Available online at www.sciencedirect.com
SCIENCE DIRECT
Applied Surface Science 252 (2006) 5538–5541
applied surface science
www.elsevier.com/locate/apsusc

Study of DNA coated nanoparticles as possible programmable self-assembly building blocks

Björn Högberg [a,*], Jing Helmersson [a,b], Svante Holm [c], Håkan Olin [a,*]

[a] Department of Engineering Physics, Mid Sweden University, Sweden
[b] Department of Physics and Astronomy, California State University Long Beach, California, USA
[c] Department of Natural Science, Mid Sweden University, Sweden

Available online 3 April 2006

Abstract

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

PACS: 81.07.−b; 81.16.Dn

Keywords: Nanoparticles; Nanostructure; DNA coated nanoparticles; Programmable self-assembly; Gold particles

1. Introduction

DNA functionalized nanoparticles have been a prospect material for the construction of self-assembled structures since first demonstrated [1,2]. So far, the main interest in these particles has been to use them in detection methods for small amounts of specific DNA [3]. The structures formed in these, and other [4] experiments, are mainly periodic, or random agglomerations of particles. To fully exploit the potential of self-assembly of DNA coated nanoparticles one needs to address the problem of assembling non-periodic structures.

To implement programmable self-assembly (PSA) one needs building blocks of a certain complexity [5–9]. In short, the requirements are: (1) unspecific bonds, that can bind to several types of different building blocks, (2) an incoming building block must interact with at least two other, already assembled blocks and (3) at least four bonds on each block to avoid blocking of the assembly. For general discussions on criteria for PSA see for example [9–11]. Using a diamond like structure, it is possible to build three-dimensional structures using building blocks with only four bonds, but a more practical approach is to use triangular prisms or cubes having at least 5, respectively, 6 bonds (6, respectively 8 bonds, if the bonds are situated at the corners).

Building blocks that have a size of micrometers can be manufactured and functionalized using conventional micro-technology, one example of a such a top-down approach for the functionalization of two distinct areas of 1 μm particles can be found in [12]. However, with reducing dimensions, it is increasingly difficult to functionalize different parts of the building blocks with different functions making a top-down procedure no longer feasible. A new approach is needed. We here propose such a new method for the fabrication of nanoscale PSA-building blocks using only bottom-up methods.

2. Making PSA-building blocks from nanoparticles

Two sets of nanospheres of a suitable material are functionalized each with two different types of single stranded DNA (ssDNA). For example, for gold [13,14] and SiO_2 [15] nanoparticles, there are standard protocols for this. These spheres are then mixed in solution together with linker

* Corresponding authors. Fax: +46 60 14 88 75.
E-mail addresses: bjorn.hogberg@miun.se (B. Högberg), hakan.olin@miun.se (H. Olin).

0169-4332/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsusc.2005.12.127

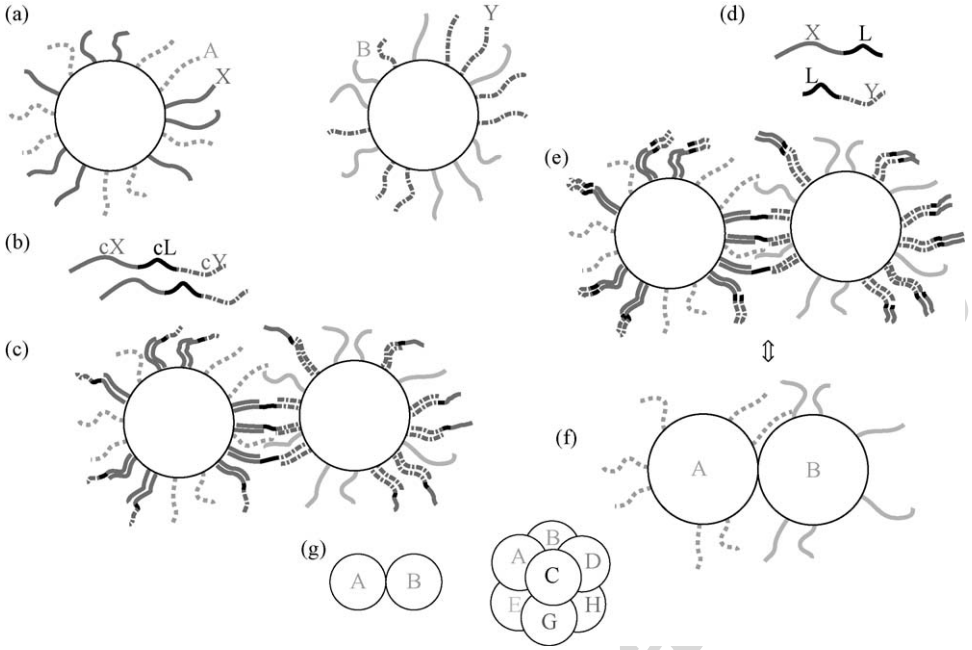

Fig. 1. Scheme for nanoparticle PSA-building block assembly. (a) Two different sets of nanospheres are each coated with two (or more) single stranded DNA sequences (A–X and B–Y). (b) Linker molecules. (c) Dimers can be extracted by mass separation. (d) Passivation of remaining sticky ends by complementary molecules (X–L and L–Y). (e) The only single stranded DNA left is of type A on one sphere and of type B on the other so the dimer in (e) is functionally equivalent to the one in (f). Using several iterations of this process will produce eight-mers (g) with eight separate and specific binding sites to be used for PSA.

molecules (Fig. 1a–e). 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 are then separated from the rest of the aggregates by mass separation. 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 (Fig. 1f and g). 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. 1g).

Following the above method one can create 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).

Programmable self-assembly require simultaneous binding of two bonds to uniquely produce the desired structures. For example, in Fig. 2, the blocks 5 and 1 can connect to the 2 block. The right position is determined by matching two bonds. The A DNA's of both blocks 1 and 5 could, in principle, make a single bond to the cA DNA of block 2 but the alignment of the connected blocks would probably be wrong. This could be avoided by setting the temperature and/or the amount of stirring to values where blocks bonded by one bond are unstable structures whereas blocks bonded by two bonds are stable.

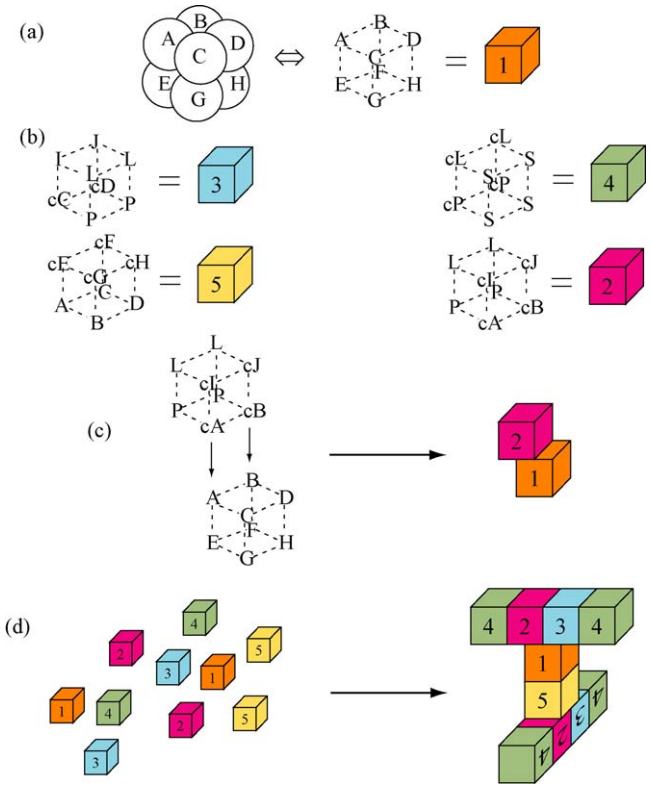

Fig. 2. Scheme of progammable self-assembly. (a) The eight-mers have eight specific ssDNA binding sites. (b) Four more eight-mers are depicted. (c) Example of binding: the cA and cB of block 2 binds to the A and B strands on block 1. (d) Structure formation. The "S" DNA of block 4 has no complement on the other blocks so it acts as a stop-block.

3. Materials and methods

Gold colloids with mean diameters ranging from 10 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 10 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 tools [16]. We have primarily used a subset of the DNA library for DNA-computing by Penchovsky and Ackermann [17]. 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 protocol [3]. Equal amounts of four 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 2 h, at an elevated sample temperature, typically 70–90 °C. 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 0.01 M 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 [18] from single particles and from larger aggregates, see Fig. 3a. 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 imagining were done using a LEO-1450 EP.

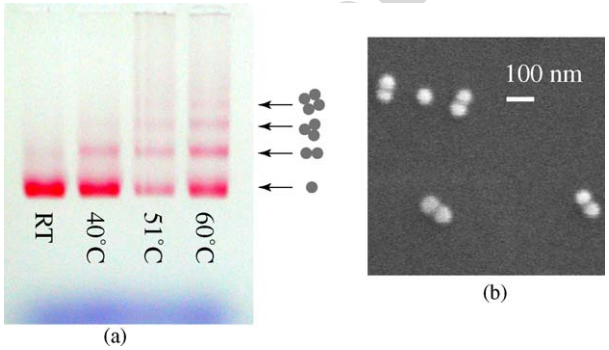

Fig. 3. (a) Photograph of the discrete lines formed by agarose gel electrophoresis of 50 nm DNA coated nanoparticles with linker molecule added. Bands of increasingly bigger aggregates are visible. The temperatures below the bands refer to the incubation temperature. (b) Electron micrograph of electrophoresis-purified nanoparticle dimers.

4. Results and discussion

The ssDNA successfully attached to the particles. This was clear from the stability of the gold colloids in high salt concentration. The formation of dimers, trimers and larger aggregates was verified by gel-electrophoresis and SEM imaging (Fig. 3a and b). The incubation temperature seemed to have an important effect. The only difference between the columns in Fig. 3a was the incubation temperature. At lower temperatures the incubation time of 1 h seemed to be inadequate to form aggregates. The samples heated to 51 and 60 °C showed 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 and 40 °C samples. Different incubation times, from 10 min to 1 h, showed no difference in the electrophoresis separation.

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. Fifty-one degree Celsius is close to the calculated DNA melting temperature for our oligos. One interesting observation was 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 not move at all in the gel. Thus, the response of coated gold particles in gel to the applied electric voltage (100 V) 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 was seemed to be working as single particles, dimers, and trimers were detected by SEM (Fig. 3b). There was still a relatively large portion of single particles in the extracted dimer and trimer samples. This might be due to breaking up of the dimers and trimers during the gel-extraction by centrifugation. There are also larger aggregates seen in the SEM micrographs 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. This would also make the dimers more resistant to elevated temperatures.

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.

5. Conclusions

A completely bottom-up 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.

References

[1] C.A. Mirkin, R.L. Letsinger, R.C. Mucic, J.J. Storhoff, Nature 382 (1996) 607.
[2] A.P. Alivisatos, K.P. Johnsson, X.G. Peng, T.E. Wilson, C.J. Loweth, M.P. Bruchez, P.G. Schultz, Nature 382 (1996) 609.
[3] J.J. Storhoff, R. Elghanian, R.C. Mucic, C.A. Mirkin, R.L. Letsinger, J. Am. Chem. Soc. 120 (1998) 1959.
[4] T.A. Taton, R.C. Mucic, C.A. Mirkin, R.L. Letsinger, J. Am. Chem. Soc. 122 (2000) 6305.
[5] E. Winfree, in: R.J. Lipton, E.B. Baum (Eds.), DNA Based Computers, vol. 27, Amer. Math. Soc., Princeton, 1996, p. 199.
[6] E. Winfree, J. Biomol. Struct. Dyn. 263 (2000).
[7] P.W.K. Rothemund, E. Winfree, Proc. of 32nd Ann. ACM Symp. Theory Comp., ACM, New York, NY/Portland, OR, USA, 2000, p. 459.
[8] Z.L. Zhang, S.C. Glotzer, Nano Lett. 4 (2004) 1407.
[9] B. Högberg, H. Olin, Programmable Self-Assembly—Unique Structures and Bond Uniqueness, J. Comput. Theor. Nanosci., in press.
[10] E. Klavins, IEEE Proc. Int. Conf. Robotics and Automation, vol. 5, 2004, p. 5293.
[11] P.W.K. Rothemund, N. Papadakis, E. Winfree, Plos Biol. 2 (2004) 2041.
[12] Z. Bao, L. Chen, M. Weldon, E. Chandross, O. Cherniavskaya, Y. Dai, J.B.-H. Tok, Chem. Mater. 14 (2002) 24.
[13] R.C. Jin, G.S. Wu, Z. Li, C.A. Mirkin, G.C. Schatz, J. Am. Chem. Soc. 125 (2003) 1643.
[14] L.M. Demers, M. Ostblom, H. Zhang, N.-H. Jang, B. Liedberg, C.A. Mirkin, J. Am. Chem. Soc. 124 (2002) 11248.
[15] Csogor, M. Nacken, M. Sameti, C.-M. Lehr, H. Schmidt, Mater. Sci. Eng. C 23 (2003) 93.
[16] Online Tools for Oligo-Design. rnature.com/oligonucleotide.html, rna.t-bi.univie.ac.at/cgi-bin/alifold.cgi, scitools.idtdna.com/Analyzer/.
[17] R. Penchovsky, J. Ackermann, J. Comput. Biol. 10 (2003) 215.
[18] D. Zanchet, C.M. Micheel, W.J. Parak, D. Gerion, S.C. Williams, A.P. Alivisatos, J. Phys. Chem. B 106 (2002) 11758.