

ADSORPTION STUDIES OF DNA ORIGAMI ON SILICON DIOXIDE

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Abstract — Self-assembled DNA nanostructures promise low-cost ways to create nanoscale shapes. DNA nanostructures can also be used to position particles with nanometer precision. Yet, reliable and low-cost ways of integrating the structures with MEMS technology still have to be developed and innovations are of great interest to the field.

We have examined in detail the adherence of DNA origami tiles on silicon oxide surfaces of wafers in dependence on pH-value and magnesium ion concentration. The results of this work will help to pursue new strategies of positioning DNA nanostructures on SiO₂. Precise control over the strength of structure-surface adhesion is a prerequisite of reliable processes.

Keywords : DNA origami, surface charge density, charge inversion, positioning

I - Introduction

Employing the DNA origami method [1], it is possible to design and produce arbitrarily shaped two-dimensional DNA nanostructures about 100×100 nm² in size by self-assembly. These structures are made of one long (approx. 7000 nucleotides) single stranded DNA molecule of natural origin and around 200 short (24 to 50 nucleotides) tailored synthetic oligonucleotides which determine the final self-assembled shape (Figure 1a, 1b).

As the position in the final nanostructure of every single base of each oligonucleotide is known, non-DNA components can be arranged on the structures with nanometer precision by chemically modifying single oligonucleotides (Figure 1c). Several attachment strategies have been demonstrated [2], and different add-ons at distinct positions of the same structure have been realized [3].

For applications, but also initially for the characterization of e.g. the electronic properties of the produced nano-scale assemblies, integration of the nanostructures with established MEMS technology is of very high interest. However, the standard substrate for the deposition of flat DNA nanostructures, commonly prior to characterization by atomic force microscopy (AFM), is mica. The best conditions with which to use silicon or silicon dioxide as a substrate have yet to be explored.

For self-assembly strategies for the arrangement of the nanostructures on surfaces, good control over the deposition process and adhesion is needed. First progress in the direction of meta-self-assembly has been made by prestructuring hydrophilicity patterns on the surface of silicon wafers.

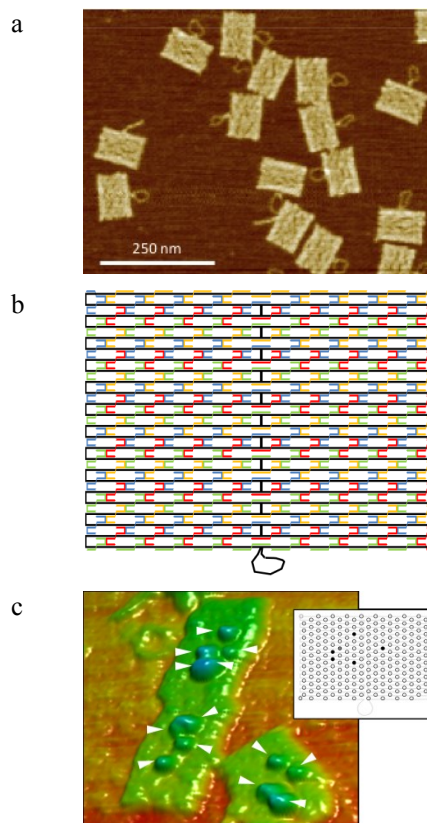


Figure 1: a) 70×90 nm² rectangular DNA origami nanostructures deposited on mica and imaged with AFM in air. The DNA loop not used in the rectangle design is clearly visible. b) Schematic of the long single stranded DNA backbone strand (black) held in desired shape after hybridization with specifically designed short oligonucleotides (colored). c) 3D representation of an AFM image of DNA origami of the same size as in a), but containing some oligonucleotides with attached biotin residues to which added streptavidin proteins are bound (white arrowheads). The inset represents the grid of modifiable positions with modifications marked by filled circles.

Kershner et al. [4] managed to deposit triangular DNA origami on specific sites by first modifying the

SiO₂ surface with a hydrophobic tetramethylsilyl (TMS) monolayer and, after electron beam structuring of patterns of similar size as the origami structures, etched through the TMS layer to the SiO₂ surface using oxygen plasma. After stripping of the resist, clean SiO₂ patches in a hydrophobic background remained. As another strategy, Sarveswaran et al. [5] also used e-beam lithography, but realized hydrophilic aminopropyltriethoxysilane (APTES) or trimethyl aminopropyltrimethoxysilyl chloride (TMAC) patches on the SiO₂ surface of a silicon wafer.

We focused on silicon with native oxide on top as substrate, as additional intermediate layers increase the roughness of the surface, which should be kept as low as possible because conventional 2D DNA origami structures are only on diameter of a DNA helix, ca. 2 nm, thick. This strategy also avoids additional parameters such as ratios of APTES and TMAC mixtures used to adjust surface charge density, a parameter that can also be adjusted via magnesium ion concentration when using SiO₂ surfaces.

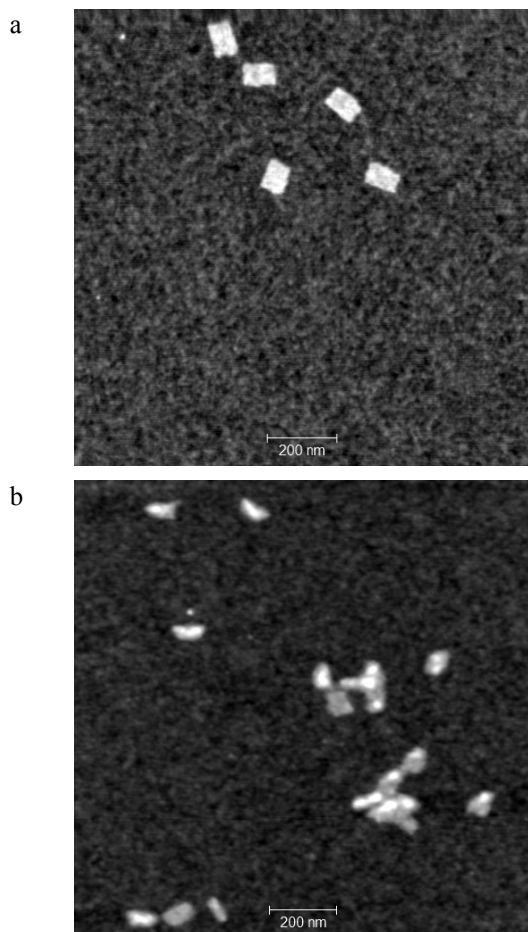


Figure 2: a) AFM image of well attached rectangular DNA origami lying flat on the SiO₂ surface. Deposited from pH 8.3 buffer with 100 mM Mg²⁺ ions. B) AFM image of the same type of origami nanostructures as in a), with many structures rolled up. Deposited from pH 7.5 buffer with 100 mM Mg²⁺ ions.

The goal of the studies presented here was to obtain a deeper understanding of the interactions between two-dimensional DNA nanostructures and SiO₂ surfaces. Therefore, we acquired quantitative data on the influences of pH and Mg²⁺ ion concentration. Since both SiO₂ surface and DNA are negatively charged, the adsorption of DNA nanostructures to the surface is caused by charge inversion [6] [7] that first occurs on the surface due to a dense layer of bivalent Mg²⁺ ions. At even higher Mg²⁺ ion concentrations, charge inversion can also occur on the DNA structures, leading to repulsion between the SiO₂ surface and the nanostructures and, supposedly, to aggregation of the nanostructures. For correct positioning of DNA nanostructures to certain spots, it is necessary to at first use conditions where the structures adhere but are still mobile, in order to enable the settlement of the structures to reach equilibrium. Afterwards, the conditions may be changed such that the structures stick in place. An adsorption at random first-contact positions is not desirable, neither is non-adsorption followed by drying, in which case the structures would again end up at random positions and in random configurations. Figure 2b shows DNA origami rectangular structures that seem to be rolled up along their length axes, which might arguably be due to either of these unsuitable conditions.

II - Experimental Details

A. Assembly of DNA origami nanostructures

DNA origami were assembled as follows. Different mixes of helper strands containing roughly the same concentration of each helper strand were prepared. These pre-mixes were then used with single-stranded M13mp18 phage DNA (M13) and TAE buffer containing Mg²⁺ ions, and brought to a final volume of 50 μ l with DI water (Millipore, 18 M Ω ·cm). Final concentrations were 240 nM per helper strand, 24 nM M13, 40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, and 12.5 mM magnesium acetate (1 \times TAE buffer w/ Mg²⁺). The sample was then heated to 95 $^{\circ}$ C in a thermal cycler (Eppendorf Mastercycler personal) and cooled at a rate of 0.5 $^{\circ}$ C per minute. The produced solution contained self-assembled DNA origami suspended in TAE buffer.

B. Buffer preparation

A stock solution of 10 \times TAE (all chemicals from VWR) buffer was prepared containing 400 mM Tris, 200 mM sodium acetate, and 10 mM EDTA. Magnesium chloride stock solution was 200 mM in DI water.

To study the effect of Mg²⁺ ion concentration, 5 \times TAE buffer with 12.5 mM, 50 mM, and 100 mM Mg²⁺ ions and 2.5 \times TAE buffer with 150 mM Mg²⁺ ions were prepared. The pH of all solutions was 8.3. The buffer containing 100 mM Mg²⁺ ions was used for the studies of the effect of pH to the adsorption. The pH was decreased by titration with hydrochloric acid to 7.1, 7.5, and 7.7.

C. Sample preparation

For the adsorption of DNA origami to clean or hydrophilic silicon dioxide surfaces, 1 cm² pieces of a n-type silicon <100> wafer were cleaned for 20 minutes with acetone and 2-propanol, then rinsed in DI water, assisted by ultrasonic agitation (all chemicals from VWR). Next, the surface was treated with oxygen plasma (FEMTO, Diener Electronic, Germany) to yield a hydrophilic surface, using standard cleaning parameters (50% power and 0.5 mbar chamber pressure for 5 min). A drop of 20 μ l 5 \times TAE buffer was dispensed onto the cleaned surface. The TAE buffer spreads completely over the cleaned surface. A 1 μ l drop of DNA origami solution was dispensed on top. The incubation time was 30 minutes in a closed petri dish. After that time the sample was dipped for 5 seconds into a solution of water and ethanol (50:50 v/v), followed by immersion for one hour in a solution of water in ethanol (10:90 v/v) [8]. All steps were done at room temperature. The sample was dried with a nitrogen gas stream before AFM imaging.

D. AFM measurements

All AFM measurements were done in tapping mode AFM with a scanning speed of 1 Hz (Veeco Multimode AFM, NanoScope III controller). The area of the scans was from 3 \times 3 μ m² up to 8 \times 8 μ m². The DNA origami per area were counted manually. Additionally, the number of DNA origami adsorbed totally flat on the surface was counted and put in relation to the total amount of DNA origami on the surface.

III - Results and Discussion

A. Effect of Mg²⁺ ion concentration

Multivalent cations are necessary to mediate the binding between the negatively charged SiO₂ surface and the also negatively charged DNA nanostructures. Our buffer contains monovalent Na⁺ ions that compete with divalent Mg²⁺ ions for the SiO₂ surface, but do not contribute to overcompensation of the negative surface charge and thus charge inversion of the surface. Therefore, higher Mg²⁺ ion concentration should help out-compete Na⁺ ions and improve DNA nanostructure adhesion. This is indeed what Figure 3a shows. The number of attached DNA origami nanostructures per area increases at a concentration of 150 mM Mg²⁺ ions. We plan to increase the density of data points between 100 mM and 150 mM Mg²⁺ ion concentration and up to 400 mM Mg²⁺.

The ratio of rectangular DNA origami structures attached flat on the surface already reaches 90 % at a Mg²⁺ ion concentration of 100 mM and does not decrease significantly when 150 mM Mg²⁺ are used.

In combination, it can be concluded that the coiled up appearance at lower Mg²⁺ ion concentrations is not

due to immediate immobilization of the nanostructures, but rather to insufficient adhesion.

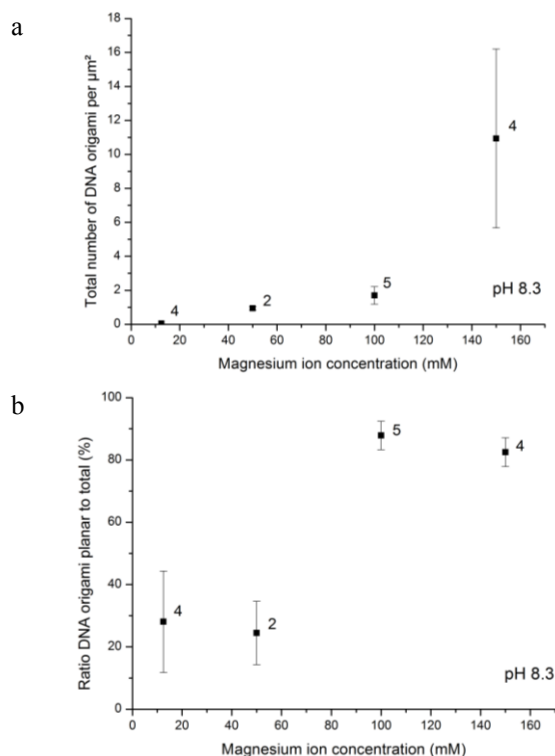


Figure 3: Dependence on Mg²⁺ ion concentration in pH 8.3 buffer during deposition. The error bars show the standard deviation. The number of samples is indicated next to each data point. a) Number of DNA origami nanostructures counted per μ m². b) Ratio of the number of DNA origami showing desired planar appearance on the substrate over total number of origami counted.

B. Effect of pH-value

Higher pH increases the density of negative charges because hydroxyl groups on the SiO₂ surface are more likely to be deprotonated. According to theoretical studies of Guldbrand, a critical surface charge density of 2.2 C/m² has to be exceeded for charge inversion to occur with divalent ions in the solution [9]. For SiO₂, this value is reached at about pH 7.5 [7]. In our experiments, the data suggest that out of the pH-values tested, good adsorption is given at pH 7.7 (Figure 4a).

The fraction of structures that are attached flat on the surface, indicating preferred interaction with the surface, clearly increases with pH (Figure 4b). This effect indicates that adsorption is not yet too high, such that the nanostructures would attach in random confirmation at first contact with the surface.

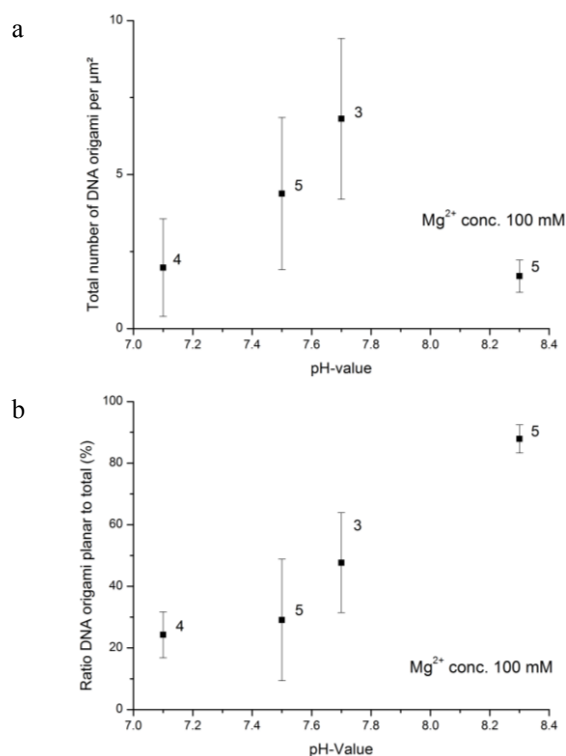


Figure 4: Dependence on the pH-value of the buffer during deposition. Mg²⁺ ion concentration was fixed to 100 mM. The error bars show the standard deviation. The number of samples is indicated next to each data point. a) Number of DNA origami nanostructures counted per μm². b) Ratio of the number of DNA origami showing desired planar appearance on the substrate over total number of origami counted.

IV - Conclusion

In this work we have shown that the Mg²⁺ ion concentration and the pH-value separately influence the adsorption of DNA nanostructures on SiO₂ surfaces. Charge inversion and the dependence on pH value are well known [7]. It is possible to take advantage of this dependence to control the adhesion of DNA nanomaterial on a silicon dioxide surface. This could be a smart way to arrange DNA material on the surface by changing pH during adsorption. An increased amount of attached DNA origami measured at pH 7.7 shows potential for the control of adsorption of DNA material, but the optimal parameters have yet to be determined.

A rapidly growing dependency of Mg²⁺ concentration up to 150 mM indicates the need for further investigation at higher concentrations. In addition, the cross-sensitivity of adsorption on pH and Mg²⁺ concentrations of the buffer solution needs to be quantified.

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