Norwegian University of
Science and Technology,
Department of Physics

EXAM I COURSE
TFY4310 MOLECULAR BIOPHYSICS

Suggested solutions

Friday, 29 November 2021
Time: kl. 9.00 - 13.00

Exercise 1.

Justify **five** of the following statements:

1. The folding of a globular protein possessing hydrophobic amino acids in water is entropically favorable.

   Answer: The folding of a protein into a globular conformation leads to a decrease in the conformational entropy of the protein and thus, should be entropically unfavorable. However, the protein possesses hydrophobic aminoacids. This means that water molecules that are close to these aminoacids, and cannot establish hydrogen bonds with them, will organize themselves leading to a large decrease in the mixing entropy of water. The collapse of the protein chain, driven by this hydrophobic effect, reduces the exposure of the hydrophobic aminoacids, which leads to the 'release' of some of the organized water molecules, leading to an increase in entropy.

2. In an aqueous salt free solution, the critical micellar concentration of ionic surfactants is higher than that of nonionic surfactants.

   Answer: Surfactants are amphiphilic molecules composed of a hydrophilic and a hydrophobic part. Above a certain concentration in solutions, that is specific for each surfactant, the surfactants self-assembly into micellar aggregates. This concentration is called the critical micellar concentration. The driving force for the formation of micelles is the hydrophobic effect and increase in water mixing entropy, as explained above. On the other hand, the formation of micelles is opposed by the mixing entropy of the surfactants and the steric interactions between adjacent surfactants. In the case of the ionic surfactants, the electrostatic repulsions between the headgroups, also oppose the formation of micelles and thus, a larger concentration of surfactants is needed to initiate micelle formation.

3. Flory-Huggins theory is not applicable to describe the thermodynamics of polyelectrolytes (polymer with charged groups).

   Answer: The Flory-Huggins theory describes the solubility of polymers in solution. One of the assumptions that the theory is based on is that the interactions of each component (*e.g.*, monomers) is limited to its nearest neighbors, that is, it assumes the interactions between all components (monomers and solvent molecules) has a short range. This is not the case with polymers containing charged groups, as the electrostatic interactions have a $1/r$ range.

4. Chromophores, groups of atoms that absorb light in the visible region, are typically conjugated systems (alternating single and double bonds).

   Answer: Chromophores typically possess a larger number of alternating single and double bonds. This leads to the delocalization of the π electrons along these regions of the molecules but, more importantly, it signifies that the energy differences between the π and $\pi^\star$ molecular orbitals present in the molecule decrease significantly. As a consequence, the light that is absorbed by these molecules, upon excitation of the electrons from lower to higher energy levels, has a longer wavelength ($\Delta E = h\nu = hc/\lambda$), in the visible range of the electromagnetic spectrum, than molecules without these groups.

5. Infra-red spectroscopy and Raman scattering are complementary techniques.

   Answer: Both Infra-red (IR) and Raman spectroscopies probe the vibrational modes of molecules and are based on the excitation of vibrational modes as a consequence of the absorption of electromagnetic radiation of the correct wavelength by the molecule. The difference between the techniques arises from the fact that the selection rules are different. In the case of IR spectroscopy one of the selection rules states that the transition dipole moment, which lies along the direction of the changing dipole moment, needs to be different than zero. In Raman spectroscopy a vibrational mode is only observable in the spectrum if there is a change in the polarizability of the molecule. Some vibrational modes will therefore be IR-active and Raman inactive, while some other will show a particular vibration mode in the Raman spectrum but not in the IR spectrum. The CO_2 molecule is a typical example. Being a symmetric molecule with no permanent dipole moment, the symmetric stretching mode will be IR-inactive but Raman active. On the other hand the asymmetric stretching mode will be IR active and Raman-inactive. Thus, the two techniques are complementary.

6. In a COSY spectrum, the number of peaks is always equal or larger than those in the corresponding 1D spectrum.

   Answer: COSY (COrrelation SpectroscopY) is a 2D NMR technique, where two frequency scans are conducted. One is conducted by varying the time between the application of two rf pulses, and the second is obtained by measuring the FID after the last pulse, that is, the equivalent to a 1D NMR experiment. By performing the frequency range between the two pulses it is possible to identify the nuclei that are J-correlated, that is, nuclei connected to neighboring atoms (up to 3 covalent bonds) that have different chemical environments.

   The diagonal peaks of the 2D spectrum will correspond to the frequencies (or chemical shifts) of the probed nuclei. Taking a simple example with nuclei A and X, this would correspond to the signals (ν_A, ν_A) and (ν_X, ν_X). If A and X are not correlated, no other peaks will be observed and the total number of peaks is equal to the number of peaks seen in a 1D NMR spectrum. If, on the other hand, A and X are correlated, two more peaks will appear (ν_A, ν_X) and (ν_X, ν_A), giving a larger number of peaks in the 2D NMR spectrum.

7. The Monte Carlo technique is useful in determining the conformational properties of polymer molecules.

   Answer: Polymers are typically composed of monomers connected by sigma bonds, that can rotate freely. This means that a polymer can adopt a large number of conformations, characterized by the relative position of the monomers. Thus, in order

to define a conformational property, such as the end-to-end distance or the radius of gyration, one needs to consider the ensemble average of these properties. The Monte Carlo technique, applied to polymer studies, is based on the random generation of polymer chains often based on random walks, and is thus able to probe a large number of conformation and to calculate the ensemble average of these properties.

Exercise 2.

Double stranded DNA in solution can be denaturated (double to single strand transition) by

i. increasing the temperature of the solution;

ii. adding ethanol to the aqueous solution.

Shortly explain the mechanism behind each of these situations.

Answer:

i. When the temperature of the solution is increased the entropy of the system increases as well, this is so because (at equilibrium and at constant pressure and temperature) $\Delta G = -T\Delta S = -Tk_{\mathrm{B}} \ln W$ and the number of available microstates/configurations (W) increases. The most relevant to the situation are the mixing entropy and the conformational entropy, and an increase in either will favor single stranded conformations over double stranded molecules.

   It can be noted that the dielectric constant varies with the temperature which will also play a role in the melting behavior of DNA, as explained below.

ii. Adding ethanol to the aqueous solution leads to a decrease in the dielectric constant of the solution (ε_0(water) = 78.4 and ε_0(ethanol) = 25.3, as indicated in the list of data and formulas). Such decrease leads to an increase in the electrostatic interactions in the solution ($V_{\mathrm{elec}}(r) \propto 1/\varepsilon$). In solution, the phosphate groups in the DNA stranded are ionized, and the strands are negatively charged. The repulsion between the chains in the double helix are balanced by the formation of H-bonds between the complementary strands and the so-called π-stacking. If the dielectric constant of the solvent decreases, the increase in the electrostatic interactions can lead to the denaturation of the double stranded DNA.

Exercise 3.

Match the statements in the rows with the values in the columns.

Answer:

	8 um	1.0 kT	0.5 kT	2.5 kT	2.0 nm	0.1 nm	170 kT	56 nm
Magnitude of the potential energy between freely rotating dipoles			✔					
Magnitude of the London dispersion potential energy between two small molecules		✔						
Length of H-O bond in water						✔		
Covalent bond energy in H-H							✔	
Bjerrum length in vacuum								✔
Magnitude of the potential energy between a sodium and a chloride ion in contact in aqueous solution				✔				
Diameter of a red blood cell	✔							
Diameter of a DNA molecule in aqueous solution					✔			

Exercise 4. Polyethylene oxide (PEO), $(\text{-CH}_2\text{-O-})_n$, is often used to coat drug carriers to prevent the interaction of the carrier with proteins that impede the delivery of the drugs to the wanted target.

1. The experimental chain dimensions of PEO are given by $\langle R_{ee}^2 \rangle_0 / M \approx 0.80$ Å^{2}mol/g.

   Calculate the characteristic ratio of PEO.

   M(EO monomer) = 40 g/mol ; EO length = 4.1 Å.

   Answer: The characteristic ratio (C_∞) describes the effect of local constraints on the chain dimension, according to:

   $$\left\langle R_{ee}^2 \right\rangle_0 = C_\infty Q^2 n$$

   with Q and n the length and number of the monomers, respectively.

   Starting with the given data

   $$\left\langle R_{ee}^2 \right\rangle_0 \approx 0.80 \cdot M = 0.80 \cdot M_{\text{mon}} \cdot N_{\text{mon}} \ ,$$

   with M_{mon} and N_{mon} , the molar mass and number of monomers, respectively. Using the definition above, and since $n = N_{\text{mon}}$:

   $$C_\infty Q^2 \approx 0.80 \cdot M_{\text{mon}}$$

$$C_\infty \approx \frac{0.80 \cdot M_{\text{mon}}}{Q^2} = \frac{0.80[\text{Å}^2\text{mol/g}] \cdot 40[\text{g/mol}]}{4.1^2[\text{Å}^2]} = 1.90$$

2. The Kuhn length of PEO is 6.0 Å. Discuss the differences between this value and the monomer (EO) length.

   Answer: Instead of using the characteristic ratio to correct the overall dimensions of the chain, when using the number and length of the monomer, we can define the statistical length of one segment (step length, Kuhn length) and a number of segments, such that the polymer chain of interest can be described by a random walk (*i.e.*, behaves like an ideal chain). The fact that the Kuhn length of polyethylene oxide is longer than the monomer length (6.0 vs. 4.1 Å) indicates that the monomer does not correctly describe the statistical behavior of the polymer and longer segments should be used instead, due to the local stiffness of the PEO chain.

Exercises 5. An enzyme with molar mass of 310 kg/mol undergoes a change in shape when the substrate (molecular mass of 500 g/mol) binds. This change can be characterized by ultracentrifugation. In the absence of the ligand the sedimentation coefficient of the enzyme in water at 20 °C is 11.7 S (1 S = 10^{-13} s). The partial specific volume of the protein is 0.732 cm^3/g.

1. Determine the hydrodynamic radius of the enzyme, assuming it is spherical.

   Answer: The Svedberg equation relates the sedimentation coefficient and the molar mass of the enzyme with the friction coefficient, f,:

   $$s = \left(1 - \overline{V}_1^{(S)}\rho\right) \frac{M_1}{N_{\text{Av}} f} \ .$$

   Assuming that the enzyme is spherical, $f = 6\pi\eta R_h$, with R_h the hydration radius, leading to

   $$s = \left(1 - \overline{V}_1^{(S)}\rho\right) \frac{M_1}{6\pi \cdot N_{\text{Av}}\eta R_h}$$

   and

   $$R_h = \left(1 - \overline{V}_1^{(S)}\rho\right) \frac{M_1}{6\pi \cdot N_{\text{Av}}\eta s} = 5.91\,\text{nm} \ .$$

2. Upon the binding of the substrate, the sedimentation coefficient increases by 3.5%. What is the (hydrodynamic) radius of the bound enzyme? Assume that there are no changes in the partial specific volume of the protein.

   Answer: Taking into account the relation between the sedimentation coefficient (s) and (R_h) presented above, and realizing that the binding of the substrate does not contribute significantly to the molecular mass of the enzyme, we can write:

   $$\frac{R_2}{R_1} = \frac{s_2}{s_1} \Rightarrow R_2 = R_1 \frac{s_1}{s_2} \ .$$

   Since $s_2 = 1.035 s_1$ and the molecular mass of the enzyme is much larger than that of the substrate, it follows that

   $$R_2 = 5.71 \cdot 10^{-9}\text{m} = 5.71\,\text{nm}$$

3. X-ray analysis showed that the ligand causes a contraction of the enzyme of about 12 Å along one axis. Why did the radius not change as much?

Answer: The radius of hydration that was calculated assumes that the enzyme is spherical. The reason why the radius did not change as much as that measured by the X-ray analysis could be due to a dramatic change in conformation (shape) of the enzyme upon the binding of the ligand, and that the new shape deviates from the spherical symmetry. The deviation from the sphericity will increase the friction coefficient, f, giving an apparent increase in R_h or, in this case, masking the large decrease in one of the dimensions.

Exercise 6.

1. Discuss the (two) molecular mechanisms behind the changes to the magnetization vector, $\vec{M}$, when a rf-pulse of 90° is applied to a sample, during a nuclear magnetic resonance experiment. Namely:

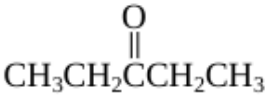
   $\vec{M}_z : \overline{M}_z \rightarrow 0$ and $\vec{M}_{xy} : 0 \rightarrow \vec{M}_{xy}$

   Answer: During a NMR experiment a magnetic field B_z is applied that orients the nuclear spins around the z-axis. This leads to the split of the spin population into different energy levels. Considering proton NMR specifically, two spin orientations are possible. The protons oriented in the direction of the magnetic field have a lower energy and those oriented in the opposite direction possess a higher energy. The difference in energy between the two populations is not large but there is a small excess of nuclear spins in the lower energy level, which is enough to induce a magnetization vector that has the same direction as $\vec{B}_z$, $\vec{M}_z$. The nuclear spins have the same angular frequency (Larmor frequency) but are out of phase so the components of the magnetization vector in the x and y direction cancel out and $\vec{M}_{xy} = 0$.

   Now, upon the application of a rf-pulse of 90 °, the following happens:

   1. During the experiment a pulse (that includes the Larmor frequency of the spin of interest) is applied to induce the excitation of some spins from the lower to the higher energy levels, which leads to a decrease in the magnitude of the magnetization in the z direction. For a 90 ° pulse, the population in the two different energy level becomes equal and $\overline{M}_z \rightarrow 0$.
   2. A second (closely related) consequence is that the excitation of the spins, induced by the pulse, also lead to the precession of the spins in phase. If the spins are precessing in phase, the x and y components of the magnetization no longer cancel and the magnetization can now be described as a vector that rotates in the xy-plane at a frequency equal to the precession frequency, $0 \rightarrow \vec{M}_{xy}$.

2. Predict the ^{1}H-NMR spectrum of the molecule below, justifying your answer in terms of relative areas, peak splitting and chemical shifts.



Answer: Firstly one should note that the molecule is symmetric, which means that the 6 protons in the CH_3 groups and the 4 protons in the CH_2 groups have the same

chemical environment. We expect thus that the ^{1}H-NMR spectrum shows two peaks with relative areas of 3 and 2, respectively.

Regarding the chemical shift, δ, we can see in the scheme of the molecule that the protons in the CH_2 group are bond to a C connected to an O. The O is an electronegative atom, gathering to itself the electrons from the atoms bound to it and leaving the protons close to it more exposed to the magnetic field that the protons in the CH_3 groups. Consequently, the peak corresponding to the CH_3 groups will appear at lower δ (closer to the peak of the reference sample, δ =0), usually at around δ =1 and the one corresponding to the CH_2 groups at larger δ.

Regarding the peak splitting or peak multiplicity, this indicates the number of protons that are J-coupled to the protons of interest, that is, the protons are in close proximity (up to 3 covalent bonds) from to the protons responsible for the peak under discussion. The splitting is a consequence of the differences in δ of the coupled protons and the different energy levels they occupy. Thus, we expect the peak corresponding to the CH_3 groups, at a lower chemical shift, to be a triplet, as there are 2 protons in the vicinity of the protons of CH_3. The peak at larger chemical shifts, corresponding to CH_2 is expected to be a quartet.

3. What is the hybridization of the carbons and the oxygen in the molecule of the exercise above? Justify your answer by describing the hybrid orbitals for each of the mentioned atoms and how these combine to form the molecular orbitals present in the molecule.

   Answer: The hybridization of the carbons in the indicated molecule is, from the end to the middle, sp^3, sp^2, and sp^2. The hybridization of the oxygen is also sp^2.

   Starting with one of the ends of the molecule, the C from the CH_3 group possess 4 sp^3 orbitals. Three of these will linearly combine with the 1s orbitals of the 3 H and the 4th will linearly combine with the sp_3 orbital from the C in CH_2 group. As a result, 4 σ and 4 $\sigma^\star$ molecular orbitals are formed. The second C, in the group CH_2 also possesses 4 sp^3. One has already been mentioned, two others combine with the 1s orbitals of the two H bound to it and the 4th combines with the sp_2 orbital from the central C. Again, 4 σ and 4 $\sigma^\star$ molecular orbitals are obtained. The central C has 3 sp_2 orbitals. Two combined and form sigma bonds with the sp_3 orbitals of the neighboring C. The third combines with one sp_2 orbital from the O. Finally, the O has also 3 sp_2 hybrid orbitals. One is engaged in the sigma bond to the C, as mentioned. The other two are occupied with electrons from the O and do not participate in bonding and antibonding orbitals. In addition to the sp_2 orbitals, both O and central C possess a 2p orbital that linearly combine to form a bonding and an anti-binding π orbitals, giving the C = O double bond.

Exercise 7.

1. Above a certain concentration, the (measured) intensity of an aqueous solutions of the surfactant sodium dodecyl sulfate (SDS), was found to change with the surfactant concentration. Use the following data to calculate the apparent molar mass of the species giving rise to the scattering. Light polarized in the z-direction is used in these experiments.

concentration $\times 10^3$ (g cm$^-$3)	2.7	4.2	7.7	9.7
measured intensity $\times 10^4$ (cm^{-1})	1.10	1.29	1.71	1.98

$\lambda_0 = 550$ nm ; $n_0 = 1.4$; $\mathrm{d}n_0/\mathrm{d}c = 0.15$ cm^3/g

Answer: Taking into account the given data, it is convenient to use

$$\frac{\langle I_S(q)\rangle}{I_0} R^2 = cM\kappa\,,$$

valid in the RGD regime, that is, assuming that the particles are small in relation to the wavelength of the radiation and/or the particles have a low contrast in relation to the solvent. The radius of micelles is typically a few nm so the first condition is met.

The first term, also called Rayleigh ratio, is the measured intensity. By plotting these data as a function of concentration, we should obtain a straight line with a slope that is proportional to M.

Let us start by calculating κ. As indicated in the table presented in the equation list, κ depends on the polarization of light (and the position of the detector). Light is polarized in the z-direction and we assume that the detector is placed in the xy-plane, to which we have info for, in the formula list. Under these condition, κ is independent of the placement of the detector within the plane, according to

$$\kappa = \frac{1}{N_{\mathrm{Av}}} \frac{4\pi^2 n_0^2}{\lambda_0^4} \left(\frac{\mathrm{d}n_0}{\mathrm{d}c}\right)^2$$

$$\kappa = \frac{1}{6.022\cdot 10^{23}\,[\mathrm{mol}^{-1}]} \frac{4\pi^2(1.4)^2}{(550\cdot 10^{-7}\,[\mathrm{cm}])^4} \cdot (0.15\,[\mathrm{cm}^3/\mathrm{g}])^2 = 3.16\cdot 10^{-7}\,\frac{\mathrm{cm}^2\cdot\mathrm{mol}}{\mathrm{g}^2}\,.$$

Let us now calculate the slope taking into account two of the point, the first and last for example:

$$\text{slope} = \frac{\Delta y}{\Delta x} = \frac{(1.98-1.10)\cdot 10^{-4}\,[\mathrm{cm}^{-1}]}{(9.7-2.7)\cdot 10^{-3}\,[\mathrm{g\,cm}^{-3}]} = 0.0126\,\mathrm{cm}^2/\mathrm{g}\,.$$

We can now calculate M:

$$M = \frac{\text{slope}}{\kappa} = \frac{0.0126\,[\mathrm{cm}^2/\mathrm{g}]}{3.16\cdot 10^{-7}\,[\mathrm{cm}^2\cdot\mathrm{mol}/\mathrm{g}^2]} = 39,870\,\mathrm{g/mol}\,.$$

2. Knowing that the molar mass of the SDS is 288 g/mol, comment the result obtained in the question above as well as the increase in intensity with concentration observed only above a certain concentration.

   Answer: The scattering of the solution changes due to the presence of surfactant aggregates (micelles) that are formed above the critical micellar concentration (cmc). Below the cmc only individual surfactants are present in solution, which likely do not scatter very much. Increasing the concentration of surfactant (above the cmc) increases the number of micelles and leads to the increase in the scattered intensity. Taking into account the molar mass of the surfactant it can be concluded that the aggregation number of the micelles, that is the number of surfactants per micelle, is about 140.