Pure Appl. Chem., Vol. 72, Nos. 1–2, pp. 229–235, 2000.

A gold nanoparticle/latex microsphere-based colorimetric oligonucleotide detection method*

Robert A. Reynolds III, Chad A. Mirkin†, and Robert L. Letsinger‡

Department of Chemistry and Center for Nanofabrication and Molecular Self-Assembly, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208 USA

Abstract: An exceptionally simple and effective DNA detection methodology based on latex microsphere and gold nanoparticle probes has been developed. The latex and gold particle probes, which were functionalized with separate oligonucleotide sequences, undergo hybridization in the presence of target strands that are complementary to both of the probes. Duplex formation thus results in linking of gold nanoparticles to the latex microspheres and a corresponding white-to-red color change, which, because of the particularly large extinction coefficient of the gold nanoparticles, is clearly visible to the naked eye. Background signal caused by unbound gold nanoparticles is significantly reduced by filtering the solution containing the sample and probes through a size-selective cellulose acetate membrane. The unbound gold probes move freely through this membrane while the larger latex particles are trapped. Therefore, if the latex and gold nanoparticles are joined together via the target oligonucleotides, the membrane appears red, indicating a positive test result. If no hybridization takes place, the membrane appears white, indicating a negative result. The lower detection limits for this system are 500 pM for a 24 base single-stranded target and 2.5 nM for a duplex target oligonucleotide.

INTRODUCTION

Most DNA detection systems rely on the molecular recognition abilities of single-stranded oligonucleotide probes that hybridize with complementary single-strand target molecules [1,2]. The oligonucleotide probes usually contain covalently linked reporter groups that provide radioactive, electrochemical, fluorescent, or colorimetric signals [1,2]. Colorimetric systems are especially attractive detection formats since they: 1) do not pose safety problems, 2) are simple to monitor, and 3) are relatively inexpensive. Previously in this laboratory, we demonstrated that 13 nm alkylthiol-functionalized oligonucleotide-modified gold nanoparticles could be used as probes for the colorimetric detection of target oligonucleotides [3,4]. In the presence of a complementary target oligonucleotide, dispersed oligonucleotide-modified gold nanoparticle probes are cross-linked via hybridization events into aggregated polymeric networks. This gold nanoparticle aggregation process occurs with a concomitant color change from red (dispersed gold nanoparticles) to blue (aggregated networks), which can be monitored spectrophotometrically in solution or with the naked eye by spotting an aliquot of the solution on a solid silica gel support.

Although the aforementioned assay works extremely well, red and blue are not colors most easily distinguished by the naked eye [5]. Additionally, the blue color of the nanoparticle aggregates in solu-

Pure Appl. Chem.* **72, 1–331 (2000). An issue of reviews and research papers based on lectures presented at the 1st IUPAC Workshop on Advanced Materials (WAM1), Hong Kong, July 1999, on the theme of nanostructured systems.
†E-mail: camirkin@chem.nwu.edu
‡E-mail: r-letsinger@chem.nwu.edu

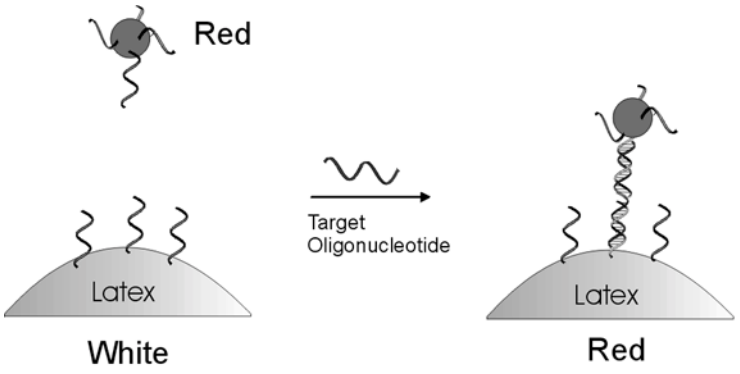

Fig. 1 Hybridization of oligonucleotide-modified gold nanoparticle probes to oligonucleotide-modified latex microsphere probes in the presence of a complementary target oligonucleotide.

tion is a factor of 1.5 less intense than the red color of the dispersed 13-nm gold probes. Herein, we describe a new detection system based upon latex and gold particle probes, which involves linking of the latex and gold probes via hybridization with a target DNA strand (Fig. 1). Therefore, if target is present the white latex particles turn red and if it is absent they remain white. Filtration of the excess Au nanoparticle probes through a size-selective cellulose membrane support provides for an easy way of visualizing the test results and virtually eliminates background signal (Fig. 2).

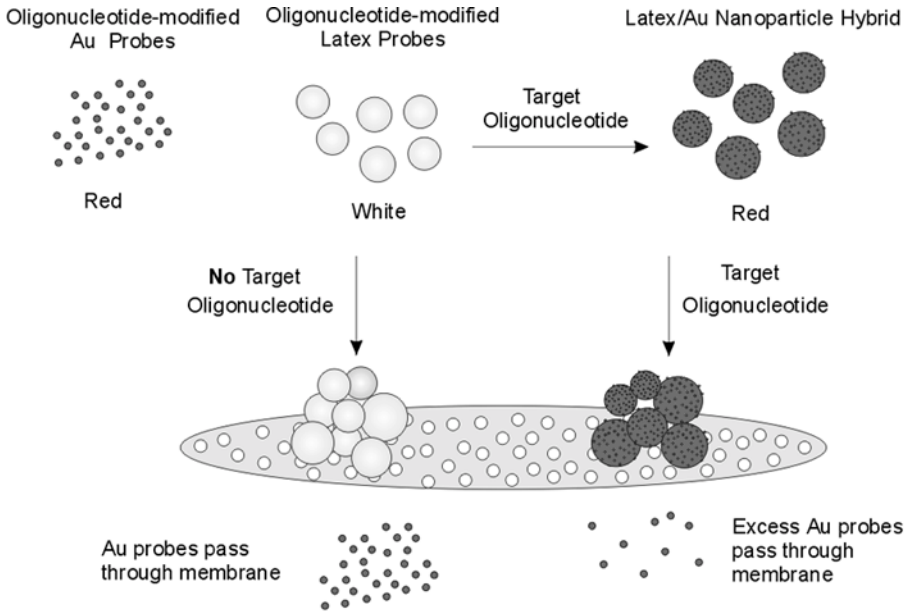

Fig. 2 Gold nanoparticle/latex microsphere-based colorimetric DNA detection method.

EXPERIMENTAL

General methods

For the purpose of consistency, the lengths and sequences of the oligonucleotides used in this study were identical to those in the gold nanoparticle system previously reported, Table 1 [3]. All hybridization experiments were performed in a 0.3 M NaCl, 10 mM phosphate, pH 7.0, 0.01 wt% sodium dodecyl sulfate (SDS) buffer solution unless otherwise stated. The filtration membranes used were 0.45 µm AcetatePlus™ purchased from Micron Separations Inc., Westboro, Massachusetts. Alkylamino-functionalized latex particles, 3.1 µm, were purchased from Bangs Laboratories, Fishers, Indiana. Reagents, phosphoramidites, and modified controlled pore glass (CPG) for oligonucleotide synthesis were purchased from Glen Research, Sterling, Virginia. The preparation of 13 $\pm$ 3 nm diameter oligonucleotide (**2**)-modified gold nanoparticle probes and the target oligonucleotides (**3** and **4**) have been reported previously [3].

Table 1 Sequences of oligonucleotides **1–5**.

1	5' TCT-CAA-CTC-GTA-TTT-TTT-TTT-T-alkylamine 3'
2	5' alkylthiol-TTT-TTT-TTT-TCG-CAT-TCA-GGA-T 3'
3	5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG 3'
4	5' CGC-ATT-CAG-GAT-TCT-CAA-CTC-GTA 3'
5	5' GCG-TAA-GTC-CTA-AGA-GTT-GAG-CAT 3'

Synthesis of probe oligonucleotide (1)

3' Alkylamine-T[10] -ATG-CTC-AAC-TCT-5' was synthesized using standard solid phase phosphoramidite oligonucleotide chemistry from 3' amino-C7-modified CPG. It was purified by reverse-phase HPLC, and final purity was confirmed by ion-exchange HPLC [6].

Attachment of 1 to amine-modified latexes [7]

A dimethylformamide (DMF) solution containing a large excess of 1,4-phenylene diisothiocyanate (25 mg, 0.13 mmol) was added to an aqueous borate buffer solution (0.1 M, pH 9.3) of the alkylamino-functionalized oligonucleotide (**1**) (10 nmol). After several hours the excess 1,4-phenylene diisothiocyanate was removed via a butanol/water extraction, and the aqueous solution containing the activated oligonucleotide was lyophilized. The activated oligonucleotide was subsequently dissolved in borate buffer and reacted with alkylamino-functionalized latex microspheres (1.0 wt%) in a carbonate buffer (0.1 M, pH 9.3, 1 M NaCl). After 12 h, the particles were isolated by centrifugation, washed three times with buffered saline solution (0.3 M NaCl, 10 mM phosphate, pH 7.0, 0.01 wt% SDS), and stored in saline buffer as a 1.0 wt% solution.

Single-strand detection

The target oligonucleotide (**3**) (4 µl, various concentrations) was added to 3 µl of oligonucleotide (**1**)-modified latex probe solution (3.1 µm; 0.1 wt%). After 5 min, 3 µl of oligonucleotide (**2**)-modified 13-nm gold nanoparticle probe solution (8 nM) was added to the assay solution. Upon standing an additional 10 min, the solution was taken up into a micro-pipet, transferred onto a cellulose acetate

membrane, and vacuum filtered. The latex particles, with or without hybridized gold nanoparticles, were trapped on the cellulose acetate while unbound gold nanoparticles passed through it.

Duplex detection

The target oligonucleotide duplex (**3:4**) (4 µl, various concentrations), 3 µl of oligonucleotide (**1**)-modified latex probe solution (3.1 µm; 0.1 wt%), and 3 µl of oligonucleotide (**2**)-modified 13 nm gold nanoparticle probe solution (8 nM) were combined and heated to 100 °C for 3 min. The entire solution was immediately frozen in a Dry Ice/isopropanol bath for 3 min, then thawed at room temperature and filtered as described above.

TEM

TEM images were obtained on a Hitachi 8100 transmission electron microscope operating at 200 kV. A typical sample was prepared by dropping 10 µl of the latex microsphere/gold nanoparticle solution onto a holey carbon grid, followed by wicking the solution away with tissue paper.

RESULTS AND DISCUSSION

Targeting single-stranded DNA

The detection of single-stranded target oligonucleotides is straightforward and rapid with this gold nanoparticle/latex microsphere probe format. In a typical experiment, target oligonucleotide (4 µl, sequence **3** in Table 1) was added to a latex microsphere probe solution (3 µl, 0.1 wt%) and allowed to hybridize for 5 min at room temperature. Then, a dilute solution of gold nanoparticle probe (3 µl, 8 nM) was added to the solution containing the target and the latex probes. After a hybridization period of 10 min, the assay solution was transferred onto a size-selective membrane (0.45 m cellulose acetate) and vacuum-filtered. This step resulted in the efficient separation of unreacted gold probes from the latex probes. In the presence of target oligonucleotide **3**, the latex probes that were transferred to the membrane appeared red, and when no target DNA was present (or the target concentration was below our detection limits) the latex probes on the membrane appeared white (Fig. 3). A cellulose acetate membrane was

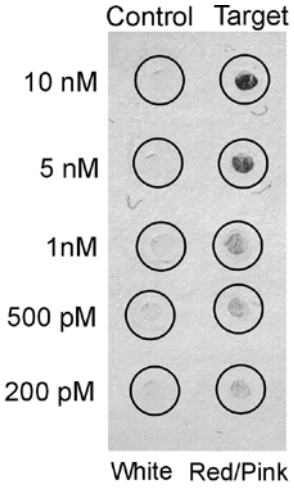

Fig. 3 Controls; noncomplementary oligonucleotide **5** from 200 pM to10 nM. Target oligonucleotide **3** from 200 pM to10 nM.

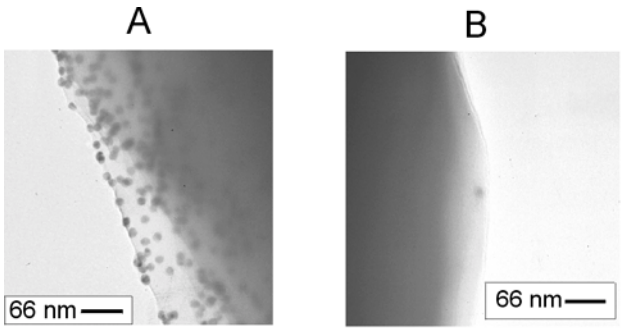

Fig. 4 TEM images of: A) Target oligonucleotide **3** (10 nM), numerous gold nanoparticle probes are attached to the surface of the latex microsphere, B) Noncomplementary oligonucleotide **5** (10 nM), on average only 1–2 gold nanoparticle probes can be seen adhered to the surface of each latex microsphere.

used as the size-selective filter since it displays little affinity for the oligonucleotide-modified gold nanoparticle probes. With this new colorimetric gold nanoparticle/latex microsphere-based detection system, the single-stranded target **3** can be detected at concentrations of down to 500 pM.

A series of experiments were performed to verify that the observed color change of the latex probes from white to red was due to selective hybridization of the target strand. As expected, no color change was observed in the absence of either target strand **3** or salt, both of which are required for duplex formation to take place. Therefore, the gold and latex probes do not bind nonspecifically to each other in solution. Importantly, a negative test also resulted when the noncomplementary oligonucleotide sequence **5** was used instead of **3**, confirming that the color change is the result of a specific hybridization event.

The hybridized conjugates were further characterized by TEM. When the complementary oligonucleotide **3** was present, many gold nanoparticles adhered to each latex microsphere (Fig. 4a). In contrast, the same system with the noncomplementary sequence **5** in place of **3** yields latex probes with a relatively small number of gold nanoparticles attached to their exteriors (Fig. 4b). These results are consistent with the assertion that specific hybridization of complementary target strands to gold nanoparticle and latex microsphere probes is responsible for the observed colorimetric change of the latex probes from white to red in our novel assay.

Targeting double-stranded DNA

Nearly all DNA detection methods, including the one reported herein, rely on the selective hybridization of a single-stranded target [1,2]. Therefore, in order to detect double-stranded DNA, the duplex target must be denatured prior to reaction with probes. Consequently, a primary challenge involved in the detection of double-stranded DNA is the development of probes that hybridize to the single-stranded target before the full complement does. The ability to significantly reduce background signal with this detection system is an attractive feature. A large excess of gold nanoparticles can be employed so that hybridization of the target to the gold probe will be kinetically favored over nonproductive recombination with the complement. Unreacted gold probes then can be removed from the system by filtration.

In a typical experiment, duplex oligonucleotide target **3:4** (4 μl, various concentrations) was combined with oligonucleotide-modified latex probe solution (3 μl, 0.1 wt%) and oligonucleotide-modified gold nanoparticle probe solution (3 μl, 8 nM). The mixture was heated briefly to 100 °C to dissociate the target duplex, then the entire solution was frozen by immersing the sample in a dry ice/isopropanol bath. After thawing slowly at room temperature, the sample was filtered through a cellulose

acetate membrane. The freezing step was necessary to increase the rate of hybridization of the target single-strand oligonucleotide, the latex microsphere probes, and the gold nanoparticle probes. The rate enhancement through freezing has been proposed to be due to the generation of higher local concentrations of probes, target, and salt [4]. No evidence of nonspecific interactions between the gold nanoparticles and latex microspheres due to freezing was observed. Currently, the lower detection limit for the 24 base pair duplex target **3:4** using this method is 2.5 nM.

CONCLUSIONS

We have developed a colorimetric detection method based on a convenient size-selective filtration procedure. In our procedure, gold and latex probes are linked together by the target DNA strand, generating a white-to-red color change. Excess/nonhybridized gold particles can then be separated from latex indicator probes, allowing the white or red color of the latex to be visualized, and the presence or absence of target determined. The filtration step significantly reduces background signal and gives the system a distinct advantage for the detection of double-stranded targets because a large excess of gold probes can be used to react with target without interfering with signal interpretation.

This proof-of-concept oligonucleotide detection method is sensitive (500 pM for single-strand target, 2.5 nM for duplex target), rapid (15 min), straightforward, and inexpensive (no special equipment is required). In addition, this system establishes a general detection methodology that can be applied to a variety of material compositions and particle sizes. In theory, any microsphere or nanoparticle with a thiol or amino reactive surface, alkylamino surface functionalities, or alkylcarboxylato surface functionalities can be modified with oligonucleotides and employed in a similar detection format. Potential materials include a variety of commercially available polymer compositions, SiO_2, silica-coated Fe_3O_4, [8] polymer-modified gold nanoparticles [9] (where the polymer can be modified to have any desirable property, i.e. fluorescent, color, redox active), and fluorescent CdS/CdSe particles [10–12].

ACKNOWLEDGMENTS

C. A. M. is grateful to the NSF (CHE-9871903) and ARO (DAA G55-97-1-0133) for supporting this work. C. A. M., R. L. L., and R. A. R. acknowledge NIH (1 R01 GM57356-01; NRSA postdoctoral fellowship 1 F32 HG00202-01) for support of this research.

REFERENCES

1. B. D. Hames and S. J. Higgins (eds.). *Gene Probes 1,* IRL Press, New York (1995).
2. L. J. Kricka (ed.). *Nonisotopic DNA Probe Techniques,* Academic Press, San Diego (1992).
3. J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin, R. L. Letsinger. *J. Am. Chem. Soc.* **120,** 1959–1964 (1998).
4. R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, C. A. Mirkin. *Science* **227,** 1078–1081 (1997).
5. A. Arditi and K. Knoblauch. In *Functional Assessment of Low Vision,* R. Cole and B. Rosenthal (eds.), pp. 129–135, Mosby, St. Louis (1996).
6. F., Eckstein (ed.). *Oligonucleotides and Analogues,* 1st ed., Oxford University Press, New York (1991).
7. M. T. Charreyre, O. Tcherkasskaya, M. A. Winnik, A. Hiver, T. Delair, P. Cros, C. Pichot, B. Mandrand. *Langmuir* 13, 3103–3110 (1997).
8. Q. Liu, Z. Xu, J. A. Finch, R. Egerton. *Chem. Mater.* **10,** 3936–3940 (1998).

9. K. J. Watson, J. Zhu, S. T. Nguyen, C. A. Mirkin. *J. Am. Chem. Soc.* **121,** 462–463 (1999).
10. G. P. Mitchell, C. A. Mirkin, R. L. Letsinger. *J. Am. Chem. Soc.* **121,** 8122–8123 (1999).
11. W. C. W. Chan and S. M. Nie. *Science* **281,** 2016–2018 (1998).
12. M. Bruchez, M. Moronne, P. Gin, S. Weiss, A. P. Alivisatos. *Science* **281,** 2013–2016 (1998).