Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

meso-methylhydroxy BODIPY: a scaffold for photo-labile protecting groups

Naama Rubinstein[a], Pei Liu[b], Evan W. Miller[b,c,d] and Roy Weinstain[a]

[a] *Department of Molecular Biology and Ecology of Plants, Life Sciences Faculty, Tel-Aviv University, Ramat Aviv, Tel-Aviv 6997801, Israel.*

[b] *Department of Chemistry, University of California, Berkeley, CA, 94720 USA.*

[c] *Department of Molecular & Cell Biology, University of California, Berkeley, CA, 94720, USA.*

[d] *Helen Wills Neuroscience Insitute, University of California, Berkeley, CA, 94720 USA.*

[*] Correspondences and requests for materials should be directed via e-mail to:

Roy Weinstain (royweinstain@post.tau.ac.il)

Table of content

Supplementary figures

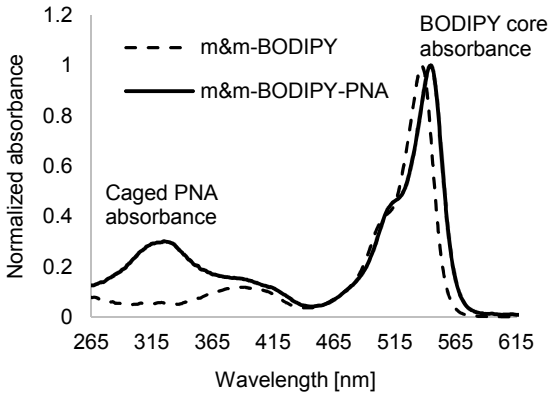


Figure S1. Absorbance spectra of m&m-BODIPY and its PNA conjugate (m&m-BODIPY-PNA). Both molecules display absorbance maxima at ca. 545 nm in PBS (pH 7.4 20 mM). In addition, m&m-BODIPY-PNA displays an added absorbance maxima at 325 nm, which arise for the conjugated PNA.

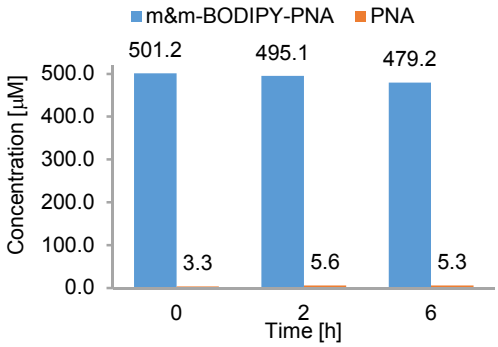


Figure S2. Dark stability of m&m-BODIPY-PNA. The conjugate (500 µM) was incubated in PBS 20 mM pH 7.4, supplemented with 10% ACN for the indicated times. At each time point, a sample was taken for HPLC-MS analysis. Less than 1% degradation was observed after 6 hours.

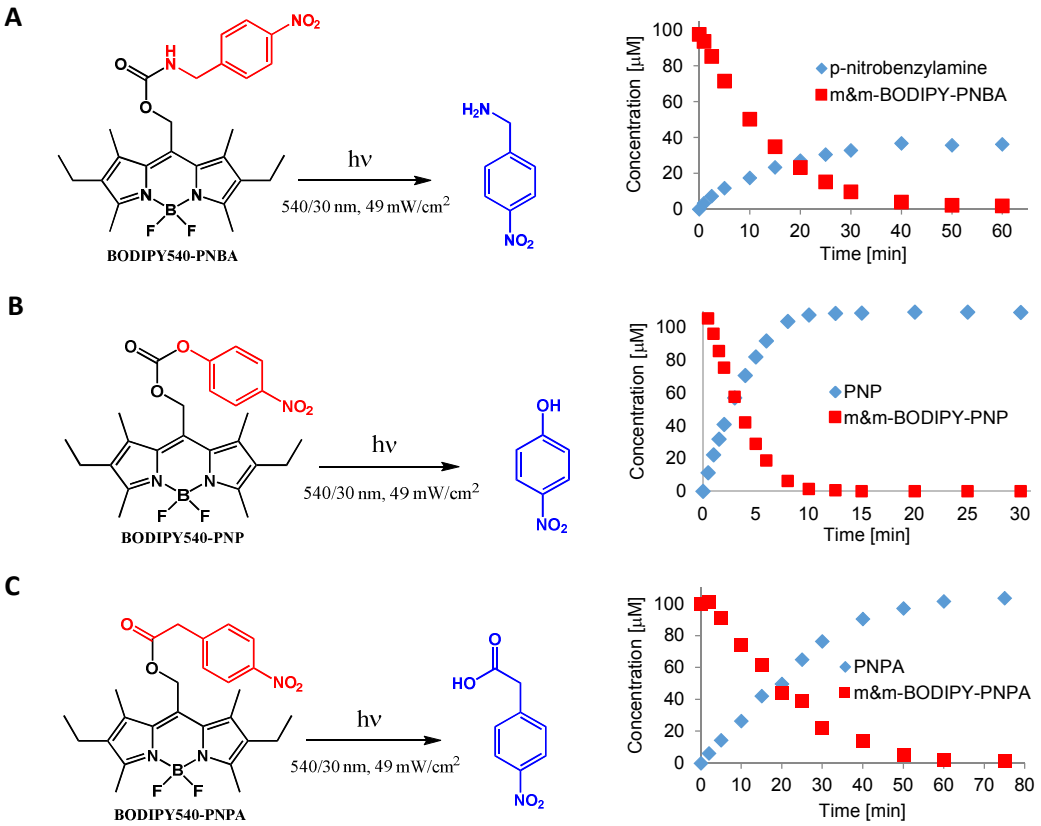


Figure S3. Light-induced release of model leaving groups from m&m-BODIPY cage. Schematic representation (*left*) and HPLC-MS monitoring (*right*) of photolysis of **A**) *p*-nitrobenzylamine, **B**) *p*-nitrophenol and **C**) *p*-nitrophenylacetic acid from the corresponding m&m-BODIPY conjugate, following 540/30 nm irradiation (100 μM in PBS 20 mM pH 7.4 supplemented with 5% ACN) for indicated times.

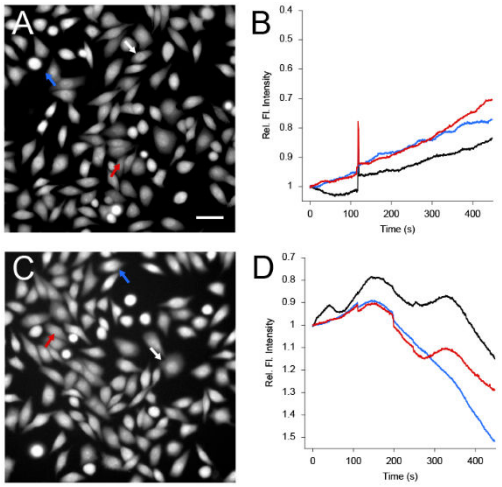


Figure S4. Additional controls for Figure 3 in main text: live cell epifluorscence imaging of Ca^{2+} release triggered by m&m-BODIPY-histamine in HeLa cells. All cells were loaded with fura-2 AM and then treated with either m&m-BODIPY and green light (**A**) or green light alone (**C**). Changes in Fura-2 fluorescence are quantified in panels **B** and **D**. Spike in **D** is from refocusing the drifting cells. Changes in **B** are from bleaching and recovery of the highly fluorescent m&m-BODIPY compound. Scale bar is 20 μm.

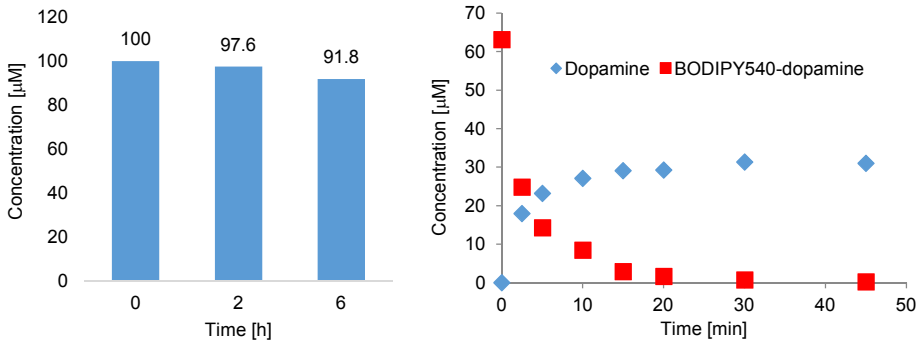


Figure S5. Dark stability of m&m-BODIPY-dopamine and light-induced release of dopamine. *Left:* Dark stability of m&m-BODIPY-dopamine (100 µM in PBS pH 7.4 20 mM, 5% ACN). Less than 9% hydrolysis was observed after 6 hours. *Right:* Accumulation and depletion of dopamine and m&m-BODIPY-dopamine, respectively, following 540/30 nm irradiation of BODIPY540-PNBA (65 µM in PBS 20 mM pH 7.4 supplemented with 5% ACN) for indicated times, as monitored by HPLC-MS.

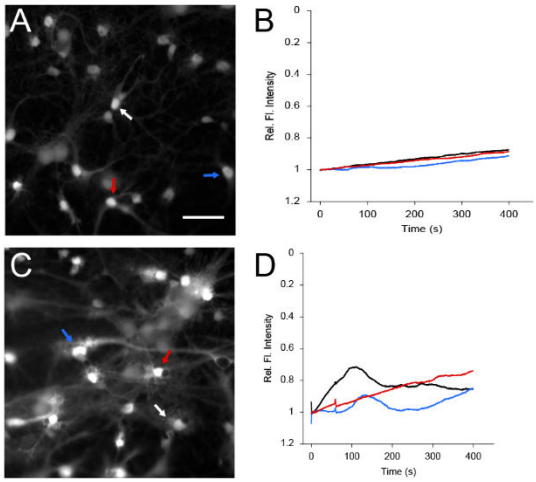
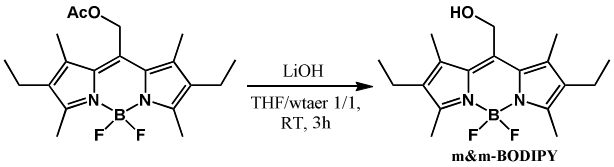


Figure S6. Additional controls for Figure 4 in main text: live cell epifluorscence imaging of Ca^{2+} release triggered by BODIPY-dopamine in KCl-primed rat cortical/hippocampal neurons. All cells were loaded with fura-2 AM and then treated with either green light alone (**A**) or m&m-BODIPY540 and green light (**C**). Changes in Fura-2 fluorescence are quantified in panels **B** and **D**. Changes in **D** are from bleaching and recovery of the highly fluorescent m&m-BODIPY compound. Scale bar is 20 µm.

General synthetic and analytical methods

Pyrromethene605 was purchased from Exciton. All other chemicals were purchased from Sigma-Aldrich and used as received unless otherwise noted. Anhydrous solvents and reagents (DCM, THF, DMF) were obtained as SureSeal bottles from Sigma-Aldrich. Thin-layer chromatography and flash chromatography were performed using EMD pre-coated silica gel 60 F-254 plates and silica gel 60 (230-400 mesh). UV absorbance spectra were recorded on a Cary 3E (Varian) fluorimeter. Fluorescence spectra were recorded on Tecan infinite 200 pro multimode reader or Fluorolog 2 (Spex) fluorimeter. Analytical and preparative HPLCs were performed on Agilent HPLCs, with Luna C18(2) columns (Phenomenex) using water (solvent A) and acetonitrile (solvent B) with 0.05% TFA as an additive. Low resolution ESI mass spectrometry was performed on an Agilent LC/MSD Trap XCT coupled to an Agilent HPLC. High resolution ESI mass spectrometry was performed on a Waters SYNAPT system. ^{1}H- and ^{13}C-NMR spectra were collected in $CDCl_3$ or CD_3OH (Cambridge Isotope Laboratories, Cambridge, MA) at 25 ºC using a Bruker Avance III spectrometer at 400 MHz and 100 MHz respectively at the Department of Chemistry NMR Facility at Tel-Aviv University. All chemical shifts are reported in the standard δ notation of parts per million using the TMS peak as an internal reference. Abbreviations: THF: tetrahydrofurane, DMF: dimethylformamide, DCM: dichloromethane, DIPEA: diisopropylethylamine, DMAP: dimethylaminopyridine, PNA: *p*-nitroaniline, PNP: *p*-nitrophenol, PNPA: *p*-nitrophenylacetic acid, PNBA: *p*-nitrobenzylamine, EtOAc: ethylacetate, Hex: hexane, RT: room temperature, DBTL: dibutyltin dilaurate, DCC: N,N'-Dicyclohexylcarbodiimide, ACN: acetonitrile,

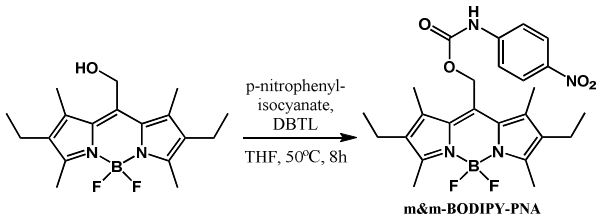
Synthetic procedures



m&m-BODIPY

m&m-BODIPY540. Pyrromethene605 (500 mg, 1.3 mmol, 1 eq) was dissolved in 26.5 mL THF to yield a 0.05 M solution. LiOH (280 mg, 6.65 mmol, 5 eq) was dissolved in 26.5 mL water, added to the THF solution and the reaction was stirred at RT for 3 hours while monitored by TLC. Upon completion, the reaction was diluted with EtOAc and washed with saturated NH_4Cl solution and brine. The water fractions were extracted with EtOAc. The combined organic phase was dried with $MgSO_4$, filtered and solvents were removed under reduced pressure. The crude product was purified by silica gel chromatography (5%-20% EtOAc in Hexane gradient) to yield **m&m-BODIPY** (280 mg, 0.84 mmol, yield 65%) as a dark orange/brown solid.

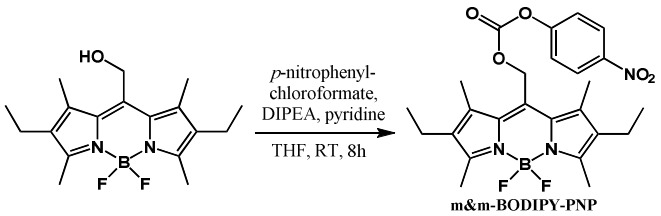
^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 1.05 (t, *J* = 7.5 Hz, 6H), 1.72 (bs, 1H), 2.40 (q, *J* = 7.5 Hz, 4H), 2.42 (s, 6H), 2.55 (s, 6H), 5.30 (s, 2H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 12.7, 14.9, 17.3, 31.1, 56.3, 131.8, 133.5, 136.5, 136.7, 154.7. ESI-MS (positive mode) calculated ($C_{18}H_{25}BF_2N_2O$) 334.2, found *m*/*z* $[M+H]^+$ 335.3. ESI-HRMS (positive mode) $[M+Na]^+$: *m*/*z* calculated: 356.1962, found: 356.1963. λ_{max} (abs.) = 537 nm, ε = 53,300 $L \cdot mol^{-1} \cdot cm^{-1}$, Φ_{fl} = 0. 0.90.



m&m-BODIPY-PNA

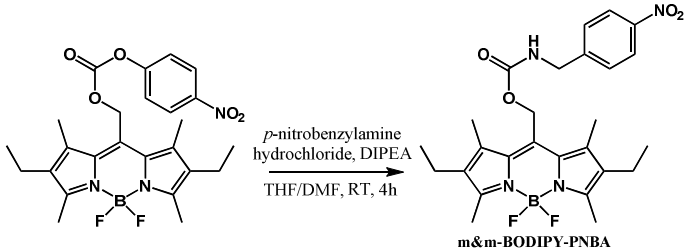
m&m-BODIPY-PNA. m&m-BODIPY (15 mg, 0.045 mmol, 1 eq) was dissolved in 3 mL toluene and *p*-nitrophenylisocyanate (29 mg, 0.180 mmol, 4 eq) and Et_3N (catalytic amount) were added. The reaction mixture was heated to 50ºC and stirred under argon atmosphere for 72 hours. Upon completion, the reaction was diluted with EtOAc and washed with saturated NH_4Cl solution and brine. The water fractions were extracted with EtOAc. The combined organic phase was dried with $MgSO_4$, filtered and solvents were removed under reduced pressure. The crude was purified by silica gel chromatography (0%-12% EtOAc in Hexane gradient) to yield **m&m-BODIPY-PNA** (16 mg, 0.032 mmol, yield 71%) as a dark orange solid.

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 1.04 (t, *J* = 7.5 Hz, 6H), 2.32 (s, 6H), 2.39 (q, *J* = 7.5 Hz, 4H), 2.51 (s, 6H), 5.48 (s, 2H), 7.44 (bs, 1H), 7.58 (d, *J* = 8.9 Hz, 2H), 8.20 (d, *J* = 8.9 Hz, 2H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 12.8, 14.8, 17.3, 31.3, 56.1, 120.1, 125.3, 131.9, 133.4, 136.5, 136.8, 143.2, 144.5, 154.6. ESI-MS (positive mode) calculated ($C_{25}H_{29}BF_2N_4O_4$) 498.3, found *m*/*z* $[M+H]^+$ 499.5. ESI-HRMS (positive mode) $[M+Na]^+$: *m*/*z* calculated: 356.1962, found: 356.1963. λ_{max} (abs.) = 545 nm, ε =34,500 L·mol^{-1} ·cm^{-1}, Φ_{fl} = 0.71.



m&m-BODIPY-PNP. m&m-BODIPY (25.5 mg, 0.076 mmol, 1 eq) was dissolved in 2 mL dry THF. Then, *p*-nitrophenylchloroformate (61 mg, 0.3 mmol, 4 eq), DIPEA (66 µL, 0.38 mmol, 5 eq) and pyridine (1.6 µL, 0.02 mmol, 0.25 eq) were added and the reaction was stirred at room temperature under argon atmosphere for 16 hours. Upon completion, the reaction was diluted with EtOAc and washed with saturated NH_4Cl solution and brine, dried with $MgSO_4$, filtered and solvents were removed under reduced pressure. The crude product was purified by silica gel chromatography (EtOAc:Hex 5:95 to 40:60 gradient) to yield **m&m-BODIPY-PNP** (25 mg, 0.051 mmol, yield 68%) as a dark orange solid. According to NMR and HPLC-MS, the product contained a small amount of *p*-nitrophenol but was nevertheless used for the subsequent reactions without further purification. A small amount was purified by prep-HPLC for spectroscopic and photochemical analysis.

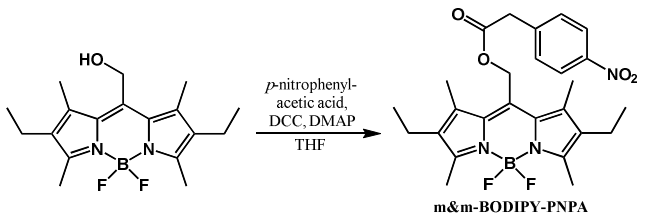
^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 1.06 (t, *J* = 7.5 Hz, 6H), 2.36 (s, 6H), 2.41 (q, *J* = 7.5 Hz, 4H), 2.53 (s, 6H), 5.60 (s, 2H), 7.41 (d, *J* = 9.1 Hz, 2H), 8.30 (d, *J* = 9.1 Hz, 2H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 12.9, 14.8, 17.3, 31.1, 62.3, 121.8, 125.5, 129.3, 132.3, 134.1, 136.7, 145.6, 152.4, 155.4, 155.8. ESI-MS (positive mode) calculated ($C_{25}H_{28}BF_2N_3O_5$) 499.3, found *m*/*z* $[M+H]^+$ 500.4. ESI-HRMS (positive mode) $[M+Na]^+$: *m*/*z* calculated: 521.2024, found: 521.2019. λ_{max} (abs.) = 547 nm, ε = 48,400 L·mol^{-1} ·cm^{-1}, Φ_{fl} = 0.54.



m&m-BODIPY-PNBA. m&m-BODIPY-PNP (25 mg, 0.051 mmol, 1 eq) was dissolved in 1 mL dry THF. *p*-nitrobenzylamine hydrochloride (29 mg, 0.153 mmol, 3 eq) and DIPEA (27 µL, 0.158 mmol, 3.1 eq) were dissolved

in 0.5 mL dry DMF and added to the THF solution. The reaction mixture was stirred at room temperature under argon atmosphere for 4 hours. Upon completion, the reaction was diluted with EtOAc and washed with saturated NH_4Cl solution and brine, dried with $MgSO_4$, filtered and solvents were removed under reduced pressure. The crude product was purified by preparative HPLC (see Preparative HPLC purification conditions below). The eluted product was immediately frozen and lyophilized to yield **m&m-BODIPY-PNBA** (19 mg, 0.037 mmol, yield 73%) as a dark orange solid.

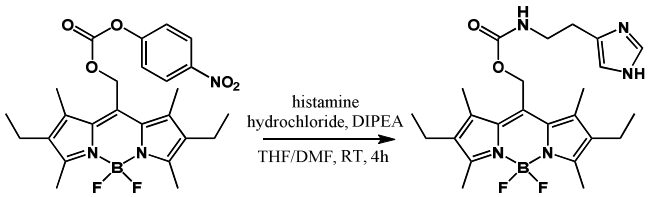
^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 1.05 (t, *J* = 7.5 Hz, 6H), 2.30 (s, 6H), 2.40 (q, *J* = 7.5 Hz, 4H), 2.51 (s, 6H), 4.51 (d, *J* = 6.0 Hz, 2H), 5.30 (t, *J* = 6.0 Hz, 1H), 5.39 (s, 2H), 7.45 (d, *J* = 8.1 Hz, 2H), 8.21 (d, *J* = 8.1 Hz, 2H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 12.8, 14.8, 17.3, 44.7, 59.1, 124.2, 128.1, 131.6, 132.4, 133.8, 136.7, 145.7, 155.3, 156.1. ESI-MS (positive mode) calculated ($C_{26}H_{31}BF_2N_4O_4$) 512.3, found *m*/*z* $[M+H]^+$ 513.6. ESI-HRMS (positive mode) $[M+Na]^+$: *m*/*z* calculated: 534.2343, found: 534.2340. λ_{max} (abs.) = 544 nm, ε = 45,000 $L \cdot mol^{-1} \cdot cm^{-1}$, Φ_{fl} = 0.69.



m&m-BODIPY-PNPA

m&m-BODIPY-PNPA. m&m-BODIPY (10 mg, 0.03 mmol, 1 eq) was dissolved in 2 mL dry DCM. Then, PNPA (6 mg, 0.033 mmol, 1.1 eq), DCC (7mg, 0.033 mmol, 1.1 eq) and DMAP (4 mg, 0.033 mmol, 1.1 eq) were added and the reaction was stirred at RT under argon atmosphere for 16 hours. Upon completion, the reaction was diluted with EtOAc and washed with saturated NH_4Cl solution and brine, dried with $MgSO_4$, filtered and solvents were removed under reduced pressure. The crude product was purified by preparative HPLC (see Preparative HPLC purification conditions below). The eluted product was immediately frozen and lyophilized to yield **m&m-BODIPY-PNPA** (13 mg, 0.026 mmol, yield 87%) as a dark orange solid.

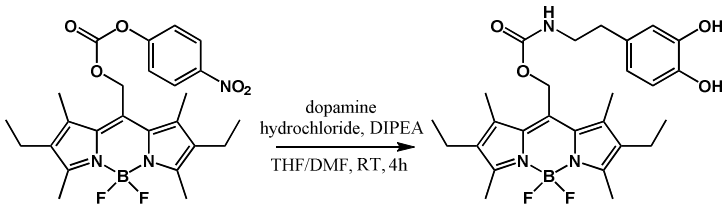
^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 1.03 (t, *J* = 7.5 Hz, 6H), 2.13 (s, 6H), 2.37 (q, *J* = 7.5 Hz, 4H), 2.51 (s, 6H), 3.80 (s, 2H), 5.36 (s, 2H), 7.46 (d, *J* = 8.6 Hz, 2H), 8.19 (d, *J* = 8.6 Hz, 2H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 12.7, 12.9, 14.8, 17.3, 41.0, 59.2, 124.0, 130.4, 130.9, 132.3, 133.9, 136.5, 140.7, 154.9, 155.5. ESI-MS (positive mode) calculated ($C_{26}H_{30}BF_2N_3O_4$) 497.3, found *m*/*z* $[M+H]^+$ 498.3. ESI-HRMS (positive mode) $[M+Na]^+$: *m*/*z* calculated: 519.2231, found: 519.2233. λ_{max} (abs.) = 545 nm, ε = 38,600 $L \cdot mol^{-1} \cdot cm^{-1}$, Φ_{fl} = 0.23.



m&m-BODIPY-histamine. m&m-BODIPY-PNP (25 mg, 0.051 mmol, 1 eq) was dissolved in 1 mL dry THF. Histamine (17 mg, 0.153 mmol, 3 eq) and DIPEA (27 µL, 0.158 mmol, 3.1 eq) were dissolved in 0.5 mL dry DMF and added to the THF solution. The reaction mixture was stirred at RT under argon atmosphere for 4 hours. Upon completion, the reaction was diluted with EtOAc and washed with saturated NH_4Cl solution and brine, dried with

$MgSO_4$, filtered and solvents were removed under reduced pressure. The crude product was purified by preparative HPLC (see Preparative HPLC purification conditions below). The eluted product was immediately frozen and lyophilized to yield **m&m-BODIPY-histamine** (15 mg, 0.034 mmol, yield 66%) as an orange solid.

^{1}H-NMR (MeOD, 400 MHz): δ = 1.06 (t, *J* = 7.5 Hz, 6H), 2.30 (s, 6H), 2.45 (bs Hz, 10H), 3.90 (t, *J* = 6.6 Hz, 2H), 3.46 (q, *J* = 6.6 Hz, 2H), 5.30 (s, 2H), 7.27 (s, 1H), 8.65 (s, 1H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.7, 13.9, 16.1, 24.6, 44.7, 56.9, 118.4, 131.1, 131.9, 132.6, 136.0, 153.5, 155.2. ESI-MS (positive mode) calculated ($C_{23}H_{30}BF_2N_5O_2$) 457.3, found *m*/*z* $[M+H]^+$ 458.5. ESI-HRMS (positive mode) $[M+Na]^+$: *m*/*z* calculated: 493.2551, found: 493.2552. λ_{max} (abs.) = 543 nm, ε = 32,800 L·mol^{-1}·cm^{-1}, Φ_{fl} = 0.72.



m&m-BODIPY-dopamine. m&m-BODIPY540-PNP (25 mg, 0.051 mmol, 1 eq) was dissolved in 1 mL dry THF. Dopamine hydrochloride (29 mg, 0.153 mmol, 3 eq) and DIPEA (27 µL, 0.158 mmol, 3.1 eq) were dissolved in 0.5 mL dry DMF and added to the THF solution. The reaction mixture was stirred at RT under argon atmosphere for 4 hours. Upon completion, the reaction was diluted with EtOAc and washed with saturated NH_4Cl solution and brine, dried with $MgSO_4$, filtered and solvents were removed under reduced pressure. The crude product was purified by preparative HPLC (see Preparative HPLC purification conditions below). The eluted product was immediately frozen and lyophilized to yield **m&m-BODIPY-dopamine** (20 mg, 0.04 mmol, yield 79%) as a dark orange solid.

^{1}H-NMR ($CDCl_3$+MeOD, 400 MHz): δ = 1.05 (t, *J* = 7.5 Hz, 6H), 2.27 (s, 6H), 2.39 (q, *J* = 7.5 Hz, 4H), 2.49 (s, 6H), 2.69 (t, *J* = 6.9 Hz, 2H), 3.41 (q, *J* = 6.5 Hz, 2H), 5.28 (s, 2H), 5.30 (t, *J* = 6.2 Hz, 1H), 6.54 (dd, *J* = 8.1, 1.8 Hz, 1H), 6.67 (d, *J* = 1.8 Hz, 1H), 6.74 (d, *J* = 8.1 Hz, 1H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 12.7, 12.8, 14.8, 17.3, 35.4, 42.5, 58.5, 115.6, 115.9, 121.3, 131.5, 132.2, 132.3, 133.7, 136.9, 142.4, 143.9, 155.0, 156.0. ESI-MS (positive mode) calculated ($C_{27}H_{34}BF_2N_3O_4$) 513.4, found *m*/*z* $[M+H]^+$ 514.6. ESI-HRMS (positive mode) $[M+Na]^+$: *m*/*z* calculated: 535.2544, found: 535.2542. λ_{max} (abs.) = 543 nm, ε = 56,501 L·mol^{-1}·cm^{-1}, Φ_{fl} = 0.69.

Preparative HPLC purification conditions.

All preparative separations were performed on Agilent HPLC system (1200 series) with Luna 10 µm PREP C18(2) column (250.0 X 21.2 mm, 100 Å), using a water-acetonitrile gradient of 25% to 100% solvent B in 20 minutes then 5 minutes at 100% solvent B at flow rate of 15 mL/min (solvent A = water, solvent B = acetonitrile, both with 0.05% TFA as an additive).

Fluorescence quantum yield and molar absorption coefficient measurements.

The quantum fluorescence yields (Φ_{fl}) of all compounds were determined in ethanol using the comparative method with Pyrromethene605 in ethanol as standard (Φ_{fl} = 0.74). Recordings were performed at room temperature.

Molar absorption coefficients (ε) and maximum absorbance wavelengths (λ_{max}) were determined in PBS (pH 7.4, 50 mM) supplemented with 5% ACN using Beer's law, from plots of absorbance vs. concentration (for example, see Figure S7A). Recordings were performed in 10 mm path length quartz cuvettes at room temperature.

General photolysis and monitoring procedures.

Compound sample (2 mL of 100 µM in PBS 20 mM pH 7.4 supplemented with 5% ACN) was placed in 10x10x30 mm quartz cuvette (10 mm path) equipped with an internal magnetic stirrer. The cuvette was placed in a Solar Simulator fitted with a 540/30 nm filter and irradiated for the indicated times while constantly stirred. Light intensity at the cuvette was measured by light meter to be 49.1 ± 0.2 mW/cm^2 in all experiments. At each time point, samples were taken for analysis by spectrophotometer and/or HPLC-MS. For spectrometry, samples were diluted 20-fold in ACN to ensure absorbance reading within the linear range. For HPLC-MS, samples were diluted 5-10-fold in ACN and span down to remove salts.

Calibration curves for all tested and reference compounds were generated in each detection method (spectrophotometer or HPLC-MS). As an example, the calibration curves generated for m&m-BODIPY-PNA photolysis monitoring are presented below, including raw data for HPLC analysis (data from spectrophotometer is presented in Figure 2B):

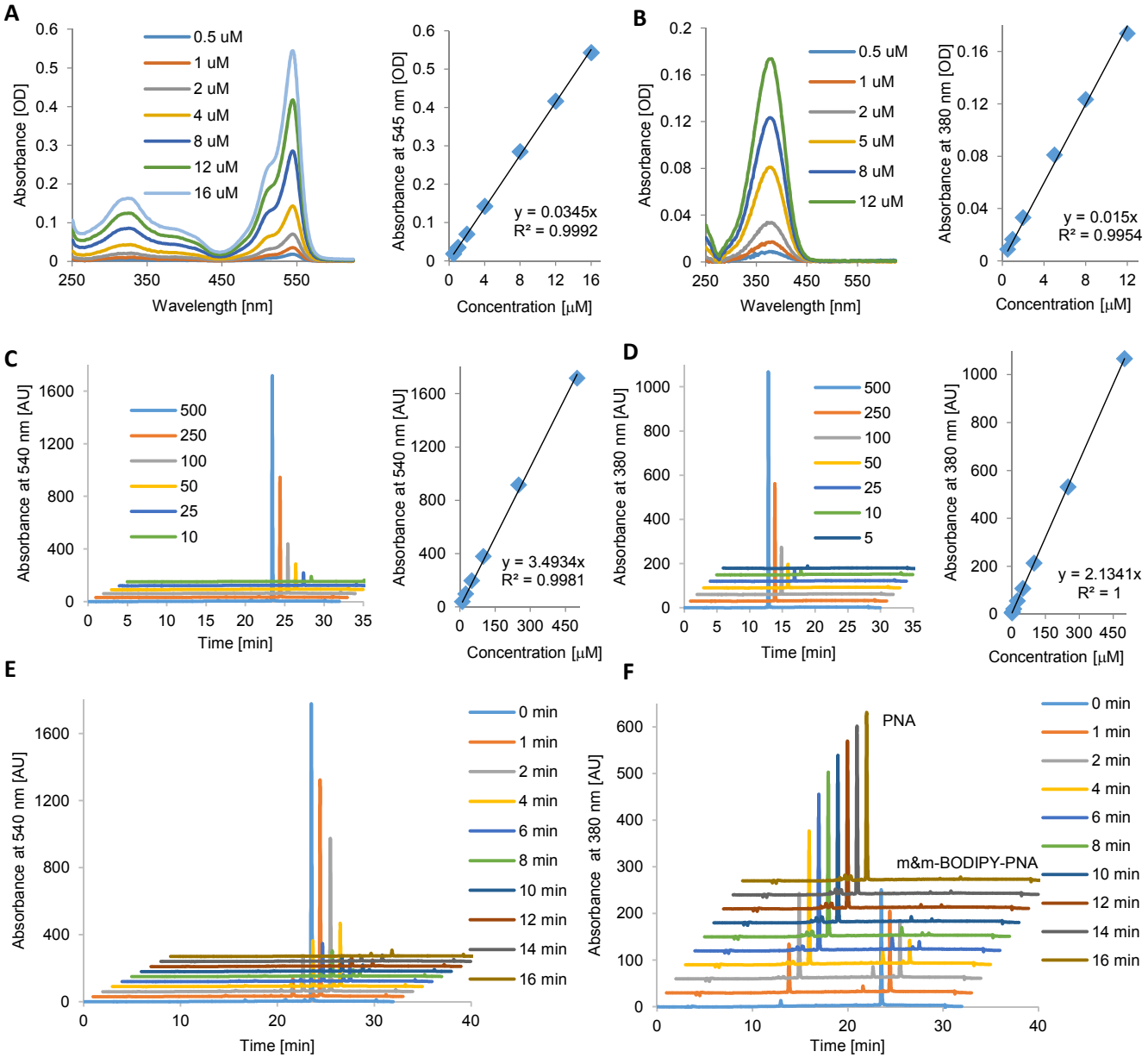


Figure S7. Calibration curves for m&m-BODIPY-PNA photolysis analysis. **A**) *Left:* absorbance spectra of BODIPY540-PNA at indicated concentrations (in PBS 20 mM pH 7.4). *Right:* Calibration curve generated from left at 545 nm. **B**) *Left:* absorbance spectra of PNA at indicated concentrations (in PBS 20 mM pH 7.4, 5% ACN). *Right:* Calibration curve generated from left at 377 nm. **C**) *Left:* HPLC chromatograms of m&m-BODIPY-PNA at indicated concentrations (μM), as monitored at 540 nm. *Right:* Calibration curve generated from left. **D**) *Left:* HPLC chromatograms of PNA at indicated concentrations (μM), as monitored at 380 nm. *Right:* Calibration curve generated from left. **E, F**) HPLC chromatograms of m&m-BODIPY-PNA (100 μM in PBS 20 mM pH 7.4) after light irradiation (540/30 nm 49 mW/cm^2) for indicated times, as monitored at **E**) 540 nm or **F**) 380 nm.

General Cell Culture Methods

HeLa cells were cultured in DMEM with 10% FBS and plated on 12 mm round poly-D-lysine coated coverslips in 24 well trays at a density of 1.6x10^5 cells/well 12-18 hours prior to imaging experiments. Prior to imaging, cells were incubated with 2 μM fura2-AM (LifeTech) in HBSS at room temperature (25 °C) for 1 hour, washed once, and transferred to a 35 mm imaging dish containing 2 mL HBSS. Compounds (m&m-BODIPY photocages) were diluted from stocks in DMSO (1000x) to 3x concentration in 1 mL HBSS. This was added to the imaging dish and then the experiment began. Pyrilamine maleate (1 μM, Sigma) was added prior to addition of the BODIPY compounds.

Neurons were harvested from rat embryonic hippocampus and cortex. After culturing for 14 days *in vitro* (DIV) in neurobasal media supplemented with B27, cells were loaded in a fashion identical to the HeLa cells described above. (+)-butaclamol (100 μM, Sigma) was added prior to treatment with dopamine (10 μM) or BODIPY compounds (10 μM). Neurons were treated with a 3x stock of KCl (150 mM stock, 50 mM final) to prime the response to dopamine. After initial Ca^{2+} transients, as measured by fura-2 reached baseline levels, dopamine or BODIPY was added and the photolysis began. All animal care and experimental protocols were approved by the Animal Care and Use Committee at UC Berkeley

Imaging Instrumentation and Experimental

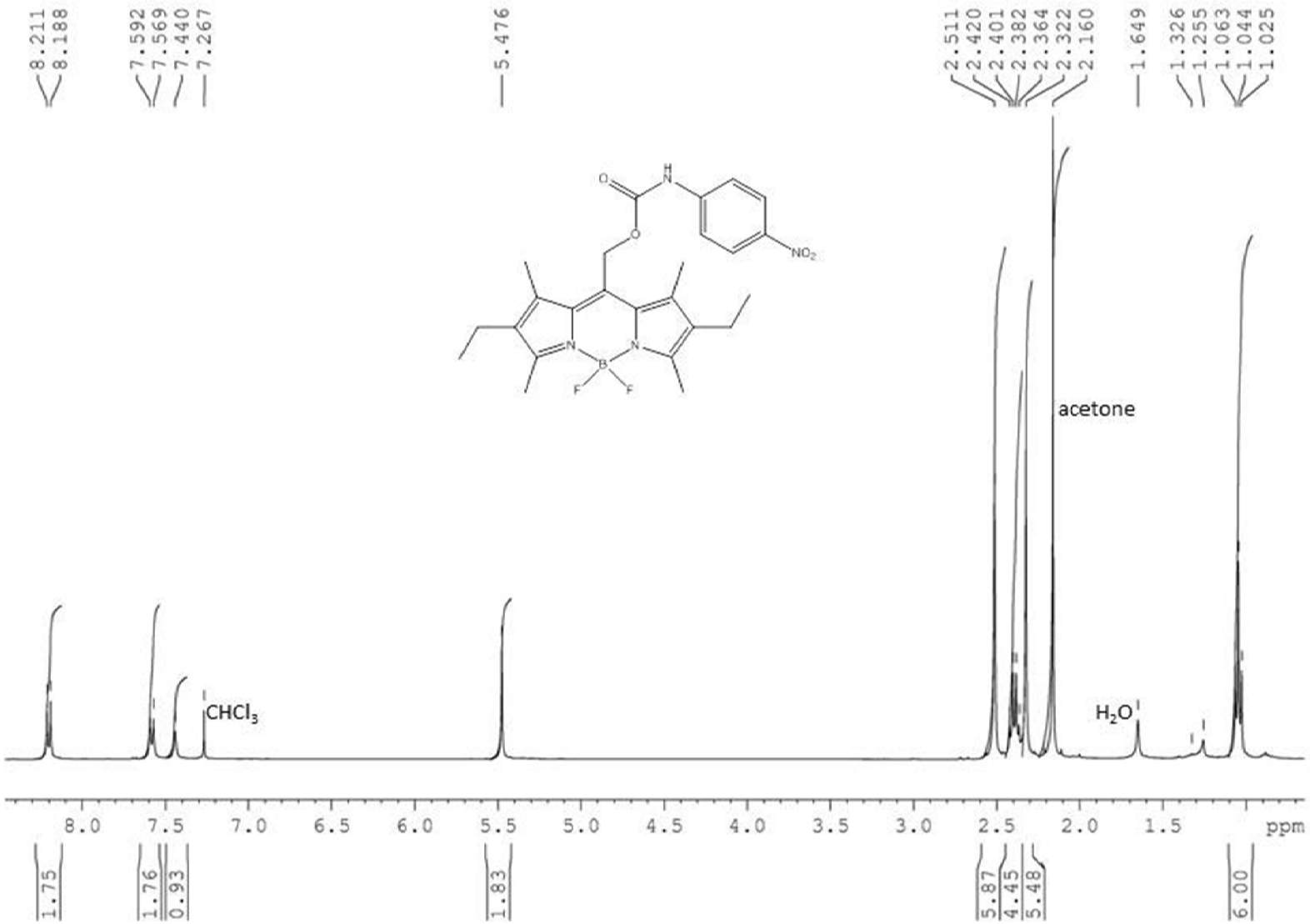
All imaging was performed on an AxioExaminer Z-1 (Zeiss) equipped with a Spectra-X Light engine LED light (Lumencor). Light was delivered through a W-Plan-Apo 20x/1.0 objective and focused onto a OracFlash4.0 sCMOS camera (Hamamatsu). Excitation for fura-2 imaging was provided at 390/22, passed through a quadruple dichroic mirror (432/38, 509/22, 586/40, 654LP), and a quadruple emission filter (430/32, 508/14, 586/30, 708/98). In this configuration, increases in $[Ca^{2+}]_i$ result in decreases in fura-2 fluorescence. Bleaching of BODIPY compounds was conducted at 542/33 nm, passing through the same dichroic and emission filter. Samples were uncaged with 542 nm light for 15 seconds at maximum power, followed by fura-2 imaging, 25 ms exposures every second for ~5 minutes after uncaging.

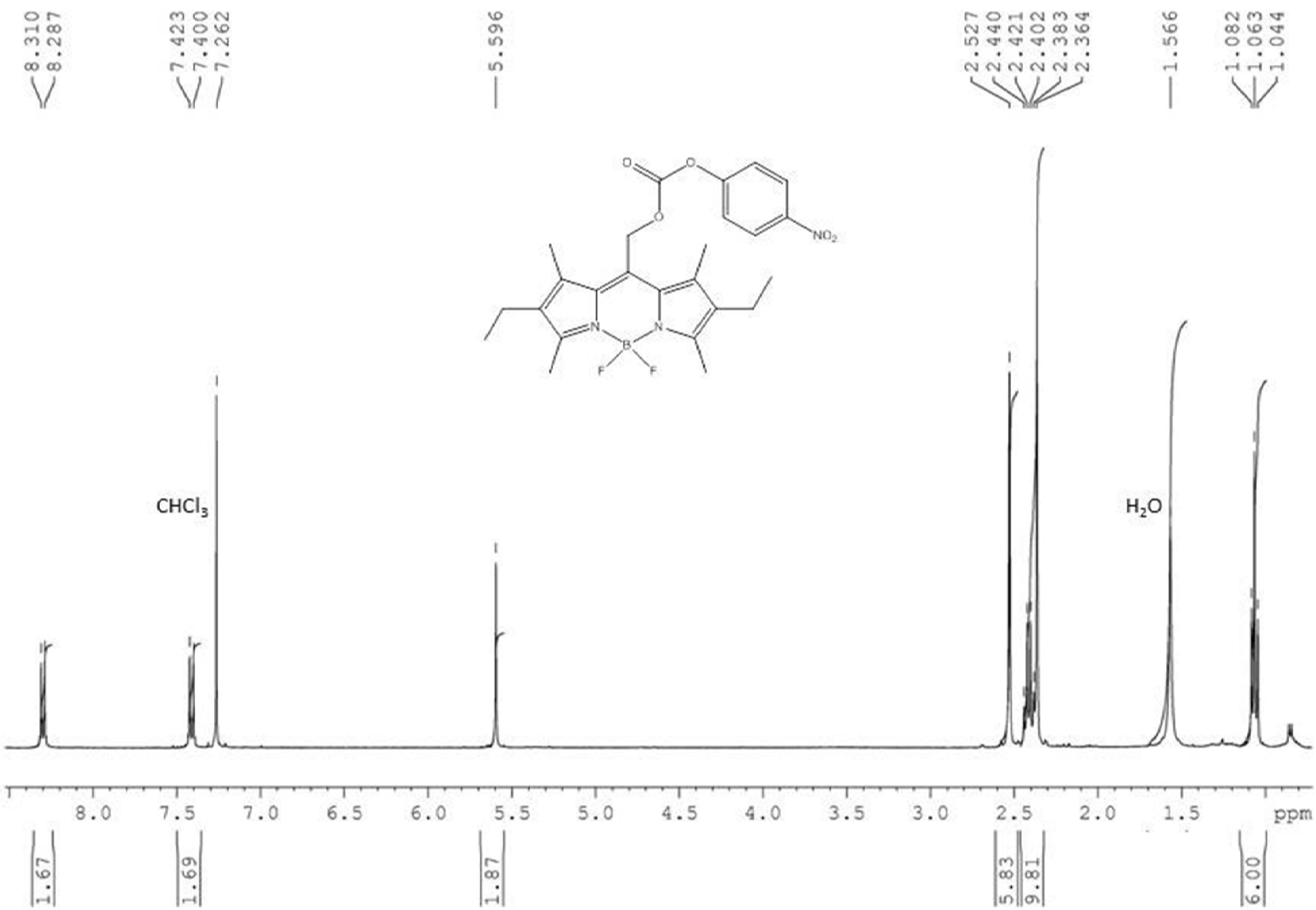
Data Analysis

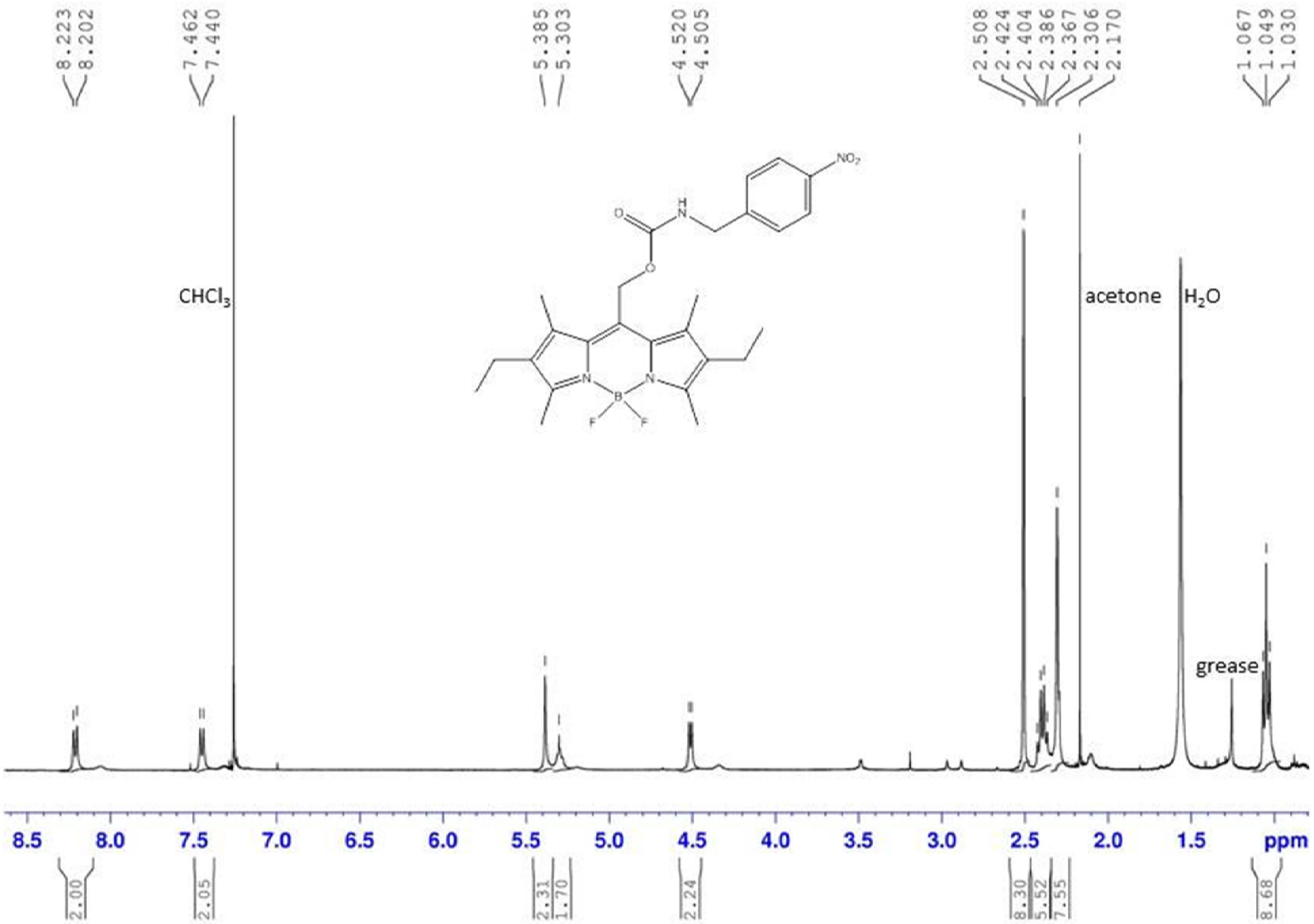
Plots of ΔF/F were constructed by creating regions of interest (ROI) around cells, subtracting background (ROI of non-cell region), and dividing by the mean fluorescence for the first 10 frames of the acquisition. These values were plotted as ΔF/F vs. time.

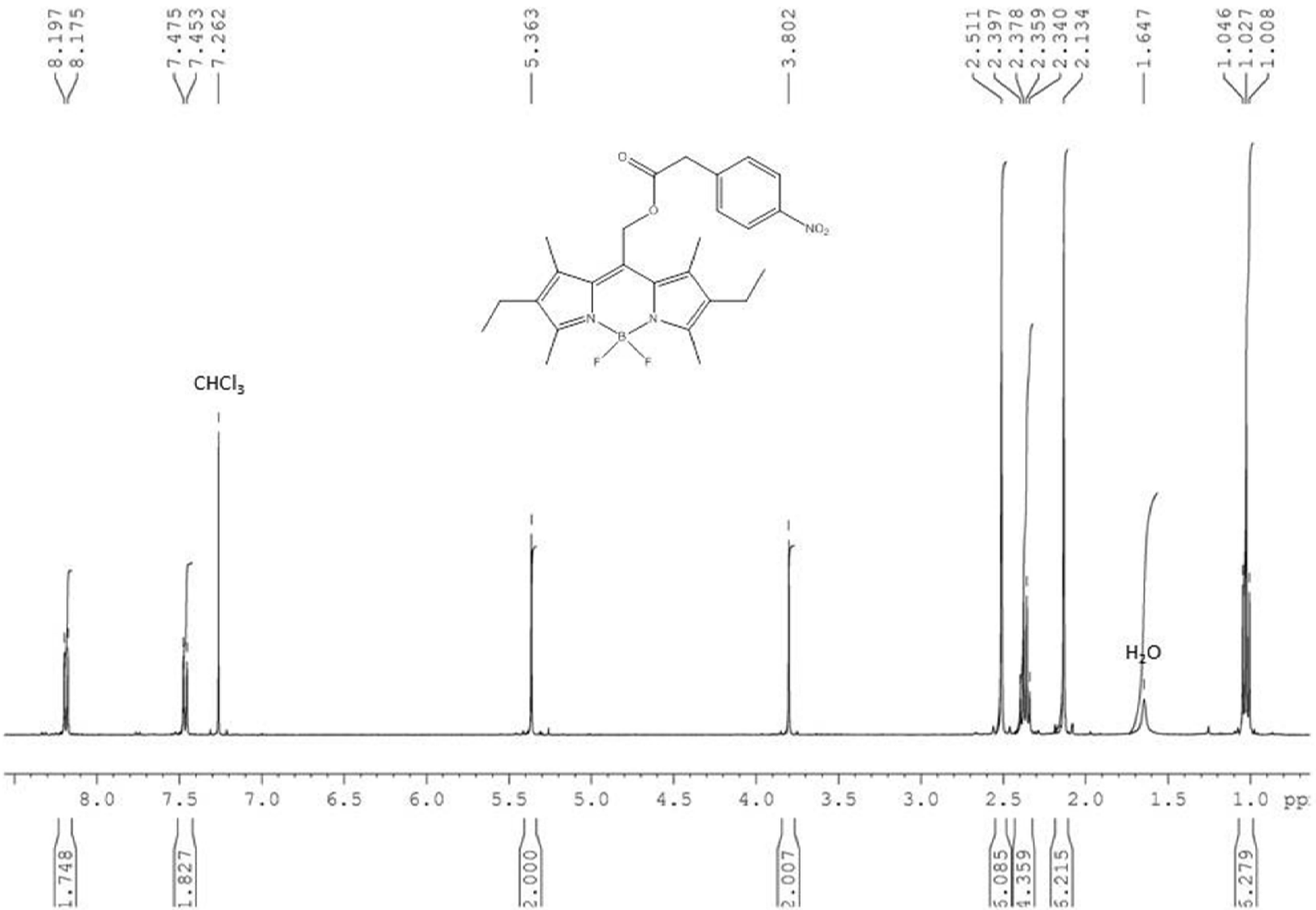
HeLa cells: Images opened in FIJI (v1.49b, FIJI is just ImageJ, NIH). Corrected drift, with Plugins>Registration>StackReg>Rigid Body Transformation. Bleaching was corrected, if necessary, with Image>Adjust>Bleach Correction>Exponential Fit. Then an average intensity Z-project was calculated for the first 10 frames (to set F_0). The Registered, Bleach-corrected stack was then divided by F_0 to give the F/F_0 movie, which was scaled from 0.75 to 1.25 in all cases, except for SI Movie 5, which was m&m-BODIPY540 control.

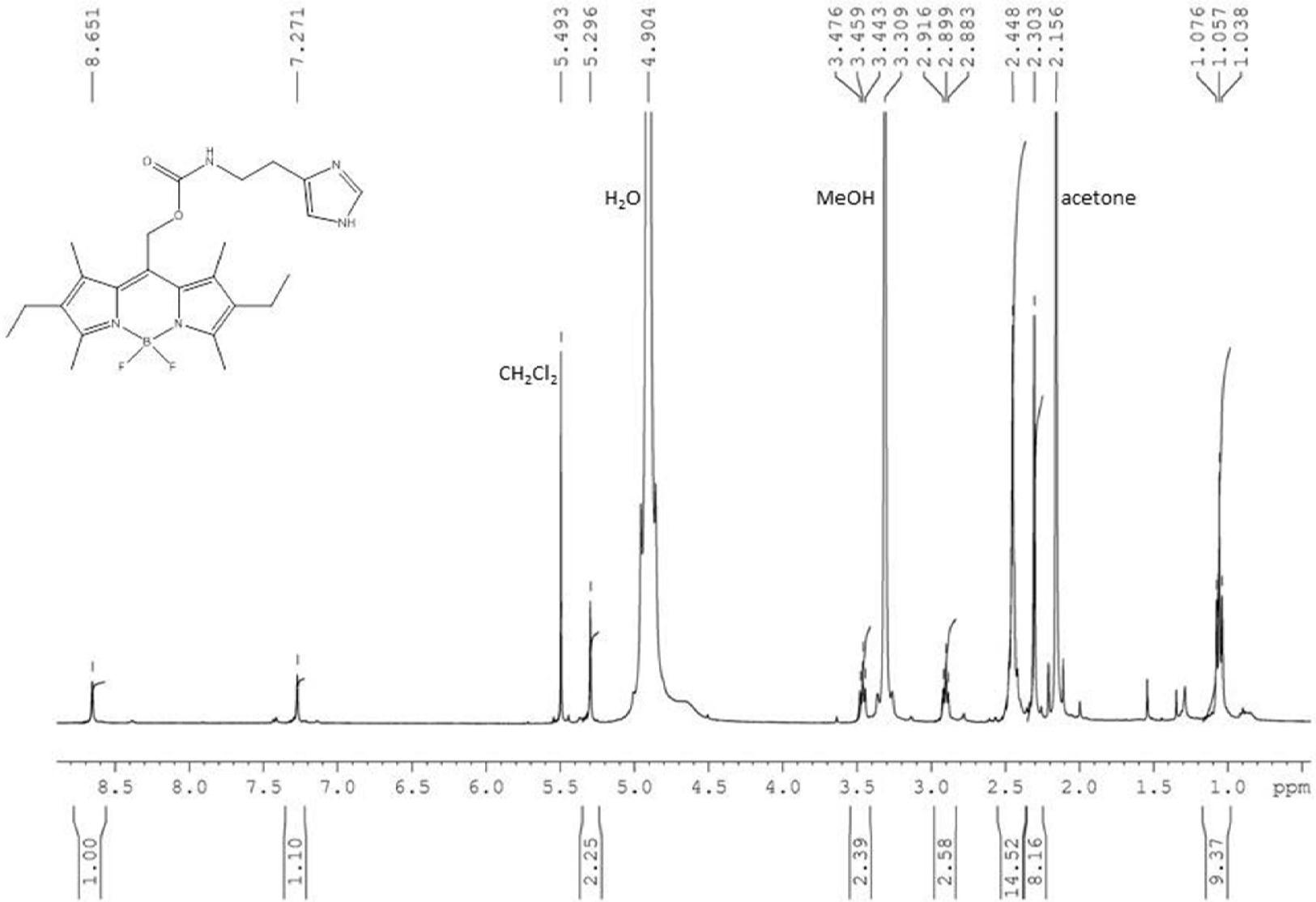
Neurons: Images opened in FIJI (v1.49b, FIJI is just ImageJ, NIH). Corrected drift, with Plugins>Registration>StackReg>Rigid Body Transformation. Bleaching was corrected, if necessary, with Image>Adjust>Bleach Correction>Exponential Fit. Then an average intensity Z-project was calculated for the 10 frames near the beginning of the acquisition (to set F_0). The Registered, Bleach-corrected stack was then divided by F_0 to give the F/F_0 movie, which was scaled from 0.75 to 1.25 in all cases except for SI Movie 11, which was m&m-BODIPY540 control.

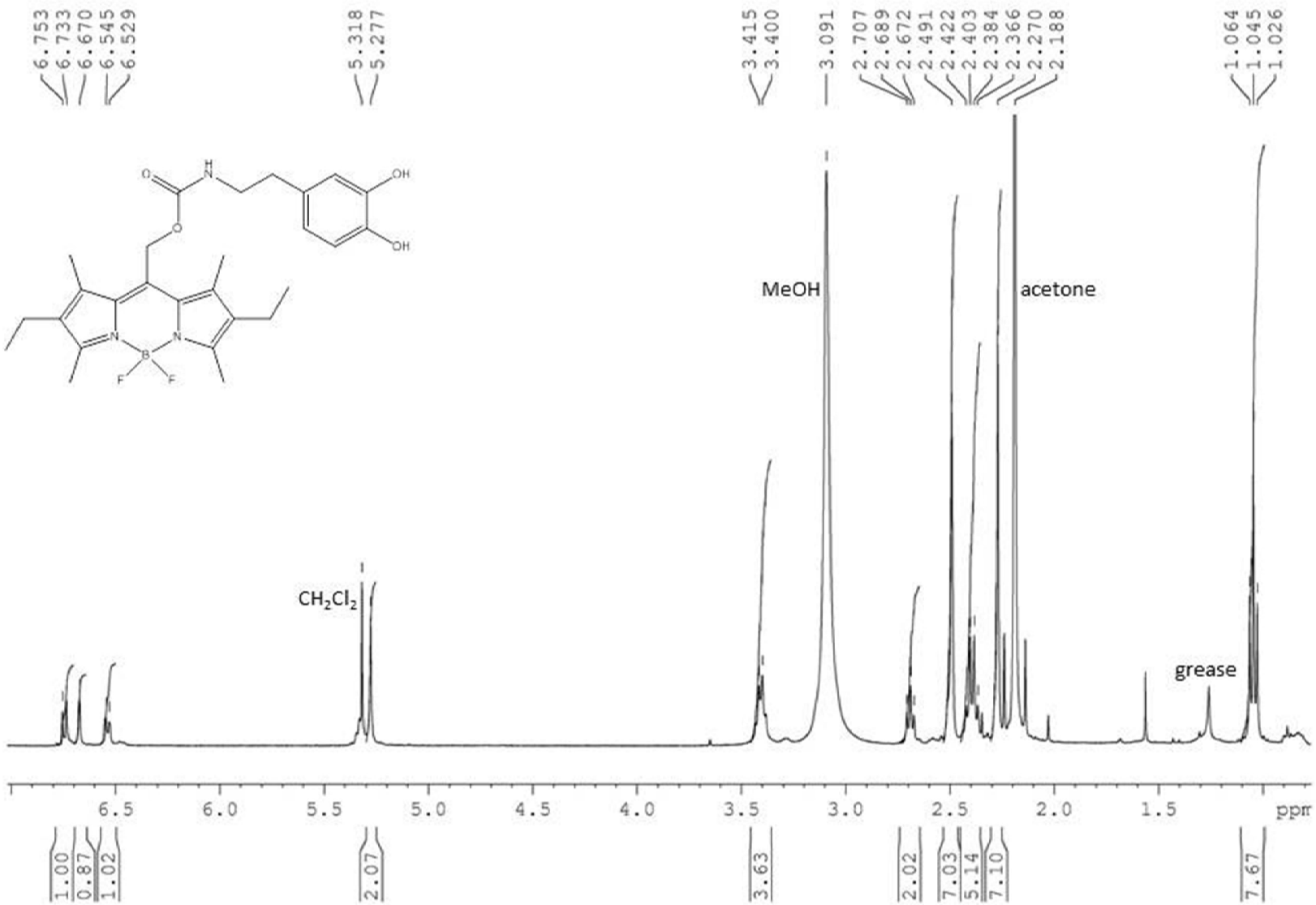

8.211
8.188
7.592
7.569
7.440
7.267
5.476
2.511
2.420
2.401
2.382
2.364
2.322
2.160
1.649
1.326
1.255
1.063
1.044
1.025
acetone
CHCl3
H2O
ppm
1.75
1.76
0.93
1.83
5.87
4.45
5.48
6.00


8.310
8.287
7.423
7.400
7.262
5.596
2.527
2.440
2.421
2.402
2.383
2.364
1.566
1.082
1.063
1.044
CHCl3
H2O
NO2
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.67
1.69
1.87
5.83
9.81
6.00


8.223
8.202
7.462
7.440
5.385
5.303
4.520
4.505
2.508
2.424
2.404
2.386
2.367
2.306
2.170
1.067
1.049
1.030
CHCl3
acetone
H2O
grease
NO2
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
2.00
2.05
2.31
1.70
2.24
8.30
5.52
7.55
8.68


8.197
8.175
7.475
7.453
7.262
5.363
3.802
2.511
2.397
2.378
2.359
2.340
2.134
1.647
1.046
1.027
1.008
CHCl3
H2O
O
O
NO2
N
B
N
F
F
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.748
1.827
2.000
2.007
5.085
4.359
5.215
5.279


8.651
7.271
5.493
5.296
4.904
3.476
3.459
3.443
3.309
2.916
2.899
2.883
2.448
2.303
2.156
1.076
1.057
1.038
H2O
MeOH
acetone
CH2Cl2
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.00
1.10
2.25
2.39
2.58
14.52
8.16
9.37


6.753
6.733
6.670
6.545
6.529
5.318
5.277
3.415
3.400
3.091
2.707
2.689
2.672
2.491
2.422
2.403
2.384
2.366
2.270
2.188
1.064
1.045
1.026
MeOH
acetone
CH2Cl2
grease
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.00
0.87
1.02
2.07
3.63
2.02
7.03
5.14
7.10
7.67

SI movies legends:

Movie 01 – HeLa cells treated with histamine

Movie 02 – HeLa cells treated with m&m-BODIPY-histamine and 540 nm light

Movie 03 – HeLa cells treated with m&m-BODIPY-histamine, 540 nm light and pyrilamine

Movie 04 – HeLa cells treated with m&m-BODIPY-histamine, no light

Movie 05 – HeLa cells treated with 540 nm light only

Movie 06 – HeLa cells treated with m&m-BODIPY-OH and 540 nm light

Movie 07 – Neuron cells treated with dopamine

Movie 08 – Neuron cells treated with m&m-BODIPY- dopamine and 540 nm light

Movie 09 – Neuron cells treated with m&m-BODIPY- dopamine, 540 nm light and (+)-butaclamol

Movie 10 – Neuron cells treated with m&m-BODIPY- dopamine, no light

Movie 11 – Neuron cells treated with 540 nm light only

Movie 12 – Neuron cells treated with m&m-BODIPY-OH and 540 nm light