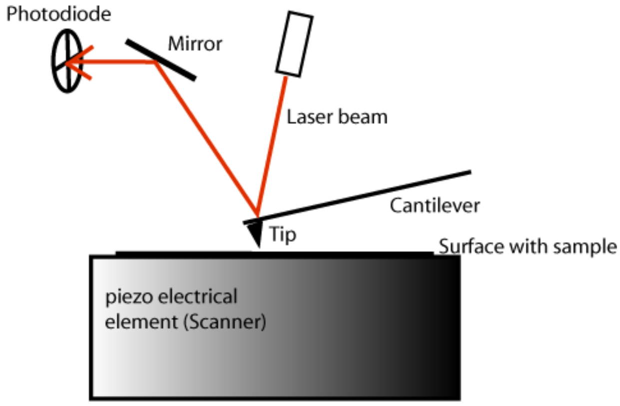
Introduction

Scanning force microscopy (SFM)

Some background

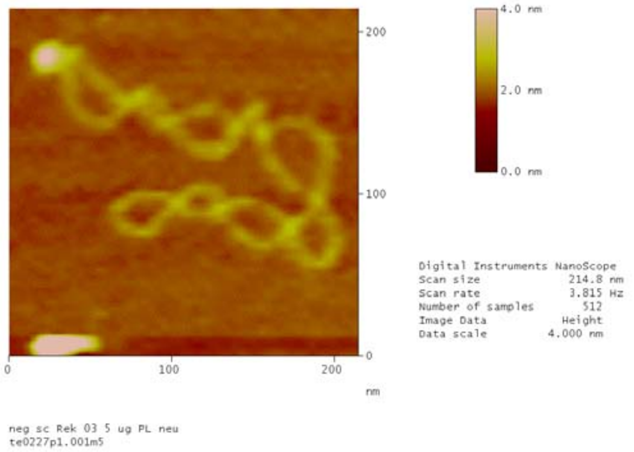
SFM technology's ancestor, STM (Scanning Tunneling Microscopy), was invented in 1981 by Gerd Binnig and Heinrich Rohrer at IBM in Zurich, Switzerland. They went on to win the Nobel Prize for physics with this discovery. Their work has formed the basis for all serious scanning probe microscopy research worldwide ever since.



SFM works like an old fashioned record player.

A scanning force microscope (SFM) basically works like an old fashioned record player, where the movement of the needle sends the recorded impulse through the amplifier and on to the speakers to produce music.

Unlike to the record player a SFM detects the movement of the needle through a laser beam, which is reflected from the top of a cantilever to a photodiode. The bending of the cantilever can be measured by using a four-quadrant photodiode. The strength of the cantilever deflection is assigned to a colour scale and the data is used to generate a map of the surface topography.



Topography map:

Superhelical plasmid DNA with one nucleosome

The surface

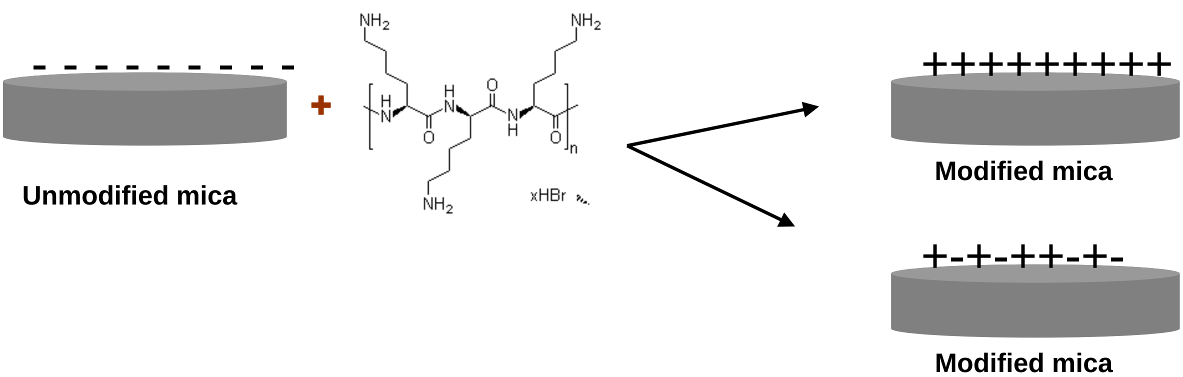
Samples that are to be measured must be deposited onto a surface, which is flat on atomic scale.
Materials that are used are e.g. glass, gold or mica. We will use mica, which belongs to the group of sheetsilicates (phyllosilicate). These minerals have the property of a highly perfect basal cleavage which is explained by the hexagonal sheet like arrangements of its atoms. Sheet-silicates like mica provide a negatively charged surface.

Unprocessed mica

Processed mica plates for AFM

Imaging of DNA on a mica surface

DNA is negatively charged due to its phosphate backbone. A mediator is needed to facilitate binding of DNA to the mica surface. Metal ions (Mg^{2+}) are being used as well as modifications of the surface properties by coating of the mica surface with positively charged substances like polylysine.
We will be using the polylysine coating method which was developed here in this Lab. Basically we will be coating the mica surface with different polylysine concentrations thereby altering the DNA binding strength of the surface.



Experimental part

We will estimate the diffusion coefficient of different DNA structures (linear, and supercoiled plasmid DNA) from their shapes as measured by AFM.

We will prepare mica surfaces with different binding abilities by using the method described above. We will then perform AFM using different DNA topologies (linear DNA, supercoiled plasmids). Using the collected data we will then perform some approximative calculations on their overall shape in 3 dimensions and calculate their diffusion coefficient.

Calculation

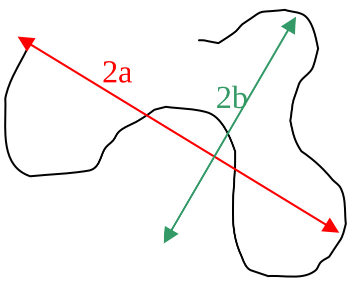
The diffusion coefficient D of a sphere with the radius r_0 is:

(1) $$D = \frac{kT}{6\pi\eta r_0}$$

k = 1.38 * 10^{-23} J/K (Boltzmann constant)
η = 0.001 Js/m^3
T = 293 K
r_0 = Stoke´s radius

The radius r_0 is obtained from the volume V_{sphere} estimated from the dimensions of the molecules bound to the scanning surface.

Example:



(2) $$V = \frac{4}{3}\pi a b^2$$ (for an ellipsoid)

(3) $$r_0 = \sqrt[3]{\frac{3V}{4\pi}} = \sqrt[3]{a b^2}$$

The frictional coeffiecient of a sphere is given by:

(4) $f_0 = 6\pi\eta r_0$ in Js/m^2

Using equation (1) and (4) we will get:

(5) $$D = \frac{kT}{f_0}$$ in m^2/s

At a given volume (V, see above) the frictional coefficient of the ellipsoid ($f_{ellipse}$) which is described through the measured values for **a** and **b** (see above) is given by the axial ration a/b:

(6) $F = \frac{f_{ellipse}}{f_0}$

Where F can be read from the following shape parameter table for ellipsoids:

Axial ratio (a/b)	**F (Prolate)**	**F (Oblate)**
1	1.000	1,000
2	1.044	1,042
3	1,112	1,105
4	1,182	1,165
5	1,250	1,244
6	1,31	1,277
8	1,433	1,374
0	1,543	1,458
15	1,784	1,636
20	1,996	1,782
30	2,356	2,020
40	2,668	2,212
50	2,946	2,375
60	3,201	2,518
80	3,58	2,765
100	4,067	2,974
200	5,708	3,735

The diffusion coefficient of the ellipsoid is then defined by:

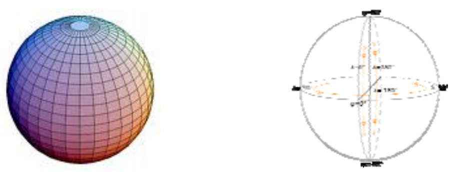
(7) $D_{ellipse} = \frac{kT}{f_{ellipse}}$

Effects of shape on translational friction properties

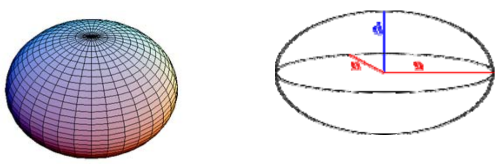
Assuming a „spherical appearance“ for a molecule the diffusion coefficient (**D**) depends only on the hydrodynamic radius or „Stokes radius“ $\mathbf{r_0}$ (at given temperature and viscocity of the medium). However, most shapes of biological macromolecules (proteins, DNA) do not resemble spheres. A significant fraction appears to be compact globular, irregular rigid bodies. For these shapes, an ellipsoid of revolution is a more realistic model than a sphere. There are two classes of such ellipsoids, both of which are limiting cases of the general ellipsoid with three different axes:

1) The **oblate** ellipsoid is a disk shape, generated by rotating an ellipse around its short semiaxis (b); the two long semiaxes (a) are identical.
2) A **prolate** ellipsoid is a rodlike shape generated by rotating an ellipse around its long semiaxis (a); here the two short semiaxes (b) are identical.

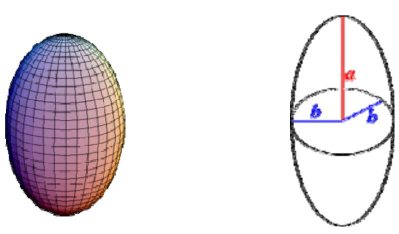
For either kind of ellipsoid the axial ratio ($\mathbf{p_r}$) is defined as a/b, the ratio of the long to the short semiaxes. For equal volumes the surface area of either ellipsoid will be greater than that of a sphere.
Due to the fact that the volume of a molecule is proportional to the molecular weight we see that (for constant mass) the more a molecule deviates from a sphere the larger its frictional coefficient and the smaller the diffusion coefficient will become.



Sphere: Perfectly round in three-dimensional space



Oblate spheroid with major axes (a) and minor axes (b).



Prolate spheroid with major (a) and minor axes (b)