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Synthetic Strategies Toward DNA-Coated Colloids that Crystallize

Yufeng Wang,[†,‡] Yu Wang,[‡] Xiaolong Zheng,[‡] Étienne Ducrot,[†] Myung-Goo Lee,[§] Gi-Ra Yi,[§] Marcus Weck,*[,‡] and David J. Pine*[,†,∥]

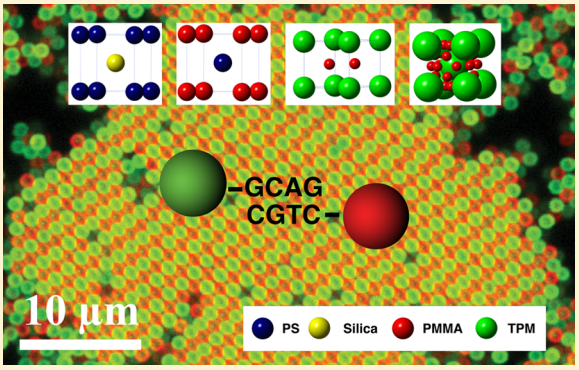
[†]Center for Soft Matter Research and Department of Physics and [‡]Molecular Design Institute and Department of Chemistry, New York University, New York, New York 10003, United States

[§]School of Chemical Engineering, Sungkyunkwan University, Suwon 440746, Republic of Korea

[∥]Department of Chemical & Biomolecular Engineering, Polytechnic School of Engineering, New York University, Brooklyn, New York 11201, United States

Supporting Information

ABSTRACT: We report on synthetic strategies to fabricate DNA-coated micrometer-sized colloids that, upon thermal annealing, self-assemble into various crystal structures. Colloids of a wide range of chemical compositions, including poly(styrene), poly(methyl methacrylate), titania, silica, and a silica-methacrylate hybrid material, are fabricated with smooth particle surfaces and a dense layer of surface functional anchors. Single-stranded oligonucleotides with a short sticky end are covalently grafted onto particle surfaces employing a strain-promoted alkyne−azide cycloaddition reaction resulting in DNA coatings with areal densities an order of magnitude higher than previously reported. Our approach allows the DNA-coated colloids not only to aggregate upon cooling but also to anneal and rearrange while still bound together, leading to the formation of colloidal crystal compounds when particles of different sizes or different materials are combined.



INTRODUCTION

DNA-coated colloids, owing to the specificity and programmability of DNA hybridization, have the potential to assemble into complex architectures for applications in plasmonics, magnetics, and photonics.[1−4] This idea has been demonstrated for DNA-coated nanoparticles, which form crystalline superlattices with tunable structures, compositions, and morphologies.[5−8] Design principles for engineering such crystals have also been established.[9] However, creating ordered crystalline structures based on larger colloids—micrometer-sized particles—coated with DNA has proven significantly more challenging.[10,11]

Unlike nanoparticles, the thickness of DNA coatings on microparticles is much smaller than the particle size. This can be a significant advantage, depending on the application. Thin coatings occupy only a few percent of available volume so that particle assemblies comprise mostly of the colloidal materials. Thick coatings, where the length of the DNA strands is comparable to the particle size, occupy most of the particle volume, in excess of 95%, for example, when the thickness of the DNA coating is equal to the particle diameter. Thin coatings are more sensitive to particle roughness and other inhomogeneities. As a consequence, when particles with thin DNA coatings bind, they often undergo a "hit and stick" or "random parking" process,[12] resulting in kinetically trapped amorphous aggregates, which lack the ability to rearrange further and anneal to reach the equilibrium structures that minimize the free energy.[13] This problem is particularly apparent when DNA is bound to particles using a streptavidin−biotin link.[14]

In one approach to this problem, DNA is conjugated to a block copolymer, which is then coated onto poly(styrene) (PS) particles through a swelling−deswelling method.[15] The use of the polymer spacer increases the overall length and flexibility of the DNA linker allowing the formation of colloidal crystals. Another approach relies on absorbing DNA onto the surface of lipid bilayer-coated solid particles, leading to DNA linkers that are fully mobile.[16] Using these methods, however, only small or two-dimensional crystalline structures have been produced.[17] Moreover, both approaches depend on the noncovalent attachment of DNA to particle surfaces, which limits the scope of particle materials that can be used.

Recently, we reported an approach for creating DNA-coated micrometer-sized colloids that readily crystallize upon annealing, forming bulk crystals from a wide range of particle diameters.[13] Our hypothesis was that in order to anneal after binding, colloids functionalized with short DNA must have smooth surfaces and a high density of DNA, both of which promote DNA-bound colloids that can diffuse on each other

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and thus anneal and crystallize. In that work, we focused our discussion on the *in situ* observation of the crystallization process of DNA-coated particles made from 3-(trimethoxysilyl)propyl methacrylate (TPM), and in particular on nucleation, restructuring, growth, and defect formation.

In this contribution, we present a comprehensive synthetic scheme for making DNA-coated micrometer-sized particles that crystallize. We show that colloids of diverse chemical compositions, including organic materials such as PS and poly(methyl methacrylate) (PMMA), inorganic materials such as titania and silica, and hybrid materials, can all be fabricated and functionalized with DNA. Colloids of each material are made with smooth surfaces and prepared with a dense layer of surface functional anchors—azides—for further functionalization, most notably with DNA. A strain-promoted alkyne−azide cycloaddition reaction (SPAAC)[18] is employed to functionalize the colloid surfaces with single-stranded DNA (ssDNA) with high coverage. This greatly expands the types of particles that can be functionalized with DNA.

Colloids of the same or different materials and sizes can be mixed and programmed to specifically bind by choosing complementary DNA binding sequences, allowing for the self-assembly of three-dimensional bulk colloidal crystals of a variety of structures, including ones with sublattices occupied by different chemical compositions. Our strategy should, in principle, provide access to many other complex colloidal structures through programmable DNA assembly.[19,20]

MATRIALS AND METHODS

General Methods. All reagents are purchased either from Sigma-Aldrich, Acros Organics, or Click Chemistry Tool and used without further purification unless otherwise noted. Styrene, 4-vinylbenzyl chloride, methyl methacrylate (MMA), and 3-chloro-2-hydroxypropyl methacrylate are allowed to pass through a column filled with inhibitor remover (Sigma-Aldrich) right before use. Colloidal particles in the dried state are imaged using a Merlin (Carl Zeiss) field-emission SEM. Flow cytometry experiments are carried out using a BD LSRII HTS cytometer. Bright-field optical images are obtained using a Nikon TE300 microscope equipped with a CCD camera. Fluorescent images are taken using a Leica SP5 or SP8 confocal fluorescence microscope. Some of the images are digitally postprocessed to improve brightness and contrast.

Microspheres Fabrication. *PS Particles.* Sulfate PS seed particles (350 nm in diameter) are synthesized using the standard surfactant-free emulsion polymerization method.[21] A two-stage swelling and copolymerization method is employed to make the chlorinated PS particles. Typically, 50 mg of the seed particles are dispersed in 25 mL of aqueous sodium dodecyl sulfate solution (SDS, 0.3% w/w), and the suspension is charged in a 50 mL two-neck flask along with a magnetic stir bar. In a separate vial, 50 μL of 1-chlorodecane and 2 mL of aqueous SDS are mixed and emulsified by ultrasonication. The emulsion is then added to the particle suspension. The resulting mixture is allowed to stir at 30 °C for 12 h. Then, 25 mg of azobis(isobutyronitrile) (AIBN), dissolved in 1.8 mL of styrene and 200 μL of 4-vinylbenzyl chloride, is added. The flask is then equipped with a condenser, at the top of which a bubbler is set. The mixture is then purged with nitrogen for 30 min and stirred for another 10 h. The temperature is elevated to 78 °C to initiate the polymerization. After 6 h, the reaction is quenched upon cooling. The particles (~500 nm) are washed (mainly to remove tiny particles formed by secondary nucleation) and stored in aqueous solution containing 0.25% w/w of Pluronic F127. By consecutive growth, larger particles are obtained.

PMMA Particles. A two-stage swelling and copolymerization method is also employed to make chlorinated PMMA particles. We describe the detailed procedure in the Supporting Information (SI).

Silica Particles. Silica particles are synthesized using the Stöber method.[22] The resulting particles are washed and redispersed in anhydrous DMF for functionalization. Typically, 1 mL of the particle suspension (~1% w/v) is charged in a glass vial along with a magnetic stir bar. Twenty μL of (3-bromopropyl)trichlorosilane is introduced at room temperature while the suspension is being stirred vigorously. This step is done in glovebox to avoid moisture. After about 2 min, the vial is capped, taken out of the glovebox, and allowed to stir for another 6 h during which the vial was ultrasonicated for three times (20 s each) to prevent particle aggregation. The reaction is quenched by repeated centrifugation/redispersion washes with solvents in the following order: DMF for twice, DMF/water (9:1) for three times, and DMF for three times. For silica particles with lower DNA grafting densities, we used (3-chloropropyl)trimethoxysilane to functionalize silica in ethanol.

Titania Particles. Titania particles of various sizes are synthesized, as described,[23] and transferred to anhydrous acetonitrile for surface functionalization. Typically, to a glass vial with a magnetic stir bar is charged 5 mL of particle suspension (~1% w/v), followed by the addition of 500 μL of (3-iodopropyl)trimethoxysilane. The mixture is heated at 65 °C for 8 h before it is quenched by cooling to room temperature.

TPM Particles. Chlorinated TPM particles are fabricated by copolymerizing 3-(trimethoxysilyl)propyl methacrylate (TPM) with 3-chloro-2-hydroxypropyl methacrylate (CHPMA). Typically, 200 μL of TPM is added to 20 mL of deionized water, followed by 20 μL of ammonium hydroxide (28%). The reaction is stirred for 4 h at room temperature, producing monodisperse TPM emulsions. Then, 40 μL of CHPMA is added and allowed to diffuse into the emulsion droplets. After 30 min, 5 mL of an aqueous SDS (5% w/w) is introduced. Ten minutes later, 10 mg of AIBN is added, and the reaction mixture is allowed to stir for another 20 min before the temperature was rapidly raised to 80 °C. The reaction is quenched upon cooling to room temperature after 4 h, resulting chlorinated TPM particles about 1 μm in diameter, which is purified by repeated centrifugation/redispersion.

Azide Functionalization. Particles with halogen groups on the surface are treated with sodium azide (NaN_3) to obtain azide functional groups. Typically, to a 20 mL of particle suspension (~1% w/w) in aqueous F127 solution (0.25% w/w) is added 100 mg of NaN_3 and trace amount of potassium iodide. The suspension is heated at 70 °C overnight. After washing by centrifugation/redispersion, the azide particles are stored in 0.1% w/w Triton X-100 solution at 4 °C.

DBCO-DNA. Single-stranded oligonucleotides with a sticky end are used in this study. 5′-NH_2-DNAs are purchased (Integrated DNA Technologies, USA), and the amine group is converted to a dibenzyl cyclooctyne (DBCO) group by treating the DNA with DBCO-sulfo-NHS (Click Chemistry Tool) in phosphate buffered saline (PBS, 10 mM, pH 7.4, 100 mM sodium chloride, same below). The DNA is also internally fluorescent labeled with Cy3 or Cy5, respectively. Both palindrome (P) and complementary (A/B) DNA are used, with the length of sticky end containing four bases. Typically, 33.3 μL of amine ssDNA (300 μM) is mixed with 50 μL of 1 mM DBCO-sulfo-NHS in PBS (freshly prepared) and vigorously stirred overnight. The DNA is purified by passing through a MicroSpin G-25 columns (GE), diluted to 100 μM, and stored in PBS at −20 °C.

DNA Grafting. Azide-functionalized particles are first dispersed in 400 μL of PBS containing Triton X-100 (0.1% w/w) with a particle concentration of about 0.1% w/w. Then, 20 μL of DBCO-DNA (100 μM) is added, and the reaction mixture is stirred at 55 °C for 24 h. The particles are washed and stored in PBS containing 1% w/w Pluronic F127 for the self-assembly experiments. Note that adding sodium chloride is necessary for DNA grafting. Control experiments in a medium without sodium chloride (0.1% Triton X-100 w/w solution) resulted in particles with very low grafting density. Those particles do not aggregate at room temperature.

Flow Cytometry. We use flow cytometry to quantify the number of DNA strands functionalized per particle. Cy5-labeled microspheres are used as cytometry standard (Quantum Cy5MESF, Bangs Laboratories Inc.). Using the provided molecules of equivalent soluble fluorochromes (MESF), a calibration curve is constructed, based on

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which the measured fluorescent intensity data for each of DNA-coated particle sample are converted to an approximate number of DNA grafted on each particle. For analysis, particle samples are dispersed in PBS containing Pluronic F127 (1% w/w).

Crystallization. The particles of interest are combined, mixed according to the stoichiometry of the target crystalline structure, and transferred to a hydrophobic glass capillary tube (2 mm × 100 μm × 10 cm, pretreated with oxygen plasma and exposed to hexamethyldisilazane vapor). This pretreatment causes some of the F127 dissolved in the particle suspension to adhere to the glass tube thus forming a dense steric layer that prevents particles from sticking to the glass. The capillary tube is sealed and attached to a microscope glass slide using wax. The slide is then mounted on a homemade microscope thermal stage with a temperature gradient. For crystallization, the sample is first heated above the melting temperature (T_m) to melt any aggregates, and then the temperature range of the stage is set such that the lower limit is about 5° below T_m, while the upper limit is about 5° above T_m. Crystallization occurs in minutes to hours, but in this study, the samples are typically allowed to anneal overnight before microscopic imaging.

RESULTS AND DISCUSSION

Figure 1 illustrates the general scheme for assembling colloidal crystals through DNA hybridization. First, particles of the

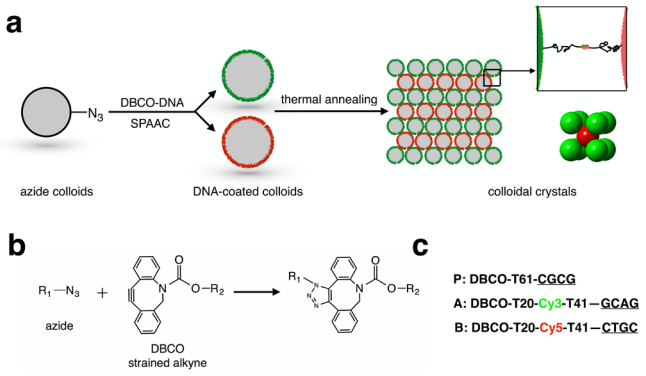

Figure 1. (a) General scheme of building colloidal crystals mediated by DNA hybridization. (b) Strain-promoted alkyne–azide cycloaddition reaction (SPAAC). (c) DNA structures and sequences for the sticky ends (underscored).

desired sizes are synthesized with halide groups on the surface, either chlorine, bromine, or iodine, that can be converted to azide groups for DNA grafting (Figure 1a). To achieve a smooth particle surface and at the same time introduce a sufficient number of surface functional handles, we employ different design and fabrication conditions tailored to each material from which the colloid is made. In all cases, we use SPAAC[18] to coat DNA on the colloid surface.

SPAAC couples azides to a strained alkyne such as DBCO forming a covalent triazole linkage (Figure 1b).[24] It has been recently utilized to coat nanoparticles with oligonucleotides.[6] Besides high efficiency and the stable nature of the covalent linkage, another advantage of SPAAC is its compatibility with high ionic strength and nonionic surfactants. When grafting negatively charged DNA strands to colloidal surfaces, a certain amount of salt needs to be introduced to screen the DNA charges,[25] thus reducing electrostatic repulsion between DNA strands and facilitating a high packing density of DNA on the surface. Typically, we use 100 mM sodium chloride. To prevent aggregation of the colloids, we add a nonionic surfactant, mostly Triton X-100 with a trace amount of Pluronic F127, when conducting the functionalization using SPAAC. Under these conditions, we can graft up to 10^5 DNA strands on each 1.0 μm particle, as measured by flow cytometry. Far fewer strands are detected when no salt is added (see Materials and Methods).

We use ssDNA that consists of three parts. At the 5′ end, we attach a DBCO group as a reactive handle for the SPAAC reaction with the azide-functionalized particles. A 61-base long "polyT" part, internally labeled with a fluorescent dye (Cy3 or Cy5), serves as a flexible spacer. The 3′ terminus contains the short sticky end (4 bases) to provide specific binding to complementary strands via DNA hybridization. The sequences for the self-complementary palindrome (P) as well as heterocomplementary strands (A and B) are listed in Figure 1c.

When combined in aqueous suspension, colloidal particles coated with complementary DNA sticky ends associate and dissociate reversibly at the melting temperature T_m. Thermally annealing the system at or slightly below T_m for an extended period of time (minutes to hours) allows crystals to form with their structures determined by particle sizes, stoichiometry, and sticky-end sequences, etc. The crystals are typically three-dimensional, with their thickness determined by the initial particle concentration, the particle size, and the particle density. (Figure 1a and Figures 2–4 and 6 for crystal structures).

The use of SPAAC is crucial to our research design; it is highly effective for attaching DNA to the particles without damaging the DNA[26] or reducing colloidal stability. We also emphasize the importance of controlling the particle synthesis.

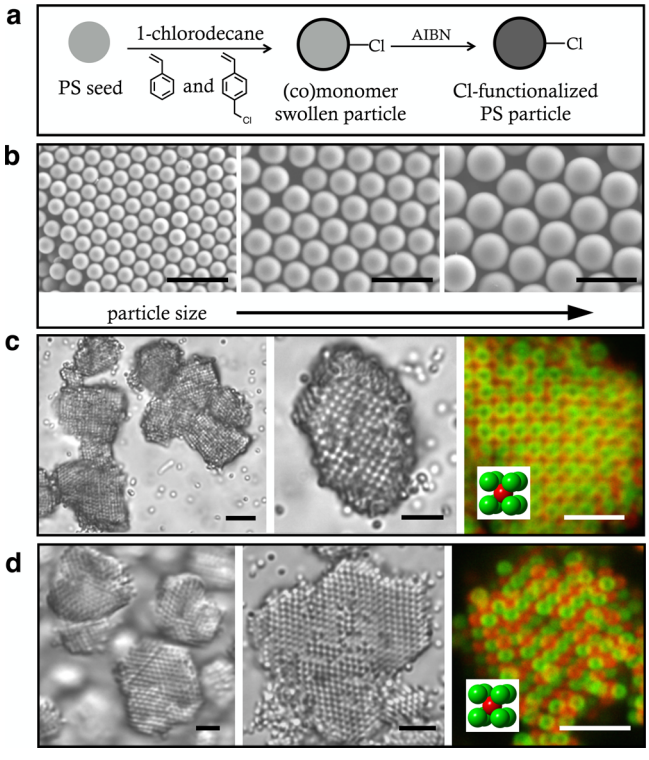

Figure 2. (a) Synthesis of Cl-functionalized PS particles. (b) SEM images of PS particles of different sizes with smooth surfaces. Scale bars, 2 μm. (c) 950 nm DNA-coated PS particles form bulk crystals of AB CsCl-like structure. The 100-plane is shown by a confocal fluorescent image. Scale bars, 5 μm. (d) 500 nm DNA-coated PMMA particles crystallize into FCC (left) or AB CsCl-type crystals (middle). A confocal fluorescent image shows multiple layers of the AB crystal. Scale bars, 2 μm.

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The challenge lies in introducing a high density of halide groups on the particle surface and, at the same time, maintaining the surface smoothness. We have not had much success using commercially available colloidal particles. Instead, we developed various synthetic strategies for colloidal particles of different composition.

PS Particles. A two-stage swelling and copolymerization method is used to fabricate chlorine-functionalized PS particles (Figure 2a). First, sulfate PS seed particles, synthesized by standard surfactant-free emulsion polymerization[21] using styrene as monomer and potassium persulfate (KPS) as initiator, are dispersed in an aqueous solution containing sodium dodecyl sulfate (SDS). 1-Chlorodecane is then introduced as an oil-in-water emulsion preswelling the seeds, followed by the addition of styrene along with an oil soluble initiator azobis(isobutyronitrile) (AIBN). A comonomer, 4-vinylbenzyl chloride, is introduced at the same time to further swell the particles. After swelling, the temperature is raised to initiate the polymerization by thermally degrading AIBN, producing chlorinated PS particles. Particles of larger sizes can be synthesized by consecutive growth in the same manner using particles previously made as seeds. All particles are spherical and have smooth surfaces, as shown from the scanning electron microscope (SEM) images in Figure 2b. Particles sizes range from 500 nm to 1.3 μm. 4-Vinylbenzyl chloride is chosen as the chlorine source because its molecular structure is similar to styrene. The ratio of 4-vinylbenzyl chloride to styrene is fixed at 10%, which ensures incorporation of enough chlorine groups on the particle surfaces while limiting the possibility of phase separation between the two monomers when they are polymerized, which can result in nonspherical particles (Figure S1).

The chlorine groups on the particle surfaces are converted to azides by treating the particles with sodium azide. Finally, ssDNA strands are attached using SPAAC, and approximately 80,000 DNA strands are grafted onto each 1 μm PS particle. The areal density of DNA is 1/39 strand/nm^2.

Thermal annealing of the DNA-coated PS particles at 0.5−2 °C below the DNA-hybridization melting temperature (T_m = 42 °C for P; T_m = 30 °C for A/B) results in bulk colloidal crystals. Figure 2c shows optical images of crystals formed by 950 nm particles functionalized with A- and B-DNA. Confocal fluorescence images suggest a CsCl-type structure. Much larger face-centered cubic (FCC) crystals are shown in Figure S2. In all cases, the crystals appear dark indicating thick crystals with multiple layers that extend into three dimensions. This can be attributed to PS particles having similar density (1.05 g/cm^3) to that of water.

PMMA Particles. Following a similar method, i.e., two-stage swelling and copolymerization, we fabricate chlorinated PMMA particles using SDS, MMA, and AIBN as the surfactant, monomer, and initiator, respectively. Sulfate PMMA seed particles are synthesized via surfactant-free emulsion polymerization.[21] 3-Chloro-2-hydroxypropyl methacrylate (CHPMA) is chosen as the comonomer and chlorine source. The resulting particles have smooth surfaces, as shown in Figure S3. The amphiphilic nature of CHPMA facilitates the enrichment of chlorine groups on the particle surface. After functionalization, a DNA areal density of 1/30 strand/nm^2 is achieved, which corresponds to 105,000 strands for each 1.0 μm particle. The FCC crystals and CsCl-type crystals created using 500 nm P-PMMA particles or 750 nm A- and B-PMMA particles are displayed in Figure 2d and Figure S4; multiple layers of the crystal can also be distinguished in the fluorescent image.

Silica Particles. We also fabricate DNA-coated silica colloids that can crystallize. Colloidal silica has Si−OH groups on its surface, which serve as anchor points for further modifications. We synthesize silica particles of various sizes using the Stöber sol−gel method.[22] The resulting particles, which have smooth surfaces, as shown in Figure 3a, are

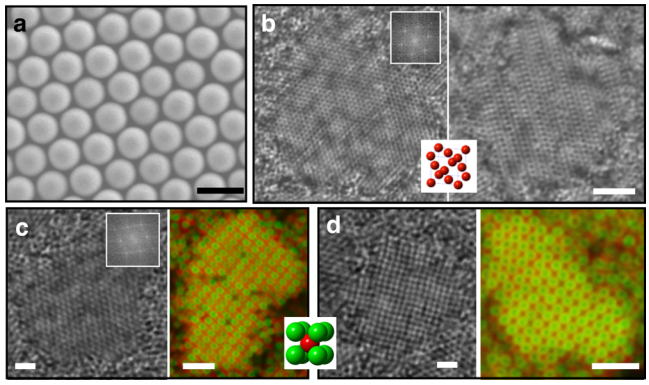

Figure 3. (a) SEM image of silica particle indicates smooth particle surface. Scale bar, 1 μm. (b) Optical images showing the 111- and 311-plane of FCC crystal structures formed by silica particles coated with P-DNA. Scale bars, 5 μm. AB CsCl-type colloidal crystals formed by silica particles coated with complementary A- and B-DNA; the 110-plane (c) and 100-plane (d) are displayed in both bright-field and confocal fluorescent images. Scale bars, 3 μm. Cartoons and selected FFT images are used to help elucidate the crystal structures.

dispersed in DMF and subsequently treated with (3-bromopropyl)trichlorosilane (BPCS). BPCS is highly reactive toward Si−OH groups; it coats the silica surfaces with bromine groups efficiently. The conversion of bromine to azide and the attachment of DNA with a sticky end using click chemistry are conducted as described above. Using this method, 65,000 DNA strands are grafted onto each 1 μm silica particle, which corresponds to an areal density of DNA strands of 1/48 strand/nm^2. Particles functionalized with ssDNA bearing complementary sticky ends are mixed in aqueous suspension, forming a variety of colloidal superlattices including FCC (Figure 3b) and AB CsCl-type crystals (Figure 3c,d). Because silica particles are heavier (2.3 g/cm^3) than particles made from organic materials (e.g., PS, 1.05 g/cm^3), the crystals obtained have flat surfaces with fewer defects. The 111-, 311-plane of FCC crystal, and the 110-, 100-plane of CsCl crystals are shown in Figure 3.

Titania Particles. Colloidal particles made from titania are investigated. Similar to silica particles, they have surface hydroxyl groups, Ti−OH, which can be modified for DNA tethering. Particles with smooth surfaces (Figure S5a) are synthesized following a sol−gel method reported in literature.[23] (3-Iodopropyl)trimethoxysilane is used to functionalize the titania surface in anhydrous acetonitrile at 65 °C, resulting in iodine groups coated on the surface. After converting the iodine to azide and attaching DNA, about 55,000 DNA strands are attached on each 1.0 μm particle; the areal density is 1/57 strand/nm^2. Crystals made from DNA-coated titania particles are shown in Figure S5.

TPM Particles. Using a method that combines a sol−gel process and emulsion polymerization, we fabricate chlorine-containing colloids based on TPM,[27] an organo-silica hybrid material (Figure 4a). In basic aqueous conditions, the TPM

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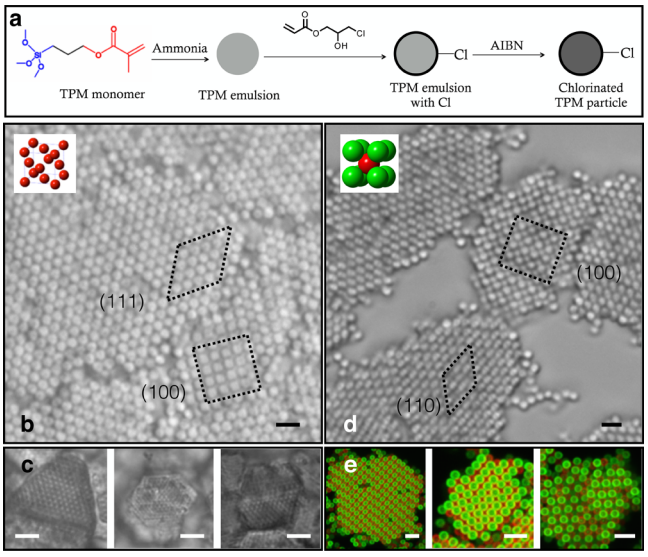

Figure 4. (a) Scheme showing the synthesis of Cl-functionalized TPM particles. (b) FCC crystals of TPM particles showing the 111-plane and 100-plane. (c) FCC crystals (500 nm particles) show facets like molecular crystals. (d) AB CsCl-type crystals are shown by bright-field and (e) confocal fluorescent images. Scale bars, 3 μm.

monomer is allowed to hydrolyze and condense, producing monodisperse micrometer-sized emulsion droplets (sol–gel). CHPMA is then introduced (20% to TPM monomer), which gradually diffuses into the emulsion droplets, as described previously.[13] Addition of SDS surfactant is followed stabilizing the emulsion against coalescence. Finally, AIBN is introduced, initializing the polymerization that solidifies the emulsion droplets resulting in chlorinated TPM particles (emulsion polymerization process). Particles of different sizes are synthesized by adjusting the amount of TPM and the reaction pH.

A DNA areal density of 1/27 strand/nm^2, or 115,000 strands on each 1.0 μm particle, is achieved for TPM particles. They can readily form bulk crystals upon annealing. Figure 4b–e shows the FCC and CsCl crystals out of DNA-coated TPM particles (500 nm or 1.0 μm); the 100-, 111-plane of the FCC crystals and the 100-, 110-plane of CsCl crystals are highlighted. Similar to molecular crystals, 500 nm particles functionalized with P-DNA form crystals with facets (Figure 4c). Various crystal planes of AB crystals are also shown in fluorescent images (Figure 4e).

A previous study showed that the areal density of ssDNA on the particle surfaces is implicated in whether or not ssDNA-coated colloids crystallize.[13] Here, we find that when we use commercial PS particles that contain fewer chlorine groups, and therefore fewer DNA strands after functionalization, we see no crystallization for A- and B-DNA coated particles.

High DNA coverage is particularly important for the assembly and crystallization of silica particles. Silica is known to bind strongly and often irreversibly through multiple forces such as electrostatic interactions to the phosphor-containing backbone of nucleotides.[28] If there is insufficient DNA coverage, DNA chains can lie on the particle surface, which, in turn, allows the surfaces of the colloids to come much closer to each other when the particles bind. In this limit, the van der Waals attraction between colloids becomes very strong, causing irreversible aggregation that does not respond to temperature cycling. Increasing the areal density of DNA so that it forms a dense polymer brush can prevent the close approach of colloid surfaces, and thus prevent irreversible aggregation. Indeed, particles having less DNA can aggregate but not dissociate upon heating. They also do not crystallize (Figure S6).

The roughness of the particle surface is also important for the crystallization of micrometer-sized particles.[13] Smooth particle surfaces make it easier to create a relatively homogeneous interaction potential, facilitating the crystallization process, while particles with relatively rough surfaces tend to have more inhomogeneous interaction potentials, which slows down or even prevents particles from annealing. Figure 5a–c shows

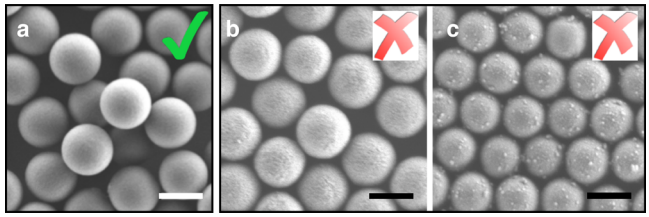

Figure 5. SEM images showing silica particles with (a) smooth, (b) slightly rough, and (c) very rough surfaces. Scale bars, 1 μm.

silica particles with various degrees of surface roughness. Surface roughness is introduced by adding poly(vinylpyrrolidone) (PVP) as a surfactant during particles fabrication. PVP promotes the nucleation of silica on the particles and leads to rough surfaces. The degree of roughness is determined by the molecular weight of PVP (SI).[29] We find that particles with smooth surfaces (Figure 5a) can crystallize. By contrast, particles with rough surfaces, when functionalized to a similar areal density of DNA, aggregate and dissociate but do not form any ordered structures. This is in contrast to DNA-coated nanoparticles where the DNA is sufficiently long, typically comparable or longer than the particle size, so that surface roughness does not play a role in the crystallization process.

An underlying challenge in materials science is to precisely control the spatial arrangement of materials at the nanometer and micrometer scales. We address this issue by employing DNA-coated colloids whose size, chemical composition, and interaction can be programmed at will. Aside from homogeneous crystal structures fabricated using particles of a single component, we can build heterogeneous structures or colloidal crystal compounds. By mixing and annealing 1.0 μm PS and 1.0 μm silica microspheres coated with complementary DNA, we obtain AB CsCl-type crystals (Figure 6a). The crystal is truly binary, comprising two different materials, organic and inorganic, arranged in an ordered array. Because the refractive index is very different for PS and silica, bright-field optical images of the crystals show sharp contrast between the two components. In Figure 6a, we show the 110- (left) and 100-plane (right) of the crystals. Since silica is heavier than PS, the 100-planes that are closest to the glass slide are silica particles. Similarly, we fabricate PS-PMMA crystals, PS-TPM crystals, and TPM-titania crystals, as shown in Figure 6b and Figure S7.

We also demonstrate that the particle compositions, sizes, and stoichiometry can be altered at the same time resulting in binary AB_2 and AB_6 crystals isostructural to aluminum boride (AlB_2) and cesium fullerene-complex (Cs_6C_{60}). In Figure 6c,d we show that such heterogeneous structures are possible by combining, at different ratios, 500 nm PMMA particle with 1.0 μm TPM or 1.5 μm TPM particles coated with complementary

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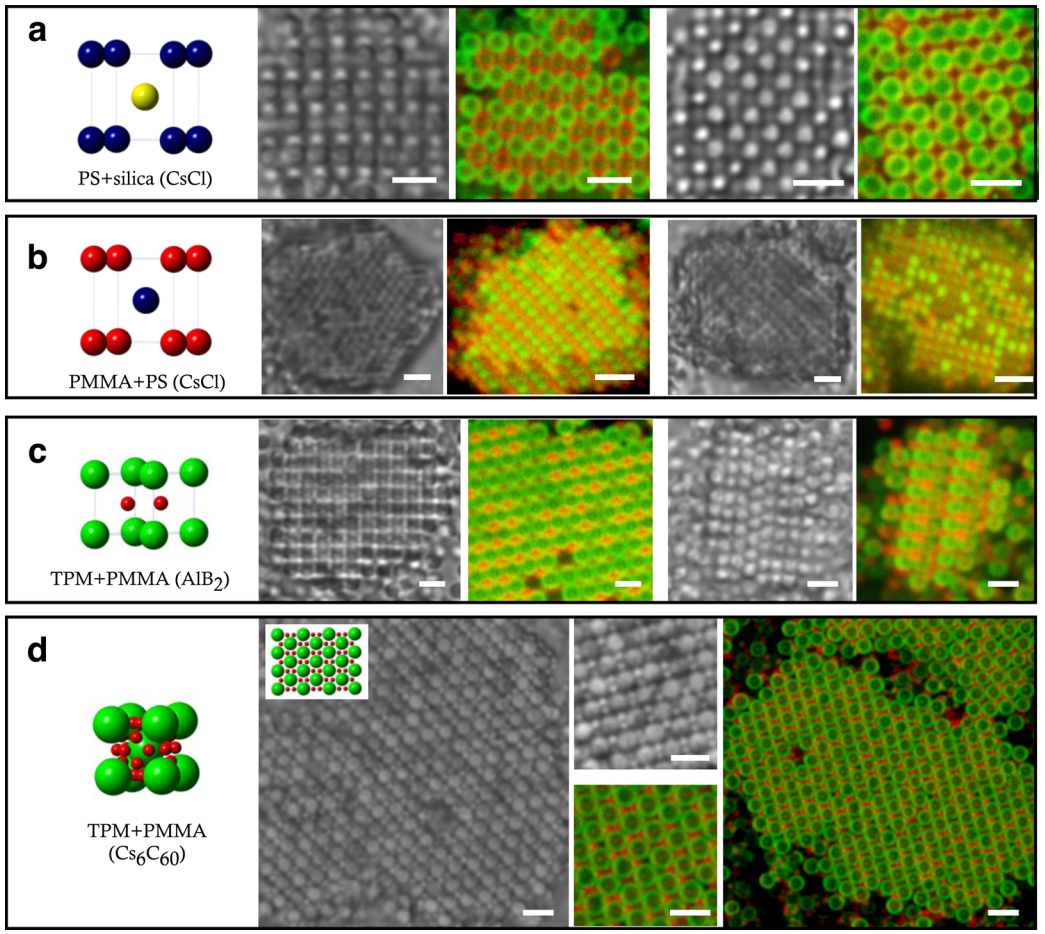

Figure 6. AB CsCl-type binary colloidal crystals formed using PS and silica particles (a) or PMMA and PS particles (b). Scale bars, 2 μm. (c) AlB_2 colloidal crystal can be fabricated using 1.0 μm TPM and 500 nm PMMA particles. (d) Cs_6C_{60} crystals are formed when 1.5 μm TPM and 500 nm PMMA particles with complementary DNA sticky ends are employed. Scale bars, 3 μm.

DNA strands. The characteristic crystal planes are shown by both optical and fluorescent images. While hybrid colloidal crystal compounds have been made previously with oppositely charged spheres,[30] the methods used here should prove more versatile, since both like-charged and oppositely charged spheres of virtually any composition can in principle be combined.

CONCLUSION

We have developed synthetic schemes to fabricate DNA-coated micrometer-sized colloids and demonstrated that colloidal crystals can be produced possessing programmed compositions and structures. More complex structures, such as tertiary structures that have been achieved by DNA-coated nanoparticles,[9,31] should be possible for micrometer-sized colloids by tuning the particles sizes and interactions.

The ability to mix and crystallize micrometer-sized colloids made from different materials should readily facilitate the fabrication of photonic crystals. For instance, the CsCl crystal made by silica and PS particles can be considered as a simple cubic lattice of matter with low and high refractive index.

The particle synthesis and coating approach we describe can, in principle, be applied to integrate other functionalities on micrometer-sized particle surface. Examples include small molecules, polymers, and biomacromolecules such as proteins.

ASSOCIATED CONTENT

Supporting Information

Additional procedures for particle synthesis; supplementary figures. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b06607.

AUTHOR INFORMATION

Corresponding Authors

*marcus.weck@nyu.edu
*pine@nyu.edu

Notes

The authors declare no competing financial interest.

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