1	Electronic Supplementary Information			
2	Au(III)-promoted polyaniline gold nanospheres with electrocatalytic			
3	recycling of self-produced reactants for signal amplification			
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EXPERIMENTAL SECTION

Materials. Monoclonal mouse anti-human IgG (Fab specific) antibody (clone GG-6, designated as Ab₁, ~5.0 mg mL⁻¹), polyclonal goat anti-human IgG (Fc specific) antibody (designated as Ab₂, ~2 mg mL⁻¹), and IgG from human serum (reagent grade, \geq 95 *wt%*, essentially salt-free, lyophilized powder) were purchased from Sigma-Aldrich (USA). HAuCl₄·4H₂O, *p*-nitrophenol (NP), *p*-aminophenol (AP), NaBH₄, and bovine serum albumin (BSA, 96–99 *wt%*) were obtained from Sinopharm Chem. Re. Co. Ltd. (Shanghai, China). Aniline monomer solution (>99.5 *wt%*) was purchased from Sangon Biotech. Co. Ltd. (China). 16-nm colloidal gold particles were synthesized consulting to our previous report.^{S1} All other reagents were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore water purification system (\geq 18 MΩ, Milli-Q, Millipore) was used in all runs. 0.1 mol L⁻¹ K₂HPO₄ and 0.1 mol L⁻¹ KH₂PO₄, and 0.1 mol L⁻¹ KCl was added as the supporting electrolyte.

Preparation and Bioconjugation of Au(III)-Promoted Polyaniline Gold Nanospheres. The Au(III)-promoted polyaniline gold nanospheres (designated as GPANGs) were synthesized according to our previous literature.^{S2} Briefly, HAuCl₄ aqueous solution (5 mL, 1.0 mmol L⁻¹) was initially dropped into HCl solution (10 mL, 0.1 mol L⁻¹) containing 0.1 mmol L⁻¹ aniline monomers with gentle stirring. With the reaction progressing, the color of the mixture gradually changed from yellow to blue, and finally to dark green. Following that, the obtained precipitate (i.e. polyaniline-protected gold nanoparticles) by centrifugation was re-dispersed into 1 mL of deionized water. Afterwards, 3 mL of gold colloids ($C_{\text{[Au]}} \approx 24 \text{ mmol L}^{-1}$) was added into the mixture, which was gently shaken on a shaker for 2 h at room temperature (RT) to make gold nanoparticles assemble on the polyaniline-protected nanogold particles *via* the -Au-NH- bonds. Subsequently, the Au(III)-promoted polyaniline gold nanospheres (GPANGs) were collected by centrifugation for 20 min at 12,000 g, and re-suspended in 1.0 mL of deionized water.



Next, the resulting GPANGs were adjusted to pH 9.0-9.5 by using Na₂CO₃ aqueous solution. And then, 150 μ L of Ab₂ (1.0 mg mL⁻¹) was added into the GPANG colloids. 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 (12,000 g) for 20 min at 4 °C. The pellet (i.e. GPANG-labelled Ab₂ antibodies (designated as bionanolabels) were re-suspended in 1.0 mL of 2 mmol L⁻¹ sodium carbonate containing 1.0 wt% BSA and 0.1% sodium azide, pH 7.4, and stored at 4 °C until use. For comparison, polyaniline nanospheres (12 nm in diameter) and gold nanoparticles (16 nm in diameter) were utilized for the label of Ab₂ by using the same method mentioned above, respectively (*Note*: 12-nm polyaniline nanospheres were synthesized by using ammonium persulfate in the absence of any protonic acids according to the literature^{S3}).

Fabrication of Immunosensor. Before modification, glassy carbon electrode (GCE, 4 mm in diameter) was polished with 0.3 μ m and 0.05 μ m alumina, followed by successive sonication in acetone, ethanol and deionized water for 5 min and dried in air. Then polyaspartic acid (PASP) membranes were formed on the surface of glassy carbon electrode in 0.02 M PBS buffer solution (pH 6.84) containing 1.0 mmol L^{-1} aspartic acid for electropolymerization by means of cyclic voltammetry. Then polyaspartic acid (PASP) modified electrode was washed by successive sonication in 0.02 M PBS buffer solution (pH 6.84) and deionized water for 2 min to remove the unreacted substances and dried in air. Next, the electrode was immersed into 0.1 mol L^{-1} PBS solution (pH 7.0) containing 50.0 mmol L^{-1} 1-ethyl-3-(3-dimethylaminopropyl) buffer 25.0 mmol L⁻¹ N-hydrosulfosuccinimide (NHS) and for 2 h to carbodiimide (EDC) and activated the carboxyl groups. Anti-HIgG (8 µL, 1.0 mg mL⁻¹) was dropped onto the activated electrode and incubated for 1 h. Then the electrode was immersed in 0.2 wt% BSA solution for 1 h at RT to block the unreacted and nonspecific sites.

Electrochemical Measurement. All electrochemical measurements were carried out with a CHI 620D Electrochemical Workstation (Shanghai, China) with a conventional three-electrode system using a modified gold electrode as working electrode, a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference electrode. Initially, 5 μ L of HIgG samples with various concentrations was thrown onto the modified electrode and incubated for 30 min at room temperature. After washing with deionized water, GPANGs-labelled Ab₂ antibodies were dropped onto the surface of the prepared immunosensor, and incubated for another 30 min under the same conditions. After rinsing thoroughly with deionized water, the electrochemical response of the immunosensor were performed in pH 8.0 PBS buffer solution containing 10.0 mmol L⁻¹ p-nitrophenol (NP) and 10.0 mmol L⁻¹ NaBH₄ by using differential pulse voltammetry (DPV) from -150 to 300 mV (*vs.* SCE) with a pulse amplitude of 50 mV and a pulse width of 50 ms. All electrochemical measurements were done in an unstirred electrochemical cell at room temperature (RT, 25 ± 1 °C). Analyses are always made in triplicate.

Electrochemical Behaviors of Variously Modified Electrodes. To verify the fabrication process of electrochemical immunosensor, electrochemical impedance spectroscope (EIS) of variously modified electrodes were monitored in 5 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl (Fig. S1). The high frequency region of the impedance plot shows a semicircle related to the redox probe $Fe(CN)_6^{3-/4-}$, followed by a Warburg line in the low frequency region which corresponds to the diffusion step of the overall process. There is a small resistance at the cleaned GCE (curve 'a' in Fig. S1). However, when PASP membranes were modified on the surface of glassy carbon electrode by electropolymerization, the resistance was decreased (curve 'b' in Fig. S1). The reason might be on account of the PASP membranes as a kind of amino acid like cysteine can facilitate the electron transfer. After the free amines of Ab₁ antibodies binding to carboxyl groups of polyaspartic acid (PASP) via the EDC and NHS, the resistance was increased (curve 'c' in Fig. S1). The reason might be the fact that Ab_1 antibodies as a kind of biomacromolecules hampered the electron transfer. As shown from curve 'd' in Fig. S1, the resistances increased again after HIgG analyte assembled on the Ab₁-PASP-GCE. The reason might be attributed to the fact that the formed immunocomplex served as an inert layer and hindered the electron transfer. Curve 'f in Fig. S1 exhibited that the resistances were increased. This is most likely a consequence that the as-prepared GPANGs were redox-active, and could favour for electron communication.

Notes and references

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Fig. S1 TEM image of the as-prepared GPANG nanostructures.



Fig. S2 UV-vis absorption spectra of (a) *p*-nitrophenol, (b-d) *p*-nitrophenol after reaction with various concentrations of NaBH₄, and (d-g) the mixture '*d*' upon addition of pure gold nanoparticles with various concentrations.



Fig. S3 EIS spectra of (a) bare GCE, (b) PASP-GCE, (c) Ab_1 -PASP-GCE, (d) HIgG/Ab_1-PASP-GCE, (e) GPANG-Ab_2/HIgG/Ab_1-PASP-GCE in pH 7.4 PBS containing 5 mmol L⁻¹ Fe(CN)₆^{-4/-3} at frequency 10⁻² to 10⁵ Hz.

Measurement protocol ^{<i>a</i>}	Linear range	LOD	Refs
DNAzyme-based electrochemical immunoassay	$0.1 \text{ pg mL}^{-1} - 100 \text{ ng mL}^{-1}$	0.1 pg mL^{-1}	[S 4]
HCR-based electrochemical immunoassay	$0.1 \text{ fg mL}^{-1} - 100 \text{ ng mL}^{-1}$	0.1 fg mL^{-1}	[\$5]
Electrochemiluminescent immunosensor	$0.1 \text{ pg mL}^{-1} - 10 \text{ ng mL}^{-1}$	87 fg m L^{-1}	[S6]
Chemiluminescence resonance energy transfer	0.2 – 4.0 nM	29 pM	[S 7]
Photoelectrochemical immunoassay	$0.5 \text{ pg mL}^{-1} - 5.0 \text{ ng mL}^{-1}$	0.5 pg mL^{-1}	[S 8]
Chemiluminescent immunoassay	$0.008 - 5.0 \ \mu g \ mL^{-1}$	1.14 ng mL ⁻¹	[S 9]
QD-based electrochemical immunosensor	$1.0 \text{ pg mL}^{-1} - 25 \text{ ng mL}^{-1}$	0.6 pg mL ⁻¹	[S 10]
Fluorescence immunoassay	$1.0 - 100 \text{ pg mL}^{-1}$	0.3 pg mL^{-1}	[S 11]
Surface plasmon-coupled emission sensor	-	1.0 ng mL^{-1}	[S 12]
Voltammetric immunoassay	0.01 pg mL ⁻¹ – 100 ng mL ⁻¹	1.0 fg mL ⁻¹	This work

 Table S1. Comparison of analytical properties of the developed immunoassay proposed here with other IgG assay methods

^{*a*} HCR: hybridization chain reaction.