Electronic Supplementary Information

Nanogold-based bio-bar codes for label-free immunosensing of proteins coupling with an in situ DNA-based hybridization chain reaction

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EXPERIMENTAL SECTION

Materials and Reagents. Carcinoembryonic antigen (CEA) was purchased from Biocell Biotechnol. Co., Ltd. (Zhengzhou, China). Monoclonal mouse anti-human CEA antibody (clone II-7, designated as mAb_1, dilution: 1:25 – 1:50) and polyclonal rabbit anti-human CEA antibody (clone HP-6001, designated as pAb_2) were purchased from Dako Diagnostics (Shanghai, China) Co., Ltd. β-Cyclodextrin (CD) was obtained from Sinopharm Chem. Re. Co. (Shanghai, China). Gold colloids with 16 nm in diameter were prepared and characterized as described.[S1] All other reagents were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all runs. The initiator strand (S0), H1 and H2 were obtained from Sangon Biotech. Co., Ltd. (Shanghai, China). The sequences of oligonucleotides are listed as follows:

S0: 3'-SH-$(CH_2)_6$-AATTGGGTGCGGCTTAGGATCTGA-5'

H1: 5'-<u>TTAACC</u>CACGCCGAATCCTAGACT*CAAAGT*AGTCTAGGATTCGGCGTG-3'

H2: 3'-<u>GTTTCA</u>TCAGATCCTAAGCCGCAC*AATTGG*GTGCGGCTTAGGATCTGA-5'

In the hairpin sequences, loops are italicized and sticky ends are underlined.

Preparation of Electrochemical Immunosensor. A glassy carbon electrode (GCE) with 2 mm in diameter 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 five 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.[S2] 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 surface.[S3] After washing with distilled water, 30 μL of mAb_1 antibodies (dilution ratio: 1:50) was thrown on the modified electrode, and incubated for 4 h at RT. During this process, mAb_1 antibodies were immobilized on the CD-modified GCE due to the capture of β-cyclodextrin.[S4] Finally, the as-prepared mAb_1-CD-GCE was stored at 4 °C when not in use.

Preparation of Initiator Strands (S0) and pAb_2-Conjugated Gold Nanoparticles (pAb_2-S0-AuNPs). The pAb_2-S0-AuNP nanocomplexes were synthesized and prepared according to our previous report.[S5] Prior to experiment, 5 mL of 16-nm gold colloids (AuNPs, $C_{[Au]}$ = 24 μM) was adjusted to pH 9.0-9.5 by directly using 0.1 M Na_2CO_3 aqueous solution. Then, 200 μL of polyclonal rabbit anti-human CEA antibody (pAb_2, 1 : 100) was added into gold colloids, and incubated for 20 min at room temperature. During this process, pAb_2 antibodies were covalently bound to gold nanoparticles via the dative binding between gold nanoparticles and free -SH groups of the antibody. Afterwards, the alkylthiol-capped barcode DNA initiator strands (S0, 0.5 OD) was injected into the mixture. After gently shaking for 5 min, the mixture was transferred to the refrigerator at 4 °C for further reaction (overnight). Following that, the mixture was centrifuged (14,000 g) for 25 min at RT. The pellet (i.e. S0/pAb_2-functionalized gold nanoparticles, designated as pAb_2-S0-AuNPs) was re-suspended in 1.0 mL of 2 mM sodium carbonate solution ($C_{[Au]}$ = 120 μM) containing 1.0 wt % BSA and 0.1% sodium azide, pH 7.4, and stored at 4 °C until use.

Electrochemical Measurements. All electrochemical measurements were performed on a CHI 604D Electrochemical Workstation (Shanghai CH Instruments Inc., China) using a conventional three-electrode system with a modified GCE working electrode, a platinum foil auxiliary electrode, and a saturated calomel electrode (SCE) reference electrode. The assay was performed as follows:

(i) *Immunoreaction*: 10 μL of mixture solution comprising various concentrations of target CEA sample/or standards and 1.0 mg mL^{-1} pAb_2-S0-AuNP colloids was dropped onto the surface of the mAb_1-CD-GCE, and incubated for 25 min at RT. The aim of this step was to form a sandwiched immunocomplex on the GCE surface.

(ii) *Hybridization chain reaction*: After washing with pH 7.4 PBS, the resulting immunosensor was immersed (*Note*: suspended) into the hybridization solution containing 0.5 μM H1 and 0.5 μM H2, and incubated for 70 min at RT. During this process, the hybridization chain reaction was triggered and progressed to form the long nicked DNA polymers on the AuNPs.

(iii) *Intercalation of methylene blue*: After washing with pH 7.4 PBS, the modified electrode was suspended into the 0.5 mM methylene blue aqueous solution, and incubated for 30 min at RT. During this process, the methylene blue molecules were intercalated into the grooves of the double-helix.

(iv) *Electrochemical measurement*: After rinsing thoroughly with pH 7.4 PBS to remove the un-intercalated methylene blue, the electrochemical characteristics of the resulting immunosensors were investigated in pH 7.0 PBS by square wave voltammetry (SWV) from -500 mV to 0 mV (*vs.* SCE) (Amplitude: 25 mV; Frequency: 15 Hz; Increase *E*: 4 mV). Analyses are always made in triplicate.

For comparison, ferrocene-labeled H1 and H2 probes were also used for the immuno-HCR assay. The assay involved in the above-mentioned steps (i), (ii) and (iv).

Optimization of Experimental Conditions. Fig. S1 displays the effect of hybridization chain time between pAb_2-AuNP-S0 and H1 + H2 on the electrochemical signal of the immuno-HCR assay toward 10 pg mL^{-1} CEA. The current increased with the increasing hybridization time, and tended to level off after 70 min. So, 70 min was used for the HCR reaction.

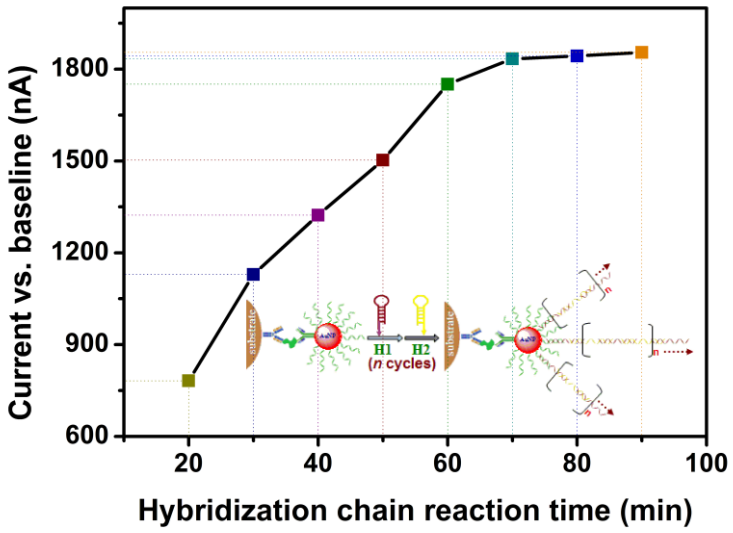

Fig. S1 The effect of hybridization chain reaction time on the electrochemical signal of the immunosensor (10 pg mL^{-1} CEA used in the case).

Usually, the antigen-antibody reaction is adequately carried out at human normal body

temperature (37 °C). Considering the possible application of the proposed immunoassay in the future, we selected room temperature (25 ± 1.0 °C) for the antigen-antibody interaction throughout the experiment. At this condition, we monitored the effect of incubation time on the currents of the immuno-HCR assay from 10 min to 40 min (*Note*: To avoid confusion, the incubation times of the immunosensor with CEA were paralleled with those of the immunosensor-CEA with S0-AuNP-pAb_2). As shown in Fig. S2, the SWV peak currents increased with the increment of incubation time, and tended to level off after 25 min. Hence, an incubation time of 25 min was selected for sensitive determination of CEA at acceptable throughput.

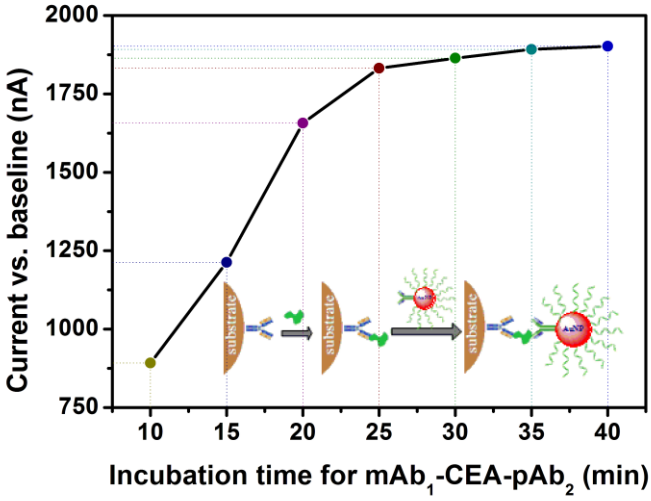

Fig. S2 The effect of immunoreaction time on the electrochemical signal of the immunosensor (10 pg mL^{-1} CEA used in the case).

In this work, the peak current mainly derives from the intercalated methylenen blue. Typically, the peak current heavily depends on the pH values of the assay solution. Fig. S3 displays the dependence of peak currents on pH of PBS toward 10 pg mL^{-1} CEA. As indicated from Fig. S3, an optimal current was obtained at pH 7.0 PBS. Thus, a pH 7.0 with PBS was chosen as the supporting electrolyte.

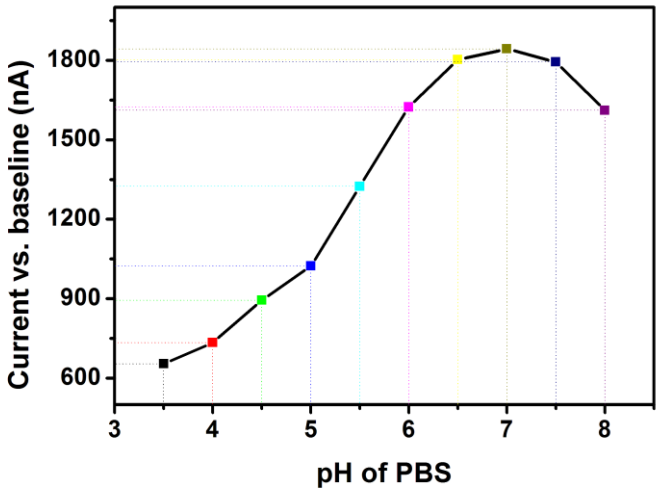

Fig. S3 The effect of pH of PBS on the electrochemical signal of the immunosensor (10 pg mL^{-1} CEA used in the case).

Reference

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