Unnatural Amino Acids and Nonnative Metal Cofactors in Metalloprotein Design and Engineering

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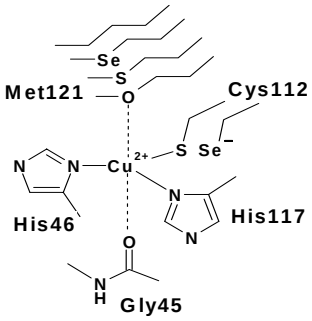
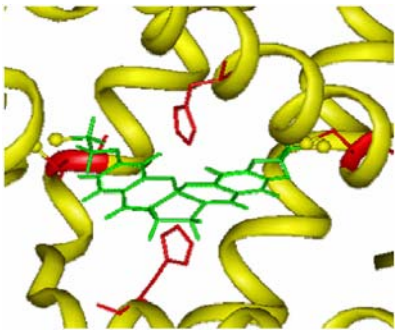
Incorporation of unnatural amino acids or nonnative cofactors into metalloproteins can provide structural and electronic structural information about the native system at an unprecedented level of detail or endow the protein host with new or enhanced reactivity. Toward these goals, express protein ligation was used to isostructurally replace copper ligands in the blue copper protein azurin with unnatural amino acids providing electronic perturbation without major structural perturbation of the metal center. Replacement of equatorial cysteine with selenocysteine dramatically modifies the UV-Vis and EPR spectra but not the reduction potential (1). However, axial methionine replacements show nearly wild type spectroscopy with >200mV variations in reduction potentials. The reduction potential is observed to correlate linearly with the hydrophobicity of the axial ligand (2). Implementation of metalloprotein components not found in nature can be extended beyond amino acids to include nonnative cofactors. Covalent dual anchoring of a manganese salen complex inside the heme pocket of myoglobin has produced a novel enantioselective oxidation catalyst with >50% enantioselectivity (3). This same attachment strategy has been applied to anchoring a ferrocene complex inside of apo azurin resulting in a nearly 400mV shift in the ferrocene reduction potential. Thus, production of semisyntetic metalloproteins may incorporate unnatural amino acids or nonnative cofactors for elucidation of native metal ligand function or for introduction of novel catalytic activity.





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