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Soft Matter and Biological Physics

Question Sheet (Physics)

Michaelmas 2014

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Question 1 Optical tweezers calibration

Consider a dielectric, spherical particle with radius R (of order of 1 micron), mass m and friction coefficient γ immersed in water, which is held in an optical trap. Which are the main forces in an optical trap? The trapping potential can be approximated as harmonic. The potential is characterised by a trap stiffness κ and corresponding frequency f_c. Write down the Langevin equation in one dimension for this particle taking into account the thermal random force $\xi(t)$. Under which conditions can one neglect the inertial acceleration term in the Langevin equation?

With the the constant power spectrum of an ideal white noise source $S_\xi = |\xi(f)|^2 = 4\gamma k_B T$ calculate the power spectrum of the particle motion in the optical trap $S_x(f) = |x(f)|^2$. Briefly, describe two techniques to register the motion of the particle in the trap.
Sketch $S_x(f)$ for several values of κ and label all axis and relevant points.
What happens for frequencies $f > f_c$ with the power spectrum when the laser power is increased? Remember that part of the laser power is absorbed in the solution.

Question 2: Force measurements with magnetic tweezers

Sketch a magnetic tweezers setup pulling on a double-stranded DNA molecule. Briefly explain how the force on the magnetic particle is generated. Give the relevant formulae.

In order to detect the DNA stiffness, the force exerted by the magnetic field gradient has to be measured. Assume that the DNA-particle system is an inverted pendulum. The DNA can be considered as an entropic spring with end-to-end distance h pulling the particle back into its equilibrium position.

Derive an expression for the restoring force that depends on $1/h$. Using equipartition, the force can be calibrated. Explain how this is achieved in an experiment.

Describe a measurement protocol for measuring the force-extension relationship of a DNA molecule using magnetic tweezers.

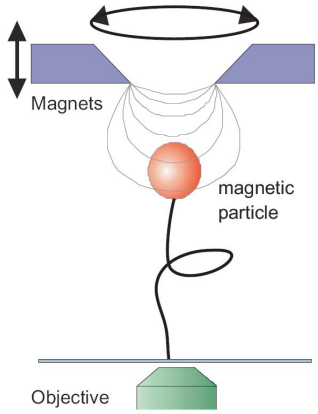
Question 3: Twisting DNA with magnetic tweezers

A double-stranded DNA molecule is stretched by magnetic tweezers as shown in the figure below. A constant force F is applied and the magnets can be rotated leading to a torque on the

DNA molecule. Upon twisting, elastic energy is stored in the DNA according to

$$E = \frac{k_B T C \vartheta^2}{2L}$$

where ϑ is the twist angle and $C \approx 100$ nm is the torsional persistence length of the DNA. After a certain number of turns n, with a total twist angle of $\vartheta = n2\pi$, the DNA molecule buckles and forms a small loop as shown below.



(i) Write down a general expression for the energy of such a loop considering the bending energy and the fact that DNA is stretched by a force F. Remember from the lecture that the energy to form a bend of $\pi/2$ is given by

$$E(R) = \frac{k_B T l_p}{2} \frac{\pi}{2R}$$

where R is the bending radius and l_p the persistence length.

(ii) What is the minimum energy cost required to form this loop? Start by taking the derivative of the expression derived in (i) with respect to the loop radius R. Compute the associated loop size for $F = 1$ pN. Assume a bending persistence length of $l_p = 50$ nm for DNA.

(iii) Calculate the energy increase associated with increasing the number of turns from n to $n + 1$.

(iv) The torque Γ on a DNA molecule is

$$\Gamma(n) = \frac{k_B T C}{L} 2\pi n$$

Assume that $n \gg 1$ and thus rewrite the expression derived in (iii) for the torsional energy in terms of torque. Use the new expression to obtain the buckling torque for DNA with $F = 1$ pN and a DNA contour length of 10,000 basepairs.

Question 4: Force dependence of protein-protein (un-)binding

The unbinding between two proteins can be interpreted as a chemical reaction with a rate

$$k \propto \exp\left(-\frac{G_S}{k_B T}\right)$$

where G_S is the free energy of the transition state S separating the bound and unbound states. The dissociation can be studied using constant hydrodynamic shear forces on a colloidal particle attached to one protein while the other protein is connected to a surface.Assuming that a constant force F can be applied, sketch the energy as a function of separation distance with and without the force, indicating the bound and unbound states as well as the position of the transition state S.

Hence show that the dissociation rate varies with the force as

$$k(F) = k_0 \exp\left(\frac{F}{F_0}\right)$$

defining F_0 and k_0.

Question 5: Loading rate dependence of protein unbinding

Protein-protein binding interactions can be studied using an atomic force microscope to apply a pulling force that increases in time as $F(t) = ft$, where f is a constant loading rate. Sketch the experimental situation.

The probability $p(t)$ to find the proteins in the bound state as time t is given by

$$\frac{dp(t)}{dt} = -k(t)p(t) + k_+(t)(1 - p(t))$$

where k_+ denotes the association constant. Explain why k_+ can be ignored in this experimental situation.

For the case where $F(t) = ft$, find an expression for $p(F)$ and sketch it for a few values of f.

Find an expression for the force $F_{1/2}$ at which half the proteins are unbound.

Question 6: Electro-osmotic flow

Assume that the surface of a cylindrical capillary of radius r is charged and has a fixed surface potential $\zeta < 0$. Under the assumption that $r \gg \lambda$ show that the fluid velocity v of the electro-osmotic flow in the centre of the capillary can be written as

$$v = -\frac{\epsilon_0 \epsilon_r \zeta E}{\eta}$$

where η is the fluid viscosity and E the applied electric field along the capillary. Explain why this velocity does not depend on r.

When should v depend on the nanopore radius r?

Question 7: Gel Electrophoresis [1]

A flexible polymer with N Kuhn segments of length b is moving inside a gel. The gel fibers are

[1] Part of this question is based on Zimm and Levene, "Problems and prospects in the theory of gel electrophoresis of DNA" Quart. Rev. Biophys (1992). Can be downloaded from the course webpage.

spaced far enough apart to only marginally affect the conformation of the polymer chain.

(i) Assume that the polymer has a drag coefficient of $\gamma = \eta N b$ in the gel, with η the viscosity of water. Find an expression for the time τ it takes for the polymer to diffuse a distance equal to its contour length $L = Nb$. Using this expression, estimate τ for double-stranded DNA ($b = 100$ nm) with a length of 30,000 basepairs.

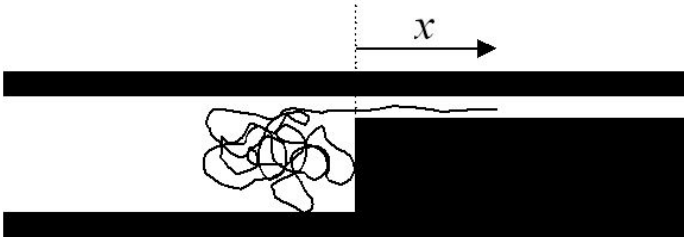
(ii) Now we apply an uniform electric field E in the gel which leads to a total force $F = fN$ on the polymer. Assuming a purely reptation-like motion, show that the drift velocity in the gel is

$$v_d = \frac{f}{\eta b N}.$$

Estimate the electric field E that you need to drive DNA molecules with 30,000 basepairs through a gel of 10 cm length in 1 hour. Calculate the distance DNA molecules with 25,000 basepairs would have traveled in the same amount of time. In both cases you may assume that the DNA has a charge of $600e$ per 100 nm segment.

Question 8: Polymers in Confinement

Consider an experiment in which a long piece of a charged polymer, with charge per unit length ρ, is situated in front of a narrow constriction, as illustrated in the sketch below. The polymer can only enter the narrow channel by adopting a straight configuration. An electric field E in the narrow part of the channel tries to pull the polymer inside the channel. The difference in entropy gives rise to an average waiting time in front of the narrow channel of the form $t = t_0 \exp(\Delta G^*/k_B T)$, where t_0 is a constant and ΔG^* is the height of the free energy barrier.



(i) Calculate the change in electrostatic potential energy $\Delta U(x)$ of the polymer when it enters the channel.

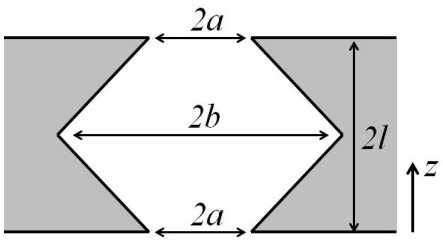
(ii) The corresponding increase of entropy is $\Delta S(x) = -\gamma x$, where γ is an experimentally determined constant. Give an argument why this is the correct sign and dependence on x. Basic thermodynamics will do the job.

(iii) Using the results obtained in (i) and (ii) calculate ΔG^* as a function of T, ρ, E and γ.

(iv) Discuss the temperature dependence of ΔG^*.

Question 9: Nanopores

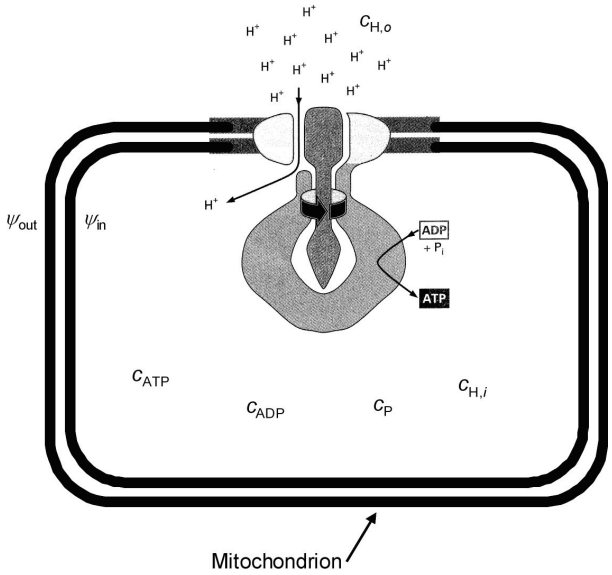
Solid-state nanopores can be used to analyze single molecules in aqueous salt solutions. Briefly summarize the principles of resistive-pulse analysis of transport through nanopores.
Consider an axially symmetric nanopore with its thickness and profile illustrated below.



The total length of the nanopore is $2l$, and the smallest and largest radii are r and R, respectively. Assuming that the surface of the nanopore is uncharged, calculate the resistance of such nanopore, $\mathcal{R}$, between $z = 0$ and $z = 2l$, when it is immersed in an ionic solution of resistivity ρ.
Use your calculation for $\mathcal{R}$ to calculate the electric field $E(z)$ along the centre of the nanopore. Sketch your result for $E(z)$ between 0 and $2l$.
On one side of the nanopore charged polymers are added to the reservoir. The applied electric field drives single polymers through the nanopore. The polymer contour length is assumed to be smaller than $2l$ and R. Sketch the ionic current as a function of time when a single polymer is passing the nanopore.

Question 10: Thermodynamics of the ATP synthase molecular motor

The F_0F_1 ATP synthase rotary motor is a protein complex found in the inner membrane of mitochondria. It converts one ADP molecule into one ATP molecule and water. The necessary free energy is obtained from a flow of protons across the membrane:



(i) Suppose that N protons must cross the membrane to convert one ADP molecule and one phosphate into ATP. For a process with no entropy change $\Delta S = 0$, derive an expression for the standard free energy ΔG^0 stored per ATP molecule. The relevant quantities for this are N, the membrane potential $\psi_m = \psi_{in} - \psi_{out}$ and the following concentrations: protons inside $c_{H,i}$ and outside $c_{H,o}$ the mitochondria, ATP and ADP inside the mitochondria c_{ATP} and c_{ADP} and phosphate inside c_p.

(ii) In (i) we assumed $\Delta S = 0$ for the process. If this is not true anymore, is your answer to (i) a lower or an upper bound for ΔG^0? Justify your answer.

Question 11: Bacterial motor proteins

Consider a rotary motor in the cell wall of bacteria. Describe the significance of the lipid membrane, the membrane potential and the proton gradient across the membrane for a rotary motor driven by protons.

Consider this rotary motor driven by a proton gradient at room temperature. The total chemical potential gradient over the membrane is $\Delta\mu_p$. Assuming a typical membrane potential $\Delta\Psi \approx -120$ mV and internal pH=$-\log_{10}[H_i]$ of 7.7, calculate at which pH value outside the proton gradient is compensated by the membrane potential, resulting in $\Delta\mu_p = 0$.

On passage of a proton through the membrane the motor can adopt one of two possible configurations, $(+)$ or $(-)$, which have free energy G_+ and G_-, respectively. These configurations lead to rotation in the anti-clockwise $(+)$ or clockwise $(-)$ directions, respectively. At $T = 37°$ C, the probability for the motor to rotate in the $(+)$-direction, for each proton, is $p_+ \approx 0.01$ (i.e., the probability $p_- \approx 0.99$ it will rotate clockwise). Estimate the energy difference G_+ and G_-.