

1. Hemoglobin allostery and oxygen transport (35 pts):

(a; 10 pts) Briefly describe three steps, in terms of changes in protein and heme structure, in the allosteric induction of the R state of hemoglobin upon binding a molecule of O₂. What one word describes the shape of the resulting binding curve?

- O₂ binds to Fe⁺² in heme

2
(+1) Sigmoidal or cooperative

- ~~Heme~~ ~~protein~~ Leads to movement of Fe⁺² that flattens out closed T-state heme to flat R state heme

- This has the effect of pulling on the distal histidine (ligand of the Fe⁺²).

- Leads to a change in the position of helix F

- And that leads to a change in the inter-subunit interfaces which promotes conversion of neighboring subunits into the R state.

(+3)
each
up
to three
+9 total

(b; 25 pts: 5 pts each) For each of the following ligands, indicate whether it binds better to the R state or the T state of hemoglobin, and briefly describe the significance of the preference to physiological oxygen transport.

O₂ (Circle one: ⁺¹R ⁺²T) Significance:

Leads to cooperative binding of O₂, as subsequent O₂ molecules bind to Hb that's already ~~in~~ in the R state. Therefore more efficient delivery of O₂ to tissues.

H⁺ (Circle one: R ⁺²T) Significance:

Bohr effect - binding of protons ~~to~~ induces T state, so low pH in tissues (from CO₂) leads to ↑ release of O₂.

CO₂ (Circle one: R ⁺¹T) Significance:

Carbamate formation allows Hb to carry CO₂ to the lungs, and also stabilizes T state → ↑ release of O₂, and also contributes via Bohr effect.

NO (Circle one: ⁺¹R T) Significance:

- Hb carries •NO and ∴ protects it from decaying. Conversion to T state leads to release of •NO → vasodilation in active tissues.

BPG (Circle one: R ⁺²T) Significance:

- ⁺² - Decreases O₂ affinity relative to stripped Hb into useful range
- ⁺² - Allows for changing O₂ affinity in response to altitude

2. Protein folding and prions (25 pts):

(a; 9 pts) What is the molten globule in protein folding? How does the activity of chaperones in unfolding molten globules enhance the overall rate of folding to the native state?

- The molten globule is a pre folding intermediate with a hydrophobic core, substantial secondary structure, but incorrect/nonnative tertiary structure. Unfolding of the molten globule can be rate-limiting for folding of some proteins to the native state. (+2)
- Chaperones help accelerate the slow process of unfolding/disrupting the molten globule and allow the protein to "try again" to fold into the native state. (+2) (+3)

(b; 8 pts) Name a protein folding disease. Give two possible general causes for disease arising from protein folding defects.

(+2) for any one

BSE, vCJD, Alzheimer's, Type II diabetes, Kuru, Huntington's disease, cystic fibrosis

- General causes for disease can be

(+3) each for any two

1. Loss of function of protein product (e.g. diabetes, CF).
2. Toxic effects of aggregated protein, for example leading to immunological attack or apoptosis (Alzheimer's plaques?).
3. Toxic effect of possible intermediates along the aggregation pathways (e.g. possible membrane pores).

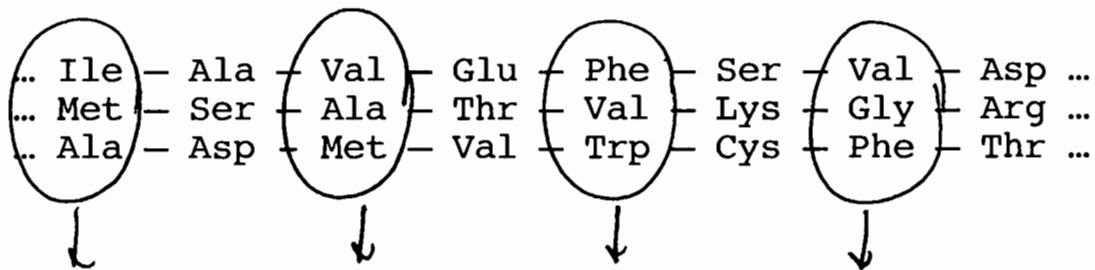
4. ?

(c; 8 pts) Give two rationales for the prevalence of independently folding 100-200 residue domains in proteins. How might you identify domain boundaries, either experimentally or by sequence analysis (pick one)?

- (+3) - Efficient use of "coding capacity" - it's the surface of the protein that does the work, no need to waste most of the amino acids by burying them.
- (+3) - Ability to evolve newer functions by mixing + matching functions encoded by domains, e.g. SH3 domains, ATP binding folds, etc.
- (+2) for either - Identify domains by increased sequence conservation (BLAST does this automatically) within a protein. -OR- Light tryptic digestion to preferentially cut inter-domain linkers

3. Protein sequence analysis and evolution (25 pts):

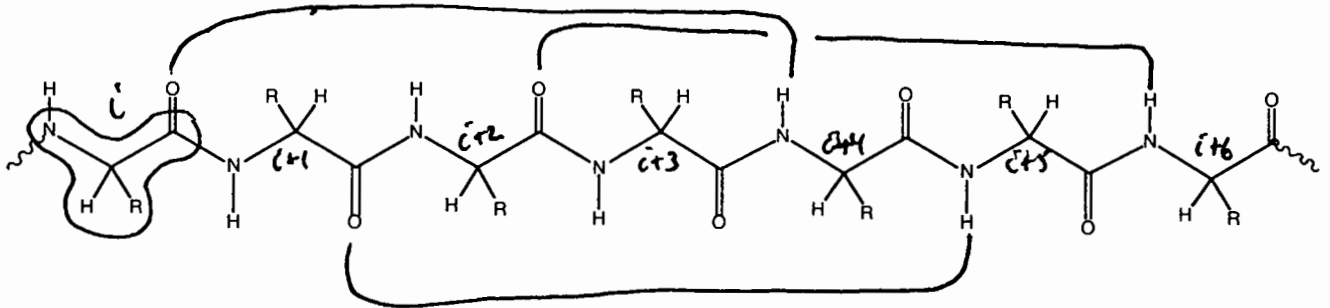
(a; 6 pts) The sequence below shows three segments of a protein region that binds retinol, a large hydrophobic ligand. Do you think the binding region is an α -helix or a β -sheet? Explain your reasoning. (Exaggerated from the actual sequence given by Branden and Tooze.)



- (+3) Alternating bulky hydrophobics and polar/charged residues
- (+3) Suggests a β -sheet, because the alternating sides would allow the h-phobic side to face in toward the ligand and the polar/charged side to be on the surface.

4. Secondary structure (15 pts):

(a; 6 pts) On the extended peptide representation below, diagram the hydrogen bonding pattern of the α -helix. In other words, draw in which three carbonyls are H-bonded to which three amides.



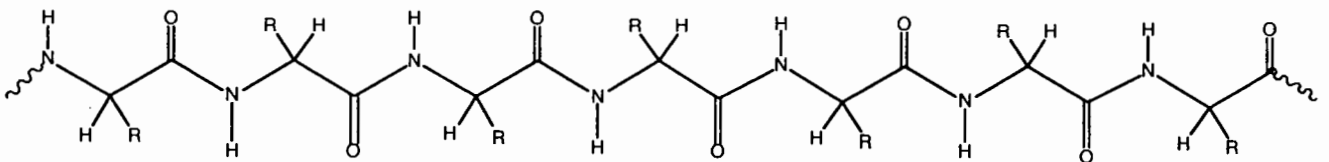
$i \rightarrow i+4$ H-bonding

(2) for each correct H-bond = +6

+3 total for consistent $i \rightarrow i+3$ or $i \rightarrow i+5$

+2 ~~total~~ total for any $N-H \cdots O=C$ H-bond

If you mess up, try again here:




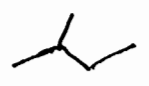
any \updownarrow

(b; 5 pts) What is the number of residues per turn for the α -helix? The $i \rightarrow i \pm$ (some number) H-bonding pattern you diagrammed in (a) connects amino acids that are one above another along the direction of the helix axis. How can this be consistent with a non-integral number of residues per turn?

(+2) 3.6 residues/turn

(+3) The "leading edge" carbonyl of residue i H-bonds to the "trailing edge" of residue $i+4$, therefore the points directly above and below each other represent ~~less than~~ less than a full four residues

(c; 4 pts) Why does leucine have a greater propensity to be found in the α -helix than isoleucine, whereas Ile has the greater β -sheet propensity of the two?

(+2) | Leu has an unbranched ~~side chain~~ ^{β carbon} $R =$ , whereas Ile is branched at C- β - $R =$ .

(+2) The β -sheet has more room near C- α , so bulky side chains, especially if β branched, encounter less steric hindrance. Leu packs better on the more crowded surface of the α -helix.

Score:	1. Hemoglobin allostery and oxygen transport (35 pts):	_____
	2. Protein folding and prions (25 pts):	_____
	3. Protein sequence analysis and evolution (25 pts):	_____
	4. Secondary structure (15 pts):	_____
Total: out of 100		_____