Supporting information

Multidrug-resistant P-glycoprotein assembly revealed by Tariquidar-probe's super-resolution imaging

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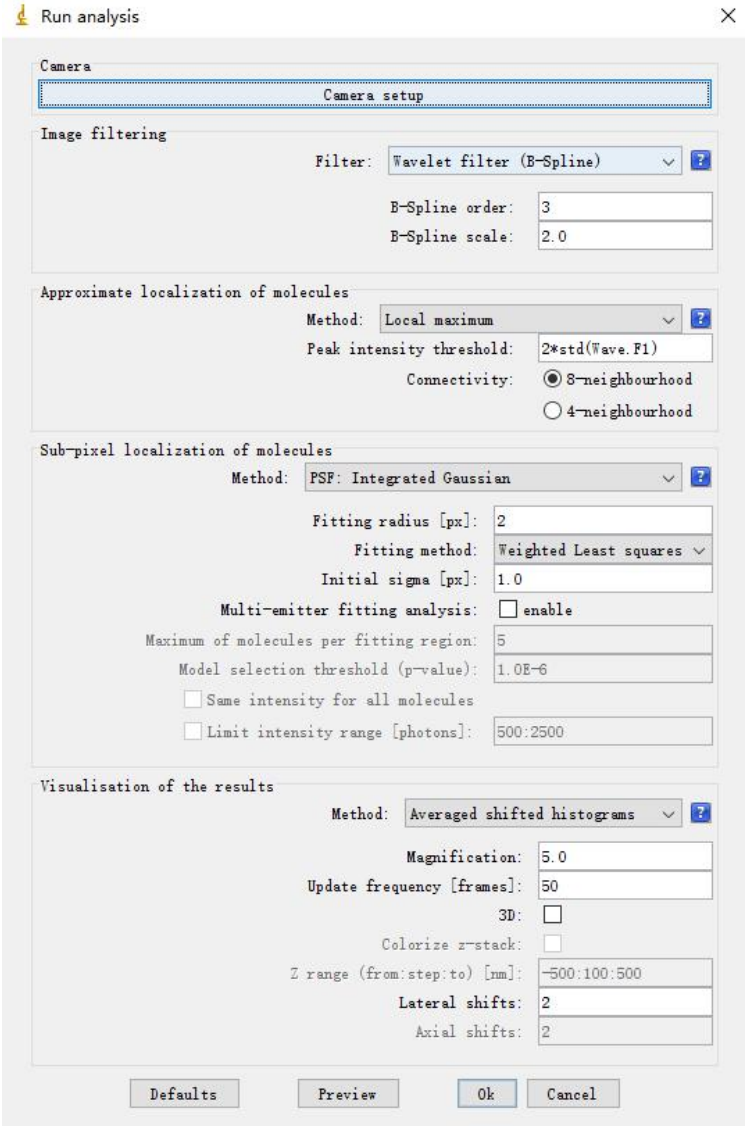


Figure S1. Parameter setting of data analysis in ThunderSTORM. It includes a wavelet B-Spline filter for feature enhancement, local maximum detection to find approximate position of single molecule and a 2D Gaussian function in integrated form using weighted least-squares method.

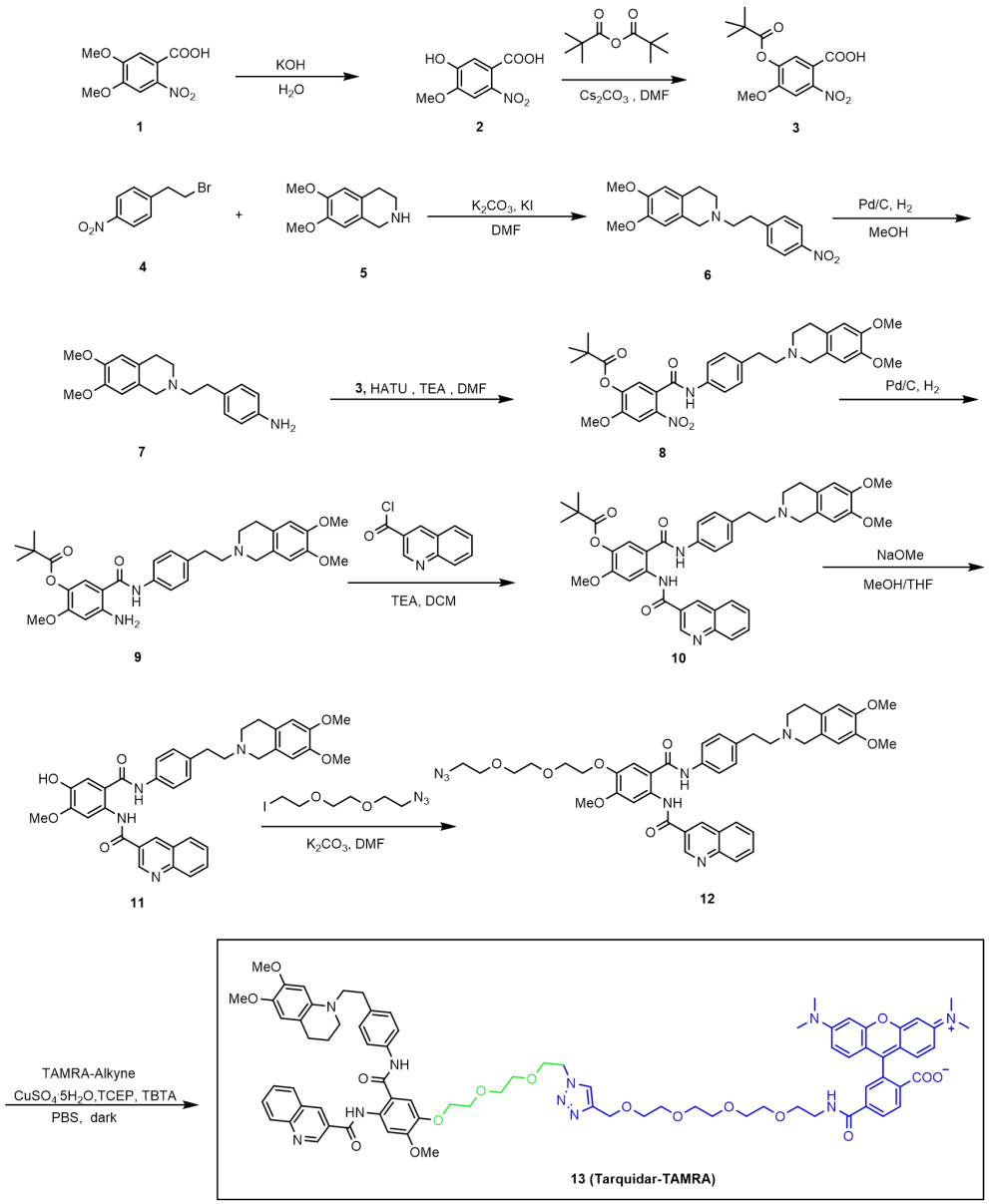


Figure S2. Synthesis of Tariquidar-TAMRA.

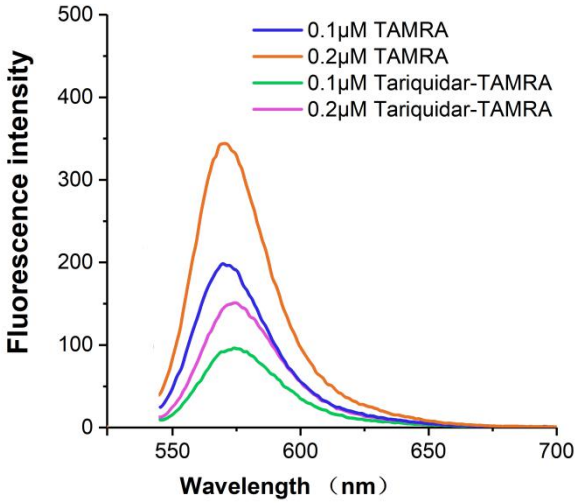


Figure S3. The fluorescence spectra of free TAMRA and Tariquidar-TAMRA.

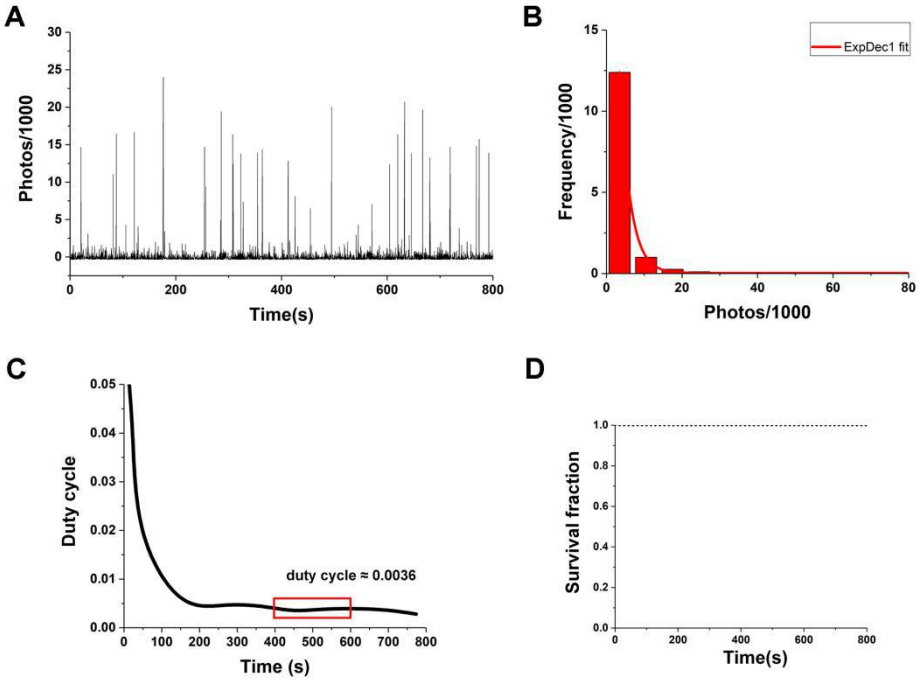


Figure S4. Characterization of switching properties of Tariquidar-TAMRA. (A) Single-molecule fluorescence time traces measured in the presence of βME and an oxygen-scavenging system (glucose oxidase with catalase (GLOX)) for Tariquidar-TAMRA. (B) The histogram of number of detected photons for each switching event, the data was acquired from many events from many molecules, with showing the mean value derived from the single exponential fit of the distribution (red curves). (C) The on-off duty cycle value was plotted versus time, with showing the average duty cycle between 400-600 s (red box). (D) The fraction of molecules that survived photobleaching.

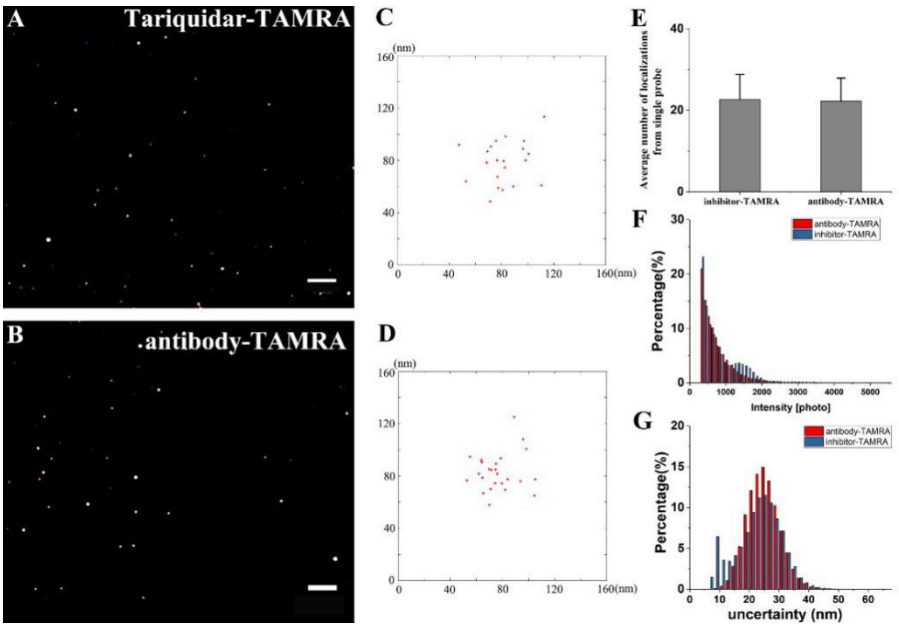


Figure S5. The single molecule information of Tariquidar-TAMRA and antiP-gp-TAMRA. (A and B) The dSTORM images of single Tariquidar-TAMRA (A) and single antiP-gp-TAMRA (B) distribution on glass slides. Scale bars are 1 μm. (C and D) The corresponding representative images of repeat localizations of single probe. (E) The histogram of average number of localizations from single probe, statistical data came from 200 single probes of three independent experiments, with showing average ± SE. (F and G) The compared distributions of intensity and uncertainty of Tariquidar-TAMRA (in blue color) and antiP-gp-TAMRA (in red color), statistical data came from 200,000-300,000 localizations of three independent experiments.

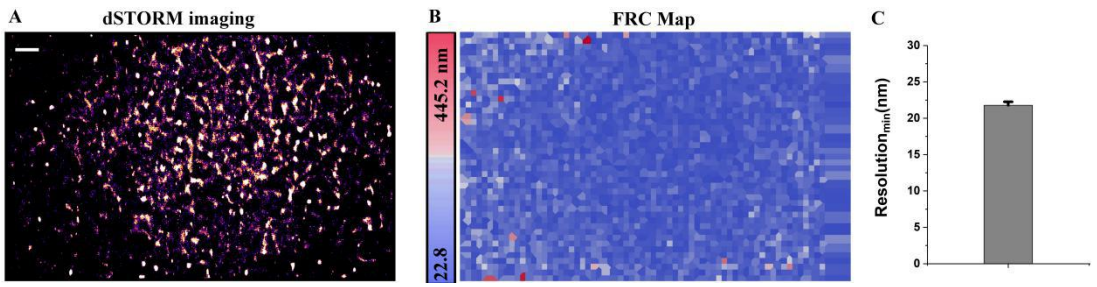


Figure S6. The minimum resolution of Tariquidar-TAMRA labeling. (A) The reconstructed dSTORM images of P-gp on MCF10A cell membranes by Tariquidar-TAMRA. (B) The FRC maps of Tariquidar-TAMRA labeling. (C) The histogram of the average minimum resolution of Tariquidar-TAMRA labeling. All statistics resulted from 10 cells in three independent experiments. Scale bar 2 μm.

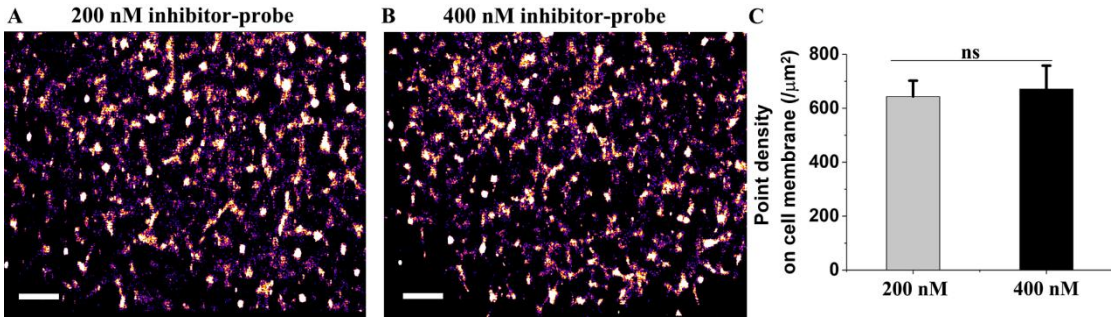


Figure S7. The similar dSTORM imaging of P-gp on MCF10A membrane labeled by different concentrations of Tariquidar-TAMRA. The similar dSTORM images of P-gp distribution on the MCF10A cell membranes stained by Tariquidar-TAMRA at the appropriate concentration (A, 200 nM) and the higher one (B, 400 nM). Scale bars are 2 μm.

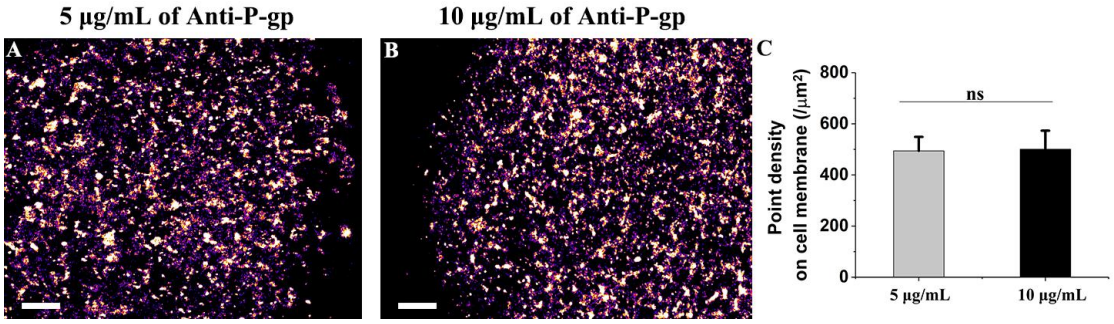


Figure S8. The similar imaging of P-gp on MCF 10A membrane by different concentrations of antibody-probe in IIF labeling. The similar dSTORM images of P-gp distribution on the MCF10A cell membranes stained by Anti-P-gp and secondary antibody-Alexa532 at the appropriate concentration (A, 5 μg/mL of Anti-P-gp) and the higher one (B, 10 μg/mL of Anti-P-gp). Scale bars are 2 μm.

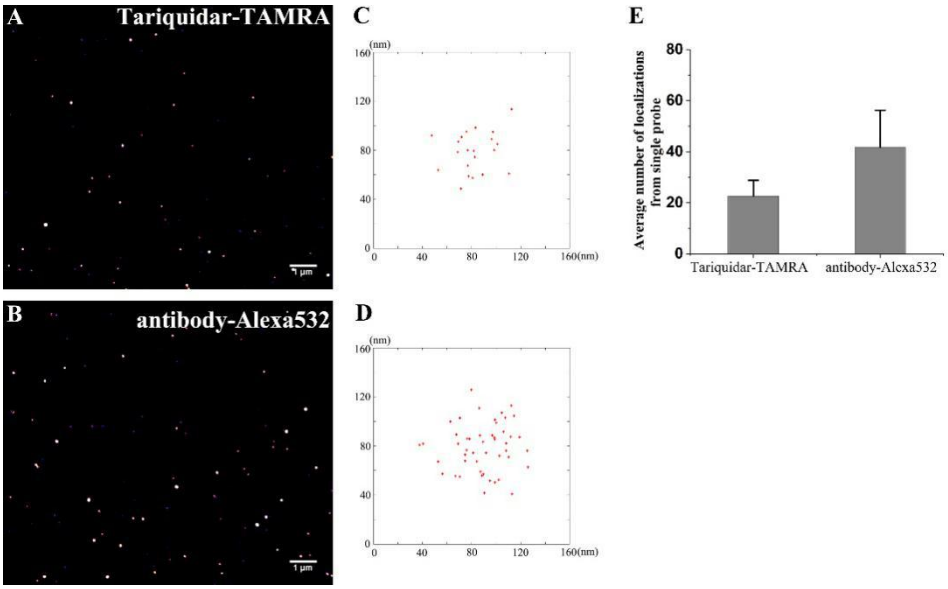


Figure S9. The repeat localizations of single Tariquidar-TAMRA and single secondary antibody-Alexa532. (A and B) The dSTORM images of single Tariquidar-TAMRA (A) and single secondary antibody-Alexa532 (B) distribution on glass slides. Scale bars are 1 μm. (C and D) The corresponding representative distribution images of repeat localizations of single probe. (E) The histogram of average number of localizations from single probe, statistical data came from 200 single probes of three independent experiments, with showing average ± SE.

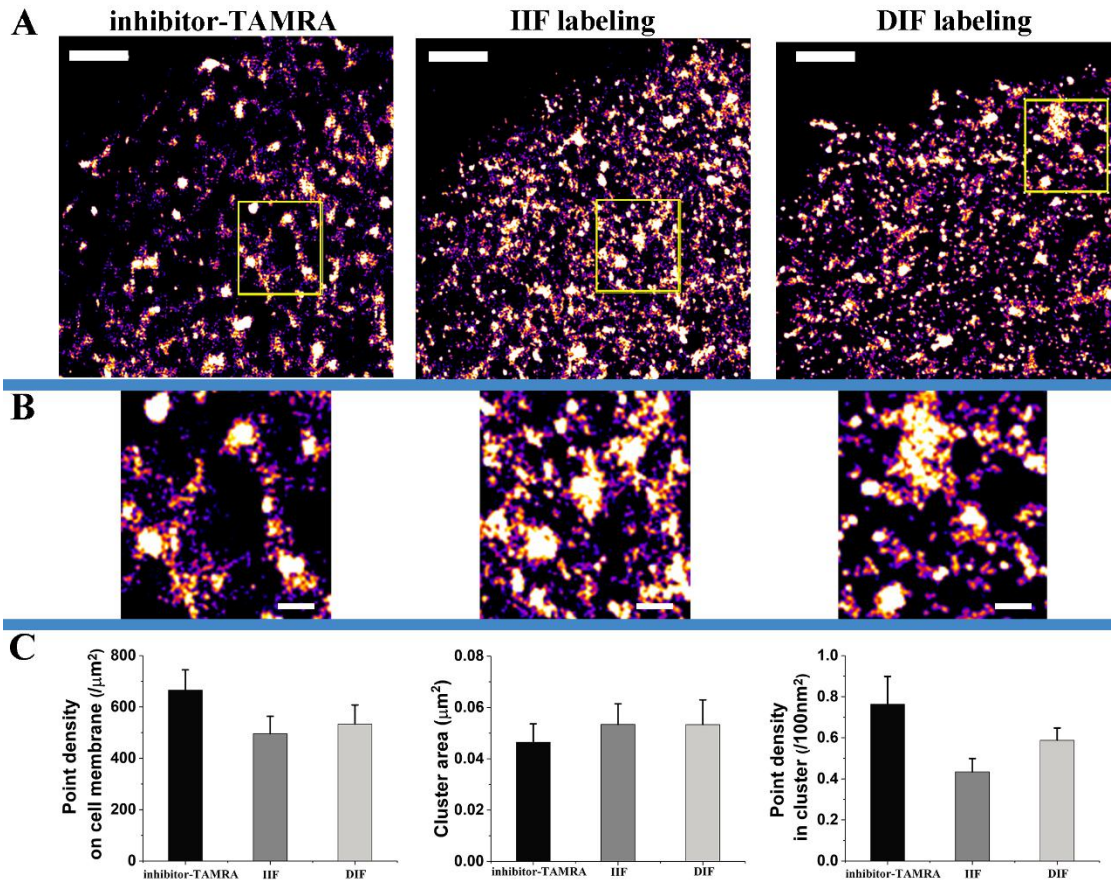


Figure S10. The compared imaging differences between the inhibitor-probe labeling and the IIF and DIF labeling. (A) The dSTORM images of P-gp distribution on MCF10A cell membranes with Tariquidar-TAMRA labeling (left), IIF labeling (middle) and DIF labeling (right). (B) The corresponding enlarged images of the boxes in the figure A. (C) The histograms of three parameters of these three labeling methods, that is, point density on cell membrane (left), cluster area (middle) and point density in cluster (right). Statistical data is from total ten cells of three independent experiments, with showing average ± SE. Scale bars are 2 μm in A and 500 nm in B.

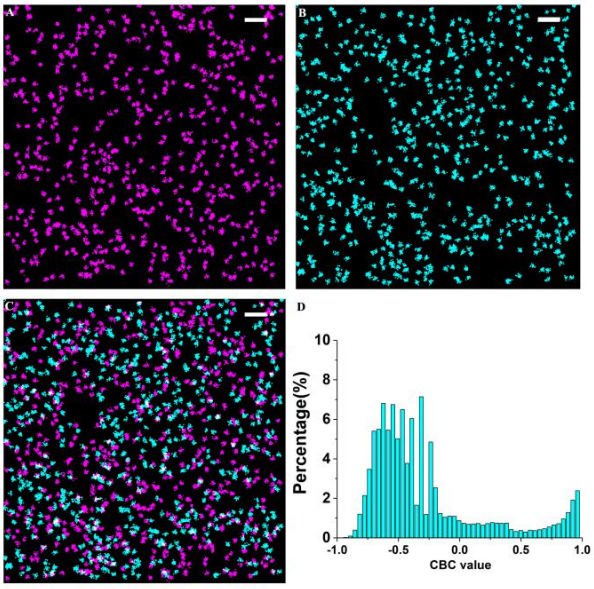


Figure S11. The CBC analysis on simulated clusters with random distribution. (A and B) The images of random distribution of simulated clusters yielded by DBSCAN. (C) The merged image of A and B shows every few colocalization distribution of these two random clusters. (D) The distribution of CBC value of the clusters in cyan color indicating that these two clusters are mainly in the anti-correlation (C_{Ai} = -1) or non-correlated distribution (C_{Ai} = 0). Scale bars are 2 μm in A-C.

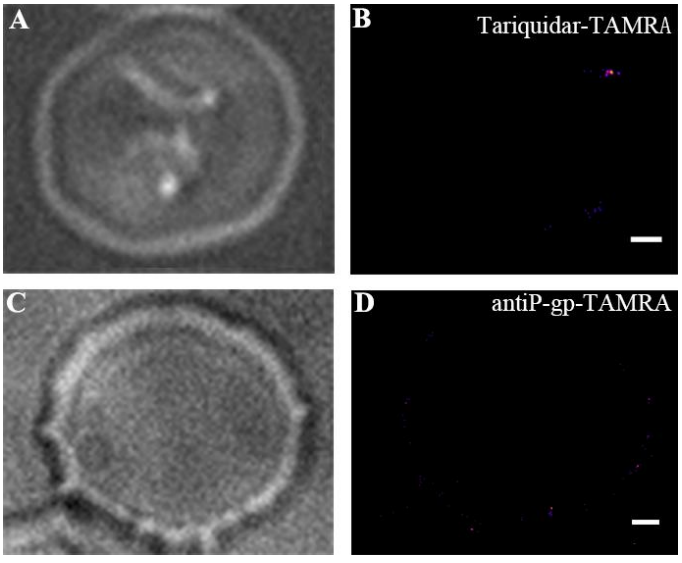


Figure S12. dSTORM imaging of P-gp negative cells by Tariquidar-TAMRA or antiP-gp-TAMRA labeling. (A and B) The bright field image and the corresponding dSTORM image of RBC by Tariquidar-TAMRA labeling. (C and D) The corresponding images from antiP-gp-TAMRA labeling. Scale bars are 1 μm in B and D.

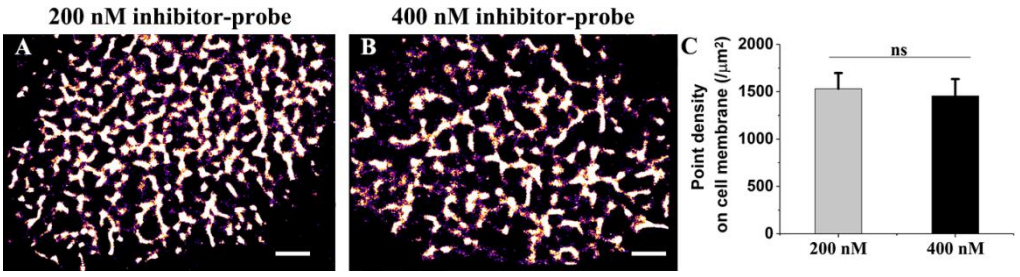


Figure S13. The similar imaging of P-gp on MDA-MB-231 membrane labeled by different concentrations of Tariquidar-TAMRA. The similar dSTORM images of P-gp distribution on the MDA-MB-231 cell membranes stained by Tariquidar-TAMRA at the appropriate concentration (A, 200 nM) and the higher one (B, 400 nM). Scale bars are 2 μm.

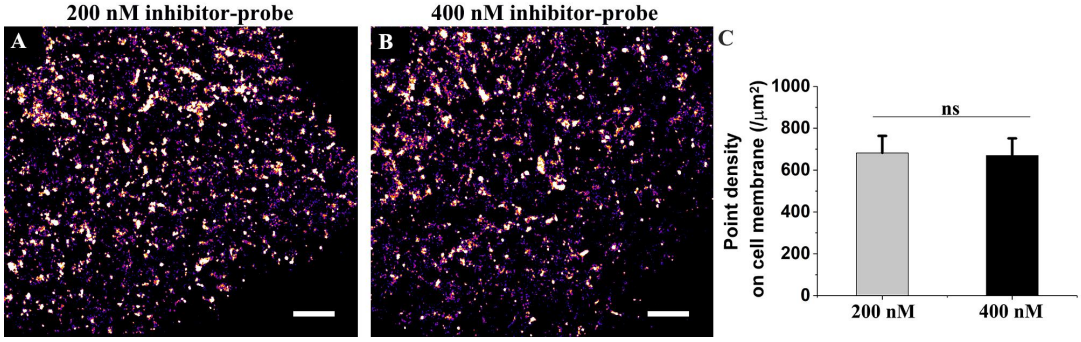


Figure S14. The similar imaging of P-gp on H71L membrane by different concentrations of Tariquidar-TAMRA. The similar dSTORM images of P-gp distribution on the H71L cell membranes stained by Tariquidar-TAMRA at the appropriate concentration (A, 200 nM) and the higher one (B, 400 nM). Scale bars are 2 μm.

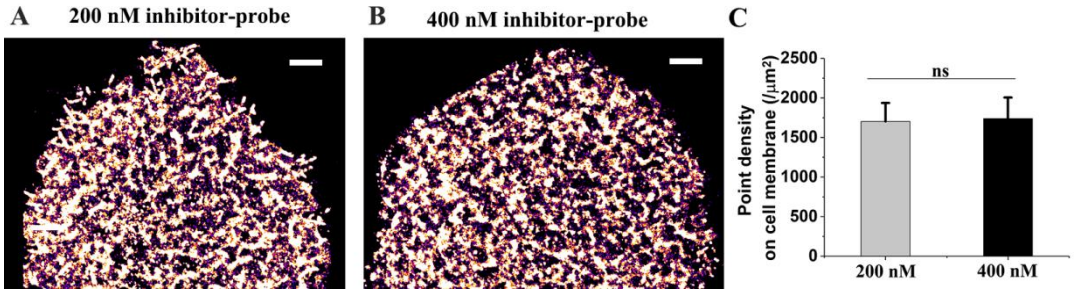
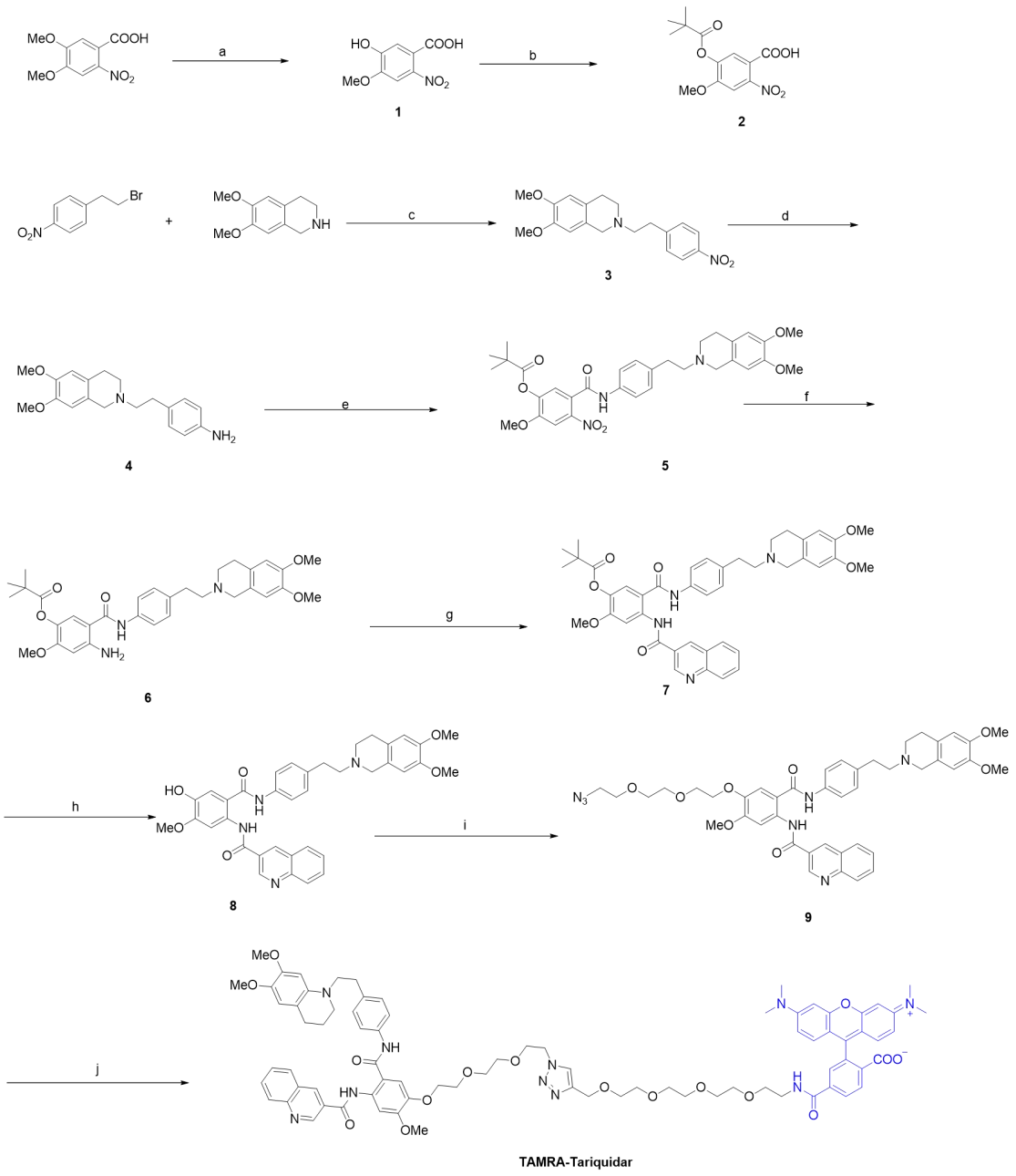


Figure S15. The similar imaging of P-gp on 149T membrane by different concentrations of Tariquidar-TAMRA. The similar dSTORM images of P-gp distribution on the 149T cell membranes stained by Tariquidar-TAMRA at the appropriate concentration (A, 200 nM) and the higher one (B, 400 nM). Scale bars are 2 μm.

Supplementary experiment section

Synthesis of Tariquidar-TAMRA probe



Scheme 1: Synthesis route of Tariquidar-TAMRA. Reagents and conditions: a) KOH (4.0 eq.), H_2O, reflux, 24 h; b) pivalic anhydride (1.1 eq.), Cs_2CO_3 (0.68 eq.), DMF, 70°C, 5 h; c) K_2CO_3 (1.1 eq.), KI (20 mol%), DMF, 70°C, 4 h; d) Pd/C (10wt%), H_2 (1 atm), EtOH, rt, 16 h; e) **2** (1.2 eq.), HATU (1.2 eq.), TEA (3.0 eq.), DMF, rt, 5 h; f) Pd/C (10wt%), MeOH/EA (1:1, v/v), rt, 16 h; g) (1)3-Quinolinecarboxylic acid (3.0), $SOCl_2$, reflux, 4h; (2) TEA (10.0 eq.), DCM, rt, 16 h; h) MeONa (1.2 eq.), MeOH/THF (1:1, v/v), rt, 16 h; i) PEG linker (1.2 eq.), DMF, 50°C, 10 h; j) TAMRA-Alkyne, $CuSO_4$, TCEP, TBTA, PBS, rt, dark ,overnight.

General Methods: Substrates and reagents are commercially available, and used as received. ^{1}H and ^{13}C spectra were recorded with Bruker AVX 400 MHz spectrometers in $CDCl_3$, D_2O and *d6*-DMSO. Tetramethylsilane (δ = 0 ppm) was used as internal standard for ^{1}H and ^{13}C spectra. The structures of known compounds were confirmed by ^{1}H NMR spectroscopy and comparison with literature data.

5-hydroxy-4-methoxy-2-nitrobenzoic acid (1)

To a stirred solution of 4,5-dimethoxy-2-nitrobenzoic acid (2.2 g, 10.0mmol) in distilled H_2O (10 mL) was added KOH (2.24 g, 40.0 mmol), then the resulted solution was heated to reflux for 24 h. After cooling to room temperature, the mixture was acidified with diluted HCl (1.0 M) to pH 1-2, during the addition of diluted HCl, precipitate was generated gradually. The precipitate was filtered off and washed with water for three times. Then, the crude product was recrystallized from H_2O to give the desired compound **1** as a yellow solid (1.50 g, 70% yield). ^{1}H-NMR (D_2O): δ 7.29 (s, 1H), 6.78 (s, 1H), 3.73 (s, 3H).

4-methoxy-2-nitro-5-(pivaloyloxy)benzoic acid (2)

To the suspended solution of compound **1** (900 mg, 4.23 mmol) in DMF (18 mL) were added pivalic anhydride (866 mg, 4.65 mmol) and Cs2CO3 (937 mg, 2.83 mmol). Then, the mixture was purged with nitrogen and heated to 50°C for 5 h. Cooled to room temperature, the solvent was removed under reduced pressure, the residue was dissolved in diluted HCl (1.0 M) and extracted with ethyl acetate for three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silical gel column chromatography with eluent (DCM/MeOH = 20:1, v/v) to afford compound **2** (500 mg, 40% yield) as an off-white solid for the subsequent reaction. ^{1}H NMR (400 MHz, *d6*-DMSO): δ 13.78 (s, 1H), 7.74 (s, 1H), 7.65 (s, 1H), 3.90 (s, 3H), 1.31 (s, 9H).

6,7-dimethoxy-2-(4-nitrophenethyl)-1,2,3,4-tetrahydroisoquinoline (3)

To a stirred solution of 4-nitrophenethyl bromide (1.5 g, 6.58 mmol) and 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (1.5 g. 6.58 mmol) in DMF (15 mL) were added K_2CO_3 (1.1 g, 7.90 mmol) and KI (218 mg, 1.32 mmol), then the mixture was heated to 70°C for 4 h. Cooled to room temperature, the mixture was filtered off and washed with DCM for three times, concentrated under reduced pressure to remove the solvent. The residue was purified by silical gel column chromatography with eluent (PE/EA = 1:1, v/v) to afford compound **3** (1.2 g, 53% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (s, 1H). 8.14 (s, 1H), 7.42 (s, 1H), 7.39 (s, 1H), 6.60 (s, 1H), 6.53 (s, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.64 (s, 2H), 3.03-2.99 (m, 2 H), 2.84-2.78 (m ,6H).

4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)aniline (4)

Compound **3** (1.0 g, 2.92 mmol) was dissolved in EtOH (25 mL), Pd/C (100 mg, 10wt%) was added. The flask was degassed with nitrogen for three times, then filled

with H_2, the resulting solution was stirred at room temperature for 16 h. The reaction mixture was filtered and washed with DCM for three times, concentrated under reduced pressure to give the compound **4** (800 mg, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.04 (s, 1H), 7.02 (s, 1H), 6.65 (s, 1H), 6.63 (s, 1H), 6.60 (s, 1H), 6.53 (s, 1H), 3.84 (s, 3h), 3.83 (s, 3H), 3.63 (s, 2H), 2.86-2.67 (m, 8H).

5-((4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)phenyl)carbamoyl) -2-methoxy-4-nitrophenyl pivalate (5)

To a stirred solution of compound **4** (500 mg, 1.60 mmol), compound **2** (572 mg, 1.92 mmol) and HATU (730 mg, 1.92 mmol) in DMF (10 mL) was added TEA (0.67 mL, 4.80 mmol), then the resulting solution was stirred at room temperature for 5 h. The mixture was diluted with water and extracted with ethyl acetate for three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silical gel column chromatography with eluent (PE/EA = 1:1, v/v) to afford compound **5** (500 mg, 53% yield) as a yellow solid. NMR (400 MHz, *d*$_6$-DMSO): δ 10.55 (s, 1H), 7.84 (s, 1H), 7.61 (s, 1H), 7.55 (d, 2H, J = 7.97 Hz), 7.24 (d, 2H, J = 8.44 Hz), 6.66 (d, 2H, J = 9.49 Hz), 3.93 (s, 3H), 3.70 (s, 6H), 3.61 (s, 2H), 2.82-2.67 (m, 8H), 1.32 (s, 9H).

4-amino-5-((4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)phenyl)ca rbamoyl)-2-methoxyphenyl pivalate (6)

Compound **5** (500 mg, 0.85 mmol) was dissolved in MeOH/EtOAc (1:1, 10 mL), Pd/C (50 mg, 10wt%) was added. The flask was degassed with nitrogen for three times, then filled with H_2, the resulting solution was stirred at room temperature for 16 h. The reaction mixture was filtered and washed with DCM for three times, concentrated under reduced pressure. The residue was purified by silical gel column chromatography with eluent (PE/EA = 1:1, v/v) to afford compound **6** (200 mg, 42% yield). ^{1}H NMR (400 MHz, *d*$_6$-DMSO): δ 9.70 (s, 1H), 7.56 (d, 2H, J = 8.34 Hz), 7.41 (s, 1H), 7.18 (d, 2H, J = 8.24 Hz), 6.65-6.63 (m, 4H), 6.41 (s, 1H), 3.71-3.69 (m, 9H), 3.54 (s, 2H), 2.81-2.62 (m, 8H), 1.29 (s, 9H).

***O*-Pivaloyl-tariquidar (7)**

3-Quinolinecarboxylic acid (154 mg, 0.89 mmol) was added to $SOCl_2$ (2 mL), then the mixture was heated to reflux for 3 h. Cooled to room temperature, the mixture was concentrated under reduced pressure to remove the excess $SOCl_2$. The acid chloride was then suspended in THF (0.50 mL) and dropwise addition to a solution of **6** (200 g, 0.36 mmol) in DCM (2 mL) containing Et_3N (0.50 mL). The resulting solution was stirred overnight at room temperature and then quenched by H_2O. The mixture was diluted with DCM and extracted with DCM for three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silical gel column chromatography with eluent (PE/EA = 2:1, v/v) to afford compound **7** (120 mg, 47% yield). ^{1}H NMR (400 MHz, *d*$_6$-DMSO): δ 12.57 (s, 1H), 10.38 (s, 1H), 9.36-9.35 (m, 1H), 8.91-8.90 (m, 1H), 8.43 (s, 1H), 8.18-8.12 (m, 2H), 7.93 (t, 1H, J

= 7.41 Hz), 7.80 (s, 1H), 7.76-7.72 (t, 1H, *J* = 7.41 Hz), 7.61 (d, 2H, *J* = 8.37 Hz), 7.25 (d, 2H, *J* = 8.36 Hz), 6.64 (d, 2H, *J* = 8.37 Hz), 3.90 (s, 3H), 3.69-3.67 (m, 6H), 3.54 (s, 2H), 2.81-2.70 (m, 8H), 1.35 (s, 9H).

_O_-Desmethyl-tariquidar (8)

To a stirred solution of **7** (120 mg, 0.17 mmol) in MeOH/THF (1:1, 2 mL) was added MeONa (0.04 mL, 0.20 mmol) under nitrogen atmosphere, then the resulting mixture was stirred at room temperature for 10 h. The mixture was quenched by diluted HCl and extracted with ethyl acetate for three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silical gel column chromatography with eluent (PE/EA = 1:1, v/v) to afford compound **8** (50 mg, 47% yield). ^{1}H NMR (400 MHz, d_6-DMSO): δ 12.07 (s, 1H), 10.29 (s, 1H), 9.34-9.32 (m, 1H), 9.28 (s, 1H), 8.87 (br, 1H), 8.18-8.10 (m, 3H), 7.91 (t, 1H, *J* = 7.56 Hz), 7.72 (t, 1H, *J* = 7.54 Hz), 7.62 (d, 2H, *J* = 8.06 Hz), 7.41 (s, 1H), 7.23 (d, 2H, *J* = 8.32 Hz), 6.65 (d, 2H, *J* = 8.06 Hz), 3.90 (s, 3H), 3.70-3.69 (m, 6H), 3.54 (s, 2H), 2.81-2.72 (m, 8H).

Compound 9

To a stirred solution of **8** (50 mg, 0.08 mmol) in DMF (2 mL) were added PEG linker (28 mg, 0.096 mmol) and K2CO3 (35 mg, 0.24 mmol), then the resulting mixture was heated to 50^{o}C for 10 h. Cooled to room temperature, the mixture was diluted with water and extracted with ethyl acetate for three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silical gel column chromatography with eluent (PE/EA = 2:1, v/v) to afford compound **9** (25 mg, 40% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.63 (s, 1H), 9.54 (s, 1H), 8.78 (s, 1H), 8.63 (s, 1H), 8.23 (s, 1H), 8.17 (d, 1H, *J* = 8.18 Hz), 7.99 (d, 1H, *J* = 7.96 Hz), 7.83-7.80 (m, 1H), 7.64-7.56 (m, 3H), 7.42 (s, 1H), 7.29-7.26 (m, 2H), 6.61 (s, 1H), 6.55 (s, 1H), 4.26-4.24 (m, 2H), 3.99 (s, 3H), 3.86-3.84 (m, 8H), 3.72-3.69 (m, 6H), 3.62-3.60 (m, 2H), 3.31-3.28 (m, 2H), 2.95-2.85 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.57, 163.78, 153.58, 149.33, 148.83, 147.69, 147.35, 143.55, 136.82, 136.48, 135.79, 131.38, 129.28, 129.19, 127.40, 126.94, 125.89, 121.50, 114.31, 111.40, 109.52, 104.98, 70.59, 70.12, 69.85, 69.81, 59.80, 56.04, 55.94, 55.43, 50.89, 50.64, 33.20, 38.32. HRMS: calcd. for $C_{43}H_{48}N_7O_8$ $[M+H]^+$ 790.35589; found 790.35250.

Tariquidar-TAMRA

In 100 μL of PBS, TAMRA-alkyne (4 mM) and compound **9** (4 mM) were allowed to react in the presence of CuSO4 (1 mM), tris(carboxyethyl)phosphine (1 mM), and ligand (2 mM). After reaction at dark for 12 hours, the reaction mixture was purified by NAP-5 columns (GE Healthcare) with PBS as eluent to obtain the desired Tariquidar-TAMRA. The combination ratio of dye to TAMRA was measured by UV-visible absorption spectroscopy assay. Finally, the qualified solution with a suitable ratio (~1.0) were collected for labeling experiment.

Preparation of antiP-gp-TAMRA probe

Stock solution of P-gp antibody was diluted to the appropriate concentration with PBS (commonly, 100 μg/ mL, 100 μL), then added 0.5 μL TAMRA (0.5 mg/mL, Invitrogen) to react with antibody in the dark for 3h at RT under continuous vortex. After reaction, the excessive free dyes were removed by filtration with illustra NAP-5 columns (GE Healthcare). The combination ratio of dye to antibody was determined by UV-visible absorption spectroscopy assay. Finally, the qualified solution with a suitable ratio (0.7-1.2) were collected for labeling experiment.

Cell culture

MCF10A cells and MBA-MD-231 cells were purchased from purchased from Stem Cell Bank, Chinese Academy of Sciences. H71L and 149T cells are donated by the research group of Professor Hongbin Ji of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. MCF10A cells were cultured in Dulbecco's Modified Eagle Medium/F12 (DMEM/F12, HyClone) with 5% Horse Serum (FBS, HyClone), 20 ng/mL EGF (Sigma), 0.5 μg/mL Hydrocortisone (Sigma), 100 ng/mL Cholera toxin (Sigma), 10 μg/mL Insulin (Sigma), 100 μg/mL Penicillin (Sigma) and 100 μg/mL Streptomycin (Sigma). MBA-MD-231 cells were cultured in 1640 medium and H71L and 149T cells were cultured in DMEM, with 10% fetal bovine serum (FBS, Gibco) and antibiotics. All cells are maintained in humidified incubator at 37℃ (with 5% CO2). For dSTORM imaging, the cells were cultured on a clean cover slip (22 mm × 22 mm, Fisher) in a dish for at least 24 hours to achieve ~60-70% confluence.

The sample preparation of human BRCs
Fresh red blood cells (RBCs) were obtained from centrifuging the whole human fingertip blood of healthy donors. In details, RBCs were washed five times with PBS by a low speed centrifuge (1,000 r/min, 1 min) to remove serum and buffy coat. Then, RBCs were resuspended and diluted by PBS into appropriate cell density. 200 μL erythrocyte suspension was dropped on the clean silanized slide to attach for 20 min at room temperature, the unabsorbed RBCs were washed away by PBS. Then, the RBC was treated as cultured cells to prepare the imaging sample.

Sample preparation

Cell stained by Tariquidar-TAMRA

After washing with pre-warm PBS for three times, well cultured cells were fixed by 4% paraformaldehyde (PFA) at room temperature for 30 min. Then, with washing for three times with PBS, cells were blocked by 3% Bovine Serum Albumin (BSA) at room temperature for 20 min, then after washing, the cells were stained with the Tariquidar-TAMRA at specific concentration (i.e.200 nM or 400 nM) in the dark for

10 min at 4°C. Finally, the sample was washed by PBS for 4-5 times to remove away the excess probes for the dSTORM imaging experiments.

Cell stained by indirect immunofluorescence (IIF) labeling and direct immunofluorescence (DIF) labeling

For IIF labeling, after the washing and fixation procedures as above, cells were blocked by 3% Bovine Serum Albumin (BSA) at room temperature for 20 min. Cells were then incubated in primary antibody of P-gp (Anti-P-gp, abcam, ab226937) solution containing 1% BSA at 4°C overnight. Next, after washing with PBS for 5 min for 3 times, the sample was stained by goat anti-rabbit IgG-Alexa532 (1 μg/mL in 1% BSA; Invitrogen) in darkness for 1 h at room temperature. Finally, it was washed with PBS for 3 min each time for 4 times, which was used for dSTORM imaging experiment.

For DIF labeling, the sample was washed, fixed and blocked as above, then stained with antiP-gp-TAMRA at the suitable concentration (5 μg/ml) for 30 min at room temperature in dark. Then, the sample was washed with PBS for 3 min each time for 4 times, which was used for dSTORM imaging experiment.

Sample preparation of dual-color dSTORM imaging

In addition to Goat anti rabbit IgG alex647 (1 μg/mL in 1% BSA; Invitrogen), the sample was firstly stained with antibody-probe as the above steps. Then, cells were stained by Tariquidar-TAMRA (200 nM) at 4°C for 10 min in the dark. Finally, the sample was washed with PBS for 4-5 times to remove the excess probes for dSTORM imaging experiment.

Sample preparation of dSTORM imaging of single probe on glass slide

Tariquidar-TAMRA, antiP-gp-TAMRA or secondary antibody-Alexa532lectins were diluted in the imaging buffer with the final concentration of 1 nM, then the 20 μL probe solution was poured on an empty large coverslip and a small coverslip was placed on it and sealed with nail polish.

dSTORM imaging

An inverted Nikon Ti-E microscope equipped with an oil-immersion objective (100×, 1.49 NA, Nikon, Japan) was used for dSTORM imaging. The sample was imaged under the approximate total internal reflection fluorescence (TIRF) illuminating mode by adjusting the excitation inclination to maximize the signal-to-noise ratio. A 532 laser (~200 mW) was applied to excite the sample in single color dSTORM imaging; and a 647 laser (~150 mW) was firstly used to image the red channel and then a 532 laser was applied to image the green channel in the dual-color dSTORM imaging. To minimize the color-crosstalk of Alexa 647 excited by a 532 laser, a band pass emission filter (FF01-595/34-25, Semrock) was added, apart from the conventional excitation filter, dichroic mirror and emission filter set. An EMCCD camera (Photometrics, Cascade II) combined with Micro-Manager based on ImageJ (U.S. National Institutes of Health) is used to acquire 5000 raw frames with 20 ms exposure time. Moreover, four-color microspheres (Invitrogen) were utilized as fiducials to

correct the x–y drift and chromatic drift in dual-color imaging. A perfect focus system (PFS) in Nikon micro imaging equipment also provides a real-time correction of the focus drift in the z axis. Besides, the drift-correct in the post-process of ThouderSTORM can correct the occasional errors.

Measurement of fluorescent emission spectra

Fluorescent emission spectra of Tariquidar-TAMRA and free TAMRA were recorded using a LS 55 luminescence spectrometer with a 1 cm-path-length microquartz cell (Perkin-Elmer Instruments, UK).

Data analysis

The reconstruction of dSTORM image

We implemented ThunderSTORM based on ImageJ to analyze the raw data and yielded qualified localizations to reconstruct dSTORM image, as described in previous study[1]. In short, with setting the private camera parameters according to our experiment conditions and selecting the appropriate analysis method and parameter thresholds, the raw data can be analyzed. After obtaining all localizations, we further set the vital parameters thresholds, including the sigma, the intensity, the offset, the uncertainty, and acquired the qualified localizations with removing the “bad localization”; then, with applying the “merge” and “drift correct”, a dSTORM image with nanoscale resolution was finally reconstructed.
As mentioned in previous study[1], we analyzed the original data by using ThouderSTORM based on ImageJ, and obtained qualified localizations to reconstruct dSTORM image. In short, according to the experimental conditions to set camera parameters, select the appropriate analysis method and parameter threshold, the original data can be analyzed. After getting all the localization results, we further set the threshold of key parameters, including sigma, intensity, offset and uncertainty, and get qualified positioning by removing "bad positioning"; then, we applied "merge" and "drift correction" to reconstruct the dSTORM image with nanometer resolution.

The calculation of point density on cell membrane

The localization density on the cell membrane was calculated by the method of the previous paper[1]. In short, an inherent image process in ImageJ is used to measure the area of the cell membrane of interest, then the total number of points on the cell membrane of interest is calculated by MatLab. Then, the point density on the cell membrane was acquired. The appropriate labeling concentration was determined by comparing the point density of the probe on the cell membrane under different labeling concentrations.

Cluster analysis by SR-Tesseler

Cluster analysis was performed by SR-Tesseler as described in our previous study[1]. SR-Tesseler[2] is an accurate cluster analysis method suitable for single-molecule localization data. It can precisely and automatically segment protein clusters based on the local density, and finally characterize protein distribution at different scales. Detailed theoretical basis can refer to the related literature[2].
Here, for our data, localization coordinates are imported to reconstruct the original dSTORM image. After selecting the region of interest, the Voronoï diagram is created based on local localization density. Next, with setting the density factor, cut distance, min area and min#locs, objects are recognized. Clusters can then be further abstracted from the objects by setting the density factor and min area and min#locs. Besides, this analysis can yield the morphological parameters, such as the cluster area, the number of points in cluster, the cluster circularity and so on.

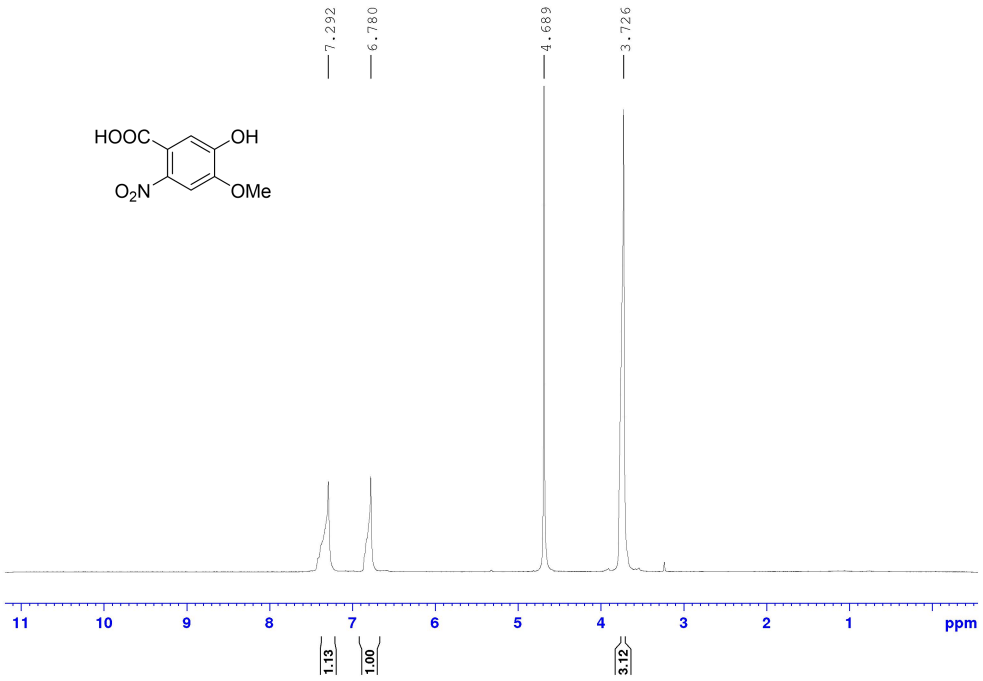
Colocalization analysis by CBC method

We applied the coordinate-based colocalization (CBC) method[3] suitable for single-molecule localization data to analysis the spatial correlation of two imaging channels. Detailed producers are similar to those described in our previous studies[1]. In this analysis, each location of each species (A or B) is assigned a CBC value C_{Ai}, which can describe the detailed spatial relationship of A to B. After the final normalization, the C_{Ai} value distributes between -1 to 1, which means that the spatial relationship of the two species changes from anti-correlated (C_A = -1) through no colocalization (C_A = 0) to fully correlated distribution (C_A = 1). Therefore, the percentage of C_A greater than 0 can be used to quantify the degree of co-localization of two probes.

The simulated independent clusters with random distribution was analyzed by CBC method. We applied the DBSCAN[4] to yield two sets of simulated cluster data. Then, after importing all localizations of two sets of data by “import results” and “import ground-truth”, CBC analysis was performed to give the corresponding CBC values of every localization of these two images.

Resolution Measurement by FRC Mapping. Fourier ring correlation (FRC)[5] is a current standard for measuring image resolution in single molecule localization microscope (SMLM) images. Here, we exploited the NanoJ-SQUIRREL[6] based on ImageJ to calculate the FRC map to measure the imaging resolution from the dSTORM images. This method compares two independently acquired super-resolution images (SRMs) of the same field-of-view, so the SMLM data will firstly be splitted into odd and even frames to acquire two SRM images, then their correlation is measured at different frequencies in Fourier space. When the correlation falls below the set threshold, it indicates the resolution of the image. Finally, the FRC map can be provided to estimate the resolution across the whole image, with the lowest FRC value representing the minimum resolution in the image.

NMR spectra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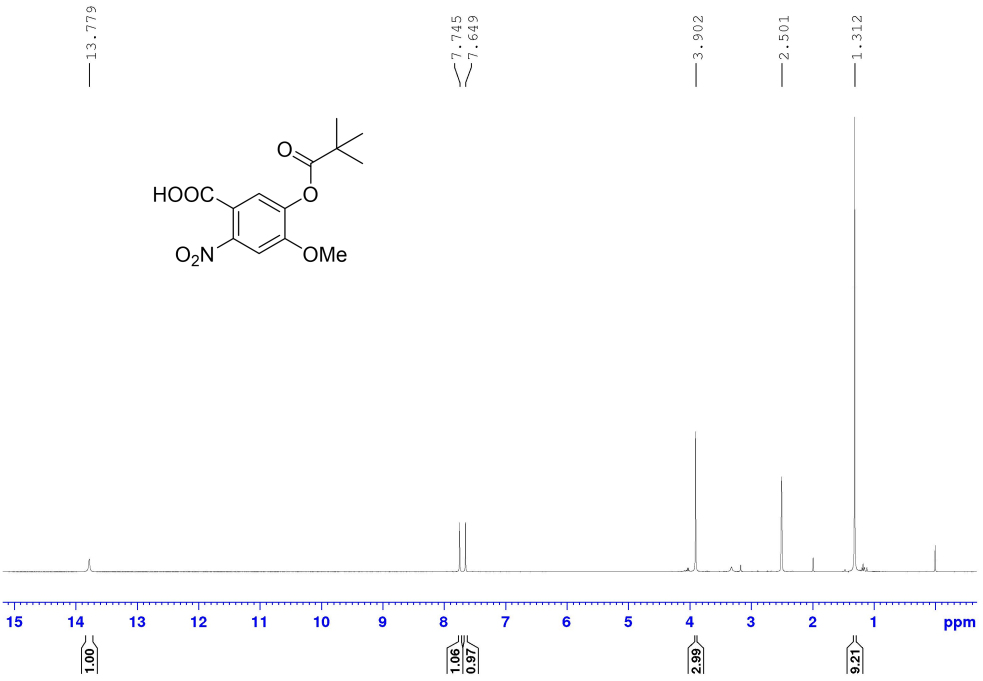
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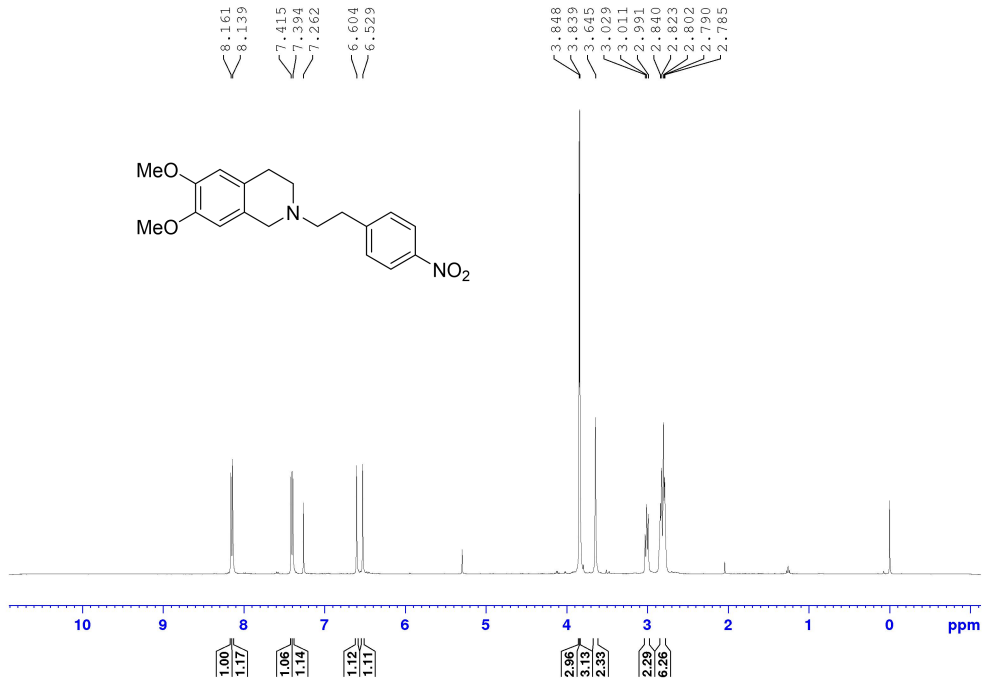
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2.823
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2.790
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MeO
N
NO2
10
9
8
7
6
5
4
3
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1
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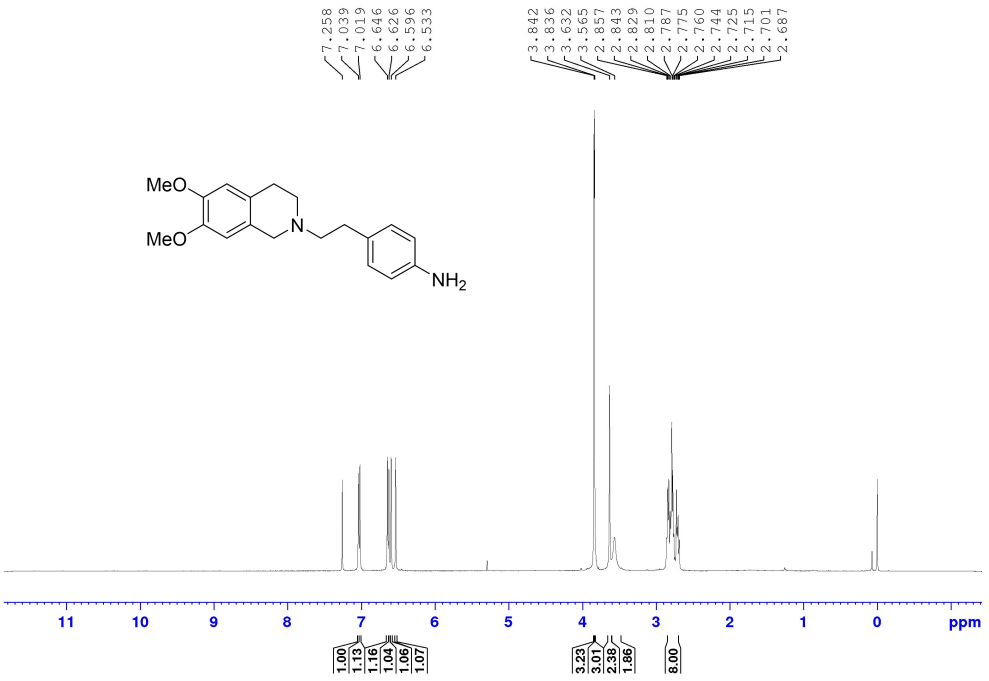
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2.715
2.701
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MeO
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NH2
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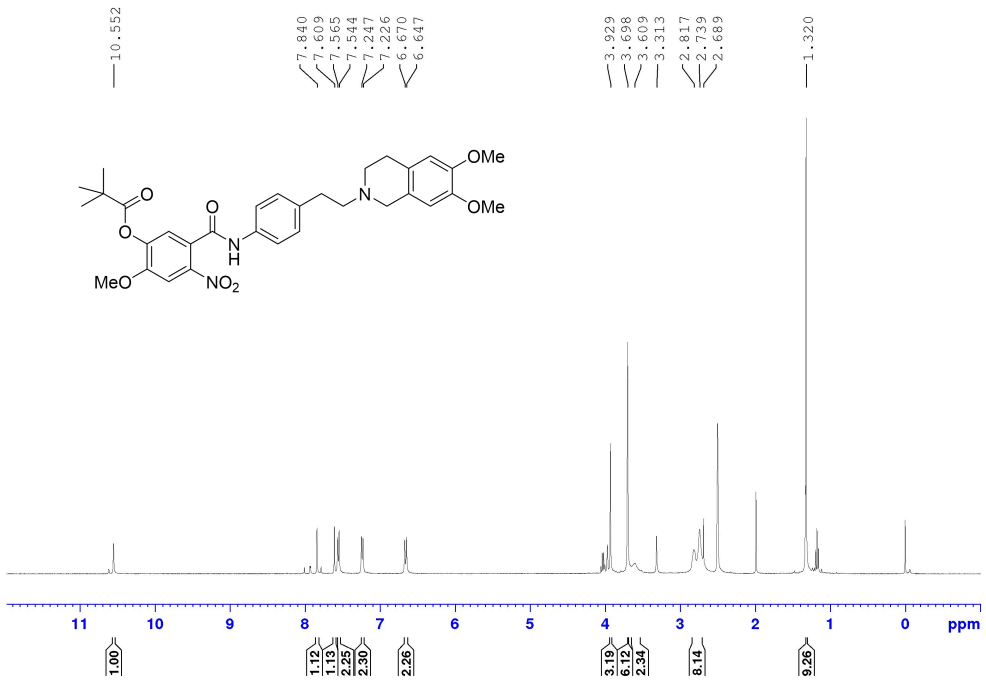
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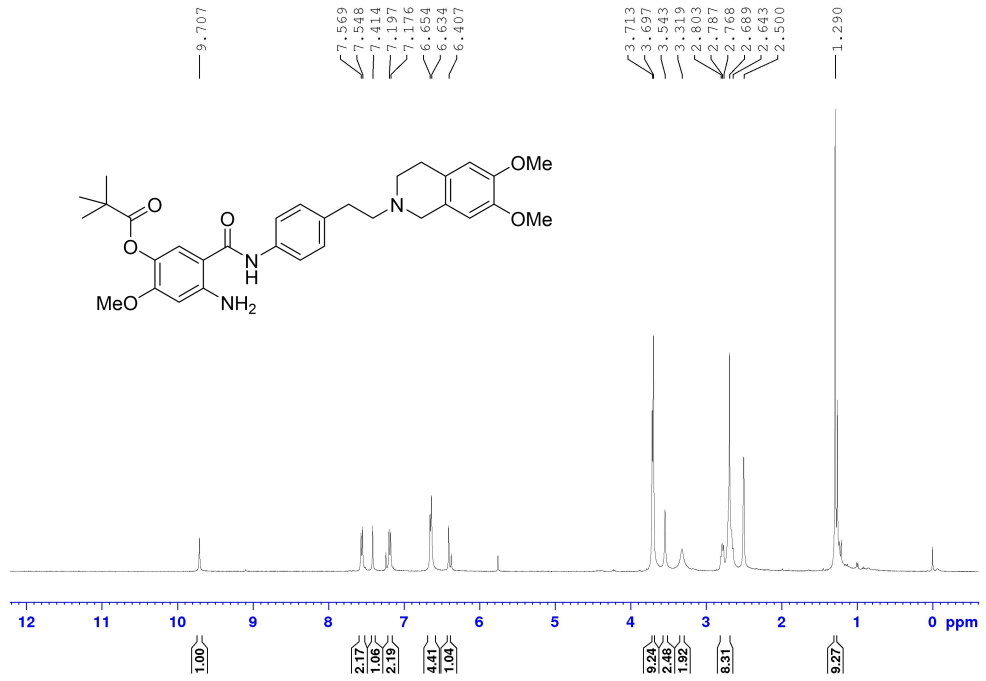
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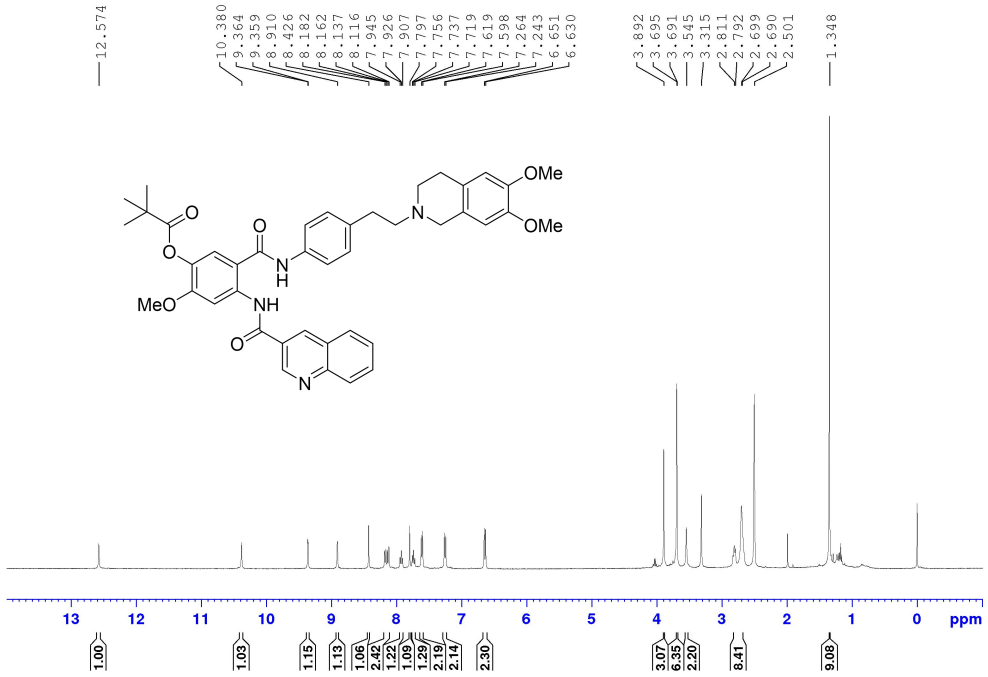
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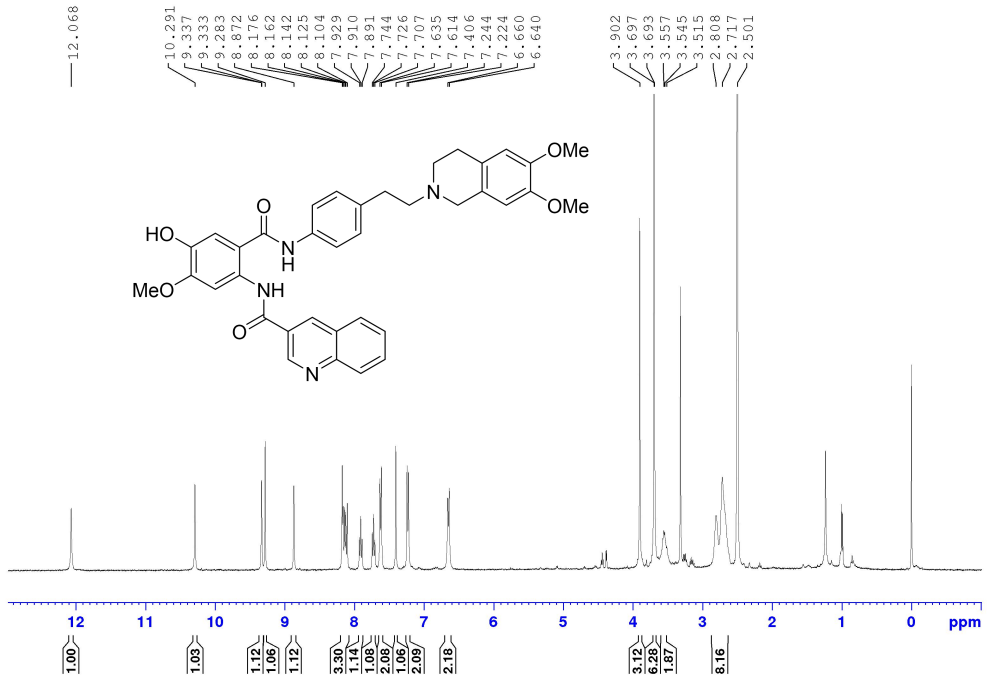
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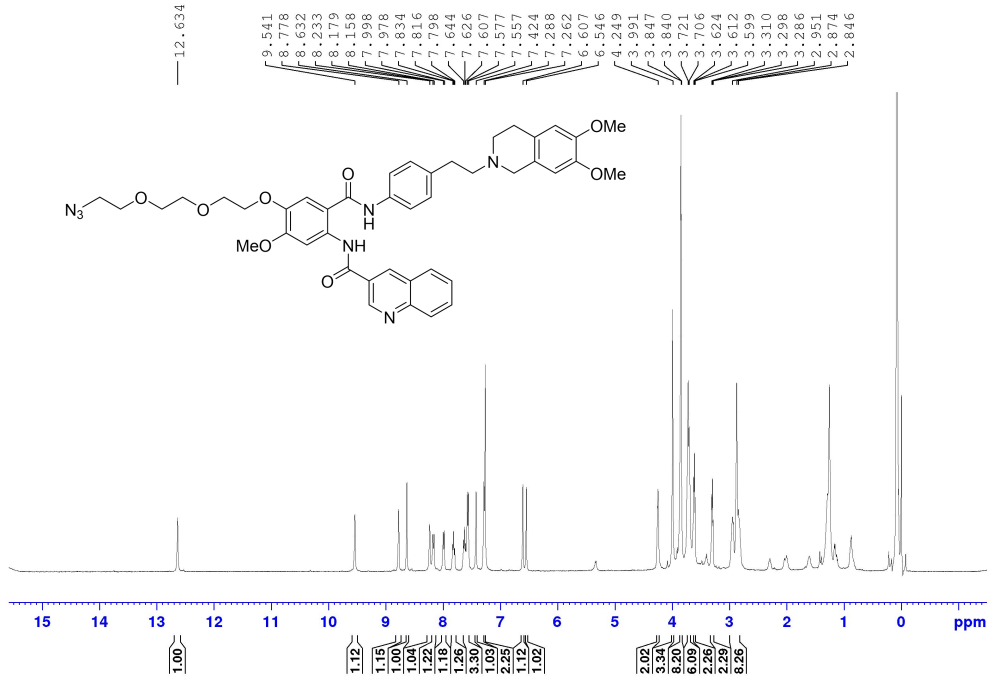
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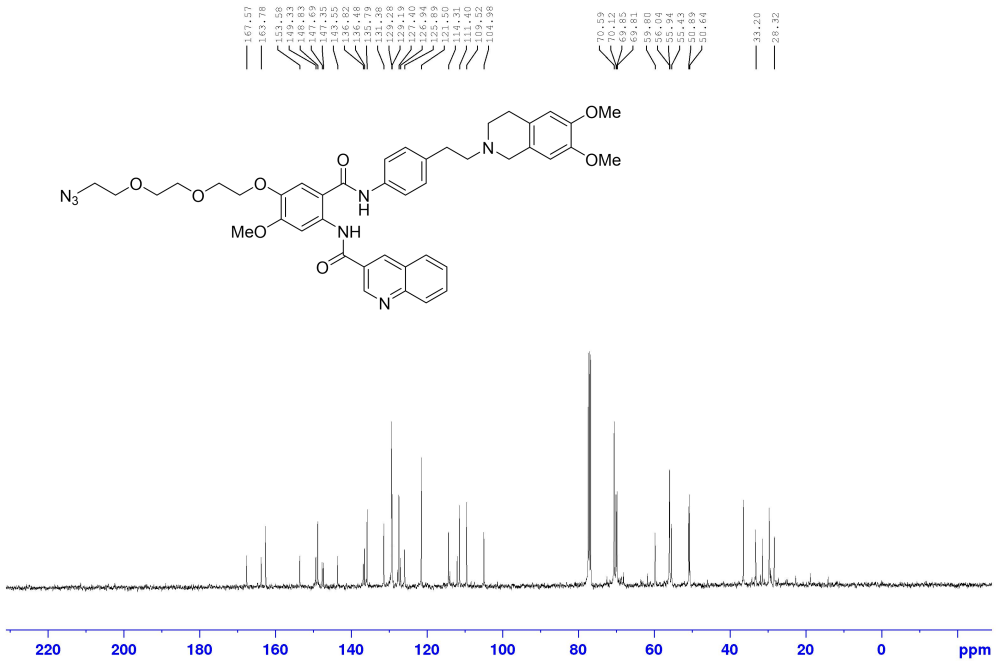
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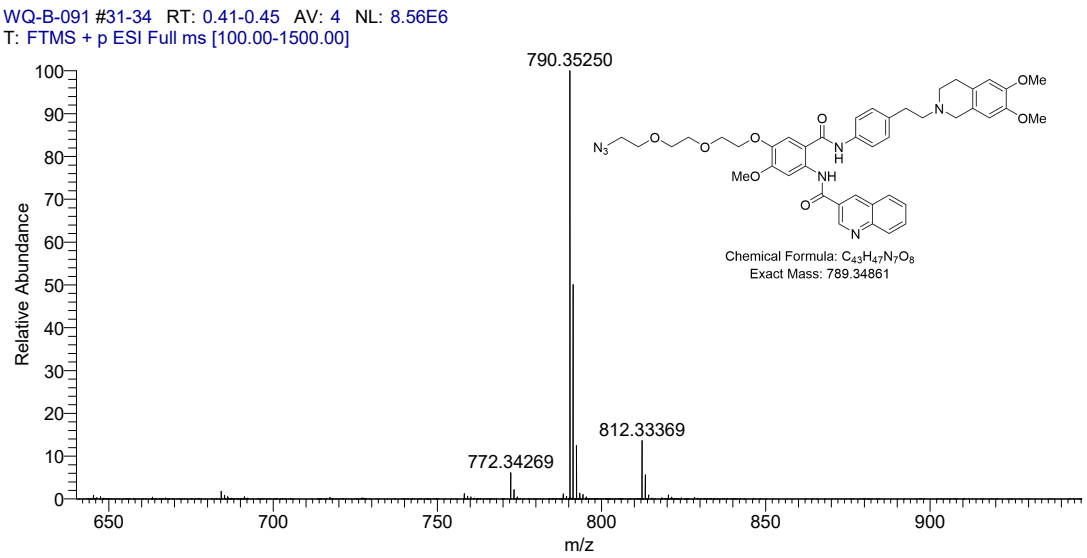

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5 S. Culley, D. Albrecht, C. Jacobs and P. M. Pereira, *Nature Methods*, 2018, **15**, 263-266.
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