Structural basis and inhibition of outer membrane protein biogenesis in pathogenic *Neisseria*

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Abstract:
Neisseria gonorrhoeae (Ngo), is an obligate human pathogen and the causative agent of the disease gonorrhea. If left untreated, gonorrhea can lead to serious health issues including ectopic pregnancy and infertility. In addition to unsuccessful vaccine development, Ngo has rapidly become resistant to almost all classes of antibiotics, which contribute to over 500,000 drug resistant cases in year in the U.S. Due to this widespread resistance, the current recommended treatment option has become limited to a single drug of last resort: ceftriaxone. However, resistant strains have now emerged and the disease will likely become untreatable in the near future. This has created a need for developing novel antibiotics and vaccines to fight this disease. Like many bacterial pathogens, Ngo is Gram-negative. The outer membrane of this diderm bacteria contains unique β-barrel outer membrane proteins (OMPs); the biogenesis of which is mediated by a multi-component protein complex: the β-barrel assembly machinery (BAM) complex. Conserved across all Gram-negative bacteria, BAM is required for viability and a powerful potential therapeutic target. In *E. coli*, this complex is comprised of five proteins: an OMP, BamA, and four lipoproteins, BamB through E. However, *Neisseria* do not possess a BamB homolog, raising questions as to how it may differ from *E. coli*. To gain insight into how *Ng*BAM may function as a four component complex, we determined its structure using cryo-EM to high resolution. Our structure revealed surprising features of the component proteins distinct from *E. coli*, particularly in the conformations of BamA and BamC. We are now focusing on characterizing a novel antibiotic that targets BamA, against *Ng*BAM both *in vivo* and *in vitro*. Using a combination of structural studies and DEER spectroscopy, we have characterized the binding site of the inhibitor on *Ng*BamA and described conformational changes that occur upon inhibitor binding. Further work to measure the binding affinity and gain higher resolution information is ongoing. This work will lay the foundation for characterizing BAM for therapeutic development against gonorrhea.

Support or Funding Information:
E.B. is supported by a fellowship through the T32 Molecular Biophysics Training Program (GM132024) and a pre-doctoral fellowship through the American Heart Association (Award# 909066). E.B. and N.N. are supported through R01 GM127884 through the NIGMS. Some of this work was performed at the National Center for CryoEM Access and Training (NCCAT) and the Simons Electron Microscopy Center located at the New York Structural Biology Center, supported by the NIH Common Fund Transformative High Resolution Cryo-Electron Microscopy program (U24 GM129539), and by grants from the Simons Foundation (SF349247) and NY State Assembly.