An article in *JACS*, “Formation and Electronic Structure of an Atypical CuA Site,” from the group of Amy Rosenzweig (*J. Am. Chem. Soc.* **2019**, *141*, 4678-4686; DOI: 10.1021/jacs.8b13610) investigated the structural and spectroscopic properties of the dicopper CuA site found in a new protein, PmoD, discovered in methane-oxidizing bacteria. This report provides a new configuration of CuA that expands the diversity of the known biological examples.

1. In a typical CuA site, the two copper centers are able to cycle from [2Cu]2+ to [2Cu]3+ and back again. How does this ability to change oxidation state contribute to the functionality of the protein where the CuA site is found?
2. Looking at Figure 1A, what is the ligation around each Cu center? How does this contrast to known CuA sites?
3. The optical spectrum for the PmoD CuA has two charge transfer (CT) bands at 475 and 530 nm and an intervalence charge transfer band (IVCT) at 770 nm (See Figure 3A for optical spectrum).
4. What are the two CT bands due to?
5. What is an intervalence charge transfer band?
6. What do the CT and IVCT bands tell you about the structure of this CuA?
7. Define isosbestic point. How does the presence of an isosbestic point contribute to the understanding of the formation of the CuA site in the PmoD bacteria? (See Figure 2A.)
8. Figure 2 shows a series of experiments where key amino acid residues necessary for the formation of the CuA site were varied. Compared to the wild type (WT), what did each variation show in regards to the formation of CuA?
9. The optical spectrum of the two Cu2+ mononuclear centers after the decay of the CuA site only has a broad d-d transition at 615 nm. It has no CT bands and is missing the band at 770 nm. (See Figure 3A, dark blue line.) What does this tell you about the ligation around the Cu2+ center?
10. The authors conclude that the PmoD protein remains as a dimer even after the CuA structure decays. What is holding the dimer together after this decay? Explain the series of experiments that allowed them to reach this conclusion.
11. What experimental evidence allows the authors to determine that this CuA does not populate the πu ground state? (See Figure 5)
12. The authors propose that some of the anomalous results obtained for the PmoD CuA site can be explained by the ligation of a methionine to both copper centers. How does this differ from typical CuA sites? How does this help to explain the spectroscopic properties observed in this paper?