Supporting Information for

A Turn-On Fluorescent Probe for Detection of Tyrosinase Activity

Changcheng Wang,[a‡] Shengyong Yan,[a‡] Rong Huang,[a] Shuo Feng,[a] Boshi Fu,[a]

Xiaocheng Weng*,[a] Xiang Zhou*[a,b]

College of Chemistry and Molecular Sciences, Key Laboratory of Biomedical Polymers of Ministry of Education, Wuhan University, Hubei, Wuhan, 430072, P. R. of China, Corresponding Author Email Address: xzhou@whu.edu.cn, xcweng@whu.edu.cn

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Materials, methods and instruments

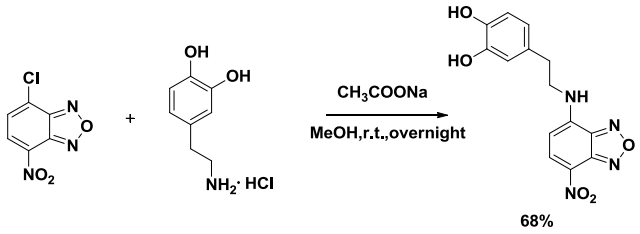
All solvents and reagents were commercially available and used without further purification unless for special needs. $CaCl_2$, $MgCl_2$, KCl, NaCl and $FeCl_3$ were bought from Sangon Biotechnology Co., Ltd. (Shanghai, China). Tyrosinase (EC 1.14.18.1, 1715 U/mg) from mushroom and Bovine serum albumin, S1 nuclease, Trypsin, APE were purchased from Sigma. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 300 spectrometers, respectively. HRMS were recorded on a Brucker APEX IV (7.0 T). HPLC chromatograms were obtained on Agilent 1100. Fluorescent emission spectra were collected from 480-700 nm on PerkinElmer LS 55 with an excitation wavelength of 460 nm, the excitation and emission slit widths were 10 and 20 nm, respectively. Quartz cuvette with 2 mL volume was used for emission measurements. Unless otherwise specified, all spectra were taken at an ambient temperature in 10 mM potassium phosphate buffered at pH 6.4.

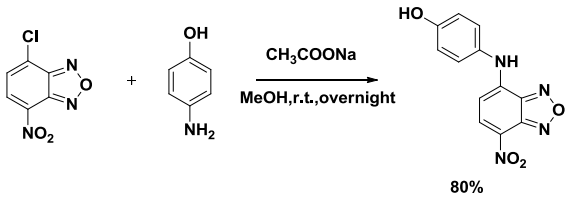
MBTH Assay

Compound 1 (50 μM) was incubated with 100 U/mL of mushroom tyrosinase and 1 mM MBTH for 1 h. MBTH solutions were freshly prepared before use

General procedure for the synthesis of compound 2 and compound 1

NBD-NH_2 was prepared by the literature method.[1]





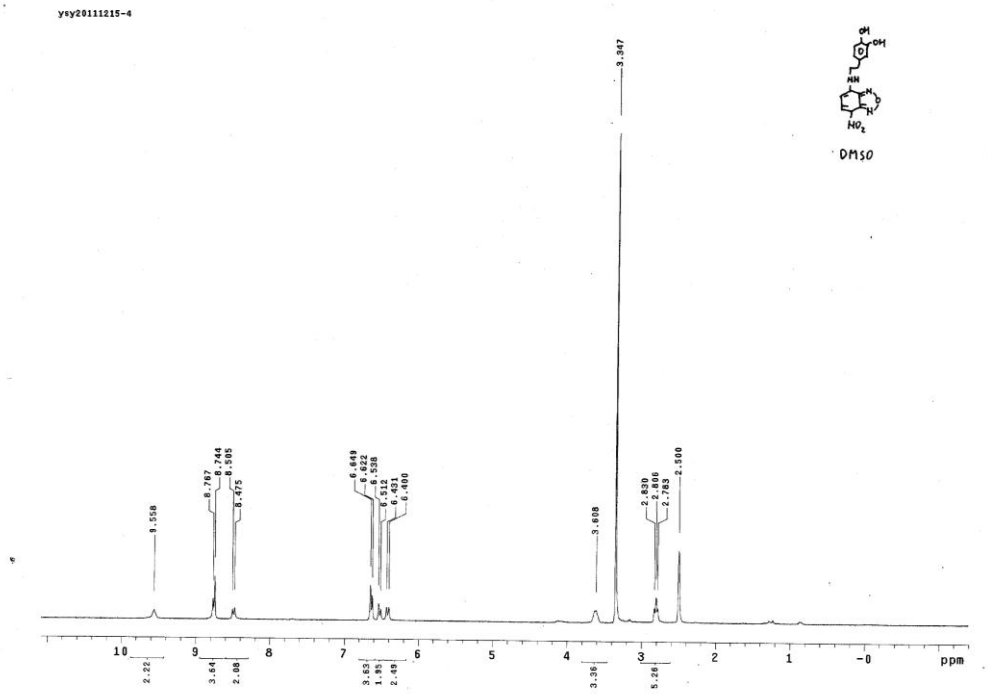
Compound 2

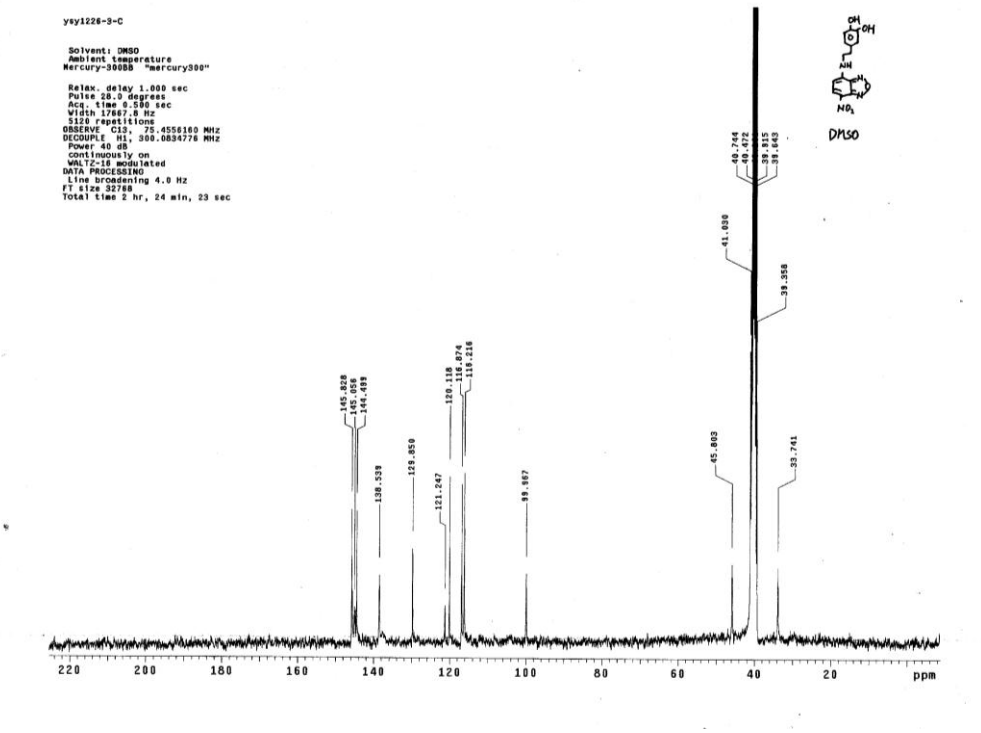
Sodium acetate (123 mg, 1.5 mmol) was added to a solution of 4-chloro-7-nitrobenzo -2-oxa-1,3-diazole (200 mg, 1 mmol) and dopamine hydrogen chloride salt (283.5 mg, 1.5 mmol) in ethanol (3 mL) in small portions. The reaction mixture was stirred at room temperature overnight, then, the solution was filtered and washed with ethanol to afford the product as a red solid (215 mg, 0.68 mmol). Yield 68%. Mp: 183-184℃. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 9.55 (br, 1 H), 8.76-8.74 (m, 2 H), 8.50 (d, 1 H, *J* = 9 Hz), 6.64 (d, 2 H, *J* = 8.1 Hz), 6.53 (d, 1 H, *J* = 7.8 Hz), 6.43 (d, 1 H, *J* = 9.3 Hz), 3.60 (m, 2 H), 2.80 (t, 2 H, J = 7.2 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 145.8, 145.0, 144.5, 138.5, 129.8, 121.2, 120.1, 116.8, 116.2, 99.9, 45.8, 33.7. HRMS (ESI) calcd for $C_{14}H_{12}N_4O_5$ $[M]^+$ 316.0808; found: 339.0702 $[M+Na]^+$

Compound 1

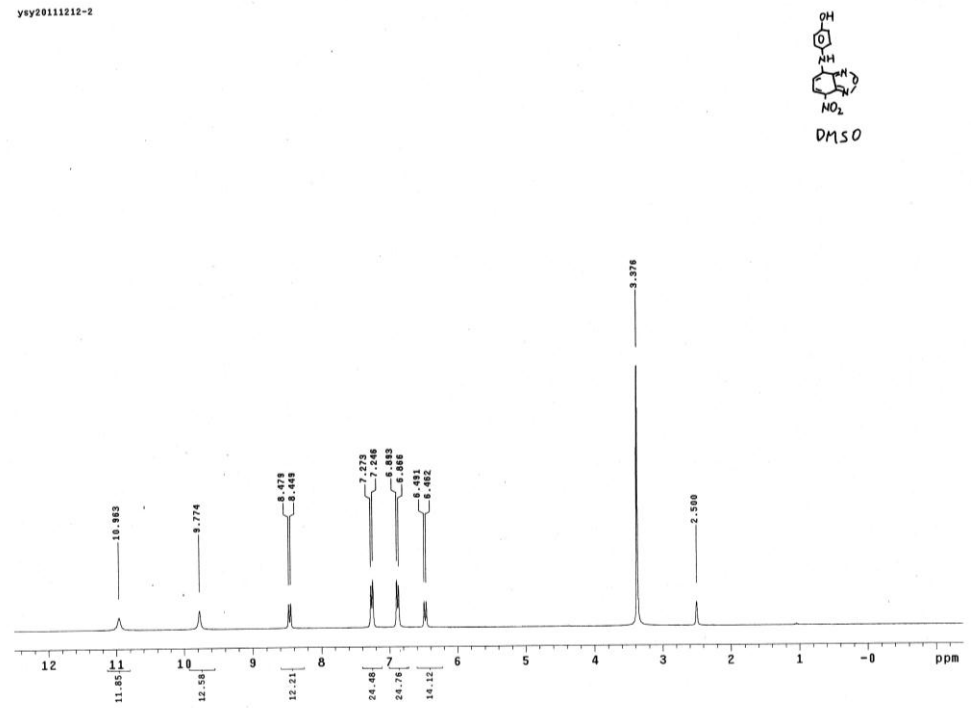
Sodium acetate (246 mg, 3 mmol) was added to a solution of 4-chloro-7-nitrobenzo -2-oxa-1,3-diazole (200 mg, 1 mmol) and 4-aminophenol (327 mg, 3 mmol) in ethanol (3 mL) in small portions. The reaction mixture was stirred at room temperature overnight; then, the solution was filtered and washed with ethanol to afford the product as a red solid (217 mg, 0.80 mmol). Yield 80%. Mp:223-224℃. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 10.96 (br, 1 H), 9.77 (br, 1 H), 8.47 (d, 1 H, *J* = 9.0 Hz), 7.27 (d, 2 H, *J* = 8.1 Hz), 6.89 (d, 2 H, *J* = 8.1 Hz), 6.49 (d, 1 H, *J* = 8.7 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 161.3, 149.9, 149.4, 148.6, 142.8, 133.8, 131.0, 126.8, 121.1, 105.9. HRMS (ESI) calcd for $C_{12}H_8N_4O_4$ $[M]^+$ 272.0546; found: 295.0436 $[M+Na]^+$

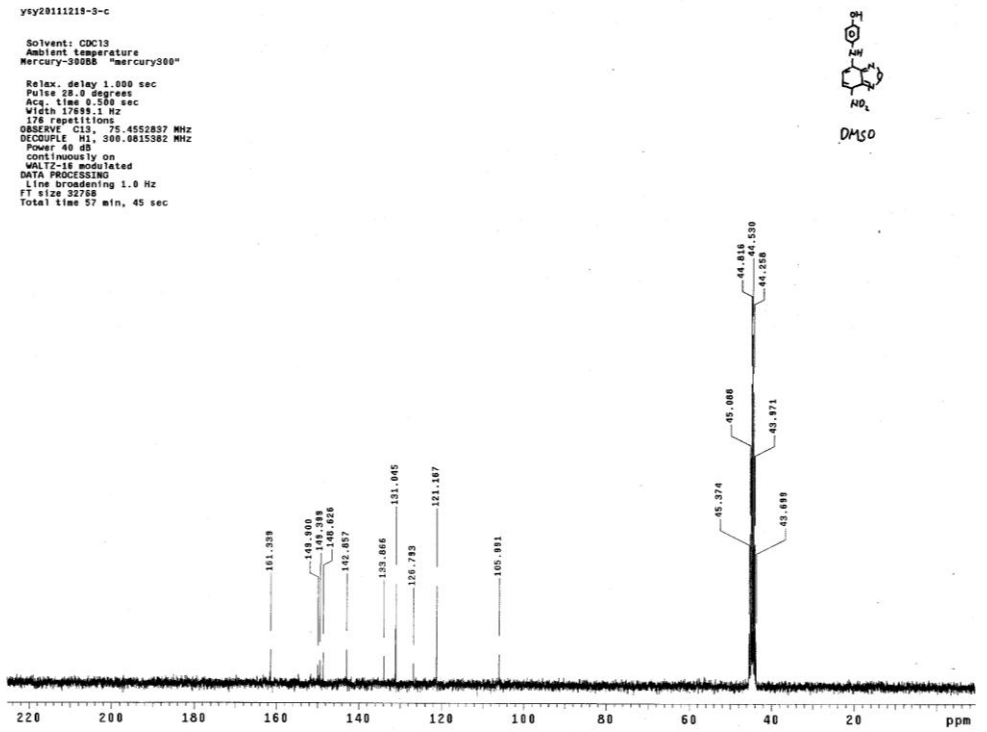
^{1}H and ^{13}C NMR spectra of compound 2 recorded in DMSO-d_6





^{1}H and ^{13}C NMR spectra of compound 1 recorded in DMSO-d_6





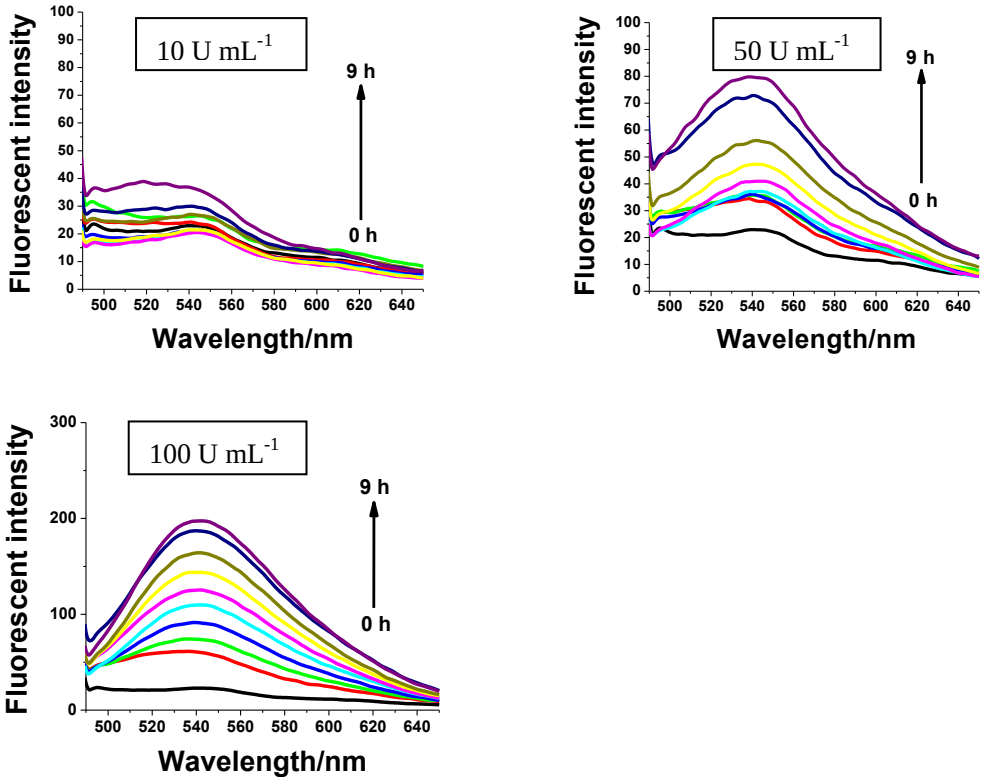


Figure S1. Time-dependent fluorescent response (from 0 h to 9 h) of probe 1 (10 μM) toward different amount of tyrosinase.

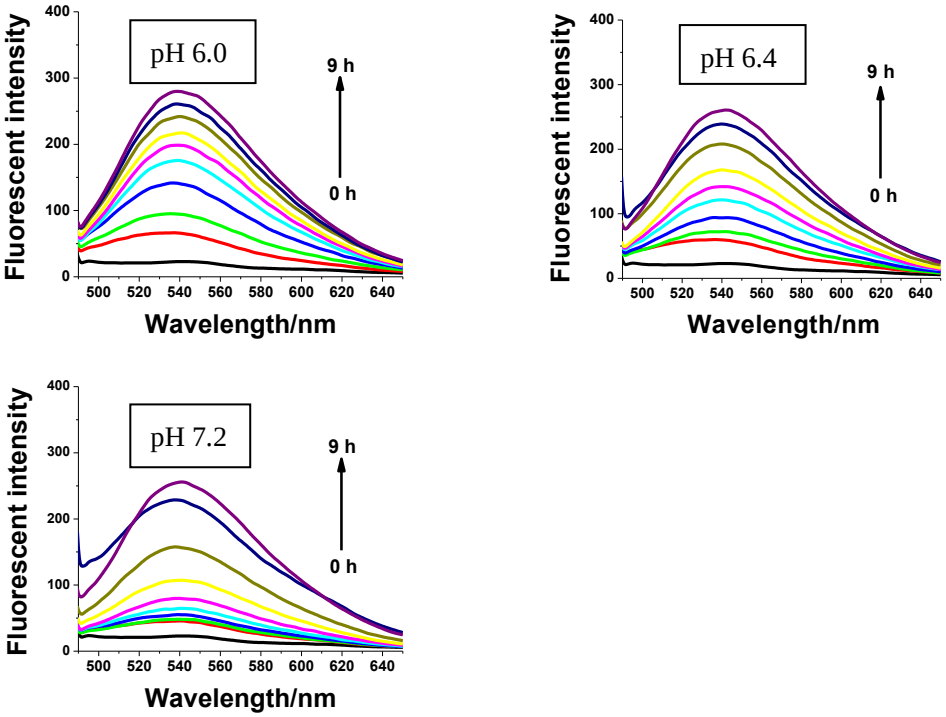


Figure S2. pH-dependent fluorescent response of probe 1 (10 μM) toward tyrosinase (100 U mL^{-1})

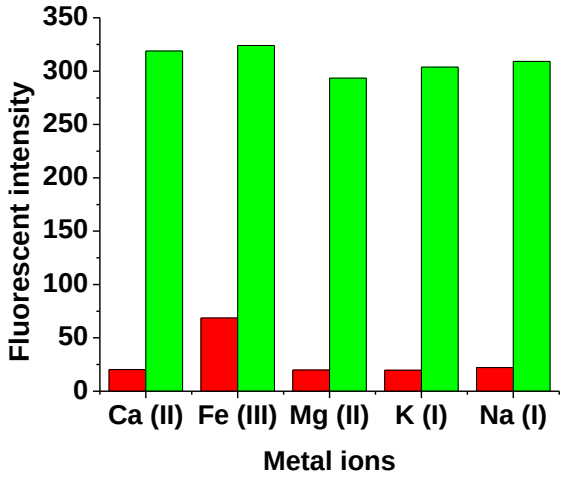


Figure. S3 Fluorescence responses of probe 1 (10 μM) toward tyrosinase in the presence of biologically relevant metal ions (Na^{+}, K^{+}, Mg^{2+}, Fe^{3+} and Ca^{2+}, 250 μM respectively). Red bars: probe 1 incubated with each of the five metal ions; green ones: probe 1 with tyrosinase in the presence of each metal ion.

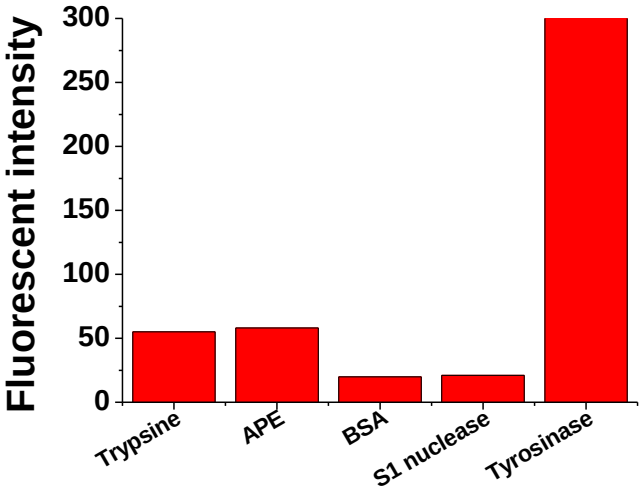


Figure. S4 Fluorescence responses of probe 1 (10 μM) toward four types of biologically relevant proteins and enzymes. Trypsin and BSA: 20 μM, APE, S1 nuclease, Tyrosinase: 100 U mL^{-1}

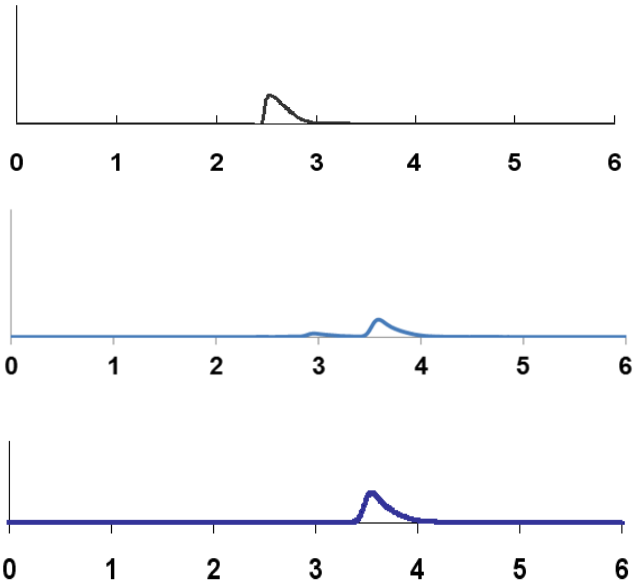


Figure S5. HPLC analysis of probe 1 alone (top), 1 incubated for 24 h at 37℃ with tyrosinase (middle), and fluorescent NBD-NH_2 only (bottom). The retention time at 3.58 min corresponds to NBD-NH_2. The signal was monitored under irradiation at 230 nm for probe 1(retention time 2.54 min). The fluorescence response at 540 nm was measured under irradiation at 460 nm for 1 incubated with tyrosinase and NBD-NH_2

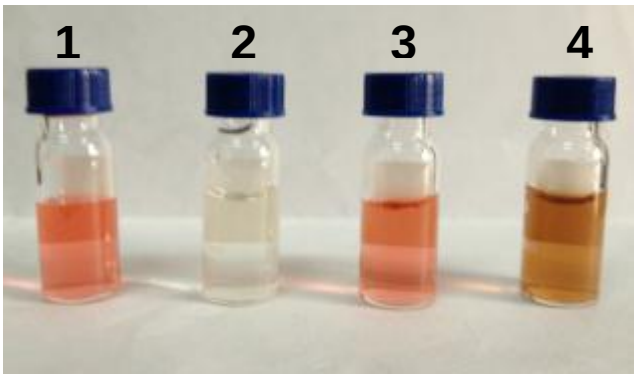


Figure S6. MBTH color test based to trap the formed intermediate orthoquinone during the tyrosinase oxidation. The concentrations of probe 1, MBTH, and tyrosinase were fixed at 50 μM, 1mM, and 100 UmL^{-1}, respectively. Images of vials containing probe 1 and MBTH (1), MBTH and tyrosinase (2), probe 1 and tyrosinase (3), and probe 1, tyrosinase, and MBTH (4) (from left to right)

Reference

1. W. Jiang, Q. Q. Fu, H. Y. Fan, J. Ho, W. Wang. *Angew. Chem. Int. Ed.* 2007, **46**, 8445-8448.