Supporting Information

Controlling the Thermally-Driven Crystallization of DNA-Coated Nanoparticles with Formamide

AUTHOR NAMES.

Theodore Hueckel, Seungyeon Woo, and Robert J. Macfarlane

AUTHOR ADDRESS.

Department of Materials Science and Engineering, Massachusetts Institute of Technology (MIT), 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, United States

*Address correspondence to rmacfarl@mit.edu

1 Materials and Methods

1.1 Oligonucleotide Designs and Nanoparticle Synthesis

All synthetic DNA sequences were purchased from Integrated DNA Technologies (IDT). PAEs were produced through a multistage process involving the attachment of DNA "anchor" strands to gold nanoparticles via thiol bonds, followed by the attachment of DNA "linker" strands to the anchors (Fig. S1). DNA anchors comprise a terminal thiol group, followed by a 12-mer polyethylene glycol (PEG) group that adds flexibility to the rigid DNA, and finally an 18-base coding region for linkers to bind to. Linker strands carry the anchor complementary 18-base sequence for hybridization, followed by variable 20-base spacer regions to set the length of the DNA. Additionally, a single unpaired adenine base is present between each spacer sequence to add flexibility. Separate, 20-base "duplexer" strands are used to hybridize to these spacer regions to provide rigidity. Finally, linkers are capped with a 6-base sticky end. The sticky end goes unhybridized within the isolated PAE architecture but finds its complement in a neighboring particle to form a colloidal bond. Alternatively, linkers may utilize a sticky end that will find no complement in the system to serve as a "dummy" strand. Two PAE binding schemes are utilized in this work, a self-complementary sticky end to produce FCC lattices, as well as a pair of complementary sticky ends to form a BCC type lattice. All sequences used here are provided in Table S1.

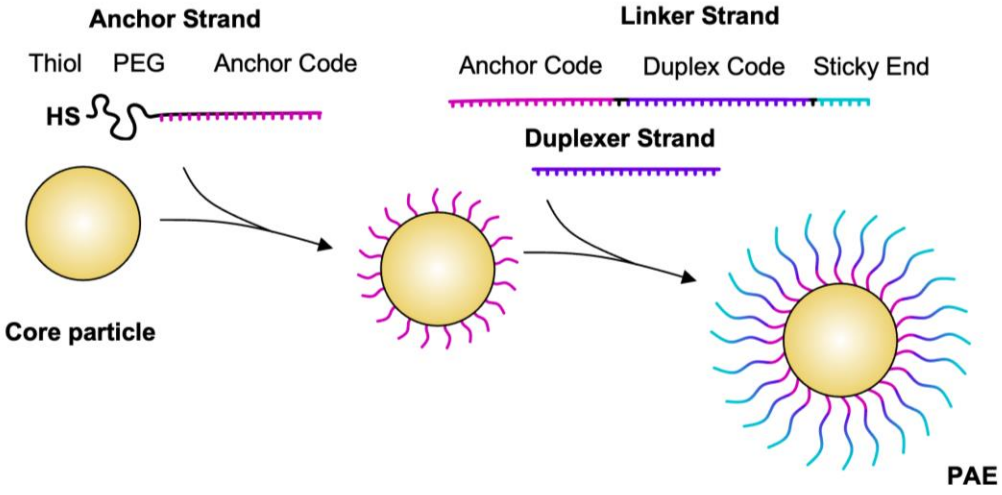

Figure S1 PAE scheme. Anchor strands attach to gold nanoparticles via sulfur groups. The anchor code provides specific sequences for linker strands to bind, providing sticky ends for binding.

Anchor Strands	Sequence (5' → 3')
Anchor X	TCA ACT ATT CCT ACC TAC–PEG–Thiol
Anchor Y	TCC ACT CAT ACT CAG CAA–PEG–Thiol
Linker Strands	**Sequence (5' → 3')**
X'–A (BCC)	GTA GGT AGG AAT AGT TGA A TTT AGT CAC GAC GAG TCA TT A TTT AGT CAC GAC GAG TCA TT A TTCCTT
Y'–A' (BCC)	TTG CTG AGT ATG AGT GGA A TTT AGT CAC GAC GAG TCA TT A TTT AGT CAC GAC GAG TCA TT A AAGGAA
X'–B" (FCC)	GTA GGT AGG AAT AGT TGA A TTT AGT CAC GAC GAG TCA TT A TAGCTA
X'–Dummy (FCC)	GTA GGT AGG AAT AGT TGA A TTT AGT CAC GAC GAG TCA TT A TTCCTT
Duplexor Strands	**Sequence (5' → 3')**
D20'	AAT GAC TCG TCG TGA CTA AA

Table S1 DNA sequences. Two types of anchors are used so that systems with mixed linkers can be prepared. For BCC type crystals, particles with anchor X, and anchor Y hybridized with two different linker strands bearing complementary sicky ends. For FCC, only one type of anchor code is needed. All duplexer regions in the linkers are hybridized with a stoichiometric concentration of duplexer strands.

1.2 Nanoparticle Synthesis

Gold nanoparticles with approximate diameters ranging from 15 to 35 nm were synthesized following a standard Turkevich style seeded growth protocol. Initial seed particles, with a 10 nm diameter, were prepared by heating a 400 mL solution comprising 0.02 mM Ethylenediaminetetraacetic acid tetrasodium salt dihydrate (EDTA, Sigma, 99%) and 2.75 mM sodium citrate buffer (242.6 mg Sodium Citrate (Sigma, ≥99.0%), 57.8 mg Citric Acid (Sigma, ≥99.5%)) to boiling in a 1 L flask. Subsequently, a solution of $HAuCl_4{:}3H_2O$ (Sigma, ACS reagent, ≥49.0% Au basis) (26.25 mg in 2 mL water) was rapidly injected while stirring vigorously. The color of the solution turned red within 5 minutes. The solution was then cooled to 90 °C and equilibrated for 30 minutes before two more injections of $HAuCl_4{:}3H_2O$ (same concentration and volume as the first), separated by 30-minute intervals. After the third injection, the particle size was 15 nm.

For further growth, 200 mL of nanoparticle solution was replaced with 200 mL of fresh 2.75 mM sodium citrate buffer. The reaction was equilibrated for 30 at 90 °C, then three more $HAuCl_4{:}3H_2O$ were performed. This process of solvent replacement and injection was then repeated once more to obtain particles with a 30 nm diameter.

1.3 Nanoparticle Functionalization Procedure

Anchors are attached to gold nanoparticles via a salt aging process. Initially anchors are treated with 100 mM dithiothreitol (DTT, Sigma, 99%) for 1 hour to cleave the 3' propyl-mercaptan protecting group. The cleaved strands are then purified using a NAP5 size exclusion column (GE Healthcare). The cleaved anchors were then added to the nanoparticle solutions in a molar ratio of

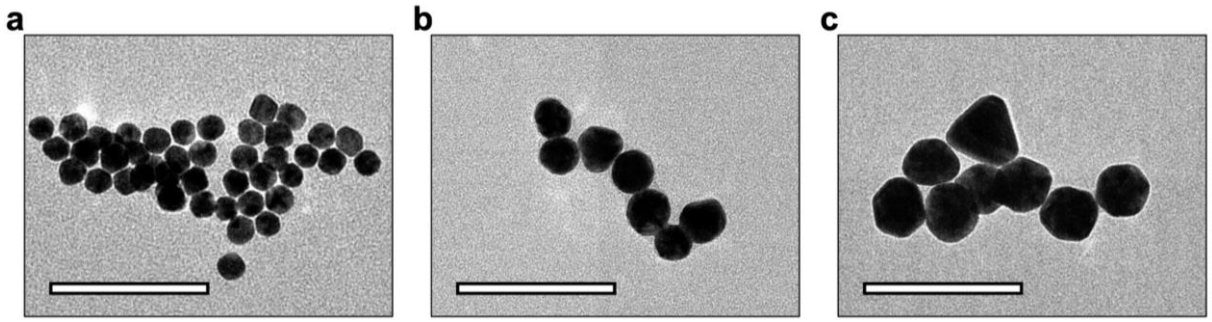

Figure S2 Gold nanoparticle cores. a-c) TEM images of gold nanoparticle cores, sized 15, 25, and 35 nm, respectively. Scale bars 100 nm.

approximately 6000:1 (equivalent to a 20-fold excess of DNA). After incubating for 1 hour, sodium dodecyl sulfate (SDS, ≥99.0%, Sigma-Aldrich) was added to a final concentration of 0.01% SDS, followed by a phosphate buffer solution (0.021 M NaH_2PO_4 (98.0%, Acros Organics), 0.079 M $Na_2HPO_4 \cdot 7H_2O$ (99.0%, Fisher Scientific)) to achieve a final concentration of 10 mM phosphate. The solution was then incubated again for additional 30 minutes. After this, the sodium chloride (NaCl, Sigma-Aldrich, 99.0%) concentration was incrementally increased to 0.5 M, over 6 half-hourly injections of 2 M NaCl in 10 mM phosphate buffer (2 M PBS). The solution was then incubated overnight. After functionalization, the particles were washed via multiple centrifugation and resuspension cycles in deionized water, until finally setting the particles in 0.5 M PBS. A Cary 50 UV-Vis-NIR spectrophotometer (Agilent) was used to measure particle concentrations, using their absorbance at 520 nm and known extinction coefficients to calculate concentration.

1.4 Linker Strand Preparation

Linker and duplexer sequences purchased from IDT, were dissolved in deionized water, and the concentration of the DNA was quantified using a Cary 50 UV-Vis-NIR spectrophotometer (Agilent), measuring absorption at a wavelength of 260 nm and using the extinction coefficients provided by IDT.

Duplexed linkers were prepared by stoichiometrically combining linkers and duplexers, with one duplexer strand for each region on the linkers. The linkers were set in 0.5 M PBS, and incubated at 35 °C before use. Linkers were added to PAEs based on a PAE grafting density of 0.25 strands/nm^2.(1,2)

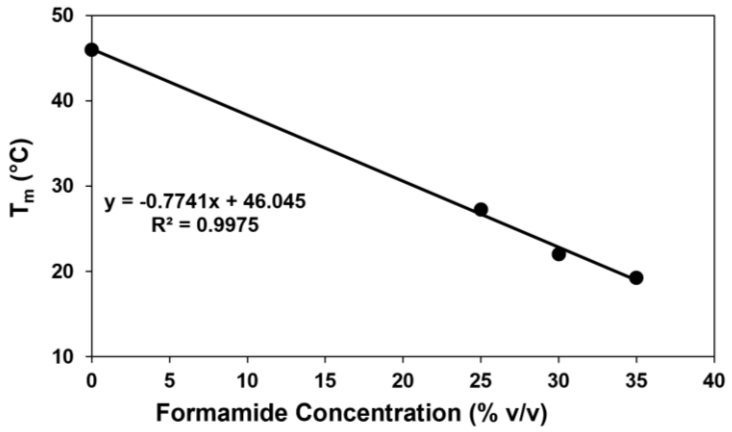

Figure S3 Formamide sensitivity of binary systems. T_m of PAEs formulated using DNA linker strands X'-A and Y'-A'. The two complimentary strands form crystals with a BCC arrangement. Additionally, these strands have an additional D20 spacer section to increase the length of the DNA brush.

1.5 Formamide Treating PAEs

When adding formamide to PAEs, linkers can denature from the anchors, which then need long a long time to diffuse back onto the particles, especially if they have been significantly diluted. To avoid this a solution of 75% v/v formamide (≥99.5%, Sigma-Aldrich) and 25% v/v 2 M PBS was first prepared, producing a final concentration of 0.5 M NaCl in the formamide. This ensures that adding formamide does not disrupt the NaCl concentration of PAEs. PAEs and the salt/formamide solution were then chilled to 2 °C before combination, ensuring that the drop in T_m is not sufficient to dissociate linkers from anchors. Due to the possibility of oxidation, formamide is prepared in small aliquots stored at -20 °C, used within 2 weeks of opening.

1.6 Melt curves of PAE assemblies

1 nM Solutions of formamide treated PAEs assemblies were then added to cuvettes at a total volume of 1.5 mL. The absorbance of solutions at 520 nm was then analyzed using a Cary 5000 UV-Vis-NIR spectrophotometer (Agilent) with temperature control. The samples were first equilibrated at the starting temperature for 30 minutes, then heated until full dissociation at a rate of 0.25 °C per minute, with data collection every 0.25 °C.

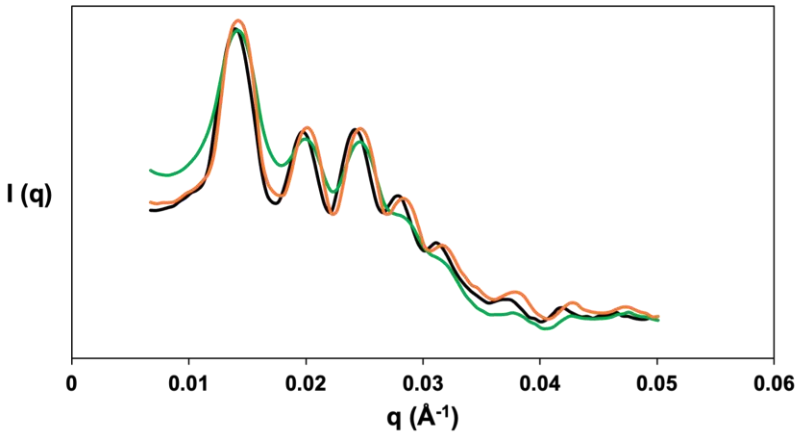

Figure S4 SAXS data of PAEs assembled under different processing conditions. 1D SAXS patterns obtained from a binary PAE system (25 nm cores), annealed from 60 °C to 10 °C. All samples were washed into 0.5 M PBS after annealing. Interparticle spacing of the BCC lattice is determined to be 53.4 nm, and surface-to-surface distance of 28.4 nm, determined from a q_0 value of 0.0144 $Å^{-1}$.(3)

1.7 PAE crystallization

PAEs were first heated to full dissociation at 60 °C, following by a slow cooling to 10 °C in a Techne Prime Thermal Cycler at a rate of 0.1 °C/min.

1.8 Silica Embedding Procedure

PAE crystals were embedded in silica following typical sol-gel protocols. First, 1.5 µL of N-trimethoxysilylpropyl-N, N, N-trimethylammonium chloride (TMSPA, Gelest, 50% in methanol) was added to 1 mL solutions of crystals in 0.5 M PBS. After a 15 minute incubation period, 1.5 µL of tetraethyl orthosilicate (TEOS, MilliporeSigma), and shaken for two days at 1400 rpm at room temperature on an Eppendorf Thermomixer R. Crystals were then sedimented and washed several times in deionized water.

1.9 Williamson-Hall Analysis

Williamson-Hall (W-H) analysis is used to separate size and strain broadening contributions in X-ray diffraction (XRD) patterns, providing insights into the crystallite size and microstrain within a material. The Williamson-Hall equation is expressed as:

$$\beta\cos\theta = \frac{K\lambda}{D} + 4\epsilon sin\theta$$

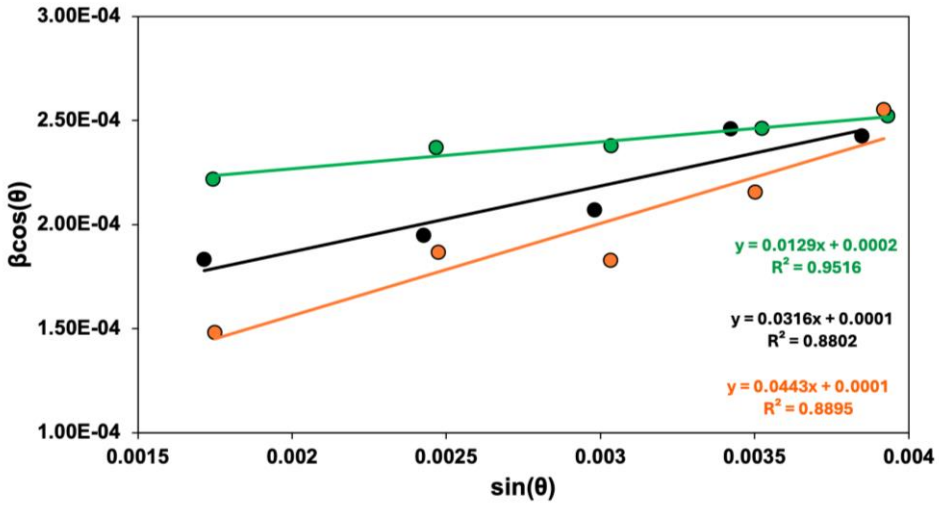

Figure S5 Williamson-Hall analysis. Traces for crystals formed under different processing conditions: low salt, green; traditional high temperature, black; formamide, orange.

where β is the full width at half maximum (FWHM) of the peak in radians, θ is the Bragg angle, K is the shape factor (0.9), λ is the wavelength of the X-ray used (1.54 Å), D is the average crystallite size, and ε is the microstrain. By plotting βcosθ against 4sinθ, the slope of the line gives the microstrain (ε), and the intercept provides the crystallite size (D). Diffraction peaks in the XRD pattern were identified, and the FWHM (β) of each peak was measured using the peak fitting software Fityk. A Williamson-Hall plot was constructed by plotting βcosθ (y-axis) against sinθ (x-axis). A linear regression analysis was performed on the Williamson-Hall plot and the y-intercept was used to calculate the crystallite size (D) using:

$$D = \frac{K\lambda}{\text{Intercept}}$$

1.10 Van 't Hoff Analysis

Van 't Hoff analysis was performed to determine the enthalpy change (ΔH) and entropy change (ΔS) of the binding interaction using melt curves obtained from UV-Vis spectroscopy. The UV-Vis spectra were recorded on a Cary-5000 spectrophotometer, monitoring the absorbance changes of the sample over a range of temperatures. T_m is the temperature where 50% of the PAEs are in solution, which is measured by normalizing the UV-vis absorbance at 520 nm relative to temperature. PAE melting behavior can then be represented by:

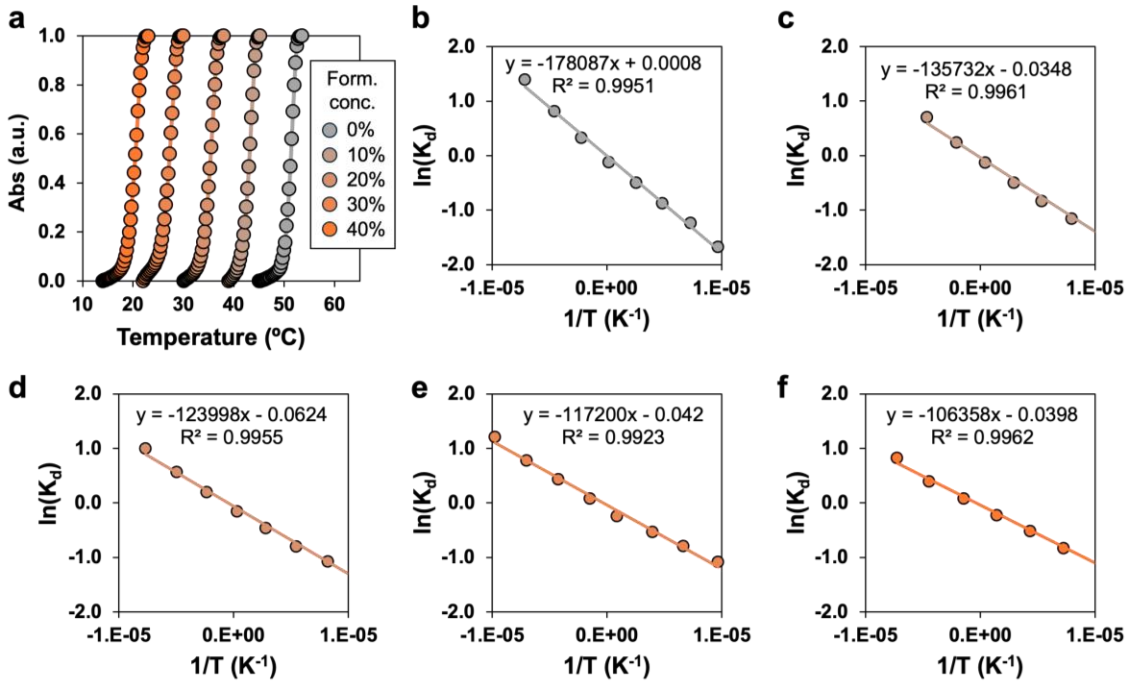

Figure S6 Van 't Hoff analysis. a) Melt curves for PAE's at varying formamide concentration. b-f) Van 't Hoff analysis and linear fit for each formamide concentration from 0-40% v/v, respectively (color correspondence with a).

$$K_d = \frac{M}{A}$$

where K_d is the equilibrium constant for the release of a PAE from the assembled state (A) into the melted state (M). By plotting the fraction of melted PAE aggregates against temperature, the Van 't Hoff equation:

$$\ln K_d = -\frac{\Delta H}{R} \times \left(\frac{1}{T} - \frac{1}{T_m}\right)$$

can be used to determine the change in enthalpy (ΔH) during the melting by fitting the data near T_m. Subsequently, the entropy change (ΔS) can be calculated using the experimentally determined T_m in the following equation:

$$\Delta S = -\Delta H / T_m$$

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3. Macfarlane RJ, Lee B, Jones MR, Harris N, Schatz GC, Mirkin CA. Nanoparticle Superlattice Engineering with DNA. Science. 2011 Oct 14;334(6053):204–8.