Supporting Information

Multiple signal amplification strategy of porous materials and novel controlled release self-on mode for ultrasensitive immunoassay

Lu Zhao [a], Xuejing Liu [a], Xiang Ren [a], Zhongfeng Gao [a], Hongmin Ma [a], Dan Wu *[a], Qin Wei [a], Huangxian Ju [a,b]

[a] School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, P. R. China.

[b] State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, P. R. China.

Corresponding author

Tel.: +86 531 82765730;

Fax: +86 531 82765969;

E-mail: wudan791108@163.com (Dan Wu)

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Chemicals and Apparatus. PSA antigen, PSA aptamer and ssDNA were both brought from Sangon Biotech (Shanghai) Co., Ltd.. $HAuCl_4 \cdot 4H_2O$, $AgNO_3$, L-ascorbic acid, $AgClO_4$, rubrene, $FeCl_3 \cdot 6H_2O$, sodium citrate, ammonium acetate, citric acid, H_3BTC, $K_2S_2O_8$, N'-(ethylcarbonimidoyl)-N,N-dimethylpropane-1,3-diamine monohydrochloride (EDC), N-Hydroxysulfosuccinimide sodium salt (NHS), KH_2PO_4, Na_2HPO_4 were brought from Macklin Biochemical Co., Ltd. (Shanghai). CH_3CN, CH_2Cl_2, I_2, ethanol and glycol were all supplied by Sinopharm Chemical. The phosphate buffered saline (PBS) was used as the detection solution which was prepared by mixing KH_2PO_4 (0.1 M) and Na_2HPO_4 (0.1 M). Furthermore, ultrapure water (18.25 $M\Omega \cdot cm^{-1}$) was employed throughout this research. DNA sequences are as follows:

PSA aptamer: 5'-ATTAAAGCTCGCCATCAAATAGC-3'

Anchor DNA: 5'-COOH-GCTATTTGACAGCTTTAAT-3'

The morphologies and sizes of as-prepared nanomaterials were characterized by scanning electron microscope (SEM, JEOL JSM-6700F microscope, Japan), transmission electron microscope (TEM, JEM-1400, Japan) and high resolution transmission electron microscope (HRTEM, JEM-2100F, Japan). X-ray powder diffraction (XRD) patterns were acquired with a D8 advance X-ray diffractometer (Bruker AXS, Germany). The pore properties were tested by specific surface area aperture analyzer (BET, TristarII, USA). Meanwhile, the elemental composition of nanoparticles was obtained by X-ray photoelectron spectroscopy (XPS, Escalab MK II, UK). The UV-vis absorption spectra were acquired by the UV-vis spectrophotometer (UV-2450, Shimadzu, Japan). In addition, the cyclic voltammetry (CV) and

electrochemical impedance spectroscopy (EIS) measurements were realized with an electrochemical workstation (Zahner Zennium PP211, Germany). The ECL signals were monitored by the MPI-E Electrochemiluminescence Analyzer (Xi'an remax Electronic Science Tech. Co. Ltd., Xi'an China).

Preparation of the Ag/Au@Rub-antigen. Firstly, 20 μL solution mixed by 40 mM EDC and 10 mM NHS was added into 1 mL sonicated Ag/Au@Rub solution, which was stirred at 4 °C for 2 h. Next, add 20 μL of PSA antigen solution with different concentrations and keep incubated for 8 h. After centrifuging, the Ag/Au@Rub-antigen was successfully prepared.

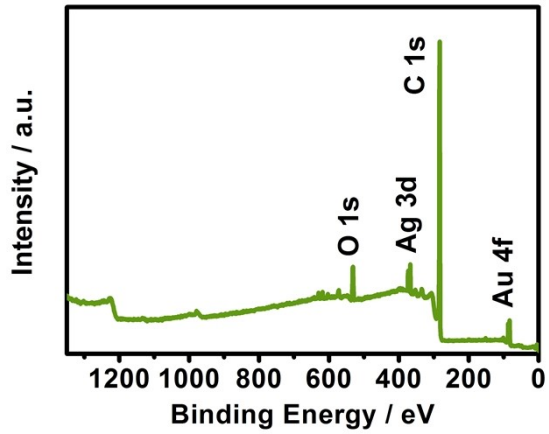

Figure S1. XPS survey spectrum of as-prepared Ag/Au@Rub PNCCS.

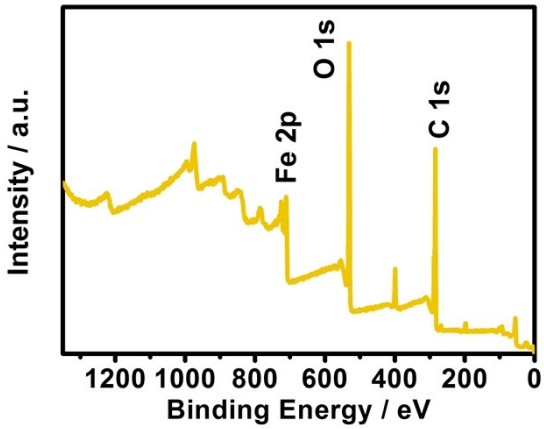

Figure S2. XPS survey spectrum of as-prepared MIL-100(Fe)@Fe_3O_4.

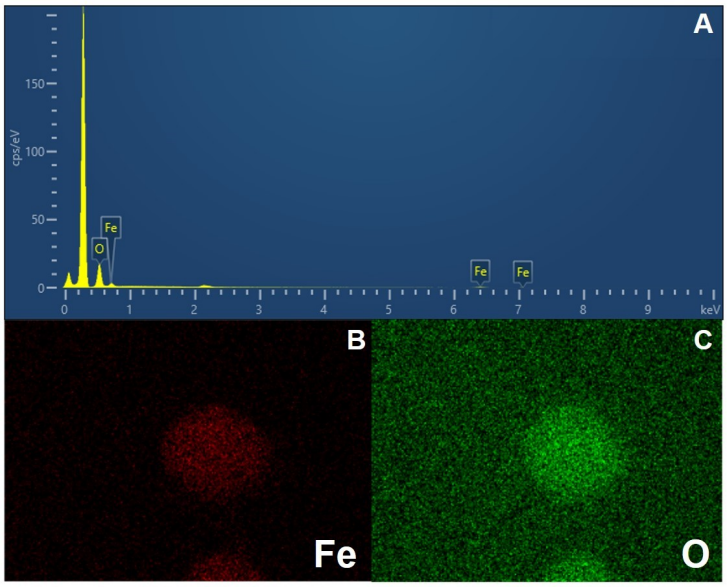

Figure S3. (A) EDS spectrum, (B-C) elemental mapping of Fe and O for as-prepared MIL-100(Fe)@Fe_3O_4.

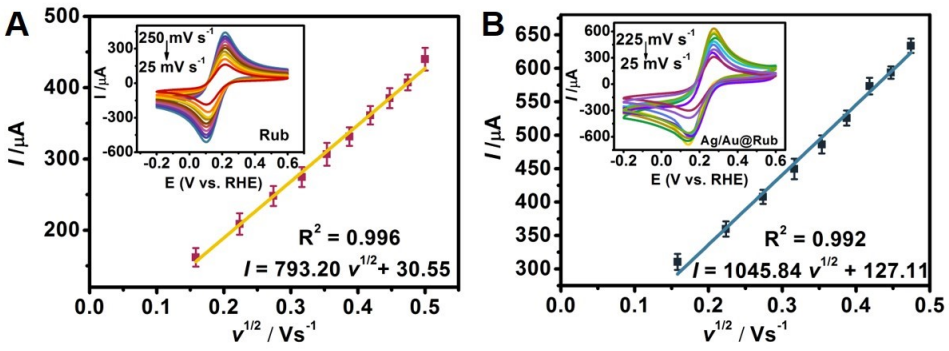

Figure S4. CV curves and linear relations of electrodes modified with (A) Rub and (B) Ag/Au@Rub in 5.0 mmol/L of $[Fe(CN)_6]^{4-/3-}$ under the scan rate of 25~250 mV/s. Error bars = SD (n = 3).

Electrochemical Performance of the Biosensor. Sequential assembly process of the ECL biosensor was judged through CV and Electrochemical impedance spectroscopy (EIS) intuitively. In terms of CV, the biosensor with every modification layer were tested in an electrolyte containing 2.5 mM $[Fe(CN)_6]^{4-/3-}$ and 0.1 M KNO_3. The test potential of CV was -0.2~0.6 V. In terms of EIS, the electrolyte was 2.5 mM $[Fe(CN)_6]^{4-/3-}$ contained 0.1 M KCl. The control potentiostat, high frequency and amplitude of EIS measurement were 5 mV, 100 kHz and 0.01 V. As shown in Figure S5A, the current peak of GCE was superior to that of Ag/Au@Rub-antigen/GCE, which demonstrated an accelerated electron shift speed of bare GCE. The subsequent dripping of MIL-100(Fe)@Fe_3O_4/ssDNA-apt with poor electrical conductivity leaded to the decline of current peak step by step. The change rule presented by EIS was to be

precisely the same with CV (Figure S5B). The impedance of GCE had a smallest value. Then, with the sequential embellishment of Ag/Au@Rub-antigen and MIL-100(Fe)@Fe_3O_4/ssDNA-apt, the resistance enhanced one by one. Results that mentioned above confirmed the successful sequential assembly process of this ECL biosensor.

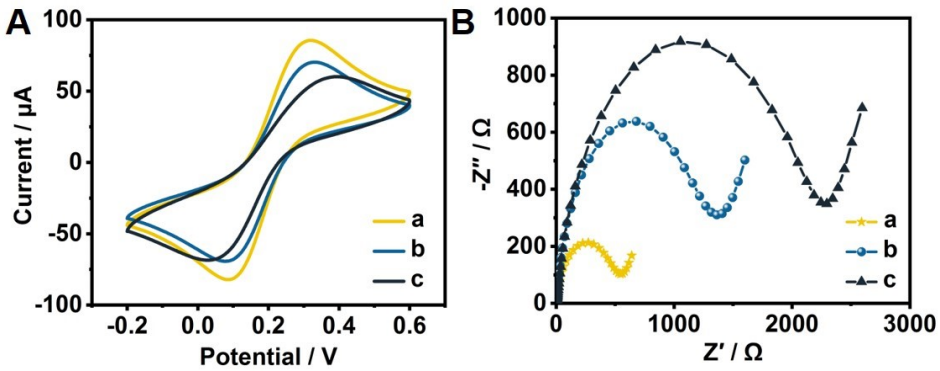

Figure S5. (A) CV and (B) EIS profiles of stepwise-modified electrodes in a 0.1 M PBS solution containing 0.1 M KCl and 5 mM $[Fe(CN)_6]^{4-/3-}$: (a) bare GCE, (b) Ag/Au@Rub-antigen/GCE, (c) Ag/Au@Rub-antigen/MIL-100(Fe)@Fe_3O_4/ssDNA-apt/GCE.

Optimization of Experimental Conditions. Optimizing the amount of Ag/Au@Rub, the concentration of MIL-100(Fe)@Fe_3O_4 and pH of PBS were for the sake of gaining better ECL performance primarily. Figure S6A-C show the ECL intensity affected the amount of Ag/Au@Rub, the concentration of MIL-

100(Fe)@Fe_3O_4 and pH, and ECL signal can reach maximum under 8 μL Ag/Au@Rub, 60 mM MIL-100(Fe)@Fe_3O_4 and pH=7.4.

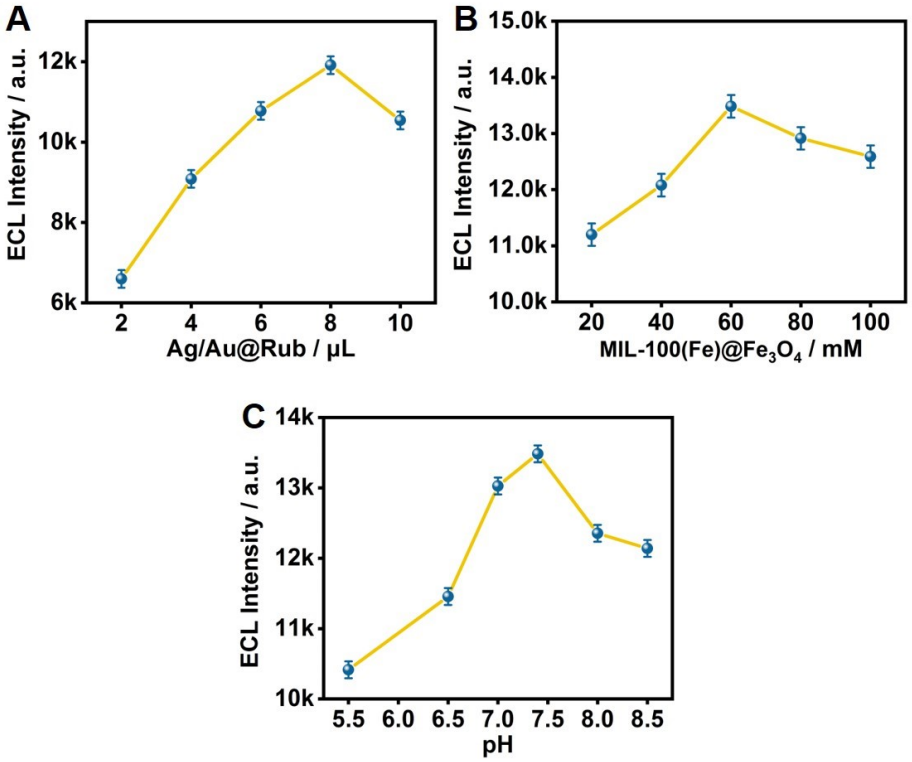

Figure S6. Optimization of (A) the amount of Ag/Au@Rub under 80 mM MIL-100(Fe)@Fe_3O_4 and pH=7.4, (B) the concentration of MIL-100(Fe)@Fe_3O_4 under 8 μL Ag/Au@Rub and pH=7.4, (C) pH value tested under 8 μL Ag/Au@Rub and 60 mM MIL-100(Fe)@Fe_3O_4. Error bars = SD, (n = 3).

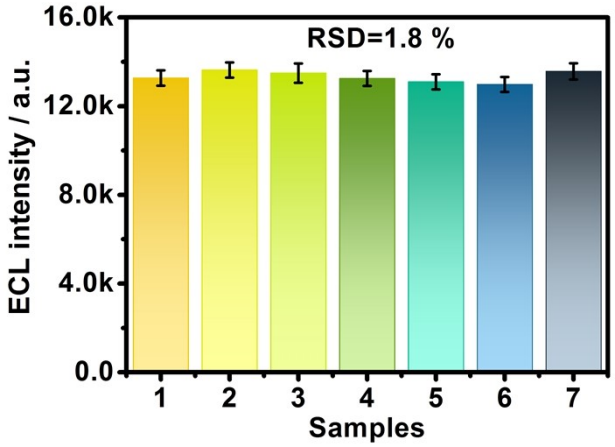

Figure S7. Reproducibility of the constructed CRE aptasensor.

Table S1. Comparison of different methods and biosensors for PSA detection.

Methods	Linear range (ng/mL)	Detection limit (pg/mL)	References
ICTS[a]	0-12.00	320.0	[1]
SIA[b]	0.001000-1000	0.1100	[2]
Magnetic electrochemical aptasensor	0.05000-50.00	28.00	[3]
Lab-on-membrane platform	0.01000-50.00	3.000	[4]
ECL	0.01000-8.000	8.000	[5]
ECL	0.001000-10.00	0.7200	[6]
ECL	0.0001000-1000	0.005010	This work

a fluorometric lateral flow immunochromatographic strip

b surface-enhanced Raman scattering (SERS)-based immunoassays

Table S2. Standard addition data of the constructed CRE aptasensor for PSA detection.

Initial Concentration (ng/mL)	Added (ng/mL)	Average Found (ng/mL, *n*=5)	RSD (%, *n*=5)	Recovery (%)
0.166	0.0400	0.206	6.0	100
	0.0800	0.244	8.6	97.5
	0.120	0.284	3.7	98.3
2.07	0.500	2.56	7.0	98.0
	1.00	3.10	4.7	103
	1.50	3.56	3.0	99.3
5.94	1.30	7.27	2.4	102
	2.60	8.55	1.5	100
	3.90	9.85	1.5	100

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