Factors Controlling Absolute Fe(III) and Fe(II) Heme Affinity and Electrochemistry in Natural and De Novo Designed Heme Proteins.

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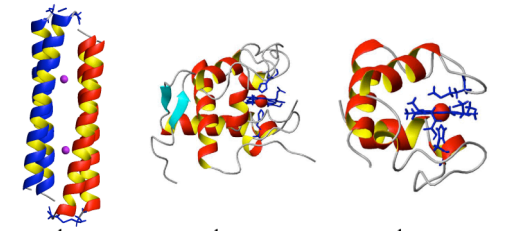

cytochrome maquette | cytochrome c_{549} | cytochrome c_{553}

Our approach to the study of metalloproteins is to engineer and fabricate peptide structures that incorporate metal cofactors toward the goal of generating molecular *maquettes*, protein-based synthetic analogues We have analyzed natural heme proteins in the PDB to design synthetic heme protein maquettes for the investigation of the fundamental design principles of natural heme proteins involved in electron transfer reactions (the cytochromes) and dioxygen transport (the globins). Using cytochrome maquette and *c*-type cytochrome scaffolds shown above, we have delineated the environmental factors which alter the heme reduction potential, a fundamental chemical property of natural cytochromes. The type of porphyrin macrocycle, the local electrostatic environment, the burial of the heme and the influence of pH all contribute to the modulation of the heme reduction potential. In designed heme proteins, we can modulate the heme reduction potential by 435 mV - *nearly half the range observed for natural heme proteins*. Evaluation of the Fe(III) and Fe(III) heme binding constants and the resultant electrochemistry provides insight into the absolute (de)stabilization of these states by the protein environment. We have expanded the repertoire of ligands available for heme protein design by using nonnatural amino acids containing pyridine, triazole and tetrazole sidechains. Altering the axial ligands leads to significant changes in heme spectroscopy (electron paramagnetic resonance, magnetic circular dichroism, resonance Raman), reduction potential (+286 mV) and Fe(III) and Fe(II) stability constants (150,000-fold). Additionally, the use of 1-methyl-L-histidine as an axial heme ligand provides a spectroscopic model for deoxymyoglobin.