Supporting Information

A novel fluorescence-electrochemiluminescence dual-mode sensing platform for high-precision BRAF gene detection

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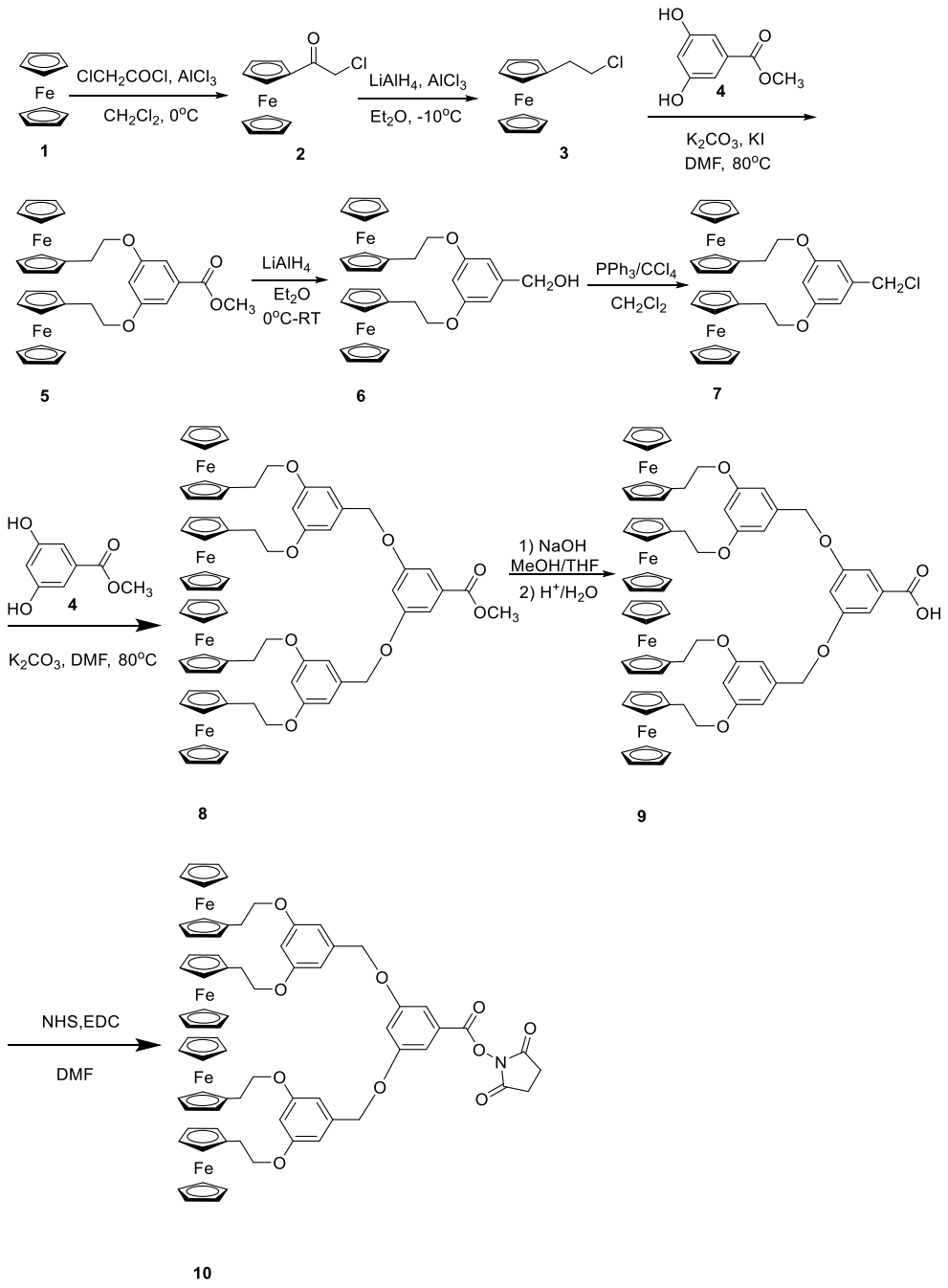
1. The synthesis of 2,5-dioxopyrrolidin-1-yl 3,5-bis((3,5-bisferrocenethoxybenzyl) oxy)benzoate.



Scheme S1. The synthesis of 2,5-dioxopyrrolidin-1-yl 3,5-bis((3,5-bisferrocenethoxybenzyl)oxy)benzoate (10).

Procedures for the synthesis of 2,5-dioxopyrrolidin-1-yl 3,5-bis((3,5-bisferrocenethoxybenzyl)oxy)benzoate (10):

α-chloroacetylferrocene (2).

To a solution of ferrocene (11.2 g, 60 mmol) in dichloromethane (60 mL) at 0 ℃, 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 (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 ℃. 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-O, t, J = 7.2 Hz, 2H), 2.84 (Fc-CH_2-, t, J = 7.2 Hz, 2H).

Methyl 3,5-bisferrocenethoxybenzoate (5).

2-Chloroethylferrocene (548.1mg, 2.21 mmol), methyl 3,5-dihydroxybenzoate (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 ℃ 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%).^{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 (Cp-H, t, J = 1.5 Hz, 4H), 4.06 (Cp-H, s, 10H), 4.04-4.02 (Cp-H, -CH_2-O, m, 8H), 3.82 (O-CH_3, s, 3H), 2.75 (Fc-CH_2-, t, J = 7.2 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, 52.3, 29.5. HRMS (ESI) Calcd. for $C_{32}H_{32}Fe_2O_4$ $[M]^+$: 592.0999; found 592.0991.

(3, 5-bisferrocenethoxyphenyl)methanol (6).

To a solution of $LiAlH_4$ (28.8 mg, 0.76 mmol) in anhydrous ether (10 mL), a solution of methyl 3, 5-bisferrocenethoxy-benzoate (50 mg, 0.084 mmol) in anhydrous ether (15 mL) was added dropwise at 0 ℃. The mixture was stirred at room temperature until the disappearance of the starting material. And then the reaction was carefully quenched by H_2O (20 mL), the aqueous layer was separated and extracted with diethyl ether (3×25 mL). The combined organic layer was washed with brine (3×50 mL), dried over anhydrous sodium sulfate, and concentrated. The residue was purified by flash column chromatography to give orange crystal **(6)**. Yields: 89.0%。^{1}H NMR ($CDCl_3$, 600 MHz)

δ 6.55 (Ar-H, s, 2H), 6.43 (Ar-H, s, 1H), 4.64 (ArCH_2-O, s, 2H), 4.20-4.13 (Cp-H, m, 18H), 4.10 (-CH_2-O, t, J = 7.2 Hz, 4H), 2.84 (Fc-CH_2-, t, J = 7.2 Hz, 4H). HRMS (ESI) Calcd. for $C_{31}H_{32}Fe_2O_3$ $[M]^+$: 564.1050; found 564.1037.

3, 5-bisferrocenethoxybenzyl chloride (7).

To a solution of PPh_3 (0.4313 g, 1.65 mmol) in CCl_4 (10 mL), a solution of (3, 5-bisferrocenethoxyphenyl)methanol (0.7739 g, 1.37 mmol) in CH_2Cl_2 (10mL) was added dropwise. The mixture solution was refluxed for 30 min and observed by TLC. The mixture was diluted with dichloromethane (40 mL) and washed with water (3×20 mL) and brine (3×20 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by a silica gel column with ethyl acetate-petroleum ether as the eluent to gave **(7)** as dark orange solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.55 (Ar-H, d, J = 1.8 Hz, 2H), 6.43 (Ar-H, t, J = 2.4 Hz, 1H), 4.53 (ArCH_2-O, s, 2H), 4.18 (Cp-H, t, J = 1.8 Hz, 4H), 4.15(Cp-H, s, 10H), 4.11 (Cp-H, t, J = 1.8 Hz, 4H), 4.09 (-CH_2-O, t, J = 7.2 Hz, 4H), 2.83 (Fc-CH_2-, t, J = 7.2 Hz, 4H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 160.3, 139.5, 107.1, 101.3, 84.6, 68.7, 68.6, 68.6, 67.5, 46.2, 29.2. HRMS (ESI) Calcd. for $C_{31}H_{31}ClFe_2O_2$ $[M]^+$: 582.0711; found 582.0731.

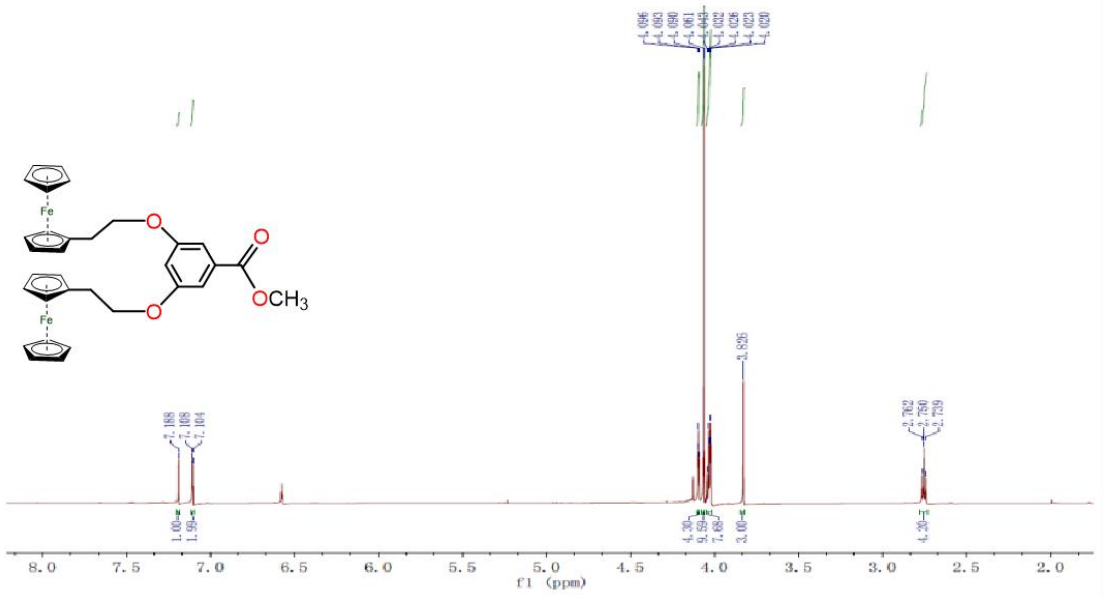
Methyl 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoate (8).

To a solution of methyl 3,5-dihydroxybenzoate (6.6 mg, 0.039 mmol) and anhydrous K_2CO_3 (21.5 mg, 0.156 mmol) in 10mL of DMF, 3, 5-bisferrocenethoxybenzyl chloride (50 mg, 0.085mmol) was added. The reaction was stirred at 80 ℃ under nitrogen atmosphere and observed by TLC. The mixture was diluted with ethyl acetate (30 mL) and washed with brine (3×15 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 **(8)** as dark yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.30 (Ar-H, d, J = 2.4 Hz, 2H), 6.82 (Ar-H, t, J = 2.4 Hz, 1H), 6.59 (Ar-H, d, J = 2.4 Hz, 4H), 6.45 (Ar-H, t, J = 2.4 Hz, 2H), 5.01 (ArCH_2-O, s, 4H), 4.18 (Cp-H, t, J = 1.2 Hz, 8H), 4.15-4.09 (Cp-H and -CH_2-O, m, 36H), 3.91 (-$COOCH_3$, s, 3H), 2.83 (Fc-CH_2-, t, J = 7.2 Hz, 8H).

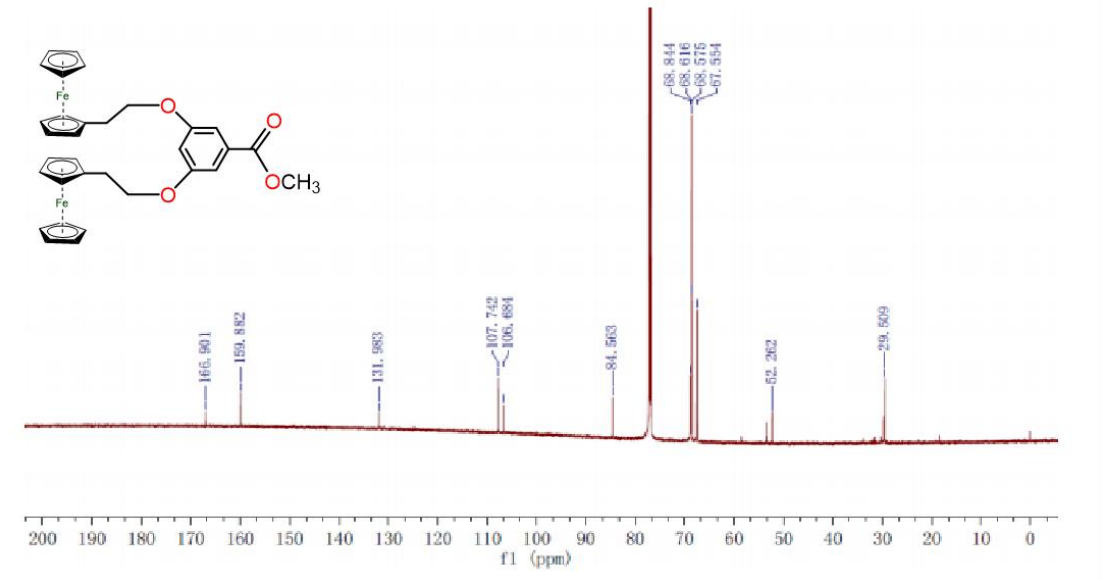
3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic acid (9).

To a solution of methyl 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoate (50 mg, 0.04 mmol) in THF/MeOH (10mL,v/v=1:1), 10% aqueous sodium hydroxide (2 mL) was added at room temperature. The mixture was kept stirring overnight, the pH was adjusted to about 5 with dilute hydrochloric acid. The resulting orange crystals were collected by filtration, washed with water, and dried under vacuum. A pure sample was obtained by recrystallization from dichloromethane/ petroleum ether to give **(9)** (32 mg, 65% yield, purity > 95%) as dark yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.32 (Ar-H, s, 2H), 6.85 (Ar-H, s, 1H), 6.59 (Ar-H, s, 4H), 6.44 (Ar-H, s, 2H), 5.01 (ArCH_2-O, s, 4H), 4.20-4.11 (Cp-H, m, 36H), 4.09 (-CH_2-O, t, J = 7.2 Hz, 8H), 2.83 (Fc-CH_2-, t, J = 6.6 Hz, 8H). HRMS (ESI) Calcd. for $C_{69}H_{66}Fe_4O_8$ [M]+: 1246.2155; found 1246.2091.

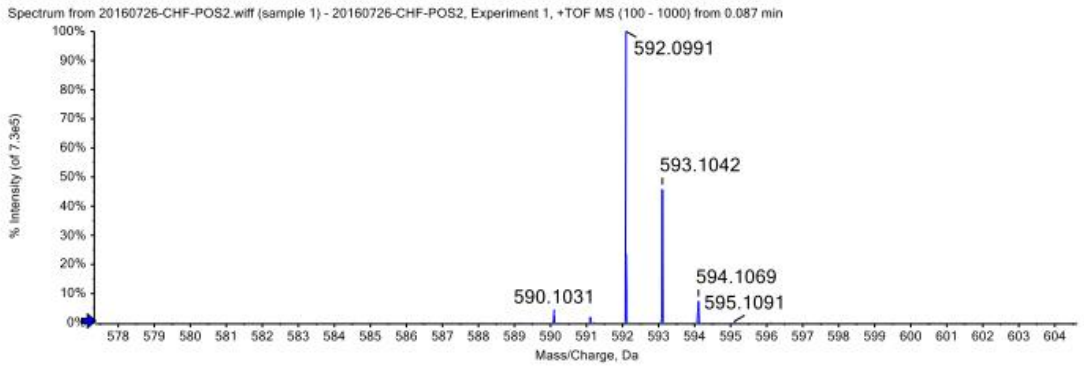
2,5-dioxopyrrolidin-1-yl 3,5-bis((3,5-bisferrocenethoxybenzyl)oxy)benzoate (10).
To a solution of 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic acid (24.22 mg, 1.96 mmol) in DMF (3 mL), NHS (0.361 g, 3.14 mmol) and EDC (0.601 g, 3.14 mmol) was added at 0℃. The mixture was diluted with H_2O (40 mL) and extracted with CH_2Cl_2 (3×25 mL) after kept stirring overnight at room temperature. The organic phase was filtered and dried in vacuum to obtain (**10**) as light yellow solid. 1H NMR ($CDCl_3$, 400 MHz) δ 8.02 (Ar-H, s, 1H), 7.35 (Ar-H, s, 1H), 6.88 (Ar-H, s, 1H), 6.56 (Ar-H, s, 4H),6.42 (Ar-H, s, 2H), 4.99 ($ArCH_2$-O, s, 4H), 4.08-4.16 (Cp-H, m, 36H), 4.08 (-CH_2-O, t, J = 8 Hz, 8H), 2.96 (CO-CH_2-CH_2-CO,s, 4H), 2.83 (Fc-CH_2-, t, J = 6.6 Hz, 8H).



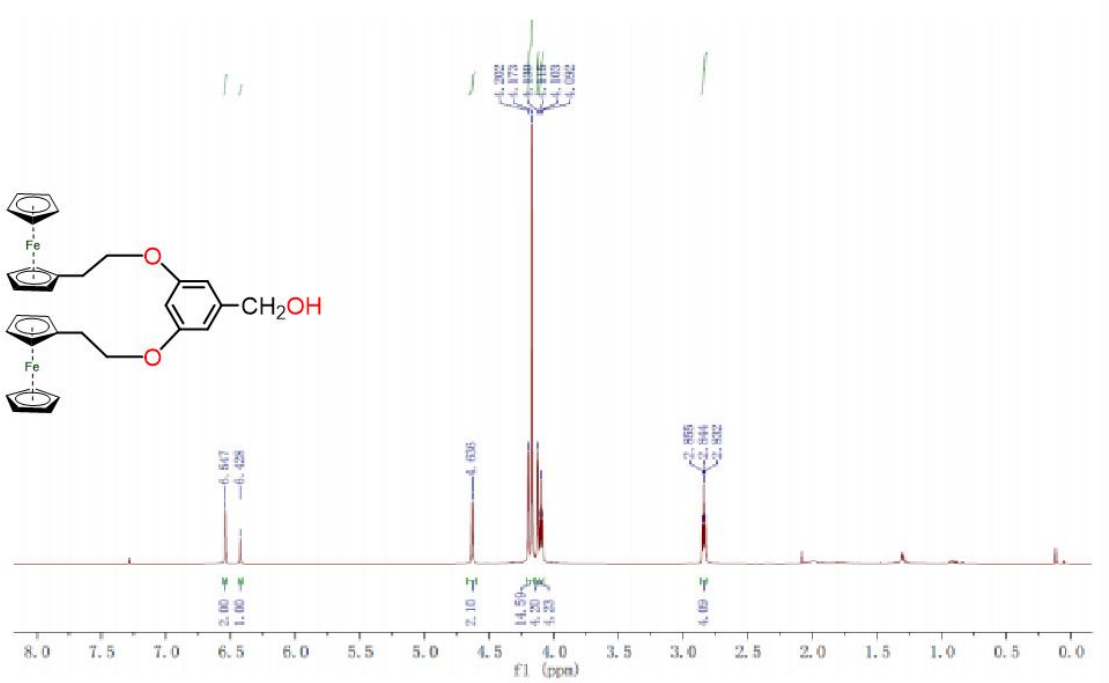
^{1}H NMR of methyl 3,5-bisferrocenethoxybenzoate (5)



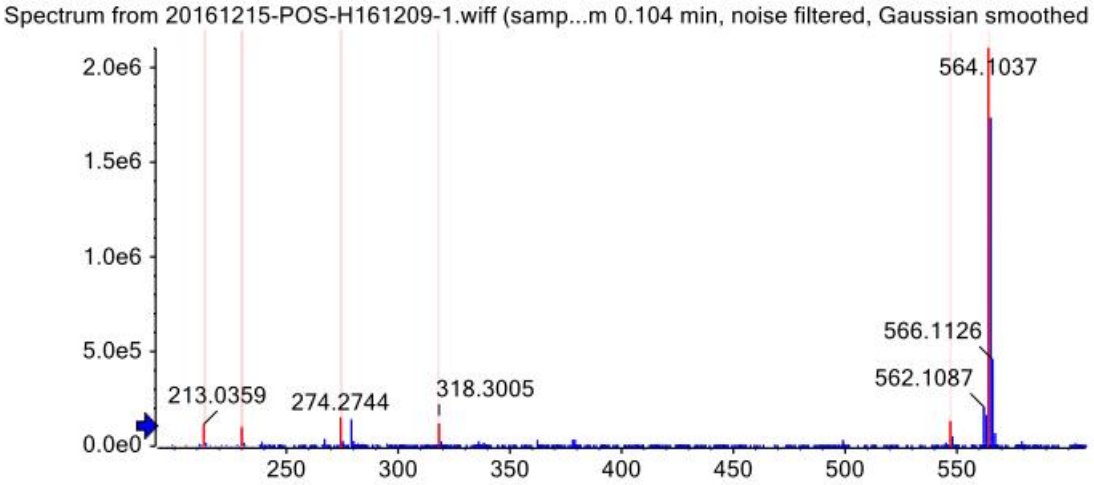
^{13}C NMR of methyl 3,5-bisferrocenethoxybenzoate (5)



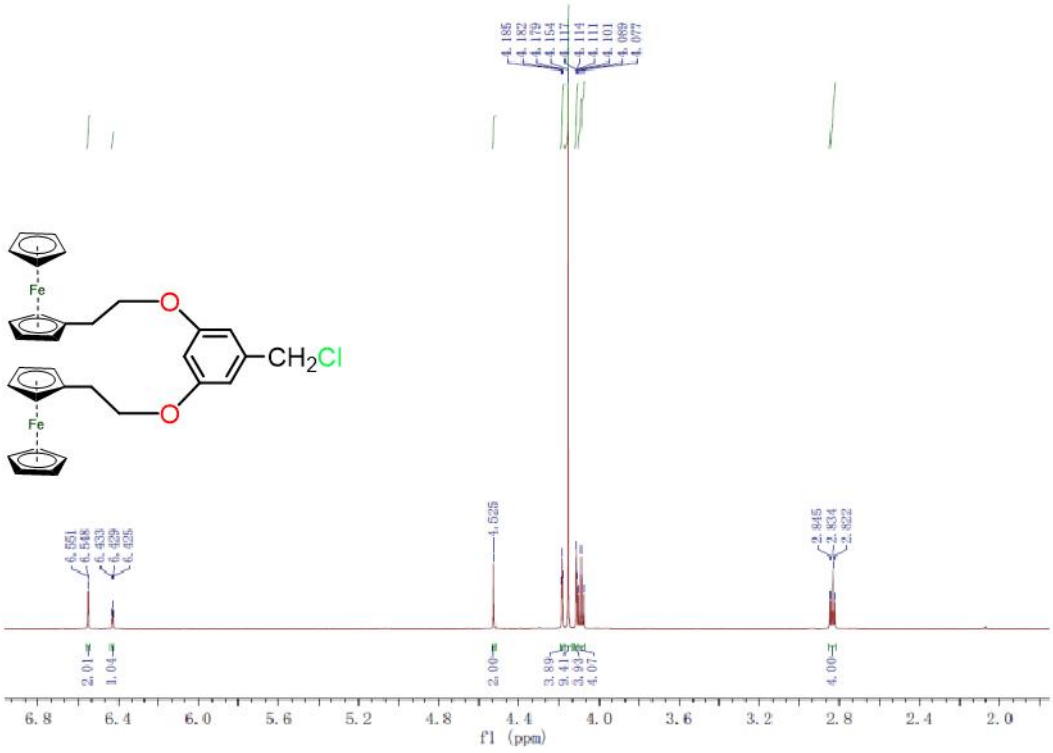
HRMS of methyl 3,5-bisferrocenethoxybenzoate (5)



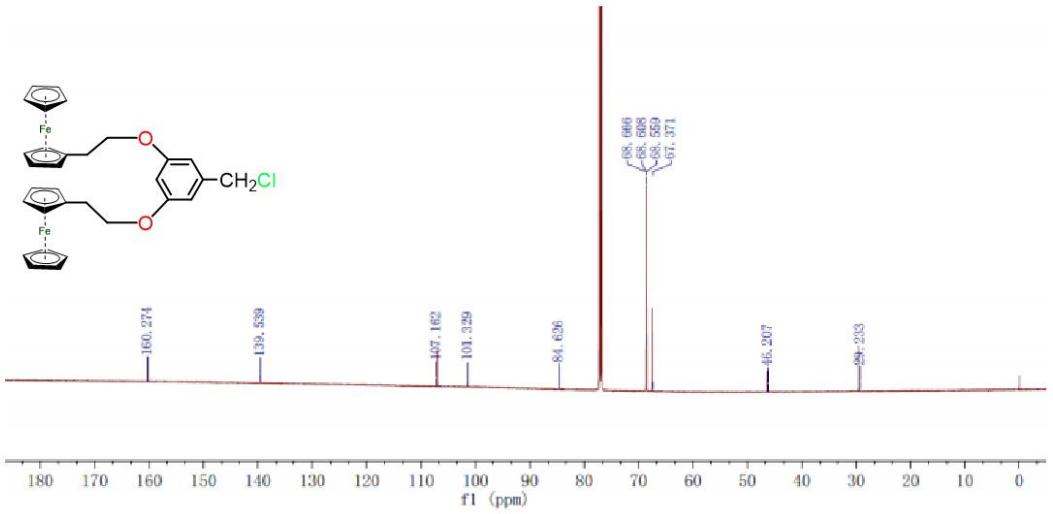
^{1}H NMR of (3, 5-bisferrocenethoxyphenyl)methanol (6)



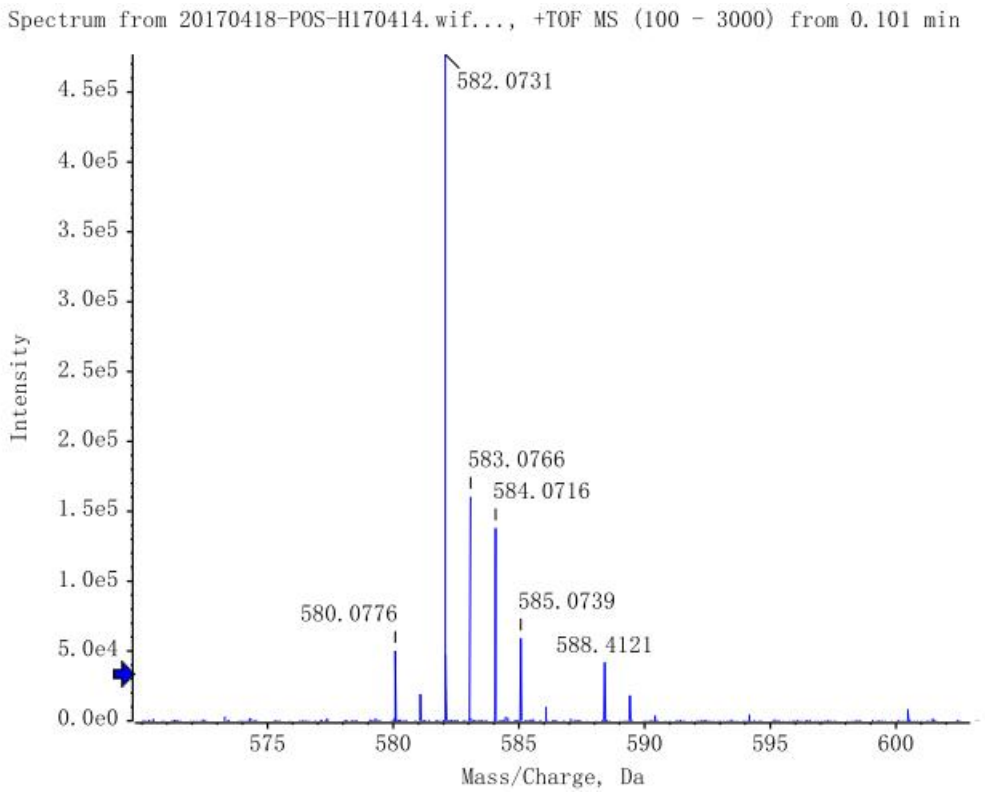
HRMS of (3, 5-bisferrocenethoxyphenyl)methanol (6)



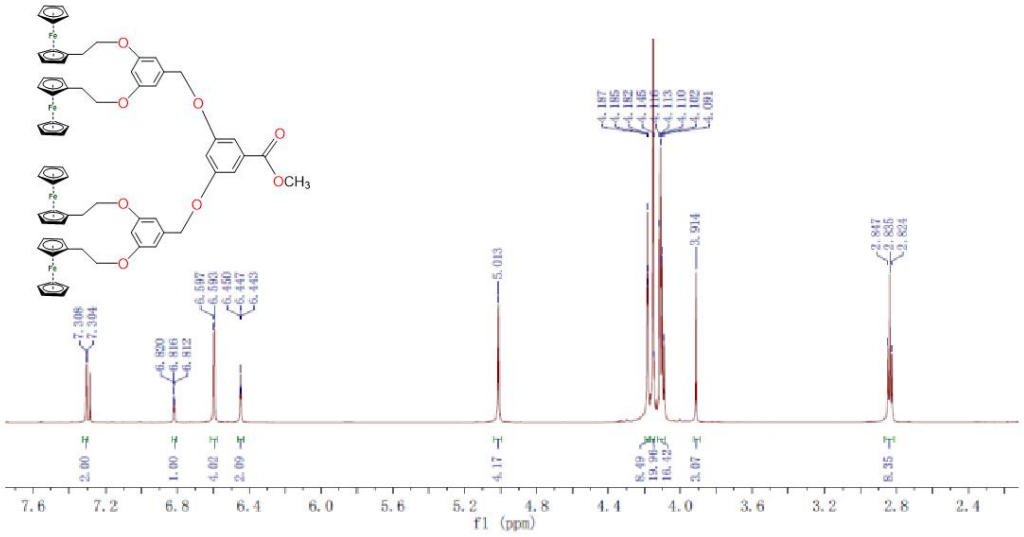
^{1}H NMR of 3, 5-bisferrocenethoxybenzyl chloride (7)



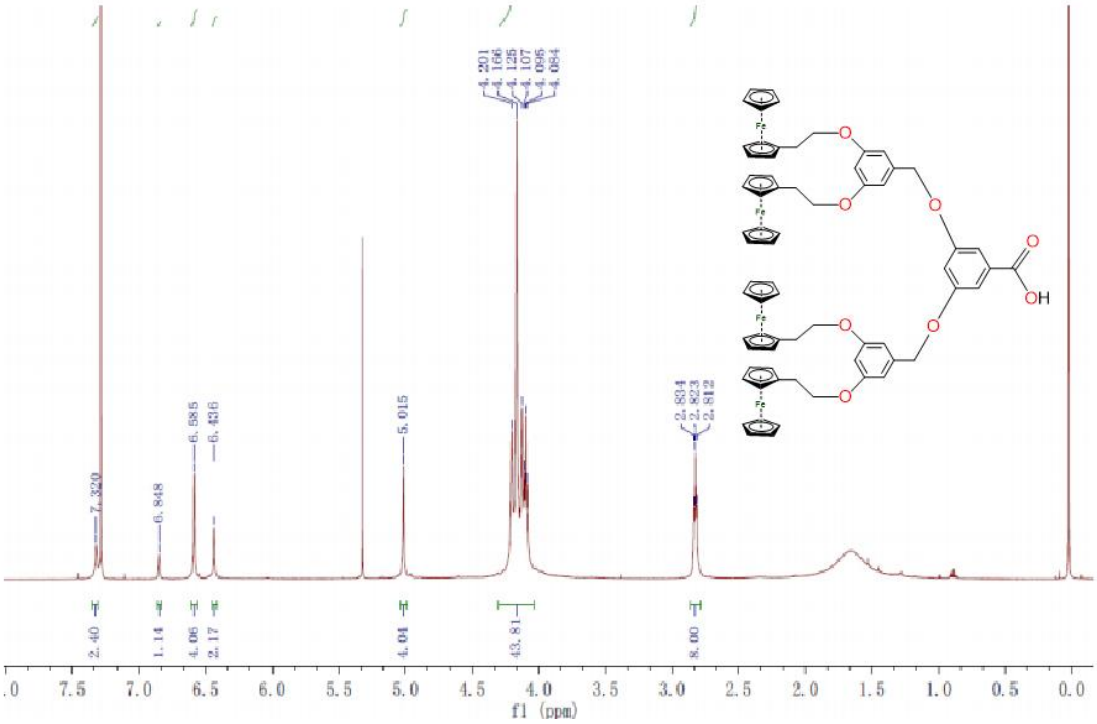
^{13}C NMR of 3, 5-bisferrocenethoxybenzyl chloride (7)



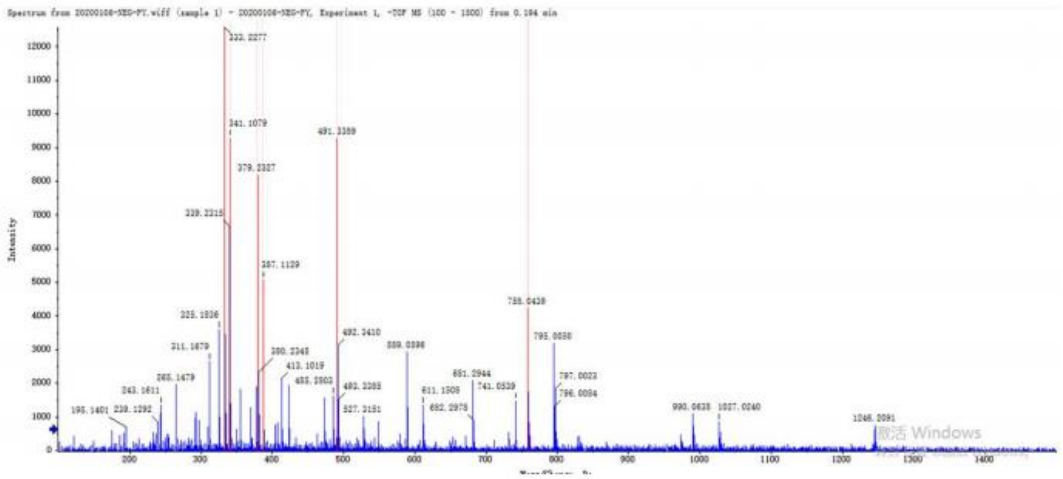
HRMS of 3, 5-bisferrocenethoxybenzyl chloride (7)



^{1}H NMR of methyl 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoate (8)



^{1}H NMR of 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic acid (9)



HRMS of 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic acid (9)

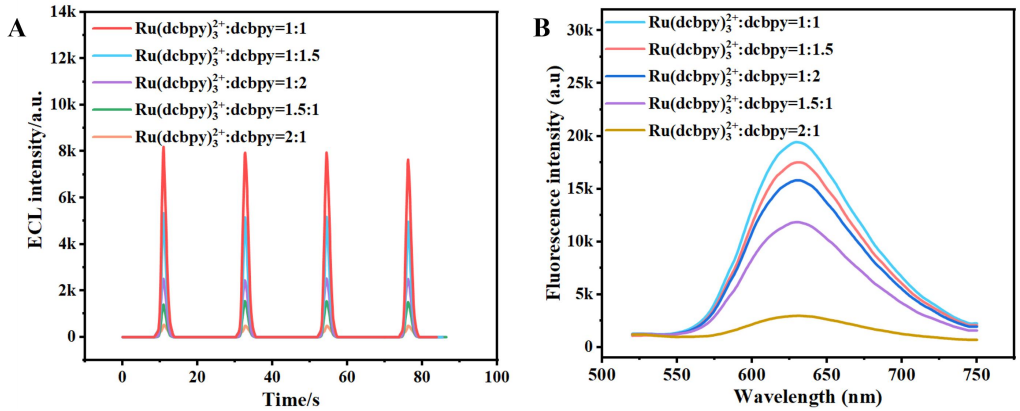


Fig. S1. FL (A) and ECL (B) intensity synthesized with different ratios of $Ru(dcbpy)_3^{2+}$ and dcbpy.

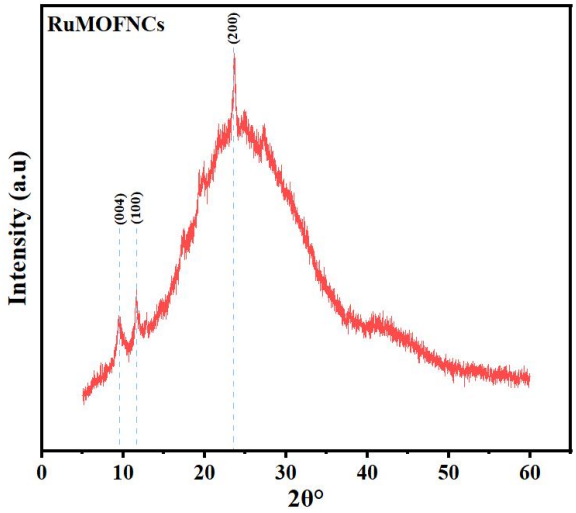


Fig. S2. X-ray Diffraction (XRD) plots of RuMOFNCs.

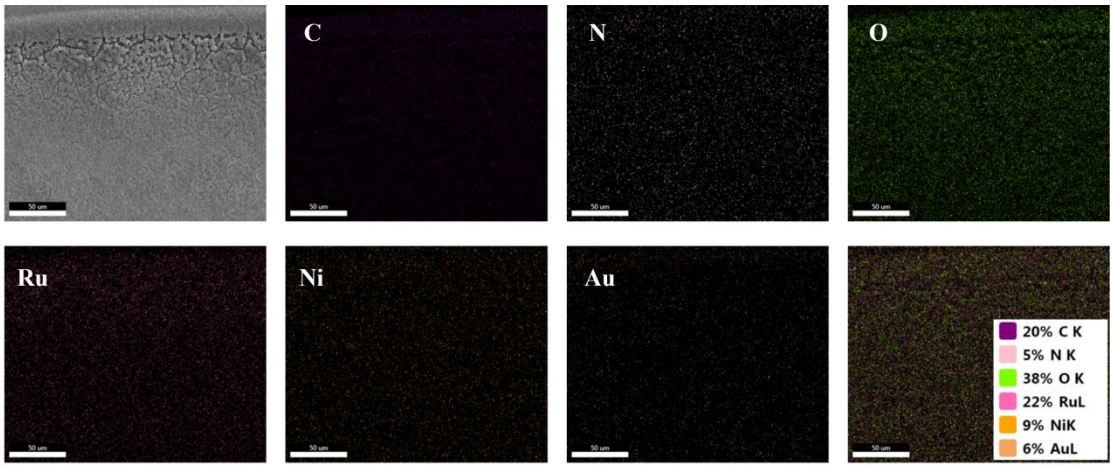


Fig. S3. The EDS spectrometer of RuMOFNCs@AuNPs, element mapping of C, N, O, Ru, Ni, Au, and sum spectrum.

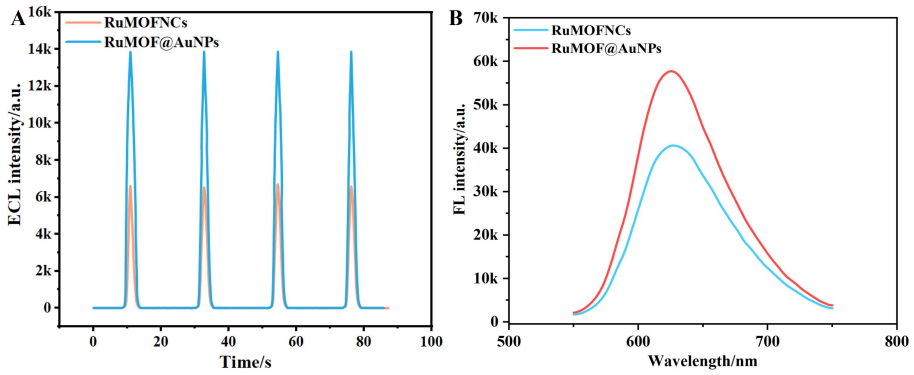


Fig. S4. AuNPs are modified to enhance the FL and ECL of RuMOFNCs based on the surface plasmon resonance effect.

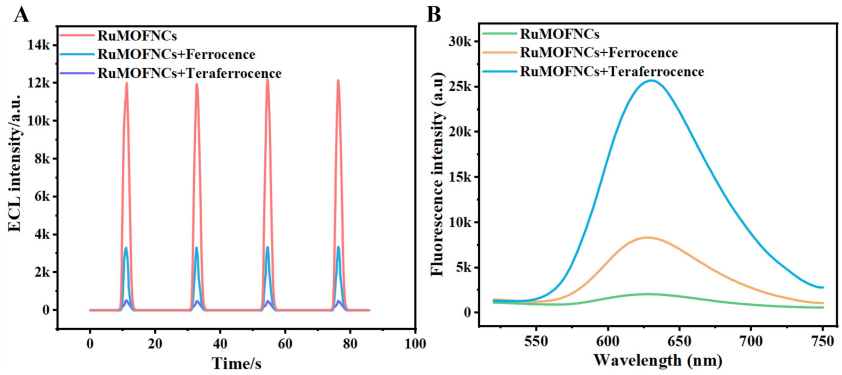


Fig. S5. FL and ECL quenching efficiency of RuMOFNCs by ferrocene and tetraferrocene.

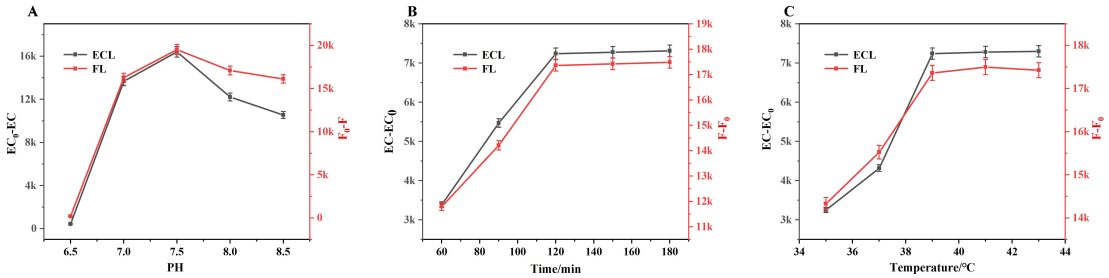


Fig. S6. Optimization of experimental conditions. pH of PBS (A), EXO III digestion time (B) and EXO III digestion temperature (C). Error bars = RSD (n = 6).

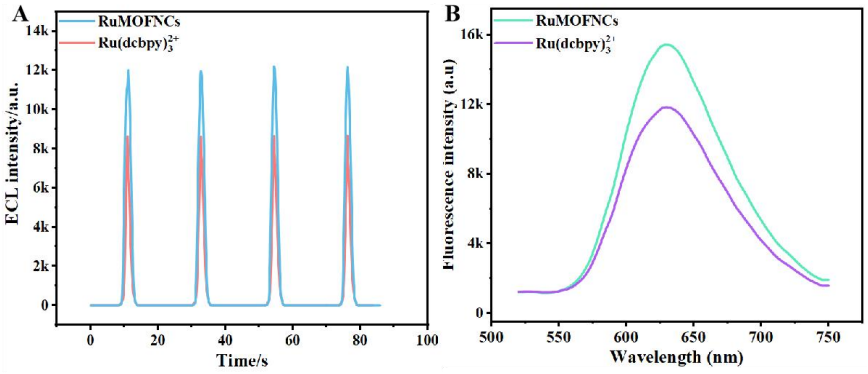


Fig. S7. The intensity comparison of ECL (A) and FL (B) of RuMOFNCs and $Ru(dcbpy)_3^{2+}$.

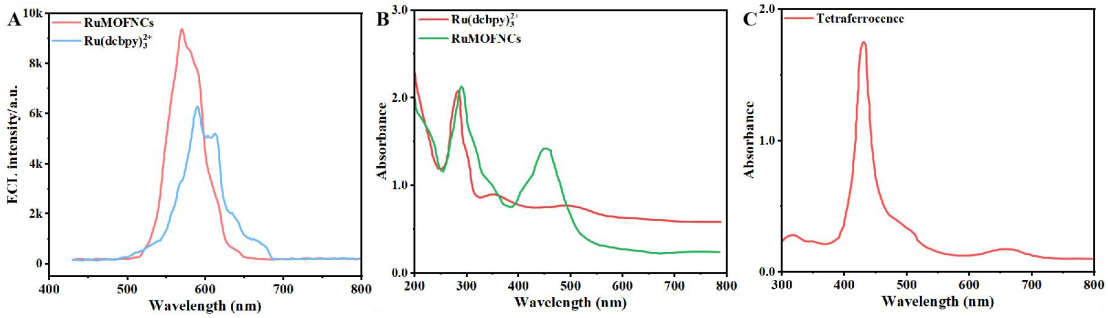


Fig. S8. The ECL spectra (A) and UV absorption spectra (B) of RuMOFNCs and $Ru(dcbpy)_3^{2+}$. UV absorption spectrum of Tetraferrocence (C).

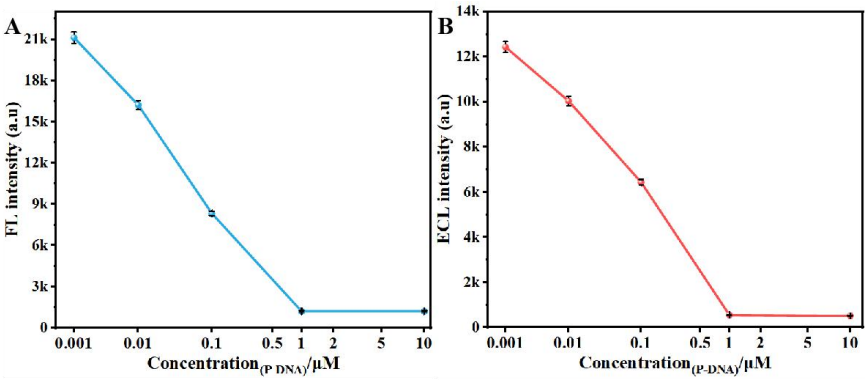


Fig. S9. Optimization of P-DNA modification in FL (A) and ECL sensing modes (B).

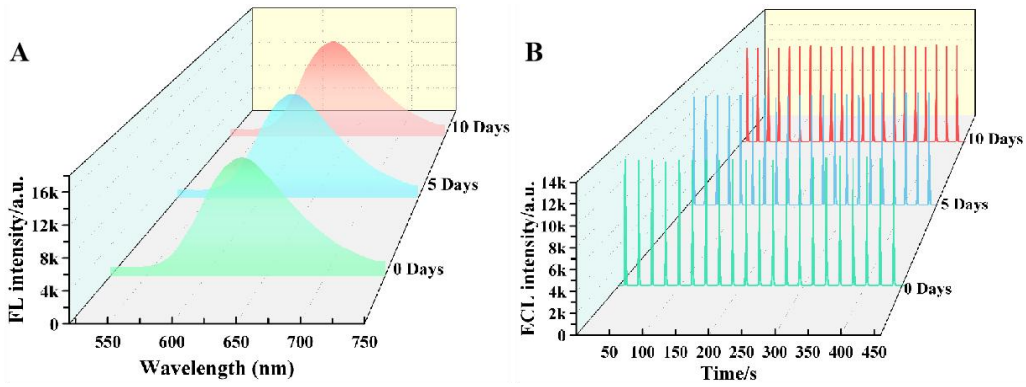


Fig. S10. Long-term stability tests in FL (A) and ECL sensing modes (B).

Table S1. Comparison of the various methods for detecting BRAF gene.

Method	Linear range	Detection Limit	Ref.
FL-ECL	0.1 fM~1 nM 0.01 fM~10 pM	10.3 aM 3.1 aM	This work
FL	200 zM~2 pM	200 zM	1
ECL	1 pM~1 nM	3.06 fM	2
SPR-ECL	0.5 pM~2000 pM	0.34 pM	3
RCA-FRET	75 fM~4.5 pM	60±10 pM	4
ECL	1 pM~1.5 nM	0.3 pM	5

Table S2. Recovery tests for BRAF gene in human serum samples.

		Measured (fM)		Recovery (%)		RSD (%)	
sample	Added(fM)	FL	ECL	FL	ECL	FL	ECL
No.1	5	4.92	4.98	98.40	99.60	0.57	0. 28
No.2	10	10.12	10.21	101.2	102.10	0.42	1.47
No.3	20	20.50	20.61	102.5	103.05	0.87	2.12

Reference

1. L. Zhang, Y. Zhang, L. Huang, Y. Zhang, Y. Li, S. Ding and W. Cheng, *Sensors and Actuators B: Chemical*, 2019, **287**, 111-117.
2. Q. Zhang, Z. Liang, Y. Nie, X. Zhang and Q. Ma, *Mikrochim Acta*, 2020, **187**, 599.
3. E. Yang, Z. Ning, F. Yin, Z. Fang, M. Chen, M. Zhang, W. Xu, Y. Zhang and Y. Shen, *Sensors and Actuators B: Chemical*, 2022, **369**.
4. M. Dekaliuk, X. Qiu, F. Troalen, P. Busson and N. Hildebrandt, *ACS Sensors*, 2019, **4**, 2786-2793.
5. Y. Liu, M. Wang, Y. Nie, Q. Zhang and Q. Ma, *Anal Chem*, 2019, **91**, 6250-6258.