

DNA Origami

Advanced Lab Course

Supervisors

Max Hohnemann

Pierre Stoemmer

Elena Willner

Massimo Kube

This Lab Course is designed to fit comfortably within one afternoon, therefore the overall duration does not exceed 4h.

1 Introduction and Goals

Deoxyribonucleic acid (DNA) has ideal features for producing artificial structures of user defined shape and functions on the nanometer scale. The folding of a long DNA scaffold strand assisted by shorter strands (brief: DNA origami [1] [2]) was a decisive breakthrough in the field of DNA nanotechnology: thousands of atoms are arranged relative to each other with an unprecedented precision due to the pair-wise interactions of the nucleotides. The production of DNA nanostructures is based on the principle of self organisation and is therefore relatively simple and efficient, making it possible to produce highly complex structures with molecular weights in the megadalton range. DNA-origami based nanotechnology has an immense potential in the production of basic applications in various fields of science and technology. The goal of this lab course is to give an insight into the field of DNA origami structures, including their fabrication, structural analysis, and an application. In the first part of the practical course the folding behaviour of an DNA origami brick is analysed via gel electrophoresis. In the second part, the energy landscape of two hybridising DNA single strands is measured by using a DNA origami force spectrometer.

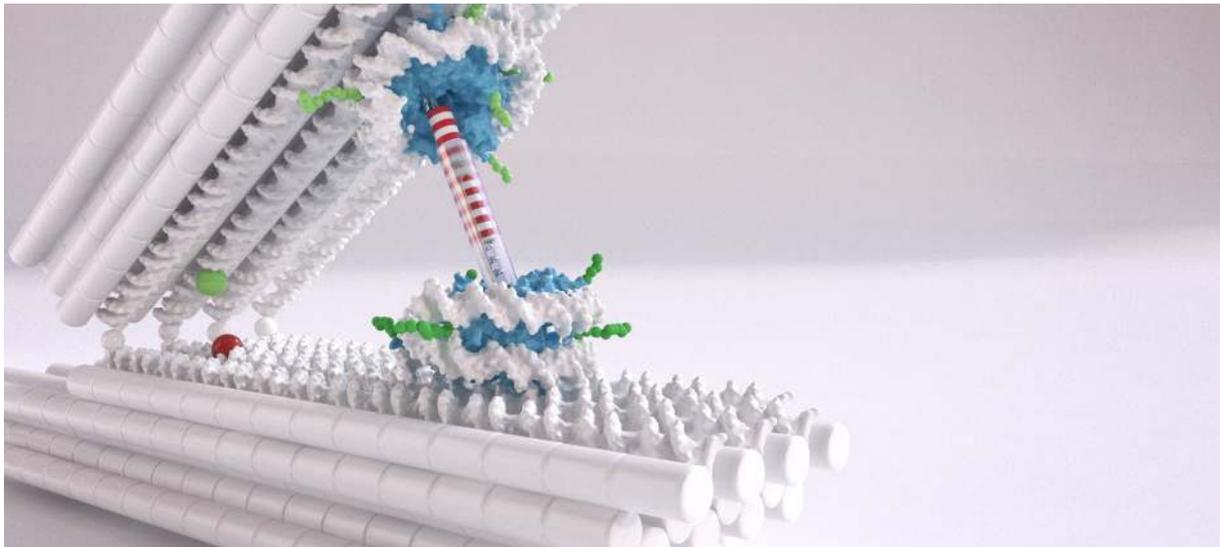


Figure 1: DNA origami force spectrometer. In this model the measurement of the interaction between two nucleosomes is shown. DNA segments and fluorescent dyes are represented by cylinders and coloured spheres, respectively. The forces between the nucleosomes are illustrated by a spring balance.

2 Theoretic Background

In many organisms, DNA serves as storage for genetic information, encoded in the sequence of its building blocks. In DNA nanotechnology, the user defined sequence of many different DNA single strands contains the structural information of the desired shape and function of the nano-object. The following chapter explains the basic properties of DNA and how they are exploited

to design DNA nanostructures.

2.1 Basic properties of DNA

DNA is a biopolymer of repeating subunits, termed nucleotides. Each nucleotide is composed of the monosaccharide 2-deoxyribose with a phosphate at its 5' carbon atom and nucleobase at its 1' carbon atom [3] In natural DNA four different nucleobases, termed adenine (A), thymine (T), guanine (G) and cytosine (C) can be found. Two neighbouring nucleotides are connected with each other via a phosphodiester bond between the 3' carbon atom of the first nucleotide and the phosphate at the 5' carbon atom of the second nucleotide. Double stranded B form DNA (in the following referred to as DNA) consists of two strands running antiparallel to each other in a double helical shape. The two backbones are located at the exterior of the double helix with the nucleobases point inwards facing each other (fig. 2.1a). Bases facing each other are complementary and form so called base pairs (bp) via hydrogen bonds (fig. 2.1b). Additionally to the inter-strand base pairing, adjacent nucleobases interact with each other via non covalent base stacking between the aromatic rings giving the major contribution to the stability of the double stranded conformation of DNA (fig. 2.1c).

In solution, DNA has a helical pitch of 35.7 Å and an inter-strand base stack distance of 3.4 Å, which results in 10.5 bp per turn and a twist angle of 34.3°. Due to the non-linear geometry of the base pairs, DNA shows a major and a minor groove. These geometric properties have to be taken into account during the design of DNA origami objects.

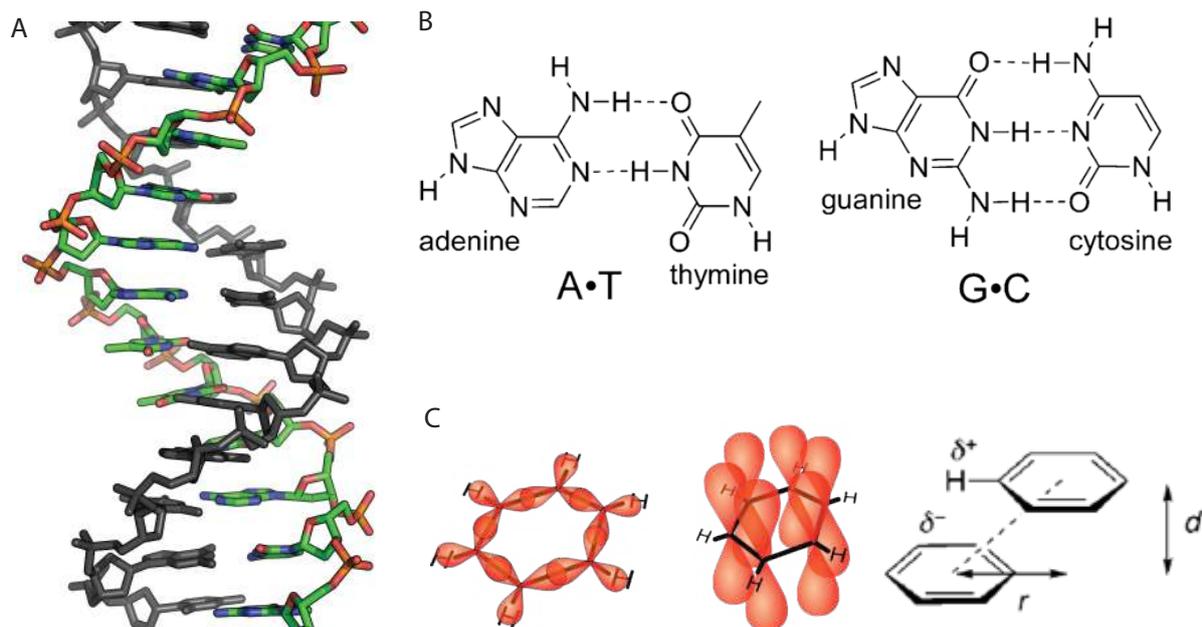


Figure 2: DNA properties. (A) Three dimensional structure of a DNA double helix (PDB 1D28) [3]. The planar bases are located in the interior between the two backbones, stabilising the double helical conformation via base pairing and base stacking interaction. (B) Base pairing. The complementary bases adenine and thymine, and guanine and cytosine form base pairs via hydrogen bonds (dashed lines). (C) Base stacking. The overlapping sp^2 orbitals of the nucleobase carbon ring atoms form a framework of δ -bonds in one plane, with the p_z orbitals building a delocalised π -system above and below the σ -plane. The energetically favoured shifted conformation of the base stacks leads to the helical structure.

2.2 DNA Origami

A typical DNA origami object is formed by a long circular “scaffold” DNA strand and multiple short “staple” oligonucleotides interacting with each other via the hydrogen bonds of the base pairs [1]. The helices are arranged on a square or a honeycomb lattice and connected with each other via Holliday Junctions (fig. 2.2a-c). These two preferred lattice arrangements result from the geometric properties of DNA allowing a connection between a helix and one of its neighbours every 7 and 8 base pairs, corresponding to a 240° and a approximately 270° turn in the honeycomb and square lattice, respectively. In general, each staple connects multiple helices with each other, contributing to the overall stability of the DNA origami object. The formation, also termed folding, takes place in a one pot reaction where the many single strands are mixed in a buffer solution. The objects self assemble via hybridisation of the individual strands whereby the well folded object displays the global minimum in the folding energy landscape. However, local energetic minima can act as kinetic traps leading to misfolded objects. The folding quality can be analysed via gel electrophoresis and transmission electron microscopy. The approach of DNA origami allows high precision design and high yield production of nanoobjects. They find application for instance in form of a nano force spectrometer to measure the interaction strength of various test specimens.

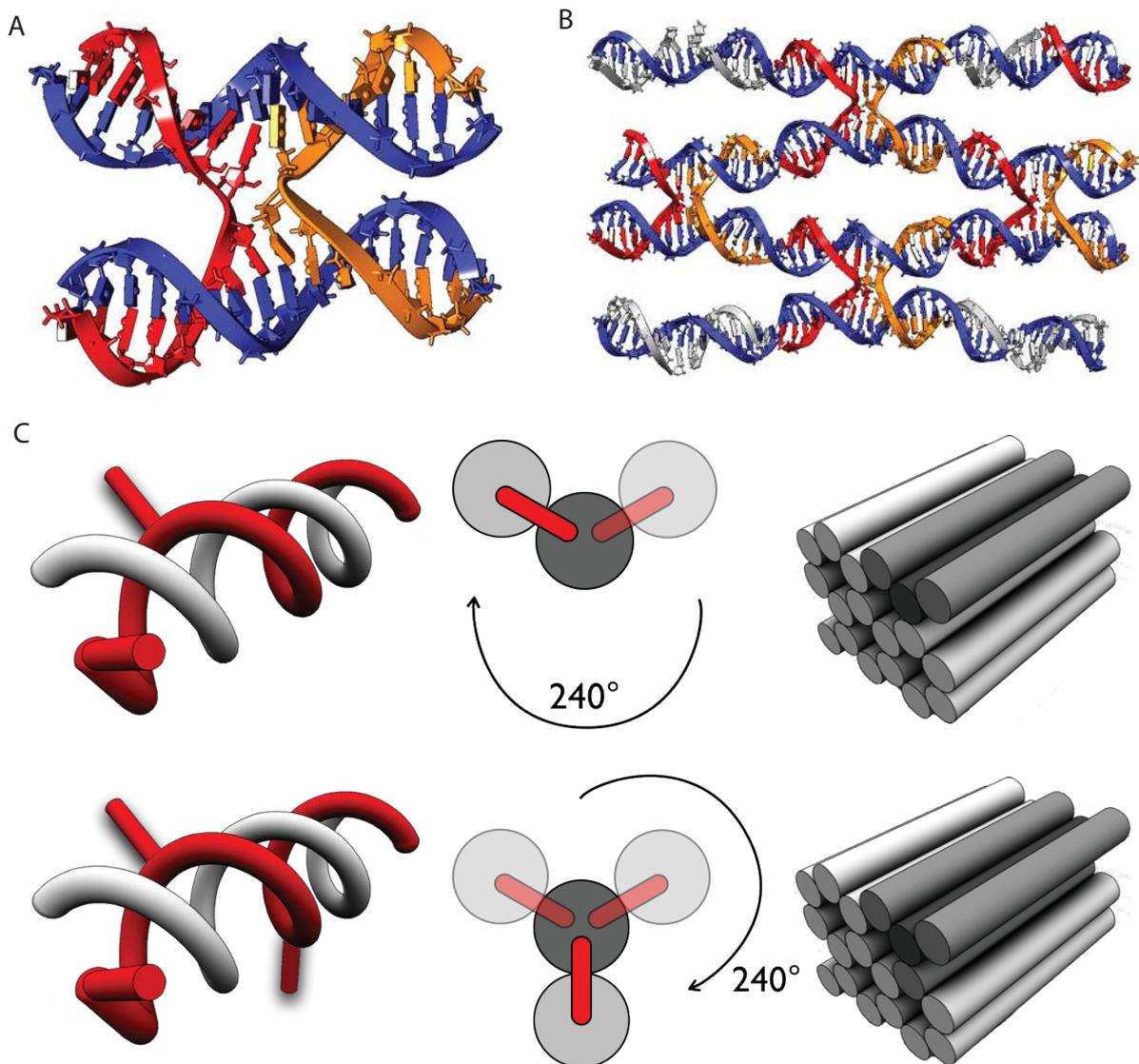


Figure 3: DNA Origami. (A) Holiday junction. Four DNA single strands, part wise complementary to each other, form a Holiday junction. (B) Network of Holiday junction. (C) Honeycomb lattice. In this arrangement a helix can be connected to one of its three neighbours every 7 base pairs corresponding to a 240° turn.

2.3 DNA Origami force spectrometer

Single-molecule force-spectroscopy methods have led to important insights into the functions and mechanical properties of proteins and nucleic acids. For example, one can measure the unfolding landscape of proteins or the force-dependent extension of ssDNA. Conventional force-spectroscopy methods are, however, challenged by their low throughput and by their requirement for connecting microscopic specimens to macroscopic instrumentation - it is like playing Jenga with an excavator. Furthermore, these methods do not allow the user to control the orientation

of mounted target molecules. To overcome these challenges, we developed a nanoscopic force spectrometer made from DNA.

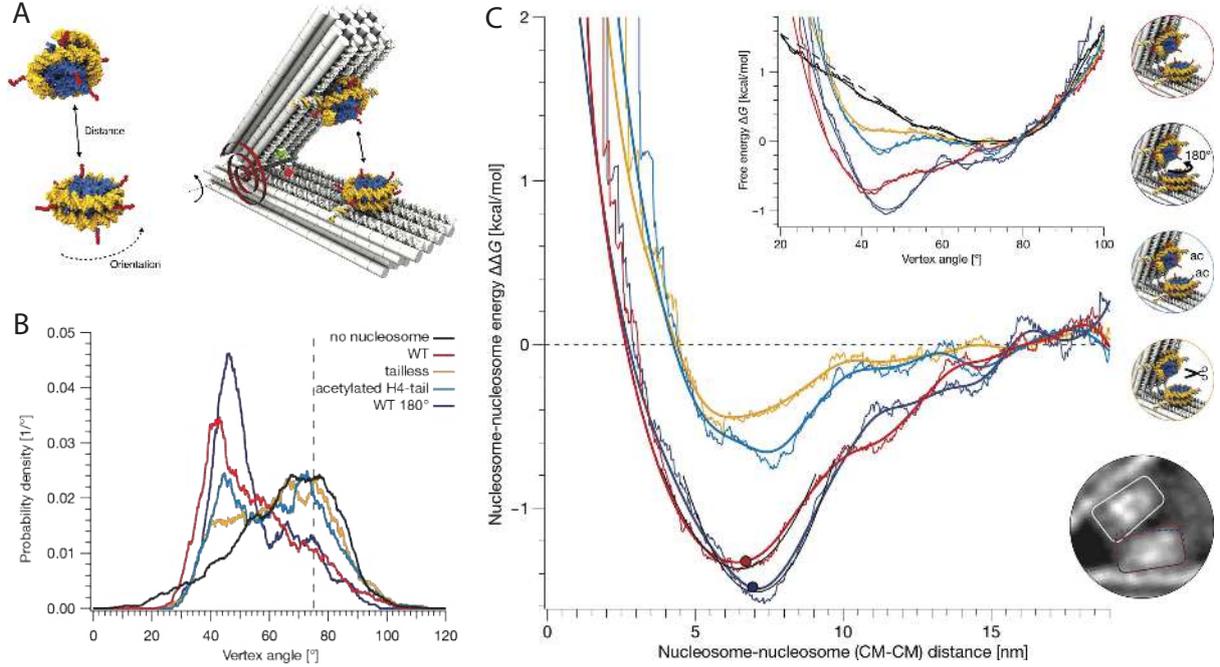


Figure 4: DNA Origami Force Spectrometer. (A) DNA origami force spectrometer with nucleosomes as test sample. Consisting of two arms connected with a hinge the force spectrometer can be used to measure the interaction between two test sample specimens, in this case between two nucleosomes. The spring-like force of the hinge is illustrated by a spring. Cylinders represent DNA helices. The histone octamer, its tails and the DNA of the nucleosome is coloured in blue, red, and yellow, respectively. (B) Angle probability density of the bare spectrometer (black) and loaded with different nucleosome test specimens (coloured). (C) Energy landscape. The nucleosome-nucleosome energy landscape is calculated from the energy landscapes of the force spectrometer with and without test specimens [4].

The DNA origami force spectrometer (Figure 5 A) was developed as a tool of similar size as the test specimen [4]. It consists of two arms connected by a hinge and a spring with attachment sides for the test specimens on each arm. In solution, the arms can open and close and therefore vary their enclosed angle. Every angle corresponds to a certain state of the underlying energy landscape (Figure 5 B,C). In turn, the probability of the force spectrometer to be in a certain state, i.e. a particular angle Θ , is determined by its free energy as described by the Boltzmann distribution:

$$p(\Theta) = \frac{1}{Z} e^{-\frac{E(\Theta)}{k_B T}} \quad , \quad Z = \int_0^{2\pi} e^{-\frac{E(\Theta)}{k_B T}} dx \quad (1)$$

with the probability $p(\Theta)$ of the particle being in the state with the arms enclosing the angle

Θ and having the energy $E(\Theta)$, and the partition function Z as a normalisation. From this, the energy distribution $E(\Theta)$ can be calculated by taking the logarithm of the probability density.

$$\frac{E(\Theta)}{k_B T} = -\ln(p\Theta) - \ln(Z) \quad (2)$$

with $\ln(Z)$ as a normalisation constant depending on the underlying energy landscape.

The potential of the test specimen (TS) pair can then be calculated from the probability densities of the force spectrometer with and without test specimen:

$$E_{TS} = E_{withTS}(\Theta) - E_{noTS}(\Theta) = k_B T [-\ln(p_{withTS}(\Theta)) + \Delta + \ln(p_{noTS}(\Theta))] \quad (3)$$

Assuming zero interaction of the two test specimens at high angles one can calculate the test specimen dependent shift constant Δ

$$\Delta = \ln \left(\frac{\int_{\Theta_0}^{2\pi} p_{withTS}(\Theta) d\Theta}{\int_{\Theta_0}^{2\pi} p_{noTS}(\Theta) d\Theta} \right) \quad (4)$$

where p_{withTS} and p_{noTS} are the probability density estimates of the spectrometer with and without test specimen, and Θ_0 as a cut off where the interaction goes to zero.

Question: Which assumptions have to be made to use this approach?

3 Experimental Procedure

3.1 General lab safety rules

The lab in which we are going to work is a S1 area. Drinking and eating in the lab is not allowed. Please pay attention to the instructions of the supervisors and do not touch anything without adequate protection (lab coat and gloves, in particular nitrile gloves in the gel area). Pay particular attention to the areas delimited with red tape (potentially contaminated areas). Change gloves every time you touch something in the contaminated areas.

During the course we will handle dangerous material, in particular EtBr for gel preparation and UFO stain during TEM sample preparation. In both cases particular attention must be taken to ensure cleanliness. The sample preparation must only be carried out on properly equipped and marked benches or fume hood. Radioactive and EtBr-containing waste must only be disposed in labelled containers.

3.2 42 helix bundle

In the first part of the lab course we will fold 42 helix-bundles (42 hb), once using all DNA strands and once leaving out 20 strands. This brick consists of 42 DNA double helices arranged in a honeycomb lattice (Figure). 140 staples fold the 7560 base long scaffold strand into the desired shape.

3.2.1 Folding of 42 helix-bundle

The staples are delivered in 96 well plates, where each kind of staple is inside a different well at a concentration of 100 μmol . The first step consists in mixing 10 μL per staple in a reaction tube. This mixture is called pre-stock. Which is the final volume and concentration of the pre-stock? For the folding itself, we need 4 different components: scaffold, staples from the pre-stock, folding buffer and magnesium chloride.

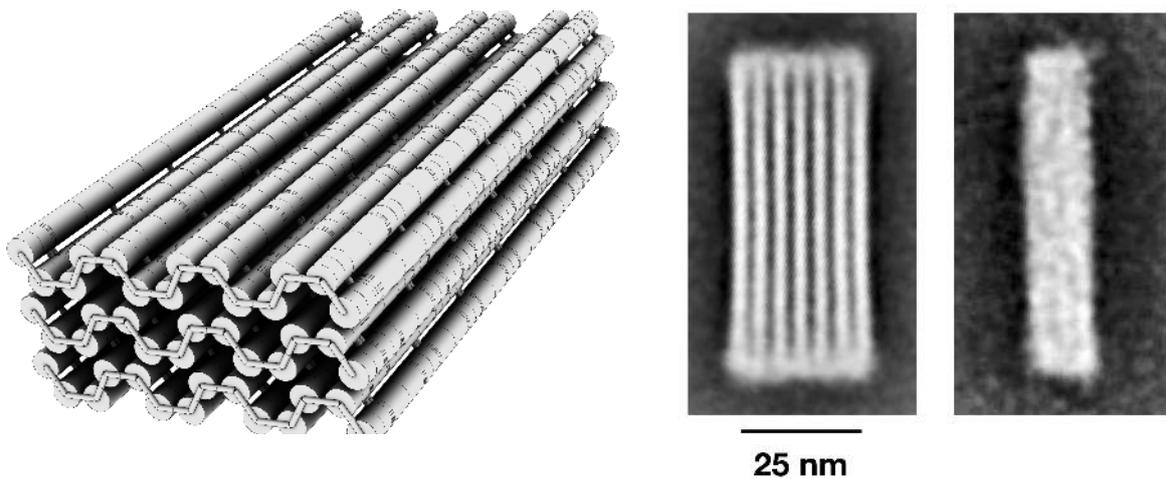


Figure 5: *42 helix bundle. Model and TEM class averages [5].*

1. Scaffold: the scaffold is available at a concentration of 100 μM . The final concentration for the folding has to be 20 nM.
2. Staples: the staples must be in a 5x excess with respect to the scaffold.
3. Folding buffer: the folding buffer consist in a mixture of 50 mM NaCl, 50 mM Tris- Base, 10 mM EDTA. For the folding we need 5 mM NaCl, 5 mM Tris-Base, 1 mM EDTA.
4. Magnesium chloride: this solution is available at 100 mM. For the folding, a final concentration of 20 mM MgCl_2 is needed.

Considering a final volume of 50 μL , calculate the necessary volume for each of the components. To reach the final volume, water may be added if needed.

SAMPLE A	initial Concentration	final concentration	final volume of reaction	volume needed
Scaffold				
Staples				
Folding Buffer				
MgCl_2				
ddH ₂ O				

Calculate the same also for the reaction in which 20 oligo are missing. Which are the differences from the first reaction?

SAMPLE B	initial Concentration	final concentration	final volume of reaction	volume needed
Scaffold				
Staples				
Folding Buffer				
MgCl ₂				
ddH ₂ O				

The two reaction mixtures need to be warmed for the components to fold correctly. First, the reaction is warmed up at 65 °C for 15 minutes in order to destroy all potential unspecific bounds among the DNA components. After that, the sample is incubated at 52 °C for 5 minutes to let the structure fold correctly.

To check the quality of the folding products, we will use gel electrophoresis analysis.

Together with the folded samples we will load into the gel two pre-prepared samples (called sample C and D) for comparison: sample C consists in a 42 hb which was folded according to the protocol written above and then heated to 65 °C for 90 minutes. Sample D is a 42 hb which underwent the same temperature steps as sample C, but afterwards was re-folded at 50 °C for 15 minutes.

3.2.2 Gel electrophoresis: product analysis

The principle of agarose gel electrophoresis consists in the separation by size of the different components present in the sample. The negative charged backbone of the DNA origami structures interacts with an applied electric field, such that the structures migrate towards the anode. Since the charge of the origamis is proportional to their mass, smaller structures migrate in general faster than bigger ones. In general, bigger aggregates remain in the pockets, while well folded structures form a sharp and bright leading band. Bands with smaller migration distance consist of partially or not well folded origami. The excess staples can be recognised as very bright and diffuse bands with the fastest migration distance.

The gel is composed of a solution containing 0.5xTBE (44.5 mM TrisBase, 44.5 mM Boric Acid, 1 mM EDTA), 2% agarose and 5.5 mM MgCl₂. In case the structures do not contain fluorescent dyes, EtBr can be added to detect the structures due to its ability to intercalate the DNA molecules. The running buffer consists in 0.5xTBE and 5.5 mM MgCl₂. Prior to loading, the samples are mixed with 20% (v/v) loading dye (containing Ficoll 400, 0.1% Bromophenole blue and 5 mM Tris PH8.8). This causes the molecules to sink in the gel pocket and allows their tracking during the migration.

After loading the samples, a constant voltage of 90 V is applied for 1.5 hours. The gel can later be visually inspected in a laser scanner, which can excite the EtBr molecules.

3.3 Force Spectrometer

You will be provided with a sample of the force spectrometer with two DNA single strands attached to the arms as test specimen. The DNA strands are partly complementary to each other. Depending on the energy landscape they adopt hybridised and de-hybridised states resulting in different angles of the force spectrometer. Prepare the sample for TEM, image it, and measure the angle distribution. You will be provided with the angle distribution of the bare force spectrometer (without a test sample). Calculate the probability distributions and the energy landscapes of the force spectrometer with and without test specimen, and the energy landscape of the DNA single strands.

3.3.1 Transmission Electron Microscopy

During sample preparation for the TEM, the samples are stained negatively using a solution of Uranyl Formate which fills the cavities of the DNA origami objects. Due to the high mass of the uranium atoms compared to the lighter atoms of DNA (H, C, O, etc) the electron are scattered more strongly than by the DNA origami objects. This leads to spatial differences of the detected intensity (contrast) in the image.

In principle, an electron microscope has similar components like a bright-field or transmitted light microscope, including an illumination source, condenser, objective and magnifying lenses, and apertures. Compared to a light microscope the information carrier in a TEM the information are electrons. They are emitted by an electron gun and accelerated in a high voltage field, in case of the TEM used in this lab course by a heated LaB6 filament and to an energy of 100 keV. Electromagnetic lenses are used to condense the electron beam to an even and parallel illumination of the sample, and to magnify and focus the image onto the detector after the electrons were scattered by the sample.

Questions:

Would it be possible to use a light microscope instead of a TEM? What happens if we would not negatively stain our sample?

Why do many DNA objects have stripes in the TEM images?

Large CAUTION:

The used Uranyl Formate is a radioactive alpha source. Handling is only allowed in the labelled area, wearing protective gear, and after the instruction of the supervisor. Waste has to be discarded in the labeled containers.

The TEM is a sensitive and expensive instrument. Please touch any components or press any knobs etc without instruction by the supervisor.

3.3.2 Energy Landscape Determination

For the determination of the energy landscape of the force spectrometer from its angular fluctuations, a library with TEM images of individual particles is provided. The following protocol allows an optimised angle measurement of many single particles using the program ImageJ:

1. Open ImageJ
2. Press “O” and open the first image
3. Measure the angle with the ”angle tool”. Set three points which define the angle. The points can be moved and adjusted with the mouse.
4. “M” measures the angle and opens a table with the measured angles. Each new value is continuously added to the table. It is recommended to save the table from time to time.
5. “Shift + O” opens the image of the next particle. After the particles have been measured, the table is saved with the measured angles.

Not all particles are suitable for angle measurement. Define selection criteria before you start the measurements. Think about which are appropriate selection rules and apply them to the angle measurement (Tip: What causes the parallel stripe pattern on the particles?).

Use a program of your choice (e.g. origin) to make a histogram for the opening-angle probability density from the measured angles. Explore what happens when you change the width and offset. Make uniform and normal kernel density estimations for the opening-angle probability density using different band width parameters. Compare the histograms and the kernel density estimations with each other. Plot the uniform kernel density estimation of the probability density of the empty and the loaded force spectrometer dataset with band width 5° . Plot the energy landscapes by taking the logarithm of the kernel density estimates: $\Delta G_i = -\ln(p_i(\Theta))$. Calculate the energy shift parameter Δ . The upper and lower integration limit depend on the test specimen and will be told you by the supervisor (is there an alternative way to calculate the shift parameter?) Calculate and plot the energy landscape of the test specimen as a function of the distance between the two DNA test specimen attachment points and discuss your results.

4 Results and Discussion

The protocol follows the classical documentation of scientific experiments. Some points are noted below for the individual experiments, which should be dealt with in the protocol.

4.1 Folding of the 42 Helix bundle

In the protocol the materials and methods should be described e.g. which are the ingredients for the folding, what role they play in the reaction and in which volume and concentration they are present in the mixture, sample preparation, etc.

In the result part, the scan of the gel has to be shown and discussed. What are the different bands present in the gel? Are there some differences among them, and why? What is the effect of leaving out 20 oligo from the folding reaction (sample B)? What is the effect of heating and re-folding the sample (samples C and D)? Which conclusions can we draw from this experiment (keywords to be discussed: pathways of folding, nucleation during folding, out-of-equilibrium assembly, hysteresis in the folding)? Are there other ways to detect miss-folded structures?

4.2 Force Spectrometer

The opening angles of the force spectrometer (FS) have to be measured. Not every particle is suitable for the measurement. Based on which criteria did you choose the particles?

Plot the distributions of the opening angle of the force spectrometer with and without test specimen as histograms and kernel density estimates. What are the effects of bin/band

width, offset, and the number of evaluated particles? Calculate the energy landscape of the test specimen. Compare the result to the value of the literature, discuss the result and possible error sources.

References

- [1] P. W. K. Rothemund, “Folding dna to create nanoscale shapes and patterns,” *Nature*, vol. 440, no. 7082, p. 297–302, 2006.
- [2] S. M. Douglas, H. Dietz, T. Liedl, B. Högberg, F. Graf, and W. M. Shih, “Self-assembly of dna into nanoscale three-dimensional shapes,” *Nature*, vol. 459, no. 7245, p. 414–418, 2009.
- [3] N. Narayana, S. L. Ginell, I. M. Russu, and H. M. Berman, “Crystal and molecular structure of a dna fragment: d(cgtgaattcacg),” *Biochemistry*, vol. 30, no. 18, p. 4449–4455, 1991.
- [4] J. J. Funke, P. Ketterer, C. Lieleg, S. Schunter, P. Korber, and H. Dietz, “Uncovering the forces between nucleosomes using dna origami,” *Science Advances*, vol. 2, no. 11, 2016.
- [5] F. Schneider, N. Möritz, and H. Dietz, “The sequence of events during folding of a dna origami,” *Science Advances*, vol. 5, no. 5, 2019.

A Experimental Details

A.1 Protocol for agarose gel electrophoresis:

CAUTION: change gloves every time you need to touch something outside the red-marked area (avoid contamination!!). The laser scanner computer must be handled without gloves

1. Gel preparation:

- (a) Weight 1.0 g agarose in a flask
- (b) Add up to 50 g 0.5xTBE
- (c) Boil in microwave until agarose dissolved completely (max power, 1.5 min, shaking every 30 s)
- (d) Replace evaporated water by adding ddH₂O (check on balance)
- (e) Cool flask under running tap water until it is cold enough to be held with gloved hands
- (f) Add 200 μ L 1.375 M MgCl₂ solution for a total conc. of 5.5 mM
- (g) Add 2 droplets EtBr
- (h) Pour into gel tray
- (i) Remove air bubbles and insert comb
- (j) Let the gel dry for 20 min

2. Sample loading and running:

- (a) Once the gel is solidified, remove comb and orient gel tray with pockets facing the cathode ("black cable plug")
- (b) Fill gel box with running buffer (0.5 x TBE + 5.5 mM MgCl₂) up to 1-5 mm above gel surface
- (c) Remove any air bubbles that may be entrapped inside pockets, then load sample
- (d) Mix an appropriate amount of your samples with 6 x loading dye
- (e) Carefully load the samples into the pockets of your gel
- (f) Set appropriate parameters at power supply and start electrophoresis: 90 V, 1 h
- (g) Clean used flask immediately while gel is running. Clean gel box (especially cathode wire!) as soon as you are done.

3. Gel scanning:

- (a) After 1.5 h, stop the gel. With clean gloves open the laser scanner, position and block the gel and close the scanner again
- (b) Make a new folder on the computer
- (c) Select EtBr, 400 PMT and 50 μm as scanning parameters
- (d) Select the appropriate scanning area and click on Start scan
- (e) If some of the bands are red (saturated signal), stop the scanning and start it again using a lower PMT
- (f) Once the scanning is finished, remove the gel and clean the laser scanner

A.2 Grid Staining Protocol

CAUTION:

The used Uranyl Formate is a radioactive alpha source. Handling is only allowed in the labelled area, wearing protective gear, and after the instruction of the supervisor. Waste has to be discarded in the labeled containers.

Grid Staining Protocol:

1. Add a 1 μL droplet of 1 M NaOH to the inner wall of a tube containing 200 μL Uranyl Formate (UFO) solution. The droplet must not touch the solution.
2. Vortex the tube for one minute using a Lab Dancer to mix the NaOH and the UFO.
3. Centrifuge the tube at 19k rcf for 6min.
4. Carefully take the grids from the box using a pair of tweezers and only touching the very edge of the grid, and place it onto a parafilm wrapped glass slide with the shiny side up.
5. Treat the grids in the glow discharge device at 35 mA for 45 s to make it hydrophilic.
6. Place filter paper and a piece of parafilm onto the bench in the labelled area. Fix the parafilm by a few water droplets.
7. Use the pipette to place two droplets of 5 μL and 20 μL UFO next to each other onto the parafilm.
8. Grab a grid with a pair of tweezers, pipette 5 μL sample on it and incubate it for 5 s.

9. Touch the filter paper perpendicularly with the grid to remove excessive sample solution and immediately pick up the small UFO droplet with the grid.
10. Remove it with the filter paper, pick up the big droplet, incubate it for 30 s, and remove it with the filter paper.
11. Let the grid dry for 15 min.
12. Clean the working area.