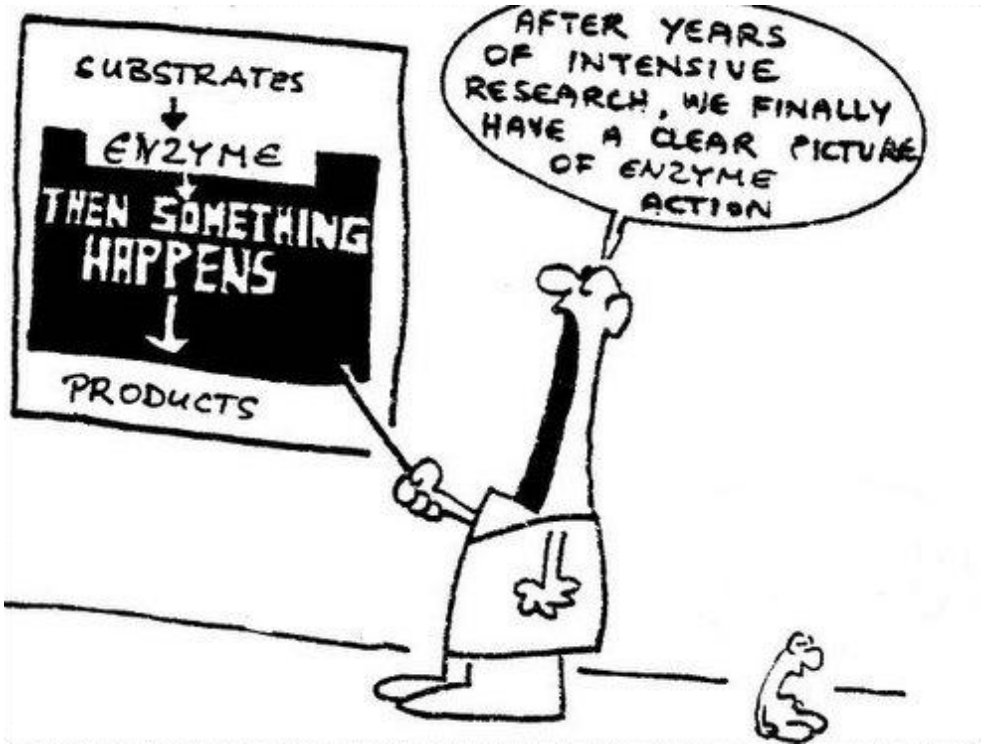
BCH2333B **Midterm 3 2015**

NAME_____**KEY**_____

Student number_____



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

1. K_M is a true dissociation constant when k_{-1} is approximately = k_2.

True

False If false, explain why. **K_M is a true dissociation constant only when $k_{-1} >> k_2$ such that $k_{-1} + k_2 \approx k_{-1}$.**

2. The Adair equation is the mathematical equation describing the Monod-Wyman-Changeux model for cooperative binding.

True

False If false, explain why. **The MWC model assumes the same K_D for the T and R states and that the equilibrium between the T and R states is changing. In the Adair equation, the K_D for each successive ligand binding is different. This is the mathematical description of the KNF model.**

3. In competitive inhibition the V_{MAX} decreases as inhibitor is added.

True

False If false, explain why. **In competitive inhibition the V_{max} remains constant and the K_M increases. The V_{max} stays the same since S can compete I from E, leading to the majority of E as E-S complex.**

4. In uncompetitive inhibition the V_{MAX} decreases as inhibitor is added.

True

False If false, explain why.

5. In the sequential model for allostery the free protein prior to ligand binding is in the T state.

True

False If false, explain why.

Define these terms (15 points)

Lock and Key:

The specificity of an enzyme (or protein) for its substrate (or ligand) arises from their geometrically complementary shapes. In this model the two shapes, enzyme and substrate are rigid, like a lock and key.

Allosteric modulation:

The regulation of a protein's function by binding of an effecter molecule at an allosteric site. Allosteric effecter binding leads to a conformational change taht impacts the proteins function.

Symmetry model for allostery

Describes the allosteric transitions of proteins made up of identical subunits. In the symmetry model the protein can exist in at least 2 states. All subunits must be in the same state (this is the symmetry part). Effecter binding changes the equilibrium between states.

Apoenzyme

Inactive enzyme. The apoenzyme requires a cofactor that must be tightly associated with Ii or covalently linked to it to be active and become holoenzyme.

Michaelis constant

K_M. This is a ratio of rate constants = $(k_{-1} + k_2)/k_1$. If there is a true pre-equilibrium for S binding E, the Michaelis constant describes the affinity of S for E.

Long answers questions

1. Derive the rate law for an enzymatic reaction that converts substrate (S) into product (P) and is inhibited by the product (P) reversibly binding to the free enzyme (E) with an inhibition constant of K_I. (10 points)

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

$v = k_2[ES]$

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

$K_I = \frac{[E][P]}{[EP]}$ therefore $[EP] = \frac{[E][P]}{K_I}$

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

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

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

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

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

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

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

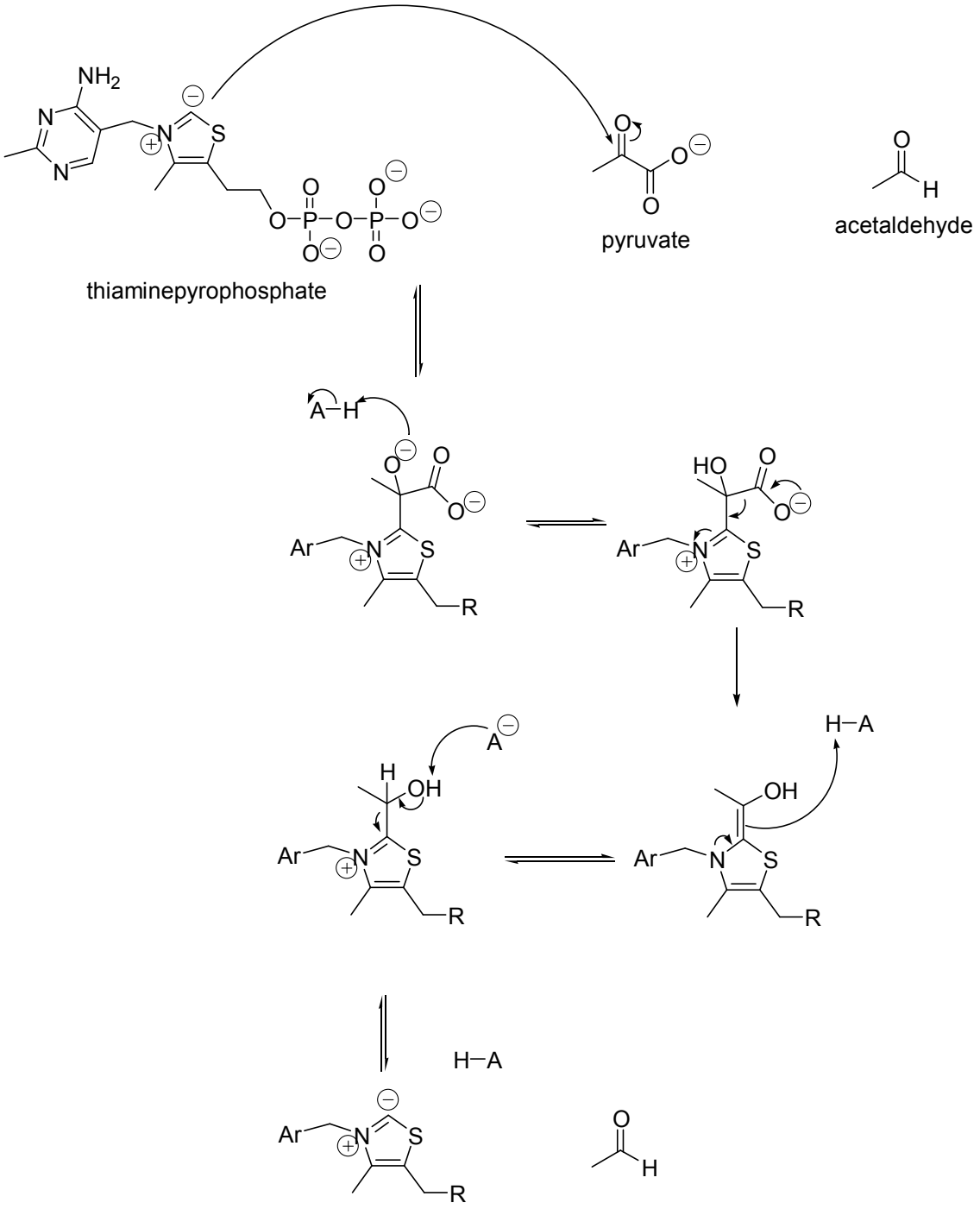
$v = \frac{k_2[E_T][S]}{[S] + K_M\left(1 + \frac{[P]}{K_I}\right)}$

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

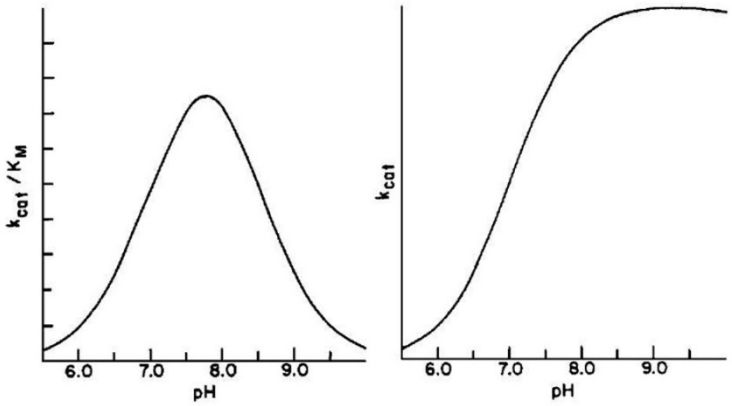
therefore

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

2. The enzyme-bound co-factor shown is the anion of thiamine pyrophosphate. It is essential for the covalent catalysis of pyruvate into acetaldehyde, which is catalyzed by the enzyme pyruvate decarboxylase. Provide a mechanism for how the cofactor catalyzes decarboxylation in this enzymatic reaction. (10 points)



3. The enzyme chymotrysin is a serine hydrolase which uses a catalytic S to hydrolyze a peptide bond. In addition to the S, the active site has two other residues that participate in catalysis, H and D (all 3 make a "catalytic triad"). This enzyme shows different pH dependencies for k_{cat} and k_{cat}/K_M.



During catalysis which residue from the catalytic triad is acting as a catalytic base (use the 3 letter code)? What data did you use to determine this?

His. Kcat, which gives information on the reaction involving attck of the Ser on the peptide substrate shows a strong pH dependence. The midpoint of this sigmodial curve is pH 7. This is closest to the pKa of His. Thus when protonated, below pH 6, the enzyme is slow since His is not a base. Above pH 8, where His is deprotonated, the enzyme is fast.

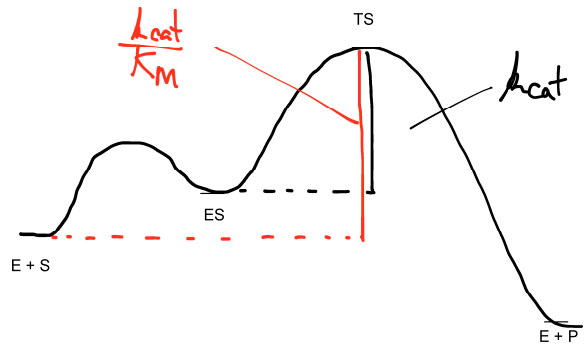
Compare the magnitude of the K_M at high pH (pH = 10) and low pH (pH = 6). At which pH does the substrate have the highest affinity?

The substrate has the highest affinity at pH 6.

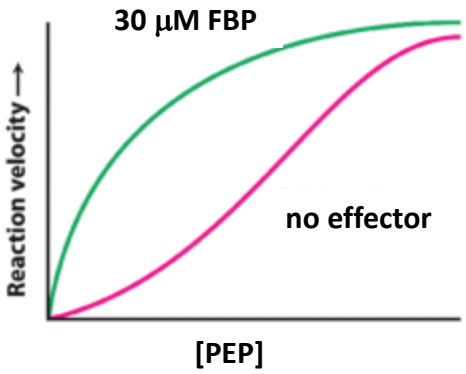
What is the pKa of the residue impacting substrate binding? Based on this pKa what residue is it?

pKa approximately 9. This is from the N-terminal α-amino group (pKa 9.5).

Draw a reaction coordinate diagram. Show and label the free enzyme (E) plus free substrate (S), the enzyme-substrate complex (ES), the transition state (TS), and the free enzyme and product (P). Indicate which energy difference corresponds to k_{cat} and which corresponds to k_{cat}/K_M.



4. The tetrameric enzyme pyruvate kinase transfers a phosphate from phosphoenoyl pyruvate (PEP) to AMP, generating ATP and pyruvate. The plot showing relative activity versus substrate concentration (PEP) is shown with and without 30 μM fructose-1,6-biophosphate (FBP) (, a (10 points)



Is pyruvate kinase in the absence of FBP cooperative for PEP? What data enables you to determine this?

YES. At low concentration of PEP these is a non-linear increases in reaction velocity as more PEP is added to the reaction. This sigmodial [sub] versus rxn velocity is indicative of cooperativity.

When FBP is added, the enzyme activity is modified. Is FBP an activator or inhibitor? What data enables you to determine this?

FBP is an activator. The reaction velocity at the same {PEP] is higher when FBP is added versus when it is absent.

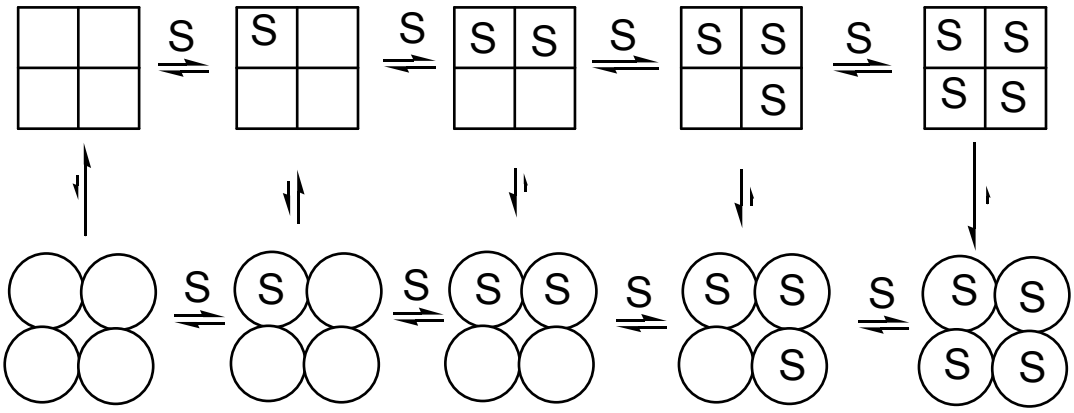
Is pyruvate kinase in the presence of FBP cooperative for PEP?

No, the PEP cooperativity is lost when FBP is added. The [sub] versus rxn velocity curve is no longer sigmoidal but rather Michaelis-Menten-like.

Use the symmetry model to explain any cooperativity or change in cooperativity for PEP and FBP.

No FBP

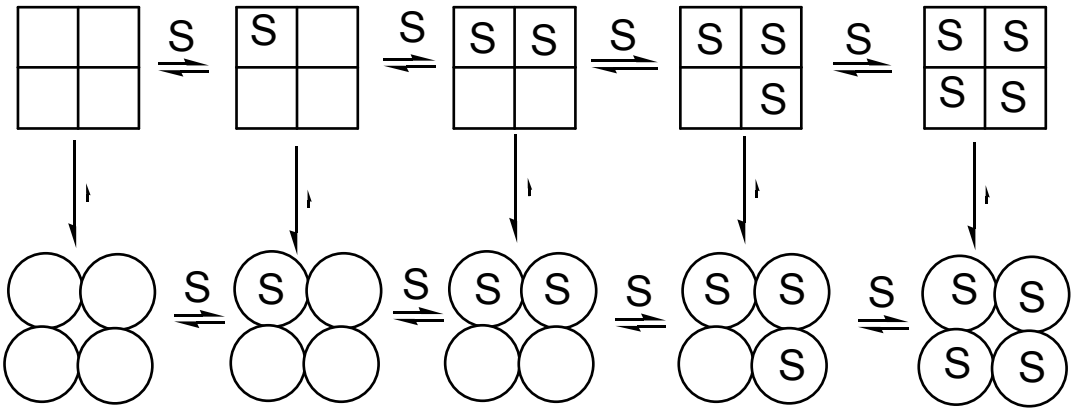
T state - low activity



R state - high activity

With FBP bound

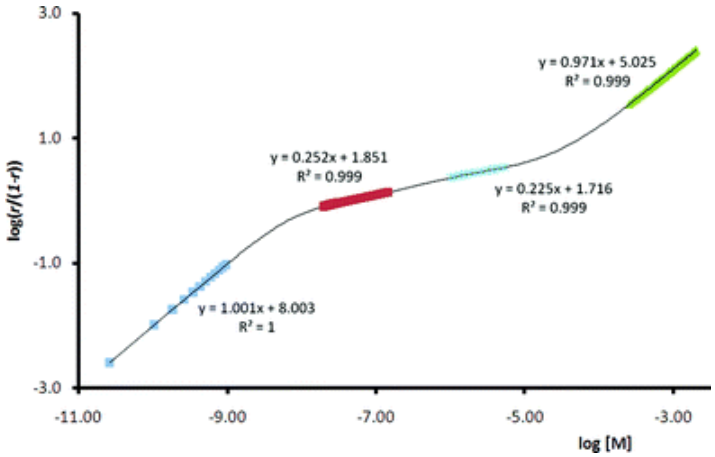
T state - low activity



R state - high activity

With No FBP the T state predominates at low substrate concentration. Substrate binding leads favours the R state increasing reactivity. With FBP present the R state is favoured. There is no cooperativiyt with substrate as all the enzyme is in the R state already.

5. The Prior protein (Prp) which is involved in some human neurodegenerative diseases can bind the ligand Cu^{2+}. A Hill plot for Cu^{2+} binding is shown. (10 points)



a) What are the K_Ds for the first Cu^{2+} binding and the last Cu^{2+} binding to PrP?

1st Cu^{2+} $K_D = 1 \times 10_{-8}$ M
Last Cu^{2+} $K_D = 1 \times 10^{-5}$ M

b) Which is the high affinity state? What type of cooperativity is this?

The first Cu^{2+} is the high affinity state. This is an example of negative cooperativity.

c) Use the KNF model to explain the cooperative binding of Cu^{2+} to Prp.