. L., and Lacy, D. B. (2019) "Structural insights into the transition of *Clostridioides difficile* binary toxin from prepore to pore." *Nature Microbiology.* **5(1).**

Jensen, J. L., Wu, Q., and Colbert, C. L. (2017) "NMR assignments of the N-terminal signaling domain of the TonB-dependent outer membrane transducer PupB." *Biomolecular NMR Assignments*. https://doi.org/10.1007/s12104-017-9785-0

Jensen, J. L. Balbo, A., Zhao, H., Neau, D. B., Chakravarthy, S., Sinha, S. C., and Colbert, C. L. (2015). "Mechanistic implications of the unique structural features and dimerization of the cytoplasmic domain of the *Pseudomonas* sigma regulator, PupR." *ACS Biochemistry.* **54(38):**5867-5877.

- Work was highlighted in Oct. 1, 2015 issue of *Argonne Today*.

Jensen, J. L., Indurthi, V. S. K., Vetter, S. W., and Colbert, C. L. (2015). "Structural insights into ligand binding of the human receptor for advanced glycation endproducts by S100B, as revealed by an S100B:RAGE-derived peptide complex." *Acta Crystallographica* D**71:**1176-1183.

Piya, G., Mueller, E. N., Haas, H., Ghospurkar, P. L., Wilson, T. M., **Jensen, J. L.**, Colbert, C. L., and Haring, S. J. (2015). “Characterization of the interaction between Rfa1 and Rad24 in *Saccharomyces cerevisiae*.” *PLoS ONE.* 10(2): e0116512.

D. Research Support

Ongoing Research Support

5T32DK007673 (Peek, R.) 07/01/2018 – 06/30/2020
NIH/NIDDK
Training in Gastroenterology
Role: Traineeship recipient

Vanderbilt Institute for Clinical and Translational Research 03/01/2020 – 06/30/2021
Resource Award: $6,338
“Probing the interactions of *C. difficile* toxin B with human colonic epithelial receptor ectodomains using bio-layer interferometry”
Role: PI

Completed Research Support

Vanderbilt Institute for Clinical and Translational Research 05/01/2018 – 02/01/2019
Resource award: $1,975
“Mass spectrometry analysis of a human colonic epithelial receptor for TcdA, a *C. difficile* toxin”
Role: PI

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: Kroh, Heather K.

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

POSITION TITLE: Research Instructor

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
Tennessee Technological University, Cookeville TN	B.S.	05/1999	Biology
Vanderbilt University, Nashville TN	Ph.D.	05/2010	Cellular & Molecular Pathology
Vanderbilt University Medical Center, Nashville TN	Postdoctoral	08/2012	Biochemistry
Vanderbilt University Medical Center, Nashville TN	Postdoctoral	12/2017	Structural Biology

A. Personal Statement

My research background involves extensive training in protein biochemistry, incorporating recombinant protein expression and purification, enzyme kinetics, equilibrium binding, and other biophysical techniques. My current work concentrates on structure-function studies of the *Clostridioides difficile* secreted toxins, which are the primary virulence factors for *C. difficile* infection, and I have several recent publications which incorporate both X-ray crystallography and electron microscopy. In addition to this project, I have established multiple collaborations with investigators who seek structural input on their research. These projects have provided multiple opportunities to expand my cryo-electron microscopy skillset while addressing a diverse array of biological questions. Subsequently, I have increased my proficiency in sample preparation and data collection/processing and work to keep current on my training through additional cryo-EM seminars and workshops.

B. Positions and Honors

Positions and Employment

2004-2009 Graduate Research Fellow, Vanderbilt University Medical Center, Nashville, TN
2010-2012 Postdoctoral Research Fellow, Vanderbilt University Medical Center, Nashville, TN
2012-2017 Postdoctoral Fellow, Vanderbilt University Medical Center, Nashville, TN
2018- Research Instructor, Pathology, Microbiology & Immunology Department, Vanderbilt University Medical Center, Nashville, TN

Other Experience and Professional Memberships

2005- Member, American Association for the Advancement of Science
2008- Member, American Chemical Society
2009- Member, American Society for Biochemistry and Molecular Biology
2019- Member, American Crystallographic Association

Honors & Awards

2005-2007 Traineeship support, NIH Training Grant 2-T32 HL007751
2007 Young Investigator Award, XXIst Congress of the International Society on Thrombosis
2012-2015 Traineeship support, NIH Training Grant 5-T32 HL007751
2019 ABRCMS Judge Travel Award, Annual Biomedical Research Conference for Minority Students (ABRCMS), Anaheim, CA

C. Contributions to Science

1. **Staphylococcal coagulases.** The main topic of my graduate work with Paul E. Bock at Vanderbilt was a newly-identified homolog of the *Staphylococcus aureus* coagulase called von Willebrand-factor binding protein (vWbp). I determined that vWbp could conformationally activate the inactive thrombin precursor prothrombin through a unique fibrinogen-dependent mechanism. In contrast to the classic coagulase, vWbp displays hysteretic activation of prothrombin, where enzymatic activity is generated only upon binding of an appropriate substrate by the complex. Characterization of vWbp as a coagulase has emphasized the importance of the coagulases as virulence factors for *S. aureus*, particularly for their roles in abscess formation and protection from the host immune system.
    a. Panizzi PR, Friedrich R, Fuentes-Prior P, **Kroh HK**, Briggs J, Tans G, Bode W, Bock PE. (2006) Novel fluorescent prothrombin analogs as probes of staphylocoagulase-prothrombin interactions. Journal of Biological Chemistry, 281(2):1169-78.
    b. **Kroh HK**, Panizzi P, Bock PE. (2009) Von Willebrand factor-binding protein is a hysteretic conformational activator of prothrombin. Proceedings of the National Academy of Science, U.S.A., 106(19):7786-91.
    c. Panizzi P, **Kroh HK**, Fuentes-Prior P, Bock PE. (2010) Procoagulant Proteins: Staphylocoagulase. Toxins in Hemostasis: From Bench to Beside. Springer Publishing (book chapter).
    c. **Kroh HK**, Bock PE. (2012) Effect of zymogen domains and active site occupation on allosteric activation of prothrombin by von Willebrand factor-binding protein. Journal of Biological Chemistry, 287(46):39149-57.

2. **Thrombin activation.** In addition to the previous contributions on pathophysiological coagulation, I defined several of the allosteric mechanisms that govern normal thrombin activity. These studies established the role of sodium in controlling ligand binding during the multiple mechanistic steps required for zymogen activation, as well as how an artificial “active-like” conformation of prothrombin can paradoxically serve as a competitive inhibitor of thrombosis. This has increased our understanding of the mechanistic basis for allosteric effectors during coagulation, a significant step for development of therapeutics that target the existing regulatory machinery of enzymes.
    a. **Kroh HK**, Tans G, Nicolaes GAF, Rosing J, Bock PE. (2007) Expression of allosteric linkage between the sodium ion binding site and exosite I of thrombin during prothrombin activation. Journal of Biological Chemistry, 282(22):16095-104
    b. **Kroh HK***, Panizzi P*, Tchaikovski S*, Baird TR*, Chang N, Tans G, Rosing J, Furie B, Furie BC, Bock PE. (2011) Active site-labeled prothrombin inhibits prothrombinase in vitro and thrombosis in vivo. Journal of Biological Chemistry, 286(26):23345-56. (*authors contributed equally)

3. **Toxin-neutralizing antibodies.** Neutralizing monoclonal antibodies produced in response to a pathogen are an abundant source of not only possible therapeutics against infection, but also tools for examining protein function on a molecular level. The three secreted toxins (TcdA, TcdB, and CDT) produced by the gram-positive pathogen *Clostridioides difficile* can elicit distinct host antibody responses during an infection. My identification and characterization of important epitopes recognized by these antibodies has revealed specific “hot-spots” for toxin function, which has clarified some of the structure-function questions that have persisted within the field.
    a. Hernandez LD, **Kroh HK**, Hsieh E, Yang X, Beaumont M, Sheth PR, DiNunzio E, Rutherford SA, Ohi MD, Ermakov G, Xiao L, Secore S, Karczewski J, Racine F, Mayhood T, Sher X, Gupta P, Lacy DB, Therien AG. (2017) Epitopes and mechanism of action of the *Clostridium difficile* toxin A-neutralizing antibody actoxumab. Journal of Molecular Biology, 429(7):1030-44.
    b. **Kroh HK**, Chandrasekaran R, Rosenthal K, Woods R, Jin X, Ohi MD, Nyborg AC, Rainey GJ, Warrener P, Spiller BW, Lacy DB. (2017) Use of a neutralizing antibody helps identify structural

features critical for binding of *Clostridium difficile* toxin TcdA to the host cell surface. Journal of Biological Chemistry, 292(35):14401-14412.

c. **Kroh HK***, Chandrasekaran R*, Zhang Z, Rosenthal K, Woods R, Jin X, Nyborg AC, Rainey GJ, Warrener P, Melnyk RA, Spiller BW, Lacy DB. (2018) A neutralizing antibody that blocks delivery of the enzymatic cargo of *Clostridium difficile* toxin TcdB into host cells. Journal of Biological Chemistry, 293(3):941-952. (*authors contributed equally)

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

I01-BX002943-04 Lacy (PI) 01/01/2016-12/31/2023
Veterans Administration
Pre-clinical evaluation of *Clostridium difficile* toxin inhibitors
This proposal is designed around the hypothesis that inhibition of toxin activity represents a therapeutic approach that can impact clinical treatment and outcome for individuals suffering from *Clostridium difficile* infection.
Role: Key personnel

Merck Foundation Lacy (PI) 11/01/2016-10/31/2020
A high-resolution structure of bezlotoxumab bound to *Clostridium difficile* TcdB
This proposal is designed to provide structural insight into the mechanism by which the monoclonal antibody bezlotoxumab provides protection against *Clostridium difficile* toxin TcdB.
Role: Senior personnel

Pfizer Lacy (PI) 01/01/2019-12/31/2021
This project involves defining the mechanism of action for a panel of antibodies that neutralize TcdA or TcdB function.
Role: Senior personnel

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Michael J. Sheedlo

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

POSITION TITLE: Postdoctoral Fellow

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
Michigan State University, East Lansing MI	B.S.	08/2006	06/2010	Biochemistry
Purdue University, West Lafayette IN	Ph.D.	08/2010	05/2016	Chemistry
Vanderbilt University, Nashville TN		06/2016		Microbiology

A. Personal Statement

The focus of my graduate and postdoctoral studies has been to understand the mechanisms employed by pathogenic bacteria to manipulate their host. Throughout my training I have garnered an expertise in structural techniques including both X-ray crystallography and cryo-EM. I have developed these skillsets while probing a number of functionally distinct systems including 1) *Legionella pneumophila* effector molecules, 2) toxins produced by *Clostridioides difficile*, and 3) type 4 secretion systems.

As a graduate student in the lab of Dr. Chitta Das at Purdue University I studied a family of bacterial effector molecules secreted by the pathogenic bacterium *L. pneumophila*, called the SidE family. I described two opposing activities within these effectors that manipulate the ubiquitination pathway of the host cell through either the removal (deubiquitination) or attachment (ligation) of ubiquitin to host cell proteins. I discovered that the mechanisms governing both of these activities are distinct from that of their eukaryotic counterparts.

As a postdoctoral fellow in the lab of Dr. Borden Lacy at Vanderbilt University I have expanded my structural skillset, mastering the technique of cryo-EM. The series of structures that I have solved depict toxins that are secreted by the pathogenic bacterium *C. difficile*. A subset of these structures paint a picture of the process of pore formation as observed for one such toxin known as the *C. difficile* transferase toxin (CDT). I now aim to use CDT as a tool to dissect the fundamental phenomenon of protein translocation. Building upon this strong structural foundation I will incorporate new techniques such as confocal microscopy and flow cytometry to understand how CDT translocation is regulated and accomplished. By acquiring this new skill I will add a new facet to my research group which will allow us to address questions that arise from our work from a cellular perspective.

B. Positions and Honors

Positions

2010-2016 – Graduate Student, Purdue University
2010-2013 – Graduate Teaching Assistant, Chemistry Department, Purdue University
2013-2014 – Head Graduate Teaching Assistant, Organic Chemistry, Purdue University
2015 – Graduate Teaching Assistant, Biochemistry, Purdue University
2016-Present – Postdoctoral Fellow, Vanderbilt University

Honors

1. Vanderbilt Institute for Infection, Immunology, and Inflammation Mini-Sabbatical Recipient, 2018
2. NIH Postdoctoral Training in Gastroenterology Recipient (Training Grant 2T32DK007673-27), 2019
3. Vanderbilt Institute for Clinical and Translational Research, 2019

C. Contributions to Science

Bacterial Effector Molecules

I started graduate school in the fall of 2010 in the lab of Dr. Chitta Das in the Chemistry Department at Purdue University. The focus of my research was on the SidE family of secreted effector molecules from the intracellular pathogen, *Legionella pneumophila*. When I began, relatively little was known about the function of this family other than that this group of effectors is essential for virulence. The work I conducted on this system focused primarily on one protein out of this group known as SdeA, though all of these molecules are thought to be functionally redundant. As a part of my graduate work, I developed a protocol for purifying a library of constructs and assayed their enzymatic activity to define their preferred substrate using a variety of ubiquitin linkages that I prepared. From these studies I described a portion of the N-terminus as the minimal deubiquitinating domain (DUB) and reported the first structure of a prokaryotic DUB in both the apo and ubiquitin-bound forms using X-ray crystallography (PDB 5CRA, 5CRB, 5CRC). These observations informed complementary studies conducted by Zhao-Qing Luo's lab at Purdue University which demonstrated the ability of this DUB to remodel the *Legionella* containing vacuole. A second aim of my thesis involved another domain encoded within SdeA that functioned as an ubiquitin ligase, independent of canonical ubiquitination machinery (E1, E2, E3, ATP). I followed up on the Luo lab's initial observation that this system did not require ATP by recapitulating this activity using a purified system. I then used this *in vitro* assay to dissect this unique ubiquitination mechanism. My proposal that this novel ligase domain used an ADP-ribosyltransferase-like activity to attach ubiquitin to target proteins through a ribose-phosphate moiety was a key element of our Nature publication in 2016. This work was profiled in a number of high impact journals and even nominated for Science Signaling breakthrough of the year in 2016. My work also served as the preliminary data for a R01 grant that I helped my PI prepare as well as the basis of a patent application that was submitted in 2017 (Application Number US20170283852A1). I have further contributed to the work of the Das lab by co-authoring a total of eight additional manuscripts. The Das lab has continued to work on this system and is in the process of preparing another manuscript based on my observations.

Please see the following publications on this topic:

1. Sheedlo MJ, Qiu J, Tan Y, Paul LN, Luo ZQ, Das C. Structural basis of substrate recognition by a bacterial deubiquitinase important for dynamics of phagosome ubiquitination. Proc Natl Acad Sci U S A. 2015 Dec 8;112(49):15090-5. doi: 10.1073/pnas.1514568112. Epub 2015 Nov 23. PM CID: PM C4679006.

2. Qiu J, Sheedlo MJ, Yu K, Tan Y, Nakayasu ES, Das C, Liu X, Luo ZQ. Ubiquitination independent of E1 and E2 enzymes by bacterial effectors. Nature. 2016 May 5;533(7601):120-4. doi: 10.1038/nature17657. Epub 2016 Apr 6. PM CID: PM C4905768.

3. Puvar K, Iyer S, Sheedlo MJ, Das C. Purification and functional characterization of the DUB domain of SdeA. Methods Enzymol. 2019;618:343-355. doi: 10.1016/bs.mie.2018.12.024. Epub 2019 Feb 1. PMID:30850059.

Please also see the following patent application:

1. Luo ZQ, Qui JQ, Das C, Sheedlo MJ. Patent Application Number: US20170283852A1

Clostridioidal Toxins

In 2016, I joined the lab of Dr. Borden Lacy to work on bacterial toxins and to gain expertise in the technique of cryo-electron microscopy (cryo-EM). I started with the aim of understanding the mode of receptor recognition by the large glucosylating toxin from *Clostridioides difficile* known as TcdB. When I began this work, it was unclear if this system would be amenable to analysis by cryo-EM due to both the small size and structural

heterogeneity of these particles. To gain experience in the field of cryo-EM, I regularly attended the Vanderbilt Cryo-EM facility user meeting as well as a workshop that was organized by the National Resource for Automated Molecular Microscopy (NRAMM) in 2017. This led to the preliminary characterization of several forms of TcdB. I was awarded a mini-sabbatical based on these preliminary data from the Vanderbilt Institute for Infection, Immunology, and Inflammation (VI4) to continue this project with the aim of solving a high-resolution structure of TcdB in complex with host cell receptors. I have since, successfully reconstructed maps of TcdB in both the apo form and in complex with a domain of one such receptor, Frizzled-2, to near-atomic resolution. These data were presented as a poster at both the Purdue Cryo-EM symposium in 2018 as well as the VI4 annual symposium at Vanderbilt University in 2019. I am currently in the process of preparing a manuscript which highlights the remarkable flexibility of this toxin in both forms. The cryo-EM workflow that I have established will serve as a basis for reconstructing these highly dynamic toxins with a number of different ligands, as the toxins are known to engage multiple host cell surface proteins.

I simultaneously began working to characterize the underappreciated binary toxin known as the *C. difficile* transferase toxin, CDT, an oligomeric assembly of two proteins called CDTa and CDTb. I determined a series of CDTb structures by cryo-EM and proposed a mechanism for CDTb pore formation that is described in a manuscript that was recently accepted to be published in Nature Microbiology. These data were selected for a presentation at the Vanderbilt Biochemistry department retreat and as a poster at the Cold Spring Harbor Microbe Pathogenesis and the Host Response meeting in 2019. I have additionally obtained a structure of the entire CDT functional complex (CDTa bound to a CDTb oligomer). The model I constructed demonstrates a recognition mode that is distinct from other binary toxins and raises new questions about the minimal domain required for toxin assembly as well as the recognition elements required for CDTa translocation across the membrane. I am in the process of preparing a manuscript describing the structure (Sheedlo et al. to be submitted to *PNAS*) and intend to develop the mechanistic questions regarding protein translocation in my independent work going forward.

1. Anderson DM*, Sheedlo MJ*, Jensen JL, Lacy DB. Structural Insights into the Transition of *Clostridioides difficile* Binary Toxin from Prepore to Pore. Nature Microbiology. 2020. (5):102-107.

2. Sheedlo MJ, Anderson DM, Thomas AK, Lacy DB. Structural elucidation of the *Clostridioides difficile* transferase toxin reveals a single-site binding mode for the enzyme. PNAS. 2020.

The following publications on this topic are in preparation:

1. Sheedlo MJ and Lacy DB. *Clostridioides difficile* toxin B is highly dynamic in both apo and receptor bound states.

Helicobacter pylori Type IV Secretion System

I worked on a supramolecular complex known as the type IV secretion system (T4SS) in a collaborative project with the laboratories of Dr. Tim Cover (Vanderbilt University) and Dr. Melanie Ohi (University of Michigan). The goal of this project was to understand the architecture of this highly complex protein translocation machine. The Cag T4SS was purified from *Helicobacter pylori* (Cover lab) and several maps were reconstructed by cryo-EM (Ohi lab). My role in this project has been to generate atomic models of the individual proteins that contribute to this complex structure using these electron density maps. To aid in this task, I attended a workshop co-hosted by the SbGrid consortium (Harvard University) and the Northeastern Collaborative Access Team (Advanced Photon Source, Argonne National Lab) aimed at outlining techniques to aid in constructing models using low resolution maps. I was co-first author on the manuscript describing our first high-resolution structure, published in the journal eLife and profiled in an eLife digest. The structure represents one of the largest bacterial assemblies ever characterized, and I am currently generating higher resolution structures for a follow up publication.

1. Chung JM*, Sheedlo MJ*, Campbell AM, Sawhney N, Frick-Cheng AE, Lacy DB, Cover TL, Ohi MD. Structure of the Helicobacter pylori Cag Type IV Secretion System. Elife. 2019 Jun 18;8. pii: e47644. doi: 10.7554/eLife.47644. PMC ID: PM C6620104.
 * *indicates Co-first authors*

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

YEAR	COURSE TITLE	GRADE
2010	Safety in Laboratory	credit
2010	Bionanotechnology	B
2011	Membrane Structure and Function	A
2011	Biochemistry Structural Aspects	A
2012	Biophysical Chemistry	A