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Robust silica-coated quantum dot–molecular beacon for highly sensitive DNA detection

Chung-Shieh Wu [a,b], Maung Kyaw Khaing Oo [a], Jay M. Cupps [b], Xudong Fan [a,*]

[a] Biomedical Engineering Department, University of Michigan 1101 Beal Avenue Ann Arbor, MI 48109 USA
[b] Department of Biological Engineering, Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA

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ABSTRACT

We synthesized and characterized small yet highly robust silica-coated quantum dots (QDs) and then used them to develop highly sensitive molecular beacon (MB) for DNA detection. As compared to the previously reported methods, our silica coating approach enabled simple and rapid synthesis of silica-coated QDs in large quantities and high concentrations with a well-controlled silica layer. The QDs such made were stable and had a high quantum yield in a wide range of pH values (1–14) and high salt concentrations (up to 2 M). They were less than 10 nm in diameter, much smaller than current silica-coated QDs, thus allowing for efficient energy transfer. The MB sensor based on these silica-coated QDs was capable of rapidly detecting the target DNA at 0.1 nM concentration within 15 min. It could also differentiate the target DNA from the single base mismatched DNA. The QD–MB developed in this work can be used for highly sensitive and selective detection of DNA and other biomolecules in homogeneous solution and inside a cell, as well as in harsh environment.

1. Introduction

DNA detection plays a very important role in many applications such as clinical diagnostics, biochemical research, environmental monitoring, homeland security, and forensics (Tan et al., 2005; Tyagi and Kramer, 1996; Wu et al., 2009; Wu et al., 2010a,b). Recently, great efforts have been made to develop rapid, simple, and selective biosensors for detecting DNA (Sun et al., 2010; Tan et al., 2005; Tyagi and Kramer, 1996; Wu et al., 2009). Among them, the molecular beacon (MB) enables real-time and label-free DNA detection in homogeneous solution as well as inside a cell (Tan et al., 2005; Tyagi and Kramer, 1996). The MB has a hairpin like stem–loop structure that contains both fluorophore and quencher moieties (Tan et al., 2005; Tyagi and Kramer, 1996). In the absence of the target DNA, the fluorophore and the quencher are in close proximity and the fluorescence from the MB is quenched. In the presence of the target DNA, the MB opens to form a double stranded DNA structure that separates the fluorophore and the quencher, leading to the fluorescence restoration. However, most of the organic fluorescent dyes used in MBs are limited by several issues such as low photo-stability, low quantum yield, and the narrow excitation band that requires multiple excitation sources (Eggeling et al., 1998; Resch-Genger et al., 2008).Quantum dots (QDs) are semiconductor nanocrystals that have a number of attractive characteristics for the MB development due to their high photo- and chemical stability, and high quantum yield, as compared to traditional organic dyes. Furthermore, the different fluorescence emissions of QDs can simply be controlled by adjusting the constituent materials or QD sizes. Finally, all colors of QDs can simultaneously be excited with a single excitation source, which makes it much easier to achieve multiplexed detection (Dabbousi et al., 1997; Resch-Genger et al., 2008).

Recently, the QD–MB has been successfully applied for DNA detection (Cady et al., 2007; Kim et al., 2004; Medintz et al., 2007; Yeh et al., 2010; Wu et al., 2010a,b). However, traditional water-soluble QDs used in the MB, for example, COOH-coated QDs, suffer from the problems such as degradation of QD fluorescence, and chemical and colloidal instabilities under harsh chemical environments (*e.g.*, low pH buffers). To overcome these issues, much attention has been paid to the transparent surface-silanization of QD, which offers many advantages such as improved optical and chemical stability of the QDs, enhanced QD fluorescence, ease of QD surface functionalization, low leakage of heavy metal ions from the QDs (Gerion et al., 2001; Jovanovic et al., 2006; Parak et al., 2002). Consequently, silica-coating methods have been applied to CdSe, CdTe, CdS, and ZnS QDs (Correa-Duarte et al., 1998; Schroedter et al., 2002; Selvan et al., 2005; Yuan, 2008). Very recently Hu et al. reported the work on synthesis of robust QDs using the silica-polymer dual coating method, which show remarkable stability in pH 1–14 solutions (Hu and Gao, 2010). Unfortunately, in

* Corresponding author. Tel.: +1 734 763 1273; fax: +1 734 647 4834.
E-mail address: xsfan@umich.edu (X. Fan).

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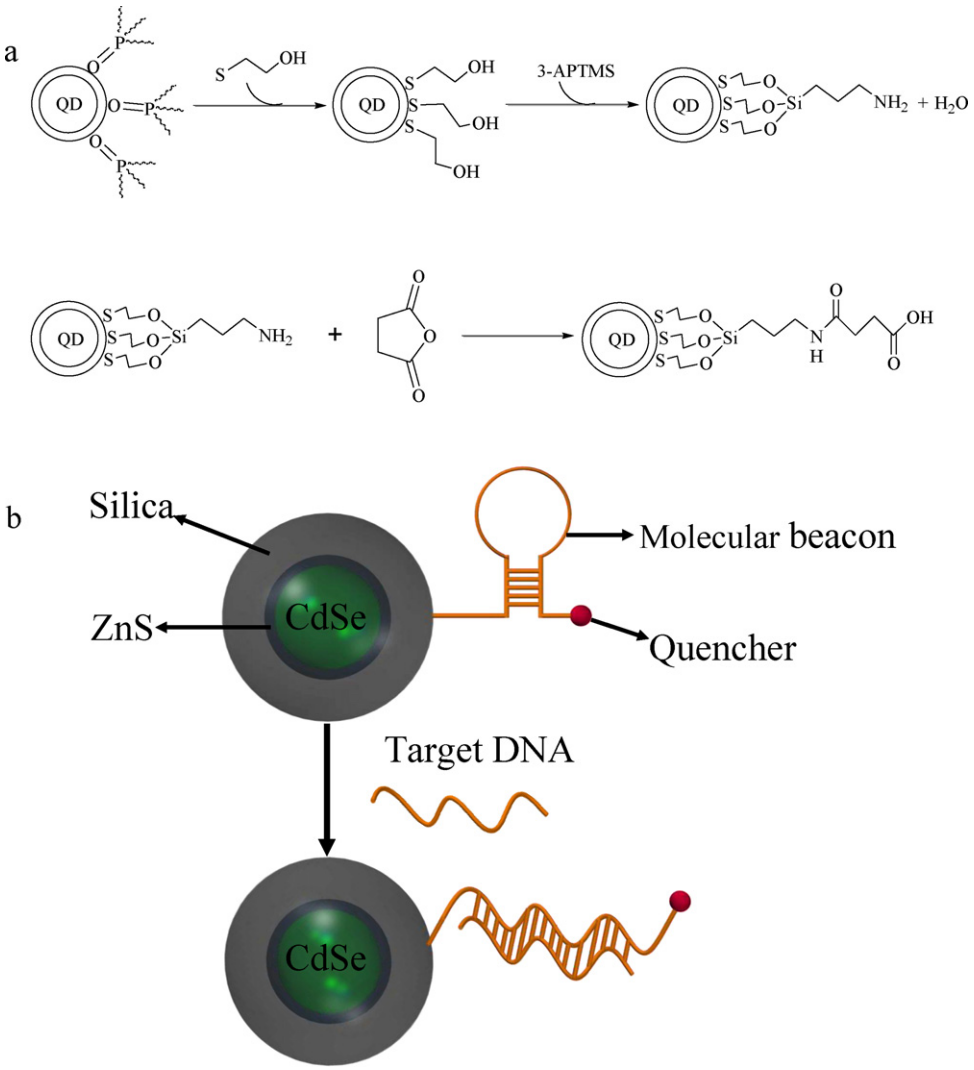

Fig. 1. (a) Reaction scheme of the silanization and functionalization of QDs. Chemically synthesized TOPO-coated QDs were replaced by mercaptoethanol using thiol-exchange reaction to introduce the hydroxyl group. Then, the QDs were silanized with 3-APTMS. Finally, the amine group was replaced by the carboxyl group by ring opening of succinic anhydride. (b) Schematic of a QD based molecular beacon. The CdSe/ZnS QD is embedded in a silica shell and covalently coupled to molecular beacons (MBs). Each molecular beacon consists of one quencher. In the absence of the target DNA, the fluorescence from the QD is quenched. The fluorescence from the QD is restored once the complementary target DNA binds to the MB.

those studies the silica coating layer on the QD was too thick to achieve efficient resonance energy transfer for MB applications. For example, the dual silica-polymer encapsulated QDs are over 50 nm in diameter (Hu and Gao, 2010), much larger than the typical Förster distance between the QD and the dye (or quencher), which is approximately only 4–7 nm (Clapp et al., 2004; Wargnier et al., 2004). In addition, those silica coating methods are quite inefficient. They involve many complicated steps, yield large thickness distribution in the silica layer, and may not be able to handle large quantity of QDs (Nann and Mulvaney, 2004; Parak et al., 2002).

In this study, we developed thin yet highly robust silica-coated QDs to overcome the limitations of aforementioned water-soluble QDs in harsh environment. Our QDs were synthesized using a simple and quick approach that produces silica-coated QDs in a large quantity with a well-controlled silica layer. The QDs were then characterized in terms of size and quantum yield, as well as salt and pH stability. Finally, the QDs were conjugated with the MB to develop a highly sensitive and selective sensor that is capable of detecting sub-nM target DNA in about 15 min.

2. Experimental

2.1. Materials

2-Mercaptoethanol (99.0%), dihydrolipoic acid (DHLA, reduced, 98%), sodium hydroxide (NaOH, 98%), hydrogen chloride (99.8%), methanol (99.8%), 3-aminopropyltrimethoxysilane (3-APTMS, 97%), chlorotrimethylsilane (99%), tetramethylammonium hydroxide (TMAH) pentahydrate (97%), chloroform (99%), succinic anhydride (99%), phosphate buffered saline (PBS), 0.2 μm syringe filter, 0.45 μm syringe filter, 30,000 MWCO Nanosep® centrifugal filter, and 100,000 MWCO Nanosep® centrifugal filter were obtained from Sigma–Aldrich (St. Louis, MO). Sodium chloride (NaCl), boric acid/potassium chloride/sodium hydroxide (200 mM, pH 9.0) buffer, potassium phosphate monobasic/sodium hydroxide (100 mM, pH 8.0) buffer and phosphate buffer (50 mM, pH 6.5) were obtained from Fisher Scientific (Pittsburgh, PA). 1-Ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride (EDC) and *N*-hydroxysulfosuccinimide (Sulfo-NHS) were obtained from PIERCE (Rockford, IL). NAP-25

columns were obtained from GE Healthcare (South Burlington, VT).

All DNAs used in experiments were of HPLC grade and were obtained from IDT (Coralville, IA). The DNA sample names and the corresponding sequences are given in AppendixBTable S1 in Supplementary data. Briefly, the MB consists of 26-base single-stranded DNA labeled with Iowa black FQ. Target DNA (16-base) and 1 mismatched DNA (16-base) served as the complementary target DNA sequence and single base mismatched DNA sequence, respectively.

2.2. Preparation of OH-coated QDs

Trioctylphosphine oxide (TOPO)-coated CdSe/ZnS QDs were synthesized according to a previously published procedure (Wu et al., 2009). To obtain OH-coated QDs, TOPO-coated QDs were dried by evaporation, then 100 mg of the QDs 1 mL of 2-mercaptoethanol and 0.5 mL of methanol was added and pH value was adjusted to 8 with 0.2 M NaOH. This mixture was stirred for 1 h at 85 °C and the solution became optically clear. Then the resulting OH-coated water-soluble QDs were precipitated by adding 3 mL of chloroform. The residue was washed 3 times by chloroform and then resulting suspension was centrifuged at 14,000 rpm for 5 min. The supernatant was discarded and the QDs were dissolved in 1 mL of pH 8 buffer and filtered through a 0.2 μm syringe filter to obtain OH-coated QDs in clear solution. After the experiment, we prepared the OH-coated QDs with the emission peak at 535 nm.

2.3. Silanization of QDs and surface functional modification

To obtain the silanized QDs, 2.5 mL 3-aminopropyltrimethoxysilane (3-APTMS) diluted with 1 mL methanol was added to 5 mL of OH-coated QDs (15 μM) slowly at 0 °C with vigorous mixing. This mixture became cloudy immediately and then turned clear within a few minutes. After 10 min of stirring, the solution was heated to ~60 °C for 30 min and then cooled to ~30 °C. To quench the silanization reaction, a mixture of 65 mL of methanol and 1.8 mL of chlorotrimethylsilane basified with 2.75 g of solid TMAH pentahydrate was added at room temperature. After ~2 h of stirring, the solution was heated to ~60 °C for 30 min and then stirred slowly under argon at room temperature for 3 h. Methanol was removed *in vacuo* for 6 h, and then the silanized QDs were dissolved in 5 mL of deionized H_2O and the solution was filtered through a 0.45 μm syringe filter. Free silane was removed *via* size-exclusion chromatography using NAP-25 column with 1× PBS buffer. Then the solution was concentrated twice with a centrifuge (8000 rpm) using a 30,000 MWCO Nanosep® centrifugal filter. Finally, the silanized QDs solution were reduced to ~1 mL. The shelf lifetime of those silanized QDs was longer than 6 months when stored in a 4 °C dark room.

Carboxyl-silanized QDs were generated by the following procedures described in the literature (Thomas et al., 2004). The amino-silanized QD solution was precipitated using anhydrous chloroform. The wet precipitate (500 mg) was suspended in 25 mL pH 9 buffer and 50 mg succinic anhydride was added under sonification. The suspension was stirred for 16 h at room temperature. The residue was filtered through a 0.2 μm syringe filter and the excessive succinic anhydride was removed by a NAP-25 column with 1×PBS buffer. The carboxyl-silanized QDs were concentrated with a centrifuge (8000 rpm) using a 100,000 MWCO Nanosep® centrifugal filter. After the experiment, we prepared the carboxyl-silanized QDs with the emission peak at 539 nm.

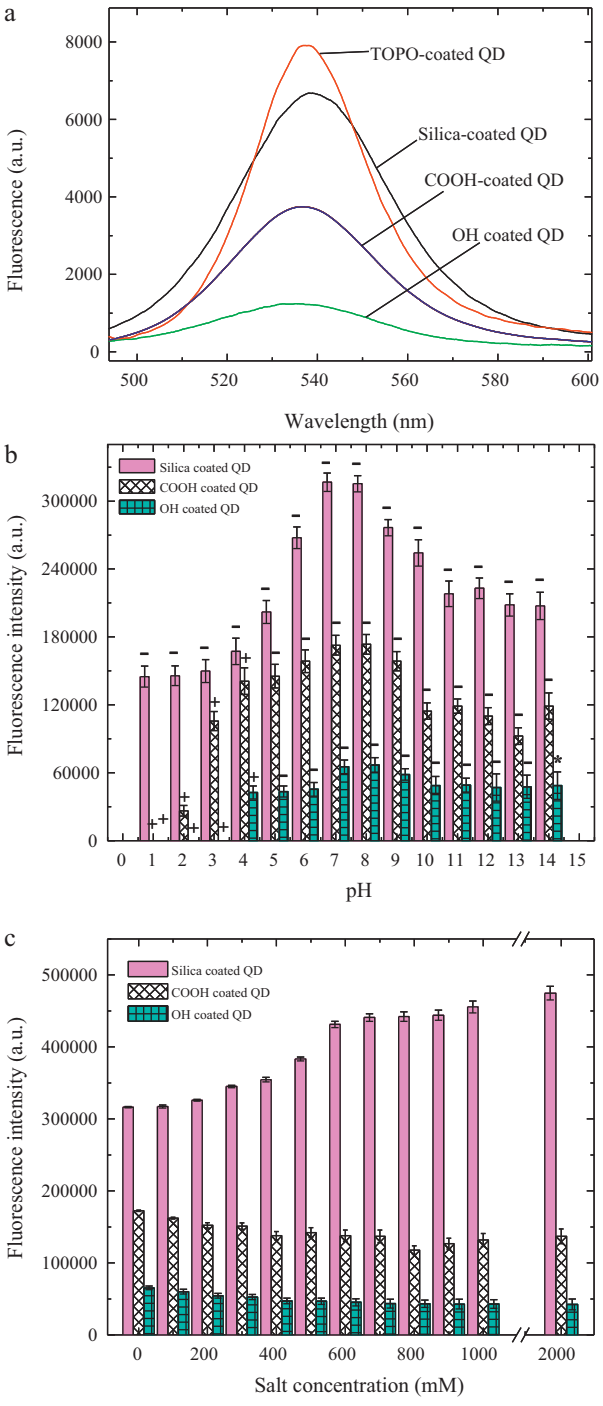

Fig. 2. (a) Fluorescence spectra of TOPO-coated QDs (in chloroform), OH-coated QDs, COOH-coated QDs, and silica-coated QDs (all in water). (b and c) pH stability and salt tolerance test of OH-coated QDs, COOH-coated QDs, and silica-coated QDs. All QD concentrations were 50 nM. '+' denotes that the QD aggregation was observed immediately. '*' denotes that the QD aggregation was observed within 1 h. '—' denotes that no QD aggregation was observed after 1 h. No aggregation was observed in salt tolerance tests after 1 h. Error bars indicate standard deviations of three measurements.

2.4. Preparation of the QD–MB sensor

A list of the names and the modifications of the DNA sequences used for QD–MB synthesis is provided in AppendixBTable S1 in Supplementary data. EDC (0.2 mg) and sulfo-NHS (0.1 mg) dissolved in 10 μL of phosphate buffer (25 mM, pH 6.5) was mixed with 125 μL carboxyl-silanized QDs (15 μM). After incubating for 10 min

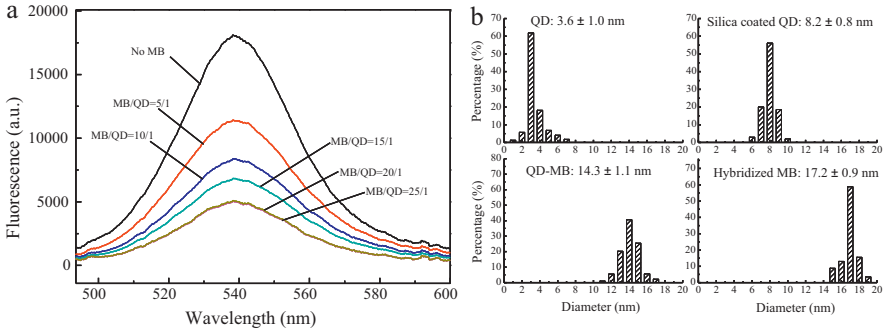

Fig. 3. (a) Evolution of fluorescence spectra when different amount of MBs were conjugated with the carboxyl silanized QDs. (b) Size distribution histogram, measured by DLS, of OH-coated QDs, silica-coated QDs, QD–MB complex, and QD–MB after hybridization with the target DNA.

at room temperature, 375 μL of 100 μM MB probes were added to the mixture. After 1 h at 30 °C, unreacted EDC/sulfo-NHS and excessive MB were removed by NAP-25 column with 1× PBS and the QD–MB was concentrated with a centrifuge (8000 rpm) using a 100,000 MWCO Nanosep® centrifugal filter to the final concentration of 100 nM. The purified QD–MB sensors had a shelf lifetime over 2 weeks when stored in a 4 °C dark room.

2.5. Preparation of COOH-coated QDs

For comparison purposes, we also prepared COOH-coated QDs. TOPO-coated QDs were dried by evaporation, then to 80 mg of the QDs 1 mL of DHLA and 0.5 mL of methanol was added. This mixture was stirred overnight at 80 °C and the resulting water-soluble QDs were then precipitated by adding 0.5 mL of chloroform. The residue was washed twice by chloroform and then resulting suspension was centrifuged at 14,000 rpm for 5 min. The supernatant was discarded and the QDs were dissolved in 1 mL of deionized H_2O and filtered through a 0.2 μm syringe filter to obtain DHLA-coated QDs in clear solution.

2.6. pH stability and salt tolerance tests

To test the QD stability of acids and bases, different pH values of solutions were tuned with HCl (5 M) and NaOH (5 M). The QDs aqueous solution (5 μM) were diluted with indicated pH values solution and incubated for 1 h with continuous shaking. To investigate the QDs stability in high salt concentration solutions, different salt solutions were prepared by diluting NaCl with deionized H_2O (0, 100–1000, 2000 mM). Then the QDs aqueous solution (5 μM) were diluted with typical concentration of NaCl solution (QDs final concentration 50 nM) and incubated for 1 h with continuous shaking. The fluorescence spectra of the QD samples were recorded by a USB 4000 Miniature Fiber Optic spectrometer (Ocean Optics, FL) at a scan rate of 350 ms for post-analysis. Spectral integration takes place from 496 nm to 600 nm.

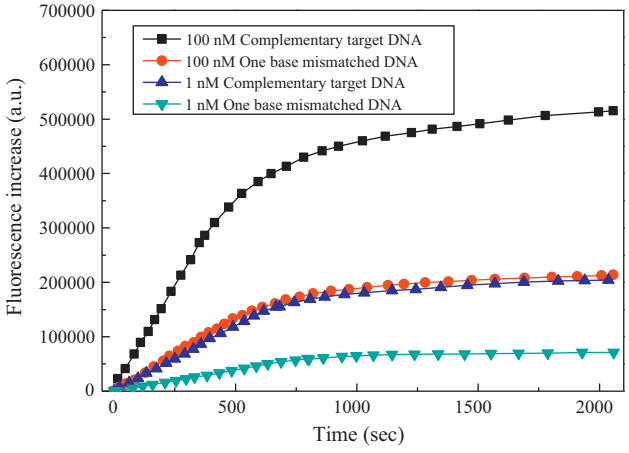

Fig. 4. Time dependent total QD–MB fluorescence increase with respect to the background in response to the target DNA (1 nM and 100 nM) and one base mismatched DNA (1 nM and 100 nM). Fluorescence intensity is integrated from 496 nm to 600 nm.

2.7. Spectroscopy of DNA detection

Hybridization of the QD–MB sensor with the target DNA or one base mismatched DNA took place by mixing by mixing 30 μL QD–MB (10 nM) with 30 μL DNA sample in a micro-quartz cuvette at room temperature. All of the hybridizations were executed in 1× PBS buffer solution. A laser at 480 nm was used for QD excitation. The fluorescence spectra were recorded by a USB 4000 Miniature Fiber Optic spectrometer (Ocean Optics, FL) at a scan rate of 10 s for post-analysis.

3. Results and discussion

The procedures for synthesis of carboxyl-silanized QDs are shown in Fig. 1a. The CdSe/ZnS QDs were synthesized by a modification of the method described previously (see Section 2) (Hines and Guyot-Sionnest, 1996; Peng et al., 1997; Wu et al., 2009). Then those water-insoluble trioctylphosphine oxide (TOPO)-coated QDs were converted to water-soluble 2-mercaptoethanol (OH)-coated QDs by using the ligand exchange reaction. To obtain robust and highly fluorescent QDs, 3-APTMS was applied to form a silica shell and to introduce the primary amine group to the QD surface *via* a silanization reaction, which was subsequently converted to the carboxyl group. The resultant carboxyl-silanized QDs could readily be covalently conjugated to the MB using the well-established cross-linking method (see Section 2) (Wu et al., 2010a,b). Since most of the QD–MB produced to date use COOH-coated water-soluble QDs (*e.g.*, dihydrolipoic acid (DHLA)-coated QDs) that are covalently conjugated with the MB (Cady et al., 2007; Kim et al., 2004; Medintz et al., 2007), we also synthesized those types of QDs for comparison purposes.

The schematic of the silica-coated QD–MB is illustrated in Fig. 1b. The MB sequence comprised 5-base pairs of the stem part and a 16-base loop part complementary to the target DNA sequence (see AppendixBTable S1 in Supplementary data). The quencher (Iowa black FQ) was attached to 3′ end of the MB, which can quench fluorescence of the QDs through the resonance energy transfer in the absence of the target DNA. In the presence of the target DNA, the hybridization between the loop part and the target was longer and

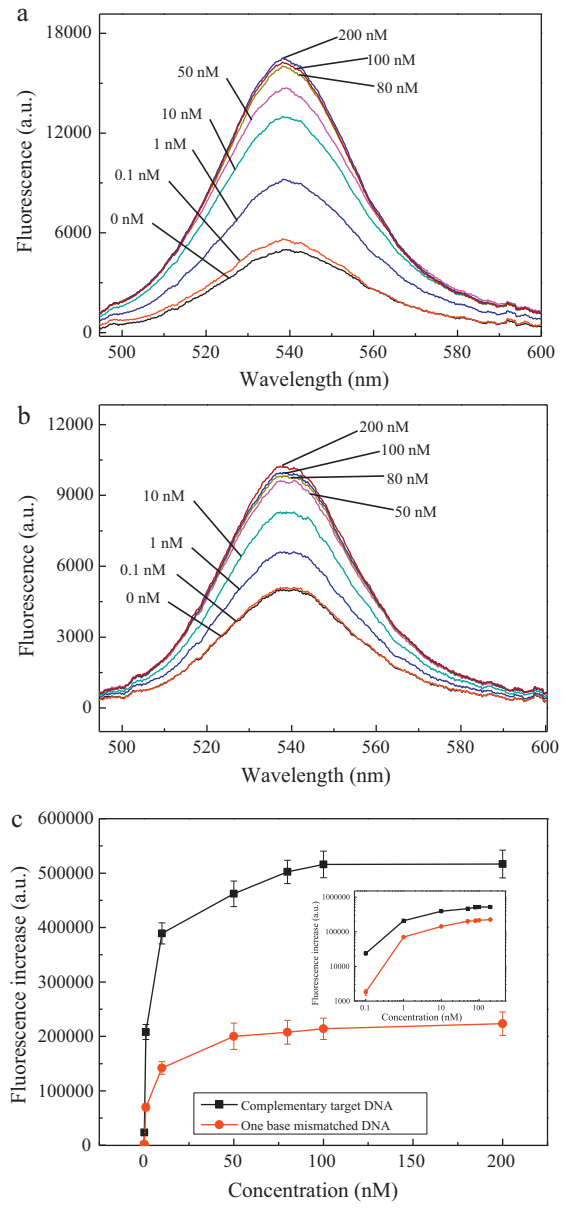

Fig. 5. Fluorescence spectra of the QD–MB as a function of (a) target DNA concentration and (b) one base mismatched DNA concentration. The spectra were taken after 30 min of incubation. (c) Total fluorescence increase of the QD–MB based on (a) and (b). Fluorescence intensity is integrated from 496 nm to 600 nm. Error bars indicate standard deviations of three measurements. Inset is the same curves plotted in the log–log scale.

more stable than the stem part. Thus, the MBs underwent spontaneous conformational change and caused the QDs and quenchers to detach from each other, resulting in the restoration of QD fluorescence.

Note that some other silica coating methods have also been developed previously by other groups using the sol–gel process and micro-emulsion process (Darbandi et al., 2005; Gerion et al., 2001; Nann and Mulvaney, 2004). However, those methods are slow, involve many preparation steps, and produce a very thick silica layer (15–35 nm). Furthermore, quantity or concentration of QDs needs to be kept low (50–200 nM, 10 μL) in order to avoid multiplicity problems, *i.e.*, multiple QDs are encapsulated in a single silica shell. In contrast, our method can be performed quickly and conveniently on high concentrations (2–20 μM) and large quantity (>10 mL) of QDs without aggregation. During the process of silanization, the thickness of silica shell could be adjusted by controlling the reaction time, temperature, and the silane concentration. For the MB development, the growth of a thin silica shell can be accomplished within an hour.

Fig. 2a compares the fluorescence spectra from TOPO-coated QDs (in chloroform solvent), OH-coated QDs, COOH (DHLA)-coated QDs, and silica-coated QDs (all in water). A dramatic drop in quantum yield was observed from TOPO-coated QDs (quantum yield = 19%) to OH-coated QDs (quantum yield = 4.2%) and COOH-coated (quantum yield = 11%); this decrease in quantum yield was also associated with a slight blue-shift of the emission spectra. In sharp contrast, after encapsulating the OH-coated QDs into the silica shell, the quantum yield increased nearly 5 fold to 20% with a slight red shift. Remarkably, the quantum yield of the silica-coated QDs were almost twice that of COOH-coated QDs, suggesting that the silica-coated QDs can provide highly fluorescent, functionalized, and water-soluble fluorophores for biological labeling applications.

To compare the stability of those water-soluble QDs, OH-coated, COOH-coated, and silica-coated QDs were respectively incubated in the solution with a pH value ranging from 1 to 14 and salt concentration of 0–2 M. As shown in Fig. 2b, in the pH tests, the COOH-coated QD showed no fluorescent emission for pH 1 and its fluorescence intensity decreased significantly for pH 2 and pH ≥ 10. In addition, severe precipitations occurred immediately when the COOH-coated QDs mixed with solutions of pH ≤ 4. Similar phenomena occurred for the OH-coated QD. Likewise, it also has been shown previously that in another type of COOH-coated QD (coated with mercaptoacetic acid) no fluorescence was observed for pH ≤ 4 and fluorescence intensity decreased drastically for pH ≥ 10 (Hu and Gao, 2010). In contrast, the silica-coated QD exhibited significantly improved pH stability. The fluorescence of the silica-coated QD at pH 1–4 still remained nearly 50% of its fluorescence emission at pH 7. All of the silica-coated QD solutions were clear and no precipitations were observed.

In the salt tolerance tests, even though no precipitations were observed in either COOH-coated or OH-coated QDs, the fluorescence intensity (or quantum yield) of both types of QDs decreased slightly from 0 mM to 800 mM and then became steady (see Fig. 2c), suggesting that the COOH-coated and OH-coated QDs were slightly unstable in high concentration salt solutions. Our study is complementary to an early investigation by Hoshino et al. (Hoshino et al., 2004), in which another type of COOH-coated QD (coated with mercaptoundecanoic acid) was tested and severe precipitations were observed in 1 M and 5 M salt solutions. In contrast, the fluorescence of silica-coated QDs increased monotonically with the increased salt concentration. Both the pH stability and salt tolerance tests clearly show that the silica-coated QDs are highly robust against harsh environment and they are far superior to their COOH-coated or OH-coated counterparts.

To form the QD–MB conjugation, we applied the crosslinkers, EDC and sulfo-NHS, to attach the aminated quencher-labeled MB with the carboxyl-silanized QD surface. To determine the optimal conjugation molar ratio between the MB and the QD, we investigated in Fig. 3a the quenching efficiency by incubating 500 μL QDs at 10 nM with 500 μL MB with different concentrations. A significant increase in the quenching efficiency from 36.8% to 72% for the QD–MB was observed when the MB-to-QD molar ratio was increased from 5:1 to 20:1. Since no further quenching was observed for the MB-to-QD molar ratio beyond 25:1, in the subsequent experiments we used QD–MB sensors prepared with the MB-to-QD molar ratio of 20:1.

In Fig. 3b, we used dynamic laser scattering (DLS) to characterize the size of the QD–MB. Comparison between the QD before and after silanization shows that the thickness of the silica layer on the QDs was only 2.3 nm, resulting in the silica-coated QD

of approximately 8.2 nm in diameter. Consequently, the distance between the QD and the quencher was well within their Förster distance (Clapp et al., 2004; Wargnier et al., 2004). With the conjugation of the MB onto the QD, the size of the QD–MB complex became 14.3 nm in diameter. Since part of the MB forms a loop on the QD, it is difficult to estimate the MB density of the QD surface directly from the QD–MB size. For more accurate estimation, we hybridized QD–MB with the complementary target DNA so that the MB was completely open to form a more rigid double stranded DNA structure. The size increase from the silica-coated QD (8.2 nm in diameter) to the QD coated with double stranded DNA (17.2 nm in diameter) shows that there were approximately 15 MB per QD on average, in agreement with what we observed in Fig. 2b where the quenching efficiency leveled off after 20:1 MB-to-QD ratio.

To apply the QD–MB sensor for DNA detection, various concentrations of target DNA were added to the QD–MB solution. Fig. 4 shows the time dependent fluorescence increase with two representative concentrations of target DNA and one base mismatched DNA. The signal (*i.e.*, fluorescence increase with respect to the background) saturated in approximately 15 min, indicative of rapid DNA hybridization and hence detection time. To further investigate the DNA detection capability of the QD–MB, Fig. 5a and b show the fluorescence spectra taken after 30 min of incubation for both target DNA and one base mismatched DNA. The corresponding sensing signal is plotted in Fig. 5c. For the target DNA the lowest concentration experimentally obtained was 0.1 nM with the signal of about 20,000 counts. For the single base mismatched DNA, the signal is about 10 times lower than that from the perfectly matched DNA for low sample concentrations and 2–3 times lower for relatively high sample concentrations.

4. Conclusion

We have synthesized highly robust silica-coated QDs with high quantum yield. The QD–MB sensor is capable of detecting sub-nM DNA in about 15 min and exhibits high detection specificity to differentiate the target DNA from the single based mismatched DNA. The QD–MB described in this work can be used in the development of DNA sensors for medical diagnostics, biochemical research, homeland security, and forensics in harsh detection environment.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.02.049.

References

Clapp, A.R., Medintz, I.L., Mauro, J.M., Fisher, B.R., Bawendi, M.G., Mattoussi, H., 2004. J. Am. Chem. Soc. 126, 301–310.
Cady, N.C., Strickland, A.D., Batt, C.A., 2007. Mol. Cell. Probes 21 (2), 116–124.
Correa-Duarte, M.A., Giersig, M., Liz-Marzán, L.M., 1998. Chem. Phys. Lett. 286 (5–6), 497–501.
Dabbousi, B.O., Rodriguez-Viejo, J., Mikulec, F.V., Heine, J.R., Mattoussi, H., Ober, R., Jensen, K.F., Bawendi, M.G., 1997. J. Phys. Chem. B 101, 9463–9475.
Darbandi, M., Thomann, R., Nann, T., 2005. Chem. Mater. 17 (23), 5720–5725.
Eggeling, C., Widengren, J., Rigler, R., Seidel, C.A.M., 1998. Anal. Chem. 70 (13), 2651–2659.
Gerion, D., Pinaud, F., Williams, S.C., Parak, W.J., Zanchet, D., Weiss, S., Alivisatos, A.P., 2001. J. Phys. Chem. B 105 (37), 8861–8871.
Hines, M.A., Guyot-Sionnest, P., 1996. J. Phys. Chem. 100, 468–471.
Hoshino, A., Fujioka, K., Oku, T., Suga, M., Sasaki, Y.F., Ohta, T., Yasuhara, M., Suzuki, K., Yamamoto, K., 2004. Nano Lett. 4 (11), 2163–2169.
Hu, X., Gao, X., 2010. ACS Nano 4 (10), 6080–6086.
Jovanovic, A.V., Flint, J.A., Varshney, M., Morey, T.E., Dennis, D.M., Duran, R.S., 2006. Biomacromolecules 7 (3), 945–949.
Kim, J.H., Morikis, D., Ozkan, M., 2004. Sens. Actuators B 102 (2), 315–319.
Medintz, I.L., Berti, L., Pons, T., Grimes, A.F., English, D.S., Alessandrini, A., Facci, P., Mattoussi, H., 2007. Nano Lett. 7 (6), 1741–1748.
Nann, T., Mulvaney, P., 2004. Angew. Chem. Int. Ed. 43 (40), 5393–5396.
Parak, W.J., Gerion, D., Zanchet, D., Woerz, A.S., Pellegrino, T., Micheel, C., Williams, S.C., Seitz, M., Bruehl, R.E., Bryant, Z., Bustamante, C., Bertozzi, C.R., Alivisatos, A.P., 2002. Chem. Mater. 14 (5), 2113–2119.
Peng, X., Schlamp, M.C., Kadavanich, A.V., Alivisatos, A.P., 1997. J. Am. Chem. Soc. 119, 7019–7029.
Resch-Genger, U., Grabolle, M., Cavaliere-Jaricot, S., Nitschke, R., Nann, T., 2008. Nat. Methods 5 (9), 763–775.
Schroedter, A., Weller, H., Eritja, R., Ford, W.E., Wessels, J.M., 2002. Nano Lett. 2 (12), 1363–1367.
Selvan, S.T., Tan, T.T., Ying, J.Y., 2005. Adv. Mater. 17 (13), 1620–1625.
Sun, Y., Shopova, S.I., Wu, C.-S., Arnold, S., Fan, X., 2010. Proc. Natl. Acad. Sci. U.S.A. 107 (37), 16039–16042.
Tan, L., Li, Y., Drake, T.J., Moroz, L., Wang, K., Li, J., Munteanu, A., James Yang, C., Martinez, K., Tan, W., 2005. Analyst 130 (7), 1002–1005.
Thomas, S., Herwig, B., Günter, E.M.T., 2004. J. Nano Nanotechnol. 4, 504–511.
Tyagi, S., Kramer, F.R., 1996. Nat. Biotechnol. 14 (3), 303–308.
Wargnier, R., Baranov, A.V., Maslov, V.G., Stsiapura, V., Artemyev, M., Pluot, M., Sukhanova, A., Nabiev, I., 2004. Nano Lett. 4, 451–457.
Wu, C.-S., Cupps, J.M., Fan, X., 2009. Nanotechnology 20 (30), 305502.
Wu, C.-S., Khaing Oo, M.K., Fan, X., 2010a. ACS Nano 4 (10), 5897–5904.
Wu, S.-M., Tian, Z.-Q., Zhang, Z.-L., Huang, B.-H., Jiang, P., Xie, Z.-X., Pang, D.-W., 2010b. Biosens. Bioelectron. 26 (2), 491–496.
Yeh, H.-Y., Yates, M.V., Mulchandani, A., Chen, W., 2010. Chem. Commun 46, 3914–3916.
Yuan, J., 2008. Nanotechnology 19 (14), 145702.