# One-Pot Colorimetric Differentiation of Polynucleotides with Single Base Imperfections Using Gold Nanoparticle Probes

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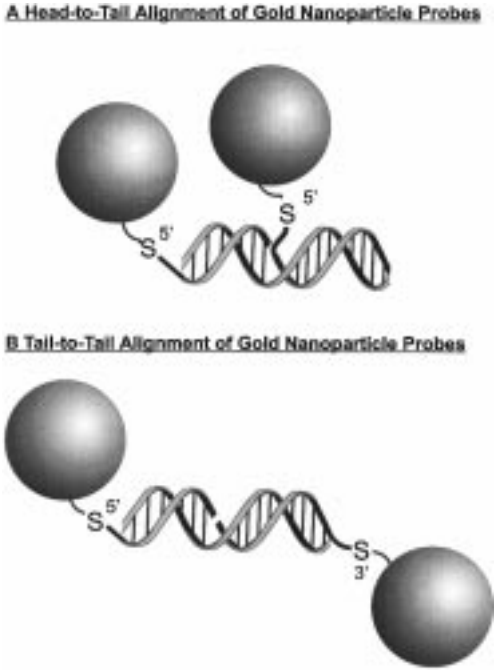
**Abstract:** Selective colorimetric polynucleotide detection based on Au nanoparticle probes which align in a "tail-to-tail" fashion onto a target polynucleotide is described. In this new nanoparticle-based detection system, Au particles (∼13 nm diameter), which are capped with 3′- and 5′-(alkanethiol)oligonucleotides, are used to complex a 24-base polynucleotide target. Hybridization of the target with the probes results in the formation of an extended polymeric Au nanoparticle/polynucleotide aggregate, which triggers a red to purple color change in solution. The color change is due to a red shift in the surface plasmon resonance of the Au nanoparticles. The aggregates exhibit characteristic, exceptionally sharp "melting transitions" (monitored at 260 or 700 nm), which allows one to distinguish target sequences that contain one base end mismatches, deletions, or an insertion from the fully complementary target. When test solutions are spotted onto a C18 reverse-phase thin-layer chromatography plate, color differentiation is enhanced and a permanent record of the test is obtained, thereby providing a better method for distinguishing the aforementioned target sequences. Significantly, one-pot colorimetric detection of the target in the presence of four strands with single base imperfections can be accomplished with this new probe system.

## Introduction

Sequence-specific DNA detection has been a topic of significant interest because of its application in the diagnosis of pathogenic and genetic diseases.[1−3] Many detection techniques have been developed which rely upon target hybridization with radioactive, fluorescent, chemiluminescent, and other types of labeled probes.[4−12] Still other detection techniques employ indirect methods that rely on enzymes to generate colorimetric, fluorescent, or chemiluminescent signals.[13] Recently, we reported a novel method for detecting polynucleotides which utilizes the distance-dependent optical properties of aggregated Au nanoparticles functionalized with 5′-(alkanethiol)-capped oligonucleotides, Scheme 1A.[14] This method has many desirable features including rapid detection, a colorimetric response, good selectivity, and little or no required instrumentation. Herein, we describe a related system based upon Au nanoparticles chemically modified with 5′- and 3′-(alkanethiol)-capped oligonucleotides, Scheme 1B. This system exhibits extraordinary selectivity and provides a simple means for colorimetric, one-pot detection of a target oligonucleotide in the presence of a mixture of oligonucleotides with sequences differing by one nucleotide, regardless of position, in the target region. The

**Scheme 1**

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(1) Razin, S. *Mol. Cell. Probes* **1994**, *8*, 497−511.
(2) Hacia, J. G.; Brody, L. C.; Chee, M. S.; Fodor, S. P. A.; Collins, F. S. *Nat. Genet.* **1996**, *14*, 441−447.
(3) Santiago, F. S.; Todd, A. V.; Hawkins, N. J.; Ward, R. L. *Mol. Cell. Probes* **1997**, *11*, 33−38.
(4) *Nonisotopic DNA Probe Techniques*; Kricka, L. J., Ed.; Academic Press, Inc.: San Diego, CA, 1992.
(5) *Gene Probes 1*; Hames, B. D., Higgins, S. J., Eds.; IRL Press: New York, 1995.
(6) Wang, J.; Palecek, E.; Nielsen, P. E.; Rivas, G.; Cai, X.; Shiraishi, H.; Dontha, N.; Luo, D.; Farias, P. A. M. *J. Am. Chem. Soc.* **1996**, *118*, 7667−7670.
(7) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303−308.
(8) Peterlinz, K. A.; Georgiadis, R. M.; Herne, T. M.; Tarlov, M. J. *J. Am. Chem. Soc.* **1997**, *119*, 3401−3402.
(9) Stimpson, D. I.; Hoijer, J. V.; Hsieh, W.; Jou, C.; Gordon, J.; Theriault, T.; Gamble, R.; Baldeschwieler, J. D. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6379−6383.
(10) Hakala, H.; Heinonen, P.; Iitia, A.; Lonnberg, H. *Bioconjugate Chem.* **1997**, *8*, 378−384.
(11) Guo, Z.; Liu, Q.; Smith, L. M. *Nat. Biotechnol.* **1997**, *15*, 331−335.
(12) Ferguson, J. A.; Boles, T. C.; Adams, C. P.; Walt, D. R. *Nat. Biotechnol.* **1996**, *14*, 1681−1684.
(13) Mansfield, E. S.; Worley, J. M.; McKenzie, S. E.; Surrey, S.; Rappaport, E.; Fortina, P. *Mol. Cell. Probes* **1995**, *9*, 145−156.

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nanoparticle probe preparation methods, origin of this unusual selectivity, and implications of this new DNA detection system are described herein.

## Experimental Section

**General Methods.** $HAuCl_4 \cdot 3H_2O$ and trisodium citrate were purchased from Aldrich Chemical Company. Thiol-Modifier C3 S-S CPG, 5′-Thiol-Modifier C6-phosphoramidite reagent, and other reagents required for oligonucleotide synthesis were purchased from Glen Research, Sterling, VA. NAP-5 columns (Sephadex G-25 Medium, DNA grade) were purchased from Pharmacia Biotech. TLC silica gel RP 18 reverse-phase plates were purchased from Alltech Associates, Deerfield, IL. For all experiments, Nanopure $H_2O$ (18.1 MΩ) purified with a Barnstead NANOpure ultrapure water system was used. An Eppendorf 5415C centrifuge was used for centrifugation of Au nanoparticle solutions. Electronic absorption spectra of the oligonucleotides were recorded using a Hewlett-Packard (HP) 8452a diode array spectrophotometer. High-performance liquid chromatography (HPLC) was performed using a HP series 1100 HPLC.

**Preparation of Au Nanoparticles.** Approximately 13 nm diameter Au particles were prepared by the citrate reduction of $HAuCl_4$.[15,16] All glassware was cleaned in aqua regia (3 parts HCl, 1 part $HNO_3$), rinsed with Nanopure $H_2O$, and then oven dried prior to use. An aqueous solution of $HAuCl_4$ (1 mM, 500 mL) was brought to a reflux while stirring, and then 50 mL of a 38.8 mM trisodium citrate solution was added quickly, which resulted in a change in solution color from pale yellow to deep red. After the color change, the solution was refluxed for an additional 15 min, allowed to cool to room temperature, and subsequently filtered through a Micron Separations Inc. 0.45 μm nylon filter. A typical solution of 13 nm diameter gold particles exhibited a characteristic surface plasmon band centered at 518−520 nm. Transmission electron microscopy (TEM) performed with a Hitachi 8100 transmission electron microscope was used to determine the size and monodispersity of the resulting nanoparticle solutions.[17] A typical sample was prepared by dropping 10 mL of nanoparticle solution onto a holey carbon TEM grid, followed by wicking the solution away. The grid was subsequently dried under vacuum and imaged.

**Synthesis and Purification of (Alkanethiol)-Modified Oligonucleotides.** The 3′-alkanethiol 12-base oligomer was synthesized on a 1 μmol scale using standard phosphoramidite chemistry[18] with a Thiol-Modifier C3 S-S CPG solid support. To aid in purification, the final dimethoxytrityl (DMT) protecting group was not removed. After synthesis, the supported oligonucleotide was placed in 1 mL of concentrated ammonium hydroxide for 16 h at 55 °C to cleave the oligonucleotide from the solid support and remove the protecting groups from the bases. Cleavage from the solid support via the succinyl ester linkage produced a mixed disulfide composed of the (mercaptopropyl)-oligonucleotide and a mercaptopropanol linker. After evaporation of the ammonia, the modified oligonucleotide was purified by preparative reverse-phase HPLC using an HP ODS Hypersil column (5 μm, 250 × 4 mm) with 0.03 M triethylammonium acetate (TEAA), pH 7 and a 1%/min gradient of 95% $CH_3CN$/5% 0.03 M TEAA at a flow rate of 1 mL/min, while monitoring the UV signal of DNA at 254 nm. The retention time of the DMT protected modified 12-base oligomer was 30 min. The DMT was subsequently cleaved by dissolving the purified oligonucleotide in an 80% acetic acid solution for 30 min, followed by evaporation; the oligonucleotide was redispersed in 500 μL of water, and the solution was extracted with ethyl acetate (3 × 300 μL). After evaporation of the solvent, the oligonucleotide was redispersed in 400 μL of a 0.1 M DTT, 0.17 M phosphate buffer (pH 8) solution at room temperature (21 ± 1 °C) for 2 h to cleave the 3′ mixed disulfide. Aliquots of this solution (<10 ODs) were purified through a desalting NAP-5 column. Purity was assessed by ion-exchange HPLC using a Dionex Nucleopac PA-100 column (250 × 4 mm) with 10 mM NaOH (pH 12) and a 2%/min gradient of 10 mM NaOH, 1 M NaCl at a flow rate of 1 mL/min while monitoring the UV signal of DNA at 254 nm. Two major peaks with retention times ($t_R$) of 17.2 and 21.1 min were observed. The main single peak at $t_R$ = 17.2 min, which has been attributed to the 3′-alkanethiol 12-base oligomer, was 82.0% by area. The second peak at $t_R$ = 21.1 min (11.8% by area) has been attributed to a disulfide formed from two (mercaptopropyl)oligonucleotides.

5′-(Alkanethiol)-modified oligonucleotides were prepared using the following protocol: (1) a CPG-bound, detritylated oligonucleotide was synthesized on an automated DNA synthesizer (Expedite) using standard procedures; (2) the CPG-cartridge was removed and disposable syringes were attached to the ends; (3) 200 μL of a solution containing 20 μmol of 5-Thiol-Modifier C6-phosphoramidite (Glen Research) in dry acetonitrile was mixed with 200 μL of standard "tetrazole activator solution" and, via one of the syringes, introduced into the cartridge containing the oligonucleotide-CPG; (4) the solution was slowly pumped back and forth through the cartridge for 10 min and then ejected followed by washing with dry acetonitrile (2 × 1 mL); (6) the intermediate phosphite was oxidized with 700 μL of 0.02 M iodine in THF/pyridine/water (30 s) followed by washing with acetonitrile/pyridine (1:1; 2 × 1 mL) and dry acetonitrile. The trityloligonucleotide derivative was isolated and purified as described for the 3′-(alkanethiol)-oligonucleotides; the trityl protecting group was cleaved by adding 150 μL of a 50 mM $AgNO_3$ solution to the dry oligonucleotide sample and letting it stand for 20 min, which resulted in a milky white suspension. The excess silver nitrate was removed by adding 200 μL of a 10 mg/mL solution of DTT (5 min reaction time), which immediately formed a yellow precipitate that was removed by centrifugation. Aliquots of the oligonucleotide solution (<20 ODs) were then transferred onto a desalting NAP-5 column for purification. The final amount and the purity of the resulting 5′-(alkanethiol)oligonucleotides were assessed using the techniques described above for 3′-(alkanethiol)oligonucleotides. Two major peaks were observed by ion-exchange HPLC with retention times of 18.5 min (thiol peak, 83.2% by area) and 22.7 min (disulfide peak, 10.0% by area).

**Preparation of 3′- or 5′-(Alkanethiol)oligonucleotide-Modified Au Nanoparticles.** Gold nanoparticle probes were synthesized by derivatizing 5 mL of an aqueous 13 nm diameter Au nanoparticle solution (≅17 nM)[15] with 2.5 OD of (alkanethiol)oligonucleotide (final oligonucleotide concentration is 3.61 μM). After standing for 16 h, the solution was brought to 0.1 M NaCl, 10 mM phosphate buffer (pH 7) and allowed to stand for 40 h, followed by centrifugation for at least 25 min at 14 000 rpm to remove excess reagents. Following removal of the supernatant, the red oily precipitate was washed with 5 mL of a stock 0.1 M NaCl, 10 mM phosphate buffer (pH 7) solution, recentrifuged, and redispersed in 5 mL of a 0.3 M NaCl, 10 mM phosphate buffer (pH 7), 0.01% azide solution. Approximately 25−30% of the original nanoparticle concentration was lost during centrifugation and workup.

**Preparation and Characterization of Target Oligonucleotides.** Target oligonucleotides were purchased in 40 nmol quantities from the Northwestern University Biotechnology Facility, Chicago, Illinois. Stock solutions were prepared by evaporating a solution containing 1 nmol of the oligonucleotide to dryness and redispersing the sample in 100 μL of a stock 0.3 M NaCl, 10 mM phosphate (pH 7) buffer solution. Aliquots of these solutions (1 μL = 10 pmol) were then used for sample preparation.

**Melting Analyses.** Melting analyses were performed using an HP 8453 diode array spectrophotometer equipped with a HP 89090a Peltier temperature controller. Gold probe/target oligonucleotide solutions were prepared by adding a 6 μL aliquot (60 pmol) of the appropriate oligonucleotide target to a solution containing 150 μL of each gold probe. After mixing, the solutions were heated to 60 °C for 5 min and then allowed to cool to room temperature and stand until full precipitation had occurred (approximately 2−3 h). The solutions were diluted to 1 mL with a 0.3 M NaCl, 10 mM phosphate buffer (pH 7) stock solution (0.06 μM final target oligonucleotide concentration). The UV−vis signature of the gold probe/target oligonucleotide ag-

(14) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078−1081.
(15) Grabar, K. C.; Freeman, R. G.; Hommer, M. B.; Natan, M. J. *Anal. Chem.* **1995**, *67*, 735−743.
(16) Frens, G. *Nat. Phys. Sci.* **1973**, *241*, 20−22.
(17) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607−609.
(18) *Oligonucleotides and Analogues*, 1st ed.; Eckstein, F., Ed.; Oxford University Press: New York, 1991.

gregates was recorded at 1 min intervals, as the temperature was increased from 25 to 75 °C with a holding time of 1 min/deg. The solution was agitated by shaking the vessel at least once every 5 min to maintain homogeneous mixtures throughout the heating period. The first derivative plot calculated from the "melting analysis " at 260 nm was used to determine the reported $T_m$ values. The melting analyses of the comparable oligonucleotide duplexes without Au nanoparticles were performed under the same experimental conditions except that the solutions were 2.2 $\mu$M in each oligonucleotide component. After 5 min of annealing at 80 °C, the solutions were cooled to room temperature for at least 30 min before analysis.

**Spot Test.** For the spot test studies, a 1 $\mu$L aliquot (10 pmol) of a solution of the appropriate oligonucleotide target was added to a 600 $\mu$L thin-wall PCR tube containing 25 $\mu$L of each gold probe. After standing for 15 min at room temperature, the solution was transferred to a temperature controlled water bath. After the set-point temperature of the bath was reached (monitored with a mercury thermometer, 0.5 °C increments), the mixture was allowed to equilibrate for 5 min at which time 3 $\mu$L aliquots of the gold probe/target oligonucleotide solution were transferred with a pipet onto the reverse-phase silica plate and allowed to dry.

## Results and Discussion

**Preparation and Properties of Au Nanoparticles Derivatized with 3′- and 5′-Alkanethiol 12-Base Oligonucleotides.** Gold nanoparticles (~13 nm diameter) were chemically modified with 5′- or 3′-alkythiol-capped 12-base oligonucleotides. The number of oligonucleotides attached to each individual nanoparticle has not yet been determined, but we have confirmed that many oligonucleotide molecules are attached to each individual nanoparticle (vide infra). These oligonucleotide-modified nanoparticles exhibit high stability (no detectable decomposition after 3 months as evidenced by TEM and UV−vis spectroscopy) in solutions containing elevated salt concentrations (0.3 M NaCl), an environment that is incompatible with unmodified particles.[19] The UV−vis spectra of unmodified Au nanoparticles in aqueous solution and Au nanoparticles modified with a 5′-hexanethiol 12-base oligonucleotide at 0.3 M NaCl are shown in Figure 1A. After modification, only a modest shift in the surface plasmon band from 519 to 524 nm was observed. This shift is not necessarily due to surface modification; centrifugation of the DNA-modified particles may affect the particle size distribution, which also could affect the position of the plasmon band.[19] In addition, the electrolyte in the case of the DNA-modified nanoparticles could affect the plasmon band due to charge screening effects and a change in the dielectric constant of the medium.[20] The apparent decrease in intensity of the plasmon band which accompanies surface modification is due to a decrease in particle concentration during the workup of the oligonucleotide-modified particles.

**Targeting Polynucleotides Using Au Nanoparticle Probes.** The nanoparticle-based detection system discussed herein was designed so that two different 12-base oligonucleotide modified Au nanoparticle probes (**1** and **2**) would align in a tail-to-tail fashion onto a complementary target polynucleotide (**4**), Figure 2C. When 60 pmol (6 $\mu$L) of a target polynucleotide strand (**4**) is introduced into a mixture containing 150 $\mu$L of each Au nanoparticle probe (**1** and **2**) at 0.3 M NaCl, 10 mM phosphate (pH 7), the solution color changes from red to purple within 5 min. This color change can be attributed to the formation of large DNA-linked three-dimensional aggregates of Au nanoparticles, which leads to a red shift in the surface plasmon resonance from $\lambda_{max}$ = 524 to 576 nm, Figure 1B.[17] If the solution is allowed to stand for over 2 h, precipitation of the aggregates is observed. A "melting analysis" of the solution with the resuspended aggregates was performed by monitoring the absorbance at 260 nm as a function of temperature, Figure 3 (−■−). Consistent with our characterization of the aggregates as an extended network of DNA-linked Au nanoparticles, a characteristic sharp transition[14,17] (full-width at half-maximum of the first derivative = ~2.2 °C) is observed with a "melting temperature" ($T_m$) of 53.0 °C. Note that, unlike normal DNA melting analyses where the optical signature at 260 nm is due primarily to a transition dipole sensitive to base stacking, the optical signature at 260 nm in these experiments is due primarily to a nanoparticle signature that is, in part, sensitive to interparticle distance.[14] The UV−vis spectra of these aggregates exhibit substantial changes in the 240−810 nm range in the temperature window associated with aggregate "melting", Figure 4. Therefore, aggregate dissociation can be monitored at a variety of wavelengths throughout the UV−vis spectrum in this system, with the largest changes in absorbance occurring at 260, 520, and 650−700 nm. It also may be noted that little change in absorbance is observed in the UV−vis spectra in the 54−75 °C temperature range, which indicates that these nanoparticle solutions are stable at elevated temperatures under these conditions.

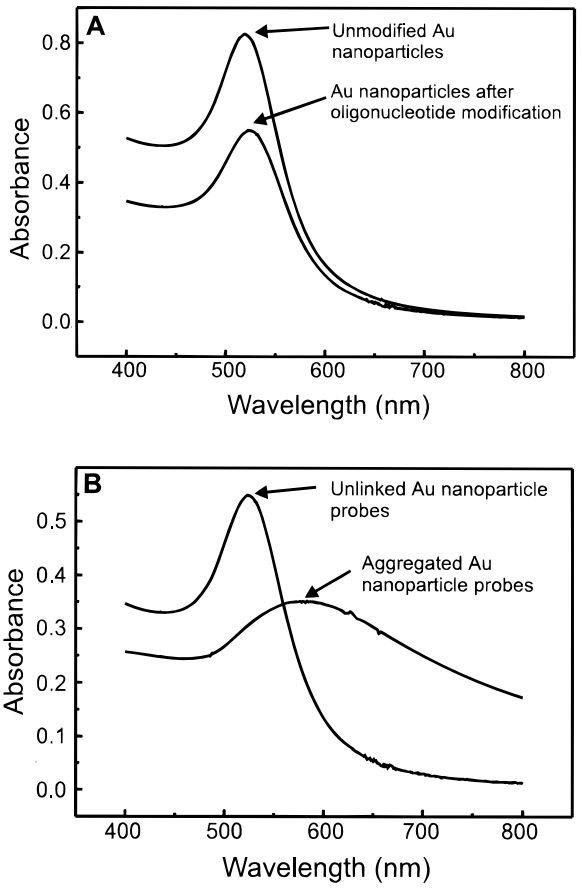


**Figure 1.** (A) Comparison of UV−vis spectra for 300 mL of ~13 nm diameter Au nanoparticles in 1 mL total of aqueous solution and 300 mL of ~13 nm diameter gold nanoparticles functionalized with 5′-hexanethiol 12-base oligonucleotides in 1 mL total of 0.3 M NaCl, 10 mM phosphate (pH 7) solution. (B) Comparison of Au nanoparticles functionalized with 5′-hexanethiol 12-base oligonucleotides (**1** and **2**, see Figure 2A) before and after treatment with a complementary 24-base oligonucleotide (see the Experimental Section under Melting Analyses for preparation procedure of the aggregate solution).

(19) *Colloidal Gold: Principles, Methods, and Applications*; Hayat, M. A., Ed.; Academic Press: San Diego, CA, 1991.
(20) Mulvaney, P. *Langmuir* **1996**, *12*, 788−800.

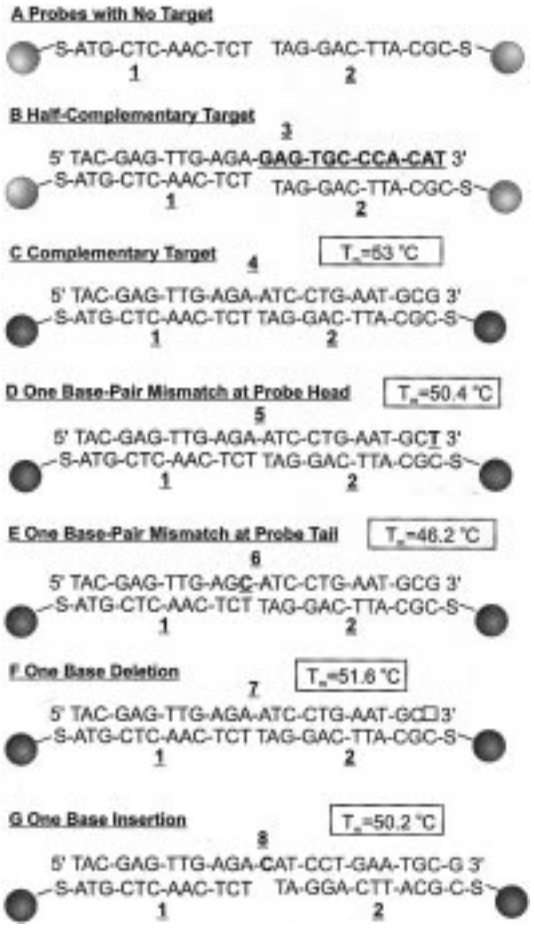


**Figure 2.** (Alkanethiol)oligonucleotide-modified 13 nm diameter Au nanoparticle probes (**1** and **2**) and polynucleotide target sequences (**3**−**8**) used for examining the selectivity of the nanoparticle-based colorimetric polynucleotide detection system. Portions of the target sequences which are underlined in bold represent mismatched bases, bold lettering represents an inserted base, and a box represents a deletion in the polynucleotide. The "melting temperatures" of the Au nanoparticle/target polynucleotide aggregate structures are shown in boxes. Only one attached oligonucleotide is shown per particle even though each particle actually has many oligonucleotides attached to it. Also, only two particles are shown aligning on a target strand; in reality, large extended networks of Au nanoparticles are formed.

**Selectivity of Au Nanoparticle Probes toward Targets Containing Single Base Imperfections.** As one test of the selectivity of this new system, we compared the $T_m$ for the aggregate formed from the nanoparticle probes **1** and **2** and their perfect complement **4** with the $T_m$ values for aggregates formed from targets that contained one base pair end mismatches, a one base deletion, or a one base insertion, Figures 2C−G and 3. The solutions containing both of the Au nanoparticle probes (**1** and **2**) and the imperfect oligonucleotides (**5**−**8**) were prepared analogously to the Au nanoparticle probe/complementary target mixture, and similar shifts in the surface plasmon band and precipitation were observed upon hybridization. All of the Au nanoparticle/DNA aggregates exhibited sharp thermal transitions, but the $T_m$ values were depressed for imperfect targets, Figures 2C−G and 3. Importantly, the solutions containing the imperfect targets could be readily distinguished from the solution containing the perfect complement by their color when placed in a water bath at 52.5 °C. Since this temperature is above the $T_m$ values of the aggregates formed from the imperfect polynucleotides, only the solution with the perfect target exhibited a purple color. For comparison,

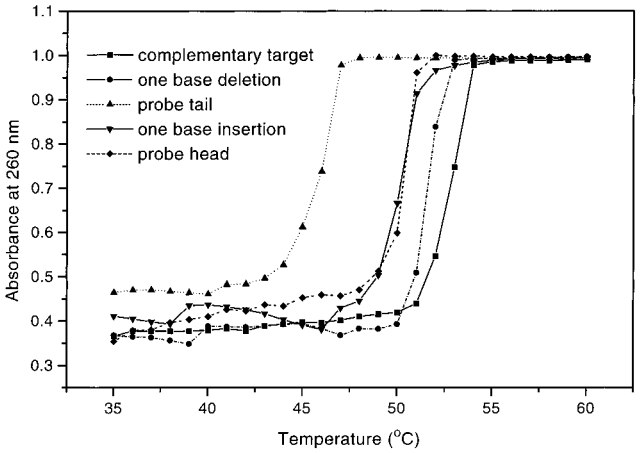


**Figure 3.** Comparison of the normalized thermal dissociation curves for Au nanoparticle probes (**1** and **2**) with a fully complementary target (**4**) and targets containing single base imperfections (**5**−**8**) in hybridization buffer (0.3 M NaCl, 10 mM phosphate (pH 7)), see Figure 2 for sequences and $T_m$ values. Absorbance values at 260 nm were recorded at 1 °C intervals with a holding time of 1 min/deg from 25 to 75 °C. Here, only the region from 35 to 60 °C is shown.

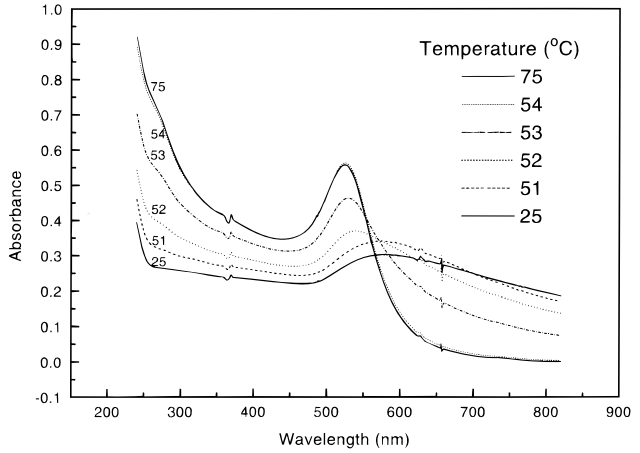


**Figure 4.** UV−vis spectra from the melting analysis of the Au nanoparticle probe/target polynucleotide aggregate solution which show the spectral changes associated with thermal dissociation of the aggregate. Here, only selected temperatures which illustrate the major changes in aggregate dissociation are shown (25, 51, 52, 53, 54, 75 °C).

dissociation curves were obtained for duplexes formed from **4**−**8** and conventional oligonucleotides isosequential to probes **1** and **2**, using higher concentrations of oligomers (2.2 $\mu$M in each oligonucleotide) in order to obtain a measurable signal at 260 nm; the experiment could not be performed at concentrations comparable to those used for the aforementioned nanoparticle probe studies since the conventional oligonucleotides do not exhibit measurable absorbance changes upon hybridization in this concentration range. Broad melting curves were observed in all these cases, with $T_m$ values of 54, 52, 48, 52, and 53 °C for oligonucleotides **4**−**8**, respectively.

A simpler way to monitor the hybridization of Au nanoparticle probes to target sequences is to spot a 3 $\mu$L aliquot of the Au nanoparticle/DNA aggregate solution onto a reverse-phase silica gel plate.[14] This technique, which we refer to as the "Northwestern Spot Test", enhances the color differentiation associated with hybridization. The probe/target aggregate structures, which have a purple color in solution, develop a blue color upon drying on the reverse-phase plate. Solutions that are heated above their dissociation temperature retain their red

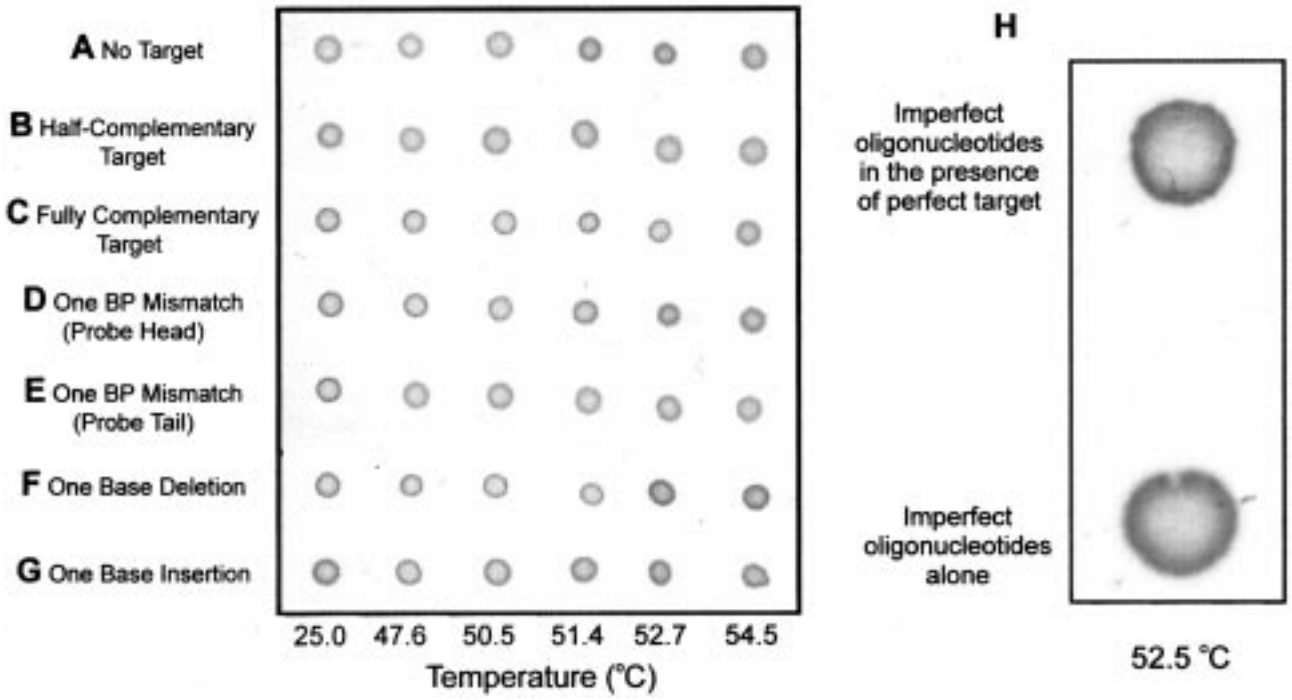


**Figure 5.** (A−G) The spot method for polynucleotide detection which demonstrates the selectivity of the Au nanoparticle based detection system toward single base imperfections. The probes and corresponding polynucleotide targets are listed in Figure 2. (H) Spot test demonstrating the detection and differentiation by color of a polynucleotide target in the presence of polynucleotides with single base imperfections.

color when deposited on the plate (i.e., rehybridization does not occur on the solid support even though the support is at room temperature and below the $T_m$); therefore, this procedure gives one a permanent record for the test. With this spotting technique, the visually detected transition occurs over a very small temperature range (less than 1 °C), thereby allowing one easily to distinguish the solution containing the perfect target **4** from the solutions with oligonucleotides containing one base-pair end mismatches (**5** and **6**), an end deletion (**7**), and a one base insertion at the point in the target where the two oligonucleotide probes meet (**8**), Figures 2C−G and 5C−G. Control experiments in which the target was omitted (Figures 2A and 5A) or was complementary to only one of the probes (Figures 2B and 5B) gave no signs of particle aggregation (Figures 5A and 5B).

The observation that the oligonucleotide with a one base insertion (**8**) can be differentiated from the complementary target **4** is striking, considering the complete complementarity of the one base insertion target with the two probe sequences, Figures 2C,G and 5C,G.[21] We attribute the greater stability of the aggregate formed from **4** and the nanoparticle probes ($\Delta T_m$ = + 2.8 °C compared to the aggregate containing the insertion target) to base stacking between the two thymidine bases where the probe tails meet, Figure 2C,G.[22,23] In our previous system, which utilized 15-base oligonucleotide/Au nanoparticle probes (see Scheme 1A),[14] oligonucleotides with one base insertions could not be distinguished from the fully complementary target. Although a definitive study has not yet been made, it appears that the probe alignment may play a significant role in determining the degree of base stacking that occurs where the two probes meet. Previous investigations by Cantor and co-workers have demonstrated that stacking interactions between a duplex probe containing a single-stranded sticky end and a complementary single-stranded target can enhance discrimination of mismatches where the duplex probe and target meet.[22,24]

**One-Pot Detection of a Target in the Presence of Polynucleotides with Single Base Imperfections Using Au Nanoparticle Probes.** To further address the issue of selectivity in this system, an experiment was designed to determine whether the complementary target polynucleotide (**4**) could be detected in the presence of polynucleotides with sequences differing by one nucleotide in the target region (**5**−**8**). For this test, two solutions were prepared. Each contained the two nanoparticle probes (**1** and **2**, 25 $\mu$L of each) and 10 picomoles of each of the polynucleotides with a single base end polymorphism (**5** and **6**), one base deletion (**7**), and one base insertion (**8**). To one of the solutions was added 10 pmol of the fully complementary target strand (**4**), and to the other was added another 10 pmol of the polynucleotide with a one base end deletion (**7**) (note that **7** is the most difficult to differentiate from **4**). The solutions were then heated to 45 °C (15 min) and equilibrated at 52.5 °C (5 min), which is above the dissociation temperatures for the aggregates formed by the imperfect polynucleotides but below the melting temperature for the aggregate formed from the complementary target. The spot test showed a blue spot for the solution containing **4** in the presence of the imperfect polynucleotides and an easily distinguished purplish red spot for the mixture lacking **4**, Figure 5H. This experiment demonstrates that the nanoparticle system can be used effectively in identifying specific polynucleotides in the presence of polynucleotides with closely related nucleotide sequences. It is reasonable to assume that hybridization with the mixture of polynucleotides at the lower temperatures affords complex aggregates in which nanoparticles are linked by duplex segments containing the imperfect as well as matched target sequences.

(21) In addition, a target containing a three-base insertion (CCC) at the same position exhibited a similar destabilization effect when hybridized to the probes under comparable conditions ($T_m$ = 50.3 °C).

(22) Fu, D.-J.; Broude, N. E.; Koster, H.; Smith, C. L.; Cantor, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 10162−10166.

(23) Kotler, L. E.; Zevin-Sonkin, D.; Sobolev, I. A.; Beskin, A. D.; Ulanovsky, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 4241−4245.

(24) Broude, N. E.; Sano, T.; Smith, C. L.; Cantor, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3072−3076.

Heating to the higher temperature breaks the weaker duplex links formed from the imperfect polynucleotides and accelerates the formation of strong links formed from the nanoparticle probes and the complementary target **4**, which results in "higher melting" aggregates linked primarily by these stronger fully complementary segments.

## Conclusions

We have demonstrated that this new nanoparticle-based detection system, which employs two 12-nucleotide Au nanoparticle probes which align in a "tail-to-tail" fashion, can distinguish nearly any mismatch, deletion, or insertion in the polynucleotide sequence from the fully complementary target sequence. In our previous system which utilized Au nanoparticles functionalized with 15-base nucleotides which align in a "head-to-tail" fashion, polynucleotides containing a one base insertion could not be distinguished from the fully complementary target. We have attributed this difference in selectivity to base stacking where the probe tails meet, which is facilitated by the "tail-to-tail" alignment. However, further experiments are needed to determine the role of probe alignment, oligonucleotide probe length, and hybridization conditions in dictating the selectivity of detection systems based upon these novel Au nanoparticle probes and network materials. Importantly, we also have demonstrated that a polynucleotide target can be detected and differentiated by color using our spot method in the presence of polynucleotide strands with single base imperfections. This extraordinary selectivity derives from the sharp colorimetric melting transitions associated with Au nanoparticle network materials. This new, one-pot colorimetric detection method and probe design may prove useful in diagnosing genetic diseases that contain single nucleotide mutations.

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