Supporting Information

Photocleavable Polyethylene Glycol for the Light-Regulation of Protein Function

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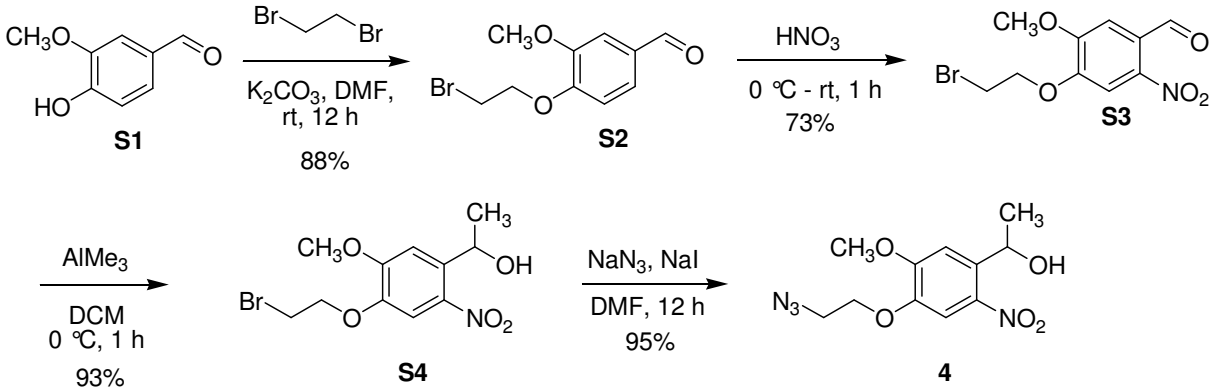
SYNTHESIS OF THE AZIDE 4

The synthesis of 1-[4-(2-azidoethoxy)-5-methoxy-2-nitrophenyl]ethanol (**4**) was accomplished in four steps starting with commercially available vanillin (**S1**). The bromide intermediate **S2** was prepared by alkylating the phenol functionality of **S1** with dibromoethane in the presence of a base (K_2CO_3, DMF; 88% yield). Subsequent nitration of **S2** to **S3** was achieved in 73% yield using concentrated HNO_3. The aldehyde **S3** was subsequently methylated to the secondary alcohol **S4** using $AlMe_3$ (DCM, 0 °C; 93% yield). The azide caging group **4** was obtained in 95% yield by nucleophilic substitution of the bromine with sodium azide (NaN_3, NaI, DMF). This molecule is very versatile since its azido and hydroxy groups can be reacted in an orthogonal fashion in order to covalently link two different molecules (e.g. a protein and a PEG), which can then be separated again using a brief light irradiation at 365 nm.



EXPERIMENTAL PROCEDURES

Synthesis of 4-(2-Bromoethoxy)-3-Methoxybenzaldehyde (S2). 1,2-Dibromoethane (7.4g, 3.4 mL, 39.3 mmol) was added to a solution of vanillin (**S1**) (2g, 13.1 mmol) in DMF (30 mL), containing K_2CO_3 (5.4g, 39.3 mmol). The reaction was stirred for 12 h at r.t. Subsequently, the reaction was filtered, diluted with ethyl ether (200 mL), and washed with 1 M NaOH (200 mL), water (2 × 200 mL), and brine (200 mL). The organic layer was dried over Na_2SO_4 and the volatiles were evaporated, affording 4-(2-bromoethoxy)-3-methoxybenzaldehyde (**S2**) in 88% yield as a white solid (2.99 g, 11.5 mmol). The analytical data matched previously reported data (^{1}H NMR, ^{13}C NMR, HRMS) [(1)].

Synthesis of 4-(2-Bromoethoxy)-5-methoxy-2-nitrobenzaldehyde (S3). 4-(2-Bromoethoxy)-3-methoxybenzaldehyde (**S2**) (3 g, 11.5 mmol) was suspended in 60 mL of ice-cold HNO_3. The solution was slowly warmed to room temperature, and the reaction was stirred until all of the material

dissolved. The solution was then poured onto ice and the precipitate was filtered off then washed with cold water. The product was recrystallized from boiling EtOH (approximately 30 mL), affording 4-(2-bromoethoxy)-5-methoxy-2-nitrobenzaldehyde (**S3**) in 73% yield as a yellow solid (2.55 g, 8.4 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.69 (q, *J* = 6.3 Hz, 2 H), 4.01 (s, 3 H), 4.47 (q, *J* = 6.3 Hz, 2 H), 7.41 (s, 1 H), 7.61 (s, 1 H), 10.43 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.0, 57.0, 69.6, 108.1, 110.6, 126.5, 143.7, 151.0, 153.9, 187.9. HRMS: *m/z* calcd for $C_{10}H_{10}NO_5Br$ $[M+H]^+$: 302.9742; found: 302.9743.

Synthesis of 1-[4-(2-Bromoethoxy)-5-methoxy-2-nitrophenyl]ethanol (S4). Trimethylaluminum (2.4 mL, 4.8 mmol; 2 M solution in hexanes) was added dropwise over 20 min to a solution of 4-(2-bromoethoxy)-5-methoxy-2-nitrobenzaldehyde (**S3**) (1 g, 3.18 mmol) in DCM (20 mL), at 0 ºC. The reaction was stirred at 0 ºC for 1 h and quenched with 2 mL of ice-cold water. 20 mL of 1 M NaOH were added and the mixture was stirred for 30 min. The reaction was taken into DCM (100 mL) and washed with 1 M NaOH (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and the volatiles were evaporated, giving 1-[4-(2-bromoethoxy)-5-methoxy-2-nitrophenyl]ethanol (**S4**) in 93% yield as a yellow solid (946 mg, 2.95 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.54 (d, *J* = 6.3 Hz, 3 H), 3.67 (t, *J* = 6.6 Hz, 2 H), 3.98 (s, 3 H), 4.38 (t, *J* = 6.6 Hz, 2 H), 6.43 (q, *J* = 6.3 Hz, 1 H), 7.32 (s, 1 H), 7.58 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.5, 28.5, 56.7, 65.9, 69.5, 109.4, 110.5, 126.2, 138.1, 146.3, 154.6. HRMS: *m/z* calcd for $C_{11}H_{14}NO_5Br$ $[M+H]^+$: 319.0055; found: 319.0054.

Synthesis of 1-[4-(2-azidoethoxy)-5-methoxy-2-nitrophenyl]ethanol (4). Sodium azide (100 mg, 1.56 mmol) and catalytic amounts of NaI (spatula tip) were added to a solution of 1-[4-(2-bromoethoxy)-5-methoxy-2-nitrophenyl]ethanol (**S4**) (50 mg, 0.156 mmol) in DMF (1 mL), and the reaction mixture was heated to 60 ºC for 16 h. After cooling to room temperature, the reaction mixture was filtered and diluted with EtOAc (5 mL). The reaction was washed with water (3 × 5 mL) and brine (5 mL). The organic layer was dried over Na_2SO_4 and the volatiles were evaporated, affording the azide 1-[4-(2-azidoethoxy)-5-methoxy-2-nitrophenyl]ethanol (**4**) as a yellow solid in 95% yield (42 mg, 0.148 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.54 (d, *J* = 6.3 Hz, 3 H), 3.67 (t, *J* = 5.4 Hz, 2 H), 3.97 (s, 3 H), 4.22 (t, *J* = 5.1 Hz, 2 H), 7.31 (s, 1 H), 7.57 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.5, 50.2, 56.6, 65.9, 68.6, 109.2, 110.0, 138.1, 139.5, 146.6, 154.6. HRMS: *m/z* calcd for $C_{11}H_{14}N_4O_5$ $[M+H]^+$: 282.0964; found: 282.0970.

Synthesis of photocleavable PEG reagent 6. The *N*-hydroxysuccinimide ester of pentynoic acid **2** (58.5 mg, 0.3 mmol)[2] and DMAP (spatula tip) were added to a solution of $mPEG_{5000Da}$-NH_2 (**1**, 300 mg, 0.06 mmol; LaysanBio) in dioxane (3 mL). The reaction was stirred at 40 °C for 12 h, and was then precipitated into Et_2O (30 mL). The suspension was centrifuged, the supernatant was poured off, and was repeated three times. Alkyne PEG **3** (302 mg, ~0.06 mmol) was obtained as a white solid, and directly dissolved in DMSO (1 mL). The azide caging reagent **4** (84 mg, 0.297 mmol), TBTA (15.6 mg, 0.0295 mmol), $CuSO_4$•$5H_2O$ (1.5 mg, 0.0059 mmol), and sodium ascorbate (13 mg, 0.068 mmol) were added. The reaction was stirred at 40 °C for 12 h, the DMSO was concentrated and the residue was re-dissolved in DCM (2mL). Et_2O (30 mL) was added and the suspension was centrifuged, the supernatant was poured off, and was repeated three times to afford PEG **5** (234 mg, ~0.044 mmol) as a tan solid. PEG reagent **5** (234 mg, 0.044 mmol) was subsequently dissolved in CH_3CN (2.6 mL) in the presence of cat. DMAP (spatula tip) and to the solution was added *N,N′*-disuccinimidyl carbonate (56 mg, 0.22 mmol). The reaction was stirred at 40 °C for 12 h, the CH_3CN was concentrated and the residue was re-dissolved in DCM (1mL). Et_2O (30 mL) was added and the suspension was centrifuged, the supernatant was poured off, and was repeated three times giving the activated PEG **6** (232 mg, ~0.044 mmol) as a tan solid.

Lysozyme PEGylation. Hen egg white lysozyme was dissolved in phosphate buffered saline (PBS, pH 7.5) to a final concentration of 450 µM. 50 equivalents of (NHS)-PEG_{5000Da} **7** or the photocleavable PEG reagent **6** were added as a solid to 100 µL of the lysozyme solution, and the reaction mixture was incubated overnight at 4 °C. The solution was then dialyzed (50 kDa MWCO, SpectraPor) over two days into PBS (pH 7.5) at 4 °C. The concentration of PEGylated lysozyme after dialysis was determined by a standard Bradford assay (3).

Lysozyme Assays. The lyophilized lysozyme substrate *Micrococcus lysodeikticus* (Sigma) was suspended in 66 mM sodium phosphate, pH 6.24, to an optical density at 450 nm of approximately 2.0, providing a 2× cell suspension stock. Wild-type or PEGylated lysozyme (100 µL of 6 µM enzyme solution in PBS) was added to the cell suspension (100 µL) for a final concentration of 3 µM enzyme. Prior to the assay, the Photo-PEG lysozyme was kept in the dark or was selectively irradiated in one well of a 96-well plate on a transilluminator (365 nm, 25 W, 5-30 min) pre-chilled to 0 °C with an ice pack. Wild type lysozyme was also irradiated in the same manner and showed no decrease in activity, indicating that irradiation causes no discernible photodamage to the enzyme. A_{450} values were measured every 12 seconds for 20 minutes (SpectraMax Plus^{384} UV plate reader, Molecular Devices), and all assays were performed in triplicate. Additionally, assays were performed with decreasing percentages of wild-type lysozyme to determine the percent activity restoration of Photo-PEGylated lysozyme. A_{450} values were then measured every 12 seconds for 20 minutes (Figure S1), and the V_{max} values calculated from the initial slope of each reaction (first 72 sec, linear regression resulted in $R > 0.998$) for wild-type lysozyme and PhotoPEGylated lysozyme activated by the optimal UV irradiation (365 nm, 25 W, 30 min). The obtained velocities were: V_{max}(wild-type) = $(263 \pm 6)\times10^{-5}$ s^{-1} and V_{max}(PhotoPEG+UV) = $(132 \pm 4)\times10^{-5}$ s^{-1}. Thus, the light-activation of Photo-PEGylated lysozyme was found to be 50% of wild-type lysozyme activity.

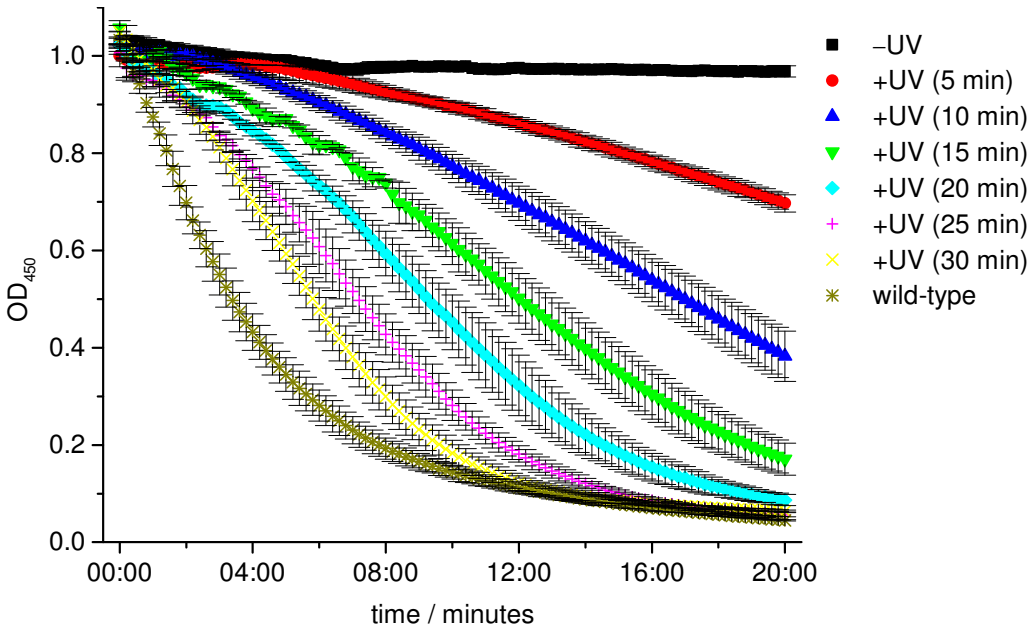


Figure S1. Time-course of the lysozyme assay for PEGylated and UV irradiated protein, as described above.

SDS-PAGE Analysis. To ensure complete cleavage of Photo-PEG upon irradiation, PEGylated lysozyme was analyzed by denaturing polyacrylamide electrophoresis (SDS-PAGE). 100 ng wild-type and PEGylated lysozyme (non-irradiated and irradiated) were mixed with 4× SDS loading buffer (final concentration 10% (v:v) glycerol, 62.5 mM Tris-HCl, pH 6.8, 2% (w:v) SDS, 0.01 mg/mL bromophenol blue, and 5% ethanethiol) and separated on a 13% SDS-PAGE running gel overlaid with a 4% stacking gel at 8 mA until the buffer loading dye was clear of the stacking gel, then at 14 mA for 45 minutes. The low amperage was determined to provide more compact protein bands and eliminate streakiness of the highly PEGylated proteins. The gel was then stained (0.006 g coomassie brilliant blue, 100 mL glacial acetic acid and 900 mL H_2O) for two hours, followed by incubation in the destaining solution (100 mL glacial acetic acid and 900 mL H_2O) for at least two hours.

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