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Electronic Supplementary Information for MS:

AIEgen-based 2D ultrathin metal-organic layer as electrochemiluminescence platform for ultrasensitive biosensing of carcinoembryonic antigen

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S-1. Materials and Reagents

Hafnium(IV) chloride ($HfCl_4$), Nafion and thrombin (TB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 4',4''',4'''',4''''''-(ethene-1,1,2,2-tetrayl)tetrakis(([1,1'-biphenyl]-4-carboxylic acid)) (H_4ETTC) was received from Beijing HWRK Chem Co., Ltd. (Beijing, China). N,N-dimethylformamide (DMF) and triethylamine (TEA) were provided from Kelong Chemical Inc. (Chengdu, China). Formic acid (HCOOH) was received from Aladdin biochemical technology Co. Ltd. (Shanghai, China). Phi29 polymerase and T4 DNA ligase were obtained from Vazyme Biotech Co., Ltd. (Nanjing, China). Deoxynucleotides (dNTPs) was bought from Genview Scientific Inc. (E1 Monte, CA, USA). Exonuclease I (Exo I) and Exonuclease III (Exo III) were brought from Thermo Fisher Scientific Inc. (Shanghai, China). Nb.BbvCI was purchased from New England Biolabs (Ipswich, MA). α-1-fetoprotein (AFP), β2-microglobulin (β2-MG), and carcinoembryonic antigen (CEA) were bought from Biocell Company (Zhengzhou, China). Human mucin (MUC1) and all the DNA oligonucleotides (Table S1) utilized in this work were provided from Sangon Biotech. Co. Ltd. (Shanghai, China). Phosphate buffered solutions (PBS, pH 7.4) were prepared with 0.1 M K_2HPO_4, 0.1 M NaH_2PO_4, and 0.1 M KCl. Tris-HCl buffer solution (pH 7.4) contained 20 mM Tris-HCl, 140 mM NaCl, 1 mM $MgCl_2$, and 5 mM KCl. TM buffer solution (pH 8.0) consisted of 20 mM Tris

and 12.5 mM $MgCl_2$ standard stock solutions. Ferricyanide solution [$Fe(CN)_6^{3-/4-}$] was prepared by dissolving potassium ferricyanide and potassium ferrocyanide with 0.1 M PBS (pH 7.0). All chemicals were analytical grade and used without further purification. All solutions were prepared with ultrapure water, which was purified by water purification system with an electrical resistance of 18.2 MΩ·cm^{-1}.

Ethical statement The human serum specimens were obtained from the Southwest Hospital (Chongqing, China). 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 study

Name	Sequences (5′ to 3′)
H1	ATACCAGCTTATTCAATTTTTTTTGAATAAGTGGT
padlock	CCACTTATTCACCTCAGCCCTACTACTAACCACTTATTCACCTCAGCCCTACTACTAA
	CCACTTATTCACCTCAGCCCTACTACTAA
assistant	TGAATAAGTGGTTAGTAGTAGG
S1-Fc	GCCCTACTACTAACCACTTATTCACCTCA-Fc

S-2. Apparatus

The electrochemistry measurements were carried out on a CHI 660C electrochemistry workstation (Shanghai Chen Hua Instrument, Shanghai, China). The ECL measurements were performed on a model MPI-A electrocheminescence analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China) Both the ECL and cyclic voltammetry (CV) measurements were equipped with a

conventional three-electrode system including GCE (glassy carbon electrode) working electrode, a platinum wire counter electrode, and an Ag/AgCl (saturated KCl) reference electrode. Powder X-ray diffraction (PXRD) patterns were collected on a XD-3 X-ray diffractometer with Cu Kα radiation (Purkinje, China). The surface morphology was characterized by scanning electron microscopy (SEM, Hitachi, Tokyo, Japan) and transmission electron microscopy (TEM, JEOL Ltd, Tokyo, Japan). The thickness was evaluated out by dimension edge atomic force microscopy (AFM, Bruker Co., Germany). The Fourier transform infrared (FTIR) spectrum was carried out using Spectrum GX FTIR spectroscopy system (PerkinElmer, USA).

S-3. Synthesis of the Hf-ETTC-MOL and Hf-ETTC-MOF

The Hf-ETTC-MOL was synthesized according to the literature[S1] with some modification. Briefly, 2 mg H_4ETTC (0.0025 mmol) was dissolved in 0.2 mL DMF solution, and 4.8 mg $HfCl_4$ (0.015 mmol) was dissolved in 0.3 mL of DMF, 0.1 mL of HCOOH and 0.2 mL of H_2O. Following that, these solutions were combined and then kept in a 120 ^oC oven for 48 h to obtain a white suspension. Finally, the Hf-ETTC-MOL was obtained through centrifugal separation, washed and then dispersed in 100 μL ultrapure water for further use. The 3D Hf-ETTC-MOF was prepared with the similar condition without the addition of water.

S-4. Preparation of circular template

The circular template was prepared by the ligation reaction. The mixed solution containing 1 μL padlock (100 μM), 3 μL assistant (100 μM) and 96 μL TM buffer was

heated to 65 °C for 10 min and slowly cooled down to room temperature for 20 min. After adding 10 μL T4 DNA ligase (2 U/μL) and 10 μL 10 × T4 DNA ligase reaction buffer, the reaction system was incubated 5 h at 16 °C to achieve intramolecular ligation. Following that, 20 U Exo I and Exo III were added at 37 °C for 2 h to cut off the extra nucleotides to form circular templates. Finally, the reaction was terminated by a thermal treatment at 85 °C for 15 min, and the resultant products were stored at 4 °C for further use.

S-5. RCA reaction

First, H1 was heated to 95 °C for 5 min and chilled to room temperature to ensure the formation of the hairpin structure. Then, the RCA reaction was conducted in a volume of 49 μL containing 10 μL H1 (1.0 μM), 10 μL circular template, 10 μL dNTPs (500 μM), 4 μL phi29 DNA polymerase (1 U/μL), 5 μL Nb.BbvCI endonuclease (1 U/μL) and 10 μL CEA (1 fg/mL to 1 ng/mL) at 37 °C for 3 h.

S-6. Fabrication of aptasensor

The GCE was first pretreated according to previous method.[S2] Bare GCE was polished carefully with 0.3 and 0.05 μm alumina powder respectively. After that, the electrode was sonicated successively in ultrapure water, anhydrous ethanol and ultrapure water for 5 min to obtain a clean electrode surface. Then, the GCE was dried in air at room temperature. Next, 3 μL of Nafion dropped onto the pretreated GCE and

dried in the air to obtain a Nafion modified electrode (Nafion/GCE). Afterward, 10 μL of Hf-ETTC-MOL was dropped on the Nafion/GCE and dried in air to get a uniform layer. Subsequently, the modified electrode (Hf-ETTC-MOL/Nafion/GCE) was incubated with Fc-S1 (15 μL) for overnight. After that, the modified electrode was washed with PBS solution to remove the unbound reagents.

S-7. ECL detection

For ECL detection, 10 μL products of RCA reaction was dropped onto the prepared electrode and incubated at 37 °C for 2 h. After incubation, the electrode was rinsed with PBS solution to remove the unbound reagents. Finally, the ECL signal was detect in 2 mL PBS solution containing 10 μL TEA, with a potential scanning from 0 to 1.6 V at a scanning rate of 300 mV / s.

S-8. PXRD patterns

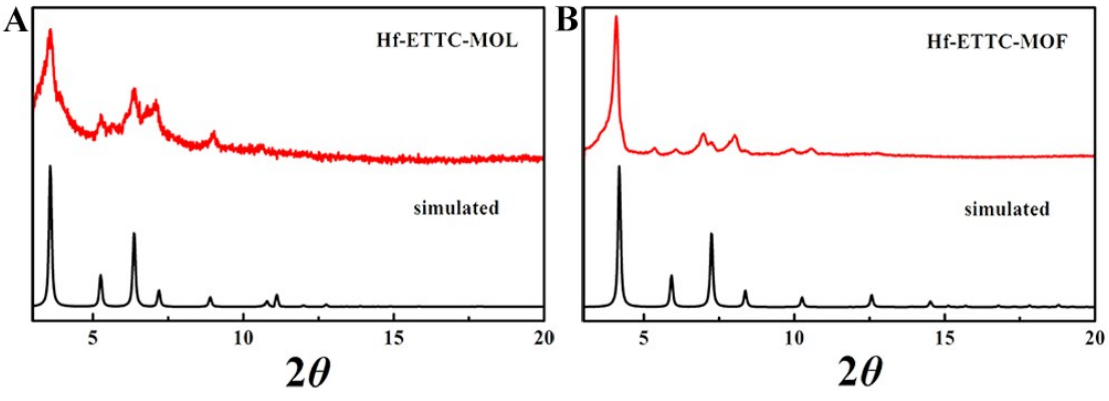

Fig. S1 (A) The experimental and simulated PXRD patterns of Hf-ETTC-MOL. (B) The experimental and simulated PXRD patterns of Hf-ETTC-MOF.

A comparison of the experimental and simulated PXRD patterns of Hf-ETTC-MOF shows that the Hf-ETTC-MOF is isostructural to PCN-94 reported by Zhou's group.[S3]

S-9. Scanning electron microscopy images

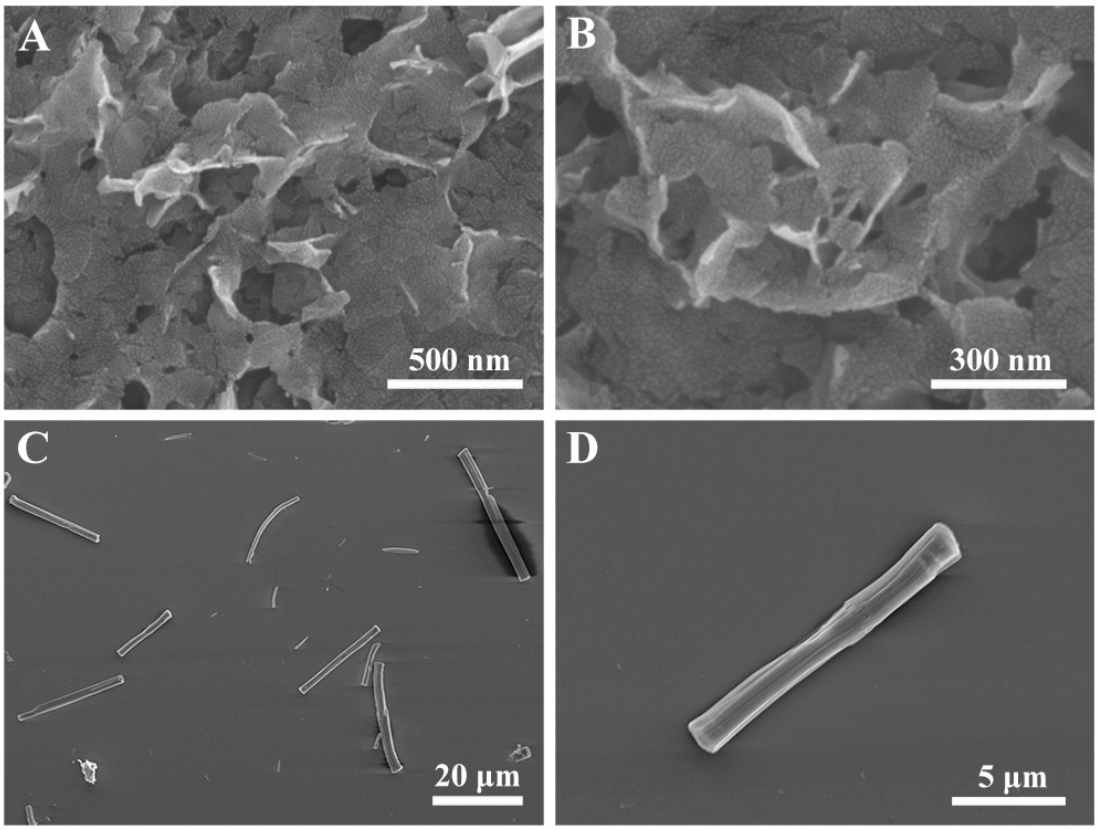

Fig. S2 (A, B) SEM images of Hf-ETTC-MOL. (C, D) SEM images of Hf-ETTC-MOF.

S-10. Transmission electron microscopy images

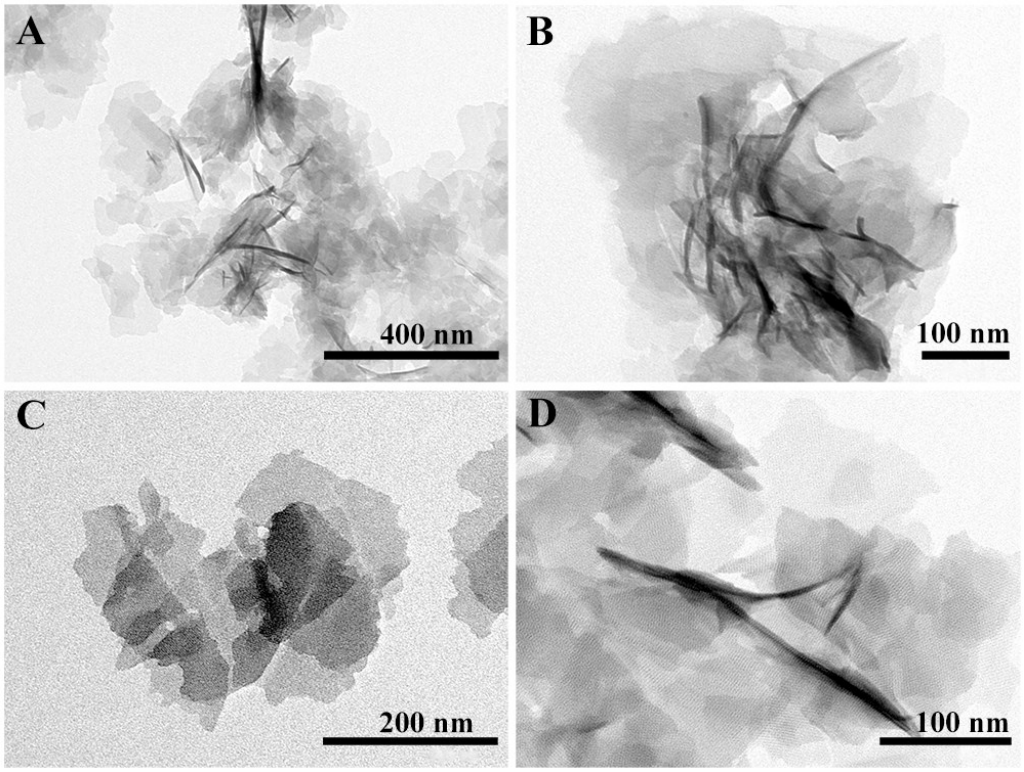

Fig. S3 TEM images of Hf-ETTC-MOL.

S-11. Atomic force microscopy images

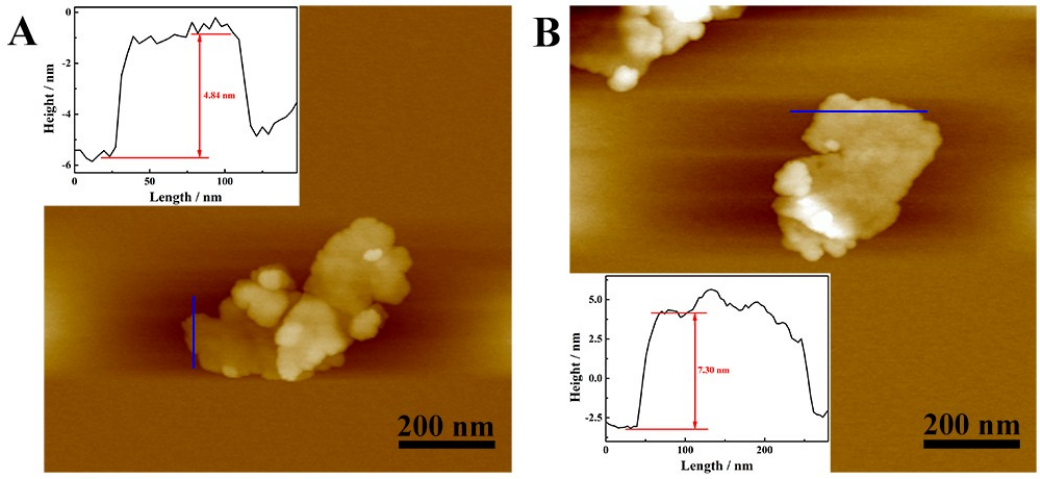

Fig. S4 AFM images and the height profiles of Hf-ETTC-MOL.

S-12. IR spectra of Hf-ETTC-MOL and Hf-ETTC-MOF

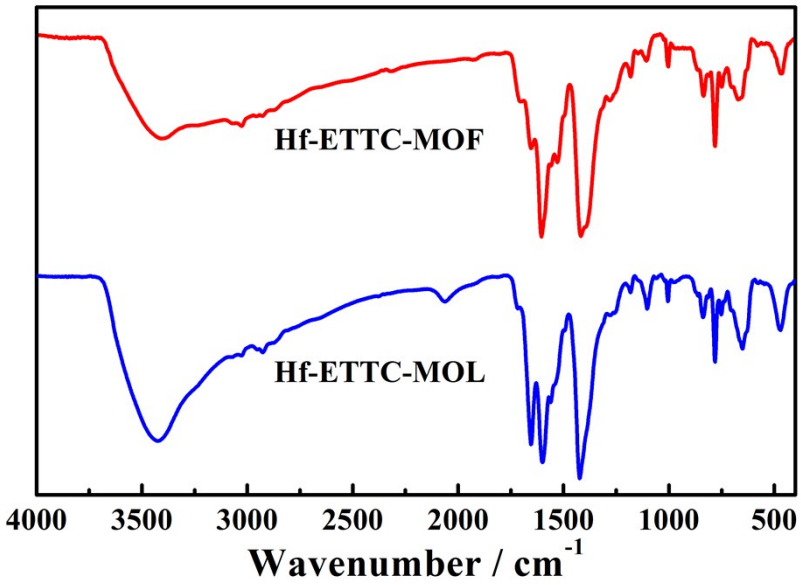

Fig. S5 The IR spectra of Hf-ETTC-MOL and Hf-ETTC-MOF.

S-13. UV-vis absorption spectroscopy of Hf-ETTC-MOL

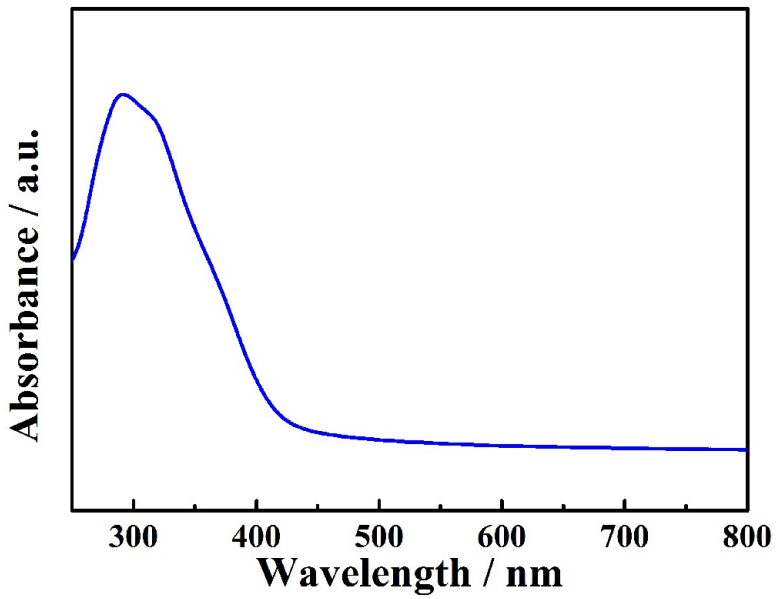

Fig. S6 UV-vis absorption spectroscopy of Hf-ETTC-MOL

S-14. ECL efficiency calculation

ECL efficiency was calculated as following equation:[S4]

$$\emptyset_x = \emptyset_{st}\left(\frac{\int_0^t Idt}{\int_0^t idt}\right)_x \Big/ \left(\frac{\int_0^t Idt}{\int_0^t idt}\right)_{st}$$

$\emptyset_{st}$ represents the ECL efficiency of $[Ru(bpy)_3]^{2+}$ (1 mM and 0.1 M $(TBA)BF_4/CH_3CN$, $(TBA)BF_4$ = tetrabutylammonium tetrafluoroborate) via annihilation, taken as 5.0%, *I* represents ECL intensity, *i* represents current value, and *x* represents the sample.

S-15. CV and ECL responses of the aptasensor

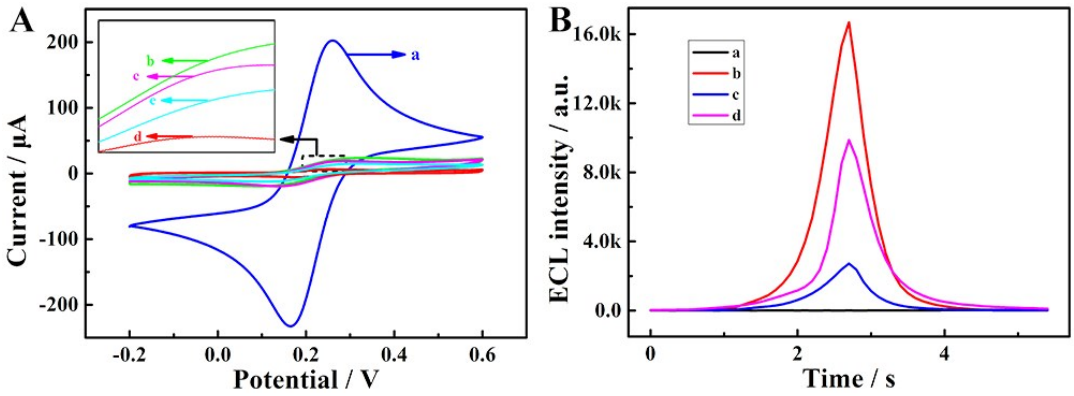

Fig. S7 (A) CV responses of the aptasensor: (a) bare GCE, (b) Nafion/GCE, (c) Hf-ETTC-MOL/Nafion/GCE, (d) S1-Fc/Hf-ETTC-MOL/Nafion/GCE, (e) S2/S1-Fc/Hf-ETTC-MOL/Nafion/GCE. (B) ECL responses of the aptasensor: (a) bare GCE, (b) Hf-ETTC-MOL/Nafion/GCE, (c) S1-Fc/Hf-ETTC-MOL/Nafion/GCE, (d) S2/S1-Fc/Hf-ETTC-MOL/Nafion/GCE.

S-16. Detection limit calculation

Detection limit was calculated as following equation:[S5]

$$I_L = I_B + k\, s_B$$

I_B represents the average ECL intensity of blank samples with three parallel tests, s_B represents the standard deviation of blank samples with three parallel tests, k represents the signal-to-noise ratio value, taken as 3, and I_L represents the smallest detectable signal.

Then the I_L value was put into the linear equation to get the detection limit.

S-17. Notes and references

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