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Electronic Supplementary Information

In situ amplified electronic signal for determination of low-abundance proteins coupling with nanocatalyst-based redox cycling

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1 **EXPERIMENTAL SECTION**

2 **Materials.** Polyclonal anti-CEA antibody produced in rabbit (anti-CEA, 1.0 mg/mL) was
3 purchased from Beijing Biosynthesis Biotechnol. Co., Ltd. (China). CEA ELISA kits with 0, 5.0,
4 10, 20, 40 and 80 ng/mL CEA standards were obtained from Biocell Biotechnol. Co., Ltd.
5 (China). *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC),
6 *N*-Hydroxysuccinimide (NHS), and ferrocenecarboxylic acid were supplied by Sigma-Aldrich
7 (USA). Cerium nitrate hexahydrate, chitosan, $NaBH_4$, *p*-nitrophenol (PNP), *p*-aminophenol
8 (PAP), and bovine serum albumin (BSA, 96–99 wt %) were obtained from Sinopharm Chem. Re.
9 Co. Ltd. (Shanghai, China). All other reagents were of analytical grade and were used without
10 further purification. Ultrapure water obtained from a Millipore water purification system ($\geq$ 18
11 MΩ, Milli-Q, Millipore) was used in all runs. 0.1 mol/L phosphate buffered saline (PBS)
12 solutions with various pH values was prepared by mixing 0.1 mol/L K_2HPO_4 and 0.1 mol/L
13 KH_2PO_4, and 0.1 mol/L KCl was added as the supporting electrolyte.

14 **Preparation and Bioconjugation of Platinum/Cerium Oxide Hybrid Nanostructures**
15 **(CeO_2/Pt).** Cerium oxide (CeO_2) nanoparticles were synthesized according to the literature with a
16 little modification.[S1] Briefly, cerium nitrate solution (5.82 mL, 0.05 mol/L) was initially dropped
17 into ammonia (3.4 mL, 25 wt %) under vigorous stirring, and then further reacted at room
18 temperature (RT) until the color of the suspension changed from purple to yellow. Following that,
19 the suspension was centrifuged for 10 min at 10,000 rpm, and the obtained precipitate was
20 re-dispersed into 3 mL of distilled water.

21 Next, the platinum/cerium oxide hybrid nanostructures were synthesized consulting to the
22 literature.[S2] 20 mg of poly(*N*-vinyl-2-pyrrolidone) (PVP) was added into the as-prepared solution,
23 and stirred for 12 h at RT. The PVP-functionalized CeO_2 nanoparticles were centrifuged, and
24 re-dispersed in 2.5 mL of distilled water. Following that, 1.0 mL of K_2PtCl_4 (21 mg/mL) was
25 added in the suspension, and heated to 95 ºC for 1 h under gentle stirring. Subsequently, 1.0 mL
26 of $NaBH_4$ (4.0 mg/mL) was added into the mixture. The reaction was maintained for another 30

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27 min at 95 ºC. Finally, platinum/cerium oxide hybrid nanostructures (designated as CeO_2/Pt) were
28 collected by centrifugation, and dispersed in 2.0 mL of distilled water for the conjugation of
29 anti-CEA antibody as follows: 200 μL of anti-CEA (1.0 mg/mL) was initially added into 1.0 mL
30 of CeO_2/Pt colloids ($C_{[CeO2]} \approx 0.14$ mM), and then the mixture was incubated for 12 h at 4 ºC with
31 gentle stirring. During this process, anti-CEA antibodies were covalently conjugated onto the
32 CeO_2/Pt nanostructures via the Pt-NH bonds. Finally, anti-CEA-functionalized CeO_2/Pt
33 (designated as CeO_2/Pt-anti-CEA) were obtained by centrifugation, and stored in 2.0 mL of pH
34 7.4 PBS at 4 ºC until use. For comparison, pure Pt and CeO_2 nanoparticles were utilized for the
35 label of anti-CEA antibodies using the same method, respectively (*Note*: Pure platinum and CeO_2
36 nanoparticles were prepared according to Ref. [S3] and Ref. [S4], respectively. The antibodies
37 were labeled onto the CeO_2 nanoparticles based on the encapsulation method by using chitosan
38 acidic suspension).

39 **Preparation of Electrochemical Immunosensor.** Initially, 1.0 mg of chitosan (CS) flakes was
40 added into 1.0 mL of 1.0 wt % acetic acid solution, and then the mixture was adequately stirred at
41 RT until chitosan flake were completely dissolved. Following that, 1.0 mg of ferrocenecarboxylic
42 acid was added into the chitosan solution with adequately stirring until a homogeneous mixture
43 was obtained (designated as Fc-CS).

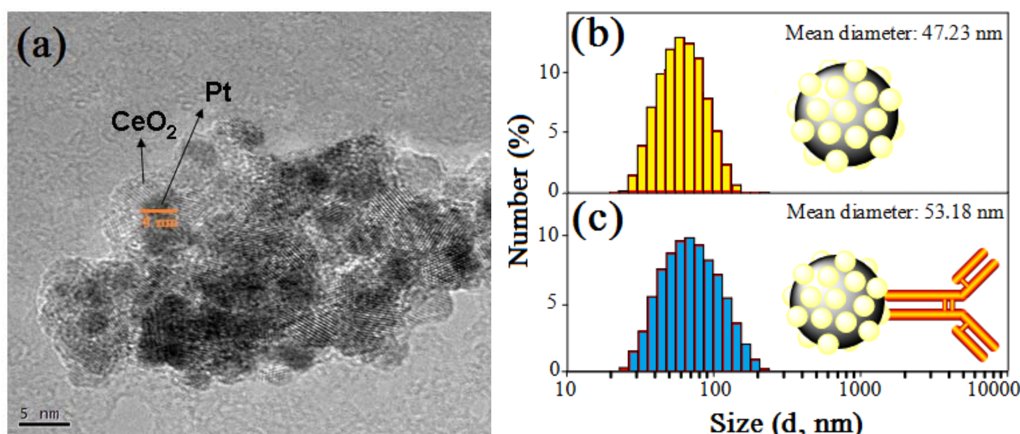
44 Next, a glassy carbon electrode (GCE, 4 mm in diameter) was polished with 0.3 μm and 0.05
45 μm alumina, followed by successive sonication in acetone, ethanol and deionized water for 5 min
46 and dried in air. Afterwards, 10 μL of the as-prepared Fc-CS suspension was cast on the surface
47 of the cleaned GCE. After dry, 5 μL of 0.025% (w/v) of glutaraldehyde was added on the
48 electrode and incubated for 1 h at RT. Excess glutaraldehyde was removed by washing with pH
49 7.4 PBS. Subsequently, the modified electrode was immersed into the anti-CEA solution, and
50 incubated for 12 h at 4 ºC. During this process, anti-CEA antibodies were conjugated onto the
51 GCE via the glutaraldehyde. Finally, anti-CEA-modified GCE was incubated with 2.5 wt % BSA
52 for 1.0 h at RT to block the unreacted and nonspecific sites. The as-prepared immunosensor was
53 used for detection of CEA.

54 **Electrochemical Measurement.** All electrochemical measurements were carried out with a

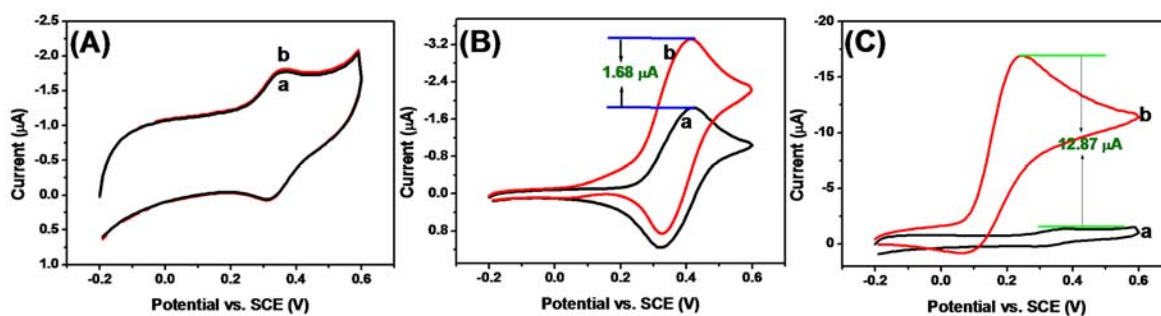
55 CHI 620D Electrochemical Workstation (Shanghai, China) with a conventional three-electrode
56 system using a modified GCE as working electrode, a platinum wire as auxiliary electrode, and a
57 saturated calomel electrode (SCE) as reference electrode. Initially, 5 μL of CEA samples with
58 various concentrations was thrown onto the modified electrode and incubated for 30 min at RT.
59 After washing with pH 7.4 PBS, 10 μL of as-prepared CeO_2/Pt-anti-CEA suspension were
60 dropped onto the modified electrode, and incubated for another 30 min under the same conditions.
61 After rinsing thoroughly with pH 7.4 PBS, the electrochemical response of the immunosensor
62 were performed in pH 8.0 PBS containing 6 mM *p*-nitrophenol and 6 mM $NaBH_4$ by using
63 differential pulse voltammetry (DPV) from -100 to 600 mV (*vs*. SCE) with a pulse amplitude of
64 50 mV and a pulse width of 50 ms. All electrochemical measurements were done in an unstirred
65 electrochemical cell at room temperature (RT, 25 ± 1.0 °C). Analyses are always made in
66 triplicate. Scheme 1 represents the assay protocol of the electrochemical immunosensor.

67 **Elucidation of Signal Amplification by Nanocatalyst-Based Redox Cycling.** To verify the
68 advantages of the as-synthesized nanolabels in the redox cycling system, we also prepared
69 another two types of nanomaterials, i.e., Pt and CeO_2 nanoparticles, which were used as the
70 nanolabels for conjugation of anti-CEA antibodies. The comparative study was carried out by
71 assaying 0.1 ng/mL CEA (as an example) on the same-batch immunosensors. The judgement was
72 based on the change in the current in the absence and presence of PNP and $NaBH_4$ after the
73 formation of sandwiched immunocomplexes. As seen from Fig. S2-A, upon addition the PNP and
74 $NaBH_4$ in pH 8.0 PBS, redox peak current of the CeO_2 based immunoassay was not almost
75 changed in comparison with the background current. The results revealed that CeO_2 could not
76 reduce the PNP to PAP. However, an obvious increase of the oxidation peak was obtained for the
77 Pt-based immunoassay (Fig. S2-B), indicating Pt nanoparticles possessed excellent catalytic
78 ability for the reduction of PNP. More inspiringly, the peak currents would be greatly improved
79 by using CeO_2/Pt as the nanolabels (Fig. S2-C). The reason might be the fact that the CeO_2
80 nanoparticles with a large surface area can load a lot of Pt nanoparticles. When one secondary
81 antibody was conjugated with one CEA antigen on the electrode, more Pt nanoparticles will

82 participate in the catalytic cycling amplification reaction.

83 

84 **Fig. S1** (a) Typical TEM image of platinum/cerium oxide hybrid nanostructures. Dynamic light scattering (DLS)
85 data of platinum/cerium oxide hybrid nanostructures before (b) and after (c) conjugation with anti-CEA
86 antibodies. Nanostructures were characterized using Hitachi H-7650 transmission electron microscope (TEM,
87 Japan). DLS measurements were used to determine the hydrodynamic sizes of the nanostructures and the
88 aggregates using a Malvern Zetasizer 3000 HS particle size analyzer (Malvern Instruments, UK).



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90 **Fig. S2** Cyclic voltammograms of the immunosensor after reaction with 0.1 ng/mL CEA using various
91 nanolabels: (A) anti-CEA-CeO_2, (B) anti-CEA-PtNP, and (C) CeO_2/Pt-anti-CEA, in pH 8.0 PBS in the absence
92 (a) and presence (b) of 6.0 mM PNP + 6.0 mM $NaBH_4$.

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Table S1 Comparison of analytical properties of various CEA immunosensors or immunoassays

Method	Linear range (ng/mL)	LOD (ng/mL)	Ref.
Nanocatalyst-based electrochemical immunoassay	0.0005 - 20	0.0005	This work
CdS/DNA nanochain-based electrochemical immunoassay	0.1 - 100	0.0033	[S5]
Chemiluminescent multiplex immunoassay	1.0-70 ng/mL	0.65 ng/mL	[S6]
Multianalyte electrochemical immunoassay	≤ 188 ng/mL	1.1 ng/mL	[S7]
Multiplexed electrical detection	-	≤0.9 pg/mL	[S8]
Time-resolved fluoroimmunoassay	-	≤70 pg/mL	[S9]
Immunofluorescence assay	0.5-1000 pmol/L	1.31 pmol/L	[S10]
Amperometric immunoassay	0.01-160 ng/mL	5.0 pg/mL	[S11]
Piezoelectric immunoassay	1.5-30 μg/mL	1.5 μg/mL	[S12]
Chemiluminescence enzyme immunoassay	2-162 ng/mL	0.69 ng/mL	[S13]
Potentiometric immunoassay	1.5-200 ng/mL	0.5 ng/mL	[S14]

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Table S2 Comparison of the assayed results for clinical serum specimens or standard samples with various dilution ratios using the developed protocol and the referenced ELISA method

Sample no.[b]	Method;[a] Concentration [mean ± SD (RSD, %), n = 3, ng mL^{-1}]		t_{exp}
	Found by immunosensor	Found by the ELISA	
1	8.4 ± 1.2 (7.6%)	7.9 ± 0.8 (10.1%)	0.48
2	15.3 ± 0.6 (3.9%)	15.4 ± 2.5 (16.2%)	0.67
3	2.6 ± 0.3 (11.5%)	2.4 ± 0.4 (16.7%)	0.69
4	5.7 ± 0.5 (8.8%)	6.5 ± 0.7 (10.8%)	1.61
5	0.8 ± 0.15 (18.8%)	0.7 ± 0.1 (14.3%)	0.96
6	18.5 ± 2.1 (11.4%)	17.5± 1.3 (7.4%)	0.70
7	6.1 ± 0.5 (8.2%)	5.8 ± 0.4 (6.9%)	0.81
8	4.1 ± 0.3 (7.3%)	3.7 ± 0.3 (8.1%)	1.63
9	17.5±2.0 (11.4%)	17.7±1.9 (11.1%)	0.25
10	10.9 ± 2.3 (17.8%)	10.4± 1.6 (15.4%)	1.54
11	9.3 ± 1.1 (11.8%)	9.8 ± 2.7 (27.0%)	0.29
12	13.9 ± 1.5 (10.8%)	13.2 ± 1.8 (13.6%)	0.52
13	19.3 ± 1.1 (5.7%)	19.7 ± 0.9 (4.6%)	0.49
14	10.2 ± 0.6 (5.9%)	9.8 ± 0.5 (5.1%)	0.89
15	4.9 ± 0.3 (6.1%)	5.1 ± 0.2 (3.9%)	0.96
16	0.51 ± 0.08 (15.6%)	0.54 ± 0.09 (16.7%)	0.43

[a] The regression equation (linear) for these data is as follows: $y = 0.99x - 0.0243$ ($R^2 = 0.9942$) (x-axis: by magnetic electrochemical immunoassay; y-axis: by the ELISA).

[b] Clinical serum samples (no.: 1-12) and CEA standard samples with various dilution ratios (no.: 13-16) were appropriately diluted if CEA levels were over the calibration ranges.

Notes and references

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