Supporting Information

Figure S11. Emission spectra of 1 (10 µM) in PBS (0.1 M; pH 8.0) with (blue) or without (red) TCEP (10 mM) after an hour incubation at room temperature. Excitation: 365 nm; step size 5 nm.

Fig. S12. Chemical structure of BG-Ru5 and in vitro kinetics of the study of BG-Ru5 with SNAP tag. The final concentrations of BG-Ru and enzyme were 5 µM and 1 µM, respectively. The reaction was carried out in a reaction buffer (pH 7.4) at room temperature. The reaction was stopped at different time points, and the formation of SNAP ligation was monitored by luminescence (ex: 488 nm; em: 605 nm).

Figure S13. Luminescence imaging of human bone cancer U2OS cells with SNAP-mito after the treatment of 5 (100 nM) and 2 (20 µM). The luminescence from ruthenium from the cells was captured with gating time 5 to 12 ns before irradiation with 455 nm LED with 1 W (excitation with 470 nm laser and emission was monitored at 600-640 nm).

Figure S14. Luminescence imaging of human bone cancer U2OS cells with SNAP-mito after the treatment of excess BG (10 mM) and followed by 5 (100 nM). The luminescence from ruthenium from the cells was captured with gating time 5 to 12 ns before irradiation with 455 nm LED with 1 W (excitation with 470 nm laser and emission was monitored at 600-640 nm).
Representative procedure for photoreactions
Stock solutions of final derivatives (2 mM in DMF for QPD derivatives and 1 mM in deionized water for ruthenium derivatives) were diluted separately with reaction buffer. Each experiment was performed in duplicates or triplicates. During the photo reaction all the samples were irradiated with a 455 nm LED (1 W, 30 cm distance). The experiments were protected from external light.

General techniques:
Anhydrous solvents were obtained by passing them through a commercially available alumina column (Innovative technology, MA). Reactions were monitored by TLC carried out on 0.25 mm E. Merck silica-gel plates (60F-254) by using UV light as visualizing agent and ninhydrin or vanillin solution and heat as developing agents. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash-column chromatography. NMR spectra were recorded on Bruker Avance-400 and 500 instruments and calibrated by using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, and br=broad. LC-MS were recorded by using a Thermo Scientific Accela HPLC equipped with a Thermo C18 (5 cm x 2.1 mm, 1.9 μm particles) Hypersil gold column coupled with A: Surveyor MSQ Plus spectrometer or B: LCQ Fleet mass spectrometer (both ESI, Thermo Scientific). Method: linear elution gradient for 95% H₂O 0.01% TFA to 90% MeCN 0.01% TFA in 4 minutes at a flow rate of 1.0 mL/min. Final compounds were purified by reverse-phase chromatography using a Biotage Isolera ONE equipped with a Biotage SNAP Cartridge KP-C18-9S (linear gradient from 100% H₂O 0.01% TFA to 100% MeCN 0.01% TFA with a flow rate of 5 mL/min).

SNAP-His-tag protein expression
The plasmid PET-28b SNAP-fast, which contains resistance to kanamycin and His-tagged SNAP-f was electroporated into the cell lines BL21. Cells were selected by growing on agar plates containing kanamycin (Calbiochem, Cat. No 420411) at 50 μg/ml. For protein expression cells were grown in Overnight Expression TM Instant TB medium 60g/l (Novagen, Cat. No 71491-4) supplemented with 20 ml/l of glycerol (Applichem, Cat. No A2926) and kanamycin at 50 μg/ml. Afterwars cells were collected by centrifugation and suspended in extraction buffer (150 mM NaCl, 5mM imidazole, 50 mM KH₂PO₄, pH 8) containing 0.14 mg/ml lysozyme (Fluka, Cat. No 62971). The mix is incubated on ice for 10 min and sonicated for 10 min at 70% of maximal power with BandelinSonopulsHD 2070 sonicator. The mix is centrifuged at 40000g for 30 min at 4°C. Purification of His-tagged SNAP-f was performed according to manufacturer recommendations using Ni-NTA agarose (Qiagen, Cat. No 30250). Purified and concentrated protein was stored in storage buffer (80 mM Na₂HPO₄, 20 mM NaH₂PO₄, 100 mM NaCl, 50% glycerol, 2 μM 2-mercaptoethanol, pH 7.3).

In vitro kinetics measurement of SNAP labelling
His-tagged SNAP-f enzyme was diluted in TBS buffer (50 mM Tris, 150 mM NaCl, pH 7.4) with 0.1 mg/ml BSA (NEB, Cat. No B9001) to the desired concentration of 1 μM. The reactions were started by the addition of a five molar excess of a substrate (as a 100 μM solution in DMSO). At each time point, 20 μl of reaction mixture were withdrawn and immediately mixed with 10 μl stop buffer (6x SDS loading buffer supplemented with 5 mM O²⁻-benzyguanine) pre-heated to 95 °C. The samples were analysed on a pre-casted 4 - 16% SDS-PAGE gradient gel (Biorad, Cat. No 456-1083) at 50 °C for 30 min in a CO₂ incubator. Finally the cells were irradiated with 455 nm LED for 1 h and finally microscopic images were acquired.

Experimental detail with cell
MCF-7 and HEK293T cells: Both cell lines that were maintained in 10% FBS in DMEM (Invitrogen) and 1% pen-strep antibiotic at 37 °C for 48 h. Then, the cells were washed three times with PBS. Labelling of estrogen receptor protein: MCF-7 cells maintained in 10% FBS in DMEM (Invitrogen) at 37 °C under 5% CO₂ were incubated at 37 °C for 48 h. Then, the cells were washed three times with PBS and incubated with 1 μM 4 for 1 h in a CO₂ incubator. After the culture medium was replaced, cells were finally treated with 5 μM of 2 for 1 h in a CO₂ incubator and irradiated with 455 nm LED for another 1 h and finally microscopic images were acquired. Background labelling in MCF-7 cells: MCF-7 cells maintained in 10% FBS in DMEM (Invitrogen) at 37 °C under 5% CO₂ were incubated at 37 °C for 48 h. Then, the cells were washed three times with PBS and incubated with 5 μM of 2 for 1 h in a CO₂ incubator. Finally the cells were irradiated with 455 nm LED for 1 h and finally microscopic images were acquired. Labelling of cell surface protein (BL-EGFR): HEK293T cells maintained in 10% FBS in DMEM (Invitrogen) at 37 °C under 5% CO₂ were transfected with the pcDNA3.1(+)-BL-EGFR plasmids using Lipofectamine 2000 (Invitrogen).[S1] After 5–6 h, the culture medium was replaced with DMEM (without phenol red), and the cells were incubated at 37 °C for 24 hours. Then, the cells were washed three times with PBS and incubated with 100 nM of 3 for 30 min in a CO₂ incubator. After the culture medium was replaced, cells were finally treated with 1 μM of 2 for 30 min in a CO₂ incubator and irradiated with 455 nm LED for 1 h and finally microscopic images were acquired.

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Background labelling in HEK cells: HEK293T cells maintained in 10% FBS in DMEM (Invitrogen) at 37 °C under 5% CO₂ were incubated at 37 °C for 48 h. Then, the cells were washed three times with PBS and incubated with 100 nM of 3 for 30 min in a CO₂ incubator. After the culture medium was replaced, cells were finally treated with 1 µM of 2 for 30 min in a CO₂ incubator and irradiated with 455 nm LED for 1 h and finally microscopic images were acquired. 

U2OS SNAP-mito cells: U2OS SNAP-mito cell lines that was maintained in 10% FBS in DMEM (Invitrogen) and puromycin antibiotic (1 µg/mL) at 37 °C under 5% CO₂ were incubated at 37 °C for 48 h. Then, the cells were washed three times with PBS. 

Labelling of SNAP-mito cells: U2OS SNAP-mito cells maintained in 10% FBS in DMEM (Invitrogen) at 37 °C under 5% CO₂ were incubated at 37 °C for 48 h in the presence of Doxycycline (0.1 µg/mL). Then, the cells were washed three times with PBS and incubated with 100 nM of 5 and with or without Hoechst 33258 (1 µg/mL) for 15 min. Then the cells were fixed with methanol and replaced with PBS. Finally the cells were treated with 20 µM of 2 for 1 h, sodium ascorbate (20 mM) was added, and the cells were irradiated with 455 nm LED for another 1 h and microscopic images were acquired. 

Background labelling in U2OS SNAP-mito cells: U2OS SNAP-mito cells maintained in 10% FBS in DMEM (Invitrogen) at 37 °C under 5% CO₂ were incubated at 37 °C for 48 h in the presence of Doxycycline (0.1 µg/mL). Then, the cells were washed three times with PBS and incubated with or without Hoechst 33258 (1 µg/mL) for 15 min. Then the cells were fixed with methanol and replaced with PBS. Finally the cells were treated with 20 µM of 2 for 1 h, sodium ascorbate (20 mM) was added, and the cells were irradiated with 455 nm LED for another 1 h and microscopic images were acquired. 

**Time gated Emission Microscopy**

Imaging of ruthenium was performed with live cell in a 35 mm glass bottom dish on a Leica SP5 confocal microscope with the white laser at 470 nm using time gating 5 to 12 ns. The emission was monitored at 600-640 nm. 

**Two photon Confocal Fluorescence Imaging**

Live and fixed cells were kept in 35 mm glass bottom dish. Two photon confocal images were acquired using a Zeiss LSM710 upright confocal microscope with a 20X/1.0 water dipping objective using the 730 nm laser. The emission was monitored at 500-550 nm. Finally the confocal images were analysed using the ImageJ software.

**Inverted Fluorescence Microscopy**

Fluorescence microscopic images were recorded using a Nikon Eclipse Ti inverted fluorescence microscope and a Photometrices Cool Snap HQ2 CCD camera (Nikon). The filter sets used were Semrock Bright Line Exciter 340-380, Dichroic 395, and Emitter 510-560 for rhodamine. Nikon NIS-Elements AR 3.10 imaging software was used to process the images.

**Syntheses**

**Synthesis of 1:**

This compound was prepared according to the literature procedure.[S2]

**Synthesis of 4-azidobenzyl alcohol:**

This compound was prepared according to the literature procedure.[S3]

**Synthesis of 2:**

$$\begin{align*}
\text{4-azidobenzyl alcohol (30 mg, 0.2 mmol, 2 equiv.) and triphosgene (58.4 mg, 0.2 mmol, 2 equiv.) were added in THF (5 mL) at room temperature. To this solution, DIPEA (49.6 µL, 0.3 mmol, 3 equiv.) was added drop wise and finally the suspension of 1 (30.6 mg, 0.1 mmol, 1 equiv.) in THF was added. The reaction mixture was stirred for overnight at room temperature. Then the desired product was isolated as white precipitate and washed with cold THF and thoroughly with water and ether. 40 mg, 83% yield; } & {}
\end{align*}$$

$^1$H NMR (400 MHz)(DMF-d7) δ(ppm) 12.78 (1H, s), 8.14 (1H, d, J = 2.6 Hz), 8.04 (1H, d, buried under solvent peak), 7.89 (1H, dd, J = 8.8 Hz, J = 2.6 Hz), 7.79 (1H, dd, J = 8.8 Hz, J = 2.6 Hz), 7.60 (2H, m), 7.44 (2H, d, J = 8.0 Hz), 7.05 (2H, d, J = 8.0 Hz), 5.28 (2H, s). $^{13}$C NMR (100 MHz)(DMF-d7) δ(ppm): 161.86, 153.75, 151.33, 148.94, 148.46, 141.25, 135.76, 133.12, 132.96, 131.99, 131.95, 131.04, 129.62, 126.21,
126.03, 123.88, 120.21, 70.85; LCMS (ESI): RT = 2.36 min; MS (ESI) m/z calcd for C_{22}H_{14}N_{5}O_{4}Cl_{2} [M+H]^+: 482.0423, found: 481.8166 [M+H]^+. 
Synthesis of RA1:
This compound was prepared according to the literature procedure\cite{54}.
$^1$H NMR (500 MHz)(DMSO-d$_6$) $\delta$(ppm): 10.33 (1H, s), 8.57-8.56 (1H, m), 7.93 (2H, br s), 7.90 (1H, s), 7.85 (1H, m), 7.53 (1H, d, $J = 7.5$ Hz), 7.38-7.35 (1H, m), 7.10 (1H, s), 6.07 (1H, q, $J = 6.5$ Hz), 4.41 (1H, s), 3.06 (2H, m), 2.81 (2H, t, $J = 7.0$ Hz), 1.64 (3H, d, $J = 6.5$ Hz). MW: (C$_{21}$H$_{20}$N$_3$O$_4$) 378.15; LC-MS (ESI) RT= 1.73 min m/z found: 378.12.
Synthesis of 3:
RA1[S4] (0.38 mg, 1 μmol, 2.0 equiv) and triethylamine (5 μL, excess) were mixed with bis(2,2′-bipyridine)-(5-isothiocyanato-phenanthroline)ruthenium bis-(hexafluorophosphate) (0.5 mg, 0.5 μmol, 1.0 equiv) in DMF and stirred for overnight at room temperature. The final compound was purified by biotage. Yield: 0.45 mg (87%); 1H NMR (500 MHz)(DMSO-d6) δ(ppm): 8.88-8.83 (4H, m), 8.67 (1H, d, J = 8.0 Hz), 8.59 (1H, d, J = 8.0 Hz), 8.57-8.54 (1H, m), 8.52-8.47 (1H, m), 8.40 (1H, s), 8.23-8.19 (2H, m), 8.13-8.09 (3H, m), 8.05 (1H, d, J = 8.0 Hz), 7.87-7.79 (5H, m), 7.51-7.59 (5H, m), 7.39-7.35 (4H, m), 7.01 (1H, d, J = 8.0 Hz), 6.07 (1H, q, J = 6.8 Hz), 4.36 (1H, s), 3.81-3.73 (2H, m), 2.82 (2H, m), 2.07 (1H, s), 1.64 (3H, dd, J = 6.5 Hz, 1.8 Hz). 13C DEPT-135 (125 MHz)(DMSO-d6) δ(ppm: 152.11, 151.40, 151.18, 148.72, 143.18, 137.70, 137.06, 136.14, 132.74, 128.26, 128.06, 127.85, 127.59, 127.01, 126.16, 126.82, 124.23, 123.87, 122.96, 120.42, 120.24, 73.67, 42.70, 20.33, 19.28. MW: (RuC44H32N10O4S) 1028.22 LC-MS (ESI) RT= 2.08 min m/z found: 514.13 [M]^2+; MALDI-TOF m/z found: 1027.63 [M-H]^+.
Synthesis of 4:
This compound was prepared according to the literature procedure.[35]

Synthesis of 5: p-Amino-4′-benzylguanine (BG, 2.7 mg, 10 μmol, 2.0 equiv) and triethylamine (50 μL, excess) were mixed with bis(2,2'-bipyridine)-[5-isothiocyanato-phenanthroline]ruthenium bis- (hexafluorophosphate) (5 mg, 5 μmol, 1.0 equiv) in 2 mL DMF and stirred for overnight at room temperature. The final compound was purified by biotage. 4.0 mg, 87% yield; 1H NMR (500 MHz)(DMSO-d6) δ(ppm): 10.29 (1H, s), 8.89-8.84 (5H, m), 8.77 (1H, d, J = 8.0 Hz), 8.59 (1H, d, J = 8.0 Hz), 8.49 (1H, s), 8.24- 8.06 (7H, m), 7.87-7.83 (4H, m), 7.59 (2H, d, J = 4.5 Hz), 7.54 (1H, d, J = 5.5 Hz), 7.49 (2H, d, J = 5.5 Hz), 7.39 (4H, m), 5.50 (2H, s), 4.81 (2H, d, J = 4.5 Hz). 13C DEPT-135 (125 MHz) (DMSO-d6) δ(ppm): 152.16, 151.21, 137.67, 136.22, 132.63, 128.53, 127.57, 127.26, 126.25, 125.81, 124.20, 123.91, 67.06, 47.13. LCMS (ESI): RT = 1.65 min; MS (ESI) m/z calcd for RuC₄₆H₃₆N₁₃O₄S: 920.19, found: 307.48 [M+H]⁺, 460.78 [M]²⁺, 920.32 [M-H]⁺; MALDI-TOF m/z found: 920.39 [M-H]⁺.
References