Supporting Information for:

A near-infrared fluorescent probe for monitoring tyrosinase activity

Xiaohua Li,*[a] Wen Shi,[a] Suming Chen,[a] Jia Jia,[a] Huimin Ma*[a] and Otto S. Wolfbeis[b]

[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; Fax: +86-10-62559373*

[b]*Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, D-93040, Regensburg, Germany*

1. Apparatus and reagents

A Hitachi F-2500 spectrofluorimeter was used for fluorescence measurements. The absorption spectra were recorded with a TU-1900 spectrophotometer (Beijing Purkinje General Instrument Co. LTD). ^{1}H NMR spectra were measured on a Bruker DMX-400 spectrometer at 400 MHz in CD_3OD with tetramethylsilane as the internal standard. ^{13}C NMR spectra were measured on a Bruker Avance 600 MHz NMR spectrometer. High resolution electrospray ionization (HRESI) mass spectra were measured with a Bruker Apex IV FTMS instrument (Bruker, Daltonics). A Delta 320 pH-meter [Mettler-Toledo Instruments (Shanghai) Co., China] was used for pH measurements.

The cyanine dye (compound **2** in Scheme 1) was obtained from FEW Chemicals (www.few.de). Tyramine and tyrosinase (25 KU, from mushroom) were purchased from Sigma-Aldrich. The lyophilized powder of tyrosinase was dissolved in pure water, and the solution was divided into 20 parts as suitable amounts for daily experiments. All the solutions were frozen immediately at −20 ^{o}C for storage and allowed to thaw before use according to the known procedure (E. Mylon and S. Roston, *Am. J. Physiol.*, 1953, **172**, 612-616), which results in no change of the enzyme activity. The stock solution (250 μM) of **1** was prepared by dissolving the probe in methanol.

All other chemicals used were local products of analytical grade. Distilled-deionized water was used throughout.

2. Synthesis of 1

The cyanine derivative **1** can be readily prepared from compound **2** and tyramine by one step (Scheme 1). Typically, compound **2** (370 mg, 0.5 mmol) and tyramine (72 mg, 0.5 mmol) were dissolved in DMF (15 mL). The solution was stirred at 40 ^{o}C overnight. After cooled to room temperature, the solution was evaporated *in vacuo* to near dryness. The crude product was purified by silica-gel column chromatography with dichlomethane/methanol (1:3, v/v) as eluent, affording 376 mg of **1** (yield 90%); mp 217-219 ^{o}C. The ^{1}H NMR and ^{13}C NMR spectra of **1** are shown below in Figures S1 and S2, respectively. ^{1}H NMR (CD_3OD, 400 MHz, 298 K) δ 7.79 (d, J = 7.5 Hz, 2H), 7.30 (m, 4H), 7.07 (m, 7H), 6.74 (d, J = 8.7 Hz, 4H), 5.67 (d, J =7.0 Hz, 2H), 4.01 (m, 5H), 3.04 (m, 4H), 2.85 (m, 9H), 1.90 (s, 7H),1.61 (s, 10H); ^{13}C NMR (d_6-DMSO, 150 MHz, 298 K) δ 166.5, 165.1, 156.5, 156.4, 143.3, 140.2, 130.0, 129.9, 128.7, 128.5, 127.9, 126.6, 122.7, 122.4, 115.7, 115.7, 109.5, 96.7, 51.3, 49.0, 48.4, 47.4, 42.7, 41.0, 34.2, 33.0, 28.2, 26.3, 25.7, 23.0. HRESI-MS: Calcd for $C_{45}H_{54}N_3O_7S_2Na_2$, 858.31931 $[M + Na]^+$; Found: 858.31988.

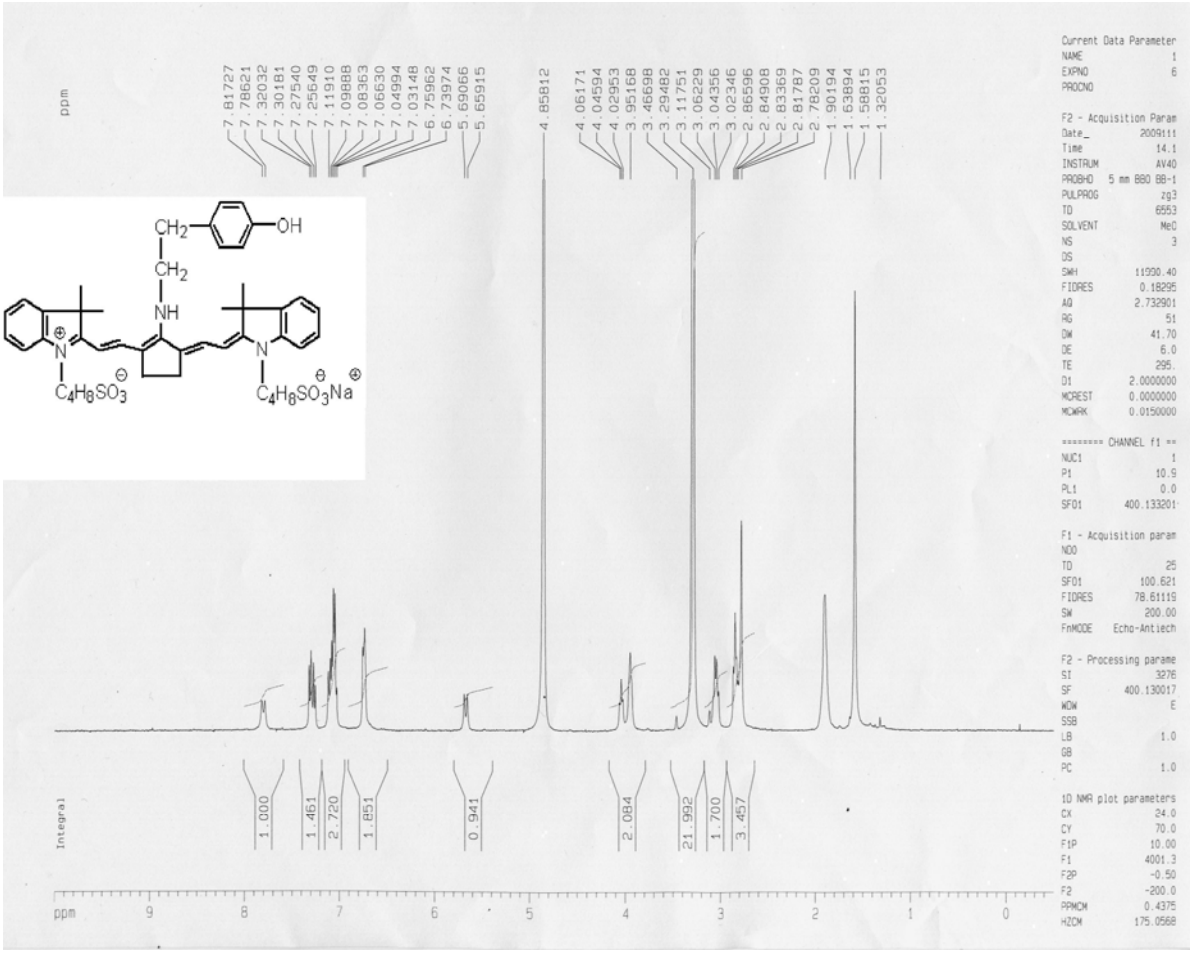

Figure S1. ^{1}H NMR spectrum of **1**.

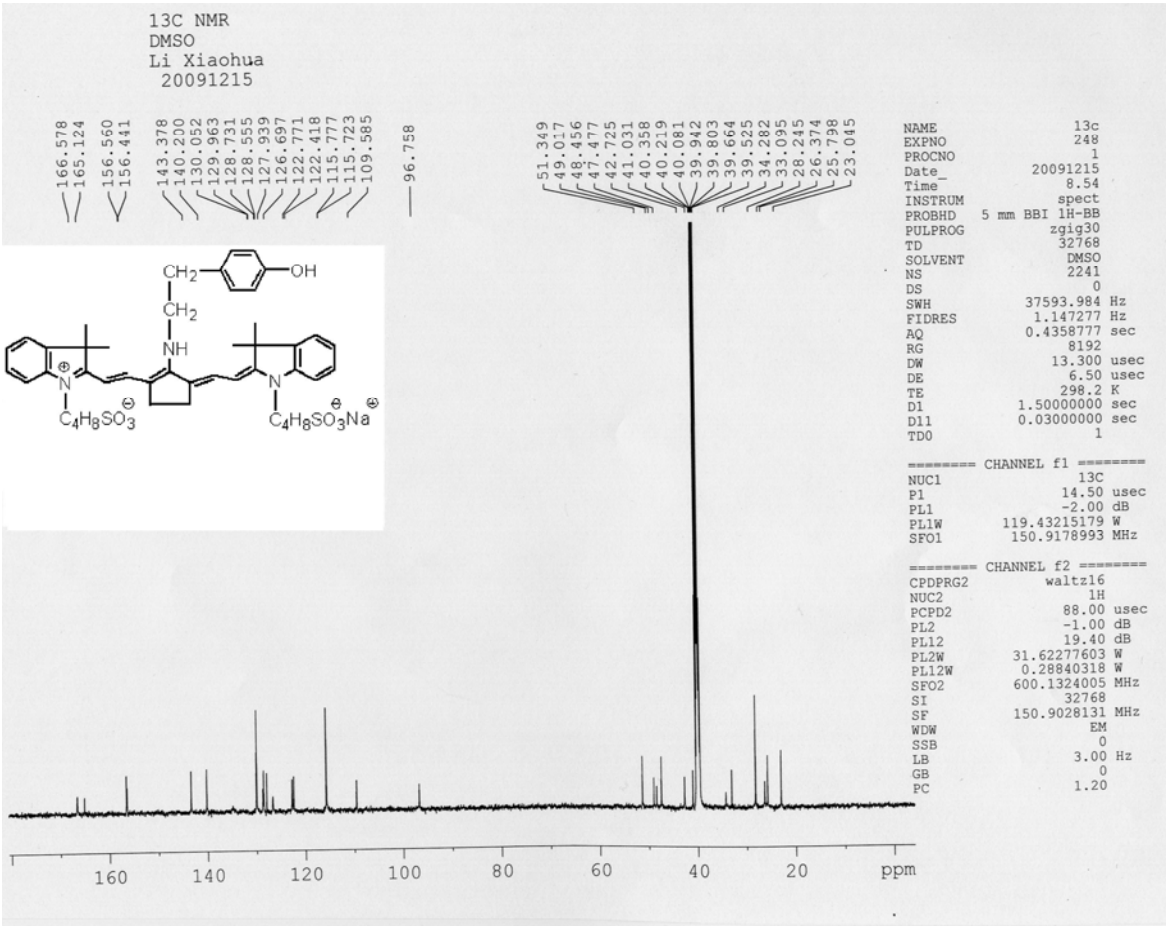

Figure S2. ^{13}C NMR spectrum of **1**.

3. General procedure for monitoring tyrosinase activity

All the measurements were made according to the following procedure. In a 10 ml tube, 5 ml of 20 mM Na_2HPO_4-NaH_2PO_4 buffer (pH 6.3) and 50 μl of the stock solution of **1** were mixed, followed by addition of an appropriate volume of tyrosinase sample solution. The final volume was adjusted to 10 ml with the phosphate buffer and the reaction solution was mixed rapidly. Then a 3-ml portion of the reaction solution was transferred to a quartz cell of 1-cm optical length to measure absorbance or fluorescence intensity/spectrum with $\lambda_{ex/em}$ = 650/720 nm and both excitation and emission slit widths of 10 nm. In the meantime, a blank solution containing no tyrosinase was prepared and measured under the same conditions for comparison.

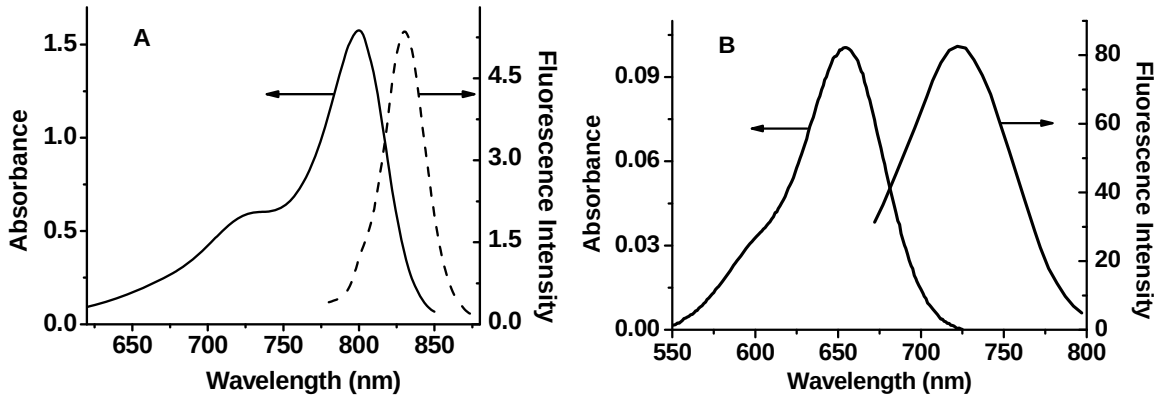

Figure S3. Absorption and emission spectra of compound **2** (5 μM, Figure S3: A) and probe **1** (0.85 μM, Figure S3: B) in 20 mM pH 6.3 phosphate buffer solution.

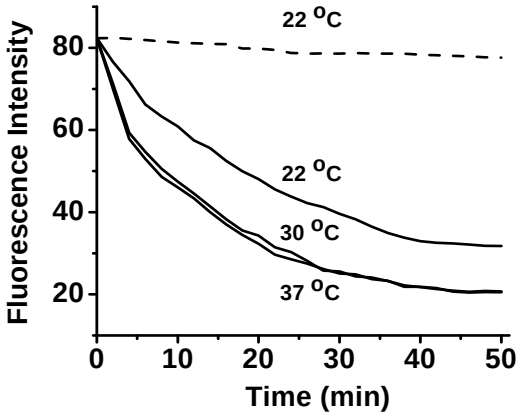

Figure S4. The change of fluorescence intensity ($\lambda_{ex/em}$ = 650/720 nm) of **1** (1.25 μM) as a function of incubating time with tyrosinase (0.16 U/ml) at different temperature (solid lines). For comparison, a control experiment with the reference compound **2** was also made (dash line). The measurements were performed in phosphate buffer solution (20 mM, pH 6.3).

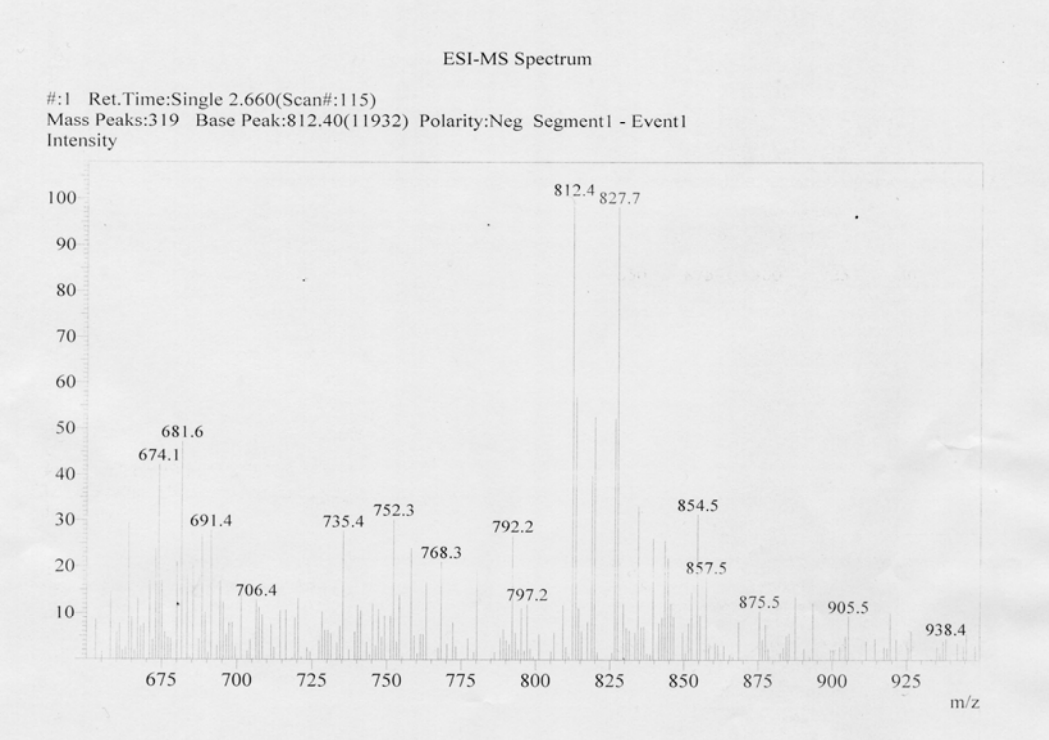

Figure S5. ESI−MS spectrum of **1** after reaction with tyrosinase at pH 6.3. The appearance of the peak at *m/z* 827 provided evidence for the quinone formation ($[M–Na]^-$); the peak at at *m/z* 812 was characterized to be the unreacted probe **1** ($[M–Na]^-$).