Supporting Information

Nanoparticle Shape Anisotropy Dictates the Collective Behavior of Surface-Bound Ligands

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Supplementary Figures

Figure S1. Calculation of the fraction of total ligands bound on a nanostructure in a face-to-face oriented association with another particle of the same shape. These values are determined for several common anisotropic nanostructures and plotted against their sphericity (a measure of how closely a shape resembles a sphere with a value of unity representing a perfect sphere). From left to right, the particles and their dimensions are as follows: sphere (22 nm diameter), rod (40 nm length, 10 nm diameter), cube (40 nm edge length), and triangular prism (40 nm edge length, 7.5 nm thick). The trend of increasing fraction of ligands bound with anisotropy suggests that nanoparticle shape is capable of modulating ligand-mediated interparticle interactions.

Figure S2. Calculation of nanoparticle sphericity, a measure of how closely a shape resembles a sphere, for gold nanorods of 10 and 12 nm diameter (the most common dimensions available)¹ and gold nanoprisms as a function of nanostructure volume. A value of unity indicates a perfect sphere, with lower values indicating greater degrees of anisotropy. In region I, the prisms have the lowest sphericity value and are therefore more anisotropic than all other particle types. In region II, prisms are more anisotropic than 12 nm diameter rods but not 10 nm diameter rods. However, these rods are greater than 80 nm in length, an aspect ratio which is not possible with standard synthesis methods. In region III, 12 nm diameter rods become more anisotropic but have lengths that are too large (245 nm) to achieve *via* normal synthesis approaches. Therefore, within the bounds of currently available techniques, nanoprisms are most anisotropic.

Figure S3. Quantification of the number of oligonucleotides per nanoparticle as a function of nanostructure size (see supplementary methods section for experimental details). In the case of spherical particles, size is represented as the diameter and in the case of prisms, it is represented as the edge length (prism thickness remains constant at ~7 nm). Both the power-function relationship and the specific values correlate well with previous reports.²⁻³ The dotted line denotes spheres (22 nm) and prisms (40 nm) that each have ~270 DNA molecules per particle that were utilized in all hybridization binding studies in this work.

Figure S4. Small angle X-ray scattering (SAXS) patterns from nanoprism aggregates with varying linker to nanoparticle ratios. The legend values denote the percentage of nanoparticle-bound oligonucleotides hybridized with linker oligonucleotides that are responsible for interparticle association. The dotted line is meant as a guide to the eye to demonstrate that the q -values, and therefore interparticle distances, remain unchanged as the number of interparticle linkages are varied. This demonstrates that the distance between particles, and therefore the rise/base-pair value, is independent of the number of connections between nanoparticles.

Supplementary Methods

Nanoparticle Synthesis and TEM Imaging

Triangular prisms (~40 nm) and spherical nanoparticles (~22 nm) were synthesized simultaneously from a single solution following literature methods.⁴ Briefly, a “seed” nanoparticle solution was first prepared by adding 0.25 mL of HAuCl₄ (0.01 M), 0.5 mL of trisodium citrate (0.01 M), and 0.3 mL of ice-cold NaBH₄ (0.01 M) to a vigorously stirring solution of water (18.95 mL). These seed NPs were then heated for 15 minutes between 40 and 45 °C,⁵ allowed to cool to room temperature, and then scanned using a Cary 5000 (Agilent Technologies, Inc.) to determine nanoparticle concentration (using the peak absorbance at the surface plasmon resonance of ~504 nm and an extinction coefficient of 9.696×10^6 , most seed solutions contained ~28 nM of nanoparticles). Meanwhile, a surfactant solution was prepared containing 0.05 M cetyltrimethylammonium bromide (CTAB) and a 50 μM concentration of NaI, which has been shown to be a crucial shape-directing agent in the synthesis of nanoprisms.⁴ A “growth” solution was then prepared by mixing 9.0 mL of the above CTAB solution, 0.25 mL of HAuCl₄ (0.01 M), 0.05 mL of NaOH (0.1 M), and 0.05 mL of ascorbic acid (0.1 M). Seed nanoparticles were then added to the growth solution such that the final concentration of seeds in the total solution volume (9.35 mL) would be ~220 pM. Addition of the seeds initiated the particle synthesis reaction, and in approximately 30 minutes a purple solution containing both 40 nm prisms and 22 nm spheres was available for further modification (Figure S5). Transmission electron microscopy images were obtained using a Hitachi 8100 operating at 200 kV in bright field mode. Samples were prepared by pipetting 10 μl of nanoparticle solution onto a carbon coated copper grid followed by drying under vacuum (Figure S5b).

Figure S5. (a) Optical extinction spectrum of an as-synthesized solution of ~22 nm spheres (532 nm plasmon resonance) and ~40 nm triangular prisms (750 nm plasmon resonance). (b) TEM image of an as-synthesized mixture of ~22 nm spheres and ~40 nm triangular prisms.

DNA Synthesis and Sequences

All oligonucleotides were synthesized in-house using a MM48 Automated Oligonucleotide Synthesizer (Bioautomation) and all phosphoramidite reagents were obtained

from Glen Research. Oligonucleotides containing a terminal thiol group were used to functionalize all nanoparticles and consisted of the following base sequence: 5' AAC AAT TAT ACT CAG CAA-A₁₀-SH 3'. Linker oligonucleotides added to nanoparticle solutions contained an 18-base sequence complementary to the nanoparticle-bound strands, a single unpaired base for flexibility, and a 4-base “sticky end” self-complementary recognition sequence to drive interparticle association and consisted of the following base sequence: 5' TTG CTG AGT ATA ATT GTT-A-TGCA 3'.

Nanoparticle DNA Functionalization

Nanoparticles were functionalized with thiolated oligonucleotides from the CTAB-containing reaction solution according to previously established protocols.⁶ Briefly, as-synthesized nanoparticle solutions were centrifuged twice (10 min, 16100 rcf) and resuspended in water. Thiolated oligonucleotides that had been treated with 0.1 M dithiothreitol (DTT) and purified with a desalting column were then added to the nanoparticle solutions at a ratio of 3 OD₂₆₀ of DNA for every 1 mL of nanoparticle solution. Sodium dodecyl sulfate, sodium phosphate, and sodium chloride were then slowly added to the nanoparticle solutions with final concentrations of 0.01%, 0.01 M, and 0.5 M, respectively. After allowing 12-18 hours for the particles to become fully functionalized, they were centrifuged three times to remove excess unreacted oligonucleotides.

DNA Loading Assay

Calculation of the number of alkylthiol modified DNA strands per particle was done with a combination of TEM, ICP-MS, and a fluorescence-based DNA quantification assay. TEM was first used to determine the particle size and the ratio of prisms to spheres in a given sample. From the particle dimensions, the number of gold atoms per particle could be estimated using the average volume a gold atom occupies in an FCC lattice. A separate sample of the nanoparticle solution was treated with concentrated nitric acid at 65 °C for ~8 hours to dissolve the particles, and ICP-MS was utilized to determine the gold ion concentration in the resulting solution. The number of DNA strands covalently attached to the nanoparticles' surfaces was subsequently determined by first dissolving a sample of the DNA-functionalized particles in a 1M solution of potassium cyanide, then using a Quant-iT OliGreen ssDNA assay (obtained from Invitrogen) and a FluoDia T70 fluorescence plate reader to quantify the concentration of DNA strands in solution, in accordance with literature protocol and the manufacturer's instructions.^{2, 7} Using these pieces of information (ratio of prisms to spheres in solution, number of gold atoms per particle, concentration of gold atoms in solution, concentration of DNA strands in solution), the number of DNA strands per particle could be calculated; these results are presented in Figure S3.

Nanoparticle Hybridization Analysis

As-prepared DNA-functionalized nanoparticle solutions containing both prisms and spheres were usually concentrated into 1 or 2 mL total volumes after the final centrifugation step. A small aliquot from this solution was diluted and used to determine the prism and sphere

nanoparticle concentrations by UV-vis spectroscopy; extinction coefficients used for the 22 nm spheres and 40 nm prisms were approximately 1.3×10^9 and 4.5×10^9 $\text{M}^{-1}\text{cm}^{-1}$, respectively. Linker oligonucleotides were then added to the concentrated nanoparticle solution at a ratio of 0.8 relative to nanoparticle-bound strands, resulting in immediate aggregation of the nanoparticles. This stock solution was then annealed at 60 °C (above the dehybridization temperature of the linker/NP-bound DNA duplex ~ 58 °C) for 30 min, and allowed to slowly cool to room temperature to ensure a homogeneous distribution of linkers on all the nanoparticles. This stock solution was then diluted to cover a range of nanoparticle concentrations for hybridization experiments (NP concentrations varied from 674 to 33.7 pM for spheres, and 134 to 6.7 pM for prisms). Thermodynamic transition temperatures (T_m) for nanoparticle association were determined by cooling solutions containing prisms, spheres, and linker oligonucleotides from 60 to 10 °C over the course of 8-9 hours, while simultaneously monitoring the surface plasmon resonance of each particle type ($\lambda_{\text{max}} = 530$ nm for spheres, $\lambda_{\text{max}} = 750$ nm for prisms, Figure S5a). Large spectral shifts in the resonance wavelength upon particle association away from those being monitored allow for accurate discrimination between the dispersed and aggregated states.⁸ This was accomplished using a Cary 5000 spectrophotometer (Agilent Technologies, Inc.) with Temperature Controller accessory using a data acquisition rate of 0.25 °C/min and a cooling rate of 0.1 °C/min. Transition temperatures were determined by the inflection point in each hybridization curve and were run in triplicate for each of 10 different prism and sphere particle concentrations.

It is important to note that the particle hybridization analysis was conducted using mixed solutions containing both prisms and spheres. Separation of the two particle types of this size is extremely challenging, as they are of similar mass. However, there are several ways in which one can confirm that using a mixture of particles does not affect the thermodynamic results. First, the hybridization data show that all the nanoprism particles are fully associated before spherical particles are capable of interacting (Figure 2a). Thus, one can conclude that there are very few, if any, relevant interactions between prisms and spheres as the reaction is essentially two-staged. Second, this result is corroborated by SAXS patterns of solutions under similar conditions, which show the appearance of prism aggregate peaks before those corresponding to spherical particle aggregates. Furthermore, scattering peaks have never been observed that can be attributed to prism-sphere connections, indicating that if such structures do exist in solution, they are a minor product compared to the prism-prism and sphere-sphere structures.

Calculation of Equilibrium Constant

Thermodynamic parameters for the hybridization of prisms and spheres were determined from a linear interpolation method based on the following equation⁹⁻¹¹:

$$\frac{1}{T_m} = \frac{R}{\Delta H^\circ} \ln(C_{\text{NP}}) + \frac{\Delta S^\circ}{\Delta H^\circ}$$

Where T_m is the hybridization transition temperature, ΔH° the enthalpy of NP hybridization, ΔS° the entropy of NP hybridization, C_{NP} the nanoparticle concentration, and R the ideal gas constant. Linear fits of plots of $1/T_m$ as a function of $\ln(C_{NP})$ allow for determination of $R/\Delta H^\circ$ from the slope, and $\Delta S^\circ/\Delta H^\circ$ from the intercept (correlation coefficients for the curves in Figure 2b are both greater than 0.95). Using these values of ΔH° and ΔS° , one can determine the Gibbs free energy (ΔG°) from the following equation:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

Finally, values of ΔG° can be used to determine the equilibrium constant (K_{eq}) from the following equation:

$$K_{eq} = e^{-\frac{\Delta G^\circ}{RT}}$$

A comparison of all the thermodynamic binding parameters for prisms and spheres is provided in table S1.

	Spheres	Prisms
ΔH° (kJ/mol)	-295.3	-857.6
ΔS° (J/mol·K)	-776	-2538
ΔG° (kJ/mol)	-63.8	-101.1
K_{eq} (M⁻¹)	1.56×10^{11}	5.33×10^{17}

Table S1. Summary of thermodynamic parameters for nanoparticle hybridization for 22 nm spheres and 40 nm prisms.

DNA Sticky End Effective Concentration Measurement

The binding constants for DNA linker sticky ends presented in Figure 3 were determined in a similar manner to the nanoparticle binding constants. However, in this case, the concentration of linker oligonucleotides was varied while the nanoparticle concentrations were kept constant at 353 pM for spheres and 67.5 pM for prisms. This allowed the number of thiolated strands bound to prisms or spheres to remain unchanged, while permitting the number of linkers, and therefore the number of sticky ends, surrounding the particles to be varied (linker strand to particle-bound strand ratios of 1, 0.8, 0.6, 0.4, and 0.2 were used to construct the curves in Figure S6). A similar hybridization analysis as described above allowed for the determination of transition temperatures as a function of sticky end effective concentration, from which K_{eq} values could be calculated. After first calculating the sphere effective concentration according to literature precedent,⁹ it was found that multiplying this value by a factor of 1.3 for the prism samples resulted in excellent agreement between the two curves (Figure S6c). Since the sticky ends should exhibit identical thermodynamic behavior regardless of the nanoparticle scaffold to

which they are anchored, this analysis allowed for the conclusion that the prism effective sticky end concentration was approximately 30% larger than that for spheres.

Figure S6. Comparison of the binding constant of individual sticky end DNA duplexes ($5'$ TGCA $3'$) linking particles as a function of particle shape (note that this is an alternative representation of the data presented in Figure 3 in the main text). Here, the “effective” DNA sticky end concentration is defined by the number of DNA strands divided by limited volume the terminal nucleotides occupy off the particle surface. In this analysis, the effective DNA sticky end concentration surrounding spheres is estimated according to literature precedent, and the prism effective concentration is chosen such that the lines overlap, indicating identical thermodynamic behavior. If the prism effective DNA concentration is chosen to be 0.7 (a) or 1 (b) times that of the spheres, then the binding curves do not match. However, if the prism effective concentration is selected to be 1.3 times that of the spheres, perfect overlap of the curves is achieved (c). This indicates that the prisms are able to elevate the DNA sticky end concentration by 30% over spheres, thus elevating the on-rate for DNA hybridization.

Time-Resolved Small Angle X-ray Scattering Measurements

In order to eliminate any interactions between prisms and spheres in the kinetics experiments, separated solutions of each particle type were obtained following a procedure outlined previously.⁶ For the particle sizes being used in this work, this process was extremely challenging and time consuming; further investigation and optimization will follow in a future publication. SAXS experiments were performed at the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) beamline of the Advanced Photon Source (APS) at Argonne National Laboratory. X-rays of wavelength 1.24 Å (10 keV) were used to probe the sample, and the scattering angle was calibrated using silver behenate as a standard. Two sets of slits were used to define and collimate the X-ray beam and a pinhole was used to remove parasitic scattering. The X-ray beam cross-section measured 1.0×1.0 mm. A Hamilton ML540 stopped-flow apparatus was used to oscillate the samples during time-resolved measurements to ensure homogeneous heat distribution and to prevent overexposure to the X-ray beam. Nanoparticle samples were first allowed to equilibrate to 60 °C to ensure complete dissociation of any DNA-linked nanoparticles, before being rapidly cooled to 20 °C at a rate of 40 °C/min, all under constant oscillation (scattering peaks due to interparticle association were only observed after reaching 20 °C). Exposure times of 0.7 and 1.2 seconds were used for spheres and prisms, respectively, with a maximum temporal resolution of 10 seconds. The scattered radiation was detected with a CCD area detector. Dark current frames were subtracted from all data; scattering of the buffer, DNA, and capillary were negligible compared to the DNA-AuNPs. 2-D SAXS patterns were then

azimuthally averaged and relative scattering intensity was plotted as a function of scattering vector q :

$$q = \frac{4\pi \sin \theta}{\lambda}$$

where θ is the scattering angle and λ is the wavelength of X-ray radiation. Each scan was then divided by the particle's form factor (the scattering pattern for particles that are completely dissociated) and analyzed as a function of time.

Kinetics Analysis

From the time-resolved scattering data, individual peaks (1st order peaks for spheres, 2nd order peaks for prisms) were extracted and analyzed. Each peak was fit to a Gaussian function of the following form using Origin 8.0 (in some cases a linear baseline was subtracted from a peak before fitting to ensure the minimum on the left and right side occurred at the same y -value):

$$y = y_0 + A \exp \left[-\frac{1}{2} \left(\frac{x - x_c}{w} \right)^2 \right]$$

where y_0 , A , x_c , and w are all independent fitting parameters (examples of diffraction peaks fit with this function are given in Figure 4a,b). Using the Gaussian functions determined for each scattering peak, the full width at half maximum (FWHM) could be calculated mathematically and used for determination of the nanoparticle aggregate size according to the Scherrer equation:

$$\tau = \frac{K\lambda}{\beta \cos \theta}$$

where τ is the average crystalline domain size, K is the shape factor and is taken to be 0.9 in all calculations, λ is the X-ray wavelength, β the full width at half maximum, and θ the scattering angle. Repeating this analysis for sphere and prism diffraction peaks at increasing time points allowed for a comparison of the growth of each nanoparticle aggregate. Dividing the domain size by the interparticle distance (determined from the q -value of a particular diffraction peak) allowed for calculation of the number of nanoparticles in an aggregate. From this, the fraction of total particles bound could be found by simply dividing each value by the number of particles in an aggregate at the final time point. The data shown in Figure 4c represents a nanoparticle concentration of 200 pM and a linker concentration of 108 nM. A range of other combinations of nanoparticle and linker concentration were analyzed in this way and all show a marked preference for prism-prism association over sphere-sphere association:

Figure S7. Quantification of fraction of particles hybridized as a function of time for nanoparticle and linker concentrations of: (a) 100 pM and 54 nM, (b) 200 pM and 27 nM, (c) 200 pM and 54 nM, (d) 400 pM and 54 nM.

Rather than look at the fraction of nanoparticles bound as a function of time, one can also compare the one-dimensional growth of an aggregate as a function of time:

Figure S8. Quantification of domain size growth as a function of time for nanoparticle and linker concentrations of: (a) 100 pM and 54 nM, (b) 200 pM and 27 nM, (c) 200 pM and 54 nM, (d) 400 pM and 54 nM.

However, this analysis makes comparison of the prisms and spheres more difficult, as prism aggregates are primarily one-dimensional while sphere aggregates extend in three-dimensions. This has the effect of artificially inflating the kinetics of sphere association, as free spherical particles can associate to any location on a three-dimensional aggregate, while prism aggregates have a directionality (are one-dimensional) and thereby impose a restriction on how free prisms can incorporate into a growing aggregate. Regardless, the data still show an obvious enhancement to the kinetics as a result of nanoparticle shape.

Analysis of Carboxylate Ligand-Functionalized Nanoparticles

Nanoparticles were modified with an oligo(ethylene glycol) carboxylic acid-terminated ligand ($\text{HS}-(\text{CH}_2)_{11}-(\text{EG})_6-\text{OCH}_2-\text{COOH}$, Prochimia Surfaces) using a protocol similar to that for DNA functionalization. First, as-synthesized nanoparticle solutions were centrifuged twice and resuspended in a 0.17 M pH 8 buffered phosphate solution. Next, the carboxylic acid terminated ligand was added to a final concentration of 50 – 100 μM and allowed to react with the

nanoparticles overnight. Removal of excess ligand was accomplished via three rounds of centrifugation and washing with the pH 8 buffer. Addition of concentrated hydrochloric acid was used to modulate the solution pH, resulting in protonation of the terminal carboxylates. To determine the pH dependence, individual solutions of the nanoparticles were brought to a range of pH values and allowed to equilibrate overnight. Extinction spectra of each solution were then correlated to their pH, showing selective aggregation of the prisms at higher pH values than the spheres (Figure S9). This allowed for the construction of Figure 5b in the main text from the peak intensity values for each particle shape.

Figure S9. Extinction spectra of nanoparticle solutions as a function of pH (labeled at right). The dotted lines are meant as a guide to the eye and are placed at the spherical particle surface plasmon resonance (532 nm, left) and the nanoprism surface plasmon resonance (750 nm, right).

Supplementary References

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