Electronic Supplementary Information for

Turn-on silicon-based fluorescent probe for visualizing endogenous CO during hypoxia

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Materials and instruments

All chemicals and solvents used in the experiment were of analytical grade and were used without further purification. 3, 3'-(1, 1, 3, 3-tetramethyldisiloxane-1, 3-diyl) bis (Propan-1-amine) purchased from Hangzhou Da di Chemical. 3-Nitro-1, 8-naphthalic anhydride was purchased from Shanghai Aladdia Biochemical Technology Co., Ltd. HepG2 cells lines were purchased from Procell Life Science&Technology Co., Ltd. The other reagents used in this work were purchased from the supplier, and the water used in the experiment was ultrapure water.

The ^{1}H NMR and ^{13}C NMR spectra were measured on an AVANCE III 400 MHz Digital NMR Spectrometer. Fluorescence spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell. Absorption spectra were obtained on a Shimadzu UV-2700 Power spectrometer. MTT was obtained from Sigma-Aldrich. Fluorescence imaging of HepG2 cells was performed with Nikon A1MP confocal microscopy. The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter.

Cytotoxicity Assays

The cytotoxicity of **SAH-N** was performed via the standard MTT assays. HepG2 cells cells were first inoculated in culture plate until they adhered to the walls. Subsequently, the culture media with different concentrations of **SAH-N** were added to the 96-well plate and cultured in an incubator (5% CO_2 and 95% air, 37 °C) for 24 h. MTT (10 μL) was added and the cells were continue cultured for 4 h. Finally, the plate was shaken for about 10 min, and each well was analyzed by the microplate reader and detected at the absorbance of 570 nm.

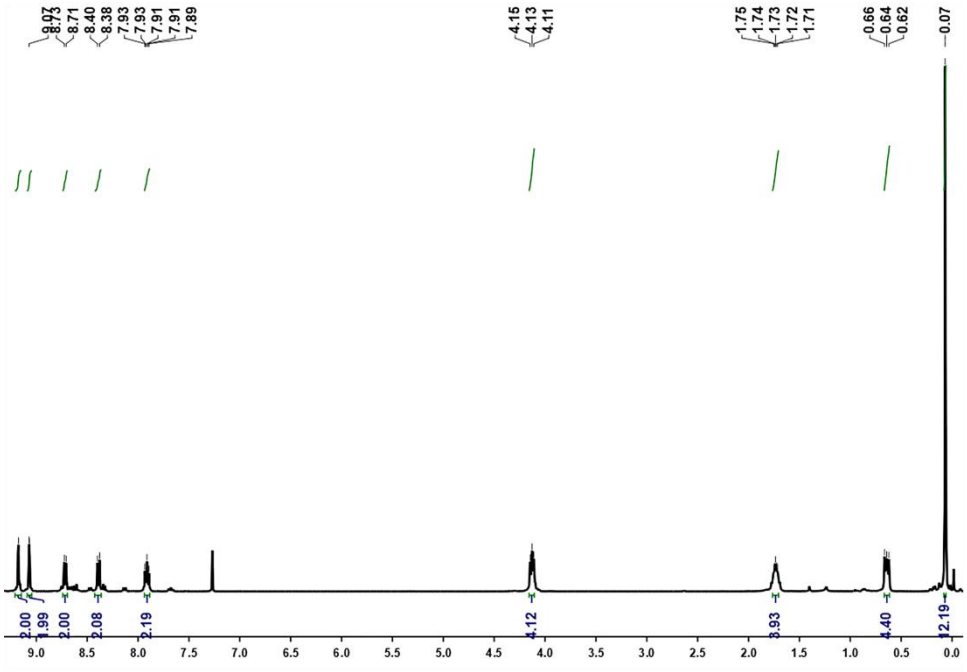


Fig. S1. The ^{1}H NMR spectrum of **SAH-N**.

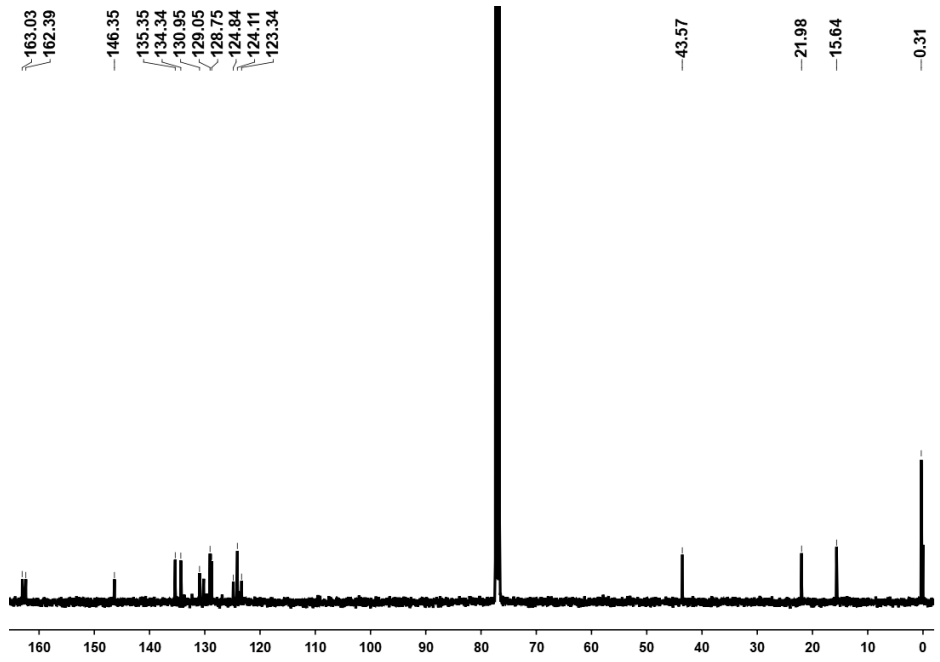


Fig. S2. The ^{13}C NMR spectrum of **SAH-N.**

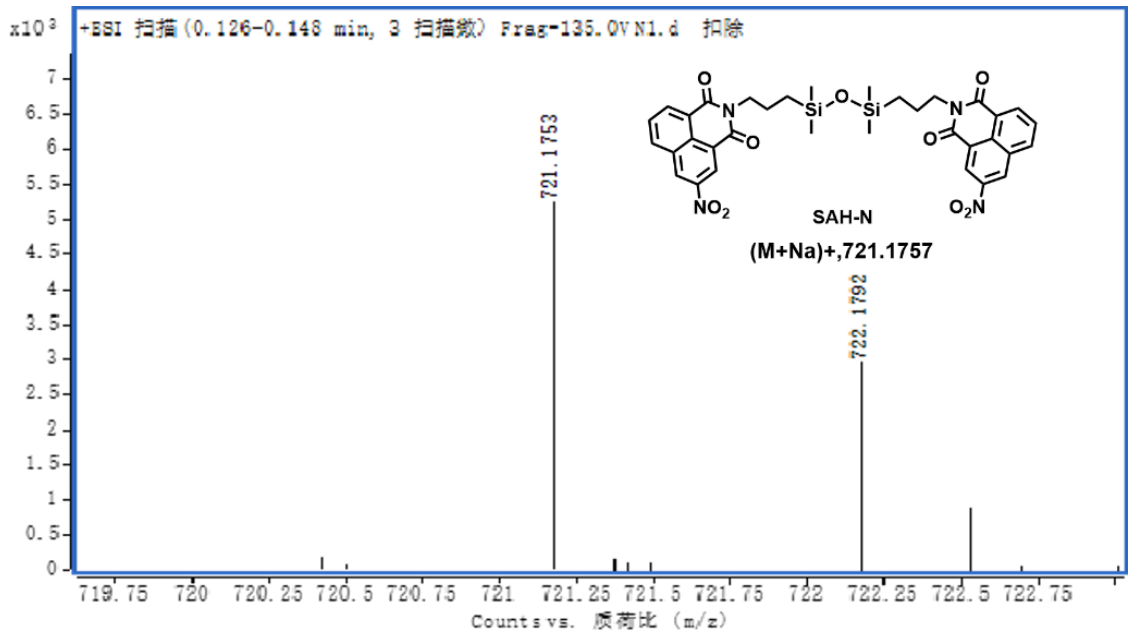


Fig. S3. The HR-MS spectrum of **SAH-N**.

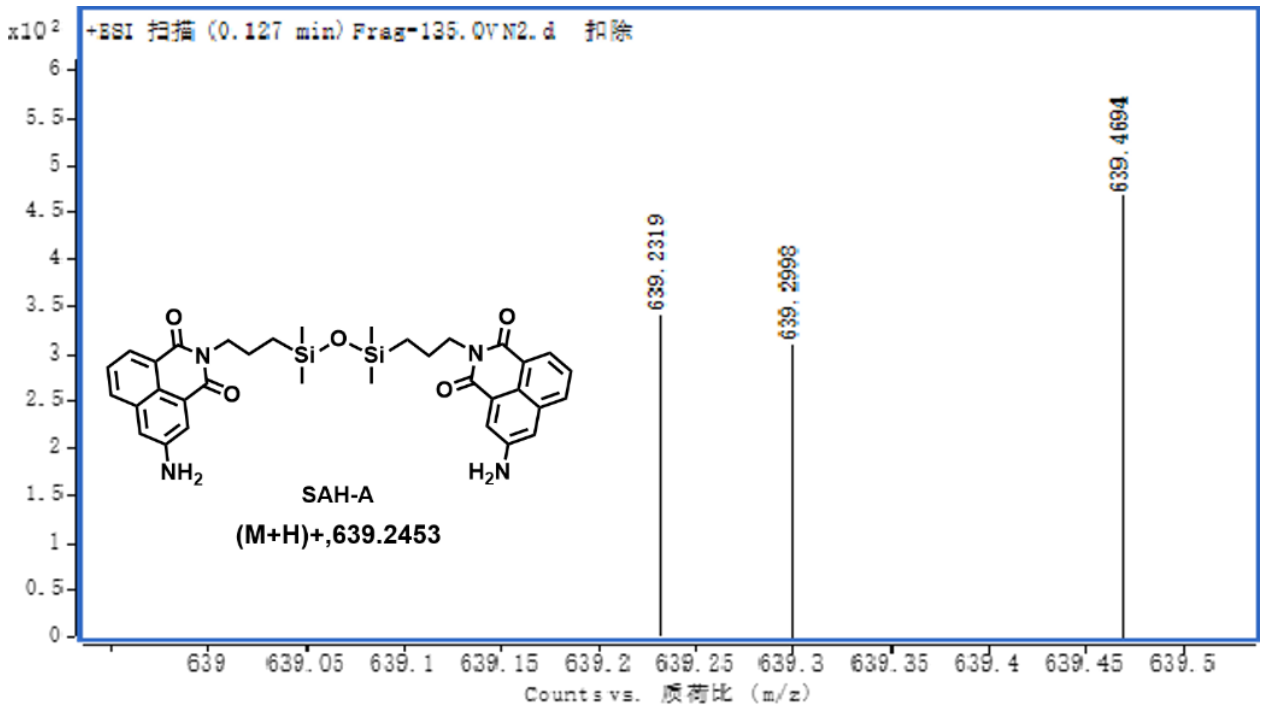


Fig. S4. The HR-MS spectrum of **SAH-N** reacted with CORM-2.

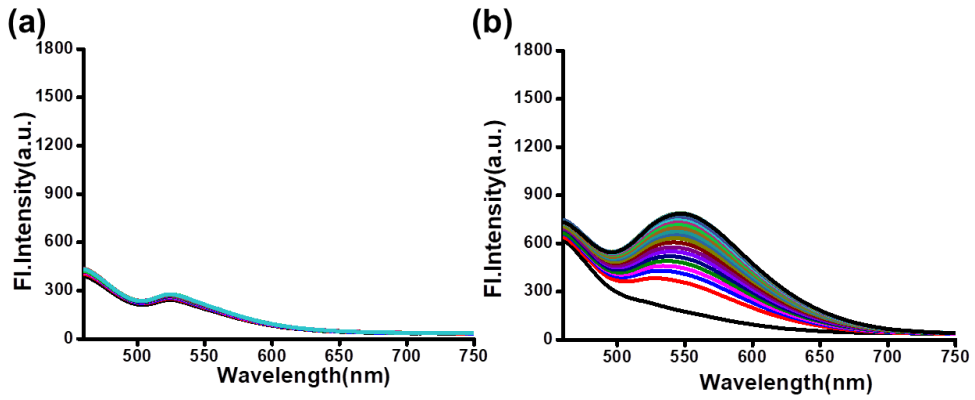


Fig. S5. (a) Fluorescence spectra of **SAH-N** (10 μM) for 60 min. (b) Fluorescence spectra of **SAH-N** (10 μM) treated with 30 μM CORM-2 for 60 min. λ_{ex} = 440 nm.

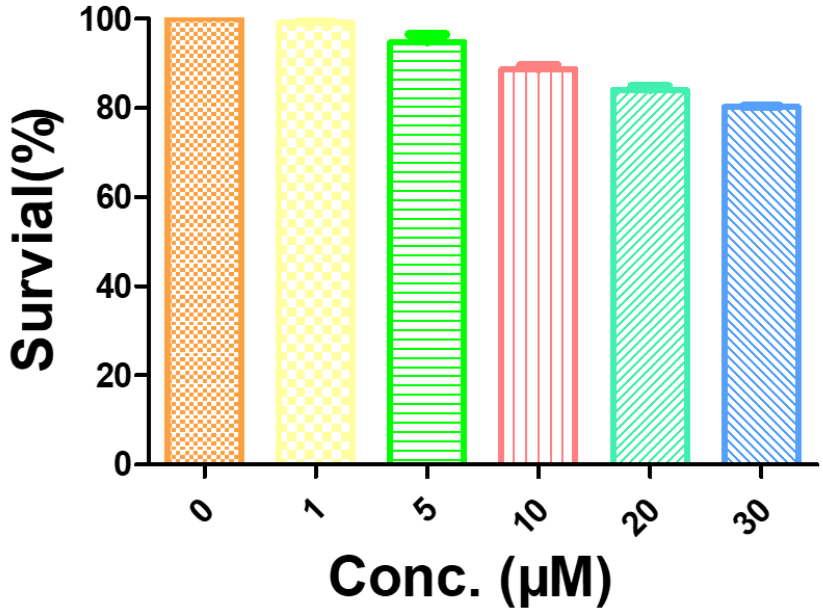


Fig. S6. HepG2 cells in the presence of **SAH-N** at various concentrations measured using MTT assay.

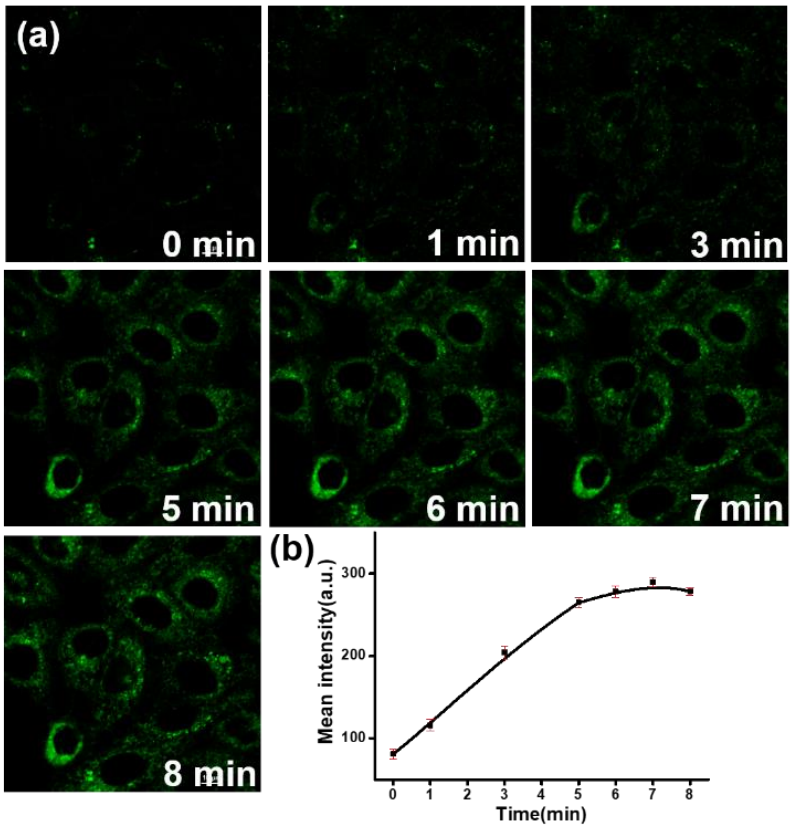
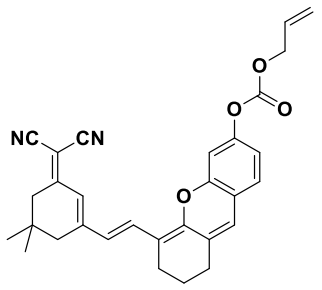
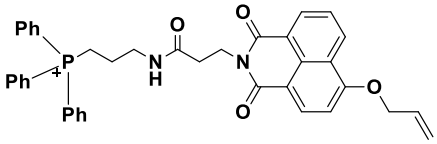
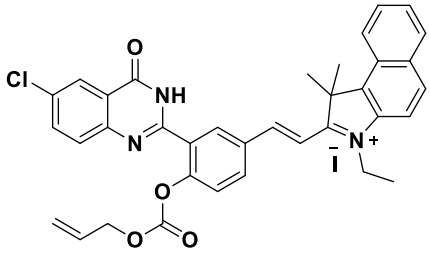
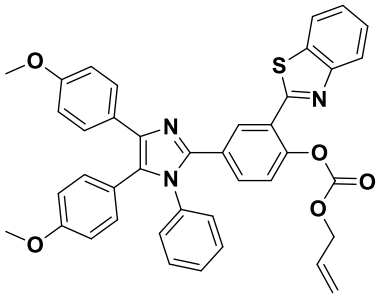
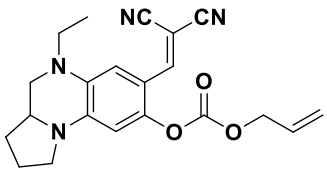
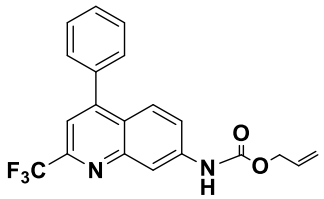
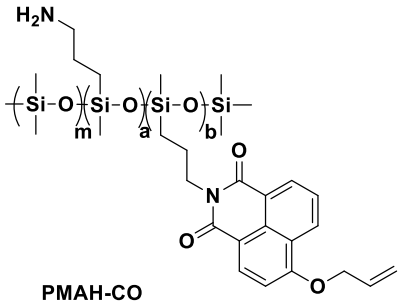
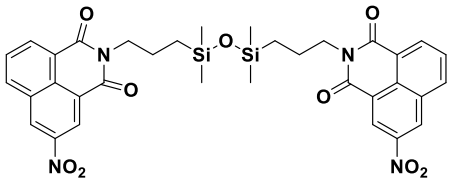


Fig. S7. (a) Fluorescent images of of HepG2 cells stained with 10 μM **SAH-N** with increasing incubation times. (b) Fluorescence intensity changes of the photos of cells in green channel. CORM-2 concentration: 30 μM; λ_{ex}: 488 nm; λ_{em}: 500 nm – 550 nm; scale bar: 10μM.

Table S1. Additional table of comparison between reported CO probes and probe **SAH-N**.

Probe	λ_{em} (nm)	λ_{ex} (nm)	Pd^{2+} free	Reaction site	Endogenous experiment	Ref.

	580	770	No	Ester bond	Heme	36
	430	549/451	No	Ether bond	LPS	37
	520	605	No	Ester bond	Heme	38
	330	552/440	No	Ester bond	Hypoxia	39
	471	608	No	Ester bond	---	40

	360	520/425	No	Urethane bond	Heme/LPS	41
 PMAH-CO	425	559/450	No	Ether bond	---	Anal. Chem. 2021, 93, 38, 12899–12905
	440	537	Yes	Nitro	Mainly hypoxia	This work