BIOGRAPHICAL SKETCH

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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.

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	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 AI095755-08 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-BX002943-04 Lacy (PI) 1/1/2016-12/31/2019
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: 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 AI069233-11 (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