Electronic Supplementary Information for

Vibration induced emission based internal standard fluorogenic probe for visualizing PTP1B in living cells

Qiuyu Gong,[a,b#] Wenjing Qin,[a#] Peng Xiao,[c] Xiang Wu,[c] Lin Li,[a*] Gaobin Zhang,[a] Renshuai Zhang,[a] Jinpeng Sun,[c*] Shao Q. Yao,[b*] Wei Huang,[a,d]

[a]Key Laboratory of Flexible Electronics (KLOFE) & Institute of Advanced Materials (IAM), Nanjing Tech University (Nanjing Tech), 30 South Puzhu Road, Nanjing, 211816, P. R. China

[b]Department of Chemistry, National University of Singapore, 3 Science Drive 3, 117543, SingaporeKey

[c]Key Laboratory Experimental Teratology of the Ministry of Education, Department of Biochemistry and Molecular Biology, Shandong University School of Medicine, Jinan, Shandong 250012, P. R. China

[d]Shaanxi Institute of Flexible Electronics (SIFE), Northwestern Polytechnical University, 127 West Youyi Road, Xi'an, 710072, P. R. China

Email: iamlli@njtech.edu.cn; sunjinpeng@sdu.edu.cn; chmyaosq@nus.edu.sg

1. Experimental

Reagents. Boron tribromide (BBr_3), sodium orthovanadate (Na_3VO_4), glutathione (GSH), lipase (Type VII, L1754), protease (P6911), phosphatase-acid (ACP, P1146), alkaline phosphatase (ALP, P7640), dimethyl sulfoxide (DMSO), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) and sodium borohydride ($NaBH_4$) were obtained from Sigma-Aldrich Merck. 4-Iodoanisole, phosphorus oxychloride ($POCl_3$) and pyridine were purchased from Alfa Aesar Chemicals. 1,3,5-Trichlorobenzene was obtained from Apollo Scientific Ltd. Copper trifluoromethanesulfonate $(CF_3SO_3)_2Cu$ was purchased from Energy Chemicals. Pyroglutamate aminopeptidase 1 (PGP-1; molecular weight, 23 kDa, ZJ-01-031) was obtained from State Key Laboratory of Antibody Medicine and Targeted Therapy, Shanghai, China. Dipeptidyl peptidase IV (DPP IV, enz-375) was obtained from Prospec. Ltd. Phosphatases were obtained in Department of Physiology, School of Medicine, Shandong University. Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Biological Industries Ltd. 10 mM phosphate buffered saline (PBS) of pH 7.4 was obtained from Invitrogen. 6,8-Difluoro-4-methylumbelliferyl

phosphate (DiFMUP, a commercial phosphatase probe) was purchased from Thermo Fisher Scientific Ltd. Rabbit polyclonal PTP1B antibody (11334-1-AP) was purchased from Proteintech, Co. Ltd., USA. Ultrapure water (over 18 MΩ·cm) from a Milli-Q reference system (Millipore) was used throughout. The stock solution (200 μM) of **Q1** was prepared by dissolving requisite amount of it in DMSO. Stock solutions of other substances were prepared by dissolving in PBS or water. All other reagents were local.

Apparatus. Fluorescence measurements were made on a Fluoromax-4 Spectrofluorometer (France) or Cytation 5 microtiter plate assay system (Bio Tek, USA). ^{1}H NMR and ^{13}C NMR spectra were measured with a Bruker Avance 300 or 500 spectrometer in DMSO-d_6. The fluorescence quantum yield (Φ) was determined by using a quantum efficiency measurement system: QE-2100 (Qtsuka Electronics Co. Ltd., Japan). Cytotoxicity assay was made on microtiter plate assay system (SynergyHTX, Bio Tek, USA). Protein quantifications were made on a Nano-300 Micro spectrophotometer. All images were acquired on a Zeiss LSM880 NLO (2+1 with BIG) Confocal Microscope System (Germany). Gel imaging was performed on Aplegen Omega Lum W imaging system (Aplegen, USA).

Synthesis. Compound **2** was obtained according to the following method: In a 100 ml one-neck flask, starting material **1** (1.8 g, 5 mmol; synthesized according to the reported literature[S1]), 4-iodoanisole (3.5 g, 15 mmol), K_2CO_3 (4.15 g, 30 mmol), $(CF_3SO_3)_2Cu$ (180 mg, 0.5 mmol) and 1,3,5-trichlorobenzene (6 g) were added. The mixture stirred for 8 h at 210 °C. After reaction, dichloromethane (DCM) was used to extract the product, and the solvent was removed by reduced pressure. The crude product was purified by column chromatography on silica (n-hexane:DCM = 1:1) to give a faint yellow solid **2** (580 mg, yield: 25 %). The ^{1}H NMR and ^{13}C NMR spectra of compound **2** are shown below in Figure S1 and Figure S2, respectively. ^{1}H NMR (500 MHz, 298K, DMSO-d_6): δ 8.911 (d, J = 3.5 Hz, 2H), 8.050-8.009 (m, 2H), 7.889-7.878 (t, 2H), 7.843-7.627 (m, 4H), 7.589-7.559 (t, 2H), 7.387-7.125 (m, 4H), 7.110-6.991 (t, 2H), 6.878-6.850 (t, 1H), 6.729-6.721 (t, 2H), 3.617 (d, J = 1.25 Hz, 3H). ^{13}C NMR (125 MHz, 298K, DMSO-d_6): δ 155.169, 147.915, 144.985, 142.580, 140.874, 137.493, 135.488, 129.656, 129.098, 128.885, 128.478, 128.194, 127.249, 127.118, 126.960, 126.633, 126.499, 125.850, 125.156, 124.827, 123.837, 123.547, 123.506, 123.477, 121.795, 120.977, 116.281, 114.246, 55.045.

Compound **2** (464 mg, 1 mmol) was dissolve in 15 mL of dry DCM, and BBr_3 (142 μL, 1.5 mmol) was added into above solution slowly at -78 °C under inert gas protection. The mixture was stirred at room temperature overnight. And then methanol (MeOH) was added to quench the reaction, solvent was removed by reduced pressure. The crude product was purified by column chromatography on silica (n-hexane:ethyl acetate = 2:1) to give a colorless solid **3** (292 mg, yield: 65 %). The ^{1}H NMR and ^{13}C NMR spectra of compound **3** are shown below in Figure S3 and Figure S4, respectively. ^{1}H NMR (500 MHz, 298K,

DMSO-d_6): δ 9.187 (s, 1H), 8.885 (d, *J* = 6.5 Hz, 2H), 8.019-7.980 (m, 2H), 7.824-7.530 (m, 6H), 7.351-7.320 (m, 2H), 7.136-6.843 (m, 7H), 6.524 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, 298K, DMSO-d_6): δ 153.721, 148.277, 145.400, 142.122, 139.518, 137.711, 134.883, 129.721, 128.965, 128.884, 128.565, 128.223, 127.288, 127.165, 126.857, 126.583, 126.380, 125.923, 124.519, 123.954, 123.457, 123.173, 120.916, 116.204, 115.564. HR-ESI-MS, calcd for $C_{32}H_{21}N_2O^-$[M-H]$^-$: *m/z* 449.1654; found [M-H]$^+$: *m/z* 449.1646.

Compound **3** (225 mg, 0.5 mmol) was dissolved in dry CH_2Cl_2 (30 mL) and stirred at 0 °C under inert gas protection. And $POCl_3$ (0.2 mL, 2 mmol) and pyridine (0.3 mL, 3.7 mmol) were added though syringe. After that, the reaction solution was stirred at room temperature for 2 h. Then, ice water (50 mL) was added, and the reaction solution was extracted with DCM/ethanol (EtOH). The solvents were removed by reduced pressure. The crude product was purified by column chromatography on silica (DCM:MeOH = 10:1) to give a white solid: **Q1** (66 mg, yield: 25 %). The ^{1}H NMR and ^{13}C NMR spectra of **Q1** are shown below in Figure S5 and Figure S6, respectively. ^{1}H NMR (500 MHz, 298K, DMSO-d_6): δ 8.887 (d, *J* = 4 Hz, 2H), 7.975 (d, *J* = 4 Hz, 2H), 7.822 (d, *J* = 4.25 Hz, 2H), 7.692-7.602 (m, 3H), 7.529 (s, 1H), 7.339 (d, *J* = 3.5 Hz, 2H), 7.017-6.875 (m, 8H), 6.792 (s, 1H). ^{13}C NMR (125 MHz, 298K, DMSO-d_6): δ 129.624, 129.513, 128.904, 128.421, 128.222, 127.291, 127.101, 127.009, 126.682, 125.791, 123.845, 123.550, 121.046, 120.193, 116.360. HR-ESI-MS, calcd for $C_{32}H_{24}N_2O_4P$[M+H]$^+$: *m/z* 531.1474; found [M+H]$^+$: *m/z* 531.1503 (Figure S7).

General procedure for phosphatase detection. Unless otherwise stated, all the fluorescence measurements were made according to the following procedure. In a test tube, 2 μL of stock solution of **Q1**, 2 μL of 1 % Tritox-100 solution and appropriate volume of PBS were mixed, followed by addition of an appropriate volume of phosphatase or other substance solutions. The mixed solution was adjusted to 200 μL with PBS. After incubation at 37 °C for appropriate time (0-60) min, the reaction solution was transferred to a quartz cell to measure fluorescence with λ_{ex} = 360 nm (both excitation and emission slit widths were set to 2.5 nm). At the same time, a blank solution without phosphatase was prepared and measured under the same conditions for comparison. Data are expressed as mean ± standard deviation (SD) of three separate measurements. For enzymatic activities, 2 μL of stock solution of DiFMUP and appropriate volume of PBS were used, followed by addition of an appropriate volume of phosphatase solutions (the finally volume is 200 μL).

Protein expression, purification and activity determination. The expression and purification of phosphatase (Gleep, PTP1B, StepCD, N12, SSH2, PPM1A, PPM1B, PPM1K) were accomplished as follows: (1) Plasmid was transformed into E. coli (BL21: competent cells) and was selected on fresh agar plates containing ampicillin or kanamycin (100 μg/mL). (2) A single colony of E. coli was inoculated into 200 mL of LB media (containing ampicillin or kanamycin, 100 μg/mL) with rotation overnight at 37 °C (200 rpm). (3) 30 mL of E. coli

culture was transferred into 1.2 L of LB media with vigorous aeration at 37 °C and the mixture was incubated until OD_{600} reached 0.6-0.8. (4) A final concentration of 0.3 mM isopropyl-b-D-thiogalactopyranoside (IPTG) was added into bacteria solution, and the solution was incubated with vigorous aeration for 10-12 h at 25 °C. (5) The cells were centrifuged at 3000 *x*g for 30 min at 4 °C and the supernatant was removed. Then the pellet was re-suspended with 40 mL of His buffer (25 mM Tris-HCl, pH=8.0, 150 mM NaCl). (6) The solution was fragmented with high pressure for 4-5 times until the solution is no longer ropy. The solution must be kept cooled on ice. (7) Cell debris was sedimentated at 120,000 *x*g for 60 min at 4 °C in an ultracentrifuge. (8) After high speed centrifugation, the supernatant was poured into 50 mL centrifuge tube. 1 mL of His beads (Roche, cOmplete™ His Tag Purification Resin) were added and the mixture was incubated at 4 °C chromatography refrigerators with rotary mixing (3 r/min) for 2 h. (9) The blinding beads were applied to His affinity column and the disturbing beads were removed flow through. (10) Beads were washed in 10 mL of His buffer for 3 times. (11) Protein purification is achieved by washing the beads via 50 mL of His buffer containing 50 mM imidazole concentration. (12) The His-tagged protein was eluted from the column with 10 mL of His buffer containing 50 mM imidazole. (13) The protein concentration of the brownish main elution fractions were immediately determined by nano-300 micro spectrophotometer. (14) The proteins were transferred to the enrichment column and centrifuged at 4000 *x*g until the final concentration were between 1 and 10 mg/mL. (15) Salt ions and imidazole in the protein were removed by molecular sieve. Glycerin was added (final concentration: 20 %) into proteins. And the proteins were flash freezed by liquid nitrogen and storaged in the -80 °C refrigerator.

Cell culture and cytotoxicity assay. HepG2 cells were cultured in DMEM and supplemented with 10% FBS under 37 °C with 5% CO_2 at an incubator. Cells were seeded in 96-well U-bottom plates with 70 %-80 % degrees of fusion per well and incubated with **Q1** or dye at varied concentrations (1-50 μM) at 37 °C for 24 h. Then, the culture media were discarded, and 10 μL of the MTT solution (0.5 mg/mL in PBS) was added to each well, followed by incubation at 37 °C for 4 h. The supernatant was abandoned, and 150 μL of DMSO was added to each well to dissolve the formed formazan. After shaking the plates for 10 min under dark, absorbance values of the wells were read with microtiter plate assay system at 490 nm. The cell viability rate (VR) was calculated according to the equation: $VR = A/A_0 \times 100$ %, where A is the absorbance of the experimental group (i.e., the cells treated with **Q1** or dye) and A_0 is the absorbance of the control group (i.e., the cells untreated). The cell survival rate from the control group was considered to be 100%.

Cell imaging. All cells were cultured DMEM and supplemented with 10 % FBS under 37 °C with 5 % CO_2 at an incubator. Before cell imaging, the culture media were removed, and the cells were washed using DMEM for three times. Then, the cells were incubated with **Q1** (2 μM) at 37 °C for appropriate time DMEM containing 1 % DMSO as co-solvent (for

inhibitor experiments, the cells were first treated with appropriate concentration of inhibitor for 30 min and then treated with **Q1**), and subjected to fluorescence imaging experiments. λ_{ex} = 360 nm, λ_{em}: green channel: 430-530 nm; red channel: 550-650 nm. Unless otherwise noted, data are expressed as mean ± standard deviation (SD) of three separate measurements.

Detection of phosphatase in cell lysate. To prepare cell lysate, HeLa/LO2 cells (~$1*10^7$) were grown for 48 h in DMEM/RPMI 1640 supplemented with 10 % FBS and 1 % penicillin-streptomycin in 10 cm tissue culture plate. After aspirating the media, the cells were washed three times with 1 mL of cold PBS buffer. 1 mL of cold PBS buffer containing 0.4 % triton was added into the plate and cell scraper was used to harvest the cells. The cells were transferred to a 1.5 mL tube and then clarified by centrifugation at 4 °C and 13,000 *x*g for 5 min. Finally, the supernatant was transferred to a clean tube on ice for subsequent fluorescence measurement.

Western blot. The western blot analysis was performed according the previous method (Liu, et al, *Sensor Actuat. B: Chem.* **2018**, *279*, 38-43).

2. Supporting Figures

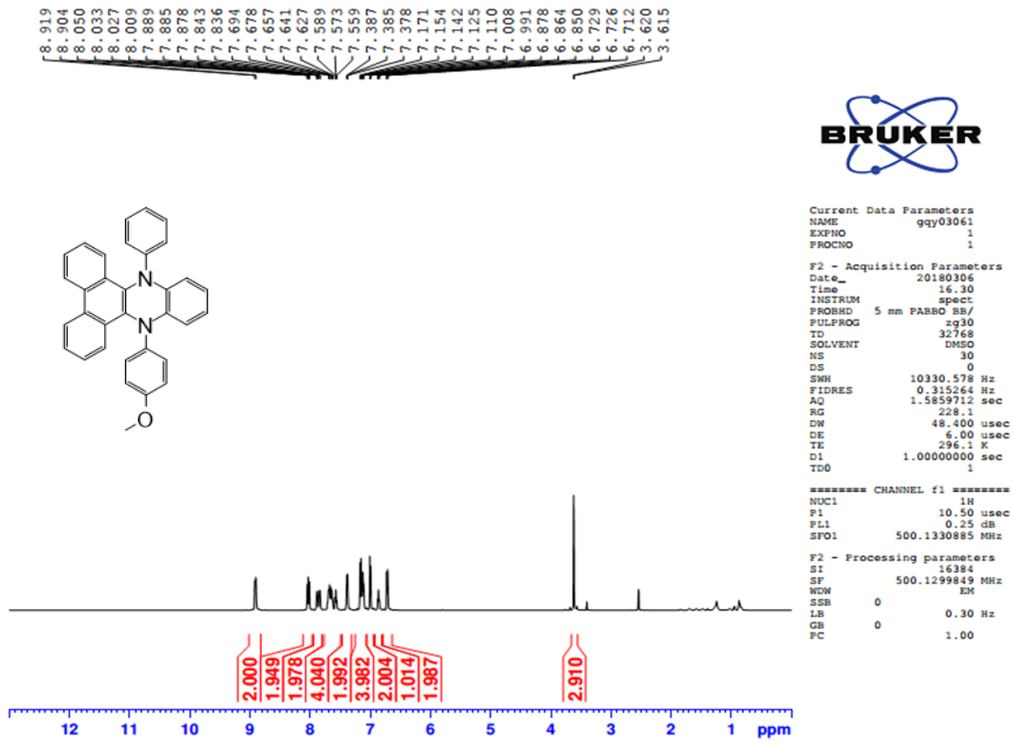


Figure S1. The ^{1}H NMR spectrum of compound **2** (500 MHz, DMSO-d_6, 298 K).

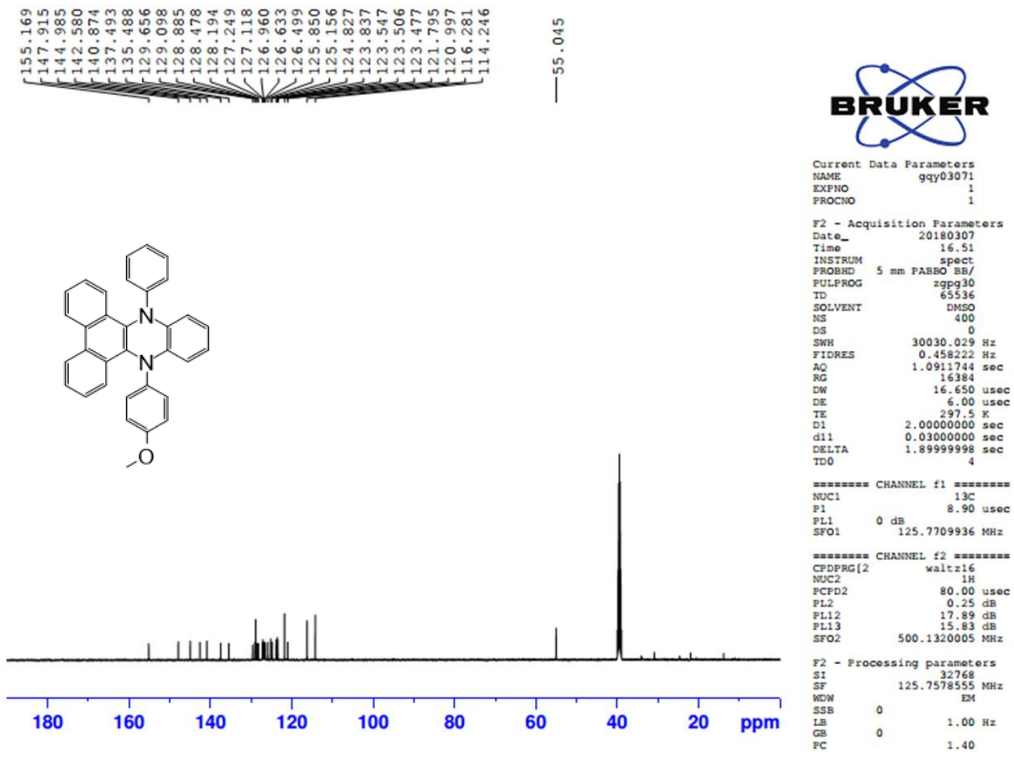


Figure S2. The ^{13}C NMR spectrum of compound **2** (125 MHz, DMSO-d_6, 298 K).

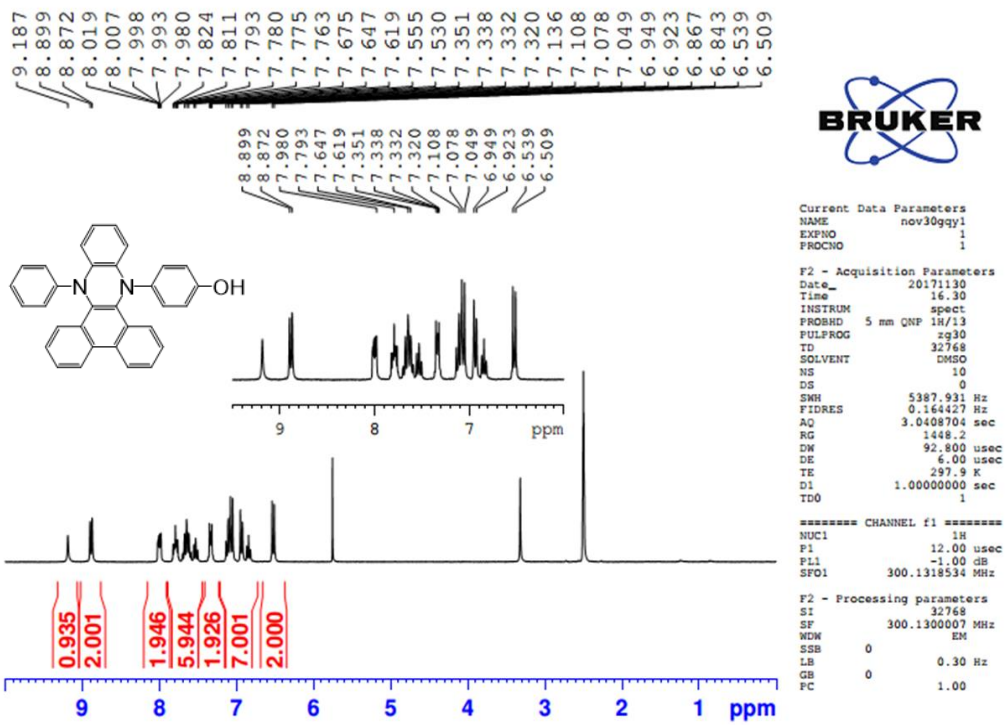


Figure S3. The ^{1}H NMR spectrum of compound **3** (500 MHz, DMSO-d_6, 298 K).

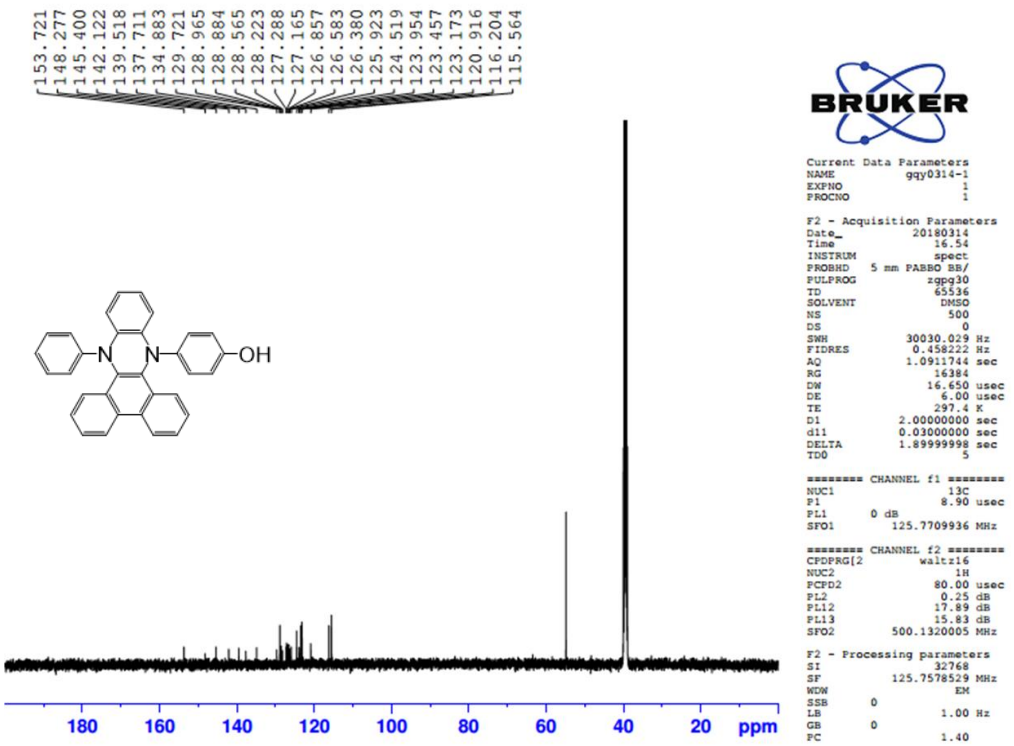


Figure S4. The ^{13}C NMR spectrum of compound **3** (125 MHz, DMSO-d_6, 298 K).

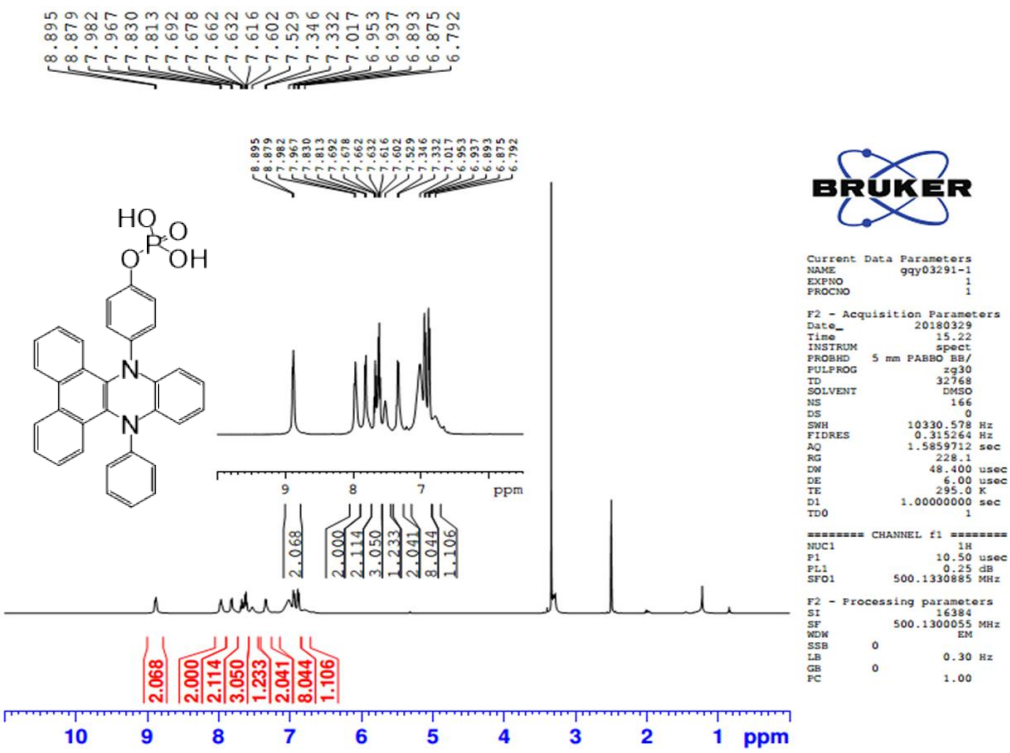


Figure S5. The ^{1}H NMR spectrum of **Q1** (500 MHz, DMSO-d_6, 298 K).

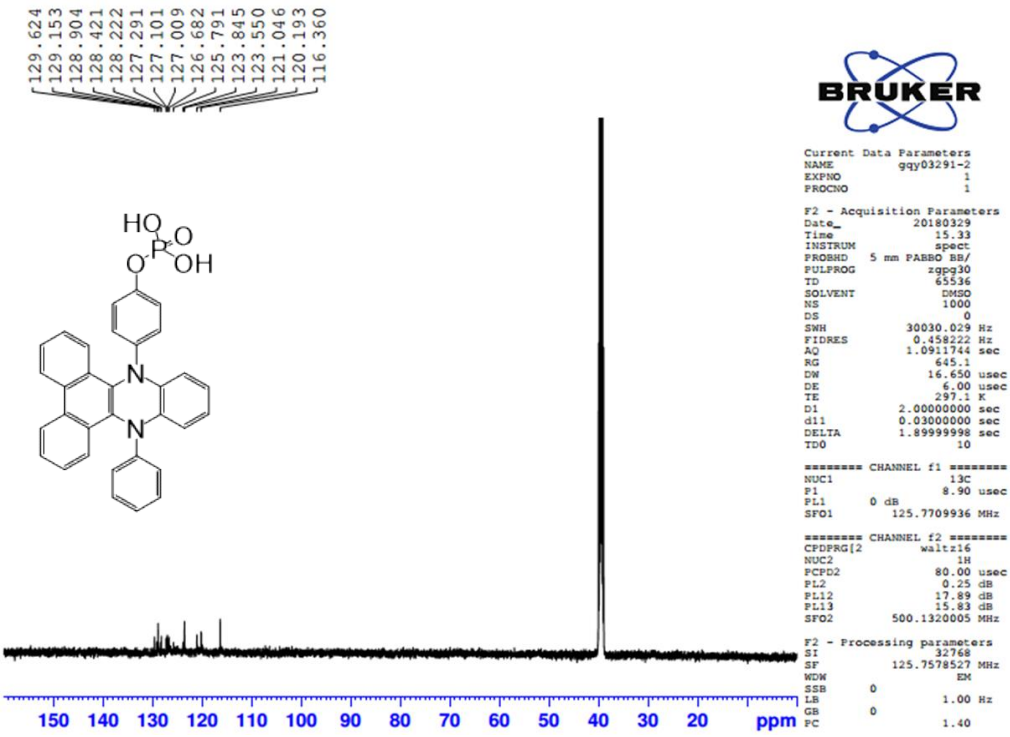


Figure S6. The ^{13}C NMR spectrum of **Q1** (125 MHz, DMSO-d_6, 298 K).

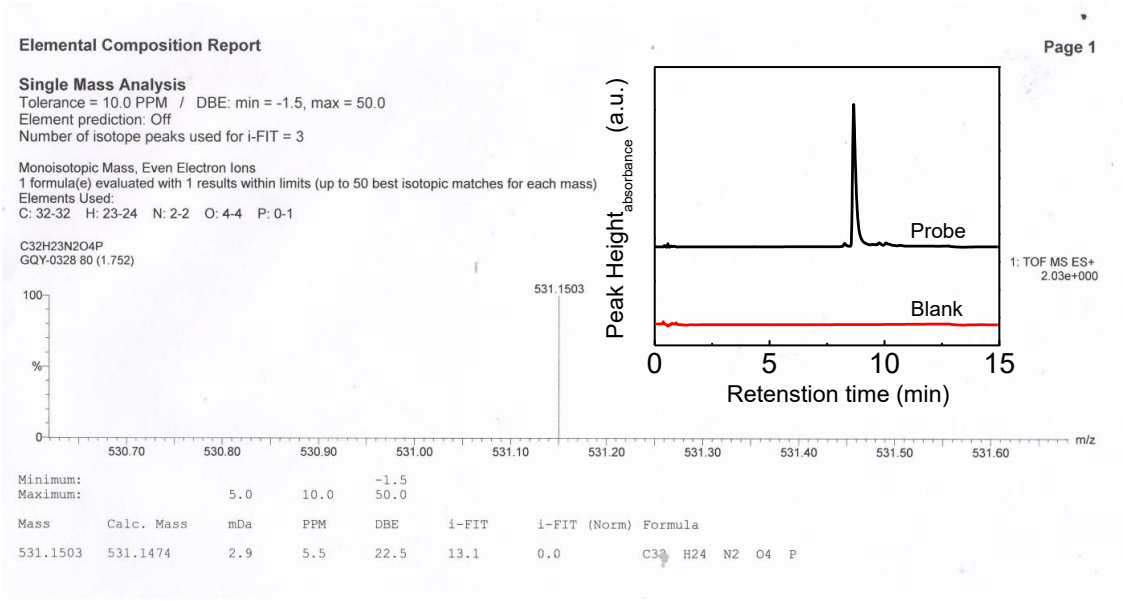


Figure S7. The HR-MS spectrum of **Q1**. The inset shows the HPLC analysis of **Q1**.

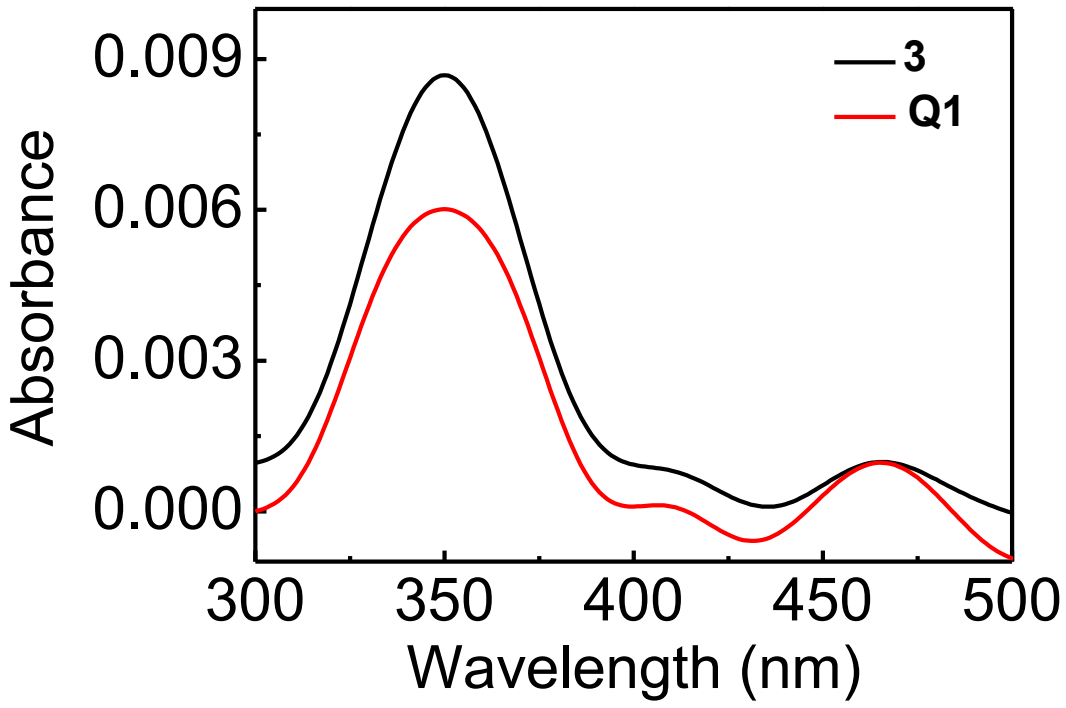


Figure S8. The absorbance spectrum of fluorophore (**3**) and probe (**Q1**).

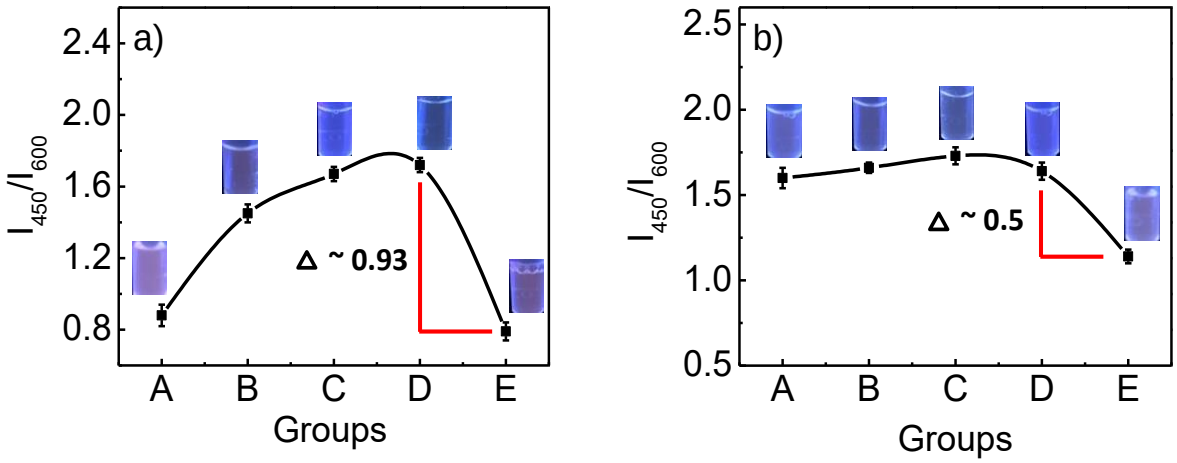


Figure S9. (a) I_{450}/I_{600} of **Q1** (2 μM) in different solutions: 10 mM PBS with A. 20 %; B. 10 %; C. 5 %; D. 1 % DMSO, respectively. E. System D with 0.1 % Triton-100. (b) I_{450}/I_{600} of compound **3** (2 μM) in different solutions: 10 mM PBS with A. 20 %; B. 10 %; C. 5 %; D. 1 % DMSO, respectively. E. System D with 0.1 % Triton-100. λ_{ex} = 360 nm.

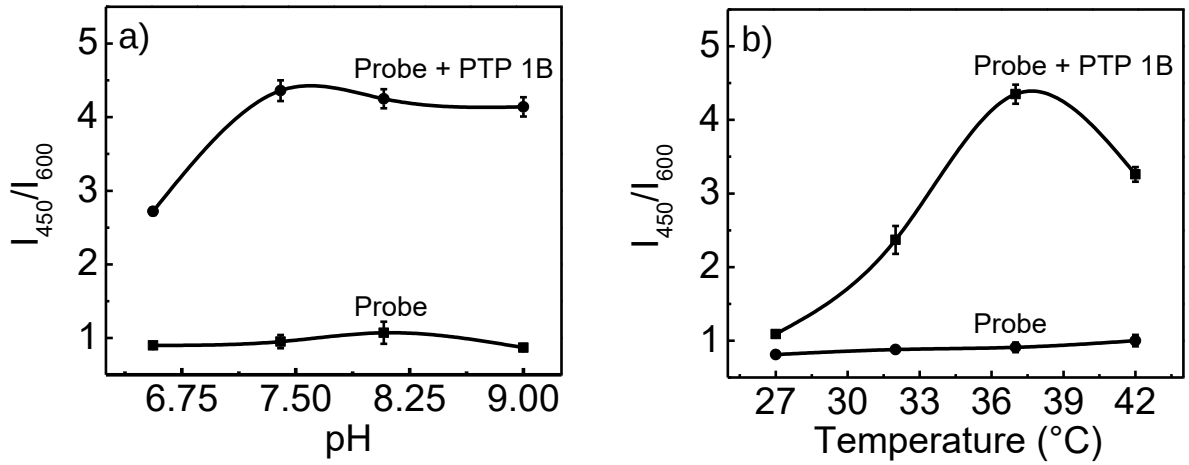


Figure S10. Effects of (a) reaction temperature and (b) pH on I_{450}/I_{600} of 2 μM **Q1** without and with PTP 1B (50 ng/μL). Conditions: (a) the reaction was performed at 37 °C in 10 mM PBS for 40 min at different pH containing 1 % DMSO and 0.01 % Triton-100 as co-solvent. (b) The reaction was performed in 10 mM PBS (pH 7.4) containing 1 % DMSO and 0.01 % Triton-100 as co-solvent for 40 min at different temperatures. λ_{ex} = 360 nm.

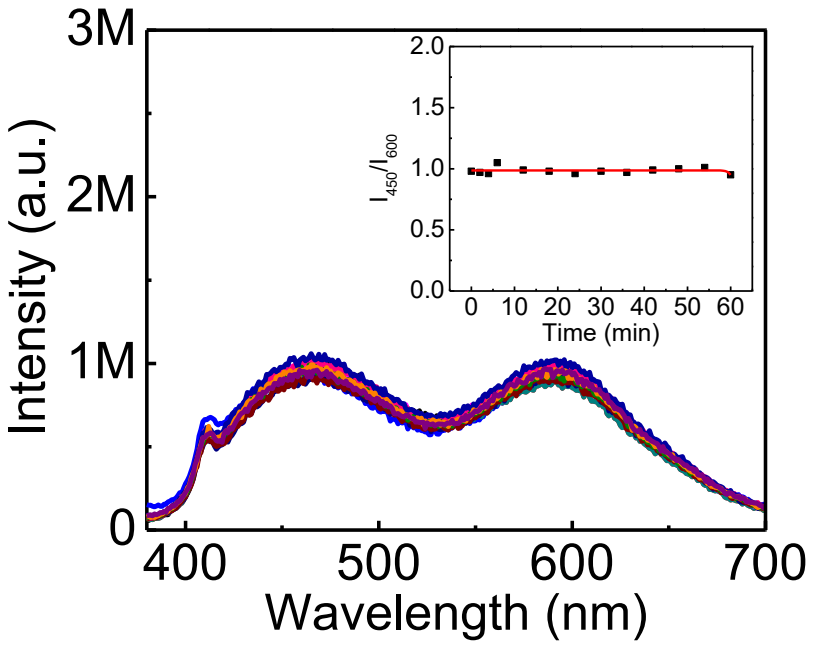


Figure S11. Effects of reaction time on the fluorescence of **Q1** (2 μM) at 37 °C in 10 mM PBS (pH 7.4) containing 1 % DMSO and 0.01 % Triton-100 as co-solvent. λ_{ex} = 360 nm. The inset shows the effects of reaction time on I_{450}/I_{600}.

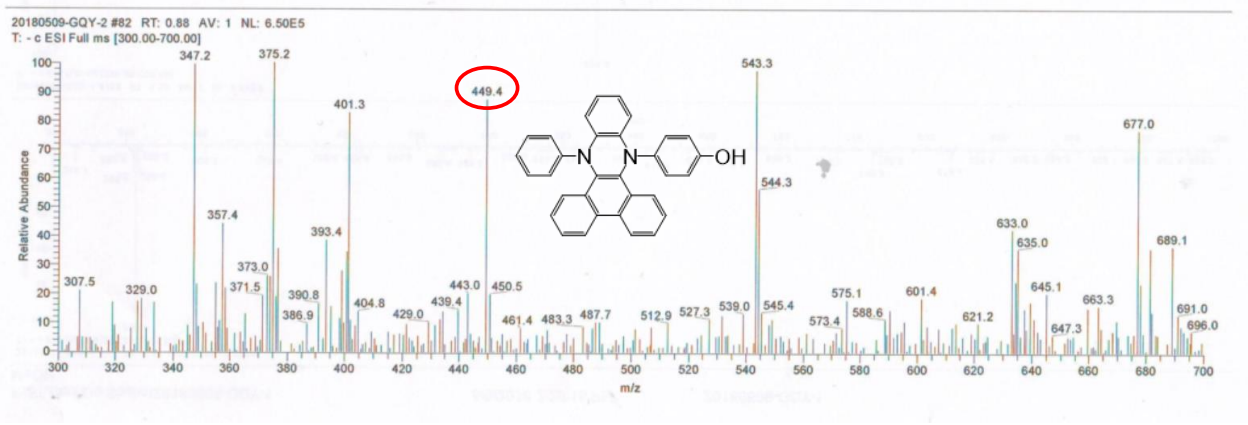


Figure S12. The MS spectrum of the reaction product of **Q1** with PTP 1B.

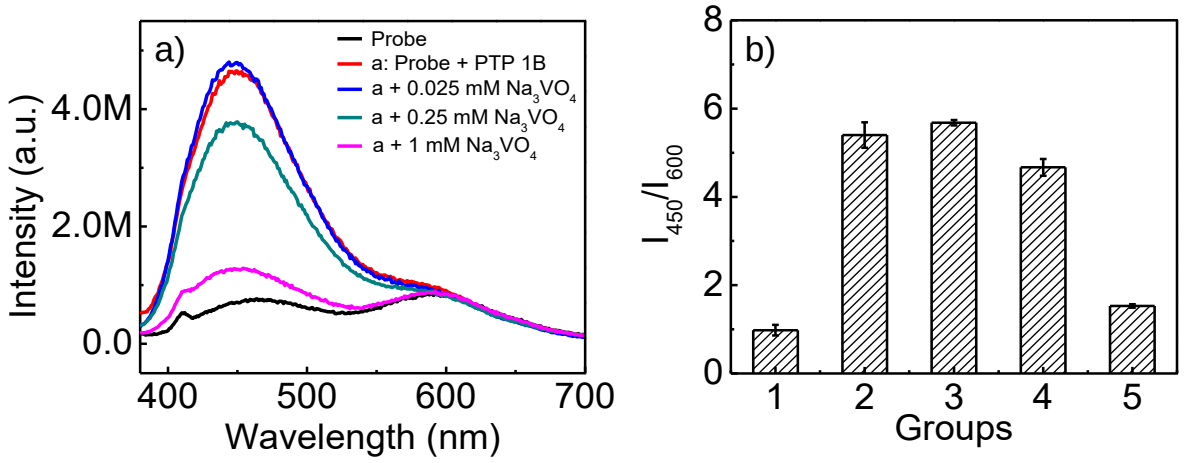


Figure S13. (a) Fluorescence emission spectra of different reaction systems. (b) I_{450}/I_{600} of different reaction systems. Groups 1 - 5 : **Q1** only (2 μM), **Q1** + PTP 1B (100 ng/μL), group 2 + Na_3VO_4 (0.025 mM), +Na_3VO_4 (0.25 mM), +Na_3VO_4 (1 mM). For inhibitor groups, the PTP 1B (100 ng/μL) was incubated with Na_3VO_4 for 60 min, and then performed with **Q1** (2 μM) at 37 °C in 10 mM PBS (pH 7.4) containing 1 % DMSO and 0.01 % Triton-100 as co-solvent for 40 min. λ_{ex} = 360 nm.

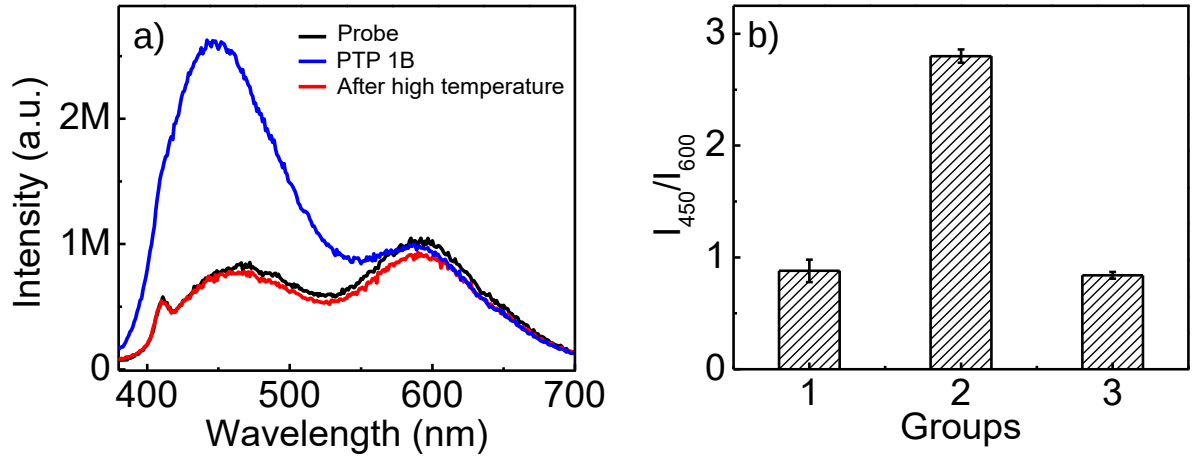


Figure S14. (a) Fluorescence emission spectra of different reaction systems. (b) I_{450}/I_{600} of different reaction systems. Groups 1 - 3 : **Q1** only (2 μM), **Q1** + PTP 1B (25 ng/μL), PTP 1B was first treated at 95 °C for 1 hour and then incubated with **Q1** at 37 °C in 10 mM PBS (pH 7.4) containing 1 % DMSO and 0.01 % Triton-100 as co-solvent for 40 min. λ_{ex} = 360 nm.

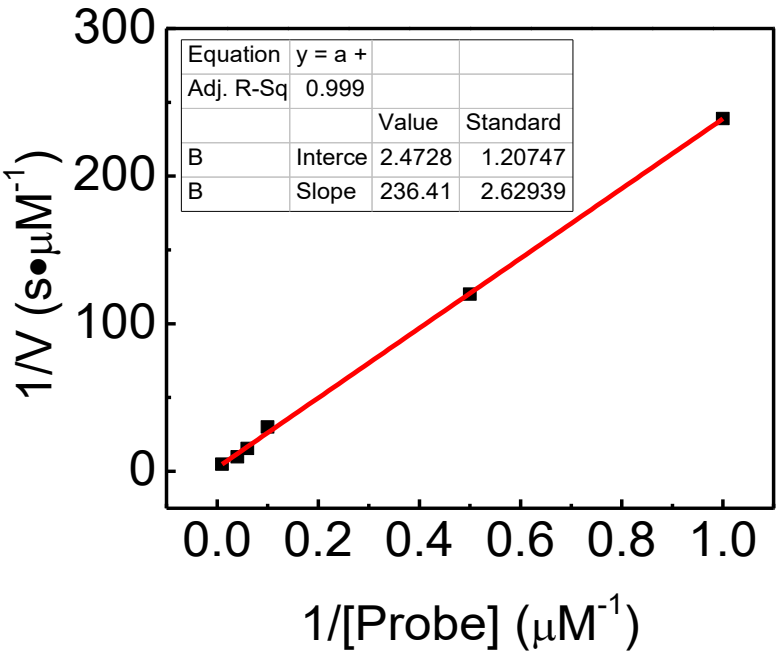


Figure S15. Lineweaver-Burk plot for the enzyme-catalyzed reaction. The Michaelis-Menten equation was described as: $V = V_{max}[probe]/(K_m+[probe])$, where V is the reaction rate, [probe] is the **Q1** concentration, and K_m is the Michaelis constant. Conditions: 50 ng/μL PTB 1B, 1 - 100 μM **Q1**, pH 7.4, temperature 37 °C. λ_{ex} = 360 nm. K_m = 94.56 μM, V_{max} = 0.4 μM/s, K_{cat} = 0.4 s^{-1}; $K_{cat}/K_m = 4.23\times10^3$ s^{-1} M^{-1}.

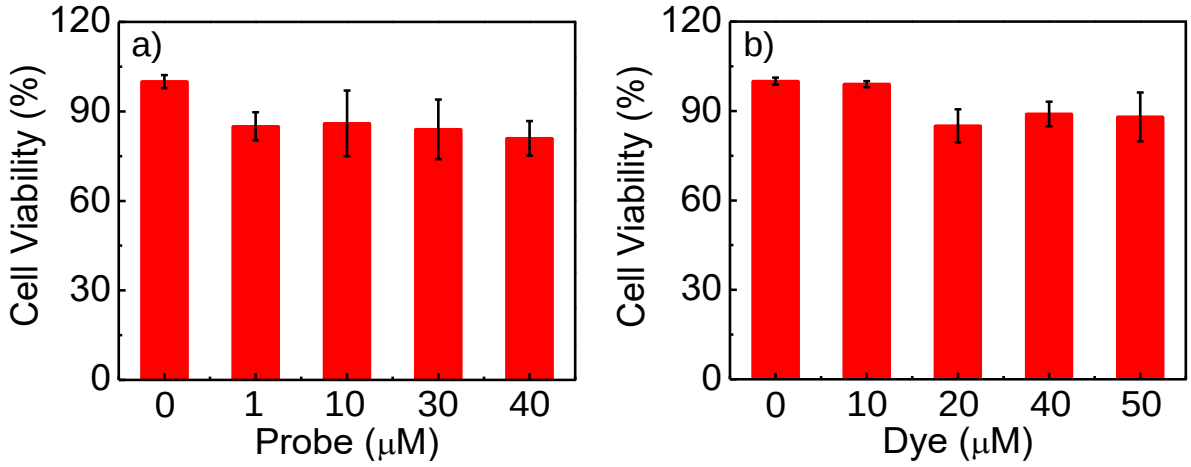


Figure S16. Effects of (a) **Q1** and (b) dye (compound **3**) at varied concentrations on the cell viability of HepG2 cells. The cell viability of cells untreated with **Q1** is defined as 100 %.

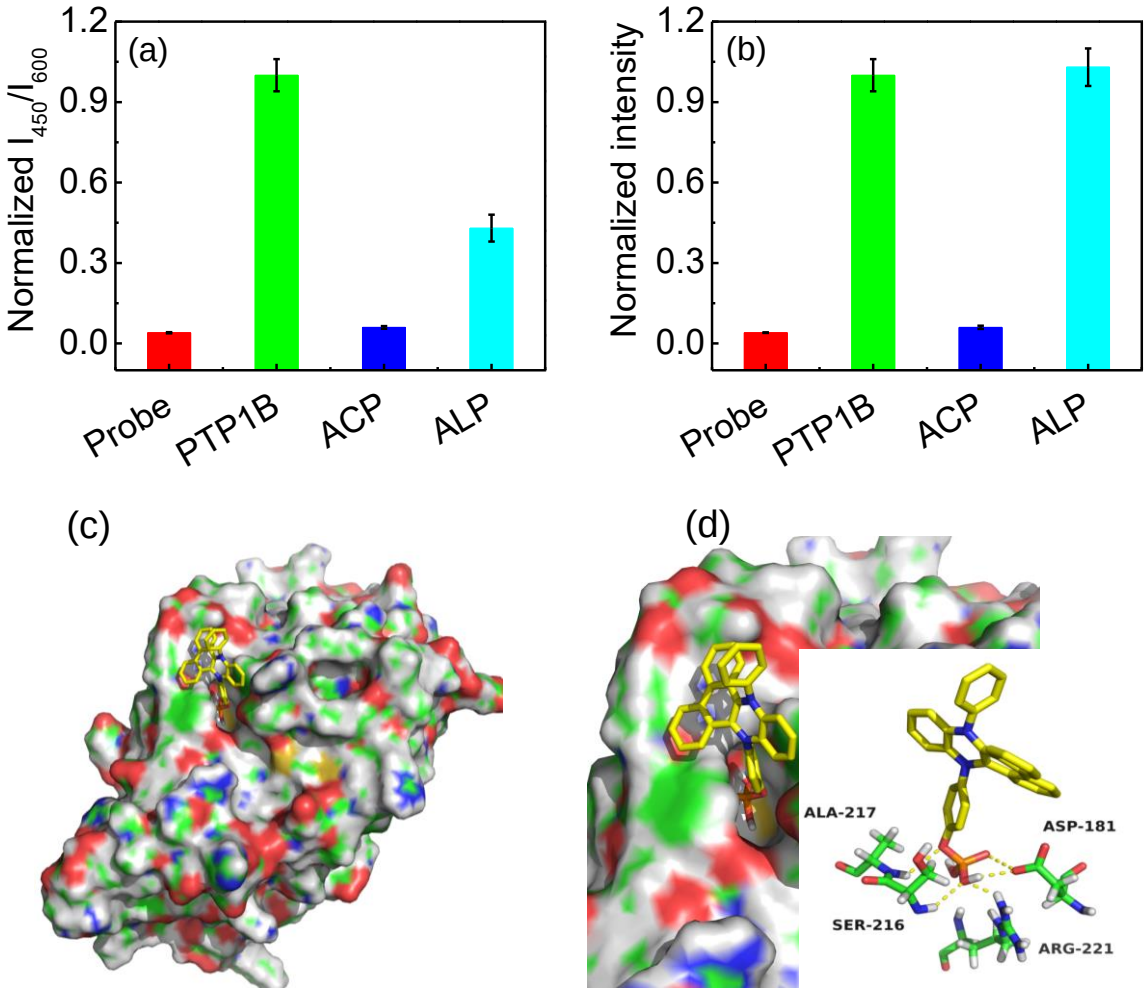


Figure S17. (a) Normalized I_{450}/I_{600} **Q1** (2 μM) in the presence of PTP1B (10 ng/μL), ACP (25 ng/μL) and ALP (10 ng/μL). (b) Normalized intensity of commercial phosphatase probe DiFMUP (2 μM) in the presence of PTP1B (10 ng/μL), ACP (25 ng/μL) and ALP (10 ng/μL). (c) and (d) The binding mode of probe with PTP1B (PDB: 1qxk). The figures were produced with PyMOL. The inset in figure d shows the hydrogen bond interaction between phosphate groups in Q1 with the amino acid residues in PTP1B.

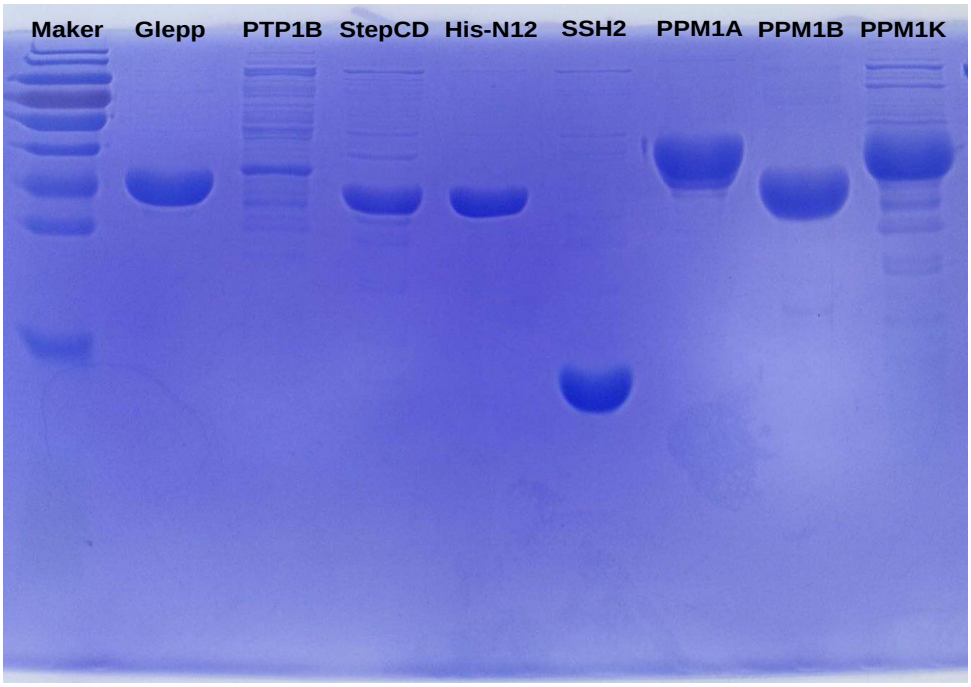
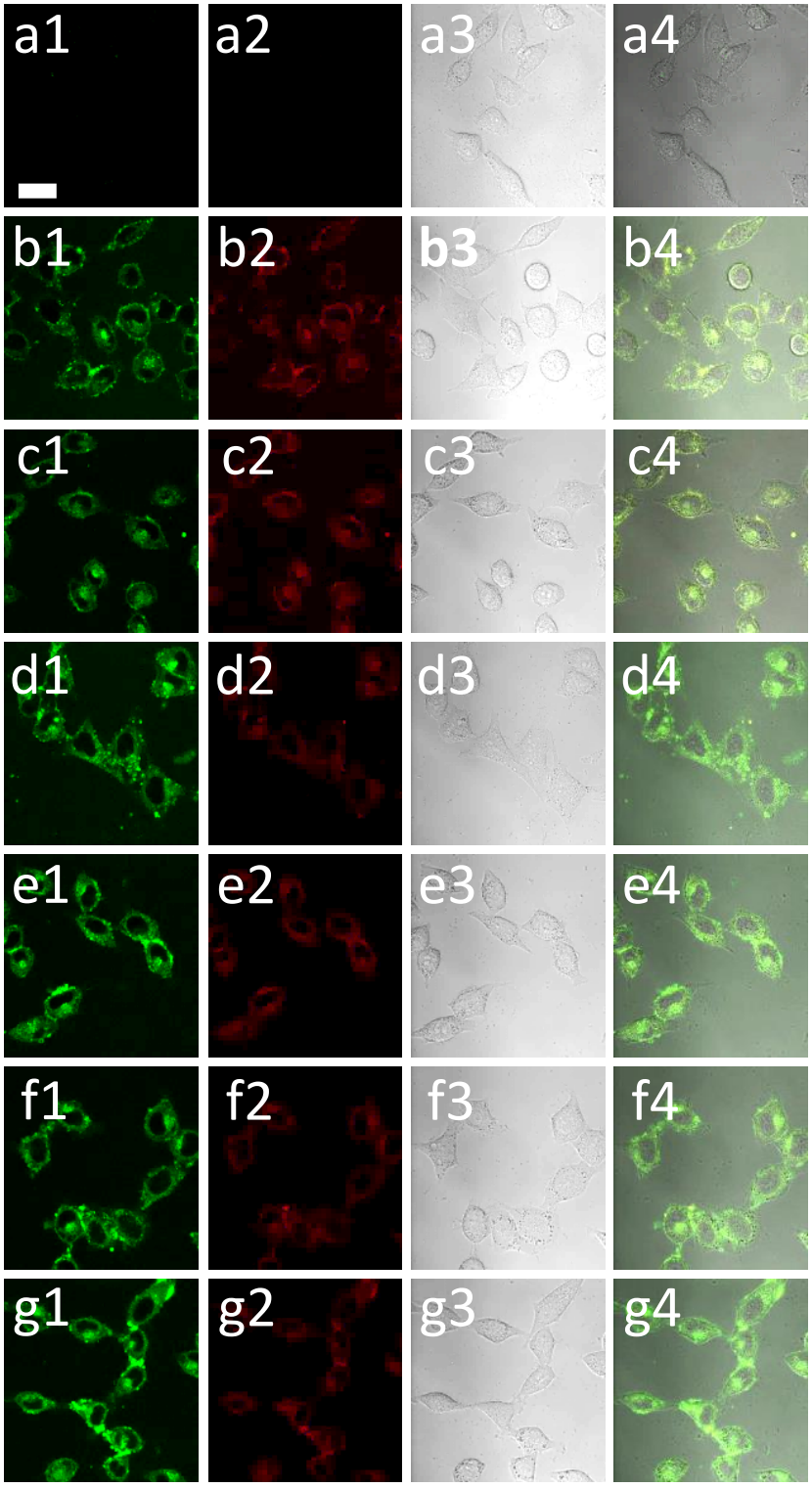


Figure S18. SDS-gel of phosphatase (Gleep, PTP1B, StepCD, N12, SSH2, PPM1A, PPM1B, PPM1K).


a1
a2
a3
a4
b1
b2
b3
b4
c1
c2
c3
c4
d1
d2
d3
d4
e1
e2
e3
e4
f1
f2
f3
f4
g1
g2
g3
g4

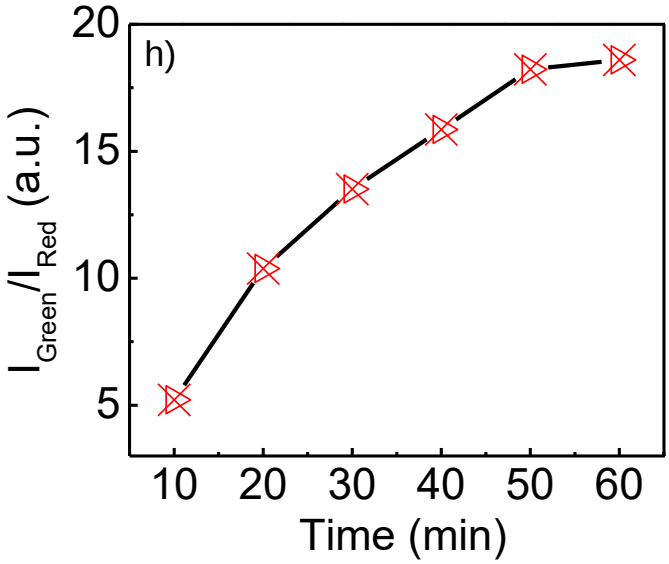
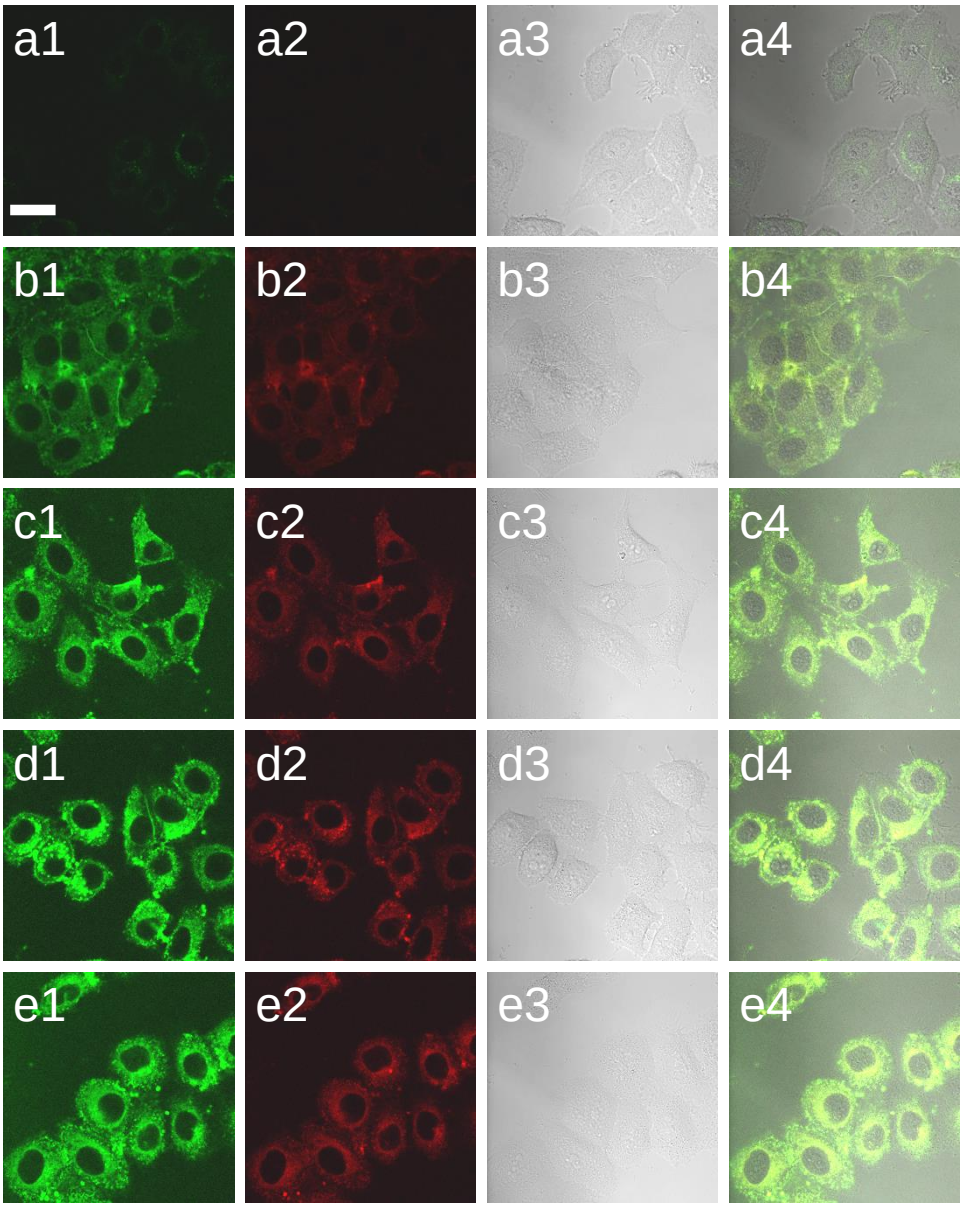


Figure S19. Fluorescence images of HeLa cells. (a1) Cells only (green channel). (b1-g1) Green channel of cells incubated with **Q1** (2 μM, 1 % DMSO as co-solvent) at 37 °C for 10, 20, 30, 40, 50 and 60 min, respectively. (a2) Cells only (red channel). (b2-g2) Red channel of cells incubated with **Q1** (2 μM) at 37 °C for 10, 20, 30, 40, 50 and 60 min, respectively. (c3-g3) The DIC images of corresponding cells. (d4-g4) The overlap images of green channel, red channel and DIC images. λ_{ex} = 405 nm; green channel: 430-530 nm; red channel: 550-650 nm. Scale bar 20 μm. (h) The ratio of green channel fluorescence intensity to red channel fluorescence intensity (I_{Green}/I_{Red}) of above cell images.


a1
a2
a3
a4
b1
b2
b3
b4
c1
c2
c3
c4
d1
d2
d3
d4
e1
e2
e3
e4

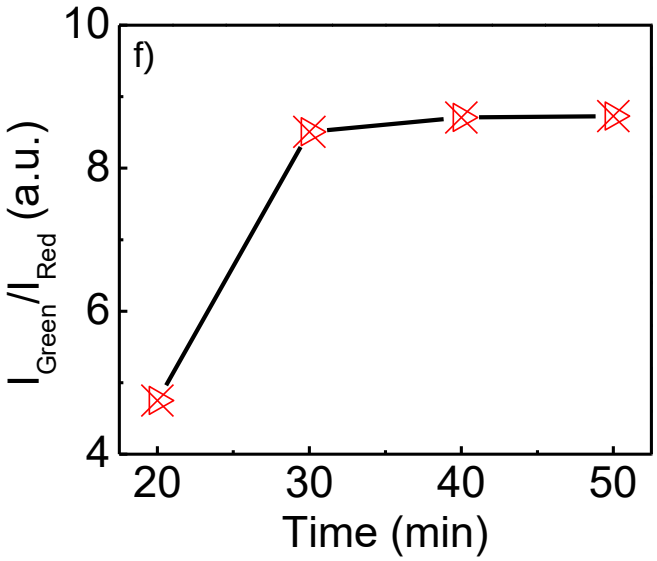


Figure S20. Fluorescence images of HepG2 cells. (a1) Cells only (green channel). (b1-e1) Green channel of cells incubated with **Q1** (2 μM, 1 % DMSO as co-solvent) at 37 °C for 20, 30, 40 and 50 min, respectively. (a2) Cells only (red channel). (b2-e2) Red channel of cells incubated with **Q1** (2 μM) at 37 °C for 20, 30, 40 and 50 min, respectively. (a3-e3) The DIC images of corresponding cells. (a4-e4) The overlap images of green channel, red channel and DIC images. λ_{ex} = 405 nm; green channel: 430-530 nm; red channel: 550-650 nm. Scale bar 20 μm. (f) The ratio of green channel fluorescence intensity to red channel fluorescence intensity (I_{Green}/I_{Red}) of above cell images.

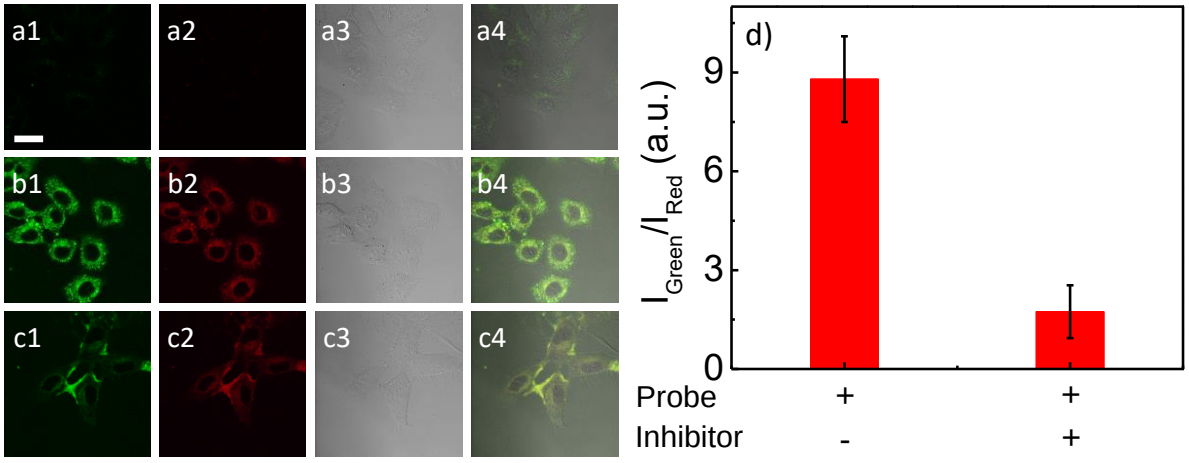


Figure S21. (a1, a2) HepG2 cells only (green and red channel, respectively). (a3) The DIC image. (a4) The overlap image a1-a3. (b1, b2) Green and red channels of cells incubated with **Q1** (2 μM, 1 % DMSO as co-solvent) at 37 °C for 50 min. (b3) The DIC image. (b4) The overlap image b1-b3. (c1, c2) Green and red channels of cells incubated firstly with inhibitor (1 mM) at 37 °C for 30 min and then with **Q1** (2 μM, 1 % DMSO as co-solvent) at 37 °C for

50 min. (c3) The DIC image. (c4) The overlap image of c1-c3. λ_{ex} = 405 nm; green channel: 430-530 nm; red channel: 550-650 nm. Scale bar 20 μm. (d) The ratio of green channel fluorescence intensity to red channel fluorescence intensity (I_{Green}/I_{Red}) of above cell images.