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# Studies on quantum dots synthesized in aqueous solution for biological labeling via electrostatic interaction

Zhangbi Lin,[a] Shuxun Cui,[b] Hao Zhang,[b] Qidan Chen,[a] Bai Yang,[b] Xingguang Su,[a,c,1] Jiahua Zhang,[c] and Qinhan Jin[a,*]

[a] *Institute of Analytical Science, College of Chemistry, Jilin University, Changchun 130023, PR China*
[b] *Key Lab for Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130023, PR China*
[c] *Laboratory of Excited State Processes, Chinese Academy of Sciences, Changchun 130021, PR China*

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**Abstract**

3-Mercaptopropyl acid-stabilized CdTe nanoparticles synthesized in aqueous solution are effectively bound to a biomacromolecule, papain, via electrostatic interaction. The conjugation between the nanoparticles and the papain is demonstrated by UV-Vis absorption, photoluminescence spectroscopy, transmission electron microscopy, and fluorescence micrographs. The biological activity of papain is maintained after the conjugation. The effects of the quantity of papain and the size of nanoparticles on the fluorescence characteristics of the CdTe–papain bioconjugates were studied.


*Keywords:* Water-soluble; Quantum dots; Papain; Electrostatic interaction; Conjugate

Colloidal semiconductor nanocrystals, often referred to as "quantum dots" (QDs),[2] have attracted considerable interest in luminescence tagging due to their unique size-dependent optical and electronic properties. Compared with traditional organic fluorophores, QDs present considerable advantages, for example, a narrow, tunable, symmetric emission spectrum, broadband excitation, high photobleaching threshold, and good chemical stability [1,2]. In 1998, Bruchez et al. [1] and Chan and Nie [2] applied QDs to label biomolecules as fluorescent probes, which initiated a new application field of QDs in life science. Recently, a major advance was made when QDs-tagged microspheres were successfully used in multiplexed optical coding of biomolecules [3]. To date, biomolecules are linked to QDs mainly by covalent attachment [1,2,4], electrostatic attraction [5,6], hydrogen bonding interaction [1], and hydrophobic force [7].

The QDs used in the above-mentioned studies, however, were generally produced with a capping layer composed of one or more organic ligands such as trioctylphosphine or trioctylphosphine oxide. These ligands are hydrophobic and thus nanoparticles capped with these coatings are not compatible with aqueous bioassay conditions. Consequently, it is necessary to substitute these organic ligands with hydrophilic capping agents for biological applications. Such processes are not only complicated, but also necessary to use hazardous organic ligands. Therefore, some simpler and more feasible synthesized methods to make directly water-soluble QDs have been reported [8–13]. The application of water-soluble QDs in conjugating with biomolecules has been studied only very recently [14,15]. The first example of such application utilized a one-pot glutardialdehyde linkage procedure to couple albumin to L-cysteine-capped CdTe nanoparticles [14]. Recently, thioglycolic acid-stabilized green- and red-emitting CdTe nanoparticles were attached to antibody and antigen via sulfo-NHS (*N*-hydroxysulfo-succinimide)

* Corresponding author. Fax: +86-431-8499805.
*E-mail addresses:* suxg@jlu.edu.cn (X. Su), qhjin@jlu.edu.cn (Q. Jin).
[1] Also corresponding author.
[2] *Abbreviations used:* QDs, quantum dots; TEM, transmission electron microscopy; MPA, 3-mercaptopropyl acid.

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coupling reaction [15]. In these cases, biomolecules were immobilized onto the surface of the quantum dots by covalent interactions.

In this paper, water-soluble QDs with different sizes, negatively charged CdTe nanoparticles, were effectively conjugated to a positively charged protein by electrostatic attraction, which was demonstrated by absorption and fluorescence spectroscopy, transmission electron microscopy (TEM), and fluorescence micrographs.

## Materials and methods

### *Materials*

3-Mercaptopropyl acid (MPA) (99+%), tellurium powder (~200 mesh, 99.8%), $CdCl_2$ (99+%), and $NaBH_4$ (99%) were purchased from Aldrich Chemical Com. Papain was used as obtained from Roche Diagnostics GmbH (Mannheim, Germany). The isoelectric point (p*I*) of papain is 8.75 [16]. All other reagents were of analytical reagent grade and used without further purification. Purified water (>18 MΩ cm) was used.

### *Preparation of water-soluble CdTe nanoparticles*

MPA-modified CdTe nanoparticles were synthesized in aqueous solution using previously described synthesis method [10,17]. In brief, freshly prepared oxygen-free NaHTe solution was added to nitrogen-saturated $1.25 \times 10^{-3}$ M $CdCl_2$ aqueous solution at pH 11.4 in the presence of MPA as a stabilizing agent. NaHTe was produced by the reaction of $NaBH_4$ with tellurium powder (2:1 molar ratio). The molar ratio of $Cd^{2+}$:MPA:$HTe^-$ was 1:2.4:0.5. The solution was refluxed for different times to control the size of the CdTe nanoparticles. The fluorescence quantum yield of CdTe nanoparticles at room temperature is ~25% (determined by comparing the integrated emission of the CdTe nanoparticles in solution to that of a solution of rhodamine 6G of identical optical density at the excitation wavelength). Nanoparticles with three different diameters 2.8, 3.0, and 3.3 nm, and with luminescence peaks at 527, 542, and 574 nm, respectively, were used in the study.

### *QDs–papain conjugate preparation*

Conjugation of CdTe nanoparticles with papain was carried out in 2 mM sodium borate buffer solution at pH 7.3. In all experiments, simply mixing the dissolved protein with diluted nanoparticles (3.0 nm, $3.125 \times 10^{-4}$ M, referring to $Cd^{2+}$) in buffered media yielded QDs–protein conjugates ready for assay within a few minutes.

### *Characterization*

UV-Vis absorption spectra were obtained using a Shimadzu 3100 UV-Vis-near-IR recording spectrophotometer. Fluorescence spectra were recorded by a Shimadzu RF-5301 PC spectrofluorophotometer. In both experiments 10-mm-optical-path cells were used to collect the absorption and fluorescence spectra. All measurements were carried out under ambient conditions.

TEM measurement was carried out on a Hitachi-8100IV transmission electron microscope operating at an acceleration voltage of 200 kV. The QDs or QDs–papain solution were dropped onto a carbon-coated copper grid, after which the grid was allowed to dry at room temperature.

Fluorescence microscopy (at 400×) was carried out using an inverted optical microscope (Leica DM IRB, Germany) equipped with a charge-coupled device camera (TOTA 500II, Japan) and a 100-W Hg excitation lamp. Samples of QDs or QDs–papain solution were spread on glass slides and dried under air.

### *Assay of papain and QDs–papain bioconjugates for enzymic activity*

The assay of papain and CdTe–papain for enzymic activity was performed following the method described [18]. The protein solution (10 μL) and *N*-benzyloxycarbonyl-glycine *p*-nitrophenyl ester in acetonitrile (50 μL, 5 mM) were added with rapid mixing to a thermally equilibrated buffer solution (3 mL, 0.1 M $NaH_2PO_4$, 1 mM EDTA, pH 6.0) in a cuvette in a Shimadzu 3100 UV-Vis-near-IR recording spectrophotometer. The release of *p*-nitrophenol was monitored by the change in absorbance at 340 nm.

## Results and discussion

The absorption spectrum of QDs–papain bioconjugates is flatter than that of the free QDs (Fig. 1); at the same time, the absorbance increases. The emission peak of the unconjugated QDs is at 542 nm; however, the emission peak of QDs–papain undergoes a red shift (557 nm), but the intrinsic spectral width is unchanged.

The changes of the absorption and fluorescence spectra result from the shortened distances between QDs owing to the electrostatic attraction between negatively charged CdTe quantum dots and positively charged protein, which increase dipole–dipole interaction between QDs and hence cause a larger Stokes' loss [19,20]. However, the fluorescence spectrum of the CdTe–papain solution has no change if the pH value of the buffered media is 9.3 (higher than the p*I* of papain). The charge of papain changes from positive to negative when the

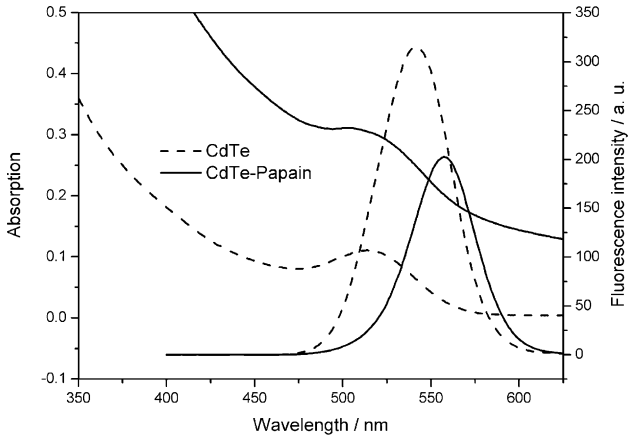


Fig. 1. Absorption and fluorescence spectra of CdTe nanoparticles solution (dashed lines) and CdTe–papain solution (solid lines). Excitation wavelength is 380 nm. The papain has no absorption at 380 nm and does not fluoresce under 380 nm excitation.

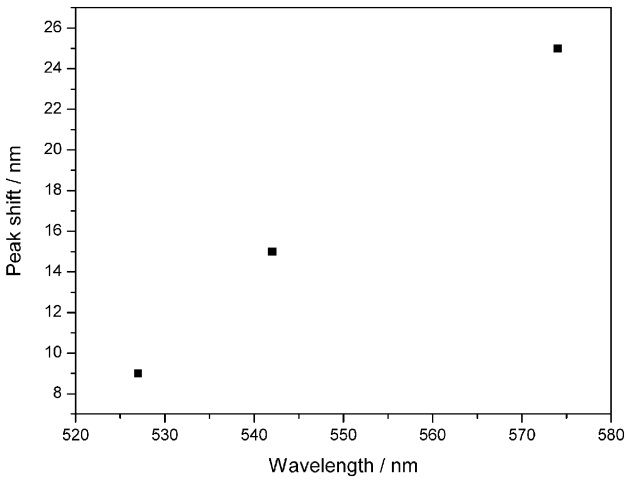


Fig. 2. The redshift in the emission peak with different sized CdTe bound to papain.

pH value of the solution varies from 7.3 to 9.3, which inhibits papain from conjugation with CdTe nanoparticles via electrostatic attraction. Furthermore, similar change in fluorescence emission spectra occurs when papain is replaced by a polycation, polylysine. These findings demonstrate undoubtedly that papain electrostatically binds to the oppositely charged surface of the capped QDs.

We also utilized QDs with different sizes (2.8 and 3.3 nm) to conjugate with papain in the same way. Interestingly, we find that the redshift of the QDs after conjugation is related to the size of the QDs: the redshift of the smaller QDs (2.8 nm) is 9 nm and that of the larger QDs (3.3 nm) is 25 nm, compared to that of the middle-sized QDs (3.0 nm in size and 15 nm in redshift) (Fig. 2). The reason is that the dipole–dipole interactions among the nanoparticles depend strongly on the particle volume [19]. With regard to larger QDs, the interaction of dipoles has a larger effect on the Stokes' loss. As a result, the phenomenon of redshift of the emission peak is more and more prominent with increasing particle size. The further characterization is mainly focused on the QDs with the size of 3.0 nm.

Morphological changes during the conjugation of CdTe nanoparticles with papain have been observed by TEM images. The average size of CdTe nanoparticles is about 3 nm and the size distribution of that is relatively uniform (Fig. 3A). However, the concentration of CdTe nanoparticles attributed to the attraction of the QDs and papain is observed (Fig. 3B). The TEM images support the above-mentioned conclusion further.

Fig. 4 shows the change of fluorescence spectrum of QDs–papain with a gradually increasing quantity of papain to a fixed amount of QDs. A break of the redshift of the emission peak appears when the quantity of papain is in the range of 40–100 μg, whereas the intensity of the emission decreases. The dipole interaction between QDs (induced by the shortening of the distance) does not increase until the quantity of papain is sufficient. Therefore, the red-shift phenomenon is distinct only when there is enough protein. However, there is no further shift of the emission peak by addition of excess papain because the interaction between QDs and papain has reached saturation. Moreover, the decrease of the fluorescence intensity could be explained in terms of the concentration effect [21].

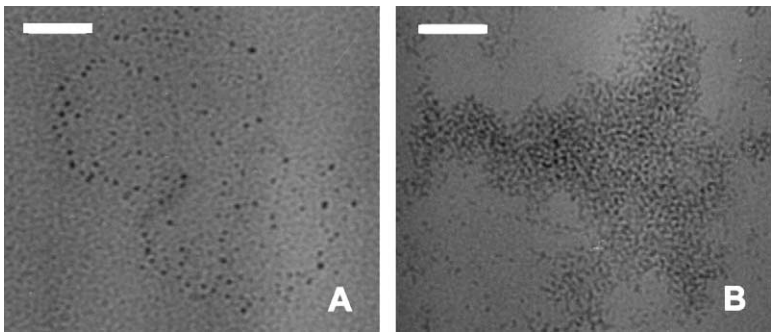


Fig. 3. TEM images of MPA-stabilized CdTe nanoparticles (A) and CdTe nanoparticles bound to papain (B). Scale bars, 50 nm.

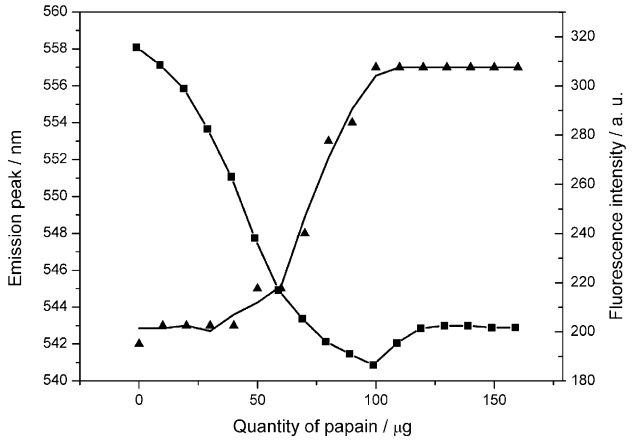


Fig. 4. Variations of the fluorescence intensity (squares) and the emission peak (triangles) of CdTe–papain solution as a function of papain quantity.

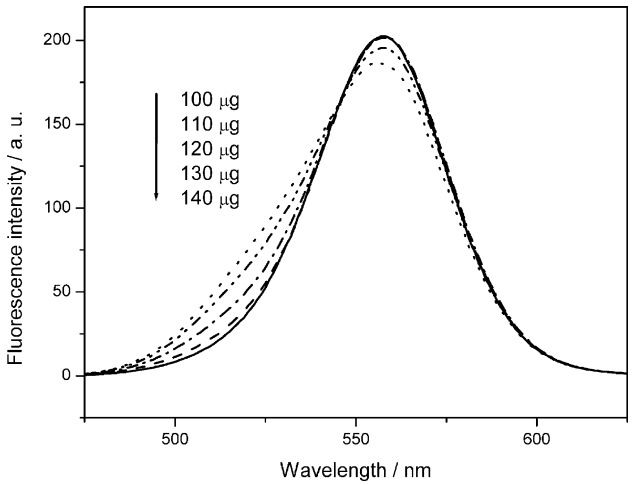


Fig. 5. Changes of the fluorescence spectra of CdTe–papain solution with increased quantity of papain from 100 to 140 μg.

This is an interesting phenomenon: although there is no shift of the emission peak with shift of the amount of papain from 100 to 140 μg, the shapes of the spectra become gradually symmetrical (Fig. 5). With further addition of papain, the fluorescence intensity in the range of 500–540 nm, corresponding to smaller QDs in crude solution, becomes less pronounced and that near 557 nm increases a little. This phenomenon suggests that papain prefers to conjugate with larger particles in the majority at the beginning. However, when a saturation situation between larger particles and papain occurs, excess papain tends to bind to smaller particles. As a result, the emission peak of smaller-sized QDs shifts to longer wavelengths with the quantity of papain exceeding 100 μg (from 100 to 120 μg). Consequently, the fluorescence intensity decreases in the range of 500 to 540 nm and increases a little at wavelengths near 557 nm. Moreover, the proportion of smaller-sized QDs is estimated to be approximately 1/6 through the "titration" curve (Fig. 4).

Further evidence for the conjugation of QDs and papain is provided by fluorescence micrographs. Fig. 6 presents a set of fluorescence micrographs of free CdTe nanoparticles and those bound to different quantities of

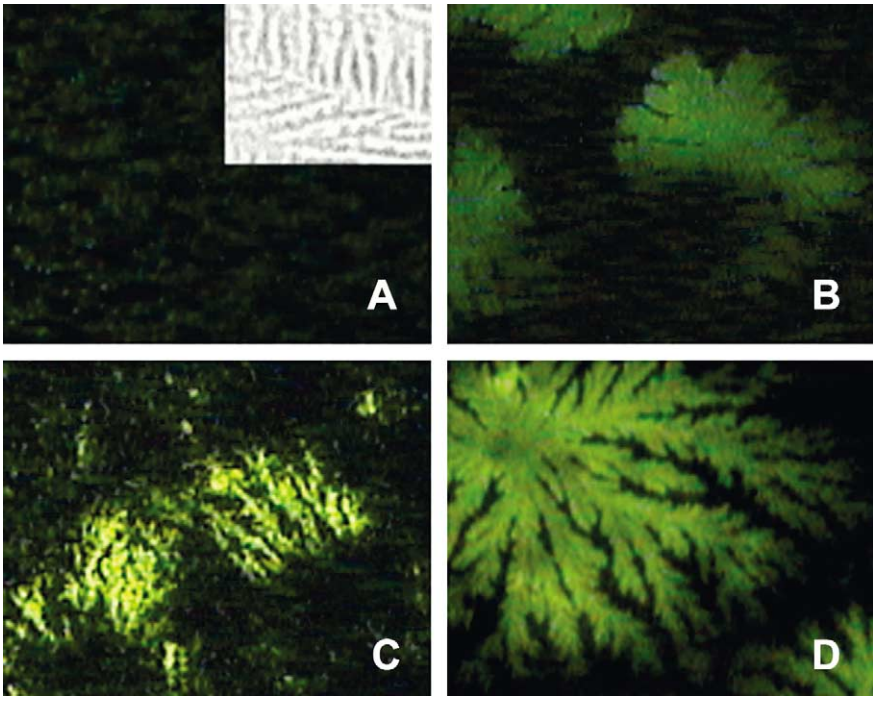


Fig. 6. Fluorescence images (35 × 30 μm) of CdTe–papain with different quantities of papain: (A) 0 μg, (B) 60 μg, (C) 90 μg, and (D) 120 μg. The inset of (A) shows the image of pure papain.

papain. The image taken with unconjugated nanoparticles shows that luminescent centers are homogeneously distributed (Fig. 6A); however, a significantly higher density and stronger brightness of QDs are observed for the samples containing papain (Figs. 6B–D). This phenomenon can be attributed to a higher local concentration of QDs in the presence of papain. From Fig. 4, we know that there is a jump of emission peak shift corresponding to the amount of papain from 40 to 100 μg. This jump causes Fig. 6C to lean toward yellow compared with Fig. 6B. With larger amounts of papain (from 90 to 120 μg), the fluorescence images tend to return to green due to the contribution of smaller-sized QDs (Fig. 6D). These fluorescence images are in good agreement with the results shown in Figs. 4 and 5.

It is necessary to study the effect of nanoparticles on the enzymic activity of papain after the conjugation. The experimental results show that the QDs–papain bioconjugates preserve the biological activity of papain, i.e., they can still hydrolyze ester linkage to hydroxyl and carboxyl. It is calculated that ~76% of the original enzymic activity is retained.

## Conclusions

CdTe nanoparticles synthesized in aqueous solution are effectively bound to a protein via electrostatic interaction. The method for labeling biomacromolecules with semiconductor nanoparticles presented in this paper is simple and feasible and can be applied to other inorganic nanomaterials with charged surfaces. These new types of nanoparticles–biomolecules conjugates could find their own potential application in cell surface labeling, intracellular tracking studies, and other imaging applications.

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