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Anisotropically DNA-functionalized nanoparticle dimers

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Abstract. Self-assembly of complex, non-periodic nanostructures can only be achieved by using anisotropic building-blocks. The building blocks need to have at least four bonds pointing in separate directions [J. Comput. Theor. Nanosci. **3**, 391 (2006)]. We have previously presented a method for the synthesis of such building-blocks using DNA-functionalized gold nanoparticles. Here, we report on the progress in the experimental realization of this scheme. The first goal, in a process to make programmable self-assembly building-blocks using nanoparticles, is the production of dimers with different DNA-functions on the two component particles. We report on the fabrication of anisotropically functionalized dimers of nanoparticles of two different sizes. As a result of their anisotropy, these demonstrator building blocks can be made to assemble into spherical structures.

PACS. 81.16.Dn Self-assembly – 81.16.Rf Nanoscale pattern formation – 82.39.Pj Nucleic acids, DNA and RNA bases

1 Introduction

A technology for the production of self-assembled nanostructures is generally regarded as an important step towards better electronics, sensors and medical technology [1]. As always in these applications, the required geometrical structures are rather complex and often far from periodic in nature. Whereas periodic self assembly in the form of monolayers, particle crystals etc has been thoroughly investigated in the past, the assembly of complex, aperiodic, structures has only recently begun to attract attention. The most complex self-assembled nanostructures produced to date are made from DNA [2,3]. Although impressive in their structural complexity, it is generally accepted that the functionality of structures made from DNA alone is rather limited. To produce functional nanodevices metals and semiconductors will probably be needed. DNA can easily be attached to gold nanoparticles [4,5] and DNA-coated gold particles have been successfully attached to pure DNA-nanostructures [6]. Our aim is to directly assemble structurally complex particle structures, without the need for a pre-fabricated DNA scaffold. In order to produce non-periodic structures from self-assembly of simple building blocks, these need to be of a certain minimal complexity. In particular, they need to have the possibility to form at least four different bonds and they need to be anisotropic so that the bonds are well directed in separate directions [7]. Some attempts to produce anisotropically functionalized particles have been made, see for example [8]. However these particles are large (1 μm) and it is hard to imagine how similar methods could be used on nanoscale particles.

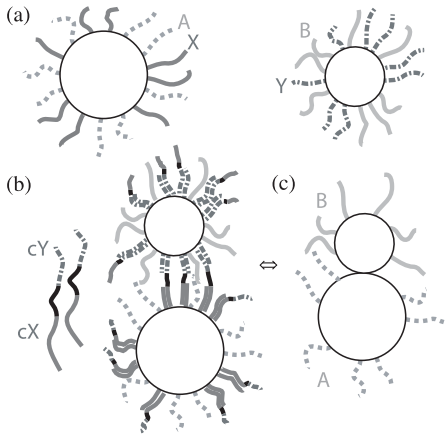

Fig. 1. (a) Two batches of nanoparticles are uniformly coated with a mix of two types of DNA, A, X and B, Y. (b) The two nanoparticle types are mixed with linker DNA, cX-cY, that binds two DNAs together (the X and Y DNAs), forming a nanoparticle dimer. The nanoparticle dimer has different kinds of unused DNA left on the different particles and is thus functionally equivalent to the anisotropic dimer shown in (c).

2 Ideas and experimental design

Instead of trying to anisotropically functionalize single particles, our approach is to make building blocks consisting of several particles. By making a heterodimer, for example, a building block is obtained which provides two

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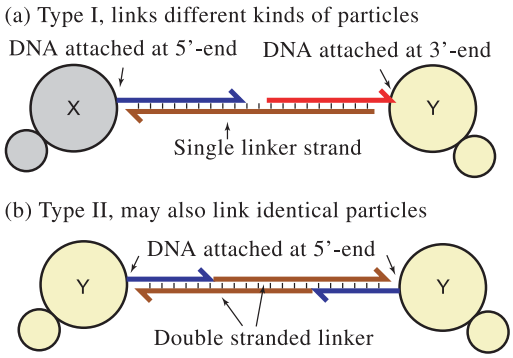

Fig. 2. The two ways of attaching a DNA strand to a particle, either at its 3'-end or its 5'-end, defines to types of possible ways of linking the particles with DNA.

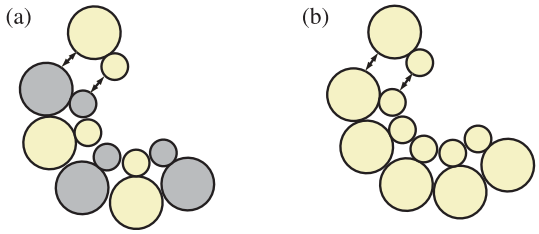

Fig. 3. Self-assembly of anisotropic dimers. If the small particles binds to small particles and large particles binds to large particles, the dimers should self-assemble into curved structures (or spherical 3-dimensional structures). In type I experiments the interacting dimers must be of two different types (a) whereas in type II experiments the dimers can all be identical (b).

different bonding options, one on each particle. The idea is briefly presented in Figure 1. This scheme can in principle be extended to produce more advanced building blocks like tetramers or octamers, see [9,10]. Note that when linker DNA is added, the formation of dimers is not the only possible reaction. One would expect a number of larger aggregates to form as well. By adding equimolar amounts of linker, particles of type one, and particles of type two, we can promote dimer formation. However, purification by gel electrophoresis is still necessary to obtain pure heterodimer samples.

After the production and purification of dimers we proceed to self-assembly using the dimers as building blocks. Two types of DNA linkage has been tested.

2.1 Type I

In type I experiments we used the same type of DNA linking during assembly as in the case of the dimer formation. Here, the gold-attached DNA strands that participated in the binding reaction had opposite orientations. One strand was attached to its particle by the DNA 5'-end and the other to its particle by the 3'-end. The linker was single stranded and bound with its 5'-end to the 3'-attached particle and the 3'-end of the linker strand bound to the 5'-attached particle. See Figure 2a.

2.2 Type II

In type II experiments the particles were linked by a double stranded DNA linker with protruding sticky ends. Here the strands attached to the particles must have the same orientation, i.e. both were attached at the 5'-end. See Figure 2b. In type II linkage, the participating particle types can be identical whereas in type I linkage the particles must be of different types since the strands attached to the particles must have different orientations.

2.3 Proving anisotropy of the dimers

As explained above, the dimers should behave like a particle with two bond types, one on each constituent particle. By mixing the dimers with linkers that make the small particles stick to other small particles and large particles stick to large particles, the dimers should self-assemble into curved structures like the ones shown in Figure 3. Other structures are also possible by multiple linkages between small or large particles. However, cooperative binding of several bonds, both on the large and the small particles, should promote formation of the curved structures. For discussions on cooperative bonding in programmable self-assembly, see [2,7] and references therein.

3 Materials and methods

3.1 Nanoparticles

Gold nanoparticles of size 10 and 20 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 created a ligand shell around the particles [11], preventing them to precipitate when exposed to salt. It also facilitated the electrophoresis by giving the particles a negative charge [12].

3.2 DNA-samples

Synthetic oligonucleotides were bought from Cybergene AB, Sweden. The strands for attachment on gold particles were equipped with a thiol modification at the 3' or 5' end.

3.3 DNA-gold conjugates

The ligand protected nanoparticles were concentrated by salt precipitation followed by centrifugation. An excess (compared to reported maximum surface coverage [13]), about 50 DNA strands/particle, of thiolated DNA were added to the particle suspension. 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

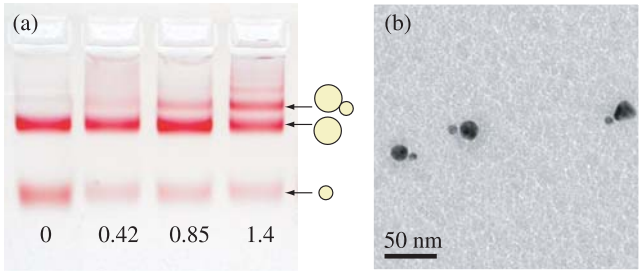

Fig. 4. (Color online) Electrophoretic separation of 10 nm and 20 nm-dimers. The numbers below the lanes in (a) indicate how many linker strands per small-large particle pairs that are added to the solution. The dimer band is strongest when about 1.4 linkers per pair are added. (b) A TEM micrograph from a dimer sample extracted from the gel.

0.3 M NaCl, 10 mM phos. buffer. The amount of particles was assessed by absorbance spectroscopy at $\lambda = 520$ nm on a Varian Cary 50 UV/Vis spectrophotometer.

3.4 Dimer formation and purification

To prepare 10 nm and 20 nm dimers, an equimolar mix (in terms of number of particles) of the two size DNA-conjugated particles, were mixed with linker DNA. The formation of dimers was verified by agarose gel-electrophoresis in TBE buffer and the dimer bands were electroeluted to a small piece of glass-fiber filter paper. The filter paper was brought to a centrifugal filter and the dimer solution collected by centrifugation.

3.5 Self-assembly

NaCl (aq., 5 M) was added to the suspensions of dimers (two kinds of dimers in type I experiments, and one kind in type II) to give a 0.3 M NaCl concentration. The resulting suspensions were mixed with appropriate DNA-linker strands, heated to around 45 °C, and allowed to cool slowly under 2 h.

3.6 TEM-sample preparation

A drop of gold suspension was placed on a silicon monoxide / formvar coated copper grid (Ted Pella Inc.), allowed to adsorb to the surface for about 10 minutes and then dried by wicking from the side with a piece of filter paper.

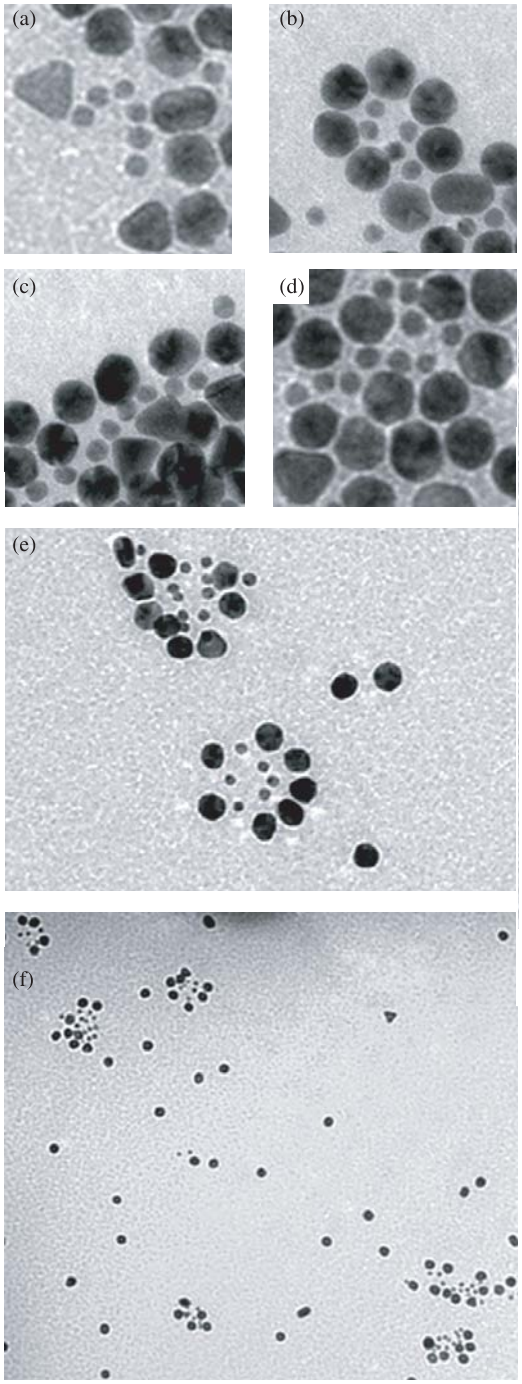

Fig. 5. TEM images of samples resulting from dimer self-assembly. (a)−(d) Type I linkage, two types of dimers. (e)−(f) Type II linkage, one type of dimers.

4 Results and discussion

The formation of dimers of 10 and 20 nm gold particles was verified by gel electrophoresis. As shown in Figure 4a the samples containing linker-DNA showed additional bands corresponding to dimers and larger aggregates. The most efficient way to produce dimers was to add slightly more than one linker par pair of 10 nm and 20 nm particles. We normally used 1.4 strands per pair. The dimers were always made using type I linkage.

We tried a few methods for retrieval of the dimers from agarose gel. When we inserted a glass fiber filter backed by a piece of dialysis membrane in front of the desired band and then electroeluted the sample onto the filter, the largest amount of dimers was collected. We estimate that only about 50% of all particles form dimers and that about 50% of the dimers are lost during gel extraction, leading to a total yield of about 25%. Figure 4b shows

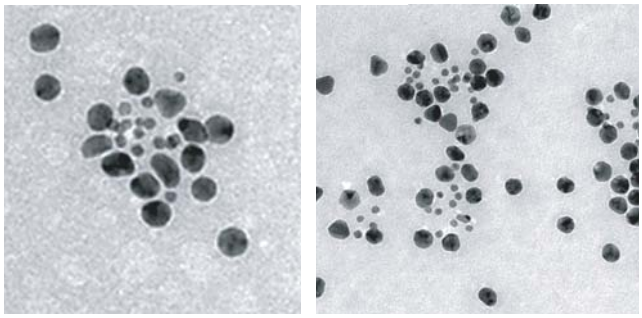

Fig. 6. TEM images of a sample where the large-large linkers have been excluded.

a TEM image from an extracted dimer sample. Most of the particles were found in dimers, however, a substantial amount (about 5−10%) of the particles were singles. We hypothesize that many of the singlets are formed from dimers breaking up during gel-extraction.

Initially, this work was performed using only type I linking, both for dimer formation and assembly of the dimers. The results are shown in Figures 5a−5d. The yield of structures were quite low, many particles were found in larger aggregates without ordered structure. Structures, like the ones found in Figures 5c−5d were unexpected. Maybe an excess of single particles could explain this sort of linear structures.

Two problems were identified in these type I experiments: stoichiometry and temperature sensitivity. Getting the right stoichiometry between the two types of dimers used, was very difficult. Because the amount of extracted dimer sample was so low, the quantization readings by spectroscopy were of poor quality. Thus, the preferred (1:1) stoichiometry of the two dimer types could not be ensured. Furthermore, to get good self-assembly we wanted to keep the sample at a temperature where the dimer-dimer bonds formed. In the initial experiments, Figures 5a−5d, the intra-dimer bonds were as strong as the bonds between dimers, i.e. the sticky ends of the DNAs that formed the bonds were of the same length. The bonds keeping the dimers together, thus had the same melting temperature as the bonds making up the dimer-dimer structures. In order to remedy this, we designed a type II experiment where only one type of dimer where involved, thus avoiding the problem of stoichiometry. Furthermore the type II experiment was designed so that the DNA sticky ends involved in the dimer-dimer assembly were much shorter than the ones keeping the dimers together. This way we could stay at the melting temperature of structure formation without risking dimer break-up.

The results of the type II experiment is shown in Figures 5e−5f. Here, a majority of all particles were found in curved structures. The particles not in curved structures were almost all single particles, so one can conclude that almost all of the correctly preserved dimers were successfully assembled into curved structures. This in all gives a clear indication that the observed structures are indeed results of anisotropic building-blocks.

To test if the structures could be tuned by changing the type of linkers added, we made a sample where no large particle-large particle linker was added, only the small-small linker. Since the large particles do not participate in the self-assembly, only as luggage for the small particles, the structures formed should be more irregular. The result is shown in Figure 6. As expected, this sample showed structures much more disordered than the previous ones but still with a clear effect of aggregation of small particles, surrounded by larger ones.

5 Conclusions

A method for the production of nanoparticle building blocks for DNA-mediated self-assembly is presented. As anisotropic building blocks are crucial for the production of complex patterns by self-assembly we have focused on the production such building blocks: anisotropic dimers. The fact that the dimers self-assemble into small curved aggregates demonstrates that the dimers are in fact anisotropic building blocks.

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