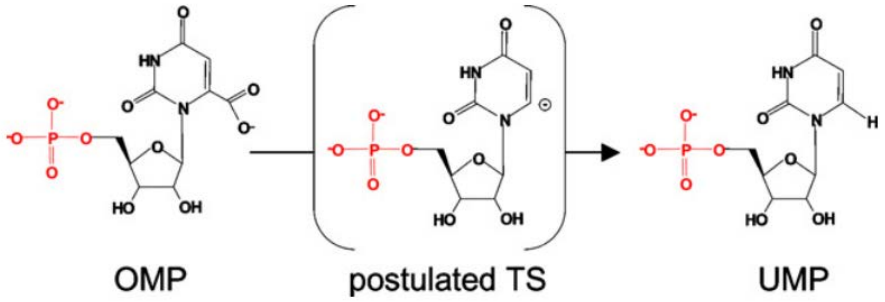
Problem Set 2

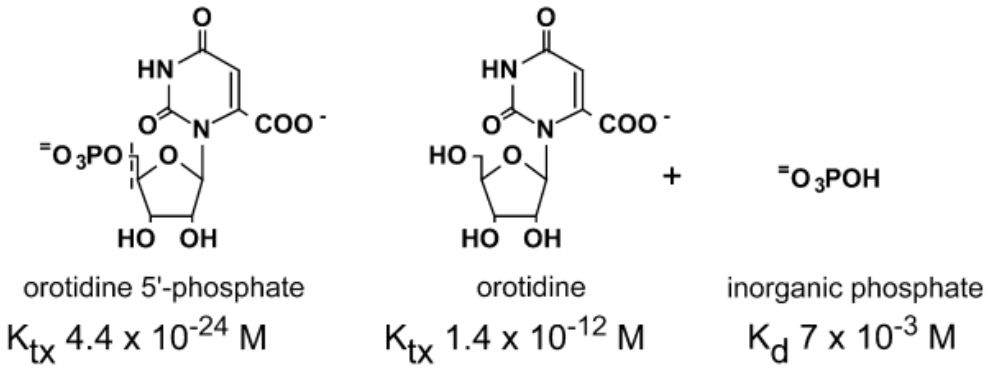
BMB178
Fall, 2016

Due 11/02/2016, noon
Office Hour: 7-9pm 11/01/2016, 121 Braun

Problem 1 (25 points):
Orotidine 5'-phosphate decarboxylase (OMP decarboxylase) catalyzes the following reaction:



(a) The estimated K_{tx} (maximal dissociation constant) for the transition state of the reaction is 4.4×10^{-24} M. Dividing the substrate into two pieces substantially weakens binding to the enzyme. For example,

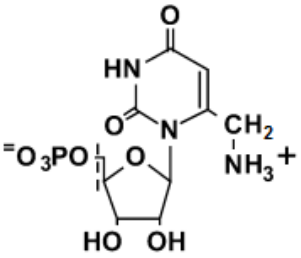


What is the effective concentration achieved by the OMP decarboxylase active site with OMP in the above case? Using a standard state of 1M, calculate the free energy change at 298K for the effect of dividing the substrate into the two pieces shown above. (6 points)

(b) Using PyMOL, create a figure of OMP decarboxylase (pdb: 1DQX) that illustrates the non-covalent binding interactions between OMP decarboxylase and an inhibitor, 6-hydroxyuridine 5'-phosphate (BMP). Using the figure and the discussions in lecture, explain how large rate enhancement can be achieved by OMP decarboxylase with OMP as a whole but not fragmented. (6 points)

(c) What type of inhibitor is BMP? Would you expect to see the same effect observed with dividing OMP for BMP? Why or why not? (5 points)

(d) Based on the crystal structure, suggest a possible role for residues 91 and 96 in the rate enhancement achieved by OMP decarboxylase. In light of this possible role, what differences would be expected between using OMP as a substrate versus the substrate illustrated on the right? (8 points)



Problem 2 (21 points):

To test how T7-DNA polymerase handles mismatches that might occur during DNA replication, the following experiment was carried out. DNA polymerase was allowed to synthesize DNA using the labeled primer/template complex:

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5′ – GCCTCGCAGCCGTCCAACCAACTCA  (labeled primer)
     CGGAGCGTCGGCAGGTTGGTTGAGTAGGTCTTGTTT – 5′  (template)
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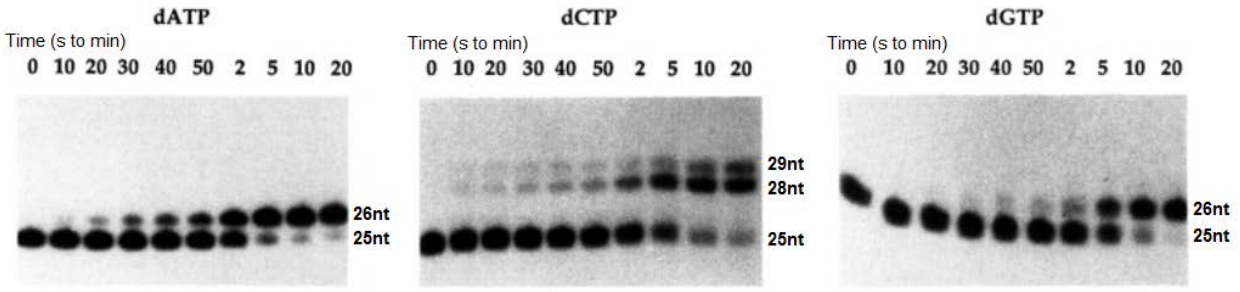
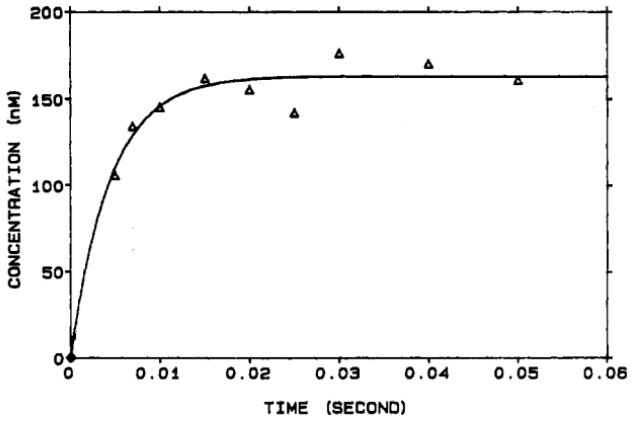
Replication reactions were carried out in the presence of only one dNTP and quenched at various time points. The DNA present at each time point was resolved via electrophoresis:

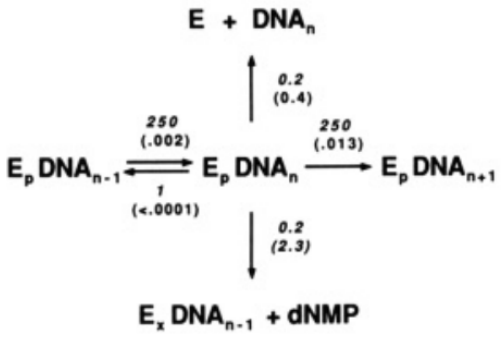


(a) What reaction(s) happened with dATP and dGTP? What reaction(s) happened with dCTP and why? Based on the data, estimate the half-time (the time where 50% of substrate is converted to product) for the reactions with dATP and dGTP. (6 points)

(b) In a separate experiment, the time course on the right was observed for the reaction using dTTP. Estimate the half-time for this reaction. What can you conclude about DNA synthesis with matched versus mismatched nucleotides? (6 points)



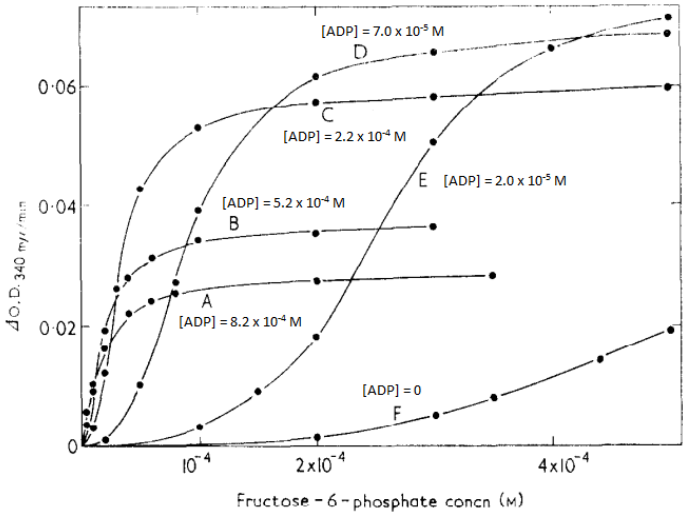
(c) In addition to the previous data, subsequent experiments were performed to build the model shown below. In this model, the left to center reaction represents incorporation of either a correct or incorrect nucleotide; the center to the right reaction indicates incorporation of an additional nucleotide after the first; the top reaction represents dissociation of the product from enzyme; and the bottom reaction represents transfer of the product to alternative sites for destruction. The rate constants for the matched nucleotide are shown in *italics*, and the rate constants for the mismatched nucleotide are shown in parentheses.



Based on the model, describe three different mechanisms DNA polymerase uses to ensure fidelity of replication. (9 points)

Problem 3 (30 points):

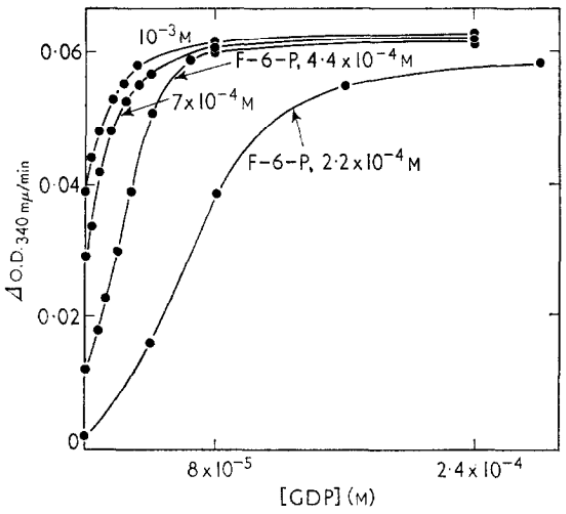
Phosphofructokinase (PFK) catalyzes the phosphorylation of fructose-6-phosphate to form fructose-1,6-diphosphate using ATP. PFK shows allosteric effect towards one of its substrates, fructose-6-phosphate (F-6-P), but not towards ATP. Monitoring the initial rates of F-6-P phosphorylation by PFK under different conditions yields the figure on the right. In this figure, the X-axis shows the concentration of substrate, i.e. F-6-P, and the Y-axis represents the reaction rate. From curve A to F, the ATP concentration is kept constant, but the ADP concentration gradually decreases from ~10^{-3} M to 0. Also note that ADP acts as a competitive inhibitor of ATP.



a) Judging from the shape of each curve, which ADP concentration gives the most allosteric effect? Which ADP concentration gives the least allostery? Please describe a qualitative trend of the Hill coefficient from curve A to F. (6 points)

b) Propose an explanation to the phenomena above based on the two-state allosteric model. Why does the allosteric effect change with different ADP concentrations? Why does the maximum reaction rate decrease at high ADP concentrations? (6 points)

c) GDP serves as an allosteric regulator of PFK analogous to ADP. The influence of GDP or ADP on the initial velocity of the reaction can also be cooperative, and the stimulatory effect of GDP is influenced by substrate (F-6-P) concentration (see figure). Based on the two-state allosteric model, explain the effect of substrate on both the shape and magnitude of the stimulatory effect of GDP. (6 points)



d) Phosphoenolpyruvate (PEP) serves as an allosteric inhibitor for PFK. What is the effect of increasing PEP concentration on PFK activity? Draw a figure to illustrate how the rate of reaction varies as a function of PEP concentration. How will the PEP inhibition curves differ at different concentrations of F-6-P? (8 points)

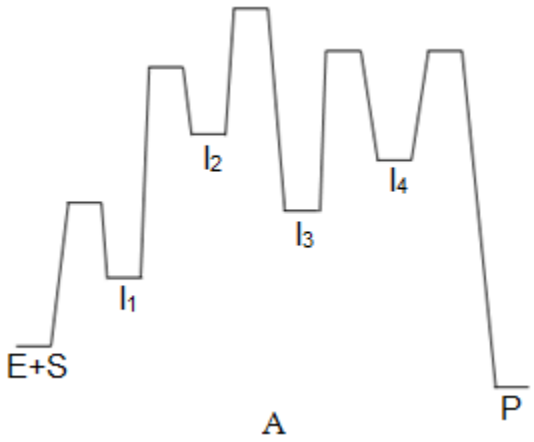
e) In the context of the glycolysis pathway, what is the importance of the fact that ADP stimulates PFK while PEP (a product of the glycolysis pathway) inhibits PFK? (4 points)

Problem 4 (24 points):

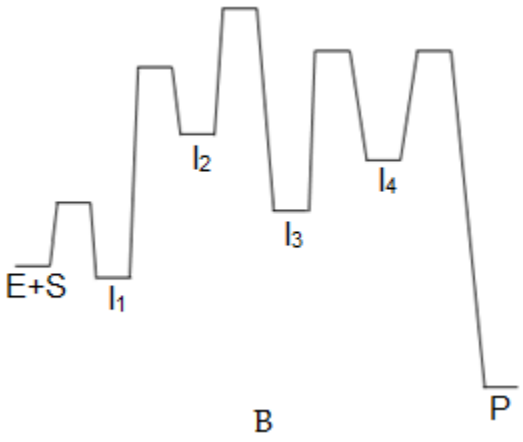
a) Consider the following reaction sequence, where substrate S is converted to product P by the enzyme E through several intermediates.

$$E + S \rightleftharpoons I_1 \rightleftharpoons I_2 \rightleftharpoons I_3 \rightleftharpoons I_4 \rightleftharpoons P$$

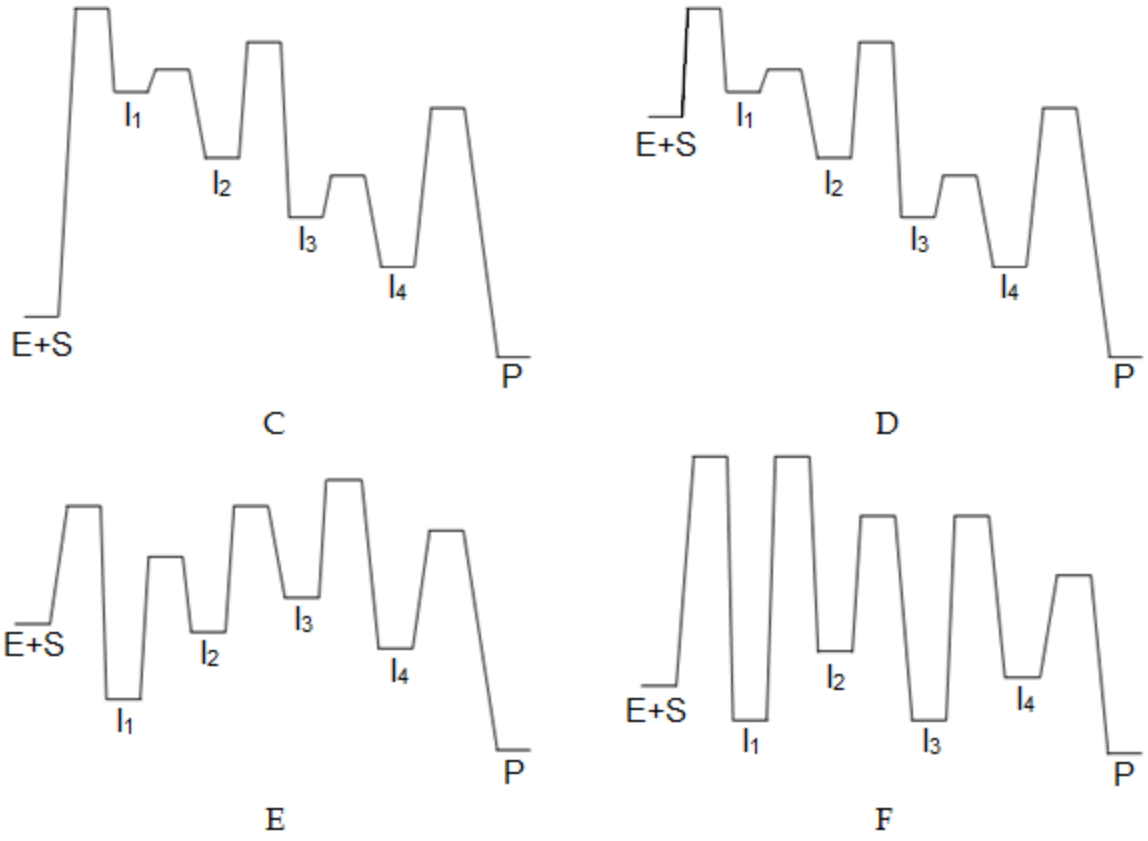
Identify the rate-limiting step in each of the following cases (A to F). Briefly explain your choices. (6 points)



A



B



b) Scenarios A and C above can be separated from scenarios B and D into two groups based on their starting reaction conditions. What distinguishes these two groups and how can a difference in starting conditions affect the rate-limiting step? Which of the two groups would scenarios E and F fall under? (6 points)

c) Consider the following reaction where two substrates, A and B, bind to the enzyme E.

$$E_1 \underset{k_2}{\overset{k_1 A}{\rightleftharpoons}} E_2 \underset{k_4}{\overset{k_3 B}{\rightleftharpoons}} E_3 \xrightarrow{k_5} E_1$$

Using the method developed by Cleland (Cleland, W.W., *Biochemistry*, **1975**, *14*, 3220), derive the overall observed rate constant for this reaction. Simplify the final answer (no fractions in the numerator or denominator). (6 points)

d) For the same reaction, assume that [A] is constant and saturating. Derive the apparent first and second order rate constants with respect to B (i.e. k_{cat} and $(k_{cat}/K_m)^B$). Explain the significance of these two rate constants. (6 points)