Question 1 Optical tweezers calibration
Consider a dielectric, spherical particle with radius \( R \) (of order of 1 micron), mass \( m \) and friction coefficient \( \gamma \) 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 \( \kappa \) 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 \( \kappa \) 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 \( \vartheta \) is the twist angle and \( C \approx 100 \text{ nm} \) 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

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

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 \( F = 1 \text{ pN} \). Assume a bending persistence length of \( l_p = 50 \text{ nm} \) for DNA.

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

(iv) The torque \( \Gamma \) 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 \( F = 1 \text{ pN} \) and a DNA contour length of 10,000 basepairs.

**Question 4:** Enzyme DNA interaction studied using magnetic tweezers (Exam January 2013)
Using magnetic tweezers, a double-stranded DNA molecule is twisted and forms plectonemes at constant force $F_z$. An enzyme is now added, cutting one strand of the DNA and allowing plectonemes to unwind freely. During unwinding, the distance between the magnetic particle (radius 1.0 $\mu$m) and the surface increases at constant speed of 10.5 $\mu$m$^{-1}$. The persistence length of the DNA is 50 nm. Sketch the experiment. Calculate $F_z$ and hence find the radius of the plectonemes. The viscosity of water is $8.9 \times 10^{-4}$ Ns$m^{-2}$.

In a second experiment, a different enzyme is used to cut the DNA and the speed of the particle is reduced to 4.1 $\mu$m$^{-1}$. Estimate the energy lost due to friction between the DNA and the enzyme per turn. Discuss what may lead to this additional dissipation.

**Question 5**: Transition state theory for protein folding

Protein folding can be described as a progression through intermediate folded states. The folding rate $k$ should approximately follow an Arrhenius-type behavior

$$k = \nu_0 \exp\left(-\frac{\Delta G}{k_BT}\right)$$

The aim is to calculate the pre-factor $\nu_0$.

Below you find a representation of a system with states $A$ and $B$ separated by an energy barrier at the transition state $S$.

![Diagram of a system with states A and B separated by an energy barrier at the transition state S.](image)

The arrows denote the reaction pathways, which are irreversible within the region $\delta$. Assuming that there is a total concentration $[A]$ of molecules in state $A$, $N_+$ denotes the part of molecules going towards $B$ while $N_-$ denotes the reverse pathway. Show that

$$\frac{dN}{dt} = N_+ \frac{v}{\delta} = \frac{1}{2} k[A] \frac{v}{\delta}$$

where $v$ is the average thermal drift velocity.

**Hint**: Write down an equation for the total particle density around $S$ and assume that the system is in equilibrium.

A good approximation for the partition function of a mixture of $i$ species is given by

$$Z = \prod_i \frac{Z_i^{N_i}}{N_i!}$$
where $Z_i$ is the single particle partition function of species $i$. Thus show that the chemical potential for species $j$ is given by

$$
\mu_j = -k_B T \ln \left( \frac{Z_j \exp(-E_j/k_B T)}{N_j} \right)
$$

The additional Boltzmann factor originates from the fact that each molecule has an energy $E_j$ with respect to the lowest energy state.

Assuming that $\mu_A = \mu_S$ calculate the reaction rate $k$ as a function of $Z_S$, $Z_A$, and $E_0 = E_A - E_S$.

Finally, evaluation of $Z_S$ is necessary. Motion from $A$ to $B$ is decoupled for all trajectories perpendicular to the path and thus $Z_S = Z_* Z_r$, where $Z_*$ is the partition function of perpendicular degrees of freedom. Calculate $Z_r$ by integrating

$$
Z_r = \frac{1}{h} \int_{-\delta/2}^{\delta/2} dx \int_{-\infty}^{+\infty} dp \exp \left[ -\frac{p^2}{2m_r k_B T} \right]
$$

here $m_r$ is the reduced mass in the potential landscape around the transition state and $p = m_r v$ is the corresponding momentum.

Using that $v = \sqrt{2k_B T/\pi m_r}$ we now can write down $\nu_0$ as seen in the lectures.

**Question 6**: Force dependence of protein-protein (un-)binding (Exam 2013)

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 7**: 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 8**: 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

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

where $\eta$ 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 9**: Gel Electrophoresis
A flexible polymer with $N$ Kuhn segments of length $b$ is moving inside a gel. The gel fibers are spaced far enough apart to only marginally affect the conformation of the polymer chain.

(i) Assume that the polymer has a drag coefficient of $\gamma = \eta Nb$ in the gel, with $\eta$ the viscosity of water. Find an expression for the time $\tau$ it takes for the polymer to diffuse a distance equal to its contour length $L = Nb$. Using this expression, estimate $\tau$ 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 10**: Polymers in Confinement
Consider an experiment in which a long piece of a charged polymer, with charge per unit length $\rho$, is situated in front of a narrow constriction, as illustrated in the sketch below. The polymer

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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 \( \Delta G^* \) 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 \( \gamma \) 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 \( \Delta G^* \) as a function of \( T, \rho, E \) and \( \gamma \).

(iv) Discuss the temperature dependence of \( \Delta G^* \).

**Question 11: Nanopores**

(i) Consider a capillary tube with radius \( r \), much larger than the Debye-Huckel screening length \( \lambda \), containing monovalent dissociated salt ions of concentration \( c_0 = c_0^+ = c_0^- \). The solution has a resistivity \( \rho \). The capillary has length \( l \) and connects two semi-infinite reservoirs. Calculate the total resistance of the capillary taking into account the access resistance given by \( \rho / 4r \).

Calculate the resistance of a nanopore in 0.1M KCl as a function of radius for a membrane of 20 nm thickness. Plot the resistance between 0 and 100 nm pore diameter.

(ii) Assume that electrodes for the ionic current measurements have to be placed in a microfluidic channel on either side of the nanopore with length 1 mm and cross section 10 by 10 \( \mu \)m\(^2\). How small does the nanopore has to be so that its resistance is 10 times larger than the resistances of the channels connecting the nanopore with the electrodes? The resistivity of 0.1M KCl at 20°C is \( \sim 0.856 \) \( \Omega \)m.

(iii) Derive the total resistance for a conical capillary with different radii at both its ends \( r_1 \) and \( r_2 \), where \( r_1 > r_2 \). Sketch the electric field and potential for the cylindrical case.

(iv) In a real experiment, the nanopore surface will be charged in solution. Consider the resistance of the nanopore upon changing the salt concentration in the reservoirs. Sketch (not calculate) the resistance of the nanopore as a function of salt concentration from 1M to 0M KCl.
**Question 12: Polymer Translocation**

Compare the translocation time for the freely-jointed and the worm-like polymer chain models. Both molecules have constant charge per segment and are pulled through a nanopore by electrophoretic forces. The chains are very long compared to the nanopore length. Calculate the drag experienced by the polymer coil shrinking in front of the nanopore by assuming that the translocation time is much shorter than all relaxation times of the polymer. How does the translocation time depend on polymer length for the two chain models?

**Question 13: Nanopores II (Exam January 2012)**

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, $R$, between $z = 0$ and $z = 2l$, when it is immersed in an ionic solution of resistivity $\rho$.

Use your calculation for $R$ 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.

The average velocity $v$ of the polymer is given by

$$ v = \frac{dz}{dt} = \mu E(z) $$

where $\mu$ is the polymer’s electrophoretic mobility. Show that the time $\Delta t$ between the polymer entering and leaving the cavity is

$$ \Delta t \propto \frac{2 \pi l r^3 - R^3}{3 \mu \frac{r - R}{r}}. $$

For polymers with contour length $L \gg 2l$ one finds that $\Delta t$ is smaller than expected, while for polymers with contour length $L \ll 2l$ data shows a longer $\Delta t$. What could give rise to these experimental results?

**Question 14: Thermodynamics of the ATP synthase molecular motor**
The F$_0$F$_1$ 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 $\Delta G^0$ 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 $\Delta G^0$? Justify your answer.

**Question 15:** Bacterial motor proteins (Exam January 2012)

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 $T = 37^o$ 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 $G_-$. Several helical filaments bundle together to form a superhelical flagellum. However, the individual filaments have a range of different lengths $S$. The superhelix of the flagellum (see figure below) has an optimal radius $R$ depending on the maximum difference in length of the filaments $S_o - S_i$, where $S_o$ is the length of the outer filament at $R + D/2$ and $S_i$ of the inner filament at
$R - D/2$. Show that $R$ is given by

$$\frac{S_o^2 - S_i^2}{S_o^2 + S_i^2} = \frac{DR}{R^2 + p^2},$$

where $2\pi p$ is the helical pitch and $D$ the diameter of the assembled filaments. You may assume that $p, R \gg D$. (Hint: A helix can be parameterised by $x = R\sin(\phi t), y = R\cos(\phi t)$ and $z = \phi t$.)

At low Reynolds number, the time-dependent torque $M(t)$ of the motor can be described by the following equation

$$M(t) = -\gamma r \omega(t)$$

where $\xi(t)$ is the thermal noise, $\omega(t)$ the angular velocity and $\gamma r$ the rotational friction coefficient of the flagellum. Estimate the energy delivered by the proton driving the motor, and argue which of the two terms can be safely neglected. Describe an experimental procedure using magnetic tweezers to measure the torque $M$ when $\gamma r$ is unknown.

**Revision Question**: Linearized Poisson-Boltzmann Equation (Exam January 2011)

Consider a charged surface in aqueous solution with surface potential $\phi_0$. The surface is immersed in a solvent containing monovalent dissociated salt ions of concentration $c_0 = c_0^+ = c_0^-$. Solve the Poisson-Boltzmann equation for the electrostatic potential $\phi(x)$ in this geometry and thus derive the formula for the Debye-Huckel screening length $\lambda$ in the limit of small surface potential $e\phi_0 \ll k_BT$. Discuss the dependence of the screening length on $c_0$. Sketch and discuss the distribution of the positive and negative ions close to the surface.

A second, identical surface is now held in close proximity to the first surface at a distance $D$. Find a solution for the Poisson-Boltzmann equation again in the limit of $e\phi_0 \ll k_BT$. The calculation is simplified if you place the origin in the centre between the two surfaces. Make a sketch of the potential between the surfaces.

What happens if $D \to \infty$ and $D \to 0$ with $\phi(x = 0)$.

By calculating the total electrostatic energy per unit area of this ionic solution between the surfaces, $E(D)$, find the added pressure between them.

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- **Bacterium**
- **Superhelical flagellum**
- **Diagram**: Bacterium and superhelical flagellum with labels $R$, $2\pi p$, and $D$.