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A photoluminescent nanocrystal-based signaling protocol highly sensitive to nerve agents and highly toxic organophosphate pesticides†

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A photoluminescent II–VI group semiconductor nanocrystal (NC)-based signaling platform composed of thioglycolic acid capped CdS NCs and acetylcholinesterase-acetylthiocholine enzyme catalytic reaction system was developed that was shown to be highly sensitive to nerve agents and toxic organophosphate pesticides with detection limits down to sub-nM levels. This new sensing protocol does not require troublesome conjugation of biomacromolecules onto the surface of NCs.

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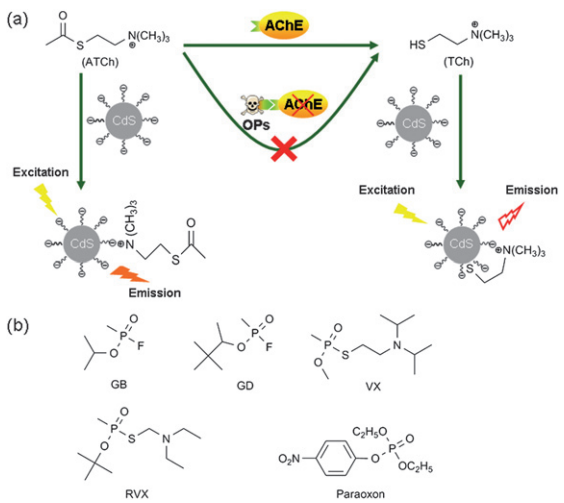
Introduction

II–VI group semiconductor nanocrystals (NCs) have attracted tremendous interest in sensing and imagining due to their unique photophysical properties.[1] A variety of biosensing strategies have been developed by using photoluminescent II–VI group semiconductor NCs.[2] Most of them rely on directly conjugating biomacromolecules onto the surface of NCs, monitoring enzyme activity and/or analyte concentration. The size of NCs at the nanometer scale is close to that of many biomacromolecules, which limits the number of biomacromolecules that can tether onto the surface of NCs. Also, bioconjugation processes, that often involve covalent and electrostatic interactions, may affect biological functions and labeling stability of the biomolecules and the photophysical properties of NCs.[3] Therefore, simple and feasible strategies for constructing biosensing systems based on photoluminescent II–VI group semiconductor NCs remains a challenging subject. Herein we report a facile signaling strategy highly sensitive for nerve agents and highly toxic organophosphate pesticides (OPs), that uses thioglycolic acid ($HSCH_2CO_2H$, TGA) capped CdS NCs (Scheme 1). In this protocol, no bioconjugation of biomacromolecules on the surface of NCs is needed.

It is known that OPs, by inhibiting the activity of acetylcholinesterase (AChE), lead to *in vivo* accumulation of acetylcholine, thereby resulting in serious impairment on nerve functions and even death.[4] Highly toxic OPs such as nerve agents and organophosphate pesticides therefore severely threaten public safety. Efficient and rapid detection of OPs has thus become very important and related research is active.[5] II–VI group semiconductor NC-based assay and detection of OPs have been documented in the literature.[6–8] For example, Willner *et al.*[6] reported several II–VI group semiconductor NC-based sensing systems for AChE inhibitors, under the mechanisms of photonic sensing, surface plasmon resonance, and biocatalytic cascade reactions. Leblanc *et al.*[7] developed two sensing systems for paraoxon by incorporating CdSe NCs into layer-by-layer films and by changing the secondary structure of an organophosphate hydrolase that was conjugated to the surface of the NCs. In addition, electrochemical resonance based approaches have also been applied for the detection of OPs.[8] All these strategies involve bioconjugation of enzymes onto the surface of NCs.

In our proposed protocol (Scheme 1a), positively charged acetylthiocholine (ATCh) was employed as a substrate for AChE that catalyses the hydrolysis of ATCh to produce thiocholine (TCh), another positively charged species that bears an additional thiol group. Both ATCh and TCh prefer to adsorb onto the surface of negatively charged TGA-CdS NCs *via* electrostatic interactions and hydrogen bonding. Yet, TCh is able to cap to a higher extent on NC surfaces by its SH group following



Scheme 1 (a) Illustration of signaling strategy for highly toxic OPs based on an AChE-ATCh/TGA-CdS NCs system and (b) chemical structures of investigated OPs, in which two are G-type (sarin (GB) and soman (GD)), two are V-type (VX and RVX), and one is a pesticide (paraoxon). All operations were carried out under chemical safety principles.

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a known chemistry of thiol capping on NCs.[9] Effects of ATCh and TCh on the amount of vacancies at NC surfaces were therefore expected to be different, leading to a change in the surface trap state photoluminescence (PL) of TGA-CdS NCs that could reflect the amount of OP that inhibits AChE activity. This forms the basis of our signaling of highly toxic OPs that affect the enzyme catalytic reaction (Scheme 1a).

Experimental

Apparatus

PL spectra were measured using a Hitachi F-4010 fluorescence spectrometer (Hitachi Ltd., Japan) controlled by Accessport software (a serial port communication software). Absorption spectra were recorded with a HP-8453 UV-Visible spectrometer (HP Ltd., USA). pH measurements were made with an Orion 420 pH meter (Orion Ltd., USA). Enzyme catalytic reactions were performed in an Infors AG CH-4103 incubator (Infors AG, Ltd., Swiss).

Reagents

$CdCl_2 \cdot 10H_2O$, $Na_2S \cdot 9H_2O$, and other inorganic compounds were purchased from Shanghai Chemicals Group (China). TGA, AChE and *S*-acetylthiocholine iodide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mercaptoethanol (ME), mercaptoethylamine (MEA) and dithiobisnitrobenzoate were obtained from Acros (New Jersey, USA). Sarin (isopropyl methylphosphonofluoridate), Soman (pinacolyl methylphosphonofluoridate), VX (*O*-ethyl-*S*-2-diisopropylaminoethyl methylphosphonothioate) and Russian (R)VX (*O*-isobutyl-*S*-2-diethylaminoethyl methylphosphonothioate) were obtained from the Institute of Chemical Defence (China). Paraoxon-ethyl (paraoxon) was purchased from Dr. Ehrenstorfer GmbH (Germany).

Procedures

TGA-CdS NC preparation[10]. 5 mmol $CdCl_2$ and 25 mmol TGA was dissolved in 40 mL doubly deionized water. After adjusting the pH to 7.0 by 1.0 M NaOH, 10 mL Na_2S aqueous solution (0.25 M) was added dropwise under vigorous stirring that then continued for 24 h. CdS NCs were precipitated from the solution by adding ethanol. The TGA-CdS NCs were then treated in three repeated cycles of precipitation, washing, and redispersion in order to remove other components. The purified CdS NC powder was dried under vacuum and finally dissolved in doubly deionized water and stored in a refrigerator at 4 °C.

Preparation of ATCh. 0.6 mmol *S*-acetylthiocholine iodide was dissolved in 5 mL doubly deionized water. 0.7 mmol $AgNO_3$ was added into the solution to remove I^- that would severely quench the PL of the CdS NCs. After removing AgI precipitates by centrifugation, 0.3 mmol NaCl was added into the solution and the formed precipitates were removed by further centrifugation. The resulting solution was immediately used for the following experiments.

Preparation of TCh[11]. The enzyme catalytic reaction of AChE with freshly prepared ATCh was allowed in 50 mM phosphate buffer of pH 7.6 for 30 min. The final concentration of TCh was determined by spectrophotometry after reacting with dithiobisnitrobenzoate. The resulting solution was immediately used for the following experiments.

Preparation of stock solutions of OPs. OPs were dissolved in 5 mL hexane and stored in a refrigerator at 4 °C. Standard solutions of OPs were freshly prepared in isopropanol.

Measurement of AChE activity in TGA-CdS NCs/ATCh system. Varying unit activity of AChE was dissolved in 250 μL 20 mM Tris-HCl pH 7.6 buffer and the solution was incubated at 37 °C for 10 min. 10 μL ATCh was then added and incubated at 37 °C for 20 min, to which 250 μL TGA-CdS NCs were added. Final concentrations of ATCh and TGA-CdS NCs were 75 μM and 500 nM, respectively. PL measurements were carried out after 10 min incubation at room temperature.

Signaling OPs in TGA-CdS NCs/ATCh system. 10 μL OPs of varying concentrations were dissolved in 240 μL 20 mM Tris-HCl buffer (pH 7.6) containing 0.008 unit activity of AChE and the solution was incubated at 37 °C for 10 min. 10 μL ATCh was then added and incubated at 37 °C for 20 min, to which 250 μL TGA-CdS NCs were added. Final concentrations of ATCh, TGA-CdS NCs, and AChE were 75 μM, 500 nM, and 0.016 units mL^{-1}, respectively. PL measurements were carried out after 10 min incubation at room temperature.

Results and discussion

The prepared TGA-CdS NCs show a well resolved exciton absorption peaked at 370 nm (Fig. 1), indicating good dispersity of the NCs. Both the particle size (2.5 nm) and concentration of NC solutions were calculated according to Peng's empirical equations, based on its absorption maximum.[12] Broad PL of TGA-CdS NCs shows a maximum of 541 nm (Fig. 1) featuring a large Stokes shift, probing the surface-trap emissive state nature.[13]

AChE is an esterase that cleaves the ester linkage in ATCh, producing TCh that bears an additional SH group compared to ATCh. The effect of AChE on the emission of TGA-CdS NCs/ATCh system was examined. It was observed that quenching and blue shift of the PL of NCs occurred with increasing

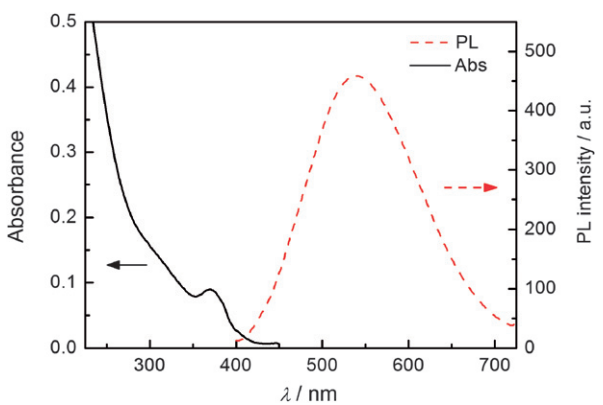

Fig. 1 Absorption (—) and PL (---) spectra of prepared TGA-CdS NCs in 10 mM Tris-HCl buffer of pH 7.6, [NCs] = 500 nM, λ_{ex} = 370 nm.

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AChE concentration (Fig. 2). The PL of TGA-CdS NCs was found to not be affected by AChE itself (Fig. 3), which means there is no appreciable interaction between TGA-CdS NCs and AChE. The AChE concentration-dependent spectral change in the TGA-CdS NCs/ATCh system was therefore attributed to the enzymolysis of ATCh. This produces TCh which bears an SH group that promotes an enhanced interaction of TCh, compared to ATCh, with the TGA-CdS NCs surface because of a strong complexation of the SR group in TCh with the TGA-CdS NCs' surface unbound and/or unsaturated Cd^{2+} ions.

Indeed, ATCh and ATCh/AChE do exert substantially different influence on the PL of TGA-CdS NCs (Fig. 3). It was noted from Fig. 3 that the PL of the TGA-CdS NCs was enhanced by 1.7-fold and the emission was red-shifted by 10 nm from 541 nm to 551 nm in the presence of ATCh. In the case of ATCh/AChE where the species interacting with the TGA-CdS NCs are ATCh and TCh, the PL was enhanced by 1.1-fold while the emission was red-shifted by 20 nm. These distinct differences in the change of PL of NCs are the first support of the different interactions of ATCh and TCh with TGA-CdS NCs as proposed in Scheme 1a. In addition, we found that the PL of TGA-CdS NCs remained unchanged in the presence of only OPs, indicating that an observable interaction between these inhibitors of AChE and the TGA-CdS NCs was absent (Fig. 3 and Fig. S1 in ESI†). It was therefore made clear that it was the difference in the species that interacted with the TGA-CdS NCs that led to changes in the PL of TGA-CdS NCs, substantializing the signaling platform that is sensitive to these highly toxic OPs.

As the activity of AChE could be inhibited by OPs,[4] quenching of the PL of TGA-CdS NCs should be suppressed and blue-shift in the emission would be observed in the presence of these inhibitors. Indeed, an increase in the concentration of OPs (GB, GD, VX, RVX, and paraoxon) at nM level in the AChE/ATCh enzyme catalytic reaction system was sensitively imparted by an enhancement in the PL and a blue-shift of the emission of TGA-CdS NCs (Fig. 4–6, and Fig. S2–S5 in ESI†). These agree well with the observed relationship of the PL intensity of the TGA-CdS NCs with AChE activity (Fig. 2). Data in Figs. 5 and 6 clearly indicate that this signaling system exhibits a high sensitivity for these toxic OPs, with excellent limits of detection (LODs) and wide dynamic ranges. LODs of four nerve agents GB, GD, VX, and RVX were calculated to be at 0.1 nM level while the LOD of paraoxon was calculated at 40 nM level; dynamic ranges for the four nerve agents were found to span two orders of magnitude, while for paraoxon it spanned across three orders of magnitude (40 nM–10 μM, Fig. 6).

We had evidence to show that the modulation of the PL of TGA-CdS NCs results from changes in the amounts of surface vacancies. In the enzyme catalytic reaction system two species, ATCh and TCh, that can interact with the surface of TGA-CdS NCs were found to lead to PL spectral changes to different extents (Figs. 3 and 7). This is because TCh, bearing an additional thiol group, has a stronger interaction with the surface unbounded or weakly bounded Cd^{2+} ions of TGA-CdS NCs. This was further verified by variations in the PL of TGA-CdS NCs in the presence of three differently charged SH-containing species, mercaptoethylammonium ($HSCH_2CH_2NH_3^+$, MEA^+), mercaptoethanol ($HSCH_2CH_2OH$, ME), and TGA^- ($HSCH_2CO_2^-$). Fig. 8 shows that these three compounds lead to PL quenching

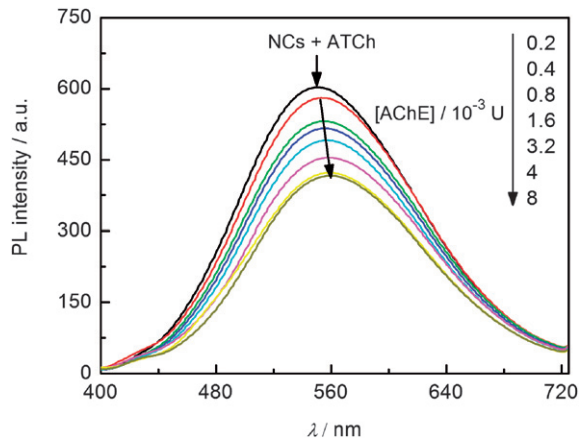

Fig. 2 PL spectra of TGA-CdS NCs in 20 mM pH 7.6 Tris-HCl buffered ATCh-AChE reaction solution, with different [AChE]. [NCs] = 500 nM, [ATCh] = 75 μM, λ_{ex} = 370 nm.

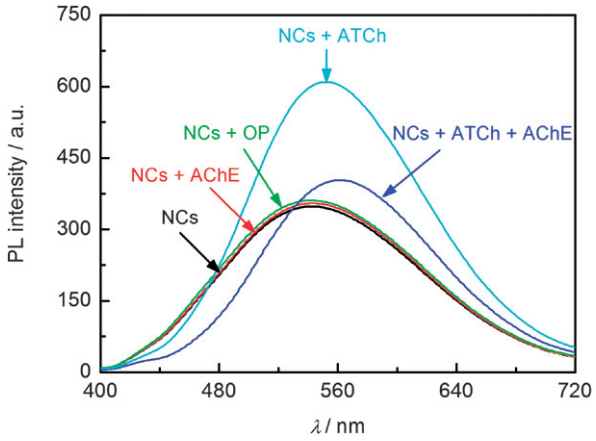

Fig. 3 PL spectra of TGA-CdS NCs in 20 mM Tris-HCl pH 7.6 buffer in the absence and presence of 0.016 Units mL^{-1} AChE, 1 μM paraoxon (OP), 75 μM ATCh, and 75 μM ATCh plus 0.016 units mL^{-1} of AChE. [NCs] = 500 nM, λ_{ex} = 370 nm.

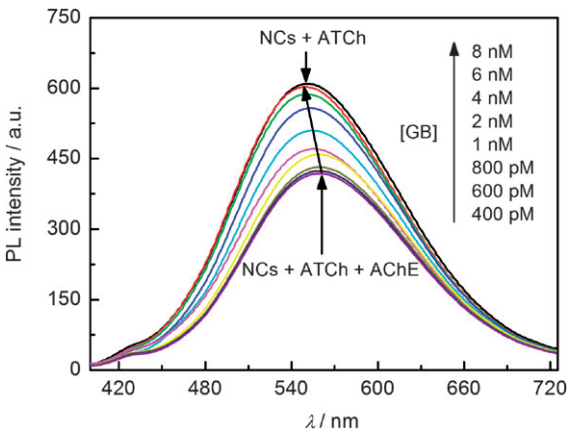

Fig. 4 PL spectra of TGA-CdS NCs in 20 mM Tris-HCl pH 7.6 buffered reaction solution of AChE, ATCh, and GB. [NCs] = 500 nM, [AChE] = 0.016 units mL^{-1}, [ATCh] = 75 μM, λ_{ex} = 370 nm.

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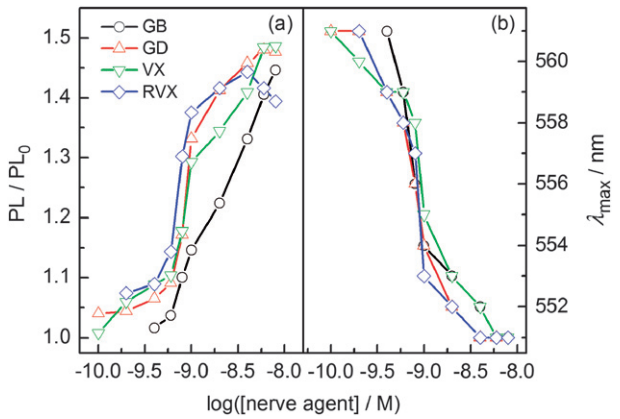

Fig. 5 Plot of PL intensity (a) and maximum wavelength (b) *versus* concentration of the four nerve agents. The buffer used was 20 mM Tris-HCl of pH 7.6, [NCs] = 500 nM, [AChE] = 0.016 units mL^{-1}, [ATCh] = 75 μM, λ_{ex} = 370 nm. PL and PL_0 represent luminescence intensity of TGA-CdS NCs in the presence and absence of OPs, respectively.

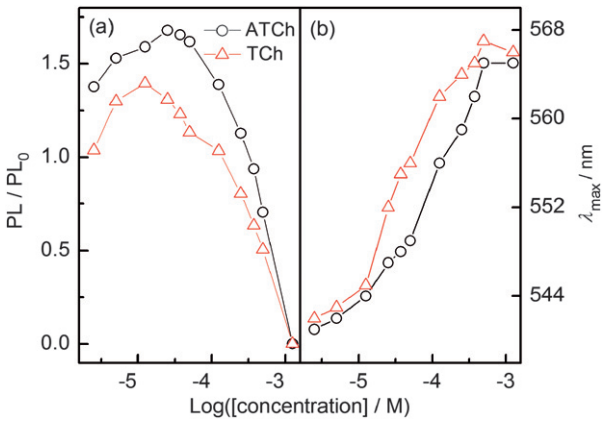

Fig. 7 Effect of ATCh and TCh on PL/PL_0 (a) and maximum PL wavelength (b) of TGA-CdS NCs. The buffer used was 20 mM Tris-HCl of pH 7.6, [NCs] = 500 nM, λ_{ex} = 370 nm. PL and PL_0 represent luminescence intensity of TGA-CdS NCs in the presence and absence of ATCh or TCh, respectively.

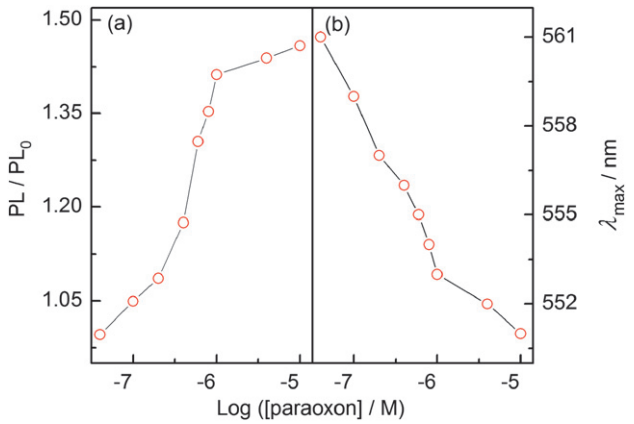

Fig. 6 Plot of PL intensity (a) and maximum wavelength (b) *versus* concentration of paraoxon in 20 mM Tris-HCl buffer of pH 7.6. [NCs] = 500 nM, [AChE] = 0.016 units mL^{-1}, [ATCh] = 75 μM, λ_{ex} = 370 nm. PL and PL_0 represent luminescence intensity of TGA-CdS NCs in the presence and absence of paraoxon, respectively.

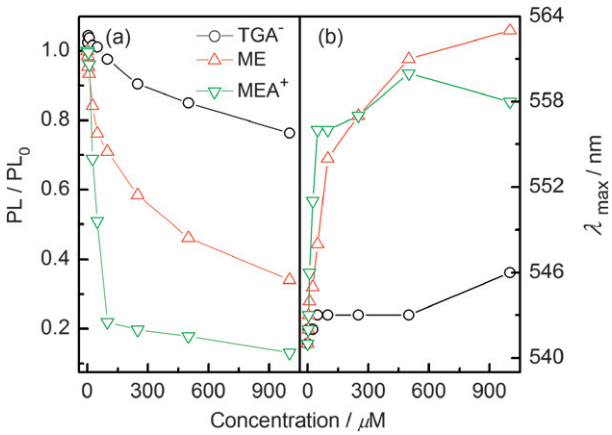

Fig. 8 Plot of PL/PL_0 (a) and maximum PL wavelength (b) of TGA-CdS NCs in the presence of MEA^+, ME, and TGA^- in 20 mM Tris-HCl buffer of pH 7.6, [NCs] = 500 nM, λ_{ex} = 370 nm. PL and PL_0 represent luminescence intensity of TGA-CdS NCs in the presence and absence of the SH-containing species, respectively.

and blue shift in the order of $MEA^+ > ME > TGA^-$, similar to that observed with ATCh and TCh (Fig. 7). It should be added that hydrogen bonding interactions between the introduced SH-containing compounds and TGA-CdS NCs surface CO_2H may also contribute to the quenching of PL.

The high sensitivity of the signaling was attributed to both the inhibition of OPs on the catalytic activity of AChE and the difference in the interaction with TGA-CdS NCs of ATCh and TCh. The latter difference is efficiently enhanced by the multivalent character of the TGA-CdS NCs surface.[14] It is understood that, compared with the cases involving conjugation of biomacromolecules, much more small-molecules such as ATCh and TCh can interact with the surface of TGA-CdS NCs, which would contribute to the observed higher sensitivity. It should be pointed out that Au nanoparticles (NPs)-based ATCh/AChE catalytic systems have recently been developed for the detection of OPs by means of the interaction of Au NPs with the SH group in the resulting TCh.[15–17] Compared with these strategies, our work represents a new entry for highly sensitive signaling OPs based on photoluminescent II–VI group semiconductor NCs that is, in principle, easily extended to other enzymatic catalytic reactions for sensitive signaling.

Conclusions

In summary, we have developed a simple yet highly sensitive strategy for detection of toxic OPs by photoluminescent TGA-CdS NCs in an AChE/ATCh enzyme catalytic reaction medium. LODs down to 0.1 nM were achieved. The observed significant signal amplification is the result of a combination of the catalytic effect of AChE[18] and the multivalent effect of the surface of TGA-CdS NCs that provide more binding sites for the small functional ligands ATCh and TCh. Differing from most of the reported biomacromolecules/NC conjugation systems, the signaling

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strategy reported here uses small-molecule substrate and product with no need of conjugation of bulky biomacromolecules on the surface of NCs. This provides a new entry for highly sensitive OPs detection, and more significantly a photoluminescent II–VI group semiconductor NC-based biosensing protocol that can, in principle, be expected for other analytes as well.

Acknowledgements

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