BMB 170 2017

Problem Set 2: Proteins II (100 Points)

Due 10/24/2017 12:00pm

Please remember to use ray tracing (set ray_shadows, 0) to generate all PyMol figures.
Turn in as a HARD COPY or as a PDF FILE by 12:00PM 10/24/2017. Points will be deducted otherwise.
PS#2 office hour:

Monday 10/23, 4:00-5:00PM, Sherman Fairchild Library (SFL) Group Study Room 231

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Send questions and PDF file to: icho@caltech.edu

1. Protein Folding [28 points]

A. Levinthal's Paradox (i): Explain Levinthal's paradox, and explain how Levinthal's paradox leads to the conclusion that protein folding pathways more efficient than a random search must exist. [5 points]

B. Anfinsen's Dogma: In his experiment, Anfinsen showed that denatured ribonuclease refolded into the active form. Describe at least three ways in which this experimental setup is different from physiological folding processes. [4 points]

C. Folding in vivo – Single molecule methods: Most protein folding studies on single molecule levels are using either atomic force microscope (AFM) or optical tweezers, for instance, as in the paper by Naqvi et al. (doi: 10.1016/j.bpj.2015.05.028). How does the single molecular technique allow the study of an intermediate state that was not observed in ensemble experiments? Can using an optical tweezer introduce artifact/errors? How so? [7 points]

D. Folding in vivo – Protein expression (i): Give three reasons why overexpression of a complex eukaryotic protein might lead to formation of inclusion bodies in a prokaryotic expression system. [6 points]

E. Folding in vivo – Protein expression (ii): Suggest three ways in which one can manipulate E.coli for better expression of properly folded proteins. [6 points]

2. Protein modifications [31 points]

A. Chaperones: Give three reasons why molecular chaperones do not violate Anfinsen's self-assembly principle while facilitating the correct fate of proteins in vivo. [4 points]

B. Psychrophiles: Most of earth's biosphere is not at 37°C; in fact about 80% of the biosphere is permanently below 5°C. Psychrophiles are extremophilic organisms that can survive and often thrive in such cold temperatures. Explain why psychrophiles cannot share the mesophilic tools of protein synthesis, in terms of how

temperature affect the activity and stability of protein structures. What can happen if mesophiles were given with a complete psychrophilic protein synthesis pathway instead of their own, in room temperature? [6 points]

C. Post-translational modifications: Post-translational modifications play crucial roles in regulating gene functions in lots of biological processes. Name one modification and briefly describe how this modification is involved in regulation of protein function. [5 points]

D. Psychrophilic post-translational modification: It is vital for psychrophiles to maintain high capacity for post-translational modification. How would (1) the optimal temperature and (2) the expression amount of psychrophilic chaperones compare to those of mesophilic chaperones? [4 points]

E. Studying post-translational modifications: You plan to express a eukaryotic protein in yeast, and you would like to know if the protein of interest requires extensive glycosylation. Assuming that you know the exact sequence (and therefore the mass) of this protein and that the protein is likely to express very well in yeast, what experiments would you conduct to verify if the protein is heavily glycosylated? [5 points]

F. O-GlcNAcylation – Drug design: Post-translationally modified proteins are novel targets in drug design. O-GlcNAcylation has been shown to play a significant role in cancer development and is an interesting area to focus on in terms of anti-cancer drug development (Fardini et al., doi: 10.3389/fendo.2013.00099). Describe how the level of O-GlcNAcylation and its cycling enzymes such as OGT (O-glycosyl transferase) relates to cancer. How might targeting OGT be an interesting approach to overcome drug resistance in breast cancer? Why is it challenging to use O-GlcNAcylation of proteins as a mean to design anti-cancer drugs? [7 points]

3. Assemblies, viruses, symmetrical assemblies [10 points]

A. Oligomers: Many of the proteins in the Protein Data Bank (PDB) are oligomeric complexes consisting of two or more subunits that associate by rotational or helical symmetries. List 3 reasons why having a protein with oligomeric structure compared to a monomeric asymmetric sequence might be beneficial for a cell. [4 points]

B. Virus assembly: Viruses have protein shells surrounding their genome, and the viral shell is often highly symmetrical. Why is it preferable for a virus to have a symmetrical shell? While it should be easy to obtain the shell structure of a virus thanks to its symmetry, it is hard to get crystals of the shell of an enveloped virus. Why is that? [4 points]

C. Quasi-equivalence: In 1962, Donald Caspar and Aaron Klug developed theoretical framework accounting for the properties of larger (>60 units) particles with icosahedral symmetry. Explain the principle of quasi-equivalence. [2 points]

4. Metals and enzymes [31 points]

A. Transition state theory: Enzymes enhance biological reaction rate by 10^5 ~ 10^{20} fold compared to uncatalysed reaction. How does enzyme achieve such high rate enhancement? How does the free energy of reactants and products change for a catalyzed reaction? How about the magnitude of activation energy? Briefly explain the transition state theory. If necessary, include a figure. [6 points]

B. Metals in enzymes – coordination number: Enzymes employ transition metal cofactors that have tetrahedral (n=4) coordination geometry much more frequently than those that have octahedral (n=6) geometry. Based on this information, name two transition metal ions that are the most likely to be found in enzymes. Why would enzymes prefer tetrahedral geometry to octahedral geometry? [4 points]

C. Metals in enzymes – crystallographic method: Although a high-resolution structure of nitrogenase Mo-Fe protein was determined in 2002, the central light atom ligand of nitrogenase Fe-Mo cofactor has long been a mystery. In 2011, Spatzal et al. (doi: 10.1126/science.1214025) presented evidence for interstitial carbon in nitrogenase Fe-Mo cofactor. They used atomic-resolution x-ray diffraction data and an electron spin echo envelope modulation (ESEEM) analysis. Describe, briefly, the basics of the techniques employed and explain why these techniques provide a more direct and conclusive evidence regarding the identity of the interstitial atom. [6

D. Metals in enzymes – mechanistic insight: In a more recent publication, Spatzal et al. (doi: 10.1126/science.1256679) gain even greater detailed insight into the mechanism of nitrogenase. Describe the tricks that the author used to crystallize the protein in this paper. Why would crystallization with the substrate be so difficult? [4 points]

E. Metals in enzymes – low-temperature adaptation: Azotobacter vinelandii, the origin of the nitrogenase studied by Spatzal et al., is a free-living aerobic soil bacteria. Azotobacter species are ubiquitous and some are even found in Arctic and Antarctic soil. The cold climate of Artic and Antarctica poses a huge challenge to the basic Azotobacter nitrogenase with Fe-Mo cofactor. What alternative do Azotobacter species have to the Fe-Mo nitrogenase, and how does this alternative let Azotobacter to keep fixing nitrogen in cold environment? [5 points]

F. Biological vs. nonbiological catalysts: On one hand, enzymes sometimes provide a much higher reaction rate enhancement than nonbiological catalysts such as palladium. On the other hand, a vast majority of industrial catalysts are metals or nonbiological small molecules. What are advantages of using enzymes instead of a chemical catalyst? Why is a massive scale-up of enzymatic reaction difficult? [6 points]