Problem Set 1

BMB178
Fall, 2016

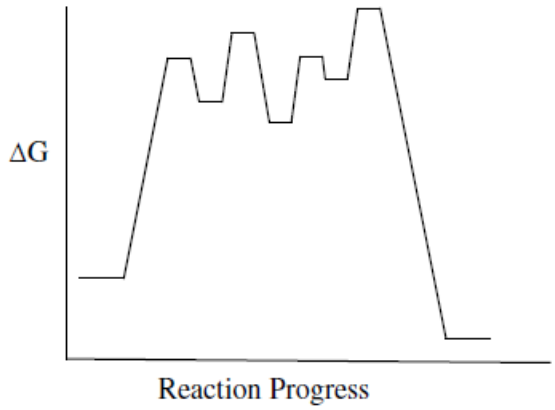
Due 10/14/2016, noon
Office Hour: 7-9pm 10/12/2016, 121 Braun

Problem 1:
Consider a general unimolecular reaction, where a substrate (S) is converted to a product (P):

$$S \underset{}{\overset{Keq}{\rightleftharpoons}} P$$

At 25°C, the equilibrium constant (Keq) is 250 and the rate constant of the spontaneous reaction, k_{uncat}, is 1.2×10^{-4} s^{-1}. The reaction can be catalyzed by an enzyme, E, which provides a 10 kJ/mol stabilization of the transition state without forming detectable intermediates. (25 points)

(a) Calculate ΔG° and ΔG‡ for both the uncatalyzed and enzyme-catalyzed reactions. (8 points)
(b) Calculate the rate enhancement k_{cat}/k_{uncat} of the enzyme. (5 points)
(c) Draw a free energy diagram to scale showing both the uncatalyzed and catalyzed reactions. Label the substrate, transition states, and product appropriately. Show and label ΔG° and ΔG‡ for both reactions. (5 points)
(d) Consider the following free energy diagram for a different enzyme catalyzed reaction. Label S, P and the three intermediates on this diagram. (2 points)



(e) The reaction progresses through three intermediates. Which intermediate would be the best structural mimic for designing an inhibitor of the enzyme? Explain your answer. (5 points)

Problem 2:
The work by Wang *et al* (*Biochemistry*, 1995) focused on the analysis of catalytic strategies taken by β-Glucosidase. (25 points)

(a) Draw the step-wise electron-pushing mechanism of a β-glucoside hydrolysis catalyzed by β-Glucosidase. The enzyme can be simplified to two amino acids at the active site in the reaction scheme. (3 points)
(b) What are the three evidences showing that Glu170 is a general acid-base catalyst? (12 points)
(c) Why did sodium azide rescue the activity of the enzyme mutant? (4 points)
(d) Considering Figure 6a, why are the two Brønsted slopes different? (3 points)
(e) In Figure 7, why isn't the rate of PNPG affected by azide concentration? (3 points)

Problem 3:
Alkaline phosphatase (AP) of *E. coli* is a very efficient enzyme catalyzing the hydrolysis of phosphate monoesters more than 10^{20} fold. (25 points)

(a) Download the structure 1B8J from PDB. This is an E. coli AP structure in complex with vanadate. What is the role of vanadate here? Is the structure more similar to the ground state or transition state? (5 points)
(b) Use Pymol to highlight the active site, and show the contacts between the vanadate and the enzyme. (3 points)
(c) According to the structure, what might be the catalytic role of R166? (2 points)

Please refer to Andrews *et al*. (PLos Biol, 2013) for the following questions.
(d) According to Table 1, what is the catalytic role of S102? Please explain based on the data. (4 points)
(e) Why did the authors measure the affinity of Pi to the enzyme instead of phosphate monoester in order to determine the ground state stability? What assumption did they make? (3 points)
(f) Why did the authors mutate R166? (3 points)
(g) In Figure 4a, why does the phosphate affinity of R166S show a bell-shaped pH dependence? How do the authors verify their prediction for the acidic pH region? (5 points)

Problem 4:
Please refer to the paper by Lassila *et al*. (PNAS, 2010). The authors analyzed the catalytic contributions of a *de novo* designed enzyme. (25 points)

(a) How were the retroaldolases designed? Please briefly describe the logic. (6 points)
(b) What are the three elements that were intentionally designed to contribute to the catalysis? According to the analysis in this study, how much does each of them contribute? (6 points)
(c) Why did the author compare the rate constants of enzyme-catalyzed reactions with that of free lysine-catalyzed reaction, instead of k_{uncat}? (3 points)
(d) In Figure 4b, why does the rate show maximum at pKa = 7.5? (4 points)
(e) What were the functions of Tyr78 and Ser87? Did they work as expected? Why or why not? (6 points)
(f) **(Bonus)** What are other possible elements that can accelerate the reaction rate? What experiments can be done to verify the assumption? (3 points)