DNA Coated Nanoparticle eight-mers as Programmable Self-Assembly Building Blocks

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Abstract

Nanoparticles coated with single stranded DNA have been shown to efficiently hybridize to targets of complementary DNA. This property can be used to implement programmable (or algorithmic-) self-assembly (PSA) to build nanoparticle structures. However, we argue that a DNA coated nanoparticle by itself cannot be used as a PSA building block since it does not have directed bonds. A scheme for assembling and purifying nanoparticle eight-mers with eight geometrically well-directed bonds is presented together with some preliminary experimental work.

Introduction

DNA functionalized nanoparticles has been a prospect material for the construction of self-assembled structures ever since they were first discovered. 1,2 So far the main interest in these particles has been using it as a detection method for small amounts of specific DNA. 3 The structures formed in these and other 4 experiments are mainly periodic-, or random agglomerations of particles. In order to fully exploit the possibilities for self-assembly of DNA coated nanoparticles one needs to address the problem of assembling nonperiodic structures.

PSA Building Blocks

It has become clear that in order to implement programmable self-assembly one needs building blocks of a certain complexity and diversity. 5-9

In addition to different types of building blocks, PSA also requires that the building blocks have some minimal geometrical complexity. PSA is not possible using parts that have the same kind of binding on all faces. Using these kind of building blocks will inevitably lead to periodic, noncomplex structures. A PSA building block needs to have at least four different functionalized surfaces that are geometrically separated. 9 In other words, the building blocks need to have at least an up-face and a down-face with at least two different open bonds on each face. Preferably they should have as many functionalized surfaces as possible in order to make complex 3D structures.

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Building blocks that are tens of micrometers big can be manufactured and functionalized using conventional micro-technology. However, as the dimensions of the building blocks get reduced, the harder it gets to functionalize different parts of the building blocks with different functions. When dealing with nanoscale objects, a top-down procedure for selective functionalization is no longer feasible.

We propose a method for the fabrication of nanoscale PSA building blocks using a bottom-up approach.

Making PSA-Building Blocks from Nanoparticles

First, nanospheres of a suitable material are functionalized with two different types of single stranded DNA (ssDNA). For example, for gold and SiO$_2$ there exists standard protocols for this. Nanospheres of a second kind are prepared in the same way but using yet another set of different DNA sequences.

![Figure 1](image-url)
These spheres are then mixed in solution, see fig. 1(a). Consider the case when nanospheres of type I have ssDNA sequences A and X and the nanospheres of type II have sequences B and Y. Linker molecules, consisting of a chain of complementary sequences to Y (cY) and X (cX) coupled by some sequence of bases that are irrelevant to the assembly process (cL) are added to the solution, see fig. 1(b). As the linker molecules are added the spheres of type I and II will adhere to one another and form aggregates of different sizes. By controlling the temperature of the solution we can promote the formation of dimers. After a while the linker DNA is rinsed away, stopping any further aggregation of the nanoparticles. The dimers could then be separated from the rest of the aggregates by centrifugation, gel electrophoresis or some other mass separation technique. If necessary, the sticky ends of the linker DNA can be made more stable by annealing.

Figure 2 Demonstration of a self-assembly process, using DNA nanosphere eight-mers as building blocks. The eight-mers have eight specific ssDNA binding sites. These eight-mers can be thought of as cubes having functionalized surfaces (a). In (b) four more eight-mers are depicted. When mixed in solution the DNA complements will bind to each other. In (c) for example, the cA and cB of block 2 binds to the A and B strands on block 1. Mixing all the building blocks of (a) and (b) will lead to the formation of structures like the one on the right in (d). Note that the “S” DNA of block 4 has no complement on the other blocks so block 4 acts as a stop-block.

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molecules could be passivated by adding a solution of LY and LX DNA (fig. 1(d)-(e)). The dimers produced in this way constitute simple PSA building blocks with ssDNA of types A and B sticking out at different ends of the dimers. By starting out with particles coated with four specific DNA sequences and using several iterations of this technique one could make four-mers and eight-mers to be used as more advanced PSA building blocks, fig. 1(g).

Following the above method one can create a great number of different basic building blocks with specific sticky ssDNA on different faces of the blocks. As the basic building blocks are mixed together with blocks having complementary DNA self-assembly will occur. Fig. 2 contains an example of an assembly process using these basic PSA nanosphere building blocks.

It is important to note that the programmable self-assembly process will require cooperative binding of two bonds in order to uniquely produce the desired structures and making erroneous structures unstable. In this report we will not go into the details of the PSA process itself but concentrate on the production of the PSA building blocks. For general discussions on criteria for PSA see for example 9,14,15.

Materials and Methods

Gold colloids with mean diameters ranging from 10 nm to 50 nm were purchased from G.Kisker GbR. Typical size distributions were about 15%, concentrations are approximately 0.07 nM for the 50 nm up to about 7 nM for the 10 nm particles. Thiol modified (5’ and 3’) and unmodified ssDNA were purchased from Cybergene AB. All oligos were HPLC-purified by the manufacturer. The length of the thiolated oligonucleotides were 16 code bases and 10 bases of consecutive T’s between the thiol group and the coding sequence. The ten T’s act as a separator between the particle and the hybridizing sequence. The coding sequences were optimized to have cross-hybridization probability as small as possible. The sequences were also checked for unwanted hairpin and dimer formation. To this end we used software developed by ourselves and some online tools16. We have also used a subset of the DNA library for DNA-computing by Penchovsky and Ackermann17. This set of oligos is well optimized for uniqueness and the sequences are also optimized to have similar melting temperatures.

The functionalization of the gold particles followed a modified version of the Storhoff protocol3. Equal amounts of 4 different thiolated oligos to a total of 1 nmole DNA are mixed separately and then mixed with 1.3 ml of the gold colloid. These samples are stored at 30°C overnight followed by the addition of a sodium phosphate buffer to 0.01 M (pH 7) and a NaCl solution up to 0.1 M. The addition of salt is done drop wise and in two steps separated by roughly two hours. Furthermore, the addition of salt is done at an elevated sample temperature, typically 70-90°C. Without this temperature increase, the gold solution easily aggregates. After another 48 h at 30°C the samples are brought to 0.3 M NaCl, again at 70-90°C. The colloidal solutions are then centrifuged twice at 14000 rpm for 10-60 min (depending on particle size) with an intermediate rinsing. Finally the pellet containing the DNA-modified particles, is redispersed in a 0.01M phosphate, 0.3 M NaCl, 0.01% Azide solution.
Particles of different DNA types were mixed together and the appropriate linker molecules added. After incubation for about 60 min at 20-60°C gel-loading buffer (dextrose) was added and the samples were allowed to cool down during gel loading. Agarose gel (0.8% w/v) electrophoresis was performed to separate the dimers from single particles and from larger aggregates, see fig. 3(a).

The dimer bands were cut out from the gel using a scalpel and diced into smaller pieces. The dimers were retrieved from the gel slices using Nanosep MF centrifugal filters (Pall Corporation).

SEM studies were performed on the 50 nm gold particles using a LEO-1450 EP electron microscope.

**Preliminary Results and Discussion**

The ssDNA successfully attached to the particles. This is clear from the fact that the gold colloids are stable in high salt concentration. The formation of dimers, trimers and larger aggregates has been verified by gel-electrophoresis, see fig. 3(a) and SEM photos, fig. 3(b). The incubation temperature seems to have an important effect. The only difference between the columns in fig. 3(a) is the incubation temperature. At lower temperatures the incubation time of 1 h seems to be inadequate to form aggregates. The samples heated to 51 °C and 60 °C show bands that may correspond to gold-particle clusters of single, dimer, trimer and even four-mer as well as a long “tail” of larger aggregates not found in 20 °C and 40 °C samples. The incubation time of 10 minutes or 1 hour shows no difference in electrophoresis.

We have performed other electrophoresis experiments where gold particles coated with two types of ssDNA also showed strong dependence on temperature. Below 46°C, the column did not separate but above 51 °C it showed the same separated bands that presumably depends on the cluster size. 51 °C is close to the calculated DNA melting temperature for our oligos. One thing interesting is that gold particles coated with only one type of ssDNA traveled furthest in electrophoresis gel compared to those coated with 4 ssDNA, whereas the gold particles without DNA coating did

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**Figure 3** (a) Photograph of the discrete red lines formed by agarose gel electrophoresis of 50 nm DNA coated nanoparticles with linker molecule added. Bands of increasingly bigger aggregates are visible. The temperatures below the bands refer to the incubation temperature. (b) Electron micrograph of electrophoresis-purified nanoparticle dimers. The concentration of single particles is still relatively high in these samples, probably due to to breaking up of some of the dimers when the particles are extracted from the gel by centrifugation.
not move at all in the gel. Thus, the response of coated gold particles in gel to the
applied electric voltage (100V) is due to electrostatic force of the coated DNA
molecules only. The separated bands are due to the balancing of the electrostatic
force that is proportional to the coated DNA density and the drag force that is
proportional to the cluster size of gold particles.

The method of separating the dimers from the agarose gel by centrifugal filtration
seems to be working. Single particles, dimers, and trimers have been detected by
SEM, see fig. 3(b). There is still a relatively large portion of single particles in the
extracted dimer and trimer samples. We hypothesise that this could be due to
breaking up of the dimers and trimers during the gel-extraction by centrifugation.
There are also larger aggregates in SEM photos at the edge of the sample area. This
may be due to aggregation during the drying process of the SEM sample droplet.

If the linking DNA strands were ligated before electrophoresis the bond should be
stronger and less single particles would be found as the dimers would not break up so
easily.

One problem with the current procedure is the low yield of dimers using the gel
separation technique, this low yield is also to be expected for the assembled four-mers
and eight-mers. Another difficulty is to control the temperature of the gel chamber.
An optimum temperature for dimers and other smaller aggregates is probably just
below the melting temperature for the oligos. A separation technique with a higher
yield and precise thermal control will eventually be needed.

Conclusions

A principal scheme for the production of building blocks with well-directed bonds for
programmable self-assembly using DNA-nanoparticles has been presented. Initial
experiments have not given any indications that the devised scheme would be
unfeasible. On the contrary we are encouraged by the fact that this method seems to
work with simple and proved methods. However, to get a higher yield from the
process a new particle separation method might be needed.

2 Alivisatos, A. P.; Johnsson, K. P.; Peng, X. G.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P.;
3 Storhoff, J. J.; Eghbalian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *Journal of the American
4 Taton, T. A.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *Journal of the American Chemical Society*
2000, 122, 6305.
5 Winfree, E. *DNA Based Computers*; Lipton, R. J., Baum, E. B., Eds.; Amer. Math. Soc.: Princeton,
1996; Vol. 27; pp 199.
7 Rothemund, P. W. K.; Winfree, E. Proceedings of 32nd Annual ACM Symposium on Theory of
Computing, 2000, New York, NY and Portland, OR, USA.
10 Bao, Z.; Chen, L.; Weldon, M.; Chandross, E.; Cherniavskaya, O.; Dai, Y.; Tok, J. B.-H. *Chemistry of
11 Jin, R. C.; Wu, G. S.; Li, Z.; Mirkin, C. A.; Schatz, G. C. *Journal of the American Chemical Society*
2003, 125, 1643.
12 Demers, L. M.; Ostblom, M.; Zhang, H.; Jang, N.-H.; Liedberg, B.; Mirkin, C. A. *Journal of the
American Chemical Society* 2002, 124, 11248.
16 Online tools for oligo-design. mature.com/oligonucleotide.html, rna.tbi.univie.ac.at/cgi-bin/alignfold.cgi, scitools.idtdna.com/Analyzer/.