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EXAM
TFY4335 BIONANOTECHNOLOGY

1st of December 2011. 09:00

Examination support materials:

- Formula sheet - see Appendix A
- Simple calculator (according to NTNU exam regulations)
- K. Rottmann: Matematisk formelsamling (eller tilsvarende)
- Carl Angell og Bjørn Ebbe Lian: Fysiske størrelser og enheter, navn og symboler (eller tilsvarende)

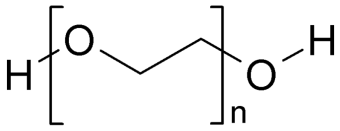
Answer must be written in English or Norwegian. **You have to answer Question 1 and two (2) out of three remaining questions.** The maximum score for the exam is **100p**.

Question 1: Short questions (30p)

1. What is define by λ_D (equation 54) and ℓ_B (equation 49). **(10p)**
   Describes how electrostatic potential will change with distance, close to large, flat surface with given charge density (derived for large flat surface and not any other object, as we do derivation in 1D (nothing is changing in the direction parallel to the surface)). Electrostatic potential is exponentially deceasing with distance and (equation 55) is e^{-1} at $x = \lambda_D$. λ_D does not depend on surface charge, but on ion concentration ($V(x)$ and the force charge particle is experiencing close to the surface obviously depends on surface charge, equation 55). ℓ_B - distance between 2 like-charges, at which the electrostatic energy is equal to k_BT
2. What are hydrophobic interactions? **(10p)**
   Attractive interaction between hydrophobic groups/molecules in solution; entropic in nature; gain in entropy of water molecules. Ordering of water molecules around hydrophobic groups in solution (in order to maximize the number of H-bonds) reduces their entropy. The total system entropy will increase if some of those molecules are realised due to hydrophobic groups coming in contact (reduces hydrophobic area in contact with water).
3. What is defined by depletion force? Describe the origin of that phenomena. **(10p)** Depletion force in a attractive force between large objects (for example large globular proteins) in a presence of small molecules. Short range interaction. Entropic in origin. If two large molecules are separated, each of them is surrounded by a small depletion zone which is not accessible for small molecules. If two large molecules stick together, the total volume of

that depletion zone is reduced and small molecules gain entropy. Inside the cell, depletion force will promote interactions between large protein molecules - helps large molecules to find each other.

Question 2: Polymer chins (35p) Imagine that you are able to stretch single protein molecule from its native conformation to fully extended conformation by somehow holding to its chain ends (for example with AFM, OT or magnetic tweezers). You are also able to monitor force needed to increase the end to end distance. The experiment is done in buffer at pH = 7 and the buffer concentration is c = 0.1M. The protein molecule is 35nm long (contains around 1000 amino acids).



Figur 1: Cheimcal structure of Polyethylene glycol (PEG).

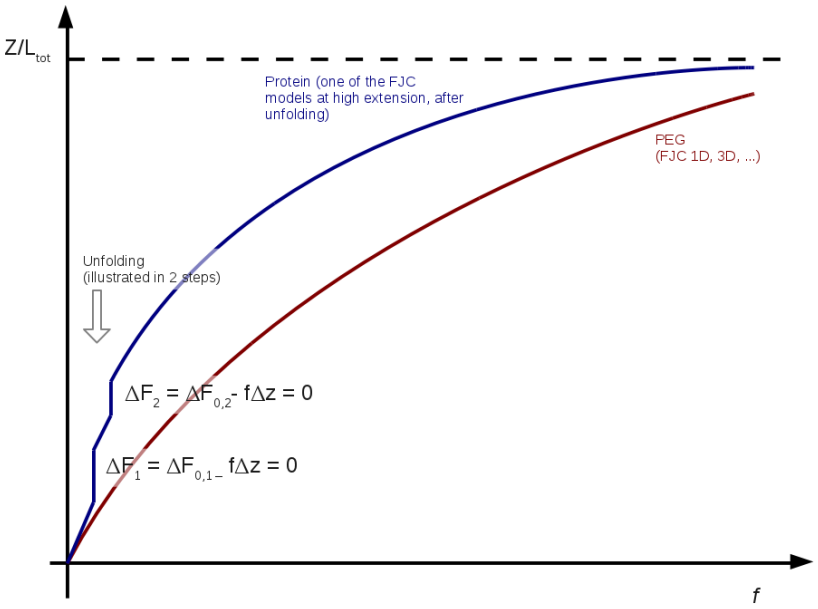
Describe what you expect to see as you increase the separation. What kind of forces you will have to overcome to increase end to end distance (hint: what forces are involve in stabilisation of native conformation). What models with different degree of simplification could you use to describe experimental observations. Describe those models and the assumption needed to derive theoretical force/extension behaviour.

What will be the main difference if you replace protein molecule by a flexible hydrophilic polymer like polyethylene glycol (PEG, see Figure 1 for chemical formula). Assume that you stretch a polymer chain also 35nm long.

At low extension we have a situation which we could compare to RNA hairpin example, where the molecule is unfolded "by force". For the protein, we have a number of intermolecular interactions (hydrogen bonds, hydrophobic interactions, S-S bonds, charge-charge interaction) which stabilize native protein conformation and those will be broken when the end to end distance is increased. This transition can be described by using difference in free energy between folded and unfolded configuration. We can envisage this process as only one transition (only two molecular states (conformations), folded at low force and unfolded at large enough force), or as a number of transitions, where the protein unfolds in a number of steps (2 steps are illustrated in Fugure 2). Each of the unfolding steps will have $\Delta F_0, i$ associated with it and at the force at which protein unfolds $\Delta F_0, i - f\Delta z = 0$. At low extension (we do need to stretch the protein very much to unfold native conformation), the force-extension curve will schematically look as the left part of the drawing in Figure 2. When all intermolecular interactions (internal structures) are broken, we can describe further stretching with a polymer model sutable for describing force extension behaviour of a flexible polymer, for example FJC (1D model which is not really correct, but illustrative, 3D-model or 3D-model with cooperativity, or one of the more complex models). The main difference between the protein and PEG molecule is that there will be no unfolding transition at low force, and whole force/extension curve can be represented by on of our FJC models. Interaction between PEG molecule and the solvent will contribute to the segment length we need to use to fit observed data to the predicted curve, but will not result in any distinctive transitions as for the protein molecule.

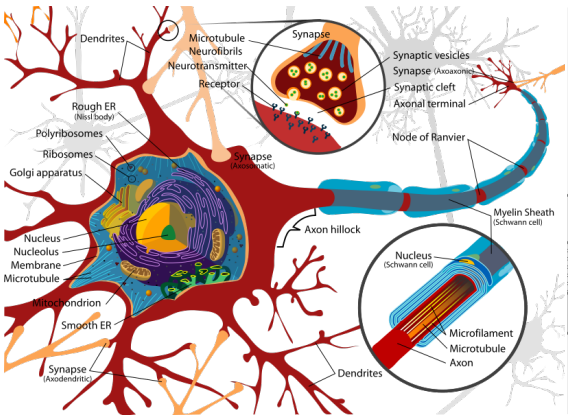
Question 3 Transport along an axon**(35p)**

The axon is a fine, often very long, cable-like projection of a neuron that carries nerve signals. The axon we study in the lab is 1m long, has a cylindrical cross-section and a diameter of 500nm

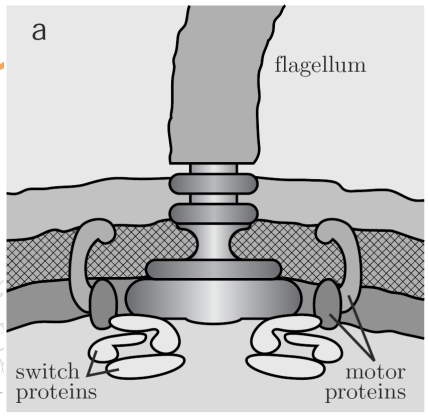


Figur 2: Schematic force extension behaviour. The shape of the curve will depend on the experimental conditions (for example, of we are precisely controlling extension or force).

(see Figure 3a). We would like to investigate transport of vesicles (100nm in diameter) which are produced at the cell body and transported along the axon. Experimental observations show that those move along the axon at 400mm/day.



(a) Anatomy of a neuron



(b) Schematic illustration of a molecular motor which is "powering" flagellum of a bacteria

Figur 3: Figures for Q3 and Q4

The vesicle concentration at one end of the tube (close to cell nucleus) is c_0, and the concentration is zero at axon terminal. Calculate and compare diffusive flux with the flux observed experientially. If you discovered that vesicles are not moved by only passive diffusion but are transported by molecular motors, calculate force which such a motor needs to delivered to achieve experimentally observed transport speed of 400mm/day. Comment on the obtained value.

First we need diffusion constant:

$$D = \frac{k_B T}{6\pi\eta R} = 4.3 \times 10^{-12} \mathrm{m^2 s^{-1}}$$

Diffusive flux is given by

$$j = -D\frac{\partial c}{\partial x} = -D\frac{-c_0}{L} = D\frac{c_0}{L}$$

The observed flux

$$j_{obs} = vc_0$$

So,

$$\frac{j}{j_{obs}} = \frac{Dc_0}{vc_0L} = \frac{D}{vL} = \frac{4.3\times10^{-12}\cdot 24\cdot 3600}{0.4} = 9\times10^{-7}$$

No points were deducted for those who used observed velocity of 400nm/day as stated in the Norwegian version of the exam. Force which a motor has to deliver:

$$f = v\zeta = 6\pi\eta Rv = 9.4\times10^{-10}\frac{0.4}{24\cdot3600} = 4.36\times10^{-15}N = 0.004\text{pN}$$

This is very low force. If we remember that $k_BT = 4.1\text{pN}\cdot\text{nm}$ (on the order of $0.5\text{pN}\cdot 8\text{nm}^{-1}$), then this can not be the real force generated by the motor, but more like a time average force. Molecular motors typically deliver force in pN range. In our case, the vesicle is not moved at constant velocity, but moves, then stops, then moves again. Motors typically make on the order of 100 steps, then they detached form the track, before attaching again and continuing.

Question 4 (35p) Figure 3b shows a schematic illustration of a molecular motor which is "powering" flagellum of a bacteria. Describe basic principle of how such motor could work based on a concept of a "ratchet" and the principle of how chemical energy is converted into mechanical work in such "device". Describe what are the requirements to use such simplification. Explain how equations 64-67 can be used to describe this model in more quantitative way, that is, predict speed as a function of load f.

We need to implement an S-ratchet concept in an enzymatic machine which will make use of chemical energy in ATP. We make S-ratchet design circular (hinges on the outside). Once the central part is rotating, the enzyme is moving down potential energy landscape, crossing small local energy bariers. The chemical energy is used to "reload" the springs, so we never get to a global energy minimum. Anisotropy of the design is used to direct Brownian motion and once the motor is moving in one direction it can do useful work. For S-ratchet the maximum velocity is a function of the diffusion constant of the motor (rotational diffusion in our case). The average velocity as a function of load (or generated torque) can be derived from the Smoluchowski equation - random walk on a potential energy surface. To solve it we need to notice that for a ratchet, the flux of "hinges" need to be independent on position along the circular motor. The equation deals with the flux both in upwards and downwards direction on the potential energy surface and the motor can cross energy barriers as long as they are not much larger then k_BT (which would be the case for high load). S-ratchet is not moving constantly down the potential energy landscape but has to cross energy barriers borrowing energy from the surrounding. The time motor needs to cross those barriers (waiting time) will slow the motor down comparing to maximum velocity, and that time is an exponential function of the load. Larger the load, larger the local energy barriers the motor will need to cross, slower the velocity

Appendix A: Equation Sheet

$$k_B = 1.38 \times 10^{-23}\text{J K}^{-1} \quad (1)$$

$$e = 1.6 \times 10^{-19}\text{coul.} \quad (2)$$

$$\varepsilon_0 = 8.9 \times 10^{-12}\text{F m}^{-1} \quad (3)$$

$$\eta_{water} = 1 \times 10^{-3}\text{Pa s} \quad (4)$$

$$v_{drift} = \frac{f}{\xi} \quad (5)$$

$$\xi = 6\pi\eta R \quad (6)$$

$$\xi D = k_B T \quad (7)$$

$$\lambda_X = \sqrt{2Dt} \quad (8)$$

$$\lambda_{3D} = \sqrt{6Dt} \quad (9)$$

$$\left\langle r^2 \right\rangle = N L_{seg}^2 \quad (10)$$

$$D = \frac{1}{\tau} \int_{-\infty}^{\infty} \frac{\Delta^2}{2} \rho(\Delta) d\Delta \quad (11)$$

$$2D\tau = \left\langle \Delta^2 \right\rangle \quad (12)$$

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \quad (13)$$

$$j_s = -D \frac{\partial c}{\partial x} \quad (14)$$

$$\frac{\partial c}{\partial t} = -\frac{\partial j}{\partial x} \quad (15)$$

$$j_s = -P_s \Delta c \quad (16)$$

$$\frac{\partial c}{\partial t} = D \nabla^2 c \quad (17)$$

$$\vec{j} = -D \nabla c \quad (18)$$

$$c(\vec{r}, t) = \frac{N}{(4\pi D t)^{3/2}} e^{-\frac{r^2}{4Dt}} \quad (19)$$

$$j = D \left(-\frac{dc}{dx} + \frac{q}{k_B T} \varepsilon c \right) \quad (20)$$

$$\Delta \left[\ln c \right] = -\frac{q}{k_B T} \Delta V \quad (21)$$

$$c\left(z\right) = C e^{\frac{-m_{\text{eff}} g z}{k_B T}} \quad (22)$$

$$j\left(r\right) = D \left(-\frac{dc}{dr} + \frac{r \omega^2 m_{\text{eff}}}{k_B T} c(r) \right) \quad (23)$$

$$c(r) = C e^{\frac{m_{net} \omega^2 r^2}{2 k_B T}} \quad (24)$$

$$v_{crit} = \frac{\eta}{\rho R} \quad (25)$$

$$f_{crit} = \frac{\eta^2}{\rho_m} \quad (26)$$

$$f_{fric} = \frac{\eta \ell^3 v}{R^2} \quad (27)$$

$$f_{inert} = \frac{\rho_m \ell^3 v^2}{R} \quad (28)$$

$$\Re = \frac{v R \rho}{\eta} \quad (29)$$

$$\frac{f}{A} = -G \frac{\Delta z}{d} \quad (30)$$

$$\frac{f}{A} = -\eta \frac{v}{d} \quad (31)$$

$$Q = \frac{\pi R^4 p}{8 L \eta} \quad (32)$$

$$\frac{k_B T}{2} = \alpha \frac{\left\langle x^2 \right\rangle}{2} \quad (33)$$

$$S \equiv k_B \ln \Omega \quad (34)$$

$$T^{-1} = \left(\frac{dS}{dE} \right) \quad (35)$$

$$\Delta U = \Delta Q + \Delta W \quad (36)$$

$$\Delta S \geq \frac{\Delta Q}{T} \quad (37)$$

$$F_a \equiv E_a - T S_a \quad (38)$$

$$G_a \equiv E_a + p V_a - T S_a \quad (39)$$

$$\frac{P_1}{P_2} = e^{\frac{\Delta E}{k_B T}} \quad (40)$$

$$P_1 = \frac{1}{1 + e^{-\frac{\Delta E}{k_B T}}} \quad (41)$$

$$P_2 = \frac{1}{1 + e^{\frac{\Delta E}{k_B T}}} \quad (42)$$

$$\tau^{-1} = C e^{\frac{-\Delta E^{\ddagger}}{k_B T}} \left(1 + e^{\frac{-\Delta E}{k_B T}} \right) \quad (43)$$

$$\Delta F = \Delta F_0 - f \Delta z \quad (44)$$

$$Z = \sum_j e^{-E_j / k_B T} \quad (45)$$

$$p_{equil} = c_{osm} k_B T \quad (46)$$

$$c_{osm} = \varphi M c \quad (47)$$

$$\Sigma = Rp/2 \quad (48)$$

$$\ell_B \equiv \frac{e^2}{4\pi\varepsilon k_B T} \quad (49)$$

$$\overline{V}(x) = \frac{eV(x)}{k_B T} \quad (50)$$

$$c_+(x) = \frac{2\pi\ell_B \left(\frac{\sigma_q}{e}\right)^2}{\left(1 + 2\pi\ell_B \frac{\sigma_q}{e} x\right)^2} \quad (51)$$

$$x_0 = \left(\frac{e}{2\pi\ell_B \sigma_q} \right) \quad (52)$$

$$\frac{\mathrm{d}^2 \overline{V}}{\mathrm{d}x^2} = -4\pi\ell_B c_0 e^{-\overline{V}} \quad (53)$$

$$\lambda_D = \left(8\pi\ell_B c_\infty\right)^{-\frac{1}{2}} \quad (54)$$

$$V(x) = -\frac{\sigma_q \lambda_D}{\varepsilon} e^{-\frac{x}{\lambda_D}} \quad (55)$$

$$\lambda_D = 0.31[\text{NaCl}]^{-1/2} \quad (56)$$

$$\frac{E}{A} \approx k_B T \left(\frac{\sigma}{e} \right) \quad (57)$$

$$\frac{E}{A} \approx k_B T \left(\frac{\sigma}{e} \right)^2 2\pi\lambda_D \ell_B \quad (58)$$

$$f = 2 k_B T b^2 r \quad b^2 \propto \frac{1}{n l^2} \quad (59)$$

$$\left\langle z / L_{tot} \right\rangle = \tanh\left(f L_{seg}^{(1d)} / k_B T \right) \quad (60)$$

$$\left\langle z / L_{tot} \right\rangle = \coth\left(f L_{seg} / k_B T\right) - \\ - \left(f L_{seg} / k_B T\right)^{-1} \quad (61)$$

$$\left\langle z / L_{tot} \right\rangle = \frac{\sinh \alpha}{\sqrt{\sinh^2 \alpha + e^{-4\gamma}}} \quad (62)$$

$$\alpha \equiv \frac{f\ell}{k_B T} \quad \alpha \equiv \frac{\Delta G}{-2 k_B T} \quad (63)$$

$$j(x) = c v_{drift} - D \frac{\mathrm{d}c}{\mathrm{d}x} \quad (64)$$

$$j^{(1D)} = -MD \left(\frac{\mathrm{d}P}{\mathrm{d}x} + \frac{1}{k_B T} P \frac{\mathrm{d}U_{tot}}{\mathrm{d}x} \right) \quad (65)$$

$$0 = \frac{\mathrm{d}}{\mathrm{d}x} \left(\frac{\mathrm{d}P}{\mathrm{d}x} + \frac{1}{k_B T} P \frac{\mathrm{d}U_{tot}}{\mathrm{d}x} \right) \quad (66)$$

$$v = \left(\frac{f\,L}{k_B T} \right)^2 \frac{D}{L} \left(e^{f L / k_B T} - 1 - \frac{f\,L}{k_B T} \right)^{-1} \quad (67)$$