Student Name : _____ 2013-10-17
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		10
2		10
3		9
4		8
5		4
6		6
7		5
8		7
Total		**59**

Short Answer:

(1) **In the space provided**, give short answers to the following questions: (10 marks)

(a) What is the purpose of the 'fragmentation step' in chemical sequencing of proteins?
Produce peptides of < 50 residues as the Edman sequencing is not reliable for longer peptides.
2 marks

(b) What are the advantages and disadvantages of traditional chemical sequencing methods for determining a proteins primary structure?
Advantages include the ability to detect quaternary structures, disulfide bonds, post-transcriptional and post-translational modifications.
Disadvantages include time and cost. (Additionally it is not always possible to sequence the entire polypeptide).
4 marks

(c) How is capillary electrophoresis an improvement over traditional electrophoresis methods?
Much faster as higher voltages can be used due to superior cooling properties. Greater resolving power and lower detection limits due to reduced convective mixing which produce exceptionally sharp peaks.
2 marks

(d) Explain the 'Levinthal Paradox' and its major conclusion?
Simple calculations indicate even the smallest proteins cannot sample all possible conformations in a search for the lowest energy state as it would take millions of years for the search to complete. Knowing proteins fold within seconds indicates proteins must fold via some type of pathway.
2 marks

Applications:

(2) You wish to synthesize the tetrapeptide HELP. You have access to following listed reagents:

Boc-Pro,	Fmoc-Glu γ-benzyl ester ,	Fmoc-Leu,	N-ethylmaleimide,	Boc-Glu,
Boc-His dibenzyloxycarbonyl ester,		PITC,	Fmoc-Lys,	Boc-His,
dicyclohexylcarbodiimide,		PTH-His,	Fmoc-Lys benzyloxycarbonyl ester,	
Chloromethyl polystyrene resin		Agarose	and any required solvents.	

Answer the following about the synthesis (use complete names). (10 marks)

(a) The last step of the synthesis is coupling the _____ Boc-His dibenzyloxycarbonyl ester__ to __Glu γ-benzyl ester – Leu – Pro_ in the presence of _triethylamine (mild base)_ .

(b) After an initial coupling reaction, unreacted materials and solvents are rinsed away and the _Boc_ protecting group is removed from the coupled amino acid in the presence of _anhydrous trifluoroacetic acid_.

(c) Peptide bond formation is _endergonic_. The _carboxylate_ must be 'activated' using _dicyclohexylcarbodiimide_ to drive the reaction to completion.

(d) Once the tetrapeptide has been synthesized, the blocking groups are removed using _Hydrofluoric acid (HF)_. This also releases the dipeptide from the _chloromethyl polystyrene resin_.

(3) Answer the following questions regarding the chemical sequencing of proteins. (9 marks)

(a) What are the products of a single cycle of the Edman sequencing reaction?
A polypeptide with the N-terminal residue removed and a PTH-amino acid. 2 marks

(b) How do we identify the amino acid sequence in this method?
Unknown PTH-amino acid is run on a reverse phase (or ion exchange) chromatography column and elution time/volume is recorded. The elution time/volume of the unknown is then compared to the known elution time/volumes of all 20 PTH-amino acids on the same column. 3 marks

(c) If an amino acid in our sequence does not correspond to any of the twenty common amino acids, what would be a reasonable explanation? How might you identify the unknown amino acid?
Most likely the PTH-amino acid does not correspond to one of the twenty common amino acids due to post-translational modifications. Comparison of the unknown amino acid to common post-translational modifications of amino acids will almost always identify these residues. 2 marks

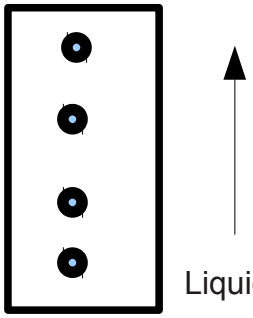
(d) N-terminal arginine residues have both an α-amine and an η-amine. What type of product will this form after a single cycle of the Edman reaction?
PTC group attached to η-amine and PTH group attached to α-amine. 2 marks

(4) Thin Layer Chromatography (TLC) is a versatile, fast and inexpensive means of identifying small organic compounds, including lipids, carbohydrates, amino acids and nucleotides. You have silica based TLC plates and a mixture of the following compounds at pH 6.0: (8 marks)

Ala-Leu-Ile, stearic acid (carboxylate containing 18:0 fatty acid),

His, FQN(one letter code), UTP

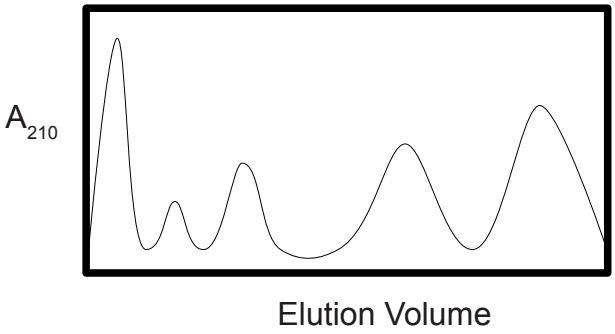
(a) Predict how the above compounds would separate using a 1:1 mixture of water:acetone as the mobile phase? Identify each spot on the resulting chromatogram after detection with a carboxylate specific dye.



Stearic acid (top) 0.5 marks for each band
Ala-Leu-Ile 1 mark for UTP (3 total)
FQN
His (bottom)
(UTP will not be observed as it does not have a carboxylate)

(b) Describe how the results may change if you used acetone alone as the mobile phase?
Improved separation of non-polar compounds and less (or no) separation of polar and charged compounds. 2 marks

(c) How might the same compounds separate if subjected to Reverse Phase Chromatography? Draw a reasonable chromatogram and identify each peak.



From left to right the compounds are
UTP ... His ... FQN ... Ala-Leu-Ile ... Stearic acid
(-1 for each error) 3 marks total

5) Western blots are among the most sensitive methods for detecting small quantities of proteins. Why is this method significantly more sensitive than the Coomassie Brilliant Blue stain? (4 marks)

Coomassie blue stain binds stoichiometrically to proteins and the signal is directly proportional to protein quantity.

Western blots utilize enzymes (or radioactivity) that can produce a signal that is not directly proportional to protein quantity. Instead the signal is primarily time or substrate dependent.

Problems:

(6) You have step-eluted a 2 mL protein sample from an anion exchange chromatography column using a solution containing 0.1 M HEPES (pH 7.0) and 2.0 M KCl. Design a dialysis experiment to produce a protein sample dissolved in an aqueous solution of 0.05 M HEPES (pH 7.0) and 0.5 M KCl, using the minimum volume of dialysis solution. (6 marks)

Since the [KCl] is being reduced by four fold it will determine the minimum volume.

(2 mL) (2.0 M KCl) + (x mL) (0 M KCl) = (2+x mL) (0.5 M KCl)

x = 6 mL

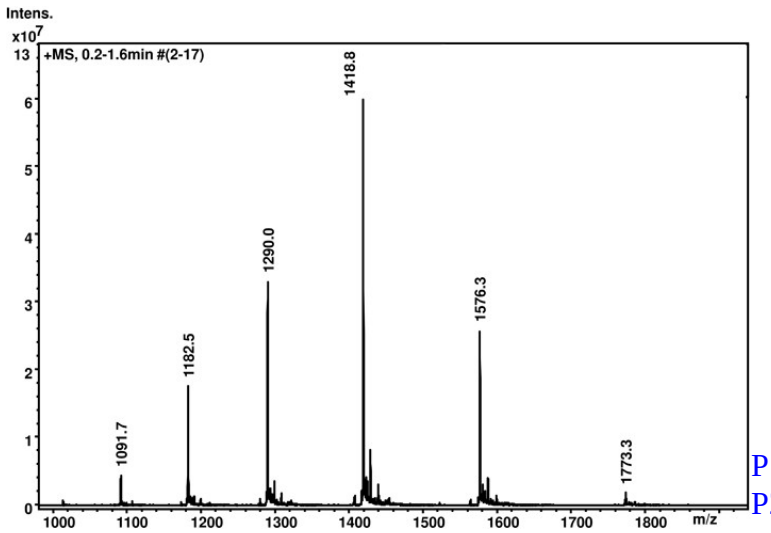
Knowing that 6 mL is the minimum volume, we need to determine the [HEPES]

(2 mL) (0.1 M HEPES) + (6 mL) (x M HEPES) = (8 mL) (0.05 M HEPES)

x = 0.03 M HEPES

We can dialyze our sample against 6 mL of 0.03 M HEPES

(7) The following is an electrospray ionization Mass spectrogram for an unknown protein. Calculate the molecular mass of the protein using the information provided in the spectrogram. (5 marks)



P1 = 1418.8 = (M+z) / z
P2 = 1576.3 = (M + z -1) / (z – 1)

1417.8 z = M substituting into our P2 expression yields
1576.3 = (1417.8 z + z -1) / (z - 1)
1576.3 z – 1576.3 = 1418.8 z -1
157.5 z = 1575.3 and z = 10 2 marks fo equations
1 mark for work
M = 14178 Da 2 marks for answer

(8) You have recently identified a potentially new transcription factor. The protein is basic (pI = 10) and only weakly binds to cation exchange column at pH 7.0. You hypothesize that this basic protein binds to the 'TATAAT' box sequence of a procaryotic gene encoding an endonuclease. Design an experiment that proves (or disproves) the specific binding of your potential transcription factor to the 'TATAAT' box sequence. You can synthesis any desired DNA sequence using the standard nucleotides and have access to standard reagents required to run chromatographic or electrophoretic separations. (7 marks) Native PAGE is the preferred technique.

Since the DNA has large negative charge and the protein has a small positive charge the only way to get the complex to enter a native PAG is using the normal basic buffers.

We need to use a DNA specific dye (Ethidium Br) as the question excludes non-standard nucleotides including those with radioactivity or fluorophores. This requirement means we will not visualize the protein on our gels (and it would migrate out of the gel due to charge anyway).

Having a native PAGE lane with the promoter sequence alone (ie. TATAAT box plus flanking sequences), a second lane with the promoter sequence AND the protein, and a third lane with the promoter sequence AND the protein AND a very short TATAAT containing oligonucleotide (double stranded) will be sufficient to prove or disprove specific binding. In the case of specific binding we would observe a single promoter DNA band in lane 1, a promoter and protein:promoter complex band in lane 2 and a single promoter DNA band in lane 3 (assumes short TATAAT oligonucleotide is migrates off the bottom of the gel). Only the lane 3 result would change if binding were non-specific.

Note: Affinity chromatography is not applicable due to question restrictions (making your own column requires non-standard reagents). Despite this restriction, this approaches was marked and a maximum of 3.5 marks would be awarded.