

Dr. Yi Lu  
University of Illinois at Urbana-Champaign

Thursday, February 28, 2008  
11:30 a.m.  
101 Biochemistry

## Designing a cytochrome c oxidase

### Research in the Lu Laboratory

Cytochrome c oxidase (CcO) is a terminal oxidase of the aerobic respiratory chain. It consists of several metal-binding sites, two of which play critical roles in its structure and function. They are the Cu<sub>A</sub> center and the heme-Cu<sub>B</sub> center. The Cu<sub>A</sub> center is a mixed-valence dinuclear [Cu<sub>2</sub>S<sub>2</sub>(Cys)] center (Fig. 1). It is the initial electron entry site to CcO and its function is electron transfer (ET). The heme-Cu<sub>B</sub> center is the site of O<sub>2</sub> reduction. It is a heteronuclear center consisting of a heme with a proximal histidine and a Cu(II) with three histidines (Fig. 2). In a complementary approach to studying native CcO and its variants, we are designing metalloproteins that contain Cu<sub>A</sub> or heme-Cu<sub>B</sub> centers. Using the designed proteins, we have elucidated structural features important for the structural and function of CcO, and provided unique insights into the reaction mechanisms.

**Design and Engineering of a Cu<sub>A</sub> Center in the Blue Copper Protein Azurin.** We have succeeded in engineering of the Cu<sub>A</sub> center into the blue copper protein azurin by using a technique called loop-directed mutagenesis (i.e., replacing a whole loop between two β-barrels with another loop).<sup>1</sup> Comprehensive spectroscopic characterizations<sup>2</sup> and a high-resolution (1.65 Å) X-ray structural study indicate that the Cu<sub>A</sub> center in azurin is identical to that in CcO.<sup>3</sup> This model protein provides a simple system for learning the principles of protein design<sup>4</sup> and for elucidating the structure and function of CcO, including possible “gate” in controlling proton-coupled electron transfer.<sup>5</sup> In addition, the engineering of the Cu<sub>A</sub> center into the same framework of the blue copper azurin allowed a direct comparison of the electron transfer properties of the two centers; electron transfer study of the two systems provided conclusive evidence that the dinuclear Cu<sub>A</sub> center is more efficient than the mononuclear blue copper center.<sup>6</sup>

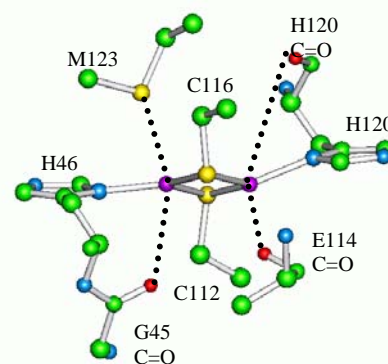


Figure 1. Cu<sub>A</sub> center.

### Design and creation of a Cu<sub>B</sub> center in myoglobin and cytochrome c peroxidase:

The Cu<sub>B</sub>-site has been designed into both cytochrome c peroxidase<sup>7</sup> and myoglobin<sup>8</sup> through a careful structural comparison between the template proteins and the target protein, computer modeling, and energy minimization. UV-vis and EPR studies of these model proteins indicate that the heme-copper center in both model proteins closely mimics that in CcO. Using the designed protein, we have defined the roles of the Cu<sub>B</sub> center,<sup>9</sup> chloride,<sup>10</sup> and heme type in the function of CcO, and demonstrated the importance of secondary coordination, specifically hydrogen bonding networks, in fine-tuning the functional properties of CcO, and showed histidine and tyrosine cross-linking in the model protein and its importance to function. Recent progress will be reported.

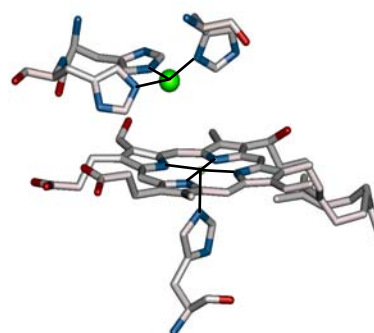


Figure 2. Heme-Cu<sub>B</sub> center.

### References

1. Hay, M.; Richards, J. H.; and Lu, Y. *Proc. Natl. Acad. Sci. U. S. A.* 93, 461-464 (1996);
2. Hay, M. T. et al. *Inorg. Chem.* 37, 191-198 (1998);
3. Robinson, H. et al. *Biochemistry* 38, 5677-5683 (1999);
4. Lu, Y.; Berry, S. M.; and Pfister, T.D. *Chem. Rev.* 2001, 101, 3047-3080;
5. Hee Jung Hwang and Yi Lu, *Proc. Natl. Acad. Sci. USA*, 100, 12842-12847 (2004);
6. Farver, O.; Lu, Y.; Ang, M. C.; and Pecht, I. *Proc. Natl. Acad. Sci. USA*. 96, 899-902 (1999);
7. Sigman, J. A.; Kwok, B. C.; Gengenbach, A.; and Lu, Y. *J. Am. Chem. Soc.* 121, 8949-8950 (1999);
8. Sigman, J. A.; Kwok, B. C.; and Lu, Y. *J. Am. Chem. Soc.* 122, 8192-8196 (2000);
9. Xuan Zhao, Natasha Yeung, Zhilin Wang and Yi Lu, *Biochemistry* 44, 1210-1214 (2005);
10. Xuan Zhao, Mark J. Nilges and Yi Lu, *Biochemistry* 44, 6559-6564 (2005);
11. Ningyan Wang, Xuan Zhao, and Yi Lu, *J. Am. Chem. Soc.* 127, 16541-16547 (2005);
12. Jeffrey A. Sigman, et al., *Proc. Natl. Acad. Sci. USA*, 100, 3629-3634 (2003).