Electronic Supplementary Information (ESI†)

Reaction-Based Bi-signaling Chemodosimeter Probe for Selective Detection of Hydrogen Sulfide and Cellular Studies

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Figure S1. ESI-MS mass spectrum of Compound 2

Figure S2. ESI-MS mass spectrum of Compound 3
Figure S3: $^1$H NMR of compound Compound 3 in DMSO-d6

Figure S4. ESI-MS mass spectrum of DPQI
Figure S5: $^1$H NMR of DPQI in CDCl$_3$

Figure S6: $^{13}$C NMR of DPQI
Figure S7. HR-MS (partial) of sample withdrawn from assay system.

Figure S8. Comparative fluorescence spectra of DPQI (at 593 nm) (1.0 x10^{-5} M) in the presence of tetrabutyl ammonium and sodium salts of different analytes (10.0 x10^{-4} M) (CN^{-}, F^{-}, Cl^{-}, Br^{-}, I^{-}, S_{2}O_{3}^{2-}, SO_{4}^{2-}, AcO^{-}, and H_{2}PO_{4}^{-}) and Cys, Hcy, GSH in DMSO-H_{2}O(1:2 V/V; pH 7.4).
Figure S9. Change in fluorescence spectra of DPQI (at 593 nm) (c= 1× 10⁻⁵ M) to 100 eqv. addition of H₂S[green big bar]and 100 eqv. of other analytes[small bars] and to the mixture of 100 eqv. of other analytes 100 eqv. addition of H₂S[big black bars].

**Calculation of Detection limit:**

The detection limit (DL) of DPQI for H₂S were determined from the following equation:

\[
DL = K \times \frac{Sb1}{S}
\]

Where \( K = 2 \) or 3 (we take 2 in this case); \( Sb1 \) is the standard deviation of the blank solution; \( S \) is the slope of the calibration curve.

From graph \( Sb1 = 1.08 \times 10^7 \), \( S = 18.89 \). DL = 3.5µM

**Figure S10:** Calibration curve for Fluorescence titration of DPQI at 593 nm(Ex@500nm) with H₂S.
Figure S11: Job’s plot [(a) is by absorbance and (b) is by fluorescence method] of sensor DPQI (1×10⁻⁵ M) with H₂S (1×10⁻⁵ M) in DMSO–H₂O (1:2 v/v; 20 mM, HEPES buffer, pH = 7.4) by UV spectroscopic method, that indicates 1:2 stoichiometry.

Figure S12: (a) Absorption spectra of DPQI (1.0 μM, 20 mM HEPES buffer PH 7.4, DMSO-H₂O; 1:2; v/v) upon gradual addition of Na₂S (0–100 equiv.). (b) (i) Fluorescence color of DPQI and (ii) fluorescence color of DPQI in presence of 100 equiv. of H₂S. (c) Structure of Quinoline-Indole (QI) required for DFT calculation to know the fluorescence behavior of the probe DPQI.

Figure S13: The visible color (top) and fluorescence changes (bottom) of receptor DPQI in aq. DMSO (DMSO: H₂O = 1:2 v/v, 20 mM HEPES buffer, pH = 7.4) upon addition of various analytes.
Kinetic Study:
The solution phase chemodosimetric reactions of DPQI in DMSO were followed by measuring the fluorescence spectra after mixing DPQI and Na$_2$S in a cubic 4-sided quartz cell of 3 ml. The reaction was carried out at 35°C under the excess amount of H$_2$S (25-100 eqv.) (initial concentration [DPQI] << [H$_2$S]) and the reaction was expected to reach 100% conversion. Separate solutions of different concentrations of DPQI and Na$_2$S in DMSO were prepared and mixed to investigate the kinetics. The excitation wavelength was 500 nm and in all cases the concentration was low enough to maintain a UV absorption that was < 0.1. The rate of the reaction was determined by fitting the fluorescence intensities of the samples to the Pseudo-First Order Equation (1):

$$\ln\left(\frac{F_{\text{max}} - F_t}{F_{\text{max}}}\right) = -\frac{k}{t} \quad \ldots(1)$$

Where $F(t)$ and $F_{\text{max}}$ are the fluorescence intensities at the monitoring wavelengths at times $t$ and the maxima values which are the last fluorescence intensities when DPQI reached the conversion of 100%. The $k$ is the apparent rate constant. Figure S14a is the pseudo first order plot of DPQI with 100 equiv. of H$_2$S. The negative slope of the plot shows the apparent rate constant = 0.232 min$^{-1}$. The apparent rate constant, $k'$, contains the concentration of H$_2$S as a constant and is related to the second-order rate constant, $k_2$ (M$^{-1}$min$^{-1}$), by equation (S2):

$$k' = k_2[H_2S] \quad \text{(S2)}$$

The second-order rate constant for this reaction is thus the slope of a linear plot of $k'$ Versus the concentration of H$_2$S (Fig. S15): $k_2 = 775.44$ M$^{-1}$min$^{-1}$
Figure S14: (a) Pseudo first-order kinetic plot of reaction of DPQI (10µM) with H₂S (100 equiv.) in DMSO. Slope = -0.23 min⁻¹. (b) Kinetic plot of DPQI with 25 equiv. H₂S, (c) Kinetic plot of DPQI with 50 equiv. H₂S(d) Kinetic plot of DPQI with 80 equiv. H₂S.

Figure S15: Plot of the observed k versus the concentration of H₂S for the pseudo first-order reaction of DPQI (10µM) with varying concentration of H₂S (25-150 eq). Slope = 775.44 M⁻¹min⁻¹.

Figure S16: The energy optimized structures of (a) DPQI and (b) QI-SH.
**Figure S17:** HOMO-LUMO distribution and energy difference of **DPQI** and **QI-SH**.

**Table S1.** Selected electronic excitation energies (eV), oscillator strengths (f), main configurations, and CI Coefficients of all the complexes. The data were calculated by TDDFT//B3LYP/6-31+G(d,p) based on the optimized ground state geometries.

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Electronic Transition</th>
<th>Excitation Energy(^a)</th>
<th>f(^b)</th>
<th>Composition(^c) (composition) %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DPQI</strong></td>
<td>(S_0 \rightarrow S_1)</td>
<td>2.5540 eV 495.45 nm</td>
<td>0.1833</td>
<td>H (\rightarrow) L             93.3</td>
</tr>
<tr>
<td></td>
<td>(S_0 \rightarrow S_2)</td>
<td>2.9837 eV 415.54 nm</td>
<td>0.6022</td>
<td>H-1 (\rightarrow) L            86.1</td>
</tr>
<tr>
<td></td>
<td>(S_0 \rightarrow S_{23})</td>
<td>4.4035 eV 281.56 nm</td>
<td>0.1559</td>
<td>H-2 (\rightarrow) L+2          53.4</td>
</tr>
<tr>
<td></td>
<td>(S_0 \rightarrow S_{33})</td>
<td>4.8286 eV 256.77 nm</td>
<td>0.2141</td>
<td>H-14 (\rightarrow) L  H-3 (\rightarrow) L+2  H-1 (\rightarrow) L+3  68.2</td>
</tr>
<tr>
<td><strong>QI-SH</strong></td>
<td>(S_0 \rightarrow S_1)</td>
<td>2.2867 eV 542.20 nm</td>
<td>0.2349</td>
<td>H (\rightarrow) L             96.7</td>
</tr>
</tbody>
</table>

[a] Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength. [b] Oscillator strength. [c] H stands for HOMO and L stands for LUMO.

**Table S2.** Energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)

<table>
<thead>
<tr>
<th>Species</th>
<th>(E_{\text{HOMO}}) (a.u)</th>
<th>(E_{\text{LUMO}}) (a.u)</th>
<th>(\Delta E) (a.u)</th>
<th>(\Delta E) (eV)</th>
<th>(\Delta E) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DPQI</strong></td>
<td>-0.34444</td>
<td>-0.23556</td>
<td>0.10888</td>
<td>2.962799</td>
<td>68.3</td>
</tr>
<tr>
<td><strong>QI-SH</strong></td>
<td>-0.04069</td>
<td>0.05442</td>
<td>0.09511</td>
<td>2.588095</td>
<td>59.7</td>
</tr>
</tbody>
</table>
Computational details:
Geometries have been optimized using the B3LYP/6-31+G(d,p) level of theory. The geometries are verified as proper minima by frequency calculations. Time-dependent density functional theory calculation has also been performed at the same level of theory. All calculations have been carried out using Gaussian 09 program.

Water specimens: Different water specimens that we have collected from different areas of West Bengal, India, are given below.

Specimen 1: Water of River Ganga (West Bengal, India).
Specimen 2: Tap water of IIEST, Shipur campus (Howrah, West Bengal, India).
Specimen 3: Water of Hooghly River (East Midnapur, West Bengal, India).
Specimen 4: Shalimar hand pump water (Howrah, West Bengal, India).

Figure S18: MTT assay to determine the cytotoxic effect of DPQI and Na$_2$S complex on Vero cell.
Figure S19: HR-MS of DPQI treated with 0.5 equiv. of H₂S.

Figure 20: HR-MS of DPQI treated with excess of H₂S.