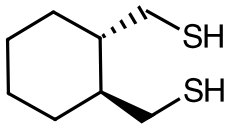
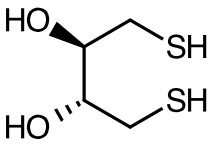
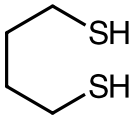
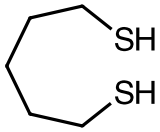
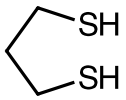
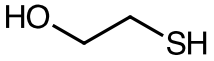
Problem Set #1x– Chem 391 **Name Solutions**

Not due. Never.

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	**Restricted motion about the central C-C bond reduces entropy loss upon disulfide formation.**
	-0.327 (DTT)	**HO groups are electron withdrawing and reduce electron density on thiol slightly (enthalpic).** **Substitution restricts conformation on central C-C bond less than above, but more than below**
	-0.316	**More entropy loss around central C-C bond than above**
	-0.277	**Seven membered ring formation requires greater loss of entropy than 6-membered ring formation.**
	-0.271	**No entropic argument here, must be enthalpic – ring strain possible with S-S bond.**
	-0.260 (βME)	**βME dimerizes in disulfide bond formation. Greatest loss of entropy of all.**

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: Glutamine substitution lowers $E^{\circ\prime}$ because polarity of the amide group provides greater electrostatic stabilization of Cu^{2+} than the thioether.

M121L: Leucine substitution raises $E^{\circ\prime}$ because non-polar side chain leave Cu^{2+} more electron starved than ever.

F114N: Asparagine is suspected to form H-bond with carbonyl of Gly45. C=O was directed towards Cu^{2+}. Loss of interaction raises $E^{\circ\prime}$ due to less stabilization of Cu^{2+}.

N47S: H-bond to backbone rigidifies loop connected to Cys112 ligand. Possible interation with thiolate reduces electron donation to Cu^{2+}. Raises $E^{\circ\prime}$.

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?

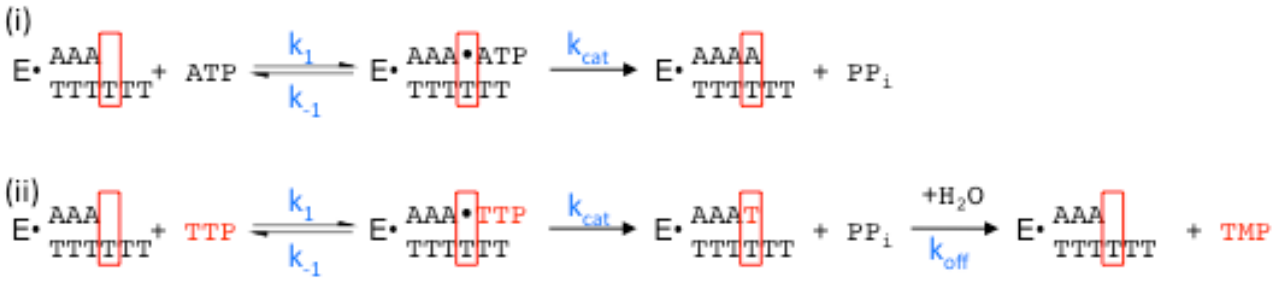
For both reducing and oxidizing reactions to be spontaneous, the following conditions must apply.

$H_2O_2 + 2\ H^+ + 2e^- \rightarrow 2\ H_2O$ $E_{red}^{\circ\prime} = +1.35$ V $H_2O_2 \rightarrow O_2 + 2\ H^+ + 2\ e^-$ $-E_{red}^{\circ\prime} = -0.28$ V

$Heme \rightarrow Heme^{2+} + 2e^-$ $-E_{red}^{\circ\prime} > -1.35$ V $Heme^{2+} + 2e^- \rightarrow Heme$ $E_{red}^{\circ\prime} > 0.28$V

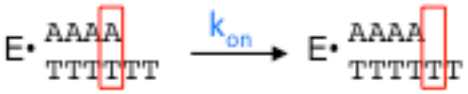
So 0.28 V < $E_{red}^{\circ\prime}$ < 1.35 V

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 enzyme moves along the primer so that the new nucleotide is no longer in the active site and everything is ready to add a new ATP. We hope.

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

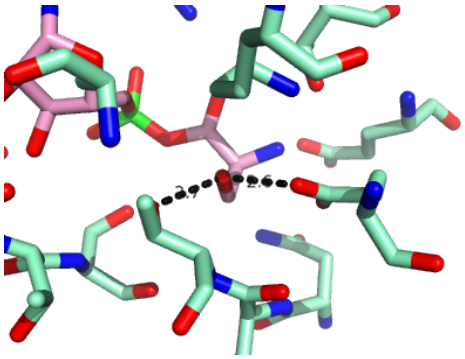
c. Determine the error frequency without proofreading (Hint: divide the efficiency of the all wrong reactions by the sum of the efficiencies of all possible reactions).
Using k_{cat}/K_m values for each, I get 99.97% correct incorporation, meaning an error rate of 3 in 10,000.

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.
According to the Hopfield mechanism, the second screening step applies an additional efficiency of k_{off}/k_{on}. Since we need to go from 3 in 10,000 to 1 in 1.1 million, we need an additional 330 x specificity. That means $k_{off}/k_{on} = 330$.

Given a k_{off} of 9 s^{-1}, $k_{on} = k_{off}/330 = 0.03\ s^{-1}$

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.



Two hydrogen bonds are made to the hydroxyl of the serine substrate (in pink), one from Glu335 and another from Thr436. Alanine would not form those H-bonding interactions and the lack of stabilizing contacts for E335 and T436 would render the complex with alanine.

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: The larger atomic radius of sulfur makes the distances to E335 and T436 (both under 2.8 Å) too tight, leading to steric clash.

Thr: The methyl group of the side chain is 2.6 Å away from the backbone of Asn434 and 2.3 Å away from both carboxylate oxygens on our friend Glu279. Since the combined vdW radii of C and O are 3.1 Å, this is a real problem sterically.