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Stable Near-Infrared Photoluminescence from Silicon Quantum Dot–Bovine Serum Albumin Composites

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KEYWORDS

silicon quantum dot, protein corona, size effect, photoluminescence

ABSTRACT

The interaction between negatively-charged all-inorganic silicon quantum dots (Si QDs) and bovine serum albumin (BSA) is studied. It is shown that a small difference in the size of Si QDs affects the structure of Si QDs-BSA composites significantly. When the diameter of Si QDs is 4 nm, a heterodimer (~20 nm) composed of one Si QD and one BSA molecule is a preferable and stable structure. On the other hand, when the diameter is 7 nm, the size of the composites increases to ~50 nm. The Si QDs-BSA composites exhibit stable photoluminescence in the near-infrared range in phosphate-buffered saline.

INTRODUCTION

Semiconductor quantum dots (QDs) have been an important fluorescent probe that complement and replace fluorescent proteins and organic dyes¹ for *in vivo* and *in vitro* biophotonics due to their superior optical properties such as the highly stable and wavelength-tunable luminescence, the broad absorption band, the large stokes shift, *etc.*²⁻⁵ In addition to widely used cadmium chalcogenide-based QDs,⁶ silicon (Si) QDs have been emerging as environmentally friendly, biocompatible and biodegradable QD phosphors exhibiting the luminescence in the near-infrared (NIR) biological window.⁷⁻¹⁰

Si QDs-based fluorescence probes developed so far can be categorized into several groups. The first one is porous Si nanoparticles (NPs) produced by electrochemical anodization of bulk Si crystal. Porous Si NPs are composed of Si QDs or Si nanowire skeletons and exhibit the luminescence in the red to NIR ranges. In porous Si NP phosphors, high contrast imaging has been achieved by using time-gated imaging¹¹ and two-photon imaging techniques.¹² Another intensively studied Si QDs-based fluorescence probe is micelle encapsulated Si QDs; Si QDs are encapsulated in phospholipid micelles to attain water dispersibility and resistance to oxidation

without invading the optical properties. Imaging *in vitro*¹³ and *in vivo*¹⁴ by using micelle encapsulated Si QDs has been demonstrated and the toxicity was carefully tested. In contrast to extensive researches on fluorescence imaging by Si QDs agglomerates, those using isolated Si QDs are rare, especially in the red to NIR range. This is due to the fact that red to NIR luminescent Si QDs soluble in aqueous media have not been developed until recently. A straightforward approach to attain hydrophilicity to Si QDs is surface modification by polar molecules such as carboxylic acid¹⁵. Another approach is to induce surface charges by inorganic ligands. It has been reported that heavily B doping on the surface makes Si QDs dispersible in water.^{16,17} In our group, we have developed water-dispersible Si QDs by heavily doping boron (B) and phosphorus (P) simultaneously.¹⁸ Doped B and P atoms are accumulated on the surface of Si QDs and induce negative potential on the surface (zeta potential: ~ -30 mV at pH 7.0).¹⁹ The B and P codoped Si QDs exhibit size-tunable luminescence in the wavelength range from 600 to 1100 nm in water in a wide pH range due to the donor-to-acceptor state transitions.²⁰ The highest luminescence quantum yield is around 30%. Because of the all-inorganic surface, the luminescence is very stable in an aqueous medium in a wide pH range. The impact of B and P codoped Si QDs on different human cells has been studied by *in vitro* fluorescence imaging.²¹ Furthermore, a process to conjugate codoped Si QDs to antibodies has been developed to utilize them as a fluorescence probe in enzyme-linked immunosorbent assay.²²

It is well-known that in biological environment, proteins are adsorbed on the surface of nanomaterials and protein coronas are formed.^{23–32} Previous studies revealed that formation of protein coronas depends on the shape, size, surface charge, surface ligand, *etc.* of nanomaterials. In II-VI QDs such as CdSe, the formation of protein coronas and the effects on the optical and physical properties of QDs have been studied.^{33–42} Similarly, in organic-functionalized Si QDs

such as allylamine-capped Si QDs, interaction between Si QDs and proteins in water was studied and the effects of proteins on the water dispersibility and the photoluminescence (PL) properties were discussed.^{43,44} In these studies, the interaction is between positively charged amine ligands and negatively charged protein domains.

In this work, we study the interaction between proteins and B and P codoped all-inorganic Si QDs. As a model protein, we employ bovine serum albumin (BSA). Serum albumins are the most abundant protein in the bloodstream.⁴⁵ Since the size of BSA is a few nm, which is very close to the size of Si QDs, a small difference in the size of Si QDs may affect the structure of the protein corona. We show that the structure of QDs-BSA composites is significantly different between Si QDs 4 nm and 7 nm in diameters. We then study the PL properties of Si QDs-BSA composites in phosphate buffered saline (PBS). We show that the composites exhibit stable PL in the NIR range in PBS during incubation at 37°C for more than 15 days.

RESULTS AND DISCUSSION

Si QDs used in this work are all-inorganic B and P codoped Si QDs developed in our group. Details of the preparation procedures are shown in the Experimental Procedure section. Figure 1 (a) and (b) show TEM images of Si QDs grown at 1100 °C and 1200 °C, respectively. The diameters estimated from TEM images are 4 nm in 1100 °C grown Si QDs and 7 nm in 1200 °C grown ones (see Figure S1 in the Supporting Information for the size distribution). Since Si QDs are well-dispersed in water, three-dimensional agglomerates are hardly observed in the TEM images. The insets are the high resolution TEM images. Lattice fringes corresponding to {111} planes of Si crystal are clearly seen. The surface of Si QDs just after preparation is terminated predominantly by hydrogen (H). During the storage in methanol, very thin native oxides are formed on the

surface.⁴⁶ In this work, we use Si QDs aged more than 30 days in methanol to ensure that native oxides are formed on the surface. The surface of Si QDs are negatively charged (zeta potential: ~ -30 mV at pH 7.0), and thus they are dispersed in water by the electrostatic repulsion.¹⁸

Figure 1(c) shows the schematic illustration of the procedure to produce Si QDs-BSA composites. 60 μ L of water solution of Si QDs (1.6 mg/mL) was mixed with 900 μ L of phosphate buffered saline (PBS) solution with different concentrations of BSA (0 to 100 μ M). The mixed solution was slowly stirred and stored for 2 hours in a refrigerator at 4 °C. Figure 1 (d) shows the transmittance spectra of water solutions of Si QDs before and after mixing with PBS solutions of BSA (100 μ M) for Si QDs 4 nm and 7 nm in diameters. The transmission loss in the blue to ultraviolet region is due to light absorption by Si QDs. In Si QDs 4 nm in diameter, the spectrum is not affected by the mixing with the BSA solution. On the other hand, in Si QDs 7 nm in diameter, the transmittance decreases in the whole wavelength range. This suggests that relatively large Si QDs-BSA composites are formed in the solution and they scatter light. Note that the transmittance spectrum is not affected if Si QDs solution is mixed with PBS solution without BSA (see Figure S2 in the Supporting Information), *i.e.*, PBS does not promote agglomeration of Si QDs.

Figure 1 (e) shows the hydrodynamic diameter of Si QDs-BSA composites measured by dynamic light scattering (DLS) as a function of BSA concentration. Without BSA, *i.e.*, Si QDs in PBS solution, the hydrodynamic diameters of Si QDs are about 10 nm in both 4 nm and 7 nm diameter (TEM diameter) Si QDs. A similar discrepancy between a hydrodynamic diameter and a TEM diameter has been reported in Si QDs prepared by other methods.⁴⁷ When Si QDs 4 nm in diameter are mixed with BSA, the hydrodynamic diameter slightly increases to around 20 nm and then it saturates; further increase of the BSA concentration does not increase the hydrodynamic diameter. Considering that the hydrodynamic diameter of Si QDs is around 10 nm and that of BSA is around

7 nm, it is very likely that a pair of a Si QD and a BSA molecule is formed and agglomeration does not proceed further even if BSA molecules are still available in the solution. The slight decrease of the hydrodynamic diameter from the BSA concentration of 50 to 100 μM indicates that isolated BSA remains in the 100 μM solution. On the other hand, in Si QDs 7 nm in diameter, the hydrodynamic diameter increases to around 30 nm when a small amount of BSA is added and then continues to increase by increasing the BSA concentration. It reaches 53 nm when the BSA concentration is 100 μM . The formation of large agglomerates is consistent with the decrease of the transmittance in Figure 1(d).

To confirm the formation of Si QDs-BSA composites, we performed the agarose electrophoresis. Figure 2 (a) shows the results for Si QDs 4 nm in diameter. The brownish band arises from light absorption by Si QDs. The image on the right is electrophoresis of BSA (without Si QDs). The gels are stained by Coomassie brilliant blue to show the position of BSA. We can see that the band shift is almost binary, *i.e.*, two Si QDs-related bands appear when BSA is mixed. Since the position of the retarded band is close to that of BSA, it can be assigned to a pair of a Si QD and BSA. The binary behavior suggests that isolated Si QDs and heterodimers composed of a Si QD and a BSA molecule coexist in the solutions and the ratio of the latter one increases with increasing BSA concentration.

The situation is different in Si QDs 7 nm in diameter. In Figure 2(b), the band shifts gradually as BSA concentration increases. This suggests that the composites become large with increasing BSA concentration. This is consistent with the DLS data in Figure 1(e).

In order to show that the agglomerates observed in the electrophoresis and the DLS are not those of Si QDs, but are composites composed of Si QDs and BSA, we performed polyacrylamide gel electrophoresis (PAGE) for the same samples. The results are shown in Figure 2(c) and (d) for Si

QDs 4 nm and 7 nm, respectively, together with the molecular rulers (NativeMark™ Unstained Protein Standard, Thermo Fisher Scientific) (left-most lane). The gels are stained by Coomassie brilliant blue to show the position of BSA. The BSA concentration is changed from 0 to 100 μ M. The data of the solutions of Si QDs-BSA composites (right) and BSA without Si QDs (left) are shown side-by-side for each BSA concentration. The bands corresponding to a monomer, dimer, trimer and larger oligomers of BSA are seen. In Figure 2(c) (Si QDs 4 nm in diameter), the distributions of oligomers are identical between the solutions of Si QDs-BSA composites and BSA when the BSA concentration is the same. This is consistent with the DLS and the agarose electrophoresis results that composites larger than one Si QD and one BSA molecule are hardly formed. On the other hand, in Figure 2 (d) (Si QDs 7 nm in diameter), we can see a clear difference in the oligomer distributions between the solutions of Si QDs-BSA composites and BSA. Larger oligomers are formed in Si QDs-BSA composites, indicating the formation of large agglomerates.

Figure 3(a) shows a TEM image of Si QDs-BSA composites prepared from Si QDs 7 nm in diameter. The BSA concentration is 100 μ M. We can see gray irregular shape objects 10 to 80 nm in size. The size distribution of the gray objects is shown in Figure 3(b). The mean size determined from the fitting by the log-normal distribution function is 46.3 nm, which is close to the hydrodynamic diameter obtained by the DLS measurement. Figure 3(c) shows a high magnification image of a composite. Although direct observation of lattice fringes of Si QDs is difficult due to a thick amorphous layer presumably composed of proteins, the fast Fourier transform (FFT) image in Figure 3(d) shows the spots corresponding to {111} planes of crystalline Si. The TEM image confirms that Si QDs-BSA composites as schematically shown in Figure 2(b) are formed.

Figure 4 (a) shows the PL spectra of Si QDs 4 nm in diameter in water and in PBS, and Si QDs-BSA composites (BSA concentration: 100 μ M) in PBS. In water, the PL maximum is around 900 nm.¹⁸ The dip around 1000 nm is due to absorption by water molecules. In PBS, the PL intensity decreases, although the spectral shape is almost identical. The mechanism of the PL quenching in PBS is not clear, but probably adsorption of Na⁺ and K⁺ ions in PBS induces non-radiative recombination channels in Si QDs. On the other hand, the formation of Si QDs-BSA composites does not affect the PL intensity. The results are similar for Si QDs 7 nm in diameter (Figure 4 (b)). In this case, the PL maximum is around 1100 nm. The dip around 1200 nm is also due to light absorption by water molecules.

We incubated Si QDs-BSA composites (BSA concentration: 100 μ M) in PBS at 37°C for 15 days to study the PL stability. Figure 4(c) shows the PL peak wavelength and the intensity as a function of incubation duration. In Si QDs-BSA composites produced from 4 nm Si QDs, the PL peak shifts to shorter wavelength within the first 2 days of the incubation and then almost stabilized. The PL shift may be due to oxidation of Si QDs surface and resultant decrease of the crystalline size. During the incubation, the PL intensity first increases and then decreases slightly. The important fact is that the PL quenching is only about 25% of the initial intensity even after incubation for 15 days. Therefore, the Si QDs-BSA composites are stable fluorescence markers usable in harsh biological environment. In Si QDs-BSA composites produced from 7 nm Si QDs, similar short wavelength shift is observed during the incubation duration. However, the PL intensity exhibits a different trend. It increases continuously during the incubation period and reaches 5-fold of the initial intensity, indicating defect passivation during incubation. Since the composites have the PL maximum around 1100 nm, they can be used as stable phosphors in the second biological window.⁴⁸

CONCLUSION

We have studied the interaction between negatively-charged all-inorganic Si QDs and BSA. We found that a small difference in the size of Si QDs affects the structure of Si QDs-BSA composites significantly. When the diameter of Si QDs is 4 nm, the maximum size of the composites is around 20 nm; the agglomeration does not proceed further even if BSA concentration is increased. This suggests that a heterodimer composed of one Si QD and one BSA molecule is a preferable and stable structure. On the other hand, when the diameter is 7 nm, the size increases to ~50 nm with increasing BSA concentration. The Si QDs-BSA composites exhibit stable PL in the NIR range in PBS; incubation at 37°C for 15 days in PBS does not strongly degrade the PL property. Considering the high biocompatibility of Si QDs, the Si QDs-BSA composites can be a promising phosphor for *in vitro* and *in vivo* bioimaging and biosensing in the NIR range.

EXPERIMENTAL PROCEDURE

Preparation of B and P codoped Si QDs dispersed in water

B and P codoped Si QDs were prepared by the sputtering method.^{18,19} A thick Si-rich borophosphosilicate glass (BPSG) film is deposited on a thin stainless steel plate by sputtering Si, B₂O₃, and phosphosilicate glass (PSG) (SiO₂: P₂O₅ = 95:5 wt%). The films were removed from the plate and ground into powder. The powder was annealed in a N₂ gas atmosphere at 1100-1200°C for 30 minutes to grow B and P codoped Si QDs in BPSG. The annealed powder was etched by a hydrofluoric acid (HF) solution (46 wt%) to liberate codoped Si QDs from BPSG matrices. Si QDs dispersed in HF solution were first transferred to methanol and then to water. To transfer Si QDs from methanol to water, a methanol solution was diluted by the same amount of

Milli-Q water and heated to evaporate methanol. All the processes were performed in an ordinary laboratory environment.

Formation of Si QDs-BSA composites

BSA (BSA heat shock fraction, protease free, fatty acid free, essentially globulin free, pH 7, $\geq 98\%$ Sigma Aldrich) was dispersed in PBS solution (PBS(-), Wako) at desired concentration (0 to 100 μM). The mixed solution was slowly stirred and then stored for 2 hours at 4 °C in a refrigerator. After that, a 60 μL water solution of Si QDs (1.6 mg/mL) was added to a 900 μL PBS solution of BSA to form Si QDs-BSA composites.

Structural and optical characterization

Optical transmittance spectra were measured by a UV-VIS-NIR spectrophotometer (UV-3101PC, Shimadzu). The PL spectra were measured by spectrofluorometer (Fluorolog-3, Horiba Jobin Yvon) equipped with a Xe lamp (450 W) and a double grating monochromator as an excitation source. The wavelength of excitation light was 405 nm. TEM observations were performed by JEM-2100F (JEOL) operating at 200 kV. Hydrodynamic diameters were measured by DLS (Zetasizer Nano ZS, Malvern).

Agarose gel electrophoresis

A solution of Si QDs-BSA composites was mixed with a loading buffer (6 $\times$ Loading Buffer, Takara bio Inc) and then 20 μL of the solution was loaded into 1% agarose gel prepared by using TAE (Tris-acetate EDTA Buffer) (UltraPure™ DNA Typing Grade™ 50X TAE Buffer, Thermo Fisher Scientific). TAE buffer was also used as a running buffer. Gels were run for 30 minutes at a constant voltage of 100 V.

Polyacrylamide gel electrophoresis

Native polyacrylamide gel electrophoresis (PAGE) was performed using a vertical electrophoresis cell (Mini PROTEAN, Bio-Rad). A solution of Si QDs-BSA composites was mixed with loading buffer (6× Loading Buffer, Takara bio Inc) and then 10 µL of the solution was loaded into precast polyacrylamide gel (Any kD™ Mini-PROTEAN® TGX™ Precast Protein Gels, BIO RAD Laboratories). Tris/glycine buffer (10x Tris/Glycine Buffer for Western Blots and Native Gels, BIO RAD) was used as a running buffer. Gels were run for 60 minutes at a constant voltage of 100 V, and subsequently stained with Coomassie brilliant blue (AE-1340 EzStain Aqua, ATTO) for 1 hour and destained in water for 24 h in a shaker.

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Supplementary Material Available: Optical transmittance spectra of Si QDs in water and in PBS, and size distributions of Si QDs obtained from TEM images.

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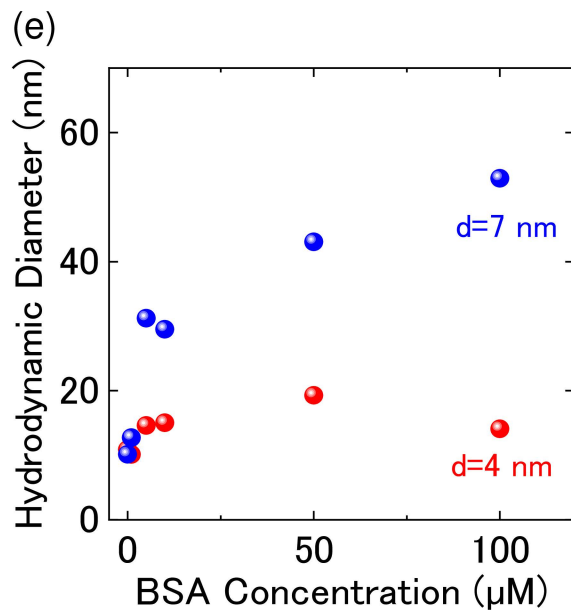
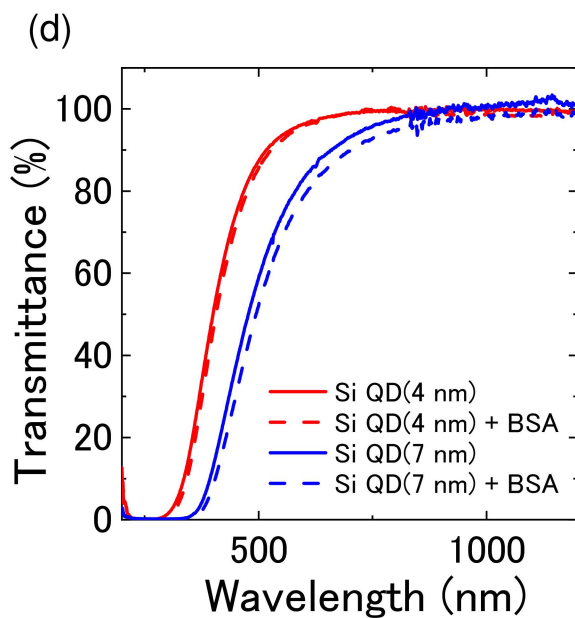
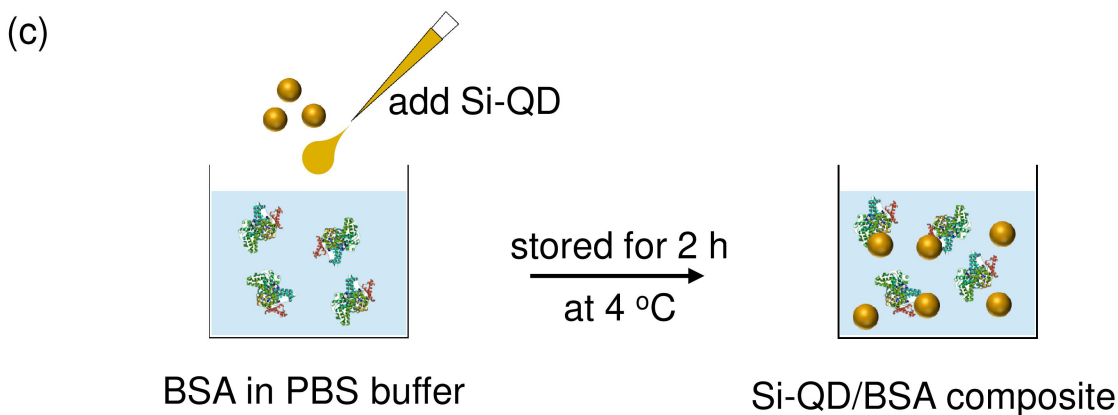
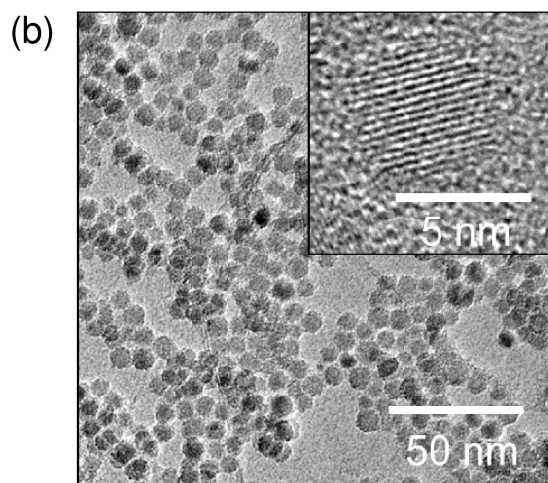
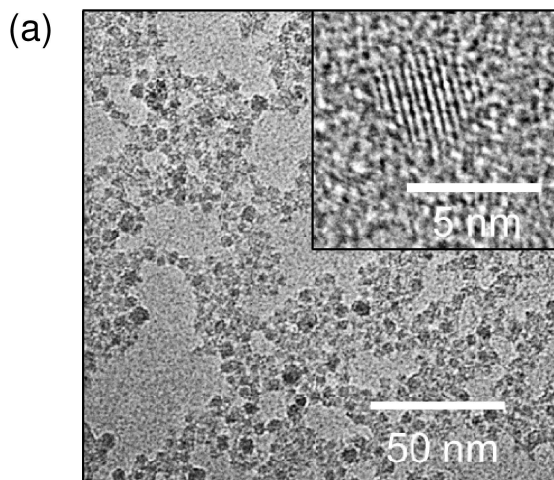
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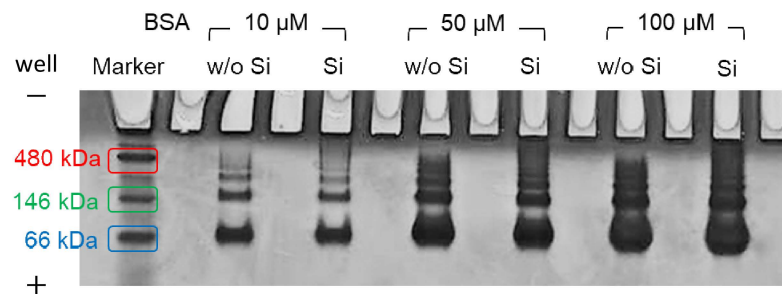
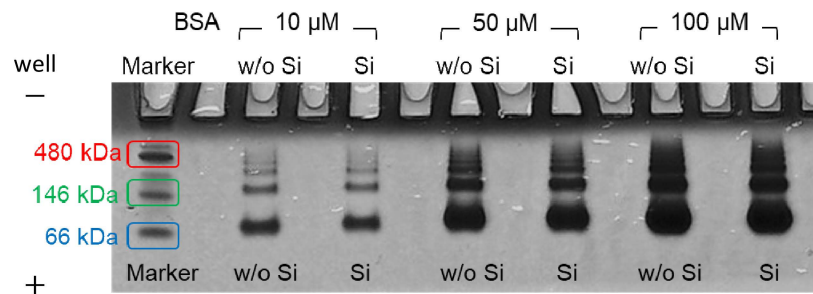
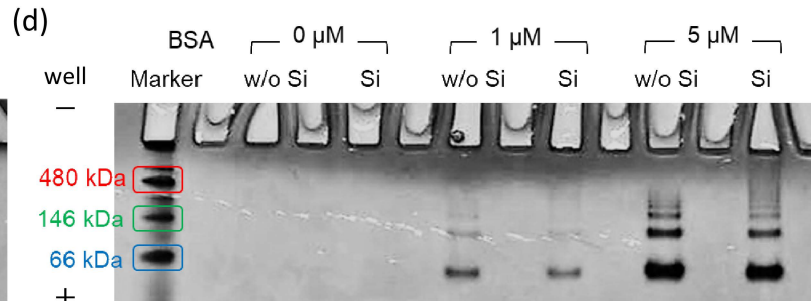
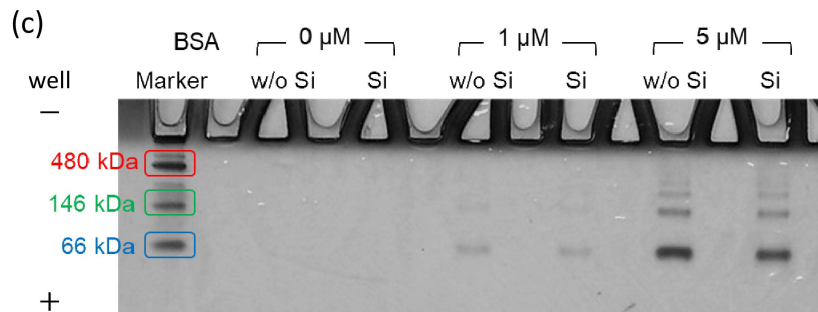
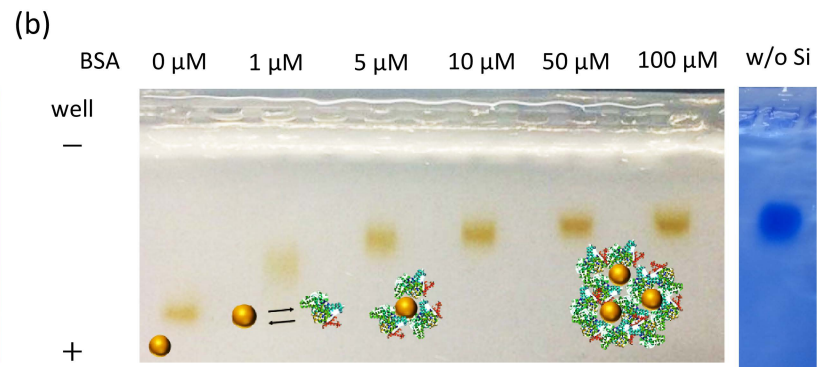
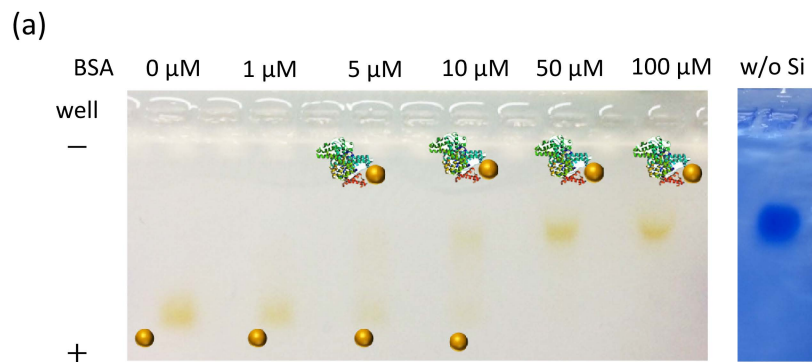
Figure 1 (a, b) TEM images of Si QDs grown at 1100°C (a) and 1200°C (b). Insets are the high resolution TEM images. (c) Procedure for the formation of Si QDs-BSA composites. (d) Transmittance spectra of solutions of Si QDs 4 nm (red) and 7 nm (blue) in diameters before and after mixing with BSA (100 µM). (e) Hydrodynamic diameters of Si QDs-BSA composites estimated from the intensity peak of dynamic light scattering as a function of BSA concentration. The data of Si QDs 4 nm and 7 nm in diameters are shown.

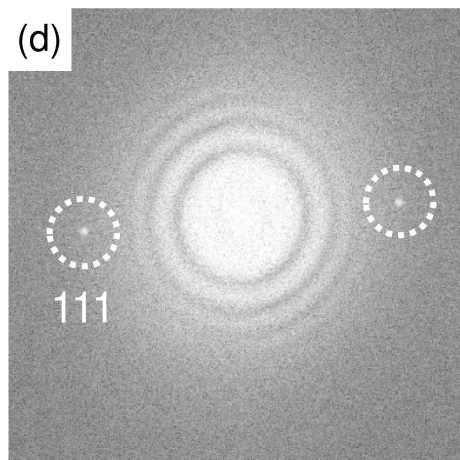
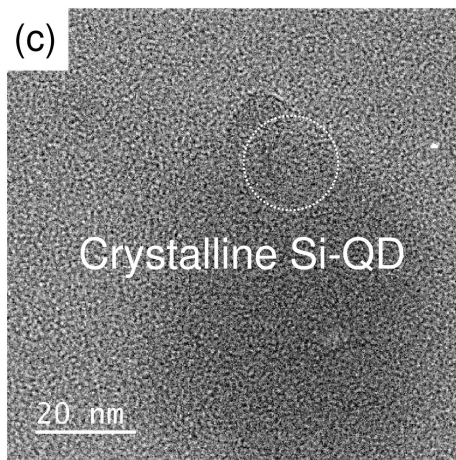
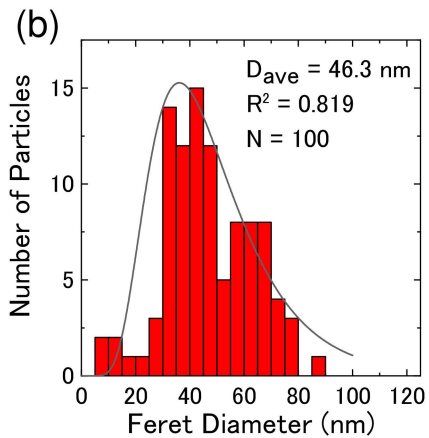
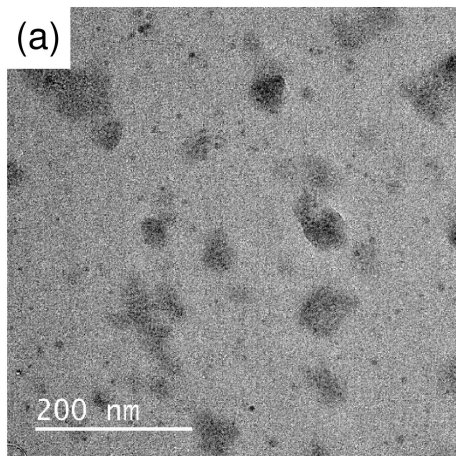
Figure 2. (a, b) Agarose gel electrophoresis of Si QDs-BSA composites. The BSA concentration is changed from 0 to 100 µM. The diameter of Si QDs is (a) 4 nm and (b) 7nm. The images on the right are electrophoresis of BSA (without Si QDs). The gels are stained by Coomassie brilliant blue to show the position of BSA. (c, d) Polyacrylamide gel electrophoresis (PAGE) of Si QDs-BSA composites. The BSA concentration is changed from 0 to 100 µM. The diameter of Si-QDs is (c) 4 nm and (d) 7nm. The left-most lanes are molecular rulers to estimate the molecular weight of protein.

Figure 3. (a) TEM image and (b) size distribution histogram of Si QDs-BSA composites. (c) High-resolution TEM image and (d) the FFT image of (c). The diameter of Si QDs is 7 nm and the BSA concentration is 100 µM.

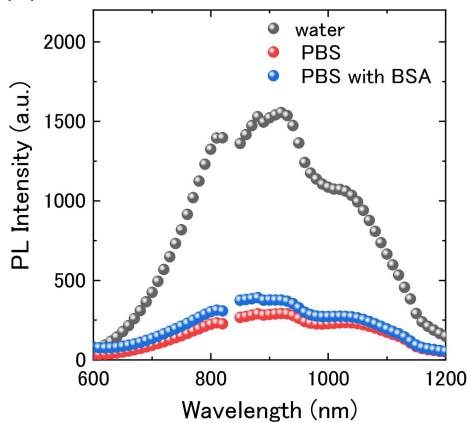
Figure 4. (a, b) PL spectra of Si QDs in water (black) and in PBS solution (red), and Si QDs-BSA composites in PBS solution (blue) excited at 405 nm. The diameters of Si QDs are (a) 4 nm and (b) 7 nm. The BSA concentration is 100 μ M. (c) PL peak wavelength and the intensity of Si QDs-BSA composites in PBS solution as a function of incubation duration.



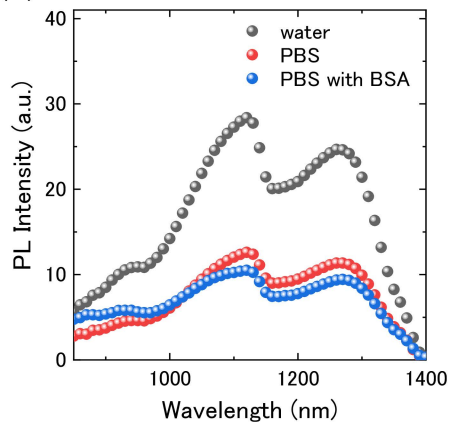




(a)



(b)



(c)

