TT 1, Fall 2006

1	B	Take one amino acid at a time and eliminate as soon as one contains N, O or S
2	C	Hydrophobic effect postulates that hydrophobic and hydrophilic substances prefer to interact as less as possible; nothing to do with secondary structure stabilization
3	A	Attempts to play on characteristic of stretching wool, denaturation of collagen only modifies non-covalent intermolecular bonds, no effects on the covalent intramolecular bonds responsible for secondary structure
4	C	At this pH, Glu / Asp are negatively charged and Arg / Lys are positively charged. Add up all the charges, don't forget the ends of the peptide, even though they cancel each other out.
5	D	β-turns are located on the outside of globular proteins. This question simply tests the characteristics of β-turns.
6	C	The Ramachandran plot graphs the ψ bond between Cα-C and φ bond between Cα-N. It gives no information about the ω and χ bonds regarding the planar amide bond and side chain rotation respectively.
7	B	The active site of RNase contains a Lys and two His residues.
8	B	Only boiling in concentrated acid and certain proteins can break the stable peptide bond. Urea can only disrupt non-covalent bonds by competing for interactions.
9	C	Pay close attention to the composition as well as occurrence. Answer must be fully right.
10	B	In gel filtration, larger molecules elute faster than smaller ones. Also pH is only relevant for ion exchange.
11	C	Trypsin cuts at the C-terminal of basic amino acids. Remember peptides are always written from N to C terminals. Thus the "EH" fragment must be at the right hand end. In addition, notice that ii and iv are the only fragments of equal length, making them the only two possible choices.
12	C	DNA ligase seals in inserted DNA but does not itself introduce the DNA into the plasmid.
13	D	The Asp residue is responsible for stabilizing the His residue. Although this is a tough question, one can more easily eliminate the other three true options.
14	D	The major CD band is the biggest clue, indicates β-sheet structure. However location or exact % composition cannot be indicated.
15	A	Edman Degradation occurs from the N-terminus. Thus the first 5 residues must be the same, eliminating "b" and "c". Tryptophan and Tyrosine cannot be in the remaining peptide as it does not fluoresce.
16	B	Use the equation: Propensity of Ala for a-helix = $\frac{\%\ of\ all\ Ala\ residues\ in\ helical\ structure}{\%\ occurence\ of\ helical\ structure}$ For (a), the % Gly in either structure is low (21% and 14% respectively). For (c) the equation will prove the statement right. (d) is the whole point of Chou-Fasman rules as they were about 80% correct in predicting structure. You'll

		find that (b) is incorrect. It is IMPORTANT that you know the % occurrence of helical and sheet structure
17	D	The letter "O" is not designated to an amino acid
18	A	The β-sheet structure of the segment allows for the aggregation of the proteins into dimers, thus anything promoting dimerization is normal and NOT an explanation for a disease.

#26

This question combines the features of Ribonculease A and the experiments on Subtilisin.

1. Using Genetic Engineering techiques, generate a mutant RNase with serine residues at all or some of the cysteine residues in the native protein. This can result in several mutants, each missing one, two or three of the disulfide bonds. This will test both the necessity and sufficiency of each disulfide bond in refolding the active enzyme.
    a. Synthesize oligonucleotide for each mutant
    b. Splice oligonucleotide into vector plasmid using restriction endonculeases, polymerases and ligases
    c. Grow bacteria and extract required protein
2. For each mutant protein, treat with β-mercaptoethanol and urea then perform dialysis to study the refolding of the mutant (and subsequently activity) relative to the native protein.

@27

This question is no longer relevant for term test 1

TT1, Fall 2007

1	C	Concept similar to chirality, difference arises due to different possible arrangement of substituents around a central atom.
2	A	(2) is not a naturally occurring amino acid, (4) is isoleucine
3	D	Pay attention to identify the answer that includes ALL the amino acids with an oxygen, not just some
4	A	Trypsin attacks at the C-terminal of basic residues Lysine (K) or Arginine (R). Thus the final peptide fragments are: K K DENYVIPLR D. The largest

		fragment contains two acidic residues (negative at about neutral pH) and one Basic residue (positive at about neutral pH) thus a net charge of -1.
5	B	The point of the hydrophobic effect is that unlike molecules would prefer to interact at little as possible. Thus less surface area of the oil droplet is less for the water to have to deal with.
6	D	Hydrogen bonds involve the carbonyl AND the amino groups, also bonding patterns differ between the two structures. Urea disrupts bonds by competing for hydrogen bonds.
7	A	A little tricky, this is the MOST right answer, one may argue for (b) or even (d), however (a) is undisputable.
8	C	Behold the most controversial question of 2007, this question may have been dropped from the test. In essence, the even distribution eliminates (a) and (b), while (d) was irrelevant. However, evidence for (c) cannot be extracted "directly" from the diagram, though by process of elimination it is right.
9	D	Water at room temperature has no effect on collagen.
10	C	A tough question, one can better solve this by process of elimination. Proline locks the φ bond in place but does not drastically change it.
11	C	Use they hydrophobic effect to explain this, head groups like water, tails like each other. Should a hydrophobic solvent be used, (d) would be correct but is the least right of the two.
12	C	The lack of β-mercaptoethanol prevents proper disulfide links, urea competes of H-bonds.
13	A	Cutting with chymotrypsin cuts at the C-terminal of residues with hydrophobic aromatic side chains (Tyr, Trp, Phe). This leaves only (a) as a possibility. (b) will have only 4 Arg-Phe dipeptides. (c) will have 4 and one with the Met attached. (d) gives various di/tripeptides
14	A	First you need a restriction endonuclease to cut, two are needed to cut at two places in order to remove the original. To "close up" the plasmid, DNA ligase is required.
15	C	Watch out for "never" and "always". Ramachandran plots display data for all angles regardless of structure. Process of elimination is easy here.
16	B	Amide bands are relative to the environment surrounding they carbonyl group. Thus the signal will differ based on hydrogen bonding or any effects on the C=O bond.
17	A	CD allows one to study the secondary structure makeup of proteins, each type of structure gives a particular signal, its strength is proportional to the structure's prevalence in the protein. No information can be gained as to where the structures are in the sample.
18	D	The method is based on the original database; it is illogical to use the method to prove its own roots wrong. Thus this circular reasoning cannot happen.
19	D	Tricky question, numbers are very close for (a), (b) and (c), however (d) is the answer on the basis that neither of the residues (His, Arg, Thr) are good at forming ANY secondary structure, thus most likely this group will be unstructured.
20	D	Easy way to answer this question quickly is to look for the vowels "U" and

		“O” as they do not represent amino acids.

21.

- Use gel filtration to separate the sample based on size. The matrix will slow down smaller molecules while allowing larger ones to pass more freely through, thus protein #2 will be eluted far quicker than any of the other three.
- Use a cation exchange column at neutral pH. This column will have negatively charged groups bound to its beads, slowing down the positively charged protein #4 due to its abundant lysine residues. Proteins 1 and 3 are not charged at neutral pH, thus will not be affected nearly as much.
- Use HPLC to separate 1 and 3, protein #3 has a higher makeup of hydrophobic lysine residues than #1, thus it will be slowed down more than #1. This will separate the two proteins based on hydrophobicity, with #1 eluting well ahead of #3.

22.

- We would expect the activity of the double mutant to be lower. Asn-155 contributes to the active site and the activity of the enzyme by hydrogen bonding with the substrate using its amino group. This is a critical step in destroying the stability of the amide bond and splitting the substrate. Methionine contains no hydrogen bond donors in its side chain and therefore cannot complete the same task. Therefore the double mutant would be less efficient than the single mutant.

23.

- Treat normal BPTI with β-mercaptoethanol and urea before performing dialysis to remove the chemicals and allow the native protein to refold. Perform the same experiment on the mutant BPTI, a loss of activity will confirm that the structure is not the same as native BPTI
- Using an affinity column bound with AAARRWWWWW peptides, introduce a solution composed of trypsin with excess mutant BPTI before a wash-off. Should the BPTI retain proper structure and properly inhibit trypsin, the column will fluoresce upon excitation with UV radiation as the fluorescent residues remain. If the mutant BPTI fails, indicating its structure is not identical to native BPTI, the trypsin will cleave the fluorescent residues which will be washed away, showing no fluorescence

TT #1, Fall 2008

1	A	This may take a while to think through, try to eliminate all the more prominent amino acids that you know definitely don't have a methyl group, (c) and (d) are easily eliminated.
\ 2	C	HKR are positively charged at pH 2. At pH 8.5, H is uncharged, DE are negatively charged and RK are positively charged
3	B	Electrons for H-bonds can come ONLY from O, N or F
4	D	The carbonyl group is responsible for most of the H-bonding stabilizing secondary structure
5	D	Every copy of the same protein will have the same structure at the "unstructured regions", these regions simply do not fall into the more common secondary structure definitions.
6	C	Less solvent required to solvate the oil droplets is more favourable entropically.
7	A	Collagen helical structure is not reliant on H-bonds from Hyp
8	D	The strands of collagen have simply been rearranged to form gelatine
9	D	The Ramachandran plot gives no information about location or side chains.
10	D	Anfinsen's experiment never specified the order of folding
11	C	Similar to a reaction diagram, an energy barrier exists in between the unfolded and folded state of a protein, preventing spontaneous changes
12	A	Analyze step by step, cutting with trypsin eliminated (c). Cyanogen Bromide eliminates (b) and (d).
13	C	Hydrophobicity is irrelevant here, larger proteins elute faster from a gel filtration column
14	B	The gene insertion is already done, so the process is past endonucleases and ligase. You simply need to insert the plasmid into a culture (transform) and grow then extract.
15	C	2' is the wash-off of the column, which does not contain the protein that the labelling antibody is specific to.
16	A	Asp acts only to stabilize His, which in turn hydrogen bonds the substrate but has no covalent interaction. Met has no direct effect on the catalytic triad.
17	D	Stronger hydrogen bonding gives a lower signal, thus β-sheets have stronger H-bonds
18	A	Use $$\text{Propensity of Ala for a-helix} = \frac{\%\ of\ all\ Ala\ residues\ in\ helical\ structure}{\%\ occurence\ of\ helical\ structure}$$ As a sample calculation to calculate Propensity for the amino acid to be in each type

		of structure. $P\alpha = 1.58$, $P\beta = 1.00$, $Pt = 0.63$
19	D	Replacing the normal Glu-6 with Asp-6 makes essentially the same molecule but missing one methylene group. This experiment does not directly enforce the fact that hydrophobicity modifications are responsible for aggregation.
20	D	"O" and "U" are easiest to find in such questions, (c) contains a "B"

21.

- With silk, the amino acid sequence is made up of small residues (Gly, Ala, Ser) that allow for extensive hydrogen bonding with minimal steric interference. This allows the silk fiber to be strong and resistant to tension
- With collagen, every third Gly residue allows for tight packing between the strands as it has minimal steric interference. Along with the inflexible structure of Pro and Hyp, the close packing and rigid collagen structure is resistant to stretching
- With keratin, the multitude of Cys residues allows for extensive disulfide bridges the form strong covalent bonds between strands. These bonds provide the major restoring force when stretched, the more concentrated these bonds are in a given substance, the harder and more rigid it is.

22.

i. The peptide contains at least one Tryptophan or Tyrosine residue, most likely Tryptophan as the fluorescence is strong.
ii. Tryptophan cuts at Arginine or Lysine residues, thus the 4th or the 6th residue is either R or K.
iii. The Tryptophan/Tyrosine residue(s) are located within the first 6 residues from the N-terminal, this does not dismiss W/Y residues in the last 4 residues that were cleaved off either.
iv. The peptide most likely contains a polar group and a non-polar group separated towards the sides of the protein
v. The fluorescent W/Y residue(s) are located within the first two residues from the N-term. In addition, the polar group may have been cleaved off as the peptide no longer dimerizes, suggesting a major change in the organization of hydrophobicity within the peptide.

General outline: Polar residue-W/Y-V-V-V-R/K-I-I-I-I

ONE of many possible sequences: E-W-V-V-V-R-I-I-I-I

23. The glucagon peptide is known to exist predominantly in a β-sheet gel-like state at high concentrations. Now we assume that this β-sheet state is biologically active *in vivo*. Should the mutations mentioned occur, the peptide sequence responsible for the transformation would be more likely to form β-sheets than the native peptide as there is a large difference between its (Pβ) and (Pα). Seeing how the β-sheet state is assumed to be responsible, the mutated peptide should still be biologically active.

Experiment: Acquire samples of both glucagon types at a biologically equivalent concentration. Perform CD on both samples. According to Chou-Fasman rules and as mentioned above, the mutated peptide should exist predominantly in a β-sheet form. This can be observed in a more prominent peak at -217 and less prominent peaks at +195, -208 and -222 for the CD spectrum of the mutated glucagon sample when compared to the native sample.