Supplementary Information for Critical Undercooling in DNA-Mediated Nanoparticle Crystallization

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A. DNA Design

The DNA design was taken from previous work, which involves two components:

1. An “anchor strand” that can be attached to the nanoparticles, composed of three regions: a 3' thiol modification that connects the DNA to the gold nanoparticle surface; an A₁₀ region designed to distance the functional portion of the DNA from the nanoparticle surface; and an 18 base recognition sequence. For binary PAE experiments, each set of nanoparticles was functionalized with an orthogonal “anchor” strand, denoted as “A” and “B” in Table S1.
2. A “linker strand” that hybridizes to the anchor strand, composed of three regions: an 18 base sequence complementary to the anchor strand; a single A “flexor” base; and a terminus, or “sticky end,” designed to link two nanoparticles together through complementary base pair interactions. Specific sequences that were used are denoted in Table S1.

In the case of binary PAE systems, these are composed of two nanoparticle populations with dense shells of DNA oriented in a surface normal fashion that allows each nanoparticle population to bind to the other, but not to itself. In the case of the single-component PAE systems, every particle can bind to each other.

Supplementary Table 1. DNA sequences used for nanoparticle assembly. Black denotes spacer regions, purple denotes thiols, blue denotes complementary regions of the anchor and linker strands, red represents the complementary regions to link “A” and “B” nanoparticles, and green represents the self-complementary linker region.

Name	ϵ_{260} (M ⁻¹ cm ⁻¹)	MW (g/mol)	Sequence (5' to 3')	# DNA Bases
"A" Anchor	286100	8729.9	TCA ACT ATT CCT ACC TAC AAA AAA AAA A - SH	28
"A" Linker	245300	7446.9	GTA GGT AGG AAT AGT TGA A TCTCT	24
"B" Anchor	290600	8763.9	TCC ACT CAT ACT CAG CAA AAA AAA AAAA - SH	28
"B" Linker	252000	7520.9	TTG CTG AGT ATG AGT GGA A AGAGA	24
"B" Linker (self-comp.)	231000	7158.7	TTG CTG AGT ATG AGT GGA A TGCA	23

B. Full Extinction Profiles for Associated and Dissociated States

An example of the full extinction profiles for 41 nm diameter spherical PAEs in the associated and dissociated phases is given in Figure S1.

Supplementary Figure 1. Full extinction profiles for 41 nm spheres in the associated (blue) and dissociated (red) states, with the position at which the extinction was monitored indicated by black and red dashed lines for the DNA and nanoparticle, respectively. Inset shows the nanoparticle LSPR region at higher magnification.

C. Conversion from Extinction to Fraction Assembled in Temperature-Dependent UV-Vis Measurements

Conversion to fraction assembled rests on an assumption borrowed from the nucleic acids community that extinction correlates linearly with fraction assembled after baseline subtraction.¹ While this assumption is not explicitly true in the context of plasmonic coupling between particles, the distances between particles are sufficiently large (~20 nm) and the volume fractions sufficiently low that most of the change in extinction likely comes from assembly size.^{2,3} In a freezing experiment, conversion to fraction assembled is also predicated on the assumption that the initial decrease in extinction with a decrease in temperature corresponds to nucleation, and that the subsequent decrease (with a markedly lower slope) corresponds to relaxation. Relaxation refers to the rearrangement, or annealing, of particles within a crystal to adopt a more thermodynamically favored state.⁴ Relaxation becomes more prominent as ramp rate increases in crystallization experiments (Figures S3-S5), as would be expected for a more kinetically formed crystal.

Supplementary Figure 2. A baseline subtraction method was used to evaluate the melting temperature (T_m).

The breadth of the transition can be evaluated by the full width at half maximum (FWHM) of the first derivative of the extinction versus temperature curve and offers a qualitative evaluation of the cooperative nature of the transition. In particular, the first derivative of the fraction assembled curve described in the previous paragraph was determined and fit to a Gaussian function in Origin. The FWHM was determined from the parameters of the Gaussian fit.

D. Freezing and melting experiments for PAEs of different size, shape, and DNA sequence

Supplementary Figure 3. Freezing (blue) and melting (red) curves for 41 nm diameter spherical PAEs are plotted for each temperature ramp rate (noted along top) and nanoparticle concentration (noted along side). Extinction values have been normalized to facilitate comparison. All samples are binary systems, unless otherwise noted (bottom self-complementary).

Supplementary Figure 4. Freezing (blue) and melting (red) curves for 8.5 nm diameter spherical PAEs are plotted for each temperature ramp rate (noted along top) and nanoparticle concentration (noted along side). Extinction values have been normalized to facilitate comparison. All samples are binary systems.

Supplementary Figure 5. Freezing (blue) and melting (red) curves for 48 nm diameter disk PAs are plotted for each temperature ramp rate (noted along top) and nanoparticle concentration (noted along side). Extinction values have been normalized to facilitate comparison. All samples are binary systems.

Freezing (T_{freezing}) and melting (T_{melting}) temperatures were determined through analyzing the data in Figures S3-S5.

Supplementary Table 2. T_f and T_m for each sample investigated in the main text, where the nanoparticle (NP) shape, size and concentration, as well as DNA “sticky end” sequence and concentration. The breadth of the melting transition is provided for each sample, as determined from the full width at half maximum (FWHM) of the first derivative of the melting curve. Error in the freezing and melting temperatures was determined by measuring the melting temperature of a single same sample prepared on three separate occasions. Error in the FWHM comes from the maximum error of a sample in the Gaussian fit.

NP: 8.5 nm diameter spheres; 1.5 nM concentration

DNA: AGAGA-TCTCT, 1000 linkers:NP

Ramp Rate ($^{\circ}\text{C min}^{-1}$)	T_f ($\pm 0.2 ^{\circ}\text{C}$)	T_m ($\pm 0.2 ^{\circ}\text{C}$)	ΔT ($\pm 0.4 ^{\circ}\text{C}$)	$\text{FWHM}_{\text{melting}}$ ($\pm 0.2 ^{\circ}\text{C}$)
0.1	29.5	33.9	4.4	5.1
0.25	27.4	34.1	6.7	5.7
0.5	25.7	34.3	8.6	6.6
1	20.4	35.1	14.7	-
2	16.2	36.7	20.5	-

NP: 8.5 nm diameter spheres; 5 nM concentration

DNA: AGAGA-TCTCT, 1000 linkers:NP

Ramp Rate (°C min⁻¹)	T_f (± 0.1 °C)	T_m (± 0.1 °C)	ΔT (± 0.2 °C)	FWHM_{melting} (± 0.1 °C)
0.1	31.6	34.4	2.7	5.6
0.25	30.4	34.5	4.2	5.5
0.5	28.8	35.1	6.4	5.4
1	26.4	35.8	9.4	5.4
2	20.0	37.0	17.0	-

NP: 8.5 nm diameter spheres; 10 nM concentration

DNA: AGAGA-TCTCT, 1000 linkers:NP

Ramp Rate (°C min⁻¹)	T_f (± 0.1 °C)	T_m (± 0.1 °C)	ΔT (± 0.2 °C)	FWHM_{melting} (± 0.1 °C)
0.1	32.4	34.5	2.1	4.8
0.25	31.5	34.5	3.0	4.7
0.5	29.9	34.8	4.9	4.6
1	27.6	35.7	8.1	4.6
2	22.0	36.9	14.9	4.9

NP: 41 nm diameter spheres; 15 pM concentration

DNA: AGAGA-TCTCT, 5000 linkers:NP

Ramp Rate (°C min⁻¹)	T_f (± 0.1 °C)	T_m (± 0.1 °C)	ΔT (± 0.2 °C)	FWHM_{melting} (± 0.1 °C)
0.1	43.3	46.7	3.4	4.1
0.25	42.5	47.0	4.4	3.7
0.5	41.6	47.4	5.8	3.3
1	39.0	48.2	9.2	3.1
2	35.0	49.4	14.4	3.2

NP: 41 nm diameter spheres; 75 pM concentration

DNA: AGAGA-TCTCT, 5000 linkers:NP

Ramp Rate (°C min⁻¹)	T_f (± 0.1 °C)	T_m (± 0.1 °C)	ΔT (± 0.2 °C)	FWHM_{melting} (± 0.1 °C)
0.1	45.8	47.6	1.8	4.0
0.25	44.8	47.8	2.9	3.6
0.5	43.9	48.2	4.3	3.3
1	41.8	49.0	7.3	3.3
2	37.0	50.2	13.2	3.3

NP: 41 nm diameter spheres; 150 pM concentration

DNA: AGAGA-TCTCT, 5000 linkers:NP

Ramp Rate (°C min ⁻¹)	T_f (± 0.1 °C)	T_m (± 0.1 °C)	ΔT (± 0.2 °C)	FWHM_{melting} (± 0.1 °C)
0.1	46.8	48.4	1.6	3.7
0.25	46.4	48.2	1.8	3.4
0.5	45.1	48.6	3.5	3.3
1	42.8	49.3	6.6	3.2
2	38.4	50.5	12.1	3.4

NP: 41 nm diameter spheres; 300 pM concentration

DNA: TGCA (Self-Complementary), 5000 linkers:NP

Ramp Rate (°C min ⁻¹)	T_f (± 0.1 °C)	T_m (± 0.1 °C)	ΔT (± 0.2 °C)	FWHM_{melting} (± 0.1 °C)
0.1	46.3	49.2	3.0	4.6
0.25	46.0	49.1	3.1	4.0
0.5	45.2	49.1	3.9	3.6
1	43.5	49.9	6.4	3.3
2	38.6	51.2	12.6	3.5

NP: 46 nm diameter, 8 nm thick disks; 15 pM concentration

DNA: AGAGA-TCTCT, 5000 linkers:NP

Ramp Rate (°C min ⁻¹)	T_f (± 0.1 °C)	T_m (± 0.1 °C)	ΔT (± 0.2 °C)	FWHM_{melting} (± 0.1 °C)
0.1	47.1	48.8	1.7	3.8
0.25	46.6	48.7	2.1	3.2
0.5	46.1	49.5	3.5	2.9
1	45.1	50.2	5.1	2.9
2	43.1	51.2	8.1	2.6

NP: 46 nm diameter, 8 nm thick disks; 150 pM concentration

DNA: AGAGA-TCTCT, 5000 linkers:NP

Ramp Rate (°C min ⁻¹)	T_f (± 0.1 °C)	T_m (± 0.1 °C)	ΔT (± 0.2 °C)	FWHM_{melting} (± 0.1 °C)
0.1	47.1	48.2	1.0	3.0
0.25	47.0	48.0	0.9	2.5
0.5	46.8	48.8	2.0	2.3
1	46.3	49.3	3.0	2.0
2	44.8	50.3	5.5	1.8

E. Effect of Ramp Rate on Peak Breadth

PAE melts show sharp transitions indicative of a “cooperative transition” (*i.e.* once a critical number of connections are broken, the remainder of the structure loses its integrity and the whole structure comes apart).⁵ It is important to note that this is distinct from “cooperative binding,” wherein each ligand-ligand binding event is enhanced with respect to the previous binding event. Peak breadths can be used to evaluate the cooperative nature of the melting transitions. This analysis was performed based on the procedure described above, but not for the freezing transitions, as these appear to undergo a two-state transition at fast ramp rates. Interestingly, greater ramp rates led to sharper transitions in the vast majority of samples. This observation indicates that the rate of temperature change is too quick for the system to equilibrate at each temperature, such that melting does not begin until above the characteristic temperature. At this elevated temperature, the driving force for dissociation is greater, and therefore the transition occurs over a narrower temperature range.

Supplementary Figure 6. Peak breadth can be evaluated from a Gaussian fit to the first derivative of the extinction versus temperature curve.

F. Linear Fit Parameters to Transition Temperature versus Ramp Rate Data

Linear fits to T_m and T_f vs. ramp rate data were used to evaluate the asymptotic T_f and T_m at infinitely slow ramp rates. The slope to this fit has been previously studied as a process sensitivity factor in crystallization processes,⁶ and in our samples, we find that the ramp rate affects this slope for freezing transitions significantly greater than melting transitions, consistent with undercooling. Furthermore, the slopes for the freezing curves correlate with concentration and valency, wherein greater concentrations and lower valency show smaller slopes.

Table S3. Linear fit parameters to the freezing and melting transition temperatures versus ramp rate data for each of the samples investigated in the main text. In the table, the fitted slope is listed as m , the intercept is listed as b , and the difference between the melting and freezing intercepts is given as ΔT .

Shape	Size (nm)	Conc.	m_{melting} (min^{-1})	b_{melting} ($^{\circ}\text{C}$)	m_{freezing} (min^{-1})	b_{freezing} ($^{\circ}\text{C}$)	ΔT ($^{\circ}\text{C}$)
Sphere	8.5	1.5 nM	2.0	33.8	-9.8	30.3	3.5
	8.5	5 nM	1.7	34.2	-5.6	31.9	2.3
	8.5	10 nM	1.3	34.2	-5.4	32.8	1.4
Sphere	41	15 pM	1.7	46.5	-3.4	43.4	3.1
	41	75 pM	1.4	46.4	-4.5	44.5	1.9
	41	150 pM	1.3	46.4	-3.0	45.1	1.3
	41	300 pM (TGCA)	1.2	48.8	-4.1	47.0	1.8
Disk	46	15 pM	1.0	48.9	-1.9	47.1	1.8
	46	150 pM	1.0	48.1	-1.4	47.4	0.7

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