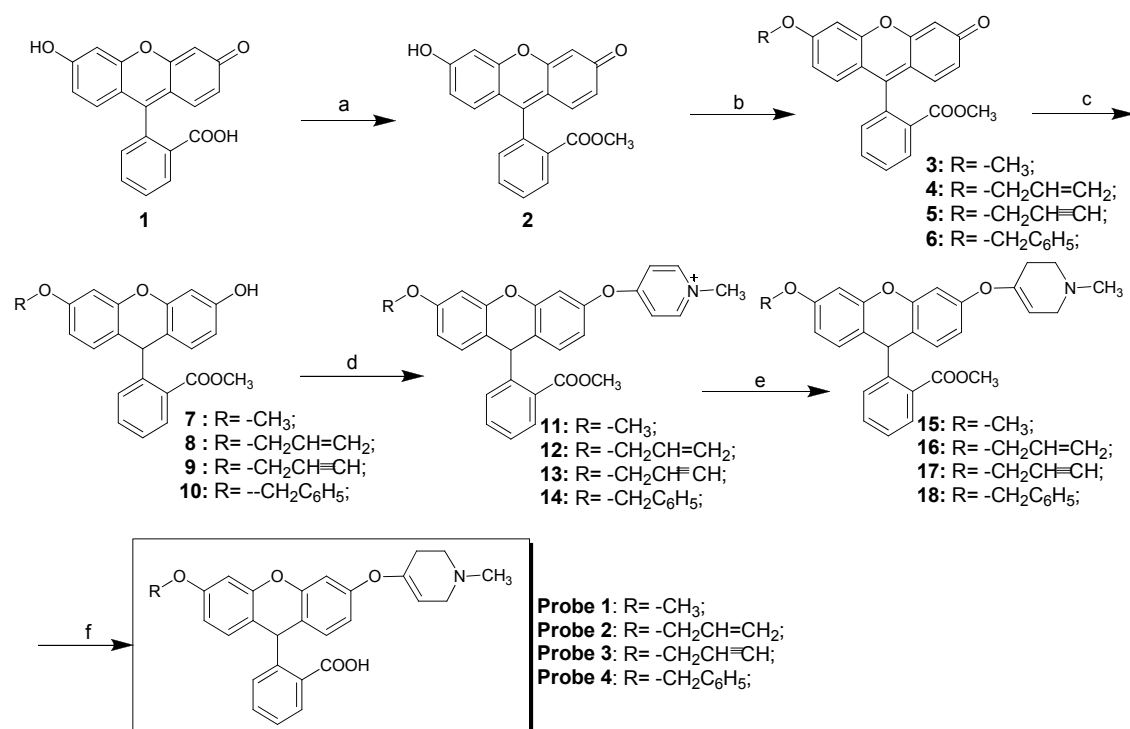


Supporting Information

Experimental Section

Part I :Chemistry

All commercially available solvents and reagents were used without further purification. All reactions were carried out under a nitrogen atmosphere. ¹H-NMR spectra and ¹³C-NMR were recorded at 400 MHz. All fluorescent and UV measurements were carried out at 37 °C.



Scheme 2 Synthesis of fluorogenic momoamine oxidase probes.

Reagents and conditions: (a) H₂SO₄/MeOH,rt,82.7%. (b) for 3:iodomethane,K₂CO₃/DMF,rt,91.5%; for 4:allyl bromide,K₂CO₃/DMF,rt,91.5%; for 5:propargyl bromide, K₂CO₃/DMF,rt, 90.5%; for 6:benzyl bromide,K₂CO₃/DMF,rt,80%. (c) NaBH₄/MeOH,0^oC,91%. (d) for 11-14:1-methyl-4-bromide-pyridine salt, MeCN/Pyridine,rt,67%. (e)NaBH₄/MeOH,0^oC,91%. (f)NaOH/MeOH-H₂O,67%.

2-(6-Hydroxy-3-oxo-3H-xantene-9-yl)-benzoic acid methyl ester (2) To a solution of fluorescein 1 (8 g, 0.24 mmol) in MeOH (45 mL) was added dropwise concentrated sulfuric acid (6 mL). The reaction was carried out at 85 °C monitored by TLC until completion (~12h). The reaction mixture was poured into 20g of ice-water. Then NaHCO₃ (24 g) was added to the solution in portions and stirred vigorously at room temperature. A red precipitate formed; this was collected by filtration, washed with water and petroleum, dried and obtained 6.9g products. Yield: 82.7%. ¹H NMR (400 MHz, CDCl₃): δ 8.05 (dd, *J* = 1.2, 0.8 Hz, 1H), 7.74 (m, 1H), 7.64 (m, 1H), 6.41 (d, *J* = 9.2 Hz, 2H), 6.03 (dd, *J* = 2.0, 2.0 Hz, 2H), 5.97 (d, *J* = 2.0 Hz, 2H), 3.55 (s, 3H). ESI-MS *m/z* 346.1 (M+1)⁺.

2-(6-Methoxy-3-oxo-3H-xantene-9-yl)-benzoic acid methyl ester (3) MeI (0.511 g, 3.6 mmol) was added to the mixture of 2 (1.04 g, 3.0 mmol) and K₂CO₃ (0.621 g, 4.5 mmol) in 30 ml of DMF at room temperature. After stirring for 24 hours, the product was extracted with CH₂Cl₂ (100 mL × 3), and the organic layer was washed with water (100 mL × 2) and brine (100 mL), dried over Na₂SO₄ and concentrated under reduced pressure giving 3.28 g of 3 (91%). ¹H NMR (400 MHz, CDCl₃):

3.80 (s, 3H), 3.88 (s, 3H), 6.22 (m, 1H), 6.38 (m, 1H), 6.77-6.91 (m, 3H), 7.21 (m, 1H), 7.48 (s, 1H), 7.73-7.88 (m, 2H), 8.17 (m, 1H). ESI-MS m/z 360.1(M+1)⁺.

2-(6-allyloxy-3-oxo-3H-xanthen-9-yl) benzoic acid methyl ester (4) This compound was prepared according to the same procedure for the synthesis of 3 by using allyl bromide (3.6 mmol) in place of MeI. Yield: 91.5 %. ¹H NMR (400 MHz, CDCl₃): δ 8.17 (dd, $J = 7.8, 1.1$ Hz, 1H), 7.68 (td, $J = 7.4, 1.3$ Hz, 1H), 7.61 (td, $J = 7.6, 1.3$ Hz, 1H), 7.25 (dd, $J = 7.4, 1.0$ Hz, 1H), 6.90 (d, $J = 2.4$ Hz, 1H), 6.82 (dd, $J = 15.2, 9.2$ Hz, 2H), 6.71 (dd, $J = 8.8, 2.4$ Hz, 1H), 6.47 (dd, $J = 9.6, 1.8$ Hz, 1H), 6.38 (d, $J = 1.8$ Hz, 1H), 5.99 (m, 1H), 5.42 (d, $J = 1.3$ Hz, 1H), 5.38 (d, $J = 1.3$ Hz, 1H), 5.30 (dd, $J = 10.4, 1.2$ Hz, 1H), 4.60 (d, $J = 5.3$ Hz, 2H), 3.61 (s, 3H). ESI-MS m/z 386.1 (M+1)⁺.

2-(6-(prop-2-ynyloxy)-3-oxo-3H-xanthen-9-yl) benzoic acid methyl ester (5) This compound was prepared according to the same procedure for the synthesis of 3 by using propargyl bromide (3.6 mmol) in place of MeI. Yield: 90.5 %. ¹H NMR (400 MHz, CDCl₃): δ 8.24 (d, $J = 7.8$ Hz, 1H), 7.73 (t, $J = 7.4$ Hz, 1H), 7.67 (t, $J = 7.6$ Hz, 1H), 7.30 (d, $J = 7.5$ Hz, 1H), 7.05 (d, $J = 2.2$ Hz, 1H), 6.90 (d, $J = 8.9$ Hz, 1H), 6.85 (d, $J = 9.6$ Hz, 1H), 6.79 (dd, $J = 8.9, 2.2$ Hz, 1H), 6.56 – 6.48 (m, 1H), 6.44 (d, $J = 1.1$ Hz, 1H), 4.79 (d, $J = 2.1$ Hz, 2H), 3.64 (s, 3H). ESI-MS m/z 384.1 (M+1)⁺.

2-(6-benzyloxy-3-oxo-3H-xanthen-9-yl) benzoic acid methyl ester (6) This compound was prepared according to the same procedure for the synthesis of 3 by using benzyl bromide (3.6 mmol) in place of MeI. Yield: 90.5 %. ¹H NMR (400 MHz, CDCl₃): δ 8.23 (d, $J = 7.8$ Hz, 1H), 7.72 (t, $J = 7.4$ Hz, 1H), 7.65 (t, $J = 7.6$ Hz, 1H), 7.37 (td, $J = 13.3, 7.4$ Hz, 5H), 7.32 – 7.02 (m, 2H), 7.00 (d, $J = 2.3$ Hz, 1H), 6.89 (d, $J = 8.9$ Hz, 1H), 6.87 – 6.77 (m, 2H), 6.52 (d, $J = 9.7$ Hz, 1H), 6.43 (s, 1H), 5.13 (s, 2H), 3.62 (s, 3H). ESI-MS m/z 436.1 (M+1)⁺.

4-(6-methoxy-9-(2-(methoxy-carbonyl)phenyl)-9H-xanthen-3-yloxy)-1-methylpyridinium (11) To a solution of 3 (1.08g, 3.0 mmol) in MeOH (100 mL) was added NaBH₄ (0.57 g, 15.0 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, and at room temperature for 2 h. After removal of the solvent, the product was extracted with CH₂Cl₂ (50 mL × 3), and the organic layer was washed with water (50 mL × 2) and brine (50 mL), dried over Na₂SO₄ and concentrated under reduced pressure to yield the air-sensitive product 7 as a yellow powder (0.882 g, 81 %).

To a solution of 7 (0.882 g, 2.42 mmol) in anhydrous CH₃CN (100 mL) were added 1-methyl-4-bromo-pyridine salt (0.87 g, 2.9 mmol) and Pyridine (5mL). The mixture was stirred at room temperature for 24 h. The remaining solid residue was removed by filtration. The filtrate was concentrated and purified by SiO₂ chromatography (CH₂Cl₂: MeOH = 20:1) to give compound 11. Yield: 78 %. ¹H NMR (400 MHz, CDCl₃): δ 9.13 (d, $J = 2.4$ Hz, 2H), 7.83 (d, $J = 7.6$ Hz, 1H), 7.38 (m, 1H), 7.31 (d, $J = 6.4$ Hz, 2H), 7.21 (m, 2H), 7.12 (d, $J = 7.6$ Hz, 1H), 6.87 (d, $J = 8.8$ Hz, 2H), 6.54-6.71 (m, 3H), 6.33 (s, 1H), 4.66 (s, 3H), 3.96 (s, 3H), 3.78 (s, 3H). ESI-MS m/z 454.2 (M+1)⁺.

4-(6-allyloxy-9-(2-(methoxy-carbonyl) phenyl)-9H-xanthen-3-yloxy)-1-methylpyridinium (12) This compound was prepared according to the same procedure for the synthesis of 11. Yield: 78 %. ¹H NMR (400 MHz, CDCl₃): δ 8.95 (s, 2H), 7.82 – 7.64 (m, 1H), 7.22 (d, $J = 17.3$ Hz, 4H), 7.09 (s, 1H), 7.08 – 7.00 (m, 2H), 6.97 (s, 1H), 6.83 – 6.71 (m, 2H), 6.57 (d, $J = 8.2$ Hz, 1H), 6.50 – 6.36 (m, 2H), 6.15 (s, 1H), 6.11 – 5.70 (m, 1H), 5.24 (s, 2H), 5.19 – 5.03 (m, 2H), 4.34 (d, $J = 7.4$ Hz, 5H), 3.79 (d, $J = 4.3$ Hz, 3H). ESI-MS m/z 454.2 (M+1)⁺.

4-(6-prop-2-ynyloxy-9-(2-(methoxy-carbonyl) phenyl)-9H-xanthen-3-yloxy)-1-methylpyridinium (13) This compound was prepared according to the same procedure for the synthesis of 11. Yield: 76 %. ¹H NMR (400 MHz, CDCl₃): δ 9.19 (d, *J* = 7.3 Hz, 2H), 9.19 (d, *J* = 7.3 Hz, 1H), 7.84 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.84 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.38 (m, 3H), 7.24 (td, *J* = 7.7, 1.1 Hz, 1H), 7.18 (d, *J* = 8.9 Hz, 1H), 7.12 (d, *J* = 7.9 Hz, 1H), 6.89 (dd, *J* = 11.0, 5.7 Hz, 2H), 6.71 (d, *J* = 2.7 Hz, 2H), 6.61 (d, *J* = 2.6 Hz, 1H), 6.60 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.59 (d, *J* = 2.6 Hz, 1H), 6.33 (s, 1H), 5.29 (s, 1H), 4.65 (d, *J* = 2.4 Hz, 2H), 4.51 (s, 3H), 3.94 (s, 3H). ESI-MS *m/z* 478.2 (M+1)⁺.

4-(6-benzyloxy-9-(2-(methoxy-carbonyl) phenyl)-9H-xanthen-3-yloxy)-1-methylpyridinium (14) This compound was prepared according to the same procedure for the synthesis of 11. Yield: 67 %. ¹H NMR (400 MHz, CDCl₃): δ 9.16 (d, *J* = 7.2 Hz, 2H), 7.82 (dd, *J* = 1.2, 1.6 Hz, 1H), 7.54 – 7.09 (m, 11H), 6.87 (dd, *J* = 10.7, 5.5 Hz, 2H), 6.75 – 6.64 (m, 7H), 6.32 (s, 1H), 5.00 (s, 2H), 4.49 (s, 3H), 3.92 (s, 3H). ESI-MS *m/z* 530.2 (M+1)⁺.

Methyl-2-(3-methoxy-6-(1-methyl-1, 2, 3, 6-tetrahydropyridin-4-yloxy)-9H-xanthen-9-yl) benzoate (15) To a solution of 11 (0.681 g, 1.5 mmol) in MeOH (50 mL) was added NaBH₄ (0.228 g, 6.0 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, after removal of the solvent, the residue was dissolved in CH₂Cl₂ (30 mL × 3). The organic layer was then washed with water (50 mL) and brine (50 mL), dried over MgSO₄, and concentrated in vacuo. Yield: 91 %. ¹H NMR (400 MHz, CDCl₃): δ 7.66 (d, *J* = 7.6 Hz, 1H), 7.15 (m, 1H), 6.98–7.06 (m, 2H), 6.79–6.86 (m, 2H), 6.67 (d, *J* = 2.4 Hz, 1H), 6.49 (dd, *J* = 2.4, 2.0 Hz, 2H), 6.38 (dd, *J* = 2.4, 2.4 Hz, 1H), 6.12 (s, 1H), 4.83 (s, 1H), 3.80 (s, 3H), 3.63 (s, 3H), 2.85 (d, *J* = 2.0 Hz, 2H), 2.50 (m, 2H), 2.25 (s, 3H). ESI-MS *m/z* 457.2 (M+1)⁺.

Methyl-2-(3-(allyloxy)-6-(1-methyl-1, 2, 3, 6-tetrahydropyridin-4-yloxy)-9H-xanthen-9-yl) benzoate (16) This compound was prepared according to the same procedure for the synthesis of 15. Yield: 91 %. ¹H NMR (400 MHz, CDCl₃): δ 7.77 (d, *J* = 7.1 Hz, 1H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.19 – 7.05 (m, 2H), 6.96 (d, *J* = 8.5 Hz, 1H), 6.90 (d, *J* = 8.6 Hz, 1H), 6.77 (d, *J* = 2.3 Hz, 1H), 6.65 – 6.58 (m, 2H), 6.51 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.23 (s, 1H), 5.99 – 5.93 (m, 1H), 5.38 (d, *J* = 1.3 Hz, 1H), 5.24 (s, 1H), 4.92 (s, 1H), 4.46 (d, *J* = 5.2 Hz, 2H), 3.90 (s, 3H), 2.96 (d, *J* = 2.7 Hz, 2H), 2.63 (t, *J* = 5.8 Hz, 2H), 2.36 (s, 5H). ESI-MS *m/z* 483.2 (M+1)⁺.

Methyl-2-(3-(1-methyl-1, 2, 3, 6-tetrahydropyridin-4-yloxy)-6-(prop-2-ynyloxy)-9H-xanthen-9-yl) benzoate (17) This compound was prepared according to the same procedure for the synthesis of 15. Yield: 91 %. ¹H NMR (400 MHz, CDCl₃): δ 7.78 (d, *J* = 7.2 Hz, 1H), 7.78–7.09 (m, 3H), 6.92 (m, 2H), 6.78 – 6.71 (m, 2H), 6.63 – 6.56 (m, 2H), 6.23 (s, 1H), 5.27 (s, 1H), 4.94 (s, 2H), 4.64 (s, 3H), 3.93 (s, 2H), 2.98 (d, *J* = 2.8 Hz, 2H), 2.70–2.64 (m, 2H), 2.38 (s, 5H). ESI-MS *m/z* 481.2 (M+1)⁺.

Methyl-2-(3-(benzyloxy)-6-(1-methyl-1, 2, 3, 6-tetrahydropyridin-4-yloxy)-9H-xanthen-9-yl) benzoate (18) This compound was prepared according to the same procedure for the synthesis of 15. Yield: 91 %. ¹H NMR (400 MHz, CDCl₃): δ 7.75 (d, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.24 (dd, *J* = 24.4, 7.2 Hz, 4H), 7.09 (dd, *J* = 12.1, 4.4 Hz, 2H), 6.96 (d, *J* = 8.5 Hz, 2H), 6.91 (d, *J* = 8.6 Hz, 2H), 6.79 (d, *J* = 2.3 Hz, 1H), 6.69 (d, *J* = 2.5 Hz, 1H), 6.61 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.55 (dd, *J* = 8.6, 2.5 Hz, 2H), 6.25 (s, 1H), 4.92 (s, 3H), 3.85 (s, 3H), 2.91 (s, 2H), 2.57 (t, *J* = 5.7 Hz, 2H), 2.38 – 2.27 (m, 5H). ESI-MS *m/z* 533.2 (M+1)⁺.

2-(3-methoxy-6-(1-methyl-1, 2, 3, 6-tetrahydropyridin-4-yloxy)-9H-xanthen-9-yl) benzoic (Probe 1)

To a solution of 15 (0.32 g, 0.7 mmol) in MeOH (50 mL) was added 2 M NaOH (10 mL), the mixture was stirred at room temperature. After complete consumption of the starting material (TLC monitoring), the mixture was concentrated under reduced pressure and then acidified with 1M HCl (pH = 2). The product was extracted with AcOEt (30 mL×3), and the combined organic layer was dried over MgSO₄ and concentrated. The crude residue was purified by SiO₂ chromatography (CH₂Cl₂: MeOH = 15:1) to give compound Probe 1. Yield: 67 %. ¹H NMR (400 MHz, DMSO): δ 7.66 (d, *J* = 5.2 Hz, 1H), 7.24 (d, *J* = 7.2 Hz, 1H), 7.17 (d, *J* = 6.8 Hz, 1H), 7.05 (d, *J* = 8.0 Hz, 2H), 6.96 (d, *J* = 8.84 Hz, 1H), 6.79 (s, 1H), 6.67 (d, *J* = 7.6 Hz, 1H), 6.57 (d, *J* = 8.4 Hz, 2H), 6.43 (s, 1H), 4.98 (s, 1H), 3.73 (s, 3H), 3.37 (s, 2H), 3.07 (s, 2H), 2.60 (s, 3H). ESI-MS *m/z* 443.2(M+1)⁺.

2-(3-(allyloxy)-6-(1-methyl-1, 2, 3, 6-tetrahydropyridin-4-yloxy)-9H-xanthen-9-yl) benzoic acid (Probe 2)

This compound was prepared according to the same procedure for the synthesis of Probe 1. Yield: 67 %. ¹H NMR (400 MHz, CDCl₃): δ 7.82 (s, 1H), 7.08 (t, *J* = 7.2 Hz, 3H), 6.82 (m, 3H), 6.69 (s, 1H), 6.44 (m, 3H), 6.27 (s, 1H), 5.92 (m, 1H), 5.21 (m, 2H), 4.65 (s, 1H), 4.41 (s, 2H), 3.65 (m, 2H), 3.91 (s, 2H), 2.96 (s, 3H), 2.70 (s, 2H); ¹³C NMR (101 MHz, CDCl₃): δ 157.83, 153.04, 151.46, 151.08, 150.79, 147.02, 132.81, 132.81, 131.63, 131.20, 130.92, 130.54, 129.37, 125.94, 121.23, 117.59, 117.59, 116.78, 115.04, 110.75, 107.91, 101.81, 95.48, 68.84, 50.88, 50.49, 42.30, 37.67, 24.32. ESI-MS *m/z* 469.2 (M+1)⁺.

2-(3-(1-methyl-1, 2, 3, 6-tetrahydropyridin-4-yloxy)-6-(prop-2-ynyloxy)-9H-xanthen-9-yl) benzoic acid (Probe 3)

This compound was prepared according to the same procedure for the synthesis of Probe 1. Yield: 68%. ¹H NMR (400 MHz, CDCl₃): δ 7.83 (d, *J* = 7.7 Hz, 2H), 7.21 (d, *J* = 7.5 Hz, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 6.90 (m, 3H), 6.69 (d, *J* = 2.0 Hz, 1H), 6.60 (d, *J* = 2.2 Hz, 1H), 6.45 (dd, *J* = 8.4, 1.9 Hz, 2H), 6.27 (s, 1H), 5.29 (s, 2H), 4.65 (s, 1H), 4.59 (d, *J* = 1.9 Hz, 2H), 3.63 (s, 2H), 3.36 (s, 2H), 2.88 (s, 3H), 2.72 (s, 2H); ¹³C NMR (101 MHz, CDCl₃): δ 156.83, 153.11, 151.65, 151.65, 151.08, 150.81, 146.75, 131.78, 131.56, 131.26, 130.98, 130.70, 129.40, 126.05, 121.24, 117.69, 115.19, 110.76, 110.76, 107.96, 102.18, 95.50, 55.99, 53.45, 50.91, 50.55, 42.40, 37.77, 24.53. ESI-MS *m/z* 467.2 (M+1)⁺.

2-(3-(benzyloxy)-6-(1-methyl-1, 2, 3, 6-tetrahydropyridin-4-yloxy)-9H-xanthen-9-yl) benzoic acid (Probe 4)

This compound was prepared according to the same procedure for the synthesis of probe 1. Yield: 61 %. ¹H NMR (400 MHz, CDCl₃): δ 7.67 (d, *J* = 6.1 Hz, 1H), 7.35 – 7.01 (m, 5H), 6.89 – 6.70 (m, 5H), 6.58 (s, 2H), 6.45 (dd, *J* = 61.8, 23.0 Hz, 2H), 6.16 (s, 1H), 5.14 (s, 1H), 4.77 (s, 2H), 4.52 (s, 2H), 4.47 (s, 2H), 3.20 (s, 2H), 2.72 (s, 3H), 2.54 (s, 2H); ¹³C NMR (101 MHz, CDCl₃): δ 172.30, 157.86, 152.99, 151.26, 150.97, 150.71, 146.46, 136.39, 132.48, 131.04, 130.90, 130.59, 128.93, 128.25, 128.25, 128.25, 127.69, 127.22, 127.22, 125.75, 121.15, 116.96, 114.91, 110.72, 107.69, 101.83, 95.75, 69.88, 53.44, 50.64, 50.26, 42.13, 24.31. ESI-MS *m/z* 519.2 (M+1)⁺.

Determination of the quantum yield

Absorption spectra, the steady-state fluorescence emission and excitation spectra were obtained by using a Molecular Devices Spectramax M2 Microplate Spectrofluorometer. For the determination of the relative fluorescence quantum yields (Φ_f) of **Probe 1-4** and *O*-methyl fluorescein, only dilute solution with an absorbance below 0.1 at the excitation wavelength λ_{ex} were used. Fluorescein in 0.1M NaOH aqueous solution (λ_{ex} =470nm, Φ_f =0.85) were used as fluorescence standard. The fluorescence quantum yield (Φ_f) was calculated from eq (1).

$$QY_X = QY_S * [A_X / A_S] * [F_S / F_X] \quad (1)$$

Here, F_S denotes the fluorescence intensity of standard material at the excitation wavelength, A_S is the absorption intensity of standard material at the excitation wavelength; QY_S denotes the quantum yield of standard material.

The quantum efficiencies of fluorescence in this work were obtained from average values of multiple (generally three) independent measurements. All spectra were recorded at 37 °C. The result was shown in **Stabe 1**.

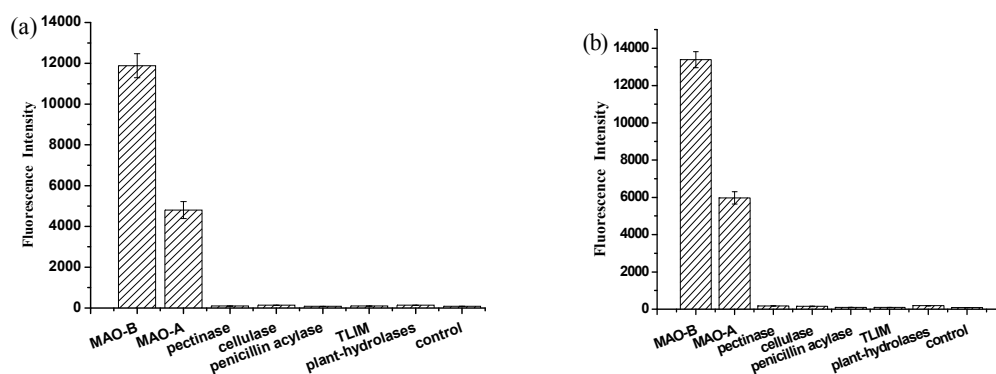
Stabe 1 The quantum yield of Probe 1-4.

Probe	Φ_f
Probe 1	0.0012
Probe 2	0.0017
Probe 3	0.0010
Probe 4	0.0007
<i>O</i> -methyl fluorescein	0.38

Part II:Enzymatic Assays

In Vitro Fluorescence Properties and Determination of MAO

To approximate in vivo environment, we employed mitochondrial preparations rather than purified enzymes. MAO-A and MAO-B were prepared from human placenta and liver, respectively. Firstly, we cut the tissue of placenta and liver, Secondly, tissue homogenate machine was used for further broken. At last, we get mitochondria preparations by Ultrasonic cell crusher instrument and determinate enzyme activity. In order to determine the selectivity of probe **1-4** toward MAO and demonstrate the MAOs activity depending characteristics, their response toward various proteins, including MAOs, pectinase(UniBioche Corp), cellulase(UniBioche Corp), penicillin acylase(Zhejiang wind HaiDeEr co., LTD), TLIM(Lipase from Thermomyces lanuginosus,Amano pharaceutical company Ltd) and plant-hydrolases(UniBioche Corp) were tested, The detection of activity of enzymes with Probes was performed in 96-well fluorescence assay plates. The stock solutions of **Probe 1-4** were prepared in DMSO(10mM) and diluted in enzyme assay buffer(50 mM Borate buffer, pH=8.4) to a final concentration 200 uM. All kinds of enzymes were added to a final protein concentration 40 ug/mL and incubation for 2h at 37 °C,respectively. Fluorescence intensity of **Probe 1-4** were collected by using Molecular Devices Spectramax M2 Microplate Spectrofluorometer after 2h. The excitation wavelength was 470nm.The result is shown in **Figure S1** and **Figure S2**.



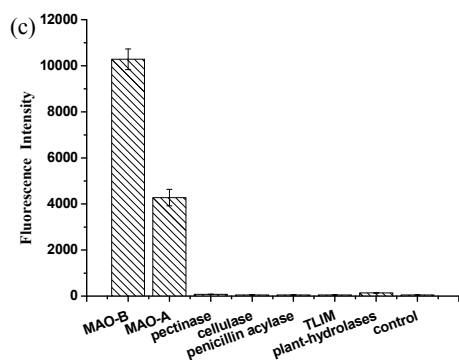


Figure S1. MAOs selectivity of **Probe 1-3** in 50 mM Borate buffer (pH=8.4). The bars represent the fluorescence intensity at 515 nm for **Probe 1-3**, after 2 h of reaction of 200 μ M probes with each type of MAO-A, MAO-B, pectinase, cellulase, penicillin acylase, TLIM, plant-hydrolases and Borate buffer (Control). The excitation wavelength was 470 nm.

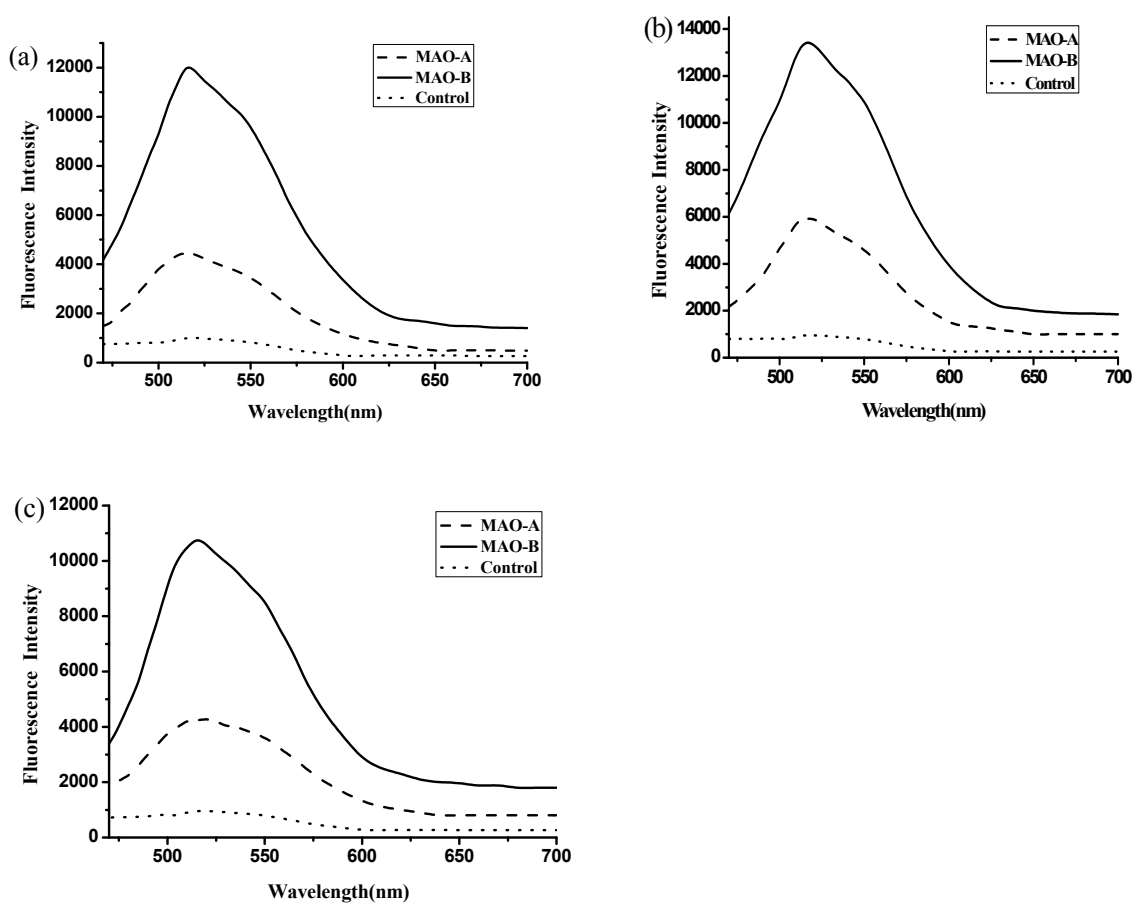


Figure.S2 Fluorescence measurement of **Probe1-3** before and after reaction with MAO-A (dotted line) and B (solid line). The spectrum were recorded after 2 h of reaction of Probe 4 with MAO-A and B in 50 mM borate buffer (pH=8.4). Excitation wavelength was 470nm.

Enzymatic Kinetics Assays

Enzyme kinetics experiments were performed in 96-well fluorescence assay plates. A series concentration of **Probe 1-4** was diluted in enzyme assay buffer(50 mM Borate buffer, pH=8.4) to a final concentration containing (0-300uM). The fluorescence intensity was collected at 515 nm(λ_{ex} =470nm) by using Molecular Devices Spectramax M2 Micropalte

Spectrofluorometer at 5min intervals from 0 to 1h at 37 °C and the rate of change of fluorescence was calculated by eq (2). Enzyme kinetics experiments with **Probe 1-4** and MAO-A or MAO-B were performed on three independent experiments.

$$v = \frac{\frac{n_{st} \times [F_t - F_0]}{F_{st}}}{\text{time}} \quad (2)$$

Here, n_{st} is the standard nano molar number of the product; F_t and F_0 denotes the fluorescence intensity at t and initial time; F_{st} is the fluorescence intensity of the product of n_{st} .

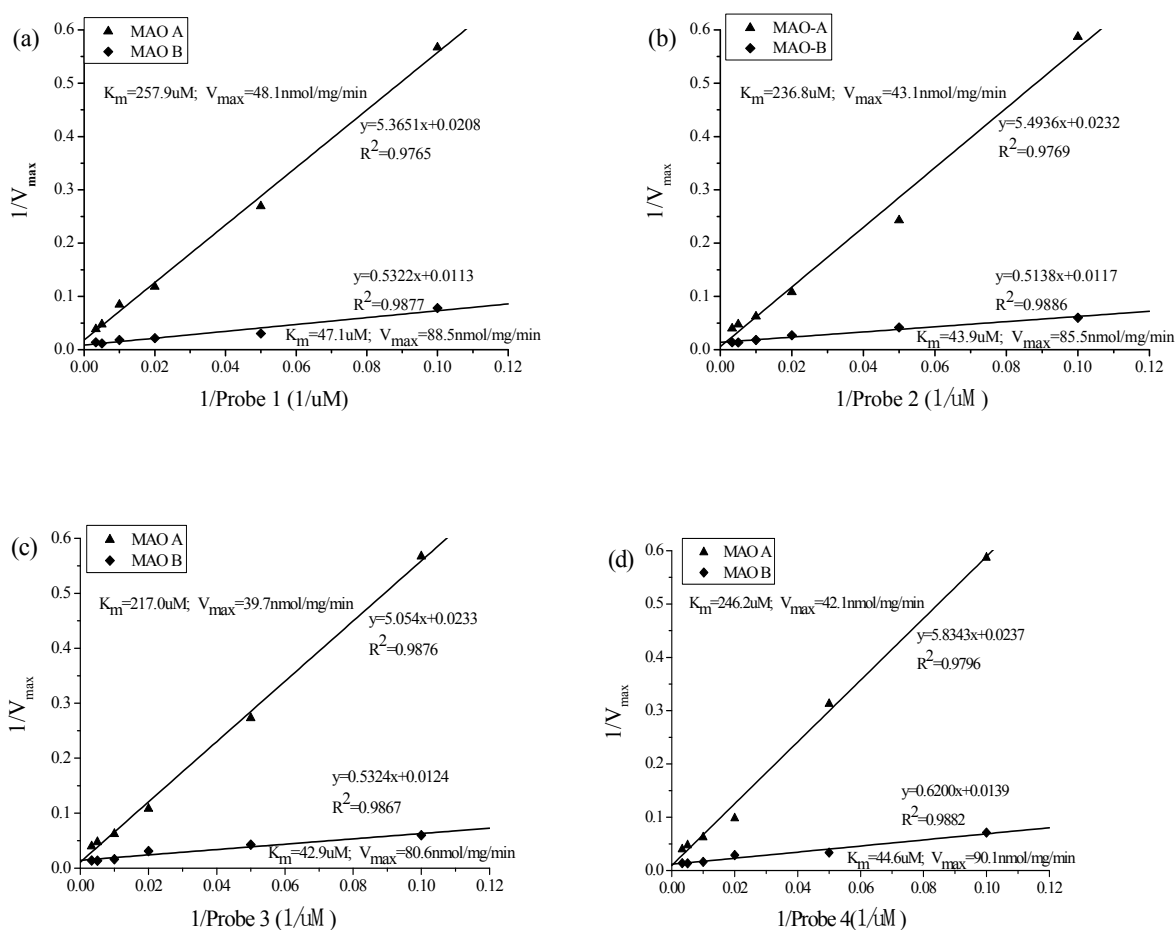


Figure S3. K_m values of **Probe 1-4**(a-d) with MAO-A or MAO-B. The K_m and V_{max} value of MAO-A or MAO-B was assessed by a series concentrations of **Probe 1-4**(0-300 μM) reaction with MAO-A or MAO-B (40 $\mu\text{g/mL}$) at 37 °C in enzyme assay buffer(50 mM borate buffer, pH=8.4). The fluorescence intensity was collected at 515nm(λ_{ex} =470nm).

Part III: Confocal imaging

In order to realize real-time MAO imaging and detection, we use R group modification for improving the lipid solubility of our probe, the value of ClogP is shown in Table S1. From the table, we know these probes can penetrate into cell.

Table S1 the cLogP value of probe 1-4

	Probe 1	Probe2	Probe 3	Probe 4	Without R
CLogP	2.348	3.292	3.118	4.286	1.932

For live cell imaging of MAO activity, MCF-7 cell was used a model to test whether we could use our probe to detect MAO. C6 glioma cell was used for negative control.

Experiments were carried out as follows. Firstly, MCF-7 and C6 cells were cultured in chamber at 37 °C, and after 80% confluence, the medium was removed followed by washing with PBS buffer twice. The different concentrations of probes (200 μM) were then added to the chamber in the growth medium. After incubation for 1 h at 37 °C, cells were washed three times with PBS buffer to remove the extracellular probes, and further fixed by 75% ethanol for 30min. The image of the cell monolayer was conducted with excitation at 470nm and emission at 515nm. To investigate its selectivity toward MAO-B, a MAO-B inhibitor, pargyline was incubated with MCF-7 cell for 3 hours before the addition of the probe.