

### DISCUSSION SESSION ON LECTURE #3: STUDY QUESTIONS

ASSIGNED PAPER: Vogel M, Mayer MP, Bukau B (2006) Allosteric regulation of Hsp70 chaperones involves a conserved interdomain linker. *J. Biol. Chem.* 281: 38705-38711.

- (1) What is the definition of a molecular chaperone? What do you think is the mechanistic basis for why some chaperones are ATP-dependent and others not?
- (2) What is Hsp70? Where does the name come from? Would the work described have been possible without the previously determined crystal structure of this protein?
- (3) What is the physicochemical basis of each of the spectroscopic techniques used in this study and what questions was each spectroscopic method used to address?
- (4) What long-standing questions about how this chaperone operates does this paper attempt to answer? Did the work presented achieve its goal?

QUANTITATIVE QUESTION: Assume, first, that any residue  $n$  has three stable conformations ("rotamers") that can be described by the value of its  $\phi$  and  $\psi$  angles. Hence, for a protein of  $n$  residues in length, the possible conformations are  $3^{2n}$ , which is  $9^n$  or  $\sim 10^n$  (to make things easier). It is estimated that if a protein could randomly explore new conformations at the rate at which atoms reorient by rotation around single bonds, then the protein could explore  $\sim 10^{13}$  conformations per second. Even at this grossly overestimated speed, the time necessary for a small (e.g. 100-residue) protein to explore all the available conformations open to it would be:  $t = 10^{100} / 10^{13} \text{ s}^{-1} = 10^{87} \text{ s}$  (an absurdly long time, given that the apparent age of the universe is only supposed to be approx. 20 billion years, i.e.  $6 \times 10^{17} \text{ s}$ ). Yet, many proteins fold into their native conformation in seconds or less. These considerations suggest that sequences in proteins have some intrinsic propensity to operate as units or segments, rather than as individual residues, and achieve the final folded state via an ordered pathway and not by a random process. If so, how long a stretch of residues ( $n$ ), on average, would be required to act as such nucleating centers so that, if one of them was rate-limiting for the overall folding of the protein, that the protein would achieve its final fold in 10 s? Given that value of  $n$ , if those residues were all in an  $\alpha$ -helix, how many turns of the helix would that represent?