analytical chemistry

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Application of Polymer Quantum Dot-Enzyme Hybrids in the Biosensor Development and Test Paper Fabrication

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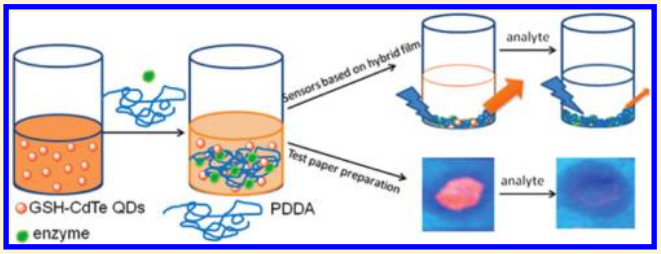
Supporting Information

ABSTRACT: Both glutathione capped CdTe quantum dots (QDs) and enzymes were encapsulated with poly(diallyldimethylammonium chloride) (PDDA) via electrostatic attraction to form hybrid films. The obtained PDDA QD-enzyme hybrids feature both high fluorescence and bio-recognition. In the obtained hybrid materials, the fluorescence emission of the QDs was stable for at least 3 months, and the structure and activity of the enzyme was also well maintained as the Michaelis constant of tyrosinase was determined to be 0.90 mmol/L, which is just 2 times higher than that of free enzyme. This hybrid material was then utilized as a platform for the development of biosensors based on the quenching effects of the enzymatic products on the emission of the QDs with a kind of phenol (catechol) and glucose as example analytes. The detection limits of catechol and glucose were 1.0×10^{-5} and 5.0×10^{-6} mol/L, respectively. Moreover, this hybrid material was applied to the fabrication of test paper for these two analytes. The test paper was very stable with respect to the fluorescence of the QDs and the activity of the enzyme maintained for at least 1 month.



Luminescent quantum dots have been investigated intensively in recent years as target-specific probes for the development of various sensors and biosensors. Their fascinating optoelectronic properties such as broad absorption and narrow emission spectra together with high emission quantum yields make QDs advantageous and interesting probes in the development of chemical sensors, especially biosensors with various detection methods including fluorescence transduction and electrochemistry or electrochemiluminescence methods.[1−7] In particular, the comparable dimension of QDs and biomolecules such as proteins and DNA enables the preparation of biocompatible QD-bioconjugates with recognition functions which are essential for the development of bioprobes and biosensors,[8−11] especially for the eventual use in various assays on solid substrates such as the optical fibers and microfluids.[12,13] Enzymes as a kind of biomolecule with high specificity and activity are used as effective chemical transformation units in the biosensors. With the combination of QDs and enzymes through electrostatic interaction, covalent conjugation, or simple mixing of various analytes has been successfully detected based on fluorescence transduction.[14−20] However, until now most biosensors with QDs as optical probes are made in the form of aqueous dispersions. For the fabrication of simple and economical sensors and biosensors using a solid platform, one of the key procedures is the efficient immobilization of the sensing indicators and recognizing biomolecules.[12] Thus, the effective incorporation of biorecognition units such as enzyme and QDs as the signaling unit in one hybrid material is essential for the fabrication of QD based biosensors. The layer by layer assembly has been developed to be an economical method for the immobilization of various charged species, such as functional nanomaterials, DNA, and proteins,[21,22] and the self-assembly of polyelectrolytes and QDs or enzymes is an attractive way to obtain uniform hybrid films on solid substrates of complex shape.[23,24] Alternatively, sol−gel methods enable the encapsulation of sensing dyes and enzymes in porous matrixes by choosing the right precursors and by the addition of the dyes prior to the polymerization.[25] Recently, polymer and peptide hydrogels containing enzyme-QD conjugates have proven to be ideal systems for biosensors as both the fluorescence and the activity of the enzyme were well maintained in the hydrogel, and the functionalized hydrogels were further used in micrototal-analysis-systems.[26−29] However, the layer by layer assembly is relatively tedious and time-consuming as many layers (on the order of 10 or more) should be assembled in order to prepare films encapsulating a suitable amount of QDs and enzymes for signaling and sensing. In some of the hydrogel encapsulation methods mentioned above a coconjugation of QDs and enzymes was performed before the gelation of the polymers through chemical reactions which, however, may cause a decrease of the enzymes activity which then is accompanied by a loss of sensitivity of the sensors.

Herein, we report a facile method for the generation of polymer QD-enzyme hybrid materials acting as a versatile platform for the development of biosensors. Poly(diallyldimethylammonium chloride) (PDDA) was used to spontaneously coencapsulate negatively charged QDs and enzymes in phosphate buffer to form PDDA-enzyme-QDs hybrid films on solid substrates as shown in Figure 1A. The

Received: March 13, 2012
Accepted: April 30, 2012
Published: April 30, 2012

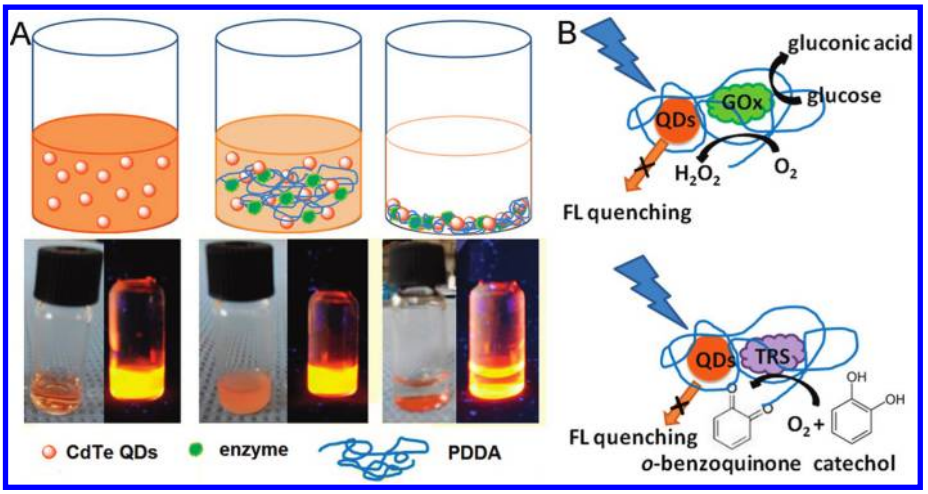


Figure 1. Schemes of the QD enzyme PDDA hybrid formation and corresponding true color images taken under room light and under 365 nm UV lamp irradiation (A) and sensing mechanisms (B).

emission of the QDs and the activity of the enzyme are well maintained and are very stable in the hybrids. Tyrosinase (TRS) and glucose oxidase (GOx) were taken as example enzymes to evaluate the utility of this kind of hybrids as platforms for biosensors for phenol and glucose, respectively (Figure 1B). Furthermore, the applicability of this hybrid material was also verified in a test paper fabrication. The as prepared test paper showed a relatively high stability, as both the fluorescence of the QDs and the activity of the enzyme encapsulated in the hybrids were maintained well for at least 4 weeks.

EXPERIMENTAL SECTION

Chemicals. All the starting materials for the CdTe QD synthesis were obtained from commercial suppliers and were used without further purification. Glutathione (GSH, reduced form), β-D-glucose, 1,2-dihydroxybenzene (catechol), mercaptosuccinic acid (MSA), poly(diallyldimethylammonium chloride) (M_w 200 000−350 000, 20 wt % in H_2O) were obtained from Aldrich. Glucose oxidase (EC. 1.1.3.4, from *Aspergillus niger*) and tyrosinase (EC 1.14.18.1, from mushrooms) were purchased from Sigma-Aldrich. The chemicals used are of at least analytical grade. All the solutions were prepared with water purified by a Milli-Q system (Millipore, Bedford, MA).

Instrumentation. UV−vis absorption spectra were recorded on a CARY 50 spectrophotometer (Varian). Fluorescence experiments were performed using a FluoroMax-4 spectrofluorimeter (HORIBA Jobin Yvon Inc.). Time resolved fluorescence measurements were performed on a Fluorolog (HORIBA Jobin Yvon Inc.) spectrometer using a 200 ps pulsed laser diode emitting at 403 nm. Transmission electron microscopy (TEM) measurements were made on an EM 208 transmission electron microscope (Philips) equipped with an Olympus Keenview digital camera. Samples for TEM characterization were prepared by placing a drop of QD colloidal solution onto a carbon-coated copper grid and subsequent drying at room temperature.

Synthesis of CdTe QDs. GSH or MSA capped CdTe QDs were synthesized through the so-called one-pot method with cadmium chloride and Na_2TeO_3 as precursors.[30] Briefly, 0.05 g of $NaBH_4$ was added to a 50 mL solution containing cadmium chloride (0.16 mmol), trisodium citrate dihydrate (0.1 g), glutathione (0.05 g) or mercaptosuccinic acid (0.1 g), and Na_2TeO_3 (0.01 mmol) with stirring. The mixture reacted at 100 °C under open-air conditions for a certain period of time. Thioglycolic acid (TGA) capped CdTe QDs were prepared according to the literature.[31] The obtained QDs were precipitated with ethanol, and the precipitates were separated by centrifugation and were redissolved in 50 mM phosphate buffer solution (pH 7.4) to eliminate the free ligands and salts from the crude CdTe QD colloids.

Preparation of PDDA CdTe QD Hybrids. A volume of 0.1 mL of PDDA solution (concentration of monomer was 10 mmol/L) was added to 1 mL of CdTe QDs sol, and the QD solution immediately turned turbid and was left at room temperature overnight to form a hybrid film on the bottom of the tube.

Preparation of PDDA QD-Enzyme Hybrids and Fluorescence Experiments. A volume of 500 μL of GSH-CdTe QDs were mixed with enzyme (GOx or TRS solution) and 10 mmol/L PDDA of a certain volume. Then the mixture was diluted to 1 mL with phosphate buffer (50 mmol/L, pH 7.4). The final concentrations of GOx, TRS, and PDDA were 1.6 mg/mL, 0.32 mg/mL, and 1 mol/L, respectively. After mixing thoroughly, the mixture was left undisturbed overnight at 4 °C. PDDA QD-enzyme hybrids form thin film precipitates on the walls of the reaction tube. These precipitates were washed several times by phosphate buffer and were kept wet before use. The ratios of QDs/enzyme in the hybrids were determined using UV−vis spectroscopy, according to a previous publication.[16] The activity of TRS in the PDDA-QDs-TRS hybrids was determined using the 410 nm absorbance of benzoquinone by incubating the hybrids in phosphate buffer (50 mmol/L, pH 7.4) containing 5 mmol/L catechol for 2 min. The activity of GOx in the PDDA QDs-GOx hybrids was determined using 3,3′,5,5′-tetramethylbenzidine (TMB) as a probe (maximum absorbance at 450 nm of TMB); the hybrids were incubated with 5 mmol/L glucose for 5 min in the presence of 0.025% TMB and 0.12 mg/mL horseradish peroxidase, and then H_2SO_4 (final concentration of 1 mol/L) was added into the above solution for another 2 min.

For the analyte sensing, these PDDA QDs-enzyme hybrid films were incubated with analytes for 20 min for fluorescence detection under the excitation wavelength of 450 nm.

Test Paper Preparation. GSH-CdTe QDs were precipitated with ethanol and then redissolved in phosphate buffer (50

dx.doi.org/10.1021/ac300714j | *Anal. Chem.* 2012, 84, 5047−5052

mM, pH 7.4). A volume of 150 μL of TRS (2.4 mg/mL) and 40 μL of PDDA (10 mmol/L) were added into 200 μL of QD sol successively. For the preparation of glucose test papers, 80 μL of GOx (2.0 mg/mL) was mixed with 200 μL of QD sol and 40 μL of PDDA (10 mmol/L). A volume of 5 μL of the hybrid mixtures was dropped onto the surface of a chromatography paper, and after the solution had evaporated, the filter paper was stored at 4 °C in the refrigerator.

RESULTS AND DISCUSSION

Effect of PDDA on CdTe QDs. With the addition of PDDA into three kinds of QD sols (TGA-CdTe QDs, GSH-CdTe QDs, and MSA-CdTe QDs), the QD colloid became turbid, and precipitates on the bottom of the tube formed after standing for several hours. During the above process, the fluorescence of the QDs in the hybrids was maintained. Photographs of hybrids of PDDA with these three kinds of QDs under room light and under 365 nm UV lamp excitation are shown in the Supporting Information (Figure S1). The stability of the GSH capped CdTe QD PDDA hybrids was studied with the hybrids being kept at 4 °C for more than 1 week. As shown in Figure 2, the fluorescence intensity of the

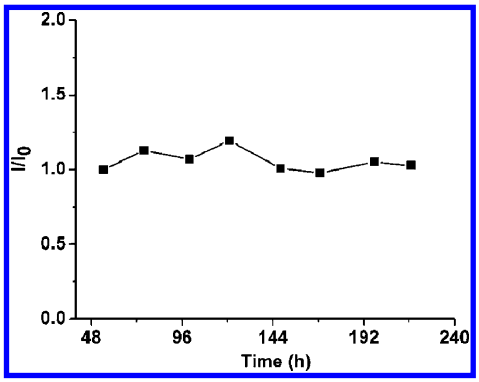


Figure 2. Stability of the fluorescence emission of the PDDA GSH CdTe QD hybrid material. The PDDA GSH CdTe QD hybrids were stored at 4 °C.

QD PDDA hybrids was very stable. Actually, the hybrid materials containing these three kinds of PDDA-QDs were still highly fluorescent also after more than 3 months. The high stability of the PDDA-QDs hybrids made it a good material for the development of solid state sensors and biosensors. In this study, we found that PDDA of different concentrations have different effects on the fluorescence and absorption spectra of the QDs. PDDA with concentrations below 1 mmol/L caused a decrease in fluorescence intensity and a red-shift of the maximum of the emission of GSH-CdTe QDs, whereas PDDA has no obvious effect on the emission and absorption spectra of the same QDs when its concentration is equal or above 1 mmol/L (Supporting Information, Figure S2). These effects are discussed in detail in the Supporting Information. As a consequence in the following experiments PDDA was used with concentrations of 1 mmol/L to form the PDDA-QDs hybrids.

Kinetic Study of Tyrosinase Encapsulated in PDDA-QD Hybrids. In order to prepare a multifunctionalized hybrid material, enzymes as biorecognition units were added in the PDDA-QD hybrids being fixed electrostatically. Tyrosinase (TRS, with an isoelectric point of 4.4) is a negatively charged enzyme in phosphate buffer (pH 7.4 or 6.5) and tends to be encapsulated by PDDA. To keep the native structure and activity of the enzyme is a challenge when trying to immobilize the enzyme on a substrate. Generally, physical encapsulation of enzymes is superior to chemical immobilization. To investigate the activity of the enzyme in the PDDA-QD enzyme hybrids, the kinetics of TRS in the hybrid materials was studied using the characteristic absorption of *o*-benzoquinone at 410 nm (ε = 1450 L mol^{-1} cm^{-1}).[32] The apparent Michaelis constant (K_m), which reflects the binding efficiency of the enzyme with the substrate, was determined using the Lineweaver–Burk plot analysis (see the Supporting Information). Figure 3 shows the

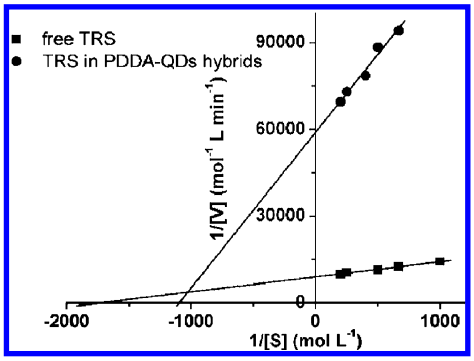


Figure 3. Lineweaver–Burk plot analysis of the enzymatic kinetics of free TRS and TRS in PDDA QD TRS hybrid materials. The concentrations of catechol were 1.0, 1.5, 2.0, 2.5, 4.0, and 5.0 mmol/L. Phosphate buffer (50 mmol/L, pH 6.5); incubation time 1 min.

comparison of this plot of free TRS enzyme and the TRS immobilized in the PDDA-QDs hybrids. The apparent K_m value of the free enzyme is determined to be 0.58 mmol/L, which is consistent with the reported value of 0.52 mmol/L.[33] It is known that the kinetics of the immobilized tyrosinase is different from that of free enzyme such that higher K_m values and a decrease in the enzymatic reaction rate were observed upon the immobilization of the enzymes.[34] In the present study, the K_m value of the TRS enzyme immobilized in the PDDA-QDs hybrids was determined to be 0.90 mmol/L, which is higher than that of free TRS enzyme, showing a lower affinity of TRS toward catechol. Also the reaction rate (maximum velocity) was 0.017 mmol/L per minute, which is decreased to about 15% of that of free enzyme (0.11 mmol/L per minute). The changes of the enzyme kinetics in the hybrid materials were mainly due to the structural change of the enzyme when encapsulated in the hybrids and the limited diffusion of substrate to the hybrids as compared to the diffusion in the phosphate solution. The changes in the TRS kinetics in the PDDA-QDs hybrid are comparable with that of TRS in the alginate-SiO_2 hybrid gel[35] and are far superior to that of the enzyme immobilized in copolymer matrixes with a 10 times increase of the K_m value.[34,36] Additionally, in the copolymer matrixes, the decrease of V_{max} was more dramatic (to about 1% of the free enzyme). The good stability of the fluorescent QDs and the high activity of the enzyme in the hybrid materials made this a good candidate for the development of QD based biosensors.

QD PDDA Enzyme Hybrids for the Development of Biosensors. As shown in Figure 1, PDDA as a polyelectrolyte tends to encapsulate both QDs and negatively charged enzymes to form a hybrid material. The PDDA QDs enzyme hybrid material serves as both a signal transforming and recording unit in the biosensor development. To validate the use of these

dx.doi.org/10.1021/ac300714j | Anal. Chem. 2012, 84, 5047–5052

materials in biosensing, catechol was taken as an example analyte. Mushroom tyrosinase in the hybrids will catalyze the oxidation of catechol by dissolved oxygen into *o*-benzoquinone, and the QDs in the hybrids served as a benzoquinone probe as their fluorescence can effectively be quenched by benzoquinone while the extent of quenching can be used for the quantification of the analytes. To obtain reproducible fluorescence signals from the hybrid films, it is of importance to fix the position of the reaction tubes in the focus of the fluorimeter. As a consequence a small relative standard deviation of 1.75% was obtained for duplicate 10 measurements (Supporting Information, Figure S4). In the PDDA QDs-TRS hybrids, the ratio of TRS/QDs was determined to be about 2.4 (UV−vis spectroscopy was used for the determination of concentration of both QDs and TRS[16]); the activity of TRS was 774 U per liter hybrids. Figure 4 shows the sensing of catechol using PDDA

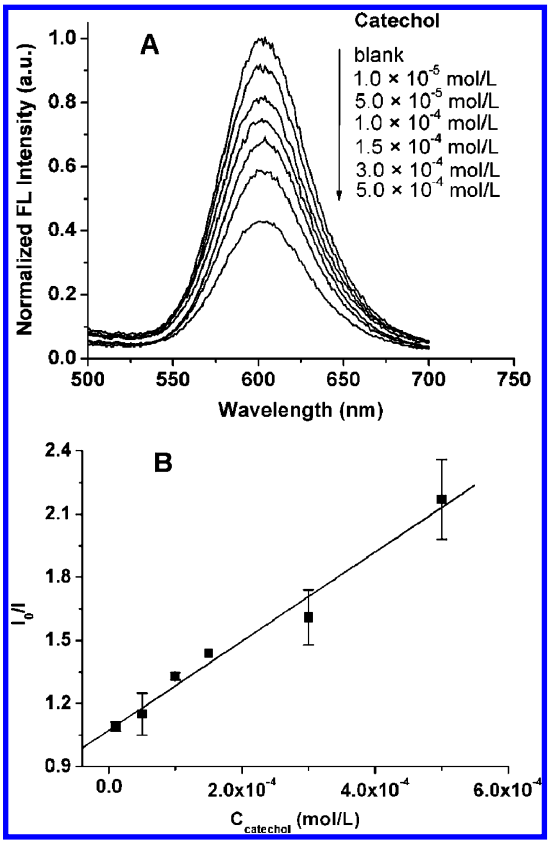


Figure 4. Catechol detection using PDDA GSH CdTe QD-TRS hybrid materials. (A) Fluorescence spectra representing the quenching effects of catechol with concentrations of 1.0×10^{-5}, 5.0×10^{-5}, 1.0×10^{-4}, 1.5×10^{-4}, 3.0×10^{-4}, and 5.0×10^{-4} mol/L on the PDDA GSH CdTe QD TRS hybrid and (B) corresponding Stern−Volmer plot.

GSH CdTe QD TRS hybrids. After the washing of the hybrid material at least 5 times with phosphate buffer to remove free enzymes in the solution, the hybrid materials were immersed in the buffer with subsequent addition of catechol of different concentrations. The hybrid materials were incubated for 20 min, and then fluorescence spectra of the hybrid materials were recorded. Figure 4A shows the quenching effects of catechol with different concentrations on the fluorescence of QDs in the hybrid materials. In a control experiment catechol had no obvious effect on PDDA QD hybrids (Supporting Information, Figure S5). As expected, the fluorescence intensity decreased with the increase of the catechol concentration. Fluorescence lifetime measurements showed that lifetime of GSH-CdTe QDs was shortened from 24.7 to 19.7 ns in the presence of 0.2 mmol/L benzoquinone (see the Supporting Information, Figure S6). Furthermore, the quenching effect of catechol (I_0/I, I_0 and I represent the fluorescence intensity of the QDs in the absence and presence of analytes) obeys the Stern−Volmer plot representing a linear relationship between the relative fluorescence intensity and the concentration of the quencher as shown in Figure 4B. The Stern−Volmer linear regression yields $I_0/I = 1.07 + 2.12 \times 10^3 C_{catechol}$ ($r^2 = 0.9788$). The detection limit of catechol is 1.0×10^{-5} mol/L, and the detection range is between 1.0×10^{-5} and 5.0×10^{-4} mol/L. The detection limit of catechol determined by the PDDA QD TRS hybrids was more than 10 times lower than that obtained by encapsulated QDs and TRS in a polymer hydrogel.[26] The detection limit and linear relationship between the quenching extent and the concentration of catechol proved the PDDA QD TRS hybrid materials to be good candidates for the fabrication of fluorescence biosensors for a variety of phenolic compounds.

The PDDA QDs can be used as a versatile platform to develop biosensors for various analytes by immobilizing in the hybrids different enzymes that can catalyze the production of substrates that influence the fluorescence of the QDs in the hybrids. In a previous study, GSH-stabilized CdTe QDs were chosen as a sensitive probe for hydrogen peroxide and further used in the detection of glucose with glucose oxidase as the catalyst to produce hydrogen peroxide.[37] Herein, glucose oxidase was also chosen to testify the utility of the new hybrid material for the biosensor development. In the prepared PDDA QDs enzyme hybrids, GOx/QDs ratio is 13; and the activity of GOx is 7.92 U per liter hybrids. Figure 5 shows the fluorescence quenching of the QDs in PDDA GSH CdTe QD GOx hybrids by different concentrations of glucose. A slight decrease in the fluorescence lifetime of GSH-CdTe QDs from 24.7 to 23.0 ns was observed (see Supporting Information, Figure S6). The Stern−Volmer plot representing the quenching effect of glucose yields $I_0/I = 1.16 + 2.60 \times 10^3 C_{glucose}$ ($r^2 = 0.990$). The detection limit of glucose is 5.0×10^{-6} mol/L, and the detection range is between 5.0×10^{-6} mol/L and 6.0×10^{-4} mol/L. The detection limit of glucose obtained using the PDDA QD GOx is comparable with that obtained with Mn-doped ZnS QDs as a phosphorescent probe[38] and is 10 times lower than the previous method in which both GSH CdTe QDs of similar size and GOx were dispersed in phosphate buffer solution.[37] Compared with the previous GOx and QD coimmobilization method with a peptide hydrogel,[27] a much lower detection limit of glucose was obtained in the present study although the concentration of the GOx in the PDDA QDs GOx hybrids was about 6 times lower than that in the peptide hydrogel.[27]

QD PDDA Enzyme Hybrids for the Development of Test Papers. The PDDA QD hybrid material is deemed to be a good candidate for test paper preparations as the polymer offers the immobilization of both QDs and enzyme in the paper. The assumption was approved by comparison of test papers made from a QD sol and PDDA QD hybrids. As shown in the Supporting Information (Figure S7), the QDs dried on the filter paper tend to diffuse with the phosphate buffer while PDDA can prevent the diffusion of the QDs with the buffer solution in PDDA QD hybrids, which is more suitable for the test paper preparation. Figure 6A,B shows the response of test

dx.doi.org/10.1021/ac300714j | *Anal. Chem.* 2012, 84, 5047−5052

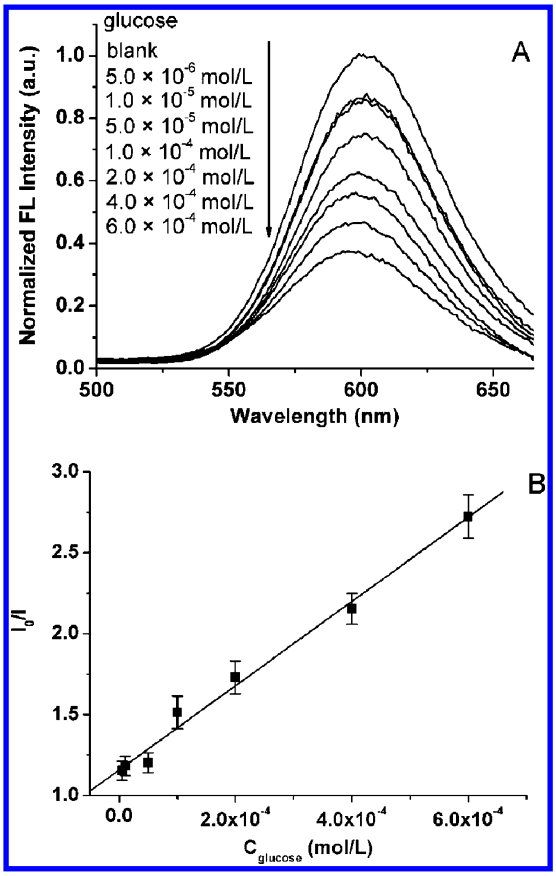


Figure 5. Glucose detection using PDDA GSH CdTe QD GOx hybrid materials. (A) Fluorescence spectra representing the quenching effects of catechol with concentrations of 5.0×10^{-6}, 1.0×10^{-5}, 5.0×10^{-5}, 1.0×10^{-4}, 2.0×10^{-4}, 4.0×10^{-4}, and 6.0×10^{-4} mol/L on the PDDA GSH CdTe QD GOx hybrid and (B) corresponding Stern–Volmer plot.

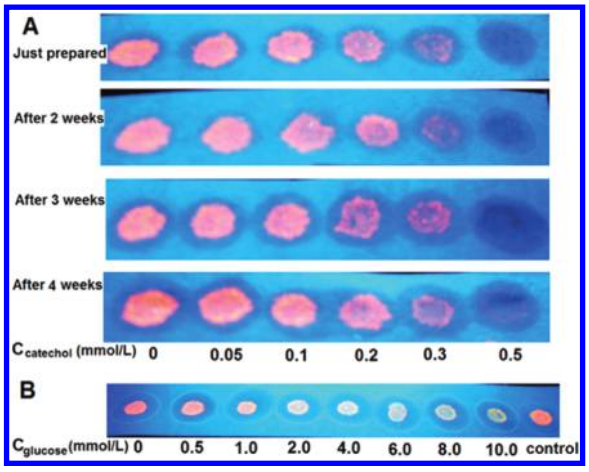


Figure 6. (A) Response of test papers prepared using PDDA GSH CdTe QD TRS hybrid materials to different concentrations of catechol. The images are made under 365 nm UV-lamp excitation. Results obtained with both freshly prepared test paper and test papers kept after preparation for 2, 3, and 4 weeks are shown. (B) Response of the test papers prepared using PDDA GSH CdTe QD GOx hybrid materials to glucose with concentrations of 0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 mmol/L; the control spot represents the PDDA QD GOx hybrids on the test paper without dipping any solution.

papers to catechol and glucose with a series of concentrations, respectively. The response of the test papers to catechol is within 1 min, while a longer response time is needed for glucose (about 10 min). Detection limit of glucose is comparable with recently reported two kinds of paper-based devices for glucose with ceria nanoparticles and iodide as colorimetric probes.[39,40] To the best of our knowledge, this is the first attempt to fabricate fluorescence test papers using QDs. The stability of the as-prepared test paper immobilizing PDDA-QD-TRS was good with both the QDs fluorescence emission and the enzyme activity lasting for at least 4 weeks as shown in Figure 6A. The PDDA-QD-enzyme hybrids based test paper combines good stability and low cost. In comparison to solution-based methods, it largely reduces the usage of cadmium containing QDs.

CONCLUSIONS

In conclusion, this work provides a simple way for the successful incorporation of the biotransformation and recognition unit of an enzyme and the signaling unit of QDs into polymer hybrid materials. The PDDA QD enzyme hybrid material proved to be a stable and versatile material for biosensors and test paper development. Low binding constant values of enzyme immobilized in these hybrids and the low detection limits showed the good maintenance of the enzyme structure and activity in the hybrids. With the advantages of simple preparation and high stability, this kind of hybrid material possesses great potential in the development of portable sensing devices.

ASSOCIATED CONTENT

Supporting Information

Detailed discussion of the effects of PDDA of different concentrations on the fluorescence and UV–vis absorption spectra of GSH-CdTe QDs, TEM images of GSH-CdTe QDs and PDDA-GSH CdTe QDs hybrids, experimental procedure as well as results concerning the reproducible detection of PDDA-QD hybrid films, detailed description of Lineweaver–Burk plot analysis, control experiments concerning the biosensing of catechol and glucose, results of fluorescence lifetime measurements, and a comparison of PDDA-QD hybrids with the QD sol on the test paper preparation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Vladimir Lesnyak for the TGA-CdTe QD synthesis, and J. Yuan appreciates the support from the Alexander von Humboldt (AvH) Foundation.

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