Supplementary Information

A dual mode turn-on probe for H_2O_2 sensing via fluorescence and colorimetry in live cells and serum

Weitian Chen [a,b,1], Hui Dong [b,*,1], Siyu Wang [b], Yitong Jiang [b], Yujia Qian [b], Aixin Chang [b], Jiahui Ding [b], Xue Yang [b], Yanli Zhou [b], Yintang Zhang [a,b,c], Zhaohui Li [a], Maotian Xu [a,b]

[a] *College of Chemistry, Zhengzhou University, Zhengzhou 450001, China*

[b] *Henan Key Laboratory of Biomarker Detection and Diagnosis for Neurodegenerative Diseases, Henan Joint International Research Laboratory of Chemo/Biosensing and Early Diagnosis of Major Diseases, College of Chemistry and Chemical Engineering, Shangqiu Normal University, Shangqiu 476000, China*

[c] *School of Chemistry & Materials Engineering, Xinxiang University, Xinxiang 453000, China*

** Corresponding authors.*

E-mail addresses: huidong607607@126.com

[1] *Hui Dong and Weitian Chen contributed equally to this work.*

Table of contents

1. Instruments.

^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra were taken on a 400 MHz NMR spectrometer (Bruker, Karlsruhe, Germany) using $CDCl_3$ as solvent, and chemical shifts were reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS, δ scale with solvent resonances as internal standards). High-resolution mass spectra (HR-MS) were obtained on Bruker solanX 70 FT-MS; Agilent 6540 TOF. mass spectrometer with negative ion mode using methanol and water (v/v = 1:1) as solvent. High-performance liquid chromatography (HPLC) measurements were conducted on Agilent Technologies 1260 Infinity II (column: Agilent SB-C18, 5 μm, 4.6 × 150 mm). Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet iS 10 Fourier spectrophotometer (Thermo Electron, Waltham, MA, USA). Optical spectroscopy of absorption spectra and fluorescence spectra was carried out on a Lambda 565 UV-Vis spectrophotometer (PerkinElmer, USA) and LS55 fluorescence spectrophotometer (PerkinElmer, USA), respectively, in a 1 cm × 0.2 cm cuvette. Fluorescence imaging was performed on a Leica TCS SP8 STED super-resolution confocal system and a Leica TCS SP8 X white light laser scanning confocal microscope system. All pH measurements were performed using a pH-3C digital pH meter (Shanghai Lei Ci Device Works, Shanghai, China).

2. Samples Preparation.

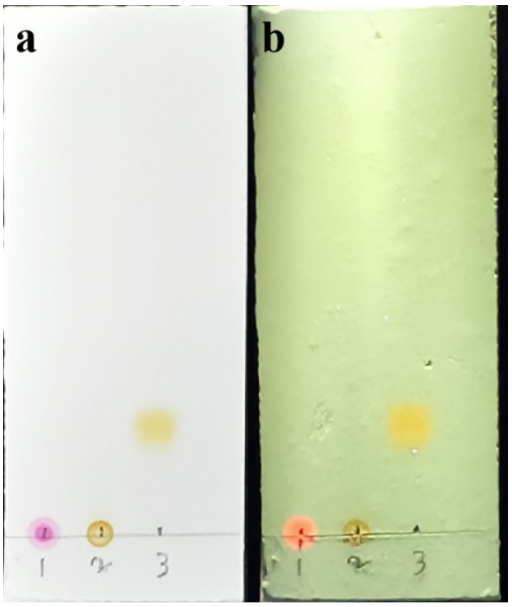
Preparation of human serum.

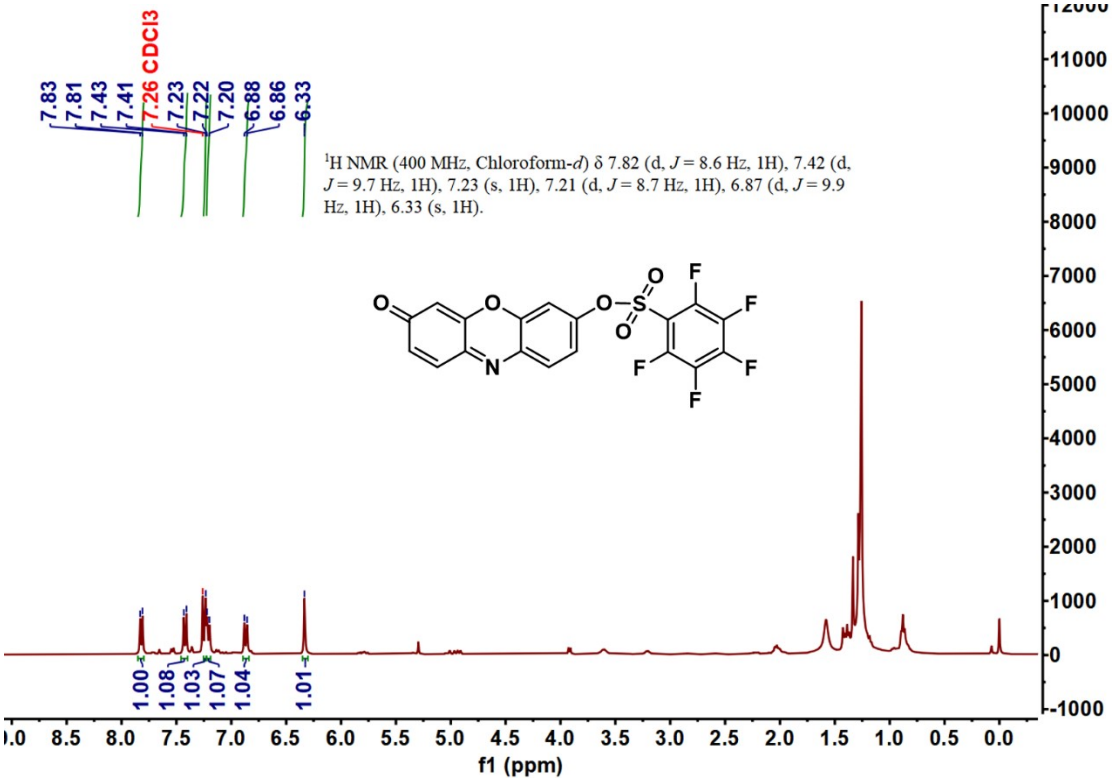
Human blood samples (obtained from Shangqiu First People's Hospital) were centrifuged at 7000g and 4 °C for 5 min. The serum was diluted 100-fold with PBS (0.1 M, pH 7.4) and added to 0.5 mL test tubes. Diluted serum samples spiked with different concentrations of H_2O_2 were mixed with 10 μM Re-FS and incubated at 37 °C for 60 min prior to spectral analysis. All procedures involving animals were conducted with the approval of the Animal Ethics Committee of Shangqiu Normal University, China.

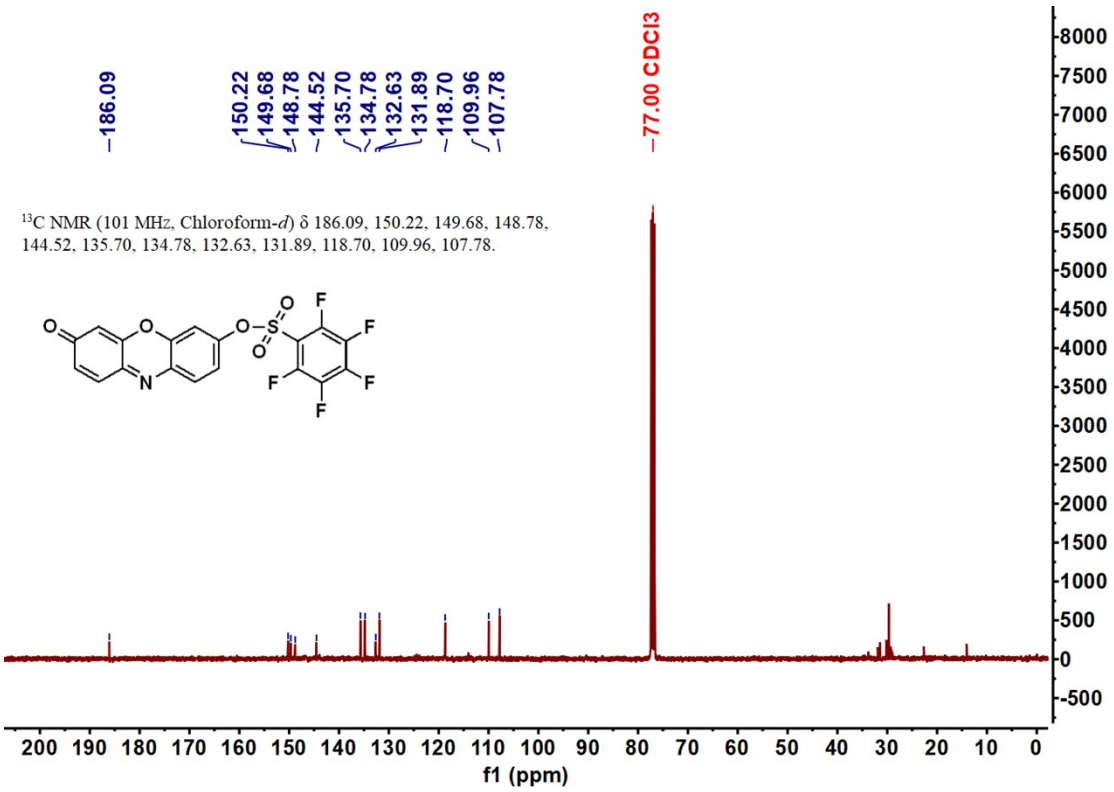
Preparation of cell.

HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotics (100.0 U/mL penicillin and 100.0 μg/mL streptomycin) at 37 °C under a 5% CO_2 atmosphere to maintain stable growth. The cells were then seeded into culture flasks containing 5 mL of medium and incubated at 37 °C with 5% CO_2. Once the cells reached approximately 80% confluence, subculture was performed. Finally, well-shaped cells were seeded into confocal dishes containing 1 mL of medium and cultured for 24 h for subsequent cell imaging experiments.

3. TLC and ^{1}H NMR, ^{13}C NMR, ^{19}F NMR of Re-FS.






186.09
150.22
149.68
148.78
144.52
135.70
134.78
132.63
131.89
118.70
109.96
107.78
77.00 CDCl3
13C NMR (101 MHz, Chloroform-d) δ 186.09, 150.22, 149.68, 148.78, 144.52, 135.70, 134.78, 132.63, 131.89, 118.70, 109.96, 107.78.
f1 (ppm)

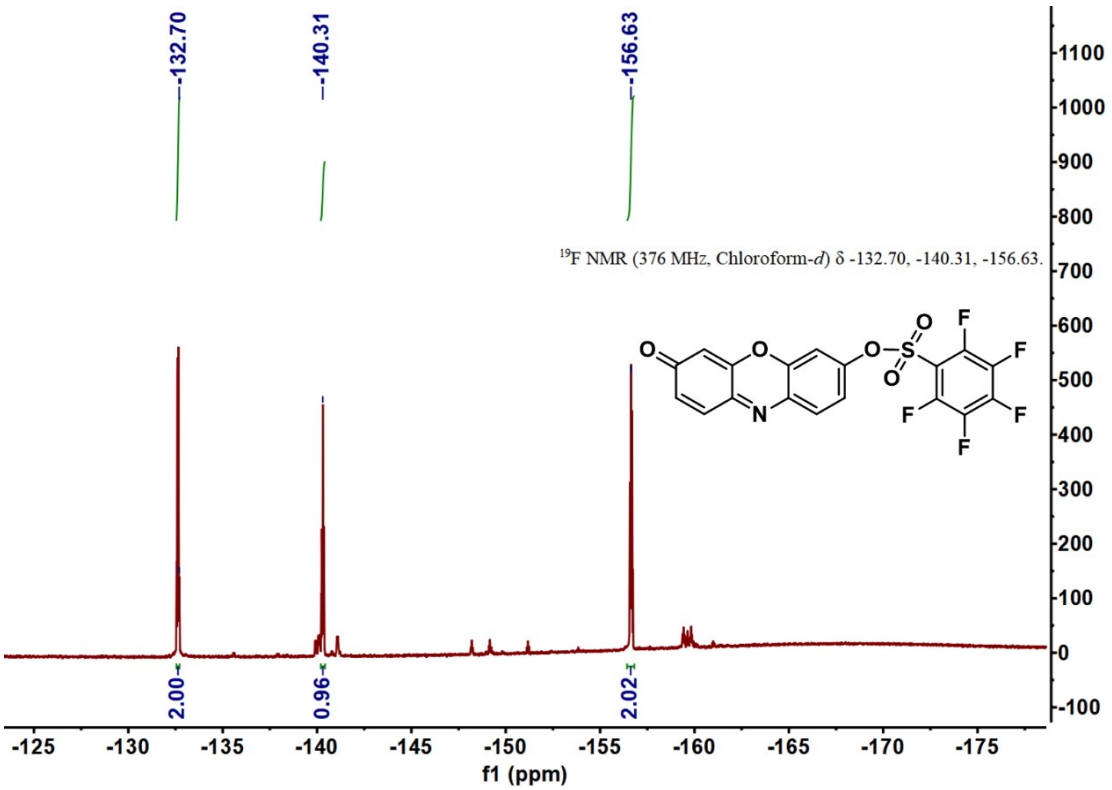


Fig. S1 (1) Re, (2) Pentafluorobenzenesulfonyl chloride, (3) Re-FS probe. Photographs of the TLC plate will be provided under two environments: the productnatural light (a) and 365 nm ultraviolet light (b) to clearly demonstrate the results under different observation modes. ^{1}H NMR, ^{13}C NMR and ^{19}F NMR.

4. Mass spectra of Re-FS.

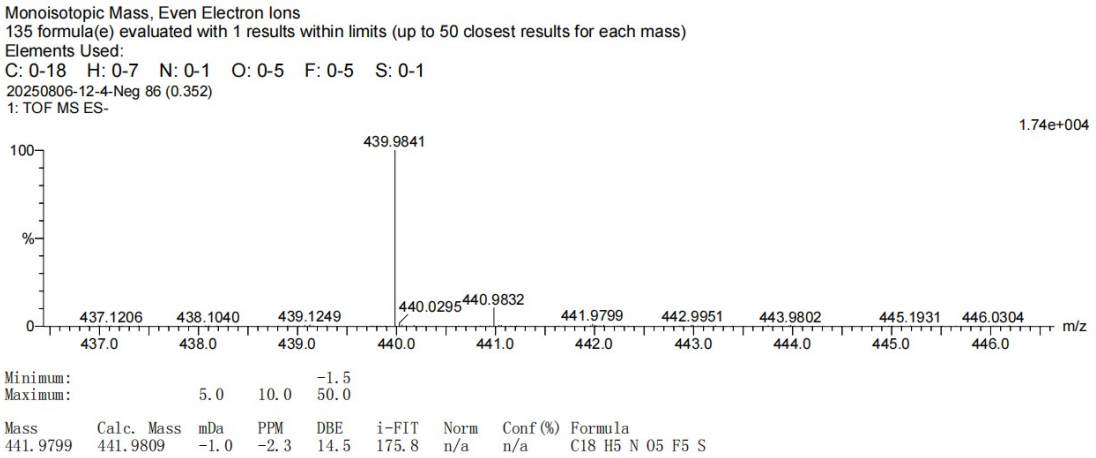


Fig. S2 HRMS (ESI-) m/z: $[M-2H]^{2-}$ calculated for $C_{18}H_5NO_5F_5S$: 440.9799, found: 440.9832.

5. Mass spectra of Re-FS with H_2O_2.

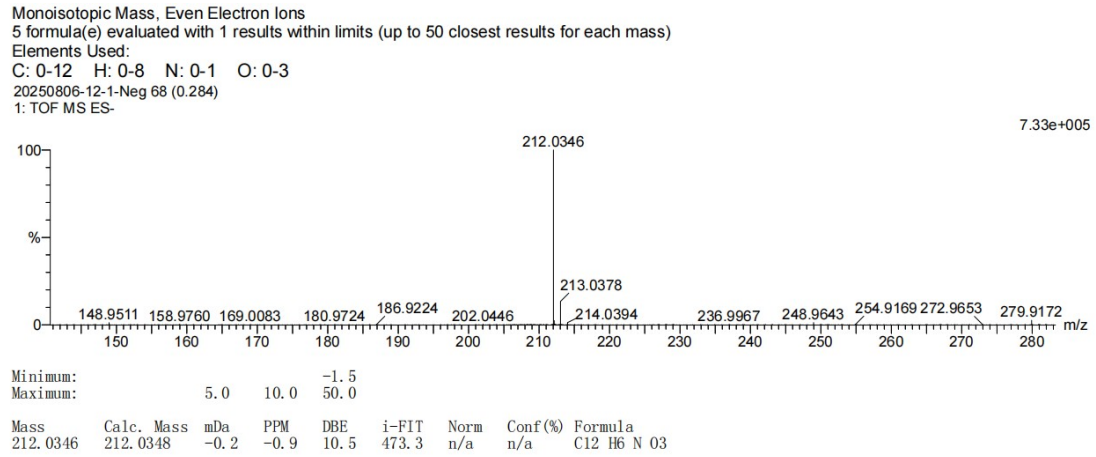


Fig. S3 HRMS (ESI-) m/z: $[M-H]^-$ calculated for $C_{12}H_6NO_3$: 212.0346, found: 212.0348.

6. HPLC.

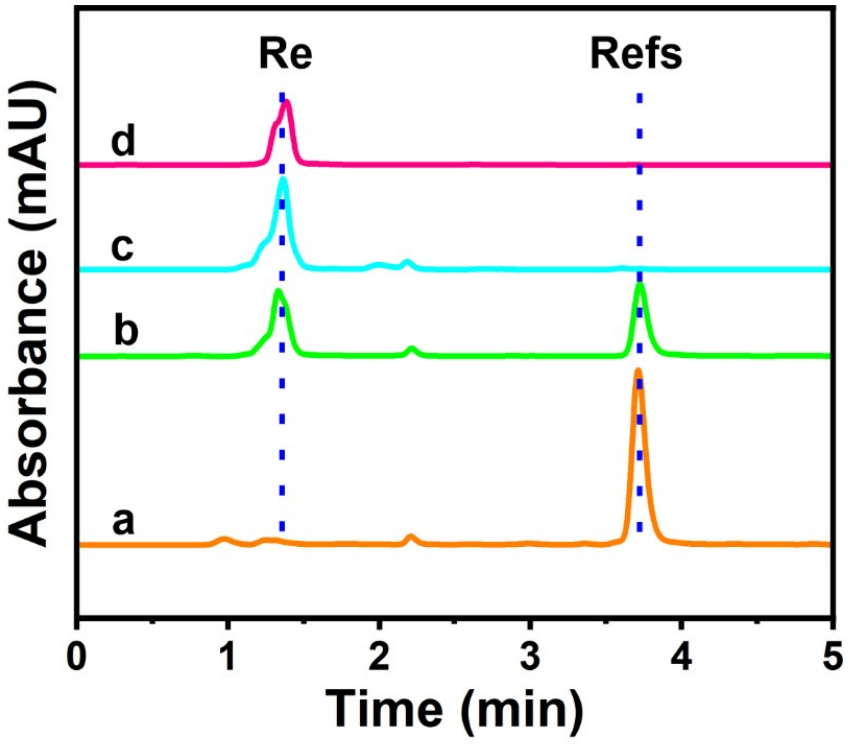


Fig. S4 HPLC analysis of (a) Re-FS, before the reaction; Re-FS reacted with 1 mM H_2O_2, (b) during the reaction, (c) react completely; (d) free resorufin (Re)

7. FTIR.

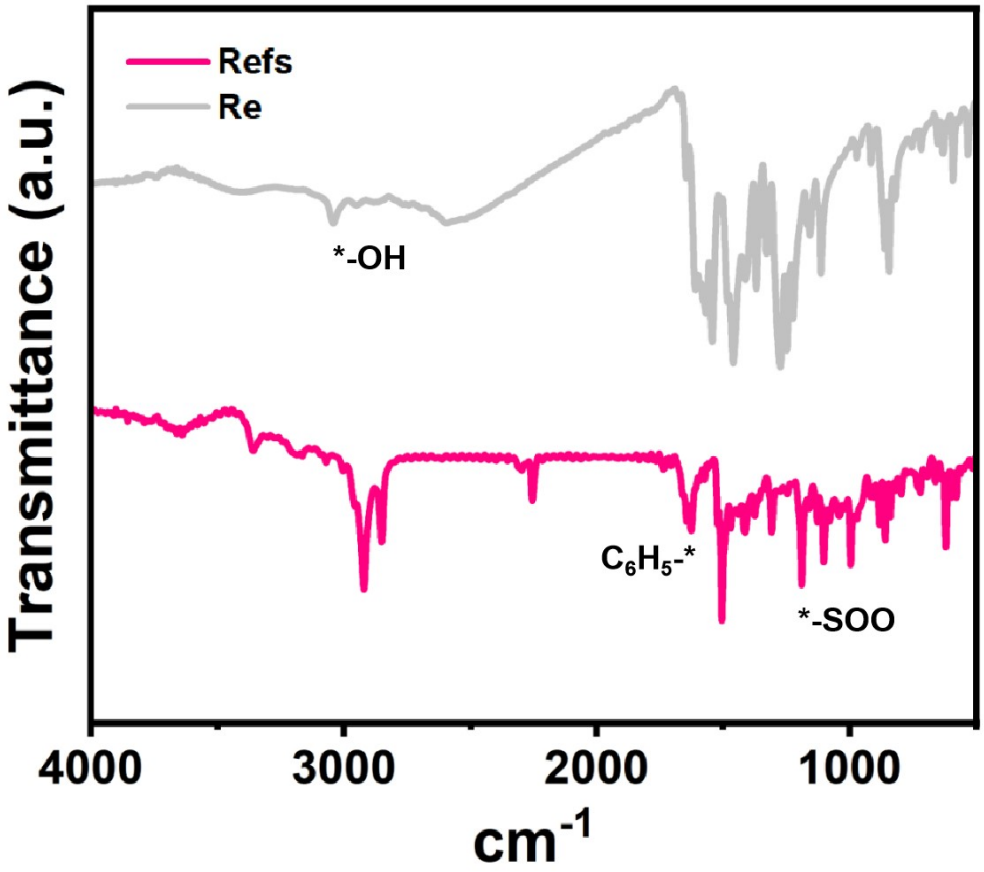


Fig. S5 FT-IR spectra of Re and Re-FS.

8. Reaction temperature.

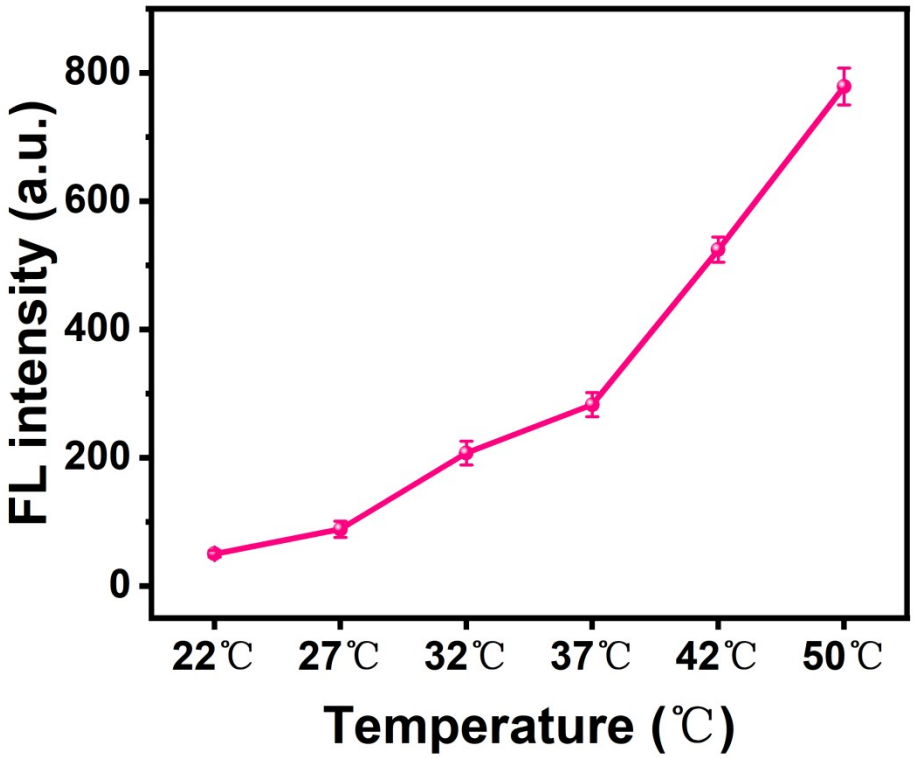


Fig. S6 Reaction temperature.

9. Selectivity and competition tests.

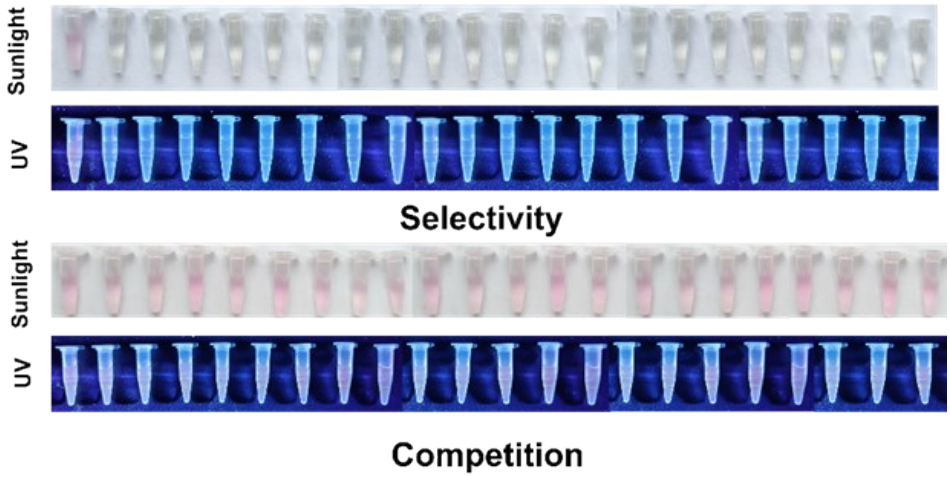


Fig. S7 Corresponding photographs in sunlight and 365 nm UV light obtained under sunlight and 365 nm UV light respectively.

10. Cell experiments

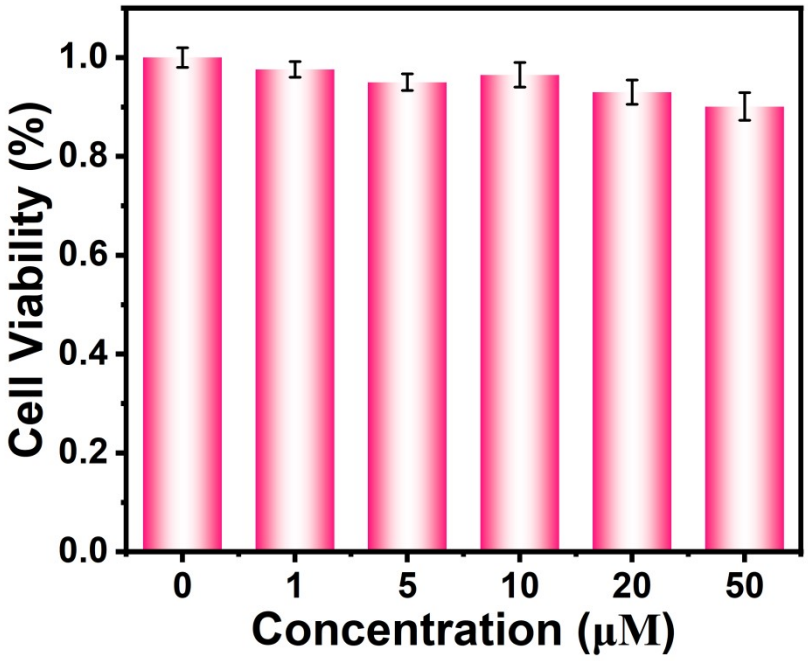


Fig. S8 CCK-8 assay for estimating cell viability (%)of HeLa cells were treated with various concentrations of Re-FS (0-50 μM). Data are shown as mean ±s.d.,n =3.

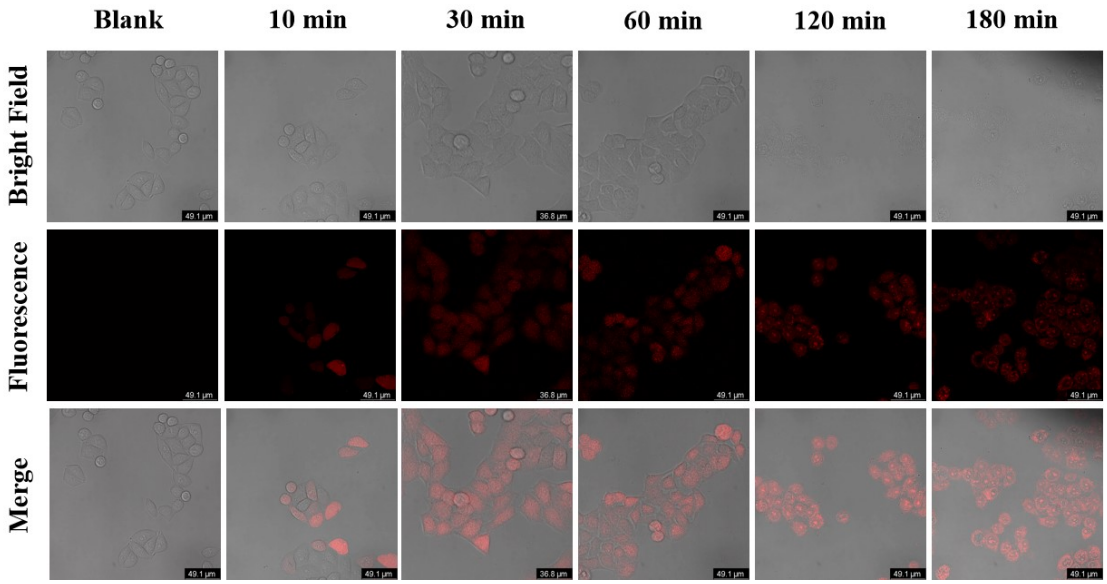


Fig. S9 Fluorescence images of HeLa cells treated with Re-FS (10 μM) at different time (10-180 min).

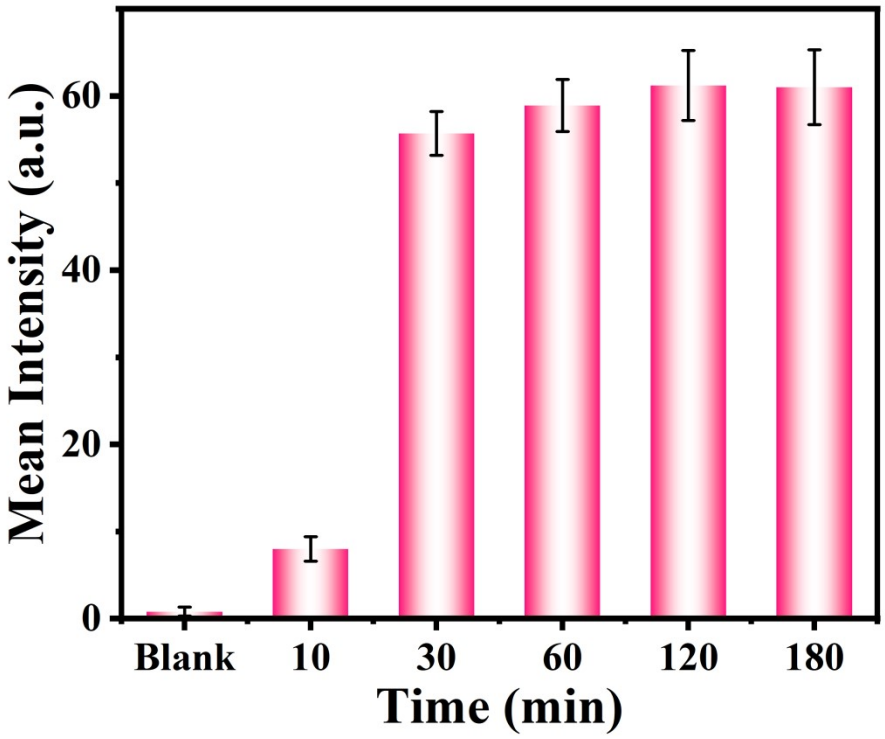


Fig. S10 Quantification of fluorescence intensity of images in Fig. S9

Table S1. Comparison of different H_2O_2 sensing systems.

Sensing methods	Materials/probe	Detection limit	Linear range	Reference
Electrochemistry	Fe@G-MWCNT	28.2 μM	0.1 – 7 mM	1
Electrochemistry	Hastelloy G35	13.5 mM	1 – 1000 mM	2
Electrochemistry	AgNp@GNR	20 μM	0.05 – 5 mM	3
Electrochemistry	Cat-HMFs/GCE	50 μM	0.1 – 10 mM	4
Electrochemistry	AuNPs-NH_2/Cu-	1.2 μM	5 – 850 μM	5
Electrochemistry	PtNP / rGO–CNT / PtNP / SPCE	4.3 μM	25 – 1000 μM	6
Electrochemistry	ZnO /ITO	14.8 μM	0.0625 – 5 mM	7
Electrochemistry	Cu-PAM	16.7 μM	0.05 – 25.31 mM	8
fluorescence	BOD-COOH	4.41 μM	25 – 125 μM	9
fluorescence	AuNPs/His-AuNCs	3.6 μM	5 – 135 μM	10
Fluorescence / Colorimetry	FeCo-CD	1.23 μM	FL: 200 – 600 μM UV-Vis:200 – 1500 μM	11
Fluorescence / Colorimetry	XH-2	10 μM	FL: 0 – 140 μM UV-Vis:0 – 140 μM	12
Fluorescence / Colorimetry	Re-FS	5 μM	0 – 1 mM	This work

Table S2. Fluorescence, colorimetric, and RGB method linear fitting.

Detection method	Linear fitting equations	Linear range	Detection limit
Fluorescence	y=17.25+468C, R^2=0.997	0 – 1 mM	5 μM
UV-Vis	y=0.293+0.44C, R^2=0.997	0 – 1 mM	10 μM
RGB	y=0.505+0.054C, R^2=0.991	0 – 1 mM	20 μM

Table S3. Detection of H_2O_2 in serum samples from normal human by Fluorescence (n=3)

Sample	Added (uM)	Fluorescence			HPLC		
		Detected (uM)	Recovery (%)	RSD (%)	Detected (uM)	Recovery (%)	RSD (%)
Serum	0	0			0.11		2.7
	10.00	9.81	98.1	2.2	10.18	100.7	2.8
	20.00	19.77	98.9	3.3	20.15	100.2	2.2
	30.00	30.52	101.7	3.0	31.15	103.5	2.2

Table S4. Detection of H_2O_2 in serum samples from normal human by UV-Vis (n=3)

Sample	Added (uM)	UV-Vis			HPLC		
		Detected (uM)	Recovery (%)	RSD (%)	Detected (uM)	Recovery (%)	RSD (%)
Serum	0	0			0.11		2.7
	10.00	9.86	98.6	2.9	10.18	100.7	2.8
	20.00	20.88	104.4	3.2	20.15	100.2	2.2
	30.00	28.92	96.4	2.7	31.15	103.5	2.2

Table S5. Detection of H_2O_2 in human serum by smartphone-based RGB extraction method (n=3)

Sample	Added	Smartphone sensing			HPLC		
	(uM)	Detected (uM)	Recovery (%)	RSD (%)	Detected (uM)	Recovery (%)	RSD (%)
Serum	0	0			0.11		2.7
	20.00	18.96	94.8	2.9	20.15	100.2	2.2
	30.00	28.33	94.4	3.2	31.15	103.5	2.2
	40.00	43.02	107.6	2.7	39.89	99.5	2.4

References

1 D. Soto, M. Alzate, J. Gallego and J. Orozco, *J. Electroanal. Chem.*, 2021, **880**, 114826.

2 A.a.M.A. Alboull, H. Liu, F. Abbas, A. Hussain, J. Jiang, B. Lou and G. Xu, *Langmuir*, 2025, **41**, 2065-2070.

3 V. Stanković, S. Đurđić, M. Ognjanović, J. Mutić, K. Kalcher and D.M. Stanković, *J. Electroanal. Chem.*, 2020, **876**, 114487.

4 M. Zhang, M. Chen, Y. Liu, Y. Wang and J. Tang, *Mater. Lett.*, 2019, **243**, 9-12.

5 W. Dang, Y. Sun, H. Jiao, L. Xu and M. Lin, *J. Electroanal. Chem.*, 2020, **856**, 113592.

6 S. Lee, Y.J. Lee, J.H. Kim and G.-J. Lee, *Chemosensors*, 2020, **8**, 63.

7 N. Ait Ahmed, K. Hebbache, A. Lahreche, S. Kerakra, K. Nasri, N. Aliouane, C. Chassigneux and M. Eyraud, *J Water Process Eng*, 2025, **78**, 108748.

8 L. Su, Y. Li, H. Chen, X. Liu, Z. Zhang, D. Bin, B. Yang, L. Sun, H. Lu and B. Liu, *Talanta*, 2025, **287**, 127689.

9 Y. Wang, Y. Liu, B. Liu, Y. Yuan, L. Wei, M. Wang and Z. Chen, *Molecules*, 2024, **29**, 229.

10 H. Pezhhan, M. Akhond and M. Shamsipur, *J. Lumin.*, 2020, **228**, 117604.

11 S. Wu, Z. Yu, J. Zhao, Y. Wang, J. Nan, L. Xu, X. Li, L. Yang and S. Dong, *Anal. Chem.*, 2025, 97, 15320-15328.

12 L. Xu, Y. Zhang, L. Zhao, H. Han, S. Zhang, Y. Huang, X. Wang, D. Song, P. Ma, P. Ren and Y. Sun, *Talanta*, 2021, 233, 122578.