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Quantum-dot-embedded silica nanotubes as nanoprobes for simple and sensitive DNA detection

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Abstract
We have developed a new technique using fluorescent silica nanotubes for simple and sensitive DNA detection. The quantum-dot-embedded silica nanotubes (QD-SNTs) were fabricated by a sol–gel reaction using anodic aluminum silica oxide (AAO) as a template. The fluorescent QD-SNTs of different colors were then immobilized with single-stranded DNA and used as nanoprobes for DNA detection. The optical and structural properties of QD-SNT nanoprobes were examined using photoluminescence spectroscopy, confocal microscopy and transmission electron microscopy (TEM). The QD-SNT nanoprobes were applied to detect dye-labeled target DNA in a solution phase. The obvious color change of the QD-SNT nanoprobes was observed visually under a simple microscope after the successful detection with target DNA. The quantitative analyses indicated that ∼100 attomole of target DNA in one nanoprobe can generate a distinguishable and observable color change. The detection results also demonstrated that our assay exhibited high specificity, high selectivity and very low nonspecific adsorption. Our simple DNA assay based on QD-SNT nanoprobes is expected to be quite useful for the needs of fast DNA screening and detection applications.

1. Introduction

The development of new sequence-specific DNA detection assays has important applications in medical research and disease diagnostics. Recently, nanomaterial-based DNA detection assays have been widely explored and shown remarkable potentials for future clinical testing [1–4]. In general, nanomaterial–DNA conjugates with unique optical or structural characteristics were designed and used as a DNA detection probe to detect specific target DNA sequences. The successful detection of target DNA sequences was usually differentiated through the changes in optical signal after the hybridization. Because of the unique physical properties of the nanomaterials [5–9], the assays have provided some advantages such as shorter detecting time and easier set-up in comparison to those of a microarray system [10]. To date, various types of nanomaterials were still being explored to be designed as a new DNA probe.

Recently, nanomaterials such as gold nanoparticles [11], silica nanoparticles and quantum dots have been applied to design new DNA detection assays. The gold nanoparticle–oligonucleotide conjugates were hybridized with the target DNA of interest and resulted in a red-to-purple color change, providing a colorimetric DNA detection method [12–17]. Also, gold nanoparticles promoting silver reduction were applied in Raman [18] and electric [19] detection assays with high sensitivity and selectivity. Furthermore, gold nanoparticles were conjugated with oligonucleotide and organic dye to form a hybrid material as a molecular beacon. In this hybrid material, gold nanoparticles were used as a

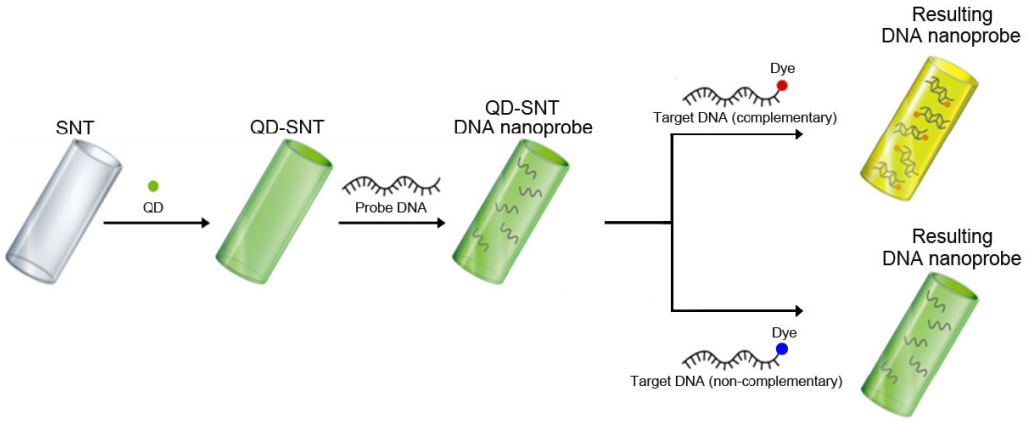

Figure 1. The silica nanotube was first incorporated with QDs to become fluorescent QD-SNTs. Then, the QD-SNT DNA nanoprobe is prepared by the immobilization of a specific probe DNA sequence onto the fluorescent QD-SNTs (green). After the hybridization with dye-labeled target DNA (red, labeled with complementary target DNA; blue, labeled with non-complementary target DNA), the resulting nanoprobes exhibited either yellow (successful hybridization) or green (unsuccessful hybridization) as judged visually under a fluorescent microscope.

(This figure is in colour only in the electronic version)

quencher, to quench the fluorescence of the organic dye into close proximity. After hybridization, fluorescence was restored when complementary target DNA was detected [20, 21]. Besides gold nanoparticles, silica nanoparticles [22] and quantum dots (QDs) [23] were designed as a signal indicator in DNA probes to achieve strong and photostable fluorescence signals. Fluorescent dye-doped silica nanoparticles were functionalized with oligonucleotides as a label for gene profiling [24–26]. QDs were used as energy donors in a FRET donor–acceptor ensemble and target concentrator in the presence of target DNA. The resulting assembly revealed significant fluorescent emission to differentiate target DNA by means of FRET on illumination of the donor [27–29]. QDs were also used as optical coding materials in DNA detection assays. By tagging specific sequence of DNA, QD-based nanoprobes were prepared and then mixed with the solution in the presence of target DNA. As a result, colorization of the two linked QD nanoprobes revealed a combined color to indicate hybridization results [30]. In addition, recent studies have demonstrated that QDs were embedded in polymeric structures and applied them for multiplexed optical coding detection assays [31–34]. In these cases, both coding and target signals can be simultaneously obtained, and massively parallel coding of the DNA sequence can be achieved.

In this work, we have prepared QD-embedded silica nanotubes (QD-SNTs) of different colors. The QD-SNTs are then immobilized with single-stranded DNA molecules and used as a DNA detection probe (QD-SNT nanoprobes). After mixing with complementary dye-labeled target DNA, the color of the QD-SNT nanoprobes is changed due to color merging with the target DNA. The color can be distinguished easily under a microscope so that the hybridization result can be judged in a simple and fast way (figure 1). Various target DNA sequences in a solution were used to test the selectivity, specificity and nonspecific adsorption of our DNA assay. The results showed that the assay can detect target DNA with high selectivity and sensitivity up to ∼100 attomole of DNA while the color of one QD-SNT nanoprobe was distinguished under a microscope. The simple and fast DNA detection assay has provided a new strategy for the development of fast and easy DNA diagnostic kits in a solution phase.

2. Experimental section

2.1. Materials

Unless specified, chemicals were purchased from Acros Organics (Geel, Belgium). Ethyl alcohol (Shimakyu's Pure Chemicals), 10× PBS buffer (aMReSCO®), 20× SSC buffer (aMReSCO®), hybridization buffer (Sigma) and sodium dodecyl sulfate (SDS, J T Baker) were used as received. All aqueous solutions were prepared with nanopure water (DI water, resistivity = 18.2 MΩ cm) obtained from a Millipore Milli-Q gradient water polisher. A commercial and ordered nanochannel array of an anodic aluminum oxide (AAO) membrane with a thickness of 60 μm and a pore diameter of 200 nm was purchased from Whatman (Anodisc 13). Anti-bleach solution was purchased from Vector Laboratories (H-100).

2.2. Synthesis of QD-SNTs

QD-SNTs were prepared according to a previous report [35]. First, a silica sol was prepared from a mixture of tetraethyl orthosilicate (52 ml, 98%), ethanol (230 ml), H_2O (18 ml) and HCl (0.27 ml). After aging the sol for 15 days, the AAO membrane was dipped into it at 4 °C for 1 min then dried at 90 °C under vacuum. After rinsing with DI water and methanol, the AAO membrane was placed in a solution containing 10% 3-(aminopropyl)trimethoxysilane (APTMS, 97%) in methanol for 12 h then dried at 90 °C for 1 h under vacuum. Second, different sizes of CdSe/ZnS nanocrystals (exhibiting different colors) were prepared [36], and the surfaces of CdSe/ZnS nanocrystals were exchanged

with mercaptoacetic acid ligands (–S(CH_2)$_2$COOH) [37, 38], making them water-soluble. The AAO membrane with an APTMS-modified silica layer was immersed into the water-soluble nanocrystal solution in a 4 °C dark room for 12 h then dried at 50 °C for 1 h under vacuum. Afterwards, the membrane was dipped into the silica sol–gel again at 4 °C for 1 min then dried at 90 °C for 1 h under vacuum. The resulting membrane was polished with a commercial polishing machine (thickness: approx. 2 μm). Finally, the resulting membrane was dissolved with HCl (1 ml, 5 M) and the QD-SNTs were collected by centrifuging then stored in a 4 °C dark room. QD-SNTs were characterized by transmission electron microscopy (TEM) (JEM-2010, JEOL, Japan; H-7100, Hitachi, Ibaragi) and imaged by a fluorescence microscope (Leica TCS SP2, Leica Microsystems GmbH, Wetzlar, Germany).

2.3. Synthesis of QD-SNT nanoprobes.

DNA (MDBio, Inc., Taipei, Taiwan) was synthesized with/without a 5′-aminohexyl group modification (–NH_2–C_6) containing the following sequences: P1 : 5′-NH_2–C_6-GGC GGT GGT GGA CAA G-3′, P1′ : 5′-GGC GGT GGT GGA ATT G-3′, P3 : 5′-NH_2–C_6-GGC GGT GGT GGA CAA GAA CAT TAT-3′. P1 and P3 served as the probe DNA and P1′ was used in a control experiment. All DNA solutions (10 μM) were stocked in DI water and were heated to 95 °C for 2 min then placed on ice for 10 min before QD-SNT nanoprobes synthesis.

To prepare QD-SNT nanoprobes, the QD-SNT-embedded membrane was immersed into APTMS solution for an additional 12 h at room temperature then dried at 90 °C for 1 h under vacuum. At present, the outer surfaces of QD-SNTs remained intact and were not modified with APTMS because of membrane protection. Only the inner surfaces of the QD-SNTs were modified with APTMS. Free-standing and APTMS-modified QD-SNTs were obtained by mechanically polishing both surfaces of the membrane with sandpaper to reach the desired length of QD-SNTs (approx. 2 μm). After dissolving the membrane in H_3PO_4, APTMS-modified QD-SNTs were collected and reacted with 1.25% (v/v) glutaraldehyde (GA) [39] at 4 °C for one day. Then, GA-linked QD-SNTs were washed with 1× PBS and DI water followed by being dried at 90 °C for 1 h under vacuum. The resulting QD-SNTs (APTMS-and GA-modified) were incubated with DNA (10 μM, 10 μl) to a final volume of 20 μl in DI water then incubated at 50 °C for 2 h. Finally, DNA was immobilized onto the inner surfaces of QD-SNTs to form QD-SNT nanoprobes, and a specific sequence of DNA was coded by a specific color of QD-SNTs. All procedures were accomplished against light.

2.4. QD-SNT-based DNA detection assay

As we mentioned before, the following procedures were also accomplished against light. Before hybridization, QD-SNT nanoprobes were washed with 0.1% Tween 20/PBS and DI water. Target DNA (T1 : 5′-TAMRA-CTT GTC CAC CAC CGC C-3′) was stocked in buffer solution (3× SSC, 0.2% SDS) at 4 °C to a final concentration of 10 μM. Hybridization was accomplished by mixing QD-SNT DNA nanoprobes and target DNA (5 μl, 1 μM) at 55 °C for 30 min, and hybridization buffer was added to a total volume of 20 μl. After the hybridization, the solution was completely washed with washing buffer (2× SSC, 0.2× SSC and DI water) in sequence to remove unhybridized target DNA. A hybridization buffer containing QD-SNTs only was used as a negative control experiment. The same protocol and conditions were used for hybridization with other sequences of target DNA (T2 : 5′-FITC-CTT GTC CTC CAC CGC C-3′, T3 : 5′-Cy5-ATA ATG TTC TTG TCC ACC ACC GCC-3′, T4 : 5′-Cy3-CCA ATC TCA CCT GTT CCT GTA ATA-3′).

2.5. Image capture under a fluorescence microscope.

Glass microscope slides were first cleaned in 1 M KOH in a sonicator for 30 min and rinsed thoroughly with DI water. After drying by N_2, a 6 μl drop containing a mixture of sample (3 μl) and anti-bleach solution (3 μl) was pipetted onto the slide and illuminated by a confocal microscope equipped with a 100× oil objective (Leica, NA = 1.3). Light and humidity were well controlled to avoid pre-bleaching of fluorescence and scattering interference of dried samples.

2.6. Calculation of target DNA in single QD-SNT DNA nanoprobe

To further quantitatively define the color change in the assay, the amount of target DNA inside each resulting nanoprobe was analyzed using software (Northern Eclipse, Empix, Toronto, Canada). Five different concentrations of target DNA T1 (from 11.36 to 568 nM) were prepared and separately mixed with QD-SNT DNA nanoprobes (P1 DNA immobilized). For each experiment, the image was captured in a size of 80 × 120 μm^2 grids from different regions for 30 pictures and repeated three times, which means 90 pictures were obtained and analyzed.

3. Results and discussion

3.1. Preparation and characterization of QD-SNTs

Figure 2 shows the optical images and fluorescence spectra of QD-SNTs after being removed from the AAO membrane. The open-ended QD-SNTs are clearly observed in the image with a uniform length of 2 μm and pore diameters of 200 nm (figure 2(a)). The outside diameter (∼200 nm) of the resulting QD-SNTs was determined by the pore sizes of the template (AAO membrane). The length of QD-SNTs was controlled by polishing the AAO membrane to reach the desired thickness before they were removed. They are well dispersed in water solution without aggregation, mainly because of the hydroxyl groups on their surfaces. QD-SNTs were stored in DI water at room temperature for more than one month without any decomposition. In addition, the EDS analyses of the samples confirmed the presence of CdSe and ZnS of QD-SNTs. Figure 2(b) showed the fluorescence image of QD-SNTs of four different colors. Each color in the image is easily distinguished visually against the background. Here, green and red QD-SNTs represented SNTs embedded with

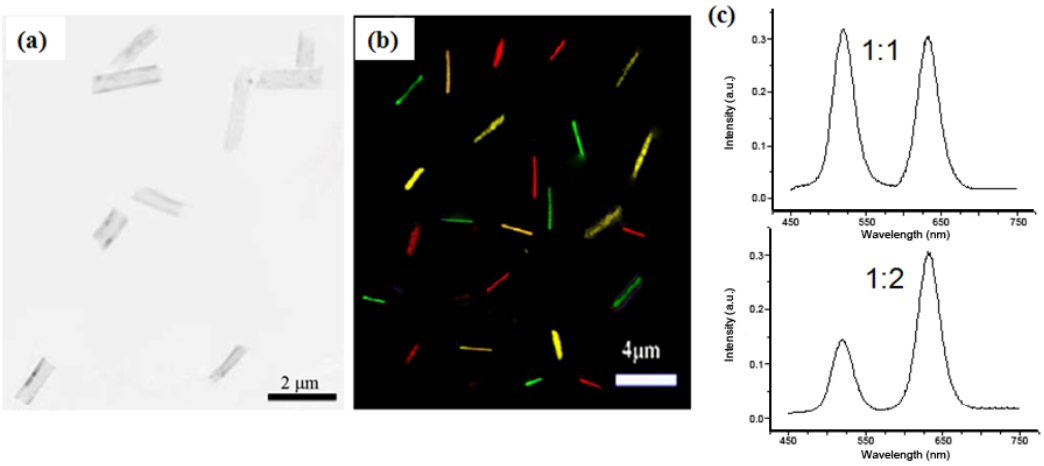

Figure 2. (a) Optical images of QD-SNTs after removal from 200 nm pore sizes of AAO membrane. The length of QD-SNTs was uniformly controlled, and the outer diameter of QD-SNTs was consistent with the pore sizes of AAO membrane. (b) Fluorescence image of a mixture of QD-SNTs. Green (or red) QD-SNTs were prepared by embedding green (or red) QDs into a single SNT, respectively. Yellow and orange QD-SNTs were prepared by embedding both green and red QDs of different concentration ratios into a single SNT in sequence. Different colors of QD-SNTs were prepared in separate batches, then mixed and spread on a glass slide. (c) PL spectra of yellow (up) and orange (down) QD-SNTs. The intensity ratios of 1:1 and 1:2 indicated the concentration ratio of green and red QDs in QD-SNTs.

green and red QDs, respectively. Yellow and orange QD-SNTs were prepared from SNTs that were embedded with green and red QDs of different concentration ratios. The ratios of green and red QDs were adjusted based on their PL intensities. Here, yellow QD-SNTs were prepared based on [green QDs]/[red QDs] $=$ 1 and orange QD-SNTs were obtained from [green QDs]/[red QDs] $=$ 1/2. The image in figure 2(b) exhibited a uniform yellow and orange color from one end to the other, and also no uneven striped color pattern was found.

The fluorescence spectra of yellow and orange QD-SNTs were shown in figure 2(c). No significant shift and broadening of the fluorescence peak in the spectra were observed before and after QDs were embedded into the QD-SNTs. Also, no obvious photo-bleaching of QD-SNTs was found under a microscope for more than a half hour. We believe that the silicon oxide layer acts as a matrix to spatially block the embedded QDs against QD aggregation and oxidation. Our simple calculation indicated that the average distance between two adjacent QDs is ∼30 nm inside QD-SNTs of 2 μm in length [31]. The distance is much larger than the Förster energy transfer radius for QDs (5–8 nm) [31]. Overall, the optical measurements suggested that green and red QDs were distributed quite evenly inside the SNTs. Also, no aggregation of QDs and no energy transfer between QDs occurred after the QDs were embedded into the silicon oxide layer. In order to test the stability of QDs embedded inside SNTs for further DNA detection, buffers containing SSC and SDS with various concentrations were added to the QD-SNT solution. No noticeable decrease of fluorescence intensity of QD-SNTs was observed in the spectrum after the solution was kept at 55 °C for 2 h. These results indicated that QD-SNTs remained physically stable and no QDs were released into solution even in high ionic strength solutions (3× SSC, 0.2% SDS). The unique optical characteristics of QD-SNTs have provided great advantages for coding colorless probe DNA. Also, the high solubility of QD-SNTs in water allowed us to design a DNA detection system in a solution phase. Because the color of each QD-SNT can be distinguished clearly under a microscope, the detection system of a DNA sequence based on the color readout of a single QD-SNT has become feasible.

3.2. Qualitative analysis of QD-SNT-based DNA detection assay

3.2.1. Specificity and selectivity testing. The DNA detection assay was designed based on water-soluble QD-SNTs. First, a defined color of QD-SNTs was conjugated with single-stranded probe DNA onto the inside walls of the QD-SNTs to generate color-and-sequence-coded QD-SNT nanoprobes. Then, target DNA was mixed with the QD-SNT nanoprobes in solution to reach the complete hybridization. Finally, the resulting nanoprobes were placed on a glass slide and observed under a microscope.

Table 1 lists the experimental details of ten representative tests using our QD-SNT-based DNA detection assay. Here, each coded DNA nanoprobe of a defined color was designed and then applied to detect dye-labeled target DNA sequences. After hybridization, the colors of the resulting nanoprobes and detected target DNA were also presented in table 1. The first experiment (Exp. 1) in table 1 demonstrated the basic concept of the assay. First, QD-SNTs of green color were conjugated with single-stranded probe DNA (P1) to form green P1-nanoprobes. Red target DNA (Red T1, TAMRA-labeled) was then mixed with the P1-nanoprobes. As a result, all of the resulting P1-nanoprobes appeared yellow after hybridization, as we can observe under a microscope (figure 3(a)). Every individual resulting P1-nanoprobe was distinct without any aggregation. As shown in figure 3, the yellow color of the resulting P1-nanoprobes is inferred to be a merged color composed of green and red fluorescence of green P1-nanoprobes and red T1 under an optical microscope

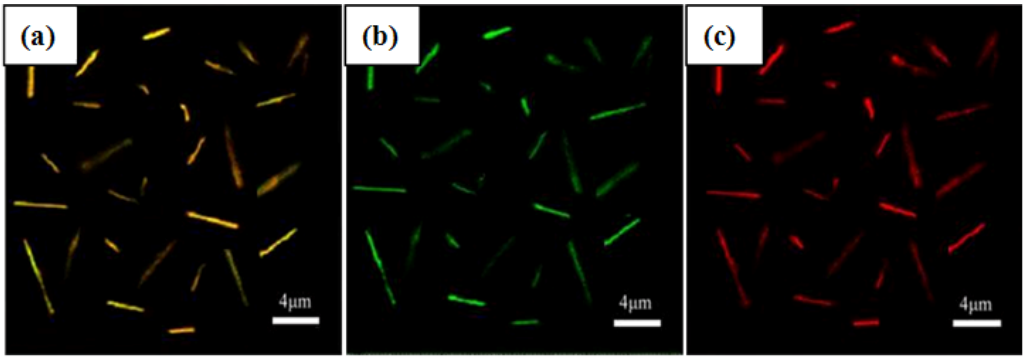

Figure 3. Fluorescence images of the resulting P1-nanoprobes. The basic principle of the QD-SNT-based DNA detection assay is demonstrated using green color of QD-SNTs P1-nanoprobes to detect red color of complementary target DNA T1 (TAMRA-labeled). After hybridization, the resulting P1-nanoprobes were observed under microscope. Three colors of the resulting nanoprobes were obtained as yellow (a), green (b) and red (c), corresponding to the merged color of the resulting P1-nanoprobes, green fluorescence of QD-SNTs and red fluorescence of target DNA T1, respectively. Each scale bar indicates 4 μm from (a) to (c).

Table 1. QD-SNT-based DNA (note: DNA sequences used here were shown in section 2) detection assay.

Exp. No.[a]	Color of QD-SNTs nanoprobes	Color of target DNA	Color of the resulting nanoprobes	Detected target DNA after hybridization
1	Green P1-nanoprobes	Red T1	Yellow	T1
2	Red P1-nanoprobes	Green T2	Red	—[b]
3	Green P3-nanoprobes	Red T3	Yellow	T3
4	Red P3-nanoprobes	Green T4	Red	—[b]
5	Yellow P1-nanoprobes	Red T1 Green T2	Orange	T1
6	Yellow P3-nanoprobes	Red T3 Green T4	Orange	T3
7	Yellow P1-nanoprobes	Red T1 Green T2 Green T4	Orange	T1
8	Green QD-SNTs + P1	Red T1	Green	—[b]
9	Green QD-SNTs + P1	Red T1	Green	—[b]
10	Green QD-SNTs + P1′	Red T1	Green	—[b]

[a] Experiment number.

[b] No distinguished fluorescence from target DNA of the resulting nanoprobes was observed. We defined this result as no target DNA was detected.

with filter panels. The images indicated that the green and red fluorescent colors of each resulting P1-nanoprobe were distributed quite uniformly throughout the entire resulting P1-nanoprobe. The yellow fluorescence of the resulting P1-nanoprobes was observed at the positions where green and red fluorescence were located on the glass slide. The results of Exp. 1 suggested that the red T1 was hybridized with green P1-nanoprobes. The successful hybridization can be simply justified by the color of the resulting nanoprobes. The uniform fluorescence in figure 3 also suggested that the hybridization probability of red T1 was equal throughout the whole tubular dimension of the resulting P1-nanoprobes. The equal hybridization probability was attributed to the uniform immobilization of P1 onto the inner wall of QD-SNTs before the hybridization.

To further test the specificity of the QD-SNT-based DNA detection assay, Exp. 2 in table 1 was performed by the hybridization of red P1-nanoprobes and green T2 (16-mer, non-complementary to probe DNA). The results showed that only red fluorescence was observed throughout the entire field of view, indicating that non-complementary green T2 was not detected by the red P1-nanoprobes. Instead of using the 16-mer probe and target DNA in Exps. 1 and 2, Exp. 3 and Exp. 4 were also performed using 24-mer probe DNA-immobilized nanoprobes and 24-mer target DNA (table 1). Furthermore, four additional tests were also performed using 16-mer P1-nanoprobes to separately detect 24-mer target DNA (T3 and T4), and 24-mer P3-nanoprobes to separately detect 16-mer target DNA (T1 and T2). The overall results of the aforementioned tests indicated that, after the hybridization, complementary target DNA can be successfully detected by the corresponding nanoprobes with high specificity, and they can be easily distinguished through the color of the resulting nanoprobes. Further, detection results remained of high specificity when different sequence lengths of nanoprobes and target DNA were used.

The selectivity tests of the QD-SNT-based DNA detection assay were performed in Exps. 5–7. Three individual target

DNA solutions were prepared by mixing two target DNA sequences (T1 and T2 in Exp. 5, T3 and T4 in Exp. 6) at 1:1, 1:3 and 1:9 ratios. In Exp. 7, three individual target DNA solutions were prepared by mixing three target DNA sequences (T1, T2 and T4) at 1:1:1, 1:2:2 and 2:9:9 ratios. The results showed that yellow P1-nanoprobes or yellow P3-nanoprobes can selectively detect complementary target DNA from those individual target DNA solutions containing two or three different DNA sequences at different ratios, even those target DNA solutions containing non-complementary target DNA with much higher ratios (almost 90% in Exp. 7) than complementary target DNA (T1).

3.2.2. Nonspecific adsorption testing. To test the extent of nonspecific adsorption of the detection assay, QD-SNTs were mixed with/without probe DNA and target DNA in Exps. 8–10. After the mixing and then washing procedures similar to those of Exps. 1–7, the results showed that the color of the QD-SNTs was unchanged, and a negligible amount of double-stranded DNA (P1 and T1 in Exps. 8–9) or single-stranded DNA (T1 in Exp. 10) was adsorbed on the resulting QD-SNTs. Obviously, the nonspecific adsorption of target DNA onto QD-SNTs was not significant. The negligible nonspecific adsorption was mainly attributed to strong repulsion between the hydroxyl groups on QD-SNTs and the phosphate groups on the DNA backbone. Overall the qualitative tests of Exps. 1–10 have demonstrated that our QD-SNT-based DNA detection assay can be easily executed on a single nanoprobe under a microscope. The assay might be further developed to be a convenient DNA detection kit.

3.3. Concentration of target DNA in single QD-SNT nanoprobe after the hybridization

Our detection assay has provided a simple method to qualitatively differentiate the target DNA sequence by judging the color of the resulting nanoprobes. To further quantitatively define the color change in the assay, the amount of target DNA inside each resulting nanoprobe was measured. Different concentrations of target DNA (red T1) were separately detected by green P1-nanoprobes, and the detection results were plotted in figure 4. Significantly reduced signal fluctuation (RSD $<$ 10%) and good linear response ($R^2 = 0.981$) was achieved, indicating that our detection assay was carefully controlled. Although approximately 2 attomole of red T1 was the lowest amount to be detected for each P1-nanoprobe and observed under a microscope, the merged color of the resulting P1-nanoprobes was not apparent and hard to judge. To reach the distinguished merged color (yellow color) similar to Exp. 1, at least 1×10^{-16} mole of complimentary T1 was detected inside each P1-nanoprobe. The data indicated that $\sim$100 attomole of target DNA in one nanoprobe can generate the distinguishable color change that was easily judged visually under a microscope in our assay. In other words, we can define the lowest amount of target DNA resulting in a distinguishable color change of our assay to be $\sim 1 \times 10^{-14}$ mole if 10 QD-SNT nanoprobes are used in each assay. To further define the hybridization results of our assay, the quantitative measurements of Exps. 1, 2, 5 and 8 in table 1 were executed. Here, we defined the hybridization ratios in Exp. 1 as 100% by the measurement of the fluorescence intensity of red T1 (and/or green T2) from the resulting P1-nanoprobes. The quantitative results of high specificity (Exp. 2) and low nonspecific adsorption (Exp. 8) experiments were found to be 0.9% and 0.08%, respectively. Also, the results of the high selectivity experiment in Exp. 5 were obtained as T1 = 86.67% and T2 = 6.68%.

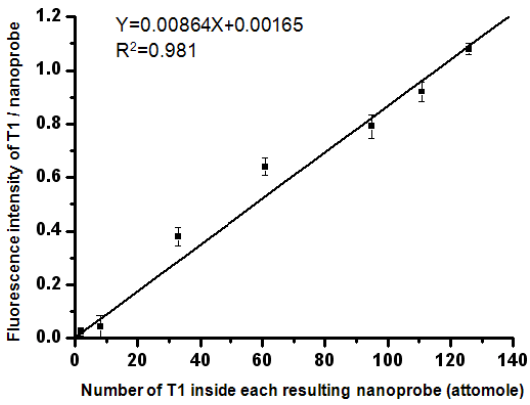

Figure 4. The amount of T1 inside each resulting nanoprobe versus fluorescence intensity ratio of T1 from each resulting nanoprobe. Ninety images were counted for each experiment, and three triplicates were prepared for each T1 concentration.

Overall, in comparison to other DNA detection assays, where the probe DNA was usually immobilized on a substrate, the hybridization processes were performed in a solution phase, in which the efficiency of hybridization can be greatly enhanced. Also, the confined space of silica nanotubes could provide some kinetic advantages to increase the hybridization efficiency. For future commercialization concerns, in practice, we are ready to produce $\sim 2 \times 10^9$ QD-SNTs from one AAO template in a batch, which is more than enough to generate up to 2×10^8 testing assays or kits.

4. Conclusions

In conclusion, the DNA detection assay using conjugates of DNA and QD-SNTs as DNA nanoprobes was demonstrated. With careful manipulation of the concentration of QDs embedded inside QD-SNTs, a variety of colors of QD-SNTs can be generated to code colorless probe DNA. After the hybridization with target DNA in solution, detection results have been easily judged by the color of the resulting nanoprobes. The fluorescence of QD-SNTs (representing probe DNA), target DNA and the merged color from the previous two were all observed when complementary target DNA was detected. The information from coding, target and detection results can be obtained simultaneously. Further, 1×10^{-14} mole of target DNA can result in a distinguishable color change (using 10 QD-SNT nanoprobes), and 2×10^8 sets of different QD-SNT nanoprobes can be generated from on-time QD-SNT synthesis. Overall, our detection assay has demonstrated a new strategy for simple, fast and reliable screening applications.

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References

[1] Rosi N L and Mirkin C A 2005 *Chem. Rev.* **105** 1547–62
[2] Alivisatos P 2004 *Nat. Biotechnol.* **22** 47–52
[3] Daniel M C and Astruc D 2004 *Chem. Rev.* **104** 293–346
[4] Niemeyer C M 2001 *Angew. Chem. Int. Edn* **40** 4128–58
[5] Alivisatos A P 1996 *Science* **271** 933–7
[6] Alivisatos A P 1996 *J. Phys. Chem.* **100** 13226–39
[7] Henglein A 1989 *Chem. Rev.* **89** 1861–73
[8] Kelly K L, Coronado E, Zhao L L and Schatz G C 2003 *J. Phys. Chem.* B **107** 668–77
[9] Schmid G 1992 *Chem. Rev.* **92** 1709–27
[10] Duggan D J, Bittner M, Chen Y D, Meltzer P and Trent J M 1999 *Nat. Genet.* **21** 10–4
[11] Hu M, Chen J Y, Li Z Y, Au L, Hartland G V, Li X D, Marquez M and Xia Y N 2006 *Chem. Soc. Rev.* **35** 1084–94
[12] Mirkin C A, Letsinger R L, Mucic R C and Storhoff J J 1996 *Nature* **382** 607–9
[13] Elghanian R, Storhoff J J, Mucic R C, Letsinger R L and Mirkin C A 1997 *Science* **277** 1078–81
[14] Storhoff J J, Elghanian R, Mucic R C, Mirkin C A and Letsinger R L 1998 *J. Am. Chem. Soc.* **120** 1959–64
[15] Taton T A, Mirkin C A and Letsinger R L 2000 *Science* **289** 1757–60
[16] Taton T A, Lu G and Mirkin C A 2001 *J. Am. Chem. Soc.* **123** 5164–5
[17] Su X D and Kanjanawarut R 2009 *ACS Nano* **3** 2751–9
[18] Cao Y W C, Jin R C and Mirkin C A 2002 *Science* **297** 1536–40
[19] Park S J, Taton T A and Mirkin C A 2002 *Science* **295** 1503–6
[20] Dubertret B, Calame M and Libchaber A J 2001 *Nat. Biotechnol.* **19** 365–70
[21] Maxwell D J, Taylor J R and Nie S M 2002 *J. Am. Chem. Soc.* **124** 9606–12
[22] Yan J L, Estevez M C, Smith J E, Wang K M, He X X, Wang L and Tan W H 2007 *Nano Today* **2** 44–50
[23] Resch-Genger U, Grabolle M, Cavaliere-Jaricot S, Nitschke R and Nann T 2008 *Nat. Methods* **5** 763–75
[24] Santra S, Zhang P, Wang K M, Tapec R and Tan W H 2001 *Anal. Chem.* **73** 4988–93
[25] Zhao X J, Tapec-Dytioco R and Tan W H 2003 *J. Am. Chem. Soc.* **125** 11474–5
[26] Zhou X C and Zhou J Z 2004 *Anal. Chem.* **76** 5302–12
[27] Zhang C Y, Yeh H C, Kuroki M T and Wang T H 2005 *Nat. Mater.* **4** 826–31
[28] Peng H, Zhang L J, Kjallman T H M, Soeller C and Travas-Sejdic J 2007 *J. Am. Chem. Soc.* **129** 3048–9
[29] Zhang C Y and Hu J 2010 *Anal. Chem.* **82** 1921–7
[30] Ho Y P, Kung M C, Yang S and Wang T H 2005 *Nano Lett.* **5** 1693–7
[31] Han M Y, Gao X H, Su J Z and Nie S 2001 *Nat. Biotechnol.* **19** 631–5
[32] Gerion D, Chen F Q, Kannan B, Fu A H, Parak W J, Chen D J, Majumdar A and Alivisatos A P 2003 *Anal. Chem.* **75** 4766–72
[33] Klostranec J M, Xiang Q, Farcas G A, Lee J A, Rhee A, Lafferty E I, Perrault S D, Kain K C and Chan W C W 2007 *Nano Lett.* **7** 2812–8
[34] Xu H X *et al* 2003 *Nucl. Acids Res.* **31** e43
[35] Chen C C, Liu Y C, Wu C H, Yeh C C, Su M T and Wu Y C 2005 *Adv. Mater.* **17** 404–7
[36] Dabbousi B O, RodriguezViejo J, Mikulec F V, Heine J R, Mattoussi H, Ober R, Jensen K F and Bawendi M G 1997 *J. Phys. Chem.* B **101** 9463–75
[37] Chan W C W and Nie S M 1998 *Science* **281** 2016–8
[38] Chan W C W, Maxwell D J, Gao X H, Bailey R E, Han M Y and Nie S M 2002 *Curr. Opin. Biotechnol.* **13** 40–6
[39] Hou S F, Wang J H and Martin C R 2005 *Nano Lett.* **5** 231–4