

Reversible Energy-Transfer Switching on a DNA Scaffold

Magnus Bälter,[†] Martin Hammarson,[†] Patricia Remón,[†] Shiming Li,[†] Nittaya Gale,[#] Tom Brown,[‡] and Joakim Andréasson^{*,†}

[†]Department of Chemical and Biological Engineering, Physical Chemistry, Chalmers University of Technology, 412 96 Göteborg, Sweden

[#]ATDBio, School of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, United Kingdom

[‡]Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Oxford, OX1 3TA, United Kingdom

Supporting Information

ABSTRACT: We show that FRET between Pacific Blue (PB) and Alexa488 (A488) covalently attached to a DNA scaffold can be reversibly controlled by photochromic switching of a spiropyran derivative. With the spiropyran in the closed spiro isomeric form, FRET occurs freely between PB and A488. UV-induced isomerization to the open merocyanine form shuts down the FRET process by efficient quenching of the PB excited state. The process is reversed by exposure to visible light, triggering the isomerization to the spiro isomer.

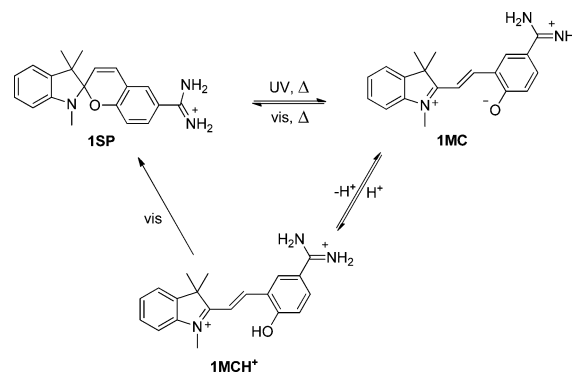
Multichromophore fluorescence resonance energy transfer (FRET) cascades on self-assembled systems using DNA as a structural platform has attracted a great deal of interest in the last decades.^{1–8} The unique recognition properties governed by the base-pairing allow for bottom-up structural addressability on a very precise level, making DNA an excellent scaffold for nanometer-scale devices upon which the chromophores/photoactive units can be spatially arranged with high precision. This approach has allowed for the realization of DNA architectures where the flow of excitation energy can be controlled in one-, two-, and three-dimensions.^{9–11} Typically, the FRET processes are regulated by the addition of molecular functionalities capable of switching energy-transfer processes *on* or *off*. The downside of these pioneering studies is the irreversible nature of the switching process. Once the functionality is introduced, there is no straightforward means to remove it from the DNA construct in order to reset the system to the initial form.

Gaining dynamic reversible control over the FRET processes by external stimuli is essential for any future nanophotonic application that requires repeated *on–off* switching (i.e., all applications apart from the “one-time use”). Photochromic molecules are very promising candidates to be used as switching units, since photoisomerization between the implicated isomers typically occurs reversibly.¹² Furthermore, the all-photonic nature of the light used to trigger the isomerization reactions makes this approach totally waste-free, as opposed to the majority of the systems triggered by chemical inputs, e.g., where DNA is being used as the “fuel”.¹³ In this context, the steadily growing research field of DNA-based logic operations and computing^{14–20} is another area where reversible switching processes will be an undeniable precondition.²¹ Here,

we present a novel approach of photochromic *on–off* switching of FRET between a Pacific Blue (PB) donor chromophore and an Alexa488 (A488) acceptor chromophore covalently attached to a 20-mer DNA double-helix. This study represents the first example where FRET-switching on a DNA scaffold is done reversibly. It should be noted that in the majority of the previously reported studies on FRET-switching on DNA scaffolds, the typical approach has been to switch *on* the energy transfer between a donor- and an acceptor dye by the addition of FRET mediators. This allows for FRET cascades over distances longer than 10 nm. The major objective of the herein presented study was not to achieve wire-like long-range FRET processes but instead to gain reversibility of the switching by shutting *on-and-off* FRET between the donor- and the acceptor dye by all-photonic means.

We base the approach on our previous studies on photocontrolled DNA-binding of spiropyran derivatives, where we found that UV-activation of the spiro isomer SP to the merocyanine forms MC and MCH⁺ is required for binding to occur.^{22–25} Scheme 1 shows the structures and the interconversion pathways for the amidine appended spiropyran derivative (1) used here.²⁴ The closed spiro form 1SP is isomerized to the corresponding open merocyanine isomer 1MC by UV light exposure (254 nm used in this study, isomerization quantum yield = 0.02 ± 0.01). The reverse

Scheme 1. Isomerization Scheme for Spiropyran Derivative 1 Used in This Study



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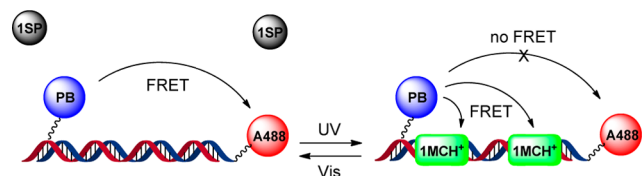
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reaction is triggered by visible light ($\lambda > 450$ nm used in this study, isomerization quantum yield = 0.014 ± 0.007). In the dark, a thermal equilibrium is established with a 20/80 [SP]/[MC] ratio and a time constant of ~ 6 h at RT. The corresponding photostationary distribution using 254 nm light is 30/70. Upon acidification, the phenolate oxygen of the merocyanine isomer is protonated, yielding IMCH^+ with a pK_a value of 5.2. This species is known to bind to DNA through intercalation with a binding constant of $3.4 \times 10^4 \text{ M}^{-1}$, whereas IMC binds ~ 35 times weaker.²⁴ ISP shows no detectable DNA binding. Upon DNA binding, the pK_a value of IMCH^+ increases to 6.8. Hence, at pH 5.4 where these studies were conducted, IMCH^+ is the dominating DNA binder. A comprehensive characterization of the DNA-binding properties of **1** has recently been reported.²⁴ For the absorption spectra of all relevant forms of **1**, see Figure S2.

Due to the dramatic differences in both the absorption spectra and the DNA-binding properties of the implicated isomers, the FRET process between a donor chromophore D and an acceptor chromophore A attached to a DNA scaffold could be affected by light-induced isomerization of **1**. **PB** and **A488** is an established D–A pair for FRET, with an R_0 value of 52 Å (see below). It is obvious from Figure S1 that the absorption of DNA-bound IMCH^+ displays a substantial overlap with the **PB** emission. The R_0 value for this D–A pair was determined to 38 Å. On the contrary, the absorption of ISP does not overlap with the absorption of **PB** nor does it show any specific binding to DNA, which implies that ISP cannot quench the excited state of **PB** by FRET.

PB and **A488** were covalently linked to complementary 20-mer sequences, referred to as **OPB** and **OA488**, respectively (see Scheme 2). Using our experimentally determined values

Scheme 2. Schematic Representation of the Reversible on–off Switching of FRET (top) and Sequences of the Dye-Labeled 20-Mer Oligonucleotides OPB and OA488 (bottom)^a



OPB: 5'-GCCGAT**PB**GTGGTCGGAGAGA

OA488: 5'-TCTCA**A488**CCGACCACAATCGGC

^a**PB** and **A488** are both on T-bases. For the conjugate structures including the linkers, see the SI.

for the molar absorption coefficients and fluorescence quantum yields in aqueous solution for **PB** in **OPB** ($17,700 \text{ M}^{-1} \text{ cm}^{-1}$ at 405 nm and 0.84) and **A488** in **OA488** ($72,500 \text{ M}^{-1} \text{ cm}^{-1}$ at 495 nm and 0.81) hybridized to the respective unlabeled complementary strand, the R_0 value for this D–A pair was determined to be 52 Å, assuming a κ^2 value of 2/3. The relevant normalized spectra are shown in Figure S1. Ideally, uninterrupted FRET will occur between **PB** and **A488** in the **OPB-OA488** double-strand. We hypothesized that the addition of ISP to the sample should not interfere with the FRET process for the reasons described above. UV exposure isomerizes ISP to IMC , and subsequent protonation yields

IMCH^+ that is expected to bind to the **OPB-OA488** double-strand. Given a sufficiently high binding density, FRET from **PB** to DNA-bound IMCH^+ will be much more efficient than the corresponding process between **PB** and **A488**, and the latter process is efficiently shut off. Finally, exposure to visible light will trigger the formation of ISP , which will dissociate from DNA, and switch on the FRET between **PB** and **A488**. This scenario is schematically depicted in Scheme 2, together with the sequences of the **OPB** and the **OA488** oligonucleotides.

Figure 1 shows the emission intensities from **OPB** (blue line) and **OA488** (red line) hybridized with the respective

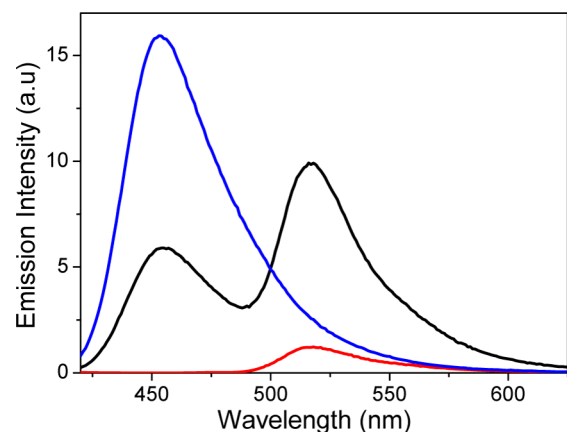


Figure 1. Fluorescence emission intensities (excitation at 410 nm) for **OPB** (blue line) and **OA488** (red line) hybridized with the respective unlabeled complementary strand. Also shown is the emission spectra of the **OPB-OA488** double-strand (black line). [**OPB**] = $1.2 \mu\text{M}$ and [**OA488**] = $1 \mu\text{M}$. The same concentrations were used also for the corresponding unlabeled single-strands.

unlabeled complementary strand upon excitation at 410 nm ([**OPB**] = $1.2 \mu\text{M}$, [**OA488**] = $1 \mu\text{M}$). It is obvious that the 410 nm light directly excites a fraction of the **A488** acceptor dye. The corresponding spectrum of the **OPB-OA488** double-strand is shown in black and clearly shows quenched emission from **PB** and sensitized emission from **A488**. The efficiency of the FRET process is estimated to 78%, judging by the **PB** quenching and taking into account the 20% excess of the **OPB** strand (see Supporting Information, SI, for details).

Given the nine base-pair separation between **PB** and **A488** in the duplex, the distance between the chromophores is around 30.6 Å. With an R_0 value of 52 Å, a FRET efficiency of 0.96 is expected from the Förster formulation. The observed discrepancy is likely a result of a κ^2 value $\neq 2/3$, well in line with the short linkers between the **PB/A488** dyes and the oligonucleotides, hindering free rotation (see SI).

Time-resolved measurements using the single photon counting (SPC) technique clearly shows that the **PB** excited state is quenched in the **OPB-OA488** double-strand. The lifetime of **PB** in the **OPB** strand hybridized with the unlabeled complementary strand is 3.4 ns, whereas the corresponding lifetime in the **OPB-OA488** double-strand is 0.62 ns (see Figures S5 and S6). This implies a FRET efficiency of 82%, which is in excellent agreement with the 78% efficiency estimated from the steady-state measurements described above.

The effect of adding the dissolved form of **1**, ISP ($25 \mu\text{M}$), to the **OPB-OA488** double-strand is shown in Figure 2. It is obvious that the emission intensity of both **PB** and **A488**

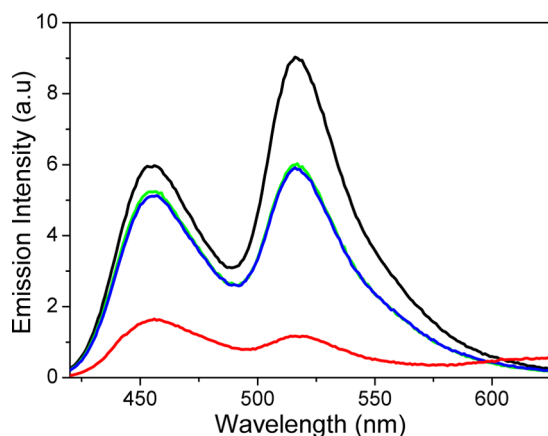


Figure 2. Emission intensities (excitation at 410 nm) for the OPB-A488 double-strand before (black line) and after (green line) the addition of 25 μM ISP. The red line shows the emission intensity after 2 min. 254 nm UV irradiation to isomerize ISP to the DNA-binding forms IMC and IMCH⁺, whereas the blue line is the emission after subsequent visible light irradiation (5 min, $\lambda > 450$ nm) to trigger the reverse isomerization to ISP.

decreases significantly (green line vs black line). We assign this effect to a weak static quenching complex between ISP and PB, as no short-lived component was observed in the time-resolved SPC fluorescence decay (data not shown). We can exclude residual amounts of IMCH⁺, formed either thermally or from incomplete isomerization to ISP in the stock solution, as the origin of the quenching (see Figures S3 and S4 for emission intensity variations with time and with different total concentrations of **1**). The red line shows the emission spectrum after 2 min. 254 nm UV exposure, triggering the isomerization to the DNA-binding form IMCH⁺. The substantially decreased emission intensity from both PB and A488 clearly shows that PB is further quenched and A488 less sensitized. Both these observations are in favor of the notion that DNA-bound IMCH⁺ efficiently interrupts the FRET process between the dyes. The emission observed at wavelengths longer than 650 nm originates from IMC.²⁶ This emission does not overlap with the absorption spectra of PB or A488 and thus does not interfere with the intended function.

SPC measurements were undertaken in order to quantify the FRET efficiency between PB and DNA-bound IMCH⁺. The results show that the PB excited state is too efficiently quenched for the lifetime to be resolved in the measurements, i.e., a lifetime significantly shorter than the instrumental response function of around 40 ps. The R_0 value for PB and DNA-bound IMCH⁺ is 38 Å, which implies that binding of IMCH⁺ within a four base-pair distance from PB will quench the lifetime to around 7 ps or shorter.²⁷ Given the concentrations of IMCH⁺ and DNA binding sites used in this experiment, virtually 100% of the OPB-OA488 double-strands are expected to display such a binding density. The residual emission from PB and A488 observed in the steady-state measurements (Figure 2) is thus explained by the 20% unhybridized OPB single-strands and directly excited A488 in the OPB-OA488 double-strand, respectively.²⁸ Hence, we can conclude that UV-induced isomerization of ISP to the IMCH⁺ form totally shuts off the FRET process between PB and A488 on the DNA scaffold. Furthermore, exposure to visible light (5 min, $\lambda > 450$ nm) triggers the isomerization back to ISP which restores the emission intensities of both PB and A488 to the

initial values (blue line in Figure 2). Thus, the process is indeed fully reversible. The repeated operation of the switching cycle is shown in Figure S7, and it is seen that the system degrades significantly after 10 UV-vis cycles. What ultimately limits the fatigue resistance of the system seems to be photocleavage of the linker between the PB/A488 dyes and the DNA oligonucleotides (see SI for details).

A few things should be commented on. Photochromic FRET switching in covalently linked molecular dyads and triads, etc., has been previously reported.^{29–35} In these studies, the FRET switching relies on the ability of only the UV generated colored isomeric form of the photoswitch to act as a FRET acceptor to the covalently attached donor chromophore, thereby switching the donor fluorescence emission on and off in concert with the isomerization processes. While this switching scheme certainly is interesting *per se*, it comes with several limitations when contrasted to multichromophoric/photochromic constructs on, e.g., DNA scaffolds: (i) With few exceptions,^{36,37} the colored isomeric forms of photoswitches from the most frequently used photochromic families (spiropyran, azobenzenes, diarylethenes, fulgimides, etc.) display very low fluorescence quantum yields. Hence, fluorescence switching is only observed for the donor chromophore, which implies that energy transfer cascades and other higher order processes are very hard to achieve. (ii) Many applications require switch-to-switch communication. With increasing level of sophistication, larger numbers of molecular switching units and chromophores need to be incorporated into the constructs. Relying on covalently linked supramolecules implies that the complexity of the synthetic chemistry rapidly grows. This is why a self-assembly approach is preferable. (iii) Finally, it is worth emphasizing that the FRET efficiency between PB and A488 in this study does not necessarily have to be switched between the two extremes. Instead it can be tuned continuously to intermediate values by varying the UV-vis exposure times to achieve variations in the binding density of IMCH⁺, resulting in a “transistor-like” FRET response. This is not possible for covalently linked dyads, where the FRET process between the donor and the photochromic acceptor can only be switched in a binary fashion.

In summary, we have presented a novel method to control the flow of excitation energy from a donor to an acceptor on a DNA scaffold using all-photonic switching of a DNA-binding photochromic spiropyran derivative. Due to the all-photonic nature of the switching stimuli, the operation leaves no chemical waste behind. The process is totally reversible, but the repeated cycling is ultimately restricted by the limited resistance to photofatigue.

■ ASSOCIATED CONTENT

📄 Supporting Information

Experimental section, oligonucleotide synthesis, labeling, purification, and characterization, spectral properties of the dyes and spiropyran derivative **1**, SPC decays, and repeated operation of the switching cycle. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*a-son@chalmers.se

Notes

The authors declare no competing financial interest.

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- (26) **IMCH⁺** is a photoacid, i.e., it deprotonates in the excited state to emit as **IMC**.
- (27) At very high binding densities, photoinduced electron-transfer reactions between **PB** and **IMCH⁺** cannot be excluded. The thermodynamic driving force of this process (if any) is not known due to the absence of redox data for the involved species.
- (28) The residual emission from **PB** is somewhat lower than what is expected from the 20% excess of single-stranded **OPB**. This is accounted for by the experimental observation that **IMCH⁺** is able to quench **PB** also in the **OPB** single-strand, but to a much less extent

(data not shown). Likewise, the residual emission intensity from **A488** is also lower than what is expected from unquenched emission of the directly excited fraction. This is accounted for by FRET from **A488** to small amounts of DNA-bound **IMC**.

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