Electronic Supplementary Information

Hemin/G-quadruplex-based DNAzyme concatamers as electrocatalysts and biolabels for amplified electrochemical immunosensing of IgG1

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EXPERIMENTAL SECTION

Materials and Reagents. Monoclonal mouse anti-human IgG1 (Fab specific) [G1M(f)] antibody (clone SG-16, designated as Ab_1, ~5.0 mg mL^{-1}), monoclonal anti-human IgG1 (Fc specific) antibody (clone HP-6001, designated as Ab_2, ~3 mg mL^{-1}), and IgG1 (Kappa from human myeloma plasma, human IgG subclasses) were purchased from Sigma-Aldrich (USA). Hemin was purchased from Tokyo chemical industry Co., Ltd (Japan). β-Cyclodextrin (CD) was obtained from Sinopharm Chem. Re. Co. (Shanghai, China). Oligonucleotides designed in this study were synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China), which were purified by HPLC and confirmed by mass spectrometry. The primer (S_0)-labeled Ab_2 antibodies (Ab_2-S_0) were synthesized and purified by Beijing Dingguo Biotechnol. Co. Ltd. (China). The sequences of the primer (S_0), S_1 and S_2 are listed as follows:

S_0: 5'-NH_2-$(CH_2)_6$-GTACTACAGCAGCTG-3'

S_1: 5'-GGGTAGGGCGGGTTGGGTATCTCCTAATAGCAGCAGCTGCTGTAGTAC-3'

S_2: 5'-ferrocence-$(CH_2)_6$-CTGCTATTAGGAGATGTACTACAGCAGCTG-3'

In the probe S_1, hemin-binding aptamers are underlined. DNA stock solution was obtained by dissolving oligonucleotides in tris-HCl buffer solutions (pH 7.4). Each oligonucleotide was heated to 90 °C for 5 min, and slowly cooled down to room temperature before usage. 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. Phosphate-buffered saline (PBS, 0.1 M) solution with various pHs was prepared by mixing the stock solutions of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4, and 0.1 M KCl was added as the supporting electrolyte. Clinical serum samples were made available by Fujian Provincial Hospital, China.

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.[1] 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.[2] After washing with distilled water, 30 μL of Ab_1 antibodies (1.0 mg mL^{-1}) was thrown on the modified electrode, and incubated for 4 h at RT. During this process, Ab_1 antibodies were immobilized on the CD-modified GCE due to the capture of β-cyclodextrin.[3] Finally, the as-prepared Ab_1-CD-GCEs were stored at 4 °C when not in 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 IgG1 sample/or standards and 1.0 mg mL^{-1} Ab_2-S_0 was dropped onto the surface of the Ab_1-CD-GCE, and incubated for 25 min at RT. The aim of this step was to form a sandwiched immunocomplex on the surface of the Ab_1-CD-GCE.

(ii) *Hybridization reaction*: After washing with pH 7.4 PBS, the resulting immunosensor was immersed (*Note*: suspended) into the hybridization solution containing 0.5 μM S_1 and 0.5 μM S_2, and incubated for 75 min at RT. During this process, the hybridization reaction was triggered and progressed to form the DNA concatamers between S_1 and S_2 in the presence of the primer S_0.

(iii) *Formation of DNAzymes on the concatamers*: After washing with pH 7.4 PBS, the modified electrode was suspended into the 0.2 mM hemin solution, and reacted for 30 min at RT to form the hemin/G-quadruplex (i.e. DNAzyme) complex.

(iv) *Electrochemical measurement*: After rinsing thoroughly with pH 7.4 PBS to remove the unbound hemin, the electrochemical characteristics of the resulting immunosensors were investigated by differential pulse voltammetry (DPV) from 550 V to 200 mV (vs. SCE) with a pulse amplitude of 50 mV and a pulse width of 50 ms in the PBS (pH 7.4) containing 3 mM H_2O_2 due to the catalytic reduction of the bound hemin towards H_2O_2 in the presence of the concatamerized ferrocene. Analyses are always made in triplicate.

Cyclic Voltammetric Characteristics. Fig. S1 shows the cyclic voltammograms of variously modified electrodes in pH 7.4 PBS without and with 3.0 mM H_2O_2. Almost no redox peak was observed at S_1+S_2/S_0-Ab_2/IgG1/Ab_1-CD-GCE in pH 7.4 PBS (curve *a* in Fig. S1-A). Despite the ferrocene redox tags in the S_2 probe, the labeled amount was relatively less. When hemin was conjugated onto the electrode '*a*', the peak currents gently increased (curve *b* in Fig. S1-A). The reason might be the fact that the hemins could facilitate the electron transfer. Importantly, when the formation of hemin/G-quadruplex-based DNAzyme concatamers on the Ab_1-CD-GCE, a pair of redox peaks was achieved (curve *c* in Fig. S1-A). The results revealed that the DNAzyme concatamers could largely improve the electrochemical response of the developed immunosensor. Vice versa, we also investigated the cyclic voltammetric characteristics after addition of 3.0 mM H_2O_2 in pH 7.4 PBS (Fig. S1-B). Compared curve *a* with curve *a*', the presence of H_2O_2 resulted in the gentle decrease in the peak. This is most likely a consequence of the fact that the added H_2O_2 changed the ionic strength of the supporting electrolyte to some extent. Significantly, an obvious catalytic characteristic with the increase of cathodic current and decrease of anodic current could be acquired upon addition of H_2O_2 in pH 7.4 PBS (curve *b* versus curve *b*', curve *c* versus curve *c*'). Alike, we also found that a large catalytic characteristic was obtained at the formed DNAzyme concatamers. The results adequately indicated the developed immunoassay could favor for sensitive detection of IgG1.

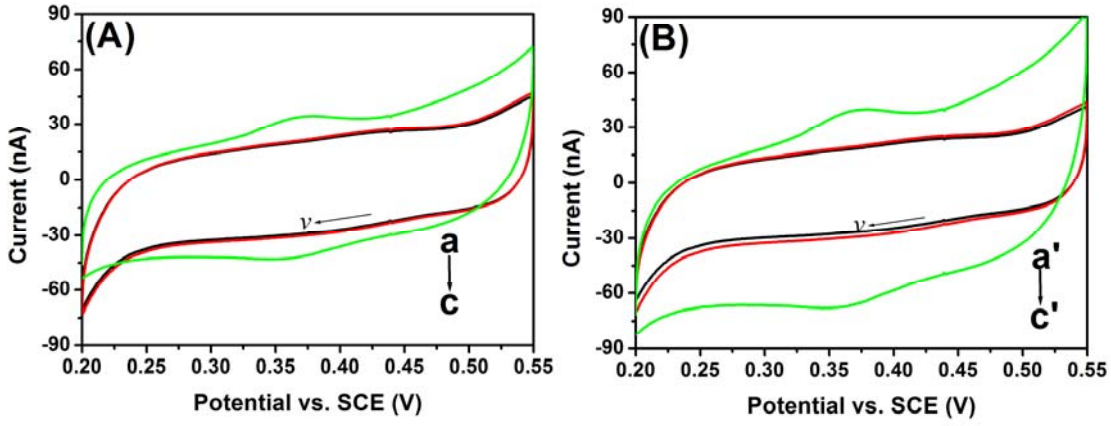

Fig. S1 Cyclic voltammograms of (a,a') S_1+S_2/S_0-Ab_2/IgG1/Ab_1-CD-GCE, (b,b') hemin/S_1+S_2/S_0-Ab_2/IgG1/Ab_1-CD-GCE, and (c,c') the hemin-binding aptamers at 50 mV s^{-1} in (A) pH 7.4 PBS and (B) pH 7.4 PBS containing 3.0 mM H_2O_2 (0.1 ng mL^{-1} IgG1 used as an example).

Optimization of Experimental Conditions. Fig. S2 displays the effect of hybridization time between S_0-Ab_2 and S_1 + S_2 on the electrochemical signal of the immunoassay toward 0.1 ng mL^{-1} IgG1. The current increased with the increasing hybridization time, and tended to level off after 75 min. Meanwhile, we also investigate the effect of incubation time between the Ab_1-CD-GCE and IgG1 + Ab_2-S_0 on the current of the immunoassay (Fig. S3). An optimal signal was acquired at 25 min. Hence, 25 min and 75 min were selected for the antigen-antibody interaction and the hybridization reaction, respectively.

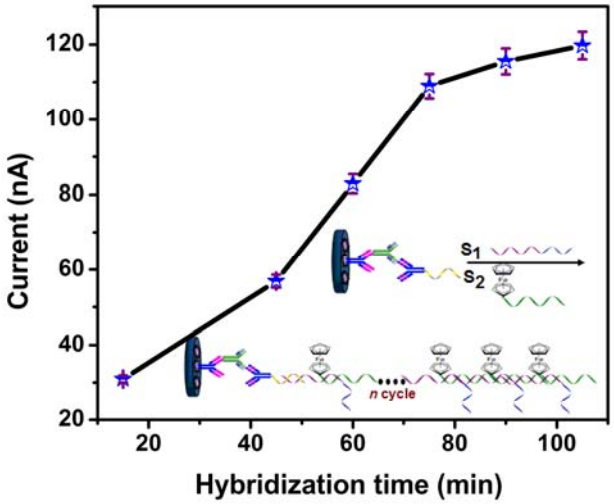

Fig. S2 The effect of hybridization time between the S_0-Ab_2/IgG1/Ab_1-CD-GCE and S_1 + S_2 on the electrochemical signal of the immunosensor (0.1 ng mL^{-1} IgG1 used in the case).

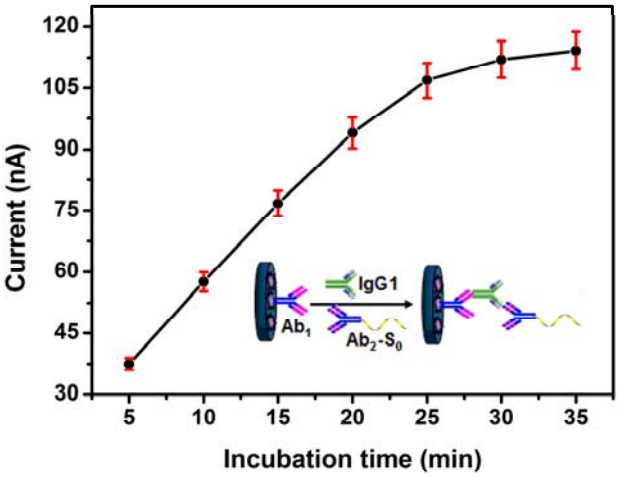

Fig. S3 The effect of incubation time between the Ab_1-CD-GCE and S_0-Ab_2 + IgG1 on the electrochemical signal of the immunosensor (0.1 ng mL^{-1} IgG1 used in the case).

Another key point is the characteristic of the DNAzyme. Usually, it takes some time for the formation of hemin/G-quadruplex. An acceptable signal was obtained at ~30 min, as shown in Fig. S4. Longer incubation time did not cause the large change in the current. Therefore, 30 min was employed for the DNAzyme construction. At the condition, we also monitored the influence of H_2O_2 concentration in the assay solution on the current of the immunosensor. As seen from Fig. S5, the catalytic current increased with the increasing H_2O_2 concentration, and reached a plateau at 3 mM. So, 3 mM of H_2O_2 was used as the enzymatic substrate for detection of target IgG1.

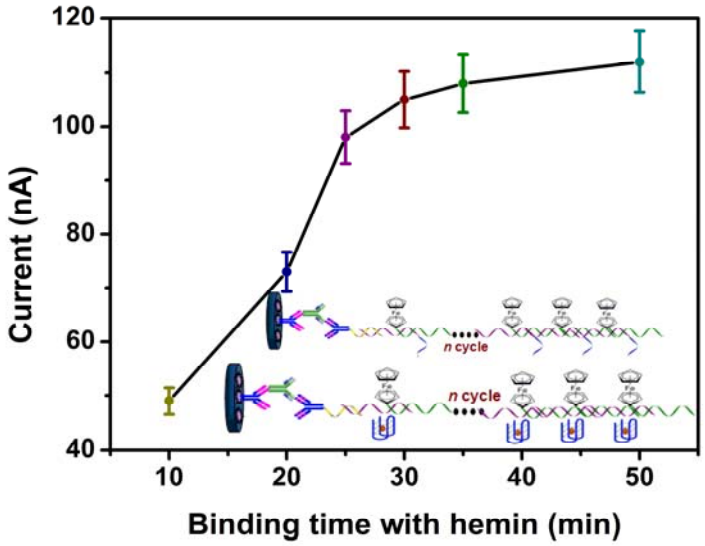

Fig. S4 The effect of the binding time of the DNA concatamers with the hemin on the electrochemical signal of the immunosensor (0.1 ng mL^{-1} IgG1 used in the case).

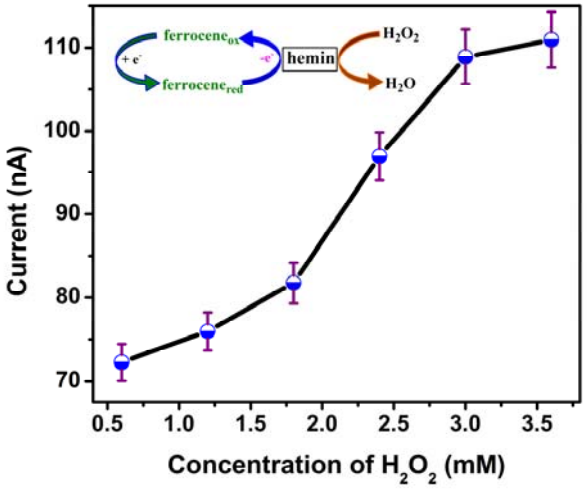

Fig. S5 The effect of H_2O_2 concentration on the electrochemical signal of the immunosensor (0.1 ng mL^{-1} IgG1 used in the case).

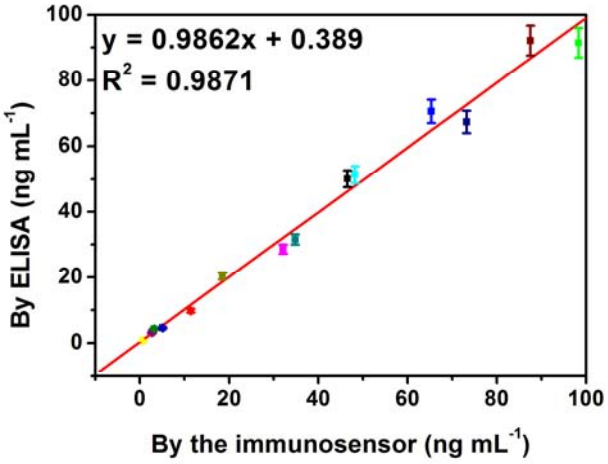

Fig. S6 Comparison of the titer results of detecting samples between the ELISA and the developed immunosensor (*Note*: Each data point represents the average value obtained from three different measurements; The error bars represent the 95% confidence interval of the mean for *y*-axis concentrations)

Table S1 Comparison of the assay results for clinical serum specimens using the developed immunoassays and the referenced ELISA method

	Method; Concentration (mean ± SD (RSD), n = 3, ng mL^{-1})		
Sample no.[a]	Immunosensor	ELISA	t_{exp}
1	46.5 ± 2.3	50.1 ± 1.4	2.32
2	11.4 ± 1.2	9.8 ± 0.7	1.99
3	98.3 ± 3.9	91.4 ± 2.8	2.49
4	65.3 ± 2.7	70.5 ± 3.4	2.07
5	48.2 ± 1.8	51.3 ± 2.2	1.89
6	32.1 ± 1.7	28.5 ± 2.1	2.31
7	0.7 ± 0.1	0.8 ± 0.1	1.22
8	18.5 ± 1.2	20.3 ± 0.9	2.08
9	73.2 ± 3.6	67.3 ± 2.4	2.36
10	0.05 ± 0.01	No application	No application
11	2.7 ± 0.7	3.2 ± 0.3	1.14
12	87.5 ± 4.5	92.1 ± 2.1	1.60
13	3.2 ± 0.9	4.3 ± 0.3	2.01
14	34.8 ± 1.4	31.5 ± 1.6	2.69
15	5.1 ± 0.8	4.6 ± 0.3	1.01

[a] These samples were diluted to various concentrations of IgG1, because their exact levels of IgG1 (undilution) are 7.0 – 10 mg mL^{-1}. Hence the data were obtained by multiplying the dilution ratio.

Reference

1 (*a*) M. Won, M. Rahman, N. Kwon, D. Shankaran and Y. Shim, *Electroanalysis*, 2005, **17**, 2231-2238; (*b*) W. Collier and T. Tougas, *Anal. Chem.,* 1987, **59**, 396-399.

2 D. Jia, J. Dai, H. Yuan, L. Lei and D. Xiao, *Talanta*, 2011, **85**, 2344-2351.

3 (*a*) L. Zeng, Q. Li, D. Tang, G. Chen and M. Wei, *Electrochim. Acta*, 2012, **68**, 158-165; (*b*) K. Ikura, J. Fujimoto, K. Kubonishi, S. Natsuka, H. Hashimoto, T. Ito and K. Fujita, *Cytotechnology*, 2002, **40**, 23-29.