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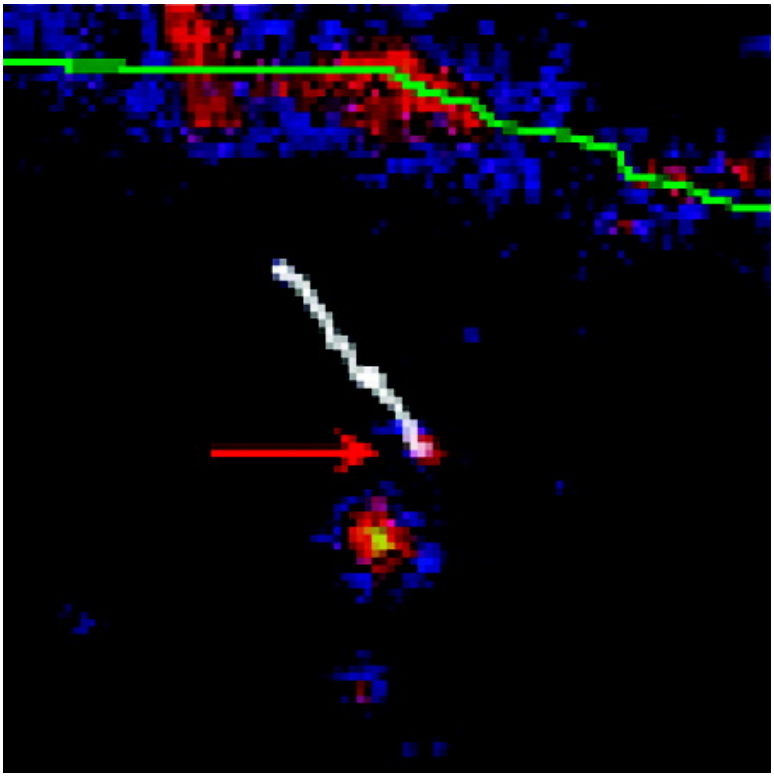

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# Imaging and Tracking of Tat Peptide-Conjugated Quantum Dots in Living Cells: New Insights into Nanoparticle Uptake, Intracellular Transport, and Vesicle Shedding

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***Abstract:*** We report the use of Tat peptide-conjugated quantum dots (Tat-QDs) to examine the complex behavior of nanoparticle probes in live cells, a topic that is of considerable current interest in developing advanced nanoparticle agents for molecular and cellular imaging. Dynamic confocal imaging studies indicate that the peptide-conjugated QDs are internalized by macropinocytosis, a fluid-phase endocytosis process triggered by Tat-QD binding to negatively charged cell membranes. The internalized Tat-QDs are tethered to the inner vesicle surfaces and are trapped in cytoplasmic organelles. The QD loaded vesicles are found to be actively transported by molecular machines (such as dyneins) along microtubule tracks. The destination of this active transport is an asymmetric perinuclear region (outside the cell nucleus) known as the microtubule organizing center (MTOC). We also find that Tat-QDs strongly bind to cellular membrane structures such as filopodia and that large QD-containing vesicles are released from the tips of filopodia by vesicle shedding. These results provide new insights into the mechanisms of Tat peptide-mediated delivery as well as toward the design of functionalized nanoparticles for molecular imaging and targeted therapy.

## Introduction

Bioconjugated semiconductor quantum dots (QDs) are a new class of fluorescent probes under intense research and development for broad applications in molecular, cellular, and in vivo imaging.[1−6] The basic rationale is that these nanometer-sized particles have unique functional and structural properties, such as size and composition tunable fluorescence emission, large absorption cross sections, and exceptional brightness and photostability compared with organic dyes and fluorescent proteins. Recent research has achieved considerable success in using QDs for labeling fixed cells and tissue specimens and for imaging cell membrane proteins.[7−11] However, only limited progress has been made in developing QD probes for molecular imaging inside living cells.[12−16] A major problem is the lack of efficient methods for delivering monodispersed (that is, single) QDs into the cytoplasm of living cells. A common observation is that QDs tend to aggregate inside living cells and are often trapped in organelles such as vesicles, endosomes, and lysosomes. As a result, little is known about the interactions of QDs with intracellular proteins and their transport behavior inside living cells.

We have used Tat peptide-conjugated QDs (Tat-QDs) as a model system to examine the cellular uptake and intracellular transport of nanoparticles in live cells. Previous work has used cell-penetrating peptides such as polyarginine and Tat to deliver QDs into living cells,[17,18] but the delivery mechanism and the

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behavior of intracellular QDs are still a matter of debate. Derived from the HIV-1 Tat protein, the Tat peptide has emerged as a novel cellular delivery vector for a wide range of cargos, due to its excellent delivery efficiency and minimal cytotoxicity.[19−24] Considerable effort has been devoted to understanding the delivery mechanism of this cationic carrier. The delivery process was initially thought to be independent of endocytosis because of its apparent independence on temperature.[20−24] However, later research showed that the earlier work failed to exclude the Tat peptide conjugated cargos bound to plasma membranes, which was largely an artifact caused by cell fixation. More recent studies based on improved experimental methods indicate that Tat peptide-mediated delivery occurs via macropinocytosis,[25] a fluid-phase endocytosis process that is initiated by Tat-QD binding to the cell surface.[26] These new results, however, did not shed any light on the downstream events or the intracellular behaviors of the internalized cargos. This kind of detailed and mechanistic investigations would be possible with QDs, which are sufficiently bright and photostable for extended imaging and tracking of intracellular events. In addition, most previous studies on Tat peptide-mediated delivery are based on the use of small dye molecules and proteins as cargos,[20−24] so it is not clear whether larger nanoparticles would undergo the same processes of cellular uptake and transport. This understanding is needed for the design and development of imaging and therapeutic nanoparticles for biology and medicine.

In this work, we have used a spinning-disk confocal microscope for dynamic fluorescence imaging of quantum dots in living cells at 10 frames per second. The results indicate that the peptide-conjugated QDs are internalized by macropinocytosis, in agreement with the recent work of Dowdy and coworkers.[26] It is interesting however that the internalized Tat-QDs are tethered to the inner surface of vesicles and are trapped in intracellular organelles. An important finding is that the QD loaded vesicles are actively transported by molecular machines (such as dyneins) along microtubule tracks to an asymmetric perinuclear region called the microtubule organizing center (MTOC).[27] Furthermore, we find that Tat-QDs strongly bind to cellular membrane structures such as filopodia and that large QD-containing vesicles are able to pinch off from the tips of filopodia. These results not only provide new insights into the mechanisms of Tat peptide-mediated delivery but also are important to the development of nanoparticle probes for intracellular targeting and imaging.

## Experimental Section

**Preparation of QD-Peptide Conjugates.** The sequence of Tat peptide (Invitrogen, Carlsbad, CA) is RRRQRRKKRGY. Tat-QDs were prepared by incubating streptavidin-coated QDs (emission peak wavelength = 655 nm, Invitrogen, Carlsbad, CA) with Tat-biotin at a ratio of 1 to 20 (that is, 20 Tat peptides are added to conjugate with 1 QD) for 1 h at room temperature. The conjugation was conducted in cell culture medium at the working concentration. Since each QD has approximately 10 streptavidin molecules on its surface, and each streptavidin has 2 binding sites available for biotin, we estimated that an average of 20 Tat molecules were conjugated to each QD.

**Live Cell Imaging.** HeLa cells (ATCC, Manassas, VA) were maintained with Eagle's minimum essential medium supplemented with 10% fetal bovine serum in a humid incubator (37 °C and 5% $CO_2$). The cells were plated onto multiple glass-bottom tissue culture plates (MatTek, Ashland, MA) at an initial confluency of 20%. After 40 h, Tat-QDs (1 nM unless specified) were added. The cells were washed with fresh culture medium after being incubated with Tat-QDs for 1 h. At each given time point, one plate of cells was examined with a spinning disk confocal microscope (Ultraview, Perkin-Elmer, Wellesley, MA) equipped with $CO_2$ and temperature control for imaging (Hamamatsu ORCA ER CCD camera, 488 nm laser excitation, 63X Plan Apo/NA = 1.3 or 100X Plan Apo/NA = 1.4 objective). Because laser illumination could cause redistribution of cell-penetrating peptides from endosomes to the cytoplasm,[28] different plates were used at different time points to minimize the interference from laser and lamp illumination; this also reduced changes caused by incubation on the microscope stage under suboptimal culture conditions. Multiple views were randomly selected for both Z-stack confocal and T-series imaging at a data acquisition rate of 10 frames per second. To label cell lipid membranes and nuclei, two green fluorescent dyes DiO (1 $\mu$g/mL) and Syto 16 (100 nM) ((Invitrogen, Carlsbad, CA) were used, respectively, by incubation with live cells for 15 min. Data were collected and transferred as a series of raw or tiff files for further analysis. Images were processed using the NIH ImageJ software. Locating and tracking QDs were conducted manually or by using the software from Harvard University (download website: http://www.rowland.harvard.edu/labs/bacteria/index_software.html).

**Uptake Inhibition Studies.** To study the effect of temperature on nanoparticle uptake, Tat-QD conjugates were prepared by mixing 5 nM Tat peptides and 0.4 nM QDs. The Tat-QDs were subsequently added to cells that were kept at either 4 °C or 37 °C for 1 h prior to confocal imaging. To study the uptake inhibition effect of cytoskeleton-disrupting drugs, Tat-QDs were prepared by mixing 25 nM Tat peptides and 1 nM QDs; then, cytochalasin D (5 $\mu$M) was used to disrupt actin filaments, and nocodazole (15 $\mu$M) was used to disrupt microtubules by treating the cells for 30 min. Tat-QDs were added to the drug-treated cells (no washing), and confocal images were taken 2 h afterward.

## Results

**Intracellular Uptake and Active Transport of Tat-QD Conjugates.** Figure 1 shows time-dependent fluorescence images of cultured HeLa cells when incubated with Tat-QD nanoparticles for a 24-h period. Within 5 min of Tat-QD addition, the cell outer surfaces (including filopodia and plasma membranes) were visibly stained. At 30 min, Tat-QDs started to appear inside the cells but remained close to the cell peripheries. At 1 h of incubation, the filopodial bridges (membrane channels between adjacent cells) were also labeled (highlighted by a box), and a small number of Tat-QDs were accumulated at a unique intracellular region (highlighted by a circle). Although they are not a focus in this study, filopodial bridges are involved in retrovirus travel from infected cells to healthy ones.[29,30] For the internalized QDs, a striking feature was a gap region between the cell peripheries and their

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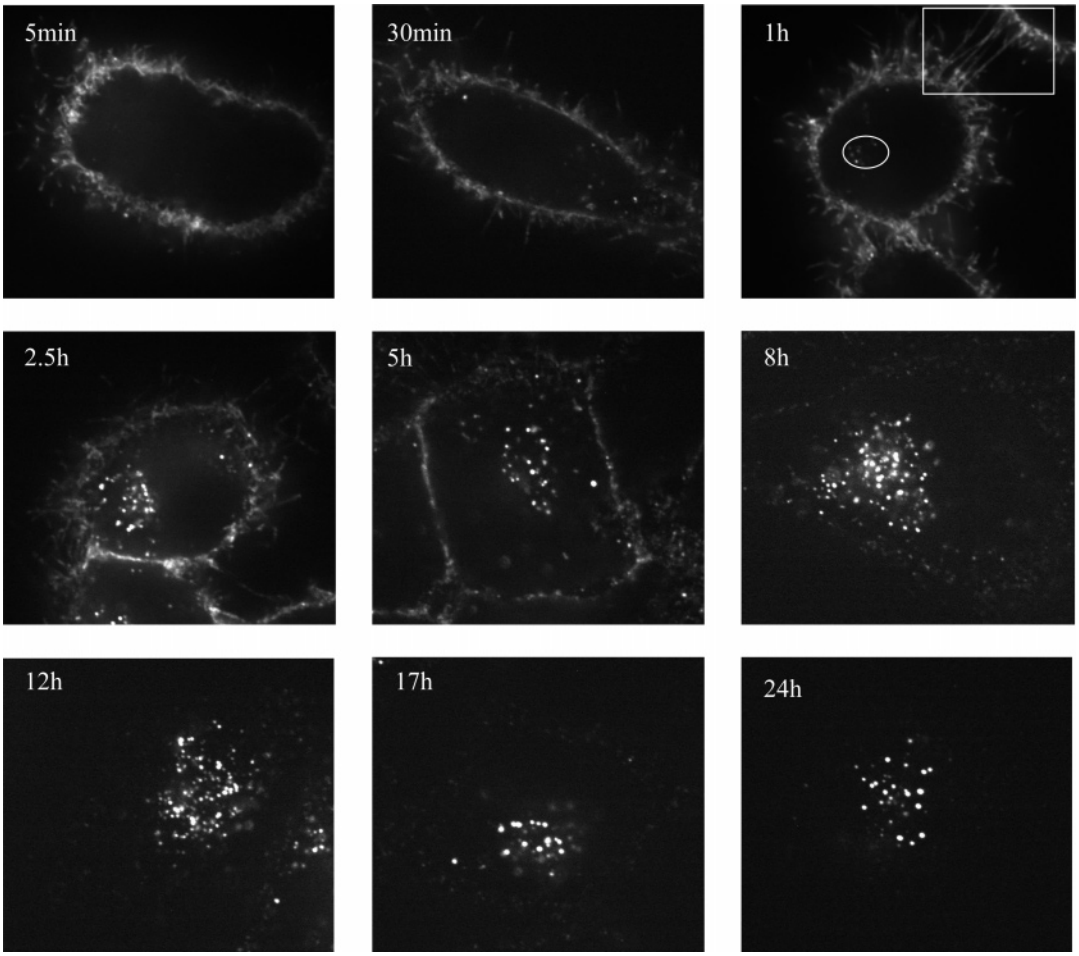


***Figure 1.*** Time-dependent imaging of Tat-QD uptake and intracellular transport in cultured HeLa cells over a 24-hour period. New cell culture plates were used for each time point, and multiple views were randomly acquired by using a spinning-disk confocal microscope at a rate of 10 frames per second. These changes were found to minimize the effects of lamp and laser illumination on cultured cells. In the image taken at 1 h, QD-stained filopodial bridges are highlighted with a rectangular box, and the microtubule organizing center (MTOC) is indicated with a circle.

accumulation site where there were no or few Tat-QDs detected. This indicates that the intracellular transport of Tat-QDs is mediated by an active process because passive diffusion would lead to a random distribution of Tat-QDs in the cytoplasm. From 1 to 24 h, more and more Tat-QDs were transported and accumulated at this intracellular region, with fewer and fewer Tat-QDs on the cell surface. By 24 h, essentially all the Tat-QDs had accumulated at the intracellular region. There were minimal adverse effects on cell functions, which remained normal for at least 3 days. This intracellular region corresponds to the microtubule organizing center (MTOC), a distinct region that is responsible for attaching and organizing the microtubules in living cells.[27] To confirm that the MTOC is indeed the site for QD accumulation, we have used GFP-encoded MCF-7 cells (stably expressing GFP-tubulin) and have found that QDs and tubulins are spatially colocalized in a perinuclear region (just outside the cell nucleus).

To determine the key factors involved in Tat-mediated QD delivery, we have carried out a series of uptake inhibition studies. First, lowering the temperature to 4 °C blocked the delivery of Tat-QDs, suggesting that it is an energy-dependent process (Figure 2a,b). Second, the use of cytoskeleton-disrupting drugs (small molecules that are commonly used to disrupt actin filaments or microtubules) led to the blocking of Tat-QD delivery, suggesting that cytoskeletons are likely involved in Tat-QD delivery (Figure 2c,d). These results are consistent with the previous finding that actin filaments are required for Tat peptide internalization via macropinocytosis.[26] Dynamic fluorescence imaging further reveals that most of the endocytosed Tat-QDs are actively transported to an intracellular region just outside the nucleus (Figure 3a,b) (also see Supporting Information, Supplementary Video 1), while directed motion also occurs along cell peripheral tracks (Figure 3c,d) (also see Supporting Information, Supplementary Video 2). The motion of Tat-QDs from the cell periphery toward the perinuclear region is most likely caused by microtubule-dependent transport, an active process that is mediated by molecular motors such as dyneins.[31−33] The destination of this transport is the microtubule organization center (MTOC). In addition to inward motion, we also observed directed motion from the perinuclear region to the cell periphery, a process that is probably mediated by different molecular motors such as kinesins.[34] The fact that eventually Tat-QDs were all accumulated at the perinuclear region suggests that the inward motion is dominant. The average speed of the trajectories shown in Figure 3A was determined to be 1.0 $\mu$m/s, similar to the value

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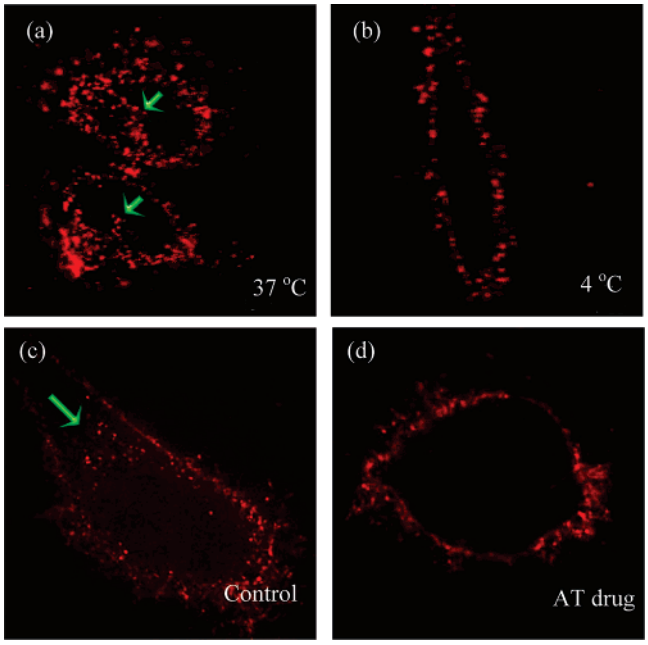


***Figure 2.*** Inhibition of Tat-QD uptake and transport by temperature and cytoskeleton-disrupting drugs. (a, b) Fluorescence images of Tat-QDs incubated with HeLa cells at 37 and 4 °C. Green arrows denote internalized QDs, also visualized with Z-stack scanning. (c, d) Fluorescence images of Tat-QDs incubated in the absence (control) and presence of the actin (AT)-disrupting drug cytochalasin D. Similar data were obtained with the microtubule-disruption drug nocodazole (data not shown). HeLa cells were treated with the drug (5 $\mu$M) for 30 min in Eagle's minimum essential medium plus 10% fetal bovine serum at 37 °C and were then incubated with Tat-QDs (1 nM QDs) for 1 h. The confocal imaging parameters were the same as those for Figure 1.

reported for dynein motors under *in vitro* conditions.[35,36] The second class of directed motion (that is, along cell peripheries) probably arises from microtubules that lie along the cell peripheries, but it is also possible that myosin motors carry Tat-QDs along actin filaments. Actin-dependent active transport of Tat-QDs could correspond to an intermediate stage in the endocytic pathway of cells, as reported recently by Zhuang and co-workers.[37] These researchers have used the infection pathway of influenza virus as a model to study the cellular endocytic pathway and found that the pathway involves a three-stage active transport process, with the first stage being transport along cell peripheries (on actin filaments), the second stage being transport from cell peripheries to the perinuclear region, and the third stage being intermittent motion in the perinuclear region including both plus- and minus-end-directed microbule-based motility.

Several lines of evidence indicate that nearly all of the endocytosed Tat-QDs were transported inside vesicles without being released into the cytoplasm. First, the brightness of nearly all the Tat-QDs was considerably higher than that of single QDs. Second, there was a gap region between the cell periphery and the perinuclear region in which few QDs were observed (see Figure 1). If QDs were released from vesicles, they would diffuse randomly in the cytoplasm in which the vesicles would be too large to diffuse effectively in the crowded cytoplasmic environment. We estimate that the time for a single QD of hydrodynamic radius 10 nm to diffuse across a 20-$\mu$m-diameter cell is ca. 30 s, whereas the time for a vesicle of hydrodynamic size 80 nm to diffuse across the same distance is 5 h or longer. This dependence of diffusion rates on particle size is explained by the fact that cytoskeletons in cytoplasm cause more hindrance to the diffusion of larger particles than that to the diffusion of smaller particles.[38,39] Third, the Tat-QDs at the perinuclear region were mostly located at one side of the nucleus (the MTOC) and stayed at this location for at least 24 h with little dispersing or motion. This indicates that the QDs are encapsulated in vesicles and are not free to diffuse (see Supporting Information, Supplementary Video 3). Fourth, Tat-QDs were colocalized with DiO, a membrane dye that is commonly used to stain intracellular vesicles and plasma membranes (Figure 4a−c), supporting the conclusion that most Tat-QDs are trapped inside intracellular organelles. Nuclear staining studies further demonstrate that the internalized Tat-QDs do not enter the cell nucleus (Figure 4d−f), because the Tat-QDs are trapped in large vesicles or are unable to interact with the protein importin $\beta$ for nuclear transport.[40]

**Tat-QD Distribution and Vesicle Shedding.** Another novel finding is that the internalized Tat-QDs are observed as a circular structure inside large intracellular vesicles (ca. 2 $\mu$m in diameter) (Figure 5a−c). This ring-shaped fluorescence pattern indicates that the Tat-QDs are not randomly distributed inside the vesicle but are bound to the inner vesicle surfaces. This "tethering" effect likely arises from electrostatic interactions between the positively charged Tat peptides and the negatively charged vesicle membranes. This distribution is clearly observed in larger vesicles but is less clear in smaller vesicles due to image blurring. We have also captured the movement of Tat-QDs on the vesicle membrane, indicating the tethered Tat-QDs can laterally diffuse in the lipid bilayers (see Supporting Information, Supplementary Video 4). It is important to note that the zeta potential of Tat-QDs is only +0.5 mV at pH = 7, because the positive charge of the Tat peptide is neutralized by negatively charged streptavidin molecules (isoelectric pH 5.5) and residual carboxylic acid groups on the QD surface. It is possible that the Tat peptide molecules are not uniformly distributed on the QD surface, but there are clusters of strongly positive charges for cellular binding and uptake. Recent work by Duan er al.[13] has also shown that the surface coating layer plays a key role in QD cellular uptake as well as in endosomal release and cytoskeleton association.

Outside the cells, large vesicles containing Tat-QDs were attached to filopodia or were observed as free structures in the culture medium (Figure 5d−f) (also see Supporting Information, Supplementary Video 5). In contrast to endocytosed Tat-QDs trapped in intracellular vesicles, these filopodia-generated vesicles are formed by Tat-QD binding to the outer membrane surface. Vesicle shedding or pinching off from filopodia leads to free vesicles with Tat-QDs bound on the outside (see illustration in Figure 5f). Vesicle shedding of Tat-QDs occurs in less than 1 h after the addition of Tat-QDs, producing QD-containing vesicles in the cell culture medium and at the end of filopodia as shown in Figure 5d.

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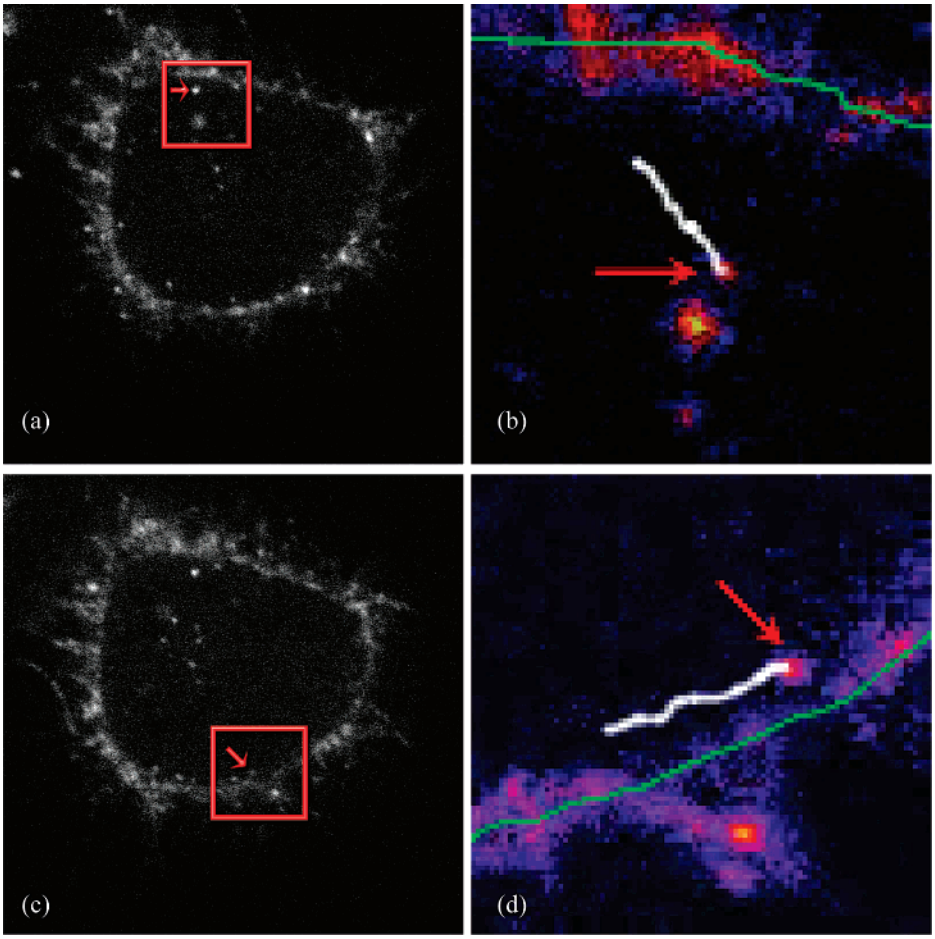


***Figure 3.*** Direct observation of active transport of endocytosed Tat-QDs inside living cells. (a, b) Directed motion from the cell periphery to an intracellular region adjacent to the cell nucleus. The red box area of image (a) is magnified in image (b). The white line is the trajectory of one Tat-QDs vesicle pointed by the red arrow (Supporting Information, Supplementary Video 1, frame 21 to 29, frame rate 249 ms/frame). The green line shows the plasma membrane boundary of the cell. (c, d) Directed motion along cell peripheral tracks. The red box area of image (c) is magnified in image (d). The white line is the trajectory of one Tat-QD vesicle pointed by the red arrow (Supporting Information, Supplementary Video 2, frame 5 to 13, frame rate 249 ms/frame). The green line shows the plasma membrane boundary of the cell.

## Discussion

Our experimental findings support a mechanistic model for Tat peptide-mediated delivery of QDs as shown in Figure 6. The delivery process starts with the binding of Tat-QDs with plasma membranes and filopodia, due to the high local charge density and flexibility of Tat peptides. Vesicles are generated from plasma membranes to internalize Tat-QDs, followed by active transport of the QD-containing vesicles from the cell periphery to an intracellular region just outside the nucleus. In the vesicles, Tat-QDs are bound to the inner lipid membrane, and secretion of Tat-QDs via vesicle shedding occurs shortly after the addition of Tat-QDs. It is not clear whether exocytosis, in which no vesicles are generated outside cells, also plays a role in the secretion of Tat-QDs. The transition from the inward transport of Tat-QDs inside cells to the secretion process might involve kinesin-mediated transport of Tat-QDs from the perinuclear region to the cell periphery.

Different cargos could lead to dramatically different intracellular fates even when they are delivered by the same carriers. It has been previously reported that Tat peptide-conjugated small molecules and macromolecules have access to the cytoplasm and eventually enter the cell nuclei.[22] In contrast, our results show that Tat-QDs are trapped in vesicles and do not enter the cell nuclei to any significant degree. This difference in the intracellular fate of Tat peptide-conjugated molecules and that of Tat-QDs is likely caused by the difference in the sizes of the cargos. QDs are much larger than standard molecules and are thus more difficult to escape from vesicles. Consistent with our results, Tkachenko et al.[41] reported that Tat peptide-conjugated gold nanoparticles similar in size to QDs failed to enter the cell nucleus. These researchers also pointed out that they were unable to distinguish between two possibilities, namely the entrapment of nanoparticles in vesicles or the alteration of physicochemical properties of the Tat peptide. Our results support the entrapment of nanoparticles in vesicles for the failure of nuclear entry. In a model proposed by Joliot and Prochiantz[42] describing the pathway of Tat peptide-mediated delivery, it is assumed that Tat peptide-conjugated cargos are located in the aqueous cores of intracellular vesicles.[42] Our results clearly show that endocytosed Tat-QDs are tethered to the vesicular membranes, indicating that the interactions between the Tat peptide and vesicular membranes should be considered in describing the delivery pathway of Tat peptides.

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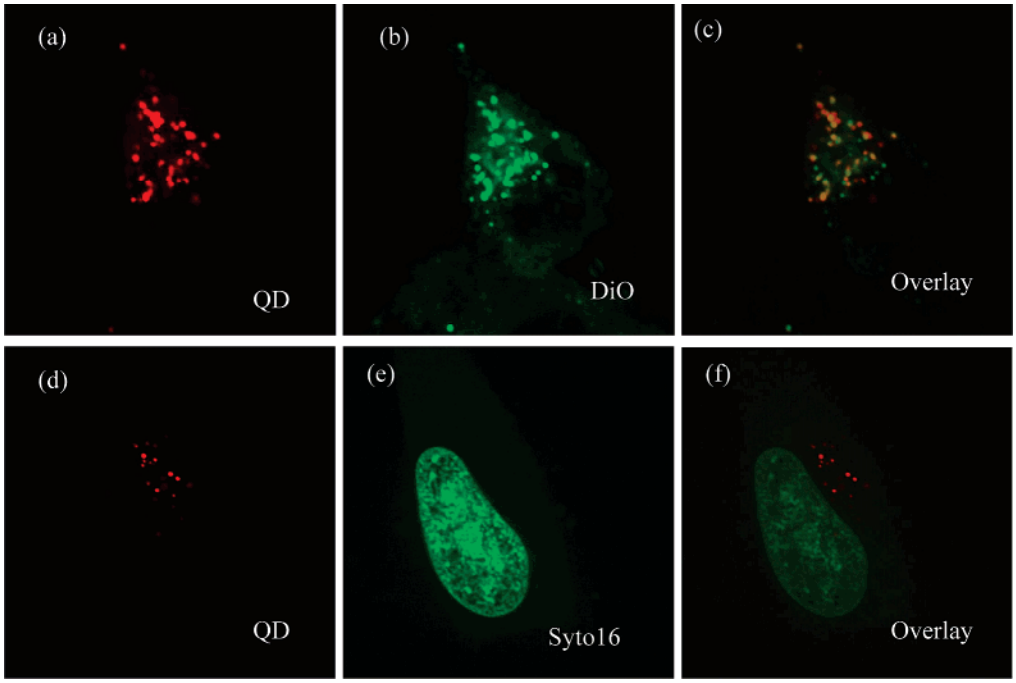


***Figure 4.*** Two-color colocalization studies showing Tat-QDs trapped in intracellular organelles and perinuclear localization outside of the cell nucleus. (a−c) Confocal fluorescence images of Tat-QDs (red), the membrane dye DiO (green), and an overlaid image showing their colocalization in organelles. (d−f) Confocal fluorescence images of Tat-QDs (red), the cell nucleus stained with (Syto 16, green), and an overlaid image showing the separation of QDs and nuclear signals. Tat-QDs were incubated with HeLa cells (cultured in Eagle's minimum essential medium plus 10% fetal bovine serum at 37 °C, 5% $CO_2$) for 24 h. DiO (1 $\mu$g/mL) or Syto 16 (100 nM) was added to the cell culture medium and incubated for 15 min before confocal images were taken at 10 frames per second.

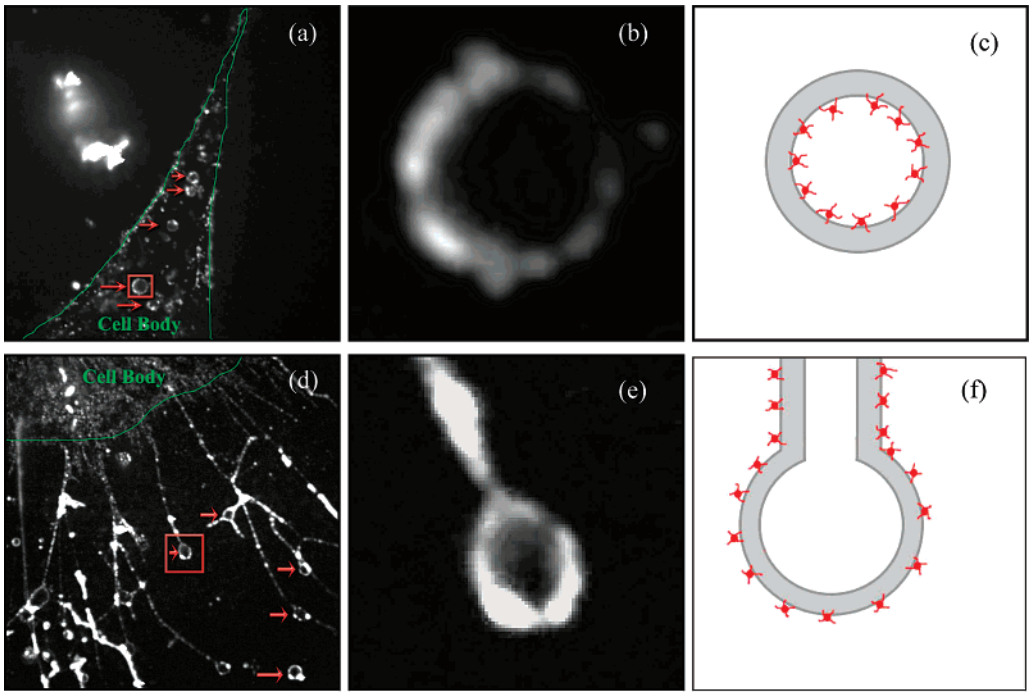


***Figure 5.*** Dynamic confocal fluorescence imaging showing Tat-QD distribution inside living cells and shedding of large vesicles from filopodia. (a−c) Internalized QDs trapped in vesicles (red arrows) and their binding to the vesicle membrane by electrostatic interactions. The vesicle highlighted by a red box in (a) is expanded in (b), and the binding of Tat-QDs (red symbol) to the vesicle membrane is depicted in (c). (d−f) QD-containing vesicles (red arrows) attached to the tips of filopodia or in a free form in the cell culture medium. One vesicle attached to a filopodia (highlighted by a red box) is expanded in (b), and the mode of Tat-QD membrane binding is shown in (f).

The observation of QD-containing vesicles in the cell culture medium and at the end of filopodia is the first report of vesicle shedding induced by Tat-QDs. Exocytosis of poly(lactide-co-glycolide) nanoparticles has been reported by Panyam and Labhasetwar.[43] However, the vesicle shedding that we observed may be a different secretion process from typical exocytosis. As mentioned earlier, in a typical exocytosis process, the contents of intracellular vesicles are directly secreted to the

(43) Panyam, J.; Labhasetwar, V. *Pharm. Res.* **2003**, *20*, 212−220.

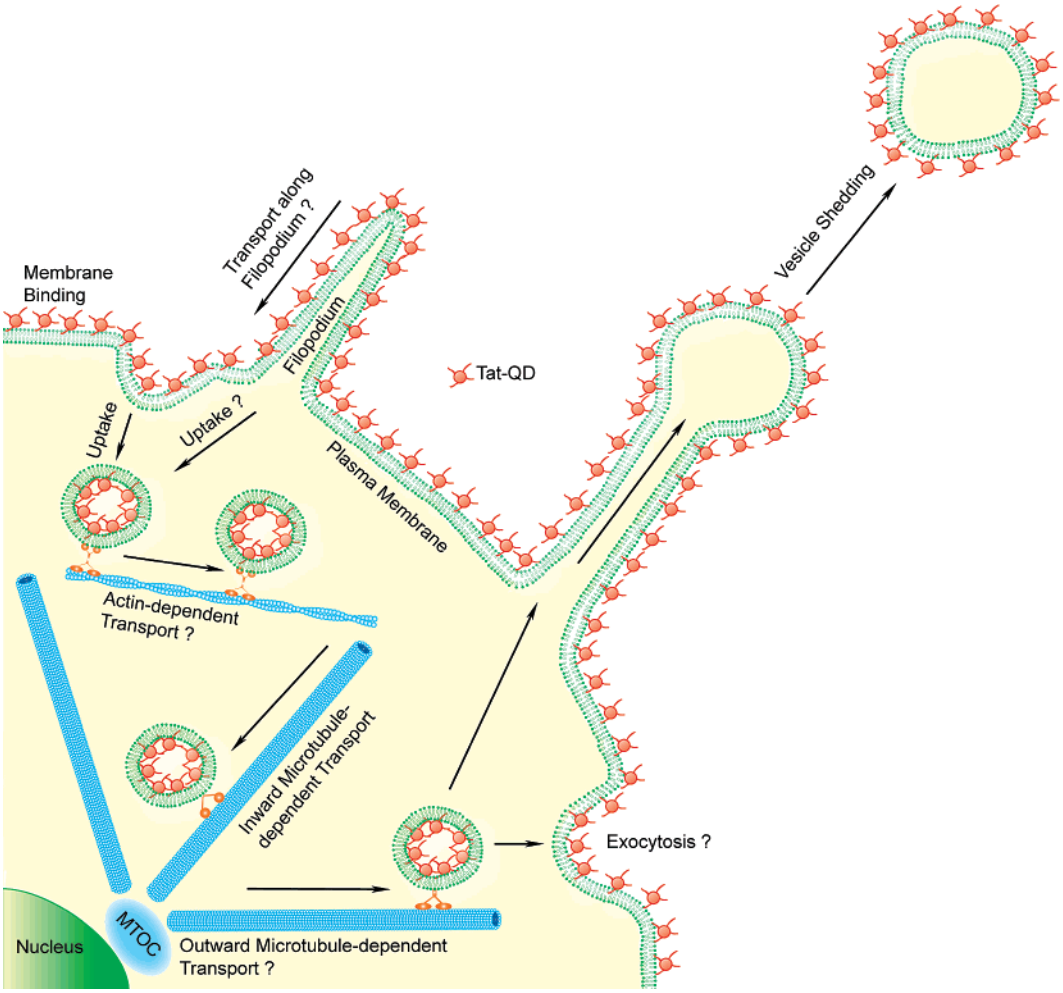


***Figure 6.*** Schematic diagram illustrating the key steps involved in Tat-QD uptake and transport in living cells. The delivery process starts with the binding of Tat-QDs with plasma membranes and filopodia, leading to internalization by macropinocytosis. This is followed by active transport of the QD-containing vesicles from the cell periphery to a perinuclear region called the microtubule organizing center (MTOC). The endocytosed Tat-QDs are tethered to the inner vesicular membranes, while Tat-QD binding to filopodia occurs on the outer membrane. Vesicle shedding is observed for both modes of QD-membrane binding.

extracellular space without forming vesicles outside the cells.[44] Vesicle shedding can occur on both plasma membranes and their extensions such as filopodia. However, in our experiments we observed that the secreted vesicles occurred mostly at the tips of filopodia. From a thermodynamic point of view, generating large vesicles from the end of filopodia would need to overcome a smaller energy barrier than from flat plasma membranes. The vesicle shedding of Tat-QDs could affect the delivery efficiency of QDs and might also be responsible for the toxicity of Tat peptides and QDs. Clearly, further studies are needed to investigate the secretion processes of nanoparticles from live cells and their potential implications in nanoparticle toxicity, delivery efficiency, and therapeutic efficacy.

The results presented are likely to impact the design and development of nanoparticles for cellular imaging and therapeutic applications. The finding that nearly all Tat-QDs are trapped in vesicles suggests that Tat peptide-mediated delivery is not suitable for labeling and imaging single molecules inside living cells. The difficulties for Tat-QDs to escape from the vesicles may, at least partially, result from the binding of Tat-QDs with the vesicular membranes because Tat-QDs are mainly located at the inner vesicle membrane. The binding of Tat-QDs with lipid membranes would make Tat-QDs not available for recognizing their intended targets in the cytoplasm even when the vesicles are disrupted or when Tat-QDs escape from the vesicles. If the vesicles are disrupted, Tat-QDs would still tend to stay bound to the fragmented lipid membranes, and the diffusion rates of these QDs would be greatly reduced. If Tat-QDs escape from the original vesicles, their binding with lipid membranes that are present throughout the cytoplasm (in organelles, plasma membranes, and other vesicles) would prevent Tat-QDs from binding to their targets. Of course, a minimal amount of Tat-QDs could escape from the vesicles and become available for binding with their targets in the cytoplasm.

On the other hand, Tat-QDs could be an ideal probe for investigating vesicular transport and molecular motors inside living cells, since these QDs are mainly trapped in vesicles and their intracellular transport is mediated by molecular motors. Vesicles and molecular motors are key components of the active transport system in cells. The current information about vesicles and molecular motors has been obtained mostly from biochemical, genetic, and *in vitro* mechanic studies.[44] The use of Tat-QD probes could thus help us gain a more sophisticated view of the active transport processes in live cells. Yet for other

(44) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*; Garland Science: New York, 2002.

applications, a small amount of free nanoparticles in cytoplasm could be sufficient. For example, Rosi et al.[45] and Won et al.[46] have used endocytosed gold nanoparticles and superparamagnetic nanoparticles for regulation of gene expression and detection of drug targets inside live cells, respectively. Our results suggest that vesicular release may be a rate-limiting step for these applications. Therefore, any approach that could aid vesicular release, such as laser illumination,[28] vesicle-disrupting drugs,[47] and fusogenic agents,[48] would significantly improve the utility of nanoparticle probes for intracellular imaging and targeting.

In conclusion, we have used QDs coupled with spinning disk confocal microscopy to follow the Tat peptide-mediated delivery of nanoparticles into live cells. We have shown that Tat-QDs are trapped in vesicles and remain tethered to the inner vesicle membranes inside the cytoplasm. Molecular motors are found to transport QD-loaded vesicles to a perinuclear region called the microtubule organizing center (MTOC). We have further observed a process called vesicle shedding in which large QD-contained vesicles pinch off from the tips of filopodia, resulting in free vesicles with Tat-QDs bound on the outside. These findings not only offer insights into the mechanism of Tat peptide-mediated delivery but will also be important toward the design and development of nanoparticle probes for intracellular imaging and therapeutic applications. By combining the brightness and photostability of QDs with the dynamic imaging capability of spinning disk confocal microscopy, we envision that the pathways of cellular endocytosis and exocytosis could be followed with QD-labeled ligands; the mechanism of viral infection could be examined with QD-tagged vectors; and the intracellular fate of nanoparticles drugs could be imaged and tracked in real time.

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**Supporting Information Available:** Supplementary videos (avi) and description of the videos (pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

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