Exploring a possible moonlighting role for global phosphatase in *S. pneumonia*

Grossoehme N.E.[1], *Sellers, H.S.[2]

[1]Associate Professor, Department of Chemistry, Winthrop University; [2]Research Assistant, Department of Chemistry, Winthrop University

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Abstract: Iron is essential to an overwhelming majority of life on Earth; however, in aerobic conditions it can take on multiple oxidation states and create harmful oxidative species that must be regulated to maintain the health of the cell. Many bacteria use a one of the common metal regulatory proteins (e.g. FUR) to maintain safe levels of iron in the cell, but genome analysis of *S. pneumonia* indicates that it lacks any of the standard sensors. Interestingly, the presence of extracellular iron triggers an intracellular uptake response; this process involves three proteins: StkP (membranous kinase), RitR (transcription factor), and PhpP (phosphatase). It is likely that the intracellular iron sensor is linked to this uptake system; in fact, we hypothesize that the intracellular sensor is built directly into this system. Noting that PhpP is a magnesium dependent enzyme, we hypothesize that perhaps PhpP is activated by intracellular iron in *S. pneumonia*, thus providing the intracellular iron sensor that it needs. Using a combination of UV-Vis and fluorescence spectroscopy, we tested this hypothesis. Using para-nitrophenylphosphate assays (PNPP, a surrogate for phosphorylated RitR) along with manganese as an aerobic condition friendly surrogate we demonstrated that PhpP is activated by manganese. Using fluorescence competition experiments with the metal binding fluorophore Mag-Fura-2, we quantified the affinity PhpP for manganese (Kd = 2.16 μM) and magnesium (Kd = 185.1 μM). Together, these results support the hypothesis. Future work will focus on testing ferrous iron activation of PhpP.