Microsecond Folding of the Cold Shock Protein Measured by a Pressure-Jump Technique

Discussion questions: may be asked on the in-class portion of the exam

1) Describe some of the goals of authors of the present paper
2) What are cold shock proteins?
3) How can one experimentally identify a protein as a cold shock protein?
4) Why is the kinetics of cold shock proteins relatively easy to study?
5) What contributions to the field of protein folding were made by the early (1970's) stopped-flow studies?
6) Why is photochemical triggering of a folding reaction suitable for the study of folding of redox enzymes such as cytochrome c, but not others, such as HIV protease?
7) What happens if a solution of unfolded protein is compressed such that the pressure increases significantly?
8) What happens is a solution of folded protein is compressed such that the pressure increases significantly?
9) What happens is a solution of unfolded protein is expanded such that the pressure decreases significantly?
10) Why does reaction volume decrease as proteins unfold even though intuitively the unfolded protein is larger than the folded protein?
11) What is the normal sea-level air pressure in bars?
12) What is a typical air pressure in car tires (hint: 1 bar = 14.5 psi)
13) What was the magnitude of pressure change in this work?
14) What is the advantage of large pressure jumps over smaller pressure jumps?
15) What is equation 1a good for?
16) In your PChem textbooks, you normally see that $\Delta G = \Delta H - T\Delta S$. However, equation 2 has additional terms besides ΔH and $T\Delta S$. Why?
17) What are piezoelectric crystals?
18) What is kaptan (kapton)
19) What is the purpose of the sapphire ring in the pressure-jump apparatus?
20) What signal was used to monitor protein folding and unfolding?
21) What is transition state theory?
22) What is the relationship between the Boltzmann constant and universal gas constant R?
23) What is the difference between Eq. 6 and Eq. 2?
24) Explain what is shown in Figures 2A and 2C.
25) What can we say about the change in reaction volume based on Fig 2B?
26) Explain the purpose of Fig 3 (A-D)
27) What is the effect of guanidinium hydrochloride on protein stability at constant temperature?
28) What is the importance of Figure 4C?
29) Briefly compare the folding behavior of Phe27Ala mutant and the wild-type protein
30) What happens to folding rates in 95% ethylene glycol? Why?

Advanced questions: may be asked for take-home exam:

1) Why is the kinetics of cold shock proteins relatively easy to study?
2) Explain and illustrate the use of photochemical triggering of a folding reaction
3) Why does reaction volume decrease as proteins unfold even though intuitively the unfolded protein is larger than the folded protein?
4) Derive Eq. 1
5) What was the usefulness of studying different Phe -> Ala mutants of CspB?
6) What are the advantages of P-jump technique over stopped-flow approach if one wants to know the numeric value of $\Delta S^{\neq}$?
7) What can you say about $\Delta C_p^{\neq}$ based on visual inspection of Fig 6B and 6C? Justify your answer.
8) What can you learn about the folding and unfolding process based on $\Delta C_p^{\neq}$ values?
9) Explain in less than 125 words what is enthalpy/entropy compensation and how is this relevant to protein folding
10) Explain what authors mean when they say that the activated state of folding of CspB follows Hammond-type behavior.