



Handout for Practical Course

Magnetic tweezers and its application to DNA mechanics

When: 9.00 am

Where: Biotec, 3rd Level, Room 317

Tutors: Ralf Seidel, Daniel Klaue (german, english language)

Aim: Imagine DNA as a flexible rod. In this experiment we want to measure the flexibility of this rod, i.e. how easy is it to bend it (determined by the bending modulus) and how easy is it to twist it (determined by the torsional modulus).

Introduction

1. Magnetic Tweezers:

Magnetic tweezers are a technique, which allows to probe the mechanical properties of single biomolecules, e.g. a DNA molecule. For this single DNA molecules are brought into a flow cell, i.e. a very small chamber filled with buffer (Fig. 1). One end of each molecule is specifically attached to the bottom of the cell, the other to a superparamagnetic bead (via an antibody (Fig. 2)). A pair of magnets above the flow cell is exerting force onto the magnetic bead and stretches the molecule. Varying the height of the magnets above the flow cell allows to apply different stretching forces, whereas turning the magnets allows to twist (supercoil) the DNA molecule. In order to measure the DNA extension at a given force, the x,y,z position of the magnetic bead is determined in real time from video images of the bead using smart image analysis and computational algorithms.

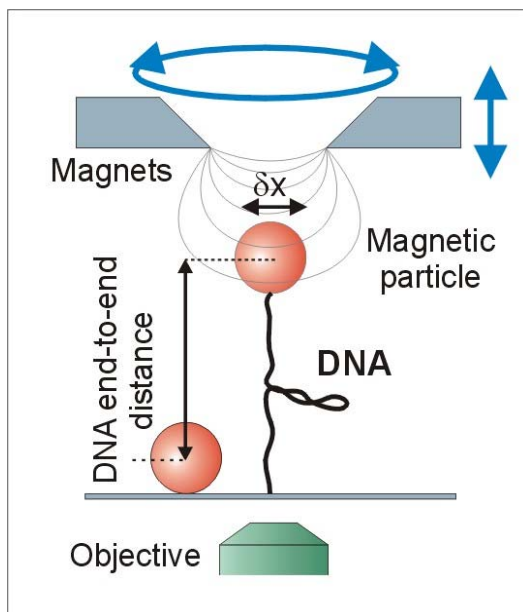


Fig. 1

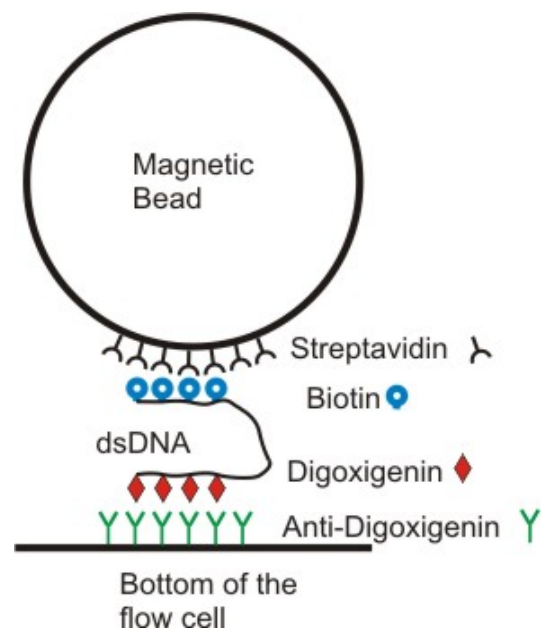


Fig. 2

For finding x and y one uses the symmetry of the bead image along these axes. To determine the z position of the bead one uses the diffraction rings at the bead, which depend on the focal position of the bead (Fig. 3). A set of reference images taken at well defined focal positions is used for calibration. During a measurement the radial profile of the actual bead image is constantly compared to the reference images and the z-position is interpolated. In order to allow measurements of the total length of a DNA molecule and to reduce drift of the system, additionally the z-position of a non-magnetic reference bead at the bottom of the flow cell is measured (Fig. 1).

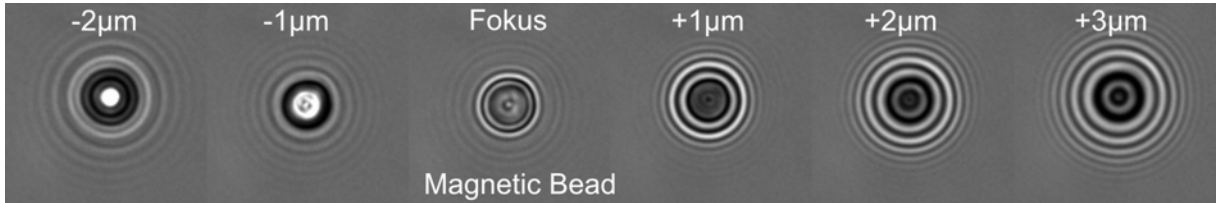


Fig. 3: Magnetic Bead under and over focus. The diffraction rings determines the z position of the bead.

2. Calibration:

Due to frequently hits by surrounding water molecules, the bead as well as the flexible DNA molecule are in permanent motion (Brownian motion). The amount of Brownian motion lets us derive the force acting on the bead. This is quite simple to imagine: As harder the magnetic force is pulling the bead as harder is it for the bead to leave the the upright stretching position and as smaller is the Brownian motion

Mathematically the bead-DNA configuration can be described as an inverted pendulum (Fig. 3). A restoring force (F_b), which is proportional to small displacements, always tends to bring the bead back to equilibrium (upright stretched DNA). This is similar to a spring with a spring constant κ , which is in this case F_m/l . According to the equipartition theorem, the mean energy of this spring is in one dimension equal to the thermal energy per degree of freedom, which is $\frac{1}{2} k_B T$. Rearranging these terms leads to a formula witch connects the pulling force (F_m) with the length of the DNA molecule (l) and the mean square displacement in x ($\langle x^2 \rangle$), according to:

$$F = \frac{k_B T \cdot l}{\langle x^2 \rangle}$$

To know at which magnet position which force is acting on the DNA it is necessary to calibrate the system. Therefore one measures the x or y positions and the length of the DNA over a long time for different magnet positions. After determination of the mean square x (or y) one can calculate the force for each magnet position. As the force decays exponentially with the distance of the magnets from the flow cell one can also interpolate forces at magnet positions, which have not been measured.

3. WLC:

In the range of forces, which we apply with the magnetic tweezers (0.01 – 30 pN) DNA does not behave like a rubber band, i.e. we hardly elastically overstretch it. However, we still need quite some force in order to stretch it. Why is this? In an environment subjected to Brownian motion the solvent molecules always try to disorganize the flexible DNA. In the absence of force a DNA molecule of few kbp in length is a random coil. Upon pulling we have to

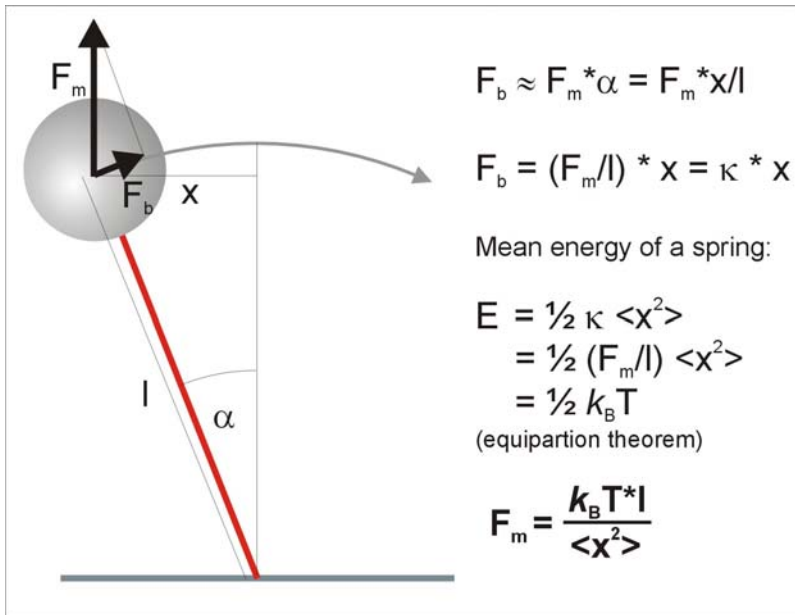


Fig. 4

counteract the water molecules, which want to drive it back into the random coil configuration, and have to actively apply force. In other words, we have to fight against entropy, which tries to randomize the molecule configuration. The amount of force we have to exert depends on the number of flexible segments we have in the molecule, i.e. how many degrees of freedom we have. A molecule, which is hardly bendable over its length, will always be rather straight. So a small stretching force is required. However, if the molecule is very flexible, i.e. it can be seen as many independent stiff segments, one has lots of degrees of freedom subjected to entropic disorder. Therefore, a high force is needed to “order” or stretch the molecule. The quantity, which describes the flexibility of a polymer like DNA is the persistence length p . It can in easy words be described as the length over which DNA can be bent to 90° by the Brownian motion. For dsDNA p equals 50 nm or 150 bp, whereas for ssDNA it equals to few nm. DNA shorter than the persistence length can be seen as a rather straight rod, whereas DNA much longer than the persistence length is a random coil. The persistence length is linked to the bending modulus M_b via: $M_b = k_B T \cdot p$, where M_b determines the bending moment M or torque, to hold a bent rod with a bending radius R according to: $M = M_b/R$. The force extension behaviour of a flexible polymer is described by the worm-like-chain model according to:

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

which only depends on the persistence length p and L the contour length of the DNA.

4. Supercoiling:

By rotating the magnets above the flow cell one can twist/supercoil the DNA molecule. Because the molecule is anchored at its ends one induces so called plectonemes comparable to an over wound phone cord (Fig. 1). Inducing supercoils (at a certain force) reduces the DNA end-to-end distance (Fig. 6). At low forces super coils are induced independent whether you turn with or against helical turns of the DNA (positive or negative supercoils). At slightly higher forces it's energetically more favourable to partially unwind the double helix into single strands while turning negative. At even higher high forces (> 5 pN)

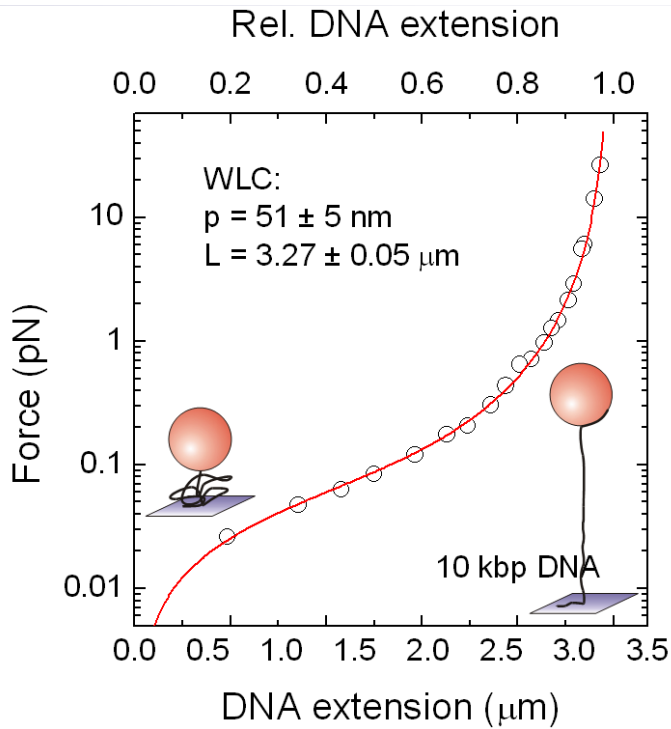


Fig. 5: Force extension curve of a 10kbp long dsDNA molecule. The red line is a fit with the WLC model to the experimental data (open circles).

one cannot anymore induce plectonemes for positive supercoils, as the DNA undergoes a structural transition, where the bases start to turn outside (Fig. 6).

5. Torque and Torsion:

Upon twisting a DNA molecule it takes a number of turns, before the DNA length reduces significantly and plectonemes are formed. The point (N_{buckling}) where DNA starts to form plectonemes with a constant length reduction per turn is called buckling instability (Fig. 7a).

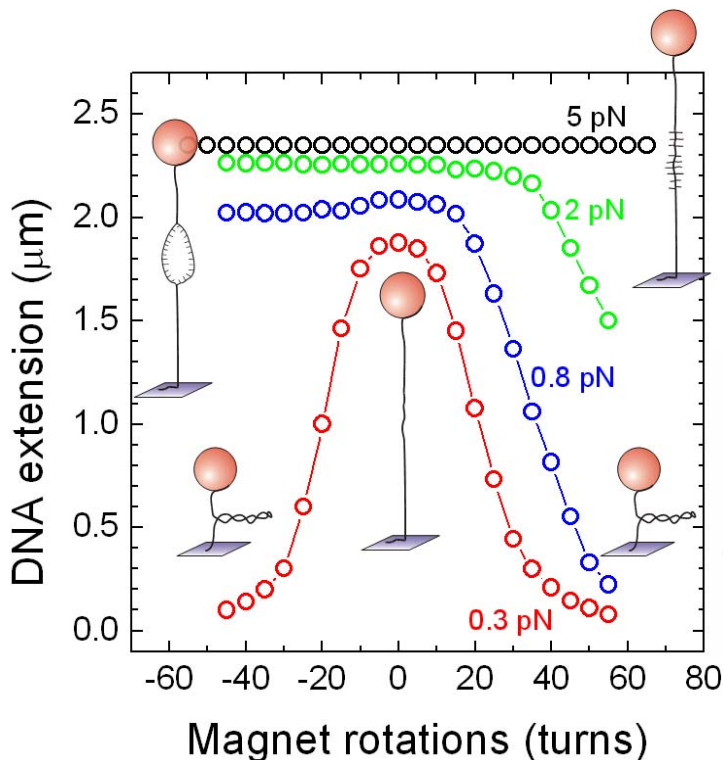


Fig. 6: Rotation extension curves for different forces. At higher forces one cannot induce supercoils but denature the DNA molecule.

Up to that point the twist or torque builds up linearly with the number of turns (Fig. 7b). Beyond the buckling instability the torque stays constant, as all supercoils which are generated is now transformed into plectonemes (Fig. 7a). This is because the energetic cost to further twist the DNA is now higher than the cost to move the bead down against magnetic force and to bend the DNA into a plectoneme. Calculating the competition between DNA twisting and plectoneme formation within a simplified model allows to estimate the critical torque (Γ_{buckling}) where plectonemes are start to form:

$$\Gamma_{\text{Buckling}} = \sqrt{2k_B T \cdot \rho \cdot F},$$

and the length decrease per turn after the buckling instability:

$$\Delta l / \text{turn} = 2\pi \sqrt{\frac{k_B T \cdot \rho}{2F}}$$

The relation between torque and induced supercoils/turns N before DNA starts buckling is determined by the torsional modulus of DNA according to:

$$\Gamma = \frac{C}{L} \cdot 2\pi \cdot N$$

where L is the crystallographic length of DNA.

In analogy to the bending modulus there is following relation between torsional modulus and the torsional persistence length ρ_{torsion} :

$$C = k_B T \cdot \rho_{\text{torsion}}$$

ρ_{torsion} indicates the length over which the DNA is considered stiff regarding torsional displacements.

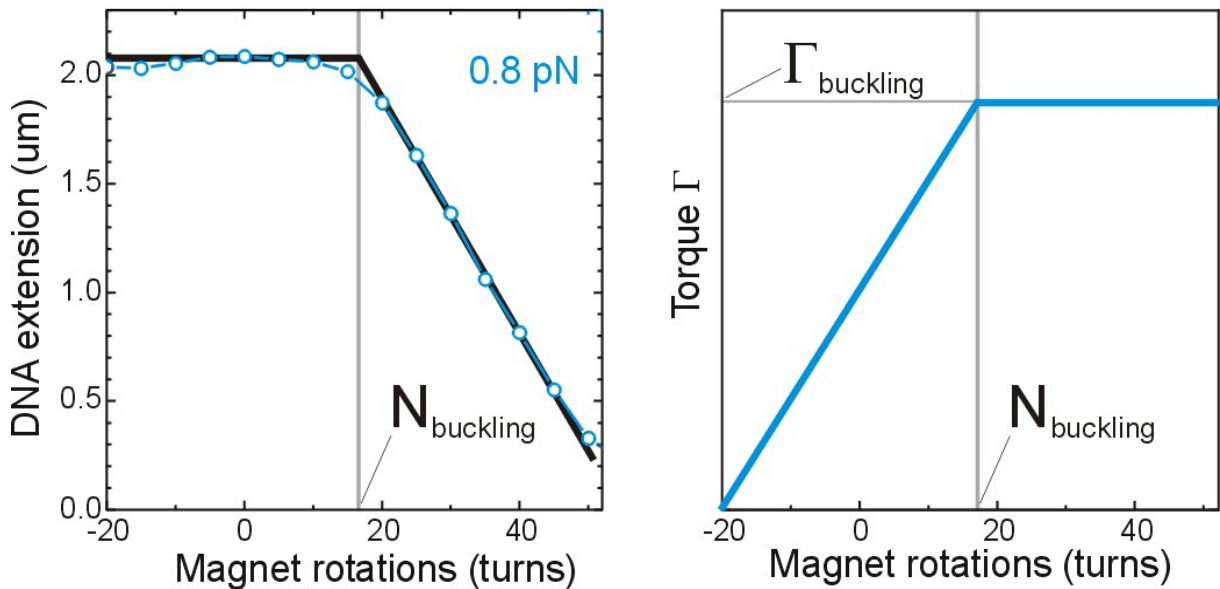


Fig. 7: **a)** From fitting a rotation-extension curve the position of the buckling instability (N_{buckling}), at which plectonemes start to form, can be determined. **b)** Schematic representation of the torque within the DNA molecule as a function of the induced supercoiling.

Experiments:

The setup will be prepared with an installed flow cell filled with an appropriate buffer, reference beads and a ~10kbp DNA molecule attached to a magnetic bead. An introduction to the software to operate the setup and evaluate the data will be given.

1. Find an unnicked, i.e. supercoilable DNA molecule.
2. Take rotation curve at about 0.3 pN (2 mm magnet for 1 μm magnetic beads, 3.5 mm for 2.8 μm magnetic beads). Determine rotational zero position and correct the software for that offset.
3. Take a force-extension curve for an appropriate DNA molecule at magnet positions of 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5. mm magnet position.
4. Calibrate the forces by calculating the mean square displacement along the y-direction.
5. Fit the force-extension curve with the WLC model. Determine the persistence length p and the contour length of the molecule.
6. Take rotation-extension curves for 6-7 different forces, e.g. 0.2, 0.3, 0.5, 0.8, 1.5, 2.5, 4 pN.
7. Fit the slopes of the rotation curves to find N_{buckling} and calculate for each force the corresponding Γ_{buckling} .
8. Plot Γ_{buckling} over N_{buckling} for all forces in order to find the torsional modulus C for DNA.

Questions:

1. What can you measure with magnetic tweezers?
2. How can you measure forces with Brownian motions?
3. How can the mechanical behaviour of DNA be described (What is the persistence length)?
4. What are plectonemes and why they do arise?
5. What happens to DNA when plectoneme formation is inhibited at elevated stretching forces ?
6. Explain the development of torque upon DNA supercoiling!

Literature:

Strick et al., Behaviour of supercoiled DNA, 1998, Biophys J.

Howard J., Mechanics of motor proteins and the cytoskeleton, 2001, Sinauer