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A molecular light switch Ru complex and quantum dots for the label-free, aptamer-based detection of thrombin†

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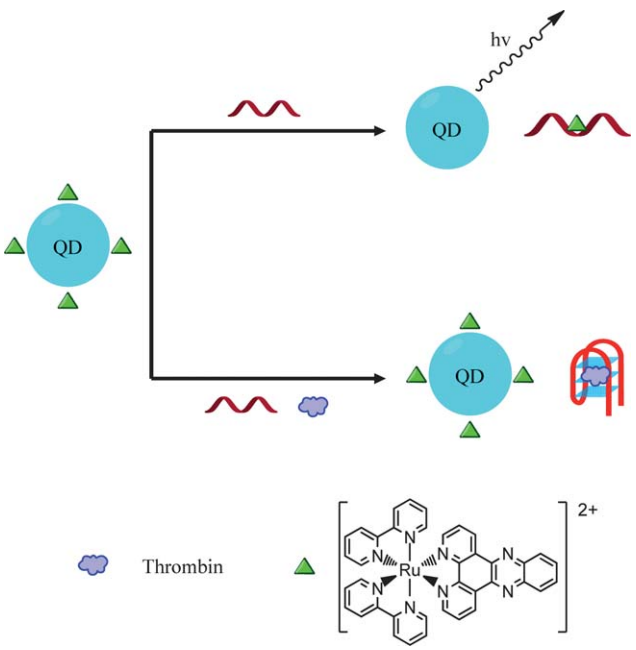
A simple, label-free method for the detection of thrombin has been developed based on the conformational transition of aptamer in the presence of the target by using a molecular light switch, Ru polypyridine complex, and quantum dots as novel fluorescence probes in aqueous solution.

Semiconductor quantum dots (QDs) are currently of great interest due to their unique optical properties, such as photostability, broad excitation spectra, and narrow, symmetric, and tunable emission spectra, compared to conventional organic dyes. Different QDs were implemented to follow enzymatic reactions and the formation of antigen–antibody complexes.[1,2] Specifically, QDs were used to analyze DNA and the formation of aptamer–substrate complexes.[3] Since the interaction of QDs with DNA molecules is weak and nonselective, direct detection of DNA through QD derivatives is not a promising process. In contrast, DNA detection based on fluorescence resonance energy transfer (FRET) between the QD-modified probe and the dye-labeled target nucleic acids has been widely documented.[4–6]

Aptamers are nucleic acid ligands which are specific to amino acids, drugs, proteins and other molecules. Aptasensors have been broadly used in the detection of drugs, cancer cells and a variety of proteins.[7] Thrombin, an important physiological protease, exists in blood and plays an essential role in some physiological and pathological processes, such as blood solidification, wound cicatrization and inflammation. Many efforts have been made to develop sensitive sensors to detect thrombin. The fluorescent aptasensor for thrombin has been developed using a conformational transition of the aptamer DNA from an unfolded structure to a thrombin-binding aptamer G-quartet structure.[8,9] However, in most cases, these methods involve an additional tagging process or sophisticated experimental techniques. Sometimes, labeling with various fluorescent and quenching molecules may even affect the target binding properties of the aptamers.

Polypyridine ruthenium complexes have prominent DNA binding properties.[10] Fluorescence enhancement of the aromatic Ru complex in the intercalation from the major groove of a double-stranded DNA was introduced by Barton and coworkers.[11] The complex $Ru(bpy)_2dppz^{2+}$ (bpy = 2,2′-bipyridine; dppz = dipyrido[3,2-*a*:2′,3′-*c*] phenazine) known as DNA "light switch" has attracted much attention. This complex does not emit luminescence in aqueous solution upon photoexcitation of the metal-to-ligand charge transfer (MLCT) band, but does emit luminescence brightly in the presence of DNA.[11] These complexes have been used in a wide variety of applications, including DNA and protein detection, cell imaging, DNA cleavage, and photoinduced electron-transfer reactions, among others.[12–14]

Metal complexes have long been used as an effective quencher for luminescent organic and polymeric materials.[15] The interactions between QDs and metal complexes have been reported.[16] Here we report a QDs-based biosensor by utilizing ruthenium complex $Ru(bpy)_2(dppz)^{2+}$ as both the quencher to QDs and a receptor to DNA (Scheme 1). $Ru(bpy)_2(dppz)^{2+}$ was utilized as it can efficiently quench the luminescence of QDs to provide a favorable "off" state for our detection. The possible mechanism could be the photoinduced electron transfer from the QDs to the Ru complex.[17] In aqueous



Scheme 1 Principles of the thrombin detection system, based on QDs/Ru complex photoinduced charge transfer.

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solution, water-soluble TGA-capped QDs carry the negatively charged carboxylate group. After the addition of Ru complex solution, the positively charged Ru complex and the negatively charged QDs form an ionic conjugate due to their electrostatic attraction. This static association induces ultra-fast photoinduced electron transfer, and results in a decrease of the fluorescent intensity of QDs. On the other hand, the Ru complex also serves as the receptor to bind specifically with DNA.[18] When the thrombin-specific aptamer was added, compared to the electrostatic association of the cationic ruthenium complex on the surface of QDs, it could remove the quencher from the surface of the nanoparticles due to the strong DNA binding propensity of the ruthenium complex, resulting in the restoring the luminescence of the QDs. In addition, in aqueous solution, free $Ru(bpy)_2(dppz)^{2+}$ has no fluorescence, but it can produce red fluorescence at 610 nm after binding to DNA. When thrombin binds to the aptamer, the induced conformational change from random coil to quadruplex dissociates the ruthenium polypyridine complexes from the DNA. Thus, a decrease in the QDs emission was observed and could be used for the label-free detection of thrombin.

TGA (thioglycolic acid)-capped CdTe QDs, synthesized in an aqueous system, were selected for our detection. Both the size and concentration of the obtained QDs could be estimated from the UV-vis absorption spectrum by Peng's empirical equations (Fig. S1, ESI†).[19] The QD solution showed a photoluminescence peak at 560 nm. The fluorescence quenching ability of $Ru(bpy)_2(dppz)^{2+}$ was evaluated *via* fluorescence measurements of 100 nM QDs in the presence of $Ru(bpy)_2(dppz)^{2+}$. As shown in Fig. 1, with the increasing concentration of Ru complex, the fluorescence intensity of the QDs decreased and trended to a minimum value at 5 μM. Thus, 5 μM Ru complex was used for analytical purposes, and it can interact with QDs (100 nM) to form a fluorescence-quenched complex, named Ru-QDs, hereafter. From the dependence of fluorescence intensity of QDs on Ru complex concentration, the fluorescence quenching could be described by the Stern–Volmer equation with a quenching constant of 9.62×10^5 M, and a linear range of 2×10^{-7} M – 1.6×10^{-6} M.

Our strategy to detect thrombin was based on aptamers; single-stranded DNA oligonucleotides that bind to their target molecules with high affinity. Therefore, a thrombin aptamer consisting of a 28-base linear DNA sequence (5′-AGCCGTGGTAGGGCAGGTTG GGGTGACT-3′) was chosen for detection. Due to the strong and

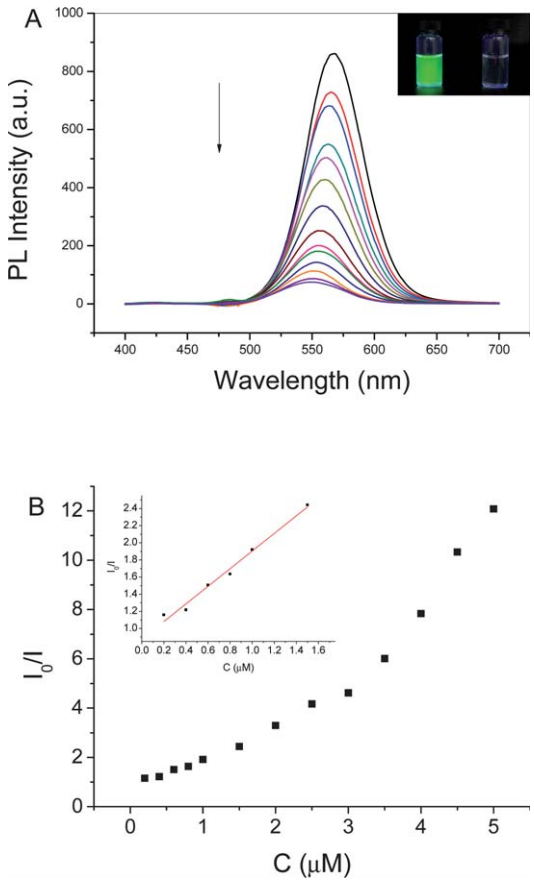

Fig. 1 (A) Changes in the fluorescence spectra of TGA-capped CdTe QDs (100 nM) in 10 mM Tris-HCl (pH = 7.4), with increasing concentration of $Ru(bpy)_2(dppz)^{2+}$, with concentrations of 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 2.5, 3, 3.5, 4, 4.5, 5 μM (from top to bottom). Inset: fluorescence image of CdTe QDs (100 nM) in the absence (left) and presence (right) of 5 μM $Ru(bpy)_2(dppz)^{2+}$. (B) Stern–Volmer plot of QD quenched by $Ru(bpy)_2(dppz)^{2+}$ in buffer solution. Excitation: 365 nm, emission: 560 nm.

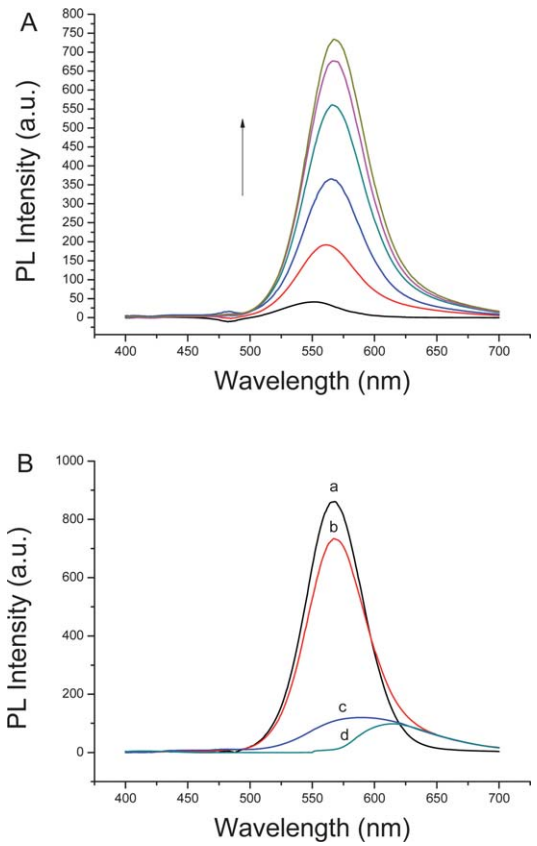

Fig. 2 (A) Fluorescent spectra of 100 nM TGA-capped CdTe and 5 μM $Ru(bpy)_2dppz^{2+}$ with the presence of aptamer with concentrations of 0, 0.5, 1, 1.5, 2, and 2.5 μM (from bottom to top). (B) Fluorescence emission spectra at different conditions: (a) 100 nM QDs in Tris-HCl buffer; (b) 100 nM QDs + 5 μM $Ru(bpy)_2(dppz)^{2+}$ + 2.5 μM DNA; (c) 100 nM QDs + 5 μM $Ru(bpy)_2(dppz)^{2+}$ + 2.5 μM DNA + 2000 nM Thrombin; (d) 5 μM $Ru(bpy)_2(dppz)^{2+}$ + 2.5 μM DNA. Excitation: 365 nm.

specific binding of DNA with $Ru(bpy)_2(dppz)^{2+}$, DNA can break up the low-fluoresced Ru-QDs and set free the luminescent QDs, enabling them to display the signal output at 560 nm. Meanwhile, in aqueous solution, free $Ru(bpy)_2(dppz)^{2+}$ has no fluorescence, but it can produce red fluorescence at 610 nm when bound to DNA. With the increasing concentration of aptamer added to Ru-QDs, the fluorescence emission intensity of QDs increased (Fig. 2A). After adding 2.5 μM aptamer, the fluorescence intensity of QDs almost restored.

In a typical experiment, thrombin was added to 2.5 μM aptamer solution and the mixture left to bind for about 30 min at room temperature. Next, Ru-QDs was mixed with this solution and incubated at room temperature for 5 min. The resulting solutions were studied by PL spectroscopy. Fig. 2B shows the fluorescence emission spectra at different conditions. When aptamer was added to Ru-QDs, the fluorescence emission of QDs at 560 nm recovered (curve a, curve b, Fig. 2B). However, in the presence of thrombin, the fluorescence of QDs was quenched by the Ru complex again due to the formation of quadruplex-thrombin complexes (curve c, Fig. 2B). It is reasonable that aptamers bind preferentially to thrombins because of the high affinity between aptamers and thrombin. It should be noted that when the Ru complex binds to DNA, a fluorescent output at 610 nm was formed (curve d, Fig. 2B). However, the fluorescence intensity is much lower than that of QDs. Therefore, the fluorescence output of the QDs at 560 nm was utilized in our assay. Fig. 3A shows the fluorescence intensity of Ru-QDs and aptamer at various concentrations of thrombin. The fluorescence intensity decreased when thrombin was added into the aptamer. Over 80% fluorescence was quenched by Ru complex when aptamer incubated with 1600 nM thrombin. A detection limit down to 50 nM at a signal-to-noise ratio (S/N) of 3 : 1 was achieved.

The selectivity of this Ru-QDs based detection system for thrombin was also investigated. As indicated in Fig. 3B, control experiments were performed using bovine serum albumin (BSA) and lysozyme. It was revealed that these proteins had little effect on the separation of Ru complex from aptamer, showing that the platform was specific to thrombin response. This system is a method that has potential for future use.

In summary, we propose a label-free, aptamer-based biosensor for the selective detection of thrombin using a molecule light switch Ru polypyridine complex and QDs. $Ru(bpy)_2dppz^{2+}$ is a classic, versatile and powerful molecular switch which can intercalate its dppz ligand between DNA base pairs.[20] Meanwhile, it can act as an excellent quencher to quench the fluorescence of QDs. Based on above considerations, the proposed aptasensor possesses high selectivity, simplicity and is cost-effective. We expect that this convenient and sensitive sensing strategy can provide a highly promising approach for label-free detection of protein in various applications.

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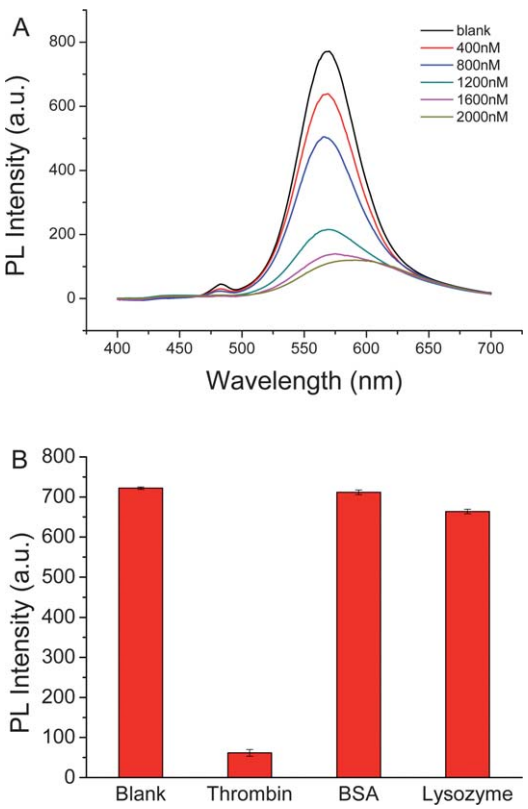

Fig. 3 (A) Fluorescence emission spectra of Ru-QDs and aptamer in the presence of different concentrations of thrombin. Black curve is the fluorescence emission of control probes (same volume of buffer added) without target. Excitation: 365 nm. (B) The PL signals of biosensor response to 2 μM thrombin, BSA and lysozyme. Excitation: 365 nm, emission: 560 nm.

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