Supplementary Material (ESI) for Chemical Communications

In situ assembly of gold nanoparticles on nitrogen-doped carbon nanotubes for sensitive immunosensing of microcystin-LR

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Experimental

Materials and Reagents. Nitrogen-doped carbon nanotubes (CNx-MWNTs) were synthesized according to the previously reported method,[1] and were further refluxed in 6 M NaOH at 110 °C for 4 h to remove the Al_2O_3 support, followed by refluxing in 1 M H_2SO_4 for 8 h to remove residual Fe catalysts.[2] The purified CNx-MWNTs was thoroughly washed with double-distilled water until the pH value of the filtrate reached 7, and then dried at 70 °C overnight for further study. Multi-walled carbon nanotubes (MWNTs) were obtained from Shenzhen Nanotech Port Company. Microcystin-LR (MC-LR), MC-LR antibody (anti-MC-LR) and horseradish peroxidase (HRP) labeled MC-LR (HRP-MC-LR) were purchased from Express Technology Co., Ltd (China). Bovine serum albumin (BSA) was obtained from Sigma (St. Louis, MO). All other reagents, including H_2O_2 and o-phenylenediamine (o-PD), were of analytical grade. Blocking buffer was 0.01 M pH 7.4 phosphate buffer saline (PBS) containing 2% BSA. To minimize unspecific adsorption, 0.05% Tween-20 was spiked into PBS as wash buffer (PBST). The detection buffer was 0.2 M pH 7.4 PBS. Ultrapure water obtained from a Millipore water purification system (≥ 18 MΩ, Milli-Q, Millipore) was used in all runs. The polluted water samples were from Tai lake in Wuxi, China.

Preparation of Au/CNx-MWNTs Nanocomposite. 7 mg CNx-MWNTs was dispersed in 40 mL aqueous solution containing 2.5×10^{-4} M trisodium citrate and then sonicated for 30 min. 0.4 mL 1% $HAuCl_4\cdot3H_2O$ was added to the solution and stirred for 10 min. 1.2 mL of 0.1 M ice cold $NaBH_4$ solution was added to the mixture solution under stirring at 0 °C. After stirring for an additional 2 h, the black solid was separated by centrifuging at a speed of 10000 rpm, washed with water for several cycles, and then dried overnight at 80 °C.

Preparation of MC-LR Immunosensor. The glassy carbon electrode (GCE, 3 mm diameter) was polished successively with 0.3- and 0.05-μm alumina slurry (Beuhler) followed by rinsing thoroughly with doubly distilled water. After successive sonication in 1:1 nitric acid, acetone and deionized water, the electrode was rinsed with deionized water and allowed to dry at room temperature. 6 μL of 2 mg mL^{-1} Au/CNx-MWNTs suspension was dropped on the pretreated GCE and dried in a desiccator. 10 μL of 1 μg mL^{-1} anti-MC-LR was dropped on its surface and then incubated for 12 h to form anti-MC-LR/(Au/CNx-MWNTs)/GCE. Following a rinse with 0.01 M pH 7.4 PBST, the formed immunosensor was blocked with 0.01 M pH 7.4 PBS containing 2% BSA for 30 min. After thorough washing with 0.01 M pH 7.4 PBST, the obtained immunosensor was stored at 4 °C prior to use. As control, anti-MC-LR/(Au/MWNTs)/GCE and anti-MC-LR/MWNTs/GCE were prepared with the same procedure.

Analytical Procedure. 10 μL MC-LR solutions with different concentrations or water samples with appropriate dilutions were mixed with 10 μL HRP-MC-LR to obtain the incubation solution. The incubation solution was dropped on the MC-LR immunosensor and incubated for 20 min at 25 °C, and then washed carefully with 0.01 M pH 7.4 PBST to obtain HRP-MC-LR/anti-MC-LR/(Au/CNx-MWNTs)/GCE. During the incubation process the immunosensor was placed in container for avoiding the evaporation of incubation solution. The electrochemical measurement was recorded in 0.2 M PBS solution containing 8.0 mM H_2O_2 and 10 mM *o*-PD. The detection solution was bubbled thoroughly with high purity nitrogen for 10 min and maintained in nitrogen atmosphere. The differential pulse voltammetric (DPV) measurements were from -300 to -850 mV with pulse amplitude of 50 mV and width

of 50 ms. The data for condition optimization and the calibration curve were the average of three measurements.

Characterization of Au/CNx-MWNTs

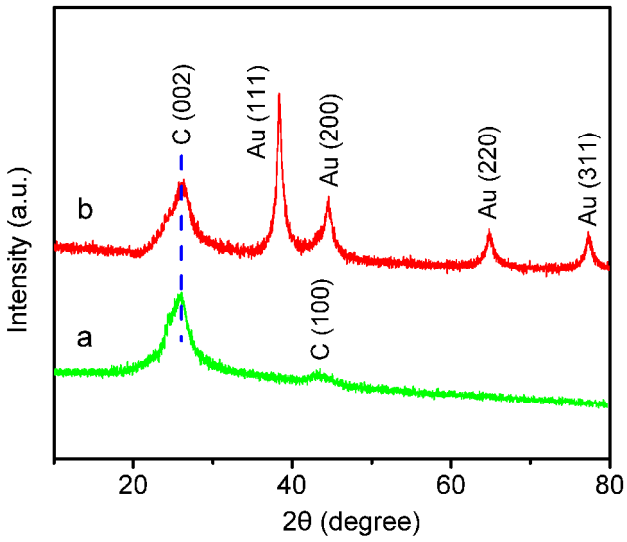

Fig. S1 XRD profiles of (a) CNx-MWNTs and (b) Au/CNx-MWNTs.

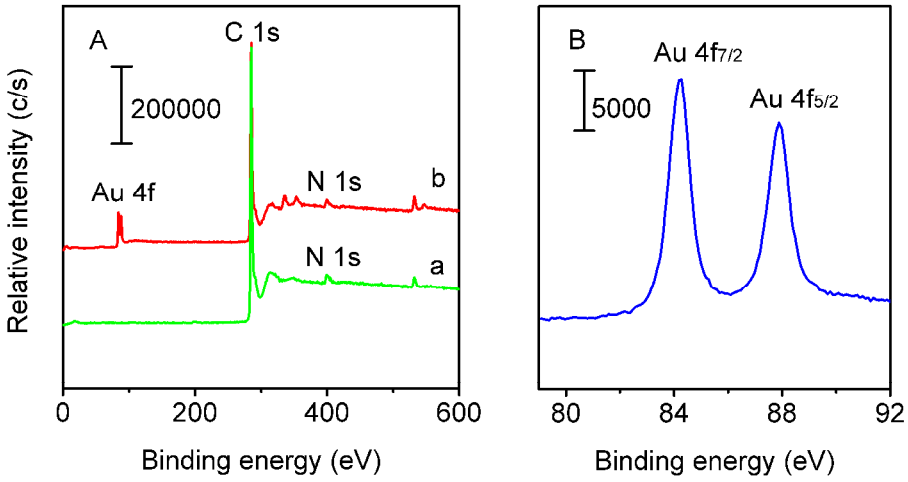

Fig. S2 (A) Survey XPS spectra of CNx-MWNTs (a) and Au/CNx-MWNTs (b), and (B) Au 4f XPS spectrum of Au/CNx-MWNTs.

Optimal Conditions for Immunoreaction

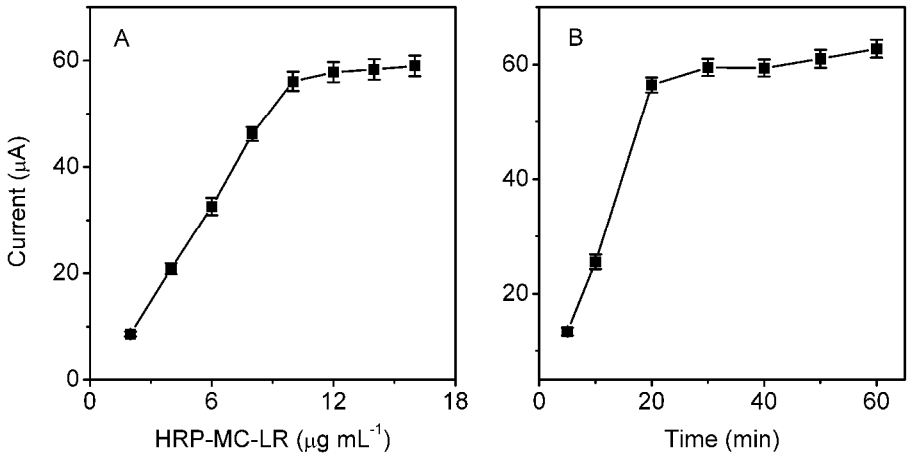

Fig. S3 Effects of (A) HRP-labeled MC-LR concentration and (B) incubation time on amperometric response of HRP-MC-LR/MC-LR/(Au/CNx-MWNTs)/GCE under other optimal conditions.

Optimal Conditions for Detection Solution

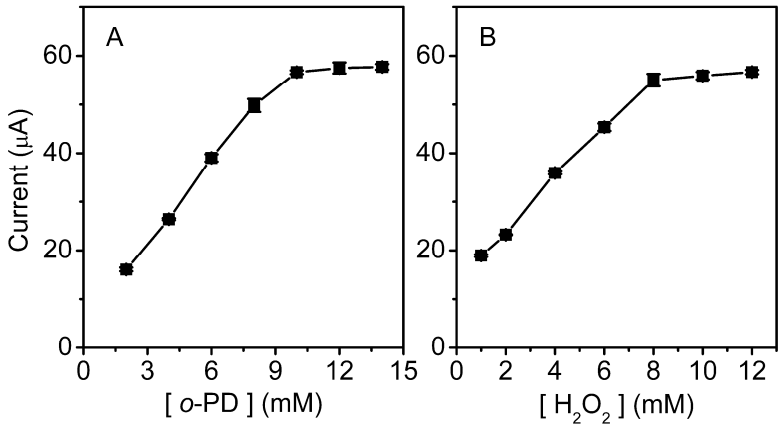

Fig. S4 Effects of (A) *o*-PD and (B) H_2O_2 concentrations on amperometric response of HRP-MC-LR/anti-MC-LR/(Au/CNx-MWNTs)/GCE under other optimal conditions.

References

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2 B. Yue, Y. W. Ma, H. S. Tao, L. S. Yu, G. Q. Jian, X. Z. Wang, X. S. Wang, Y. N. Lu and Z. Hu, *J. Mater. Chem.*, 2008, **18**, 1747–1750.