

Biliverdin An Emerging Signaling Molecule in the Regulation of Extracellular Heme Uptake and Virulence in *Pseudomonas aeruginosa*.

Angela Wilks, Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore

The opportunistic pathogen *P. aeruginosa* encodes two extracellular heme uptake systems, the *Pseudomonas* heme uptake (*phu*) and the heme assimilation systems (*has*). (1) Consistent with recent studies indicating PhuR as the primary heme receptor in clinical infection, our laboratory has shown Phu system is the major heme transporter with the hemophore dependent Has system acting primarily in heme sensing. (2) ¹³C-heme isotopic labeling and bacterial genetics combined with biochemical and biophysical studies further show the intracellular heme chaperone PhuS is critical in linking heme flux through the biliverdin IX β and IX δ regioselective HemO to post-transcriptional regulation of heme utilization. (3, 4) Complementation of the Δ *hemO* deletion strain with a BVIX- α selective (*hemO α*) or a catalytically inactive (*hemOin*) *hemO* mutant down regulates expression of the hemophore HasA and its cognate receptor HasR. Mechanistic aspects the PhuS-HemO coupled regulation of heme flux have been studied by in vitro and in vivo methods employing hydrogen deuterium exchange (HDX) mass spectrometry techniques combined with resonance Raman spectroscopy. Furthermore, we present the first evidence for BVIX β -dependent post-transcriptional regulation of the ECF sigma-factor heme dependent cell surface signaling system. Coupling the regioselective metabolic flux of heme through PhuS-HemO with the extracellular heme sensing and regulatory network, provides a novel mechanism for the cell to rapidly respond and adapt to changes in extracellular heme and iron availability. (5)

1. Ochsner UA, Vasil ML. 1996. Gene repression by the ferric uptake regulator in *Pseudomonas aeruginosa*: cycle selection of iron-regulated genes. *Proc Natl Acad Sci U S A* 93: 4409-14
2. Smith AD, Wilks A. 2015. Differential contributions of the outer membrane receptors PhuR and HasR to heme acquisition in *Pseudomonas aeruginosa*. *J Biol Chem* 290: 7756-66
3. O'Neill MJ, Bhakta MN, Fleming KG, Wilks A. 2012. Induced fit on heme binding to the *Pseudomonas aeruginosa* cytoplasmic protein (PhuS) drives interaction with heme oxygenase (HemO). *Proc Natl Acad Sci U S A* 109: 5639-44
4. O'Neill MJ, Wilks A. 2013. The *P. aeruginosa* heme binding protein PhuS is a heme oxygenase titratable regulator of heme uptake. *ACS Chem Biol* 8: 1794-802
5. Mourino S, Giardina BJ, Reyes-Caballero H, Wilks A. 2016. Metabolite-driven Regulation of Heme Uptake by the Biliverdin IX β /delta-Selective Heme Oxygenase (HemO) of *Pseudomonas aeruginosa*. *J Biol Chem* 291: 20503-15