"Clickable" elastins : Elastin-like polypeptides functionalized with azide or alkyne groups - Supplementary information

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Nomenclature

The ELP construct can be described using the notation ELP[$V_5L_2G_3$-90][1], where the capitals between the brackets represent the single letter amino acid code replacing guest residue Xaa in the pentapeptide ValProGyXaaGly. The subscript stands for the number of guest residues in the monomer gene, and the ELP consists of 90 pentapeptide repeats.

Cloning

Standard molecular biology protocols were used for gene synthesis and oligomerization. Digested inserts and linearized vectors were purified by agarose gel electrophoresis (QIAquick Gel Extraction Kit, Qiagen, Valencia, CA). All clones were maintained in *E. coli* XL1-Blue.

ELP gene synthesis

A synthetic gene for a 10-polypentapeptide ELP was constructed from four $X_iY_jZ_k$ synthetic oligonucleotides (Fig. S1). The oligonucleotides were annealed to form double-stranded DNA with BsmBI- and EcoRI-compatible ends, phosphorylated, and ligated into BsmBI/EcoRI linearized, and dephosphorylated pMTL23-δ-BsaI-aIII. This vector was constructed from two synthetic aIII oligonucleotides (Fig. S1), that were annealed to form double-stranded DNA with XhoI- and EcoRI-compatible ends, and ligated into

XhoI/EcoRI linearized pMTL23-δ-BsaI[2] (BsaI site removed from Amp gene). The DNA sequence of the insert was verified by DNA sequencing. For a typical oligomerization, the vector was linearized with BsmFI, and enzymatically dephosphorylated. The insert was doubly digested with BsmFI and FokI, and ligated into the linearized vector. This was repeated until the ELP gene had 90 repeats of the pentapeptide, resulting in vector pMTL23-δ-BsaI-aIII-ELP. Colonies were screened by PCR, and the DNA sequence of inserts was verified by DNA sequencing.

Adapter gene synthesis

The genes for adapterXX were constructed from synthetic Adapter oligonucleotides (Fig. S1). The oligonucleotides were annealed to form double-stranded DNA with XhoI- and EcoRI-compatible ends, and ligated into XhoI/EcoRI linearized pMTL23-δ-BsaI. Colonies were screened by PCR and restriction analysis with SmaI, and the DNA sequence of inserts was verified by DNA sequencing. The ELP genes were doubly digested from pMTL23-δ-BsaI-aIII-ELP with BsmFI and EcoRI, and ligated into BsmFI/EcoRI linearized and dephosphorylated pMTL23-δ-BsaI-XX, resulting in pMTL23-δ-BsaI-XX-ELP. Colonies were screened by PCR, and the DNA sequence of inserts was verified by DNA sequencing.

Expression vector pET15b

The ELP gene was doubly digested with XhoI/BamHI and ligated into XhoI/BamHI linearized expression vector pET15b (Novagen). Colonies were screened by restriction analysis with XhoI/BamHI.

Resulting ELP sequence

M G S S H H H H H H S S G L V P R G S H **M** L E K R E A E A G P (V P G G G V P G V G V P G V G V P G G G V P G L G V P G V G V P G V G V P G V G V P G G G V P G L G)$_9$V P G G G A

aIII

Sense 5'-TCGAGAAAAGAGAGGCTGAAGCGGGACGTCTCGGTGCCTAACATCCG-3'

anti-sense 5'-AATTCGGATGTTAGGCACCGAGACGTCCCGCTTCAGCCTCTCTTTTC-3'

$V_5L_2G_3$-10

Sense I

5'-
GTGCTGGTGGTGTTCCGGGCGTCGGTGTTCCTGGAGTCGGTGTTCCAGGTGGAGGTGTTCCAGGATTGGGTGTTCCTGG
TGTAGGTG-3'

Anti-sense I

5'-
GGAACACCTACACCAGGAACACCCAATCCTGGAACACCTCCACCTGGAACACCGACTCCAGGAACACCGACGCCCGGA
ACACCACCA-3'

Sense II

5'-
TTCCTGGTGTTGGTGTTCCAGGTGTTGGTGTTCCAGGTGGAGGTGTTCCTGGTTTGGGAGTTCCTGGTGGTGGTGCCTAA
CATCCG-3'

Anti-sense II

5'-
AATTCGGATGTTAGGCACCACCACCAGGAACTCCCAAACCAGGAACACCTCCACCTGGAACACCAACACCTGGAACAC
CAACACCA-3'

AdapterXX

Sense 5'-TCGAGAAAAGAGAGGCTGAAGCGGGACCAGTTCCTGGTGGTGCCTAACATCCG-3'

Anti-sense 5'-AATTCGGATGTTAGGCACCACCAGGAACTGGTCCCGCTTCAGCCTCTCTTTTC-3'

Adapter FP

Sense 5'-GTGCTGGTGGACCGGTGTAACATCCGAGCGGCCGCCATCCG-3'

Anti-sense 5'-AATTCGGATGGCGGCCGCTCGGATGTTACACCGGTCCACCA-3'

Figure S1. DNA sequences of the used oligonucleotides (Eurogentec, Seraing, Belgium).

Protein expression

PET15b-ELP was transformed into the *E. coli* expression strain BLR(DE3) (Novagen). For a typical expression a 100 mL Luria Broth culture, supplemented with 100 μg/mL ampicillin and 12.5 μg/mL tetracycline, was inoculated with a single colony and incubated at 37 °C overnight. The overnight culture was diluted to an OD_{600} of 0.1 in a 0.5 L LB culture supplemented with 100 μg/mL ampicillin and 12.5 μg/mL tetracycline,

and incubated at 37 °C. At an OD_{600} of 0.6 the expression was induced by the addition of IPTG to a final concentration of 1 mM. After incubation at 25 °C overnight the cultures were harvested by centrifugation (18,000 x g, 4 °C). The cell pellet was re-suspended in PBS, and incubated with lysozyme (1 mg/mL in PBS) at 4 °C for 30 min. The cells were then lysed by ultrasonic disruption at 4 °C. The lysate was centrifuged (15 min, 4600 rpm, 4 °C Minifuge RF, Heraeus Sepatech, Germany) to remove insoluble material.

Auxotroph expression

PET15b-ELP was transformed into the methionine auxotroph expression strain B834(DE3)pLysS (Novagen). For a typical expression a 100 mL Luria Broth culture, supplemented with 100 μg/mL ampicillin and 50 μg/mL chloramphenicol, was inoculated with a single colony and incubated at 37 °C overnight. The overnight culture was diluted to an OD_{600} of 0.1 into 0.5 L M9 minimal medium supplemented with all 20 natural amino acids (40 mg/L each), thiamine (0.0005%), ampicillin (100 μg/mL) and chloramphenicol (50 μg/mL), and incubated at 37 °C to OD_{600} of 0.8. To induce synthesis of T7 polymerase in the presence of methionine, isopropyl-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM, and the culture was incubated for 15 min at 37 °C. Cells were spun down (10 min, 4600 rpm, 4 °C Minifuge RF, Heraeus Sepatech, Germany), washed twice in cold NaCl (0.9%), and re-suspended in 0.5 L M9 minimal medium supplemented with 19 natural amino acids (40 mg/L each, no methionine), thiamine (0.0005%), ampicillin (100 μg/mL) and chloramphenicol (50 μg/mL). After incubation for 10 min at 37 ºC, the culture was supplemented with azidohomoalanine (AHA)[3] or homopropargylglycine (HPG; Chiralix, Nijmegen, The Netherlands) (40 mg/L) and IPTG (1 mM), followed by incubation overnight at 25 °C. Cells were harvested by centrifugation (18,000 x g, 4 °C).

ELP purification

The ELPs were purified by inverse transition cycling.[4] In short, ELPs were aggregated by adding NaCl to a concentration of 1M and increasing the temperature of the cell lysate to 65 °C. The aggregated protein was separated from the solution by centrifugation at 40 °C (10 min, 4600 rpm, Multifuge, Heraeus Sepatech, Germany). The supernatant was

decanted and discarded while the pellet containing the fusion protein was re-suspended in cold PBS. The re-solubilized pellet was then centrifuged at 4 °C (10 min, 4600 rpm) to remove any remaining insoluble material. The inverse transition cycling was repeated, yielding typically 10 mg purified protein/L culture.

Characterization of proteins

The SDS-PAGE analysis used a Mini-PROTEAN system (Bio-Rad, Hercules, CA) with 7.5% or 10% gels, stained with Coomassie brilliant blue. Protein concentrations were determined by bicinchonic acid assay (Pierce Chemical Co., Rockford, IL).

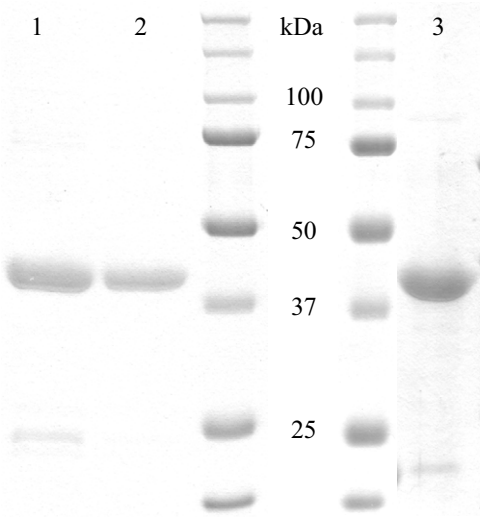


Figure S2. Typical SDS-PAGE coomassie stained gel after purification. 1) AHA-ELP, 2) HPG-ELP, 3) Met-ELP.

MALDI-TOF mass spectrometry measurements on the whole proteins were performed on a Bruker Biflex III spectrometer with 2,5-dihydroxyacetophenone (DHAP) as matrix.[5]

Table S1. Calculated and measured molecular weights of proteins, determined by MALDI-TOF.

Protein	Calculated (Da)	Measured (Da)
Met-ELP	39802	39390
AHA-ELP	39792	39497

HPG-ELP	39758	39551

To detect incorporation of AHA and HPG tryptic digests were performed and analyzed by MALDI-TOF mass spectrometry. Sequencing-grade modified trypsin (0.5 µg, Promega) was added to the ELPs in PBS. After incubation for 3h at RT the tryptic digests were analyzed with α-cyanohydroxycinnamic acid (Sigma) as matrix. In all samples the *N*-terminal methionine deletion[6, 7] was clearly observed.

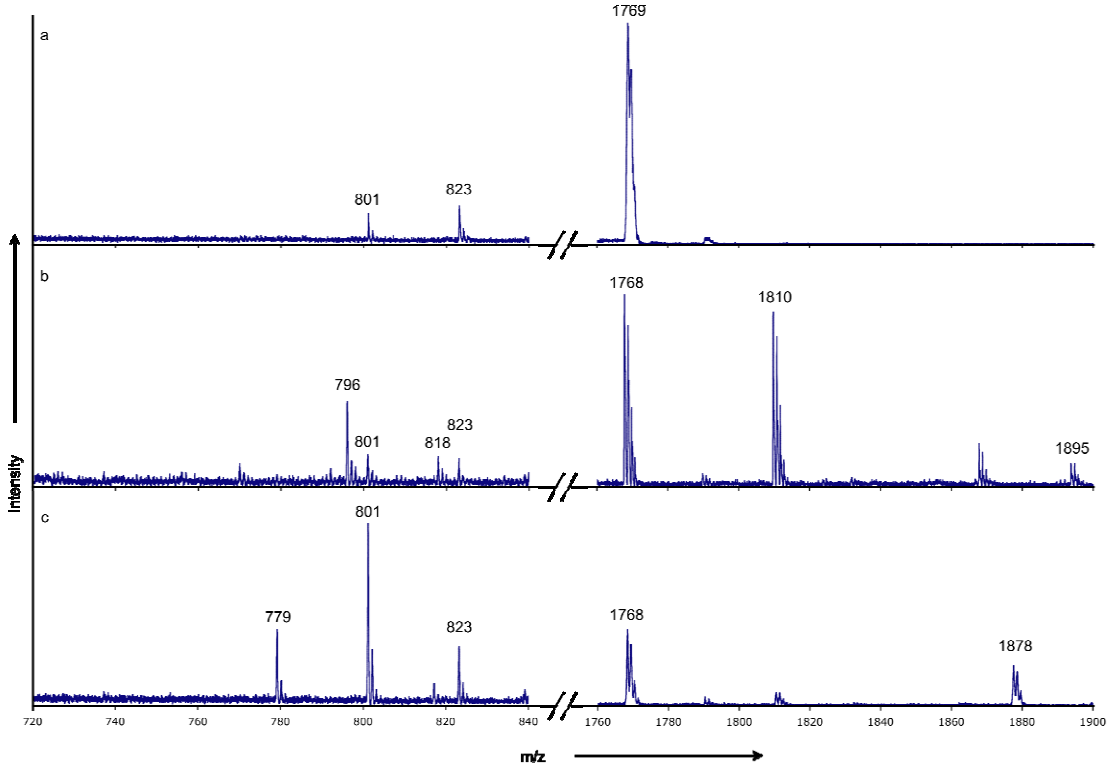


Figure S3. MALDI-TOF spectra of two peptide fragments derived from tryptic digest of Met-ELP (a), AHA-ELP (b), HPG-ELP (c). The peak at 801/796/779 originates from the peptide fragment with residue 18-24. The peak at 1769/1895/1878 comes from the peptide fragment with residue 1-17.

The optical absorbance at 350 nm of ELPs was measured in the 15-65 °C range on a Jasco J-810 spectropolarimeter (band width: 1 nm, response: 1 sec., sensitivity: standard,

heating rate: 1 °C min^{-1}) equipped with a PFD-425s Peltier temperature controller (Jasco).

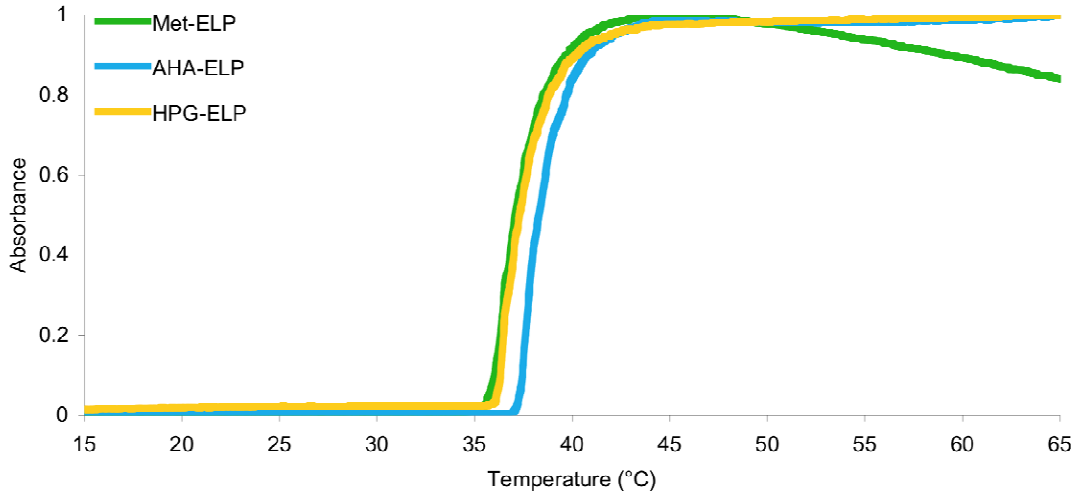
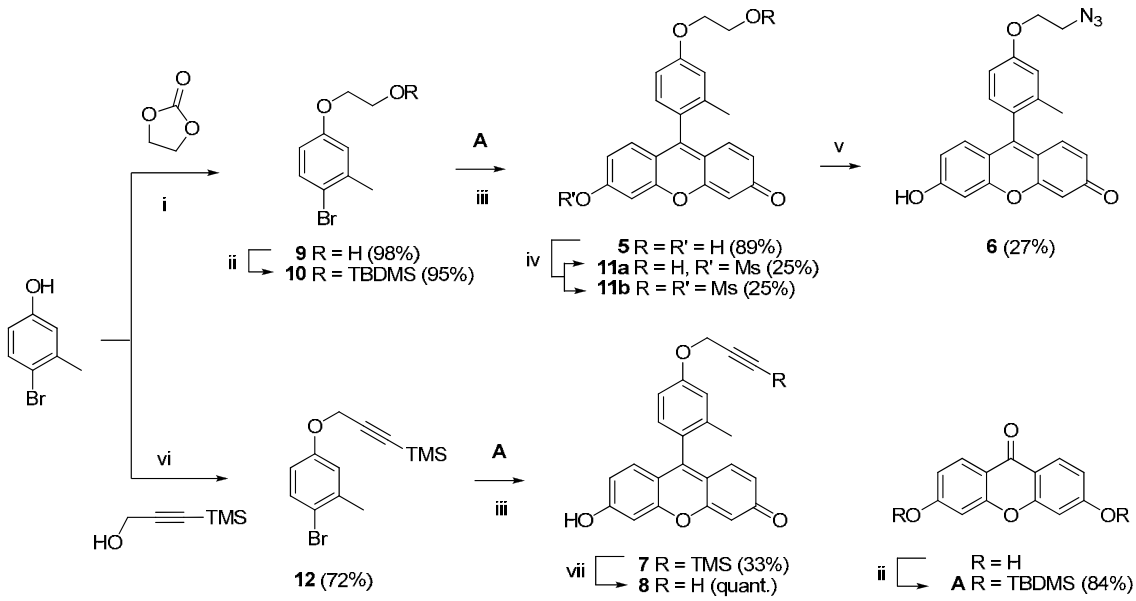


Figure S4. Normalized turbidity profile of Met-ELP, AHA-ELP, and HPG-ELP (each 0.1 mg/mL) in PBS measured at 350 nm.

Syntheses of fluorescent probes

The synthesis of ClickGreen derivatives **6** and **8** commenced with the functionalization of 4-bromo-3-methylphenol (Scheme S1). As a result compound **9** was obtained in excellent yield (95%) by reacting 4-bromo-3-methylphenol with ethylene carbonate. The alcohol moiety was subsequently protected with a silyl protecting group yielding **10** (95%). Next, a Grignard reaction between **10** and TBDMS protected xanthone **A** was performed followed by the *in situ* deprotection resulting in desired product **5** in excellent yield (89%). Transformation of primary alcohol into a good leaving group was envisioned to proceed smoothly using a small excess mesyl chloride. Surprisingly, the phenolic hydroxyl group turned out to be more reactive than the primary alcohol resulting in a mixture of mono and di-mesylated compounds **11a** and **11b** (both in 25%). Subjecting compound **11a** to NaN_3 at a temperature of 60 °C resulted in mesyl substitution and *in situ* deprotection generating azido-ClickGreen **6** (27%).



Scheme S1. Synthesis of alkyne and azido fluorophores. *Reagent and conditions*: i) K_2CO_3, toluene, Ar-atm., 115 °C, 24 h; ii) TBDMS-Cl, DMF, imidazole, r.t. 2 h; iii) a) Mg, $EtBr_2$, Et_2O, r.t., 2 h, b) **A** (at 0 °C), THF, r.t., 1.5 h, c) MeOH, TFA, r.t., 30 min; iv) Ms-Cl, CH_2Cl_2, DMAP, 0 °C to r.t., 4 h; v) NaN_3, DMF, 60 °C, 36 h; vi) DiAD, PPh_3, CH_2Cl_2, 0 °C to r.t. 48 h; vii) K_2CO_3, MeOH, r.t., 18 h.

Introduction of the TMS-protected propynol onto 4-bromo-3-methylphenol was achieved *via* a Mitsunobu reaction yielding **12** in a good yield (72%). Performing the Grignard reaction with **12** and xanthone **A** and subsequent hydrolysis of the TBDMS group gave TMS-protected compound **7**. In the final step the TMS group was removed resulting in acetylene-ClickGreen **8** in quantitative yield.

Materials

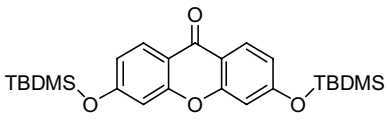
All chemicals were obtained from commercial sources and used without further purification, unless otherwise stated. Analytical thin layer chromatography (TLC) was performed on *Merck* precoated silica gel 60 F-254 plates (layer thickness 0.25 mm) with the indicated eluent and visualization by ultraviolet (UV) irradiation at λ = 254 nm and/or λ = 366 nm. Preparative thin layer chromatography (Prep-TLC) was performed on *Merck* precoated silica gel 60 F-254 plates (layer thickness 1.00 mm) with concentration zone

and visualization by UV irradiation at λ = 254 nm and/or λ = 366 nm. Purification by silica gel chromatography was carried out using *Acros* (0.035 – 0.070 mm, pore diameter ca. 6 nm) silica gel. THF was distilled under nitrogen from sodium/benzophenone. CH_2Cl_2 was distilled under nitrogen from CaH_2.

General analytical techniques

NMR spectra were recorded on *Bruker DMX300* (300 MHz and 75 MHz for ^1H and ^{13}C, respectively) and *Varian Inova 400* (400 MHz for ^1H) spectrometers. ^1H-NMR chemical shifts (δ) are reported in parts per million (ppm) relative to a residual proton peak of the solvent; δ = 7.26 for $CDCl_3$ or δ = 3.31 for CD_3OD. Multiplicities are reported as: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), or m (multiplet). Broad peaks are indicated by the addition of br. Infrared (IR) spectra were recorded on an *ATI Matson Genesis Series FTIR* spectrometer fitted with an ATR cell. The vibrations (ν) are given in cm^{-1}. Matrix assisted laser desorption/ionisation time-of-flight (MALDI-ToF) spectra were measured on a *Bruker Biflex III* spectrometer and samples were prepared from MeOH solutions using indoleacrylic acid (IAA) (20 mg/mL) as a matrix. LCQ/MS analysis was performed using *Thermo scientific Advantage LCQ* Lineair-Iontrap Electrospray (ESI-MS). Electrospray ionisation time-of-flight (ESI-ToF) spectra were measured with a *JEOL AccuToF*.

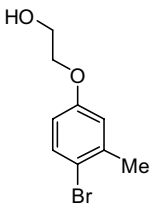
3,6-bis(*tert*-butyldimethylsilyloxy)-9H-xanthen-9-one (**A**)



3,6-Dihydroxy-9H-xanthen-9-one (0.500 g, 2.20 mmol) was dissolved in dry DMF (45 mL) where after TBDMS chloride (1.99 g, 13.2 mmol) and imidazole (1.50 g, 22.0 mmol) were added. After stirring at room temperature for 2h, the reaction mixture was diluted with toluene, washed extensively three times with water and dried over Na_2SO_4. Evaporation *in vacuo* left a light brown solid, which was recrystallized from ethanol to give **A** as off white needle crystals (0.841 g, 84% yield). R_F = 0.90 (*n*-heptane/EtOAc, 1:2); ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.20 (td, *J* = 9.1, 1.2, 1.2 Hz, 2H), 6.85 (dd, *J* = 9.2, 2.2 Hz, 2H), 6.84 (s, 2H), 1.01 (s, 18H), 0.29 (s, 12H) ppm.

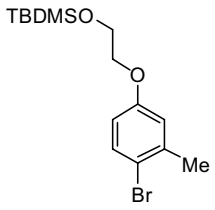
^{13}C NMR ($CDCl_3$, 50 MHz) δ: 161.3, 159.1, 157.7, 128.2, 117.6, 116.4, 107.3, 25.5, 18.3, -4.4 ppm. FT-IR ν_{max}: 2924, 16.15, 1279,1270, 850, 840 cm^{-1}. HRMS (CI+) *m/z* calcd for $C_{25}H_{37}O_4Si_2$ 457.2234, found 457.2230 $[M+H]^+$.

2-(4-Bromo-3-methoxyphenoxy)ethanol (**9**)



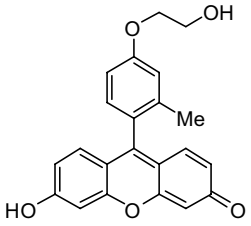
Modified literature procedure[8]: Under an Ar-atmosphere, K_2CO_3 (207 mg, 1.50 mmol) was added to a solution of 3-methyl-4-bromophenol (134 mg, 0.75 mmol) and ethylene carbonate (264 mg, 3.00 mmol) in dry toluene (5 mL). The mixture was heated to 115 °C and stirred for 24 hours. After completion, water (20 mL) was added and the emulsion was extract with EtOAc (2 × 25 mL). The organic layers were combined, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. Further purification was accomplished by column chromatography (*n*-heptane/EtOAc, 2:1) to afford **9** as an off-white semisolid (169 mg, 98%). R_F = 0.48 (*n*-heptane/EtOAc, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 (d, *J* = 8.7 Hz, 1H), 6.81 (d, *J* = 3.0 Hz, 1H), 6.63 (dd, *J* = 8.7, 3.0 Hz, 1H), 4.05 (m, 2H), 3.95 (m, 2H), 2.36 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 157.7, 138.9, 132.8, 117.1, 115.8, 113.5, 69.3, 61.3, 23.1 ppm. FT-IR ν_{max} film: 3382 (br), 2911, 2358, 2336, 1476 (s), 1238, 1027 cm^{-1}. HRMS (EI+) *m/z* calcd for $C_9H_{11}O_2Br$ $[M]^{+\bullet}$ 229.9942, found 229.9945.

(2-(4-Bromo-3-methylphenoxy)ethoxy)(*tert*-butyl)dimethylsilane (**10**)



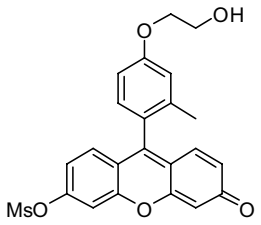
To a stirred solution of 2-(4-bromo-3-methylphenoxy)ethanol (**9**, 1,00 g, 4.35 mmol) in DMF (30 mL) were added TBDMS-Cl (978 mg, 6.52 mmol) and imidazole (888 mg, 13.0 mmol). The reaction was finished after 1.5 hours stirring at room temperature. DMF was evaporated *in vacuo* and column chromatography (*n*-heptane/EtOAc, 2:1) afforded **C** as a colorless oil (1.48 g, 99%). R_F = 0.86 (*n*-heptane/EtOAc, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38 (d, *J* = 8.7 Hz, 1H), 6.80 (d, *J* = 3.0, 1H), 6.62 (dd, *J* = 8.7, 3.0 Hz, 1H), 4.00 (m, 2H), 3.95 (m, 2H), 2.36 (s, 3H), 0.91 (s, 9H), 0.10 (s, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ : 158.2, 138.7, 132.7, 117.2, 115.4, 113.6, 69.5, 62.0, 25.9, 23.1, 18.4, -5.2 ppm. FT-IR ν_{max} film: 2923, 1473 (s), 1241, 1128, 828, 776 cm^{-1}. HRMS (ESI+) *m/z* calcd for $C_{15}H_{26}O_2BrSi$ $[M+H]^+$ 345.0885, found 345.0907.

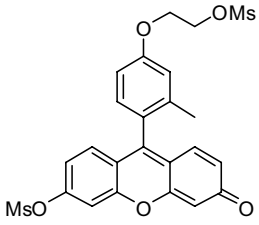
6-Hydroxy-9-(4-(2-hydroxyethoxy)-2-methylphenyl)-3H-xanthen-3-one (**5**)



Modified literature procedure[9]: In a flame-dried Schlenk tube under an Ar-atmosphere, dried magnesium powder (36.5 mg, 1.50 mmol) was suspended in a minute quantity of dry Et_2O. After activation of the Mg with a drop of 1,2-dibromoethane, compound **10** (329 mg, 0.96 mmol) dissolved in dry Et_2O (1.5 mL) was slowly added to the mixture while gas formation was maintained by interval warming with a heat gun. When no more gas formation was observed, the mixture was stirred for 30 min at room temperature and was then cooled to 0 °C. Compound **A** (325 mg, 0.71 mmol) dissolved in dry THF (4 mL) was added drop wise to the reaction mixture. Upon warming to room temperature, the color changed from yellow to brownish to deep purple in 1.5 hours. The mixture was quenched with CH_3OH and the remaining Mg was filtered off. Deprotection of the TBDMS groups with aqueous HCl (2 M, 8 mL) took 10 min and was followed by TLC. After complete deprotection, H_2O (20 mL) was added and the mixture extracted with EtOAc (3 × 40 mL). The organic layers were combined and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and further purification was performed by column chromatography over silica gel (CH_3OH/CH_2Cl_2, 1:19). The resulting orange sticky oil was lyophilized to obtain compound **5** as a red-orange fluffy solid (211 mg, 82%). R_F = 0.30 (CH_3OH/CH_2Cl_2, 1:9); ^{1}H NMR (400 MHz, CD_3OD) δ: 7.24 (d, J = 9.2 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 7.09 (d, J = 2.3 Hz, 1H), 7.05 (dd, J = 8.4, 2.5 Hz, 1H), 6.86 (d, J = 2.2 Hz, 1H), 6.84 (dd, J = 9.2, 2.2 Hz, 1H), 4.17-4.15 (m, 2H), 3.94-3.92 (m, 2H), 2.04 (s, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ: 161.8, 159.7 (2C), 158.6, 158.5, 139.1, 132.9 (2C), 131.6, 125.6, 122.5, 122.5, 117.8, 117.2 (2C), 113.5, 104.2 (2C), 70.8, 61.7, 20.1 ppm. FT-IR v_{max} film: 3377 (br), 2915, 1592 (s), 1461, 1382, 1244, 1207, 1109, 621, 609 cm^{-1}. HRMS (ESI+) *m/z* calcd for $C_{22}H_{18}NaO_5$ $[M+Na]^+$ 385.1052, found 385.1024.

9-(4-(2-Hydroxyethoxy)-2-methylphenyl)-6-(mesylate)-*3H*-xanthen-3-one (**11a**) and 9-(4-(2-hydroxyethyl mesylate)-2-methylphenyl)-6-(mesylate)-*3H*-xanthen-3-one (**11b**)



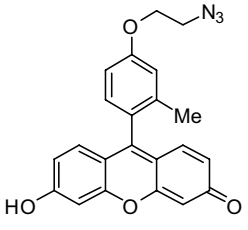


Compound **5** (100 mg, 0.28 mmol) was suspended in dry CH_2Cl_2 (12 mL) in a flame-dried Schlenk tube under an N_2-atmosphere. The suspension was cooled to 0 °C and DMAP (135 mg, 1.10 mmol) was added. After 5 min methanesulfonyl chloride (MsCl, 85.4 μL, 1.10 mmol) was added. The suspension was stirred for 30 min at 0 °C, warmed to room temperature and then stirred for an additional 16 hours to obtain a clear solution. Additional CH_2Cl_2 (15 mL) was added and the solution was washed with H_2O (15 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL) and the organic layers were combined, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude mixture was purified by gradient column chromatography over silica gel (CH_3OH/CH_2Cl_2, 1:40 to 1:15) and afforded **11a** and **11b** separately as orange sticky oils (30 mg (25%) and 35 mg (25%), respectively).

Analytical data for compound **11a**: R_F = 0.55 (CH_3OH:CH_2Cl_2, 1:9); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.40 (dd, *J* = 1.9, 0.8 Hz, 1H), 7.16-7.12 (m, 2H), 7.08 (d, *J* = 8.2 Hz, 1H), 7.01 (d, *J* = 9.8 Hz, 1H), 6.97-6.96 (m, 1H), 6.94 (dd, *J* = 8.0, 2.6 Hz, 1H), 6.59 (dd, *J* = 9.8, 1.9 Hz, 1H), 6.43 (d, *J* = 1.9 Hz, 1H), 4.20-4.16 (m, 2H), 4.06-4.02 (m, 2H), 3.25 (s, 3H), 2.06 (s, 3H) ppm. LRMS (ESI+) *m/z* calcd for $C_{23}H_{21}O_7S$ $[M+H]^+$ 441.1, found 441.2.

Analytical data for compound **11b**: R_F = 0.60 (CH_3OH:CH_2Cl_2, 1:9); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.41-7.40 (m, 1H), 7.18-7.13 (m, 1H), 7.13-7.12 (m, 1H), 7.09 (d, *J* = 8.2 Hz, 1H), 7.01-6.96 (m, 2H), 6.93 (dd, *J* = 8.1, 2.5 Hz, 1H), 6.59 (dd, *J* = 9.5, 1.9 Hz), 6.44 (d, *J* = 1.9 Hz, 1H), 4.66-4.62 (m, 2H), 4.36-4.34 (m, 2H), 3.26 (s, 3H), 3.15 (s, 3H), 2.07 (s, 3H) ppm. LRMS (ESI+) *m/z* calcd for $C_{24}H_{23}O_9S_2$ $[M+H]^+$ 519.1, found 519.1.

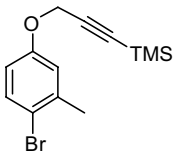
9-(4-(2-Azidoethoxy)-2-methylphenyl)-6-hydroxy-3H-xanthen-3-one (**6**)



Compound **11a** (30 mg, 0.068 mmol) and sodium azide (22 mg, 0.338 mmol) were dissolved in DMF (8 mL). The reaction mixture was warmed to 60 °C and stirred for 36 hours. A similar workup procedure utilized for the previous methods afforded azido-ClickGreen **6** as an orange-red solid after lyophilization from

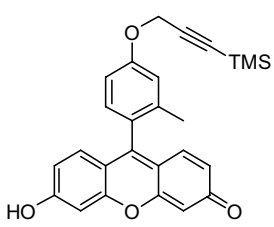
dioxane and H_2O (7 mg, 27%). R_F = 0.35 (CH_3OH/CH_2Cl_2, 1:9); ^1H NMR (400 MHz, CD_3OD) δ: 7.18 (d, *J* = 8.4 Hz, 1H), 7.11 (d, *J* = 9.8 Hz, 2H), 7.08 (d, *J* = 2.5 Hz, 1H), 7.03 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.71 (d, *J* = 2.0 Hz, 2H), 6.70 (ddd, *J* = 9.7, 2.2, 0.4 Hz, 2H), 4.29 (t, *J* = 4.8 Hz, 2H), 3.66 (t, *J* = 4.8 Hz, 2H), 2.04 (s, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ: 184.5, 161.0, 159.5 (2C), 156.1 (2C), 139.2, 132.3 (2C) 131.6, 126.5, 123.11 (2C), 117.7, 116.2 (2C), 104.5 (2C), 68.6, 51.4, 20.0 ppm. FT-IR ν_{max} film: 3317 (br), 2915, 2107, 1593 (s), 1502, 1462 (s), 1282, 1242, 1204, 1104, 845, 616 cm^{-1}. HRMS (ESI+) *m/z* calcd for $C_{22}H_{18}N_3O_4$ $[M+H]^+$ 388.1295, found 388.1297.

(3-(4-bromo-3-methylphenoxy)prop-1-ynyl)trimethylsilane (**12**)



Under an N_2-atmosphere, 3-methyl-4-bromophenol (93 mg, 0.50 mmol), propargyl alcohol (64 mg, 73.1 μL, 0.50 mmol) and PPh_3 (137.6 mg, 0.525 mmol) were dissolved in dry CH_2Cl_2 (5 mL). The mixture was cooled to 0 °C and diisopropyl azodicarboxylate (DiAD, 107 μL, 0.55 mmol) was added drop wise. The reaction was allowed to warm to r.t. and was subsequently stirred for 48 h. After completion, HCl (1M, 5 mL) was added. The water layer was washed once with CH_2Cl_2 (5 mL) and the combined organic layers were subsequently washed with aq. $NaHCO_3$ (sat. 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. Further purification was accomplished by column chromatography (*n*-heptane/EtOAc, 3:1) to afford **12** as colorless oil (106 mg, 72%). R_F = 0.78 (*n*-heptane/EtOAc, 3:1); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.41 (d, *J* = 8.8 Hz, 1H), 6.87 (d, *J* = 2.7 Hz, 1H), 6.69 (dd, *J* = 8.8, 3.0 Hz, 1H), 4.63 (s, 2H), 2.37 (s, 3H), 0.17 (s, 9H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ: 156.9, 138.8, 117.6, 116.2, 113.9, 99.7, 93.0, 56.8, 23.1, -0.3 (3C) ppm. Both HRMS and LRMS techniques were employed to acquire the mass of the described compound. Unfortunately, none of the techniques used gave a comprehensible mass spectrum.

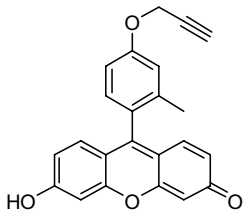
9-(4-(3-(TMS)-prop-2-ynyloxy)-2-methylphenyl)-6-hydroxy-3H-xanthen-3-one (**7**)



In a flame-dried Schlenk tube under an Ar-atmosphere, dried magnesium powder (8.6 mg, 0.36 mmol) was suspended in a

minute quantity of dry Et_2O. After activation of the Mg with a drop of 1,2-dibromoethane, compound **12** (106 mg, 0.36 mmol) dissolved in dry Et_2O (2 mL) was slowly added to the mixture while gas formation was maintained by interval warming with a heat gun. When no more gas formation was observed, the mixture was stirred for 30 min at r.t. and then cooled to 0 °C. Compound **A** (114 mg, 0.25 mmol) dissolved in dry THF (2 mL) was added drop wise to the reaction mixture. Upon warming to room temperature, the color changed from yellow to brownish to deep purple during 1.5 hours. The mixture was quenched with CH_3OH and the remaining Mg was filtered off. Deprotection of the TBDMS groups with aqueous HCl (2 M, 5 mL) took approximately 10 min and was followed by TLC. After complete deprotection, H_2O (20 mL) was added and the mixture extracted with EtOAc (3 × 20 mL). The organic layers were combined and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and further purification was performed by gradient column chromatography over silica gel (CH_2Cl_2/CH_3OH, 95:5 / 9:1). The resulting orange sticky oil (*i.e.* compound **G**, (35 mg, 33%)) was taken trough to the next step without extensive characterization. R_F = 0.46 (CH_3OH/CH_2Cl_2, 1:9). LRMS (ESI+) *m/z* calcd for $C_{26}H_{25}O_4Si$ $[M+H]^+$ 429.6, found 429.3.

6-Hydroxy-9-(2-methyl-4-(prop-2-ynyloxy)phenyl)-3H-xanthen-3-one (**8**)



Compound **7** (35 mg, 0.08 mmol) was dissoveld in CH_3OH (5 mL) followed by the addition K_2CO_3 (40 mg, 0.29 mmol). The reaction mixture was stirred for 18 hours at r.t. Since no R_F difference was observered for the product and the starting material, the reaction was followed by MS-analysis. The crude reaction mixture was purified by preparative TLC (CH_2Cl_2/CH_3OH, 9:1) resulting in the acetylene-ClickGreen **8** as a orange fluffy solid after lyophilization from H_2O/dioxane (10 mL, 1:0.5 v/v) (28 mg, 99%). R_F = 0.46 (CH_3OH:CH_2Cl_2, 1:9). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.11-7.09 (m, 3H), 7.00-6.97 (m, 2H), 6.87 (d, *J* = 2.1 Hz, 2H), 6.83 (dd, *J* = 9.2, 2.1 Hz, 2H), 4.78 (d, *J* = 2.4 Hz, 2H), 2.60 (t, *J* = 2.4 Hz, 1H), 2.03 (s, 3H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ: 178.4, 160.2, 159.6 (2C), 156.1, 139.1, 132.3 (2C), 131.5, 126.7, 123.2 (2C),

118.0 (2C), 115.9, 113.7 (2C), 104.5, 79.5, 77.2, 56.8, 14.5. HRMS (ESI+) *m/z* calcd for $C_{23}H_{17}O_4$ $[M+H]^+$ 357.1127, found 357.1118.

Click conditions for AHA-ELP and acetylene-ClickGreen

To a mixture of AHA-ELP (10 μL, 0.37 mg/mL in PBS buffer, pH = 7.4) and acetylene-ClickGreen **8** (1.8 μL, 1 mM in PBS buffer, pH = 7.4) was added 2 μL of Cu(I)Br/**4** mixture. The Cu(I)Br/**4** mixture contained CuBr in MeCN (40 mM) and **4** (80 mM) in mixed in a 1:1 v/v ratio. MiliQ (6.2 μL) was added to obtain a total reaction volume of 20 μL. The reaction mixture was gently shaken at r.t. for 16 hours. The reaction mixture was analyzed by SDS-PAGE analysis in combination with fluorescence imaging.

Click conditions for HPG-ELP and azido-ClickGreen

To a mixture of HPG-ELP (10 μL, 0.58 mg/mL in PBS buffer, pH = 7.4) and azido-ClickGreen **6** (1.8 μL, 1 mM in PBS buffer, pH = 7.4) was added 2 μL of Cu(I)Br/**4** mixture. The Cu(I)Br/**4** mixture contained CuBr in MeCN (40 mM) and **4** (80 mM) in mixed in a 1:1 v/v ratio. MiliQ (6.2 μL) was added to obtain a total reaction volume of 20 μL. The reaction mixture was gently shaken at r.t. for 16 hours. The reaction mixture was analyzed by SDS-PAGE analysis in combination with fluorescence imaging.

Click conditions HPG-ELP and N_3-Coumarin-PEG2000

To a mixture of HPG-ELP (20 μL, 0.58 mg/mL in PBS buffer, pH = 7.4) and azido-coumarin-PEG2000 (20 μL, 2.1 mg/mL in PBS buffer, pH = 7.4) was added 3 × 2 μL of Cu(I)Br/**4** mixture over a period of 15 minutes. The Cu(I)Br/**4** mixture contained CuBr in MeCN (40 mM) and **4** (80 mM) in mixed in a 1:1 v/v ratio. MiliQ (4.0 μL) was added to obtain a total reaction volume of 50 μL. The reaction mixture was gently shaken at r.t. for 16 hours. The excess copper and **4** was removed by spin-filtration using a Millipore 10 kDa NMWL membrane. The reaction mixture was analyzed by SDS-PAGE analysis in combination with fluorescence imaging.

Click conditions HPG-ELP and AHA-CalB

To a mixture of HPG-ELP (20 µL, 0.587 mg/mL in PBS buffer, pH = 7.4) and AHA-CalB (20 µL, 2.61 mg/mL in PBS buffer, pH = 7.4) was added 3 × 2 µL of Cu(I)Br/**4** mixture over a period of 15 minutes. The Cu(I)Br/**4** mixture contained CuBr in MeCN (40 mM) and **4** (80 mM) in a 1:1 v/v ratio. MiliQ (4 µL) was added to obtain a total reaction volume of 50 µL. The reaction mixture was gently shaken at r.t. for 16 hours. The excess copper and **4** was removed by spin-filtration using a Millipore 10 kDa NMWL membrane. Next, the mixture was subjected to aqueous NaCl (66 µL, 5M) whereupon the CalB-ELP conjugate precipitated from solution. Removal of remaining HPG-ELP in the precipitate was achieved by FPLC (Pharmacia SMART system, Superdex 75 PC 3.2/30 column, eluent: PBS buffer pH = 7.4).

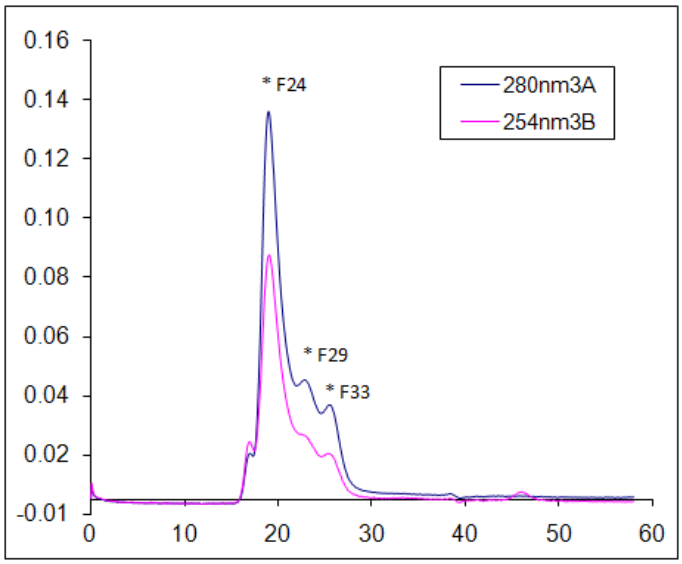


Figure S5. FPLC trace of removal of HPG-ELP (F29-F33) from CalB-ELP conjugate (F24), measured at 280 and 254 nm.

Enzyme activity assay

Lipase activity was analyzed by the hydrolysis of *para*-nitrophenol butyrate (*p*-NPB, Sigma). The reaction mixture (50 µL, pH 7) was composed of 50 mM NaH_2PO_4, 150 mM NaCl, enzyme (100 nM), isopropanol (5%), Triton (0.1%) and *p*-NPB (1 mM). The production of *para*-nitrophenol was monitored at 25 °C for 2 h with 2 minute intervals by

measuring absorbance at 405 nm in a Multicounter Wallac Victor2 (PerkinElmer Life Science). This experiment was carried out *in triplo*. The slope of the curve was taken as a measure of hydrolytic activity.

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