Supporting Information

Detection of uridine diphosphate glucuronosyltransferase 1A1 for pancreatic cancer imaging and treatment via a "turn-on" fluorescent probe

Lingxiao Wang,[a] Lingyun Wang,[d] Xiao Sun,[a] Lili Fu,[a,b] Xinlei Wang,[a] Xiaoyan Wang,[a,b] Lingxin Chen,*[a,b,c] Yan Huang,*[a,b]

a. School of Pharmacy, Binzhou Medical University, Yantai, 264003, China.

b. CAS Key Laboratory of Coastal Environmental Processes and Ecological Remediation, Shandong Key Laboratory of Coastal Environmental Processes, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China.

c. Laboratory for Marine Biology and Biotechnology, Qingdao Marine Science and Technology Center, Qingdao 266237, China.

d. Jinan Zhangqiu District People's Hospital, Jinan 250000, China.

E-mails: lxchen@yic.ac.cn (L. Chen); yhuang@yic.ac.cn (Y. Huang)

List of Contents:

1. General Experimental Section

1.1 Instruments.

Mice imaging was performed on Perkinelmer IVIS Lumina XRMS Series III In Vivo Imaging System. Fluorescence spectra were determined using a HORIBA Scientific Fluoromax-4 spectro fluorometer with a Xenon lamp and 1.0-cm quartz cells. Absorption spectra were measured on a Thermo Scientific NanoDrop 2000/2000C spectrophotometer. All pH measurements were performed with a basic pH-Meter PH-3C digital pH-meter (Lei Ci Device Works, Shanghai) with a combined glass-calomel electrode. Mass spectra were taken on the LCQ Fleet LC-MS System (Thermo Fisher Scientific). ^{1}HNMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer. The fluorescence images of cells and tissue sections were taken using a confocal laser scanning microscope (Japan Olympus Co. Ltd) with an objective lens (× 40). Intracellular fluorescence detection was carried out on flow cytometry (Aria, BD) with excitation at 550 nm and emission at 600-700 nm.

1.2 Materials

All reactions were performed under argon protection and dark, monitored by TLC (Hailang, Yantai). Flash chromatography was carried out using silica gel (300-400 mesh). The purity of BCy-panc was separated on a Shimadzu LC-20A T HPLC system equipped with fluorescence and UV-vis absorption detectors. When it was used for imaging, the purity of BCy-panc was greater than 95%. All chemicals used in synthesis were analytical reagent grade and were used as received. 1,1,2-methyl-3H-benzo[g]indole, iodoethane,3-chloro-4-hydroxybenzaldehyde were obtained from Energy Chemical. Human UGT1A1 recombinase was purchased from iPhase Biosciences. Chrysin and nilotinib were purchased from Sigma-Aldrich.

1.3 Cell Culture

HPDE6-C7 cells (human normal pancreatic cells) and Panc-1 cells (human pancreatic cancer cells) were obtained from the Committee on Type Culture Collection of the Chinese Academy of Sciences. All cells were cultured in DMEM supplemented with 10 % fetal bovine serum, 1% penicillin, and 1% streptomycin at 37 °C (w/v) in an incubator in 5 % CO_2 / 95 % air. One day before imaging, the cells were detached and placed in glass-bottomed dishes.

1.4 Fluorescence Analysis.

Add the BCy-panc probe to a 10.0 mL cuvette tube. After dilution to 10 μM with 5 mM HEPES buffer, add different concentrations of UGT1A1. Measure after 120 min of incubation at 37 °C. Fluorescence spectra were obtained with 1.0 cm quartz cells under a xenon lamp.

1.5 Cell Imaging

Cell imaging fluorescence images were acquired using the Olympus FV1000 confocal laser scanning microscope, objective (× 40). The choice of excitation wavelength is consistent with the text. Cells are placed on a dish (Φ=20 mm) and imaged after adhesion for 24 h. Add probes to plates filled with 1 ml of fresh complete medium.

1.6 Western blot analysis.

All pretreated cells are lysed in cell lysis buffer containing the protease inhibitor PMSF (Solarbio, China). Centrifuge the cell lysate at 15,000 rpm for 10 min at 4 °C. Protein concentration was determined with the BCA Protein Assay Kit (Biogot, China), and a standard curve was established. After denaturation, equal amounts of protein are electrophoresis on a 10-12% SDS-polyacrylamide gel (Bio-Rad, USA) and transferred to a PVDF membrane. Then close the membrane with 5% milk and incubate overnight with primary antibodies at 4 °C. Proteins were quantified using a suitable horseradish peroxidase (HRP) conjugated secondary antibody (Cell Signaling Technology, USA), and the signal was detected with an enhanced chemiluminescence (ECL) detection system. The results were analyzed using Image J software.

1.7 Establishment of mouse models

Establishment of Panc-1 transplanted tumor nude mice: 5-week-old pathogen-free nude mice were housed in individually ventilated cages and fed SPF-grade laboratory chow and water. 2×10^6 Panc-1 cells were suspended in culture medium and then implanted subcutaneously in nude mice. Panc-1 xenografts were established in nude mice until the tumor volume typically reached approximately 200 cubic mm. All experiments were performed in accordance with the guidelines established by the Animal Research Policy Committee of Binzhou Medical College.

1.8 Imaging Mice in vivo.

The PerkinElmer IVIS Lumina XRMS Series III In Vivo Imaging System in vivo imaging system was used to image normal nude mice and tumor-bearing nude mice. The choice of excitation and emission wavelengths is consistent with those described in the text. Mice are anesthetized before injection and during imaging.

2. Synthesis of BCy-panc

1,1,2-methyl-3H-benzo[g]indole (15.67 g, 75 mmol) and iodoethane (11.62 g, 75 mmol), 40 mL acetonitrile mixed, refluxed at 130 °C for 12 h, to obtain compound a. Compounds a (0.238 g) and 3-chloro-4-hydroxybenzaldehyde (0.157 g, 1 mmol) were dissolved in ethanol and refluxed and stirred at 80 °C for 48 h. Vacuum concentrate to obtain the purple product Bcy (0.393 g, yield: 84%). ^{1}H NMR (500 MHz, CD_3OD-D_4) δ (ppm): 9.86 (s, 1H), 8.12-8.10 (t, 1H), 7.95-7.94 (m, 1H), 7.79-7.76 (m, 1H), 7.60-7.62 (m, 1H), 7.49-7.46 (m, 1H), 7.32-7.28 (m, 3H), 6.68-6.66 (m, 1H), 6.28-6.25 (m, 1H), 4.15-4.13 (d, 1H), 2.07-2.01 (m, 2H), 1.28-1.27 (d, 6H), 0.90-0.88 (m, 3H). ^{13}C NMR (125 MHz, CD_3OD-D_4) δ(ppm): 189.9, 182.1, 153.1, 150.8, 139.2, 137.6, 133.9, 132.2, 131.9, 130.3, 128.7, 127.8, 125.1, 122.8, 113.2, 112.4, 63.7, 54.3, 45.0, 36.7, 31.9, 29.7, 26.6, 22.7, 14.6, 14.1. LC-MS (ESI): m/z calcd for $C_{24}H_{23}ClNO^{+}$ $[M]^{+}$ 376.14, found 376.10.

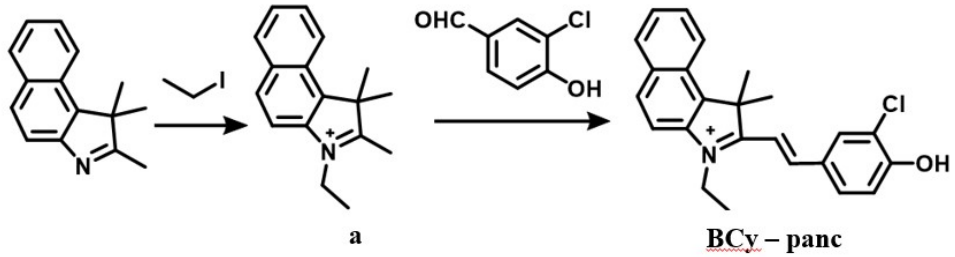
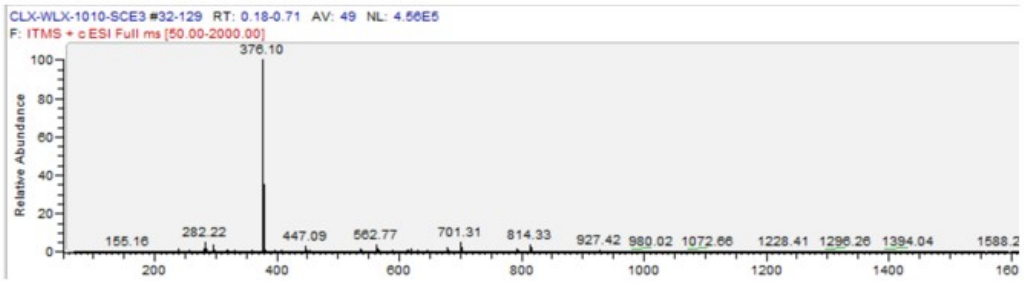
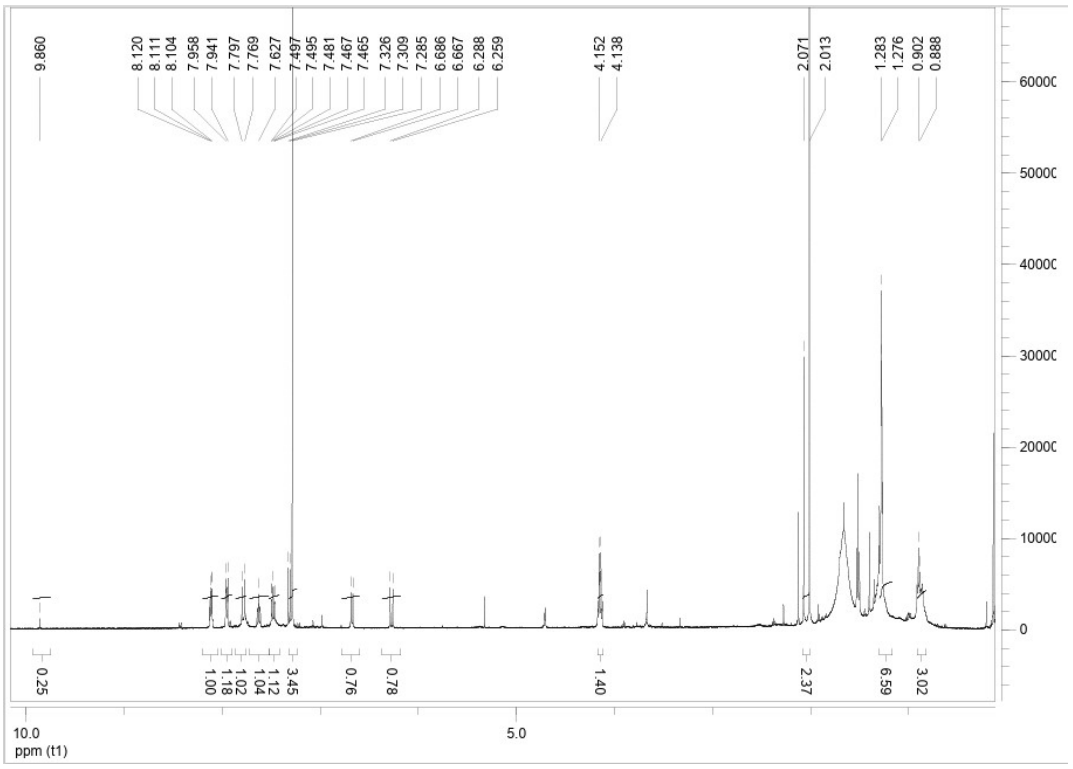


Figure S1. Synthesis method of BCy-panc prob.

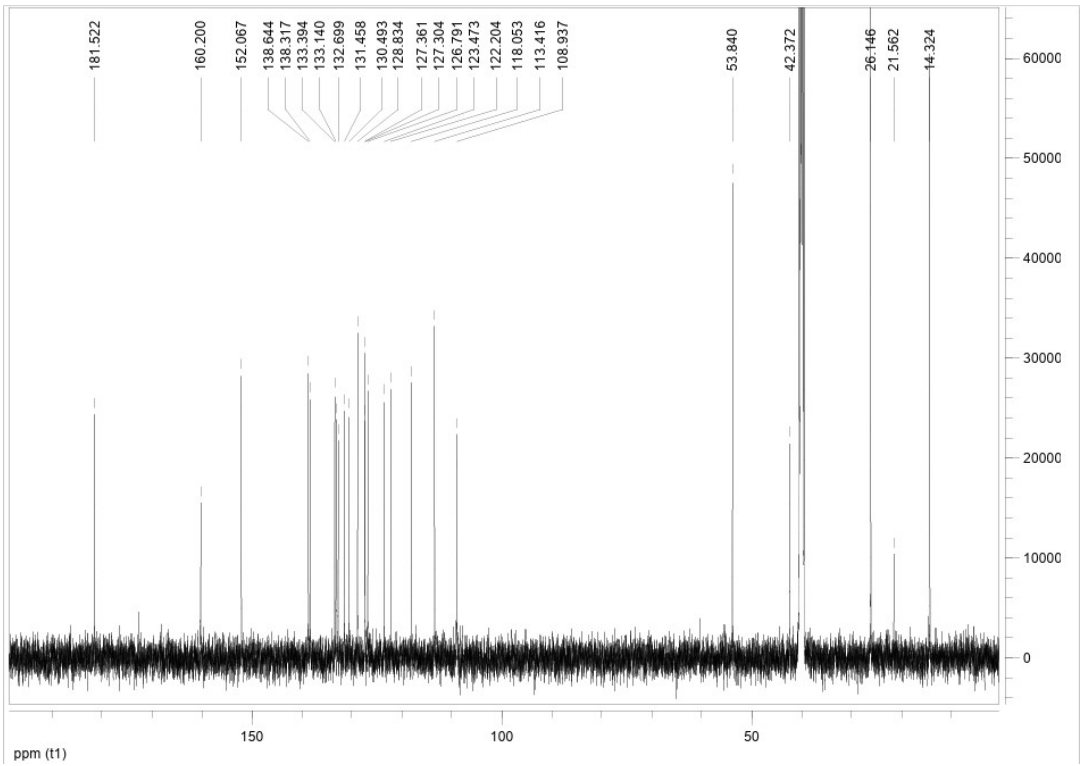
3. Characterization (MS, ^{1}H NMR and ^{13}C NMR) of BCy-panc



MS of BCy-panc



^{1}H-NMR of BCy-panc



^{13}C-NMR of BCy-panc

Figure S2. BCy-panc by ^{1}H-NMR, ^{13}C-NMR and HRMS.

4. Cytotoxicity of BCy-panc

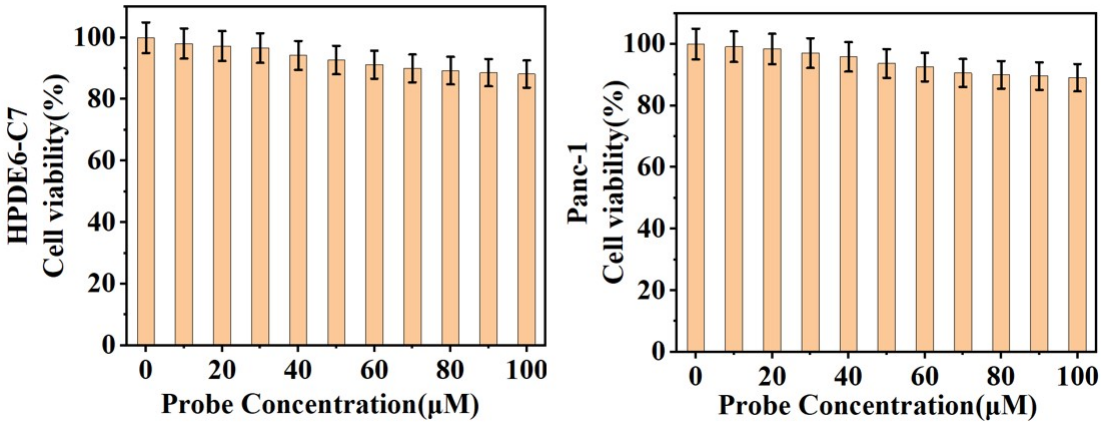


Figure S3. CCK-8 Assay for BCy-panc. The 24 h cell viability of HPDE6-C7 cells and Panc-1 cells for BCy-panc, the concentration of BCy- panc was 0, 20, 40, 60, 80,100 μM.

5. Bright-field cell images of those shown in Figure 2a, Figure 3a and Figure 4a. (in manuscript)

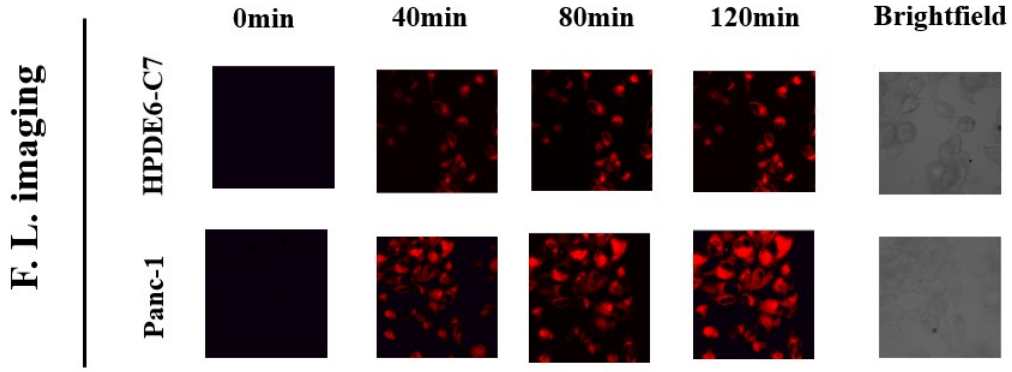


Figure S4. Bright-field Images of Figure 2a.

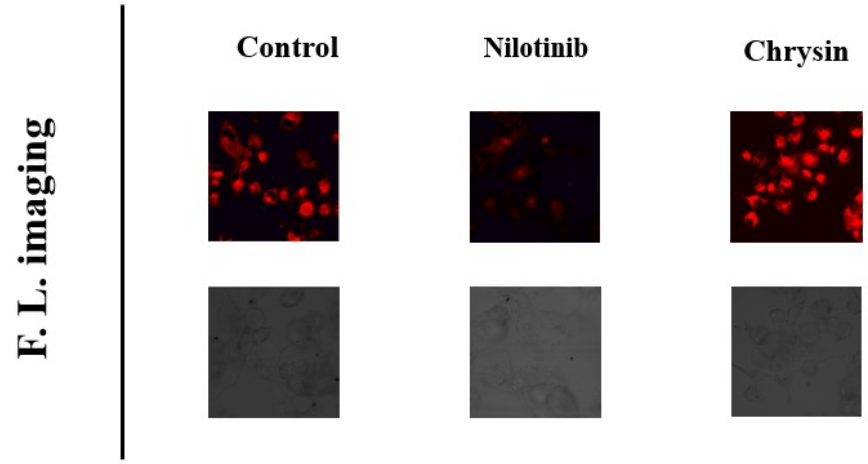


Figure S5. Bright-field Images of Figure 3a.

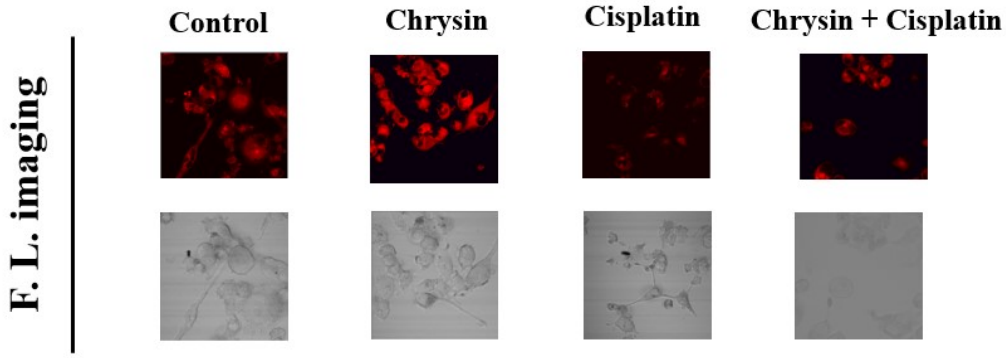
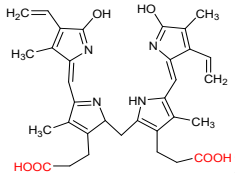
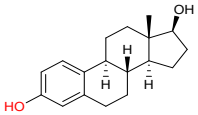
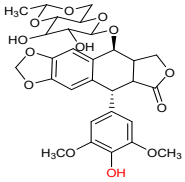
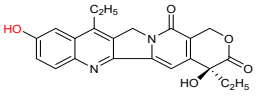
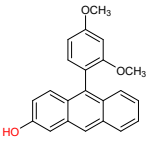
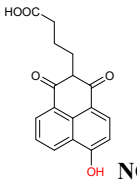
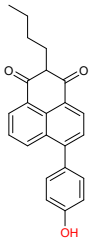
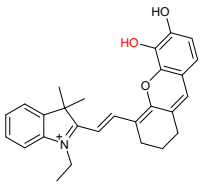


Figure S6. Bright-field Images of Figure 4.

6. A summary of currently used probes for UGT1A1

Table S1. A summary of currently used probes for UGT1A

Substrate	Selectivity	Kinetic behavior (*Km*, μM)	Detector	Sensitivity LOD
 **Bilirubin**	**UGT1A1** UGT1A3 UGT1A8 UGT1A10	Michaelis-Menten (0.2)	LC-MS-MS	6.1 mg/L (UGT1A1)
 **β-estradiol**	**UGT1A1** UGT1A3 UGT1A8 UGT1A10	Hill (23)	LC-MS-MS	5.0 mg/L (UGT1A1)
 **Etoposide**	**UGT1A1** UGT1A3 UGT1A8 UGT1A10	Michaelis-Menten (285)	LC-MS-MS	5.3 mg/L (UGT1A1)
 **SN-38**	**UGT1A1** UGT1A8 UGT1A9 UGT1A10	Michaelis-Menten (7.5)	LC-MS-MS	39 mg/L (UGT1A1)
 **2-Me -4-OMe TG**	**UGT1A1** UGT1A9	Michaelis-Menten (2.7)	Florescence	0.2 mg/L (UGT1A1)
 **NCHN**	**UGT1A1**	Substrate-inhibition (126.7)	Florescence	0.1 mg/L (UGT1A1)
 **NHPN**	**UGT1A1**	Michaelis-Menten (0.7)	Florescence	0.48 μg/L (UGT1A1)
 **HHC**	**UGT1A1**	Michaelis-Menten (0.7)	Florescence	10 μg/L (UGT1A1)

7. Experimental study of BCy-panc in the presence of mixed disturbances of various substances in living organisms

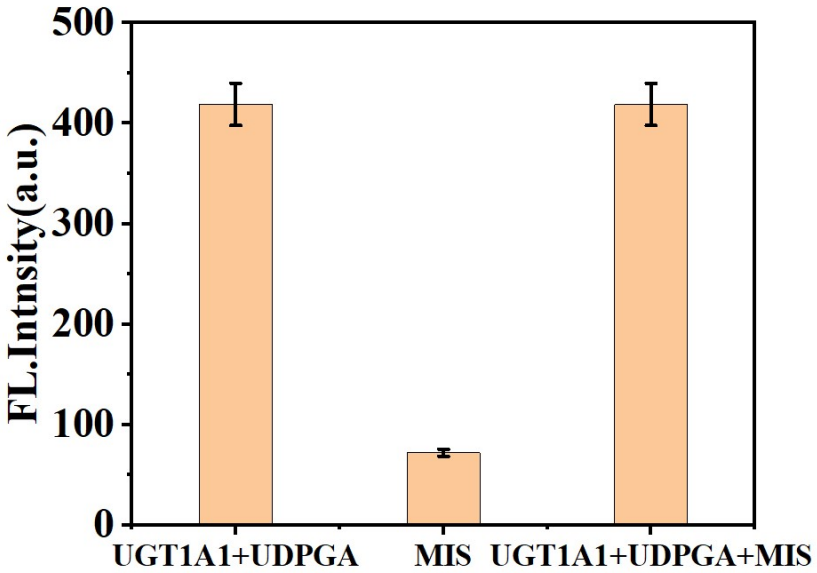
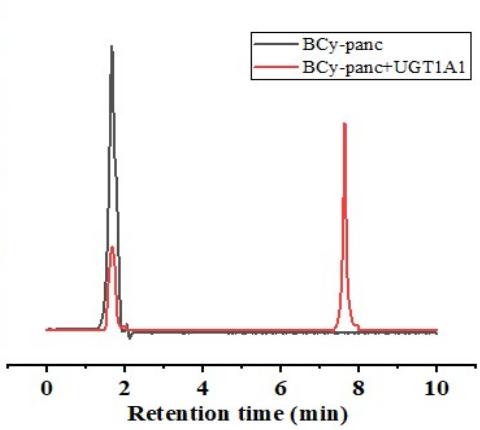


Figure S7. Mixed interference experiments with various substances in living organisms

Mixed Interference Substances (MIS): glutamate; glutamine; cysteine; tryptophan; lysine; tyrosine; glycine; serine; vitamin C; glutathione; bilirubin; glucose; myristic acid; Ca^{2+}; Mg^{2+}; K^{+}; Al^{3+}; Fe^{3+}; Mn^{2+}; Cu^{2+}; Zn^{2+}; Co^{2+}.

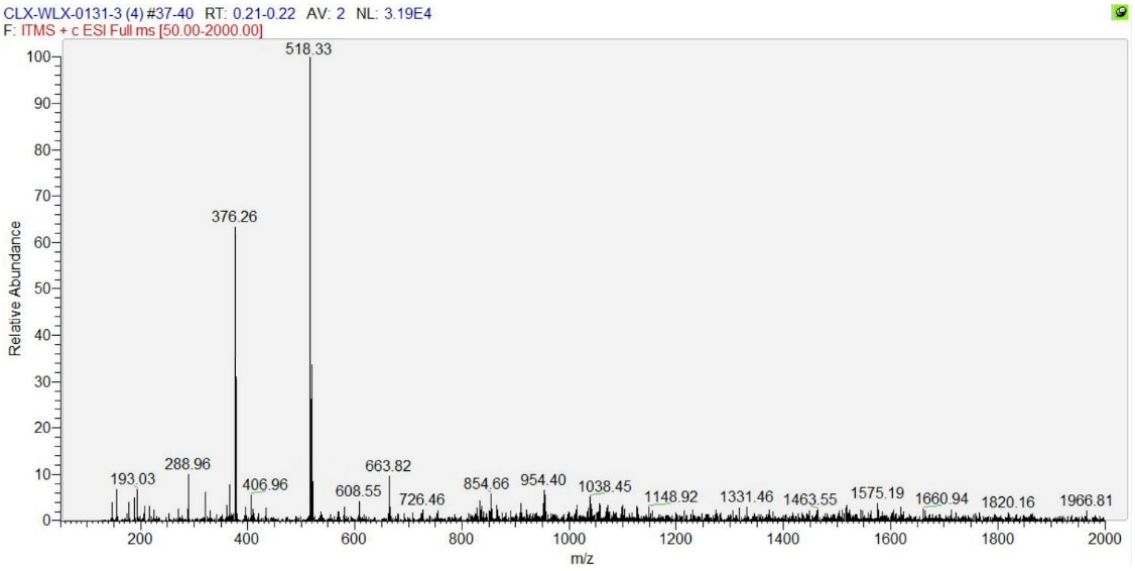
Measurements were carried out in 10 mM HEPES (pH 7.4), 37 ℃. The results showed that the fluorescence intensity in the MIS group without UGT1A1+UDPGA was much lower than that in the group with UGT1A1+UDPGA; the fluorescence was significantly enhanced by adding UGT1A1+UDPGA to the MIS group and was consistent with the fluorescence intensity of the control group (UGT1A1+UDPGA group). In conclusion, the interfering substances mixed in vivo had no effect on the actual detection of the probe.

8. Comparison of liquid chromatograms before and after addition of UGT1A1



In the simulated environment (pH=7.4, adequate amount of UDPGA), the chromatogram after the addition of UGT1A1 showed new peaks, indicating the production of new substances catalysed by UGT1A1.

9. Changes in mass spectra after addition of UGT1A1



In the simulated environment (pH=7.4, adequate amount of UDPGA), the mass spectrogram after addition of UGT1A1 showed new peaks, indicating the production of new substances catalysed by UGT1A1.