BIOGRAPHICAL SKETCH

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NAME: Spiller, Benjamin Winston, Ph.D.

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

POSITION TITLE: Associate 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 California at Davis	B.S.	12/1994	Biochemistry
University of California at Berkeley	Ph.D.	08/1999	Molecular and Cellular Biology

A. Personal Statement

I have extensive training and experience (>20 years) in structural biology and structural immunology. My research has focused on structural studies at the interface between the immune system and foreign proteins. Although the projects being studied in my laboratory are quite diverse, they interest us because they all address novel mechanisms and new paradigms at this interface. Our work ranges from epitope discovery, including bacterial and viral systems as well as newer work to identify IgE epitopes on common food allergens, to development of novel uses for conventional and single chain antibodies. I have particular interests in molecular pathogenesis, host-pathogen interactions, and antibody affinity maturation. A principal interest of my research is the host's response to pathogens. Toward this end, we are studying multiple viral and bacterial antigens to determine the mechanism of antibody neutralization. For many of our projects we collaborate with other groups, including structural studies of bacterial toxins and viral glycoproteins and their neutralization by antibodies (collaborations with the Lacy and Crowe labs, respectively). These projects have led to us to begin studies, with Scott Smith in the Department of Medicine, to elucidate the mechanism by which human IgE antibodies cause allergies. Our interests in epitope discovery has also led us to study single chain camelid antibodies, which are more amenable to phage display methods and inherently much smaller and more able to bind in small crevices than traditional antibodies. The two topics that excite me most for the next phase of my career are novel applications of nanobodies and IgE epitopes.

Select Publications Relevant to the current application:

Kroh HK, Chandrasekaran R, Zhang Z, Rosenthal K, Woods R, Jin X, Nyborg AC, Rainey GJ, Warrener P, Melnyk RA, Spiller BW, Lacy DB. A neutralizing antibody that blocks delivery of the enzymatic cargo of Clostridium difficile toxin TcdB into host cells. J Biol Chem. 2018 Jan 19;293(3):941-952. doi: 10.1074/jbc.M117.813428. PMCID: PMC5777265.

Kroh HK, Chandrasekaran R, Rosenthal K, Woods R, Jin X, Ohi MD, Nyborg AC, Rainey GJ, Warrener P, Spiller BW, Lacy DB. Use of a neutralizing antibody helps identify structural features critical for binding of Clostridium difficile toxin TcdA to the host cell surface. J Biol Chem. 2017 Sep 1;292(35):14401-14412. doi: 10.1074/jbc.M117.781112. PMCID: PMC5582835.

Chumbler NM, Rutherford SA, Zhang Z, Farrow MA, Lisher JP, Farquhar E, Giedroc DP, Spiller BW, Melnyk RA, Lacy DB. Crystal structure of Clostridium difficile toxin A. Nat Microbiol. 2016 Jan 11;1:15002. doi: 10.1038/nmicrobiol.2015.2. PMCID: PMC4976693.

Katie L. Winarski, Natalie J. Thornburg, Yingchun Yu, Gopal Sapparapu, James. E. Crowe Jr, and **Benjamin W. Spiller**. A vaccine-elicited antibody that neutralizes influenza H5N1 and variants binds the receptor site and polymorphic sites. Proc Natl Acad Sci U S A. 2015 Jul 13. PMCID: PMC4522792.

B. Positions and Honors

Positions and Employment

1991-1993	Undergraduate researcher, Dept of Genetics UCDavis
1993-1994	Undergraduate researcher, Dept of Biological Chemistry UCDavis
1995-1999	Graduate student, Dept of Molecular and Cellular Biology UCBerkeley
1999-2000	Post-doctoral Fellow, Dept of Molecular Biophysics and Biochemistry, Yale University
2000-2006	Post-doctoral Fellow, Harvard University and Harvard Medical School
2006-2015	Assistant Professor, Dept. of Pharmacology, Vanderbilt Medical School
2015-present	Associate Professor, Dept. of Pharmacology, Vanderbilt Medical School

Honors

2001	Irvington Institute Fellowship (declined)
2001-2004	Helen Hay Whitney Fellow
2001-2004	Agouron Foundation Fellow
2001-2004	Paul B. Sigler Memorial Fellow
2006-2008	Digestive Disease Development award (VUMC)
2016-2018	TN Center for Aids Research Development Award

C. Contribution to Science

1. Bacterial Pathogenesis. A focus of my independent career has been the mechanisms by which type three secretion effectors promote pathogenesis. Our first efforts in this field involved two proteins, VirA and EspG that had been erroneously identified as tubulin specific proteases. These proteins have been implicated in microtubule disruption as well as in Rac and Rho activation. We determined structures of both VirA and EspG and completed an *in vitro* functional analysis indicating that neither disrupt microtubules nor degrade tubulin. This work highlighted serious errors in numerous published reports. Subsequent to our work, three other labs have confirmed our results and extended them to show that VirA and EspG function as scaffolds that organize cellular GTPase and Kinase signaling hubs.

Germane KL, Ohi R, Goldberg MB, **Spiller BW**. Structural and functional studies indicate that *Shigella* VirA is not a protease and does not directly destabilize microtubules. Biochemistry. 2008 Sep 30;47(39):10241-3. PMCID: PMC4096815.

Germane KL, **Spiller BW**. Structural and functional studies indicate that the EPEC effector, EspG, directly binds p21 activated kinase. Biochemistry 2011 Feb 15;50(6):917-9. PMCID: PMC3040069.

2. Subsequently, we determined that CopN, a *Chlamydial* type three secretion gatekeeper protein (a class of proteins that regulate secretion), functions as an effector that directly binds tubulin and prevents microtubule formation. This was the first example of bacteria directly modulating the host's microtubule network. Unexpectedly, CopN targets the same interface on tubulin that eukaryotic tubulin regulatory proteins bind despite sharing no structural or sequence homology with such proteins. These proteins are likely the first bacterial proteins to reach the target cell cytoplasm, and may be involved in early steps in pathogenesis. Our CopN structure, determined as a complex of CopN and a secretion chaperone that is essential for early steps in type three secretion, revealed that Scc3, which had been erroneously described as a chaperone for CopN actually forms an integral part of a molecular scaffold that includes CopN, Scc3, and translocators. We subsequently showed that the interaction between Scc3 family chaperones and gatekeepers is conserved in *Shigella* (and likely many or all type three secretion systems; the essential residues are highly conserved), that

the gatekeeper-chaperone interaction is essential to establish the secretion hierarchy, a term used to describe the observation that effectors are not secreted until after translocators (molecules that form the conduit for effectors to enter the host cell).

Archuleta TL, Du Y, English CA, Lory S, Lesser C, Ohi MD, Ohi R, **Spiller BW**. The Chlamydia effector chlamydial outer protein N (CopN) sequesters tubulin and prevents microtubule assembly. J Biol Chem. 2011 Sep 30;286(39):33992-8. PMCID: PMC3190796.

Archuleta T and **Spiller BW**. A Gatekeeper-Chaperone Scaffold Directs Translocator Secretion by the Type Three Secretion System. In Press. PLoS Pathogens. 2014 Nov 6;10(11). PMCID: PMC4222845.

3. A second area of interest has been the molecular mechanism by which affinity is encoded in protein-protein interactions, specifically the mechanism by which antibodies recognize antigens. This interest began with my graduate work, where I determined the structure of a polyspecific antibody and showed that in the case of Vκ1A heavy chains, which comprise ~10% of the naïve repertoire in mice, retained substantial polyspecificity after affinity maturation. This work contributed to the now prevailing view that specificity arises from gaining affinity toward a single target, rather than from loosing affinity for other targets. A second series of experiments focused on understanding how networks of interacting amino acid substitutions could evolve and dramatically alter protein characteristics. For this second project, I initiated a collaboration with a leader in the in vitro evolution field and completed all structural aspects of the project independently.

Spiller B, Gershenson A, Arnold FH, and Stevens RC (1999). A Structural view of Evolutionary Divergence. Proc Natl Acad Sci U S A., 96, 12305-12310. PMCID: PMC22912.

Romesberg FE, Santarsiero BD, **Spiller B**, Yin J, Barnes D, Schultz PG, Stevens RC (1998) Structural Evidence for Strain in Biological Catalysis. Biochemistry 37, 14404-14409. PMID: 9772166.

Romesberg FE,* **Spiller B**,* Schultz PG, Stevens RC (1997) Immunological Origins of Binding and Catalysis in a Diels-Alderase Antibody. Science 279, 1929-1933. PMID: 9506942. *(authors contributed equally)

4. I have continued working on antibody specificity and have branched out to recognition of viral pathogens. Our first efforts were to understand how a series of interacting mutations allow two similar and genetically related antibodies to bind their antigen (VP6). This was the first example of related antibodies binding the same antigen in different orientations. A second project has focused on neutralization of influenza strain H5. H5 is not currently widespread in humans (unlike my graduate work in the 1990s, we are now able to study human monoclonal antibodies) so it allows us to study how the naïve immune system responds to influenza. This is critically different than the majority of studies, which focus on antibodies that arise from repeated exposure, often over decades, to related antigens. Such antibodies are highly mutated, with many interacting networks of mutations. Highly mutated antibodies, although immensely impressive in the breadth of their neutralizing capacities, have proven impossible to elicit through standard immunization. Our unpublished work in this area highlights retained germline character in vaccine elicited anti H5 antibodies. The implication from this work is that immunization strategies for new targets need to include multiple immunization boosts. Although not a new idea in immunology, we hope our work will be the first structural image of this phenomenon.

Katie L. Winarski, Natalie J. Thornburg, Yingchun Yu, Gopal Sapparapu, James. E. Crowe Jr, and **Benjamin W. Spiller**. A vaccine-elicited antibody that neutralizes influenza H5N1 and variants binds the receptor site and polymorphic sites. Proc Natl Acad Sci U S A. 2015 Jul 13. PMCID: PMC4522792.

Thornburg NJ, Nannemann DP, Blum DL, Belser JA, Tumpey TM, Deshpande S, Fritz GA, Sapparapu G, Krause JC, Lee JH, Ward AB, Lee DE, Li S, Winarski KL, **Spiller BW**, Meiler J, Crowe JE Jr. Human antibodies that neutralize respiratory droplet transmissible H5N1 influenza viruses. J Clin Invest. 2013 Sep 3. PMCID: PMC3784541.

Aiyegbo MS, Sapparapu G, **Spiller BW**, Eli IM, Williams DR, Kim R, Lee DE, Liu T, Li S, Woods VL Jr, Nannemann DP, Meiler J, Stewart PL, Crowe JE Jr. Human rotavirus VP6-specific antibodies mediate intracellular neutralization by binding to a quaternary structure in the transcriptional pore. PLoS One. 2013 May 9;8(5):e61101. PMCID: PMC3650007.

Aiyegbo MS, Eli IM, **Spiller BW**, Williams DR, Kim R, Lee DE, Liu T, Li S, Stewart PL, Crowe JE Jr. Differential Accessibility of a Rotavirus VP6 Epitope in Trimers Comprising Type I, II, or III Channels as Revealed by Binding of a Human Rotavirus VP6-Specific Antibody. J Virol. 2013 Oct 23. PMCID: PMC3911710.

5. We have a long-standing collaboration with the Lacy and Cover labs (both at Vanderbilt University) to understand the mechanisms bacterial toxins use to cause disease. Within this collaboration we have focused on structural studies of toxins and of antibody-toxin complexes. We have developed novel alpaca-derived nanobodies that recognize multiple domains of different Clostridial toxins and have begun evaluating these for breadth and specificity. It is clear that within our panels there are multiple "broadly neutralizing" nanobodies. In addition, we have identified and begun characterization of nanobodies that bind and neutralize the H. pylori toxin, VacA.

Kroh HK, Chandrasekaran R, Zhang Z, Rosenthal K, Woods R, Jin X, Nyborg AC, Rainey GJ, Warrener P, Melnyk RA, Spiller BW, Lacy DB. A neutralizing antibody that blocks delivery of the enzymatic cargo of Clostridium difficile toxin TcdB into host cells. J Biol Chem. 2018 Jan 19;293(3):941-952. doi: 10.1074/jbc.M117.813428. PMCID: PMC5777265.

Kroh HK, Chandrasekaran R, Rosenthal K, Woods R, Jin X, Ohi MD, Nyborg AC, Rainey GJ, Warrener P, Spiller BW, Lacy DB. Use of a neutralizing antibody helps identify structural features critical for binding of Clostridium difficile toxin TcdA to the host cell surface. J Biol Chem. 2017 Sep 1;292(35):14401-14412. doi: 10.1074/jbc.M117.781112. PMCID: PMC5582835.

Chumbler NM, Rutherford SA, Zhang Z, Farrow MA, Lisher JP, Farquhar E, Giedroc DP, Spiller BW, Melnyk RA, Lacy DB. Crystal structure of Clostridium difficile toxin A. Nat Microbiol. 2016 Jan 11;1:15002. doi: 10.1038/nmicrobiol.2015.2. PMCID: PMC4976693.

González-Rivera C, Campbell AM, Rutherford SA, Pyburn TM, Foegeding NJ, Barke TL, Spiller BW, McClain MS, Ohi MD, Lacy DB, Cover TL. A Nonoligomerizing Mutant Form of Helicobacter pylori VacA Allows Structural Analysis of the p33 Domain. Infect Immun. 2016 Aug 19;84(9):2662-70. doi: 10.1128/IAI.00254-16. Print 2016 Sep. PMCID: PMC4995914.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/10qnhhv9n8yQo/bibliography/46069021/public

D. Research Support

Ongoing Research Support

5R01 AI108778-05 (Spiller) 07/01/2014-06/30/2020 in NCE
NIH/NIAID
CopN Mechanisms as a key to understanding Type Three Secretion in bacteria
We have discovered that a novel molecular scaffold is a critical feature of T3SS and will further elucidate its role in the hierarchy of secretion in multiple gram-negative bacteria as well as test the hypothesis that this molecular scaffold is a useful target for novel antibiotics.

5R01AI095755-10 (Lacy) 03/01/2017-04/30/2021
NIAID (VUMC subcontract)
Structural Mechanisms of Clostridium Difficile Pathogenesis
The incidence, severity, and costs associated with Clostridium difficile infection (CDI) are increasing, making C. difficile a significant public health concern. The goal of the proposed project is to delineate the structural and molecular mechanisms by which the two primary toxins, TcdA and TcdB, gain access to host cells.

Completed Research Support

Medimmune Contract (Spiller, B. PI) 01/03/2018-01/03/2020
Epitope determination for SAN481 and SAN177.

The Spiller lab will crystalize and determine molecular structures for Fab fragments from SAN481, SAN177, as well as a W100a mutant of SAN481.

BIOGRAPHICAL SKETCH

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NAME: Andrew Reed Jones

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

POSITION TITLE: Research Assistant I

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
Rice University	B.S.	05/2014	Electrical Engineering
Rice University	B.A.	05/2014	Computer Science

A. Personal Statement

I have a base of knowledge in computational methods that complements the biological expertise of others in the Spiller research group. Having undergone basic training in the use of techniques and equipment required in Cryogenic Electron Microscopy, I am prepared to make contributions to the study of protein structures using these developing methods. In addition to my being in a position to contribute to research in this specific area, I have been building sufficient knowledge of foundational concepts and techniques in molecular and structural biology in order to be able to communicate about the work that is required prior to sample preparation for imaging. In doing so, I have developed some working knowledge in PCR mutagenesis, bacterial transformation, protein extraction, and protein analysis by electrophoresis and column chromatography. Prior to joining the group, I have developed skills in the areas of software development and signal processing in an engineering capacity. Both of these areas are relevant to biological imaging and the processing of biological data for research purposes. I am eager to make use of imaging and computation to answer questions of interest to the group. These interests relate broadly to epitope discovery, host-pathogen interactions, and the roles of IgE antibodies in human responses to allergens.

B. Positions and Honors

Positions and Employment

2009 High School Research Asst., Spiller Lab, Dept. of Pharmacology, Vanderbilt Medical School
2010 High School Research Asst., Lacy Lab, Depts. Of Micribiology, Immunology, and Biochemistry, Vanderbilt University Medical School
2011 Undergraduate Research Assistant, Electrical Engineering at Rice University
2011 Course Assistant, Fundamentals of Electrical Engineering at Rice University
2012 Lab Assistant, Digital Logic Design at Rice University
2012 Research Assistant, HTWK Leipzig, Germany
2013 Software Development Intern, National Instruments Corporation, Austin, TX
2014-2017 Software Engineer, National Instruments Corporation, Austin, TX
2019- Research Assistant, Spiller Lab, Dept. of Pharmacology, Vanderbilt Medical School

Honors

2011-2014 Academic Fellow, McMurtry Residential College, Rice University
2012 DAAD RISE Scholar
2014 Rice Univ. Undergraduate Research Symposium Ken Kennedy Institute First Place Award

C. Contributions to Science

Radio Frequency Research and Test Equipment
Employed in a software role in industry, I worked with a large team to develop new Vector Signal Generators and Analyzers necessary to build higher-frequency and higher-bandwidth communications systems. Among my contributions were features enabling the storage, manipulation, and retrieval of waveforms in device memory. I also managed aspects of the team's automated code compilation and testing. In this way, I contributed to the project by helping to validate and integrate the components created by all team members. The largest project I worked on was the hardware/software product NI PXIe-5840, which is a combined Vector Signal Transceiver capable of 6 GHz frequencies and 1 GHz of instantaneous bandwidth.

Wireless Communications Research
As an undergraduate research assistant I worked to help deploy, test, and manage a wireless network utilizing UHF television-band whitespace links to provide internet to an area in Houston, TX as part of a non-profit initiative. The network served both as a functioning service for homes in the neighborhood, as well as a platform for research about mesh networks. I developed a user authentication system for integration with the network. I also helped evaluate design considerations for wireless nodes to be deployed in the network by processing meta-data about network usage and testing the hardware nodes.

Development of Contact-Free Vital Signs Monitor
I worked in a small team to develop a monitor capable of accurately measuring the heart rate of human patients under many conditions. Our team used open-source computer vision software in combination with our own software and chassis. The system employed commercially available camera and time-of-flight sensors, and was intended for use in environments where a contact-free method of monitoring is necessary due to the sensitivity of the patient. This is often the case in neonatal intensive care units.

Software Development For Neuroscience Research
I developed features for the OpenWalnut open-source software project, used by researchers solving visualization problems in neuroscience research. The package is a C++ based tool for real-time processing of EEG, EKG, and MEG signals, with the potential to extend to additional modalities. I developed a module capable of performing dimensionality reduction on real-time data using Principle Component Analysis. I worked with a small team of programmers using common tools for development and version control.

High School Laboratory Assistant
As a high school student in the Spiller and Lacy labs at Vanderbilt University and Medical Center, I assisted with investigations of the function of homologous proteins VirA and EspG from the bacterium *Shigella flexneri*.

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

Coursework at Rice University, Houston, TX

COURSE	YEAR	GRADE
Linear Algebra	2010	A-
Electronic Materials	2010	B
Algorithmic Thinking	2011	A-
Fund. Computer Engineering	2011	A
Multivariable Calculus	2011	A-
Wireless Networking	2011	A
Random Signals	2011	B+
Digital Logic Design	2011	A+

COURSE	YEAR	GRADE
Fund. of Parallel Programming	2012	A
Intro to Physical Electronics	2012	A
Digital Signal Processing	2012	B
Implementation of Digital Systems	2012	A+
Advanced Object-Oriented Programming	2012	A
Computer Systems Architecture	2012	A-
Intro To Communications Networks	2012	A
Design and Analysis of Algorithms	2013	A-
Intro to Computer Vision	2013	A
Digital Communication	2013	A
Advanced Topics in Computer Networking	2013	A+
Mobile and Embedded Systems	2013	A-
Principles of Programming Languages	2014	B
Senior Design Project	2014	A