Supporting Information

Two-Photon Uncaging of Bioactive Thiols in Live Cells at Wavelengths Above 800 nm

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Figure S1. X-ray crystal structure of *bis*(boc)-protected synthetic intermediate 6. The structure confirms that the C-N bond is at desired C7 position on the NDBF core.
Figure S2. UV-Vis spectra of cDMA-NDBF and DMA-NDBF derivatives. Spectra were obtained by injecting a sample of each derivatives into an LC-MS equipped with a diode array detector set to acquire UV-Vis spectra at a frequency of 1 Hz with scanning between 300 and 600 nm and a 0.5 nm step size. An elution profile consisting of isocratic elution for 5 min with 1% buffer B, followed by a gradient to 100% buffer B was used. LC peaks displaying the correct m/z of the product were used to obtain the UV-Vis spectra. The absorbance values of DMA-NDBF-OH (11b) and cDMA-NDBF-CA (10) were normalized to the \( \lambda_{\text{max}} \) of cDMA-NDBF-FTI (12) and hence do not reflect the actual extinction coefficients. DMA-NDBF-OH (11b) and cDMA-NDBF-FTI (12) elute at 53% buffer B, while cDMA-NDBF-CA (10) elutes at 44% Buffer B.
Figure S3. Calculated UV-Vis spectra of cDMA-NDBF, DMA-NDBF and NDBF derivatives obtained using the Dalton quantum chemistry suite. These compounds include cDMA-NDBF-CA (10), NDBF-CA (11a), DMA-NDBF-CA (11c) and DMA-NDBF-EA (11d).
Figure S4. Calculated total polar surface area and CLogP for simple NDBF, DMA-NDBF and cDMA-NDBF derivatives obtained using ChemDraw.
Figure S5. Representative RP-HPLC traces of NDBF-CA (left) and cDMA-NDBF-CA (right) photolysis. UV absorption measured at 260 nm. Solvent A: H₂O-0.1%TFA, Solvent B: CH₃CN-0.1% TFA, Solvent gradient: 0%B-5 min followed by 0-100%B over 30 min. The NDBF-CA (left) starting material peak at 23 min (SM) rapidly disappears with concomitant growth of product (P) peak at 25 min. Alternatively, the cDMA-NDBF-CA (right) starting material peak remains constant over the time course.

Figure S6. Photolysis apparatus equipped with 8 x 350 nm LEDs arranged in a radial manner. Left: Apparatus without top. Right: Apparatus with top showing reaction tube inserted into the center.
Figure S7. One photon photolysis using LED apparatus of **cDMA-NDBF-CA** (red, 10 µM) and **NDBF-CA** (blue, 10 µM) after irradiation at 350 nm (left) and 428 nm (right). Photolysis reactions were performed in photolysis buffer (15 mM DTT, 20% CH$_3$CN/50 mM PB, pH 7.4). Illumination intensity values of 2.05E-09 ein·cm$^{-2}$·sec$^{-1}$ and 4.75E-09 ein·cm$^{-2}$·sec$^{-1}$ were obtained for the 350 nm and 428 nm reactors, respectively, via potassium ferrioxalate actinometry.
Figure S8. Two photon photolysis of DMA-NDBF-N-Peptide (11e) at 800 nm. Two-photon photolysis was performed using a 1 kHz Ti:Sapphire laser (125 mW, 80 fs pulse width). Photolysis reactions were performed in 0.1 mM NMM, 0.1 mM isoamylamine, 10 mM NaCl, 15 mM DTT at pH 6.8. The percent starting material was quantified using HPLC analysis of individually photolyzed samples. This graph was prepared using data previously reported by Ball and coworkers (Mangubat-Medina, A. E.; Trial, H. O.; Vargas, R. D.; Setegne, M. T.; Bader, T.; Distefano, M. D.; Ball, Z. T. Red-shifted backbone N–H photocaging agents. *Org. Biomol. Chem.*, 2020,18, 5110-5114).
Figure S9. High rep-rate laser setup for performing two-photon photolysis at wavelengths 750-900 nm.

Figure S10. Two photon photolysis of **cDMA-NDBF-CA** (10, red, 100 µM) (left) and **NDBF-CA** (11a, blue, 100 µM) (right) after 750 (■), 800 (●), 850 (▲), and 900 nm (▼) irradiation with an 80 MHz tunable Ti:sapphire mode-locked oscillator laser (450 mW, 80 fs pulse width). Photolysis reactions were performed in photolysis buffer (1 mM DTT, 20% CH₃CN/50 mM PB, pH 7.4). The percent starting material was quantified using HPLC analysis of individually photolyzed samples.
Figure S11. MDCK cells expressing GFP-H-Ras fusion protein after a) DMSO vehicle treatment showing Ras localization in the plasma membrane, b) FTI treatment (15 μM) showing Ras cytosolic accumulation after inhibition of farnesyltransferase. Cells were imaged 17 hours after treatment. Scale bar = 50 μm.

Figure S12. Structure of caged FTIs cDMA-NDBF-FTI (12) and NDBF-FTI (13). The thiol of FTI is critical for its inhibitory activity since it interacts with a Zn$^{2+}$ ion in the farnesyltransferase active site.
Figure S13. MDCK cells expressing GFP-H-Ras fusion protein after a) DMSO vehicle treatment, b) **cDMA-NDBF-FTI (12, 15 μM, no irradiation)**, and c) **NDBF-FTI (13, 15 μM, no irradiation)**. Normal Ras localization is observed in the cell plasma membrane. Cells were imaged 17 hours after treatment. Scale bar = 50 μm.
Figure S14. TP photobleaching of GFP-H-Ras in MDCK cells. a) Cells were irradiated using either 750 nm (500 mW), 800 nm (500 mW), 850 nm (1000 mW), or 900 nm (1000 mW) light for 32 sec/frame repeated four times. b) The total fluorescence intensity in each image was quantified using ImageJ software. Scale bar = 50 μm.
Synthetic Scheme

Scheme S1: Synthesis of cDMA-NDBF-Br and general conditions for thiol protection.
**Actinometry:** Actinometry was conducted using a solution of potassium ferrioxalate at 150.00 mM in 0.5 M H\textsubscript{2}SO\textsubscript{4}. 200 µL aliquots of this solution were irradiated for 30, 60, 90, 150, and 180 sec. Irradiation of this solution converts Fe(III) to Fe(II), which can then be chelated by phenanthroline, resulting in a red colored solution. The irradiated aliquots were subsequently diluted to 5 mL using 2.3 mL 0.5 M H\textsubscript{2}SO\textsubscript{4}, 2 mL 0.6 M NaOAc in 0.18 M H\textsubscript{2}SO\textsubscript{4}, and 0.5 mL of 1 mg/mL phenanthroline in H\textsubscript{2}O and allowed to incubate for 30 min, after which the absorbance of solution at 512 nm was measured in a 96-well plate. A standard curve was constructed using ferrous sulfate heptahydrate as a source of Fe(II), and diluted in the same buffer system to yield stock solutions with Fe(II) concentrations of 0.100, 0.0800, 0.0600, 0.0400, 0.0300, 0.0200, and 0.0100 mM. The absorbance of these solutions was measured in the same 96-well plate and used to determine the mols of Fe(II) produced after irradiation. The mols of Fe(II) produced were plotted against the time of irradiation in sec, and the slope of that plot was used as the rate of mols produced per second irradiation (mols/sec). The rate was divided by the quantum yield of potassium ferrioxalate at 350 nm (Hatchard, C. G.; Parker, C. A. Proc. R. Soc. Lond. A 1956, 235, 518-536), to yield I in units of ein\textperiodcentered cm\textsuperscript{-2}\textperiodcentered mol\textsuperscript{-1}. This was repeated three times using three freshly made solutions, yielding an average intensity of 1.93E-09 ein\textperiodcentered cm\textsuperscript{-2}\textperiodcentered mol\textsuperscript{-1}.
NMR Spectra

Methyl dibenzofuran acetate (2).
Methyl 2-(nitrodibenzofuran-2-yl)acetate (3).
Methyl 2-(7-aminodibenzofuran-2-yl)acetate (4).
Methyl 2-(7-((Boc)amino)dibenzofuran-2-yl)acetate (5).
Methyl 2-(7-\(\text{bis}(\text{Boc})\text{amino}\))dibenzofuran-2-yl)acetate (6).
Methyl 2-bromo-2-(7-(bis(Boc)amino)dibenzofuran-2-yl)acetate (7).
Methyl 2-bromo-2-(7-(dimethylamino)dibenzofuran-2-yl)acetate (8).
cDMA-NDBF-Br (9).
cDMA-NDBF-CA (10).
cDMA-NDBF-FTI (12).
NDBF-FTI (13).