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Communication

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DNA-Triggered Dye Transfer on a Quantum Dot

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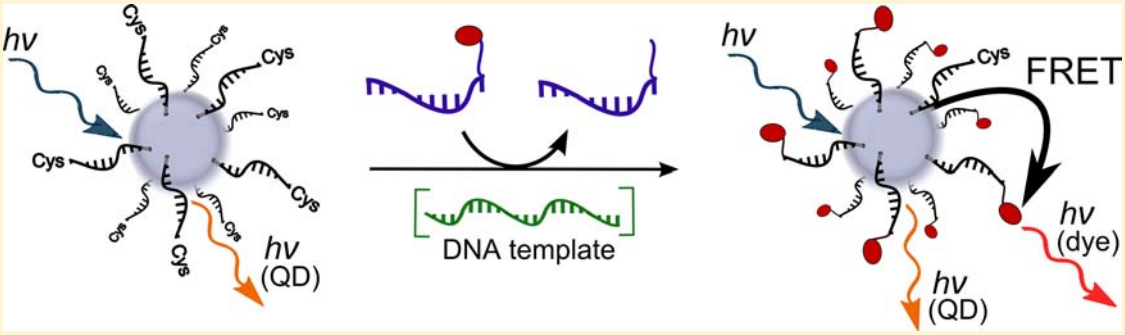
Supporting Information



ABSTRACT: Nucleic acid-templated reactions are frequently explored tools in nucleic acid diagnosis. To enable a separation-free DNA detection, the reactive probe molecules require conjugation with reporter groups that provide measurable changes of an observable parameter upon reaction. A widely used, generic read-out method is based on fluorescence resonance energy transfer (FRET) between two appended dyes. Yet, spectral cross-talk usually limits the achievable enhancements of the FRET signal in DNA-directed chemistries. We describe a DNA-triggered transfer reaction which provides for strong increases of a fluorescent signal caused by FRET. The method may involve DNA- and PNA-based probes and is based upon a proximity-triggered transfer reaction which leads to the covalent fixation of a fluorescence dye on the surface of a quantum dot (QD). The transfer reaction brings the dye closer to the QD than hybridization alone. The resulting FRET signal is a specific monitor of the reaction and allows efficient discrimination of single base mismatched templates. Of note, the 35-fold increase of the FRET signal is measured at 310 nm apparent Stokes shift and turnover in template provides a means for signal amplification.

INTRODUCTION

Nucleic acid-directed reactions provide fascinating opportunities to translate sequences into functions relevant to applications in the materials sciences and the life sciences.[1−3] For example, DNA templated chemistry has been used to facilitate or encode the discovery of reactions and drugs,[4,5] and nucleic acid-directed chemistry has frequently been explored as a tool in bioanalytical research for the detection of DNA and RNA targets.[2,6−20] This approach facilitates, in principle, high target specificity because the chemical reaction requires adjacent annealing of two rather short and appropriately functionalized oligonucleotide probes. Nucleic acid-templated chemistry offers the unique feature that reactions can proceed with turnover in the DNA/RNA template.[21] According to this, a single template molecule may instruct the formation of many signaling molecules.

The read-out mode is an important issue in the design of nucleic acid-templated reactions. Most bioassays rely upon fluorescence signals, which can be measured rapidly and with high sensitivity by using low cost equipment. Many fluorogenic detection chemistries typically are based on the release or cleavage of fluorescence quenchers induced, e.g., by S_N2 displacement,[22,23] nucleophilic aromatic substitution (S_NAr),[24,25] Staudinger reduction,[26,27] or alkene/alkyne chemistry.[28] Such reactivity-based fluorogenic probes have been used with great success in the detection of RNA targets within cells and in cell lysates.[7,14−16,27,29,30] Recently, templated *de novo* synthesis of fluorophores was introduced as a read-out method that is insensitive to background caused by nontemplated reactions.[31−33] Given the commercial availability of a large variety of different fluorophores in conjugatable form, approaches based on fluorescence resonance energy transfer (FRET) should arguably provide the most versatile detection platform. In fact, nucleic acid-triggered reactions have been used to bring two FRET partners into proximity.[13,14,34−38] However, the commonly used organic fluorophores often show spectral cross-talk and both the direct excitation of the acceptor dye and the donor emission at the acceptor wavelength are frequently occurring nuisances. Furthermore, many reactions do not lead to significant changes in the donor−acceptor distance and it is therefore difficult to distinguish reaction-based FRET from hybridization-based FRET.

We assumed that nucleic acid-triggered dye transfer reactions[13,39,40] that proceed on the surface of a nanosized quantum dot (QD) should provide significant improvements of FRET-based reaction monitoring. Quantum dots are excellent

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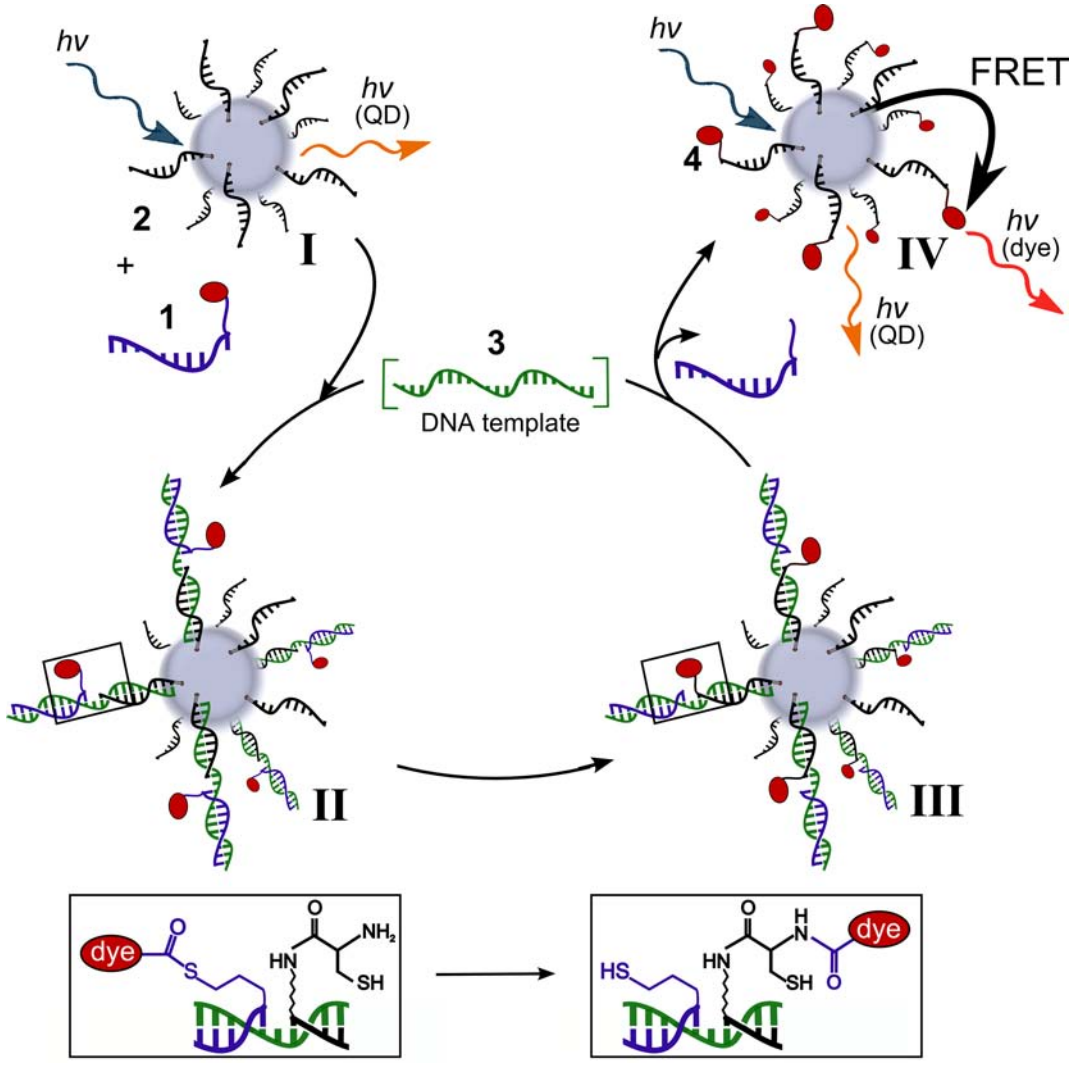
Scheme 1. Overview of the DNA-Templated Transfer Reaction from the Fluorophore-Donating (1) to the Accepting DNA Conjugate (2) that is Immobilized on a Quantum Dot



fluorescent donors in sensor schemes based on FRET due to broad absorption spectra, high extinction coefficients, large Stokes shifts, narrow emission bands, and high quantum yields.[41] Given an appropriate design, transfer reactions can be designed to lead to decreases in the distance between a transferred dye and the quantum dot. This should be accompanied by a reaction specific FRET signal provided that the chromophore distance in an assembly before reaction exceeds the Förster radius. We speculated that the nanosized dimensions of functionalized quantum dots help the distance adjustments. However, the high density of oligonucleotide probes on the surface of a functionalized quantum dot may lead to molecular crowding, and it was unclear whether templated reactions proceed under these conditions. In this Communication, we show that a DNA-triggered transfer reaction on a QD afforded 35-fold FRET-signal enhancement. Both DNA-based and peptide nucleic acid (PNA)-based reactions proceeded smoothly. Of note, FRET signaling was reaction specific, as neither hydrolysis nor hybridization alone provided strong FRET signals. We will discuss that turnover in template provides opportunities for the amplification of signals.

RESULTS AND DISCUSSION

The reaction system involved two reactive oligonucleotide probes. A 3′-thiolated oligonucleotide (**1**) served as donor probe and was armed with a thioester-linked cyanine dye (Scheme 1). The acceptor oligonucleotide (**2**) was equipped with a cysteine moiety at the 5′-end and a biotin at the 3′-terminus. The latter was used for immobilization of the acceptor probe onto the surface of a streptavidin-modified quantum dot. In the presence of a complementary nucleic acid template, the QD-bound acceptor probe recruits the donor probe **1**. Adjacent annealing in complex **II** was envisioned to trigger a chemical reaction, i.e., the transfer of the thioester-linked fluorophore from the donor **1** to the acceptor **2**, which proceeds via a native chemical ligation-type mechanism.[40] This transfer reaction brings the dye in **III** and **IV** into closer vicinity of the quantum dot and energy transfer should become more efficient.

We chose quantum dots (QD 605) consisting of a CdSe-ZnS core−shell nanocrystal as FRET donor and IDCC (a Cy5 analog) as the acceptor dye (see Figure S14, ESI for absorption and emission spectra).[42] The quantum dots consist of an inner amphiphilic coating to which streptavidin is directly attached instead of the usual PEG linkage. This variation was used as it

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leads to a smaller overall particle size and to a higher number of streptavidin units per quantum dot enabling a better FRET efficiency.[43]

On the basis of the Förster formalism, the Förster distance (R_0) for this FRET pair was calculated to be 72 Å (see Figure S14, ESI). Previous reports had suggested a 50−75 Å radius of the streptavidin-functionalized QD.[42] We reasoned that a duplex comprising the 12-nt-long acceptor probe **2** and a complementary Cy5-reference probe **5** (5′-GCGCTGTAGG-C_3H_6-Cy5) would arrange the dye within distance sufficient to obtain efficient FRET. In orienting experiments, we varied the concentrations of oligonucleotides ranging from 35 to 500 nM at 5 nM quantum dot concentration (Figure S9, ESI). The relative FRET signal $(F_{665}/F_{615})_{dye+QD}/(F_{665}/F_{615})_{QD}$ increased from 12-fold at 35 nM duplex **2•5** until saturation was obtained at 160-fold for 300 nM **2•5**. Assuming that each streptavidin unit can bind four biotin units, we inferred that the quantum dots carry about 15 streptavidin units on average.

We next examined FRET signaling upon incubation of the reactive probes **1** and **2** in the absence or presence of complementary template **3** (Figure 1). The templated transfer

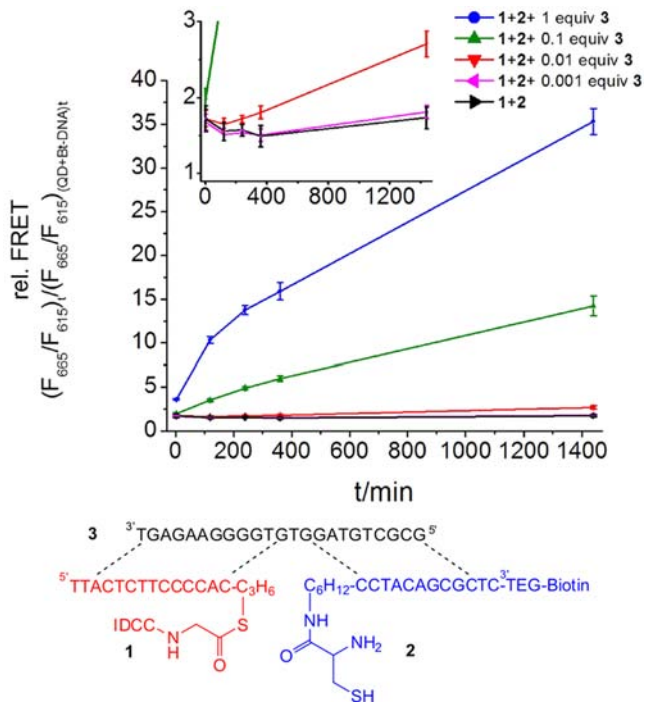

Figure 1. Templated acyl transfer reaction between **1** and **2** (TEG = PO_2^-−CH_2CHOH−$CH_2(OCH_2CH_2)_4CH_2$−NH−) at various loads of template **3**. Conditions: 5 nM QD, 100 nM **1**, 200 nM **2**, 100 mM NaCl, 10 mM NaH_2PO_4, 2 mM TCEP, pH 7.4, 30 °C, Ex: 355 nm; Em: 615 (QD) and 665 nm (IDCC).

reactions provided for marked and time dependent increases of the FRET signal. A survey of different probe concentrations revealed that the background from off-template reactions was negligible when the reactions were performed at 100 nM donor **1** and 200 nM acceptor **2** (see also Figure S10, ESI). After 24 h reaction in the absence of template, the FRET signal showed an insignificant 1.01-fold increase, indicating the absence of background reactions. By contrast, a 35-fold increase in FRET was measured when the reaction was performed on stoichiometric template. Such strong increases in FRET signals are only rarely obtained and probably are a hallmark of the reaction design (*vide infra*). A useful 10-fold signal enhancement was obtained after 120 min reaction time. Reactions on substoichiometric template still provided robust FRET signals. The presence of 0.01 equiv template induced a 2.7-fold increase in FRET, which could be detected well above the background. The fact that 10% of the maximum signal was obtained at 1% template load points to the role of signal amplification which probably is the result of turnover in template.

A multitude of nucleic acid detection methods rely upon FRET and adjacent annealing of two fluorescence-labeled hybridization probes.[9] However, the FRET enhancement observed after hybridization usually is much smaller than 35-fold. We attribute the high FRET efficiency to the reaction design. The dye transfer that follows hybridization brings the dye closer to the QD. This effect was examined in control experiments which involved two sets of probe combinations (Figure 2). Conjugate **7** lacked the cysteinyl residue required

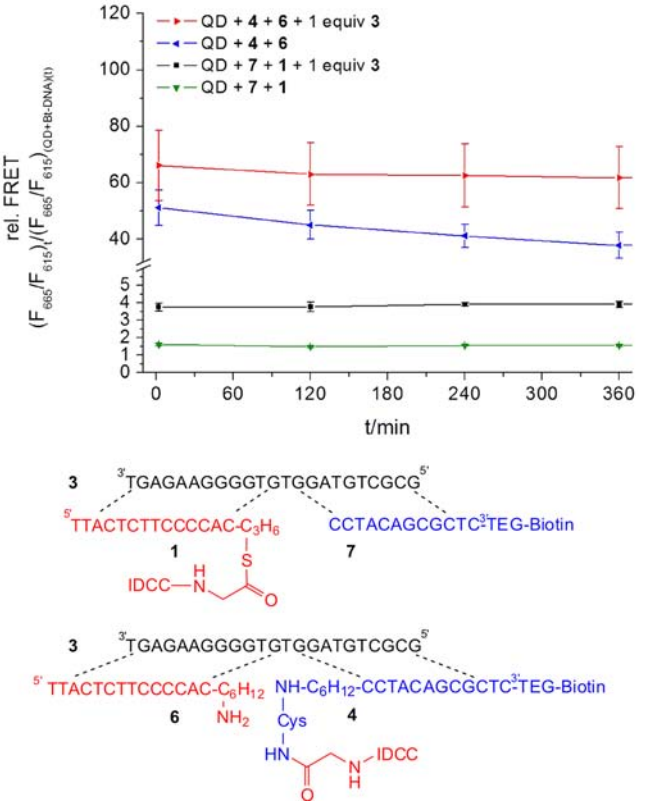

Figure 2. Relative FRET provided by nonreactive combinations of probes **1** + **7** + **3** or by the transfer product **4** in the DNA duplex (**4** + **6** + **3**) (TEG = PO_2^-−CH_2CHOH−$CH_2(OCH_2CH_2)_4CH_2$−NH−). Conditions: (a) 5 nM QD, 200 nM **7**, 100 nM **1**; (b) 5 nM QD, 100 nM **4**, 100 nM **6** in 100 mM NaCl, 10 mM NaH_2PO_4, 2 mM TCEP, pH 7.4, 30 °C, Ex: 355 nm; Em: 615 (QD) and 665 nm (IDCC).

for transfer. The complex comprising this "non-reactive" acceptor mimic (**7**), the thioester-linked IDCC−oligonucleotide conjugate **1**, and the template **3** emulated the reaction phase at the hybridization step before transfer had occurred. The addition of this complex to the QD resulted in a relatively modest 3.8-fold increase of the FRET signal. The situation after dye transfer was reenacted when product-like probes **4** and **6** were incubated with the QD in the presence or absence of template. The 40- to 60-fold increase in FRET provides unambiguous evidence for the increase in FRET efficiency

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conferred by the transfer reaction. We concluded that the FRET signaling shown in Figure 1 is, in fact, a specific monitor of product formation.

The DNA-analogous peptide nucleic acids (PNA) are known to bind complementary DNA and RNA strands with improved affinity.[44,45] PNA is noncharged and, therefore, suited to assess the role of charge in transfer reactions on the crowded surface of QDs. We envisioned that the use of PNA might lead to higher reaction rates based on our previous findings of rapid DNA-templated transfer reaction employing PNA probes.[13] The reactive PNA conjugates **8** and **9** were designed to provide similar affinities for the template as the DNA-based reaction probes **1** and **2**. The transfer of IDCC from **8** to **9** yielded a relative FRET signal of 10 in the presence of 1 equiv template DNA after 24 h (Figure 3). This signal increase is considerably

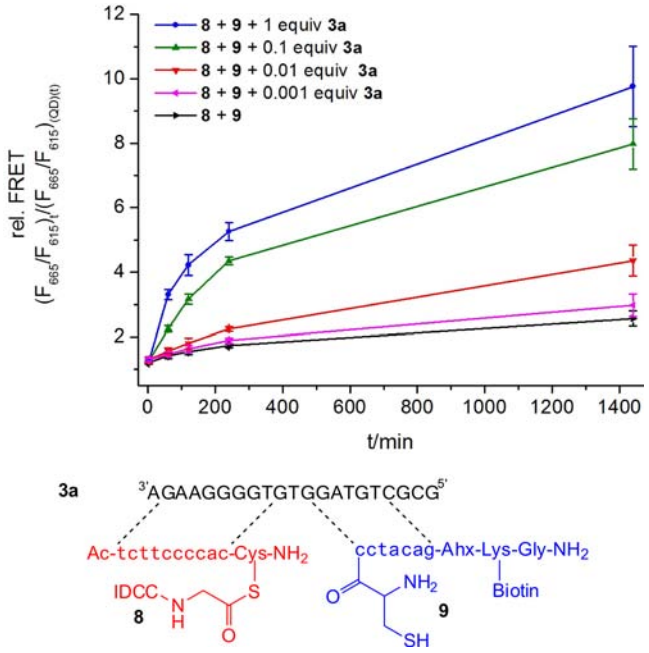

Figure 3. Templated acyl transfer reaction between PNA conjugates at various loads of the template **3a**. Conditions: 5 nM QD, 100 nM **8**, 200 nM **9**, 100 mM NaCl, 10 mM NaH_2PO_4, 2 mM TCEP, Roche blocking reagent (0.1 mg/mL), pH 7.4, 30 °C, Ex: 355 nm; Em: 615 (QD) and 665 nm (IDCC).

smaller than that obtained with reactions of DNA conjugates (35-fold). As a result, the signaling rate provided by PNA probes **8** and **9** was 37% smaller than signaling by DNA-based probes **1** and **2** (see Figure S11, ESI). One possible explanation could be the intermolecular aggregation of the hydrophobic PNA probes and dyes on the surface of the QDs which may be aided by molecular crowding. Such an aggregation may affect FRET signaling by inducing self-quenching.

Next, we evaluated the sequence specificity of the transfer reaction. The assessment of two alternative nucleic acid scaffolds showed that DNA-based probes better suited the environment provided by the quantum dot than PNA-based reactivity probes. Consequently, the specificity test was performed by exploring the reactivity of **1** and **2** on different single base mismatched DNA templates. The mismatch sites were placed either opposite the donating probe **1** or opposite the acceptor probe **2**. At 30 °C the mismatched templates gave 20- to 27-fold fluorescence signal increase indicating only modest sequence specificity for the perfectly matched template (Figure S12, ESI). Raising the reaction temperature near to or above T_M (see Figure S12, ESI) of the probe−template complexes led to a notable increase in sequence specificity (Figure 4). The reaction on the matched template proceeded

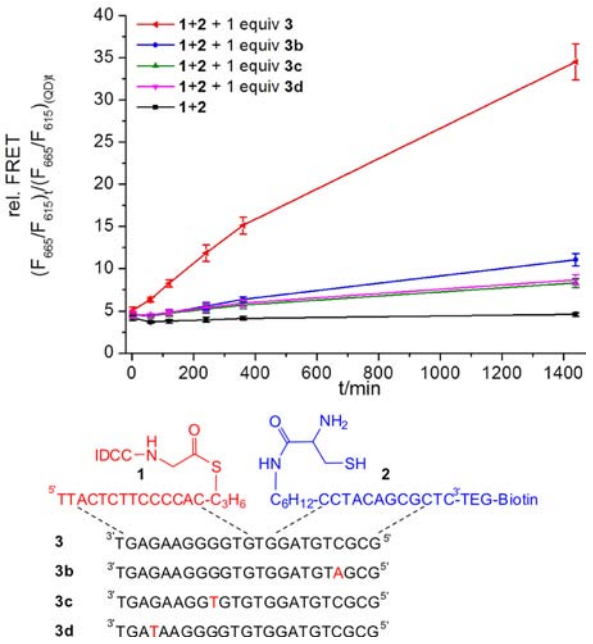

Figure 4. Sequence specificity of the DNA-templated acyl transfer reaction between **1** + **2** (TEG = $PO_2^--CH_2CHOH-CH_2(OCH_2CH_2)_4CH_2-NH-$) in the presence of matched target **3** or the mismatched targets (**3b**−**d**) at 37 °C. Conditions: 5 nM QD, 100 nM **1**, 200 nM **2**, 100 mM NaCl, 10 mM NaH_2PO_4, 2 mM TCEP, pH 7.4, 37 °C, Ex: 355 nm; Em: 615 (QD) and 665 nm (IDCC).

significantly faster than the reaction on the single mismatched templates. The lowest reactivity was obtained on templates **3c** and **3d** containing the most destabilizing mutations. We concluded that the criteria for the design of sequence specific transfer reactions, i.e., the selection of probes that provide the highest hybridization specificity, also apply for transfer reactions on the quantum dot surface.

The work described here offers a potentially general approach to monitor a DNA-templated transfer reaction based on FRET-signals. The nucleic acid-triggered transfer reaction brings the dye closer to the quantum dot than hybridization of reactive probes before reaction. This provides an efficient means to distinguish increases caused by hybridization from increases caused by the reaction. The 35-fold gain in the FRET signal induced by the reaction on 1 equiv target compares favorably to FRET enhancements furnished by other reaction systems (9−12-fold for dye pairs such as FAM and TAMRA[36] or ALEXA 488 and Cy5).[46] Non-FRET based methods such as the *de novo* fluorophore synthesis through a Wittig transformation[33] or the Staudinger reactions[47] provided for 100- and 370-fold increases of fluorescence intensity. These methods require special, noncommercial fluorophores or precursors thereof. By contrast, a QD based FRET-system allows the unrestricted choice of a multitude of fluorophores because the QD emission can readily be tuned by variations of size.[41,48] This may facilitate the design of multiplexing assays. The large Stokes shift is advantageous as the acceptor excitation is negligible at the QDs excitation maximum. Furthermore, the

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very high extinction coefficient and large quantum yield of the quantum dots along with the enrichment of the detected reporter groups enable extremely low detection limits when special instrumentation such as flow cytometry or confocal fluorescence spectroscopy is applied.[42,49] With the DNA-triggered transfer reaction on a QD, we have shown signal amplification by approximately 1 order of magnitude which is the result of turnover in the template. We assume that the combined use of DNA-triggered transfer reactions and advanced instrumentation could possibly lift the state of the art detection methodology to an even more sensitive level.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details on preparation and use of reactive probes in transfer reactions, characterization data, and calculation of the Förster radius. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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