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Dual-Mode Fluorescence and Electrochemiluminescence Sensor Based on Ru-MOF Nanosheets for Sensitive Detection of apoE Genes

Hao Fan[a#], Huiting Hu[a#], Xia Yin[b], Qiqi Fan[a], Hai Shuai[a], JingZhang[a], Fusheng Liao[a]， Wei Xiong[a], Hedong Jiang[a],Wenming Liu [a]*,Hanfeng Cui [a]*, Guobing Wei[a]*

[a] JiangXi University of Chinese Medicine, Nan Chang, JiangXi 330004, China

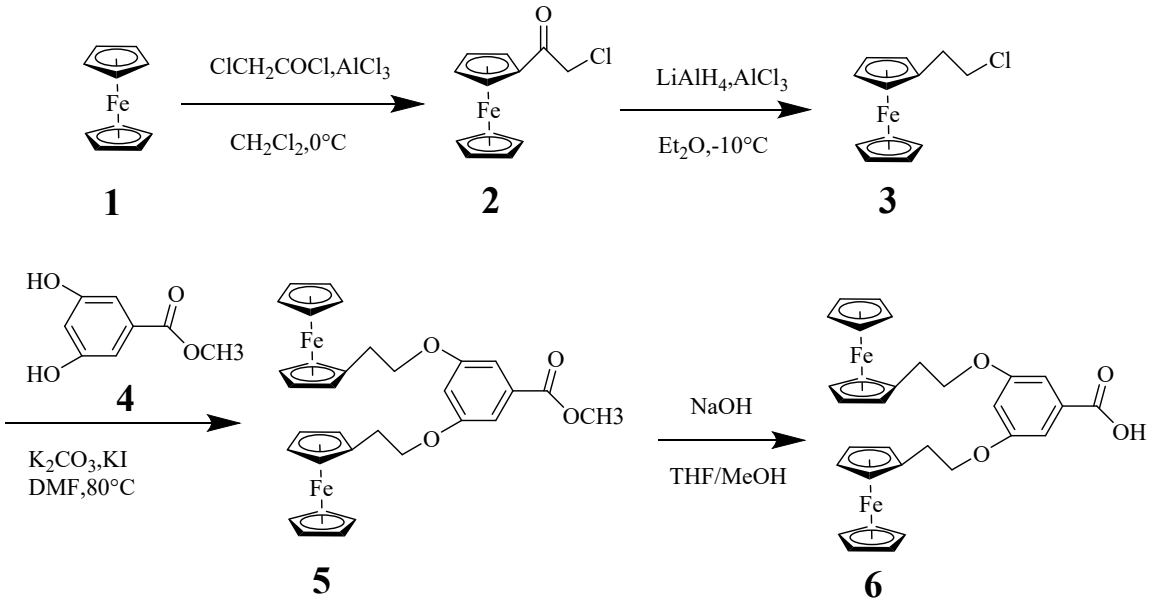
[b] JiangXi Province Hospital of Integrated Chinese and Western Medicine, Nan Chang, JiangXi 330004, China

# Hao Fan and Huiting Hu contributed equally to this paper.

* E-mail address: abc2481264364@163(W.Liu); cuihangfeng@126.com (H.Cui);

weigb@163.com(G.Wei)

1.Synthesis of 3,5-bisferrocenethoxybenzoic acid.



SchemeS1. Synthesis of 3,5-bisferrocenethoxybenzoic acid

α-chloroacetylferrocene (2).

To a solution of ferrocene (**1**) (11.2 g, 60 mmol) in dichloromethane (60 mL) at 0°C, a solution of chloracetyl chloride (3.8 mL, 50 mmol) and anhydrous aluminum trichloride (6.6 g, 50 mmol) in dichloromethane (80 mL) was added dropwise. After stirred for 8 h at room temperature, brine (100 mL) was added. The organic phase was separated and washed by brine. The resulting solution was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by flash column chromatography to give orange crystal in 41.5% yield. ^{1}H NMR ($CDCl_3$, 600MHz): δ 4.88 (Cp-H, t, *J* = 2.0 Hz, 2 H), 4.62 (Cp-H, t, *J* = 2.0 Hz, 2 H), 4.46 (CH_2, s, 2 H), 4.25 (Cp-H, s, 5 H).

2-Chloroethylferrocene (3).

A solution of α-chloroacetylferrocene (**2**) (1.2 g, 4.58 mmol) in Et_2O (120 mL) was added dropwise to a solution of $LiAlH_4$ (180 mg, 4.7 mmol) and $AlCl_3$ (609 mg, 4.58

mmol) in Et_2O (90 mL) at -10 °C. After 1 h, the reaction was carefully quenched by addition of H_2O (20 mL) and diluted with brine (100 mL). The aqueous layer was extracted with ethyl acetate (3×50 mL). The combined organic layer was washed with brine (3×50 mL), dried over anhydrous sodium sulfate, and concentrated. The residue was purified by a silica gel column with ethyl acetate-Petroleum ether (1:15) as the eluent to afford yellow product **3** (0.76 g, 67%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.16 (Cp-H, s, 7H), 4.13 (Cp-H, s, 2H), 3.62 (CH_2, t, J = 7.2 Hz, 2H), 2.84 (CH_2, t, J = 7.2 Hz, 2H).

Metyhl 3,5-bisferrocenethoxybenzoate (5).

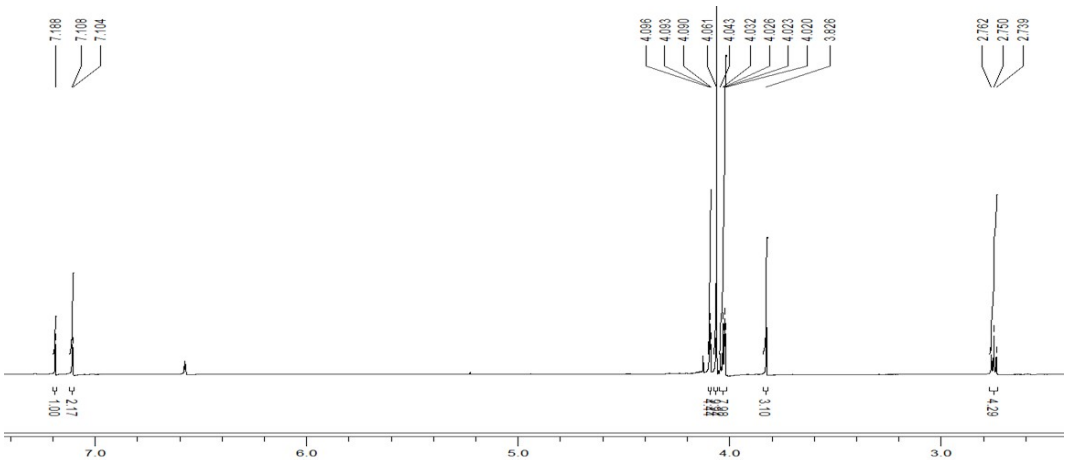
2-Chloroethylferrocene (**3**) (548.1mg, 2.21 mmol), methyl 3,5-dihydroxybenzoate (**4**) (154.6 mg, 0.92 mmol), anhydrous potassium carbonate (552 mg, 4 mmol) and potassium iodide (10 mg, 0.06 mmol) in DMF (4 mL) was mixed together and stirred at 80 ^{o}C until disappearance of starting material was observed by TLC. The mixture was diluted with ethyl acetate (80 mL) and washed with water (3×30 mL) and brine (3×30 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent was removed in vacuum. The residue was purified by a silica gel column with ethyl acetate-petroleum ether as the eluent to gave **5** as yellow solid (398 mg, 73%).

As shown in Supporting Information Figure S1, ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.19 (Ar-H, s, 1H), 7.11 (Ar-H, d, J = 2.4 Hz, 2H), 4.09 (-CH_2-O, t, J = 1.5 Hz, 4H), 4.06 (Cp-H, s, 10H), 4.04-4.02 (Cp-H, m, 8H), 3.82 (O-CH_3, s, 3H), 2.75 (-CH_2-, t, J = 6.0 Hz, 4H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.9, 159.9, 131.9, 107.7, 106.8, 84.6, 68.8,

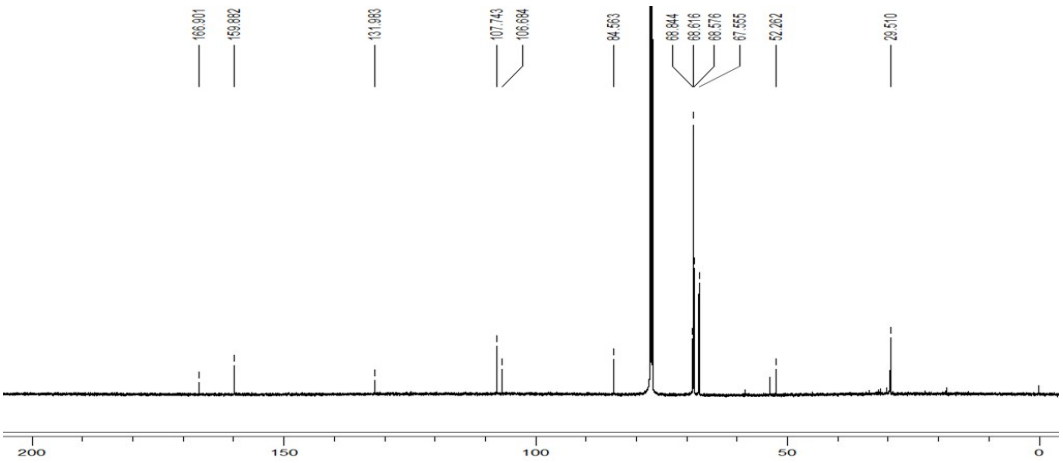
68.6, 68.6, 67.5, 52.2, 29.5. HRMS (ESI) Calcd. for C32H32Fe2O4 [M]+: 592.0999; found 592.0991.

3,5-bisferrocenethoxybenzoic acid (6)

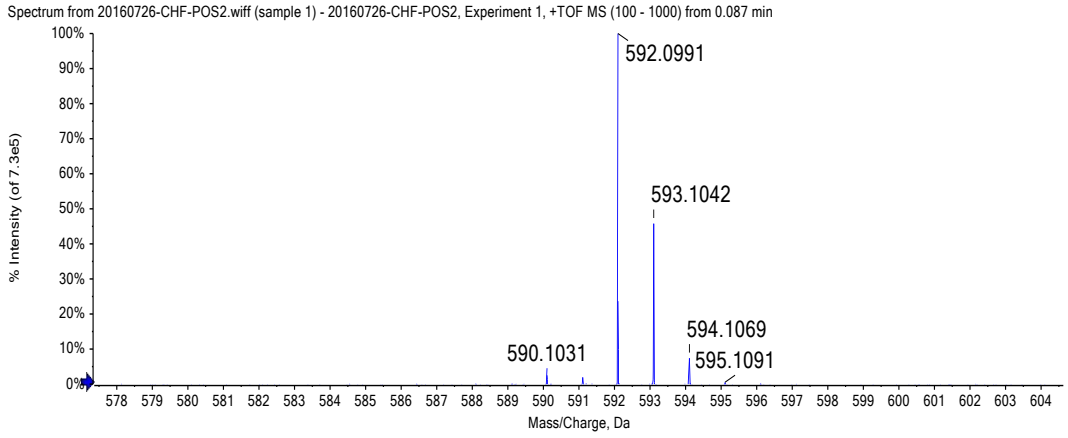
To a solution of methyl 3,5-bisferrocenethoxybenzoate (**5**) (100 mg, 0.17 mmol) in THF/MeOH (20mL, v/v = 1: 1) was added 10% aqueous sodium hydroxide (4 mL) at room temperature. The mixture was kept stirring overnight, after which time complete disappearance of the starting material was observed by TLC. The organic solvents were removed and the aqueous phase was neutralized with aqueous HCl (2 N) at 0 ^{o}C. The resulting orange crystals were then filtered, washed with water, and dried under vacuum. A pure sample was obtained by recrystallization from dichloromethane/ petroleum ether to give 3, 5-bisferrocenethoxybenzoic acid (**6**) (59 mg, 60% yield) as yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.26 (Ar-H, s, 2H), 6.71 (Ar-H, s, 1H), 4.20-4.13 (Cp-H and -CH_2-O, m, 22H), 2.85 (-CH_2-, s, 4H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.1, 159.9, 131.0, 108.2, 107.5, 84.5, 68.8, 68.6, 68.6, 67.5, 29.5. HRMS (ESI) Calcd. for C31H30Fe2O4 [M]+: 578.0843; found 578.0858.



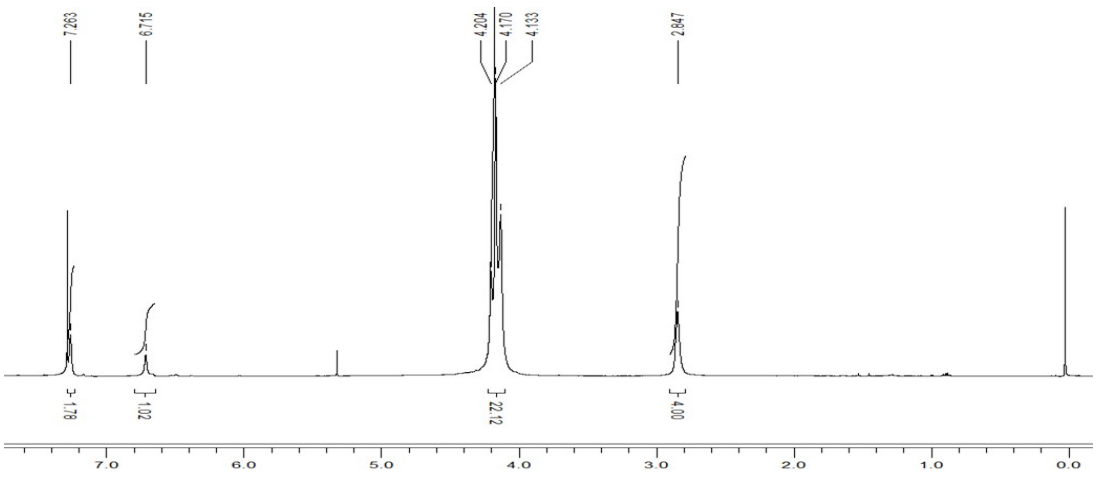
^{1}H NMR of methyl 3,5-bisferrocenethoxybenzoate (**5**)



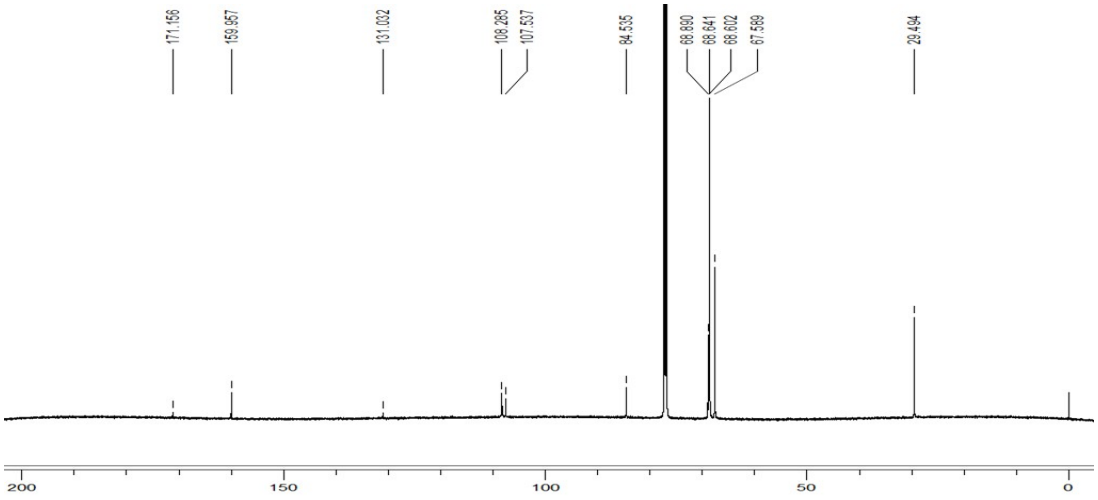
^{13}C NMR of methyl 3,5-bisferrocenethoxybenzoate (**5**)



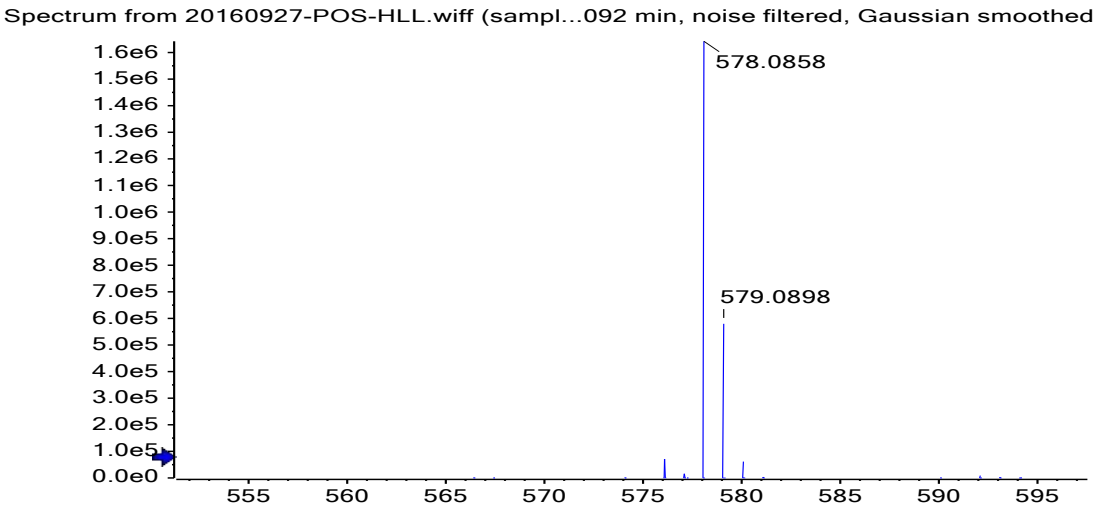
(C) HRMS of methyl 3,5-bisferrocenethoxybenzoate (**5**)



^{1}H NMR of 3,5-bisferrocenethoxybenzoic acid (**6**)



^{13}C NMR of 3,5-bisferrocenethoxybenzoic acid (**6**)



HRMS of 3,5-bisferrocenethoxybenzoic acid (**6**)

2.ECL measurements

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) measurements were performed using an IGS4030 electrochemical workstation (Insen Sensing Technology Co., Ltd., Guangzhou, China). CV analysis was performed in 10-3 M potassium ferricyanide solution in the potential range of 0.6 V to -0.2 V at a scan rate of 0.1 V/s. EIS parameters were 0.2 V onset, 0.005 V amplitude, and the scanning range was 0.1 Hz to 100000 Hz. The ECL-detected electrolyte was PBS solution (1 mL), containing 150 μL (0.1 M) K2S2O8 was added as the co-reactant. The experimental parameters were an initial potential of 0.2 V, a high potential of 1.3 V, a scanning potential of 0.1 V/s, and a high voltage of 800 V for the photomultiplier tube.

3. Characterization of RuMOFNSs

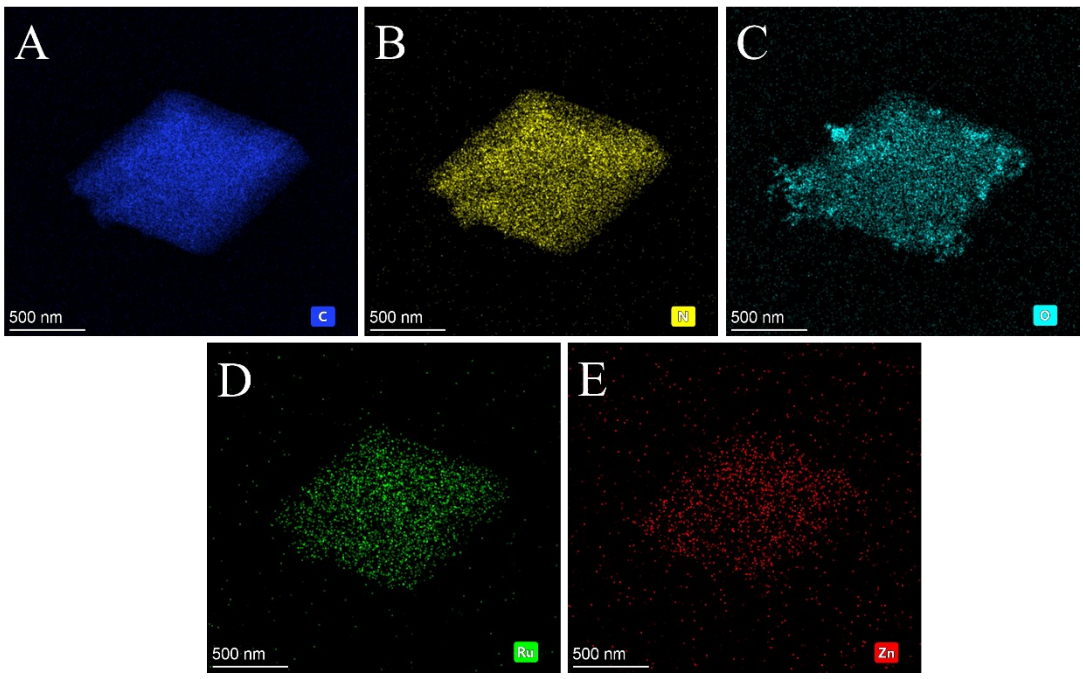


Fig. S1 (A-E) EDS elemental mapping of RuMOFNSs.

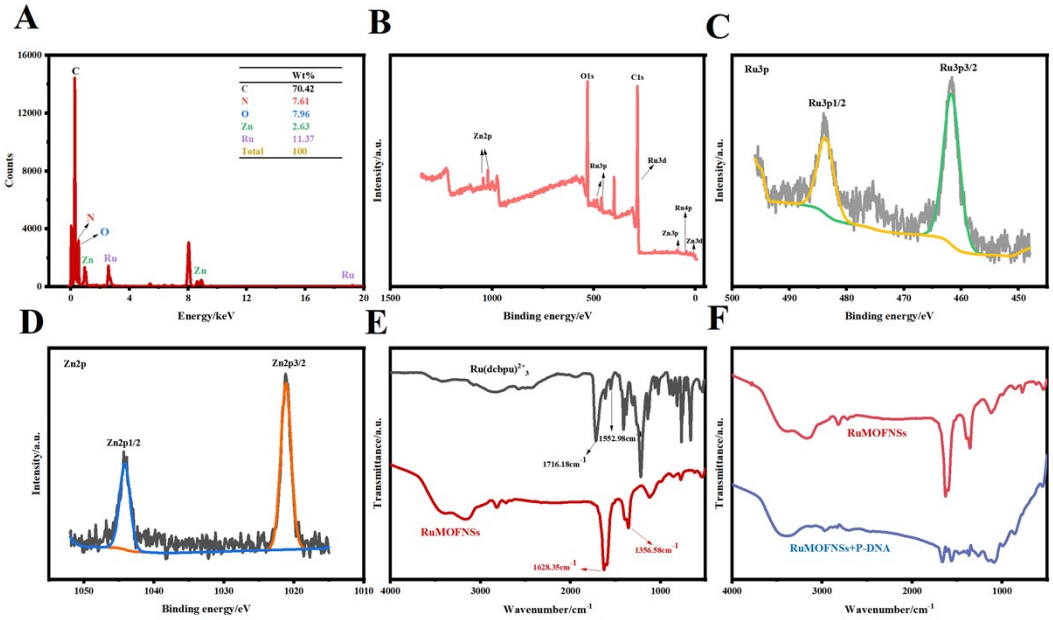


Fig.S2. (A) EDS spectrum image, (B) Full spectrum of XPS measurements, (C) High-resolution Ru 3p region, (D) Zn 2p region, (E) FT-IR mapping of RuMOFNSs, and (F) RuMOFNSs/P-DNA.

4.Efficiency comparison of bisferrocene quench RuMOFNSs and comparison of FL and ECL signals of RuMOFNSs and Ru(bpy) $_3^{2+}$

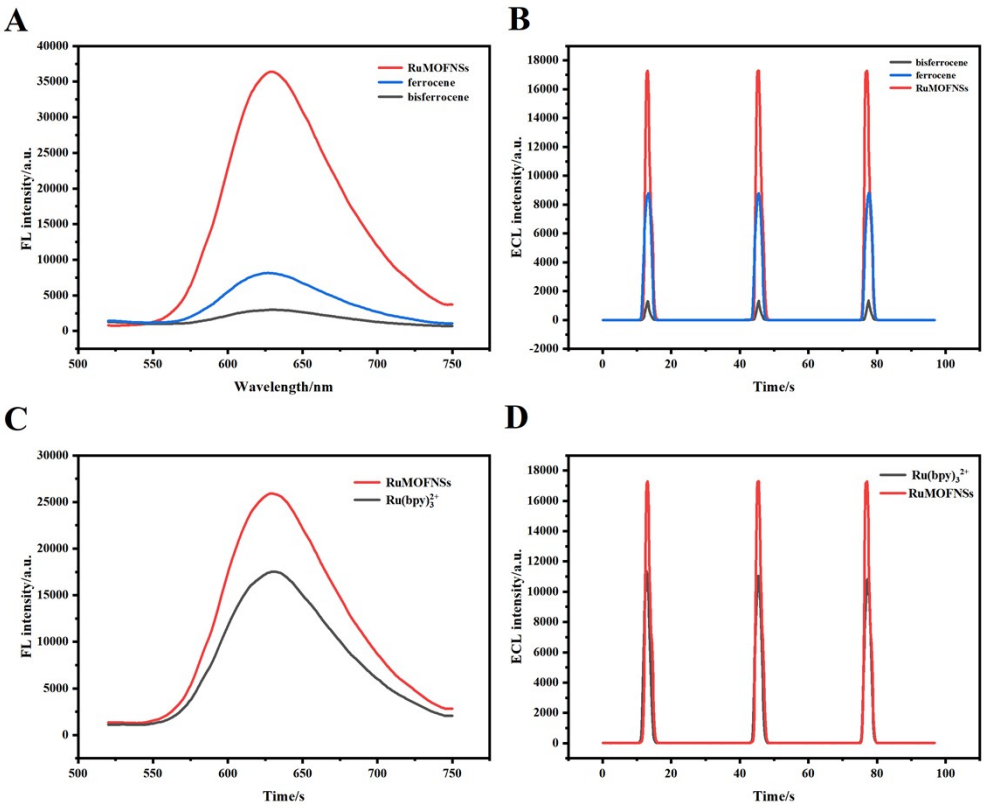


Figure .S2 Comparative plots of the effect of bisferrocene and ferrocene quenches on the luminescence intensity of RuMOFNSs FL (A) and ECL (B);Comparative plots of FL (C) and ECL (D) signals of Ru(bpy) $_3^{2+}$ and RuMOFNSs.

5. Optimization of experimental conditions

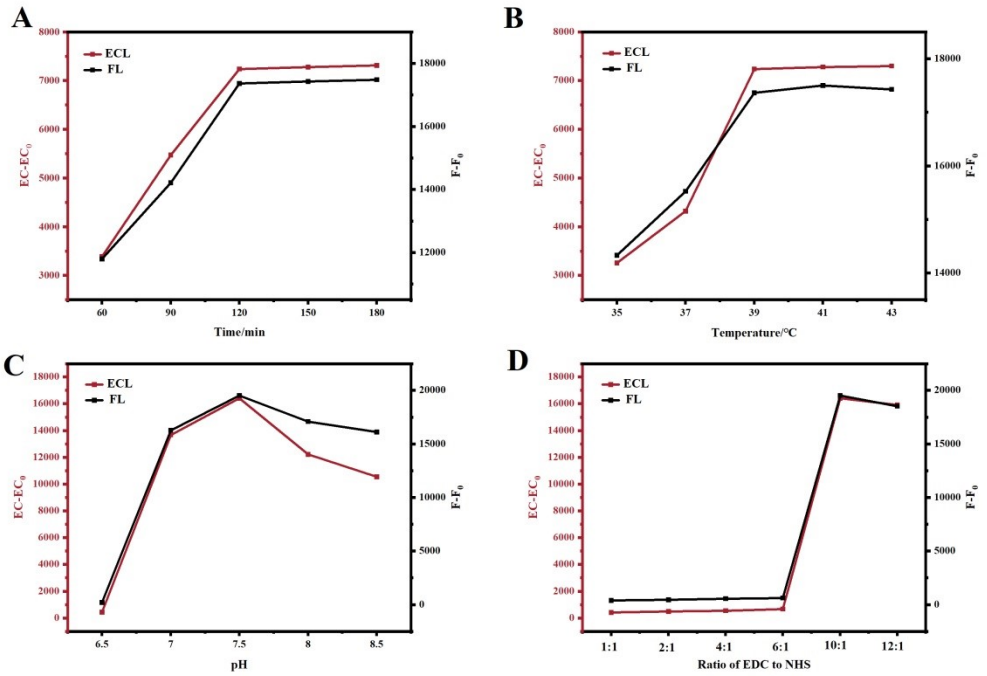


Fig.S3 Optimization of experimental conditions. (A)EXO III digestion time, (B)EXO III digestion temperature, (C)pH of PBS, (D)ratio of EDC to NHS. Error bars = RSD (n = 6).

Fig.S3A-B shows the relationship between digestion time, digestion temperature, and FL and ECL intensity change ($\Delta I_F = I_F - I_{F0}$, $\Delta I_E = I_E - I_{E0}$, I_F, and I_E are FL intensity and ECL intensity, I_{F0} and I_{E0} are blank values). The highest digestion efficiency is achieved at 120 min and 39℃ when the P-DNA is catalytically cleaved by Exo III, moving the bisferrocene fragments away from RuMOFNSs, and allowing the maximum recovery of both signals. Fig.S3C, D shows the relationship between the buffer pH, the ratio of EDC to NHS, and the FL intensity, ECL intensity change ($\Delta I_F = I_{F0} - I_F$, $\Delta I_E = I_{E0} - I_E$, I_F and I_E are FL intensity and ECL intensity, I_{F0} and I_{E0} are FL intensity and ECL intensity of RuMOFNSs). The ΔI_F and ΔI_E are very few before or after pH 7.5, while the values reach the maximum at pH 7.5, suggesting that an acidic or basic environment inhibits the attachment efficiency of RuMOFNSs to P-DNA, resulting the inability of bisferrocene to quench the luminescence intensity of RuMOFNSs effectively. Similarly, the ratio of activator EDC to NHS is also very

important in ligating RuMOFNSs to P-DNA. Fig. 2D shows that the FL and ECL signal changes reach the maximum when at a 10:1 ratio, and the luminescence intensity is quenched to the maximum extent. In addition, the digestion temperature and time affect the degree of optical signal recovery, thus, the sensor's analytical performance.

6. Real sample analysis

Table S1. Recovery tests for apoE gene in human serum samples.

		Measured (fM)		Recovery(%)		RSD(%)	
sample	Added(fM)	FL	ECL	FL	ECL	FL	ECL
No.1	5	5.11	4.89	102.2	97.8	1.85	0.88
No.2	10	9.82	10.21	98.2	102.1	2.40	1.89
No.3	20	20.37	20.35	101.8	101.7	2.21	2.19

Table S2. Oligonucleotide sequences for Corydalis yanhusuo gene analysis.

Oligo Name	Sequence (5' to 3')
Target DNA (PCR product)	GAGTCGCCCCCATCCCTCTATTTACCGAGAGGGGGGGGGAGAGGAGAATGG CCCCCCGTGTGCACTCCGCGCGGCCGGCCCAAACACAGGCCCCGGGAGGCC GACGTCACGATCCGTGGTGGTTGTAAAAGACACGCCCTCGAAACCGGATCC CGTGCACGCCGCGCCGCGAAACCCGGAGGGCCACAG
Forward primer	AGTCGCCCCCATCCCTCTA
Reverse primer	CTGTGGCCCTCCGGGTTT
Probe DNA(P-DNA)	H2N-TTTCACCACGGATCGTGACGTCGGCCTCCCG-bisferrocene

PCR Detection

We selected the detection of PCR amplicons from the Corydalis yanhusuo gene to test the real applicability of the constructed the dual-mode sensor. The PCR amplifications program was set up as 94 ℃ for 2 minutes, followed by 30 cycles of 94 ℃ for 10 s, 68 ℃ for 5 s, and 72 ℃ for 5 min. By using PCR protocol, we obtained

DNA targets of 189 bp that could be directly detected by the sensor. We used NanoDrop 2000/2000C to determine the concentration of PCR product.