Structural Basis of Cell-Surface Signaling by the Sigma-Regulator PupR in *Pseudomonas putida*.

Christopher L. Colbert[1], Jaime L. Jensen[1], and Sangita C. Sinha[1]

[1]Department of Chemistry and Biochemistry, North Dakota State University, Fargo, ND 58108

Cell-surface signaling (CSS) pathways in Gram-negative bacteria that allow the cell to respond to extracellular stimuli are highly conserved. CSS pathways usually involve three distinct proteins: 1) an outer membrane (OM) receptor responsible for sensing the stimuli, 2) an inner membrane (IM) protein sigma-regulator that transduces the signal from the periplasm to the cytoplasm, and 3) a cytosolic extracytoplasmic function sigma-factor, which regulates transcription of stimulus response genes. Our model system is the *Pseudomonas putida* WCS358 pseudobactin BN7/BN8 Fe^{3+}-signaling axis which comprises: 1) the OM TonB dependent transporter, PupB that binds and transports Fe^{3+}-laden siderophore 2) the IM sigma-regulator, PupR, and 3) the sigma-factor, PupI. Signaling across the periplasm is mediated by the interaction between the PupB N-terminal signaling domain and the PupR C-terminal CSS domain (CCSSD). Here we report the PupR CCSSD : PupB NTSD complex to 1.6 Å resolution. The structure of the complex reveals that the CCSSD consists of two subdomains: the C-terminal juxtamembrane domain (CJ), comprising residues 110 – 250 that has a novel beta-fold, and a Secretin and TonB N terminus short domain (STN), comprising residues 251 – 325. The CCSSD binds the PupB NTSD with micromolar affinity (K_d = 1.4 micromolar). Further, our biochemical analysis of the CCSSD indicates it is metastable when it is not in complex with the NTSD. Thus, it is likely that the complex serves to prime the CSS mechanism prior to binding of Fe^{3+}-laden siderophore. Upon siderophore-binding, a conformational change in the CCSSD enables signal transduction via PupR, ultimately resulting in transcriptional regulation.