Supporting information

Covalently Labeled Fluorescence–MRI Dual–Modal Polystyrene Microspheres for Imaging and Analysis of Microplastics in Biological Systems

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Experiment

Materials

N,*N*'-dimethylethylenediamine, 4-chloromethylstyrene, *N*,*N*-diisopropylethylamine, styrene, sodium dodecyl sulfate, potassium persulfate, 2,2'-azobis(isobutyronitrile), trifluoroacetic acid, and thiazolyl blue tetrazolium bromide were purchased from Energy Chemical. *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate,polyvinylpyrrolidoneK30, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid tri-tert-butyl ester, and gadolinium(III) chloride were obtained from Bide Pharmatech. Mouse breast cancer cells (4T1), human breast cancer cells (MCF-7), human cervical cancer cells (HeLa), and human normal mammary epithelial cells (MCF-10A) were obtained from the Cell Bank of the Chinese Academy of Sciences. Dulbecco's Modified Eagle Medium, MCF-10A specific medium, phosphate-buffered saline, fetal bovine serum, and penicillin-streptomycin solution were purchased from Titan Scientific Co., Ltd. Mito-Tracker Green, Lyso-Tracker Green, Golgi-Tracker Green, trypsin-EDTA solution (0.25%), and trypsin-EDTA solution (0.05%) were obtained from Beyotime Biotechnology. Zebrafish embryos, E3 medium, and MS-222 were procured from the China Zebrafish Resource Center.

Synthesis of Fluorescent Dye RhSt

Under an ice-water bath, a 500 mL single-neck flask was charged with 100 mL of anhydrous dichloromethane (DCM) and 2.6 mL (20 mmol) of *N*,*N*'-dimethylethylenediamine. A solution of 0.7 mL (5 mmol) of 4-chloromethylstyrene in 50 mL of anhydrous DCM was added dropwise to the reaction mixture. The reaction was then allowed to warm to room temperature and stirred magnetically for 4 h. Upon completion, the solvent was removed by rotary evaporation under

reduced pressure. The crude product was purified by silica gel column chromatography using a mixture of DCM and methanol (V_{DCM}: V_{MeOH} = 15: 1) as the eluent. After vacuum drying, the product was obtained as a pale-yellow solid (0.72 g, 3.56 mmol), yielding 71%.

In the subsequent step, dye precursor 2 (500 mg, 1.16 mmol) and O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) (661 mg, 1.74 mmol) were dissolved in a mixed solvent of 2 mL N,N-dimethylformamide (DMF) and 8 mL anhydrous DCM. The mixture was stirred magnetically in an ice-water bath for 10 min. Then, N,N-diisopropylethylamine (DIPEA) (0.6 mL, 3.48 mmol) was added dropwise, and stirring was continued in the ice-water bath for another 30 min. Subsequently, intermediate 1 (260 mg, 1.27 mmol) was added. The reaction was allowed to warm to room temperature and stirred overnight. After the reaction was complete, DCM was removed by rotary evaporation under reduced pressure. The resulting residue was triturated with 50 mL of methyl tert-butyl ether for 1 h. The resulting precipitate was collected by filtration and dried, yielding a deep red solid. This crude product was dissolved in a small amount of methanol and purified by medium-pressure liquid chromatography (MPLC) using a mixture of methanol and water (V_{MeOH}:V_{H2O} = 3:2) as the eluent. The methanol was subsequently removed by rotary evaporation, and the final product was obtained as a deep red solid (390 mg, 0.63 mmol) after freeze-drying, corresponding to a yield of 54.4%. ^{1}H NMR (400 MHz, DMSO-d_6): δ= 13.29 (s, 1H), 7.91 (dd, 1H), 7.70 (dd, 1H), 7.37 (m, 3H), 7.19 (2H, d), 6.69 (1H, m), 6.47 (6H, m), 5.77 (1H, dd), 5.21 (1H, d), 3.59 (2H, d), 3.32 (5H, s), 2.90 (12H, s), 2.62 (2H, s), 2.24 (3H, s). ppm; 13C NMR (101 MHz, DMSO): δ= 170.06, 168.86, 152.68, 152.40, 139.28, 138.79, 136.80, 136.27, 134.24, 129.44, 128.88, 128.66, 127.29, 126.40, 124.71, 123.14, 114.22, 109.42, 106.07, 98.34, 61.54, 54.31, 48.87, 44.88, 42.17, 39.89, 33.24. ppm; HRMS(ESI): m/z calc. for $[C_{38}H_{41}N_4O_4]^+$ 617.3122, found 617.3125.

Spectroscopic Characterization of RhSt

The UV-vis absorption and fluorescence spectra of compound RhSt were recorded using a Duetta fluorescence and absorption spectrometer. The dye was vacuum-dried, and a stock solution (2 mmol/L) was prepared in DMSO with precise weighing, then stored at 4 °C for subsequent use. For measurements, an aliquot of the stock solution was diluted with different solvents to appropriate concentrations. The UV-vis absorption spectra (300–800 nm) and fluorescence spectra (excitation at 550 nm; emission collected from 560 to 800 nm) of RhSt were measured in water, PBS, methanol, ethanol, and dichloromethane using a quartz cuvette. All measurements were performed at room temperature.

The photostability of RhSt was evaluated using the same Duetta spectrometer. An aqueous solution of RhSt at a specific concentration was placed in a quartz cuvette and irradiated with a 520 nm laser at a power density of 10 mW/cm² for 2 h. The UV-vis absorption spectrum was recorded at 20 min intervals throughout the irradiation period. All measurements were conducted at room temperature. The absolute fluorescence quantum yield of RhSt in water was determined using a HAMAMATSU absolute photoluminescence quantum yield spectrometer, applying the same excitation conditions as those used for the fluorescence spectral measurements.

Preparation of Polystyrene Fluorescent Microspheres

Emulsion Polymerization

Since styrene is prone to spontaneous polymerization at room temperature, commercial styrene typically contains an inhibitor, predominantly p-tert-butylcatechol (TBC). Prior to polymerization,

the styrene was pretreated by passing it through a chromatography column packed with basic alumina. The collected styrene was stored in a brown reagent bottle at 4°C for subsequent use.

In a typical procedure, RhSt (5 mg), sodium dodecyl sulfate (6 mg), and ultrapure water (8 mL) were added to a 100 mL three-neck flask. The mixture was sonicated for 5 min to facilitate dissolution, followed by magnetic stirring at room temperature for 30 min. The flask was equipped with a condenser, and the system was purged with nitrogen gas using a diaphragm pump and a double-manifold line. This process involved three cycles of evacuating for 2 min and refilling with nitrogen to remove oxygen. The flask was then immersed in a preheated oil bath at 80°C under continuous stirring and nitrogen protection. Styrene (1 g) was added dropwise to the reaction mixture via syringe, and stirring was continued for 1 h. Subsequently, 1 mL of an aqueous potassium persulfate solution (5 mg/mL) was rapidly added as an initiator to initiate the polymerization, which was allowed to proceed for 12 h. After completion, the reaction mixture was subjected to gradient centrifugation at 6000 rpm, 8000 rpm, and 10000 rpm for 5 min each to remove precipitates. The resulting supernatant emulsion was dialyzed against ultrapure water for 48 h to obtain the polystyrene fluorescent microspheres dispersed in water, which were stored at room temperature for further use.

Dispersion Polymerization

The inhibitor in styrene was removed using the method described above. The purchased 2,2'-azobis(isobutyronitrile) (AIBN) was dissolved in anhydrous methanol at room temperature and then recrystallized at –20°C. The purified AIBN was obtained by filtration and drying. In a typical procedure, anhydrous ethanol (8.5 g), ultrapure water (1.5 g), polyvinylpyrrolidone (1 g), styrene (2.5 g), AIBN (80 mg), and RhSt (5 mg) were added to a 100 mL three-neck flask. The mixture

was sonicated for 5 min to ensure homogeneity, followed by magnetic stirring at room temperature for 30 min. The flask was equipped with a condenser and purged with nitrogen using the same procedure as described above. The flask was then placed in a preheated oil bath at 70°C and stirred for 8 h. After the reaction, the mixture was centrifuged at 6000 rpm. The precipitate was washed sequentially with ethanol and ultrapure water several times, and then dialyzed against ultrapure water for 48 h. The final product, polystyrene fluorescent microspheres dispersed in water, was stored at room temperature for further use.

Gel Permeation Chromatography

The number-average molecular weight and weight-average molecular weight of the synthesized polystyrene fluorescent microspheres were determined using a Shimadzu gel permeation chromatograph. Each sample solution was first freeze-dried to obtain a solid. Subsequently, 5 mg of each solid sample was dissolved in 0.5 mL of tetrahydrofuran (THF). Chromatography-grade THF was used as the mobile phase. Using a microsyringe, 50 μL of each sample solution was injected sequentially into the injection port for analysis. All tests were performed at room temperature.

Fourier Transform Infrared Spectroscopy

Structural analysis of the prepared polystyrene fluorescent microspheres was performed using a PerkinElmer Spectrum Two Fourier Transform Infrared Spectrometer. A small amount of the dried sample was thoroughly ground with potassium bromide until no visible particles remained. The mixture was then pressed into a pellet, which was placed in the instrument for measurement. The spectral data were collected over a wavenumber range of 4000 cm^{-1} to 500 cm^{-1}.

Photostability Test

The photostability of the prepared polystyrene fluorescent microspheres was evaluated using a Duetta fluorescence and absorption spectrometer. The sample solution was diluted with ultrapure water, and 2 mL of the diluted solution was placed in a quartz cuvette. The sample was then irradiated with a 520 nm laser at a power density of 10 mW/cm² for 2 h. The UV-vis absorption spectrum of the solution was recorded at 20 min intervals throughout the irradiation period. All measurements were conducted at room temperature.

Calibration Curve and Dye Content Determination

A mass concentration-absorption intensity calibration curve for compound RhSt was established using a Duetta fluorescence and absorption spectrometer. A stock solution of RhSt in DMF at a concentration of 1 mg/mL was prepared and subsequently diluted to a series of gradient concentrations. The UV-vis absorption spectra of these RhSt solutions at different concentrations were recorded. A linear standard curve was then constructed by plotting the concentration of RhSt against the maximum absorption intensity at 550 nm. Separately, a DMF solution of the polystyrene fluorescent microspheres was prepared at a concentration of 10 mg/mL, and its UV-vis absorption curve was measured. The dye content within the polystyrene fluorescent microspheres was calculated according to the following formula: $C = (A_{PS} / k) / 10$. C is the mass percentage of the dye in the polystyrene fluorescent microspheres; A_{PS} is the absorption intensity of the polystyrene fluorescent microsphere DMF solution at a wavelength of 550 nm; k is the slope of the RhSt mass concentration-absorption intensity standard curve.

Dye Leakage Rate Test

The dye leakage rate of the prepared polystyrene fluorescent microspheres under acidic and alkaline conditions was assessed using a Duetta fluorescence and absorption spectrometer. A 200 μL aliquot of the sample was diluted in 4 mL of simulated gastric fluid. Four parallel experiments were set up for each condition. At 1 h intervals over a total period of 4 h, the diluted solutions were centrifuged and filtered, and the supernatant was collected for fluorescence spectrum measurement. The same procedure was followed to evaluate the leakage of the fluorescent dye from the polystyrene fluorescent microspheres in simulated intestinal fluid, with the experimental duration extended to 6 h.

Acid-Base Stability Test

The stability of the prepared polystyrene fluorescent microspheres in acidic and alkaline solutions was investigated using an Anton Paar Litesizer 500 nanoscale particle size and zeta potential analyzer. Three parallel experiments were conducted. A 20 μL aliquot of the sample was mixed uniformly with 1.5 mL of simulated gastric fluid. The hydrodynamic diameter of the particles was measured at 1 h intervals over a total period of 4 h. The same procedure was followed to monitor the changes in particle size of the polystyrene fluorescent microspheres in simulated intestinal fluid, with the experimental duration extended to 6 h.

Synthesis of Gadolinium Complex Gd-DOTASt

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid tri-tert-butyl ester (500 mg, 0.87 mmol) and HATU (498 mg, 0.96 mmol) were dissolved in 20 mL of anhydrous dichloromethane (DCM). The mixture was magnetically stirred in an ice-water bath for 10 min. DIPEA (0.46 mL, 2.62 mmol) was then added dropwise, and stirring was continued in the ice-water bath for 30 min. Subsequently, intermediate **1** (260 mg, 1.27 mmol) was added. The reaction mixture was allowed

to warm to room temperature and stirred overnight. Upon completion, DCM was removed by rotary evaporation under reduced pressure. The residue was dissolved in 30 mL of ethyl acetate and washed sequentially with 30 mL of 1M dilute hydrochloric acid, saturated sodium carbonate solution, and water. The organic phase was collected, dried over anhydrous sodium sulfate, and concentrated by rotary evaporation. The crude product was purified by silica gel column chromatography using a mixture of DCM and ethyl acetate (V_{DCM}: V_{EA} = 8: 1) as the eluent. After vacuum drying, the compound **3** was obtained as a pale-yellow solid (456 mg, 0.60 mmol), yielding 68.8%.

Compound **3** (456 mg, 0.60 mmol) was dissolved in a mixed solvent of 10 mL anhydrous DCM and trifluoroacetic acid (V_{DCM}: V_{TFA} = 1: 1). The reaction mixture was stirred magnetically at room temperature for 4 h. After completion, the solvent was removed by rotary evaporation under reduced pressure, and the residue was dried under vacuum to afford DOTASt as a pale-yellow solid (325 mg, 0.55 mmol), with a yield of 91.6%. 1H NMR (400 MHz, CD_2Cl_2) δ 7.45 – 7.16 (m, 4H), 6.70 (dd, J = 17.6, 10.9 Hz, 1H), 5.74 (d, J = 17.6 Hz, 1H), 5.24 (dd, J = 11.1, 2.2 Hz, 1H), 3.58 – 2.03 (m, 36H), 1.44 (dd, J = 7.9, 5.6 Hz, 27H). 13C NMR (101 MHz, CD_2Cl_2) δ 172.60, 172.57, 170.91, 136.38, 136.35, 129.04, 128.98, 126.05, 126.02, 113.70, 113.57, 81.65, 81.53, 81.42, 62.23, 62.02, 55.50, 55.44, 54.82, 54.29, 54.22, 46.70, 45.78, 42.92, 42.32, 34.71, 33.96, 27.83, 27.78. HRMS(ESI):m/z calc.for[$C_{41}H_{70}N_6O_7$]758.5306,found781.5200[M+Na]$^+$.

DOTASt (200 mg, 0.34 mmol) and gadolinium chloride (100 mg, 0.38 mmol) were dissolved in 20 mL of ultrapure water. The mixture was sonicated for 10 min and then stirred magnetically overnight to obtain an aqueous solution of Gd-DOTASt for subsequent use. 1H NMR (400 MHz, D_2O) δ 7.69 – 7.45 (m, 4H), 6.82 (dd, J = 17.7, 11.0 Hz, 1H), 5.92 (d, J = 17.8 Hz, 1H), 5.40 (d,

J = 11.3 Hz, 1H), 4.13 – 2.81 (m, 36H). 13C NMR (101 MHz, D_2O) δ 162.87, 139.25, 135.73, 131.75, 127.59, 126.83, 120.62, 117.72, 115.95, 114.82, 111.93, 59.55, 42.87, 39.87, 33.83. HRMS(ESI):m/z calc.for[$C_{29}H_{46}N_6O_7$]590.3428,found589.3350 $[M-H]^-$.

Preparation of Fluorescent-Magnetic Polystyrene Microspheres

In a three-neck flask, fluorescent dye RhSt (5 mg), gadolinium-based agent Gd-DOTASt (5 mg), sodium dodecyl sulfate (10 mg), and ultrapure water (8 mL) were combined. The mixture was sonicated for 5 min to aid dissolution, followed by stirring at room temperature for 30 min. The flask was equipped with a condenser, and the system was purged with nitrogen using a diaphragm pump and a double-manifold line, with each cycle consisting of 2 min of evacuation to remove air from the system. The flask was then immersed in an oil bath preheated to 80°C under continuous stirring. Under nitrogen protection, styrene (1 g) was added dropwise to the reaction system via a syringe, and stirring was continued for 1 h. Subsequently, 1 mL of an aqueous potassium persulfate solution (5 mg/mL) was rapidly added as an initiator, and the reaction was allowed to proceed for 12 h. Upon completion, the reaction mixture was subjected to gradient centrifugation at 6000 r/min, 8000 r/min, and 10000 r/min for 5 min each to remove precipitates. The resulting supernatant emulsion was dialyzed against ultrapure water for 48 h to obtain the microsphere solution, which was stored at room temperature for further use.

Relaxation Time Measurement of Fl-MRIPS

Aqueous solutions of Fl-MRIPS were prepared at concentrations of 0 mg/mL, 1 mg/mL, 2 mg/mL, and 4 mg/mL. The longitudinal relaxation time (T_1) of these solutions at different

concentrations was measured using a nuclear magnetic resonance (NMR) imaging system. The longitudinal relaxivity (R_1), defined as the reciprocal of T_1 ($R_1 = 1/T_1$), was then calculated. Theoretically, a linear relationship exists between R_1 and the concentration of Fl-MRIPS. This relationship was subsequently determined by performing a linear regression fit of R_1 against concentration.

Cellular Uptake Experiment

4T1, MCF-7, and HeLa cells were seeded in confocal dishes containing 1 mL of DMEM medium, while MCF-10A cells were seeded in confocal dishes containing 1 mL of MCF-10A-specific medium. All cells were cultured in a 37°C, 5% CO_2 environment until they reached approximately 50% confluence. The culture medium was then removed, and the cells were washed three times with PBS. Subsequently, the cells were incubated with DMEM medium containing 0.5 mg/mL Fl-MRIPS for 2 h under the same culture conditions. After incubation, the cells were washed three times with PBS to remove excess microspheres and observed under a laser scanning confocal microscope. The excitation wavelength was set at 550 nm, and the emission signal was collected in the range of 560 nm–700 nm.

Cytotoxicity Assay

The cytotoxicity of Fl-MRIPS was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The principle of the MTT assay is based on the reduction of yellow tetrazolium salt to purple formazan crystals by mitochondrial succinate dehydrogenase in metabolically active cells. The resulting formazan crystals are solubilized in dimethyl sulfoxide (DMSO), and the absorbance of the solution is measured at 490 nm using a microplate reader. Cell

viability is calculated by comparing the absorbance of treated cells with that of untreated control cells.

4T1, MCF-7, and HeLa cells were seeded in a 96-well plate at a density of 5,000 cells per well and cultured for 24 h, while MCF-10A cells were seeded under the same conditions and cultured for 48 h. The culture medium was then replaced with DMEM medium containing Fl-MRIPS at a series of concentrations (1.000 mg/mL, 0.500 mg/mL, 0.250 mg/mL, 0.125 mg/mL, 0.063 mg/mL, 0.031 mg/mL, 0.016 mg/mL, and 0 mg/mL), and the cells were incubated for an additional 24 h. After treatment, the medium was carefully removed, and the cells were incubated with MTT-containing medium (0.5 mg/mL) for 4 h. The MTT medium was then aspirated, and DMSO was added to dissolve the formazan crystals. The absorbance at 490 nm was measured using a microplate reader, and cell viability was calculated accordingly.

Zebrafish Fluorescence Imaging

All the animal experiments were in accordance with the Dalian Medical University Animal Care and Use Committee (Ethics Approval Number: DUTSCE240305-08). Purchased zebrafish embryos were placed in 10 cm diameter round cell culture dishes and incubated in E3 medium supplemented with 0.1% methylene blue (composition: 5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, and 0.33 mM $MgSO_4$) at a constant temperature of 26°C. The culture medium was replaced daily, and white or developmentally arrested abnormal embryos were discarded.

For larval fluorescence imaging, 3 days post-fertilization larvae were selected and transferred to a 6-well plate. The larvae were incubated in E3 medium containing 0.2 mg/mL Fl-MRIPS for 2 h, then transferred to fresh E3 medium for washing. The larvae were anesthetized using 0.02% Tricaine (MS-222, prepared in E3 medium) until respiratory arrest with sustained heartbeat was

achieved (approximately 3 min–5 min). For *in vivo* imaging, the larvae were transferred to pre-warmed 1% low-melting-point agarose (dissolved in E3 medium) and observed under a laser scanning confocal microscope. The excitation wavelength was set at 550 nm, and emission signals were collected within the range of 560 nm–700 nm.

Mouse Magnetic Resonance and Fluorescence Imaging

The purchased mice were acclimated for 7 d under controlled conditions of 23°C–25°C temperature and 50%–60% humidity before the experiment. A 100 μL of FI-MRIPS solution (65 mg/mL) was administered via subcutaneous injection into the axillary region of mice. Subsequently, fluorescence imaging and magnetic resonance imaging were performed using a small animal *in vivo* imaging system and a small animal MRI system, respectively.

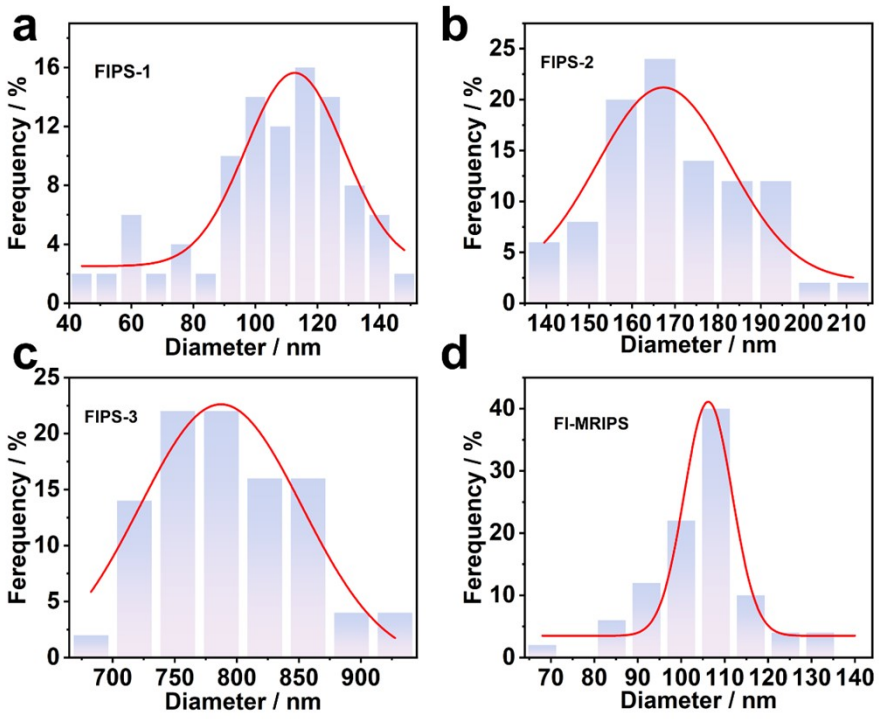

Fig. S1 The particle size distribution of the microspheres obtained from TEM image analysis: (a) FlPS-1, (b) FlPS-2, (c) FlPS-3, (d) Fl-MRIPS.

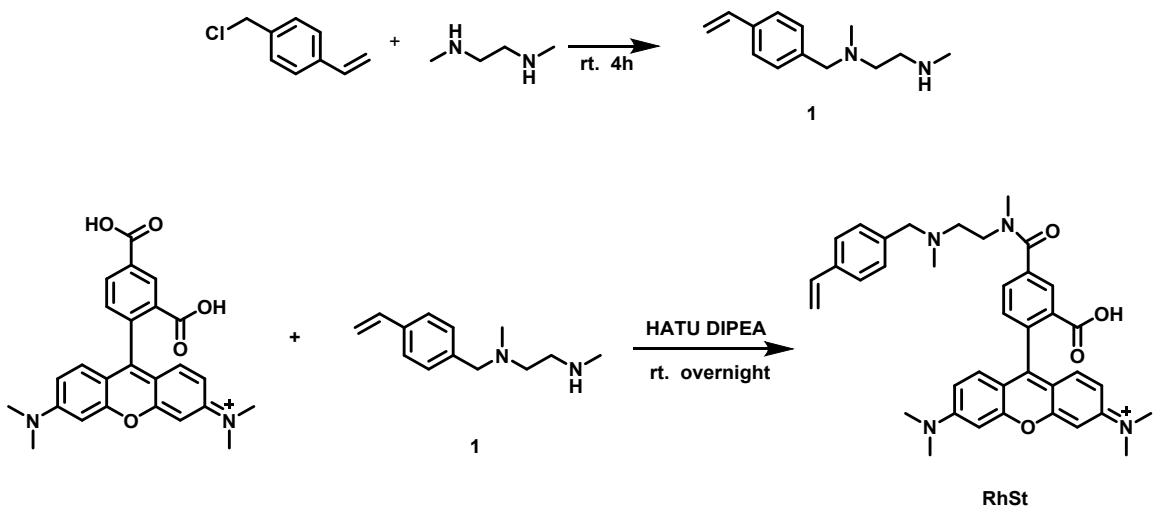

Fig. S2 Synthesis route of RhSt

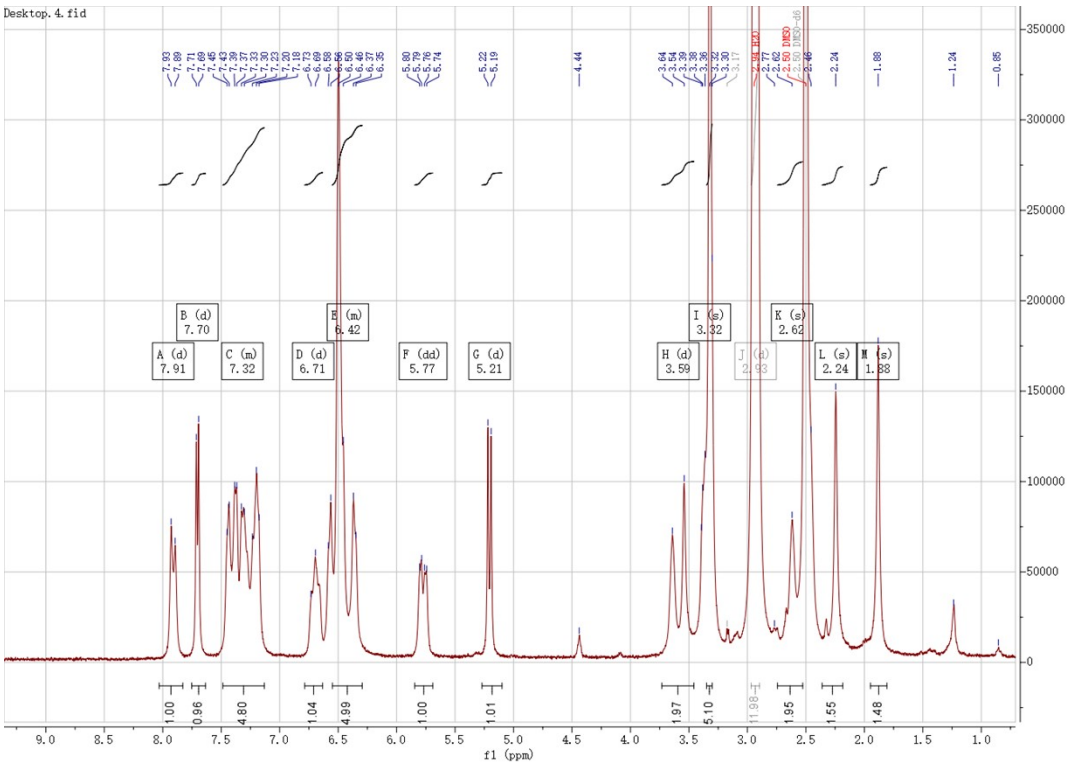

Fig. S3 ^{1}H NMR spectrum of RhSt (DMSO-d_6).

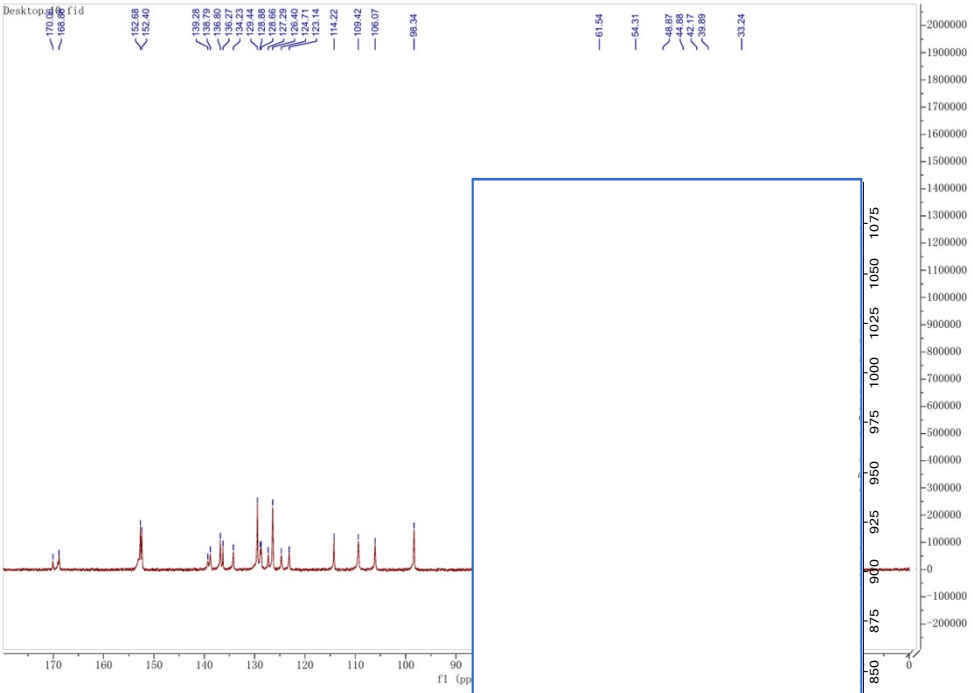

Fig. S4 ^{13}C NMR spect

Fig. S5 HR-MS spectrum of RhSt.

Table S1 The molecular weight of FlPS-1 , FlPS-2 and FlPS-3

Sample	M_n	M_w	M_w/M_n
FlPS-1	6.3×10^4	1.2×10^5	1.90
FlPS-2	8.0×10^4	2.8×10^5	3.55
FlPS-3	5.4×10^4	2.0×10^5	3.80

Fl-MRIPS	4.7×10^4	2.5×10^5	5.30

Table S2 The corresponding maximum absorption and emission wavelengths

Solvent	λab /nm	λem /nm	Absolute Photoluminescence Quantum Yield φ_f
Water	550	580	0.404
PBS	550	580	-
Methanol	540	570	0.445
Ethanol	540	574	-
Dichloromethane	550	574	0.787

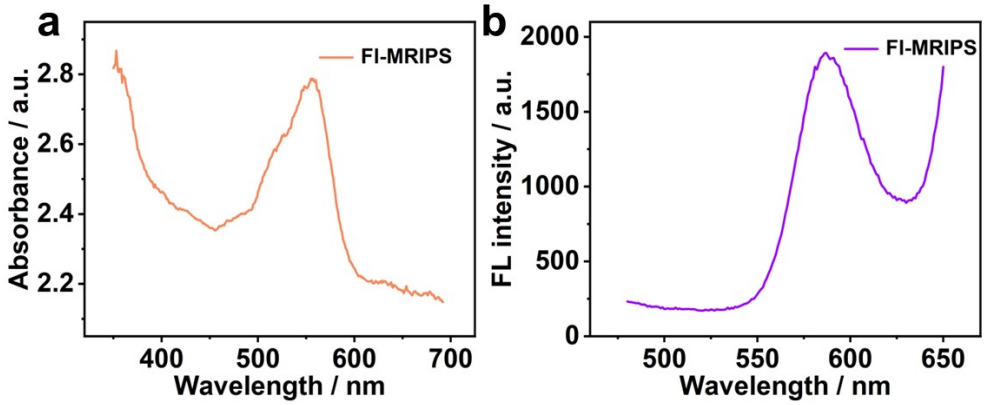

Fig. S6 UV-vis Absorption (a) and Fluorescence (b) Spectra of Fl-MRIPS.

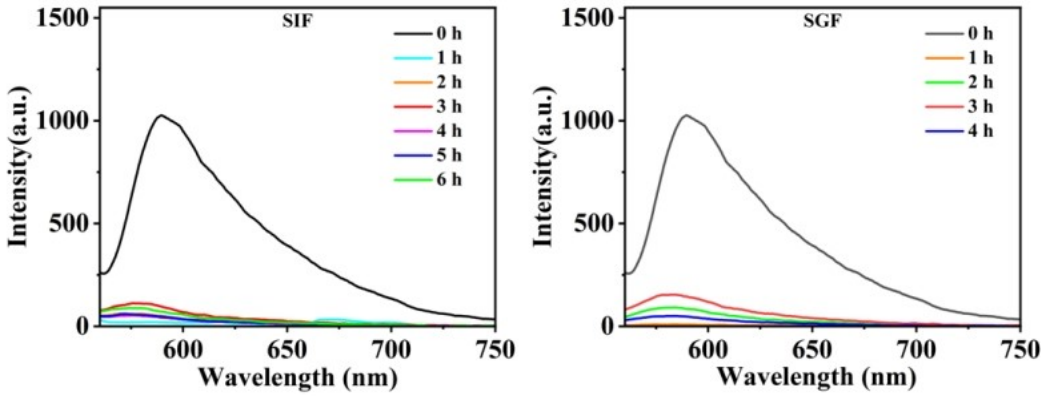

Fig. S7 Dye leakage test of FlPS-1 in SIF and SGF.

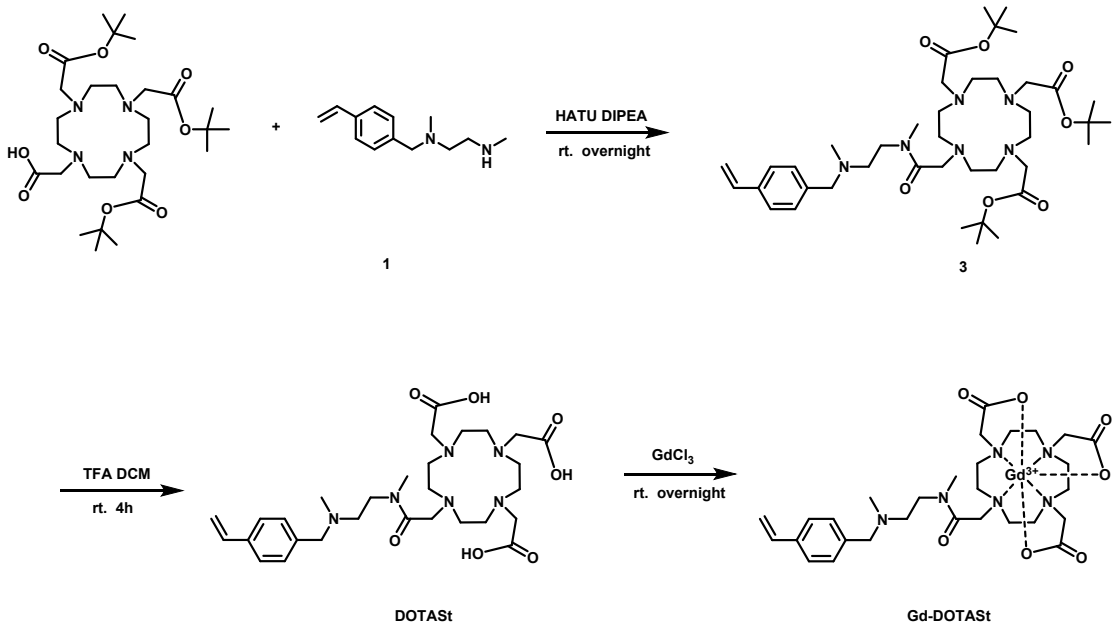

Fig. S8 Synthesis route of Gd-DOTASt.

Fig. S9 ^{1}H NMR spectrum of compound **3** (CD_2Cl_2).

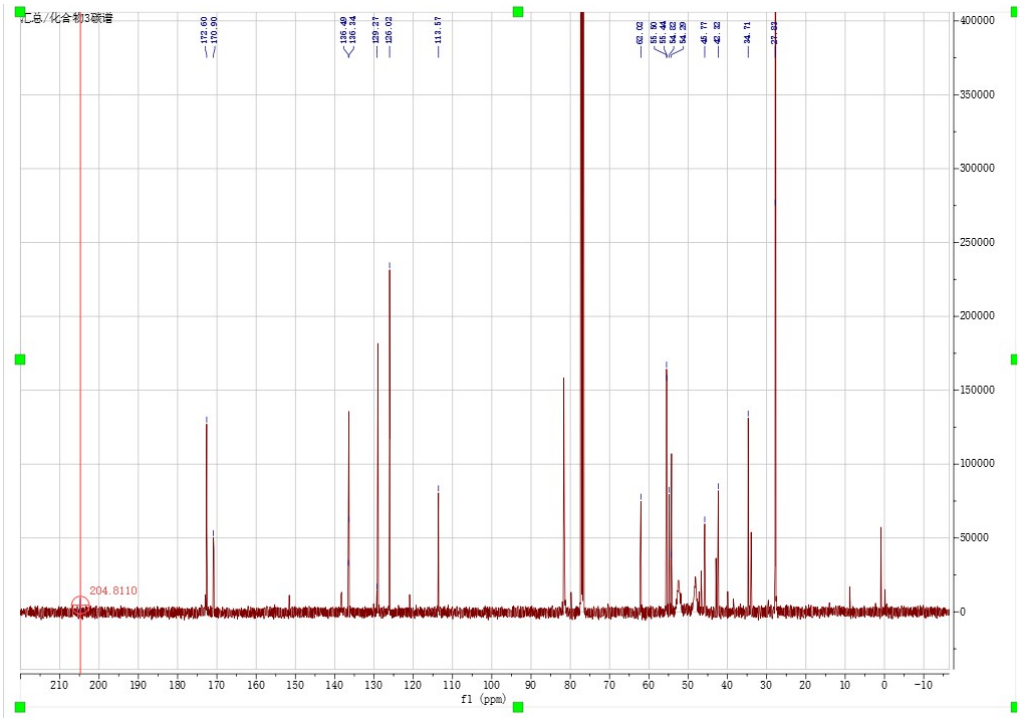

Fig. S10 ^{13}C NMR spectrum of compound **3** (CD_2Cl_2).

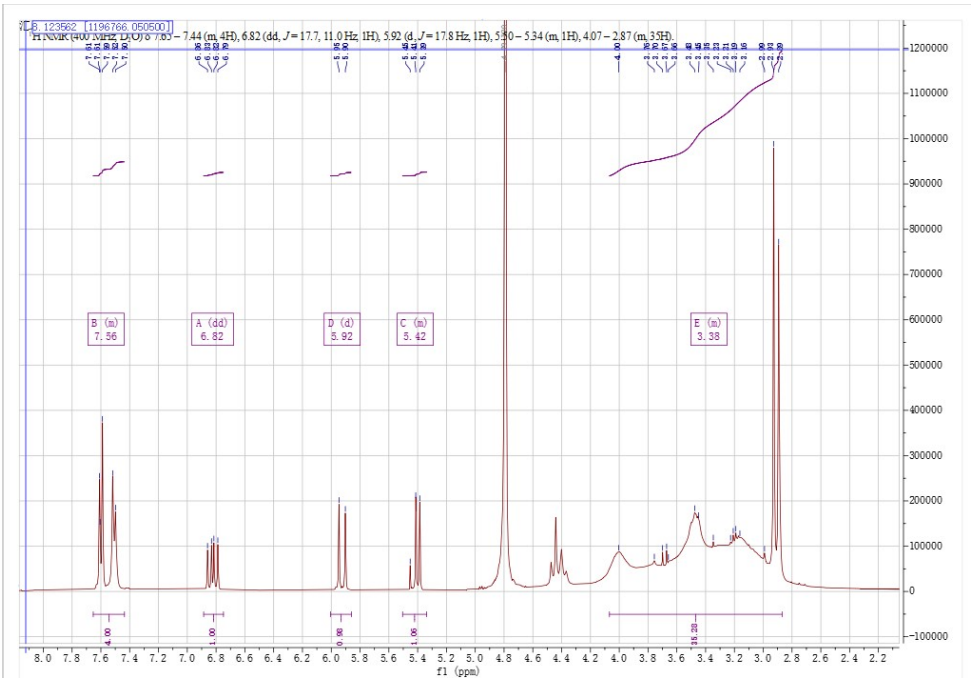

Fig. S11 ^{1}H NMR spectrum of DOTASt (D_2O).

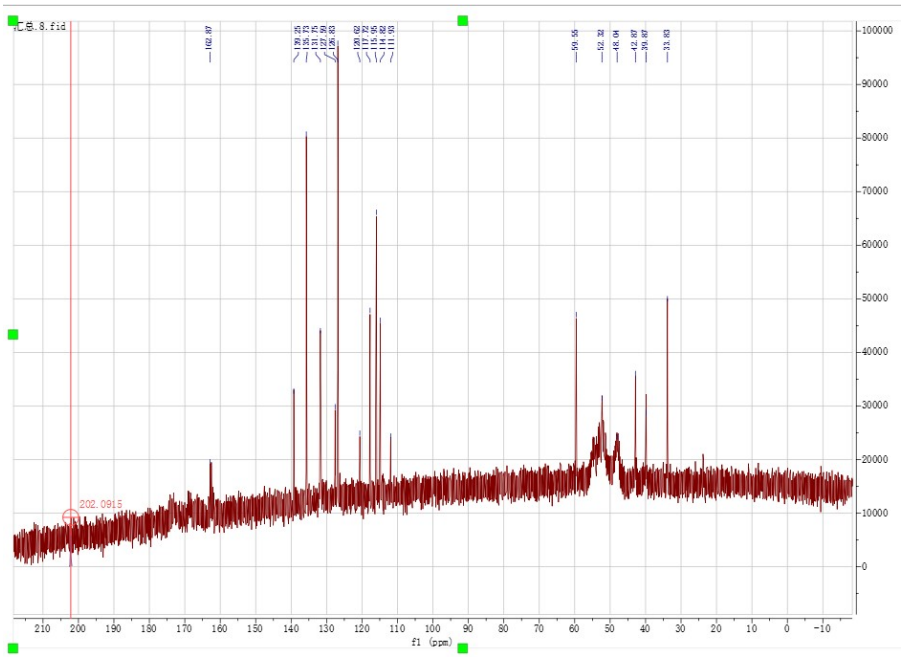

Fig. S12 ^{13}C NMR spectrum of DOTASt (D_2O).

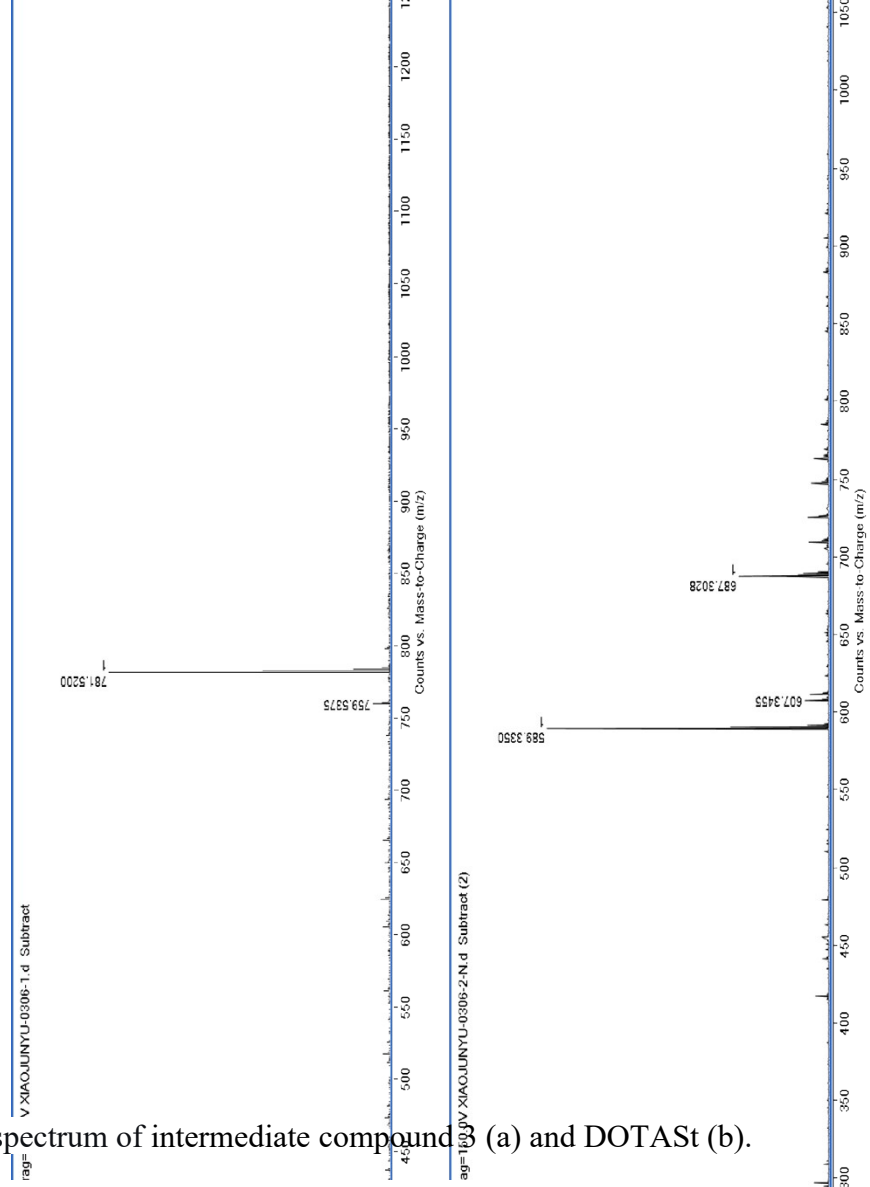

Fig. S13 HR-MS spectrum of intermediate compound 3 (a) and DOTASt (b).

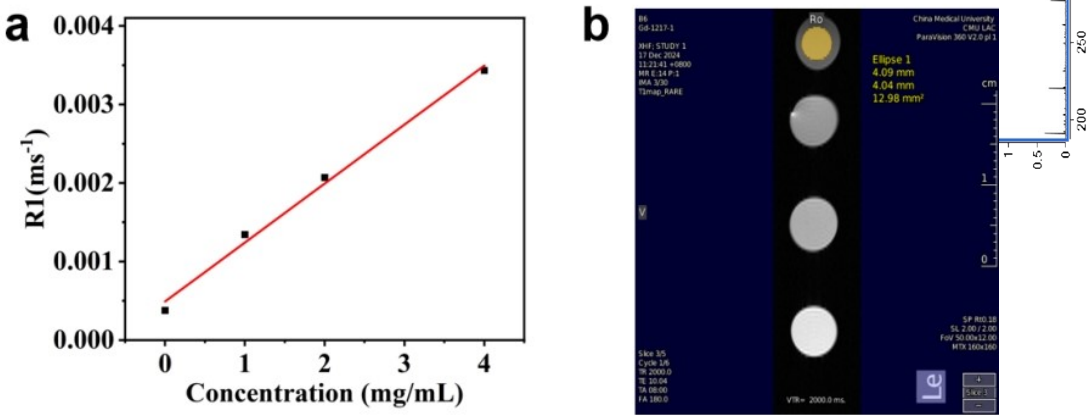

Fig. S14 (a) Relaxation rate-concentration relationship graph of Fl-MRIPS, (b) Magnetic resonance imaging of Fl-MRIPS at different concentrations.

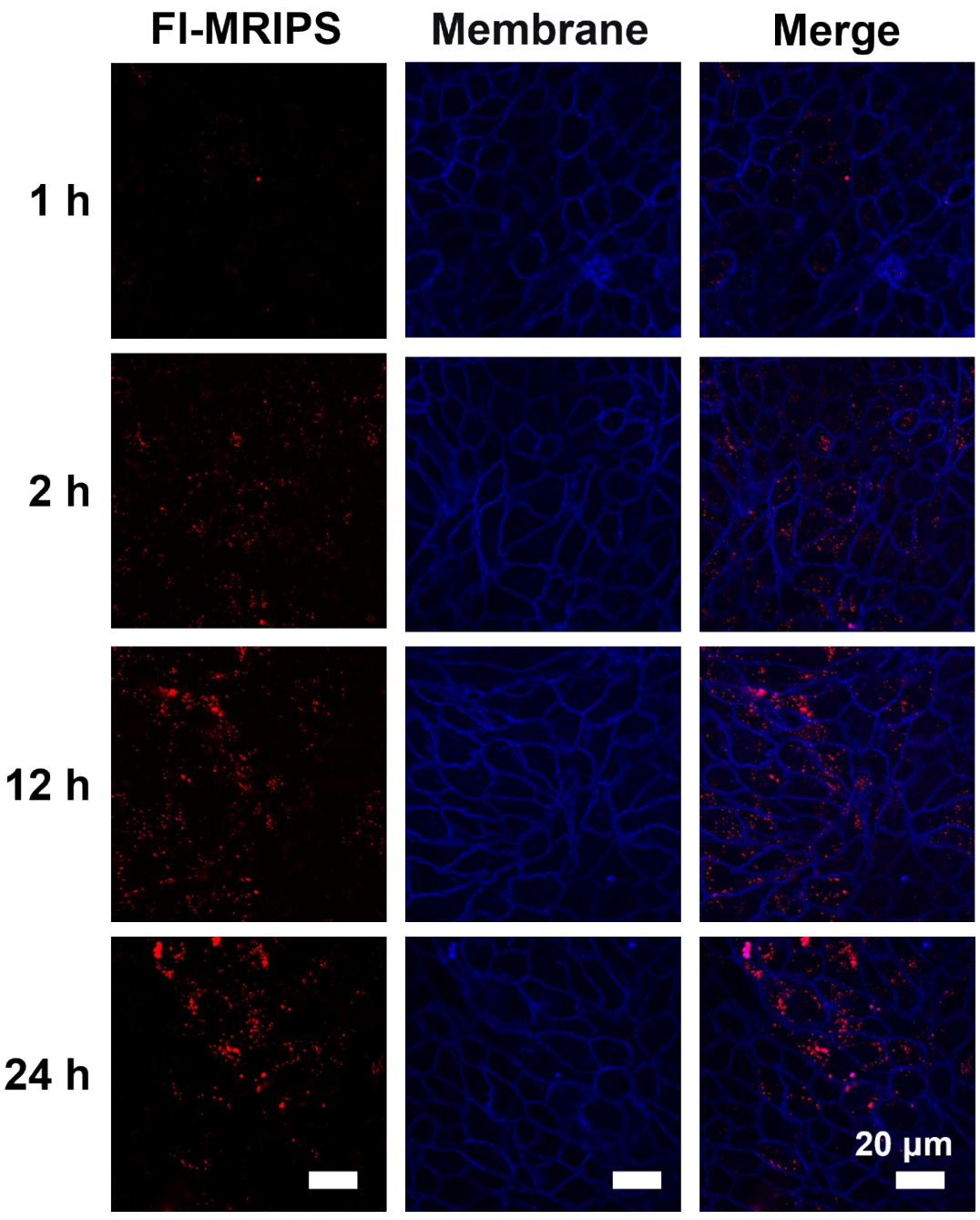

Figure S15. Cellular uptake of FI-MRIPS by 4T1 cells at different time points.

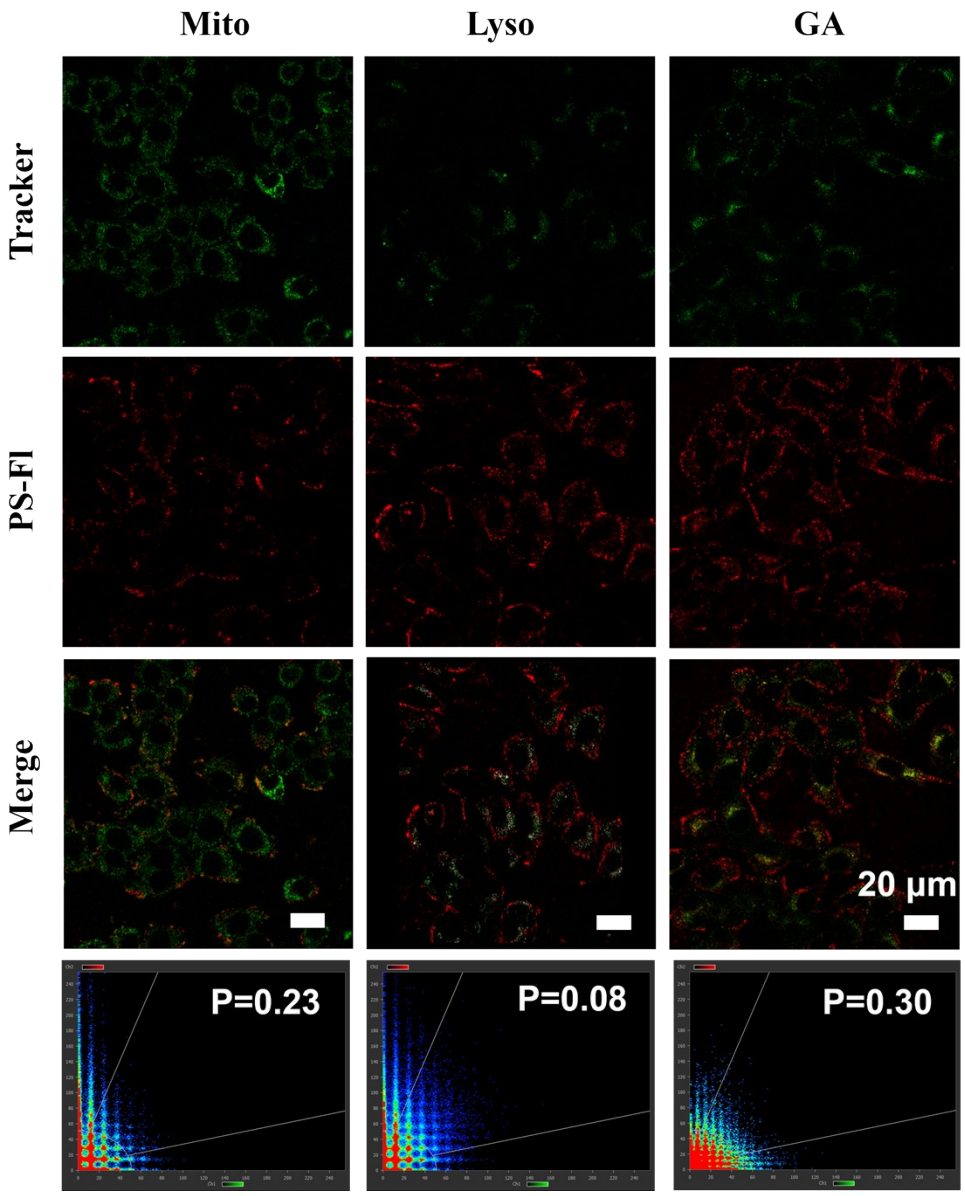

Figure S16. Co-localization of Fl-MRIPS with Mitochondria, Lysosomes, and the Golgi Apparatus in 4T1 Cells

Table S3. Comparison with other imaging strategies.

Materials	Imaging Modalities	Labeling Method	
YIGSR-Gd-DTPA-RhB、RGD-Gd-DTPA-RhB	**MRI + FL**	**Covalent conjugation**	[1]
Protein-based probe and LBT-Gd complex	**MRI + FL**	**Biosynthetic fusion + non-Covalent chelation**	[2]
CIZS@FeOOH	**MRI + FL**	**Physical coating + in situ reaction**	[3]
DEPN@MMT	**FL**	**Physical composite**	[4]
Fe_3O_4@SiO_2@$NaYF_4$:Er^{3+}	**MRI + FL**	**---**	[5]
AuNS@M@Ag	**SERS + PRRS**	**Covalent conjugation + physical/chemical deposition**	[6]
Au@PEI-Gd-AAG NPs	**CT + MRI**	**Covalent conjugation + in situ reduction**	[7]
MoO_2@SiO_2-Cy5.5	**FL + XRF**	**Physical coating + covalent conjugation**	[8]
QD-PDDA-Apt/ AuNS-cDNA-PEG	**SERS + FL**	**Covalent conjugation**	[9]
Fl-MRIPS	**MRI + FL**	**Covalent conjugation**	**This work**

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