Design and Engineering of Heteronuclear Metal Binding Sites in Heme Proteins

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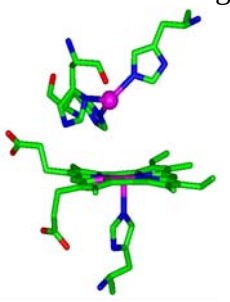
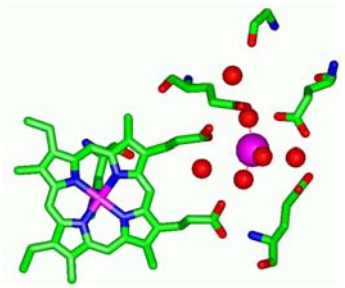
Heme proteins catalyze a wide range of reactions with numerous substrates. The type of reaction that is catalyzed is controlled by the environment around the heme center including presence of heteronuclear metal binding sites such as heme-manganese center in manganese peroxidase (MnP), heme-copper center in cytochrome oxidase (HCO), and heme-non-heme iron center in nitric oxide reductase (NOR). To gain insights into the structure and catalytic mechanism of these enzymes, we have been able to use stable, easy-to-produce, and well-characterized heme proteins (cytochrome *c* peroxidase (C*c*P) and myoglobin (Mb)) as scaffolds and introduced into them novel manganese, copper, and non-heme iron binding sites to mimic MnP,[1] HCO[2,3] and NOR. These biosynthetic model enzymes have been characterized by UV-vis, EPR, NMR, XAS, and Resonance Raman spectroscopy as well as by X-ray crystallography. This endeavor allowed us to compare the different metal-binding sites in the same protein framework and helps identify and study the role of key residues at the heteronuclear metal binding site. New insights have been obtained such as the role of metal ion, proton, and heme types in modulating structure and functions of these heteronuclear metal binding sites. These projects will be summarized, including discussion of the latest results.



Cu_BMb



MnCcP.1

(1) Pfister, T. D.; Gengenbach, A. J.; Syn, S.; Lu, Y. *Biochemistry* **2001**, *40*, 14942-14951.
(2) Sigman, J. A.; Kwok, B. C.; Lu, Y. *J. Am. Chem. Soc.* **2000**, *122*, 8192-8196.
(3) Sigman, J. A.; Kim, H. K.; Zhao, X.; Carey, J. R.; Lu, Y. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3629-3634.