Supporting Information

Evaluation of Nitric Oxide Fluctuation *via* a Fast-responsive Fluorescent Probe in Idiopathic Pulmonary Fibrosis Cell and Mice Models

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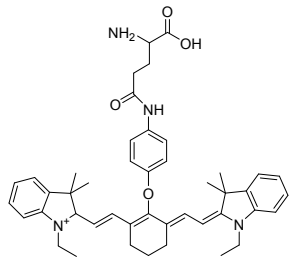
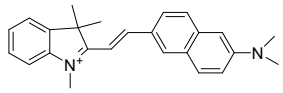
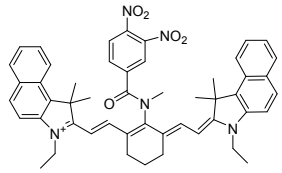
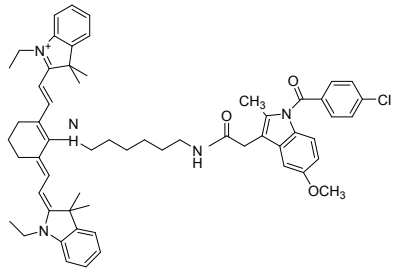
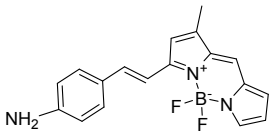
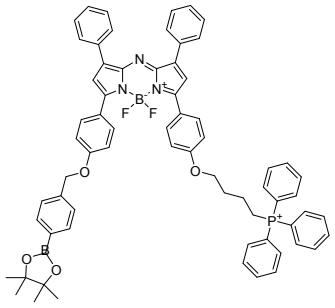
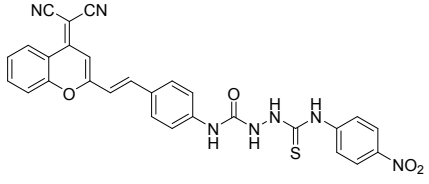
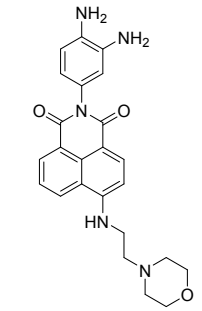
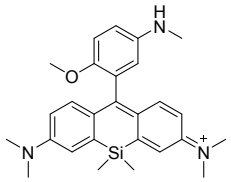
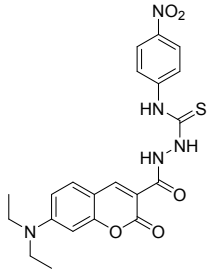
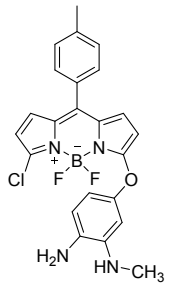
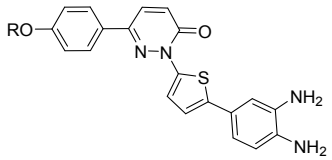
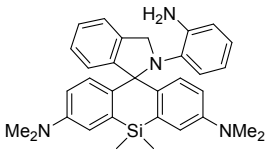
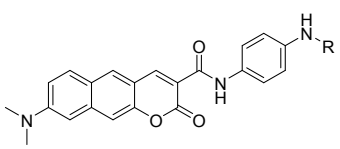
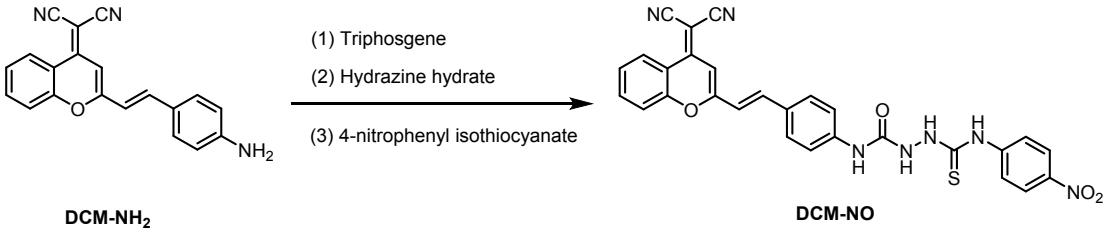
1. Table S1 Fluorescent probes for IPF.

Probe	Response time (min)	analyte	Stokes shift (nm)	detection limit	Literature
	30	GGT	10	7.6 mU/L	Sensor. Actuat. B-Chem. 2020, 322, 128565
	4	$ONOO^-$	160	85 nM	Anal. Chem. 2019, 91, 11461-11466
	15	GSTs	80	10 μg/L	Anal. Chem. 2019, 91, 5424-5432
	60	COX-2	120	11 μg/L	J. Mater. Chem. B, 2021, 9, 6226-6233
	60	NO	14	57 nM	Anal. Chem. 2020, 92, 699-706
	25	H_2O_2	30	100 nM	ACS Sens. 2021, 6, 1228-1239
	1	NO	198	17 nM	This work

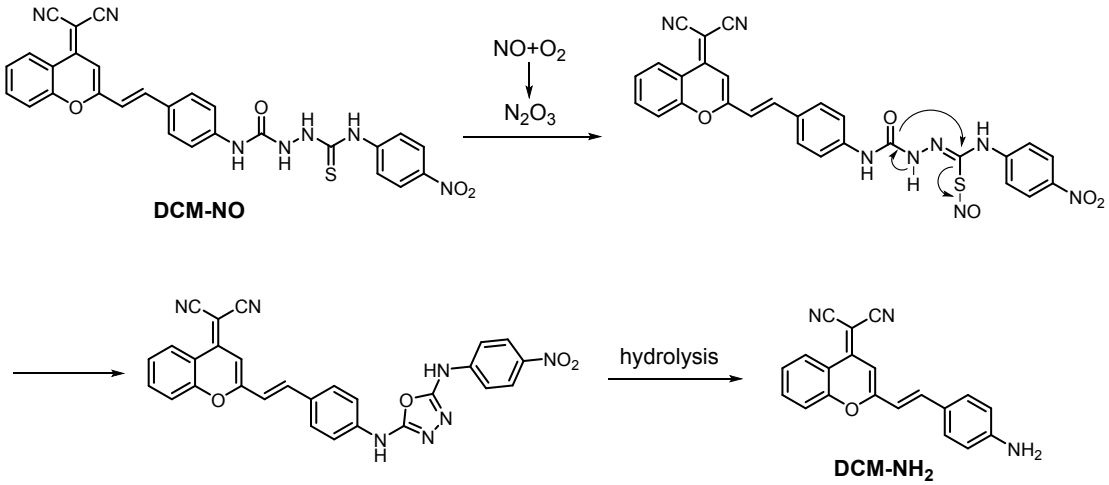
2. Table S2 Fluorescent probes for NO.

Probe	Response time (min)	Stokes shift (nm)	detection limit	Literature
	-	90	5 nM	J. Am. Chem. Soc. 2012, 134, 17486-17489
	1.5	19	14 nM	Anal. Chem. 2017, 89, 9620-9624
	0.3	39	47.6 nM	ACS Sens. 2019, 4, 309-316
	30	23	56 nM	Anal. Chem. 2019, 91, 4301-4306
	<1	179	242 nM	Anal. Chem. 2020, 92, 5064-5072
	<1	35	0.12 nM	Chem. Sci., 2017, 8, 6857-6864
	2	140	37 nM	Chem. Sci., 2017, 8, 4533-4538

3. Experimental Procedures



Scheme S1. Synthetic route of **DCM-NO**.



Scheme S2. The proposed response mechanism of probe **DCM-NO** to NO.

Materials and Instruments.

NMR spectra were measured with a Bruker 400 MHz spectrometer. High-resolution mass spectra were taken on a Bruker Daltonics MICROTOF-Q II mass spectrometer. The fluorescence spectra were recorded by a Hitachi F-4600 spectrometer. UV-vis absorption spectra were recorded by an Agilent Cary 60 spectrophotometer. HPLC analyses were performed on an Agilent 1200 HPLC system. pH values of the aqueous media were measured with a Leici PHS-3C meter. The fluorescent images were measured on a TCS SP8 II (Nikon C2plus, Germany) confocal laser scanning microscope. The vivo imaging was performed by an IVIS Lumina LT vivo imaging system. MRC-5 and MH-S cells were obtained from the Fuheng Biotechnology Co., LTD. All male C57BL/6 mice were purchased from Wuhan Servicebio Biotechnology Co., LTD. All reagents were obtained from commercial suppliers and were used without further purification.

Preparation of Analytes.

The testing solutions of NaClO, H_2O_2, Na_2S, Cys, Hcy, GSH, $MgCl_2$, $CaCl_2$, $CuSO_4$, $(CH_3COO)_2Pb$, $FeCl_2$, $FeCl_3$, $ZnCl_2$, Na_2CO_3, $NaNO_2$, $NaNO_3$, KOH, Na_2SO_3, KI were prepared by dissolving or diluting each of them in double-distilled water. $ONOO^-$ was prepared according to previous literature and the concentrated was determined by absorbance at 302 nm. Hydroxyl radical (•OH) was generated in the Fenton system from $FeSO_4$ and H_2O_2. [1]

H&E and Masson Staining

Histology analysis of lung from each group was carried out by sacrificing the mice. The lungs were fixed in 10% paraformaldehyde solution, and then embedded in paraffin. The lung sections were observed by an optical microscope after stained with H&E and Masson.

Imaging of NO in Lung Sections

Lung sections of each group were cultured with probe **DCM-NO** (10 μM) for 30 min, washed three times with PBS and imaged with confocal laser scanning microscope.

4. Supplementary Figures

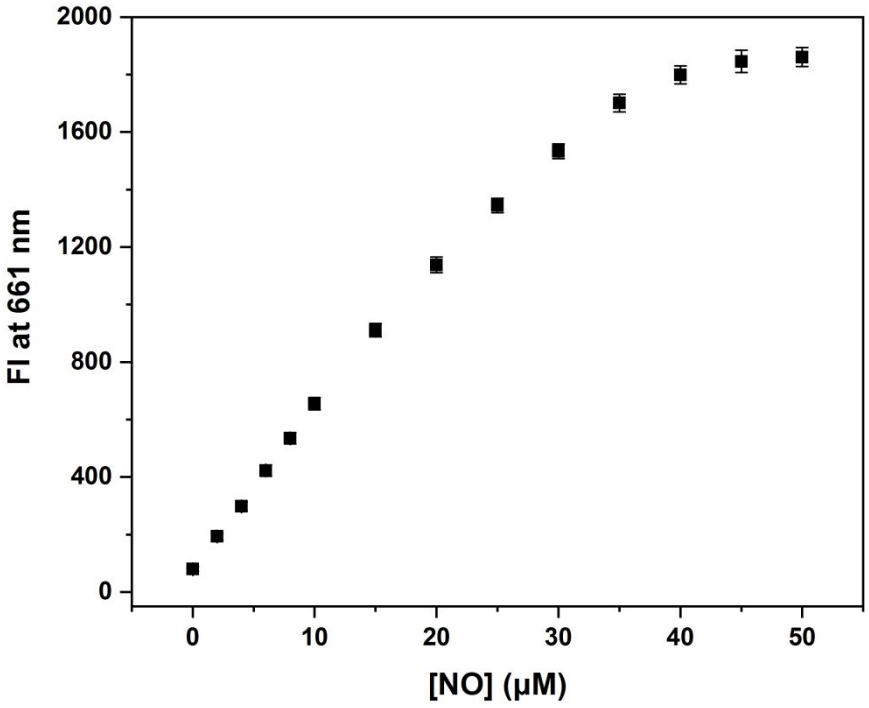


Figure S1. Plot of emission intensity at 661 nm of probe **DCM-NO** (10 μM) upon the addition of NO (0-50 μM) in PBS buffer (10 mM, pH 7.4, including 30% DMSO).

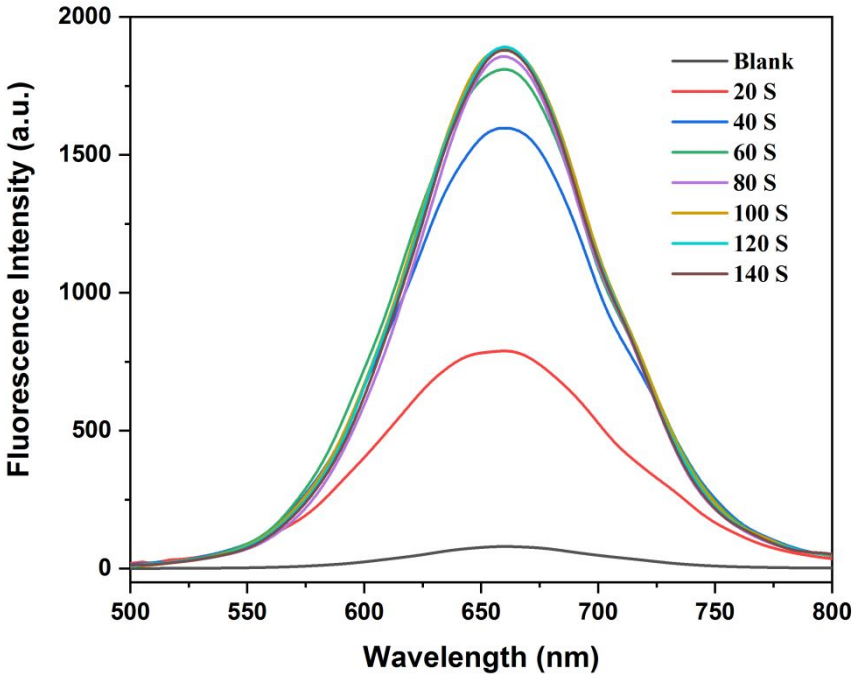


Figure S2. Time-dependent fluorescence spectra of probe **DCM-NO** (10 μM) with NO (50 μM) in PBS buffer (10 mM, pH 7.4, including 30% DMSO).

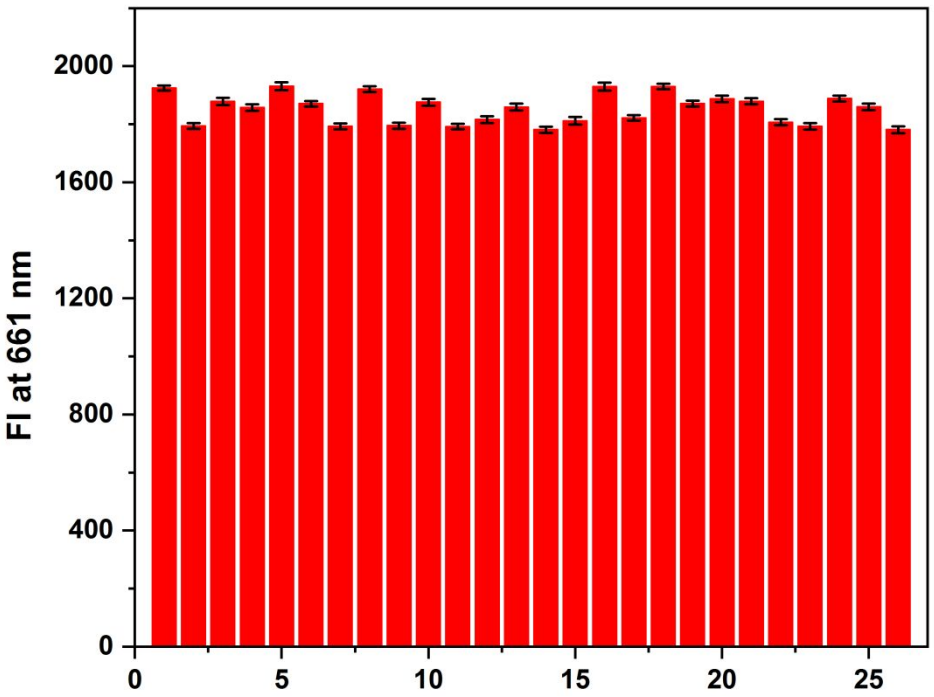


Figure S3 Fluorescence intensity of probe **DCM-NO** (10 μM) with NO (50 μM) in the presence of various relevant analytes (50 μM) in PBS buffer (10 mM, pH 7.4, including 30% DMSO). The analytes include Na^+, Mg^{2+}, Ca^{2+}, K^+, Cu^{2+},Fe^{2+}, Fe^{3+}, Zn^{2+}, Pb^{2+}, AcO^-,SO_4^{2-}, CO_3^{2-}, NO_2^-, Cl^-, NO_3^-, OH^-, SO_3^{2-}, I^-, S^{2-}, Cys, Hcy, GSH, ClO^-, $ONOO^-$, H_2O_2, •OH.

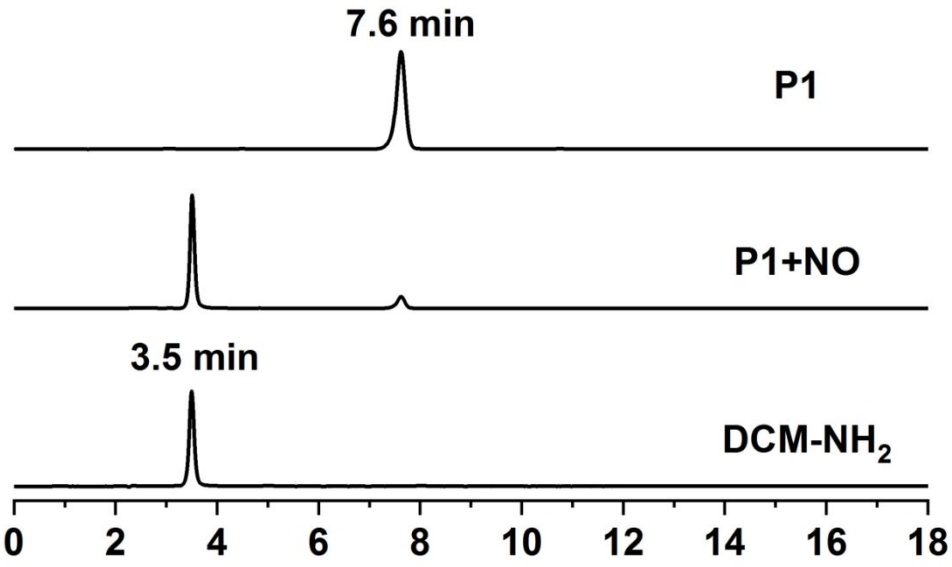


Figure S4. HPLC chromatograms of probe **DCM-NO**, compound **DCM-NH$_2$**, and the reaction mixture of probe **DCM-NO** with NO. Conditions: eluent, H_2O/CH_3CN (v/v, 1/9), flow rate, 0.8 mL/min; temperature, 25 ℃; detection wavelength, 455 nm; injection volume, 10 μL.

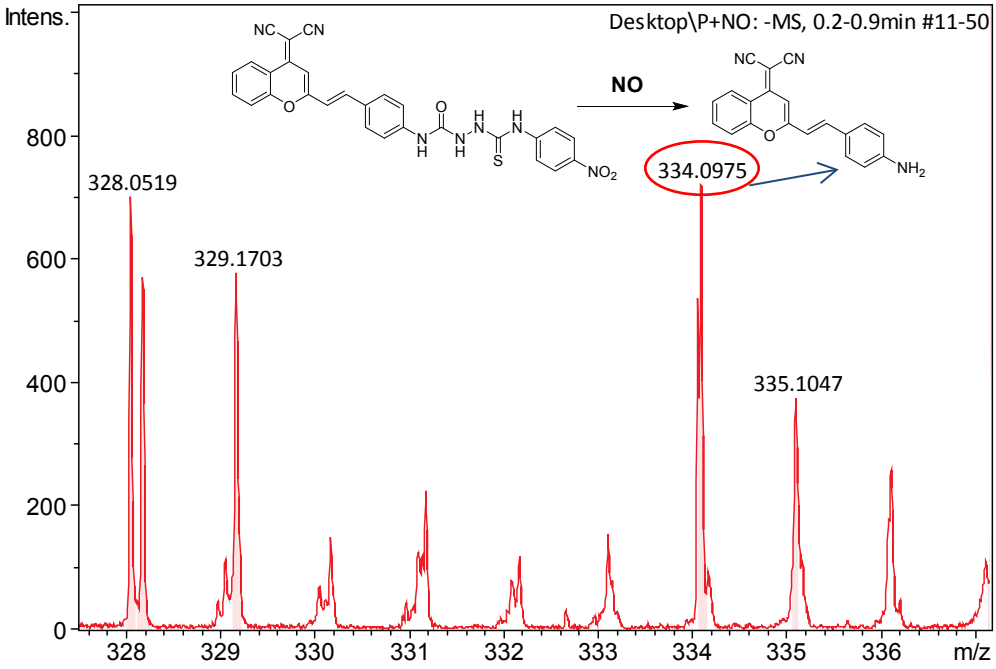


Figure S5. HRMS spectrum of the reaction product from probe **DCM-NO** with NO.

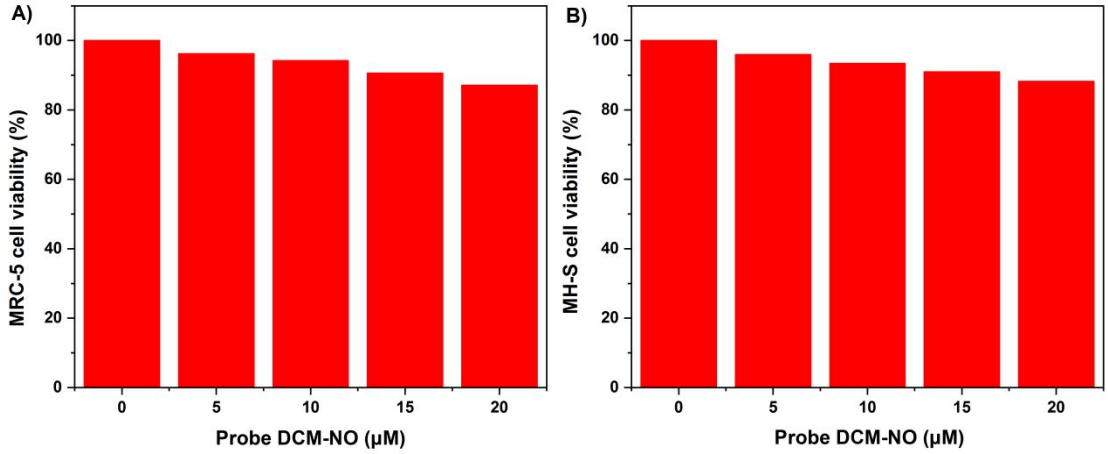


Figure S6. Cytotoxicity assay of probe **DCM-NO** at different concentration for MRC-5 (A) and MH-S (B) cells.

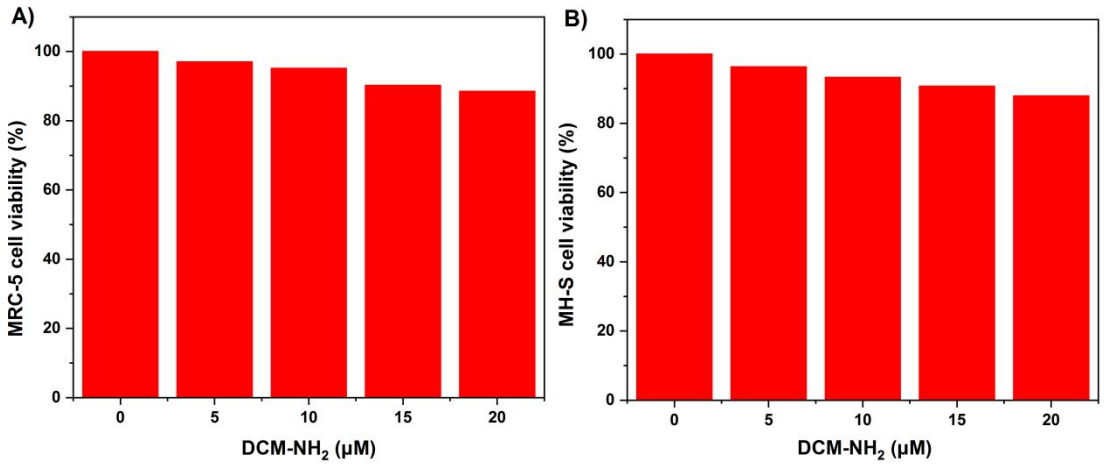


Figure S7. Cytotoxicity assay of compound **DCM-NH_2** at different concentration for MRC-5 (A) and MH-S (B) cells.

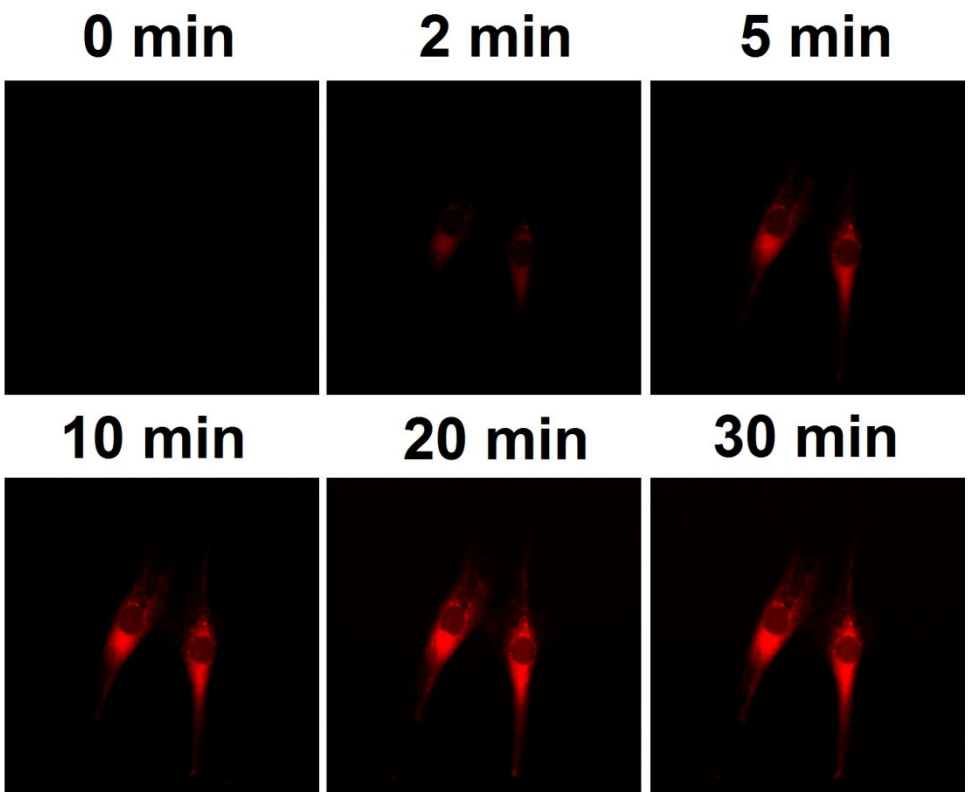


Figure S8 Confocal fluorescence images of NO in IPF cell models at diverse time points: 0, 2, 5, 10, 20, and 30 min as control.

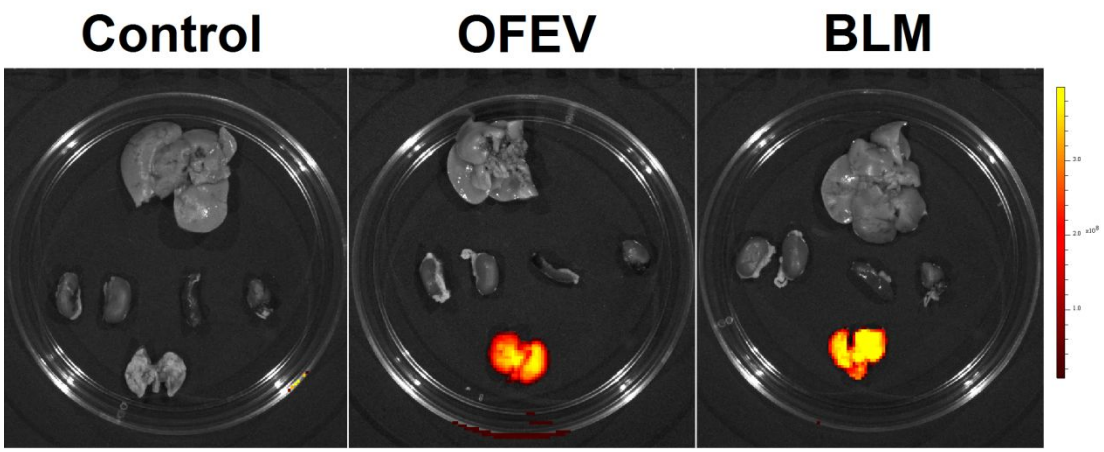


Figure S9. Images of isolated organs, involving heart, liver, spleen, lung, and kidney.

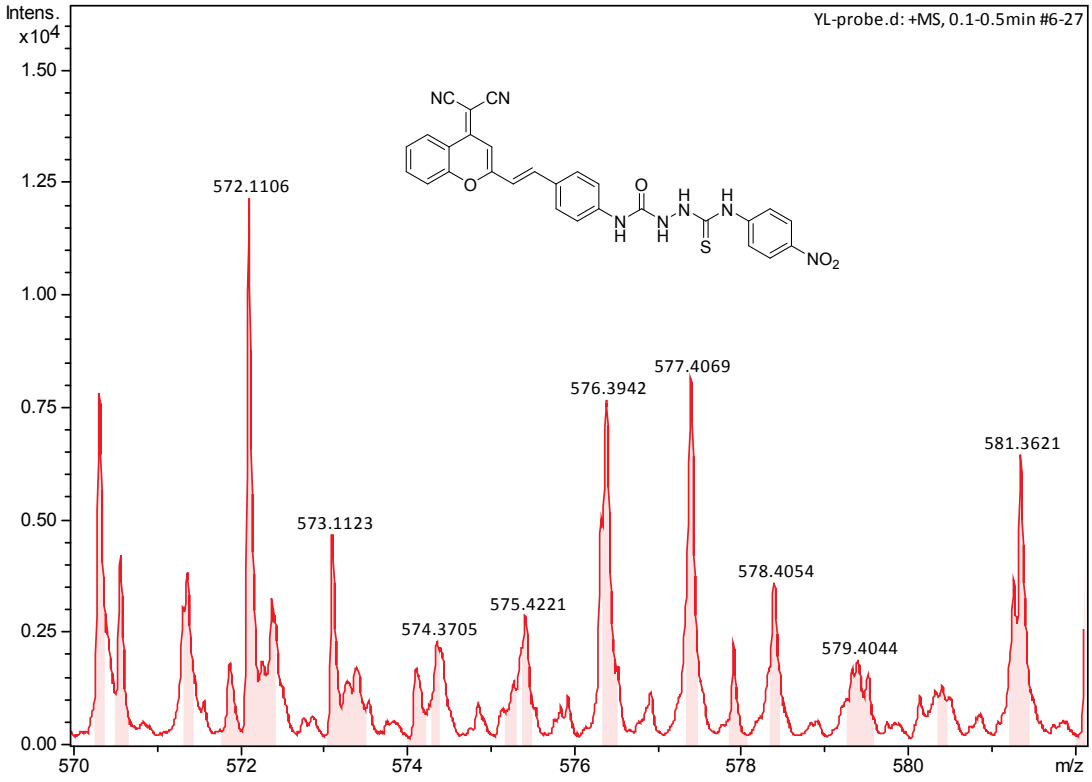


Figure S10. HRMS spectrum of probe **DCM-NO**.

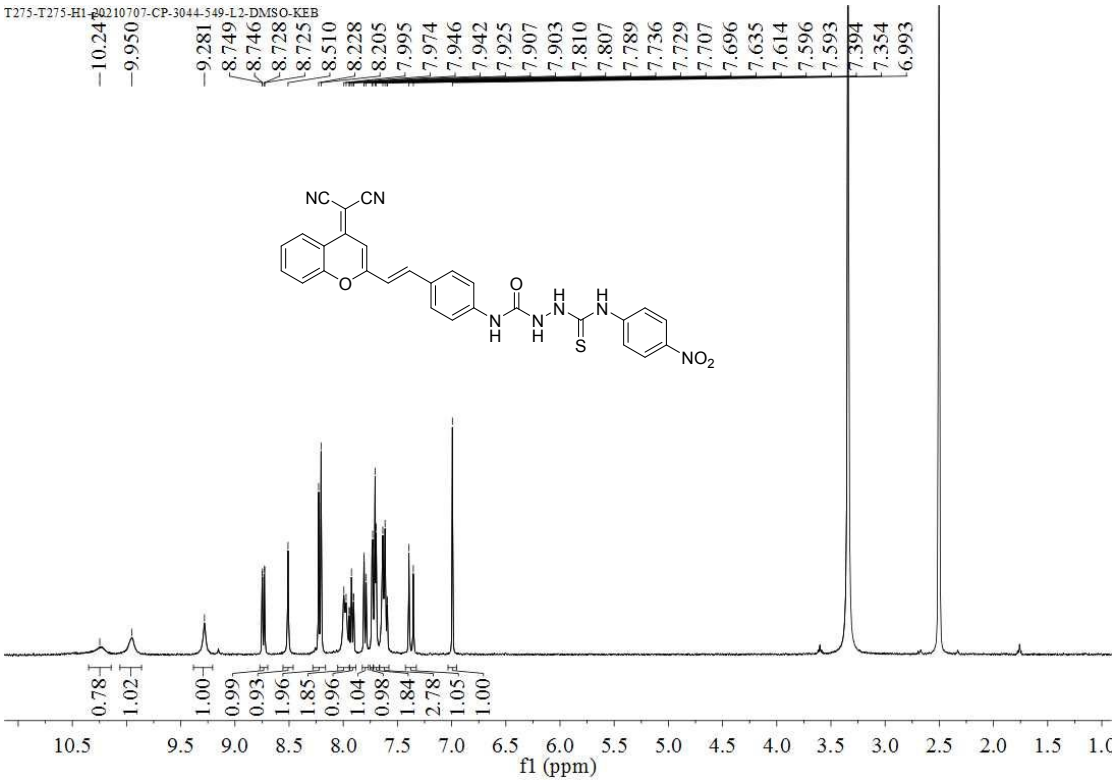


Figure S11. ^{1}H NMR spectrum of probe **DCM-NO** in DMSO-d_6.

(1) Mao, Z.; Jiang, H.; Li, Z.; Zhong, C.; Zhang, W.; Liu, Z. An N-nitrosation reactivity-based two-photon fluorescent probe for the specific in situ detection of nitric oxide. *Chem. Sci.* **2017**, *8*, 4533-4538.