Supporting Material:

Table of Contents

Supporting Material and Methods

Synthesis and Characterization of Coum-3:

Synthesis of coumarin-caged-rhodamine110 2: A 20% phosgene solution (1.0 ml, 1.75 mmol) was added via syringe to a solution of 7-(diethylamino)-4-(hydroxymethyl)-2H-chromen -2-one (0.0700 g, 0.29 mmol) in dry THF (5 ml) under nitrogen. The solution was stirred overnight and then purged with nitrogen to cause evaporation of the solvent from coumarinchloroformate **1**. Rhodamine 110 (0.1000 g, 0.3 mmol) was dissolved in anhydrous DMF (1 mL) under nitrogen. NaH (0.0144 g, 0.36 mmol) was added slowly, and the resulting purple solution was stirred at ambient temperature for 1 h. Coumarin chloroformate **1** dissolved in DMF (1 mL) was added dropwise, and the reaction mixture was stirred at ambient temperature for 24 h. The reaction was quenched with a few drops of glacial acetic acid and solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel, 3–5% v/v gradient of MeOH in $CHCl_3$). The solvent was removed by rotary evaporation to a give a solid (39 mg, 23%); ^{1}H NMR (400 MHz, MeOD) δ 1.12 (t, *J* = 7.6 Hz, 6H), 3.39 (dd, *J* = 6.8, 4.8 Hz, 4H), 5.31 (s, 2H), 6.04 (s, 1H), 6.38 (m, 2H), 6.47 (m, 2H), 6.63 (m, 2H), 7.12 (d, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 9.2 Hz, 1H), 7.43 (d, *J* = 9.2 Hz, 1H), 7.56 (m, 2H), 7.88 (s, 1H), 7.92 (m, 2H).

Synthesis of 6-oxohexanoic acid: To a solution of 2-hydroxycyclohexanone dimer (0.3420 g, 3.0 mmol) in a 60% aqueous THF solution (30 mL) was added sodium periodate (0.6420 g, 3.0 mmol). The solution was stirred overnight at room temperature. Thereafter, sodium periodate (0.3210 g, 1.5 mmol) was again added, and the solution was stirred for an additional 6 h. The reaction mixture was diluted with ethylacetate (30 mL), and washed with brine. The organic phase was extracted and dried over $MgSO_4$. Flash chromatography of the residue (eluent: $CHCl_3$/MeOH 98/2) provided 6-oxohexanoic acid as a clear oil (0.160 g, 40%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68 (m, 4H), 2.38 (m, 2H), 2.48 (m, 2H), 9.77 (s, 1H), [Lit.[1] ^{1}H NMR 400 MHz, δ 1.65-1.80 (m, 4H), 2.35-2.55 (m, 4), 9.79 (t, 1H)].

Synthesis of coumarin-caged-rhodamine110-carboxylic acid **3**: Caged-rhodamine 110 **2** (0.0360 g, 0.06 mmol) was added to a DMF (2 ml) solution of 6-oxohexanoic acid (0.0120 g, 0.12 mmol) and was stirred at room temperature for 1 h. Then $NaBH_3CN$ (0.0200 g, 0.3 mmol) and a drop of acetic acid were added and the solution was stirred for another 2 h. The reaction was quenched by 1 mL TFA and diluted with 5 mL DMSO. The product was purified by reverse-phase HPLC (semiprepative C-18 column) using a linear gradient binary solvent system (solvent A: 0.1% TFA/H_2O; solvent B: 0.l% TFA/CH_3CN) with a ratio of A:B that varied from 100:00 (0 min) to 5:95 (40 min). Removal of solvent by lyophilization afforded a red solid (0.0250 g, 60%); ^{1}H NMR (400 MHz, MeOD) δ 1.12 (t, *J* = 7.6 Hz, 6H), 1.42 (m, 2H), 1.59 (m, 2H), 1.67 (m, 2H), 2.24 (t, *J* = 7.2 Hz, 2H), 3.39 (m, 6H), 5.37 (s, 2H), 6.04 (s, 1H), 6.47 (s, 1H), 6.66 (d, *J* = 5.2 Hz, 1H), 6.92 (m, 2H), 7.10 (d, *J* = 9.6 Hz, 1H), 7.14 (d, *J* = 9.2 Hz, 1H), 7.30 (d, *J* = 7.2 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.43 (d, *J* = 7.2 Hz, 1H), 7.75 (m, 2H), 8.18 (s, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), FAB HRMS cald. for $C_{41}H_{39}N_3O_9Cs$ ($M+Cs^+$) *m/z* = 850.1741, found 850.1729.

Synthesis of coumarin-caged-rhodamine110-maleimide **4**: Caged-rhodamine 110-carboxylic acid **3** (9 mg, 0.013 mmol), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBop, 0.0130 mg, 0.026 mmol) and DIPEA (0.0020 mg, 0.026 mmol) were dissolved in DMF (5 ml) and the solution was stirred at room temperature for 5 min. The TFA salt of aminoethylmaleimide (0.0060 g, 0.026 mmol) was added and the solution was stirred for another 2 h. The reaction was quenched by 0.1 mL TFA diluted with 5 mL DMSO. The product was purified by reverse-phase HPLC (semiprepative C-18 column) using a linear gradient binary solvent system (solvent A: 0.1% TFA/H_2O; solvent B: 0.l% TFA/CH_3CN) with a ratio of A:B that varied from 100:00 (0 min) to 5:95 (40 min). Removal of solvent by lyophilization afforded a red solid (0.0050 g, 60%); FAB HRMS calcd. for $C_{47}H_{45}N_5O_{10}Na$ ($M+Na^+$): *m/z* = 862.3064, found 862.3035.

Synthesis of cell-permeable peptide **5**: A standard peptide coupling protocol on a peptide synthesizer was used for all amino acid couplings. Amino acid (5 equiv/equiv resin), HCTU/6-Cl-HOBt (5 equiv/equiv resin) and DIPEA (10 equiv/equiv resin) were mixed with resin in DMF and reacted for 5 min x 2 at temperature. Fmoc deprotection was achieved using 20% piperidine in DMF for 4 min x 3 at temperature. The N-terminus was capped using acetic anhydride with 20% DIPEA for 5 min. Resins were dried and subsequently cleaved in a solution of 87.5% TFA, 5% thioanisole, 2.5% phenol, 2.5% H_2O and 2.5% EDT. The peptide was purified by HPLC (semiprepative C-18 column) using a linear gradient binary solvent system (solvent A: 0.1% TFA/H_2O; solvent B: 0.l% TFA/CH_3CN) with a ratio of A:B that varied from 100:00 (0 min) to 30:70 (40 min). The major fraction for the peptide was collected and lyophilized to remove solvent; FAB HRMS calcd. for $C_{79}H_{146}N_{20}O_{16}S$ ($M+2H^{2+}$): *m/z* = 831.5061, found 831.4645.

Synthesis of cell-permeable caged TAMRA conjugate Coum-3**:** Coumarin-caged-rhodamine110-maleimide **4** (3 mg, 3.6 µmol) was mixed in the PBS buffer-DMF solution (3:1, 2 mL, pH 7.5) with cell-permeable peptide **5 (**3 mg, 1.8 µmol) and was stirred for 1 h. The solution was diluted by 3 mL DMSO and HPLC (semiprepative C-18 column) purified using a linear gradient binary solvent system (solvent A: 0.1% TFA/H_2O; solvent B: 0.l% TFA/CH_3CN) with a ratio of A:B that varied from 100:00 (0 min) to 30:70 (40 min). Removal of solvent by lyophilization afforded a red solid (5 mg, 60%); FAB HRMS calcd. for $C_{126}H_{191}N_{25}O_{26}S^{2+}$ ($M+2H^{2+}$) *m/z* = 1251.2060, found 1251.2522.

Synthesis and Characterization of Cob-4:

Synthesis of β-(3-aminopropyl)cobalamin **6**: Prepared from hydroxocobalamin and 3-chloropropylamine hydrochloride according to a literature procedure.[2] After completion of the alkylation reaction the mixture was gravity filtered to remove the zinc dust and was dripped into cold ether resulting in a red suspension. The suspension was centrifuged and the supernatant was discarded. The desired compound was purified by reverse phase flash chromatography (C-18 column) using a linear gradient binary solvent system (solvent A: 0.1% TFA/H_2O; solvent B: 0.l% TFA/CH_3CN) with a ratio of A:B that varied from 97:3 to 50:50 over 10 column volumes. Removal of solvent by lyophilization afforded an orange solid; ESI MS calcd. for $C_{65}H_{98}N_{14}O_{14}PCo$ (M^{1+}): *m/z* = 1388.7, found 1388.7; calc. for (M^{2+}) *m/z* = 694.3, found 694.5; calcd. for (M^{3+}): *m/z* = 463.1, found 462.9.

Synthesis of cobalamin-TAMRA conjugate **7**: *N*,*N*,*N*′,*N*′-Tetramethyl-*O*-(*N*-succinimidyl)uranium tetrafluoroborate (TSTU, 0.0233 g, 77 µmol), 5-carboxytetramethylrhodamine (TAMRA, 0.0054 g, 12 µmol) and DIPEA (0.0292 g, 220 µmol), were mixed for 1 h in a 2:2:1 dimethylformamide:dioxane:water solution. β-(3-aminopropyl)cobalamin **6** (0.0187 g, 14 µmol) was added and the reaction was mixed for 18 h. The desired was purified by HPLC (semiprepative C-18 column) using a linear gradient binary solvent system (solvent A: 0.1% TFA/H_2O; solvent B: 0.l% TFA/CH_3CN) with a ratio of A:B that varied from 97:3 (0 min) to 10:90 (40 min). Removal of solvent by lyophilization afforded a red solid (0.0154 g, 68%); ESI MS calcd. for $C_{90}H_{118}N_{16}O_{18}PCo$ (M^{2+}): *m/z* = 900.4, found 900.5; calcd. for (M^{3+}): *m/z* = 600.3, found 600.3.

Due to the light sensitivity of Cob-4, all experiments involving Cob-4 were performed in a darkroom.

Confocal Microscopy:

All microscopy studies were performed on an Olympus FV1000 point scanning confocal with an IX81 microscope base. A Coherent Genesis CX355-100 STM 355 nm, 100 mW continuous wave UV laser was coupled to the confocal via an installed UV port. Laser and region of photolysis was controlled via Fluoview software.

Photolysis of NB-1, NB-2, Coum-3 and Cob-4 in microwells on the confocal:

Ibidi 30 µL, 35 mm dishes were loaded with 1 µM NB-1, Coum-3 or Cob-4 for individual photolysis experiments and 1 µM NB-2 + 1 µM Cob-4 for dual photolysis experiments. Photolysis was performed using the stimulation mode of the Fluoview software with images collected before and after stimulation. A photolytic dwell time of 100 µs/pixel was employed for the defined region of interest using the following settings: a) 0 - 100 frames at 355 nm (100 mW) for NB-1 and Coum-3 b) 0 - 25 frames at 355 nm (5 mW) for Cob-4 c) 0 - 100 frames at 440 nm (25 mW) for Cob-4 d) 0 - 25 frames at 559 nm (0.3 mW) for Cob-4 e) 25 frames at 559 nm (0.3 mW) followed by 25 frames at 355 nm (100 mW) for a mixture of NB-2 and Cob-4 f) 0 - 100 frames at 355 nm (5 mW) for a mixture of NB-2 and Cob-4. All imaging was performed with a 4X UPlan S-Apo objective using 559 nm (0.3 mW) for NB-1 and Coum-3; 559 nm (0.03 mW) for Cob-4; 488 nm (0.6 mW) for NB-2. TMRE (Invitrogen) was used to generate photobleaching curves for all photolysis experiments. Image J software was employed for all image analyses. RF (relative fluorescence of the entire well) changes were determined by the equation $RF= (F_t - F_0)/F_0$, F_t is the average fluorescence at time t of the entire well and F_0 is the average initial fluorescence of the entire well subtracting out the photobleaching curve. All experiments were performed in triplicate.

Cell culture experiments:

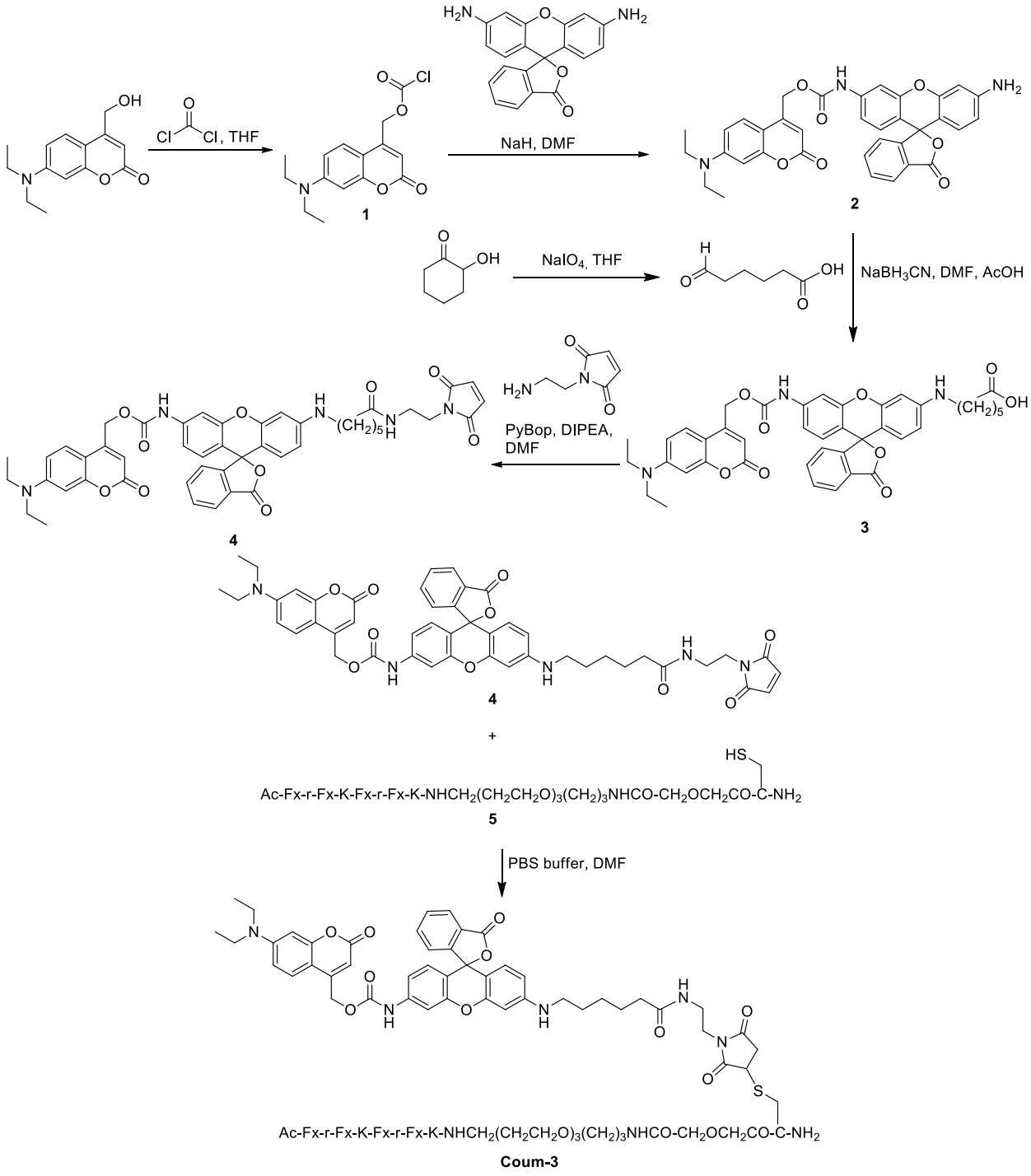
Cell culture media and solutions were from Invitrogen. HeLa cells were from the tissue culture facility at UNC Chapel Hill. HeLa cells were passaged by treatment with 0.5% trypsin + 0.53 mM EDTA before reaching confluence and maintained in Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum (FBS) supplemented with gentamycin and kanamycin and incubated at 37 ºC in 5% CO_2. One day prior to microscopy experiments, cells were plated onto MatTek 35 mm 1.5 glass bottom dishes. On the day of the microscopy experiments, the media was replaced with Leibovitz's L-15 without phenol red supplemented with 2% FBS.

References

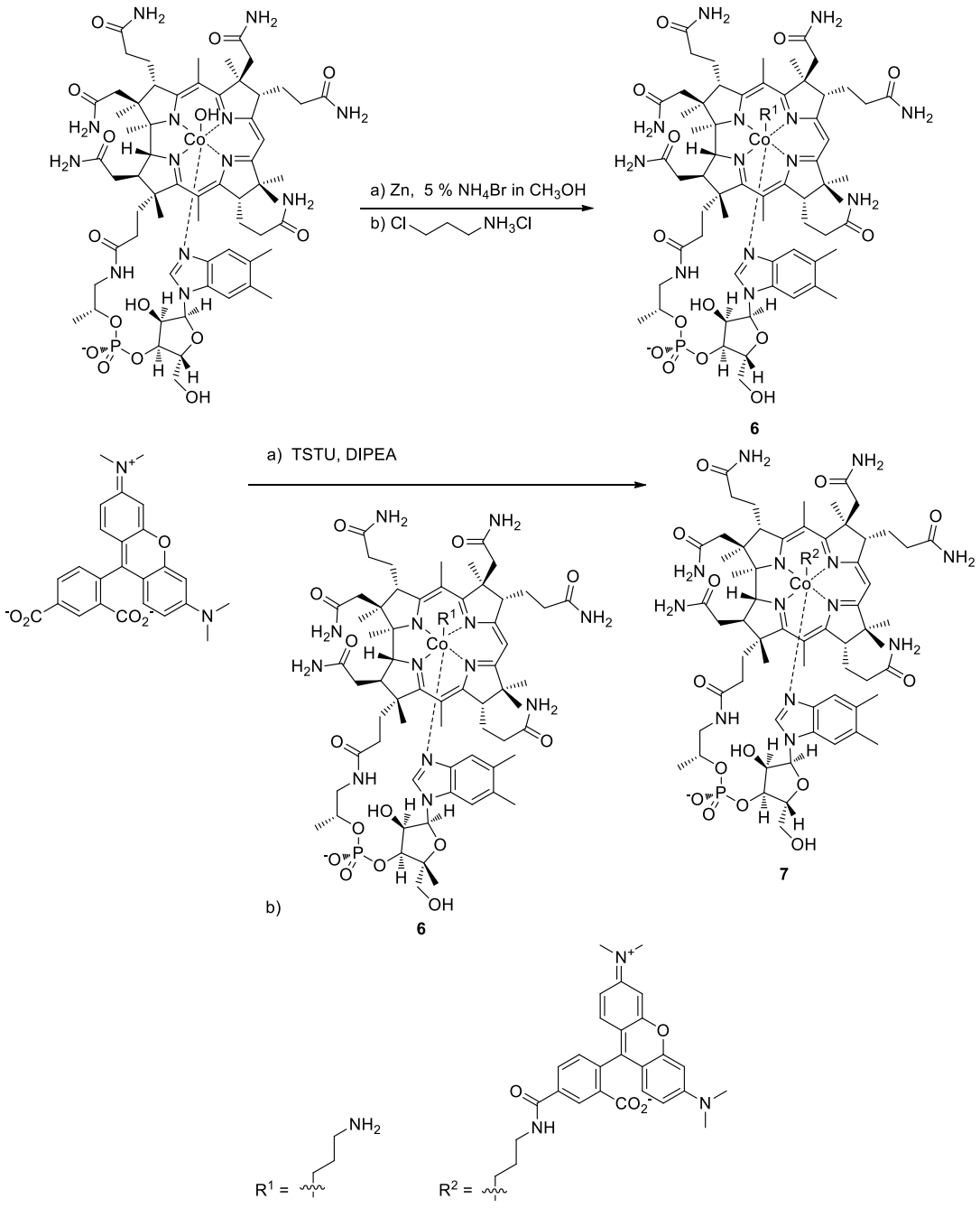
1. Floresca, R.; Kurihara, M.; Watt, D. S.; Demir, A. *J. Org. Chem.* **1993**, 58, 2196-2200.
2. Smeltzer, C. C.; Cannon, M. J.; Pinson, P. R.; Munger, J. D., Jr.; West, F. G.; Grissom, C. B *Org. Lett.*, **2001**, 3, 799–801.

Schemes

Scheme S1. Synthesis of Cell-Permeable Caged TAMRA Conjugate. Fx = cyclohexylalanine and r = D-arginine



Scheme S2. Synthesis of Cob-4.



Figures

Figure S1. Absorbance spectra of caging moieties: nitrobenzyl (black), amino-coumarin (blue) and cobalamin (red) moieties. RA is the relative absorbance where the maximum peak is set to 100.

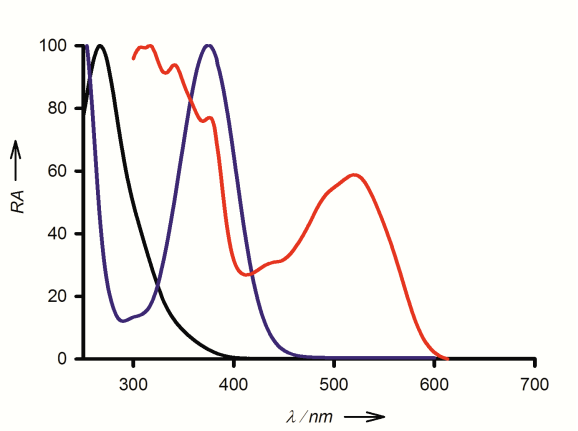
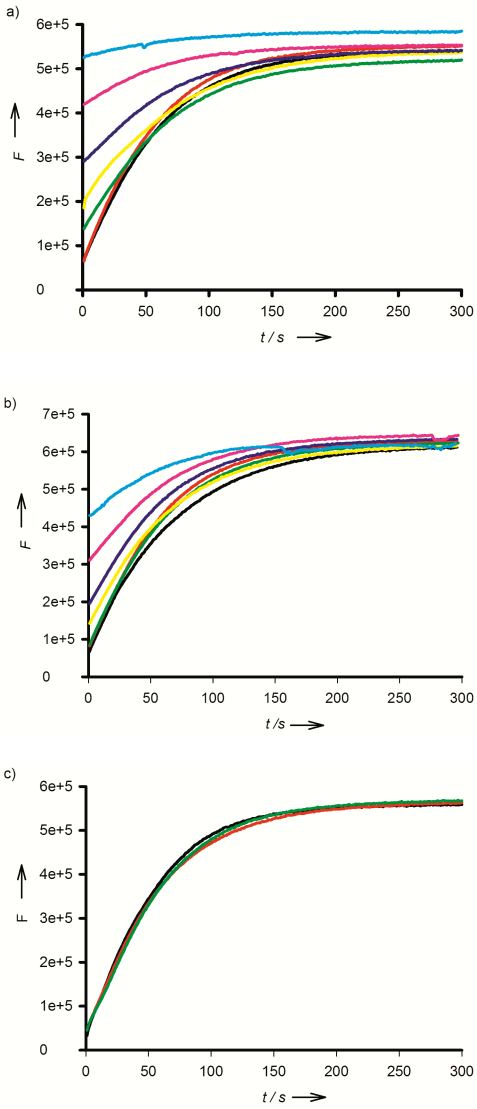


Figure S2. Fluorescence of photolyzed Cob-4 using a fluorimeter by excitation at 360 nm (a), 440 nm (b) or 560 nm (c). Photolysis at 360 nm and 440 nm was carried out for 5 (black), 10 (red), 20 (green), 40 (yellow), 80 (purple), 160 (pink), and 300 (cyan) seconds then monitored by excitation at 560 nm and emission at 585 nm. Data are represented as averages with standard errors of three independent assays. F is an abbreviation for fluorescence.



Rhodamine 110
Coum-3 photolysis product

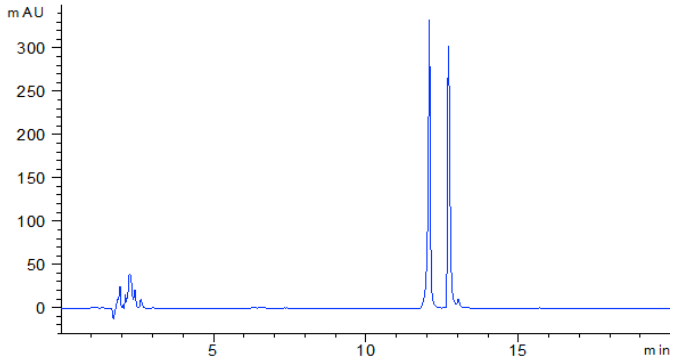
Rhodamine 110
Coum-3

Retention Time (min)	Area	Observed Mass	Compound	% Yield
11.907	1255.6	1115.8 (M^{2+}), 743.7 (M^{3+}), 557.8 (M^{4+}), 446.7 (M^{5+})	Coum-3 photolysis product	92

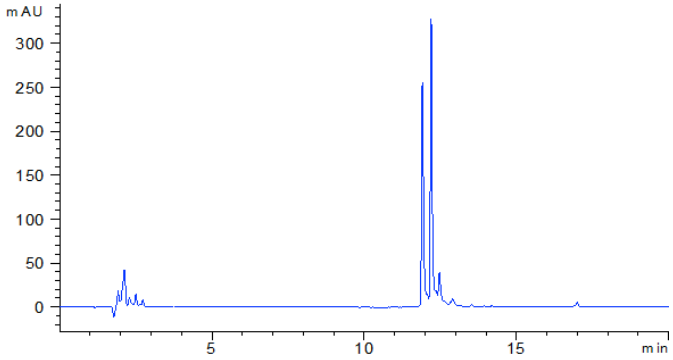
Retention Time (min)	Area	Observed Mass	Compound	% Yield
12.074	1791.3	331.1 (M^{+})	Rhodamine 110	NA
12.194	1568.1	331.1 (M^{1+})	Rhodamine 110	NA
12.755	1707.4	1258.1 (M^{2+}), 834.8 (M^{3+}), 626.4 (M^{4+}), 501.3	Coum-3	

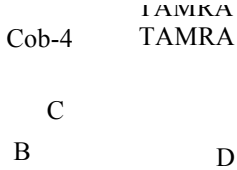
Figure S3. Photolytic conversion yield of Coum-3. (a) LC-MS of Coum-3 (5 µM) spiked with Rhodamine 110 (5 µM) as a standard. (b) LC-MS of a Coum-3 (5 µM) spiked with Rhodamine 110 (5 µM) as a standard that was exposed to 440 nm light for 3 h. The mixtures were analyzed by monitoring absorbance (498 nm) and using a linear gradient binary solvent system (solvent A: 0.1% formic acid/H_2O; solvent B: 0.l% formic acid/CH_3CN) with a ratio of A:B that varied from 97:3 (0 - 5 min) to 3:97 (5 - 18 min).

(a)



(b)

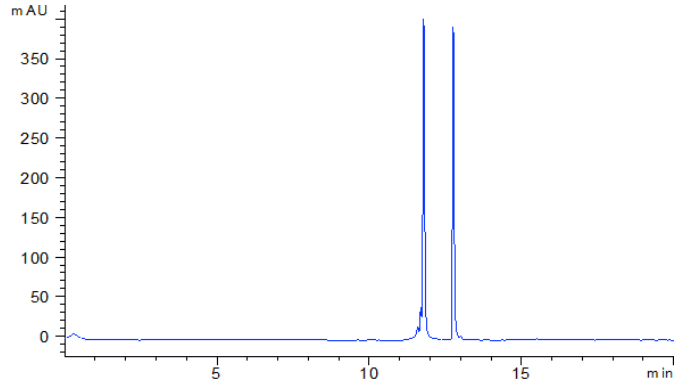




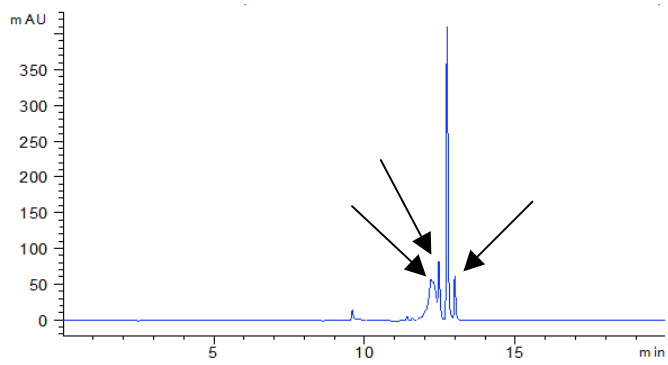
Time (min)	Area	Observed Mass	Mass	Compound[1]		% Yield
9.591	1.785	112.6	1625.8	865.0 (M^{2+})	900.1 (M^{2+}), 600.8 (M^{3+})	A Cob-4	NA
12.306	927.7			486.2 (M^{1+})		B	57
12.755			1570.0	431.1 (M^{1+})		C TAMRA	
12.465	387.4			504.4 (M^{1+})			24
12.734	1623.5			431.1 (M^{1+})		TAMRA	NA
12.992	261.2			470.2 (M^{1+})		D	16
						Total	97

Figure S4. Photolytic conversion yield of Cob-4. (a) LC-MS of Cob-4 (5 µM) spiked with TAMRA (5 µM) as a standard. (b) LC-MS of a Cob-4 (5 µM) spiked with TAMRA (5 µM) as a standard that was exposed to 546 nm light for 1 min. The mixtures were analyzed by monitoring absorbance (550 nm) and using a linear gradient binary solvent system (solvent A: 0.1% formic acid/H_2O; solvent B: 0.1% formic acid/CH_3CN) with a ratio of A:B that varied from 97:3 (0 - 5 min) to 3:97 (5 - 18 min). (c) Structures of photolytic products C, D, and E.

(a)

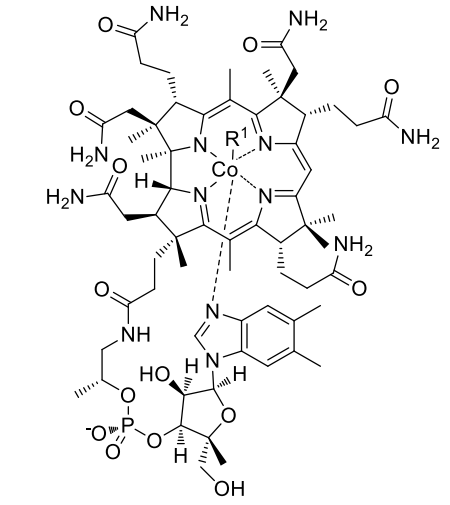


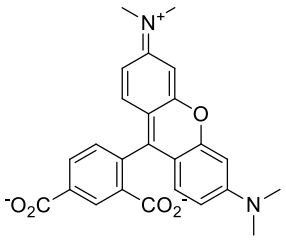
(b)



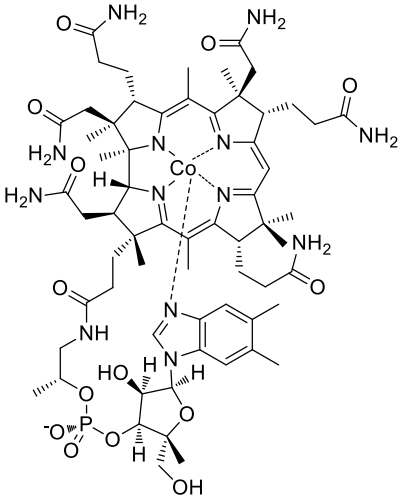
[1]Structures are assigned to peaks based on the observed masses of the ions and the known products of alkylcobalamin photolysis (Schwartz, P. A.; Frey, P. A. *Biochemistry-US* **2007**, *46*, 7284-92).

(c)



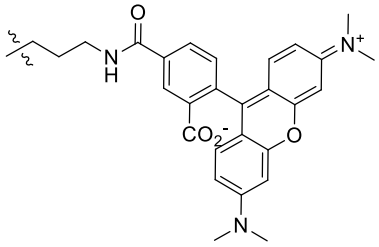


TAMRA

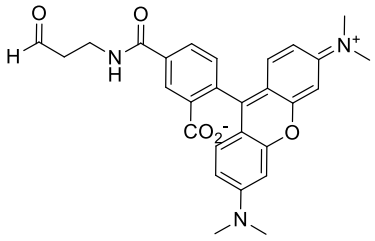


A

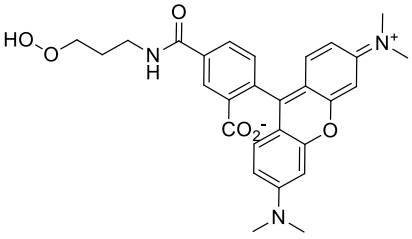
R^1 =



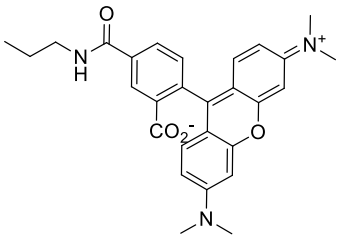
Cob-4



B



C



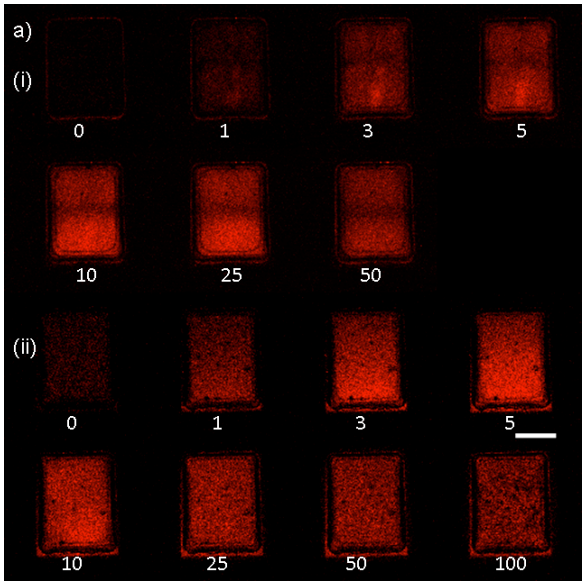
D

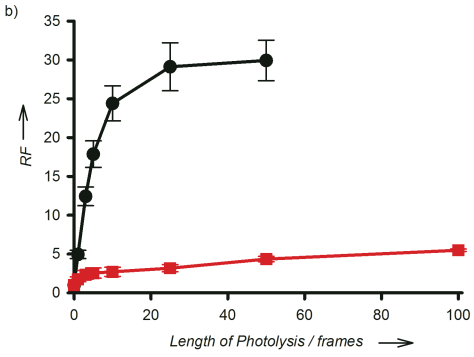
NB-1

Photolysis at 355 nm

Coum-3

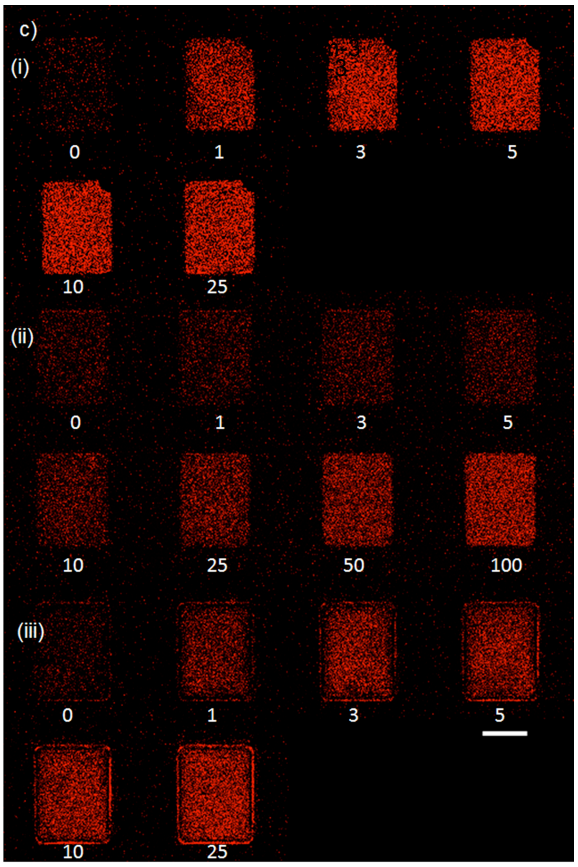
Figure S5. Photolysis of NB-1, Coum-3, and Cob-4 in microwells on the confocal microscope. (a) Photolysis of NB-1 (i) and Coum-3 (ii) using a 355 nm laser at 100 mW for 0 - 100 frames. (b) Quantification of the photolysis (at 355 nm) of NB-1 (●), and Coum-3 (■). (c) Photolysis of Cob-4 using a (i) 355 nm laser, (ii) 440 nm laser, and (iii) 559 nm laser for 0 - 100 frames. (d) Quantification of the photolysis of NB-1 (●), Coum-3 (♦) and Cob-4 (▲) using a 355 nm laser at 5 mW for 0 - 25 frames. (e) Quantification of the photolysis of NB-1 (●), Coum-3 (♦) and Cob-4 (▲) using the 440 laser at 25 mW for 0 - 100 frames. (f) Quantification of the photolysis of NB-1 (●), Coum-3 (♦) and Cob-4 (▲) using the 559 laser at 0.3 mW for 0 - 25 frames. All photolysis reactions on the confocal microscope employed 10 µL well plates. Data are represented as averages of the entire well with standard errors of three independent assays. Scale bars are 1 mm. RF is an abbreviation for relative fluorescence.

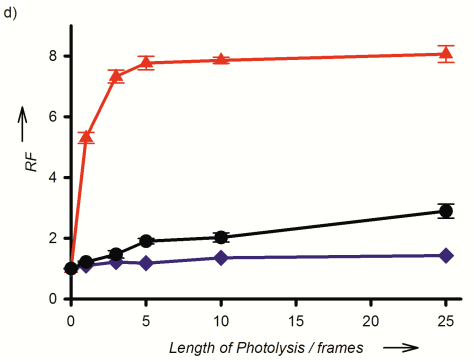


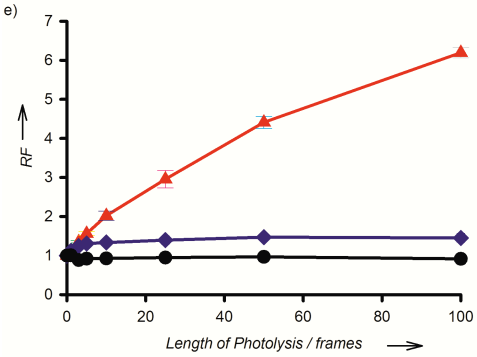


Photolysis at 440 nm

Photolysis at 355 nm







Photolysis at 559 nm

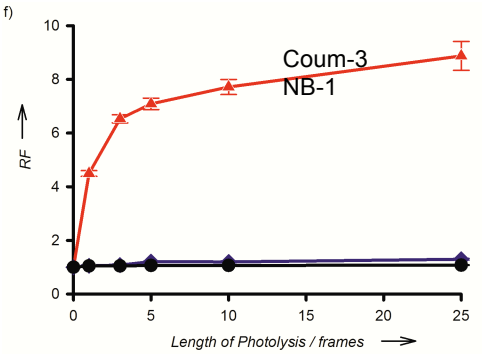
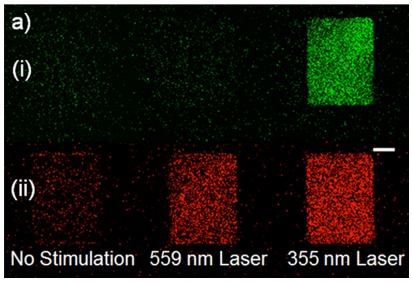
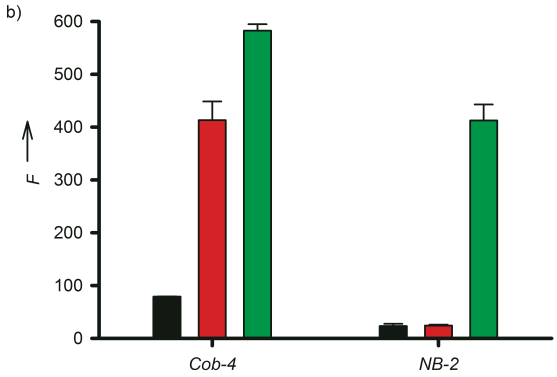


Figure S6. Wavelength selective photolysis of Cob-4 in the presence of NB-2 via confocal microscopy. (a) Photolysis conducted at 559 nm for 25 frames followed 355 nm exposure for 25 frames. NB-2 and Cob-4 were simultaneously imaged by excitation at (i) 488 nm (fluorescein/NB-2) and (ii) 559 nm (TAMRA/Cob-4), respectively, and data collected using spectral PMTs set for the fluorophores. 559 nm photolysis only activates Cob-4 [cf. (ii) with (i)]. Subsequent 355 nm photolysis activates NB-2 [see (i) with the 355 nm laser]. (b) Fluorescence prior (black) to photolysis, following photolysis at 559 nm (red; 25 frames), and following photolysis as 355 nm (green; 25 frames) of an NB-2/Cob-4 mixture. As shown in (a), Cob-4 is selectively photolyzed at 559 nm in the presence of NB-2 (cf. green bars for Cob-4 and NB-2). Note: images are pixilated due to the short dwell time (2 µs/pixel) and low laser power (0.03 mW) for image acquisition required to prevent photolysis of Cob-4. Scale bars = 1 mm.

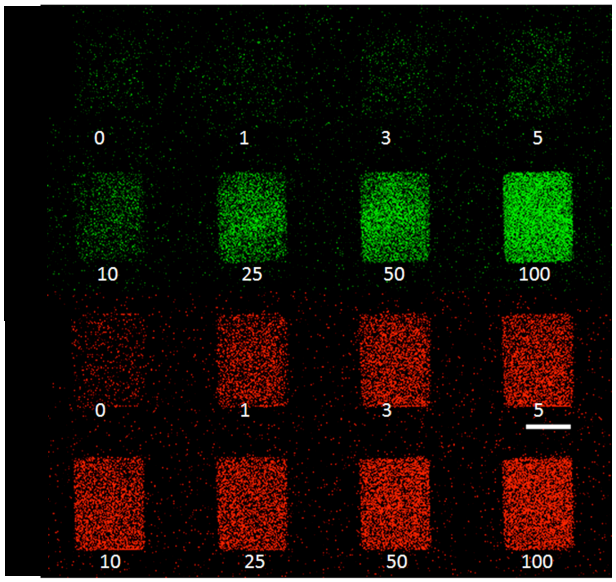




Cob-4 photolysis product emission

NB-2 photolysis product emission

Figure S7. 355 nm selective photolysis of Cob-4 in the presence of NB-2 under the confocal microscope. (a) Photolysis conducted at 355 nm for 0 - 100 frames. NB-2 and Cob-4 were simultaneously imaged by excitation at (i) 488 nm and (ii) 559 nm, respectively, and data collected using spectral PMTs set for the FITC and TAMRA fluorophores. (b) Quantification of the fluorescent increase for NB-2 (▼) and Cob-4 (●) following 355 nm. Data are represented as averages of the entire 10 µL well with standard errors of three independent assays. Note that within 5 frames of 355 nm illumination, Cob-4 is nearly completely photolyzed whereas NB-2 remains unphotolyzed.



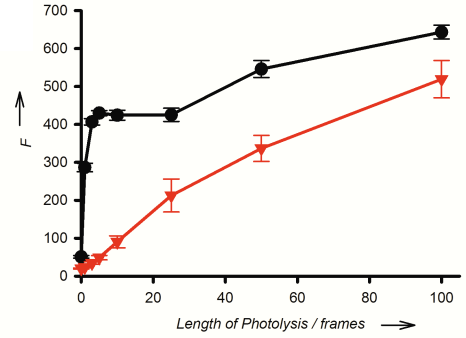
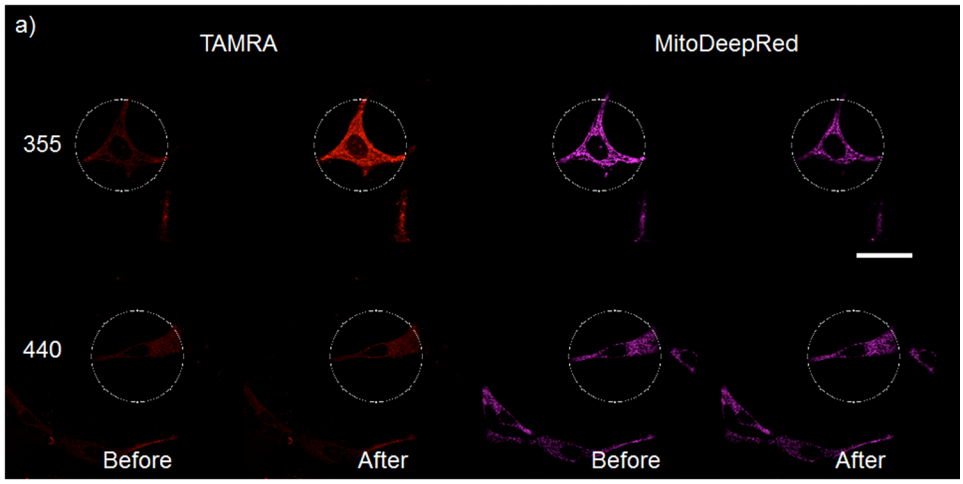
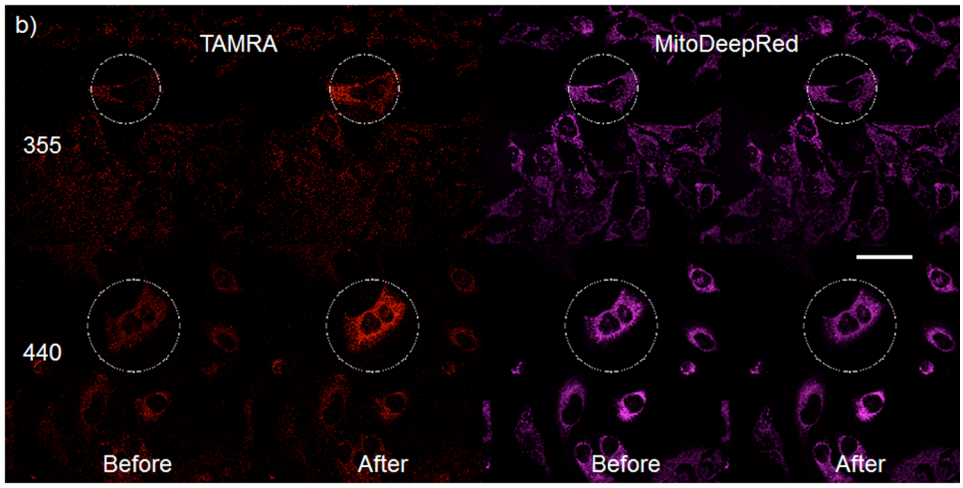
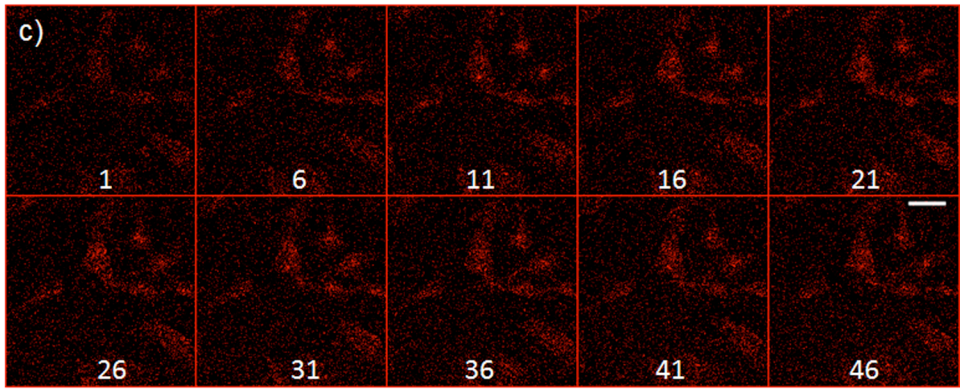
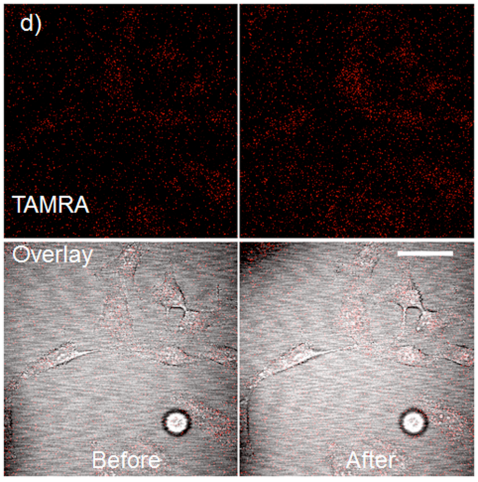


Figure S8. Photolysis of NB-1, Coum-3, and Cob-4 in HeLa cells. (a) Fluorescence of NB-1 and MitoTracker Deep Red in HeLa cells before and after 25 frames of photolysis with 355 nm laser at 5 mW or 440 nm laser at 30 mW. (b) Fluorescence of Coum-3 and MitoTracker Deep red in HeLa cells before and after 25 frames of photolysis with 355 nm laser at 5 mW or 440 nm laser at 30 mW. (c) Fluorescence of Cob-4 in HeLa cells imaged from 1 - 50 frames with 559 nm laser at 0.03 mW. (d) Fluorescence of Cob-4 in HeLa cells before and after 15 frames of imaging with 559 nm laser at 0.03 mW: (i) TAMRA fluorescence and (ii) overlays of TAMRA with transmitted light images. (e) Fluorescence of Cob-4 in HeLa cells after photolysis with 559 nm laser for 50 frames. Imaging was performed with a 559 nm laser at 0.75 mW. Data are represented as averages of 7 - 20 cells with standard errors. Scale bars are 50 µm. White circle represents the region of photolysis.








d)
TAMRA
Overlay
Before
After

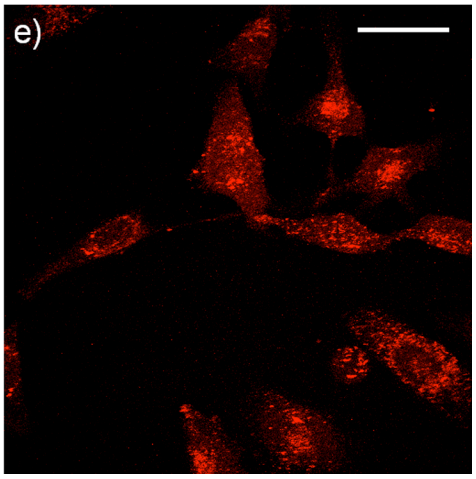

e)

Figure S9. Predicted mitochondrial localization of NB-1, Coum-3 and Cob-4 photolysis products, where supernatant is non-mitochondrial- and pellet is mitochondrial-associated. Note: MLS = Mitochondria localization sequence.

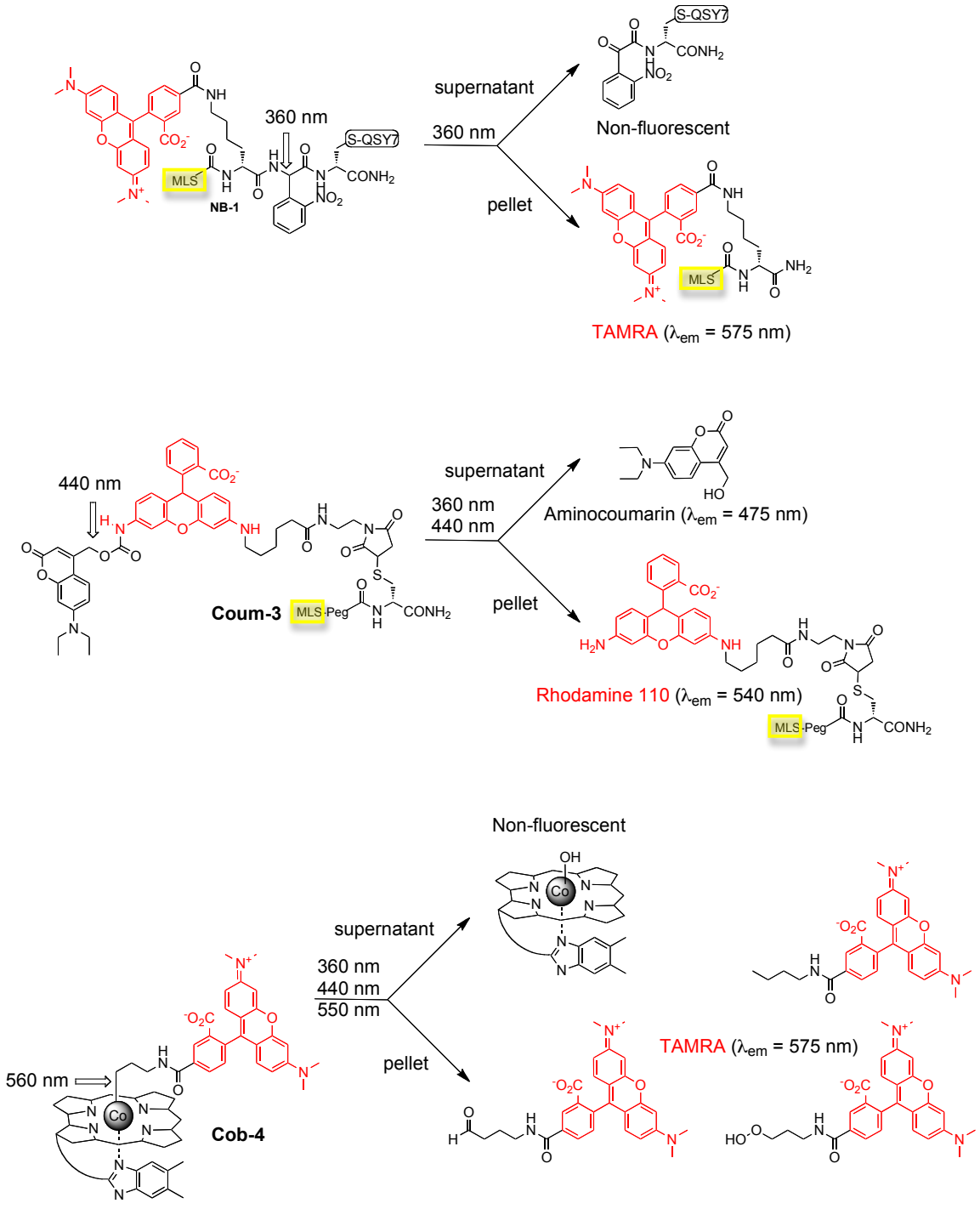
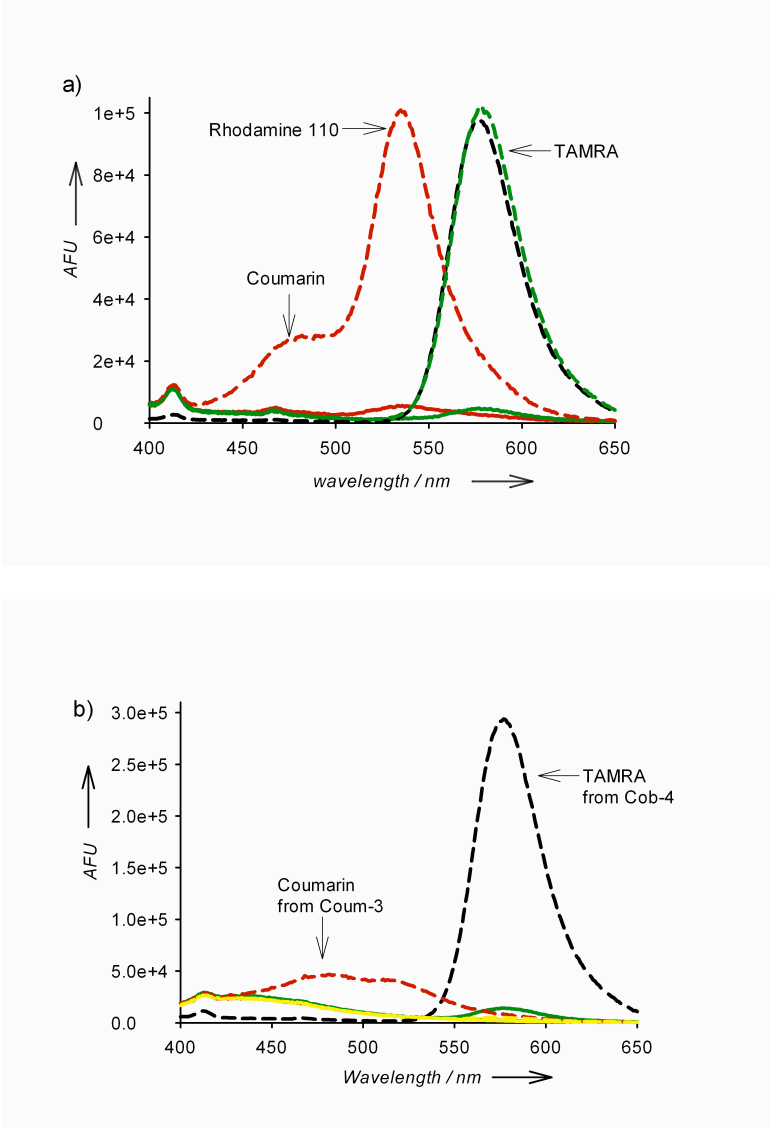


Figure S10. Mitochondrial localization of NB-1, Coum-3 and Cob-4 photolysis products. (a) Spectral scans of *individual samples* of 0.5 µM NB-1 (green), 0.5 µM Coum-3 (red) or 0.5 µM Cob-4 (black) before (solid line) and after (dashed line) photolysis at 360 nm, 440 nm and 550 nm, respectively. The unleashed fluorescent products from photolysis (dashed lines) of NB-1 (TAMRA), Coum-3 (Rhodamine 110/coumarin), and Cob-4 (TAMRA) are highlighted. (b) Supernatant from the incubation of mitochondria with no compound (yellow), 0.5 µM NB-1 (green), 0.5 µM Coum-3 (red) or 0.5 µM Cob-4 (black) before (solid line) and after (dashed line) photolysis at 360 nm, 440 nm and 550 nm, respectively. Note that only the photolyzed product from Cob-4, which lacks an MLS, is observed in the supernatant. (c) Resuspended pellet from the incubation of mitochondria with no compound (yellow), 0.5 µM NB-1 (green), 0.5 µM Coum-3 (red) or 0.5 µM Cob-4 (black) before (solid line) and after (dashed line) photolysis at 360 nm, 440 nm and 550 nm, respectively. Samples were excited at 360 nm while monitoring emission from 400 - 650 nm in order to observe the fluorescence of all products at the same time. Note that the photolyzed products from NB-1 and Coum-3, which contain an MLS, display fluorescence in the pellet (i.e. associated with the mitochondria).



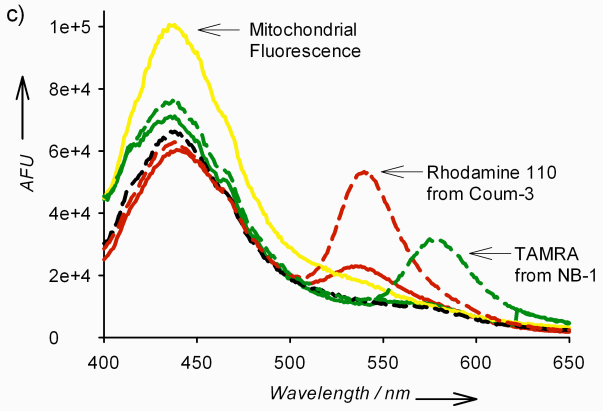
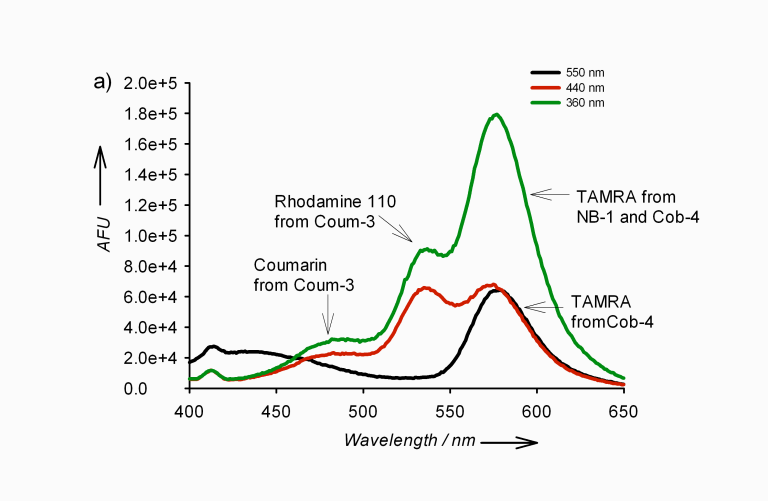
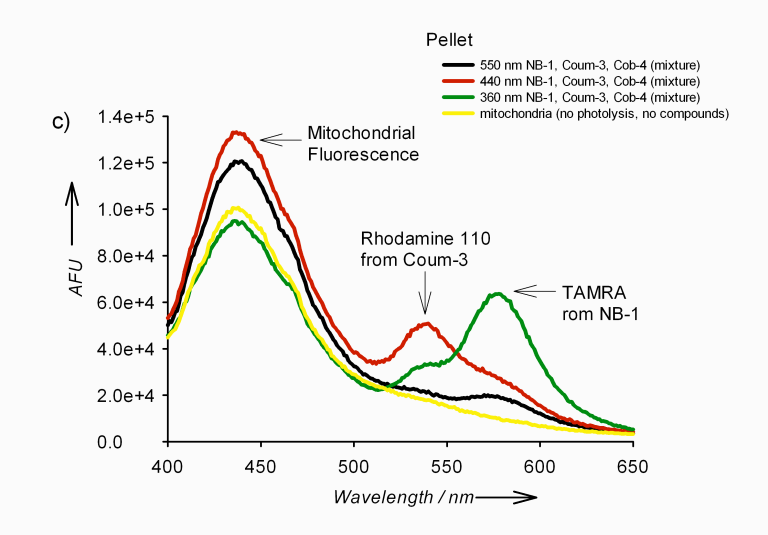

c)
1e+5
8e+4
6e+4
4e+4
2e+4
0
AFU
400
450
500
550
600
650
Wavelength / nm
Mitochondrial
Fluorescence
Rhodamine 110
from Coum-3
TAMRA
from NB-1

Figure S11. Wavelength-selective activation of NB-1, Coum-3 and Cob-4. (a) Spectral scans of a *mixture* of 0.5 µM NB-1 + 0.5 µM Coum-3 + 0.5 µM Cob-4 after 550 nm (black), 440 nm (red) and 360 nm (green) photolysis. 550 nm photolysis (black): only TAMRA fluorescence is observed. 440 nm photolysis: both TAMRA and Rhodamine fluorescence are observed. 360 nm photolysis: TAMRA fluorescence is doubled in size. (b) Supernatant from the incubation of mitochondria with no compound (yellow) or a mix of 0.5 µM NB-1 + 0.5 µM Coum-3 + 0.5 µM Cob-4 after 550 nm (black), 440 nm (red) and 360 nm (green) photolysis. 550 nm photolysis (black): only TAMRA fluorescence is observed in the supernatant (from Cob-4). 440 nm photolysis: both TAMRA and coumarin-byproduct (from Coum-3) fluorescence are observed in the supernatent. 360 nm photolysis: No change TAMRA fluorescence size relative to 550 nm photolysis. (c) Resuspended pellet from the incubation of mitochondria with no compound (yellow) or a mix of 0.5 µM NB-1 + 0.5 µM Coum-3 + 0.5 µM Cob-4 after 550 nm (black), 440 nm (red) and 360 nm (green) photolysis. 550 nm photolysis (black): no TAMRA fluorescence is observed in the pellet. 440 nm photolysis: Rhodamine 110 (from Coum-3) fluorescence is observed in the pellet. 360 nm photolysis: TAMRA fluorescence is observed in the pellet (from NB-1). Samples were excited at 360 nm monitoring emission from 400 - 650 nm in order to observe the fluorescence of all products at the same time.




Pellet
550 nm NB-1, Coum-3, Cob-4 (mixture)
440 nm NB-1, Coum-3, Cob-4 (mixture)
360 nm NB-1, Coum-3, Cob-4 (mixture)
mitochondria (no photolysis, no compounds)
c)
1.4e+5
1.2e+5
1.0e+5
8.0e+4
6.0e+4
4.0e+4
2.0e+4
0.0
AFU
Mitochondrial
Fluorescence
Rhodamine 110
from Coum-3
TAMRA
rom NB-1
400
450
500
550
600
650
Wavelength / nm