# Quantum Dots Protected with Tiopronin: A New Fluorescence System for Cell-Biology Studies

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Nanocrystals of semiconducting materials, otherwise included in the term quantum dots (QDs), have fascinated physicists, chemists and electronic engineers since the 1970s. The most striking feature of these materials is that their chemical and physical properties differ markedly from those of the bulk solid.[1] Since their quantum size effects are understood, fundamental and applied research on these systems has become increasingly popular. One of the most interesting applications is the use of nanocrystals as luminescent labels for biological systems.[2–5] Quantum dots have several advantages over conventional fluorescent dyes: they emit light at a variety of precise wavelengths depending on their size and have long fluorescent lifetimes.

Numerous methods exist for the syntheses of semiconductor nanocrystals, but most processes are costly, require sophisticated equipment or extreme reaction conditions and result in low product yields.[2,3,4,6] These synthetic methods are impractical for applications requiring larger quantities or higher concentrations of nanocrystals. However, *Candida glabrata* yeasts can produce CdS nanocrystallites by chelating Cd with phytochelatins (PCs) or glutathione (GSH) to form a peptide–Cd complex.[7] Then, labile sulfide is introduced to produce the peptide-capped CdS nanocrystallites.

Based on the yeast's use of peptides as naturally powerful chelators of foreign metal species and our previous experiments on sulfide-protected glyconanoparticles,[8] we have developed a simple production method that yielding gram quantities of stable, water-soluble CdS nanocrystals by using the non-natural amino acid tiopronin (*N*-2-mercaptopropionylglycine) as a capping agent. Tiopronin is a pharmaceutically important drug used for the treatment of cystinuria and rheumatoid arthritis.[9] Importantly, tiopronin has a free terminal -$CO_2H$ group that provides a handle for further reactivity. The chemical functionality of this capping agent gives the nanocrystals a very high stability and has allowed us to functionalize the QDs with a HIV-1 Tat protein-derived peptide sequence. This pep-

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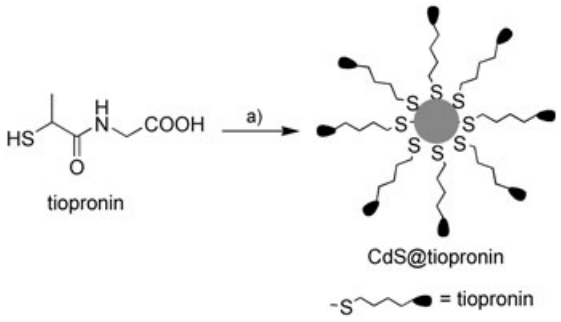
tide sequence has been used as an efficient way of internalizing a number of marker proteins into cells.[10,11] We hypothesized that entire semiconductor nanocrystals derivatized with similar sequences could be efficiently ferried into cells. We have also demonstrated the biocompatibility of CdS@tiopronin nanocrystals in hTERT-BJ1 human fibroblasts.

We used a single-step procedure to prepare CdS nanocrystals that were functionalized and protected with the non-natural amino acid tiopronin as tools for targeting specific cell sites. Tiopronin has been successfully used as a capping agent to protect gold and silver nanoparticles.[12,13] The thiol group of the tiopronin is directly attached to the CdS nanocrystals. Tiopronin acted as the stabilizer, controlling particle size and aggregation and, at the same time, provided water solubility and active groups for specific labelling.

The functionalized CdS nanocrystals were obtained by adding sodium sulfide to an aqueous solution of tiopronin and cadmium nitrate at room temperature by following a modification of the procedure of Spanhel et al.[14] The CdS@tiopronin QDs (Scheme 1) thus prepared gave a yellow solution and under ultraviolet illumination ($\lambda = 360$ nm) emitted light in the green region (550 nm).




**Scheme 1.** Preparation of CdS@tiopronin: a) $Cd(NO_3)_2 \cdot 4H_2O$, $Na_2S$, pH 10, $H_2O$.

The molecules were purified by precipitation with ethanol and characterized by $^1$H NMR, FTIR, UV-visible and fluorescence spectroscopy. After lyophilization, the CdS@tiopronin was water-soluble and stable for a year in the absence of light at 4 °C.

The UV-visible absorption and the fluorescence emission spectra for CdS@tiopronin are shown in Figure 1. The UV-visible spectrum of these QDs showed an excitonic transition with a band-gap energy ($E_g$) at 3.22 eV (385 nm). The emission spectra of the CdS@tiopronin particles presented a band at 540 nm when the excitation wavelength was 380 nm.

Biocompatibility studies of CdS@tiopronin QDs were undertaken by evaluating the cell viability of hTERT-BJ1 human fibroblasts by two different cell methods. Cell viability staining with calcein AM/ethidium homodimer[15] showed that cells exposed for 24 h to CdS@tiopronin QDs were more than 99% viable. Cell viability was also assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[16] This assay relies on the mitochondrial activity of fibroblasts and represents a parameter for their metabolic activity. The metabolic activity and proliferation of fibroblasts was thus measured after 24 hours' culture, and the values reached 80% compared to untreated controls (see Supporting Information).

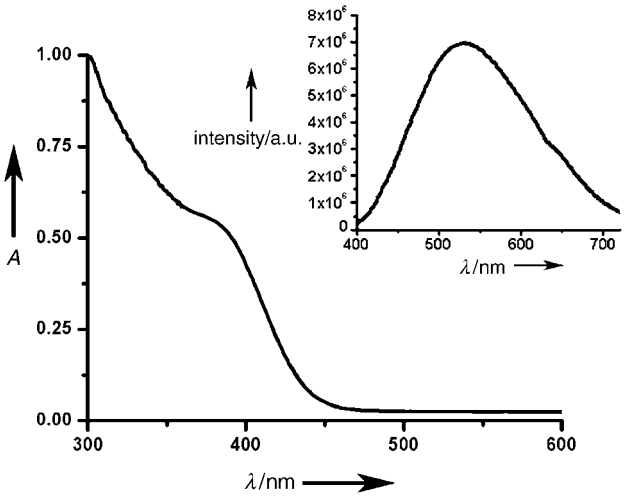


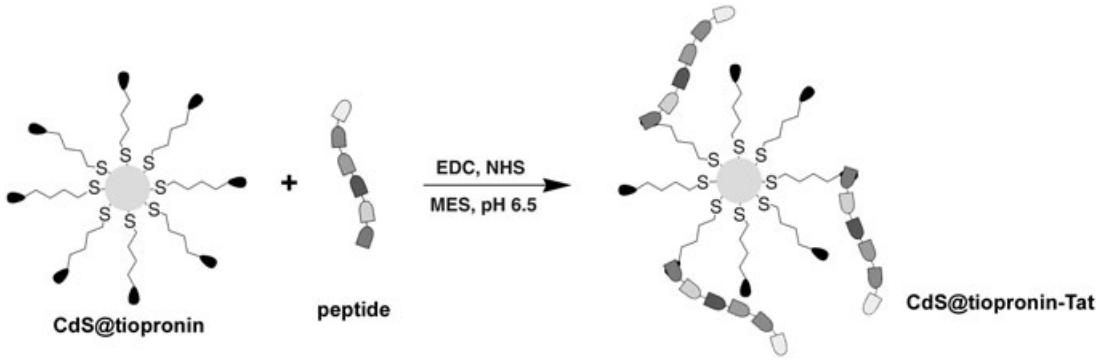

**Figure 1.** UV/Vis and fluorescence (inset; $\lambda_{exc} = 380$ nm) spectra of CdS@tiopronin in water.

To demonstrate the utility of CdS@tiopronin QDs in cell-biology studies, these QDs were functionalized with a Tat protein-derived peptide sequence (GRKKRRQRRR). As previously stated, the free carboxyl group of the tiopronin is available for covalent coupling to various biomolecules (such as proteins, peptides and nucleic acids) by cross-linking to reactive amine groups. In addition, this carboxylic layer is expected to reduce passive protein adsorption on QDs. We used this reactivity for the functionalization of the quantum dots with a Tat protein-derived peptide sequence. The reactions utilize the water-soluble carbodiimide *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC)[17] to catalyze reactions between CdS@tiopronin acid groups and Tat protein-derived peptide sequence amine groups. We included *N*-hydroxysulfosuccinimide in the reaction mixture to improve the efficiency of the carbodiimide-mediated amide-forming reaction by producing hydrolysis-resistant active ester reaction intermediates (Scheme 2).[18]

In this study, CdS@tiopronin QDs coupled to Tat protein-derived peptide sequences were used to achieve nuclear targeting of the nanoparticles in hTERT-BJ1 human fibroblasts. The CdS@tiopronin–Tat QDs were added to a cell suspension for 15 minutes. Excess QDs were removed by cell centrifugation, and the cells were cultured for 24 h. The general morphology of the fibroblasts incubated with CdS@tiopronin–Tat QDs, after cell fixation, is shown in Figure 2c. The figure shows that the cells were well spread, with no distinct change in morphology before (Figure 2a) or after incubation (Figure 2c). Fluorescence staining was observed around the cell nucleus; this shows the translocation of the CdS@tiopronin-Tat QDs to the nucleus. No fluorescence staining was observed when naked CdS@tiopronin QDs were incubated with the cells (Figure 2b).

In conclusion, we have prepared stable, water-soluble CdS nanocrystals functionalized with tiopronin by using a straightforward and economical methodology. The biocompatibility of these QDs has been demonstrated. The functionalization of




**Scheme 2.** Synthesis of CdS@tiopronin–Tat. NHS = *N*-hydroxysuccinimide (5 mM), EDC (2 mM), MES = 2-morpholinoethanesulfonic acid (pH 6.5).

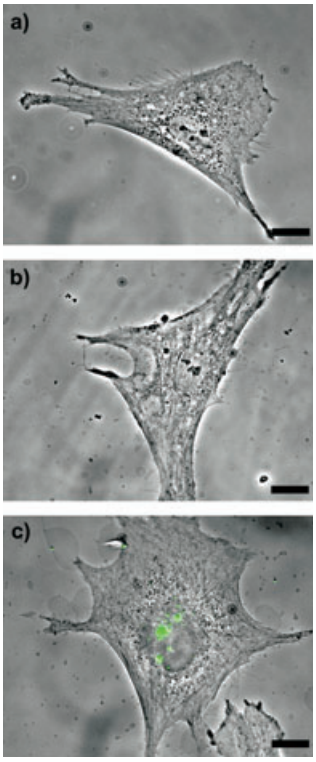



**Figure 2.** Overlay of the fluorescence (green) and phase-contrast images of: a) hTERT-BJ1 human fibroblasts (control experiment); b) hTERT-BJ1 human fibroblasts incubated with CdS@tiopronin; c) hTERT-BJ1 human fibroblasts incubated with CdS@tiopronin–Tat. Scale bars = 20 μm.

our QDs with a translocation peptide has allowed them to penetrate the cell membrane and target the nucleus. At the present time, different peptides and proteins are being conjugated to these quantum dots to improve staining methodologies for cell-biology studies.

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