

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.

Marks

Booklet 1

- 3 1a) What do “ppm”, “Hz” and “MHz” stand for?
- 4 1b) What does it mean to “lock” an NMR spectrometer and how is this accomplished?
- 4 1c) What is a ³J-coupling constant and how can it be used to deduce structural information about a protein?
- 4 1d) What is an NOE and how can it be used to deduce structural information about a protein?
- 12 2) In the CHEM 4700 laboratory you purified a fusion protein made of green fluorescent protein and calmodulin. Based on what you learned, describe a method for the purification of calmodulin and explain the principles underlying each of the steps. Would you expect GFP-calmodulin to behave differently from calmodulin in any of the purification steps you have just described?
- 6 3a) You measured the Circular Dichroism spectrum of ApA dissolved in a 40/60 (v/v) water/ethylene glycol mixture at 25°C. You also measured the CD spectra of ApA dissolved in water at 10°C, 25°C, and 60°C. Using the results of those experiments suggest how CD could be used to follow the folding and unfolding of the DNA double helix.
- 2 3b) Define Circular Dichroism.
- 8 3c) 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.
- $$y_{obs} = \frac{(\theta_N + m_N * x) + (\theta_U + m_U * x) \cdot e^{-[(\Delta H_m + x * \Delta S_m)/(R * x)]}}{1 + e^{-[(\Delta H_m + x * \Delta S_m)/(R * x)]}}$$
- 5 4a) Explain how saturation of the water resonance affects the NH intensities in the hydrogen exchange experiment.
- 4 4b) Explain what is meant by a first-order and a second-order rate constant. What are the units for each?
- 8 5) Prior to the invention of the ESI and MALDI methods to introduce proteins into the vacuum of a mass spectrometer proteomics was difficult. Using examples explain how mass spectrometry can be used to rapidly and accurately identify large numbers of proteins in a cell or organelle.

Marks

Booklet 2

- 8 6a) Outline the steps by which, starting with the amino acid sequence of a protein from a *mus musculus* (common house mouse), you could use BLAST database searching techniques to identify the gene for a homologous protein in humans. In your answer, clearly indicate what a BLAST search does; for each step in the search, what kind of BLAST search is needed, and what kind of database you would search; and what you would expect the search output to look like.
- 4 6b) Do you expect to get complete and accurate information about the gene at the end of the search? If not, what factors may cause difficulties?
- 3 6c) What is the Protein Data Bank, and what additional information might be available from this database?
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Booklet 3

- 7) You have recently purified and crystallized a protein, and found that it 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?
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Booklet 4

- 5 8a) Define FRET (fluorescence resonance energy transfer).
- 5 8b) In the experiment “*monitoring the unfolding of cytochrome c with fluorescence*”, what is the energy donor species? What is the energy acceptor species?
- 5 8c) Explain how FRET distinguishes between the folded and unfolded forms of cytochrome c.