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: 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. **My lab focuses on studying 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.

Gram-negative bacteria, mitochondria, and chloroplasts contain an inner and outer membrane. 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. The structures of BamB, BamC, BamD, BamE and a large portion of the periplasmic domain of BamA were reported, providing some initial insight into how the BAM complex may function, but lacking any specifics. In my postdoc, I solved the structure of BamA and BamB, which provided the first evidence that BamA may function by a lateral gate, much like what has been proposed with the α-helical Sec translocon for the biogenesis of α-helical membrane proteins. And since being here at Purdue, my lab has solved the structure of the fully assembled BAM complex from *E. coli*, which has revealed unprecedented conformational changes with the BamA protein. Together, our studies suggest that BamB-E may regulate the conformational plasticity of BamA in mediating OMP biogenesis. We are also currently pursing targeting BAM for the development of novel antibiotics.

Another major focus of my lab is the lactoferrin binding protein (Lbp) complex in Neisseria, consisting of two receptors called LbpA and LbpB, both virulence factors mediating pathogenesis in Neisseria. As a postdoc, I determined the first structures of the related proteins TbpA and TbpB from *N. meningitidis*, both in complex with human transferrin. 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. We are able to express and purify both LbpA and LbpB, are able to make complexes of both with lactoferrin and currently characterizing these complexes using structural methods. Additionally, we are specifically investigating the role of LbpB as an antimicrobial peptide sink, adding further protection to Neisseria.

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

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

Honors

2018 Excellence in Research Award, College of Science, Biological Sciences, Purdue University
2017 College of Science Team Award, Biological Sciences, 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

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)
2011 American Crystallographic Association Annual Meeting
2011 NIDDK Fellows' Scientific Retreat
2011 Membrane Protein Special Interest Group Symposium
2010 Gordon Conference: Protein Transport Across Cell Membranes

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.

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.

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 MyBibliography:
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

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

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)

Completed Support

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

Purdue Univ. Award (Barker) 05/01/15 - 12/31/16
Structural dynamics of psychostimulants at dopamine transporters
To study the dynamics of dopamine transporters upon ligand recognition and signal transduction. Role: co-PI

Purdue U. (Noinaj) 05/01/15 - 12/31/16
Bringing Crystallography Back into Focus with a Suite of State-of-the-Art Microscopes
Internal Purdue Univ. equipment grant for the purchase of department shared microscopes for the crystallization core facility. Role: PI