Problem Set 1

BMB 178
Fall 2017

Due: 10/13/2017 (Friday), Noon
Office Hours: 10/11/2017
7-9pm 121 Braun

Problem 1 (20 Points):
In the study by Matouschek et al. (Nature 340: 122-126, 1989), the authors mapped the structure of the transition state of the unfolding reaction of barnase.

a) The approach involved careful selection of single residues for mutagenesis. In selecting these residues, what did the authors look for, and why was this important for the study? (6 Points)

b) The authors concluded that the transition state for unfolding of barnase is compact and retains considerable secondary structure. State specific evidence that allowed them to draw this conclusion. (6 Points)

c) In a similar study, the authors studied the unfolding of a small protein, p20. The free energy of unfolding is linearly dependent on urea concentration according to:

$$\Delta G = \Delta G_U^{H_2O} - m_U[Urea] \quad (1)$$

where m is a proportionality constant that measures the degree of solvent exposure. A similar equation relates the urea concentration and the activation energy of unfolding:

$$\Delta G_U^{\ddagger} = (\Delta G_U^{H_2O})^{\ddagger} - m_U^{\ddagger}[Urea] \quad (2)$$

The value of m_U from equilibrium unfolding experiments is 3.2. The data for the kinetics of unfolding are shown below, where k_U is the rate constant of unfolding. Calculate the fractional increase in solvent exposure ($m_U^{\ddagger}/m_U$) for the protein in the transition state of unfolding. What can you conclude about the structure of the transition state? (8 points)

[Urea] (M)	k_U (s^{-1})
3	3.16
4	5.01
7	12.0
8	15.8

Problem 2 (30 points):
The catalytic center of serine proteases is universally conserved. A crucial step in the reaction mechanism for serine proteases is the formation of an acyl-enzyme intermediate, during which the substrate becomes covalently linked to a catalytic serine in the enzyme active site.

a) Download the crystal structure of porcine pancreatic elastase complexed with human beta-casomorphin-7 (PDB: 1QIX). Create an image using PyMOL that shows the active site interactions that assist in the *breakdown* of the acyl-enzyme intermediate. Start by showing and labeling all the residues in the catalytic triad (Ser195, His57, Asp102) and the substrate (VEPI). Label the nucleophilic water and measure the distance between: (1) the nucleophilic water and the electrophilic carbonyl group in the substrate; and (2) the nucleophilic water and the general base that facilitate proton transfer. Lastly, suggest and label a reasonable proton donor that can help regenerate the hydroxyl group on Ser195 during the breakdown of the acylenzyme intermediate. (8 points)

b) The breakdown of the acyl-enzyme intermediate is energetically taxing due to charge accumulation on the reactive carbonyl of the substrate (forming an oxyanion). Elastase and other serine proteases circumvent this issue by placing <u>two</u> "stabilizing" residues in close proximity. Locate these residues using PyMOL and create an image showing the interactions. Briefly explain how the interactions aid in stabilization. (5 points)

c) Steady-state rate constants for pancreatic elastase and mutants are as follows:

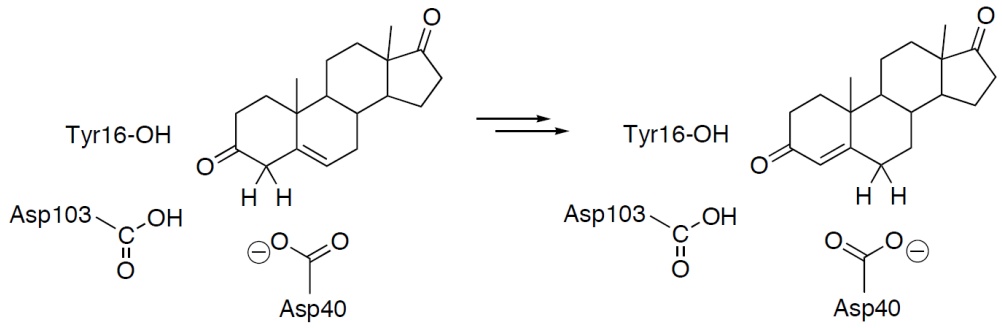
Protein	k_{cat} (s^{-1})	K_m (μM)
wild type	$(2.0 \pm 0.1) \times 10^{1}$	400 ± 25
D102V	$(5.5 \pm 0.3) \times 10^{-2}$	1250 ± 25
H57V	$(5.5 \pm 0.2) \times 10^{-4}$	900 ± 25
S195V	$(2.3 \pm 0.1) \times 10^{-5}$	700 ± 25
D102E	$(5.7 \pm 0.2) \times 10^{-1}$	410 ± 35
S195T	$(2.5 \pm 0.1) \times 10^{-1}$	590 ± 44
G193A	??	??

i) Consider the k_{cat} and K_m for the residues in the catalytic triad. What can you conclude about their roles in binding and catalysis? (5 points)

ii) Consider the mutations D102E and S195T. Why do these mutations cause a modest defect compared to the corresponding D102V and S195V mutations*?* What is likely to be the primary source of defect associated with these mutations ?(6 points)

iii) Imagine that a new student wants to engineer a mutation to *destabilize* the oxyanion hole during breakdown of the covalent intermediate. The student suggests that G193 be mutated to an alanine. Is this a good suggestion? Explain why or why not. If not, suggest an alternate amino acid to which you would mutate G193. (6 points)

Problem 3 (30 points):
Ketosteroid isomerase catalyzes the following reaction using three key residues: Tyr16, Asp103, and Asp40. (30 points)



a) Download PDB: 1OH0. Generate a figure showing the substrate analogue, key residues involved in substrate binding and enzymatic activity. (5 points)

b) Generate electrostatic view of the enzyme showing the substrate in substrate binding pocket. What can you infer from this diagram? What kind of interactions are mainly involved for binding substrate? (3 points)

c) Describe what effective molarity means and how it relates to enzyme function. (2 points)

d) Refer to Lamba V et al. (JACS 2016, 138, 9902-9909). What are the limitations of studying catalytic contribution from general base by using a mutant enzyme that can be rescued by exogenous general base? What did authors do to overcome this issue? (5 points)

e) Authors determined k_{base} values across different mutants and different exogenous bases. What was the reason that they excluded some of the values for determining 'real' effective molarity? (5 points)

f) Download PDB file 5DRE. This structure has D38G/P39G/D99N Mutations. Align this mutant to the structure 3M8C and make a figure showing the mutations. (5 points)

g) How did the authors determine if the mutation did not cause change in the mechanism? What was the prediction and what did they observe? (5 points)

Problem 4 (20 points):
Refer to the study by Andrews et al. (PLoS Biol 11(7)). The authors studied interactions between Alkaline Phosphatase (AP) and substrates to elucidate the possible mechanism of enormous rate enhancement achieved by this enzyme. (20 points)

a) What are some of the strategies used by AP to provide its enormous rate enhancement based on previous studies? (4 points)

b) Compare the scenarios in Figure 2C and 2D. By destabilizing the ground state, the barrier for reaction from E•S to E•P is reduced to allow the reaction to proceed faster. What additional effect does ground state destabilization have in enzyme activity? Why is this important? Think in terms of enzyme recycling. (6 points)

c) Why did the authors make mutations on R166? What is the function of R166 based on their structural analysis? (5 points)

d) The authors tested the pH dependence of P_i binding to AP. Why does R166S AP show a bell shaped pH dependence? How do the authors verify their prediction for the acidic region of this pH dependence? (5 points)