

BMB170c
Problem set 1
Regulated proteolysis

- 1.) Kenniston et al. (2003) studied the mechanistic details of protein degradation by bacterial ClpXP. Read the paper carefully and answer the following questions.
 - A.) State the authors' main hypotheses of the paper.
 - B.) A hypothetical protein composed of 120 amino acids (comparable length to Titin I27) has ΔG_u of 7 kcal/mole. When an ssrA tag is attached to the C-terminal end of this protein, ClpXP degrades it at an overall rate of $k_{deg} = 0.7 \text{ min}^{-1}$. Based on the results in this paper, estimate, for this hypothetical substrate, the binding affinity to ClpXP, the rate of translocation k_{trans} , the rate of denaturation k_{den} , the number of ATP hydrolyzed per molecule degraded, and the ATPase rate. Show your work/reasoning.
 - C.) Based on the results in this paper, do you think different protein substrates will bind to ClpX with similar or different affinity? Why or why not?
 - D.) From the results in the paper, which step(s) provide the main difference in the ATP usage for degradation of different substrates? Which step(s) is the rate-determining step in degradation of native protein substrates by ClpXP?

- 2.) Kisselev et al. (2006) reported the extent of inhibition of the protease activity in the proteasome core.
 - A.) What is the main conclusion of the paper?
 - B.) What could be potential problems of targeting protease active sites?
 - C.) From the results of this paper, and others that you have read, where would you target if you want to design a novel inhibitor of the proteasome? Why do you think your new inhibitors could be more superior than these existing ones in the paper?

- 3.) Wang et al. (2008) solved the structure of ClpS bound to the N-end rule substrate and elucidated the mechanism by which the substrate is specifically recognized by ClpS.
 - A.) Look at the structure (PDB: 3DNJ) and zoom in around the N-end recognition moiety. Show in a structural snap shot (similar to Fig 4A) and briefly explain how the structure rationalizes the results in Figure 4D. When you create the snap shots using the mutagenesis function of your

favorite PDB-rendering program, report % occurrence of the rotamer you pick. Discuss if their results in Figure 4D are obvious to you considering your *in silico* mutagenesis experiments, and propose plausible explanation for any discrepancy.

- B.) Propose biochemical experiments that would test the model in Figure 5, especially addressing the movements of substrate-ClpS-N domain of ClpA toward the ClpA chamber or the specific interaction that is replaced afterward.

4.) In 2000, the structure of a heterologous proteasome complex consisting of the 11S regulatory particle and the 20S proteasome was solved by x-ray crystallography (Whitby et al., 2000). The structure provided structural basis of the activation of the 20S particle, specifically the pore opening that presumably let substrates in or products out.

- A.) Where do most of the conformational changes in the 20S occur?
- B.) Describe the mechanism of 20S activation proposed by the authors, based on the structural changes they observed.
- C.) Propose a biochemical experiment to test this model that the author proposed. In other words, how do you test whether the movement of the N-terminus of the alpha subunits is important to the activation of proteasome.
- D.) Based on your answer in C, predict what the results of the experiment in each case would be if the substrates were small peptides (<4 amino acids long), denatured proteins, or folded proteins with degradation tags?

5.) Based on the results and the conclusions in Thrower et al. (2000), predict how the kinetic parameters (K_m and k_{cat}) would change (higher, lower, unaffected) in each scenario given below. Provide a brief explanation for each of your answer.

The experimental setup would be like that in Figure 2B, and, for comparison, K_m and k_{cat} values are 50 nM and 0.05 min^{-1} as reported by the authors. If the scenario does not involve change in substrate, take Ub₅DHFR as the substrate in that reaction.

- A.) If Uba1 is omitted.
- B.) If the substrate is Ub₉DHFR
- C.) If the substrate is Ub₃DHFR
- D.) If the reaction contains high concentration of MTX
- E.) If the substrate is Ub₅DHFR*, where DHFR* denotes a DHFR mutant that has lower stability.
- F.) If the substrate is Ub₅*DHFR, where Ub₅* denotes chain 11 from Table IIB of the paper.
- G.) If a hypothetical “extraproteasomal chaperone” is present (mentioned in the discussion part of the paper; assume this extraproteasomal chaperone

has been identified and purified; assume that the purified 26S proteasomes used in the experiments are intact and completely active and that each experiment is done side by side with the standard (like that in Figure 2E) to minimize the variation.).