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# CdTe quantum dots as a highly selective probe for prion protein detection: Colorimetric qualitative, semi-quantitative and quantitative detection

Ling-Yan Zhang[a], Hu-Zhi Zheng[a,*], Yi-Juan Long[a], Cheng-Zhi Huang[b], Jian-Yu Hao[a], Dong-Bo Zhou[a]

[a] College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China
[b] College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, PR China

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ABSTRACT

CdTe quantum dots (QDs) were used as a highly selective probe for the detection of prion protein. Orange-emitting precipitates appeared within 30 s of the addition of recombination prion protein (rPrP) to a solution of green-emitting CdTe QDs. This allowed colorimetric qualitative and semi-quantitative detection of rPrP. The decrease in fluorescence intensity of the supernatant could be used for quantitative detection of rPrP. The fluorescence intensity of the supernatant was inversely proportional to the rPrP concentration from 8 to 200 nmol $L^{-1}$ ($R^2$ = 0.9897). Transmission electron microscopy results showed that fibrils existed in the precipitates and these were partly transformed to amyloid plaques after the addition of rPrP.



## 1. Introduction

Transmissible spongiform encephalopathies (TSEs), which are commonly known as prion diseases, can lead to fatal neurodegenerative disorders. All prion diseases share the same pathogenic mechanism, which is based on the conversion of misfolded normal cellular prion protein ($PrP^C$) into β-sheet-rich, protease-resistant prion protein ($PrP^{Sc}$) [1–4]. The misfolded protein has a high tendency to aggregate into amyloid plaques, which will finally develop into spongiform degeneration diseases of the brain such as Creutzfeldt–Jakob disease (CJD) and Gerstmann–Straussler–Scheinker syndrome (GSS) [5–8]. Approximately, 10–15% of human prion diseases are caused by mutations in the coding region of the germ line prion gene [9]. Using molecular genetics, several important point mutations of the PrP gene at codons 102, 117, 178, 180 and 198 have been reported in CJD and GSS [10–14]. Analysis of the PrP gene of a patient with CJD revealed a mutation point at codon 180 (Val-Ile) [15]. In this regard, the development of a simple, sensitive, and rapid method to detect this prion protein before the onset of clinical symptoms is of the utmost importance.

Currently, there are several methods available for prion protein detection. These generally involve conformational detection by labeling [16], fluorescence correlation spectroscopy (FCS) assay [17], protein misfolding cyclic amplification [18], capillary electrophoresis-based immunoassay [19], and aptamer-mediated fluorescence assay [20]. For example, Bieschke et al. [21] reported a sensitive detection method for $PrP^{Sc}$ using dual-color FCS based on the strong aggregation of $PrP^{Sc}$. This technique is approximately ten-times more sensitive than Western Blotting. However, the equipment for this method is expensive. Schmerr et al. [22] developed a capillary electrophoresis-based non-competitive immunoassay, but it included complicated functionalization steps and required expensive apparatus.

Quantum dots (QDs) have many attractive features, including broad excitation, narrow and symmetric emission, and high photostability [23,24]. QDs have been used in biological applications for cellular labeling and imaging [25,26], and tissue imaging [27,28]. Recently, many researchers have investigated QDs for the analysis of biomolecules. For example, Mattoussi et al. applied QDs to maltose binding protein (MBP), which was detected based on fluorescence resonance energy transfer [29]. Ma et al. utilized QDs to determine bovine serum albumin (BSA) by covalent attachment [30].

The aim of this research was to develop a novel fluorescence assay method using CdTe quantum dots as a probe to detect rPrP. The change in the color of fluorescence on interaction of CdTe QDs and rPrP was used for colorimetric qualitative and semi-quantitative detection by visual inspection. Quantitative detection of rPrP could be achieved by measuring the change in fluorescence intensity of the supernatant. This

* Corresponding author. Tel.: +86 23 68252360; fax: +86 23 68254000.
*E-mail address:* zhenghz@swu.edu.cn (H.-Z. Zheng).

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technique shows promise for clinical diagnosis of prion disease.

## 2. Experimental

### 2.1. Chemicals

Analytical grade $CdCl_2{\cdot}2.5H_2O$ was purchased from Chengdu Chemical Reagent Factory (Chengdu, China). Tellurium was purchased from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China). Mercaptoacetic acid was purchased from Shanghai Qiangshun Chemical Co. Ltd. (Shanghai, China). All regents used for purification of rPrP were purchased from Genview. Other proteins such as human serum albumin (HSA), hemoglobin from bovine blood (Hb), chicken egg white lysozyme, horseradish peroxidase (HRP), and glucose oxidase (GOD) were all purchased from Sigma-Aldrich (St. Louis, MO). BSA was purchased from Shanghai Bio Life Science & Technology Co. Ltd. (Shanghai, China). L-lactate dehydrogenase (LDH) was purchased from Livzon Pharmaceutical Group (Zhuhai, China). Snailase was purchased from Beijing Biodee Biological Technology Co. Ltd. (Beijing, China). Pepsin was purchased from Shanghai Institute of Biological Products (Shanghai, China). Cellulase was purchased from Dongfeng Biochemistry Technology Co. Ltd. (Shanghai Institute of Biological Science, China). Thrombin was purchased from Heilongjiang Dilong Pharmaceutical Co. Ltd. (Suihua, China). $His_6$-tagged MBP was a gift from Professor Kwok-Yin Wong (Hong Kong Polytechnic University).

### 2.2. Preparations

#### 2.2.1. Synthesis of CdTe QDs

Preparation of the CdTe QDs and their capping with mercaptoacetic acid followed an established method [31,32]. Briefly, under a nitrogen atmosphere and magnetic stirring, tellurium (0.0191 g) was mixed with an excess of sodium borohydride in water to prepare sodium hydrogen telluride (NaHTe). $H_2Te$ gas was then generated by mixing the NaHTe with dilute $H_2SO_4$ (0.5 mol $L^{-1}$). The $H_2Te$ gas in a stream of $N_2$ gas was bubbled into an aqueous solution of $CdCl_2{\cdot}2.5H_2O$ (0.0685 g) and mercaptoacetic acid (0.72 mmol $L^{-1}$), which had been adjusted to pH 9 by dropwise addition of aqueous NaOH. The resulting solution was refluxed under nitrogen at 100 °C to produce CdTe QDs. The reflux time could be used to control the size of the CdTe QDs.

#### 2.2.2. Purification of rPrP

Recombinant prion protein with a mutation at codons 180 was separated from freshly transformed *Escherichia coli* BL21 (DE3) (Novagen) containing the plasmid pET-rPrP (a gift from Professor Geng-Fu Xiao, Wuhan Institute of Virology, Chinese Academy of Science), and purified using a nickel ion-charged Sepharose column as described previously [33]. The protein concentration was determined with a Bio-Rad protein assay kit.

#### 2.2.3. Fluorescence spectra measurement

Fluorescence spectra were recorded using an F-4500 spectrofluorophotometer (Hitachi, Japan). The excitation wavelength was 360 nm, and the excitation and emission slits were set to 5.0 nm. The fluorescence spectra of the precipitates were recorded using the standard solid sample holder of the F-4500 spectrofluorophotometer.

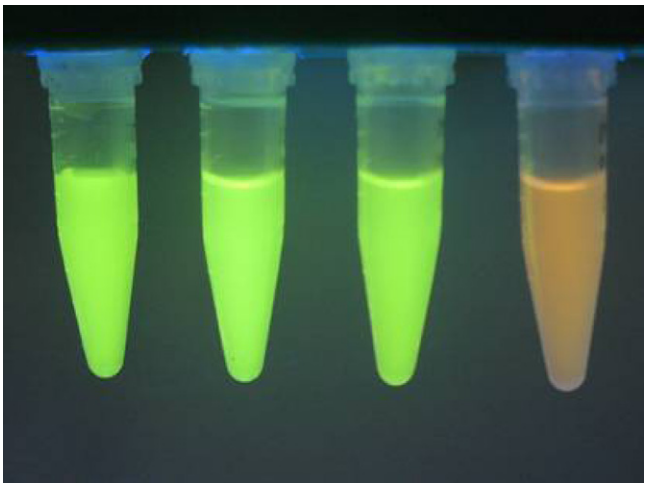


**Fig. 1.** Colorimetric qualitative detection of rPrP (6.7 μg $mL^{-1}$) with CdTe QDs ($1.0 \times 10^{-6}$ mol $L^{-1}$). The excitation wavelength of the UV lamp was 302 nm. From left to right: CdTe QDs, CdTe QDs with BSA, CdTe QDs with $His_6$-tagged MBP, CdTe QDs with rPrP.

## 3. Results and discussion

### 3.1. Colorimetric qualitative detection of rPrP with CdTe QDs

The fluorescence of $1.0 \times 10^{-6}$ mol $L^{-1}$ CdTe QDs in pH 7.0 phosphate buffer solution (PBS) was measured under UV lamp illumination (Shanghai Jiapeng Technology Co. Ltd.) with an excitation wavelength of 302 nm. Fluorescence images were recorded with a Canon A630 digital camera. The solution emitted bright green fluorescence. After addition of 6.7 μg $mL^{-1}$ rPrP to the CdTe QDs solution, the solution immediately became turbid and the color of the fluorescence changed to orange. This change could be clearly detected by visual observation of the solution (Fig. 1). Remarkably, the intensity of the orange fluorescence was stable for at least 1 day. To confirm the selectivity of rPrP detection, we also added 6.7 μg $mL^{-1}$ of BSA or $His_6$-tagged MBP to the CdTe QDs solution. The turbidity was not observed and fluorescence color did not change at all (Fig. 1). Other common proteins, such as HSA, Hb, chicken egg white lysozyme, snailase, pepsin, cellulase, thrombin, rabbit IgG, L-LDH, HRP, and GOD, also did not cause the turbidity or emission change. Therefore, these proteins did not interfere with the qualitative detection of rPrP.

To further study the change in emission, 320 nmol $L^{-1}$ rPrP was added to the $1.0 \times 10^{-6}$ mol $L^{-1}$ CdTe QDs solution. The precipitate that formed was collected by centrifugation (10,000 rpm, 10 min) and its fluorescence spectra were measured. The maximum emission of precipitate was 588 nm, while the maximum emission of the original CdTe QDs solution was 551 nm (Fig. S1). As a control, CdTe QDs solution in the absence of rPrP was centrifuged. No change in the fluorescence intensity or emission wavelength was found. This suggested that the red shift in the fluorescence spectra of the precipitates resulted from the shorter distances between the CdTe QDs in the precipitate than between the free CdTe QDs. This increased the dipole-dipole interaction between the CdTe QDs, and caused a larger Stoke's shift [34]. Accordingly, qualitative detection of rPrP could be realized based on the change in the emission wavelength.When a sample solution containing lysate from *E. coli* expressing rPrP was mixed with CdTe QDs, it became turbid immediately and produced orange fluorescence under the UV lamp. These responses were not observed when a solution containing lysate from *E. coli* not expressing rPrP (Fig. S2) was placed under the UV lamp. This showed that the interaction between rPrP and the CdTe QDs was highly selective. The mechanism of selective interac-

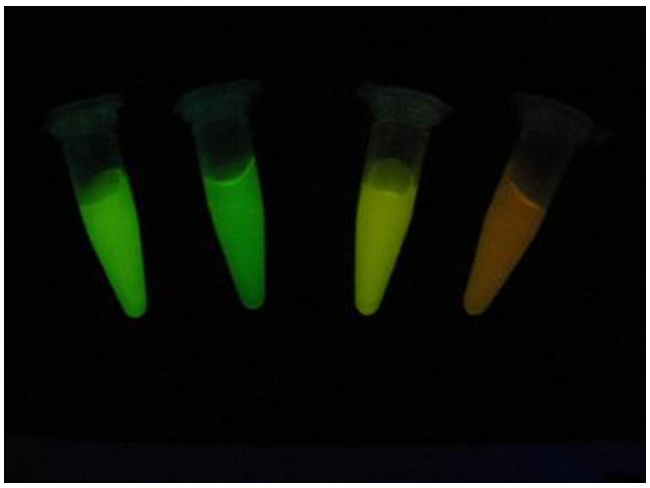


**Fig. 2.** Colorimetric semi-quantitative detection of rPrP with CdTe QDs ($1.0 \times 10^{-6}$ mol $L^{-1}$). From left to right: 0, 80, 240, 480 nmol $L^{-1}$ rPrP.

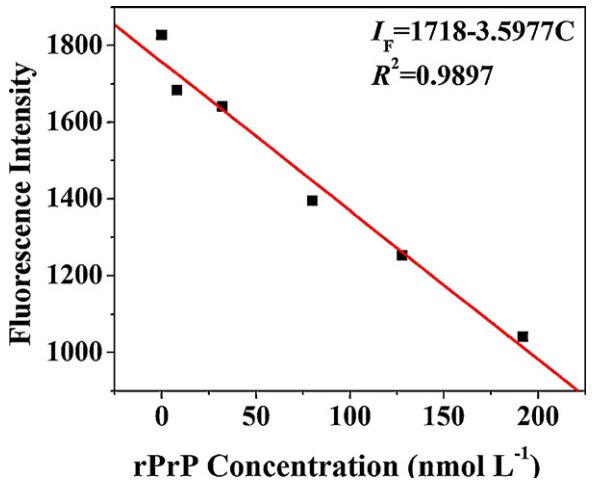



**Fig. 3.** Linear plot of fluorescence intensity of green-emitting CdTe QDs ($1.0 \times 10^{-6}$ mol $L^{-1}$) against rPrP concentration. The excitation wavelength was 360 nm and the emission wavelength was 551 nm.

tion is discussed in Section 3.6. This selectivity means that the CdTe QDs can act as a rPrP probe for colorimetric qualitative detection even in *E. coli* lysate solution.

### 3.2. Colorimetric semi-quantitative detection of rPrP with CdTe QDs

As the concentration of rPrP mixed with CdTe QDs solution increased, the color of the fluorescence changed gradually from green to yellow, and then orange (Fig. 2). Because of the strong fluorescence of the CdTe QDs, this color change could be detected directly by visual observation of the solution. Hence, the CdTe QDs could be used as a fluorescent probe for sensitive, rapid and simple colorimetric semi-quantitative detection of rPrP.

The color of the fluorescence was related to the precipitate that formed in the CdTe QDs solution in the presence of rPrP. As mentioned above, the free CdTe QDs produced green fluorescence while the precipitate produced orange fluorescence. When the rPrP concentration was low, only a small amount of precipitate was formed. In this case, the orange fluorescence from the precipitate was very weak compared to the green fluorescence from the CdTe QDs. Consequently, green fluorescence was observed. By contrast, with a high rPrP concentration, most of the CdTe QDs reacted with rPrP and a large amount of precipitate was produced. In this case, the orange fluorescence from the precipitate was stronger than the green fluorescence from the free CdTe QDs. As a result, orange fluorescence was observed. When the rPrP concentration was between these two extremes, the orange and green fluorescence were comparable, and yellow-green fluorescence was observed.

### 3.3. Quantitative detection of rPrP based on fluorescence intensity of the supernatant

When the CdTe QDs were mixed with rPrP, the concentration of CdTe QDs in the supernatant decreased because of precipitation. This reduced the fluorescence intensity of the supernatant. This change could be used for quantitative detection of rPrP. CdTe QDs with different sizes and concentrations were investigated to optimize the conditions for quantitative detection of rPrP.

The sensitivity of rPrP detection was studied with red-emitting CdTe QDs (average size 3.4 nm) and green-emitting CdTe QDs (average size 2.9 nm) [35]. After adding 100 nmol $L^{-1}$ rPrP, the fluorescence intensity of the supernatant of the red-emitting CdTe QDs decreased much more than that of the green-emitting CdTe QDs on addition of 100 nmol $L^{-1}$ rPrP (Table S1). This suggests that the red-emitting CdTe QDs have higher sensitivity for detection of rPrP than the green-emitting CdTe QDs.

For both the red- and green-emitting CdTe QDs, the sensitivity of rPrP detection increased as the CdTe QDs concentration decreased. The $1.0 \times 10^{-6}$ mol $L^{-1}$ CdTe QDs solution was chosen for rPrP detection because its fluorescence intensity was sufficient to detect high concentrations of rPrP.

While the red-emitting CdTe QDs ($1.0 \times 10^{-6}$ mol $L^{-1}$) had high sensitivity for the detection of rPrP, their linear range was not very wide (Fig. S3). The green-emitting CdTe QDs ($1.0 \times 10^{-6}$ mol $L^{-1}$) had a better linear relationship for the fluorescence intensity of the supernatant against the concentration of rPrP (8–200 nmol $L^{-1}$) (Fig. 3). The linear equation was $I = 1718–3.5977C$ ($R^2 = 0.9897$), relative standard deviation (RSD) = 2.1% ($n = 11$), where $I$ is the fluorescence intensity of the supernatant of the CdTe QDs solution after addition of rPrP, and $C$ is the concentration of rPrP. The lower limit of detection was 3 nmol $L^{-1}$ ($3\sigma$).

### 3.4. Effects of coexisting substances

A number of coexisting substances (Table S2) were studied for potential interferences with the CdTe QDs probe for rPrP. With these substances, a relative error of less than ±5% was considered to be acceptable. Most of the amino acids and metal ions tested did not affect the determination of rPrP. Although $Hg^{2+}$ presented higher effect on the fluorescence intensity of CdTe QDs, it is usually present at very low concentrations in *E. coli*. Consequently, its effect could be ignored.

### 3.5. Detection of rPrP in a solution of E. coli lysate

To demonstrate the potential application of the CdTe QDs system, we detected rPrP in dilute *E. coli* lysate solutions after spiking them with standard solutions of purified rPrP (0–200 nmol $L^{-1}$). This was used to construct a standard curve (Fig. 4). From this curve, the concentration of rPrP in the dilute solution of lysate from *E. coli* expressing rPrP was determined to be 99 nmol $L^{-1}$, and that in the original lysate from *E. coli* expressing rPrP was 22 μmol $L^{-1}$.

### 3.6. Selectivity of the interaction between CdTe QDs and rPrP

The selectivity of the interaction between the CdTe QDs–rPrP and the resulting precipitate is important for rPrP detection.

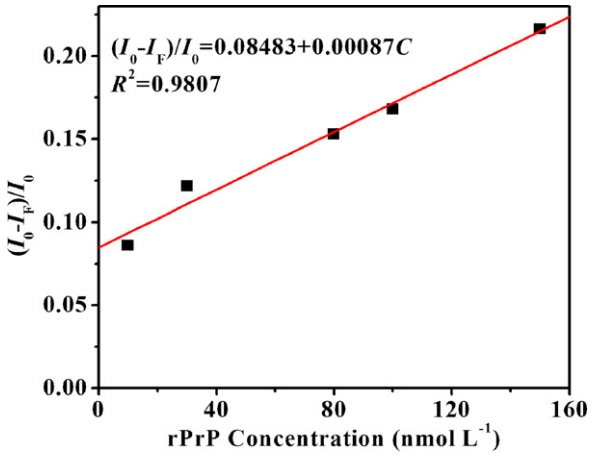



**Fig. 4.** Detection of rPrP spiked in a solution of lysate from *Escherichia coli*. $I_F$ is the fluorescence intensity of the supernatant of the CdTe QDs solution after adding lysate from *E. coli* spiked with standard solutions of purified rPrP and $I_0$ is the fluorescence intensity of the original CdTe QDs solution. The concentration of CdTe QDs was $1.0 \times 10^{-6}$ mol L$^{-1}$. The excitation wavelength was 360 nm and the emission wavelength was 551 nm.

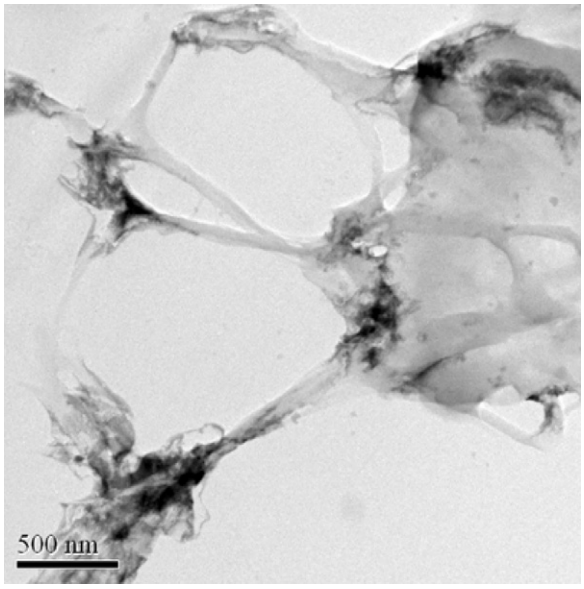
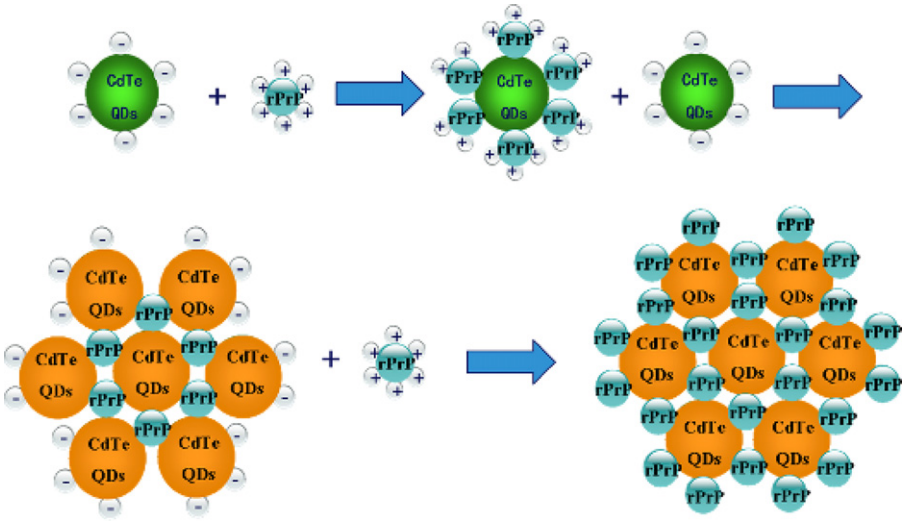



**Fig. 5.** TEM images of the fibrous precipitates. Fibrils were stained with 0.5% phosphotungstic acid, scale bar = 500 nm.

Histidines exist at positions 61, 69, 77, 85 in the octapeptide repeat region of rPrP, and these can strongly coordinate $Ni^{2+}$, $Zn^{2+}$ and other metal ions [36]. After mixing with $His_6$-tagged MBP, the CdTe QDs solution still produced green fluorescence even after 24 h. This suggested that the interaction between the CdTe QDs and rPrP was not because of coordination of $His_6$ with metal ions such as $Cd^{2+}$.The isoelectric point (pI) of rPrP is 10. In the experimental conditions (pH 7.0), the rPrP will be positively charged and there should be an electrostatic interaction between it and the electronegative CdTe QDs modified with mercaptoacetic acid [37,38]. When the pH is increased to >10.0, the rPrP will be negatively charged, and the interaction between it and the CdTe QDs will change from electrostatic attraction to electrostatic repulsion. Consequently, distance between the CdTe QDs will increase. This re-disperses the precipitate in solution. This suggested the electrostatic interaction will be important in determining the interaction between rPrP and CdTe QDs. Chicken egg white lysozyme (pI 11) was mixed with the CdTe QDs solution and used to investigate this further. After this mixture was incubated by 1 day, only a few orange-emitting precipitates were observed on the internal walls of centrifuge tube. The solution still appeared to produce green fluorescence. However, after mixing fibrils of the chicken egg white lysozyme with CdTe QDs, the color of the fluorescence changed to orange within seconds (Fig. S4). These results indicated that the change of emission of the CdTe QDs and rPrP mixture was not caused only by the electrostatic interaction but also by the formation of fibrils of rPrP.

Because some mutations result in significant destabilization of rPrP, some oligomers with fibrillar structures and less ordered aggregates will form even under physiologically relevant buffer conditions [39] such as with pH 7.0 PBS. Nanoparticles (NPs) can significantly enhance the rate of protein fibrillation, or the formation of fibrils [40]. Their potential to induce protein fibrillation is a function of both the NPs surface charge, which promotes adherence of the protein, and its large surface area, which acts as a platform for protein association to induce significant changes in the protein structure [41]. NPs surfaces had been shown to nucleate the formation of inorganic structures, and became incorporated as a core into the final product [42]. The CdTe QDs are NPs, and their opposite charge to rPrP and large surface area could result in rPrP adhering onto the surface of the CdTe QDs. This would induce oligomer for-




**Scheme 1.** The interaction of CdTe QDs and rPrP.

mation. Oligomer formation could then lead to aggregation of the rPrPs. The electrostatic interaction between rPrP and the free CdTe QDs then results in further absorption of the CdTe QDs onto the surface of the rPrP aggregate. This process then continues and a larger aggregate forms (Scheme 1). When the aggregates become large enough, this results in precipitate formation, which occurred very rapidly in the solution. In these precipitates, the CdTe QDs were closer together than when free in solution, and this caused a red shift in the emission [34]. The aggregation of rPrP formed fibrils and amyloid plaques (Fig. 5 and Fig. S5). Because other proteins did not form fibrils in pH 7.0 PBS, this method has high selectivity for rPrP.

## 4. Conclusions

CdTe QDs were successfully used as a highly selective probe for rPrP detection. The detection was sensitive, rapid and simple. Qualitative detection and semi-quantitative detection could be accomplished by visual observation of the solution based on changes in its emission. Quantitative detection of rPrP was achieved by measuring the fluorescence intensity of the supernatant of the CdTe QDs.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.11.075.

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