CH-311 Macromolecular structure and interactions

January 17th, 2020

Formula collection and a pocket calculator may be used

08h15 – 11h15, CM012

Important Remarks:

- **The exam contains 6 question (8 pages), check that you have them all.**
- **Read all questions carefully before answering.**
- **You can use 2 pages of formulae and a calculator**
- **Your answers for every question have to be presented on the provided sheets of paper (you can write on both sides)**
- **Be brief (keywords) and concise in your answers and use a sketch when applicable**
- **In numerical answers, round to 3 significant digits and include correct units (e.g. $\Delta G^0{}_f = 2.02$ kJ/mol)**
- **You can answer in French or English.**

Write your name on each page.

NAME:

Constants and helpful parameters

Boltzmann constant: $k_b = 1.3806488 \times 10^{-23}\ \mathrm{m^2\,kg\,s^{-2}\,K^{-1}}$

Avogadro's constant: $N_a = 6.02214129 \times 10^{23}\ \mathrm{mol^{-1}}$

Gas constant: $R = 8.3144621\ \mathrm{J\,K^{-1}\,mol^{-1}}$

Unit electric charge $e = 1.60217662 \times 10^{-19}$ C

$\pi = 3.141592654$

Amino acid residue length: $l = 0.38$ nm

Average molecular weight of an amino acid residue: 110 Da

Average density of a protein: 1.3 g / $\mathrm{cm^3}$

Flory characteristic ratios:

residue	C_∞
Gly	2.16
Ala	9.27
Pro	116

Fundamental energy scale (at 298 K)

$k_BT = 4.11\times10^{-21}$ J

$k_BT = 4.114$ pN·nm

$k_BT = 9.83\times10-22$ cal

$k_BT = 25.7$ meV

$k_BT/e = 25.7$ mV

Question 1: (10 points)

1. **You are investigating the stability of a protein domain composed of 50 amino acids. You label the first amino acid using a FRET donor dye and the last amino acid with a FRET acceptor dye, forming a FRET pair (Förster radius R_0 = 6 nm).**

a. Calculate the expected FRET value of the unstructured protein chain, assuming all amino acids behave like alanine? (2 points)

b. A fluorescence measurement of the protein with only a FRET donor dye yields a donor emission of 12000 cps (counts per second). The protein with both donor and acceptor dyes present exhibits a donor emission of 6000 cps. Determine the real characteristic ratio of the protein. (2 points)

c. Give an explanation, how the characteristic ratio determined in b) might arise? (1 point)

2. **At 25°C, the protein is exactly 90% folded. A mutant of this protein, where an internal glutamate residue is mutated to lysine, is only 50% folded.**

a. Propose what type of interaction is most likely disrupted by the mutation (1 point)

b. Determine the strength of this interaction in kJ/mol. (3 points)

c. Propose a mutation in the protein at a different position, that could restore the native stability of the protein (1 point).

Question 2: (10 points)

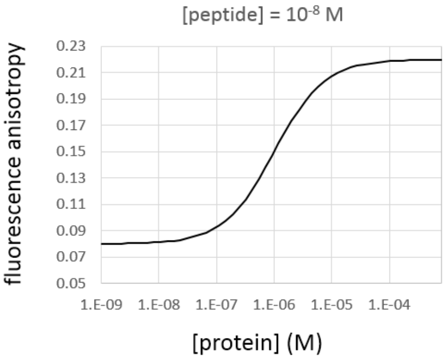
1. **The binding of a peptide (labeled with fluorescein (FSC), fluorescence lifetime τ = 4 ns) to a protein partner is measured using fluorescence anisotropy.**

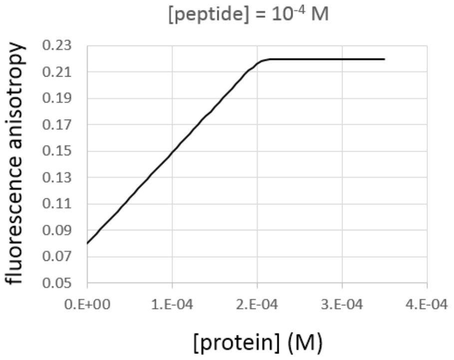
a. In the absence of molecular motion, FSC exhibits a fluorescence anisotropy r = 0.4. Calculate the ratio of light intensity, orthogonal ($I_{\perp}$) and parallel (I_{II}) to the polarized excitation beam. $I_{\perp} / I_{II}$ = ? (1 point)

b. Explain why the ratio $I_{\perp} / I_{II} > 0$ even without of molecular motion. (1 point)

c. For the labeled peptide in solution, r = 0.08 is measured. Calculate the rotational correlation time of the peptide (1 point)

2. **A titration of a protein (unlabeled) binding to the FSC-labeled peptide (at 10 nM and 100 µM) is performed. The results for the two concentrations are shown:**





a. What is the approximate dissociation constant of the interaction? (1 points).

b. What is the binding stoichiometry? Explain your answer. (1 point)

c. Why are the plots different? Explain how you arrive at your conclusion. (2 points)

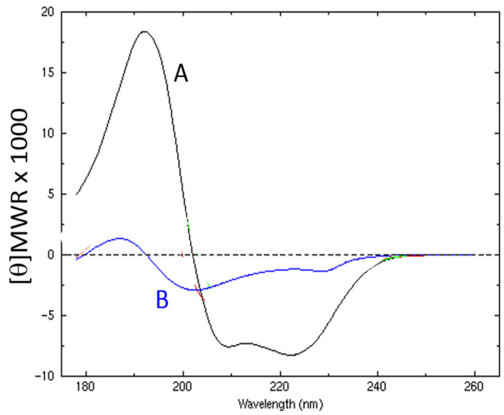
3. In a study, a binding reaction is described as showing a Hill coefficient n = 2. What is understood by the term "Hill coefficient" and what does n = 2 indicate for the binding interaction? (2 points)

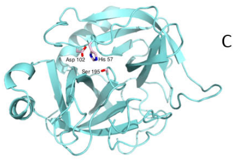
Question 3: (10 points)

You use circular dichroism (CD) to study the structure of a protein domain.

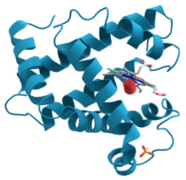
1. **The production of circular polarized light requires a "λ/4" plate. Describe its role in the process (2 points).**

2. **Shown are two CD spectra (A, B) and two proteins (chymotrypsin, myoglobin). Which CD spectrum belongs to which protein and why? (2 points)**





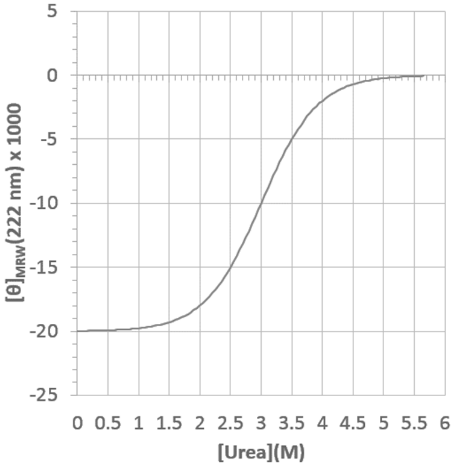
Chymotrypsin



Myoglobin

3. **Draw the chemical structure of <u>urea</u> and explain how it results in protein denaturation. (2 points)**

4. **The protein A is subjected to urea denaturation and the transition is observed via CD spectroscopy.** Determine **<u>ΔG⁰</u>** (at 0M Urea) and the **<u>m-value</u>** of the transition from the plot to the right. (4 points)



Question 4: (10 points)

1. **Accurate FRET measurements depend on the correct value for R_0, the Förster radius. R_0 depends on properties of the donor and acceptor dyes: $J(\lambda)$, Q_D and κ^2.**

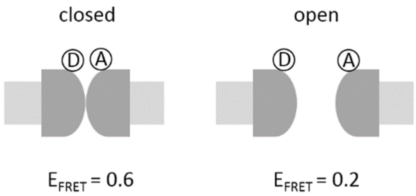
a. Explain how $J(\lambda)$ is determined by using a sketch. (2 points)

b. When fluorophores are attached to biomolecules, Q_D and κ^2 can change. Give an explanation for this behavior, for both **i) Q_D** and **ii) κ^2.** (2 points)

i)

ii)

2. FRET between a donor (D) and acceptor (A) dye is used to measure the behavior of a voltage-gated channel. The expected FRET values for the closed and open channel are given to the right.



a. In an actual experiment, for a membrane potential of -60 mV, an E_{FRET} value of 0.5 is observed. For -40 mV, an E_{FRET} value of 0.3 is detected. Calculate the gating charge of the channel. (4 points)

b. In this experiment, the membrane potential (**-60 mV**) is established by different K^+ concentrations across the membrane. What is the intracellular concentration of K^+ for this potential, when the extracellular concentration amounts to $[K^+]$ = 4mM? (2 points)

Question 5: (10 points)

1. **In Zimm-Bragg (ZB) theory for helix formation, two parameters are of key importance, s and σ.** (6 points)

a. What reaction step are characterized by s and σ, respectively? (2 points)

b. Which helix-coil transition is more cooperative? **Transition 1** with s = 1.3 and $\sigma = 1$; or **transition 2** with s = 1.01 and $\sigma = 10^{-2}$. Explain your answer. (2 points)

c. Different amino acids have different propensities to form an α-helix. Which two amino acids have the lowest helix propensity, and why? (2 points)

2. **What is the <u>helix dipole</u>? Explain and use a sketch if applicable (2 points).**

3. The introduction of a <u>covalent crosslink</u> between amino acids i and i+4 has been proven to greatly stabilize helical structure in peptides (“stapled helices”). Explain why such a stable results in helix stabilization. (2 points)

Question 6: (10 points)

1. What are the origins of the hydrophobic effect? Use a sketch to explain. (2 points)

2. Explain the phenomenon of cold denaturation observed in proteins.
a. What is cold denaturation? (1 point)

b. What is the thermodynamic parameter responsible for cold denaturation? (1 point)

c. Propose an experimental method to measure this thermodynamic parameter for a given protein. (1 point).

d. Through which molecular mechanism is cold denaturation related to the hydrophobic effect? (1 points)

3. The hydrophobic effect is also critical for the formation the cell membrane.
a. Use a sketch of a membrane to display how the hydrophobic effect, in combination with H-bonds and ionic interactions organize a cell membrane (2 points).

b. Proteins are often anchored in the membrane by a single trans-membrane helix domain. How can such a helix domain be identified from the protein sequence alone? (2 points)