BIO-212. Lecture 4.
Exercises

Question 1.

Which of the following statements are TRUE, and which are FALSE?

a) Secondary structural elements of a domain generally pack so that a hydrophobic core is formed.
b) Most protein conformational changes involve breaking and reforming several covalent bonds along the polypeptide chain.
c) Two proteins that share more than 50% sequence identity over a 100-residue stretch are likely to have the same three-dimensional fold.

During a protein folding event, what happens to the entropy of water molecules? Explain your answer.

a) It increases
b) It decreases
c) It is equal to the protein entropy change
d) It does not change

A protein has two conformational states with different energy. Which of the following is not true? Explain your answer.

a) If the energy difference between the two states is equal to k_BT, they will be equally populated
b) With higher temperature it is more probable to visit the state with higher energy
c) The occupancy of the two states depends on the temperature
d) The occupancy of the two states depends on the energy difference between the two states

Question 2.

Trp	Phe	Leu	Ile	Met	Tyr	Val	Cys	Pro	His^0	Thr	Ser	Ala	Gln	Asn	Gly	Arg	His^+	Lys	Glu	Asp
−8.8	−7.1	−5.0	−4.5	−2.9	−2.9	−2.1	0.0	+0.4	+0.4	+0.8	+2.1	+2.1	+3.3	+3.8	+4.5	+7.5	+9.5	+11.7	+15.0	+15.0

Using the hydrophobicity scale above, calculate the hydrophobicity index of the central amino acid in the sequence and the average hydrophobicity for the 19 contiguous residue window defined by the following sequence:

Pro-Gly-Ala-Val-Val-Ile-Trp-Phe-Val-Val-Met-Ser-Ala-Ile-Ile-Phe-Tyr-Ala-Thr

Could this segment be part of a transmembrane helix?

Question 3.

The TIM barrel structure is a protein fold that occurs in approximately 10% of all enzymes. Evolved from an ancient ancestor, TIM barrels today can catalyze a wide range of biochemical reactions. Hence, protein designers are interested in building a TIM barrel from scratch (de novo) to understand how this fold works and to potentially unlock new functions (We will talk about protein design later). It took protein designers 25 years to tackle this problem, but in 2016 Huang et al. published the first crystal structure of a completely de novo designed TIM barrel (see Figure 1).

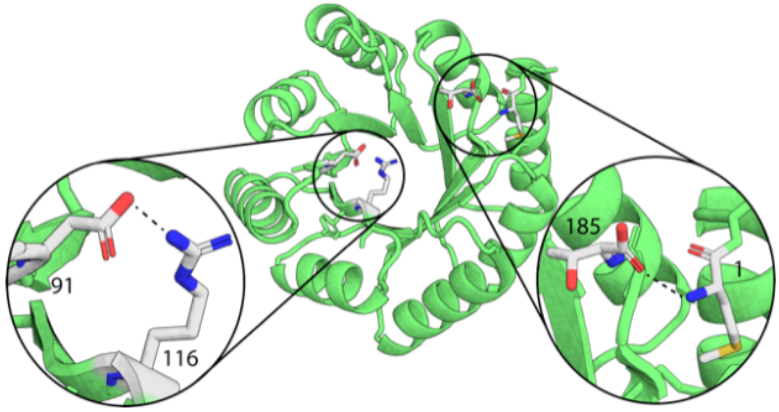


Figure 1. The first completely de novo designed TIM barrel (PDB ID: 5BVL)

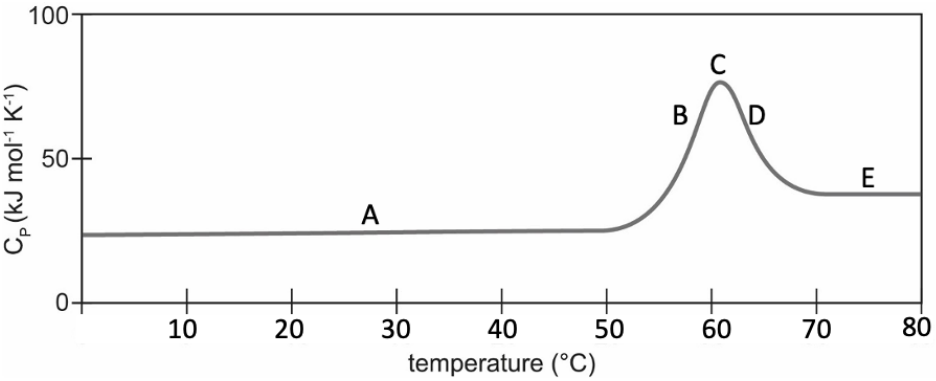
a. What secondary structural elements is a TIM-barrel composed of?
b. To which CATH class does this protein belong? Search online what the CATH classification is about?
c. Identify the amino acids and their interactions in the two zoomed region. Reason how pH conditions can affect these interactions

Question 4.

The two accessible conformations of a protein differ by 2 kJ/mol. What percentage of protein molecules will be in the higher energy state at 270K?

Question 5.

You are studying a recombinant protein you've designed, expressed, and purified in the lab. You are unsure of its thermal stability so you perform a calorimetry experiment and obtain themelting curve below.



A. What is the Tm?

B. At point 'D' on the graph, approximately what percent of proteins are still folded and what percent are unfolded?

C. Estimate roughly how much energy it takes to melt the protein.

Question 6.

The Boltzmann distribution describes the most probable distribution of particles in different energy states for a given system. Thinking about each particle as being a protein and remembering that each different possible conformation of a protein has a different energy, use the Boltzmann distribution to explain why at extremely high temperatures proteins are unfolded.

Question 7.

A protein can transition between two conformations which differ by 5 kJ/mol. Which of the following statements is false?

a. 15 % of the protein is in the higher energy state at 350 K
b. 10 % of the protein is in the higher energy state at 270 K
c. The protein can change conformation at room temperature
d. 90 % of the protein is in the lower energy state at 300 K

Question 8.

You are interesting in the protein called CLIMP-63. Search the web and use uniprot.org to characterize its cellular localization, structural classification, interaction with the membrane.