Supporting Information

What Controls the Hybridization Thermodynamics of Spherical Nucleic Acids?

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Contents:

S2 – S6: Supporting Experimental Methods

S6 – S7: Supporting Discussion

S7 – S13: Supporting Theoretical Model Development

S14 – S24: Supporting Figures and Tables

S25: References

Supporting Experimental Methods:

DNA Synthesis and Sequences

Oligonucleotides were synthesized using standard solid-phase phosphoramidite chemistry (MerMade-48, BioAutomation). All reagents were purchased from Glen Research and the oligonucleotides were purified by reverse-phase high performance liquid chromatography (HPLC Pro Star, Varian). The oligonucleotide sequences used in this study are shown below.

Sequence Name	DNA Sequence
Thiolated DNA	5' - CTT GAG AAA GGG CTG CCA – A ₁₀ – propylthiol – 3'
9-mer	5' – Fluorescein – TGG CAG CCC – 3'
12-mer	5' – Fluorescein – TGG CAG CCC TTT – 3'
15-mer	5' – Fluorescein – TGG CAG CCC TTT CTC – 3'
18-mer	5' – Fluorescein – TGG CAG CCC TTT CTC AAG – 3'

SNA Synthesis

Spherical Nucleic Acid (SNA) gold nanoparticle conjugates were prepared by combining thiol-modified DNA (3 μ M) with a solution of 13 ± 1 nm citrate-capped gold particles (10 nM, prepared via the Frens method)¹ in Nanopure™ water (18.2 M Ω) for 1 hour at room temperature. Next, Tween 20 (Sigma-Aldrich P9416), phosphate buffer (pH = 7.4), and sodium chloride (NaCl) were added to a final concentration of 0.01%, 5 mM, and 100 mM, respectively. After a 2 hour incubation period, the solution was further aged with additions of NaCl over 12 hours to bring the final NaCl concentration to 300 mM to

complete the functionalization process. Finally, the solution containing the conjugates was centrifuged (13,000 rpm, 25 min) and resuspended in NanoPure water three times to produce the purified SNAs used for all hybridization studies.

Thiolated DNA Strand Quantification

To determine the number of thiolated DNA loaded on each particle, the concentration of gold nanoparticles and the concentration of thiolated DNA in an aliquot of purified conjugates were measured. The concentration of the gold nanoparticles was determined by performing UV-visible spectroscopy measurements (Cary 5000, Agilent). The measured absorbance was related to the concentration of gold particles using Beer's Law ($A = \epsilon bc$), where $\epsilon = 2.77 \times 10^8$. Subsequently, 20 mM KCN (final concentration) was added to oxidatively dissolve the gold nanoparticles and release all thiolated DNA from the gold surface. The concentration of DNA in the aliquot was measured using the QuantiTTM OliGreen[®] ssDNA Reagent and Kit (Life Technologies #07582) in a spectrofluorometer (Jobin Yvon FluoroLog FL3-22, Horiba Scientific). This process was repeated in triplicate for three individual aliquots of SNAs.

Complementary Strand Hybridization

Varying concentrations of fluorophore-labeled complementary strands were hybridized to different aliquots of purified SNA (10 nM). To facilitate hybridization, NaCl and Tween 20 were added to each aliquot to achieve final concentrations of 150 mM and .01%, unless otherwise noted, respectively, and the mixtures were heated up to 70 °C for 30 minutes and slowly cooled to room temperature over 24 hours. At this point,

each mixture contains hybridized as well as unhybridized complementary strands. To purify the SNAs and remove unhybridized strands from the solutions, the SNA mixtures were centrifuged at room temperature (13,000 rpm, 30 minutes), and without disturbing the pellet of gold nanoparticles, the supernatant was removed, and the SNAs were resuspended in a 150 mM NaCl and .01% Tween 20 buffer. Repeating this process three times resulted in the removal of all unhybridized complementary strands.

Fluorescence Assay to Quantify the Number of Hybridized Strands

To quantify the number of strands hybridized to the SNAs, 1 ml of 1 nM hybridized and unpurified SNA mixtures were centrifuged at room temperature (13,000 rpm, 30 minutes). Without disturbing the gold nanoparticle pellet, the supernatant was removed, and the SNAs were resuspended in a 150 mM NaCl and 0.01% Tween 20 buffer. The above process was repeated a total of three times to ensure all unhybridized strands were removed from the mixture. After the last centrifugation and re-suspension in 1 ml of buffer, 20 mM KCN was added to dissolve the gold particles. The total fluorescence of the sample was measured using the spectrofluorometer, and by comparing the sample's fluorescence values against a standard curve, the concentration of complementary strands in the sample was determined. The number of complementary strands hybridized per particle was found by dividing the concentration of complementary DNA strands by the concentration of SNAs in the measured sample. All measurements were made in triplicates from 3 independent samples for each condition.

Assay to Measure Stepwise Changes in Binding Affinity

In order to probe the stepwise changes in binding affinity of 9-mer strands as a function of number of already-hybridized strands, 0, 5, 10, 20, and 25 equivalents of non-fluorophore labeled 18-mer complementary strands were prehybridized to SNAs in 150 mM NaCl and 0.01% Tween 20 buffer. These mixtures were heated to 70 °C for 30 minutes, slowly cooled over 24 hours to room temperature, and purified to remove all unbound 18-mers (see complementary strand hybridization section for details). Next, 1 equivalent of fluorescein-labeled 9-mer strands was added to each of the prehybridized SNAs, and the solutions were equilibrated at room temperature for 24 hours. Fluorescence melts were conducted on each prehybridized SNA conjugate as described in the following section.

Fluorescence Melts

For all melting experiments, the SNAs and complementary strands were allowed to incubate at 10 °C (the starting temperature for the melts) for 2 hours to ensure equilibrium. Melting experiments were carried out on a Jobin Yvon Fluorolog FL3-22 spectrofluorometer at 1 degree increments with a 5 minute equilibration time at each temperature from 10 °C to 75 °C. At least four separate experiments were carried out for each system at each concentration. The average melting temperatures with the standard deviations are shown in Table S2.

40 ss-DNA SNA Synthesis

To synthesize SNAs functionalized with 40 single stranded DNA, 1.5 μ M of thiolated DNA was added in solution with 15 μ M of hexaethylene glycol terminated thiol (OEG, Sigma Aldrich #259268) to 10 nM AuNPs, and the functionalization process was carried out as described in the SNA synthesis section. OEG was chosen as the thiol diluent because of its charge neutrality, water solubility, and minimal steric hinderance; even in a fully extended state, the molecule would be less than 1 nm in length, substantially lower than the thiolated DNA strands. It should be noted that diluent molecules, such as OEG, must be included to keep the AuNP surface fully passivated and particles stable. This OEG diluent allowed SNAs to be synthesized with 40 ± 2 thiolated DNA strands per particle that were stable under the conditions required for all assays in this work.

Supporting Discussion:

It is important to differentiate the negative cooperativity observed in this work for the hybridization of sub-stoichiometric quantities of free oligonucleotides to SNAs from the cooperativity that has been observed previously in the hybridization of two or more SNAs via the addition of linker DNA strands that form polyvalent connections between particles.²⁻³ In the latter case, it is the recruitment of multiple DNA linkages and the entropic stabilization around these strands that accounts for the increase in temperature and sharpness of particle-particle based dehybridization events.³ Increasing the number of linkages between particles results in an increased stability of particle-particle interactions,

which is in contrast to the decreasing stability of individual DNA duplexes on isolated SNAs probed in the current work.

Supporting Theoretical Model Development

We generate SNA conformations via molecular dynamics trajectories using an detailed electrostatic coarse-grained DNA model based on treating DNA as a bead-spring polymer.⁴⁻⁷ Here, we are interested in the hybridization thermodynamics of tethered DNA (tDNA) loaded onto a 13 nm gold nanoparticle at high loading densities (80 strands). At high tDNA loading densities, the concentration of the counterions (ρ) is quite high ~300-700 mM and a weak electrostatics treatment such as simple Debye screening is no longer appropriate. Further, the entropic penalty of the complementary free DNA (cfDNA) strands bringing more counterions to the nanoparticle needs to be accounted for in the binding thermodynamics. For these reasons, we have developed a model that explicitly models tDNA hybridization/melting and has incorporated discrete ions into the model. All hydration forces (entropic and enthalpic) are implicitly included in order to keep the model tractable.

The tDNA and cfDNA are described by the following Hamiltonian:

$$H_{DNA} = \sum_b^{Nb} \frac{k_b}{2} (r_b - l)^2 + \sum_a^{Na} \frac{k_\theta}{2} (\theta_a - \theta_o)^2 + \sum_{i,j \notin b,a}^N \frac{1}{4\pi\epsilon_o} \frac{q_i q_j}{r_{ij}} + \sum_{i,j \notin b,a}^N 4\epsilon \left[\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right] + U_{ions} \quad (1)$$

where b and a is the bond and angle between adjacent beads ($i, j, (k)$) and U_{ions} is the interaction between the DNA and free ions. N_b , N_a , and N are the number of bonds, angles, and beads in the model. The first term represents: (1) the phosphodiester bond

along the DNA backbone, and (2) the thiolate bond between the DNA and the gold surface. The second term is the three-body angle term that accounts for the stiffness of the chain. Unlike previous models, the three-body angle constant k_θ was set to $7k_B T$, since the stiffness of the chain is almost entirely derived from the charged backbone.⁸ The third term is the Coulomb interaction between the ($-1e$) charged backbone beads (excluding bonded neighbors and next-nearest neighbors in angles). A uniform dielectric of $\epsilon=78$ was used in all simulations to screen the charges in an aqueous environment. The fourth term is a Lennard-Jones (LJ) potential to account for the excluded volume of each DNA bead, as well as for the short-range attraction of complementary pairs of DNA beads (see Fig. S3 and Table S3). The LJ potential is also excluded in bonded, neighboring beads. Non-complementary beads interact via the Weeks-Chandler-Andersen (WCA) potential,⁹ which is just the LJ potential shifted to zero at $r=r_{\min}=2^{1/6}\sigma$. The final term is the interaction between the DNA beads and the free ions: Na^+ counterions and Cl^- co-ions. U_{ions} is of the form of the second and third terms in H_{DNA} , except no bonds and angles are excluded.

Each DNA bead has been adopted from the flanking bead model of Knorowski et al.,⁵ a size accurate model that uses flanking beads to require that hybridization events bond in the typical (anti-)parallel configuration. Although the model is not sufficiently chemically detailed to account for the helical secondary structure, it preserves the persistent lengths of dsDNA (~ 50 nm). Further, the beads are decorated with a binding region (H-bond donor/acceptors) and flanking beads to prevent multiple H-bond acceptors from binding. The model has been used to previously predict the phase diagram of SNA assemblies,^{5,8} predict design rules and melting properties of DNA linked prisms,⁶

and crystallization of DNA linked nanocubes.¹⁰ The parameters used for the tDNA and cfDNA are shown in Table S3. All Au-Au bonds were constrained using rigid body dynamics.¹¹

Molecular Dynamics simulations were performed using HOOMD-Blue simulation package.¹² The NVT ensemble (with rigid body dynamics)¹¹ was employed to simulate the binding events between tDNA and cfDNA. A range of cfDNA concentrations (1-130 strands per cell) were studied to investigate the effects of the chemical potential on hybridization. For all the results in this paper, an excess of 50 cfDNA were in each cell. The simulation cell was kept fixed at $L=37.4$ nm for all trajectories. The Coulombic forces were calculated using a pppm-Ewald summation¹³ to separate the long-range and short-range contributions. A short-range Ewald cut-off was set to 13.6 Å, and the mesh size was 3 Å. All calculations were done with a bulk salt concentration of $\rho_{\infty}=150$ mM.

Hybridization Analysis

We use molecular dynamics to thermodynamically sample the tDNA binding events on the SNA surface with all interactions governed by the above model. The tDNA configurations generated in the MD trajectories are separated into ssDNA (single stranded DNA) sites and dsDNA (double stranded DNA) sites, based on the number of base-pairing per strand e.g. the complement needs to be bound to at least 50% the total number of bases. The radius of gyration for each of the sites, both duplexed and ssDNA, are calculated using the gyration tensor and finding the principal axes. The R_G is then defined as

$$R_G = \langle \sqrt{\text{Tr}(S_{lk}^i)} \rangle \quad (2)$$

where S_{lk}^i is the diagonalized gyration tensor of the i^{th} strand, $\text{Tr}(\dots)$ is the trace and $\langle \dots \rangle$ is the ensemble average over all the strands in each set. The R_G of the ssDNA for increasing hybridization fraction is plotted as a normalized probability distribution function (PDF) is shown in Fig. S4.

We fit the $n=0$ (0 %) (no dsDNA) distribution to a generalized Gamma distribution p_n

$$p_n(x) = \frac{2e^{-\frac{(x-\mu)^2}{\beta^2}} \left(\frac{x-\mu}{\beta}\right)^{2\alpha-1}}{\beta\Gamma(\alpha)} \quad (3)$$

where μ , β , and α are the location, scale and shape parameters of the distribution, respectively. $\Gamma(\alpha)$ is the gamma function of the shape parameter. The generalized Gamma distribution is a parent distribution to many of other distributions such as the one parameter exponential and Poisson distributions. We keep fixed both the shape and scale parameters (α , β) allowing only the location parameter (μ) to vary as n increases. The fit parameters are shown in Table S4. The 0% fit was subtracted from the {12%, 23%, 35%, 46%, 58%, 69%, 81%} distributions. If a secondary peak emerged, a second distribution p_n was fit to it. The subtracted PDFs are plotted in Fig. 2b of the main text.

The probability distribution of the hybridized tDNA strands follows the maximally stretched polymer distribution (see Fig. S5). The radius of gyration of a tethered dsDNA molecule can be predicted through the gyration tensor of an extended rod of radius R and length L . The radius of gyration R_G is taken from the eigenvalues of the gyration tensor $\mathbf{S}_{kl}$

$$\mathbf{S}_{kl} = \frac{\int \rho(\vec{r}) r_l r_k d\vec{r}}{\int \rho(\vec{r}) d\vec{r}} \quad (4)$$

The squared radius of gyration R_G^2 then is the sum of the gyrations around the principal axes

$$R_G^2 = S_{xx} + S_{yy} + S_{zz} \quad (5)$$

In the case of a rod, $S_{xx} = S_{yy} = R^2/4$, which can be computed from Eq. 4. Similarly, $S_{zz} = L^2/12$. Taken together, R_G for an extended rod is given by

$$R_G = \sqrt{\frac{R^2}{2} + \frac{L^2}{12}} \quad (6)$$

Reasonable R and L parameters for 18bp dsDNA B-form DNA are $R=12 \text{ \AA}$, $L=61.2 \text{ \AA}$, which leads to an $R_G=19.6 \text{ \AA}$ for an extended rod. In the coarse grained model, the helical conformation of the dsDNA has been smeared out, but the radius of gyration of a helix requires only a small correction to Eq. 6.

$$R_G = \sqrt{R^2 + \frac{L^2+W^2}{12}}, \quad (7)$$

where W is the width of the sugar-phosphate backbone. By comparison, using Eq. 7 and taken with $W=3.3 \text{ \AA}$, the helix has an $R_G=21.4 \text{ \AA}$, a difference of less than 10%. Comparing to Fig. S5, the maximum of the $H(R_G)$ is found to be $\langle R_G \rangle = 27.9 \text{ \AA}$, which includes a 10-base spacer between 18bp dsDNA and Au surface (see Fig. S3). After including a 10 base spacer to the rigid rod prediction, with dsDNA rise per base of $3.4 \text{ \AA}$, yields an $R_G=28.8 \text{ \AA}$, which agrees well with the rigid limit predicted from Eq. 6.

Chain Entropy Analysis

The fit PDFs $p_n(R_G)$ were related to the configurational entropy S_c of each DNA strand via $S_c = k_B \ln p_n$. We plot the change in configurational entropy of the ss-tDNA (including the entropy loss of the $N=18$ counterions per cfDNA strand going from bulk to

the SNA, $\Delta S_{ions} = Nk_B \ln \frac{V_{ds}}{V_{ss}} \approx -1.2Nk_B$) going from 0 occupied strands to n bound targets (Fig. 2b inset). We note that Flory theory predicts there will be two contributions to the configurational entropy: an ideal chain elastic term and an chain-chain interaction term due to the high SNA surface density.¹⁴ In Fig. S6 we show snapshots that distinguish between the high and low- n ds-tDNA. The magnified portion shows the ss-tDNA (purple strands) elongated when surrounded by ds-tDNA (orange strands) in Fig. S6b, while at low- n (< 35) the ss-tDNA configurations are not greatly affected by the ds-tDNA.

In taking the ΔS between states with different bound strands n , we are directly probing the interaction term. At small n ($n < 35$) the p_n distributions were indistinguishable from $n=0$. This could manifest from a limitation in the MD sampling, where the distance between hybridized strands is on average not close enough to interact. Experiments show a change in ΔS by $n \sim 10$. To connect the Van't Hoff measured entropies (small occupation fractions) to the computed entropies (large occupation fractions), we assume $\Delta S_n - \Delta S_0$, to first order, scales with the change in configurational entropy of the *unhybridized* strands,

$$\Delta S_n - \Delta S_0 \sim S_c^n - S_c^0 + \Delta S_{ions} = k_B \ln(p_n(R_G)^\gamma) \quad (8)$$

where S_c^n is the configurational entropy of an unhybridized DNA (ssDNA) strand with n pre-hybridized strands in the SNA. Using the scaling predicted by Daoud and Cotton for polymer brushes, we plot Eq. 8 with $\gamma=3/5$ in Fig. 2b. The scaling behavior predicts well both experimental and simulation data of the entropic loss of the ss-tDNA for the Van't Hoff and p_n data, respectively (Fig. 2b inset - green line). The simplicity of the model belies the complex mechanisms of the cooperativity the SNA displays, with both

electrostatic interactions of the ion clouds and configurations of the bound sites modulating the microstates of the unbound sites. A mechanistic study, using a detailed DNA model, could elucidate the exact mechanism and range of the collective interactions leading to the elongation.

Ion Distributions

The ion distribution that surrounds the tDNA gives insight into the electrostatic environment of highly confined DNA. Previous work has shown that the radial gradient in the “corona” surrounding the gold nanoparticle can be substantial (5x increase from bulk).¹⁵ In this work, we have monitored the radial ion profile for three hybridization fractions {0%; 0 strands, 25%; 20 strands, 75%; 60 strands}. In Fig. S7, we plot the difference between the local and bulk salt concentration ($\rho - \rho_\infty$) as a function of the distance from the surface of the nanoparticle ($\rho_\infty = 0.15$ M). We see that already at 20 strands hybridized there is beginning to be an overlap between the neighboring ion clouds and a corresponding increase from the bulk ion concentration. At 75% coverage the radial decay elongates due to the majority of the tDNA being dsDNA ($l_{\text{DNA}} \sim 15$ nm). This is in agreement with the results for 100% hybridized shown in Ref. 15. We find a $\sim 3x$ increase for 75% coverage, which is substantial enough to change the melting properties of the tDNA in the radial direction, but not enough to see any correlation effects between the ions (hence a renormalization of the entropy).¹⁶

Supporting Figures and Tables:

Table S1: Thermodynamic Analysis from Van't Hoff Plots^a

N	ΔH° (kcal/mol)	ΔS° (kcal/mol·K)	$\Delta G^\circ_{298\text{ K}}$ (kcal/mol)	$K_{\text{eq}, 298\text{ K}}$ (M ⁻¹)
0	-109.7 ± 3.3	-0.305 ± 0.009	-18.8 ± 0.5	$8.5 \times 10^{13} \pm 8.3 \times 10^{13}$
5	-107.0 ± 0.9	-0.302 ± 0.004	-17.0 ± 0.2	$2.7 \times 10^{12} \pm 1.1 \times 10^{12}$
10	-99.9 ± 0.6	-0.281 ± 0.002	-16.2 ± 0.2	$7.4 \times 10^{11} \pm 2.4 \times 10^{11}$
20	-90.1 ± 1.1	-0.251 ± 0.003	-15.3 ± 0.2	$1.4 \times 10^{11} \pm 7.0 \times 10^{10}$
25	$-88.7 \pm .3$	-0.248 ± 0.001	-14.8 ± 0.3	$6.2 \times 10^{10} \pm 2.5 \times 10^{10}$
20*	-93.5 ± 1.2	-0.255 ± 0.003	-17.5 ± 0.2	$6.0 \times 10^{12} \pm 1.8 \times 10^{12}$

^a Thermodynamic quantities determined for the hybridization of a free oligonucleotide to an SNA as a function of the number of pre-hybridized strands, n . 20* Refers to the OEG-modified conjugates (~40 thiolated DNA/SNA) prehybridized with $n = 20$ complementary strands.

Table S2: Melting Data^a

nM	0	5	10	20	25	20 (40-DNA)
0.05	36.60 ± 0.12	31.28 ± 0.09	28.73 ± 0.04	25.84 ± 0.10	24.08 ± 0.02	33.46 ± 0.09
0.10	37.56 ± 0.10	32.28 ± 0.06	30.22 ± 0.04	27.87 ± 0.03	25.47 ± 0.07	35.29 ± 0.12
0.20	39.45 ± 0.18	33.55 ± 0.05	31.55 ± 0.06	28.73 ± 0.06	26.88 ± 0.08	36.19 ± 0.12
0.50	40.60 ± 0.08	35.51 ± 0.07	32.83 ± 0.07	30.71 ± 0.06	28.75 ± 0.06	38.20 ± 0.09
0.70	40.99 ± 0.14	35.76 ± 0.05	33.65 ± 0.10	31.38 ± 0.05	29.44 ± 0.03	38.88 ± 0.10
0.80	41.42 ± 0.10	36.08 ± 0.04	33.89 ± 0.12	31.65 ± 0.06	29.63 ± 0.08	39.15 ± 0.15
0.90	41.62 ± 0.06	36.29 ± 0.05	34.11 ± 0.05	31.89 ± 0.04	29.75 ± 0.04	39.39 ± 0.14
1.00	41.80 ± 0.18	36.39 ± 0.06	34.30 ± 0.04	32.10 ± 0.06	30.60 ± 0.06	39.60 ± 0.09
2.00	42.61 ± 0.13	37.57 ± 0.07	35.73 ± 0.07	33.72 ± 0.10	31.39 ± 0.09	41.12 ± 0.16
5.00	44.98 ± 0.08	39.33 ± 0.13	37.44 ± 0.04	35.37 ± 0.09	33.45 ± 0.04	43.10 ± 0.09

^a 0, 5, 10, 20, and 25 refers to the number of complementary 18-mer strands prehybridized to SNAs functionalized with 80 thiolated DNA strands. 20 (40-DNA) refers to the system where 20 complementary 18-mer strands are prehybridized to SNAs functionalized with 40 thiolated DNA strands.

Figure S1. Melts were conducted on SNAs hybridized with either one 9-mer or twenty-four 18-mer fluorophore-labeled complementary strands. According to the normalized melt profiles of each system and the full width at half maximum plots of each, SNAs hybridized with one 9-mer complementary strand completely dehybridize before SNAs hybridized with twenty-four 18-mer complementary strands start to dehybridize. This indicates that the melting transition of the 9-mer does not overlap with the melting transition of the 18-mer strands. Therefore, in a system incorporating both 9-mer and 18-mer strands, it is possible to determine the thermodynamic transition temperature of the 9-mer from SNAs as a function of the number of pre-hybridized 18-mer strands.

Figure S2. Melting experiments were conducted using 1) SNAs hybridized with 1 equivalent of fluorophore labeled 9-mer complementary strand and 2) free DNA hybridized to the identical fluorophore labeled 9-mer complementary sequence. The normalized melting transition (a) as well as the binding constant (b) for a single 9-mer target to an SNA with no pre-hybridized strands ($n=0$ system) are enhanced relative to the free-strand association of the same duplex.

Figure S3. Model DNA snapshot. The tDNA strands are shown in orange with the first 10 beads closest to the surface being non-binding spacer beads. The next 18 bases have binding beads (magenta center bead) and 2 flanking beads (brown). The terminal bead has 3 flanking beads. The cfDNA strands are shown in teal and have the same structure as the tDNA strands. The counterions, distributed around the cell, are shown in grey. The gold particle is represented by a regular triangular lattice of gold beads projected onto a spherical surface.

Table S3: Potential energy parameters for the tDNA and cfDNA used in Equation 1^a

Parameter	Value
l (DNA-DNA)	3.4 Å
k_b (DNA-DNA)	330 $k_B T$
l (DNA-Au)	6.8 Å
k_b (DNA-Au)	330 $k_B T$
k_θ (DNA-DNA-DNA)	7 $k_B T$
k_θ (Au-DNA-DNA)	0 $k_B T$
ϵ DNA-DNA (non-complementary)	1 $k_B T$
ϵ DNA-DNA (complementary)	5.5 $k_B T$
ϵ DNA- x	1 $k_B T$
σ DNA-[Na,Cl]	5.44 Å
σ DNA-DNA	7.48 Å
σ DNA-Au	6.3 Å
σ Au-Au	5.1 Å
σ [Na,Cl]-[Na,Cl]	3.4 Å
N_{beads} (tDNA)	28 beads (18 binding region)
N_{beads} (cfDNA)	18 beads

^a All Au-Au bonds were constrained using rigid body dynamics.¹¹

Figure S4. The probability distribution function p_n of the radius of gyration for single-stranded tDNA for increasing fractions of hybridization. There are 80 total tDNA strands on the 13 nm Au particle.

Table S4: Gamma distribution fit parameters from Equation 3

Parameter	$n=0\%$	$n=12\%$	$n=23\%$	$n=35\%$	$n=46\%$	$n=58\%$	$n=69\%$	$n=81\%$
α	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
β	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
μ	1.1	1.1	1.1	1.1	1.77	1.80	1.83	1.90

Figure S5. Histogram of the *hybridized* strands for four increasing fractions of hybridized strands $\{n=18, n=46, n=55, n=64\}$. Blue dashed line is the prediction from Equation 5 in the principal moments analysis of a rigid rod $R_G=2.88 \text{ nm}$.

Figure S6. Representative snapshots from MD simulations of SNA hybridized with (a) (10 dsDNA) duplexes and another with 50 dsDNA (b) 18-mer duplexes. Magnified insets show that as the number of duplexes (orange) per SNA increase, the surrounding single strands (purple) become more outstretched (green complement strands have been masked out).

Figure S7. Radial ion profiles from nanoparticle surface to bulk for three hybridization amounts ($n=0$, $n=20$, $n=60$). Bulk salt concentration is $\rho_{\infty}=0.150$ M.

Supporting Information References:

- (1) Frens, G. *Nature-Phys Sci* **1973**, *241*, 20.
- (2) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607.
- (3) Jin, R.; Wu, G.; Li, Z.; Mirkin, C. A.; Schatz, G. C. *J Am Chem Soc* **2003**, *125*, 1643.
- (4) Knotts, T. A.; Rathore, N.; Schwartz, D. C.; de Pablo, J. J. *J Chem Phys* **2007**, *126*.
- (5) Knorowski, C.; Burleigh, S.; Travesset, A. *Phys Rev Lett* **2011**, *106*.
- (6) Kohlstedt, K. L.; de la Cruz, M. O.; Schatz, G. C. *J Phys Chem Lett* **2013**, *4*, 203.
- (7) McCullagh, M.; Prytkova, T.; Tonzani, S.; Winter, N. D.; Schatz, G. C. *J Phys Chem B* **2008**, *112*, 10388.
- (8) Li, T. I. N. G.; Sknepnek, R.; de la Cruz, M. O. *J Am Chem Soc* **2013**, *135*, 8535.
- (9) Weeks, J. D.; Chandler, D.; Andersen, H. C. *J Chem Phys* **1971**, *55*, 5422.
- (10) Knorowski, C.; Travesset, A. *J Am Chem Soc* **2014**, *136*, 653.
- (11) Nguyen, T. D.; Phillips, C. L.; Anderson, J. A.; Glotzer, S. C. *Comput Phys Commun* **2011**, *182*, 2307.
- (12) Anderson, J. A.; Lorenz, C. D.; Travesset, A. *J Comput Phys* **2008**, *227*, 5342.
- (13) LeBard, D. N.; Levine, B. G.; Mertmann, P.; Barr, S. A.; Jusufi, A.; Sanders, S.; Klein, M. L.; Panagiotopoulos, A. Z. *Soft Matter* **2012**, *8*, 2385.
- (14) Daoud, M.; Cotton, J. P. *J Phys-Paris* **1982**, *43*, 531.
- (15) Zwanikken, J. W.; Guo, P. J.; Mirkin, C. A.; de la Cruz, M. O. *J Phys Chem C* **2011**, *115*, 16368.
- (16) Garcia, G. I. G.; de la Cruz, M. O. *J Phys Chem B* **2014**, *118*, 8854.