Electronic Supplementary Information

A dual-signal electrochemical immunosensor for detection of HPV16 E6 oncoprotein based on PdBP dendritic ternary nanospheres and MBSi-Chi nanocomposites

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The synthesis of MSN

An aqueous solution (12 mL) that contained CTAB (25 mg), NaOH (7 mg) was added into a flask, and then the flask was heated to 80℃ in a silicone oil bath for 1 h. Then, 125 μL of TEOS was added into the flask dropwise, keeping heating for 2 h. The resultant solution was centrifuged at 10000 rpm for 10 min and washed three times with methanol. Subsequently, the precipitation was dispersed in ethanol. Pore system can be opened via extraction. The extraction solution was composed of ethanol (7.2 mL) and concentrated hydrochloric acid (0.8 mL). 4 ml of extraction solution was added into the ethanol dispersion and then heated at 90℃ for 45 min. Following centrifugation and washing with ethanol/H_2O, the previous step was repeated. Ultimately, MSN was prepared and dried in vacuum at 37℃ for about 20 h.

Optimization of experimental conditions

Some factors will affect the performance of immunosensor. In this work, certain crucial parameters were taken into consideration, like volume of PdBP NSs, antibody capture time, incubation time between antigen and antibody and concentration of H_2O_2. The results were presented in Fig. S3.

The volume of PdBP NSs was a critical factor that offered an ideal platform for antibody binding, which was analyzed in Fig. S3A. The current value increased gradually ranging from 6 to 10 μL, and then decreased slightly when the volume was raised to 12 μL. Thus, 10 μL was selected as the optimal volume.

The capture time at which the antibodies were bound to the modified electrode was also a rather important factor in performance. When we gradually extended capture time to 2h, current change appeared to increase and then began to level off (Fig. S3B). Hence, 2 h was decided as the optimal antibody capture time.

To maximizing the binding time, we optimized incubation time between antigen and antibody at 37 ℃. The incubation time was tested in the range from 0.5 h to 2.5 h. In Fig. S3C, current change was augmented from 0.5 to 2 h, and if we continued to extend incubation time, the current change does not change obviously. Thus, 2 h was used as the optimal combination time.

In addition, the concentration of H_2O_2 also plays a key part. 10 μL of PdBP NSs were dropped on the cleaned GCE and dried at room temperature. Then, i-t amperometric curves was used to record the current values ($\Delta Current = Current_{100s} - Current_{40s}$) with different concentration of H_2O_2 (20 μL) adding into a 5 mL PBS buffer. As shown in Fig. S3D, current signal increased rapidly with the concentration increasing from 2.2 to 3.0 mol/L and reached maximum at 3.0 mol/L. After dropping 3.2 mol/L of H_2O_2, current signal declined instead. Hence, 3.0 mol/L was determined as the optimal concentration of H_2O_2.

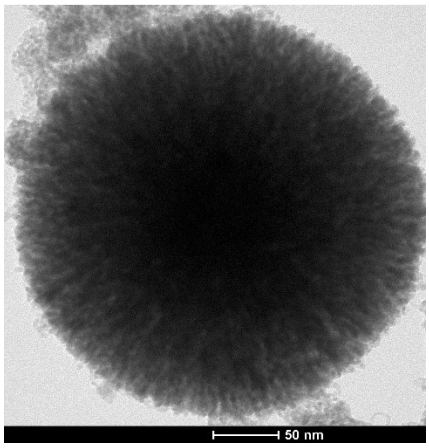

Fig. S1 FE-TEM image of PdBP NSs after electrochemical analysis in a 5 mL PBS buffer with H_2O_2 adding into the solution.

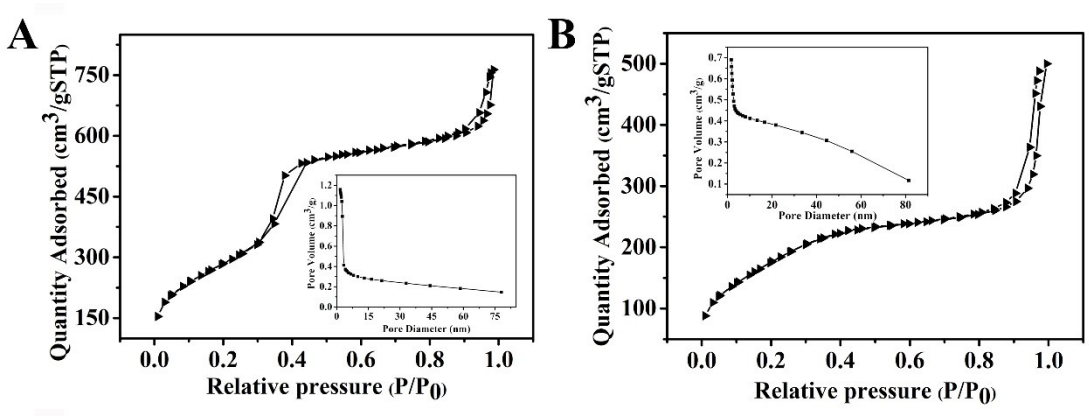

Fig. S2 N_2 absorption-desorption isotherm of (A) MSN, (B) MBSi-Chi (inset is its BJH desorption pore distribution curve).

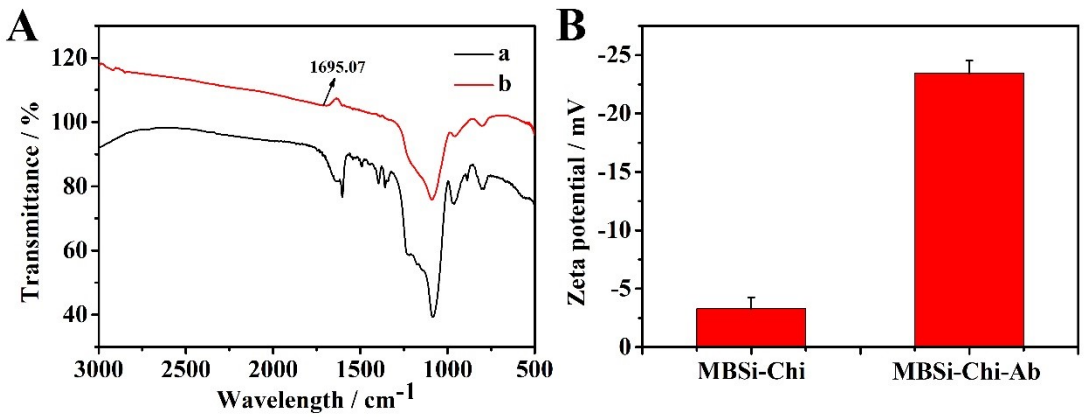

Fig. S3 (A) FT-IR spectrum of (a) MBSi-Chi, (b) MBSi-Chi-Ab nanocomposites. (B) Zeta potential of (a) MBSi-Chi, (b) MBSi-Chi-Ab nanocomposites.

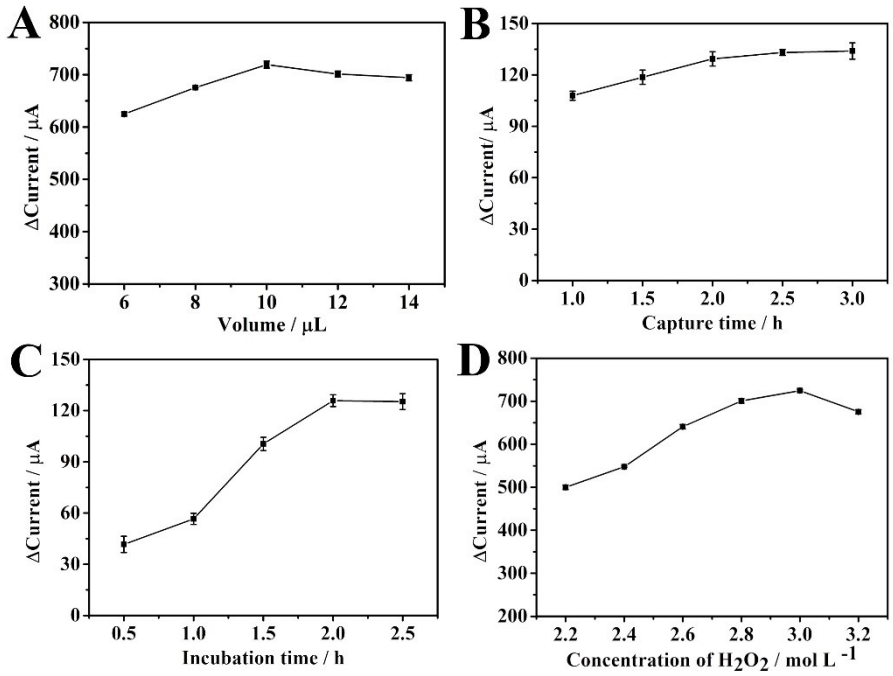

Fig. S4 Optimization: (A) volume of PdBP NSs, (B) capture time, (C) incubation time, (D) concentration of H_2O_2.

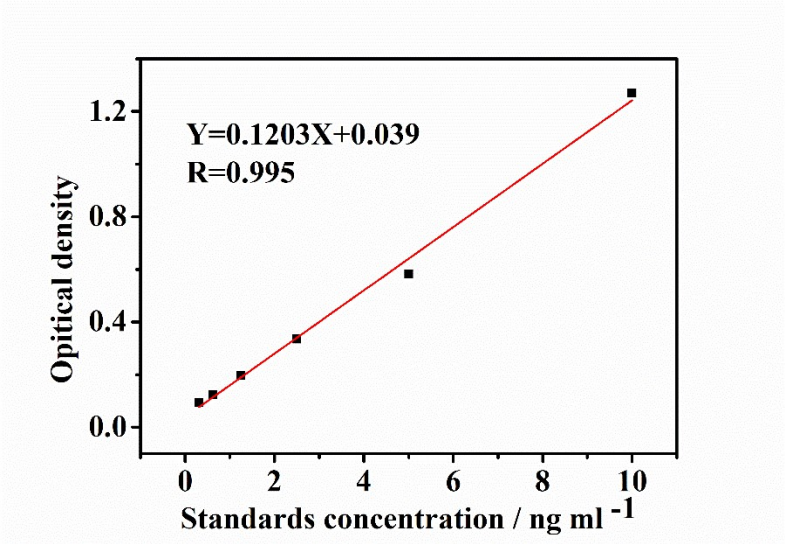

Fig. S5 The calibration curve of the HPV16 E6 oncoprotein ELISA kit.

Table S1. ICP-OES of ternary PdBP NSs.

Element type	Instrument reading	Final content	Unit
Pd	0.7317	7.317	mg/L
B	0.0066	0.066	mg/L
P	0.0086	0.086	mg/L

Table S2. Comparison of analytical performance with previous reported immunosensors

Materials	Target	Linear range	Limit of detection	Reference
516-MOF/Anti-vomitoxin	Vomitoxin	0.001 ~ 0.5 ng/mL	0.7 pg/mL	1
BP-PAMI modified LBG	IL-6	0.003 ~ 75 ng/mL	1 pg/mL	2
In2O3/ In2S3/CdIn2S4, PDA@CNTs	CYFRA 21–1	0.5 pg/mL ~ 50 ng/mL	0.16 pg/mL	3
SiO2-Fc-COOH-Au, UiO-66-TB	Procalcitonin	1 pg/mL ~ 100 ng/mL	0.3 pg/mL	4
g-C3N4/Au/WO3	Aflatoxin B1	1.0 pg/mL ~ 100 ng/mL	0.33 pg/mL	5
Au NFs, Fc-aptamer, MB-cDNA	Aflatoxin B1	0.1 pg/mL ~ 1 ng/mL	0.32 pg/mL	6
Fc-DNA, MB	Ochratoxin A	10 pg/mL ~ 10 ng/mL	3.3 pg/mL	7
ns@gold	EGFR receptor	10 pg/mL ~ 100 ng/mL	6.9 pg/mL	8
SA-β-Gal-CaHPO4 Nanoflower	AFP	0.1 pg/mL ~ 10 ng/mL	0.17 ng/mL	9
Au NPs/SnO_2/$ZnIn_2S_4$	RNase A	1 pg/mL ~ 10 ng/mL	0.2 pg/mL	10
Molybdophosphate precipitate		10 pg/mL ~ 5 ng/mL	2 pg/mL	
PdBP NSs, MBSi-Chi nanocomposites	HPV16 E6 oncoprotein	100 fg/mL ~ 4 ng/mL	72.8 fg/mL	This work (label-free)
		50 fg/mL ~ 4 ng/mL	34.1 fg/mL	This work (sandwich)

Table S3. Detection of HPV16 E6 oncoprotein in five real samples

Sample no.	ELISA	This work (label-free) (pg/mL)	Relative error (%)	This work (sandwich) (pg/mL)	Relative error (%)
1	436.3	457.09	4.77	426.56	-2.23
2	808.8	794.33	-1.79	776.25	-4.02
3	1379	1445.44	4.82	1445.44	4.82
4	2020	2137.96	5.84	2089.3	3.43
5	2760	2951.21	6.93	2818.38	2.12

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