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Electronic Supporting Information

A one-step structure-switching electrochemical sensor for transcription factor detection enhanced with synergistic catalysis of PtNi@MIL-101 and Exo III-assisted cycling amplification

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Experimental section

Reagent and materials

NF-κB p50 was bought from Enzo Life Sciences (NY, U.S.A.). Methylene blue (MB), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), ethylenediaminetetraacetic acid (EDTA), bovine serum albumin (BSA), human serum albumin (HSA) and human immunoglobulin G (IgG) were supplied by Sigma-Aldrich (St. Louis, MO, U.S.A.). Exonuclease III (Exo III) was obtained from New England Biolabs (Ipswich, USA). Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), terephthalic acid (BDC), potassium platinochloride (K_2PtCl_4), nickel (II) chloride hexahydrate ($NiCl_2 \cdot 6H_2O$), sodium borohyride ($NaBH_4$), acetic acid (HAc) and sodium acetate (NaAc) were received from Aladdin Chemicals Co. Ltd. *N,N*-dimethylformamide (DMF) and other chemical reagents are analytical reagent (A.R.) grade.

The buffers involved in this work were prepared as follows: Phosphate buffered solution (PBS, 0.1 M, pH 7.4) containing KH_2PO_4 (0.1 M), Na_2HPO_4 (0.1 M) and KCl (0.1 M) was used as the electrolyte solution. HAc-NaAc (0.1 M, pH 5.5) was served as working buffer solution. 5 mM $[Fe(CN)_6]^{4-/3-}$ solution containing 0.1 M KCl was employed as a redox probe for electrochemical characterization. DNA hybridization buffer (HB, pH 7.4) containing 10 mM Tris-HCl, 1.0 M NaCl and 1.0 mM EDTA was used for preparing the oligonucleotide solutions. Probe immobilization buffer (IB, pH 7.4) contained 10 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 10 mM Tris-HCl, 1.0 mM ethylenediaminetetraacetic acid (EDTA) and 0.1 M NaCl. Ultrapure water was used in all experiments. All

oligonucleotides (including MB-labeled DNA) were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and were dissolved in Tris-HCl buffer (10 mM, pH 7.4) to get the stock solution. The sequences were listed as follows (The bold regions denote the specific recognition sequences of NF-κB p50):

Signal probe (SP): 5′-MB-$(CH_2)_6$-ACCCTCTGTAGGTTGTATAGAAAACAGAGGGT-$(CH_2)_6$-SH-3′

Assistant probe (AP): 5′-AACCTTTTCTATACAACCTACTACCTCA-3′

ON1: 5′-TGAGGTAGTAGGTTGTATAGAAAAGGTTAAAGGTT**$G_3ACT_3C_2$**ATCT-3′

ON2: 5′-AGAT**$G_2A_3GTC_3$**AACCTTTAACCTTTTCTATACAACCTACTACCTCA-3′

Apparatus

Electrochemical measurements, including cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and square wave voltammetry (SWV) were implemented by a CHI660E electrochemical workstation (Chenhua Instruments Co., Shanghai, China). A three-electrode system including a modified glassy carbon electrode (GCE, Φ = 4 mm) was used as working electrode, a saturated calomel electrode (SCE) acted as the reference electrode and a Pt wire served as auxiliary electrode. The morphologies, structural and composition analysis of different materials were characterized by scanning electron microscopy (SEM, Carl Zeiss Microscopy GmbH., Gemany), powder X-ray diffraction (PXRD, Bruker D8), X-ray photoelectron spectra (XPS, Perkin-Elmer) and inductively coupled plasma atomic emission spectroscopy (ICP-AES, 725-ES).

Synthesis of MIL-101 (Fe) and PtNi@MIL-101

MIL-101 (Fe) was synthesized according to a previous report [1] with some modification. Typically, 0.268 g $FeCl_3 \cdot 6H_2O$ and 0.082 g BDC were dissolved in 8.0 mL DMF with ultrasonic dispersion. Then, the homogeneous solution was transferred to a 10 mL Teflon-liner autoclave and heated at 105 °C for 20 h. After cooling down to room temperature, the yellow products were isolated by centrifugation and washed with DMF and ethanol. Finally, the product was dried overnight at 60 °C for further use.

PtNi@MIL-101 was prepared *via* a facile impregnation method. Briefly, MIL-101 (Fe) (25 mg), K_2PtCl_4 (10.38 mg), $NiCl_2 \cdot 6H_2O$ (5.94 mg) were suspended in H_2O (5 mL) under ultrasonication. After that, the above suspension was vigorously stirred for 9 h to impregnate the Pt^{2+} and Ni^{2+} precursors into the pores of the MIL-101. Subsequently, 1 mL freshly prepared 0.6 M aqueous $NaBH_4$ solution was quickly added the above solution while vigorous stirring, resulting in the generation a celadon suspension. After reaction for 20 min, the synthesized samples were collected by centrifuging and dried at vacuum freeze-drying.

Fabrication of the electrochemical biosensor interface

Before the fabrication of biosensor, 50 μL SP (2 μM, 1 mM TCEP) were mixed with 50 μL AP (2 μM, HB). Then, the mixture was heated to 95 °C for 5 min and cooled down to room temperature to obtain the duplex probe (SP-AP, 1 μM). The sensor fabrication process is as follows. Firstly, the GCE was polished with 0.3, and 0.05 μm alumina powder, respectively, followed by sonication with ultrapure water, and dried in the air to obtain a mirror-like surface. After that, 15 μL the prepared

PtNi@MIL-101 was assembled onto the cleaned GCE over 6 h at room temperature. Next, 10 μL as-prepared duplex probe (SP-AP, 1 μM) was dropped onto the electrode and incubated at 4 °C overnight. Lastly, the modified electrodes were treated with BSA (0.25%) for 30 min at room temperature to prevent any non-specific adsorption. Followed by each modification, the electrodes were washed with ultrapure water to avoid physical adsorption. Finally, the prepared electrodes (BSA/SP-AP/ PtNi@MIL-101/GCE) were stored at 4 °C when not in use.

Electrochemical NF-κB p50 detection

NF-κB p50 detection procedure: Initially, the complete complementary DNA duplex (ON1/ON2, 1 μM) containing a NF-κB p50 recognition site was prepared by mixing 50 μL ON1 (2 μM, HB) and 50 μL ON2 (2 μM, HB). Then the mixed sequences were heated to 95 °C for 5 min, cooled down to room temperature and distributed to various microcentrifuge tubes. For the assay of NF-κB p50, various concentrations of NF-κB p50 were added into the mixture to incubate at room temperature for 40 min to allow NF-κB p50 protein binding with its recognition DNA sequences. After that, the digestion reaction was started at 37 °C for 40 min with the addition of 1 μL Exo III (2 U) and 10 μL buffer. Finally, the reacted solution was dropped onto the modified electrode and further incubated at 37 °C for about 1 h to actuate the signal amplification procedure before the electrochemical test.

All measurements were performed with a CHI660E electrochemical workstation. SWV experiment was carried out in 0.1 M HAc-NaAc buffer (pH 5.5) at a potential range from -0.5 V to 0.1 V (*vs.* SCE). The fabrication process of the electrode was

111 characterized by CV and EIS in 0.1 M PBS (pH 7.4) containing 5 mM $[Fe(CN)_6]^{4-/3-}$
112 as redox probe. CV experiment was carried out with a potential window from -0.2 V
113 to 0.6 V at a scan rate of 0.1 V s^{-1}. EIS measurement was determined at a frequency
114 window from 0.01 Hz to 100 KHz with the 5 mV alternative voltage.

115 **Design of electrochemical biosensor for NF-κB p50 assay**

116 A structure-switching electrochemical method was designed, and the two-part
117 formed mechanism of this assay was outlined in Scheme 1. The partially hybridized
118 probe complex (SP-AP) is first self-assembled on the electrode *via* thiol-PtNi alloy
119 affinity, which maintains the signal probe with a rigid linear structure, keeping the
120 electroactive reporter MB far away from the electrode surface. In the signal converter
121 part, ON1 and ON2 are complete hybridized dsDNA, which contained the binding
122 sequence (red regions of ON1 and ON2) of NF-κB p50. In the presence of NF-κB p50
123 and Exo III, ON3 attached with the Exo III's digestion product NF-κB p50/dsDNA
124 complex produced due to the protection of NF-κB p50 against the continually
125 digestion of the recognition sites by Exo III. In this way, NF-κB p50 signal translates
126 into the ON3. Then, ON3 was introduced into the reaction system and further
127 hybridize with AP, which results in SP return to hairpin-like conformation and thus
128 brings MB in proximity to the electrode surface. More importantly, ON3/AP with
129 blunt 3′ terminus would trigger the digestion of Exo III and thus released ON3, which
130 realizes the Exo III-assisted cycling amplification. As a result, a large amount of MB
131 bring in proximity to the electrode surface and can further be catalyzed by
132 PtNi@MIL-101, realizing an highly efficient 1:N target-responsive strategy.

Eventually, a significantly amplified current is obtained.

Results and discussion

PXRD measurement

The structural characterization of the PtNi@MIL-101 above is confirmed by PXRD measurements. As displayed in Fig. S1, the experimental diffractogram of MIL-101 (Fe) is in good agreement with the simulated one of MIL-101 (Fe), the synthetic PtNi@MIL-101 is completely overlapped with that of MIL-101 (Fe), demonstrating that the sample of MIL-101 (Fe) has high purity and no crystallinity loss after the formation of PtNi@MIL-101.

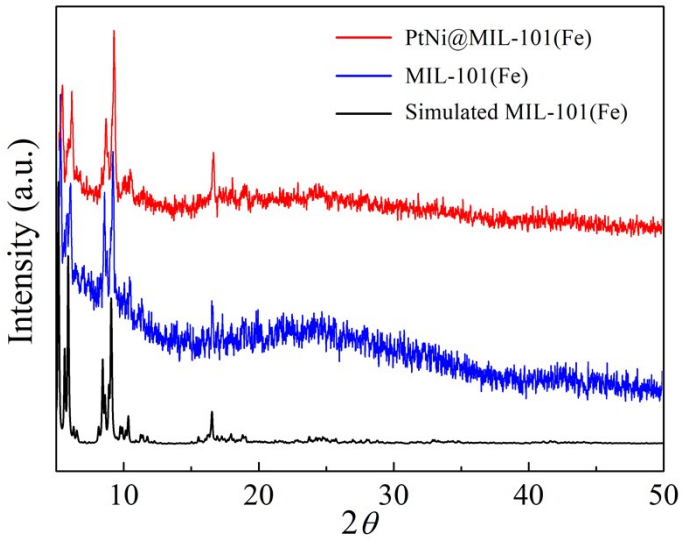

Fig. S1. XRD patterns of MIL-101 (Fe) and PtNi@MIL-101 compared with simulated MIL-101 (Fe).

Characterization of the electrochemical biosensor

CV and EIS measurements were used to investigate the modification process of prepared electrode in 5.0 mM $[Fe(CN)_6]^{4-/3-}$ solution, and the results are presented in Fig. S2A and B. As we can see from Fig. S2A, the bare GCE exhibits a couple of

149 apparent quasi-reversible redox peak of $[Fe(CN)_6]^{4-/3-}$ (curve a). After PtNi@MIL-101
150 was assembled onto the cleaned GCE, a larger peak current than that of bare GCE
151 appears (curve b), which can be attributed to the satisfactory electro-conductivity of
152 PtNi alloy nanoclusters. Then, the following introduction of non-electroactive SP-AP
153 onto the resulting electrode surface caused the decrease of the peak current (curve c).
154 Subsequently, a dramatic dropped peak current can be obtained when the electrode
155 surface was blocked with BSA (curve d). After modification with NF-κB p50 reacted
156 solution (the mixture containing ON1/ON2, NF-κB p50 and Exo III), an increased CV
157 response is obtained (curve e), which may be originated from that the ON3 can trigger
158 a strand displacement reaction by forming AP/ON3 duplex that is then released into
159 the bulk solution, which led to the AP away from the electrode surface. Furthermore,
160 EIS of every modification step is displayed in Fig. S2B, the diameter of the semicircle
161 can manifest electron transfer resistance (R_{et}) of the electrode surface. It is found that
162 the bare GCE exhibits a small semicircle (curve a). After modification of PtNi@MIL-
163 101 on the GCE, we can see that the R_{et} value is gradually decreased (curve b), owing
164 to the good conductivity of PtNi alloy nanoclusters. However, when the negatively
165 charged SP-AP duplex and BSA were successively immobilized on the electrode
166 surface, the diameter increases in order (curve c and d), because DNA sequences and
167 protein as electron transfer blocking layer can retard the electron transfer. In the end,
168 there is an apparent increase of the diameter (curve e) after incubation of NF-κB p50
169 reacted solution, which is attributed to the fact that the AP is taken away from the
170 electrode surface. All the results obtained reveal the successful fabrication of the

constructed sensor.

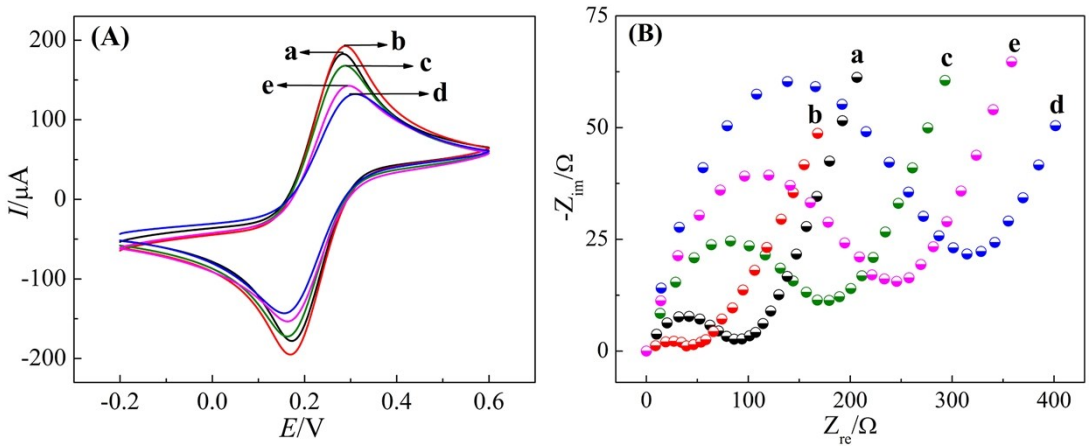

Fig. S2. CV (A) and EIS (B) responses of the fabricated procedures for the biosensor performed in PBS (pH 7.4) containing 5 mM $[Fe(CN)_6]^{4-/3-}$ as a redox probe: (a) bare GCE, (b) PtNi@MIL-101 /GCE, (c) SP-AP/PtNi@MIL-101/GCE, (d) BSA/SP-AP/PtNi@MIL-101/GCE, and (e) after being incubated with NF-κB p50 reacted solution.

Optimization of biosensor preparation conditions

To obtain the best analytical performance for NF-κB p50 detection, several factors were taken into consideration, including the concentration of SP, the concentration and incubation time of Exo III. As shown in Fig. S3A, the concentration of SP is an important parameter in response of the prepared biosensor, SWV peak current gradually increased as the concentration of SP elevated from 0.1 to 1.0 μM, and then decreased after over 1 μM. The reason could be that higher density of SP would induce steric hindrances and thus prevent SP from forming the hairpin structure on the electrode surface. Hence, the concentration of SP at 1.0 μM was optimized. Fig. S3B depicts the effect of the concentration of Exo III, it is found that the peak current gradually exhibits a positive correlation with the concentration of Exo III, and reaches

188 its plateau when the concentration was 2.0 U μL^{-1}. Thus, 2.0 U μL^{-1} of Exo III is used

189 as the optimal concentration. In addition, the incubation time of Exo III also plays an

190 important role in constructed biosensor. From Fig. S3C, SWV response increases

191 clearly with the increasing incubation time of Exo III from 20 to 60 min, and remains

192 unchanged after 60 min. Therefore, the optimal incubation time of Exo III is 60 min.

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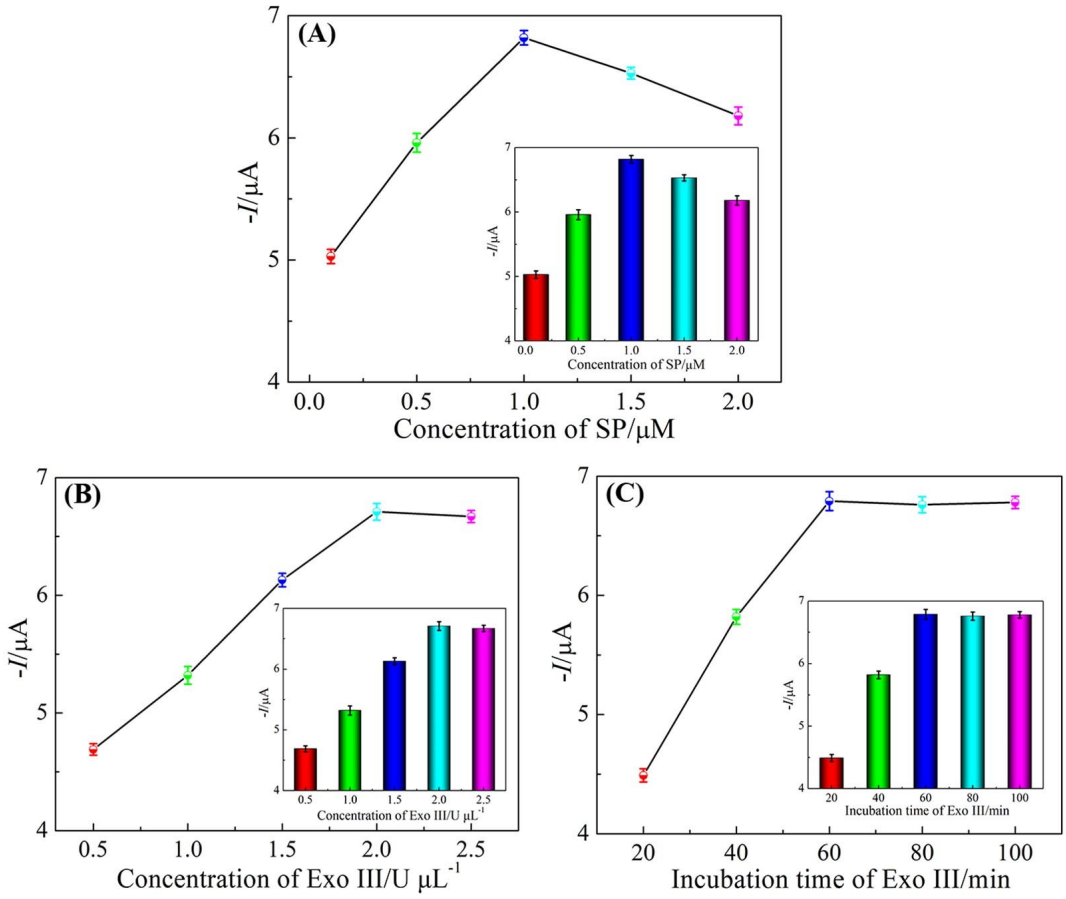

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196 **Fig. S3.** The optimization for experimental conditions of the concentration of SP (A), the

197 concentration (B) and incubation temperature (C) of Exo III.

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Optimization of the ratio of Pt/Ni

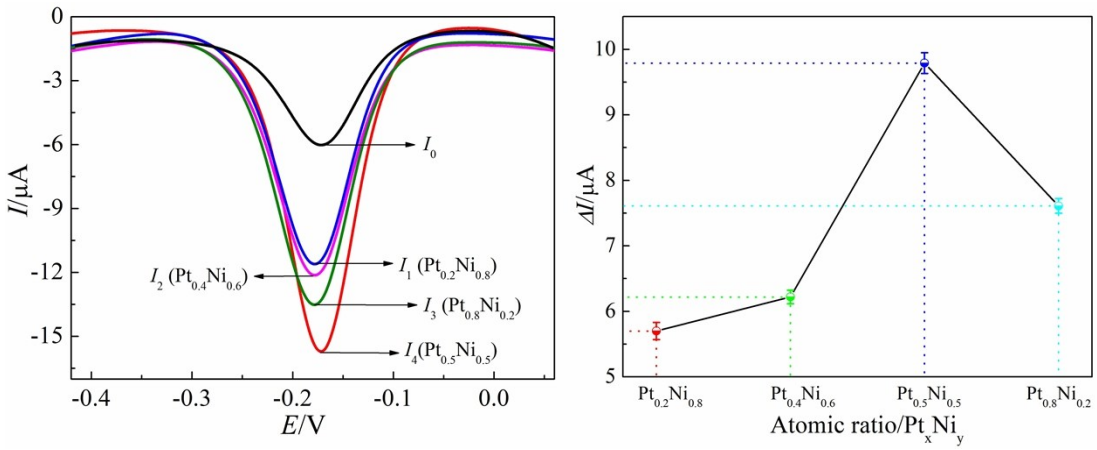

Fig. S4. Electrocatalytic performance of different the Pt:Ni atomic ratio toward MB ($\Delta I = I_n - I_0$, I_0 is the initial peak current value of MB, while the peak current after catalysis of Pt_xNi_y is recorded as I_n).

Table S1.

Comparison with other published methods for NF-κB p50 detection.

Analytical method	Linear range	Detection limit	References
Square wave voltammetry	200 pM−1 nM	40 pM	[2]
Fluorescent assay	40 pM−550 pM	25 pM	[3]
Differential pulse voltammetry	10 pM−5 nM	10 pM	[4]
Fluorescent assay	30 pM−1.5 nM	10 pM	[5]
Square wave voltammetry	0.5 nM−200 nM	0.08 nM	[6]
Colorimetric assay	5 pM−2 nM	3.8 pM	[7]
Fluorescent assay	38 pM−15 nM	20 pM	[8]
Square wave voltammetry	1 pM−5 nM	0.23 pM	this work

Table S2.

Results of the recovery test of NF-κB p50 in human serum.

Sample No.	Actual/pM	Detected/pM	Recovery/%
1	1.00	1.07	107.0
2	5.00	4.96	99.2
3	10.00	10.47	104.7
4	50.00	53.32	106.6
5	100.00	98.59	98.6

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