GBG Ph.D. DEFENSE

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Johnson Cancer Research Center
Conference Room
36 Chalmers Hall, 10:00 a.m.
Refreshments available

TonB-dependent Transport of Ferric Enterobactin through FepA in Gram-negative Bacteria

Siderophore uptake systems are one the most prominent methods of Fe^{3+}-iron acquisition in Gram negative bacteria. The catecholate siderophore enterobactin is synthesized and utilized by many members of *Enterobacteriaceae* as well as several of the ESKAPE pathogens. The outer membrane (OM) transporter of ferric enterobactin (FeEnt), FepA is a ligand-gated porin (LGP) that requires interaction with the inner membrane (IM) protein TonB in order to accomplish active transport. TonB is thought to transduce the electrochemical energy created by the proton gradient across the IM to LGPs like FepA in the OM, to promote siderophore transport through their occluded channels. However, we do not yet have a clear picture of either how TonB transfers energy to FepA, or what kind of conformational changes occur in the occluding domain of FepA to allow ligand passage. The experiments described herein investigate these two questions, building on previously outlined models and observations. Using fluorescence labeling of strategically substituted cysteines in the surface loops of FepA, we unraveled a hierarchy of loop motion during binding of FeEnt to FepA. Additionally, by rendering parts of the FepA protein immobile as a result of engineered disulfide bonds, I identified residues or regions within its occluding domain that may normally unfold to open a size-specific channel for FeEnt. I also elucidated the role of the peptidoglycan polymer beneath the OM a framework for protein-protein interactions between IM and OM proteins. This includes the proposed interaction between a rotating TonB and FepA, or other LGPs, that may transfer kinetic energy to the OM transporter.

The role of iron in microbial survival and pathogenesis makes iron-uptake pathways an attractive target for therapeutic intervention. Using the FeEnt-FepA uptake system as a model, we used a fluorescence based high-throughput screening method to identify novel small molecule inhibitors of TonB action in *E. coli*. The approach used can be potentially adopted to screen bigger chemical libraries as well as used to find inhibitors of ESKAPE pathogens that use FeEnt such as, *Acinetobacter baumannii*, *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*. Finally, we discoverd a TonB-dependent OM transporter of heme/hemoglobin called HutA in the oligotrophic bacterium *Caulobacter crescentus*.

Major Professor: Dr. Phillip Klebba