

**Agard Protein Folding: Discussion Paper P20**


**Discussion Questions:**

1. Why is there a need for active degradation?

2. What is an ssrA tag and why/how does it get point on proteins?

3. Fig 1: architecture of ClpX protein showing separation of unfolding function (clpX) and proteolytic cavity (ClpP). Discuss why is it important to control entrance into protease?

4. Fig 2. Why titan? Why carboxymethylated?

5. Fig 2. Discuss measurements of protein unfolding, residual structure, etc.

6. Why no dip at around 222nm?
   That is where \( \alpha \)-helices absorb, but this is All \( \beta \)

7. What is difference between 2D and 2E
   D. Uses tryp fluorescence to get at native vs denatured

8. How do structure and stability affect degradation rates? See fig 3A

9. If the effect of structure on kcat or km for interaction? See Fig 3B

10. What governs maximal degradation rate of unfolded protein?
    Time it takes to translocate, shown by peptide vs denatured titan

11. What is the role of ATP in degradation? Is it binding or hydrolysis? Why use ATP\( \gamma \)S?

12. How efficiently does ClpXP use energy of ATP hydrolysis?

13. Related to Fig 4B what are the two steps in the degradation process? Which is sensitive to mutation in the protein?

14. Discuss Fig 5. What do we think is the most relevant parameter that would match the degradation rate? Stability? Unfolding rate?

15. Why are the correlations so bad in Fig 6?