Supporting Information

A near-infrared fluorescent probe that can image endogenous hydrogen polysulfides in vivo in tumour-bearing mice

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**General Information.** Thin layer chromatography was performed on silica gel 60 F$_{254}$ plates (250 μm) and column chromatography was conducted over silica gel (300-400 mesh). Visualization of the developed chromatogram was accomplished by a UV lamp. Unless noted otherwise, reagents and solvents were obtained from commercial suppliers and employed without further purification. Nuclear magnetic resonance (NMR) spectra were obtained on JEOL ECZ-400S operated at 400/100 MHz for $^1$H NMR and $^{13}$C NMR, respectively. High-resolution mass spectra (HRMS) were measured by using a mass spectrometer. The pH measurements were measured with a Mettler-Toledo Delta 320 pH meter. Absorbance in the MTT assay was recorded using a spectrophotometer (Thermo Fisher Scientific, Finland). All fluorescence measurements were recorded on a Hitachi F4600 Fluorescence Spectrophotometer. All fluorescence imaging experiments were conducted on a FV1000 confocal laser scanning microscope (Olympus, Japan). The in vivo imaging was performed using a Night OWL IILB 983 small animal in vivo imaging system.

![Scheme S1. Response mechanism of NIR-CPS to H$_2$S$_n$.](image-url)
Synthesis and Characterisation of compounds.

**Scheme S2.** Synthetic route of NIR-CPS.

**Synthesis of compound 2.** Compound 2 was synthesized according to the method reported by Liu et al.\(^1\) \(p\)-hydroxybenzaldehyde (0.2 g, 1.64 mmol) was dissolved in 20 L dichloromethane followed by the addition of triethylamine (274.1 \(\mu\)L, 1.97 mmol) to the solution. The mixture was cooled in an ice-bath and then 2-fluoro 5-nitrobenzoyl chloride (0.4 g, 1.97 mmol) in 10 mL dichloromethane was added dropwise. The mixture was stirred at 0 \(^\circ\)C for 30 min, and then stirred at room temperature for 1 h. The solvent was removed under reduced pressure to obtain the crude product. The crude product was purified by recrystallization with ethyl acetate (20 mL) to afford compound 2 (0.36 g, 75.1 %). TLC (silica, PE:EA, 5:1 \(v/v\)): \(R_f = 0.5\). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 10.04 (s, 1H), 9.01 (dd, \(J = 6.0, J = 2.8\) Hz, 1H), 8.49 -8.53 (m, 1H), 7.98 -8.01 (m, 2H), 7.40 - 7.46 (m, 3H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 190.82, 166.90, 164.12, 160.05, 154.71, 134.68, 131.47, 130.72, 130.59, 128.70, 128.68, 122.37, 119.06, 118.96.

**Synthesis of NIR-COH.** Compound 1 (0.2 g, 0.53 mmol), 4-hydroxybenzaldehyde (77.90 mg, 0.64 mmol) and piperidine (5.2 \(\mu\)L, 0.05 mmol) dissolved in dry ethanol (15 mL). The mixture was reflux for 5 h. The solvent was removed under reduced pressure, then the crude product was dissolved in 60 mL dichloromethane, washed with 30 mL water. The crude product was purified by silica gel column to afford
compound NIR-COH as a black solid (145 mg, 56.8 %). TLC (silica, CH₂Cl₂:CH₃OH, 10:1 v/v) :Rᵣ = 0.4. 

¹H NMR (400 MHz, CD₃OD): δ 8.18-8.20 (m, 1H), 8.11 (s, 1H), 7.64-7.73 (m, 2H), 7.56 (d, J = 8.8 Hz, 2H), 7.21-7.24 (m, 1H), 7.18 (t, J = 2.0 Hz, 1H), 7.09-7.15 (m, 2H) 6.89 (d, J = 8.8 Hz, 2H), 3.67 (q, J = 7.2 Hz, J = 14.4 Hz, 4H), 2.93-2.97 (m, 2H), 2.40-2.44 (m, 2H), 1.78-1.85 (m, 2H), 1.29 (t, J = 7.2 Hz, 6H). HRMS(ESI⁺): (M⁺)calcd. for C₃₁H₃₀NO₄, 480.2169; found, 480.2170.

Absorption analyses. UV-Vis absorption spectra were detected at room temperature on a Shimadzu PharmaSpec UV-2401PC UV-Visible spectrophotometer. NIR-CPS probe solution (DMSO) was added to a quartz cuvette. With the probe diluted to 10 μM with 20 mM PBS buffer, Na₂S₄ was added. The resulting solution was incubated for 20 min prior to measurements (n = 3), with the mean ± SD expressed.

Evidence of mechanism detection. NIR-CPS (64.70 mg, 0.10 mmol) was dissolved in DMSO (15 mL), and then the solution of Na₂S₄ (348 mg, 2.0 mmol) in PBS buffer (30 mL, 20 mM, pH = 7.4) was added. After stirring at 37 °C for 30 min, the resultant mixture was extracted by EtOAc. The fluorescent product was thereafter purified by column chromatography and further characterised by HRMS and ¹H NMR. The NMR and HRMS spectra of fluorescent product were consistent with those of compound NIR-COH, hence confirmation of the fluorescent product as compound NIR-COH.

Determination of the detection limit. The detection limit was calculated based on previous method.² The fluorescence emission spectrum of NIR-CPS without Na₂S₄ was measured by 10 times and the standard deviation of blank measurement was determined. Then the probe solution was added with Na₂S₄ of concentration from 0 to 20 μM. A linear regression curve was then achieved according to the fluorescence intensity in the range of Na₂S₄ from 0 to 20 μM. The detection limit was calculated with the following equation: Detection limit = 3σ/k. Where σ is the standard deviation of blank measurements, k is the slope between the fluorescence intensity ratios versus Na₂S₄ concentrations.

Quantum Yields. Quantum yields were determined by using ICG as fluorescence standard (Φᵣ = 0.13 in DMSO). The quantum yield was calculated according to the equation: Φ_sample = Φ_standard (Grad_sample/Grad_standard)(η²_sample/η²_standard); where Φ is the quantum yield, Grad is the slope of the plot of absorbance versus integrated emission intensity, and η is the refractive index of the solvent. Absorbance of sample and standard at their respective excitation wavelengths was kept below 0.05.

MTT assay. The in vitro cytotoxicity of NIR-CPS were measured using a colorimetric MTT assay kit (Sigma-Aldrich). MCF-7 cells were seeded in 96-well plates at a density of 50,000 cells/well and then maintained at 37 °C in a 5 % CO₂ incubator. The cells were incubated with different concentrations of NIR-CPS for 24 h, respectively. Cells in culture medium without NIR-CPS were used as control. After the incubation time, 20 μL of MTT dye (3-[4, 5-dimethylthiazol-2-yl]- 2, 5-diphenyl tetrazolium bromide, 5 mg/ml in phosphate buffered saline), was added to each well, and the plates were incubated for 4 h at 37
°C. Then, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to
dissolve the formazan crystals. The plate was shaken for 10 min and the absorbance was measured at 570
nm on a microplate reader (ELX808IU, Bio-tek Instruments Inc, USA). Each sample was performed in
triplicate, and the entire experiment was repeated three times. The cell viability of NIR-CPS (0 μM, 5 μM,
10 μM, 15 μM, 20 μM) at 0 h, 6 h, 12 h, 18 h, 24 h and 48 h further demonstrated that the NIR-CPS was
of low toxicity to cultured MCF-7 cells.

**Preparation of the test solution.**

(1) NIR-CPS stock solution preparation: NIR-CPS (6.47 mg, 0.01 mmol) was dissolved into DMSO (10
mL) to get 1.0 mM stock solution.

(2) Na₂S₄ stock solution preparation: Na₂S₄ (17.42 mg, 0.1 mmol) was dissolved in 10 mL 20 mM PBS
(pH= 7.4) solution under nitrogen. The resulting solution was 10 mM Na₂S₄, which was then diluted to
1.0 mM-100 μM stock solution for general use. The solution was freshly prepared before each use.

(3) Na₂S₂ stock solution preparation: Na₂S₂ was prepared according to previous method³. Na₂S₂ (11.01
mg, 0.1 mmol) was dissolved in 10 mL 20 mM PBS (pH= 7.4) solution under nitrogen. The resulting
solution was 10 mM Na₂S₂, which was then diluted to 1.0 mM-100 μM stock solution for general use. The
solution was freshly prepared before each use.

(4) Na₂S stock solution preparation⁴: 5 mg EDTA was dissolved in 10 mL DI H₂O in a 25 mL Schlenk
tube. The solution was purged vigorously with nitrogen for 15 min. Then 24.0 mg sodium sulfide
(Na₂S·9H₂O) was dissolved in the solution under nitrogen. The resulting solution was 10 mM Na₂S, which
was then diluted to 1.0 mM-100 μM stock solution for general use.

(5) CTAB stock solution: 3.64 mg CTAB (Hexadecyl trimethyl ammonium bromide) was dissolved in 10
mL 20 mM PBS (pH= 7.4).

(6) Cys (L-Cysteine) stock solution preparation: Cys (12.0 mg, 0.1 mmol) was dissolved into DI H₂O (10
mL) to get 10.0 mM stock solution, which was then diluted to 1.0 mM and 100 μM solution for general
use.

(7) Hcy (Homocysteine) stock solution preparation: Hcy (13.5 mg, 0.1 mmol) was dissolved into DI H₂O (10
mL) to get 10.0 mM stock solution, which was then diluted to 1.0 mM and 100 μM solution for general
use.

(8) GSH (Glutathione) stock solution preparation: GSH (30.7 mg, 0.1 mmol) was dissolved into DI H₂O (10
mL) to get 10.0 mM stock solution, which was then diluted to 1.0 mM and 100 μM solution for general
use.

(9) Stock solutions of other reactive sulfur species, including GSSG, S₈, Na₂S₂O₃, Na₂SO₃, Na₂SO₄, Cys-
polysulfide,⁵,⁶ NaHSO₃ and S-nitroso glutathione were prepared in DI H₂O. The stock solution of
CH₃SSSSCH₃ was prepared in CH₃CN. S₈ stocking solution was prepared with ethanol.
(10) Stock solutions of other biological analytes $\text{H}_2\text{O}_2$, $\text{ClO}^-$, $\text{BuOOH}$, $\cdot \text{OH}$, $^1\text{O}_2$, $\text{O}_2^-$, $\text{NO}_2^-$, $\text{ONO}_2^-$, $\text{NO}$, $\text{NO}_3^-$, $\text{Na}^+$, $\text{K}^+$, $\text{Cu}^{2+}$, $\text{Ca}^{2+}$, $\text{Mg}^{2+}$, $\text{Zn}^{2+}$, $\text{Fe}^{3+}$, $\text{Fe}^{2+}$, $\text{CO}_3^{2-}$, $\text{HCO}_3^-$, $\text{Cl}^-$, $\text{Br}^-$, $\text{I}^-$, $\text{HPO}_4^{2-}$, $\text{H}_2\text{PO}_4^-$, $\text{OAc}^-$, glucose and ascorbic acid were prepared in DI $\text{H}_2\text{O}$. Superoxide radicals ($\text{O}_2^-$) were generated according to the previous reported method. $^7$ $\cdot \text{OH}$ was generated by Fenton reaction between $\text{Fe}^{II}$($\text{EDTA}$) and $\text{H}_2\text{O}_2$ quantitively. $^8$ NO is generated in form of $3$-(Aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC-5, 50 μmol/ml). $\text{NO}_2^-$ was provided by NaNO$_2$.

References


**Figure S1.** (A) Fluorescence spectra of NIR-COH, NIR-CPS and Na$_2$S$_4$ + NIR-CPS in PBS buffer (20 mM, pH = 7.4, 1 % DMSO, containing 1 mM CTAB). (B) Absorption spectra of NIR-COH, NIR-CPS and Na$_2$S$_4$ + NIR-CPS in PBS buffer (20 mM, pH = 7.4, 1 % DMSO, containing 1 mM CTAB).

**Figure S2.** Fluorescence spectra of NIR-COH (10 μM) and NIR-CPS (10 μM) in PBS buffer (20 mM, pH = 7.4, 1 % DMSO) (A), DMSO (B), Acetonitrile (C).
Figure S3. Absorption spectra of NIR-COH (10 μM) and NIR-CPS (10 μM) in PBS buffer (20 mM, pH = 7.4, 1 % DMSO) (A), DMSO (B), Acetonitrile (C).

Figure S4. (A) Fluorescence spectra of NIR-CPS (10 μM) with Na₂S₄ (100 μM) in PBS buffer (20 mM, pH = 7.4, 1 % DMSO, containing 1 mM CTAB) at 37 °C for 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30 and 60 min. (B) Time profile of NIR-CPS (10 μM) toward Na₂S₄ (100 μM) in PBS buffer (20 mM, pH = 7.4, 1% DMSO, containing 1 mM CTAB) at 37
°C for 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30 and 60 min. Data are presented as the mean ± SD (n = 3).

Figure S5. (A) Fluorescence spectra of NIR-CPS (10 μM) with Na₂S₄ (100 μM) in different pH buffer (20 mM, pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.4, 7.5, 8.0, 8.5, and 9.0, 1% DMSO, containing 1 mM CTAB) at 37 °C for 30 min. (B) Fluorescence responses of NIR-CPS (10 μM) with Na₂S₄ (100 μM) in different pH buffer (20 mM, pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.4, 7.5, 8.0, 8.5, and 9.0, 1% DMSO, containing 1mM CTAB) at 37 °C for 30 min.

Figure S6. Fluorescence intensities of NIR-CPS (10 μM) alone in PBS buffer (20 mM, pH = 7.4, 1 % DMSO) for 10,20, 30, 40, 50 and 60 min.
Figure S7. The selectivity of NIR-CPS for $H_2S_n$. (A) Fluorescence spectra of NIR-CPS (10 μM) towards $Na_2S_4$ (100 μM), $Na_2S_2$ (100 μM) and various reactive oxygen species ($H_2O_2$, $ClO^-$, $t$BuOOH, $^{1}OH$, $^{1}O_2$, 100 μM), reactive nitrogen species ($NO_2^-$, $ONOO^-$, NO, $NO_3^-$, 100 μM) in PBS buffer (20 mM, pH = 7.4, 1 % DMSO, containing 1 mM CTAB) at 37 °C for 30 min. (B) Fluorescence responses of NIR-CPS (10 μM) towards $Na_2S_4$ (100 μM), $Na_2S_2$ (100 μM) and various reactive sulfur species at 37 °C for 30 min. In each group, the red bars represent relative responses at 670 nm of NIR-CPS to ROS, RNS and the mixture of ROS/RNS with 100 μM $Na_2S_4$, respectively. 1. blank + $Na_2S_4$ (100 μM); 2. blank + $Na_2S_2$ (100 μM); 3. $H_2O_2$ (100 μM)+ $Na_2S_4$ (100 μM); 4. $ClO^-$ (100 μM)+ $Na_2S_4$ (100 μM); 5. $t$BuOOH (100 μM)+ $Na_2S_4$ (100 μM); 6. $^{1}OH$ (100 μM)+ $Na_2S_4$ (100 μM); 7. $^{1}O_2$ (100 μM)+ $Na_2S_4$ (100 μM); 8. $O_2^-$ (100 μM)+ $Na_2S_4$ (100 μM); 9. $NO_2^-$ (100 μM) + $Na_2S_4$ (100 μM); 10. $ONOO^-$ (100 μM)+ $Na_2S_4$ (100 μM); 11 NO (100 μM)+ $Na_2S_4$ (100 μM); 12. $NO_3^-$ (100 μM)+ $Na_2S_4$ (100 μM). Data are presented as the mean ± SD ($n$ = 3).

Figure S8. The selectivity of NIR-CPS for $H_2S_n$. (A) Fluorescence spectra of NIR-CPS (10 μM) towards $Na_2S_4$ (100 μM), $Na_2S_2$ (100 μM) and various ions ($Na^+$, $K^+$, $Cu^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Fe^{3+}$, $Fe^{2+}$, $Mn^{2+}$, $CO_3^{2-}$, $HCO_3^-$, $Cl^-$, Br, I, $HPO_4^{2-}$, $H_2PO_4^-$, OAc, glucose, ascorbic acid, 1 mM) in PBS buffer (20 mM, pH = 7.4, 1 % DMSO, containing 1 mM CTAB) at 37 °C for 30 min. (B) Fluorescence responses of NIR-CPS (10 μM) towards $Na_2S_4$ (100 μM), $Na_2S_2$ (100 μM) and various ions at 37 °C for 30 min. In each group, the red bars represent relative responses at 670 nm of NIR-CPS to ions and the mixture of ions with 100 μM $Na_2S_4$, respectively. 1. blank + $Na_2S_4$ (100 μM); 2. blank + $Na_2S_2$ (100 μM); 3. $Na^+$ (1 mM)+ $Na_2S_4$ (100 μM); 4. $K^+$ (1 mM)+ $Na_2S_4$ (100 μM); 5. $Cu^{2+}$ (1 mM)+ $Na_2S_4$ (100 μM); 6. $Ca^{2+}$ (1 mM)+...
Na$_2$S$_4$(100 μM); 7. Mg$^{2+}$(1 mM)+ Na$_2$S$_4$(100 μM); 8. Zn$^{2+}$(1 mM)+ Na$_2$S$_4$(100 μM); 9. Fe$^{3+}$(1 mM)+ Na$_2$S$_4$(100 μM); 10. Fe$^{2+}$(1 mM)+ Na$_2$S$_4$(100 μM); 11. Mn$^{2+}$(1 mM)+ Na$_2$S$_4$(100 μM); 12. CO$_3^{2-}$(1 mM)+ Na$_2$S$_4$(100 μM); 13. HCO$_3^{-}$(1 mM)+ Na$_2$S$_4$(100 μM); 14. Cl$^{-}$(1 mM)+ Na$_2$S$_4$(100 μM); 15. Br$^{-}$(1 mM)+ Na$_2$S$_4$(100 μM); 16. I$^{-}$(1 mM)+ Na$_2$S$_4$(100 μM); 17. HPO$_4^{2-}$(1 mM)+ Na$_2$S$_4$(100 μM); 18. H$_2$PO$_4^{-}$(1 mM)+ Na$_2$S$_4$(100 μM); 19. OAc$^{-}$(1 mM)+ Na$_2$S$_4$(100 μM); 20. glucose (1 mM)+ Na$_2$S$_4$(100 μM); 21. ascorbic acid(1 mM)+ Na$_2$S$_4$(100 μM). Data are presented as the mean ± SD (n = 3).

**Figure S9.** Cell viability of different concentrations of NIR-CPS (0 μM, 5 μM, 10 μM, 15 μM, 20 μM) at 24 h in MCF-7 cells. Data are presented as the mean ± SD (n = 3).

**Figure S10.** Cell viability of NIR-CPS (10 μM) at different times (0 h, 6 h, 12 h, 18 h, 24 h, 48 h) in MCF-7 cells. Data are presented as the mean ± SD (n = 3).
Figure S11. The corresponding bright images of Fig. 3, panels A, B, C, D and E.

Figure S12. The corresponding bright images of Fig. 4, panels A and B.

Figure S13. Representative fluorescence images of visualizing H_2S_n levels at different times in living mice using NIR-CPS. The mice were i.p. injected with LPS (10 μg/mL, 100 μL in saline) for 24h, followed by i.p. injection of NIR-CPS (2 mM, 100 μL DMSO). Images were taken after incubation of NIR-CPS at: 0 min; 1 min; 5 min; 10 min; 15 min; 20 min; 25 min; 30 min; 35 min; 40 min. Quantification of the fluorescence emission intensities from the abdominal area of the mice of the above groups. Data are presented as the mean ± SD (n = 3).
Figure S14. $^1$H NMR spectrum of compound 2.

Figure S15. $^{13}$C NMR spectrum of compound 2.
Figure S16. $^1$H NMR spectrum of compound NIR-CPS.

Figure S17. $^{13}$C NMR spectrum of compound NIR-CPS.
Figure S18. HRMS identification of NIR-CPS (calculated for C_{38}H_{32}FN_{2}O_{7} (M)^{+} 647.2118; found 647.2190).

Figure S19. \textsuperscript{1}H NMR spectrum of compound NIR-COH.
Figure S20. HRMS identification of NIR-COH (calculated for $\text{C}_{31}\text{H}_{30}\text{NO}_4$ (M)$^{+}$480.2169; found 480.2170).

Figure S18. $^1$H NMR spectra of the isolated fluorescent product of NIR-CPS + Na$_2$S$_4$. 
Figure S22. HR-MS identification of isolated fluorescent product of NIR-CPS + Na₂S₄ (calculated for C₃₁H₃₀NO₄ (M)⁺ 480.2169; found 480.2167).

Table S1. Summary of the reported H₂Sn fluorescent probes

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Chem Commun, 2019, 55(56): 8130-8133. 511/576 65 60 Cells

Dyes Pigm, 2019, 172: 107818. 比率计 F_{596}/F_{471} - 21 Zebrafish


Sens Actuators B Chem, 2019, 281: 871-877. λ_{em} = 633 - 95.2 Tissues


$\lambda_{em} = 635$  no  100  Mice

Sens Actuators B Chem, 2019, 284: 30-35.  
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305/520  215  84  Cells

430/506  76  100  Mice  (PD model)

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<td>700/720</td>
<td>20</td>
<td>22 Mice</td>
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<td>J Am Chem Soc</td>
<td>2014, 136(20)</td>
<td>7257-7260</td>
<td>λ&lt;sub&gt;em&lt;/sub&gt; = 515</td>
<td>no</td>
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<td>Anal Chem.</td>
<td>2015, 87(5)</td>
<td>3004-3010</td>
<td>362/534</td>
<td>172</td>
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<td>Anal Chem.</td>
<td>2016, 88(14)</td>
<td>7206-7212</td>
<td>432/542</td>
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<td>Sens Actuators B Chem.</td>
<td>2016, 232</td>
<td>531-537</td>
<td>λ&lt;sub&gt;em&lt;/sub&gt; = 548</td>
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<th>Chemical Structure</th>
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<th>Volume/Issue</th>
<th>Title</th>
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<td><img src="image1.png" alt="Chemical Structure 1" /></td>
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<td>258</td>
<td>125-132</td>
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<td>2016</td>
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<td>2016</td>
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<td>2015</td>
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<td>2017</td>
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<td>2017</td>
<td>8(2)</td>
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<td>2015</td>
<td>54(47)</td>
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770/780 10 nm 50 Mice