SUPPORTING INFORMATION

"Three-Dimensional Hybridization" with Polyvalent DNA-Gold Nanoparticle Conjugates

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Supporting Information 1

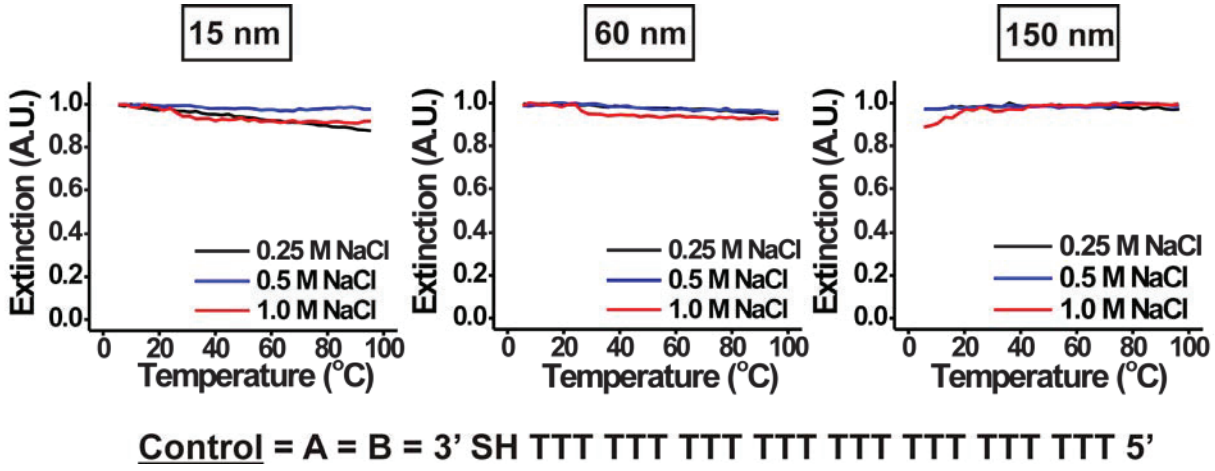


Figure S1. Normalized melting curves (monitored at 260 nm) for 15, 60, and 150 nm DNA-Au NPs functionalized with a control poly-T DNA sequence at 0.25 M, 0.5 M, or 1.0 M NaCl.

Supporting Information 2

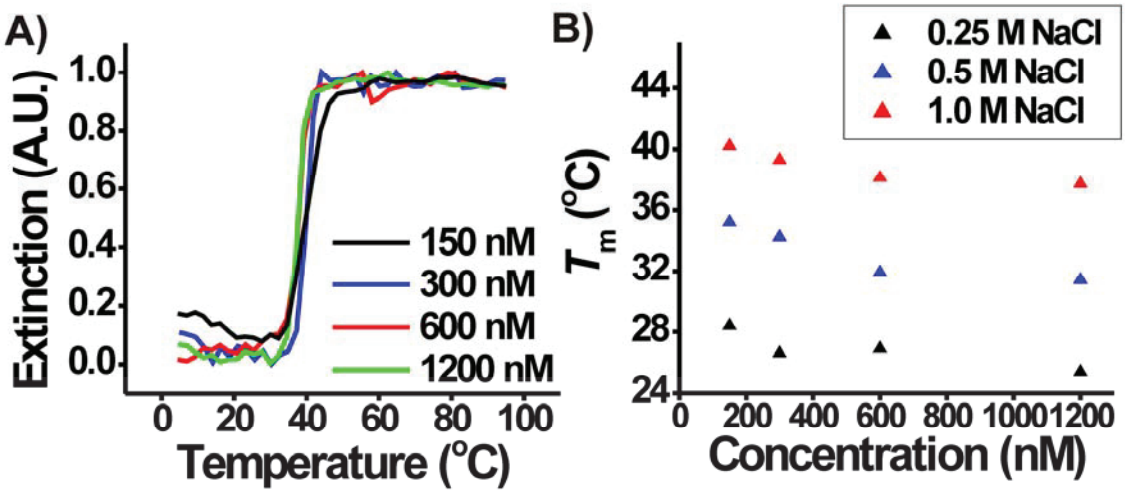


Figure S2. A) Normalized melting curves (monitored at 260 nm) for 15 nm DNA-Au NP aggregates at 1.0 M NaCl for 4 different concentrations (150, 300, 600, 1200 nM) of nanoparticle-bound DNA. **B)** Graph of T_m (°C) vs. nanoparticle-bound DNA concentration (nM) for 15 nm DNA-Au NPs at 0.25 M, 0.5 M, and 1.0 M NaCl. Note that the T_m of the DNA-Au NPs does not change significantly with nanoparticle-bound DNA concentration. All melting information is for the 3 BP DNA sequence.

Supporting Information 3

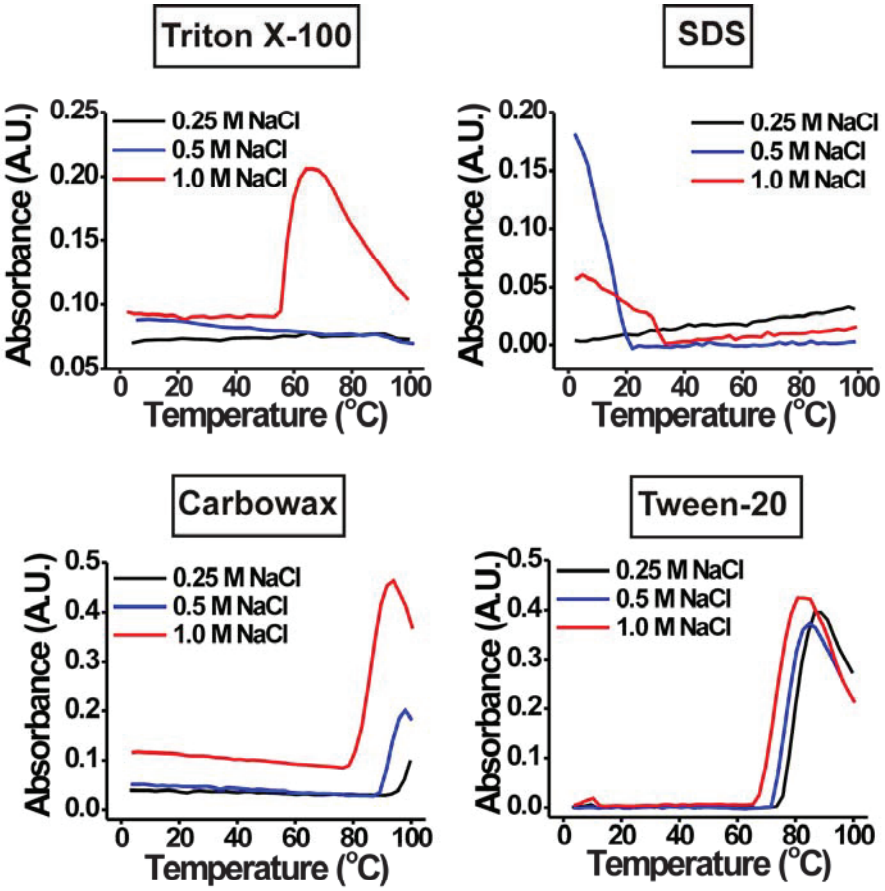


Figure S3. Temperature profiles (monitored at 260 nm) for dilute (0.01 %) aqueous solutions of Triton® X-100, SDS, Carbowax, or Tween-20 in 0.25 M, 0.5 M, and 1.0 M NaCl.

Supporting Information 4

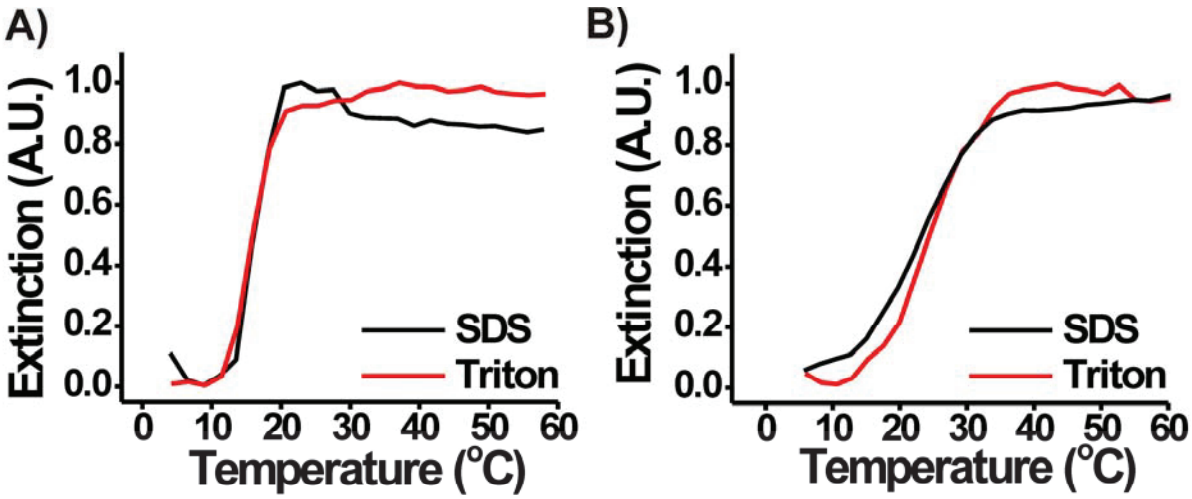


Figure S4. Normalized melting curves (monitored at 260 nm) for 60 nm DNA-Au NP aggregates at 1.0 M NaCl using either 0.01 % SDS or 0.01 % Triton® X-100 as a surfactant. Melting information is for 2 BP DNA sequence with either a **A)** poly-T or **B)** PEG spacer. Note that the T_m of the DNA-Au NPs does not change significantly with the type of surfactant. In **A)**, the T_m of the DNA-Au NP aggregates is 16.3 °C and 15.6 °C for SDS and Triton® X-100, respectively. In **B)**, the T_m of the DNA-Au NP aggregates is 23.1 °C and 24.4 °C for SDS and Triton® X-100, respectively.

Supporting Information 5

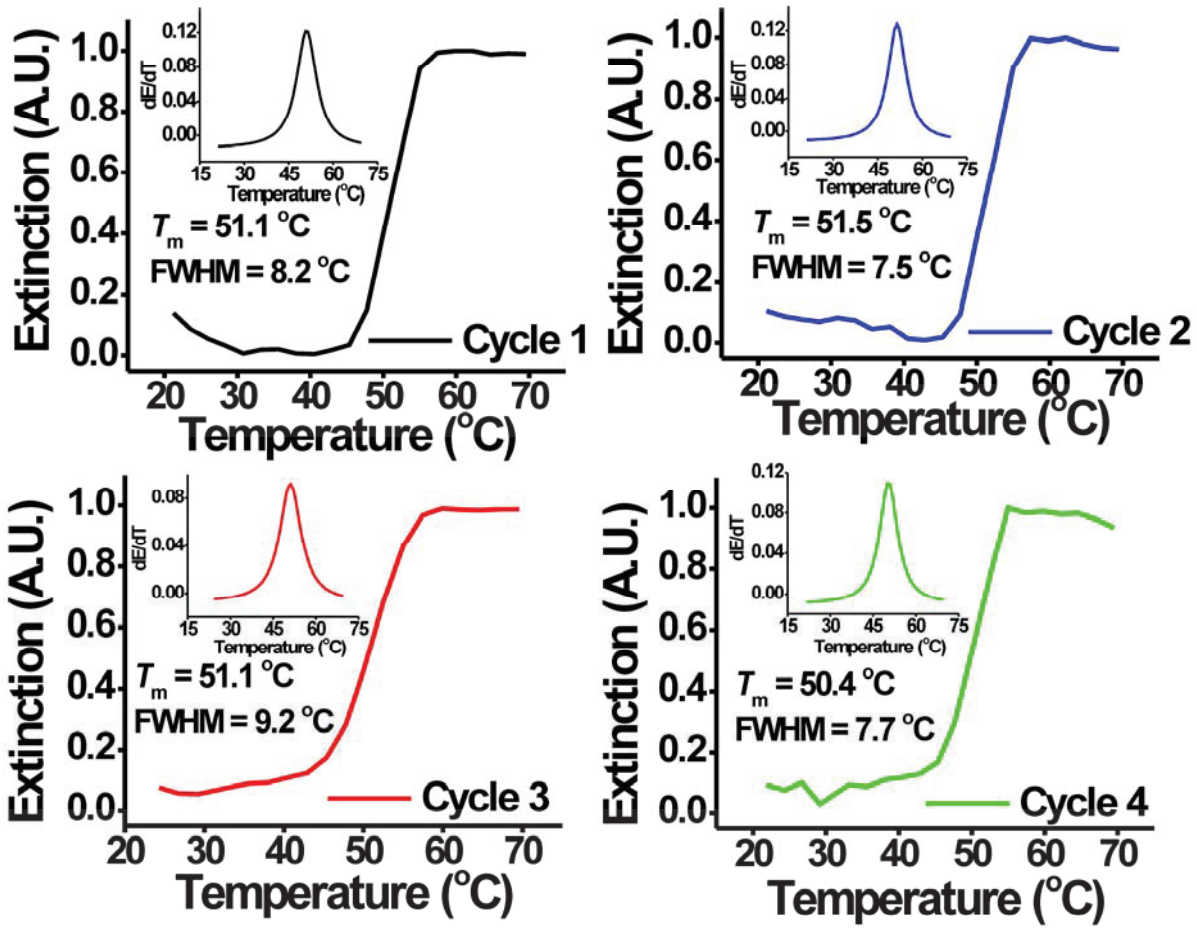


Figure S5. Melting transitions (monitored at 260 nm) for the same sample of 60 nm DNA-Au NP aggregates cycled repeatedly. The salt concentration is 1.0 M NaCl and the 3 BP DNA sequence was used. The inset in each graph shows the Lorentzian fit to the first derivative of the melting transition. The T_m and the FWHM of each curve is listed. Note that the values for the T_m do not change significantly over four cycles, indicating the stability of the DNA-Au NPs to heat and degradation in aqueous media. The same trend was observed for 2 BP using 60 nm nanoparticles.

Supporting Information 6

	T_m (°C)								
	1 BP			2 BP			3 BP		
	0.25 M	0.5 M	1.0 M	0.25 M	0.5 M	1.0 M	0.25 M	0.5 M	1.0 M
15 nm	X	X	X	X	X	X	25.4	31.4	37.7
30 nm	X	X	X	X	X	X	31.2	40.5	46.8
60 nm	X	X	X	X	X	15.6	34.0	45.4	54.1
80 nm	X	X	X	X	11.2	22.6	35.5	48.4	58.7
150 nm	X	X	18.2	X	13.1	28.2	42.9	51.2	63.8

Figure S6. Melting temperatures for all DNA-Au NPs.

Supporting Information 7

	FWHM (°C)								
	1 BP			2 BP			3 BP		
	0.25 M	0.5 M	1.0 M	0.25 M	0.5 M	1.0 M	0.25 M	0.5 M	1.0 M
15 nm	X	X	X	X	X	X	4.2	4.1	4.2
30 nm	X	X	X	X	X	X	0.9	1.6	3.7
60 nm	X	X	X	X	X	5.9	0.7	2.4	4.3
80 nm	X	X	X	X	5.9	6.7	3.5	4.6	5.1
150 nm	X	X	17.3	X	9.5	15.7	3.8	5.5	18.0

Figure S7. FWHMs for all DNA-Au NPs. These values generally increase with increasing salt concentration and nanoparticle size and decrease with increasing number of base pairings.

Supporting Information 8

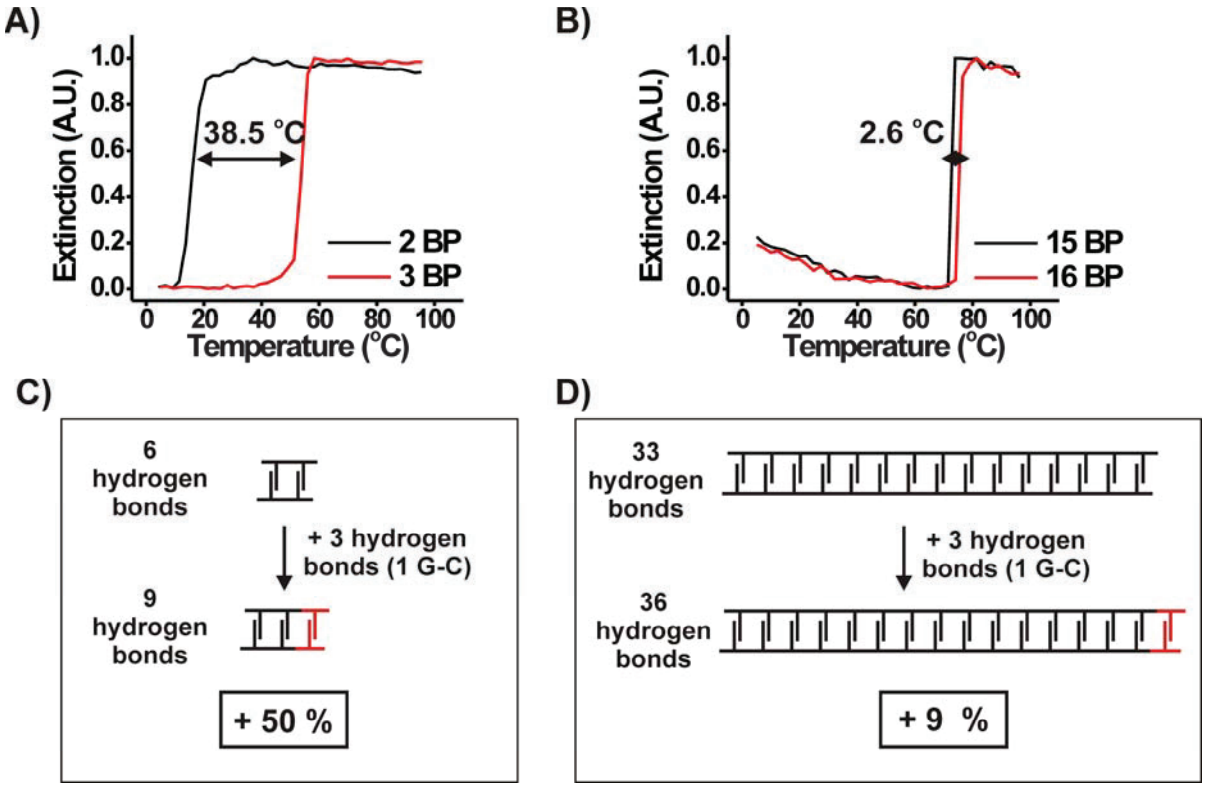
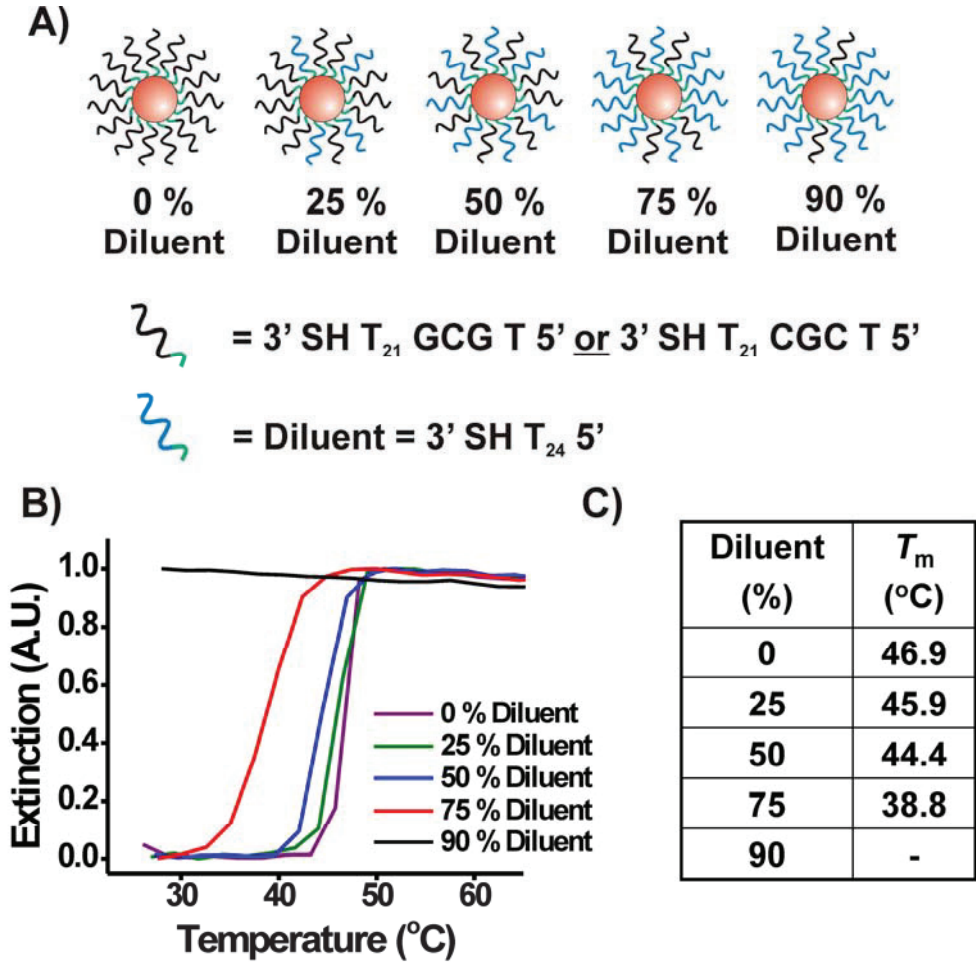


Figure S8. Melting transitions for 60 nm DNA-Au NP aggregates in 1.0 M NaCl. **A)** Melting transitions (monitored at 260 nm) for 2 BP and 3 BP. **B)** Melting transitions (monitored at 260 nm) for complementary 15-mer DNA (A = 5′ SH A_{10} ATC CTT TAC AAT ATT 3′, B = 5′ SH A_{10} AAT ATT GTA AAG GAT 3′) and complementary 16-mer DNA (A = 5′ SH A_{10} ATC CTT TAC AAT ATT C 3′, B = 5′ SH A_{10} G AAT ATT GTA AAG GAT 3′). **C)** and **D)** depict the relative percent increase in the number of potential hydrogen bonds per connection (50 % and 9 %) by adding one additional G-C pair (3 hydrogen bonds) to a 2 BP and 15 BP duplex DNA sequence, respectively. These respective increases in melting temperature as a result of these additions are 38.5 °C and 2.6 °C.

Supporting Information 9



Diluent (%)	T_m (°C)
0	46.9
25	45.9
50	44.4
75	38.8
90	-

Figure S9. **A)** Schematic showing the relative coverage of the diluent DNA strand and the complementary DNA strand on the surface of the gold nanoparticle. **B)** Melting transitions (monitored at 260 nm) for 60 nm DNA-Au NPs with different concentrations of diluent strands on their surfaces (0.5 M NaCl, 10 mM PB, 0.01 % SDS). **C)** Melting temperatures corresponding to the curves shown in **B)**.

Calculation of Equilibrium Binding Constants (K_{eq})

The equilibrium constants (K_{eq}) for the DNA-Au NP systems were calculated using the theoretical model outlined in Ref. 12. Briefly, the total enthalpy of the system (ΔH_{tot}) was determined by fitting the experimental melting curves to

$$f = 1/[1 + \exp[(\Delta H_{tot}/R)*((1/T)-(1/T_m))]],$$

where f is the fraction of the total aggregate in the dispersed state, R is the universal gas constant, and T_m is the measured melting temperature. Then, since

$$K_{eq} = \exp[(\Delta H_{tot}/R)*((1/T)-(1/T_m))],$$

the values of ΔH_{tot} (fitted) and the T_m (measured) could be used to calculate K_{eq} at a given temperature T (298 K). Supporting Table 1 shows the ΔH_{tot} and K_{eq} values obtained using this method for 15 nm DNA-Au NPs (0.25 and 1.0 M NaCl), 60 nm DNA-Au NPs (0.25 M NaCl) and 150 nm DNA-Au NPs (0.25 M NaCl) at 298 K. The melting temperatures used for these calculations are listed in Figure S6.

Supporting Table 1		**ΔH_{tot} (kcal/mol)**	**K_{eq}**
15 nm DNA-Au NPs	**0.25 M**	-311.1	2×10^0
	1.0 M	-138.5	1.4×10^4
60 nm DNA-Au NPs	**0.25 M**	-290.2	2×10^6
150 nm DNA-Au NPs	**0.25 M**	-215.2	9×10^8

To calculate the K_{eq} for the free DNA system, the thermodynamic values determined by Honda et al (Ref. 29) were used (Supporting Table 2).

Supporting Table 2	**$\Delta H°$ (kcal/mol)**	**$\Delta S°$ (cal/mol•K)**
dGC/dCG	-10.5	-26.4
dCG/dGC	-11.8	-29.0
Initiation Penalty	0.6	-9.0
Total for free 3 BP	-21.7	-64.4

We then calculated ΔG using the Gibbs free energy equation:

$$\Delta G° = \Delta H° + T\,\Delta S°,$$

where $\Delta G°$ is the free energy of the system, $\Delta H°$ is the enthalpy of the system, and $\Delta S°$ is the entropy of the system at a given T (298 K). This calculation gave us a value of $\Delta G°^{free} = -2.5 \times 10^3$ cal/mol for free 3 BP (1.0 M NaCl, 298 K). We then calculated the equilibrium binding constant using the following equation:

$$K_{eq} = \exp(\Delta G/-RT).$$

This calculation resulted in a value of $K_{eq}^{free} = 7 \times 10^{1}$ for free 3 BP (1.0 M NaCl, 298 K). Since this value is greater than unity, it suggests that this sequence will form at room temperature under these conditions. However, we have not observed this hybridization/melting behavior experimentally. Likely, this method is overestimating the stability of free 3 BP duplexes. As a result, this might suggest that the differences between the free DNA and DNA-Au NP systems are even larger than we predict here.