Supporting information for

Novel Porphyrin Zr Metal−Organic Frameworks (PCN-224) Based Ultrastable Electrochemiluminescence System for PEDV Sensing

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Table of Contents:

EXPERIMENTAL DETAIL

1. Materials and Reagents.

N, N-dimethylformamide (DMF), benzoic acid, potassium peroxydisulfate ($K_2S_2O_8$), absolute ethyl alcohol, acetone, zirconium(IV) chloride, ammonium tetrabutyl hexafluorophosphate and acetonitrile were purchased from Sinopharm Chemical Reagent Company (China). Meso-Tetra (4-carboxyphenyl) porphine was purchased from Aladdin. BSA was purchased from Sigma-Aldrich Chemicals Co. (Beijing, China). Phosphate buffer (PBS) is prepared by adding 0.9% NaCl to phosphate buffer. PBST solution was prepared by adding 0.02% Tween 20 into PBS

solution at pH 7.4. All reagents were used directly without any further purification.

The rabbit anti-human IgG (1 mg·mL^{-1}) was purchased from Sangon biotech Co. Ltd. (Shanghai, China). PEDV（Ag, 2.8 mg/mL), EV71, PCV, PRRSV, TGEV and PEDV antibody (Ab1) were gotten from the State Key Laboratory of Agricultural Microbiolgy of Huazhong Agricultural University (Wuhan, China). Deionized (DI) water produced by a Milli-Q Millipore system (18.2 MΩ cm-1 resistivity, Millipore Corp.) was used throughout this work.

2. Measurements.

ECL measurements were carried out on a model MPI-EII electroluminescence analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., China) and the voltage of the photomultiplier tube (PTM) was set at 800 V in the process of detection. CHI 600B electrochemical work-station (Shanghai CH Instruments, China) was used for cyclic voltammograms (CVs) and electrochemical impedance spectroscopy (EIS) measurements. Transmission electron micrographs (TEM) were measured on a FEI Tecnai G20 transmission electron microscope, using an accelerating voltage of 200 kV. Ultraviolet-visible (UV-vis) absorption spectra were obtained by PerkinElmer Lambda 25 UV/VIS Spectrometer (Thermo Nicolet, US). Photoluminescence (PL) spectra were obtained on an Edinburgh FLS920 spectrometer with an integrating sphere attachment under excitation of 365 nm light. The zeta potential was measured with a Zeta-sizer Nano ZS90 DLS system (Malvern, England). Fourier transform infrared (FT-IR) spectra were collected on a Nicolet Avatar-330 spectrometer.

3. Synthesis of TiO_2-PAA composite. TiO_2-PAA composite was performed according to reference[1]. Specific methods were as follows: PAA was dissolved in distilled water at 25□ and the temperature was maintained for 2 h. Then, the PAA/TiO_2 dispersion was prepared, in which 0 %, 1 %, 2 %, 3 %, 4 % and 5 % PAA were added into 0.5 mg/ mL TiO_2 solution, respectively. The dispersion was treated on a magnetic stirrer with a rotation speed of 900 r/min for 24 h to increase the PAA molecules adsorbed on the surface of TiO_2. In order to improve the dispersity of TiO_2, ultrasound was conducted for 1 h before use.

4. Preparation of PCN-224. The synthesis of PCN-224 was based on the method

reported by Feng's groups [2]. Generally, 10 mg TCPP, 30 mg $ZrCl_4$ and 0.4 mg benzoic acid were homogeneously mixed in 3 mL DMF under the condition of ultrasound, and then transferred to a high-pressure reactor for reaction at 120□ for 24 hours. After the reaction, the product was naturally cooled, and the resulting dark purple cubic crystals were washed with DMF and acetone for freeze-drying. The prepared PCN-224 was stored at 4 □ for later use. During the construction of the sensor, 0.1 M pH 7.4 PBS was used to dissolve PCN-224 into a solution of 4 mg/mL. Before the construction of the sensor, 400 mM EDC and 100 mM NHS were used to activate the carboxyl group and the mixture underwent centrifugation.

5. Calculation of electrochemical active surface area[3]. In order to calculate the electrochemical active surface areas of bare GCE and various modified electrodes, the cyclic voltammetry (CV) of potassium ferrocyanide, as a redox probe, was performed by Randles-Sevcik equation:

$$Ip = (2.69 \times 10^5)\, n^{3/2}\, A\, C^*\, D^{1/2}\, \upsilon^{1/2}$$

Where Ip refers to the anodic peak current, n is the total number of electron transferred (n =1), A is the effective surface area of the electrode, D is the diffusion coefficient for $K_3[Fe(CN)_6]$= 7.6×10^{-6} cm^2 S^{-1}, C* is the concentration of $K_3[Fe(CN)_6]$ and ν is the scan rate. The effective surface areas of various electrodes were calculated from the slope of the Ip versus $v^{1/2}$ plot. The surface area of GCE (0.07 cm^2) is less than surface areas of other electrodes. The calculated surface areas for PCN-224/GCE, TiO_2/GCE, TiO_2-PCN224/GCE were 0.15, 0.17, 0.13 cm^2, respectively.

6. PEDV isolation and sampling procedure. The Vero cells in good condition were used for PEDV isolation. The culture medium was discarded, and the cells were washed with PBS solution for 3 times to remove serum completely. 5 mL virus suspension was added into 75 cm^2 cell culture flask, and trypsin was added at the final concentration of 10 μg/mL. The flask was left in CO_2 incubator at 37 ℃ for 1 h. Then, the liquid was discarded and 15 mL DMEM solution containing 10 μg/mL trypsin without serum was added. The flask was continued to culture in CO_2 incubator at 37 ℃ until 90% of the cells were infected under an inverted microscope. The virus was collected by repeated freeze-thaw the cells for 3 times. The virus suspension was

centrifuged at 12000 rpm for 10 min. The supernatant was collected and divided into small tubes, stored at -80℃ until use.

The real samples were gotten from the State Key Laboratory of Agricultural Microbiolgy of Huazhong Agricultural University. The real samples were the fecal samples. Twenty-five fecal samples were taken from pigs with clinical diarrhea from different pig farms in Hubei between 2017 and 2019. The fecal samples were diluted with PBS and then frozen and thawed repeatedly for three times. The supernatants were collected by centrifugation stored at -80°C before utilization.

7. The ECL detection mechanism on TiO_2-PCN-224 in $S_2O_8^{2-}$ solution is elucidated as below[4]:

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{2-} + SO_4^{\cdot -} \quad S1$$

$$SO_4^{\cdot -} \rightarrow SO_4^{2-} + h^+ \quad S2$$

$$TiO_2 + h^+ \rightarrow TiO_2(h^+) \quad S3$$

$$TiO_2(h^+) + e^- \rightarrow TiO_2^* \quad S4$$

$$TiO_2^* \rightarrow TiO_2 + hv \quad S5$$

$$PCN\text{-}224 + e^- \rightarrow PCN\text{-}224(e^-) \quad S6$$

$$PCN\text{-}224(e^-) + TiO_2(h^+) \rightarrow PCN\text{-}224 + TiO_2^* \quad S7$$

$$PCN\text{-}224(e^-) + TiO_2(h^+) \rightarrow PCN\text{-}224^* + TiO_2 \quad S8$$

Supporting Figures:

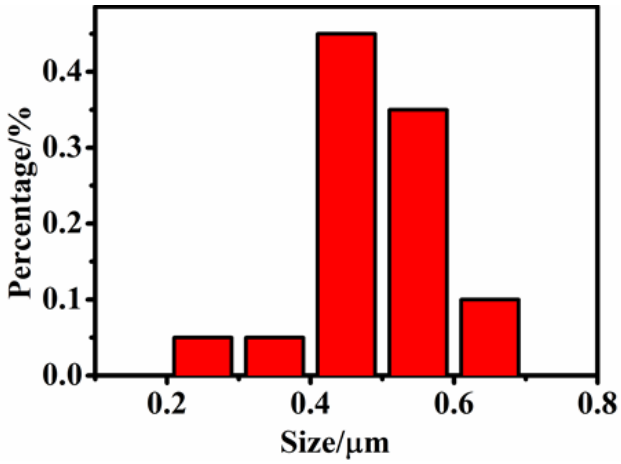

Figure S1. Size distribution of PCN-224.

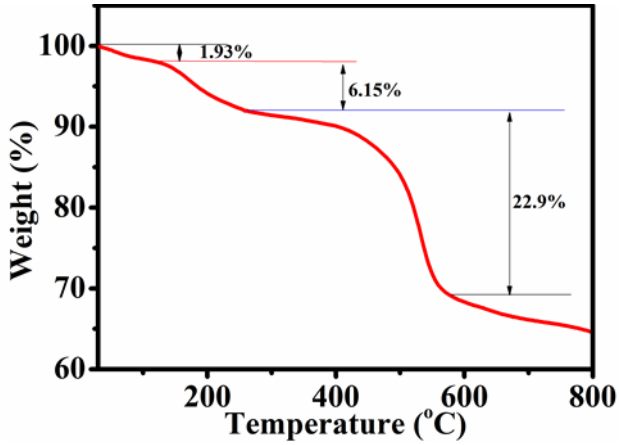

Figure S2. Thermogravimetric analysis of PCN-224.

Before 158 ℃, the weight loss rate of PCN-224 is 1.93 % due to the evaporation of water and organic solvents trapped in the hole. The second weight loss is from 158 ℃ to 284 ℃, which is mainly caused by the removal of coordination water on Zr6 cluster, and the loss rate is 6.15%. Finally, there is no significant weight loss in the temperature range of 290 ℃~ 400 ℃. The destruction of organic bonds is at temperature more than 400 ℃, leading to a large loss of weight, with a loss rate of 22.9% [5,6]. This result demonstrates that PCN-224 is relatively stable among MOFs material.

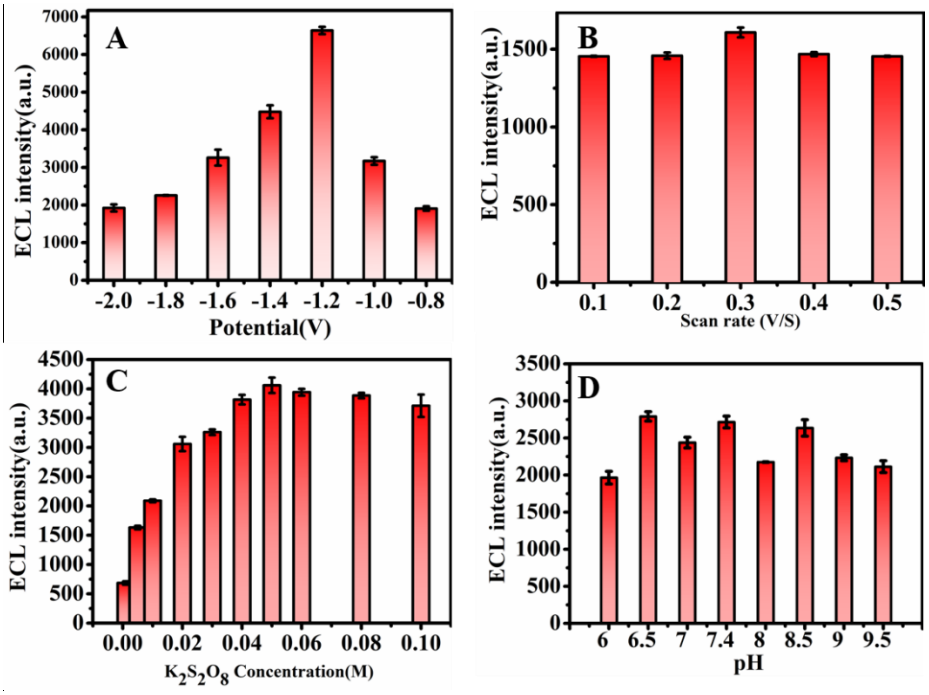

Figure S3. ECL intensity of PCN-224 (A) in different potential windows(0 V～-0.8 V, 0 V～-1.0 V, 0 V～-1.2 V, 0 V～-1.4 V, 0 V～-1.6 V, 0 V～-1.8 V, 0 V～-2.0 V); (B) with different scan rate (0.1 V/s，0.2 V/s，0.3 V/s，0.4 V/s，0.5 V/s); (C) with different concentration of $K_2S_2O_8$ (0.005M、0.01M、0.02M、0.04M、0.05M、0.06M、0.08M and 0.10M); (D) under different pH (pH 6.0, 6.5, 7.0, 7.4, 8.0, 8.5, 9.0, 9.5).

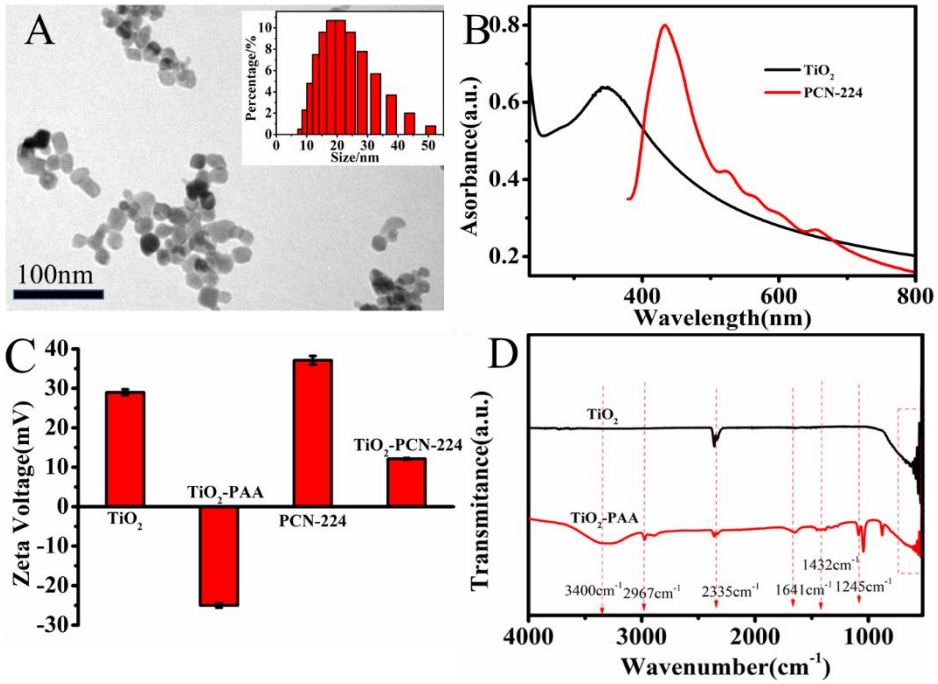

Figure S4. (A)TEM image of TiO_2 NPs (Inset is the size distribution); (B) UV- vis spectra of TiO_2 NPs and PCN-224; (C) Evolution of Zeta potential of TiO_2 NPs, TiO_2-PAA NPs, PCN-224 and TiO_2-PCN-224; (D)FT-IR spectra of TiO_2 and TiO_2-PAA.

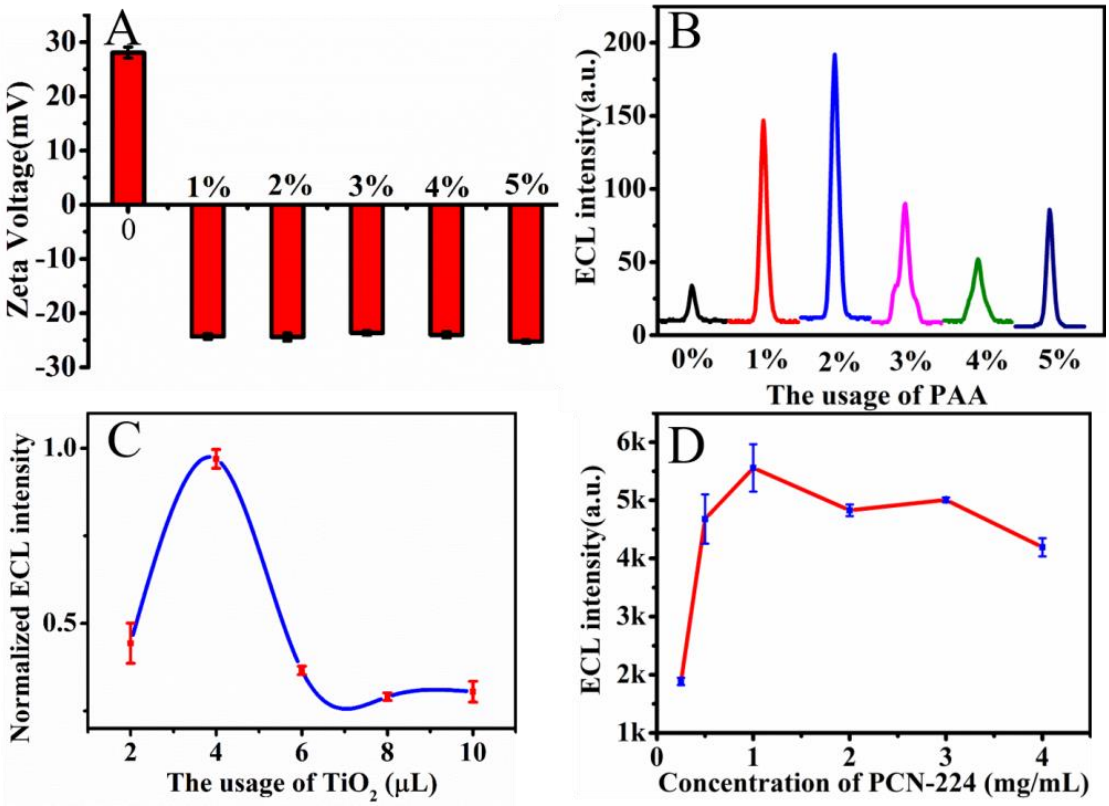

Figure S5. Influence of PAA dosage to the Zeta potential (A) and ECL intensity (B) of TiO_2; The usage of TiO_2 (C) and the concentration of PCN-224 (D) on ECL response of immunosensor.

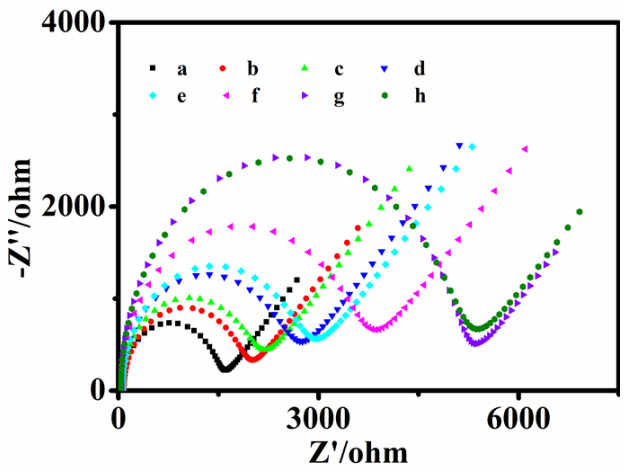

Figure S6. Immunosensor Nyquist plots of the proposed biosensor to PEDV with varying concentrations：(a) 0.001 ng/mL; (b) 0.01 ng/mL; (c) 0.1 ng/mL; (d) 1 ng/mL; (e) 2 ng/mL ;(f) 6 ng/mL; (g) 8 ng/mL; (h) 10 ng/mL.

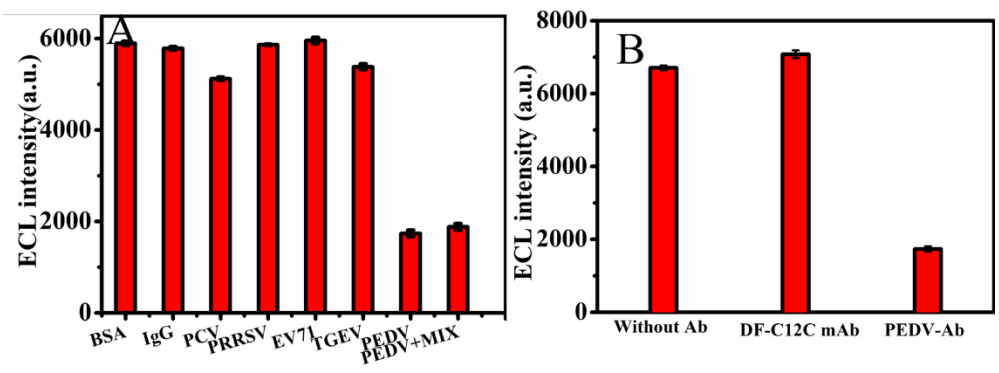

Figure S7. (A) Histogram for the specificity of this method for PEDV detection: BSA (1 ng/mL), IgG (3 ng/mL), PCV (3 ng/mL), PRRSV (3 ng/mL), EV71(3 ng/mL), TGEV (3 ng/mL), PEDV (3 ng/mL), and a mixture containing 1 ng/mL BSA, 3 ng/mL IgG, 3 ng/mL PCV, 3 ng/mL PRRSV, 3 ng/mL EV71, 3 ng/mL TGEV and 3 ng/mL PEDV. (B) ECL response of the proposed biosensor to PEDV with varying condition: Without PEDV Ab, DF-C12CmAb (Huang-long-bing antibody) and PEDV Ab.

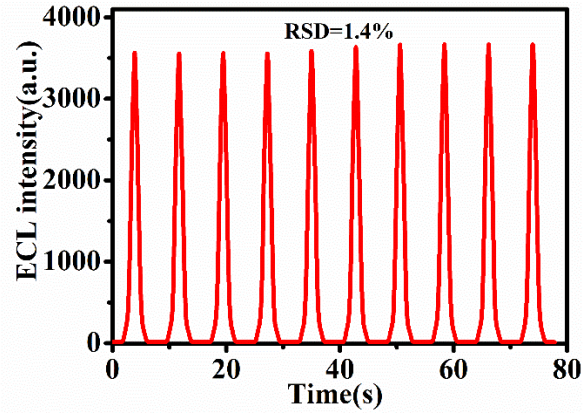

Figure S8. Stability of the proposed method under consecutive 10 cyclic potential scans. The potential scanning was set from -1.2 V to 0 V with a scan rate of 0.3 V/s.

Supporting Tables:

Table S1. Recoveries of PEDV from the excrement detected by the proposed immunosensor (n = 3).

Sample NO.	Added (pg/mL)	Found(pg/mL) (x± s, n = 3)	R.S.D (%)	Recovery (%)
1	10	11± 4	1.7	110.0%
2	100	103± 37	1.5	103.0%
3	1000	940± 31	7.2	94.0%

Table S2. Comparison between our proposed ECL platform with indirect ELISA assay (25 pig excrement samples).

Detection methods	**Negative samples**	**Positive samples**	**Total**
ECL	20	5	25
ELISA	18	7	25

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