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# EXAM I COURSE
# TFY4310 MOLECULAR BIOPHYSICS

**Suggested solutions**

Friday, 30 November 2018
Time: kl. 09.00 - 13.00

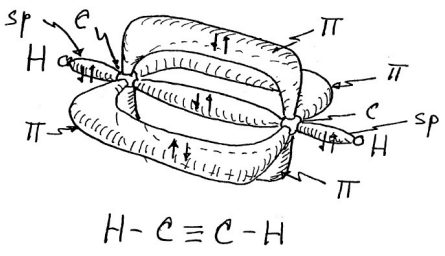
All questions have the same weight. None of the questions require lengthy answers so answer as precisely and concisely as possible. Good luck!

## Exercise 1.

Justify **six** (6) of the following correct sentences:

1. A triple bond between carbon atoms (HC ≡ CH) consists of one sigma and two pi molecular orbitals occupied with electrons.

   **Answer:** HC ≡ CH is a linear molecule. The carbons are *sp* hybridization, that is, one 2p atomic orbital and the 2s atomic orbital of each carbon hybridize into two *sp* bonding orbitals. One of the *sp* orbitals combines linearly with the 1s from the hydrogen to form the bonding and anti-bonding $\sigma$ molecular orbitals (where only the bonding orbital is occupied), and the other combined with the *sp* orbital from the other carbon to form the sigma orbital that binds the two carbons. Two atomic orbitals are left on the carbon, $p_y$ annd $p_z$, if we define the $\sigma$ bond to be position along the $x-$axis. Each $p$ orbital will combine into a bonding and an anti-bonding $\pi$ molecular orbitals. Thus, the triple bond in HC ≡ CH consists of one (occupied) sigma and two pi molecular orbitals:

2. Absorption of a polymer to a nanoparticle decreases the conformational entropy of the polymer.

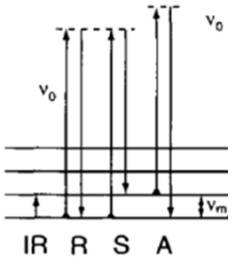
**Answer:** When a polymer absorbs onto a nanoparticle (or any surface) it looses degree of conformation since it cannot occupy the space that the nanoparticle occupies. Therefore the conformational entropy of the polymer is decreased.

3. When performing molecular modeling of concentrated systems it is desirable to use periodic boundary conditions.

   **Answer:** When modeling concentrated systems it is desirable to work with relatively small simulation boxes to keep the number of particles relatively small and the computational times relatively short. If the simulation box is too small, on the other hand, the surface effects will be too large. To avoid this, periodic boundary conditions are implemented so when a particle is moved towards the wall of the cell, it enters a neighboring cell instead. In practice, the particle enters the same cell but from the opposite side.

4. Both Raman scattering and IR spectroscopy probe the vibrational states of molecules. In Raman scattering however, one typically illuminates the sample with a laser beam and does not need to scan all frequencies in the IR spectra to obtain a spectrum.

   **Answer:** In IR spectroscopy, as in other absorption techniques, one measures the transmittance (or absorbance) as a function of the wavelength, by scanning the sample with electromagnetic radiation with varying frequency (or wavelength). If a sample is irradiated with electromagnetic radiation in the infra red region of the spectrum, one probes the excitation of the vibrational modes of the molecules, that appear as bands for the wavelength that corresponds to the different between the vibrational levels, according to $\Delta E = h\nu = hc/\lambda$. Raman scattering is a technique that probes the vibrational levels of a molecule, not by exciting them from fundamental to excited vibrational states but by exciting electrons (typically) from the electronic ground state to virtual states and measuring the emitted photon as the electron relaxes to the ground state. In most cases the excitation and emitted photon will have the same frequency (Rayleigh scattering) but if there is a change in polarizability with the equilibrium




   separation of the nuclei, Stokes (S) and anti-Stokes (A) lines can be observed. The former refers to transitions that start as the ground vibrational level and end in the excited vibrational level, and the latter corresponds to transitions that start in the first vibrational state and finish in the ground vibrational level (see scheme above). In this way it is possible to study the vibrational modes of the molecules without doing a scan of light at different frequencies.

5. In a common transient electric birefringence set-up the analyser is oriented 90° relatively to the polariser.

   **Answer:** With transient electric birefringence one can measure the rotational diffusion coefficient of long (and somewhat stiff) macromolecules. The set-up is built so that the

macromolecules are firstly oriented in solution, using an electric field. When the the electric field is switched off, the molecules relax to a random orientation in the sample, which is measure in terms of changes in birefringence. The birefringence is detected by using an optical system with two crossed polarisers. If there is no birefringence in the sample the intensity coming out of the analyzer will be zero. If there is birefringence there will be a phase difference between light polarised in y-direction and x-direction, which will be detected, in the form of intensity reaching the detector.

6. Spin-spin ($T_2$) relaxation does not involve the flipping of spins between levels.

   **Answer:** In a typical 1D-NMR experiment we can define two relaxation times: The spin-lattice relaxation ($T_1$) occurs when the radio frequency pulse is turned off and the magnetization that was centered in the $xy$-plane returns to the $x$-axis. During this period the spins return to their fundamental state, that is, it involves the flipping of spins between levels; The spin-spin relaxation ($T_2$), on the other hand, occurs because different spins will have slightly different precessing frequencies due to differences in their chemical environment. This leads to the unbundling of the spins in the $xy$-plane that leads to a decrease in the magnetization in the $xy$-plane, but no changes in the magnetization in the $z$-axis, $M_z$; that is, there is no variation in the spin level population.

7. The $^1$H-NMR spectrum of $CH_3$–$CH_2$–Br possesses a quadruplet (intensities of 1:3:3:1) and a triplet (intensities of 1:2:1) at the chemical shifts of 3.5 and 1.7 ppm (in relation to TMS), respectively.

   **Answer:** The splitting of the peaks is due to spin-spin coupling between a nucleus (proton in this case) and the protons in a neighbouring group. In the case of the $CH_3$ group, the number of equivalent nearest neighbour protons is two, which gives rise to a multiplet with three peaks (triplet) with relative area
   $1:2:1 = (\uparrow\uparrow) : (\uparrow\downarrow, \downarrow\uparrow) : (\downarrow\downarrow)$.
   For the $CH_2$ group, the number of nearest neighbour protons is three giving rise to a quadruplet with relative area
   $1:3:3:1 = (\uparrow\uparrow\uparrow) : (\uparrow\uparrow\downarrow, \uparrow\downarrow\uparrow, \downarrow\uparrow\uparrow) : (\downarrow\downarrow\uparrow, \downarrow\uparrow\downarrow, \uparrow\downarrow\downarrow) : (\downarrow\downarrow\downarrow)$.

   The protons of the carbon that is closer to the bromide atom are more deshielded, due to the fact that bromide is an electronegative atoms and drags the electrons of the molecule to itself. This leads to that the protons closer to the Br field the magnetic field more strongly than the other protons and so they precess at a faster pace. Thus, these peaks will be shifted downfield, that is, to higher $\delta$ values.

8. In the most usual configuration for light scattering, the incident and scattered beams lie in the $xy-$plane, which is horizontal. In most modern systems the incident beam is provided by a laser source which is vertically polarized.

   **Answer:** From a practical point of view it is convenient to have the detector mounted and moving in the horizontal plane, here defined as the $xy-$ plane, as well as the incident light. In this case, having light polarized in the $z-$direction, perpendicular to the $xy-$plane assures that the intensity of the scattered light will not change with the angle when performing light scattering experiments, as can be seen by the table in the end of the exam: the Rayleigh ratio, and thus the intensity of the scattered light has no dependence with the scattering angle, $\theta$.

**Exercise 2.** Cationic surfactant X has 14 carbon atoms, $X^+$-$(CH_2)_{13}$-$CH_3$ $Cl^-$, in the alkyl chain and forms spherical micelles in aqueous solution above the critical micellar concentration. Assume that the bare radius of the headgroup is 0.32 nm.

1. What is the hybridization of the carbons in the surfactant alkyl chain? Justify.

   **Answer:** The hybridization of the carbons in the alkyl chain is $sp^3$. Each carbon is bound to 4 other atoms in a tetrahedral configuration. This is achieved by hybridizing the $2s$ atomic orbital with the 3 $p$ atomic orbitals of the carbon, which give rise to 4 $sp^3$ bonding orbitals with the same energy, oriented according to the corners of a tetrahedron.

2. What is the driving force for micelle formation?

   **Answer:** The formation of micelles is driven by hydrophobic forces. This is an entropically driven force that occurs to minimize the contact between the hydrocarbon tails of the surfactants, which are apolar and form no hydrogen bonds, and water. The hydrocarbon tails are then said to be hydrophobic. Above a certain concentration the surfactant molecules are pushed towards each other to decrease the hydrophobic area exposed to water, and releasing thus some of the water molecules that were organized around the individual surfactant tails. The larger the hydrophobic hydrocarbon groups the stronger this effect will be and thus, surfactants with longer chains lengths have a lower critical micellar concentration.

3. Calculate the radius of the micelle.

   **Answer:** The radius of the micelle corresponds to approximately the length of the surfactant chain length given by

   $$l_c \leq l_{\max} \approx (0.154 + 0.1265n)$$

   where $n$ is the number of carbons. The radius of the micelle is then approximately 1.92 nm.

   **Note:** Some students added the diameter of the headgroup to the calculation. This was also considered correct.

4. Calculate the root-mean-square (rms) of the end-to-end distance of the surfactant assuming that the hydrophobic tail behaves as an ideal freely jointed chain. Assume that the Kuhn length is 1 nm, corresponding to 7 $CH_2$ units. Ignore the contribution from the headgroup.

   **Answer:** The mean square end-to-end distance is given by

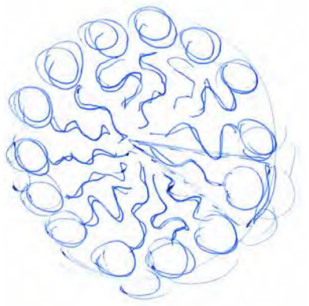
   $$\left\langle R_{\text{ee}}^2 \right\rangle^{1/2} = C_n^{1/2} Q n^{\alpha} \,,$$

   where $C_n = 1$ for freely jointed models and $\alpha$, the scaling factor is $1/2$ for ideal chains. Since we can ignore the headgroup, and the surfactant has 14 carbons, $n$ the number of Kuhn segments is 2:

   $$\left\langle R_{\text{ee}}^2 \right\rangle^{1/2} = 1 \times 2^{1/2} = 1.41\,\text{nm} \,.$$

5. Draw a schematic drawing of a spherical micelle. Discuss the results obtained in questions 2.3 and 2.4 and consider them in your drawing.

   **Answer:** The schematic drawing of the cross-section of a sherical micelle can be seen below.



The value obtained in 3., 1.9 nm, corresponds to a stretched surfactant chain. Typically one or few chains will be stretched to occupying the center of the micelle. However it is not favorable, from an entropic point of view, for the surfactant to have a very stretched conformation. There is a larger probability to possess more compact conformations and so the majority of the chains will have more compact structures, as depicted by the ideal chain (see also the micelle drawing). The chain might not be a compact as calculated (1.41 nm) since there are restrictions on the position of one of the ends (the headgroup) and they will also feel steric (overlap) repulsions from the neighboring surfactant molecules.

6. Increasing the salt concentration of the solution leads to a change in the shape of the micelles. Which shape do you predict it will form and why?

   **Answer:** Increasing the salt concentration of the aqueous solution will lead to a decrease in the repulsive interactions between the surfactant headgroups which will lead to a decrease in the area of the surfactants and an increase in the critical packing parameter (CPP). The increase in salt will thus lead to the change from spherical to elongated (rod-like) micelles. Further increase of salt may lead to the formation of surfactant bilayers.

7. Calculate the potential energy between the headgroup of the surfactant and a water molecule for the shorter possible separation. Assume a conformation that maximizes the interaction. Consider water to be a dipole with a radius of 0.14 nm and a dipole moment of 1.85 D (1 D = 3.336 $\times 10^{-30}$ Cm). Due to the proximity between water and headgroup assume that $\varepsilon = 1$.

   **Answer:** The charge-dipole potential energy can be calculated according to:

$$V(r) = -\frac{(ze)u\cos\theta}{4\pi\varepsilon_0\varepsilon r^2} \ .$$

   In order to maximize the interaction we consider that the water dipole and the surfactant headgroup are as close as they can be without overlapping ($r = 0.32 + 0.14 = 0.46$ nm) and that the dipole is oriented with the charge, $\cos\theta = 1$ (all other orientations will decrease the strength of the interaction). Taking the data from the formula in the end of the exam:

$$V(r) = -\frac{(1.602\times10^{-19})1.85\times3.336\times10^{-30}}{4\pi(8.854\times10^{-12})(0.46\times10^{-9})^2} = -4.20\times10^{-20} \quad \text{J} \ .$$

8. Is this interaction strong enough to orient the water molecule at room temperature (T=20 °C)?

   **Answer:** To assess if the interaction is strong enough to orient the water molecule, we start by calculating the thermal energy: $1\ k_{\mathrm{B}}T = 1.38\times10^{-23}\times293.15 = 4.045\times10^{-21}$

J. The interaction energy between the charged headgroup and the water molecule (assuming water to be a dipole) calculated in the previous question is larger than 1 $k_BT$. In practice this means that the interaction energy will be large enough to oppose the thermal motion and orient the water.

9. To learn more about the surfactant aqueous solution you performed dynamic light scattering experiments were using light with a wavelength of 500 nm and a scattering angle of 40° at 20 °C. Plotting the results as $\ln[g^{(2)}(q,\tau)-1]$ as a function of $\tau$ gives a straight line with a slope equal to $-14,423\ \mathrm{s}^{-1}$.

   Calculate the hydrodynamic radius of the micelle. Comment the result taking into account your previous answers.

   **Answer:** From the equations

$$g^{(2)}(q,\tau) = 1 + [g^{(1)}(q,\tau)]^2 \qquad \text{and} \qquad g^{(1)}(q,\tau) = \exp(-q^2 D_T \tau)$$

   One can deduce the equation

$$\ln\left[g^{(2)}(\tau) - 1\right] = -2q^2 D_T \tau.$$

   Since we are given the slope of the graph of $\ln[g^{(2)}(q,\tau)-1]$ as a function of $\tau$, we can calculate the translational diffusion from the relation:

$$\text{slope} = -2q^2 D_T \ .$$

   We need to calculate the scattering vector using $q = \frac{4\pi}{\lambda}\sin(\frac{\theta}{2})$, where $\lambda$ is the wavelength of light in the solution and $\theta$ is the angle between the incident light and the measured scattered light (detector):

$$q^2 = \left(\frac{4\pi}{\lambda}\sin\left(\frac{\theta}{2}\right)\right)^2 = \left(\frac{4\pi}{500\times10^{-9}[\mathrm{m}]}\sin\left(\frac{40}{2}\right)\right)^2 = 7.389\times10^{13}\ \mathrm{m}^{-2}\ .$$

   Finally, we can write

$$D_T = -\frac{\text{slope}}{2q^2} = -\frac{-14,423\ [\mathrm{s}^{-1}]}{2\cdot 7.389\times10^{13}\ [\mathrm{m}^{-2}]} = 9.76\times10^{-11}\ \mathrm{m}^2/\mathrm{s}\ .$$

   From the translation diffusion coefficient we can calculate the hydrodynamic radius according to:

$$f = \frac{k_B T}{D_T} = 6\pi\eta R_h$$

$$R_h = \frac{1.38\times10^{-23}\times 293.15}{6\pi 0.001\times 9,76\times10^{-11}} = 2.2\times10^{-9} \quad \mathrm{m}\ .$$

   In the previous question we found that the strength of the potential energy between the surfactant headgroups and water is sufficiently strong to align the water molecules. It is also to be expected that the water molecules will be sufficiently bound to diffuse with the micelle. It is thus not surprising that the radius of the micelle is large than the value calculated in question 2.3.

**Exercise 3.**

Consider a mixture of two proteins of molecular weight $M_1 = 20,000$ g/mol and $M_2 = 200,000$ g/mol in water. Assume that both are unhydrated and have the same partial specific volume of 0.74 $\mathrm{cm}^3/\mathrm{g}$. Consider that the average viscosity of the medium is $\eta = 1.5 \times10^{-3}$ kg $\mathrm{m}^{-1}\mathrm{s}^{-1}$ and the density 1.05 $\mathrm{g/cm}^3$.

1. Show that, for a unhydrated mixture of proteins, the following relation is valid: $s_2/s_1 = (M_2/M_1)^{2/3}$.

**Answer:** As the proteins have the same specific volume , we apply

$$s_i = \frac{M_i\,(1 - \overline{V}^{(S)}\rho)}{N_A f_i} \quad \text{with} \quad i = 1, 2,$$

allowing for

$$\frac{s_2}{s_1} = \frac{M_2}{M_1}\frac{f_1}{f_2}$$

Since $f = 6\pi\eta R$

$$\frac{s_2}{s_1} = \frac{M_2}{M_1}\frac{R_1}{R_2}$$

For an unhydrated protein sphere

$$\frac{4}{3}\pi R_i^3 = \frac{M_i \overline{V}^{(S)}}{N_A} \quad \Rightarrow \quad R_i \propto M_i^{1/3}.$$

Therefore:

$$\frac{s_2}{s_1} = \frac{M_2}{M_1}\left(\frac{M_1}{M_2}\right)^{1/3} = \left(\frac{M_2}{M_1}\right)^{2/3}$$

2. Calculate the ratio of the sedimentation coefficients of the proteins.

   **Answer:** Ratio of the sedimentation coefficients of the proteins is $s_2/s_1 = 4.64$.

3. Consider a sedimentation experiment where the two proteins are placed on top of a centrifuge tube filled with aqueous buffer containing a linear sucrose gradient from 5% to 20%, and then the tube is spun. The top of the tube is 4 cm from center, the bottom is 8 cm from the center. When the larger protein has sedimented a distance of 3 cm, how far has the smaller protein traveled (neglect any changes in viscosity and density associated with the sucrose gradient)? Is this a good method to separate the proteins?

   **Answer:** We can use the equation:

   $$\ln\left(\frac{r}{r_0}\right) = \omega^2\, s\,(t - t_0),$$

   to find:

   $$\frac{\ln(r_2/r_0)}{\ln(r_1/r_0)} = \frac{s_2}{s_1} = 4.64.$$

   Since at $t_0 = 0$ , $r_0 = 4$ cm:

   $$\ln(r_1/4) = \frac{1}{4.64}\ln(7/4) \quad \Rightarrow \quad r_1 = 4.5\,\text{cm}.$$

   Since the small protein has moved only 0.5 cm, this is a very good way to separate them.

4. Calculate the diffusion coefficients for each of the two proteins at 298 K. Note that a simple relation between $D_i$ and $M_i$ should also be valid in this case.

   **Answer:** We can use the Stokes law and, consequently we also need the radius $R_2$.

   Therefore,

   $$R_2 = \left(\frac{3}{4\pi}\frac{M_2\,\overline{V}^{(S)}}{N_A}\right)^{1/3} = 3.89 \times 10^{-7}\text{cm},$$

$$D_2 = \frac{k_B T}{6\pi\eta R_2} = 3.74 \times 10^{-7}\mathrm{cm}^2/\mathrm{s}.$$

We could calculate $D_1$ in a similar way or simply realize that, for an unhydrated sphere:

$$\frac{D_1}{D_2} = \frac{f_2}{f_1} = \left(\frac{M_2}{M_1}\right)^{1/3}.$$

Therefore:

$$D_1 = D_2 \left(\frac{M_2}{M_1}\right)^{1/3} = 3.74 \times 10^{-7} \left(\frac{200,000}{20,000}\right)^{1/3} = 8.06 \times 10^{-7}\,\mathrm{cm}^2/\mathrm{s}.$$

5. As the proteins sediment, the concentrated layer of protein will spread out due to diffusion. If the distance over which the molecules spread is larger than the distance separating the two proteins, then it will not be possible to use this method to separate the two proteins, because they will overlap. Calculate the average (root-mean-square) distance the two proteins move due to diffusion at 298 K if the experiment lasts 12 hours. Is this spreading distance due to diffusion significant compared to the separation of the two proteins due to sedimentation?

   **Answer:** The bands will spread out in the course of the centrifugation experiment due to diffusion, and the width of the bands is given by the root-mean-square displacement:

   $$r_{rms} = \langle r^2 \rangle^{1/2} = \sqrt{2Dt}.$$

   We obtain

   $$\langle r_1^2 \rangle^{1/2} = \sqrt{2D_1 t} = 0.26\,\mathrm{cm},$$

   and

   $$\langle r^2 \rangle^{1/2} = \sqrt{2D_2 t} = 0.18\,\mathrm{cm}.$$

   Since $r_2 - r_1 = 7 - 4.5 = 2.5$ cm, the broadening will not degrade resolution markedly.