

Supporting Information

A Bright Fluorescent Probe Enables Analyte-Responsive H₂S, 3D Imaging in Live Zebrafish using Light Sheet Microscopy

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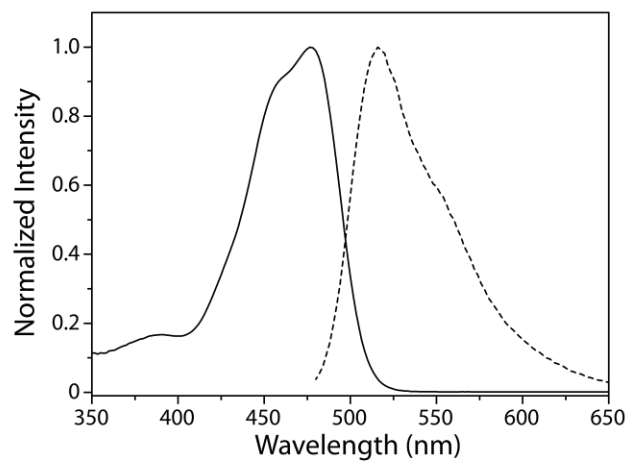


Figure S1. Normalized absorption (solid line) and emission (dashed line) spectra of **MeRho**.
Conditions: 5 μ M **MeRho**, PIPES buffer (50 mM, 100 mM KCl, pH 7.4), $\lambda_{\text{ex}} = 476$ nm

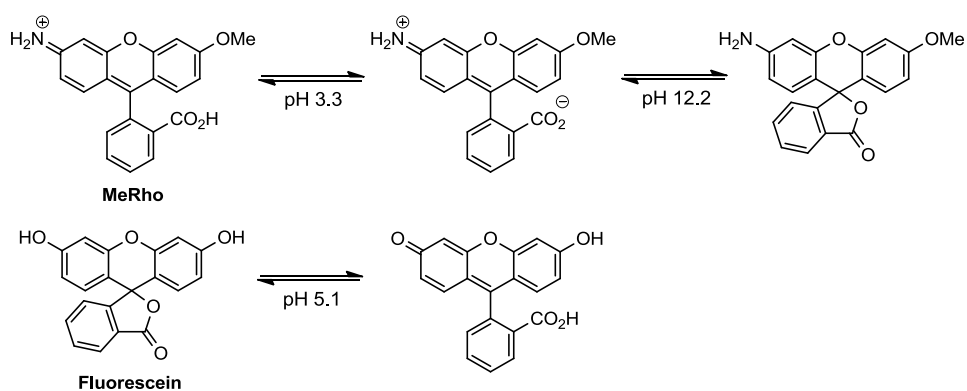
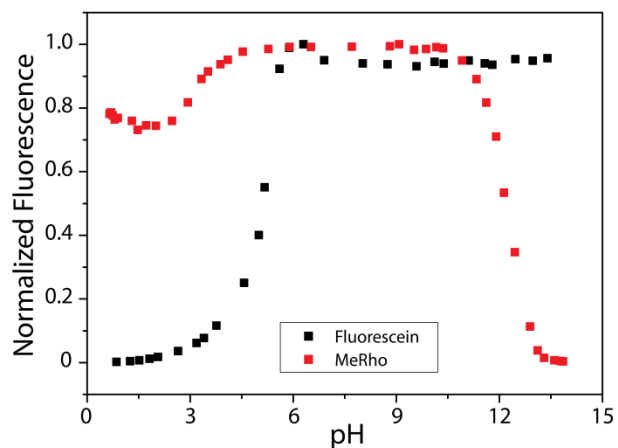


Figure S2. (top) Integrated fluorescein (20 μM , $\lambda_{\text{ex}} = 494 \text{ nm}$, $\lambda_{\text{em}} = 500\text{-}650 \text{ nm}$) and **MeRho** (20 μM , $\lambda_{\text{ex}} = 476 \text{ nm}$, $\lambda_{\text{em}} = 480\text{-}650 \text{ nm}$) fluorescence in aqueous solution at various pH (100 mM KCl). (bottom) Proposed protonation/deprotonation transitions of **MeRho** and fluorescein at various pH.

<u>[H₂S] (μM)</u>	<u>Integrated Fluorescence</u>	<u>Standard Deviation</u>
Blank	612234	141173
0.10	1093559	74073
2.5	13564824	2539964
5.0	24469632	4032106
7.5	30622452	4228960
15	58199116	8051924

Table S1. Tabulated detection limit data of toward H₂S. Conditions: 5 μM **MeRho-Az**, PIPES buffer (50 mM, 100 mM KCl, pH 7.4), $\lambda_{\text{ex}} = 476 \text{ nm}$, $\lambda_{\text{em}} = 480\text{-}650 \text{ nm}$, 37 $^{\circ}\text{C}$. Each concentration represents the average of at least three trials.

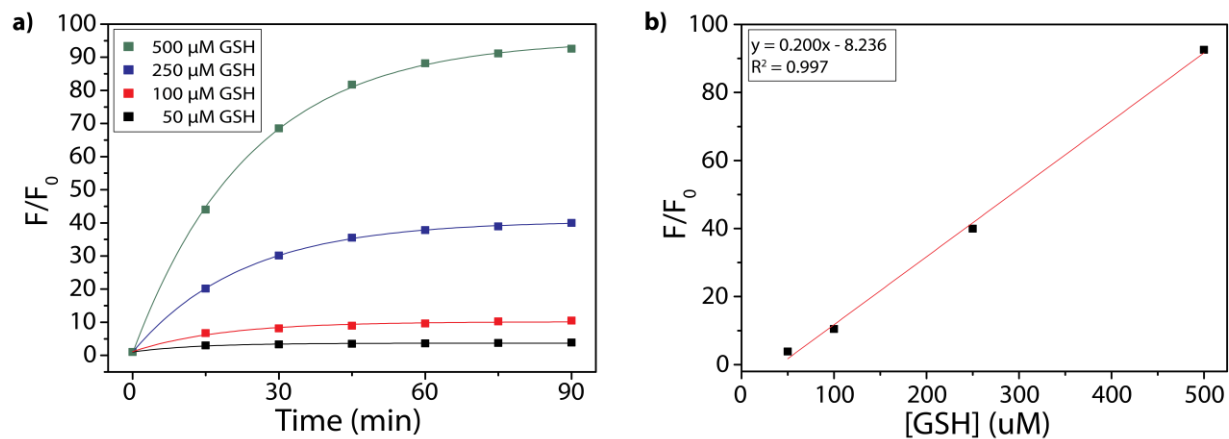
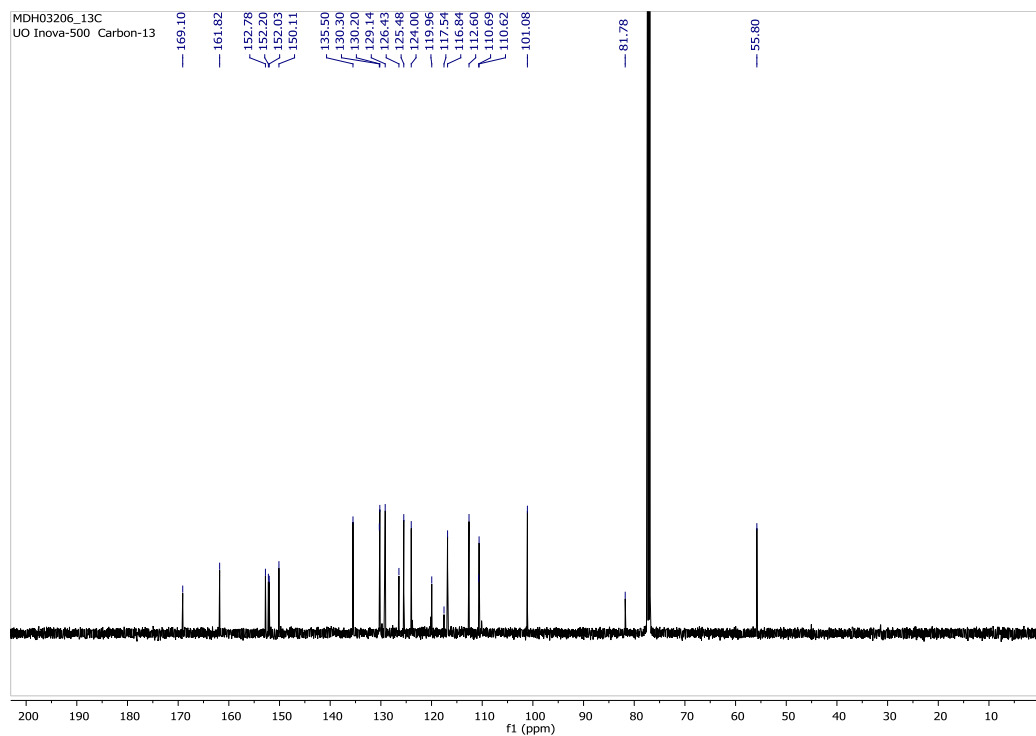
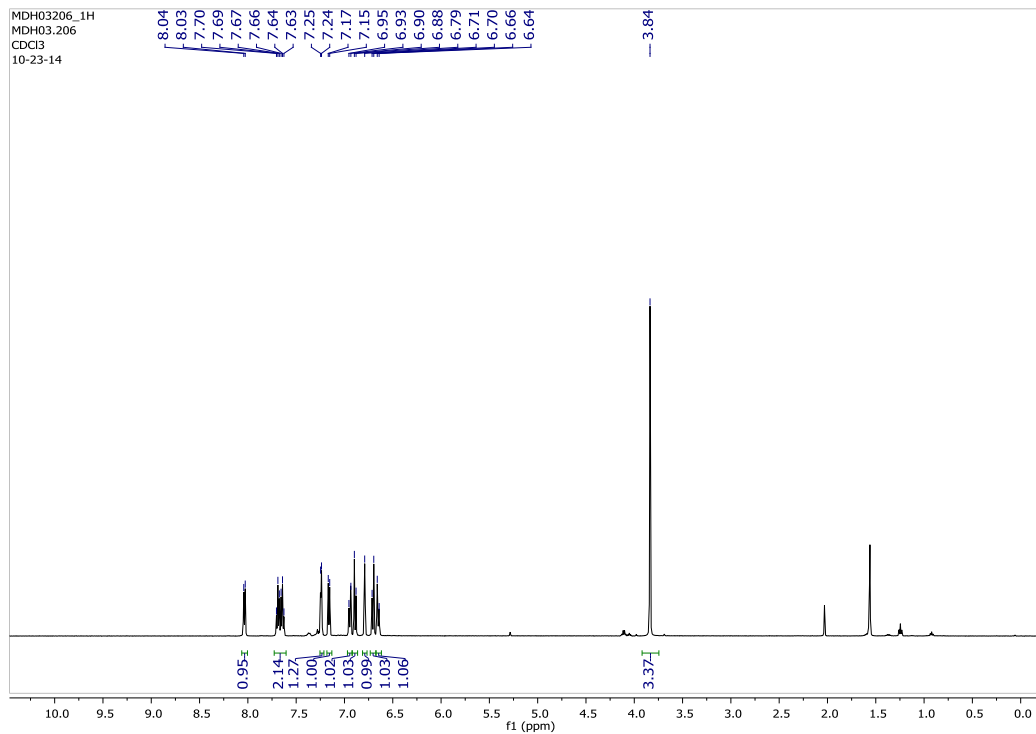
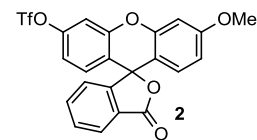


Figure S3. a) Fluorescent response of **MeRho-Az** to H₂S released from DATS with various [GSH] over 90 minutes. Conditions: 5 μM **MeRho-Az**, 300 μM DATS, 50-500 μM GSH, PIPES buffer (50 mM, 100 mM KCl, pH 7.4), $\lambda_{\text{ex}} = 476$ nm, $\lambda_{\text{em}} = 480$ -650 nm, 37 °C. b) Linear relationship between observed fluorescence turn-on from H₂S release and [GSH].

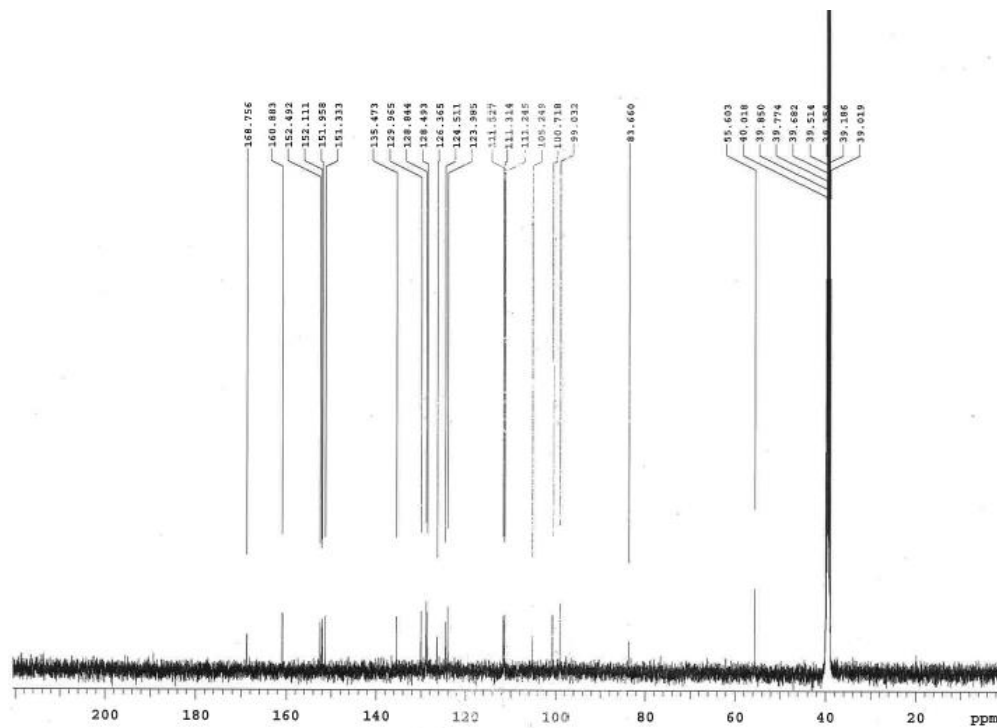
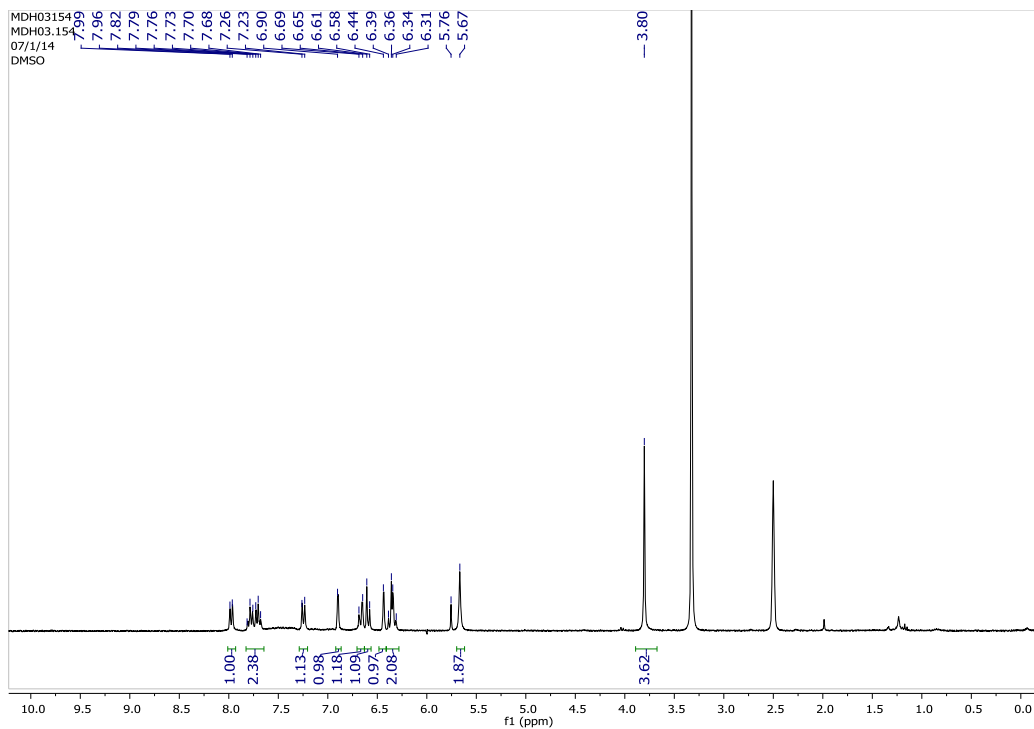
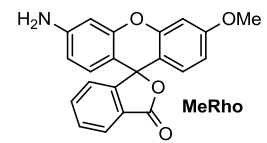
Video S1. Three dimensional scan of the intestinal bulb of a 7 dpf zebrafish gavaged with a solution of 5uM MeRho-Az, 250 uM DATS, and 250 uM GSH (intensity false color, as in Figure 5b). Field of view is 412 x 230 μm^2 and each frame represents a 1 μm step deeper into the larva. Shadows seen at ~18 seconds result from pigment cells distributed along the ventral midline. Autofluorescent cells external to the gut can also be seen.

NMR Spectra

^1H (500 MHz, CDCl_3) and $^{13}\text{C}\{^1\text{H}\}$ (125 MHz, CDCl_3) NMR Spectra of **2**



^1H (500 MHz, CDCl_3) and $^{13}\text{C}\{^1\text{H}\}$ (125 MHz, CDCl_3) NMR Spectra of **MeRho**



^1H (600 MHz, CDCl_3) and $^{13}\text{C}\{^1\text{H}\}$ (125 MHz, CDCl_3) NMR Spectra of **MeRho-Az**

