# MKS (SI-units) 🗇 CGS

- See pages 107-109 in "Van der Waals Forces" by Adrian Parsegian for full overview of conversion
- First example: Coulomb forces in SI units

Force =  $\frac{q_1 q_2}{4\pi \varepsilon_0 r^2}$  N,  $q_1$ ,  $q_2$  in coulombs C, r in meters,  $\varepsilon_0 = 8.85 \times 10^{-12}$  C<sup>2</sup> N<sup>-1</sup> m<sup>-2</sup> or  $(1/4\pi\varepsilon_0) = 8.992 \times 10^9$  N m<sup>2</sup>/C<sup>2</sup>; CGS units Force =  $\frac{q_1q_2}{r^2}$  dyn,  $q_1$ ,  $q_2$ in statcoulombs, r in centimeters.

• Second example: Poisson equation

 $\nabla \cdot (\varepsilon \mathbf{E}) = \rho_{\rm free} / \varepsilon_0, \qquad \nabla \cdot (\varepsilon \mathbf{E}) = 4\pi \rho_{\rm free}.$ 

• Other conversion please see text book by A. Parsegian

# Physics of single molecule experiments

- Optical tweezers:
  - Why optical traps?
  - Physics of optical trapping
  - Calibration of optical traps using equipartition and fluctuations

# Scale of molecular forces

We discussed already that the thermal energy at room temperature is equivalent to  $\sim 10^{-21} \text{ J} \Leftrightarrow \sim 25 \text{ meV} \Leftrightarrow \sim 4 \text{ pNnm}.$ 

Polymers like DNA or other macromolecules in solvents are constantly exposed to the bombardment of the surrounding molecules or atoms.

One very nice example is the entropic stiffness of a polymer chain. We discussed this earlier in the course briefly as freely jointed chain in the section on fluctuation induced forces. This means that living organisms need to work in noisy environments where many forces are on similar order.

The value of 4 pNnm also gives us already an indication what kind of capabilities any technique would need to assess processes on this molecular scale.

In essence, we would like to be able to measure forces in the range of 10<sup>-12</sup> Newton or even lower, and if possible with (sub-)nanometre spatial resolution.

There are three complementary single-molecule techniques that are widely used an lead to a revolution in biological physics since the 1990s.

Starting with *optical tweezers*, we will learn how they are experimentally implemented and for which kind of experiments both in soft matter and biological physics they can be used. Then we will discuss *magnetic tweezers* and their use to twist DNA. Finally we will look into the *atomic force microscope* and its use for protein unfolding studies.

### **Optical Tweezers: Photonic Forces**



Photons carry momentum p depending on their wavelength  $\lambda$ 

$$\left|\vec{p}\right| = \frac{h}{\lambda}$$

Where h is Planck's constant. This can be observed by the two tails of comets. One containing the heavier particles following the path of the comet and a second tail containing lighter atoms and molecules. From the observation of bent tails of comets, one can conclude that photons can interact with particles or even single atoms and transfer momentum.

One of the first quantitative explanations can be found in a publication from 1909 by Peter Debye. This is remarkable as the fundamental principles for optical tweezers are already laid out in the early days of quantum mechanics when the discovery that photons in fact carry momentum was just being discovered. One should also remember in this context that Einstein got the Nobel prize for the photoelectric effect - in 1921.

#### **Estimating Photonic Forces**

From the observations on comets one can deduce that photons carry momentum which can be transferred to atoms and molecules. To get a rough idea about the magnitude of the forces we can do a quick calculation. Assuming that photons are reflected by a perfect mirror, we can write down the light pressure exerted on the mirror. Let's define the intensity *I* as the power *P* per area *A* on the mirror  $I = \frac{P}{A}$ We can then write down the light pressure on the mirror  $p_r$   $p_r = 2\frac{I}{c}$ 

where c is the speed of light. The factor of takes account of the fact that the light is reflected.

Assuming that our light source (laser) has an output power of P=500 mW we can estimate the force on the mirror  $F_r$  as

$$F_r = p_r A = 2\frac{P}{c} \approx 3.5 \text{ nN}$$

This is more than three orders of magnitude larger when compared to forces in the range of 10<sup>-12</sup>N we want to measure. It is even more important to note that the gravitational force on a bacterium is in the range of 10 fN.

#### **Optical Tweezers in Action**

Before we discuss in detail how optical tweezers can be build and used, perhaps we can already look at a nice demonstration of the strong effects that light can have on



In the movie an infrared laser beam at a wavelength of 1064 nm is focussed into a diffraction limited spot with diameter of roughly 1 micron. In this experiment the power of the laser is 500 mW.

Here comes bacterium

single bacterial cells.



# Optical Trapping in the Ray Optics Regime



One straightforward approach to understand optical trapping can be discussed in the context of ray optics (Mie Regime).

Assuming that the particle held in the laser focus is much larger than the wavelength of light, we can avoid Gaussian optics but simply use Snellius' law to understand the acting forces.

Upon hitting the interface between particle and surrounding medium the light is refracted at the interface. For trapping, the refractive index  $n_m$  of the medium should be lower than  $n_p$  of the particle. The change in direction of the ray leads to a change in momentum of the photons and thus a momentum acting on the particle. Depending on the initial position of the particle relative to the focal spot (indicated a black spot) the net force always pulls the particle back towards the focal point. This establishes a stable three-dimensional trapping, if these restoring forces are larger than the thermal forces and the scattering force (light pressure). The latter is neglected in this simple example, obviously.

#### **Optical Tweezers: Typical setup**



There is a wide range of possible experimental configurations for optical tweezers (OT).

In essence an OT setup needs a tightly focussed laser beam, usually achieved by focussing the beam through a microscope objective with high numerical aperture NA>1. For an optimal quality of the imaging, all elements should be adapted, apertures etc. This is achieved bv introducing optically conjugated planes which are shown in the figure by the crosshatched planes. If planes are optically conjugated it means that objects are in focus in all these plans at the same time. In the example on the left, two microscope objectives are used. One for creating the trap and the second one for illumination and detection of the motion of the particle in the optical trap.

#### **Reminder: Numerical Aperture**



Numerical aperture determines resolution  $d_0$  of the objective. This is the minimal distance when two objects can be distinguished. For fluorescent objects one can go beyond this limit by turning the molecules on and off (Nobel prize for chemistry 2014).

Numerical aperture (*NA*) is defined as  $NA=n \sin(\alpha)$ , where *n* is the refractive index of the medium and  $\alpha$  the opening angle of the objective lens.

The factor of 0.61 due to diffraction at round aperture which leads to the so called Airy disc that has minima at  $0.61\lambda/R$ ,  $1.116\lambda/R$ ,  $1.619\lambda/R$ , ... with  $\lambda$  the wavelength and R the radius of the objective lens. The solutions of a plane wave incident on a round aperture leads to an intensity distribution described by Bessel functions of the first kind with poles at the positions given above. The resolution (for distinguishing objects) of any microscope is limited by the wavelength and the diffraction pattern of the lens.

#### **Optical Tweezers: Position Detection in Reflection**



We will discuss two different strategies for the measurement of positions of a particle in an optical trap. Both methods we are discussing are extremely relevant for all techniques discussed in the lecture and are widely used in the tracking of single particles or molecules.

On the left, the detection of the position using a second laser beam, that is reflected off the particle is depicted. This is also the method of choice employed in the atomic force microscope. In general this technique allows for resolution down to 0.01nm and below. It is important to note here, that spatial resolution in particle tracking is NOT limited by the resolution determined by the NA of the used objective. The NA only determines at what distance two particles can be distinguished. This is important remember, especially when modern to developments like STED (stimulated emission depletion microscopy) are discussed which can overcome the limitations by NA.

#### **Optical Tweezers: Quadrant Photodetectors**



X position  
$$X = \frac{(A+D) - (B+C)}{A+B+C+D}$$

Y position  
$$Y = \frac{(A+B) - (C+D)}{A+B+C+D}$$

In order to detect the position of the red laser beam and thus determine the position of the trapped particle, one can use quadrant photodetectors . This approach is ubiquitous in experimental systems.

Four independent photodiodes (A,B,C,D) are combined into a single quadrant photodetector. Each element has its on amplifying circuit converting the incoming photons into a voltage A,B,C,D. The relative position of the laser beam can then be simply determined by comparing the incident power C+D on two of the sectors A+B with the two opposite ones C+D, while dividing by the total incident laser power.

Position measurements can be taken every 10<sup>-6</sup>s and below. Most applications in soft and biological physics it is not necessary to go beyond due to the low Reynolds number environment.

Calibration of the detector response is necessary. One method to do this is the use of the powerspectrum of the optical trap that we will discuss next. Detection of sub-nm movements is possible. Obvious limitations of the resolution are related to electronic noise of detector, light level, background light, and size of laser spot on detector.

### Video Based Particle Tracking



#### <u>(Otto 2010)</u>

An alternative to quadrant photodetectors (QPDs) is using video cameras. There are several advantages with this approach, namely the possibility to follow several particles in parallel, easier calibration of the position information and reduction of drift (1/f-noise source) or electrical interference.

With an intense light source illuminating the sample, a fast camera is mounted behind the tube lens of the imaging microscope objective and the video recorded. Modern digital cameras can reach frame rates of 10,000 or more per second. The stream of images is captured by a computer for analysis of the position of the particles in the field of view. It is also possible to analyse the data on the fly in real time, removing the need to store GBs of video data and rather just saving two numbers for the x, y, z position of the particles in the image.

# Cross-correlation position tracking



#### Region of interest

Yellow lines denote lines that are averaged to produce profiles on top and right. This reduces noise due to electronic noise in the electronics of the pixels. Since the typical amplitude of the motion of a trapped colloid is of the order of tens of nanometres, and typically one pixel on a camera corresponds to a few hundred nm, position determination must be carried out with subpixel accuracy.

In particle tracking we use a number of assumptions to obtain a sub-pixel resolution: (i) the particles should be rotationally symmetric and produce a symmetric diffraction pattern on the camera that is (ii) constant over time.

For the determination of the particle position in the region of interest one can use a cross-correlation based tracking algorithm. Sub-pixel accuracy is achieved by calculating the cross-correlation of the mean intensity distribution with its reverse by applying the convolution theorem. The convolution theorem uses that the Fourier transforms of signals f and g is equal to the point wise product of each their Fourier transforms.

#### (Otto 2010)



After calculation of the mean intensity profile along an  $4 \times 30px2$  rectangular array of the  $30 \times 30px2$  sub-ROI (see inset in (a)), the intensity profile is depicted in *x*- and *y*- direction (a). Cross-talk between the directions is minimal, this is another advantage over QPD tracking. Part (b) shows 1-dimensional cross-correlation transformation and 2nd-order polynomial fit of  $\pm 3px$  around the maximum. The fit shows why we can achieve sub-pixel resolution with this technique. As we assume that the shape of the intensity profile is constant over time and the cross-correlation can be approximated by a simple polynomial, even with a fit of only seven points we achieve a resolution of a few nm in this case. The inset in (b) shows a time trace for a colloid tracked for 80 s at 10,000 fps in real-time.

#### Resolving nm steps with video tracking



One can test the tracking routine by investigating the motion of of a surface attached colloidal particle that is moved in defined steps. In order to determine the resolution in the instantaneous position of a particle a 3.47  $\mu$ m silica particle was adhered to the surface of a sample cell and a piezoelectric stage was moved in 2nm steps.

The plot on the left shows the change in the detected position signal of the particle following a 2 nm step of the piezoelectric stage. Analysis of the data reveals that our tracking algorithm can resolve step size with an accuracy of down to 0.2 nm. The red line is calculated using a moving, adjacent average filtering over 10 data points.

## Brownian Motion of a Particle in an Optical Trap



On the left, a example for the Brownian motion of a spherical particle in an optical trap is shown. The position was measured with a calibrated quadrant photo detector. The position-axis already shows the signal in micrometres. The conversion of the voltage output of the photo detector is usually achieved by moving a stuck particle with a calibrated piezoelectric stage in the nanometre range. This is raw data, without taking care about filtering, background corrections or other analysis methods.

We will now investigate how this type of data can be used to calibrate the forces acting in the optical trap. To first approximation we assume that the trap is harmonic for small deviations from the trap centre, i.e. the restoring force driving the particle back into the equilibrium position is proportional to distance. This is of course just Hooke's law  $F=-\kappa\Delta x$ , where  $\kappa$  is the trap stiffness in that direction and  $\Delta x$  the distance from the trap centre. In general, optical traps are not isotropic but have to be described with a two-dimensional matrix.

#### Calibration: Equipartition - Position Histograms



Using the equipartition theorem we can extract information from the data to determine the trap stiffness  $\kappa$  of the optical trap. At constant temperature T, a particle in a harmonic potential has a thermal energy of  $\frac{1}{2}k_BT$ . Assuming an harmonic potential we can directly write down the expected width of the random particle positions in the optical trap to be

$$< x^2 > = k_B T / \kappa$$

However, in the case of a real data, there are complications with this method. The position histograms shown on the left are clearly asymmetric, depending on recording 0.010 times and time resolution of measurements. This is due to other sources of error like 1/f-noise etc.

# **Optical Trapping & Single Chains**

- Calibration of optical traps
- Single stranded DNA is an extensible freely jointed chain
  - Structure of nucleic acids
  - Revision of ideal chains
  - Comparison with experiments

#### **Calibration: Using Power Spectrum**



# Power Spectra as a function of Laser Power



The trap stiffness should not be confused with the maximum force optical tweezers can exert. These are in the range of a few 10s of pN. Obviously force and position resolution critically depend on the stiffness. By tuning of the laser power one can easily choose the required stiffness. This allows for adaption to the forces that should be measured. To first approximation we would expect that the trap stiffness depends linearly on the applied laser power. In practice this holds for most situations, as shown by the data below. Corrections due to heating are usually in the range of a few per cent or even less and do not show up at the low frequency range however can sometimes be observed at higher frequencies.



### Test of Calibration of Optical Trap



One straightforward check of the trap stiffness calibration is by using Stokes drag on the particle. By dragging a particle of known diameter through the liquid at known speed v we have force  $F = \gamma v$ . One has just to compare the measured force with the expected force. As in the trap calibration, the biggest uncertainties these in particle measurements are the diameter and the temperature of the liquid in the optical trap. Depending on the laser wavelength, it might heat liquid. the As the viscosity is temperature dependent this influences the drag coefficient of the particle and thus the Stokes drag.

Laser induced changes of the temperature around the particle can be detected with sensitive detection especially in the high frequency range of the powerspectra (see question sheet) where the particle is 'freely' diffusing in the optical trap.

#### Influence of Bandwidth on Powerspectra



The bandwidth or number of samples/second of the optical trap system is crucial for an accurate determination of the trapping stiffness using the powerspectral analysis method. If the same signal is digitized with a range of different samples/s but with the same temporal resolution this has a profound influence on the powerspectrum.



Sampling with the same temporal resolution but lower bandwidth leads to an increase of power in the low frequency part of the spectrum. This is the well known effect of aliasing, leading to so-called ghost frequencies in the spectrum. Obviously, this fundamentally changes the shape of the powerspectrum, rendering it useless for calibration.

# Issues with REAL Data in Video Tracking

Video tracking produces aliasing as pixels are not exposed during the full time. The camera electronics needs a finite amount of time to read the data from the pixel into the memory of the camera/computer.

A typical example is shown below for three different trapping laser powers imaged at 10,000 fps. The nominal shutter time would be  $\Delta t$ =100 µs, however the real time (temporal) resolution is  $\Delta t$ =95 µs which makes the real bandwidth is 5,263 Hz (undersampling leads to aliasing).



# REAL Data: 1/f noise and bandwidth

To avoid problems with aliasing quadrant photo detector electronics have inbuilt low-pass filters which limit the bandwidth. This shows up as a faster drop in the powerspectrum at frequencies larger than the filter drop off. In the ideal case the recording bandwidth should be always at least 2 times higher than measurement bandwidth.



Another feature seen at very low frequencies is the so called 1/f noise. This can be due to thermal drift, mechanical or electrical interference or other noise sources interfering with the There measurement. is not one single source for this 1/f-noise however it severely limits the long stability of the term system and is the reason equipartition for often predict failing to the correct stiffness.

#### More possibilities for Optical Tweezers

#### Tetris with multi beam optical tweezers

http://www.youtube.com/watch?v=jCdnBmQZ6\_s&feature=player\_embedded

## Optical Tweezers work in air and vacuum ...

Water droplet trapped in air imaged at 2000 frames per second



Padgett group Glasgow

# DNA structure and worm-like chains

- Double-stranded DNA structure double helix
- Single-stranded DNA is an extensible freely jointed chain
  - Structure of nucleic acids
  - Revision of ideal chains
  - Comparison with experiments

## Double Stranded DNA forms a Double Helix

Calladine & Drew Chapter 2 (1997)



DNA is a co-polymer with a phosphate backbone and functional side-chains consisting of the four bases, Adenosine (A), Thymine (T), Guanine (G) and Cytosine (C). Hydrogen bonding (Watson-Crick) between the bases (A-T, G-C) holds the two backbone strands together. The formation of the double helix (shown here is the B-form) minimizes water between hydrophobic basepairs by twisting the basepair against each other. This is possible due to the flexibility of the backbone structure and its phosphate group. The phosphate group also leads to the negative charge of DNA in aqueous solution at pH>5.

# Helix possible due to Rotating Bonds Calladine & Drew, Chapter 2 (1997) 0 (a) (b) Figure 2.10 (a) Tetrahedral arrangement of phosphate group: atom types are phosphorus (P) and oxygen (O); (b) freedom of rotation for adjacent links of the chain, with atom type carbon (C).

The double helix is a consequence of the freedom of the C-atom to "swivel" around the P-O bond. This makes the DNA-backbone flexible to rotate and adapt its structure. It is also interesting to note that the backbone has a direction. As shown above, the C atoms in the sugar ring a labelled from 1-5. The phosphate group is either attached at the 5' or 3' end of the sugar and thus DNA ends a labeled as the 3' or 5'. In the double helix the strands are usually running anti-parallel. The flexibility of the backbone and in fact of the hydrogen bonding makes DNA a highly adaptable molecule depending on the conditions.

#### Some Forms of ds-DNA

DNA can be found not in just the standard B-double helix but also take different forms. There are many structural variants known which are usually labelled by letter а A, B, C, D, E, P, Z, ... Most of these are right-handed double-helices, however as seen on the right, they can have pitch, diameter different and differences in the size of the major and minor grooves. Even the formation of a left handed helix is possible, as shown by the example for the Z-DNA. Since DNA structure was analysed by the crystallography, most x-ray prevalent forms (A,B) were discovered first as they can be easily crystallized. This illustrates that double-stranded DNA is a dynamic and adaptable building block of the living cell. It should also be clear that the different forms have distinctive mechanical properties which can be determined with optical tweezers.

Calladine & Drew Chapter 2 (1997)

http://www.damtp.cam.ac.uk/user/gold/teaching\_biophysicsIII.html

B

#### dsDNA shows Intrinsic Curvature

Calladine & Drew, Chapter 5 (1997)









The double helix is due to hydrogen bonding, hydrophobic interactions, structure of the bases and also influenced by Coulomb interactions of the charged backbone. All interactions together can lead to an intrinsic bending of the DNA depending on the basepair composition. In these transmission electron microscopy images, the bending of the DNA molecule can be clearly seen.

These bends are implicated in playing a role in gene regulations and thus might play an important structural role in the functioning of cells, underlining again that DNA is not simply a B-form double helix.

### Single-Stranded DNA (ssDNA)



Single-stranded (ss)DNA is a copolymer consisting of phosphodiester backbone (purple), linked by single bonds and allowing to bind side chains or bases (red,yellow). The distance between the bases is 0.34 nm. ssDNA is not very often found freely in cells. In general hydrogen bonding between the bases will lead to formation of more complicated structures like hairpins or even more complex molecular complexes.



Even ssDNA can have a helical form, depending on the exact base sequence, but it is freely rotatable due to the PO-C bonds. One specific molecule forming a helix is consisting of long stretches of Adenine bases, which then, due to hydrophobic interactions giving rise to base-stacking, forms a compact single helix with an outer diameter of . It can even be found as a double-helix under acidic conditions.

## Stretching a One Dimensional Chain

Phillips et al., Chapter 8 (2009)



After discussing the background and operations of optical trapping we will discuss now the main use of these instruments in polymer and biolgical physics, namely the stretching of polymer chains. First we will discuss the ideal case with overlapping elements, no constraints and ideal bonds and later discuss more complex models like the worm-like chain already known from the Part II soft matter course (P. Cicuta).

# Force Extension of the 1D, 2D and 3D chains Phillips et al., Chapter 8 (2009)



With the model we just developed and we can calculate the force extension or as shown here, the time averaged *<L>* extension as a function of force, for 1D to 3D chains with extension normalized by  $L_{tot} = Nb$  which is the contour length with N number of segments, at constant temperature. <u>http://www.damtp.cam.ac.uk/user/gold/teaching\_biophysicsIII.html</u>

# Single-stranded DNA is an Extensible FJC

(Bustamante et al. Science 1996)



Force-extension measurements on ssDNA can be also performed with optical tweezers. The data can be easily fit with a model describing the freely-jointed chain (FJC, dashed line) at low forces, however higher forces and long at extensions the model significantly deviates and overestimates the results. This can be taken into account by making the length of the monomers force dependent b(F), which leads to an almost perfect description of the data. Note that the charge of the DNA backbone and thus the Coulomb interactions significantly changes the behaviour at low salt concentrations as we will see later also for dsDNA.

http://www.damtp.cam.ac.uk/user/gold/teaching\_biophysicsIII.html


It is interesting to note that all real polymer chains can be made ideal if they are long enough. This can be understood by redefinition of the segment length to fulfil the condition  $\langle R^2 \rangle = N'b'^2$ . Here we introduce *b* or *b*' as the 'Kuhn'-segment length with b=2p. The Kuhn length is commonly used, however, we will use more often *p* which is the persistence length as defined in the worm-like chain model that we will discuss now in more detail.

The main difference between classical springs (Hooke's law) and polymer chains is that, depending on the dimensionality and nature of the bonds, the force is a power law as a function of the extension.

## Worm-like chain model (WLC)

Nelson, Chapter 9 (2006)

We discussed the freely-jointed chain as a good description of single-stranded DNA. However now we will try to understand the more complicated case of double-stranded DNA. The complications arise from the helical structure of the molecule as we discussed before. But the system contains so many atoms that calculations of the mechanical properties from first principles is too computationally expensive. So we will have to use some major simplifications that nevertheless can produce predictions that will be experimentally accessible. The idea is to describe dsDNA as a continuous rod, comparable to a beam of steel. We will test three different distortions of the dsDNA molecule, bending, stretching and twisting. These can be described within the following coordinate system.



## Four Parameters Characterize dsDNA (WLC)

Nelson, Chapter 9 (2006)

We will use three quantities to describe the dsDNA beam. First we will use the scalar stretch u(s) which measures the fractional change in length of the segment, where s denotes the position along the DNA. The bend deformation  $\beta(s)$  describes the change of the tangent vector t, when the molecule is distorted (a).  $\beta(s)$  is a vector with dimension [1/L]. Finally we will use the twist density  $\omega(s)$  which describes the torsional deformation of the molecule. With this we describe the change of the angle along the length of the dsDNA. Obviously, u(s),  $\beta(s)$  and  $\omega(s)$  are local quantities so we will have to integrate over the total unstretched length of the molecule to obtain the change in energy:



## Making a bend in DNA

 $dE = \frac{1}{2}k_BT \Big[A\beta^2 + Bu^2 + C\omega^2 + 2Du\omega\Big]ds$  Nelson, Chapter 9 (2006)

To calculate the change in energy dE, we introduce now four parameters A,B,C and D. All four parameters can be experimentally determined. The obvious interpretation of A is the persistence length of the molecule. C is the twist persistence length that, as we will see in the next section, describes the buckling transition of the DNA molecule.  $Bk_BT$  and  $Dk_BT$  are the stretch stiffness and twist-stretch coupling. In the following discussion we will assume that – at the forces we are working at – B&D=0. For the case of single-stranded DNA we can also set C=0 as the molecule can freely rotate around its axis. Thus twisting will not lead to any distortions in the molecule.

Thus, in the simplest case we will treat DNA as an inextensible rod and, assuming that we do not twist the molecule, we can calculate the energy needed for making bending the molecule  $1 \frac{L}{c}$ 

$$E = \frac{1}{2} k_B T \int_0^2 A\beta^2 ds$$

which is known as the simplified elastic rod model. (This is also named Kratky-Porod model.) With this equation we can now easily calculate the energy needed to bend the molecule by 90deg. This can be used to estimate the energy needed to make a plectoneme or supercoil into the DNA molecule (see question sheet). It is important to note that despite the major simplifications, the worm-like chain model works extremely well for real polymers.

#### Video pulling single chains

<u>video</u>

## Twisting dsDNA

- Magnetic tweezers
- Testing models for Worm-like-chain with magnetic tweezers
- Molecular friction in enzymes

## Twisting DNA using Magnetic Tweezers



(Phillips 2009)

Until now we discussed optical tweezers as one of the methods that allows to interrogate the physics of single molecules. Although they revolutionized single molecule biophysics, optical tweezers have obvious limitations. By construction, optical tweezers are defining the position of a particle, this is also known as **position clamping**. OTs can move dielectric particles around but they cannot exert torque onto a molecule. However, twisting a molecule is a very important process in cells as protein motors walking along the double-helix of the DNA molecule induce significant torque. Thus we require a technique that enables to exert torque on the molecule. One obvious choice for a technique is the magnetic fields. The idea of magnetic tweezes is as simple as elegant. A gradient in a magnetic field leads to a force on a magnetic particle with magnetic moment  $\mu_r$ . This leads to a force towards the magnets, while the direction of the magnetic moment allows for rotating of the particle with a rotation of the magnets. It is also important to notice that magnetic tweezers are force clamps, in contrast to OT which are position clamps.



Biological background: in the cell specialized enzymes have to regulate supercoiling to enable replication of DNA (see movie). During the replication of DNA the replication forc (composed of several enyzmes) moves through the DNA inducing negative and positive supercoils. These have to be removed (in a controlled way) by specialized enzymes known as topoisomerases, gyrases. Supercoiling can also be induced by many other DNA binding enzymes and studied by magnetic tweezers.

## Magnetization of the beads is anisotropic



It is essential to have magnetic particles with a finite magnetic moment  $\mu_r$ . This is acheived by using many small ferromagnetic nanoparticles that are inserted into a polymeric colloidal particle.

The energy E of the magnetic dipole in the magnetic field B is then:

$$E = -\overrightarrow{\mu}_r \cdot \overrightarrow{B}$$

and thus we can calculate the force in z direction

$$\frac{dE}{dz} = -\frac{d}{dz} \left( \vec{\mu}_r \cdot \vec{B} \right)_z$$

Obviously, the particle orients itself along a preferred axis determined by the outside field. With typical particles with diameters of a few microns, one can create pN forces and thus rotate the particle and any attached (bio)molecules. As mentioned before, this **allows twisting/supercoiling of DNA molecules** by rotating the magnets.

## Schematic of Magnetic Tweezers



Magnetic tweezers are basically an inverted microscope, with a light source (LED). Two permanent magnets are mounted in a rotatable holder, the gap determines the gradient and thus the force. The force can be controlled by moving the magnet holder up or down with respect to the molecule attached (tehtered) to a microscope slide and the magnetic particle. This is usually achieved by employing receptor-ligand interaction with proteins extracted from cells. http://www.damtp.cam.ac.uk/user/gold/teaching\_biophysicsIII.html

## Magnetic Tweezers in the Lab \*



Magnetic tweezers can be build in very compact form. They are amazingly simple if one considers the exceptionally high resolution of forces. They can detect forces of less than 10 fN. However, the name tweezers is a bit misleading as strictly speaking they are only applying a force and do not define a position.

#### Magnetic Tweezers Position Detection in Z \*

Gosse, Croquette 2002



One of the challenges in magnetic tweezers is the detection of the distance between the colloidal particle and the surface of the sample cell. Here, the diffraction pattern of the particle on the camera can be used to determine the relative position of the particle to a pre-recorded look-up table shown in part E of the above figure. In practice, two particles are used to measure the length of the molecules by detecting their distance. One particle is stuck to the glass indicating where the molecule is tethered to at one end while the other end is labeled with the second colloidal particle. This also eliminates drift of the microscope stage from the measurements.

Х

## Magnetic Tweezers Force Measurements

(Seidel 2005)



The movie demonstrate the functions of magnetic tweezers. The lower left shows the video microscopy image of a magnetic particle connected to the surface by a single double-stranded DNA molecule. The magnets are placed at three different heights above the sample and thus the DNA molecule is stretched by applying three different forces. Obviously, as the DNA molecule is relaxed, its stiffness decreases. This is indicated by the increase of the Brownian fluctuations of the particle, which directly reflect the change in stiffness of the DNA molecule and the higher applied forces.

## Magnetic Tweezers Calibration: Forces in x,y



Since the DNA stiffness depends on the applied force, with higher applied magnetic stretching force we expect less movements of the colloid in the x, y direction. This is very similar to a pendulum. In fact this movement can be used to calibrate the forces applied by the magnetic tweezers. The approach is similar to the one we discussed for the optical tweezers, the position histogram will directly reflect the stiffness of the pendulum, allowing to extract the force.

#### Magnetic Tweezers Forces in x,y



Brownian motion of the magnetic particle reveals the stiffness of the DNA. In real experiments, the fluctuations in x- and y-direction are often not symmetric. This has a variety of reasons, one is that the DNA is not necessarily centrally connected to the particle. The force on the DNA molecule depends exponentially on the distance of the magnets from the sample. For all practical purposes, the force remains constant if the magnet position is fixed since the gradient is small. This is the reason that magnetic tweezers are force clamps.

#### Entropic stretching forces



#### Deriving force extension relations

Freely jointed chain (FJC)

**Boltzmann factor** 



#### Entropic stretching forces



Freely jointed chain (FJC) (no bending energy)

$$I = I_0 \left( \operatorname{coth} \left( \frac{Fb}{k_B T} \right) - \frac{k_B T}{Fb} \right)$$

Worm-like chain (WLC)

$$F = \frac{k_B T}{p} \left[ \frac{1}{4} \left( 1 - \frac{x}{L} \right)^{-2} - \frac{1}{4} + \frac{x}{L} \right]$$

#### Stretching a single DNA molecule



## dsDNA is a Worm-Like-Chain



Force extension measured for DNA with contour length L=3.2 μm und 7.0 μm
Red curve: approximated WLC (Marko Siggia)
Black curve: ,exact' WLC (Croquette et al.)
Difference of up to 8% between 3 pN and 8 pN (relevant force region for most proteins)

# DNA charge important for persistence length

Czerwinski (2007)



Figure 3.10: Force-extension curves of the same dsDNA molecule in buffer with low salt concentration (monovalent sodium ions  $[Na^+]=1$  mM, black data points) and with high salt conditions ( $[Na^+]=50$  mM, blue data points) normalized to the contour length  $L_0$  of the DNA molecule. The lines are fits with the exact WLC model yielding values for the persistence lengths of 53 nm (low salt) and 38 nm (high salt). The buffer was TRIS-HCl [50 mM] at pH 7.0.

## DNA forms supercoils under Torque



Twisting dsDNA with magnetic tweezers leads to torque on the backbone of the DNA molecule. We can charactersize the state of the DNA with two parameters. The Twist (Tw) and writhe (Wr) describe linking number of DNA L=Tw+Wr. The twist denotes the number of helical turns, while writhe counts the number of times DNA crosses itself. Twist can be converted to writhe, when DNA is forming supercoiled structures.

# DNA forms supercoils under Torque

Employing magnetic tweezers one is able to study the properties of twisted DNA molecules. Rotating the magnets with the magnetic tweezers leads to torque  $\Gamma$ . The total torsional energy is

 $E{=}(k_{B}TC/2L)\theta^{2}$  ,

which is a direct consequence from our treatment with the WLC-model. *C* is the torsional persistence length and  $\theta=2\pi n$  counts the number of turns. We can then calculate the torque to be

 $\Gamma = (k_B T C/L) \theta$ .

At critical torque which is reached at the buckling number  $n_b$ , DNA buckles and forms supercoils with radius R as indicated on the right. As soon as the first supercoils formed, the torque remains constant, and additional rotations lead to more supercoils. In other words, for  $n > n_b$  energy is stored  $\Gamma_b$  in plectonemes (writhe) not in twist. As this is done with a force clamp provided by the magnetic tweezers the total energy cost for forming a plectoneme is given by



 $E = 2\pi RF_z + \pi k_B TA/R$ <u>http://www.damtp.cam.ac.uk/user/gold/teaching\_biophysicsIII.html</u>

## **Energetics of supercoiling**





Length change per turn of magnets

$$\Delta \left\langle z \right\rangle / turn = \sqrt{\frac{k_B T \cdot l_p}{2F}}$$

Buckling torque

$$\Gamma_{B} = \sqrt{2k_{B}T \cdot l_{p} \cdot F}$$

$$\Gamma_{B} = M_{twist} / \langle z \rangle \cdot 2\pi N_{B}$$



Twisting dsDNA at Different Forces

The critical torque for buckling depends on force: hence at higher force one needs to apply more turns before seeing DNA buckling and formation of supercoils. The dashed lines indicate the position when the buckling of the DNA starts. For the lowest force the buckling starts directly.

#### Structural DNA Transitions due to Torque



Double-stranded DNA is usually a right handed helix. Twisting the molecule should reveal the asymmetry in the structure. When the DNA is twisted counter clock-wise (CCW) we always observe the formation of supercoils indicated by the shrinking length. However at higher forces, negative turns clockwise (CW), against the natural helicity, leads to a denaturing of the hydrogen bonds and a total change in the structure. No supercoils can be formed anymore.

#### Structural DNA Transitions due to Torque



At even higher forces, another structural transition of the DNA can seen. At very high stretching the hydrogen bonds denature and P-DNA forms, which is a left-handed helix with the bases turned to the outside. This structure of DNA was proposed by Pauling in the same Nature issue of Watson and Crick's famous paper eraning them the Nobel prize. Of course, the WLC does not include these structural transitions such as denaturation or other DNA forms.

## Following a single protein with Magnetic Tweezers



Koster et al. (2005)

Until now we discussed the magnetic tweezers as instrument to study the structural properties of macromolecules. However, they can be also used to gather quantitative information on the functions of enzymes that change the state of DNA. One striking example are the topoisomerases which can change the linking number of DNA by cutting one of the backbone strands of DNA. This enables the other strand to rotate in the cavity formed by the protein. The chemical energy to break the bond is stored in the molecule and later used to restore the intact backbone to stop the rotation.

In the following we will see that single protein-DNA interaction can be probed by magnetic tweezers and used to clarify microscopic interactions.

#### Investigate DNA-enzyme interactions

Koster et al. (2005)



## **Topoisomerase (TopIB) Removes Supercoils**



The time resolution of the video detection system allows for studies of the relaxation speed, i.e. how quickly the DNA extension increases for each event. This gives insight about the molecular interactions in the enzyme-DNA system. A single topoisomerase enzyme can remove all supercoils in the DNA molecule. Analysis of the velocity reveals a most probable velocity.



## Topoisomerase: friction on molecular level

By doing these experiments with a range of different enzymes which perform the same action as the topoisomerase (nicking enzymes create also a double strand break in the DNA) it is possible to prove that the velocity of the DNA relaxation is indeed dominated by the interaction between the DNA and the enzyme. The data shows that for the nicking enzyme the relaxation velocity is almost twice as fast when compared to the topoisomerase enzyme (labelled as TopIB, above). Only conclusion: friction on molecular level slows down relaxation. <u>http://www.damtp.cam.ac.uk/user/gold/teaching\_biophysicsIII.html</u>

#### Interaction between DNA and topoisomerase



#### Koster et al. (2005)

One can make an idealized model energy landscape for the DNA moving within the protein with several minima that reflect the interaction between DNA and protein. Since this is rotary motion it will be periodic with periodicity of  $2\pi$ . Assuming a rate k to cross from one well into the other is given by  $k \approx exp(-\Delta G / k_B T)$  where  $\Delta G$  is the height of the barrier. The torque  $\Gamma$  applied by MT drives the removal of any supercoils.

One can assume the lowering of the barrier to be linear function of torque,  $\Gamma\theta$ 

#### $k' \approx exp(-(\Delta G - G d\theta / k_B T))$

The torque depends on the applied stretching force as  $\Gamma(F) = (2 k_B T l_p F)^{1/2}$ 

## Force-dependence of step size



#### Koster et al. (2005)

With this simple, qualitative model of the potential landscape we can try to understand the step size in DNA extension curves. As the religation probability p depends now on applied torque  $\Gamma$ :

#### $p(\Gamma(F))$

In the simplest case we have again a Boltzmann factor depending on the barrier  $\Delta G$  in the potential landscape:  $p(F) \approx \exp(-\Delta G / k_B T)$ 

The torque  $\Gamma$  applied by the MT drives removal of supercoils, if we assume this to be linear in  $\theta$  it follows:  $p(F) \approx \exp(-\Gamma(F) \ \delta\theta \ / k_B T)$ 

The step size given by the change in linking number  $\Delta Lk$  is now given by:

$$\Delta Lk(F) \rangle = \langle \Delta Lk(F) \rangle_{F=0} \exp\left(\delta \theta \sqrt{2l_p F / k_B T}\right)$$

The model fits the experimental data very well, strengthening the interpretation.

## Proteins – Structure and Folding

- Amino Acids: properties
- Proteins: primary, secondary, tertiary and quarternary structure
- Protein unfolding

## Proteins: Primary structure

- 20 typical amino acids (AA) side groups for peptide chain, main building blocks of proteins
- Positively or negatively charged (peptides can be polyelectrolytes, separation and manipulation by electric fields possible)
- Side chains (charge, hydrophobicity, polarity) dictate chemical and physical properties of folded protein chains
- Non-standard amino acids are used by bacteria/plants/microorganisms

**Table 2.1** The pK values for the  $\alpha$ -carboxyl,  $\alpha$ -amino groups and side chains found in the individual amino acids

Amino acid	р <i>К</i> 1	р <i>К</i> 2	pK <sub>R</sub>	Amino acid	<b>p</b> <i>K</i> <sub>1</sub>	p <i>K</i> <sub>2</sub>	pK <sub>R</sub>
Alanine	2.4	9.9		Leucine	2.3	9.7	100 <u>-</u> 900
Arginine	1.8	9.0	12.5	Lysine	2.2	9.1	10.5
Asparagine	2.1	8.7	-	Methionine	2.1	9.3	-
Aspartic Acid	2.0	9.9	3.9	Phenylalanine	2.2	9.3	-
Cysteine	1.9	10.7	8.4	Proline	2.0	10.6	-
Glutamic Acid	2.1	9.5	4.1	Serine	2.2	9.2	-
Glutamine	2.2	9.1		Threonine	2.1	9.1	-
Glycine	2.4	9.8		Tyrosine	2.2	9.2	10.5
Histidine	1.8	9.3	6.0	Tryptophan	2.5	9.4	-
Isoleucine	2.3	9.8	-	Valine	2.3	9.7	-
#### **Proteins: Secondary Structure**

(Whitford 2005)

Main structural elements of proteins:  $\alpha$ -helices and  $\beta$ -strands or sheets

Main element are the hydrogen bonds between side chains that give rise to these



## Secondary Structure: $\beta$ -Sheets and Hairpins

(Whitford 2005)



Figure 3.11 Two adjacent  $\beta$  strands are hydrogen bonded to form a small element of  $\beta$  sheet. The hydrogen bonds are inter-strand between neighbouring CO and NH groups. Only the heavy atoms are shown in this diagram for clarity

#### **Protein Tertiary Structure**

Example for protein assembly: example movie for beta-barrel



## **Proteins: Quaternary Structure**

Proteins with several (identical) subunits

mouse cholera antibody





Toxin from staphylococcus aureus heptameric 7 identical subunits

<u>nup://www.gamip.cam.ac.uk/user/gold/teaching\_biophysicsIII.html</u>

## **Protein Folding**

Even small proteins consists of many atoms, and already in the classical description each atom has six degrees of freedom (three for dimensionality, and corresponding three for momentum). Considering that proteins are found in polar solvent (water) with salt ions complicating the situation, protein folding has to be described in a highly dimensional phase space. Just assuming that we have a small protein with 200 atoms has a phase space with 12,000 dimensions. The interactions (H-bonds, hydrophilic-/phobic, electrostatic,...) between the AA lead to the formation of a complex energy landscape for protein folding.

Directly related to this there are two main questions for protein folding: (1) How are conformations which are separated by barriers in the energy landscape are explored by thermally activated processes. (2) What is the overall structure of the energy landscape.



## **Folding Funnel**

(Fersht 1999)



Figure 19.16 Cross section through a folding funnel. *E* corresponds to free energy. http://www.damtp.cam.ac.uk/user/gold/teaching\_biophysicsIII.html

## Folding Funnel for Proteins: Example



**Figure 19.13** Structures of denatured, intermediate, major transition, and native states of barnase from molecular dynamics, Φ values, and NMR. [Coordinates from C. J. Bond, K. B. Wong, J. Clarke, A. R. Fersht, and V. Daggett, *Proc. Natl. Acad. Sci. USA* **94**, 13409 (1997).]

(Fersht 1999) for the folding of

One example for the folding of a protein and representing a nice example for the folding funnel is presented by Barnase.

Experimentally, protein folding sub-states of Barnase can be determined with molecular dynamics simulations, magnetic resonance imaging and analysis. In the biochemical *denatured state* the protein has alpha-helices as main two structured elements, while in the *intermediate state* we can already see the formation of beta-sheets in the protein. The transition state is already close to the *native* conformation of the protein.

## Folding Funnel: Connection to Physics



(Fersht 1999)

The whole folding process can be rationalized in plotting a free enthalpy G associated to the energy of each of the state as a function of the order parameter Q or reaction coordinate r.

In this way it becomes possible to describe the process of protein folding as a succession of barrier crossings which can be formally described with the wealth of mathematical techniques developed in statistical physics.

**Figure 19.17** Reduction of the folding funnel to a conventional reaction coordinate diagram. This reconciles the classical view of a pathway with the new view of an energy landscape and an ensemble of conformations. [After W. A. Eaton, P. A. Thompson, C. K. Chan, S. Hagen, and J. Hofrichter, *Structure* 4, 1133 (1996).]

## Rate Constants in Protein Folding

Two states (A,B) of the protein should be characterized by a difference in energy  $\Delta G$ . The rate constant k to change from state A to B can then be given by an Arrhenius type equation:  $k = v_0 \exp(-\Delta G / k_B T)$  where  $\Delta G$  is the energy difference between the



states and  $v_0$  is a pre-factor.

We will now discuss two approaches to determine  $v_0$ , and at the same time investigate  $\Delta G$ . A chemical reaction runs along the path with the lowest enthalpy connecting the two states, which was before given in the schematics as the reaction coordinate r. In general the system will try to find the nearest stable minimum in the energy landscape. Since we want to know k we will have to make some assumptions about the exact shape of the energy landscape. This can be illustrated by considering a landscape with two minima which are connected by a saddle point. As soon as the protein reaches a point beyond the saddle point it will end up in state B. For calculating k we will need to know the details of state A and the saddle but not of the final state.

## Reaction Rate from Transition State Theory

One approach to get the rates is to assume that all parts of the reaction path can be approximated as parabolas, which means that we can regard all degrees of freedom along the reaction coordinate as harmonic oscillators. The saddle point has the lowest probability to be occupied as the enthalpy is in all other points raising or falling. With the following assumptions:

- 1) If the systems is past the saddle it will end up in state 2, this is non-reversible.
- 2) If the system is in the saddle point (transition state) it can be described classically and is separable from all other coordinates.

The exact calculation will be shown in one of the questions on the questions sheet. The rate dN/dt of reactions from particles over the barrier can be shown to follow

$$\frac{dN}{dt} = \frac{k_B T}{h} \frac{Z_S}{Z_A} \exp\left(-\frac{E_0}{k_B T}\right) [A] = k [A]$$

Where  $Z_A$  is the partition function of the starting point A and  $Z_S$  the partition function of the saddle point. For the calculation of  $Z_S$  one has to fix the reaction coordinate to S and integrate over all other degrees of freedom.

For the reaction rate for a single molecule we can now write down k

$$k = \frac{k_B T}{h} \frac{Z_S}{Z_A} \exp\left(-\frac{E_0}{k_B T}\right)$$

## Reaction Rate from Transition State Theory

There are a few remarks regarding this result:

$$k = \frac{k_B T}{h} \frac{Z_S}{Z_A} \exp\left(-\frac{E_0}{k_B T}\right)$$

- 1) The separation of the degrees of freedom at the transition point from the rest leads to the fact that  $Z_S$  only contains reactions for which S is stable.
- 2) The rate depends only on the characteristics of the start and transition states.
- 3) The rate should get faster at higher temperatures, if this is not the case then this points to a situation where other intermediate states have to be considered.
- 4) The equation assumes that  $E_0$  can be interpreted as the thermal (measured) activation energy. However, this is only a good approximation for small proteins. For larger molecules it is not always possible to separate the degrees of freedom. Then one has to take these effects into account and entropy becomes important. The activation energy is then a free enthalpy.
- 5) The main result is the pre-factor which gives a fundamental rate as  $k_B T/h$ =6.13x10<sup>12</sup>Hz

at room temperature. This defines a fundamental timescale for chemical reactions in the range of picoseconds. This estimation also indicates that with ultra-short laser pulses (10<sup>-18</sup> s) chemical reaction can be imaged.

The transition rate theory does not take into account that the motion in the complex energy landscape is usually overdamped. One obvious reason is that there are so many degrees of freedom in the system that as soon as momentum is transferred from one molecular group to another in the molecule, it is extremely unlikely that this will be transferred back into the reaction pathway.

The thermal activated passage over the barrier connecting two states can again be described as the motion of a point in the energy landscape. We will assume that we look at the over damped case only assuming that at every point along the reaction the system is in equilibrium. This means that we will be able to describe the system in analogy to a diffusion problem.

We will now assume the Gibbs free energy of the system is given by G(r), where r is the reaction coordinate of the system and write down the chemical potential

$$\mu(r) = G(r) + k_B T \ln x(r)$$

With x(r) is the probability density to find the system at position r. Assuming that our particle diffusing in this landscape has a friction coefficient  $\gamma$ , we can write down the average drift velocity as

$$u = -\gamma^{-1} \frac{\partial \mu(r)}{\partial r}$$

The flux is now given by

$$j = xu$$

and as expected for diffusion problems we have continuity

$$\frac{\partial j}{\partial r} + \frac{\partial x}{\partial t} = 0$$



all equations are already given in one-dimensional form to simplify the system. Combining the three last equation we find that the time development of x can be written as

$$\frac{\partial x}{\partial t} = -\frac{\partial j}{\partial r} = \frac{\partial}{\partial r} \left( \frac{x}{\gamma} \frac{\partial G}{\partial r} + \frac{k_B T}{\gamma} \frac{\partial x}{\partial r} \right)$$

This is also known as the Smoluchowski equation which is the overdamped case of the Fokker-Planck equation. If *G* is constant along the reaction coordinate then we would get the simple diffusion equation with the diffusion constant  $k_B T/\gamma$ . With this equation we can now calculate the reaction rate *k*.

The reaction rate is given by the particle current over the potential barrier. Since we assume that at the lowest point the particles vanish and there is now flow beyond the origin at A, the current is  $r_1$ 

$$\frac{\partial}{\partial t} \int_{r_0}^{r_1} x(r) dr = j(r_0) - j(r_1) = -j(r_1)$$

We also assume that the barrier is high, which means that particles near A are in thermal equilibrium. With the following boundary conditions for x(r):

$$x(r) = \frac{\exp(-G(r) / k_B T)}{\int_A \exp(-G(r) / k_B T) dr}, \text{ for } r \text{ around } A$$
$$x(r) = 0, \text{ for } r = B$$

the current  $j_{0}$ , in the stationary case is given by:

$$j_0 = \frac{k_B T}{\gamma} \frac{1}{\int\limits_A \exp(-G(r)/k_B T) dr} \frac{1}{\int\limits_A^B \exp(+G(r)/k_B T) dr}$$

This is the general form for any shape of G(r).

With the assumption of a near parabolic profile for G(r)

$$G(r) \approx \frac{m_{eff}\omega_A^2}{2}r^2$$

Where  $m_{eff}$  is again the effective mass of the particles diffusing in G(r) and  $\omega_A$  is the angular frequency of the state at A.

We can then simplify the expression for  $j_0$  and obtain

Kramers result: 
$$k = j_0 = \frac{m_{eff}\omega_A\omega_S}{2\pi\gamma}\exp\left(-\frac{G_S}{k_BT}\right)$$

This result is similar to the one obtained with the transition state theory we discussed earlier:  $k T Z = \begin{pmatrix} E \\ E \end{pmatrix}$ 

Transition state 
$$k = \frac{k_B T}{h} \frac{Z_S}{Z_A} \exp\left(-\frac{E_0}{k_B T}\right)$$

In both cases, k is determined by the height of the barrier, as expected. The main difference is that  $E_0 - k_B T \ln Z_s$  which is the free energy. The difference between free energy and free enthalpy only plays a role if the density of the protein changes substantially. The prefactors are also different. The Kramers approach combines damping and effective mass, which thus cannot be separated. Due to the damping term, k can be much lower if calculated by the Kramers theory.

## **Protein Unfolding**

- Atomic force microscope as single-molecule tool
- Imaging of membrane proteins
- Unfolding of proteins under variable loads



The atomic force microscope consists of three key elements. The imaging system (laser and photodetector), tip for imaging the sample mounted on a cantilever arm and a piezoelectric stage which controls sample position. The force is controlled by detecting the bending of the cantilever arm which can be approximated as a classical spring. The topography of the sample is measured by keeping the photodetector signal (force) as constant as possible. This is achieved by moving the sample with sub-nm accuracy in three dimensions using the piezoelectric stage. Spatial resolution depends on sharpness of tip and force resolution on the total mass of the cantilever.

## Tips for atomic force microscopes \*



The easiest approach to obtain tips for AFM is using a wet (anisotropic) wet-chemical etch of silicon. The etching rates along the different crystal axis is different and leads to a pyramidal tip as shown above with diameters at the sharp end of below 10 nm. With these tips atomic resolution can be achieved. However, there are more sophisticated approaches for making AFM tips, which can now be functionalized with carbon nanotubes to achieve higher resolution, and imaging samples with high aspect ratios (height/width). It is interesting to note that imaging of single atoms even at room temperature is possible. The AFM is Ideally suited for soft (polymers) or biological samples due to possibility to work in liquids especially water.

# AFM: Imaging biological membranes and proteins

(Engel 2007)



The interactions between tip and sample are critical for the resolution. Taking into account that there are a number of interactions we have to take into account, electrostatic, van der Waals, hydrophobic, etc. this is a complex problem. However, in aqueous solutions containing salt the tip-sample interaction can be tuned by adjusting the salinity and the type of ions in the solution surrounding the sample to minimize long-range interactions and enable a direct contact between AFM tip and sample surface resulting in the highest spatial resolution. http://www.damtp.cam.ac.uk/user/gold/teaching\_biophysicsIII.html

## Imaging biological membranes

(Engel 2007)



One of the most striking examples for the imaging of membranes and their proteins are the images obtained for bacteriorhodopsin. Bacteriorhodopsin forms a densely packed hexagonal "crystal" in biological membranes. These can be spread out on atomically flat mica surfaces as a support. For the high resolution imaging the aqueous buffer has to be adjusted. To minimize damage to the surface and reduce changes in the proteins the maximum force on the sample is minimized and is around ~100 pN. By overlaying the individual images a high resolution image (insets in middle and right) can be reconstructed.

## Biology: Muscle contraction requires Titin \*

Titin (aka connectin) is a protein found in muscles and is responsible for passive tension when muscle is stretched



Imunoglobulin (Ig) sub domain responsible

for rubber like behaviour of titin



## Full Titin 'I-Band' unfolding with AFM



Dynamic force spectroscopy can be used to study the unfolding patterns of proteins. Ig domains of Titin are ideally suited to demonstrate the capabilities of AFM unfolding spectroscopy due to its regular structure with identical sub-domains. Saw tooth pattern in the force-distance plots indicates unfolding of the ingle protein sub-domains in sequential order.



Using biochemical techniques, recombinant Ig domains with a well-defined number of 4 and 8 repeats can be engineered as control experiment. Data fits to expectations perfectly, showing up to 8 unfolding in the upper and up to 4 unfolding peaks in the lower panel.

#### Refolding of proteins with dynamic force spectroscopy



(Gaub 1997)

With the AFM based dynamic force spectroscopy one can also probe the refolding of the domains. Protein refolds only in low force state after the extended chain is relaxed to low extension and thus lower forces. This is an essential proof that the process is reversible induce and does not fundamental changes in the protein. Refolding is force dependent. In high force state refolding occurs no on experimental time scale (lower panel).

Using force spectroscopy with the AFM we can study unfolding and refolding of proteins, the time scales and force dependence of the process.

#### Unfolding force depends on loading rate



#### (Gaub 1997)

The maximum unfolding force detected in the process depends on the pulling speed of the AFM retracting from the surface and stretching the protein. We can thus define a loading rate with of [N/s]units which measures how quickly we apply forces to the molecular bonds in the protein. The question is now if we can understand the dependence of the unfolding force on the pulling rate.

This can be understood by simplifying the system into breaking a bond that can either be open or closed. In the next slide we will develop a model for the loading rate dependence using this two-state system.

#### Unfolding force depends on loading rate



Probability p(F) for a bond to break depends on loading rate  $\dot{f}$ 

With the time dependence of the force given by F(t) = ft