Supporting Information

Entropy-driven crystallization behavior in DNA-mediated nanoparticle assembly

Ryan V. Thaner,^{§,†,▽} Youngeun Kim,^{§,‡,▽} Ting I.N.G. Li,^{§,‡,▽} Robert J. Macfarlane,^{‡,||,▽} SonBinh T. Nguyen,^{‡,▽} Monica Olvera de la Cruz,^{‡,▽} and Chad A. Mirkin^{,‡,‡,▽}*

[†]Department of Chemistry, [‡]Department of Materials Science and Engineering, [▽]International Institute of Nanotechnology, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3113, United States

Corresponding Author

* Email: chadnano@northwestern.edu

§ These authors contributed equally

|| Present address: Department of Chemistry, California Institute of Technology, 1200 East California Boulevard, Pasadena, California 91125, United States

Keywords: DNA, nanoparticle superlattice, nanomaterials, colloidal crystals, self assembly

Experimental Procedure

Oligonucleotide Design and Synthesis

All oligonucleotides used in this work were synthesized on a Mermade 48 (MM48) DNA synthesizer (BioAutomation) using reagents purchased from Glen Research (Sterling, VA) unless noted otherwise. All syntheses were carried out from the 3' direction using controlled pore glass (CPG) beads possessing 1 μ mol of either adenine, cytosine, or thymine attached to the surface (1000 Å or 2000 Å pore sizes were used depending on the length of strand). Standard β -cyanoethyl phosphoramidites were used for coupling bases onto the growing strands (dA-CE, Ac-dC-CE, dmf-dG-CE, dT-CE) as well as two modifications for the thiolated strand (3'-Thiol-Modifier C3 S-S CPG; DMT-hexaethyloxy-Glycol phosphoramidite, ChemGenes (Wilmington, MA)).

Each oligonucleotide linker strand contained a (i) “recognition region” that is complementary to the particle-bound propyl thiol-modified strand, (ii) a “sticky end” that engages in interparticle hybridization, and (iii) a “spacer region” situated between the “recognition region” and “sticky end” to control the overall length of the linker strand.¹

All oligonucleotide linkers had self-complementary sticky ends (-GCGC) in this work. A single adenine base “flexor” was located between the recognition region, spacer region, and the sticky end, flanking each segment of the linker to provide flexibility during PAE assembly. The length of the linker strand was increased by incorporating 20-mer blocks between the recognition region and the sticky end. To impart rigidity to the linker strands, complementary duplexed blocks were added in the appropriate stoichiometry based on the linker used (i.e., $n = 0 - 4$) and allowed to hybridize to the spacer region.

Table S1. Oligonucleotide Sequences Used for AuNP Crystallization

Name	Sequence (5' → 3')
Thiolated strand	AAC AAT TAT ACT CAG CAA [EG ₆] ₂ (-SH)
Linker strand	TTG CTG AGT ATA ATT GTT A (TTT AGT CAC GAC GAG TCA TT A) _n GCGC
20-base block	AAT GAC TCG TCG TGA CTA AA
41-base block	AAT GAC TCG TCG TGA CTA AAT AAT GAC TCG TCG TGA CTA AA

* [EG₆] designates one DMT-hexaethyloxy-Glycol phosphoramidite incorporated into the sequence

** n indicates integer values ranging from 0 to 4 used in this work.

Oligonucleotide Purification

Synthesized oligonucleotides were deprotected according to manufacturer-recommended standard deprotection protocols. Short oligonucleotides (approx. 45 bases or less) were synthesized with the 5' dimethoxytrityl (DMT) protecting group still attached and then purified using reverse-phase high performance liquid chromatography (HPLC, Varian ProStar). Purifications were performed on a Varian Microsorb C18 column using a gradient method starting with 30 mM triethylammonium acetate aqueous buffer (TEAA) at pH 7 ramping at 1% acetonitrile (CH₃CN) per minute to pure acetonitrile at a flow rate of 3 or 15 mL/min, while monitoring the UV signal of the oligonucleotides at 254 nm and 280 nm. After HPLC purification, the oligonucleotides were lyophilized and resuspended in nanopure water (Milli-Q Water Purification System, Millipore).

All oligonucleotides longer than 45 bases were purified using denaturing polyacrylamide gel electrophoresis (PAGE). An 8% PAGE gel stock (500 mL; 8 M urea) was prepared with 240 g of Urea (Invitrogen), 50 mL of 10x Tris/Borate/EDTA buffer (TBE), and 100 mL of 19:1 acrylamide:bisacrylamide (Bio-Rad; Hercules, CA). In order to induce polymerization, 60 mL of the 8% PAGE gel stock was pulled into a syringe, then 440 µL of a 10% ammonium persulfate solution (APS; Sigma-Aldrich; Milwaukee, WI) followed by 44 µL of N,N,N',N'-tetramethylethylenediamine (TEMED; Bio-Rad) were added and mixed by gently inverting the syringe several times. The solution was then

poured between two gel plates (20 cm x 20 cm x 3 mm glass plates; Bio-Rad) and allowed to polymerize for 30 minutes. Once it solidified, the gel was clamped into a vertical electrophoresis chamber, filled with 1X TBE, and preheated at 350V for 15 minutes. Meanwhile, the oligonucleotide samples were mixed with an equal volume of denaturing loading dye (8 M urea, bromophenol blue; Bio-Rad) and heated to 70°C for 15 minutes. After preheating, the well was cleaned by gently pipetting the running buffer to remove loose gel pieces and precipitated salt before the oligonucleotides were loaded, then run at 350V for 70 minutes. Upon completion, the gel was removed from the plates and stained (SYBR Gold, Life Technologies) then imaged (Fujifilm FLA-5100; Figure S1) so that the desired band could be cut out and extracted in nanopure water over the course of 24-48 hours. The extracted solution was then concentrated down using Amicon Ultra centrifugal filters (10 K MWCO, Millipore) and washed multiple times to remove excess salt.

Figure S1. Representative image (n=2 linker, 65 bases, used as an example) of a purification scale polyacrylamide gel used for strands longer than 45 bases. A nucleic acid stain and imaging system were

used to visualize the bands prior to the product band (top, dark band shown here) being cut out and extracted in water.

All strands were characterized using matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) on a Bruker AutoFlex III MALDI-ToF mass spectrometer (Bruker Daltonics, Billerica, MA) using negative ions in the linear mode. The matrix was prepared by dissolving 30 mg of 3-hydroxypicolinic acid (Fluka) in 1 mL of a 1:1 H₂O:MeCN solution, which then was used to dissolve 10 mg of ammonium citrate dibasic (Sigma). Equal portions (2 µL) of this matrix were then mixed with each strand and then an aliquot of the resulting mixture (2 µL) was dropped on a steel MALDI-ToF plate and allowed to dry.

Nanoparticle Functionalization and Assembly

All gold nanoparticles (AuNPs) were purchased from Ted Pella (BB International; Cardiff, UK). Thiolated strands were treated with a solution of 0.1 M dithiothreitol (DTT, pH=8) for 1 hour and purified using size exclusion columns (illustra NAP columns, GE Healthcare) to separate the DTT from oligonucleotides.² The eluted strands were then added to the gold sols in a ratio of 1 optical density unit (OD) at 260 nm per mL of particles and allowed to incubate overnight. These DNA-AuNP conjugates were then “salted” via stepwise additions of 1% sodium dodecyl sulfate (SDS), 1.0 M sodium phosphate (pH = 7.4), and aliquots of 2.0 M NaCl, followed by 10 seconds of sonication until the final salt concentrations of 0.5 M NaCl, 10 mM NaPO₄, and 0.01% SDS were reached. The solutions were allowed to sit overnight in order to increase the density of oligonucleotides on the particle surface by shielding against electrostatic repulsion between strands.³ Excess oligonucleotide strands were removed by centrifugation, 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 NaCl. The absorbance of AuNPs and oligonucleotides were measured on a Cary 5000 UV-Vis-NIR

spectrophotometer (Agilent; Santa Clara, CA) using known extinction coefficients from the Ted Pella and Integrated DNA Technologies (IDT) websites.

Linker stocks were prepared at 10-20 μM in 0.5 M NaCl by adding the linker, the appropriate number of complementary blocks based on the linker length, 2 M NaCl (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 NaCl. To ensure that the complementary blocks fully hybridized with the linkers, the solutions were either heated at approximately 45°C for an hour or left overnight at room temperature. Linkers were then added to DNA-functionalized AuNPs to assemble the particles and aggregate formation was typically visible to the naked eye via a color change from red to purple within a few minutes of mixing. These aggregates were then annealed in two separate ways: (1) a “slow-cool” annealing using a temperature cyclers (Life Technologies) in which the initial temperature is raised above the samples’ characteristic melting temperatures (T_m) and slowly ramped (0.1°C per 10 minutes) to room temperature, and (2) a “regular” thermal annealing in which the sample is heated to a temperature slightly below its T_m . The T_m of a particular sample was determined using a thermal application on the abovementioned UV-Vis-NIR spectrophotometer, monitoring the extinction of the AuNPs at 520 nm while increasing the temperature from 25°C to 65°C at a 0.25°C/min temperature and data collection rates. Cuvettes equipped with stir bars were employed to ensure that the sample did not precipitate out of the path of the beam and the T_m was determined by taking the maximum value of the first derivative of the melting curve.

Small Angle X-ray Scattering (SAXS) Characterization

SAXS characterization was performed on all samples at the Argonne National Laboratory’s Advanced Photon Source (APS), at the DuPoint-Northwestern-Dow Collaborative Access Team (DND-CAT) beamline. 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.⁴ To obtain the structure factor $S(q)$, the raw scattering data, $I(q)$, was divided by the form factor, $P(q)$. Form factor data was collected via SAXS 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.¹

Dynamic Light Scattering (DLS) Measurements

The size and size distribution of particles were measured using a Nano ZS Zetasizer system (Malvern Instruments). Since the self-complementary sequences employed in this study induce aggregation almost immediately upon mixing of the linker with the DNA-coated AuNPs, non-self-complementary linkers were used in this experiment such that the sizes of discrete particles in solution could be measured. This strand contained the exact same sequence as the linker listed in Table S1, except that the sticky end was 5' – TTCCTT – 3', which allowed us to probe the effects of both complementary block types on the hydrodynamic radii. To mimic the PAES used in the SAXS experiments with both types of complementary blocks (i.e., two 20-base blocks or one 41-base block), only 10 nm AuNPs were tested. Before each DLS measurement, the sample was diluted in 0.5 M NaCl to an approximate concentration of 30 nM, loaded into a disposable microcuvette, and five measurements were performed for which the mean intensity result was plotted (Figure S2). The 41-base block sample had an average size of 51.3 ± 0.5 nm (Z-average of 47.3 ± 0.45 nm) while the 20-base block sample measured as 45.9 ± 2.1 nm (Z-average of 42.0 ± 1.44 nm).

Figure S2. DLS data of one 41-base block and two 20-base blocks (using a non-self-complementary linker) show that there is a ~ 5 nm difference in the hydrodynamic sizes of PAEs. The 41-base block samples had a larger hydrodynamic size because of the rigid rod-like oligonucleotide linker strands. On the other hand, the 20-base block samples had more flexible oligonucleotide linkers that could access more conformational states, and therefore the overall hydrodynamic size was slightly smaller and the deviation was larger.

Figure S3. SAXS data for the same type of system (10 nm AuNP and $n=2$ linker) but different types of complementary blocks incorporated to modulate the relative rigidities of the linkers. (A) and (B) are

systems shown in Figure 3 of main text where (A) contains two 20-base blocks and yielded a bcc lattice, (B) utilizes the same linker with one 41-base block and formed an fcc lattice, and (C) is the exact same linker design used in previous work: a 65-base linker was hybridized with a 40-base complementary duplexing block, which matched the previously reported experimental observation of fcc lattice formation.⁵

Figure S4. Various linker equivalents were added for each nanoparticle size to test if linker loading affected the observed phase behavior, but only 15 nm AuNPs demonstrated a dependence as seen in the SAXS data. When 100 equivalents of linker strands were added, a bcc lattice formed while the addition of 175 and 250 equivalents led to fcc lattice formation.

Supporting Information References

1. Macfarlane, R. J.; Lee, B.; Jones, M. R.; Harris, N.; Schatz, G. C.; Mirkin, C. A. *Science* **2011**, 334, 204.
2. Hill, H. D.; Mirkin, C. A. *Nat. Protoc.* **2006**, 1, 324.
3. Hurst, S. J.; Lytton-Jean, A. K. R.; Mirkin, C. A. *Anal. Chem.* **2006**, 78, 8313.
4. Cullity, B. D.; Stock, S. R., *Elements of X-Ray Diffraction*. 3 ed.; Prentice Hall: 2001; p 664.
5. Macfarlane, R. J.; Jones, M. R.; Senesi, A. J.; Young, K. L.; Lee, B.; Wu, J.; Mirkin, C. A. *Angew. Chem., Int. Ed.* **2010**, 49, 4589.