[Supporting Information to accompany:]

The significance of multivalent bonding motifs and “bond order” in DNA-directed nanoparticle crystallization

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S1. Materials and instrumentation. Unless otherwise stated, all reagents and reagent-grade solvents were purchased from Acros Organics (part of Thermo Fisher Scientific, New Jersey, NJ), Strem Chemicals, Inc. (Newburyport, MA), Aldrich Chemical Company (Milwaukee, WI), or Glen Research Corp. (Sterling, VA), and used as received. 10 nm (5.7×10^{12} particle /mL) and 15 nm (1.4×10^{12} particle /mL) gold nanoparticles were purchased from British BioCell International (shipped from Ted Pella, Inc., Redding, CA). Quartz capillaries for small-angle X-ray scattering (SAXS) experiments were purchased from Charles Supper Company, Inc. (1.5 mm; Natick, MA). Ultrapure deionized H₂O (18.2 MΩ cm resistivity) was obtained from a Millipore system (Milli-Q Biocel).

Synthesis of DNA sequences were performed on an Expedite 8909 Nucleic Acid system. All strands were purified on an Agilent 1100 HPLC equipped with semi-preparative (Dynamax, 250 mm × 10 mm, Microsorb 300 Å/10 μm/C18, Agilent #R083213C10) and analytical columns (Dynamax, 100 mm × 4.6 mm, Microsorb 100 Å/3 μm/C18, Agilent #R0080200E3). Absorption spectra and melting analyses of DNA and DNA-NP materials were recorded on a Varian Cary 300 Bio UV-vis spectrophotometer using a masked quartz cell (path length = 10 mm, Starna cells, Inc., catalog #29B-Q-10-MS, Atascadero, CA). Fluorescence emission spectra were obtained on a Horiba Jobin Yvon Fluorolog fluorometer (λ_{ex} = 495 nm, λ_{em} = 518 nm for fluorescein attached to DNA and λ_{ex} = 480 nm, λ_{em} = 520 nm for OliGreen[®] ssDNA assay, slit width = 3 nm for all measurements) controlled by Florescence software. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) data was collected on a Bruker AutoFlex III MALDI-ToF mass spectrometer (Bruker Daltonics, Billerica, MA). Data from Agilent HPLC and Bruker MALDI-ToF instruments were process using Mestrenova software version 8.1.1-11591.

S2. General synthesis procedure for single-stranded (ss) disulfide-modified DNA. In a typical procedure, DNA syntheses were carried out from the 3' direction using controlled porosity glass (CPG) beads possessing 1 μmol of either adenine (Glen Research, #20-2001-10, 1000 Å, 38 μmol/g) or thymine attached to the surface (Glen Research, #20-2031-10, 1000 Å, 26 μmol/g). The CPG beads were placed in a 1 μmol synthesis column and 3'-phosphoramidites (Glen Research, dA-CE phosphoramidite #10-1000-C5, Ac-dC-CE phosphoramidite #10-1015-C5, dmf-dG-CE phosphoramidite #10-1029-C5, dT-CE phosphoramidite #10-1030-C5), spacer phosphoramidite 18 (PEG6, Glen Research, #10-1918-90), and thiol-modifier C6 S-S phosphoramidite (Glen Research, #10-1936-90) were then added using the standard 1 μmol protocol on an Expedite 8909. At the end of the synthesis, the beads were dried and placed in AMA deprotecting solution (1 ml of 1:1 v/v mixture of 28-30 wt % aq NH₃ and 41 wt % aq methyl amine) at 65 °C for 15 minutes. (CAUTION: Only fresh AMA solutions that are not more than two weeks old and have been kept in the refrigerator below 0 °C should be used). Afterwards, ammonia and methyl amine were removed by passing a stream of N₂ over the AMA solution until the smells of ammonia and methyl amine are no longer present. The remaining solution was diluted to 1 mL with ultrapure deionized H₂O, filtered through a 0.45 μm nylon syringe filter (Acrodisc[®] 13 mm syringe filter PN 4426T), and purified using semi-preparative RP-HPLC.

DMT-containing fractions (around 45 min, see purification section for more details) were collected, lyophilized, dissolved in AcOH:H₂O mixture (8:2, v/v), incubated for 1 h, and then lyophilized again to afford the pure product (isolated yield = 40-50%), which was confirmed by analytical HPLC and MALDI-ToF mass spectroscopy (see Figures S1-S7 for details).

General synthesis procedure for multivalent linkers. In the first step, the DNA stem (18 bp sequence, see Table S1 for details) of the linkers were synthesized on CPG beads possessing 1 μ mol of either adenine or thymine attached to the surface in the 3' direction using the standard 1 μ mol protocol on an Expedite 8909 synthesizer. In the last step of the synthesis, a branching phosphoramidite (Glen Research, symmetric doubler phosphoramidite #10-1920-90 or long trebler phosphoramidite #10-1925-90) was introduced using a 15-minute-long coupling protocol and 4,5-dicyanoimidazole (DCI, 0.25 M in dry MeCN) as an activator to improve the coupling yield between the branching phosphoramidite and growing DNA strand. The DNA synthesis protocol was then changed based on the branching phosphoramidite employed (2 μ mol protocol for doubler, 3 μ mol for trebler) to synthesize multiple arms simultaneously after the branching point. At the end of the synthesis, the beads were dried followed by deprotection and purification of the stands as described above. DMT-containing fractions (between 65-70 min, see purification section for more details) were collected, lyophilized, redissolved in AcOH:H₂O mixture (8:2, v/v), kept in this mixture for 1 h, and then lyophilized again to afford the pure product (isolated yield = 5-20%), which was confirmed by analytical HPLC and MALDI-ToF mass spectroscopy (see Figures S8-S41 for details).

Table S1. DNA sequences and abbreviations used for nanoparticle assembly. Note that A and B sequences contained the [PEG6]₂-C₆-S-S-C₆ modifications at their 5' termini. When employed, branching points were incorporated at the 5' termini of A' and B' followed by a flexor region (A, T₃, T₆, PEG6, [PEG6]₂, [PEG6]₃) and sticky-end sequences (3'→5') TTTCCTT (A') and AAGGAAA (B').

DNA Sequences (3'→5')	Abbreviation
AAC AAT TAT ACT CAG CAA	A
AAG AAT TTA TAA GCA GAA	B
TTG CTG AGT ATA ATT GTT	A'
TTC TGC TTA TAA ATT CTT	B'

We note in passing that since PAEs consist of two primary oligonucleotides, the thiolated strand and the linker, it is possible to incorporate flexible moieties into the various regions of either strand. The original demonstration of DNA-directed NP crystallization (reference 9a in the main text) also modified the linker by using a PEG6 moiety in the flexor region. Although this work represented a single data point in the visual comparison of the SAXS patterns of systems employing different flexor types, it prompted us to study the effects of different flexor types in a detailed and quantitative fashion through the use of microstrain analysis in the current work. Another previous study focused on the thiolated oligonucleotide and incorporating multiple oligo(ethylene glycol) chains into the spacer region between the thiol-modification and the recognition sequence (reference 9b in the main text). While this design indeed improves the crystal quality of various systems, particularly those using large nanoparticle cores, it does have some notable limitations when compared to our current design that focuses on the linker strand. From a practical standpoint, the solid-phase synthesis of multiple oligo(ethylene glycol) units in succession suffers from poor yields. Since the thiol strand is used in large excess during nanoparticle functionalization, the ability to generate a necessary amount of these materials for these types of studies is seriously limited. In contrast, by using the linker as a means to incorporate flexibility, the amount of material needed for a given assembly experiment decreases significantly and facilitates modular experimentation since the linkers can be easily hybridized with complementary particles of various sizes.

S3. DNA purification and AuNP functionalization

Purification of disulfide DNA strands and multivalent linkers. In a typical protocol, the reaction mixture from a DNA synthesis was first analyzed using a reverse-phase (RP) analytical HPLC column (Dynamax, 100 $\times$ 4.6 mm, Microsorb 100 Å/3 μ m/C18, Agilent #R0080200E3) and a gradient method that begins with 95:5 v/v 0.1 M aq

TEAA:MeCN (aq TEAA = triethylammonium acetate, aqueous solution), and increases to 60:40 v/v 0.1 M aq TEAA:MeCN over 35 minutes (1% MeCN/minute), with a flow rate of 1 mL/min. Then, all strands were purified by semi-preparative RP-HPLC column (Dynamax, 250 × 10 mm, Microsorb 300 Å/10 µm/C18, Agilent #R083213C10) and a gradient method that begins with 95:5 v/v 0.1 M aq TEAA:MeCN and increases to 60:40 v/v 0.1 M aq TEAA:MeCN over 70 minutes (0.5% MeCN/minute, a slower gradient rate was used for semi-preparative RP-HPLC to ensure adequate separation of the peaks), with a flow rate of 3 mL/min. DMT-containing fractions (see Figures S2 and S5 for details) showing UV-vis signals from the DNA bases (260 nm) and with elution times that correspond to those in the analytical RP-HPLC runs were collected, lyophilized, and dissolved in H₂O (1 mL). Upon MALDI-ToF analysis, the collected fractions containing the desired products were combined, lyophilized, and redissolved in water. Analysis of the dissolved pure products by analytical RP-HPLC (using the 1% MeCN/minute gradient method with the flow rate of 1 mL/min) confirms the purity of the sample (see Figures S3-S4, S6-S41 for details).

Synthesis of thiol-modified DNA and functionalization of AuNPs. The 5'-hexyldisulfide-modified oligonucleotides (50-55 nmole measured by UV absorbance at 260 nm) were converted into the more reactive thiol/thiolate species prior to nanoparticle functionalization. Briefly, the disulfide DNA strands were reduced with 100 mM dithiothreitol (DTT, Sigma-Aldrich; Milwaukee, WI) in 170 mM sodium phosphate buffer (pH = 8.0) for approximately one hour then subsequently purified using size-exclusion chromatography (illustra NAP5 column; GE Healthcare) to separate the DTT from the reduced oligonucleotides. The desired eluate was collected in a 1.5 mL Eppendorf (Newark, NJ) centrifuge tubes and quantified (45-50 nmole, 80-90% yield), which was then added to colloidal solutions of gold nanoparticles (~ 3 nmol DNA per mL AuNPs using known extinction coefficients; 500 or 2000 equivalents of thiol DNA per 10 or 15 nm AuNP, respectively) and allowed to incubate overnight. The solutions were then slowly salted via stepwise additions of aliquots of 1% aq sodium dodecyl sulfate (SDS), 1 M aq sodium phosphate (pH = 7.4), and 2 M aq NaCl, followed by 10 seconds of sonication. Once the final buffer concentrations of 0.5 M NaCl, 10 mM NaPO₄, and 0.01% SDS were reached, the solution was allowed to sit for 16 h to maximize DNA loading. Excess DNA strands were removed by centrifugation (10 nm AuNPs, ~21000 rcf for 60 minutes; 15 nm AuNPs, ~17000 rcf for 30 minutes), followed by removal of the supernatant and redispersal of the pellet in 0.02% SDS. After three rounds of centrifugation and removal of the supernatant, the pellet was redispersed in 0.5 M phosphate-buffered saline (PBS). The absorbance of AuNPs and oligonucleotides were measured and calibrated against known extinction coefficients from the Ted Pella and Integrated DNA Technologies (IDT) websites.

MALDI-ToF experiments of all DNA strands. The matrix was prepared by addition of aqueous ammonium hydrogencitrate (0.6 µL of a solution of 15 mg in 30 µL of H₂O) to a solution of 2-hydroxypicolinic acid (Fluka #56297, 2 mg in H₂O:MeCN (30 µL, 1:1 v/v). An aliquot (1 µL, 10-100 pmol) of the isolated pure DNA-containing sample was then mixed with a portion of this matrix (5 µL). A small amount of the resulting mixture (0.3-3 µL) was dropped on a steel MALDI-ToF plate and dried at 25 °C before being analyzed on a Bruker AutoFlex III MALDI-ToF mass spectrometer (Bruker Daltonics, Billerica, MA) as negative or positive ions using the linear mode. The instrument was equipped with Smartbeam™ laser technology operated at 30-90% power with a sampling speed of 10 Hz. Five hundred spectra were averaged for the corresponding mass spectrum. The instrument was operated using the following parameters: ion source voltage 1 = 20 kV, ion source voltage 2 = 18.5 kV, lens voltage = 8.5 kV, linear detector voltage = 0.6 kV, deflection mass = 3000 Da. The MALDI-ToF spectra of strands were shown in the inset of the pure analytical traces.

S4. Preparative and analytical HPLC and MALDI-ToF traces for DNA strands

Figure S1. Analytical RP-HPLC trace of crude synthesis mixture of 3'-A-[PEG6]₂-C₆-S-S-C₆-ODMT, which was run using a 0.5% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The fraction at 39 min is the DMT-containing final product.

Figure S2. Semi-preparative RP-HPLC trace of the crude synthesis mixture of 3'-A-[PEG6]₂-C₆-S-S-C₆-ODMT, which was run using a 0.5% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The fraction between 43.9-45.2 min is the desired DMT-containing product (see Figure S3 for the analytical HPLC data and MALDI-ToF spectra of the DMT-deprotected pure product).

Figure S3. Analytical RP-HPLC trace of pure 3'-A-[PEG6]₂-C₆-S-S-C₆-OH, which was obtained by DMT deprotection of the 43.9-45.2 min fraction collected from a semi-preparative RP-HPLC run (Figure S2) with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 6477.1$ (6476.7 theoretical).

Figure S4. Analytical RP-HPLC trace of pure 3'-A-[PEG6]₂-C₆-SH, which was obtained by DTT treatment of the pure disulfide product (Figure S3) with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 6346.1 (6344.4 theoretical).

Figure S5. Semi-preparative RP-HPLC trace of crude synthesis mixture of 3'-B-[PEG6]₂-C₆-S-S-C₆-ODMT, which was run using a 0.5% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The fraction between 43.9-45.5 min (desired DMT-containing product, see Figure S6 for analytical HPLC and MALDI-ToF spectra of the DMT-deprotected pure product) was collected.

Figure S6. Analytical RP-HPLC trace of pure 3'-B-[PEG6]₂-C₆-S-S-C₆-OH, which was obtained by DMT deprotection of the 43.9-45.5 min fraction from a semi-preparative RP-HPLC run (Figure S5) with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 6467.9 (6471.7 theoretical).

Figure S7. Analytical RP-HPLC trace of pure 3'-B-[PEG6]₂-C₆-SH, which was obtained by DTT treatment of the pure disulfide product (Figure S6) with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 6341.4 (6339.4 theoretical).

Figure S8. Analytical RP-HPLC traces of pure 3'-fluorescein-A'-A-TTTCCTT linker run with 1% MeCN/min gradient method. The top and bottom traces are the signals from the diode detector set at 490 nm and 260 nm, respectively. The inset shows the MALDI-ToF spectra of the pure product: m/z = 8525.3 (8516.7 theoretical).

Figure S9. Analytical RP-HPLC trace of pure 3'-A'-A-TTTCCTT linker run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 7970.6$ (7947.2 theoretical).

Figure S10. Analytical RP-HPLC trace of pure 3'-B'-A-AAGGAAA linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 8109.4$ (7967.3 theoretical).

Figure S11. Analytical RP-HPLC trace of pure 3'-A'-T₃-TTTCCTT linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 8548.8$ (8546.6 theoretical).

Figure S12. Analytical RP-HPLC trace of pure 3'-B'-T₃-AAGGAAA linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 8688.9 (8676.7 theoretical).

Figure S13. Analytical RP-HPLC trace of pure 3'-A'-T₆-TTTCCTT linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 9456.7 (9459.2 theoretical).

Figure S14. Analytical RPHPLC trace of pure 3'-B'-T₆-AAGGAAA linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 9583.6 (9589.3 theoretical).

Figure S15. Analytical RP-HPLC trace of pure 3'-A'-[PEG6]-TTTCCTT linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 7989.0$ (7978.3 theoretical).

Figure S16. Analytical RP-HPLC trace of pure 3'-B'-[PEG6]-AAGGAAA linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 8113.3$ (8108.5 theoretical).

Figure S17. Analytical RP-HPLC trace of pure 3'-A'-[PEG6]₂-TTTCCTT linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 8326.4 (8322.6 theoretical).

Figure S18. Analytical RP-HPLC trace of pure 3'-B'-[PEG6]₂-AAGGAAA linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 8454.1 (8452.7 theoretical).

Figure S19. Analytical RP-HPLC trace of pure 3'-A'-[PEG6]₃-TTTCCTT linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 8669.8 (8666.9 theoretical).

Figure S20. Analytical RP-HPLC trace of pure 3'-B'-[PEG6]₃-AAGGAAA linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 8793.0 (8797.0 theoretical).

Figure S21. Analytical RP-HPLC trace of pure 3'-A'-doubler-(TTTCCTT)₂ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 10083.3 (10085.6 theoretical).

Figure S22. Analytical RP-HPLC trace of pure 3'-B'-doubler-(AAGGAAA)₂ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 10338.0$ (10340.9 theoretical).

Figure S23. Analytical RP-HPLC trace of pure 3'-A'-doubler-(T₃-TTTCCTT)₂ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 11872.4$ (11910.8 theoretical).

Figure S24. Analytical RP-HPLC trace of pure 3'-B'-doubler-(T₃-AAGGAAA)₂ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 12172.4$ (12166.1 theoretical).

Figure S25. Analytical RP-HPLC trace of pure 3'-A'-doubler-([PEG6]-TTTCCTT)₂ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 10757.9 (10774.2 theoretical).

Figure S26. Analytical RP-HPLC trace of pure 3'-B'-doubler-([PEG6]-AAGGAAA)₂ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 11003.2 (11029.5 theoretical).

Figure S27. Analytical RP-HPLC trace of pure 3'-A'-doubler-([PEG6]₃-TTTCCTT)₂ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 12137.9$ (12151.4 theoretical).

Figure S28. Analytical RP-HPLC trace of pure 3'-B'-doubler-([PEG6]₃-AAGGAAA)₂ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 12401.9$ (12406.7 theoretical).

Figure S29. Analytical RP-HPLC traces of pure 3'-fluorescein-A'-trebler-(T₃-TTTCCTT)₃ linker, run with 1% MeCN/min gradient method. The top and bottom traces are signals from the diode detector set at 490 nm and 260 nm, respectively. The inset shows the MALDI-ToF spectra of the pure product: m/z = 15541.9 (15568.4 theoretical).

Figure S30. Analytical RP-HPLC trace of pure 3'-A'-trebler-(TTTCCTT)₃ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 12278.3 (12261.1 theoretical).

Figure S31. Analytical RP-HPLC trace of pure 3'-B'-trebler-(AAGGAAA)₃ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 12670.8 (12641.5 theoretical).

Figure S32. Analytical RP-HPLC trace of pure 3'-A'-trebler-(T₃-TTTCCTT)₃ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 14821.1 (14998.9 theoretical).

Figure S33. Analytical RP-HPLC trace of pure 3'-B'-trebler-(T₃-AAGGAAA)₃ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 15454.0 (15379.4 theoretical).

Figure S34. Analytical RP-HPLC trace of pure 3'-A'-trebler-(T₆-TTTCCTT)₃ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 17808.0$ (17736.4 theoretical).

Figure S35. Analytical RP-HPLC trace of pure 3'-B'-trebler-(T₆-AAGGAAA) linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 18175.2$ (18117.4 theoretical).

Figure S36. Analytical RP-HPLC trace of pure 3'-A'-trebler-([PEG6]-TTTCCTT)₃ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: $m/z = 13277.7$ (13294.4 theoretical).

Figure S37. Analytical RP-HPLC trace of pure 3'-B'-trebler-([PEG6]-AAGGAAA)₃ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 13722.0 (13674.4 theoretical).

Figure S38. Analytical RP-HPLC trace of pure 3'-A'-trebler-([PEG6]₂-TTTCCTT)₃ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 14315.5 (14328.4 theoretical).

Figure S39. Analytical RP-HPLC trace of pure 3'-B'-trebler-([PEG6]₂-AAGGAAA) linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 14726.0 (14707.4 theoretical).

Figure S40. Analytical RP-HPLC trace of pure 3'-A'-trebler-([PEG6]₃-TTTCCTT)₃ linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 15427.8 (15359.8 theoretical).

Figure S41. Analytical RP-HPLC trace of pure 3'-B'-trebler-([PEG6]₃-AAGGAAA) linker, run with 1% MeCN/min gradient method. The trace is the signal from the diode detector set at 260 nm. The inset shows the MALDI-ToF spectra of the pure product: m/z = 15794.2 (15740.1 theoretical).

S5. Nanoparticle lattice assembly and DNA loading studies

Nanoparticle assembly.

In a typical experiment, linker stocks were prepared at 10 μM concentrations in 0.5 M aq NaCl solution by adding the linker, 2 M aq NaCl solution (to bring the volume of DNA added in water to 0.5 M, roughly 1/3 of the total volume of DNA added), and diluting to a target volume with 0.5 M aq NaCl solution. The linkers were then added to the appropriate DNA-functionalized AuNP solutions to form the two complementary NPs (Figure S42). Mixing these solutions assemble the particles and aggregate formation was typically visible to the naked eye via a color change from red to purple. Precipitation occurs within a few minutes of mixing, affording a small disordered aggregates and a clear solution. These aggregates were annealed by placing the sample at a temperature slightly below or above its T_m until a “softening” of the precipitate is observed without total melting of the aggregates.

Figure S42. Schematic illustration of two-component DNA-programmed nanoparticle assembly. After each nanoparticle stock was prepared as described above, complementary linker stocks (10 μM in 0.5 M aq NaCl solution) were added to the corresponding nanoparticle solution. After a brief incubation period (approximately 30 minutes), the two pre-hybridized particle solutions were combined. Duplex formation between sticky ends on neighboring particles leads to aggregate formation, which was typically visible to the naked eye via a color change from red to purple and precipitation within a few minutes of mixing. Thermal annealing of this disordered aggregate is necessary for the particles to reorganize into a PAE-based superlattice.

Thiol-loading studies using the OliGreen Assay. Fluorescent calibration curves (Figure S43, $\lambda_{\text{ex}} = 480 \text{ nm}$, $\lambda_{\text{em}} = 520 \text{ nm}$) for thiol-modified strands (3'-A-[PEG6]₂-C₆-SH and 3'-B-[PEG6]₂-C₆-SH) were prepared. DNA loading was found to be ~175 strands for the 10 nm particle and ~315 strands for the 15 nm nanoparticles, consistent with previous reports.^{S1} Aliquots (2 μL) of DNA-functionalized AuNPs (10 and 15 nm) with known concentrations of particles were dissolved in 2 M KCN (10 μL) and then combined with the OliGreen reagent (2 μL). Each solution was then diluted with 1 $\times$ TE buffer (970 μL) and water (16 μL). The fluorescence data was collected and used to calculate the total loading for each sequence (Table S2) according to the calibration curves in Figure S43

Figure S43. Fluorescent calibration curves for 3'-A-[PEG6]₂-C₆-SH (A) and 3'-B-[PEG6]₂-C₆-SH (B) sequences. $\lambda_{\text{ex}} = 480$ nm, $\lambda_{\text{em}} = 520$ nm.

Table S2. Loading of 3'-A-[PEG6]₂-C₆-SH and 3'-B-[PEG6]₂-C₆-SH sequences on 10 and 15 nm AuNPs.

		Thiol Loading			Average
Particle Size	Sequence	Aliquot 1	Aliquot 2	Aliquot 3	
10 nm	A	179	173	164	172 ± 6.2
	B	186	185	181	184 ± 2.2
15 nm	A	337	337	324	333 ± 6.1
	B	319	309	294	307 ± 10.3

Linker-loading studies with fluorescein-modified linkers. Aliquots (30 μL) of each component A and B, consisting of DNA-functionalized nanoparticles of the same diameter, were combined in the manner described in Figure S42 to yield aggregates. The sequences listed in Table S1 were used such that one of the linkers (always the “A” linker) is functionalized with fluorescein at the 3' position and various linker loadings were added based on nanoparticle size (60, 100, 120, 180 equivalents per 10 nm AuNP; 120, 200, 240, 360 equivalents per 15 nm AuNP). After precipitation, the resultant aggregate was allowed to incubate for 1 h. Each mixture is then centrifuged for 10 seconds at 6000 rpm (VWR™ Galaxy Mini Microcentrifuge), leaving a red precipitate in a tiny pellet at the bottom of the tube and a clear supernatant. An aliquot (50 μL) of the supernatant was removed and placed in a new tube then combined with an aliquot (50 μL) of 2M KCN solution. The resulting mixture was kept at rt for 1 hour then diluted with an aliquot (900 μL) of 10 mM PBS (pH = 7) buffer containing 0.5 M NaCl followed by fluorescence measurements.

The number of linker equivalents in the supernatant were calculated based on calibration curves prepared with pre-determined amounts of the corresponding fluorescein-labeled strand (3'-fluorescein-A'-A-TTTCCTT or 3'-fluorescein-A'-trebler-(T₃-TTTCCTT)₃; also prepared in a 1:18 v/v mixture of 2M KCN (50 μL) and 10 mM PBS (pH = 7) buffer containing 0.5 M NaCl (900 μL)). The remainder of the initially added linker equivalents are then assumed to be in the pellet. This approach showed that all aggregates contained nearly 90% of the linkers added (Figure S44), except when high linker equivalents above the total capacity of AuNP were tested (i.e., 180 and 360 equivalents for 10 and 15 nm NPs, respectively). The hybridization efficiency between linkers and particle-bound strands were measured to be similar for linear and trebler systems, confirming that the linker uptake amount is independent of its steric demand for these high-curvature NP systems. By extrapolation, we will assume that the hybridization efficiency for doubler systems will also be similar.

Figure S44. Fluorescent linker loading for 10 nm linear systems (A), 10 nm trebler systems (B), 15 nm linear systems (C), and 15 nm trebler systems (D).

S6. Optical melting experiments

Disordered AuNP aggregates (Tables S3-S7), where both components contain AuNPs of the same diameter, were formed in the manner described in Figure S42. The resulting mixtures were diluted 33 times with 10 mM PBS (pH = 7) buffer containing 0.5 M NaCl and denatured by heating from 20 °C to 60 °C at a ramp rate of 0.5 °C per minute while monitoring the UV absorbance at 260 nm at 0.1 °C intervals to observe the melting progress. Cuvettes equipped with stir bars were employed to ensure that the sample did not precipitate out of the path of the beam. Alpha curves of melting data were generated using the Varian Thermal Application software (version 3.0(182), Varian Australia Pty., Ltd.), which also provided the melting temperature (T_m , Table S3-S7) of the aggregates. The full-width-at-half-maximum (FWHM, Table S3-S7) of the derivative of each melting curve was calculated using Origin 6.1 (v6.1052 (B232), OriginLab Corporation, Northampton, MA 01060, USA, as described in prior literature.^{S2} Briefly, to accurately calculate the FWHM of the derivative of the melting curves, Gaussian fitting analysis in Origin 6.1 were first carried out for the derivative curves using the equation:

$$y = y_0 + \frac{A}{w\sqrt{\frac{\pi}{2}}} e^{-\frac{2(x-x_0)^2}{w^2}}$$

where y_0 is baseline offset, A is total area under the curve from the baseline, x_0 is center of the peak, w is 2σ , approximately 0.849 of FWHM, so

$$\text{FWHM} = 2\sqrt{2\ln 2}\sigma = 2.3548\sigma = 1.1774w$$

The FWHM value for each derivative curve is then obtained by multiplying the width (w) value of the Gaussian fitting with 1.1774.

Table S3. Thermal properties (T_m and FWHM values obtained from UV-vis melting analysis) of pre-bcc disordered aggregates containing 10 nm AuNPs with various linker loadings, branching units, and flexor regions.

Equiv added	Linear-A		Linear-[PEG6] ₂		Treble-T ₃		Treble-[PEG6] ₂	
	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)
100/100	45.1	2.1	40.0	1.6	46.6	0.9	47.0	1.0
80/80	42.2	2.8	37.8	2.3	45.2	1.1	46.2	1.0
100/50	39.8	4.4	36.2	3.1	43.7	1.4	45.2	1.4
80/40	38.4	3.1	33.2	4.2	41.9	2.0	44.0	1.4
60/60	38.7	3.7	34.8	3.9	43.4	1.3	44.4	1.3

Table S4. Thermal properties (T_m and FWHM values obtained from UV-vis melting analysis) of pre-bcc disordered aggregates containing 15 nm AuNPs with various linker loadings, branching units, and flexor regions.

Equiv added	Linear-A		Linear-[PEG6] ₂		Treble-T ₃		Treble-[PEG6] ₂	
	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)
200/200	49.1	1.5	45.5	1.1	47.8	0.9	48.7	0.8
200/100	45.8	2.6	42.1	1.3	47.0	0.9	48.1	0.8
160/160	47.4	1.9	43.3	1.1	48.0	0.7	48.6	0.7
160/80	44.0	3.0	40.8	1.5	46.0	1.0	47.1	0.9
120/120	45.3	2.4	40.9	1.4	47.1	1.0	47.6	0.7
70/70	39.6	3.8	34.6	2.6	43.9	1.2	44.9	1.2

Table S5. Thermal properties (T_m and FWHM values obtained from UV-vis melting analysis) of pre-CsCl disordered aggregates containing 15 nm-10 nm AuNPs with various linker loadings, branching units, and flexor regions.

Equiv added	Linear-A		Linear-[PEG6] ₂		Treble-T ₃		Treble-[PEG6] ₂	
	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)
180/90	45.8	2.1	41.0	1.7	46.4	0.8	47.5	1.0
120/100	44.5	2.2	40.0	1.5	46.8	0.9	47.8	0.7
100/100	43.6	2.6	39.2	1.6	46.2	1.0	47.8	0.9
120/60	41.9	2.8	36.4	2.5	45.2	1.0	45.6	1.3
80/80	41.7	2.8	37.0	2.0	44.9	1.2	46.3	0.8
90/60	40.7	3.1	36.0	2.4	44.5	1.1	45.2	1.3

Table S6. Additional thermal properties (along with Table S3; T_m and FWHM values obtained from UV-vis melting analysis) of pre-bcc disordered aggregates containing 10 nm AuNPs (100 linker equivalents added to each component with various branching and flexor designs other than those presented in Table S3. Also see Figure S46 A and B for plotted data.

Flexor (# bonds) ^a	Linear		Doubler		Trebler	
	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)
None ^b (6, 10, 8)	45.1	2.1	43.0	1.2	40.4	1.2
T ₃ (18, 28, 26)	40.0	2.1	45.1	1.0	46.6	0.9
T ₆ (36, 46, 44)	36.7	2.3	-	-	45.9	0.9
[PEG6] (20, 30, 28)	37.7	2.3	42.9	1.6	46.3	1.0
[PEG6] ₂ (40, 50, 48)	40.0	1.6	-	-	47.0	1.0
[PEG6] ₃ (60, 70, 68)	39.9	1.4	44.6	1.0	46.4	0.9

^aTotal number of bonds between the branching point and the sticky end for linear, doubler, trebler, respectively. ^bLinear system has a single A unit as flexor.

Table S7. Additional thermal properties (along with Table S4; T_m and FWHM values obtained from UV-vis melting analysis) of pre-bcc disordered aggregates containing 15 nm AuNPs (200 linker equivalents added to each component) with various branching and flexor designs other than those presented in Table S4. Also see Figure S46 C and D for plotted data.

Flexor (# bonds) ^a	Linear		Doubler		Trebler	
	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)	T_m (°C)	FWHM (°C)
None ^b (6, 10, 8)	49.1	1.5	46.6	0.7	42.7	0.5
T ₃ (18, 28, 26)	44.6	2.0	46.9	0.8	47.8	0.9
T ₆ (36, 46, 44)	42.2	1.5	-	-	47.7	0.7
[PEG6] (20, 30, 28)	44.2	1.5	46.5	0.8	48.5	0.7
[PEG6] ₂ (40, 50, 48)	45.5	1.1	-	-	48.7	0.8
[PEG6] ₃ (60, 70, 68)	43.7	1.0	47.4	0.7	48.4	0.7

^aTotal number of bonds between the branching point and the sticky end for linear, doubler, trebler, respectively. ^bLinear system has a single A unit as flexor.

Figure S45. Based on the chemical structures of the branching units, it is not clear whether the enhanced cooperative melting behavior (see Figure 1B in main text) in the trebler system is best attributed to the additional sticky ends or the flexibility imparted by these strand modifications. To determine which of these characteristics was causing such behavior, variations to the length and identity of the flexor region were tested through the incorporation of hexa(ethylene glycol) units ([PEG6]₂; spacer phosphoramidite 18, Glen Research) before the sticky ends in place of the more rigid A- and T₃-bases used previously for the linear and trebler linkers, respectively. (A) Comparison of T_m 's for binary 10 nm systems consisting of linear and trebler linkers upon the incorporation of different flexor types (i.e., unpaired bases or hexaethylene glycol units). (B) Melting transition breadths (full-width-at-half-maximum, FWHM) for same systems compared in (A).

Analysis of the corresponding melting transitions showed minor differences between the trebler systems (blue traces, T₃ flexor; green traces, PEG flexor) in terms of transition temperatures and breadths, both of which possess an elevated T_m and sharpened transition with respect to the traditional linkers (black traces, A flexor). In contrast, the incorporation of a PEG flexor into a linear linker system (red traces) led to a noticeable decrease in T_m while maintaining a similar transition breadth. This drop can be attributed to a decrease in the local concentration of strands at the periphery of the particle since the sticky ends are now able to sample a larger volume than when using an A-flexor.^{S3} However, when branching is introduced such a decrease in melting temperature is not observed. This can be attributed to the high local concentration of sticky ends regardless of the additional source of flexibility in the linker. Modulations in the number of linkers added per particle reinforced these general trends: trebler systems always have higher T_m values and sharper melting transitions than linear ones.

However, variations to the number of linkers added also provide an example of the increased tolerance of multivalent designs; namely, treblers systems are much less sensitive to changes in linker loading than linear analogs (Figure S45; see Tables S4-S8 for full values). When screening a variety of linker equivalents added, the T_m and full-width-at-half-maximum values (FWHM) values of trebler systems only changed at most by 3 °C and 0.4 °C, respectively, while changes in the linear systems are much more significant (6.4 °C and 2.3 °C). The observation that the T_m of a traditional linear-A system (Figure S45, T_m = 45.1 °C, FWHM = 2.1 °C) approaches that of a trebler when using high loadings (100 linker equivalents added to each component) further emphasize the strong correlation between local sticky end concentration and T_m . However, when more flexibility is added to the linear system, this effect diminishes: while the more flexible linear-[PEG6]₂ systems (T_m = 40.0 °C, FWHM = 1.6 °C) also shows an increasing T_m with linker loading, its T_m never approaches that of the trebler analogs. Similar results and patterns were observed for other particle sizes, specifically 15-15 nm and 15-10 nm aggregates (Tables S4-S5). Taken together, these data clearly show the advantage of the trebler systems: they melt at high temperatures and with sharp transition breadths, regardless of the linker loading and flexor type. System with linear linkers, on the other hand, have melting transitions which are more sensitive to these design parameters, showing a large drop in T_m values upon the incorporation of [PEG6]₂ flexors.

Taken together with the SAXS data, the new crystals made with the trebler linkers may appear to have an increased thermal stability that accompanies increased intrinsic repulsions (see SAXS data and discussion in Section S7). This apparent contradiction can be resolved when one considers that the melting experiments are carried out

with an initially disordered aggregate. Such a system is most likely undergoing reorganization to a crystalline state and aggregate melting simultaneously during the temperature ramp, which occurs over a relatively short time frame (i.e., one to a few minutes depending on the breadth of the melting transition). This combination of processes makes it difficult to discern if the suggested repulsive effects are also manifesting themselves in the melting experiments. In contrast, the crystallization experiments were carried out under different thermal conditions since the tube is held for minutes to hours at a temperature a few degrees below the T_m . Under these conditions, the DNA chains can sample many microstates during equilibration and therefore “feel” the repulsive environment within the lattice. Nevertheless, the fact that the melting temperature goes up indicates that the attractive interaction between treblers is stronger than the attractive interaction between linear linkers. One potential explanation for this data is that when bound, the interaction is stronger due to the increased density. However, prior to binding, there are more steric/entropic barriers to overcome in order for binding to occur. This is akin to a typical reaction coordinate where the binding between treblers is more exothermic but has a higher energy barrier than linear DNA binding.

Figure S46. Thermal melting data for 10 nm (A and B) and 15 nm (C and D) systems containing different types of flexors. Both the melting temperatures (T_m 's, A and C) and full-width-at-half-maximum (FWHM, B and D) are plotted as a function of the type of flexor and the linker type is distinguished by the data point shape and color. Notably, the branched linkers have higher melting temperatures than linear ones for all flexor types (except for the system where the flexor is absent) yet maintain similar transitions breadths.

S7. Small-angle X-ray scattering (SAXS) characterization and data analysis

SAXS characterization was carried out on all samples at the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) beamline at the Argonne National Laboratory's Advanced Photon Source (APS). Collimated X-rays with a wavelength of 1.24 Å (10 keV) were used, and exposure times of 0.1-0.2 s to minimize damage to the

sample. A CCD area detector collected scattered radiation to obtain 2-dimensional scattering data that was subsequently radially averaged to yield 1-dimensional plots in the form of intensity versus scattering vector, q :

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

where θ is half of the scattering angle, 2θ , and λ is the wavelength of the X-ray radiation. To obtain the structure factor $S(q)$, the raw scattering data, $I(q)$, was divided by the form factor, $P(q)$. Form factor SAXS data was collected by scanning the free NP building blocks (i.e., without the linker added such that all PAEs are completely dissociated) since scattering from buffer and DNA are negligible compared to that due to the nanoparticles.

Figure S47. Comparison of 1-D SAXS patterns of 10 nm (A) and 15 nm (B) bcc superlattices composed of traditional linear-A (top black traces) and trebler- T_3 (bottom red traces) linkers. The insets are depictions of the respective unit cells.

Figure S48. SAXS data of analogous linear, doubler, and trebler systems (CsCl-type superlattices composed of 10 and 15 nm AuNPs, 60 linker equivalents added per NP) containing various types of flexors: (A) either an unpaired A-base for linear systems or T_3 flexors for doublers or treblers; (B) either two PEG flexors units for linear linkers or three units for doublers and treblers ((PEG6) $_2$ and (PEG6) $_3$, respectively)

Williamson-Hall analysis of SAXS patterns

In a typical W-H analysis, lineshape analysis of each SAXS pattern was carried out and the integral breadth value of each peak was plotted as a function of the scattering vector, q . Specifically, the scattering intensity vs. q data was loaded into MATLAB (The Mathworks, Inc.) and divided by the form factor to obtain the structure factor. Next, up

to the first nine peaks were fit simultaneously to the sum of nine Lorentzian functions on a cubic background using a non-linear least squares fitting protocol (Figure S49A). This fit had up to twenty three free parameters (i.e., the amplitude and width of each peak, four coefficients of the cubic background, and the location of the q_0 peak). Because the relative position of each scattering peak is known for a given crystal symmetry, the position of each peak beyond the first scattering peak is not a free parameter. For a fit with so many free parameters to be successful, seed values for each of the free parameters were rigorously determined by fitting each peak independently. Following determination of the breadth of each peak, the breadth as a function of location in q -space was fit to a line (Figure S49B). This analysis, which forms the heart of the W-H analysis, allows one to separate the broadening due to grain size, which is q -independent, from the q -dependent broadening originating from microstrain. Specifically, the y-intercept of the line quantifies the average grain size and its slope represents the amount of strain in the lattice. We note that the error bars for strain are based on the curve fitting to the integral breadth peaks. However, because the error associated with each of these points is taken into account during fitting, the error bars represent the propagated errors from the whole analysis process and thus a confidence interval of the fitting.

Figure S49. Images demonstrating the steps involved in Williamson-Hall analysis. (A) Lineshape analysis of each SAXS pattern was carried out such that up to the first nine peaks were fit and the corresponding integral breadths were calculated. (B) These integral breadth values were then plotted as function of their respective positions in q -space and fit to a line where the y-intercept of the line quantifies the average grain size and its slope represents the amount of strain in the lattice.

Figure S50. Plot of microstrain values obtained from W-H analysis for 10 nm (A) and 15 nm (B) bcc superlattices, when both particles were loaded with linkers in a 1:1 ratio, as a function of ΔT (i.e., the temperature at which each sample was annealed relative to its T_m). The use of treblers enables high-quality crystal formation when annealed several degrees below the T_m , whereas linear linkers must be annealed right at or just above their respective T_m values. Each data point represents an individual system, distinguished by point shape and color for the various linker types employed. (In such a plot, systems that appear to have different strains at the same ΔT value are unique assemblies that were annealed at different absolute temperatures.) The error bars correspond to the error associated with the W-H analysis.

Figure S51. Depiction of the Th_3P_4 unit cell and “coordination sphere” of 15 nm particle in the lattice. When A and B denote 10 and 15 nm particles, the existence of the Th_3P_4 structure indicates that the interactions between adjacent A particles (A to A) are different from those between B particles (B to B), which cannot be realized when linear or doubler linkers are employed and form a CsCl-type structure. For these two linker types, the energy gained from DNA hybridization is greater than the energy loss associated with the minor contributions and differences from like-particle repulsions (i.e., A-A and B-B). In contrast, the formation of Th_3P_4 with trebler linkers suggests that the notable asymmetry (or difference) between A-A and B-B interactions are appreciably large such that the energy loss due to like-particle repulsions, that was previously neglected in the case of CsCl, can no longer be ignored and potentially contributes energetically at a level similar to the energy gained by DNA hybridization. Altogether, this means that the formation of this structure may be due to a balance of the overall number of A-B pairs being reduced to accommodate the different A-A and B-B repulsions.

Figure S52. Small-angle X-ray scattering (SAXS) data probing the role of linker equivalents added in the formation of the Th_3P_4 lattice using both T_3 (A) and $[\text{PEG}6]_2$ flexors (B) after the trebler branching point on 10 and 15 nm AuNPs. The notation of each trace, from left to right, includes the observed symmetry, the total number of linkers added, and the number of linkers added to the 10 and 15 nm particles. These data show that CsCl structure is favored in the low-loading regime while Th_3P_4 lattice is favored when higher loadings are employed. However, the thresholds for the shift in observed symmetry differ as a function of flexor type: the more rigid T_3 flexors induce Th_3P_4 formation at lower linker values relative to the more flexible $[\text{PEG}6]_2$ flexors. Given the fact that Th_3P_4 is a less-dense structure than CsCl, these trends suggest that a repulsive force, most likely steric in nature, induces formation of the less-dense lattice when the particles are densely loaded with linkers.

Figure S53. Small-angle X-ray scattering (SAXS) data for the in-situ experiment that was carried out on a thermal stage to probe the thermal stability of the Th_3P_4 lattice. The sample (15 nm AuNP with 180 linkers added, 10 nm AuNP with 90 linkers added) was assembled at room temperature and exposed to the X-ray beam (top pattern) before being heated to a temperature just below its characteristic T_m (determined by UV-vis melting). Upon increasing the temperature from 40.0 °C to 43.5 °C, a gradual sharpening and delineation of peaks was observed, indicating superlattice formation. The peak highlighted in the grey box that disappears upon annealing corresponds to a CsCl structure while the other peaks can be indexed to the Th_3P_4 lattice. These data confirm that this new phase is indeed the thermodynamically preferred structure compared to CsCl.

S8. References.

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