Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Light Induced Protein-DNA Conjugation

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Content

1) Experimental procedures

1.1) Materials and methods

Unless otherwise mentioned, all chemicals were purchased from Sigma-Aldrich and used without further purification. UV- Vis spectra were obtained using VARY 300 Scan UV-Visible Spectrometer (Varian Inc., Germany). Fast protein liquid chromatography (FPLC) was performed using Äkta explorer system, which was connected to Mono Q 5/50 anion exchange column (GE Healthcare, Germany). High-performance liquid chromatography (HPLC) was done by using HPLC system 1200 series of Agilent Technologies connected to a Eclipse XDB-C18 column. Gel filtration NAP5 and NAP 10 columns and Vivaspin filtration columns were also purchased from GE Healthcare (Germany) and D1-NH_3 on solid support, SH-D1 ssDNA and horse heart myoglobin (Mb) was obtained from Sigma Aldrich (Germany). The microtiterplates (MTPs) for kinetic measurements in solution were purchased from NUNC (Denmark) whereby the DDI was realized in NUNC high absorp MTPs from thermo Fischer scientific (Germany). Solutions were removed in vacuum using concentrator plus system and the incubations for coupling procedures were done by using a Thermomixer compact system (both from eppendorf, Germany). PAGE characterizations were done by

using a Mini-Protean® Tetra System which was connected to PowerPac™ voltage source (BioRad, Germany).

Setup for Light-Triggered Reactions

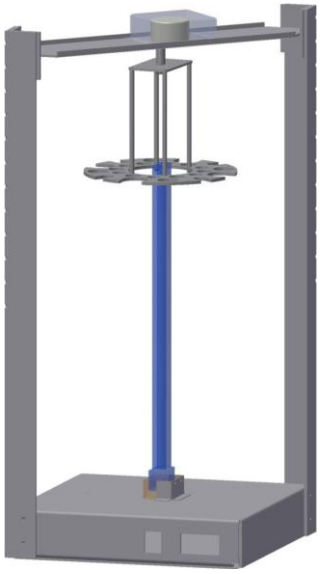


Figure S1. Drawing of the custom-built photoreactor employed in the current study.

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 S1**) 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 S2**).

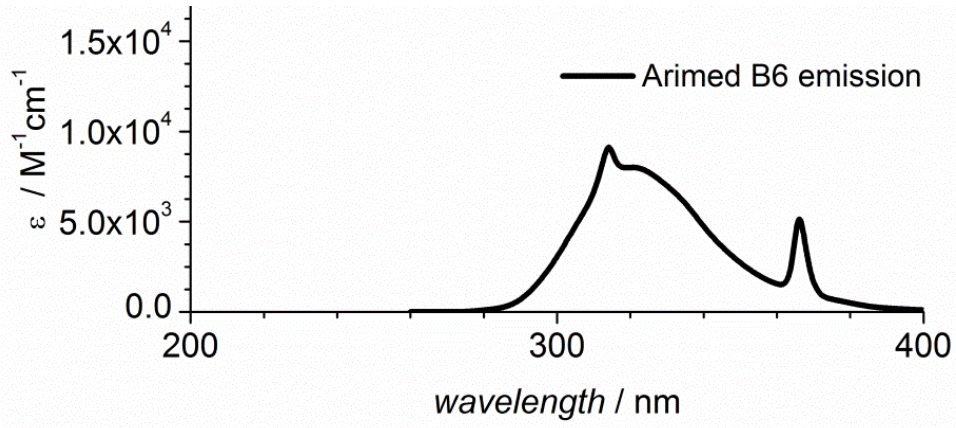
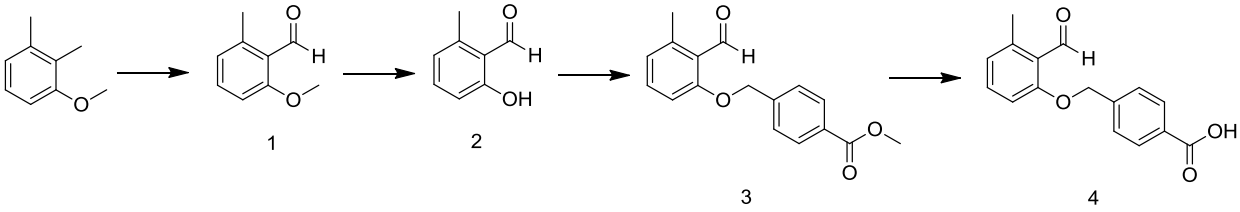


Figure S2. Emission spectrum of the employed compact low-pressure fluorescent lamp (36 W, Arimed B6, λ_{max} = 320 nm).

1.2.) Synthesis of photoenol precursor



Scheme S1: Synthesis of photoenol precursor.

Synthesis of 2-methoxy-6-methylbenzaldehyde 1:

The synthesis of 1 was performed according to a literature procedure.[1] A vigourously stirred mixture of 4.91 g 2,3-dimethylanisole (36.0 mmol), 9.02 g copper(II) sulfate pentahydrate (110.0 mmol) in 250 mL acetonitrile/water 1:1 is heated at reflux. After 15-30 min, the mixture becomes dark green in color and TLC analysis shows that no starting material is present. The mixture is cooled to ambient temperature and 100 mL CH_2Cl_2 are added. The layers are separated and the aqueous phase is further extracted with additional CH_2Cl_2 (2x50 mL). The combined organic solutions are dried ($NaSO_4$), filtered and evaporated at reduced pressure to give the crude product **1** that is suitable for further reaction; yield: 5.20 g (96%). ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 3H), 3.87 (s, 3H), 7.10 (m, 3H), 10.62 (s, 1H).

Syntheses of 2, 3 and 4 were performed according to literature.[2]

Synthesis of 2-hydroxy-6-methylbenzaldehyde 2:

5.20 g (34.6 mmol, 1 eq.) of **1** was dissolved in 50 mL dichloromethane. 13.87 g (104.0 mmol, 3 eq.) $AlCl_3$ were added to the yellow solution and the mixture was stirred at ambient temperature overnight. The color changed from yellow over red to a dark brown. Excess of aluminum chloride was cautiously quenched with water; the organic layer was extracted three times with dichloromethane and dried over sodium sulfate. The solvent was removed under reduced pressure and 3.06 g (22.5 mmol, 65 %) of a dark brown oil was obtained. In order to obtain an increased overall yield for **2**, the raw product was used directly in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 2.60 (s, 3H), 6.68-6.84 (m, 2H), 7.32-7.42 (m, 1H), 10.32 (s, 1H), 11.90 (s, 1H).

Synthesis of methyl 4-((2-formyl-3-methylphenoxy)methyl)benzoate 3:

3.06 g (22.5 mmol, 1 eq.) of **2** and 5.14 g (23.7 mmol, 1.02 eq.) of 4-(bromomethyl) benzoate were dissolved in 150 mL of acetone (the acetone has been pre-dried over sodium sulfate). To this solution 4.64 g (33.5 mmol, 1.5 eq.) of potassium carbonate and 91.0 mg (0.35 mmol, 0.015 eq.) of 18-crown-6 were added. The mixture was stirred at 40 °C overnight. After filtration, the solvent was removed under reduced pressure, the residue was re-dissolved in 100 mL dichloromethane/water (1:1) and acidified with aqueous HCl (3%). The aqueous layer was extracted two more times with dichloromethane (2×30 mL) and the combined organic layers were dried over sodium sulfate. The solvent was removed under reduced pressure, 50 mL of 7:1 v/v hexane/ethyl acetate was added and 3.71 g (13.1 mmol, 58%) pure **3** precipitated after cooling in the fridge. The pure solid was obtained after filtration and drying under reduced pressure. ^{1}H NMR (acetone-d^{6}) δ 2.52 (s, 3H), 3.89 (s, 3H), 5.38 (s, 2H), 6.89 (d, *J* = 7.7 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 7.45 (t, *J* = 8.2 Hz, 1H), 7.68 (d, *J* = 8.3 Hz, 1H), 8.06 (d, *J* = 8.4 Hz, 1H), 10.73 (s, 1H).

Synthesis of 4-((2-formyl-3-methylphenoxy)methyl)benzoic acid 4:

2.83 g (9.95 mmol, 1 eq.) of **3** was dissolved in 150 mL of dichloromethane and 1.20 g sodium hydroxid (29.9 mmol; 3 eq.) dissolved in 15 mL methanol were added. The reaction mixture was stirred over night at ambient temperature. The solvents were removed under reduced pressure and the residue was subsequently redissolved in 140 mL dichloromethane/water 1:1. The organic layer was extracted with water, all water layers were combined and acidified with aqueous HCl (3%) to pH 3. The aqueous layer was subsequently extracted 3 times with dichloromethane and the combined organic layers were dried over sodium sulfate. The solvent was removed under reduced pressure and 1.88 g (6.96 mmol, 70%) of a white powder was obtained. ^{1}H NMR (acetone-d^{6}) δ 2.52 (s, 3H), 5.39 (s, 2H), 6.89 (d, *J* = 7.6 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 7.45 (t, *J* = 8.1 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 8.05 (d, *J* = 8.4 Hz, 1H) , 10.74 (s, 1H).

1.3) Coupling of photoenol (PE) onto D1-ssDNA using solid phase methodology

The dimethoxytrityl (DMT) protected D1 ssDNA on controlled pore glass (CPG) solid support was deprotected with 1 mL dichloracetic acid solution (successful deprotection could be followed by color change from yellow to colorless). After deprotection, the solid phase columns were washed 3 times with CH_3CN and 3 times with DMF, dried and removed from the column holder. For the photoenol (PE) carboxyl activation, 45 µmol PE was mixed with 45 µmol HOBt, 45 µmol HBTU and 10 µL DIPEA and incubated for 30 min at r.t. in 300 µL

DMF/CH_3CN mixture (ratio 1:1). Subsequently, the solution was added to the dried solid support, incubated for 1.5 h at r.t. and washed with DMF and CH_3CN. The modified ssDNA was cleaved from solid support by incubation with 1 mL 25 % ammonia solution at 55 °C over night and was purified by reversed phase HPLC (Puffer A: 0.1 M ammonium acetate, Buffer B: CH_3CN) using C18 column. Concentrations of modified and unmodified DNA were calculated with UV-Vis spectroscopy, whereby the main fractions were characterized with native PAGE and MALDI-TOF.

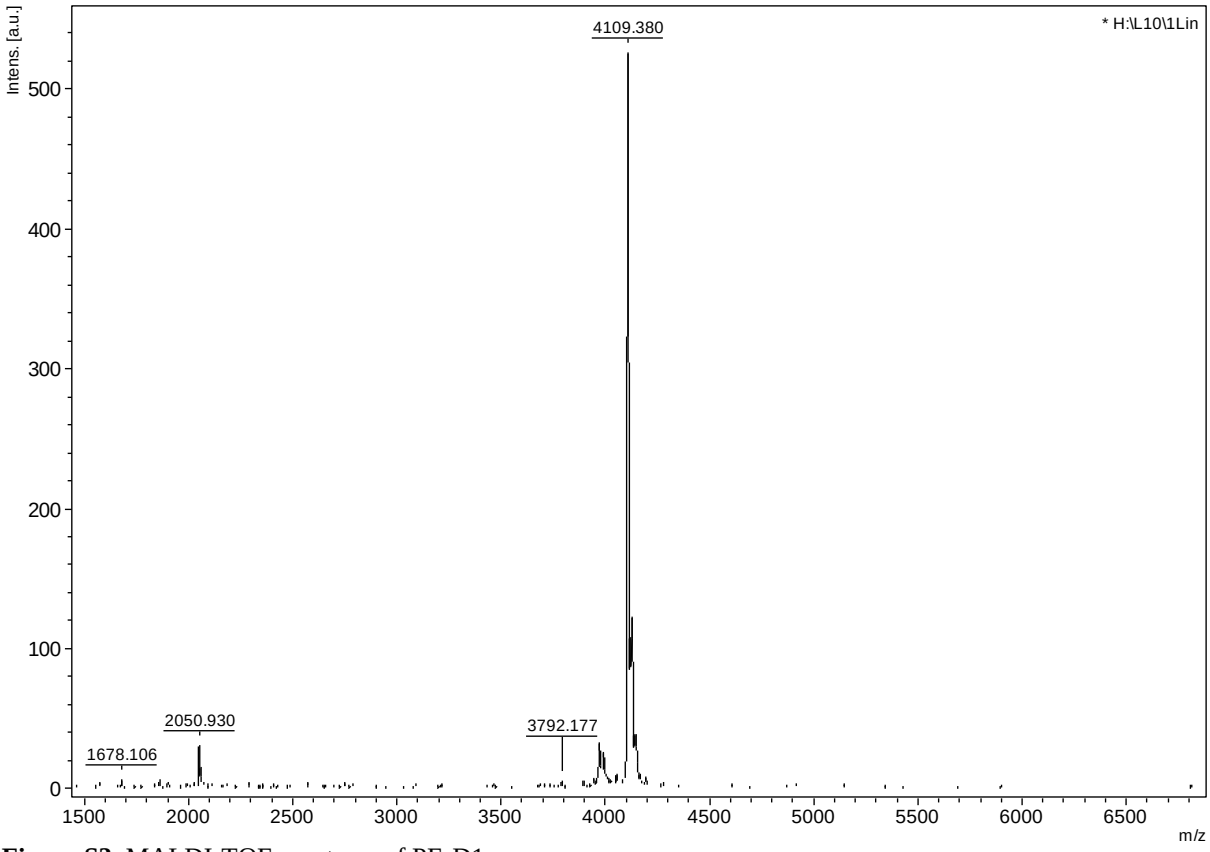


Figure S3. MALDI-TOF spectrum of PE-D1.

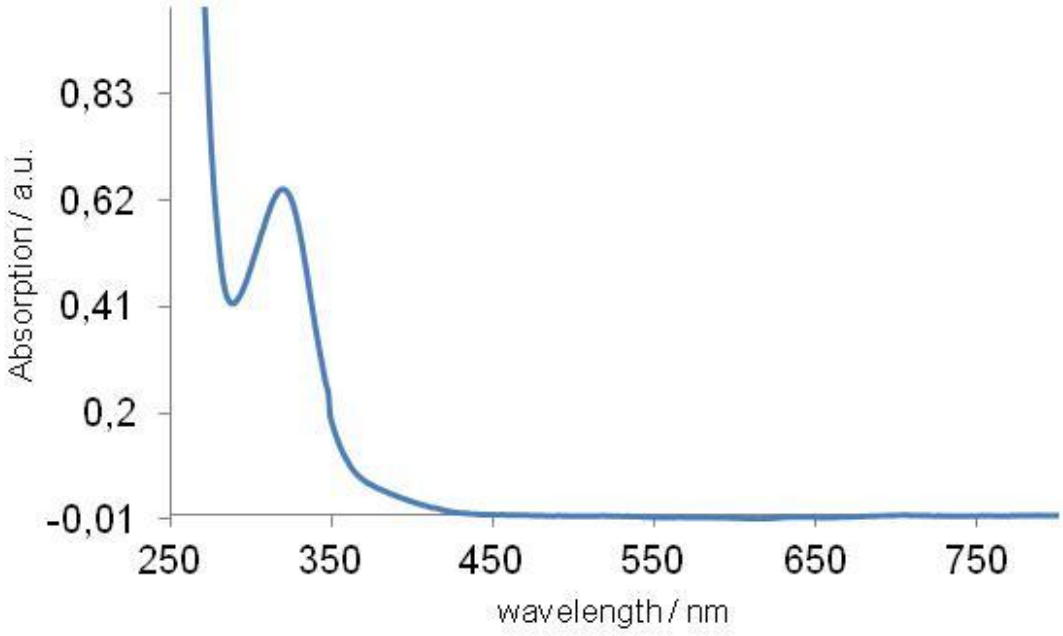


Figure S4. UV-Vis spectrum of molecule 4 (0.25 mM) in PBS/MeCN (ratio 1:1).

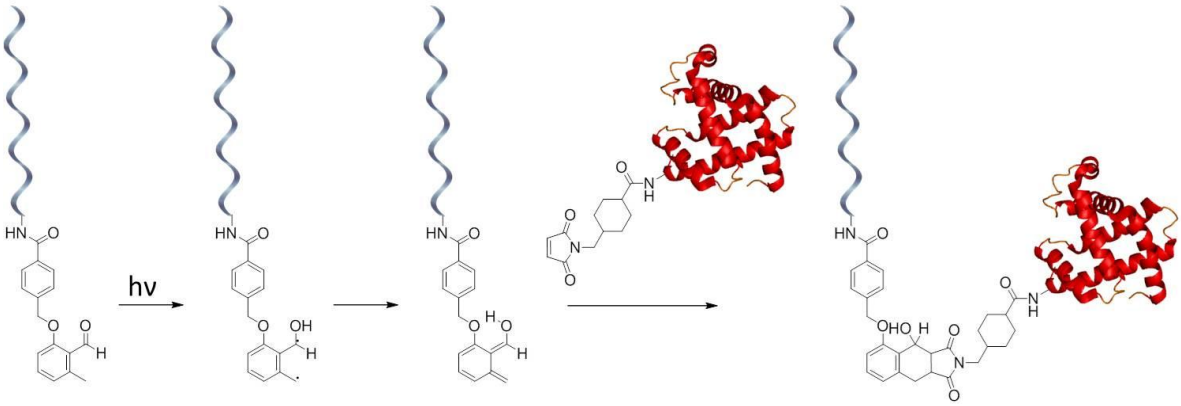
Table S1. MS (MALDI-TOF, 3-HPA) results of unmodified **D1**ssDNA and photoenol modified **PE-D1** ssDNA.

#	M_{cal} [g/mol]	M_{exp} [g/mol]
D1 (not shown)	3861	3858
PE-D1	4113	4109

1.4) Preparation of Mal-Mb

For the preparation of Mb-Mal, 2 mg of sSMCC was dissolved in 60 µL DMF [66,7 mM] and 600 nmol of ssMCC (10 fold excess) was added to 200 µL of Mb in PBS buffer [300 uM, 60 nmol] and incubated for 2 h at r.t. (DMF ratio ca. 4.5 %). After incubation, the reaction mixture was filtered with NAP5 and NAP10 columns to remove the excess sSMCC and eluted with PBS buffer. The concentration was calculated using UV-Vis spectroscopy and the freshly prepared **Mb-Mal** was used for further coupling procedures immediately.

1.5) Coupling of PE-D1 onto Mal-Mb



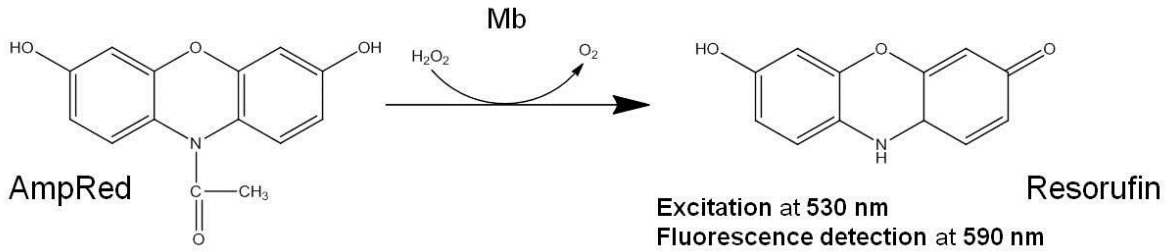
Scheme S2. Irradiation of **PE-D1** leads to intramolecular hydrogen abstraction followed by bond reorganization to diene formation. This reactive diene is stabilized by hydrogen bond formation and undergoes Diels-Alder reaction with maleimide functionalized **Mal-Mb**.

For the coupling procedure, 20 nmol of PE-D1 (1 eq) and 25 nmol of freshly prepared Mal-Mb (1.25 eq) were mixed in 900 µL acetonitrile/PBS 1:1 v/v and placed into the headspace vial, which was crimped air-tight using SBR seals with PTFE inner liner. The solution was deoxygenated by purging with nitrogen for 10 min and subsequently irradiated overnight in the photoreactor at 320 nm. After irradiation, the reaction mixture was treated with 5 kDa Vivaspin and the buffer was exchanged to 20 mM Tris/HCl. The conjugate Mb-D1 was purified by using anion-exchange-chromotography using Mono Q 5/50 column (Buffer A: 20 mM Tris, Buffer B: 20 mM Tris, 1 M NaCl, Gradient: Linear increase of buffer B to 100 % in 30 min, Flowrate: 1 mL / min) and the main fractions were characterised with native PAGE.

1.6) Activity study of Mb-D1 conjugate

The proxidase activity of native **Mb**, **Mb-D1** and **Mb-D1chem** conjugates were measured by using a Synergy multiwellplate reader (Biotek) and were carried out in black 96 well microtiterplates. For the measurements, 1 pmol of **Mb** or **Mb-D1**/**Mb-D1chem** conjugates respectively in 50 µL NaP_i-buffer (pH = 6.0) were added to the wells (8 wells per sample). A solution of AmplexRed dye and H_2O_2 were prepared according to manufacturers instructions (Invitrogen) and were added to each well just before the measurements. Final concentrations of all reagents in 100 µL volume were: 50 µM AmplexRed, 1 mM H_2O_2 and

10 nM of protein or conjugates respectively. Each measurement was done over a time period of 1 h by using an excitation wavelength at 530 nm and fluorescence detection at 590 nm. The sensitivity at the Synergy plate reader was set to 100.



Scheme S3. Reaction pathway of AmpexRed (AmpRed) in the presence of myoglobin (Mb) and H_2O_2. AmpRed is a non fluorescent reagent, which is oxidized by H_2O_2 in the presence of peroxidases to the fluorescent molecule resorufin (at 590 nm after excitation at 530 nm).

1.7) DDI of Mb-D1 conjugate

DNA directed immobilization (DDI) was carried out in black 96-well microtiterplates (MTPs). The streptavidin (STV) coated plates were prepared according to published protocols.[3] Freshly prepared STV coated MTPs were incubated with 50 µL of complementary biotinylated strand **bcD1** in TETBS buffer [240 nM, 1.2 nmol] for 45 min at r.t. under shaking. Afterwards, the wells were washed 2 times with 200 µL NaPi buffer (pH=6) and 2 times with 200 µL TETBS buffer [20 mM Tris, 150 mM NaCl, 5 mM EDTA, 0,05 % Tween] and were incubated with 1 pmol of **Mb-D1**, **Mb-D1chem** or **Mb** respectively for 1 h at r.t. in the dark (4 wells for each sample). The wells were washed again 2 times with 200 µL TETBS and 2 times with 200 µL NaPi-buffer and were filled with 50 µL NaPi-buffer (pH=6.0) for the kinetic measurements. The AmplexRed/H_2O_2 solution was prepared as described in manufacturer's instructions (Invitrogen) and 50 µL were added in every well before the measurements (Final concentrations: 50 µM AmplexRed, 1 mM H_2O_2). Measurements were done over 1 h using an excitation wavelength at 530 nm and fluorescence detection at 590 nm, while the sensitivity of the plate reader was set to 100.

2.) Supplementary data and figures

2.1) DNA sequences

Table S2. Sequences of used ssDNA.

Indication	Sequence and 5´-modification
D1-NH_2	5´- [AminoC6]-GGCGTATAACAA-3´
D1-SH	5´-[ThioC6]- GGCGTATAACAA-3´
$cD1_{TAMRA}$	5´-[TAMRA]- TTTTTT**TTGTTATACGCC**-3´
cD1	5´-**TTGTTATACGCC**-3´
bcD1	5´-[Biotin]- TTTTTT**TTGTTATACGCC**-3´

2.2) Stability study of Mb and D1 under light irradiation

For the stability assays, native Mb was incubated under the same experimental conditions as used for light induced coupling of **Mb-Mal** with **PE-D1** at 320 nm over night. SDS-PAGE characterisation showed no fragmentation of myoglobin after the irradiation procedure (Figure S4A). The preserved function was also verified by AmplexRed peroxidase activity tests, which was done using nMb after irradiation. The ssDNA **D1-NH_2** was also irradiated for different time periods at 320 nm and the samples were characterised with native PAGE. No fragmentation could be observed after irradiation (Figure S4B).

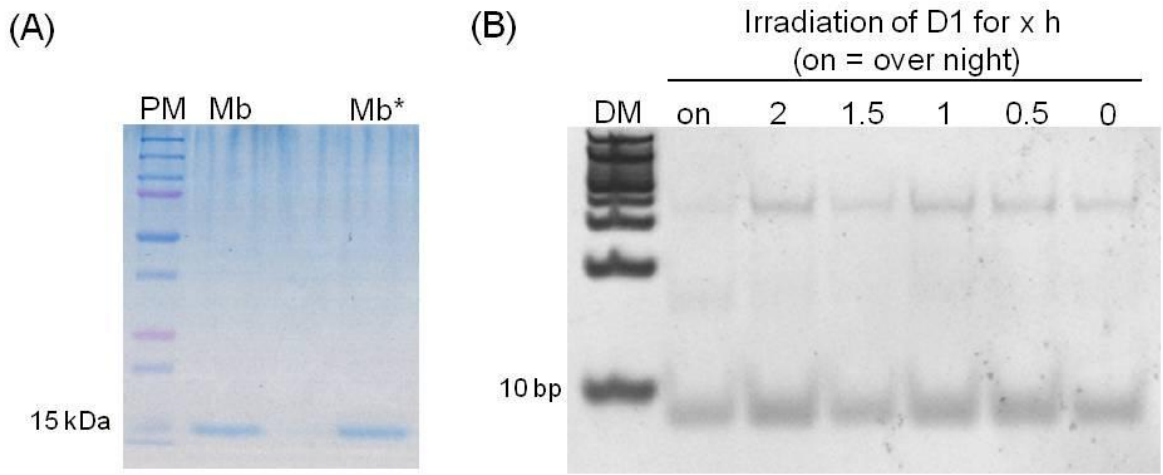


Figure S5. Stability tests of Mb (A) and D1 ssDNA (B) upon light irradiation. **(A)** 12 % SDS PAGE characterisation of native myoglobin (Mb) in comparison with light irradiated n Mb(Mb*). **PM:** Protein marker. **(B)** 21 % native PAGE characterisation of D1 ssDNA in comparison to irradiated D1 ssDNA at 320 nm after different irradiation times (0 h, 0.5 h, 1 h, 1.5 h, 2 h and over night (on)). **DM:** DNA marker.

To confirm additionally that a) no further DNA fragments, which cannot be detected by gel electophoresis, were produced upon irradiation and b) that T-T dimers often obtained upon UV irradiation are not formed, we have irradiated both D1 and cD1 sequence containing two pairs of TT bases ((5´-**TT**G**TT**ATACGCC-3´) overnight using 320 nm light. HPLC chromatograms (Fig. S6 and S7) were performed using non irradiated and irradiated (D1* and cD1*) and no side products of photoirradiation could be detected.

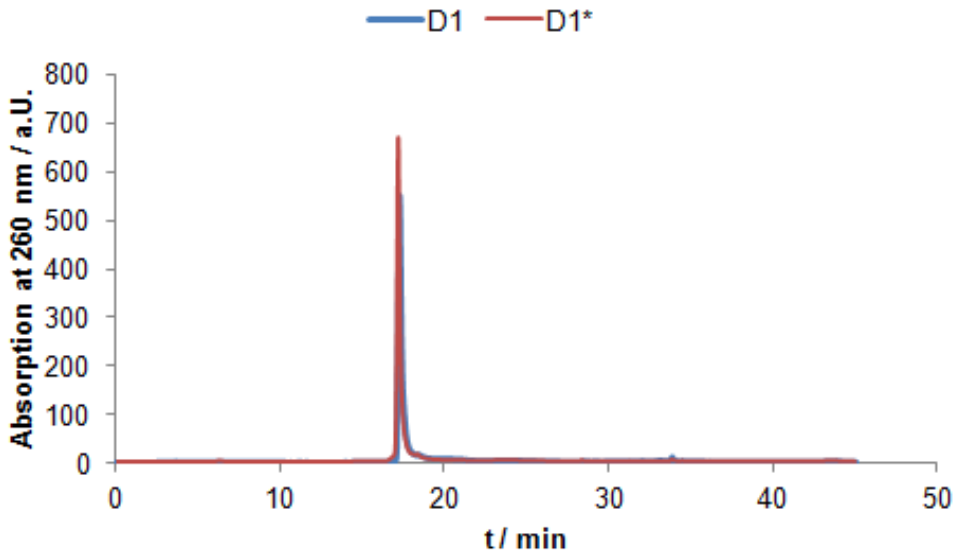


Figure S6: HPLC spectra of the sequence D1, before (D1) and after (D1*) irradiation at 320 nm over night.

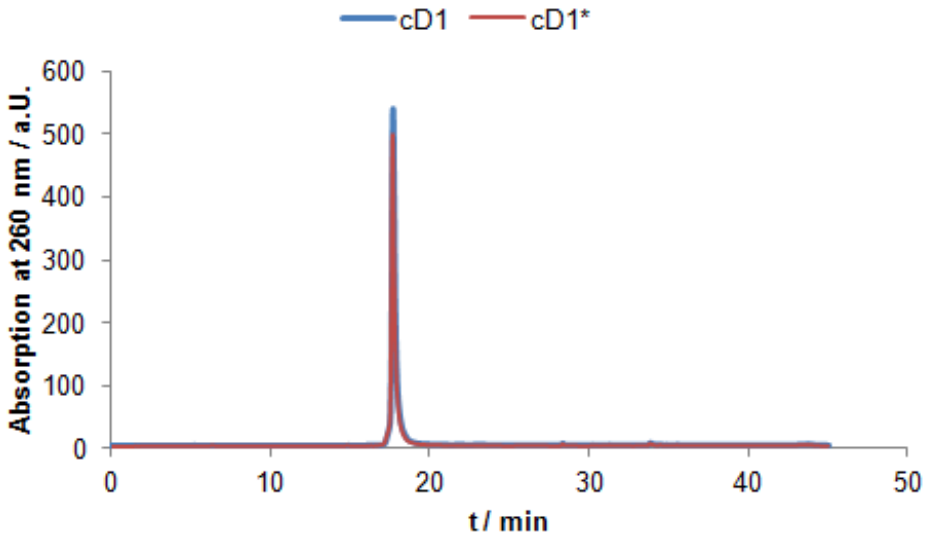


Figure S7: HPLC spectra of the sequence cD1, before (D1) and after (D1*) irradiation at 320 nm over night.

In addition, the hybridization tests were conducted with both irradiated DNA sequences to confirm that they are fully functional after the irradiation. 1:1 ratio between DNA and cDNA was used and clear shifts are observed when complementary sequences are added (Fig. S8).

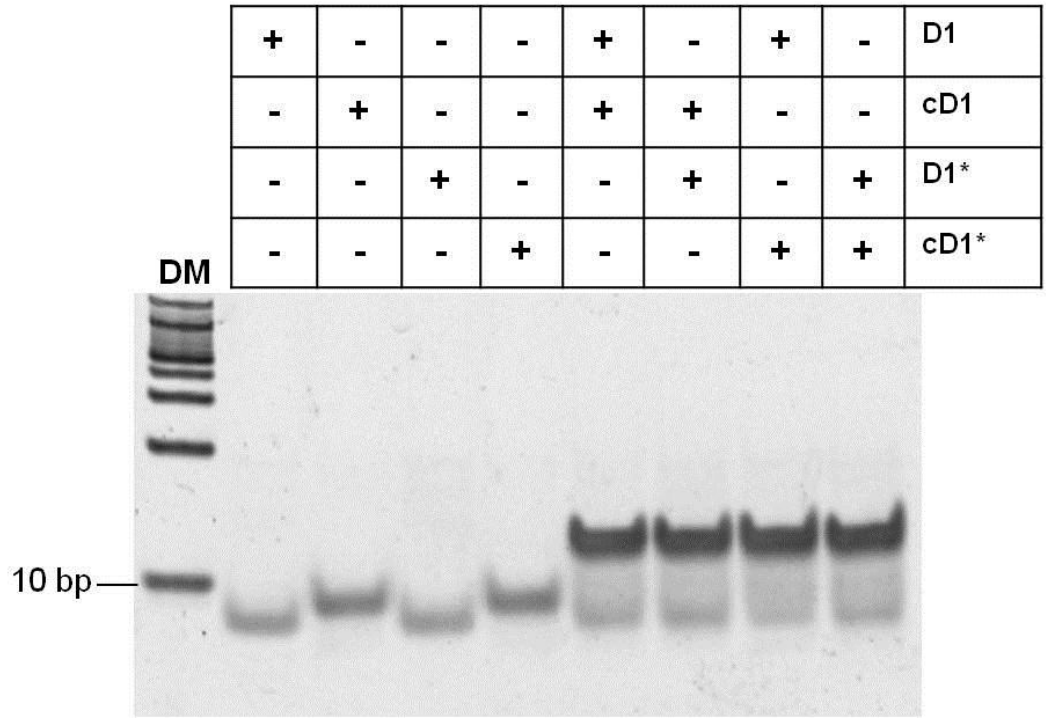


Figure S8: Figure 1: Characterization of the hybridisation efficiency of non irradiated D1 / cD1 and irradiated D1* / cD1* samples in 1:1 ratio. D1* and cD1* were irradiated overnight under the same experimental conditions, which are used for protein coupling. All samples could be hybridized successful. **Gel conditions:** 21% native PA-gel, **Running buffer**: 1xTBE.

2.3) Negative controls

To explore if any non specific reactions between unmodified molecules take place, we preformed different control reactions: **Mb-Mal** conjugate was incubated with **PE-D1** overnight in the dark without irradiation (figure S9A), native **Mb** was irradiated with unmodified **D1** (figure S9B) or **PE** modified (Figure S9D) at 320 nm over night and finally, **Mb-Mal** with unmodified **D1** was irradiated at 320 nm under the same experimental conditions as used for preparation of Mb-D1 conjugate.

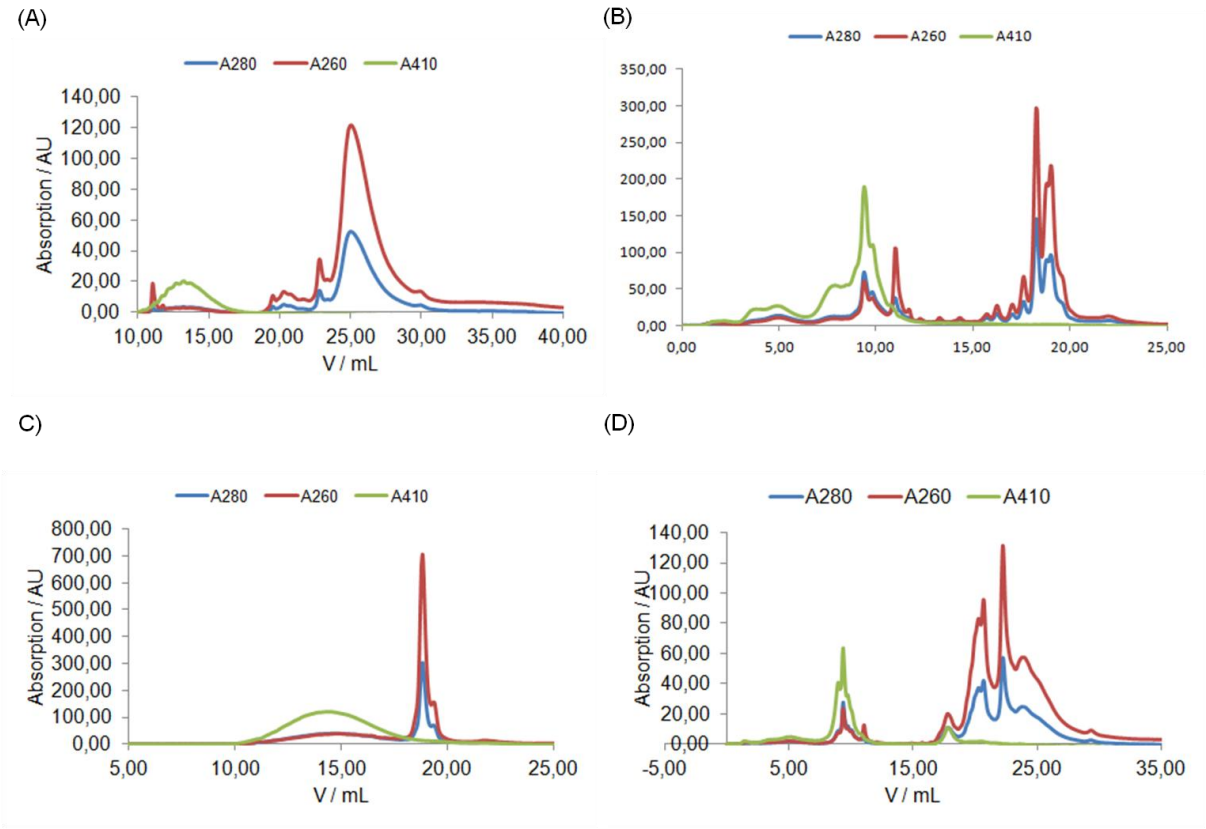


Figure S9. FPLC chromatogram of negative controls: Chromatogram **(A)** after incubation of **Mb-Mal** with **PE-D1** overnight without irradiation; **(B)** after incubation of **Mb** and **D1** under irradiation at 320 nm over night; **(C)** after incubation of **Mb-Mal** and **D1** under irradiation at 320 nm over night and **(D)** after incubation of **Mb** and **PE-D1** with irradiation at 320 nm over night.

References

[1] F. M. Hauser, S. R. Ellenberger, *Synthesis* **1987**, *1987*, 723.

[2] T. Pauloehrl, G. Delaittre, V. Winkler, A. Welle, M. Bruns, H. G. Börner, A. M. Greiner, M. Bastmeyer, C. Barner-Kowollik, *Angew. Chem. Int. Ed.* **2012**, *51*, 9181–9184.

[3] C.M. Niemeyer, M. Adler, D. Blohm, *Anal. Biochem.* **1997**, 246, 140-145.