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Effect of protein molecules on the photoluminescence properties and stability of water-soluble CdSe/ZnS core-shell quantum dots

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The fact that the photoluminescence properties of quantum dots are always strongly influenced by the environment limits the scope of further progress in the field of QD' bio-applications. In this paper, the effects of immunoglobulin G (IgG) on the photoluminescence properties and stability of water-soluble CdSe/ZnS core-shell quantum dots coated with amphiphilic poly (acrylic acid) (PAA) are studied. Photoluminescence (PL) spectra, UV-vis spectra and excited state lifetime measurements are used to characterize the influence of different protein molecules, such as IgG (goat anti-human IgG, rabbit anti-human IgG, human IgG, and goat anti-human IgG-human IgG conjugates), avidin and bovine serum albumin (BSA) on the PL properties of QDs. The PL intensity and stability of CdSe/ZnS are largely enhanced compared to that of pure CdSe/ZnS QDs when the IgG molecules are added into the QD solution. The PL intensity increases with increasing the IgG concentration, but there appears no influence on the PL peak and a full width at half maximum (FWHM). The PL evolution of QDs as a function of different protein molecules depends on the structure of protein molecules, which is used as a sensor to recognize human IgG. It is inferred that the interaction between PAA coating layer and IgG molecules results in the enhancement of PL intensity. The study of the effect of pH and ion strength on optical properties of QD-IgG mixed solution, compared with the pure QD solution, suggests that pH value and ion strength do not destroy the interaction between the PAA coating layer and IgG. Excited state lifetime analysis indicates that the PL enhancement comes from the passivation of surface of the QDs with the PAA coating layer. IgG molecules have no effects on the properties of the biological system but can increase the stability and PL intensity of CdSe/ZnS QDs, which will enlarge the application of QDs in biomedicine and other fields.

quantum dots, photoluminescence, stability

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Colloidal semiconductor quantum dots (QDs) have unique photoluminescence (PL) properties due to the quantum confinement effect [1]. Compared with conventional organic fluorescent dyes, QDs possess advantages such as higher PL quantum efficiency, tunable luminescence depending on the size, wide continuous absorption, narrower PL band, and higher photostability [2]. Since hydrophilic QDs are first used as fluorescence probes in cellular labeling in 1998 [3,4], QDs have attracted widespread attention from the fields of biology and medicine and achieved remarkable progress in biomedical applications [5–8].

To make them suitable for biological applications, QDs must be passivated [9]. The best effective method to passivate QDs is that a dot (the core) is surrounded by another material of a larger optical bandgap (the shell). CdSe dots are commonly passivated with a ZnS shell to improve the optical properties of the QDs [10]. These QDs are synthesized through an organic method to hamper their biological applications. Then new chemical strategies are established to solve this problem. Recent progress in developing func-

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tional, biocompatible QDs has allowed demonstration of their use in several applications, including immunoassays, single-molecule tracking, live cell and tissue imaging [5–8].

One distinct feature of QDs is the large surface to volume ratio due to the decreasing particle size. The high surface to volume ratio makes PL properties variable and sensitive to local environments and light illumination [11], although the CdSe dots are passivated with a single ZnS shell or multi-shell (CdS/CdZnS/ZnS) [12]. To obtain a better biocompatiblity of QDs, it is very important to understand the effects of biomolecules on the PL intensity and stability of QDs. Several groups have focused on the interaction between biomolecules and QDs. Most of the work in this area has centered on improving the luminescent property and stability of highly capping QDs with proteins, DNA, or other biological molecules by passivating the surface of QDs by the interaction between biomolecules and surface of QDs [13,14]. According to our knowledge, no works are discussed about the effect of biomolecules on the PL and stability of QDs coated with amphiphilic PAA, although this kind of QDs is the most early obtained commercial QDs. More importantly, the constancy of the PL signal of QDs under various chemical environments has to be established before quantitative interpretation of the fluorescence signal can be reliably drawn.

In this work, the water soluble CdSe/ZnS QDs coated with amphiphilic PAA was used to research the effect of protein molecules on the PL properties and stability of QDs. Here, we selected some usual protein molecules (IgG, BSA and avidin) to exclude their influence on the PL properties of QDs in the future bioapplication such as assay and imaging. The coating layer-PAA provided additional protecting layer which weakened or avoided directly the interactions between protein molecules and surface of QDs. The interaction mechanism between QDs and protein molecules was discussed from another way. The results showed that the IgG molecules could enlarge the PL properties and stability of QDs compared to that of pure QDs. The effect of protein molecules on the PL properties of QDs depended on the structure of protein molecules, which demonstrated that the interaction between protein and coating layer PAA resulted in the changes in PL properties of QDs. It is believed that the application of IgG as a new stabilization agent will extend the biological applications of QDs.

1 Experimental

1.1 Preparation of water-soluble CdSe/ZnS core-shell nanoparticles

The detailed preparing method was described in our articles published [15,16]. Organic-soluble CdSe/ZnS core-shell nanocrystals emitting at 650 nm were precipitated as the method lightly improved in ref. [12] from the initial butanol stock solution with methanol, rinsed with methanol, and dried under vacuum. QDs were redispersed in a 5 mg/mL octylamine (OCT)-modified polyacrylic acid solution in chloroform. The molar ratio of polyacrylic acid and QDs was kept above 500∶1. The tube containing the mixture of polymer and QDs was evaporated. The residue was dissolved in water, and purified from excess polymer by gel filtration. The purified solution of QDs coating with polymer could then be stored in the dark for at least 2 months without any aggregate or precipitate formation. QDs with emission maxima at 650 nm were ellipsoid, with a core/shell diameter 6 nm (minor axis)×12 nm (major axis). The hydrophilic coats increased QD size several-fold in aqueous solution as a result of solvation effects, an increase that was reflected in the hydrodynamic diameter. The hydrodynamic diameter was 24 nm.

1.2 Preparation of samples

Stock solutions of the human IgG were prepared by diluting them with 5 mmol/L sodium borate buffer solution. The appropriate amounts were then added by micropipet to 3 mL aliquots of water soluble CdSe/ZnS nanoparticles that had been diluted to a uniform concentration of 8.4×10^{-9} mol/L, simply mixing dissolved protein with quantum dots ready for assay after twenty minutes. In the above experiments the quantum dot solutions were firstly prepared and then proteins were added to the solutions in order to make concentration of quantum dots identical. The same experiments were performed using the other proteins (bovine serum albumin (BSA), avidin, goat anti-human IgG, rabbit anti-human IgG, and denaturalized human IgG). The denaturalized human IgG was obtained by adding dodecyl sulfonic acid sodium salt (SDS) in the human IgG solution. The superfluous SDS was removed by dialyzing the SDS-human IgG solution.

1.3 Apparatus

Ultraviolet-visible absorption and fluorescence spectra were measured at room temperature using UV-3101 spectrophotometer and a Hitachi F-4500 fluorescence spectrofluorimeter, respectively. The QY of nanocrystal solutions was determined using Rhodamine 6G in ethanol as a standard. The optical densities (OD) at the excitation wavelength (480 nm) for the reference dye and the QDs were set to be identical at 0.05. The low absorption value should keep at a negligible level reabsorption of the emitted light by the sample. The obtained QYs of oil and aqueous QDs were 35% and 13%, respectively. The sizes of QDs at oil and water phase were determined by a transmission electron microscope (TEM) (JEOL-3010) and a dynamic light scattering (DLS) apparatus (Malvern, UK 3000HS), respectively. Fluorescence lifetimes were measured using the time-correlated single photon counting technique with FL920-fluorescence lifetime spectrometer (Edinburgh instrument) with the instru-

ment response of ~1 ns. The excitation source was an nF900 ns flash lamp. The recorded decay curves were fitted with a multiexponential function deconvoluted with the system response. The excitation wavelength of samples was 350 nm.

2 Results and discussion

For biological and medical applications, it is of particular importance to study the effect of protein on the PL properties of QDs to exclude environment effect and obtain enlarged PL intensity and stability of QDs. Figure 1 shows the absorption and PL spectra of CdSe/ZnS QDs and QDs-human IgG. The absorption spectrum (Figure 1(a)) indicates that both QDs and QDs-human IgG have a broad range of absorption. The absorbance of the QD-IgG is higher than that of QDs in the shorter wavelength. Compared to the QDs, the absorption wavelength of QDs-IgG is no change. The difference in absorption spectra comes from the absorption of IgG. The PL spectra of QDs and QD-IgG in Figure 1(b) are measured at the excitation wavelength of 350 nm. The PL spectra show the peak position and a full width at half maximum (FWHM) are no change. The results indicate that the inherent properties of QDs hold no change when IgG molecules are added into the QD solution. Compared with the QDs, the QD-IgG shows a higher photoluminescence quantum yield, as illustrated in Figure 1(b).

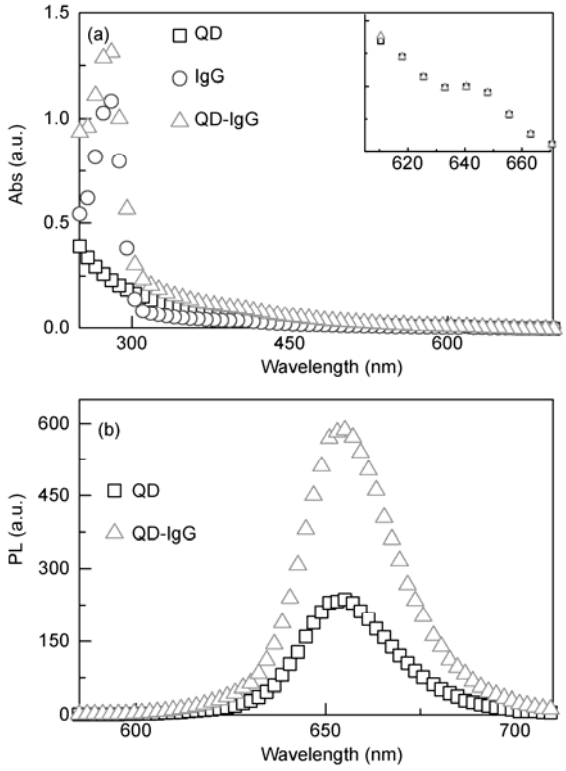


Figure 1 (a) UV-vis absorption spectrum and (b) PL spectrum of CdSe/ZnS QDs and QD-human IgG solution with identical QD concentrations of 8.4×10^{-9} mol/L. The inset in (a) is the enlarged absorption spectra near the first exciton absorption peak of QDs.

Figure 2 shows the PL spectra of a serial of aqueous solutions with the same QD concentration but different IgG-to-QD ratios. These samples were incubated at room temperature for one hour after the addition of IgG. The inset depicts the change of the relative intensities as functions of IgG/QD molar ratios. No new PL peak is observed in the emission spectra when the concentration of IgG varies in the QD solution. The PL intensity is enhanced with increasing protein concentration.

For biological applications, it is important to study the influence of pH on the PL intensity of QDs. In the most cases, it will be better to perform the biological applications at pH between 6 and 8. Figure 3 shows the PL spectra of QDs and QD-IgG at different pH. The inset presents the corresponding PL intensity as a function of pH. The intensity of QDs firstly increases slightly as the pH increase from 7.4 to 9.1, and then decreases when the pH gets higher. For the QD-IgG, the change is consistent with that of QDs. All these results indicate that the PL efficiency of the QD-IgG slightly depends on the pH of the solution. This will provide a wide application in bio-systems. The data also show that the QD solutions with the protein always enhance the PL intensity in comparison with that of QDs at different pH. This proves the pH do not influence the interaction between QDs and IgG.

The plots of PL intensity of QDs and QD-IgG with different ionic strength are shown in Figure 4. It reveals a relatively insignificant salt dependence up to 1 mol/L of NaCl. The above results indicate when the Na^+/Cl^- ions are added into the solution, they can not influence the surface of QDs. A 2.5-fold PL intensity difference for identical numbers of QDs and QD-IgG is maintained across the entire range of ionic strength studied. This demonstrates that varying the ion strength can not destroy the interaction between QDs and IgG.

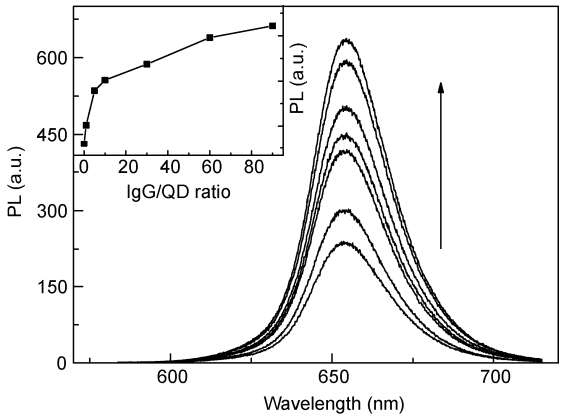


Figure 2 PL spectra of CdSe/ZnS QDs capped with PAA in PBS buffer with different concentration of human IgG. Arrow represensts the increase of human IgG concentration (The human IgG to QD molar ratios is 0, 1, 5, 10, 30, 60, and 90). The inset shows the plots of PL peak intensity versus human IgG to QD molar ratios.

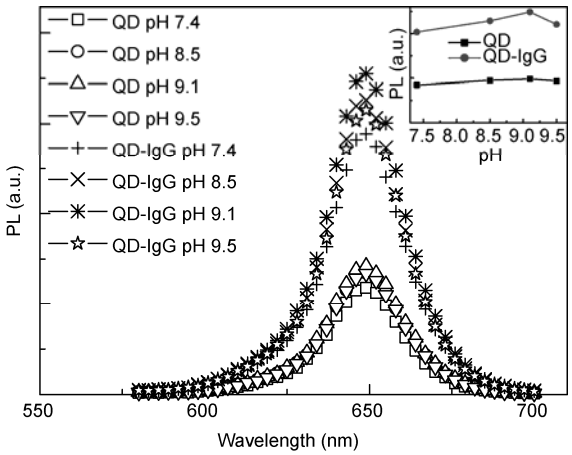


Figure 3 PL spectra of QD and QD-human IgG solution at different pH. The inset shows the plots of PL peak intensity versus pH.

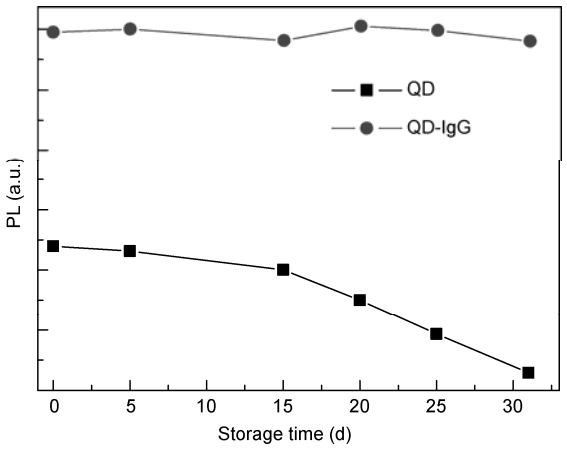


Figure 5 Plots of PL intensity of QDs and QD-human IgG solution as a function of storage time.

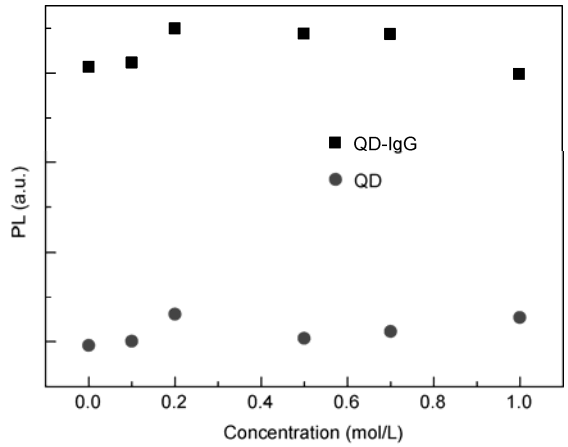
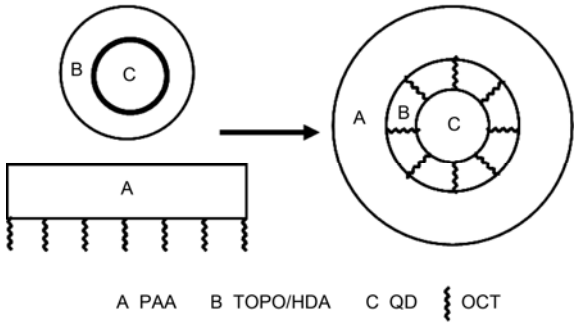


Figure 4 Plots of PL peak intensity of QDs and QD-human IgG solution under different ionic strength controlled by sodium chloride solution.



Scheme 1 Surface modification of TOPO/HDA-stabilized QDs by wrapping with the amphiphilic PAA.

Before QDs are used for biological and medical applications, it is necessary to understand the effect of QDs storage time on their PL QY and stability. We select QD-IgG samples with the IgG to QD ratio 60, compared with original QDs, for tracking the variation of optical properties with their storage time. Figure 5 shows the PL spectra of samples incubated in a refrigerator at 4°C for 31 d. The PL intensity of QD-IgG keeps constant in the first 25 d and then slightly decreases with the increase of storage time. Additionally, the PL intensity of pure QDs keeps constant for the first 5 d, then decreases more dramatically. The results indicate that the QD-IgG has much higher stability than that of QDs.

Scheme 1 gives the structure of water-soluble QDs prepared by hydrophobic interaction between TOPO/HDA-coated QDs and PAA modified with hydrophobic chains. The hydrophilic coating enlarged the size of the QDs in aqueous solution, reflected in an increase of the hydrodynamic diameter from ~16 nm for TOPO/HDA-coated QDs in $CHCl_3$ to ~24 nm for PAA-coated QDs in aqueous solution. The structure of water-soluble QDs contains three parts: CdSe/ZnS core (C), TOPO/HDA/OCT hydrophobic layer (B), and PAA hydrophilic layer (A). Region B is bound to region C by the molecular level interaction between the surface atoms and the head group of TOP/HDA [17]. Region A is bound to region B by hydrophobic interaction between hydrocarbons [17]. When the IgG molecules were added into the QD solution, the structure of PAA-coated QDs hampered the IgG molecules to directly contact the surface of QDs due to the PAA layer. Therefore, we inferred the interaction between IgG and PAA-coating layer resulted in the enhancement of QD PL intensity. The different conformations of PAA-coating layer can vary the optical properties of QDs has been reported in the ref. [18].

Many articles have reported that nano-material could rapidly adsorb proteins and form what is known as the protein "corona" when a nano-material enters a physiological environment [19]. The structure and composition of the protein corona depend on the nano-material (size, shape, and composition), the nature of the physiological environment, and the duration of exposure [20]. Therefore, if the influence of IgG on the PL intensity of QDs is attributed to the interaction between the PAA coating layer and IgG, the protein molecules with different structure should appear

different effect of PL properties of QDs. To demonstrate it and further exclude the effect of the other usual protein molecules on the PL properties of QDs, we researched the effect of BSA, avidin, rabbit anti-human IgG, goat anti-human IgG, goat anti-human IgG and IgG conjugates (GII) and SDS-human IgG on the PL of QDs. Figure 6 shows the PL peak intensities of QDs with different protein as a function of protein to QD ratios. For goat anti-human and rabbit anti-human IgG, there appear the same results with human IgG. For BSA and avidin, the evolution behaviors of PL intensity are much different from that of IgG. When SDS-denaturalized IgG is added in the QD solution, the PL enhanced efficiency is much bigger than that of natural IgG. We also researched the effect of the SDS on the PL of QDs in the same conditions. The enhanced PL of QDs is only very small. Therefore, the influence of SDS on the PL of QDs can be excluded. For the goat anti-human IgG and human IgG conjugates (GII), the PL enhanced ratio is smaller than that of IgG. All the results demonstrate the influence of protein on the PL intensity of QDs depends on the structure of protein. This demonstrates the interaction between the PAA coating layer and protein influences the optical properties of QDs.

Because the PL evolution behavior of QDs with protein depends on the structure of protein, based on this, we can design a sensor to recognize IgG molecules. We firstly added the goat anti-human IgG into the QD solution with a protein to QD ratio 60, and then added different protein into the solution. At last, we measured the PL spectra of the above solution to recognize the different protein (see Figure 7). The PL intensities of goat anti-human IgG and QD solution (Goat-QD) increase when rabbit anti-human IgG is added into the solution. Because goat and rabbit anti-human IgG have the same structure, they hold the same effect on the PL of QDs. For BSA and avidin, they appear no influence on the PL of Goat-QD. When the human IgG is added into the Goat-QD, the PL intensity decrease, which is attributed that the goat anti-human IgG and human IgG form

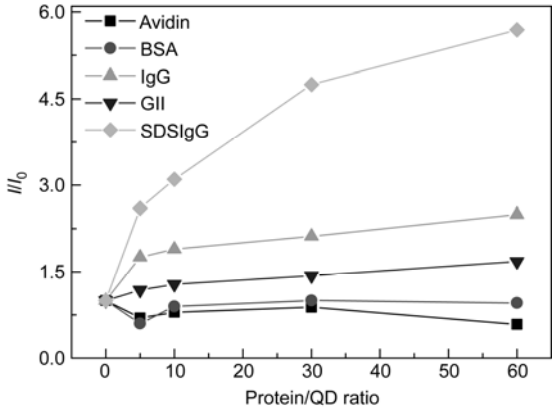


Figure 6 I/I_0 of QD-protein solutions as a function of protein to QD molar ratios.

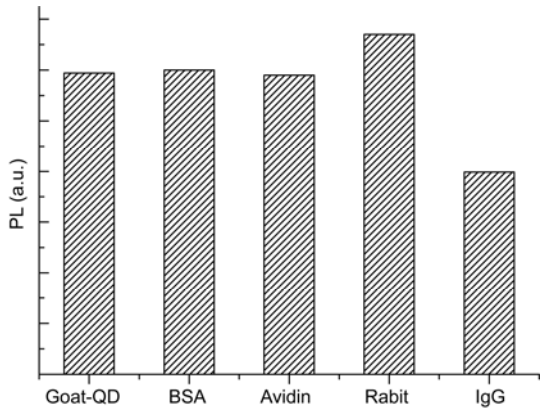


Figure 7 PL intensity of goat anti-human and QD solution in the presence of different protein molecules (BSA, avidin, Rabbit anti-human IgG (Rabbit) and human IgG (IgG)).

conjugates. All the results demonstrate a sensor to recognize IgG molecules is performed.

To gain physical insight into the PL of QDs evolution behavior, we have performed fluorescence lifetime measurements. The PL decay dynamics of QDs in the absence and presence of different concentrations of IgG are shown in Figure 8. The QDs exhibit slight PL lifetime lengthening with increasing the amount of IgG. The PL decay curves of QDs at different concentrations of protein can be well fitted by a bi-exponential function defined as $I(t)=A_1\exp(-t/\tau_1)+A_2\exp(-t/\tau_2)$ (see Figure S1), where τ_1 and τ_2 are the time constants, and A_1 and A_2 are the normalized amplitudes of the components, respectively [21]. The detailed fitting data are showed in Table S1. The average lifetimes τ_{AV} determined by the expression: $\tau_{AV}=(A_1\tau_1^2+A_2\tau_2^2)/(A_1\tau_1+A_2\tau_2)$ are showed inset in Figure 8, where it is clearly shown that adding the IgG into CdSe core/shell QD solution results in an increase of the average PL lifetime. The results indicate

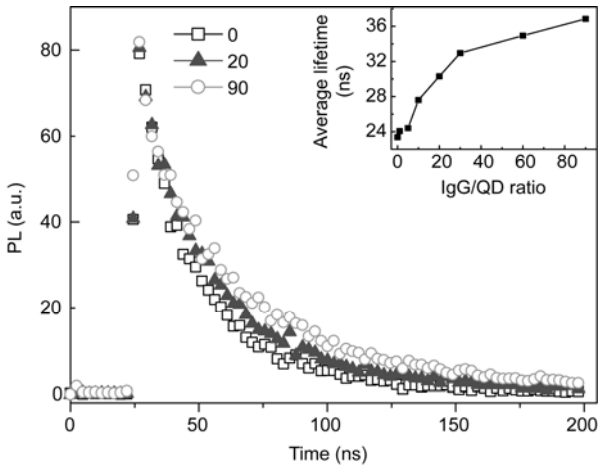


Figure 8 PL decays as a function of time for QDs with different human IgG concentration. The human IgG to QD molar ratio is 0, 20 and 90. The inset shows the plots of average lifetime versus the human IgG to QD ratios.

the PAA coating layer can passivate the surface of QDs [22], resulting in the enhancement of PL intensity of QDs when the IgG is added into the solution.

3 Conclusions

In conclusion, we have shown that IgG molecules can enhance the PL intensity and stability of CdSe/ZnS QDs effectively. The enhancement of PL intensity of QDs increases as increasing protein concentration. In addition, the effect of different protein molecules on the PL intensity of QDs depends on the structure of protein. Based on this, a sensor to recognize the human IgG molecules is designed. The PL evolution behavior of QDs with protein depending on the structure of protein demonstrates the interaction between the PAA coating layer and protein results in the change of PL intensity of QDs when protein molecules are added into the QD solution. The decay dynamics reveal that the PAA coating layer can passivate the surface of QDs. This study provides PAA coated CdSe/ZnS QDs with more opportunities for applications in either biological or medical fields.

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Supporting Information

Figure S1 The fitting example for PL lifetime.
Table S1 The fitting PL lifetime of QDs in protein/QDs mixture with identical concentrations of 8.4×10^{-9} mol/L

The supporting information is available online at csb.scichina.com and www.springerlink.com. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.