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EXAM I COURSE
TFY4310 MOLECULAR BIOPHYSICS

Friday, 16 December 2016
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 **five** (5) of the following sentences:

1. In the presence of trivalent ions, long DNA chains adopt more condensed conformations that those predicted by a simple neutralization of the phosphate charges along the chain.

2. The width of the probability distribution of the end-to-end distance $(P(R_{ee}))$ of a polymer chain is larger taking into account the freely jointed model than the rotationally-hindered statistical model, but the root-mean-square of the end-to-end distance $\langle R_{ee}^2 \rangle^{1/2}$ is lower.

3. When a cross-linked rubber is stretched by a dead load and is heated its extension decreases.

4. Brownian dynamics is a suitable computer modelling technique to follow adsorption of long polymers to oppositely charged surfaces.

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

6. In Raman scattering, the Stokes lines are stronger than the anti-Stokes lines, at room temperature.

7. X-ray scattering ($\lambda = 0.1$ nm) radiation with a scattering angle of 0.1° is better suited to study proteins with around 60 nm in diameter than visible light ($\lambda = 400$ nm) at a scattering angle of 50°. Note that the reciprocal of the scattering vector is defined as $2\pi/q$.

8. Small-angle neutron scattering (SANS) is a powerful tool to study the structure of DNA-protein complexes such as the nucleosomes.

Exercise 2.

1. Shortly describe the three forces that contribute to the van der Waals force.

2. van der Waals forces are predicted to decrease with increasing ε of the medium, however the strength of the interaction between two small apolar molecules increases when these are taken from air to water. Justify.

3. Figure 1(a) shows the scheme of a lipid membrane in water and the respective lateral pressure (or stress) profile. Taking into account the pressure profile, discuss the forces that act within the bilayer.
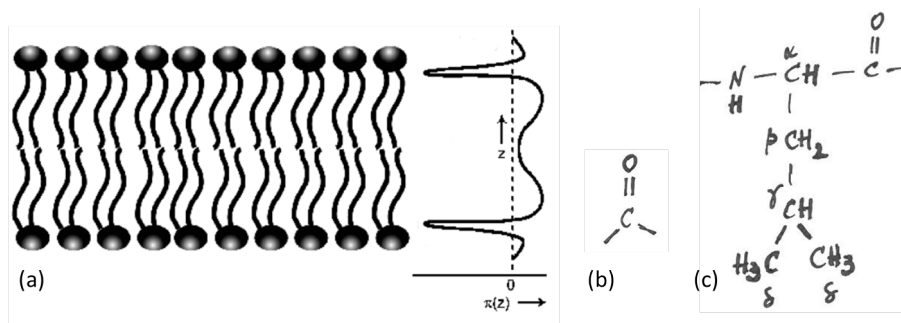

Figure 1: (a) Scheme of a lipid bilayer and lateral pressure profile (question 2.3). (b) Molecular structure of the carbonyl group (question 3.1). (c) Molecular structure of amino acid leucine (question 3.3).

4. Assuming that the headgroups of the lipids are negatively charged, that there is one lipid molecule per 0.8 nm^{-2} at 293 K, and that the concentration of ions in the bulk tend to zero, what will be the surface density of counterions at the membrane surface? Assume these occupy a thickness of 0.2 nm.

Exercise 3.

1. Draw the bonding and molecular orbital diagram of the carbonyl group in the peptide bond (Figure 1(b)) and discuss the hybridization scheme of the carbon and oxygen atoms.

2. Why does the extinction coefficient $(\varepsilon(\lambda))$ of a molecule depend on the wavelength?

3. The molecular structure of the amino acid leucine is depicted in Figure 1(c). Protons α to δ give rise to NMR peaks at chemical shifts around 3.3, 2.4 and 2.6 (each β proton experiences a different chemical environment due to the chiral group they are attached to), 1.9 and 1.0, respectively.

   i. Which multiplicity do you expect to observe for each peak?
   ii. Draw the two-dimensional COSY spectrum for leucine taking into account solely the indicated protons.

Exercise 4.

The variation in solute concentration with distance from the rotation axis r and time is given by the Lamm equation:

$$\frac{\partial c(r,t)}{\partial t} = D_{\mathrm{T}} \left(\frac{\partial^2 c(r,t)}{\partial r^2} + \frac{1}{r} \frac{\partial c(r,t)}{\partial r} \right) - s\omega^2 \left(r \frac{\partial c(r,t)}{\partial r} + 2c(r,t) \right)$$

1. Draw the evolution of the solute concentration along the cell with time.

2. Starting from the Lamm equation, explain how the sedimentation coefficient, s, is obtained experimentally.

3. The data given below describe the variation of the sedimentation coefficient and diffusion coefficient for protein A as a function of pH. Explain what happens to the protein at low and high pH, assuming that it is in the native (functional) state between pH 5 and 6.

pH	$s_{20,w}$ ($\times 10^{-13}$ s)	$D_{20,w}$ ($\times 10^{-7}$ cm^{2}s^{-1})
3	3.02	4.85
4	3.89	4.85
5	4.41	3.58
6	4.40	3.59
7	4.15	3.40
8	3.60	2.95
9	2.25	1.85

4. Calculate the molecular weight of protein A ($\overline{V}_1^{(S)} = 0.72$ cm^3/g) at pH 5.

5. Calculate the hydrodynamic radius of protein A at pH 5.

6. Protein A was additionally studied using small-angle X-ray scattering, where X-rays of $\lambda = 0.154$ nm were used, and the following data recorded:

θ (mrad)	$\ln I_s$
1.41	70.76
2.50	70.71
3.40	70.66
4.00	70.61

Calculate the radius of inertia (gyration). The data showed that the proteins have a nearly spherical shape, what is their diameter?

7. Comment on the values obtained in question 5 and 6.

The following formulas and data may or may not be of use in answering the preceding questions. You do not need to derive any of the formulas but all parameters must be defined, if used.

Electron charge: $\qquad e = 1.602 \times 10^{-19}$ C

Avogadro constant: $\qquad N_{\mathrm{Av}} = 6.022 \times 10^{23}$ mol^{-1}

Boltzmann constant: $\qquad k_{\mathrm{B}} = 1.380 \times 10^{-23}$ m^2 kg s^{-2} K^{-1}, J K^{-1}

Permittivity in vacuum: $\qquad \varepsilon_0 = 8.854 \times 10^{-12}$ C^2 J^{-1} m^{-1}

Properties of water at 20 °C:
$$\varepsilon = 78.4; \quad \eta = 0.01 \text{ g cm}^{-1}\text{s}^{-1}; \quad \rho = 1.02 \text{ g/cm}^3$$

Temperature: $\qquad [\mathrm{K}] = [°\mathrm{C}] + 273.15$

Atomic orbitals: H: $1s^1$; C: $[\mathrm{H}]2s^2 2p_x^1 2p_y^1$; N: $[\mathrm{H}]2s^2 2p_x^1 2p_y^1 2p_z^1$; O: $[\mathrm{H}]2s^2 2p_x^2 2p_y^1 2p_z^1$

Atomic weights: $\mathrm{A}_r(\mathrm{H}) = 1.0$; $\mathrm{A}_r(\mathrm{C}) = 12.0$

Thermodynamics
$$G = H - TS \qquad A = U - TS \qquad \vec{F} = -\vec{\nabla}A$$
$$S = k_{\mathrm{B}} \ln W$$

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

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

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

Coulomb potential $\qquad V(r) = \dfrac{z_1 z_2\, e^2}{4\pi\epsilon_0 \epsilon r}$

Screened Coulomb potential
$$V(r) = \frac{z_1 z_2\, e^2}{4\pi\epsilon_0 \epsilon r} \exp\left(-\frac{r}{\lambda_{\mathrm{D}}} \right)$$

Debye screening length $\qquad \lambda_{\mathrm{D}}^2 = \dfrac{\epsilon k_{\mathrm{B}} T}{\sum_i (e Z_i)^2\, n_{i\infty}}$

Density of ions at a charged surface $\qquad \rho_{\mathrm{s}} = \rho_0 + \dfrac{\sigma^2}{2\epsilon\epsilon_0 k_{\mathrm{B}} T}$

Friction coefficients $\qquad \vec{F} = -f\vec{v}, \qquad \vec{M} = -\xi\vec{\omega}$

Stokes formula $\qquad f = 6\pi\eta R_h, \qquad \xi = 8\pi\eta R_h^3$

For long chains and the random walk model $\qquad \left\langle R_{\text{ee}}^2 \right\rangle = 6\left\langle R_G^2 \right\rangle$

Radius of gyration of a sphere $\qquad R_{G,\text{sph}} = \sqrt{3/5}\,R_{sph}$

Hydrodynamic volume $\qquad v_{\text{h},1} = \left(\overline{V}_1^{(S)} + \delta\overline{V}_0^{(S)}\right)\dfrac{M_1}{N_{\text{Av}}}$

Specific volume (per mass) $V_1^{(S)} = v_1\left(\dfrac{N_{\text{Av}}}{M_1}\right)$

Fick's laws $\qquad \dfrac{\partial c}{\partial t} = -\vec{\nabla}\cdot\vec{J}, \qquad \vec{J} = -D_{\text{T}}\vec{\nabla}c, \qquad \dfrac{\partial c}{\partial t} = D_{\text{T}}\dfrac{\partial^2 c}{\partial x^2}$

Nernst-Einstein relations $\quad fD_{\text{T}} = k_{\text{B}}T, \qquad \xi D_{\text{R}} = k_{\text{B}}T$

Lamm-equation $\qquad \dfrac{\partial c(r,t)}{\partial t} = D_{\text{T}}\left(\dfrac{\partial^2 c(r,t)}{\partial r^2} + \dfrac{1}{r}\dfrac{\partial c(r,t)}{\partial r}\right) - s\omega^2\left(r\dfrac{\partial c(r,t)}{\partial r} + 2c(r,t)\right)$

Svedberg equation $\qquad s = \left(1 - \overline{V}_1^{(S)}\rho\right)\dfrac{M_1}{N_{\text{Av}}f}$

Equilibrium centrifugation: $\qquad m_1(r) = m_1(r_{\text{m}})\exp\left\{\dfrac{M_1(1 - \overline{V}_1^{(\text{S})}\rho)\omega^2(r^2 - r_{\text{m}}^2)}{2RT}\right\}$

Electrically-induce birefringence
$$I(t) = \dfrac{I_0}{4}\delta_0^2\exp(-12D_R t)$$

UV/Vis spectrophotometry
$$\log(I_0/I) = A(\lambda) = C\varepsilon(\lambda)l$$

Raman spectroscopy
$$P = \alpha_0 E_0\cos 2\pi\nu_0 t + \dfrac{1}{2}\left(\dfrac{\partial\alpha}{\partial q_i}\right)_0 q_{i0}\left[\cos(2\pi(\nu_0 + \nu_m)t) + \cos(2\pi(\nu_0 - \nu_m)t)\right]$$

Nuclear spin $\qquad \vec{m} = \gamma\vec{L}, \qquad (\vec{m})^2 = \gamma^2\hbar^2\ell(\ell+1), \qquad m_{\text{z}} = m_\ell\,\gamma\,\hbar$

Gyromagnetic ratio

Nucleus	^{1}H	^{2}H	^{13}C	^{14}N	^{19}F	^{31}P
$\gamma\left(10^7\frac{\text{rad/s}}{\text{T}}\right)$	26.753	4.107	6.728	1.934	25.179	10.840

Larmor frequency $\qquad \nu = \dfrac{\gamma}{2\pi}B_0$

Small-angle scattering

$$q = \frac{4\pi}{\lambda} \sin\left(\frac{\theta}{2}\right)$$

Guinier approximation

$$I_s(q) = I_0 \exp\left(-\frac{1}{3}q^2 R_G^2\right)$$

Discrete identical
homogeneous particles

$$\langle I_s(q) \rangle = N b^2(0) P(q) S(q)$$

Static light scattering
RGD regime

$$\frac{\langle I_S(q) \rangle}{I_0} = cM\kappa \frac{1}{R^2},$$

Large systems

$$\frac{\kappa c}{R_\theta} = \frac{1}{M}\left[1 + \frac{16\pi^2}{3\lambda^2} R_G^2 \sin^2 \frac{\theta}{2}\right] \cdot [1 + 2B_2 c],$$

Dynamic light scattering
Siegert relation

$$g^{(2)}(q, \tau) = 1 + [g^{(1)}(q, \tau)]^2$$

$$g^{(1)}(q, \tau) = \exp(-q^2 D_T \tau)$$

Scattering length density

Substance	H_2O	D_2O	proteins	nucleic acids	lipids
ρ (10^{-4} nm^{-2})	-0.55	6.36	3.11	4.44	-0.01