Molecular basis of bacterial heme thievery

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Gram-negative pathogenic bacteria require iron for use in metabolic enzymes, and have various means of obtaining it from their hosts. In the common human pathogen Pseudomonas aeruginosa, one major pathway involves theft of heme from hemoglobin, followed by oxidation of the porphyrin ring to release the central iron atom. This heme acquisition system includes a heme-binding protein (or hemophore) referred to as HasA. HasA binds heme extremely tightly, but readily releases it to a receptor on the bacterial cell surface (HasR). HasA in Pseudomonas aeruginosa has two amino acids that serve as ligands to heme iron, His-32 and Tyr-75 (Figure A). The Tyr ligand environment is virtually identical in the heme-bound and heme-free (apo) forms of the protein. In contrast, the His ligand resides in a region of the polypeptide that is quite flexible when heme is absent. Current understanding of the heme-binding mechanism is that the Tyr ligand and surrounding residues create a well-ordered and sticky hydrophobic "platform" for the heme (Figure B). Docking of heme on this platform triggers relatively slow closure of the loop containing the His ligand, and ultimate formation of the His32-Fe bond. While Tyr-75 is an invariant residue in HasA proteins, His-32 is not.

Recent studies in the Rivera group with HasA from P. aeruginosa (referred to as HasAp) have shown that replacing His-32 with alanine only modestly decreases heme affinity, but sets up an equilibrium between a monomeric and a dimeric form of the heme-bound protein. In the dimeric form the bound heme molecules are stacked on top of one another. This suggests the possibility that naturally occurring HasA variants lacking His-32, such as the protein from Yersinia pestis (the organism responsible for the bubonic plague), may utilize dimer formation in the process of heme acquisition. An REU student working on this collaborative project in the Rivera and Benson laboratories would investigate this possibility. The participant would gain experience in protein expression and purification, site-directed mutagenesis, kinetic assays, X-ray crystal structure analysis, and various forms of spectroscopy including NMR and EPR.

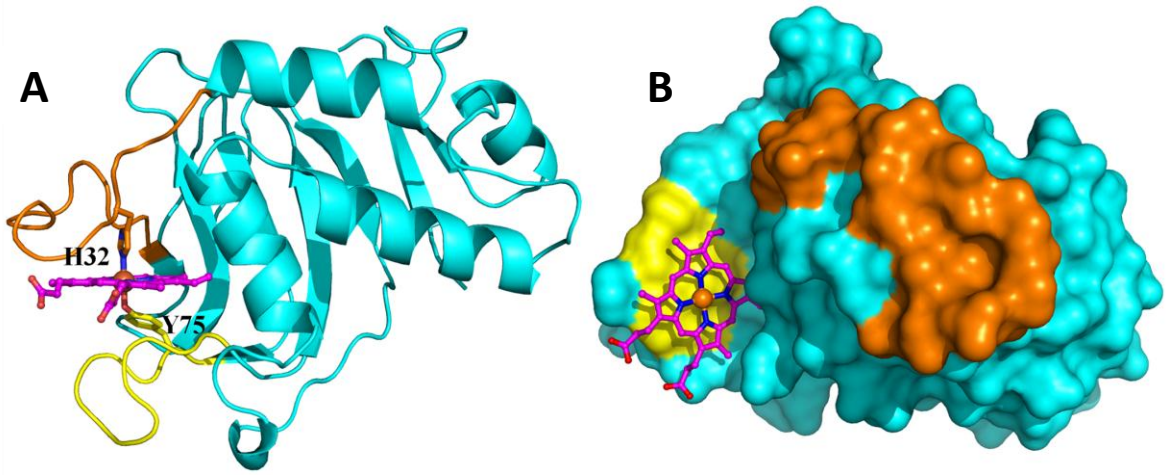

Figure (A) Holo-HasAp (PDB: 3ELL) showing secondary structure in cyan; extended loops that coordinate heme iron are shown in orange and yellow. Residues H32 and Y75 involved in coordination of the heme are shown in stick representation **(B)** Surface representation of subunit A of H32A Holo-HasAp (PDB: 3MOL) showing H32 loop in orange, Y75 loop in yellow and associated heme in magenta