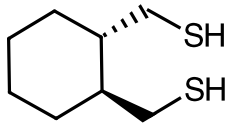
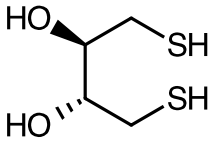
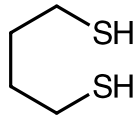
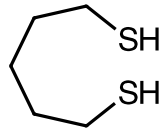
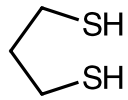
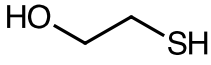
Problem Set #1x – Chem 391 **Name**_____

Not due. Never. But you should be able to do these.

1. The following compounds (shown with the standard reduction potentials for their oxidized forms) have been studied as potential reductants. In the space provided provide a brief explanation for why that compound might have the relative strength as a reductant that it does. For example, why is a given compound a better reductant that one and worse than another. Terms like enthalpy and entropy may find favor. For your edification, two common lab reductants, dithiothreitol and β-mercaptoethanol are ID'd.

Compound	**E°' (V)**	**Why it is where it is on the list**
	-0.354	
	-0.327 (DTT)	
	-0.316	
	-0.277	
	-0.271	
	-0.260 (βME)	

2. The following question requires the PyMOL session file **azurin.pse**. The display will show you a model of the copper-binding protein azurin, which serves a role in electron transport. The wild-type protein as a reduction potential of 265 mV:

$Azurin{\bullet}Cu^{2+} + e^- \rightarrow Azurin{\bullet}Cu^+$ $E^{\circ\prime} = +265$ mV

A study by the Lu lab at Illinois (Marshall et al. (2009) *Nature* **462**, 113-116; linked on web site) indicates that the reduction potential of azurin can be "tuned" by mutagenesis of residues near the copper ion. Explain how the following mutations affect the reduction potential of the copper site (you will need to refer the paper and/or the PyMOL file to assist your understanding).

M121Q:

M121L:

F114N:

N47S:

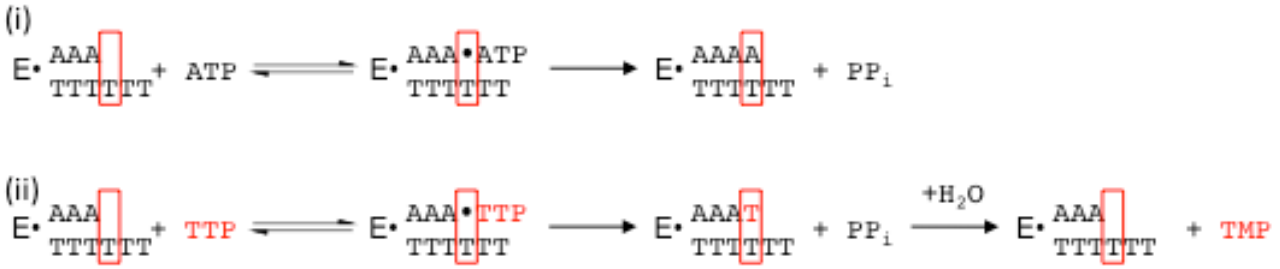
3. Catalase is an enzyme that catalyzes the dispropotionation of H_2O_2 to O_2 and water. The following half-reactions are relevant.

$O_2 + 2\ H^+ + 2\ e^- \rightarrow H_2O_2$ $E_{red}^{\circ\prime} = +0.28$ V

$H_2O_2 + 2\ H^+ + 2e^- \rightarrow 2\ H_2O$ $E_{red}^{\circ\prime} = +1.35$ V

The enzyme uses a heme co-factor (heme is an iron ion embedded in a porphyrin ring). What range of reduction potentials for the heme group are acceptable if this reaction is to be catalyzed via two spontaneous reactions, one in which heme reduces H_2O_2 in the first half of the reaction and oxidizes it in the second half?

4. Consider mitochondrial DNA polymerase, which progressively adds nucleotides to a growing chain. It has an error frequency of 1 in 1.1 x 10^6, which arises in part from a proofreading reaction, in which incorrectly added nucleotides are hydrolyzed from the growing DNA strand rather than allowing the strand to be extended. Case (i) below shows an enzyme bound to template (TTTTTT) and growing strand (AAA) and about to add the cognate ATP. Case (ii) shows an enzyme adding TTP in the same situation. The red box indicates the position of the active site for addition.



a. Think about the Hopfield mechanism, suggest the chemical events that take place in the above steps with rate constants: k_1, k_{-1}, k_{cat}, and k_{off}. Label them by the above arrows.

b. Suggest what happens in the k_{on} step for DNA polymerase. It is not shown above, so you must propose it as an additional step.

The following represents the kinetics of addition of a dNTP to a growing chain, where the template is polyT when the proofreading domain is not present.

Substrate	**k_{cat} (s^{-1})**	**K_m (μM)**	**k_{cat}/K_m ($\mu M^{-1}s^{-1}$)**
dATP	45	0.8	56
dTTP	0.013	57	0.00023
dCTP	0.038	360	0.00011
dGTP	1.16	71	0.016

c. Determine the error frequency without proofreading (Hint: divide the efficiency of the all wrong reactions by the sum of the efficiencies of all possible reactions).

d. When the exonuclease domain is present, selectivity increases. The excision of a mis-matched base by hydrolysis proceeds with a rate constant of 9 s^{-1} (correctly matched bases are excised at 0.05 s^{-1}). Given those hydrolysis rates, predict the value of k_{on}, based on the observed error frequency of 1 in 1.1 million.

5. Seryl tRNA synthetase (SerRS) activates serine with ATP to form a seryl-adenylate (Ser~AMP) and then subsequently transfers serine from the adenylate 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. 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.

a**.** Sketch the interactions between Ser~AMP and the enzyme SerRS that allow the enzyme to exert high specificity for serine over alanine. Indicate distances.

b. How does the enzyme exclude Cys and Thr as substrates without using kinetic proofreading? (You may wish to hide Ser~AMP and show Cys~AMP or Thr~AMP.) Cite specific atom groups and distances. (Hint: Consider the relative atomic radii of oxygen and sulfur.)

Cys:

Thr: