

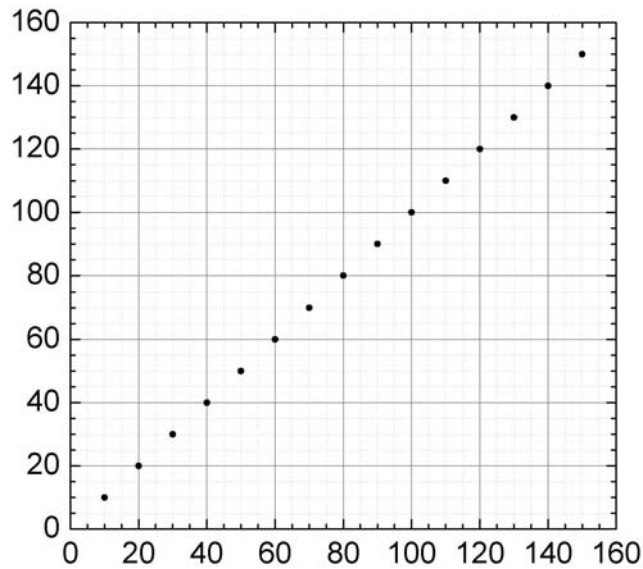
Name: \_\_\_\_\_

# BIOCHM 623 EXAM 3

## April 25 2007

- place name on all pages
- show all reasoning and work clearly, lettering each part of the answer
- exams will be collected at 11AM at the latest

1) (10 points) Sketch the self-dot matrices of a 150 residue polypeptide with 3 nearly identical segments spanning its residues 40-60, 90-110, and 120-140.



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2) (15 points) Define

a) Correlated mutation (as used in Anthrax PA paper discussion)

b) Homologues proteins

c) % sequence identity

3) (10 points) a) For a new isolated and sequenced protein, what **reliable** information can you get when you do multiple sequence alignment with homologous proteins

b) What amino acid side chains are more usually found in  $\beta$ -strands than in  $\alpha$ -helices?

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4) **(17 points)** A new bacterial chaperone was identified, cloned, overexpressed in *E. coli* and purified to apparent homogeneity. The sequence analysis revealed that the protein has 5 Trp, 17 Tyr, 8 Phe, 3 His, and no Cys. The purified protein in PBS buffer has an absorbance of 1.7 at 280 nm. When diluted 1:2, 1:3, 1:10, and 1:50 in PBS the absorbance (280 nm) values were 1.2, 0.88, 0.26, and 0.05, respectively.

a) Estimate the extinction coefficient for the protein. b) What is the concentration ( $\mu\text{M}$ ) of the original protein solution? Justify.

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5) **(18 points)** A protein has two Trp. Three X-ray diffractionists working in the Lederle-GRT 10<sup>th</sup> floor are studying the protein. The first is sure that there is an internal Trp and a surface exposed Trp surrounded by a Glu, and Asp, and a Tyr. The second person thinks that the external Trp it is surrounded by Gln, two Asn, and a Tyr. The third person believes that both Trp are on the surface.

You perform a fluorescence measurement and discover that:

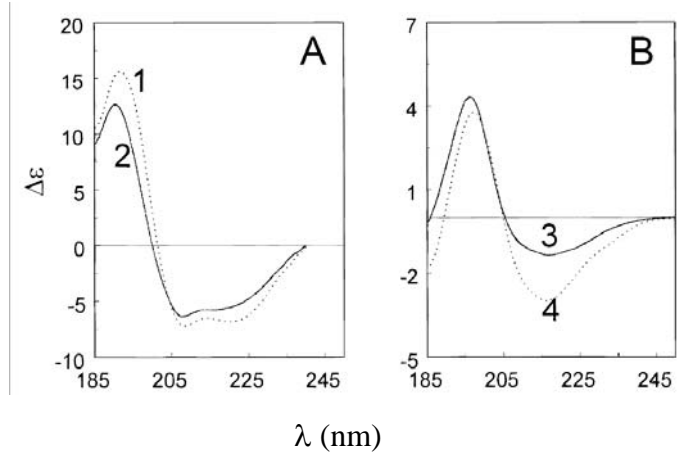
- a) addition of  $\Gamma$  or  $\text{Cs}^+$  (quenchers) decreases the Trp fluorescence to half of the initial value, and
- b) addition of  $\Gamma$  causes a shift of  $\lambda_{\text{max}}$  to shorter wavelengths.

Which of the three diffractionists is correct? Justify.

Name: \_\_\_\_\_

6) (15 points) You have isolated a new 200 amino acids protein secreted by recently discovered Gram-negative bacteria, and its amino acid sequence has 38% identity with the pore-forming domain of bacterial colicins.

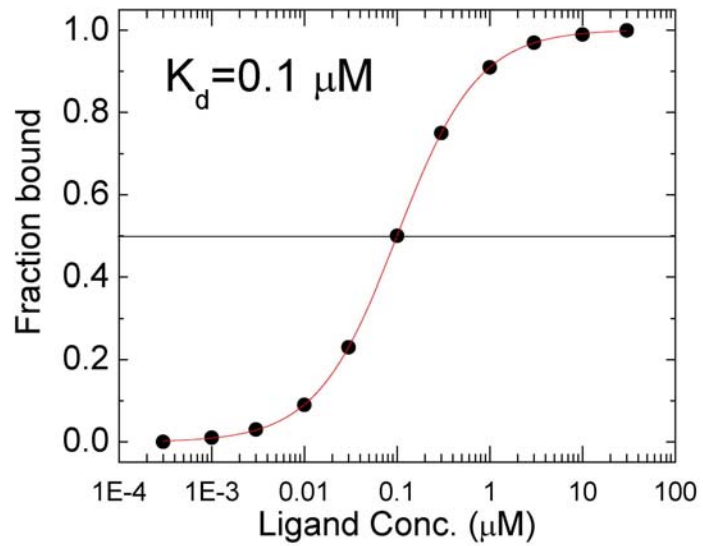
a) Which of the following CD spectra, A1 or B4, do you expect to observe for the new protein in solution? Justify.



b) With all the above mentioned information, can you confidently predict if the new protein will have pore-forming activity? Justify.

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7) (15 points) The binding curve for a protein-ligand interaction in a buffer A (pH 7.4 and 100 mM NaCl) is shown in the figure. If the affinity of the ligand is dictated mainly by electrostatic interactions between charged groups, a) sketch (qualitatively) the binding curve you will obtain if you increase the NaCl concentration to 2M. Justify.



b) In buffer A and in the presence of 1 μM ligand, the protein will be more, equally, or less stable against unfolding? Justify.

Name: \_\_\_\_\_

Formulas:

$$K_{app} = K_F (1 + [L]/K_d^L); \quad K_F = [N]/[U]; \quad K_d^L = [N].[L]/[NL]$$

$$\Delta G = \Delta H - T\Delta S$$

$$\epsilon^{280} (\text{M}^{-1} \cdot \text{cm}^{-1}) = \#\text{Trp } 5500 + \#\text{Tyr } 1490 + \#(-\text{S-S-}) 125$$

$$\text{Protein (mg/ml)} = 1.55 A_{280} - 0.76 A_{260}$$

$$E = R_0^6 / (R_0^6 + r^6); \quad F/F_0 = 1 + K_{SV} [Q] \quad A = \log(I_0/I) = \epsilon \cdot c \cdot l$$

Extra space for work if needed: