Supporting information

Silicon Oxide Microchips Functionalized with Fluorescent Probes for Quantitative Real-Time Glutathione Sensing in Living Cells

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S1. Materials and methods

S1.1. Materials

4-Hydroxybenzaldehyde, propargyl bromide, boron trifluoride etherate, potassium carbonate, pyrrole-2-carboxaldehyde, diethyl malonate, sodium acetate, methylamine hydrochloride, sodium chloride, p-toluenesulfonic acid, sodium bicarbonate, 4,5-dichloro-3,6-dioxo-1,4-cyclohexadiene-1,2-dicarbonitrile (DDQ), phosphate buffered saline (PBS), sodium ascorbate, and deuterated chloroform ($CDCl_3$) were purchased from Sigma-Aldrich Co. (USA). Acetonitrile, tetrahydrofuran, dichloromethane, toluene, hexane, methanol, and ethyl acetate were purchased from VWR Chemicals Co. (France). 11-Azidoundecyltriethoxysilane was also provided from Sikemia Co. (France). The water used in the experiments was HPLC grade, produced by a MilliQ plus system from Millipore (Milli-Q water). Coated F254 silica gel plates and aluminium oxide 60 F254 plastic sheets purchased from Merck Co. were applied for performing thin layer chromatography (TLC). Silica gel (40-60 μm, VWR CHEMICALS Co.) and Aluminum oxide 150 basic (0.063-0.200 mm, Merck Co.) was used for column chromatography.

S1.2. General methods

A Varian Mercury 400 MHz spectrometer was used for recording ^{1}H NMR (400 MHz). A Varian Mercury 400 MHz spectrometer was used for recording ^{13}C NMR (100 MHz). Thermo Nicolet Avatar 320 FTIR was also applied for the FT-IR. FT-IR samples were prepared by dissolving a sufficient quantity of the samples in chloroform ($CHCl_3$), then adding one drop of the solution to a NaCl disk (round crystal window, NaCl, diameter = 25 mm, thickness = 4 mm). After waiting for the solvent to evaporate, the sample was run in the equipment. ESI-MS mass analyses were performed using an LC/MSD-TOF mass spectrometer (Agilent Technologies, 2006).

Contact angle measurement was done (CA goniometer (THETALITE100 with the software OneAttension, Finland)) to show the difference in the hydrophilic and hydrophobic properties of the silicon oxide surfaces before and after functionalization. For each experiment, 3 µL drop of Milli-Q water was added to the prepared surfaces. In order to give statistical significance, contact angle measurements have been done at least three times for each surface.

Flow cytometry was used for the evaluation of the fluorescent intensity of functionalized microchips and counting the number of microchips existing in a suspension. Released non-functionalized SOµC and SOµC-Bdpy1 in suspension were analyzed using a Cytek® Aurora spectral flow cytometer (Cytek Biosciences, Inc., Fremont, CA) configured with four lasers (16V-14B-10YG-8R). These microchips were detected based on their scatter parameters (FSC/SSC profile), and their emission was recorded at λ_{em} = 498-531 nm upon excitation at λ_{ex} = 488 nm.

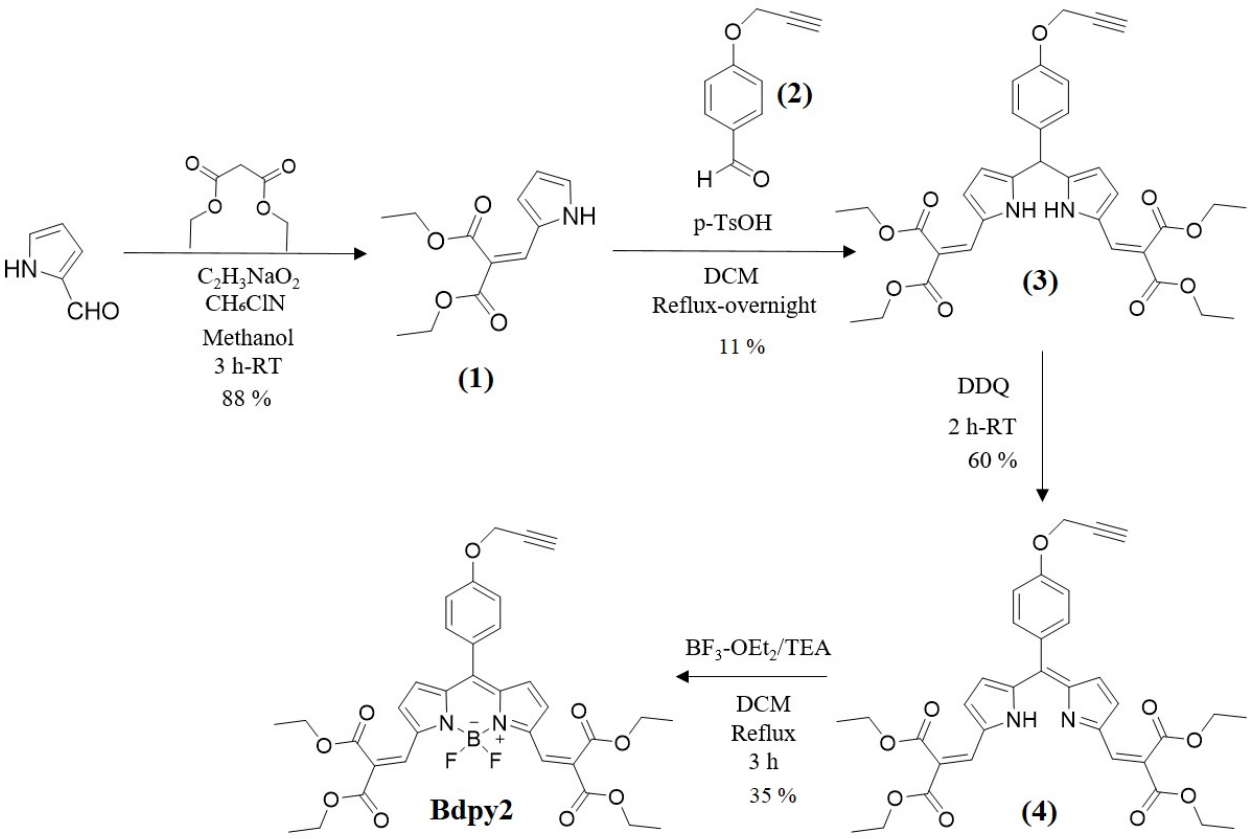
S1.3 Optical characterization of Bdpy1 and Bdpy2 of synthesized compounds

UV–vis absorption spectroscopy (UV-1800 Shimadzu UV spectrophotometer) and fluorescence spectroscopy (Hitachi F-4500 fluorescence spectrometer) were applied to study the behavior of Bdpy1 and Bdpy2 in the absence and presence of GSH in solution. Absorption quartz cuvettes (Hellma® absorption cuvettes, standard cells, Macro) and fluorescence quartz cuvettes (Hellma® fluorescence cuvettes, standard cells, Macro) were used for UV–vis absorption and fluorescence experiments, respectively. For this purpose, Bdpy1 was prepared at a concentration of 10 µM with a solvent proportion of PBS:MeCN (9:1), pH 7.2, and a constant temperature of 37 °C. To study Bdpy1 in the presence of GSH, the concentration was adjusted to 1 mM GSH with the same solvent proportion of PBS:MeCN (9:1) for 4 h incubation time. Regarding Bdpy2, the concentration was 10 µM, with addition of 1 mM GSH at pH 7.2, maintained at a constant temperature of 37 °C for 4 hours of incubation. In this case, the solvent proportion was PBS/MeCN (8:2).

S1.4 Reversibility assessment of Bdpy2

UV–vis absorption spectroscopy (UV-1800 Shimadzu UV spectrophotometer) was utilized to evaluate the reversibility of Bdpy2 in the presence of GSH and N-ethylmaleimide (NEM), a GSH scavenger that reacts irreversibly with GSH molecules. For this purpose, Bdpy2 was prepared at a concentration of 10 μM in a solvent mixture of PBS/MeCN (8:2) at pH 7.2 and maintained at a constant temperature of 37 °C. Stock solutions of GSH and NEM were prepared using the same solvent proportions as Bdpy2 (PBS/MeCN (8:2)). The concentrations of Bdpy2 solutions were adjusted to include 1 mM GSH, 0.5 mM and 1 mM NEM, followed by the subsequent addition of GSH to a total concentration of 2 mM. The incubation time after addition of each concentration of GSH and NEM was 4 h.

S1.3. Synthesis of Bdpy2



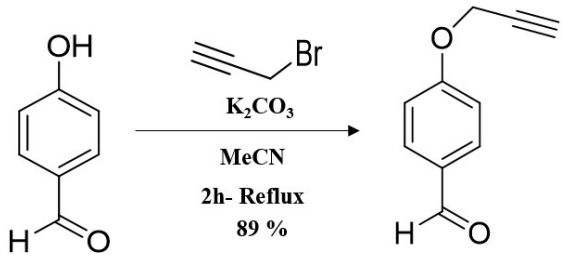
Scheme S1. Synthesis steps of Bdpy2 preparation

Diethyl 2-((1H-pyrrol-2-yl)methylene)malonate (1)

Pyrrole-2-carboxaldehyde (4 g, 42 mmol) was dissolved in methanol (40 mL), followed by addition of diethyl malonate (6.38 mL, 42 mmol), sodium acetate (3.52 g, 41.8 mmol) and methylamine hydrochloride (2.84 g, 42 mmol). The mixture was stirred for 3 h at room temperature. Then, brine (40 mL) and water (60 mL) were added to the mixture of reaction. The mixture was extracted with dichloromethane (3 x 40 mL), and dried using anhydrous Mg_2SO_4. After removing the solvent under vacuum, the remaining oil was purified by column

chromatography using silica gel (100 % dichloromethane). Yellow oil (2.2 g). Yield: 88 %; ^{1}H NMR (400 MHz, $CDCl_3$): 1.32-1.37 (m, 6 H), 4.25-4.36 (m, 4 H), 6.32-6.34 (m, 1 H), 6.72-6.74 (m, 1 H), 7.08-7.10 (m, 1 H), 7.66 (s, 1 H), 11.42 (s, 1 H, pyrrole). ^{13}C NMR (75 MHz, $CDCl_3$): 14.1, 14.6, 61.1, 61.7, 111.4, 113.6, 123.1, 125.9, 127.6, 137.4, 166.8, 168.2.

4-(Prop-2-yn-1-yloxy)benzaldehyde (2)



(2)

4-Hydroxybenzaldehyde (2.00 g, 16.37 mmol) was added to a 250 mL round bottom flask equipped with a magnetic stirrer and dissolved in 150 mL of dry acetonitrile. 2.9 g (24.5 mmol) of propargyl bromide was added to the solution under an Ar atmosphere. Afterward, potassium carbonate (2.5 g, 18.1 mmol) was added to the reaction. The reaction was refluxed and the progress of the reaction was monitored by TLC using DCM as the eluent. After 2 hours, the reaction was completed and the mixture was cooled to room temperature. Potassium carbonate was removed from the mixture of the reaction by filtration and washed with acetonitrile (5 mL). The solvent was evaporated under vacuum, resulting in 2.33 g of **2** as a yellowish solid (yield: 89 %). Melting Point: 74-76 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 9.88 (s, 1H), 7.87 (d, J = 8.8 Hz, 2H), 7.13 (d, J = 8.8 Hz, 2H), 4.84 (d, J = 2.4 Hz, 2H), 2.86 (t, J = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.9, 76.4, 77.5, 115.2, 130.6, 131.8, 162.4, 190.7.

Tetraethyl2,2'-((((4-(prop-2-yn-1-yloxy)phenyl)methylene)bis(1H-pyrrole-5,2-diyl))bis(methaneylylidene))dimalonate (3)

2 (0.5 g, 3.1 mmol) and DPMM (1.5 g, 6.2 mmol) were dissolved in dry dichloromethane (30 mL) under Ar atmosphere. p-Toluenesulfonic acid (4 %, 0.06 g, 0.31 mmol) was added to the solution. The mixture was stirred overnight at room temperature. Then, the mixture of reaction was washed with saturated sodium bicarbonate (30 mL) and extracted with, dichloromethane (3 × 30 mL), and the organic phase was dried using $MgSO_4$. Then, solvent was evaporated under reduced pressure. For the purification of compound 3, column chromatography (Alumina) was used with a solvent mixture containing Hexane (70 %) and Ethyl acetate (30 %). resulting in 211 mg of solid (yield: 11 %). ^{1}H NMR (400 MHz, $CDCl_3$): 11.18 (s, 2H), 7.58 (s, 2H), 7.18 (d, *J* = 8.9 Hz, 2H), 6.97 (d, *J* = 8.8 Hz, 2H), 6.68 (dd, *J* = 3.8, 2.4 Hz, 2H), 6.10 (dd, *J* = 4.4, 2.5 Hz, 2H), 5.49 (s, 1H), 4.68 (d, *J* = 2.4 Hz, 2H), 4.26 – 4.17 (m, 8H), 2.51 (t, *J* = 2.4 Hz, 1H), 1.28 (dt, *J* = 17.1, 7.1 Hz, 12H).

Diethyl (Z)-2-((2-((5-(3-ethoxy-2-(ethoxycarbonyl)-3-oxoprop-1-en-1-yl)-1H-pyrrol-2-yl)-(4-(prop-2-yn-1-yloxy)phenyl)methylene)-2H-pyrrol-5-yl)methylene)malonate (4)

A suspension of DDQ (72 mg, 0.32 mmol) in dichloromethane (3 mL) was added to a solution of **3** (0.2 g, 0.32 mmol) dichloromethane (27 mL) under an Ar atmosphere. The reaction mixture was stirred for 2 h at room temperature. After solvent evaporation, the residue was chromatographed (Alumina) by a mixture of solvent Hexane (80 %) and Ethyl acetate (20 %) which resulted in 0.12 g of **4** (yield: 60 %). Melting point: 115-120 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 12.95 (s, 1H), 7.96 (s, 2H), 7.43 (d, *J* = 8.8 Hz, 2H), 7.06 (d, *J* = 8.8 Hz, 2H), 6.72 – 6.64 (m, 4H), 4.78 (d, *J* = 2.4 Hz, 2H), 2.58 (t, *J* = 2.4 Hz, 1H), 1.34 (q, *J* = 7.2 Hz, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): 14.1, 14.5, 55.4, 62.5, 62.67, 76.6, 77.5, 114.5, 119.3, 127.5, 130.3, 131.9, 132.9, 133.8, 136.1, 146.4, 150.9, 158.3, 162.8, 165.4. MS (ESI) *m/z*: $[M+NH_4]^+$ 632.485, $[M+Na]^+$ 637.415.

Tetraethyl 2,2'-((5,5-difluoro-10-(4-(prop-2-yn-1-yloxy)phenyl)-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine-3,7-diyl)bis(methaneylylidene))dimalonate (Bdpy2)

0.1 g (0.16 mmol) of **4** were dissolved in dry dichloromethane (10 mL) under Ar atmosphere. Then, 10 eq. of triethylamine (0.162 g, 1.6 mmol) were added to the solution, and the mixture was stirred for 30 min at room temperature. Afterward, 15 eq. of boron trifluoride etherate (0.34 g, 2.4 mmol) were added dropwise to the mixture. After 2 hours, 1 M of NaOH (10 mL) solution was added to the mixture of reaction. Then, 1 M HCl solution was added to the mixture to bring the pH of the solution to 6. The organic phase was extracted with dichloromethane (3 × 10 mL), and the solvents were evaporated under residue pressure. The residue was chromatographed (silica gel) with dichloromethane (99 %) and ethyl acetate (1 %) as the eluent, resulting in 38 mg of purple solid. (Yield: 35 %). Melting point: 183-188 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 8.22 (s, 2H), 7.49 (d, J = 8.8 Hz, 2H), 7.14 (d, J = 8.9 Hz, 2H), 6.93 (d, J = 4.6 Hz, 2H), 6.79 (d, J = 4.6 Hz, 2H), 4.80 (d, J = 2.4 Hz, 2H), 4.36 (q, J = 7.2 Hz, 8H), 2.60 (t, J = 2.4 Hz, 1H), 1.35 (dt, J = 16.4, 7.1 Hz, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): 13.9, 14.2, 56.1, 62.1, 62.2, 76.4, 77.7, 115.1, 120.6, 126.7, 129.7, 130.5, 131.5, 132.4, 137.4, 145.7, 149.7, 160.2, 163.4, 166.2. MS (ESI) *m/z*: $[M+NH_4]^+$ 685.215, $[M+Na]^+$ 680.260.

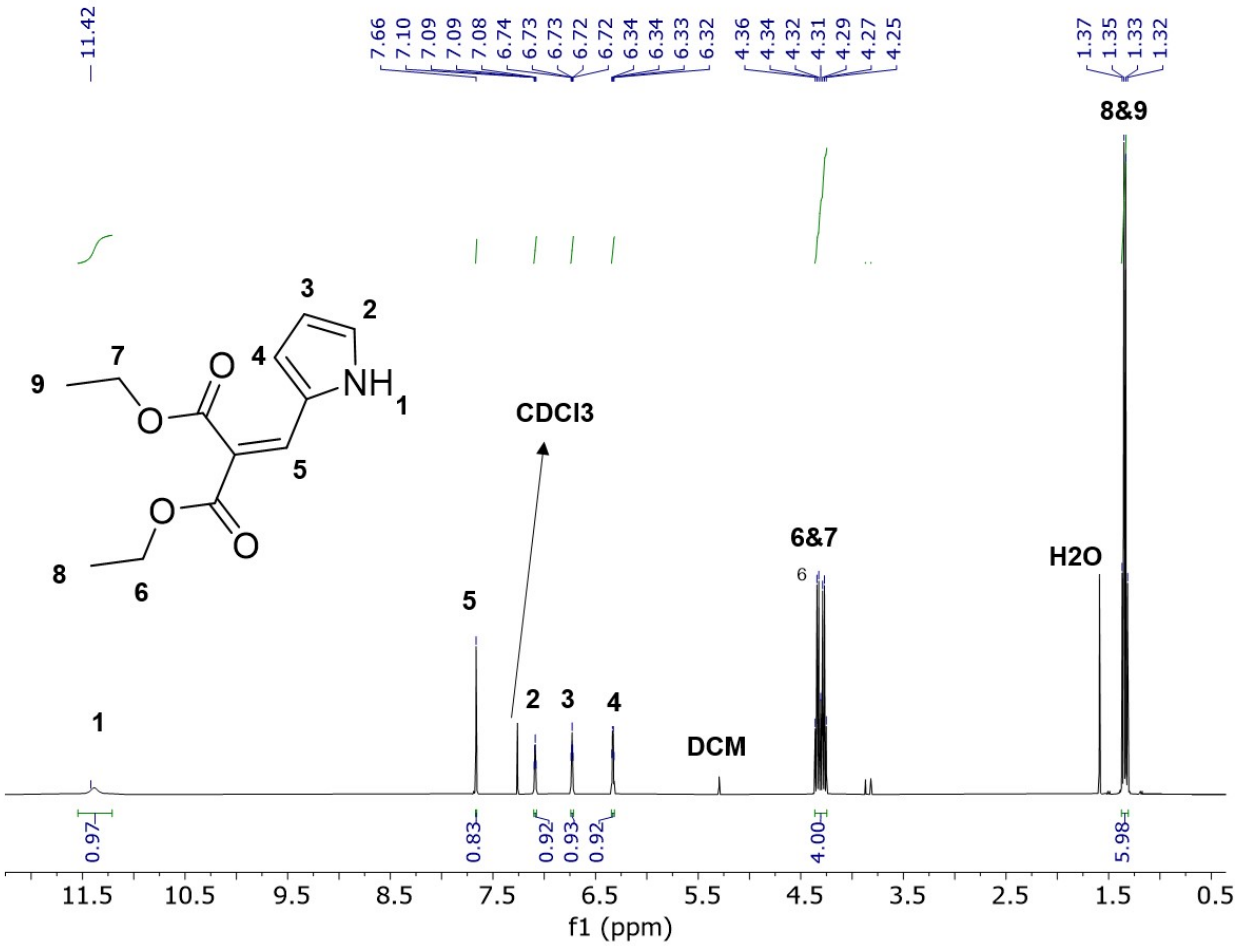


Fig. S1-1. ^{1}H NMR spectrum of **1** in $CDCl_3$

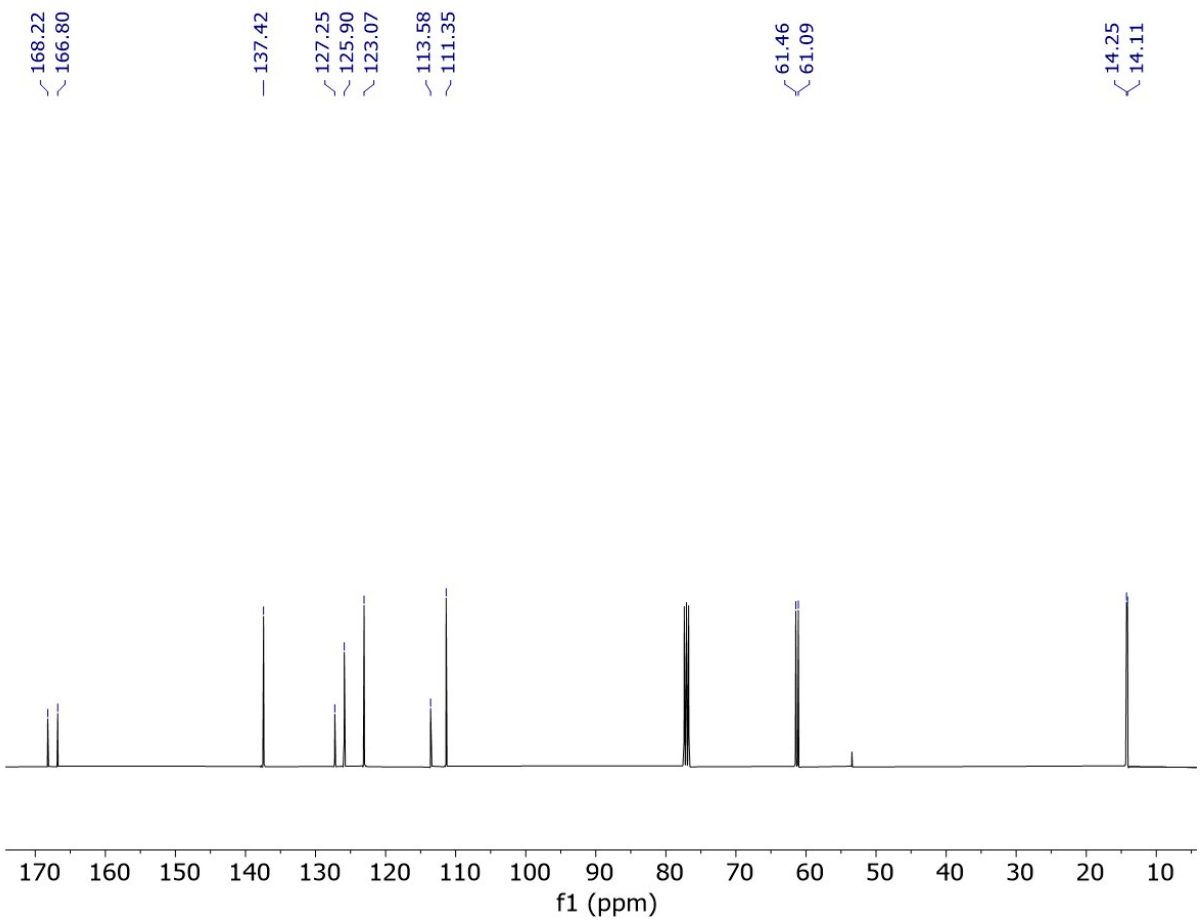


Fig. S1-2. ^{13}C NMR spectrum of **1** in $CDCl_3$

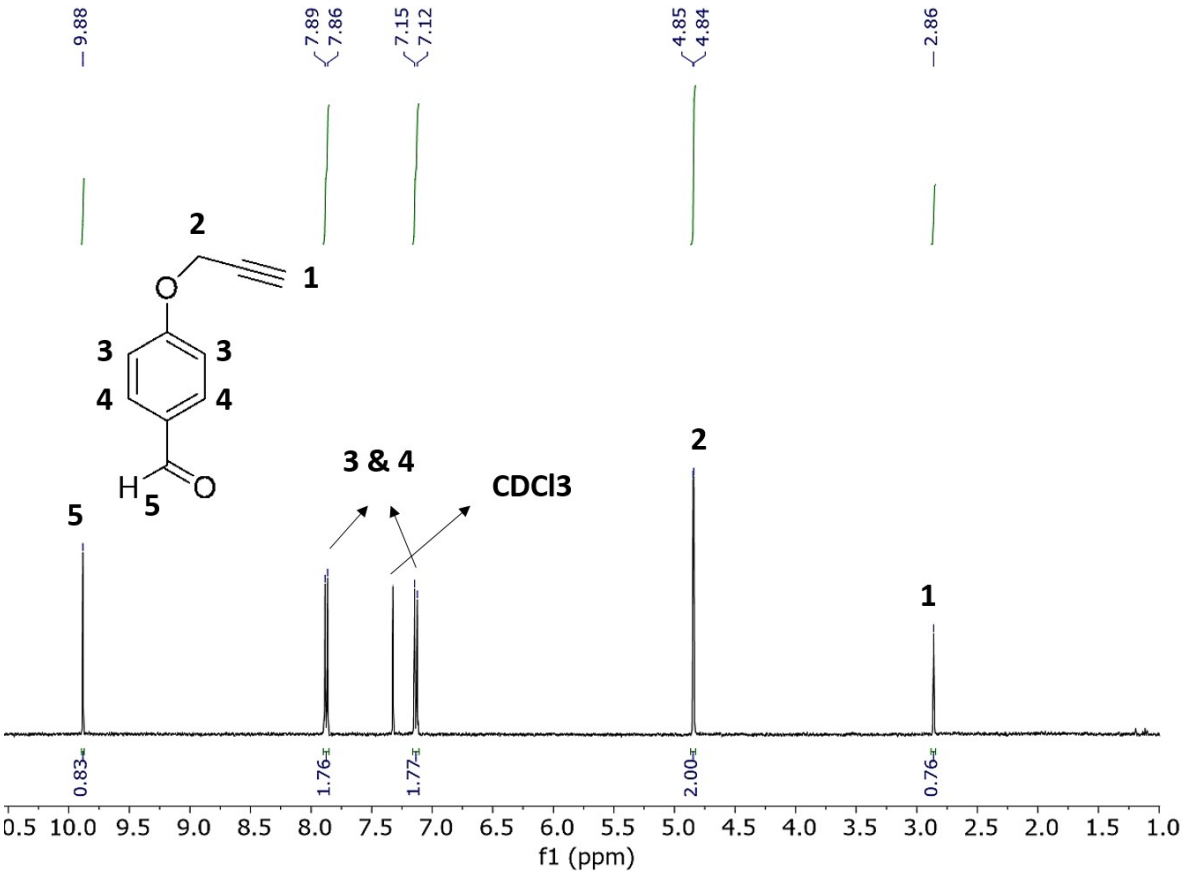


Fig. S1-3. ^{1}H NMR spectrum of **2** in $CDCl_3$

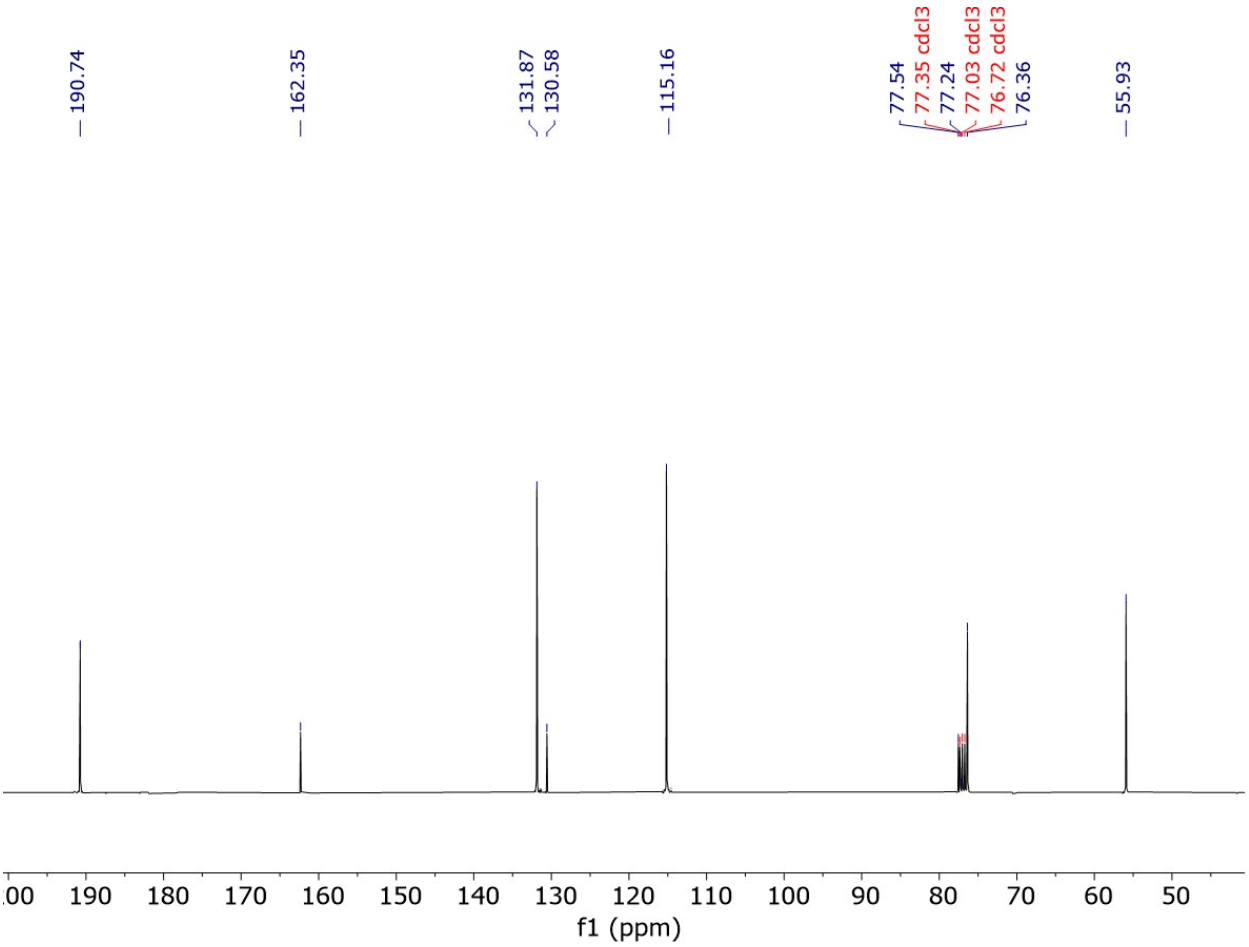


Fig. S1-4. ^{13}C NMR spectrum of **2** in $CDCl_3$

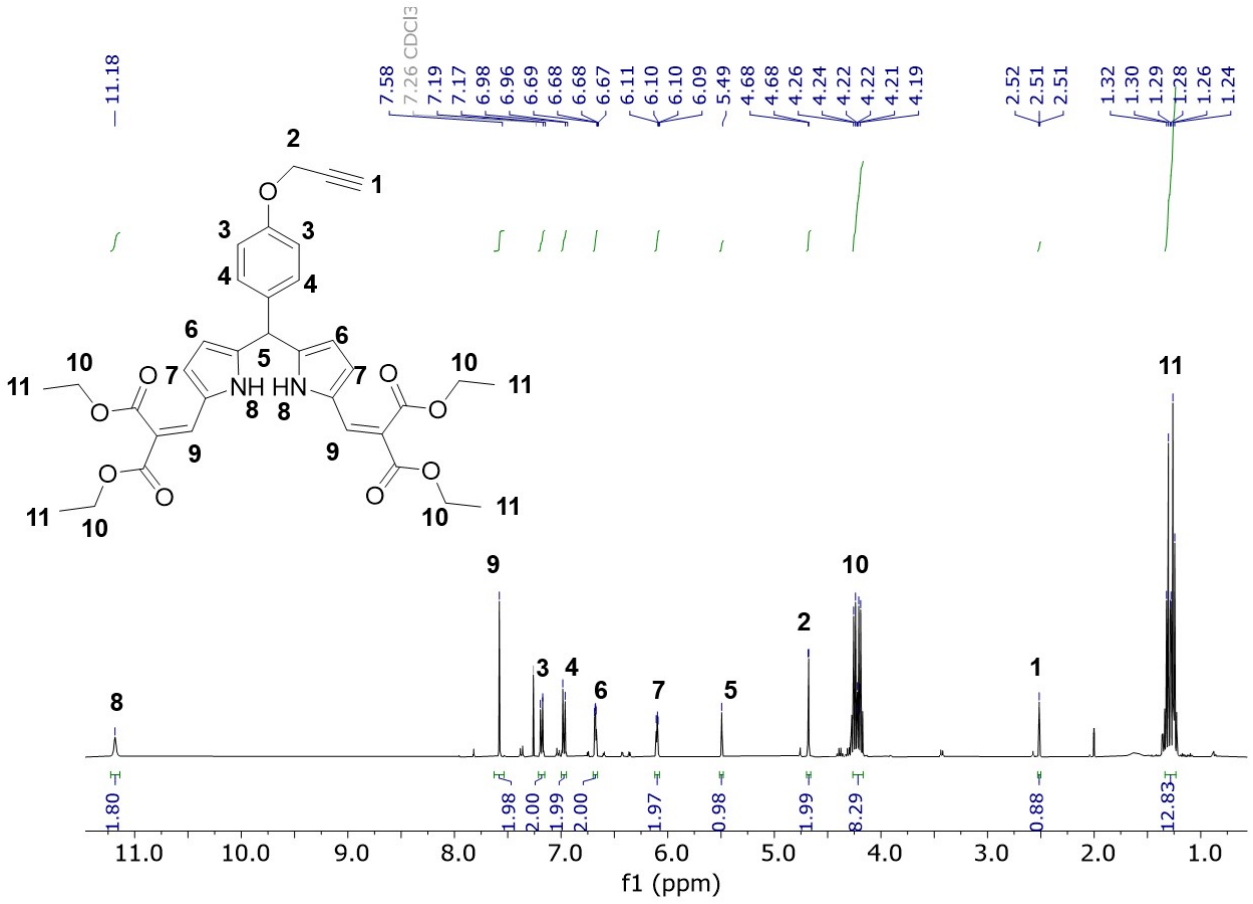


Fig. S1-5. ^{1}H NMR spectrum of **3** in $CDCl_3$

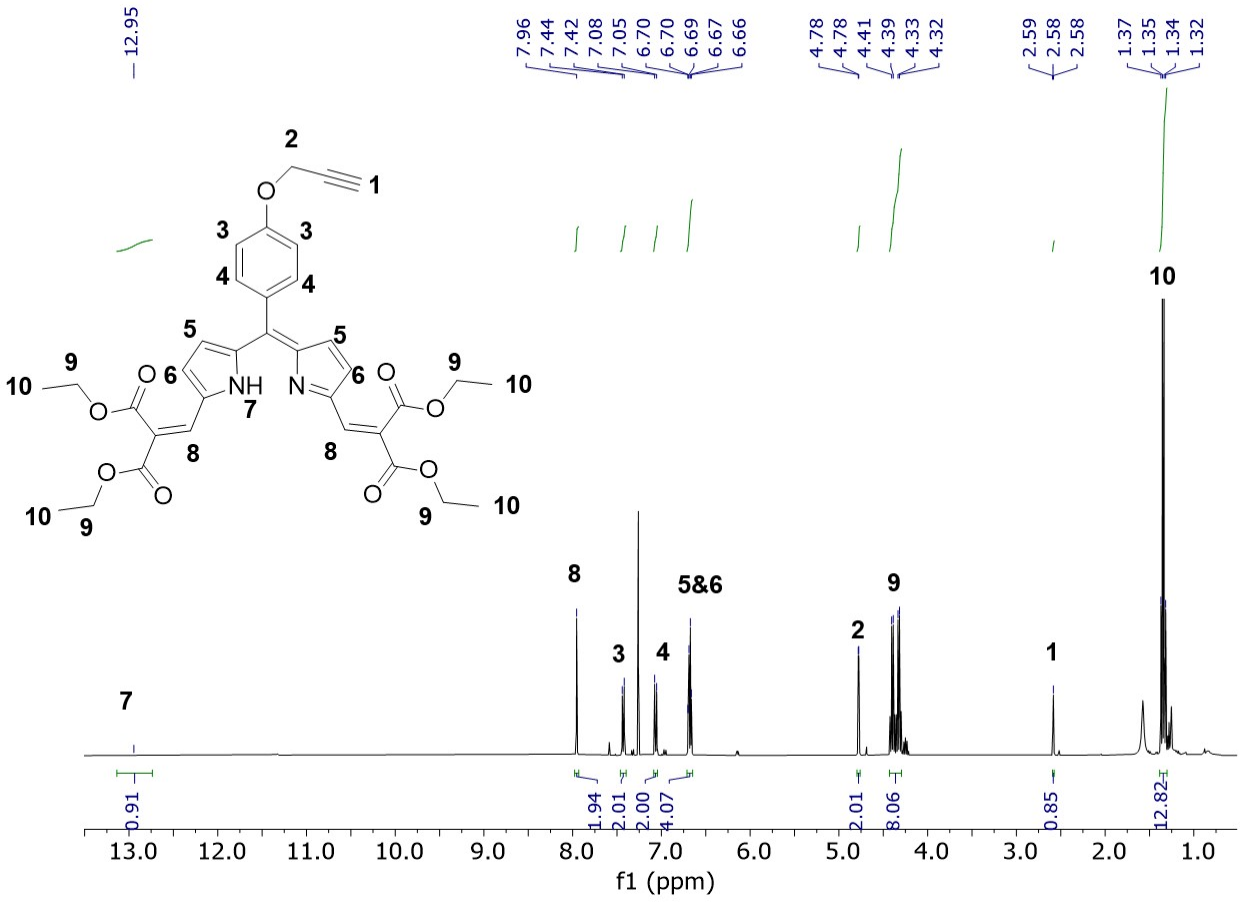


Fig. S1-6. ^{1}H NMR spectrum of **4** in $CDCl_3$

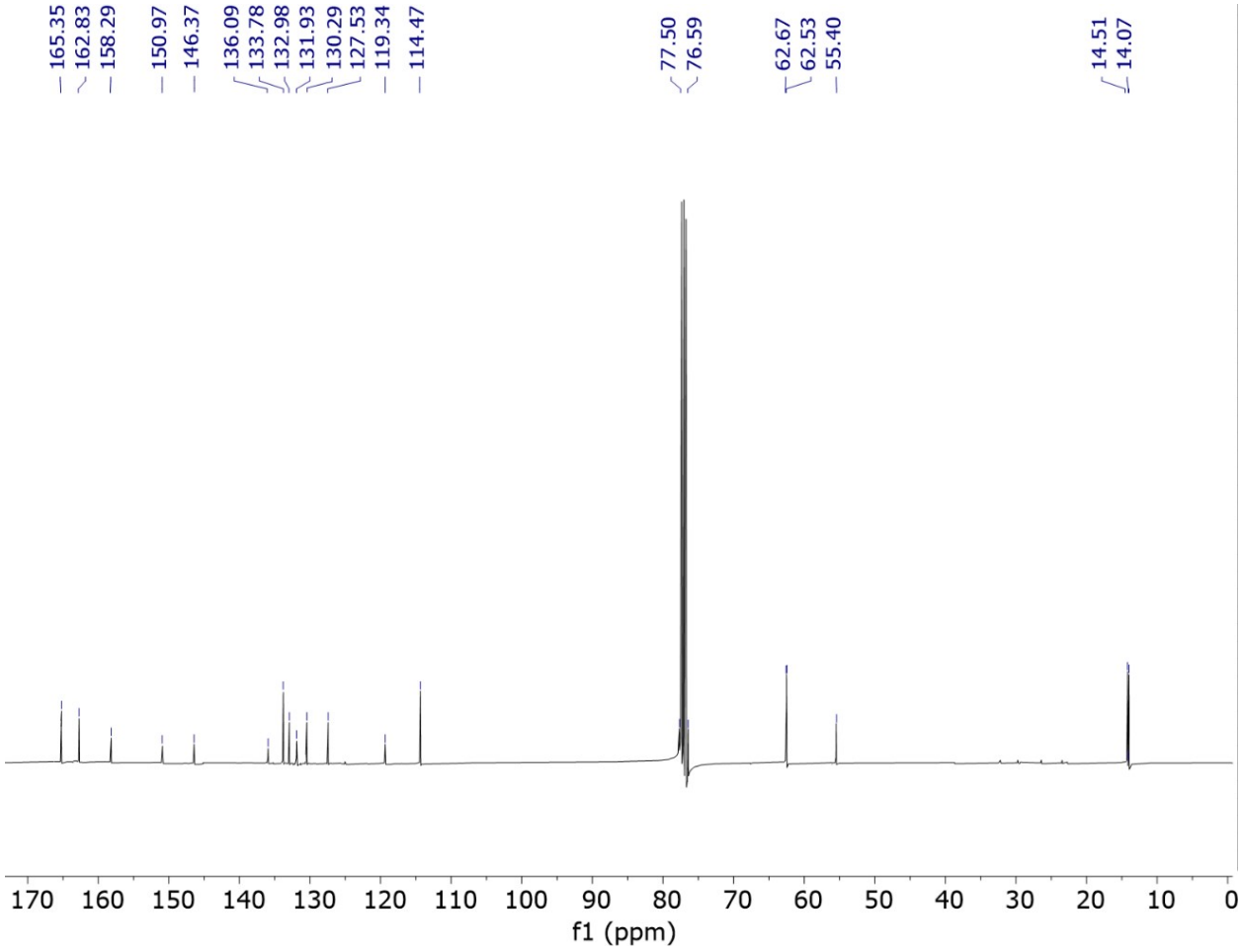


Fig. S1-7. ^{13}C NMR spectrum of **4** in $CDCl_3$

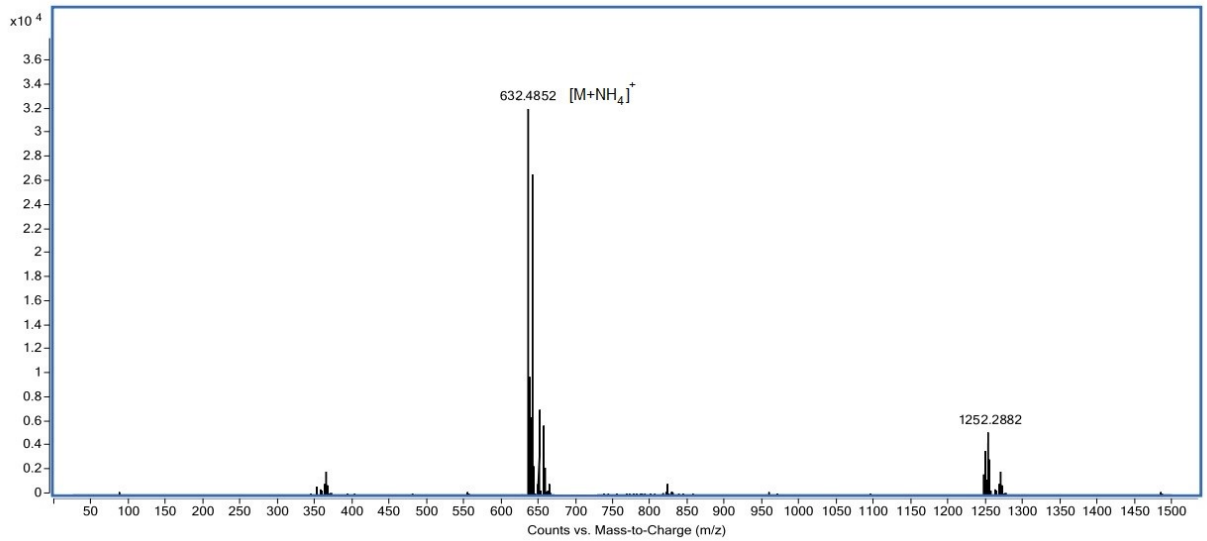


Fig. S1-8. ESI-MS mass spectrum of **4**

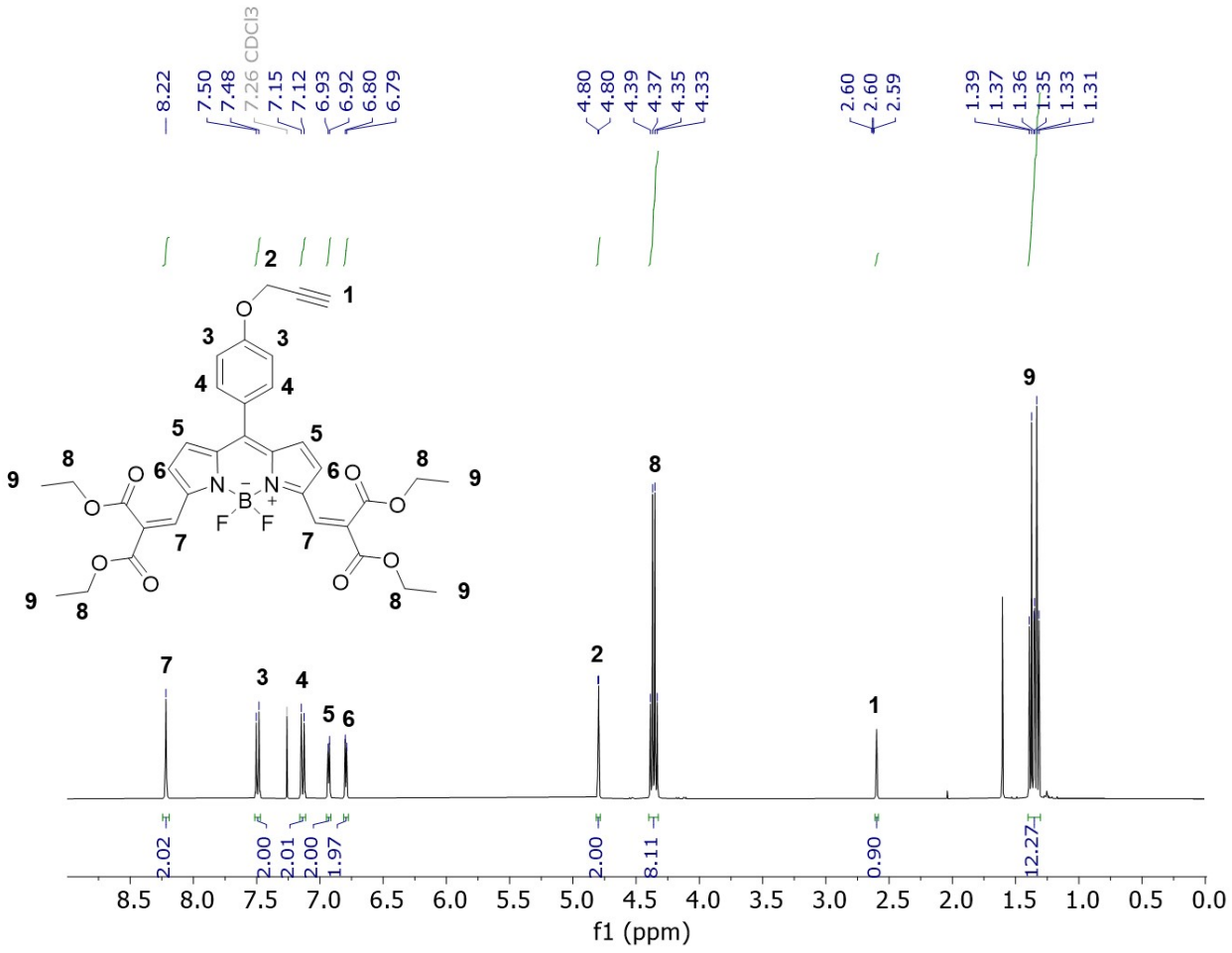


Fig. S1-9. ^{1}H NMR spectrum of **Bdpy2** in $CDCl_3$

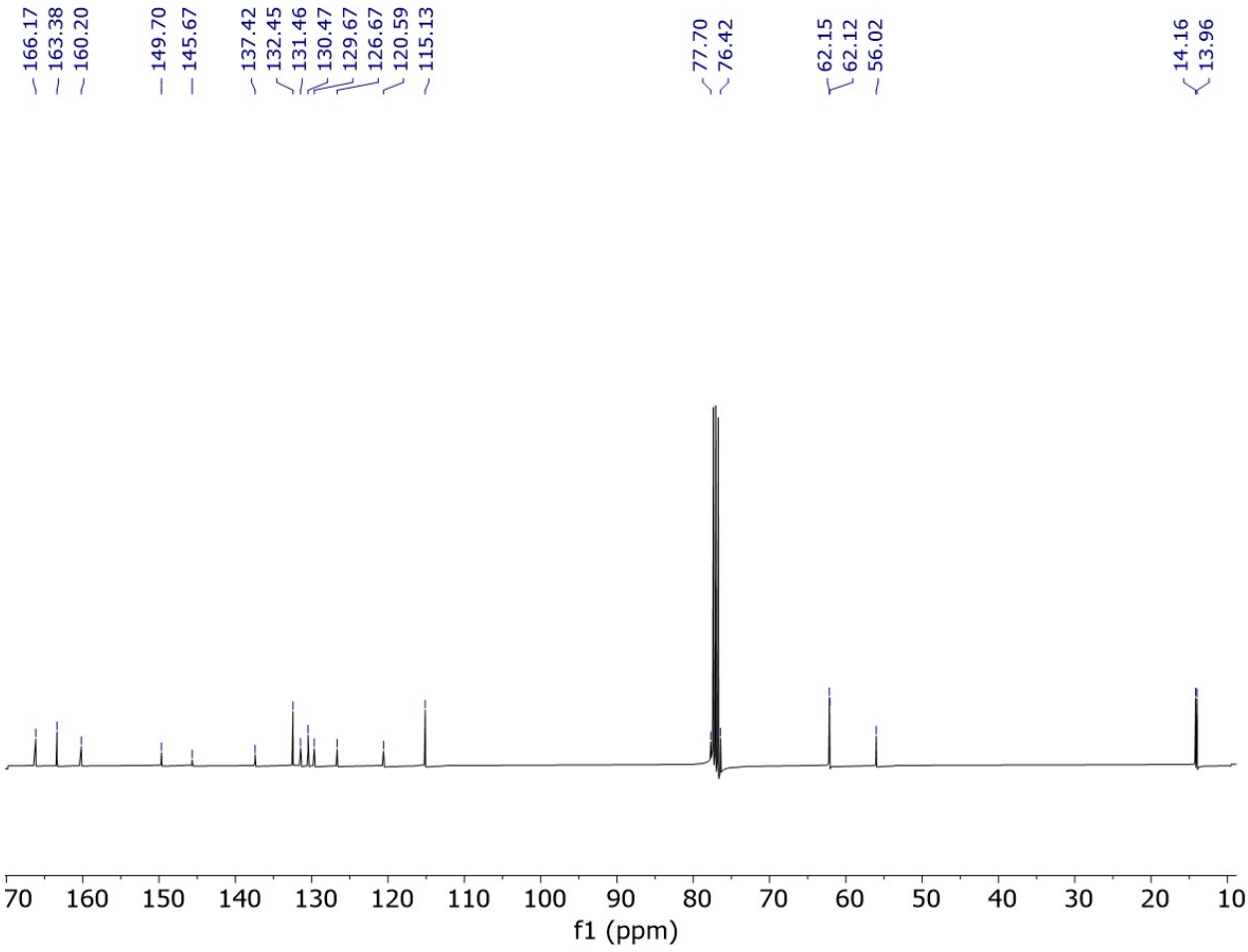


Fig. S1-10. ^{13}C NMR spectrum of **Bdpy2** in $CDCl_3$

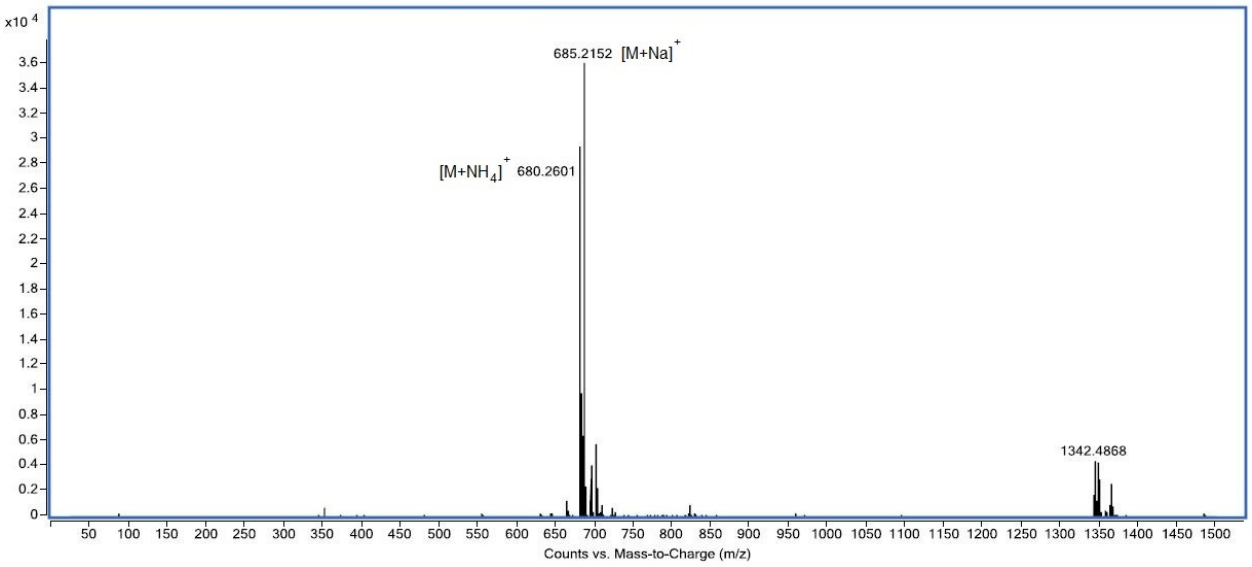


Fig. S1-11. ESI-MS mass spectrum of **Bdpy2**

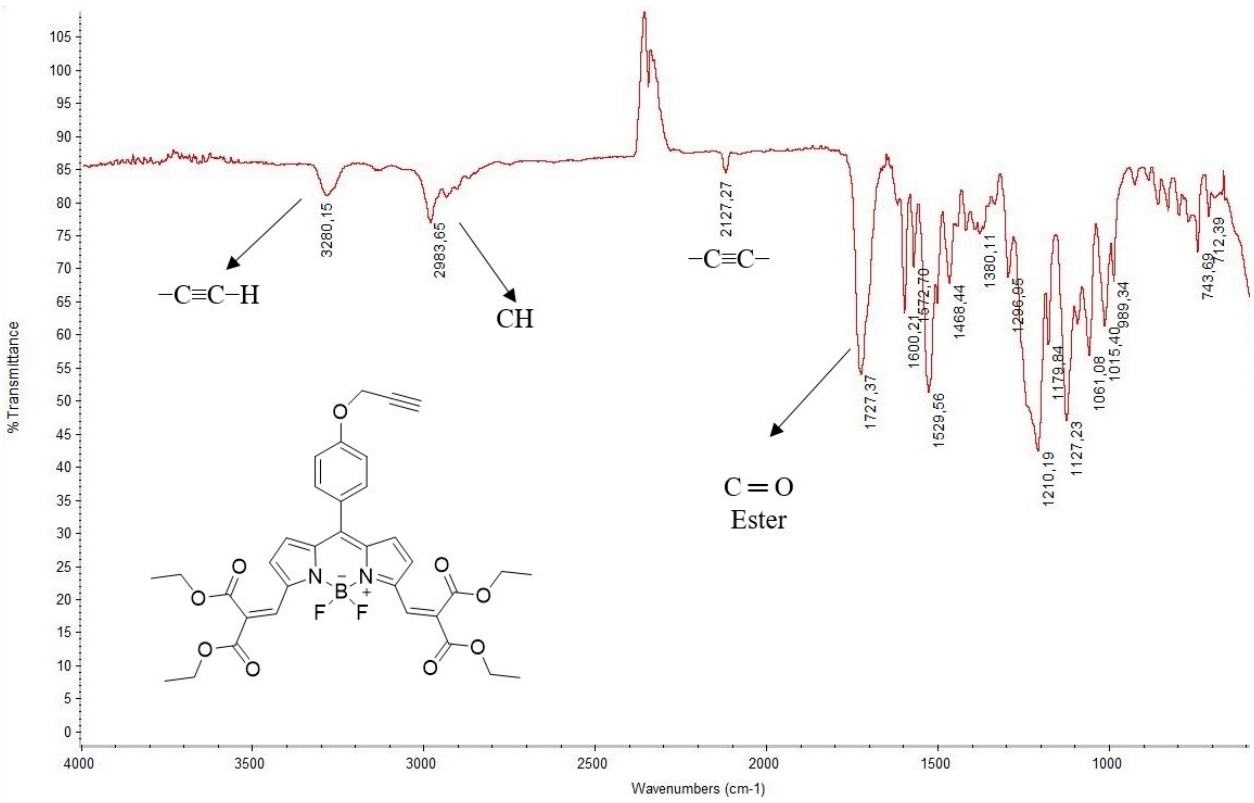


Fig. S1-12 FT-IR spectrum of **Bdpy2**

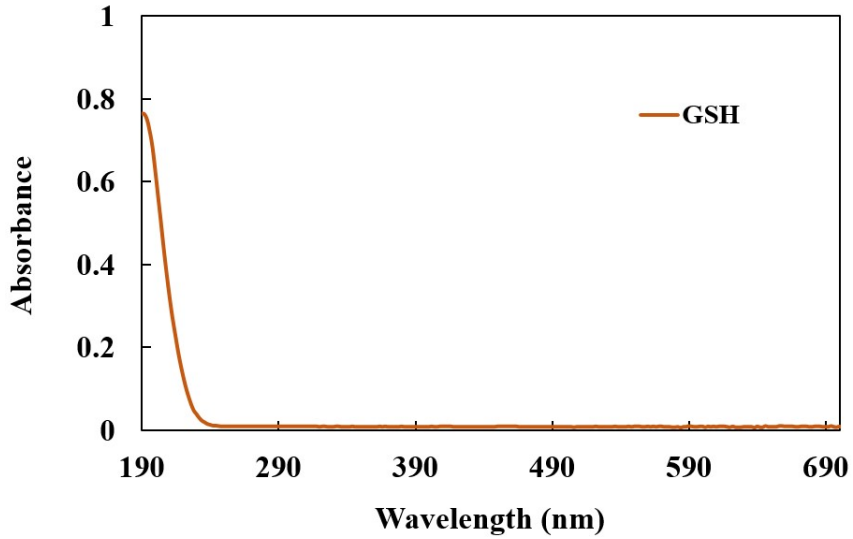


Fig. S2. Absorption spectrum of 20 μM of GSH (PBS: MeCN (9:1), pH: 7.2, 37 °C)

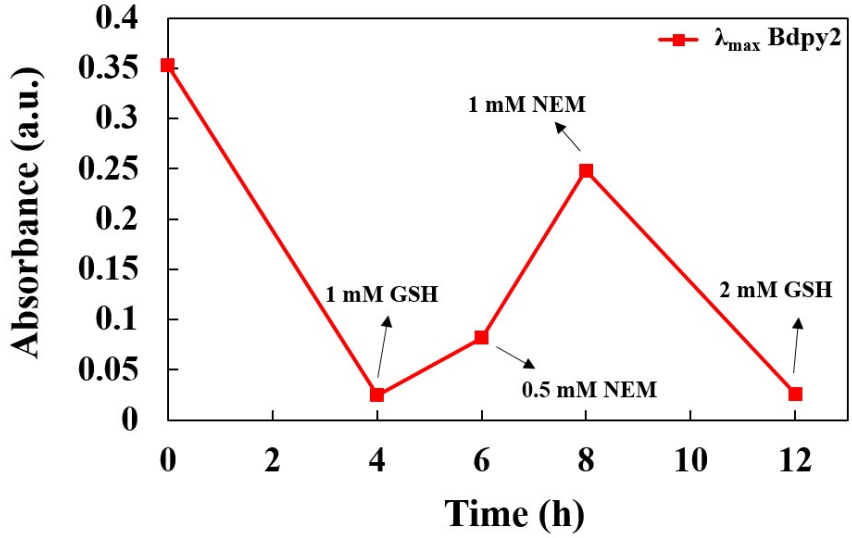


Fig. S3. Maximum absorption of Bdpy2 upon the addition of different concentrations of GSH and NEM over 12 h time intervals.

Table S1. Contact angle **(°)** measurements for non-functionalized silicon oxide surface (SOS), functionalized silicon oxide surface with azide linker (SOS-N3), Bdpy1 conjugated surface (SOS-Bdpy1), Bdpy2 conjugated surface (SOS-Bdpy2), SOS-Bdpy1 incubated in GSH aqueous solution (SOS-Bdpy1-GSH), and SOS-Bdpy2 incubated in GSH aqueous solution (SOS-Bdpy2-GSH). Contact angle (°) measurements for non- functionalized silicon oxide surface immersed in Bdpy1 (SOS′) and Bdpy2 (SOS″) solutions with the reactions for the conjugated surface of SOS-Bdpy1 and SOS-Bdpy2.

Surface	Contact angle (°)
SOS	39±3
SOS-N3	90±2
SOS-Bdpy1	72±3
SOS-Bdpy2	68±2
SOS-Bdpy1-GSH	54±3
SOS-Bdpy2-GSH	51±2
SOS′	40±2
SOS″	41±3

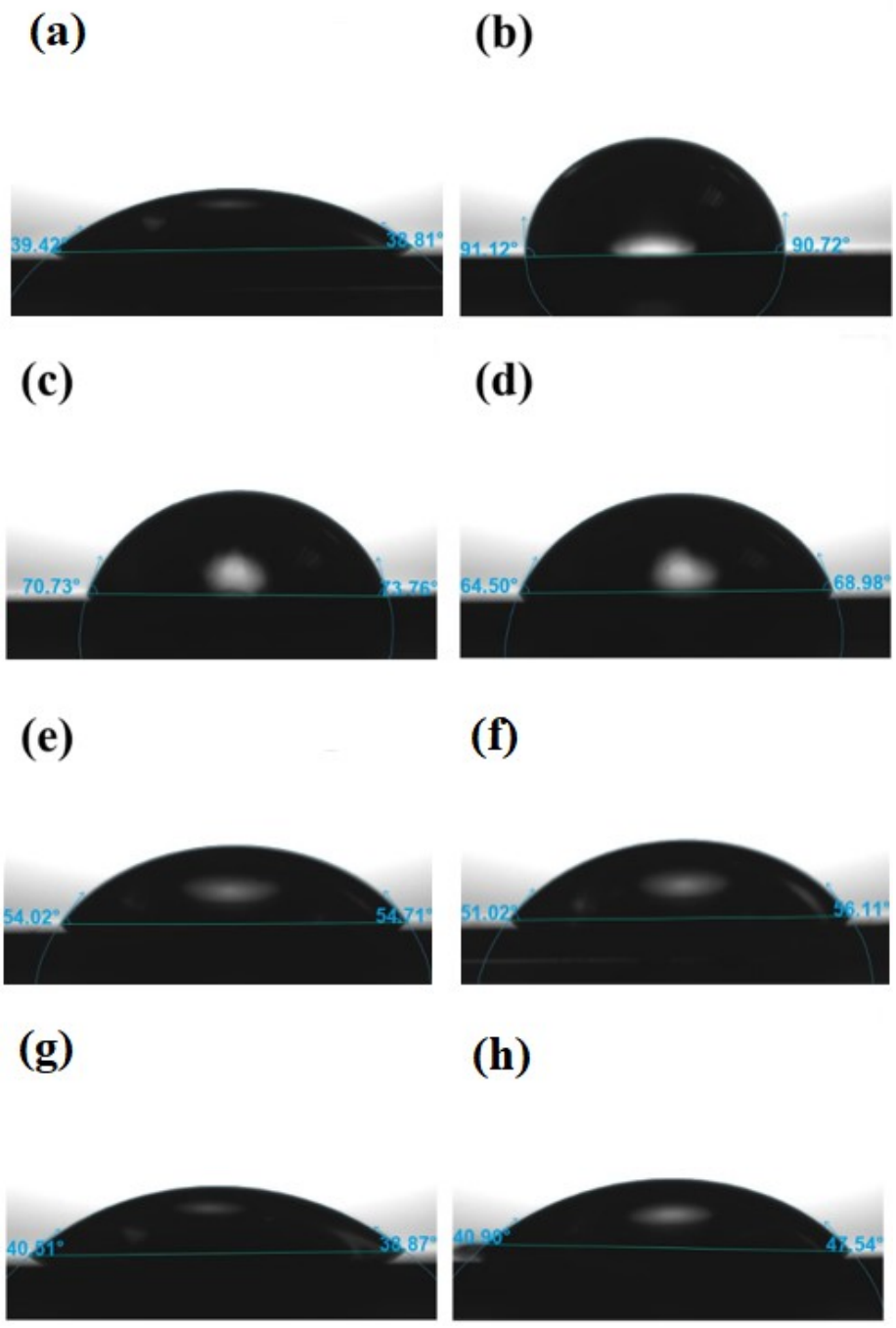


Fig. S4. Contact angle images of (a) SOS, (b) SOS-N3, (c) SOS-Bdpy1, (d) SOS-Bdpy2, (e) SOS-Bdpy1-GSH, (f) SOS-Bdpy2-GSH, (g) SOS′, and (h) SOS.

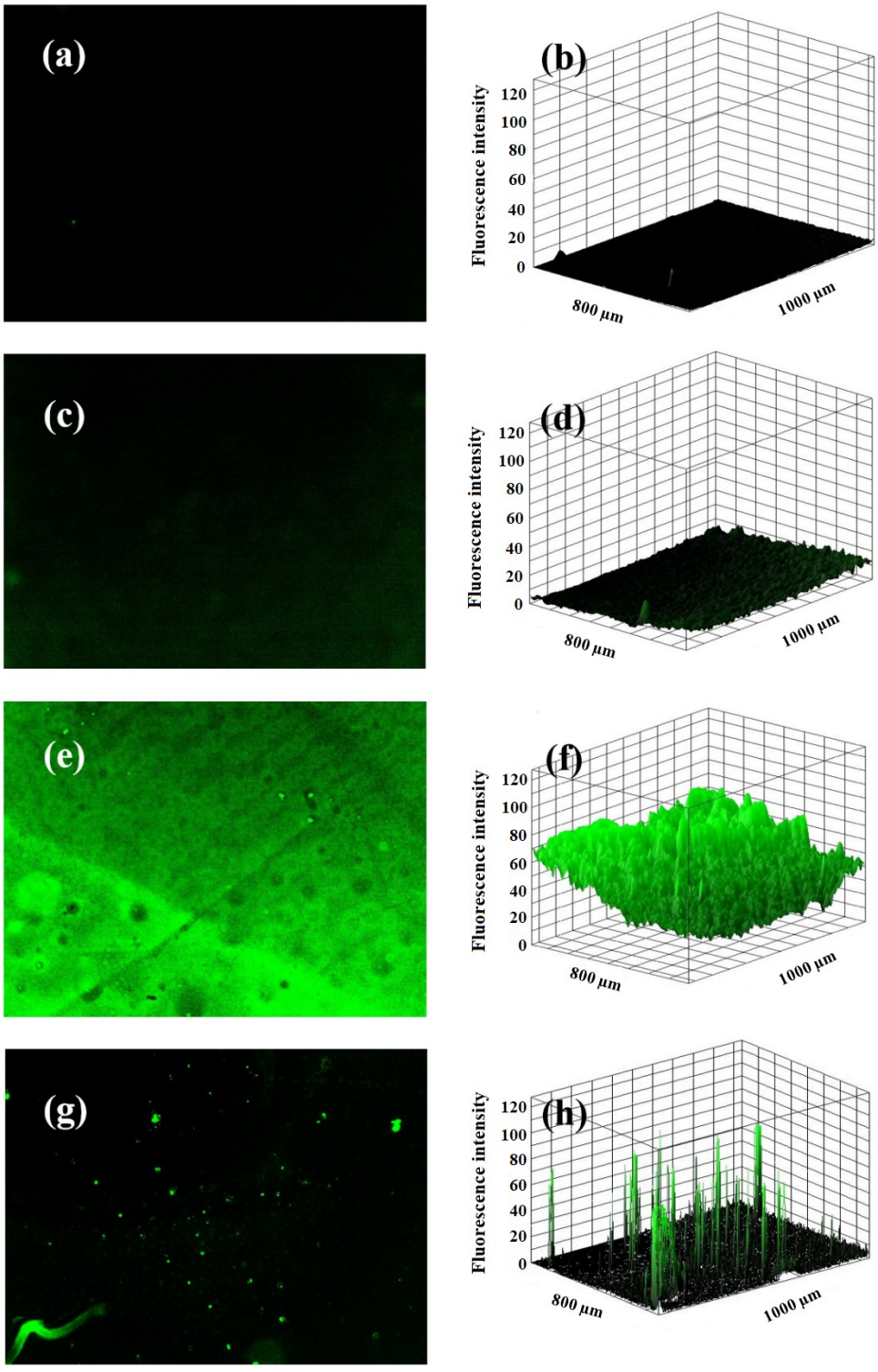


Fig. S5. Fluorescence microscopy images and their corresponding 3D surface plots of (a-b) SOS, (c-d) SOS-N3, (e-f) SOS-Bdpy1, and (g-h) the non-functionalized silicon oxide surface immersed in Bdpy1 solution as a negative control (SOS′).

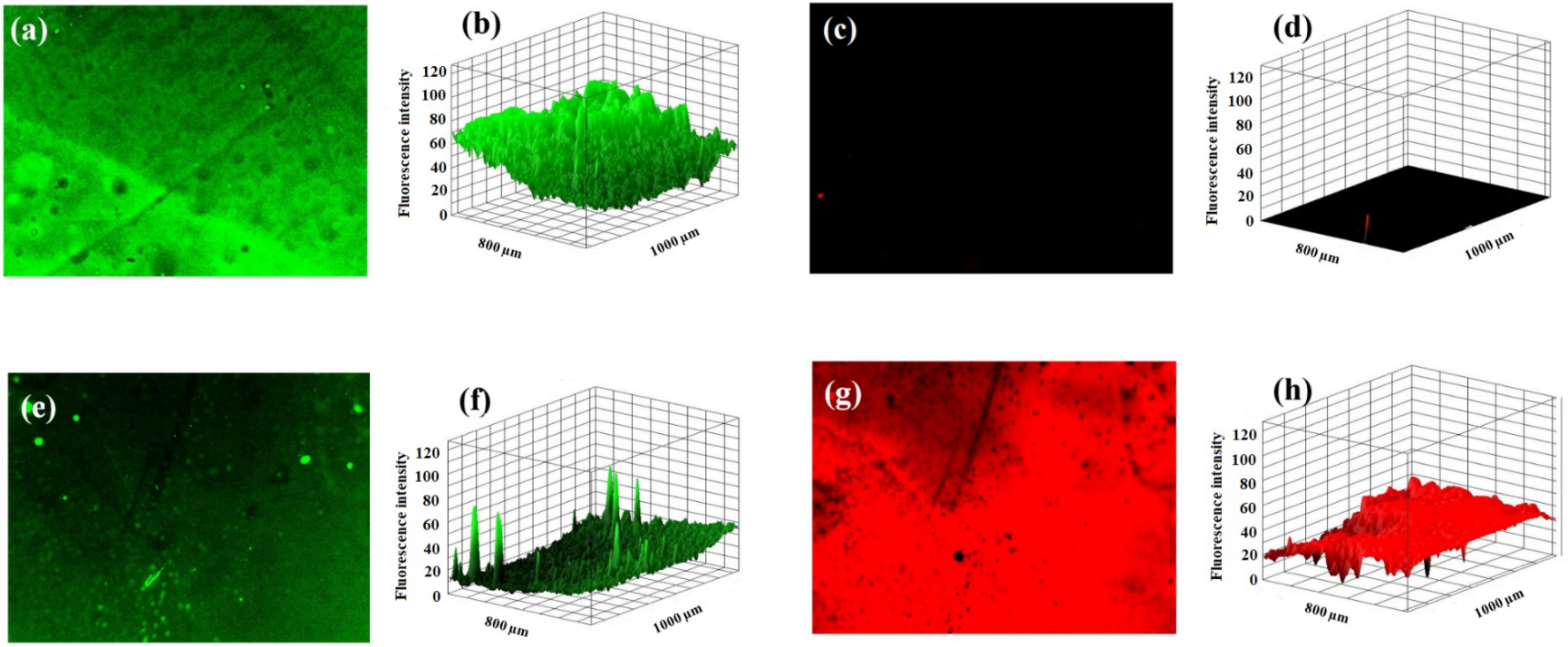


Fig. S6. Fluorescence microscopy images and their corresponding 3D surface plots of (a-b) SOS-Bdpy1 in the absence of GSH in λ_{em} = 498-531 nm, (c-d) SOS-Bdpy1 in the absence of GSH in λ_{em} = 565–632 nm, (e-f) SOS-Bdpy1 in the presence of GSH (SOS-Bdpy1-GSH) in λ_{em} = 498-531 nm, and (g-h) SOS-Bdpy1 in the presence of GSH (SOS-Bdpy1-GSH) in λ_{em} = 565–632 nm

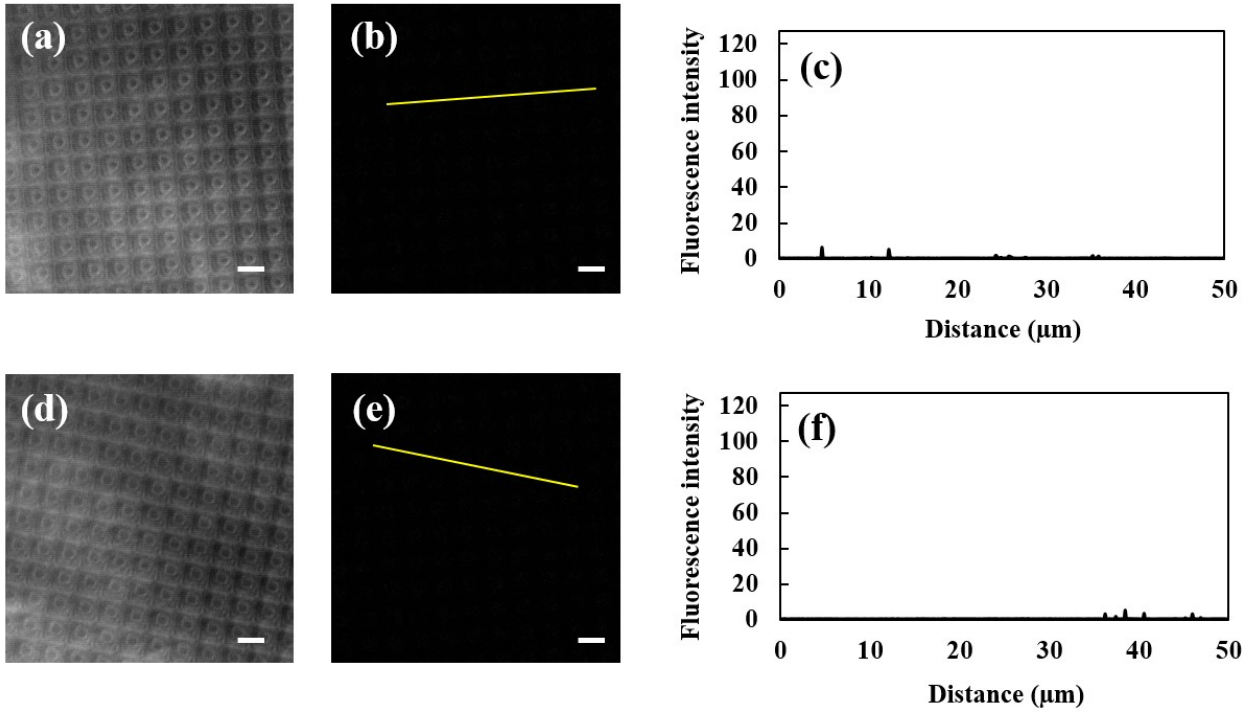
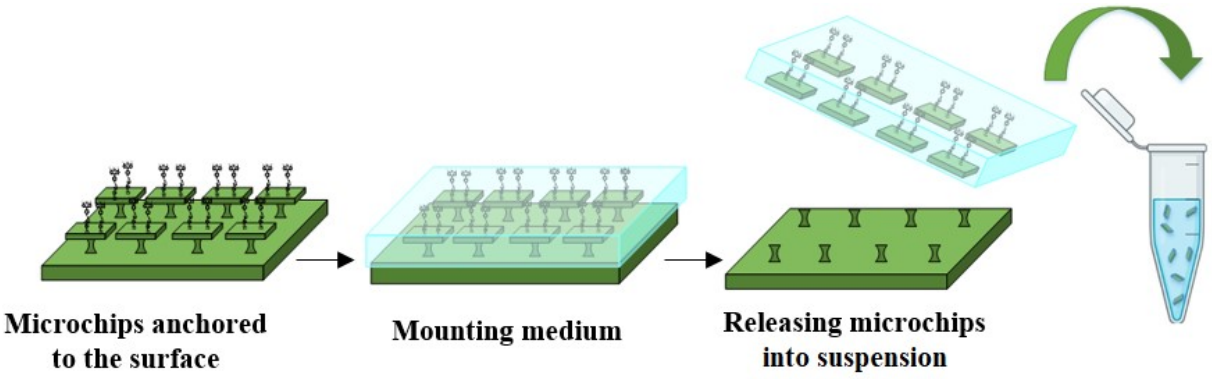


Fig. S7. (a and d) Bright-field, (b) fluorescence microscopy images in λ_{em} = 498-531 nm, (e) fluorescent image in λ_{em} = 595-630 nm for the non-functionalized SOµC. (c) and (f) fluorescence intensity profile of the yellow lines in (b) and (e) respectively. (Scale bar = 5 µm)



Scheme S2. Releasing microchips from surface using a mounting medium.

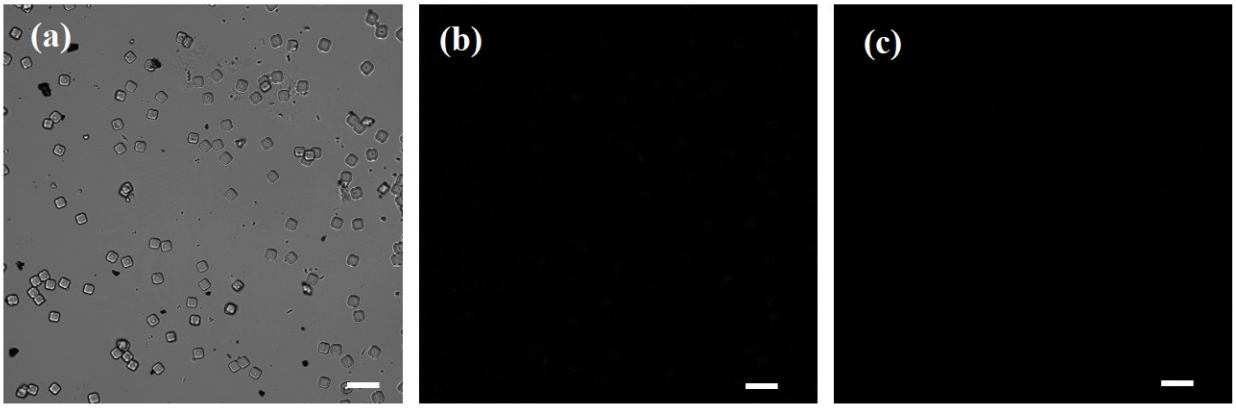


Fig. S8. (a) Bright-field, (b) fluorescent image in λ_{em} = 498-531 nm, and (c) fluorescent image λ_{em} = 595-630 nm for the non-functionalized released SOμC. (Scale bar = 10 μm)

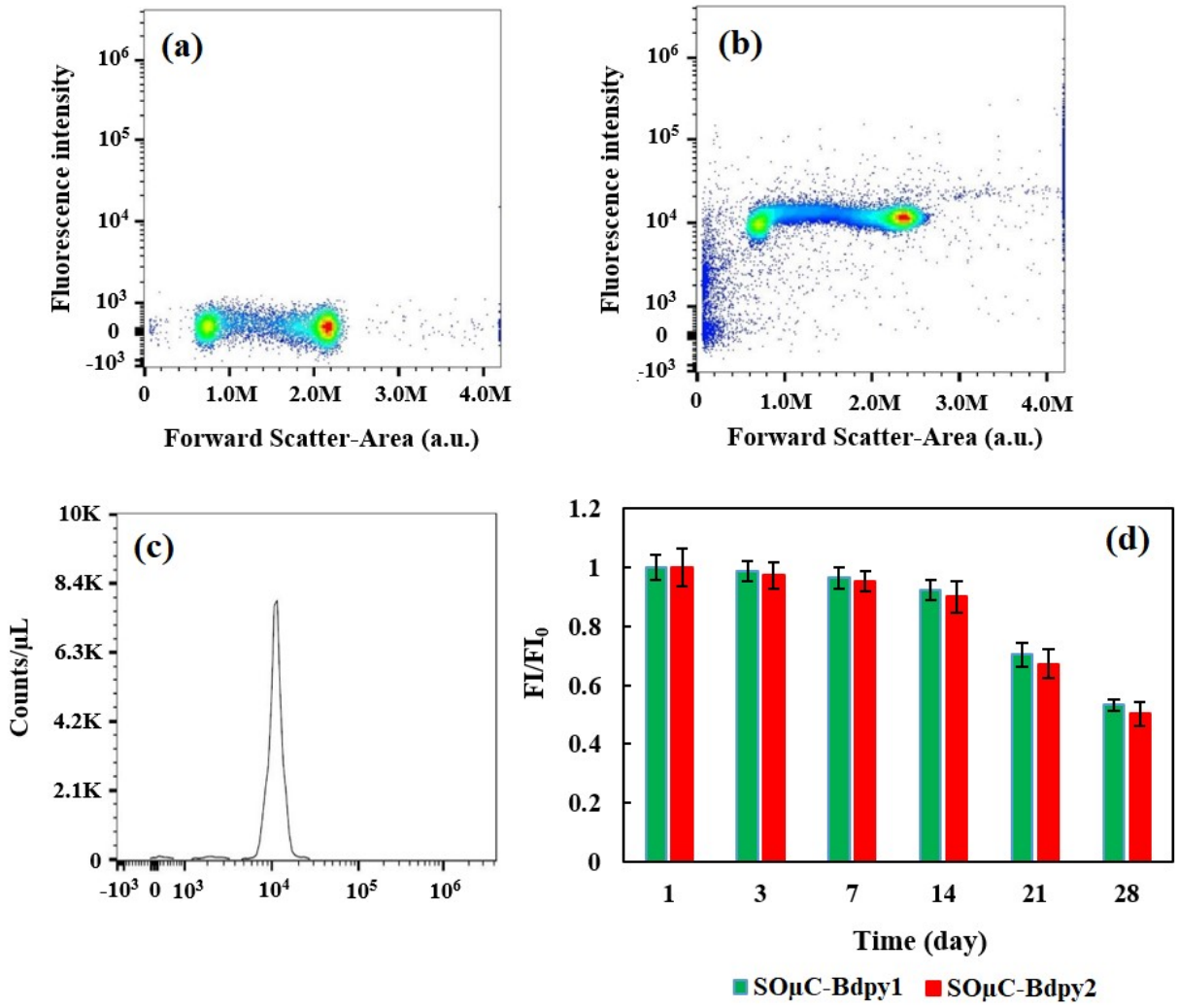


Fig. S9. Plots of relative fluorescence intensity in terms of the forward scattering using flow cytometry technique for (a) SOµC and (b) SOµC-Bdpy1. (c) Number of SOµC-Bdpy1 in terms of fluorescence intensity. (d) Variation in fluorescence intensity for SOµC-Bdpy1 and SOµC-Bdpy2 in the PBS suspension (room temperature) during different time intervals using fluorescence microscopy technique.

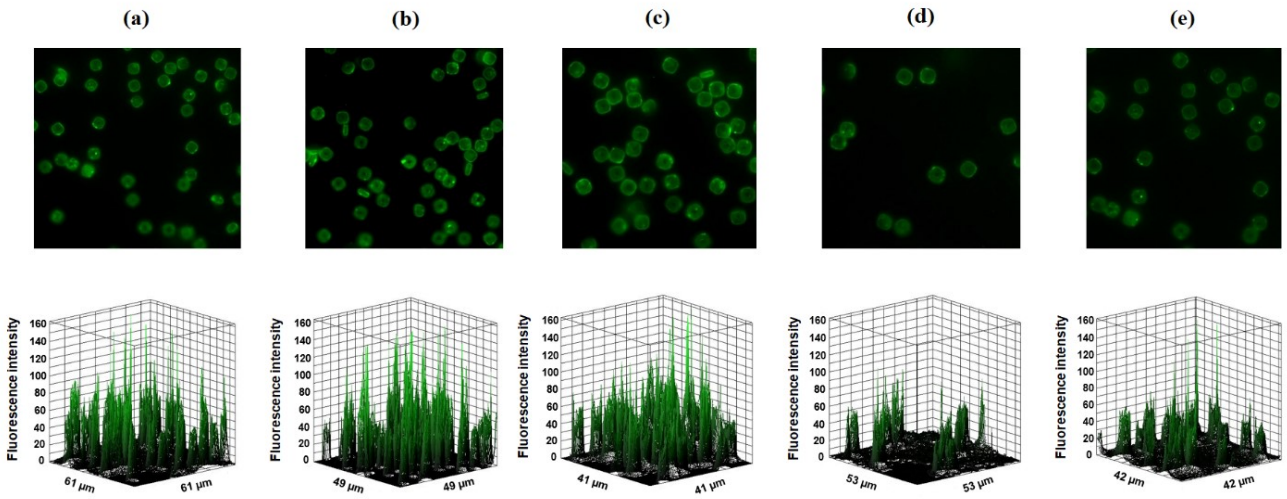


Fig. S10. Fluorescence microscopy images and the corresponding 3D surface plots(below) of SOµC-Bdpy1 in PBS after (a) 3 day, (b) 7 days, (c) 14 days, (d) 21 days, and (e) 28 days

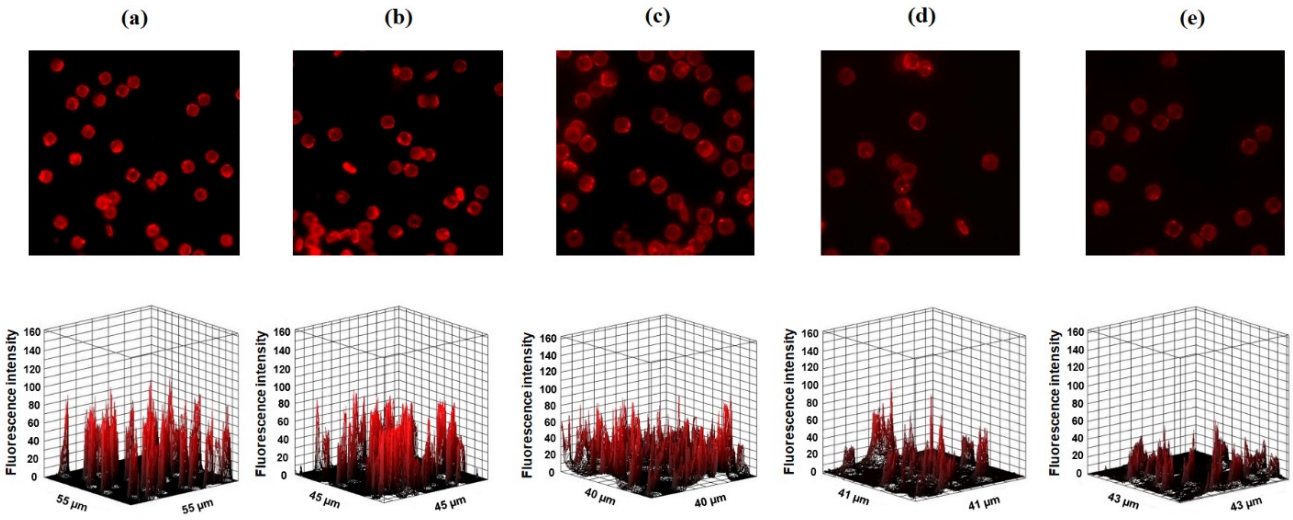


Fig. S11. Fluorescence microscopy images and 3D surface plots of SOµC-Bdpy2 in PBS after (a) 3 day, (b) 7 days, (c) 14 days, (d) 21 days, and (e) 28 days

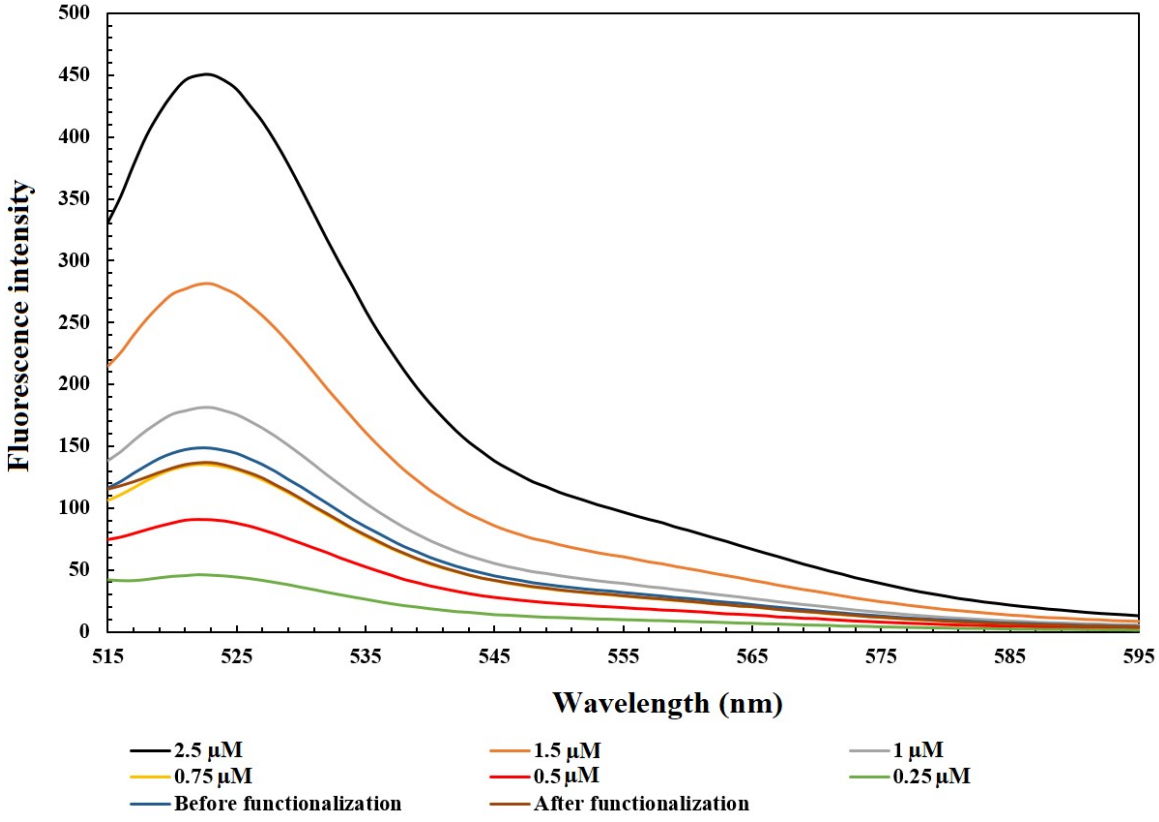


Fig. S12. Fluorescence spectra of different concentration of Bdpy1, as well as solution of reaction before and after surface functionalization process with Bdpy1. Solvent proportion is (DMF (8) / H_2O (2)) which is used for conjugation of Bdpy1 to the surface of microchips.

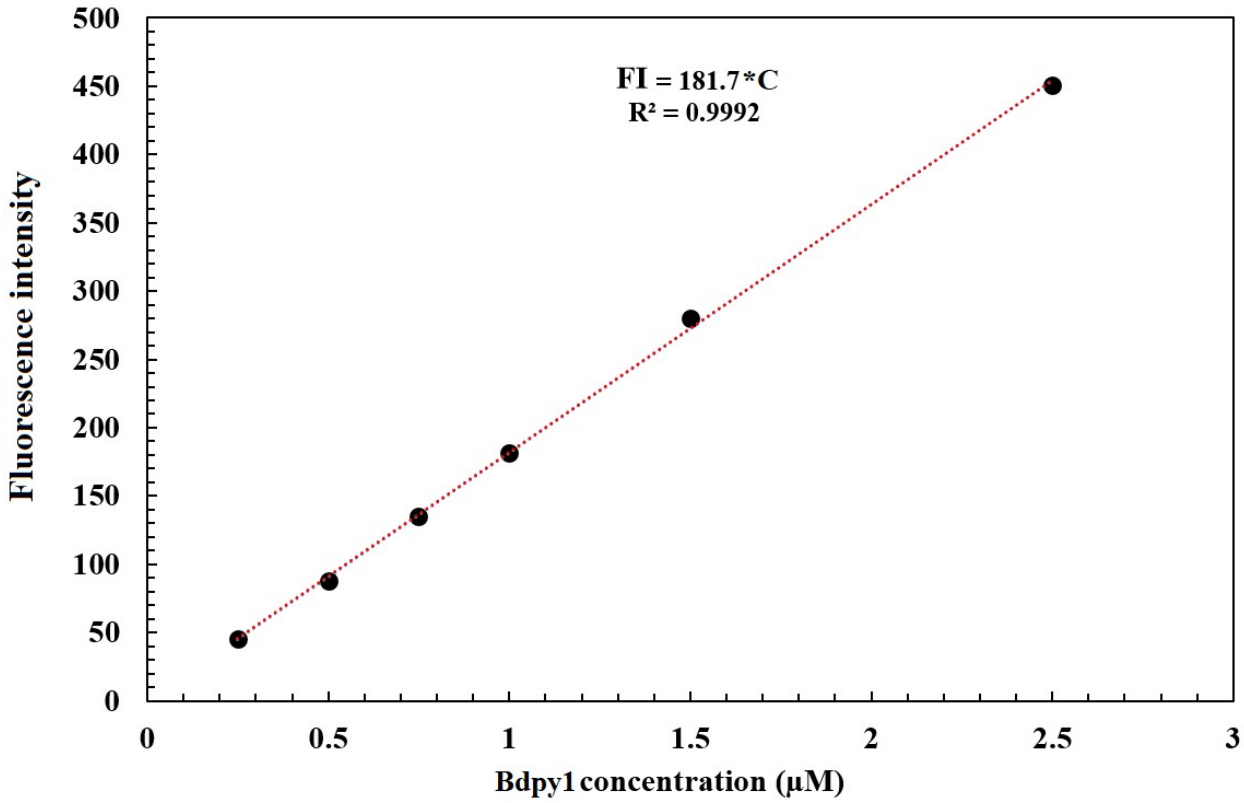


Fig. S13. Fluorescence intensity calibration curve resulting from different concentrations of Bdpy1. In the equation FI is fluorescence intensity of Bdpy1 solution in different concentration, and C is the concentrations of Bdpy1 solutions.

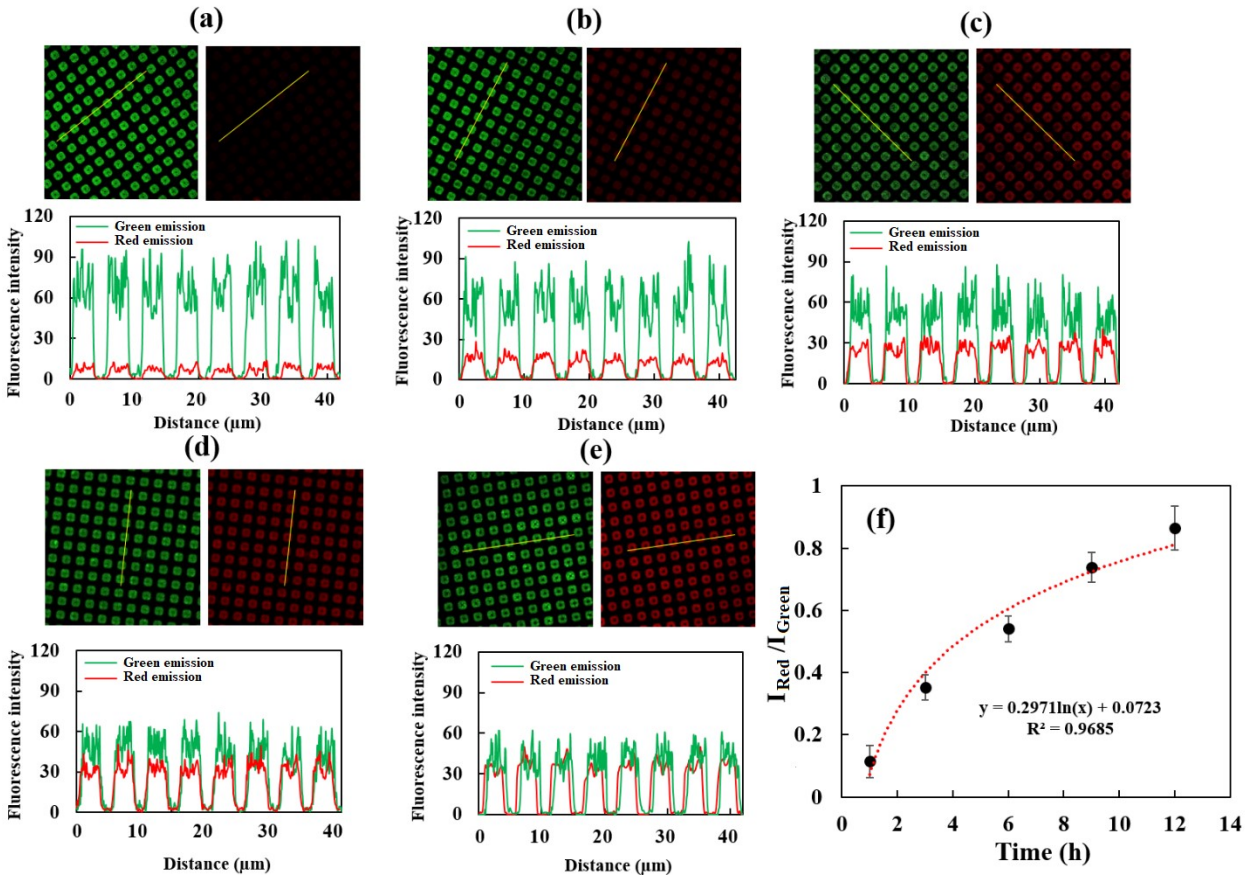


Fig. S14. Fluorescence microscopy images and fluorescence intensity profiles SOμC-Bdpy1 in the presence of 1 mM GSH solution (PBS, pH= 7.4, 37 °C) after (a) 1 h, (b) 3 h, (c) 6 h, (d) 9 h, and (e) 12 h incubation time. λ_{em} = 498-531 nm and λ_{em} = 565-632 nm. Yellow lines exhibit the fluorescence intensity profiles. (f) The ratiometric fluorescent intensity in terms of time for SOμC-Bdpy1.

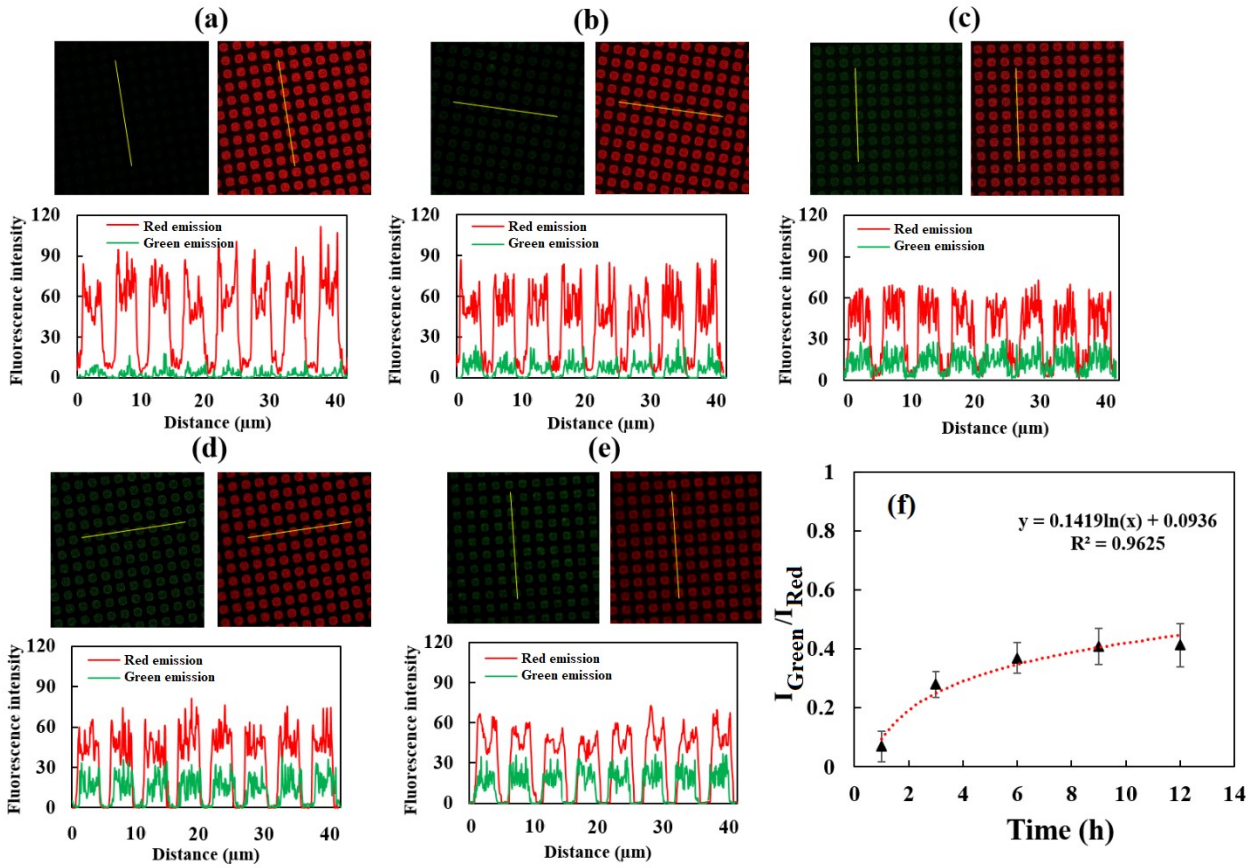


Fig. S15. Fluorescence microscopy images and fluorescence intensity profiles SOμC-Bdpy2 in the presence of 1 mM GSH solution (PBS, pH= 7.4, 37 °C) after (a) 1 h, (b) 3 h, (c) 6 h, (d) 9 h, and (e) 12 h incubation time. λ_{em} = 530-580 nm and λ_{em} = 595-630 nm. Yellow lines exhibit the fluorescence intensity profiles. (f) The ratiometric fluorescent intensity in terms of time for SOμC-Bdpy2.

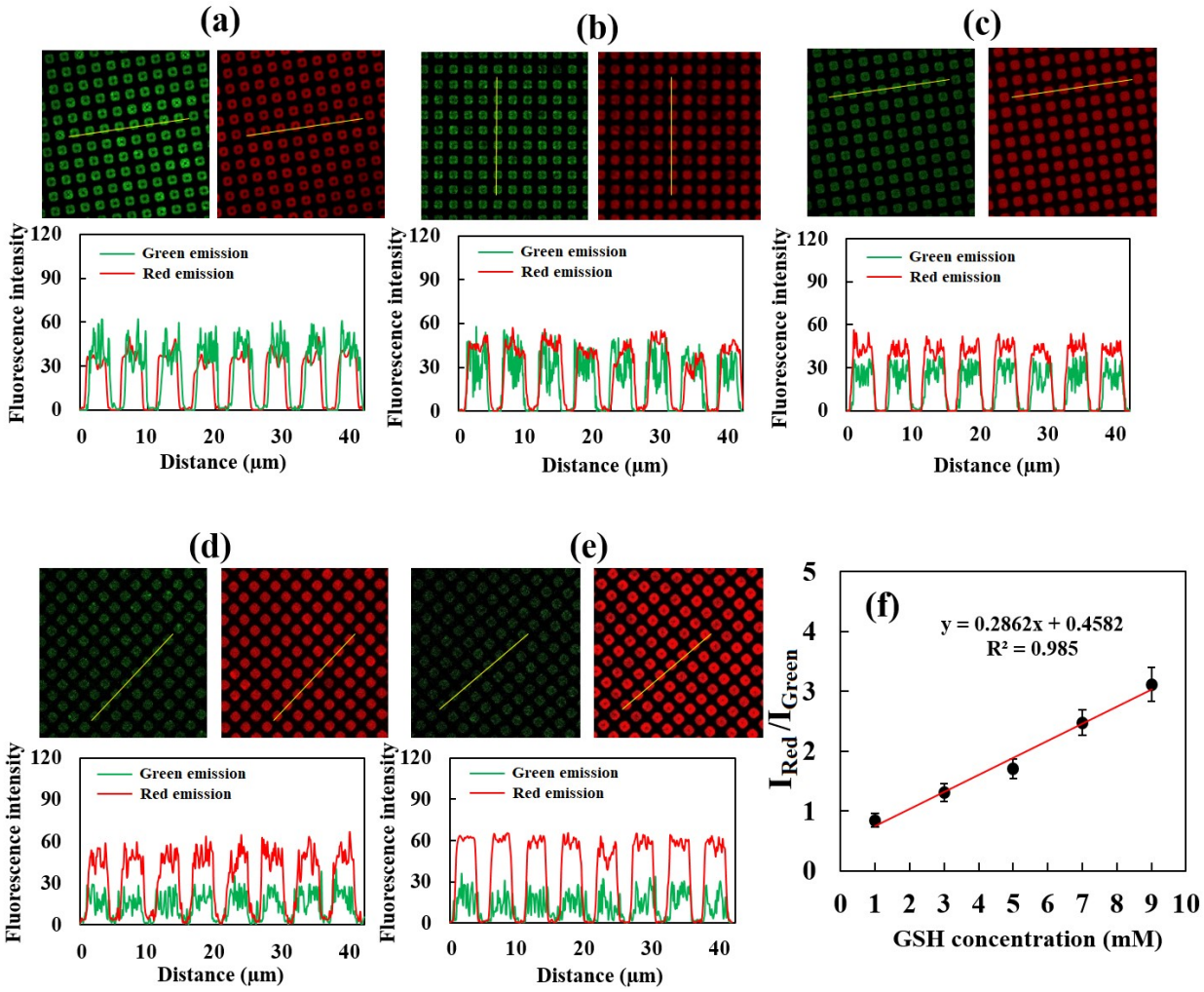


Fig. S16. Fluorescence microscopy images and fluorescence intensity profiles SOμC-Bdpy1 in different concentrations of GSH solution (PBS, pH= 7.4, 37 °C) during overnight h incubation time. (a) 1 mM, (b) 3 mM, (c) 5 mM, (d) 7 mM, and (e) 9 mM concentration of GSH. λ_{em} = 498-531 nm and λ_{em} = 565-632 nm. Yellow lines exhibit the fluorescence intensity profiles. (f) The ratiometric fluorescent intensity in terms of GSH concentration for SOμC-Bdpy1 after overnight incubation time.

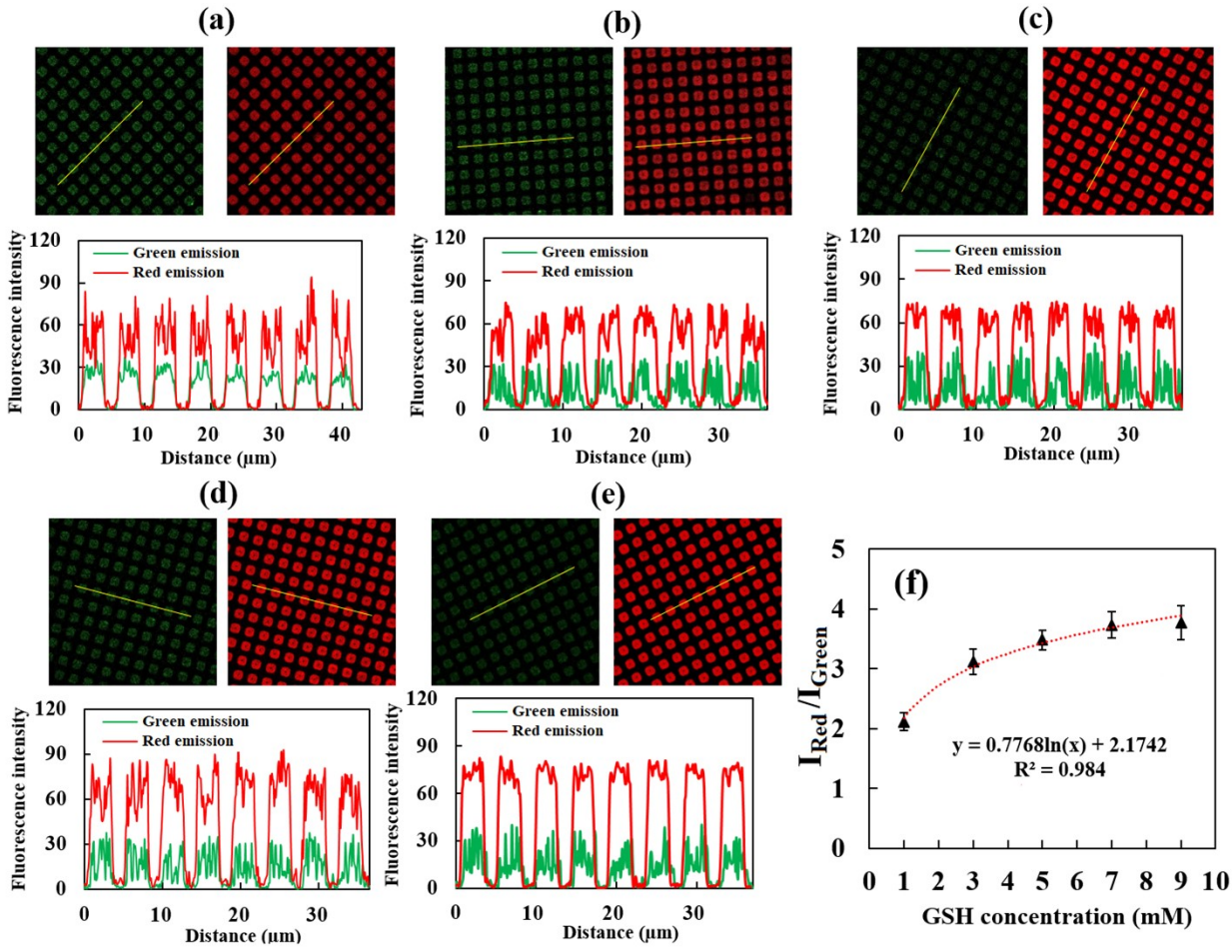


Fig. S17. Fluorescence microscopy images and fluorescence intensity profiles SOμC-Bdpy1 in different concentrations of GSH solution (PBS, pH= 7.4, 37 °C) during 24 h incubation time. (a) 1 mM, (b) 3 mM, (c) 5 mM, (d) 7 mM, and (e) 9 mM concentration of GSH. λ_{em} = 498-531 nm and λ_{em} = 565-632 nm. Yellow lines exhibit the fluorescence intensity profiles. (f) The ratiometric fluorescent intensity in terms of GSH concentration for SOμC-Bdpy1 after 24 h incubation time.

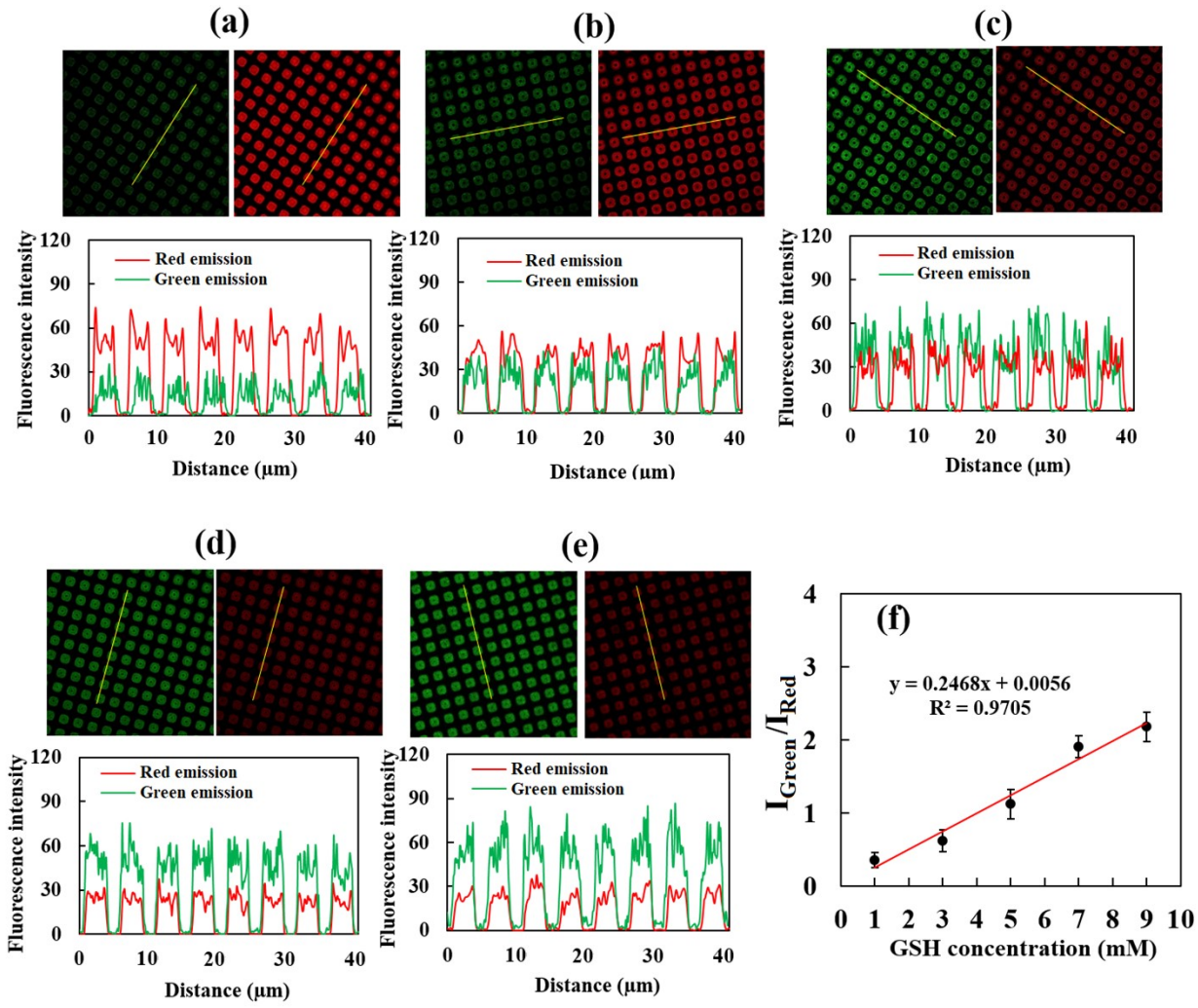


Fig. S18. Fluorescence microscopy images and fluorescence intensity profiles SOμC-Bdpy2 in different concentrations of GSH solution (PBS, pH= 7.4, 37 °C) during 12 h incubation time. (a) 1 mM, (b) 3 mM, (c) 5 mM, (d) 7 mM, and (e) 9 mM concentration of GSH. λ_{em} = 530-580 nm and λ_{em} = 595-630 nm. Yellow lines exhibit the fluorescence intensity profiles. (f) The ratiometric fluorescent intensity in terms of GSH concentration for SOμC-Bdpy2 after overnight incubation time.

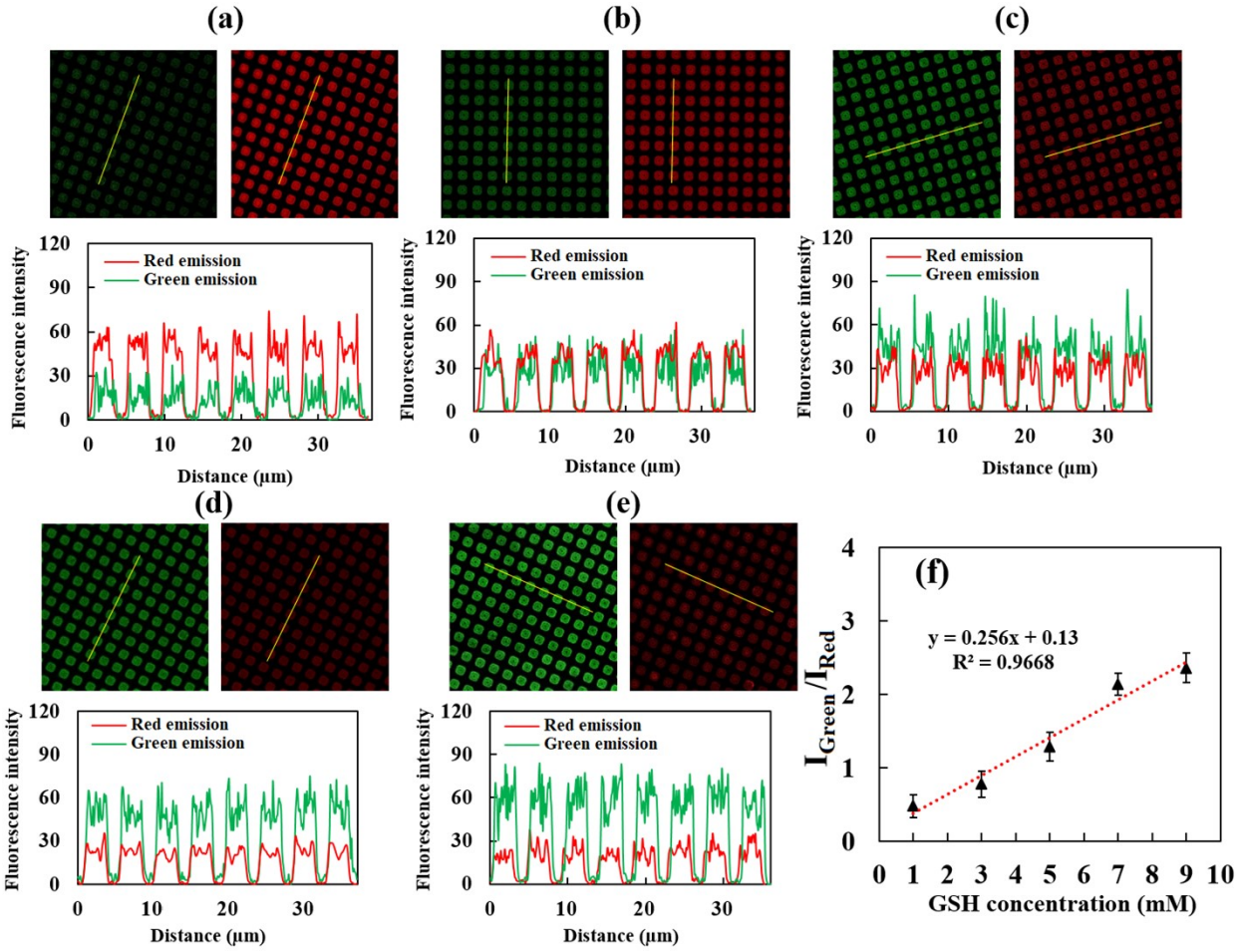


Fig. S19. Fluorescence microscopy images and fluorescence intensity profiles SOμC-Bdpy2 in different concentrations of GSH solution (PBS, pH= 7.4, 37 °C) during 24 h incubation time. (a) 1 mM, (b) 3 mM, (c) 5 mM, (d) 7 mM, and (e) 9 mM concentration of GSH. λ_{em} = 530-580 nm and λ_{em} = 595-630 nm. Yellow lines exhibit the fluorescence intensity profiles. (f) The ratiometric fluorescent intensity in terms of GSH concentration for SOμC-Bdpy2 after 24 h incubation time.

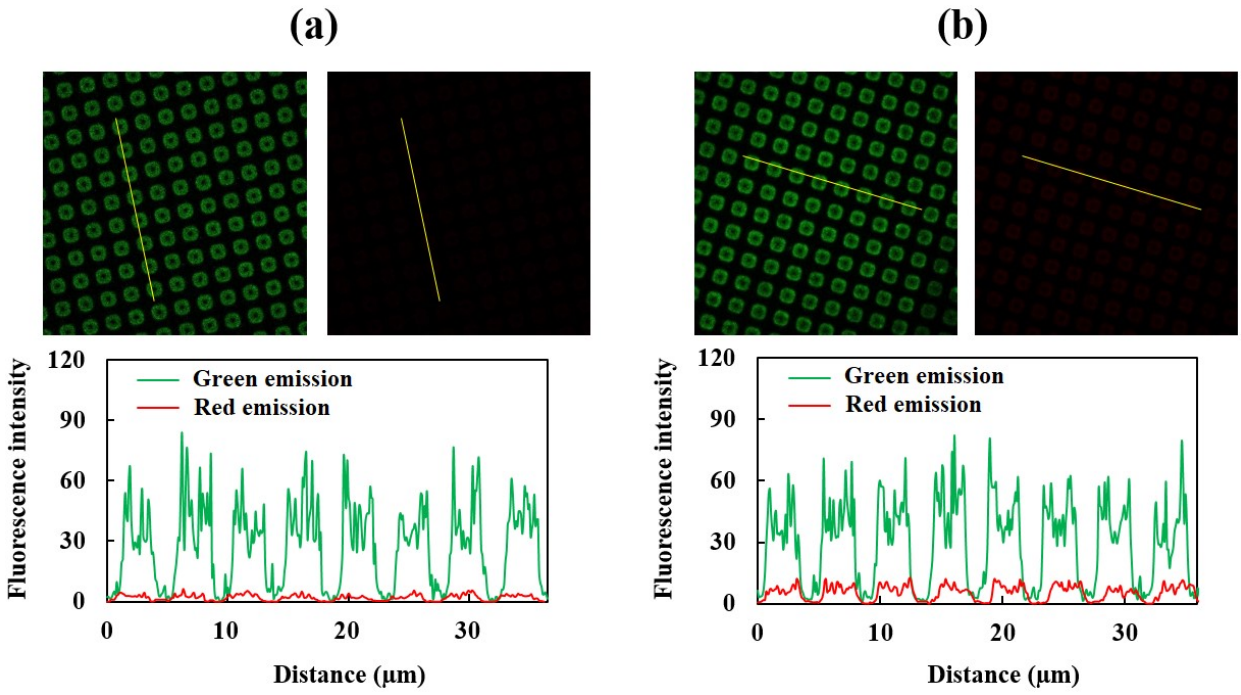


Fig. S20. Fluorescence microscopy images of incubated SOμC-Bdpy1 in 1 mM of (a) Cys solution and (b) Hcy in overnight (PBS, pH= 7.4, 37 °C). λ_{em} = 498-531 nm and λ_{em} = 565-632 nm. Yellow lines exhibit the fluorescence intensity profiles.

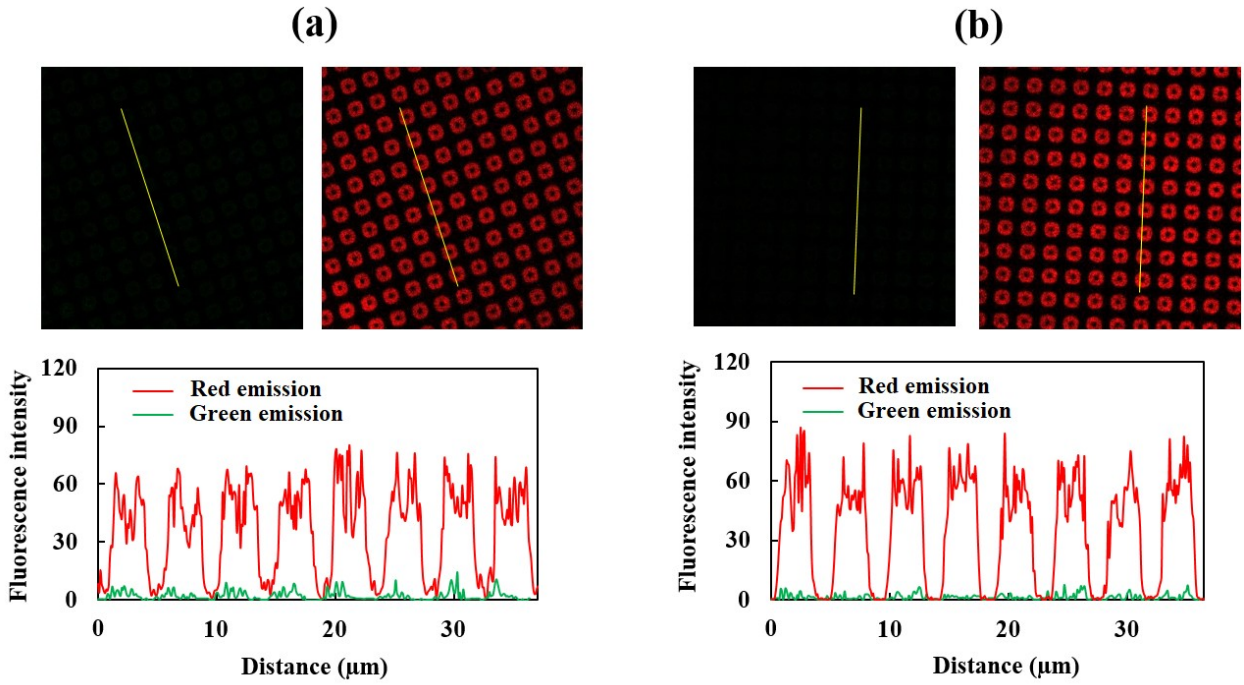


Fig. S21. Fluorescence microscopy images of incubated SOμC-Bdpy2 in 1 mM of (a) Cys solution and (b) Hcy in overnight (PBS, pH= 7.4, 37 °C). λ_{em} = 530-580 nm and λ_{em} = 595-630 nm. Yellow lines exhibit the fluorescence intensity profiles.

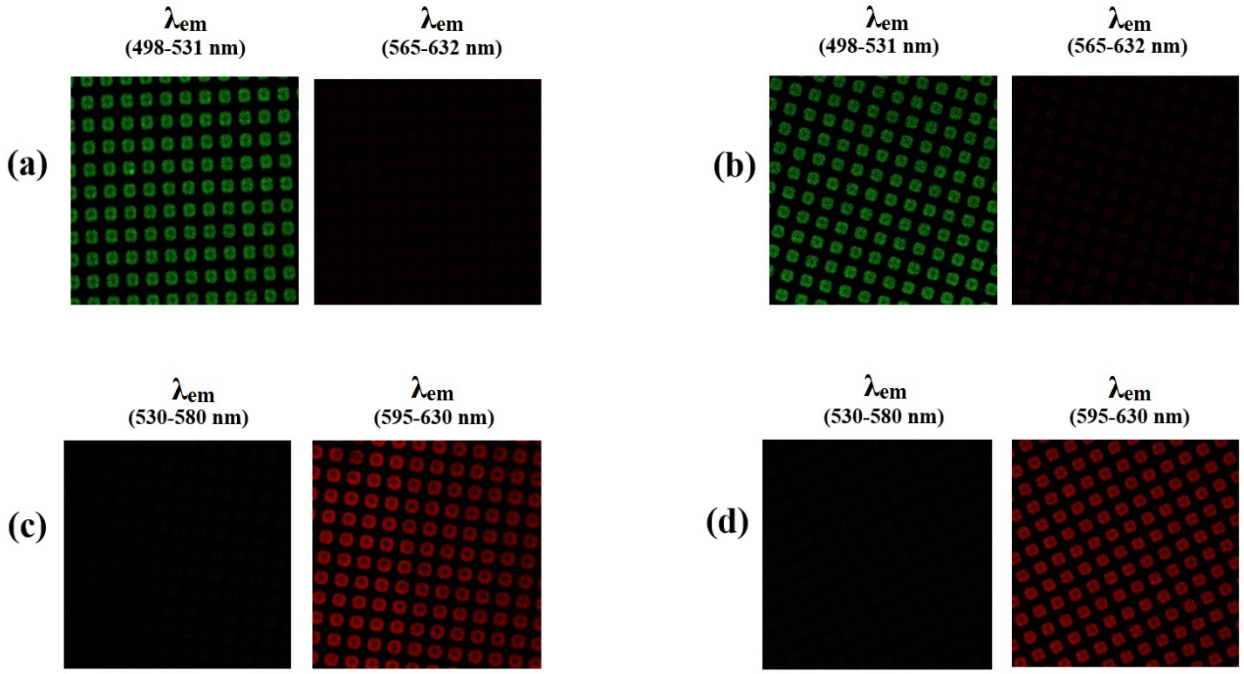


Fig. S22. Fluorescence microscopy images of incubated SOμC-Bdpy1 in (a) PBS, pH= 7.4, 37°C and (b) in cell culture medium, 37°C for overnight. Incubated SOμC-Bdpy2 in (c) PBS, pH= 7.4, 37°C and (d) in cell culture medium, 37 °C for overnight.

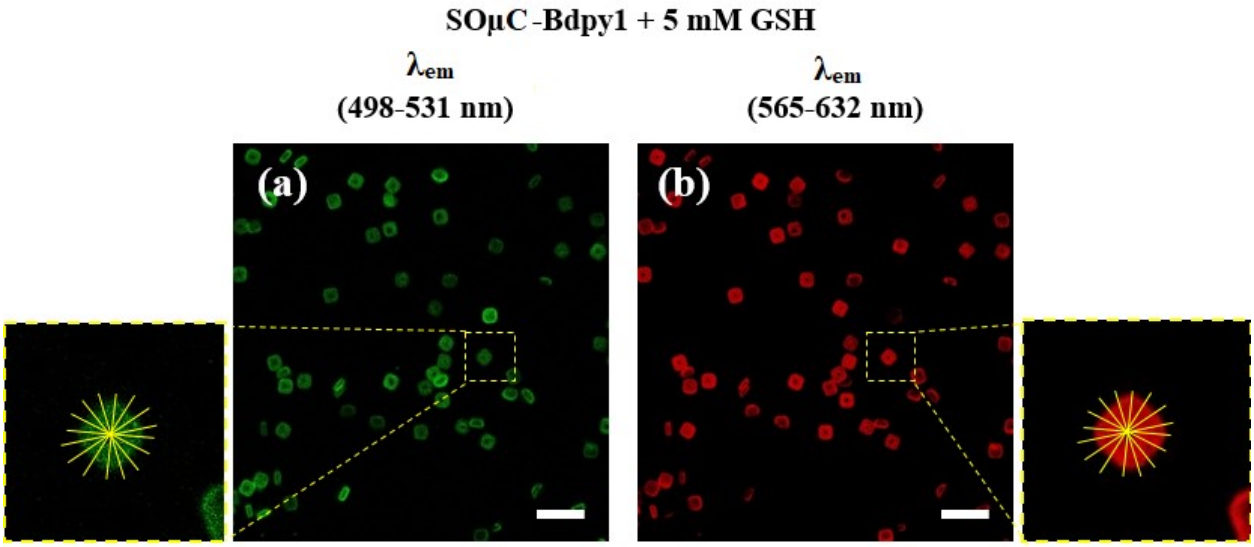


Fig. S23. Fluorescence microscopy images of released microchips of (a-b) SOμC-Bdpy1 in 5 mM GSH solution (cell culture medium, 37 °C, and overnight). Yellow lines are fluorescence intensity profiles used for the ratiometric signal calculations. (Scale bar = 10 μM)

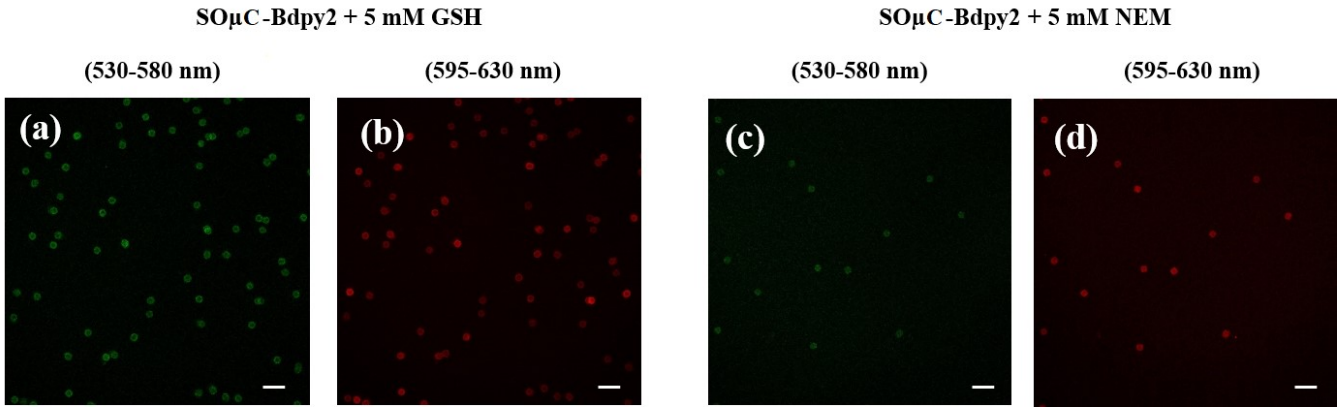


Fig. S24. Fluorescence microscopy images of SOμC-Bdpy2 in (a-b) 5 mM GSH and (c-d) 5 mM NEM (cell culture medium, 37 °C, and overnight).

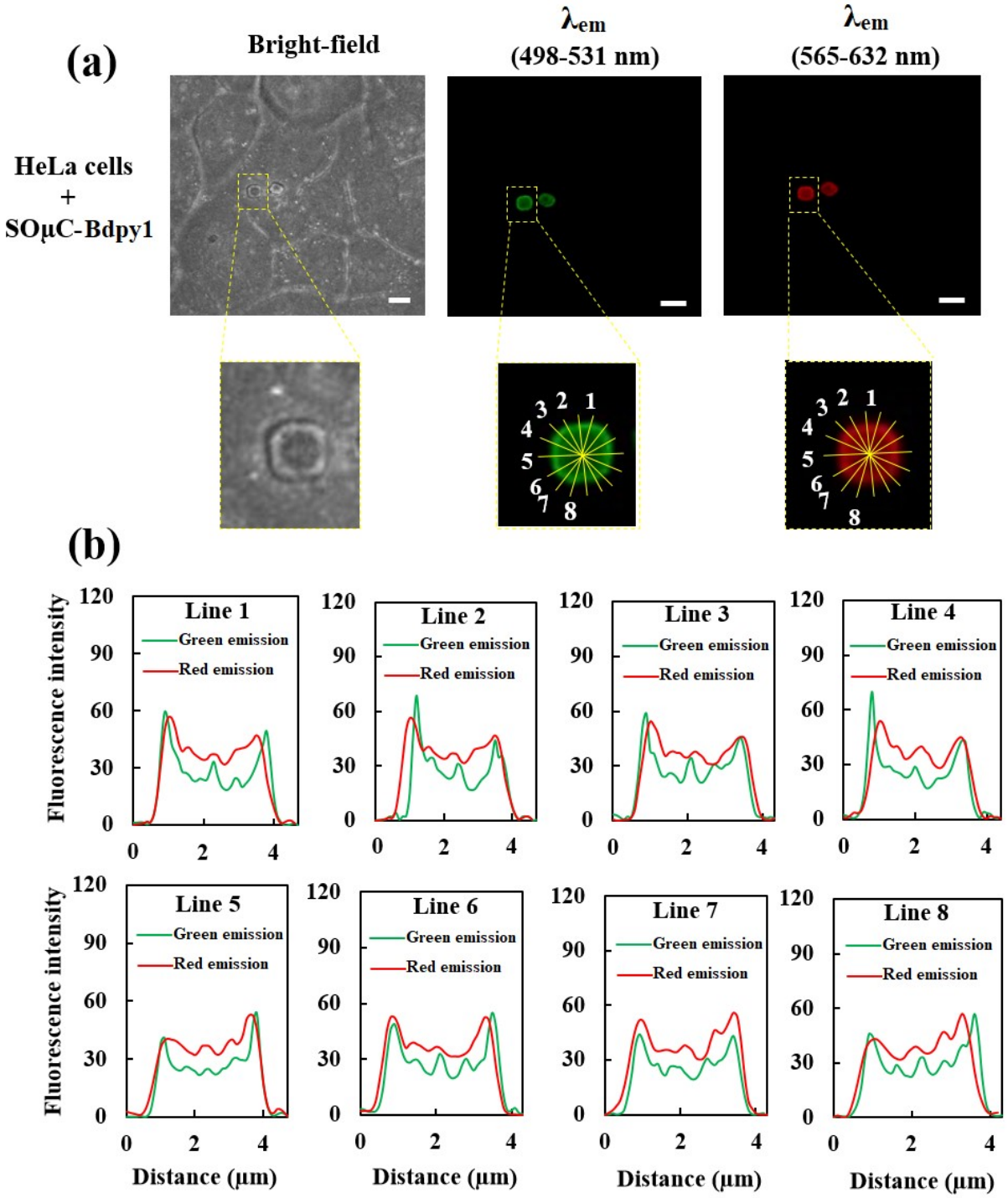


Fig. S25. Confocal laser scanning microscopy images of internalized (a) SOμC-Bdpy1 in Hela cells during overnight incubation. (b) Fluorescence intensity profile related to each profile line for internalized SOμC-Bdpy1 in λ_{em} = 498-531 nm and λ_{em} = 565-632 nm. Yellow lines are fluorescence intensity profiles used for the ratiometric signal calculations. Scale bar = 10 μm.

Table S2. Measurement of the ratiometric signal (ratiometric fluorescence intensity) for the example internalized SOμC-Bdpy1

Plot profile number	Average signal in λ_{em} = 498-531 nm	Average signal in λ_{em} = 565-632 nm	Ratiometric signal calculation
Line 1	21.523	25.822	1.312
Line 2	19.678	27.505	1.278
Line 3	22.276	27.225	1.222
Line 4	19.829	26.539	1.338
Line 5	20.785	26.052	1.253
Line 6	22.954	28.572	1.245
Line 7	20.815	29.110	1.399
Line 8	23.457	29.232	1.246
Average of the ratiometric signal for the example internalized SOμC-Bdpy1 = **1.287 ± 0.055**			
Intracellular GSH concentration for the example cell = **2.9 ± 0.2 mM**			

Table S3. The ratiometric signal average of the internalized SOμC-Bdpy1 and corresponding intracellular GSH concentration calculation

Ratiometric Signal Average For Internalized SOμC-Bdpy1	Intracellular GSH concentration (mM)	Ratiometric Signal Average For Internalized SOμC-Bdpy1	Intracellular GSH concentration (mM)
1.522	3.717	1.158	2.447
1.186	2.543	1.333	3.057
1.245	2.750	1.141	2.387
1.362	3.158	1.210	2.628
1.428	3.387	1.287	2.896
1.441	3.434	1.498	3.632
1.393	3.266	1.231	2.699
1.636	4.114	1.472	3.541
1.096	2.229	1.618	4.053
1.259	2.806	1.479	3.566
1.032	2.005	1.243	2.742

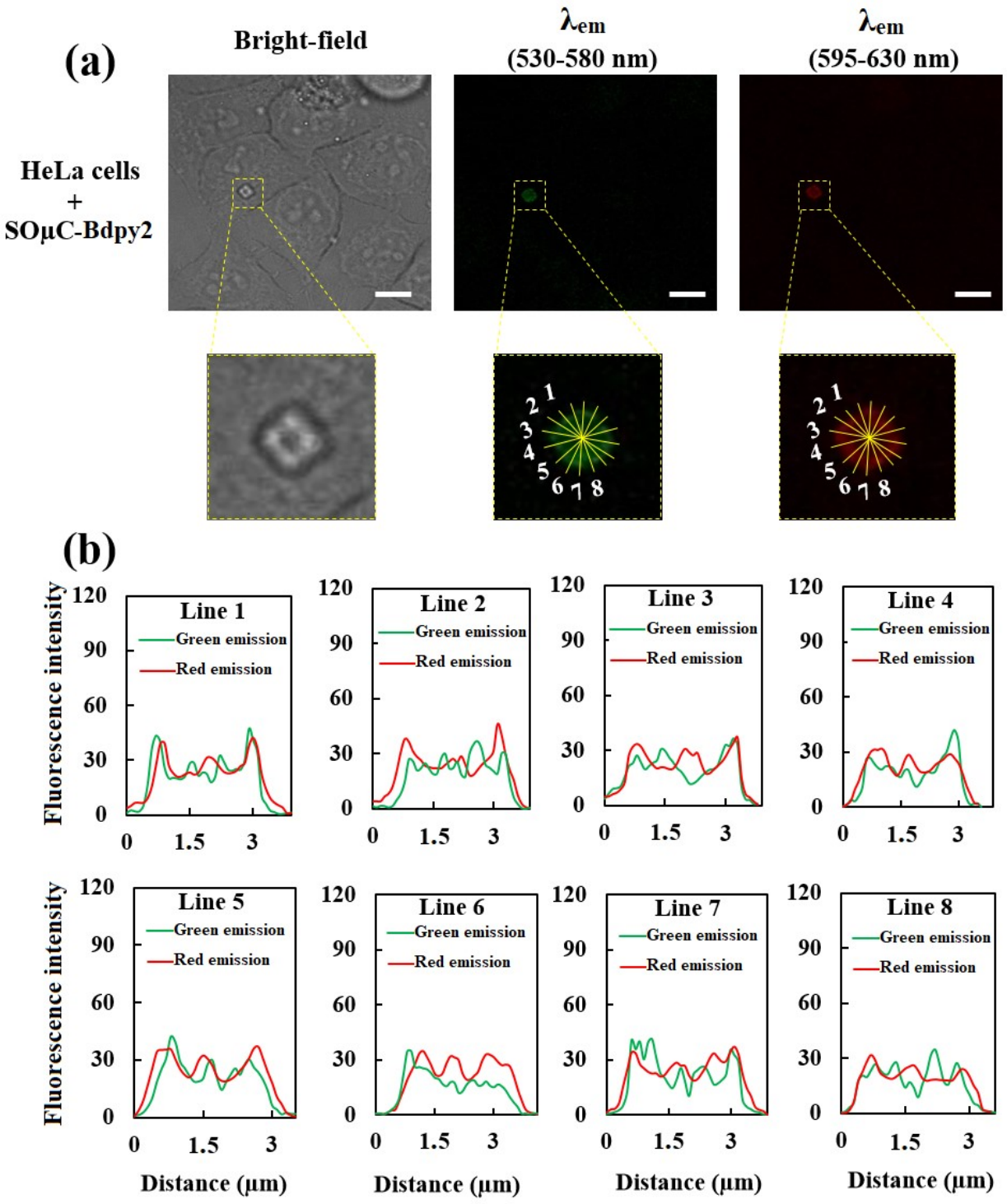


Fig. S26. Confocal laser scanning microscopy images of internalized (a) SOμC-Bdpy2 in Hela cells during overnight incubation. (b) Fluorescence intensity profile related to each profile line for internalized SOμC-Bdpy2 in λ_{em} = 530-580 nm and λ_{em} = 595-630 nm. Yellow lines are fluorescence intensity profiles used for the ratiometric signal calculations. Scale bar = 10 μm.

Table S4. Measurement of the ratiometric signal (ratiometric fluorescence intensity) for the example internalized SOμC-Bdpy2

Plot profile number	Average signal in λ_{em} = 530-580 nm	Average signal in λ_{em} = 595-630	Ratiometric signal calculation
Line 1	20.457	21.339	0.959
Line 2	18.570	21.888	0.848
Line 3	18.799	21.858	0.860
Line 4	16.973	20.208	0.840
Line 5	19.002	23.572	0.806
Line 6	17.188	20.363	0.844
Line 7	20.777	21.936	0.947
Line 8	20.081	21.489	0.934
Average of the ratiometric signal for the example internalized SOμC-Bdpy2 = **0.879 ± 0.054**			
Intracellular GSH concentration for the example cell = **3.6 ± 0.2 mM**			

Table S5. The ratiometric signal average of the internalized SOμC-Bdpy2 and corresponding intracellular GSH concentration calculation

Ratiometric Signal Average For Internalized SOμC-Bdpy2	Intracellular GSH concentration (mM)	Ratiometric Signal Average For Internalized SOμC-Bdpy2	Intracellular GSH concentration (mM)
1.212	4.973	1.088	4.458
0.771	3.125	0.812	3.295
0.762	3.091	0.657	2.653
1.215	4.983	0.863	3.516
0.711	2.872	0.708	2.864
1.428	5.882	1.425	5.868
0.640	2.576	0.765	3.124
1.272	5.227	0.893	3.633
0.795	3.228	0.807	3.276
1.197	4.916	0.661	2.624
0.873	3.556	0.880	3.584

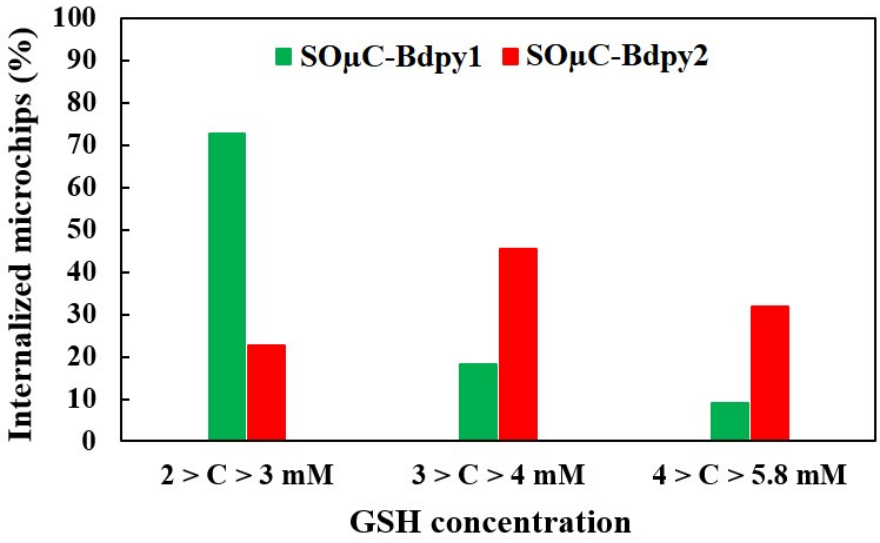
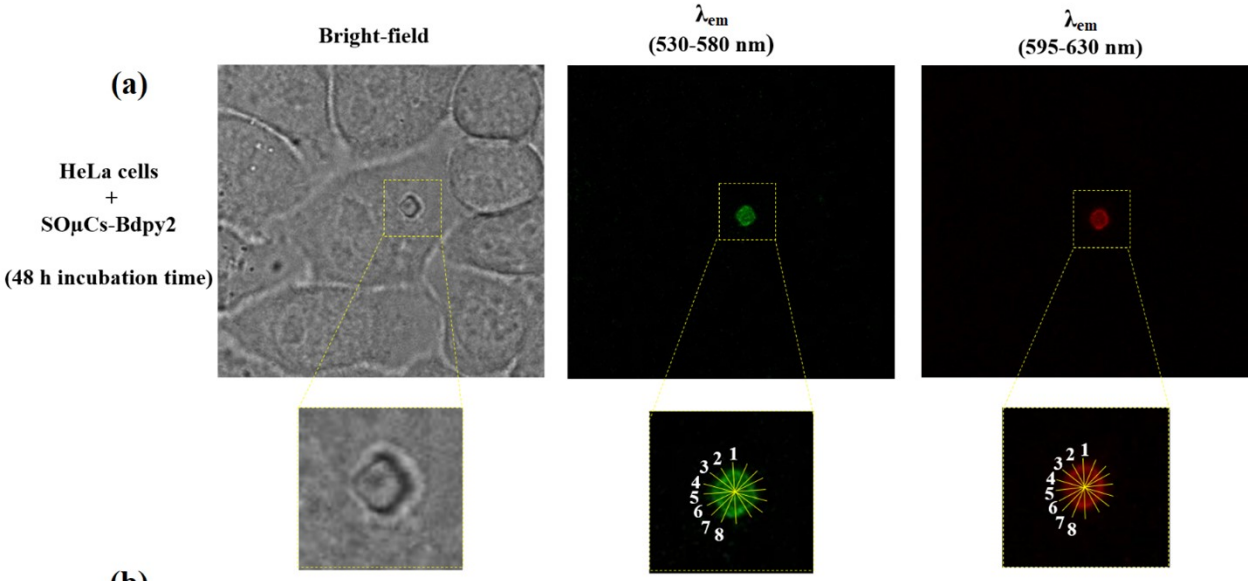


Fig. S27. Percentage of internalized SOμC-Bdpy1 and SOμC-Bdpy2 in terms of the quantified intracellular GSH concentration using the calculated ratiometric signals



(b)

Plot profile number	Average signal in λ_{em} = 530-580 nm	Average signal in λ_{em} = 595-630 nm	Ratiometric signal calculation
Line 1	21.069	21.466	0.982
Line 2	23.435	21.126	1.109
Line 3	20.913	22.459	0.931
Line 4	22.695	22.787	0.996
Line 5	18.981	21.214	0.895
Line 6	20.481	19.423	1.054
Line 7	20.046	22.431	0.894
Line 8	21.841	22.322	0.978
Average of the ratiometric signal for the example internalized SOμCs-Bdpy2 after 48 h incubation time = **0.981 ± 0.07**			
Intracellular GSH concentration for the example cell after 48 h incubation time = **3.9 ± 0.3 mM**			

Fig. S28. Confocal laser scanning microscopy images of internalized (a) SOμC-Bdpy2 in Hela cells after 48 h incubation time. λ_{em} = 530-580 nm and λ_{em} = 595-630 nm. Yellow lines are fluorescence intensity profiles used for the ratiometric signal calculations. Scale bar = 10 μm. (b) Measurement of the ratiometric signal for the example internalized SOμC-Bdpy2 after 48 h incubation time.

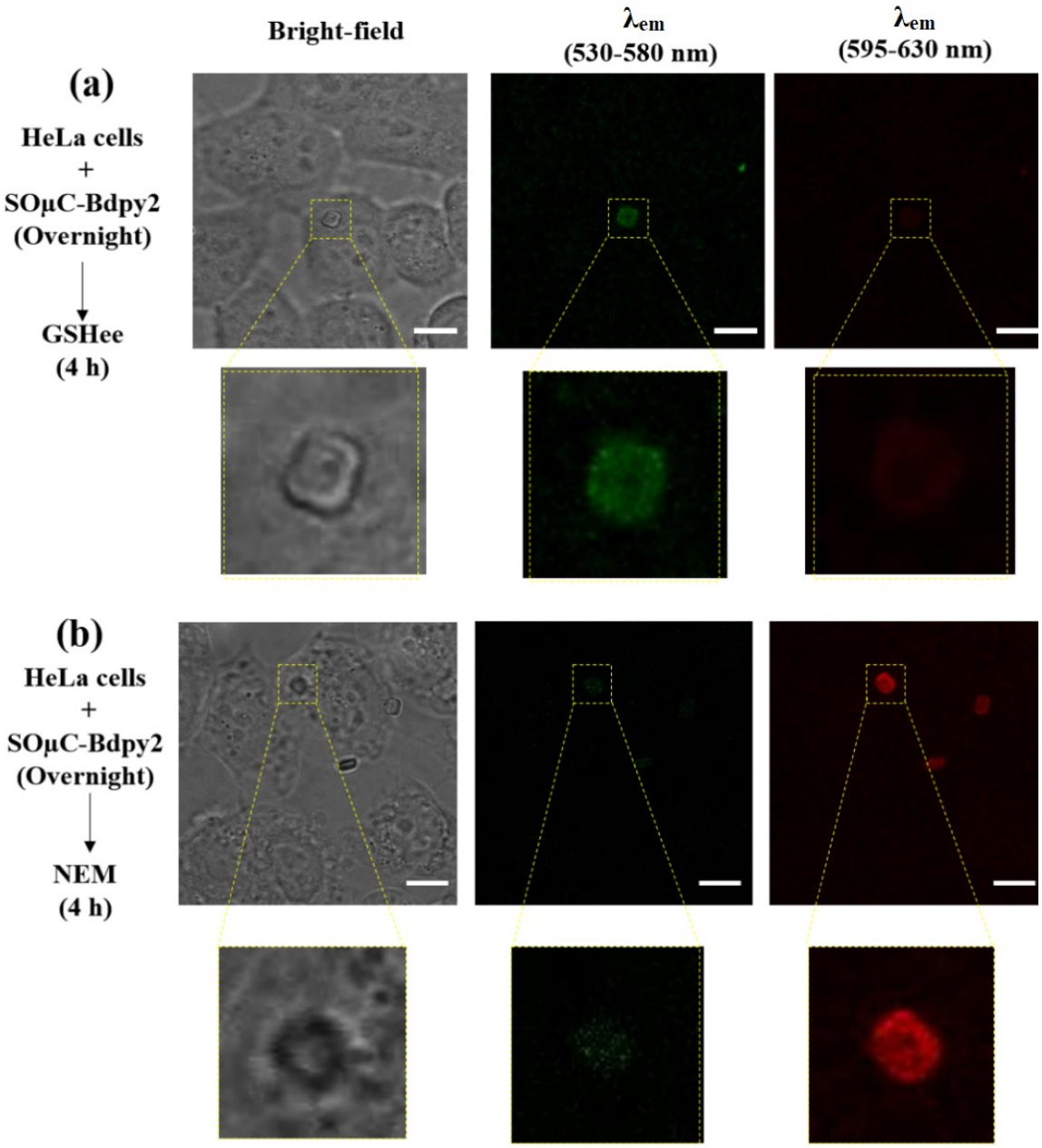


Fig. S29. Confocal laser scanning microscopy images of Hela cells incubated with (a) SOμC-Bdpy2 overnight followed by incubation with 10 mM GSH for 4 h and incubated with (b) SOμC-Bdpy2 overnight followed by incubation with 100 μM of NEM for 4 h. Scale bar = 10 μm.

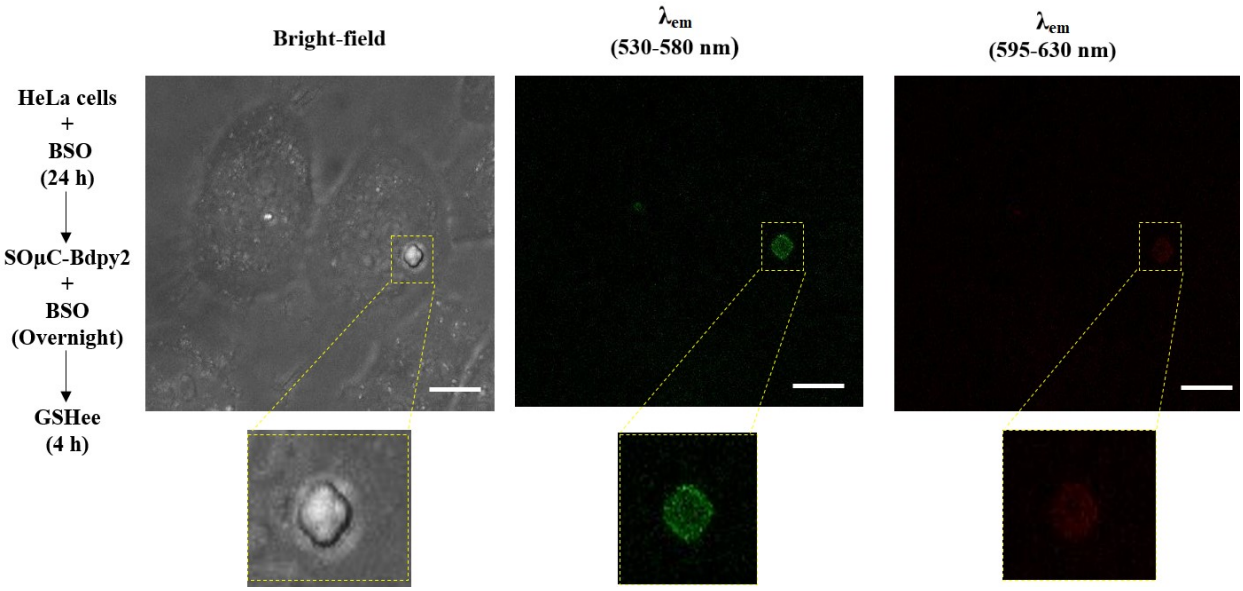


Fig. S30. Hela cells incubated with 1 mM of BSO for 24 h followed by incubation SOμC-Bdpy2 in the presence of BSO for overnight and subsequent incubation with 10 mM of GSHee for 4 h. (Scale bar = 10).

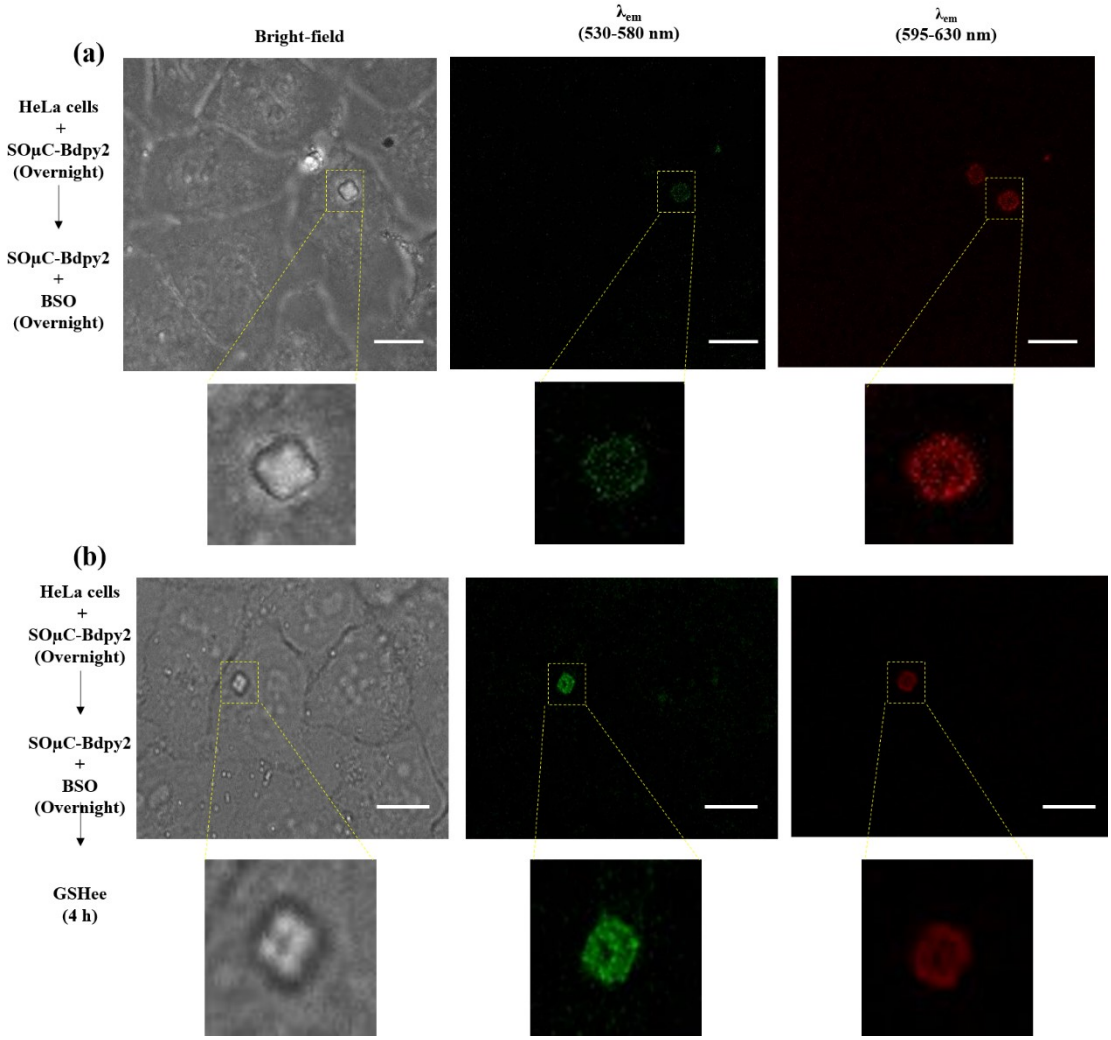


Fig. S31. Confocal laser scanning microscopy images of Hela cells incubated with (a) SOμC-Bdpy2 overnight followed by incubation with 1 mM BSO in the presence of SOμC-Bdpy2 for overnight (SOμC-Bdpy2 48 h). (b) SOμC-Bdpy2 overnight followed by incubation with 1 mM BSO for overnight (SOμC-Bdpy2 48 h), and subsequent incubation with 10 mM of GSHee for 4 h. Scale bar = 10 μm.