A Biotin-Guided Hydrogen Sulfide Fluorescent Probe and Its

Application in Living Cell Imaging

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Fig. S2 ¹³C NMR spectrum (DMSO-*d*₆) of NP-Biotin



Fig. S3 Fluorescence spectra (a) and F/F_0 at 550 nm (b) of 10 μ M NP-Biotin with Na₂S in the presence of various species (GSH, Cys, Hcy, Na₂S₂O₄, KNO₃, Na₂S₂O₃, NaOCN, Na₂S₂O₅, KBr, NaNO₂, NaN₃, K₂P₂O₇, NaI, Na₂SO₄, CH₃COONa, KF, Na₂SO₃, KSCN and NaHSO₃) under excitation at 480 nm. (F₀ represents the fluorescence intensity of NP-Biotin and F represents the fluorescence intensity of NP-Biotin with Na₂S in the presence of other guests respectively).



Fig. S4 HRMS study of NP-Biotin (100 μ M) in MeOH at room temperature



Fig. S5 HRMS study of the product of NP-Biotin (100 μ M) with Na₂S (1 mM) in MeOH at room temperature.



Fig. S6 Cytotoxicity of NP-Biotin towards HepG2. The cell viability was measured by CCK-8 assay.



Fig. S7 (a) Fluorescence spectra of NP-Biotin at different concentrations in dimethyl sulfoxide(DMSO); (b) The linear relationship between absorbance and the NP-Biotin concentration. NP-Biotin showed maximal absorption at 490 nm (

 $\varepsilon = 2.395 \times 10^4 L / (mol \ cm)$).

Optical properties of NP-biotin was examined in DMSO on a Cary Eclipse spectrophotometer and Fluorolog fluorescence spectrophotometer. For determination of the quantum efficiency (Φ_f) of fluorescence, Rhodamine 6G (Φ =0.95 in ethyl alcohol) was used as standards and the Φ_f value was calculated according to Equation (1)

 $\Phi_{\rm x} / \Phi_{\rm st} = [A_{\rm st} / A_{\rm x}][n_{\rm x}^2 / n_{\rm st}^2][D_{\rm x} / D_{\rm st}] \qquad (1)$

Where st refers to the standard, x refers to the sample, A is the absorbance at the excitation wavelength, and n is the refractive index.

We calculated that the fluorescence quantum yield of NP-Biotin was 0.05 in the absence of hydrogen sulfide.



Fig. S8 Photobleaching of LO2 cells after NP-Biotin labeling. LO2 cells were imaged from 0 minute to 120 minutes after incubation with 10 μ M NP-Biotin for 1 h and washing by PBS.