TFY 4310 MOLECULAR BIOPHYSICS

Exam Wednesday 11 May 2022

SUGGESTED SOLUTIONS

EXERCISE 1

1

Gi en vurdering av om eksplisitt og implisitt representering av løsningsmiddelmolekyler i molekylære simulering er å foretrekke for modellering av organisering av proteiner som kan være integrert / transmembrane i cellemembraner.

Cellmembranes and insertion transmembrane proteins are large governed by hydrophobic effects. To be able to account for hydrophobic effects in molecular simulations, the change in entropy of solvent (water) molecules need to be provided. The entropic facet requires explicit representation of the water molecules in the simulations.

The hydrofobic effect (interactions/bonds) is connected to the fact that it is energetically favorable 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.

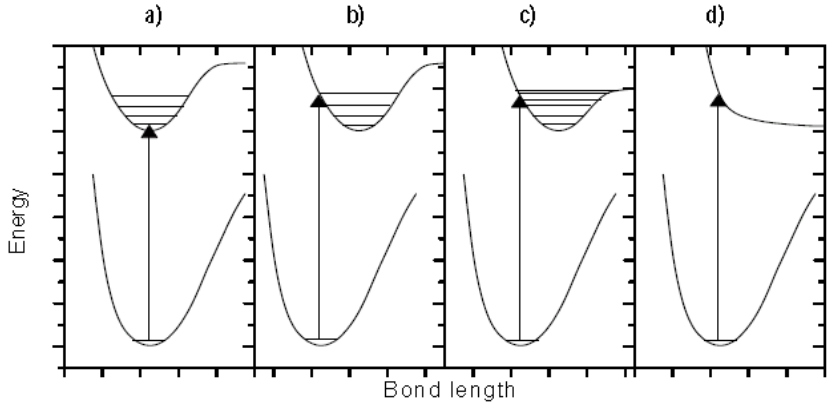
The organization of cell membranes are characterized by the hydrophobic effect on the molecular amphiphilic constituents, resulting in the membrane with hydrophilic headgroups of the lipids are facing towards the outer aqueous phase on the surface of the membrane, and the lipid hydrophobic tails in the interior – each facing or hydrophobic tails.

For the insertion of the transmembrane proteins in the lipid bilayer:

in the organisation of the proteins relative the lipid part: transmembrane proteins with hydrophobic protein domain on the surface is embedded in the hydrophobic part of the lipid bilayer; and with hydrophilic parts exposed to the water. Thus, the patches of various hydrophobic/hydrophobic nature determine the orientation of the protein in the membrane.

2

Figuren under viser ulike elektroniske transisjoner i et to-atomig molekyl i et energi diagram.



Identifiser underfigur(er) som viser elektroniske transisjoner som følger:
2i) til en tilstand som fører til dissosiering av molekylet
2ii) til en tilstand hvor bindingslengden i den eksiterte tilstand er større enn grunntilstanden
2iii) hvor intensiteten til transisjonen mellom 0-0 vibrasjonstilstander er størst.
Gi også en kortfattet begrunnelse.

The panel d) shows a monotonic decreasing energy for the exited state of the diatomic molecule. This implies that excitation leads to a state where a process towards increasing bond length, eventually yielding no bonding, is initiated. The panels a-c, all show that there is a minimum energy for a given bond length for the exited stated; thus, there is no dissociation being initiated on excitation.

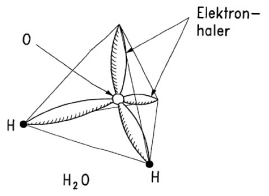
2ii) The graph show that this is the case for figures b) and c) (and 2d - but this is not considered to be binding any longer..)

2iii) This is a) since the likelihood of hitting 0 vibration ground level of the first exited electronic stated is largest in this case.

3

Rett eller galt: analyser utsagnet: Det er kun en elektronhale i hydrogenionet H_3O^+ i forhold til to elektronhaler i vannmolekylet

There are total 10 electrons in one H_3O^+ ion/molecule. Two electrons are in the 1s non-bonding orbital of the O-atom. Six of the electrons are located in three σ-molecular orbitals (formed by the three sp^3 – s) yielding three bonds between the oxygen atom and the three hydrogens; and the remaining two electrons are in the sp^3 orbital that is not binding a hydrogen, thus yielding one electron tail. In the water molecule, the there are two σ-molecular orbitals between the oxygen and the hydrogen, and the remaining four electrons are within two sp^3 hybridized orbitals of O not forming bonds to H, thus being electron tails. Thus, the claim is correct.



4

Rett eller galt: vurder følgende påstand: Flory-Huggins teori angir at blanding av løsningsmiddel og polymer er mindre gunstig for lange (mange monomerer i hver polymerkjede) enn korte (færre monomerer i hver polymerkjede) polymerer.

The Flory-Huggins theory specify 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 (given the same volume fraction). Or in other words: increased entropy for the collection of shorter polymers since they can be selected to independently.

It is also possible to use the mathematical expression to look into this statement:
The free energy of mixing of a polymer (component 2) with degree of polymerization x in a solvent (component 1) is given by):

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

Where N = N1 + xN2 is the molar number of lattice sites; v1 and v2 are the fraction of the lattice occupied by solvent and polymer, respectivly, x is the number of segments per polymer molecule, and kji 12 the Flory Huggins interaction parameter. For the good solvent conditions, we assume kji = 0 here.
Thus:

$$\frac{\Delta G_{mix}\left(polymer\ A\right)}{N} - \frac{\Delta G_{mix}\left(polymer\ B\right)}{N} = RT\left(\frac{v_2}{x_A} \ln v_2 - \frac{v_2}{x_B} \ln v_2 \right) = RTv_2 \ln v_2 \left(\frac{1}{x_A} - \frac{1}{x_B} \right)$$

This difference is < 0 when $x_A^{-1} - x_B^{-1} > 0$ (since ln v2 < 0 due to v2< 1). Thus: $x_A < x_B$ to (polymer A need to be shorter than polymer B) for polymer A to be more favorable than polymer B in the mixing.

5.

Rett eller galt: vurder følgende utsagn: Overlapp konsentrasjonen øker og skalerer med antall segmenter n i en polymer som $n^{-4/5}$ når polymeren overføres fra et godt løsningsmiddel til et ideelt løsningsmiddel.

In an ideal solvent the interactions between the polymer segments and the solvent is equivalent to the interactions between the polymer segments. In a good solvent, the interactions between the polymer and the solvents is preferred and so the polymer chain will expand relative to the ideal solvent;
If the polymer adopts a more expanded conformation the overlap concentration c* will be less/decrease, as lower concentrations are required for the polymers to start overlapping. For the prosess presented here: transfer from a good to an ideal solvent: the polymer will reduce its size, thus resulting in an increase in the overlap concentration.

Regarding the scaling, the root-mean-square (rms) of the end-to-end distance scales 1/2, as so does the rms of the radius of gyration,

$$\left\langle R_{ee}^2 \right\rangle_0^{1/2} \approx Qn^{1/2}$$

The radius of gyration, as entered in the expression for the overlap concentration:

$$C^* = \frac{3N_p}{4\pi N_{Av}} \frac{10^{-3}}{R_G^3} \approx \frac{n}{R_G^3} \approx \frac{n}{n^{3/2}} \approx n^{-1/2}$$

in the ideal solvent.

Thus, the first part of the statement: «Overlapp konsentrasjonen øker» is correct, whereas the latter: «og skalerer med antall segmenter n i en polymer som $n^{-4/5}$ når polymeren overføres fra et godt løsningsmiddel til et ideelt løsningsmiddel.» is not correct. Thus, overall: the statement is not correct.

6

Begrunn følgende utsagn: En surfaktant med to hydrofobe karbonkjeder bundet til samme hydrofile hodegruppe har en større tendens til å bidra til flate ampfifile aggregater enn surfaktant med samme hodegruppe og en hydrofob karbonkjede.

The surfactant with the two hydrophobic tails will have a critical packing parameter (CPP) closer to 1, whereas the the one with one tail will have a smaller CPP. The latter will then occupy a con-like space as in a spherical micelle more easily than the two-tail case. Thus, the two-tail amphiphile will to a larger extent form a planar lipid bilayer structure, whereas the one tail, is likely to adopt a spherical micelle structure.

EXERCISE 2

Beregn elektrostatisk potensiell energi mellom to motsatt ladede punktladninger i en fysiologisk vandig løsning som inneholder 150 mM NaCl og 3 mM $CaCl_2$. Anta at de to punktladningen har følgende
Protein 1: Z_1 = 12 e, R_1 = 3.0 nm
Protein 2: Z_2 = -20 e, R_2 = 1.8 nm
Anta at temperaturen er 20 ^oC og beregn denne energien nå punktladningen er 4.8 nm fra hverandre.

For the actual case, we apply the screened Coulomb potential since the electrostatic potential is screened by the presence of the salts:

$$V(r) = \frac{z_1 z_2 e^2}{4\pi\varepsilon_0 \varepsilon r} \exp(-\kappa r)$$

In this expression, parameter κ is is the inverse of Debye screening length λ_D given by

$$\lambda_D = \kappa^{-1} = \left(\frac{\varepsilon_0 \varepsilon k_B T}{2eIN_{Av} \times 1000} \right)^{1/2}$$

Here, *I* is the ionic strength of the solution:

$$I = \frac{1}{2} \sum_{i=1}^{N} z_1^{\,2} c_{\infty,i}$$

where $c_{\infty,i}$ is the bulk concentration of the ion type i. For the particular solution

$$I = \frac{1}{2}\left(150 + 150 + (-2)^2 3 + 6)\right) \text{mM} = 159 \text{mM}$$

The Debye screening length in the solution becomes:

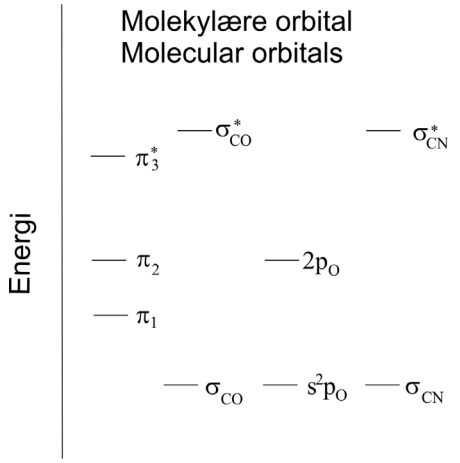
$$\lambda_D = \left(\frac{8.854 \times 10^{-12} \times 78.4 \times 1.38 \times 10^{-23} \times 293.15}{2(1.602 \times 10^{-19})^2 0.159 \times 6.02 \times 10^{23} \times 1000} \right)^{1/2} = 7.56 \times 10^{-10} m$$

For a separation that correspond to a separation r=4.8 nm

$$V(r) = \frac{z_1 z_2 e^2}{4\pi\varepsilon_0 \varepsilon r} \exp(-\kappa r) = \frac{(12)(-20)(1.609 \times 10^{-19})^2 \text{ J}}{4\pi \times 8.854 \times 10^{-12} \times 78.4 \times 4.8 \times 10^{-9}} \exp\left(-\frac{4.8}{0.756} \right) = -2.57 \times 10^{-22} \text{ J}$$

EXERCISE 3

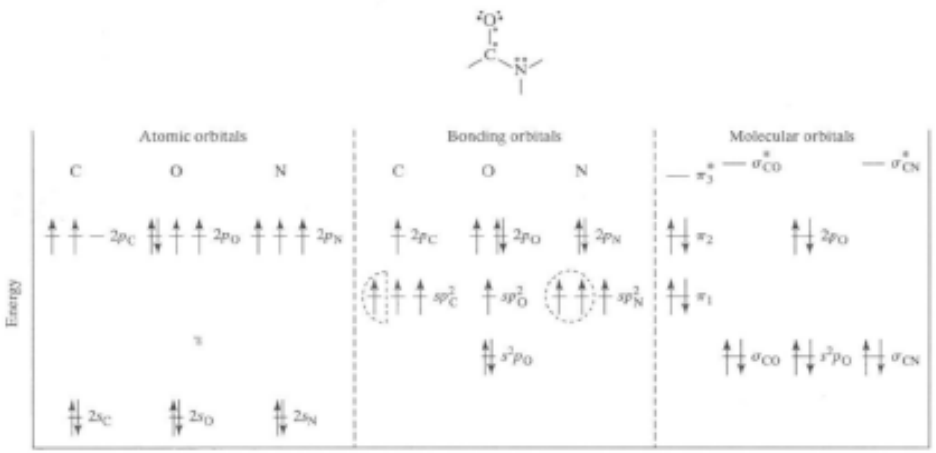
Amid gruppen er sentral i proteiner. Figuren under viser skjematisk energinivåene til molekylære orbitaler som forekommer i denne gruppen.



Figur. Energinivåer til molekylære orbital forekommende i amid gruppen.

Amid gruppen kan angis som $R_1CONR_2R_3$ hvor R (med indeks) beskriver et atom som inngår med enkeltbinding til C for R_1 og N for R_2 og R_3. Er de molekylære orbitalene vist i figuren ulikt det som forventes basert på atomære orbital som utgangspunkt? (begrunn svaret). Identifiser overgang fra elektronisk grunntilstand til den første elektronisk eksiterte tilstanden i amid gruppen

The expected orbitals based on atomic orbitals:
The electronic configuration of the C, N and O and are: C: $1s^2\,2s^2 2p_x^1\,2p_y^1$; N: $1s^2\,2s^2 2p_x^1\,2p_y^1 2p_z^1$; and O: $1s^2\,2s^2 2p_x^2\,2p_y^1 2p_z^1$. The 2 electrons in the 1s orbitals are not involved in the formation of molecular orbitals.
The electronic configuration of the atoms are depicted in the left hand part of the figure below



The hybridization of the oxygen and the carbon are sp^2. Furthermore, it is known that the N-C-O structure is planar, thus the hybridization of the nitrogen must also be sp^2. Using this hybridization, we will have the following occupancies of the valence electrons:
C: 3 in sp^2, 1 in 2p orbital
N: 3 in sp^2, 2 in 2p orbitals
O: 3 in sp^2, 3 in 2p orbitals
See figure above middle panel

The molecular orbitals formed in the covalent bonds to R_1 R_2 and R_3 requires electrons from the sp2 orbitals, but their energy level is not shown on the diagram. Using the C=O as the initial bond to consider, we expect that one of sp2 orbitals of the carbon linearly combines with one of the sp2 orbitals of the oxygen to form a σ bonding and a σ* antibonding molecular orbitals.

The lower level energy level σ_{CO} (on the diagram) is occupied by the each of the electrons from the sp2 orbitals from C and O forming this bond. For oxygen, the other two sp2 orbitals are expected to be fully occupied and do not contribute to molecular orbitals. The double bond between C and O are arises from the combination of 2p atomic orbitals of the C and O, are expected to yield a π bonding and an anti-bonding π* molecular orbitals. The second sp2 orbital of the carbon combines with another atomic orbital to give two molecular orbitals with R1; and the third combines with a sp2 from the nitrogen to form two more MOs (σ_{CN} and a σ^*_{CN}). This are overall expected to leave the 2e in the 2p atomic orbitals of N.

This differ from the observation as outlined:
The 2e thought to be in 2p of N, is involved in π and π* orbitals, not only located between the C and N off axis, but also resonates with the π and π* between C and O (overall yielding π_1 and π_2 binding, and corresponding antibinding orbitals). Moreover, the e of O not involved in molecular orbitals do no remain in sp2, but redistribute to s2p

EXERCISE 4

a). Beskriv hva som menes med spin-gitter og spin-spin relaksasjon i NMR spektrosopi. Beskriv hva som menes med en 90^o rf puls som brukes for i et proton NMR eksperiment, og hvordan netto magnetisering i prøven påvirkes som følge av denne pulsen.

The spin – lattice relaxation depicts the process for relaxation of the longitudinal M_z component of the magnetization vector towards its equilibrium following an out-of equilibrium perturbation. This process include interaction with the environment (lattice). The time constant for this is T_1 : spin-lattice relaxation time constant (longitudenal relaxation time constant).

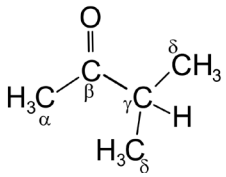
T_2 : spin-spin relaxation time constant (transversal relaxation time constant)

T_2 is the time constant of relaxation of the transverse component of the magnetization vector relaxes (by interactions between spins)

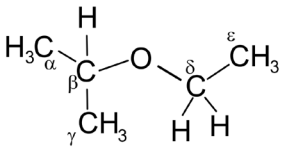
A 90 deg pulse is a pulse of the rf field with a duration that flips the net magnetization from being parallel to the (static) external magnetic field to be orthogonal (90 deg) to it.

For the candidate molecules i)-iii), the carbons in the structured are labelled alfa-eps, for reference in the analysis:

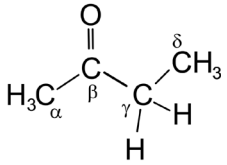
i)



ii)



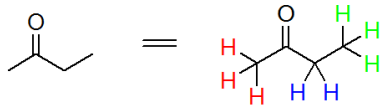
iii)

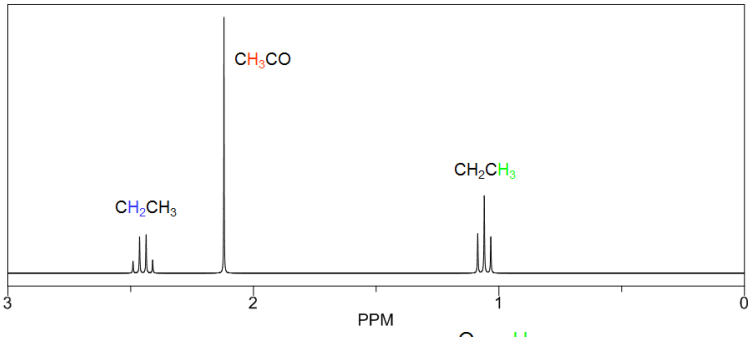


The two spectra contain one proton signal (at about 2 ppm) that is not split. This can occur only for the three protons on the carbon labelled α in structure i) and iii). In these structures, the three H on C (α) are equivalent.
For further analysis of i):
The total 6 H's bound to the two C denoted δ are equivalent and split into two due to the local magnetization contribution from the two possible spin states of H on C (γ). The total signal intensity of these two peaks correspond to the 6 H.
The H on C (γ) are in the neighborhood of the 6 H on C (δ); with each of these being in either of the two possible spin states, gives a septet of the signal for the H on C (γ). Due to the overall signal intensity of this septet corresponding to 1 H, it can bit challenging to see the lowest intensity ones.
Thus, this show that molecule i) gives rise to the spectrum in Figure b).

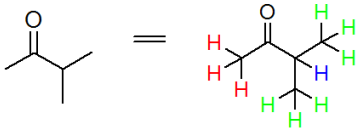
For iii):
The total 3 H's bound to the two C denoted δ are equivalent and split into three due to the local magnetization contribution from the two possible spin states of the two H on C (γ). The total signal intensity of these three peaks correspond to the 3 H.

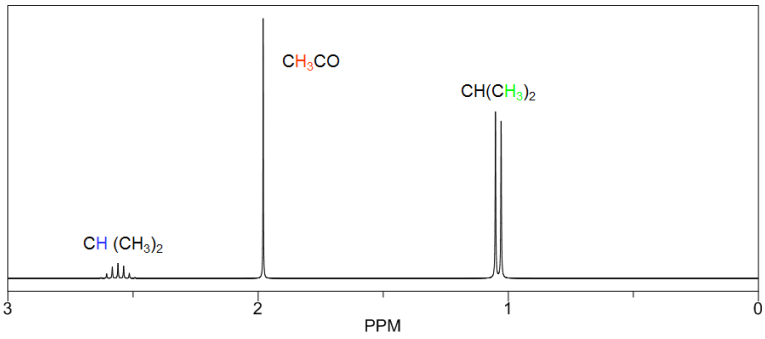
The two H on C (γ) are in the neighborhood of the 3 H on C (δ); with each of these being in either of the two possible spin states, gives a quartet of the signal for the two H on C (γ). The overall signal intensity of this corresponding to 2 H.
Thus, this show that molecule iii) gives rise to the spectrum in Figure a).





a - iii

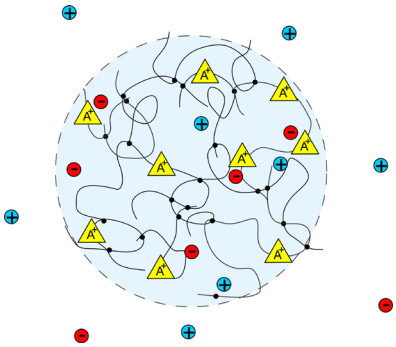




b) i

EXERCISE 5

Figur 1 viser en skjematisk illustrasjon av en hydrogel med ladede gruppe på polymerkjendene i et overskudd av volum av vandig løsningsmiddel.



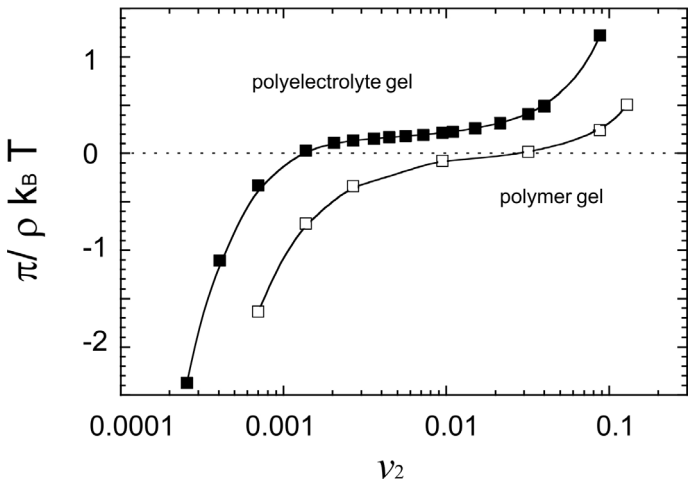
Figur 1. Skjematisk skisse av hydrogel (i området farget lyseblått) med ladede kjemiske grupper (gule trekanter) i et overskuddsvolum av vann. Blå og rød sirkler med + og - tegn angir positivt og negativt ladede ioner (monovalente).

a) Vurder helheten i Figur 1 og angi om det er noen mangler i skissen (med begrunnelse). Beskriv prinsippet for likevektsvolum av en ladet hydrogel i et overskudd av vann og beskriv prinsippene for hvordan likevekt oppnås fra en tilstand utenfor likevekt.

The graph in figure 1 depict an ionic/polyectrolyte hydrogel in an excess of solvent (water), with counterions and other ions. Tue to strict requirements of electroneutrality, there is need for a electric charge balance in the hydrogels and

in the outside solution. For the hydrogel domain: There is fewer counterions than charged groups on the polymers (missing 4 in the schematic), and since there are 3 positive ions in the hydrogel domain: they should also be balanced by the presence of further negative charges. For the outside: not charged balanced.
Principle for equilibrium swelling volume: This is corresponding the minimum free energy of the system. Or, equivalent, zero osmotic pressure inside the hydrogel domain in excess of solvent eventually with ions.
Principle of equilibration: moveable species, e.g. solvent molecules and ions, will exchange between the excess immersing solvent (water) bath and the hydrogel domain until equilibrium is achieved.
In a situation with ionic hydrogel and not too much salt: water will tend to stream into the hydrogel to "dilute" the counterion concentration inside the hydrogel domain (since the counterions cannot escape the hydrogel due to requirement of electroneutrality). Thus, the charge concentration inside the hydrogel will be reduced. This is the part referred to as the Donnan term in the three additive contributions to the osmotic swelling pressure. As the solvent molecules invade the hydrogel domain, the elasticity of the network polymers will increasingly oppose the stretching, and set up a restoring contribution to the overall swelling pressure. The change in the mixing term will also integrate into the overall osmotic pressure.¨

Figur 2 viser beregnet redusert osmotisk trykk som funksjon av volumfraksjon av polymer, v_2 i to typer hydrogeler. Den ene har uladet polymer (merket "polymer gel") og den andre har en polymer som har ladede grupper (merket "polyelektrolyte gel").



Figur 2. Redusert osmotisk trykk som funksjon av polymer volumfraksjon til to typer hydrogeler.

Anta at polymerene i de to hydrogelene er kryssbundet ved volumfraksjon $v_2 = 0.1$ Denne volumfraksjonen svarer til ustrekt tilstand. Beskriv likevektsbetingelsen for svelletilstand til de to hydrogelene og avled et numerisk estimat av volumfraksjon av polymerene for de to hydrogelene ved likevekt svelletilstand basert på data i figur 2. Beregn hvor mye polymerkjedene er strekt ved likevekt svelletilstand ved uttrykket:

$$\frac{\left\langle R_{ee}^2 \right\rangle_0^{1/2}}{\left\langle R_{ee}^2 \right\rangle^{1/2}}$$

for de to type hydrogelene. Indeks 0 i uttrykket over svarer til tilstanden der kryssbindingene ble introdusert.

Vurder om en ideell Gaussisk polymer model med n=15 segmenter anvendt på polymerene (mellom kryssbindingspunktene) ved kryssbindingstilstanden kan brukes for de aktuelle tilfellene.

The graph provide estimate of the volume fraction at equilibrium swelling volume (given by osmotic pressure equal 0 in the graph): for polymer gel: v2 = 0.03 (OK within 0.027 – 0.033), and for polyeletrolyte hydrogel: 0.0014 (ok between 0.0011 og 0.0016)

The ratio between the polymer volume fraction at swelling equilibrium and the reference states 0.1:
Polymer gel:

$$\frac{v_{2,eq}}{v_2} = \frac{0.03}{0.1} = 0.3$$

And polyelectrolyte hydrogel:

$$\frac{v_{2,eq}}{v_2} = \frac{0.0014}{0.1} = 0.014$$

The stretching ratio of the individual polymer chains at equilibrium swelling volume: For the polymer gel:

$$\frac{\left\langle R_{ee}^2 \right\rangle^{1/2}}{\left\langle R_{ee}^2 \right\rangle_0^{1/2}} = \left(\frac{v_2}{v_{2,eq}} \right)^{1/3} = \left(\frac{1}{0.3} \right)^{1/3} = 1.49$$

and the polyelectrolyte gel:

$$\frac{\left\langle R_{ee}^2 \right\rangle^{1/2}}{\left\langle R_{ee}^2 \right\rangle_0^{1/2}} = \left(\frac{v_2}{v_{2,eq}} \right)^{1/3} = \left(\frac{1}{0.014} \right)^{1/3} = 4.15$$

For Gaussian chain chain model with 15 segments:

$\left\langle R_{ee}^2 \right\rangle_0 = nQ^2$ and countour length : $L_c = nQ$

Thus, at reference condition: $\frac{\left\langle R_{ee}^2 \right\rangle_0^{1/2}}{L_c} = \frac{n^{1/2}Q}{nQ} = n^{-1/2}$

and at the equilibrium swelling condition:

$\frac{\left\langle R_{ee}^2 \right\rangle^{1/2}}{L_c} = \frac{\left\langle R_{ee}^2 \right\rangle_0^{1/2}}{L_c} \frac{\left\langle R_{ee}^2 \right\rangle^{1/2}}{\left\langle R_{ee}^2 \right\rangle_0^{1/2}}$ $\frac{\left\langle R_{ee}^2 \right\rangle^{1/2}}{L_c} = 1.49n^{-1/2}$ for hydrogel and $\frac{\left\langle R_{ee}^2 \right\rangle^{1/2}}{L_c} = 4.15n^{-1/2}$ for

polyelectrolyte hydrogel. Numerically with n=15: for hydrogel: 0.385 (of Lc) and 1.07 (of Lc); the latter: larger than 1implying larger than Lc – thus the n=15 segment model cannot be an appropriate model for the polyelectrolyte hydrogel but can be for the polymer hydrogel.

EXERCISE 6

Et protein blir sentrifugert ved 12 000 rpm (omdreininger pr minutt) til likevekt ved i en fortynnet vandig løsning. Netto absorbans ved 280 nm til proteinløsning er bestemt i prøvecellen som funksjon av avstand fra rotasjonsaksen som følger:

r(cm)	*4.00*	*4.05*	*4.10*	*4.15*	*4.20*	*4.25*	*4.30*	*4.35*
A(280 nm)	*0.161*	*0.195*	*0.237*	*0.290*	*0.354*	*0.433*	*0.532*	*0.655*

Anta at det partielle spesifikke volumet til proteinet er 0.740 ml/g.

Beregn molar masse (enhet g/mol) til proteinet.

At sedimentation equilibrium, the processes of sedimentation and concentration driven diffusion are balanced so that the concentration of the macromolecule is only position (along the sample cell) and not time dependent. In the compendium, this is derived to yield:

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

where m is the molality of component 1; r the actual radial position, rm the radial positon of the meniscus, V1(S) is the partial specific volume of the protein, rho the density, R the molar gass constant and T absolute temperature.
In the present case, the measure absorbance is proportional to the concentration (mass/volume), which is proportional to the molality.
Based on the form of data, we recast the eqation to :

$$\ln(m_1(r)) = \ln(m_1(r_m)) + \frac{M_1(1-\overline{V}_1^{(S)}\rho)\omega^2(r^2 - r_m^2)}{2RT}$$

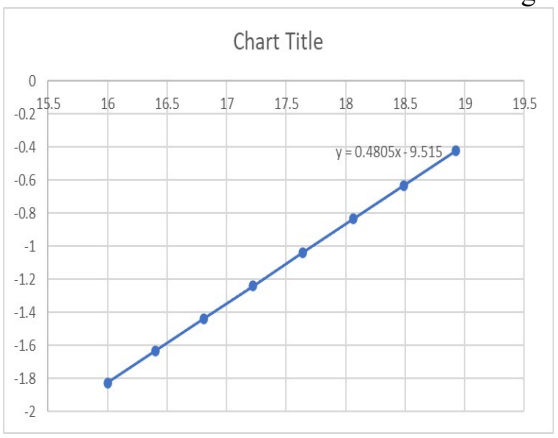
Or:

$$\ln(A(r)) = konst + \frac{M_1(1-\overline{V}_1^{(S)}\rho)\omega^2}{2RT} r^2$$

Thus, plotting ln A(r) vs r^2 will give an estimate of the slope, and using the expression that this is equal to

$$\frac{\Delta(\ln(A(r)))}{\Delta r^2} = \frac{M_1(1-\overline{V}_1^{(S)}\rho)\omega^2}{2RT}$$

allow us to calculate the molar mass from the given data. Plotting of the data



Yields a slope: $\frac{\Delta(\ln(A(r)))}{\Delta r^2} = 0.4805\,\text{cm}^{-2}$.

$$M_1 = \frac{\Delta(\ln(A(r)))}{\Delta r^2}\frac{2RT}{(1-\overline{V}_1^{(S)}\rho)\omega^2}$$

$$= 0.4805\,cm^{-2}\frac{2x8.314\,JK^{-1}mol^{-1}x277K}{(1-0.74\,x1)(12\,000\,rpm)^2}$$

$$= 0.4805\,cm^{-2}\frac{2x8.314\,JK^{-1}mol^{-1}x277K}{(1-0.74\,x1)(12\,000)^2(2\pi/60\,rad/s)^2}$$

$$= 0.0053907\frac{kgm^2s^{-2}mol^{-1}}{cm^2\,s^{-2}} = 53907\frac{g}{mol}$$

Herein, the unit rpm (revolutions per min) correspond to 2 pi rad per 60 seconds.

EXERCISE 7

Anta følgende instrumentering er tilgjengelig: En sentrifuge som kun kan opereres med en rotasjonshastighet 20 000 rpm eller 40 000 rpm (ved romtemperatur 20 degC). Til denne sentrifuge er det kun tilgjengelig en rotor, hvor prøvecellen er i horisontal avstanden mellom 5 og 10 cm, for henholdsvis menisk og bunn av prøvecellen, begge avstander målt fra rotoraksen. Du skal finne fram til en foretrukket bruk av dette utstyret for å behandle en løsning av to makromolekyler som er i løsningen slik at du får mest mulig av det ene makromolekylet tilgjengelig uten at det er «forurenset» av det andre.

Følgende molekylære parametre er kjent for de to makromolekylene:
MolA har sedimentasjonskoeffesient s_A = 4.31 S og diffusjonskonstant D_A = 6.3 10^{-7} cm^2/s
MolB har sedimentasjonskoeffesient s_B = 132 S og diffusjonskonstant D_B = 1.15 10^{-7} cm^2/s

a) *a) Anta at diffusjon av makromolekylene kan ses bort fra. Foreslå en prosedyre basert på sentrifugen som er beskrevet slik at prosedyren gir en løsning hvor du har mest mulig av det ene makromokylet og minst mulig av det andre. Forslaget til prosedyre(r) skal begrunnes. Hvilken av de to rotor hastighetene vil du anbefale for dette formålet?Consider what is the effect of diffusion on your suggested procedure.*

The two possible procedures to consider:

i) running the centrifuge and consider what is accumulated at the bottom of sample cell,
ii) running the centrifuge and consider what is remaining in the solution (e.g. that is not accumulated at the bottom)

In the present case, where it is first assumed that we can disregard the diffusion, these two options correspond both to the moving boundary method, where the definition of the sedimentation coefficient describes key features for the process:

$$s = \frac{u}{\omega^2 r}$$

Here, u is sedimentation velocity, ω is the angular velocity of the rotor and r is the distance to rotational axis. The sedimentation velocity can be estimated from the moving step-change in concentration as the macromolecules are forced to sediment:

$$s = \frac{u}{\omega^2 r} \approx \frac{1}{\omega^2}\frac{1}{\overline{r}}\frac{d\overline{r}}{dt} = \frac{1}{\omega^2}\frac{d\ln(\overline{r})}{dt}$$

where $\overline{r}$ is the position of the moving boundary. Solved for the time it takes for the migration of the moving boundary from the meniscous to the bottom of the cell:

$$\Delta t = \frac{1}{\omega^2 s}\Delta \ln(\overline{r})$$

For the actual centrifuge, the following numerical estimates of Δt are obtained:
For MolA 20 000 rpm:

$$\Delta t = \frac{1}{\left(20000 rpm\, 2\pi / 60\, rad\, s / \min\right)^2 4.31\cdot 10^{-13} s^{-1}}\Delta \ln(\overline{r}) = 3.67\cdot 10^5 s = 102\, hours$$

and overall

Spinning speed	molecule	s	Time to sediment to bottom
20 000 rpm	MolA	4.31 S	102 hours
20 000 rpm	MolB	132 S	3.33 hours (3 hours, 20 min)
40 000 rpm	MolA	4.31 S	25.5 hours
40 000 rpm	MolB	132 S	0.83 hours (50 min)

From this we deduce under the assumption a) that we can either run the centrifuge for 3 hours and 20 min to sediment molecule B (all of them if diffusion is ignored) while MolA mostly remain – and as the only component that is not sedimented. Although we also lose some of MolA in the centrifugation procedure (e.g. sedimented to the bottom of the tube), what is left throughout the sample cell is not containing MolB. One can also obtain the same situation by centrifugation at 40 000 rpm for 50 min. When disregarding the diffusion, there is no preference of using 20 000 rpm for 3 hours 20 min or 40 000 rpm for 50 min based on the requirements for the sample to be obtained (the argument that the shorter duration can be more efficient for other reasons is not considered here).

b. Vurder hva som er effekten av diffusjon av makromolekylene på foreslått prosedyre(r) i oppgave a) og om det fører til endret forslag til prosedyre.

Also taking diffusion into account, the assumption that there is step function in the concentration driven towards increasing distance with increasing centrifugation time is not valid. Instead, there will be a diffusional broadening where the width is determined by sedimentation duration and diffusion constant of the macromolecules. Thus, we see that the longer duration of the protocol (at 20 000 rpm) will result in more MolB diffusing "back" to the solution than for the shorter duration protocol (at 40 000 rpm). Thus, for the actual duration of the procedures suggested under a), there is preference for the 40 000 rpm protocol since there will be less MolB still in the solution than for the 20 000 rpm protocol.

EXERCISE 8 Scattering – molecular conformation

Et protein med ukjent geometri/konformasjon er karakterisert ved lav-vinkel røntgen spredning og ved dynamisk lysspredning. Oppgaven går ut på å analysere data fra disse to eksperimentelle teknikkene, og vurdere resultatene i sammenheng for å komme fram til mulig type geometri/konformasjon til det ukjente proteinet. Alle oppgitte data er ved 20 ^{0}C.

Det er benyttet en lav konsentrasjon av proteinet ved dataopptak ved lav-vinkel røntgen spredning med en bølgelengde λ= 0.16 nm, og følgende data er oppnådd:

Spredevinkel (mRadianer)	*1.0*	*2.0*	*3.0*	*4.0*	*5.0*
I(theta)/I(0)	*0.9887*	*0.9554*	*0.9025*	*0.8332*	*0.7520*

For the low angle scattering data, the Guinier approximation is used to analyze the data:

$$\left\langle I_S(q)\right\rangle = I_0 \exp\left[-\frac{q^2\left\langle R_G^2\right\rangle}{3}\right]$$

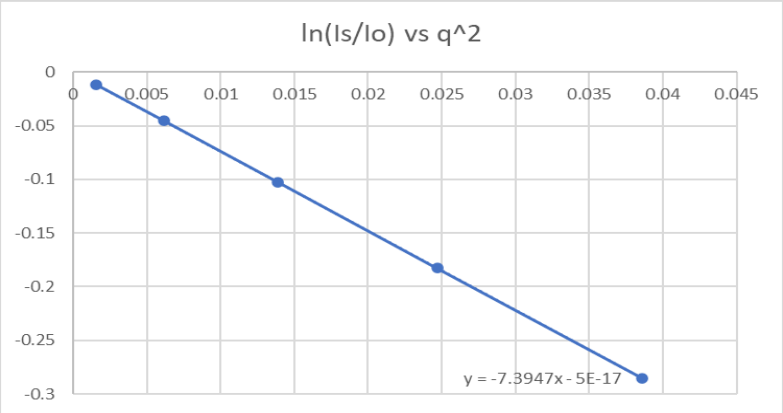
The q-dependence is equivalent to a dependence on the scattering angle. Recasting the expression to

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

we see that plotting the data $\ln\left(\langle I_S(q)\rangle / I_0\right)$ versus q^2, we would expect to get a straight line, and where the slope is $-\langle R_G^2\rangle / 3$.

Calculation of scattering vector: $q = \frac{4\pi}{\lambda}\sin(\theta/2)$

Analysis of the given data:



yields a slope $\frac{\Delta \ln\left(\langle I_S(q)\rangle / I_0\right)}{\Delta q^2}$ =- 7.3947nm^2. The low angle scattering data thus gives an estimate of size parameter of the protein:

$$\langle R_G^2\rangle = 3 \times 7.3947\,\text{nm}^2 = 22.1814\text{nm}^2\text{; or}$$
$$\langle R_G^2\rangle^{1/2} = \sqrt{22.1814\text{nm}^2} = 4.71\text{nm}$$

Dynamisk lysspredningsdata oppnådd ved bølgelengde

Det er også benyttet lav konsentrasjon av proteinet ved dynamisk lysspredning. I dynamisk lysspredningsoppsettet er det benyttet = 633 nm (i løsningen), og spredevinkel er 45 grader.

Følgende data er observert for intensitetskorrelasjonsfunksjonen:τ

τ (μs)	*10*	*20*	*30*	*40*	*60*	*80*	*100*	*200*
$g^{(2)}$	*1.961*	*1.923*	*1.887*	*1.852*	*1.787*	*1.726*	*1.679*	*1.449*

b) Beregn en parameter for størrelse til det samme proteinet basert på dynamisk lysspredningsdata

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:

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

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:

$$D_0 = \frac{k_B T}{6\pi\eta R_p}$$

Where R_p is the effective radius of the particle; kb Boltzmanns constant, T absolute temperature and η the viscosity of the solvent.

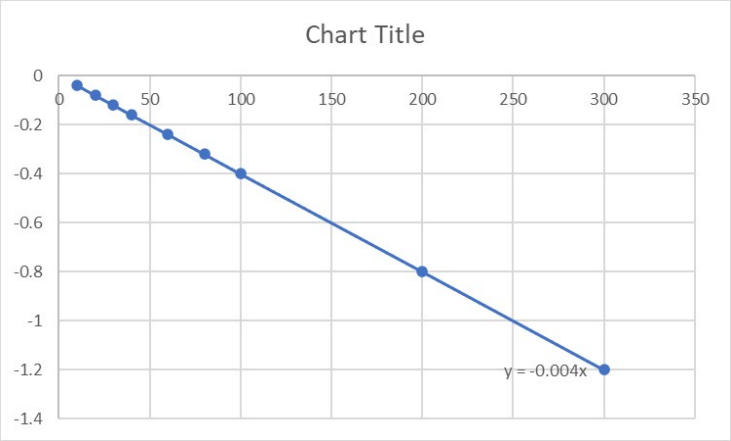
Thus,

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

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

The latter expression gives the basis: plot of ln[ ] vs. τ should yield a straight line with slope – $2q^2D_0$.

Using the tabulated data, the plot of plot of ln[ ] vs. τ:



Yields a slope: -0.004 μs $^{-1}$ = - 4.0x10^{3} s^{-1}
With the specifications:

$$\frac{\Delta \ln\left[g^{(2)}(q,\tau)-1\right]}{\Delta\tau} = -4.0\times10^{3}\ \mathrm{s}^{-1} = -2q^{2}D_{0}$$

The scattering vector in the dynamic light scattering is calculated to q=7.6x10^{6} m^{-1}.
The diffusion constant is then obtained with the value:

$$D_{0} = \frac{4.0\times10^{3}\ \mathrm{s}^{-1}}{2\times\left(7.6\times10^{6}\ \mathrm{m}^{-1}\right)^{2}} = 3.47\times10^{-11}\mathrm{m}^{2}\ \mathrm{s}^{-1}$$

Using :

$$D_{0} = \frac{k_{B}T}{6\pi\eta R_{p}}$$

we obtain the estimate for the radius:

$$R_{p} = \frac{k_{B}T}{6\pi\eta D_{0}} = \frac{1.38\times10^{-23}\ \mathrm{JK}^{-1}293\mathrm{K}}{6\pi\,1.0\times10^{-3}\mathrm{kgm}^{-1}\mathrm{s}^{-1}3.47\times10^{-11}\mathrm{m}^{2}\ \mathrm{s}^{-1}} = 6.2\mathrm{nm}$$

c) Vurder de to parameterne for størrelse til proteinet i sammenheng, diskuter ut fra det hva som kan være en mulig konformasjon og begrunn dette.

The two size parameters obtained by the techniques is the radius of gyration and overall radius. For the various conformations and geometries, we have information as follow:
For the ideal random coil chain, the relation between the average of the end-to-end distance and the radius of gyration:

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

Thus, one would expect a ratio between overall radius and R_G:

$$\frac{R_{overall}}{\left\langle R_{G}^{2}\right\rangle^{1/2}} = \frac{\frac{1}{2}\left\langle R_{ee}^{2}\right\rangle^{1/2}}{\left\langle R_{G}^{2}\right\rangle^{1/2}} = \frac{\frac{1}{2}\sqrt{6}\left\langle R_{G}^{2}\right\rangle^{1/2}}{\left\langle R_{G}^{2}\right\rangle^{1/2}} = \frac{1}{2}\sqrt{6} = 1.22$$

Other possible models can be a compact sphere. In this case, the overall radius is related to the radius of gyration:

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

Thus

$$R_{sphere}/R_{G} = \sqrt{5/3} = 1.29$$

For the experimental data, we have

$$\frac{R_{p}}{\left\langle R_{G}^{2}\right\rangle^{1/2}} = \frac{6.2}{4.71} = 1.31$$

From the comparison the ratios for the actual models: it is close to a sphere with homogeneous mass density.