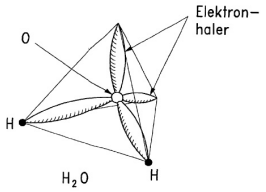
TFY 4310 MOLECULAR BIOPHYSICS

FINAL Monday 10. dec 2012

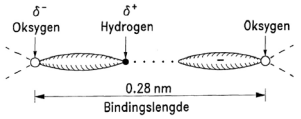
SUGGESTED SOLUTIONS

EXERCISE 1

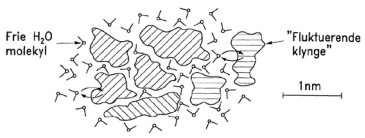
a) There are total 10 electrons in one H_2O molecule. Two electrons are in the 1s non-bonding orbital of the O-atom. Four of the remaining electrons are located in two sp^3 non-binding electron tails and the remaining four in the two s-sp^3 -σ-bonds between the oxygen atom and the two hydrogen atoms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



Hydrogen bonds are interactions stronger than van der Waals interactions existing 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).

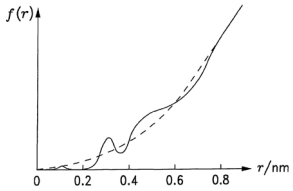


A model where H_2O can be viewed as a highly dynamic ice slurry where the lifetime of a given hydrogen bond is about 10^{-11} seconds and average size of the ”ice pieces”is just a few nanometers can account for the observed large fraction of the hydrogen bonds (85%) in water compared to ice. This often also referred to as a system consisting of fluctuating clusters. At any time there is in average only app. 15% of the water molecules that are free water molecules. Lifetime of H_2O molecule cluster membership (10^{-11} s) and cluster size decreases with T. Cluster size: from 90 (2 ºC) to 25 at 90 ºC.



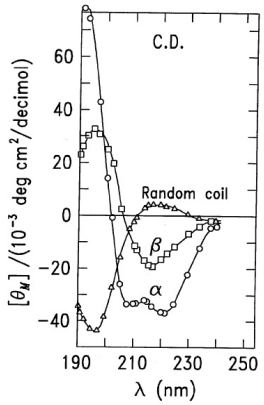
Fluctuating cluster model for water

The experimentally determined radial electron density distribution (solid line, f (r) , figure right, obtained from Xray diffraction) deviating from the dotted line indicates structuring of probable electron distributions.

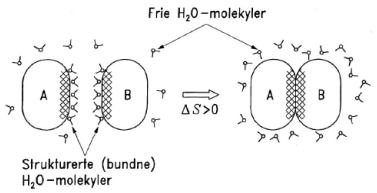


b) The term primary sequence in biological macromolecules refers to the order of the residues covalently connected (e.g. amino acide sequence, base sequence). Secondary structure refers to local geometric organisation of residues relative to neighbouring units, e.g. various angles between connecting bonds (examples: alpha helix in proteins). Tertiary structure refers to geometry of posistions of various secondary structural elements relative to each other, ex: beta pleated sheet in proteins. The term quarternary structure refers to positioning of separate molecules relative to each other.

Circular dichroism CD refers to optical properties of a sample, in our case: e.g. a solution of a biological macromolecule. In specific, CD refers to difference in absorbtion of left and right circular polarized light passing throug the sample. This can be view as incident linear polarized light are seen as the net of oppositely rotating (left and right handed) circular polarized light components. After passing the sample, asymmetry in the absorption yield a net elliptically polarized wave, with ellipticity used to define the extent of asymmetry. The specfic ellipticity depends different on wavelength for varios higher order structural elements in protens (figure to the right). The wavelength dependent ellipticity of a macromolecule can be decomposed to various fractions of the structures using a linear combination of these spectra.



c) The hydrofobic effect (interactions/bonds) is connected to the fact that it is energetically favourable for water molecules to form more regular structures near apolar surfaces than in free aqueous solution. This ordering, however, is entropically unfavorable. When two such apolar surfaces with structured water molecules are brought together, the apolar surfaces will not be accessible for structuring of water molecules, and thus, a larger fraction of the water is not stuctured. Or in other words: the association of the apolar surfaces is driven by an entropy gain in the aqueous system.



Schematic illustration of entropy gain of water occurring on association of apolar surfaces

The hydrophobic effect contributes to the stability/organisation of the membrane of red blood cells as follow:

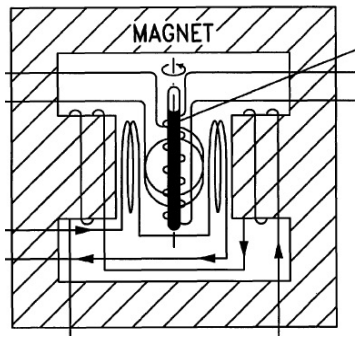
- in the lipid bilayer by polar headgroups of lipids facing towards water phase (both intra and extracellular) and tail (lipophilic) towards the interior in the double layer.
- in individual proteins (globular) by the hydrophobic amino acids being located where appriate: water exposed surface: interior, trannsmembrane: part of the surface; and polar/charged on the surface exposed to water.
- in theorganisation of the proteins relative the lipid part, in particular transmembrane proteins with hydrophobic domain on the surface that is embedded in the hydrophobic part of the lipid bilayer.

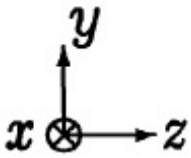
EXERCISE 2

a) The parameters in the Bloch equations are:

M_i: Macroscopic magnetization in direction indicated by index i = x,y,z. Additional subscript 0 depicts the equilibrium magnetization in the i = x,y,z directions.
B_i: Magnetic flux density in direction indicated by index i = x,y,z.
γ: gyromagnetic ratio
T_1 : spin-lattice relaxation time constant (longitudenal relaxation time constant)
T_2 : spin-spin relaxation time constant (transversal relaxation time constant)

T_1 depicts the time constant for relaxation of the longitudinal M_z component of the magnetization vector towards its equilibrium following an out-of equilibrium perturbation.
T_2 is the time constant of relaxation of the transverse component of the magnetization vector relaxes (by interactions between spins)





The direction of the x,y and z components relative to the cross-section of part of set-up for NMR spectroscopy is given at the right of the graphic illustration above. The z-axis is identified based on the design of the magnet setting up the magnetic flux density of the static field in the z-direction. The coils associated with the x and y directions are detection coil in the xy plane, 90 deg relative to the coil used for the radiofrequency coil. The static magnetic field set up along the z direction is used for generation of a static flux density:

$$\vec{B} = B_0 \vec{\delta}_z \qquad (1)$$

along the z –aksis. The coil for the radiofrequency excitation and oberservations are used for perturbation of the molecular system (pulse sequences) and observing the resulting response.

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:

$$\omega_0 = \gamma B_0 \tag{2}$$

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:

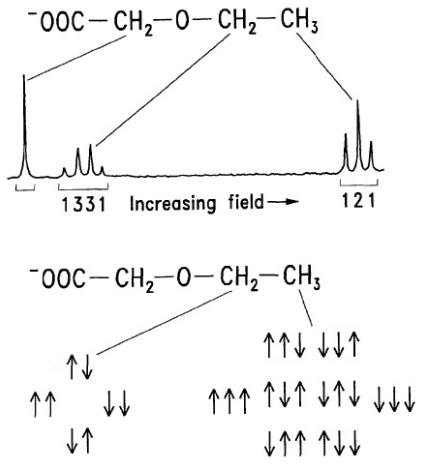
$$\vec{B}_{local} = \vec{B}_0 + \vec{B}_{el} + \vec{B}_{dipol} = \vec{B}_0 \left(1 - \sigma\right) \tag{3}$$

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:

$$\delta = \frac{\omega - \omega_{ref}}{\omega_{ref}} = \frac{f - f_{ref}}{f_{ref}} \tag{4}$$

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.



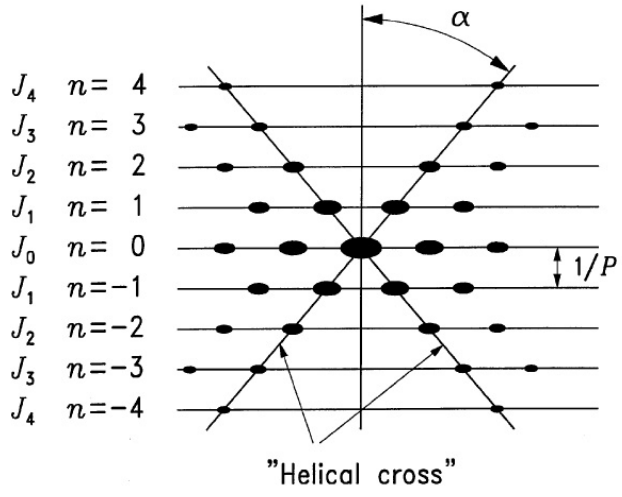
Schematics of proton (60 MHz) spectrum of ethoxyacetic acid

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) The equation

$$H(\vec{R}) = \frac{1}{P} \cdot \sum_{n=-\infty}^{\infty} J_n(\chi) \exp\{in(\psi + \pi/2)\} \delta(w - n/P) \qquad (5)$$

where J_n are the n'th order Bessel function, parameter $\chi = 2\pi R r_0$ and δ is the Dirac delta function, provided for a continuous helix with radius r_0 and pitch P is its Fourier transfom.



-→ $\chi = 2\pi R r_0$

The figure above illustrates schematically a X-ray fiber diffraction diagram of a continuous helix, where the intensity dots at the various layer lines (n) are depicted as function of $\chi = 2\pi R$. The X-ray fiber diffraction from a discontinuous helix will be stacked helical cross with distances between the "stacks" equal to 1/p, where p is the projected distance along the helical axis of the residues.

EXERCISE 3

a) In the equation:

$$V(r) = -\frac{A}{r^6} + \frac{B}{r^{12}} \quad , \text{A>0; B>0} \qquad (6)$$

V is the potential between to atoms separated at interatomic distance r and A is a parameter depending on the atomic pair describing the attraction and B a parameter describing the repulsion (also depending on the atomic pair being considered). $-A/r^6$ decribes the dispersion forces. The inverse sixth-power of the interatomic separation can be deduced by considering the following line of argument:

Spontaneous fluctuations within the electron cloud of one atom results in an electric dipole field. This induces an induced electric dipole in neighboring atoms. The potential energy of the induced electric dipole in the field set up by the spontaneous fluctuating electron cloud yields the inverse sixt-power dependence of the interatomic distance.

The equilibrium distance (minimum in the potential) is calculated as follow:

$$F(r) = \left.\frac{dV(r)}{dr}\right|_{r=r_{\min}} = 0; \qquad \frac{dV(r)}{dr} = 6\frac{A}{r^7} - 12\frac{B}{r^{13}}; \qquad 6\frac{A}{r_{\min}^{\ 7}} - 12\frac{B}{r_{\min}^{\ 13}} = 0 \qquad (7)$$

yielding an equilibrium distance: $r_{\min} = (2B/A)^{1/6}$ (8)

(and potential) $V(r_{\min}) = -\frac{A^2}{4B}$

b) The electrostatic potential $V(\vec{r})$ in space with charge density $\rho(\vec{r})$ is given by Poisson's eq:

$$\nabla^2 V(\vec{r}) = -\rho(\vec{r}) / \varepsilon \qquad (9)$$

where ε is the permitivity of the medium. The charge density is given by the sum of the charge concentration from N ionic species present at the actual locations:

$$\rho(\vec{r}) = \sum_{i=1}^{N} eZ_i n_i(\vec{r}) \tag{10}$$

Here, *e* is the electron charge, Z_i is the valence of ionic species of t, and $n_i(\vec{r})$ is the concentration of species *i* at position $\vec{r}$. At thermodynamic equilibrium, the distribution of each type of charged species adopts a boltzmann distribution due to the electrostatic potential energy of the specie in the $V(\vec{r})$: $eZ_iV(\vec{r})$:

$$n_i(\vec{r}) = n_{i\infty}(\vec{r}) \exp\left\{-\frac{eZ_iV(\vec{r})}{k_BT}\right\} \tag{11}$$

where $n_{i\infty}$ is the concentration of species of type i at the reference state (at infinite distance from the point charge being considered), k_B is Boltzman constant, and T the absolute temperature. Insertion of eqs 11 and 10 in eq 9 yields:

$$\varepsilon \nabla^2 V(\vec{r}) = -\sum_{i=1}^{N} eZ_i n_{i\infty} \exp\left\{-\frac{eZ_iV(\vec{r})}{k_BT}\right\} \tag{12}$$

Q.E.D.

This equation is referred to as the Poisson-Boltzman eq.

Equation (12) can be solved using the series expansion of $\exp(x) = 1 + x + x^2/2! + x^3/3! + \ldots$. Applying this expansion for the exponential in eq 12 and retaining the three first terms yields:

$$\varepsilon \nabla^2 V(\vec{r}) = -\sum_{i=1}^{N} eZ_i n_{i\infty} \left\{1 - \frac{eZ_iV(\vec{r})}{k_BT} + \left(-\frac{eZ_iV(\vec{r})}{k_BT}\right)^2 \Big/ 2 + \right\} \tag{13}$$

Assuming that the potential energy in the electrostatic field is much less than k_BT imply that the last term in eq. 13 can be omitted:

$$\varepsilon \nabla^2 V(\vec{r}) = -\sum_{i=1}^{N} eZ_i n_{i\infty} \left\{1 - \frac{eZ_iV(\vec{r})}{k_BT}\right\} = -\sum_{i=1}^{N} eZ_i n_{i\infty} + \frac{V(\vec{r})}{k_BT} \sum_{i=1}^{N} e^2 Z_i^2 n_{i\infty} \tag{14}$$

The requirement of overall electroneutrality yields:

$$\sum_{i=1}^{N} eZ_i n_{i\infty} = 0 \tag{15}$$

Inserting eq 15 in 14 yields:

$$\varepsilon \nabla^2 V(\vec{r}) = \frac{1}{k_BT} \sum_{i=1}^{N} e^2 Z_i^2 n_{i\infty} V(\vec{r}) \tag{16}$$

Equation 16 can be recast into

$$\nabla^2 V(\vec{r}) = \frac{1}{\lambda_D^2} V(\vec{r}) \tag{17}$$

provided that

$$\lambda_D^2 = \varepsilon k_B T \Big/ \left(\sum_{i=1}^{N} e^2 Z_i^2 n_{i\infty}\right) \tag{18}$$

Parameter λ_D is the Debye shielding distance, and describes the screening of the electrostatic potential due to the added ions in solution. At the distance r = λ_D, the electrostatic potential is screened to 37% of value of the unscreened potential. Various values of this parameter can be obtained experimentally by changing salt concentration (ionic strength) of the solution.

c) Manning- Oosawa counterion condensation describes reduction of effective charge density of a charged, extended (rodlike) macromolecule due to non-dissociable counterions on dilution. For linear polyelectrolytes of length L and P charged groups each of valence Z_p , the linear charge density is given as:

$$\rho_l = PZ_Pe / L = Z_Pe / b \tag{19}$$

where b is the spacing between the charges. Counterion condensation occurs when b becomes less than a critical distance that for monovalent counterions is given by:

$$b_{kritisk} = \frac{e^2}{4\pi\varepsilon k_B T} \quad \textbf{(20)}$$

In water at 25°C, $b_{kritisk}$ = 0.714 nm. The fraction of neutralized charges on the polymer due to the counterion condensation is given as:

$$\theta_m = 1 - \xi^{-1}$$

where paremeter ξ is given by

$$\xi = \frac{e^2}{4\pi\varepsilon k_B T b}$$

In the above eq, e is the elementary charge, ε permittivity, k_B Boltzmann constant, and T absolute temperature. For the given DNA molecule, we get:

$$\theta_m(10\ ^0C) = 1 - \xi^{-1} = 1 - \frac{b}{b_{kritisk}}\frac{273+10}{273+25} = 0.774$$

$$\theta_m(40\ ^0C) = 1 - \xi^{-1} = 1 - \frac{b}{b_{kritisk}}\frac{273+40}{273+25} = 0.750$$