Supporting Information

A New Approach for Turn-on Fluorescence Sensing of L-DOPA

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**Materials and General Experimental Methods**

All chemical reagents for the synthesis were obtained from Sigma Aldrich, Alfa Aesar, or MERCK, and used without further purification unless otherwise specified. Column chromatography was carried out on Merck Silica Gel 60 (0.040-0.064 mm, 230–400 mesh). Synthetic reactions and analytical characterization were monitored by HPLC-MS (shimadzu, LCMS-2010) with a single quadrupole mass spectrometer. Mobile phase condition (water/acetonitrile): 0 min, 95% water; 1 min, 90% water; 14 min, 5% water; 15 min. Mobile phase contains 0.1% formic acid. NMR spectra (\(^1\)H 600 MHz and \(^{13}\)C-151 MHz) were recorded on Bruker Avance 600 NMR spectrometers. High-resolution mass-spectra were acquired on an Ultimate3000 (Dionex, Inc) module coupled to a Orbitrap Velos Pro mass spectrometer (ThermoFisher, Inc.) operating in ESI\(^+\) or ESI\(^-\) mode. For high mass accuracy, all spectra were obtained with Orbitrap operating at a resolution of 100,000. Spectroscopic data were measured on spectroscopic measurements, performed on Spectra Max M2 by Molecular Device (The slit width: 1 nm). pH Value was determined by a Mettler Toledo S220 SEVENCOMPACT pH meter (Columbus, OH).

**Experimental HPLC Condition for L-DOPA Detection**

The experiment was performed on HPLC-MS (model: shimadzu, LCMS-2010) with a single quadrupole mass spectrometer. The analytical method is mobile phase A: H\(_2\)O (0.1% HCOOH), mobile phase B: CH\(_3\)CN (0.1% HCOOH), gradient: 0-0.5 min 5 % B, 0.5-13 min gradient from 5% B to 95% B, 13 -15 min, 95 % B. Column: C18 (2) Luna column (250 x 4.6 mm, 5 \(\mu\)m particle size). Data was collected at 500 nm.
Figure S1. General synthetic scheme of sensors. Reagents and conditions: a: K$_2$CO$_3$, DMF, 60°C; b: Phenol, K$_2$CO$_3$, DMF, 60°C; c: K$_2$CO$_3$, DMF, 60°C.

Figure S2. The fluorescent response of Resa-Con (10 µM) to different analytes (100 µM) as function of time and pH. Analytes: 1: PBS buffer; 2: dopamine; 3: L-DOPA; 4: Epinephrine; 5: Norepinephrine; 6: NADH; 7: Phenol; 8: Catechol. Measurement conditions: PBS buffer with 50% DMSO as co-solvent; $\lambda_{ex}$=480 nm, $\lambda_{em}$=570 nm.
Figure S3. Fluorescence spectra of (A) Resa-Sulf (20 µM) and (B) Resa-Con (20 µM) during the titration with L-DOPA as function of concentration (0-400 µM). Measurement conditions: PBS buffer with 1% or 50% DMSO as co-solvent; Incubation time: 2 min; λ<sub>ex</sub>=480 nm.

Figure S4. Images of Resa-Sulf and Resa-Con at different concentrations (10-100 µM) in PBS buffer with 1% DMSO as co-solvent in 96-well plate. (A) Images were taken using iPhone SE camera. (B) Images were taken using Ts2-FL Cell Culture Microscope (Nikon Instruments Inc).
Figure S5. Time-resolved fluorescence responses of Resa-Sulf (50 μM) to L-DOPA (0-100 μM). Measurement conditions: PBS buffer with 1% DMSO as co-solvent; $\lambda_{\text{ex}}$=480 nm.

Figure S6. Concentration-resolved fluorescence responses of Resa-Sulf (20 μM) to L-DOPA, Measurement conditions: PBS buffer with 1% as co-solvent; Incubation time: (A) 1 min; (B) 10 min; $\lambda_{\text{ex}}$=480 nm.
Figure S7. Job plot shows a 1:1 binding stoichiometry between Resa-Sulf and L-DOPA. Total concentration: 50 µM. Incubation time: 10 min.

Figure S8. The reaction rate constants for Dopamine (A), L-DOPA (B), Epinephrine (C) and Norepinephrine (D).
Figure S9. HPLC-MS characterization of Resa-Sulf upon addition of L-DOPA. (A) ESI-positive MS spectra at 8.1 min and ESI-positive MS spectra at 8.5 min, respectively.
**Figure S10.** L-DOPA concentration-fluorescence intensity standard calibration curve. X axis is L-DOPA concentration (µM) and Y axis is the corresponding fluorescence intensity. Resa-Sulf concentration is 50 µM. Values are represented as means and error bars as standard deviations (n = 3). The fluorescence intensity of sample from health care products is 391.77±8.66.

![L-DOPA concentration-fluorescence intensity standard calibration curve](image1)

\[ Y = 18.56X + 66.19 \]
\[ R^2 = 0.9997 \]

**Figure S11.** L-DOPA concentration-HPLC data standard calibration curve. X axis is L-DOPA concentration (mM) and Y axis is L-DOPA peak area appeared on HPLC at 254 nm. Values are represented as means and error bars as standard deviations (n = 3). The intensity of sample from health care products is 5686±297.6.

![L-DOPA concentration-HPLC data standard calibration curve](image2)

\[ Y = 7090X - 415.1 \]
\[ R^2 = 0.995 \]
Figure S12. L-DOPA concentration in DOPA Mucuna Veg Capsules. Y axis is L-DOPA peak area appeared on HPLC at 254 nm. The intensity of sample from health care products is 5686± 297.6. Sample preparation: 9.9 mg sample was dissolved in 7.5 mL DI water (stock sample solution), strenuous vibration for 5 min, then filter to remove insoluble substances. HPLC Method: 50 µL stock sample solution was injected to HPLC for L-DOPA peak area calculation.
Synthesis and Characterization

Preparation of Resa-Sulf. To a stirred solution of Resazurin (200 mg, 0.87 mmol) and potassium carbonate (400 mg, 2.89 mmol) in 10 mL dimethylformamide (DMF), 1,4-Butane sultone (1 mL, 9.77 mmol) was added under argon atmosphere. The mixture was stirred at 60 °C for overnight. The reaction was monitored by LC-MS and a red product with reduced polarity was observed. After cooling to room temperature, the DMF reaction solution was dropped into ethyl acetate and let the solution stand for 5 min. Then the precipitate was filtered and washed with ethyl acetate and dichloromethane for 3 times respectively. Later, the precipitate was collected and purified by preparative TLC with a solvent system (acetonitrile : water = 100 : 15) and brown solid products were obtained.

Resa-Sulf. Yield: 163.1 mg (51.3%)

\[ \delta \text{H NMR (600 MHz, D}_2\text{O)} \delta 7.98 (t, J = 9.9 Hz, 2H), 7.03 – 6.94 (m, 2H), 6.77 (d, J = 9.9 Hz, 1H), 6.27 (s, 1H), 4.13 (t, J = 5.3 Hz, 2H), 3.00 (t, J = 7.0 Hz, 2H), 1.94 (s, 4H). \]

\[ \delta \text{C NMR (151 MHz, D}_2\text{O)} \delta 186.12, 164.66, 154.21, 149.24, 131.28, 130.32, 124.28, 123.36, 121.34, 115.68, 104.20, 100.78, 69.14, 50.55, 26.99, 20.80. \]

HRMS m/z (C\text{16H14NO7S}-), calculated: 364.0496, found: 364.0491[M-].

Preparation of Resa-Con. To a stirred solution of Resazurin (200 mg, 0.87 mmol) and potassium carbonate (400 mg, 2.89 mmol) in 10 mL dimethylformamide (DMF), 1,3-Dibromopropane (874.22 mg, 4.35 mmol) was added under argon atmosphere. The mixture was stirred at 60 °C for 6 h. The reaction was monitored by LC-MS and a red product with reduced polarity was observed. After cooling to room temperature, 50 mL DCM was added and the mixture was extracted with water (3 x 100 mL). The organic phase was collected and removed under vacuum condition. Then the mixture was purified by preparative TLC with a solvent system (DCM : MeOH = 100 : 5) and red solid Resa-Br was obtained. The product was used in the next step directly. Resa-Br (100 mg, 0.28 mmol), potassium carbonate (200
mg, 1.44 mmol) and phenol (131.6 mg, 1.4 mmol) were added into 5 mL dimethylformamide (DMF). The mixture was stirred at 60 °C for 6 h. The reaction was monitored by LC-MS and a red product with reduced polarity was observed. After cooling to room temperature, 20 mL DCM was added and the mixture was extracted with water (3 x 50 mL). The organic phase was collected and removed under vacuum condition. Then the mixture was purified by preparative TLC with a solvent system (DCM : MeOH = 100 : 5) and red solid Resa-Con was obtained.

**Resa-Con.** Yield: 72.7 mg (71.6%)

$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.18 (d, $J = 9.3$ Hz, 1H), 8.09 (d, $J = 10.0$ Hz, 1H), 7.31 – 7.27 (m, 2H), 7.02 (dd, $J = 9.3$, 2.5 Hz, 2H), 6.98 – 6.93 (m, 2H), 6.91 (d, $J = 7.8$ Hz, 2H), 6.58 (s, 1H), 4.34 (t, $J = 6.1$ Hz, 2H), 4.18 (t, $J = 5.8$ Hz, 2H), 2.37 – 2.31 (m, 2H).

$^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 184.98, 163.86, 158.64, 152.98, 149.04, 132.66, 130.87, 129.68, 124.51, 122.92, 122.00, 121.13, 114.48, 114.17, 105.70, 101.06, 65.96, 63.64, 29.05

HRMS m/z (C$_{21}$H$_{17}$NO$_5$), calculated 364.1185, found: 364.1172 [M + H$^+$].

**Preparation of Reso-Sulf.** Resa-Sulf (10 mg, 0.027 mmol) and L-DOPA (10.8 mg, 0.04 mmol) were dissolved in 5 mL PBS buffer (pH 11.2). After stirring for 60 min at 25 °C, the solution was removed under vacuum. The product was purified by preparative TLC with a solvent system (acetonitrile : water = 100 : 15) Yield: 8.48 mg (90.5%).

$^1$H NMR (600 MHz, D$_2$O) $\delta$ 7.45 (d, $J = 9.0$ Hz, 1H), 7.30 (d, $J = 9.7$ Hz, 1H), 6.87 (dd, $J = 9.0$, 2.6 Hz, 1H), 6.76 (d, $J = 2.5$ Hz, 1H), 6.73 (dd, $J = 9.7$, 2.1 Hz, 1H), 6.15 (d, $J = 2.1$ Hz, 1H), 4.02 (t, $J = 5.5$ Hz, 2H), 2.96 – 2.90 (m, 2H), 1.92 – 1.81 (m, 4H).

$^{13}$C NMR (151 MHz, D$_2$O) $\delta$ 187.93, 164.01, 150.78, 145.84, 143.21, 134.53, 132.88, 130.97, 127.75, 115.45, 105.46, 100.60, 68.89, 50.58, 27.06, 20.82.

HRMS m/z (C$_{16}$H$_{14}$NO$_6$S$^-$), calculated: 348.0541, found: 348.0547, [M$^-$].
NMR Spectra