

EXAM I COURSE
TFY4310 MOLECULAR BIOPHYSICS

Wednesday, 10 December 2014

Suggested solutions

Exercise 1. [total: 36 p]

Justify **nine** (9) of the following sentences [4 pts each]:

1. The angle between hydrogen atoms in a water molecule is 104.5° .

Answer: The binding orbitals of O in the water molecule are sp^3 hybridised and so one would expect the water molecule to have a tetrahedral shape with angles of 109.5° . Because two of the molecular orbitals are occupied with the 4 unpaired electrons, these will be more repulsive than the HO covalent bonds and so the angle between those will be somewhat smaller: 104.5° .

2. Ice is less dense than liquid water.

Answer: Because the angle between the sp^3 orbitals in H_2O is roughly 109° (see Ex. 1.1), water at low temperatures adopts a tetrahedral coordination (four nearest neighbours per molecule) rather than a high packing density. Therefore the ice crystal structure is very open and full of voids. Water in the liquid state does not possess such an organised structure due to the thermal motion of the molecules. In these case the “water structure” collapses; the number of nearest neighbours is larger (about 5) and the number of hydrogen bonds is smaller, about 3.5. Ice is thus less dense than liquid water.

3. Hydrophobic interactions are entropic in nature.

Answer: Water molecules are able to establish four hydrogen bonds (H-bonds) with neighbouring water molecules. As mentioned in Ex. 1.2, in liquid water, and due to thermal motions, the average number of H-bonds that a water molecule forms with its neighbours is around 3.5. When a hydrophobic (apolar) particle (or molecule) is placed in water, water molecules in the vicinity of the particle, who cannot establish H-bonds with it, will organise themselves to avoid losing H-bonds with the neighbouring water molecules. While this situation often leads to the establishment of even more H-bonds than in the case of pure water, the entropic cost is very large. Hydrophobic interactions between hydrophobic particles, and the concomitant association of these particles, arise not due to an effective attraction between the hydrophobic particles but to the fact that the systems wants to minimise the hydrophobic area exposed to water, and thus the number of “ordered” water molecules. Of course, this costs the mixing entropy of the hydrophobic particles but this is well compensated by the large increase in the mixing entropy of water.

4. The model describing the chain with hindered rotations is more realistic than the freely-jointed chain model.

Answer: In a freely-jointed chain model all angular variables are allowed to assume any value with equal probability. Commonly, the bond angles in polymers are fixed or narrowly fixed at constant values. For example, in polyethylene the bond rotational angles are all close to 112° . This is so because it takes more energy to distort bond angles than it does to induce rotations about single bonds (the freely-rotating chain model takes this into account by considering all bond angles constant and equal to θ). It is also so that the dihedral angle (ϕ) is not completely free to assume all possible values. Instead, the angle is restricted by sterical interactions with the neighbouring groups (see Figure 1). The model describing the chain with hindered rotations is thus

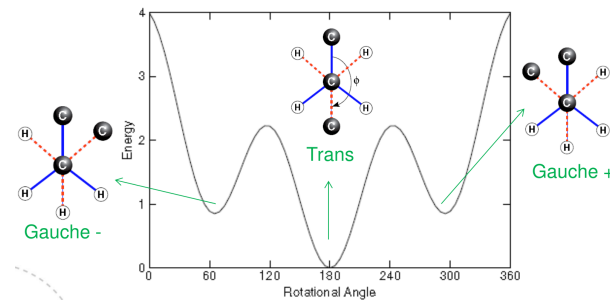


Figure 1: Potential energy diagram for the rotation about backbone bonds in a hypothetical, symmetrical polymer. Φ is the dihedral angle (named as rotational angle in the x -axis), from Exercise 1.4.

more realistic, leading to values of the polymer chain end-to-end distance that are closer to the experimentally determined values, than the freely-jointed chain model.

5. When a rubber is stretched by a dead load and is heated its extension decreases.

Answer: Rubber is made of polymer chains. When polymer chains are stretched, their conformational entropy decreases (we decrease the number of available conformational states). When the rubber is heated, the entropy of the polymer chains will increase which leads to the decrease in the extension of the polymer chains and, thus, the extension of the rubber

6. According to the Flory-Huggins theory, the entropy of mixing decreases with the length of the polymer chain.

Answer: If we consider two different systems, one containing one polymer with 25 monomers and the other containing 5 polymers of 5 monomers, it is intuitive that the former has less mixing entropy than the second one. In fact, there is a large loss in mixing entropy when the monomers are connected to form a polymer.

The Flory-Huggins theory is based on the ideal mixing model, where the entropy of mixing (total number of possibilities for the distribution) of A and B molecules in a lattice is calculated. The ideal mixing model has been developed for molecules with low molecular weight but can also be used to describe polymers, by connecting the low molecular weight particles (Figure 3). Due to the connectivity between the monomers, there are restrictions in the placement of the polymers. In other words, the first monomer can be placed in any available place in the grid but the second monomer is restricted to the neighbouring positions in the grid, and so on... If we have shorter chains, we will have a large entropy of mixing, as compared to one long polymer which will have all but one restricted placements.

Note: Mixing entropy should **not** be confused with conformational entropy. The mixing entropy decreases with chain length whereas the conformational entropy increases with chain length.

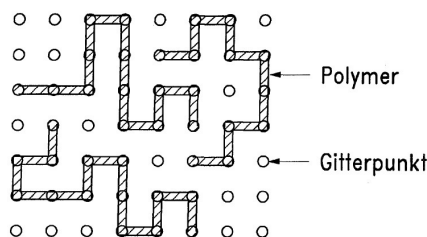


Figure 2: Representation of the placement of polymers on a grid, according to the Flory-Huggins theory, from Exercise 1.6.

7. In sedimentation velocity, a high rotor speed maximises resolution.

Answer: During a sedimentation velocity experiment a high rotor speed, and thus a high centrifugal force, is used to “push” the particles to the bottom of the column, and one follows how the boundary between the solvent and the solute moves with time. The solute particles (or molecules) and the solvent molecules in the boundary are subject to a concentration gradient that increases as the boundary moves towards the bottom of the column, that is, as the solution beyond the boundary becomes more and more concentrated. The boundary formation leads thus to the diffusion of the solute particles to the dilute part of the sample, and that of the solvent molecules to the concentrated part of the sample, reducing the resolution of the experiment. If the rotor speed is high the particles and molecules will diffuse more slowly along the concentration gradient, which will increase the resolution of the experiments.

8. Rotational friction coefficient is more sensitive to the shape of a molecule than the translational friction coefficient.

Answer: In most hydrodynamic measurements, one typically observes net mass transport and so an explicit knowledge of the orientation of the molecule during transport is not necessary. However, measurements of the rotational motion must include the detailed distribution of orientations. It is impossible to speak of just a single rotational friction coefficient and, for the case of ellipsoids, for example, both rotational coefficients for rotation around the short and the long semi-axes need to be defined. Furthermore, the coefficients of the different semi-axes of the ellipsoids are different whether the particle is an oblate or a prolate. Therefore, if it would be possible to measure independently those two coefficients one would have a much better description of the particle than just the translational friction coefficient. Unfortunately it is very difficult to measure those two coefficients independently.

Finally we can also look at the Stokes’ relations and realize that the R^3 dependence of the rotational friction coefficient, ξ , makes it more sensitive to small variations in the dimensions of the particles than the translational friction coefficient.

9. The $^1\text{H-NMR}$ spectrum of $\text{CH}_3\text{-CH}_2\text{-Br}$ 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 CH₃ 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 CH₂ 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 δ values.

10. The extinction coefficient (ϵ) of a molecule depends on the wavelength.

Answer: The extinction coefficient contains the wavelength or frequency dependence of the absorption spectrum of a particular molecule, that is, it describes how a particle absorbs light. A molecule absorbs light when an electronic transition occurs, which happens when the wavelength of light corresponds to the energy gap of two electronic states, and the transition selection rules are met. Each molecule, with its own chemical structure, will have its own electronic structure, and thus a different wavelength-dependent extinction coefficient.

11. The CO₂ molecule has four modes of vibration but only three will contribute to bands in the infra-red spectrum.

Answer: The modes of vibration of the CO₂ molecule are the symmetric and anti-symmetric stretching and the symmetric and anti-symmetric bending. One of the vibrational selection rules states that the transition dipole moment (which lies along the direction of the changing dipole moment) needs to be different than zero. The symmetric stretching of the CO₂ molecule has no net dipole moment and, therefore, does not contribute with a band to the infra-red spectrum.

12. Static light scattering allows to determine the molecular weight of a macromolecule even using concentrated samples.

Answer: When dealing with concentrated macromolecular solutions, one has to consider the intermolecular interactions between different macromolecules, and how such interaction affect the scattering of light, in this case. In this respect, the Laurentz-Mie regime, defined by the relation:

$$\frac{\kappa C}{R_\theta} = \frac{1}{M_w} \left[1 + \frac{2\pi}{3} S^2 \cdot R_G^2 \right] \cdot [1 + 2B_2 C]$$

is very useful since it accounts for the interactions between particles in the second virial coefficient term, B_2 . The procedure to determine the M_w consists in preparing solutions at different concentrations and measuring the solutions at different angles. With this information it is possible to draw a Zimm plot, as shown schematically. Extrapolating the data for $C = 0$ and $S = 0$ yields the inverse of the molecular weight.

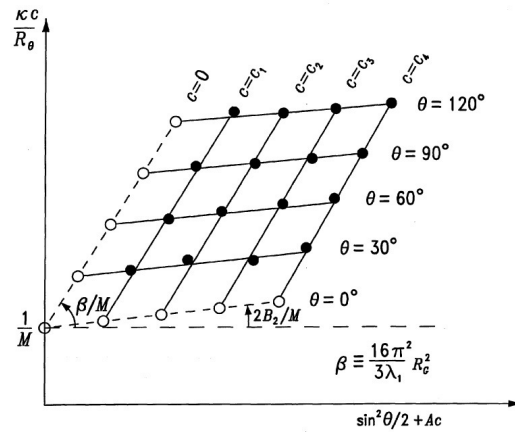


Figure 3: Zimm plot, from Exercise 1.12.

1. A protein has been 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). If the particles are spherical, what is their diameter?

Answer: To calculate the radius of inertia we resort to the Guinier approximation given by:

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

where q , the scattering angle is defined as:

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

With the information given we see that a graph of $\ln(I_s(q)/I_0)$ vs. q^2 gives a straight line with a slope of $-\frac{1}{3}R_G^2$, from where we can calculate the R_G .

To calculate the slope lets us take two values from the table, say the first and the last.

$$q(\theta = 1.41 \text{ [mrad]}) = \frac{4\pi}{0.154 \text{ [nm]}} \sin\left(\frac{1.41 \times 10^{-3}}{2}\right) = 0.0575 \text{ nm}^{-1}$$

$$q(\theta = 4.00 \text{ [mrad]}) = 0.163 \text{ nm}^{-1}$$

$$\text{Slope} = \frac{\Delta y}{\Delta x} = \frac{70.61 - 70.76}{0.163^2 - 0.0575^2} = -6.45 \text{ nm}^2$$

$$R_G = \sqrt{3 \times 6.45} = 4.40 \text{ nm}$$

To calculate the diameter of the particle we take the relation:

$$R_{G,\text{sphere}} = \sqrt{3/5} R_s$$

and get

$$R_s = R_G/\sqrt{3/5} = 4.40/0.774 = 5.68 \text{ nm}.$$

The diameter of the sphere is thus 11.36 nm.

Note: In reality the Guinier approximation is only valid for $qR < 1$, which is not the case.

2. A few other studies were performed with the same protein sample. Sedimentation velocity measurements yield a sedimentation coefficient of 18.1 S (10^{-13} s). Data obtained from dynamic light scattering, and plotted on a graph of the $\ln(g^{(1)}(q, \tau))$ versus τ , was found to be linear with a slope: -8.368×10^3 . The scattering angle was 90° and the wavelength of the light through the medium was 500 nm. The temperature was 20°C . Assume that the solution has a viscosity of 1.0×10^{-3} Ns/m² and a density of 1.0 g/cm^3 and that the protein has a specific partial volume of $0.73 \text{ cm}^3/\text{g}$.

Calculate the molecular weight and the hydrodynamic radius of the protein.

Answer: To calculate the molecular weight of the protein we can use the Svedberg equation. The only value missing for this is the translational friction coefficient. This can be obtained from the dynamic light scattering measurements. It is mentioned that the graph of the $\ln(g^{(1)}(q, \tau))$ versus τ , was found to be linear with a slope: -8.368×10^3 . Using

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

we see that slope = $-q^2 D_0$. Knowing that the scattering angle was 90° and the wavelength of the light through the medium was 500 nm we calculate q according to

$$q = \frac{4\pi}{\lambda} \sin\left(\frac{\theta}{2}\right) = 1.777 \times 10^7 \text{ m}^{-1}$$

The translational diffusion coefficient is then:

$$D_0 = 8.368 \times 10^3 / q^2 = 2.65 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$$

We can use the Nerst-Einstein relation to obtain the friction coefficient:

$$f = k_B T / D_0 = 1.527 \times 10^{-10} \text{ kgs}^{-1},$$

and finally, the molecular weight, from the Svedberg equation:

$$M_w = \frac{s N_{\text{Av}} f}{\left(1 - \bar{V}_1^{(S)} \rho\right)} = 616.6 \text{ kg/mol}$$

The hydrodynamic radius of the particle is calculated using the Stokes formula:

$$f = 6\pi\eta R_h, \quad \xi = 8\pi\eta R_h^3$$

$$R_h = 8.10 \text{ nm}$$

3. Assuming that the protein is a sphere, calculate the required hydration.

Answer: Assuming that the protein is a sphere we can calculate the volume of hydration according to:

$$v_h = \frac{4}{3}\pi R_h^3 = 2.23 \times 10^{-24} \text{ m}^3$$

To calculate the hydration factor, δ , we resort to the relation:

$$v_{h,i} = \left(\bar{V}_i^{(S)} + \delta \bar{V}_0^{(S)}\right) \frac{M_1}{N_{\text{Av}}}$$

with i referring to the protein and $\bar{V}_0^{(S)} = 1/\rho$ the partial specific volume of the solvent.

Replacing with the given or above calculated values we get a hydration factor, $\delta = 1.45$.

4. Calculate f/f_0 . Assuming that the protein is a prolate ellipsoid with no hydration, estimate the axial ratio of the protein.

Answer: f is the translation friction coefficient obtained experimentally ($f = 1.527 \times 10^{-10} \text{ kgs}^{-1}$). f_0 is the friction coefficient of an unhydrated sphere. The deviations of f_0 maybe due to the hydration of the protein or to deviations from sphericity. Since it is mentioned that the protein has no hydration one takes that the difference is solely due to a deviation from the sphericity, that is, f/f_0 . Resorting to the Stokes formula, where f is calculated from the measured “hydrodynamic radius” and f_0 from the unhydrated sphere, we can simple write:

$$\frac{f}{f_0} = \frac{R_h}{R_0} = \frac{8.10}{5.68} = 1.43$$

where R_0 is R_s calculated in Exercise 2.1.

Inspecting the table given in the end of the exam we see that the axial ratio of the protein, assumed to be a prolate with no hydration, is 8.

5. A 2D COSY ^1H NMR spectrum was made to the protein in question. A particular amino acid of the protein showed the following peaks in the 1D ^1H -NMR spectrum: NH = 8.4 for both protons, $\alpha\text{H} = 4.3$, $\beta\text{H} = 2.1$ and 1.9, $\gamma\text{H} = 2.3$ for both. At which 2D coordinates do you expect COSY interaction? (*Hint:* the structure of the amino-acid is the following: $\text{H}_2\text{NC}_\alpha\text{H}(\text{COO}^-)\text{C}_\beta\text{H}_2\text{C}_\gamma\text{H}_2$).

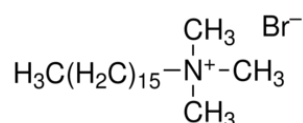
Answer: In 2D COSY ^1H NMR the applied pulse sequence probes the pairs of nuclei that are coupled via spin-spin coupling. That is, nuclei that are, typically 3 bonds, apart. In this particular case, we will observe in the diagonal of the COSY spectrum the proton peaks that are observed in the 1D ^1H NMR spectrum: 8.4 (NH), 4.3 (αH), 2.1 and 1.9 (βH), and 2.3 (γH).

The cross-peaks give information about pairs of neighbouring protons. For example, the protons from the NH group will couple with the proton from the αH . Since the two protons from NH have the same chemical shift only two cross-peaks will appear as the result of this coupling at the coordinates: 8.4 – 4.3 and 4.3 – 8.4.

Considering now all the protons of the mentioned amino-acid, and using the same reasoning, we would expect cross-peaks at the following coordinates: 8.4–4.3, 4.3–8.4, 4.3–2.1, 2.1–4.3, 4.3–1.9, 1.9–4.3, 2.1–1.9, 1.9–2.1, 2.1–2.3, 2.3–2.1, 1.9–2.3, 2.3–1.9.

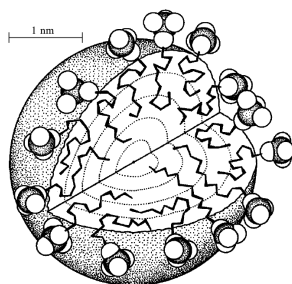
Exercise 3. [total: 28 p]

Cetyltrimethylammonium bromide (CTAB) surfactant (see structure below) forms spherical micelles in solution, at relatively low surfactant concentrations.



1. Make a schematic drawing of a spherical micelle.

Answer: A normal spherical micelle can be schematically drawn as:



2. Name the main intermolecular forces involved in the stabilisation and destabilisation of a surfactant micelle in solution.

Answer: Stabilization of surfactant micelles in solution is given mostly by the hydrophobic interactions between the surfactant alkyl chain and concomitant increase of the entropy of water. Entropy of mixing acts to destabilise the micelles, as well as the electrostatic repulsions between the headgroups in the case of charged surfactants.

3. The addition of salt leads to the transition from a sphere to a long cylinder-like (rod-like) micelles. Why?

Answer: Since the given surfactant is (positively) charged, the addition of salt will “reduce” the area of surfactant headgroups, since it screens the electrostatic repulsions between different headgroups. Thus, the shape of the surfactant changes from a cone to a cylinder, which, from a packing point of view, favours the formation of cylindrical micelles.

Note: Nothing is said about the aggregation number, that is, on the number of surfactants that constitutes a micelle. Since the radius of the spherical and the rod micelles will normally be identical (it is determined by the chain length of the hydrophobic part which is not affected by the addition of salt), the aggregation number increases upon the sphere-to-rod transition.

4. Which of the following computer simulation techniques would you use to follow this sphere-to-rod transition? Justify.
 - Molecular dynamics
 - Brownian dynamics
 - Monte Carlo simulations

Answer: If we want to follow the sphere-to-rod transition we have to use a computational technique that follows dynamics. Therefore Monte Carlo simulations, which probe systems in equilibrium is excluded.

Molecular dynamics deals with the computation of *quasi*-atomistic models. In order to follow the transition from a spherical micelle to a rod one, we need to calculate a significant number of surfactant molecules and an enormous amount of water molecules. This makes the calculation very time consuming. In addition, the time scales that

are typically probed by this technique, μs , may not be sufficiently long to follow the transition.

The best way of calculating these systems would thus be to coarse-grain the system and use a brownian dynamic program.

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: $e = 1.602 \times 10^{-19} \text{ C}$

Values for dielectric constants
at 25°C $\epsilon(\text{water}) = 78.4$; $\epsilon(\text{ethanol}) = 19.9$; $\epsilon(\text{chloroform}) = 4.81$

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

Boltzmann constant: $k_{\text{B}} = 1.38 \times 10^{-23} \text{ m}^2 \text{ kg s}^{-2} \text{ K}^{-1}$

Temperature: $[\text{K}] = [^{\circ}\text{C}] + 273.15$

Atomic orbitals: H: $1s^1$; C: $1s^2 2s^2 2p_x^1 2p_y^1$; O: $1s^2 2s^2 2p_x^2 2p_y^1 2p_z^1$

Atomic weights: $A_r(\text{H}) = 1.0$; $A_r(\text{C}) = 12.0$

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

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

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

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

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

Debye screening length $\lambda_{\text{D}}^2 = \frac{\epsilon k_{\text{B}} T}{\sum_i (eZ_i)^2 n_{i\infty}}$

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

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

For long chains and the random walk model

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

Fluid dynamic volume

$$v_{h,i} = \left(\bar{V}_i^{(s)} + \delta \bar{V}_0^{(s)} \right) \frac{M_1}{N_{Av}}$$

Fick's laws

$$\frac{\partial c}{\partial t} = -\vec{\nabla} \cdot \vec{J}, \quad \vec{J} = -D_T \vec{\nabla} c, \quad \frac{\partial c}{\partial t} = D_T \frac{\partial^2 c}{\partial x^2}$$

Nernst-Einstein relations

$$f D_T = k_B T, \quad \xi D_R = k_B T$$

Lamm-equation

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

Sedimentation centrifugation:

$$s = \frac{\ln \{c_0/c_p(t)\}}{2\omega^2 t}$$

Svedberg equation

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

Equilibrium

centrifugation:

$$m_1(r) = m_1(r_m) \exp \left\{ \frac{M_1 (1 - \bar{V}_1^{(s)} \rho) \omega^2 (r^2 - r_m^2)}{2RT} \right\}$$

Perrin shape parameters for ellipsoids of revolution

Axial ratio	prolate	oblate
1	1.000	1.000
2	1.044	1.042
4	1.182	1.165
6	1.314	1.277
8	1.433	1.374
10	1.543	1.458

Nuclear spin

$$\vec{m} = \gamma \vec{L}, \quad (\vec{m})^2 = \gamma^2 \hbar^2 \ell(\ell + 1), \quad m_z = m_\ell \gamma \hbar$$

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

Small-angle scattering:

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

$$\frac{\langle I_s(q) \rangle}{I_0} = c M \kappa \frac{1}{R^2},$$

Large systems $\frac{\kappa c}{R_\theta} = \frac{1}{M} \left[1 + \frac{16\pi^2}{3\lambda^2} R_G^2 \sin^2 \frac{\theta}{2} \right] \cdot [1 + 2B_2c],$

with :

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

$$R_{G,\text{sphere}} = \sqrt{3/5} R_s$$

Dynamic light scattering:

Siegert relation:

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

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