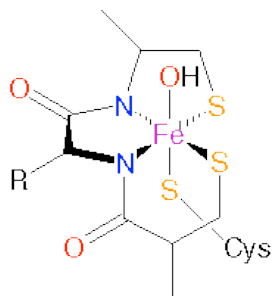


## Problem Set #10 – Chem 391

Due Thursday, Nov. 28<sup>th</sup> in class

1. Nitrile hydratase is an  $\text{Fe}^{3+}$ -dependent enzyme that catalyzes the hydrolysis of nitriles to amides. It's used in the industrial production of acrylamide. The active site iron is bound as follows.

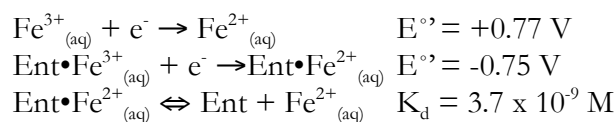
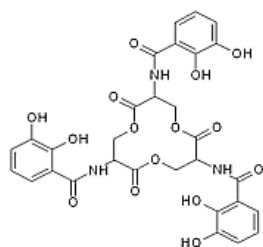


a. The nominal  $\text{pK}_a$  of water bound to  $\text{Fe}^{3+}_{(\text{aq})}$  is 2.2. Would you predict the  $\text{pK}_a$  to be higher or lower than 2.2? Why?

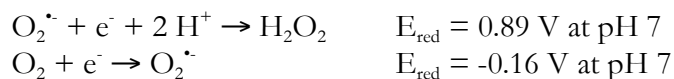
b. Draw an arrow-pushing mechanism for the hydrolysis of a simple nitrile ( $\text{CH}_3\text{CN}$ ) to an amide, in which the metal-bound hydroxide is used as a nucleophile. How does this ligand environment affect the nucleophilicity of the bound hydroxide bound to  $\text{Fe}^{3+}$  (compared to hydrated  $\text{Fe}^{3+}$ )?

c. Assuming the only role of this iron center is to provide an active site nucleophile, predict how the substitution of  $\text{Fe}^{3+}$  with  $\text{Co}^{3+}$  (a stronger Lewis acid) will affect the kinetic parameters  $k_{\text{cat}}$  and  $K_m$  for the enzyme.

2. Siderophores are small molecules used by bacteria and fungi to bind  $\text{Fe}^{3+}$  in the environment, where it's soluble concentration is less than  $10^{-18}$  M, and import it into the cell. The dissociation constants of these compounds are extremely low and difficult to measure. Instead, one can calculate the  $K_d$  value for  $\text{Fe}^{3+}$  by measuring the reduction potential of the siderophore-bound ferric ion and the  $K_d$  of  $\text{Fe}^{2+}$  from the siderophore. Use the following data to calculate the dissociation constant of  $\text{Fe}^{3+}$  from enterobactin (Ent), a hexacoordinate siderophore produced by *E. coli*. (Hint: determine the equilibrium constant for the reaction of  $\text{Fe}^{2+}_{(\text{aq})}$  with  $\text{Ent}\cdot\text{Fe}^{3+}$ .)

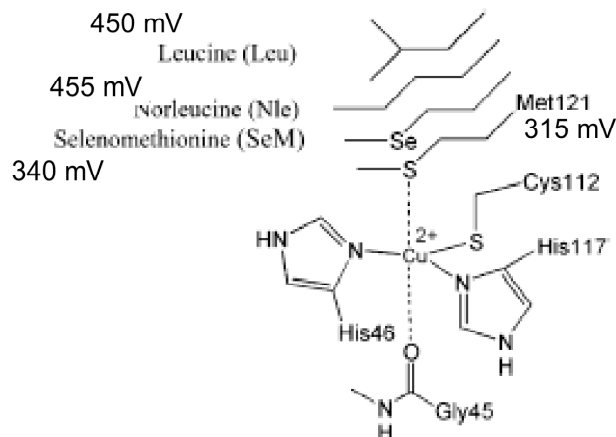


3. Based on the reduction potential shown in “2” above, show that  $\text{Ent}\cdot\text{Fe}^{3+}$  is not capable of catalyzing the disproportionation of superoxide.



*more on the back, of course...*

4. Azurin is a copper-containing protein involved in bacterial denitrification – converting nitrite and nitrate to  $N_2$ . A study has been performed to test the role of an active site methionine (Met121) in binding the copper ion. Methionine was replaced by leucine and the unnatural amino acids, norleucine and selenomethionine:



Standard reduction potentials were measured for each form of azurin, ranging from 315 mV for the WT (Met121) to 455 mV for norleucine (Nle). Explain these results in the context of the metal binding geometry shown. Ref: Berry et al. (2003) *JACS* **125**, 8760.

5. Seryl tRNA synthetase (SerRS) activates serine with ATP to form a seryl-adenylate (Ser~AMP) and then subsequently transfers serine from the adenylyate to tRNA. To answer this question about the specificity of SerRS for serine as a substrate, you will need to download **SerRS.pse** from the 391 web site (in the problem sets page). When you initially open the file, you will see a ribbon cartoon, but to answer questions, you will need to hide the cartoon and focus on the active site and Ser~AMP.

- What interactions between Ser~AMP and the enzyme SerRS allow the enzyme to exert high specificity for serine over alanine?
- What interactions between *Cys*~AMP and the enzyme SerRS allow the enzyme to exert high specificity for serine over cysteine? (You may wish to hide Ser~AMP and show Cys~AMP.) Cite specific atom groups and distances.
- What interactions between *Thr*~AMP and the enzyme SerRS allow the enzyme to exert high specificity for serine over threonine? (As above, you may wish to close the view of Cys~AMP and show Thr~AMP.) Cite specific atom groups and distances.