Supporting Information

Reversible Energy Transfer Switching on a DNA Scaffold

Magnus Bälter, Martin Hammarson, Patricia Remón, Shiming Li, Nittaya Gale, Tom Brown, and Joakim Andréasson*

Experimental Section

All experiments with DNA were performed in aqueous solution contaning 7.5 mM of NaCl buffered with 7.5 mM of Na_2HPO_4/NaH_2PO_4 buffer set to pH 5.4. The buffers were set by mixing Na_2HPO_4 and NaH_2PO_4 in different ratio and pH was adjusted by droplets of 0.1 M NaOH or 0.1 M HCl. Ground state absorption spectra were recorded on a Cary 5000 Spectrometer spectrophotometer. Corrected fluorescence spectra were recorded on a SPEX Fluorolog-3 spectrofluorimeter.

The photoinduced isomerization reactions of **1** were performed in the UV using a UVP lamp model UVGL-25 (254 nm, 700 μW/cm^2) or in the visible with a 500 W Xe lamp equipped with a hot mirror (A=1.8 at 900 nm) to reduce IR intensity and a 450 nm long-pass filter. Using the visible light only 1/2 of the sample volume was exposed to the light, whereas the whole sample volume was exposed to the 254 nm UV light. The samples were continously stirred during all irradiation processes. Prior to the irradiation, the samples were argon-bubbled for 10 minutes to reduce the oxygen concentration.

Sample preparation: 2 μM of the Alexa488 labeled 20-mer **OA488** (TCTCYCCGACCACAATCGGC; Y = Aminoethoxy dT labeled with Alexa488, see below) was mixed with a 20% excess of the corresponding Pacific Blue labeled 20-mer **OPB** (GCCGATXGTGGTCGGAGAGA; X = Propagylamine dT labeled with Pacific Blue, see below) in the preparation of the **OPB-OA488** double-strand. This sample was diluted to half the concentration before the spectroscopic measurements. The singly labeled double-strands were also prepared following this procedure, including the 20% excess of the (unlabeled) **OPB** strand. Annealing was performed by heating the samples to 80 °C for 20 minutes and allowing them to cool down to room temperature over 3 hours.

For fluorescence quantum yield determinations, singly labeled double-strands were used at 1 μM concentration. The reference compound used for determination of the Alexa488 quantum yield was fluorescein in 0.1 M NaOH aqueous solution (Φ_F = 0.89)[1] and for determination of Pacific Blue quantum yield Coumarin 102 in ethanol (Φ_F = 0.76)[2] was used.

Fluorescence lifetimes were measured using a single photon counting (SPC) set up. The excitation pulse (1-2 ps broad) was provided at a repetition rate of 82 MHz by a Tsunami Ti:sapphire laser (Spectra Physics) that was pumped by a Millenia Pro X laser (Spectra Physics). The Tsunami output was set to 820 nm and its repetition rate was subsequently reduced to 4 MHz using a pulse picker (Spectra Physics, Model 3985) before frequency-doubling to yield λ_{exc}= 410 nm as excitation wavelength. The emitted photons were collected at magic angle (54.7°) at 455 nm by a micro-channel plate photomultiplier tube (MCP-PMT R3809U-50; Hamamatsu) with a spectral bandwidth of 10 nm. The signal was digitalized using a multi-channel analyzer with 4096 channels (SPC-300, Edinburgh Analytical Instruments) and to get a good statistics 10 000 counts in the top channel were recorded. The measured fluorescence decays were fitted using the program FluoFit Pro v.4 (PicoQuant GmbH) after deconvolution of the data with the instrument response function (IRF) with FWHM~40 ps.

The electrophoresis was performed at room temperature using 20% poly-acrylamide gels containing 7 M urea to ensure denaturation of the double-stranded DNA. The buffer used was TBE (50 mM boric

acid, 50 mM tris pH 8.2, 1.25 mM EDTA). The gels were post-stained with Sybr-gold and scanned with excitation at 488 nm. The emission was monitored using a 520 nm band-pass filter.

Synthesis

The synthesis of spiropyran **1** has been reported.[3]

Oligonucleotide synthesis, labeling, purification, and characterization

Oligonucleotide synthesis

Standard DNA phosphoramidites, solid supports, and additional reagents were purchased from Link Technologies and Applied Biosystems. Aminolink dT monomers were synthesized in house (2′-aminoethoxy dT, 5-propagylamino dT)[4]. All oligonucleotides were synthesized on an Applied Biosystems 394 automated DNA/ RNA synthesizer using a standard 1.0 μmol phosphoramidite cycle of acid-catalyzed detritylation, coupling, capping, and iodine oxidation. Stepwise coupling efficiencies and overall yields were determined by the automated trityl cation conductivity monitoring facility and in all cases were >98.0%. All β-cyanoethyl phosphoramidite monomers were dissolved in anhydrous acetonitrile to a concentration of 0.1 M immediately prior to use. The coupling time for normal A, G, C, and T monomers was 35 seconds, and for non-standard monomers (2′-aminoethoxy dT, 5-propagylamino dT) coupling time was extended to 560 seconds. Cleavage of oligonucleotides from the solid support and deprotection was achieved by exposure to concentrated aqueous ammonia solution for 60 minutes at room temperature followed by heating in a sealed tube for 5 hours at 55 °C before HPLC purification as explained below.

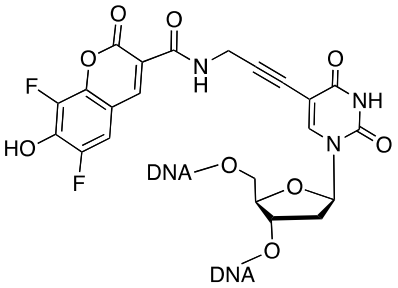
Purification of oligonucleotides (DNA)

The fully deprotected oligonucleotides were purified by reversed-phase HPLC on a Gilson system using a Luna 10 μ C8 100 Å pore Phenomenex 10 x 250 mm column with a gradient of acetonitrile in triethylammonium acetate buffer (0% to 35% buffer B (before labeling and to 40% after labeling) over 20 min, flow rate 4 mL/min), (buffer A: 0.1 M triethylammonium acetate, pH 7.0, buffer B: 0.1 M triethylammonium acetate, pH 7.0, with 50% acetonitrile). Elution was monitored by UV absorption at 295 nm. After HPLC purification, oligonucleotides were desalted using NAP-25 then NAP-10 columns (GE Healthcare).

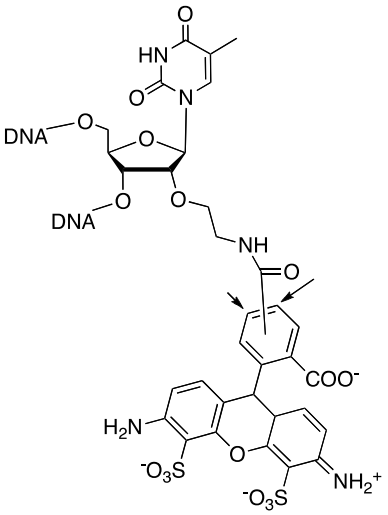
General method for dye labeling and purification of labeled oligonucleotides

The above amino-modified oligonucleotides were labeled *via* amide bond formation using succinimidyl ester of the fluorescent dyes. Both dyes were purchased from Life Technologies Ltd (UK, Cat. No: P-10163 for Pacific™ blue and A-20000 for Alexa Flour® 488)

The dye succinimidyl ester (1 mg) in *N,N* dimethylformamide (50 μL) was added to a solution of purified amino-modified oligonucleotide (150-250 nmol) in buffer (100 μL, 0.5 M Na_2CO_3/$NaHCO_3$, pH 8.75) and the reaction was agitated at 180 rpm, 37 ˚C, for 4 hours. The reaction mixture was desalted with G-25 gel-filtration column (NAP-25, GE Healthcare, UK) to remove free dyes and the oligonucleotide was then purified by RP-HPLC (as described above).



Structure of oligonucleotide containing 5-propagylamino dT labeled with Pacific Blue (**OPB)**.



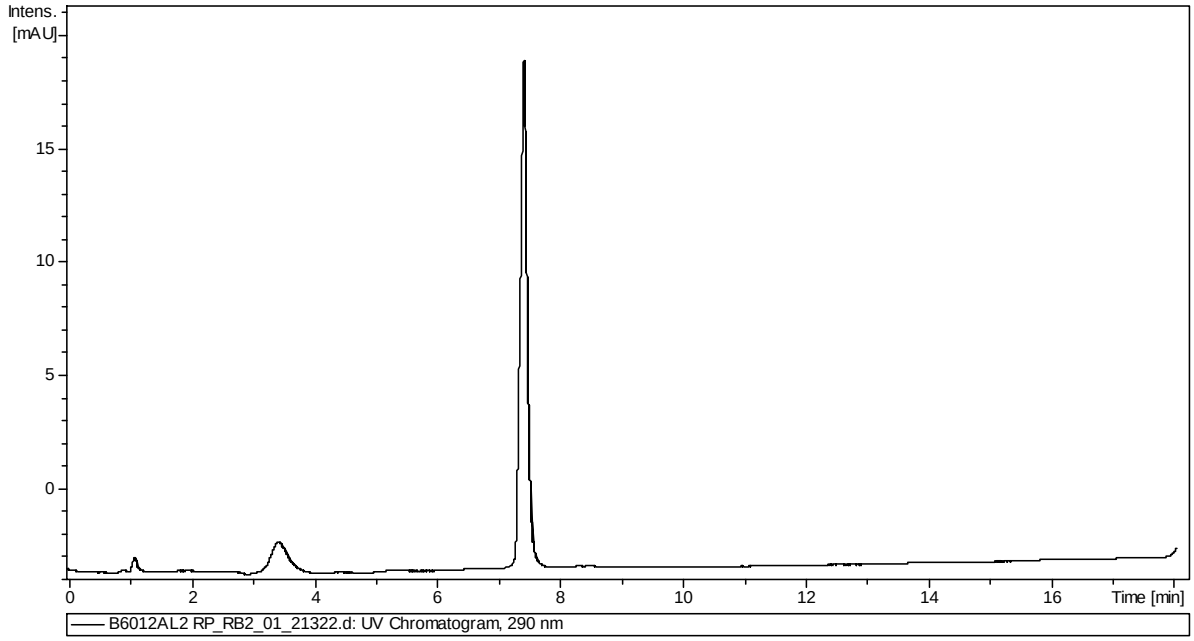
Structure of oligonucleotide containing 2'-aminoethoxy dT labeled with Alexa488 (**OA488)**.

Oligonucleotide characterisation

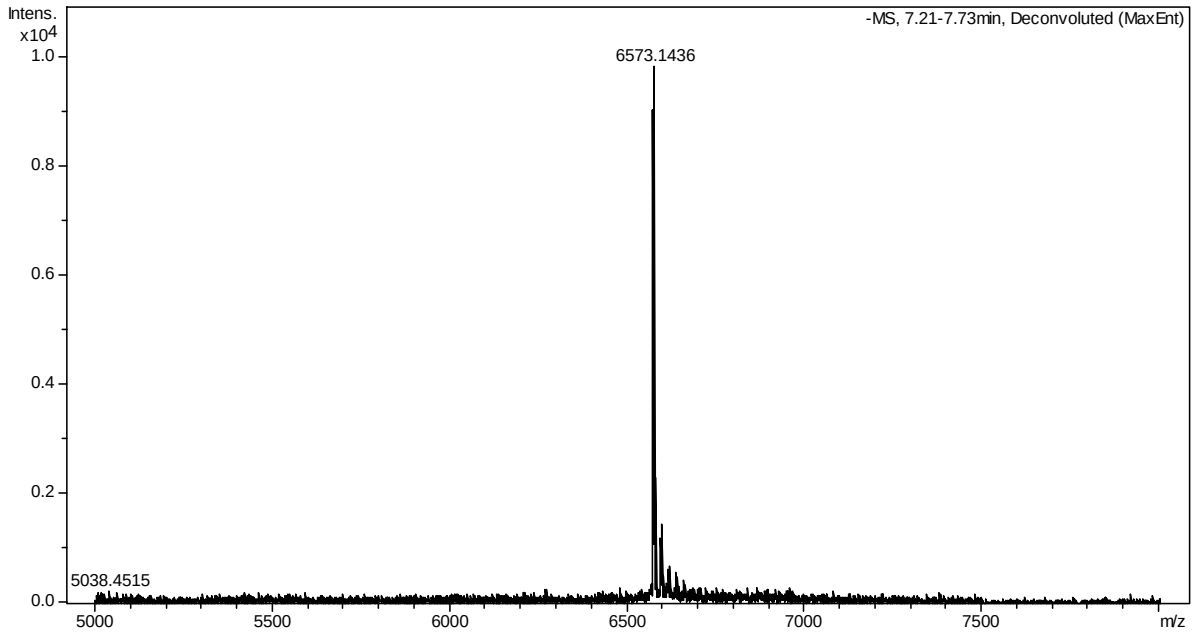
The purity of the oligonucleotides was determined by HPLC-ESI MS analysis by injecting 2 μL of the oligonucleotides (~10-20 μM in water) to a HPLC-mass spectrometry (Bruker Daltronics micrO-TOF mass spectrometer), equiped with a Dionex UltiMate 3000 liquid chromatography system, UV/visible detector, heated column compartment, chilled autosampler (Dionex, Hemel Hempstead, UK) and an Acquity UPLC BEH C18 (1.7 μm, 1 mm × 100 mm column, Waters, Milford, MA, USA). The column temperature was set to 40 ˚C and UV absorbance was measured at 290 nm. A binary phase solvent system was used a gradient of acetronitrile in triethylammonium acetate (TEAA) buffer pH 7 (5-40% buffer B) over 14 minutes, with a flow rate of 0.1 mL min^{-1}. Buffers used: Buffer A: 10 mM TEAA, 100 mM HFIP (hexafluoroisopropanol) in water; Buffer B: 20 mM TEAA in acetronitrile. Negative ion ESI data were acquired over the m/z range 250 – 3500. Data were analysed using Data Analysis™ software v4.0. Raw data were processed/deconvoluted using the DataAnalysis function of the Bruker Daltronics Compass™ 1.3 software package.

OA488 HPLC-MS analysis

HPLC spectra at 290 nm

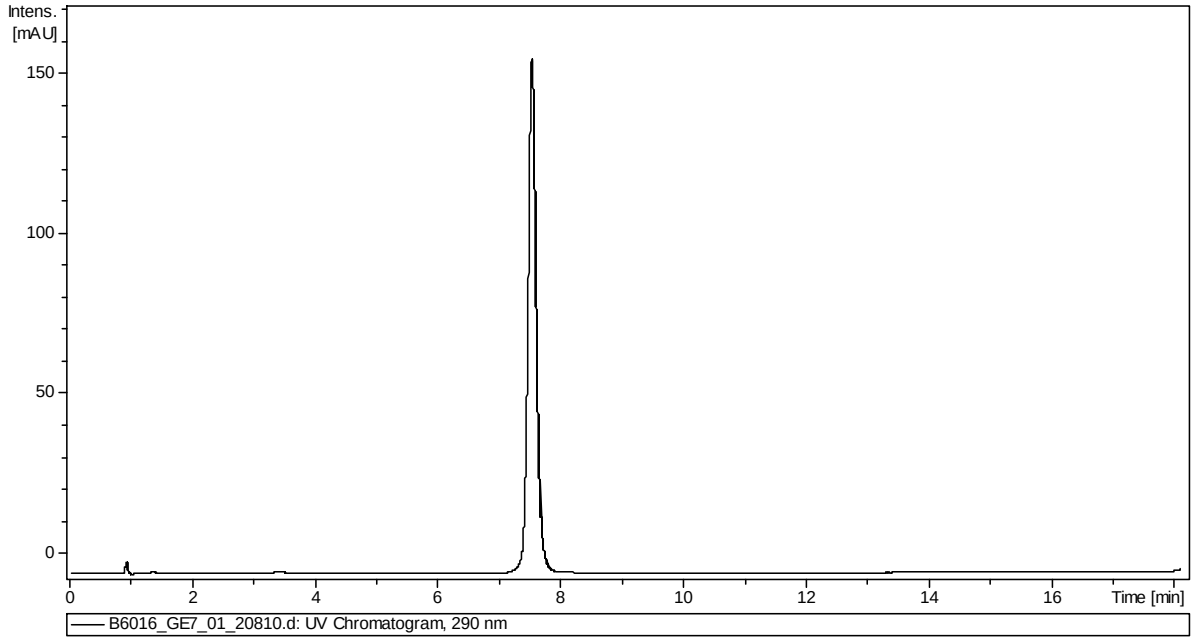


MS: calculated MS = 6573.11 Da, found MS = 6573.1436

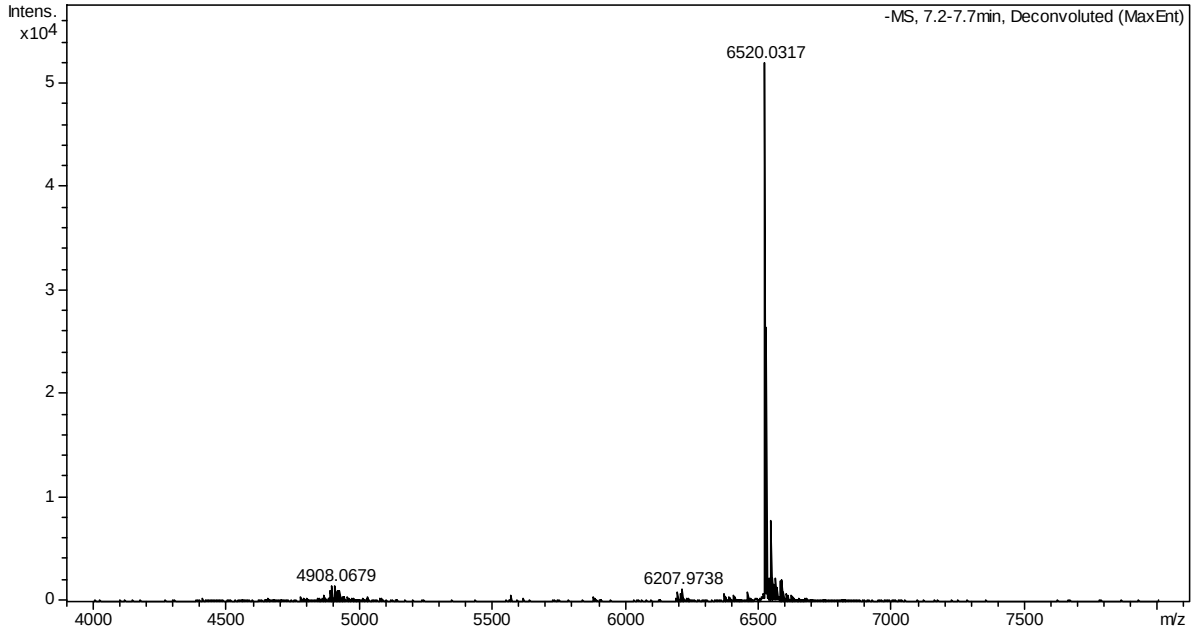


OPB HPLC-MS analysis

HPLC spectra at 290 nm



MS: calculated MS = 6521.14 Da, found MS = 6520.0317



Spectral properties of the dyes and spiropyran derivative 1

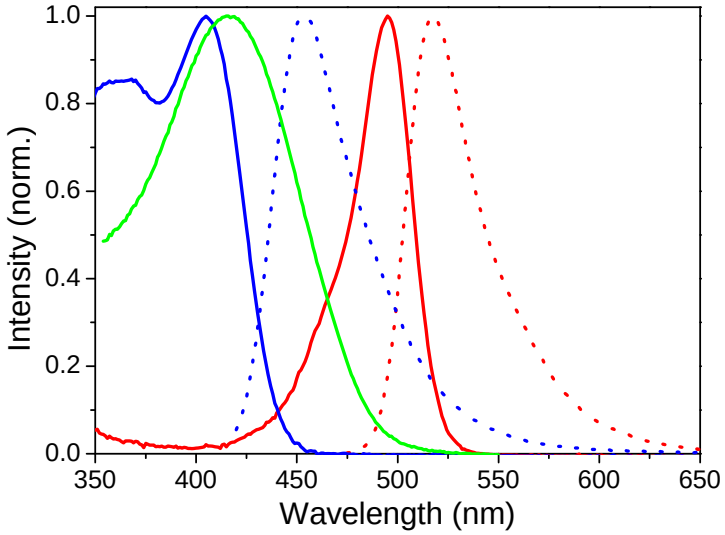


Figure S1. Absorption (solid lines) and emission (dotted lines) of **OPB** (blue) and **OA488** (red) hybridized to the respective unlabeled single-strand, together with the absorption spectrum of DNA-bound **1MCH**$^+$ (green).

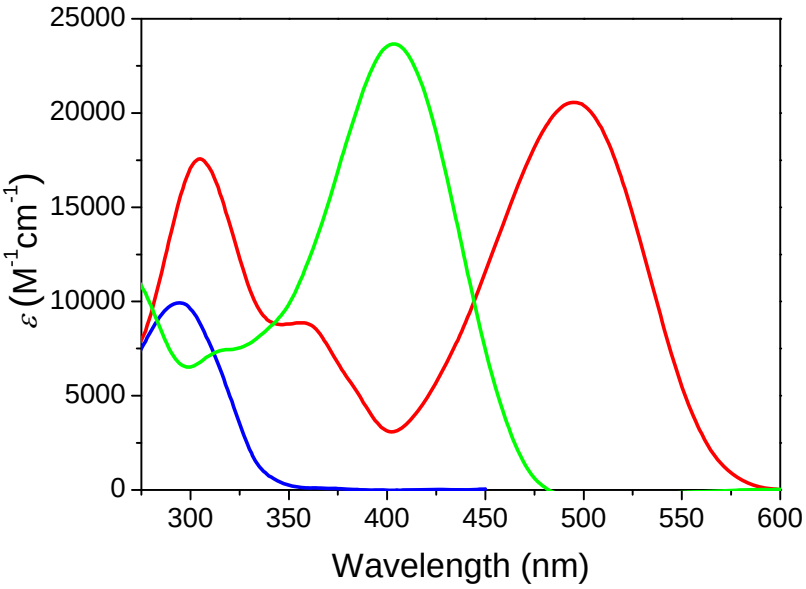


Figure S2. Absorption spectra of **1SP** (blue line), **1MC** (red line), and **1MCH**$^+$ (green line).

Determination of the FRET efficiency from steady-state emission measurements

The FRET efficiency between **PB** and **A488** in the **OPB-OA488** double strand was determined according to the following (see Figure 1):

The emission intensity of **OPB** hybridized with unlabeled complementary strand (blue line) contains a 20% contribution from the excess of **OPB**. Hence, the corrected emission intensity is achieved by subtracting 20% of the experimentally recorded intensity. This yields a value of 0.8×15.91 = 12.73 at 453 nm. The corresponding emission intensity in the **OPB-OA488** double-strand (black line) must also be corrected for the 20% excess **OPB**. This yields a value of 5.90 - 0.2×15.91 = 2.72.

The FRET efficiency thus equals 1-2.72/12.73 = 0.78.

The effect of thermal isomerization 1SP→1MC/1MCH$^+$ on the FRET process

As **1SP** can isomerize thermally to the merocyanine forms **1MC** and **1MCH**$^+$, we recorded the emission intensities of **PB** and **A488** as a function of time after the addition of **1SP** to a sample of the **OPB-OA488** double-strand left in the dark. It is seen that during the first 15 minutes, the emission intensities are reduced by less than 10 % due to the thermal isomerization. After 60 minutes, the corresponding decrease is around 35 %. It is seen that the quenching observed after the addition of **1SP** at t = 0 is comparable to the quenching observed 60 minutes after the addition (red line vs. magenta line). This is why we can exclude that the initial quenching induced after the addition of **1SP** originates from small amounts of thermally formed **1MCH**$^+$.

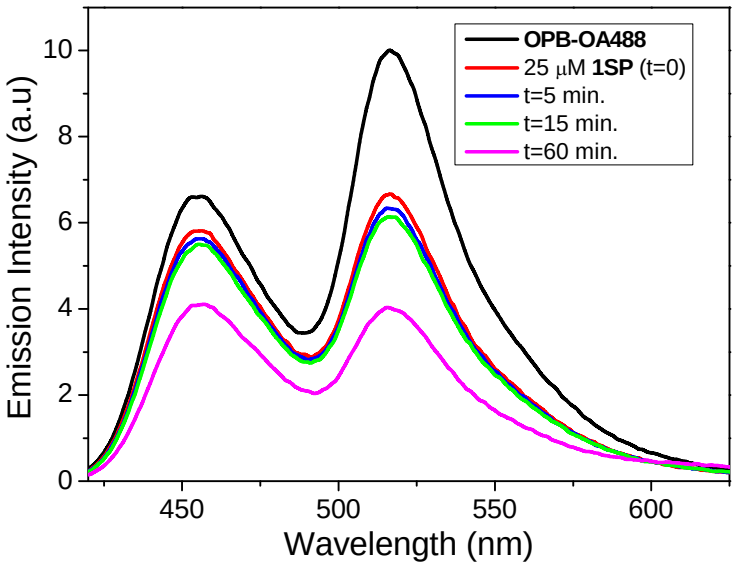


Figure S3. Emission intensities (excitation at 410 nm) for the **OPB-A488** double strand before (black line) and after (red line) the addition of 25 μM **1SP**. The blue, green, and magenta lines are the corresponding intensities after 5, 15, and 60 min. in the dark, respectively. [**OPB**] = 1.2 μM, [**OA488**] = 1 μM.

The effect of varying the concentration of 1 on the FRET process

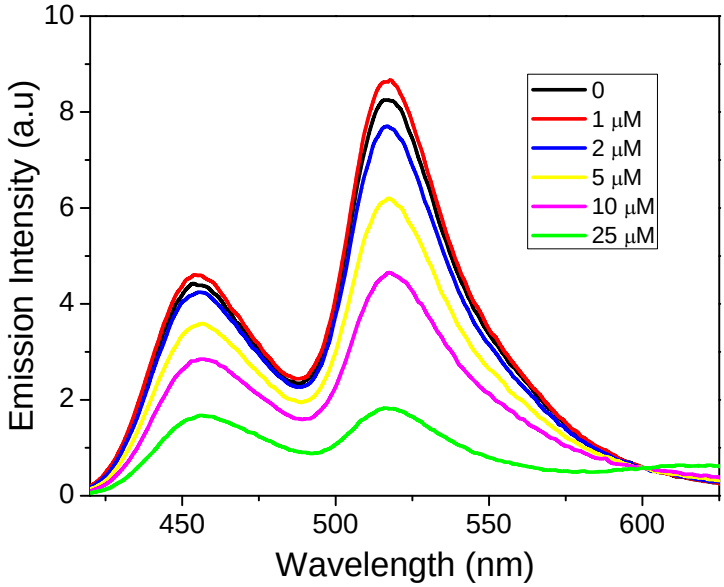


Figure S4. Emission intensities (excitation at 410 nm) for the **OPB-A488** double strand before (black line) and after the addition of 1 μM (red line), 2 μM (blue line), 5 μM (yellow line), 10 μM (magenta line), and 25 μM (green line) **1** at thermal equilibrium. [**OPB**] = 1.2 μM, [**OA488**] = 1 μM.

From Figure S4 it is seen that the quenching that results after the addition of **1SP** (Figures 2 and S3) is comparable to the quenching induced by ca. 5 μM **1MC**/**1MCH**$^+$. Therefore, we can exclude that the initial quenching induced after the addition of **1SP** originates from small amounts of **1MCH**$^+$.

Single photon counting decays

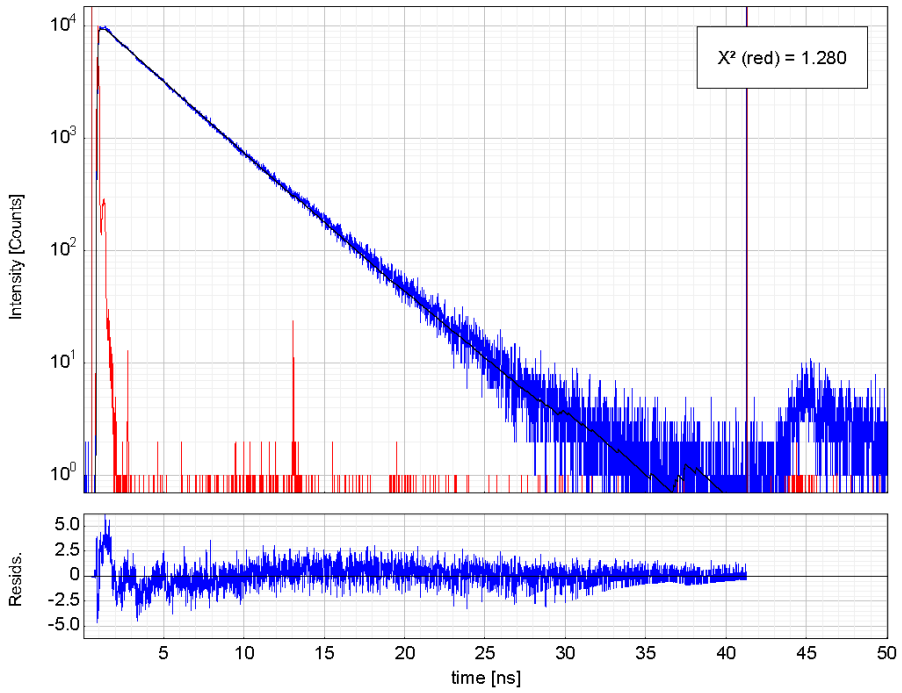


Figure S5. Fluorescence decay of **PB** in the **OPB** single-strand hybridized to its unlabeled complementary strand. $\tau = 3.4$ ns, $\chi^2 = 1.28$.

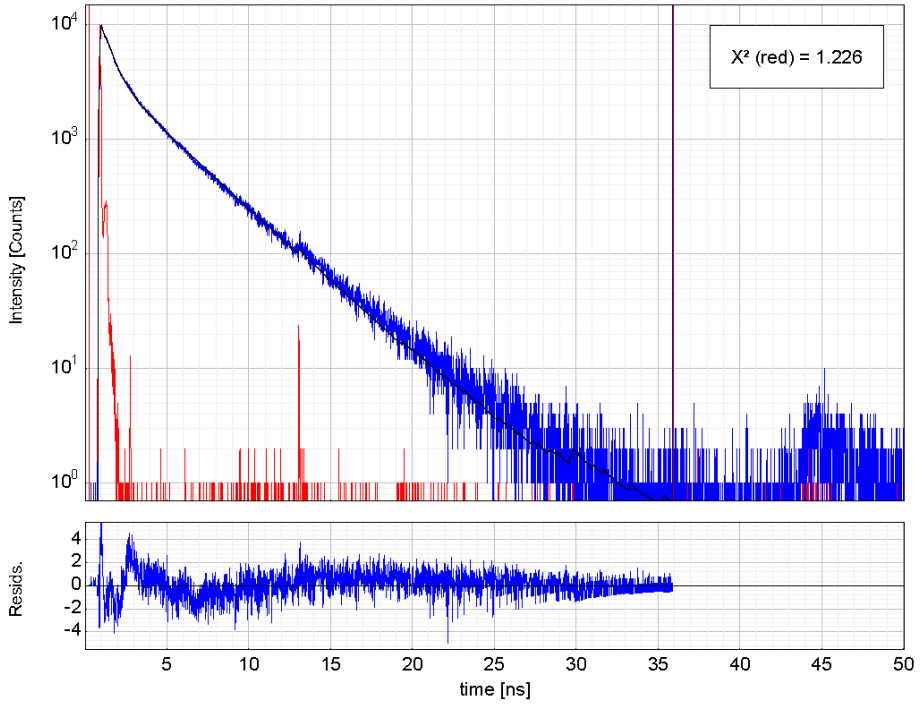


Figure S6. Fluorescence decay of **PB** in the **OPB-A488** double-strand. $\tau_1 = 0.62$ ns (67 %), $\tau_2 = 3.3$ ns (33 %), $\chi^2 = 1.23$. τ_2 originates from **PB** in the excess **OPB** single-strands (see text for details).

Repeated operation of the switching cycle

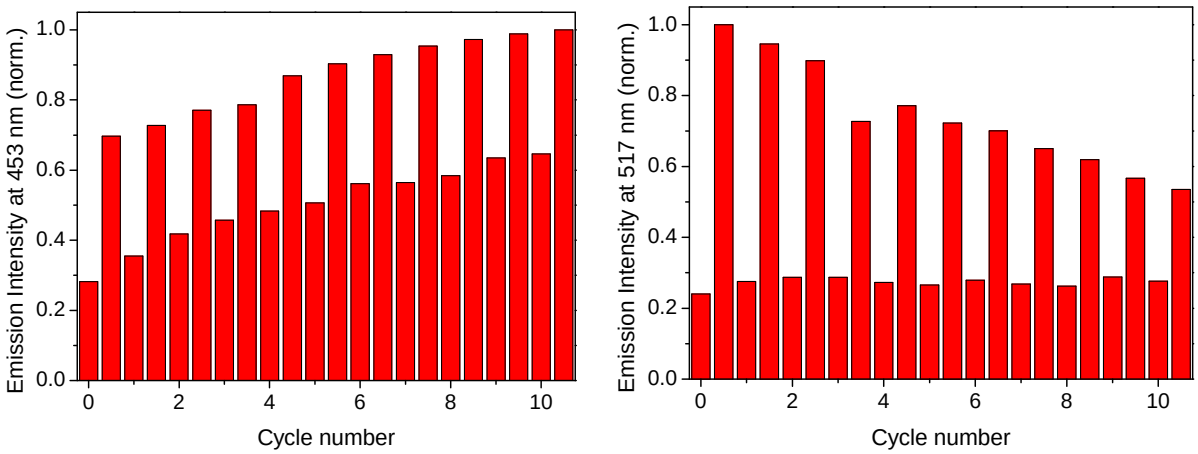


Figure S7. Photocycling of the **OPB-OA488** double-strand ([**OPB**] = 1.2 μM, [**OA488**] = 1 μM) in the presence of **1 (**25 μM). The emission intensities of **PB** (left) and **A488** (right) were monitored at 453 nm and 517 nm, respectively, upon excitation at 410 nm. Each cycle consists of exposure to 254 nm UV (2 min.) and visible light (λ > 450 nm, 5 min.).

In order to investigate the origin of the performance loss during the cycling, the photodegradation of the individual building blocks of the system was examined. The cycle described in Figure S7 (2 min. 254 nm UV and 5 min. visible light) was used to expose in separate experimens:

a) **1** alone (15 μM). See Figure S8.

b) **1** (15 μM) together with salmon sperm DNA (1mM). See Figure S9.

c) **OPB** and **OA488** hybridized to the respective unlabeled complementary strand (1 μM). See Figure S10.

d) Unlabeled 20-mer double-strand (1 μM). See Figure S11.

e) **OPB-OA488** double-strand (1 μM) together with **1** (25 μM). See Figures S11 and S12.

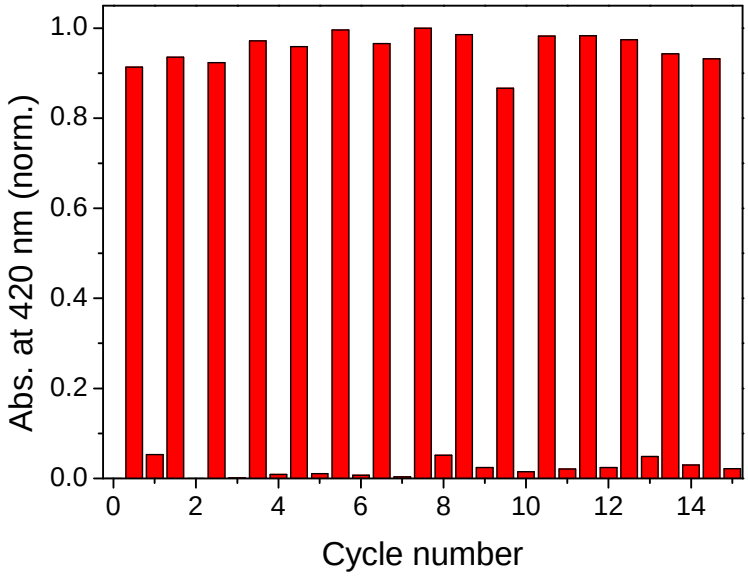


Figure S8. Photocycling of **1** alone (15 μM).

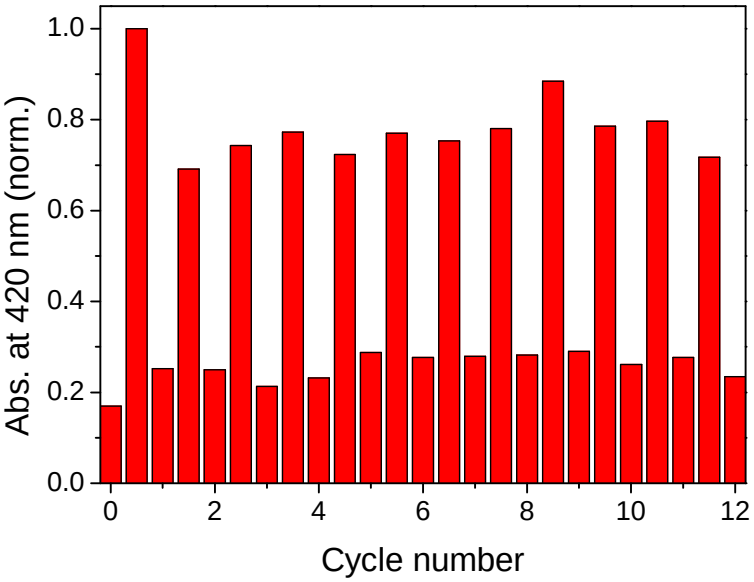


Figure S9. Photocycling of **1** (15 μM) together with salmon sperm DNA (1mM). The smaller dynamic range observed in this experiment compared to **1** alone is a result of a slower isomerization process upon DNA binding.

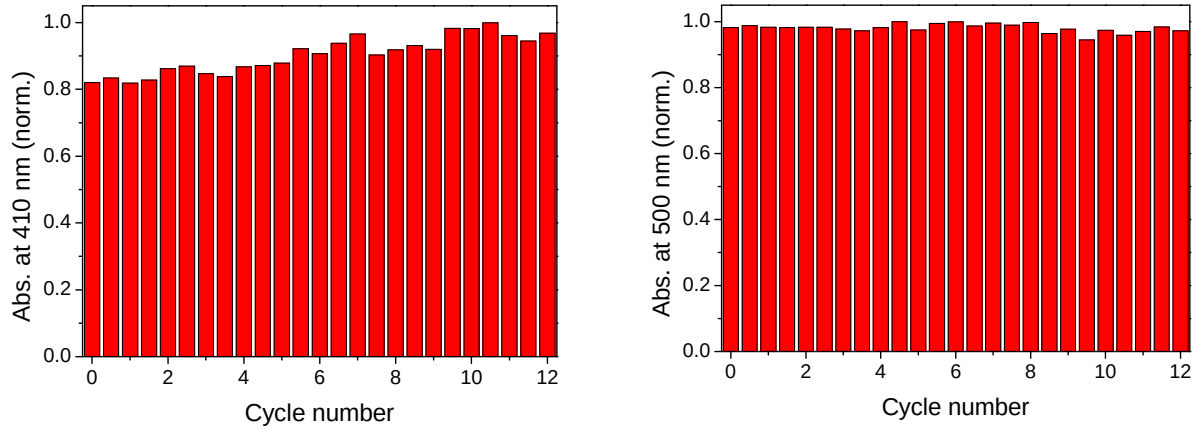


Figure S10. Photocycling of **OPB** (left) and **OA488** (right) hybridized to the respective unlabeled complementary strand. [**OPB**] = 1.2 μM, [**OA488**] = 1 μM.

In a-c (Figures S8-S10 above), no significant degradation is observed. This implies that **1** is not sensitive to photodegradation, irrespective of if it is free in solution (a) or bound to DNA (b). Furthermore, the dyes **BP** and **A488** in **OPB** and **OA488** also withstand more than ten cycles without degradation (c).

Gel electrophoresis was used to assess the photostability of the DNA oligomers in d and e. Figure S11 shows one representative denaturing gel (a total of four separate gels were analysed) containing the following samples:

The leftmost lane (20 & 10) contained unlabeled 20- and 10-mer single-stranded DNA used as reference.

Samples #1 - #3 contained the unlabeled 20-mer double-strand after 1, 10, and 15 cycles, respectively (d above).

Samples #4 - #9 are the **OPB-OA488** double-strand with **1** added (e above), exposed to 0, 1, 3, 5, 10, and 15 cycles, respectively.

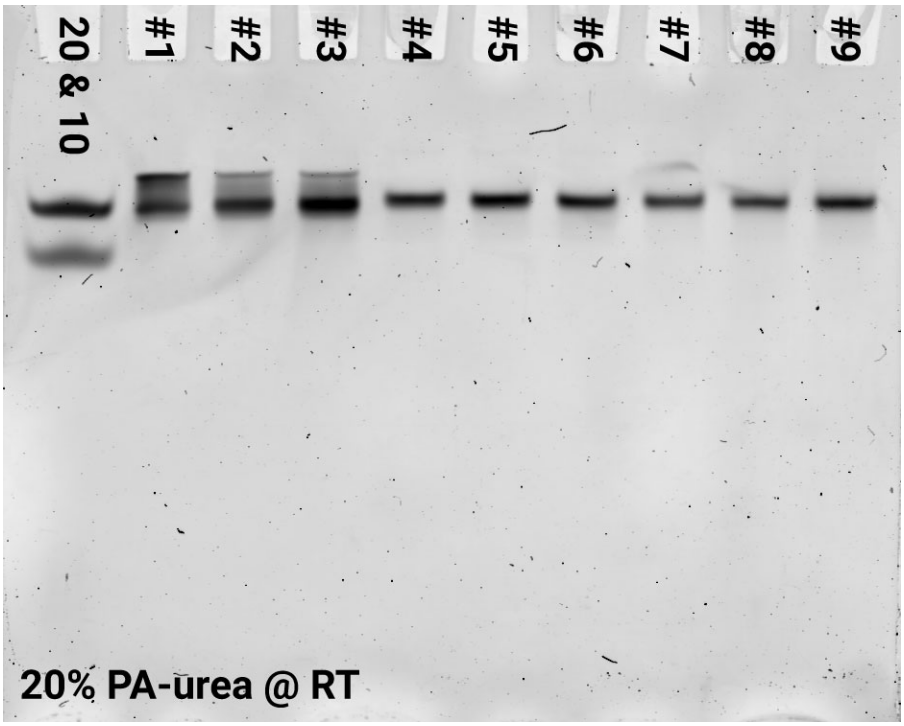


Figure S11. The results from gel electrophoresis. The gels were post-stained with Sybr-gold and scanned with excitation at 488 nm. The emission was monitored using a 520 nm band-pass filter. Samples #4 - #9 are the **OPB-OA488** double-strand with **1** added, exposed to 0, 1, 3, 5, 10, and 15 cycles, respectively. No significant fragmentation of the DNA is observed.

Samples #1 - #3 display an unexpected behaviour. All gels show that these samples produce two bands, one that migrates at the same rate as the reference 20-mer, and a slower band. The samples also exhibit a trend with increasing exposure time, where longer exposure produces more of the expected 20-mer. If degradation would have occurred, we would expect DNA migrating faster than the reference 20-mer, which is not observed. This observation, however, is unrelated to the results from the relevant **OPB-OA488** samples (#4 - #9) described below.

Samples #4 - #9 all migrate at the same rate as the 20-mer reference, and the band profiles do not suggest any degradation to smaller fragments. This shows that no photodecomposition (fragmentation) of the DNA strands occurs, even after 15 cycles. The mean integrated emission intensities from four gels of samples #4 - #9 are shown in Figure S12. The error bars show the ±1 SD of the four different measurements. The results also support the notion that no significant fragmentation occurs, as the integrated emission intensities do not change with the number of cycles.

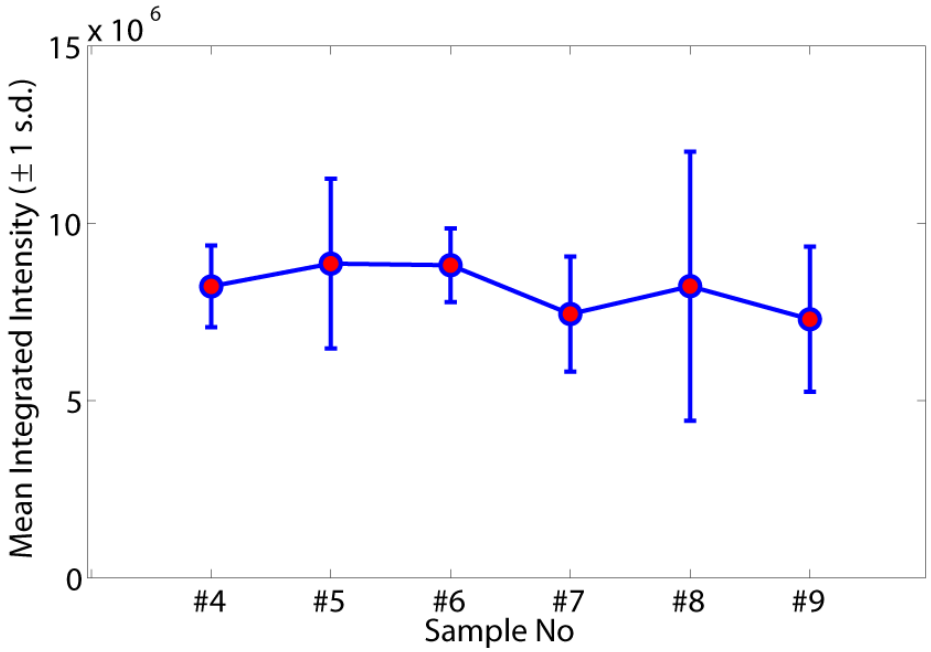


Figure S12. Mean integrated emission intensities from four gels of samples #4 - #9 (the **OPB-OA488** double-strand with **1** added, exposed to 0, 1, 3, 5, 10, and 15 cycles, respectively). The error bars show the ±1 SD of the four different measurements.

We interpret the data from Figures S7 – S12 as follows: As **PB**, **A488**, and **1** are all stable toward photodegradation and no fragmentation of the DNA occurs, the variations in the emission intensities with cycle numbers seen in Figure S7 are likely to originate from photocleavage of a linker between the **PB**/**A488** dyes and the oligonucleotides. The amide bond in the linkers (see structures on page S3) is conjugated to the dye chromophores which absorb quite strongly at 254nm. The energy at this wavelength could be high enough to cause this bond to cleave.

This seems particularly likely for the **PB** donor chromophore, judging from the emission intensity variations in Figure S7. From the high-intensity values (FRET occurs freely between **PB** and **A488** with no interruption from DNA-bound **1MCH**$^+$) it is seen that the emission from **PB** is increasing with the cycle number, signalling less FRET quenching by **A488**. Moreover, the emission intensity of **A488** is decreasing with increasing cycle number, signalling less sensitization by FRET from **PB**. These observations are consistent with the cleavage of the linker to one or both dyes.

From the low-intensity values (FRET between **PB** and **A488** is interrupted by FRET from **PB** to DNA-bound **1MCH**$^+$) it is seen that the **PB** emission intensity is increasing with the cycle number. This observation clearly signals the cleavage of the linker to **PB**. If the linker would have been intact, the emission from **PB** would have stayed low and constant over the cycles, due to quenching induced by DNA-bound **1MCH**$^+$. This is clearly not observed, why we ascribe the performance degradation to the photocleavage of the **PB** linker, alternatively in combination with cleavage of also the **A488** linker.

References

(1) Würth, C.; Grabolle, M.; Pauli, J.; Spieles, M.; Resch-Genger, U. *Nat. Protoc.* **2013**, *8*, 1535–1550.

(2) Rurack, K.; Spieles, M. *Anal. Chem.* **2011**, *83*, 1232–1242.

(3) Nilsson, J. R.; Carvalho, C. P.; Li, S.; Da Silva, J. P.; Andréasson, J.; Pischel, U. *ChemPhysChem* **2012**, *13*, 3691-3699.

(4) The two monomers were synthesized in house following: (a) Lou, C.; Shelbourne, M.; Fox, K. R.; Brown, T. *Chem. Eur. J.* **2011**, 17, 14851-14856. (b) Davison, A.; McClean, J.; Rao, V. M.; Brown, T. *Biomed. Pept. Proteins Nucleic Acids* **1996**; 1, 1-6 and (c) Bijapur, J.; Keppler, M. D.; Bergqvist, S; Brown, T.; Fox, K. R. *Nucleic Acids Res.* **1999**, 27, 1802-1809.