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EXAM
TFY4335 BIONANOTECHNOLOGY

Tuesday 27th of May 2008. 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. Exam consists of five (5) main questions with sub-questions. Number of points given to each sub-question is given in bold font. The maximum score for the exam is **75p**.

Question 1: Diffusion of small and large drug molecules

You have synthesized new type of drug based on nanoparticles with sizes 5nm, 10nm and 50nm (referred to as `P5`, `P10` and `P50`). You wonder how efficiently those particles can be delivered to tissue which need treatment through diffusion from blood vessels. You want to compare transport of your new nano-medicine with conventional drug based on small protein molecule with a radius of 1nm (referred to as `C1`).

1. calculate diffusion constant for particles `P5`, `P10` and `P50` in water at room temperature and compare it with the one calculated for `C1`. Use viscosity of water at 20℃ $\eta_{20^oC} = 1 \times 10^{-3}$ kg m^{-1}s^{-1}. (**7p**)
2. How the diffusion constants will change when particles are diffusing at 37℃ ($\eta_{37^oC} = 6.9 \times 10^{-4}$ kg m^{-1}s^{-1}). Calculate for `P5` and `C1`. (**3p**)

3. To reach cells which need treatment, particles will need to diffuse through extracellular matrix (ECM). You have made a model of ECM from a biopolymer gel. In the first approximation, you assume that diffusion of the particles in the gel can be described as diffusion in a viscous media. For your model gel $\eta^{gel}_{37^oC} = 1 \times 10^{-2}$ kg m^{-1}s^{-1}. If diffusion is the only transport mechanism, how much time is needed for a single particle to diffuse a distance of 10μm. Calculate and compare results for `P50` and `C1`. (**5p**)

Question 2: Optical tweezers

1. Briefly describe main concept and technical solutions (critical components) used for manipulation and trapping of small objects with light. (**6p**)
2. Can you explain the origin of the trapping force for particles in the μm size range. (**3p**)
3. How optical tweezers are used to measure forces? (**3p**)
4. You use your optical tweezers setup to study forces between particles in solution. Consider following situations:
   (a) two 1μm particles with positively charge surface in pure water.
   (b) two 1μm particles with positively charge surface in 1M solution of NaCl.
   (c) two uncharged 1μm particles in pure water.
   (d) two uncharged 1μm particles in solution containing high concentration of globular protein molecules 2nm in diameter.

   You perform your experiment in a following manner. One particle is fixed by a glass pipette, the other one you can move using optical tweezers. You change the distance between particles and measure force between them. You can control movement of the particle in the optical trap with 0.1 nm precision.

   Compare situations (a) and (b) and describe what will be the main difference between those two situations.

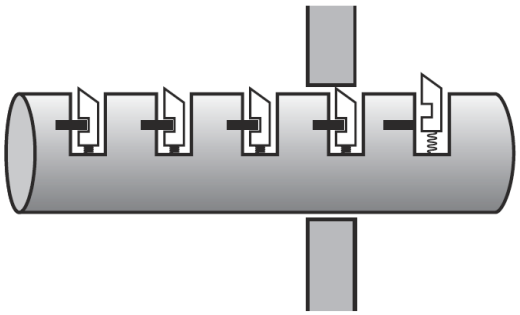
   Are you expecting to find any attractive or repulsive forces between particles in situations (c) and (d)? If so, explain nature of those interactions. In all cases ignore all properties of the particle surface, appart from the mentioned charge. (**6p**)

Question 3: Protein stability

1. Describe main interactions involved in stabilizing conformations of protein molecules. (**4p**)
2. Some mechanical properties of single protein and polymer chains can be described using freely jointed chain (FJC) model which contains only one adjustable parameter L_{seg}. Describe main concepts of FJC model. (**4p**)
3. Suggest how one could determine the parameter L_{seg} experimentally using optical tweezers or AFM. (**4p**)

Question 4: Molecular Machines

1. Why cells need to be able to do mechanical work. Mention few examples. What is a usual source of energy which is converted to the mechanical work. (**8p**)
2. Describe how a molecular machine with a design illustrated below can generate mechanical force. If we want it to pull a load, on which side the load should be attached. What are the factors limiting its maximum speed and how it will depend on the size of the "moving" parts, solution properties and temperature? (**8p**)



Question 5: Bionanosensors

Describe consequences of size which you have to consider while designing nanotechnology based sensors which are to function in aqueous, biological environment. Consider fluid properties, thermal motion and interactions between objects with a nm-size. (**14p**)

Appendix A: Equation Sheet

$$k_B = 1.38 \times 10^{-23}\text{J K}^{-1} \quad (1)$$
$$e = 1.6 \times 10^{-19}\text{coul.} \quad (2)$$
$$\varepsilon_0 = 8.9 \times 10^{-12}\text{F m}^{-1} \quad (3)$$
$$v_{drift} = \frac{f}{\xi} \quad (4)$$
$$\xi = 6\pi\eta R \quad (5)$$
$$\xi D = k_B T \quad (6)$$
$$\lambda_X = \sqrt{2Dt} \quad (7)$$
$$\lambda_{3D} = \sqrt{6Dt} \quad (8)$$
$$\left\langle r^2 \right\rangle = N L_{seg}^2 \quad (9)$$
$$D = \frac{1}{\tau}\int_{-\infty}^{\infty} \frac{\Delta^2}{2}\rho(\Delta)d\Delta \quad (10)$$
$$2D\tau = \left\langle \Delta^2 \right\rangle \quad (11)$$
$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \quad (12)$$
$$j_s = -D\frac{\partial c}{\partial x} \quad (13)$$
$$\frac{\partial c}{\partial t} = -\frac{\partial j}{\partial x} \quad (14)$$
$$j_s = -P_s \Delta c \quad (15)$$
$$\frac{\partial c}{\partial t} = D\nabla^2 c \quad (16)$$
$$\vec{j} = -D\nabla c \quad (17)$$
$$c(\vec{r}, t) = \frac{N}{(4\pi Dt)^{3/2}} e^{-\frac{r^2}{4Dt}} \quad (18)$$
$$j = D\left(-\frac{dc}{dx} + \frac{q}{k_B T}\varepsilon c\right) \quad (19)$$
$$\Delta\left[\ln c\right] = -\frac{q}{k_B T}\Delta V \quad (20)$$
$$c\left(z\right) = Ce^{\frac{-m_{eff} g z}{k_B T}} \quad (21)$$
$$j\left(r\right) = D\left(-\frac{dc}{dr} + \frac{r\omega^2 m_{eff}}{k_B T}c(r)\right) \quad (22)$$
$$c(r) = Ce^{\frac{m_{net}\omega^2 r^2}{2k_B T}} \quad (23)$$
$$v_{crit} = \frac{\eta}{\rho R} \quad (24)$$
$$f_{crit} = \frac{\eta^2}{\rho_m} \quad (25)$$
$$f_{fric} = \frac{\eta \ell^3 v}{R^2} \quad (26)$$
$$f_{inert} = \frac{\rho_m \ell^3 v^2}{R} \quad (27)$$
$$\Re = \frac{vR\rho}{\eta} \quad (28)$$
$$\frac{f}{A} = -G\frac{\Delta z}{d} \quad (29)$$
$$\frac{f}{A} = -\eta\frac{v}{d} \quad (30)$$
$$Q = \frac{\pi R^4 p}{8L\eta} \quad (31)$$
$$\frac{k_B T}{2} = \alpha\frac{\left\langle x^2 \right\rangle}{2} \quad (32)$$
$$S \equiv k_B \ln\Omega \quad (33)$$
$$T^{-1} = \left(\frac{dS}{dE}\right) \quad (34)$$
$$\Delta U = \Delta Q + \Delta W \quad (35)$$
$$\Delta S \geq \frac{\Delta Q}{T} \quad (36)$$
$$F_a \equiv E_a - TS_a \quad (37)$$
$$G_a \equiv E_a + pV_a - TS_a \quad (38)$$
$$\frac{P_1}{P_2} = e^{\frac{\Delta E}{k_B T}} \quad (39)$$
$$P_1 = \frac{1}{1 + e^{-\frac{\Delta E}{k_B T}}} \quad (40)$$
$$P_2 = \frac{1}{1 + e^{\frac{\Delta E}{k_B T}}} \quad (41)$$
$$\tau^{-1} = Ce^{\frac{-\Delta E^{\ddagger}}{k_B T}}\left(1 + e^{\frac{-\Delta E}{k_B T}}\right) \quad (42)$$
$$\Delta F = \Delta F_0 - f\Delta z \quad (43)$$
$$Z = \sum_j e^{-E_j/k_B T} \quad (44)$$
$$p_{equil} = c_{osm} k_B T \quad (45)$$
$$c_{osm} = \varphi M c \quad (46)$$
$$\Sigma = \frac{Rp}{2} \quad (47)$$
$$\ell_B \equiv \frac{e^2}{4\pi\varepsilon k_B T} \quad (48)$$
$$\overline{V}(x) = \frac{eV(x)}{k_B T} \quad (49)$$
$$c_+(x) = \frac{2\pi\ell_B\left(\frac{\sigma_q}{e}\right)^2}{\left(1 + 2\pi\ell_B\frac{\sigma_q}{e}x\right)^2} \quad (50)$$
$$x_0 = \left(\frac{e}{2\pi\ell_B\sigma_q}\right) \quad (51)$$
$$\frac{\mathrm{d}^2\overline{V}}{\mathrm{d}x^2} = -4\pi\ell_B c_0 e^{-\overline{V}} \quad (52)$$
$$\lambda_D = \left(8\pi\ell_B c_\infty\right)^{-\frac{1}{2}} \quad (53)$$
$$V(x) = -\frac{\sigma_q\lambda_D}{\varepsilon}e^{-\frac{x}{\lambda_D}} \quad (54)$$
$$f = 2k_B T b^2 r \quad (55)$$
$$b^2 \propto \frac{1}{nl^2} \quad (56)$$
$$\langle z/L_{tot}\rangle = \tanh\left(fL_{seg}^{(1d)}/k_B T\right) \quad (57)$$
$$\langle z/L_{tot}\rangle = \coth\left(fL_{seg}/k_B T\right) - \left(fL_{seg}/k_B T\right)^{-1} \quad (58)$$
$$\langle z/L_{tot}\rangle = \frac{\sinh\alpha}{\sqrt{\sinh^2\alpha + e^{-4\gamma}}} \quad (59)$$
$$\alpha \equiv \frac{f\ell}{k_B T} \quad (60)$$
$$j(x) = cv_{drift} - D\frac{\mathrm{d}c}{\mathrm{d}x} \quad (61)$$
$$j^{(1D)} = -MD\left(\frac{\mathrm{d}P}{\mathrm{d}x} + \frac{1}{k_B T}P\frac{\mathrm{d}U_{tot}}{\mathrm{d}x}\right) \quad (62)$$
$$v = \left(\frac{f\,L}{k_B T}\right)^2\frac{D}{L}\left(e^{fL/k_B T} - 1 - \frac{f\,L}{k_B T}\right)^{-1} \quad (63)$$

Question 1: Diffusion of small and large drug molecules

1. We use Einstein-Stokes equation to calculate the diffusion constant (equation 6). For 5nm particles:

$$\begin{aligned} \xi D &= k_B T \\ D &= \frac{k_B T}{\xi} \\ D &= \frac{k_B T}{6\pi\eta R} \\ D_{5nm} &= \frac{1.38\times10^{-23}\text{J K}^{-1}\cdot 293\text{K}}{6\pi\cdot 1\times10^{-3}\text{ kg m}^{-1}\text{s}^{-1}\cdot 5\times10^{-9}\text{ m}} \\ D_{5nm} &= 2.15\times10^{-10}\text{J kg}^{-1}\text{s}^{-1} = 4.3\times10^{-11}\text{kg m}^2\text{ s}^{-2}\text{ kg}^{-1}\text{s}^{-1} = 4.3\times10^{-11}\text{m}^2\text{s}^{-1} \end{aligned}$$

   For particles with other sizes, D can be determined easily since $D \propto R^{-1}$. So:

   - $D_{10nm} = D_{5nm}/2 = 2.15\times10^{-11}\text{m}^2\text{s}^{-1}$
   - $D_{50nm} = D_{5nm}/10 = 4.3\times10^{-12}\text{m}^2\text{s}^{-1}$
   - For C1: $D_{1nm} = D_{5nm}\cdot 5 = 2.15\times10^{-10}\text{m}^2\text{s}^{-1}$

2. At 37℃, η and T will change. At those conditions $D_{5nm} = 6.5\times10^{-11}\text{m}^2\text{s}^{-1}$ and $D_{1nm} = 3.2\times10^{-10}\text{m}^2\text{s}^{-1}$. This gives an increase of 51%.

3. For a model of the ECM, again η and T will change. To get the time needed for the particles to diffuse, we need first to calculate the diffusion constant. Then we can calculate average time which a particle need to diffuse a distance $\lambda_{3D} = 10\mu$m from equation 8.

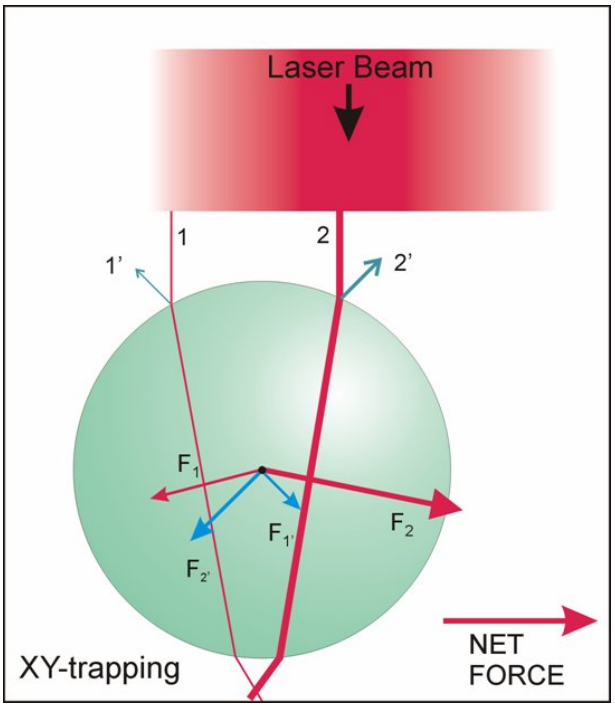
R [nm]	D [m^2s^{-1}]	t [s]
1	2.27E-011	7.34E-001
50	4.54E-013	3.67E+001

Question 2: Optical tweezers

1. Main components of a simple optcal tweezers setup: high power laser, microscope objective lens with a short focal length for focusing of the laser light into a small spot, holder for object to be trapped mounted in the focal plane of the objective lens, camera to observe trapped objects and the sample, some method of moving the focal point of the lase in the sample plane (mechanical or optical). Typically, setup is based on an optical inverted microscope.

   More advanced setups include presice particle position detection systems (especially important for force measurements), holographic steering of the trap position,

2. this can be explain considering the momentum of incoming photons - geometric optics. Only valid for particles with $R \gg \lambda$



3. If an external force is pulling on a particle in an optical trap, it will move away from the tarp centre,
4. In pure water, there will be diffuse charge layer close to the charged surface formed by the charges which has escaped from the surface and which make our system charge neutral. Moving two charged surfaces close together will result in compression of diffuse charge layers and therefore in a repulsive force which is entropic in nature. Energetically, all neutralising charges should be placed on the surface, but this result in a large decrease in the entropy and therefore increase in the free energy of the system. Free energy is at the minimum when charges form thin (few nm) diffuse layer. This layer will oppose compression and crate repulsive force at the length scale comparable with its thickness. In the pure water the diffuse charge layer is called Gouy-Chapman layer, and its thickness is given by equation 51.

   Once we add salt to the solution we will shrink the diffuse charge layer, therefore the repulsive force will be felt at shorther distance between particles. Shrinking of the charge layer can be explained as a consequence of lowering the gain in entropy by moving a charge away from the surface - there are other ions in the solution, so there is less configurational freedom for charges from the surface. Diffuse charge layer in a presence of salt is describe by Debye screening lengths λ_D given by equation 53. For 1M NaCl this lengths is below 0.5nm.

   Uncharged particle will experience depletion forces while they are in the slution containing protein molecules and no interaction should be observed in pure water (Van der Walls interactions will be important at a very short separation ≈ 0.2 nm). Depletion forces will be observed at the separation distance smaller then the size of protein particles - in this case at the separation below 2nm.

Question 3: Protein stability

1. Interaction crucial for protein stability
   - hydrophobic interactions
   - hydrogen bonding
   - charge-charge interactions (between aa with opposite charge)

2. FJC chain model
   - straight inextensible and not bendable segments connected by fully flexible joints
   - conformation and properties of a polymer chain can be described in terms of a random walk in 3D with a step size L_{seg}. For real polymers the situation is slightly different from a pure random walk because of the excluded volume effect - no more then one polymer chain segments can occupy any given position at any given time (in contrast to a particle doing a random walk which can "come back" to the same place in space) - see BP p 122.
3. This can be determined from force-extension curve recorded with optical tweezers or AFM, which is the fitted with 3D FJC model - equation 58. For optical tweezers the polymer chain has to be attached to two larger particles, one of which is held in place by mechanical constraint (for example a pipette) and the other one is moved by optical tweezers. The polymer will act as a mechanical spring, pulling the particles towards each other. The force can be measured as described above (Q2). For AFM, polymer chain is usually attached with one end to an AFM tip and with the other end to a sample surdace. Then it is stretched by moving the tip away from the surface, and the force is determined by observing bending of the AFM cantilever.

Question 4: Molecular Machines

1. active transport needs some form of mechanical work - ion pumping,creating concentration gradients, opposing diffusion and osmotic pressure. cells division, adhesion, movement, etc. also need mechanical energy. Muscles.
2. Energy stored in hinges which are presses down can converted to mechanical work. Central part will move by diffusion, and when a pressed down hinge wonders to the right of the obstruction it goes up, and therefore stops the central part from moving back to the left. When no load is attached, the maximum speed will be limited by diffusion of the central part - this is a function of its size, temperature and solution viscosity.

   If we want this machine to do mechanical work, load needs to be attached on the left side. In that case the speed of the motor will be determined by diffusion (Brownian motion) on a potential energy landscape (load is a source of that potential energy). That mootion in general is described by Smoluchowski equation 62 and for a machine in the figure the speed will depend on load force f as given by equation 63.

Question 5: Bionanosensors

- fluid properties at the nm scale are very different from mm scale, viscosity is dominating all behaviour, fluid flow is laminar with mixing occurring only through diffusion. Viscous drag force on a moving nano-particle is very large; even for large speeds the particles will stop as soon as there is no force to push them further (particle momentum can be neglected).
- depletion forces will make things sticky; electostatic interactions are short range due to screening;
- Brownian motion will cause small parts of the sensors to move, rotate and vibrate. Even rigid structures will undergo bending vibrations due to Brownian motion.
- diffusion - effective transport at the nm - μm range but very slow for longer distances;