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Click-Functionalized Compact Quantum Dots Protected by Multidentate-Imidazole Ligands: Conjugation-Ready Nanotags for Living-Virus Labeling and Imaging

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Supporting Information

ABSTRACT: We synthesized a new class of mutifunctional multidentate-imidazole polymer ligands by one-step reaction to produce conjugation-ready QDs with great stability and compact size. Furthermore, combined with strain-promoted click chemistry, we developed a general strategy for efficient labeling of living-viruses with QD probes.

The development of fluorescent probes to visualize and track an individual virus particle is a key step for investigating viral infection routes and characterizing the dynamic interactions between viruses and target cells.[1] Effective labels must be stable, bright, and small. Compared to traditional organic dyes and fluorescent proteins, quantum dots (QDs) show great promise as fluorescent tags in biolabeling due to their superior brightness and photostability.[2] Despite its many successes in virus tracking and imaging,[3] QD-labeling is confronted with fundamental barriers. The relatively large size of QDs has a large impact not only on their own dynamic behavior in cellular environments, but also on that of the labeled biomolecules or organisms.[4] Moreover it is also notable that the infectivity of viruses coupled with QDs is somewhat impaired.[3d]

The size of QDs could be attributable to several factors, including the size of the inorganic core, the organic surface coating, and the functional moieties on the QD surface for bioconjugation. The surface coating of QDs is certainly the most important aspect that affects not only the particle size but also the stability of QDs.[5] Hydrophilic QDs have been achieved via ligand exchange with thiol-bearing small molecules[6] or encapsulation with amphiphilic copolymers.[7] However, these approaches faced a trade-off between size and stability. Recently, multidentate polymer ligands (using thiol/imidazole as QD-coordinating groups) have been proposed to not only enhance the stability but also minimize hydrodynamic sizes of QDs by compact surface coating.[8]

QD bioconjugates usually rely upon the traditional bioconjugate chemistries (carbodiimide and biotin/avidin coupling). Click chemistry provides another more efficient and facile conjugation strategy in mild condition to eliminate undesirable side reactions, minimize steric hindrance, and improve reproducibility in production.[9] The most popular reaction which fulfills "click reaction" criteria is the copper-catalyzed or metal-free strain-promoted cycloaddition of azides and alkynes (or cyclooctynes). The cytotoxic copper ions have the irreversible photoluminescence (PL) quenching effect on QDs. In this respect, the copper-free click reaction is ideal for QD bioconjugation since no addition reagents are required.[10] In addition, these click functional groups are biologically unique, stable, inert, and small.

In the present study, we synthesized a new class of multidentate−imidazole ligands by the one-step method, and produced the highly bright and conjugation-ready QDs with superior stability and small hydrodynamic size. Furthermore, we successfully developed a general living-virus QD-labeling method by metal-free strain-promoted click chemistry. More importantly, labeling a virus in this manner does not affect the viral infection.

The multidentate polymer ligand with imidazole pendant groups (PMAH or N_3−PMAH) was produced by reacting poly(maleic anhydride) with either pure histamine or a mixture of histamine and N_3−PEG−NH_2 (Scheme 1 or Supporting Information, Figure S1). The high reactivity of anhydride with primary amines facilitated the quantitatively controlled one-step reaction and incorporation of specific reactive groups. These multidentate polymers were soluble only in strongly polar solvents such as water, DMSO, and DMF. Characterization of the final products was carried out using ^{1}HNMR spectroscopy (see Figure S2). The one-step polymer synthesis was facile and flexible in comparison with a previous method which involved an elaborate multistep synthesis with time- and labor-intensive consumption.[8b] Water-soluble QDs capped with PMAH or N_3−PMAH (noted as PMAH-QDs or N_3−PMAH-QDs, here after) were prepared from the initial oil-soluble QDs via ligand exchange by displacing the native hydrophobic ligands with the imidazole groups along the polymer backbone, which can participate in multiple binding interactions with the Zn-rich QD surface.

First, we examined the effects of PMAH coating on the hydrodynamic and optical properties of CdSe/ZnS QDs with different inorganic core size (525 nm emission corresponding to 3.5 nm in size, 605 nm emission corresponding to 6.5 nm in size and 705 nm emission corresponding to 10.0 nm in size).

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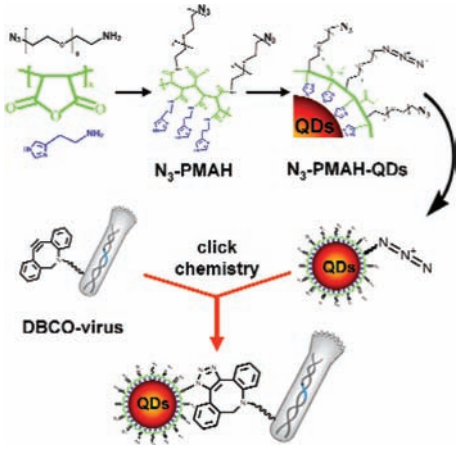
Scheme 1. Schematic Illustration of the One-Step Synthesis of the Azido-Derivatized Multidentate–Imidazole Polymer Ligands (N_3–PMAH) along with the Structures of QDs Protected by the Multidentate Ligands and the General Strategy for the Specific Labeling of Viruses with QDs via Strain-Promoted Metal-free Click Chemistry



Complete ligand displacement was achieved within 10 min, and was confirmed by FT-IR spectroscopy (Supporting Information, Figure S3). TEM analysis of QDs after ligand exchange with PMAH showed that the QDs were well dispersed (see Figure S4a–c). The hydrodynamic sizes of the series of QDs capped with PMAH were measured by gel filtration chromatography (GFC) and dynamic light scattering (DLS) and found to be ~8.7, ~11.2, and ~13.0 nm for PMAH-QD525, PMAH-QD605, and PMAH-QD705, respectively (Figure 1b and Supporting Information, Figure S4d). The

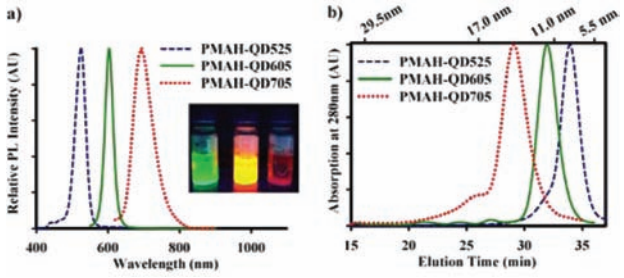


Figure 1. Optical and hydrodynamic properties of QDs solubilized in water with PMAH. (a) Normalized PL spectra of the three different-sized QDs. QD525 (3.5 nm core, dashed line, blue), QD605 (6.5 nm core, solid line, green), and QD705 (10 nm core, dotted line, red) after ligand exchange with PMAH in water. Inset: photos of PMAH-QDs under 365 nm UV irradiation. (b) GFC of PMAH-QDs showing direct size comparison with protein standards (see Supporting Information, Figure S5 for protein standards).

results showed a systematic increase in the measured diameter with increasing the inorganic core size. The hydrodynamic radii of the PMAH-QDs were only about 1.5–2.5 nm larger than their inorganic cores. The hydrodynamic diameters of the QDs were comparable between the GFC and DLS method. The single peak of GFC and the monomodal size distribution of DLS indicated that these QDs formed well-dispersed aggregate-free solutions, which was important for biological labeling and single-particle tracking applications. Upon ligand exchange with PMAH, the PL intensities of different QDs were maintained or even slightly enhanced, except for QD525 (coated with thin layers of ZnS), which showed a decreased fluorescence (Figure S4e–h). The final PL intensity of the hydrophilic core/shell QDs was strongly associated with the thickness of the shell grown on the CdSe core, confirming that the quality of the shell was essential for maintaining optical properties of QDs.[6b,c,7e]

Next, the effects of the nature and spatial extension of the capping ligands on the hydrodynamic sizes of QDs were investigated. As shown in Figure 2, GFC measurements

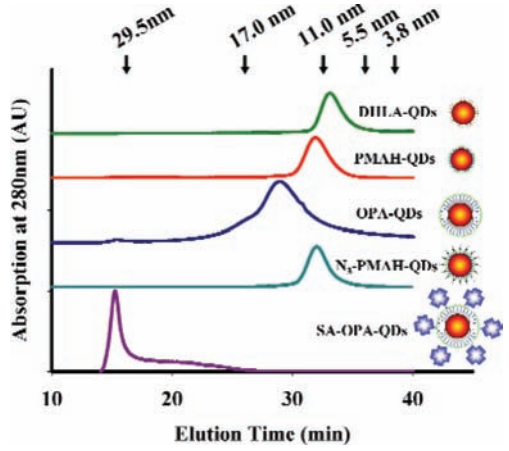


Figure 2. Hydrodynamic sizes comparison of QDs (6.5 nm core) coated with thiol ligand (DHLA-QDs, green), multidentate polymer ligand (PMAH-QDs, red), amphiphilic polymer (OPA-QDs, blue), azido-derivatized PMAH-QDs (N_3–PMAH-QDs, cyan), and commercial streptavidin-conjugated QDs (SA-OPA-QDs, purple) obtained from GFC. All samples were dissolved in PBS.

indicated that the hydrodynamic diameter was 10.5 nm for DHLA-QDs, 11.5 nm for PMAH-QDs, and 14.6 nm for OPA-QDs. As compared with the size of bare CdSe/ZnS core/shell QDs (~6.5 nm), the thickness of the multidentate polymer coating was only ~2.3 nm. The hydrodynamic thickness value of approximately 2 nm was consistent with the theoretical prediction of a "loops–trains–tails" binding conformation for a monolayer of this multidentate polymer on the nanocrystal surface.[8a] However, the amphiphilic polymer coating thickness was about 4.5 nm. Therefore, the size of PMAH-QDs was considerably smaller than traditional amphiphilic polymer-coated QDs, but similar to those capped with thiol ligands (~2 nm in thickness). Moreover, the incorporation of click reactive groups did not dramatically alter the size of the QDs. The GFC results demonstrated that the size of N_3–PMAH-QDs was 11.6 nm, comparable to that of PMAH-QDs, and was only $^1/_3$ that of commonly used commercial streptavidin-conjugated QDs (~30.6 nm). The significantly reduced size of N_3–PMAH-QDs allows us to pursue a more appropriate and efficient strategy of living-virus labeling and single-virus tracking.

It was of interest that PMAH-QDs had a superior stability (Figure 3). Storage of PMAH-QDs at room temperature under ambient condition for one month did not significantly change their intensity or cause precipitation (see Figure 3a). Furthermore, the photostability tests indicated that the PL intensity of QDs increased more than 30% after 10 min excitation with 365 nm UV. As shown in Figure 3b, the enhanced fluorescence was retained for at least 1 h, which was consistent with previous studies.[8b] Furthermore, thermal stability tests indicated that the PMAH-QDs were also stable

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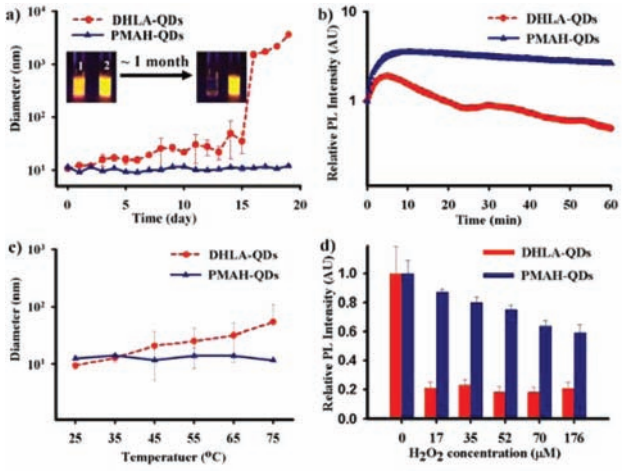

Figure 3. The stability experiments of PMAH-QDs (imidazole-capped QDs). DHLA-QDs (thiol-capped QDs) was used as control. (a) The storage stability experiment of PMAH-QDs. Inset: Fluorescence photographs of DHLA-QDs (1) and PMAH-QDs (2) solutions in PBS before and after 1 month storage at ambient conditions. DHLA-QDs precipitated, while PMAH-QDs retained fluorescence for at least 1 month. (b) The photostability experiment of the PMAH-QDs. The samples were continuously irradiated for 1 h by a xenon lamp (365 nm, 450 W). (c) The thermal stability tests of PMAH-QDs. (d) The dose-dependence of the normalized PL intensities of PMAH-QDs and DHLA-QDs against different concentration of H_2O_2 etching. The excitation wavelength is 400 nm. Error bars represent standard deviations from multiple trials.

at up to 75 °C (Figure 3c). In addition, we investigated the tolerance of PMAH-QDs against chemical oxidation by H_2O_2 (Figure 3d). Although the presence of H_2O_2 decreased the PL intensity of both DHLA-QDs and PMAH-QDs, QDs capped with imidazole (PMAH-QDs) appeared to be more resistant to the oxidation. For example, there was above 80% of luminescence decreased in the DHLA-QDs, while only 20% of the PL intensity of the PMAH-QDs was dropped after 10 min of incubation with 17 μM H_2O_2. The instability of thiol-capped QDs has already been studied.[11] The photo-oxidation of the thiol ligands in the presence of oxygen and light produced disulfides, which thus detached from the surface of nanocrystals and lead to aggregation. As a result, new surface defects were generated. The PL intensity of QDs decreased, and finally the QDs are bleached. In contrast, imidazole is resistant to degradation by oxidation and its multidentate binding motif can greatly enhance stability of QDs. Therefore, the stability of imidazole-capped QDs was superior to that of thiol-capped ones. Also, PMAH-QDs stayed stable in buffers over a broad range of pH (pH 6–11), in the presence of added excess salt and in a wide range of polar solvents (Supporting Information, Figure S6 and Table S1).

The biocompatibility of PMAH-QDs was investigated in different cell lines (A549, Hela, MB-MDA-231, 293T, and RAW 254.7 cells) using a MTS cell proliferation assay. There was no significant change in cell proliferation for PMAH-QDs (400 nM QDs highest concentration tested, see Supporting Information, Figure S7) in all five types of cell lines relative to the untreated control, suggesting that as-prepared QDs were biocompatible.

Baculovirus, a widely applied vector of gene delivery, was chosen as the model system for the living-virus labeling study.[12] First, the viruses were modified with a clickable group (DBCO, 4-dibenzocyclooctynols) using DBCO-PEG_4-NHS. The tetraethylene-glycol moiety was used as a hydrophilic linker to space the group from the viral surface and minimize possible steric hindrance. Because click chemistry can be conducted under mild conditions and allows a selective conjugation within the extremely complex biological systems, further addition of N_3–PMAH-QDs allowed the specific QD-labeling of the viral particle by cycloaddition between the azide moieties on the QDs and the DBCO tag on the virus. To ensure the QDs were coupled to the virus, the virus particles labeled with QDs were further stained with SYBR gold (a fluorescent dye to label the viral nucleic acids). Virus-QDs conjugations were confirmed by two-color colocalization fluorescence imaging studies (Figure 4a). The fluorescence signal of the QDs (red) on the viral

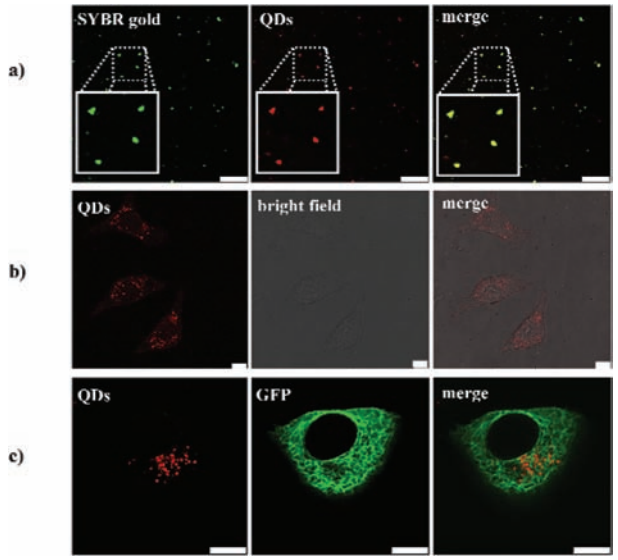

Figure 4. (a) Two-color colocalization fluorescence imaging studies to confirm virus–QDs conjugations. Virus particles labeled with QDs (red) and the viral nucleic acids stained with SYBR gold (green). The colocalization of the green fluorescence and the red fluorescence indicated efficient labeling virus with QDs. (b) Confocal imaging analysis to confirm the entry and internalization of virus into cells. (c) Gene expression analysis by confocal microscopy. The resulting GFP expression on tubulin was analyzed by confocal imaging. The gene expression begins within 4–6 h of transduction and is completed after an overnight period. Scale bars: 10 μm.

surface was readily detected, and most of QDs were colocalized with the signals generated by the SYBR gold staining (green).

We further examined whether the QD-labeled viruses remained actively infectious. The labeled viruses carrying a Tubulin-green fluorescent protein (GFP) reporter gene were added into A549 cells. After incubation at 37 °C for 1 h, QD labeled-virus effectively entered into the cytoplasma of A549 cells (Figure 4b). The viral-infected cells were then incubated for another 24 h to ensure the expression of a reporter gene in the host cells. As shown in Figure 4c, there was a significant expression of GFP gene in the cells, indicating that viruses labeled with these compact QDs by click chemistry successfully maintained their infectivity.

In summary, we have synthesized a novel class of multifunctional imidazole-based multidentate polymer ligand to yield conjugation-ready QDs with superior stability, while minimizing the hydrodynamic size. Furthermore, we successfully developed an efficient living-virus labeling method by QDs

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via strain-promoted click chemistry, which could also be readily adapted to label other types of viruses. As compared to the conventional QD probes, labeling living-viruses with these much smaller compact QDs can potentially offer a promising means to the real time of tracing viral particles with a minimal disturbance of virus-host interaction during virus tracking studies.

■ ASSOCIATED CONTENT

Supporting Information

Figure S1−Figure S7, Table S1, experiment procedures and characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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