DNA Origami

Advanced Lab Course

FoPra takes place on Wednesdays starting at 11 am.
We meet at ZNN, second floor, seminar room.

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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-organization 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 and structural analysis. In the first part of the practical course the folding behavior of an DNA origami brick is analysed via gel electrophoresis. In the second part, the nano-object is analyzed by transmission electron microscopy (TEM).
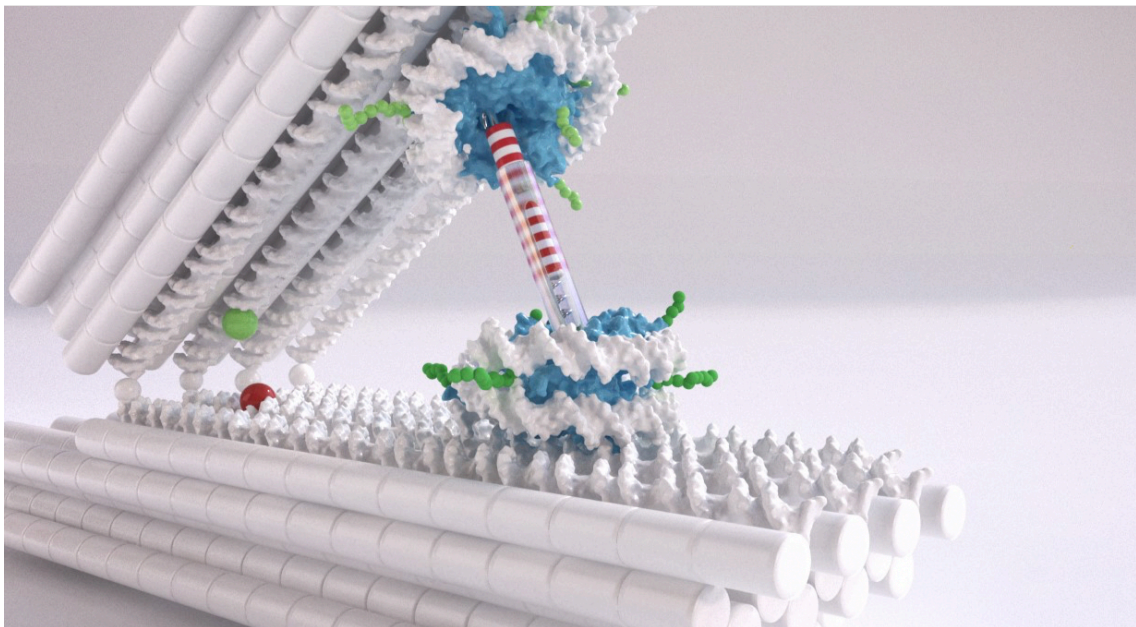

Fig 1.1: Example of DNA origami. In this model the DNA origami is used to measure the interaction between two nucleosomes.[3] DNA segments and fluorescent dyes are represented by cylinders and colored spheres, respectively. The forces between the nucleosomes are illustrated by a spring balance.

2 Theoretical 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 sequences of many different DNA single strands contain 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 [4]. In natural DNA four different nucleobases, termed adenine (A), thymine (T), guanine (G) and cytosine (C), can be found. Two neighboring 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.
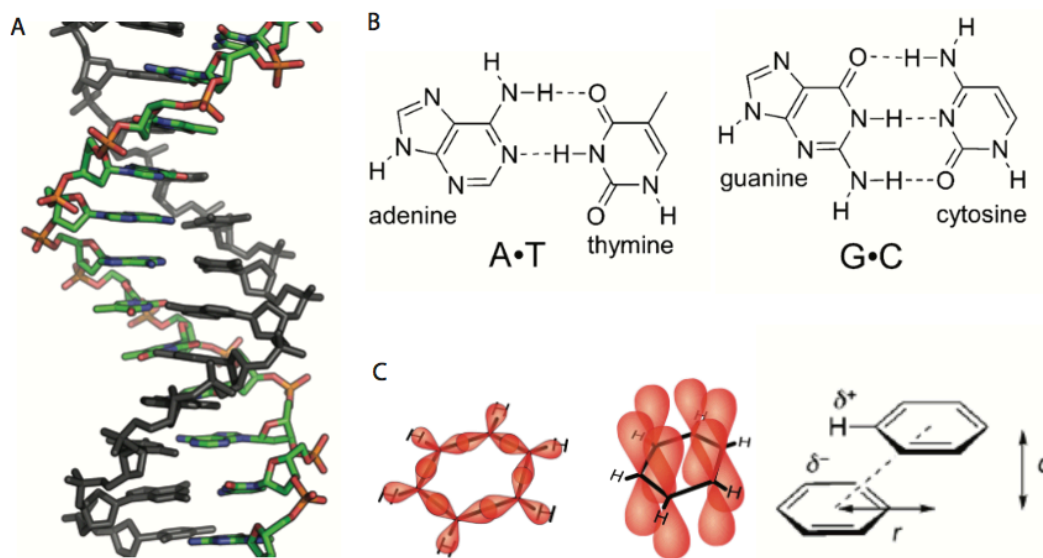
Fig 2.1: DNA properties. (A) Three-dimensional structure of a DNA double helix (PDB 1D28) [4]. The planar bases are located in the interior between the two backbones, stabilizing the double helical conformation via base pairing and base stacking interaction. **(B)** Base pairing. The complementary bases adenine and thymine, and guanine and cytosine form base pairs via hydrogen bonds (dashed lines). **(C)** Base stacking. The overlapping sp2 orbitals of the nucleobase carbon ring atoms form a framework of σ-bonds in one plane, with the p_z orbitals building a

delocalized π-system above and below the σ-plane. The energetically favored shifted conformation of the base stacks leads to the helical structure.

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). In addition 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.

2.2 DNA Origami

A typical DNA origami object is formed by a long, circular, single-stranded "scaffold" DNA strand and multiple short "staple" oligonucleotides ("oligos") interacting with each other via hydrogen bonds between paired bases [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 an 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 of a DNA origami object ("folding") takes place in a one pot reaction where the many single strands are mixed in a buffer solution. The objects self-assemble via pairing ("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 nano-objects. They find applications for instance in form of a nano force spectrometer to measure the interaction strength of various test specimens.
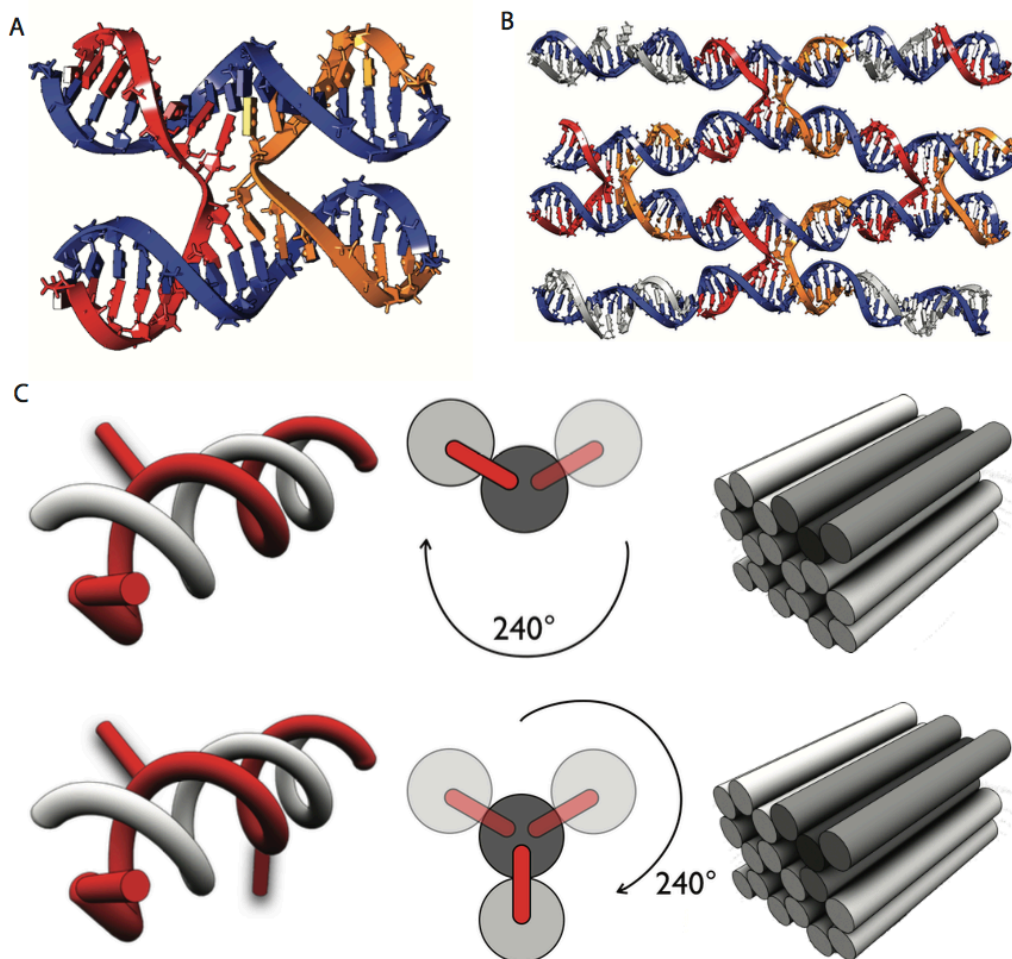
Fig 2.2: DNA properties. (A) Holiday junction. Four DNA single strands, part wise complementary to each other, form a Holiday junction. **(B)** Network of Holiday junction. **(C)** Honeycomb lattice. In this arrangement a helix can be connected to one of its three neighbors every 7 base pairs corresponding to a 240° turn.

3 Experimental procedures

3.1 General lab safety rules

The lab in which we are going to work is an 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. Many hazardous chemicals penetrate gloves within minutes or seconds, thus gloves are no replacement for a vigilant and careful working style. **Do not touch your face or phone when wearing gloves to avoid cross contamination.**

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. 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 a 42 helix-bundle (42 hb), brick-like structure. This origami object consists of 42 DNA double helices arranged in a honeycomb lattice (Fig. 3.1). The scaffold is 7560 bases long and requires 140 staples to fold the desired shape.

25 nm
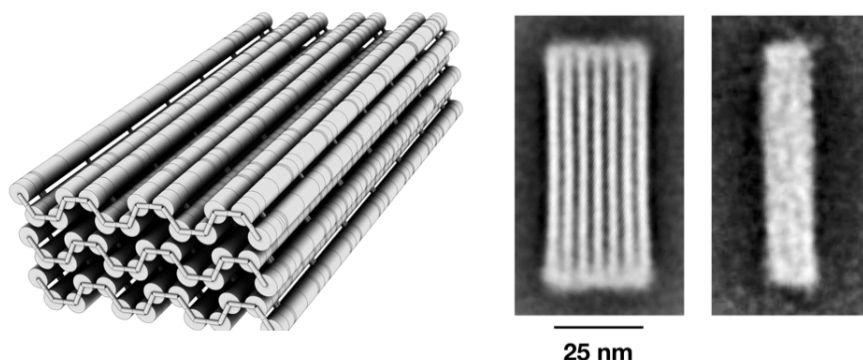
Fig 3.1: 42 helix bundle. Model and TEM class averages of a 42 helix bundle [5].

3.2.1 Folding of 42 helix-bundle

The staples come in 96 well plates, where each kind of staple is inside a different well at a concentration of 100 µM (micromolar, or micromoles per litre). In the first step 10 µL (microlitres) of each staple solution is mixed in a reaction tube. This mixture is called *prestock*. **What is the final volume and concentration of the prestock?**

For the folding of the complete 42hb, we need 4 different components:

1. Scaffold: available at a concentration of 100 nM. The final concentration for the folding has to be 20 nM.
2. Staple prestock: each individual staple within the prestock mixture must be added to the folding reaction at a 5x excess concentration with respect to the scaffold. What is the starting concentration of each individual staple inside the prestock? What is the desired final concentration of each staple in the folding? How much prestock solution is required to reach the desired concentration?
3. Folding buffer: for pH stabilization. Mixture of 50 mM NaCl, 50 mM Tris-Base, 10 mM EDTA. For the folding reaction we need 5 mM NaCl, 5 mM Tris-Base, 1 mM EDTA.
4. Magnesium chloride: 100 mM stock solution. 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 (double-distilled, "ddH$_2$O") may be added if needed.

SAMPLE	Initial concentration	Final concentration	Required volume
Scaffold			
Prestock			
Folding Buffer			
$MgCl_2$			
ddH$_2$O	-	-	

The reaction mixture needs to be warmed for the components to fold correctly. First, the reaction is warmed up to 65°C for 15 minutes in order to destroy all potential unspecific bonds among the DNA components. After that, the sample is incubated at 50 °C for approx. 1.5 hours to let the structure fold correctly.

The quality of the folding will be determined using agarose gel electrophoresis.

The supervisors have prepared a few other samples that will also be analysed in the gel for comparison:

- Sample -20S: incomplete 42hb missing the 20 slowest binding staples, folded normally.
- Sample -20F: incomplete 42hb missing the 20 fastest binding staples, folded normally.
- Sample 1: complete 42hb folded normally and then heated to 65 °C for 15 min.
- Sample 2: Like Sample 1, but additionally kept at 50 °C for 1.5 hours following incubation at 65 °C.

3.2.2 Gel electrophoresis: product analysis

In agarose gel electrophoresis, charged sample molecules are pulled into an agarose gel by an electric field where they are separated by size. The negatively charged DNA origami structures interact with the applied electric field such that they migrate towards the anode. Since the charge of the origami is proportional to its mass, smaller structures generally migrate faster than bigger ones. Bigger aggregates remain in the loading pockets, while well folded structures form a sharp and bright leading band. Bands at smaller migration distances in many cases represent partially or not well folded origami. Excess staples can be recognised as very bright and diffuse bands with the fastest migration distance.

The agarose gel is made by preparing a solution 2% (w/w) agarose in running buffer (0.5 x TBE buffer (44.5 mM Tris Base, 44.5 mM Boric Acid, 1 mM EDTA) and 5.5 mM $MgCl_2$). A plastic comb is inserted into the liquid gel while it hardens to form pockets for sample loading. In case the structures are not fluorescently labelled, the DNA intercalator Ethidium Bromide (EtBr) is added to the gel for DNA detection.

Prior to loading, the samples are mixed viscous loading dye (containing Ficoll 400, 0.1% Bromophenole blue and 5 mM Tris pH 8.8) to.a final concentration of 16.6% (v/v). This facilitates sample loading by promoting the sinking of the sample solution into the gel pocket and allows tracking during electrophoresis. Finally, the gel is immersed in running buffer, the samples are loaded into the gel pockets and a constant voltage of 90 V is applied for 1-1.5 hours. The gel can later be visually inspected in a laser scanner, which excites intercalated EtBr molecules.

Protocol for agarose gel electrophoresis:

CAUTION:

Change gloves every time you need to touch something outside the red-marked area (avoid contamination!!). ***Do not touch your face, hair or phone while working with EtBr!*** *Microwaved agarose solutions frequently superheat, causing potentially dangerous boiling delays ("bumping"). When handling heated agarose solutions, always point the opening of the flask against the bench wall to prevent rapid expulsion of boiling liquid onto your colleagues or yourself. The laser scanner computer must be handled without gloves (EtBr free zone).*

1. Gel preparation:

    1. Weigh 1.00 g agarose in a flask
    2. Add up to 50 g with running buffer
    3. Boil in microwave until agarose is dissolved completely (max power, 1.5 min, shaking every 30 s; Do not point the opening of the flask against other people! Boiling delay!)
    4. Replace evaporated water by adding ddH$_2$O (check on balance)
    5. Cool flask under running tap water until it is cold enough to be held with gloved hands
    6. Add 2 droplets EtBr
    7. Pour into gel tray
    8. Remove air bubbles and insert comb
    9. Let the gel harden for at least 20 min

2. Sample loading and running:

    1. Once the gel hardened, remove comb and orient gel tray with pockets facing the cathode ("black cable plug")

2. Fill gel box with running buffer (0.5 x TBE + 5.5 mM MgCl$_2$) up to 1-5 mm above gel surface

3. Remove any air bubbles that may be entrapped inside the pockets

4. Mix 10 µl of your samples with 6x loading dye

5. Carefully load the samples into the pockets of your gel (see images below)

6. Set appropriate parameters at power supply and start electrophoresis: 90 V, 1 h

7. Clean used flask immediately while gel is running. Clean gel box (especially cathode wire!) as soon as you are done.
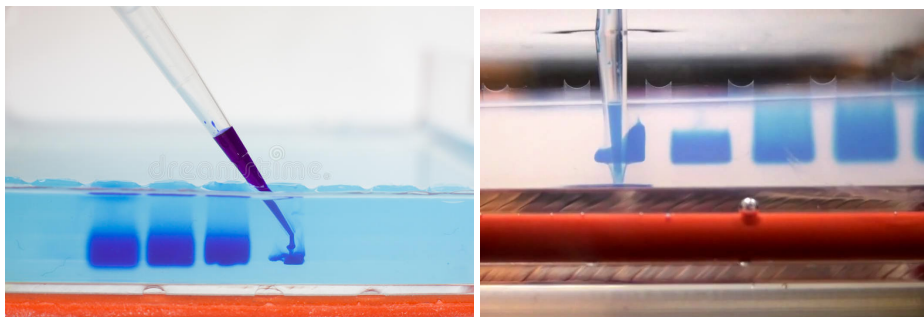
Fig 3.2.2.2: Agarose gel loading technique:

Left: correct loading technique. Insert the pipette tip into the well and carefully/slowly push the plunger. Make sure not to pipette too quickly or to make any rapid movements, which could expel the sample out of the pocket.

Right: Pipette tip was inserted beyond the well depth into the gel. This could lead to well leakage, low quality bands and sample loss. If you push the plunger but cannot see your sample solution leaving the pipette tip, you have most likely pricked the gel!

3. Gel scanning:

1. After 1-1.5 h, stop the gel. With clean gloves open the laser scanner, position the gel, and close the scanner again. Note down the scanning area.

2. Make a new folder on the computer to store the image

3. Select *EtBr*, *400 V PMT* and *50 µm* as scanning parameters

4. Select the appropriate scanning area and click on *Start scan*

5. If some of the bands are red (saturated signal), stop the scanning and start it again using a lower PMT voltage

6. Once the scanning is finished, remove the gel and clean the laser scanner

3.3.1 Transmission Electron Microscopy

During sample preparation for transmission electron microscopy (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 electrons are scattered more strongly by it 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 components similar to bright-field or transmitted light microscopes, including an illumination source, condenser, objective and magnifying lenses, and apertures. The information carrier in a TEM is electrons, which 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 LaB_6 filament and to an energy of 100 keV. Electromagnetic lenses are used to condense the electron beam into an even beam for 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? (see e.g. Fig. 3.1)

CAUTION:
*Uranyl Formate (UFO) 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. **Do not touch your face, hair or phone while working with UFO!***

Grid Staining Protocol:

1. Pipette 1 µl of 5 M NaOH to the inner wall of a tube containing 200 µl Uranyl Formate (UFO) solution. The droplet must not touch the solution.
2. Briefly vortex the tube to rapidly mix both solutions.
3. Centrifuge the tube at max speed for at least 5 min.

4. Carefully pick up a grid from the box using a pair of tweezers 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 grid in the glow discharge device at 35 mA for 45 s to make its surface hydrophilic.

6. Prepare filter paper and a piece of parafilm in the labelled area.

7. Pipette 25 µl stain solution onto the parafilm, forming two droplets

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 the sample solution and immediately hold the grid into one of the stain droplets.

10. Immediately blot off the stain with the filter paper, pick up the second droplet and incubate for 30 s

11. Blot off the stain using filter paper and let the grid dry for 15 min.

12. Clean up the working area.

The TEM is a sensitive and expensive instrument. Do not touch any components or press any knobs without instruction by the supervisor.

5 Bibliography

[1] P. Rothemund, Folding DNA to create nanoscale shapes and patterns, *Nature*, 2006.

[2] S. M. Douglas, H. Dietz, T. Liedl, B. Hogberg, F. Graf, and W. M. Shih, Self-assembly of DNA into nanoscale three-dimensional shapes, *Nature*, 2009.

[3] Jonas J. Funke, Philip Ketterer, Corinna Lieleg, Sarah Schunter, Philipp Korber, Hendrik Dietz, Uncovering the forces between nucleosomes using DNA origami, *Science Advances*, 2016.

[4] N. Narayana, S. L. Ginell, I. M. Russu, and H. M. Berman, Crystal and molecular structure of a DNA fragment: d(CGTGAATTCACG), *Biochemistry*, 1991.

[5] F. Schneider, N. Möritz and H. Dietz, The sequence of events in DNA origami folding, *accepted for publication in Science Advances*, 2019.

Protocol requirements

Please follow the instructions below and send the finished protocol to your instructors. If any questions pop up, feel free to contact us via email.

Title page

Please include your team number, degree program (biochemistry/physics), full names, email addresses and matriculation numbers of all members in your team.

Introduction (0.5 - 1 page)

Briefly describe DNA, its relevant physicochemical properties, DNA Origami and the experiments we have conducted during the lab course. Explain agarose gel electrophoresis & TEM. What information are you hoping to obtain to further characterize your structure using these methods?

Results (1 - 3 pages)

Present and explain the results you have obtained. In particular, your protocol should include:

1. Image of your gel:
   a. Please label each lane.
   b. Digitally adjust the brightness & contrast of your image using appropriate software, e.g. Fiji (https://imagej.net/Fiji.html#Downloads) for better visibility of the individual bands & smears, where necessary. Auto-levelling should yield reasonable results. If it does not, you may need to oversaturate your image for clarity, but be careful not to distort the image too much and document this.
   c. Interpret the gel. What do you see? Are your bands clearly defined or smeared, and what does this tell you? Are your structures aggregated, and how do you see this? Compare the different lanes and draw conclusions. Is there anything else that's noteworthy?
2. 2-4 representative TEM images:
   a. Digitally adjust the brightness & contrast of your image if necessary
   b. Interpret your images. What do you see?

Discussion & conclusions (0.5 - 1 page)

What conclusions do you draw from your experiment? Are the results reasonable, sound and satisfactory? Do your results match your expectations? Does the data obtained by TEM match the gel data? Is there anything you would do differently next time, and if so, can you think of an adequate follow-up experiment?