

**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. **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.

A major focus of my lab are 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*. As a postdoc, I determined the first structures of the related proteins TbpA and TbpB from *N. meningitidis*, both in complex with human transferrin. However, many questions remain unanswered mechanistically here, which we hope studying the Lbp system might also answer. 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*.

Another major focus of my lab has been in 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. 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  $\alpha$ -helical Sec translocon for the biogenesis of  $\alpha$ -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 currently pursuing targeting BAM for the development of novel antibiotics.

## **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**

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 (45 <sup>th</sup> ) 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	11 <sup>th</sup> 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 (<sup>1</sup>co-first author, \*corresponding author)

- 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.
  - Noiraj N**, Easley N, Oke M, Mizuno N, Gumbart JC, Boura E, Steere A, Zak O, Aisen P, Tajkhorshid E, Evans RW, Goringe AR, Mason AB, Steven AC, and Buchanan SK. (2012). Structural basis for iron piracy in pathogenic *Neisseria*, *Nature* (Research Article), 483 (7387):53-8.
  - Noiraj N**, Cornelissen CN, Buchanan SK. (2013). Structural insight into the lactoferrin receptors from pathogenic *Neisseria*. *J Struct Biol*. 2013 184(1):83-92.
  - Noiraj N**, Buchanan SK, and Cornelissen CN, (2012). The transferrin–iron import system from pathogenic *Neisseria* species. *Mol Micro*, 86 (2):246-257.
  - Yadav R, **Noiraj 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.
- 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 GPCR 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.
  - White JF, **Noiraj 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.
- 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 *Haemophilus 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  $\beta$ -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  $\beta$ -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  $\beta$ -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<sup>\*</sup>, **Noinaj N**<sup>\*</sup>, 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](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  $\beta$ -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 NgBAM 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

