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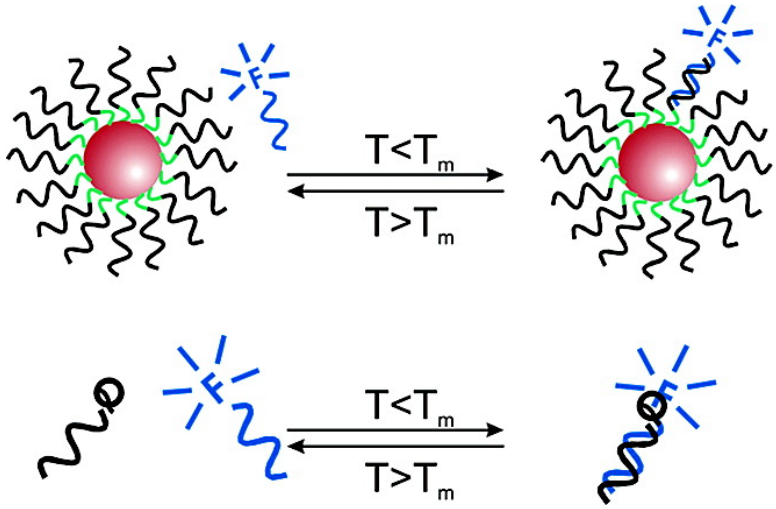

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A Thermodynamic Investigation into the Binding Properties of DNA Functionalized Gold Nanoparticle Probes and Molecular Fluorophore Probes

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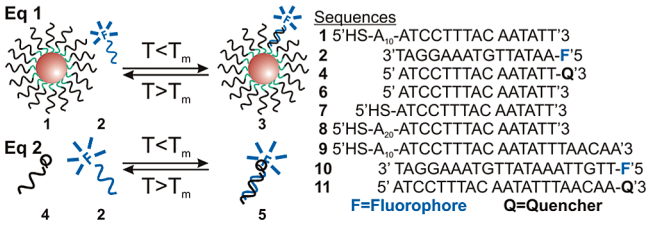
Oligonucleotide functionalized gold nanoparticles, or nanoparticle probes, have become the basis for an increasing number of diagnostic applications that compete with molecular fluorophores in certain settings.[1−12] For these types of systems, detection relies upon the binding events between a DNA target sequence and the probe sequence. For such an associative equilibrium process, a decrease in target concentration will decrease the amount of duplex formed and, therefore, the melting temperature, T_m, for the system. Thus, the equilibrium binding constant between the target and probe can be a fundamental limiting factor for a high sensitivity detection system that does not involve target amplification. Several studies have qualitatively determined that the presence of gold nanoparticle probes results in cooperative melting properties of the probe/target complex, which both sharpen and increase the melting transition of DNA-linked nanoparticle aggregates.[2,13,14] Surprisingly, there have been no thermodynamic studies of such systems aimed at quantifying the differences between the binding properties of molecular fluorophore probes and gold nanoparticle probes. Herein, we report the first study which compares, on a sequence-for-sequence basis, the melting properties of nanoparticle probes and molecular fluorophore probes. These are the first analytical benchmarks for understanding the fundamental and technological differences between gold nanoparticle probes and molecular fluorophores.

Thermodynamic properties were derived through concentration-dependent melting studies.[15−17] In a typical experiment, 13 nm diameter Au nanoparticles functionalized with a 5′-thiol-modified 15-base DNA recognition sequence, containing an A_{10} spacer, **1**, were allowed to hybridize to one equivalent of a 5′-fluorescein-modified 15-base complementary DNA sequence, **2** (eq 1). The concentrations of the nanoparticle probe and complementary fluorophore sequences were varied while maintaining a 1:1 ratio. To obtain comparable melting data for a molecular quencher/molecular fluorophore system, similar experiments were performed with **2**, now acting as the probe, and a complementary 15-base DNA sequence modified with a 3′-dabcyl molecular quencher, **4** (eq 2). All experiments were allowed to equilibrate for over 24 h in 0.3 M NaCl 10 mM PBS buffer. To investigate the effects of the poly-A spacer and the length of the recognition sequence on the binding properties of nanoparticle probes, melting experiments were carried out with multiple types of probes. Poly-A spacers are commonly used to stabilize gold nanoparticle probes and increase their hybridization efficiency by moving the target recognition sequence further from the particle surface. To test the influence of spacers on hybridization thermodynamics, 15-base probes were designed with and without A_{20} spacers, **7** and **8**. To investigate the effects of DNA recognition strand length on such properties, nanoparticle probes were designed with an A_{10} spacer and a 21-base recognition sequence, **9**. Finally, all of these systems were studied and compared with data from analogous molecular quencher/fluorophore systems with identical recognition sequences.

Scheme 1



Binding of nanoparticle probes to a complementary target sequence modified with a molecular fluorophore resulted in quenching and decreased fluorescence intensity.[18] Subsequent heating resulted in dissociation of the probe/target complex and an increase in fluorescence intensity, providing a way to spectroscopically monitor the melting transition (Figure 1A). Melting temperatures, T_m, were determined by taking the maximum of the first derivative of a melting transition measured by fluorescence spectroscopy (Figure 1A). As the concentration of probe and target increased, a corresponding increase in T_m was observed.

Comparison of the A_{10}-15-base nanoparticle/fluorophore and 15-base molecular quencher/fluorophore melting experiments revealed that the nanoparticles typically melted approximately 5 °C higher than the corresponding molecular system. Melting data were analyzed according to literature procedures[15−17] for molecular systems by graphing $1/T_m$ as a function of concentration according to the following equation:

$$\frac{1}{T_m} = \frac{R}{\Delta H^\circ} \ln C_T + \frac{\Delta S^\circ - R \ln 4}{\Delta H^\circ}$$

where T_m is the melting temperature, R is the gas constant, and C_T is the total concentration of nanoparticles plus fluorophore or quencher plus fluorophore (Figure 1B and Table 1).

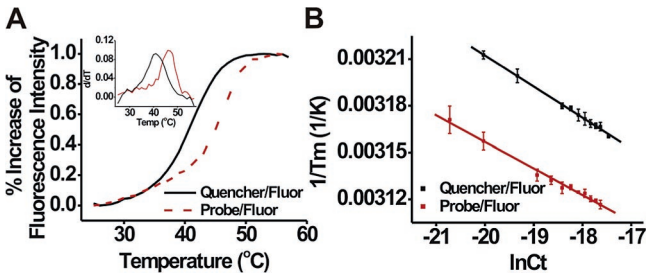


Figure 1. (A) Melting curves of (red) 3 nM A_{10} 15mer nanoparticle probe hybridized to 3 nM 15mer fluorophore, T_m = 46 °C, and (black) 3 nM 15mer quencher hybridized to 3 nM 15mer fluorophore, T_m = 40 °C. (B) Thermodynamic analysis of concentration-dependent melting data for A_{10} 15mer nanoparticle/fluorophore and 15mer molecular quencher/fluorophore experiments.

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Table 1. Thermodynamic Values of Nanoparticle/Fluorophore and Molecular Quencher/Fluorophore Systems

	15mer Q/F	A_{10} 15mer probe/F	A_{20} 15mer probe/F	21mer Q/F	A_{10} 21mer probe/F
$\Delta H°$ (kcal/mol)	-98 ± 2.0	-117 ± 3.9	-109 ± 3.3	-136 ± 9.7	-144 ± 3.9
$\Delta S°$ (kcal/mol·K)	-0.272 ± 0.0056	-0.326 ± 0.011	-0.301 ± 0.0093	-0.377 ± 0.027	-0.397 ± 0.011
$\Delta G°$ (kcal/mol)	-16.7 ± 2.0	-19.4 ± 3.9	-19.2 ± 3.3	-23.8 ± 9.7	-25.5 ± 3.9
K_{eq} (M^{-1} cm^{-1}) at 298 K	1.8×10^{12}	1.8×10^{14}	1.2×10^{14}	2.8×10^{17}	4.9×10^{18}

Analysis of the A_{10}-15-base probe melting data reveals that the nanoparticle probes have a binding constant of 1.8×10^{14}, 2 orders of magnitude higher than the binding constant for the molecular quencher/fluorophore system under identical conditions. At room temperature, this translates to binding of the nanoparticles at concentrations as low as 20 fmol. For the equivalent fluorophore probe, the concentration must be increased to at least 2 pmol before target binding will begin to occur.

There are approximately 100 strands of DNA per gold particle (30 pmol/cm^2).[19] To determine if the enhanced binding strength of the nanoparticle probes was due to the additional DNA bound to the nanoparticle surface or to some other property unique to the nanoparticles, analogous experiments were performed using 100 nm silica particles functionalized with the same sequence as DNA **1** (Supporting Information, ~200 strands of DNA/particle, 1 pmol/cm^2). These particle/fluorophore complexes were found to melt at the same temperature as that of the duplex structures in the case of the molecular quencher/fluorophore experiments. This observation is consistent with the conclusion that the increased binding strength of the gold nanoparticle probes is due to the high density of DNA bound to the gold surface and not the absolute amount of DNA on a particle probe surface.

The presence of the poly-A spacer is important, as predicted earlier in qualitative analyses.[14,19] Removal of the A_{10} spacer dramatically reduced the binding efficiency such that melting transitions were indistinguishable from background fluorescence. On the other hand, increasing the poly-A spacer from A_{10} to A_{20} had very little effect on T_m. This demonstrates that there is an optimum distance between the particle and the DNA necessary to achieve the maximum enhancement of the binding strength. In the absence of the poly-A spacer, the DNA strands are close to the particle surface and to each other, reducing the ability of the target sequence to bind to the probe. Introduction of the A_{10} moves the DNA further away from the particle and alleviates steric hindrance, dramatically increasing the binding strength. Extending the poly-A spacer to A_{20} does little to affect target binding. Increasing the DNA recognition length dampens the enhanced binding strength provided by the nanoparticles. Analysis of the 21-base nanoparticle/fluorophore and molecular quencher/fluorophore experiments determined equilibrium binding constants of 4.9×10^{18} and 2.5×10^{17}, respectively. This is an enhancement of the nanoparticle binding strength of just over 1 order of magnitude.

It is important to note that in actual detection systems nanoparticle and molecular fluorophore probes would not bind to fluorophore/quencher modified DNA. Our model systems do not account for effects of DNA modification; however, it has been shown that modifications may effect duplex stability in some cases.[20] To test this, we examined the melting properties of duplexes with and without modifications. We found that, overall, the relative binding properties of the nanoparticle and molecular fluorophore probes are not significantly affected (Supporting Information).

In conclusion, we have quantitatively determined and compared the thermodynamic values of oligonucleotide functionalized gold nanoparticle probes and molecular fluorophore probes of the same sequence. Nanoparticle probes have a higher binding constant, which increases the sensitivity of assays based upon them, as compared with their molecular counterparts. As the length of the recognition sequence increases, the enhancement of the binding strength diminishes. In designing an effective probe, a balance must be maintained between binding strength and selectivity. Moving to a longer recognition sequence can increase the binding strength but at the cost of selectivity. Nanoparticle probes with a shorter 15-base recognition sequence and the appropriate spacer provide a greater sensitivity than a molecular fluorophore, while maintaining a high selectivity without additional amplification of target sequence.

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Supporting Information Available: Experimental conditions, melting data, calculations, DNA functionalized 100 nm silica particles, and effects of fluorophore/quencher modifications. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607.
(2) Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757.
(3) Rosi, N. L.; Mirkin, C. A. *Chem. Rev.* **2005**, *105*, 1547.
(4) Nam, J.-M.; Stoeva, S. I.; Mirkin, C. A. *J. Am. Chem. Soc.* **2004**, *126*, 5932.
(5) He, L.; Musick, M. D.; Nicewarner, S. R.; Salinas, F. G.; Benkovic, S. J.; Natan, M. J.; Keating, C. D. *J. Am. Chem. Soc.* **2000**, *122*, 9071.
(6) Weizmann, Y.; Patolsky, F.; Willner, I. *Analyst* **2001**, *126*, 1502.
(7) Maxwell, D. J.; Taylor, J. R.; Nie, S. *J. Am. Chem. Soc.* **2002**, *124*, 9606.
(8) Zhao, X.; Tapec-Dytioco, R.; Tan, W. *J. Am. Chem. Soc.* **2003**, *125*, 11474.
(9) Sato, K.; Hosokawa, K.; Maeda, M. *J. Am. Chem. Soc.* **2003**, *125*, 8102.
(10) Li, H.; Rothberg, L. J. *J. Am. Chem. Soc.* **2004**, *126*, 10958.
(11) Su, M.; Li, S.; Dravid, V. P. *Appl. Phys. Lett.* **2003**, *82*, 3562.
(12) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303.
(13) Storhoff, J. J.; Lazarides, A. A.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L.; Schatz, G. C. *J. Am. Chem. Soc.* **2000**, *122*, 4640.
(14) Jin, R.; Wu, G.; Li, Z.; Mirkin, C. A.; Schatz, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 1643.
(15) Morrison, L. E.; Stols, L. M. *Biochemistry* **1993**, *32*, 3095.
(16) Marky, L. A.; Breslauer, K. J. *Biopolymers* **1987**, *26*, 1601.
(17) SantaLucia, J. J.; Turner, D. H. *Biopolymers* **1997**, *44*, 309.
(18) Dulkeith, E.; Morteani, A. C.; Niedereichholz, T.; Klar, T. A.; Feldmann, J.; Levi, S. A.; van Veggel, F. C. J. M.; Reinhoudt, D. N.; Moller, M.; Gittins, D. I. *Phys. Rev. Lett.* **2002**, *89*, 203002−1.
(19) Demers, L. M.; Mirkin, C. A.; Mucic, R. C.; Reynolds, R. A.; Letsinger, R. L.; Elghanian, R.; Viswanadham, G. *Anal. Chem.* **2000**, *72*, 5535.
(20) Moreira, B. G.; You, Y.; Behlke, M. A.; Owczarzy, R. *Biochem. Biophys. Res. Commun.* **2005**, *327*, 473.

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