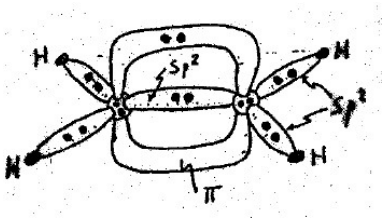
EXAM I COURSE
TFY4310 MOLECULAR BIOPHYSICS
December 2013

Suggested resolution

Exercise 1. [total: 25 p]

a) [t: 5 p] Describe the bonding [1.5 p] and the molecular orbitals [1.5 p] of the ethylene ($H_2C{=}CH_2$) and make a sketch that shows the electron density distribution [2 p].

Answer: $H_2C{=}CH_2$ (ethylen): Total # of electrons = 2·1+2·6+2·1=16



Molecular/binding orbital	# orbitals	# electrons
1s C (atomic orbital)	2	4
σ / sp^2 C-C	1	2
σ / sp^2 C-H	4	8
π /$2p_z$ C-C	1	2

b) [t: 2 p] The ethylene is a planar molecule with bond angles of about 120 °. Justify [1+1 p].

Answer: The bonds form an angle of 120 ° due to the formation of the three sp^2 orbitals in the Carbon atoms. The planarity is justified by the fact that the rotation of the molecule along the C-C bond would result in the loss of overlap of the $2p_z$ bonding orbitals and consequent breaking of the π molecular orbital which would be very energetically unfavourable, i.e., due to the stiffness of the π bond.

c) [t: 6.0 p] The melting temperature of a lipid membrane is directly proportional to the entropy of the lipid hydrocarbon chain. How do you expect the melting temperature to change with an increase in the chain length [1 p] and with the presence of unsaturated bonds [1 p]? Justify [2+2 p].

Answer: It is mentioned that the melting temperature is directly proportional to the entropy of the lipid hydrocarbon chain. The increase in the lipid chain length leads to the increase in the entropy since more conformation (states) will be available. The presence of unsaturated bonds (double bonds) leads to a restriction of the rotation of the chain around those particular bonds leading to an decrease in the conformational entropy of the chain and concomitant decrease in the melting temperature of the lipids.

d) [t: 12 p] The denaturation of a charged globular protein can be achieved by (i) an increase in the temperature, (ii) an increase in salt concentration, (iii) the addition of 6 M of urea (H_2-CO-NH_2), and (iv) the addition of alcohol. Discuss in detail the intramolecular interactions involved in the stabilization and destabilization of a globular protein in solution and how these are affected by the variations described above [4 x 3.0 p (of which 1.0 is for the description of the intra-molecular interactions and 1.0 for good argumentation (even if the rest is wrong)].

Answer: The main intra-molecular interactions involved in the stabilization of a globular protein are: (i) hydrophobic interactions between different hydrophobic residues that lead to the folding into a globule, (ii) hydrogen bonds between some of the residues, (iii) electrostatic interactions between oppositely charged residues in the protein (ionic bridges). Those that destabilize the protein conformation may be: (i) conformational entropy of the residue chain which opposes folding, and (ii) electrostatic interaction between residues with the same charge.

The denaturation of the protein may be achieved by

(i) Increasing the temperature: increase in the conformational entropy of the chain and lead to its unfolding.

(ii) Increasing the salt concentration leads to the screening (reduction) of electrostatic interactions in the system. Since it is indicated that the protein undergoes denaturation with the increase in salt, it indicates that the ion bridges are more important in stabilising the protein than the repulsion between equal charges are in destabilising it.

(iii) Urea is a chaotropic molecule. It forms strong hydrogen bonds with water, but do not have the same symmetry as the water molecules and will give rise to an increase in the overall structuring of the water. Hydrophobic interactions appear because the water molecules located near apolar surfaces are, on average, more structure than the rest of the water molecules. Chaotropic substance make this difference smaller, which gives rise to a weakening of the hydrophobic forces and consequent unfolding (denaturation) of the protein.

(iv) Alcohols, due to their carbon tail, can interact with the hydrophobic part of the proteins are therefore weaken the hydrophobic interactions between the different residues of the protein. We expect here that the effect is larger the larger the alcohol is.

Exercise 2. [total: 25 p]

a) [t: 6 p. Minus 0.5 for mistakes in calculations or units.] Calculate the molecular weight of a polyethylene polymer (CH_2 repeating unit) assuming that it takes the form of a spherical-like gaussian chain in solution. Dynamic light scattering measurements give an hydrodynamic radius of 275.8 Å and sedimentation velocity measurements yield a sedimentation coefficient of 613.1 S (10^{-13} s). Assume that the solution has a viscosity of 1.0 $\times 10^{-3}$ Ns/m^2 and a density of 1.0 g/cm^3 and that the polymer has a specific partial volume of 0.73 cm^3/g.

Answer: The sedimentation coefficient s can be written as

$$s = \left(1 - \overline{V}_1^{(S)} \rho_0\right) \frac{M_w}{N_{\mathrm{Av}} f}\,, \tag{1}$$

where $\overline{V}_1^{(S)} = 1/\rho_{\mathrm{protein}}$ is the protein's partial specific volume, M_w is the molecule weight of the protein and f is the translational friction coefficient. For spherical particles we have $f = 6\pi\eta R_h$ (Stokes formula).

We start by calculating the translational friction coefficient, $f = 6\pi\eta R_h = 5.199 \times 10^{-10}$ Ns/m.

Inserting the numerical values in the Svedberg equation we can calculate the molecular

weight according to:

$$M_w = \frac{sN_{\mathrm{Av}}f}{\left(1-\overline{V}_1^{(S)}\rho_0\right)} = 71093.2 \quad \mathrm{Kg/mol}\,,$$

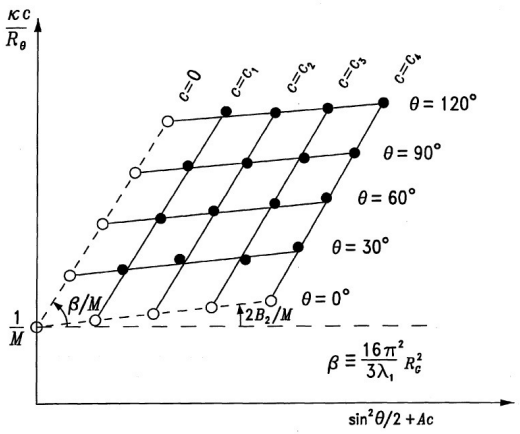
b) [t: 6 p: 2 points for naming the technique and 4 points for the experimental set-up.] The relations used in a) assume that the solution is very dilute. Describe the experimental procedure that allows to determine the molecular weight of a macromolecule more accurately.

Answer: Static light scattering can be used to obtain a more accurate measurement of the molecular weight of particles. Since the particle is not very small compared to the wavelength we have to consider the Laurenz-Mie regime and the relation:

$$\frac{\kappa C}{R_\theta} = \frac{1}{M_w}\left[1+\frac{2\pi}{3}S^2\cdot R_G^2\right]\cdot[1+2B_2C]$$

where κ is a constant that depends on experimental parameters (such as wavelength and the refractive index of the solution), C the concentration, R_θ the Rayleigh factor, which depends on the scattering angle, S the scattering vector, R_G the radius of gyration, and B_2 the second virial coefficient.

The procedure to determine the M_w consists in preparing solutions are different concentrations and measure the solutions at different angles. With this information it is possible to draw a Zimm plot as schematically shown below. Extrapolating the data for $C = 0$ and $S = 0$ yields the inverse of the molecular weight. Small angle X-ray scattering (SAXS) can



also be used using a similar experimental procedure.

c) [t: 13 p] Assuming that the bond length, 1.53 Å corresponds to one CH_2 unit, calculate the R_G of the polyethylene polymer considered above using the freely-jointed, the freely-rotating and the hindered-rotating chain models. Take $\theta = 112°$ and $\langle\cos\phi\rangle$ = -0.4. Give a brief description of the selected models and compare the results with the experimental value obtained above (take $R_h \approx R_G$). [2 p for reaching the value of n, 1 p for the description and 2 p for the calculation of each of the three chain models, and 2 p for a (reasonable) final comment regarding the results (even if these are wrong).]

Answer: Taking that the bond length corresponds to one CH_2 unit, we can calculate the number of bonds dividing the molecular weight of the polymer (obtained in (a)) with the

molecular weight of the unit (14 g/mol). This yields 5.08×10^6 bonds.

(i) **Freely-jointed chain model:** All 2(n+1) angular variables are allowed to assume any value with equal probability since he direction of any bond is equally likely to occur in any possible directions of space and the joints at each bond move freely to allow all possible conformations.

The end-to-end distance can be calculated according to:

$$\left\langle R_{\mathrm{ee}}^2 \right\rangle = Q^2 n \,,$$

where Q is the bond length and n the number of bonds. Replacing with the numerical values we get $\langle R_{\mathrm{ee}}^2 \rangle = 1.19 \times 10^{-13}$ m. Taking the relation $\langle R_{\mathrm{ee}}^2 \rangle = 6 \langle R_{\mathrm{G}}^2 \rangle$ we get $\langle R_{\mathrm{G}}^2 \rangle = 1.98 \times 10^{-14}$ m, and $R_{\mathrm{G}} = 140.8$ nm.

(ii) **Freely-rotating chain model:** More commonly, the bond angles in polymers are fixed or narrowly fixed to constant values, since it takes a lot more energy to distort bond angles than to induce rotations about single bonds, which are considered free. The conformation of a given chain in then reduced to a specifying the dihedral angles for n-1 bonds (all except the first). Polyethylene molecules, in particular, have a tetrahedral geometry due to the sp^3 bonds ($\theta = 109.5°$ or $\cos\theta = -1/3$).

The end-to-end distance can be calculated according to:

$$\left\langle R_{\mathrm{ee}}^2 \right\rangle = Q^2 n \left(\frac{1 - \cos\theta}{1 + \cos\theta} \right) ,$$

where θ is the bond angle. Replacing with the numerical values we get $\langle R_{\mathrm{ee}}^2 \rangle = (1.53 \times 10^{-10})^2 \cdot 5.078 \times 10^6 \cdot 2.20 = 2.62 \times 10^{-13}$ m. Taking $\langle R_{\mathrm{ee}}^2 \rangle = 6 \langle R_{\mathrm{G}}^2 \rangle$ we get $\langle R_{\mathrm{G}}^2 \rangle = 4.37 \times 10^{-14}$ m, and $R_{\mathrm{G}} = 209.0$ nm.

(ii) **Hindered-rotating chain model:** The dihedral angle is not really free to assume all possible values. Instead the angle is restricted by sterical interactions, with the trans conformation being the one with the lowest energy and the gauche+ and gauche-, other conformations with a minimum of energy. All rotations are perhaps possible, but the dihedrical angles will have a preference for low energy states.

The end-to-end distance can be calculated according to:

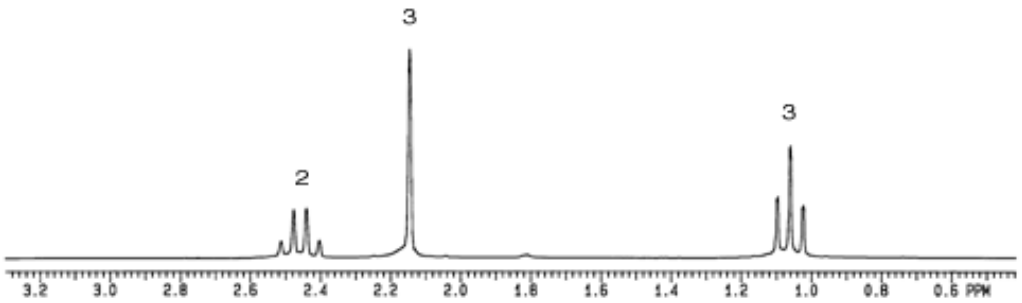
$$\left\langle R_{\mathrm{ee}}^2 \right\rangle = Q^2 n \left(\frac{1 - \cos\theta}{1 + \cos\theta} \right) \left(\frac{1 - \cos\phi}{1 + \cos\phi} \right) ,$$

where ϕ is the rotational (or dihedral) angle. Replacing with the numerical values we get $\langle R_{\mathrm{ee}}^2 \rangle = (1.53 \times 10^{-10})^2 \cdot 5.078 \times 10^6 \cdot 2.20 \cdot 2.33 = 6.09 \times 10^{-13}$ m. Taking $\langle R_{\mathrm{ee}}^2 \rangle = 6 \langle R_{\mathrm{G}}^2 \rangle$ we get $\langle R_{\mathrm{G}}^2 \rangle = 1.01 \times 10^{-13}$ m, and $R_{\mathrm{G}} = 318.6$ nm.

We see from the results that the more restrictions we consider in the models, the more extended the polymer chain will be. However, it can be seen that the results obtained by the models, are about one order of magnitude above that obtained experimentally (275.8 Å), when the opposite would be expected from a random coil in solution, indicating that the chain is in a more compact conformation, a globule perhaps, and so we can speculate that it is placed in solvent that is not good for the chain.

Exercise 3. [total: 24 p]

a) [t: 11 p]Below is a ^{1}H NMR spectrum of a given molecule, where the chemical shift is given relatively to TMS (not included). The number correspond to the area under each peak. Discuss the structure of the molecule [Discussion of the spectra in terms of the area under the peaks (3 p), the chemical shift of the peaks (3 p) and the spin-spin coupling of the peaks (3 p). 2p for writing the correct formula].



Answer: We can start by noticing that we have three groups of absorption peaks with the following information:

Group number	1	2	3
Relative area	2	3	3
Chemical shift (ppm)	2.45	2.15	1.05
# of peaks	4 (1:3:3:1)	1	3 (1:2:1)

The relative area under the absorption peaks in the different groups tells us the relative number of protons within each of the three groups.

The splitting of the peaks is due to spin-spin coupling between the nuclei (protons in this case) and of the respective groups and protons in a neighbouring group. If the number of equivalent nearest neighbor protons is two, this gives splitting into three peaks with relative area
$1:2:1 = (\uparrow\uparrow) : (\uparrow\downarrow, \downarrow\uparrow) : (\downarrow\downarrow)$.
If the number of nearest neighbor protons is three, this gives splitting into four peaks with relative area
$1:3:3:1 = (\uparrow\uparrow\uparrow) : (\uparrow\uparrow\downarrow, \uparrow\downarrow\uparrow, \downarrow\uparrow\uparrow) : (\downarrow\downarrow\uparrow, \downarrow\uparrow\downarrow, \uparrow\downarrow\downarrow) : (\downarrow\downarrow\downarrow)$.
This means that the number of splittings equals the number of possible values of nuclear spin of the equivalent nearest neighbour protons. Equivalent nearest neighbour protons are protons bound to a neighbouring C–atom that all have the same chemical shift. Protons bound to the same C–atom are equivalent and are not included as nearest neighbour.

Having this is mind we can conclude that group 2, composed of 2 protons, is the neighbour of a CH_3 group, group 3 is composed of 3 protons and it is connected to an atom that is not connected to protons, due to the lack of splitting. Finally group 3 is composed of 3 protons neighbour to a CH_2 group. We can further speculate that we have a molecule with a -CH_2-CH_3 group and a -CH_3 group, with a group lacking protons in between.

The chemical shift depends on the local chemical environment. By looking at a table with the typical values of chemical shifts of diverse groups, given in the end of the exam, we should be able to find the missing group. We see that the chemical shifts of groups 1 and 2

fit nicely with a ketone group.

The structure of the molecule is thus: $H_3C(=O)$-CH_2-CH_3, butanone.

b) [t: 13 p] We can write, from the Bloch equations:

$$\text{Signal} = \text{const}\, N \left(\frac{\mu_z^2 B_z^2}{k_B T}\right)\left(\frac{\gamma^2 B_{xy} T_2}{1 + T_2^2(\omega_0 - \omega)^2 + \gamma^2 B_{xy}^2 T_1 T_2}\right)$$

Define all parameters in this equation [0.5 p each]. Explain in detail why the NMR signal decreases with an increase in temperature [4 p]. Explain the difference between T_1 and T_2 [4 p].

Answer: The parameters are the following:

N: Number of nuclei in the sample volume;

μ_z: Magnetic moment along the field axis;

B_z: Magnetic field along the z-axis;

B_{xy}: Magnetic field in the xy-plane, which rotates at a frequency ω_0 in the same direction of the precessional motion of the nuclei;

T: Temperature in the experimental set-up;

T_1: Spin-lattice (longitudinal) relaxation time;

T_2: Spin-spin (transverse) relaxation;

γ: Gyromagnetic (magnetogyric) ratio;

ω_0: Larmor frequency;

ω: Frequency of B_{xy}.

NMR probes the alignment of the magnetic moment with the external field, B_z. If the temperature is increased, the net orientation decreases, leading to a decrease in the magnetization of the sample and, therefore, a decrease in the signal.

T_1 is a measure of time is takes for the nuclei in the sample to reach the average magnetization equilibrium, $\overline{M}_z$, after the magnetic field, B_z, is turned on. T_2 is a measure of time it takes the z component of M, M_z, to spontaneously return to its equilibrium value after the field B_{xy} has been turned off. M_{xy} will, in turn, decay to zero.

Exercise 4. [total: 26 p]

a) [t: 9 p] Large DNA molecules showing a coil (gaussian) conformation can undergo compaction to smaller globular structures by the addition of oppositely charged polymers (polycations). If the concentrations of DNA and polycations is sufficiently large the (neutralized) DNA-polycation complexes precipitate out of solution.

Name three techniques that can be used to study the compaction of DNA in solution and/or precipitation of DNA-polycation complexes [1 p each]. Justify your choice by describing, for each of the three techniques, the molecular properties that are determined [1 p each], as well

as one advantage [0.5 p] and disadvantage [0.5 p].

Answer: The answer to this question is very broad. I have basically accepted all the suggested techniques as long as they were reasonably justified. Same with the properties.

b) [t: 17 p] Some experimental work has been done on DNA gels, for studying DNA – polycation interaction.

i) Name the advantages [1 p] and disadvantages [1 p] of using this methodology.

Answer: Advantage: it is easy to visualise the changes in the polymer conformation by following the swelling/de-swelling of the gel. Disadvantage: The kinetics of swelling/de-swelling can be very slow but this can be partially solved by using gels with small volumes.

ii) The swelling equilibrium of ionic networks can be written in a simplified way, according to:

$$\Pi_{tot} = \Pi_{mixt} + \Pi_{elas} + \Pi_{ionic}$$

Describe qualitatively each of the three terms [2 p each]. Refer, justifying, two properties of the system that may influence each of the terms [1 p for each property and for each term (6 p in total)].

Answer: Π_{mixt}**:** Entropic mixing contribution to the osmotic pressure that leads to the swelling of the polymer chains. The temperature and quality of the solvent influence the swelling of the gels.

Π_{elas}**:** Elastic contribution to the osmotic pressure. The fact that the polymer chains are connected into a network restrains the swelling of the gels. The increase in the flexibility of the polymer chain and a low number of cross-linkers are parameters that contribute to a larger swelling of the gel.

Π_{ionic}**:** Contribution to the osmotic pressure that arises from the charges in the polymer chains and the presence of the respective counterions. The addition of simple salt (higher ionic strength) or decrease in the dielectric constant of the medium should lead to the de-swelling of a polyelectrolyte in solution.

iii) Do you expect the DNA gel to swell or deswell in the presence of the polycations [1.5 p]? If the gel was prepared from single-stranded DNA molecules, would you expect the swelling/deswelling to be larger or smaller? Justify [1.5 p].

Answer: The DNA gel is expected to deswell in the presence of the polycation, by a similar mechanism than the compaction of single DNA molecules in solution, ion-correlation effects. Mentioning the neutralization of the DNA chains and/or release of the counterions from the gel into solution is also fine.

There are two opposing effect when we consider gels made of ssDNA gels. The most relevant in this context is the fact that ssDNA molecules are more flexible than dsDNA which leads to a greater elastic contribution to the swelling/deswelling behavior and, therefore, a stronger swelling/deswelling behaviour. On the other hand, the ssDNA has a lower charge density when compared to the dsDNA molecules, meaning that to a smaller ionic contribution the osmotic pressure and a lower swelling/deswelling. As said, the first (stronger deswelling) is the most predominant but both answers were considered to be correct.