

Enhancement of Intracellular Delivery of CdTe Quantum Dots (QDs) to Living Cells by Tat Conjugation

F. L. Xue · J. Y. Chen · J. Guo · C. C. Wang ·
W. L. Yang · P. N. Wang · D. R. Lu

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Abstract Quantum dots (QDs), as novel fluorescence probes, have shown a great potential for bio-molecular labeling and cellular imaging. To stain cellular targets, the sufficient intracellular delivery of QDs is required. In this work the tat, a typical membrane-permeable carrier peptide, was conjugated with thiol-capped CdTe QDs to form CdTe Tat-QDs, and the intracellular deliveries of CdTe QDs or CdTe Tat-QDs were compared in human hepatocellular carcinoma (QGY) cells and human breast cancer (MCF7) cells *in vitro* by means of confocal laser scanning microscopy. Added into the cell dishes, both QDs and Tat-QDs adhered to the outer leaflet of the plasma membrane of cells within a few minutes, but the binding amount of Tat-QDs was obviously higher than that of QDs. Then both QDs and Tat-QDs can penetrate into cells, and their cellular contents increased with incubation time but both saturated after 3 hours incubation. However the cellular levels of Tat-QDs were higher than those of QDs, with the ratio of 2.1 (± 0.3) times in QGY

cells and 1.5 (± 0.2) times in MCF7 cells, demonstrating the enhancing effect of Tat conjugation on the intracellular delivery of QDs.

Keywords Quantum dots · Fluorescence probes · Fluorescence images · Cells

Introduction

With the development of cell and molecular biology, the fluorescence labeling has become an important tool to visualize the cellular structure, study the dynamic cellular processes and even track the path of single molecules in cells [1, 2]. The organic fluorophores, such as rhodamine and fluorescein isothiocyanate (FITC), are well established and widely used in cellular labeling experiments [3]. However these organic fluorophores have some obvious disadvantages such as narrow excitation band, broad emission spectrum and relative low fluorescence efficiency, especially the photo-instability which make them easier to be photobleached, resulting in a major limitation for cellular visualization particularly for long term imaging [4, 5]. Recently the semiconductor nanocrystals, called quantum dots (QDs), have demonstrated the encouraging characteristics as new type fluorescence probes [6–8]. Due to the quantum confinement effect, QDs have high luminescence efficiency with their emission peak tunable depending on their size. In addition, the excellent photostability of QDs makes them have the potential in cellular labeling and bioimaging, and a number of works have shown these characteristics in different kinds of biological systems [4, 5, 9–13]. However, when used as biological probes, QDs must be soluble in aqueous buffers. While most QDs with the core-shell structure, such as CdSe and CdS, are synthesized in organic solvents having the hydrophobic surfaces, so that their surface

J. Y. Chen (✉)
Department of Physics, Fudan University,
Shanghai, China
e-mail: jychen@fudan.edu.cn

J. Y. Chen · P. N. Wang
State Key Laboratory for Advanced Photonic Materials and
Devices, Fudan University,
Shanghai, China

F. L. Xue · D. R. Lu
Institute of Genetics, Fudan University,
Shanghai, China
drlu@fudan.edu.cn

J. Guo · C. C. Wang · W. L. Yang
Department of Macromolecular Science and Key Laboratory of
Molecular Engineering of Polymers, Fudan University,
Shanghai, China

ligands should be replaced by the biofunctional ligands such as mercaptoacetic acid to become hydrophilic [6]. In an alternative way, the QDs also can be synthesized directly in aqueous solutions with a hydrothermal route to have hydrophilic surfaces [14]. The hydrophilic surfaces of these QDs only meet the primary requirement for biological applications. To label subcellular targets, the intracellular delivery of QDs to living cells is the next challenge [15]. Though QDs could be engulfed into the living cells [16], the intracellular concentration of QDs is probably not so high. Our previous work showed that the photostability of cellular QDs is concentration-dependent, and became less photostable when the intracellular concentration of QDs was low [17]. To optimize the cellular labeling, the intracellular delivery of QDs should be enhanced certainly. Toward this goal, some efforts have made with the way of QD bio-conjugates such as biotin-QDs and peptide-QDs, and the progress on QD delivery has been achieved [4, 13, 15, 18, 19]. Nevertheless more work on QD bio-conjugation is needed to further improve the intracellular delivery of QDs and promote the QD biological applications.

Recently, several membrane-permeable carrier peptides have been successfully used to deliver exogenous proteins into living cells [20]. Tat of Human immunodeficiency virus (HIV)-1 is one of the most representative carrier peptides, which has been proved to effectively translocate proteins across cell membrane [21]. In the present work, the Tat (position 48–60), an arginine-rich peptide segment derived from the HIV-1, was selected as the bioconjugate of QDs. The enhancement of the Tat conjugation on intracellular delivery of QDs was studied in human hepatocellular carcinoma (QGY) and human breast carcinoma (MCF7) cell lines *in vitro*.

Materials and methods

Thiol capped CdTe quantum dots were made in our lab by the hydrothermal route, which is believed as a simple and efficient method [14]. Briefly, a typical reaction is as follows: by a molar ratio of 2:1, sodium borohydride was used to react with tellurium in water to prepare the sodium hydrogen telluride (NaHTe). Fresh solutions of NaHTe were diluted by N₂-saturated deionized water to 0.0467 M for a further use. CdCl₂ (1 mmol) and thioglycolic acid (1.2 mmol) were dissolved in 50 mL of deionized water. Stepwise addition of NaOH solution adjusted the precursor solution to pH = 9. Then, 0.096 mL of oxygen-free solution containing fresh NaHTe, cooled to 0°C, was added into 10 mL of the precursor solution and vigorously stirred. Finally, the solution with faint yellow color was put into a Teflon-lined stainless steel autoclave with a volume of 15 mL. The autoclave was maintained at the reaction temperature for a certain time and then

cooled to the room temperature by hydro-cooling process. The details of the procedure can be found in our previous work [22].

Tat (N¹-RKKRRQRRR-C¹), derived from the HIV-1, was purchased from Shanghai PeProSyn Co.,Ltd.. Tat conjugation with QDs was performed by the EDC (1-Ethyl-3-(3-dimethylamino-propyl)-carbodiimide, hydrochloride) coupling reaction. The reaction was hold in 20 mM borate buffer, pH 6.0. The QDs, Tat and EDC in molar ratio of 1:20:20 were mixed in the buffer and reacted for 4 h at room temperature. After reaction the surface of QDs was conjugated with Tat forming Tat-QDs. Because of the Tat capping, the Tat-QDs are bigger than un-conjugated QDs. Tat-QDs and un-conjugated QDs can be separated with ultrafiltration, and the cut off 100 Kd ultrafiltration film (MILLIPORE) was found to be the appropriate film to remain the Tat-QDs and filter out the un-conjugated QDs. After several times ultrafiltration, the obtained Tat-QDs were dispersed in PBS (pH7.2) and stored at 4°C. The luminescence spectra of Tat-QDs and un-conjugated QDs were measured in a Hitachi F-2500 fluorescence spectrophotometer in the same condition. The results show (Fig. 1) that their spectra are very similar with the same luminescence efficiency and spectral width. However a red shift of the luminescence peak (635 nm) of Tat-QDs was observed as compared with that (625 nm) of un-conjugated QDs. Such a small spectral shift of the luminescence peak seems the characteristic of the ligand absorption on the QD surface. It has been reported recently that the decylamine conjugation to CdSe QDs as well as the addition of mercaptan to the amine-capped CdSe surfaces all caused a luminescence spectral red-shift of about 4 nm [23], due to the electronic effects of the ligand [23].

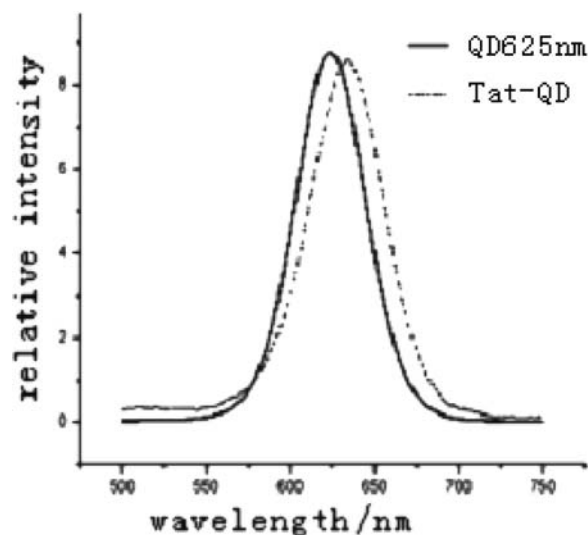


Fig. 1 The luminescence spectra of QDs and Tat-QD in PBS (pH 7.2) solution. The concentrations of both QDs and Tat-QD are 20 μ g/ml. Excitation: 400 nm

The QGY cells (Human Hepatocellular Carcinoma Cell Line 7701) and MCF7 cells (human breast cancer cell line) were obtained from the cell bank of Shanghai Science Academe. The cells were seeded in culture dishes containing RPMI1640 medium with 10% calf serum, 100 units/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin, and 100 $\mu\text{g/ml}$ neomycin and incubated in a fully humidified incubator at 37°C with 5% CO₂.

When cells reached 80% confluence with normal morphology, the QDs or Tat-QDs were added to the cell dishes with the concentration of 50–100 $\mu\text{g/ml}$ respectively. These cell dishes were put in an incubator for a desirable incubation time. Under such incubation concentrations of QDs or Tat-QDs, no detectable damage to cells was observed. After incubation, the QD loaded cells were washed with PBS three times to remove unbound QDs or Tat-QDs, and then these cell samples were ready for luminescence imaging measurements. The luminescence images of cellular QDs were acquired in an Olympus confocal laser scanning microscope (FV-30, IX71 microscope) with a water immersion objective (60 \times), excited by a 488 nm laser beam. The luminescence image was recorded in a fluorescence channel with a 590–640 nm band-pass filter and a sensitive PMT, and the corresponding differential interference contrast (DIC) image was detected simultaneously in a transmission channel to show the cell morphology. A matched pinhole in the system was used in measurements to ensure that the image obtained on x - y dimensions has a resolution of about 0.4 μm in z direction. Using the z -scan function of the machine, the images in different layers along the z direction can be recorded to see the luminescence distribution in cells in three dimensions. From these images, the intracellular distribution of QDs and the relative luminescence intensities in cells were obtained. The luminescence intensity is proportional to the cellular concentration of QDs. By measuring the luminescence intensities in different cell groups that have been incubated with QDs or Tat-QDs respectively, the ratio of cellular QD amounts between the Tat-QD incubated and QD incubated cells was determined statistically.

To study the fast course of cellular binding, the QDs or Tat-QDs were added to the cell dishes, respectively, where the cells adhered on the bottom of the dishes. These dishes were immediately put in the microscope to measure a series of luminescence images with the way of acquiring one image in every 5 s for 2 min. Thus the binding course of QDs to plasma membrane of cells could be observed.

Results and discussion

Delivery of QDs to living cells consists of two phases: QDs are initially bound to plasma membrane of cells, and then are

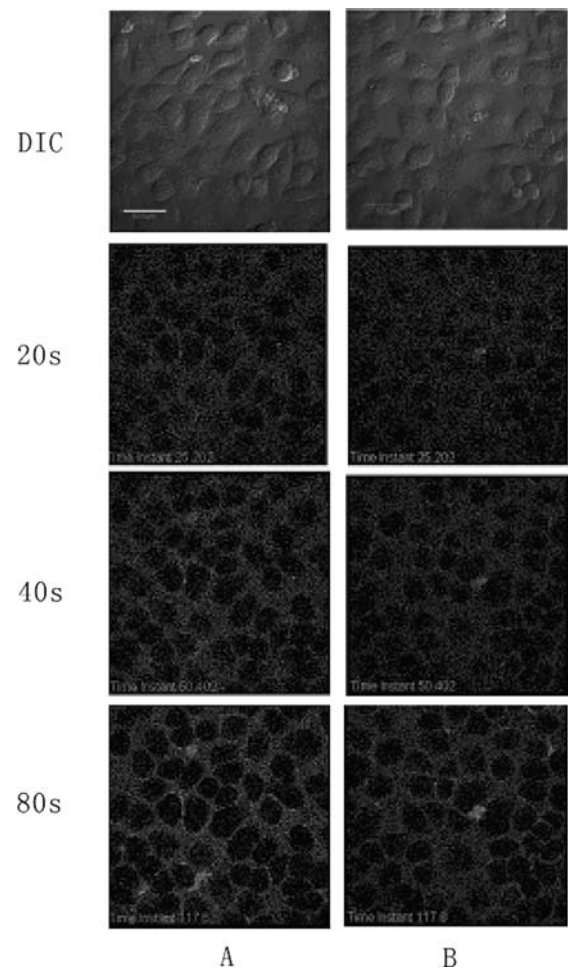


Fig. 2 The luminescence image series of QD gathering to QGY cells and adhering on the outer leaflet of the plasma membrane after the Tat-QDs or QDs have been added to cell dishes. Column A: Tat-QDs; Column B: QDs. Excitation: 488 nm. The bar is 50 μm

gradually internalized by cells. Figure 2 shows the typical luminescence images of the QD fast binding course in QGY cells. The Tat-QDs and QDs seem to have the affinity for the plasma membrane of cells. Once being added in the cell dish, they gathered around the cell quickly and adhered on the outer leaflet of the plasma membrane within about one minute. As expected, the amount of the membrane adhered Tat-QDs is higher than that of the adhered QDs as seen in Fig. 2, demonstrating that the Tat conjugation enhanced the membrane-bound ability of Tat-QDs providing a favourable condition for the cellular internalization. Tat, on the surface of Tat-QDs, is a basic peptide with arginine-rich segment bearing cationic charges. Some surface areas of the plasma membrane are anionic. The enhancement of the membrane-bound Tat-QDs may be explained by the interaction of the cationic part of Tat with the anionic membrane. In addition, the Tat is the bio-material, therefore the bio-compatibility of

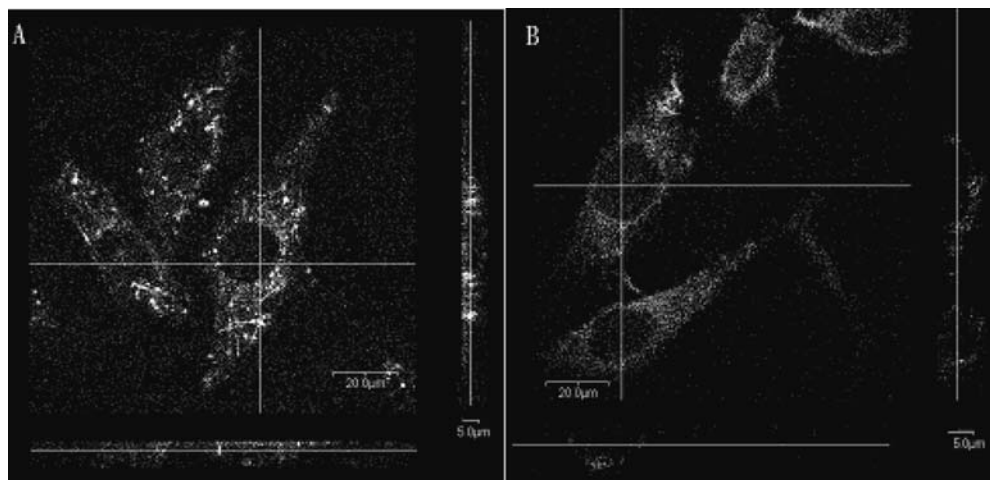


Fig. 3 Three-dimensional confocal projection of Tat-QDs (A) and QDs (B) in QGY cells. Cells have been incubated with Tat-QDs or QDs of 100 $\mu\text{g/ml}$ for 4 h, respectively. A series of the luminescence

images in different layers of cells obtained from the *z*-scanning were combined to show the *x-z* and *y-z* profiles. Excitation: 488 nm

Tat-QDs to the plasma membrane should be better than that of QDs.

After being incubated with Tat-QDs or QDs for 4 h, respectively, the cellular internalization happened and the cellular Tat-QDs or QDs were seen. Figure 3a and b demonstrate the intracellular distribution of Tat-QDs or QDs in QGY cells in three dimensions, respectively, where the main images show the cellular Tat-QDs or QDs in *x-y* plane and the right profiles exhibit the distributions of cellular Tat-QDs or QDs in *y-z* dimensions along the marked line parallel to the *y* axis (see main images) while the bottom profiles show the distributions in *x-z* dimensions along the marked line (parallel to *x* axis) in main images. These results indicate that the cells can internalize the Tat-QDs or QDs. The Tat-QDs diffusely localize in the cytoplasm with a number of patches particularly in the perinuclear region but not in the nucleus. The QDs have the similar pattern of the cellular localization with the relative low concentration in contrast with Tat-QDs, confirming that the Tat conjugation can enhance the cellular internalization of Tat-QDs.

The courses of the uptake of Tat-QDs and QDs by cells were studied in the cell samples incubated with Tat-QDs or QDs for different time intervals from 0.5 to 4.5 h. The luminescence images of these treated cells were acquired subsequently. Figure 4 presents a typical slow course of the cellular QD accumulation in QGY cells. The amount of cellular quantum dots increases with incubation time in both cases of Tat-QDs and QDs. Comparing Tat-QD treated cells with un-conjugated QD treated cells; the delivery enhancement of Tat-QDs can be clearly seen. Thirty minutes incubation of Tat-QDs made the cellular Tat-QDs visualized, while it required ninety minutes incubation in the case of QDs. After 3 h incubation, the amount of cellular Tat-QDs or QDs

almost saturated. By measuring the luminescence intensities on confocal images for about one hundred cells, the average intensity ratio between the group of Tat-QDs and that of QDs was determined to be $2.1 (\pm 0.3)$, demonstrating an enhancement of Tat on intracellular delivery of QDs in QGY cells.

Similar to the QGY cells, the delivery enhancement of Tat-QDs was also seen in MCF7 cells. However the enhancing ratio was found to be $1.5 (\pm 0.2)$, slightly lower than that in QGY cells, reflecting that the enhancing effect of the Tat conjugation on cellular QD delivery is cell line dependent.

For un-conjugated QDs, endocytosis was suggested as the pathway of the intracellular delivery [5], while for Tat-QDs the mechanism of the delivery enhancement is not clear at the moment. Though Tat is known to possess the strong ability to effectively translocate exogenous proteins across cell membrane and has been widely used in such kind experiments [21, 23], the mechanism has been debated for a long time. A possible explanation is that the peptides may increase the membrane-permeation, but the hypothesis of enhanced endocytosis for these carrier peptides was just denied in recent experiments [24, 25]. The real mechanism is still under investigation [26].

Due to special luminescence advantages, QDs have a great potential for biological labeling and cellular imaging. However no matter the targets are the cellular organelles or biomolecules, QDs must enter the cells first. The intracellular delivery of QDs is one of main problems encountered. Since the common water-soluble QDs are not well biocompatible, their membrane-permeation is low. To effectively stain cellular targets, the enhancement of intracellular delivery is of importance. Here, the Tat conjugation provides a feasible way to enhance the QD intracellular delivery.

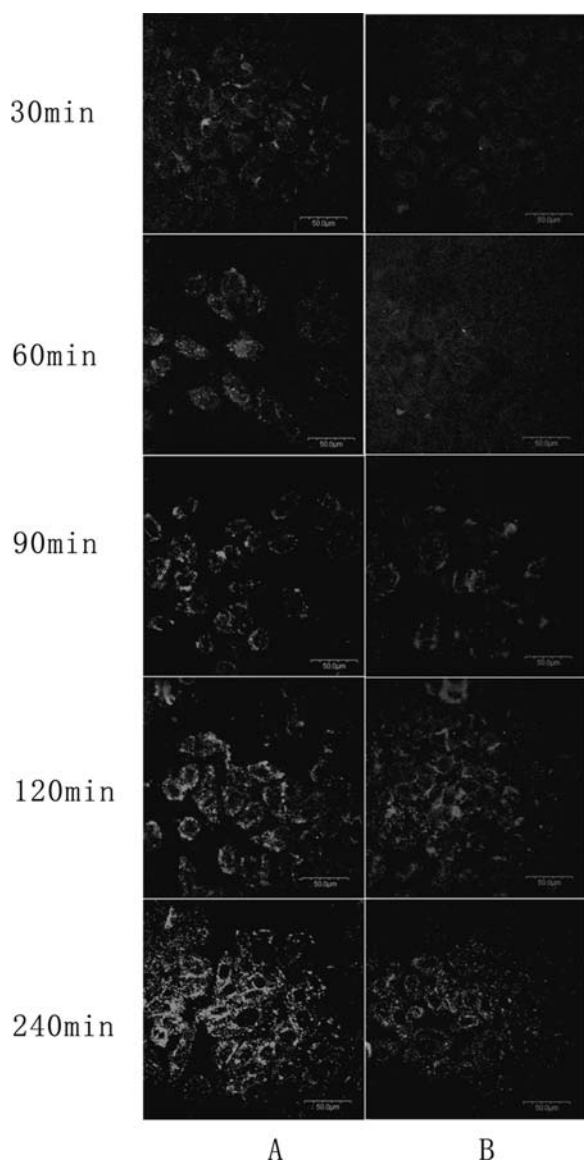


Fig. 4 The accumulation of cellular Tat-QDs (column A) and QDs (column B) in QGY cells. The cells have been incubated with 50 $\mu\text{g/ml}$ Tat-QDs or QDs for 30 min, 60 min, 90 min, 120 min, 240 min, respectively. The bars in images represent 50 μm

Conclusion

With the Tat conjugation, the enhancement of QD intracellular delivery was found in both QGY cells and MCF7 cells, suggesting that Tat may be useful to improve uptake of QDs by cells.

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