Synthesis and characterization of quantum dot chains: Self-assembly of mercaptopropionic-acid-capped quantum dots conjugated with short single-stranded DNA

M Oda¹, ², K Sasano², A Nishi², T Zako³ and T Tani⁴
¹ Department of Basic Sciences, Faculty of Engineering, ² Department of Applied Science for Integrated System Engineering, Graduate School of Engineering, Kyushu Institute of Technology, 1-1 Sensui-cho, Tobata-ku, Kitakyushu, Fukuoka, 804-8550 Japan
³ Department of Chemistry and Biology, Graduate School of Science and Engineering, Ehime University, 2-5 Bunkyo-cho, Matsuyama, Ehime 790-8577 Japan
⁴ Department of Applied Physics, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Kogane-i, Tokyo 184-8588, Japan
Email: odamasa@mns.kyutech.ac.jp

Abstract. In this paper, we describe the synthesis and characterization of quantum dot (QD) chains. We used small QD-DNA conjugates, i.e., short-ligand-capped QDs conjugated with short (15-mers) single-stranded DNA, as the building blocks. The small conjugates were separated based on the valence of the DNA, i.e., the amount of DNA per particle, using agarose gel electrophoresis. The conjugates formed self-assemblies composed of closely spaced (inter-particle spacing of 0.6–2 nm) QDs. We report the results of our investigation of the electrophoresis and hybridization of the conjugation.

1. Introduction
DNA-functionalized quantum dots (QDs) are QDs conjugated with single-stranded (ss) DNA oligomers. They have been used as building blocks for self-assemblies in a variety of fields, such as materials science and engineering. In particular, self-assemblies composed of closely spaced QDs have received considerable attention due to their novel optical and electronic properties, which arise from energy transfer/coupling between neighboring QDs [1,2].

To increase the efficiency of energy transfet/coupling in the assemblies, it is necessary to decrease the inter-particle spacing between adjacent QDs. Short-ligand-capped QDs conjugated with short ssDNA particles are believed to be suitable for this purpose, although there are some practical difficulties. For example, it is easy to quench their photoluminescence (PL) during synthesis procedures [3] and difficult to control the valence of the DNA [4] due to the small charge and molecular size of short DNA.

We recently investigated the synthesis of one-dimensional (1D), closely spaced QD chains based on short mercaptopropionic-acid (MPA)-capped QDs conjugated with short (15-mer) ssDNA. In this paper, we present the results of this investigation.

2. Experiments

2.1. Conjugation of ssDNA onto QD surface
DNA-functionalized QDs were fabricated according to the following procedure: first, CdSe/ZnS core-shell QDs capped with trioctylphosphineoxide (TOPO) were synthesized via pyrolytic decomposition of organometallic compounds (or QDs provided by NS Materials Inc.) [5]. Next, the TOPO ligand was exchanged with MPA in chloroform using Pong’s method [6]. The QDs were rendered water-soluble, so that a droplet containing QDs capped with MPA floated on chloroform. The droplet was washed several times with fresh chloroform, and then diluted in borate buffer solution (pH 8.3). QDs capped with MPA are known to be stable in the pH range between 7.0 and 9.5 [6]. Thus, all procedures described in §2.1–2.3 were performed at a pH of 8.3.

Separately, thiolated short ssDNA oligomers (5’-HS-(CH2)6-TACGCCACCGGCTCC-3'; Eurofins Genomics K.K.) were dispersed in borate solution. Then, 3 mL of mixed borate solution containing the QDs capped with MPA (10^5–10^7 mol/L) and the ssDNA (10^5–10^7 mol/L) was prepared and left for 12 hours at room temperature to conjugate the ssDNA oligomers onto QD surface.

2.2. Agarose gel electrophoresis
Agarose gel electrophoresis was used to separate the DNA-functionalized QDs based on the DNA valence (N_DNA). Just prior to the electrophoresis, the DNA-functionalized QD solution (100 μL−1 mL) was concentrated with a centrifugal filter device to a volume of 50 μL. Then, electrophoresis was performed using agarose gel (NuSieve GTG agarose; Lonza) in 0.5× tris-borate buffer solution with an electrophoresis system (Mupid-2plus; Takara Bio Inc.). Some parts of the gel containing the separated QDs were cut and mashed after electrophoresis, then loaded in separate microtubes with 500 μL borate buffer solution. We extracted the QDs from the gel by rotating the microtubes using a 12 cm rotator at 20 rpm for 12 h.

2.3. Hybridization of DNA-functionalized QDs
Here, 1 mL of the DNA-functionalized QD solutions were mixed with 1 mL of solutions containing similarly prepared QDs conjugated with complementary DNA oligomers (5’-HS-(CH2)6-ATGCCGGTGGTCTGAGG-3'). We fabricated the QD self-assemblies based on DNA hybridization by gradually cooling the mixed solutions to room temperature following by heating them to 75 °C.

2.4. Characterization of QD self-assemblies
We characterized the structures of the QD self-assemblies using transmission electron microscopy (TEM; H-9000; Hitachi, Ltd.). The samples for the TEM analysis were obtained as follows: first, TEM grids (NPC15; Okenshoji Co., Ltd.) were hydrophilized by plasma treatment. Droplets of self-assembled QD solutions were dropped onto plastic films, and then covered by TEM grids for 20 minutes to adsorb the assemblies onto the TEM grid. Finally, the grids were dried by evacuation for 2 days.

3. Results and discussions
DNA-functionalized QDs with N_DNA = 2 can be used to build 1D chains, as shown in Figure 1(a). First, we describe our results regarding the optimization of the mixing molar ratio of ssDNA to QDs used for the conjugation process. Figure 1(b) shows a typical TEM image of QD self-assemblies fabricated by using DNA-functionalized QDs prepared with a molar ratio of 8:1. This was the best ratio for the fabrication of chain-like assemblies in the case without using electrophoresis, although the assemblies had branched structures, as shown in Figure 1(b). The branched structures suggest that QDs with N_DNA = 3 or more were also fabricated, even when using the best molar ratio for obtaining those with N_DNA = 2. Monomers and dimers of QDs were obtained in the ratio of 1:1–2:1 (not shown here). The apparent ratio dependence of the assembled structure confirms that both the conjugation of thiol-modified DNA and crosslinking of the DNA-functionalized QDs works properly. The average inter-particle spacing was estimated to be 0.6 nm, although it was several times shorter than the DNA molecule. We believe that this shortness is due to the deformation of the DNA by drying in a vacuum and/or adsorbing into the grid prior to the TEM observations.

We also found a novel structure in assemblies prepared at the molar ratio of 8:1, although its production
Figure 1. (a) Schematic diagram of the formation process of one-dimensional closely spaced quantum dots (QDs). (b) Typical transmission electron microscopy (TEM) image of QD self-assemblies fabricated from QD-DNA conjugates. The molar mixing ratio, r, of the sample was 8. Average diameter of the QDs was 4.0 ± 0.4 nm.

Figure 2. TEM image of circle-like QD structures. Average diameter of the QDs was 2.8 ± 0.4 nm. The lattice spacing was 0.37 nm, which matches with the (100) plane of bulk cadmium selenide (CdSe).

efficiency and reproducibility were quite low. It seems that the QDs formed circle-like structures with short inter-particle distance, and that their lattice fringes aligned along the circle, i.e., the crystal orientations of the QDs aligned with the circle, as shown in Figure 2. Further investigations of the structure may provide important information not only on how best to fabricate well-aligned QD assemblies but also on optical properties of QD assemblies exhibiting highly efficient energy transfer/coupling.

Next, we describe the electrophoresis separation of the DNA-functionalized QDs before hybridization. Figure 3 shows a typical PL image of agarose gel after electrophoresis. The QDs capped with MPA (without DNA conjugation) migrated towards an anode, as shown in lane 1, which means that the MPA ligands attached to the surfaces of the QDs have negative charges. A broad PL band appeared, and the most luminous position, i.e. the place most densely populated by QDs, was around A on line 1. We also measured the PL spectra of the QDs at different migration distances in the broad PL band (not shown here) and observed only a small change in the peak PL energy, although the PL energy depended on the size of the QD. Therefore, the origin of the broadness of the PL band is assumed to be charge dispersion due to the numerical dispersion of MPA ligands on the surfaces of the QDs.

It has been noted that PL quenching of the QDs sometimes occurs during and after electrophoresis when a buffer is used. We found that buffers containing ethylenediaminetetraacetic acid (EDTA), such as tris/borate/EDTA (TBE) buffer, caused such quenching. The DNA-functionalized QDs prepared at mixing ratios of 4:1 and 8:1 migrated in lanes 2 and 3, respectively. The QDs were separated into α, β and γ bands, as shown in Figure 3, although the tails of the bands overlapped slightly. The probability of separation into slow migration bands (β and γ) increased with the mixing ratio. Thus, we assume that the migration speed decreases with increasing N_{DNA}, as shown in Figure 3, although this migration order was opposite to that of conjugates between streptavidin-coated QDs and relatively long DNA [4]. The bandwidth was not as broad as that of the QDs in lane 1, which suggests that the charge on the short DNA oligomer was larger than the total charge of the MPA ligands bound to the surfaces of the QDs.

Then, we describe the structural features of self-assemblies of the QDs after hybridization. Figures 4 (a) and (b) show typical TEM images of the self-assemblies of the QDs extracted from band α (prepared at the molar ratio of 8:1) and similarly prepared QDs conjugated with complementary DNA oligomers. Figure 4 (c) shows a histogram of the particle number per assembly obtained from the TEM images. As expected, the most frequent particle number was 2 (the black bar in Figure 4 (c)), which confirms that the main component in band α was QDs with N_{DNA} = 1. We also observed 1D QD chains without blanched structures, as shown in Figure 4 (b) and by the red bars in Figure 4 (c).
Figures 3. Photoluminescence (PL) image of agarose gel under 365-nm light irradiation. The QDs without DNA, with DNA \((r = 4)\), and with DNA \((r = 8)\) migrated in lanes 1, 2 and 3.

Figures 4. (a) and (b) show typical TEM images of self-assemblies of the QD-DNA conjugates extracted from band \(\alpha (r = 8)\). The lines represent the outlines of the particle. (c) Histogram of the particle number per assembly. Average diameter of the QDs was \(5.8 \pm 0.8\) nm.

particle spacing was estimated to be approximately 1–2 nm, although accurate estimation was difficult due to the low spatial resolution of the TEM images. The low resolution was probably due to the existing of agarose molecules. The mixing of the QDs with \(N_{DNA} = 2\) into the band \(\alpha\) is believed to contribute to the formation of the 1D closely spaced QD chains without blanched structures.

We observed QD chains with blanched structures in self-assemblies fabricated using QDs extracted from band \(\beta\) (not shown here). The shape of the QD chains was similar to those in Figure 1 (b). This suggests that band \(\beta\) did not only contain QDs with \(N_{DNA} = 2\), but also those with \(N_{DNA} = 3\) (and 1). Thus, it is necessary to improve the separation resolution to fabricate 1D assemblies using QDs from band \(\beta\). We recently found that we can narrow each band by removing excess MPA ligands from the MPA-capped QDs prior to conjugating them with the ssDNA oligomers. The improvement will be described elsewhere in the near future.

4. Summary

We conjugated short-ligand-capped QDs, i.e., MPA-capped QDs with thiol-modified short (15-mer) ssDNA. The small QD-DNA conjugates were used as building blocks for self-assemblies of 1D QD chains. We fabricated closely spaced assemblies with inter-particle spacings of 0.6–2 nm. The small QD-DNA conjugates were roughly separated by \(N_{DNA}\) using agarose gel electrophoresis. Some of the conjugates obtained after the electrophoresis formed 1D closely spaced chains without branched structures. We did not achieve precise control of the shape and particle number. We plan to improve this method for fabricating 1D closely spaced chains in the near future.

Acknowledgments

This work was partly supported by grants-in aid from the Ministry of Education, Science, Sports and Culture, Nos. 25286015 / 16K04878.

References

[1] Tikhomirov G, Hoogland S, Lee P, Fischer A, Sargent E, and Kelley S, 2011, *Nat. Nanotechnol.*, 6, 485
[2] Koole R, Liljeroth P, Donega C, Vanmaekelbergh D, and Meijerink A, 2006, *JACS*, 128, 10436
[3] Banerjee A, Pons T, Lequeux N, and Dubertret B, 2016, *Interface Focus* 6, 20160064
[4] Carstairs H, Lymeropoulos K, Kapanidis A, Bath J, and Turberfield A, 2009, *ChemBioChem*, 10, 1781
[5] Hashizume K, Matsubayashi M, Vacha M, and Tani T, 2002, *J. Lumin.*, 98, 49
[6] Pong B, Trout B, and Lee J, 2008, *Langmuir*, 24, 5270