
Please place your answers to each section in a separate Exam Booklet.
You have 3 hours to complete the exam so you can spend about 3 - 4 minutes on a 2-mark question and 18 minutes on a 10-mark question.
Wherever possible use diagrams to enhance your answers.

Booklet 1

Marks

15 1a) Outline how NMR spectroscopy can be used to determine the 3-dimensional structure of a protein. Be sure to explain the roles of COSY and NOESY experiments, ^{3}J-coupling constants and chemical shifts.

12 2) In the CHEM 4700 laboratory you attempted to purify a fusion protein made of green fluorescent protein and calmodulin making use of the following buffers:

Buffer A
- 250 mM Tris
- 1 mM 2-mercaptoethanol
- 1 mM EDTA
- pH 7.5

Buffer I
- 250 mM Tris
- 1 mM 2-mercaptoethanol
- 0.1 mM $CaCl_2 \cdot 2H_2O$
- pH 7.5

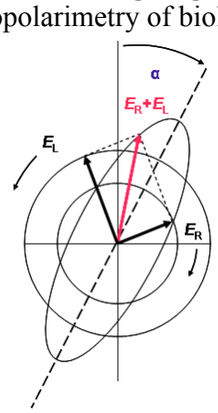
Buffer I with salt
- 250 mM Tris
- 1 mM 2-mercaptoethanol
- 0.1 mM $CaCl_2 \cdot 2H_2O$
- 500 mM NaCl
- pH 7.5

Buffer 2
- 250.0 mM Tris
- 1 mM 2-mercaptoethanol
- pH 7.5

Buffer 2 with EGTA
- 250.0 mM Tris
- 1 mM 2-mercaptoethanol
- 1 mM EGTA
- pH 7.5

Explain how each buffer solution was used and the purpose of each component.

8 3a) What is illustrated in the following diagram? Relate the main features of this diagram to CD spectropolarimetry of biological molecules.



6 3b) Explain how temperature, viscosity, and solvent can alter the structure and circular dichroism spectrum of ApC.

Marks

5 4a) Explain how Na_2SO_4 alters the thermal stability of proteins.

3 4b) What is an isodichroic point?

3 4c) What structural feature of GFP yields its green fluorescence?

2 5a) Explain the purpose of the D_2O and DSS present in the N-acetyl-Trp-amide solution that you measured in the hydrogen exchange experiment.

4 5b) What properties of poly-*d,l*-alanine make it a useful reference material for protein hydrogen exchange measurements?

10 5c) Explain the symbols in the following equation and describe how you used the equation to analyze the hydrogen exchange measurements for N-acetyl-Trp-amide.

$$F = \frac{1}{1 + (k_{OH} 10^{pH - pK_W} + k_H 10^{-pH}) T_{1NH}}$$

Booklet 2

Marks

6 6a) Outline the general procedure whereby a protein is overexpressed in insect cells using the baculovirus system. Please include in your answer (using a specific example) how an affinity tag is typically used in the process.

4 6b) Briefly outline two column chromatography techniques (other than affinity chromatography) that are used to purify proteins.

Booklet 3

Marks

10 7a) First, by writing down a simple equation show how the efficiency of fluorescence resonance energy transfer (FRET) depends on the distance between donor and acceptor moieties.

7b) Second, explain how R_0 (the Forster distance) is calculated;
report the value of the Forster distance that you calculated between the tryptophan and heme in cytochrome c.

7c) Third, report what were the FRET efficiencies between tryptophan and heme in the folded and unfolded states of cytochrome c and estimate the average distance between tryptophan and heme when the protein is unfolded.

Booklet 4

Marks		
7	8a)	What is a vapour diffusion experiment in protein crystallography? Describe the individual steps (Scheme required [3 marks]) and outline the establishment of a concentration gradient for the crystallizing agent [4 marks].
3	8b)	What is the role of a cryo-protecting agent?

98