

Stepwise Evolution of DNA-Programmable Nanoparticle Superlattices**

Andrew J. Senesi, Daniel J. Eichelsdoerfer, Robert J. Macfarlane, Matthew R. Jones, Evelyn Auyeung, Byeongdu Lee,* and Chad A. Mirkin*

Colloidal crystals can be assembled using a variety of entropic,^[1–3] depletion,^[4,5] electrostatic,^[6–8] or biorecognition forces^[9–12] and provide a convenient model system for studying crystal growth. Although superlattices with diverse geometries can be assembled in solution and on surfaces, the incorporation of specific bonding interactions between particle building blocks and a substrate would significantly enhance control over the growth process. Herein, we use a stepwise growth process to systematically study and control the evolution of a body-centered cubic (bcc) crystalline thin-film comprised of nanoparticle building blocks functionalized with DNA on a complementary DNA substrate. We examine crystal growth as a function of temperature, number of layers, and substrate–particle bonding interactions. Importantly, the judicious choice of DNA interconnects allows one to tune the interfacial energy between various crystal planes and the substrate, and thereby control crystal orientation and size in a stepwise fashion using chemically programmable attractive forces. This is a unique approach since prior studies involving superlattice assembly typically rely on repulsive interactions between particles to dictate structure, and those that rely on attractive forces (e.g. ionic systems) still maintain repulsive particle–substrate interactions.

In addition to providing a model for crystallization, the field of particle assembly has garnered considerable interest because materials generated from ordered particle arrays can have novel optical,^[1,3,13–17] electronic,^[13,18] and magnetic properties.^[19,20] These properties can be sensitive to the composition, symmetry, and distance between nanoparticles, in addition to the number of layers and orientation.^[9,15,16] DNA-mediated nanoparticle crystallization is particularly attractive for preparing these materials because the nanoparticle building blocks can be considered a type of “pro-

grammable atom equivalent” with tailorable size, composition, shape, and bonding interactions.^[9–11,14,21–27] This tunability allows one to access a diverse class of crystal symmetries,^[10,25] tailor lattice parameters with sub-nanometer resolution,^[22] and create structures that have no known mineral equivalent.^[27] Indeed, to date, 17 unique symmetries have been realized and over 100 unique crystal structures have been synthesized, all of which conform to a key hypothesis: these atom equivalents assemble into structures that maximize the total number of hybridized DNA interconnects between particles.^[25]

While these structures have enormous potential, their use is limited because they are typically formed in solution as polycrystalline aggregates with little control over crystal size or orientation. Consequently, it is difficult to measure their properties or integrate them with other device elements using existing microfabrication techniques. The development of thin-film superlattices is therefore necessary to fully realize the potential of these structures as metamaterials, photonic crystals, and data storage elements.

The growth of DNA-mediated mono- and multi-layered nanoparticle structures was first examined by our group^[28] and later by Niemeyer and co-workers.^[29,30] However, the use of strong DNA interactions prevented nanoparticle crystallization. Herein, we exploit multiple weak DNA interactions for superlattice growth to examine the development of crystal orientation (texture) and control film thickness. Body-centered cubic colloidal crystals composed of spherical nucleic acid gold nanoparticle conjugates (SNA-AuNPs)^[21] were used as a model system since these structures require two complementary particle types^[10] and therefore allow the stepwise introduction of each layer. Alternatively, other crystal symmetries such as face-centered cubic (fcc) require

[*] A. J. Senesi, D. J. Eichelsdoerfer, R. J. Macfarlane, Prof. C. A. Mirkin
Department of Chemistry, Northwestern University
2190 Campus Drive, Evanston, IL 60208 (USA)
E-mail: chadnano@northwestern.edu

M. R. Jones, E. Auyeung, Prof. C. A. Mirkin
Department of Materials Science and Engineering
Northwestern University
2190 Campus Drive, Evanston, IL 60208 (USA)

Dr. B. Lee
X-ray Science Division, Argonne National Lab
9700 S. Cass Ave., Argonne, IL 60439 (USA)
E-mail: blee@anl.gov

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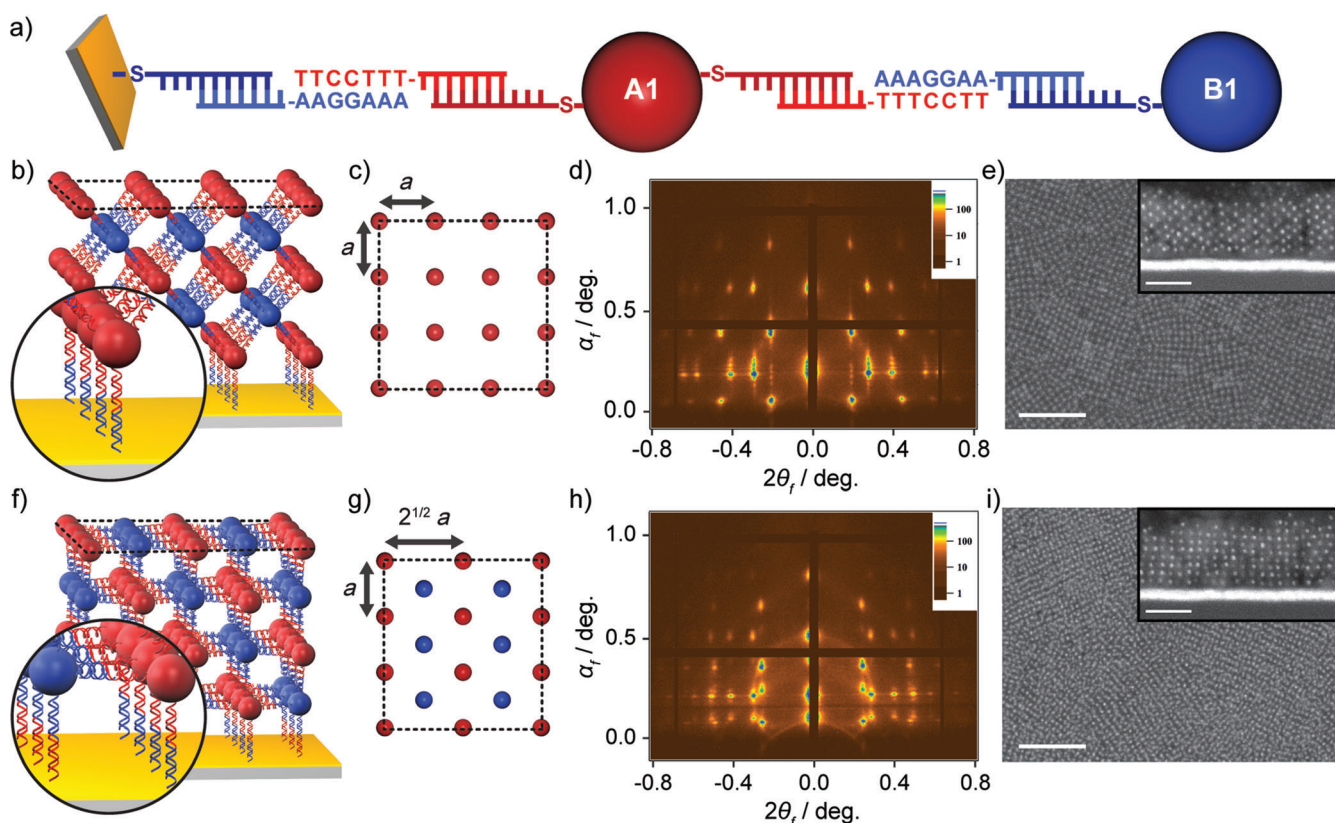


Figure 1. Crystallographic orientation can be programmed by controlling the type of bonding interactions between the superlattice and substrate. a) A binary DNA sequence design enables the stepwise growth of DNA–nanoparticle superlattices with either (100) (b,c,d,e) or (110) orientation (f,g,h,i). Schematic illustrations of (100)- and (110)-oriented bcc superlattices grown on mono- or bifunctionalized substrates, respectively, are shown in (b,f). The (100) orientation formed on substrates that displayed a single type of linker (blue) while the (110) orientation formed on substrates that displayed both types of linkers (blue and red), matching the complementary DNA-functionalized nanoparticles in the (100) (c) and (110) planes (g). d,e) 2D GISAXS scattering pattern and SEM of (100)-oriented bcc superlattices. h,i) 2D GISAXS scattering pattern and SEM of (110)-oriented bcc superlattices. Scale bars for the SEM top-down and cross-section views are 200 nm and 100 nm, respectively (e,i).

self-complementary particles and therefore one cannot introduce particle layers one at a time.

Attractive interparticle forces were mediated with DNA-linkers that displayed hetero-complementary pendent “sticky ends” (Figure 1 a). In a typical experiment, superlattices were prepared by successive immersion of the DNA substrate into a suspension of particle “A” and then particle “B” (defined as one growth cycle) at various temperatures (Figure 1 a). The samples were characterized by synchrotron grazing incidence small angle X-ray scattering (GISAXS),^[31] a powerful solution-compatible technique that allows one to extract orientation, symmetry, lattice parameter, crystallite size and electron density for thin-film superlattices. These data were corroborated with scanning electron microscopy (SEM) of superlattice films embedded in silica.^[32]

We first analyzed surface-confined growth of superlattices on DNA substrates that presented a B-type linker. For this mono-functionalized substrate, superlattices grown through five half-cycles resulted in a (100)-oriented polycrystalline bcc film (Figure 1 b–e; Figures S5–S7 in the Supporting Information show GISAXS indexing and simulated diffraction patterns). This observation can be understood in terms of maximizing DNA duplexes at the substrate–superlattice interface. In principle, planes with higher packing densities

result in increased bonding between the superlattice and substrate. For a bcc crystal, the (110) plane has the highest packing density, followed closely by the (100) plane. Note, however, that the crystal symmetry of these superlattices is defined by the positions and types of inorganic cores; thus, while the AuNPs form a bcc lattice, the binary linker design results in a SNA lattice isostructural with CsCl. The interfacial energy is then inversely proportional not to the planar packing density of all particles in the system, but solely to the density of particles that can bind to the substrate. For B-type substrates, the planar packing density of complementary A-type particles with bcc (100) texture is $1/a^2$ (where a is the lattice parameter) and is therefore favored over the (110) texture, which has a density of $1/(\sqrt{2}a^2)$ (compare Figures 1 c and g). Another way to state this is that the (100) plane, although less densely packed, is comprised entirely of particles that can hybridize to the B-type substrate. While particles in the (110) plane are more densely packed, only half can engage in attractive hybridization interactions.

In contrast to the previous system, the surface can also be functionalized with both A- and B-type linkers. For these bifunctionalized substrates that can bind both particle types, the (110) plane has the highest packing density, $2/(\sqrt{2}a^2)$, of substrate-binding particles (Figure 1 g). Therefore, these sur-

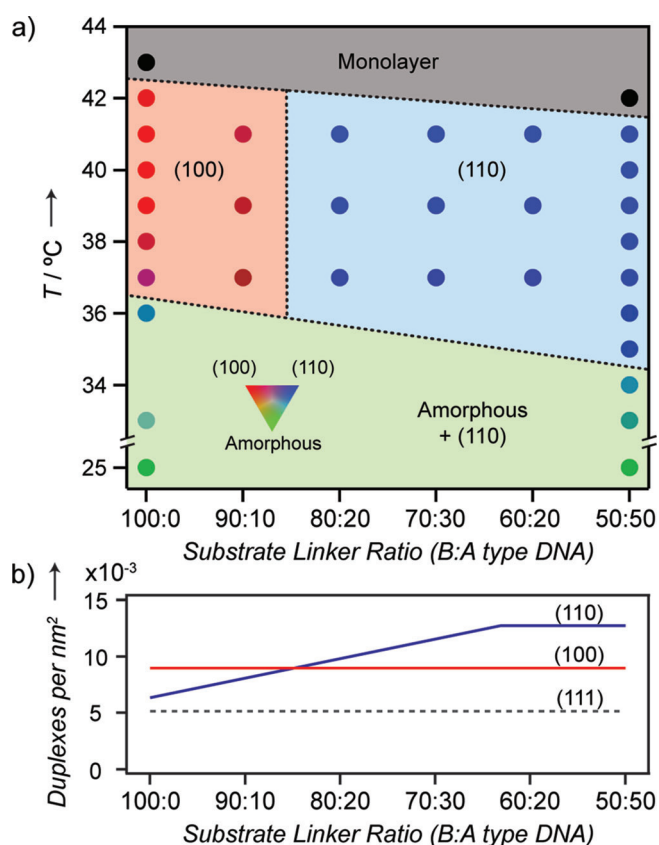


Figure 2. a) Crystallographic phase behavior for bcc thin-film superlattices depends on both growth temperature and the substrate linker ratio. All data are for five half-cycles of crystal growth. The relative concentration of (100), (110) and amorphous phases were determined by fitting experimental GISAXS data and set to a RGB color scale. Under annealing conditions (red, blue regions), the orientation depends on the type of interfacial DNA-bonding interactions, while at low temperatures amorphous structures are formed. The dashed lines and coloring are guides for the eye. b) The relative stability of each orientation can be determined using the CCM model, and confirmed experimentally in (a). For reference, the (111) orientation with the next highest planar packing density is included.

faces should direct the growth of crystals with (110) texture, which matches experimental observations (Figure 1 f–i).

An interesting aspect of this novel system is that one can track the crystal phases present as a function of the substrate linker ratio (Figure 2). Here, the substrate linker ratio is defined as the ratio of B- to A-type linkers on the substrate. At low growth temperatures, kinetically trapped structures are formed that consist of mostly amorphous aggregates (green region in Figure 2a), while crystal growth near the melting temperature (T_m , the temperature at which the superlattice dissociates) results in monolayer formation (gray region in Figure 2a). Superlattice growth at several degrees below T_m resulted in (100)- or (110)-oriented crystals depending on the substrate linker ratio (red and blue regions in Figure 2a; Figures S9, S11, S16 for GISAXS data). The relative stabilities of each orientation can be determined by comparing the number of superlattice–substrate duplexed interconnects for each orientation using the Complementary

Contact Model (CCM),^[25] modified to include interfacial interactions (Figure 2b, Figure S24). This simple geometrical model based on complementary hybridization interactions can be used to predict various crystal phases under ideal annealing conditions. Significantly, the model predicts a crossing point in the relative stability of (100)- and (110)-orientated superlattices at a substrate linker ratio of 85B:15A, which was confirmed experimentally. This result demonstrates that these structures are likely the thermodynamic products. Interestingly, (110)-textured phases are observed at low temperatures on monofunctionalized substrates, which can be attributed to strain and low particle mobility at these growth temperatures (see below, Supporting Information).

To better understand the growth process of the nanoparticle thin-film superlattices, GISAXS was used to monitor each step of the assembly process (Figure 3). Several distinct nanoparticle arrangements were observed on B-type substrates during growth at optimal temperatures (40 °C), and all were found to maximize the number of particle–substrate and particle–particle DNA interconnects. For a two-dimensional assembly of repulsive spheres (a single layer of particles), a hexagonal arrangement maximizes particle density.^[33] Indeed, after one half-cycle the in-plane scattering peaks at $q = 0.020$ and $0.040 \text{ \AA}^{-1}$ can be attributed to a disordered hexagonal structure with an average interparticle distance of 36 nm (Figure 3a,b).

After the second half-deposition cycle on a B-type substrate, three-dimensional ordering consisting of two interpenetrating square lattices was observed (Figure 3a,b), which is equivalent to a bcc (100) crystal with two particle layers. This structure results from particles in the first layer simultaneously maximizing the number of nearest neighbors (4) and the areal density of particle–substrate interactions. SEM imaging of these two-layer samples shows two-dimensional crystallization with fractal morphology (Figures S20, S21). During the transition from a disordered hexagonal monolayer to a square array, the particles in the first layer must densify. If this reorganization process is hindered by low particle mobility, the structure will become strained, which results in a larger in-plane lattice parameter for these two-layer structures ($35.0 \pm 0.5 \text{ nm}$) than would be expected from both multilayered films ($32.7 \pm 0.1 \text{ nm}$) and bulk 3D superlattices ($30.8 \pm 0.1 \text{ nm}$)^[25] with the same nanoparticle size and DNA sequence. An increase in this tensile strain (by using lower growth temperatures) promotes the growth of the lower-density (110) orientation (Figure 2, 100B:0A, 36 °C).

Remarkably, after only three half-cycles, the GISAXS scattering pattern is dominated by (100)-oriented crystals consisting of a single unit cell in the out-of-plane direction. Simultaneously, the in-plane lattice parameter contracts as additional duplexed DNA linkages stabilize the crystal (Figure S12). Though some instability in the first few layers results in the presence of (110)-textured particle arrangements, subsequent growth resulted in exclusively (100) oriented superlattice films (Figure 3b,c). We observed a linear increase in film thickness with deposition cycles, in addition to a narrowing of the scattering peaks. The assemblies therefore form more ordered structures and grow in size as additional

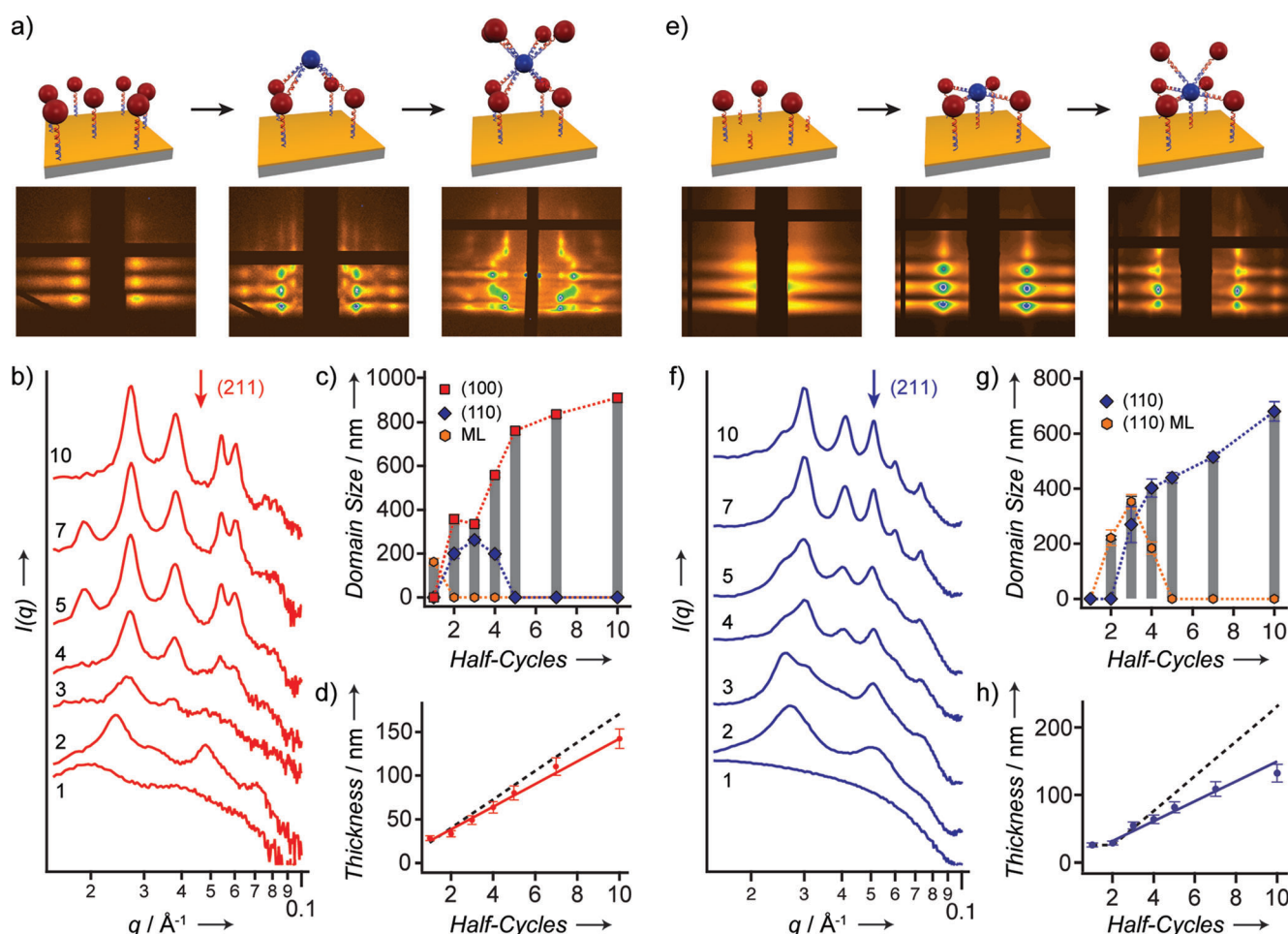


Figure 3. Stepwise GISAXS characterization of (100)- and (110)-textured bcc superlattices. a,e) Idealized schematic and 2D GISAXS scattering patterns after 1, 2 and 3 half deposition-cycles on monofunctionalized (100)-directing and bifunctionalized (110)-directing DNA substrates, respectively. b,f) Normalized GISAXS horizontal linecuts at $\alpha_f = \alpha_i$ after successive half-cycles on monofunctionalized and bifunctionalized DNA substrates, respectively. The curves are offset for clarity and plotted on a log-log scale. Note the systematic absence (b) or presence (f) of the (211) peak, which can be used as a diagnostic for determining crystallographic orientation. The peaks at $q = 0.02$ ((b), 5 and 7 half-cycles) and $q = 0.023$ ((f), 7 and 10 half-cycles) are from diffuse scattering and are not attributed to the particle arrangement. The crystal grain sizes increase with deposition cycles, both in the plane of the substrate (c,g) and out of the plane (d,h), for (100)- and (110)-textured superlattices, respectively. The gray bars in (c,g) show average domain size, while the colored markers show various structures deconvoluted from the GISAXS line cuts ("ML" denotes the presence of a monolayer). Error bars are determined from the standard deviation of at least three separate measurements, and in many cases are smaller than the marker size. The dashed lines in (d,h) show the expected film thickness if a full layer were deposited per half-cycle.

particle layers are added, as expected from standard thin-film crystal growth models (Figure 3c,d, Figure S8).^[34]

The same step-by-step analysis for bifunctionalized substrates with a 50B:50A substrate linker ratio was used to elucidate the difference in growth mechanisms on substrates that could bind both particles (Figure 3e–h). We observed a sub-monolayer of particles after a single half-deposition-cycle with interparticle distances that were too large to be observed by GISAXS (> 150 nm). This low particle density is attributed to the two-fold decrease in substrate-bound DNA linkers, which makes particle–substrate interactions less favorable. The decrease in attractive interaction is also observed by monitoring the thermal desorption transition, which broadens and shifts to lower temperatures compared to the desorption of SNA-AuNPs from monofunctionalized DNA substrates (Figure S2). In the context of superlattice growth, this sub-monolayer acts as a seed layer, such that after

two half-cycles, only two-dimensional aggregation occurs with no increase in film thickness. The structure is consistent with the (110) plane of a bcc crystal, and the characteristic first-order scattering peak at $q = 0.024 \text{ \AA}^{-1}$ was observed through the first four half-cycles (Figure 3e,f). The presence of this monolayer after several deposition cycles indicates island formation, which is often observed when substrate–particle interactions are weaker than particle–particle interactions (Figures S17, S18). Three-dimensional ordering with bcc symmetry (q_0 at $0.028 \text{ \AA}^{-1}$) was first observed with as little as two crystal layers, which here occurred after three half-cycles. As before, subsequent growth resulted in a linear increase in film thickness with deposition cycles and crystallite in-plane growth (Figure 3g,h, S11).

We have demonstrated that DNA-mediated crystallization can be used to provide a simplified model for understanding crystal growth in which adlayers display direct

bonding interactions with a substrate but lack the periodic potential inherent to atomic systems. The interfacial energy between a thin-film superlattice and the substrate, and consequently the orientation, can be controlled by appropriate choice of DNA interconnects. This work creates a new design rule for these structures: the orientations of such programmable crystalline thin-films will be dictated by the crystal planes that maximize complementary interactions with the substrate. This strategy could easily be applied to other binary crystal symmetries, or to surfaces patterned with DNA for lithographically templating nanoparticle superlattices. Furthermore, the ideas set forth in this work suggest a route for growing single-crystal nanoparticle superlattices by controlling epitaxial processes. We have also shown that the number of layers in SNA-NP superlattice thin films can be controlled, which is useful for determining thickness-dependent material properties. The additional level of control extended to the system through direct substrate–adlayer bonding interactions will be important for the development of materials that take advantage of the periodicity of the inorganic core material, such as optical metamaterials, photonic bandgap materials, and magnetic storage media.

Experimental Section

Superlattice growth. DNA sequences and detailed procedures can be found in the Supporting Information. Nanoparticles (10 nm diameter AuNPs) were functionalized with DNA according to literature procedures.^[10] Au-coated silicon wafers (8 nm Au, 2 nm Cr adhesion layer) were diced into 7.5 × 15 mm chips and functionalized overnight at various molar ratios of B:A DNA from a 2 mM total concentration of thiolated DNA in 1M phosphate buffer saline (PBS, 10 mM phosphate, 1M NaCl). After washing 3 × in 0.5M PBS, linker DNA (0.5 μM total concentration) was hybridized to the DNA substrates at the same molar ratio as the thiolated sequences in 0.5M PBS by slowly cooling from 75 °C to room temperature over 2 h. SNA-AuNP films were grown by sequential immersion in B- and A-type SNA-AuNPs in 0.5M PBS for 1–1.5 h per half-cycle at various temperatures. After each half-cycle, unbound SNA-AuNPs were carefully removed in 0.5M PBS (5 ×).

X-ray scattering. All GISAXS experiments were conducted at the 12ID-B station at the Advanced Photon Source (APS) at Argonne National Laboratory using collimated 12 keV (1.033 Å) X-rays. See Supporting Information for further data interpretation, indexing and modeling.

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