Interfacial Transduction of Nucleic Acid Hybridization Using Immobilized Quantum Dots as Donors in Fluorescence Resonance Energy Transfer

W. Russ Algar and Ulrich J. Krull*

Chemical Sensors Group, Department of Chemical and Physical Sciences, University of Toronto Mississauga, 3359 Mississauga Road North, Mississauga, Ontario L5L 1C6, Canada

Received September 19, 2008. Revised Manuscript Received November 4, 2008

Fluorescence resonance energy transfer (FRET) using immobilized quantum dots (QDs) as energy donors was explored as a transduction method for the detection of nucleic acid hybridization at an interface. This research was motivated by the success of the QD-FRET-based transduction of nucleic acid hybridization in solution-phase assays. This new work represents a fundamental step toward the assembly of a biosensor, where immobilization of the selective chemistry on a surface is desired. After immobilizing QD-probe oligonucleotide conjugates on optical fibers, a demonstration of the retention of selectivity was achieved by the introduction of acceptor (Cy3)-labeled single-stranded target oligonucleotides. Hybridization generated the proximity required for FRET, and the resulting fluorescence spectra provided an analytical signal proportional to the amount of target. This research provides an important framework for the future development of nucleic acid biosensors based on QDs and FRET. The most important findings of this work are that (1) a QD-FRET solid-phase hybridization assay is viable and (2) a passivating layer of denatured bovine serum albumin alleviates nonspecific adsorption, ultimately resulting in (3) the potential for a reusable assay format and mismatch discrimination. In this, the first incarnation of a solid-phase QD-FRET hybridization assay, the limit of detection was found to be 5 nM, and the dynamic range was almost 2 orders of magnitude. Selective discrimination of the target was shown using a three-base-pairs mismatch from a fully complementary sequence. Despite a gradual loss of signal, reuse of the optical fibers over multiple cycles of hybridization and dehybridization was possible. Directions for further improvement of the analytical performance by optimizing the design of the QD-probe oligonucleotide interface are identified.

1. Introduction

Since the first breakthrough applications of water-soluble colloidal quantum dots (QDs),[1,2] the majority of the interest in QDs has focused on their use as labels to replace conventional fluorophores, particularly in imaging applications. However, there are a growing number of assays that use QDs as donors in fluorescence resonance energy transfer (FRET).[3−5] Our interest has been in developing a QD-FRET-based approach for the detection of nucleic acid hybridization. QD-FRET assays for nucleic acids have included QD-quencher molecular beacons,[6,7] a single-molecule spectroscopy approach for measuring hybridization-mediated FRET between QDs and Cy5,[8] and a multiplexed approach using two donor−acceptor pairs developed in our laboratory.[9] All of these approaches have been single-use solution-based assays. There is desire to move the advantages of the QD-FRET assays to a reusable format. One goal in such research would be a QD-FRET nucleic acid biosensor. To create a reusable assay or biosensor, it is necessary to immobilize the biorecognition chemistry on a solid substrate. This allows the chemistry to be exposed to different conditions for hybridization and regeneration as well as allowing for integration into flow cells, microfluidics, or other sample-handling devices. Although it could be argued that using QDs for target labeling in solid-phase hybridization could satisfy this criterion, it is a disadvantage to use the QDs as a disposable component of the assay. Despite advances over the last several years, high-quality QDs are still more difficult and expensive to produce in comparison to fluorescent dyes and remain less widely available. Similarly, immobilized QDs potentially allow the development of selective sensing approaches without the need to label the target. There are also environmental and toxicity concerns over releasing QDs into the environment. Therefore, integrating QDs into the assay via immobilization is preferable. To date, only a limited number of solid-phase bioassays have accomplished this, none of which were for nucleic acids and none of which explored reusability.[10−12] In this article, we report a method for the interfacial detection of nucleic hybridization using immobilized QDs and FRET, including a strategy for overcoming nonspecific adsorption issues and addressing the reusability of the immobilized chemistry.

QDs are highly advantageous because of their optical properties and ability to act as a scaffold for the immobilization of biomolecules. Optically, the broad absorption spectrum and narrow symmetric photoluminescence (PL) spectrum make QDs ideal for multiplexing. As an example, our previous work made use of the ability to excite multiple QDs using a single excitation wavelength to detect multiple nucleic acid targets simultaneously without the use of discrete sensor elements, multiple assay wells,

* Author to whom correspondence should be addressed. E-mail: ulrich.krull@utoronto.ca.

(1) Chan, W. C. W.; Nie, S. *Science* **1998**, *281*, 2016–2018.
(2) Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013–2016.
(3) Algar, W. R.; Krull, U. J. *Anal. Bioanal. Chem.* **2008**, *391*, 1609–1618.
(4) Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H. *Nat. Mater.* **2005**, *4*, 435–446.
(5) Medintz, I. L.; Deschamps, J. R. *Curr. Opin. Biotechnol.* **2006**, *17*, 17–21.
(6) Kim, J. H.; Morikis, D.; Ozkan, M. *Sens. Actuators, B* **2004**, *102*, 315–319.
(7) Kim, J. H.; Chaudhary, S.; Ozkan, M. *Nanotechnology* **2007**, *18*, 195105.
(8) Zhang, C. Y.; Yeh, H. C.; Kuorki, M. T.; Wang, T. H. *Nat. Mater.* **2005**, *4*, 826–831.
(9) Algar, W. R.; Krull, U. J. *Anal. Chim. Acta* **2007**, *581*, 193–201.
(10) Medintz, I. L.; Sapsford, K. E.; Clapp, A. R.; Pons, T.; Higashiya, S.; Welch, J. T.; Mattoussi, H. *J. Phys. Chem. B* **2006**, *110*, 10683–10690.
(11) Sapsford, K. E.; Medintz, I. L.; Golden, J. P.; Deschamps, J. R.; Uyeda, H. T.; Mattoussi, H. *Langmuir* **2004**, *20*, 7720–7728.
(12) Tran, P. T.; Goldman, E. R.; Anderson, G. P.; Mauro, J. M.; Mattoussi, H. *Phys. Status Solidi B* **2002**, *229*, 427–432.

10.1021/la803082f CCC: $40.75
Published on Web 12/11/2008

single-molecule spectroscopy, or sorting technology.[9] QDs are also advantageous in that they tend to have greater photostability than organic dyes. This is an important consideration in moving toward the development of a reusable assay or biosensor, where greater photostability should improve assay/sensor lifetime or potentially allow for the development of continuous monitoring technology. Although organic dyes are generally used as acceptors in QD-FRET methods, indirect excitation via FRET greatly reduces the likelihood of photobleaching the dye.[13] Another significant advantage of a FRET approach is that organic dyes can be multiplexed as easily as QDs by coupling their excitation and emission through energy transfer. This coupling also provides an inherent mechanism for detecting binding events based on the distance dependence of energy transfer. Finally, FRET with a fluorescent donor and acceptor allows for ratiometric detection, which compensates for photobleaching or the effects of environmental changes on the donor. Developing an immobilized QD-FRET-based assay for nucleic acid hybridization would incorporate all of these advantages while providing the potential for reusability. In this article, we report a design for the transduction of nucleic acid hybridization at an interface using immobilized QDs and FRET. Two major limitations in developing a QD-based solid-phase assay for nucleic acid hybridization have been the immobilization of QDs and the challenge of nonspecific adsorption. With respect to immobilization, some approaches have relied on peptide-,[10] protein-,[11] or antibody-based[11] surface tethers for QDs. Our approach in this work is much simpler, using a thin film of thiol ligands to immobilize QDs.[14] With respect to the challenge of nonspecific adsorption,[9,15] the use of an intercalating dye[9] or specially synthesized polyethylene glycol ligands[16] has been shown to ameliorate these issues in solution-phase assays. However, a continuous interface of immobilized QDs does not present the same surface issues as single QDs in bulk solution. In this work, the challenge of nonspecific adsorption at the interface was overcome by blocking active adsorption sites with a protein. The design of our transduction strategy is shown in Figure 1. Hybridization events were detected via excitation of an immobilized film of QD-probe oligonucleotide conjugates and energy transfer to fluorophore-labeled target oligonucleotides. The result was a combination of QD donor and acceptor emission that represented the amount of hybridized target. Energy transfer can occur when (1) the QD donor and fluorophore acceptor have sufficient spectral overlap and (2) the QD donor and fluorophore acceptor are in close proximity to one another (a few nanometers). Spectral overlap was ensured by using QDs with an emission peak at 538 nm in combination with Cy3 as an acceptor fluorophore. The proximity required for FRET arose when the immobilized QD-probe oligonucleotide conjugates hybridized with complementary Cy3-labeled target oligonucleotides. A layer of denatured bovine serum albumin blocks nonspecific adsorption, preventing false positive signals for noncomplementary sequences. This nonoptimized signal transduction method allows the detection of hybridization in nanomolar sample solutions, which is comparable to most molecular beacon technologies. The ability to discriminate mismatches and reuse immobilized QD-probe oligonucleotide conjugates over multiple dehybridization and rehybridization cycles was also evaluated. This report provides an important first disclosure of a framework for the further development and optimization of a variety of QD−nucleic acid solid-phase assays, ultimately leading to reusable diagnostic technologies by taking advantage of the unique optical and multiplexing properties of QDs.

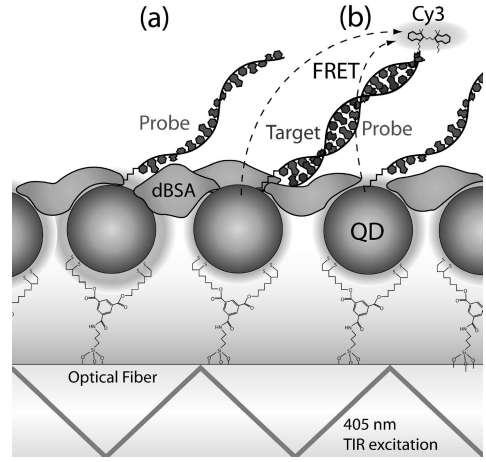

Figure 1. (a) Architecture for the transduction of interfacial nucleic acid hybridization: a layer of QDs was immobilized on a multidentate-ligand-modified fused silica optical fiber with an overlayer of oligonucleotide probes and denatured bovine serum albumin (dBSA). (b) Upon hybridization with Cy3-labeled target oligonucleotides, a FRET-sensitized emission signal was generated as energy was transferred from the QD donor to the Cy3 acceptor. Excitation light was provided via total internal reflection.

2. Experimental Section

Reagents. Fused silica optical fiber was obtained from Polymicro Technologies, LLC (Phoenix, AZ). The specifications were a 400-μm-diameter OH fused-silica core, 430 μm hard polymer cladding, and 730 μm buffer. Trioctylphosphine (TOPO)-capped CdSe/ZnS QDs were obtained from Professor Warren C. W. Chan at the University of Toronto. The synthetic methods used by Professor Chan have been published previously.[17−19] Sodium borohydride (98%), tris(2-carboxy-ethyl)phosphine hydrochloride (TCEP), and bovine serum albumin (fraction V, >96%) were from Sigma-Alrdich (Oakville, ON, Canada). Modified oligonucleotides were from Integrated DNA Technologies (Coralville, IA) and were HPLC purified by the manufacturer. Illustra NAP-10 columns were from GE Healthcare (Baie d'Urfe, PQ, Canada). Buffers were prepared using autoclaved double-distilled water. These included phosphate-buffered saline (0.1 M PBS, pH 7.4) and borate buffer (pH 9.5). All other water was deionized and purified with a Milli-Q cartridge purification system (Millipore Corp., Mississauga, ON, Canada).

Fiber Preparation. The fiber buffer was removed by mechanical stripping, and the fibers were cut to the desired length (usually 40 mm). The cladding on the cut fibers was dissolved in piranha solution (50% v/v 18 M H_2SO_4 and 50% v/v 30% hydrogen peroxide) over 72 h. The fibers were washed with copious amounts of water and rinsed with acetone. The fibers were then sonicated twice in chloroform for 30 min. Fused-silica optical fibers were modified with multidentate thiol ligands, and QDs were immobilized according to our previously published protocol.[14] This included both fiber cleaning and the four-step synthesis. The QDs were made water-soluble by ligand exchange with 3-mercaptopropionic acid (MPA) using a previously published method.[14]

Thiol-modified probe oligonucleotides (HS-C_6H_{12}-5′-ATT TTG TCT GAA ACC CTG T-3′) were received in the disulfide form. The disulfide was reduced by incubating the oligonucleotides with a 1000-fold excess of TCEP in aqueous solution for 1 h. The

(13) Medintz, I. L.; Clap, A. R.; Melinger, J. S.; Deschamps, J. R.; Mattoussi, H. *Adv. Mater.* **2005**, *17*, 2450–2455.
(14) Algar, W. R.; Krull, U. J. *Langmuir* **2008**, *24*, 5514–5520.
(15) Algar, W. R.; Krull, U. J. *Langmuir* **2006**, *22*, 11346–11352.
(16) Zhou, D.; Ying, L.; Hong, X.; Hall, E. A.; Abell, C.; Klenerman, D. *Langmuir* **2008**, *24*, 1659–1664.
(17) Hines, M. A.; Guyot-Sionnest, P. *J. Phys. Chem.* **1996**, *100*, 468–471.
(18) Fischer, H. C.; Liu, L. C.; Pang, K. S.; Chan, W. C. W. *Adv. Funct. Mater.* **2006**, *16*, 1299–1305.
(19) Jiang, W.; Mardyani, S.; Fischer, H.; Chan, W. C. W. *Chem. Mater.* **2006**, *18*, 872–878.

oligonucleotides were then purified using NAP-10 columns according to the manufacturer's protocol. Freshly purified thiol-terminated oligonucleotides were typically dissolved in PBS buffer to a final concentration of 1.0−2.0 μM. Fibers were incubated in these solutions for 1 to 2 h. The fibers were then rinsed in water, sonicated for 5 min in a 0.1% solution of SDS in borate buffer, and rinsed in water again.

Denatured bovine serum albumin (dBSA) was prepared using a method similar to that published previously.[20] A mass of 160 mg of BSA was dissolved in 50 mL of water and stirred with 7 to 8 mg of sodium borohydride for 1 h at room temperature. The solution was then heated to >90 °C and stirred for 20−30 min, filtered hot by gravity, and plunged into an ice bath to cool. The solution of dBSA was stored in the refrigerator. Fresh solutions were prepared every few days. Fibers modified with QD−oligonucleotide conjugates were immersed in a 0.5 mg/mL solution of dBSA in PBS buffer for 30−60 min. The fibers were then rinsed in water, sonicated for 10 min in a 0.1% solution of SDS in borate buffer, and rinsed in water again. Fibers could be stored immersed in buffer at room temperature.

Interfacial Hybridization Experiments. Fully modified fibers were immersed in 1.0−1.25 mL of a solution of the desired Cy3-labeled sample oligonucleotide (fully complementary target, Cy3−5′-ACA GGG TTT CAG ACA AAA T-3′; three-base-pairs mismatched target, Cy3-5′-A*T*A GGG TTT C*G*G ACA AA*G* T-3′; or non-complementary, 5′-AAA AAA AAA AAA AAA AAA AA-3′, i.e., dA_{20}) for 20 min. The fibers were removed, quickly rinsed with PBS buffer, and scanned in a spectrofluorimeter. For experiments above room temperature (25 °C), the solutions of desired oligonucleotide were placed in a block heater at 40 or 50 °C and equilibrated for 30 min prior to immersion of the fibers. Higher temperatures are used to destabilize partially mismatched hybrids.

In hybridization−dehybridization experiments, the hybridization step was carried out as previously described. The regeneration step was carried out by immersing the fibers in 1.5−1.75 mL of an 85:15 v/v formamide/PBS buffer for 30 min at room temperature. The fibers were then rinsed in water, immersed in PBS buffer for 15 min, rinsed in water again, and measured with a spectrofluorimeter.

Data Analysis. The PL spectra obtained were normalized to the peak QD emission intensity. This avoids variations in PL intensity due to variations in the length of fiber covered with QD–oligonucleotide conjugates and also variations in the laser power coupled to different optical fiber samples. Both of these sources of variation can affect the absolute PL intensities measured but do not affect the relative intensity observed. Spectral data could be reduced to numerical data via a FRET ratio. The FRET ratio represents the relative amounts of Cy3 luminescence and QD luminescence. For each spectrum, a ratio R was calculated as eq 1

$$R = \frac{\sum_{\lambda=560}^{590} F(\lambda)}{\sum_{\lambda=528}^{558} F(\lambda)} \quad (1)$$

where $F(\lambda)$ is the measured fluorescence intensity at wavelength λ. The wavelength ranges were selected such that QD emission was the major component of the denominator and Cy3 emission was the major component of the numerator. It should be noted that using larger or smaller wavelength ranges changed the numerical value of the ratio but did not change the trends observed. Raw spectra were processed by subtracting the background fluorescence from fibers without QDs (small compared to the QD signals in most experiments) and computing R. This was done for fibers without exposure or prior to exposure to sample sequences (R_0) and after exposure to sample sequences (R'). The R_0 measurement should *not* be confused with the Förster distance, which is a different quantity. The analytical parameter ΔFRET ratio was calculated as $R' - R_0$ to correct for emission overlap between QD and Cy3 PL. The ΔFRET ratio served as a numerical parameter to measure the amount of FRET. Measurements based on FRET ratios have been used in other applications as well.[21,22] Estimates of FRET efficiency were obtained by making measurements on the *same* fiber, before and after hybridization, to minimize variations in QD intensity due to variations in fiber coverage. The efficiency was calculated according to eq 2

$$E = 1 - (I_{DA}/I_D) \quad (2)$$

where E is the FRET efficiency, I_{DA} is the QD PL (donor, D) intensity in the presence of Cy3 (acceptor, A), and I_D is the QD PL intensity in the absence of FRET. Variations in the laser power coupled into each fiber for separate measurements may contribute to the relatively large uncertainty associated with these measurements. The FRET efficiency was calculated only in cases where the fiber surface was considered to be saturated with Cy3-labeled target oligonucleotides. For partial coverage, there will be a distribution of FRET efficiencies based on QD proximity to a bound acceptor. This is an additional reason to prefer the FRET ratio as an analytical parameter in these experiments.

The potential Förster distances for the QD−Cy3 pair were calculated according to the equations used in our previously published work.[9] Values of $n = 1.33$ and $\kappa = {}^2/_3$ were used for the refractive index and the orientation factor, respectively. These values correspond to water as a medium and a random orientation of the transition dipoles. In the case of an immobilized film of QDs and biomolecules, both assumptions have questionable validity. Nonetheless, these values are useful for exploring the new method in this work and drawing comparisons to solution-phase studies. The spectral overlap integral was calculated using the experimentally measured QD emission and Cy3 absorption spectra. The solution-phase QD quantum yield was estimated using the method of our previous work, with fluorescein as a standard.[9]

Instrumentation. Photoluminescence (PL) spectra were obtained with a QuantaMaster PTI spectrofluorimeter (Photon Technology International, Lawrenceville, NJ) equipped with a red-sensitive photomultiplier tube (R928P; Hamamatsu, Bridgewater, NJ). The fibers were illuminated by total internal reflection using a Coherent (Santa Clara, CA) radius 405 diode laser (407 nm, 25.0 mW) and a holder that was custom built to fit the sample compartment of the spectrofluorimeter. The optical components for coupling the laser to the fused silica fiber were from Thorlabs (Newton, NJ; SMA connector multimode patch cable, 0.22 NA, 200 μm core, part no. M25L05; SMA collimator, NA 0.25, 400−600 nm, f. 11 mm, part no. F220SMA-A; BK7A biconvex lens, diameter 12.7, f. 15.00, part no. LB1092-A; adapter for 11 mm collimator, part no. AD11F; SM05 to SMA adapter plate, part no. SM05SMA; assorted SM05 lens tubes and SM05RR retaining rings).

3. Results and Discussion

The transduction of nucleic acid hybridization at the surface of the optical fiber was first tested using a film of immobilized QD−oligonucleotide conjugates without a protein layer. It has been previously reported that adsorbed oligonucleotides tend to wrap themselves around the surface of a QD in bulk solution.[9] However, in an immobilized film of QDs, some of the surface area of the QDs is unavailable to oligonucleotides. Therefore, when comparing an immobilized film of QDs to QDs in bulk solution, it is not clear that adsorption will occur to the same extent. Changes in the normalized emission spectra for QD−oligonucleotide films without a protein layer are shown in Figure 2 for both the hybridization of a complementary Cy3-labeled oligonucleotide and the nonspecific adsorption of a

(20) Wang, J. H.; Wang, H. Q.; Zhang, H. L.; Li, X. Q.; Hua, X. F.; Cao, Y. C.; Huang, Z. L.; Zhao, Y. D. *Anal. Bioanal. Chem.* **2007**, *388*, 969–974.

(21) Cissell, K. A.; Campbell, S.; Deo, S. K. *Anal. Bioanal. Chem.* **2008**, *391*, 2577–2581.

(22) Nobles, M.; Benians, A.; Tinker, A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 18706–18711.

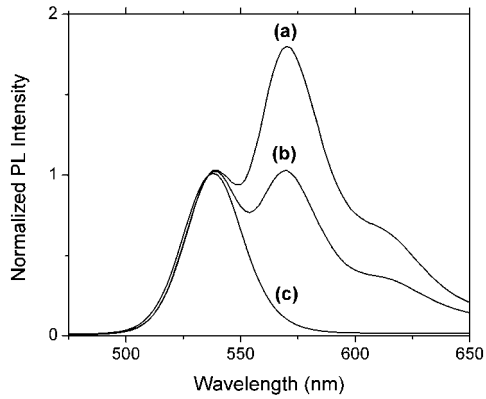

Figure 2. Response of the unpassivated QD-FRET interface to (a) a 0.1 μM complementary sequence, (b) a 0.1 μM noncomplementary sequence, and (c) no sample sequences. The spectral areas are normalized to the QD emission for comparison purposes.

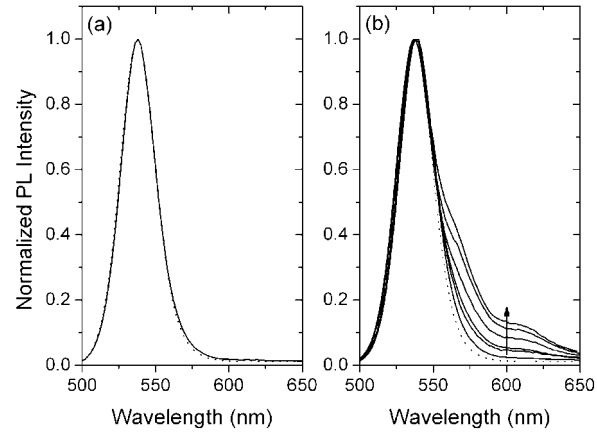

Figure 3. Response of the dBSA-passivated QD-FRET interface to (a) a 0.1 μM dA_{20} (noncomplementary) solution and (b) 0.001, 0.003, 0.006, 0.01, 0.025, 0.050, and 0.100 μM solutions of complementary sequence. In graph a, very little FRET was observed. In graph b, the amount of FRET increased with the target concentration. In both graphs, the dashed line represents the initial state. The spectra are normalized to the QD emission for comparison purposes.

noncomplementary Cy3-labeled oligonucleotide. Clearly, the problems previously encountered with nonspecific adsorption in solution-phase assays[9] transfer to this solid-phase approach. The FRET signal from the noncomplementary polyadenine sequence ranged from 50 to 100% of that of the target sequence. Given the overall magnitude of the FRET-sensitized Cy3 emission, it is probable that the oligonucleotides are strongly adsorbing to the surface of the QD film, as has been previously reported in solution.[15] On the basis of the change in QD photoluminescence, the FRET efficiency was in the range of 70−90%. It was also observed that the difference in efficiency between having the Cy3 acceptor on either terminus of the target was smaller than the variation between measurements (cf. 10% vs 15−20%). For an upright hybrid, the 3′ acceptor should be closer to the surface because the probe is anchored by its 5′ terminus. Thus the data suggests that the duplex lies across the surface of the QD. A 5′ Cy3 acceptor was used in most experiments because it typically yielded more FRET-sensitized emission. This was due to its larger quantum yield but not necessarily a higher FRET efficiency. We measured the 5′ acceptor dye to be twice as bright as the 3′ acceptor.

To eliminate signal from the nonspecific adsorption of oligonucleotides, it was necessary to use a passivating layer of protein. In our experiments, the best results were achieved with a layer of adsorbed dBSA. As shown in Figure 3a, with this modification the fibers showed no significant response to noncomplementary Cy3-labeled polyadenine oligonucleotides. In contrast, Figure 3b shows increasing FRET-sensitized Cy3 emission with increasing amounts of complementary Cy3-labeled target oligonucleotides in solution. On the basis of this data, it should be possible to detect hybridization from sample solutions with as low a sample concentration as 5 nM, a level that is comparable to many molecular beacons.[23−25] This limit of detection is based on the signal level that exceeds 3 SD above the mean baseline spectra. The response range spanned almost 2 orders of magnitude, with the fibers typically saturating in signal beyond 250 nM sample solutions. This compares favorably to the 1 order of magnitude seen when using data from previous solution-phase experiments based on a similar QD-FRET system.[9] The upper limit of the dynamic range is set by either the number of probe oligonucleotides available for hybridization or the upper limit of energy that can be transferred from the QDs. Given the higher FRET efficiencies observed without any passivation, it appears that the upper limit of the dynamic range is set by the number of probe oligonucleotides available. Typical reports[26−28] of immobilized oligonucleotide densities are $(2-6) \times 10^{12}$ molecules/cm^2. Previous measurements[14] indicate that immobilized QD densities are closer to 10^{13} particles/cm^2, suggesting less than one probe oligonucleotide conjugated per QD. In addition to reducing false positive signals greatly, the passivation appears to be advantageous with respect to hybridization time. No significant changes in FRET signals were observed after 25 min of hybridization. This is in contrast to full hybridization occurring over several hours in bulk solution for QD systems subject to nonspecific adsorption.[9,15]

Fibers modified with dBSA and saturated with the Cy3-labeled target have FRET efficiencies of approximately 30−50% based on the decrease in QD PL. In solution-phase experiments, FRET efficiencies have been previously reported to range from approximately 50 to 90%[8,9,16,29] and can be improved by >30% using microfludic flow.[30] We calculate the spectral overlap integral for this system to be 6.5×10^{-10} cm^6. For QD quantum yields varying from 5 to 25%, the Förster distance varies from 4.0 to 5.2 nm using the assumptions described in the Experimental Section. Measurements of QDs in solution suggest that their quantum yield is closer to 1%, corresponding to a Förster distance of 3 nm. However, previous work has shown a change in QD lifetime upon immobilization, suggesting a concomitant change in quantum yield. Furthermore, protein adsorption on QDs typically increases their quantum yield. The enhancements reported are observed to be in the range of several percent to values of up to 200%.[31,32] Therefore, the solution-phase quantum

(23) Sunkara, V.; Hong, B. J.; Park, J. W. *Biosens. Bioelectron.* **2007**, *22*, 1532–1537.
(24) Zuo, X.; Yang, X; Wang, K.; Tan, W.; Li, H.; Zhou, L.; Wen, J.; Zhang, H. *Anal. Chim. Acta* **2006**, *567*, 173–178.
(25) Sassolas, A.; Leca-Bouvier, B. D.; Blum, L. J. *Chem. Rev.* **2008**, *108*, 109–139.
(26) Su, X.; Wu, Y. J.; Knoll, W. *Biosens. Bioelectron.* **2005**, *21*, 719–726.
(27) Jordan, C. E.; Frutos, A. G.; Thiel, A. J.; Corn, R. M. *Anal. Chem.* **1997**, *69*, 4939–4947.
(28) Watterson, J. H.; Piunno, P. A. E.; Wust, C. C.; Krull, U. J. *Sens. Actuators, B* **2001**, *74*, 27–36.
(29) Zhou, D.; Piper, J. D.; Abell, C.; Klenerman, D.; Kang, D. J.; Ying, L. *Chem. Commun.* **2005**, 4807–4809.
(30) Zhang, C. Y.; Johnson, L. W. *Agnew. Chem., Int. Ed.* **2007**, *46*, 3482–3485.
(31) Wang, Q.; Kuo, Y.; Wang, Y.; Shin, G.; Ruengruglikit, C.; Huang, Q. *J. Phys. Chem. B* **2006**, *110*, 16860–16866.
(32) Medintz, I. L.; Clapp, A. R.; Mattoussi, H.; Goldman, E. R.; Fisher, B.; Mauro, J. M. *Nat. Mater.* **2003**, *2*, 630–638.

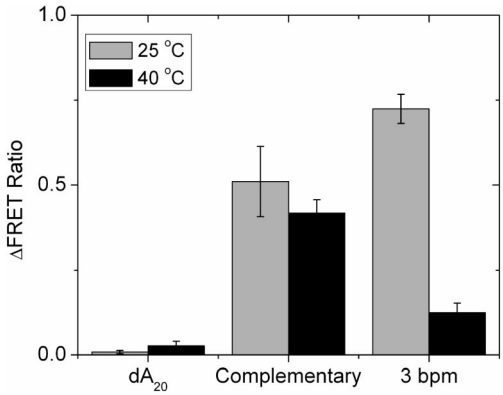

Figure 4. At 25 °C, the sensor responded strongly to both fully complementary sequences and a three-base-pairs mismatched sequence (3 bpm). At 40 °C, the mismatched sequence was significantly destabilized, and much better discrimination was achieved between the two sequences. At both temperatures, the response to noncomplementary dA_{20} was minimal.

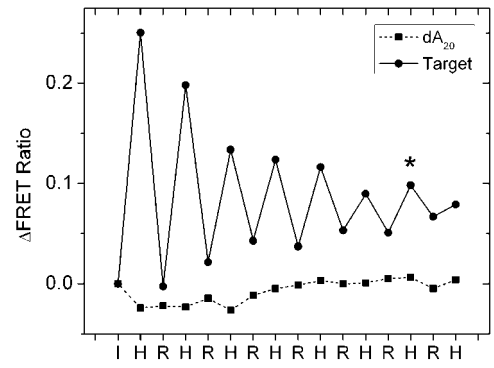

Figure 5. Reuse of the QD-FRET interface was possible over multiple cycles of regeneration (R) and hybridization (H). The response of the sensor to noncomplementary dA_{20} was small throughout the experiments. Only after the seventh hybridization (*) and dehybridization cycle was the response to the complementary sequence statistically insignificant relative to the noncomplementary response. The initial state is I. Each sample oligonucleotide concentration was 0.05 μM.

yield of the QDs is not necessarily reflective of the quantum yield of the immobilized and conjugated QDs in our experiments. Our observations of the PL intensity of immobilized QDs suggest that there is an enhancement, and we expect the Förster distance to be in the range of 3.5−4 nm.

The FRET signal for a 0.10 μM solution of Cy3-labeled target typically ranged from 20−60 times greater than for an equal concentration of a noncomplementary polyadenine sequence. The key to the reduction of false positive signals was the passivating layer of dBSA. The nonspecific adsorption of oligonucleotides on thioalkyl acid-capped QDs is well known.[15] dBSA was able to block these active adsorption sites. Although we found that native BSA was also able to passivate the modified fibers against nonspecific adsorption, the FRET signals for the target were generally 3-fold less than for sensors using dBSA passivation. Nonetheless, in comparing Figures 2 and 3, it is seen that the FRET signals were approximately 3-fold lower in intensity with the dBSA layer compared to that without any dBSA layer. Although the increased sensitivity for dBSA compared to that for native BSA does not move the limit of detection of this approach into the realm of PCR-less detection, it represents a very important observation. It was possible to alleviate nonspecific adsorption issues through protein passivation. It was then possible to tune the protein layer (i.e., by using denatured protein) to improve the sensitivity while still blocking adsorption. These results indicate that optimizing the design of this new approach may eventually bring its limit of detection down to levels more competitive with methods that have been well established through many years of optimization and research.

For the development of future assay technologies, it is important to have a robust transduction platform so that the stringency of hybridization conditions can be altered to discriminate between fully complementary and partially mismatched sequences. Furthermore, it would be a considerable advantage to regenerate and reuse a solid-phase assay platform. With these considerations in mind, we investigated the ability of this transduction platform to discriminate between fully complementary and partially mismatched (three base pairs) sequences and the ability to regenerate and reuse the transduction platform. As shown in Figure 4, strong FRET signals were observed from both fully complementary and mismatched target at 25 °C. The relative signal intensities were approximately 1.0:1.4. Again, the polyadenine control samples showed almost no response. When the temperature was increased to 40 °C, the signal from the mismatched target was greatly decreased compared to that from the fully complementary target. The relative signal intensities are 1.0:0.3, indicating much better selectivity for the fully complementary sequence over the mismatched sequence. Some thermal destabilization of the target occurred, resulting in a signal decrease of 18% compared to that at 25 °C. Increasing the temperature from 40 to 50 °C yielded relative intensities of 1.0: 0.2 for fully complementary and mismatched targets, respectively. However, the absolute signal from the fully complementary target decreased by 67% compared to the signal measured at 25 °C. A slight increase in sensor response to the polyadenine control was observed upon moving to 40 °C, suggesting some thermal rearrangement or destabilization of the dBSA layer. However, no further increase was observed upon moving to 50 °C. In this study, the purpose of the three-base-pairs mismatch is to demonstrate that selectivity can be improved by increasing the temperature. Retaining selectivity as temperature is increased could allow for an assay that can discriminate two-base-pairs mismatches and, ideally, single mismatches. However, in this preliminary work the loss of signal from the fully complementary sequence as the temperature increased indicates that additional refinement of the transduction strategy is required to achieve this goal.

To reuse the fibers, it was necessary to denature the double-stranded DNA formed during hybridization and wash away the sample sequences. For this purpose, modified fibers were regenerated by immersion in a solution of 85:15 v/v formamide/ PBS buffer for 30 min at room temperature. The formamide disrupts hydrogen bonding between the two complementary oligonucleotides but also had the unanticipated effect of greatly decreasing the QD photoluminescence (>80%). This could be largely reversed by immersing the fibers in aqueous buffer for 15 min following formamide exposure. Modified fibers were subjected to multiple rounds of hybridization and dehybridization. Control experiments with noncomplementary polyadenine were run in parallel. As shown in Figure 5, there is a clear trend of signal increase and decrease with successive hybridization and dehybridization steps, respectively. However, there was a steady increase in the background signal following regeneration and a steady decrease in the hybridization signal. This may have been due to the reorganization or loss of the dBSA layer during the regeneration or loss of probe oligonucleotides. Such processes are likely to expose active sites for strong adsorption, gradually raising the baseline intensity following regeneration. The loss of dBSA could also lead to the strong adsorption of probe

oligonucleotides, resulting in long hybridization times as reported elsewhere.[15] Therefore, the decrease in hybridization signal could be due to slower kinetics. Ellipsometry experiments on dBSA films adsorbed to oxidized silicon wafers suggested the loss of the dBSA film after two regeneration cycles. Although the oxidized silicon surface is not necessarily a good mimic of a QD film, both surfaces are negatively charged, and the results could be indicative of what is occurring on the QD film over a more extended time scale. The physisorption of the protein is expected to be stronger on the QDs than on the silicon surface and could cause slower destabilization of the dBSA layer. Despite the limitations of dBSA stability, the relative response to the complementary sequence versus polyadenine was statistically significant over seven hybridization cycles. No significant FRET signals were observed from the control fiber exposed to the noncomplementary sample. However, there does appear to be a small gradual increase in the signal from the noncomplementary material. Changes in the dBSA layer may be driven by the hybridization events themselves. An increase in charge density or a change in oligonucleotide conformation upon hybridization could help initiate the removal of dBSA. Furthermore, complementary oligonucleotides are bound at the fiber surface when each regeneration cycle begins. This is a locally high concentration that could potentially lead to very fast adsorption kinetics as the quality of the dBSA layer deteriorates, resulting in the failure to remove all of the bound complementary material. Because noncomplementary samples have an insignificant local concentration in each regeneration cycle, the adsorption kinetics may be much slower. It is clear that optimizing the design of the QD-probe oligonucleotide interface could lead to significant improvements in the analytical performance of this approach. In the case of regeneration, designing a more robust connectivity for the protein layer should lead to QD-FRET technology capable of being reused over many cycles with no significant loss of analytical performance.

The passivation effects of dBSA were also evident in the regeneration experiments. Without dBSA, the regeneration of the modified fibers took in excess of 8 h in 100% formamide. This was likely due to the strong adsorption of oligonucleotides on the surface of thioalkyl acid-capped QDs. Thus in addition to greatly reducing false positives, dBSA passivation also allowed for much more practical regeneration times. Yet again, it is evident that control over the QD-probe oligonucleotide interface could lead to the control and optimization of hybridization kinetics.

As a final note, it was found during numerical data analysis (e.g., Figures 4 and 5) that the FRET ratio was a preferred numerical value compared to the FRET efficiency. The FRET ratio exhibited approximately half the variation of the FRET efficiency. The FRET efficiency is dependent on the QD quantum yield, which may vary between individual fibers, along the length of the fiber, or between QD preparations. There will also be a distribution of FRET efficiencies for fibers with only partial coverage of hybridized material, greatly complicating potential measurements of FRET efficiency using the QD lifetime. Furthermore, when the QD quantum yield decreases, so does energy transfer and thus acceptor emission. Because the FRET ratio considers both of these parameters, it is able to provide a partial correction. The FRET ratio may also provide some correction for variation in donor−acceptor stoichiometry. Comparisons between different fibers may need to account for differences in immobilized QD densities (and thus the number of probe binding sites as well). The FRET ratio will do this better than the FRET efficiency. In general, we expect the ratiometric measurement to be a better analytical parameter than the FRET efficiency, even when comparisons are made for the same fiber rather than between fibers.

4. Conclusions

An interfacial transduction strategy for nucleic acid hybridization using QDs as energy donors in FRET has been investigated. Upon hybridization of Cy3-labeled target oligonucleotides with immobilized QD-probe oligonucleotide conjugates, energy was transferred from the QD donor to the Cy3 acceptor. FRET-sensitized Cy3 fluorescence was the analytical signal. Passivation of the immobilized chemistry with dBSA allowed for the suppression of false positive signals from noncomplementary sequences at room temperature whereas at elevated temperature it was possible to obtain some discrimination between fully complementary sample sequences and three-base-pairs mismatched sequences. Although transduction gradually degraded, it was possible to use the interface over multiple hybridization and dehybridization cycles. This work has provided an important framework for future developments in solid-phase assays for nucleic acid hybridization using immobilized QDs. There are a number of key findings: (1) a QD-FRET solid-phase hybridization assay is viable, and in this first unoptimized incarnation, it is comparable in performance to what is typically reported for molecular beacons; (2) designing a protein layer in the QD-probe oligonucleotide interface can alleviate nonspecific adsorption issues; and (3) in the absence of nonspecific adsorption, there is the potential for a reusable assay format and mismatch discrimination. The key to further refinement of this technology appears to be incorporating greater organization and stability into the protein layer, which may ultimately lead to a QD-FRET biosensor for nucleic acid detection. Work is currently underway in our laboratory to create such an interface.

Acknowledgment. We gratefully acknowledge the financial support of this research by the Natural Sciences and Engineering Council of Canada (NSERC). WRA is also grateful to NSERC for a graduate fellowship. We also thank Mr. Dawei Li in the Chan Laboratory at the Institute of Biomaterials and Biomedical Engineering (IBBME), University of Toronto, for synthesizing the QDs.

LA803082F