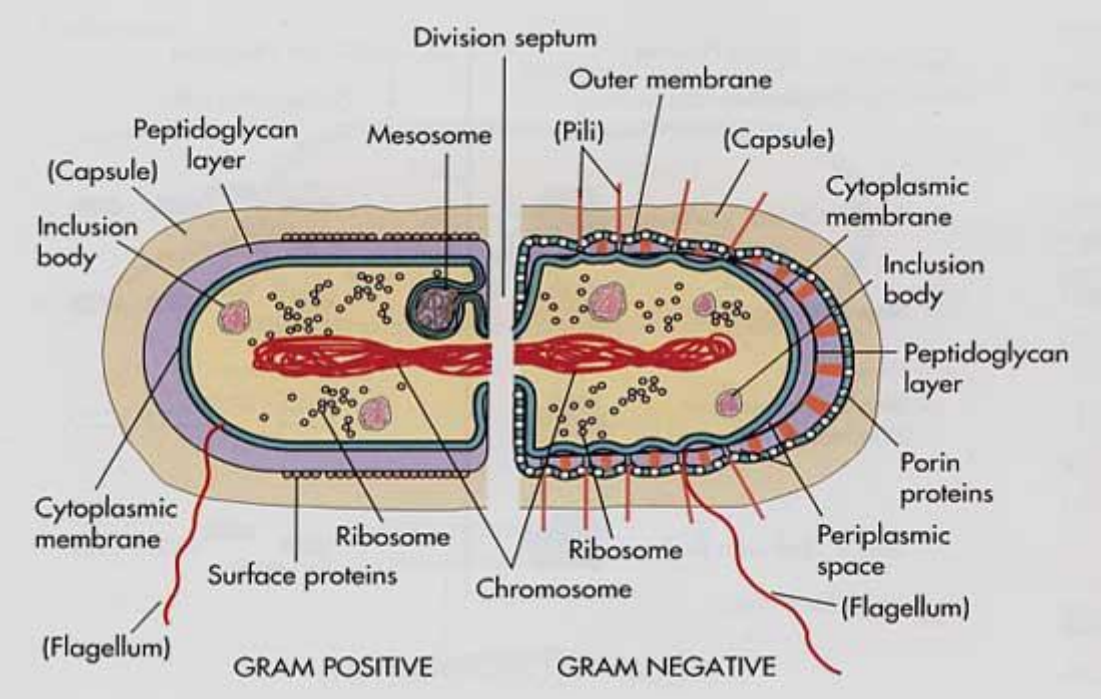
**Chemistry 5995(133)/8990(013)**
**Bioinorganic Chemistry: The Good, the Bad, and the Potential of Metals in Biology**
**Assignment 4**

Reading: Bertini Ch. 4, 5, Ch. 10 (Section 1), Ch. 13 (Sections 1-4 and Supplemental Reading on ribonucleotide reductase available on the website), and Ch. 14 (Sections 1-3).

For the following questions, please cite your references using the format previously described. Keep your answers as concise as possible.
* Indicates that you may have to use the primary literature to answer the question.

1. In class, we talked about how bacteria use siderophores to mobilize $Fe^{3+}$ and I gave an example of transport through a gram negative bacteria, in which a siderophore binds $Fe^{3+}$ in the extracellular space before transport through the outermembrane. How do gram positive bacteria mobilize $Fe^{3+}$ via siderophores?*




2. What are the bacterial ferric binding proteins and how are they similar to transferrin?*

3. Using the molecular orbital theory handouts that I gave you in class:

a. Why does $N_2$ have a higher number of bonds than $O_2$? (Note: The molecular orbital diagrams for these two homonuclear diatomic molecules will be different so please draw them correctly.)

b. Show how you might obtain singlet $O_2$.

c. Can two oxygens in the form of oxides form a bond? (Hint: Predict the bond order.)

4. In class, I showed you my proposed arrow pushing mechanism for the class I (and II) members of this family at the active site. Show me your proposed arrow pushing mechanism for the class III member and remember the one/two arrow head rule.

5. a. Why is $Ga^{3+}$ considered a biomimic of $Fe^{3+}$?*

b. Why do you think $Ga^{3+}$ would be redox inactive in biology whereas $Fe^{3+}$ is not?

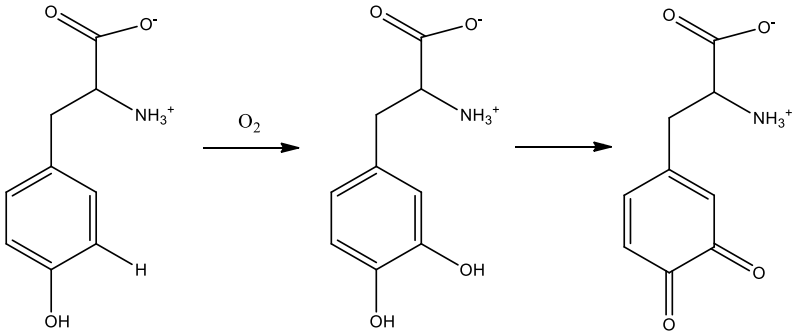
c. $Ga^{3+}$ can displace $Fe^{3+}$ from class I ribonucleotide reductase. Assuming a mechanism where both $Fe^{3+}$ ions must first dissociate before two $Ga^{3+}$ ions bind at the R2 site, why would $Ga^{3+}$ binding inhibit the enzyme?

6. What are zinc finger domains and what role do they play in DNA contacts?

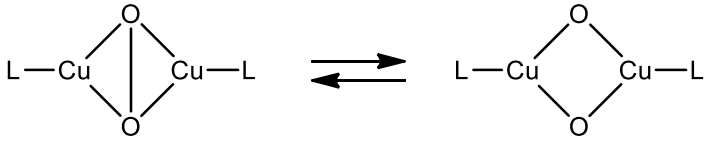
7. How does $Ca^{2+}$ interaction with calmodulin result in enyme activation?

8. Tyrosinase is a type of dinuclear copper monooxygenase.

a. Explain the reaction below that is catalyzed by tyrosinase and be thorough about identifying relevant changes in oxidation states.

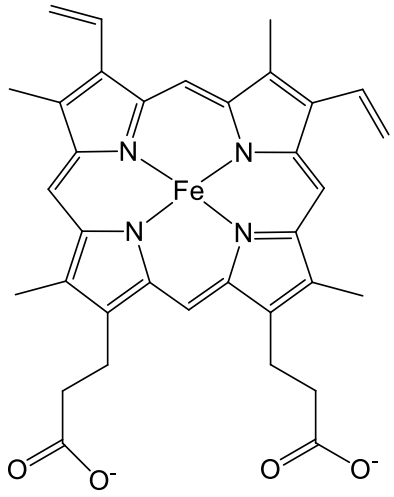



b. Small molecule structural mimics have been synthesized to determine the identity of the active form of tyrosinase. By comparing the spectroscopic signals for these mimics with those of the oxytyrosinase form of the enzyme, it is clear that molecular oxygen must coordinate to both copper ions but it is not clear whether O-O bond breaking must occur before transformation of the substrate. Using the incomplete figures below of small molecule mimics of the active oxytyrosinase form of the enzyme, identify the copper oxidation states in both structures and the coordination of the oxygen molecule (on the left side). Use molecular orbital theory to explain why the oxygens might lose their bond in the structure to the right.



c. The equilibrium shown for the two forms of the active dicopper center is an unrealistic representation of how the small molecule mimics may exist but it is drawn to represent the idea that the active form of the enzyme may be a mixture of the two forms. Using your explanation of molecular orbital theory and assuming L in the drawings is a chelating ligand, what electronic properties of the chelating ligand may favor the existence of the form on the left?

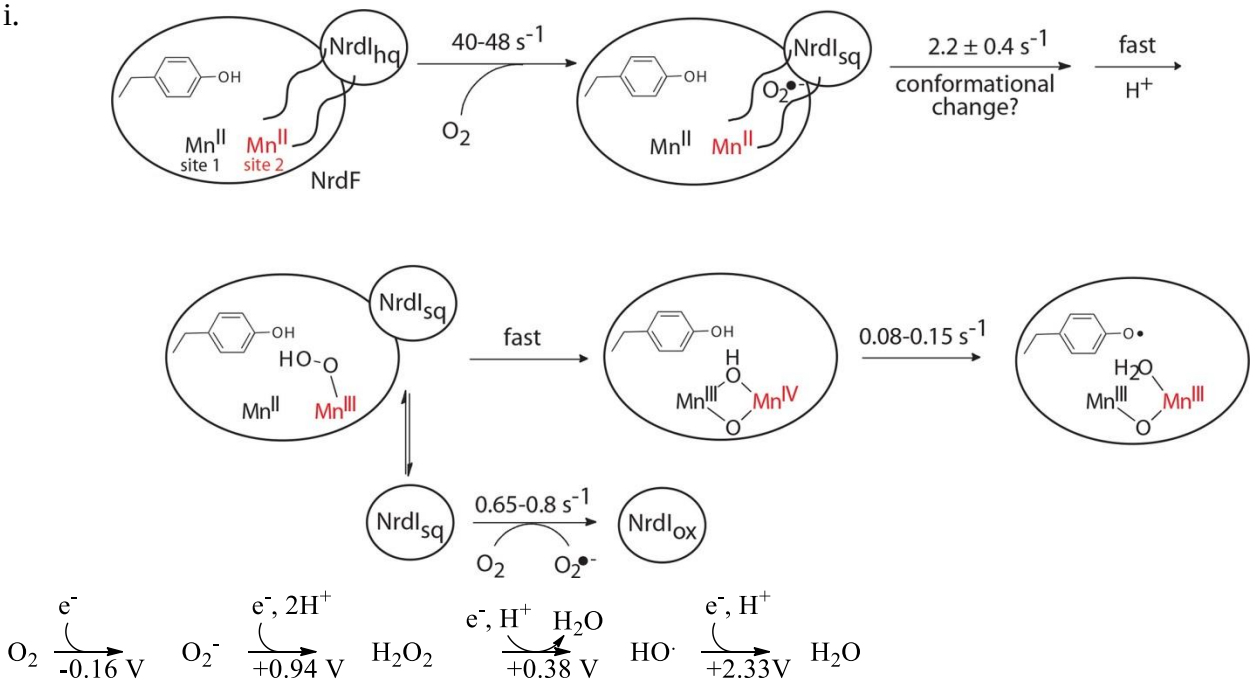
9. Researchers sometimes mutate proteins to alter their activities and to gain insight into important amino acid residues that influence the activities of the proteins. The heme group depicted below is of iron protoporphyrin IX and it is commonly employed in proteins. Under what conditions in a protein binding site would this group serve as a dioxygen carrier? Serve as a poor dioxygen carrier but as a peroxidase or a catalase?




Extra Credit. In our discussions of the ribonucleotide reductases (RNRs) we looked at the rich diversity of coenzymes and cofactors involved in the radical catalyzed mechanisms. For the class I RNRs, a stable tyrosyl radical (Y·) in the enzymes β2 subunits (R2 site) is the oxidizing agent for the reversible thiyl radical generation. In the class Ia RNRs, the active cofactor is a diferric-Y· ($Fe^{III}_2$-Y·), which is assembled by $O_2$ as oxidant. In the class Ib RNRs, an active $Fe^{III}_2$-Y· cofactor can be assembled. At MIT, J. Stubbe et al. (*Biochemistry* **2010**, *49*, 1297-1309) found that an active dimanganese(III)-Y· cofactor ($Mn^{III}_2$-Y·) can be generated as well and they believe it is physiologically relevant. The mechanism of the formation of $Mn^{III}_2$-Y· has some significant differences from that of $Fe^{III}_2$-Y·. In light of our mechanistic discussions and using the proposed mechanism by J. Stubbe below for *B. subtilis*, explain what is going in each step on the next page.

Use the following information to help you make sense of the mechanistic steps:

i.




ii. NrdI$_{hq}$ is a flavodoxin-like protein that contains a reduced flavin in the hydroquinone form. NrdI$_{sq}$ is the semiquinone form and NrdI$_{ox}$ is the quinone form.