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] The 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 sulphide 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 sulphide protected glyconanoparticles,[8] we have developed a simple production yielding gram-quantity of water-soluble and stable CdS nanocrystals, using the non-natural aminoacid tiopronin (*N*-2-mercaptopropionylglycine) as a capping agent (figure 1).[9] Tiopronin is a pharmaceutically important drug used for the treatment of cystinuria and rheumatoid arthritis.[10] Importantly, tiopronin has a free terminal $-CO_2H$ group that provides a handle for further reactivity. The chemical functionality of this capping agent provides a very high stability to the nanocrystals and has allowed us to functionalize the QDs with a HIV-1 Tat protein-derived peptide sequence. The UV-visible spectrum of these QDs showed an excitonic transition with band-gap energy 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.

The biocompatibility of these nanomaterials has been demonstrated by evaluating the cell viability of hTERT-BJ1 human fibroblasts by two different cell methods: calcein AM/ethidium homodimer and MTT assay. The metabolic activity and proliferation of fibroblasts was thus measured after 24 hours' culture, ant the values reached 80 % compared to untreated controls.

The functionalization of our QDs with the translocation peptide has allowed them to penetrate the cell membrane and target the nucleus (figure 2). 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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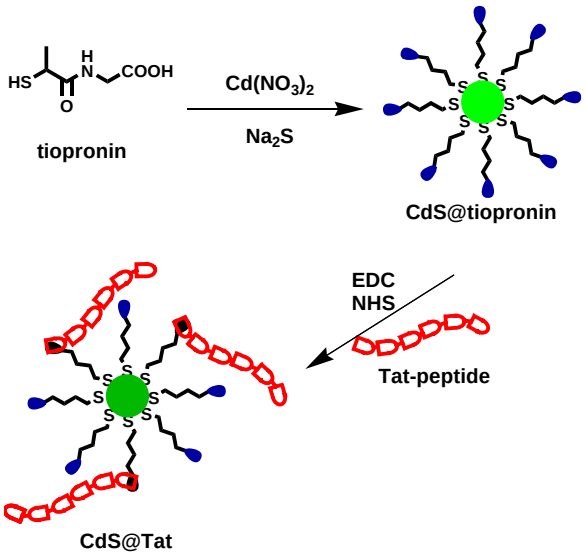

Figure 1. Preparation of CdS *quantum dots* functionalized with a Tat protein-derived peptide sequence.

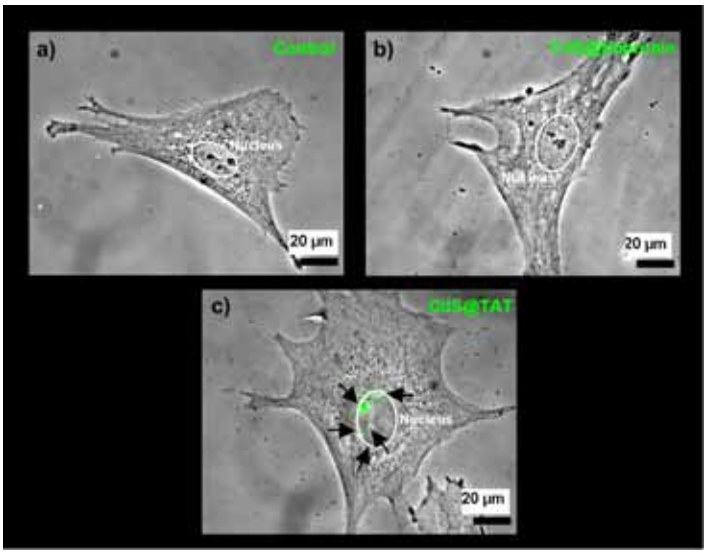

Figure 2. Overlay of the fluorescence (green, black arrows) and phase-contrast images of: a) hTERT-BJ1 human fibroblasts (control experiment); b) hTERT-BJ1 human fibroblasts incubated with CdS@tiopronin; c) hTERT-BJ1 human fibroblasts incubated with CdS@tiopronin-Tat. Scale bar –20 µm