
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 3D structure of a protein in solution. Be sure to explain the roles of COSY and NOESY experiments, coupling constants, chemical shifts, and computation.
5	1b)	Write down the fundamental equation of NMR spectroscopy and briefly explain the meanings of the symbols.
2	2a)	What is the purpose of repeated freezing and thawing of *E. coli* cells during the purification of a protein?
2	2b)	When extracting protein from *E. coli*, what is the purpose of adding DNase and RNase to the cell extract?
1	2c)	What does sonication do to *E. coli* cells?
2	2d)	What is the purpose of adding mercaptoethanol to cell extraction and chromatography buffers during the purification of a protein?
1	2e)	Why are cell extracts kept at 4^{o}C during protein purifications?
2	2f)	What purpose would heating a cell extract to 80^{o}C serve during the purification of a protein?
2	2g)	Following hydrophobic interaction chromatography why is the chromatographic resin washed with 20% ethanol?
3	3a)	Name, and draw the structure of the chromophore in proteins whose CD spectrum is sensitive to secondary structure changes. At what wavelengths does it absorb?
2	3b)	Define Circular Dichroism.
6	3c)	Draw the expected circular dichroism spectra for a protein in the α-helix, the 3_{10}-helix, and the β-sheet.
8	3d)	Following is the equation you used to fit the temperature dependence (x-variable) of the ellipticity (y-variable) of ribonuclease. Sketch a graph of the equation. Explain the meanings of the symbols and the model represented by the equation.

$$y_{obs} = \frac{(\theta_N + m_N * x) + (\theta_U + m_U * x) \bullet e^{-[(\Delta H_m + x*\Delta S_m)/(R*x)]}}{1 + e^{-[(\Delta H_m + x*\Delta S_m)/(R*x)]}}$$

2	4a)	What was the purpose of the D_2O in the hydrogen exchange experiment?
7	4b)	Explain what is meant by "*sequence-dependent inductive effects*" and how they can affect hydrogen exchange rates. What other techniques are available that could be used to measure the structure and / or dynamics of the peptide?

Marks

Booklet 2

7 5a) Explain, using a specific example, how an affinity tag is typically used in protein purification.

3 5b) Why is an affinity chromatography step typically used *before* a gel filtration (size exclusion) column in the multistep purification of a protein from lysed E.coli cells.

Booklet 3

4 6a) Define FRET and explain how FRET can be used to monitor the folding of proteins.

3 6b) In your experiment investigating the unfolding of cytochrome c, define the donor and acceptor moieties.

3 6c) What is the major limitation of FRET for determining protein folding intermediates?

Booklet 4

7) You have recently purified and crystallized a protein, and found that is diffracts X-rays to a maximum resolution of 5 Å.

8 7a) Describe a method that can be used to grow protein crystals and suggest parameters that can be adjusted in the search for conditions that yield crystals with enhanced diffraction characteristics.

2 7b) Given your knowledge of X-ray diffraction, what is the minimum resolution you would consider acceptable when screening your newly optimized crystals for their ability to diffract X-rays?

Booklet 5

10 8a) Explain two potential strategies for an advanced structure-based drug design approach! Why is structural bioinformatics a strong alternative to experimentally determined structural methods?

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