

Supporting Information

A mitochondria-targetable colorimetric and far-red fluorescent probe for sensitive detection of carbon monoxide in living cells

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1. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence spectra of free probe **FR-CO** were measured by five times and its standard deviation was obtained. To gain the slope, the fluorescence intensities at 630 nm were plotted as the increasing concentrations of CO. So the detection limit was calculated with the following equation (1):

$$\text{Detection limit} = 3\sigma/k \quad (1)$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensities versus the concentrations of CO.

2. The detailed procedure of cell culture and bioimaging

The living Hela cells were cultured in DMEM containing 10% fetal bovine serum, 1% penicillin, and 1% streptomycin at 37 °C (w/v) in a 5% CO₂ / 95% air incubator MCO-15AC (Sanyo, Tokyo, Japan). The concentrations of counted cells were adjusted to 1×10^6 cells mL⁻¹ for confocal imaging in high-glucose DMEM (4.5 g of glucose/L) supplemented with 10% fetal bovine serum (FBS), NaHCO₃ (2.0 ng/L), and 1% antibiotics (penicillin/streptomycin, 100.0 U/mL). Cultures were maintained at 37 °C under a humidified atmosphere containing 5% CO₂.

After 24 h, the cells were incubated with 10 μ M **FR-CO** in culture media for 30 min at 37 °C, and washed with PBS three times. Then the fluorescence imaging of the cells was carried out. Similarly, the cells were incubated with 10 μ M **FR-CO** and 10 μ M Pd²⁺ in culture media for 30 min at 37 °C, and washed with PBS three times. Then the fluorescence imaging of the cells was carried out. The cells were pretreated with 10 μ M **FR-CO** and 10 μ M Pd²⁺ in culture media for 30 min at 37 °C, and incubated with 10 μ M or 20 μ M CORM-2 for another 30 min at 37 °C. Then the fluorescence imaging of the cells was carried out after washing three times with PBS.

The fluorescence imaging of living HeLa cells was observed under an Olympus IX81 confocal fluorescence microscope using a 488nm light source.

3. Cytotoxicity assays

The living HeLa cells (1×10^6 cells mL^{-1}) were dispersed within replicate 96-well microtiter plates to a total volume of 200 μL well $^{-1}$. Plates were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 24 h. Then the living HeLa cells were incubated for 12 h upon different probe concentrations of 5, 10, 20, and 30 μM . 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg mL^{-1} , HEPES) was then added to each well. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 490 nm in a Triturus microplate reader. Similarly, the cytotoxicity of probe **FR-CO** with PdCl_2 (10 μM) was also estimated.

4. Preparation of reactive oxygen species

Hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl), and *tert*-butylhydroperoxide (TBHP) were diluted from the commercially available solution to 0.1 M in ultrapure water. Hydroxyl radical ($\cdot\text{OH}$) and *tert*-butoxy radical ($\cdot\text{O}^t\text{Bu}$) were generated by Fenton reactions. Superoxide anion ($\text{O}_2^{\cdot-}$) was prepared from KO_2 in DMSO. The concentration of H_2O_2 was determined from the absorbance at 240 nm ($\varepsilon = 43.6 \text{ M}^{-1} \text{ cm}^{-1}$). The concentration of $\cdot\text{OCl}$ was determined from the absorbance at 292 nm ($\varepsilon = 350 \text{ M}^{-1} \text{ cm}^{-1}$).

5. Characterization of probe FR-CO

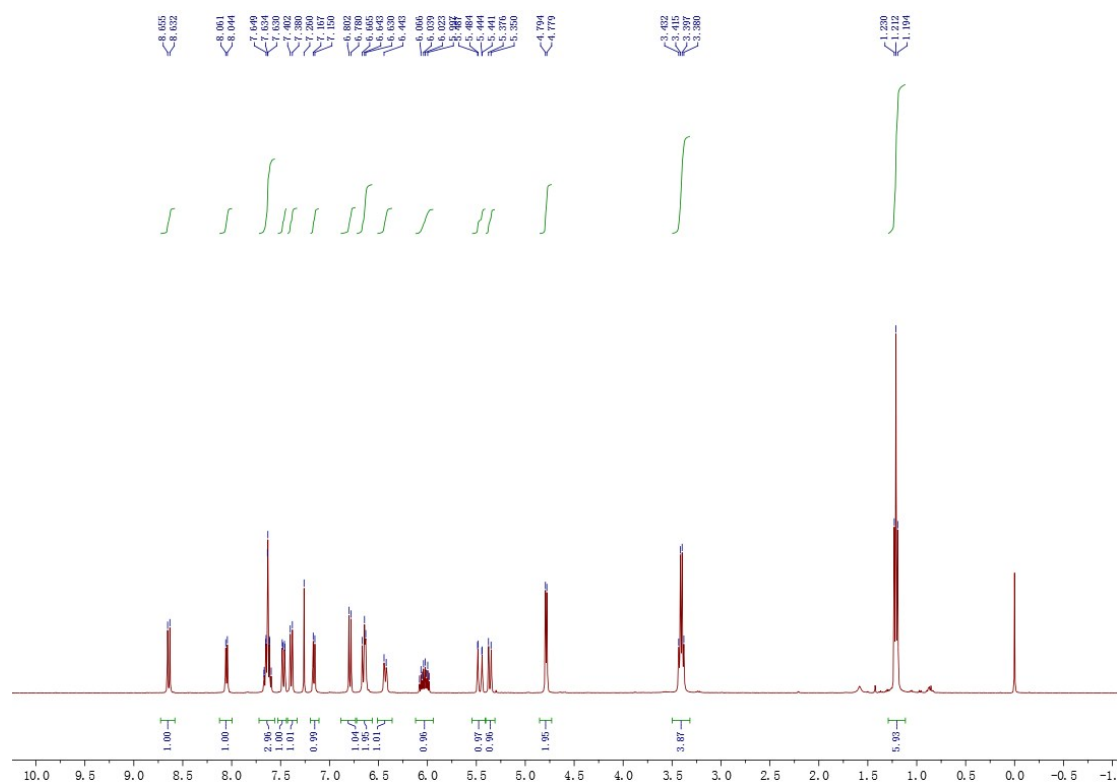


Fig. S1 ^1H -NMR spectrum of probe **FR-CO**

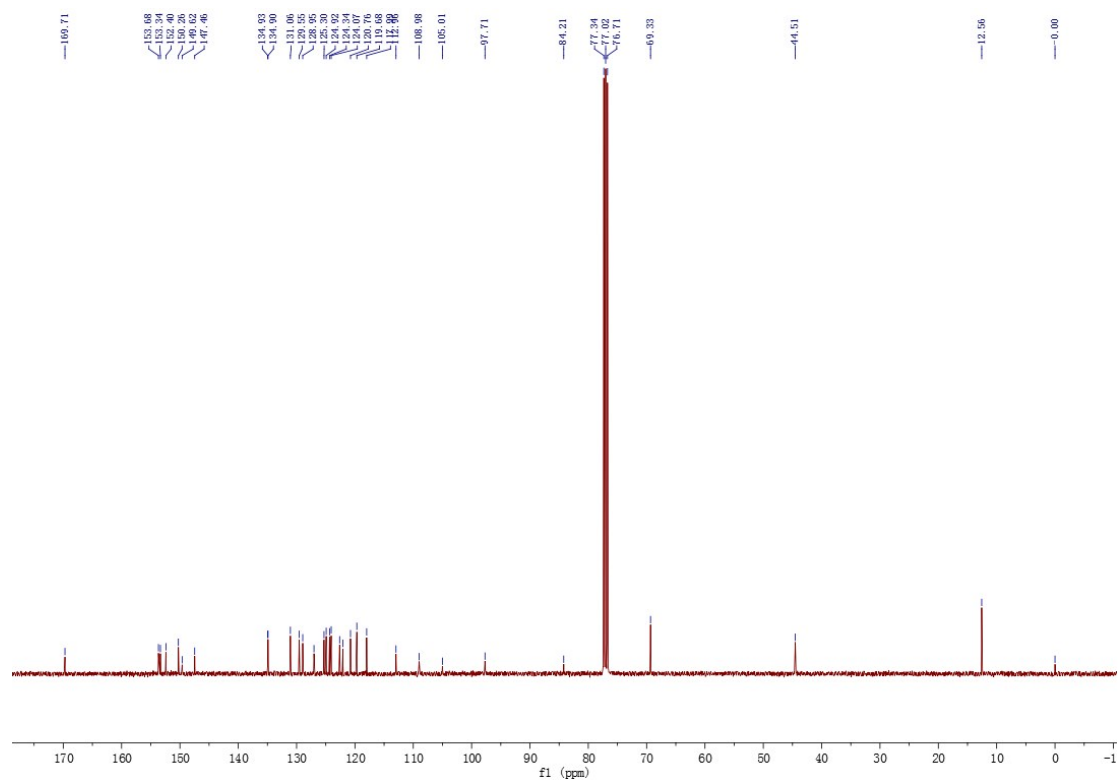


Fig. S2 ^{13}C -NMR spectrum of probe **FR-CO**

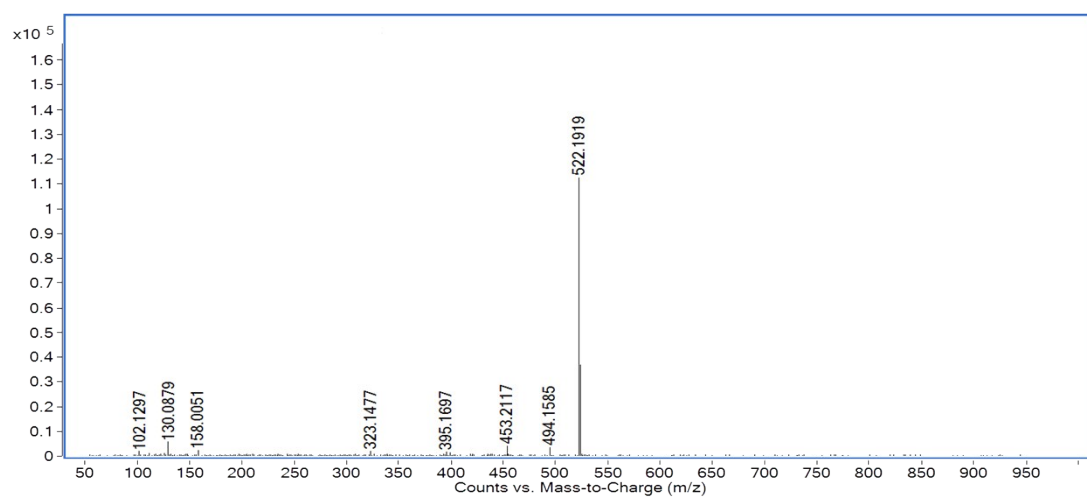


Fig. S3 HRMS of probe **FR-CO**

6. HRMS proof for the reaction mechanism of probe **FR-CO** with CO

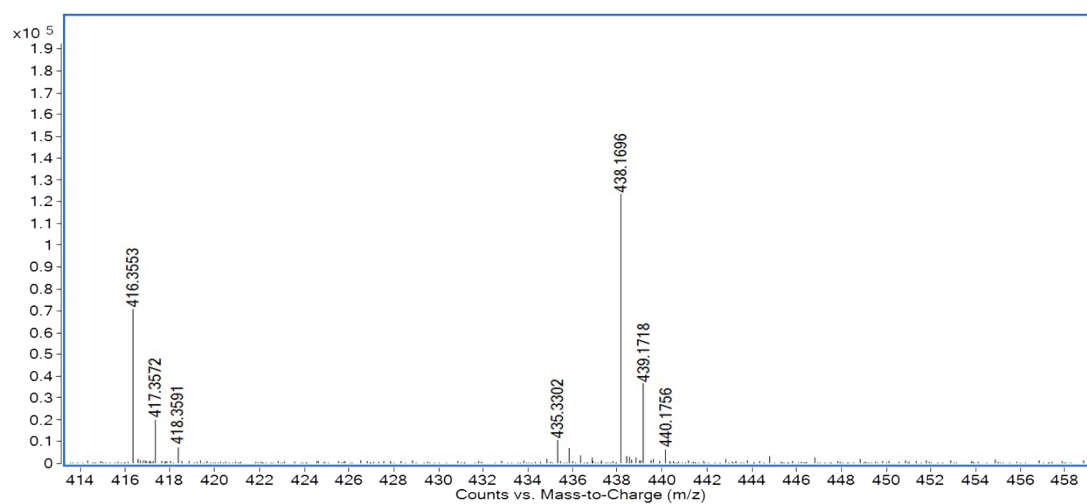


Fig. S4 HRMS proof for the reaction mechanism of probe **FR-CO** with CO