Atomic force microscopy of self-assembled DNA-micro pores.

Stephan Drothler

Abstract— **Atomic force microscopy (AFM) is a powerful tool to resolve molecular structures up to a small nanoscale. The method is widley used in biophysics for topographical analysis of surfaces and quality control of synthetic nanoconstructs. The recent advances in molecular biology led to the ability to design self-assembling, higher-order structures from small DNA sequences. The experimental setup aimed to analyse the structure of 9-duplex DNA nanopores on mica (1), a 6-duplex DNA nanobarrel on mica (2) and cholesterol-anchored 6-duplex DNA nanobarrels in a DPhPC lipid bilayer (3). The topography data provides sufficient data points for height and diameter analysis in experiment (1) and (2), while experiment (3) yielded no data hence data from another group was analyzed. The resulting data appears to be consequently smaller ((1)=-21.4%, (2) = -8%, (3) = -0,9 %) in width and up to 76% smaller in height than the theoretically predicted values from the DNA folding software, conveyed by the supervisor.**

I. INTRODUCTION

DNA origami is broadly used to create functional nanostructures that mimic or enhance current biological systems. The sequence dependent hybridization allows for software-guided design of complex molecular devices. These have a thermodynamically determined assembly characteristic, manly based on hydrogen bonding. The mechanical components are often regulated by specific molecules or external stimuli. (Endo and Sugiyama 2018) Since channels and pores fulfill various essential roles in homeostasis, cell signalling and extracellular, molecular exchange, pore-constructs are of high research interest. They may provide new medical approaches for drug delivery, perform as specific key-lock systems or act as size-selective filters. In order to make them integratable to membranes they have to be modified with hydrophobic chains, to allow anchoring within the unpolar tails of biological membranes. This paper examines the structure of a 9-duplex DNA nanopore immobilized on mica (1), as well as a 6-duplex DNA microbarrel modified with cholesterol immobilized on mica (2) and anchored within a lipid membrane (3). The structural examination is performed via atomic force microscopy (Methods), via tapping mode (AAC) in respective imaging buffers (Chemicals). The theoretical structural profile of the 6-duplex barrel describes a 15 nm width with a 2.2 nm high hydrophobic belt. The outer diameter around the barrel measures 5.5 nm which results in an inner channel width of 1.5 nm. The 9-duplex pore has a computational width of $\approx$ 10 nm and a inner pore of $\approx$ 1-2 nm, with no information about the diameter. For increased readability and due to the evaluation of only two dimensions the residual paper refers to the diameter as height.

II. METHODS AND MATERIALS

A. MATERIALS

1) Tools:

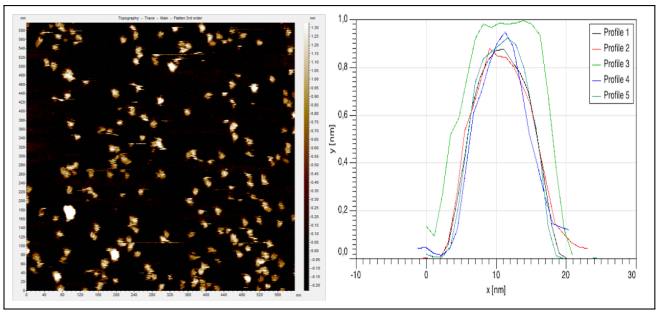
- scalpel
- tweezers
- eppendorff-pipettes (200 μL, 1000 μL)
- greiner-tubes (12 *mL*)
- AFM PicoPlus (Molecular Imaging)
- Muscovite mica
- milliQ water
- isopropanol
- SDS
- Cantilever - Tip F (k = 0,6 $\frac{N}{m}$)

2) Chemicals:

- Buffer N 10 *mM* Tris 10 *mM* $NiCl_2$ pH 8.0
- Buffer I 10 *mM* Tris 1 *mM* $NiCl_2$ pH 8.0
- Imaging Buffer 10 *mM* Tris-HCl pH 7.7
- Lipid-imaging Buffer 1xTAE 16.0 mM $MgCl_2$
- NN pore (9-Duplex DNA micro-pore)
- Ring0 pore (6-Duplex DNA micro-barrel)
- DPhPC (E.coli polar lipid extract)

B. METHODS

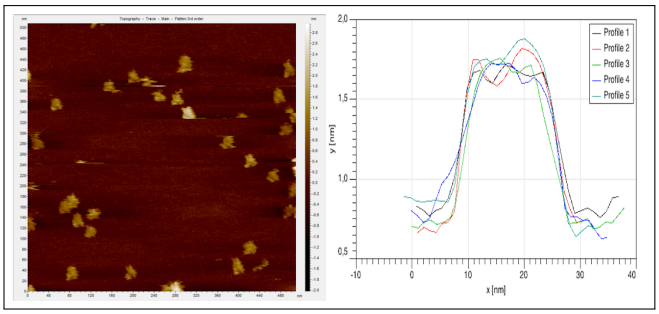
The analysis method used in this procedure was atomic force microscopy (AFM). It is based on a tip, mounted on a cantilever, which interacts with the sample scanning over it. This motion of the is monitored through a laser beam reflected off the cantilever backside. The deflection is tracked photodiode that transfers up the motion values to a computer. The sample is moved in the xyz direction by a piezoscanner, which allows for tiny movement steps. A feedback loop is used to control the distance between the tip and the surface, therefore the AFM can generate a precise topographic map of the surface features. In tapping mode, the cantilever oscillates with constant amplitude by correction via the feedback loop while in contact mode the tip is dragged with constant force over the analyte. Tapping mode was chosen as the imaging method, in order to minimize sample damage caused by frictional forces inflicted by contact mode. The preparation of the mica surfaces and the aqueous solution was performed according to the standard protocol (Saanfor Suh 2019). The assembly of the DNA pores was already carried out prior to the experminent. The AFM setup PicoPlus (Molecular Imaging) is operated with PicoView 1.2 (Windows) and analyzed with Gwyddion (V 2.53 Nefffdfffdas and Klapetek 2012). The data processing was performed via R studio (V 1.2.1335) and visualized via the ggplot2 package (R Core Team 2019, Wickham 2016).



(a) Topography of the NN pore on mica obtained via AFM imaging.

(b) Extracted sample profiles (n=5) via Gwyddion.

Fig. 1: NN pore topographical data and width /height profiles obtained on a mica surface



(a) Topography of the R3C pore on a cleaved mica surface

(b) Extracted sample profiles (n=5) via Gwyddion.

Fig. 2: R3C pore topographical data and width /height profiles obtained on a mica surface

III. Results

The length of the NN pore (n=5) is centered around the mean of 7,86 nm with a standard deviation of 1.6 nm and an average height of 0,899 ± 0.063. The R3C barrel data on mica (n=5) derived from Figure 2b has a mean length of 13.8 nm (± 0.86 nm) and an average height of 1.78 nm (± 0.068 nm) (Tab. II). On the lipid membrane, the R3C/R0 pore (n=6) has an average length of 14.86 nm (± 2.06 nm) with a height of 1.21 nm (± 0.063 nm) (Fig. 3b (Tab. III). The arithmetic means of the three measurements are plotted in a bar chart, allowing for easy visual distinction of the experiments. The smallest pore in the chart is the NN pore, while the R3C/R0 pore appears to be wider in size but smaller in height on the lipid membrane, compared to mica (Fig. 5b).

	Length (nm)	Height (nm)
profile 1	7,260787993	0,88
profile 2	7,823639775	0,80
profile 3	10,18761726	0,95
profile 4	5,797373358	0,95
profile 5	8,217636023	0,92
	mean	mean
	7,86 ± 1.6	0,899 ± 0.063

TABLE I: Data from the NN pore immobilized on mica with the calculated arithmetic mean and the standard deviation.

(a) Topography of the R3C pore on DPhPC.

(b) Extracted sample profiles (n=6) via Gwyddion.

Fig. 3: NN pore topographical data and width /height profiles obtained on a DPhPC.

	Length (nm)	Height (nm)
profile 1	15,15947467	1,718
profile 2	13,58348968	1,8184
profile 3	12,90806754	1,75684
profile 4	13,9587242	1,7271
profile 5	13,35834897	1,878
	mean	mean
	13,8 ± 0.85	1,78 ± 0.068

TABLE II: Data from the R3C pore immobilized on mica with the calculated arithmetic mean and the standard deviation.

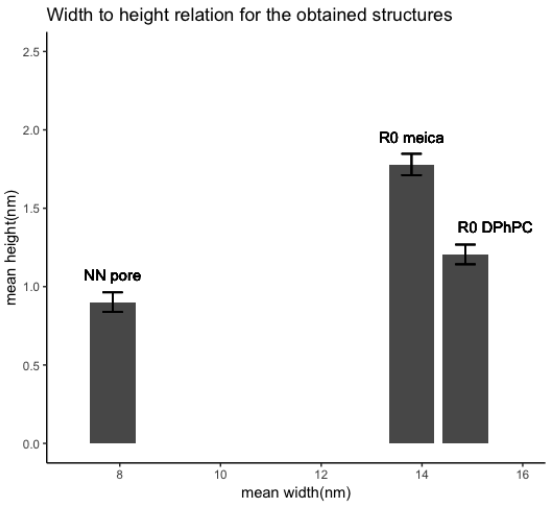


Fig. 4: Plot of the various height over width means with a visualized standard deviation of the height parameter.

	Length (nm)	Height (nm)
profile 1	16,85950413	1,1879
profile 2	17,52066116	1,1691
profile 3	13,88429752	1,1529
profile 4	11,90082645	1,1783
profile 5	14,7107438	1,2161
profile 6	14,3	1,33
	mean	mean
	14,86 ± 2.06	1,21 ± 0.063

TABLE III: Data from the NN pore on DPhPC with the calculated arithmetic mean and the standard deviation.

IV. Discussion

The data from the NN pore suggests a 21.4% (Theo = 10nm, 7,86 $\pm$ 1.6) smaller size than expected. The RC3 pore was 8% smaller on mica and 0,9 % smaller on the lipid (Theo = 15nm, $R3C_{mica}$ = 13,8 $\pm$ 0.85, $R3C_{DPhPC}$ = 14,86 $\pm$ 2.06. The height parameters are a lot smaller than expected for the R3C pore ((2) = -68% , (3) = -78%), while there is no height information for NN. The deviations might be due to an incorrect profile placement for the measurement or pore disassembly. The resolution doesn'tt allow an easy distinction of the dimensions of the respective pores and might lead to singlet observations. The height of the profiles could therefore easily be the height of a DNA singlet, instead of a fully assembled pore. The width aberrations might be explained by the flexibility of the pores. Since they are no rigid, they could appear smaller than expected and might not be locked into the computed dimensions.

The mica surface is more densly incubated on mica than on DPhPC (Fig. 2b, Fig. 3b). Our examined sample showed to pores, which was most likely due to a low concentration of the solution. The low density in the evaluated substitue data might be due to the fact that the cholesterol region is relatively small. With short incubation times or temperature fluctuations, R3C could no fully anneal to the hydrophobic membrane part. Unbound R3C would then be washed away before imaging. For enhanced data analysis the experiment could be re-performed with slower imaging times and better resolution.

V. Appendix

A. Standard protocol

1) NN pore preparation:

- Add 6 μl NNpore strand, 25 μl NNpore folding buffer and 69 μl H_2O to a PCR Tube
- Heat up the sample in a cycler to 65C for 5min
- Cycle from 65°C to 5°C at 2$\frac{°C}{min}$ for 2h
- Store at 4°C until pick-up.

2) R0C & R3C pore preparation:

- Add 6 μl ring0 pore strand, 10 μl 10x Ring folding buffer and 84 μl H_2O to a PCR Tube
- Heat up the sample in a cycler to 65C for 5min
- Cycle from 65°C to 25°C at 20$\frac{°C}{min}$; 25°C to 5°C at 1$\frac{°C}{min}$ for 14h
- Store at 4°C until pick-up
- cholesterol modification: add 2 μl cholesterol DNA to 100 μl R0C; incubate for 10 min. R0C now has 3 cholesterol tags (R3C).

3) Immobilisation of pores to mica:

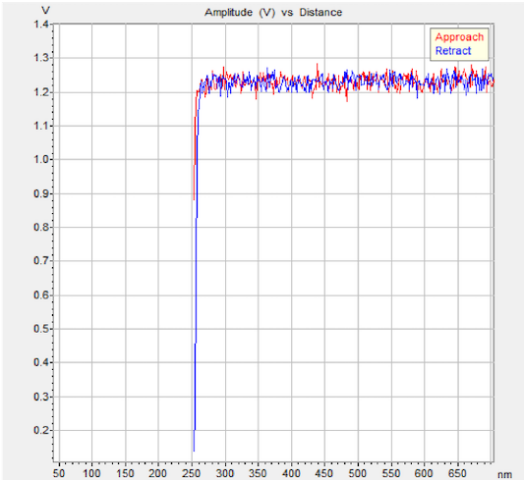
- Cleave mica
- Treat cleaved mica with buffer N for 5 mins
- Rinse with buffer I three times and allow in air for 1 min
- Add 100 μl of 1 μl R0C in 99 μl Tris-HCl buffer and incubate for 2 mins
- Dissolve the cell in 1 ml PBS. Let the unsolved tissue particles settle and take 100l for the staining.

4) Pore insertion into the lipid bilayer membrane:

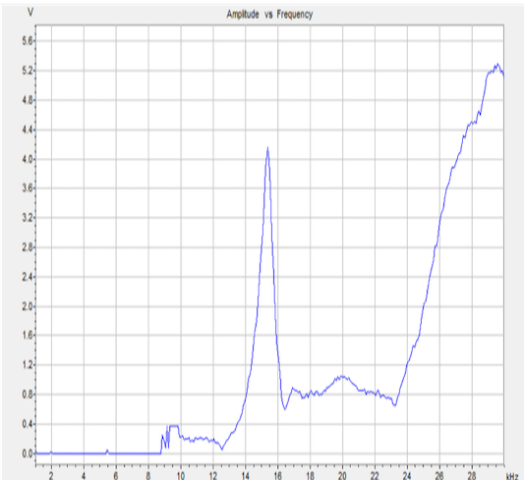
- Place 40 μl DPhPC SUV solution (1$\frac{mg}{ml}$) in 160 μl magnesium buffer onto freshly cleaved mica (final lipid conc 200 μl) for 15 mins.
- Wash the surface 5 times with the same buffer
- Image the surface to visualise vesicle spreading
- After complete coverage is ensured, wash 5 times with total of 500 l buffer
- Add 10 l of R3C and incubate for 30 minutes
- Rinse to remove free nanopores
- Image in 600 l buffer using tapping mode

5) AFM setup:

- Set drive type to AAc
- Define phase to be zero at driving frequency.
- Set servo gain settings close to one
- Set stop at values at $\approx$ 80 - 95%
- Setup speed at correct cantilever calibration
- Find cantilever resonance and set Drive Frequency (15,6 khz, Fig. 5a) and Setpoint ($\approx$ 0.98, Fig. 5b)
- Recheck values, selct size and approach



(a) Amplitude over distance plot (Min Max sweep) for setpoint determination, with the restriction of $\frac{contact\,point}{set\,point} \leq 1$



(b) Determination cantilever resonance frequency at $\approx$ 15,6 kHz

Fig. 5: Plots for variable tuning of AFM setup.

B. Experimental environment

The experiment was performed at Gruberstrae 40, Linz, Austria on 14.05.19 under the supervision of Hubert Saanfor Suh. The experiment was carried out by Caroline Rieser, Lukas Schartel and Stephan Drothler.

References

[ES18] Masayuki Endo and Hiroshi Sugiyama. "DNA origami nanomachines". In: *Molecules* 23.7 (2018), p. 1766.

[NK12] David Nefffdfffdas and Petr Klapetek. "Gwyddion: an open-source software for SPM data analysis". In: *Central European Journal of Physics* 10 (1 2012), pp. 181–188. ISSN: 1895-1082. DOI: 10.2478/s11534-011-0096-2.

[R C19] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria, 2019. URL: https://www.R-project.org/.

[Saa19] Hubert Saanfor Suh. "Biophysic II Practical Course". 2019.

[Wic16] Hadley Wickham. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York, 2016. ISBN: 978-3-319-24277-4. URL: https://ggplot2.tidyverse.org.