Supporting Information for:

**Contraction and Expansion of Stimuli-
Responsive DNA Bonds in Flexible Colloidal
Crystals**

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J. Am. Chem. Soc.

Nanoparticle Synthesis and Characterization. Gold spherical nanoparticles capped with citrate ligands were purchased from Ted Pella, Inc. with nominal diameters of 15 and 30 nm. Two techniques were used to determine the actual size distribution of the nanoparticles (Table S1). First, algorithmic analysis of transmission electron microscopy (TEM) images was performed for > 2000 particles from each sample. This data returns an average diameter and standard deviation for nanoparticles, assuming a spherical nanoparticle shape.¹ Second, small angle X-ray scattering (SAXS) experiments were used to measure an experimental form factor for discrete nanoparticles. Measured form factors were then fit with simulated form factors for spherical particles using the following parameters: diameter and diameter dispersity.² In all cases, the simulated form factors demonstrate excellent agreement with the experimental form factors (Figures S4, S5). Note that the diameters determined by TEM and SAXS are in very good agreement, and the average values from SAXS were used to calculate all interparticle gaps reported in this work.

Ted Pella Gold Colloid	Average diameter (TEM)	Average diameter (SAXS)
15 nm	14.3 nm $\pm$ 1.4 nm	13.9 $\pm$ 1.3
30 nm	30.3 nm $\pm$ 4.5 nm	29.4 $\pm$ 2.9

Table S1. Size distribution of nanoparticles used in this work.

DNA Synthesis and Characterization. All oligonucleotides used in this work were synthesized on a solid-support MM48 synthesizer (BioAutomation) using reagents purchased from Glen Research. Oligonucleotides were synthesized with a 5' trityl group and purified with reverse-phase high-performance liquid chromatography (HPLC; Agilent), followed by standard deprotection procedures. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry was used to confirm the molecular weight of the HPLC-purified oligonucleotides. The OligoAnalyzer tool from Integrated DNA Technologies was used to determine an extinction coefficient for each DNA strand, and UV-vis spectroscopy was used to determine concentrations.

DNA Design. The DNA design in this work follows previous literature protocols.^{3,4} Briefly, gold nanoparticles were densely functionalized with one of two unique single-stranded oligonucleotides composed of three regions (from 3' to 5'): 1) 3' alkylthiol moiety ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{SH}$) to anchor the DNA to the nanoparticle surface, 2) an A_{10} spacer region to distance the DNA from the surface and provide some degree of flexibility, and 3) an 18-base pair sequence. To each of these “anchor” strands, we hybridized a second “linker” strand composed of three regions (from 5' to 3'): 1) an 18-base pair sequence complementary to the anchor strand, 2) a single unpaired A base for DNA flexibility, and 3) a 5-base terminus, or “sticky end”, designed such that the terminus on nanoparticle “A” is complementary to the terminus on nanoparticle “B”. Specific sequences used in this work are listed in Table S2 and Figure S1.

DNA Description	DNA Sequence (5' to 3')
“A” Anchor	TCA ACT ATT CCT ACC TAC AAA AAA AAA A $(\text{CH}_2)_3\text{SH}$
“A” Linker	GTA GGT AGG AAT AGT TGA ATC TCT
“B” Anchor	TCC ACT CAT ACT CAG CAA AAA AAA AAA A $(\text{CH}_2)_3\text{SH}$
“B” Linker	TTG CTG AGT ATG AGT GGA AAG AGA

Table S2. DNA sequences used in this work.

Nanoparticle A

3'-S-(CH₂)₃-A AAA AAA AAA CAT CCA TCC TTA TCA ACT **AGAGA** A AGG TGA GTA TGA GTC GTT
GTA GGT AGG AAT AGT TGA A **TCTCT** TCC ACT CAT ACT CAG CAA AAA AAA AAA A-(CH₂)₃-S-3'

Nanoparticle B

Figure S1. Schematic of DNA sequences used in this work. The complementary 5-base sticky ends for nanoparticles A and B are highlighted in blue and red, respectively.

Nanoparticle Functionalization with DNA. Nanoparticles were functionalized with 3' thiolated oligonucleotides as previously reported.⁵ Briefly, thiolated oligonucleotides were treated with a 100 mM solution of dithiothreitol (DTT) in 170 mM sodium phosphate buffer (pH = 7.4) for 1 h. Residual DTT was removed using Nap-5 size exclusion columns (GE Healthcare), and the DNA was added directly to the spherical nanoparticle colloids (5 nmol and 8 nmol of DNA were added per mL of 15 nm and 30 nm nanoparticle solution, respectively). After 12 h, each nanoparticle solution was brought to 0.01 M sodium phosphate buffer (pH = 7.4) and 0.01 wt % sodium dodecyl sulfate (SDS) in water, briefly sonicated, and then placed on a shaker for 2 h. After this step, 2 M NaCl (aq) was added to the nanoparticle solutions every half hour such that the final concentration of each solution was 0.05 M, 0.1 M, 0.2 M, 0.3 M, 0.4 M, and 0.5 M NaCl after each successive addition. After each addition of 2 M NaCl (aq), the nanoparticle solution was briefly sonicated. Following the last NaCl addition, the nanoparticles were placed on a shaker at 1000 rpm for 12 h to ensure a dense loading of oligonucleotides. After this time, each nanoparticle solution was centrifuged three times to remove excess DNA, with the supernatant removed each time and replaced with 0.5 M NaCl, 0.01 M sodium phosphate buffer (pH = 7.4), and 0.01 wt % SDS. Centrifugation conditions used in this process were 25 min at 21,130 and 9,400 rcf for 15 nm and 30 nm particles, respectively.

Linker DNA Hybridization. Linker strands were hybridized to nanoparticles by adding 500 and 5000 equivalents per 15 nm and 30 nm particle, respectively.

Superlattice Assembly. A stock solution was prepared by combining concentrated solutions of DNA-functionalized nanoparticles and DNA linker strands A and B (at the above ratios) in 0.5 M NaCl, 0.01 M sodium phosphate buffer (pH = 7.4), and 0.01 wt % SDS such that the final concentration of each particle type (A and B) was 12 nM (15 nm particles) or 3 nM (30 nm particles). Aggregates formed within 5 minutes. The solution was then heated > 50 °C to dissociate the aggregates. While maintaining a temperature > 50 °C, this solution was transferred in 100 µL aliquots to 200 µL PCR 8-tube strips (Life Technologies). The solutions were then slowly cooled in a Life Technologies PCR Thermocycler from 55 °C to 20 °C at a rate of 0.01 °C/min.⁶

Preparation of Superlattice Samples in Ethanol. After assembly, the as-synthesized superlattices were combined in a 1.5-mL Eppendorf tube and washed repeatedly with an aqueous solution of 0.3 M NaCl. Aliquots of superlattices (50 µL) were then added to a series of 0.5-mL Eppendorf tubes. A stock solution of 0.3 M NaCl in 80% EtOH and 20% H₂O was then added incrementally to bring each sample sequentially to 10%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, and 70% EtOH. A sample in 80% EtOH was prepared from a superlattice suspended in 70% EtOH by replacing the supernatant with 50 µL of the 80% EtOH stock solution. After reaching 80% EtOH, a stock solution of 0.3 M NaCl in H₂O was then added incrementally to bring a series of samples sequentially to 60%, 50%, 40%, 30%, 20%, and 10% EtOH. A sample in 0%

EtOH was then prepared from a superlattice suspended in 10% EtOH by replacing the supernatant with 50 μL of 0.3 M NaCl in H_2O . Note that, as expected, experiments performed at different concentrations of superlattice crystals showed similar results for a given volume percentage of EtOH in H_2O , and the superlattice concentrations were thus not held constant during these experiments.

Silica Encapsulations of Superlattices. For all 0% EtOH samples, silica embedding and electron microscopy (EM) sample preparation was performed as previously reported.⁷ Briefly, four 100 μL aliquots of superlattice samples were combined from PCR 8-tube strips after slow cooling. The samples were then brought to 0.3 M NaCl, 0.01 M sodium phosphate buffer (pH = 7.4), and 0.01 wt % SDS in 1 mL total volume. 1.5 μL of N-trimethoxysilylpropyl-N,N,N-trimethylammonium chloride (50% in methanol, Gelest, Inc.) was then added to the solution and placed on a shaker at 1000 rpm for 10 min. After 10 min, 4 μL of triethoxysilane (Sigma-Aldrich) was added, and the solution was returned to the shaker for 12 h. After 12 h, the solution was washed by briefly centrifuging (~ 10 sec), removing the supernatant and resuspending in ~ 1 mL nanopure water. This process was repeated 1-2 more times to remove excess silica. 9 μL of solution was drop cast on a copper TEM grid (Ted Pella) and wicked away using Qualitative 413 filter paper (VWR). This was repeated 3-5 times. The samples were imaged within 1 week of preparation.

Silica embedding and EM sample preparation of 41%, 45%, and 80% EtOH samples were performed similar to the above procedure with some changes. After combining four 100 μL aliquots of solutions containing slow cooled superlattices, the solution was brought to the desired percent EtOH using a stock of 80% EtOH and 0.3 M NaCl in a volume of 1 mL total. For 80% EtOH samples this required brief centrifugation (~ 10 seconds) and removal of supernatant. As above, 1.5 μL of N-trimethoxysilylpropyl-N,N,N-trimethylammonium chloride (50% in methanol, Gelest, Inc.) was then added. The solution was placed on a shaker at 1000 rpm for 10 min. After 10 min, 4 μL of triethoxysilane (Sigma-Aldrich) was added and the solution was returned to the shaker for 12 h. Unlike 0% EtOH samples, samples containing EtOH were not washed. 9 μL of solution was directly drop cast on a copper TEM grid (Ted Pella) and wicked away using Qualitative 413 filter paper (VWR). This was repeated 2-3 times. The samples were imaged within 1 week of preparation.

Electron microscopy (EM) images were obtained at the Northwestern University Atomic and Nanoscale Characterization Experimental Center (NUANCE) on a Hitachi HD2300 Scanning Transmission Electron Microscope (STEM) in z-contrast and SE mode at an accelerating voltage of 200 kV.

Small Angle X-Ray Scattering (SAXS) Experiments. Synchrotron small angle X-ray scattering (SAXS) experiments were conducted at the Dupont-Northwestern Dow Collaborative Access Team (DND-CAT) beamline of the Advanced Photon Source at Argonne National Laboratory. The X-ray wavelength for all experiments was 1.24 $\text{\AA}$ (10 keV). The sample angle was calibrated with a silver behenate standard. Two sets of slits were used to define, and collimate the beam, and parasitic scattering was removed with a pinhole. The X-ray beam cross-section measured 200 μm , and exposure times varied from 0.1 to 0.5 seconds. Scattered radiation was detected with a CCD area detector, and dark current frames were subtracted from all data.

1D SAXS data was obtained by an azimuthal average of the 2D scattering patterns. All SAXS data is presented as scattering intensity, $I(q)$, as a function of the scattering vector, q :

$$q = 4\pi \sin(\theta)/\lambda$$

where q is half of the scattering angle 2θ and λ is the wavelength of X-ray radiation. Scattering from the solution, capillary, and DNA were assumed to be negligible.

Note that all SAXS data presented in this work are a combination X-ray scattering from the discrete nanoparticles described as a form factor, $P(q)$, and scattering from nanoparticle ordering described as the structure factor, $S(q)$. Together these compose the overall scattering for a given nanoparticle superlattice sample, $I(q)$, where $I(q) = P(q) \times S(q)$. Due to the orders of magnitude difference in the scattering associated with the gold nanoparticles relative to the DNA, one cannot draw conclusions about DNA structure using this technique.

Experimental form factors and structure factors were simulated to reconstruct $I(q)$ for each sample (Figures S4, S5).² As discussed in the main text, there is an increase in SAXS peak widths during the EtOH-induced transition of superlattices composed of both 30 nm and 15 nm PAEs. Peak broadening is typically indicative of an increase in lattice strain or decrease in crystallite size. Since the SAXS peak widths return to their original values upon superlattice re-expansion, the peak broadening is likely the result of increased lattice strain, rather than a decrease in crystallite size, during superlattice contraction. As a result, the primary SAXS simulation parameters changed when simulating diffraction patterns from 0 to 80% EtOH were: 1) unit cell dimension and 2) crystal microstrain. The unit cell dimensions and space group assignment ($Im\bar{3}m$ for all samples) were confirmed by comparing the positions of predicted and experimental diffraction peaks for each sample.

In the superlattice samples composed of 15 nm PAEs, we note that there appears to be some SAXS peak splitting that is particularly evident in the region of the EtOH-induced transition (Figures S29-S32). Although it is difficult to identify its exact origins, this peak splitting can likely be attributed to two different populations of superlattice crystals – perhaps a population of larger, micron-sized single crystals and a population of sub-micron crystallites – going through the transition at slightly different EtOH percentages.

Kinetics of Phase Transition. Although it was not possible to directly measure the kinetics of the EtOH-induced transition *in situ* given our experimental setup, the speed of the transition was probed by soaking a fully expanded superlattice in 70% EtOH and collecting a SAXS pattern as rapidly as possible. The superlattice had fully contracted by the 3 minutes required to prepare a sample and collect SAXS data, demonstrating that the transition occurs faster than 3 minutes (Figure S43).

	15 nm PAE superlattice (BCC)			30 nm PAE superlattice (BCC)		
% EtOH in H ₂ O (by volume)	<i>a</i> (nm)	<i>V</i> (nm ³)	DNA bond length (nm)	<i>a</i> (nm)	<i>V</i> (nm ³)	DNA bond length (nm)
0	36.1	47,000	17.4	52.5	144,000	16.1
10	35.9	46,300	17.2	51.8	139,000	15.5
20	35.4	44,400	16.8	51	133,000	14.8
30	34.8	42,100	16.2	50.2	127,000	14.1
40	33.1	36,300	14.8	46.5	101,000	10.9
45	30.4	28,000	12.4	41.9	73,600	6.9
50	27.3	20,300	9.7	40.6	66,900	5.8
55	25.9	17,400	8.5	40.0	64,000	5.2
60	25.5	16,600	8.2	39.0	59,300	4.4
70	24.1	14,000	7.0	38.1	55,300	3.8
80	22.9	12,000	5.9	37.2	51,500	2.8
60 (re-expansion)	25.4	16,400	8.1	39.4	61,200	4.7
50 (re-expansion)	27.8	21,500	10.2	41.0	68,900	6.1
40 (re-expansion)	33.5	37,600	15.1	47.7	109,000	11.9
30 (re-expansion)	34.9	42,500	16.3	50.0	125,000	13.9
20 (re-expansion)	35.3	44,000	16.7	50.6	130,000	14.4
10 (re-expansion)	35.9	46,300	17.2	51.3	135,000	15.0
0 (re-expansion)	36.3	47,800	17.5	52.0	141,000	15.6

Table S3. Summary of unit cell parameters and nanoparticle gap distances as determined from *in situ* SAXS experiments.

UV-Vis Melting Experiments. Superlattices of 30 nm spherical nanoparticles were suspended in solutions containing 0 to 80% of EtOH in H₂O and 0.3 M NaCl. For melting experiments, 1.5 mL of each sample was loaded in quartz cuvettes with a small cavity at the bottom for a magnetic stir bar and placed in a Varian Cary 5000 UV-vis spectrophotometer. Samples were continuously stirred throughout the experiment to facilitate thermal diffusion and to ensure that assemblies remained suspended in the beam path. Each sample was capped tightly to prevent solution evaporation over the course of the experiment. Samples were then heated from 25 °C to 70 °C at 0.1 °C/min. Temperature was regulated from within the Varian software with an external temperature controller, which drives a Peltier heat pump attached to a six-cell holder. Thermocouples in the sample holder are used to provide feedback to regulate the sample temperature. Extinction was monitored at the nanoparticle LSPR. The melting transition temperature is calculated as the point of inflection on the extinction vs. temperature curve.

Addition of EtOH to DNA-Functionalized Nanoparticles. To investigate the effect of EtOH on single-stranded DNA-functionalized nanoparticles dispersed in solution, rather than bound within a superlattice, 30 nm Au spheres were functionalized with single-stranded DNA (see “B” anchor strand in Table S2 for the specific sequence used) and suspended in H₂O at 0.3 M NaCl. A solution of 80% EtOH and 20% H₂O at 0.3 M NaCl was then added to bring the suspended nanoparticles to 0, 30, 35, 40, 45, 50, and 60% EtOH by volume. UV-vis extinction spectra were measured at each EtOH percentage (Figure S53). Up to 30% EtOH, there are no changes to nanoparticle localized surface plasmon resonance (LSPR). However, at higher EtOH

percentages, the LSPR broadens and shifts to higher wavelengths (Figure S55). Additionally, the color of the nanoparticle solution changes from pink to clear (Figure S54). Both of these phenomena are consistent with agglomeration, or precipitation, of the DNA-functionalized nanoparticles at higher EtOH percentages. While it is difficult to define precisely, nanoparticle agglomeration appears to begin at ~30-40% EtOH, which is slightly lower than the percentage of EtOH (40-45%) observed to induce a phase transition in the nanoparticle superlattices (Figure 2B). Note that the LSPR does not shift or broaden when citrate-capped, rather than DNA-functionalized, Au nanoparticles are suspended in 60% EtOH (Figure S54)

Molecular Dynamics (MD) Simulations.

System setup and equilibration. The initial A-DNA structure, which served as the starting point for all MD simulations, was generated using the LEAP module of AMBER 14.⁸ The two 3' DNA strands (see Table S2 for sequences) were attached to two flat Au(111) slabs with a dimension of 29 Å X 30 Å X 5 Å. A hole of 7 Å X 7 Å X 5 Å was made on one slab to enable the solvent to move freely between the two sides of the Au slab. Each system was charge-neutralized with Na⁺ ions and solvated with a specific number of water and ethanol molecules in a 29 Å X 30 Å X 245 Å box. Exact numbers of water and ethanol molecules are listed in Table S4. The GAFF and ff14SB force fields,^{9,10} which contain the parmbsc0 backbone modification to the ff99 DNA parameters,¹¹ were used in this study. Gold and ethanol force fields (W-46 project) were adopted from other studies.^{12,13} The partial charges of the alkythiolate linker were calculated using GAMESS with the HF/6-31G* basis set,¹⁴ followed by the RESP fitting procedure.¹⁵ MD simulations were carried out using NAMD.¹⁶ Each system was equilibrated as follows. First, the system was energy minimized in 5000 steps. Then, the system was gradually heated from 100 to 300 K in 200 ps using Langevin dynamics in a constant volume condition, followed by a 200 ps constant volume simulation at 300 K. Next, the system was switched to constant pressure (1 atm) with a damping coefficient of 1 ps⁻¹ at 300 K. Constant surface area at the X-Y plane was turned on so the box volume was only changed along the z axis. A 10.0 kcal/mol Å⁻² Cartesian restraint was applied on the DNA and alkythiolate linker during the previous steps. Lastly, the restraints were gradually decreased from 10.0 kcal/mol/Å² to 1.0 kcal/mol/Å² in a 500 ps run.

Simulations of Na⁺ distribution and A-B DNA transformation. A 100 ns unrestrained production run under 1 atm and 300 K condition was performed for every percentage of ethanol considered. The last 50 ns of the trajectories were taken for radial distribution function (RDF) and A-B conformation analyses (Figures 4B, S3). The same strand C1'-C1' distance (Figure S2B) was used to distinguish the A or B form of DNA.¹⁷ For canonical A and B DNA, the distances are 5.52 Å and 4.95 Å, respectively. Analyses were carried out using cpptarj.¹⁸

Simulations of DNA bend angle. To match the experimental distance between the Au slabs under different percentages of ethanol, a steered MD simulation approach was used. This was done by gradually moving the distance between the two Au slabs from 188 Å to the specific distance listed in Table S3 in 50 ns with a 100 kcal/mol/Å² force constant under 1 atm and at 300 K. Because this distance suddenly decreased as the amount of EtOH exceeds 40% by volume, simulations were only run in the range of 0-40% EtOH. An 150 ns unrestrained production run was performed for each percentage of ethanol considered. The last 50 ns trajectories were taken for the bend angle analyses (Figure S2).

	% EtOH (by volume)						
	0	10	20	30	40	60	80
H ₂ O	5600	5040	4480	3920	3360	2240	1120
EtOH	0	172	345	517	690	1035	1379

Table S4. Number of water and ethanol molecules used in the MD simulations.

Figure S2. (A) Schematic showing the model of two DNA strands between two gold surfaces used in MD simulations. The bend angle, θ , represents the amount of bending at the sticky end overlap region between the two strands. (B) The bend angle is plotted for different volume percentages of EtOH in H₂O calculated using MD simulations at 200 ns.

Figure S3. (A) MD simulations predict that DNA remains in the B-form from 0 to 60% EtOH and transitions to the A-form at 80% EtOH. (B) Definition of C1'-C1' distance.

Figure S4. Experimental (black) and simulated (blue) form factors are plotted for the 30 nm spherical nanoparticles used in this work. The simulated sphere radius was 147 Å with a diameter dispersity of 10%.

Figure S5. Experimental (black) and simulated (blue) form factors are plotted for the 15 nm spherical nanoparticles used in this work. The simulated sphere radius was 69.5 Å with a diameter dispersity of 9.5%.

Figure S6. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 100% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 52.5$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S7. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 10% EtOH, 90% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 51.8$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S8. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 20% EtOH, 80% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 51.0$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S9. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 30% EtOH, 70% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 50.2$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S10. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 40% EtOH, 60% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 46.5$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S11. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 45% EtOH, 55% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 41.9$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S12. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 50% EtOH, 50% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 40.6$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S13. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 55% EtOH, 45% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 40.0$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S14. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 60% EtOH, 40% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 39.0$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S15. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 70% EtOH, 30% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 38.1$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S16. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 37.2$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S17. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 60% EtOH, 40% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 39.4$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S18. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 50% EtOH, 50% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 41.0$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S19. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 40% EtOH, 60% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 47.7$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S20. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 30% EtOH, 70% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 50.0$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S21. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 20% EtOH, 80% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 50.6$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S22. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 10% EtOH, 90% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 51.3$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S23. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 100% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 52.0$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S24. Experimental scattering pattern (black) is shown for a BCC superlattice of 30 nm spherical nanoparticles in 100% H₂O, 0.3 M NaCl after five cycles of equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl followed by 100% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 51.9$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S25. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 100% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 36.1$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S26. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 10% EtOH, 90% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 35.9$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S27. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 20% EtOH, 80% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 35.4$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S28. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 30% EtOH, 70% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 34.8$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S29. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 40% EtOH, 60% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 33.1$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S30. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 45% EtOH, 55% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 30.4$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S31. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 50% EtOH, 50% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 27.3$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S32. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 55% EtOH, 45% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 25.9$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S33. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 60% EtOH, 40% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 25.5$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S34. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 70% EtOH, 30% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 24.1$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S35. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 22.9$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S36. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 60% EtOH, 40% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 25.4$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S37. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 50% EtOH, 50% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 27.8$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S38. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 40% EtOH, 60% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 33.5$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S39. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 30% EtOH, 70% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 34.9$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S40. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 20% EtOH, 80% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 35.3$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S41. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 10% EtOH, 90% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 35.9$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S42. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 100% H₂O, 0.3 M NaCl after equilibrating in 80% EtOH, 20% H₂O, 0.3 M NaCl. A simulated scattering pattern (red) is shown for $a = 36.3$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S43. Experimental scattering pattern (black) is shown for a BCC superlattice of 15 nm spherical nanoparticles in 70% ETOH, 30% H₂O, 0.3 M NaCl after equilibrating for 3 min. A simulated scattering pattern (red) is shown for $a = 24.3$ nm along with a simulated form factor (blue). Calculated peak positions are indicated by gray lines.

Figure S44. Experimental scattering patterns for a BCC superlattice of 30 nm spherical nanoparticles in 80% ETOH, 20% H₂O, 0.3 M NaCl at 25, 50, 60, 70, or 80 °C. Calculated peak positions for $a = 37.2$ nm are indicated by gray lines. Samples were equilibrated at each temperature for 3 min.

Figure S45. Variable-temperature UV-vis spectroscopy melting curves for BCC superlattices of 30 nm spherical nanoparticles suspended in 0% to 80% EtOH in H₂O at 0.3 M NaCl. Extinction was monitored at 260 nm. Note that the extinction values are offset to facilitate comparison.

Figure S46. Variable-temperature UV-vis spectroscopy melting curves for BCC superlattices of 30 nm spherical nanoparticles suspended in 0% to 80% EtOH in H₂O at 0.3 M NaCl. Extinction was monitored at the nanoparticle localized surface plasmon resonance frequency of 526 nm. Note that the extinction values are offset to facilitate comparison.

Figure S47. Additional STEM images of silica-encapsulated BCC superlattices of 30 nm spherical nanoparticles previously suspended in 100% H₂O at 0.3 M NaCl. Images were obtained in scanning mode.

Figure S48. Additional STEM images of silica-encapsulated BCC superlattices of 30 nm spherical nanoparticles previously suspended in 41% EtOH and 59% H₂O at 0.3 M NaCl. Top images were obtained in scanning mode, while bottom images were obtained in transmission mode.

Figure S49. Additional STEM images of silica-encapsulated BCC superlattices of 30 nm spherical nanoparticles previously suspended in 80% EtOH and 20% H₂O at 0.3 M NaCl. Top images were obtained in scanning mode, while bottom images were obtained in transmission mode.

Figure S50. Additional STEM images of silica-encapsulated BCC superlattices of 15 nm spherical nanoparticles previously suspended in 100% H₂O at 0.3 M NaCl. Images were obtained in scanning mode.

Figure S51. Additional STEM images of silica-encapsulated BCC superlattices of 15 nm spherical nanoparticles previously suspended in 45% EtOH and 55% H₂O at 0.3 M NaCl. Top images were obtained in scanning mode, while bottom images were obtained in transmission mode.

Figure S52. Additional STEM images of silica-encapsulated BCC superlattices of 15 nm spherical nanoparticles previously suspended in 80% EtOH and 20% H₂O at 0.3 M NaCl. Top images were obtained in scanning mode, while bottom images were obtained in transmission mode.

Figure S53. UV-vis spectra of 30 nm Au spheres functionalized with single-stranded DNA (sequence corresponds to the “B” anchor strand in Table S2) at different volume percentages of EtOH in H₂O at a constant 0.3 M NaCl. As the EtOH percentage is increased, the localized surface plasmon resonance (LSPR) shifts to higher wavelengths and broadens, which is indicative of particle agglomeration. Note that all spectra were normalized to the maximum intensity of the LSPR.

Figure S54. UV-vis spectra of citrate-capped 30 nm Au spheres suspended in H₂O (blue) or 60% EtOH and 40% H₂O (red). As expected, the position and width of the LSPR are not significantly affected by the change in solvent dielectric constant. Note that the spectra were normalized to the maximum intensity of the LSPR.

Figure S55. Wavelength (blue) and full width at half maximum (FWHM, green) of the LSPR of 30 nm Au spheres functionalized with single-stranded DNA (sequence corresponds to the “B” anchor strand in Table S2) at different volume percentages of EtOH in H₂O at a constant 0.3 M NaCl. The red-shift and peak broadening of the LSPR is indicative of a transition from suspended nanoparticles to agglomerated nanoparticles at 30-60% EtOH.

Figure S56. Images of 30 nm Au spheres functionalized with single-stranded DNA (sequence corresponds to the “B” anchor strand in Table S2) at 0% or 60% of EtOH in H₂O at a constant 0.3 M NaCl. The change in color is consistent with the red-shift and peak broadening of the nanoparticle LSPR observed by UV-vis at 60% EtOH and is indicative of particle agglomeration.

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