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Aptamer-Capped Nanocrystal Quantum Dots: A New Method for Label-Free Protein Detection

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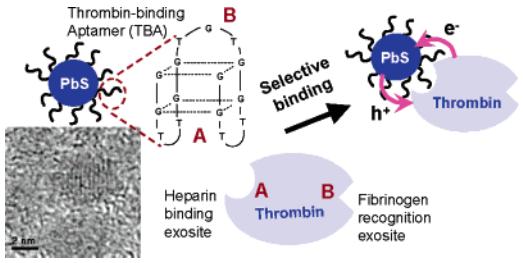
Nanocrystal quantum dots (QDs) are semiconductor materials where electrons are confined, yielding narrow, tunable, and highly stable photoluminescence (PL) compared to organic dyes.[1] As part of their stable synthesis, the QD surfaces are usually capped by long chain organic moieties such as trioctylphosphine (TOP) and trioctylphosphine oxide (TOPO).[1,2] This capping serves two purposes: it saturates dangling bonds at the exposed crystalline lattice, and it prevents irreversible aggregation by stabilizing the colloid through entropic repulsion.[1] These QDs can be dissolved in water by ligand exchange or overcoating and have been utilized for in vivo imaging.[3,4] QDs can be directly grown with biomolecules, such as DNA,[5−8] or conjugated with proteins through amide bond formation[9] and cross-linking using glutaric dialdehyde.[10]

The capping chemistry might also serve the additional purpose of providing selective binding to a target, such as a protein (Scheme 1). We have discovered that a DNA aptamer can passivate PbS QDs, rendering them water-soluble and stable against aggregation, and retain the secondary structure needed to selectively bind to its target (in this case, thrombin). Importantly, we find that when the aptamer-functionalized QD binds to its target, and only its target, there is a highly selective quenching of the PL. We attribute this quenching to charge transfer from functional groups on the protein to the QD itself. This selective detection of an unlabeled protein is distinct from previously reported schemes utilizing electrochemistry,[11] absorption,[12] and fluorescence resonance energy transfer (FRET).[13] In this work, the target detection by a unique, direct PL transduction is observed even in the presence of high background concentrations of interfering negatively or positively charged proteins. We show that extraneous proteins adsorb but do not modulate the PL and are rapidly displaced by the target analyte. This mechanism is the first to selectively modulate the QD PL directly, enabling new types of label-free assays and detection schemes.

To achieve the aptamer capping chemistry, we devised a novel, one-step synthesis of near-infrared (nIR) PbS QDs in aqueous solution at room temperature in open air: 15-mer thrombin-binding aptamer (TBA) and lead acetate were mixed in TAE buffer (pH 8), followed by the injection of sodium sulfide with vigorous stirring (molar ratio of TBA:Pb:S = 1:4:2). The resulting TBA-capped QDs are stable for several months and have diameters of 3−6 nm (Scheme 1 and Figure S1). The PL quantum yield is approximately 23%, calibrated with IR-1048 dye using a 785 nm laser. The particle concentrations used are approximated at 430 and 860 nM, assuming a uniform diameter of 5 nm. Absorption measurements reveal that approximately 20% of total TBA used passivates the QD surface (Figure S2).

Scheme 1 shows a TEM image (inset) of the TBA-capped QDs and their binding scheme to blood-clotting human α-thrombin. TBA forms a quadruplex structure in the presence of divalent cations[14] and retains its structure on the QD surface, which is necessary for thrombin binding (confirmed by circular dichroism in Figure S3).

Scheme 1. Schematic of TBA-Capped PbS QD Interaction with Thrombin[a]



[a] The TEM image indicates that the majority of the QDs have a diameter of 3−6 nm. The aptamer on the QD surface has a stable quadruplex structure that can bind to thrombin via either the heparin-binding or the fibrinogen recognition exosites. Charge transfer in the QD/thrombin complex induces photobleaching.

The TBA−thrombin interaction has been investigated thoroughly, and the chair-structured TBA binds to thrombin via either heparin-binding or fibrinogen recognition exosites.[15,16] Thrombin is a serine protease which converts fibrinogen into clottable fibrin, and its catalytic activity can be inhibited by blocking the fibrinogen recognition exosite.[15]

Figure 1 presents spectroscopic measurements of thrombin using PL transduction of the QD. The steady-state PL spectrum has a peak near 1050 nm and systematically decreases with increasing thrombin concentration. Adding free TBA in these mixture solutions did not recover the PL intensity, indicating that the binding process is irreversible at moderate time scales. From the inner panel of Figure 1b, we determine that the detection limit of thrombin is ∼1 nM under these conditions. The PL kinetic measurement at two different mixture concentrations shows that steady state is achieved within 1 min (Figure 1c), similar to the time scale observed for thrombin inhibition with TBA.[17]

The cause of PL quenching is examined by absorption spectroscopy for the QD with 0 and 180 nM thrombin (Figure 1d). A narrow absorption peak is not observed since these QDs are not sufficiently monodisperse. The decrease in the absorption with thrombin indicates that the PL modulation is caused by charge transfer (CT): we rule out FRET because thrombin absorbs only in the UV. The CT occurs most likely in a way that an electron is transferred from a functional group in thrombin (e.g., amine) to the QD conduction band, and a hole moves in the opposite direction (Scheme 1), resulting in a decrease of the absorption and PL intensities. CT-induced photobleaching of QDs and carbon nanotubes has been observed with amine groups,[18−20] Ru−polypyridine complexes,[21] and azobenzene compounds.[22]

A useful property of this scheme is that the TBA-capped QDs do not optically respond to other interfering proteins even at much higher concentrations (Figure 2). We selected control proteins with a variety of isoelectric points (pI): bovine serum albumin (BSA, pI 4.7), streptavidin (pI 5.0), proteinase K (pI 8.9), and lysozyme (pI

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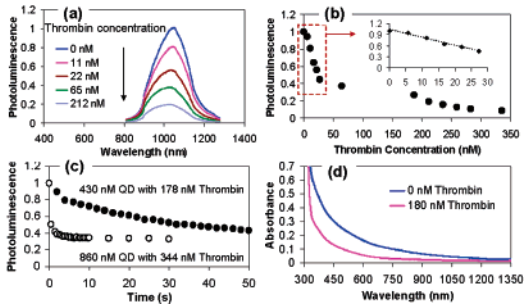

Figure 1. (a) Photobleaching of TBA-capped PbS QDs (~860 nM) with various amounts of thrombin. The peak wavelength near 1050 nm remains constant. (b) The PL gradually decreases with increasing thrombin concentration. The inner panel shows the linear region from 0 to 30 nM of thrombin, yielding the detection limit of ~1 nM. (c) Photobleaching kinetics of the QD/thrombin complex at two different concentrations measured at the peak wavelength. (d) Absorption spectra of the QDs (~430 nM) with 0 and 180 nM thrombin. The lower absorption with thrombin indicates that charge transfer occurs in the QD/thrombin complex, resulting in photobleaching.

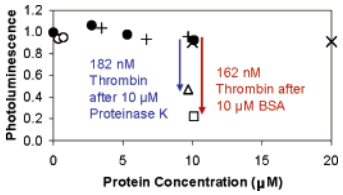

Figure 2. PL of the TBA−QD shows no response to other proteins: BSA (●), streptavidin (○), proteinase K (+), and lysozyme (×). In the presence of 10 μM BSA and 10 μM proteinase K, injection of thrombin to the QD solution immediately induces the PL decrease.

11.0). Although the negatively charged QDs can electrostatically interact with the latter two positively charged proteins,[23] the PL intensity does not change significantly during the measurement period (<10 min). We added 162 nM thrombin to the QD solution with 10 μM BSA and 182 nM thrombin to the QD with 10 μM proteinase K. The PL signals immediately decrease to 23 and 47%, respectively, which confirms selective binding/charge transfer in the TBA−QD/thrombin complex. The lower degree of photobleaching in the case of proteinase K is attributed to the electronic screening by the protein.

We further examine the QD−protein interaction by dynamic light scattering that provides diffusion coefficients, D: at protein to QD concentrations of ~8, D (μm^2/s) for initial QD = 4.13, QD + thrombin = 2.65, QD + lysozyme = 0.84, and QD + BSA = 3.92. The QD−thrombin complex has D approximately 36% smaller than that of the initial QD, indicating that the PL decrease is not due to protein-induced QD aggregation. In contrast, the strong electrostatic interaction of lysozyme with the QD results in the decrease of D by almost an order of magnitude. This is consistent with the observation that the lysozyme−QD solution becomes turbid several hours after the protein injection, implying mixture aggregation over time. Since BSA has overall negative charge in the mixture solution, it does not interact with the QD significantly, so the diffusion coefficient is similar to the initial QD D value as the scattering from the QD dominates. Hence, extraneous proteins can adsorb on the QD (depending on pH of the solution), but *only selective binding/charge transfer leads to photobleaching*.

We found that prostate-specific antigen (PSA, pI 6.8−7.5) also induced the PL response (Figure S6). This is anticipated by the structural similarity between PSA and thrombin: PSA also has the heparin-binding exosite[24−27] that can capture the TBA-capped QD, leading to charge transfer in the protein/QD complex. A six base long DNA sequence (GGTTGG) was reported to selectively bind to thrombin,[17] which implies that this sequence may be also used with QD for selective detection. Alternatively, we observed that the QD capped by 30-mer DNA alternating G and T bases quenched upon thrombin injection probably because d(GT)$_{15}$ could also form a quadruplex structure,[28] but the QD with other DNA sequences such as d(GGGGT)$_6$ did not photobleach. In summary, we have found a unique mechanism to selectively and directly modulate the PL of a QD with an aptamer capping. This chemistry may lead to more nanoparticle optical probes that can be activated in a highly chemoselective manner.

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Supporting Information Available: Details of the synthesis, characterization including TEM images, and control experiments are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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