**Electronic Supplementary Information for:**

# A highly sensitive and selective fluorescence off-on probe for the detection of intracellular endogenous tyrosinase activity

Xiaofeng Wu,[ab] Xiaohua Li*,[ab] Hongyu Li,[a] Wen Shi[a] and Huimin Ma*[ab]

[a] *Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China. E-mail: lixh@iccas.ac.cn*; *mahm@iccas.ac.cn*

[b] *University of Chinese Academy of Sciences, Beijing 100049, China.*

## Table of contents

## 1. Apparatus and reagents

$^{1}$H-NMR and $^{13}$C-NMR spectra were measured with a Bruker DMX-400 spectrometer in DMSO-$d_6$. Electrospray ionization mass spectra (ESI-MS) were implemented with a Shimadzu LC-MS 2010A instrument (Kyoto, Japan). High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed on an APEX IV FTMS instrument (Bruker, Daltonics). High-performed liquid chromatography (HPLC), absorption and fluorescence spectral analyses were conducted as described previously.[1] Fluorescence imaging was conducted on an FV 1200-IX83 confocal laser scanning microscope (Olympus, Japan), with an excitation wavelength of 488 nm and an optical section of 0.5 μm. The incubation was performed in a Shaker incubator (SKY-100C, China).

Resorufin sodium salts, monoamine oxidase A (MAO-A), monoamine oxidase B (MAO-B), tryosinase from mushroom, leucine aminopeptidase, kojic acid, and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich. 3-Hydroxybenzyl alcohol, $PBr_3$, and $CH_3CN$ were obtained from Beijing InnoChem Science & Technology Co., Ltd. KCl, $MgCl_2$, $FeCl_3$, $K_2CO_3$ and $CaCl_2$ were obtained from Beijing Chemicals, Ltd. Anti-tyrosinase antibody was from Abcam Trading (Shanghai) Co., Ltd., and tyrosinase rabbit polyclonal antibody from Beijing OriGene Technology Co., Ltd. Phosphate buffered saline (PBS: 155.2 mM NaCl, 2.97 mM $Na_2HPO_4$, 1.05 mM $KH_2PO_4$; pH 7.4) was purchased from Invitrogen, Three cells lines (B16, HepG2 and MCF-7) and Dulbecco's modified Eagle's medium (DMEM) were obtained from KeyGEN BioTECH Co. LTD, Nanjing, China. The stock solution (1 mM) of probe **1** was prepared in DMSO. Ultrapure water (over 18 MΩ·cm) was used throughout.

## 2. Synthesis of probe 1

The starting material of 3-(bromomethyl)phenol was prepared following the reported procedure.[2]

To a suspension of resorufin sodium salt (0.24 g, 1.0 mmol) in anhydrous $CH_3CN$ (15 mL), $K_2CO_3$ (0.28 g, 2 mmol) was added, followed by stirring at 60 $^{o}$C for 10 min under Ar atmosphere. Then, a solution of 3-(bromomethyl)phenol (0.56 g, 3 mmol) in $CH_3CN$ (2 mL) was added dropwise. After stirring at 60 $^{o}$C for 24 h, the solution was cooled and diluted with $CH_2Cl_2$ (20 mL). The resulting solution was then washed with brine water (15 mL × 3). The organic layer was separated and dried over anhydrous $Na_2SO_4$. The solvent was removed by evaporation, and the residue was subjected to silica gel chromatography eluted with $CH_2Cl_2$/$CH_3OH$ (v/v, 60:1), affording probe **1** as an orange solid (35 mg, yield 11%). $^{1}$H-NMR and $^{13}$C-NMR spectra of probe **1** are shown below in Figs. S1 and S2, respectively. $^{1}$H-NMR (400 MHz, 298 K, DMSO-$d_6$): $\delta$ 9.51 (s, 1H), 7.79 (d, 1H, J=8 Hz), 7.54 (d, 1H, J=8 Hz), 7.21 (d, 1H, J=8 Hz), 7.17 (d, 1H, J=4 Hz), 7.13 (d, 1H, J=8 Hz),

6.89 (s, 1H), 6.87 (d, 1H, J=8 Hz), 6.80 (d, 1H, J=12 Hz), 6.74 (d, 1H, J=8 Hz), 6.27 (s, 1H), 5.21 (s, 2H). $^{13}$C-NMR (100 MHz, 298 K, DMSO-$d_6$): $\delta$ 185.8, 162.8, 158.0, 150.2, 145.7, 145.7, 137.9, 135.4, 134.2, 131.8, 130.1, 128.5, 118.7, 115.6, 114.9, 114.8, 106.2, 101.7, 70.7. HR-ESI-MS: *m/z* calcd for probe **1** ($C_{19}H_{12}NO_4^-$, $[M-H]^-$), 318.07719; found, 318.07718.

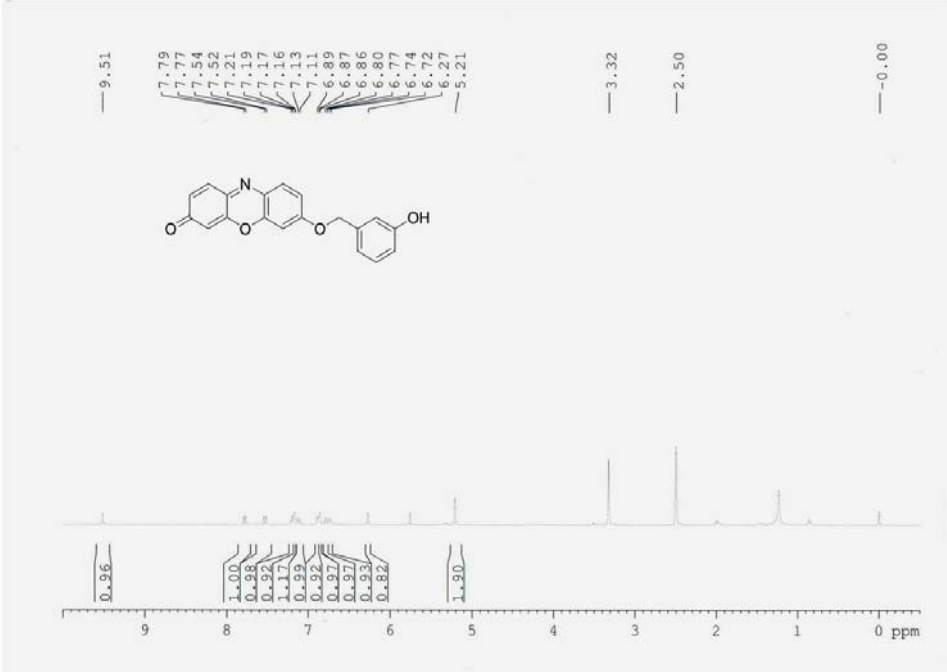


**Fig. S1.** $^{1}$H-NMR spectrum of probe **1** (400 MHz, 298 K, DMSO-$d_6$).

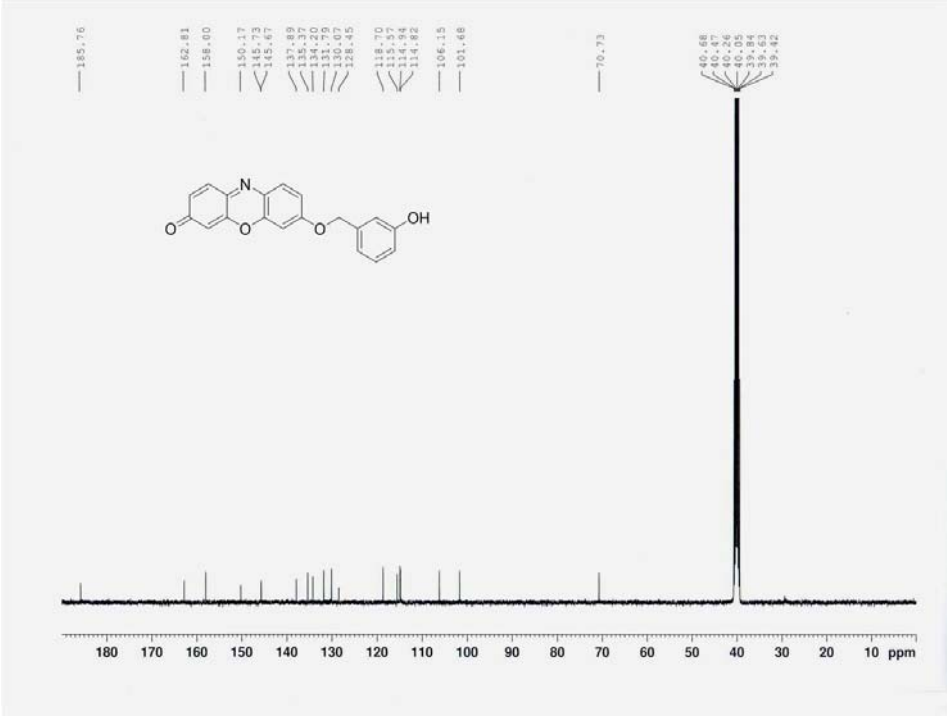


**Fig. S2** $^{13}$C-NMR spectrum of probe **1** (100 MHz, 298K, DMSO-$d_6$).

## 3. General procedure for analysis

Unless otherwise specified, the analyses were made according to the following procedure. In a tube, 4 mL of PBS (pH 7.4) and 50 μL of 1 mM probe **1** were mixed, followed by the addition of an appropriate volume of tyrosinase sample solution. The final volume was adjusted to 10 mL with PBS and the reaction solution was mixed well. After incubation at 37 $^{o}$C for 3 h, a 3-mL portion of the reaction solution was transferred to a quartz cell of 1-cm optical length to measure absorbance or fluorescence with $\lambda_{ex/em}$ = 550/586 nm (both excitation and emission slit widths were set to 10 nm). Under the same conditions, a blank solution containing no tyrosinase (control) was prepared and measured for comparison. Superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}$OH), singlet oxygen ($^1O_2$), and $ONOO^-$ were prepared according to the reported literature.[3]

## 4. Determination of fluorescence quantum yield

Fluorescence quantum yield (Φ) was determined by using resorufin (Φ = 0.75 in water solution) as a standard.[4]

## 5. Western blot analysis

Western blot analysis was made according to our previous method.[5]

## 6. Optimization of experimental conditions

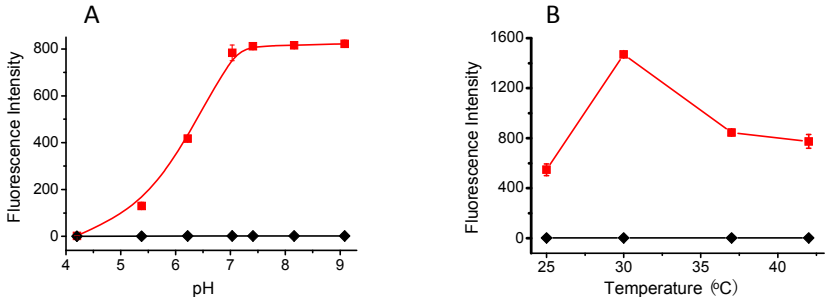


**Fig. S3** Effects of (A) pH and (B) temperature on the fluorescence intensity of probe **1** (5 μM) with (■) and without (◆) tyrosinase (100 U/mL). The results are expressed as the mean ± standard deviation of three separate measurements. $\lambda_{ex/em}$ = 550/586 nm.

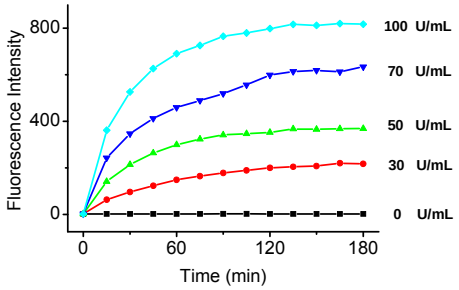


**Fig. S4** Plots of fluorescence intensity vs. the reaction time of probe **1** (5 μM) with varied concentrations of tyrosinase (0-100 U/mL). The measurements were performed at 37 °C in PBS (pH 7.4) with $\lambda_{ex/em}$ = 550/586 nm.

## 7. Linear relationship

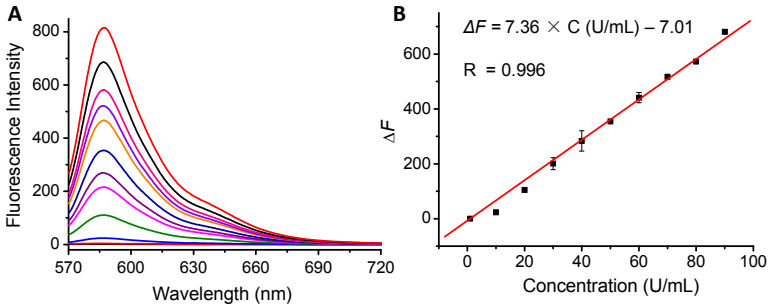


**Fig. S5** Fluorescence response of probe **1** (5 μM) to tyrosinase at different concentrations: (0, 1, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 U/mL). (B) The linear fitting curve between Δ*F* and the concentrations of tyrosinase (1 – 90 U/mL). The measurements were performed at 37 °C with $\lambda_{ex/em}$ = 550/586 nm.

## 8. Kinetic experiment

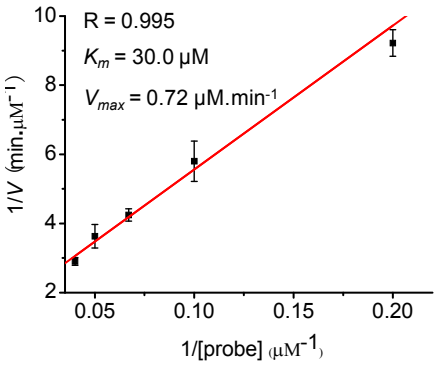


**Fig. S6** Lineweaver-Burk plot for the enzyme-catalyzed reaction. The Michaelis-Menten equation was described as: $V = V_{max}$ [probe]/($K_m$ + [probe]), where $V$ is the reaction rate, [probe] is the probe concentration (substrate), and $K_m$ is the Michaelis constant. Conditions: 20 U/mL tyrosinase, 5-25 μM of probe **1**. Reaction at each probe concentration was repeated three times, and the error bars represent standard deviations. The measurements were performed at 37 °C with $\lambda_{ex/em}$ = 550/586 nm.

## 9. Studies on reaction mechanism of probe 1 with tyrosinase

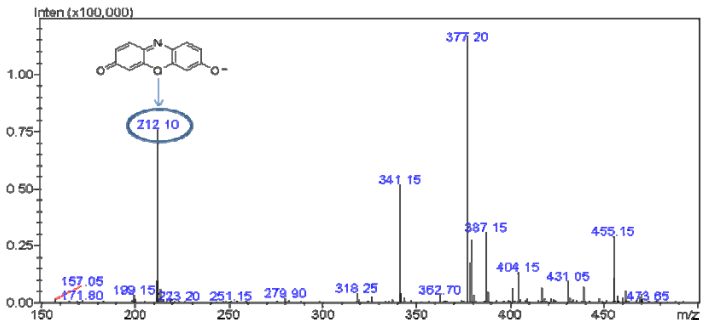


**Fig. S7** ESI mass spectrum of the reaction solution of probe **1** (200 μM) with tyrosinase (100 U/mL). The peak at *m/z* = 212.1 indicates the generation of resorufin.

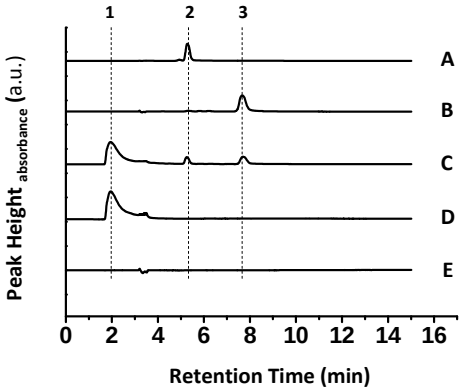


**Fig. S8** Chromatograms of different reaction systems. (A) resorufin (200 μM); (B) probe **1** (200 μM); (C) the solution of probe **1** (200 μM) reacting with 500 U/mL tyrosinase for 3 h; (D) tyrosinase (500 U/mL); (E) water (control). The assignments of the peaks: (1) 1.95 min, tyrosinase; (2) 5.28 min, resorufin; (3) 7.68 min, probe **1**. The peaks eluted from the column were monitored at 254 nm with methanol (flow rate, 0.7 mL/min) and water (flow rate, 0.3 mL/min) as eluents.

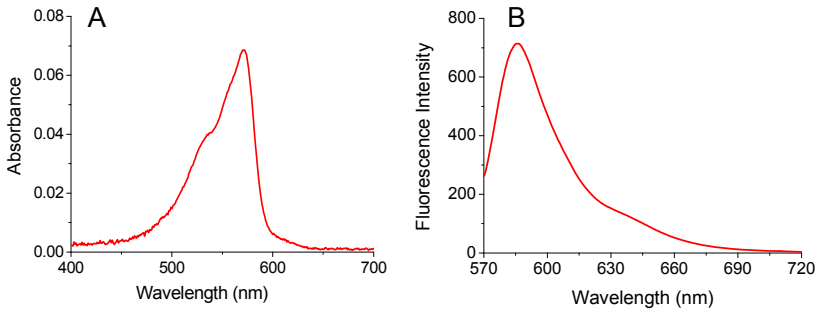


**Fig. S9** (A) Absorption and (B) fluorescence emission spectra of the isolated resorufin from reaction solution of probe **1** (5 μM) with tyrosinase (100 U/mL). $\lambda_{ex}$ = 550 nm.

## 10. Effect of inhibitor on the fluorescence intensity of the reaction system

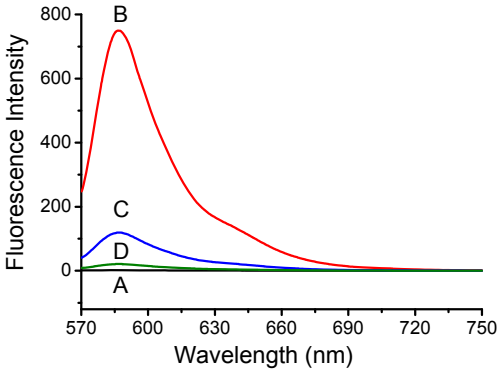


**Fig. S10** Fluorescence intensity of different reaction systems. (A) Control (5 μM probe **1** in PBS of pH 7.4); (B) system (A) + tyrosinase (100 U/mL); (C) system (B) + kojic acid (100 μM); (D) system (B) + kojic acid (200 μM). $\lambda_{ex/em}$ = 550/586 nm.

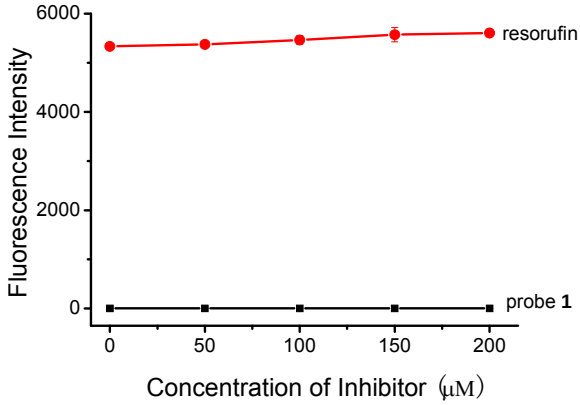


**Fig. S11** Effects of inhibitor (kojic acid) at different concentrations (0, 50, 100, 150, and 200 μM) on the fluorescence ($\lambda_{ex/em}$ = 550/586 nm) of resorufin (5 μM) and probe **1** (5 μM). The results are the mean ± standard deviation of three separate measurements.

## 11. Evaluation of the cytotoxicity of probe 1 to B16 cells

The cytotoxicity of probe **1** to B16 cells was evaluated.

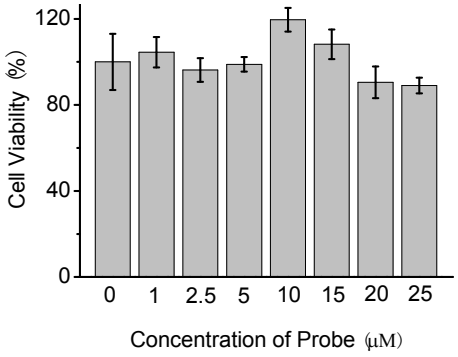


**Fig. S12** Effects of probe **1** at varied concentrations (1 - 25 μM) on the viability of B16 cells. The viability of the cells without probe **1** is defined as 100%. The results are expressed as the mean ± standard deviation of five separate measurements.

## 12. Fluorescence imaging of tyrosinase in cells

The fluorescence imaging experiment was preformed following our previous approach.[1]

## 13. References

1. Z. Li, X. H. Li, X. H. Gao, Y. Y. Zhang, W. Shi and H. M. Ma, *Anal.Chem.*, 2013, **85**, 3926.
2. J. N. Hamann, M. Rolff and F. Tuczek, *Dalton Trans.*, 2015, 44, 3251.
3. (a) X. Y. Zhu, M. Y. Xiong, H. W. Liu, G. J. Mao, L. Y. Zhou, J. Zhang, X. X. Hu, X. B. Zhang and W. H. Tan, *Chem. Commun.*, 2016, **52**, 733; (b) R. M. Uppu, *Anal.* Biochem., 2006, **354**, 165.
4. C. Bueno, M. L. Villegas, S. G. Bertolotti, C. M. Previtali, M. G. Neumann and M. V. Encinas, *Photochem. Photobiol.*, 2002, **76**, 385.
5. Q. Y. Gong, W. Shi, L. H. Li, X. F. Wu and H. M. Ma, *Anal.Chem.*, 2016, **88**, 8309.