BCH2333A **Midterm 3 2015**

NAME__________**KEY**_______________________

Student number__________________________

True or false questions. If false explain why. (15 points)

1. The binding constant K_D describes the equilibrium shown below and is in the units M^{-1}.

$$P + L \rightleftharpoons PL$$

True

False If false, explain why.

The K_D is for the dissociation reaction where PL dissociates into P and L. The units are M, not M^{-1}.

2. The equation the Lineweaver–Burk plot is based on assumes infinite cooperativity in ligand binding and is the most common plot used to look for cooperative binding.

True

False If false, explain why.

LB is a double reciprocal plot. Infinite cooperativeity is from the Hill plot.

3. In competitive inhibition the K_M increases as inhibitor is added.

True

False If false, explain why.

4. In uncompetitive inhibition the K_M increases as inhibitor is added.

True

False If false, explain why.

An uncompetatiev inhibitor causes the apparent KM to decrease by a factor of 1/alpha'.

5. In the symmetry model for allostery the free T and R states are in equilibrium with the T state favoured.

True

False If false, explain why.

Define these terms (10 points)

Induced fit:

Flexible interaction between ligand and protein induces a conformational change in the protein, which results in increased ligand binding affinity.

Allosteric modulation

Binding of an effector molecule to a protein modifies the physical properties of the protein, for example causing a structural change in the protein that enable the protein to bind ligands more or less tightly, or causing a structural change that enables an enzyme to be more or less active.

Sequential model for allostery

Each subunit can exist in two conformations. Binding of an effector causes a conformational change to the subunit, from the T to the R state, which destabilizes neighbouring subunits. The neighbouring destabilized subunits then have intermediate properties between the T and R state.

Holoenzyme

Enzyme with tightly bound or covalently attached cofactor. Holo enzymes are active enzymes.

Specificity constant

k_{cat}/K_M from michealis-menten equation. This ration constant provides information on the difference in energy between the free E and S and the TS for the rate determining step.

Long answers questions

1. Derive the rate law for an enzymatic reaction that converts substrate (S) into product (P) and is inhibited by the substrate (S) reversibly binding to the free enzyme (E) at a site other than the active site, generating an inactive enzyme-substrate' complex (ES') with an inhibition constant of K_I. (10 points)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$E + S \overset{K_I}{\rightleftharpoons} ES'$$

$$v = k_2[ES]$$

$$[E_T] = [E] + [ES'] + [ES]$$

$$K_I = \frac{[E][S]}{[ES']} \quad \text{therefore} \quad [ES'] = \frac{[E][S]}{K_I}$$

$$\text{thus } [E_T] = [E]\left(1 + \frac{[S]}{K_I}\right) + [ES]$$

$$\text{from steady state } [E][S]k_1 = (k_{-1} + k_2)[ES]$$

$$\text{therefore} \quad [E] = \frac{(k_{-1} + k_2)[ES]}{K_1[S]} = \frac{K_M[ES]}{[S]}$$

$$\text{thus } [E_T] = \frac{K_M[ES]}{[S]}\left(1 + \frac{[S]}{K_I}\right) + [ES]$$

$$[E_T] = [ES]\left(1 + \frac{K_M}{[S]}\left(1 + \frac{[S]}{K_I}\right)\right)$$

$$\text{Therefore } [ES] = \frac{[E_T]}{\left(1 + \frac{K_M}{[S]}\left(1 + \frac{[S]}{K_I}\right)\right)}$$

$$\text{thus } v = \frac{k_2[E_T]}{\left(1 + \frac{K_M}{[S]}\left(1 + \frac{[S]}{K_I}\right)\right)}$$

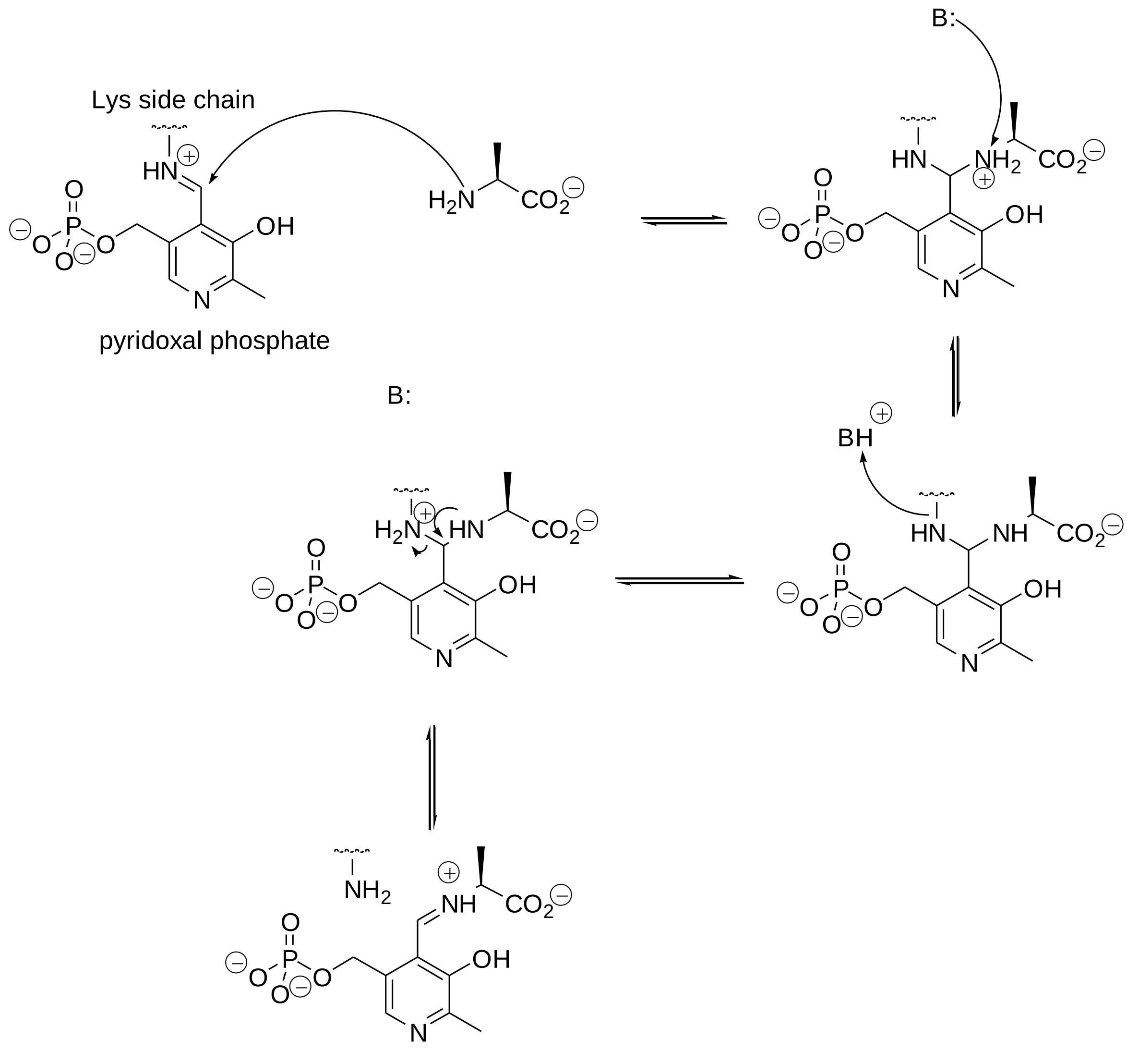
$$v = \frac{k_2[E_T][S]}{[S] + K_M\left(1 + \frac{[S]}{K_I}\right)}$$

but $k_2 = k_{cat}$ and $V_{max} = k_{cat}[E_T]$

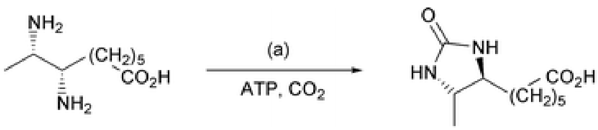
therefore

$$v = \frac{V_{max}[S]}{[S] + K_M\left(1 + \frac{[S]}{K_I}\right)}$$

2. The enzyme-bound co-factor shown is pyridoxal phosphate. It is essential for the covalent catalysis of alanine into pyruvate, which is catalyzed by a transaminase. Provide a mechanism for how the cofactor becomes covalently linked to the substrate in this reaction. (10 points)



3. The enzyme Dethiobiotin synthetase is involved in the biosynthesis of the co-factor biotin. It uses ATP and 7,8-diaminononanoic acid (DAPA) to make dethiobiotin. Biochemists identified the residues T11 (Thr at position 11), E12 and K15 as playing a role in the active site. Three site specific mutants were generated, T11V (T11 is mutated to valine), E12A, and K15Q. These mutants and the un-mutated wild type enzyme were characterized kinetically as shown below. (10 points)



enzyme	K_M (DAPA) μM	K_M (ATP) μM	k_{cat} min^{-1}
wt	0.3	0.39	3.73
T11V	0.34	9.5×10^3	1.51
E12A	1.13	2.38	4.61
K15Q	11	701	5.4×10^{-4}

Which residue (use 3 letter code) is involved in:

DAPA binding? What data tells you this? What type of non-covalent interaction is disrupted by the mutant?

E12 – The KM in the E12A mutant goes up indicating that DAPA binds with lower affinity in the mutant. Note K15 is not involved in DAPA binding. It is involved in catalysis. Ionic interaction between E carboxylate and DAPA ammoniums.

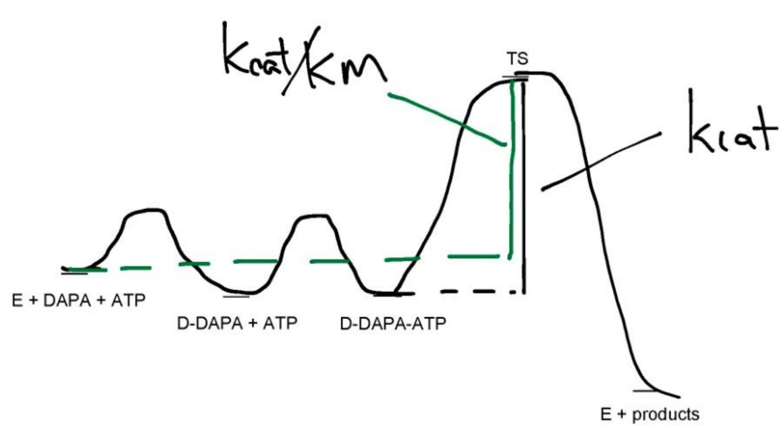
ATP binding? What data tells you this? What type of non-covalent interaction is disrupted by the mutant?

T11 is involved in ATP binding. The KM for ATP is dramatically increased when T11 is mutated to Alanine. Hydroge nbonding interaction between side chain alcohol and ATP.

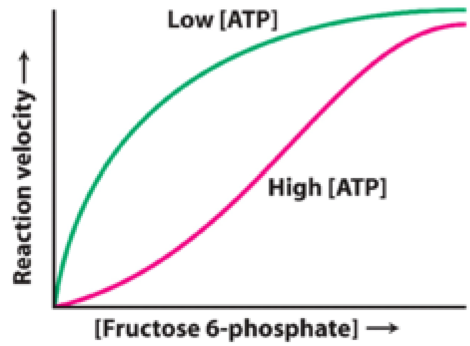
Catalysis? Explain why (explanations > 140 characters may not be graded).

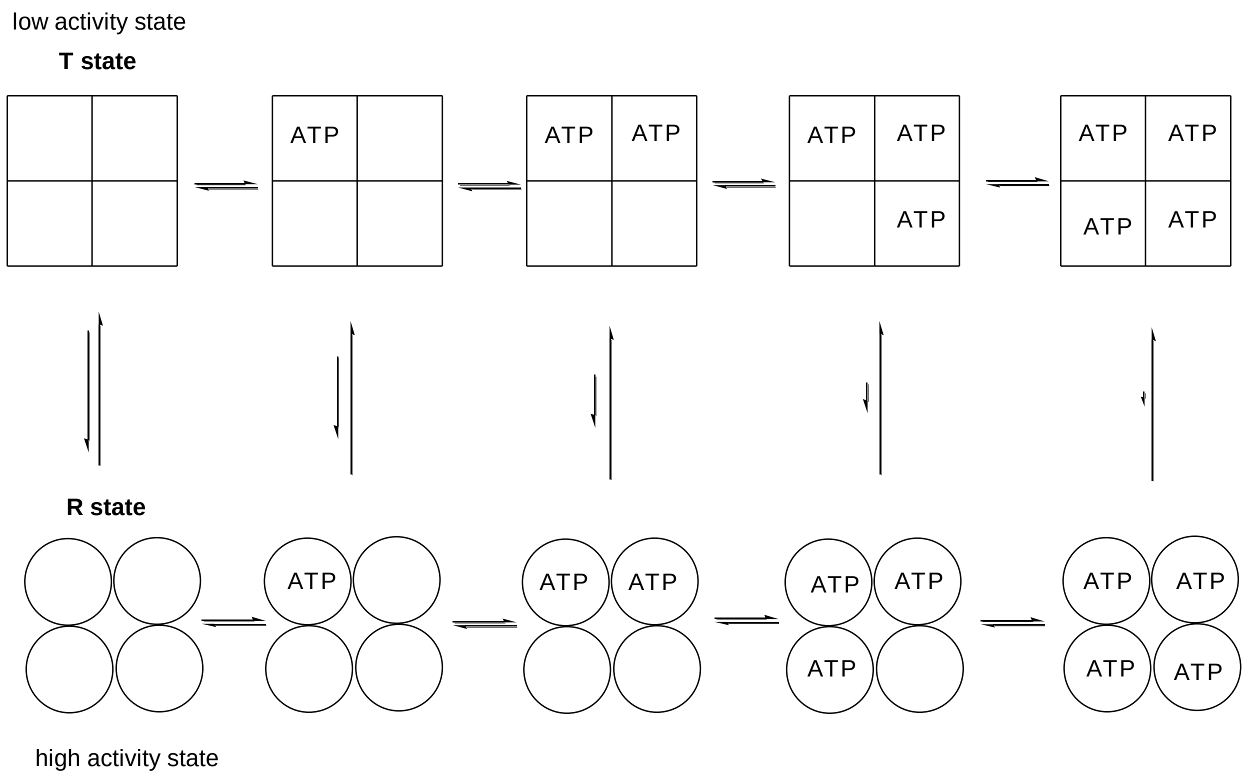
K15. Mutation to Q decreases kcat by 4 orders of magnitude. Lys is likely playing a role as a catalytic base in the enzyme.

Draw a reaction coordinate diagram. Show and label the free enzyme plus free DAPA and ATP, the E-DAP-ATP ternary complex, the TS and the free enzyme plus dithiobiotin. Indicate which energy differences correspond to k_{cat} and k_{cat}/K_M.



4. The enzyme phosphofructokinase uses ATP to convert fructose-6-phosphate into fructose-1,6-bisphosphate. In addition to being a substrate for this tetrameric protein, ATP is a negative allosteric effector as shown below. Explain the negative allosteric regulation based on the T and R states. Be sure to indicate which states are active and which are low activity and the relative stability of the states prior to effector binding. How many binding sites for ATP must each subunit have? (10 points)

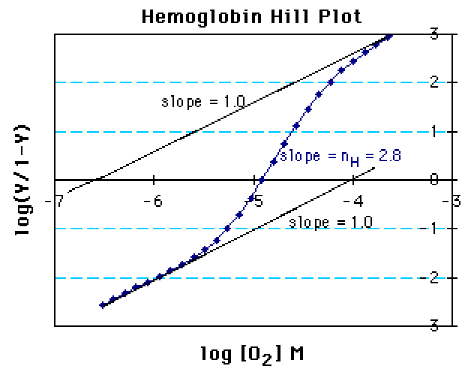




As O2 binds to the allosteric site the low activity state of the enzyme is stabilized.

Number of ATP biding sites: each subunit must have at least 2 sites. 1 for substrate ATP to bind and 1 for the allosteric negative regulation by ATOP. Note these cannot be the same sites since it is negative regulation with the symmetry model. If it was positive regulation, they could both be the same site.

5. Hemoglobin has an $\alpha_2\beta_2$ quaternary structure and binds oxygen cooperatively. Its Hill plot is shown. (10 points)



a) From this Hill plot, what are the K_Ds for oxygen binding to the T and R states.

T state $K_D = 10^{-4}$ M

R state $K_D = 3 \times 10^{-7}$ M.

b) The maximum observed slope of the Hill plot is n= 2.8. What is the maximum predicted slope for Hemoglobin? What determines the maximum possible slope and when does it occur?

Max n = 4. The number of binding sites.

c) Use the KNF model to explain the cooperative binding of oxygen to hemoglobin.

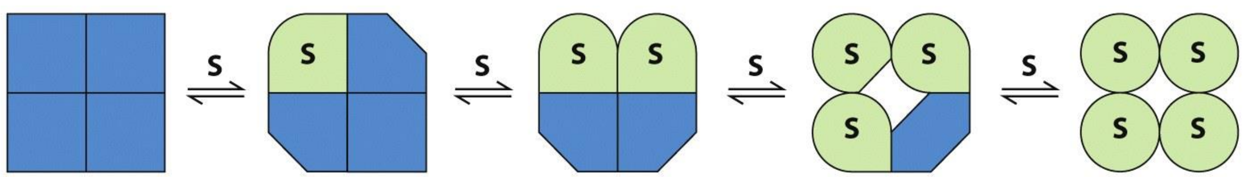


Figure 10-34

Binding of the first molecule of oxygen occurs on the T state. The induced fit binding generates the R state. The neighbouring subunits are distorted by the R state into an intermediate state with higher affinity O2 binding. The next molecule of O2 thus binds to a higher affinity state, as does the 3rd and 4th molecules of O2.