Chemistry 391 - Problem Set #7 **Name**__________________________

Due on 11/1/18 in class

1. Cytidine deaminase catalyzes conversion of cytidine to uridine.

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

$V_{max} = k_{cat}[E]_{tot} = 300\ \mu M/s$

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

$v = V_{max}[S]/(K_m + [S]) = 225\ \mu M/s$

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

$\Delta\Delta G^{\neq} = -RT\ln(k_{cat}/k_{uncat}) = -16.8$ kcal/mol

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

$K_d \approx K_m$; $\Delta G^{\circ} = -RT\ln(100 \times 10^{-6}\ M) = 5.6$ kcal/mol

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

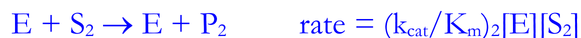
$k_{cat}/K_m = 3 \times 10^6\ M^{-1}s^{-1}$

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.

To compare reaction of E with either S_1 or S_2, compare the rates of the following two reactions:

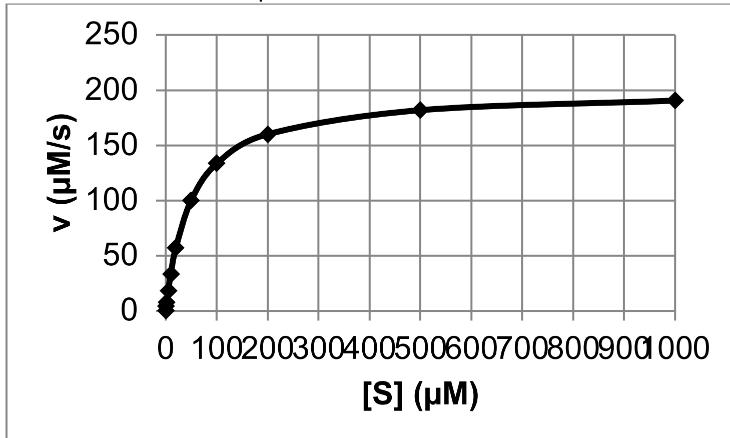
$E + S_1 \rightarrow E + P_1$ rate $= (k_{cat}/K_m)_1[E][S_1]$

Vs.



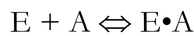
$E + S_2 \rightarrow E + P_2$ rate $= (k_{cat}/K_m)_2[E][S_2]$

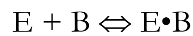
3. By inspection of this plot predict values for V_{max}, K_m, and k_{cat}, assuming an enzyme concentration of 10 μM.

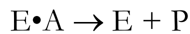


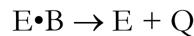
V_{max} = 200 μM/s
K_m = 50 μM (v = ½ V_{max} at that substrate conc)
k_{cat}= $V_{max}/[E]_{tot}$ = 200 μM/s divided by 10 μM = 20 s^{-1}

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









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.

$$\frac{v}{V_{max}} = \frac{[EA]}{[E]_{tot}} = \frac{[EA]}{[E]+[EA]+[EB]}$$

$$\frac{v}{V_{max}} = \frac{[E][A]/K_{MA}}{[E]+[E][A]/K_{MA}+[E][B]/K_{MB}} = \frac{[A]}{K_{MA}+[A]+K_{MA}\left([B]/K_{MB}\right)}$$

$$\frac{v}{V_{max}} = \frac{[A]}{K_{MA}\left(1+[B]/K_{MB}\right)+[A]}$$

Since $V_{max} = k_{catA}[E]_{tot}$

$$v = \frac{k_{catA}[E]_{tot}[A]}{K_{MA}\left(1+[B]/K_{MB}\right)+[A]}$$

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

$$v = \frac{k_{catA}[E]_{tot}[A]}{K_{MA}\left(1+[B]/K_{MB}\right)+[A]} \approx \frac{k_{catA}[E]_{tot}[A]}{[A]} = k_{catA}[E]_{tot}$$

That's kind of consoling…

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

The arginine substitution will run afoul of several non-polar residues from the heavy chain: Leu96, Ile100B, Ile100C (oddball numbering system)

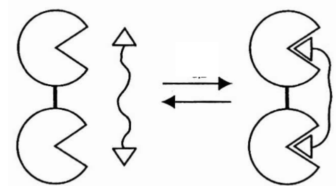
b. D63Y

Asp63 makes a beautiful pair of H-bonds to Arg94 of the heavy chain. A tyrosine wouldn't.

c. P273L

A little less obvious, but Leu273 clashes with Thr31 of heavy chain & neighboring residues. Also, Leu273 has an unpaired amide NH, not proline…

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.

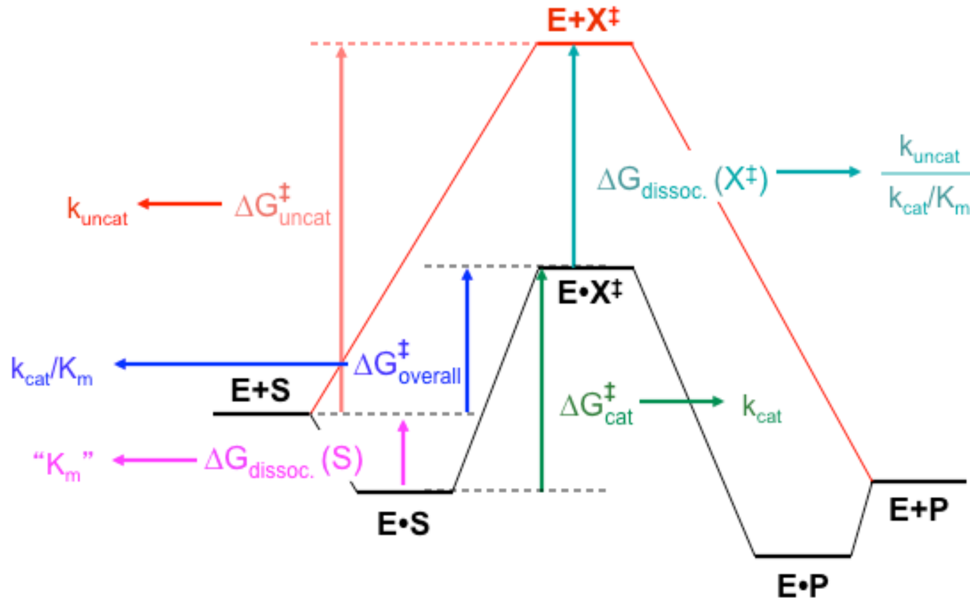
You'd have **twice the enthalpic benefit of binding**, since each binding site would be able to interact with a separate ligand groups, but you would have **less than twice the entropic cost** of binding two molecules to the two binding sites, since some of the entropy has been pre-removed via the tether.

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

Too long: Doesn't actually constrain the second ligand group to be anywhere near the binding site, so entropy loss is almost as much as two separate molecules.

Too short: Would create strain (poor enthalpy) for two ligands trying to reach both binding sites.

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}.

$\Delta G_{cat}^{\neq} - \Delta G_{uncat}^{\neq} = -RT\ln(k_{cat}) + RT\ln(k_{uncat}) = -RT\ln\left(\frac{k_{cat}}{k_{uncat}}\right)$

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:

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

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

$$k_{overall} = \nu^{\neq} e^{-(\Delta G^{\neq}_{cat} - \Delta G^{\circ}{}_{diss})/RT}$$

$$k_{overall} = \frac{\nu^{\neq} e^{-(\Delta G^{\neq}_{cat}/RT)}}{e^{-(\Delta G^{o}_{diss}/RT)}} = \frac{k_{cat}}{K_m}$$

Assuming of course, that $k_{-1} >> k_{cat}$.

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:

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

Easiest way to show this is that $\Delta G_{diss}(X^{\neq}) = \Delta G^{\neq}_{uncat} - \Delta G^{\neq}_{overall}$

$K_d(X^{\neq}) = \exp(-\Delta G_{diss} \text{ of } X^{\neq})/RT = \exp(-\Delta G^{\neq}_{uncat} + \Delta G^{\neq}_{overall})/RT =$

$$K_d(X^{\neq}) = \frac{\nu^{\pm}\exp\left(-\Delta G^{\pm}_{uncat}/RT\right)}{\nu^{\pm}\exp\left(-\Delta G^{\pm}_{overall}/RT\right)} = \frac{k_{uncat}}{k_{cat}/K_m}$$