From Bioinorganic Chemistry to Single Molecule Biophysics: O_2 Activation by Binuclear Cu Sites and Single Motor Tracking in Living Cells

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Part I. O_2 activating binuclear Cu proteins can be classified into coupled and noncoupled binuclear sites based on the Cu···Cu magnetic interaction. Coupled binuclear Cu proteins include hemocyanin, tyrosinase, and catechol oxidase, and have two Cu centers strongly magnetically coupled that provide a mechanism for the 2-electron reduction of O_2 to a well known μ-η^2:η^2 side-on peroxide bridged $Cu^{II}_2(O_2^{2-})$ species. This $Cu^{II}_2(O_2^{2-})$ species is activated for electrophilic attack on the phenolic ring of substrates. Noncoupled binuclear Cu proteins include peptidylglycine α-hydroxylating monooxygenase and dopamine β-monooxygenase, and have binuclear Cu active sites that are distant, that exhibit no exchange interaction, and that activate O_2 at a single Cu center to generate a reactive Cu^{II}/O_2 species for H-atom abstraction from the C–H bond of substrates. Possible intermediates in noncoupled binuclear Cu proteins can be defined through correlation to mononuclear Cu^{II}/O_2 model complexes. The different intermediates in these two classes of binuclear Cu proteins exhibit different reactivities that correlate with their different electronic structures and exchange coupling interactions between the binuclear Cu centers. These studies provide insight into the role of exchange coupling between the Cu centers in their reaction mechanisms. (Ph.D. work with Edward I. Solomon at Stanford University)

Part II. Much has been learned about motor proteins at the single molecule level through *in vitro* experiments. However, little has been done at the single molecule level *in vivo*. Here we report our recent effort in tracking single motor proteins in living cells through combination of quantum dots and high speed spinning disk confocal imaging. Individual steps associated with single enzymatic turnovers of dynein and kinesin motors moving along microtubules were visualized inside a living cell. While kinesin exhibits 8 nm steps, as expected, dynein shows velocity dependent step sizes that are integer numbers of 8 nm. Analysis of dynein's displacement trajectories, including infrequent backward steps and dwell time distributions, led to a model for the mechanism for dynein operation, and kinetic information for dynein ATP hydrolysis *in vivo*. (Postdoctoral work with X. Sunney Xie at Harvard University)