Dual-channel Lanthanide-doped Nanoprobe for Reliable Multi-signal Ratiometric Detection of H_2S in Whole Blood

Method

Materials & Characterization. All lanthanide oxides were purchased from STREM Chemicals, Inc. USA. Lanthanide chlorides were prepared by dissolving the corresponding metal oxide in HCl solution at elevated temperature and then evaporating the water completely under reduced pressure. All other chemicals were purchased from Alfa Aesar Chemical Co. Ltd. All chemical reagents were of analytical grade and were used directly without further purification. Deionized water was used throughout. The nanoprobe morphology and element distribution were determined by a Tecnai G^2F30 high-resolution transmission electron microscopy. The powder X-ray diffraction pattern was measured with a Brucker D8 advance X-ray diffractometer from 10° to 70° (Cu Kα radiation, λ = 1.54 Å). Dynamic light scattering (DLS) and zeta potential experiments were carried out on an ALV-5000 spectrometer goniometry equipped with an ALV/LSE-5004 light scattering electronic and multiple tau digital correlator and a JDS Uniphase He–Ne laser (632.8 nm) with an output power of 22 mW. The hydrodiameter distribution was measured at 25 °C with a detection angle of 90°. Inductively coupled mass spectroscopy (ICP-MS) analysis was performed on Agilent 7500ce ICP-MS. UV-vis-NIR absorption spectra are obtained on a Shimadzu UV-3600 UV-vis-NIR spectrophotometer.

Preparation of Ln-CuMOF. The $NaErF_4$@$NaLuF_4$ nanophosphors were prepared and modified with polyvinylpyrrolidone according to the previous reports [1, 2]. In a typical experiment, a mixture of 1 mmol $ErCl_3$, 15 mL OA, and 15 mL ODE are added into a 100 mL three-necked flask. Under the vacuum, the mixture is heated to 160 °C to form a clear solution, and then cooled to room temperature. After the solution cooling down, 0.025 mmol NaOH (0.1 g) and 0.04 mmol NH_4F (0.1481 g) are added into the flask directly and stirred for 30 minutes. The solution is slowly heated with gently stirred, degassed at 100 °C, and then heated to 300 °C and maintained for 1 hour under the Argon atmosphere. After the solution is cooled naturally, the $NaErF_4$ nanoparticles are separated *via* centrifugation (10000 rpm) and washed with ethanol/cyclohexane (1:1 v/v) three times. The $NaLuF_4$ shell was coated by the same procedure by replacing the lanthanide chloride with $LuCl_3$. The as-obtained $NaErF_4$@$NaLuF_4$ were redispersed in 10 mL chloroform (0.5 mg mL^{-1}). A solution of polyvinylpyrrolidone (Mw = 30,000) in 15 mL chloroform (0.1 g mL^{-1}) was added into the $NaErF_4$@$NaLuF_4$ suspension and the mixture was stirred at 40°C for 24 hours. The polyvinylpyrrolidone-modified $NaErF_4$@$NaLuF_4$ were collected by centrifugation at 9,000 rpm for 10 min. The polyvinylpyrrolidone-modified $NaErF_4$@$NaLuF_4$ was cleaned with ethanol three times. Then, 10 mg polyvinylpyrrolidone-modified $NaErF_4$@$NaLuF_4$ was dispersed in 15 mL $CuCl_2$ solution (1.5 mM) and ultrasonically treated for 5 min. The dispersion was added to a 15 mL dimethylformamide solution of benzene-1,3,5-tricarboxylate (6 mM). The solution was degassed, slowly heated to 100 °C with gently stirring, and maintained for one hour under the argon atmosphere. After the solution was cooled naturally, the Ln-CuMOF were separated by centrifugation (12000 rpm) and washed with ethanol and water three times, respectively. The Ln-CuMOF was stored in water at 4 °C.

H_2S detection. The preparation and quantification of standard H_2S solution followed the previous reports [3]. Due to the neutral nature of serum, the H_2S would not only dissolve but ionize to be S^{2-} and HS^-. To simulate the existence of H_2S in physiological conditions, H_2S standard solution was

prepared by adjusting the pH of freshly formulated Na_2S solution to neutral (pH = 7.0) with diluted hydrochloric acid solution in medical saline. All H_2S standard solutions were hermetically stored at 4 ^oC and used within 60 min after formulation. The H_2S standard solutions were pre-quantified by using the standard methylene blue colorimetric protocol to ensure the accuracy of H_2S concentration for further study. Additional hemin and human serum albumin were added to the final solution to simulate the whole blood. To set up the standard curve for H_2S detection, 0.5 mL standard H_2S solution was mixed with 0.5 mL dispersion of Ln-CuMOF (5 mg mL^{-1}) and incubated at 37 ^oC under gently shaking for 15 min. After that, the temperature and fluorescence signals were collected under 808 nm laser irradiation (1.5 W cm^{-2}) with the infrared camera and fluorescence spectroscopy, respectively.

Ethical statement. The establishment of acute pancreatitis mice was performed by Beijing Vital River Laboratory Animal Technology Co., Ltd. following the Guidelines for Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of the Vital River Institutional Animal Care and Use Committee.

Serodiagnostic study. The blood of mice was collected from the femoral vein and sealed with the addition of heparin before tests. Serum samples were collected by separating the blood by centrifugation and used for comparison. Twenty AP mice and twenty normal mice were used for receiver-operating characteristic analysis. For the quantification of H_2S in biological fluid samples, 0.5 mL standard sample was mixed with 0.5 mL dispersion of Ln-CuMOF (5 mg mL^{-1}) and incubated at 37 ^oC under gently shaking for 15 min. After that, the temperature and fluorescence signals were collected under 808 nm laser irradiation (1.5 W cm^{-2}) with the infrared camera and fluorescence spectroscopy, respectively. The receiver-operating characteristic analysis was performed according to previous reports [3].

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2. Y. X. Liu, Q. W. Guo, X. J. Zhu, W. Feng, L. Wang, L. Y. Ma, G. Zhang, J. Zhou and F. Y. Li, *Adv. Funct. Mater.*, 2016, **26**, 5120-5130.
3. Y. X. Liu, Z. Wei, J. Zhou and Z. F. Ma, *Nat. Commun.*, 2019, **10**, 1491.

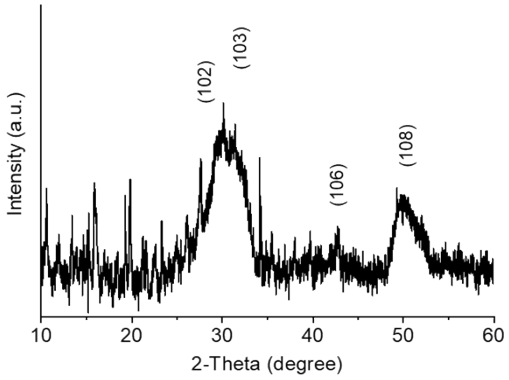

Figure S1. XRD patterns of the separated product from the reaction of Ln-CuMOF with H_2S. The referenced standard card is 06-0464, corresponding to the hexagonal CuS.

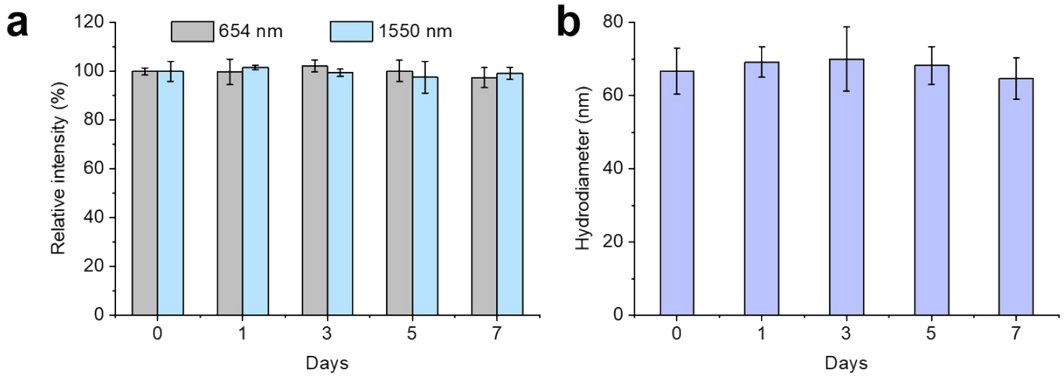

Figure S2. Relative fluorescence intensity a) and hydrodiameter b) of Ln-CuMOF over a storage period of seven days.

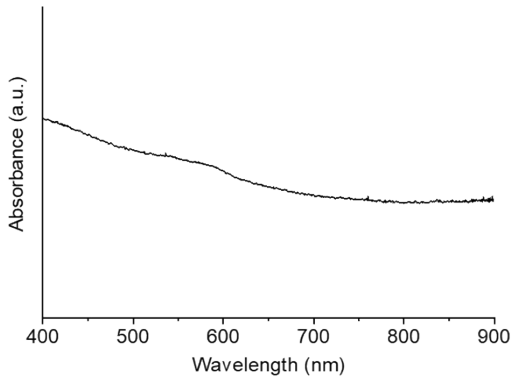

Figure S3. UV-vis-NIR spectrum of Ln-CuMOF after reaction with H_2S.

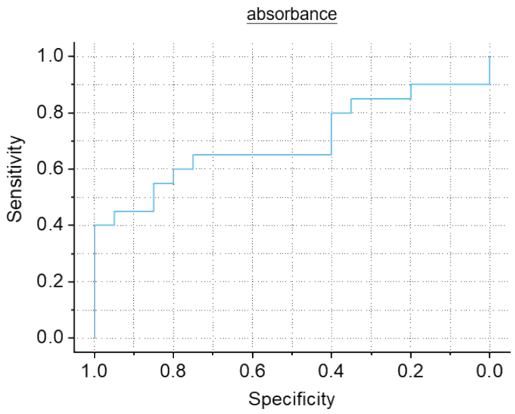

Figure S4. The receiver-operating characteristic curve presents the assay's probability of correctly distinguishing between normal and AP cases based on the plasma H_2S concentration in the whole blood samples determined by the typical colorimetric method.

Table R1. ICP-MS results of Ln-CuMOF and separated product after reaction with H_2S.

	Cu (ng mL^{-1})	S (ng mL^{-1})	Cu:S (mol/mol)
Ln-CuMOF	124.3 ± 18.6	not detected	-
Separated product	323.6 ± 24.3	157.4 ± 15.1	1.037