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Quantum Rod Bioconjugates as Targeted Probes for Confocal and Two-Photon Fluorescence Imaging of Cancer Cells

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ABSTRACT

Live cell imaging using CdSe/CdS/ZnS quantum rods (QRs) as targeted optical probes is reported. The QRs, synthesized in organic media using a binary surfactant mixture, were dispersed in aqueous media using mercaptoundecanoic acid (MUA) and lysine. Transferrin (Tf) was linked to the QRs to produce QR-Tf bioconjugates that were used for targeted in vitro delivery to a human cancer cell line. Confocal and two-photon imaging were used to confirm receptor-mediated uptake of QR-Tf conjugates into the HeLa cells, which overexpress the transferrin receptor (TfR). Uptake was not observed with QRs that lacked Tf functionalization or with cells that were presaturated with free Tf and then treated with Tf-functionalized QRs.

Semiconductor nanocrystals (NCs) have emerged as an important class of materials because of their tunable optoelectronic properties that arise from quantum size effects.[1,2] Over the past two decades, a growing array of well-developed synthetic methodologies have been used to produce nearly monodispersed spherical NCs, also called quantum dots (QDs).[3] Recently, QDs have received enormous attention for their potential applications ranging from physics to medicine. QDs have great promise as optical probes because they are brighter than traditional organic chromophores, are resistant to photobleaching, have narrow and size-tunable emission wavelength, and have broad excitation spectra. The use of QDs as optical probes in cell imaging has grown explosively since the first examples from Alivisatos's and Nie's groups.[4,5] For example, the use of CdTe/CdSe core/shell[6] and CdSeTe alloy[7] QDs for near-infrared (NIR) bioimaging was studied. CdSe QDs have also found application in photodynamic therapy of cancer.[8]

The effects of NC shape have recently attracted considerable attention because the unique behavior expected in the evolution from zero-dimensional (0-D) QDs to one-dimensional (1-D) quantum rods (QRs) or quantum wires (QWs).[9−15] Though use of QDs as probes for in vitro or in vivo imaging has been studied intensively for the past few years, there are very limited reports describing use of QRs as biological markers.[16] Very recently, Alivisatos and co-workers first reported the use of water-soluble surface silanized quantum rods for nonspecific cell tracking and specific cellular targeting.[16] CdSe QRs show several advantages over spherical QDs for serving as novel functional probes for biological and medicinal applications. For example, color control is achievable by tuning the rod diameter that was found to govern the band gap energy of CdSe QRs.[17] The Stokes shift is strongly dependent on the aspect ratio (length/diameter)

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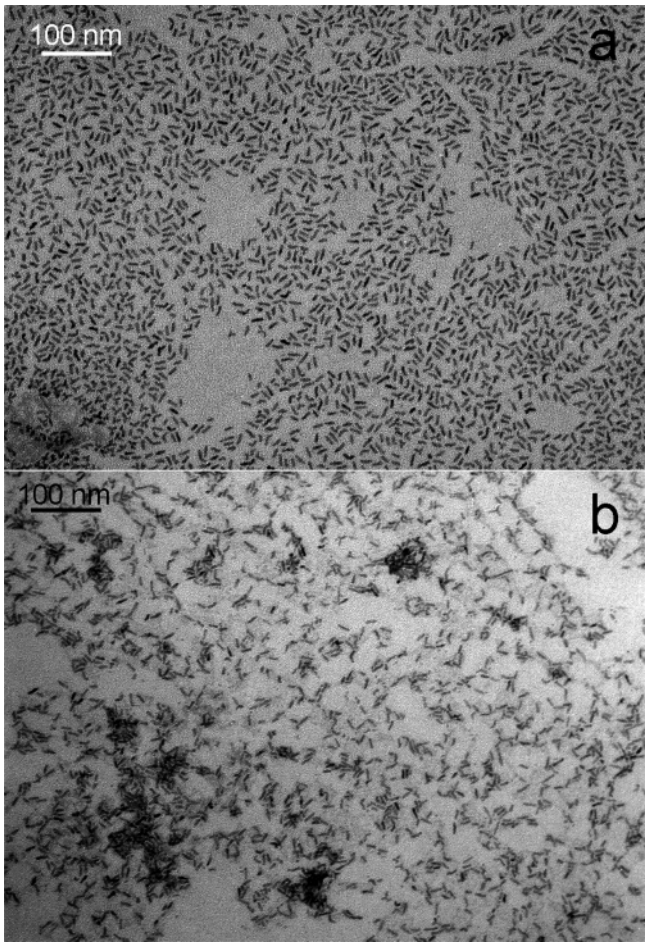

Figure 1. TEM images of CdSe/CdS/ZnS QRs cast from (a) nonpolar organic solvent and (b) aqueous dispersions. The average diameter and length of the nanorods are 14 and 4 nm, respectively. The aspect ratio of the nanorods is 3.5.

of the rod.[18,19] Quantum rods are brighter single molecule probes as compared to quantum dots. Furthermore, it was reported that CdSe QRs emitted light that was linearly polarized along the *c*-axis of the crystallites and the degree of polarization was dependent on the aspect ratio of the NCs.[20] These unique characteristics of QRs as biological markers may provide an avenue for further improvements in ultrasensitive imaging strategies.

In this paper, we report live cell imaging using colloidally synthesized CdSe/CdS/ZnS QRs as targeted optical probes. The CdSe/CdS/ZnS QRs were prepared by growing a CdS/ZnS graded shell on CdSe rods in surfactant solution.[20−24] The moderately polar surfactant mercaptoundecanoic acid (MUA) was used to displace the hydrophobic surfactants used in the nanorod synthesis from the QR surface, thereby rendering them dispersible in dimethyl sulfoxide (DMSO). Lysine was then used to cross-link the carboxylic acid ends of the MUA on the QR surface via carbodiimide chemistry, generating a hydrophilic shell displaying both carboxyl and amine groups on its surface.[25] Transferrin (Tf) was then linked to this surface, again using carbodiimide chemistry, to produce QR-Tf bioconjugates. Tf is a protein with a high affinity for TfR, which is overexpressed in cancer cells.

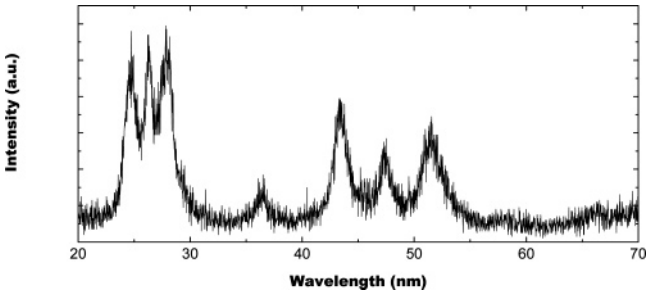

Figure 2. Powder X-ray diffraction pattern of CdSe/CdS/ZnS nanorods.

Confocal and two-photon fluorescence imaging techniques were used to confirm receptor-mediated uptake of QR-Tf conjugates into HeLa cells, which are known to overexpress the transferrin receptor (TfR).

Figure 1a presents transmission electron microscopy (TEM) images of the CdSe/CdS/ZnS QRs deposited from organic media. They are approximately 14 nm in length and 4 nm in diameter. Figure 1b shows the lysine cross-linked MUA-coated QRs deposited from aqueous media. Comparison of parts a and b of Figure 1 shows that the size and aspect ratio of the lysine-coated CdSe/CdS/ZnS QRs remain unchanged. This suggests that neither coalescence nor ripening of the QRs occurred during the ligand exchange and lysine cross-linking process. The water dispersible lysine-coated QRs were colloidally stable for months at 4 °C. Details of the preparation procedure are in the Supporting Information. The powder X-ray diffraction (XRD) pattern from the CdSe/CdS/ZnS QRs is shown in Figure 2. All observed peaks can be indexed to the wurtzite structure, with a lattice constant slightly compressed from that of bulk CdSe due to the CdS/ZnS coating. Though the 002 peak is narrow, it is lower in intensity than that for bare CdSe rods of comparable aspect ratio. The decrease in intensity of the 002 peak and the shift of all peaks to higher angle are consistent with previous reports.[26]

Figure 3a shows the absorption and photoluminescence (PL) spectra, in chloroform, from the largely monodispersed CdSe/CdS/ZnS QRs. The absorption spectrum features an excitonic peak around 594 nm. The PL spectrum of the monodispersed QRs shows a band edge emission at 610 nm. The PL quantum yield (QY) of the QRs is estimated to be ∼38%. The high QY is not surprising because of the quantum confinement and passivation provided by the epitaxial CdS/ZnS graded shell coated on the CdSe core. The QY value is sufficiently high for live cell imaging purposes.

Previously, our group has successfully shown that the efficient two-photon excitation of QDs can be used for two-photon bioimaging.[27] Here, we extend this approach to QRs, which may have larger two-photon-absorption cross section and may therefore provide brighter probes for two-photon imaging. First, we measured the two-photon spectrum of these QRs. The two-photon excited PL of the QRs is shown in Figure 3b. The two-photon PL spectrum was centered at 608 nm. The slight narrowing of PL spectra may be due to

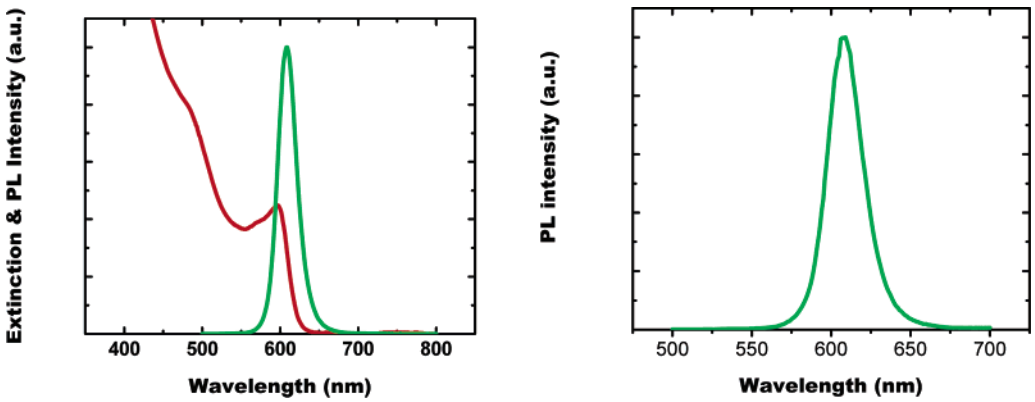

Figure 3. (a) UV−vis absorption (red) and PL (green) spectra and (b) two-photon PL spectrum of monodispersed CdSe/CdS/ZnS QRs.

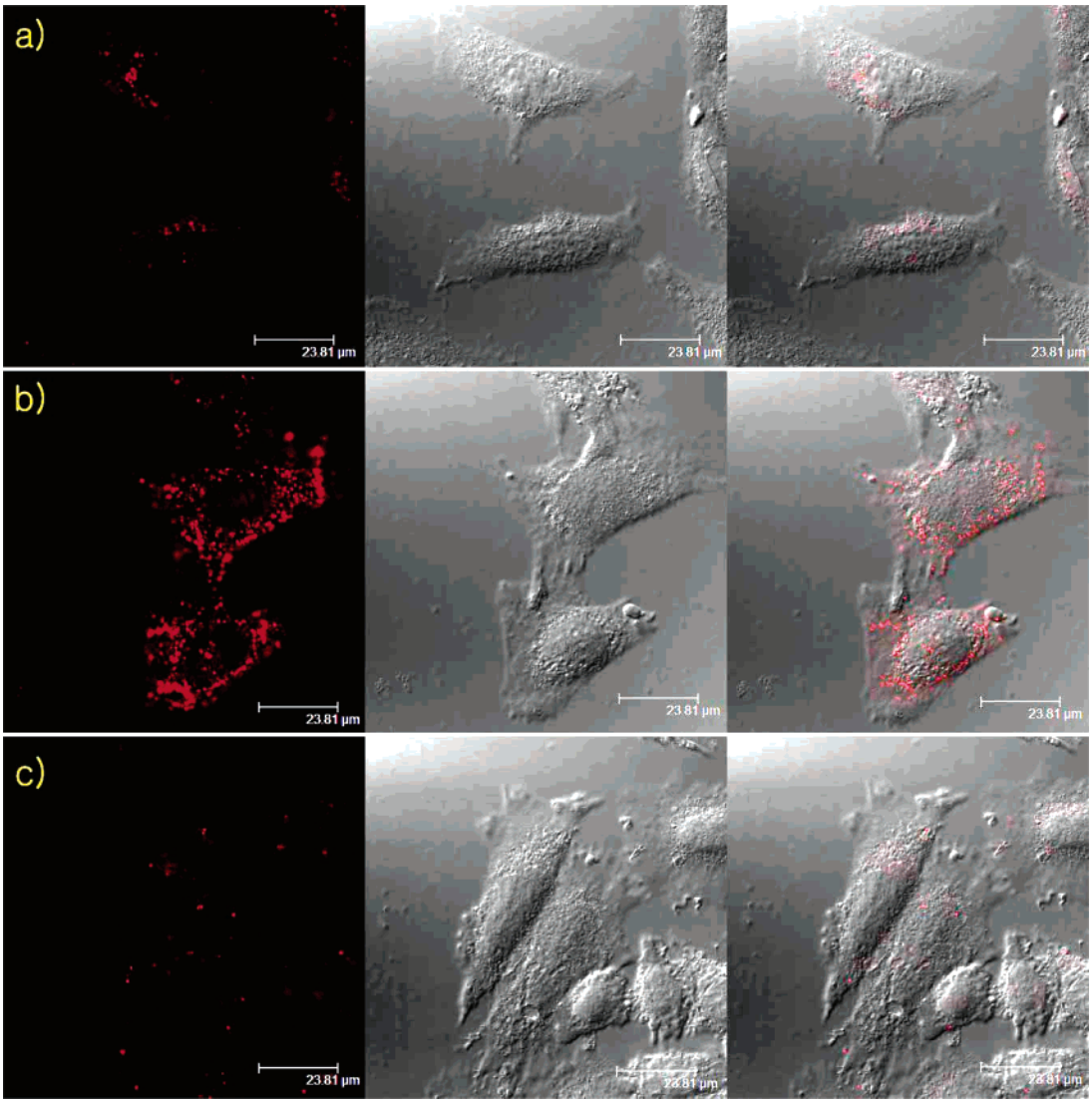

Figure 4. Confocal microscopic images of HeLa cells, treated with (a) lysine-coated CdSe/CdS/ZnS QRs. (b, c) Tf-conjugated CdSe/CdS/ZnS QRs. Cells in (c) were saturated with free Tf for 2 h, before treatment with QR−Tf. The panel to the left displays the florescence images and their corresponding transmission images are shown in the middle panel. The panel to the right shows the overlays of florescence and transmission images. Confocal microscopy images were obtained with laser excitation at 442 nm.

size-selective two-photon excitation. The emission from these QRs, under excitation at 884 nm, was sufficiently bright for them to be used effectively in two-photon imaging.

To employ QRs as an efficient targeted contrast agent for in vitro or in vivo imaging, the QRs must be conjugated with specific biorecognition molecules. Here, we present the

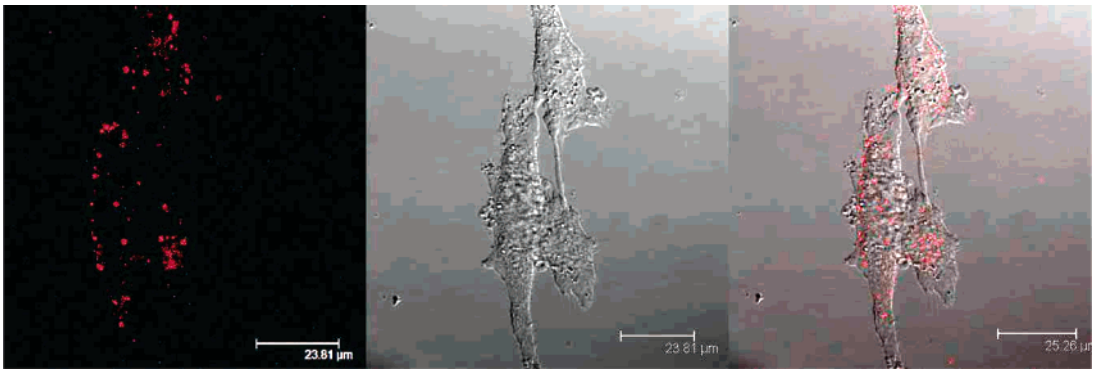

Figure 5. Two-photon images of Hela cells treated with Tf-conjugated CdSe/CdS/ZnS QRs. Two-photon microscopy images were obtained with laser excitation at 884 nm.

conjugation of QRs with a biomolecule, using transferrin (Tf) to fabricate QR−Tf bioconjugates. HeLa cells are chosen as the target cell line, which is known to overexpress transferrin receptors (TfRs).[25] Since TfRs are minimally distributed in normal cells/tissues, Tf serves as an excellent ligand for preferentially targeting cancerous cells/tissues in vitro and in vivo. The Tf-conjugated nanoprobes are transported into cells through the transferrin receptor TfR-mediated endocytosis pathway.

Figure 4 shows confocal microscopy images of HeLa cells stained with the QR−Tf bioconjugate. As seen in the images, the QR−Tf bioconjugates (panel b) appear to accumulate in vesicles within the cells, suggesting endocytotic uptake. To prove that QR−Tf bioconjugates are specifically targeted through the Tf−TfR interaction, two control experiments were performed. First, cells were incubated with unconjugated QRs (panel a). The second control experiment involved saturating the cells with free Tf for 2 h in order to block the available TfRs on the cell surface, followed by treatment with QR−Tf (panel c). In both of these cases, minimal signal from the cells was observed, confirming that the QR uptake occurred predominantly via the specific Tf−TfR interaction. Moreover, we did not observe any sign of morphological damage to the cells upon treatment with the QR bioconjugates, demonstrating their nontoxicity.

Since significant two-photon PL was observed from CdSe/CdS/ZnS QRs, direct two-photon imaging was attempted with QR−Tf bioconjugates. Figure 5 shows the two-photon images of HeLa cells labeled with QR−Tf. This figure suggests that the QRs accumulate in vesicles within the cells. This multiphoton imaging technique provides the possibility of long-term imaging of cellular processes with reduced photodamage compared to UV-excited imaging. This enhances the potential of QRs for use as biological probes for multiplex imaging and for multiphoton microscopy, where bright and photostable probes are desired for various complex multi-imaging tasks.

In conclusion, we have prepared photostable water-soluble lysine-coated CdSe/CdS/ZnS QRs for bioimaging. The lysine-coated QRs can be readily conjugated with Tf for specific targeted bioimaging. The internalization of QR−Tf bioconjugates in HeLa cells was demonstrated. The receptor-mediated uptake of QR−Tf bioconjugates into cancer cells was confirmed by presaturating the cells with free Tf in order to block the available TfRs on the cell surface, thereby drastically lowering the uptake of QR−Tf bioconjugates. We believe this study provides encouraging support for the future development of QR-based bioconjugates as targeted optical probes for localizing and studying ligand−receptor activity of cancer cells in vitro and in vivo.

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Supporting Information Available: Detailed materials and experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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