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Mechanochemical Delivery and Dynamic Tracking of Fluorescent Quantum Dots in the Cytoplasm and Nucleus of Living Cells

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ABSTRACT

Studying molecular dynamics inside living cells is a major but highly rewarding challenge in cell biology. We present a nanoscale mechanochemical method to deliver fluorescent quantum dots (QDs) into living cells, using a membrane-penetrating nanoneedle. We demonstrate the selective delivery of monodispersed QDs into the cytoplasm and the nucleus of living cells and the tracking of the delivered QDs inside the cells. The ability to deliver and track QDs may invite unconventional strategies for studying biological processes and biophysical properties in living cells with spatial and temporal precision, potentially with molecular resolution.

Studying biological processes at the molecular level inside a living cell is a major challenge in cell biology. Recent advances in single-molecule techniques have enabled the understanding of biological mechanisms in unprecedented detail. However, the existing techniques are mostly limited to in vitro studies due to the difficulties in imaging individual molecules in the optically noisy cellular environment and accessing directly the interior of living cells. On the other hand, nanoparticles, such as quantum dots (QDs) and magnetic nanoparticles (MNPs), have shown great promise in this regard because of their bright and stable fluorescence and/or manipulability and their small size relative to that of individual proteins. For example, QDs have been used to study the dynamics of individual membrane proteins, to measure the motion of individual molecular motors in the cytoplasm, to monitor antigen uptake by dendritic cells, and to study the transport of nerve growth factors and the membrane fusion of synaptic vesicles in neurons; and MNPs have been used to physically manipulate individual membrane receptors to activate signal transduction of living cells.

The unique advantages of nanoparticles for investigating biological problems at the molecular level in living cells however have not been fully realized. The main problems include the relatively large sizes of biomolecule-conjugated nanoparticles and nanoparticle–target molecule complexes, the instability of antibody-mediated targeting, and the difficulty of delivering nanoparticles into the cytoplasm. For instance, because of the lack of efficient ways of delivering dispersed and single QDs into living cells, except in a few demonstrations, the use of QDs has been limited to visualizing cell membrane molecules and biological processes related to the endocytosis. Although various approaches involving the use of cell-penetrating peptides, electroporation, ballistic nanoparticle delivery method, and more recently nanoneedles have been explored, nanoparticles internalized into cells are often trapped in the endocytic pathway or form aggregates in the cytoplasm, or in other cases, uncontrollable amounts of nanoparticles are delivered into unspecified locations in the cytoplasm, which introduces undesirable fluorescent background noise and precludes subsequent targeted labeling of endogenous molecules and thus single-molecule studies. Overall, such approaches have their unique characteristic advantages and disadvantages depending on specific applications, and among them the direct microinjection of QDs has shown better performance and has enabled a homogeneous labeling of the entire cytoplasm in a dispersed form without the need for endosomal escape. However, to take advantage of the full potential of nanoparticles for living cell
We then coated the nanotube with a thin layer of Au (10–20 nm in thickness); the thin Au layer facilitated the use of surface chemistry for attaching QDs and increased the mechanical strength of the nanoneedle. To attach designated QDs onto the Au-coated nanotube (via disulfide bonds), we developed a general procedure for engineering the surface of the nanoneedle as detailed later. To release the QDs from the nanoneedle we exploited the regulatory mechanism of cells that maintains the redox equilibrium in the cytoplasm, in which most disulfide bonds are reduced into thiol groups (R–SS–R + 2H⁺ + 2e⁻ → R–SH + SH–R).28–30 Thus, upon the entry of the nanoneedle into the cytoplasm, the QDs conjugated on the nanoneedle via disulfide bonds could be released by the reductive cleavage of the disulfide bonds (Figure 1a).

This approach possesses several distinct technical benefits for delivering QDs into cell. With the use of a small-diameter nanotube as a needle, it introduces minimal intrusiveness in penetrating through the cell membrane and accessing the interior of live cells. By exploiting surface chemistry to attach and release QDs, it avoids the use of the carrier solution and the pressure-driven injection device required in micro-injection-based delivery and circumvents related problems in using small injection needles (e.g., clogging of injection needles and high-pressure required for injection). By making the nanoneedle compatible with micromanipulators commonly installed in an optical microscope, it can be readily adopted in many laboratories without the need of technically demanding equipments, such as the scanning probe microscope used in a prior study.24 Furthermore, the whole delivery process, including the positioning of the nanoneedle, the penetration of the cell membrane, and the reach of the nanoneedle to different intracellular compartments, can be precisely controlled, monitored and recorded in situ, not achieved in prior studies.22,24,25 While this approach might limit the amount of nanoparticles delivered in a single procedure, the local concentration of the delivered nanoparticles could be sufficiently high in the vicinity of the targeted release site to facilitate efficient molecular targeting if specifically functionalized nanoparticles were used in certain applications, besides lowering the overall fluorescent background, for instance, from the QDs of no desired interest.

The general procedure for attaching QDs onto the nanoneedle via disulfide bonds (Figure 1b) consists of three steps: forming a NH₂-terminated self-assembly monolayer (SAM) on the Au-coated nanoneedle by the chemisorption of thiols on gold,31 conjugating a linker molecule containing a disulfide bond within its spacer onto the SAM, and attaching streptavidin-conjugated QDs by the binding of streptavidin and biotin (see Supporting Information for the detailed procedure). This approach is potentially extendable for attaching other species, such as DNAs, RNAs, proteins, and nanoparticles of various sizes by tuning the surface functionalization with different molecular building blocks. It would also be possible to simultaneously attach different species with controlled densities, for example, by using mixed SAMs with different terminal functional groups.31 However, the subsequent imaging and tracking of such
We demonstrated the described delivery strategy by delivering fluorescent QDs into living HeLa cells. Figure 2 shows a typical QD delivery experiment targeting the cytoplasm. We manipulated the nanoneedle by using a common piezoelectric micromanipulator (InjectMan NI 2, Eppendorf) integrated in a Leica inverted epifluorescence microscope. The nanoneedle was manipulated to approach the cell membrane along its axial direction (around 10 nm in diameter), which excludes the possibility of detecting QDs at the top or bottom of the nucleus, we identified the equator of the nuclear envelope in the bright-field mode and imaged the cell on the same focal plane in the fluorescence mode (Figure 3a–c). The size of the passive entry to the nucleus has been reported to be smaller than or around 10 nm in diameter, which excludes the possibility of diffusive introduction of QDs (around 20 nm in diameter) from the cytoplasm into the nucleus. To further verify the nuclear delivery, we expressed the green fluorescence protein (GFP) on the nuclear envelope of living HeLa cells following the general protocol and performed the nuclear delivery experiment on GFP-expressed cells (Figure 3d–f). The delivered QDs were seen to be confined within the nuclear envelope. We next questioned whether our method could deliver single QDs into living cells. We measured the fluorescence intensity of some stationary QDs delivered into living HeLa cells over a period of time (Figure 4a,b and Supporting Information, Movies S1 and S2). We measured the fluorescence intensity of some stationary QDs delivered into living HeLa cells over a period of time (Figure 4a,b and Supporting Information, Movies S1 and S2).
mean value of $D_0 = 17 \mu m^2/s$), due to molecular crowding in the cytoplasm. The immobility of some QDs (10%) is likely due to their fast-moving QDs than slow ones in the three-dimensional environment of the cytoplasm. The $D$ values are $4\times$ to 200-fold smaller than that in aqueous solution ($D_0 = 17 \mu m^2/s$), due to molecular crowding in the cytoplasm; the reported $D/D_0$ values are $0.001$ to $0.5$ for macromolecules. The immobility of some QDs (10%) is likely due to their trapping to the intracellular structures, such as cytoskeleton.

**Figure 3.** Delivery of QDs into the nucleus of living HeLa cells. (a) Overlay of bright-field and fluorescence images of the cell after the nuclear delivery. (b) Enlarged fluorescence image of the region marked in (a). (c) Overlay of bright-field and fluorescence images of the region marked in (a). (d,e) Delivery of QDs into the nucleus of a living HeLa cell expressing GFP on the nuclear envelope. The nuclear envelope (d) of the cell (green) was identified with the GFP filter and the cell (e) was imaged at the same focus with the QD filter. (f) Overlay of (d) and (e). The dotted lines locate the boundary of the nucleus. The arrows indicate QDs (red). Scale bars, 10 $\mu m$ in (a) and (d), and 5 $\mu m$ in (b).

**Figure 4.** Time trace of fluorescence intensity and tracking of QDs inside living HeLa cells. (a) Typical time trace of the fluorescence intensity of stationary QDs (red) in living cell plotted with the background signal of neighboring areas (black), showing the blinking pattern (see also Supporting Information, Videos 1 and 2). For comparison, the intensity variation of a single QD on a glass slide prepared by spreading a highly diluted QD dispersion (0.1 nM) on a glass slide is shown as labeled. (b) A fluorescence image sequence showing the QD in the living cell during the time period of 19–25 s in (a). (c) Tracking of a QD inside living cells (see also Supporting Information, Video 3). A series of fluorescence images shows the trajectory (the green line) of a tracked QD. (d) Mean-square displacement (MSD) versus time data show three types of characteristic motion of QDs: free diffusive (red square), confined (blue square), and stationary (black square). The solid red line and blue line are the line fit on the data based on a free diffusion model and a confined diffusion model, respectively. (e) Diffusion of QDs in the cytoplasm. The diffusion coefficient $D$ (0.08–3.8 $\mu m^2/s$, mean $0.8 \mu m^2/s$, n = 20) was determined by fitting a free diffusion model MSD(t) = 4Dt to the initial few data points of each MSD versus time curve acquired from tracking of different QDs. The fitted lines are shown in solid red. The dashed blue line indicates the MSD expected for freely diffusing QDs in aqueous solution ($D_0 = 17 \mu m^2/s$). The dashed green lines are reference lines for $D$ of 1.0 and 4.0 $\mu m^2/s$ as labeled. Scale bars, 500 nm in (b) and 1 $\mu m$ in (c).
the size of typical nuclear microdomains.\textsuperscript{36,37} However, we
the normal operational time interval needed in practice from
\[ t \gg 20 \text{ min} \] as seen in Figure 2c), we assigned a maximum time
to be detected even with the presence of transmission light
eedle overshadowing the vicinity of the release site (the
bright fluorescence of the QDs attached on the nanon-
3). As we could not practically observe the release and
\[ r \approx 0.5 \mu m \]
Figure 5. Confined diffusion of a QD in the nucleus of a living
HeLa cell. (a,b) Confined diffusion of a QD in the region marked
in (a) inside the nucleus of a living HeLa cell and the trajectory of
the QD (b) (see also Supporting Information, Video 4). (c)
Corresponding MSD versus time curve (blue) for this QD (A). The
data for other stationary QDs (B) (black) are also shown for
comparison. The solid line (blue) in (c) is the fit based on a confined
diffusion model. The fit estimates the diffusion coefficient of the
QD (A) to be 0.01 \( \mu m^2/s \) and the size of the confined domain \( L_a \)
to be \( \sim 300 \) nm, consistent with the typical size of the nuclear
microdomains.
and endoplasmic reticulum; the sizes of single QDs or small
QD clusters are comparable to the pore sizes in the
cytoplasmic meshwork (\( \sim 30-100 \) nm).\textsuperscript{31}

The recorded dynamics of QDs can also be used to
quantify the local biophysical properties of the intracellular
environment by the biomicrorheology method.\textsuperscript{35} According
to the Stokes–Einstein relation \[ D = kT/(6\pi r\eta) \], where \( k \) is
the Boltzmann’s constant, \( T \) is the absolute temperature, \( \eta \)
is the viscosity, and \( r \) is the radius of the particle (QDs), the
apparent viscosity \( \eta \) of the region where the QD travels can
be calculated from the measured \( D \) values. Assuming that \( r \)
of the QDs is the hydrodynamic radius of QDs (12.8 nm
obtained from \( D_0 = 17 \mu m^2/s \)),\textsuperscript{34} the apparent “nanoscale”
viscosity in the cytoplasm is estimated to span from \( \sim 4 \) to
\( \sim 200 \) cP in different regions of the cytoplasm, indicating
the high physical heterogeneity of the intracellular environ-
ment (see also Supporting Information, Figure S4).

In contrast to the QDs in the cytoplasm, most QDs
introduced into the nucleus were immobilized after the
delivery at the time when fluorescence images were acquired.
For some QDs, we observed small movement, as shown in
Figure 5, in a confined domain of \( L = \sim 300 \) nm, similar to
the size of typical nuclear microdomains.\textsuperscript{36,37} However, we
also observed that some delivered QDs were dispersed as
far as \( \sim 10 \mu m \) from the site of release in the nucleus (Figure
3). As we could not practically observe the release and
movement of QDs in real-time from the nanoneedle due to
the bright fluorescence of the QDs attached on the nanon-
needle overshadowing the vicinity of the release site (the
fluorescence of QDs on the nanoneedle was bright enough
to be detected even with the presence of transmission light
as seen in Figure 2c), we assigned a maximum time \( t \) of
\( \sim 20 \text{ min} \) as the time allowed for such diffusion, which was
the normal operational time interval needed in practice from
the instant of penetrating the nanoneedle into the cell to the
moment of performing the fluorescence imaging after
withdrawing the nanoneedle from the cell. A simple random
walk model \( d = (2Dt)^{1/2} \) with \( d \approx 10 \mu m \) and \( t \leq 20 \) min
then estimates that the diffusion coefficient of QDs in the
nucleus can be larger than \( \sim 0.04 \mu m^2/s \). This estimated \( D \)
compares favorably to the \( D \) values determined for the
transport of mRNA-protein complexes (0.01–0.09 \( \mu m^2/s \))\textsuperscript{36,38}
and nuclear proteins (\( \sim 0.2–0.5 \mu m^2/s \))\textsuperscript{39} in the nucleus
through simple diffusion. As suggested for the transport of
nuclear components by diffusion, QDs might also diffuse
over an extended distance (\( \sim 10 \mu m \)), most likely through
interchromatin domains at a similar rate as in the cytoplasm,
but eventually be trapped in nuclear compartments, such as
chromatin-dense domains, probably due to their nonmem-
brane structures of intermingled fibers.\textsuperscript{40}

The ability to deliver a small number of monodispersed
nanoparticles into living cells with spatial and temporal
precision may make feasible numerous new strategies for
biological studies, which would otherwise be technically
challenging or even impossible. For example, in combination
with effective molecular targeting strategies\textsuperscript{11,13,14} using QDs
and MNPs as molecular probes, this method can potentially
enable simultaneous observation and manipulation of indi-
vidual molecules in both the cytoplasm and the nucleus of
living cells, and afford a broad range of new biological
experiments at the single-molecule level. As the release of
such nanoparticles is local at the site of release and at the
time of release (due to the relatively low diffusivity of QDs
inside cells), this local concentration can facilitate efficient
targeting of intended region and molecules and thus potent-
ially allow spatially resolved molecular experiments inside
cells. For some cellular and molecular mechanics studies
(e.g., mechanotransduction) inside living cells, spatially
resolved delivery of one or a traceable number of force
probes (e.g., MNPs) would be desirable to pinpoint applied
forces, which would then be uniquely achievable with this
method.\textsuperscript{41} The direct delivery of only a small number
of nanoparticles would also minimize the effect of internalized
nanoparticles on cell physiology.\textsuperscript{42} Furthermore, the delivery
process can be done repeatedly at a desired time through
the cell cycle and in conjunction with other cellular
manipulations and measurements. An obvious limitation of
this method is that one functionalized nanoneedle can only
be used to deliver QDs into one cell (or at most, several
cells if the nanoneedle is reused until the attached QDs are
totally released). Beyond delivery, the nanoneedle-based
approach can also be extended in many ways for single cell
studies, for example, as an electrochemical probe\textsuperscript{27} or an
optical biosensor using QDs attached on the nanoneedle.\textsuperscript{43}

Altogether, the nanoneedle-based delivery technique offers
a powerful nanotechnology-based tool for studying biological
processes and biophysical properties inside living cells.

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