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A Facile One-Step in situ Functionalization of Quantum Dots with Preserved Photoluminescence for Bioconjugation

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Quantum dots (QDs) have emerged as a new class of fluorescent label with unique optical properties.[1] Recent advances in water-soluble and bioconjugated QDs have made them very appealing for biosensing and bioimaging.[2−11] Among these, QDs with CdSe core and ZnS shell have been the most used optical material for bioconjugation. However, CdSe/ZnS core−shell QDs are synthesized in organic medium and contain a layer of outside capping ligand of tri-*n*-octylphosphine oxide (TOPO),[12,13] which makes them insoluble in aqueous solution. Different methods have been developed to make CdSe/ZnS QDs water-soluble so that biomolecules can be either covalently or noncovalently attached to the surface of QDs.[9,10] In the early development of water-soluble QDs, water-soluble CdSe/ZnS QDs were generated by ligand exchange of TOPO with mercaptocarbonic acid,[3,9] which ought to be greatly advantageous due to its simplicity. The carboxylic acid group was further coupled with various biomolecules. However, it was found that the quantum yield (QY) of the QDs after the ligand exchange dropped significantly.[14] Although the mechanism of the PL decrease is still not clear, it has been attributed to ligand exchange between the new ligand and the original capping TOPO molecules, during which surface defects might be generated.[15]

Herein we report a new and simple method that allows simultaneous *in situ* functionalization of CdSe QDs by mercaptocarbonic acid and surface passivation by a thin ZnS shell. This method avoids the second-step ligand exchange after the core−shell formation and yet preserves high QY and stability of the QDs. We demonstrated that such prepared QDs can serve as an excellent nanomaterial for bioconjugation. Different antibodies were conjugated to the mercaptopropyl acid (MPA)-functionalized QDs and resulted in highly stable and highly photoluminescent nanoparticles for cell labeling purposes.

Scheme 1 illustrates our new scheme of QD preparation (Scheme 1b) and its comparison to the conventional TOPO−MPA ligand exchange method (Scheme 1a). In this new method, the starting oleylamine-capped CdSe QDs were prepared using our previously reported low-temperature synthesis method;[16] the preparation of the CdSe/ZnS core−shell QDs capped with MPA is achieved in a single step by *in situ* shell formation and ligand capping. This new strategy allows that ZnS shell formation and MPA functionalization to be executed simultaneously. By doing so, the ligand exchange step (Scheme 1a) can be eliminated. We found that this method preserves the photoluminescence (PL) intensity of the core−shell QDs in contrast to the case of the water-soluble QDs prepared by the conventional method through ligand exchange.

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Scheme 1. Preparation of MPA-Capped CdSe/ZnS Core−Shell QDs through (a) the Conventional Two-Step Surface Passivation and Ligand Exchange Method and (b) Our One-Step in situ Surface Functionalization Method

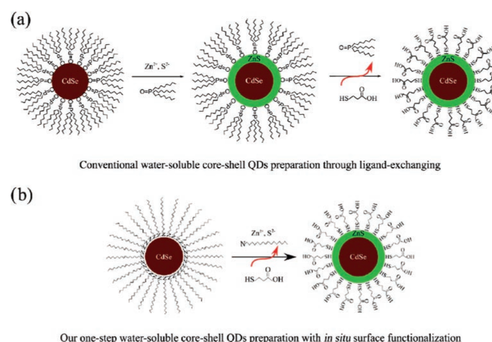

Figure 1a compares the QY of the MPA-functionalized CdSe/ZnS prepared by the two methods along with TOPO-capped core/shell product. The QY of MPA-capped CdSe/ZnS QDs is comparable with TOPO-capped CdSe/ZnS QDs produced by our new method. In contrast, the QY of MPA-capped CdSe/ZnS QDs produced using the ligand exchange method was lower by 5-fold (13 vs 62%). Photobleaching test was also carried out on the samples in solution to compare their photostability. When the QDs in water solution were exposed to a high-power continuous laser beam (2.2 W/cm^2, 400 nm) over a period of 2 h, the MPA-capped QDs from the one-step method were more stable by 250% than the ones prepared by the ligand exchange method in terms of PL intensity decrease (Figure 1b). The above results clearly indicate that the MPA-functionalized QDs prepared with the new strategy are better both in QY and photostability compared to the ones produced from the ligand exchange method. The presence of MPA on the surface of both QDs is verified by the FTIR spectrum, showing a peak at 1710 cm^{-1} which corresponds to the carbonyl group. Furthermore, ζ-potential measurement shows that MPA-capped QD prepared from our one-step method has more MPA

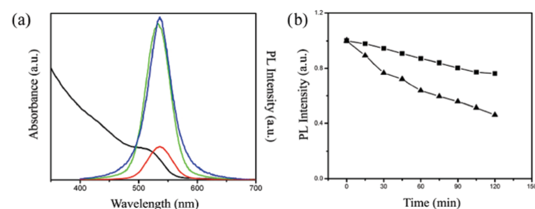

Figure 1. (a) Absorbance (black) and emission spectra of TOPO-capped CdSe/ZnS QDs (blue), MPA-capped QDs from the ligand exchange method (red), and MPA-capped QDs from the one-step method (green) by normalizing at the first excitonic peak position. (b) Photobleaching test: squares (our method); triangles (conventional method).

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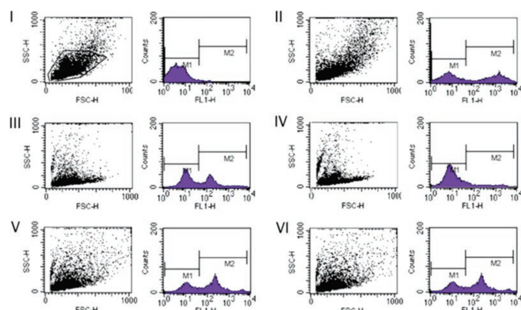

Figure 2. (I) Spleen cell only; (II) spleen cell labeled with FITC-conjugated Ab, positive control; (III) spleen cell treated with free MPA-capped QDs; (IV) spleen cell treated with free MPA-capped QDs in the presence of BSA; (V) spleen cell labeled with QD−Ab conjugate; (VI) QD−Ab conjugate labeled spleen cell in the presence of BSA.

molecule covered on the QD surface, which may result in its more robust properties (see Supporting Information).

To prove that the MPA-capped QDs using our new strategy are good materials for conjugating biological molecules, antibodies were conjugated to the QDs through a coupling reaction between the carboxylic group of MPA and an amino group from the proteins using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) as the coupling activator. We have tested our bioconjugation for cell labeling using both flow cytometry and cancer cell imaging through antibody/antigen binding.

Figure 2 shows the flow cytometry results (see Supporting Information for experimental details). We used mouse spleen cells as an example. MPA-capped CdSe/ZnS QDs are conjugated with goat anti-mouse IgG F(ab′)$_2$, which are targeted to IgM on the surface of the mouse spleen cells. The behavior of cell recognition through an antibody (Ab)-conjugated QD with mouse spleen cell is compared to that of fluorescein isothiocyanate (FITC)-conjugated Ab (positive control) and free QDs without Ab conjugations (negative control). Data in I and II show the unlabeled cell alone and FITC-labeled cells, respectively. In the histograms, M1 and M2 are the two populations of cells that are unlabeled and fluorescent-labeled, respectively. When plain QDs (without Ab) are mixed with the cells, two populations of the cells showed up (in III). When BSA is added to the solution, the QD on the cell surface can be displaced completely (IV); therefore, this binding is due to nonspecific interactions of QDs with the cell surface. In contrast, the QDs labeled with the Ab can specifically and tightly target the cell surface through the antibody−antigen interaction, which cannot be displaced by BSA as shown in V and VI. These results clearly manifest that goat anti-mouse IgG F(ab′)$_2$ is successfully conjugated to the QD with intact activity.

We further verified the bioconjugation using fluorescent imaging of cancer cells stained by the MPA-capped QDs (prepared using our method) conjugated with antibody AIF (D-20). As shown in Figure 3, the anti-AIF conjugated green QDs recognized the apoptotic induced factor (AIF) antigens in the cytoplasm of an esophageal squamous cell carcinoma (ESCC) cell line, EC-0516 (Figure 3b). The nuclei are stained by 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI), which is an organic fluorophore showing a blue fluorescence when bound to DNA (Figure 3a−c). Nonspecific binding of the MPA-capped QD with the cells is minimal, showing a low fluorescence background (Figure 3a). A photobleaching experiment was performed on the cells which have the cytoplasm labeled with the green anti-AIF QDs and the nuclei labeled with DAPI. It is obvious that DAPI was bleached after 5 min while the green anti-AIF QDs were still bright, showing robust photostability of the QD−Ab conjugates using our functionalization method (Figure 3c). In our experience, the MPA-functionalized QDs

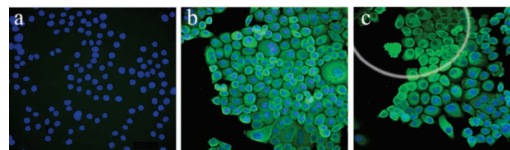

Figure 3. Fluorescence microscope images of EC-0516 cells. (a) The negative control experiment: the cell nucleus was labeled with DAPI, and the cell was nonspecifically labeled with the MPA-capped QDs (no Ab), showing a low green fluorescence background. (b) MPA-capped QDs conjugated with anti-AIF Ab. The target protein AIF located specifically in the cytoplasm, and the nuclei were labeled with DAPI. (c) Photobleaching experiment indicates that the organic dye was bleached while the QD survived. The circled area was illuminated for 5 min before the sample stage was translated halfway to a nearby area.

from our method are stable in buffer for more than 6 months stored at 4 °C. The QD−Ab is still active for cell labeling even after 2 months of the conjugation.

In summary, we presented a novel approach for MPA functionalization of CdSe/ZnS core−shell QDs. The functionalized QDs prepared using this approach do not reduce the photostability and quantum yield. Furthermore, the *in situ* approach is appealing since any biomolecules carrying or premodified with a thiol group may be directly conjugated with QDs in a single step without using MPA as a media. In this case, coupling chemistry on the QD surface could be avoided.

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Supporting Information Available: Experimental methods, TEM images, EDXS, dynamic light scattering, ζ-potential, and FTIR results. This material is available free of charge via the Internet at http://pubs.acs.org.

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