NTNU Dept of Physics

Exam TFY4310 Molecular Biophysics 24 may 2025
Suggested solutions

Exercise 1

Which of the following statements are not correct with respect to hybridization of atomic orbitals?

Select one alternative:

always results in three orbitals in a plane
reduces the electrostatic repulsion between electrons
Results in molecules with lower (more negative) potential energy
Can be viewed as linear combinations of the orbitals that underpin the specific hybridization

Exercise 2.

Increasing the concentration of salt in an aqueous solution leads to:

Select one alternative:

the strengthening of electrostatic repulsion
the Debye screening length is increasing
the weakening of electrostatic interactions
a decrease in the Bjerrum length

Exercise 3.

Which of the following statements is not correct with respect to the Poisson-Boltzmann (PB) equation:

Select one alternative:

Solutions containing ions are treated as a continuum in the PB equation
There is more than one type of ions in the PB equation
Temperature is a parameter of the PB equation
The concentration profile of ions in a solution in contact with a surface of high charge density is described by the linearized PB equation

Exercise 4

Which of the following statements is correct with respect to water/water molecules?

Select one alternative:

All hydrogen bonds between water molecules in the solid state (ice) is broken at the transition to the liquid state
There are more hydrogen bonds on average per water molecule in the liquid state than solid state.
There are fewer hydrogen bonds and more nearest neighbour molecules in liquid state of water compared the solid state (ice).
The density of water in the solid state is larger than the liquid state as most other compounds

Excerise 5

Which of the following statements are correct with respect to surfactant (amphiphilic) molecules:

Select one alternative:

- Amphipilic molecules aggregate in aqueous solutions since this reduces the contact between hydrocarbon chains and water
- Amphiphilic molecules form aggregates in aqueous solutions since this reduces their mixing entropy
- Amphiphilic molecules form aggregates in aqueous solutions due to the high permittivity of water compared to vacuum
- Amphiphilic molecules form aggregates in aqueous solutions due to van der Waals attractions between hydrocarbon chains

Exercise 6

Which of the below statements related to the critical packing parameter is not correct?

Select one alternative:

The critical parameter is related to the geometry of amphiphilic molecules when they are a part of a larger structure

The critical packing parameter describes the transition from dilute to semi-dilute polymer solution
The critical packing parameter is not used to describe the fluid character of molecular aggregates
The critical parameter is larger for a amphiphilic molecule with two hydrocarbon tails as compared to one with one tail

Exercise 7.

Which of the following sentences is not correct?

Select one alternative:

The mixing contribution of the free energy of polymer gel swelling is only valid for ideal chains.
The statistical mechanical theory (SMT) of rubber elasticity assumes that the gel network has no defects
When a rubber band, stretched by the action of a weight, is heated up, the band elongation decreases
To best describe the elastic contribution to the free energy of polymer gel swelling a term describing volume variation must be included in the SMT of rubber elasticity

Exercise 8

Polymer networks composed of neutral polymers swell less in aqueous solution than networks with charged polymers because:

Select one alternative:

-water is a poorer solvent for charged polymers
polyelectrolytes are more flexible than neutral polymers
-The osmotic pressure due to imbalance in the ion concentration inside and outside of the hydrogel is not present for the neutral hydrogel
The hydrogel with the neutral polymers has a higher crosslink density

Exercise 9.

Which of the following statement related to molecular modelling using periodic boundary conditions is correct?

Select one alternative:

Each particle only interacts with particles within the same box
-The interaction for a given particle with another one is constrained to be within a distance of half the length of the periodic box
The step length towards a new trial state is limited to the length of the periodic box
-The number of molecules at the interface between the periodic boxes are inversely proportional to the square root of the box length

Exercise 10.

Which of the below statements, regarding Metropolis sampling used in molecular modelling, is correct?

Select one alternative:

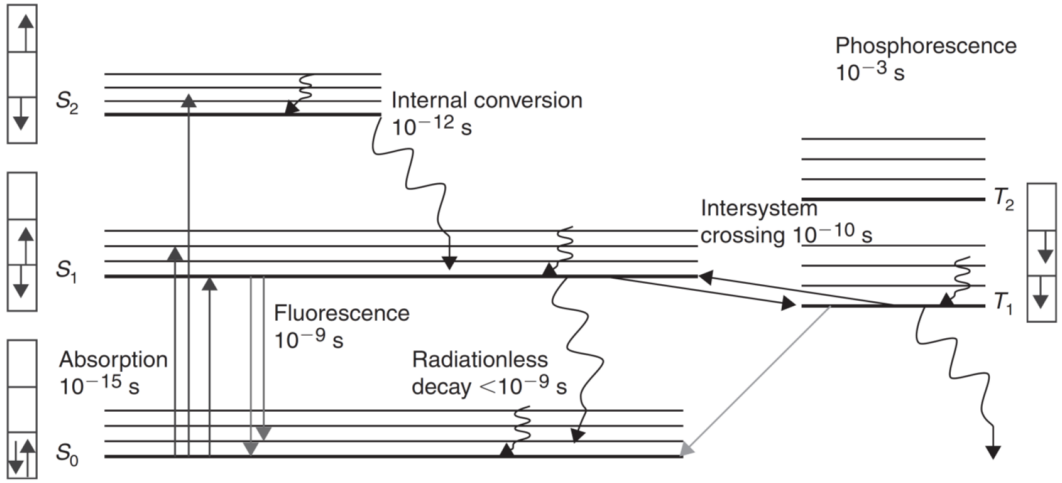
A transition to a state with higher potential energy is not always forbidden
Cannot be realized within periodic boundary conditions
The method is used for accellerated molecular dynamics
Is not including the use of random numbers

Exercise 11

a) *The Jablonski diagram is a graphical representation used to illustrate excitation and de- excitation processes when fluorescence is described. Make a drawing of a Jablonski diagram depicting relevant energy levels for excitation and de-excitation processes when fluorescence is occurring. Illustrate relevant transitions on such a diagram and describe them. Describe the assumption that is beyond the representation of an absorption process as a vertical line in a diagram that also include information of internuclear distance. Why is the fluorescence emission nearly independent of the excitation?*

Suggested solution

A Jablonski diagram:

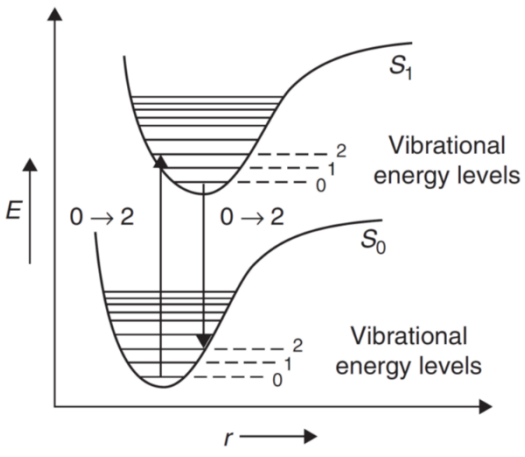


A Jablonski diagram in a version without eplicitly depicting the internucluear distance depict energy level for a molecule with ground state of electrons (S_0), first and second electronic excitated state (S_1 and S_2), and triplet states (T_1 (and excited triplet state…). On each of these energy levels of the electrons, there are overlaid vibration levels. The relevant transitions are : adsorption of electrons that induces a transition from the ground state of the electrons (S_0) to a vibration level overlaid the first electronic excited state (S_1), or from S_0 to a vibration level overlaid the second electronic excited state (S_2). These are illustrated with arrows in the above diagram – where the spins of the electrons occupying the various energy levels of the electronic state also are illustrated.

Fluorescence is a radiative decay processes that an excited system return to the ground level by. Fluorescence is return of an excited molelar system from S_1 to S_0, mostly from a low vibrational level of S_1 to a vibriationel level of S_0 with the concomitant release of the energy difference as a photon (lifetime of the order of nanoseconds). There are also radiationless decays, stated as internal conversion, and depicted by the curly arrows in the diagram, to vibrational states within the same electronic excitation level, and from a electronic excitaion level to an exciation level with lower energy, e.g., S_2 -> S_1. The reason fluorescence emission is nearly independent of exitation is that the internal conversion towards S_1 (e.g. from S_2, or also vibrational relaxation) is much faster (picoseconds) compared to fluorescence (nanoseconds). This imply that the energy of the emitted photon occurring in fluorescence correspond to the range of S_1 to S_0, independent on whether the initial excitation was to S_1 or S_2

The transition to the triplet state by intersystem crossing is a competing process to other de-exciation processes, and may thus reduce the quantum efficiency of the fluorescence.

The below graph shows a Jablonski diagram in a version the depict energy levels as function of internuclear distance. The absorption (excitation) is shown as a vertical arrow on such a diagram because the absorption of the electron is fast as compared to changes in internuclear distance. Relevant here is the Frank Condon principle: as stated in the compendium: "According to the Born-Oppenheimer approximation, electronic transitions are much faster than atomic motion; upon excitation, electronic transitions occur in about 10^{-15} s, which is much faster than the characteristic time scale for molecular vibrations, 10^{-10} to 10^{-12} s. Frank-Condon stated that electronic transitions are most likely to occur without changes in the position of the nuclei in the molecule, that is, the transition to an excited electronic state can be to any of the vibrational levels. The Frank-Condon principle states that because the nuclei are so much more massive than the electrons, an electronic transition takes place very much faster than the nuclei can

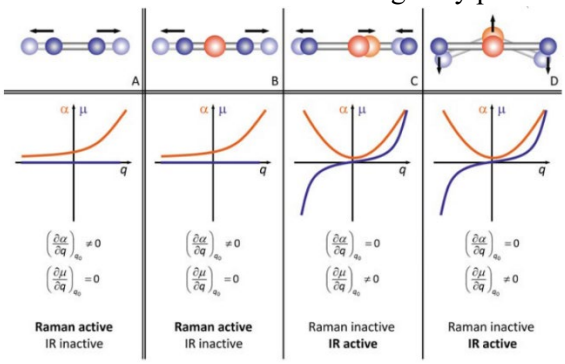


respond."

b) *Infra-red spectroscopy and Raman scattering are described as techniques giving complementary information of the samples being characterized. What is the basis for the statement that these techniques are giving complementary information*?

Infra red spectroscopy and Raman scattering give both signals in the infrared – far infrared region, and are complementary due to different impact of molecular structure on the absorption process in these two techniques. Absorption in the infrared region (IR) induces transition betweeen the vibrational states of the characterized molecules, and is depending on the transition dipoles (expectation value of the dipole moment induced by the relevant radiation) must be different than zero. In cases where the dipole moment of the molecule us altered during vibration, IR absorption will occur.

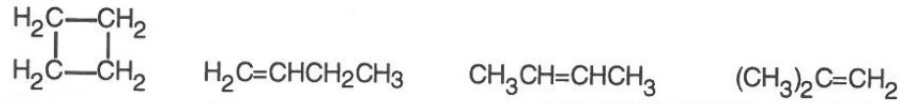
The mechanism of Raman scattering is by polarizing the molecule by the incident radiation, thus signals are dependent on that the polarizability (α) changes with vibration is different from zero:



$$\left(\frac{\partial \alpha}{\partial q}\right)_{q_0} \neq 0$$

Thus, vibrations of molecules can either be IR or Raman active depending on the mode of the vibrations (see Fig above).

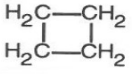
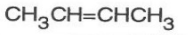
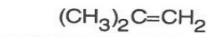
c) *Within the overall chemical composition C_4H_8 there are several possible structures as indicated in Fig 1.*



i) ii) iii) iv)

Analyze these structures with respect to the number of peaks you expect to observe from the pure forms of each of these 4 (i, - iv)) structures by ^{1}H NMR og ^{13}C NMR.

It is possible to distinguish between these isomers based on experimental characterization by either ^{1}H NMR and ^{13}C NMR. In either case, it is first necessary to find the number of different kinds of of protons or carbon atoms:

Sample				
Kind of protons	1	5	2	2
Kind of carbon atoms	1	4	2	3
Number of 1H NMR peaks	1	5	4	2
Number of 13C NMR peaks	1	4	2	3

For compound i): All H and C are equivalent, giving rise to one peak for H-NMR in the proton spectrum and one peak in the C-NMR spectrum

For compound 2: the two protons on the leftmost C, H_2C-, gives rise to two different peaks in the 1H NMR spectra since their proximity to H on the double bonded C are different. The proton the second leftmost C gives a third peak; and the two protons on the subsequent single-bonded C are equivalent, giving rise to one peak, and the three methyl protons are also equivalent. Thus, there are five peaks expected, and in the order mentioned: with intensities (area under the peaks): 1:1:1:2:3.

For compound 3, there are two isomers around the double bound, where the protons bound the double bonded protons are either cis (on the same side) or trans (on opposite sides). These two different isomers will have different resonance frequencies for these protons as well as for the methyl protons. Thus, for a mixture of the two isomers: there will be 4 peaks.

For compound 4, there are two type of protons, where each proton within a group is equivalent. Thus, there will be two peaks in the 1H-NMR spectrum with intensity ratio 6:2 between the methyl protons and those of the $=CH_2$group.

^{1}H NMR can be used to distinguish among the isomers due to different number of expected peaks (see table). This can be further substantiated by looking into the splitting (but not needed). ^{13}C NMR is the simplest method for identifying these compounds because each isomer differs in the number of adsorptions in its ^{13}C NMR spectrum.

Exercise 12

a) *Polyethylene glycol (PEG) (-CH2-CH2-O-)n is a polymer used to improve biocompatibility of e.g. drugs and nanoparticles. The action mechanism is believed to be related to its action to prevent direct interactions of the drug/nanoparticle with proteins when the drug/nanoparticle is functionalized with PEG.*

For a series of PEG samples, a colleague has provided the following corresponding experimental data of molecular weight, M, and radius of gyration, R_G:

Sample	*PEG1*	*PEG2*	*PEG3*	*PEG4*
M (in g/mol)	*$2.12x10^5$*	*$5.70x10^5$*	*$8.38x10^5$*	*$11.0x10^5$*
R_G (in nm)	*30.4*	*50.8*	*59.4*	*69.0*

(the sample identification PEGX, X=1,4, is introduced for easier reference if needed).
Your colleague asks you to analyze this information with respect to polymer properties of the PEG.
Assume that the molecular weight of the repeating unit of PEG, (-CH_2-CH_2-O-) is 44 g/mol, and the length of this repeating unit is 0.45 nm.
Calculate the characteristic ratio of PEG. The Kuhn length of PEG is 2.8 nm. Discuss the difference between the Kuhn length and length of the repeating unit of PEG.

Suggested solution:
The following equation describes the characteristic ratio:

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

Where C is the characteristic ratio, here with the index in the limit of infinitely long chain; Q is the segment length, and n is the number of segments. The data provided is the radius of gyration and not the end-to-end distance, and the relation to R_G is given by:

$$\left\langle R_{ee}^2 \right\rangle_0 = 6\left\langle R_G^2 \right\rangle$$

The experimental data provides us with corresponding values of Rg and M, and analysis indicates that the ratio R_G^2 / M and not R_G / M are independent of molecular weight:

Sample	PEG1	PEG2	PEG3	PEG4
M (in g/mol)	$2.12x10^5$	$5.70x10^5$	$8.38x10^5$	$11.0x10^5$

R_G (in nm)	30.4	50.8	59.4	69.0
R_G^2 / M (in nm^2 g^{-1} mol)	4.36x10^{-3}	4.53x10^{-3}	4.21x10^{-3}	4.45x10^{-3}
R_G / M (in nm g^{-1} mol)	1.34x10^{-4}	8.91x10^{-5}	7.09x10^{-5}	6.37x10^{-5}

The average value of R_G^2 / M for the four PEGs is 4.39 x10^{-3} nm^2 g^{-1} mol. In the following, we are using this value related to the notation $\langle R_G^2 \rangle$ here. The molecular weight of the PEGs can be written as M = M_{rep} n, where M_{rep} is the molecular weight of the repeating unit (44 g/mol), and n the number of repeating units. Inserting the information in the equations, we can write:

$$6\langle R_G^2 \rangle = C_\infty Q^2 n$$

$$C_\infty = \frac{6\langle R_G^2 \rangle}{Q^2 n} = 6\frac{\langle R_G^2 \rangle}{M}\frac{M}{Q^2 n} = 6\frac{\langle R_G^2 \rangle}{M}\frac{M_{rep} n}{Q^2 n} = 6\left(\frac{\langle R_G^2 \rangle}{M}\right)\frac{M_{rep}}{Q^2}$$

Where the bracket in the last term is introduced to group the parameters corresponding to the data. Inserting the values, including Q = 0.45 nm, yields:

$$C_\infty = 6\left(\frac{\langle R_G^2 \rangle}{M}\right)\frac{M_{rep}}{Q^2} = 6\left(4.39\times10^{-3} nm^2 g^{-1} mol\right)\frac{44 g\, mol^{-1}}{(0.45nm)^2} = 5.7$$

Thus, in providing a feedback to your colleague, one can state that the data of the PEG samples indicate a random coil like structure with a characteristic ratio of 5.7.

In addition to the characteristic ratio (as focused on above), there are other parameters that can be used as basis for characterizing chain stiffness. The Kuhn length, being one of these, is the segment length of an equivalent polymer chain model, there the contour length is kept as that based on the structure, and the chain extension, e.g., the end-to-end distance, is described as a freely hinged model (statistical model, randoom walk model). The Kuhn length is then alternatively phrased as a statistical segment length.
With the current statement the Kuhn length is stated to be 2.8 nm, which is far larger than the length of the repeating unit of 0.45 nm, indicates that the repeating units do not correctly describe the statistical behavior of the polymer as a statistical chain, and longer segments should be used instead. The reason for this is the local stiffness of the polymer (PEO) chain.

b) *Two colleagues are working with the same polymer, but they have ordered their polymer samples from different suppliers. When preparing their samples for further experimental characterization, colleague A experiences that the polymer is not soluble in water at 10% (volume fraction) polymer, while colleague B experiences that the polymer sample dissolves at 10% (volume fraction) in aqueous solution. The colleagues are puzzled by this and ask you for an explanation. Discuss why the two colleagues experience this difference*

Suggested solution
The Flory-Huggins theory specifies that the free energy of mixing is less favorable for long (many monomers in each polymer) than shorter (fewer monomers in each polymer chain) polymers since the longer chain will give fewer starting points for the first monomers, e.g. less entropy, in the lattice used as basis for the theoretical deduction of mixing free energy (given the same volume fraction). Or in other words: increased entropy for the collection of shorter polymers since they can be selected independently when putting them on the model lattice.
Based on this theory, it is suspected that the two colleagues have obtained polymer samples with different molecular mass, the one with high molecular weight not being soluble, whereas the one with a lower molar mass being soluble.
Based on equation of free energy of mixing of a polymer (component 2) with degree of polymerization x in a solvent (component 1) we can make some estimates. This equation is:

$$\frac{\Delta G_{mix}}{N} = RT\left(v_1 \ln v_1 + \frac{v_2}{x}\ln v_2 + \chi_{12} v_1 v_2\right)$$

where $N = N_1 + x\,N_2$ is the molar number of lattice sites; v_1 and v_2 are the fraction of the lattice occupied by solvent and polymer, respectively, x is the number of segments per polymer molecule, and kji 12 the Flory Huggins interaction parameter. Difference in free energy for the dissolution: the term $(v_2/x)\ln v_2$ vanishes in the case of x

Excercise 13

a) *In the microscopic description of the diffusion process, the mean square displacement of a macromolecule with diffusion constant D_T is described by the equation:*

$$\langle r^2 \rangle = 4 D_T\, t$$

This is valid for the two-dimensional (2-D) description. Parameter t is the time in this equation. What is the RMS of the displacement for the following biological macromolecules with diffusion constants as specified within 30 min?

Macromolecule	D_T
RNAse	*11.2 x 10^{-7} cm^2/s*
Fibrinogen	*1.98 x 10^{-7} cm^2/s*
DNA	*0.4 x 10^{-7} cm^2/s*

(Here: RMS: is an abbreviation for «root of mean-square»)

Suggested solution:

Performing the numerical calculations for the above proteins one get for RMS diffusion distance within 30 min:

Makromolekyl	D_T	$\sqrt{\langle r^2 \rangle} = 2\sqrt{D_T\, t}$
RNAse	11.2 x 10^{-7} cm^2/s	898 µm
Fibrinogen	1.98 x 10^{-7} cm^2/s	378 µm
DNA	0.4 x 10^{-7} cm^2/s	169 µm

b) *While the above expression of $\langle r^2 \rangle$ is valid for diffusion in a domain without boundaries, the equation describing $\langle r^2 \rangle$ for the diffusion within circular 2-D constraint with diameter d is given by:*

$$\langle r^2 \rangle = \frac{d^2}{3}\left(1 - \exp\left(-\frac{12 D_T\, t}{d^2}\right)\right)$$

Is this equation consistent with the description for free diffusion at small values of t?
How much are the diffusion driven RMS distance reduced for the above macromolecules compared to free diffusion for a circular domain with d = 300 µm (0.3 mm)?

Concerning the first question here, the limit of constrained diffusion eq for small t can be obtained using a serial expansion of the exponential (e.g. in the limit that $12\,D_T\,t << d^2$):

$$\exp(x) = 1 + x + \frac{x^2}{2!} + \frac{x^3}{3!} + \ldots.$$

Inserted with $x = -\frac{12 D_T t}{d^2}$ in the equation for $\langle r^2 \rangle$ yields:

$$\langle r^2 \rangle = \frac{d^2}{3}\left(1 - \left(1 - \frac{12 D_T\, t}{d^2} + \frac{1}{2!}\left(-\frac{12 D_T\, t}{d^2}\right)^2 + \ldots.\right)\right) = \frac{d^2}{3}\frac{12 D_T\, t}{d^2}\left(1 - \frac{1}{2}\left(\frac{12 D_T\, t}{d^2}\right) + \ldots.\right)$$

$$= 4 D_T\, t\left(1 - \frac{1}{2}\left(\frac{12 D_T\, t}{d^2}\right) + \ldots.\right)$$

This equation converges to the one for free diffusion in the limit of small t ($12\,D_T\,t << d^2$):

$$\lim_{t \to 0} 4 D_T\, t\left(1 - \frac{1}{2}\left(\frac{12 D_T\, t}{d^2}\right) + \ldots.\right) = 4 D_T\, t = \langle r^2 \rangle_{Free}$$

For the numerical values:

Macromolecule	D_T	$\sqrt{\langle r^2 \rangle} = 2\sqrt{D_T\, t}$	Constrained	% red/ constrained
RNAse	11.2 x 10^{-7} cm^2/s	898 μm	173 μm	80.7%
Fibrinogen	1.98 x 10^{-7} cm^2/s	378 μm	172 μm	54.3%
DNA	0.4 x 10^{-7} cm^2/s	169 μm	136 μm	19.8%

c) A macromolecule is characterized by dynamic light scattering (DLS) in aqueous solution at low concentration. The instrument used for DLS has a laser with a wavelength of 512 nm (in air), and the data are observed at a scattering angle of 45 degrees.

The following data are observed for the intensity correlation function as a function of τ:

τ (μs)	5	10	40	100	200
$g^{(2)}$	1.947	1.896	1.646	1.335	1.111

Calculate a parameter for the size of the macromolecule based on this data.

Suggested solution:

For the dynamic light scattering data, the normalized time correlation function of the scattered intensity is related to $g^{(1)}(q,\tau)$:

$$g^{(2)}(q,\tau) \equiv \frac{\langle I(q,0)\, I^*(q,\tau)\rangle}{\langle I(q)\rangle^2} = 1 + \left[g^{(1)}(q,\tau) \right]^2$$

where:

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

The diffusion constant here, is the one referred to as the free particle diffusion constant, e.g. the one obtained at dilute solution. This is related to the size of the particle:

$$D = \frac{k_B T}{6\pi\eta R_H}$$

Where R_H is the effective (hydrodynamic) radius of the particle, e.g. assuming it is spherical; k_B Boltzmanns constant, T absolute temperature and η the viscosity of the solvent.
Thus,

$$g^{(2)}(q,\tau) - 1 = \left(g^{(1)}(q,\tau) \right)^2 = \left(\exp(-q^2 D_0 \tau) \right)^2 = \exp(-2q^2 D_0 \tau)$$

$$\ln\left[g^{(2)}(q,\tau) - 1 \right] = -2q^2 D_0 \tau$$

For the actual data:

τ (μs)	5	10	40	100	200
$g^{(2)}$	1.947	1.896	1.646	1.335	1.111
$\ln\left[g^{(2)}(q,\tau) - 1 \right]$	-0.0547	-0.1094	-0.438	-1.094	-2.189
$\ln\left[g^{(2)}(q,\tau) - 1 \right] / \tau$ (μs^{-1})	-0.0109	-0.0109	-0.0109	-0.0109	-0.0109

Thus, the data indicate: $D_0 = \frac{0.0109\,(10^{-6}\,\mathrm{s})^{-1}}{2q^2} = \frac{0.0109\,(10^{-6}\,\mathrm{s})^{-1}}{2\times(1.26\times10^{7}\,\mathrm{m}^{-1})^2} = 3.43\times10^{-11}\,\mathrm{m^2 s^{-1}}$

(The scattering vector in the dynamic light scattering is calculated to q=1.26x10^7 m^{-1}.Note here: estimate of q requires conversion of the wavelength to that in water using the refractive index)

Using the above equation for D, we obtain the estimate for the radius:

$$R_H = \frac{k_B T}{6\pi\eta D_0} = \frac{1.38\times10^{-23}\,\mathrm{JK^{-1}}293\mathrm{K}}{6\pi\,1.0\times10^{-3}\,\mathrm{kgm^{-1}s^{-1}}\,3.43\times10^{-11}\,\mathrm{m^2\,s^{-1}}} = 6.2\mathrm{nm}$$

Exercises 14.

An antibody with a molecular weight of 150 kg/mol changes its geometry when the ligand (molecular weight 250 g/mol) binds to it. This change in geometry can be characterized by analytical ultracentrifugation (AUC). Antibody without bound ligand is characterized in water at 20 degrees Celsius by AUC, and a sedimentation coefficient of 6.4 S (1S = 10-13 s) is found. The partial specific volume of the antibody is 0.689 cm3/g.

a) *Calculate the hydrodynamic radius of the antibody without the bound ligand. Assume a spherical geometry in this calculation.*

Suggested solution:

The Svedberg equation is given in the set of formulas and equations and describes the how the sedimentaion coefficient (s) depends on partial specific volume of the molecule ($\bar{V}_1^{(S)}$), the density of the displaced fluid (ρ_0), the molar mass of the molecule (M_1) and the friction coefficient, *f:*

$$s = \left(1-\bar{V}_1^{(S)}\rho_0\right)\frac{M_1}{N_{Av}f}$$

The additional constant in this equation is the Avogadro' number (N_{Av}). To get to the hydrodynamic radius (R_h), the relation between the friction coefficient and the R_h is needed:

$$f = 6\pi\eta R_h$$

where η is the viscosity of the solvent at the actual conditions. Inserting this in the Svedberg eq:

$$s = \left(1-\bar{V}_1^{(S)}\rho_0\right)\frac{M_1}{N_{Av}6\pi\eta R_h}$$

and rearranging by solving for the hydrodynamic radius:

$$R_h = \left(1-\bar{V}_1^{(S)}\rho_0\right)\frac{M_1}{N_{Av}6\pi\eta s}$$

Inserting the actual numerical values:

$$R_h = \left(1-0.689\,cm^3\,g^{-1}1.0\,g\,cm^{-3}\right)\frac{150\,000\,\mathrm{g\,mol^{-1}}}{6.022\times10^{23}\,\mathrm{mol^{-1}}\,6\pi\,1.0\times10^{-3}\,\mathrm{kg\,m^{-1}\,s^{-1}}\,6.4\times10^{-13}\,\mathrm{s}} = 6.42\times10^{-9}\,\mathrm{m}$$

The sedimentation data correspond to a hydrodynamic radius of 6.42 nm for a spherical geometry.

b) *The sedimentation coefficient is observed to increase by 5.0% for the antibody-antigen complex (in the aqueous solution) as compared to the antibody. Calculate the hydrodynamic radius of the antibody-antigen complex. Assume that the partial specific volume of antibody- antigen complex is the same as the antibody.*

Suggested solution:

Taking into account the relation between the sedimentation coefficient (s) and (R_h) presented above, we can write the product of s and Rh:

$$s\,R_h = \left(1-\bar{V}_1^{(S)}\rho_0\right)\frac{M_1}{N_{Av}6\pi\eta}$$

And furthermore:

$$\frac{s\,R_h}{M_1} = \frac{1-\bar{V}_1^{(S)}\rho_0}{N_{Av}6\pi\eta}$$

In this expression, the right hand-side is constant for the two cases in characterized in this case. Thus;

$$\frac{1-\bar{V}_1^{(S)}\rho_0}{N_{Av}6\pi\eta} = \left(\frac{s\,R_h}{M_1}\right)_{antibody} = \left(\frac{s\,R_h}{M_1}\right)_{antibody+antigen}$$

In this notation, the paramter values of s, Rh and M1 in the brackets need to correspond to the antibody, ra or the antibody+antigen, respectively. Inserting the numerical values:

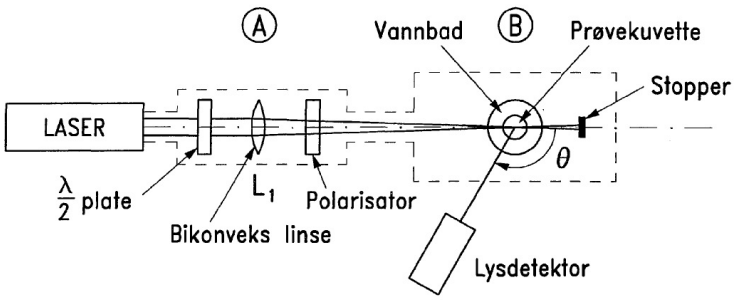
$$R_{h,antibody+antigen} = 6.42\,\text{nm}\frac{6.4}{6.4\times1.05}\frac{150\,000+250}{150\,000} = 6.12\text{nm}$$

Exercise 15 Scattering

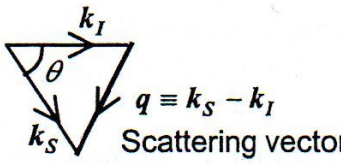
a) *Make a schematic drawing and briefly explain the layout of an instrument for characterization of biological macromolecules in solution by static light scattering. As part of this, define the scattering vector.*

The graph below provides a schematic illustration overview of an instrument suitable for static light scattering. This is a "birds eye view" of the layout. The main parts are: (A) Light source with defined wavelength (laser), lenses in the optical path to direct the incident light to the core part of the sample (scattering volume), optional optical elements in the optical path to control polarization of the incident light. (B) sample cuvette with circular cross-section (cylindrical like) to ensure reduced refraction of incident and scattered light when traversing the waterbath – sample cell boundary, thermostated bath for maintaining temperature and a beam stopper for forward direction of the light. There is a light detector mounted on a goniometer to detect light intensity at various scattering angles θ

Additionally, a computer properly interfacing to the various part for their control and data collection is usually included.



The scattering vector q is the difference between the wavevector of the scattered light, ks, and the incident light, k_I. as show in the graph to the right



The absolute value of the scattering vector:

$$q = \left|\vec{q}\right| = \frac{4\pi}{\lambda_1}\sin\frac{\theta}{2}$$

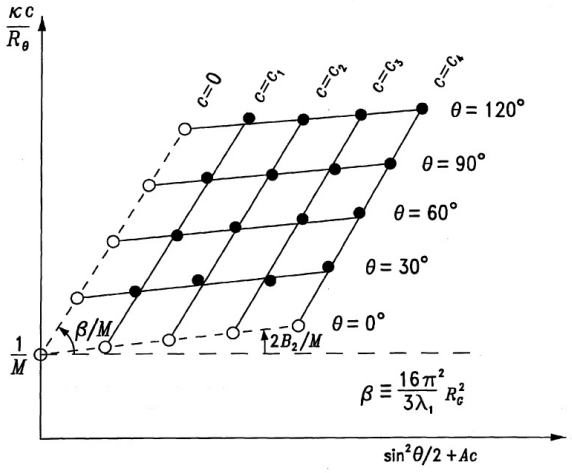
Here, θ is the scattering angle, and λ_1 the wavelength in the sample

b) A Zimm plot is a graphical representation of data obtained by static light scattering used to analyze scattering data of biological macromolecules in solution. Make a sketch of light scattering data presented as a Zimm plot and describe how the data as presented there is used to determine parameter values for the characterized macromolecule. Include the equations underlying the analysis.

Suggested solution

In the Zimm plot, the actual experimental data of from light scattering expressed as $\kappa c/R_\theta$ is presented vs.

$\sin^2\theta/2 + Ac$, for each of the c and θ and where A is a numerical constant adjusted to provide a reasonable spread of the data. An example of this is shown to the right. In this realization: the filled spherical points depict actual datapoints, and open circles extrapolated values.



The Zimm plot is a graphical representation of light scattering data according to the equation:

$$\frac{\kappa c}{R_\theta} = \frac{1}{M}\left[1 + \frac{16\pi^2}{3\lambda_1^2}R_G^2\sin^2\frac{\theta}{2}\right]\cdot\left[1 + 2B_2 c\right]$$

The parameters here (and thus, also the ones used in the Zimm plot are:

$\kappa = \dfrac{4\pi^2 n_L^2 \left(d\tilde{n}/dc\right)^2}{N_A\lambda_0^4}$, where n_L is the refractive index of the solvent; $d\tilde{n}/dc$ the refractive index increment of the solution when adding biopolymer to the solution; N_A Avogadro's number, and λ_0 the wavelength of the monochromatic light in vacuum. Parameter κ is a constant for a given biopolymer and wavelength. This is not on the formula sheet – not requiring details, but should state it is a constant for a given sample/solution)

c: is the (bio)polymer concentration

$R_\theta = I(\theta)r^2/I_0$ (valid for incoming polarized light in the "y-direction"), where I_0 and $I(\theta)$ are the intensity of the incident light and scattered light at the angle θ, and r is the distance from the scattering volume to the detector. The R_θ is referred to as the Rayleigh ratio.

M is the molecular weight of the macromolecule

λ_1 is the wavelength of the scattered light in the solution

R_G is the radius of gyration

B_2 is the second virial coefficient.

The molecular parameters that can be determined are the molecular weight (M), the radius of gyration (R_G) (and the second virial coefficient, B_2). Experimental data are analyzed according to the equation in the Zimm plot; the molecular weight is obtained as the inverse of the double extrapolation along the constant θ=0 and c=0

extrapolated points. The radius of gyration is obtained from the angular dependence of $\kappa c/R_\theta$ extrapolated to c=0. and the second virial coefficient is obtained from the concentration dependence of $\kappa c/R_\theta$ extrapolated to θ=0.

c) Present the characterization of a biological molecule using low-angle scattering, and compare this method with light scattering analyzed using a Zimm plot.

In low angle scattering, it is only the angular dependence that is exploited, and in most cases: in the limit of dilute solution. The following equation describes the low-angle scattering:

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

Provide data over a range (small angle) of, one can analyze them according to:

$$\ln\left(\frac{I_S(q)}{I_0}\right) = -\tfrac{1}{3} q^2 R_G^2$$

Or: plotting the left hand side vs q^2 would yield a slope to be used as basis to obtain the radius of gyration.

In this approach, there is only small angles used, and most conventionally, smaller λ than used in light scattering.

Nevertheless, in the case $\tfrac{1}{3} q^2 R_G^2 << 1$, the exponential can be expanded:

$$I_S(q) = I_0 \exp\left(-\tfrac{1}{3} q^2 R_G^2\right) \approx I_0\left(1 - \tfrac{1}{3} q^2 R_G^2\right)$$

This can be recasted to:

$$\frac{I_0}{I_S(q)} \approx \frac{1}{1 - \tfrac{1}{3} q^2 R_G^2} \approx \left(1 + \tfrac{1}{3} q^2 R_G^2\right) = \left[1 + \tfrac{1}{3}\left(\frac{4\pi}{\lambda}\sin\left(\frac{\theta}{2}\right)\right)^2 R_G^2\right] = \left[1 + \frac{16\pi^2}{3\lambda^2} R_G^2 \sin^2\left(\frac{\theta}{2}\right)^2\right]$$

Thus, the latter term on the right hand side is equal in functional form to a term used in the Zimm plot.

Both of these are providing the basis for analyses of the angular depedence of that scattered radiation, with the result of estimate radius of gyration. (there may be different types of radius of gyrations obtained, but that is beyond the scope here).