Calixarene-coated water-soluble CdSe–ZnS semiconductor quantum dots that are highly fluorescent and stable in aqueous solution

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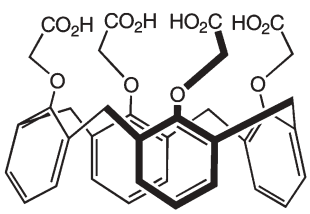
A simple method for the preparation of highly fluorescent and stable, water-soluble CdSe–ZnS quantum dots is reported using calix[4]arene carboxylic acids as surface coating agents; the coating of the surface with the calixarene and the conjugation of antibodies to the quantum dots are confirmed by fluorescence correlation spectroscopy.

Colloidal semiconductor quantum dots (QDs) have great potential as a new class of fluorophores for biological and biomedical imaging because of high brightness, long-term photostability and single-light source excitation for multi-colored QDs.[1] The syntheses of monodispersed fluorescent QDs are generally performed in organic solvents with surface passivation by alkyl phosphine oxides such as trioctylphosphine oxide (TOPO).[2] The resulting QDs are soluble only in nonpolar solvents, making them difficult to use for biological application. So far, many synthetic methodologies for the preparation of water-soluble QDs have been developed by surface modifications with amphiphilic compounds including thiols,[1,3] polymers,[4] and phospholipids.[5a] For example, thiol compounds such as mercaptopropionic acids (MPA) or mercaptoundecanoic acids (MUA) have been widely used as surface coating agents for the preparation of water-soluble CdSe–ZnS QDs.[1,3] However, thiol coating of the CdSe–ZnS QDs causes a significant decrease in the quantum yield of QD fluorescence,[6] and the resulting QDs show poor stabilities in water.[3c,6] Surface coating with polymer and lipids can preserve the quantum yield of QD fluorescence, but the size of the QDs tends to be much larger than that of the initial QDs.[4b,5] The large size of QDs is not suitable for use in fluorescence resonance energy transfer (FRET)-based research.[7] Here, we report a very simple method for the preparation of water-soluble semiconductor CdSe–ZnS QDs using calix[4]arene carboxylic acids as surface coating agents. The calixarene coating secures the high emission efficiency (*ca.* 30%), the smaller size of QDs (<10 nm in diameter), and the coupling of biomolecules to the surface of the QDs.



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Calixarene-coated CdSe–ZnS QDs were prepared by mixing calix[4]arene carboxylic acids[8] (**1**) and TOPO capped CdSe–ZnS QDs[2b,c] in tetrahydrofuran at room temperature. After deprotonation of the carboxylic groups of the calixarene, water-soluble CdSe–ZnS QDs could be obtained.[9] Fig. 1 shows the fluorescence spectra of water-soluble CdSe–ZnS QDs coated by **1**, MPA, and MUA. The **1**-coated QDs exhibit higher emission efficiency compared to the MPA- and MUA-coated QDs by a factor of 3.5–20. The quantum yields are estimated to be 0.28 and 0.34 for the **1**-coated QDs with emission peaks at 575 nm and 610 nm, respectively.[10] It is well known that the calix[4]arene **1** can selectively bind Na^+ cations.[8] We checked the effect of Na^+ and K^+ cations on the fluorescence intensity of **1**-coated QDs (610 nm emission) in water. The fluorescence intensity was almost constant up to 10 mM NaCl (or KCl), and significant effects of the cations on the fluorescence spectra of **1**-coated QDs were not observed. However, in the presence of large amounts of Na^+ or K^+ (100 mM), fluorescence quenching of *ca.* 10% was observed.

To estimate the Stokes–Einstein hydrodynamic size of the QDs, fluorescence correlation spectroscopy (FCS) was used. FCS measures the fluorescence autocorrelation function $G(\tau)$[11] which gives the diffusion rates of fluorescent molecules in solution. The $G(\tau)$ curves are measured by using a compact FCS system (C9413, Hamamatsu Photonics K. K., Japan). Fig. 2 shows the $G(\tau)$ of **1**- and thiol-coated QDs which have an emission peak at 610 nm in tetraborate buffer. For comparison, a $G(\tau)$ curve for green fluorescent protein (GFP) is also shown. All of the $G(\tau)$ curves are fitted using a simple one-component model.[12] The diffusion time[12] of the **1**-, MUA- and MPA-coated QDs, and GFP was

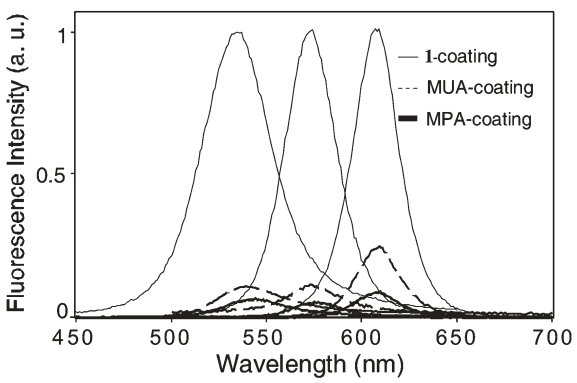

Fig. 1 Fluorescence spectra of **1**- and thiol-coated CdSe–ZnS QDs in water. The QDs are prepared from TOPO capped QDs with emission peaks at 535, 575, and 610 nm. The absorbance at excitation wavelengths (440 nm for 535 nm emission, or 480 nm for 575 and 610 nm emission) is adjusted to be 0.05 for all QDs.

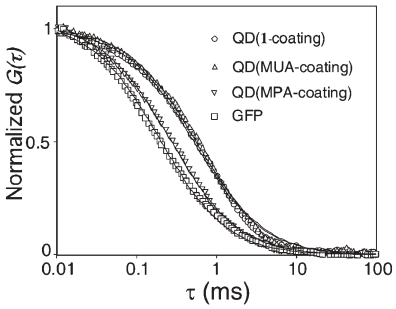

Fig. 2 Fluorescence autocorrelation curves for the water-soluble QDs with an emission peak at 610 nm and GFP in tetraborate buffer (pH = 9.2). The correlation curves are fitted by using one-component model (ref. 12*c*).

found to be 0.52, 0.53, 0.25, and 0.17 ms, respectively. By using the value of the diffusion time (0.74 ms) measured for 14 nm fluorescent latex beads (Molecular Probes, Inc. USA), the hydrodynamic sizes are calculated[13] to be 9.8, 10, 4.7, and 3.2 nm in diameter for **1**-,[14] MUA- and MPA-coated QDs, and GFP,[15] respectively. The difference in the size of the MPA- and MUA-coated QDs suggests the ligand-exchange of TOPO molecules with the thiols. The size of **1**-coated water-soluble QDs is similar to that of MUA-coated QDs and is two-times larger than that of MPA-coated QDs, indicating that the calix[4]arene carboxylic acids form a bilayer structure with TOPO molecules that stabilize the surface of QDs. The high emission efficiency of **1**-coated QDs may result from the surrounding of the QD surface by the benzene units of the calixarene. The hydrophobic benzene layer would make a high shield towards the access of water molecules to the QD surface. As a result, surface quenching of excitons in the QD can be reduced.[3*d*]

The colloidal stability of the **1**-coated QDs in tetraborate buffer is estimated by fluorescence intensity after surface coating as a function of time at 25 °C, as shown in Fig. 3. The fluorescence intensity of the **1**-coated QDs gradually increases and becomes constant after *ca.* 100 h. It should be noted that the spectral widths (the full width at half maximum) of the **1**-coated QDs are almost constant (29 nm) over time. This indicates that no surface deterioration and aggregation occurs in the **1**-coated QDs, and these QDs are stable in aqueous solution. For thiol-coated CdSe–ZnS QDs, poor stabilities resulting from the aggregation and

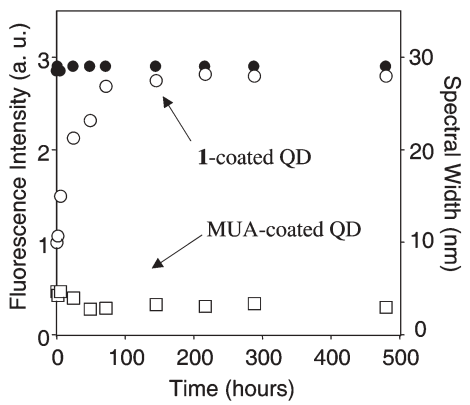

Fig. 3 Changes in fluorescence intensity (open circles and squares) of water-soluble CdSe–ZnS QDs (610 nm emission) after surface coating in tetraborate buffer (pH = 9.2). For **1**-coated QDs, changes in spectral widths are also shown (closed circles).

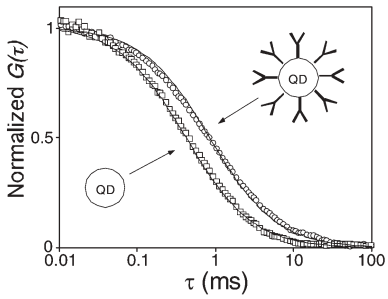

Fig. 4 Fluorescence autocorrelation curves for **1**-coated water-soluble CdSe–ZnS QDs with an emission peak at 610 nm before (open squares) and after (open circles) the addition of GFP antibody (PBS buffer, pH = 7.4) in the presence of EDC.

precipitation of the QDs have been reported in aqueous solution.[3*c*,6] In fact, it was observed that fluorescence intensity of the MUA-coated QDs gradually decreases and loses 37% of its initial fluorescence intensity after 4 days (Fig. 3).

To test the utility of calixarene-coated QDs for biological application, the coupling of antibodies to the calixarene-coated QDs was performed using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC).[16] Fig. 4 shows the fluorescence autocorrelation curves of **1**-coated QDs before and after the addition of GFP antibody (Mouse monoclonal IgG to GFP, Abcam Ltd, UK). Upon addition of the antibody, the autocorrelation curve of the **1**-coated QD immediately shifts to the right and the curve becomes stationary within 10 min. The diffusion time of the **1**-coated QDs changes from 0.52 ms to 1.0 ms. This change in the diffusion time indicates that the size of the antibody-labeled QD increases to be about twice that of the initial QD. From the value of the diffusion time, the size of the antibody-labeled QD can be estimated as *ca.* 20 nm in diameter, suggesting that the antibodies bind to the surface of the QD and form a single monolayer.

In conclusion, we have presented a simple and convenient method for the preparation of biocompatible CdSe–ZnS QDs using calix[4]arene carboxylic acids as surface coating agents. The calixarene-coated QDs have high emission efficiency and long-term stability in aqueous solution. In addition, the size of the calixarene-coated QDs is relatively small (<10 nm in diameter) in comparison with the water-soluble QDs earlier reported.[5*b*] In the calixarene-coated QDs, calixarene molecules are bound to TOPO capped QDs by hydrophobic interaction. To avoid possible disintegration of the calixarene-coating in the case of interaction with the cell membrane (lipid bilayer), cross linking between the intramolecular carboxylic groups at the QD surface should be necessary. So far, many kinds of amphiphilic calixarene derivatives attaching sugars, peptides, chiral residues, and so on, have been designed.[17] We believe that calixarenes will offer a variety of new functions for the surface architectures of colloidal semiconductor QDs.

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7 The rate of FRET depends strongly on the donor–acceptor distance r, being inversely proportional to r^6.

8 The calix[4]arene carboxylic acid **1** was synthesized according to the literature methods: (*a*) A. Arduini, A. Pochini, S. Reverberi and R. Ungaro, *J. Chem. Soc., Chem. Commun.*, 1984, 981; (*b*) S.-K. Chang and I. Cho, *J. Chem. Soc., Perkin Trans. 1*, 1986, 211.

9 1 mg of the TOPO capped CdSe–ZnS QDs was dispersed in 1 mL of tetrahydrofuran and 30 mg of calix[4]arene carboxylic acids **1** was added. After the mixed solution was sonicated for 30 s using a bath type sonicator, 3 mL of dimethylformamide was added. Then, 30 mg of potassium *tert*-butoxide was slowly added for the deprotonation of the carboxylic groups of the calix[4]arene. The resulting precipitate consisting of the QDs coated by **1** was separated using a centrifuge. The sedimented precipitate was then dispersed in 20 mL water. The aqueous QD dispersion was sonicated for 5 min and filtered using a 0.2 μm disposal filter.

10 The quantum yield of the **1**-coated QDs with an emission peak at 535 nm is not estimated because the absorption of the QDs does not show a distinct peak.

11 $G(\tau)$ is defined as $G(\tau) = <\delta I(\tau)\delta I(0)>/<I(\tau)>^2$, where $I(\tau)$ is the fluorescence intensity at the time τ, and $\delta I(\tau) = I(\tau) - <I(\tau)>$. The brackets denote ensemble average.

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13 Assuming a spherical body of fluorophores, the size of the fluorophores can be evaluated by the following relationship: $r_1/r_2 = \tau_1/\tau_2$, where r_i and τ_i are the radius and diffusion time of the fluorophore i.

14 For the **1**-coated QDs with emission peaks at 535 nm and 575 nm, the hydrodynamic size was determined to be 6.2 nm and 7.9 nm in diameter, respectively.

15 The GFP molecule forms a nearly perfect cylinder 4.2 nm long and 2.4 nm in diameter. See: M. Ormö, A. B. Cubitt, K. Kallio, L. A. Gross, R. Y. Tsien and S. J. Remington, *Science*, 1996, **273**, 1392.

16 10 μL of an aqueous dispersion of the QDs (*ca.* 1 μM) coated by **1** was diluted to 80 μL by addition of 70 μL PBS buffer (pH = 7.4) and 10 μL of EDC aqueous solution (5 mg mL^{-1}) was added. Then 10 μL of GFP antibody (5 μM) was added to the QDs aqueous solution and coupling reaction was allowed to proceed at room temperature. From the measurements of fluorescence autocorrelation curves of the QDs, it was found that the reaction was completed within 10 min. For the coupling reaction using EDC, see: G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, NewYork, 1996.

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