Biosensors

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# Enzyme-Encapsulating Quantum Dot Hydrogels and Xerogels as Biosensors: Multifunctional Platforms for Both Biocatalysis and Fluorescent Probing**

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Quantum dots (QDs) have gained great interest in both fundamental research and technical applications owing to their unique size-dependent physical and electronic properties.[1] The development and the evolution in QD synthesis play a critical role in the progress of QD applications such as biological imaging, photovoltaic and light-emitting devices, and optical sensors.[2] However, many applications and devices are based on nanoparticle assemblies in the solid state instead of nanoparticles in solutions; as a result, nanoparticle assembly is a necessary step in the utilization of nanomaterials.[3] The assembly of QDs as building blocks into functional architectures and the applications of these architectures have been amongst the priorities of QD research.[3b]

Gels and aerogels manufactured from silica and metal nanoparticles available in colloidal solutions have recently been proven to provide an opportunity to marry the nanoscale world with that of materials of macro dimensions; these gels can be easily manipulated and processed, whilst maintaining most of the nanoscale properties. Their extremely low density and high porosity provide access to the capacious inner surface of the interconnected nano-objects they consist of and make these gels enormously attractive for applications.[4] In the past few years, great attention is given to the formation of 3D networked QD aerogels. Quantum-confinement effects retain in such large interconnected monoliths,[3,5] thus making them promising candidates for further use in LEDs, photovoltaics, sensors, etc.[6] Particularly, sol–gel-derived materials have been used increasingly in the biosensor development, because the pore sizes throughout the meso and macro regimes are appropriate for molecular transport or infiltration of secondary components and also provide potential for encapsulation of a variety of biomaterials from proteins to whole cells.[7] Thus, QD gels will provide suitable media for encapsulating enzymes or other biomolecules. Both biorecognition units (enzymes) and signaling units (QDs) are integrated in such hybrid material, which is essential for the fabrication of QD-based biosensors. However, most reported enzyme-encapsulating materials used in the biosensing field are silica-gel-based and aerogel-based materials that are manufactured through the sol–gel process by addition of biomolecules into the silica precursors.[8] Up to now, only a few reports on QD-gel-based sensors are published,[9] although QDs have been widely utilized as a fluorescence (FL) probe in bioanalysis.[10] The formation of 3D networked QD aerogels through the sol–gel method is mainly based on the partial removal of thiolate ligands by oxidants or the photochemical treatment.[3a,5b,11,12] However, oxidants or irradiation will harm structure and activity of the biomolecules during immobilization in the QD gels. Recently, Kotov and co-workers reported on self-assembly of CdTe nanoparticles under ambient conditions and ultrasound-induced switching between gel and sol.[13] We consider sol–gel switching to be an appropriate way to immobilize enzymes in QD gels, but the long gelation time (months)[13] of the QDs in water hinders their application in the biosensor development.

Here we have developed an enzyme-encapsulating CdTe QD gel as a multifunctional platform for biosensors. By choosing a neutral phosphate buffer (PB) to dissolve the precipitated mercaptosuccinic acid (MSA)-capped CdTe QDs, the gelation time of the CdTe QDs is sufficiently shortened to several days, and the sol–gel switching was also observed in the as-prepared QD gels. CdTe QD hydrogels encapsulating the enzyme tyrosinase (TRS) were further manufactured and applied in the biosensing of dopamine (Figure 1).

The MSA-capped CdTe QD hydrogel was prepared by dispersion of the CdTe QDs precipitate, from which the MSA ligand had partially been removed, in PB (50 mmol $L^{-1}$) at room temperature and room light. In the FL spectra of QD sol and the corresponding gel in PB solution with pH 7.1 a red shift of the emission maximum from 589 nm to 596 nm was

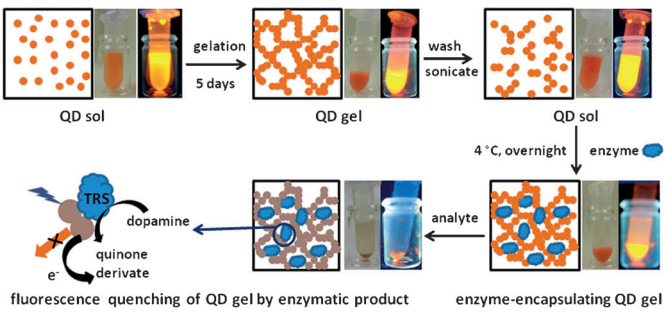


***Figure 1.*** Scheme of the encapsulation of enzymes in a QD gel using sol–gel switching process of MSA-capped CdTe QDs; corresponding true color images taken under room light and upon 365 nm UV lamp irradiation; and the sensing mechanism of the enzyme–QD hydrogel.

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observed after gelation (Figure 2A). Gelation of QDs is found to be highly pH-dependent (Figure 2B and Figure S1 in the Supporting Information). Experimental results suggested that the pH range between 6.5 and 7.1 is suitable for a relatively fast formation of stable and highly fluorescent

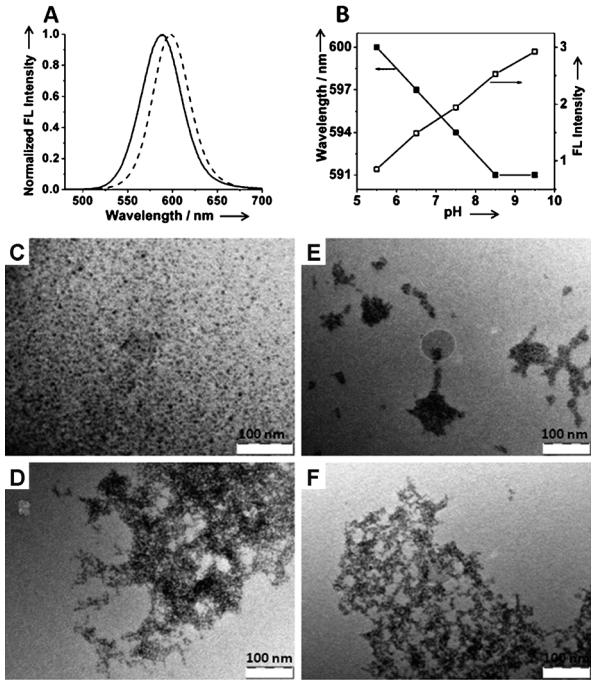


***Figure 2.*** A) FL spectra of MSA-capped CdTe QD sol (——) and MSA-capped CdTe QD gel (– – –); B) pH-dependent FL maximum wavelength (■) and intensity (□) of CdTe QD gels; C–F) TEM images of CdTe QD sol as prepared, CdTe QD hydrogel, sol obtained from sonication of the hydrogel, and TRS-encapsulating CdTe QD hydrogel, respectively. Scale bars: 100 nm.

QD gels, and is also an optimal pH range for many enzymes. Since pores with diameters ranging from 10–50 nm are observed in the TEM images shown in Figure 2, it is possible that enzymes can be encapsulated in the mesopores of the CdTe QD gel network during the gelation process. Similar to the previous report,[13] the obtained hydrogel can undergo sol–gel switching. The QD sol from sonicating the gel is the dispersion of small QD aggregates, and the FL emission maximum wavelength is same as that of the QD gel. The facile gelation and the sol–gel switching allowed the QD hydrogel to be a suitable platform for the immobilization of enzymes.

In AFM images (Figure 3), beside the network structure of QD gel, elliptically-shaped features with sizes of about 10 nm were also observed, which is consistent with the shape and size of TRS.[14] We assume that the observed two ellipsoidal features are protein aggregates. To further validate the encapsulation of TRS in QD hydrogel, the test reaction of TRS-catalyzed oxidation of catechol was applied. During the gelation, the QD gel framework grows around the enzyme molecules; thus parts of the enzyme molecules were encapsulated by QDs, and those not encapsulated dispersed into the supernatant. Figure 4A shows the absorption of catechol (1.0 mmol $L^{-1}$) after incubation with supernatant and hydrogel. The highly catalytic effect of the supernatant indicates that not all of the enzyme molecules are encapsulated. To remove free enzyme, the hydrogel was washed thoroughly with PB solution and immersed in PB solution for storage. After washing, the supernatant showed no obvious enzymatic activity on catechol, while enzyme activity in hydrogel was well maintained. Furthermore, the enzyme activity of TRS in hydrogel was maintained even after immersion in PB for at least one week (Figure 4B). The networks of the QDs effectively serve to "cage" the enzymes, preventing them from leaching.

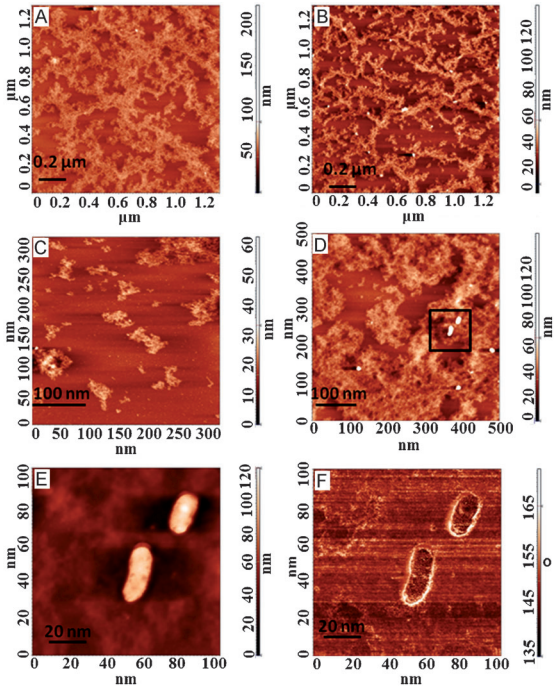


***Figure 3.*** AFM images of CdTe QD gel (A, C) and TRS-encapsulating CdTe QD gel (B, D). (E) and (F) are the height and phase images of the marked area in (D). o = phase angle degree.

It has been proven that during the sol–gel process, despite the entrapment in a solid material, enzymes are able to retain many of their solution-phase characteristics. The large surface area and the porosity of the microstructures can be easily tuned to maximize the activity of the entrapped enzyme.[7c] A kinetic study showed that the Michaelis constant of the TRS in the hydrogel (0.44 mmol $L^{-1}$) is only 1.38-fold higher than that of the free TRS (0.32 mmol $L^{-1}$), the maximum velocity of TRS in the hydrogel was 0.25 mmol $L^{-1}$ per min (92.5% of that of free TRS; Figure S2 in the Supporting Information). Compared with the polymer/QD hybrid material (in which the maximum velocity of TRS decreased to 15% of that of the free enzyme),[15] the hydrogel provides a good matrix to encapsulate TRS without obvious structural changes and allowing for relatively fast diffusion of substrate to the enzyme.

With both quantum confinement effect and high enzymatic activity well retained, the enzyme-encapsulating CdTe QD hydrogel serves simultaneously as signal-transforming and -recording unit in a biosensor. Dopamine was taken as an

  

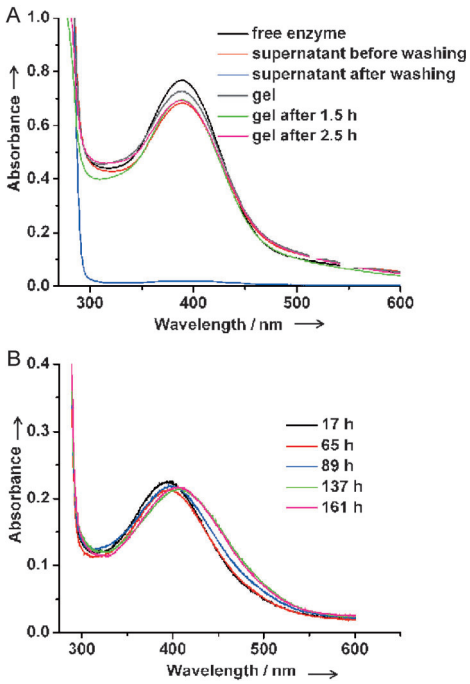


***Figure 4.*** A) Absorption spectra of catechol (1.0 mmol $L^{-1}$) after incubation with free TRS (4.0 μg), supernatant (20 μL) before and after washing the TRS-encapsulating QD gel, TRS-encapsulating QD gel (20 μL) just after washing, and 1.5 and 2.5 h after washing; incubation time, 2.5 min. B) Absorption spectra of catechol (1.0 mmol $L^{-1}$) after incubation with TRS-encapsulating CdTe QD gel (15 μL) after washing and storage in PB for 17, 65, 89, 137, and 161 h. TRS solution (100 μL of 2.0 mg $mL^{-1}$) was added into QD sol (1 mL) for gelation. PB solution (50 mM, pH 6.8).

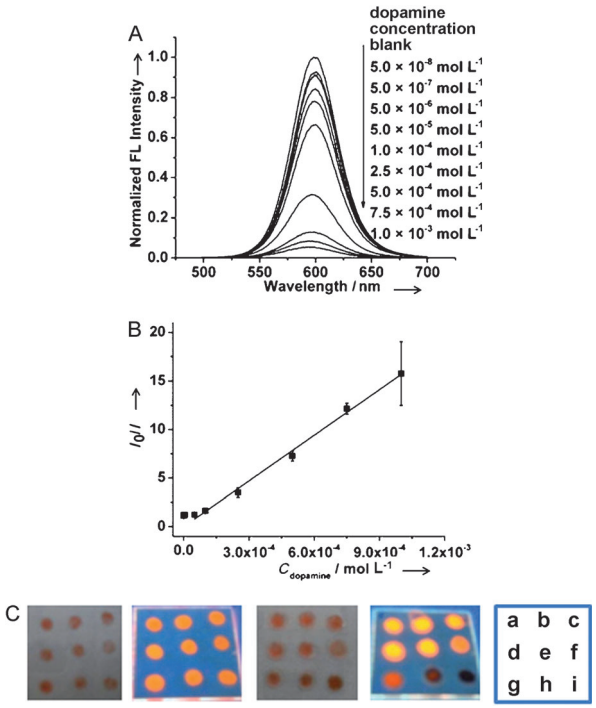


***Figure 5.*** Dopamine sensing using TRS-encapsulating CdTe QD hydrogel and xerogel. A) FL spectra representing the quenching of the QD fluorescence by a series of dopamine concentrations in TRS-encapsulating QD hydrogel. B) Relationship between $I_0/I$ and dopamine concentration. C) Photos of TRS-encapsulating QD xerogel arrays and their responses to dopamine solution (3 μL) with concentrations of 0, $1.0\times10^{-7}$, $1.0\times10^{-6}$, $1.0\times10^{-5}$, $5.0\times10^{-5}$, $1.0\times10^{-4}$, $5.0\times10^{-4}$, $1.0\times10^{-3}$, $2.0\times10^{-3}$ mol $L^{-1}$ (spots a–i, respectively) under room light and 365 nm excitation.

example analyte in this study. Figure 5 A shows the quenching of the QD FL upon different concentrations of dopamine, and dopamine showed no obvious effect on the pure QD hydrogel in the absence of TRS (Figure S4 in the Supporting Information). The ratio $I_0/I$ ($I_0$ and $I$ are QD FL intensity in the absence and presence of analytes, respectively) was proportional to the dopamine concentration, with the linear regression equation being $I_0/I = -0.021 + 1.57\times10^4\ C_{\text{dopamine}}$ ($r^2 = 0.994$). The detection limit of dopamine is $5.0\times10^{-8}$ mol $L^{-1}$, and the detection range is between $5.0\times10^{-5}$ and $1.0\times10^{-3}$ mol $L^{-1}$. This detection limit of dopamine determined for the TRS-encapsulating CdTe QD hydrogel is four times lower than that determined for the CdTe QD sol,[16] and comparable to some recently reported electrochemical methods.[17] The detection limit and the wide detection range prove that the TRS-encapsulating QD hydrogel is an excellent candidate for the fabrication of FL biosensors for a variety of phenolic compounds. It is still a challenge to use the dried enzyme-encapsulating gels (aerogel or xerogel) in aqueous medium, because the porous texture of the dry gel collapses under the capillary contraction forces when the gel is redipped into water after drying.[18] Herein, xerogel sensing arrays were fabricated by drying enzyme-encapsulating QD gels on a solid substrate. QD FL is maintained for the arrays formed on both glass slides and filter paper. However, TRS activity in the QD xerogel on filter paper was lost, since dopamine (1.0 mmol $L^{-1}$) showed no obvious quenching on the QD gel (Figure S5 in the Supporting Information). This may be due to a collapse of the gel structure and thus the inactivation of the enzyme during the fast water absorption by filter paper. Moderate enzyme activity loss was also observed when drying the hydrogel on glass slides, but fortunately, the response of the xerogel to dopamine at higher concentrations was maintained (Figure 5 C). Moreover, the sensing response (both fluorescence and enzyme activity) was proven to be reproducible for at least six weeks (Figure S6 in the Supporting Information). This method provides a way to use xerogels in aqueous sensing systems and has potential for the application in microfluidic systems.

In summary, an enzyme was successfully encapsulated in QD hydrogel networks through the sol–gel technique. The as-prepared enzyme-encapsualting QD gel turned out to be a multifunctional biosensing platform owing to the incorporation of a biorecognition unit (an enzyme) and a signaling unit (QDs). Both enzyme-encapsulating hydrogels and xerogels exhibited a good sensing ability to the example analyte. As a versatile enzyme entrapment matrix, the QD gels offer great potential in the development of various enzyme-based biosensors and portable sensing devices.

## Experimental Section

Gelation of MSA-capped CdTe QDs into hydrogel: The obtained MSA-capped CdTe QDs (3 mL) were precipitated with ethanol (1:1 QDs/ethanol volume ratio); the resulting precipitate of the QDs was separated from the supernatant by centrifugation (6000 rpm × 5 min), dried under air flow, and redissolved in PB solution (1 mL, 50 mM). The resulting CdTe QD sol was left at room temperature to form the QD gel.

Encapsulation of enzymes in CdTe QD hydrogel: The CdTe QD gel was washed for several times with PB (50 mmol $L^{-1}$, pH 6.8). Sonication in an ultrasonic cleaner bath was carried out to induce the gel to sol transition. TRS solution (100 μL of 2.0 mg $mL^{-1}$) was added into the obtained QD sol (1 mL), and the mixture was left at 4 °C overnight to receive a TRS-encapsulating CdTe QD gel.

For the analyte sensing, the TRS-encapsulating CdTe QD gel was washed with PB (50 mmol $L^{-1}$, pH 6.8), and then a portion of the TRS-encapsulating QD gel (15 μL) was immersed in PB containing analyte for 14 min for FL detection under the excitation wavelength of 450 nm.

Enzyme-containing xerogel sensing arrays on glass slide: The obtained TRS-encapsulating QD hydrogel (2.5 μL) was dropped on glass slides, dried by evaporation, and stored at 4 °C. For analyte sensing, dopamine solution (3 μL) of various concentrations was dropped on the xerogel arrays and left to interact for 10 min. FL of the xerogel array was observed under excitation with 365 nm light of a UV lamp.

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