A selenium-contained aggregation-induced “turn-on” fluorescent probe for hydrogen peroxide

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1. General experimental section

1.1 Materials:

Unless otherwise indicated, all reagents, and solvents were obtained from commercial suppliers, and were used without further purification. Column chromatography was performed on silica gel (Qingdao haiyang) 300-400 mesh. All solvents used in test systems was chromatographically pure. Ultrapure water was used throughout.

Stock solutions of probes D-HMSe and HMSe was prepared by dissolving the corresponding compound in chromatographically pure DMSO. H$_2$O$_2$ stock solution was prepared by diluting 30% H$_2$O$_2$ solution, and the concentration was determined from absorption at $\lambda$=240 nm ($\varepsilon$=43.6 M$^{-1}$·cm$^{-1}$). $^1$BuOOH was prepared by diluting 70% $^1$BuOOH solution. ·OH was generated by Fenton reaction between FeSO$_4$ and H$_2$O$_2$, and concentration of ·OH was determined by H$_2$O$_2$.

$^1$BuOO· was generated by reaction between FeSO$_4$ and $^1$BuOOH, and concentration of $^1$BuOO· was determined by $^1$BuOOH. $^1$O$_2$ was generated by the reaction of H$_2$O$_2$ with NaClO.$^1$O$_2$· was generated from KO$_2$ solid diluted in DMSO. ONOO$^-$ was generated by the reaction of H$_2$O$_2$ and NaNO$_2$ and stocked at -20 °C, the concentration was determined from absorption at $\lambda$=302 nm ($\varepsilon$=1670 M$^{-1}$·cm$^{-1}$) in 0.1 M NaOH solution. ClO$^-$ was prepared by diluting 5% NaClO aqueous solution, and the concentration was determined from absorption at $\lambda$=292 nm ($\varepsilon$=350 M$^{-1}$·cm$^{-1}$). The fluorescence quantum yields in solution were calculated using a quinine bisulfate as a standard material (5x10$^{-6}$ M, 0.05 M H$_2$SO$_4$, $\Phi_s$=0.55).

1.2 Instruments

$^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AMX-400 with chemical shifts expressed in parts per million (in deuteriochloroform or DMSO-d$_6$, Me$_4$Si as internal standard). Fluorescence spectra and fluorescence quantum yields in solid state were determined using a FluoroMax-4 Spectrofluorometer (HORIBA Jobin Yvon). UV/Vis absorption spectra were determined by a Hitachi PharmaSpec UV-1900 UV-Vis spectrophotometer. Mass spectral data were recorded on a Finnigan LCQDECA and a Bruker Daltonics Bio TOF mass spectrometer. High performance liquid chromatography (HPLC) were perform on a Waters e2695 Separatins Module using Waters 2998 PDA detector equipped with an Symmetry C18 column (4.6 X 150mm, 5µm), CH$_3$CN (0.3%TFA) were used as eluents with a flow rate of 1ml/min. 280nm was used as wavelength. The measurements of dynamic light scattering (DLS) were carried out at 25 °C using a Zetasizer Nano-ZS 3690 system from Malvern Instruments equipped with a 633 nm He–Ne laser using backscattering detection with a fixed detector angle of 90°. TLC analyses were performed on silica gel GF 254. pH values were determined by a Leici pHS-25 (digital display) pH meter.

2. Synthetic procedures

Compound 1a,$^4$, $^5$ and $^6$ was prepared according to the early literature. And compound 4 was used without further purification to next step after prepared.

**Compound 1b**

A mixture of compound 1a (1.50 g, 6.27 mmol), n-bromododecane (1.72 g, 6.89 mmol) and 33% (w/w) aqueous NaOH (1 g) in DMF (10 mL) was stirred at 60 °C for 3.5 h. Then solvent was removed in vacuo, and the residue was diluted with water and extracted with ethyl acetate (3 times). The organic layer was collected and washed water, then dried over anhydrous Na$_2$SO$_4$, and
concentrated in vacuo. The residue was purified by silica gel column chromatography using petroleum ether and ethyl acetate as eluent, and giving a yellow liquid as product (1.58 g, yield 61.8%).\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) 8.24 (d, 1 H, \textit{J} = 8.40 Hz), 7.79 (t, 2 H, \textit{J} = 7.40 Hz), 7.73 (t, 1 H, \textit{J} = 7.72 Hz), 7.65 (d, 1 H, \textit{J} = 7.40 Hz), 7.44 (d, 1 H, \textit{J} = 7.76 Hz), 7.36-7.29 (m, 2 H), 3.99 (t, 2 H, \textit{J} = 7.52 Hz), 1.73 (t, 2 H, \textit{J} = 6.82 Hz), 1.29-1.17 (m, 18 H), 0.88 (t, 3 H, \textit{J} = 13.2 Hz). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): 149.3, 148.8, 143.2, 135.0, 133.3, 132.8, 131.0, 126.5, 124.9, 123.1, 122.4, 120.2, 110.1, 44.7, 31.9, 29.6, 29.5, 29.4, 29.30, 29.0, 26.7, 22.7, 14.1. HRMS (ESI) calcd for C\textsubscript{25}H\textsubscript{34}N\textsubscript{3}O\textsubscript{2} [M+H]\textsuperscript{+}, m/z 408.2646; found, m/z 408.2650.

**General synthetic process of compound 2**

A mixture of compound 1 (4.9 mmol) and 20\% (w/w) Pd/C (Pd, 10 wt\% on carbon powder) in 30 mL CH\textsubscript{3}OH was stirred vigorously under a hydrogen atmosphere at room temperature overnight. After the reaction completed, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo to afford crude product. 2\textsuperscript{a} was used to next step without further purification. 2\textsuperscript{b} was purified by silica gel column chromatography using petroleum ether and ethyl acetate as eluent, and giving a white solid as product (988 mg, yield 96%).\textsuperscript{1}H NMR (400 MHz, DMSO-d\textsubscript{6}): \textit{\delta} 7.68-7.65 (m, 1 H), 7.61 (d, 1 H, \textit{J} = 7.40 Hz), 7.29-7.18 (m, 4 H), 6.85 (d, 1 H, \textit{J} = 8.00 Hz), 6.70-6.66 (m, 1 H), 5.61 (s, 2 H), 4.20 (t, 2 H, \textit{J} = 7.24 Hz), 1.63-1.60 (m, 2 H), 1.29-1.07 (m, 18 H), 0.87-0.84 (t, 3 H, \textit{J} = 6.86 Hz). \textsuperscript{13}C NMR (100 MHz, DMSO-d\textsubscript{6}): \textit{\delta} 152.6, 148.1, 143.1, 135.4, 130.8, 130.4, 122.5, 122.1, 119.2, 116.1, 116.0, 113.7, 111.2, 44.2, 31.6, 29.4, 29.3, 29.2, 29.1, 28.7, 22.6, 14.4. HRMS (ESI) calcd for C\textsubscript{25}H\textsubscript{36}N\textsubscript{3} [M+H]\textsuperscript{+}, m/z 378.2904; found, m/z 378.2907.

**General synthetic process of compound 5**

A mixture of compound 2 (1.33 mmol), triethylamine (2.00 mmol) and dry DCM (10 mL) in a 50 mL round-bottom flask was stirred in an ice/NaCl bath for 5 min, and a solution of compound 4 (2.00 mmol) prepared before in 10 mL dry DCM was added in 30 min. Then the reaction was kept in -15 °C bath over night. When the reaction completed, solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using petroleum ether and ethyl acetate as eluent, and gave the product. Compound 5\textsuperscript{a}: yellow solid (84.5 mg, yield 16.2%).\textsuperscript{1}H NMR (400 MHz, DMSO-d\textsubscript{6}): \textit{\delta} 12.48 (s, 1 H), 7.97-7.95 (d, 1 H, \textit{J} = 8.00 Hz), 7.89-7.87 (d, 1 H, \textit{J} = 7.52 Hz), 7.82-7.80 (dd, 1 H, \textit{J} = 7.72 Hz, \textit{J} = 0.82 Hz), 7.69-7.58 (m, 3 H), 7.56-7.52 (m, 1 H), 7.46-7.41 (m, 3 H), 7.16-7.08 (m, 2 H). \textsuperscript{13}C NMR (100 MHz, DMSO-d\textsubscript{6}): \textit{\delta} 166.8, 149.9, 144.2, 140.8, 138.1, 135.2, 132.4, 131.4, 130.7, 130.1, 129.5, 128.4, 128.1, 128.0, 126.3, 126.2, 122.8, 121.8, 119.4, 112.0. HRMS (ESI) calcd for C\textsubscript{25}H\textsubscript{34}N\textsubscript{3}O\textsubscript{2}Se [M+H]\textsuperscript{+}, m/z 392.0297; found, m/z 392.0300.

Compound 5\textsuperscript{b}: white solid (513.3 mg, yield 69.1%).\textsuperscript{1}H NMR (400 MHz, DMSO-d\textsubscript{6}): \textit{\delta} 7.89-7.87 (dd, 1 H, \textit{J} = 0.84 Hz, \textit{J} = 7.76 Hz), 7.84 (d, 1 H, \textit{J} = 8.00 Hz), 7.70-7.63 (m, 4 H), 7.57-7.52 (m, 3 H), 7.43-7.39 (m, 1 H), 7.24-7.18 (m, 2 H), 3.91 (t, 2 H, \textit{J} = 7.26 Hz), 1.45-1.43 (m, 2 H), 1.29-0.97 (m, 18 H), 0.85 (t, 3 H, \textit{J} = 6.88 Hz). \textsuperscript{13}C NMR (100 MHz, DMSO-d\textsubscript{6}): \textit{\delta} 166.7, 151.1, 140.8, 138.9, 135.4, 132.5, 131.1, 129.5, 128.5, 127.5, 126.4, 122.8, 119.9, 111.5, 44.0, 31.8, 29.4, 29.3, 29.2, 28.7, 22.6, 14.4. HRMS (ESI) calcd for C\textsubscript{25}H\textsubscript{36}N\textsubscript{3}O\textsubscript{2}Se [M+H]\textsuperscript{+}, m/z 560.2175; found, m/z 560.2180.

**Compound D-HMSeO**
20 mL HEPES buffer (20 mM, pH 7.40) was added to a methanol (5 mL) solution of compound D-HMSe (300 mg, 0.54 mmol), white solid appeared. 400 μL 30% H₂O₂ aqueous solution was then added. The reaction was stirred at ambient temperature for 2 h. Then reaction was filtered, and residue was collected and further purified by silica gel column chromatography using petroleum ether and ethyl acetate as eluent. A white solid was obtained (233 mg, yield: 75%). ¹H NMR (400 MHz, DMSO- d₆): δ 8.01 (t, 2 H, J = 9.10 Hz), 7.87 (d, 1 H, J = 6.64 Hz), 7.81-7.73 (m, 3 H), 7.63-7.59 (m, 2 H), 7.50-7.43 (m, 4 H), 4.27 (m, 2 H), 1.73-1.71 (m, 2 H), 1.27-1.04 (m, 18 H), 0.85 (t, 3 H, J = 6.76 Hz). ¹³C NMR (100 MHz, DMSO- d₆): δ 168.3, 150.3, 148.2, 136.6, 135.2, 134.9, 132.8, 131.5, 131.4, 131.3, 130.5, 129.3, 129.0, 128.4, 127.8, 123.0, 122.4, 119.3, 111.5, 44.5, 31.8, 29.4, 29.3, 29.2, 28.8, 28.6, 26.5, 22.6, 14.4. HRMS (ESI) calcd for C₃₂H₃₈N₃O₂Se [M+H]+, m/z 576.2124; found, m/z 576.2126.

3. UV-visible Absorbance Measurements

UV spectra of D-HMSe and HMSe were recorded at the concentration of 20 μM in DMSO/water (1/99, v/v) buffered by 20 mM HEPES at pH 7.40.

Figure S1 UV/Vis absorption spectra of D-HMSe (20 μM) and HMSe (20 μM) in DMSO/water (1/99, v/v) buffered by 20 mM HEPES at pH 7.40.

4. Effect of different solvents

Different solvents including water, THF, CH₃CN, CH₃OH, DMF, and DMSO were used as the test solvents and fluorescence emission spectra were recorded. It exhibited significant fluorescence emission in water and shown very weak fluorescence emission in other solvents.

Figure S2 Effect of different solvents to relative fluorescence intensity of D-HMSe (10 μM) before (blue bar) and
after treated with H₂O₂ (200 μM) at ambient temperature for 1 h at 460 nm. The testing solvents include: 1. H₂O, 2. DMSO, 3. CH₃OH, 4. CH₃CN, 5. THF, 6. DMF. All of the solvents include 1% DMSO.

5. Fluorescence spectra of D-HMSe and D-HMSeO in solid state

Fluorescence emission spectra of D-HMSe and D-HMSe and D-HMSeO in solid state was recorded. D-HMSeO exhibits a strong fluorescence emission at 494 nm and D-HMSe shows nearly no fluorescence.

![Fluorescence spectra of D-HMSe and D-HMSeO in solid state.](image)

Figure S3 Fluorescence spectra of D-HMSe and D-HMSeO in solid state. λₑₓ=347 nm, slit: 5/5 nm.

6. Effect of different ratio of DMSO and H₂O

Different ratios of HEPES buffer and DMSO (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10) were prepared as the test solvents. D-HMSe (10 μM) and H₂O₂ (200 μM) were added and kept at ambient temperature for 1 h. Then emission spectra were recorded (Figure S4).

![Fluorescence responds of the reaction between D-HMSe (10 μM) and H₂O₂ (200 μM) in different ratios of DMSO and H₂O as solvent at ambient temperature for 1 h.](image)

Figure S4 Fluorescence responds of the reaction between D-HMSe (10 μM) and H₂O₂ (200 μM) in different ratios of DMSO and H₂O as solvent at ambient temperature for 1 h.

7. HRMS of the reaction product between D-HMSe and H₂O₂

Oxidative product D-HMSeO was prepared from the reaction between D-HMSe and H₂O₂ at ambient temperature over night and further purified by column chromatography on silica gel. ESI-MS result shown that a major peak at m/z=576.2126 was found, which calcd for C₃₂H₃₈N₃O₂Se-[H⁺] m/z=576.2124.
8. Dynamic light scattering (DLS) result

DLS was performed in DMSO/water (1/99, v/v) buffered by 20 mM HEPES at pH 7.40 at ambient temperature. After the addition of H₂O₂ to D-HMSe, the solution was kept at ambient temperature for 1 h.

9. Effect of pH value

pH solutions were prepared by using 50% NaOH solution to adjust the pH values of HEPES buffer (20 mM). The solutions of D-HMSeO, D-HMSe before and after treated with H₂O₂ in different pH solutions were prepared and kept at ambient temperature for 1 h, and spectra were
recorded then.

**Figure S7** Effect of pH on the relative fluorescence intensity for 10 μM D-HMSe before (●), after (■) treated with 200 μM H₂O₂ and 10 μM D-HMSeO (▲) in DMSO/water (1/99, v/v) buffered by 20 mM HEPES at pH 7.40 for 1 h at ambient temperature. (The investigated pH values include 3.01, 4.02, 4.51, 4.97, 5.60, 5.97, 6.50, 7.00, 7.38, 7.99, 8.56, 8.97, 9.55, 10.05, 10.95 and 11.91).

10. Time-dependent UV spectra of D-HMSe reacted with H₂O₂

UV spectra of the process of D-HMSe reacting with H₂O₂ were recorded. The result is shown in Figure S8. With time going on, the three maximum absorption peaks changed gradually.

**Figure S8** Time-dependent UV spectra of D-HMSe (20 μM) reacted with H₂O₂ (400 μM) in 60 min at ambient temperature in water (1% DMSO)

**References**

Figure S9 $^1$H NMR spectra of compound 1 in CDCl$_3$.

Figure S10 $^{13}$CNMR spectra of compound 1 in CDCl$_3$. 
Figure S11 $^1$H NMR spectra of compound 2b in DMSO-d$_6$.

Figure S12 $^{13}$C NMR spectra of compound 2b in DMSO-d$_6$. 
Figure S13 \(^1\)H NMR spectra of compound HMsSe in DMSO-\(d_6\).

Figure S14 \(^1\)H NMR spectra of compound HMsSe in DMSO-\(d_6\).
Figure S15 $^1$H NMR spectra of compound D-HMSe in DMSO-$d_6$.

Figure S16 $^{13}$C NMR spectra of compound D-HMSe in DMSO-$d_6$. 
Figure S17 $^1$H NMR spectra of compound D-HMSeO in DMSO-d$_6$.

Figure S18 $^{13}$C NMR spectra of compound D-HMSeO in DMSO-d$_6$. 
Figure S19 HRMS of compound HMSe.

Figure S20 HRMS of compound D-HMSe.