Chiral Quantum Dots

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Circularly Polarized Luminescent CdS Quantum Dots Prepared in a Protein Nanocage**

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Semiconductor quantum dots (QDs) have attracted a great deal of attention because of their optically tunable light emissions, which are derived from the quantum confinement effect.[1] Unlike bulk materials, QDs utilizing these unique optical properties have a wide range of potential applications, especially in light-emitting devices, sensory materials, and bioassays. More recently, several attempts to develop QDs with optical activity have been reported both experimentally[2] and theoretically.[3] Although QDs prepared with chiral stabilizers, such as CdS or CdTe, showed significant circular dichroism (CD), their circularly polarized luminescence (CPL) was inactive. Density functional calculations revealed that the chiral stabilizer distorted the QD surface only, transmitting an enantiomeric structure to the surface layers. However, the QD core with hexagonal phase remained undistorted and achiral.[3] Herein, we hypothesize that if the whole QD crystal adopted a chiral structure, emission from the QD would express CPL activity.

Within this context, we focused on a QD prepared in a rhombic dodecahedral protein, horse spleen ferritin, as a hollow chiral template. The ferritin is composed of 24 α-helix-rich subunits with exterior and interior diameters of 12 nm and 8 nm, respectively (Figure 1 a). The ferritin ubiquitously regulates intracellular iron homeostasis, in which an iron ion is temporally stored as ferrihydride.[4] Recently, sophisticated structural analysis has revealed that the ferrihydride in the ferritin was structurally distorted, reflecting a chiral nanosphere comprised of the α-helix-rich subunits.[5] Along with the ferrihydride, a variety of QDs were prepared within the ferritin, in which the flow of ion precursors in the hollow core took place through specific channels at the interface of the apoferritin subunits.[6] Here 72 glutamate residues on the interior surface of the apoferritin shell are thought to promote the formation of the QDs (Figure 1 b).[6f] Therefore, we expected that the QD prepared in the hollow chiral template would adopt a chiral crystal structure, resulting in CPL activity.[7]

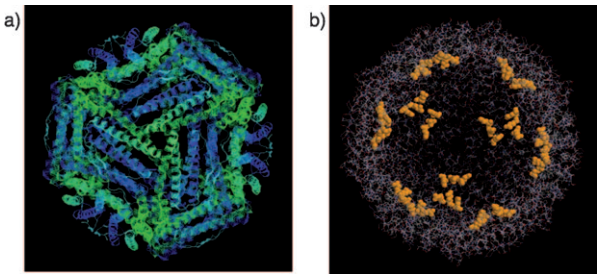

Figure 1. a) Illustration of apoferritin from a threefold channel (ribbon model) and b) a cross-sectional view (slab 65 %). The 72 glutamate residues on the interior surface are depicted as a yellow space-filling model.

We first demonstrated that a water-soluble CdS QD prepared in ferritin (CdS@ferritin) exhibits significant left-handed CPL emissions from direct transition and surface-trapping sites of the CdS QDs. Furthermore, wavelengths of the photoluminescence (PL)/CPL were modulated by laser photoetching. Figure 2 a,b shows PL and CPL spectra of the apoferritin and CdS@ferritin with an excitation wavelength at 325 nm. The apoferritin exhibited an intense PL band with λ_{max} at 396 nm, which originated from post-translationally modified dityrosine in the ferritin shell (Figure 2 a, blue line; Figure 2 c),[7] whereas its CPL signal was negligibly small (Figure 2 b, blue line). On the other hand, CdS@ferritin afforded a broad PL band with λ_{max} at 780 nm and a shoulder peak at ca. 498 nm, although the PL signal from ferritin disappeared, which was probably due to intramolecular energy migration from the ferritin shell to the CdS QD (Figure 2 a, red line; Figure 2 d). Furthermore, the CPL of CdS@ferritin showed an intense band at 498 nm and a broad band at 780 nm (Figure 2 b, red line). To quantify these PL/CPL spectra, Kuhn's anisotropy factor (g_{Lum}) was calculated: g_{Lum} is defined as $g_{Lum} = 2(I_L - I_R)/(I_L + I_R)$, where I_L and I_R indicate the signals for left- and right-handed CPL, respectively (Figure 2 e).[8] Consequently, the g_{Lum} values at 498 nm

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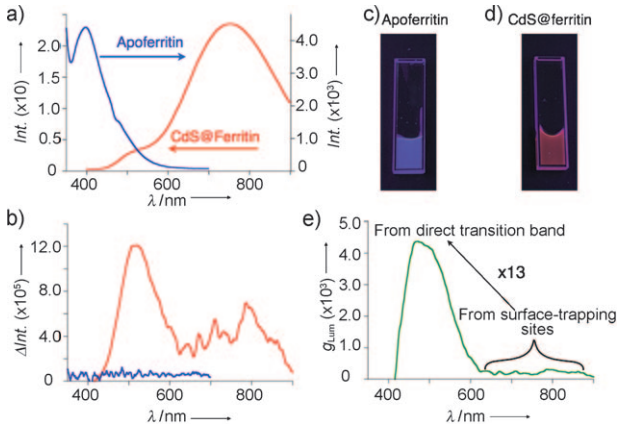

Figure 2. a) PL and b) CPL spectra of apoferritin (blue) and CdS@ferritin (red). All spectra were obtained using excitation at 325 nm. Photographs of c) apoferritin and d) CdS@ferritin. The samples were irradiated by a UV lamp with an emission wavelength of 310 nm. e) Kuhn's anisotropy factor (g_{Lum}) as a function of wavelength.

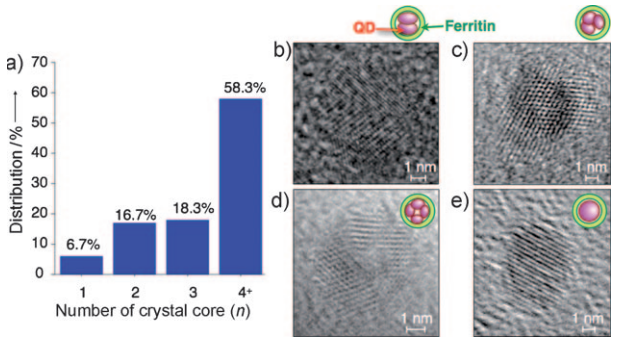

Figure 3. a) Distribution of numbers of CdS QD cores in CdS@ferritin and b–e) HRTEM images of individual CdS@ferritin containing b) two, c) three, d) four, and e) one single crystal.

and at 780 nm were determined to be about 4.4×10^{-3} and 3.5×10^{-4}, respectively. Thus, the quantified CPL signal from the direct transition band was approximately thirteen times greater than that from the surface-trapping sites, although apparent emission was dominated by the surface-trapping sites. Furthermore, these g_{Lum} values were identical to those of organic molecules excited through n–π* transitions, such as camphorquinone.[8]

The question arises as to why CdS@ferritin exhibited double CPL emissions from both the direct transition and surface-trapping sites. If the surface-trapping sites existed in CdS@ferritin, intramolecular energy migration from the direct transition band to these sites would occur within the fluorescence lifetime, resulting in emission only from these sites. This led us to hypothesize that CdS@ferritin consists of single and multiple crystals, resulting in emission from the direct transition and surface-trapping sites, respectively.

To further clarify the origins of the double CPL emissions of CdS@ferritin, we performed HRTEM on individual particles, and numbers of crystal cores were calculated for 160 CdS@ferritins (Figure 3a). As expected, the vast majority of CdS@ferritin particles were made of multiple crystals with two (Figure 3b), three (Figure 3c), and four (Figure 3d) crystal cores. The CdS QDs were fully packed in the ferritin cavities; therefore, the CdS QDs appear to be passivated with chelating amino acid residues, such as glutamate.

On the other hand, approximately 7% of the CdS@ferritin consisted of single crystals only (Figure 3e). Therefore, we concluded that the CPL double emissions of the CdS@ferritin were derived from the direct transition band of single crystals and the surface-trapping sites of polycrystals. In the case of single-crystal CdS@ferritin, an exciton exists in the single-crystalline CdS@ferritin along with the anisotropic crystal lattice, resulting in relatively intense CPL emission with great g_{Lum} value. On the other hand, both circular polarity and PL intensity of polycrystalline CdS@ferritins may be compensated at the grain boundaries of the CdS QDs owing to their lattice mismatch. Furthermore, XRD measurements revealed an unusual crystal phase behavior of the CdS QD prepared in the ferritin. Thus, although CdS QDs prepared with chemical methods generally form hexagonal phase crystal structures,[1c] those prepared in ferritin formed cubic-phase crystals (Supporting Information, Figure S1). Similar crystal-phase control of CdS QDs has been reported in biosynthesis with yeasts, in which short chelating peptides controlled the nucleation and growth of CdS QDs.[9] Thus, chiral configurations of the chelating amino acids in the ferritin cores may be transferred to the QD crystal lattices during an anisotropic crystal growth.

Although protein-templated QD preparation allows diameters to be precisely defined by the inner diameters of the proteins, uniform and discrete molecular sizes of the template proteins may sometimes cause problems for tailor-made manipulations of the optophysical properties. To overcome this shortcoming, we applied a photoetching technique to control the size of CdS QDs, as photoetching was developed as a size control method of silica-coated CdS QDs.[10] In principle, semiconducting chalcogenide QDs are photodegraded in aqueous solution when light irradiation of energy sufficient to cause band gap excitation is made. Thus, the CdS@ferritin was irradiated at 370 nm for 1 hour with a titanium–sapphire laser (150 fs, 1 μW).

From HRTEM observations, it was confirmed that the core–shell feature of the CdS@ferritin was fairly unaffected even after laser irradiation. Figure 4a shows histograms of the diameter of CdS QDs before and after laser photoetching. An average diameter of the original CdS@ferritins was calculated to be 7.1 nm, reflecting the inner diameter of the ferritin. After laser photoetching, the average diameter decreased to 6.0 nm, suggesting that the CdS QDs were selectively photoetched by laser irradiation with no effect to the protein shells (Supporting Information, Figure S1). Subtle changes in CdS size by laser photoetching drastically affected the PL/CPL behavior (Figure 4b,c). Thus, with a decrease in QD size, the PL emission from surface-trapping sites blue-shifted by 43 nm with an apparent reddish emission (Figure 4b, inset), whereas that from the direct transition band with λ_{max} at 498 nm became dull (Figure 4b, green). This indicated that photodegradation was initiated at the interface between the CdS QD and the ferritin, and the resulting voids may have become surface-trapping sites in which the CdS QD was detached from chelatable glutamate residues on the interior

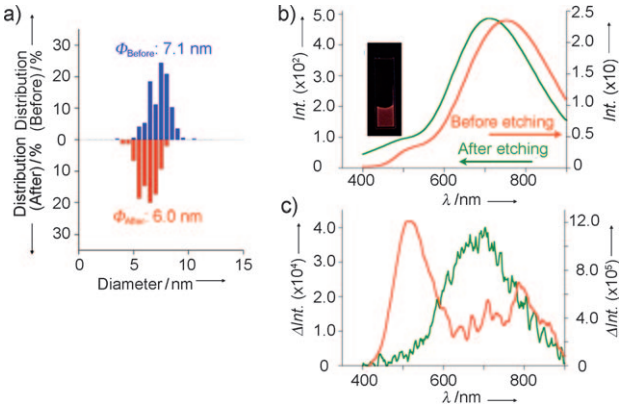

Figure 4. a) Size distributions of CdS@ferritins before (blue) and after (red) laser photoetching. ϕ = average diameter. b) PL and c) CPL spectra of CdS@ferritin before (red) and after (green) laser photoetching. All spectra were obtained using excitation at 325 nm. Inset in b): Photograph of CdS@ferritin after laser photoetching. The sample was irradiated at 310 nm.

surface of the apoferritin. This was further supported by the fact that CPL from the surface-trapping sites blue-shifted at a g_{Lum} value of 8.0×10^{-3}, whereas that from the direct transition band disappeared (Figure 4c, green). Thus, once chiral configurations of chelating amino acid residues were transferred to the QD crystal lattice, the distorted crystal structures could be kept, even after photoetching, resulting in the CPL activity. It is noteworthy that g_{Lum} value from the surface-trapping sites after photoetching consisted of mostly g_{Lum} from direct transition band of single-crystalline CdS QD before laser photoetching (4.4×10^{-3}). Considering that the g_{Lum} value of polycrystalline CdS QD was approximately thirteen times less than that from single-crystalline CdS QDs, the increase in CPL intensity at the surface trapping sites may be mainly attributed to the surface-trapping sites of the single-crystalline CdS QDs generated by laser photoetching. This implies that a circular polarity in CPL emission from the direct transition band may be conserved in the surface-trapping sites in the single-crystalline CdS@ferritins through photoetching (Figure 5).

In conclusion, we have demonstrated that CdS QDs prepared in ferritin, an α-helix-rich rhombic dodecahedral protein, showed left-handed CPL from both direct transition and surface-trapping sites with relatively large g_{Lum} values.

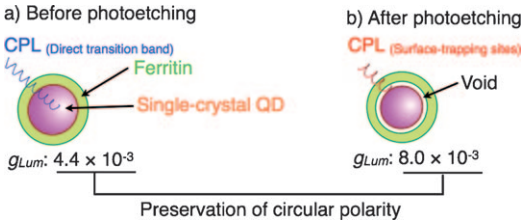

Figure 5. a) Illustration of wavelength modulation and preservation of circular polarity of CPL emission by laser irradiation. a) Single-crystalline CdS QD emitted from direct transition band, and b) that after laser irradiation with emission from surface-trapping sites. The g_{Lum} values in (a) and (b) are very similar.

Utilizing laser photoetching, the PL/CPL bands from surface-trapping sites blue-shifted with a decrease in QD size, whereas that from the direct transition band disappeared. Emission and modulation of CPL properties will likely lead to novel applications in chiroptical memory, emitting devices, and biomedical use based on inorganic nanoparticles.

Experimental Section

Horse spleen apoferritin was purchased from Sigma–Aldrich (A3641). Cadmium acetate, thioacetate, ammonium acetate, and aqueous ammonia for the synthesis of CdS QDs were purchased from Wako Pure Chemical Industries, Ltd. All other reagents were of analytical grade or the best grade available and used without further purification.

All samples were adjusted to be 1.0 mg mL^{-1}. UV spectra were measured by a JASCO V-570 spectrophotometer (Tokyo, Japan). CPL and PL spectra were recorded simultaneously on a JASCO CPL-200 spectrofluoropolarimeter (Tokyo, Japan). The magnitude of circular polarization of the excited state (CPL) is defined as $g_{Lum}=2(I_L-I_R)/(I_L+I_R)$, where I_L and I_R indicate the output signals for left and right circularly polarized light. Experimentally, the value of g_{Lum} was defined as $\Delta I/I$ = [ellipticity/(32980/ln10)]/unpolarized PL intensity at the CPL extremum.

HRTEM images of the CdS QDs were taken with a JEOL JEM-3100 FEF transmission electron microscope (Tokyo, Japan) without stain. An aqueous solution of the CdS@ferritin (0.3 mg mL^{-1}) was poured on a carbon-coated TEM grid, followed by drying under an ambient atmosphere. Diameters of the CdS QDs were calculated by Sumitomo Metal Technology, Inc. RYUSHI-KAISEKI software (Tokyo, Japan) with 0.3 nm of a resolution. Numbers of samples counted for size distribution before and after laser photoetching were 340 and 150, respectively.

For XRD measurements, CdS@ferritin was applied for HPLC purification with a TOSOH TSK-GEL BIOASSIST G4SWXL gel-filtration column (Tokyo, Japan). The obtained crude CdS@ferritin was centrifuged by high-speed centrifugation (120000 *g*). After centrifugation, the precipitated pellets were washed thoroughly with pure water. This purification operation was repeated several times. Obtained wet CdS@ferritin samples were dried under a gentle nitrogen stream, and the resulting samples were ground with mortar. XRD measurements were carried out with a Rigaku RINT-TTRIII/PC X-ray generator (Tokyo, Japan) at 50 kV and 300 mA with a copper target.

Preparation of CdS QDs: Thioacetic acid (1.0 M) was added dropwise into a horse spleen apoferritin/reaction solution (pH 6.5) with cadmium acetate (1.0 mM) in aqueous ammonia (75 mM), and then stirred overnight at room temperature. The color of the solution gradually changed from transparent to pale yellow, resulting in the formation of the CdS QDs in the apoferritin cavities. Formation of the CdS QDs was confirmed by direct observation with TEM. The obtained sample solution was centrifuged at 12000 rpm for 10 min to remove bulk precipitates of the CdS QDs. The supernatant was repeatedly replaced with 150 mM NaCl by a gel filtration membrane (Amicon Ultra-15, MILLIPORE, Billerica, MA). The concentrated solution was applied to gel-filtration column chromatography equipped with a TOSOH TSK-GEL BIOASSIST G4SWXL column (Tokyo, Japan). Here the column was equilibrated with 150 mM NaCl. The fractions with CdS@ferritin were condensed with a gel-filtration membrane (Amicon Ultra-15, MILLIPORE, Billerica, MA). To eliminate the unreacted apoferritin, the crude CdS@ferritin was further ultra-centrifuged at 45000 rpm for 60 min. The precipitated CdS@ferritin was used for all experiments. The CdS@ferritin was fairly stable under wide ranges of pH. Only when pH was below 2 or above 10, did precipitation of the CdS@ferritin occur due to

disruption and/or denature of the protein shells (Supporting Information, Figure S2).

Laser photoetching: The samples were excited by second harmonics (370 nm) of titanium: Sapphire laser (Coherent, Mira 900-F, pulse width = 150 fs, λ = 740 nm) extended with a cavity dumper (APE, PulseSwitch, repetition rate = 100 Hz) for 1 hour. During laser irradiation, the fluorescence profiles were measured with Streak Scope (Hamamatsu photonics, C4780, time resolution = 250 ps).

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