Supporting Information

Amino-Functionalized Lanthanide-Organic Framework for Ratiometric Detection of $ONOO^-$

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# H. Z. and X. L. contributed equally to this work.

Experimental Section

1. Materials and methods

Except for special statements, all regents were purchased from commercial sources without further purification. 2'-Amino-[1,1':4',1"-terphenyl]-3,3",5,5"-tetracarboxylic acid (H_4TPTC-NH_2) was obtained from Nanchang Chouhepharm Co., Ltd. $Eu(NO_3)_3 \cdot 6H_2O$ and $Gd(NO_3)_3 \cdot 6H_2O$ were purchased from Shanghai Aladdin Biochemical Technology Co,. Ltd. Ammonium iron (II) sulfate, Urea, Glucose ,NaBr, Na_2CO_3, KCl, $MgCl_2$, $NaHCO_3$, $CaCl_2$, NaOH, Glucose, L-cysteine, NH_4Cl, L-serine, Na_2SO_4, Glutathione, L-proline, and tert-butyl hydroperoxide were purchased from Beijing HWRK Chem Co., Ltd. H_2O_2, H_2SO_4, $NaNO_2$, L-Threonine, Glycine, Fructose, MnO_2, NaClO, and 3,3'-(naphthalene-1,4-diyl) dipropionic acid were purchased from Anhui Senrise Technology Co., Ltd. N, N-dimethylformamid and ethanol were obtained from Guangshunda Chemical Reagent Co., Ltd (Tianjin, China).

^{1}H NMR spectra were recorded on a Bruker AVANCE400 instrument at 400Hz, 25℃. ^{1}H NMR sample was prepared as follow: **EuTPTC-NH_2** (6.0 mg) was suspended in 3.0 mL of $ONOO^-$ solution (11.2 mM) and incubated in the dark for 6 h. The obtained precipitate was filtered, washed with water and dried under vacuum, then monitored with DMSO-d_6 and DCl (5/1, v/v) as the solvent. Liquid chromatograph-mass spectrometer (LC-MS) was recorded on a Bruker Scientific instrument with a negative ionization mode. LC-MS sample was prepared in a similar way to the ^{1}H NMR sample as expected for the use of acid digestion with hydrochloric acid. Powder X-ray diffraction (PXRD) patterns were carried on an X-ray powder diffractometer (Bruker D8 Focus) complying Cu Kα radiation (λ = 1.5418Å), operating at 40 kV and 40 mA. Fourier transform infrared spectrum (FT-IR) was recorded on a Thermo Nicolet 5700 FTIR instrument from 4000 to 400 cm^{-1}. X-ray photoelectron spectroscopy (XPS) was obtained from a Shimadzu/Krayos AXIS Ultra DLD spectrometer. The photoluminescence measurements (excitation and emission spectra) were tested by an Edinburgh Instruments FS1000 near-infrared spectrometer, with a 450 W Xenon lamp as the steady-state excitation source, a double excitation monochromator (1800 lines·mm^{-1}), an emission monochromator (600 lines·mm^{-1}), a semiconductor cooled

Hamamatsu RMP928 photomultiplier tube. UV-Vis spectra were recorded in a quartz cell (light path 10 mm) on an Agilent Carry 100 UV spectrometer.

2. Synthesis

Synthesis of $ONOO^-$: According to the reported literature,[1] the mixed solution of $NaNO_2$ (0.6 M, 5 mL) and H_2O_2 (0.7 M, 0.3mL) was reacted with sulfuric acid (0.3 M, 80 μL). Next, NaOH (1.5 M, 10 mL) was added quickly (about 2 s) to make the mixture alkaline. The excess hydrogen peroxide was removed by passing the solution through a short column of manganese dioxide. The concentration of $ONOO^-$ solution was calibrated by monitoring the absorbance at 302 nm (ε = 1670 M^{-1} cm^{-1}) in NaOH (0.1 M, 3 mL). The resulting solution was split into small aliquots and stored at lower than -18°C. The aliquots were thawed immediately before use, and the concentration of $ONOO^-$ was determined by measuring the absorption of the solution at 302 nm.

Synthesis of ·OH: According to the reported literature,[2] it was made from a mixture of H_2O_2 and 10 equiv. $(NH_4)_2Fe(SO_4)_2$.

Synthesis of $^1O_2$: According to the reported literature,[3] singlet oxygen was generated from 3,3'-(naphthalene-1,4-diyl)dipropionic acid (10 mM).

Synthesis of **EuTPTC-NH_2**: According to the reported literature,[4] 2'-amino-[1,1':4',1"-terphenyl]-3,3",5,5"-tetracarboxylic acid (21.0 mg, 0.05 mmol) and $Eu(NO_3)_3·6H_2O$ (75.0 mg, 0.168 mmol) were dissolved in the mixture of 2.5 mL DMF, 1.5 mL deionized water and 25 μL H_2SO_4 in a Teflon vessel in a stainless steel autoclave, then placed in an oven at 90°C for 3 d. It was cooled to room temperature, yellow clustered crystals can be separated out. The crystals were collected and washed with deionized water three times and dried under vacuum at 50°C overnight. The **GdTPTC-NH_2** was synthesized similarly to **EuTPTC-NH_2** expect for the use of $Gd(NO_3)_3·6H_2O$. The **EuTPTC** was synthesized similarly to **EuTPTC-NH_2** expect for the use of [1,1':4',1"-terphenyl]-3,3",5,5"-tetracarboxylic acid.

3. Determination of $ONOO^-$

The emission spectra of **EuTPTC-NH_2** saline suspension (0.02 mg/mL) were measured after adding different concentration of $ONOO^-$ and waiting 50 s (λ_{ex} = 313 nm). This procedure was repeated three times with three different **EuTPTC-NH_2**.

4. Selectivity and anti-interference experiments

The selective experiment was as follow: the emission spectra of **EuTPTC-NH_2** saline suspension (0.02 mg/mL) after adding $ONOO^-$ (112 μM) or other substances were recorded, including biologically relevant RNS, peroxide, and reactive oxygen species (ClO^-, H_2O_2, NO_2^-, $^1O_2$, ·OH, and tert-butyl hydroperoxide). The anti-interference experiment was as follow: the emission spectra of **EuTPTC-NH_2** saline suspension (0.02 mg/mL) simultaneously upon addition of $ONOO^-$ (112 μM) and serum components were recorded, including urea, glucose, L-serine, L-threonine, glycine, NH_4Cl, NaCl, $MgCl_2$, $CaCl_2$, KCl, $MgSO_4$, Na_2SO_4, Na_2HCO_3, NaH_2CO_3 and Na_2HPO_4.

5. Establishment of acetaminophen (APAP)-induced acute liver injury (ALI) model

Female C57/BL6 mice (18-20 g) were purchased from SiPeiFu Biotechnology Co., Ltd. (Beijing, China). The mouse liver injury model was established by intraperitoneal injection of APAP. Briefly, APAP was dissolved in warm saline at a concentration of 20 mg/mL. Mice were fasted overnight and then injected intraperitoneally with APAP at a dose of 300 mg/kg. Mice injected with saline were used as the control group. After 8 h, the mice were anaesthetised, blood was collected and serum was separated for photoluminescence testing. The remaining serum was used for biochemical tests to assess the liver function of the mice. Liver tissues were harvested for pathological evaluation by hematoxylin-eosin staining. All the protocols were approved by the Animal Care and Use Committee of the Animal Ethical and Welfare Committee of Tianjin Medical University Cancer Institute & Hospital (Approval No. 2024051).

6. Clinical Samples

Healthy human serum was separated from blood left over from a medical examination of a healthy donor from Cancer Prevention Center of Tianjin Medical University Cancer Institute & Hospital. Liver injury serum samples were obtained from the remainder of routine laboratory tests on clinical patients diagnosed with drug-induced liver injury (DILI) from Tianjin Third Central Hospital. All the protocols were approved by the Ethics Committee of Tianjin Medical University Cancer Institute & Hospital (Approval No. bc20241742), and the informed consents were obtained.

7. Detection of $ONOO^-$ in biological environments

A **EuTPTC-NH_2** suspension (0.02 mg/mL) was prepared by replacing saline with mice serum and human serum, and the emission spectra were recorded with the addition of different concentration of $ONOO^-$ (λ_{ex} = 313 nm). This procedure was repeated three times with three different **EuTPTC-NH_2**. The measurements were plugged into the equation of the standard curve to get the apparent concentration values which were obtained the $ONOO^-$ concentration data in the original serum specimens. After that, different concentrations of $ONOO^-$ were added into the serum samples to further assess the reliability of **EuTPTC-NH_2**. The recoveries of $ONOO^-$ in mice and human serum samples were calculated.

8. Supplementary Figures and Tables

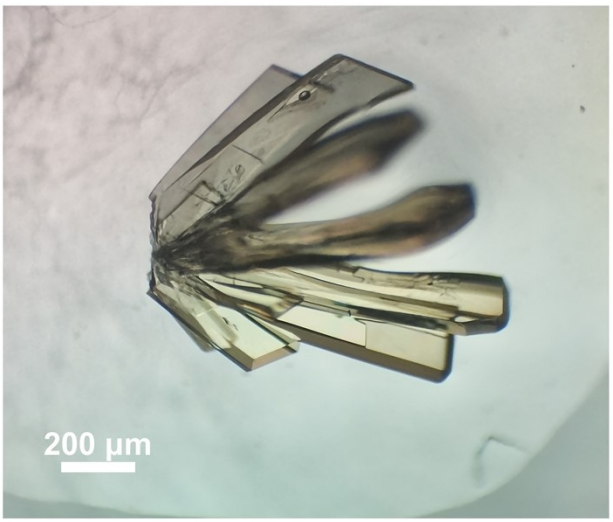


Fig. S1. Optical microscope image of the **EuTPTC-NH_2** crystal.

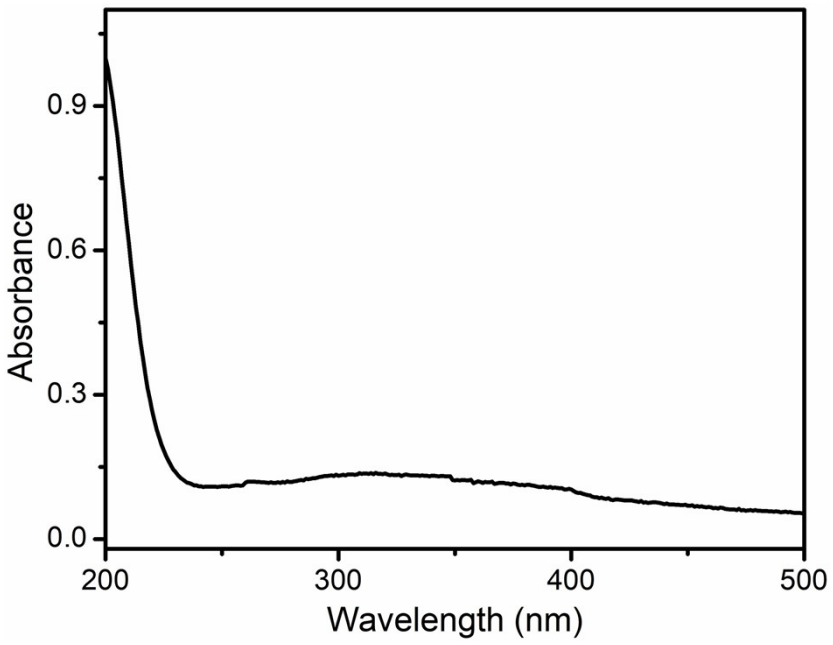


Fig. S2. UV–Vis absorption spectrum of **EuTPTC-NH_2** suspension.

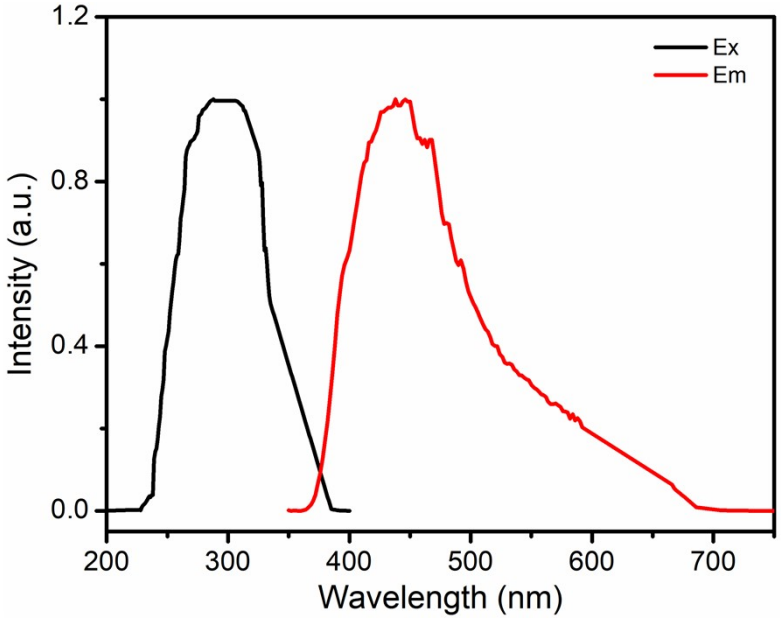


Fig. S3. Normalized excitation (λ_{mon} = 410 nm) (left) and emission (λ_{ex} = 313 nm) (right) spectra of H_4TPTC-NH_2.

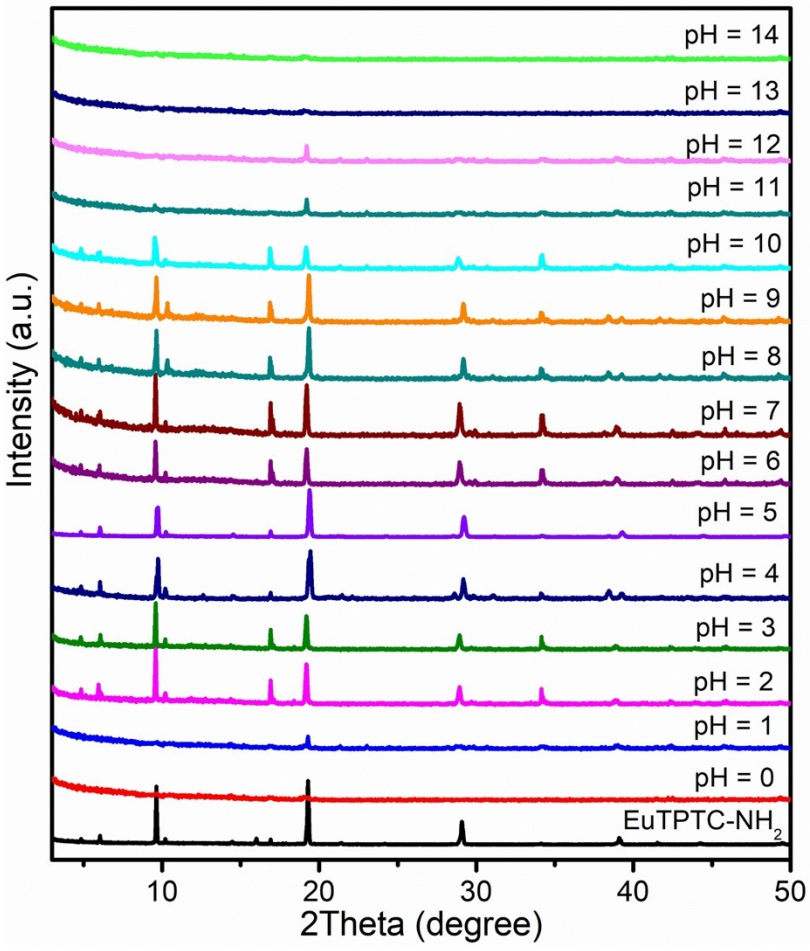


Fig. S4. PXRD patterns of **EuTPTC-NH_2** in acidic and alkaline aqueous solution (pH

0-14) for 6 h.

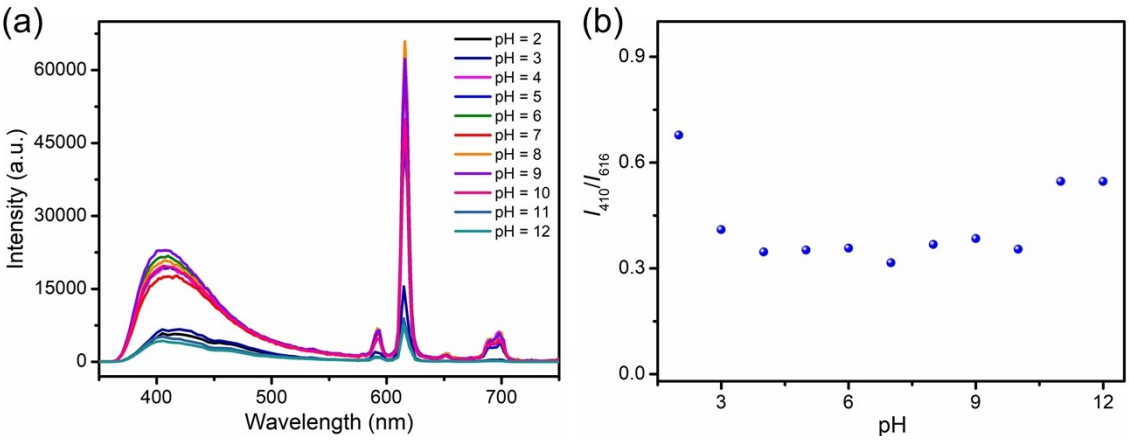


Fig. S5. (a) Emission spectra of **EuTPTC-NH$_2$** after immersion in acidic and alkaline aqueous solution (pH 2-12) for 6 h. (λ_{ex} = 313 nm). (b) Emission intensity ratio (I_{410}/I_{616}) of **EuTPTC-NH$_2$** at different pH in the range of 2-12.

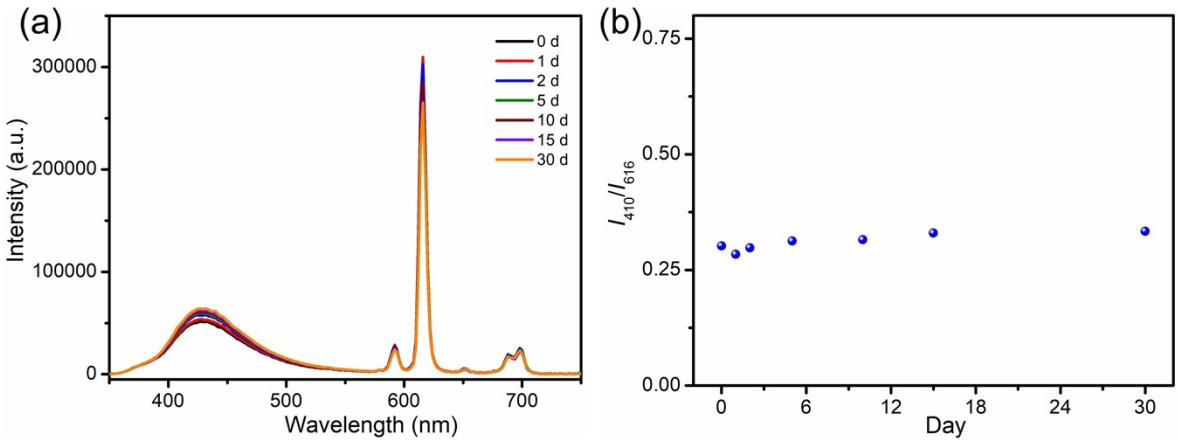


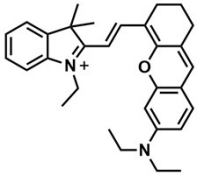
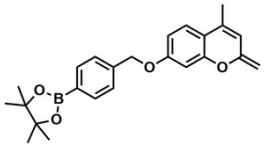
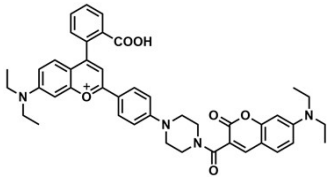
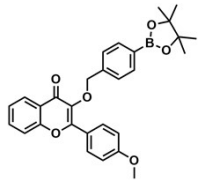
Fig. S6. (a) Emission spectra of **EuTPTC-NH$_2$** suspension in saline for different periods (λ_{ex} = 313 nm). (b) Emission intensity ratio (I_{410}/I_{616}) of **EuTPTC-NH$_2$** at different periods.

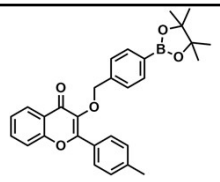
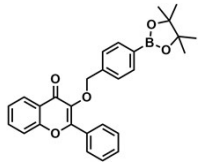
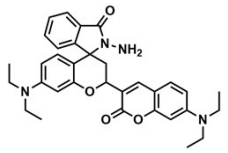
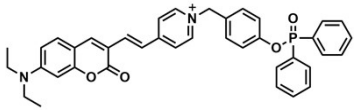
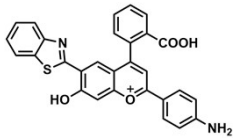
Table S1. The Lab values (L1*, a1*, b1*, L2*, a2*, and b2*) of ratiometric probes.

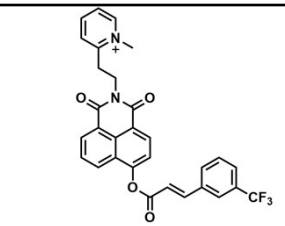
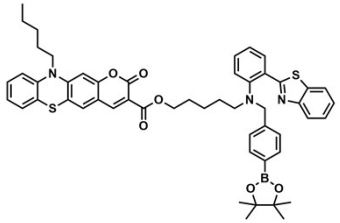
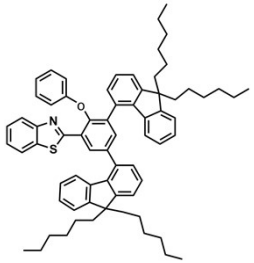
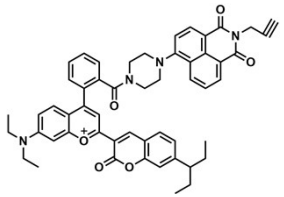
Name	L1*	a1*	b1*	L2*	a2*	b2*
4-MB	18	20	-50	94	-31	-8

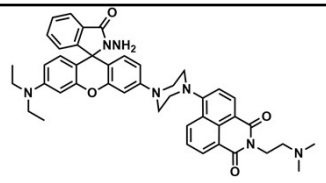
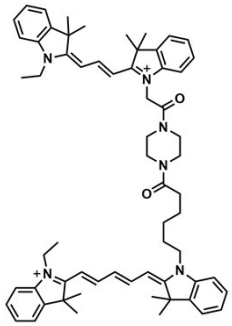
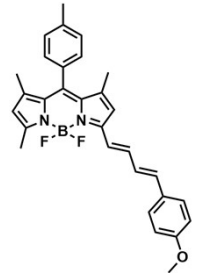
MITO-CC	37	46	-24	38	-17	-23
CS-ONOO	35	-23	25	15	32	18
CPC	21	-11	12	16	29	18
AHC	33	36	-84	67	58	-6
Mito-NA	11	10	-36	41	-3	33
PTZ-H	33	38	34	47	39	-39
HBT-FI-BnB	47	39	-37	85	3	73
NpRh-ONOO	53	-14	-5	68	-2	5
F_{482}	55	77	66	48	-20	28
ABAH-LW	9	20	-39	60	-21	18
RTFP	54	-13	-6	70	-3	5
3a	49	22	14	58	-25	-28
CHCN	36	23	13	41	-10	-6
MG-ONOO	23	2	-37	29	36	-13
Mito-CM-CD	23	36	-36	38	6	-44
NX	2	12	-32	37	23	6
GYP	84	1	27	40	64	42
CSU-FT	22	24	-36	81	6	30
NTC	20	31	-21	45	-35	4
EuTPTC-NH_2	46	70	5	21	54	-89

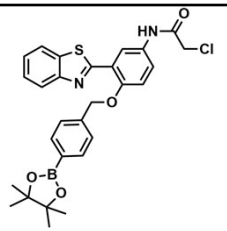
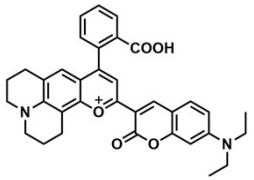
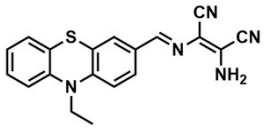
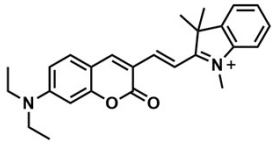
Table S2. Comparison of ratiometric probes in terms of structure/ligand, linear range, LOD and ΔE_{ab}^{*}.

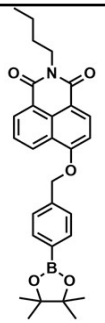
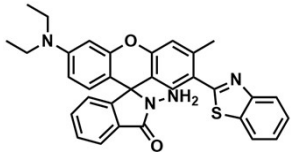
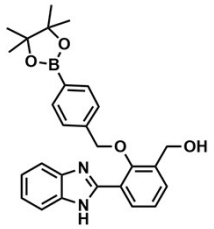
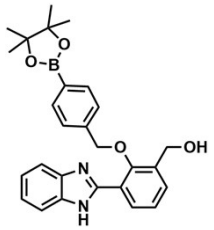
Structure/Ligand	Categorization	Name	Range (μM)	LODs (μM)	ΔE_{ab}^{*}	Ref.
	Small molecule luminescent probe	Cy-NEt_2	0.5-1.5	0.17	-	[5]
		4-MB	0-10	0.0298	101	[6]
		MITO-CC	0-7.5	0.0113	63	[7]
		3-HF-OMe	0-2.5	0.0655	-	[8]

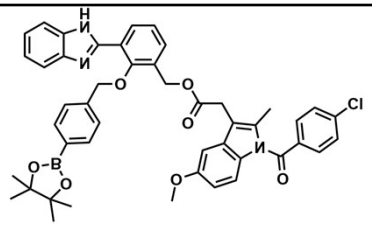
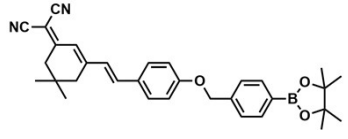
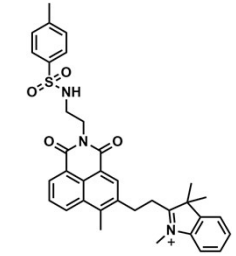
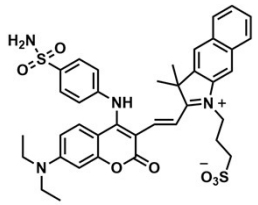
	3-HF- PhMe	0-5	0.021	-	
	3-HF-Ph	0-5	0.2556	-	
	CS-ONOO	0-50	0.059	59	[9]
	CPC	0-18	0.016	41	[10]
	AHC	5-6	0.0018	88	[11]

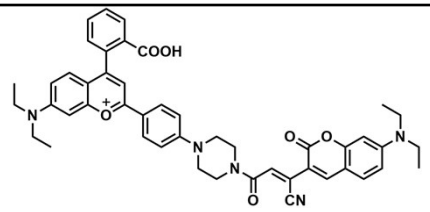
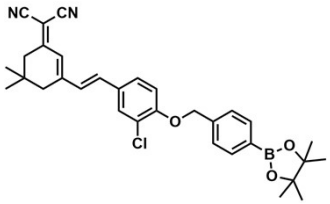
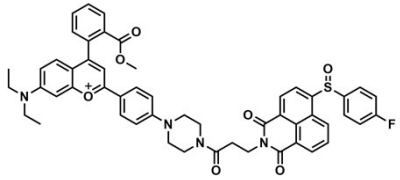
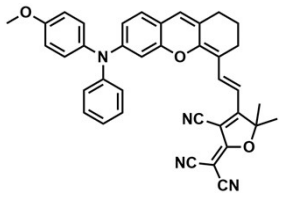
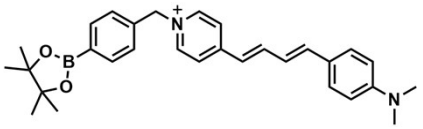
	Mito-NA	0-30	0.12	104	[12]
	PTZ-H	0-30	0.021	74	[13]
	HBT-FI-BnB	0-25	2.1	12	[14]
	MULTI-ONOO	0-20	0.0116	-	[15]

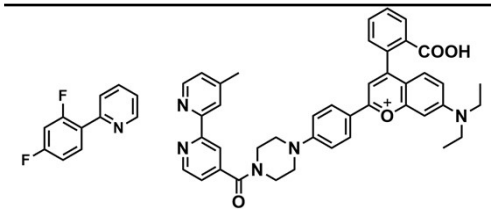
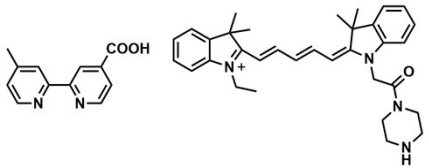
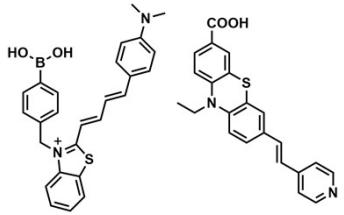
	NpRh-ONOO	0-1	0.00333	22	[16]
	PNCy3Cy5	0-0.7	0.00065	-	[17]
	F_{482}	0-20	0.15054	104	[18]

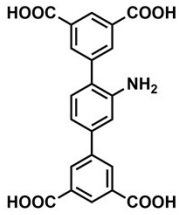
	ABAH-LW	0-10	0.0214	69	[19]
	RTFP	0-7	0.0041	22	[20]
	3a	0-2	0.0194	50	[21]
	CHCN	0-25	0.0497	38	[22]

	RTP-PN	0-1.4	0.0014	-	[23]
	RH-PN	0-18	0.093	-	[24]
	MO-E1	0-3.5	0.8	-	[25]
	MO-E2	0-2.5	6.53	-	

	MO-E3	0-8	0.28	-	
	JQ-2	5-9	5.3	-	[26]
	WND-1	0-50	0.93	-	[27]
	MG-ONOO	0-2	0.013	61	[28]

	Mito-CM-CD	2-7	0.0429	34	[29]
	K-ONOO	0-15	0.212	-	[30]
	CD-NA	0-200	0.015	-	[31]
	NX	15-40	0.082	53	[32]
	GYP	0-15	0.27	78	[33]

	Ir^{3+}-complex	Ir-CBM	0-40	0.093	-	[34]
	Ru^{3+}-complex	Ru-Cy5	0-30	0.28	-	[35]
	Polymer probe	PB-PVA	0-6	0.3	-	[36]
-	Multicolor fluorescent nanoprobe	CSU-FT	0-1	0.0117	90	[37]
-	Near-infrared ratiometric fluorescent	NTC	0-1 1-30	0.0153	75	[38]

	nanoprobe					
-	Nanoprobe	PA	0-10	0.1	-	[39]
-	Lanthanide-doped Upconversion nanoprobes	UCNPs@PEI@E-CC	0-90	0.154	-	[40]
		UCNPs@PEI@H-CC	0-100	0.241	-	
	Lanthanide metal-organic frameworks	**EuTPTC-NH_2** (in saline)	0-11	0.0053	100	This work
		EuTPTC-NH_2 (in mice serum)	0-3	0.0173	-	
		EuTPTC-NH_2 (in human serum)	0-2	0.0291	-	

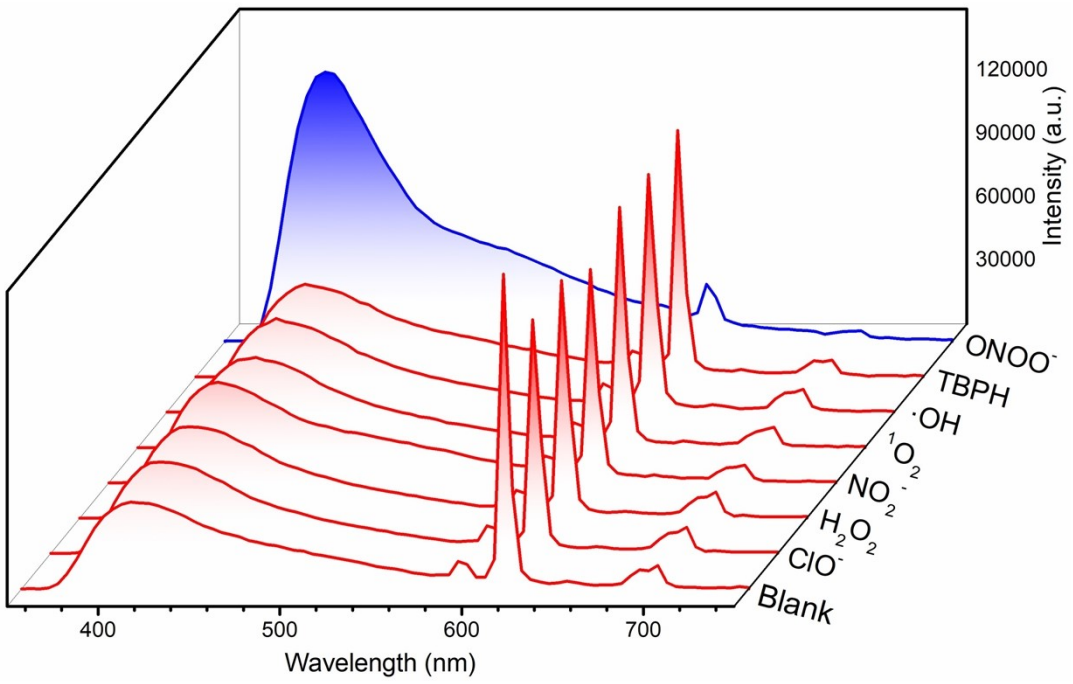


Fig. S7. Emission spectra of **EuTPTC-NH_2** suspension (0.02 mg/mL) in the presence of different interferents (λ_{ex} = 313 nm).

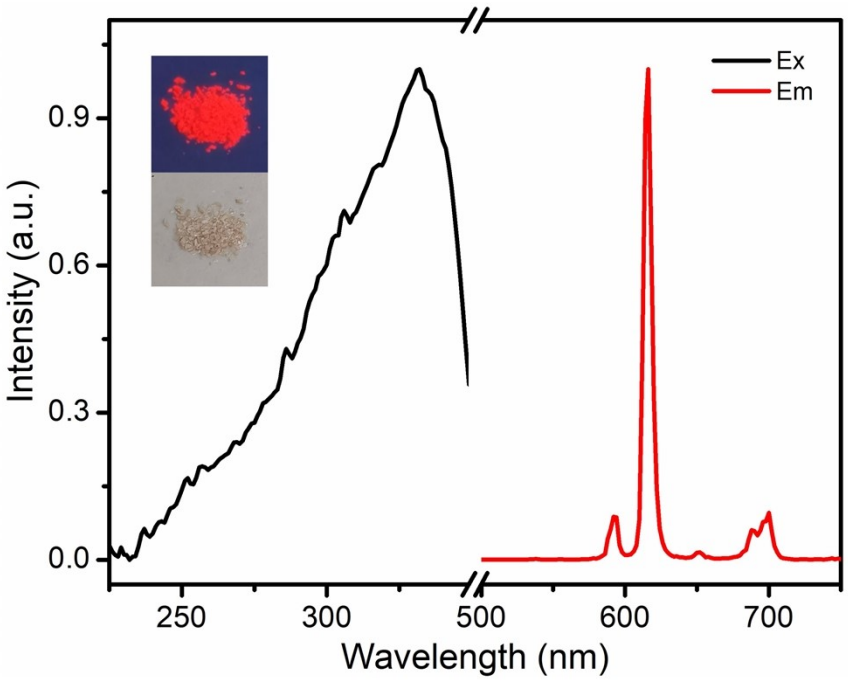


Fig. S8. Normalized excitation (λ_{mon} = 616 nm) (left) and emission (λ_{ex} = 313 nm) (right) spectra of **EuTPTC**, inset: the corresponding photographic images under 365 nm UV light (upper) and daylight (lower).

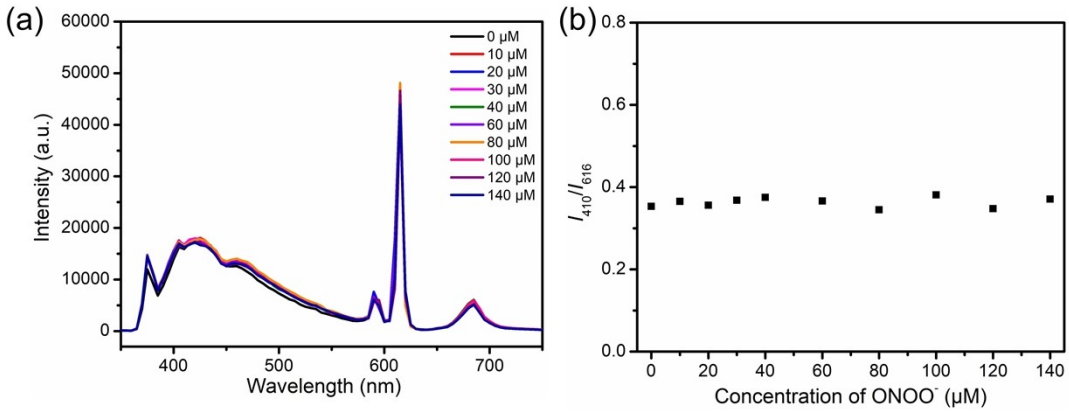


Fig. S9. (a) Emission spectra of **EuTPTC** suspension (0.02 mg/mL) in saline upon addition of $ONOO^-$ (0-140 μM) (λ_{ex} = 313 nm). (b) Changes in the emission intensity ratio (I_{410}/I_{616}) of **EuTPTC** upon addition of $ONOO^-$ in saline (λ_{ex} = 313 nm).

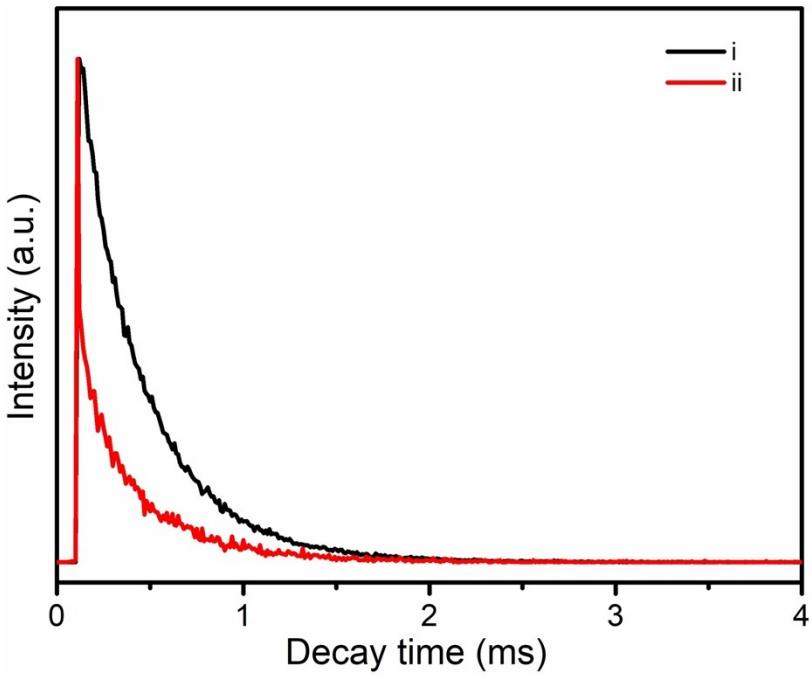


Fig. S10. Emission decay profiles of **EuTPTC-NH_2** suspension before (i) and after (ii) adding 112 μM $ONOO^-$ (excited at 313 nm and monitored at 616 nm).

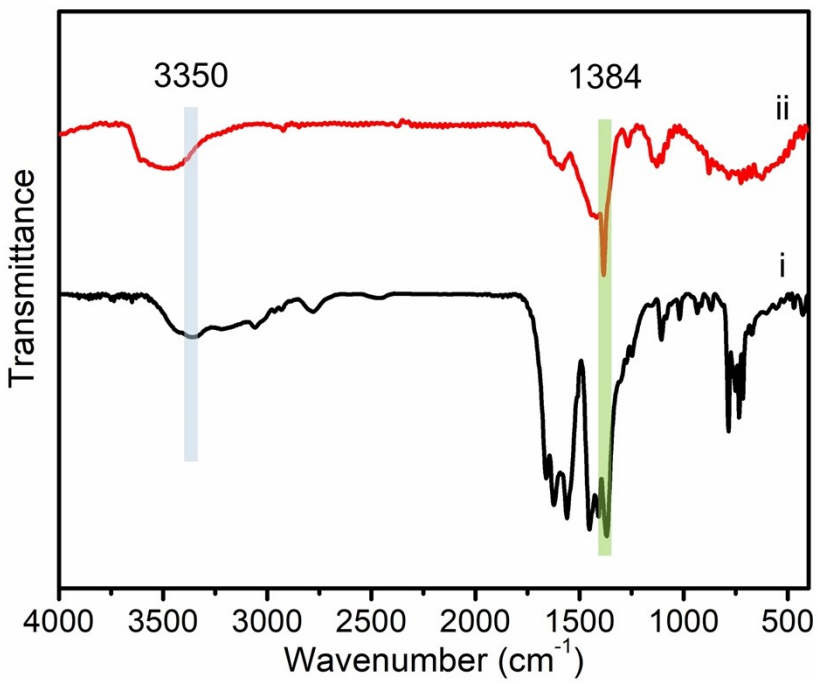


Fig. S11. FT-IR spectra of **EuTPTC-NH$_2$** before (i) and after (ii) adding $ONOO^-$.

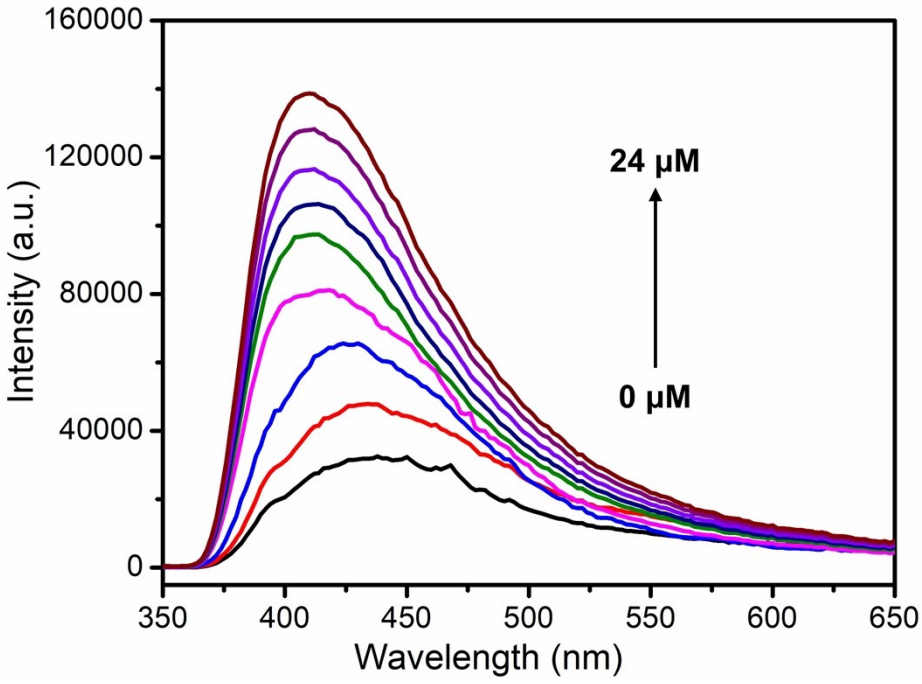


Fig. S12. Emission spectra of H_4TPTC-NH_2 suspension in saline upon addition of $ONOO^-$ (0-24 μM) (λ_{ex} = 313 nm).

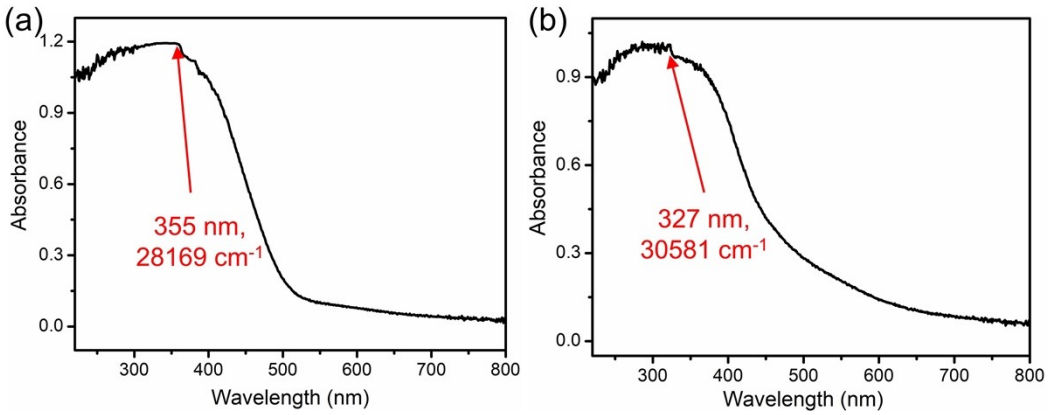


Fig. S13. Solid-state UV-Vis absorption spectra of H_4TPTC-NH_2 before (a) and after (b) adding $ONOO^-$.

According to Reinhoudt's empirical rule,[41] intersystem crossing process (ISC) is effective when the energy gap between S_1 and T_1 of the ligand is greater than 5000 cm^{-1}.

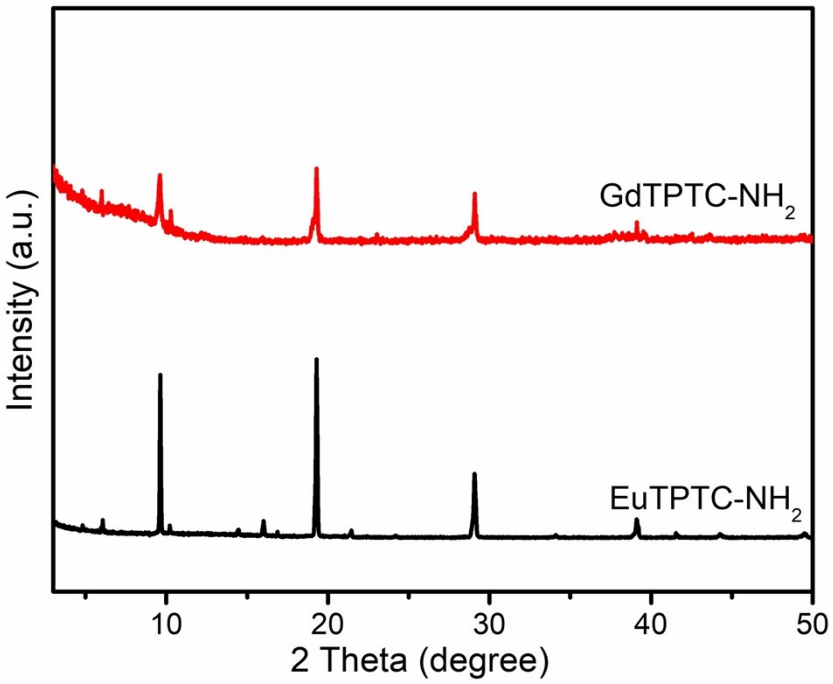


Fig. S14. PXRD patterns of **GdTPTC-NH_2** and **EuTPTC-NH_2**.

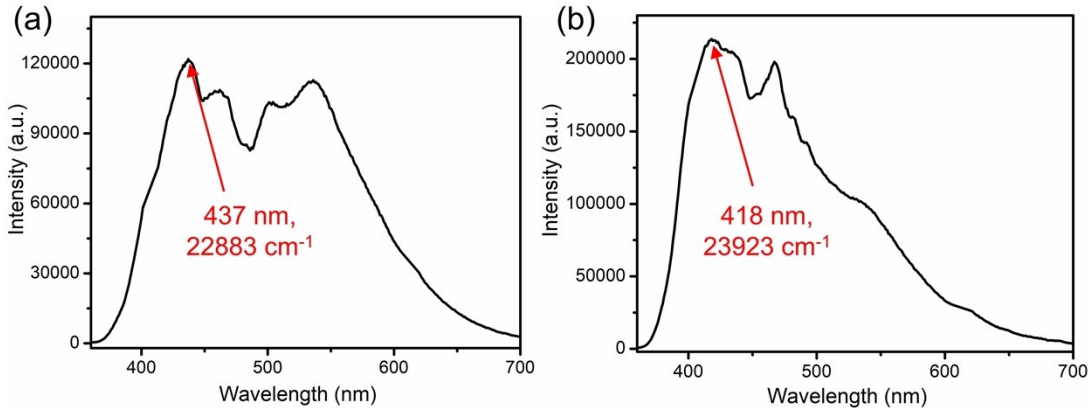


Fig. S15. Phosphorescence spectra of **GdTPTC-NH_2** before (a) and after (b) adding $ONOO^-$ at 77 K.

The triplet energy level (T_1) of the ligand H_4TPTC-NH_2 was calculated to be 22883 cm^{-1}, higher than $^5D_0$ (17500 cm^{-1}) of Eu^{3+}, which confirmed H_4TPTC-NH_2 is an "antenna chromophore" to sensitize Eu^{3+} ions.[42]

Table S3. Serum biochemical assay data for samples from healthy and liver-injured mice (The abnormal indicator values are marked in red).

Indicators	Normal reference range (U/L)	Healthy mouse （U/L）	ALI mouse-1 (U/L)	ALI mouse-2 (U/L)	ALI mouse-3 (U/L)
ALT	10.06-96.47	45.320	850.953	1026.314	1779.76
AST	36.31-235.48	174.390	377.483	645.891	2395.645

Table S4. Quantification of $ONOO^-$ in mice serum samples with RSD (n = 3).

Samples	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
Healthy mouse	0	0.67±0.01	-	1.50
	2	2.71±0.04	101.50	1.50
	4	4.74±0.06	101.50	1.17
	6	6.83±0.05	102.5	0.67

ALI mouse-1	0	76.83±0.05	-	0.11
	2	78.90±0.05	103.50	0.05
	4	80.85±0.07	100.50	0.09
	6	82.89±0.05	101.00	0.06
ALI mouse-2	0	78.91±0.05	-	0.05
	2	80.85±0.07	97.00	0.07
	4	82.92±0.05	100.25	0.05
	6	84.87±0.12	99.33	0.12
ALI mouse-3	0	87.82±0.06	-	0.06
	2	89.83±0.05	100.50	0.06
	4	91.80±0.03	99.50	0.03
	6	93.91±0.04	101.50	0.04

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Table S5. Routine blood parameters and serum biochemical assay data for healthy donor and clinical patients with DILI (The abnormal indicator values are marked in red).

Indicators	Normal reference range	Unit	Healthy donor	DILI patient-1	DILI patient-2	DILI patient-3
Gender	-	-	Female	Male	Female	Female
WBC	3.5-9.5	$\times 10^9$/L	4.21	3.2	3.44	4.06
HGB	130-175 115-150	g/L	137	135	103	125
HCT	40-50 35-45	%	41.6	39.6	31.1	36.6
PLT	125-350	$\times 10^9$/L	175	127	124	157
ALT	0-40	U/L	10	257	70	276
AST	0-42	U/L	14	196	374	1539
ALP	40-150	U/L	54	62	139	193
γ-GT	10-60	U/L	20	123	120	170

TBIL	0-23	μmol/L	11.7	165.2	165.3	129.9
TP	60-80	g/L	69.3	59.6	57.5	72.6
ALB	40-55	g/L	47.3	37.7	34.5	39.1
GLB	20-40	g/L	22	21.9	23	33.5
BUN	1.8-7.1	mmol/L	4.4	4.4	5.12	4.01
CREA	57-111 (males) 41-81 (females)	μmol/L	66	66	53	42
UA	210-430 (males) 150-360 (females)	μmol/L	270	207	246	277

WBC: white blood cells; HGB: hemoglobin; HCT: hematocrit; PLT: blood platelet; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; γ-GT: γ-glutamyl transpeptidase; TBIL: total bilirubin; TP: total protein; ALB: albumin; GLB: globularproteins; BUN: blood urea nitrogen; CREA: creatinine; UA: uric acid

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