

Controlled Assembly of Mesoscale Structures Using DNA as Molecular Bridges

Carissa M. Soto,* Amritha Srinivasan, and Banahalli R. Ratna

Naval Research Laboratory, Center for Bio/Molecular Science and Engineering,
4555 Overlook Avenue SW, Washington, D.C. 20375

Received November 30, 2001

This paper describes a method to prepare mesoscale polyhedral structures from binary mixtures of microspheres of specific size ratios using DNA as a molecular bridge. Such nonspherical building blocks can lead to the development of complex colloidal crystals of lower symmetry than is possible with uniform spherical particles.

Self-assembled colloidal crystals of microspheres have been used either by themselves or as a template to develop a number of applications in chemical sensing and photonics.^{1,2} However, their usefulness is limited by the simple opaline cubic structures that are only possible to achieve with monodisperse spherical particles.³ For example, it has been theoretically shown that an fcc lattice is not the most appropriate structure for achieving full optical band gap in a photonic crystal.^{4,5} Superlattice structures of binary mixtures of colloidal particles have been observed under certain conditions.⁶ Therefore, we may ask whether one can build complex superlattices by first building a mesoscale subcell⁷ followed by self-assembly to form crystals. Recently, Whitesides and co-workers demonstrated that self-assembly of nonspherical objects using capillary interactions can form large, regular arrays.⁸ We show in this paper another approach to build two nonspherical subcells. A biological molecule, DNA, has been used as a cohesive entity to hold the structure together.

Utilization of DNA as a molecular bridge allows control of particle periodicity, interparticle distance and size, and chemical identity of the particles in the macroscopic structure.^{9,10} We describe herein a general approach that uses DNA to link two different size polystyrene beads to construct tetrahedron and octahedron building blocks.

Geometrical calculation shows that a tetrahedron is formed by an A_4B mesoassembly involving one B and four A spheres (Figure 1a) when the diameter ratio of the two spherical particles d_B/d_A is about 0.23. Similarly, an octahedral A_6B mesoassembly requires a size ratio of 0.42 (Figure 1b). On the basis of this calculation and the commercial availability of the microspheres, we chose carboxy-modified polystyrene spheres (CM-PS) of 0.818 and 0.211 μm for the tetrahedron assembly and 0.818 and 0.364 μm CM-PS spheres for the octahedron assembly.

A 41 bp oligonucleotide (oligo A, Figure 2) was designed to contain three basic elements: (i) coupling spacer, (ii) hybridization unit, and (iii) detection probe. The coupling spacer is composed of a primary amine for coupling, a C7 acyl linker chain, and *Pst*I overhangs to create a spacer prior to the hybridization point. The hybridization unit, 36 bp long, codes for a portion of the amino terminal end of the T7 major capsid protein;¹¹ it was chosen on the basis of prior experience in cloning similar sequences.¹² The hybridization unit contains restriction sites to cleave the resultant double-stranded DNA (dsDNA) at specific sites by enzymatic

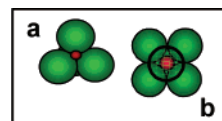


Figure 1. Regular polyhedra formed from microspheres. (a) Tetrahedron formed by A_4B assembly. (b) Octahedron formed by A_6B assembly.

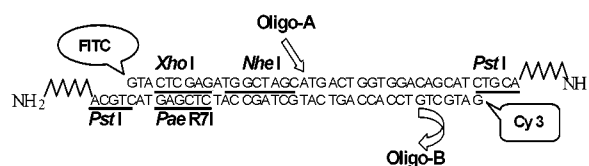


Figure 2. DNA molecular bridge. Oligo A written from left to right in the 5' to 3' direction.

digestion using *Xho*I, *Pae*R7I, or *Nhe*I. The third entity consists of fluorescent tags at corresponding 5' ends, fluorescein (FITC) in oligo A and indocarbocyanine-3 (Cy3) in oligo B (oligo A complementary strand) for identification of DNA-containing polystyrene beads.

Oligo A was coupled to a 0.818 μm CM-PS bead via carboxy-diimide chemistry (e.g., cross-linking reagent EDC, 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride).^{13,14} Similarly, oligo B was attached to 0.211 and 0.364 μm CM-PS (**PS/0.2-B**; **PS/0.3-B**) beads. Confocal fluorescence images of DNA-containing PS beads indicate that the beads are properly modified because fluorescence was detected at the corresponding wavelength for FITC and Cy3 for beads A and B, respectively.

A series of mixtures of **PS/0.8-A** with either **PS/0.2-B** or **PS/0.3-B** was prepared by titrating the smaller beads into a solution containing the larger ones. All of these mixtures were annealed by cooling them slowly from 90 $^{\circ}\text{C}$. The mixtures were observed under a confocal microscope, both in the fluorescence and in the reflection modes. We find that in all of the mixtures, the B bead was always associated with an A bead.

Fluorescence resonance energy transfer (FRET) provides valuable long-range distance information about macromolecules in solution.¹⁵ FITC and Cy3 form a donor–acceptor pair of fluorophores, previously used to determine distance information in DNA duplexes within a range of 10–80 \AA .¹⁶ In our experimental setup, FRET studies were carried out by irradiating the sample at 488 nm with the emission detection at 600–650 nm. Our results show fluorescence coming from annealed samples of **PS/0.8-A** and **PS/0.2-B** mixtures, indicating that donor acceptor fluorophores are in close proximity as a result of DNA hybridization because FRET was not detected on unannealed samples. Similar results were obtained for the mixtures of **PS/0.8-A** and **PS/0.3-B**. From these fluorescence studies, we conclude that CM-PS beads were properly decorated

* To whom correspondence should be addressed. E-mail: cmsoto@cbmse.nrl.navy.mil.

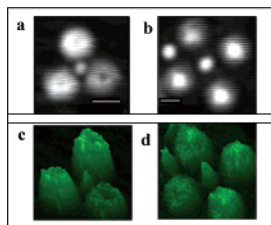


Figure 3. Confocal image of a z -section of partially formed (a) tetrahedron (scale bar, 500 nm) and (b) octahedron (scale bar, 250 nm). Parts (c) and (d) are the pseudo 3-D visualizations with the intensity plotted in the third dimension.

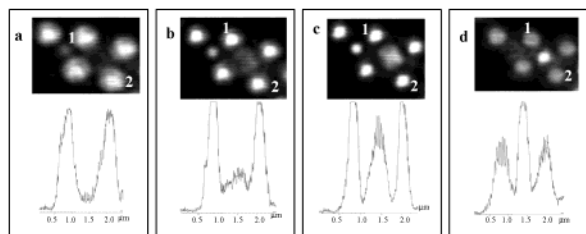


Figure 4. Representative z -scans (a) 0.2 μm , (b) 0.3 μm , (c) 0.4 μm , and (d) 0.7 μm of an octahedral assembly with the corresponding line profiles measured along a line across the center of particles 1 and 2. A total of 14 scans, each one 0.1 μm thick, was performed over a z -thickness of 1.3 μm .

with fluorescently labeled ssDNA and that on annealing mixtures dsDNA was bridging oligo A beads with oligo B beads.

Confocal laser scanning microscopy enables optical slicing and construction of three-dimensional images.¹⁷ We took advantage of this capability to probe the three-dimensional structure of the mesostructures. A series of z -scans at 0.1 μm step size was taken covering the whole thickness of the sample cell. Figure 3a shows a z -section of a partially formed tetrahedron with a 0.211 μm bead in the middle of a triangle formed by three 0.818 μm beads. A similar figure for the octahedron is shown in Figure 3b. A pseudo three-dimensional plot of these figures (Figure 3c and d) aids in visualizing the sizes of the particles and their relative positions along the z axis.

Four representative sections from a series of micrographs obtained by scanning in the z direction are depicted in Figure 4 for the octahedron. Under each micrograph, we have shown a line profile taken across two diagonally opposite particles. Note that as we move up in z (from panel A to D, Figure 4), the middle peak caused by the top particle of the octahedron starts to appear and grows brighter. Such line-profile analyses allow us to measure the size of different particles as well as the distances between the centers of adjacent particles and compare them with a physical model. The average distance between the two diagonally opposite particles, which is also the distance between the two outer peaks in profiles A–D (Figure 4), is measured to be 1.16 μm . This value is very close to what is expected (1.145 μm) with a square lattice arrangement of 0.818 μm beads in an octahedral assembly. Thus, the confocal data provide evidence to confirm the formation of the desired building blocks.

Scanning electron micrographs of the building blocks are shown in Figure 5. We observed isolated building blocks and some as part of larger aggregates. Flow cytometry is the method of choice

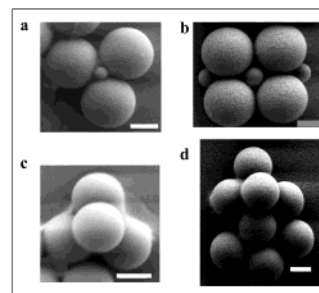


Figure 5. A sample set of building blocks seen by SEM (a, b) partially formed, (c) completely formed, and (d) larger aggregates. Scale bar, 400 nm.

to quantify the products in solution and will be used to isolate such aggregates from the mixture. In all of the samples studied, we observed two peaks, one corresponding to the unassociated beads (0.818 μm) and a broader one in the 2–3 μm region which we assigned to the mesoscale assemblies and their aggregates. A systematic study of **PS/0.8-A** and **PS/0.3-B** mixtures allowed us to estimate the yield to be 20%.

Building microstructures in three dimensions (3-D) presents significant challenges when working with binary systems. We demonstrate here that DNA can be used to bridge two sizes of polystyrene beads. By using specific size ratios, tetrahedron and octahedron structures were created.

Acknowledgment. We thank J. D. Andreadis, B. Martin, M. L. Breen, E. R. Welsh, D. Maric, and K. Shaffer for their contribution. This work has been supported by the Office of Naval Research and a postdoctoral fellowship from the National Research Council.

Supporting Information Available: Experimental procedures, fluorescence and FRET confocal images (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Joannopoulos, J.; Meade, R.; Winn, J. *Photonic Crystals Molding the Flow of Light*; Princeton University Press: Princeton, NJ, 1995.
- (2) Asher, S. A.; Holtz, J.; Weissman, J.; Pan, G. *MRS Bull.* **1998**, Oct, 44.
- (3) Zhang, Z.; Satpathy, S. *Phys. Rev. Lett.* **1990**, 65, 2650.
- (4) Biswas, R.; Sigalas, M. M.; Subramania, G.; Ho, K.-M. *Phys. Rev. B* **1998**, 57, 3701–3705.
- (5) Bush, K.; John, S. *Phys. Rev. E* **1998**, 58, 3896.
- (6) Bartlett, P.; Ottewill, R. H.; Pusey, P. N. *Phys. Rev. Lett.* **1992**, 68, 3801–3804.
- (7) Yin, Y.; Lu, Y.; Xia, Y. *J. Am. Chem. Soc.* **2001**, 123, 771–772.
- (8) Clark, T. D.; Tien, J.; Duffy, D. C.; Paul, K. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **2001**, 123, 7677–7682.
- (9) Alivisatos, A. P.; Johnson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P.; Schultz, P. G. *Nature* **1996**, 382, 609–611.
- (10) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, 382, 607–608.
- (11) Studier, F. W.; Rosenberg, A. H.; Dubendorff, J. W. *Methods Enzymol.* **1990**, 185, 60–89.
- (12) Welsh, E. R.; Tirrell, D. A. *Biomacromolecules* **2000**, 1, 23–30.
- (13) Taniuchi, M.; Clark, H. B.; Johnson, E. M., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, 83, 4094–4098.
- (14) Grabarek, Z.; Gergely, J. *Anal. Biochem.* **1990**, 185, 131–135.
- (15) Eisenberg, D.; Crothers, D. *Physical Chemistry with Applications to the Life Sciences*; The Benjamin/Cummings Publishing Co., Inc.: Menlo Park, CA, 1979.
- (16) Norman, D. G.; Grainger, R. J.; Uhrin, D.; Lilley, D. M. J. *Biochemistry* **2000**, 39, 6317–6324.
- (17) Paddock, S. W. *Mol. Biotechnol.* **2000**, 16, 127–149.

JA017653F