Supporting Information

Photo-Induced Chemistry for the Design of Oligonucleotide Conjugates and Surfaces

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1 Experimental

1.1 Specification of used oligonucleotides

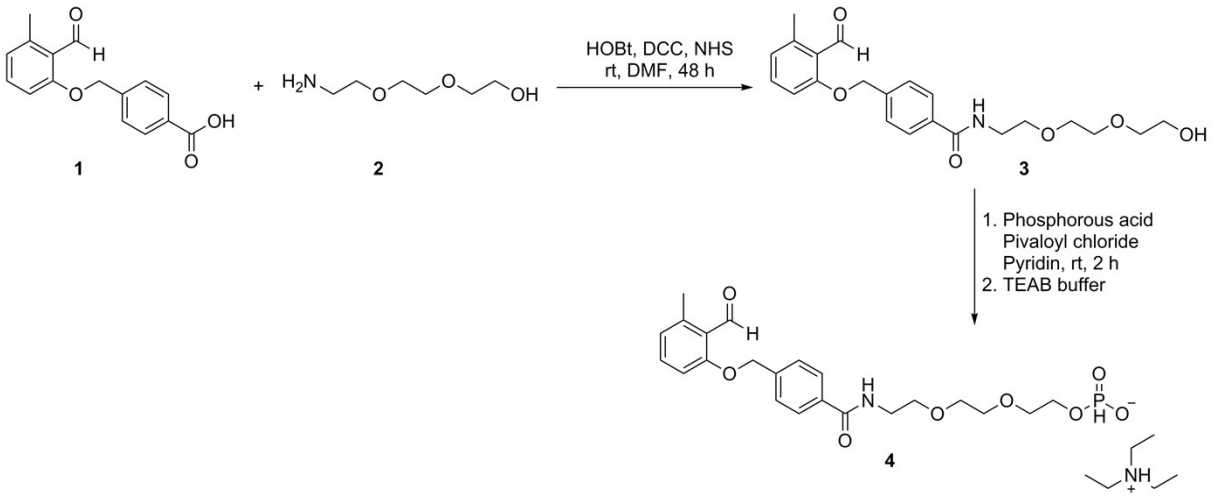
Table S1: Sequences, molecular weights and extinction coefficients (ε) of used oligos.

Indication	Sequence (5'-3')	MW [g/mol]	ε [L*mol^{-1}*cm^{-1}]
DNA1	GTGGAAAGTGGCAATCGTGAAG	6892	228100
DNA2	GGTGAAGAGATC	3732	127600
cDNA1	CTTCACGATTGCCACTTTCCAC	6584	191700
6-FAM-cDNA1	[6FAM] CTTCACGATTGCCACTTTCCAC	7122	191700
Cy®3-cDNA1	[Cyanine3] CTTCACGATTGCCACTTTCCAC	7092	191700
Cy®3-DNA3	[Cyanine3]CCTGCTTATGTTTCCGATGTGC	7190	193400

2 Experimental procedures and figures

2.1 Spectroscopic characterization of PE H-phosphonate 4

IR (ATR Platinum):



Scheme S1 Synthesis of the PE H-phosphonate 4.

ῡ = 3456 (w, ν (O-H)), 3319 (w, ν (N-H)), 2890 (w, ν (C-H)), 1688 (m, ν (C-N), ν (C=O)), 1644 (m, ν (NHC=O), ν (C=C)), 1537 (m, ν (C-H)), 1271 (s, ν (C-O)).

^{1}H-NMR (300 MHz, $CDCl_3$):

δ (ppm) = 2.56 (s, 3 H, CH_3), 3.56 – 3.59 (m, 2 H, NHCH_2), 3.65 – 3.68 (m, 10 H, 5 × CH_2), 5.17 (s, 2 H, $C_{arom}CH_2OC_{arom}$), 6.83 (t, ^3J = 8.0 Hz, 2 H, 2 × Ar-*H*), 7.34 (t, ^3J = 8.0 Hz, 1 H, Ar-*H*), 7.46 (d, ^3J = 8.2 Hz, 2 H, 2 × Ar-*H*), 7.84 (d, ^3J = 8.2 Hz, 2 H, 2 × Ar-*H*), 10.71 (s, 1 H, C*H*O).

^{13}C-NMR (400 MHz, $CDCl_3$):

δ = 21.40 (*C*H_3), 39.71 (NH*C*H_2), 61.47 (*C*H_2OH), 70.02 (4 × CH_2), 110.31 (*C*-Ar), 123.52 (C_{quart}, C_{arom}CHO), 124.56 (*C*-Ar), 127.00 (2 × *C*-Ar), 127.47 (2 × *C*-Ar), 134.19 (C_{quart}, C_{arom}CONH), 134.40 (*C*-Ar), 139.63 (C_{quart}, $C_{arom}CH_2OC_{arom}$), 142.17 (C_{quart}, $C_{arom}CH_3$), 161.89 (C_{quart}, $C_{arom}OCH_2$), 167.09 (C_{quart}, *C*ONH), 192.09 (C_{quart}, *C*HO).

HR-MS (FAB, matrix: 3-NBA), *m/z* $[M+H]^+$ calculated for $C_{22}H_{28}O_6N_1$ 402.1911; found, 402.1912.

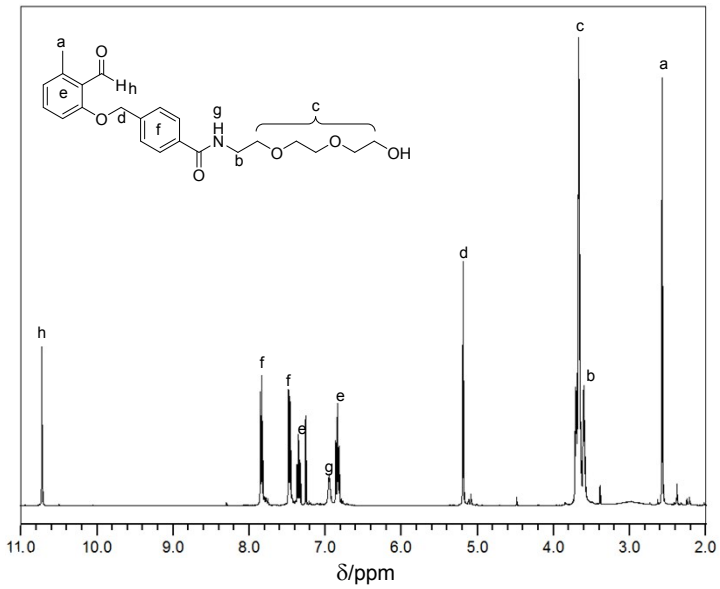


Fig. S 1 ^{1}H-NMR spectrum of **PE-TEG-OH 3** in $CDCl_3$ at 400 MHz.

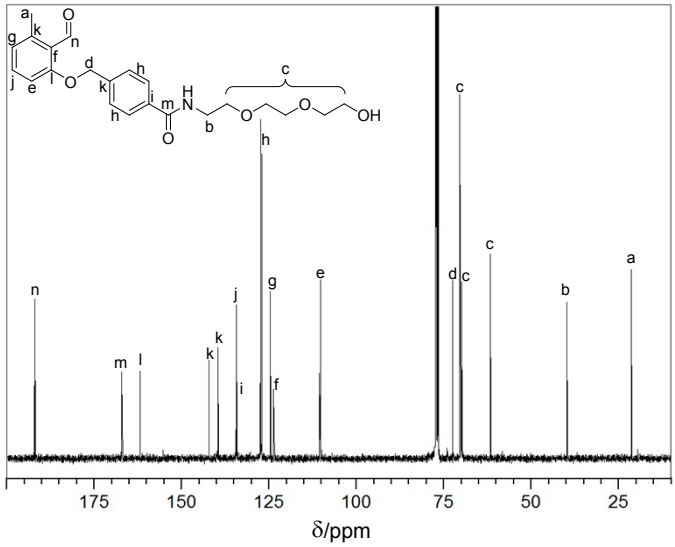


Fig. S 2 ^{13}C-NMR spectrum of **PE-TEG-OH 3** in $CDCl_3$ at 100 MHz.

2.1.1 ^{31}P-NMR of 2-(2-(2-(4-((2-Formyl-3-methylphenoxy)methyl)benzamido)ethoxy)-ethoxy)ethyl phosphonate (PE H-phosphonate 4)

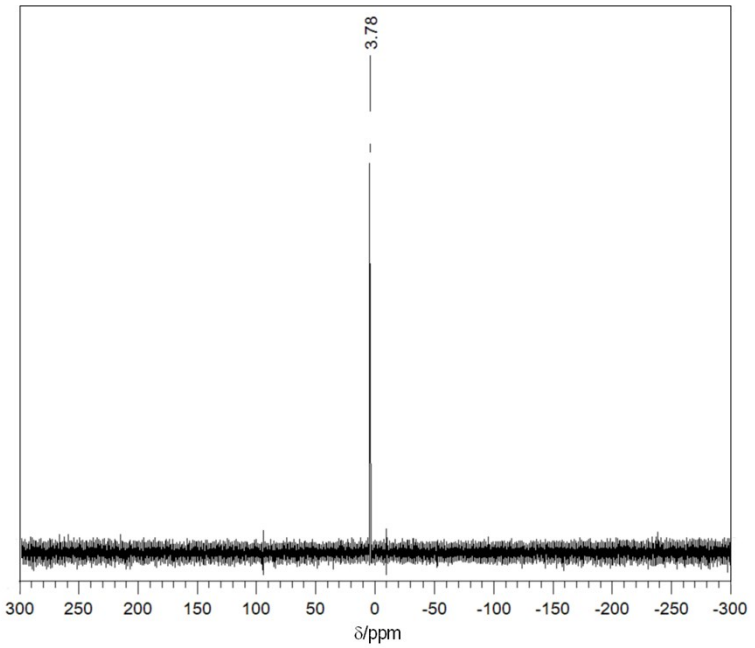


Fig. S 3 ^{31}P-NMR spectrum of **4** in CD_3CN at 101 MHz.

2.2 Gel electrophoresis and MALDI-ToF analysis of PE-DNA1 and PE-DNA2

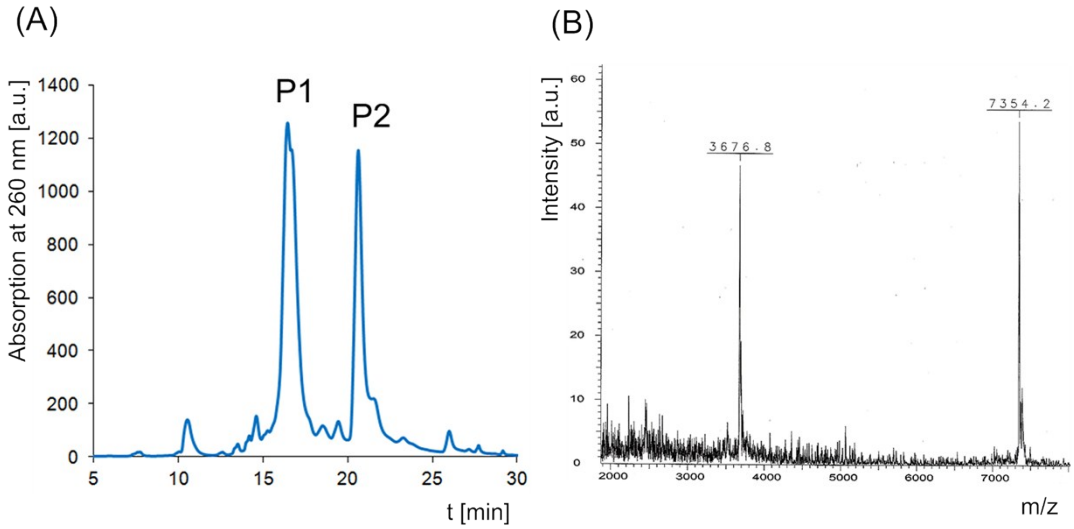


Fig. S 4 (A) HPLC chromatogram of **PE-DNA1** after cleavage from the CPG; P1 corresponds to non-modified **DNA1** and P2 to the **PE-DNA1**; (B) MALDI-TOF spectrum of **PE-DNA1**, $[M]_{theo}$ = 7356.4 Da, $[M]_{found}$ = 7354.2 Da, $[M/2]_{theo}$ = 3678.2 Da, $[M/2]_{found}$ = 3676.8 Da.

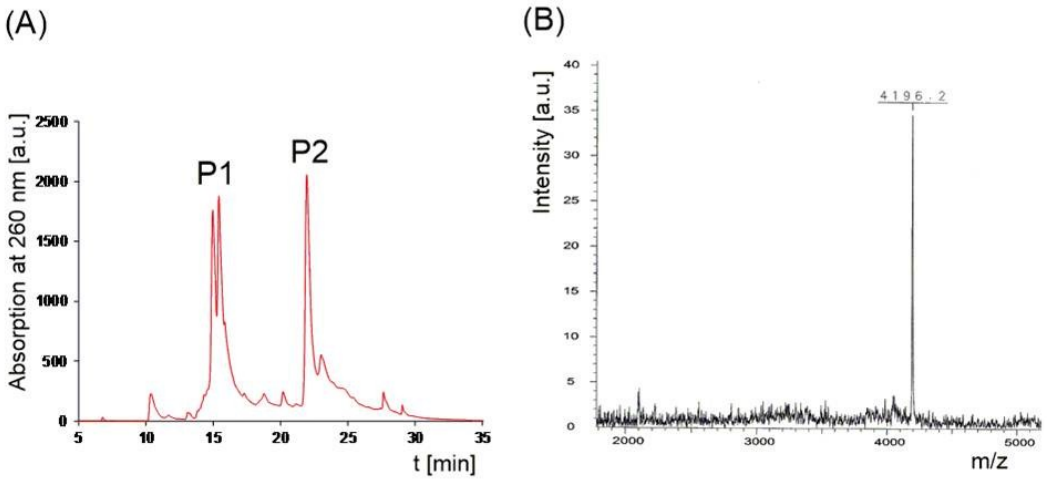


Fig. S 5 (A) HPLC chromatogram of **PE-DNA2** after cleavage from the CPG; P1 corresponds to non-modified **DNA2** and P2 to the **PE-DNA2**; (B) MALDI-TOF spectrum of **PE-DNA2**, $[M]_{theo}$ = 4197.1 Da, $[M]_{found}$ = 4196.2 Da.

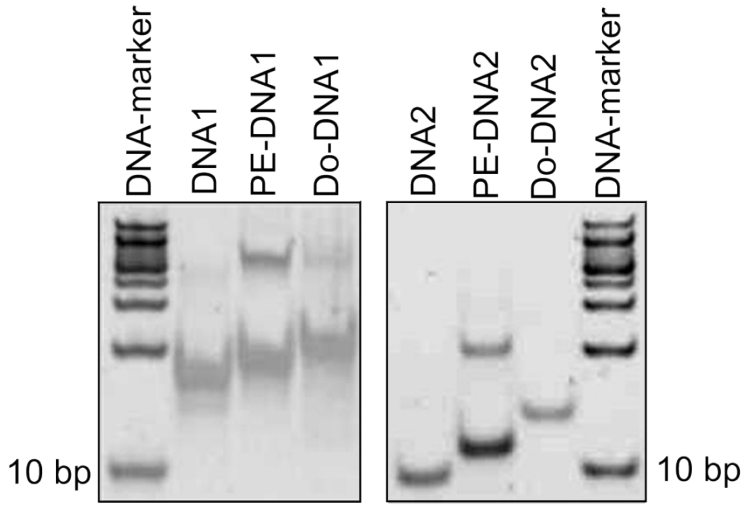


Fig. S 6 21% TBE gel of non-modified oligonucleotides **DNA1** (left gel) and **DNA2** (right gel), photoenol modified oligonucleotides **PE-DNA1** (left gel) and **PE-DNA2** (right gel) and the photoproducts with **Do-TEG-Mal Do-DNA1** (left gel) and **Do-DNA2** (right gel), DM-marker: 10 bp.

The gel electrophoresis shows a shift between non-modified oligonucleotides **DNA1**/**DNA2** and photoenol modified oligonucleotides **PE-DNA1/PE-DNA2** as well as the photoproducts **Do-DNA1**/**Do-DNA2**. The decrease of electrophoretic mobility of **PE-DNA1/PE-DNA2** and **Do-DNA1**/**Do-DNA2** can be explained by the increased mass of the modified oligonucleotides and photoproducts. The second band in the gel pocket **PE-DNA1** and **PE-DNA2** with much lower electrophoretic mobility represents a side product that could not be separated by the used HPLC purification method.

2.3 Setup for Light-Triggered Reactions

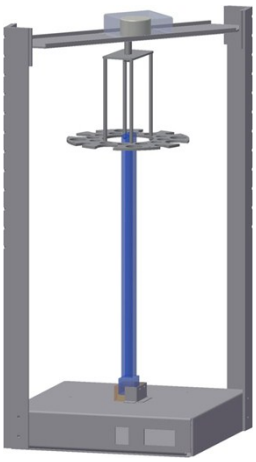


Fig. S 7 Drawing of the custom-built photoreactor employed in the current study.

The samples to be irradiated were crimped air-tight in headspace vials (20 mm, VWR, Germany) using SBR seals (VWR, Germany) with PTFE inner liner. The photoreactions were performed in a custom-built photoreactor (Figure S7), consisting of a metal disk which revolves at a distance of 40-50 mm around a compact low-pressure fluorescent lamp with λ_{max} = 320 nm ± 30 nm (36 W, Arimed B6, Cosmedico GmbH, Germany) (Figure S8). For spatially controlled surface immobilization of **PE-DNA1** maleimide functionalized surfaces were mounted into sample holders with mask before being immersed with the reaction solution.

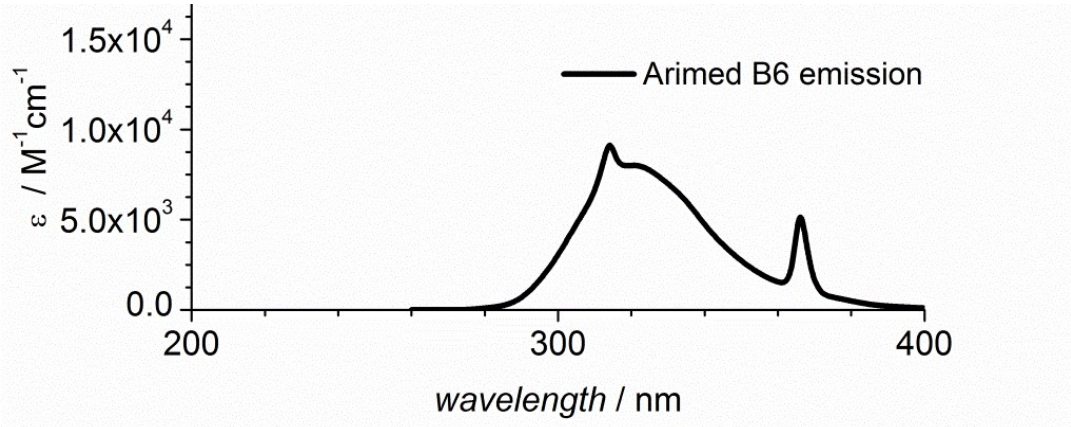


Fig. S 8 Emission spectrum of the employed compact low-pressure fluorescent lamp (36 W, Arimed B6, λ_{max} = 320 nm).

2.4 Characterization of the Photoproduct Do-DNA1

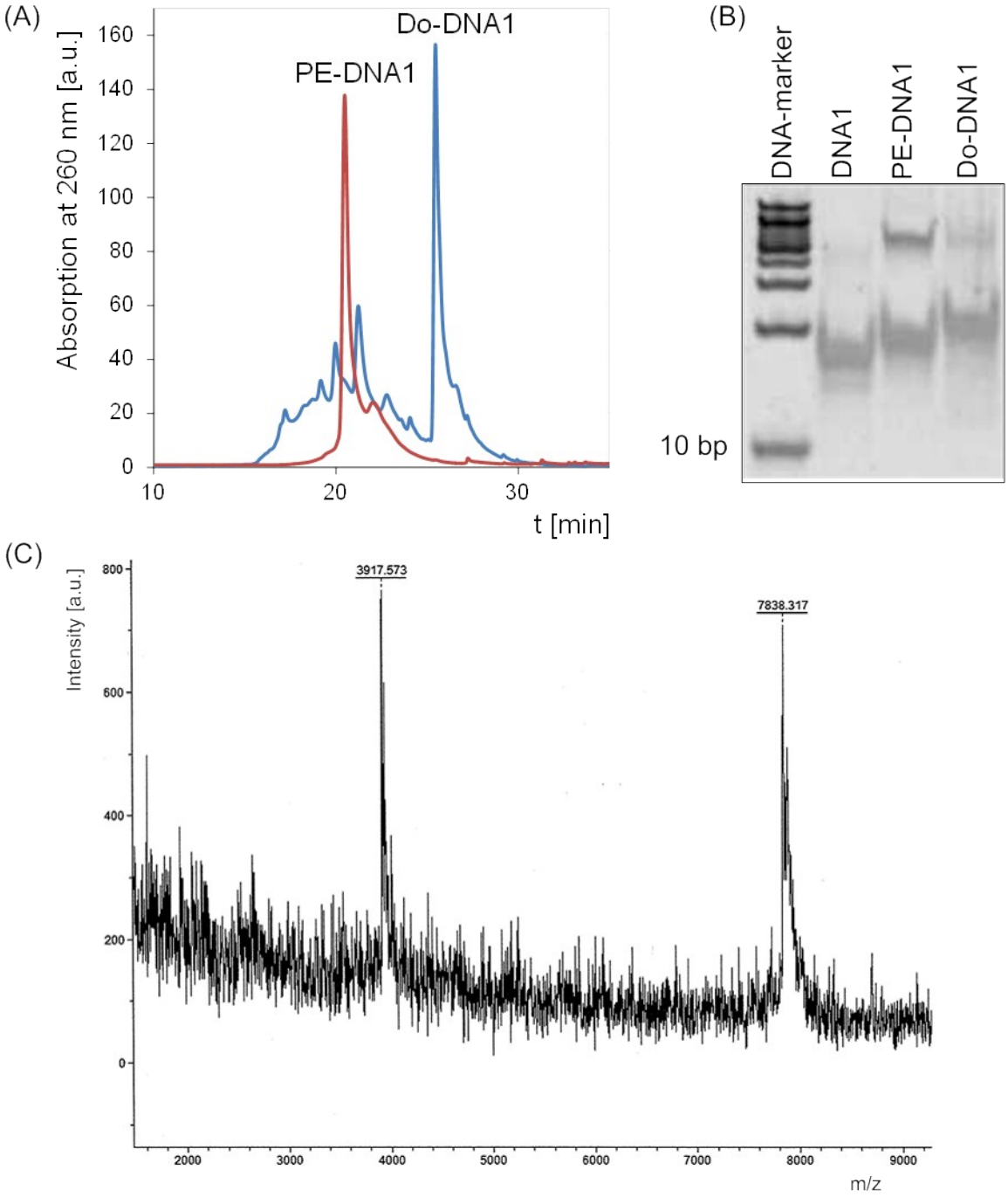


Fig. S 9 (A) HPLC chromatogram of purification of photoproduct **Do-DNA1** (blue) and HPLC chromatogram of **PE-DNA1** (red); (B) 21% TBE gel characterization of **Do-DNA1** (the line with **Do-DNA1** shows the decrease of electrophoretic mobility due the increase of mass of **PE-DNA1** after photoreaction with **Do-TEG-Mal**); (C) MALDI-TOF spectrum of **Do-DNA1**: $[M]_{theo}$ = 7832.9 Da, $[M]_{found}$ = 7838.3 Da, $[M/2]_{theo}$ = 3916.4 Da, $[M/2]_{found}$ = 3917.6 Da.

2.5 Photoreaction between Do-TEG-Mal and PE-DNA2

The mixture of 2.00 nmol **PE-DNA2** and 2.00 nmol **Do-TEG-Mal** in 1.00 mL H_2O/CH_3CN (1:1, v/v) was placed into the headspace vial, which was crimped air-tight as described above, degassed by purging with nitrogen for 15 minutes and subsequently irradiated for 16 hours in the photoreactor at $\lambda_{max} = 320$ nm. After the irradiation the solvents were removed under reduced pressure, the residue redissolved in water and purified by reversed phase HPLC using C18 column (eluent A: 0.100 M ammonium acetate, eluent B: CH_3CN). The collected fractions were characterized by MALDI-TOF and native PAGE (Fig. S10).

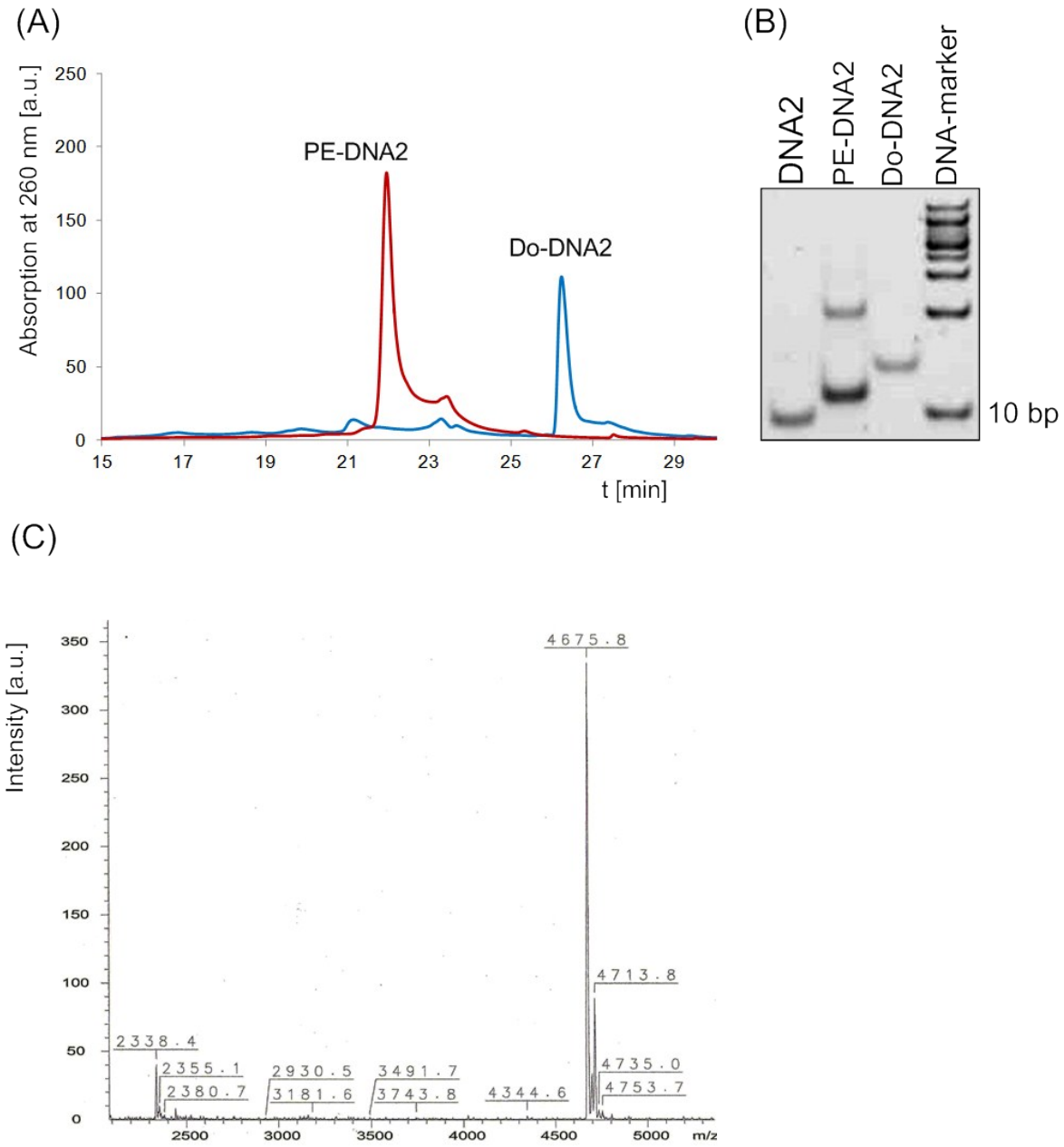


Fig. S 10 (A) HPLC chromatogram of purification of photoproduct **Do-DNA2** (blue) and HPLC chromatogram of **PE-DNA2** (red); (B) 21% TBE gel characterization of **Do-DNA1**; the line with **Do-DNA1** shows the decrease of electrophoretic mobility due the increase of mass of **PE-DNA1** after photoreaction with **Do-TEG-Mal**; (C) MALDI-TOF spectrum of **Do-DNA1**: $[M]_{theo}$ = 4673.7 Da, $[M]_{found}$ = 4675.8 Da.

2.5.1 Negative control reactions

The specificity of the photoreaction was investigated by performing different control reactions and analyses of the products by HPLC.

Reactions with non-modified **DNA1**:

(A) photoreaction between **DNA1** and **Do-TEG-Mal** (Fig. S11 A)

(B) incubation of **DNA1** and **Do-TEG-Mal** overnight in the dark (Fig. S11 B)

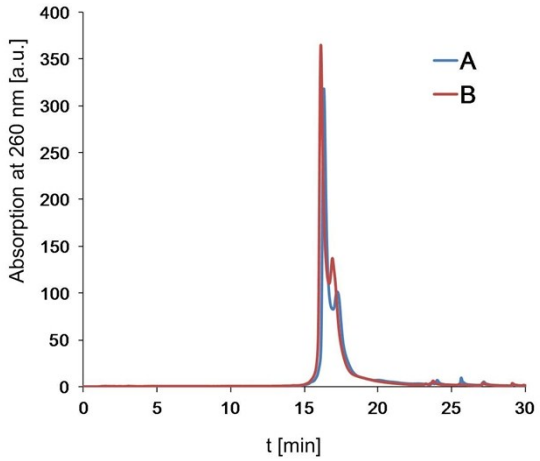


Fig. S 11 HPLC chromatograms of (A) photoreaction between **DNA1** and **Do-TEG-Mal** (blue curve); (B) incubation of **DNA1** and **Do-TEG-Mal** overnight in the dark (red curve).

Reactions with **PE-DNA1**:

(A) photoreaction between **PE-DNA1** and **Do-TEG-Mal** (Fig. S12 A);

(B) **PE-DNA1** and **Do-TEG-Mal** were incubated for 16 h in the dark (Fig. S12 B);

(C) **PE-DNA1** was irradiated for 16 h without Do-TEG-Mal (Fig. S12 C).

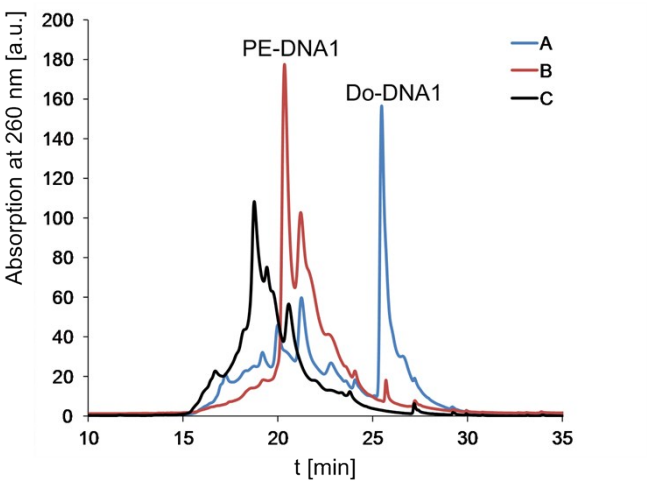


Fig. S 12 HPLC chromatograms of (A) photoreaction between **PE-DNA1** and **Do-TEG-Mal** (blue curve); (B) incubation of **PE-DNA1** and **Do-TEG-Mal** overnight in the dark (red curve); (C) irradiation of **PE-DNA1** overnight (black curve).

2.6 Photoreaction between HRP-Mal and PE-DNA1

First **HRP** was functionalized with maleimide group in the coupling reaction with sulfo-SMCC. Sulfo-SMCC (2.00 mg, 4.58 µmol) was dissolved in 100 µL DMF and added to 200 µL **HRP** solution (166 µM in PBS buffer, pH 6.0) and incubated for 1 hour at room temperature. The excess sulfo-SMCC was removed by filtration of the reaction mixture through NAP5 and NAP10 columns. The buffer was exchanged using PBS buffer pH 7.1 as eluent.

For the photoreaction 2.00 nmol of **PE-DNA1** and 2.20 nmol of fresh prepared **HRP-Mal** were dissolved in 500 µL of PBS/CH_3CN solution (3:2, v/v) and placed into the headspace vial, which was crimped air-tight as described above, degassed by purging with nitrogen for 15 minutes and subsequently irradiated for 16 hours in the photoreactor at 320 nm. The reaction mixtures were filtered through NAP5 and NAP10 columns to exchange the buffer to 20 mM Tris/HCl pH 8.3, concentrated by using 5 kDa Vivaspin and purified by anion exchange chromatography using MonoQ 5/50 GL column (buffer A: 20 mM Tris, buffer B: 20 mM Tris, 1 M NaCl; gradient: linear increase of buffer B to 100 % in 30 minutes, flowrate: 1 mL/min). The concentration and buffer exchange to PBS of collected fractions were carried out by using 5 kDa Vivaspin, the characterization of product fraction was performed by native PAGE.

2.6.1 Negative control reactions for PE-DNA1 and HRP-Mal

In order to investigate the specificity of the photoreaction between the maleimide and the photoenol moieties different control reactions were performed at the same reaction conditions:

(A) **PE-DNA1** and **HRP-Mal** were incubated for 16 h in the dark (Fig. S13 A);

(B) **PE-DNA1** and **HRP** were irradiated for 16 h (Fig. S13 B);

(C) non-modified **DNA1** and **HRP** were irradiated for 16 h (Fig. S13 C);

(D) non-modified **DNA1** and **HRP-Mal** were irradiated for 16 h (Fig. S13 D).

The reaction mixtures were filtered through NAP5 and NAP10 columns to exchange the buffer to 20 mM Tris/HCl pH 8.3, concentrated by using 5 kDa Vivaspin and purified by anion exchange chromatography as described above.

In case of the reaction (B), the irradiation of **PE-DNA1** and **HRP**, a small amount of conjugate **HRP-DNA1** was obtained, indicated that a low yielding side reaction occur between photoenol moiety and amino acids of protein under irradiation. A small yield of the side reaction and no conjugates in other cases indicates the high specificity of the photoreaction between photoenol and maleimide moieties.

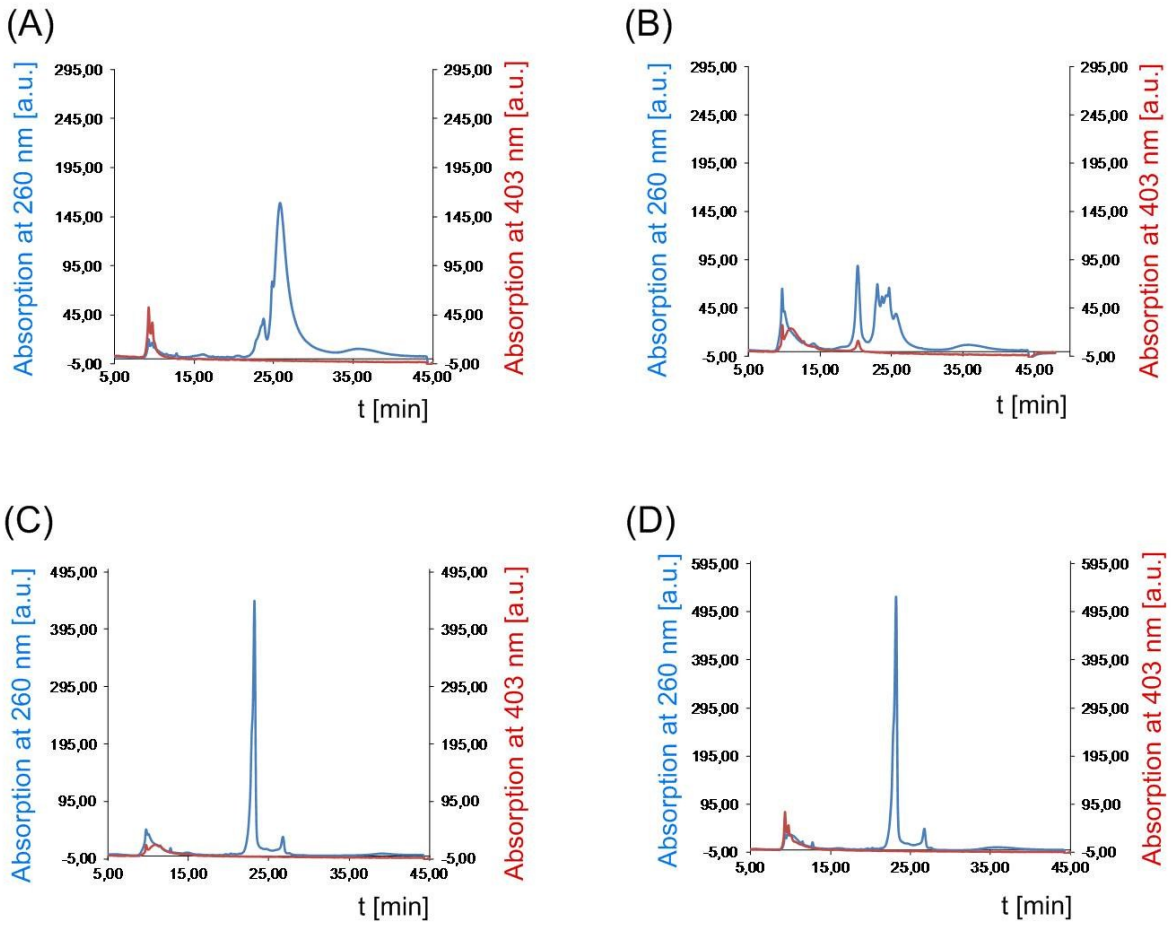


Fig. S 13 FPLC chromatograms of negative control reactions: (A) incubation of **PE-DNA1** and **HRP-Mal** for 16 h in the dark; (B) irradiation of **PE-DNA1** and **HRP** for 16 h at 320 nm; (C) irradiation of **DNA1** and **HRP** for 16 h at 320 nm; (D) irradiation of **DNA1** and **HRP-Mal** for 16 h at 320 nm. The blue curve corresponds to the absorption at 260 nm, which is typical for the **DNA**[1] and the red curve corresponds to the absorption at 403 nm, which is typical for the **HRP**.[2]

As a comparison between the formed amounts of main product and side product both FPLC chromatograms were plotted in one graph (Fig. S14). As mentioned above the amount of the formed side product is insignificant.

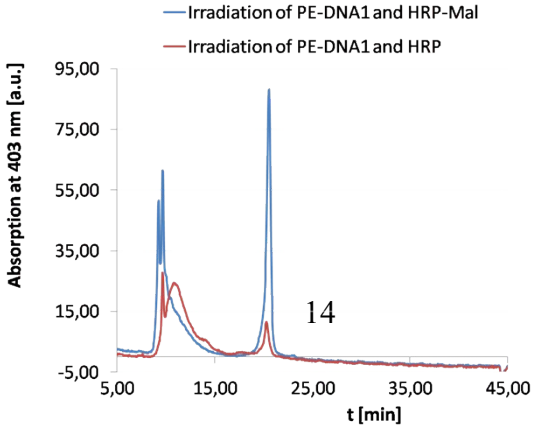
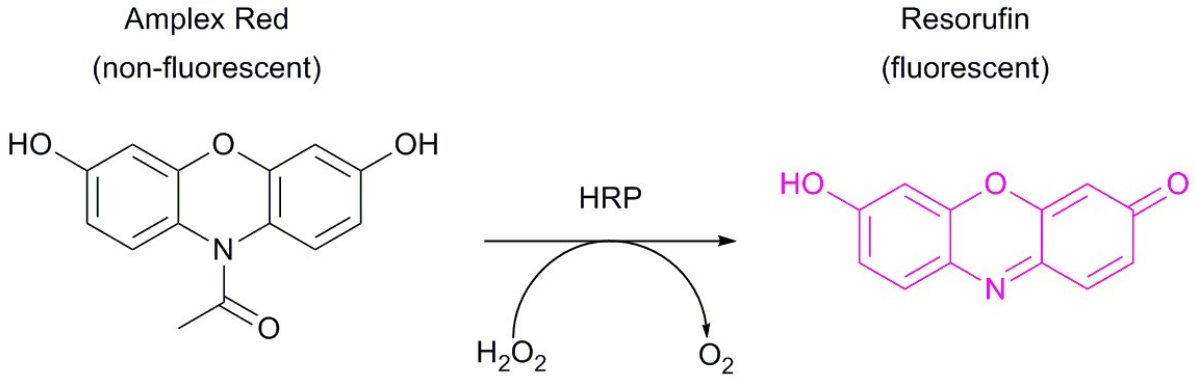


Fig. S 14 FPLC chromatograms of product **HRP-DNA1** obtained in the photoreaction between **PE-DNA1** and **HRP-Mal** (blue curve) and in the photoreaction between **PE-DNA1** and **HRP** (red curve).

2.6.2 Peroxidase activity test for HRP-DNA1 conjugate (Amplex®Red Assay)

Peroxidase activity tests for **HRP** and **HRP-DNA1** conjugate were performed in black 96 well microtiterplates using a Synergy microplate reader (BioTek) to record the fluorescence signals of resorufin (λ_{ex} = 530 nm, λ_{em} = 590 nm, sensitivity = 70), which was formed by the oxidation of Amplex Red (Scheme S2). The mixture of 2 pmol **HRP** or **HRP-DNA1** in sodium phosphate buffer (pH 6.0) and 1 nmol H_2O_2 was added to the wells (5 wells per sample). To each sample 5 nmol Amplex Red were added and the final volume of each sample was adjusted to 100 µL with sodium phosphate buffer (pH 6.0). The measurements were done over a time period of 1 h for both **HRP** and **HRP-DNA1** conjugates.



Scheme S 2 Principle of the peroxidase activity assay using Amplex Red and **HRP** in the presence of H_2O_2.

2.6.3 Stability test of HRP upon UV irradiation

For the stability test, native **HRP** was irradiated in a mixture of PBS/CH_3CN (3:2) with 320 nm light overnight. SDS-PAGE analysis showed no fragmentation of **HRP** after the irradiation.

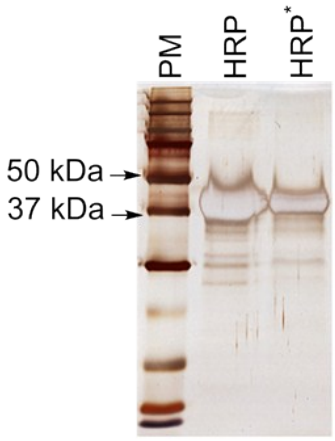


Fig. S 15 12% SDS-PAGE characterization of native horseradish peroxidase (**HRP**) in comparison to the UV light irradiated **HRP***; PM: protein marker (Precision Plus Protein Dual Xtra Standards, Bio-Rad).

2.6.4 Spatially controlled surface functionalization with PE-DNA1 and subsequent hybridization with complementary DNA labeled with Cy®3 dye (Cy®3-cDNA1)

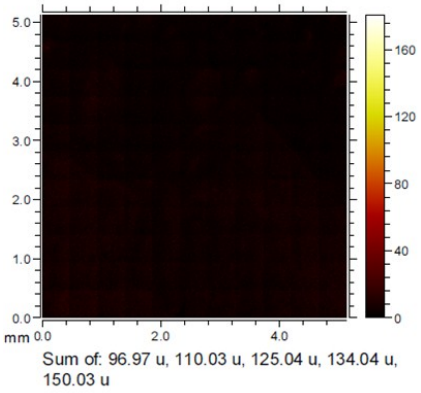


Fig. S 16 ToF-SIMS image of a maleimide terminated surface irradiated in the presence of non-functionalized **DNA1**, displaying the sum of the signals detected at 96.95 ($H_2PO_4^-$), 150.03 (G^-), 125.02 (T^-), 134.03 (A^-) and 110.03 (C^-) assigned to fragments of the corresponding oligonucleotides.

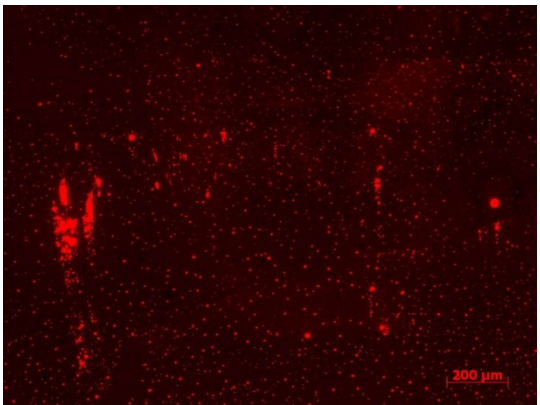


Fig. S 17 Fluorescence microscopy of hybridized **Cy®3-DNA3** (λ_{Esc}=550 nm, λ_{Em}=570 nm) on the surface patterned with **PE-DNA1**.

3 References

1. M. Barbatti, A. J. Aquino and H. Lischka, *Phys. Chem. Chem. Phys.*, 2010, **12**, 4959-4967.
2. P. I. Ohlsson and K. G. Paul, *Acta Chem Scand B*, 1976, **30**, 373-375.