

DNA-scaffolded nanoparticle structures

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Abstract. DNA self-assembly is a powerful route to the production of very small, complex structures. When used in combination with nanoparticles it is likely to become a key technology in the production of nanoelectronics in the future. Previously, demonstrated nanoparticle assemblies have mainly been periodic and highly symmetric arrays, unsuited as building blocks for any complex circuits. With the invention of DNA-scaffolded origami reported earlier this year [1], a new route to complex nanostructures using DNA has been opened. Here, we give a short review of the field and present the current status of our experiments where DNA origami is used in conjunction with nanoparticles. Gold nanoparticles are functionalized with thiolated single stranded DNA. Strands that are complementary to the gold particle strands can be positioned on the self-assembled DNA-structure in arbitrary patterns. This property should allow an accurate positioning of the particles by letting them hybridize on the lattice. We report on our recent experiments on this system and discuss open problems and future applications.

1. Introduction

DNA nanotechnology [2] is the art of building nanoscale objects using DNA. This field has evolved rapidly in recent years, from periodic DNA-lattices [3] via algorithmic assemblies [4] to recent experiments where each strand has its pre-determined position in a lattice (*uniquely addressed* assemblies) [1, 5]. DNA nanotechnology has also been used to produce three dimensional objects like a 22 nm truncated octahedron [6]. To produce useful objects for electronics however, this range of complicated nanoscale objects will need integration with metals and semiconductors. DNA can easily be attached to gold nanoparticles [7, 8]. And a few groups have now started to focus on the attachment of functionalized nanoparticles on the pure DNA structures [9, 10]. The work presented in [9] and [10] deals with nanoparticles on DNA *tilings*, where each tile is about 15-20 nm. This distance is the same as the spatial resolution for patterns on these type of assemblies. The newly invented DNA-origami [1] has a much smaller spatial resolution on the order of 5 nm. Our work focus on the attachment of nanoparticles on DNA origami.

2. DNA origami

We have used a "tall rectangle" origami, designed by Rothemund [1] as a test lattice for the attachment of nanoparticles and proteins. The origami is self-assembled by letting a long circular DNA strand hybridize with 225 shorter *staple* strands, the principle is described in fig. 1. The long circular strand was DNA from a M13mp18 virus and was bought from Sigma Aldrich, Germany. The short staple strands were synthetic, cartridge-purified, oligos, bought from DNA

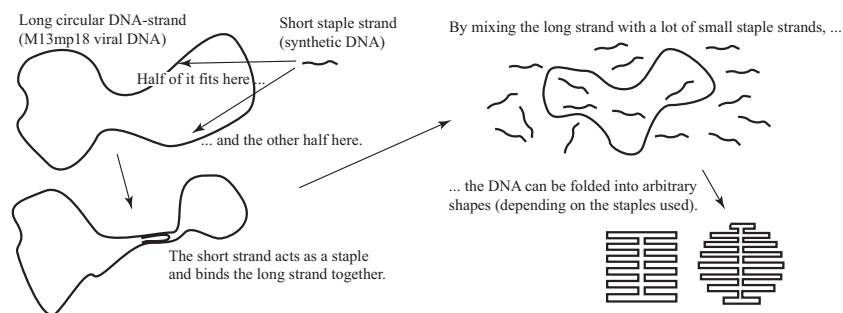


Figure 1. The principle behind DNA-scaffolded origami [1]. Since each staple has its predetermined position in the finished assembly, the staples can be used to address specific locations in the lattice (like in fig. 3(a)).

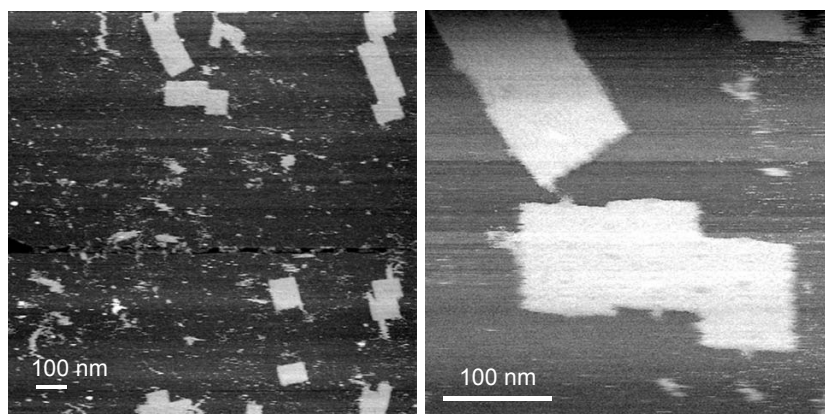


Figure 2. AFM images of the square, $\sim 70 \times 90$ nm, DNA origami's used in our experiments. AFM performed on mica under liquid.

Technology, Denmark. The origami's were self-assembled by mixing 0.05 pmole of the M13mp18 scaffold DNA with 5 pmole of each of the 225 staple strands in a $50 \mu\text{l}$ volume of Tris-HCl buffer, pH 8, 10 mM, with 1 mM EDTA and 12.5 mM MgCl_2 . Final concentration of viral scaffold was $0.001 \text{ pmol}/\mu\text{l}$. The solution was heated and allowed to cool slowly (about 1h) in a PCR-thermocycler from 95 to 20°C . Samples for atomic force microscopy (AFM) were produced by placing a drop of the solution on a piece of freshly cleaved mica. Liquid AFM was performed directly under buffer or under isopropanol after rinsing with distilled water, the result is shown in fig. 2.

3. Attaching proteins to the origami

To test the addressability of the DNA origami we exchanged some of the staples with oligos modified with a biotin molecule at the 5'-end. These oligos were purchased from Cybergene, Sweden. The biotin-oligos were exchanged for some of the staples to produce a pattern of a walking man, see fig. 3(a). After assembly of the origami's, streptavidin protein (Sigma Aldrich), was added to the solution and incubated overnight at 4°C . Streptavidin binds to biotin and creates a contrast in the AFM because of the ~ 2 nm diameter of the streptavidin protein. Since the biotin staples were present in a large excess over the origamis, streptavidin must also be added in a large excess, this results in the high streptavidin background that can be seen in the AFM micrograph (fig. 3(b))

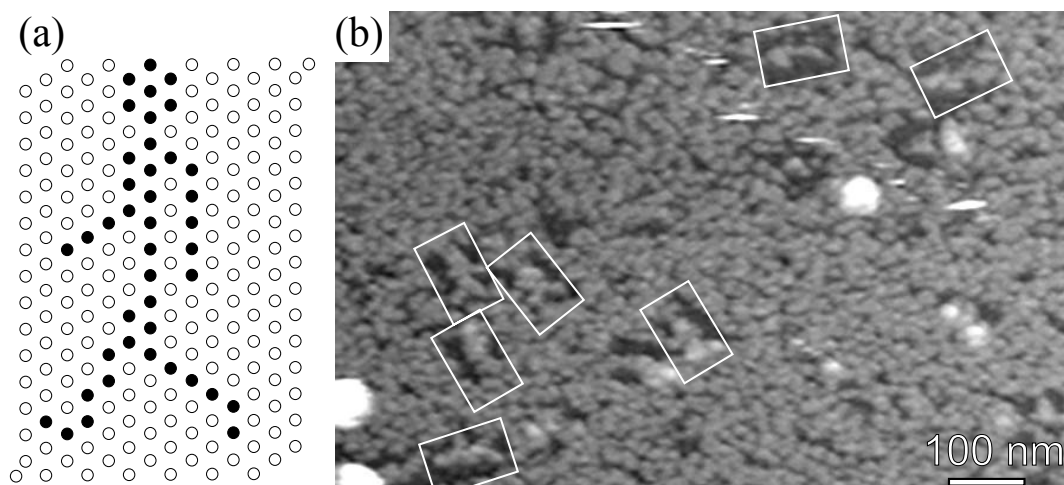


Figure 3. (a) The pattern of staples in the rectangle origami. The black staples were exchanged with biotin-modified DNA. (b) AFM image of biotin modified origami's where streptavidin proteins have been attached to produce the walking man pattern. White rectangles have been drawn around some of the walking men as a guide to the eye. AFM performed in isopropanol.

4. DNA functionalized gold nanoparticles

In order to attach nanoparticles to the DNA lattice, some of the staples are exchanged for staples that have prolonged sequences at the 5'-end. The extended part of the staple sequence is CTCTCCTTCCCTTT, and the nanoparticles are functionalized with the complementary strand AAAGGGAAGGAGAG. So instead of a biotin group in the case of the biotin-streptavidin experiment above, the origami's now contain some staples with sticky ends of DNA protruding from the lattice for attachment of functionalized nanoparticles.

Three types of DNA-functionalized gold nanoparticles were used, schematically depicted in fig. 4. We used particles that either had only one coding DNA strand per particle, like type I(a) and I(b) in fig. 4. With coding strand, we mean that the sequence of that strand is the complement to the sticky ends protruding from the origami lattice. We hypothesized that the advantage of type I particles would be that they could be mixed directly with the scaffold- and staple-DNA in a one-pot experiment. Whereas the particles of type II, with a coverage of many coding DNA strands, a one-pot experiment would probably fail, since each of the DNA strands of the particle would bind a staple and thus prohibit correct assembly of the origami. The type II particles with many coding strands were used exclusively in two-pot experiments where the origami first is produced and the particles added subsequently. The addition of a shell of non-coding DNAs as in type I(b) has the effect of stabilizing the particles in Mg^{2+} solutions, see below.

4.1. Nanoparticles

Gold nanoparticles of size 5 nm were bought from G.Kisker GbR, Germany. The red gold suspensions (100 ml) were mixed with 4,4'-(Phenylphosphinidene)bis(benzenesulfonic acid) dipotassium salt (about 10 mg), and stirred overnight. This step is created a ligand shell around the particles [11], preventing them to precipitate when exposed to salt. [12]

4.2. Thiolated DNA strands

Synthetic, modified oligonucleotides were bought from Cybergene AB, Sweden. The strands for attachment on gold particles were equipped with a thiol modification at the 3' end.

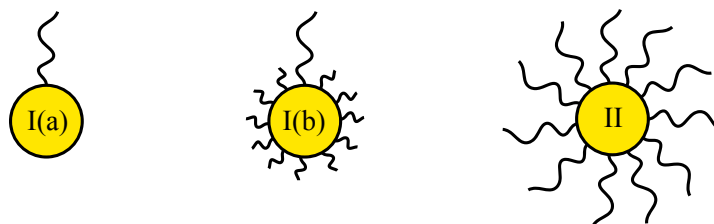


Figure 4. The three types of DNA-functionalized nanoparticles used. I(a) a single DNA strand attached to each particle. I(b) a single long, coding, DNA strand attached and many small, non-coding AAAAA sequences to prohibit particle aggregation. Type II nanoparticles uses a homogenous coverage of coding DNA strands.

4.3. DNA-Gold Conjugates

The ligand protected nanoparticles were concentrated by salt precipitation followed by centrifugation. In the production of type I(a) particles, a one-to-one ratio of particles and thiolated DNA was mixed, promoting the formation of particles conjugated to exactly one DNA strand. To produce type I(b) particles we took type I(a) particles and added a large excess of 3'-thiolated AAAAA strands. In the case of the type II particles, a large excess (about 50 DNA strands/particle) of thiolated DNA were added to the original ligand-particle suspension. In all cases the solution was brought to 0.1 M NaCl and 10 mM Phosphate buffer, pH 7, and left at room temperature for two days. After conjugation, excess DNA was washed away by repeated centrifugation and re-suspension of the pellet in 0.3 M NaCl, 10 mM phos. buffer. The amount of particles was assessed by spectroscopy at 520 nm.

4.4. One-pot experiments

In one-pot experiments, particles of type I(a) or (b) were mixed with staples and the viral, scaffold DNA in the following fashion: Twice as many functionalized particles were added as "pattern"-staples, *i.e.* the staples that were prolonged with a coding sequence at the 5'-end for attachment of nanoparticles. And the amount of each of the staples were about 20-50 times the amount of M13mp18 viral DNA. The final concentration of scaffold DNA was generally around $3 \cdot 10^{-4}$ pmol/ μ l, about 3 times lower than in the pure DNA experiment.

We found that the type I(a) particles were stable in NaCl but unstable in MgCl₂ solutions. To try the ability of forming origami's in NaCl we made a few runs with pure DNA origami's like in sect. 2 but with the Mg-ions substituted for a Na-ions at different concentrations (0.1-0.5 M). Non of the experiments yielded as clear, well-formed, origami's as with Mg-ions, (fig. 2). We therefore abandoned the experiments with particles of type I(a) in favor of particles of type I(b) where a shell of non-coding DNAs keeps the nanoparticles from precipitating in MgCl₂ [13]. We found that the type I(b) particles were stable up to about 4.5 mM MgCl₂ and 0.5 M NaCl. To try the efficiency of origami formation, DNA origami's were made in a solution of 4.5 mM MgCl₂, 0.5 M NaCl, 10 mM Tris-HCl (pH7) and 1 mM EDTA. These origami's were as reproducible and as well formed as the ones made in 12.5 mM MgCl₂, so a one-pot experiment with type I(b)-particles was expected to produce the desired nanoparticle patterned origami's. However, when type I(b) particles were added to the mixture, no traces of patterns or origami's could be found by AFM or TEM.

4.5. Two-pot experiments

The two-pot experiments were conducted by first assembling the DNA-origami's, like described in sect. 2, fixing them on a piece of freshly cleaved mica, then rinsing the mica with distilled

water, and subsequently adding a few drops of a suspension of particles of type II. As with the one-pot experiments, initial results are discouraging. We observed no clear patterns of particles.

5. Conclusions and Open Problems

As shown in the original experiment by Rothmund [1], and in our trials with streptavidin proteins it is straightforward to address different locations on a DNA-origami and make complex nanoscale patterns. However, judging from our initial experiences, it seems more difficult to attach non-biological components with the same accuracy. One reason why our experiments with nanoparticles fail to produce the desired patterns might be that the effective diameter of the nanoparticles is much greater than 5 nm. In reports dealing with periodic patterns of particles on DNA lattices [9, 10, 13], the smallest distance between two 5 nm particles is normally about 20 nm, even if the underlying lattice has more closely spaced hybridization points as in [9] and [10]. Furthermore, the apparent width of a 5 nm particle as measured in AFM is also around 20 nm. If the effective diameter of a functionalized 5 nm particle is in fact around 20 nm, then it is clear that it would be hard to prove the assembly of any complex patterns on a $\sim 70 \times 90$ nm large rectangle. We have tried to assemble nanoparticles in sparse patterns on the origami rectangles, using only a few particles one each origami. Since the rectangles end up in a non-periodic way on the mica, and the DNA lattice becomes undetectable in AFM when particles are numerous on the surface, we have not been able to prove that the particles seen in AFM really rests on an origami. The use of smaller nanoparticles needs to be tested.

Things are further complicated by the fact that the bond that attaches the particle to the origami is a rather floppy chain of DNA, about 5 nm in length (the coding sequence is 14 base-pairs long). This makes a correctly hybridized particle mobile within a 5 nm radius and could produce distortions in the desired pattern. One could try a shorter coding strand but this would make the bond more unstable. One could also try a periodic coding sequence, like a sequence of T's alone as is done in [10], this would give the particles a bit more freedom to move along the coding strand and may make it easier to closely pack particles. However, a periodic nucleotide sequence would make it harder to extend the technology to involve different coding sequences for different types of particles.

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