

BMB170c, Problem Set 2, Protein folding and chaperone

(1) Folding potential energy pathway of ubiquitin.

- a. How do the authors detect changes in protein conformation?
- b. What is the origin of the fast ( $\mu\text{s}$ ) and slow (ms) unfolding populations?
- c. What are the differences between Figure 5a and 5b? Sketch the first panel of Figure 5b and label the energy wells with N (native), I (intermediate), and U (unfold).
- d. Using the first panel in Fig. 5b, please diagram the potential surface (i) if there are less interactions between strands I&II; or (ii) the intermediate is destabilized.
- e. What do you think mutant g does not show a fast unfolding population?

(2) Single molecule analysis of protein folding.

- a. How do the authors prove that the observed intermediate is on-pathway to the native state? Describe the three-state folding model proposed by the authors.
- b. Draw the theoretical force v.s. extension curves for a molten globule (non-cooperatively folded intermediate) protein, for a protein with multiple domains, and for a disulfide bonded-protein (with and without a reducing reagent).
- c. The observed intermediate on the folding pathway of a globular protein would be interesting to study. Please propose an experimental scheme to reveal the structural property of this intermediate.

(3) Polymorphism in amyloid fibrils.

- a. State the authors' main conclusion of this paper. What evidence did the authors provide to test their hypothesis?
- b. Based on the results in this paper, what residues and interactions are critical to form a different amyloid fibril? Why? What interactions distinguish the two types of fibrils?
- c. Both sets daughter fibrils have the characteristics of their parent fibrils, even though both sets of daughter fibril were grown under the same conditions. How would you find out which form of fibrils is the most stable under the conditions for forming daughter fibrils?

(4) How Gro EL folds protein.

- a. Based on the results in this paper, what role does ATP binding and hydrolysis play in Gro EL/S folding process?
- b. Rhodanese is a stringent substrate for Gro EL/S chaperone. If a less stringent substrate were used in this study, what results would you expect? Why?
- c. The Gro EL/S reaction cycle allows a substrate about 10 seconds to fold. How would the substrate folding be affected if this duration were much shorter or longer?
- d. Suppose that the authors used Gro EL mutants that have disrupted cooperativity in their assays. How would you expect the recovery efficiency of Rhodanese to be? How about assays using Gro EL with mixed subunits with non-hydrolyzable ATP analog (ATP- $\gamma\text{s}$ ), or ADP? Please answer this question based on Fig. 5F, G and H.