Cellular internalization and targeting of semiconductor quantum dots†

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Peptide-mediated internalization and organelle targeting of quantum dots.

Semiconductor quantum dots (QDs) are increasingly gaining popularity as robust fluorescent probes for *in vivo* applications. Compared to organic fluorophores, semiconductor QDs exhibit size-tunable narrow emission, photostability and high luminance.[1,2] The quantum size confinement, where the physical size of the nanoparticle is smaller than the exciton Bohr radius, results in the unique optical, electronic and catalytic properties of nanoparticles. In contrast, organic fluorophores exhibit photobleaching and have broad emission spectra. The emission properties of QDs can be tuned by size and composition with a range of wavelengths from the blue to near infrared. However, issues regarding the cytotoxicity of nanoparticles have been raised.[2,3] Recent studies have suggested that the composition and the surface coatings of the nanoparticles might be responsible for their *in vivo* toxicity.[4] Nonetheless, QDs appear to be an attractive tool for *in vivo* applications such as in real-time imaging of cellular signaling pathways. Other nanoparticles such as carbon nanotubes and gold have also been suggested as probes for *in vivo* applications.[5]

One of the major hurdles in the use of nanoparticles for *in vivo* applications is the delivery and organelle-specific targeting of QDs. The entry of nanoparticles into the cell has been achieved by using a variety of approaches.[5] This includes the use of translocation peptides, liposomes, electroporation, or through endocytic uptake. In the case of translocation peptides, the peptides have to be conjugated to the surface of the QDs. Here we demonstrate the use of a non-covalent peptide carrier (Pep-1) to facilitate the transport of QDs conjugated with organelle-specific peptides to intra-cellular compartments. The Pep-1 peptide carrier has been shown to be extremely efficient in the targeting of proteins into cells independent of endocytosis.[6] In addition, Pep-1 does not need to be covalently coupled to the cargo that it ferries across the cell membrane, therefore making it an attractive tool for delivery of nanoparticles.

Using commercially available streptavidin conjugated QDs CdSe@ZnS QDs (Quantum Dot Corporation, CA, USA), we first investigated the ability of Pep-1 to facilitate the uptake of QDs into mammalian cells (Jurkat, HeLa and fibrobast cells). We chemically synthesized the 21-residue peptide carrier, Pep-1 (Lys-Glu-Thr-Trp-Trp-Glu-Thr-Trp-Trp-Thr-Glu-Trp-Ser-Gln-Pro-Lys-Lys-Lys-Arg-Lys-Val), consisting of the hydrophobic tryptophan-rich domain for efficient cell membrane translocation and the hydrophilic lysine-rich domain derived from the SV-40-T antigen nuclear localization sequence to facilitate solubility.[6] For the Pep-1 mediated delivery of QDs into cells (Fig. 1), Pep-1:QD complexes (**1**) were first formed in phosphate saline buffer (PBS) and overlaid onto cells (see Supplemental Information). After incubation for 30–60 min at 37 °C, the medium was replaced with fresh tissue culture medium and incubated for an additional 6–18 h. The cells were extensively washed with PBS and observed under the microscope (Fig. 2A). QDs that were complexed with the Pep-1 peptide were internalized into the cells. In contrast, cells treated with a similar concentration of QDs but without the Pep-1 peptide carrier showed a 3-fold decrease in the uptake of QDs. The internalization of QDs was verified by confocal microscopy and flow cytometry (Supplemental Information). Though the QDs appear to aggregate in the cytosol (Fig. 2B), the aggregation of QDs is not due to the streptavidin coating, since QDs lacking streptavidin were also observed to aggregate within cells (Supplemental Fig. S4). Cellular uptake of Pep-1:QDs was also observed at 4 °C, but was reduced when compared to uptake at 37 °C.

In order to target the QDs to cellular organelles, we adopted two approaches. In the first approach, we functionalized the surface of the streptavidin conjugated QDs with the nuclear localization sequence (NLS) of the simian virus 40 T-antigen. The NLS peptide (Biotin-Lys-Gly-Gly-Pro-Lys-Lys-Lys-Arg-Lys-Val-) was conjugated to the QDs *via* the biotin–streptavidin linkage (**2**). The NLS conjugated QDs were pre-incubated with Pep-1 peptide and then introduced to HeLa cells. As shown in Fig. 2C, we observed nuclear localization of the NLS-QDs in a significant number of cells (< 10%). The inefficient nuclear targeting of complex (**1**) could be explained by the fact that Pep1 is known to dissociate

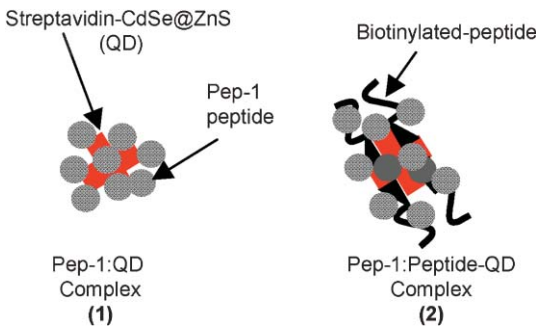

Fig. 1 Schematic of the QD complexes.

† Electronic supplementary information (ESI) available: experimental methods, confocal microscope images, mitochondrial localization, MTT assay. See http://www.rsc.org/suppdata/cc/b4/b418454h/

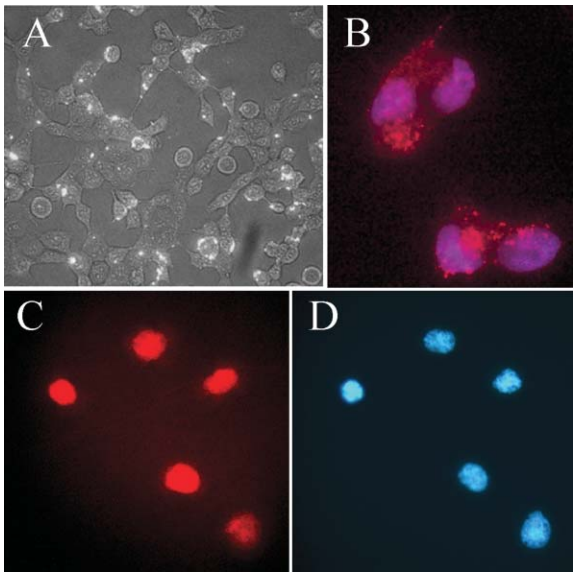

Fig. 2 Peptide-mediated uptake of QDs into mammalian cells. (A) Low magnification fluorescence/phase micrograph image of Jurkat cells loaded with QDs. (B) Distribution of the QDs in the cytoplasm of HeLa cells. The nucleus is stained with DAPI (4′,6-diamidino-2-phenylindole). (C) Localization of NLS-conjugated QDs to the nucleus. (D) DAPI staining showing the nuclei of the cells shown in (C).

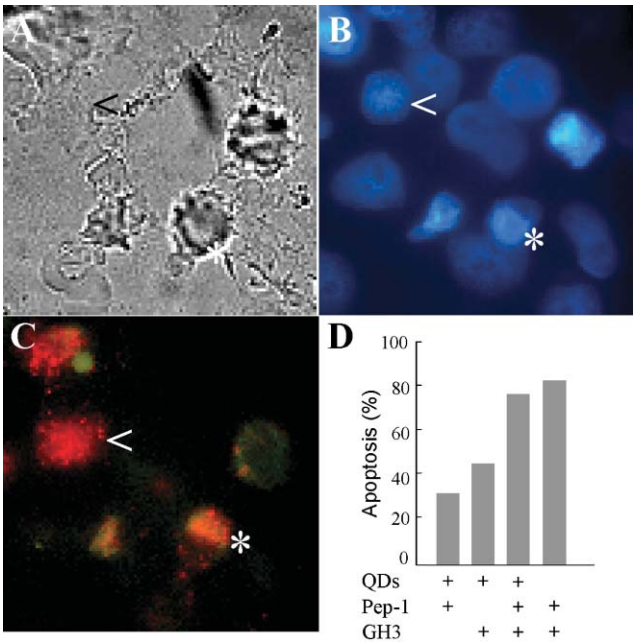

Fig. 3 Induction of membrane blebbing and nuclear condensation in cells that have taken up QDs functionalized with the GH3 pro-apoptotic peptide. (A) Light micrograph of HeLa cells. (B) Changes in nuclear morphology of DAPI stained cells. The nucleus of apoptotic cells undergoes condensation and fragmentation (asterisk). A cell exhibiting nuclear condensation but no membrane blebbing (arrow). (C) Fluorescence micrograph showing the localization of QDs functionalized with the pro-apoptotic peptide. (D) The percentage of cells with an apoptotic nucleus and membrane blebbing was determined with epifluorescence of DAPI-stained cells and phase contrast microscopy, respectively. The data represented in the graph is an average of two independent experiments.

from the cargo once it crosses the cell membrane.[6] Therefore, the conjugation of the NLS sequence to the surface of the QD is required for efficient nuclear targeting.

In the second approach, we used an apoptotic trigger known to induce mitochondrial-mediated cell death. The GH3 domain from the Grim protein has been shown to be the first proapoptotic domain responsible for mitochondrial-mediated cell death in mammalian cells.[7] The GH3 domain (-Lys-Ser-Glu-Phe-Gly-Cys-Trp-Asp-Leu-Leu-Ala-Gln-Ile-Phe-Cys-Tyr-Ala-Leu-Arg-Ile-Tyr-) was conjugated to the streptavidin conjugated QDs *via* a biotin linkage. The GH3-conjugated QDs were pre-incubated with Pep-1 and introduced into HeLa cells. A significantly large number of cells that had internalized the GH3-conjugated QDs after 18 h exhibited severe morphological changes such as membrane blebbing and nuclear condensation, known markers of apoptosis. Cells that become rounded, exhibit nuclear condensation (Fig. 3B) and also contain the GH3-conjugated QDs were considered apoptotic (Fig. 3C). The amount of cell death was determined by counting cells that exhibited all of these characteristics (membrane blebbing, nuclear condensation). Although cells that internalized GH3-conjugated QDs alone were also apoptotic, Pep-1 mediated uptake of GH3-conjugated QDs resulted in a significant increase in the number of cells undergoing apoptosis (Fig. 3D). Since QDs by themselves are also able to translocate across the cell membrane, apoptosis due to GH3-QD in the absence of the Pep1 carrier is not surprising. Nonetheless, apoptosis can be attributed to the GH3 domain because Pep-1 mediated uptake of the GH3 peptide alone also triggered significant cell death. Similar to other transfection protocols, a basal level of cell death was evident. The GH3 domain has been previously shown to be required for the targeting of the Grim protein to mitochondria.[7] We confirmed that the GH3-conjugated QDs had indeed been targeted to the mitochondria (Supplemental Fig. S7). Together, our results suggest that QDs conjugated with the GH3 domain were targeted to the mitochondria and able to cause cell death. The mechanism of cell death by the GH3 domain appears to be induced *via* a mitochondrial mediated pathway.

Based on the work presented here, we demonstrate that a peptide carrier (Pep-1) that is not covalently coupled to QDs can significantly facilitate cellular uptake of QDs. QDs functionalized with targeting sequences were shown to localize to intracellular organelles. The conjugation of an apoptotic triggering domain to the QDs was shown to be functionally active as demonstrated by the apoptotic activity of the QD functionalized complexes. This opens the door to using QDs to not only track the cellular localization of proteins but possibly to activate signaling events in cells. However, further studies are required in order to assess the cytotoxic effects of QDs. In particular, chemical composition and surface coatings are critical parameters that would have to be evaluated before QDs can be used for *in vivo* applications.

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