Chemistry 391 - Problem Set #7 **Name**__________________________
Due on 11/1/18 in class

1. Cytidine deaminase catalyzes conversion of cytidine to uridine.

$k_{uncat} = 3.0 \times 10^{-10}\ s^{-1}$ $k_{cat} = 300\ s^{-1}$ $K_m = 100\ \mu M$

a. What is V_{max} when the concentration of cytidine deaminase is 1 μM?

b. What would the rate be if the concentration of substrate were 300 μM and enzyme concentration was still 1 μM?

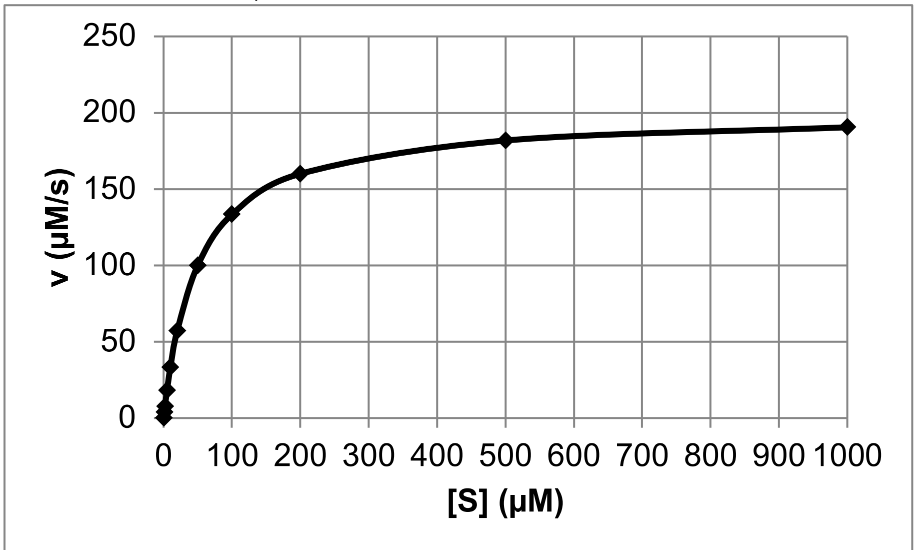
c. By how much does cytidine deaminase stabilize the transition state relative to the substrate?

d. What, very roughly, is the free energy of dissociation of cytidine from the enzyme (assuming $k_{cat} << k_{-1}$ of course)?

e. At low concentrations of cytidine, what is the second order rate constant for conversion to product?

2. k_{cat}/K_m is sometimes refered to as the "specificity constant". It is used to describe the preference of one substrate "S_1" vs. a second possible substrate "S_2". Explain why k_{cat}/K_m is a better measure of specificity than k_{cat} alone.

3. By inspection of this plot predict values for V_{max}, K_m, and k_{cat}, assuming an enzyme concentration of 10 μM.



4. Consider an enzyme that can act on two different substrates, A and B, where P and Q are the two products

$E + A \Leftrightarrow E{\bullet}A$ $\quad\quad$ $E + B \Leftrightarrow E{\bullet}B$

$E{\bullet}A \rightarrow E + P$ $\quad\quad$ $E{\bullet}B \rightarrow E + Q$

Assume that there are separate k_{cat} values for each process (k_{catA} and k_{catB}) and separate K_M values (K_{MA} and K_{MB}).

a. Derive a rate law for the production of P when the enzyme is in the presence of A and B.

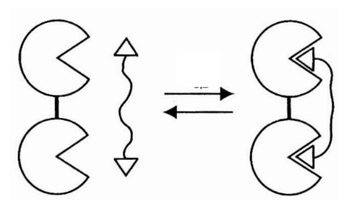
b. What is the maximum rate of the formation of P at high [A]?

5. The hemagglutanin.pse file, available on the web site, contains a structural model for the interaction of an Fab fragment on an antibody (pink light chain + green heavy chain) with hemagglutinin (yellow), the viral protein responsible for the infectivity of human influenza. The epitope of hemagglutanin and the idiotope of the Fab, can be zoomed on.

There are three mutations in the hemagglutanin epitope that allow the influenza virus to avoid immune recognition. The Fab is a stand in for all possible antibodies that recognize the flu virus, but it explains specificity. For each of the following mutations (which you can prepare in PyMOL using the mutagenesis wizard), sketch the interaction that leads to reduced affinity of the Fab for the viral protein. Note that the residues listed below are colored orange.

a. I62R b. D63Y c. P273L

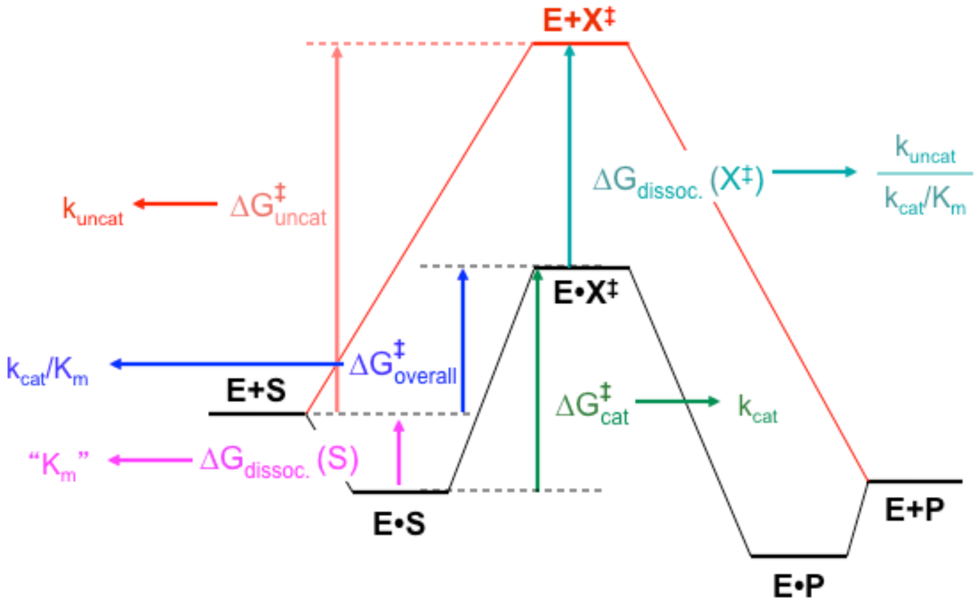
6. Medicinal chemists have been using multivalency to generate more potent drugs. The idea is to use tethered molecules that can bind more tightly to a dimeric receptor than 1:1 binding at each site would permit. A simple prediction would say that ΔG_{diss} for the tethered drug should be 2x bigger than for one drug molecule alone.



a. Provide a thermodynamic argument (enthalpy and/or entropy) for why the tethered drug might bind with greater than two-fold affinity vs. the single drug molecule.

b. Provide a thermodynamic argument for why tether length is important in maximizing binding of the drug. Consider <u>briefly</u> situations where it is too long or two short and invoke enthalpy and entropy.

7. Consider the free energy reaction profile shown below. **Note well:** the arrows indicate an equation linking a ΔG value to a kinetic parameter! *Big ΔG still means small parameter. They are not equal.*



a. $\Delta\Delta G^{\neq}$ is the difference between $\Delta G_{cat}^{\neq} - \Delta G_{uncat}^{\neq}$. Show algebraically that it can be calculated from the ratio of k_{cat}/k_{uncat}.

b. I have said that the overall rate constant for an enzyme catalyzed reaction is k_{cat}/K_m. From the above plot, it should be visible that:

$$\Delta G^{\neq}_{overall} = \Delta G^{\neq}_{cat} - \Delta G_{dissoc}(S) \qquad \text{(Eq. A)}$$

Assuming that ΔG_{dissoc} is the free energy change associated with K_m, derive the following relationship from Eq. A: $k_{overall} = k_{cat}/K_m$

c. Using similar logic, find an expression for $\Delta G_{dissoc}(X^{\neq})$ using $\Delta G^{\neq}_{uncat}$, $\Delta G^{\neq}_{cat}$ and $\Delta G_{dissoc}(S)$ that allows you to derive the following expression:

$$K_d(X^{\neq}) = k_{uncat}/(k_{cat}/K_m)$$