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Supporting Information for

Ultrasensitive and Ratiometric Two-photon Fluorescence Imaging of Golgi Polarity during Drug-Induced Acute Kidney Injury

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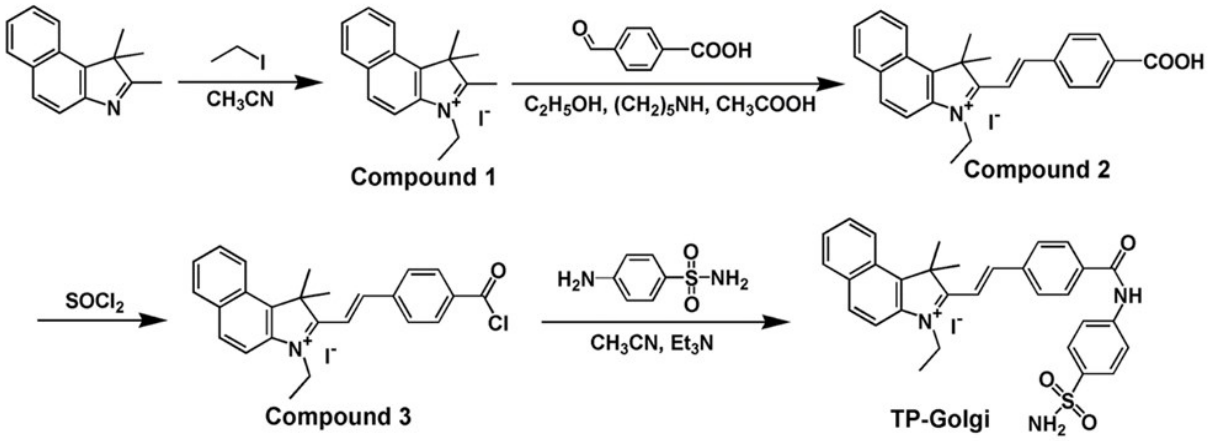
1. Materials and Instruments

All materials were commercially available and could be used without purification. LPS (Lipopolysaccharide) and MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sangon Biotech. Various organelle trackers were obtained from Beyotime. Bleomycin were purchased from HISUN (Zhejiang, China). Creatinine Assay Kit was obtained by Nanjing Jiancheng Bioengineering Institute. Sulfanilamide and monensin sodium salt were purchased from aladdin. Iodoethane and 1,1,2-trimethylbenz[e]indole were obtained from Shanghai Macklin Biochemical Co., Ltd. 4-formylbenzoic acid were obtained from Bidepharm.

Bruker NMR spectrometers were used to detect ^{1}H NMR at 400MHz and ^{13}C NMR at 100MHz. The mass spectra were recorded with BrukerMaxis ultrahigh-resolution TOF MS system. Fluorescence spectra were measured on FLS-920 Edinburgh fluorescence spectrometer. Absorption spectra were examined on TU-1900 UV-vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.). Fluorescence imaging was performed in cells using Leica TCS Sp8 (one photon) confocal laser scanning microscope and in vivo using Zeiss LSM 880 NLO (two photon) confocal laser scanning microscope.

2. Synthesis of TP-Golgi



Scheme S1 Synthesis of TP-Golgi.

Compound 1 was obtained according to the previously reported synthetic scheme. [1]

Synthesis of Compound 2. In the presence of nitrogen, 4-formylbenzoic acid (0.18 g, 1.2 mmol) and compound 1 (0.24 g, 1.0 mmol) were dissolved in absolute ethanol (10 mL), then piperidine (10 μL) and acetic acid (10 μL) were added, the reaction mixture was refluxed at 80 °C for 6 hours. Removed the solvent with a rotary evaporator, and then CH_2Cl_2/CH_3OH (v/v, 10:1) was used as eluent for silica gel column chromatography purification. The compound 2 was obtained (0.56 g, 76 %).

Synthesis of Compound 3 and TP-Golgi. In the presence of nitrogen, Compound 2 (0.13 g, 0.35 mmol) was dissolved in thionyl chloride (5.2 mL, 72 mmol), and then add a drop of DMF, slowly warmed to 70 °C and stirred for 2 hours. After cooling to room temperature, the solution was removed under reduced pressure to gain compound 3. Compound 3 was redissolved with acetonitrile (5 ml), then triethylamine (300 μ L, 2.2 mmol) and sulfonamide (0.24 g, 1.4 mmol) were added. Then the mixture was then reacted at room temperature for 12 h. The solvent was removed by a rotary evaporator. The R_f value of TP-Golgi was 0.44 in TLC analysis (CH_2Cl_2/CH_3OH, 10:1), and the TP-Golgi was purified by silica gel chromatography (CH_2Cl_2/CH_3OH, 20:1). The probe TP-Golgi was obtained (55 mg, 30 %).^{1}H NMR (400 MHz, DMSO) δ = 11.01 (s, 1H), 8.63 (d, J=16.4, 1H), 8.47 (d, J=8.4, 2H), 8.34 (d, J=9.0, 1H), 8.25 (d, J=8.3, 3H), 8.20 (d, J=9.0, 1H), 8.07 (d, J=8.7, 2H), 7.92 (d, J=16.5, 1H), 7.84 (d, J=8.8, 2H), 7.79 – 7.74 (m, 1H), 7.36 (s, 2H), 4.95 (dd, J=13.9, 6.8, 2H), 2.07 (s, 6H), 1.56 (t, J=7.2, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 182.58, 165.47, 151.47, 142.48, 139.72, 139.46, 138.56, 137.93, 137.88, 133.88, 131.71, 130.79, 130.55, 129.10, 129.02, 128.00, 127.18, 126.95, 123.76, 120.63, 114.38, 113.86, 54.63, 49.04, 43.43, 29.48, 29.14, 25.71, 22.54, 14.63, 14.41. HRMS (ESI) m/z: [M^+] calculated for $C_{31}H_{30}N_3O_3S^+$, 524.2008, found 524.2046.

3. Spectroscopic analysis

The probe TP-Golgi was dissolved in DMSO. 10 μL TP-Golgi (1.0 mM) was added to 990 μL of different polar solvents or different proportions of water–dioxane mixture to prepare the solution to be tested. After the probe was fully mixed with the appropriate testing species, immediately recorded the fluorescence spectrum and absorption spectrum. For fluorescence spectrum, the excitation wavelength was 380 nm and the collection range were 400–700 nm. For absorption spectra, the collection range was 250–500 nm.

3.1 Computational methods

UV/Vis absorption spectra and emission spectra of TP-Golgi were calculated using density functional theory (DFT) and time-dependent

density functional theory (TD-DFT) methods at the PBE1PBE[2]/6-311G(d) level. Solvation effect was modeled by IEFPCM.[3] Water and 1,4-dioxane were employed as solvent, respectively. All calculations were performed using the Gaussian 09 program package.[4] Frontier molecular orbitals were visualized with Multiwfn.[5]

3.2 Selectivity of TP-Golgi for Polarity

The fluorescence intensity of TP-Golgi at 600nm and 440nm was measured under excitation at 380nm. After TP-Golgi (10 μM) was mixed with different species (100 μM or 10 μM) in PBS (pH 7.4), the fluorescence response of TP-Golgi to different species was detected. The species include $ZnCl_2$, $FeCl_2$, $CuCl_2$, $MgCl_2$, $CaCl_2$, KCl, $FeCl_3$, $AlCl_3$, $CdCl_2$, $LiCl_2$, $MnCl_2$, CuCl, NaCl, $ONOO^-$ (10 μM), $^1O_2$, $O_2^{\cdot-}$, H_2O_2, $ROO^\bullet$, t-BuOOH, ClO^-, Asn, Gln, Thr; Ser, GSH, Cys.

3.3 Effect of pH and viscosity on TP-Golgi

TP-Golgi (1 mM, 10 μL) was added to PBS solution with different pH values (100 mM, 990 μL, pH = 5.2, 5.8, 6.2, 6.6, 7.2), or added to methanol-glycerol system with different viscosity (990 μL, the proportion of glycerol was 10%, 20%, 30%, 40%, 50%), and the mixtures were immediately detected. Fluorescence intensity values at 400 nm and 600 nm were collected at 380 nm excitation.

3.4 Fluorescence quantum yields (Φ) measurement[6]

Rhodamine B (Φ_f = 0.97 in ethanol) was selected as a fluorescence standard for TP-Golgi. The calculation formula used for the fluorescence quantum yield of TP-Golgi:

$$\Phi_x = \Phi_s (A_s F_x/A_x F_s) (n_x/n_s)^2$$

Φ: the quantum yield, F: the integrated area, A: the absorbance at 380nm, n: the refractive index of the solvent, the subscripts x: the analyte, the subscripts s: the standard.

3.5 Two-photon absorption cross-section (δ) measurement [7]

We used the two-photon-induced fluorescence measurement technique to measure the two-photon absorption cross section (δ). Using fluorescein（45 GM in 0.1 M NaOH solution at 760 nm） as a reference [8], TP-Golgi was dissolved in different solvents, the two-photon-induced fluorescence intensity was measured at 760 nm. The two-photon absorption cross sections (δ) was calculated by two-photon excitation fluorescence spectra, according to the equation:

$$\delta_s = \delta_r (\Phi_r / \Phi_s) (C_r / C_s) (n_r / n_s) (F_s / F_r)$$

δ: the two-photon absorption cross section value, Φ: the fluorescence quantum yield, C: the concentration of solution, n: the refractive index of the solution, F: two-photon excited fluorescence integral intensity, the subscripts x: the analyte, the subscripts s: the standard.

4. Cell experiments

Human liver cells HL-7702, human cervical carcinoma cells HeLa, breast cancer cells MCF-7 and glioblastoma cells U87 cells were cultured in DMEM containing 10% fetal bovine serum (FBS), 1% penicillin and 1% streptomycin at 37 °C in 5% CO_2 and 95% air. Renal cells HEK 293 were cultured in MEM containing 10% fetal bovine serum (FBS), 1% penicillin and 1% streptomycin at 37 °C in 5% CO_2 and 95% air.

4.1 Cytotoxicity Assays

The effect of different concentrations of TP-Golgi on the cell viability of HL-7702 cells was detected by MTT assay. One day before treatment, the HL-7702 cells were implanted into 96-well plates (approximately 10^5 cells per well). After 24 h, the cells were treated with different concentrations of TP-Golgi (0.1, 1, 5, 10 and 20 μM) for another 12 h. Then, MTT solution (5 mg mL^{-1}, 10 μL) and DMEM (90 μL) were added to each well and treated for an additional 4 h. Then, removed the upper culture medium, and added DMSO (150 μL $well^{-1}$). After shaking and mixing, record the absorbance at 570 nm.

4.2 Cell imaging

The confocal fluorescence imaging experiments were performed on Leica TCS SP8 (one-photon) or Zeiss LSM 880 NLO (two-photon) Confocal Laser Scanning Microscope. TP-Golgi in live cells was excited with a 405 nm laser (one-photon) or a 760 nm laser (two-photon), and two groups of channels were collected (430–500 nm for green channel, 550–650 nm for red channel). The ratiometric imaging was obtained between red channel and green channel. One day before imaging, implanted the cells into glass-bottomed dishes. For imaging, the cells were incubated with TP-Golgi for 20 min. Then, removed the incubation solution, the cells were washed three times and then the new medium was replaced, the cells were imaged.

4.3 Co-localization experiment

HL-7702 and Hela cells were used for co-localization experiments. TP-Golgi (10 μM) and 2 μM commercial fluorescent dyes were co-incubated with cells for 20 min before imaging, and then washed three times. After the new medium was replaced, the cells were imaged.

4.4 Image of cell polarity changes

Polarity of Golgi apparatus in different cells: HL-7702 cells, HEK 293 cells, HeLa cells, MCF-7 cells and U87 cells were used. Cells were incubated with TP-Golgi (10 μM) for 20 min, and then washed at least three times before imaging.

Polarity change of Golgi apparatus in cells under stimulation: Two kinds of stimulation experiments were performed with HL-7702 cells and HEK-293 cells. (1) LPS or APAP stimulation: In the stimulation group, HEK-293 cells were pre-treatment with LPS (10 μg mL^{-1})or APAP

(10 μM) for 2 h and the incubation solution was removed. The cells were subsequently incubated with TP-Golgi (10 μM) for 20 min, washed with PBS three times, and then added to the new medium for imaging. In the control group, HEK-293 cells were pre-incubated in MEM. (2) Monensin stimulation: HL-7702 cells were pre-incubated with monensin (10 μM, in DMEM) for 7 h and the incubation solution was removed. The cells were subsequently incubated with TP-Golgi (10 μM) for 20 min, washed with PBS three times, and then added to the new medium for imaging. In the control group, HL-7702 cells were pre-incubated in DMEM without monensin.

4.5 Flow Cytometry

Before flow cytometry, HL-7702 cells were pre-treatment with monensin (10 μM, in DMEM) for 7 h and the incubation solution was removed. Then the cells were treated with 500 μL of 0.05 % trypsin for 2 min to obtain cell suspension. The Imagestreamx Mark II flow cytometer (Merck Millipore, Seattle, WA) was used to collect fluorescence signals (435-505 nm and 595-642 nm) of cells (more than 5000 cells) under the excitation of 405 nm.

5. Mice experiment

All KM mice (female, 18–20 g) were obtained from the Laboratory Animal Center of Shandong University. All the animal experiments were conducted in accordance with the relevant laws and guidelines issued by the Ethical Committee of Shandong University and were in agreement with the guidelines of the Institutional Animal Care and Use Committee.

5.1 Acute kidney injury model mice and Bleomycin treatment mice.

Acute kidney injury model mice: The mice were randomly divided into the acute kidney injury group and the control group. Mice in the acute kidney injury group (AKI-mice) were constructed by intraperitoneal injection of LPS (10 mg kg^{-1}, normal saline solution) or APAP (250 mg kg^{-1}, normal saline solution), while mice in the control group (blank mice) were intraperitoneally injected with normal saline (5 ml kg^{-1}). After 24 hours, mice were randomly selected and euthanized with chloral hydrate. The kidney tissues of AKI mice and blank mice were collected, and H&E staining of the kidney tissues were performed to ensure the occurrence of kidney injury in AKI mice.

Bleomycin treatment mice: The mice were randomly divided into the Bleomycin treatment group and the control group. The mice of Bleomycin treatment group (b-stimulated mice) were constructed by injecting 100 μL bleomycin (37.5 mg mL^{-1}, in normal saline) into tail vein, and the control group mice were injected with saline (control mice). After 28 days, three b-stimulated mice and three control mice were randomly selected and euthanized with chloral hydrate. The kidney tissues of mice were collected, and H&E staining of the kidney tissues were used to analyze the pathological changes of the kidney in Bleomycin mice.

5.2 Two-photon fluorescence imaging in living tissues

After TP-Golgi (50 μM, 100 μL) was injected into mice through tail vein for 30 min, two-photon fluorescence imaging of the mouse kidneys was performed. The Zeiss LSM 880 NLO confocal laser scanning microscope were used for two-photon fluorescence imaging. TP-Golgi in the kidneys of mice was excited with a 760 nm laser, and two groups of channels were collected (Green Chanel: 430–500 nm, Red Chanel: 550–650 nm).

6. Supplementary Figures

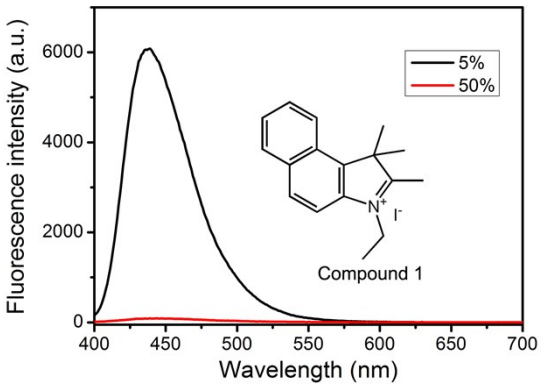


Fig. S1 Fluorescence spectra of compound 1 (10 μM) in water–dioxane mixtures (water volume fractions of 5% and 50%). λ_{ex} = 380 nm.

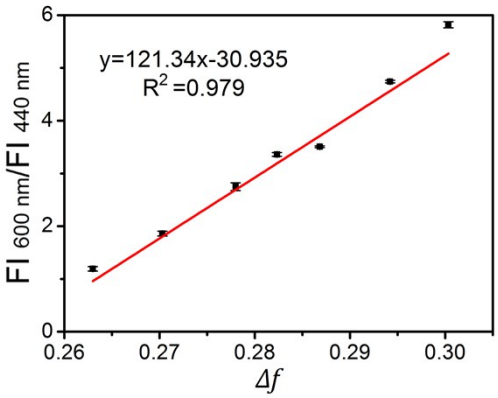


Fig. S2 The linear relationship between the fluorescence intensity ratio of TP-Golgi (10 μM) and Δf (0.26–0.30). (n = 3).

Table S1. Solvent parameters of water–dioxane mixed solvents and photophysical data of TP-Golgi in mixed solvents.

H_2O Percent (Volume Fraction)	Dioxane Percent (Volume Fraction)	ε_{mix} [a]	n^2_{mix} [b]	Δf [c]	λ_{Abs}/nm	λ_{em}/nm	Stokes Shift/nm	Φ_f [d]
5	95	6.017	2.01	0.18	378	438, 573	60, 195	0.10, 0.03
10	90	9.824	2.00	0.23	376	439, 583	63, 207	0.08, 0.05
15	85	13.63	1.98	0.25	376	441, 592	65, 216	0.06, 0.07
20	80	17.44	1.97	0.26	376	442, 599	66, 223	0.06, 0.07
25	75	21.25	1.96	0.27	374	442, 600	68, 226	0.02, 0.09
30	70	25.05	1.95	0.28	373	442, 602	69, 229	0.01, 0.10
35	65	28.86	1.94	0.28	371	441, 602	70, 231	0.01, 0.07
40	60	32.67	1.92	0.29	370	441, 602	71, 232	0.01, 0.13
50	50	40.28	1.90	0.29	370	443, 604	73, 234	0.01, 0.17
60	40	47.89	1.88	0.30	371	443, 605	72, 234	<0.01, 0.15

70	30	55.51	1.85	0.31	372	444, 605	72, 233	<0.01, 0.13
80	20	63.13	1.83	0.31	372	444, 604	72, 232	<0.01, 0.12

[a] The dielectric constant of solvents. [b] The refractive indices of solvents. [c] The Lippert–Mataga polarity parameter. [d] Fluorescence quantum yields of TP-Golgi.

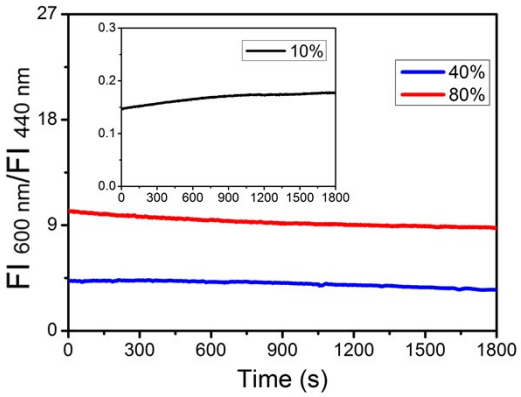


Fig. S3 Photostability experiments of TP-Golgi (10 μM) under different polarity condition.

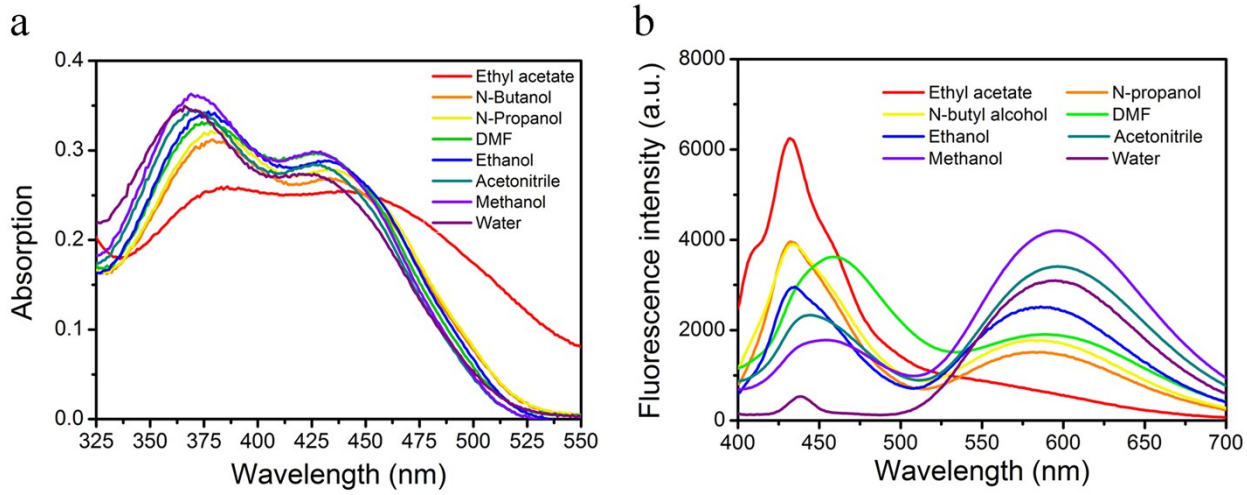


Fig. S4 Absorption and fluorescence spectra of TP-Golgi (10 μM) in different solvents. (a) Absorption spectrum. (b) Fluorescence spectrum of TP-Golgi in different solvents with 380 nm excited.

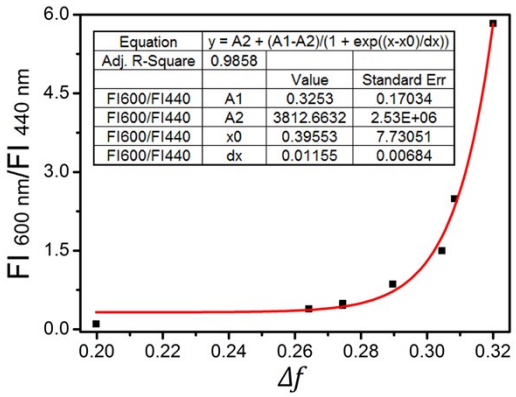


Fig. S5 Fitting curve of the fluorescence intensity of TP-Golgi and *Δf* in different solvents.

Table S2 Photophysical data of TP-Golgi in various solvents.

Solvents	ε[a]	n² [b]	Δf[c]	λ_{Abs}/nm	λ_{em}/nm	Stokes Shift/nm	Φ_f[d]	
Dioxane	2.21	2.02	0.02	382	446 None	64, None	0.12	None
Ethyl acetate	6.02	1.88	0.20	386	432, 577	46, 191	0.08	0.06
n-Propanol	17.80	1.92	0.26	384	432, 580	48, 196	0.01	0.09
DMF	37.22	2.05	0.28	380	459, 590	79, 210	0.02	0.09
Ethanol	24.85	1.85	0.29	380	435, 586	55, 206	0.01	0.12
Acetonitrile	37.50	1.81	0.30	369	444, 597	75, 228	<0.01	0.10
Methanol	33.61	1.77	0.31	369	454, 596	85, 227	<0.01	0.10
Water	80.40	1.78	0.32	366	438, 595	72, 229	<0.01	0.09

[a] The dielectric constant of solvents. [b] The refractive indices of solvents. [c] The Lippert–Mataga polarity parameter. [d] Fluorescence quantum yields of TP-Golgi.

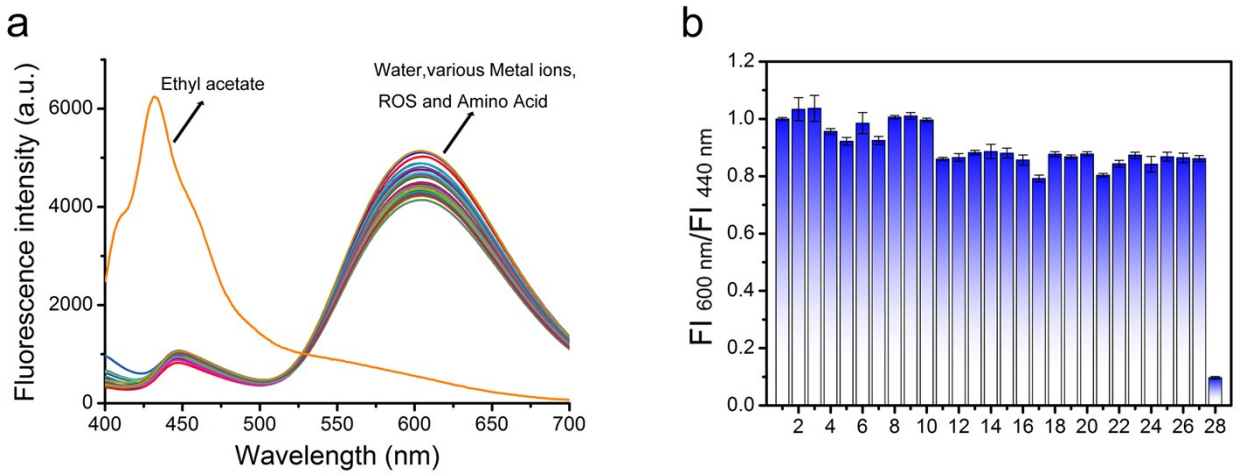


Fig. S6 Fluorescence spectrum (a) and fluorescence intensity ratio $FI_{600\ nm}$ / $FI_{440\ nm}$ (b) of TP-Golgi (10 μM) toward various analytes (100 μM unless otherwise stated): 1, H_2O; 2, GSH; 3, Cys; 4, Asn; 5, Gln; 6, Thr; 7, Ser; 8, t-BuOOH; 9, ClO^-; 10, O^{2-}; 11, $^1O_2$; 12, H_2O_2; 13, $ROO\cdot$; 14, $ONOO^-$(10 μM); 15, Zn^{2+}; 16, Fe^{2+}; 17, Cu^{2+}; 18, Mg^{2+}; 19, Ca^{2+}; 20, K^+; 21, Fe^{3+}; 22, Al^{3+}; 23, Cd^{2+}; 24, Li^{2+}; 25, Mn^{2+}; 26, Cu^+; 27, Na^+; 28, ethyl acetate. (n = 3).

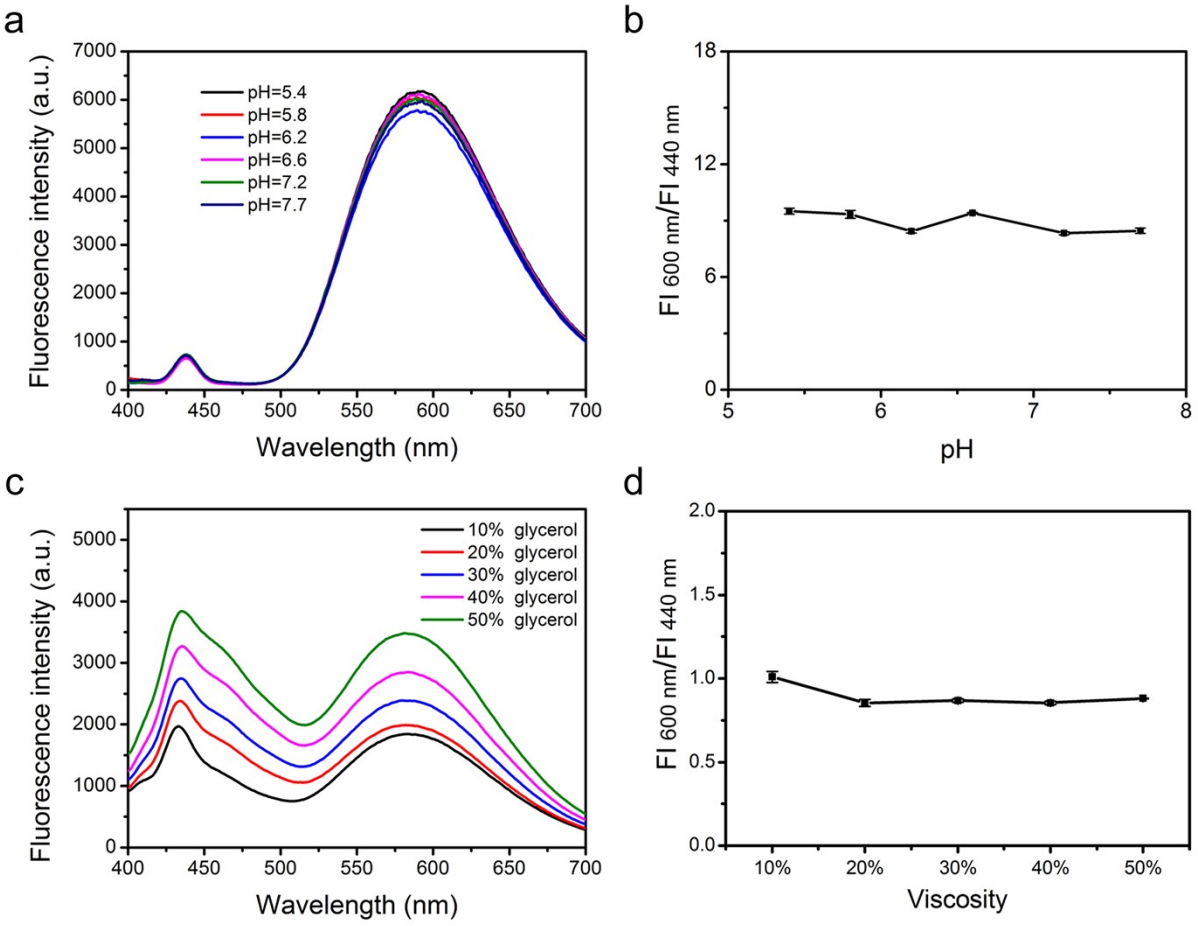


Fig. S7 (a–b) Fluorescence spectrum and fluorescence intensity ratio $FI_{600\ nm}$ / $FI_{440\ nm}$ of TP-Golgi (10 μM) in PBS with different pH values. (c–d) Fluorescence spectrum and fluorescence intensity ratio $FI_{600\ nm}$ / $FI_{440\ nm}$ of TP-Golgi (10 μM) in methanol-glycerol system with different viscosity (n = 3).

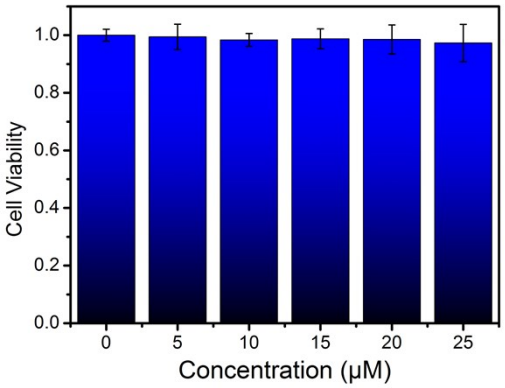


Fig. S8 Cell viability of cells after incubation with various concentrations of TP-Golgi (0, 5, 10, 15, 20, 25 μM).

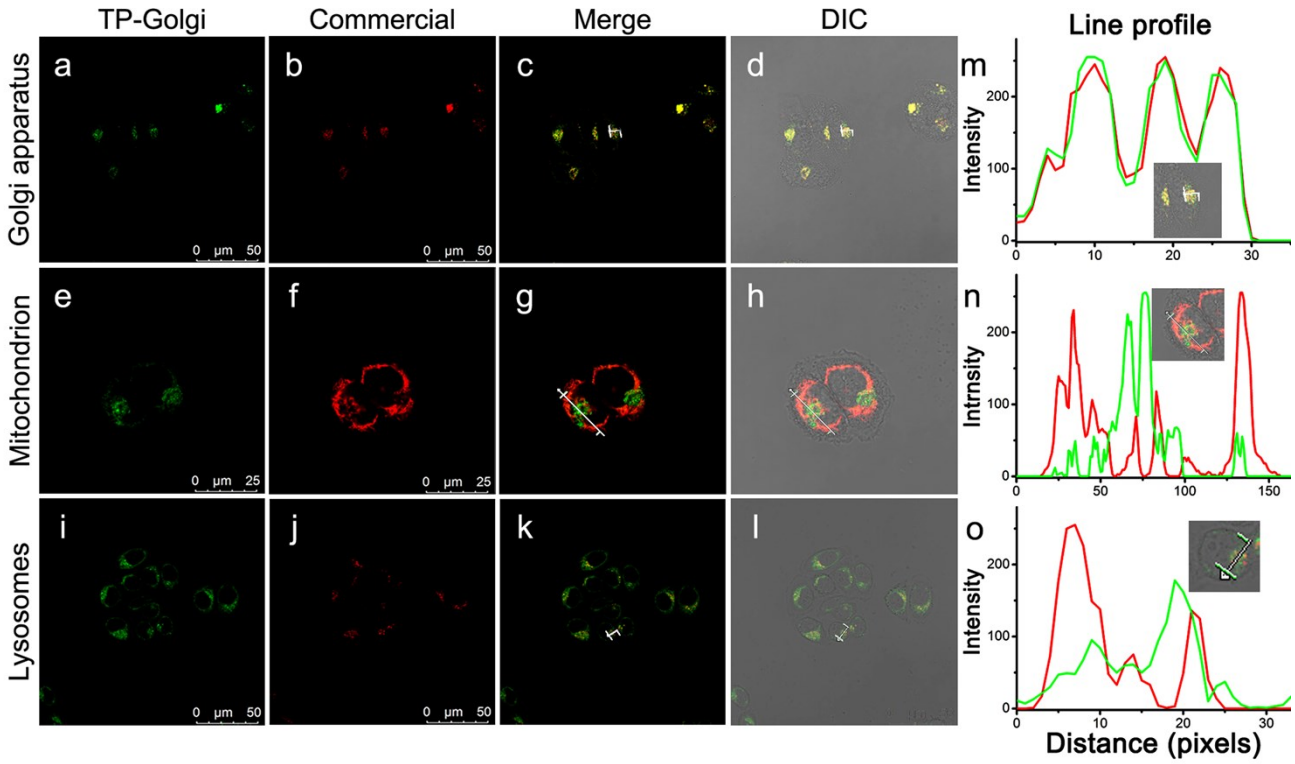


Fig. S9 Golgi-targeting properties of TP-Golgi in HL-7702 cells. (a–l) Confocal fluorescence images of TP-Golgi (10 μM, λ_{ex} = 405 nm, λ_{em} = 550–650 nm) and commercial dyes in cells, including (b–d) Golgi-Tracker Red (0.5 μM, λ_{ex} = 561 nm, λ_{em} = 610–630 nm), (f–h) Mito-Tracker Red (0.5 μM, λ_{ex} = 633 nm, λ_{em} = 650–680 nm), (j–l) Lyso-Tracker Red (0.5 μM, λ_{ex} = 633 nm, λ_{em} = 650–680 nm), respectively. (m–o) The fluorescence intensity profiles at the white line mark in (c), (g) and (k).

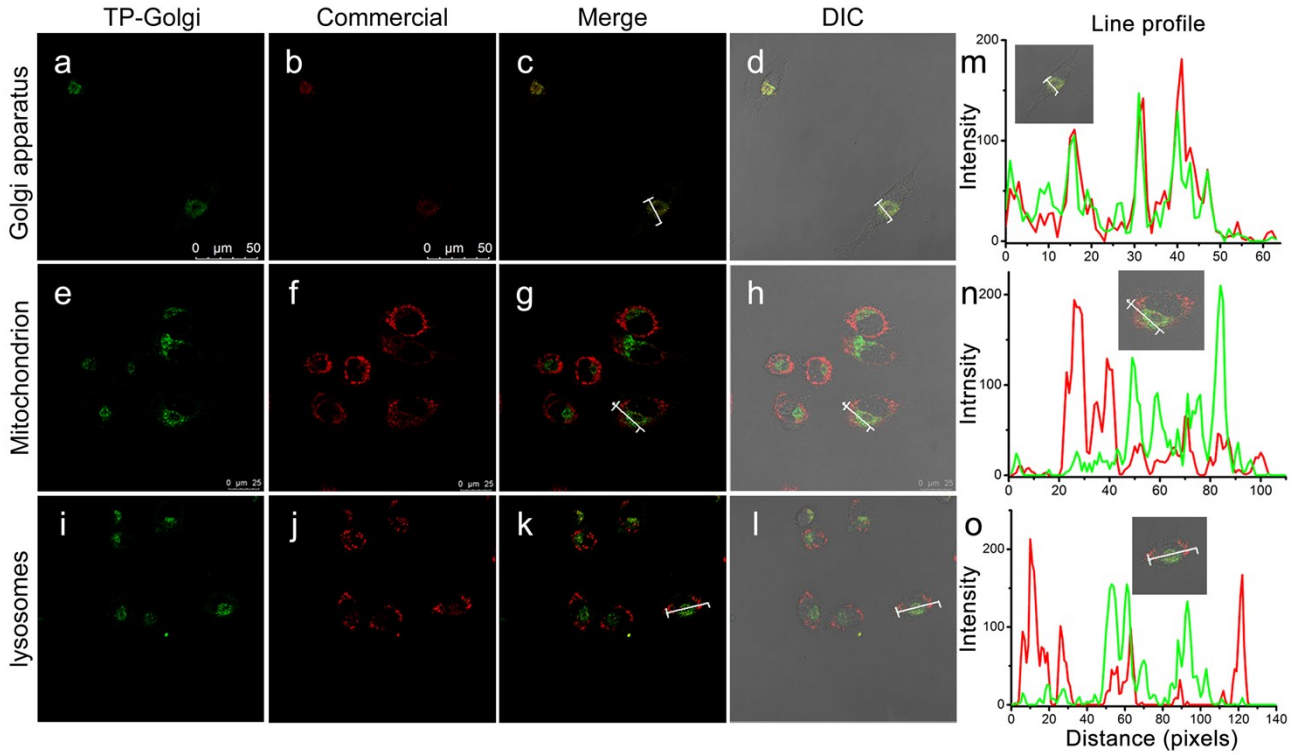


Fig. S10. Golgi-targeting properties of TP-Golgi in HeLa cells. (a–l) Confocal fluorescence images of TP-Golgi (10 μM, λ_{ex} = 405 nm, λ_{em} = 550–650 nm) and commercial dyes in cells, including (b–d) Golgi-Tracker Red (0.5 μM, λ_{ex} = 561 nm, λ_{em} = 610–630 nm), (f–h) Mito-Tracker Red (0.5 μM, λ_{ex} = 633 nm, λ_{em} = 650–680 nm), (j–l) Lyso-Tracker Red (0.5 μM, λ_{ex} = 633 nm, λ_{em} = 650–680 nm), respectively. (m–o) The

fluorescence intensity profile at the white line mark in (c), (g) and (k).

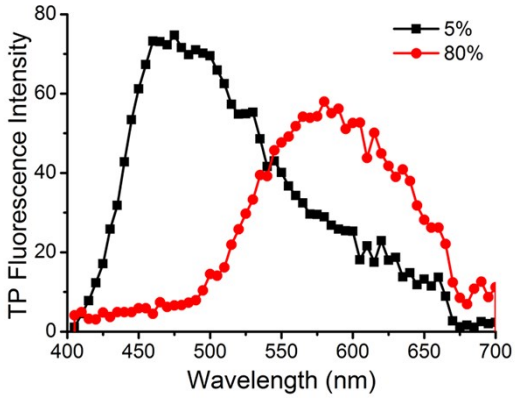


Fig. S11. TP fluorescence emission spectra of TP-Golgi in different water–dioxane mixtures (water volume fractions of 5% and 80%). TP excited: 760 nm.

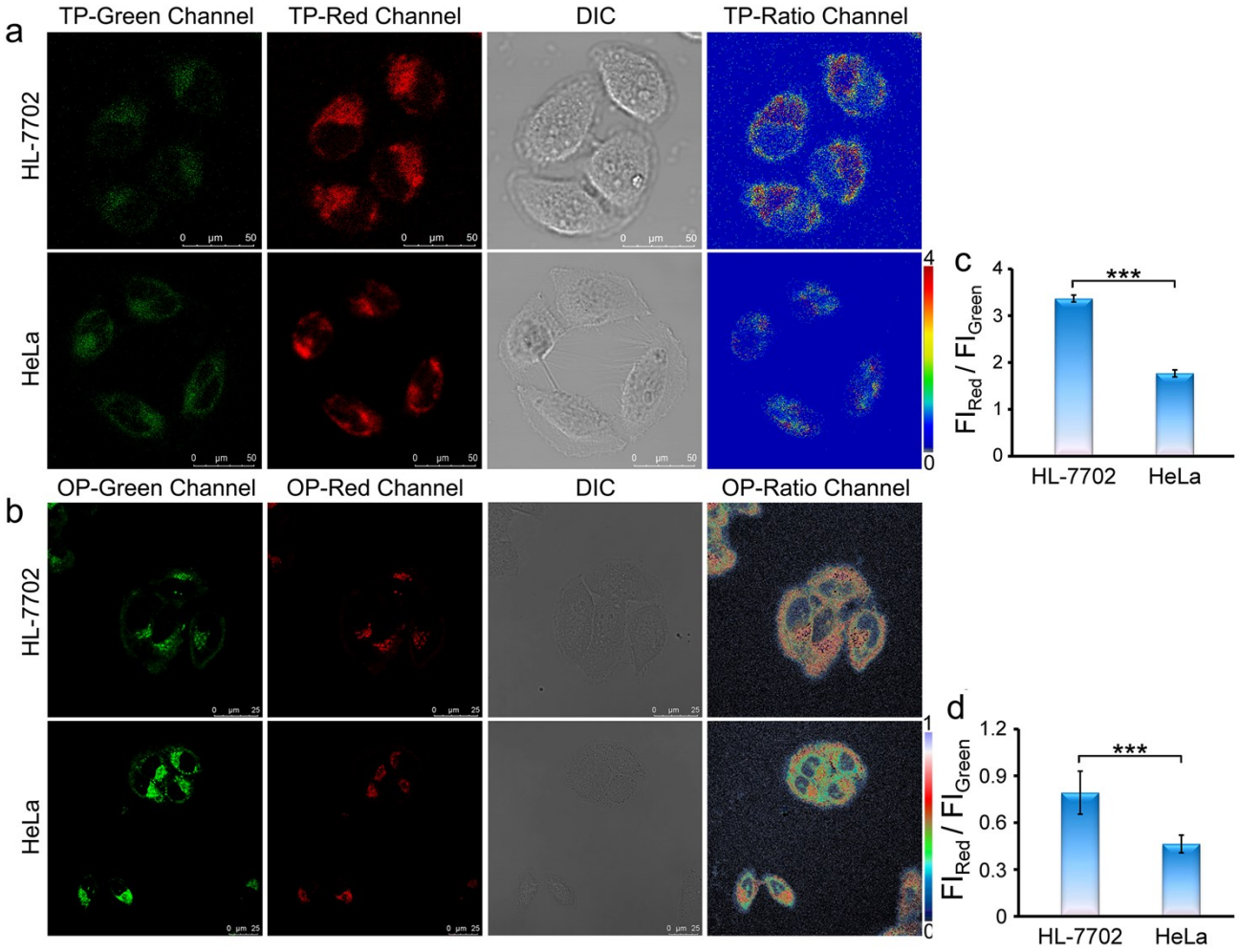


Fig. S12. Fluorescence imaging of TP-Golgi (10 μM) in HL-7702 and HeLa cells. (a) Two-photon fluorescence imaging of HL-7702 and HeLa. TP λ_{ex} = 760 nm. (b) One-photon fluorescence imaging of HL-7702 and HeLa. OP λ_{ex} = 405 nm. Ratio channel was Red channel (λ_{em} = 550–650 nm) / Green channel (λ_{em} = 430–500 nm). (c) The TP fluorescence intensity ratio (FI_{Red} / FI_{Green}) in different cells. (d) The OP fluorescence intensity ratio (FI_{Red} / FI_{Green}) in different cells. The values are the mean ± S.D. for n = 3, ***P < 0.001.

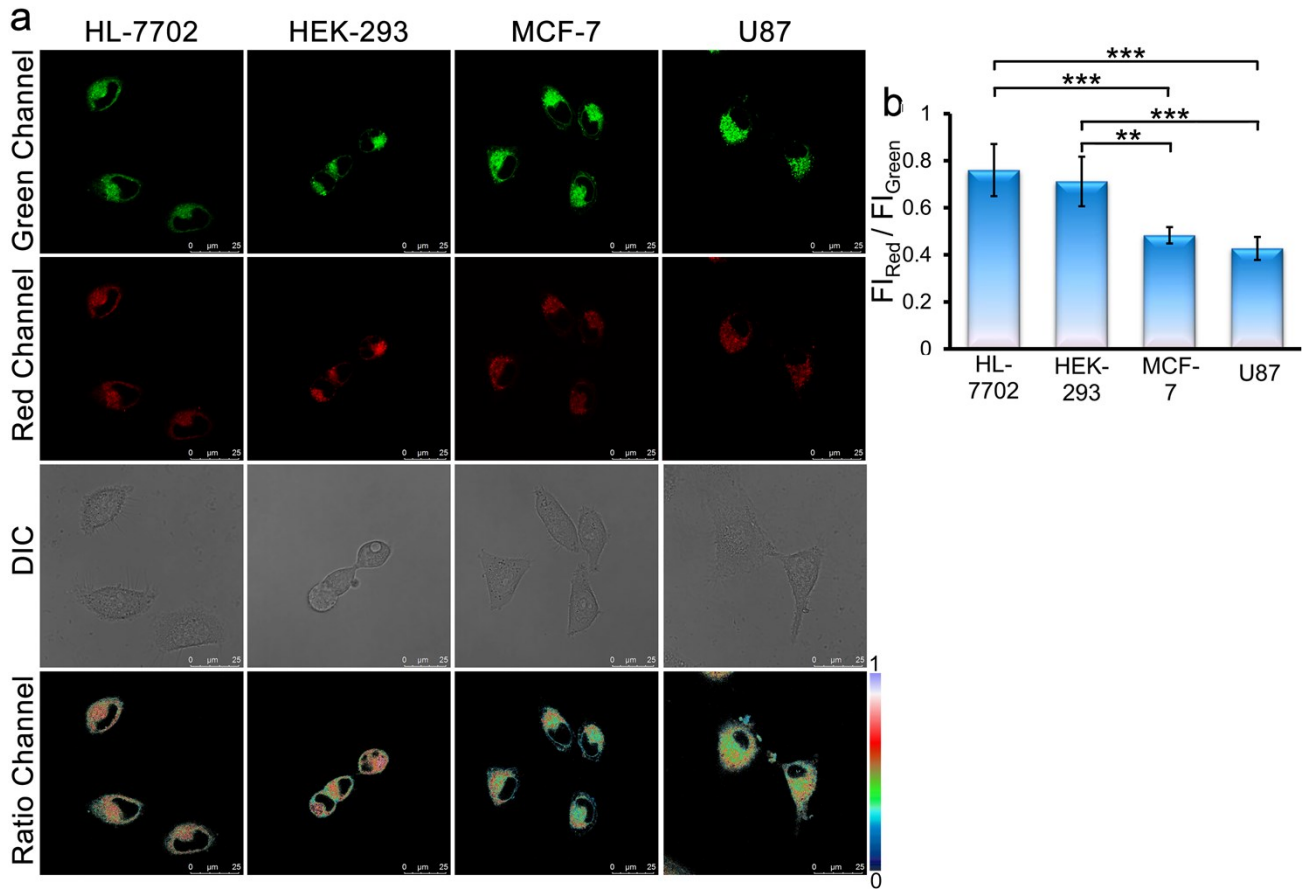
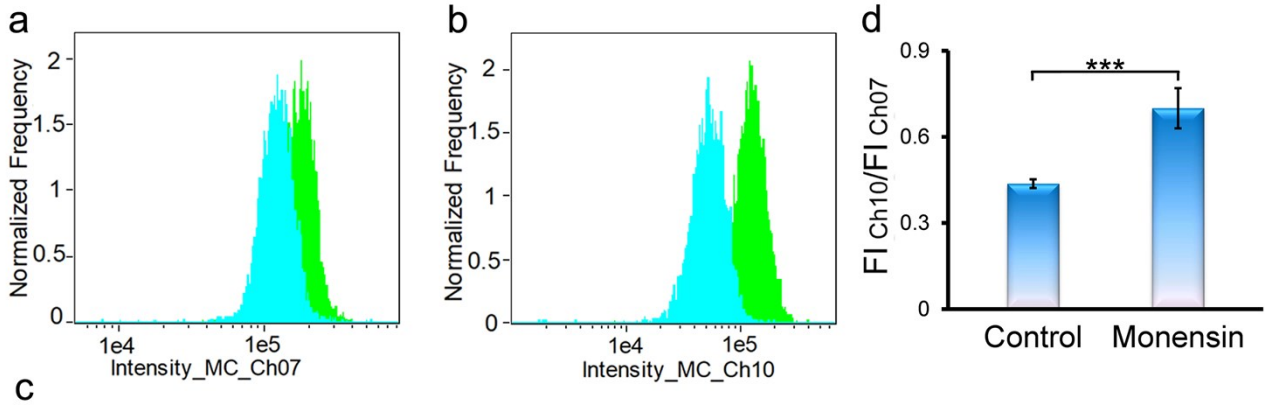


Fig. S13. Fluorescence imaging of TP-Golgi (10 μM) in HL-7702, HEK-293, MCF-7 and U87 cells. (a) Fluorescence imaging of cells. λ_{ex} = 405 nm. Ratio channel was Red channel (λ_{em} = 550–650 nm) / Green channel (λ_{em} = 430–500 nm). (b) The fluorescence intensity ratio (FI_{Red} / FI_{Green}) in different cells. The values are the mean ± S.D. for n = 3, $^{**}P < 0.01$, $^{***}P < 0.001$.

HL-7702 cells were pretreated with monensin (10 μM in DMEM) or DMEM (control cells) for 12 h, and then incubated with TP-Golgi (10 μM, 30 min) before confocal imaging and fluorescence imaging by imaging flow cytometry.



c

Population	Count	%Gated	Intensity-Ch07	Intensity-Ch10
Control group	5585	100	125023.57	55182.78
Monensin stimulated	4981	100	174071.88	126312.56

Fig. S14. Fluorescence images of TP-Golgi (10 nM) in HL-7702 cells by imaging flow cytometry under Golgi oxidative stress. (a, b) Flow cytometry analysis: the fluorescence intensities of TP-Golgi in (a) channel 07 (435 nm–505 nm) and (b) channel 10 (595 nm–642 nm) in control cells (blue) and monensin-stimulated cells (green). (c) Data output of (a) and (b). (d) The fluorescence intensity ratio of TP-Golgi in (a) and (b). λ_{ex} = 405 nm. The values are the mean ± S.D. for n = 3, $^{***}P < 0.001$.

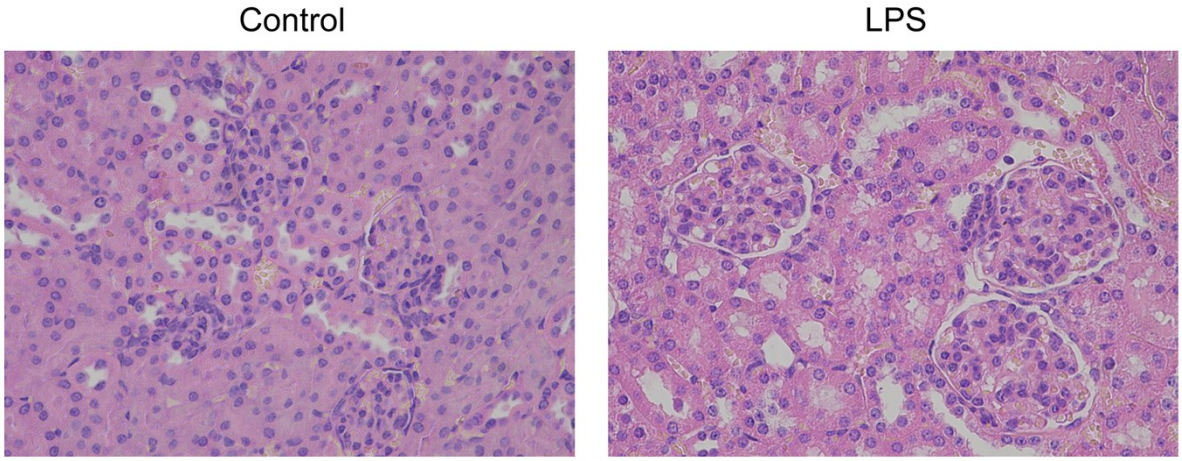


Fig. S15. The H&E staining analysis of the kidneys in control mice and LPS-induced mice.

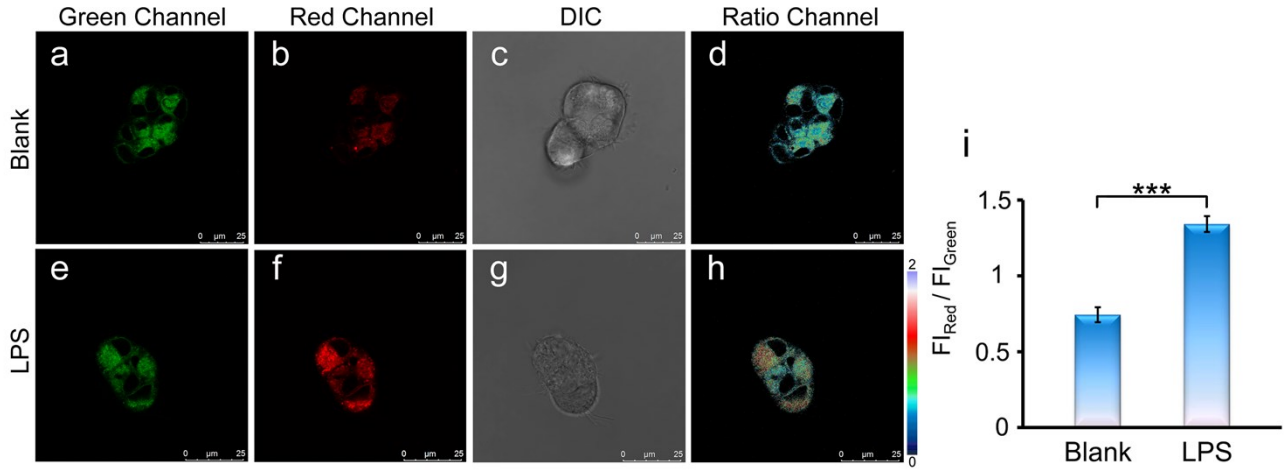


Fig. S16. Confocal ratiometric fluorescence imaging of TP-Golgi in HEK-293 cells after treatment with or without LPS (10 μM). (a–d) Blank group: HEK-293 cells. (e–h) LPS group: LPS-induced HEK-293 cells. (i) The fluorescence intensity ratio (FI_{Red} / FI_{Green}) in different cells (n = 3). λ_{ex} = 405 nm. The values are the mean ± S.D. for n = 3, ***P < 0.001.

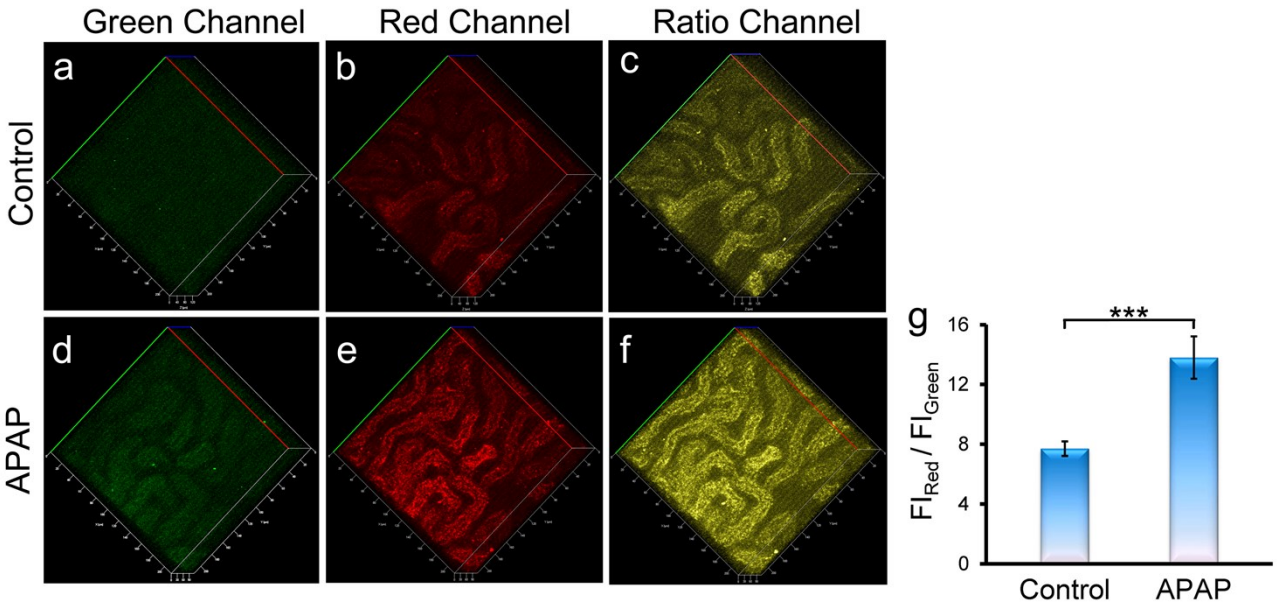


Fig. S17 TP confocal fluorescence imaging was performed on kidney tissues of control mice and APAP-induced mice with TP-Golgi (50 μM, 100 μL). (a–c) 3D images of kidney tissue in control mice. (d–f) 3D images of kidney tissue in APAP-induced mice. TP excited: 760 nm. (g) The fluorescence intensity ratio (FI_{Red} / FI_{Green}) in mice. The values are the mean ± S.D. for n = 3, ***P < 0.001.

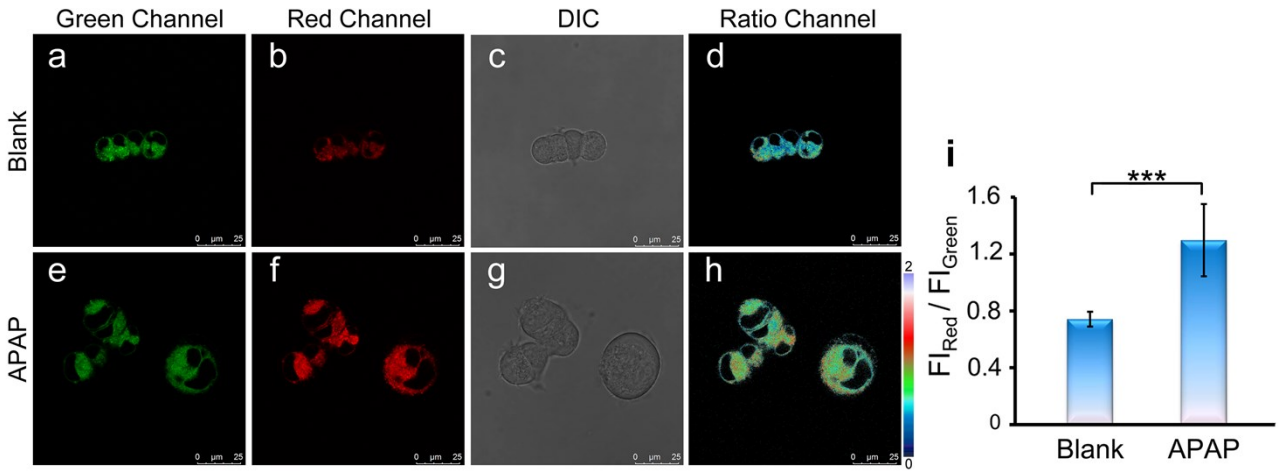


Fig. S18 Confocal ratiometric fluorescence imaging of TP-Golgi in HEK-293 cells with or without APAP (10 μM). (a–d) Blank group: normal HEK-293 cells. (e–h) APAP-induced HEK-293 cells. (i) The fluorescence intensity ratio (FI_{Red} / FI_{Green}) in normal and APAP-induced HEK-293 cells (n = 3). λ_{ex} = 405 nm. The values are the mean ± S.D. for n = 3, ***P < 0.001.

In order to further study the optical response of TP-Golgi to polarity, the electron distribution of the probe in water (high polarity) and dioxane (low polarity) was calculated by time-dependent density functional theory (TD-DFT). The results of TD-DFT showed that the TP-Golgi had two absorption peaks at 398 nm ($S_0 \rightarrow S_2$, oscillator strength, f = 0.6564) and 496 nm ($S_0 \rightarrow S_1$, f = 0.8581) in water, and at 401 nm ($S_0 \rightarrow S_3$, f = 0.6589) and 520 nm ($S_0 \rightarrow S_1$, f = 0.6709) in dioxane, respectively (Fig. S6a). In addition, the calculated emission wavelengths of the TP-Golgi in water and dioxane were 630 nm ($S_1 \rightarrow S_0$, f = 0.9338) and 462 nm ($S_1 \rightarrow S_0$, f = 0.6329), respectively (Fig. S6b). All these data corroborated the experimental results.

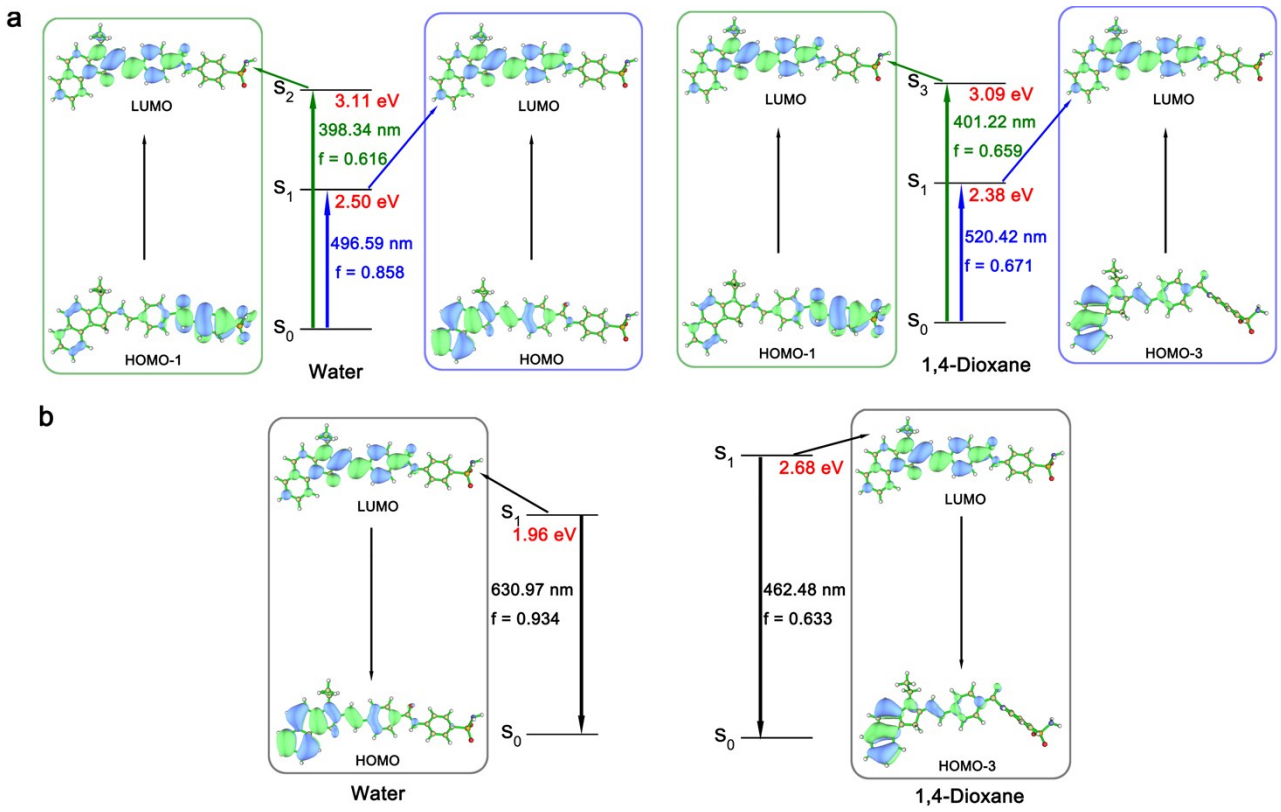


Fig. S19 Electron distribution of the probe in water and 1,4-dioxane and the electron transition corresponding to the absorption (a) and emission (b) peaks.

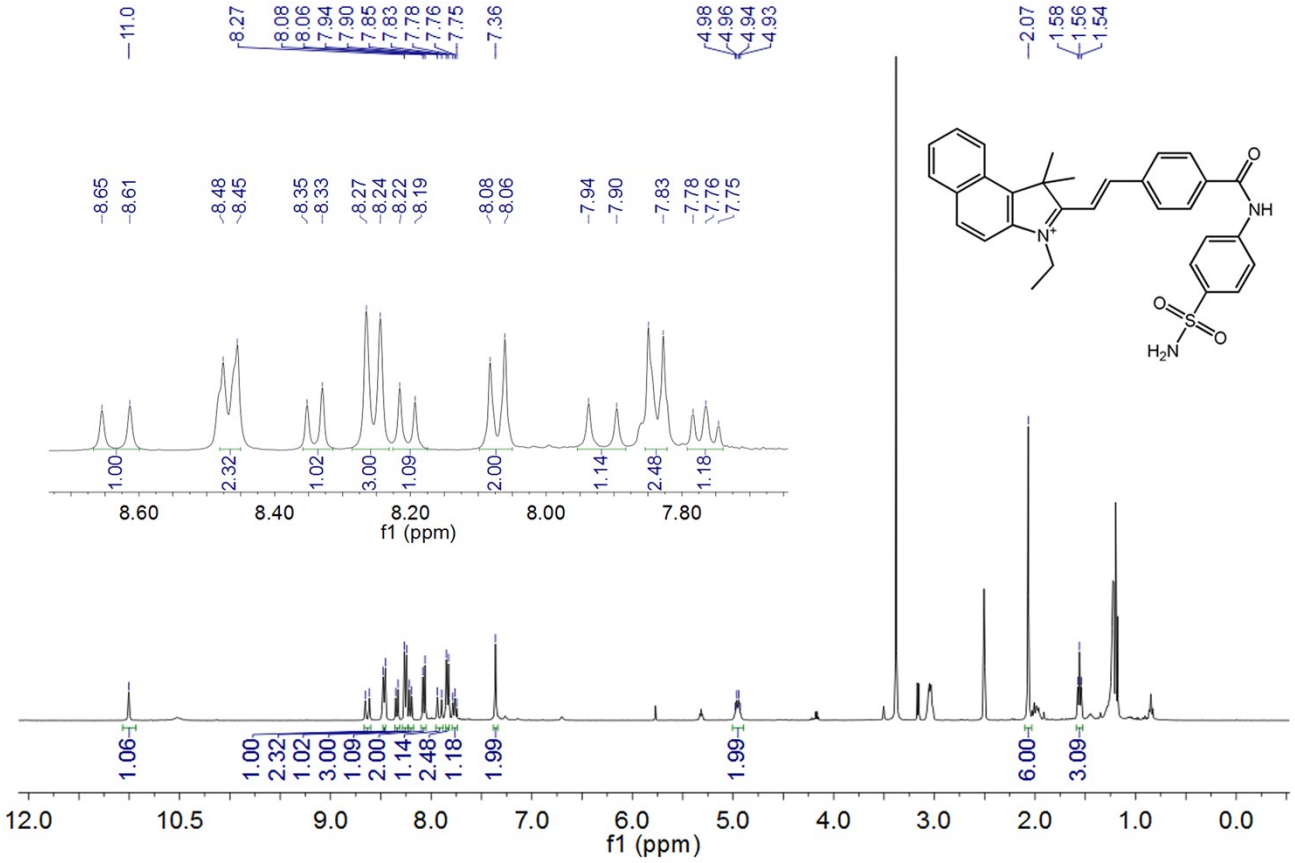


Fig. S20 ^{1}H NMR spectrum of TP-Golgi.

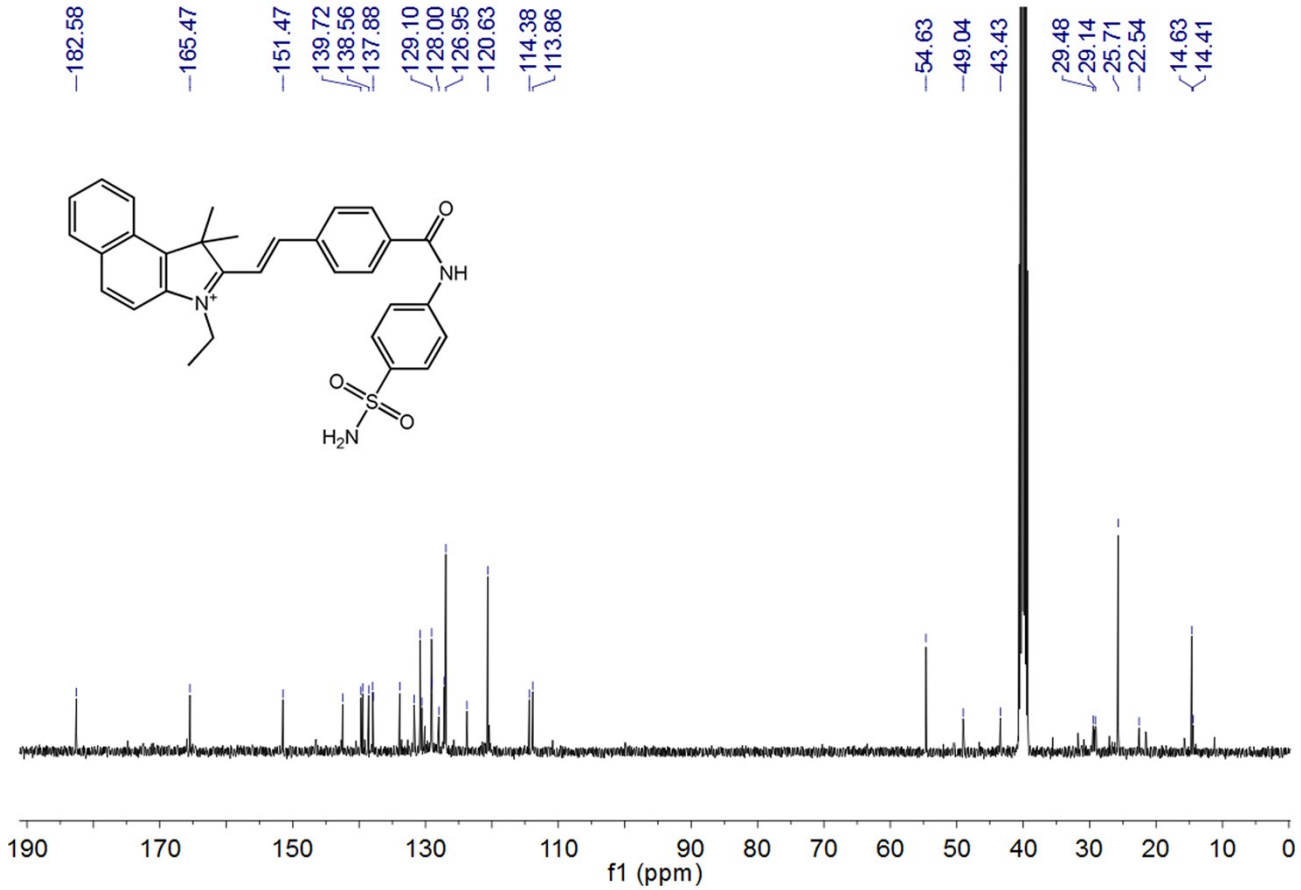


Fig. S21 ^{13}C NMR spectrum of TP-Golgi.

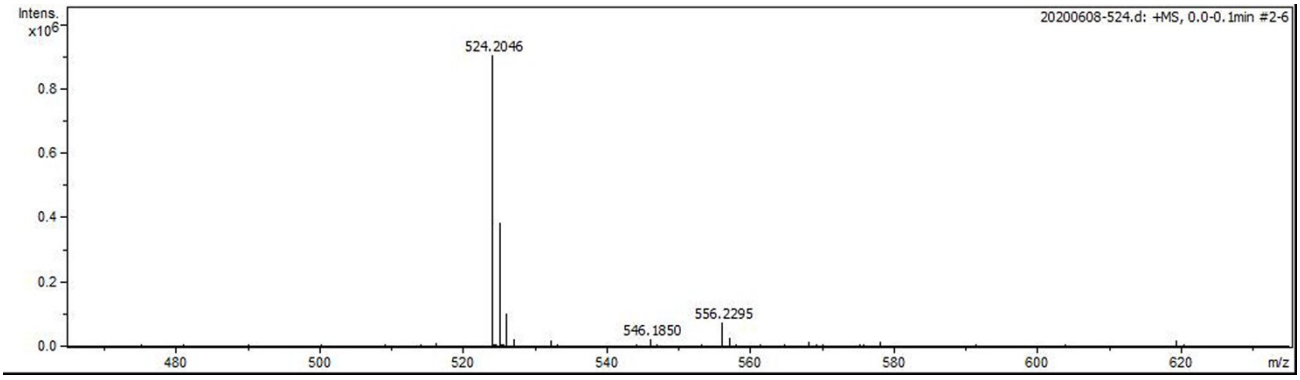


Fig. S22 Mass spectrum of TP-Golgi.

7. References

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