Supplementary information

A Nucleic Acid Directed, Red Light-Induced Chemical Reaction

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General

Commercially available chemicals of the best quality from Aldrich/Sigma/Fluka (Germany) were obtained and used without purification. The reagents for DNA synthesis were obtained from Glen Research (USA) or Link Technologies (UK). HPLC purified DNAs were purchased from IBA GmbH (Germany). MALDI-TOF mass spectra were recorded on a Bruker BIFLEX III mass spectrometer. The matrix mixture was prepared from 2,4,6-trihydroxyacetophenone (THAP, 0.3 M in acetonitrile) and diammonium citrate (0.1 M in water), 2/1, v/v. Samples for mass spectrometry were prepared by a dried droplet method using 1/2 probe/matrix ratio. Mass accuracy with external calibration was 0.1 % of the peak mass, i.e. + 3.5 at m/z 3500. Preparative and analytical HPLC was performed at 60 °C on a Shimadzu liquid chromatograph equipped with a UV-detector and a Macherey-Nagel Nucleosil C4 250 x 4.6 mm column. UV-visible spectra were measured on a Varian Cary 100 Bio UV-Vis spectrophotometer using 1 cm optical path black wall absorption semimicrocuvettes (Hellma GmbH, Germany) with a sample volume of 0.7 mL. Fluorescence spectra were acquired on a Varian Cary Eclipse fluorescence spectrophotometer using black wall fluorescence semimicrocuvettes (Hellma GmbH) with a sample volume of 0.7 mL.

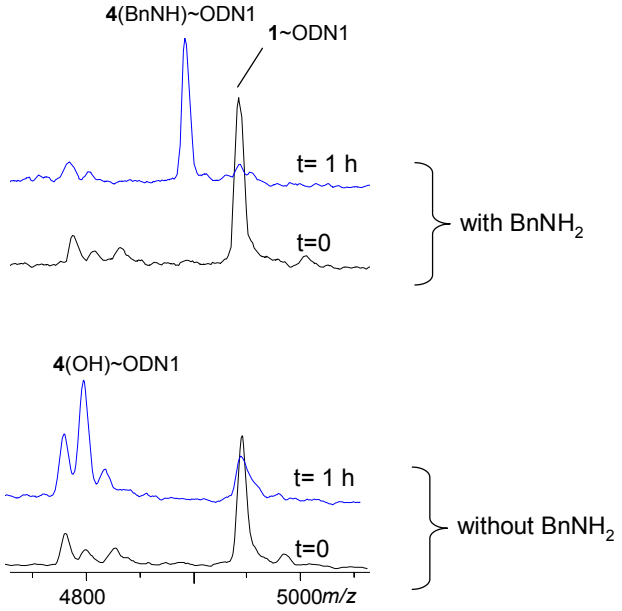


Figure S1. MALDI-TOF spectra of mixtures consisting of **1**~ODN1 (1 μM), ODN6~**P2** (1 eq) and T-1 (1 eq) with (upper two spectra) or without benzyl amine (lower two spectra). These mixtures were exposed to red light for the time shown in the plot. Buffer: NH_4OAc 0.1 M, pH 7. T= 30 °C.

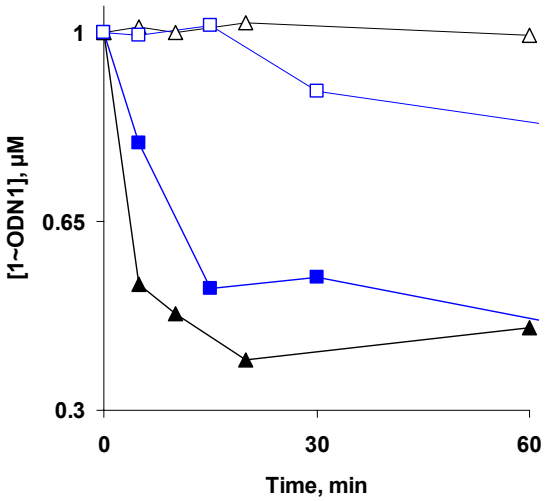


Figure S2. Time-dependent change of concentration of **1**~ODN1 (1 µM) upon its exposure to red light: in the presence of ODN5~**P2** (1 eq) (black open triangles); in the presence of ODN5~**P2** (1 eq) and T-1 (1 eq) (black filled triangles); in the presence of ODN5~**P3** (1 eq) (blue open squares); in the presence of ODN5~**P3** (1 eq) and T-1 (1 eq) (blue filled squares); buffer – NH_4OAc 0.1 M, pH 7, 22 °C.

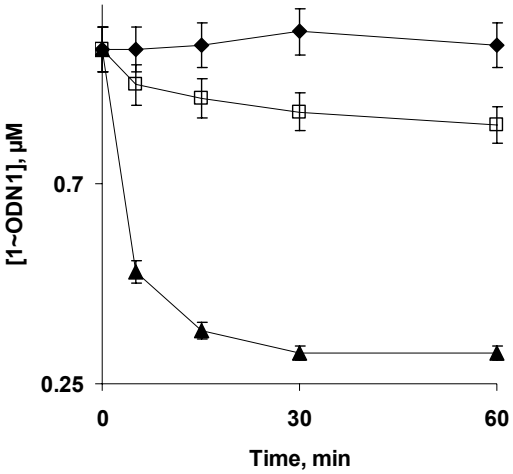


Figure S3. **1**~ODN1 (1 µM) disappearance in the presence of ODN6~**P2** (1 µM) and upon red light exposure in NH_4OAc buffer (0.1 M, pH 7) at 30 °C; filled diamonds – no template was added; filled triangles – in the presence of fully matched template T-1 (1 eq); open squares – in the presence of a template with a single mismatch T-1m (1 eq); sequences of the templates are given in Figure 1.

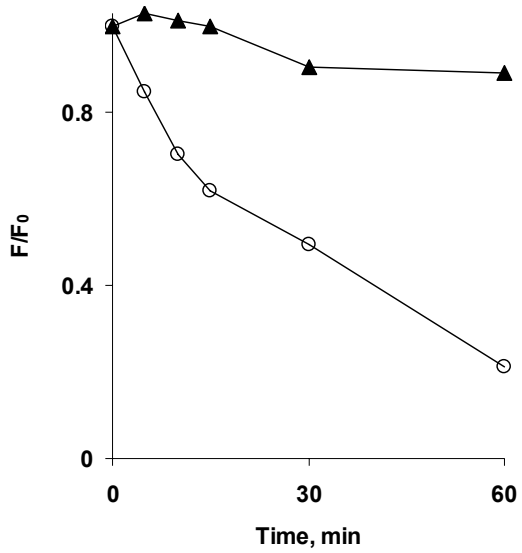


Figure S4. Monitoring bleaching of photosensitizers in conjugates ODN5~**P2** (open circles) and ODN5~**P3** (filled triangles); solutions of these conjugates (1 μM) in NH_4OAc 0.1 M (pH 7) buffer were exposed to red light at 22 °C; the fluorescence intensity was measured at 680 nm (**P2**) and 667 nm (**P3**) at the specified time; it was divided by the corresponding fluorescence intensity at t=0 (F/F_0) and plotted as a function of time

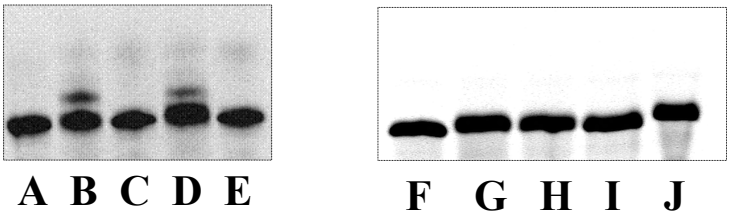
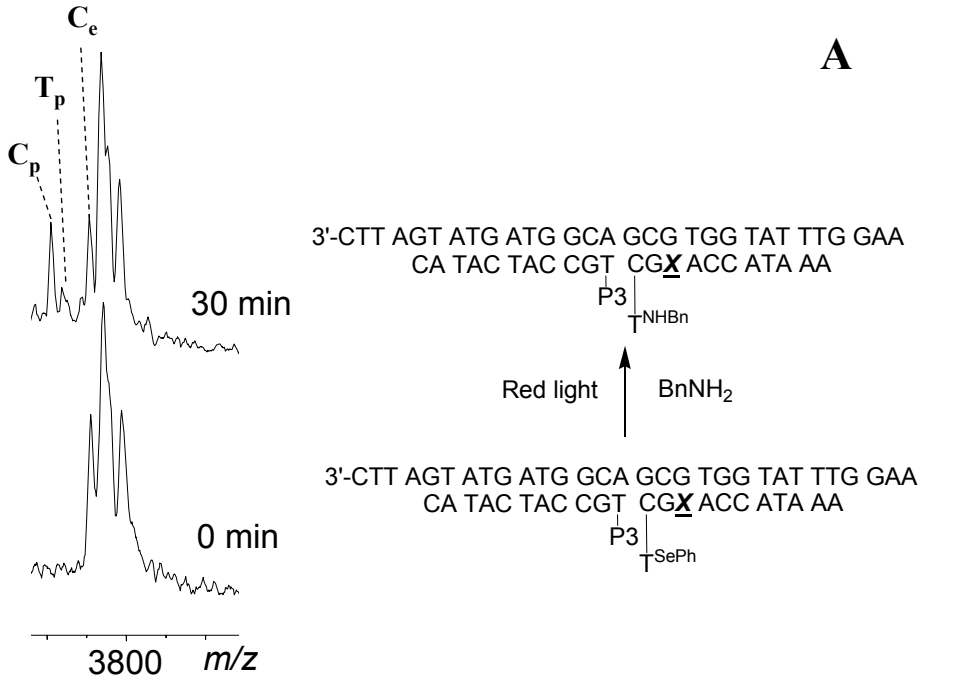
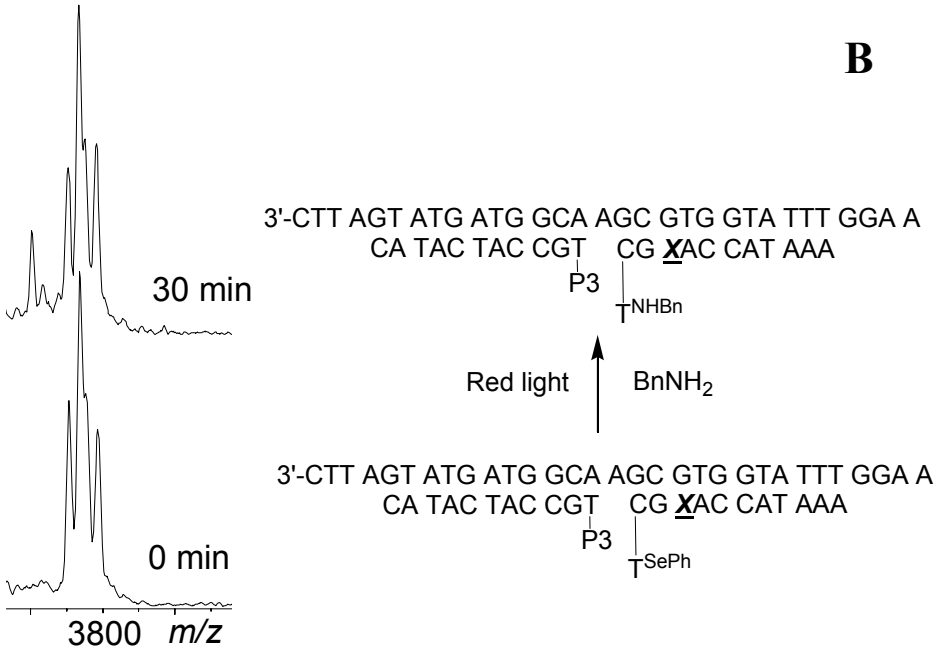
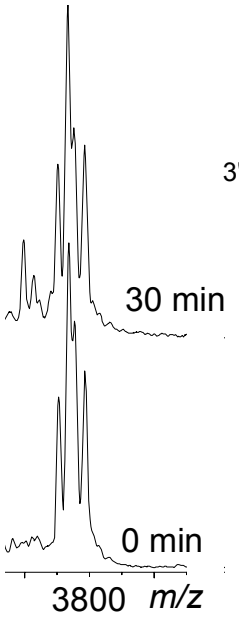


Figure S5. Denaturing gel electrophoresis; Lane A: 5'-TAMRA-labeled T-1 template (10 μM); Lane B: ODN2~**1**~ODN3 (10 μM), 5'-TAMRA-labeled T-1 template (10 μM), eosin (50 μM) exposed to green light for 30 min; Lane C: the same as in B, only kept for 30 min in the dark; Lanes A-C – phosphate, 0.1 M, pH 7 was used as a buffer; Lanes D and E are the same as Lanes B and C correspondingly, except that NH_4OAc, 0.1 M, pH 7 was used in place of the phosphate buffer; Lane F: 5'-TAMRA-labeled T-1 template (10 μM) in the acetate buffer; Lane G: **1**~ODN1 (10 μM), 5'-TAMRA-labeled T-1 template (10 μM), eosin (50 μM) in the acetate buffer exposed to green light for 30 min; Lane H: **1**~ODN1 (10 μM), 5'-TAMRA-labeled T-1 template (10 μM), eosin (50 μM) in the phosphate buffer exposed to green light for 30 min; Lane I: **1**~ODN1 (10 μM), ODN5~**P2** (10 μM), 5'-TAMRA-labeled T-1 template (10 μM) in the acetate buffer exposed to red light for 30 min; Lane J: **1**~ODN1 (10 μM), ODN5~**P2** (10 μM), 5'-TAMRA-labeled T-1 template (10 μM) in the phosphate buffer exposed to red light for 30 min.

Figure S6 (see below). Plots A-E: optimization of the photochemical reaction for sequence specific detection of nucleic acids by MALDI-TOF mass spectrometry: each mixture contains four **1**~ODN4_X4 probes (0.9 μM of each component), ODN6~**P3** (1 μM), $BnNH_2$ (50 mM) and a corresponding template nucleic acid (1 μM, sequences are given on the corresponding plots); buffer NH_4OAc, 100 mM, pH 7; T= 30 °C. Times of exposure of the mixtures to red light are given in the plots. A peak corresponding to a probe complementary to T-1 (**1**~ODN4_C4) is labeled C_e, whereas peaks corresponding to products **4**(BnNH)~ODN4_C4 and **4**(BnNH)~ODN4_T4 are labeled C_p and T_p correspondingly.





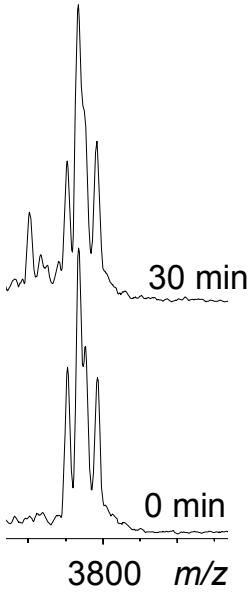


C

3'-CTT AGT ATG ATG GCA TAG CGT GGT ATT TGG AA
CA TAC TAC CGT C G***X*** A CCA TAA A
P3
T^{NHBn}

Red light ↑ $BnNH_2$

3'-CTT AGT ATG ATG GCA TAG CGT GGT ATT TGG AA
CA TAC TAC CGT C G***X*** A CCA TAA A
P3
T^{SePh}



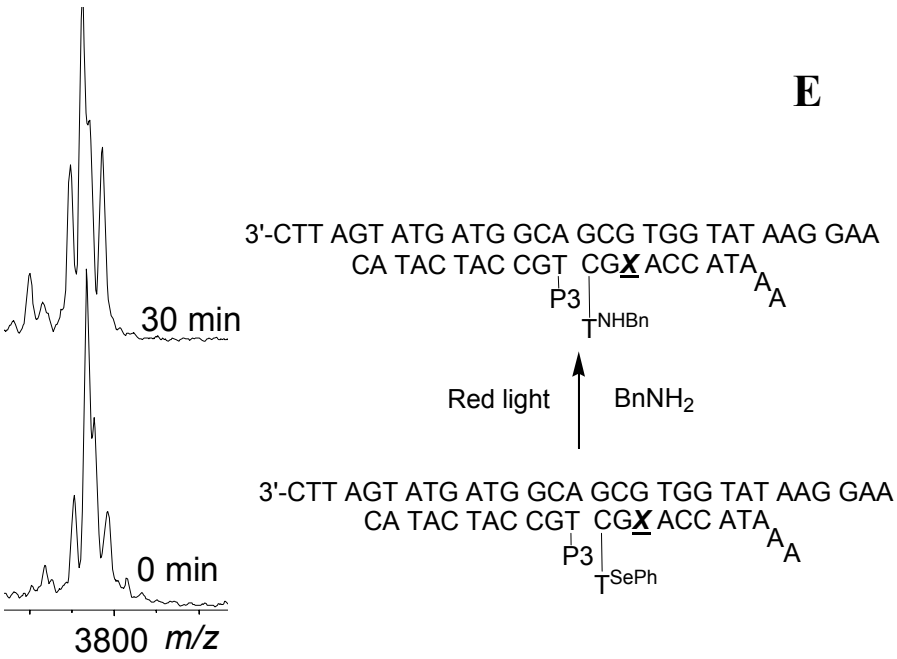
D

3'-CTT AGT ATG ATG GCA GCG TGG TAT TAG GAA
CA TAC TAC CGT CG***X*** ACC ATA A$_A$
P3
T^{NHBn}

Red light ↑ $BnNH_2$

3'-CTT AGT ATG ATG GCA GCG TGG TAT TAG GAA
CA TAC TAC CGT CG***X*** ACC ATA A$_A$
P3
T^{SePh}

E


30 min
0 min
3800 m/z
3'-CTT AGT ATG ATG GCA GCG TGG TAT AAG GAA
CA TAC TAC CGT CGX ACC ATA
A
A
P3
T
NHBn
Red light
BnNH2
3'-CTT AGT ATG ATG GCA GCG TGG TAT AAG GAA
CA TAC TAC CGT CGX ACC ATA
A
A
P3
T
SePh

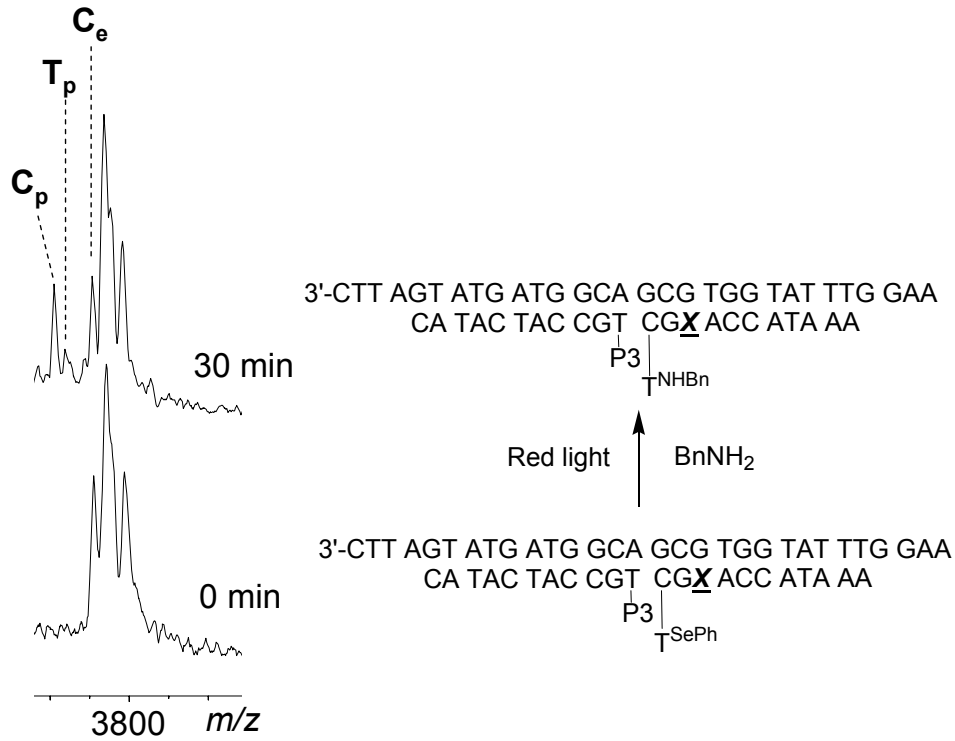


Figure S7. Test of the sensitivity of the photochemical reaction in the detection of nucleic acids: the mixture contains four **1**~ODN4_X4 probes (50 nM of each component, absolute amount 50 fmoles), ODN6~**P3** (50 nM), $BnNH_2$ (50 mM) and T-1 as a template (50 nM); buffer NH_4OAc, 100 mM, pH 7; T= 30 °C. Times of exposure of the mixtures to red light are given in the plot. A peak corresponding to a probe complementary to T-1 (**1**~ODN4_C4) is labelled C_e, whereas peaks corresponding to products **4**(BnNH)~ODN4_C4 and **4**(BnNH)~ODN4_T4 are labelled C_p and T_p correspondingly.