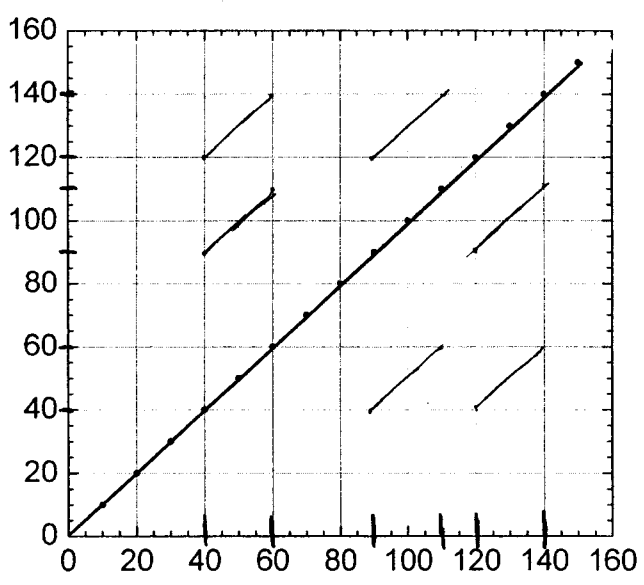


BIOCHM 623 EXAM 3

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.



2) (15 points) Define

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

Residues that show an identical conservation pattern.

b) Homologues proteins

Protein with marked similarity that suggest they derived by evolution from the same ancestral.

c) % sequence identity

% of only identical matched residues in a pairwise alignment between two amino acids or nucleic acids sequences.

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

From this alignment you will get information about protein's residues that are essential to its function (invariants), which are of lesser significance (conservatively substituted), and which have little specific function (hypervariable).

b) What amino acid side chains are more usually found in β -strands than in α -helices?

Residues whose side chains are branched at the β -carbon, such as Val, Iso, Phe, are more often found in beta-strands, because every other side chain in a sheet is pointing in the opposite direction, leaving room for β -branched side chains to pack.

Val, Ile, Tyr, Cys, Trp, Phe, Thr

Name: _____

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 (μM) of the original protein solution? Justify.

$$5 \times 5500 + 17 \times 1490 = 52830 \text{ M}^{-1} \text{ cm}^{-1}$$

$$A = \epsilon \cdot c \cdot l$$

$$c = \frac{A}{\epsilon \cdot l} = \frac{0,26}{52830 \cdot 1} = 4,92 \mu\text{M} \xrightarrow{1/10} 49,2 \mu\text{M}$$

You should use an absorbance value that follows the Beer's law (linear range). And among these values, the closest to 0,3 has the best accuracy (50% of absorption) -

5) (18 points) A protein has two Trp. Three X-ray diffractionists working in the Lederle-GRT 10th 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 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:

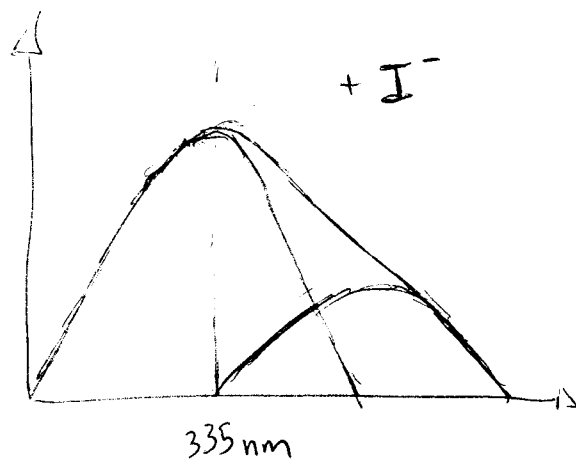
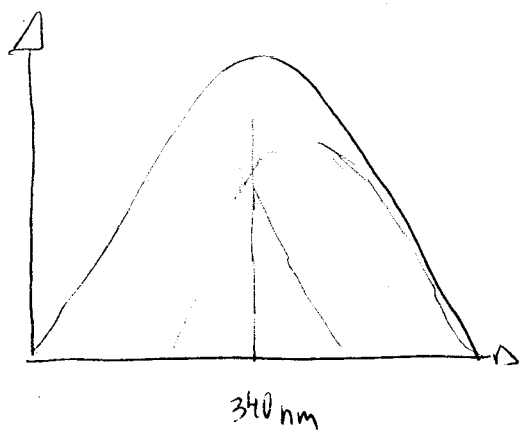
- addition of I^- or Cs^+ (quenchers) decreases the Trp fluorescence to half of the initial value, and
- addition of I^- causes a shift of λ_{max} to shorter wavelengths.

Which of the three diffractionists is correct? Justify.

- a) and b) support the hypothesis that one Trp is internal (not exposed to water-soluble quenchers).

a) \rightarrow The Trp fluorescence intensity decreases to half of the initial value

b) \rightarrow The exposed Trp contribute more to the part of the spectra at higher wavelength (red-edge). Quenching of this Trp only will produce a blue shift on the λ_{max} of the spectra.

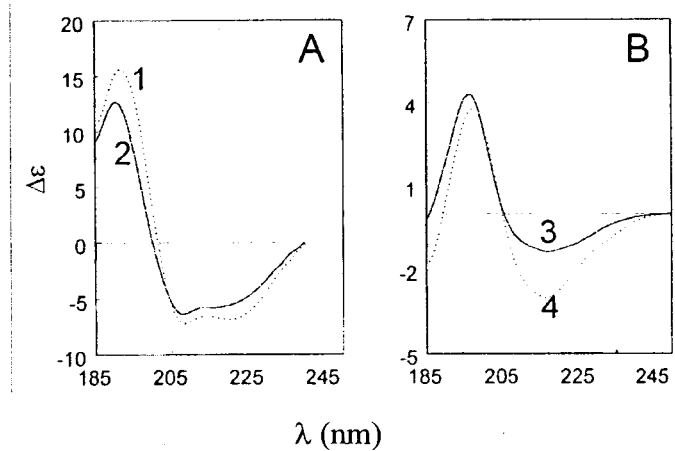


- Since a \oplus charged and a \ominus charged quencher are able to reduce the fluorescence intensity of the exposed Trp, no net \oplus or \ominus charge are expected to be present around the Trp, supporting the 4 neutral amino acid hypothesis (Gln, Asn, Tyr).
The 2nd person is correct.

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.

Colicins' pore-forming domain is completely α -helical.
38% identity suggest the fold of the protein is conserved, therefore



you expect a CD spectra typical of α -helical structure \rightarrow A1

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

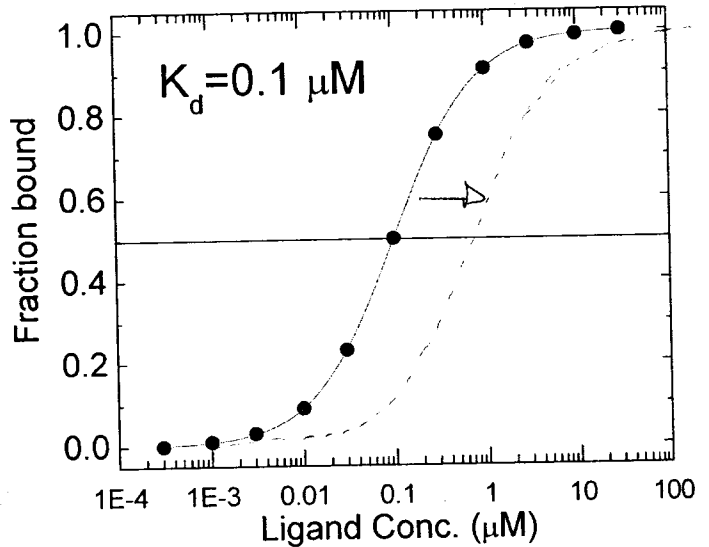
You need more than 40% identity to reliably predict the fold and function from sequence comparison.

High % of identity may suggest they have the same function, but you always need to corroborate the prediction experimentally before classifying the new protein as a pore-forming toxin.

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,

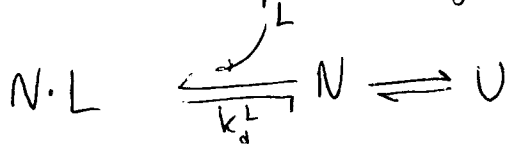
a) sketch (qualitatively) the binding curve you will obtain if you increase the NaCl concentration to 2M. Justify.

The dielectric constant of the buffer solution will be higher in the presence of NaCl 2M. Therefore, the electrostatic interactions will be weaker. The affinity of the ligand decrease, K_d increase.



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

The presence of the ligand at this concentration will stabilize the protein against unfolding.



$$K_{app} = \frac{[NL] + [N]}{[U]}$$

$$K_F = \frac{[N]}{[U]}$$

$$= K_F \left(1 + \frac{[L]}{k_d^L} \right)$$