Electro kinetic Phenomena

- Electro-osmosis
- Electrophoresis
- Gel electrophoresis, polymer dynamics in gels

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Electric Double Layer

In aqueous solutions we have to deal with situations where every surface is charged. Not only the constituents (proteins, molecules, DNA, metals or any other surface) but also the water molecules are charged. Water can dissociate into $\text{H}_3\text{O}^+$ and $\text{OH}^-$ and at pH=7, there are $10^{-7}$ M of both ions present in solution. Interactions, van der Walls, hydrophobic, chemical and Coulomb, give rise to a structure close to any charged surface, composed of ions and water, known as the **electric double layer** (EDL). The EDL determines the behaviour of charged polymers or biological molecules in aqueous solutions.

http://www.damtp.cam.ac.uk/user/gold/teaching_biologylll.html
**Full Electric Double Layer**

One of the complications of the structure of the EDL is the complex structure of the polar water molecules which usually form a hydration shell around ions or macromolecules in aqueous solution. However, in close proximity to any surface other interactions like the van der Waals hydrophobic or even chemical interactions can disrupt the hydration layer and then give rise to the complex structure of the EDL. Although this is beyond the scope of this course it is useful to remember that in a realistic situation the exact structure of the electric double layer will be determined by all molecular interactions. The ions closest or adsorbed on the surface are often regarded as bound, however they are still in equilibrium with the surrounding medium. The EDL can be decomposed into the **Outer Helmholtz** or **Stern** layer and the inner Helmholtz layer. The latter is composed of adsorbed ions that partly lost their hydration shell and may carry the same charge as the surface.

Adapted from Hamann and Vielstich
Electrokinetic Effects

The EDL is one of the key elements for all following discussions. Surfaces i.e. particles that are hydrophilic are usually charged in aqueous solutions. We will now discuss two closely related electrokinetic phenomena, *electrophoresis and electro-osmosis*, which are due applying an electric field to the system. We will discuss both electrokinetic effects in parallel as an understanding is only possible taking into account both effects.

*Adapted from Hamann and Vielstich*

**Electrophoresis/-osmosis**

*Electrophoretic (EP)* movement of negative particle due to electrophoresis

*Electro osmotic flow (EOF)* of oppositely (positively) charged counter ions

[http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html](http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html)
EOF: Visualization of electro-osmotic flow

Visualization performed using a molecular tagging technique (caged fluorescence visualization) and shows the reduced sample dispersion for (a) EOF in a capillary with a rectangular cross section 200 mm wide and 9 mm deep; (b) pressure-driven flow in a rectangular cross-section 250 mm wide and 70 μm deep.

http://microfluidics.stanford.edu/Projects/Archive/caged.htm

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Optical Tweezers: Single particle electrophoresis

We will start with an investigation of single particle electrophoresis. In order to study the electrophoretic mobility, of a single particle, optical tweezers are ideal candidates as they not only allow to follow the movement of the particle in the electric field but also can determine the forces acting on the particle. This is a unique feature allowing for a complete understanding of the system.

Otto (2008)

Here, we will more discuss an experimental realization that uses several of the approaches we discussed earlier in the course. The position of the particle will be monitored by single particle tracking with video microscopy, while the forces are determined by analysis of the power spectrum. The main trick employed here is to move the particle with an alternating field allowing to determine the motion of the particle even when the amplitude is smaller than Brownian fluctuations.

http://www.damtp.cam.ac.uk/user/gold/teaching_biologyIIII.html
Microfluidic Cell Design *

To make interpretation of the experimental results straightforward it is worth to discuss and rationalize the experimental geometry. We want to examine and determine the mobility in a homogeneous electric field of known magnitude. This can be achieved in practice by designing a long and relatively thin channel connecting two fluidic reservoirs on either end. A schematic is shown in the circle on the left. The advantage of this geometry is that we can easily calculate the electric field distribution.

In the extreme case of a very long channel we would expect that the applied voltage $U$ over the channel leads to an electric field $E$ given by

$$E = \frac{U}{l}$$

Where $l$ is the length of the channel. Here we assume that the material surrounding the channels does not have a finite dielectric constant and ignore entrance effect.

http://www.damtp.cam.ac.uk/user/gold/teaching_biology.html
**Fluidic Cell Design – Field Distribution** *

Using numerical simulations we can test our simple description. After applying a voltage $U$ and calculating the electric field distribution in this channel with an aspect ration of 100, we find that the electric field is close to the expected value of $E=4.2 \text{ V/cm}$. The main deviation is due to the electric field extending into the channel at the entrance.

![Simulated potential drop](image1.png) ![Electric field distribution](image2.png)

Figure 3.4: Simulated potential drop (left) and electric field distribution (right) within a fluidic cell having a long channel ($l = 30 \text{ mm}$, $d = 300 \mu\text{m}$). The finite-element calculations assumed an external voltage of $U_{\text{appt}} = 12.6 \text{ V}$. Both graphs show a longitudinal cut along the middle axis of the cell. More than 90% of the applied potential drops along the channel which results in a very uniform electric field $E_{\text{Channel}}$ varying less than 2% within a length of 10 mm in the middle of the channel.

[http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html](http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html)
For a typical measurement, the particle is subject to an electric field with applied voltages of up to 60 V. One annoying complication of these high voltages is the electrochemical decomposition of water into $\text{H}_2$ and $\text{O}_2$ at the electrodes. However, over short time scales (few seconds) the oscillatory motion of the particle due to the electrophoretic force can be detected giving rise to a nice oscillation. The Brownian fluctuations of the particle in the trap are readily visible even at these relatively high forces. We can detect forces around 1-2 pN easily.

The decomposition of water limits the applicability of high voltages in this type of measurement. One solution is to use again the frequency analysis using the Fourier transforms we discussed earlier in the context of force calibration.

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Optical Tweezers: Detect forces in fN – range

Applying lower voltages and thus lower electric fields pushes the movement of the particle below the fluctuations of the thermal fluctuations in the trap.

However, due to the well-defined frequency we should be able to determine the amplitude of the motion by analysing the data on the left using the amplitude spectrum. We would expect to observe a clear peak at the frequency given by the AC voltage.

http://www.damtp.cam.ac.uk/user/gold/teaching_biphoticsIII.html
Detect forces in fN – range *

Amplitude peak indicates electrophoretic motion of particle in optical trap
Electrophoretic Force depends linearly on Voltage

With this simple approach we can easily detect 50 femto Newton forces on the particles. One obvious expectation would be that the maximum force should depend linearly on the applied voltage (electric field) and this is exactly what we find. The reason for the high resolution despite the considerable Brownian fluctuations is that we average over many periods in our signal and thus see even smallest amplitudes in the amplitude spectrum.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Charge Inversion is Possible *

Another important parameter that we can get from this type of measurement is the polarity of the charge of the particle. The phase of the motion with respect to the applied AC field indicates directly the apparent charge of the particle. The complexity of the system is largely increased if the valency of the counter-ions is increased. If we add highly charged ions to the solution we observe at certain concentrations a reversal of the particle charge from being negative, as expected, to positive. This is known as charge inversion. Charge inversion of biological molecules (e.g. DNA) is relevant for problems like DNA packing and condensation in viruses and even in living cells.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Practical application: Gel Electrophoresis

After the brief discussion on electrokinetic effects, we will now introduce gel electrophoresis. Gel electrophoresis is one of the most important techniques for the characterization of biomolecules (proteins, DNA, RNA) based on electrophoretic movement of polymers in a matrix of almost uncharged molecules forming a porous structure also called “gel”. The main purpose is to sort molecules by their molecular weight employing their charge. Due to the presence of the gel we do not have to take into account complications arising from electro-osmotic flows as the gels fibers effectively stop any major electrically driven fluid flows in the system.
One example for a gels: Agarose

In order to stop electro-osmotic flows and enable sorting of polymers by their molecular mass (length) one can form entangled polymer gels. Mixing Agarose monomers heating them to around 100\(^{\circ}\)C and cooling them down to room temperature, they form a network of small pores as shown in the electron micrograph above. The density and distance of the polymers forming the mesh of pores can be easily tuned by the amount of agarose in the solution. The mesh can be regarded as very similar to concentrated polymer solutions.

The movement of polymers in this mesh can be interpreted as driven diffusion due to an externally applied electric field. The mobility of polyelectrolytes is controlled by the effective pore diameters and is tuned with regards to the length of the polymers to be sorted.

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Polymer Dynamics in Gel Electrophoresis

- **Rouse** – polymer is string of $N$ beads with radius $R$, is moving freely through chain (free draining, solvent not relevant)
  - Friction coefficient: $N\gamma$
  - Curve-linear diffusion coefficient: $D_R = \frac{k_B T}{N\gamma}$
- Rouse time $\tau_R \Leftrightarrow$ time polymer diffuses over distance equal to its end-to-end distance $R_N$
- For any chain one gets:
  $$\tau_R = \frac{1}{6\pi^2} \frac{\gamma b^2}{k_B T} N^{1+2\nu} \approx \tau_0 N^{1+2\nu}$$
- Characteristic time for monomer in an ideal chain:
  $$\tau_0 \approx \frac{\gamma b^2}{k_B T} \Rightarrow \tau_R \approx N^2 \tau_0$$
- Problems with Rouse model include: unrealistic hydrodynamics, no knots

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Polymer Dynamics

- **Zimm** – similar to Rouse model but solvent moves with chain (no slip on chain), long range hydrodynamic interactions, so we have now typical size of segments $R$ and viscosity of solvent $\eta$. The total friction coefficient of the chain is then given by size of the chain $R$ and depends on the fluid viscosity.

- Stokes friction (neglecting all pre-factors of order 1 as usual):
  \[ \gamma \approx \eta R \]

- With Stokes-Einstein we can then write down the Zimm diffusion coefficient:
  \[ D_Z = \frac{k_B T}{\eta R} \approx \frac{k_B T}{\eta b N^\nu} \]

- Remember: The exponent $\nu$ is depending on chain, $\nu = 0.5$ ideal, $\nu = 0.588 \approx 3/5$ self avoiding chain (Flory exponent– see Cicuta Soft matter course), $\nu = 1/3$ for collapsed chains

- Consequently the Zimm relaxation time is then $\tau_Z$:
  \[ \tau_Z \approx \frac{R^2}{D_Z} \approx \frac{\eta}{k_B T} R^3 \approx \frac{\eta b^3}{k_B T} N^{3\nu} \approx \tau_0 N^{3\nu} \]

- Main difference to Rouse is the weaker dependence on $N$ due to $\tau_0$.

- Rouse model works well in polymer melts and gels while Zimm is better for describing polymers in dilute solution.
Polymer Dynamics

- Sub-chains behave in the same way as the entire chain
- There are $N$ relaxation modes of the chain

$$\tau_p = \tau_0 \left( \frac{N}{p} \right)^2 \text{ with } p = 1, 2, \ldots, N$$

- Mean square displacement of a segment with $p$ monomers:

$$< |\mathbf{r}_j(\tau_p) - \mathbf{r}_j(0)|^2 > \approx b^2 \frac{N}{p} \approx b^2 \left( \frac{\tau_p}{\tau_0} \right)^{\frac{1}{2}}$$

- Mean square displacement of a monomer in chain with $N \gg 1$ for times $t < \tau_R$

$$< |\mathbf{r}_j(t) - \mathbf{r}_j(0)|^2 > \approx b^2 \left( \frac{t}{\tau_0} \right)^{\frac{1}{2}} \text{ for } \tau_0 < t < \tau_R$$
Polymer Dynamics

• Now compare to free diffusion (Fick)

\[
< |\mathbf{r}_j(t) - \mathbf{r}_j(0)|^2 > \approx b^2 \left( \frac{t}{\tau_0} \right)^{1/2} \quad \text{for} \quad \tau_0 < t < \tau_R
\]

\[
< |\mathbf{r}(t) - \mathbf{r}(0)|^2 > 6Dt
\]

• Conclusion: Rouse mean-square displacement is sub-diffusive

• With Zimm model we get a slightly different answer in the exponent:

\[
< |\mathbf{r}_j(t) - \mathbf{r}_j(0)|^2 > \approx b^2 \left( \frac{t}{\tau_0} \right)^{2/3} \quad \text{for} \quad \tau_0 < t < \tau_Z
\]
Polymer Dynamics

Both Zimm and Rouse models assume that the chain is free to move, completely independently of the others. In a gel the chain cannot move freely and is entangled in the gel fibres. The chain cannot cross the gel fibres. A very similar situation is found in high density polymer solutions.

- Idea (Sir Sam Edwards):
  chains are confined in a tube made of the fibres, tube has radius:

  \[ r_t \approx b\sqrt{N_e} \text{ with } N_e \text{ No. of monomers per entanglement} \]
  and \( r_t \) istheentanglementlength

- Coarse grained chain length is

  \[ R_0 \approx r_t \sqrt{\frac{N}{N_e}} \approx b\sqrt{N} \]

- Coarse grained contour length:

  \[ <L> \approx r_t \frac{N}{N_e} \approx \frac{b^2 N}{r_t} \approx \frac{bN}{\sqrt{N_e}} \]

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Polymer Dynamics

• Simplified picture in gel:

\[ R_0 \approx r_t \sqrt{\frac{N}{N_e}} \approx b \sqrt{N} \]

\[ < L > \approx r_t \frac{N}{N_e} \approx \frac{b^2 N}{r_t} \approx \frac{bN}{\sqrt{N_e}} \]

\[ r_t \approx b \sqrt{N_e} \text{ with } N_e \text{ No. of monomers per entanglement} \]

and \( r_t \) is the entanglement length

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Diffusion in Tube: “Reptation”

We can now use the models we discussed before to understand the diffusion in the gel. The diffusion coefficient in the tube is just given by the Rouse model \( D_R = D_C = k_B T / N \beta \).

The reptation time is the time to diffuse along the complete tube length

\[
\tau_{\text{rep}} \approx \frac{<L>^2}{D_C} \approx \frac{\beta b^2}{kT} \frac{N^3}{N_e} = \frac{\beta}{kT} \frac{b^2}{N_e} N_e^2 \left( \frac{N}{N_e} \right)^3
\]

The lower time limit for reptation is given for the Rouse mode with \( N=N_e \)

\[
\tau_e \approx \frac{\beta b^2}{k_B T} N_e^2
\]
1. For $t < \tau_e$, Rouse diffusion:
   \[ < |r_j(t) - r_j(0)|^2 > \approx b^2 \left( \frac{t}{\tau_0} \right)^{\frac{1}{2}} \]

2. For $\tau_e < t < \tau_R$, motion confined in tube $\Leftrightarrow$ displacement only along the tube, this is slower than unrestricted Rouse motion (as expected)
   \[ < |s_j(t) - s_j(0)|^2 > \approx b^2 \left( \frac{t}{\tau_0} \right)^{\frac{1}{2}} \approx r_t^2 \left( \frac{t}{\tau_e} \right)^{\frac{1}{2}} \]
   The tube itself is a random walk
   \[ < |r(t) - r(0)|^2 > \approx r_t \sqrt{< |s_j(t) - s_j(0)|^2 >} \approx r_t^2 \left( \frac{t}{\tau_e} \right)^{\frac{1}{4}} \]

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
3. For $\tau_R < t < \tau_{rep}$, motion of all segments is correlated, polymer diffuses along the tube

$$< |s(t) - s(0)|^2 > \approx D_c t \approx b^2 N \frac{t}{\tau_R} \approx r_t^2 \frac{N}{N_e} \frac{t}{\tau_R}$$

Random walk of tube is now

$$< |r(t) - r(0)|^2 > \approx r_t \sqrt{< |s_j(t) - s_j(0)|^2 >} \approx r_t^2 \left( \frac{N}{N_e} \right)^{\frac{1}{2}} \left( \frac{t}{\tau_R} \right)^{\frac{1}{2}}$$

4. For times much longer than the reptation time $t > \tau_{rep}$, free diffusion is recovered with the expected diffusion coefficient

$$< |r(t) - r(0)|^2 > 6D_{rep} t$$
Timescales in Gels

Four different regimes

1. For $\tau_0 < t < \tau_e$, \(< |r(t) - r(0)|^2 > \sim t^{1/2}$

2. For $\tau_e < t < \tau_R$, \(< |r(t) - r(0)|^2 > \sim t^{1/4}$

3. For $\tau_R < t < \tau_{rep}$, \(< |r(t) - r(0)|^2 > \sim t^{1/2}$

4. For $t > \tau_{rep}$, \(< |r(t) - r(0)|^2 > \sim t^{1}$

Polymers behave like simple liquids only when probed on time scales larger than the reptation time. On very short timescales polymer dynamics is slowed because of the connectivity of the chain segments (Rouse, Zimm), on intermediate time scales the slow-down arises from the entangled nature of the chains (reptation tube disengagement).
Gel Electrophoresis (EP)

• Reptation time is time to diffuse along its own length

• For experiments much longer than reptation time free diffusion (Fick) is recovered now with diffusion constant depending on length of the polymer
After discussing the dynamics in a gel we can now look at the experimental data. We would expect that the mobility depends also on distance of gel fibres (size of the pores the DNA has to migrate through), which is clearly observed in the range of molecular weights shown above. We would also expect that the drift velocity should inversely depend on the DNA length, which we find is true for this range of molecular weight.
Electric Field Dependence

At low electric fields the mobility is almost independent of the magnitude of the field. However, for fields bigger than 1V/cm nonlinearities occur for longer DNA molecules more pronounced than for shorter ones. In this regime the reptation Model breaks down due to “herniating” of the chains $\Leftrightarrow$ force on segments high enough to pull segments out of the reptation tube.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
The reptation model for gel electrophoresis works if the length of polymers is much longer than the Debye screening length. Typically, the DNA should be longer than several persistence lengths. Another important condition is that the chains have to be longer than the typical pore diameter in the gel, otherwise they can freely move through the gaps. Finally, for very long polymers, the reptation model also breaks down as trapping and knots become very important for the mobility and a simple, driven diffusive motion is not a good description any more.
Entropic Forces and Single Molecules

(Craighead et al. 2002)

Following our discussion of gel electrophoresis we briefly mentioned the trapping of long DNA molecules in voids in the gel. This is an interesting problem which can be studied in a more controlled geometry derived from nanotechnology, so called nanofluidic devices.

The aim is to follow the pathway of a single DNA molecule when it is partly trapped in a region with low entropy and at the same time is exposed to a region of high entropy as shown in the scheme on the right. This will allow us to determine the entropic forces acting on the molecule.

FIG. 1. The fluidic device consists of a quasi-two-dimensional gap between a floor and ceiling approximately 60 nm in height. Some regions of the device are populated with nanopillars.

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Entropic Forces and Single Molecules

(Craighead et al. 2002)

The „Nanofluidic“ device is made by „glueing“ two pieces of glass together with a distance of 60 nm. The pillars are separated by 160 nm, have an effective diameter of 35 nm, which yields an effective distance of 115 nm, which is roughly equal to two persistence lengths of each DNA molecule. All surfaces are negatively charged to reduce sticking of the DNA to the surfaces, this can be ensured by keeping the pH above 6 where glass is negatively charged.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
At the beginning of the experiment, DNA in solution is pulled into the pillar region by applying an electric field. The DNA is labeled with a fluorescent dye which makes it visible and easy to trace. The data shows that if part of the molecule is in the pillar-free region it recoils, otherwise it stays in the pillar region.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Entropic Recoiling of DNA reveals Entropic Force
(Craighead et al. 2002)

Following the trajectories of several molecules one can see that the curve follows a square root dependence. The spread in the data is what is expected for single molecule data in environments where $k_B T$ is the dominating energy scale.

These experiments allow to establish that entropy is a local quantity which affects the retraction only if a finite party of the molecule is in the high entropy region. However, the equilibrium position at infinite times would lead to all molecules ending up in the high entropy region. However, the diffusion in the pillar region is very slow on the time scale of the experiments and thus is not observed.
Solid-State Nanopores

- Resistive-pulse technique
- DNA translocation dynamics

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Resistive-Pulse Technique

The resistive-pulse technique was developed with the aim to develop a label-free counting technique for particles in (aqueous) solution. The main advantage of this technique is that it is possible to detect any analyte without labeling (chemically) altering the particles or molecules. Historically, this was first used for the counting of blood cells in samples (1953 patented by Coulter) and since then developed into a major technique for sizing and counting particles and cells.

Main idea: use orifice in glass with a diameter of tens of microns detecting particles down to several tenths of a micron by pressure driven flow. Typical applications include blood cell counting (1958) or bacterial cell counting, with special emphasis on cell-volume distributions.

- Tenths of micron diameter capillary
- particles with dimensions down to 60 nm can be detected
  - Virus counting
  - Bacteriophage particles (1977)

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Nanopore Fabrication

Detection limit obviously depends on the diameter of the orifice that is used. In the last decade the technique was further developed with the goal to count and analyze single polymers (DNA, RNA, proteins, etc.) in solution. Ideally detect not only the presence of the molecule but also the structure: bends, kinks, bound proteins, ...

Obvious challenge: the typical diameter of double-stranded DNA is only $\sim 2$ nm so standard technology is not good enough to control the orifice diameter. In teh following we wil call the orifice “nanopore”. For spatial resolution along the molecules one would also require the nanopore as short as possible. One solution is silicon-based nanotechnology.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Nanopore Fabrication

(Dekker, 2007)

Step 2: Drill small hole in free standing membrane with focused electron beam

Focused beam of an transmission electron microscope (TEM)

Image with transmission electron microscope

Nanopore with a diameter of ~10 nm

Free standing SiNx membrane with thickness of 20 nm

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Nanopore Fabrication

Step 3: Adjust diameter of nanopore by using the glassy characteristics of SiN$_x$

Focused beam of an transmission electron microscope (TEM)

Image with transmission electron microscope

Initial nanopore is elliptical with a diameter of ~20 nm

Final nanopore is circular with a diameter of ~5 nm

Sculpting at the nm-scale!

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Nanopore Fabrication

(Dekker, 2007)

Image with transmission electron microscope

Initial nanopore is elliptical with a diameter of ~20 nm

Final nanopore is circular with a diameter of ~5 nm

Sculpting at the nm-scale!

- SiNx is an amorphous (not crystalline) material
- Electron beam deposits energy into the sample
- Local temperature is increased and material can start to flow
- Surface tension wants to minimize free energy
- Free energy gain $\Delta F$ is just

$$\Delta F = \gamma \Delta A = 2\pi \gamma (rh - r^2)$$

$\gamma$ surface tension

$r$ pore radius

$h$ pore length

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Ionic Resistance of Nanopores

The simplest model for a nanopore is a cylinder filled with liquid. You can then write down the resistance $R_{\text{pore}}$ for the ionic current:

$$R_{\text{pore}} = \frac{1}{\sigma_{\text{KCl}}(T)} \left( \frac{h}{\pi r^2} \right)$$

$h$ = membrane thickness
$r$ = nanopore radius
$\sigma(T) = 1/\rho$ conductivity

However, if the membrane thickness is in the range of the diameter of the nanopore we have to take into account we have to take into account the so-called access resistance –field lines into the pore:

$$R_{\text{pore}} = \frac{1}{\sigma_{\text{KCl}}(T)} \left( \frac{h}{\pi r^2} \right)$$

$$R_{\text{access}} = \frac{1}{\sigma_{\text{KCl}}(T)} \left( \frac{1}{2r} \right) = 2 \times \frac{1}{\sigma_{\text{KCl}}(T)} \left( \frac{1}{4r} \right)$$

$$R_{\text{nanopore}} = \frac{1}{\sigma_{\text{KCl}}(T)} \left( \frac{h}{\pi r^2} + \frac{1}{2r} \right)$$

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Understanding Access Resistance

• Diffusive current $I$ (particles per second) to a perfect spherical absorber with radius $r$ is ($C=0$ at absorber and $C_0$ at infinity)

$$I = 4\pi DrC_0$$

• Thus for a hemisphere we get

$$I = 2\pi DrC_0$$

• It is interesting to note here that the diffusive current is proportional to $r$ and not to $r^2$. The reason is that as $r$ increases the surface increases as $r^2$ but the gradient is getting smaller as $1/r$

http://www.damtp.cam.ac.uk/user/gold/teaching_biologyphysicsIII.html
Understanding Access Resistance

One of the simplest ways to understand origin of the access resistance can be obtained by looking at diffusive current driven by concentration gradients.

Assuming that there is a diffusive current $I$ (particles per second) to a perfect disk like absorber with radius $r$ is ($C=0$ at absorber and $C_0$ at infinity) one can calculate the current as

$$I = 4DrC_0$$

We can the easily conclude that the diffusive current through a hole (assuming it is a perfect absorber) with radius $r$ in a membrane of zero thickness is thus just half the above value

$$I = 2DrC_0$$

It is interesting to note here that the diffusive current is proportional to $r$ and not to $r^2$. The reason for the dependence on the radius is that as $r$ increases the surface increases as $r^2$ but the gradient is getting smaller as $1/r$. This absolutely equivalent to the situation in driven ionic current when the concentration gradient is replaced by the driving potential.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Access Resistance

• For driven ionic currents through nanopores we are able to simply rewrite the equations for the diffusion current on the preceding pages by changing concentration gradient into $\Delta V$, diffusion constant $D$ into ionic conductivity $\sigma(T)$ and thus write

Resistance of a hemisphere:  
$$
R = \frac{1}{2\pi \sigma(T) r} = \frac{\rho(T)}{2\pi r}
$$

Resistance of a circular absorber:  
$$
R = \frac{1}{4\sigma(T) r} = \frac{\rho(T)}{4r}
$$

Resistance of a circular pore:  
$$
R = \frac{1}{2\sigma(T) r} = \frac{\rho(T)}{2r}
$$

• This explains the additional term in the nanopore resistance, could be also interpreted as enhanced length of the nanopore with implications for the spatial resolution of sensing applications

$$
R_{\text{nanopore}} = \frac{1}{\sigma_{KCl}(T)} \left( \frac{h}{\pi r^2} + \frac{1}{2r} \right)
$$

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Diffusion Limited Ionic Currents

The diffusion towards the nanopore entrance sets a limit to the ionic current flowing through a nanopore. For this discussion we are neglecting any potential drops in the solution surrounding the nanopore.

Assuming that we are in steady-state, we can estimate the diffusive current to a hemispherical pore mouth with radius of 5 nm is:

\[ I = 2\pi DrC_0 \]

\[ I = 2\pi \cdot 5 \cdot 10^{-9} m \cdot 2 \cdot 10^{-9} m^2 s^{-1} \cdot 0.1 \text{mol/l} \]

\[ I \approx 3.8 \cdot 10^9 \text{ions} / s = 610 \text{pA} \]

This indicates that nanopores at high bias voltage should be diffusion limited, which is not the case \(\Leftrightarrow\) there is a finite potential drop - due to the access resistance - outside of the nanopore pushing ions in.

For biological channels their radius is often below or around 1 nm:

\[ I = 2\pi \cdot 5 \cdot 10^{-10} m \cdot 2 \cdot 10^{-9} m^2 s^{-1} \cdot 0.1 \text{moll}^{-1} \approx 3.8 \cdot 10^8 \text{ions} / s = 61 \text{pA} \]

at typical membrane potentials of 100 mV this is larger than the current through most biological nanopores which indicates that they are usually not diffusion limited.
Diffusion constant and conductivity

(CRC Handbook 2000)

• For a given aqueous solution the diffusion constant of the ionic species $D_+$ and $D_-$, for the positive and negative ions respectively, is directly linked to the conductivity of the salt solution:

$$\sigma(T) = \frac{1}{\rho(T)} = \frac{z^2 e}{k_B T} (D_+ + D_-) = z^2 e (\mu_+ + \mu_-)$$

where $\mu_+$ and $\mu_-$ are the mobility for the respective ionic species

• Some diffusion constants for ions in aqueous solution (infinite dilution, $T=25^\circ C$, $10^{-9} \text{m}^2/\text{s}$):

<table>
<thead>
<tr>
<th>Ion</th>
<th>Diffusion Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>K+</td>
<td>1.957</td>
</tr>
<tr>
<td>Na+</td>
<td>1.334</td>
</tr>
<tr>
<td>Li+</td>
<td>1.029</td>
</tr>
<tr>
<td>Cs+</td>
<td>2.056</td>
</tr>
<tr>
<td>H+</td>
<td>9.311</td>
</tr>
<tr>
<td>Mg2+</td>
<td>0.706</td>
</tr>
<tr>
<td>Cl-</td>
<td>2.032</td>
</tr>
<tr>
<td>F-</td>
<td>1.475</td>
</tr>
<tr>
<td>OH-</td>
<td>5.273</td>
</tr>
</tbody>
</table>
Nanopores as DNA Detectors

- Reservoirs contain salt solution
- Connected by a nanopore
- DNA added on one side
- DNA is detected by ionic current

(Dekker, 2007)

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
DNA Translocation

- DNA translocation measured in 1M KCl with nanopore of 10 nm diameter
- Current decreases, indicating DNA passing through the nanopore
- Microsecond time resolution allow for detection of event structure

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Typical Events in Nanopores

- Linear translocation of the DNA through the nanopore
- Events are characterized by dwell time $t_{dwell}$ and averaged current blockade level $I_{block}$

(Smeets et al. 2006)

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Typical Events in Nanopores

- Doubling of the current blockade: DNA can be folded when going through the nanopore (diameter 10 nm)

http://www.damtp.cam.ac.uk/user/gold/teaching_biologyIII.html
Typical Events in Nanopores

- Combination of both events are also observed
- DNA can fold in nanopores with diameters of several nm

(Smeets et al. 2006)

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Analyzing DNA Translocations

- Each point represents one single-molecule measurement
- High throughput analysis is easily possible
- ‘Type 2’ twice the blockade of ‘Type 1’ and half the dwell time
- ‘Type 21’ lies in between, as expected
- Tertiary structure of DNA can be detected \( \Leftrightarrow \) folds and kinks, bound proteins, ...

(Smeets et al. 2006)

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Event area scales with DNA length

\[ \int I(t) dt = Area \]

- Integrated charge scales linearly with length of translocating DNA
- Prove that DNA is actually going through the nanopore

(Storm 2005)

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Polymer physics with Nanopores

(Storm 2005)

• Long DNA molecules are pulled through much faster than their Zimm relaxation time

\[
\tau_Z \approx \frac{R^2}{D_Z} \approx \frac{\eta}{k_BT} R^3 \approx \frac{\eta b^3}{k_BT} N^{3\nu} \approx \tau_0 N^{3\nu}
\]

\[
\tau_Z \approx \frac{0.001 \text{ Ns m}^{-2} (100 \times 10^{-9} \text{ m})^3}{k_BT} 160^{3 \times 0.588} \approx 2 \text{s}
\]

• Translocation time for 16 micron long DNA (48 kbp) \(\sim 1 \text{ ms}\) \(\Leftrightarrow\) polymer coil should be detectable in translocation time?

http://www.damtp.cam.ac.uk/user/gold/teaching_biologyI.html
Experimental results: fast translocations

• Fast translocation: dwell time $\tau$ larger than Zimm time $\tau_Z$.
• Polymer cannot reach a new equilibrium configuration during translocation of each segment b.
• DNA length varied between 6.6 kbp and 96 kpb $\Leftrightarrow$ length increased by factor of 16, $t$ increases 0.2 ms to 5 ms, factor of 25.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Experimental results: fast translocations

- Translocation time scales with $L^{2\nu}$ with $\nu \sim 0.588$ the Flory exponent for dsDNA – very nice fit to calculations

http://www.damtp.cam.ac.uk/user/gold/teaching_biosystemsIII.html

(Storm 2005)
Variation of Ionic Strength

For salt concentration larger than 400 mM ionic current through nanopore is DECREASED when DNA is in the nanopore.

For salt concentration smaller than 400 mM current through nanopore is INCREASED when DNA is in the nanopore.

DNA is a polyelectrolyte with charge and counterions.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Variation of Ionic Strength

a) 'Type 1' 'Type 21' 'Type 2'

500 mM KCl

b) 'Type 1' 'Type 21' 'Type 2'

100 mM KCl

(Smeets et al. 2006)

(c) 500 mM KCl

(d) 100 mM KCl
Change in nanopore conductance $\Delta G$

$\Delta G = \frac{e}{L_{\text{pore}}} \left( -\frac{\pi}{4} d_{\text{DNA}}^2 (\mu_K + \mu_{\text{Cl}}) n_{\text{Bulk}} + 2 \frac{\mu_K^*}{a} + 2 \frac{\mu_{\text{DNA}}}{a} \right)$

Conductance reduction due to DNA in Nanopore

- Change in nanopore conductance $\Delta G$ due to DNA with diameter $d_{\text{DNA}}$
- DNA pushes ions out of the nanopore
- DNA counter ions are brought into nanopore
- Opposite effects depending on bulk concentration of ions $n_{\text{bulk}}$
- With $\mu_K$ and $\mu_{\text{DNA}}$ the mobility of counterions and DNA, respectively

Counter ions on DNA

Conductance due to the moving DNA

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Change in nanopore conductance $\Delta G$

(Smeets et al. 2006)

$$\Delta G = \frac{e}{L_{\text{pore}}} \left( - \frac{\pi}{4} d_{\text{DNA}}^2 (\mu_K + \mu_{\text{Cl}}) n_{\text{Bulk}} + \frac{2\mu^*_K + 2\mu_{\text{DNA}}}{a} \right)$$

$$\Delta G = \frac{1}{L_{\text{pore}}} \left( - \frac{\pi}{4} d_{\text{DNA}}^2 (\mu_K + \mu_{\text{Cl}}) n_{\text{Bulk}} e + \frac{\mu_K q_{\text{eff},\text{DNA}}}{a} \right)$$

- Our assumption: all of the counter ions are movable, however some have lower mobility
- Account for this by reducing DNA charge by “attaching” part of the potassium counter ions to the DNA backbone
- Mobility of potassium is higher than DNA mobility

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Change in nanopore conductance $\Delta G$

(Smeets et al. 2006)

$\Delta G_- \sim \left( \frac{\pi d_{DNA}^2}{4} (\mu_K + \mu_{Cl}) n_{KCl} \right)$

$\Delta G_+ \sim \left( \mu_K^* q_{l,\text{DNA}}^* \right) \rightarrow \mu_K q_{l,\text{DNA}}^*$

$q_{l,\text{DNA}}^* = 0.58 \pm 0.02 \, \text{e/bp}$

- Simple model can be used to fit the data
- Extract the line charge density of DNA $q_{l,\text{DNA}}^*$
- Bare DNA has a line charge density of $2e$/bp

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
After discussing free DNA translocation experiments we can now combine optical tweezers with nanopores and current detection. We will now try to fully understand the physics governing the electrophoretic translocation through nanopores. The main variable we need for this is the force acting on the molecule in the nanopore. We will again employ optical tweezers, now in combination with a nanopore to measure translocation speed, force and position.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Two measurements: current and force  
(Keyser et al. 2006)

A single colloidal particle is coated with DNA and is held in close proximity to a nanopore in the focus of the optical trap. An applied electric potential will drive the DNA into the biased nanopore. When the DNA enters the nanopore we will see that both the ionic current through the nanopore as well as the position (force) or the particle will change at the same time:

(1) the current changes \( \Leftrightarrow \) (2) the bead position changes

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Time-Resolved Events

- Conductance step indicates capture of DNA in nanopore.
- Only when DNA is pulled taut the force changes.
- Time to pull taut $\Delta t$ is consistent with free translocation speed of DNA.
- DNA is stalled in the nanopore and allows for force measurements on the molecule, and other things.

(Keyser et al. 2006)

http://www.damtp.cam.ac.uk/user/gold/teaching_biologyIII.html
Force on DNA

- Linear force-voltage characteristic as expected
- Absence of nonlinearities indicate that equilibrium formulae can be used
- Poisson-Boltzmann should work fine in this situation
- (Navier-)Stokes should also work
- Force does not depend on distance nanopore-trap
- Extract the gradient and vary salt concentration

(Keyser et al. 2006)
Hydrodynamics Should Matter

$F_{elec} = F_{bare} - F_{drag}$

Hydrodynamic interactions matter

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Force on a charged wall in solution?

(Keyser et al. 2010)

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Poisson Boltzmann describes screening

- Distribution of ions $\Leftrightarrow$ Boltzmann distributed

$\quad n_{\pm}(x) = n_0 e^{\mp e\phi(x) / kT}$

- When we have

$\quad |e\phi(x) / kT| \ll 1$

- Taylor expansion yields

$\quad n_{\pm}(x) = n_0 \left( 1 \mp e\phi(x) / kT \right)$

- Calculate $\phi(x)$ self consistently with the Poisson eq.

$\quad \nabla^2 \phi(r) = -\rho(r) / \varepsilon_w$

$\quad \rho(r) = e \left[ n_+(r) - n_-(r) \right]$
Poisson Boltzmann describes screening

- This yields a simple differential equation

\[ \frac{d^2 \phi(x)}{dx^2} = \frac{kT \varepsilon_w}{2e^2 n_0} \phi(x) = \frac{\phi(x)}{\lambda^2} \]

- And we have the Debye screening length

\[ \lambda \equiv \left(\frac{kT \varepsilon_w}{2e^2 n_0}\right)^{1/2} \]

- Solution for the differential equation

\[ \phi(x) = Ae^{-x/\lambda} + Be^{+x/\lambda} \]

- Boundary conditions:

\[ \left. \frac{d\phi(x)}{dx} \right|_{x=0} = -\frac{\sigma}{\varepsilon_w} ; \quad \left. \frac{d\phi(x)}{dx} \right|_{x=d} = 0 \]
Poisson Boltzmann describes screening

• This yields the solution for $\phi(x)$

$$
\phi(x) = \frac{\sigma \lambda}{\varepsilon_w} \left( e^{-x/\lambda} - e^{-2d/\lambda} e^{x/\lambda} \right)
$$

• Assuming that $d \gg \lambda$ we get

$$
\phi(x) = \frac{\sigma \lambda}{\varepsilon_w} e^{-x/\lambda} \quad (d \gg \lambda)
$$

• Uncharged wall does not influence the screening layer!

$$
n_{\pm}(x) = n_0 \mp \frac{\sigma}{2e\lambda} e^{-x/\lambda}
$$

(Keyser et al. 2010)

http://www.damtp.cam.ac.uk/user/gold/teaching_biologyIII.html
Potential for a slightly charged wall

- Uncharged wall does not influence the screening layer!

(Potential graph with Keyser et al. 2010 reference)

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Ion distribution for a slightly charged wall

Uncharged wall does not influence the screening layer!

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Electroosmotic flow along charged wall

(Keyser et al. 2010)

• Excess of counterions near surface leads to electroosmotic flow

\[
\frac{d^2 v_z(x)}{dx^2} + \frac{\rho(x)E}{\eta} = 0
\]

\(\rho(x)E\) force exerted by electric field \(E\), \(\eta\) viscosity of water

• This leads to velocity of water \(v(x)\) assuming no-slip boundaries

\[
v(x) = -\frac{E\sigma\lambda}{\eta} \left(1 - e^{-x/\lambda} - \frac{x}{d}\right)
\]

• Uncharged wall DOES influence electroosmotic flow!

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Electroosmotic flow along charged wall

(Keyser et al. 2010)
Forces on the DNA and Nanopore wall

(Keyser et al. 2010)

- Bare force $F_{\text{bare}}$ is just product of area $A$, charge density and electric field $E$

$$F_{\text{bare}} = A\sigma E$$

- The drag force $F_{\text{drag}}$ exerted by the flowing liquid

$$F_{\text{drag}} = A\eta \left| \frac{dv(x)}{dx} \right|_{x=0} = -AE\sigma \left( 1 - \frac{\lambda}{d} \right) = -\left( 1 - \frac{\lambda}{d} \right) F_{\text{bare}}$$

- The force required to hold the charged wall stationary is thus

$$F_{\text{mech}} = -F_{\text{elec}} = -\left( F_{\text{bare}} + F_{\text{drag}} \right) = -AE\sigma \frac{\lambda}{d}$$
Part of the force goes to the uncharged wall

\( F_{\text{mech}} \) depends on the distance \( d \) between the walls

(Keyser et al. 2010)

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
DNA - High charge densities

(Keyser et al. 2010)

• For high charge densities linearized PB does not work:

\[
\frac{d^2 \phi(x)}{dx^2} = \frac{2en_0}{\varepsilon_w} \sinh \left( \frac{e\phi(x)}{kT} \right)
\]

• With two infinite walls can still be solved:

\[
\phi(x) = \frac{2kT}{e} \ln \left( \frac{1 + \gamma e^{-x/\lambda}}{1 - \gamma e^{-x/\lambda}} \right)
\]

\[
\gamma = -\lambda_{GC} / \lambda + \left(1 + \lambda_{GC}^2 / \lambda^2\right)^{1/2}
\]

• Introducing the Gouy-Chapman length

\[
\lambda_{GC} = \frac{2kT \varepsilon_w}{e |\sigma|}
\]

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Gouy-Chapman solution of PB equation

\( \sigma \) (C/m²)

1.0

0.5

0.1

DNA

\( \sigma \sim 0.16\text{C/m}^2 \)

0.05

0.025

0.01

0.005

0.003

0.001

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html

(Keyser et al. 2010)
PB in cylindrical coordinates $\Leftrightarrow$ nanopore, DNA

(Keyser et al. 2010)

- Electrostatic potential $\Phi$ and distribution of ions $n_{\pm}$:

$$\nabla^2 \Phi(r) = \lambda_D^{-2} \sinh \Phi(r) \quad n_{\pm}(r) = n_0 e^{\pm \Phi(r)}$$

with $\bar{\Phi} = -e\Phi/k_BT$ as normalized potential

- Boundary conditions:

$$d\Phi/dr = 0 \quad \text{Insulating nanopore walls (uncharged)}$$

$$d\Phi/dr = -\lambda_{bare}/2\pi a\varepsilon \quad \text{on DNA surface}$$

- Simplification: access resistance is neglected
- Only possible to solve numerically

http://www.damtp.cam.ac.uk/user/gold/teaching_biologyphysicsIII.html
Finite Element Calculation

- Combining Poisson Boltzmann and Stokes
- Main result: Force on DNA depends on pore diameter
- Change in pore diameter by factor 10 increases drag by a factor of two

(Keyser et al. 2009)
Hydrodynamics Should Matter Here!

(Keyser et al. 2009)

- Test hydrodynamic interactions by increasing nanopore diameter

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Detection of a single DNA molecule still possible? YES

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Force Dependence on Nanopore Radius

- Force is proportional to voltage as expected
- For larger nanopore force is roughly halved as expected from model
- Measure for a range of nanopore sizes and compare with numerical results

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Comparison: Model $\leftrightarrow$ Data

- Force depends on nanopore radius $R$
- Data can be explained by numerical model solving full Poisson Boltzmann and Navier-Stokes equations
- Hydrodynamics matters

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Explanation: Newton’s Third Law

\[ F_{bare} \quad F_{\text{drag}} \]

\[ F_{\text{mech}} \]
Membranes and proteins

• Membrane proteins and ion channels
• Rotary motors in cell membranes
• Rotary motors for swimming bacteria

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Molecular Machines – Ion Pumps/Motors

- Ion concentration differences lead to potentials across cell membranes, Nernst equation
- In case the membrane is slightly selective for one of the ions we get a current until a stable double layer is formed
- Thus with the Nernst equation we have a membrane potential $\Delta V$ in equilibrium which is given by

$$\Delta V = V_2 - V_1 = V_{\text{Nernst}} = -\frac{k_B T}{z e} \ln \left( \frac{c_2}{c_1} \right)$$

- Membrane potentials can be measured by patch-clamping

(Nelson 2006)

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Molecular Machines – Ion Pumps/Motors

(Nelson 2006)

• Donnan equilibrium, in the case of more than a single ionic species we have to take into account all their concentrations

• With Na, K, Cl in and out of the cell we have for outside \((c_1)\) and inside \((c_2)\) of the cell because of charge neutrality

\[
c_{1,Na} + c_{1,K} - c_{1,Cl} = 0
\]

\[
c_{2,Na} + c_{2,K} - c_{2,Cl} + \rho_{macro} / e = 0
\]

taking into account the charged macromolecules in the cell \(\rho_{macro} / e\)

• In the cell the concentration \(c_2\) will be different from outside, in addition we have the membrane potential \(\Delta V\) to take into account

• All three species have to obey the Nernst equation so we get the Gibbs-Donnan relations with \(\Delta V\) now the Donnan potential

\[
\text{const} = \left( \frac{c_{1,Na}}{c_{2,Na}} \right) = \left( \frac{c_{1,K}}{c_{2,K}} \right) = \left( \frac{c_{1,Cl}}{c_{2,Cl}} \right) = \ldots \quad \text{and} \quad \Delta V = -\frac{k_B T}{e} \ln \left( \frac{c_{1,Na}}{c_{2,Na}} \right) = \ldots
\]

• Membrane potentials can be measured by patch-clamping

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Fabrication of Glass Microcapillaries*

1. Glass capillary placed in puller

2. Laser heats up capillary and force applied to both sides: Glass softens and shrinks

3. Strong pull separates glass in two parts

Sutter P-2000

1. Glass capillary

2. Laser

3. Two glass capillaries

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Patch clamping

Current measurement through a SINGLE membrane pore possible

Patch-clamping modes of operation

http://www.damtp.cam.ac.uk/user/gold/teaching_biologyIII.html
Detection of Biological Membrane Potentials

(E. Neher Nobel lecture)

Glass capillary is pulled into a small tip with diameters of a few 10s-100s of nm and attached to the surface of the cell

First true single-molecule measurements – done in the early 1970s!

Figure 2. Early single-channel currents from denervated frog (Ranpipiens) cutaneous pectoris muscle. The pipette contained 0.2 μM suberyldicholine, an analogue of acetylcholine which induces very long-lived channel openings. Membrane potential – 120 mV; temperature 8°C. Reproduced from Neher & Sakmann 1976.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Patch clamping was a true scientific revolution allowing for studies of voltage gating in single protein channels, nerve transduction and many other biological phenomena. One of the papers of Sakmann and Neher is cited more than 16,000 times!

http://www.damtp.cam.ac.uk/user/gold/teaching_biologyIII.html
Membrane potential for sodium is not explained by the Donnan equilibrium $\Leftrightarrow$ active process (energy) needed to keep this membrane potential up.

Ion pumps use ATP to pump sodium out of the cell while the same pump (ATP driven) import potassium into the cells, hence sodium-potassium pump was discovered.

Function can be tested in the same lipid bilayer systems as described for the nanopores for DNA detection.

http://www.damtp.cam.ac.uk/user/gold/teaching.biophysicsIII.html
Generating ATP in mitochondria

- ATP is the main energy carrier in the cell, it is also used to make DNA and can be easily changed into GTP.
- Mitochondria convert a proton gradient into the rotary motion of a transmembrane protein called $F_0F_1$ATPase.
- Lipid membrane is needed to uphold the proton gradient which is created by deprotonation of NADH and generation of water.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Molecular Machines – Ion Pumps/Motors

(Nelson 2006)

- ATP production process is very close to energy production in power plants
- Free energy $\Delta G$ is provided here by the proton gradient
- There are several chemical sub-steps involved which are not explained here but can be found for instance in Nelson chapter 11.3

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Hydrogen and Oxygen to water

• After a number of reactions three educts yield water, NADH (Nicotinamide adenine dinucleotide), protons, oxygen

\[
\text{NADH} + H + O \rightarrow \text{NAD}^+ + H_2O
\]

• It can be measured that \( DG \) of this reaction is up to \(-88k_B T\) which is obviously an upper bound as in the real system we have to take the concentrations of the molecules and thus their chemical potentials into account adjusting \( DG \)

• This cycle keeps the proton gradient over the mitochondria membrane up and thus provides the energy for \( F_0F_1\)ATPase which finally generates ATP from ADP and

• Interestingly this process can also be reversed – in the absence of a protein gradient this \( F_0F_1\)ATPase burns ATP and creates ADP
Molecular Machines – Ion Pumps/Motors

- It is possible to image the rotary motion of the F$_0$F$_1$ATPase by attaching an actin filament which is fluorescently labelled to the top of the motor
- Rotary motion in three steps can be observed
- Highly efficient molecular machine with efficiency close to 1!

(Nelson 2006)

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Proton-driven Flagellum Motor of E.coli

Motor is driven by a proton gradient over the inner and outer membranes. MotA and MotB are proton channels allowing for the passage and converting the electric energy into rotational motion.

Bacteria use not ATP but ion currents for driving their rotary motors.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Change of Flagellum during Swimming/Tumbling

(Berg 2003)

E.Coli swims in straight lines with intermittent tumbling motion.

During straight line swimming the motors rotate counter clockwise (CCW) and during tumbling clockwise (CW).

Flagella conformation depends on rotation direction. In CCW the flagella form a bundle while in CW they rotate separately.

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Change of Flagellum during Swimming/Tumbling

(Berg 2003)

Single flagellar filament of E.Coli, imaged by fluorescent microscopy. Frame numbers indicate video frame numbers with ~17ms between frames.

Direction switches from CCW to CW after frame 2, changing conformation to semi coiled (frame 10) and then to the ‘curly’ helix.

Switch back to CCW (after frame 26) leads to transformation back to the normal helical confirmation (see 2).

http://www.damtp.cam.ac.uk/user/gold/teaching_biophysicsIII.html
Characteristics of the Flagella Motor

MotA is important for motor function.

Using a deletion mutant of E.coli, i.e. removing the protein from the genome, it can be shown that MotA is the relevant subunit. It can be reincorporated by gene transfer using bacteriophages. Each additional motor unit increases the rotation frequency in quantized steps.

Determine motor characteristics by attaching a single bacterium to a surface. Attaching a colloid of known diameter you can determine the rotation frequency and use drag force to extract torque $M$.

\[ M = \gamma_{\text{colloid}} \omega \approx 3 \cdot 10^{-18} \text{Nm} \]

Change of colloid diameter can be used to measure torque, as direct torque measurements are difficult with MT. The torque is independent over a wide range of frequencies.