Supplementary information

An Autocatalytic Chromogenic and Fluorogenic Photochemical Reaction Controlled by Nucleic Acids

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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. + 5 at m/z 5000. Preparative and analytical HPLC was performed at 22 °C on a Shimadzu liquid chromatograph equipped with a UV-detector and a Macherey-Nagel Nucleosil C4 250 x 4.6 mm column using the gradient of solution B (CH_3CN) in solution A ((Et_3NH)(OAc), 0.1 M in water): for 5 min at 0 % B, in 30 min to 50 % B, in 10 min to 70 % B. 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. Mercury lamp (150 W) combined with the filter (500-550 nm) was used as a green light source.

Synthesis of 5'-amino group modified oligonucleotides

ODN sequence was synthesized at standard conditions using a DNA synthesizer. 5'-MMT-amino modifier C6-CE phosphoramidite was then attached to this DNA in accordance with manufacturer recommendations (Link Technologies, UK). The MMT group was removed by treatment of the solid support bound DNA with 1 % CF_3CO_2H solution in CH_2Cl_2: 1 mL of this solution per ~20-30 mg of the solid support, ca. 1 μmol of DNA.

*Synthesis of **E**~ODN1*

Eosin-5-isothiocyanate (7.1 mg, 10 μmol) was dissolved in DMF (200 μL) and diisopropylethylamine (3.8 μL, 22 μmol) was added. The resulting mixture was added to 5'-amino group modified ODN1, which was fully protected, except of the terminal primary amino group, and bound to the controlled pore glass (3 mg, 100 nmol of NH_2-groups). The slurry obtained was shaked for 4 h, then filtered, washed with DMF (2 x 1 mL) and CH_3CN (2 x 1 mL) and dried in vacuo (0.01 mbar) for 2 h. The conjugate was cleaved from the solid support and deprotected by the treatment with aqueous ammonia solution (25 %) for 8 h at 22 °C. Then, the CPG was filtered off and the filtrate was evaporated to dryness. Water (150 μL) was added to the crude conjugate and the solution obtained was purified by HPLC. Yield 21.5 %; HPLC (Figure S1) R_t = 23.8 min; MALDI-TOF MS, negative mode, calculated for $C_{171}H_{202}Br_4N_{56}O_{94}P_{15}$ $[M-H]^-$: *m/z* 5362, found 5364 (Figure S2).

*Synthesis of nucleic acids modified with black-hole-quencher-2 (**Q**) at 3'-terminus*

3'-BHQ-2 CPG (Glen Research, USA) were used as a solid support in synthesis of 3'-modified DNAs. DNA strand was synthesized off this support at standard conditions on a DNA synthesizer.

*Synthesis of **E**~ODN4~**Q** (**Q**= black-hole-quancher-2)*

5'~amino modified ODN4 attached to the solid support and fully protected except of the terminal amino group was prepared by using approaches described above for synthesis of 3'-**Q** and 5'-amino modified oligonucleotides. Eosin was attached as described in the protocol for synthesis of **E**~ODN1. Yield 6.5 %; HPLC (Figure S3) R_t = 27.5 min; MALDI-TOF MS, negative mode, calculated for $C_{312}H_{373}Br_4N_{113}O_{171}P_{28}S$ $[M-H]^-$: *m/z* 9660, found 9670 (Figure S4).

HPLC purified conjugate ODN2~**Q** (**Q**= black-hole-quencher-3) and unmodified ODN3, ODN5 and ODN6 were purchased from IBA GmbH, Germany.

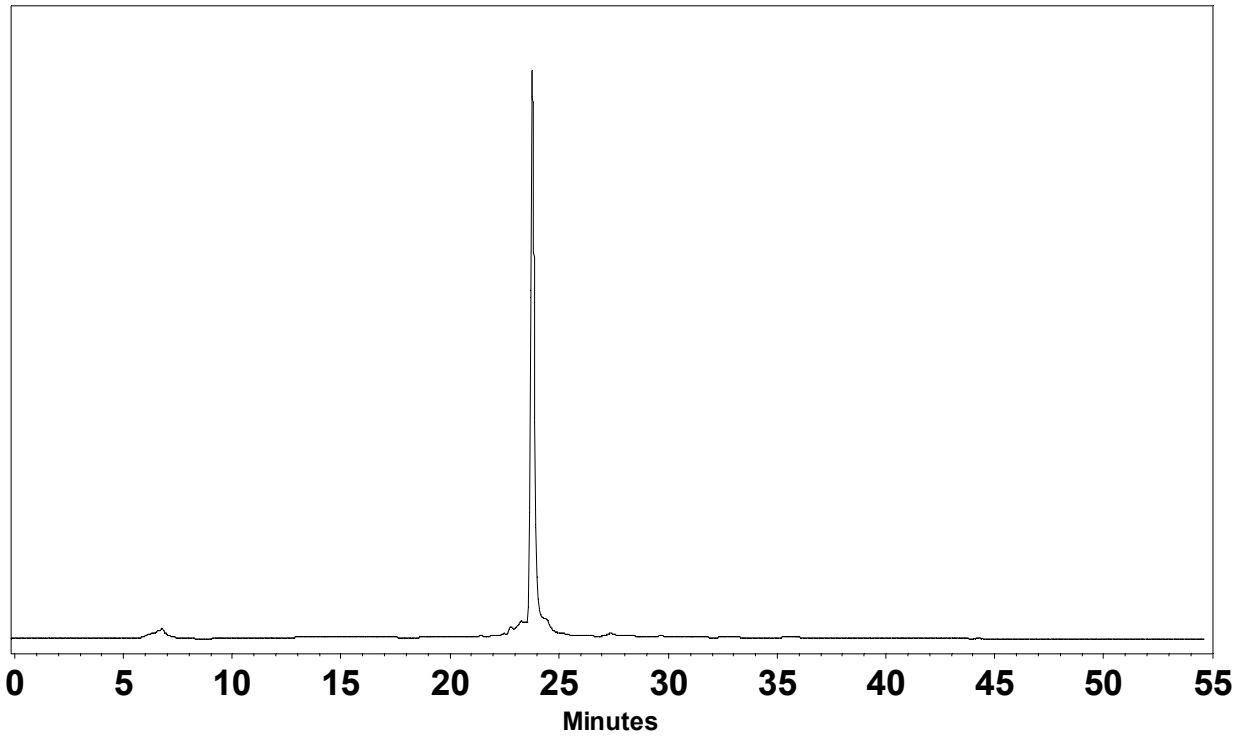

Figure **S1**. HPLC profile of purified **E**~ODN1

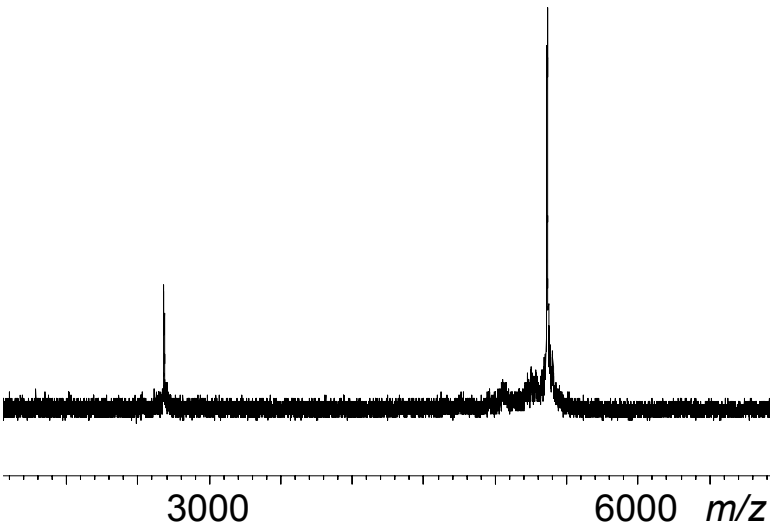

Figure **S2**. MALDI-TOF mass spectrum of purified **E**~ODN1

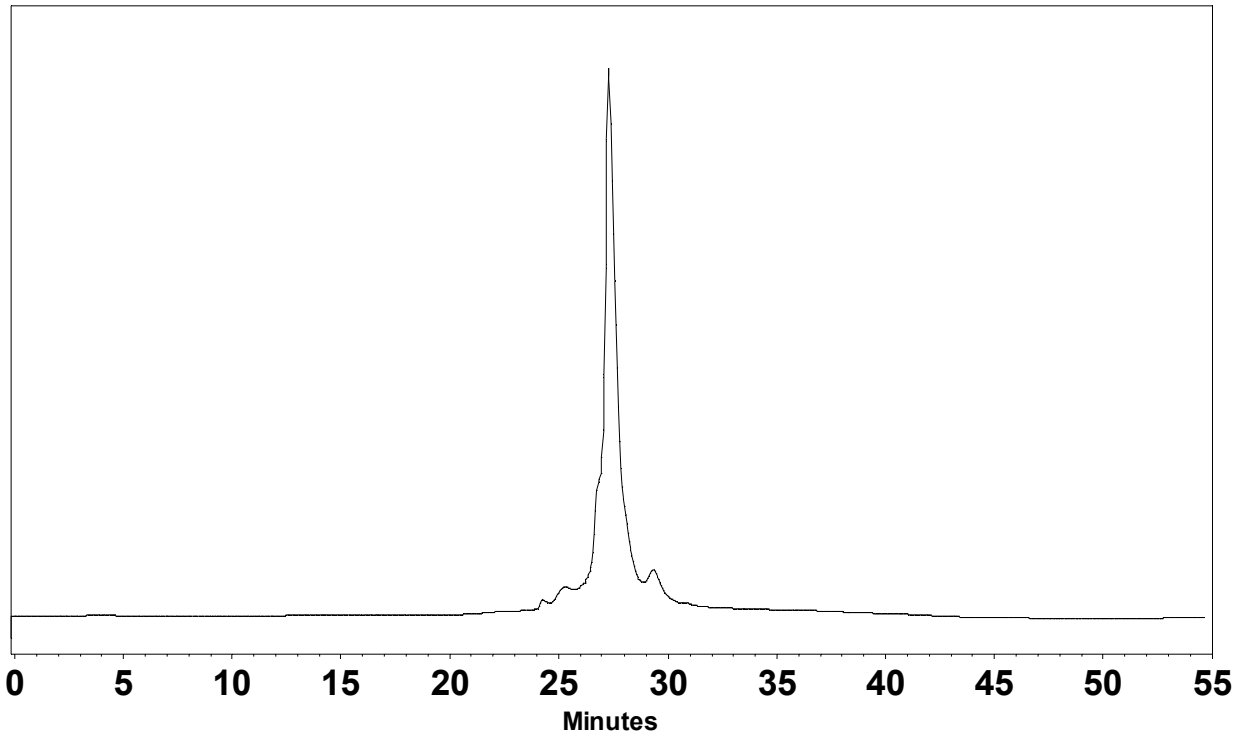

Figure **S3**. HPLC profile of purified **E**~ODN4~**Q**

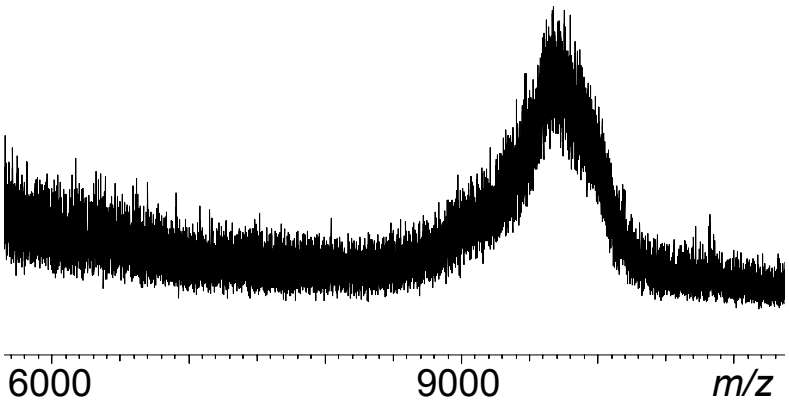

Figure **S4**. MALDI-TOF mass spectrum of purified **E**~ODN~**Q**

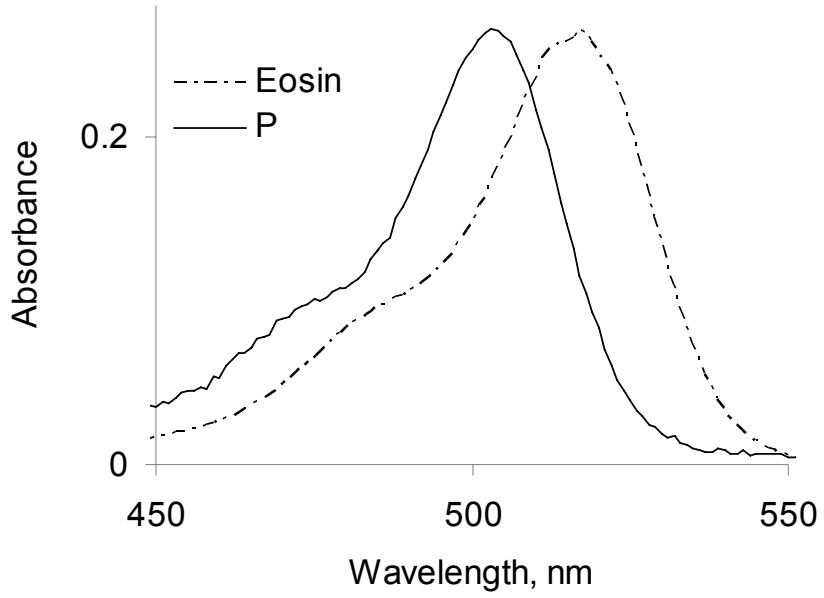

Figure **S5**. UV-visible spectra of photosensitizers eosin and **P** acquired in MOPS 10 mM (pH 7.5) buffer containing NaCl (150 mM) at 22 °C.

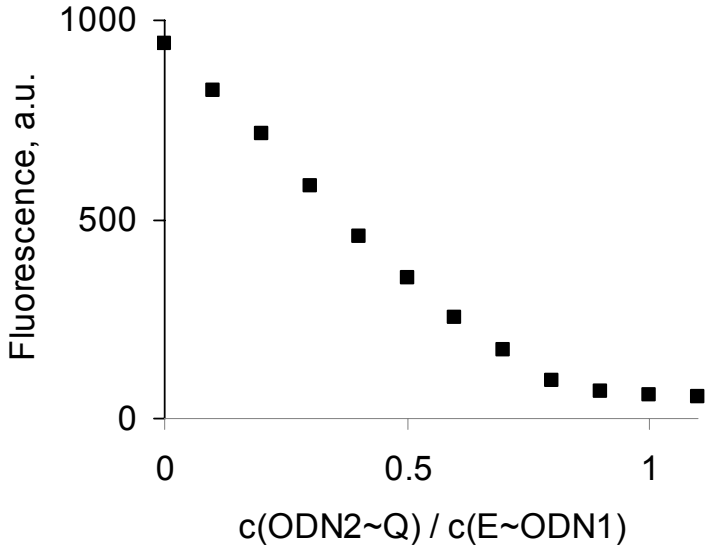

Figure **S6**. Titration of **E**~ODN1 (0.2 μM) with ODN2~**Q** monitored by using fluorescence spectroscopy; λ_{ex}= 524 nm; λ_{em}= 545 nm; buffer: MOPS 10 mM (pH 7.5), NaCl 150 mM; 22 °C

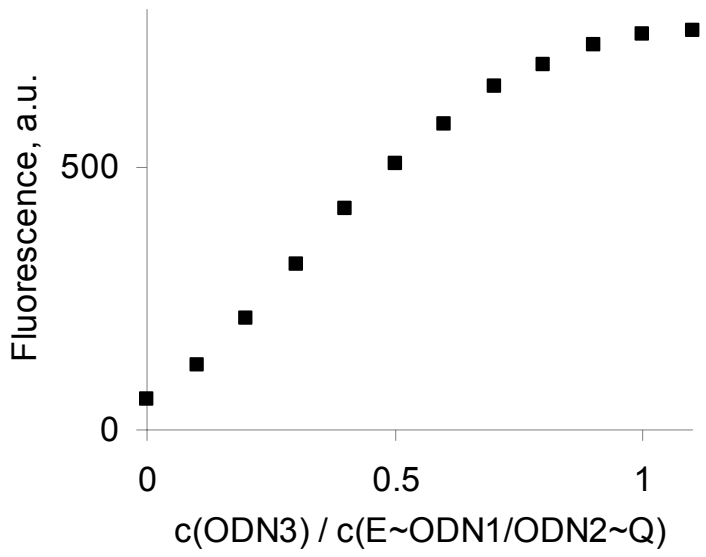

Figure **S7**. Titration of **E**~ODN1/ ODN2~**Q** duplex (0.2 μM) with ODN3 monitored by using fluorescence spectroscopy; λ_{ex}= 524 nm; λ_{em}= 545 nm; buffer MOPS 10 mM (pH 7.5), NaCl 150 mM; 22 °C

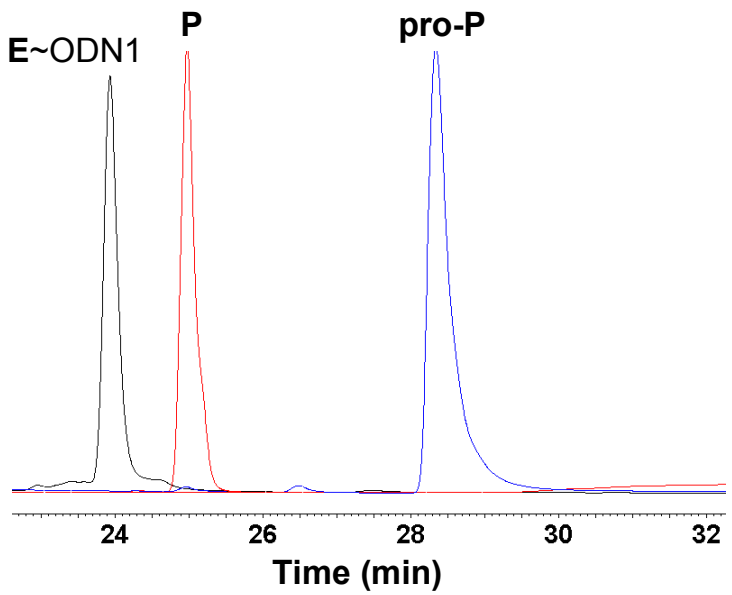

Figure **S8**. HPLC profiles of **E**~ODN1, 2',7'-dichlorofluorescein (**P**) and 2',7'-dichlorofluorescin (**pro-P**)

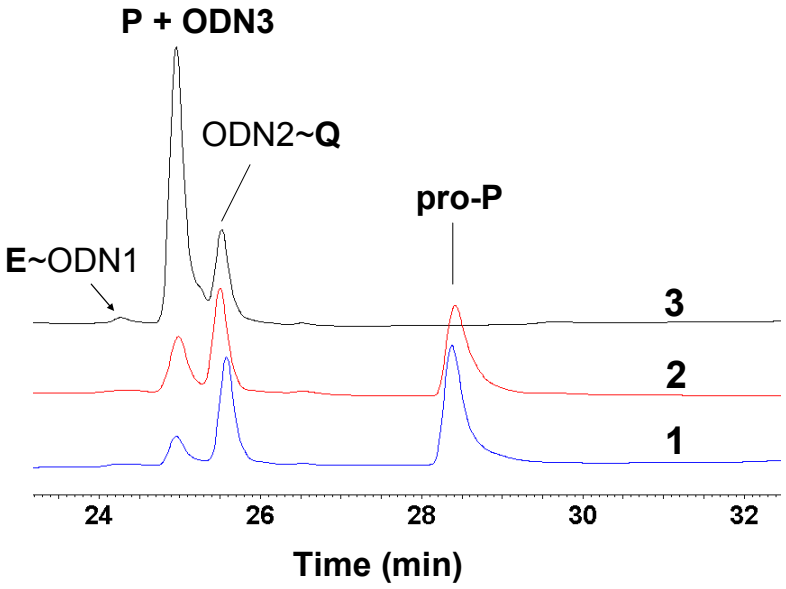

Figure **S9**. HPLC profiles of mixtures containing **E**~ODN1 / ODN2~**Q** duplex (0.2 μM), trigger ODN3 (0.2 μM) and substrate 2',7'-dichlorofluorescin (**pro-P**, 15 μM) in the buffer containing MOPS (10 mM, pH 7) and NaCl (150 mM); the mixtures were exposed to green light for 0 (profile **1**), 45 (profile **2**) and 270 min (profile **3**); peaks corresponding to ODN3 and the product of this reaction (2',7'-dichlorofluorescein, **P**) overlap with each other

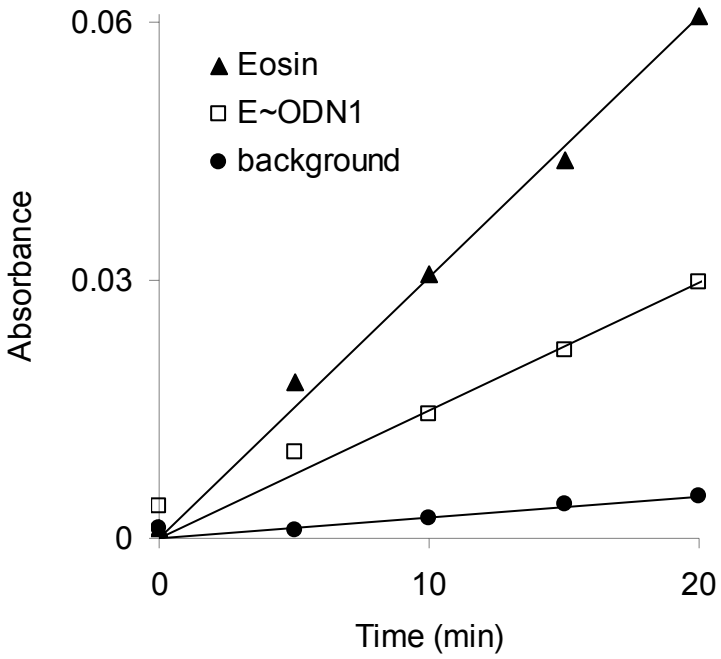

Figure **S10**. Photooxidation of 2',7'-dichlorofluorescin (**pro-P**, 5 μM) in MOPS buffer (10 mM), NaCl (150 mM) in the presence of either eosin (0.2 μM, filled triangles) or **E**~ODN1 conjugate (0.2 μM, open squares) or in the absence of any photosensitizer (filled circles)