Student Name : _____ 2014-02-14
Student ID : _____

Instructions:

Write neatly and clearly. Cross out with a single line any material you do not wish to have marked. Marks will be deducted for incorrect statements. Students must work independently and may not knowingly utilize resource materials or share resource materials with other students. Students may use pens, pencils, erasers and calculators only.

Electronic devices including cell phones, personal information managers and audio devices are prohibited.

Question	Mark	Total Marks
1	62%	6
2	67%	8
3	88%	3
4	48%	4
5	51%	11
6	73%	8
7	71%	3
8	70%	4
9	69%	3
10	56%	9
Total	62%	**59**

1 – In the space provided, give unique definitions or descriptions of the following biochemical terms and phrases: (6 marks)

(a) electrophoresis Migration of ions in an electric field

(b) homologous Derived or evolved from a common ancestor

(c) neutral drift A random mutation in a well adapted protein that does not affect function

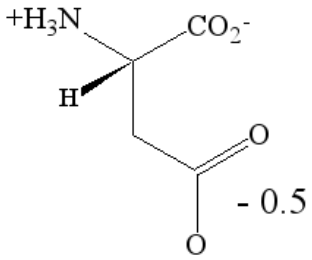
(d) domain Structurally independent units with characteristics of small globular proteins

(e) zwitterion Molecule containing a positive and negative charge functional group at the same time

(f) amphipathic β-sheet Sheet with both a non-polar and a polar surface.

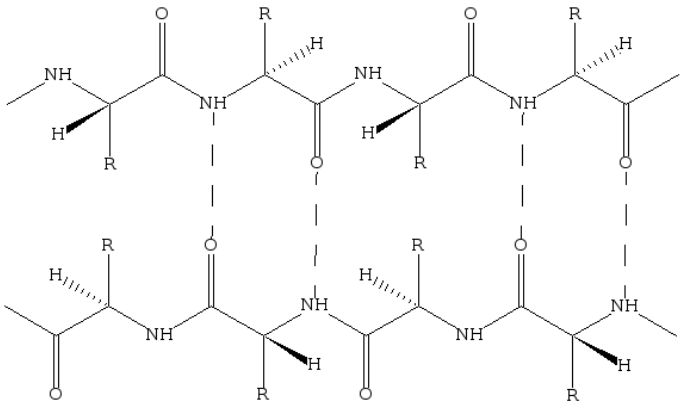
2 – Draw the following structures: (8 marks)

(a) L-aspartate at pH 3.9 (note: make sure you draw the L-isomer)



Structure, L-isomer, charge – 1 each

(b) An antiparallel β-sheet composed of two β-strands, each of four residues in length. Represent each of the side chains with the generic symbol R. Indicate all H-bonds between main-chain atoms.

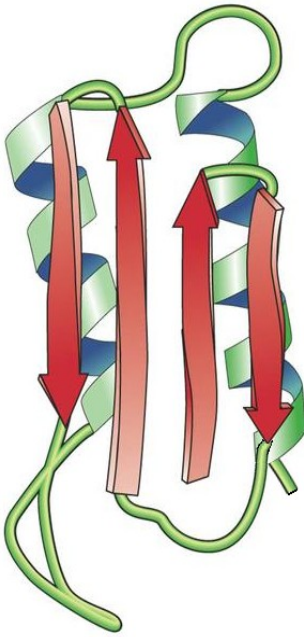


Backbone & R, antiparallel, 4 residues, H-bonds (two sets) – 1 each

3 – The following is a fragment of a primary sequence alignment of an inositol phosphatase from several microbial organisms. Classify each of the primary sequence positions as I (invariant), C (conservative substitutions) or NC (non-conservative substitutions). (3 marks)

Glu	Gln	Val	Ile	Val	Phe
Glu	Pro	Val	Ile	Ala	Phe
Asp	Lys	Val	Ile	Ile	Phe
Glu	His	Thr	Ile	Leu	Trp
_C_	NC_	NC_	_I_	_C_	_C_

4 – Consider the structure of the prion (PrP_{sc}) in its disease causing form. Answer the following question: (4 marks)



(a) Classify the domain fold of the prion protein.
α + β

(b) Identify the β-sheet type.
Mixed

(c) Once a domain fold has been classified, the protein structure is given a descriptive name that communicates the relative disposition of its secondary structures (eg. αβ-barrel). Give a reasonable descriptive name for the prion protein domain fold.
2 layer αβ open sandwich

5 – Provide short answers or fill in the blanks for the following questions: (11 marks)

(a) What is the pI of the Ile-Tyr-His-Pro tetrapeptide?

Between pH 6.0 and 9.4, the predominant ionizable species are:
α-carboxyl negative; His neutral; Tyr neutral; α-amine positive and the net charge is near zero.
PI = (6.0 + 9.4)/2 = 7.7

(1 mark each for ionizable groups, pH range with net charge zero and answer)

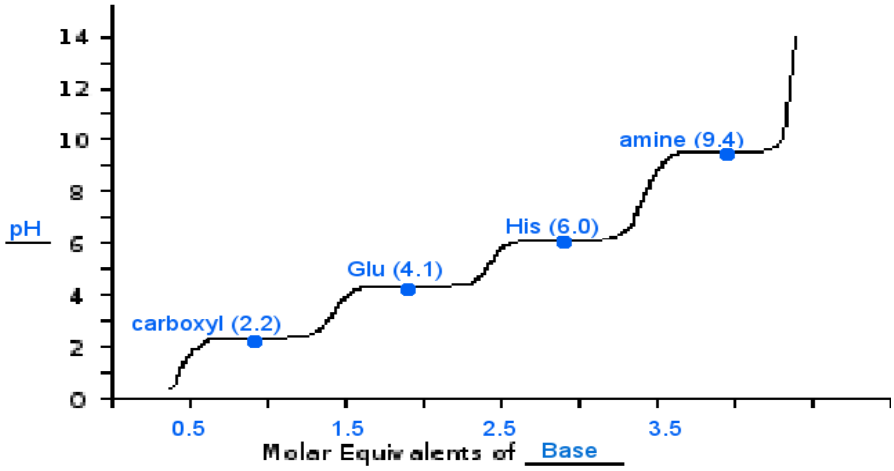
(b) The pleat in a β-sheet minimizes __steric conflict_ between _main-chain_ and _side-chain_ atoms.

(c) In long α-helices, the __3rd_ residue of the helix forms a _hydrogen bond_ with the 7th residue of the helix.

(d) Linus Pauling was able to predict the existence of polypeptide _secondary structures_ by assuming the backbone must adopt _a low E conformation_ that satisfies its _main-chain H-bonding potential_ and efficiently fills space.

6 – You have been provided with the following titration curve of an unknown oligopeptide. Answer each of the following questions. (8 marks)

1 mark pH
1 mark OH^-
1 mark for x axis numbers
0.5 marks for each pKa identified (number or name)

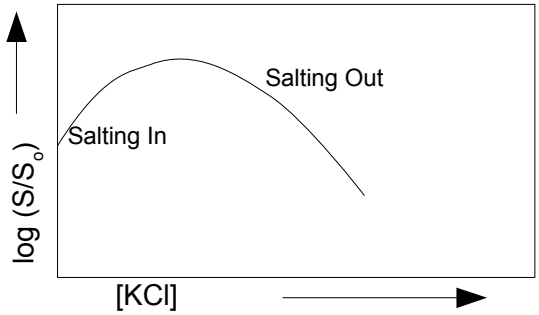


(a) Label all significant features of the titration curve. Fill in the blanks and complete the labeling of the x axis.

(b) What can be concluded about the identity of residues that comprise the oligopeptide?

One histidine and one glutamate (or aspartate) are present. There may be any number of additional non-ionizable residues (up to ~20 residues) that would not be detectable.
(1 mark for his, 1 mark for glu, 1 mark for additional non-ionizable)

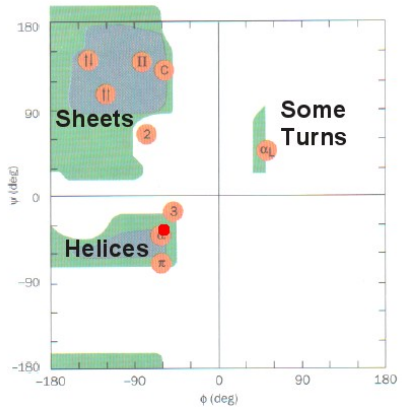
7 – Draw a protein solubility vs. [KCl] curve for a typical globular protein. Clearly label each part of the curve. (3 marks)



1 mark curve
1 mark salting in
1 mark salting out

8 – Assume a Phe residue has main-chain torsion angles of ϕ=-60° and ψ= -40°. Draw a Ramachandran plot (with labeled axis) and place the Phe residue on the plot with a circle. Is this an energetically allowed conformation for a Phe residue? (4 marks)

2 marks for plot, 1 for Phe, 1 for energetically allowed



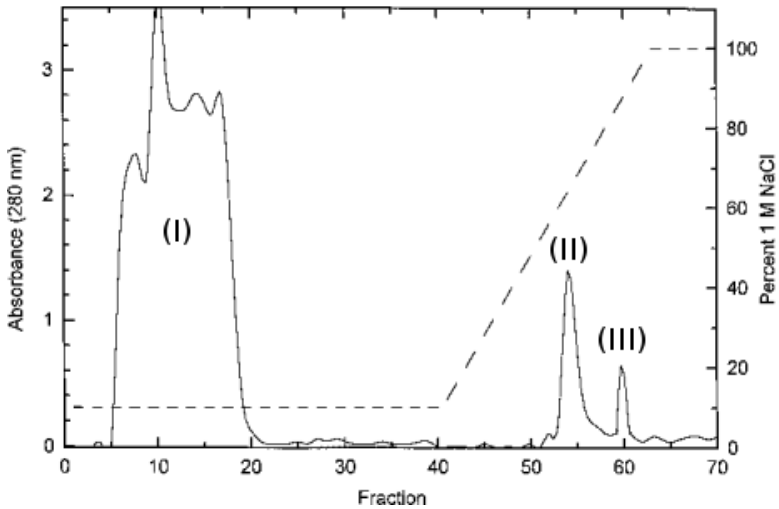
9 – Amino acid residues are not randomly distributed within the tertiary structures of proteins. What is the characteristic distributions of polar charged and non polar residues within protein tertiary structures? (3 marks)

Polar charged residues are almost exclusively found on the surface of tertiary structures. Only rarely are polar charged residues located within the hydrophobic core and always for functional reasons.

Non-polar residues make up almost all of the hydrophobic core and roughly half of the surface of tertiary structures.

10 – You want to study the protein methionyl aminopeptidase which is a crucial enzyme in tissue repair and protein degradation. It catalyzes the removal of amino terminal methionine residues from proteins that are being degraded. (9 marks)

First, you have to purify the enzyme from the cell extract. You are starting with an cation exchange chromatography at pH 6.5 resulting in the following chromatogram:

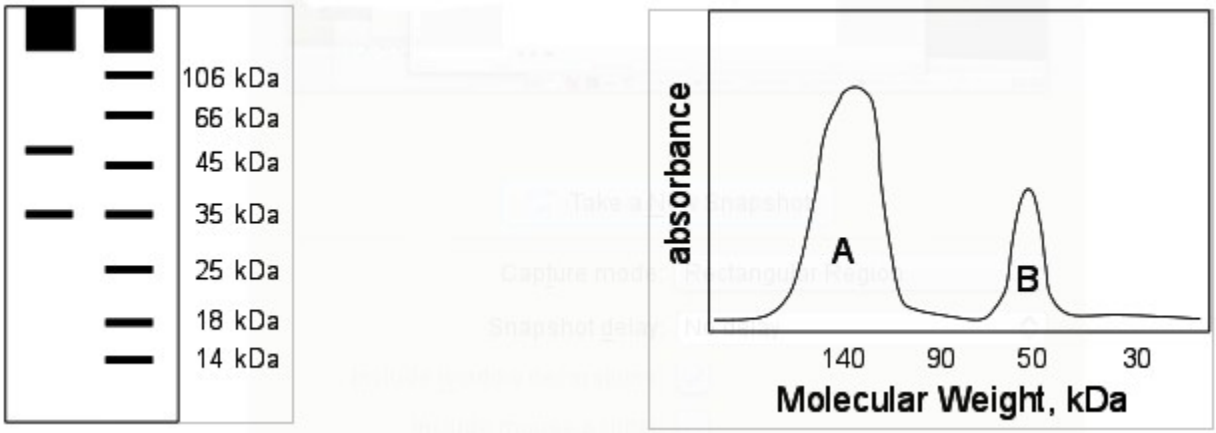


(a) What can be concluded regarding the pI and relative charge of the proteins in peaks (I), (II) and (III) of the chromatogram?

Peak I - neutral or negative charge at pH 6.5; pI is 6.5 or lower
Peak B - relatively smaller net positive charge at pH 6.5; pI > 6.5
Peak C - relatively larger net positive charge at pH 6.5; pI > 6.5

2 marks for each peak (1 for charge and 1 for pI)

Using an assay for methionyl aminopeptidase activity, you find out that your enzyme of interest has eluted in peak (II) (above). Next, you have combined these fractions (ie. peak II) and analyzed them by SDS-PAGE (left) and size-exclusion chromatography (right) with the following results.



Using the above mentioned assay, you can detect methionyl aminopeptidase activity in peak A, but not in peak B.

(b) What can you conclude about the composition of each of the peaks in the chromatogram?

Peak A contains the methionyl aminopeptidase activity which has an overall mass of about 140 kDa whereas peak B contains an unkown protein of about 50 kDa. On the SDS-PAGE, i.e. under denaturing conditions only bands of 50 kDa and 35 kDa can be detected for the sample loaded onto the size exclusion chromatography. This indicates that the unkown protein is a monomer of 50 kDa. Methionyl aminopeptidase must consist of multiple subunits to account for the oberseved mass in SEC. Most likely, it is a tetramer of 35 kDa subunits (4 x 35 kDa = 140 kDa).