# The detection of platelet derived growth factor using decoupling of quencher-oligonucleotide from aptamer/quantum dot bioconjugates

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# The detection of platelet derived growth factor using decoupling of quencher-oligonucleotide from aptamer/quantum dot bioconjugates

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**Abstract**

High-sensitivity, high-specificity detection of platelet derived growth factor (PDGF)-BB was realized using the change in fluorescence resonance energy transfer (FRET) occurring between quantum dot (QD) donors and black hole quencher (BHQ) acceptors. CdSe/ZnS QD/mercaptoacetic acid (MAA)/PDGF aptamer bioconjugates were successfully synthesized using ligand exchange. Black hole quencher (BHQ)-bearing oligonucleotide molecules showing partial sequence matching to PDGF aptamer were attached to PDGF aptamers and photoluminescence (PL) quenching was obtained through FRET. By adding target PDGF-BB to the bioconjugates containing BHQs, PL recovery was detected due to detachment of BHQ-bearing oligonucleotide from the PDGF aptamer as a result of the difference in affinity to the PDGF aptamer. The detection limit of the sensor was ∼0.4 nM and the linearity was maintained up to 1.6 nM in the PL intensity versus concentration curve. Measurement of PL recovery was suggested as a strong tool for high-sensitivity detection of PDGF-BB. Epidermal growth factor (EGF), the negative control molecule, did not contribute to PL recovery due to lack of binding affinity to the PDGF aptamers, which demonstrates the selectivity of the biosensor.

S Supplementary data are available from stacks.iop.org/Nano/20/175503

(Some figures in this article are in colour only in the electronic version)

## 1. Introduction

Quantum dots (QDs) have been of great interest to many researchers due to their versatility in high quantum yield, tunable light emission and photochemical stability compared to common organic fluorophores [1–3]. CdSe QDs have been the most studied because they show almost full visible-range light emission by quantum confinement effect when they shrink down to a size below the so-called Bohr radius (∼5 nm) [4, 5]. However, in general they suffer from low quantum yield arising from a high surface-to-volume ratio. The surface comprises atoms having dangling bonds that can act as traps for excitons, and thus it significantly suppresses light emission by disturbing edge-to-edge energy radiation. Hence, to improve their optical property, bare CdSe QDs should be overlaid with another semiconductor material having a larger band gap than CdSe QDs, such as ZnS, to tailor a type I energy band structure. In this way the light emission intensity of the CdSe/ZnS core/shell QDs can be magnified by about

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10 times compared to that of bare CdSe and thus they have found wide applications in various photonics fields [6]. In addition, CdSe/ZnS QDs have been known as sensor materials suitable for biological signal transduction, and various methods have been developed for the precise detection of proteins, DNA or other biomolecules [7–9]. Compared to common organic dyes, inorganic dyes such as CdSe/ZnS QDs have demonstrated long life time and high-intensity light emission owing to their chemical and photochemical stability and high quantum yield, respectively. Fluorescence resonance energy transfer (FRET) is a state-of-the-art technique that can be used for high-sensitivity and high-specificity detection of a target protein, DNA and other specific molecules. FRET describes energy transfer from a donor to an acceptor, and thus it occurs only when their respective energy emission and absorption bands overlap with each other. The use of FRET to detect biomolecules such as proteins, DNA or RNA has been conducted by several groups. For example, the shift in PL peak position was used for the detection of bacteria as they bind to the QDs [10] and the sensing of cancer cells based on FRET together with drug delivery was reported [11]. Recently, multiple sensing of analytes with high sensitivity has been a major challenge for biosensors. Liu *et al* [12] successfully conducted one-pot simultaneous detection of cocaine and adenosine as quenchers using gold nanoparticles. Also, Goldman *et al* developed a sensor for the explosive 2,4,6-trinitrotoluene (TNT) in aqueous environments using the FRET phenomenon [13]. Other groups tried to detect thrombin as well as lysozyme using a gold electrode with QDs [14]. Previously, Wargnier *et al* [15] revealed the quenching of light emission from CdSe QDs by gold nanoparticles and proposed a sensor application. Levy *et al* [16] also reported detection of thrombin using FRET between CdSe QDs and quenchers. Because this FRET phenomenon is highly sensitive to the distance between donors and acceptors, aptamers (artificial single-stranded nucleic acid ligands showing strong conformational change in coupling with target molecules) have been widely investigated for use in FRET-based biological detection. For instance, the conformational change of aptamers from a hair-pin to a linear structure has been used for detection of ATP, protein and HIV-1 Tat [17, 18]. Moreover, aptamers have many advantages over antibodies because they are easier to control, less costly and more stable against denaturation.

Platelet derived growth factor (PDGF) is a protein growth factor that is concerned with controlling the proliferation or differentiation of cells including fibroblasts, smooth muscle cells and glial cells [18, 19]. PDGF can be classified into two homodimeric glycoproteins, AA and BB. Specifically, PDGF-BB is generated by most types of solid tumors, inducing stroma formation and a neovascular response [20]. Huang *et al* have reported a FRET-based PDGF biosensor that consists of aptamers conjugated directly with the organic fluorophore N,N′-dimethyl-2,7-diazapyrenium (DMDAP) and a gold nanoparticle quencher [21–23]. However, the biological sensing may suffer from relatively weak signal transduction arising from low uniformity in aggregations of gold nanoparticles or an irregular distance between donors and acceptors. Until now, there have been no reports on biosensors for the detection of PDGF molecules using FRET on–off semiconductor QDs according to the detachment of quenchers.

In this study, we employed a new approach to PDGF protein targeting using QD-PDGF DNA aptamer (35 mers) bioconjugates and short oligonucleotides showing partial binding (15 out of 35 mers) to PDGF aptamers labeled with a black hole quencher (BHQ). When target PDGF proteins are added to a system, they detach quencher-bearing oligonucleotides and couple with PDGF aptamers due to a strong affinity. The PDGF aptamer has reasonable affinities to the PDGF-BB ($K_d \sim 0.1$ nM) [21]. This replacement makes the QDs free from quenchers that suppress FRET and hence it allows noticeable photoluminescence (PL) recovery of the QDs. This strategy could be more effective than biosensing based upon conformational change. Measurement of the degree of PL recovery was proposed as a tool for the high-precision quantitative analysis of PDGF.

## 2. Experimental details

CdSe/ZnS core/shell QDs capped with trioctylphosphine oxide (TOPO) surfactant were synthesized according to the previous literature by Murray *et al* [3] and Dabbousi *et al* [5]. CdO (0.050 g), stearic acid (SA) (0.600 g), TOPO and hexadecylamine (HDA) (7.275 g) were loaded into a three-necked flask and heated to ∼310 °C. Then a Se–tributylphosphine (TBP) stock solution, prepared by mixing Se (0.210 g) and TBP (0.630 g), was swiftly injected into the flask containing Cd precursor to nucleate CdSe QDs via supersaturation. To overcoat CdSe QDs with ZnS, $(ZnCH_3)_2$ (0.133 g), hexamethyldisilathiane $(TMS)_2S$ (0.138 g) and TBP (5.366 g) were mixed and dropped into the flask as slowly as possible after the temperature was reduced to ∼230 °C. An identical cleaning method was employed as in the previous papers [3, 5, 6].

TOPO capping the surface of as-prepared CdSe/ZnS QDs was exchanged for mercaptoacetic acid (MAA) having a carboxylic group to make them water soluble, and the QDs were redispersed in a phosphate buffered saline (PBS) solution. Typically, 15 ml of MAA was added to 3 ml of a QD-containing solution and it was vigorously stirred for ∼5 h. QDs were rinsed several times to remove excess MAA by centrifugation. Finally, compacted CdSe/ZnS QDs were redispersed in 10 ml of PBS solution. To obtain bioconjugated QDs, prepared 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC; 0.950 g, 1 M) was added to the above solution and the solution was shaken for about 1 min. Then *n*-hydroxysulfo-succinimide (NHS; 0.5 g, 1 M) was added to the water-soluble QD solution to prepare these QDs conjugated with PDGF aptamers through an EDC–NHS coupling reaction for 15–20 min. To make CdSe/ZnS QDs/MAA/PDGF aptamers, 100 $\mu$l of PDGF aptamer (100 ng ml$^{-1}$) was poured into 1 ml of CdSe/ZnS-MAA solution ($5 \times 10^{-8}$ M) at a ratio of about 1:10 and the mixture was well stirred. This solution was gently shaken for 12 h. Compacted QDs were obtained in this solution by

centrifugation, and they were rinsed and redispersed in 10 ml of PBS solution with a proper amount of EDTA [24].

The BHQs were first immobilized to the PDGF aptamer-bearing water-soluble CdSe/ZnS QDs to quench the fluorescence of the QDs. Briefly, 100 $\mu$l of BHQs (100 ng ml$^{-1}$) were mixed with 1 ml of QDs solution for about 5 h at 36.5 °C and washed with PBS solution followed by centrifugation three times. The morphological features and crystallinity of core/shell QDs were investigated by high-resolution transmission electron microscopy (HRTEM; Tecnai G2 F20, FEI, Amsterdam, Netherlands) and x-ray diffraction (XRD; Rigaku Ultima-2000, Tokyo, Japan). Optical properties were examined using UV–visible absorption (Jasco UV–visible spectrophotometer, V530, Tokyo, Japan) and photoluminescence (PL; Hitachi F-4500, Tokyo, Japan). Also, quenching of FRET by the reaction between PDGF aptamer–QD bioconjugates and PDGF was measured by monitoring the variation in PL intensity.

## 3. Results and discussion

As-prepared CdSe/ZnS QDs capped with TOPO/TBP were analyzed by XRD to identify crystallinity, and they showed strong crystallinity with wurtzite structure (see supporting information figure S1 available at stacks.iop.org/Nano/20/175503). From the Scherrer formula the particle size of the QDs was estimated as ∼4.97 nm. The HRTEM image in figure 1 shows that as-prepared CdSe/ZnS QDs capped with TOPO/TBP are well dispersed owing to the TOPO capping. The wurtzite crystallinity of CdSe/ZnS QDs was confirmed and their average particle size was ∼5 nm, which is in good agreement with XRD data. The CdSe/ZnS core/shell QDs showed an enhanced photoluminescence (PL) intensity compared to bare CdSe QDs (see supporting information figure S2 available at stacks.iop.org/Nano/20/175503), indicating effective surface passivation and suitability for use as a donor for FRET biosensing. Also, the ligand exchange was successfully achieved by two functional groups, mercapto (−SH) and carboxylic acid (−COOH), in MAA. Because the highly nucleophilic sulfur in the mercapto group has strong affinity to zinc in the ZnS shell, TOPO can be readily replaced with MAA. At the same time, the carboxylic acid groups in MAA showing very strong hydrophilicity give water solubility to the CdSe/ZnS QDs. There was no apparent difference in the UV–visible absorption between TOPO-capped QDs and MAA-capped QDs (see supporting information figure S3 available at stacks.iop.org/Nano/20/175503). Simultaneously, the carboxylic acid groups in MAA providing water solubility for the QDs act as reactive functional groups for the immobilization of the 5′-amine (C6) modified PDGF aptamer (5′-amino (C6)-CAG GCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TG-3′) (M-Biotech, Inc., Seoul, Korea). The carboxylic acid that is the functional group capping the surface of the QDs reacts with EDC to form an active intermediate, and this intermediate can combine with amine-containing PDGF aptamers. However, as this intermediate tends to be quite unstable with a half-life of seconds, NHS was added to form a relatively stable intermediate. Finally, PDGF aptamers having amine groups can be immobilized onto the CdSe/ZnS QDs by the EDC–NHS coupling reaction through amide bonding (figure 2(a)) [24]. The number of assembled BHQ-DNA per CdSe/ZnS QD was theoretically estimated, by calculation, to be ∼31. To detect PDGF molecules (Abcam, Cambridge, UK) through the FRET mechanism, QDs were first quenched by complementary combining of BHQ-bearing oligonucleotide (5′-ACG TGC CGT AGC CTG-3′) and the PDGF aptamers (figure 2(b)). As shown in figure 3, the fluorescence of CdSe/ZnS core/shell QDs and the absorbance of BHQs nicely overlap. When light illuminates, the energy emitted from QDs transfers to BHQs that have an absorption band similar to the emission band of QDs. Consequently, it is clear that BHQ is a suitable acceptor for tailoring FRET-based biosensors through combining with CdSe/ZnS QD donors.

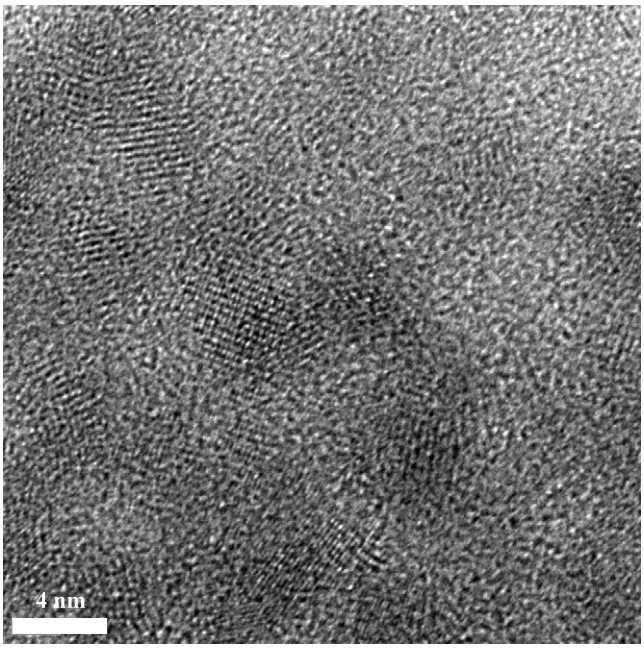


**Figure 1.** HRTEM image of CdSe/ZnS nanocrystal quantum dots.

As PGDF proteins are added to a complex of QDs and BHQs in PBS buffer solution, quenched states become released since BHQ-bearing oligonucleotides are detached from the QDs and thus FRET phenomena decrease (figure 2(c)). Indeed, since PDGF aptamers (35 mers) have a strong affinity to the PDGF proteins, quencher-oligonucleotide (15 mers) compounds can be readily detached by conformational change of PDGF aptamer after PDGF protein binding. As a result, FRET phenomena can be effectively suppressed through the detachment of quenchers from QDs. As presented in figure 4(a), there is an apparent recovery in PL intensity with the addition of PDGF protein and the degree of PL recovery shows nearly linear increase with PDGF concentration. More importantly, PL intensity was very sensitive to the addition of very small traces of the targets, as shown in figure 4(b), which allows the the bioconjugate system to detection with high precision extremely low concentrations of PDGF. Our biosensors show a detection limit of ∼0.4 nM, superior to previously reported ones (∼1.0 nM) [21].

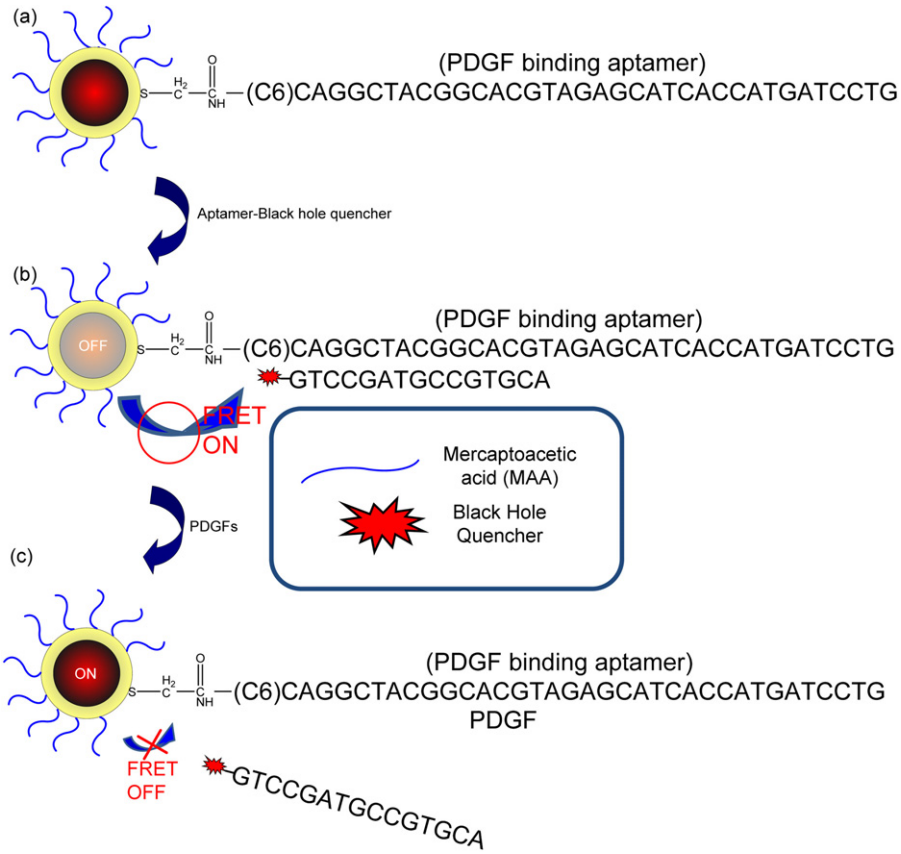


**Figure 2.** Schematic showing the procedure for detecting PDGF by suppressing FRET.

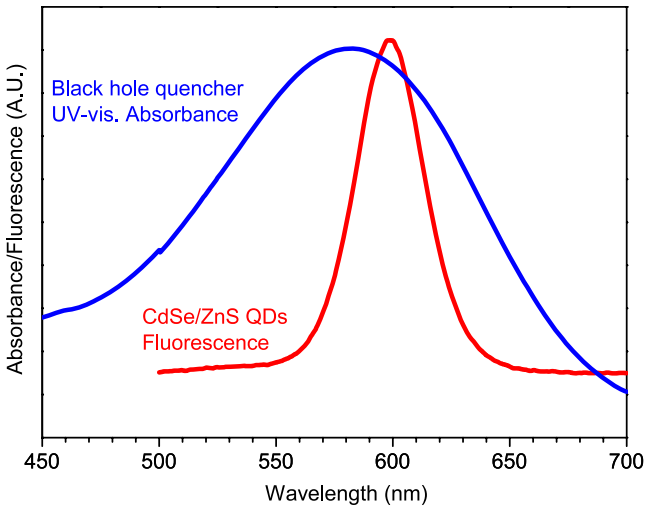


**Figure 3.** Fluorescence of CdSe/ZnS QDs and light absorbance of black hole quencher.

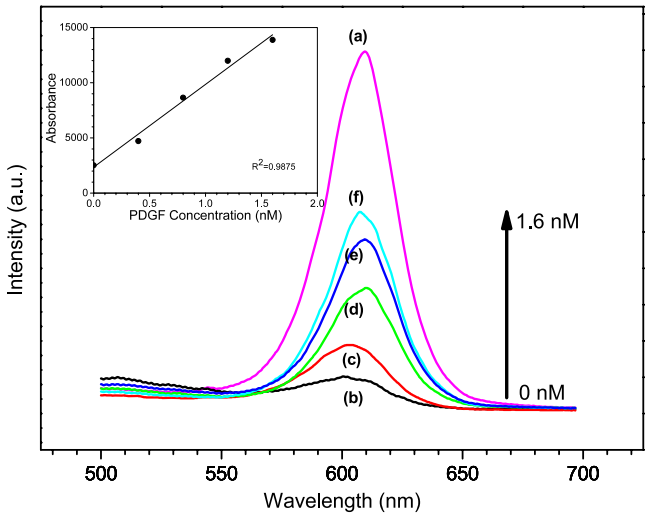


**Figure 4.** PL intensity variation in QD/aptamer bioconjugates (a) without and (b) with quenchers. The PDGF concentration was varied at (b) 0, (c) 0.4, (d) 0.8, (e) 1.2 and (f) 1.6 nM.

To exclude other influences on the PL intensity recovery, the operating temperature and pH were fixed at 25 °C and 7.4, respectively. To identify the specificity of PDGF aptamer in our system, a further experiment was conducted. Epidermal growth factor (EGF; Abcam, Cambridge, UK) was used to confirm specificity of the bioconjugate system to PDGF. EGF is a kind of growth factor related to the regulation of cell growth and proliferation like PDGF but has a different molecular structure. As shown in figure 5, the addition of EGF could not contribute to recovery of the PL intensity of the system because EGF does not have an affinity to PDGF-binding aptamers. As a result, the addition of the EGF could not contribute to the PL recovery by detachment of BHQs from the CdSe/ZnS QD/PDGF aptamer bioconjugates. Therefore, it can be concluded that the increase in PL peak intensity comes

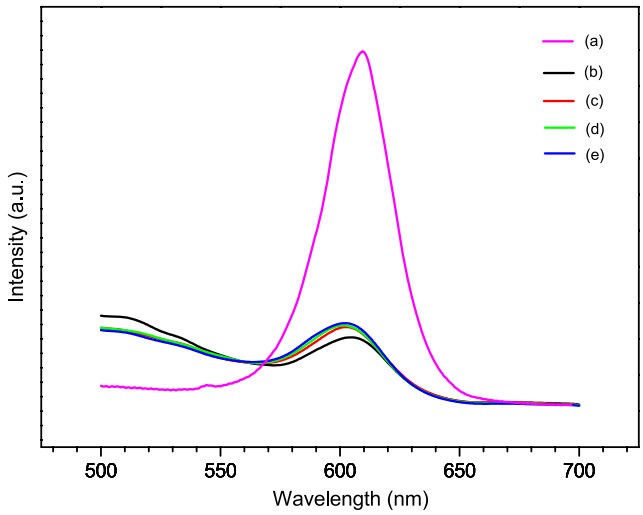


**Figure 5.** PL intensity variation in QD/aptamer bioconjugates (a) without and (b) with quenchers. The EGF concentration was varied at (b) 0, (c) 0.4, (d) 0.8 and (e) 1.2 nM.

solely from the reaction with PDGF, and the biosensor system possesses strong selectivity.

## 4. Conclusion

We demonstrate the successful synthesis of QD/MAA/PDGF aptamer bioconjugates. BHQ immobilized by oligonucleotides showing a partial sequence match to PDGF aptamer was attached to PDGF aptamers to quench the PL of the QDs. PDGF-BB having complete sequence matching with PDGF aptamers was able to detach BHQ-oligonucleotides by affinity deference, resulting in recovery of the PL intensity. We developed PDGF biosensors showing a limit of detection of 0.4 nM and a linear sensing range from 0 to 1.6 nM. The biosensor did not show PL recovery upon the addition of EGF, which implies that it has strong specificity to PDGF. The strategy of this decoupling of quencher-bearing oligonucleotide could be extended to other precision DNA or protein sensing applications.

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