Structural Insight Into a Heme Relay from Human Hemoglobin to SbnI, a Regulator of Siderophore Biosynthesis in *S. aureus*.

Anson C.K. Chan, Catherine F.M. Bowden, Meghan M. Verstraete, and Michael E.P. Murphy

Department of Microbiology and Immunology, Life Sciences Institute, The University of British Columbia, Vancouver, BC, Canada

The Isd cluster of *Staphylococcus aureus* encodes seven genes to enable growth on heme or hemoglobin (Hb) as a sole iron source. We have determined a crystal structure of a complex between Hb and IsdB, the *S. aureus* surface receptor that extract heme from Hb. In this complex, the α subunits of Hb are refolded with the heme displaced to the interface with IsdB. We also observe that atypical residues of Hb, His58 and His89 of αHb, coordinate to the heme iron, which is poised for transfer into the heme-binding pocket of IsdB. A Y440F/Y444F IsdB variant we produced was defective in heme transfer, yet formed a stable complex with Hb ($K_d = 6 \pm 2$ μM) in solution with spectroscopic features of the bis-His species observed in the crystal structure. The Isd heme uptake system terminates with heme bound to the cytosolic protein IsdG. SbnI is a heme-dependent regulator of the expression of the genes encoding for the biosynthesis of the siderophore staphyloferrin B (SB). We show by heme transfer kinetics that IsdG can actively transfer heme to SbnI. Determination of the crystal structure of SbnI revealed homology to a family of free serine kinases that produce O-phosophoserine, a precursor of SB. Biochemical studies show SbnI is an ATP dependent free serine kinase. Inspection of the structure and docking experiments, suggest heme binds at a site near the C-terminus distinct from the kinase active site and this model is supported by site-directed mutagenesis. Together, our data supports a model of a heme relay from Hb through to SbnI to repress the production of the SB providing a mechanism for the preference of heme as an iron source by *S. aureus*.