Nano Research
DOI 10.1007/s12274-013-0348-0
Research Article
ISSN 1998-0124
CN 11-5974/O4

Enhanced and tunable fluorescent quantum dots within a single crystal of protein

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Nano Res., **Just Accepted Manuscript** • DOI: 10.1007/s12274-013-0348-0
http://www.thenanoresearch.com on July 10 2013

Just Accepted

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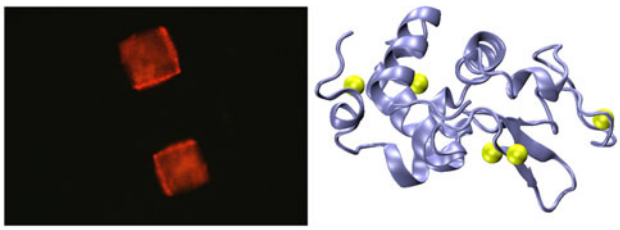

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We have developed a general approach to prepare fluorescent quantum dots within protein single crystals without disturbing the protein crystalline lattice. The quantum dots are three-dimensionally incorporated within the protein crystals, and their fluorescent properties are tunable.

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Received: day month year / Revised: day month year / Accepted: day month year (automatically inserted by the publisher)

ABSTRACT

The design and synthesis of bio-nano hybrid materials can not only provide new materials with novel properties, but can also advance our fundamental understanding of interactions between biomolecules and their abiotic counterparts. Here, we report a new approach to achieving such a goal by growing CdS quantum dots (QDs) within single crystals of lysozyme protein. This bio-nano hybrid emitted much stronger red fluorescence than its counterpart without the crystal, and such fluorescent properties could be either enhanced or suppressed by the addition of Ag(I) or Hg(II), respectively. The three-dimensional incorporation of CdS QDs within the lysozyme crystals was revealed by scanning transmission electron microscopy with electron tomography. More importantly, since our approach did not disrupt the crystalline nature of the lysozyme crystals, the metal and protein interactions were able to be studied by X-ray crystallography, thus providing insight into the role of Cd(II) in the CdS QDs formation.

KEYWORDS

Functional Bio-nanomaterials, Quantum Dots, Protein Single Crystals, X-ray Crystallography, Tomography,

1. Introduction

Bio-nanomaterials combine the merits of both biomolecules and nanomaterials, and have found applications in many areas, from catalysis, energy conversion and storage, to artificial tissues, sensing, imaging, and drug delivery [1-10]. To achieve the goal of preparing hybrid materials, many biomolecules such as DNA, proteins, carbohydrate, viruses, and bacteria have been explored [11-27]. Among them, proteins have been successfully utilized to construct a variety of bio-nanomaterials due to their nanoscale sizes comparable to most nanomaterials, their structural and functional diversities, and their recognition and assembly capabilities [28-37]. Most

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previous research, however, has focused on proteins in solution as templates for nanomaterial synthesis; for example, caged proteins have been employed as nanocontainers for directing preparation of nanomaterials in solution [38-40]. Protein single crystals, on the other hand, have highly-ordered three-dimensional structures and thus can be regarded as porous materials [41, 42]. The unique characteristics of protein crystals, such as three-dimensional assemblies at both macroscale and nanoscale, and nanosized porous structures, allows controlled growth of nanomaterials at a time scale by which the kinetics and mechanism of the growth can be readily studied using microscopic methods. Although the synthesis and characterization of nanomaterials within protein single crystals have been recently reported [42-50], this method's full potential has not been reached, especially for those reagents and conditions that do not disrupt the crystalline lattice. This latter feature is especially important for high-resolution three-dimensional structural study of the protein interactions with metal ions during the nanomaterials growth.

We have recently reported the synthesis and characterization of gold nanoparticles (AuNPs) grown within intact lysozyme single crystals [46]. Because the protein single crystals provided a well-defined three-dimensional matrix where each protein inside the crystal can be studied at an atomic level by x-ray crystallography, we were able to reveal mechanisms of AuNP formation directed by the protein template. Given the initial success, we wondered whether such an approach can be applied to synthesize and study other nanomaterials within single protein crystals. Quantum dots (QDs) are promising inorganic nanomaterials, that have been studied extensively during the past decades due to their unique optical properties for use in areas such as bioimaging, optoelectronic devices, and solar cells [51-66]. Growing QDs within the intact protein single crystals will allow us to study not only the mechanism of the QDs formation directed by the protein, but also the influence of the protein crystals on the optical properties of QDs. In this study, we demonstrate that CdS QDs can be formed *in situ* within single crystals of proteins, resulting in fluorescent nanomaterial-in-crystal hybrids. We also show that the fluorescent properties of the hybrids can be fine-tuned by the addition of selected chemical species.

2. Results and Discussion

The CdS QDs within the lysozyme single crystals (called CdS@Lysozyme) were prepared by growing the crystals from cadmium acetate, sodium sulfide, and lysozyme at room temperature. The crystals of CdS@Lysozyme could be observed after about one day of growth. As shown in Figure 1, the crystals were yellow in color and emitted red fluorescence with a peak centered at ~ 700 nm under FITC (~450-500 nm) illumination (also see Figure S1). This Stokes-shifted emission band of 700 nm was due to the trapped charge carriers' radiative recombination [67]. In contrast, when the crystals were grown under the same condition but in the absence of sodium sulfide, the crystals of Cd(II)@Lysozyme were colorless and non-fluorescent. These results suggest that the color and fluorescence may be due to the formation of CdS QDs inside the protein crystals.

Although CdS QDs have been synthesized, only limited reports have demonstrated fluorescence properties of biosynthesized CdS QDs in aqueous solution, partly due to the intrinsically weak fluorescence from the CdS QDs' broad trap emission [68-71]. For example, as a control experiment, when CdS QDs were synthesized in the buffer solution containing lysozyme under conditions that no crystal was formed, little red fluorescence emission was observed (Figure S2). Moreover, when the as-prepared CdS@Lysozyme crystals were re-dissolved in buffer solution, red fluorescence emission disappeared (Figure S3). Instead, insoluble yellow-colored aggregates were observed in both control experiments described above. To enhance the crystal stability in buffers, glutaraldehyde can be used to cross-link the crystals [44], and such cross-linked crystals containing Au nanoparticles have shown interesting catalytic properties in solution [49]. These results suggest that the protein crystals provided a unique microenvironment for formation of CdS QDs with enhanced fluorescent properties.

To verify that the CdS QDs were indeed formed within the lysozyme crystals, we used electron microscopy to study the as-prepared CdS@Lysozyme. After 8 days of growth, the size of the QDs within the lysozyme crystals was found to be ~9.8 ± 2.2 nm (Figure 2). To further characterize the chemical composition of the CdS@Lysozyme, single-particle energy-dispersive X-ray spectrum (EDS) analysis was carried out (Figure S4). The peaks of Cd, S, Cl, C, and Cu were present, confirming the formation of CdS QDs (the peak of Cl originated from the NaCl in the crystallization buffer and the peaks of C and Cu originated from the TEM grid, respectively).

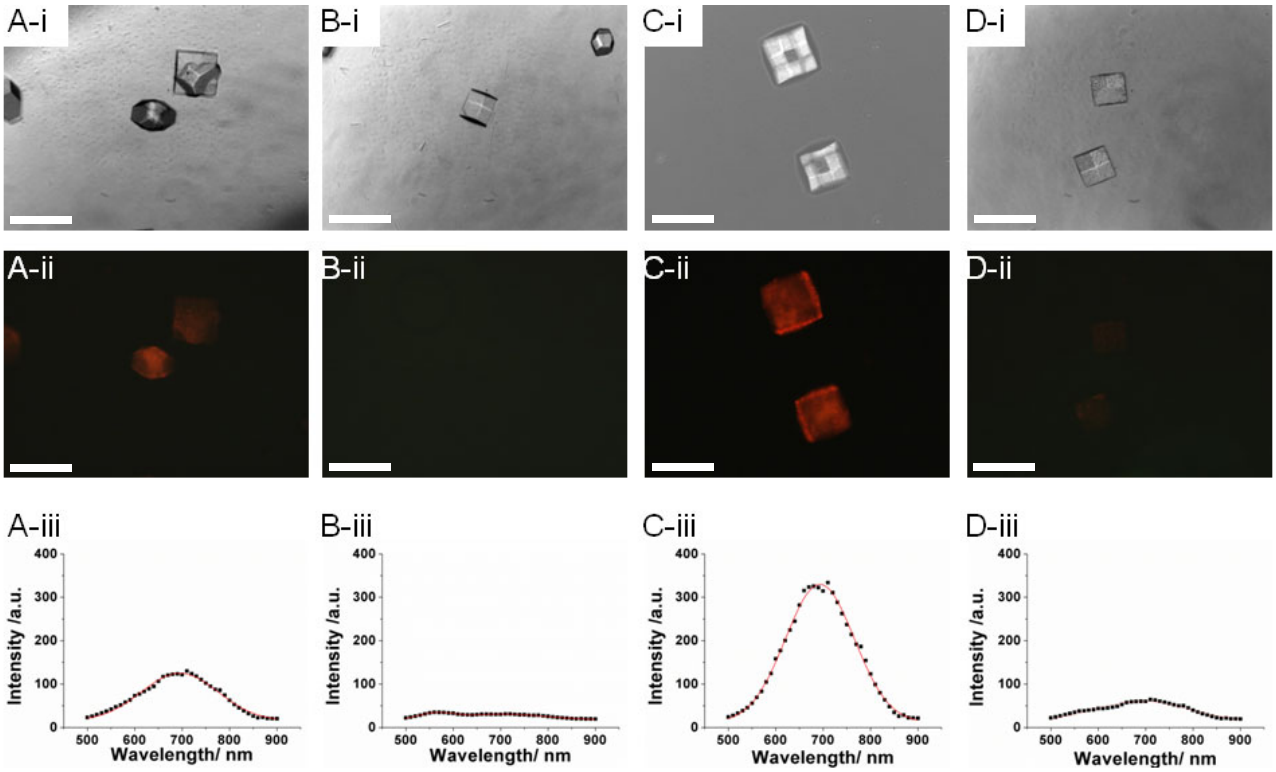

Figure 1. Bright field (i), fluorescent images (ii), and emission spectra (iii) of lysozyme single crystals. From left to right: CdS@Lysozyme (A), Cd(II)@Lysozyme (B), CdS@Lysozyme with Ag(I) (C), and CdS@Lysozyme with Hg(II) (D). The fluorescent images were collected on a Nuance multispectral imaging system with a FITC filter for excitation and a long pass filter for emission. Exposure time=500 ms. Scale bar=500 μm.

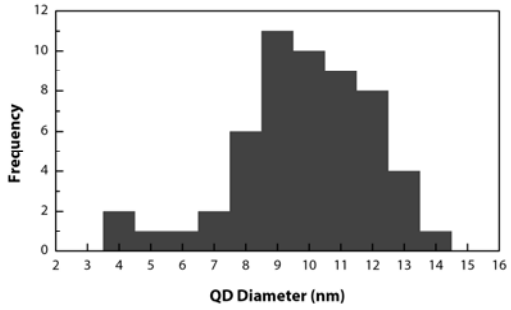

Figure 2. Size distribution of CdS QDs in the crystal used for the tomographic study. The diameters were measured from the source micrographs in the tilt series.

To determine the locations of the CdS QDs entrapped within the lysozyme single crystals, we performed an electron tomographic study. Electron tomography, an emerging technique for three-dimensional imaging [72, 73], enables us to visualize how the small objects are distributed three-dimensionally in the matrix. High-angle annular-dark-field scanning transmission electron microscopy (HAADF-STEM) was used to collect a series of images at different tilt angles for tomographic reconstruction due to its effective

suppression of coherent diffraction and Z-contrast imaging mechanism [73]. Because the Z difference between the CdS QDs and the lysozyme protein matrix is large enough, the contrast is sufficient to distinguish between them in a tomographic reconstruction. A tilt series of a relatively thin sample area was acquired over ±44°, with an image taken every 2°, and used to construct a tomogram. Movies of the aligned tilt series and the reconstructed tomogram are included in the supplementary information: Alignment and Reconstruction, respectively. Figure 3A shows a single HAADF-STEM image of CdS QDs within the lysozyme single crystals at 0° tilt. Figures 3B is a cross-sectional slice through the reconstructed tomogram, demonstrating how the 3D reconstruction clearly reveals the CdS QDs to be inside the lysozyme crystal. Figures 3C and 3D are the corresponding images to Figure 3A from the tomogram. The CdS QDs in Figure 3A overlays well with those in Figures 3C and 3D, supporting the fidelity of the reconstruction.

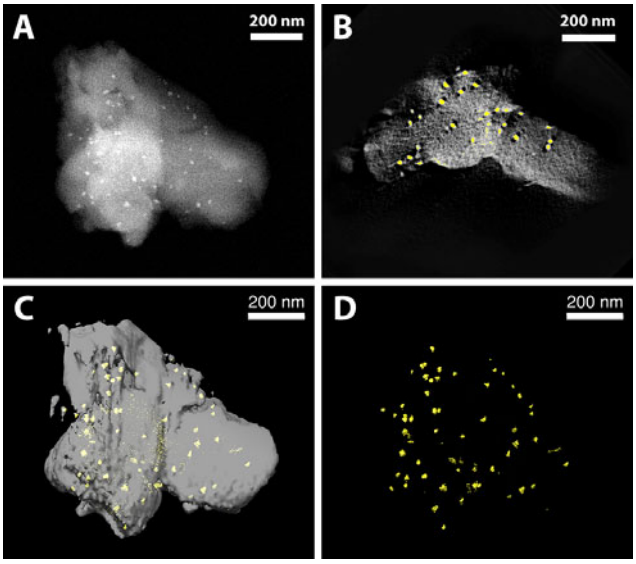

Figure 3. HAADF-STEM image of CdS@Lysozyme single crystals at 0° tilt (A) and the corresponding snapshot from the three-dimensional tomographic reconstruction (C). (D) is the same snapshot as (C) without lysozyme. (B) is a cross-sectional slice through the reconstructed tomogram. In (A), the white dots show the CdS quantum dots incorporated within the lysozyme matrix, which is slightly darker. In (B-D), the CdS quantum dots are yellow; the lysozyme crystals are white.

To quantify the encapsulation, the depth of each CdS QD inside the lysozyme crystal was measured from the reconstructed tomogram (Figure 4). The depth distribution appeared rather uniform throughout the crystal, with no strong preference for any particular depth. No QDs were observed outside the lysozyme surface; at their shallowest, the QDs were flush with the surface, and even then only 3 of the 58 QDs were this close to the surface. The vast majority was entirely encapsulated within the crystal to a depth of multiple QD diameters. Only 2 QDs were observed at a depth >100 nm because there were very few regions of the crystal that were >100 nm from any surface.

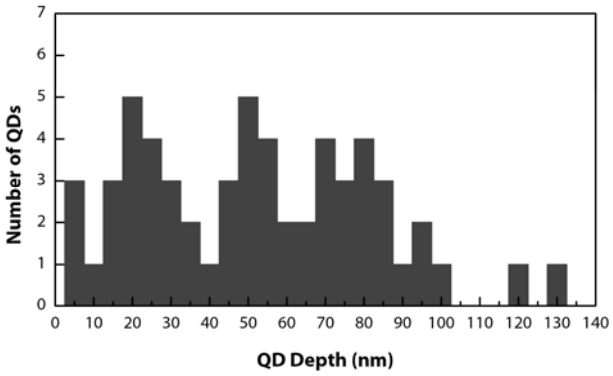

Figure 4. Distribution of depths below the lysozyme crystal's surface of the CdS QDs. Depths were measured from the center of the QD to the nearest surface.

Remarkably, despite the encapsulation of hundreds of CdS QDs within the protein matrix, the lysozyme single crystals remained intact, resulting in x-ray diffraction patterns similar to those of lysozyme alone. This feature allowed us to study the Cd(II) ions' binding sites in the protein and to elucidate its role in directing the CdS QDs growth by using high-resolution X-ray crystallography. Figure 5 shows the high-resolution X-ray

crystallographic structures of Cd(II)@Lysozyme and CdS@Lysozyme obtained. Nine Cd ions were observed in the crystal structure of Cd(II)@Lysozyme. One Cd ion, named Cd1, was coordinated by the ε-N of His15; the same residue was involved in coordination with a Au(I) ion in a previously reported AuNP@Lysozyme [46]. The other eight Cd ions, Cd2 to Cd9, interacted with N (or O) atoms of several residues, such as Tyr23, Ser24, Phe34, Gln41, Thr43, Gly49, Thr51, Asp52, Tyr53, Leu56, Gln57, Asn65, Asp66, Arg68, Thr69, Ser72, and Ser91 (see Figure S5 for detailed binding motifs). In the case of CdS@Lysozyme, however, only five Cd ions, Cd2' to Cd6', were observed, which bound to N (or O) atoms of Tyr23, Ser24, Gln41, Thr43, Asp52, Gln57, Asn65, Thr69, and Ser72 (see Figure S6 for detailed binding motifs). These five Cd ions have almost identical binding sites as Cd2 to Cd6 of Cd(II)@Lysozyme. The other four Cd ions, Cd1, and Cd7 to Cd9, of Cd(II)@Lysozyme initially bound to His15 while other residues disappeared when CdS QDs formed. These results indicate that the binding of Cd(II) ions to His15 and several other residues (i.e., Phe34, Gly49, Thr51, Tyr53, Leu56, Asp66, Arg68, and Ser91) may direct the growth of CdS QDs.

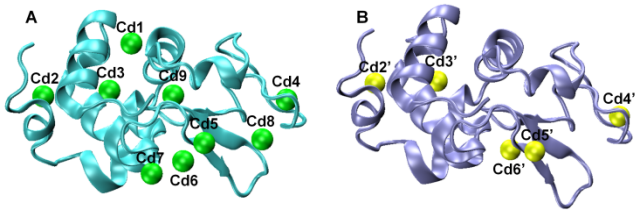

Figure 5. X-ray crystallographic structures of Cd(II)@Lysozyme (A) and CdS@Lysozyme (B). Cd ions are shown as balls.

To demonstrate control of the fluorescent properties of CdS@Lysozyme, we added different metal ions. Interestingly, we found that Ag(I) ions enhanced the fluorescent emission of CdS@Lysozyme crystals significantly (Figure 1C), while Hg(II) ions quenched the fluorescent emission (Figure 1D). The enhancement of fluorescent emission by Ag(I) ions could be due to the formation of new radiative centers and the blocking of nonradiative defect sites on the CdS QDs' surface [74], while the quenching induced by Hg(II) ions could originate from their binding to the CdS QDs' surface and further electron transfer from surface traps of QDs to Hg(II) ions [75]. To further confirm the enhancement or suppression of the fluorescent intensity of the CdS@Lysozyme, we added 1.5 μL and 3μL of 1 mM Ag(I) to the solution containing the CdS@Lysozyme. As shown in Figure S7, increased enhancements of fluorescent intensity were observed with increasing concentrations of the Ag(I). Similarly, addition of increasing concentrations of Hg(II) caused a decrease in fluorescent intensity of the crystals. These results suggest that it is possible to fine-tune the fluorescent properties of CdS@Lysozyme hybrids by using additional chemical species.

3. Conclusions

In summary, we have developed an approach to prepare CdS QDs within lysozyme single crystals without disturbing the protein crystalline lattice. Fluorescent imaging showed the as-prepared hybrids could emit red fluorescence. The fluorescent properties of the hybrids could be further fine-tuned by additional different metal ions. The CdS QDs' three-dimensional distribution and the cadmium ions' interactions with lysozyme were revealed by STEM with tomography and X-ray crystallography, respectively. This study may provide a new approach to synthesis of functional nanomaterial-in-crystal hybrids, which could find potential applications in catalysis, optical and plasmonic devices, and stimuli-responsive materials. It also allows us to further understand the chemistry of biomineralization, and the interactions between biomolecules and nanomaterials.

Acknowledgements

This work was supported by the US National Science Foundation (CMMI 0749028 and DMR-0117792). The

authors thank C. Lei and W. Swiech for help with the STEM imaging, C.M. Bee and D. Zhang for fluorescent microscopic measurements, S.M. Nie for the use of Nuance system and A.M. Smith for insightful discussions. S.H. and I.M.R. acknowledge support from the US Department of Energy (grant DE-FC36-05GO15064). STEM experiments were carried out in part in the Frederick Seitz Materials Research Laboratory Central Facilities, University of Illinois. X-ray crystallographic data for this study were measured at beamline X12C of the National Synchrotron Light Source, Brookhaven National Laboratory. Financial support comes principally from the Offices of Biological and Environmental Research and of Basic Energy Sciences of the US Department of Energy, and from the National Center for Research Resources (P41RR012408) and the National Institute of General Medical Sciences (P41GM103473) of the National Institutes of Health.

Electronic Supplementary Material: Supplementary material (experimental details, additional figures and movies, and X-ray crystallographic data) is available in the online version of this article at http://dx.doi.org/10.1007/s12274-***-****-* (automatically inserted by the publisher).

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