Supporting Information

Cascade reaction based rapid and ratiometric detection of H₂S/S²⁻ over bio-thiols with live cell-imaging: demasking of ESIPT approach

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1. Calculation of the detection limit:

The detection limit DL of FBBP for $S^{2-}$ was determined from the following equation. [S1]

$$DL = K \cdot \frac{S_{b1}}{S}$$

Where $K = 2$ or 3 (we take 2 in this case);

$S_{b1}$ is the standard deviation and $S$ is the slope of the calibration curve.

From the graph we get slope $= 85402$, and $S_{b1}$ value is $0.021958$.

Thus using the formula we get the Detection Limit $= 0.51 \, \mu M$ i.e. FBBP can detect $S^{2-}$ in this minimum level.

**Figure S1:** Fl. Intensity ratio ($I_{462 \text{ nm}} / I_{359 \text{ nm}}$) Vs. Conc. of $S^{2-}$ plot.
2. Calculation of rate constant:
From Fl. Intensity ratio \(\frac{I_{462}}{I_{359}}\) vs. time (sec.) plot using first order rate equation (Figure S2), we get rate constant \(K = \text{slope} \times 2.303 = 0.0086 \times 2.303 = 1.9 \times 10^{-2} \text{ sec}^{-1}\)

![Graph showing the relationship between time and Fl. Intensity ratio.](image)

**Figure S2:** The time vs. Fl. Intensity ratio \(\frac{I_{462 \text{ nm}}}{I_{359 \text{ nm}}}\) plot between 20 to 160 second and the full plot is in inset.

3. Fluorescence responses of FBBP + various species + S\(^2\)-:

![Bar graph showing fluorescence responses.](image)

**Figure S3:** Ratiometric fluorescence responses \(\frac{I_{462\text{nm}}}{I_{359\text{nm}}}\) of FBBP (c = 1.0 x 10\(^{-5}\) M) to S\(^2\)- (1 equiv) containing 10 equiv of various metal species.
4. $^1$H NMR, $^{13}$C NMR and ESI MS spectra:
$^1$H NMR spectrum and its expansion of Receptor i.e. FBBP in CDCl$_3$: 

![NMR Spectrum](image)
$^1$H NMR spectrum and its expansion of Receptor i.e. FBBP in $d_6$-DMSO:

$^{13}$C NMR spectrum of Receptor i.e. FBBP:
ESI MS Mass Spectrum of FBBP:

$^1$H NMR spectrum and its expansion of FBBP + Na$_2$S in $d_6$-DMSO:
ESI MS Spectrum of FBBP + Na₂S:
5. Fluorescence emission spectra of FBBP with different species such as Cysteine, Glutathione, F\(^-\), CN\(^-\), N\(_3\)\(^-\), SCN\(^-\), SO\(_3\)\(^2-\), S\(_2\)O\(_3\)\(^2-\), H\(_2\)O\(_2\), HSO\(_3\), Hydroxyl radical, Hypochlorite, Ascorbic Acid, NH\(^{4+}\), Co\(^{3+}\), Hg\(^{2+}\) and Cu\(^{2+}\) in CH\(_3\)CN : H\(_2\)O (2:8, v/v) (The solutions of anions and oxidants were prepared from Cysteine, Glutathione, TBAF, KCN, NaN\(_3\), KSCN, KSO\(_3\), K\(_2\)S\(_2\)O\(_3\), NaHSO\(_3\), H\(_2\)O\(_2\), Femton’s reagent, NaOCl, Ascorbic Acid, NH\(_4\)Cl, CoCl\(_2\), Al(NO\(_3\))\(_3\), 9H\(_2\)O, HgCl\(_2\) and CuCl\(_2\) respectively in H\(_2\)O).

(a) Cysteine

(b) Glutathione

(c) F\(^-\)

(d) CN\(^-\)

(e) N\(_3\)\(^-\)

(f) SCN\(^-\)
(g) $\text{SO}_3^{2-}$

(h) $\text{S}_2\text{O}_3^{2-}$

(i) $\text{H}_2\text{O}_2$

(j) $\text{HSO}_3^-$

(k) $\text{OH}$

(l) $\text{OCl}^-$
**Figure S4-S22:** Fluorescence emission spectra of FBBP with different species such as Cysteine, Glutathione, F, CN, N₃, SCN, SO₃²⁻, S₂O₃²⁻, H₂O₂, HSO₃⁻, Hydroxyl radical, Hypochlorite, Ascorbic Acid, NH₄⁺, Co²⁺, Al³⁺, Hg²⁺ and Cu²⁺ in CH₃CN : H₂O (2:8, v/v) respectively.

6. Fluorescence emission spectra of FBBP with Na₂S (excitation at 405 nm):

![Fluorescence emission spectra of FBBP with Na₂S](image)

**Figure S23:** Fluorescence emission spectra of FBBP (c = 1.0 x 10⁻⁵ M) with Na₂S (c = 1.0 x 10⁻⁵ M) at pH 7.5 in CH₃CN: H₂O (2:8, v/v) (excitation at 405 nm).
7. MTT assay of the probe FBBP:

![Graph showing MTT assay with different concentrations of FBBP after 24h.](image)

**Figure S24:** MTT assay with different concentration of probe (FBBP) after 24h.

8. References: