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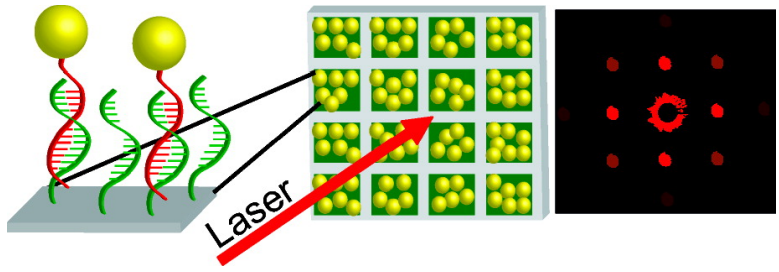

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DNA and DNAzyme-Mediated 2D Colloidal Assembly

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Abstract: DNA-mediated interactions present a significant opportunity for controlling colloidal self-assembly. Using microcontact printing to achieve spatial control of DNA-surface patterning and DNA-functionalized polystyrene colloids, we report that DNA hybridization can be utilized for sequence-specific reversible self-assembly of well-ordered 2D colloidal arrays. Two essential indicators of DNA-hybridization mediated assembly were confirmed: thermal reversibility and sequence specificity. The arrays melted at 50 °C and reassembled when introduced to fresh colloid suspension, and sequence specificity with <1% nonspecific binding was confirmed using fluorescent polystyrene colloids. The real-time assembly of the colloids onto the periodically patterned substrate was monitored by simple laser diffraction to obtain assembly kinetics. Maximum surface coverage of DNA-mediated assembly was determined to be 0.593 for DNA-functionalized 100 nm polystyrene colloids, and 90% of the assembly was complete after 6.25 h of hybridization in 50 mM NaCl Tris buffer. We also demonstrate that DNAzymes, catalytic DNA molecules, can be incorporated into the design, and in the presence of 10 μM Pb^{2+}, the hybridization-induced array assembly can be disrupted via DNAzyme activity.

Introduction

The sequence-specific interactions of DNA make it a powerful molecule for directing the bottom-up assembly of materials. DNA is quite versatile and can be modified with different functional groups for attachment to various inorganic and organic building blocks including metal nanoparticles and surfaces,[1–9] semiconductor quantum dots,[10,11] polystyrene colloids,[12–22] and glass surfaces.[17,18,20,21,23,24] Through selection of appropriate DNA sequences, these building blocks can be programmed to assemble in two and three-dimensional structures inaccessible through conventional self-assembly processes.[6,25–28] In the most intricate cases, DNA can be used as a scaffold for

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Table 1. Modified DNAzyme Oligonucleotide Sequences Tethered to PS

Am-PEG-5T(7)17E	5′-/5AmMC6/iSp18/ TTTTTAAAGAGACATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT-3′
Am-PEG-17DS(7)	5′-/5AmMC6/iSp18/ACTCACTATrAGGAAGAGATGTCTCTTT-3′
Am-PEG-17DSnc(7)	5′-/5AmMC6/iSp18/ACTCACTATAGGAAGAGATGTCTCTTT-3′

the periodic arrangement of gold nanoparticles.[25,28] Tuning of DNA-hybridization by sequence and heating can provide programmable self-assembly to yield body-centered cubic arrangements of gold nanoparticles in solution.[26,27] Furthermore, the functionality of DNA is not limited to its hybridization ability; catalytic DNA molecules known as DNAzymes, can be incorporated into traditional gold nanoparticle assemblies for both sensing[29] and enhanced self-assembly.[29–33] DNAzymes have demonstrated the ability to "proofread" DNA-assembled nanoparticle assemblies with great accuracy.

In particular, there has been considerable interest in using DNA to direct the assembly of colloidal particles. There are numerous routes to obtaining polystyrene colloids either covalently[12–18] or noncovalently[19–22] modified with ssDNA. Once functionalized with ssDNA, DNA hybridization has been used to mediate the assembly of suspensions of DNA functionalized PS into two-dimensional arrays on surfaces[17,20,21,24] and into three-dimensional structures in the bulk.[8,9,12–16,19–22,34] Colloids can also be assembled onto glass substrates covalently[17,18,24] or noncovalently[20] functionalized with complimentary ssDNA. As colloidal suspensions, these particles can be assembled via DNA hybridization to form binary aggregates[12,19,22] as well as small-FCC crystals.[15,16] Investigation of bulk DNA-mediated colloidal assembly has optimized DNA-colloidal grafting as well as provided key techniques for preventing nonspecific binding interactions.[12,14,19] Fluorescent-dyed polystyrene colloids have been used to demonstrate the sequence specificity and thermal reversibility of DNA-mediated assembly in bulk.[12,19] Colloids modified with DNA and assembled into various structures[12–22,24,34–36] can be investigated using optical, fluorescence, and confocal microscopy[12] techniques, which have long been invaluable for the study of biological systems.

Although many protocols have been developed for using DNA to direct the assembly of colloidal particles, in only a few cases, have the distinctive attributes of DNA directed assembly, sequence selectivity and thermal reversibility, been demonstrated. Among the best we have observed is the method of Schwartz and co-workers,[12] where both selectivity and thermal reversibility were demonstrated. In particular, they selected specific DNA sequences to minimize self-complimentarity and included a PEG-spacer between the DNA sequence and the amine-functional group, which was covalently linked to carboxylated-polystyrene colloids (PS), to provide the free volume necessary to permit hybridization. Here we demonstrate, for the first time, assembly of DNA-modified PS onto micro-contact-printed glass substrates functionalized with complimentary DNA in a periodic array. Array assembly occurs with both sequence specificity and thermal reversibility, essential hallmarks of DNA hybridization. The DNA-mediated colloidal assembly was demonstrated to be sequence-specific by using two sets of DNA-functionalized fluorescent colloids. Since the patterning method produces DNA-directed assembly of two-dimensional periodic colloidal arrays, colloidal assembly kinetics is followed using laser diffraction.

When conventional DNA sequences are utilized, DNA mediated particle−particle interactions are only controlled by DNA sequence complimentarity, temperature, pH, and ionic strength. To enhance assembly control beyond traditional routes, we have incorporated DNAzymes, catalytic DNA molecules that cleave specific nucleic acid sites within a complementary strand in the presence of metal ion cofactors.[29–32,37–40] The DNAzyme provides a chemically responsive tether that can mediate the assembly of particles dependent on the presence or absence of analyte to which the DNAzyme is sensitive. Here, a DNAzyme previously developed for Pb-sensor applications[29–32,37,39,41,42] was demonstrated to enable analyte-mediated colloidal assembly.

Experimental Methods

Chemicals and DNA. All chemical reagents were purchased from Sigma-Aldrich, Inc. and used without additional purification. Buffer solutions were prepared with as-received reagents, chelated with Chelex 100 beads for 1 h to remove contaminating divalent metal ions, filtered, and titrated with glacial acetic acid to adjust the pH as desired. Concentrated Pb(II) solution was prepared using Pb(II) acetate to a concentration of 10 mM in 10 mM acetic acid to assist in solubility. Yellow-green fluorescent Fluospheres (100 nm and 1 μm diameter) (YG-PS) and orange fluorescent Fluorspheres (1 μm diameter) (OR-PS) with carboxylate-modified surfaces were purchased from Invitrogen, Inc. Sylgard 184 poly(dimethyl siloxane), PDMS, monomer and cure were purchased from Dow Corning, Inc.

Oligonucleotides with modification were purchased from Integrated DNA Technologies, Inc. with HPLC purification and used without additional purification. The 12mer sequences (B-DNA, B′-DNA, and D-DNA) were taken directly from Schwartz.[12] For fluorescent imaging of DNA on glass and PS, a 5′-FAM (carboxyfluoroscein) was included. Enzyme strand, Am-PEG-5T(7)17Ea, was labeled with 5′-amine and six polyethylene glycol spacer. Cleavable substrate strand, Am-PEG-17Sa(7), was labeled with 5′-amine. Noncleavable internal control strand, Am-PEG-17Sanc(7), was labeled with 5′-amine. Oligonucleotide sequences and modification are shown in Table 1. 17Ea and 17Sa have a few changed bases in the 5′ arm of the enzyme and complimentary region of substrate to decrease self-complimentarity as predicted by M-fold

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for the original extended sequence. This change was necessary to increase the stability of PS functionalized with the DNA. When the previous unaltered sequences were used, the DNA would cross-link leading to colloidal aggregation. Amine modifications were used for N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) coupling of oligos to carboxyl groups patterned on the glass surface and on the PS. The poly(ethylene glycol), PEG, spacer was used to space the oligo away from the colloid surface and decrease nonspecific binding to the PS.[12]

Microcontact Printing Stamp. A microcontact printing stamp was prepared by curing PDMS over a TEM grid-based (25 μm pitch) silicon master. A thoroughly mixed 10:1 monomer to cure ratio was used, poured over the master, placed under vacuum until all bubbles were removed and placed in an 80 °C oven overnight. PDMS was carefully cut and removed from the master wafer and used as the microcontact printing stamp with elevated TEM grid lines and recessed TEM grid windows.

Microcontact Printing of DNA Patterns on Glass. Microcontact printing procedures were modified from previously reported methods[43] and PDMS stamps were prepared by gentle cotton swab application of 1% octadecyltrichlorosilane (OTS) in hexane and allowing the hexane to evaporate. The microcontact stamp was then carefully pressed against Piranha cleaned glass for 10 s and pulled off, being careful not to allow the stamp to slip on the surface. (*CAUTION: Piranha is a vigorous oxidant and should be used with extreme caution!*) The stamped glass is then soaked in 15 mL of 0.05% OTS in hexane for 5 min. Backfilling nonstamped areas with dilute OTS facilitated homogeneous surface coverage of the carboxy-silane (see below) as well as further reduced nonspecific binding sites. The stamped glass is then rinsed thoroughly in successive volumes of acetone, ethanol and deionized water (18 MΩ cm). The stamped glass was then soaked in a solution of 30 μL of 45% *N*-(trimethoxysilylpropyl)ethylenediamine triacetic acid trisodium salt (carboxy-silane) in 50 mL deionized water for 1.5 h followed by thorough rinsing in deionized water. The stamped glass was then soaked in 25 mL of 50 mM MES (2-(*N*-morpholino)ethane sulfonic acid) buffer at pH 4.6 containing 200 nM Am-PEG-5T(7)17E and 6 mM EDC (from 1 mL of freshly prepared 157 mM EDC in MES stock solution) for 6 h. A second EDC addition of 1 mL of 157 mM EDC in 50 mM MES was then added to the soak solution and allowed to react overnight. The patterned glass was then soaked in 25 mL of 2% Tween20 in 50 mM MES for 2 days.

Functionalization of PS with DNA. DNA functionalization of 1 μm PS was based on methods previously reported.[12] For preparation of 100 nm functionalized PS, 40 μL of the 2% 100 nm Fluospheres were suspended in 1 mL of deionized water, centrifuged at 13.2 krpm for 18 min, supernatant removed, solids resuspended in 1 mL 50 mM MES, recentrifuged and supernatant removed. The Fluospheres were then redispersed in 700 μL 50 mM MES containing 200 nM Am-17S(7) and 214 μM EDC (from 30 μL of freshly prepared 5 M EDC in MES stock solution). The colloidal suspension was vortexed for 30 min, a second addition of 30 μL of 5 M EDC in 50 mM MES, followed by vortexing; 750 μL of 2% Tween20 in 50 mM MES and 200 μL ammonium hydroxide were then added to the colloidal suspension, followed by vortexing. The colloidal suspension was then centrifuge-rinsed as described above but using 50 mM Tris acetate pH 7.7 buffer with 50 mM NaCl and finally redispersed in 10 mL of 50 mM Tris acetate pH 7.7 and 50 mM NaCl.

Hybridization of DNA Functionalized Colloids to DNA-Functionalized Surface. Final preparation of the tethered PS on patterned glass was accomplished by soaking overnight two patterned glass surfaces (8 TEM grid patterned regions total) in 10 mL of the functionalized colloids. Immediately prior to imaging, the assembled surfaces were "swirled" on a Stoval Belly Button Shaker in fresh 50 mM Tris acetate with 50 mM NaCl for 30 min.

Sequence Specificity. One micrometer of YG-PS and OR-PS were functionalized with one of two DNA sequences, B and D. Both B and D sequences were selected from Schwartz et al.[12] The B sequence is complementary to the B′ sequence tethered to the glass surface and the D sequence is noncomplementary to both B and B′. Two sets of glass slides were patterned as described previously with the B′ DNA. For one set of patterned surfaces, YG-PS were functionalized with complimentary DNA, B, and OR-PS with noncomplimentary DNA, D. They were mixed simultaneously in hybridization buffer with the surface patterned with B′. For the other set of patterned surfaces, OR-PS were functionalized with complimentary DNA, B, and YG-PS with noncomplimentary DNA, D. They were mixed simultaneously in hybridization buffer with the surface patterned with B′. Both separate sets of samples were allowed to hybridize overnight and transferred to fresh PBS buffer for imaging via wet-state fluorescence microscopy.

Pb-Mediated Colloidal Assembly. Colloidal assembly in the presence of 10 μM Pb(II) was explored for both DNAzyme (17Ea) cleavable-functionalized (17Ds) colloids and control (17Dsnc) colloids which did not contain the cleavable RNA base by addition of 10 μL of 10 mM Pb(II) stock solution to the Tris buffer solution. The same concentration of Pb(II) was also added during the rinse swirl to ensure no opportunity for the hybridization between the PS and the substrate. Using the previous methods for producing DNA-patterned glass surfaces, the enzyme strand, Am-PEG-5T(7)17Ea, was attached to glass substrates. 100 nm YG-PS were functionalized with a cleavable substrate strand, Am-PEG-17Sa(7), and allowed to assemble in Tris hybridization buffer without Pb^{2+} while swirling. On a separate set of Am-PEG-5T(7)17Ea patterned surfaces, cleavable Am-PEG-17Sa(7)-functionalized 100 nm YG-PS were hybrized in Tris buffer with 10 μL of 10 mM Pb^{2+} while swirling. For the noncleavable control samples, Am-PEG-5T(7)17Ea patterned surfaces were hybridized with noncleavable Am-PEG-17Sanc(7)-functionalized 100 nm YG-PS in Tris buffer with 10 μL of 10 mM Pb^{2+} while swirling. Samples of the noncleavable Am-PEG-17Sanc(7)-YG-PS with patterned surfaces hybrized in the absence of Pb^{2+} were also prepared.

Fluorescence Microscopy. A Ziess Axiovert 100 inverted fluorescence microscope equipped with a Black and White CCD camera and a Leica DMR microscope equipped with a color CCD camera were used for the experiments reported here. Both microscopes were equipped with a 100 W mercury lamp and a FITC filter set. Patterned samples remained immersed in Tris hybridization buffer during imaging to decrease drying effects when imaged on the Leica.

Diffraction Pattern Imaging. The diffraction imaging system was comprised of a 0.5 mW red HeNe laser (633 nm), a 0 − 5.0 variable neutral density filter wheel, a sample holder and a color CCD camera. A wet cell was mounted on the sample holder; the wet cell consisted of a sandwich formed by a 1 mm thick O-ring held between the DNA patterned slide and a blank slide. The cavity formed by the O-ring was filled with a dilute suspension (0.1% v/v) of the DNA functionalized PS in Tris hybridization buffer. A computer controlled system consisting of a CCD camera coupled to an Epix input card and the XCAP imaging collection software was used to collect diffraction images at set time intervals over 24 h. The DNA patterned glass surface was placed 48 mm from the lens of the CCD camera for optimal diffraction pattern collection. The intensity of the diffraction spots were determined using Photoshop. Following background subtraction, images were converted to gray scale images, and the intensity of individual diffraction spots were determined by masking a selected region and averaging the intensity of the spot.

Scanning Electron Microscopy. A Philips XL30 ESEM-FEG environmental scanning electron microscope operating at 5.0 kV in high-vacuum mode was used to image tethered colloids after sputter coating with Au.

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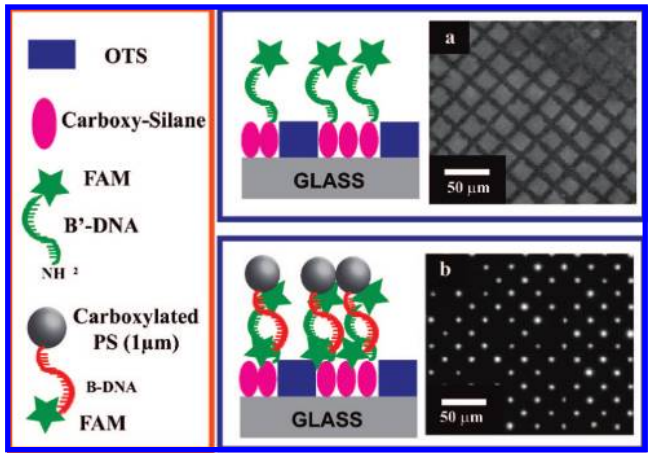

Figure 1. Wet-state fluorescence micrographs of (a) fluorescent-tagged B′-DNA in μCP patterns: stamp pitch is 25 μm. The adjacent scheme depicts the spatial functionalization of the glass surface with lines of OTS and squares of DNA. (b) One micrometer nonfluorescent PS with fluorescent-tagged B-DNA hybridized to surface-patterned B′-DNA. The adjacent scheme indicates the areas of the surface to which the colloids can assemble via hybridization. Note, colloids are brighter than surface DNA (a), and thus the surface DNA is not observed in (b).

Thermal Reversibility. The wet cell described previously was placed so that the sample was in contact with the heating element of a Linkam THMS600 heating chamber, which was not sealed due to the size of the wet cell. The heating chamber was placed on the Leica DMR stage. Due to heat transfer across the glass slide and heating loss through the open chamber, temperature was monitored independently with a thermocouple and reader. A fully assembled array of 1 μm YG-PS in a 25 μm pitch pattern was placed in the wet cell; the buffer for thermal cycling was 50 mM PBS with 1% Tween. The heating chamber temperature controller was set to ramp up to 70 °C over the course of 5 min with a final internal temperature of 55 °C. Once an internal temperature of 55 °C was reached, the temperature controller was set to 55 °C and adjusted manually to maintain a constant internal temperature.

Results and Discussion

Microcontact printing was used to form grid-based patterns of ssDNA in order to assemble two-dimensional well-ordered arrays of ssDNA functionalized PS. These arrays were demonstrated to assemble via sequence-specific DNA hybridization. Furthermore, the other trait of DNA-mediated assembly, thermal reversibility was also demonstrated. Since the patterns were of sufficient specificity and index contrast for optical diffraction, the hybridization and formation of the patterns could be observed using a simple laser diffraction setup. The diffraction data gives insight into the kinetics of colloidal assembly by DNA hybridization. We also demonstrate that DNA-assembled colloidal arrays can be controlled by nonconventional means: Pb^{2+}-activated DNAzymes were incorporated into the colloidal assembly and enzymatic activity was observed.

DNA Functionalization and Colloidal Assembly onto Patterned Surfaces. Among the many protocols for immobilization of DNA onto PS, the method of Schwartz et al. was selected.[12] For functionalization of DNA onto glass surfaces, while DNA can be directly patterned onto glass surfaces,[23] microcontact printing of octadecyltrichlorosilane (OTS) SAMs[43] enabled simultaneous patterning and blocking of the glass surface[1] (Figure 1). A grid of OTS lines was printed using a TEM grid-derived PDMS stamp; unfunctionalized regions of the substrate were backfilled with N-ethylenediamine triacetic acid trisodium salt (carboxy-silane). Blocking with additional OTS[1] and Tween-20[12] eliminated potential sites for nonspecific adhesion of colloids. Then amine-terminated 12-mer DNA[12] was immobilized to the carboxylated regions of the glass surface and PS via EDC-coupling chemistry. Exposure of the patterned substrate to the functionalized colloids in 50 mM PBS buffer produced well-ordered two-dimensional arrays of fluorescent-DNA-labeled PS (Figure 1b).

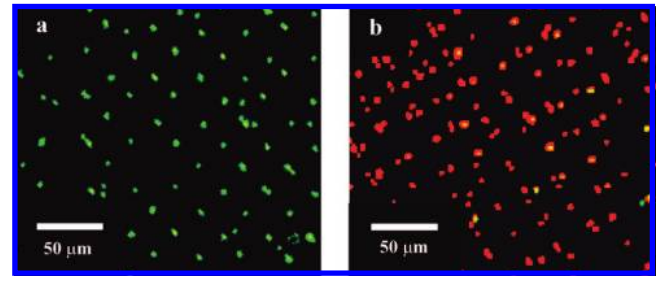

Figure 2. Fluorescent micrographs of (a) surface-patterned with B′-DNA exposed to a mixture of 1 μm B-DNA-YG-PS and 1 μm D-DNA-OR-PS and (b) surface-patterned with B′-ssDNA exposed to a mixture of 1 μm B-DNA-OR-PS and 1 μm D-DNA-YG-PS. There are a few D-DNA-YG-PS (1%) in (b) due to nonspecific binding.

Investigation of Sequence Specificity. If colloidal assembly on the patterned substrate is due to DNA hybridization, then only colloids functionalized with strands complimentary to the DNA on the patterned substrate will assemble. However, if assembly is due to nonspecific binding such as electrostatic or hydrophobic interactions,[44] both complimentarily functionalized and noncomplimentarily functionalized colloidal particles will assemble on the patterned surface. Confirmation of sequence specific assembly was achieved using DNA functionalized YG-PS and OR-PS. In Figure 2a, YG-PS were functionalized with complimentary DNA, B, and OR-PS with noncomplimentary DNA, D. A mixed suspension of both complimentary and noncomplimentary colloids was introduced over a surface patterned with B′ DNA. If assembly is sequence-specific, only the complementary-B-DNA functionalized YG-PS will hybridize with the surface-tethered DNA. As seen in Figure 2a, this is indeed the case, and only the YG-PS were assembled in DNA-patterned regions. Some noncomplimentary D functionalized OR-PS settled or adsorbed onto the surface, however the simple act of transferring the substrate into fresh buffer removed nearly all of them. In Figure 2b, the functionalization of the OR-PS and YG-PS was switched, and as expected, exposure to a B′ patterned surface yielded OR-PS arrays with a negligible quantity (1%) of noncomplimentary YG-PS. Sequence-specific assembly, one of the important hallmarks of DNA-directed assembly, was clearly observed.

Test of Thermal Reversibility. Along with sequence specific assembly, another important hallmark of DNA mediated assembly is the potential for thermal reversibility. The sequences investigated here contain 12 bases active in hybridization, and thus should dehybridize or melt at 49.4 °C at [NaCl] = 50 mM.[12] The colloidal arrays were assembled at room temperature and transferred to the wet cell for heating. After placing in the heating chamber, the temperature of the cell was increased over a period of 5 min to an internal temperature of 55 °C. During this temperature ramp (Figure 3b) no perceptible change in colloid surface coverage is detected. However, after 7 min at 55 °C, colloids could be observed lifting off and moving away

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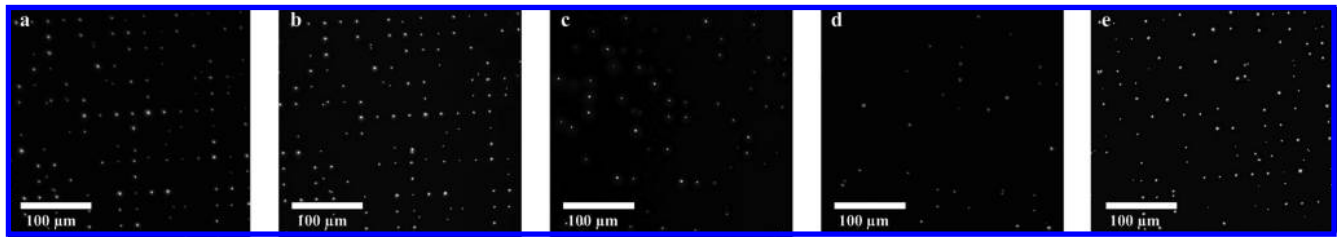

Figure 3. Fluorescence micrographs of melting-induced disassembly of 12mer DNA-hybridized colloidal arrays: (a) $T = 25$ °C, (b) $T = 55$ °C; $t = 0$ min, (c) $T = 55$ °C; $t = 7$ min, and (d) $T = 55$ °C; $t = 15$ min. (e) Reassembled colloidal array: $T = 25$ °C.

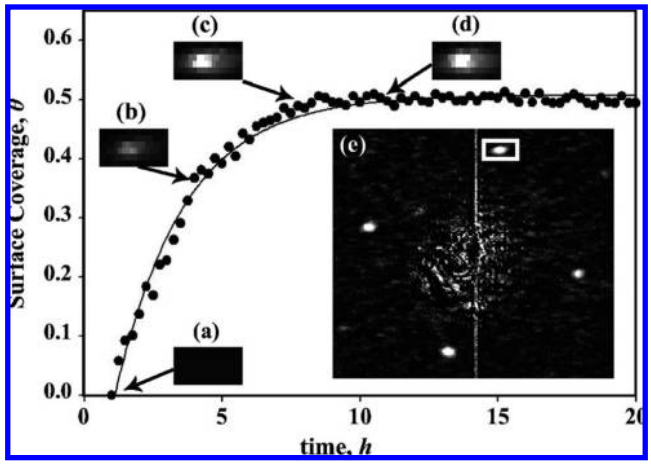

Figure 4. Surface coverage vs time from real time hybridization and assembly of 100 nm B-DNA-YG-PS onto a B′-DNA patterned glass surface (25 μm pitch) observed by laser diffraction, $\lambda = 633$ nm, as captured by a CCD camera. The central beam spot was removed by background subtraction. Insets: diffraction spot image at (a) 1 h; (b) 4 h; (c) 8 h; (d) 12 h; (e) 12 h diffraction image with white rectangular box representing diffraction spot sampling area. The vertical white line is an artifact arising from the central laser beam.

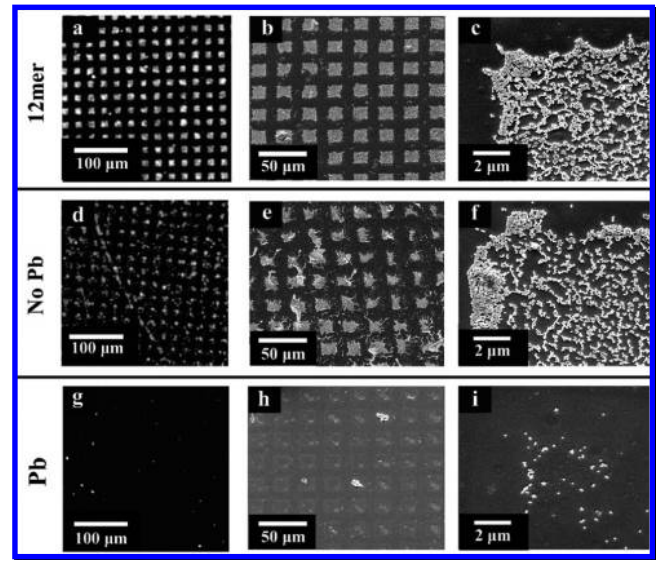

Figure 5. One hundred nanometer B-DNA-YG-PS on B′-DNA patterned surface imaged via (a) fluorescence microscopy, (b−c) SEM; 100 nm 17Sa-DNA-YG-PS on 17Ea-DNA patterned surface in hybridization buffer observed with (d) fluorescence microscopy, (e−f) SEM; 100 nm 17Sa-DNA-YG-PS on 17Ea-DNA patterned surface in hybridization buffer containing 10 μM Pb^{2+} observed with (g) fluorescence microscopy, (h−i) SEM.

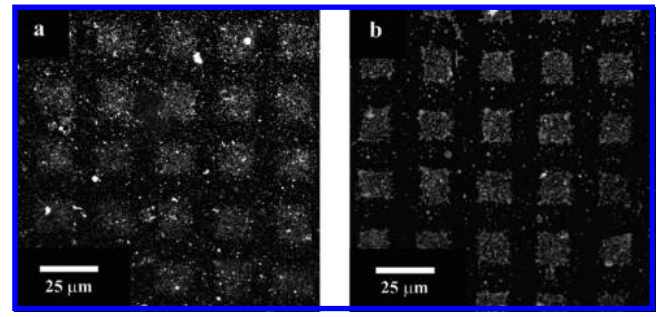

Figure 6. Confocal micrographs of 25 μm pitch arrays of 100 nm colloids (a) imaged in buffer and (b) after drying for 15 min in air.

from the surface due to internal convection in the cell (Figure 3c). At 15 min, 90% of colloids were removed (Figure 3d). The sample was removed from the heating chamber, rinsed with fresh buffer, and returned to the wet cell which now was filled with 50 mM PBS with 2% Tween for 12 h. The blocking buffer was removed from the cell and replaced with fresh buffer (50 mM PBS) and 50 μL of freshly functionalized colloids. These colloids were allowed to assemble overnight in the wet cell and imaged (Figure 3e). The imaged spot is the same TEM-grid based area of the pattern.

Diffraction Based Real-time Monitoring of Colloidal Assembly. The periodic nature of the 2D colloidal arrays enabled the use of laser diffraction to probe the real-time DNA mediated assembly of colloids. 100 nm B-DNA-PS were injected into a sealed cell containing a B′-patterned substrate immersed in 50 mM NaCl Tris hybridization buffer. 100 nm colloids were selected rather than larger 1 μm colloids to minimize light scattering by the colloids on the solution as the laser passes through the solution prior to hitting the substrate and gravity induced sedimentation. The substrate was oriented vertically so that any sedimentation did not impact colloid-substrate interactions. Because the PS have a greater refractive index than the solution (1.59 vs 1.33), once assembled into a periodic array, they will generate a diffraction pattern. Initially, the diffraction pattern was quite weak; however by 6.25 h, it reached 90% of its final intensity (t_{90}) (Figure 4). Such diffraction based imaging has an advantage over a fluorescence imaging technique as photobleaching is not an issue, thus the laser can remain on the entire experiment. After 25 h, the substrate was removed and SEM imaging was performed. Drying effects can be seen in Figure 5, where colloids are pulled away from the OTS-DNA boundary and other colloids in the DNA region tend to aggregate. To confirm that drying effects are the cause of the colloidal pile-up near the OTS boundaries, confocal micrographs of assembled arrays were imaged before and after drying. In Figure 6a, the wet sample clearly shows homogeneous distribution of the colloids within the DNA functionalized regions. The submonolayer coverage is expected due to the low concentration of colloids in suspension and is discussed in our kinetic analysis. In Figure 6b, the same sample was dried for 15 min in air and

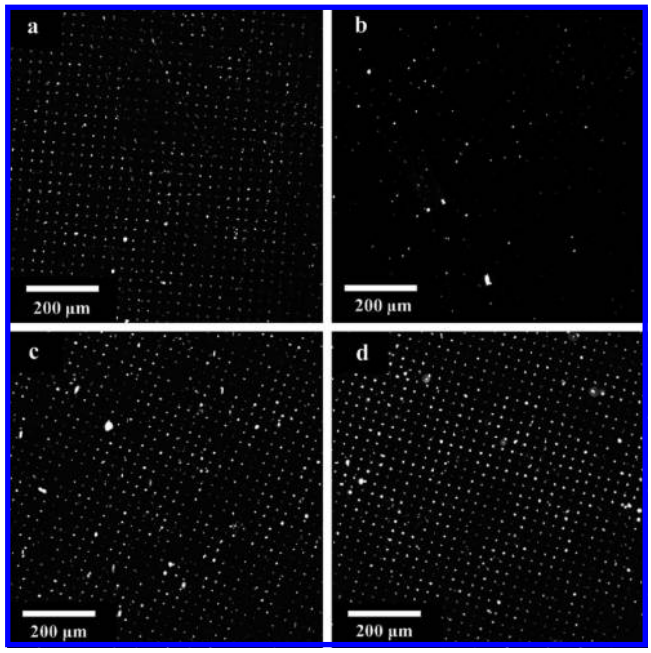

Figure 7. Fluorescence micrographs of Pb^{2+}-mediated assembly of 100 nm YG-PS on 17Ea-DNA microcontact printed glass surfaces. (a) and (b) show assembly of 100nm 17DSa-DNA-YG-PS only in the absence (a) and not in the presence (b) of Pb^{2+}. Use of noncleavable substrate functionalized 17DSanc-DNA-YG-PS shows assembly both in the absence (c) and presence (d) of Pb^{2+}.

reimaged: the dried sample clearly shows the "pulling" away from the OTS-DNA boundary that is also observed in the SEM. Furthermore, wet-state fluorescence microscopy as well as laser diffraction allowed imaging of the colloids while hybridized in buffer solution (Figure 5a).

To obtain kinetic information on DNA-hybridization mediated assembly of colloidal particles, first we determined the terminal surface coverage of the PS in the patterned regions. Based on the SEM imaging, there were ~63 colloids/μm^2 in the patterned regions, which corresponds to a surface coverage of 49% (Figure 5c). If the thickness of the hydrated DNA brush on the surface of the colloids is included, the effective surface coverage is ~59%. Because the refractive index of the hydrated DNA layer is similar to water, we do not include it in our diffraction calculations. Assuming 49% is the terminal surface coverage, the surface coverage at intermediate times can be estimated from a volumetric treatment of a sinusoidal phase grating and its diffraction efficiency.[45] The diffraction pattern can then be quantitatively analyzed to yield information on assembly kinetics. Diffraction is caused by the periodic variation of the refractive index across the surface of the substrate caused by hybridization of DNA-tethered PS. The refractive index profile can thus be treated as a phase grating. The diffraction efficiency of a phase grating can be approximated, using the sinusoidal phase grating equation,[45] as:

$$\eta = \left(\frac{\pi}{\lambda} L \Delta n\right)^2 \tag{1}$$

where η is the diffraction efficiency, λ is laser wavelength, 633 nm, L is the height of the grating, which in our case is 100 nm, the diameter of the colloidal particles, and Δn is the index contrast between the index of refraction of the filled square patch and the aqueous buffer. To calculate,

$$\Delta n_{\text{square}} = \frac{V_{\text{particles}} \cdot n_{\text{polystyrene}} - [V_{\text{square}} - V_{\text{particles}}] \cdot n_{\text{water}}}{V_{\text{square}}} \tag{2}$$

$$V_{\text{particles}} = (\#\ \text{particles}) \frac{4}{3} \pi r^3 \tag{3}$$

where $V_{\text{square}} = 20\ \mu m \times 20\ \mu m \times 100$ nm, $n_{\text{polystyrene}} = 1.59$, and $n_{\text{water}} = 1.33$. It follows that the intensity of a diffraction spot corresponds to a specific diffraction efficiency; and, from eqs 1 and 2, a particular diffraction efficiency corresponds to some colloid surface coverage. The diffraction efficiencies were calculated for counts of 25 000 colloids per DNA functionalized square and repeated for decreasing increments of 100 colloids. The corrected diffraction data was compared to the calculated diffraction efficiencies to obtain colloid surface coverage for each diffraction intensity data point.

This method allows us to extract information about the DNA-mediated assembly at intermediate times. For example, at 4 h, as calculated from the intensity of the diffraction spot, the surface coverage is 36.7% (ignoring the DNA brush). From the diffraction data at intermediate surface coverages, the behavior of the hybridization induced assembly was determined. The assembly was then modeled as an irreversible self-limiting monolayer process,[46,47]

$$\theta(t) = \Lambda(1 - e^{-St/\Lambda}) \tag{4}$$

which gives the surface coverage, θ, as a function of time, t, and the maximum surface coverage, Λ. The best fit, $R^2 = 0.9856$, was found by setting Λ to 0.511, and S to 1.96×10^{-4} s^{-1}. The tethered ssDNA increases the effective colloid diameter to 116 nm, giving an effective maximum surface coverage (Λ) of 0.593. The calculated limit for Λ, assuming random sequential absorption, is 0.547.[46] The fact that the effective surface coverage is slightly greater indicates the colloids have some mobility on the surface. The deposition rate, S, is a factor of 3 less than that calculated assuming diffusion limited absorption (~5.8×10^{-4} s^{-1}), indicating the sticking coefficient is less than 1. The surface coverage as determined from the diffraction intensity and the best fit to eq 4 is presented in Figure 4. The correlation between the diffraction intensity and surface coverage enables quantification of hybridization-driven assembly kinetics. This approach is quite general, and could be used to obtain the kinetics from a diversity of 2D assembly processes.

DNAzyme Mediated Assembly. DNA directed assembly is broadly useful, however additional functionalities are possible if the assembly can be regulated via specific triggers. As a proof of concept, we functionalized the carboxylated glass substrate with a Pb^{2+}-specific DNAzyme and the PS with a complementary strand containing a single RNA linkage.[29–32,37,39,41,42] The enzymatic strand (17Ea) was immobilized to the patterned glass substrate, while the RNA containing strand (17DSa) was attached to 100 nm YG-PS. Combination of the functionalized surface and YG-PS in Tris hybridization buffer in the absence of Pb^{2+} resulted in well-ordered 2D arrays, which were observed in the wet-state using fluorescence microscopy and laser diffraction. SEM revealed

(45) Goodman, J. W. *Introduction to Fourier Optics*, 2nd ed.; McGraw-Hill: New York, 1996.
(46) Piech, M.; George, M. C.; Bell, N. S.; Braun, P. V. *Langmuir* **2006**, *22* (4), 1379–1382.
(47) Privman, V.; Frisch, H. L.; Ryde, N.; Matijevic, E. *J. Chem. Soc., Faraday Trans.* **1991**, *87* (9), 1371–1375.

PS tethering densities comparable to that of the original 12mer system (Figure 5a−f).

The stimuli-response of the DNAzyme regulated assembly is dramatic. Addition of 10 μM Pb^{2+} into the solution inhibited colloidal assembly. DNA hybridization still occurs; however, in the presence of Pb^{2+}, it is followed by DNAzyme mediated cleavage, releasing the colloid before irreversible tethering occurs. The grid pattern does not develop (Figure 5g−i and Figure 7b) and diffraction was not observed. SEM indicates a 1000-fold decrease in colloid density in the presence of Pb^{2+}, compared to the assembly in the Pb^{2+}-free state.

To confirm the observed effect is due to Pb^{2+}-specific DNAzyme cleavage, not Pb^{2+} itself, a noncleavable substrate strand was functionalized on the PS. The noncleavable strand is identical to the cleavable substrate except the RNA base is replaced by a DNA base at the cleavage site,[48] rendering the DNAzyme complex inactive. Figure 7c presents the assembly of the noncleavable-functionalized YG-PS in the absence of Pb^{2+}. As expected, the arrays are comparable to the assembly of the cleavable-functionalized YG-PS in the absence of Pb^{2+} in Figure 7a. In Figure 7d, noncleavable-functionalized YG-PS were also assembled in the presence of Pb^{2+} and the resulting arrays were comparable both in ordering and density to both the noncleavable without Pb^{2+} and the cleavable without Pb^{2+}. The patterned assembly of noncleavable colloids even in the presence of Pb(II) indicates that the DNAzyme and Pb^{2+} cofactor were required for the prevention of colloidal assembly.

(48) Brown, A. K.; Pavot, C. M.-B.; Li, J.; Lu, Y. *Biochemistry* **2003**, *42*, 7152–7161.

Conclusions

We have demonstrated that DNA hybridization can be utilized for sequence-specific assembly of 2D colloidal arrays. In a new approach for mediating DNA-colloidal interactions, array assembly was controlled by a Pb^{2+} activated DNAzyme. Through laser diffraction, real-time monitoring of 2D assembly was demonstrated. Along with determining the kinetics of DNA-mediated colloidal assembly, such a read-out modality may prove useful for future generations of DNAzyme sensors.

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