Supporting Information

A highly specific and sensitive ratiometric fluorescent probe for carbon monoxide and its bioimaging applications

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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 Ratio-CO were measured by five times and its standard deviation was obtained. To gain the slope, the fluorescence intensity ratio at 545 nm and 455 nm ($F_{545}/F_{455}$) were plotted as the increasing concentrations of CO. So the detection limit was calculated with the following equation (1):

\[
\text{Detection limit} = \frac{3\sigma}{k}
\]

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

2. The NMR ($^1$H and $^{13}$C) spectra of probe Ratio-CO

![Figure S1. The $^1$H NMR spectra of probe Ratio-CO](image)
3. Preparation of reactive oxygen species

Hydrogen peroxide (H₂O₂), sodium hypochlorite (NaOCl), and tert-butylhydroperoxide (TBHP) were diluted from the commercially available solution to 0.01 M in ultrapure water. Hydroxyl radical (·OH) and tert-butoxy radical (·O' Bu) were generated by reaction of 1 mM Fe²⁺ with 1 mM H₂O₂ or 1 mM TBHP respectively. Superoxide anion (O₂⁻) was prepared from KO₂ in DMSO. The concentration of H₂O₂ was determined from the absorbance at 240 nm (ε = 43.6 M⁻¹ cm⁻¹). The concentration of ·OCl was determined from the absorbance at 292 nm (ε = 350 M⁻¹ cm⁻¹).

4. The mechanism of probe Ratio-CO for detection of CO

When the hydroxyl group at the 4-site of 1,8-naphthalimide fluorophore was protected with allyl moiety, its electron-donating ability was suppressed.¹ While Pd²⁺ was reduced to Pd(0) by CO,²,³ and Pd(0) removed the allylic ether bond by the
Tsuji-Trost reaction. As a result, the hydroxyl group at the 4-site of 1,8-naphthalimide was released, leading to the generation of stronger intramolecular charge transfer (ICT) structure. Consequently, a large red-shifted fluorescence spectrum was obtained upon the addition of CO in the presence of Pd$^{2+}$.

**Scheme S1.** The recognition mechanism of probe Ratio-CO for detection of CO.

5. References


