

## **Electronic Supplementary Information for MS:**

### **High-sensitive self-enhanced aptasensor based on stable ultrathin 2D metal-organic layer with outstanding electrochemiluminescence property**

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## Content

S-1. Experimental section.....	3
S-2. The preparation of the Ru-L-Lys-Zr-MOF complexes.....	10
S-3. The PXRD patterns of Zr-MOL and Ru-L-Lys-Zr-MOL .....	10
S-4. SEM images of Zr-MOL and Ru-L-Lys-Zr-MOF.....	11
S-5. SEM images of the surface of electrodes .....	11
S-6. TEM images of Ru-L-Lys-Zr-MOL.....	12
S-7. The IR spectra of Ru(bpy) <sub>2</sub> (mcpbpy) <sup>2+</sup> , L-Lys, Zr-MOL/MOF, L-Lys-Zr-MOL/MOF, and Ru-L-Lys-Zr-MOL/MOF.....	13
S-8. The ECL stability of Ru-L-Lys-Zr-MOL/MOF .....	14
S-9. Effects of volume of AuNPs solution on ECL response of the aptasensor .....	15
S-10. Effects of incubation time of MUC1 on ECL response of the aptasensor.....	16
S-11. Notes and references .....	16

## S-1. Experimental section

### Reagents and apparatus

ZrCl<sub>4</sub>·8H<sub>2</sub>O, 1,3,5-Tris(4-carboxyphenyl)benzene (H<sub>3</sub>BTB), N-N'-dimethylformamide (DMF), formic acid, acetone, Nafion, gold chloride (HAuCl<sub>4</sub>), and hexanethiol (HT) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). N-hydroxy succinimide (NHS) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimidehydrochloride (EDC) were received from shanghai Medpep Co. Ltd. (Shanghai, China). Zirconyl chloride octahydrate was purchased from Shanghai Macklin Biochemical Co. Ltd. (Shanghai, China). L-Lys was bought from Chengdu kelong chemical Industry (Chengdu, China). Ru(bpy)<sub>2</sub>(mcpbpy)Cl<sub>2</sub> (bpy = 2,2'-bipyridine, mcpbpy = 4-(4'-methyl-[2,2'-bipyridin]-4-yl)butanoic acid) was brought from Suna Tech Inc. (Suzhou, China). α-1-fetoprotein (AFP), β2-microglobulin (β2-MG), and carcinoembryonic antigen (CEA) were obtained from Biocell Company (Zhengzhou, China). Exonuclease I (Exo I) and 10× Exo I buffer (67 mM glycine-KOH (pH 9.5 at 25 °C), 6.7 mM MgCl<sub>2</sub>, 10 mM 2-mercaptoethanol) were obtained from the New England Biolabs (USA). Human mucin (MUC1) and the DNA oligonucleotides utilized in this work (see Table S1) were provided from Sangon Biotech. Co. Ltd. (Shanghai, China). Phosphate buffered solution (PBS, pH 7.4, 0.1 M) was prepared by mixing standard stock solutions of 0.1 M K<sub>2</sub>HPO<sub>4</sub>, 0.1 M

NaH<sub>2</sub>PO<sub>4</sub>, and 0.1 M KCl and adjusting the pH with 0.1 M NaOH, then diluting with double distilled water. Tris-HCl buffer (pH 7.4) consisted of 20 mM Tris-HCl, 140 mM NaCl, 1 mM MgCl<sub>2</sub>, and 5 mM KCl. Ferricyanide/ferrocyanide mixed solution ([Fe(CN)<sub>6</sub>]<sup>3-/4-</sup>, pH 7.0, 5.0 mM) was employed for cyclic voltammetric (CV) investigation. All chemicals were analytical grade and used without further purification. The human serum specimens were obtained from 60 healthy volunteers in Southwest Hospital (Chongqing, China). All volunteers gave informed consent. This study was performed in strict accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects (WHO/CIOMS, 2002) and was approved by the Southwest University Institutional Review Board (IRB).

**Table S1** Sequence of oligonucleotides in this work

Name	Sequences (5' to 3')
Fc-DNA1	Fc-GTA ACC ATG TTT TTT-(CH <sub>2</sub> ) <sub>6</sub> -HS
Fc-DNA2	SH-(CH <sub>2</sub> ) <sub>6</sub> -TTA TTC GCG TCA GTA-Fc
Aptamer	SH-(CH <sub>2</sub> ) <sub>6</sub> -GCA GCA GTT GAT CCT TTG GAT ACC CTG GTG
Bridge DNA1	AAA ACA TGG TTA CCG ATC CAA GTC ACC AGG GTA TCC
Bridge DNA2	AAA ACA TGG TTA CAC TTG GAT CGT ACT GAC GCG AAT

The ECL emission was monitored with a model MPI-A electrocheminescence analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China) in 0.1 M PBS (pH 7.4) with the voltage of the photomultiplier tube (PMT) at 800 V and the potential scan from 0.2 V to 1.25 V with scan rate of 300 mV/s in the process of detection. Cyclic voltammetric (CV) measurements were taken with a CHI 660C

electrochemistry workstation (Shanghai CH Instruments, China). In all experiments, a conventional three-electrode system was used, in which an Ag/AgCl (sat. KCl) was the reference electrode, a platinum wire was the counter electrode and the modified glassy carbon electrode (GCE) was the working electrode. Powder X-ray diffraction (PXRD) patterns were collected on an XD-3 X-ray diffractometer with Cu  $K\alpha$  radiation (Purkinje, China). The surface morphology of Ru-L-Lys-Zr-MOL was characterized by scanning electron microscopy (SEM, Hitachi, Tokyo, Japan) and transmission electron microscopy (TEM, JEOL Ltd, Tokyo, Japan). The Fourier transform infrared (FTIR) spectrum was carried out using Spectrum GX FTIR spectroscopy system (PerkinElmer, USA). The morphology of the Ru-L-Lys-Zr-MOL was evaluated out by dimension edge atomic force microscopy (AFM, Bruker Co., Germany). Inductively coupled plasma-optical emission spectrometry (ICP-OES) analyses were performed on a Thermo Scientific iCAP 7400 ICP-OES instrument.

## **Synthesis**

**[Zr-MOL]** The Zr-MOL was synthesized according to the literature.<sup>S1</sup> Briefly, 50 mg  $ZrCl_4 \cdot 8H_2O$  and 62.5 mg  $H_3BTB$  were dissolved in a mixed solvent of 7.5 mL of DMF, 3.75 mL of formic acid and 0.75 mL of  $H_2O$ . Then the mixture was kept at 120 °C for 72 h to obtain a white suspension. The Zr-MOL was obtained by centrifugation and washed with DMF and acetone, respectively.

**[Zr-MOF]** The Zr-MOF was synthesized according to the literature.<sup>S2</sup> Briefly, 48 mg  $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$  and 65 mg  $\text{H}_3\text{BTB}$  were dissolved in a mixed solvent of 7.5 mL of DMF, 4.5 mL of formic acid. Then the mixture was kept at 120 °C for 72 h. The Zr-MOF was obtained by centrifugation.

**[L-Lys-Zr-MOL]** Firstly, 12 mg Zr-MOL was placed into a 10 mL beaker. Subsequently, 6 mg L-Lys dissolved in DMF was added to the reaction beaker, and the mixture was heated at 60 °C for 36 h with stirring. The L-Lys-Zr-MOL was obtained by centrifuging and dispersed in 3 mL double distilled water for further use (L-Lys-Zr-MOL suspension).

**[Ru-L-Lys-Zr-MOL]** 30 mg  $\text{Ru}(\text{bpy})_2(\text{mcpbpy})\text{Cl}_2$  was dissolved in 3 mL double distilled water, and 0.5 mL EDC/NHS (4:1) was added into the solution with a stirring for 2 h. And then, the mixtures were added into the 3 mL as-synthesized L-Lys-Zr-MOL suspension, followed by stirring for about 4 h. The Ru-L-Lys-Zr-MOL was obtained by centrifugation and dispersed in 1 mL double distilled water (Ru-L-Lys-Zr-MOL suspension).

**[Ru-L-Lys-Zr-MOF]** The Ru-L-Lys-Zr-MOF was obtained with the same synthesis steps as Ru-L-Lys-Zr-MOL except Zr-MOF was used.

**[AuNPs]** AuNPs were synthesized according to a previous report with some modifications.<sup>S3</sup> Briefly, 2.5 mL sodium citrate (1%, w/v) was added into 100 mL

boiled  $\text{HAuCl}_4$  (0.01%, w/v) solution under violent stirring. The mixture was continued boiling for 10 min under stirring when the color turned from yellow to red violet. Then, the mixture solution was cooled to room temperature, and AuNPs were thus synthesized. The prepared AuNPs solution was stored in brown glass bottles at 4 °C for further use.

[AuNPs@Ru-L-Lys-Zr-MOL] 100  $\mu\text{L}$  as-prepared AuNPs solution was added into above Ru-L-Lys-Zr-MOL suspension (1 mL) and stirred at room temperature for 12 h. The obtained AuNPs@Ru-L-Lys-Zr-MOL was stored at 4 °C for further use.

#### **Preparation of ferrocene labeled bridge DNA-AuNPs (bridge DNA-AuNPs-Fc)**

Two ferrocene labeled probes (Fc-DNA1 and Fc-DNA2) were dissolved in Tris-HCl buffer solution (10 mM, 10 mM TCEP, 1 mM EDTA, 0.1 M NaCl, pH 7.4). 20  $\mu\text{L}$  Fc-DNA1 (100  $\mu\text{M}$ ) and 10  $\mu\text{L}$  Fc-DNA2 were incubated in 1mL AuNPs. After 16 h, the conjugates were aged in PBS buffer solution for 24 h. The Fc-DNA-AuNPs was obtained by centrifugation and redispersed in 1 mL PBS buffer solution.

Bridge DNA was formed by the hybridization of two single-stranded DNA probes. Bridge DNA1 and bridge DNA2 were separately dissolved in PBS buffer solution. The two solutions (8  $\mu\text{M}$ ) were mixed with equal volumes and heated to 95 °C for 2 min. After cooled to room temperature, 300  $\mu\text{L}$  bridge DNA was mixed with above

Fc-DNA-AuNPs (1 mL) for 2 h. The obtained bridge DNA-AuNPs-Fc was centrifuged and dispersed in 1 mL PBS (0.1 M) and stored at 4 °C for future use.

### **Fabrication of aptasensor**

Pretreatment of the glassy carbon electrode (GCE): GCE was polished carefully with 0.3  $\mu\text{m}$  and 0.05  $\mu\text{m}$  alumina powders, and then sonicated in double distilled water, anhydrous ethanol and double distilled water for 5 min separately to obtain the cleaned GCE.

The fabrication process of electrode: firstly, 3  $\mu\text{L}$  Nafion and 10  $\mu\text{L}$  AuNPs@Ru-L-Lys-Zr-MOL were respectively added into the bare GCE and dried in air to get the AuNPs@Ru-L-Lys-Zr-MOL/Nafion modified GCE. Then, the obtained electrode was incubated with 10  $\mu\text{L}$  aptamer (2  $\mu\text{M}$ ) for 16 h. Subsequently, 10  $\mu\text{L}$  hexanethiol (HT) (1 mM) was dropped onto the surface of the aptamer/AuNPs@Ru-L-Lys-Zr-MOL/Nafion modified GCE and incubated for 40 min to block the nonspecific binding sites. Finally, 10  $\mu\text{L}$  bridge DNA-AuNPs-Fc was added onto the obtained electrode surface and incubated for overnight to obtain bridge DNA-AuNPs-Fc/HT/aptamer/AuNPs@Ru-L-Lys-Zr-MOL/Nafion modified GCE. After each fabrication step, PBS solution was utilized to wash the modified GCE to remove excess reagents.

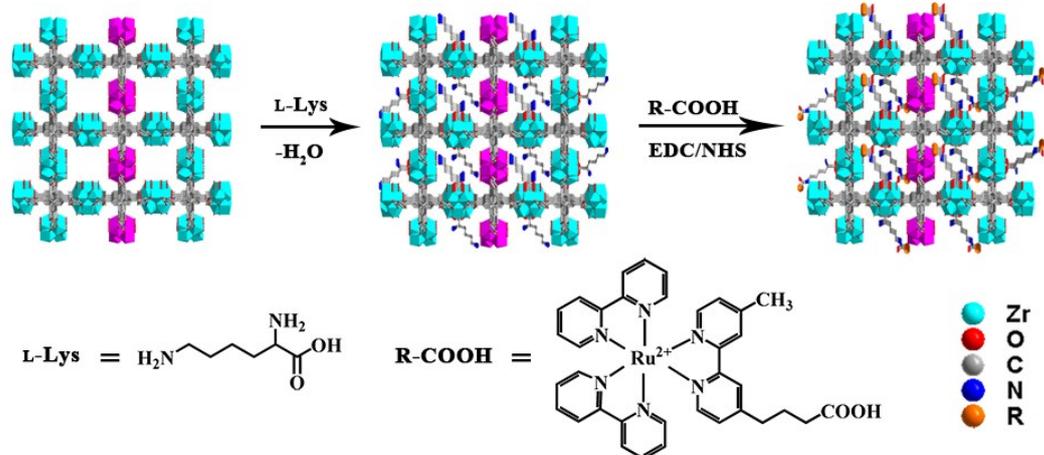
### **Measurement procedure for the analysis of MUC1 in PBS**

Firstly, different concentrations of MUC1 standard solutions were prepared utilizing PBS as solvent. Then, 10  $\mu\text{L}$  of MUC1 standard solutions with different concentrations and 6 U Exo I were dropped onto the obtained electrode surface and incubated for 90 min at 37  $^{\circ}\text{C}$ . Finally, the prepared aptasensor was rinsed with PBS to remove extra reagent and put in an ECL detector cell containing PBS (3 mL) to detect the ECL signal.

### **Measurement procedure for the analysis of MUC1 in human serum**

The recovery experiments were performed by standard addition methods in human serum to assess the feasibility of the proposed ECL aptasensor in real samples. Firstly, a series of samples containing different concentrations of MUC1 (defined as Added) were prepared utilizing human serum as solvent. Then, the proposed ECL aptasensor was used to detect MUC1 in the same way as described above. Based on the obtained ECL intensity, the corresponding concentrations of MUC1 (defined as Found) were calculated according to the obtained linear equation. The recovery was defined as the ratio between the found concentration and the added concentration, which would directly reflect the detected accuracy of the obtained ECL aptasensor.

## S-2. The preparation of the Ru-L-Lys-Zr-MOF complexes



Scheme S1 Preparation of the Ru-L-Lys-Zr-MOF complexes.

## S-3. The PXRD patterns of Zr-MOL and Ru-L-Lys-Zr-MOL

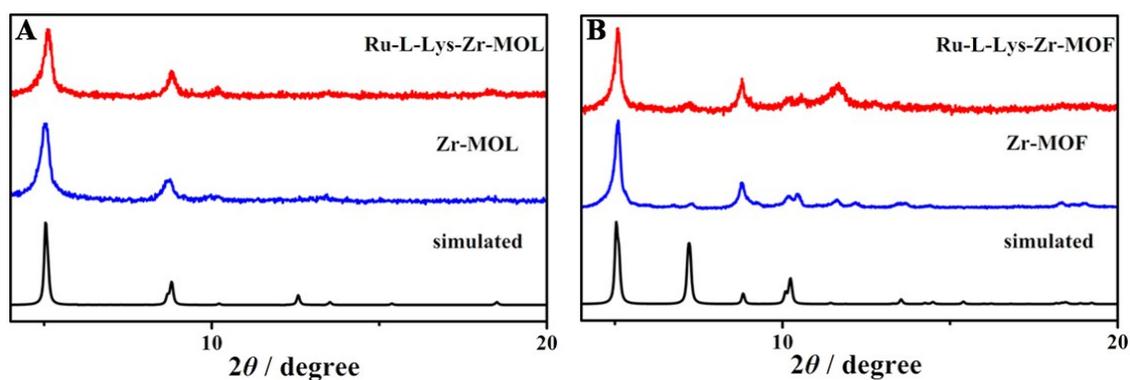
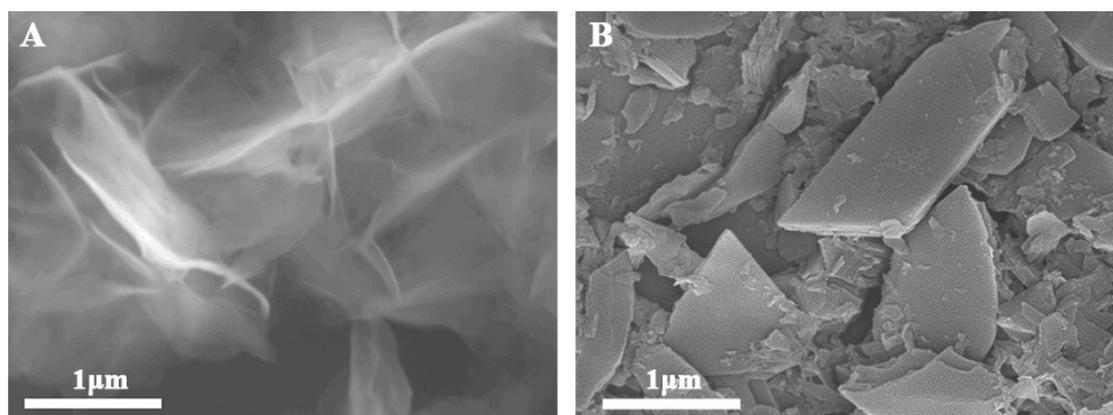


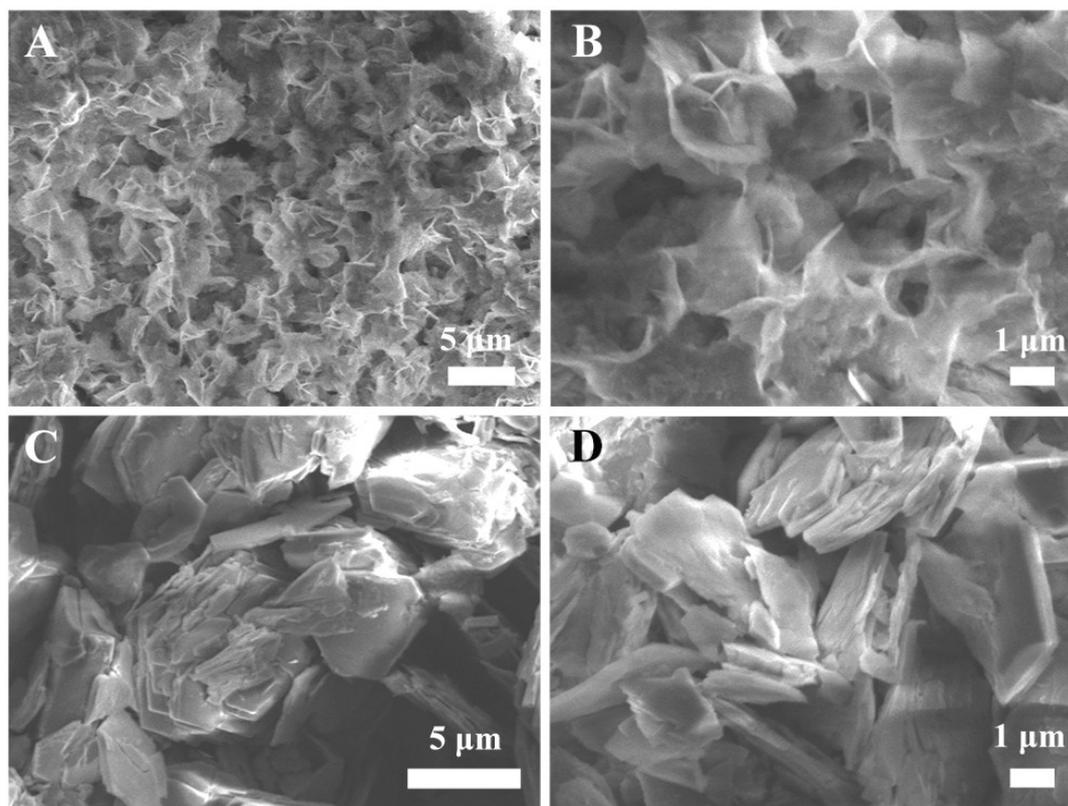
Fig. S1 (A) The powder X-ray diffraction (PXRD) patterns of Zr-MOL and Ru-L-Lys-Zr-MOL. (B) The PXRD patterns of Zr-MOF and Ru-L-Lys-Zr-MOF.

#### S-4. SEM images of Zr-MOL and Ru-L-Lys-Zr-MOF



**Fig. S2** (A) SEM image of Zr-MOL. (B) SEM image of Ru-L-Lys-Zr-MOF.

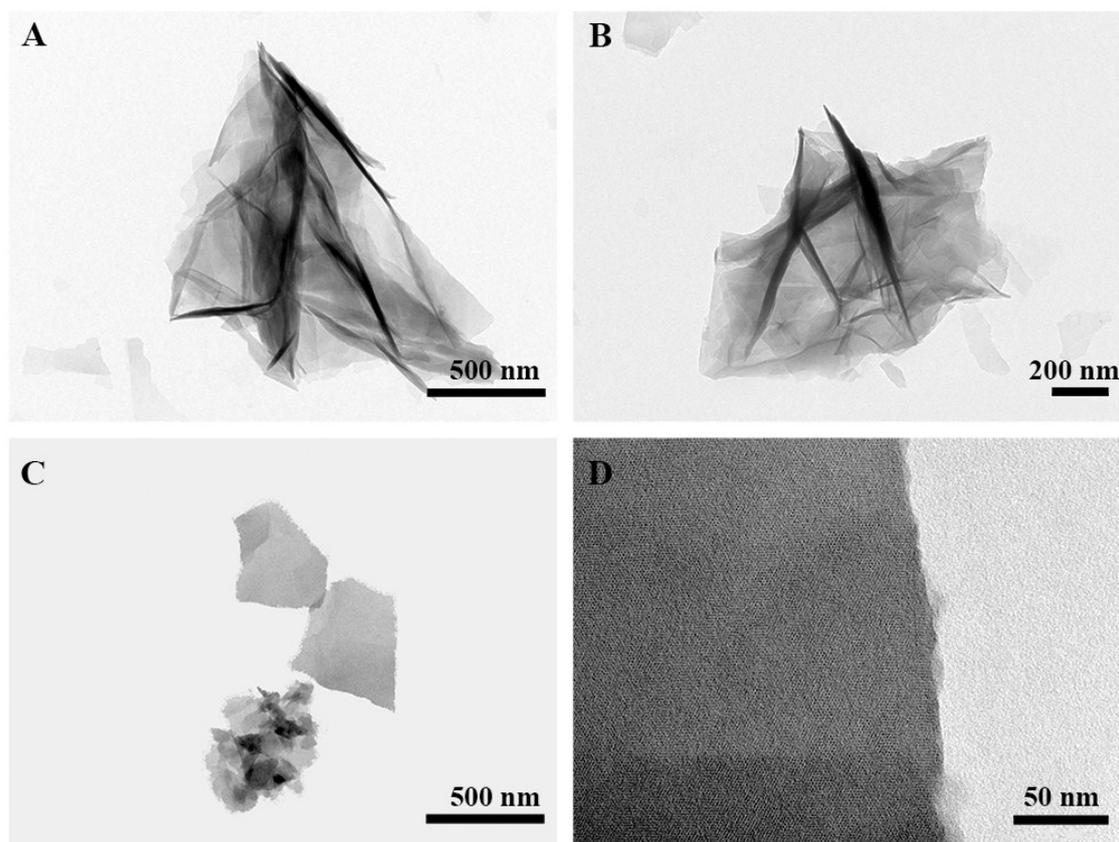
#### S-5. SEM images of the surface of electrodes



**Fig. S3** (A and B) SEM images showing the surface morphologies of AuNPs@Ru-L-Lys-Zr-MOL/Nafion modified GCE. (C and D) SEM images showing the surface morphologies of AuNPs@Ru-L-Lys-Zr-MOF/Nafion modified GCE.

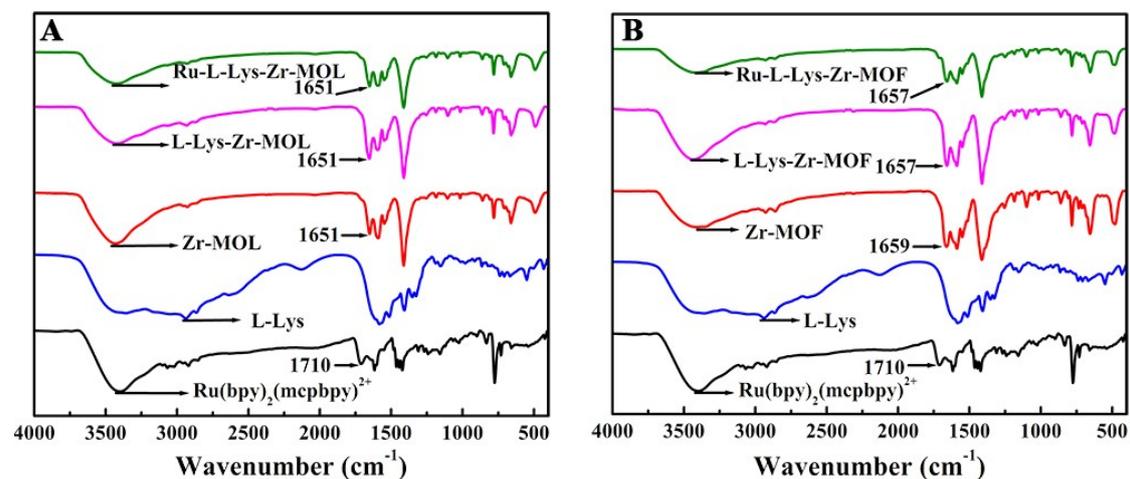
As shown in Fig. S3, the surface of AuNPs@Ru-L-Lys-Zr-MOL/Nafion modified GCE was relatively smooth, homogeneous and possessed larger surface areas compared with that of AuNPs@Ru-L-Lys-Zr-MOF/Nafion modified GCE. Owing to the ultrathin nature and large surface areas of 2D Ru-L-Lys-Zr-MOL, the AuNPs@Ru-L-Lys-Zr-MOL/Nafion modified GCE could shorten the ion/electron-transport distance, which made more Ru-L-Lys complexes could be excited and thus led to the high utilization ratio of Ru-L-Lys complexes and strong ECL signal.

#### S-6. TEM images of Ru-L-Lys-Zr-MOL



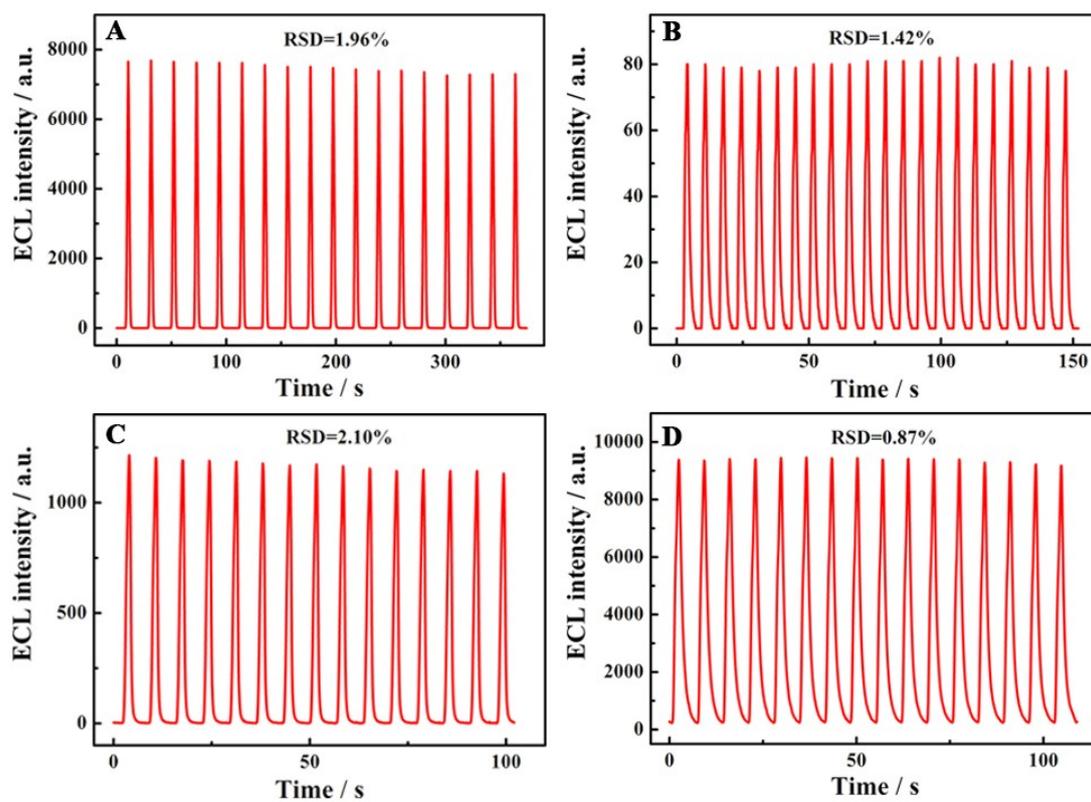
**Fig. S4** (A-C) TEM images of Ru-L-Lys-Zr-MOL. (D) HRTEM image of Ru-L-Lys-Zr-MOL.

**S-7. The IR spectra of  $\text{Ru}(\text{bpy})_2(\text{mcpbpy})^{2+}$ , L-Lys, Zr-MOL/MOF, L-Lys-Zr-MOL/MOF, and Ru-L-Lys-Zr-MOL/MOF**



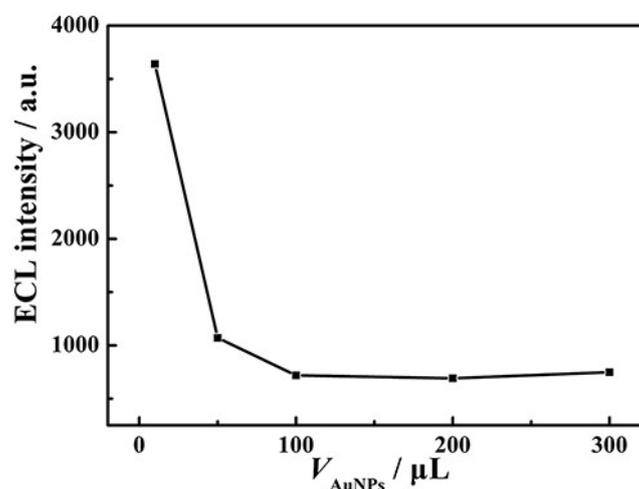
**Fig. S5** (A) The IR spectra of  $\text{Ru}(\text{bpy})_2(\text{mcpbpy})^{2+}$ , L-Lys, Zr-MOL, L-Lys-Zr-MOL, and Ru-L-Lys-Zr-MOL. (B) The IR spectra of  $\text{Ru}(\text{bpy})_2(\text{mcpbpy})^{2+}$ , L-Lys, Zr-MOF, L-Lys-Zr-MOF, and Ru-L-Lys-Zr-MOF.

### S-8. The ECL stability of Ru-L-Lys-Zr-MOL/MOF



**Fig. S6** (A) The ECL stability of Ru-L-Lys-Zr-MOL (Amplifier series was held at 2).  
(B-D) The ECL stability of Ru-L-Lys-Zr-MOF (Amplifier series was held at 2, 3 and 4, respectively).

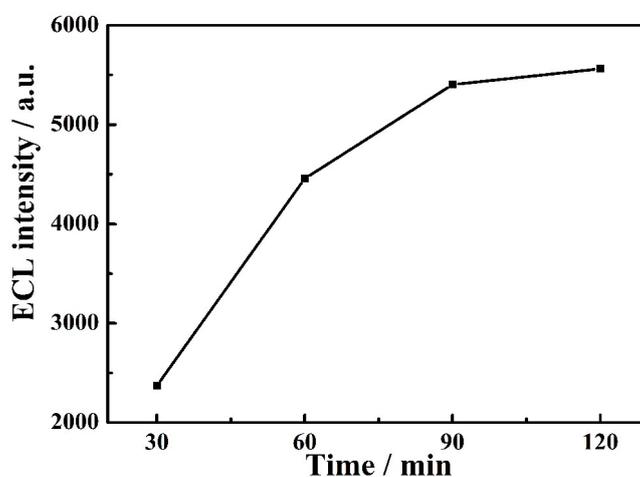
### S-9. Effects of volume of AuNPs solution on ECL response of the aptasensor



**Fig. S7** Effects of the volume of AuNPs solution on the ECL intensity of bridge DNA-AuNPs-Fc/HT/aptamer/AuNPs@Ru-L-Lys-Zr-MOL/Nafion modified GCE.

To achieve the optimum performance of the proposed ECL aptasensor for MUC1 detection, the volume of as-prepared AuNPs solution used in the synthesis of AuNPs@Ru-L-Lys-Zr-MOL was optimized. As shown in Fig. S7, as the volume of AuNPs solution increased, the ECL intensity of bridge DNA-AuNPs-Fc/HT/aptamer/AuNPs@Ru-L-Lys-Zr-MOL/Nafion/GCE decreased gradually and the relative stable value was obtained at 100  $\mu\text{L}$ . Therefore, 100  $\mu\text{L}$  was chosen as the optimal volume of AuNPs solution in the synthesis of AuNPs@Ru-L-Lys-Zr-MOL, which made the ECL emission of the subsequently prepared bridge DNA-AuNPs-Fc/HT/aptamer/AuNPs@Ru-L-Lys-Zr-MOL/Nafion /GCE could be effectively quenched by ferrocene (signal “off” state) and resulted in a low background signal.

## S-10. Effects of incubation time of MUC1 on ECL response of the aptasensor



**Fig. S8** Effects of the incubation time of MUC1 (100 pg/mL) on ECL response of the aptasensor.

## S-11. Notes and references

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(S2) L. Y. Cao, Z. K. Lin, F. Peng, W. W. Wang, R. Y. Huang, C. Wang, J. W. Yan, J. Liang, Z. M. Zhang, T. Zhang, L. S. Long, J. L. Sun and W. B. Lin, *Angew. Chem. Int. Ed.*, 2016, **55**, 4962-4966.

(S3) L. L. Pang, J. S. Li, J. H. Jiang, G. L. Shen and R. Q. Yu, *Anal. Biochem.*, 2006, **358**, 99-103.