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# Bioconjugated Gold Nanodots and Nanoparticles for Protein Assays Based on Photoluminescence Quenching

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This study describes the first instance of the use of two differently sized Au nanoparticles (Au NPs), acting separately as donor and acceptor, in homogeneous photoluminescence quenching assays developed for the analysis of proteins. Introduction of a breast cancer marker protein, platelet-derived growth factor AA (PDGF AA), to a solution of 11-mercaptopundecanoic acid-protected, 2.0-nm photoluminescent Au nanodots ( $L_{AuND}$ ) led to the preparation of PDGF AA- $L_{AuND}$  as the donor. Thiol-derivative PDGF binding aptamers (Apt) and 13-nm spherical Au NPs were used to synthesize the Apt- $Q_{AuNP}$  acceptor. The photoluminescence of PDGF AA- $L_{AuND}$  at 520 nm decreased when photoluminescence quenching occurred between Apt- $Q_{AuNP}$  and PDGF AA- $L_{AuND}$ . We used the PDGF AA- $L_{AuND}$ /Apt- $Q_{AuNP}$ -based molecular light switching system to analyze PDGFs and PDGF  $\alpha$ -receptor in separate homogeneous solutions. In the presence of PDGFs, the interaction between Apt- $Q_{AuNP}$  and PDGF AA- $L_{AuND}$  decreased as a result of competitive reactions between the PDGFs and Apt- $Q_{AuNP}$ . Similarly, the interaction between Apt- $Q_{AuNP}$  and PDGF AA- $L_{AuND}$  reduced as a result of competitive reactions between PDGF  $\alpha$ -receptor and PDGF AA- $L_{AuND}$ . The limits of detection (LODs) for PDGF AA and PDGF  $\alpha$ -receptor were 80 pM and 0.25 nM, respectively, resulting from a low background photoluminescence signal. When using the Apt- $Q_{AuNP}$  as selectors for (a) the enrichment of PDGF AA and (b) the removal of matrixes possessing intense background fluorescence from cell media and urine samples, the LOD for PDGF AA decreased to 10 pM. Unlike quantum dots, the  $L_{AuND}$  provide the advantages of biocompatibility, ease of bioconjugation, and minimal toxicity.

The application of photoluminescence quenching is a powerful technique for protein analysis.<sup>1</sup> Intermolecular and intramolecular photoluminescence quenching between the donor and acceptor through specific binding of fluorophore-labeled probes toward

target analytes is sensitive and of interest.<sup>2</sup> One interesting example is the use of fluorophore-labeled aptamers for the analysis of proteins such as platelet-derived growth factor (PDGF),<sup>2g,h</sup> which was associated with a variety of disorders, including atherosclerosis; fibroproliferative diseases of lungs, kidneys, and joints; and neoplasia.<sup>3</sup> The PDGF also found is an important protein for cell transformation and tumor growth and progression.<sup>4</sup> Aptamers are advantageous over antibodies because they can be engineered, are more easily labeled with signal moieties such as fluorophores and enzymes, are more stable, and are of a relatively lower cost.<sup>5–7</sup> When using fluorophore-labeled aptamers, tedious

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processes for labeling the probes or target analytes and the use of expensive fluorophores or coupling reagents (or both) are required.<sup>6</sup> Thus, label-free and highly sensitive assays are highly demanded for trace analysis of proteins.<sup>7</sup>

Recently, bioconjugated gold nanoparticles (Au NPs) and quantum dots have been demonstrated as quenchers and fluorescent donors, respectively, for photoluminescence quenching.<sup>8</sup> Quantum dots, such as CdSe@ZnS and CdTe@ZnS, offer several advantages over organic fluorophores for use as donors, including narrow band widths (usually 20–30 nm at the half-maximum) and size-dependent fluorescence emissions when excited at a particular wavelength.<sup>9</sup> Photoluminescence quenching assays employing pairs of quantum dots with organic fluorophores, chromophores, other types of quantum dots and Au NPs have been demonstrated.<sup>8e–h,10</sup> Concerns have been raised, however, regarding the toxicity of quantum dots; several studies have suggested that the cytotoxicity of quantum dots might be mediated by their release of Cd<sup>2+</sup>.<sup>11</sup> Thus, there is a demand for the development of nontoxic photoluminescent NPs to replace potentially toxic quantum dots in bioassays.

In this study, we developed new competitive homogeneous photoluminescence quenching assays for analyzing proteins using bioconjugated photoluminescent Au nanodots (L<sub>AuND</sub>) as donors and bioconjugated spherical Au NPs as acceptors. We prepared

a breast cancer marker protein, platelet-derived growth factor AA (PDGF AA)-bound L<sub>AuND</sub> (PDGF AA–L<sub>AuND</sub>), from 11-mercaptopundecanoic acid (11-MUA)-protected L<sub>AuND</sub> (2.0 ± 0.1 nm).<sup>12</sup> Thiol-derivatized aptamer (Apt) molecules and 13-nm spherical Au NPs were used to prepare Apt–Q<sub>AuNP</sub> acceptors. The aptamer sequence was obtained through systematic evolution of ligands using an exponential enrichment (SELEX) process.<sup>5</sup> It was reported to have a 700-fold higher affinity for PDGFs when compared with those of random DNA sequences;<sup>13</sup> we confirmed this behavior in previous studies.<sup>14</sup> Employing our two types of bioconjugated, Au-based nanomaterials, we have developed homogeneous assays for the selective and highly sensitive detection of PDGFs and one of their receptors (PDGF α-receptor).

## EXPERIMENTAL SECTION

**Materials.** The 5'-thiol-modified PDGF binding aptamer (5'-CAG GCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TG-3') and 5'-thiol-modified α-thrombin binding aptamer (5'-ACC CGT GGT AGG GTA GGA TGG GGT GGT-3') were purchased from Integrated DNA Technology, Inc. (Coralville, IA). Recombinant PDGF AA, BB, and AB and PDGF α-receptor were purchased from R&D Systems, Inc. (Minneapolis, MN). The PDGFs were dissolved in 4 mM HCl, and PDGF α-receptor was prepared in 5 mM phosphate solution (pH 7.4; 135 mM NaCl). The proteins insulin, β-casein, cytochrome *c*, carbonic anhydrase, trypsin inhibitor, BSA, trypsinogen, and lysozyme were obtained from Sigma (St. Louis, MO). Hydrogen tetrachloroaurate(III) trihydrate and all other reagents used in this study were purchased from Aldrich (Milwaukee, WI).

**Synthesis of 13-nm Spherical Au NPs.** The 13-nm spherical Au NPs were prepared through citrate-mediated reduction of HAuCl<sub>4</sub>.<sup>15</sup> A 250-mL aqueous solution consisting of 1 mM HAuCl<sub>4</sub> was brought to a vigorous boil and stirred in a round-bottom flask fitted with a reflux condenser; 38.8 mM trisodium citrate (25 mL) was then added rapidly to the solution. The solution was heated for another 15 min, during which time its color changed from pale yellow to deep red. The solution was cooled to room temperature with continuous stirring. The sizes of the Au NPs were verified through TEM analysis (H7100, Hitachi High-Technologies Corporation, Tokyo, Japan); they appeared to be nearly monodisperse, with an average size of 13.3 ± 1.2 nm. A double-beam UV–vis spectrophotometer (Cintra 10e, GBC, Victoria, Australia) was used to measure the absorption of the Au NP solution. The particle concentration of the Au NPs (~15 nM) was determined according to Beer's law using an extinction coefficient of ~10<sup>8</sup> M<sup>-1</sup> cm<sup>-1</sup> at 520 nm for the 13-nm Au NPs.<sup>15c</sup>

**Preparation of Apt–Q<sub>AuNP</sub>.** The thiol-modified 35-mer DNA oligonucleotides were attached to the 13-nm Au NPs according

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to modified literature procedures.<sup>16</sup> The 5'-thiol-modified oligonucleotides were received in disulfide form [ $\text{HOCH}_2(\text{CH}_2)_5\text{S}-\text{S}-5'$ -oligo], protected by mercaptohexanol groups. These oligonucleotides were reacted directly with the Au NPs through attachment of both the  $\text{HOCH}_2(\text{CH}_2)_5\text{S}-$  and oligo-S- units onto the Au NP surface. Two aliquots of aqueous Au NP solutions (800  $\mu\text{L}$ ) in 1.5-mL tubes were mixed separately with the thio-oligonucleotides (5.0  $\mu\text{M}$ , 200  $\mu\text{L}$ ) to obtain a final concentration of 12 nM Au NPs and 1.0  $\mu\text{M}$  oligonucleotides. After reaction for 24 h at room temperature, the mixtures were centrifuged for 25 min at 19000g to remove the excess thiol-DNA. Following removal of the supernatants, the oily precipitates were washed with 5 mM trisodium citrate. After two wash/centrifuge cycles, the Apt-QAuNP samples were resuspended separately in 5 mM trisodium citrate and stored in a refrigerator (4 °C). Finally, the Apt-QAuNP samples were equilibrated separately with 0.1% poly(ethylene glycol) (PEG; Mw: 8000) for 2 h at room-temperature prior to use in the detection of PDGFs. The PEG-modified Apt-QAuNP samples prevented nonspecific binding with interference proteins.<sup>14</sup> Moreover, the Apt-QAuNP samples were stable in solutions containing NaCl at concentrations up to 250 mM. To determine the number of oligos on each Apt-QAuNP, a solution of 2-mercaptoethanol (1.0 M, 10  $\mu\text{L}$ ) was used to displace the oligonucleotide molecules from the surfaces of the Apt-QAuNP (12 nM, 990  $\mu\text{L}$ ). The concentration of displaced oligonucleotides in the supernatant was used to calculate the quantity on each Apt-QAuNP particle. Our calculations indicated that ~40 oligonucleotide molecules were attached to each Apt-QAuNP.

**Synthesis of PDGF AA-L<sub>AuND</sub>.** The L<sub>AuND</sub> samples were first synthesized through reduction of  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  with tetrakis-(hydroxymethyl)phosphonium chloride (THPC).<sup>17</sup> NaOH (1.0 N, 0.5 mL) was added to water (45 mL), followed by the addition of a THPC solution (1 mL), which had been prepared by adding 80% THPC solution (12  $\mu\text{L}$ ) to water (1 mL). The mixture was stirred for 5 min, followed by rapid addition of 1.0 wt %  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  (1.5 mL). The color of the solution turned brown over the course of 1 min. Prior to use, the solution was stored at 4 °C. The sizes of the as-prepared spherical Au NPs were determined using high-resolution transmission electron microscopy (HRTEM; Tecnai 20 G2 S-Twin TEM, Philips/FEI, Hillsboro, OR); the average size was 2.9 ( $\pm 0.5$ ) nm. The particle concentration of the as-prepared Au NP solution ( $\sim 0.94 \mu\text{M}$ ) was determined according to a procedure described previously.<sup>12</sup>

The Au NPs were self-assembled with 11-MUA molecules by introducing 11-MUA stock solutions (100 mM) into the as-prepared Au NP solution at a concentration of 0.47  $\mu\text{M}$ . The as-prepared Au NP solution (5.0 mL), DI water (3.0 mL), sodium tetraborate (50 mM, pH 9.2, 1.0 mL), and 11-MUA (1.0 mL, 100 mM) were added, in that order, to a 10-mL volumetric flask. The mixture was left to react for 72 h in the dark at room temperature. The resulting L<sub>AuND</sub> with strong photoluminescence were designated as 11-MUA-L<sub>AuND</sub>. The excess 11-MUA in the solution was removed through centrifugal filtration (13500g) for 40 min through a filter having a cutoff of 10 kDa. The 11-MUA-L<sub>AuND</sub> solution

was then resuspended in 5 mM sodium phosphate solution (pH 7.4). By comparing the absorbance of the original 11-MUA-L<sub>AuND</sub> solution at 375 nm to that of the resuspended solution, ~95% of the 11-MUA-L<sub>AuND</sub> was deemed to have been collected (data not shown). The corresponding particle concentration in the resuspended solution was estimated to be 1.5  $\mu\text{M}$ , provided that the 11-MUA-L<sub>AuND</sub> had a uniform diameter of 2.0 ( $\pm 0.1$ ) nm, which was estimated from a count of 100 11-MUA-L<sub>AuND</sub> particles in a TEM image. The purified 11-MUA-L<sub>AuND</sub> sample was stable for at least 3 months when stored at 4 °C in the dark. The absorption and photoluminescence spectra of the as-prepared 11-MUA-L<sub>AuND</sub> solutions were measured using UV-vis absorption and fluorescence spectrophotometers (Cary Eclipse; Varian, CA), respectively.

For preparation of the PDGF AA-L<sub>AuND</sub> stock solution, a mixture of purified 11-MUA-L<sub>AuND</sub> (40 nM) and PDGF AA (80 nM) in 5 mM sodium phosphate (pH 7.4) was left for 2 h at room temperature. PDGF AA molecules readily conjugated to L<sub>AuND</sub> through electrostatic and hydrophobic interactions. A similar method was applied to the preparation of thrombin-L<sub>AuND</sub>; the concentration of thrombin was 200 nM. The as-prepared PDGF AA-L<sub>AuND</sub> and thrombin-L<sub>AuND</sub> solutions were stable for at least 3 days when stored at 4 °C in the dark.

**Cell Culture.** The breast cancer cell line HTB-26 was used in this study. Cells were cultured in Leibovitz's L-15 medium (Gibco BRL, New York) plus 10% fetal bovine serum (FBS; Gibco). Cells were grown at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub> (in air). The medium was changed every 3 days. At ~80% confluency, the cells were trypsinized with trypsin-EDTA solution (0.25% trypsin, 1 mM EDTA) for 5–10 min at 37 °C to detach the cells for reseeding and growing in new culture dishes at a density of  $\sim 2.2 \times 10^5$  per culture dish. After 24 h, the medium was removed, fresh serum-free conditioned media (Minimum Essential Medium Alpha Medium) was added, and the cells were incubated at 37 °C for 40 h. The serum-free cell medium was then collected and filtered through a 0.2- $\mu\text{m}$  filter.

**Urine Samples.** A urine sample from a healthy volunteer was filtered through a 0.2- $\mu\text{m}$  membrane and then diluted 5-fold with buffer (5 mM sodium phosphate, 200 mM NaCl; pH 7.4). The solution was then analyzed using the described probe following the processes similar to that used for the cell media.

**Competitive Assays of PDGFs, Thrombin, and PDGF  $\alpha$ -Receptor.** Aliquots (0.2 mL) of 0.2 M phosphate solutions containing 200 mM NaCl and PDGF AA-L<sub>AuND</sub> (4.0 nM) and Apt-QAuNP (0.4 nM) in the presence of PDGFs (0–2.0 nM) were equilibrated at room temperature for 1 h. All solutions were then transferred into 0.4-mL quartz cuvettes; their photoluminescence spectra were measured when operating the fluorescence spectrophotometer at an excitation wavelength of 375 nm. The real sample solutions were analyzed using the described probe following the processes similar to that used for cell media.

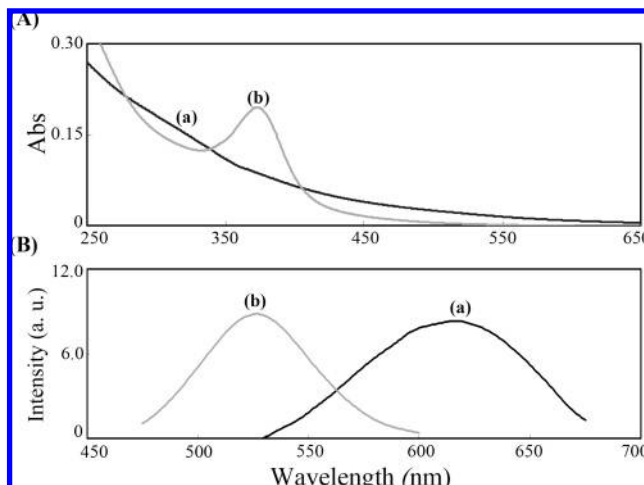
## RESULTS AND DISCUSSION

**Optical Properties of PDGF AA-L<sub>AuND</sub>.** In a previous study, we prepared water-soluble alkanethiol-L<sub>AuND</sub> having tunable photoluminescence wavelengths (501–618 nm), when excited at 375 nm, through modification of the chain length of the alkanethiol.<sup>12</sup> Because 11-MUA-L<sub>AuND</sub> photoluminescence at the wavelengths ( $\lambda_{\text{max}}^{\text{em}}$ ) was centered at 520 nm, which overlaps to a

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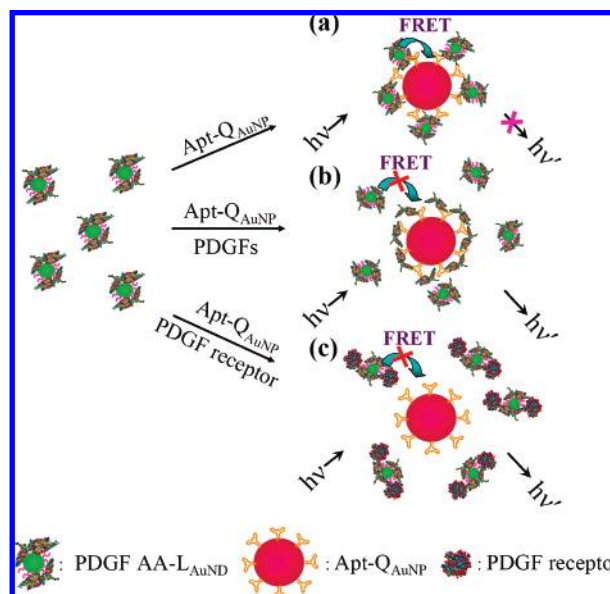




**Figure 1.** (A) UV-vis absorbance and (B) photoluminescence spectra of (a) Au NPs and (b) 11-MUA-L<sub>AuND</sub>. The photoluminescence intensities are plotted in arbitrary units (a. u.); excitation wavelength: 375 nm. The concentrations of the Au NPs and 11-MUA-L<sub>AuND</sub> in (B) were 0.94  $\mu$ M and 0.15 nM, respectively.

great extent with the surface plasmon resonance (SPR) band of the 13-nm Q<sub>AuNP</sub>, we chose them for use as donors. From HRTEM measurements, we determined the sizes of the as-prepared Au NPs and 11-MUA-L<sub>AuND</sub> from a count of 100 particles to be 2.9 ( $\pm 0.5$ ) and 2.0  $\pm$  0.1 nm, respectively (data not shown). The decreased size of the Au NPs in the presence of 11-MUA is due to the very strongly covalent, distinctly directional Au-S bond and preference of dissociation into very smaller Au and gold thiolate clusters (on the basis of fragmentation energies), as opposed to breaking the Au-S bond and detaching the Au cluster from the thiolate radical.<sup>18</sup> Although the 11-MUA-L<sub>AuND</sub> exhibited an absorption band centered at a wavelength ( $\lambda_{\text{max}}^{\text{abs}}$ ) of 375 nm, no apparent absorption band appeared for the Au NPs (Figure 1A). The absorption bands for 11-MUA-L<sub>AuND</sub>s originate from metal-center (Au 5d<sup>10</sup> to 6sp interband transitions), ligand-metal charge-transfer transitions, or both.<sup>19,20</sup> The photoluminescent bands of the Au NPs and 11-MUA-L<sub>AuND</sub> were centered at wavelengths of 618 and 520 nm, respectively (Figure 1B). Upon decreasing the size of the protected Au nanoclusters, the emission wavelength underwent a blue shift.<sup>12,21</sup> We note that the photoluminescence intensity of the 11-MUA-L<sub>AuND</sub> was ~60 000-fold

### Scheme 1. Schematic Representations of PDGF and PDGF Receptor Nanosensors That Operate Based on the Modulation of the Photoluminescence Quenching between PDGF AA-L<sub>AuND</sub> and Apt-Q<sub>AuNP</sub><sup>a</sup>



<sup>a</sup> h, Planck's constant;  $\nu$ , frequency of light.

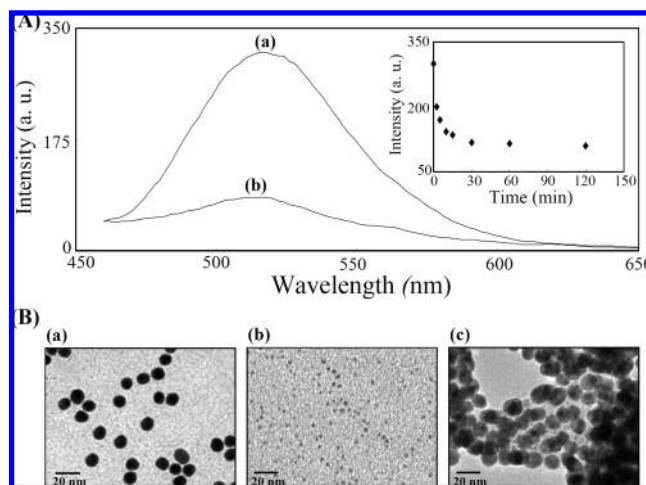
higher than that of the Au NPs. The quantum yield of the as-prepared 11-MUA-L<sub>AuND</sub> was determined to be 3.1% through comparison with quinine (QY 53%). This QY is comparable to those of the best currently available water-soluble alkanethiol ligand-protected Au NPs.<sup>19e</sup>

The purification of 11-MUA-L<sub>AuND</sub> was accomplished by centrifugal filtration (13500g) for 40 min through a filter having a cutoff of 10 kDa. Comparison with the original 11-MUA-L<sub>AuND</sub> solution showed that there is very weak photoluminescence in the removed solution, which can be detected at an excitation wavelength of 375 nm. It means the photoluminescence signal is truly from 11-MUA-L<sub>AuND</sub>. The 11-MUA-L<sub>AuND</sub> pellets were then resuspended in 5 mM sodium phosphate solution (pH 7.4). By comparing the absorbance of the original 11-MUA-L<sub>AuND</sub> solution at 375 nm to that of the resuspended solution, ~95% of the 11-MUA-L<sub>AuND</sub> was deemed to have been collected (data not shown). Our prepared 11-MUA-L<sub>AuND</sub> solution was stable in aqueous solution at 4 °C for at least 3 months. From a practical point of view, it is important to test the stability of the as-prepared 11-MUA-L<sub>AuND</sub> solution under physiological conditions. We found that our prepared 11-MUA-L<sub>AuND</sub> solution was stable in sodium phosphate (10 mM; pH 4.00–11.00) solutions containing NaCl at concentrations of up to 250 mM (Figure S1, Supporting Information). The 11-MUA-L<sub>AuND</sub> sample was then used to prepare PDGF AA-L<sub>AuND</sub>, in which PDGF AA molecules were bioconjugated with 11-MUA-L<sub>AuND</sub> through electrostatic and hydrophobic interactions.<sup>12,22</sup>

The photoluminescence intensity of PDGF AA-L<sub>AuND</sub> increased initially upon increasing the concentration of PDGF AA before reaching a plateau (Figure S2, Supporting Information). PDGF AA protected the 11-MUA-L<sub>AuND</sub> from quenchers present

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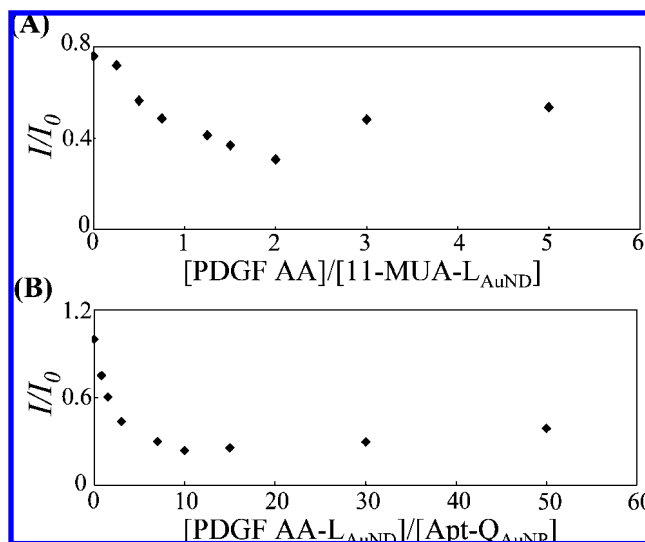


**Figure 2.** (A) Photoluminescence spectra of solutions containing PDGF AA-L<sub>AuND</sub> (4.0 nM) in the (a) absence and (b) presence of Apt-Q<sub>AuNP</sub> (0.6 nM) in 5 mM sodium phosphate (pH 7.4) containing 200 mM NaCl. Inset: Time-course measurement of the photoluminescence intensity at 520 nm for PDGF AA-L<sub>AuND</sub> after the addition of Apt-Q<sub>AuNP</sub>. (B) HRTEM images of (a) Apt-Q<sub>AuNP</sub>, (b) PDGF AA-L<sub>AuND</sub>, and (c) mixture of Apt-Q<sub>AuNP</sub> and PDGF AA-L<sub>AuND</sub>. Other conditions were the same as those described in Figure 1.

in solution, thereby increasing the photoluminescence of 11-MUA-L<sub>AuND</sub>. The saturated binding ratio [PDGF AA]/[11-MUA-L<sub>AuND</sub>] was close to 2, suggesting that two PDGF AA molecules were present on each 11-MUA-L<sub>AuND</sub>. The as-prepared PDGF AA-L<sub>AuND</sub> solution was even more stable than the 11-MUA-L<sub>AuND</sub> solution under physiological conditions (up to 1.0 M NaCl).

**Assay Strategy.** Scheme 1 illustrates that, by employing aptamer-protein interactions, the concentrations of PDGFs and their receptors in sample solutions can be determined. The photoluminescence of PDGF AA-L<sub>AuND</sub> at 520 nm decreased when photoluminescence quenching occurred between Apt-Q<sub>AuNP</sub> and PDGF AA-L<sub>AuND</sub> (route a). In the presence of PDGFs (route b), the interaction between Apt-Q<sub>AuNP</sub> and PDGF AA-L<sub>AuND</sub> decreased as a result of competitive reactions between the PDGFs and Apt-Q<sub>AuNP</sub>. As a consequence, the photoluminescence quenching efficiency between Apt-Q<sub>AuNP</sub> and PDGF AA-L<sub>AuND</sub> decreased, and photoluminescence at 520 nm occurred to a greater extent. Similarly, the interaction between Apt-Q<sub>AuNP</sub> and PDGF AA-L<sub>AuND</sub> decreased as a result of competitive reactions between PDGF  $\alpha$ -receptor and PDGF AA-L<sub>AuND</sub> (route c), resulting in decreased photoluminescence quenching efficiency between Apt-Q<sub>AuNP</sub> and PDGF AA-L<sub>AuND</sub> and increased photoluminescence at 520 nm.

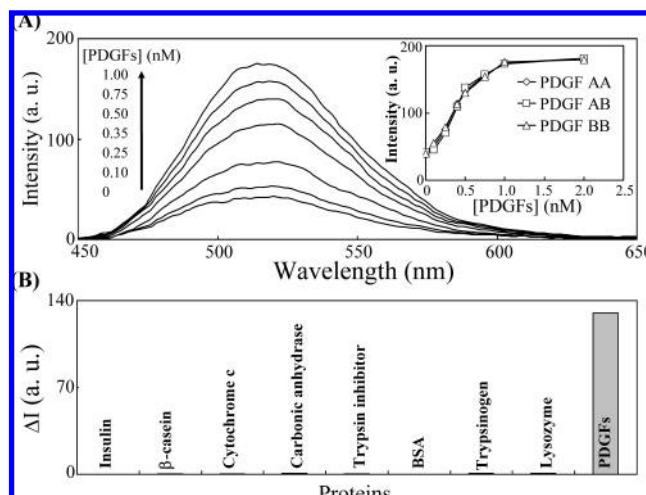
**Photoluminescence Quenching of PDGF AA-L<sub>AuND</sub> by Apt-Q<sub>AuNP</sub>.** To test our photoluminescence quenching assay, we added PDGF AA-L<sub>AuND</sub> into a solution of Apt-Q<sub>AuNP</sub>. As a result of specific interactions between the aptamer and PDGF AA molecules, the PDGF AA-L<sub>AuND</sub> sample photoluminesced more weakly in the presence of Apt-Q<sub>AuNP</sub> (Figure 2A). The photoluminescence at 520 nm of the mixture reached a state of equilibrium within  $\sim 30$  min (the inset in Figure 2A). TEM images of Apt-Q<sub>AuNP</sub>, PDGF AA-L<sub>AuND</sub>, and their mixture support our reasoning (Figure 2B). At a constant concentration of Apt-Q<sub>AuNP</sub> (0.6 nM), the mixtures at different molar ratios of [PDGF AA]/[11-MUA-L<sub>AuND</sub>] had various photoluminescence intensities at 520



**Figure 3.** (A) Quenching efficiency ( $I/I_0$ ) of PDGF AA-L<sub>AuND</sub> (4 nM) at 520 nm plotted with respect to the concentration ratio of [PDGF AA]/[11-MUA-L<sub>AuND</sub>] at a fixed concentration of Apt-Q<sub>AuNP</sub> (0.6 nM). (B) Quenching efficiency ( $I/I_0$ ) of PDGF AA-L<sub>AuND</sub> (4 nM) at 520 nm plotted with respect to the concentration ratio of [PDGF AA-L<sub>AuND</sub>]/[Apt-Q<sub>AuNP</sub>]. Buffer: 5 mM sodium phosphate (pH 7.4) containing 200 mM NaCl.  $I$  and  $I_0$  are the photoluminescence intensities of the PDGF AA-L<sub>AuND</sub> in the absence and presence of Apt-Q<sub>AuNP</sub>. Other conditions were the same as those described in Figure 1.

nm, reaching a plateau at a molar ratio of 2 (Figure 3A). Figure S3 (Supporting Information) displays that the SPR absorption of the Apt-Q<sub>AuNP</sub> solutions are different in the absence and presence of various amounts of PDGF AA-L<sub>AuND</sub>. The specific interactions between the PDGF and aptamer molecules induced interparticle cross-linking among PDGF AA-L<sub>AuND</sub> and Apt-Q<sub>AuNP</sub>. The degree of aggregation of PDGF AA-L<sub>AuND</sub> and Apt-Q<sub>AuNP</sub> is highly dependent on the molar ratios of [PDGF AA]/[11-MUA-L<sub>AuND</sub>] and coincide with the photoluminescence quenching of PDGF AA-L<sub>AuND</sub> by Apt-Q<sub>AuNP</sub>, as shown in Figure 3A.<sup>14a</sup> We thus suggest that photoluminescence quenching is through resonance energy transfer and aggregation of particles.<sup>10d,e,12,15c</sup> At higher PDGF concentrations, aggregation occurred to a lesser extent as a result of electrostatic repulsion among the PDGF AA-L<sub>AuND</sub>.<sup>14a</sup> At a constant concentration of PDGF AA-L<sub>AuND</sub>, we found that the optimum ratio of [PDGF AA-L<sub>AuND</sub>]/[Apt-Q<sub>AuNP</sub>] was 10 (Figure 3B). We suggest that the saturated binding with PDGF AA-L<sub>AuND</sub> of each Apt-Q<sub>AuNP</sub> particle was  $\sim 10$ , with this value arising mainly from the binding of PDGF AA and aptamer (1:2) and the relative size of L<sub>AuND</sub> and Q<sub>AuNP</sub> (2:13).

**Competitive Assays of PDGFs.** We used the optimum conditions (4 nM PDGF AA-L<sub>AuND</sub> (two PDGF molecules on each L<sub>AuND</sub>) and 0.4 nM Apt-Q<sub>AuNP</sub> in sodium phosphate solution (5 mM, pH 7.4, 200 mM NaCl)<sup>14a</sup> to determine the concentrations of PDGFs in solution. The photoluminescence of the mixtures increased upon increasing the concentration of PDGFs over the range 0.1–2.0 nM (Figure 4A). The linear relationships for all three PDGFs were from 0.1 to 1.0 nM, with correlation coefficients  $> 0.94$ . The limit of detection (LOD) for each of the three PDGFs was 80 pM, on the basis of a signal-to-noise ratio (S/N) of 3; these values are comparable with those obtained using other signaling aptamer reporters.<sup>6d,e,7b,g</sup> The highly sensitive detection of PDGFs by PDGF AA-L<sub>AuND</sub>/Apt-Q<sub>AuNP</sub> is due to a low background signal



**Figure 4.** (A) Competitive binding assay for PDGFs using the PDGF AA- $L_{AuND}$ /Apt- $Q_{AuNP}$  probe. (B) Photoluminescence changes of the PDGF AA- $L_{AuND}$  probe toward PDGFs and other proteins. Other conditions were the same as those described in Figure 3B.

and a signal enhancement of up to 3-fold upon addition of PDGFs at the saturated concentration. PDGFs exist as disulfide-linked dimers of two polypeptide chains (A and B) in three isoforms (PDGF AA, BB, and AB);<sup>23a</sup> they are growth factor proteins in human platelets and have growth-promoting activity for fibroblasts, smooth muscle cells, and glial cells.<sup>4,23b-d</sup>

Interestingly, the sensitivities of PDGF AA, PDGF AB, and PDGF BB were almost the same, a situation that does not correlate with the order of the stabilities of the Apt-PDGF complexes in free solution: PDGF BB > PDGF AB > PDGF AA.<sup>13</sup> We believe that this behavior is due to changes in the structures of the aptamer molecules or the PDGFs on the Au NP surface and a multivalent binding effect (ultrahigh densities of aptamer on the local surface of  $Q_{AuNP}$ ) that increases the binding affinity of PDGFs to Apt- $Q_{AuNP}$ .<sup>14,24</sup>

Our PDGF AA- $L_{AuND}$ /Apt- $Q_{AuNP}$  system can detect total PDGF concentrations in real samples because the sensitivities for the three PDGFs are identical. To test the specificity of this assay toward PDGFs, we incubated aliquots of the mixtures of PDGF AA- $L_{AuND}$  (4 nM) and Apt- $Q_{AuNP}$  (0.4 nM) in the presence of PDGFs (1.0 nM) and some possible interfering proteins (including insulin,  $\beta$ -casein, cytochrome *c*, carbonic anhydrase, trypsin inhibitor, BSA, trypsinogen, and lysozyme (1.0 nM)) that might be present in biological samples, such as blood. The responses to photoluminescence changes of PDGF AA- $L_{AuND}$ /Apt- $Q_{AuNP}$  with and without the interfering proteins are negligible relative to those from PDGFs (Figure 4B). In addition, the PDGF AA-

$L_{AuND}$ /Apt- $Q_{AuNP}$  system allowed the detection of PDGF AA at concentrations as low as 0.5 nM in the presence of BSA at 10  $\mu$ M, that is, a 20 000-fold higher concentration (Figure S4, Supporting Information). This highly specific sensor displays high potential for detecting PDGFs in complex, real samples.

**Competitive Assays of  $\alpha$ -Thrombin.** To illustrate whether our new signaling strategy was applicable to the detection of other proteins, we tested another 27-nt DNA aptamer (Apt<sub>thrombin</sub>) that binds to Exosite II of  $\alpha$ -thrombin (an endoprotease protein that has many effects in the coagulation cascade). This aptamer has a unique structure, with G-quartet conjugated stem base pairs that allow binding to  $\alpha$ -thrombin.<sup>25</sup> Again, we obtained a linear relationship (Figure S5, Supporting Information) between the photoluminescence intensity and the concentration of  $\alpha$ -thrombin added to a mixture of thrombin- $L_{AuND}$  (4.0 nM) and Apt<sub>thrombin</sub>- $Q_{AuNP}$  (0.4 nM). The linear calibration curve for quantitation of  $\alpha$ -thrombin extended from 0.25 to 4.0 nM, with a correlation coefficient of 0.96. The LOD for  $\alpha$ -thrombin at an S/N ratio of 3 was 0.15 nM.

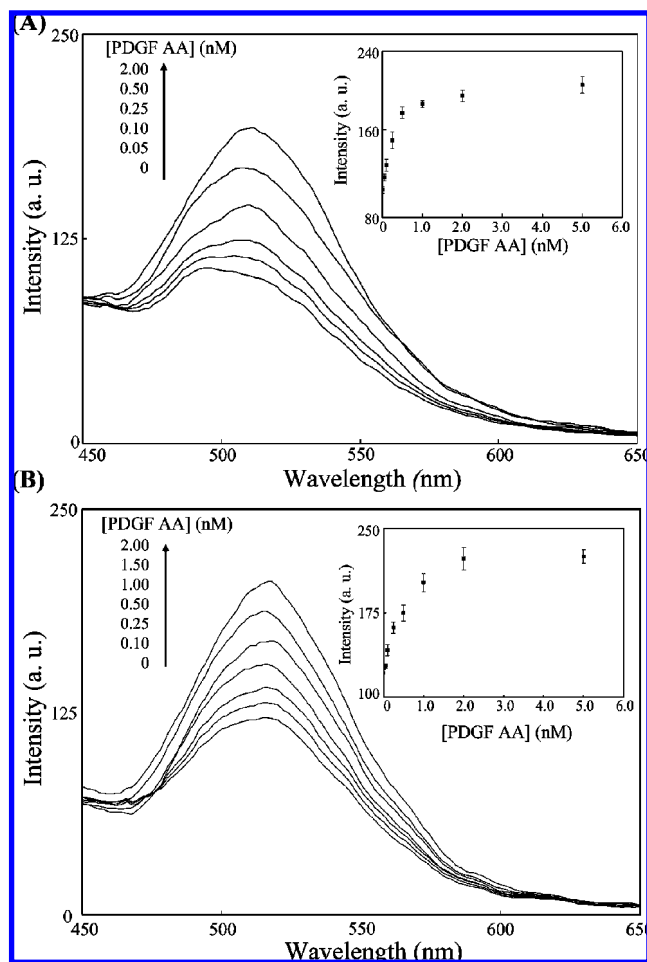
**Competitive Assays of PDGF Receptor.** The three isomeric PDGF molecules bind specifically, with different degrees, to two receptors: the PDGF  $\alpha$ - and  $\beta$ -receptors.<sup>26</sup> The dissociation constants ( $K_d$ ) for the complexes of PDGF  $\alpha$ -receptor with PDGF BB, AB, and AA are 0.5, 0.1, and 0.2 nM, respectively.<sup>26,27</sup> Binding of the receptors to PDGF activates intracellular tyrosine kinase, leading to autophosphorylation of the cytoplasmic domain of the receptor as well as phosphorylation of other intracellular substrates.<sup>27</sup> The aptamer can inhibit the binding of PDGFs to their receptors and suppress the regulation of their receptors.<sup>13,28</sup> Therefore, we believed that PDGF  $\alpha$ -receptor would bind to PDGF AA- $L_{AuND}$  and inhibit their Apt- $Q_{AuNP}$ -induced photoluminescence quenching (Scheme 1C). To test this hypothesis, we conducted a competitive assay using mixtures of 4 nM PDGF AA- $L_{AuND}$  and 0.4 nM Apt- $Q_{AuNP}$  to detect PDGF  $\alpha$ -receptor. As indicated in Figure S6 (Supporting Information), the photoluminescence of the mixture increased upon increasing the concentration of PDGF  $\alpha$ -receptor from 0 to 10 nM. The inset in Figure S6 displays a linear relationship between the photoluminescence intensity of the mixture at 520 nm and the PDGF  $\alpha$ -receptor concentration over the range 0.5–5.0 nM, with a correlation coefficient of 0.93. On the basis of an S/N ratio of 3, we estimated the LOD for PDGF  $\alpha$ -receptor to be 0.25 nM.

**Analyses of PDGFs in Biological Samples.** To test the practicality of this assay, we conducted analyses of PDGFs in biological samples. Our first representative example was a serum-free conditioned cell medium (MEM) that had been collected from the human breast carcinoma HTB-26. It has been reported that

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**Figure 5.** Validation of the use of PDGF AA- $L_{AuND}$ /Apt- $Q_{AuNP}$  probes for the detection of PDGF AA (0–5.0 nM) spiked in (A) cultured cell media and (B) urine. The inset displays the increase in photoluminescence of PDGF AA- $L_{AuND}$  upon increasing the concentration of PDGF AA. Error bars are standard deviation values across four repetitive experiments. Other conditions were the same as those described in Figure 3B.

PDGFs secrete in culture media at a level of  $\sim 0.2$  nM.<sup>29</sup> Unfortunately, an intense background fluorescence, contributed by some indigenous species in the cell medium, caused serious problems; the background fluorescence was over 10-fold greater than the photoluminescence intensity of PDGF AA- $L_{AuND}$  (4 nM). To minimize interference, we developed an NP-assisted protein enrichment method to remove the matrix. Serum-free cell media (1 mL) diluted by a factor of 10, PDGF AA at concentrations ranging from 0 to 5.0 nM, and Apt- $Q_{AuNP}$  (0.04 nM) in buffer (5 mM sodium phosphate, 200 mM NaCl; pH 7.4) were mixed at 25 °C for 1 h. Subsequently, the samples were centrifuged, and the supernatants were removed. Finally, the precipitates were resuspended in 100- $\mu$ L solutions (pH 7.4) of 5 mM sodium phosphate, 100 mM NaCl, and 4 nM PDGF AA- $L_{AuND}$  and left for another 1 h. Figure 5A displays photoluminescence spectra of the mixtures recorded at various concentrations of PDGF AA. These spectra indicate that the photoluminescence responses increased upon increasing the concentration of PDGF AA. The

LOD, at a S/N ratio of 3, for PDGF AA spiked in HTB-26 cell media was 10 pM. We achieved a recovery of 96% for PDGF AA and determined the concentration of PDGFs in the collected cell media to be  $0.18 (\pm 0.04)$  nM, which is in good agreement with the reported value.<sup>29</sup>

We also utilized our sensor system to determine PDGFs in urine samples, because their levels can be indicative of certain diseases. For example, the concentrations of PDGF in urine samples from patients with myelofibrosis and essential thrombocythemia are  $0.25 (\pm 0.08)$  and  $0.41 (\pm 0.08)$  nM, respectively, both significantly higher than that from healthy people [ $0.08 (\pm 0.01)$  nM].<sup>30</sup> Again, we applied the Apt- $Q_{AuNP}$ -assisted PDGF AA enrichment method because of serious matrix interference in urine samples (Figure 5B). We achieved a recovery of 95% for PDGF AA and determined the concentration of PDGFs in the urine sample from a healthy man (31 years old) to be  $0.10 (\pm 0.02)$  nM, which agrees well with the reported value.<sup>30</sup> We also try to detect PDGFs in human serum samples by the PDGF AA- $L_{AuND}$ /Apt- $Q_{AuNP}$  system, but a high concentration of an interfering protein such as HSA in serum is 30–50 mg/mL, which results in strong nonspecific binding with Apt- $Q_{AuNP}$  to cause a failure in the performance of PDGF AA- $L_{AuND}$ /Apt- $Q_{AuNP}$  for detecting PDGFs. The thiol-PEG  $CH_3O-(CH_2CH_2O)_n-CH_2CH_2SH$ -modified or polymer blocked Apt- $Q_{AuNP}$ , and controlling the aptamer density on  $Q_{AuNP}$  in our further work may reduce the nonspecific binding and allow detection of PDGFs in serum samples.<sup>31</sup>

## CONCLUSION

To the best of our knowledge, this paper describes the first instance of the use of two differently sized AuNPs, acting separately as donor and acceptor, in homogeneous assays developed for the analysis of proteins. Our PDGF AA- $L_{AuND}$ /Apt- $Q_{AuNP}$ -based molecular light-switching system allowed the analyses of PDGFs and PDGF  $\alpha$ -receptor in homogeneous solutions. This method takes advantage of the dramatic increase in the intensity of the turn-on photoluminescence signal of PDGF AA- $L_{AuND}$ /Apt- $Q_{AuNP}$  upon its binding with PDGFs. The PDGF AA- $L_{AuND}$ /Apt- $Q_{AuNP}$  system allowed the detection of PDGF AA at concentrations as low as 0.5 nM in the presence of BSA at 10  $\mu$ M. We have also demonstrated that the Apt- $Q_{AuNP}$  are effective selectors for enriching PDGF AA from large-volume cell media and urine samples. This approach allows removal of most of the background fluorescence, leading to detection of PDGF AA at concentrations as low as 10 pM, although ELISA's PDGF kits can separately detect to picograms per milliliter of PDGF AA, AB, and BB.<sup>30</sup> Our present method can detect total PDGFs in biological samples, such as cultured cell medium and urine samples. With its advantages of rapidity and specificity, this present approach holds great potential for use in protein analysis and cancer diagnosis.

## ACKNOWLEDGMENT

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#### **SUPPORTING INFORMATION AVAILABLE**

Photoluminescence intensities at 520 nm of 11-MUA- $L_{AuND}$  (10 nM) prepared in (A) 10 mM sodium phosphate (pH 3.0–11.0) and (B) 5 mM sodium phosphate (pH 7.4) containing 0–1.5 M NaCl (Figure S1). Photoluminescence spectra of 11-MUA- $L_{AuND}$  (4 nM) in the presence of PDGF AA (0–8.0 nM) (Figure S2). UV-vis absorption spectra of the Apt- $Q_{AuNP}$  (0.6 nM) in 5 mM phosphate solution (pH 7.4) containing 200 mM NaCl and 11-MUA- $L_{AuND}$  (4.0 nM) at various concentration ratios of [PDGF

AA]/[11-MUA- $L_{AuND}$ ] (Figure S3). Photoluminescence spectra of PDGF AA- $L_{AuND}$  (4.0 nM), Apt- $Q_{AuNP}$  (0.4 nM), and PDGF AA (0–2.5 nM) in the presence of 10  $\mu$ M BSA (Figure S4). Competitive binding assay for  $\alpha$ -thrombin (0–25 nM) using the thrombin- $L_{AuND}$ /Apt<sub>thrombin</sub>- $Q_{AuNP}$  probe (Figure S5). Competitive binding assay for PDGF  $\alpha$ -receptor using the PDGF AA- $L_{AuND}$ /Apt- $Q_{AuNP}$  probe (Figure S6).

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