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Specific Nucleic Acid Detection Using Photophysical Properties of Quantum Dot Probes

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In the present study, we report a novel separation-free method to detect and quantify avian influenza virus A (H5N1) nucleic acid without amplification, based on the alteration of photophysical parameters of quantum dot (QD) probes after hybridization with specific complementary target DNA. The target DNA was quantified in a custom-made portable device by simultaneously measuring lifetime and quenching of the QD probes. QD probes (25-mer) showed a 30% lifetime reduction and 40% fluorescence quenching when hybridized with complementary 25-mer target DNA. In comparison with a conventional QD-based assay, this assay provides a simple quantitation of nucleic acids with a single labeling step.

The need to detect and quantify specific nucleic acid sequences has increased in recent years. Methods utilizing polymerase chain reaction (PCR),[1] microarrays,[2] electrochemical detection with gold nanoparticles,[3] and flowcytometric detection with fluorescent particles[4] have been developed for the detection and quantitation of nucleic acids. However, these conventional approaches depend on a skilled technique, separation of unhybridized DNA, and semiquantitative amplification of target DNA. As an alternative, fluorescent semiconductor nanocrystals, or quantum dots (QDs), have been used as probes in a broad range of applications including immunoassays, in vivo biological imaging, and fluorescence resonance energy transfer (FRET)-based DNA hybridization.[5−7] Due to their high fluorescence, low photobleaching, and more importantly narrow emission peaks capable of effective separation of donor (QD) and acceptor (fluorescence dye) fluorescence, QD/FRET systems have been developed as a nucleic acid quantitation tool, enabling sensitive and specific detection and quantitation of DNA and protein. However, a FRET-based detection system performed as a conventional QD-based DNA assay is complicated, due to the requirement for dual labeling of two nucleic acids with QD and fluorescent dye. In addition, its applications to comparatively long target sequence lengths are limited due to the Förster distance (R_0); for efficient FRET, the distance between the QD probe (donor) and the dye (acceptor) should be within 4−7 nm.[6,7]

Furthermore, in the case of QD-streptavidin applications in FRET, multistep and complicated labeling and hybridization procedures are required.[8] To overcome these limitations, the development of simpler and more efficient methods, using a single labeling step in a separation-free format, for the rapid and sensitive detection of DNA is necessary.

The fluorescence decay time or, as is commonly used, the lifetime, τ, is the average time that a fluorophore remains in the excited state after excitation. Suhling et al.[9] reported that, in addition to the intrinsic characteristics of the fluorophore itself, fluorescence lifetime also depends in a measurable way upon local environmental variables, such as viscosity, pH, or refractive index, and on interactions with other molecules. The energy relaxation dynamics of a QD and, consequently, its optical emission characteristics, are highly sensitive to its surface charge. The electric field effect introduced by such charge, normally derived from hybridization with charged molecules, causes deactivation of the electron−hole pair states via nonradiative Auger recombination.[10,11] According to this scheme, an exciton in a charged QD does not release the recombination energy as a photon but transfers it to either an electron or a hole in the QD. Thus, the QD is re-excited to a higher energy state from which it relaxes nonradiatively.

Any alteration of the radiative or nonradiative components after surface modification will also alter the decay rate, which is inversely proportional to the sum of these components.[12] However,

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(1) Medrano, M. L.; Medrano, J. F. *Biotechniques* **2005**, *39*, 1–10.
(2) Shalon, D.; Smith, S. J.; Brown, P. O. *Genome Res.* **1996**, *6*, 639–645.
(3) Baptista, P. V.; Koziol-Montewka, M.; Paluch-Oles, J.; Doria, G.; Franco, R. *Clin. Chem.* **2006**, *52*, 1433–1434.
(4) Spiro, A.; Lowe, M.; Brown, D. *Appl. Environ. Microbiol.* **2000**, *66*, 4258–4265.
(5) Agrawal, A.; Sathe, T.; Nie, S. *J. Agric. Food Chem.* **2007**, *55*, 3778–3782.
(6) Zhou, D.; Ying, L.; Hong, X.; Hall, E. A.; Abell, C.; Klenerman, D. *Langmuir* **2008**, *24*, 1659–1664.
(7) Lockett, M. R.; Shortreed, M. R.; Smith, L. M. *Anal. Chem.* **2007**, *79*, 6031–6036.
(8) Zhang, C. Y.; Yeh, H. C.; Kuroki, M. T.; Wang, T. H. *Nat. Mater.* **2005**, *4*, 826–831.
(9) Klaus, S.; French, P. M. W.; David, P. *Photochem. Photobiol. Sci.* **2005**, *4*, 13–22.
(10) Klimov, V.; Mcbranch, D.; Leatherdale, C.; Bawendi, M. *Phys. Rev. B* **1999**, *60*, 13740–13749.
(11) Efros, A.; Rosen, M. *Phys. Rev. Lett.* **1997**, *78*, 1110–1113.
(12) Komarala, V.; Rakovich, H.; Bradley, A.; Byrne, S.; Corr, S.; Gunko, Y. *Nanotechnology* **2006**, *17*, 4117–4122.

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at present, the exact mechanisms involved in these electron−hole recombination dynamics are not fully understood.

Since the QD fluorescence lifetime can be affected by interaction with nucleic acids, changing the configuration of the QD surface, we propose that fluorescence lifetime can be distinguished spectrally and interpreted as the concentration of target DNA on QD probes and target DNA hybridization. The fluorescence quenching of a QD was used as an additional detection parameter for nucleic acid quantitation. Our approach is different from others reported previously in that hybridization of QD probes with target DNA changes photophysical properties of the QDs, leading to simultaneous target detection and quantitation.

In the present study, we introduce an approach for single-step labeling and separation-free target detection with only QD probes using a custom-made portable system and report its application to avian influenza virus A (H5N1) nucleic acid detection. QD probes were used to recognize and detect specific complementary target DNA through a single, simple labeling reaction. Hybridization of target DNA and QD probes was analyzed and quantified by lifetime and quenching-based measurements in a portable device.

We also investigated whether lifetime and quenching-based hybridization detection is affected by oligonucleotide density on the QD surface or by probe length.

MATERIALS AND METHODS

Preparation and Verification of QD probes. QD_{605} (polymer shell, Q 21301 MP, Invitrogen, USA) was used as a fluorescent probe. Conjugation of COOH functionalized QDs with 18-mer or 25-mer length oligonucleotides with 3′-terminal NH_2 and 18-carbon chain spacers (Bioneer, Korea) was performed in 10 mM PBS (Sigma, USA) at pH 7.2 or HEPES buffer (Sigma, USA) at pH 9.5 using a modified amide-coupling protocol (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS), Sigma, USA).[13] The probe sequences were derived from a conserved region of the hemagglutinin (HA) gene of the A (H5N1) avian influenza clade 1 virus isolated from a Cambodian patient in 2005 (access number: ISDN185503). The sequences of the probes were 5′ AGT GCT AGG GAA CTC GCC 3′ (18-mer probes) and 5′ AGT GCT AGG GAA CTC GCC ACT GTA G 3′ (25-mer probes). The complementary target sequences were 5′ GGC GAG TTC CCT AGC ACT 3′ (18-mer target) and 5′ CTA CAG TGG CGA GTT CCC TAG CAC T 3′ (25-mer target). The noncomplementary 18-mer and 25-mer sequences were taken from pBluescript SK (+). The sequences were 5′ GTA ATA CGA CTC ACT ATA 3′ (18-mer noncomplementary strand) and 5′ GTA ATA CGA CTC ACT ATA GGG CGA A 3′ (25-mer noncomplementary strand). The effect of buffer pH variations on the conjugation and hybridization was investigated. Briefly, 16 pmol of QDs were mixed with 1 μL of 5 mM EDC and 1 μL of 8.6 mM of NHS in 26 μL of 10 mM PBS buffer at pH 7.2 and 10 mM HEPES buffer at pH 9.5, respectively, and the resulting mixture was incubated for 15 min at room temperature. Then, 2000 pmol of oligonucleotides were added, and the mixture was shaken at 1000 rpm overnight. After conjugation, free oligonucleotides were removed by microfiltration (Microcon 100K, Millipore, USA), and each QD probe preparation was resuspended in 160 μL of PBS buffer (pH 7.2) and HEPES buffer (pH 9.5). The efficiency of the oligonucleotide conjugation was determined by OliGreen (excitation at 485 nm and emission at 530 nm, Invitrogen, USA) quantitation of the amount of oligonucleotides on the QD surface using a fluorometer (Victor, Perkin-Elmer, USA). Images of QD probes were taken using a fluorescence microscope (IX-71, Olympus, Japan) equipped with a charge-coupled device (CCD) camera.

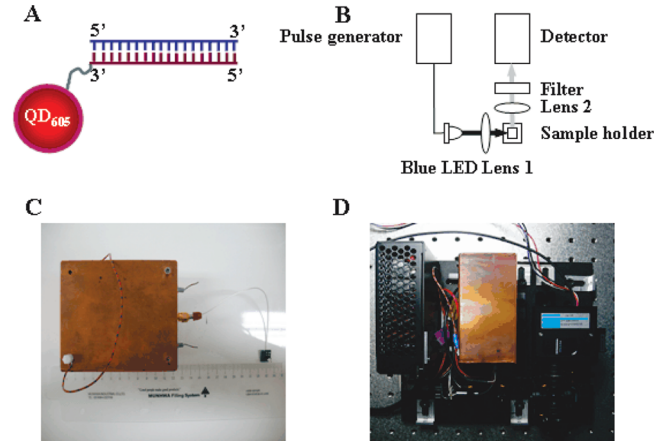

Figure 1. Schematic procedure of lifetime and quenching-based QD probes and target DNA hybridization assays. (A) Hybridization of QD probes and target DNA. (B) Scheme of the experimental setup for lifetime and quenching-based detection. (C) Prototype of the light source consisting of a blue LED and a pulse generator. (D) Experimental setup of the measuring device.

Hybridization of QD Probes and Target DNA. To investigate the effect of buffer pH variations on hybridization, two different mixtures of QD probes prepared in 10 mM PBS buffer (pH 7.2) and 10 mM HEPES buffer (pH 9.5) were compared for hybridization. To optimize hybridization time, a kinetic study of the hybridization of QD probes and target DNA was performed. One microliter of 20 μM 25-mer target DNA was hybridized with 3 μL of 0.15 nM QD probes (prepared in HEPES buffer, pH 9.5) in PBS (pH 7.2) buffer for different incubation times from 1 min to 9 h. After conjugation, free oligonucleotides were removed by microfiltration, and the probes were stained with PicoGreen (excitation at 485 nm and emission at 530 nm, Invitrogen, USA) and analyzed by a fluorometer.

To verify the hybridization of QD probes and DNA, 1 μL of 25-mer target DNA ranging from 0.8 to 25 μM (a 2-fold dilution) was hybridized with 3 μL of 0.15 nM QD probes in PBS buffer (total volume 50 μL) shaken at 1000 rpm for 3 h at 25 °C (Figure 1A); further purification and staining were performed as described above. For a quantitative analysis of hybridization, a calibration curve was obtained by measuring the fluorescence of QD probes and target DNA hybrids. Hybridization efficiency was calculated as follows: the fluorescence intensities of QD probe/target DNA hybrids were corrected for the background fluorescence of control QD probes, and the data were plotted with the QD probe/DNA hybrid fluorescence intensity as a function of target DNA concentration.

(13) Bakalova, R.; Zhelev, Z.; Ohba, H.; Baba, Y. *J. Am. Chem. Soc.* **2005**, *127*, 11328–11335.

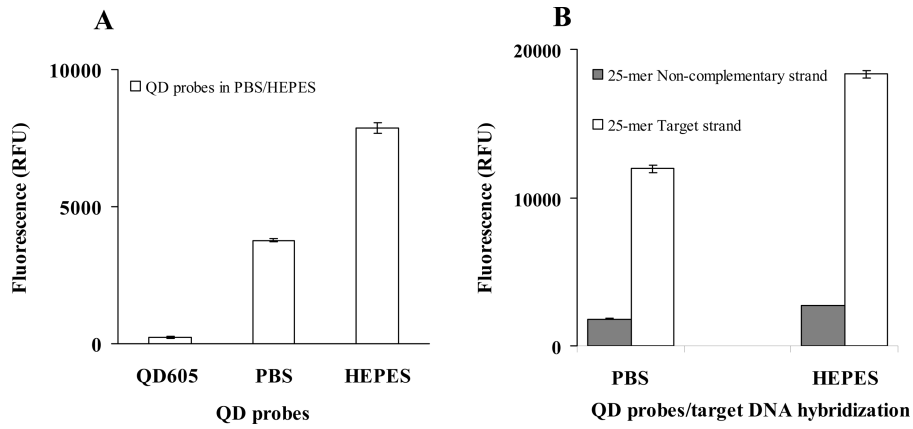

Figure 2. Verification of QD probes. (A) Conjugation of oligonucleotides to QDs and oligonucleotide binding on the surface of QD in PBS (pH 7.2) and HEPES (pH 9.5) buffer. After conjugation and purification, QD probes were stained with OliGreen and analyzed by a fluorometer. (B) Hybridization efficiency of 25-mer target DNA and 25-mer QD probes prepared in PBS (pH 7.2) and HEPES buffers (pH 9.5). Fluorescence of QD probes/target DNA hybrid was measured by staining with PicoGreen in a fluorometer.

QD Probes and Target DNA Hybridization Analysis in a Lifetime- and Quenching-Based Portable Device. First, to investigate the effects of parameters such as oligonucleotide density and probe length on hybridization, 1 μL of 18-mer or 25-mer 20 μM target DNA was reacted using the same methods as described above (Figure 1A), and 5 μL was analyzed by a custom-made portable device (Figure 1B).

Second, to quantify QD probes and target DNA hybridization, 1 μL aliquots of 25-mer target DNA ranging from 8 to 400 μM were hybridized with 3 μL of 0.15 nM QD probes (15.6 oligonucleotides/QD) in PBS buffer according to the methods described above and analyzed in the portable device (5 μL, Figure 1B). The QD probe/DNA hybrid lifetime and quenching values were plotted as a function of target DNA concentration. Figure 1B shows a scheme of the device for time-resolved measurements of the QD probe and target DNA hybridization. A 470 nm light-emitting diode (LB340, Seoul Semiconductors, Korea) was used as an excitation light source. The diode was driven by a custom-made pulse generator adjusted to produce 6 ns bright optical impulses with a short back front at repetition rate of 0.5 MHz. Average power of the pulsed LED source was 3.5 μW. The portable pulse generator (Figure 1C) was constructed of two interconnected avalanche transistors. High-brightness radiation of the LED was reached by a direct short impulse, injecting significant amount of superfluous carriers into the p-n junction of the diode, following with opposite impulse which sweeps-out, nonrecombined carriers from the transition, providing steepness of back front of the light impulse and its small width. Amplitude and width of the direct and opposite impulses and the time interval between them are independent from each other and adjustable for a given type of LED. The accuracy of the recurrence time of the sequence of impulses was provided by an operating processor and a fast comparator forming the starting impulse. The scheme can also work in self-oscillating mode.

The emitted beam was focused by lens 1 at the center of sample holder. Fluorescence light was collected by lens 2, passed through a band-pass filter (605WB20, Omega Optical, Inc., USA) and detected by a photomultiplier-tube module (H9305, Hamamatsu Photonics, Japan). A prototype of the custom-made portable system is shown in Figure 1D. The signal was measured using a 500 MHz digital oscilloscope (TDS3052B, Tecktronix, Inc., USA).

RESULTS AND DISCUSSION

Characterization of QD Probes. To prepare stable and homogeneous QD probes, EDC and NHS concentrations were optimized. We found that excess concentrations of EDC (1 mM) and NHS (1.7 mM) in the preparation caused significant aggregation of the QDs (see Supporting Information, S1A), in contrast to the cases of other microparticles, which are reportedly unaffected by the linker amount.[14] Aggregates could cause serious errors, e.g., giving false positive results in the FRET assay due to quenching.[15] Aggregation caused by inappropriate component concentration in conjugation was controlled using fluorescence microscopy. In this study, 30 000- and 50 000-fold ratios of EDC and NHS to the available COOH number per QD were shown to make homogeneous probes without any aggregates (see Supporting Information, S1B). EDC, NHS, and unbound oligonucleotides were removed by the microfiltration, and nonattachment of QD to the filter surface was checked under the UV lamp. Thus, QD probes were recovered very completely without any attachment to the filter surface by adjusting centrifugal speed and time.

Conjugation of oligonucleotides to QDs has been conventionally done in PBS buffer.[16] To make more effective QD probes, QD probes prepared in PBS (pH 7.2) and HEPES buffer (pH 9.5) were compared for their conjugation efficiency. Interestingly, QD probes prepared in HEPES buffer showed excellent conjugation efficiency (71 oligonucleotides per QD, Figure 2A), binding to nearly twice as much DNA as those prepared in PBS (34 oligonucleotides per QD, Figure 2A), showing the requirement of a buffer with high pH to increase the conjugation efficiency. Furthermore, hybridization efficiency was compared in differently prepared QD probes (Figure 2B). Hybridization efficiency was increased in QD probes prepared in HEPES buffer by 1.5 times compared to those in PBS buffer. This result demonstrates that highly stable prepared QD probes improve hybridization efficiency significantly (Figure 2B). Therefore, QD probes prepared in HEPES buffer were chosen as probes in the subsequent hybridizations.

(14) Lund, V.; Schmid, R.; Rickwood, D.; Hornes, E. *Nucleic Acids Res.* **1988**, *16*, 10861–10880.
(15) Algar, W. R.; Krull, U. J. *Anal. Chim. Acta* **2007**, *581*, 193–201.
(16) Gill, R.; Willner, I.; Shweky, I.; Banin, U. *J. Phys. Chem. B* **2005**, *109*, 23715–23719.

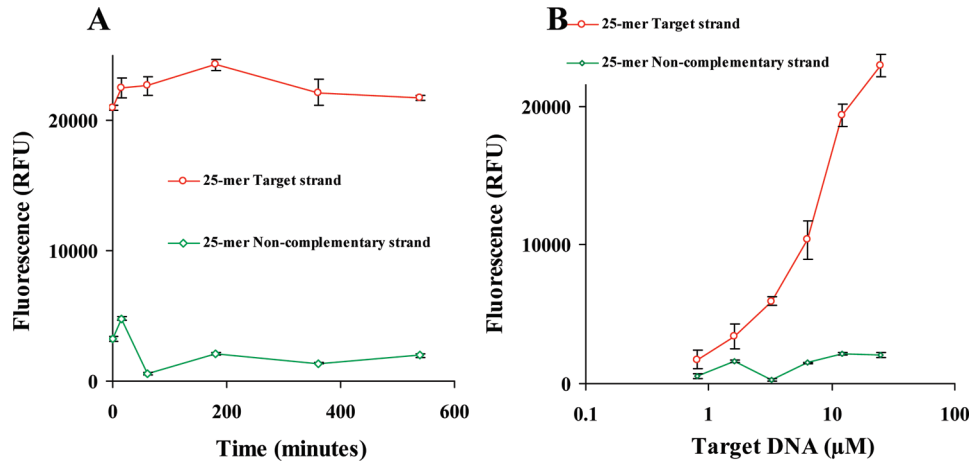

Figure 3. Hybridization kinetics and quantitative analysis of QD probe and target DNA hybridization. (A) Kinetic study of QD probe (0.45 nM) and target DNA (3 μM) hybridization. (B) Hybridization of 25-mer target DNA and 25-mer QD probes. Fluorescence was measured by staining QD probe/target DNA hybrid with PicoGreen. Fluorescence intensity was plotted as a function of target DNA concentration.

Hybridization Kinetics and Quantitative Analysis of QD Probes and Target DNA Hybridization. To develop novel strategies to detect QD probes and target DNA hybridization, comparable and more convincing proof for hybridization was required. Previous studies utilizing techniques such as melting-curve analysis and fluorescence-dye staining to evaluate QD hybridization with DNA have been reported.[13,15,17] However, these methods have limits due to the thermal stability of QDs and the high background noise caused by remaining unhybridized target DNA.[13,18,19] To verify hybridization of QD probes and target DNA, unhybridized DNA was removed by microfiltration after hybridization, and complete hybridization between QD probes and target DNA was determined.

Figure 3A shows that hybridization of 25-mer QD probes and 25-mer target DNA increased for up to 3 h and that hybridization efficiency changed very slightly even after 9 h, showing faster hybridization in comparison with earlier reports.[13,17] Based on this optimized result (Figure 3A), a 3 h hybridization time was used in the following experiments.

Figure 3B shows a quantitative analysis of QD probe and target DNA hybridization. A calibration curve was obtained by measuring the fluorescence of the QD probe/target DNA hybrid after PicoGreen staining and separation of unhybridized DNA. Fluorescence intensity increased rapidly with target concentration. From these results, QD probes were proven to hybridize with target DNA.

Effect of Oligonucleotide Density and Length on Hybridization in a Lifetime- and Quenching-Based Measurement. To further evaluate the suitability of the biophysical parameters of the QD probes in DNA quantitation, oligonucleotide density and length effects on quenching- and lifetime-based detection were analyzed and compared in a portable device. The density of conjugated oligonucleotides on the QDs using 500, 1000, and 2000 pmol of oligonucleotides were 3.5, 15.6, and 71 molecules/QD, respectively, as indicated by the measurement of oligonucleotide fluorescence on QD surfaces after conjugation. When oligonucleotide density was low, the number of hybridization events on the QD surface was also low, and the change in photophysical properties of the QDs was negligible (see Figure 4A,B; 3.5 oligonucleotides/QD gave a 13% lifetime reduction and a 4% fluorescence quenching). As the oligonucleotide density increased, the target capturing rate correspondingly increased, yielding stronger quenching and shortening the lifetime (Figure 4A,B; 15.6 oligonucleotides/QD resulted in a 30% lifetime reduction and a 40% fluorescence quenching). However, if the probe density was very high, the efficiency of hybridization dropped, probably due to repulsive forces from the net charge created by the probes on the QD surface (Figure 4A,B; 71 oligonucleotides/QD showed an 11% lifetime reduction and a 17% fluorescence quenching). QD probes having 15.6 oligonucleotides/QD were used as probes in the following hybridizations.

The results above are in agreement with reports by other groups. For example, Peterson et al.[20] observed that oligonucleotide density when immobilized on a solid support strongly influences the efficiency of target capture. It was found that essentially 100% of the probes can be hybridized in the lowest probe-density regimes and that the binding rate follows Langmuir-like kinetics, whereas at high density, the efficiency drops to nearly 10% and the binding rate is also slower.

We also investigated probe length effects on hybridization in the lifetime- and quenching-based detection method (Figure 4C,D). QD probes (18-mer) showed a 40% lifetime reduction and 50% fluorescence quenching when hybridized with complementary 25-mer target DNA (Figure 4C,D). On the other hand, 25-mer QD probes showed a 30% lifetime reduction and 40% fluorescence quenching (Figure 4C,D).

Surface coverage with probes and the probe organization depends on the probe length.[21] Steel et al.[21] reported a notable decrease in surface coverage for oligonucleotides longer than 24 bases. Shorter probes have a tendency to pack in a rodlike configuration, while longer probes have a less ordered arrange-

(17) Algar, W. R.; Krull, U. J. *Langmuir* **2006**, *22*, 11346–11352.
(18) Bras, F.; Boucaud, P.; Sauvage, S.; Fishman, G. *Appl. Phys. Lett.* **2002**, *80*, 4620–4622.
(19) Heitz, R.; Mukhametzhanov, I.; Madhukar, A.; Hoffmann, D.; Bimberg, D. *J. Electron. Mater.* **1999**, *28*, 520–527.
(20) Peterson, A. W.; Heaton, R. J.; Georgiadis, R. M. *Nucleic Acids Res.* **2001**, *29*, 5163–5168.
(21) Steel, A. B.; Levicky, R. L.; Herne, T. M.; Tarlov, M. J. *Biophys. J.* **2000**, *79*, 975–981.

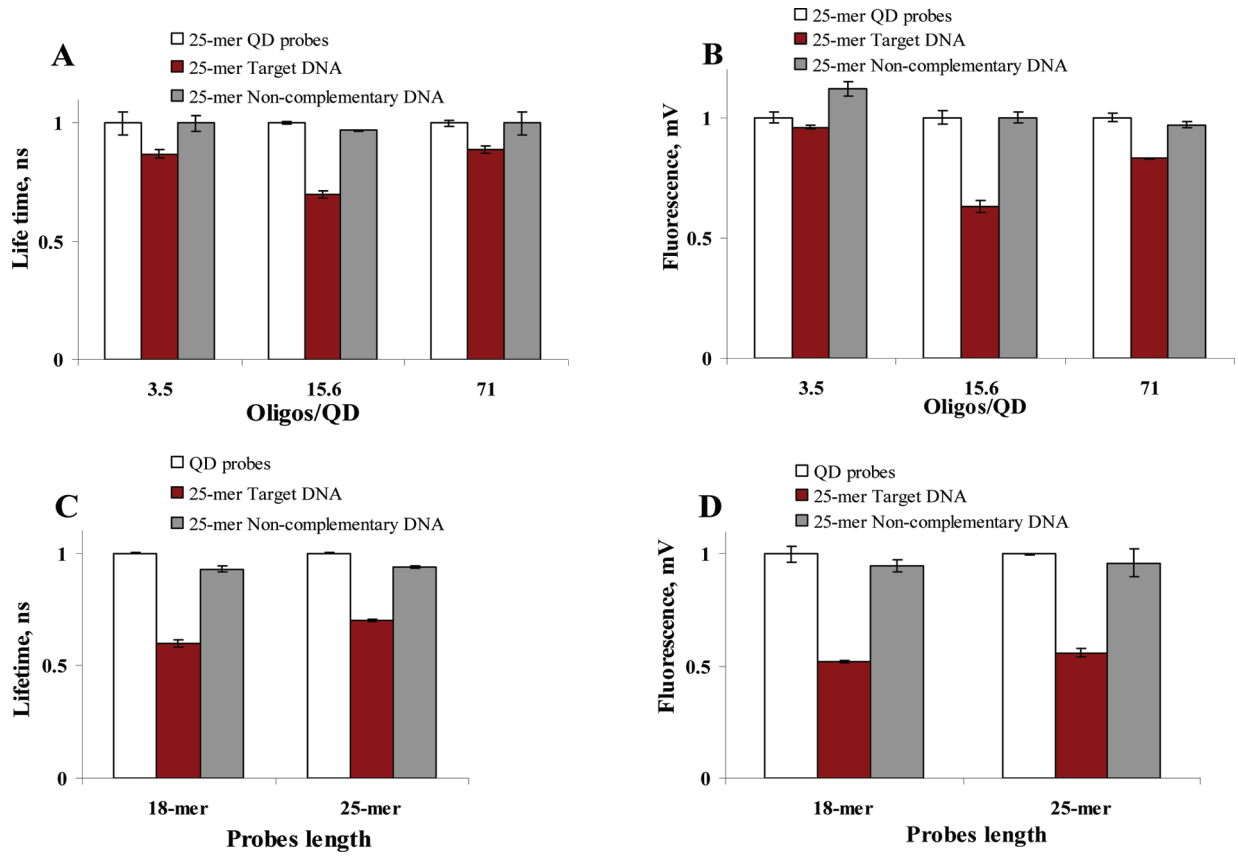

Figure 4. Effect of oligonucleotide density (A, B) and oligonucleotide length (C, D) on hybridization in lifetime and quenching-based portable device (0.45 nM QD probes and 400 μM target DNA). The hybridization complexes were quantified by the portable device and lifetime and quenching values plotted as a functions of oligonucleotide density and probe length.

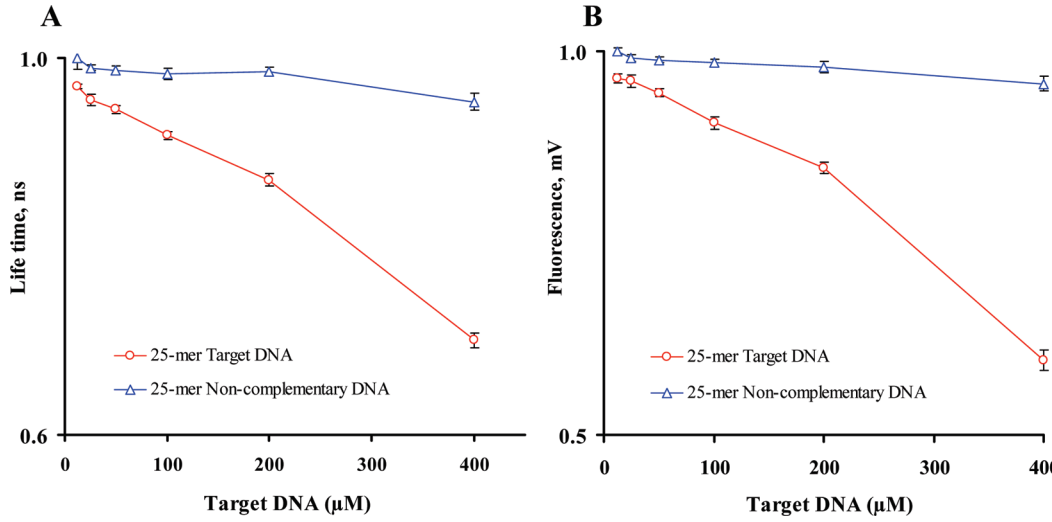

Figure 5. (A) Lifetime- and (B) quenching-based quantitative analysis of QD probes and target DNA hybridization in the portable device. The hybridization complexes were quantified by the portable device and lifetime and quenching values plotted as functions of target DNA concentration.

ment on the surface, presumably due to increased polymeric behavior. Thus, lower oligonucleotide density on the surface is associated with reduced hybridization efficiency and consequently lower changes in measured signals.

Lifetime- and Quenching-Based Quantitative Analysis of QD Probes and DNA Hybridization in a Portable Device. A calibration curve was obtained by measuring the lifetime and quenching of QD probe/target DNA hybrids in a separation-free format (Figure 5). Lifetime and quenching variations with the target DNA concentration were shown. Normalized lifetime and quenching values for control QD probes decreased after hybridization with different concentrations of 25-mer target DNA. For example, after adding 400 μM of target DNA, the lifetime and fluorescence decreased to about 30% and 40%, respectively, relative

to their initial values. This nonspecific binding is likely caused by the COOH groups on the QD surface. Using the current experimental setup, 25-mer target DNA was detected at 12.5 μM (corresponding to 1 μL of 12.5 μM DNA).

The slope in Figure 3B is significantly sharper than in Figure 5B probably because the processes of the fluorescence enhancement of PicoGreen dye and the quenching of the photoluminescence of semiconductor crystals are based on different photophysics and consequently have different dependences on the target DNA concentration. According to Schweitzer,[22] fluorescence enhancement of the monomethine cyanine dyes upon binding to DNA is due to a decreased rotational mobility around the internuclear bridge between the two aromatic ring systems, benzoxazole and quinoline. The quenching of QD is due to the nonradiative Auger recombination process.[11]

The true limit of the detection sensitivity for the target genes, captured by the QD probes, depends on sensitivity of the reading device. The problem of sensitivity can be solved by utilizing a system equipped with high-collection-efficiency optics and a single-photon-detection module featuring high quantum efficiency and low dark noise together with subnanosecond time response such as Single-Photon Avalanche Diode (also known as Geiger-mode APD).

The advantage of our system is that the detection requires only single-step hybridization, minimizing the overall time for sample analysis.

(22) Schweitzer, C.; Scaiano, Y. *Phys. Chem. Phys.* **2003**, *5*, 4911–4917.

CONCLUSIONS

We developed an innovative, simple, and portable target detection system as a single-label and wash-free platform using the QD photophysical properties of fluorescence lifetime and quenching. Preparation and characterization of stable and homogeneous probes and interactions of QD probes and target DNA were investigated. The strength of our techniques is the ability to extend the application of the analytical device to biological samples, which could allow for fast target DNA detection in point-of-care diagnostics and field analysis. This article describes a new method and this technique should obviously be tested against well-characterized specimens.

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SUPPORTING INFORMATION AVAILABLE

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