Nanocomposites

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Synthesis of Silica-Coated Semiconductor and Magnetic Quantum Dots and Their Use in the Imaging of Live Cells**

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Colloidal semiconductor nanocrystals or quantum dots (QDs) exhibit interesting optical properties (sharp emission and broad absorption), which are governed by the quantum confinement effect. They are of great interest for applications in electronics[1] (single-electron transistors, lasers, QD glass, light-emitting diodes) and in biology (biolabeling and bioimaging).[2] Conventional QD systems have a core–shell architecture, consisting of a lower-band-gap QD (e.g. CdSe) capped with a higher-band-gap material (e.g. ZnS).[3]

The applications of QDs and magnetic nanoparticles (MPs) in biolabeling[2] and magnetic resonance imaging,[4] respectively, have led to major recent advances in the field of biological and biomedical imaging. A combination of optical and magnetic properties in a single material would enable simultaneous biolabeling/imaging and cell sorting/separation.[5] Nanocomposites consisting of semiconductor and magnetic nanoparticles, known as magnetic quantum dots (MQDs),[6] are of great interest as a new class of materials. Hybrid nanocomposites,[7a] such as Fe_3O_4–Au and CoPt–Au, have revealed the potential application of such bifunctional materials.[7b,c]

Herein, we describe a facile synthesis of a bifunctional nanocomposite system consisting of Fe_2O_3 magnetic nanoparticles and CdSe quantum dots. CdSe QDs were grown onto preformed Fe_2O_3 cores at high temperature (300 °C) in the presence of organic surfactants, yielding either heterodimers or a homogeneous dispersion of QDs around the cores. The resulting MQDs exhibited superparamagnetism and tunable optical emission properties. The plain QDs and MQDs (without ZnS capping) were coated with a thin silica shell and used for the labeling of different live cell membranes through a simple bioconjugation method. The approaches described in this study would be useful and cost-effective for biological and biomedical applications requiring both fluorescence and magnetic characteristics.

Our MQD system is a Fe_2O_3–CdSe nanocomposite prepared by a facile synthesis method. It exhibits an efficient quantum yield over a broad range of colors without the need for ZnS capping of the CdSe QDs. The Fe_2O_3–CdSe MQDs are rendered water-soluble and non-toxic by using a simple silica-coating process.

In cell biology, the cell membrane acts as the defining principle for many biological processes. Significant efforts have been devoted towards understanding the mechanisms used by cells to allow proteins, DNA, and ions to directly traverse biological membranes.[8] This study describes a new bioconjugation approach for the QDs and MQDs to target the cell membrane for the imaging of live cells.

CdSe QDs were synthesized using stearic acid, trioctylphosphine oxide (TOPO), and hexadecylamine (HDA) according to reported procedures.[9] The TOPO/HDA-passivated QDs in growth solution displayed a high quantum yield, almost comparable to that of ZnS-capped CdSe QDs. Although ZnS capping improved the quantum yield significantly by providing more surface passivation, it involved the use of noxious reactants, such as diethylzinc (which is highly air-reactive) and hexamethyl disilathiane (which is pungent).[3] In contrast, TOPO/HDA-passivated QDs were less expensive and less time-consuming in synthesis.

In the synthesis of the MQDs, CdSe QDs were grown onto Fe_2O_3 magnetic cores. First, Fe_2O_3 nanoparticles were synthesized in oleic acid and dioctyl ether by the decomposition of iron pentacarbonyl, according to a slightly modified published procedure[10] (see Supporting Information). MQDs were synthesized as described in the Experimental Section. Figure 1 a and b show photographs of MQDs taken before and after magnetic harvesting, illustrating the effective separation of the nanocomposite particles with the use of a magnetic field. The fluorescence of the magnetically harvested MQDs under UV excitation at 365 nm is illustrated in Figure 1 c and d. MQDs with different emission colors were achieved, indicating the tunability of the size of the CdSe dot in the MQD system.

There have been a few reports on magnetic heterodimers[7a] (Fe_3O_4–Au,[7b] CoPt–Au,[7c] Fe_2O_3—CdS,[7d] and FePt–CdS[11]) and core–shell particles (Co–CdSe),[12] but the synthesis of MQDs with tunable optical and magnetic properties has not yet been reported. MQDs have also been prepared by synthesizing separately the individual nanoparticles (i.e. non-aqueous QDs and aqueous MPs,[13] or non-aqueous QDs and non-aqueous MPs[14]) and then linking them together through suitable ligands.[13,14] However, there has been little control

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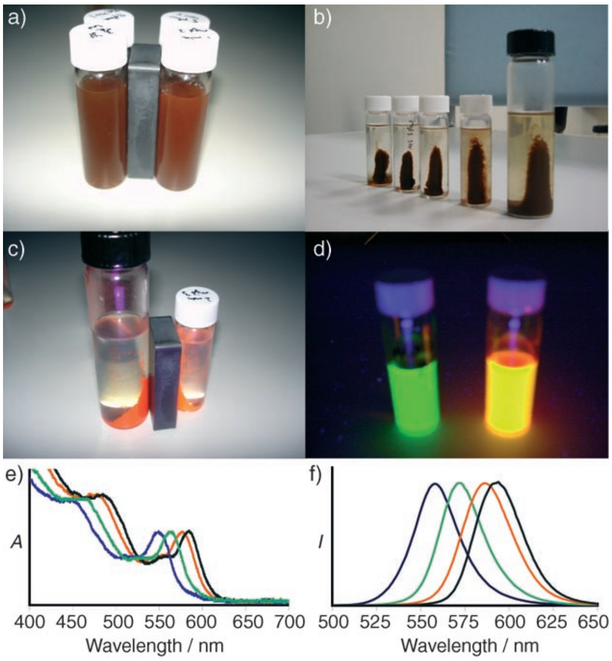


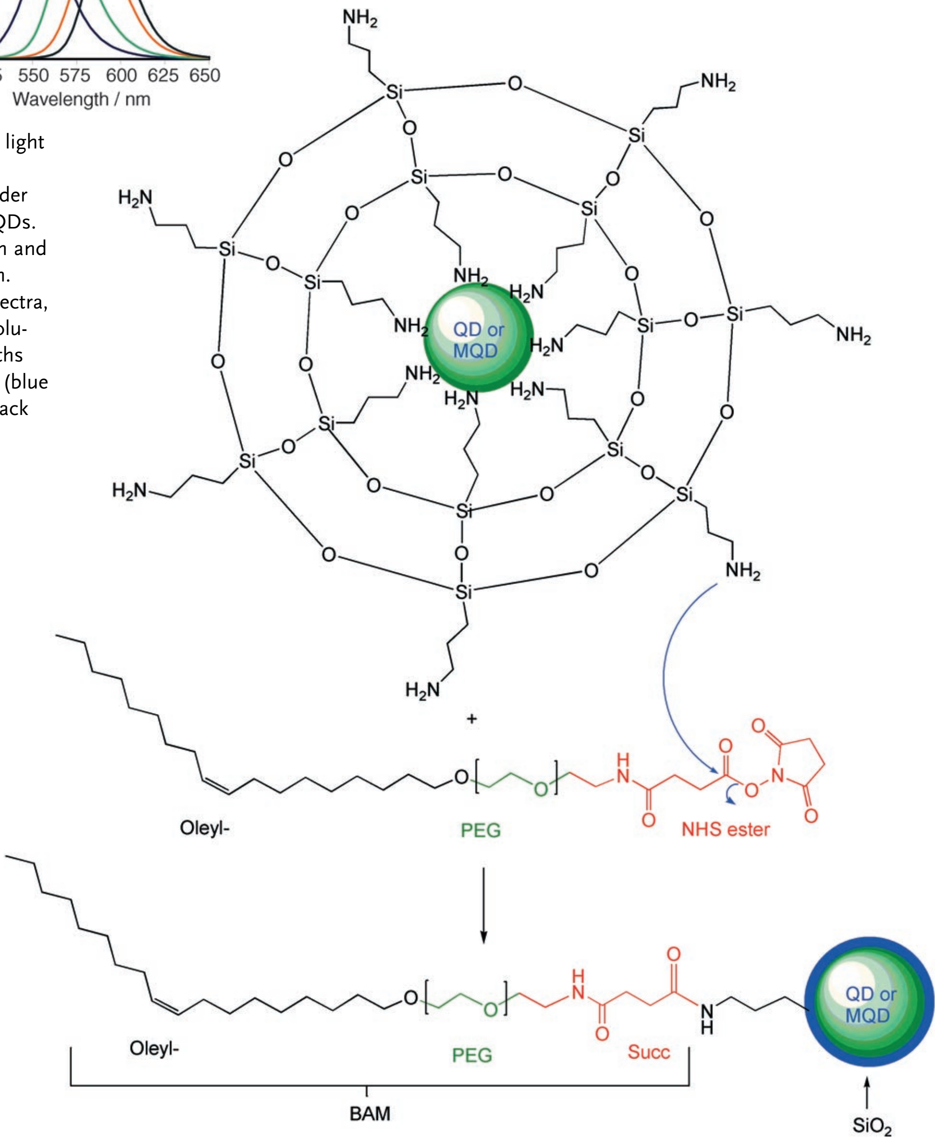
Figure 1. Photographs of MQDs under white light before (a) and after (b) magnetic harvesting. c) Magnetic and luminescence properties under UV excitation at 365 nm of harvested red MQDs. d) Harvested and chloroform-dispersed green and orange MQDs under UV excitation at 365 nm. e, f) Normalized absorption and emission spectra, respectively, of various SiO_2-MQDs in PBS solution. The absorption and emission wavelengths are characteristic of the size of the CdSe dot (blue 2.0 nm, green 2.7 nm, orange 3.6 nm, and black 4.0 nm).

over the linking of the two materials. In contrast, our present approach to MQDs allows the controlled tuning of both luminescence and magnetic properties.

Silanization in a reverse microemulsion produced a thin silica coating on bare CdSe QDs or MQDs with surface NH_2 groups (see Scheme 1). When QDs or MQDs capped with HDA were dispersed in micelles, there was a tendency for the HDA to leach from the CdSe surface. The addition of a silane precursor, aminopropyl trimethoxysilane (APS), introduced amine groups that were attached to the surface of the QDs or MQDs. With the sequential addition of tetramethylammonium hydroxide (TMAH) in 2-propanol/methanol and water, a reverse microemulsion was formed. The methoxy groups in the APS were hydrolyzed and condensed with another APS, exposing surface amine groups on the silanized QDs or MQDs (SiO_2-QDs or SiO_2-MQDs).

Figure 1 e and f show the absorption and emission spectra, respectively, of SiO_2-MQDs in phosphate buffer saline (PBS) solution. These spectra did not change with silanization, which confirms that the optical properties of the QDs were retained after silica coating. The emission spectra were narrow, with a full-width at half-maximum (FWHM) of less than 40 nm. The quantum yields of the MQDs before and after silica coating were 13–18 % and 8–10 %, respectively, and increased with increasing QD size (≈2–4 nm) and emission wavelength (≈550–600 nm). These values were significantly higher than the quantum yield of 3.2 % reported for FePt–CdS MQDs in the organic growth solution.[11]

High-resolution transmission electron microscopy (HRTEM) illustrates the heterodimer structure in the MQD system (Figure 2). Fe_2O_3 nanoparticles and CdSe QDs were



Scheme 1. Schematic of a CdSe QD or Fe_2O_3–CdSe MQD and its silanization in a reverse microemulsion. The surface NH_2 groups are then treated with a bio-anchored membrane (BAM) to form a covalent amide bond. The oleyl groups are used to anchor the quantum dots to the cell membrane during biolabeling. PEG = poly(ethylene glycol); NHS = *N*-hydroxysuccinimide; Succ = succinimide.

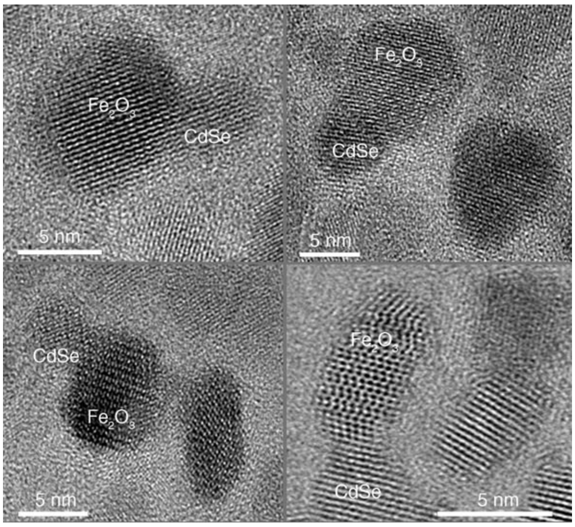


Figure 2. TEM images of heterodimers of MQDs after magnetic harvesting. The larger particles are Fe_2O_3 magnetic nanoparticles (8–10 nm), and the smaller particles are CdSe quantum dots (4–5 nm).

approximately 8–10 nm and 4–5 nm in diameter, respectively. A detailed analysis of the HRTEM images is provided in Figure S2–S4 in the Supporting Information. In a recent study, it was shown that γ-Fe_2O_3 and II–VI QDs (e.g. CdS) have a substantial lattice mismatch and are therefore composed of both dimers and isolated particles.[7d] In the present case, in addition to dimers, QDs were also found to be highly dispersed around the magnetic nanoparticles (Figure S4 in the Supporting Information). Scanning transmission electron microscopy (STEM) confirmed the presence of Cd, Se, and Fe in the MQD sample (Figure S5 in the Supporting Information). The magnetic properties of the as-harvested MQDs (Figure 1c and Figure 2) were investigated with a superconducting quantum interference device (SQUID). A superparamagnetic behavior characteristic of small magnetically ordered structures was clearly observed.[15]

The silica-coating method developed for metals (e.g. Au and Ag)[16] is not readily applicable to hydrophobic semiconductor QDs, as the latter are coated with surfactants such as TOPO and HDA. Although the silanization approach was reported earlier for preparing water-soluble QDs, the conventional methods were complex and in some cases difficult to reproduce. Also, all the silanization and silica-coating procedures were applied mostly to ZnS-capped CdSe QDs. In carrying out the reported coating procedures,[17] the fluorescence of amine-capped QDs was found to be quenched in the first step of exchange with mercaptopropyl trimethoxysilane (MPS), a common silane precursor used for silanization. Recently, we developed a reverse microemulsion method for deriving water-soluble QDs. While these silica-coated amine-capped QDs exhibited less cytotoxicity, they showed a low quantum yield owing to the thick silica coating and still suffered from a poor stability in buffer, which prevented their further application in bioconjugation.[18]

In this study, the silanized QDs or MQDs were conjugated to oleyl-*O*-poly(ethylene glycol)succinyl-*N*-hydroxysuccinimidyl ester, denoted as bio-anchored membrane (BAM; see Scheme 1). The surface amine groups of the silanized QDs or MQDs were used to bioconjugate them with a heterofunctional polyethylene glycol (PEG)[19] consisting of an *N*-hydroxysuccinimide (NHS) ester at one end and an oleyl group at the other end. The reaction between the amine group and the NHS ester resulted in covalent amide bond formation, leaving the exposed oleyl group for the effective targeting of a cell membrane. The PEG groups not only enhanced water solubility but also reduced the nonspecific adsorption of the particles.

BAM[19] was obtained from the NOF Corporation, Tokyo. We devised two methods of conjugating BAM with SiO_2-QDs and SiO_2-MQDs (see Experimental Section). Both bioconjugation methods were found to be effective. Figure 3a–c and Figure 3d show the confocal laser scanning microscopy (CLSM) images of different cell membranes labeled with BAM-SiO_2-CdSe QDs and BAM-SiO_2-MQDs, respectively. The specific labeling of live cell membranes (HepG2 human liver cancer cells, NIH-3T3 mouse fibroblast cells, and 4T1 mouse breast cancer cells) indicated the successful bioconjugation of silica-coated QDs and MQDs with a bio-anchored membrane.

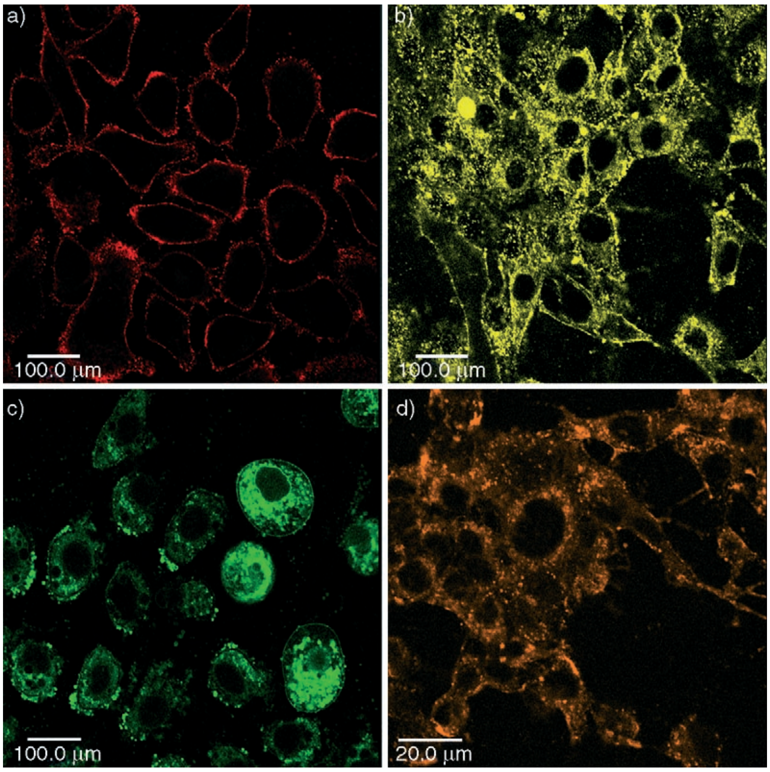


Figure 3. CLSM images showing the labeling of cell membranes with BAM-SiO_2-CdSe QDs (a–c) and BAM-SiO_2-MQDs (d). a,c) HepG2, b) NIH-3T3, and d) 4T1 cell membranes.

In conclusion, we have prepared biocompatible silica-coated QDs and MQDs that effectively target cell membranes. The synthesis, bioconjugation, and materials properties described herein give rise to a new class of multifunctional materials that allow for specific biolabeling, successful imaging of live cells, and efficient bioseparation.

Experimental Section

Synthesis of magnetic quantum dots (MQDs). In preparing the MQDs, CdO (0.05 g, 0.39 mmol) and stearic acid (0.456 g) were first heated to 150–200 °C and then cooled to room temperature. MPs (0.025 g, 0.156 mmol), TOPO (7.76 g), and HDA (7.76 g) were added to this mixture, which was then heated to 280–300 °C. Se (0.32 g, 4 mmol) dissolved in trioctylphosphine (TOP; 9.6 mL) was quickly injected into this mixture within a few seconds, and the resulting CdSe QDs were allowed to grow for different time periods (1–5 min) to yield different sizes of dots that corresponded to green, yellow, orange, and red emissions. The aliquots of growth solution were quenched by the addition of chloroform. Methanol was then added to the growth solution, and a magnet was applied to the sample-containing vial. All the particles were attracted to the magnet, leaving behind a clear solution. The harvested particles were both magnetic and fluorescent, confirming the successful synthesis of the MQD nanocomposite particles.

Silica coating of QDs and MQDs. CdSe QDs or MQDs passivated with TOPO/HDA were precipitated once with methanol, and the precipitate was dried under normal conditions at room temperature. The precipitated QDs or MQDs (4 mg) were dispersed in chloroform (1 mL). Micelles were prepared by dissolving Igepal-CO520 (polyoxyethylene(5)nonylphenyl ether; 0.2 g) in cyclohexane (4 mL) and stirring the mixture vigorously for 30 min. The QDs or MQDs in chloroform were added to the micelles along with APS (10–50 μL), and the mixture was stirred for 1 h. Next, TMAH in 2-propanol/methanol (5–20 μL) was added to the mixture. After 1 h of stirring, deionized water (20 μL) was added. The mixture was stirred for another 30 min until the bulk organic phase turned colorless, with the formation of orange/red globules on the surface of the glass vial. The colorless organic phase was then discarded, leaving behind the QDs or MQDs on the surface of the glass vial. The QDs or MQDs were washed with chloroform 3–5 times to ensure the complete removal of excess surfactants and other unreacted reagents from their surface. The silica-coated CdSe QDs and MQDs (SiO_2-CdSe and SiO_2-MQDs) were then dispersed in PBS solution (1 mL) and immediately used for bioconjugation to prevent precipitation. The second addition of APS (10–50 μL) improved the stability of the SiO_2-CdSe QDs and SiO_2-MQDs in buffer solution.

Bioconjugation of SiO_2-QDs and SiO_2-MQDs. Method 1: BAM (10 mg) was added to SiO_2-QDs or SiO_2-MQDs (4 mg mL^{-1}) in buffer. The NHS ester of BAM could readily react with the surface amine groups of SiO_2-QDs or SiO_2-MQDs to form an amide linkage between the BAM and the QDs or MQDs. Method 2: BAM (10 mg) was dissolved in anhydrous dichloromethane (1 mL), and APS (10–50 μL) was added. The mixture was allowed to react for 1 h with stirring. The solvent was evaporated, and the residue was dissolved in an aqueous dispersion (1 mL) of QDs or MQDs coated with a thin silica shell. The surface silanol groups on the silica-coated particles were reacted with the APS conjugated to BAM. The final aqueous solution was filtered through a 0.2-μm filter to remove any large aggregates.

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