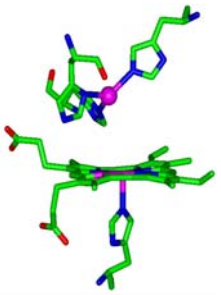
# Roles of Copper, Proton, Chlorides and Heme Types in Heme-coppper Oxidases: Kinetic and Electrochemical Studies of an Engineered Heme-Copper Center in Myoglobin

Xuan Zhao, Ningyan Wang, Natasha Yeung, and Yi Lu*

*Department of Chemistry, University of Illinois-Urbana/Champaign*

Heme-copper oxidases (HCOs) are large membrane-bound proteins that catalyze the proton-coupled reduction of $O_2$ to water. The active site of HCOs contains a high-spin heme-$Cu_B$ dinuclear site where $Cu_B$ is coordinated to three histidines with one of the histidines cross-linked to a tyrosine. Using a well-characterized sperm whale myoglobin as a template and through mutation of Leu29 and Phe43 to histines, a $Cu_B$ binding site has been created in the distal site of heme *b* in myoglobin ($Cu_B$Mb).[1] The kinetic studies on the reaction of $O_2$ with $Cu_B$Mb suggested $Cu_B$ center plays a critical role in $O_2$ binding and reduction, and that proton delivery during the $O_2$ reduction is important to avoid heme degradation and to promote the HCO reaction.[2] Further studies showed the replacement of heme *b* with a heme *o* mimic can significantly reduce the rate of heme degradation reaction. The electrochemical studies of $Cu_B$Mb showed the binding and the charges of metal ions in the designed $Cu_B$ site can have a significant effect on the heme redox potential only when the two metal centers are coupled. This study suggests that the redox-coupled proton transfer may be crucial to regulating the heme $a_3$ redox potential in HCOs.[3] In the presence of both Cu(II) and chloride, a low spin heme Fe(III)-Cu(I) intermediate was observed during the redox titration of $Cu_B$Mb, indicating that binding of chloride to the $Cu_B$ center can induce redox-dependent structural changes, and the bound chloride and hydroxide in the heme-copper center may play different roles in the redox-linked enzymatic reactions of heme-copper oxidases.[4] Latest results in further characterization of this protein model system will be presented and insight gained into the heme-copper center in HCOs will be discussed.



**$Cu_B$Mb**

(1) Sigman, J. A.; Kwok, B. C.; Lu, Y. *J. Am. Chem. Soc.* **2000**, *122*, 8192-8196. (2) Sigman, J. A.; Kim, H. K.; Zhao, X.; Carey, J. R.; Lu, Y. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3629-3634. (3) Zhao, X.; Yeung, N.; Wang, Z.; Guo, Z.; Lu, Y. *Biochemistry* **2005**, *44*, 1210-1214. (4) Zhao, X.; Nilges, M. J.; Lu, Y. *Biochemistry* **2005**, ASAP.