TITLE: Axial Ligands of the Heme Uptake Protein HmuT from *Corynebacterium diphtheriae*

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Introduction: The causative organism of diphtheria in humans*, Corynebacterium diphtheriae*, requires iron to live; the heme binding protein HmuT plays a major role in this transport. Sequence alignment indicates three potential axial ligands: H136, M292, and Y235. Homology modeling using I-TASSER confirms that these residues are indeed near the proposed heme pocket. Work in our laboratory has shown that some of these mutants are isolated with protoporphyrin as well as heme. Current work involves expression of the M292A and Y235A mutants in an effort to isolate protein with only hemin at the binding site.

Method: The possible heme axial ligands were determined sequence alignments with other heme binding proteins using ClustalW. We expressed the proteins under various conditions. Purification used fast protein liquid chromatography (FPLC). UV/visible and magnetic circular dichroism spectral signatures were compared to establish the axial ligands. Heme extraction and reconstitution were performed to increase the amount of heme bound protein in the expressed protein.

Results: The spectra of Y235A are consistent with a histidine-ligated heme. The spectra of M292A are very similar to wild type, indicating that methionine is not an axial ligand. Hemin incorporation is a more lengthy process than expected.

Conclusion: H136 and Y235 are the axial ligands of HmuT. Tyrosine is not a common axial ligand in heme proteins, but is found in a number of proteins in heme uptake pathways. The reasons for Nature's choice of this axial ligand are explored.