A Fluorescent Turn-on H$_2$S-responsive Probe: Design, Synthesis and Application

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1 Solubility in HEPES buffer containing 5% DMSO

Figure S1 Plot of fluorescence intensity (A) and absorbance (B) against the total amount of DP-NBD in HEPES (pH=7.4, 0.02 M) containing 5% DMSO.

2 Spectra upon addition of H$_2$S depending on time

Figure S2 (A) Fluorescent spectra and (B) Absorption spectra of DP-NBD (10 uM) upon addition of H$_2$S (10 equiv) depending on time in a mixed solution of DMSO-HEPES(v/v=10:90, pH=7.4, 0.02M). (C) Time-dependent fluorescence changes of DP-NBD (10 μM) upon addition of H$_2$S, Cys, Hcy and GSH (10 equiv) within 120 min in a mixed solution of DMSO-HEPES(v/v=10:90, pH=7.4, 0.02 M). (λex = 365 nm, λem = 595 nm, slit: 10/10 nm.). (D) The $k_{obs} = 2.75 \times 10^{-4}$ s$^{-1}$ calculated from the slope of the plot of ln [(Fmax – Ft)/Fmax] (measured at 595 nm vs time using excitation wavelength 365 nm).
3 Fluorescent titration of DP-NBD upon addition of H$_2$S

![Fluorescence Spectra](image)

**Figure S3** Fluorescence spectra of DP-NBD (10 μM) upon addition of H$_2$S (0 μM-300 μM for A and 0 μM-40 μM for C) in a mixed solution of DMSO-HEPES (v/v=10:90, pH=7.4, 0.02 M) and the corresponding relationship between the fluorescent intensity and H$_2$S concentration (0 μM-300 μM for B and 0 μM-40 μM for D). (λ$_{ex}$ = 365 nm, λ$_{em}$ = 595 nm, slit: 10/10 nm.)

4 Fluorescence response of DP-NBD upon addition of H$_2$S depending on pH

![Fluorescence Response](image)

**Figure S4** Fluorescence response of DP-NBD (10 μM) upon addition of H$_2$S (10 equiv) depending on pH (2-12) in a mixed solution of DMSO-HEPES (v/v=5:95, pH=7.4, 0.02M). Black line: only DP-NBD. Red bar: DP-NBD+ H$_2$S. (λ$_{ex}$ = 365 nm, λ$_{em}$ = 595 nm, slit 10/10 nm.)
5 Cell viability assay

![Cell viability assay graph]

Figure S5 Cell viability of HeLa cells treated with probe DP-NBD at various concentrations

6 The MOLDI-TOF mass spectra

![MOLDI-TOF mass spectra diagram]

Figure S6 The MOLDI-TOF mass spectra after treating DP-NBD and H₂S
7 Fluorescent spectra of NBD-P and DP

Figure S7 (A) The fluorescent spectra of NBD-P and (B) DP (10 uM) to H₂S (10 equiv) in a mixed solution of DMSO-HEPES(v/v=10:90, pH=7.4, 0.02M) (λex = 365 nm, λem = 595 nm, slit: 10/10 nm.)
8 TDDFT calculations of DP-NBD, DP and NBD-P

![Diagram showing frontier molecular orbital profiles of molecules based on TDDFT (B3LYP/6-31G*) calculations.](image)

**Figure S8** Frontier molecular orbital profiles of molecules based on TDDFT (B3LYP/6-31G*) calculations.

**Table S1.** Major electronic excitations for DP-NBD, DP and NBD-P.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Excited state</th>
<th>λ/nm [eV]</th>
<th>Osc. str (f)</th>
<th>Major contributions</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP-NBD</td>
<td>$S_0\rightarrow S_2$</td>
<td>457.25 [2.71]</td>
<td>0.3238</td>
<td>HOMO-1→LUMO (69%)</td>
</tr>
<tr>
<td></td>
<td>$S_0\rightarrow S_{11}$</td>
<td>325.01 [3.81]</td>
<td>0.1680</td>
<td>HOMO-1→LUMO+2 (53%)</td>
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<tr>
<td></td>
<td>$S_0\rightarrow S_1$</td>
<td>344.04 [3.60]</td>
<td>0.0985</td>
<td>HOMO→LUMO (69%)</td>
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<tr>
<td>DP</td>
<td>$S_0\rightarrow S_6$</td>
<td>248.38 [4.99]</td>
<td>0.0914</td>
<td>HOMO-2→LUMO+1 (45%)</td>
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<tr>
<td></td>
<td>$S_0\rightarrow S_8$</td>
<td>229.96 [5.39]</td>
<td>0.1008</td>
<td>HOMO→LUMO+2 (54%)</td>
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<tr>
<td></td>
<td>$S_0\rightarrow S_1$</td>
<td>497.92 [2.49]</td>
<td>0.1675</td>
<td>HOMO→LUMO (69%)</td>
</tr>
<tr>
<td>NBD-P</td>
<td>$S_0\rightarrow S_4$</td>
<td>342.60 [3.62]</td>
<td>0.1737</td>
<td>HOMO→LUMO+1 (66%)</td>
</tr>
<tr>
<td></td>
<td>$S_0\rightarrow S_8$</td>
<td>295.12 [4.20]</td>
<td>0.0991</td>
<td>HOMO-1→LUMO+1 (69%)</td>
</tr>
</tbody>
</table>
Figure S9. TD-DFT calculations of DP-NBD, DP and NBD-P at B3LYP/6-31G* level by using Gaussian 09 program.
Appendix: NMR and Mass spectra