Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information

Synthesis of sunflower-like gold nanostructure and its application in the electrochemical immunoassay using nanogold-triggered hydrogen evolution reaction

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EXPERIMENTAL SECTION

Materials. Monoclonal mouse anti-human neuron-specific enolase (NSE) antibody (designated as mAb), polyclonal rabbit anti-human NSE antibody (designated as pAb) and NSE standards with various concentrations were purchased from Beijing Biosynthesis Biotechnol. Co., Ltd (Beijing, China). Chitosan, ascorbic acid, bovine serum albumin (BSA), β-Cyclodextrin (CD), hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4$ $4H_2O$), poly(vinyl pyrrolidone) (PVP) and sodium dodecyl sulfate (SDS) were purchased from Sinopharm Chem. Re. Co. Ltd. (Shanghai, China). All other reagents were of analytical grade and were used without further purification. All solutions were prepared with deionized water obtained from a Milli-Q water purifying system (18.2 MΩ cm^{-1}, Milli-Q, Millipore). Phosphate-buffered saline (PBS) solution with various pH values were prepared by mixing 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4, and 0.1 M KCl was used as the supporting electrolyte. Binging and washing buffer was 0.1 M PBS (pH 7.4) containing 0.05% (w/v) Tween 20. The blocking buffer was 0.1 M PBS (pH 7.4) containing 2.5 wt% BSA.

Synthesis of Sunflower-Like Gold Nanostructures (AuNFs). Prior to synthesis, all glassware used in the synthesis was cleaned in a bath of freshly prepared 3 : 1 (v/v) HNO_3-HCl and rinsed thoroughly prior to use. Sunflower-like gold nanostructures (designated as AuNFs) with an average size of 50 nm in diameter along its horizontal or longitudinal axis were synthesized in aqueous solution with poly(vinyl pyrrolidone) (PVP)-sodium dodecyl sulfate (SDS) aggregations similar to the literatures [Y. Ren, C. Xu, M. Wu, M. Niu, Y. Fang, *Colloids Surf. A* **2011**, *380*, 222.]. Briefly, 50-mg PVP and 35-mg SDS were added into 10-mL distilled water in a beaker under vigorous stirring in sequence. Afterwards, the mixture was heated up to 40 ºC and continuously stirred for 60 min. 50 μL of 10 mM $HAuCl_4$ aqueous solution was rapidly injected to the mixture. Following that, 1.0 mL of 50 mM NaOH solution was slowly dropped to the resulting suspension under the same conditions (*Note*: The color of the mixture changed from pale white to dark blue during this process). After that, AuSFs were collected by centrifugation (15 min at 10,000 g).

Bioconjugation of pAb Antibody with AuSFs (AuNF-pAb). AuNF colloids were washed and purified by centrifugation to remove the impurities including PVP and SDS. The purified AuNF colloids were dissolved in 1.25 mL of distilled water ($C_{[Au]} \approx 6$ mM), and were conjugated with pAb antibody. The reaction is based on the interaction between $–NH_2$/or –SH groups on the pAb and AuNF. Briefly, 1.25 mL of AuNF was initially adjusted to pH 9.0-9.5 using Na_2CO_3, and then 250 μL of pAb (0.5 mg mL^{-1}) was added into the AuNF. The mixture was gently shaken for 5 min, and transferred to the refrigerator for an overnight reaction. The suspension was centrifuged at 4 °C for 30 min at 13,000 rpm. The purified AuNF-pAb conjugates ($C_{[Au]} \approx 7.5$ mM) were stored in 1.0 mL of pH 7.4 PBS containing 1.0 wt % BSA at 4 °C for further use.

Preparation of Electrochemical Immunosensor. A glassy carbon electrode (GCE) with 2 mm in diameter (Electrode area: 3.14 mm^2) was polished with 0.3 μm and 0.05 μm alumina, followed by successive sonication in bi-distilled water and ethanol for 5 min and dried in air. The well-polished electrode was cycled in a 0.1 M H_2SO_4 solution for 5 times in the potential range from 0 to 2 V. During this process, the anodization of the GCE surface resulted in a multilayer oxide film having –OH groups or –COOH groups [J. Tang, L. Hou, D. Tang, B. Zhang, J. Zhou and G. Chen, *Chem. Commun.* 2012, **48**, 8180]. Following that, 5 μL of β-cyclodextrin (CD) aqueous solution (50 mg mL^{-1}) was cast onto the surface of the pretreated GCE and dried for about 2 h at room temperature (RT) to form a CD-modified GCE. After washing with distilled water, 10 μL of mAb antibodies (1.0 mg mL^{-1}) was thrown on the modified electrode, and incubated for 4 h at RT. During this process, mAb antibodies were immobilized on the CD-modified GCE owing to the β-cyclodextrin capture [L. Zeng, Q. Li, D. Tang, G. Chen and M. Wei, *Electrochim. Acta* 2012, **68**, 158; K. Ikura, J. Fujimoto, K. Kubonishi, S. Natsuka, H. Hashimoto, T. Ito and K. Fujita, *Cytotechnology* 2002, **40**, 23]. Subsequently, the modified electrode was treated with a solution of 2.5 wt % BSA for 60 min at 37 ºC to eliminate the nonspecific effect. Finally, the as-prepared mAb-CD-GCE was stored at 4 °C when not in use.

Electrochemical Measurement. All electrochemical measurements were carried out with a

CHI 660C Electrochemical Workstation (Shanghai, China) with a conventional three-electrode system using a modified GCE as working electrode, a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference electrode. Scheme 1 gives the electrochemical immuno-HER measurement protocol toward NSE. Initially, 10 μL of NSE standards/or samples was dropped to the immunosensor, and incubated for 30 min at room temperature to form the antigen-antibody complex. After being washed with the washing buffer, 10 μL of above-prepared AuNF-pAb was thrown on the immunosensor, and incubated for another 30 under the same conditions to construct a sandwiched immunocomplex. Following that, 250 μL of 2 M HCl solution was added and a potential of +1.35 V was applied for 50 sec (electrochemical pretreatment). Thereafter, a potential of -1.0 V was applied for 50 sec in chronoamperometric mode. Under these conditions, H^+ ions were reduced to H_2 thanks to the catalytic effect of the gold nanoparticles [M. Maltez-de Costa, A. de la Escosura-Muniz and A. Merkoci, *Electrochem. Commun*., 2010, **12**, 1501; M. Maltez-de Costa, A. de la Escosura-Muniz, C. Nogues, L. Barrios, E. Ibanez and A. Merkoci, *Small*, 2012, **8**, 3605;]. The value of the catalytic current registered at 10 sec was collected as the sensor signal, being this value proportional to the quantity of gold nanoparticles and, consequently, to the concentration of target NSE. All measurements were carried out at room temperature (25 ± 1.0 ºC). Analyses are always made in triplicate.

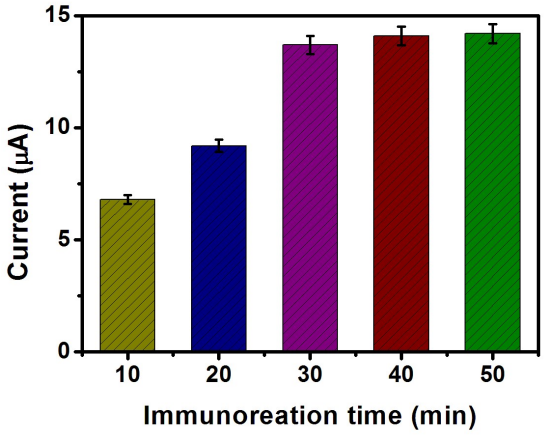

Fig. S1. Effect of immunoreaction time on the signal of AuSF-based immuno-HER assay by using 1.0 ng/mL

NSE as an example.

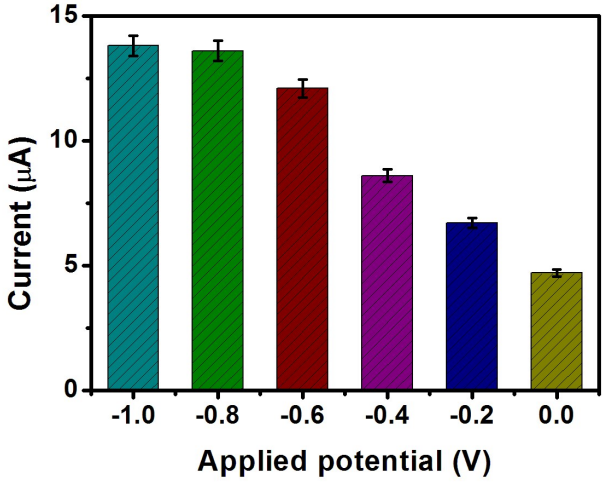

Fig. S2. Effect of the applied potential for HER reaction time on the signal of AuSF-based immuno-HER assay by using 1.0 ng/mL NSE as an example.