Supporting Information for “Reconstitutable Nanoparticle Superlattices”

I. Experimental Section:

The DNA sequences used in this study (outlined in Table S1) were synthesized on a BioAutomation MerMade 48 (MM48) automated oligonucleotide synthesizer using standard phosphoramidite chemistry on solid-state supports. After synthesis, all sequences were purified by reverse-phase high-performance liquid chromatography and characterized by matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

Spherical gold nanoparticles (diameter ~9.7 nm) were commercially bought from British Biocell International through TedPella. Unless otherwise specified, all other reagents used were purchased from Sigma Aldrich. The functionalization of the nanoparticles with thiolated DNA was performed following literature precedent. Briefly, the protecting group of the thiolated DNA strand was cleaved with 0.1 M dithiothreitol in 170 mM sodium phosphate buffer (pH 7.4) for one hour, followed by desalting on a NAP5 size exclusion column (GE Life Sciences). Freshly cleaved thiolated DNA was then added to nanoparticles in a ratio of 2 OD₂₆₀ per milliliter of as-received nanoparticles. After allowing the thiolated DNA to attach to the nanoparticle surface for one hour, NaCl, sodium dodecyl sulfate (SDS), and sodium phosphate buffer (pH = 7.4) were added stepwise over several hours until final concentrations of 0.5 M, 0.01%, and 10 mM, respectively, while sonicating the nanoparticle solution for 10 seconds after each salt addition. After the final concentrations are reached, the nanoparticle solution was allowed to sit overnight. To remove the excess DNA from solution, the nanoparticles were centrifuged three times, the supernatant removed and replaced with 0.01% SDS. The final solution was again brought to 0.5 M NaCl and 10 mM sodium phosphate buffer. To hybridize linker strands to DNA-functionalized nanoparticles, the nanoparticle solution was first brought to 0.5 μ M. Then, linkers were annealed by slowly cooling through the melting transition from 75 °C to 45 °C, followed by heating at 45 °C for one hour.

Triangular prisms were synthesized according to a protocol reported by Jones, et al.¹ Prisms were separated from the spherical nanoparticle impurities through a previously reported depletion force method, in which the addition of salt induces the selective assembly and subsequent sedimentation of triangular prisms.² DNA-functionalization of prisms was performed according to Jones, et al.³

The substrates used for the GISAXS experiments were Si with native oxide (Silicon Quest International). For circular dichroism and melt experiments, quartz substrates (Ted Pella) were used. Prior to use, all substrates were rinsed with solvent (acetone and isopropyl alcohol), dried with nitrogen, and cleaned with an oxygen plasma at 300 mtorr and 30 mW for 15 minutes. On these substrates, a thin film of gold (~8 nm) was deposited on top of a 2 nm chromium adhesion layer by PVD 75 E-beam evaporator (Kurt J. Lesker) at a base pressure of 3.5×10^{-7} torr and an evaporation rate of 0.2 Å/s. The gold layer thus deposited was smooth enough for GISAXS measurements and sufficiently transparent to enable optical measurements. The substrates were diced into 0.6 x 2 cm pieces and functionalized with 1 μ M thiolated DNA (after DTT treatment, as described above) in 1 M NaCl and 5 mM phosphate buffer. After allowing the substrates to functionalized overnight at room temperature, they were washed three times with 0.01% SDS, followed by resuspension in 0.5 M NaCl, 0.01% SDS, and 0.01 M sodium phosphate buffer. Linkers were hybridized to the substrate in the same manner as described above for the nanoparticles.

Nanoparticle monolayers were assembled by submersing linker-hybridized, DNA-functionalized substrates into linker-hybridized, DNA-functionalized nanoparticle solutions at 40 °C for one hour, while shaking at 1000 RPM. The concentration of the nanoparticles during this assembly process was 5 nM and 100 pM for spheres and prisms, respectively. For the medium and long DNA sequences (Table S1), the assembly was performed at 35 °C. {100}-oriented BCC superlattices were assembled according to a protocol reported by Senesi, et *al.*,⁴ by sequential dipping of the DNA/substrate into nanoparticle solutions with complementary linkers and incubating at the melting temperature ($43 \pm 0.5^\circ\text{C}$) for three hours. Free-standing solution-assembled superlattices were assembled via one of two methods: annealing just below the melting temperature⁵ or slow-cooling from above the melting temperature to room temperature (to produce single crystalline superlattices).⁶ Monolayer and superlattice dehydration was performed at room temperature through slow evaporation under ambient conditions. To rehydrate, the samples were immersed in 0.5 M PBS buffer.

Desorption analysis was performed on a Cary 5000 UV-Vis spectrophotometer by monitoring the localized surface plasmon resonance (835 nm for prisms) from 25 to 65 °C, in 0.5 °C increments. For this analysis, nanoparticles assembled onto quartz substrates were placed in the path of the beam. Circular dichroism was performed on a JASCO J-815 CD spectrometer at 20°C over the wavelength range of 300-200 nm with an interval of 1 nm and scan speed of 100 nm/min.

GISAXS experiments were performed at the 12ID-B station at the Advanced Photon Source at Argonne National Laboratory. The synchrotron X-ray energy was 14 keV (0.8856 Å). The scattering angle and sample-detector distance was calibrated using silver behenate as a standard. Two sets of slits were used to define and collimate the X-ray beam and a pinhole was used to remove parasitic scattering. The beam cross-section was 200 x 9 µm. The scattered radiation was detected with a Pilatus2M detector. Sample alignment was performed using a motorized stage by moving in x, y, and z- directions in addition to α_i (rotation along y-axis). Compared to gold nanoparticles, scattering of the buffer and DNA were negligible.

Table S1. DNA sequences for Monolayer Work.

Description	DNA Sequence (5' to 3')
Thiolated DNA on substrate	TCA ACT ATT CCT ACC TAC AAA AAA AAA A SH
Thiolated DNA on particle	TCC ACT CAT ACT CAG CAA AAA AAA AAA A SH
Linker on surface	GTA GGT AGG AAT AGT TGA ATC TCT
Linker on particle	TTG CTG AGT ATG AGT GGA AAG AGA
Spacer sequence	TTT TTT TTT TTT ACT GAG CAG CAC TGA TTT TTT TTT TTT T
Duplex sequence	AAA AAA AAA AAA ATC AGT GCT GCT CAG TAA AAA AAA AAA A

DNA sequences for the layer-by-layer assembly were the same as used by Senesi, et *al.*⁴ and for the solution-phase assembly were the same as used by Auyeung, et *al.*⁶

To probe the effect of DNA length on rehydration, a spacer region was added to zero, one, or both of the linkers, in between the sticky end and the sequence anchored to the nanoparticle (Figure 1 in the manuscript). Specifically, the spacers were changed in 40 base pair increments, from the initial state with no spacers, to an intermediate length with one 40 base-pair spacer on the prisms, to a maximum length with one 40 base pair sequence on both the prisms and substrate. After dehydrating and

rehydrating prisms assembled with these three DNAs, we find that rehydration recovers the initial state for all three DNA lengths.

Table S2. Details of the DNA linker systems used in the present study.

Spacer region	Single stranded bases	Duplexed bases	Predicted length of DNA (nm)
No on Surface No on Particle	22	41	21.44
No on Surface Yes on Particle	22	81	35.04
Yes on Surface Yes on Particle	22	121	48.64

II. DWBA theory for GISAXS:

The details of the distorted wave Born approximation (DWBA) theory of GISAXS can be found in the literature^{4, 7, 8}. Briefly, the scattering intensities (Fig. S1) were calculated by following DWBA based GISAXS formulae:

$$I = \left| e^{ik_{||}r_{||}} (T_i e^{ik_{i,z}r_z} + R_i e^{-ik_{i,z}r_z}) (T_f e^{-ik_{f,z}r_z} + R_f e^{ik_{f,z}r_z}) \right|^2 \quad (1)$$

where $\mathbf{k} = (\mathbf{k}_{||}, k_z)$ is the wave vector of x-ray beam, and the subscripts i and f on the z -component denote the incident and out-going beams, respectively. $\mathbf{r} = (\mathbf{r}_{||}, r_z)$ denotes the position of either Au prism or sphere (for Fig. S1, $\mathbf{r}_{||} = 0$). The wave amplitudes T and R were calculated for the z direction electron density profile including the layers of Au nanoparticles. The essence of the DWBA is to calculate the amplitudes of x-ray wave fields T and R separately for an ideal layered system and include them in scattering calculations.⁷⁻¹⁰ In the wave field calculation, particles on the substrate were considered as a monolayer whose electron density is uniform in the layer. The specular scattering occurring around the reflected beam was neglected.

The distance between the monolayer of Au nanoparticles (regardless of shape) and the substrate surface is calculated by fitting the vertical interference (Kiessig) fringe, shown in Figure S1 and S2, to equation (1). The frequency is inversely proportional to the distance r_z . In other words, longer distances between the nanoparticle and substrate result in finer oscillations. For further information please see references 4-10.

Number of Spacers	Hydrated (nm)	Dehydrated (nm)	Rehydrated (nm)
0	19.6±1.5	4.6±0.3	19.8±1.8
1	34.6±0.9	6.6±1.3	35.1±2.7
2	46.1±0.9	9.9±0.4	45.1±4.6

Table S3. Measured gap lengths for the three DNA linker systems using DWBA simulation. It is to be noted that we noticed batch to batch variation using the shortest DNA sequence.

Figure S1. GISAXS vertical linecuts from the scattering patterns of monolayers of prisms immobilized with linkers with either (A) zero, (B) one, or (C) two spacers at various incident angles (symbols). Different colored symbols in the graphs correspond to different incident angles (shown in the inset). The predicted linecuts from calculated scattering pattern by DWBA are also overlaid (solid line). The missing data in the experimental linecuts is due to the beam stop. The peak minima and maxima, indicative of the distribution in heights of prism monolayers seem to match well with the experimental data at 20-45 % height distribution.

Figure S2. Fitting of vertical cuts from data shown in Figure S11A and B. The gap distances (distance between the substrate surface to the surface of particles) for the monolayer and bilayer are ca 1.9nm and 5.5nm, respectively.

III. Melting analysis of prism monolayers:

Prism monolayers (in both hydrated and rehydrated states) were assembled on a semi-transparent quartz/Au substrate and heated from room temperature to above the DNA melting temperature while monitoring the prism extinction at 835 nm.

Figure S3. Thermal desorption (or DNA dehybridization) behavior of nanoprisms with (A) short, (B) medium, and (C) long DNA gap lengths. Extinction at the localized surface plasmon resonance of the prisms (835 nm) was monitored as a function of temperature. The melting temperatures were derived from the first derivatives of the above curves and the values are tabulated below.

Table S4. Melting temperatures of the monolayers of prisms immobilized on quartz substrate with linkers of varied lengths, as-made (hydrated) and after rehydration.

DNA linker system	Hydrated		Rehydrated	
	Melting temperature (°C)	1 st derivative FWHM (°C)	Melting temperature (°C)	1 st derivative FWHM (°C)
Short	54.0 ± 0.1	5.1 ± 0.8	54.0 ± 0.1	4.8 ± 0.4
Medium	46.8 ± 0.2	8.0 ± 1.5	46.2 ± 0.3	5.9 ± 1.3
Long	37.9 ± 0.1	8.5 ± 0.8	38.4 ± 0.3	7.3 ± 1.2

IV. In-situ rehydration of prism monolayers:

Figure S4. In-situ rehydration of prism monolayers with the long DNA system, monitored by GISAXS. 2D-maps of GISAXS vertical linecuts along the interference peaks were plotted to determine the change in the gap length over time. Upon buffer addition, the gap length increases from its dehydrated (collapsed) to fully rehydrated (extended) state in less than 20 ms, (the resolution limit of our setup). After the initial height increase, the scattering pattern does not change even after 30 minutes, demonstrating that the reorganization of DNA to its fully extended form happens in the early onset.

V. GISAXS Analysis of the sphere monolayers:

Figure S5. IAR-GISAXS of the sphere monolayers (diameter, 9.7 nm) with the short DNA system. The gap length returns to the same value after rehydration as can be noticed from the similar frequency of the Kiessig fringes in the hydrated and rehydrated states. From DWBA simulation, the gap length (with 41 duplexed base-pair DNA sequence) was observed to change from 17.9 ± 0.7 nm in (A) hydrated state to 1.9 ± 0.7 nm in (B) dehydrated state and back to 17.4 ± 0.5 nm after (C) rehydration.

Figure S6. Monolayer of spherical nanoparticles (diameter, 9.7 nm) in hydrated state: (A) structure factor obtained from GISAXS. The d-spacing, i.e., the distance between the nearest scattering planes (of Au atoms) for the monolayer was 20.7 nm. (B) Pair distribution function calculated from GISAXS structure factor indicates the nearest neighbor distance to be 25.3 nm. The ratio of these values, $25.3/20.7 \sim 2/\sqrt{3}$, implies that the particles are arranged with 2D hexagonal symmetry in the plane.

Figure S7. Pair distribution analysis i.e., the distribution of distances between pairs of particles in the nanoparticle monolayer, in dehydrated state. From the (A) SEM image, nearest neighbor distance is measured to be 24 nm by (B) pair distribution function. (C) The d-spacing, from the calculated scattering curve is about 20.7 nm. The ratio between d-spacing and the nearest neighbor distance indicates 2D hexagonal symmetry. Therefore the dehydrated state still possesses the symmetry although disorder has increased.

Figure S8. Comparison of pair distribution functions of the spherical nanoparticle monolayer in hydrated versus dehydrated state. The curves are normalized along y-axis for comparison.

VI. Analysis of two-layer samples:

Figure S9. Pair distribution analysis on the nanoparticle bilayer in dehydrated state. The measured curves in bottom two panels are from GISAXS data. The rest of the curves are calculated from the SEM image.

VII. Indexing of BCC Structure for three-layer samples:

Figure S10. (A) Locations of 110 diffraction peaks for (100) (green closed circles) and (110) orientations (blue open squares). Indexing of three-layer sample in buffer. (B) (110) orientation with lattice parameter of 45.8 nm. (C) (100) orientation with lattice parameters $a=45.8$ nm and $c=43.6$ nm.

In the case of the two-layer and three-layer samples, the GISAXS image in the original hydrated state displays a mixture of both (100) and (110) orientated domains. For the two-layer sample, each red particle is connected to four blue particles. The in-plane interparticle spacing, i.e., center-to-center distance between blue and red particle changes from 25.9 nm to 18.4 after dehydration and back to 25.5 after rehydration and annealing (Table S5). In the dehydrated state, hexagonal ordering is observed (Figure S9).

VIII. Comparison of layer-by-layer samples:

Figure S11. GISAXS scattering patterns of (A) monolayer, (B) two-layer, (C) three-layer and (D) ten-layer BCC structure in hydrated, dehydrated, and rehydrated states.

Figure S12. Large area SEM images of the nanoparticle superlattices.

Table S5. In-plane gap lengths, i.e., surface to surface distance between the nanoparticles in the layer-by-layer assembled nanoparticle superlattices.

# of Layers	Hydrated (nm)	Dehydrated (nm)	Rehydrated (nm)	Annealed (nm)
2	25.9	18.4	22.6	25.5
3	25.5	19.2	23.9	24.6
10	23.9	14.5	24.3	24.7

Figure S13. Two-dimensional GISAXS scattering patterns for 10-layer samples in the hydrated (A, B) and dehydrated (C, D) states. The scattering patterns have been indexed to a BCC lattice (A ($a = 44.8$ nm), C ($a = 34.1$ nm)) or a body centered tetragonal (BCT) lattice (B ($a = b = 44.8$ nm, $c = 37.7$ nm), D ($a = b = 34.1$, $c = 28.7$ nm)), with peaks from the direct beam in red and the reflected beam in blue. Note that the peak positions line up correctly for BCC hydrated and BCT dehydrated indexing.

IX. Annealing of surface-assembled BCC lattices after rehydration:

Upon rehydration, superlattices regained the initial BCC structure with an observed lattice parameter of 45.3 nm, however the quality of the ordering was slightly lost, with more randomly oriented, amorphous structure. To fully recover the initial ordering, the superlattices were annealed for 30 min at 40 °C.

Figure S14. (A) Circular cut along the GISAXS pattern of 10-layer BCC superlattice. In the magnified view shown in (B), it can be observed that the peak width indicative of domain size, increases after rehydration, and decreases after annealing. The scattering background, indicative of the disordered domains in the sample is also reduced after annealing.

X. Solution assembled BCC superlattices:

Figure S15. (A) Scattering patterns and (B) 1D-linecuts of free-standing nanoparticle superlattices assembled in solution. In the SEM images of the dehydrated BCC lattices, the lattices look wavy due to dried salt crystals underneath, but the local order is well preserved.

Figure S16. Additional SEM images of dehydrated single crystals.

References:

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