BIOGRAPHICAL SKETCH

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NAME: Noinaj, Nicholas

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

POSITION TITLE: Associate Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Berea College, Berea, KY	BA/BA	08/2003	Chemistry/Mathematics
Univ of Kentucky College of Medicine, Lexington, KY	PhD	08/2008	Biochemistry
NIDDK, NIH, Bethesda, MD	PostDoc	2008-2014	Membrane protein crystallography

A. Personal Statement

Infectious diseases cause widespread sickness and death throughout the world each year and are the second leading cause of death, particularly in underdeveloped countries. And with the emergence of multidrug resistance strains of Gram-negative bacteria, the necessity for new, more effective, and more sustainable therapies is immediate and vital to protect against any future pandemics. The primary focus of my lab is the study of membrane proteins and protein complexes that are promising targets for the development of new therapies (both antibiotics and vaccine) against these multidrug resistant pathogens. In doing so, much of our attention lies in surface proteins found within the outer membranes of the bacteria, which often serve essential roles in mediating pathogenesis. A secondary focus of my lab is dedicated to projects targeting cancer-related systems, both soluble and membrane proteins, through projects in my own lab and collaborations across Purdue's campus.

One set of projects involve metal-acquisition systems, particularly in Neisseria, including the transferrin binding protein (Tbp) complex, the lactoferrin binding protein (Lbp) complex, and the calprotectin binding protein TdfH zinc transporter. The Lbp complex consists of two receptors called LbpA and LbpB, both virulence factors mediating pathogenesis in Neisseria. We are currently structurally characterizing LbpA and LbpB from Neisseria, having recently collected a 2.85 Å dataset of *Nm*LbpB in complex with holo-lactoferrin. We are using X-ray crystallography and cryoEM to study the LbpA and LbpB receptors with and without lactoferrin. Further, we are keenly interested in determining the role that each of the Lbp proteins play in mediating pathogenesis, particularly in *N. gonorrhoeae*, where strains have been isolated which are resistant to all known antibiotics. Additionally, we are specifically investigating the role of LbpB as an antimicrobial peptide sink, adding further protection to Neisseria.

Another set of projects explore the biogenesis of membrane proteins in Gram-negative bacteria and chloroplasts, where the outer membrane contains a host of beta-barrel proteins commonly called outer membrane proteins (OMPs), which serve essential functions in cargo transport and signaling and are also vital for membrane biogenesis. One focus of my lab has been on the beta-barrel assembly machinery (BAM) which folds and inserts the new OMPs into the outer membrane. BAM is conserved across all Gram-negative bacteria and is essential for viability. In *E. coli*, BAM consists of five components called BamA-E, with BamA being an OMP itself and BamB-E being lipoproteins. Exactly how the BAM complex is able to accomplish its function remains unknown, however, our recent studies using cryoEM have provide evidence favoring a hybrid-barrel mechanism mediating OMP biogenesis by BAM.

My lab also studies the pathogenesis of *Fusobacterium nucleatum*, which has been implicated in colorectal cancer. Here, we are structurally and functionally characterizing the role of BAM in the biogenesis of surface receptors that may be contributing to the development of cancer. Further, my lab collaborates with several labs across the Purdue campus (Yang, Huang, Zhang, Flaherty, etc) that study a variety of cancers; in particular, assisting with structure determination of drug:enzyme complexes using X-ray crystallography and other methods to determine how the compounds interact with their target and how they can be further improved.

B. Positions and Honors

Positions and Employment

2013-14 Research Fellow, NIDDK/NIH, Dr. Susan Buchanan
2014-2019 Assistant Professor, Purdue University, Department of Biological Sciences
2019 – pres Associate Professor, Purdue University, Department of Biological Sciences

Other Experience and Professional Memberships

2020-pres Protein Society
2020-pres Biophysical Society
2008 Member of the Delta Epsilon Iota Academic Honor Society
2012 American Society for Microbiology
2006-pres American Crystallographic Association

Honors

2020 Excellence in Research Award, Purdue University
2019 Showalter Faculty Scholar, College of Science, Purdue University
2019 Mentoring Award, College of Science, Purdue University
2018 Excellence in Research Award, College of Science, Biological Sciences, Purdue University
2017 Team Award, College of Science, Purdue University
2013 Fellows Award for Research Excellence (FARE) Award Winner
2012 Fellows Award for Research Excellence (FARE) Award Winner
2008 Member of the Delta Epsilon Iota Academic Honor Society
2007 X-Ray Methods in Structural Biology, Cold Spring Harbor Laboratory (Full Scholarship)
2006 Best Poster - University of Kentucky Molecular and Cellular Biochemistry Symposium
2006 American Crystallographic Association Annual Meeting Travel Award
2004 National Institute on Drug Abuse (NIDA) Training Grant (2-years)
2006 National Institute on Drug Abuse (NIDA) Training Grant (2-years)
2003 Class of 1953 Chemistry Scholarship Award, Berea College 2004

Conference/Meetings

2020 Lorne Research Conference (45th) on Protein Structure and Function, Lorne, Australia
2019 Hitchhiker's Guide to the Biomolecular Galaxy symposium, Purdue Univ.
2019 14th Annual Midwest Protein Folding symposium, Notre Dame
2019 Missouri Symposium in Molecular Biophysics, Univ. of Missouri
2018 Purdue CryoEM Symposium, Purdue Univ.
2018 Hitchhiker's Guide to the Biomolecular Galaxy symposium, Purdue Univ.
2018 13th Annual Midwest Protein Folding symposium, Notre Dame
2018 Gordon Research Conference - Protein Transport Across Membranes (Invited talk)
2017 American Crystallographic Association Meeting (Invited talk)
2017 Tessman symposium, Purdue Univ.
2017 Hitchhiker's Guide to the Biomolecular Galaxy symposium, Purdue Univ.
2017 12th Annual Midwest Protein Folding symposium, Notre Dame
2016 Zing Conference – Protein Secretion Across Membranes
2016 FASEB Meeting – Molecular Biophysics of the Membrane (Invited talk)
2016 11th Annual Midwest Protein Folding symposium, Notre Dame (Plenary talk)
2015 Membrane Proteins Symposium at APS/Argonne IL
2014 International Union of Crystallography (Invited talk)
2014 American Crystallographic Association (Invited talk)
2014 Gordon Research Conference - Protein Transport Across Membranes (Invited talk)
2014 44th Mid-Atlantic Macromolecular Crystallography meeting (Invited talk)
2013 Janelia Farm Symposium – Structure determination of membrane proteins (Invited talk)
2013 Annual ASBMB Meeting, Boston, MA
2013 MPIG/SBIG Postdoc Symposium (Invited talk)
2012 American Crystallographic Association (Two Invited talks)
2012 Gordon Research Conference – Protein Transport Across Membranes
2012 Gordon Research Conference – Ligand Recognition and Molecular Gating (Invited talk)
2011 NIH Research Festival (Invited talk)

C. Contribution to Science ([1]co-first author, [*]corresponding author)

1. During the first few years of my postdoctoral studies, I wanted to study membrane proteins using X-ray crystallography due to their importance in biology and since they are very challenging. Therefore, I began working on the receptor transferrin binding protein A (TbpA) from *Neisseria meningitidis* to determine how it interacts with human transferrin to mediate iron hijacking and pathogenesis. Here, I was able to determine the structure of TbpA bound with human transferrin, identifying residues important for receptor binding and iron extraction and import. I also determined the crystal structure of the Neisserial co-receptor TbpB and used SAXS analysis to determine the structure of the complex with human transferrin. Further, I also worked with EM collaborators to determine a low resolution structure of the triple complex of human transferrin bound to both receptors. These studies significantly advanced our understanding of how these receptors are able to specifically bind human transferrin, extract its iron and import it across the Neisserial outer membrane for survival during infection within the human host.
    a. **Noinaj N**, Easley N, Oke M, Mizuno N, Gumbart JC, Boura E, Steere A, Zak O, Aisen P, Tajkhorshid E, Evans RW, Gorringe AR, Mason AB, Steven AC, and Buchanan SK. (2012). Structural basis for iron piracy in pathogenic Neisseria, *Nature* (Research Article), 483 (7387):53-8.
    b. **Noinaj N**, Cornelissen CN, Buchanan SK. (2013). Structural insight into the lactoferrin receptors from pathogenic Neisseria. *J Struct Biol.* 2013 184(1):83-92.
    c. **Noinaj N**, Buchanan SK, and Cornelissen CN, (2012). The transferrin–iron import system from pathogenic Neisseria species. *Mol Micro*, 86 (2):246-257.
    d. Yadav R, **Noinaj N**, Ostan N, Moraes T, Stoudenmire J, Maurakis S, Cornelissen CN. (2020). Structural Basis for Evasion of Nutritional Immunity by the Pathogenic Neisseriae. *Front Microbiol*. 2020 Jan 10;10:2981.

2. Midway into my postdoctoral studies, I became involved in a project aimed at determining the crystal structure of neurotensin receptor 1 (NTS1), a GPCR that is the receptor for the neuropeptide neurotensin. I was able to determine the structure of NTS1 which was the first GPRC of its class, which revealed exactly how the peptide interacts with the receptor for downstream conformational changes and signaling. With many structures now available for different classes of GPCRs, we were able to contribute the first from this class which showed how the endogenous substrate binds.
    a. White JF, **Noinaj N**, Shibata Y, Love J, Kloss B, Xu F, Gvozdenovic-Jeremic J, Shah P, Shiloach J, Tate CG, Grisshammer R. (2012). Structure of the agonist-bound neurotensin receptor NTS1. *Nature* (Research Article), 490 (7421):508-513.

3. During the later stages of my postdoctoral studies, I began to make progress on my primary project, to determine the crystal structure of BamA, an essential outer membrane protein in Gram-negative bacteria required for the biogenesis of all outer membrane proteins, in particular, virulence factors for pathogenic strains. Over the course of several years, I was able to determine the crystal structure of BamA from *Neisseria gonorrhoeae* and *Haemophillius ducreyi*. These structures significantly advanced our knowledge of how it, as the core of a larger complex called the BAM complex, it is able to fold and insert nascent outer membrane proteins into the outer membrane. From the structures, we were able to propose a mechanism whereby a lateral opening into the membrane is required for direct insertion. Crosslinking experiments further verified this mechanism which has served as the basis for now testing how this folding/insertion occurs. Given the essential role of BamA, these studies have a huge impact on how these bacteria are able to survive and mediate pathogenesis. While at Purdue University, my lab has solved the structure of the assembled BAM complex, providing high resolution details about the binding interfaces of the individual components with one another, and providing the first structure to suggest that binding of BamCDE may regulate BamA by inducing an unprecedented conformational change of the barrel domain of BamA, which primes the barrel in an activated state for insertion. Currently our lab studies BAM from E*. coli*, *Neisseria gonorrhoeae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Fucobacterium nucleatum*.
    a. Lundquist K, Bakelar J, **Noinaj N**, Gumbart JC. (2018). C-terminal kink formation is required for lateral gating in BamA. *PNAS*. 115(34):E7942-E7949.
    b. Sikora AE, Wierzbicki IH, Zielke RA, Ryner RF, Korotkov KV, Buchanan SK, **Noinaj N**. (2018). Structural and functional insights into the role of BamD and BamE within the β-barrel assembly machinery in *Neisseria gonorrhoeae*. *J Biol Chem* 293(4):1106-1119.
    c. Bakelar J, Buchanan SK, **Noinaj N**[*]. (2015). The structure of the β-barrel assembly machinery complex. *Science* 351(6269):180-6.

d. **Noinaj N**, Kuszak AJ, Gumbart JC, Lukacik P, Chang H, Easley NC, Lithgow T, and Buchanan SK. (2013). Structural insight into the biogenesis of β-barrel membrane proteins. *Nature* (Research Article), 501(7467):385-90.

4. I continue to collaborate on another project to structurally characterize the Ton complex, which acts as an energy transducing machine within the inner membrane of Gram-negative bacteria to provide energy to drive ligand gating at the outer membrane. While here at Purdue, I was able to solve the structure of the complex which is the first of this complex, composed of a pentamer of ExbB and a single ExbD within the pore. We went on to fully characterize the fully assembled Ton complex with other methods including EM, DEER, crosslinking, and electrophysiology to show it consist of a pentamer of ExbB, a dimer of ExbD and a single TonB. My lab continues to collaborate on this project, working towards determining the full structure of the Ton complex bound with a TonB-dept transporter.
   a. Celia H[*], **Noinaj N[*1]**, Zakharov SD, Bordignon E, Botos I, Cramer WA, Lloubes R, and Buchanan SK. (2016). Structural insight into the role of the Ton complex in energy transduction. *Nature* 538(7623):60-65.
   b. Celia H, **Noinaj N**, Buchanan SK. (2020). Structure and Stoichiometry of the Ton Molecular Motor. Int J Mol Sci. 2020 Jan 7;21(2).

5. Protein trafficking across membranes is an essential function in cells; however, the exact mechanism for how this occurs is not well understood. In the endosymbionts, mitochondria and chloroplasts, the vast majority of proteins are synthesized in the cytoplasm as preproteins and then imported into the organelles via specialized machineries. In chloroplasts, protein import is accomplished by the TOC (translocon on the outer chloroplast membrane) and TIC (translocon on the inner chloroplast membrane) machineries in the outer and inner envelope membranes, respectively. TOC mediates initial recognition of preproteins at the outer membrane and includes a core membrane channel, Toc75, and two receptor proteins, Toc33/34 and Toc159, each containing GTPase domains that control preprotein binding and translocation. Progress in the field has been hindered by the lack of structural information on the Toc proteins. Our goal is to use X-ray crystallography and cryoEM to structurally and functionally characterize the full TOC complex. Recently, we determined the structure of the POTRA domains of Toc75, the core component of Toc75.
   a. O' Neil P, Richardson LGL, Paila YD, Piszczek G, Chakravarthy S, **Noinaj N**, Schnell DJ. (2017). The POTRA domains of Toc75 exhibit chaperone-like function to facilitate import into chloroplasts. PNAS, 114(24):E4868-E4876.

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1redk8eN4_C5u/bibliography/47938167/public/?sort=date&direction=descending

D. Research Support

Active Support

NIGMS 1R01GM127896-01 (Noinaj) 05/01/18 - 03/31/23
Structural Characterization of the TOC Protein Translocon Machinery
The goal of this proposal is to structurally and functionally characterize the TOC complex, the gateway complex for import into the chloroplast. Role: PI

NIAID 1R01AI127793-01 (Cornelissen) 07/01/17 - 09/30/21
Neisseria gonorrhoeae metal transporters that subvert nutritional immunity
The goal of this proposal is to determine how Neisseria use surface proteins to evade host immunity. Role: Sub-contractor (Structural studies of TdfH)

NIGMS 1R01GM127884 (Noinaj) 07/08/19 - 05/31/23
Unraveling the mechanism by which the BAM complex mediates OMP biogenesis
The goal of this proposal is to determine substrate interactions of the BAM complex which is responsible for folding OMPs into the outer membrane of Gram-negative bacteria. Role: PI

NIGMS SC1 (Sun) 05/01/19 – 12/31/21
Membrane interaction of Mycobacterium tuberculosis virulence
The goal of this project is to characterize the structure and function of ESAT6 which is essential for pathogenesis. Role: Sub-contractor (Structural studies of ESAT6)

Indiana CTSI Pilot Funding (Noinaj) 01/01/20 – 12/31/21
Targeting the Lbp system to combat iron piracy in Neisseria
The goal of this project is to characterize the structure of the Lbp receptor system in Neisseria. Role: PI

Purdue U. (Noinaj) 07/01/19 – 06/31/20
The role of LbpB in mediating Neisserial pathogenesis
The goal of this project is to determine the dual role LbpB plays in iron piracy and in evading the host immune system during Neisserial pathogenesis. Role: PI

Completed Support

Purdue U. (Noinaj) 05/01/19 - 12/31/19
State-of-the-Art Crystallization at Purdue with Rock Imager 1000 Duo
Internal Purdue Univ. equipment grant for the purchase of a state-of-the-art crystallization imaging and plate hotel system, can automatically record high-resolution images of all these experiments on a user-designed schedule, both documenting and analyzing each condition for crystal growth. Role: PI

Sponsored Support - Achaogen (Noinaj) 11/31/17 - 12/01/18
Expression and purification of the BAM complex from Acinetobacter baumannii
The goal of this proposal is to express and purified active BAM complex from *A. baumannii* for downstream therapeutics development and for structural studies. Role: PI

Purdue U. (Noinaj) 01/01/18 - 12/31/18
Acquisition of Automated Robotics for Innovative Crystallization Screen Preparation
Internal Purdue Univ. equipment grant for the purchase of a department shared 96-channel automated pipetting instrument and automated microplate heat sealer for crystallization screen preparation for the crystallization core facility. Role: PI

NIAID/NIH K22 AI113078-02 (Noinaj) 07/15/15 - 06/30/18
The role of BamA in the biogenesis of beta-barrel membrane proteins
The goal of this project is to determine the structural features of BamA which directly play a role within the BAM complex for the folding and insertion of OMPs sin Gram-negative bacteria. Role: PI

Indiana CTSI Pilot Funding (Noinaj) 05/01/15 - 06/30/18
Investigating substrate recognition by the β-barrel assembly machinery complex
The goal of this proposal is to gather preliminary data on substrate recognition by the BAM complex using the cryoEM facilities at Purdue University. Role: PI

Purdue U. EVPRP (Noinaj) 06/01/18 - 5/31/19
The role of LbpB in mediating Neisserial pathogenesis
Internal Purdue Univ. award that is intended to assist in gathering preliminary data for future NIH funding. Role: PI

Showalter Trust Award (Noinaj) 07/01/16 - 09/30/17
Targeting the BAM Complex for Antibiotic Development against Neisseria Gonorrhoeae
The goal of this proposal is to develop an assay for drug discovery targeting the *Ng*BAM complex. Role: PI

Sponsored Support - Achaogen (Noinaj) 08/01/16 - 07/31/17
Expression and purification of the BAM complex from Acinetobacter baumannii
The goal of this proposal is to express and purified active BAM complex from *A. baumannii* for downstream therapeutics development and for structural studies. Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME:Billings, Evan Michael

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

POSITION TITLE: Graduate Student, NIH T32 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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Purdue University	B.S.	08/2014	05/2018	Biochemistry, minors in biology and philosophy
Purdue University	PhD	08/2018	05/2023	Membrane protein structural biology

A. Personal Statement

Since I began my training in structural biology, I have developed a strong interest in the structure and mechanism of protein machinery complexes and motor proteins. I therefore soon began seeking undergraduate research opportunities in structural biology and protein biochemistry. Ultimately this research led me to make the late decision to change my major to biochemistry halfway through my junior year. During my undergraduate tenure, I worked with Dr. Nicholas Noinaj because his lab studies the structure and function of various membrane bound machinery complexes in Gram-negative bacteria. Working in his lab gave me extensive experience in protein biochemistry and crystallography. I was gratefully granted the opportunity to work full time during the summers of 2017 and 2018, which allowed me to be immersed in research full time. I found I learned exponentially better when working in a lab performing experiments, rather than attending a lecture and memorizing information. The time I spent working in his lab was perhaps the most fulfilling experience of my undergraduate studies and cemented into me the decision to purse research in biochemistry, structural biology, and biophysics as a career.

After making that decision to attend graduate school, I received offers from several programs, but ultimately choose to stay at Purdue in part due to their extensive facilities and resources in structural biology and biophysics. My first year I rotated in four different structural biology labs, ranging from cancer biology to virology, but ultimately, I felt that Dr. Noinaj's research catered best to my interest. I returned to his lab as a graduate student in the spring of 2019, independent of the fact that I worked with him as an undergraduate. As a member of his lab, I am receiving ongoing training and building expertise in membrane protein biochemistry, biophysics, and cryo-electron microscopy. I am currently working to study the architecture and mechanism of the BAM complex from *Neisseria gonorrhoeae*. The BAM complex mediates the biogenesis of outer membrane proteins in Gram-negative bacteria and represents a novel antibiotic target for a human pathogen that has rapidly developed multidrug resistance. Within the last decade alone, we have seen an explosion of research in the protein complexes responsible for the biogenesis of bacterial membranes, lipoproteins, LPS, and membrane proteins! However, the mechanisms behind much of these processes is still poorly understood. My long term goal is to obtain an academic position at a large research university and bring together structural biology, *in vivo* techniques, and biophysics to better understand membrane proteins related to human health and disease. How did these complexes evolve such interesting mechanical capabilities? How can we best elucidate their mechanism using structural biology and biophysics? How can we exploit these mechanisms to further aid rational drug design and vaccine development against human pathogens? These are all questions that I am extremely interested in and would like to work to answer as they pertain to the BAM complex and other protein machinery.

B. Academic Positions

ACTIVITY/ OCCUPATION	START DATE (MM/YY)	END DATE (MM/YY)	FIELD	INSTITUTION/ COMPANY	SUPERVISOR
NIH T32 Trainee	06/19	Present	Molecular Biophysics	Purdue University	John Tesmer, PhD
Graduate Student	08/18	Present	Membrane Protein Structural Biology	Purdue University	Nicholas Noinaj, PhD
Graduate Teaching Assistant	08/18	05/19	Cell and Molecular Biology	Purdue University	--
Research Assistant	05/18	08/18	Protein Biochemistry	Purdue University	Nicholas Noinaj, PhD
Research Assistant	05/17	08/17	Protein Biochemistry	Purdue University	Nicholas Noinaj, PhD

Academic and Professional Honors

2020 College of Science Travel Award
2019-21 T32 Molecular Biophysics Training Program Trainee
2017 College of Agriculture Undergraduate Research Scholarship

Professional Memberships

2020- Member, Biophysical Society
2019- Member, American Crystallographic Association
2018- Member, certified with distinction, American Society for Biochemistry and Molecular Biology

Meetings and Conferences

2021 ASBMB PDB50 Symposium, attended virtually
2021 Experimental Biology Annual Meeting, attended virtually
2021 S^2C^2 Cryo-EM Specimen Preparation and Data Collection Workshop, attended virtually
2021 65th Biophysical Society Meeting, attended virtually
2020 6th MicroED Imaging Center Course, attended virtually due to COVID-19
2020 American Crystallographic Association Annual Meeting, attended virtually due to COVID-19
2020 S^2C^2 Image Processing Workshop, virtually hosted by SLAC due to COVID-19
2019 Everything BioSAXS V Workshop, APS, Argonne National Lab, Lemont, Illinois
2019 American Crystallographic Association Annual Meeting, Cincinnati, Ohio
2019 Hitchhikers Guide to the Biomolecular Galaxy Symposium, Purdue University
2018 Purdue Cryo-EM Symposium, Purdue University
2018 Hitchhikers Guide to the Biomolecular Galaxy Symposium, Purdue University
2017 Hitchhikers Guide to the Biomolecular Galaxy Symposium, Purdue University

C. Contributions to Science

1. **Undergraduate Research:** After enrolling as an undergraduate at Purdue University, I was initially interested in ecology and evolutionary biology. This led me to join the lab of Dr. Jeff Lucas for a semester studying the social complexity hypothesis in the Carolina chickadee. However, my research interests later changed after realizing ecology was not where my passion was and I began working with Dr. Nicholas Noinaj in the Fall of 2016. His lab primarily works to characterize the structure of membrane proteins in gram-negative, pathogenic bacteria that contribute to their virulence. In the *Neisseria* family

of bacteria, only two species are pathogenic, *N. gonorrhoeae* and *N. meningitidis*, which cause gonorrhea and meningitis, respectively. To survive, these bacteria have evolved the ability acquire ferrous iron externally from their host by binding to iron transporting proteins such as transferrin and lactoferrin. For almost three years, I worked primarily independently to structurally characterize the transferrin importing system from *N. gonorrhoeae*, which consists of transferrin binding protein A (*Ng*TbpA), a 92 kDa transmembrane receptor, and its 82 kDa lipid anchored coreceptor, transferrin binding protein B (*Ng*TbpB). I was able to express, purify, and crystallize *Ng*TbpB, which diffracted to 1.8 Å. However, we were unable to solve the structure and before I graduated.

a. Billings, E., Noinaj, N. (2017, April 11).*The crystal structure of the transferrin binding complex in N. gonorrhoeae*. [Poster] Purdue University Undergraduate Research Symposium, West Lafayette, IN.

b. Billings, E., Noinaj, N. (2017, May 10-11). *The crystal structure of the transferrin binding complex in N. gonorrhoeae*. [Poster]. The Hitchhikers Guide to the Biomolecular Galaxy Symposium, West Lafayette, IN.

2. **Graduate Research:** My current graduate work under Dr. Noinaj, studies the β barrel assembly machinery (BAM) complex, a 200 kDa protein complex in the outer membrane of gram negative bacteria that is responsible for the biogenesis of outer membrane proteins, and thus survival. In *E. coli*, this machine consists for 5 component proteins, a β barrel insertase BamA, and 4 accessory lipoproteins, BamB, C, D, and E. The structure of this complex in its entirety has been reported in recent years, however models for its mechanism have only been proposed and lack experimental evidence. My research focuses on the BAM complex from *Neisseria gonorrhoeae* (*Ng*BAM). Interestingly this complex only consists of 4 component proteins, as no homolog of BamB has been found in the neisserial genome. The structures of *Ng*BamA, D, and E have been solved using X-ray crystallography, but structural information has not been reported for *Ng*BamC, or the intact *Ng*BAM complex. In order to fill this gap of information, my current approach is to solve the structure of the intact *Ng*BamACDE complex using cryo-EM. I was able to build a reconstruction to 6.5 Å and I am currently working to improve the sample and EM conditions to get a high resolution structure. Our current working model has already revealed interesting architectural differences in the BAM complex between the two species. Even though this is the first step in understanding its mechanism, elucidating the structure will make clear how its architecture differs from that of *E. coli*. I plan to complement our structural studies with *in vivo* characterization in *Neisseria* and DEER spectroscopy in order to examine the specific role the *Ng*BAM proteins have in BAM function and into how one might develop small molecule inhibitors or vaccine candidates against the complex.

a. Billings, E. Lundquist, K. Overly, C. Srinivasan, K. and Noinaj, N. (2021). Structural Determination of Membrane Proteins Using X-Ray Crystallography. *Methods in Molecular Biology*. 2302, 101-136. DOI: 10.1007/978-1-0716-1394-8_7

b. Lundquist, K. Billings, E, Bi, M., Wellnitz, J., and Noinaj, N. (2021). The assembly of β-barrel membrane proteins by BAM and SAM. *Molecular Microbiology*, 115,(3). 425-435. DOI: 10.1111/mmi.14666

c. Billings, E., Noinaj, N. (2021, April 27-30). Structural insights into outer membrane protein biogenesis in *Neisseria gonorrohoeae*. [Virtual Poster]. Experimental Biology Annual Meeting, hosted virtually.

d. Billings, E. (2018, Oct 30). *The Magic of Molecular Machines: The Type IV Pilus and its Assembly Machinery in Gram-negative Bacteria*. [Oral Presentation] BIOL 696: Membrane Protein Supercomplexes Seminar, West Lafayette, IN

D. Additional Information: Scholastic Performance

Purdue University (Undergraduate, 2014-2018)

YEAR	SCIENCE COURSE TITLE	GRADE	YEAR	OTHER COURSE TITLE	GRADE
2015	General Chemistry	B	2014	First-Year Composition	A-
2015	Development, Structure, and Function	B-	2014	Spanish II	A-
2015	Cell Structure and Function	A-	2014	Introduction to Philosophy	A-
2015	Cell Structure and Function Lab	B	2015	Intro. to Biological Anthropology and Human Evolution	A

2016	Intro. to Ecology and Evolution	B
2016	Organic Chemistry I	B+
2016	Organic Chemistry I Lab	A
2016	Macromolecules	B-
2016	Organic Chemistry II	B
2016	Organic Chemistry II Lab	A
2016	Physics for Life Science I	B
2017	Physics for Life Science II	A
2017	Experimental Design Seminar	A
2017	Molecules	B-
2017	Analytical Biochemistry	A
2017	Metabolism	B
2018	Biological and Structural Aspects of Drug Design and Action	B
2015	Ethics	A-
2016	Biomedical Ethics	B-
2016	History of Modern Philosophy	A-
2017	Religions of the West	B+
2017	The Movies	A-
2017	Elementary Statistical Methods	B
2017	Principles of Economics	A-
2017	Science Writing and Presentation	A
2018	Philosophy of Mind	A

Purdue University considers grades above a C- passing.

Purdue University (Graduate, 2018-Present)

YEAR	COURSE TITLE	GRADE	YEAR	COURSE TITLE	GRADE
2018	Methods and Measure in Biophysical Chemistry	A+	2019	Professional Development Seminar I	A
2018-2019	Research Rotations	P/P/P/P	2019	Professional Development Seminar II	A
2018	Membrane Supercomplexes Seminar	A+	2019	Grant Writing	A
2019-present	Structural Biology Research Seminar	S/S/S/S	2020	CryoEM 3D Reconstruction	AU
2019	Macromolecules	B	2020	Biophysics Grant Writing	A
2019	Membrane Proteins	A	2020	Biophysical Methods	A
2019	X-ray Crystallography	A-			

The Department of Biological Sciences Graduate Program considers grades above B passing. AU indicates courses that were audited. Some courses are graded as Pass (P) or Fail (F). Others are graded as Satisfactory (S) or Unsatisfactory (U).

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: Lundquist, Karl

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

POSITION TITLE: Postdoctoral Research Associate

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)	END DATE MM/YYYY	FIELD OF STUDY
University of Michigan, Ann Arbor, Michigan	BS	05/2012	Physics
Georgia Institute of Technology, Atlanta, Georgia	MS	08/2015	Physics
Georgia Institute of Technology, Atlanta, Georgia	PHD	05/2019	Physics

A. Personal Statement

I am focused on advancing my career in academics with an aim at studying the structure and function of membrane proteins to advance the treatment of disease. As an undergraduate at the University of Michigan I was fascinated by the study of the universe on the most fundamental level. My coursework was focused on mathematics and physics and I spent four years working in an atomic physics laboratory. In my final year, I was set on doing a PhD in atomic physics, and while I was applying for graduate schools, I took a course in Biophysics mostly on a whim and it changed my career. My eyes were opened to the ways in which biological systems, in their vast complexity were merely following the laws of physics on the most basic level, and not only could that behavior be studied, but it could have a real impact on human health. At Georgia Tech, I found the ultimate playground for a physicist aspiring to learn more about biology in molecular modeling and molecular dynamics simulations. With Dr. James Gumbart I started working on the fascinating problem of outer-membrane protein biogenesis in Gram-negative bacteria. These proteins contain a unique ß-barrel fold as membrane proteins, and in the absence of traditional energy sources like ATP or a PMF, likely rely on the energetics of folding alone to accomplish their functions. I spent my PhD primarily working on studying the energetics of the ß-barrel assembly machinery (BAM), the complex responsible for catalyzing the assembly of outer membrane proteins (OMPs) and worked on a number of fascinating collaborations as well. While I thoroughly enjoyed the work as a molecular dynamics researcher, I found myself desiring to expand my skillset to learn experimental techniques of molecular and structural biology to probe protein dynamics and function in a greater number of ways. At Purdue University I found the perfect opportunity to hone these skills through continuing my investigation of BAM-mediated assembly of OMPs with one of the world's leading researchers in this field, Dr. Nicholas Noinaj. Purdue has world-class facilities for performing structural biology with two Titan Krios electron microscope facilities and is a short distance away from Argonne national lab for frequent X-ray data collection trips to the Advanced Photon Source. In addition, the Markey center for structural biology at Purdue University boasts a large number of prominent researchers and a highly collaborative environment for a high level of support. In this environment, I am confident that I will be able to take my academic career to the next level by learning effective protein expression and purification for biochemistry experiments, cryogenic electron microscopy, X-ray crystallography, and electron-paramagnetic resonance, all while uncovering mysteries of BAM-meditated OMP assembly, such as how BAM recognizes substrates, and the molecular details of substrate binding and conformational changes in BAM.

B. Positions and Honors

Positions and Employment

2008 - 2012 Undergraduate Researcher, Department of Physics, University of Michigan, Ann Arbor, MI
2012 - 2014 Graduate Teaching Assistant, School of Physics, Georgia Institute of Technology, Atlanta, GA
2014 - 2018 Graduate Research Assistant, School of Physics, Georgia Institute of Technology, Atlanta, GA
2018 - 2019 Postdoctoral Researcher, School of Physics, Georgia Institute of Technology, Atlanta, GA

2019 - Postdoctoral Research Associate, Department of Biological Sciences, Purdue University, West Lafayette, IN

Other Experience and Professional Memberships

2014 - 2018 Member, Biophysical Society

Honors

2013 - 2015 GAANN Award: Molecular Biophysics Training Program, Graduate Assistantship in Areas of National Need

C. Contribution to Science

1. Gram-negative bacteria, mitochondria, and chloroplasts possess two membranes. The outer of these membranes is host to almost exclusively ß-barrel transmembrane proteins also known as outer-membrane proteins (OMPs). Two elements necessary for the effective assembly of OMPs are the ß-barrel assembly machinery (BAM) and the translocation and assembly module (TAM). The central and essential members of both machineries consist of a 16-stranded ß-barrel domain of the Omp85 protein family. The structure of the ß-barrel domain of both BamA and TamA were released in 2013, and perhaps the most notable feature of both structures was a ß-barrel seam (interface between ß1 and ß16) held together by relatively few hydrogen bonds. Furthermore, in BamA, molecular dynamics simulations indicated spontaneous opening of the seam, and locking the seam together via double cysteine mutagenesis was lethal to the cell. These results placed the ß-barrel seam at the center of models describing OMP assembly. Lundquist et al, 2018 describes a detailed analysis of over 10 μs of equilibrium simulations including the extent to which gating occurs in a variety of membrane environments including bacterial OM as well as analysis of the extent to which BamA deforms its local membrane environment. Alongside the equilibrium simulations, extensive free-energy calculations of lateral gating in BamA were performed along with mean-first passage-time calculations which are able to accurately connect our observations in equilibrium simulations to the free-energy calculations. These results indicate that the formation of a C-terminal kink is a prerequisite to the formation of a lateral gate in BamA. Furthermore, mutations of the glycine residue which acts as a hinge residue in our simulations resulted in antibiotic susceptibility and OMP folding defects. Bamert et al, 2017 shows evidence that peptides from the fimbrial usher protein FimD selectively interact with TamA along with molecular dynamics simulations establishing the plausibility of these interactions as well as free-energy calculations which indicate that lateral gating occurs and is likely important for TamA as well. These findings have contributed to the understanding of OMP assembly and will likely be useful for the design of novel antibiotics targeting this process. I carried out all simulations, and analysis for the cited work, and generated the manuscript for Lundquist et al 2018.

    a. Lundquist K, Bakelar J, Noinaj N, Gumbart JC. C-terminal kink formation is required for lateral gating in BamA. Proc Natl Acad Sci U S A. 2018 Aug 21;115(34):E7942-E7949. PubMed PMID: 30087180; PubMed Central PMCID: PMC6112699.

    b. Bamert RS, Lundquist K, Hwang H, Webb CT, Shiota T, Stubenrauch CJ, Belousoff MJ, Goode RJA, Schittenhelm RB, Zimmerman R, Jung M, Gumbart JC, Lithgow T. Structural basis for substrate selection by the translocation and assembly module of the β-barrel assembly machinery. Mol Microbiol. 2017 Oct;106(1):142-156. PubMed PMID: 28752534; PubMed Central PMCID: PMC5607099.

2. Gram-negative bacteria possess two membranes. While the inner membrane is a symmetric phospholipid membrane, the outer membrane is asymmetric with a phospholipid inner leaflet and an outer leaflet that consists of large lipopolysaccharide (LPS) molecules and protects the bacterium from harsh environments and toxic agents. The machinery responsible for delivering LPS from the inner membrane to the outer membrane is called the lipopolysaccharide transport (LPT) machinery. It is composed of seven unique protein elements, of which LptB2FG is an ABC transporter in the inner membrane which thrusts LPS into a ß-jellyroll hydrophobic track. This track, formed by LptC, LptA, and the N-terminal domain of LptD, stretches from the inner membrane to the outer membrane to shuttle LPS to the outer membrane. At the outer membrane, LptE forms the plug domain of the 26-stranded ß-barrel of LptD. It is suggested that in order to insert LPS from the hydrophobic track intro the outer membrane, a separation between the N- and C-terminal strands, or a lateral gate, forms allowing LPS to pass. Performing double cysteine mutation at

adjacent residues in the lateral gate causes death in E. coli suggesting an important role played by lateral gating in LPS insertion. Botos et al, 2016 debuted four unique structures of LptDE from novel bacterial species. This manuscript also demonstrated that three proline residues adjacent to the LptD gating region act to destabilize interactions at the LptD ß-barrel seam. This was shown by demonstrating that mutation of these proline residues to alanine resulted in severe phenotypes and molecular dynamics simulations which indicated that the same mutations result in an increase in the strength of hydrogen bonds and additional secondary structure formation at the ß-barrel seam. Lundquist et al, 2020 provides a more detailed analysis of dynamics in LptDE highlighting several conformational changes which may be relevant to LPS insertion including that the N-terminal domain can stably accommodate LPS and that its presence acts to induce LptD lateral gating. These findings help to clarify the LPT delivery model for LPS. I performed all simulations and their analysis for both publications and wrote the manuscript and generated all figures for Lundquist et al, 2020.

a. Lundquist KP, Gumbart JC. Presence of substrate aids lateral gate separation in LptD. Biochim Biophys Acta Biomembr. 2020 Jan 1;1862(1):183025. PubMed PMID: 31351059; PubMed Central PMCID: PMC6899170.
b. Botos I, Majdalani N, Mayclin SJ, McCarthy JG, Lundquist K, Wojtowicz D, Barnard TJ, Gumbart JC, Buchanan SK. Structural and Functional Characterization of the LPS Transporter LptDE from Gram-Negative Pathogens. Structure. 2016 Jun 7;24(6):965-976. PubMed PMID: 27161977; PubMed Central PMCID: PMC4899211.

A complete list of publications can be viewed on either my NCBI bibliography or Google Scholar profile:
https://www.ncbi.nlm.nih.gov/myncbi/karl.lundquist.1/bibliography/public/
https://scholar.google.com/citations?user=3HhHtg0AAAAJ&hl=en

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

Scholastic Performance

YEAR	COURSE TITLE	GRADE
	UNIVERSITY OF MICHIGAN	
	GEORGIA INSTITUTE OF TECHNOLOGY	
2012	Classical Mechanics I	B
2012	Electromagnetism I	B
2012	Quantum Mechanics I	B
2013	Electromagnetism II	B
2013	Quantum Mechanics II	B
2013	Statistical Mechanics I	B
2013	Special Problems	A
2014	Biophysical Chemistry	A
2014	Macromolecular Structure	B
	GEORGIA INSTITUTE OF TECHNOLOGY	
2016	Special Topics Biophysics	B
2017	Computational Chemistry	B
2017	Special Topics Soft Matter	B
2017	Data & Visual Analytics	A

Graduate courses at Georgia Institute of Technology required a "C" grade to pass. No graduate scientific courses were taken while attending University of Michigan.