Flavodiiron Oxygen Reductase

1. Write balanced chemical equations for the reactions catalyzed by flavodiiron proteins. (4 pts)

2. Using words and/or pictures, briefly describe the structure of the catalytic diiron center in this enzyme. (3 pts)

3. In 2-3 sentences, summarize the hypothesis that forms the basis for the work in this paper and a brief description of their approach to test the hypothesis. (5 pts)

4. What is the role of the flavin mononucleotide (FMN) in this enzyme? Where is it located relative to the catalytic diiron site and why is its location important? (3 pts)

5. Why did the authors choose Tyr^{271} and Lys^{53} as the residues to mutate in *E. histolytica*? What did they mutate them to, and why? (For full credit, support your answer with direct reference to information in the paper; *e.g.*, Fig. X shows….) (5 pts)

6. How would the mutations they made alter the structure and/or electronic environment of the diiron active site? (6 pts)

7. The author's stated that "[t]heWTEhFdp1 and single mutants contained ~2 Fe and 1 FMN per monomer…" How did they determine this? (6 pts)

8. What was the goal of the experiments whose results are shown in Figure 3? What did the authors conclude from the results? (3 pts)

9. The following questions related to the EPR studies of the diiron center.
   a. Give the oxidation states and d-electron counts of the iron atoms in the diiron center in **the full oxidized state, the mixed valence state, and the fully reduced state** (noting which is which). For which, if any, of these states would you expect to observe an EPR signal? Explain your answer. (6 pts)

   b. Why does the lack of a signal at g ~ 4.3 indicate that the iron sites are not partially occupied? (4 pts)

   c. The authors use the EPR redox titrations to determine two potentials, E1 = +170 mV and E2 = 132 mV. Explain what process these electron potentials correspond to. (4 pts)

   d. The authors use report a decrease in the reduction potentials (particularly the second reduction potential) in the mutants, with the largest effect in the double mutant. However, they note that "because the standard reduction potential for the reduction of NO to N_2O is +1,175 mV, it is unlikely that the observed negative shift will modulate the enzyme reactivity. Explain the reasoning behind this statement. (3 pts)

e. The authors claim that "…tyrosine substitution leads to a structural rearrangement in the diiron site vicinity." Explain what data led them to this conclusion. (3 pts)

10. How did the authors monitor O_2 and NO reactivity in the enzymes? (What technique did they use and what *specifically* did they monitor.) (3 pts)

11. Explain the results presented in Figure 6. What conclusions do the authors make based on the data? (3 pts)

12. What role(s) do the authors postulate for Tyr^{271} in the reactivity of the enzyme? What evidence do they have in support of the role(s)? (6 pts)

13. Overall, what did the researchers learn from these studies? (3 pts)

14. Suggest an experiments/research idea that could be a follow-up study to the work presented in this paper and explain why it would be useful/interesting. (6 pts)