TFY 4310 MOLECULAR BIOPHYSICS

FINAL Tuesday 13. dec 2011

SUGGESTED SOLUTIONS

EXERCISE 1

a) σ- and π-orbitals are molecular orbitals that bind together two atoms. σ-bonds consist of linear combination of two atomic orbitals located at separate nuclei:

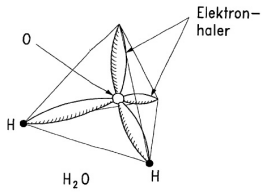
1) s-orbitals located at separate nuclei,
2) of one s-orbital and one p-orbital oriented along the line connecting the nuclei,
3) of one s-orbital and hybrid-orbital oriented along the line connecting the nuclei,
4) of two p-orbitals oriented along the line connecting the nuclei,
5) of one p-orbital and one hybrid-orbital oriented along the line connecting the nuclei,

σ-bonds display rotational symmetry about the line connection the two atom nuceli. σ-bonds are e.g. the inner half of the double bond in the N_2 and O_2 molecule, the bond between H and C in CH_3 etc.
π-orbitals consist of linear combinations of two atomic p-orbitals localized one at separate nuclei and with the p-orbitals direction normal to the line between the atom nuclei. π-bonds are e.g. the other half of the double bond in the N_2 and O_2 molecule.
Binding and anti-binding orbitals are linear combinations of two orbitals located at separate nuclei. For binding orbitals the associated electron energy in negative, i.e. stablizing. For anti-binding orbitals the associated electron energy in positive, i.e. destablizing. Both binding and anti-binding orbitals are found in e.g. N_2 and O_2.
sp^3-orbitals are hybridized orbitals of the atomic s and p orbitals involved in covalent bonding. They can be seen as linear combinations of the s and p orbitals localized at the same nuclei, and can generally be written

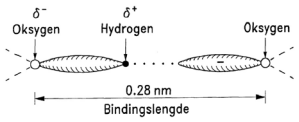
$$\Psi = \alpha_1 \Psi_{ns} + \alpha_2 \Psi_{np_x} + \alpha_3 \Psi_{np_y} + \alpha_4 \Psi_{np_z}$$

sp^3-orbitals: Coefficients α_1 α_2, α_3 and α_4 are all non-zero. Found e.g. in metan (CH_4) and ethanol (H_3CCHOH).

H_2O contains a total of 10 electrons. Two of these are locate in a 1s non-bonding orbital of the O-atom. Four of the remaining electrons are located in two sp^3 non-binding electron tails and the remaining four in the two s-sp^3 -σ-bonds between the oxygen atom and the two hydrogen atoms (Figure).An electron tail is e.g. a sp^3 orbital that contains two electrons, but is not part of and covalent bond (Figure to the right).

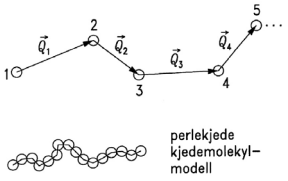


Hydrogen bonds are interactions stronger than van der Waals interactions excisting between a hydrogen atom covalently linked to an electronegative atom that is interacting with the electron tail of another electronegative atom. Can be explained based on a substantial electrostatic contribution between the proton (δ^+, figure) and the electron tail of the (δ^-) other atom (as supported by the water molecule).



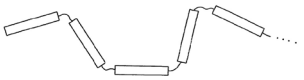
b)

Kramers chain: Spheres are connected by equally long, massless rods that are freely jointed at the speheres. All mass are associated with the spheres. The degrees of freedom are: $N_F = 3+2(N-1) = 2N+1$ where N is the number of spheres.

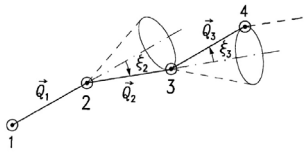


In the needle-chain, the polymers are represented as needles with

mass and thickness. These needles are connected by massless, freely jointed connections. The degrees of freedom are: $N_F = 3+2+2(N-1) = 2N+3$ where N is the number of needles



The Kirkwood-Riseman modell is also known as the freely rotating chain model. The model resembles the Kramers model, but there is a fixed angle, ξ_i, between adjacent chain segments. There is no energy barriers in the rotational angle ϕ. The degrees of freedom are $N_F = 3+2+(N-2) = N+3$ where N is the number of links.



The Rouse chain describes polymers as massless springs between spheres containing all mass. There are free rotation around the springs and the distance between the spheres varies according to the spring potential. The degrees of freedom are $N_F = 3N$ where N is teh number of spheres

c) The parameters in the probability density distribution of the end-to-end vector of an equivalent statistical chain given by:

$$P_{eq}(\vec{r}_{e-e}) = \left(\frac{3}{2\pi(N-1)Q^2}\right)^{3/2} \exp\left\{-\frac{3r_{e-e}^2}{2(N-1)Q^2}\right\} \quad (1)$$

are: $P_{eq}(\vec{r}_{e-e})$: probability density; $\vec{r}_{e-e}$: end-to-end vector of polymer chain, N: Number of spheres (N-1): number of links, Q: Length of each link.

The force acting on the two ends of the statistical chain is derived based on the general relation between force being the gradient of the potential:

$$\vec{F} = -\vec{\nabla}A \quad (2)$$

where A is the Helmholtz free energy depending on the end-to- end vector. Helmholtz free energy is given by:

$$A\left(\vec{r}_{e-e}\right) = U\left(\vec{r}_{e-e}\right) - TS\left(\vec{r}_{e-e}\right) \quad (3)$$

where U is the inner energy, T the absolute temperature and S the entropy. Because A does not depend on the direction for the statistical chain:

$$F\left(\vec{r}_{e-e}\right) = -\frac{d}{dr_{e-e}} A\left(\vec{r}_{e-e}\right)\vec{\delta}\left(\vec{r}_{e-e}\right) = T\frac{d}{dr_{e-e}} S\left(\vec{r}_{e-e}\right)\vec{\delta}\left(\vec{r}_{e-e}\right) \quad (4)$$

where we have implemented that U is independent on $\vec{r}_{e-e}$ in the last part of the equation. Boltzman's relation:

$$S = k_B \ln W \quad (5)$$

where k_B is the Boltzman constant and W is the number of possible states, is then applied. The number of possible states W is represent by the number of conformations of the statistical chain and is therefore proportional to $P_{eq}(\vec{r}_{e-e})$, e.g.:

$$W(\vec{r}_{e-e}) = const \cdot P_{eq}(\vec{r}_{e-e}) \quad (6)$$

where const is a constant independent of $\vec{r}_{e-e}$. Inserting eqs 6 and 5 in 4 yields:

$$F\left(\vec{r}_{e-e}\right) = k_B T\frac{d}{dr_{e-e}} \ln W\left(\vec{r}_{e-e}\right)\vec{\delta}\left(\vec{r}_{e-e}\right) = k_B T\frac{d}{dr_{e-e}} \ln\left(const \cdot P_{eq}\left(\vec{r}_{e-e}\right)\right)\vec{\delta}\left(\vec{r}_{e-e}\right) \quad (7)$$

Further calculus and using eq. 1 yields:

$$F\left(\vec{r}_{e-e}\right)=k_BT\frac{d}{dr_{e-e}}\ln\left(P_{eq}\left(\vec{r}_{e-e}\right)\right)\vec{\delta}\left(\vec{r}_{e-e}\right)=k_BT\frac{d}{dr_{e-e}}\left(-\frac{3r_{e-e}^2}{2(N-1)Q^2}\right)\vec{\delta}\left(\vec{r}_{e-e}\right)$$
$$=-\frac{3k_BTr_{e-e}}{(N-1)Q^2}\vec{\delta}\left(\vec{r}_{e-e}\right)=-\frac{3k_BT}{(N-1)Q^2}\vec{r}_{e-e} \quad (8)$$

Q.E.D.

The qualitative description embodied in the equation for F is that the force increases on increasing separation of the ends of the polymer chain due to the reduced entropy of the chains. The reduced entropy of the chain with increasing end-to-end distance are due to reduction of possible pathways while spanning an increasing end-to-end distance.

d) The parameters in the eq describing the free energy of deformation per unit volume:

$$\Delta A=\frac{3}{2}nk_BT\frac{\left(l_{x,0}/N_x\right)^2}{N_sQ^2}\left(\lambda_x^2+\lambda_y^2+\lambda_z^2-3\right) \quad (9)$$

are: A is the Helmholtz free energy; n: number of chains per unit volume, k_B is the Boltzman; T is the absolute temperature, $l_{x,0}$ is the macroscopic length of the network of along the x-direction in the undeformed state, N_x is the number of junction points s along the x-direction, N_s is the number of segments-1 of each polymer chain spanning two neighbouring junction points, Q the length of each segment, and λ_i, i=x,y,z is the stretching ratio between the deformed and undeformed state along the directions i.

In addition to the term emerging from eq 9, the theory of swelling of hydrogels need a theoretical account for mixing of polymers with the solvent molecules.

When polymers and solutes are mixed you will get a change in the total free energy that originate from two effects:
A) The entropy rises when you mix two components (entropy of mixing).
B) The interaction energy in general is different between the polymer segments mutually, and between the polymer segments and the solute molecules.

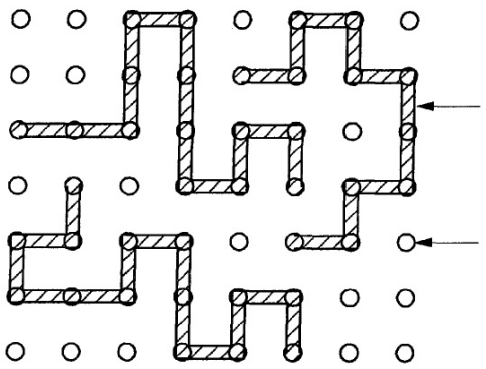


Figure 1. Lattice occupied partly by solvent molecules and polymers

The entropy of mixing can be found by considering the total number of possible combinations of polymers and solvent molecules can occupy a lattice.

For the 2-D lattice (Fig 1) consisting of total N_{gp} lattice points, the filled lattice have all lattice points either occupied by a polymer segment or solvent molecule:

$$N_{gp}=n_sN_p+N_1 \quad (10)$$

where n_s is the number of segments per polymer, N_p the total number of polymers and N_1 the total number of solvent molecules. To calculate the entropy of mixing, one start with $i<N_p$ polymers already introduced on the lattice, and consider the next polymer to be introduced. The first segment of polymer i+1 can be introduced to any of the $N_{gp}-n_si$ vacant lattice site, the second segment of polymer i+1 to any of the Z closest lattice sites that does not contain any polymer segments. The relative number of unoccupied lattice sites are: $n_{free,1}=\left(N_{gp}-n_si-1\right)/N_{gp}$, yielding an estimated number of free lattice sites for segment 2 being: $n_{free,2}=Z\left(N_{gp}-n_si-1\right)/N_{gp}$. Similarily, we estimate the number of free

lattice sites for segment 3 being: $n_{free,3} = (Z-1)\left(N_{gp} - n_s i - 2\right)/N_{gp}$ where Z-1 emerges instead of Z as for the 2nd segment is occupied by segment 1. Thus, the total number of conformations are for polymer i+1 are:

$$W_p^{(i+1)} = \frac{1}{2} n_{free,1} n_{free,2} n_{free,3} \cdot\cdot n_{free,n_s} = \frac{1}{2}\left(N_{gp} - n_s i\right)\frac{Z}{Z-1} n_{free}^{\;n_s - 1} \tag{11}$$

where

$$n_{free} = (Z-1)\left(N_{gp} - n_s i\right)/N_{gp} \tag{12}$$

is approximated by ignoring fractional occupancy decrease during the introduction of each segment. The total number of conformations for all polymers are then given by combinatorics:

$$W = \frac{1}{N_p!}\prod_{i=1}^{N_p} W_p^{(i)} \tag{13}$$

and the entropy of mixing obtained from the Boltzman eq $S = k_B \ln W$ as:

$$S = S_0 - k_B\left[N_1 \ln\left(\frac{N_1}{N_1 + n_s N_p}\right) + N_p \ln\left(\frac{N_p}{N_1 + n_s N_p}\right)\right] + k_B\left(n_s - 1\right) N_p\left[\ln(Z-1) - 1\right] - k_B N_p \ln 2 \tag{14}$$

The change in entropy on mixing is then given as:

$$\Delta S = S(N_1) - S(N_1 = 0) = -k_B\left[N_1 \ln v_1 + N_p \ln v_p\right] \tag{15}$$

where v_1, v_p are the volume fraction of solvent and polymer, respectively. This can be further developed to also include an enthalpic contribution. (NOTE: Does not require to include precise mathematical formulas in the answers to get top scores to the latter part of this question).

EXERCISE 2

a) Methods applied in molecular biophysics employ electromagnetic waves in various wavelength ranges as indicated. The corresponding energy differences are also indicated:

		Energy difference
Nuclear magnetic resonance resonans(NMR)	50-1000 MHz	
Electron spin resonance (ESR)	10-15 GHz	
Microwave	1-300 GHz	4-1000 μeV
Infra red	1-100μm,	1-1800 meV
Visible light	400 – 7 00 nm	1.8-3 eV
Ultraviolett	180-400 nm	3-1200 eV
X-ray	1-0.05nm	10 - 100 keV

b) Chemical shift and spin-spin coupling are two observable phenomena in NMR that are important information sources of the sample investigated. The question posed in the final limit these phenomena to observables for protons and hence, the suggested solution will do the same. Chemical shifts refer to differences in resonance frequency (Larmor frequency) of the proton under consideration relative to protons in a known chemical compound. The Larmor frequency ω_0 of the precessing nuclei (proton) in an external magnetic field is conventionally given by:

$$\omega_0 = \gamma B_0 \tag{17}$$

where B_0 is the magnetic flux density and γ the gyromagnetic coefficient of the nuclei. However, the local magnetic flux density experienced by an atomic nucleus is composed of several contributions:

$$\vec{B}_{local} = \vec{B}_0 + \vec{B}_{el} + \vec{B}_{dipol} = \vec{B}_0(1-\sigma) \quad (18)$$

where B_0 are the external imposed magnetic field, B_{el} is the change in local field due to electron orbitals close to the nucleus, and B_{dipol} is the magnetic flux density from other magnetic dipoles. The contribution B_{el} and B_{dipol} are much smaller than B_0 at resonance conditions, and is also expressed in terms of the screening constant σ as defined in eq. 18. The resonance condition, e.g. the Larmor frequency depends solely on B_{local}, and since the screening constant differ from compound to compound, the resonance condition will occur at slightly different frequencies for a given B_0 (or conversely).
The relative displacement of an NMR resonance peak because of differences in B_{el} and B_{dipol} is referred to as chemical shift, δ, and defined as:

$$\delta = \frac{\omega - \omega_{ref}}{\omega_{ref}} = \frac{f - f_{ref}}{f_{ref}} \quad (19)$$

where ω and ω_{ref} are the resonance frequencies of the proton investigated and reference, respectively (in radians) and f the corresponding ones in Hertz. The chemical shifts are usually given in parts per million due to their small magnitude. Chemical shifts originate from perturbations of local magnetic fields that are specific to the environment of each proton – and can thus provide information related to the chemical composition of the compounds.

Spin-spin coupling accounts for splitting of sharp resonance peaks into multiplets. The phenomenon is understood by considering the effect of different combinations of magnetic spins on protons on neighbouring atoms on the local magnetic field.

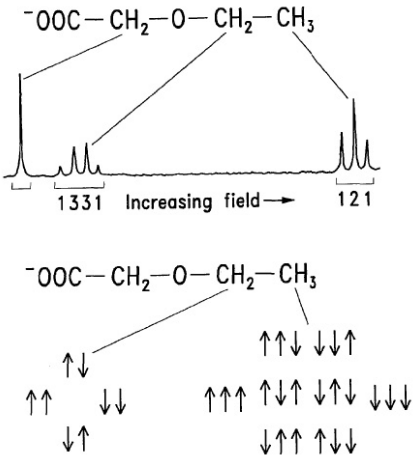


Figure 2 Schematics of proton (60 MHz) spectrum of ethoxyacetic acid

As an example, proton (60 MHz) spectrum of ethoxyacetic acid in aqueous solution (Fig 2.), shows splitting of the terminal methyl protons into three with intensity ratios 1:2:1, and adjacent CH_2 group into four peaks with intensity ratios 1:3:3:1. The splitting of protons of the methyl group are due to the three possible combinations of magnetic spins (either spin up or spin down) on the adjacent CH_2 group. Since there are two combinations of spin up and spin down, on only one for either parallel spins, the intensity ratios of the methyl protons will be 1:2:1. The argument is similar for the splitting of the proton resonances of the CH_2 group adjacent to the methyl group – i.e. due to the magnetic spin combinations of the methyl protons. Note that a similar influence of the CH_2 adjacent to the carboxyl group is not seen – this is due to it's larger distance.
Spin-spin splitting thus provides structural information.

c) Raman spectroscopy is an experimental spectroscopic technique where incoming incident electromagnetic radiation are adsorbed as induced electromagnetic dipoles with overlayed frequency corresponding to bond molecular vibrations. The polarizability $\alpha(\omega,t)$ for a given molecular bond undergoing oscillations is given as:

$$\alpha(\omega,t) = \alpha_0(\omega) + \alpha'\cos(\omega' t) \quad (20)$$

where α_0 and α' are constants for a given frequency ω of the exciting electromagnetic radiation and ω' is the vibration frequency of the specific molecular bond. Exposing the molecule with an harmonic electromagnetic field

$$E(\omega,t) = E_0\cos(\omega t) \quad (21)$$

thus yield an electric dipole

$$\begin{aligned} p_{ind} &= \alpha E = \alpha(t)\, E_0 \cos(\omega t) = \left[\alpha_0(\omega) + \alpha' \cos(\omega' t)\right] E_0 \cos(\omega t) \\ &= \alpha_0(\omega) E_0 \cos(\omega t) + \alpha' \cos(\omega' t) E_0 \cos(\omega t) \\ &= \alpha_0(\omega) E_0 \cos(\omega t) + \tfrac{1}{2}\alpha' E_0 \left[\cos((\omega+\omega')t) + \cos((\omega-\omega')t)\right] \end{aligned} \tag{22}$$

This shows that the polarization also contains two components that oscillate with slightly different frequency than the incident radiation. Following the classical theory of oscillating dipoles, this implies that the associated electromagnetic dipole radiation contains three components: one with frequency ω, one with frequency ω+ω' and one with frequency ω-ω'. For the frequency shifted components the phenomena can alternatively be viewed as a two-photon phenomena where two incoming photons with total energy $2\hbar\omega$ are emitted as two photons with energy $\hbar(\omega+\omega')$ and $\hbar(\omega-\omega')$. These emitted photons can thus be used to determine the vibration frequency ω' of the molecular bond. This information is also obtainable applying infrared spectroscopy. Raman spectroscopy is given its name from the indian scientist discovering the phenomena.

EXERCISE 3

a) Sedimentation coefficient of a macromolecule is defined as the ratio between the sedimentation velocity v (stationary) and the acceleration a acting on the macromolecule:

$$s = \frac{v}{a} = \frac{v}{\omega^2 r} \tag{23}$$

where it is assumed that the sedimentation is realized in a centrifuge in the last part of the equation. Here, w is the angular velocity of the centrifuge rotor and r the distance from the rotational axis to the macromolecule. At stationary sedimentation, the net force acting on the macromolecule gives rise to the sedimentation:

$$F_{net} = \zeta_T v \tag{24}$$

where ζ_T is the translational friction coefficient of the macromolecule. The net force acting on the macromolecule is given by

$$F_{net} = F_A - F_0 = ma - m_{water} a = \left(\frac{M}{N_A} - \frac{M}{N_A}\overline{V}_i^{(S)} \rho_0\right)\omega^2 r \tag{25}$$

wher F_A is the force acting on the macromolecule due to the sentrifugal action, F_0 is the buoyancy, M the molecular mass of the macromolecule, N_A Avogadros number, $\overline{V}_i^{(S)}$ partial specific volume of the macromolecule in the solution, and ρ_0 the density of the solvent. Eq 24 and 25 yields:

$$F_{net} = \frac{M}{N_A}\left(1 - \overline{V}_i^{(S)} \rho_0\right)\omega^2 r = \zeta_T v \tag{26}$$

Inserted in 23):

$$s = \frac{v}{\omega^2 r} = \frac{M}{N_A \zeta_T}\left(1 - \overline{V}_i^{(S)} \rho_0\right) \qquad \text{Q.E.D.} \tag{27}$$

b) The parameters in the Lamm equation:

$$\frac{\partial c(r,t)}{\partial t} = D_T\left(\frac{\partial^2 c(r,t)}{\partial^2 r} + \frac{1}{r}\frac{\partial c(r,t)}{\partial r}\right) - s\omega^2\left(r\frac{\partial c(r,t)}{\partial r} + 2c(r,t)\right) \tag{28}$$

are: c: biopolymer concentration; r: distance from rotational axis; t: time; D_T: translational diffusion coefficient; s: sedimentation coefficient and ω the angular velocity of the centrifugation

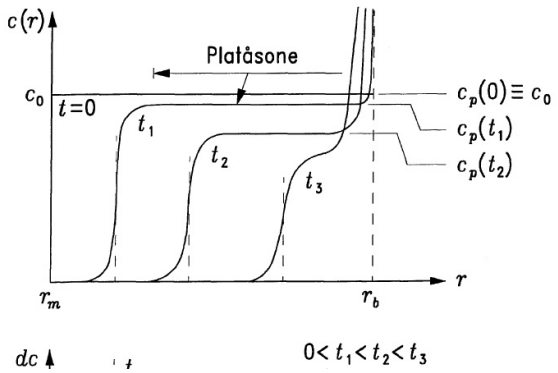


Figure 3. Schemativ illustration of biopolymer concentrationprofiles at increasing times t1, t2 and t3 after t=0 with a homogeneous concentration profile.

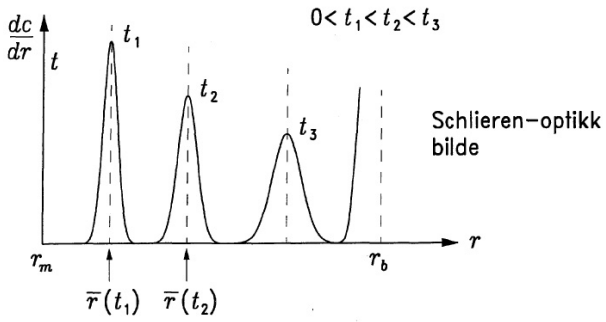


Figure 3 right panel. Gradient of concentration profiles shown in left panel.

Figure 3 shows schematically the concentration profile of a biopolymer that is analyzed by sedimentaion (figure 3 right panel depict the concentration gradient $\partial c/\partial r$). The concentration at t=0, c(t=0) is constant with increasing distance from the center, r. The concentration at increaseing time $t_1 < t_2 < t_3$ shows drainage of macromolecules at the smalles r, reduction of the concentration in the central, zone where there is essentially no concentration gradient ($\partial c/\partial r$ =0, the plateau zone), and increasing concentration at the bottom of the tube (larger r).

The plateau zone is characterized by having $\partial c(r,t)/\partial r = 0$ and also $\partial^2 c(r,t)/\partial r^2 = 0$. Inserted in the Lamm-equation, this yield for the analysis based on data collected from plateau zone

$$\frac{\partial c_p(t)}{\partial t} = -2s\omega^2 c_p(t) \tag{29}$$

where $c_p(t)$ is the concentration of the plateau zone (independent of r). Solving this for $c_p(t)$:

$$c_p(t) = c_{p,0} \exp\left(-2s\omega^2 t\right) \tag{30}$$

where $c_{p,0}$ is the plateau concentration at t=0, yields the following expression for the sedimentation coefficient:

$$s = \frac{\ln\left\{c_{p,0}/c_p(t)\right\}}{2\omega^2 t} \tag{31}$$

The moving boundary method utilizes the time dependence of the boundary directly:

$$s = \frac{u}{\omega^2 r} \frac{d\bar{r}/dt}{\omega^2 \bar{r}} = \frac{1}{\omega^2} \frac{d\left(\ln \bar{r}\right)}{dt} \tag{32}$$

where $\bar{r}$ is the r value at the maximum gradient of c.

c) Figure 4 shows a schematic overview of an instrument suitable for static and dynamic light scattering. The main parts are: Light source with defined wavelength (laser), sample cuvette, thermostated bath for maintaining temperature, light detector mounted on a goniometer to detect light intensity at various angles θ. Additionally, a computer properly interfacing to the various part for their control and data collection is usually included. A correlator is necessary for the dynamic light scattering experiments.

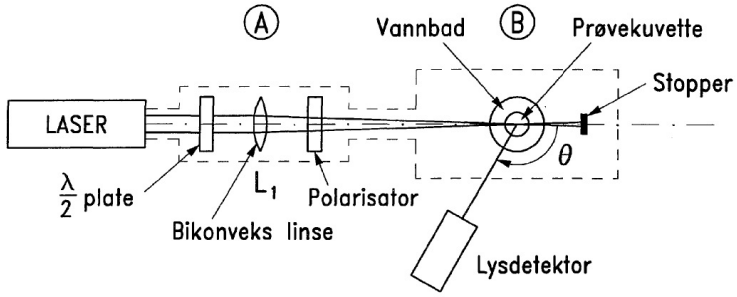


Fig 4. Schemativ illustration of instrument for light scattering.

The parameters in the eq:

$$\frac{\kappa c}{R_\theta}=\frac{1}{M}\left[1+\frac{16\pi^2}{3\lambda_1^2}R_G^2\sin^2\frac{\theta}{2}\right]\cdot\left[1+2B_2c\right] \qquad (33)$$

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.

c: is the (bio)polymer concentration

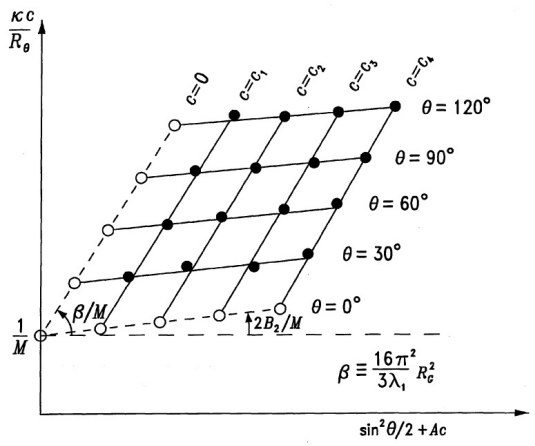
$R_\theta = I(\theta)r^2/I_0$ (valid for incoming polarized light in the "y-direction"), where I_0 and $I(\theta)$ are the intensity of the incident light and scattered light at the angle θ, and r is the distance from the scattering volume to the detector.

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.



Schematic illustration of a Zimm plot

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 eq 33, where a necessary experimental basis include determination of $I(\theta)$ for a range of θ, and for a set of concentrations c. The experimental data of $\kappa c/R_\theta$ is presented vs. $\sin^2\theta/2+Ac$, for each of the c and θ, and where A is a numerical constant. In this socalled Zimm-plot (Figure 7), 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 otained from the angular dependence of $\kappa c/R_\theta$ extrapolated to c=0.

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