Electronic Supplementary Information

An amplified electrochemical aptasensor for thrombin detection based on pseudobienzymic Fe_3O_4-Au nanocomposites and electroactive hemin/G-quadruplex as signal enhancers

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S1. Optimization of experimental conditions

The analytical performance of the prepared aptasensor had been greatly influenced by some experimental parameters, such as NH_2-TBA I concentration, TB incubation time and glucose concentration. Through optimizing these experimental conditions, the proposed aptasensor could be capable of more efficiency for the detection of thrombin.

20 μL of NH_2-TBA I with different concentrations from 0.5 to 3.0 μM was attached onto the electrode surface and measured in 1 mL $[Fe(CN)_6]^{3-/4-}$ (5.0 mM, pH 7.4). From Fig. S1A, the peak current decreased gradually with the increasing of the NH_2-TBA I concentration, and tended to level off at the concentration of 2.5 μM. So the optimal NH_2-TBA I concentration was 2.5 μM.

The TB incubation time was an important parameter affecting the specific binding with the secondary aptamer. At room temperature, the electrochemical response for TB (5 nM) decreased with the reaction time up to 40 min, then the signal began to level off (Fig. S1B), predicting the saturated binding between TB target and NH_2-TBA I. Thus, incubation time of 40 min was selected for the sandwich-type assay.

The amount of glucose in electrolytic cell played an important role to increase sensitivity and selectivity of the aptasensor. After incubation of 5 nM TB, the aptasensor was tested in 1 mL PBS (0.1 M, pH 7.0) containing glucose with different concentrations of 10, 20, 30, 40, 50, 60 μM, and the results are shown in Fig. S1C. As could be seen, the peak current increased with the increasing of glucose concentration,

and reached plateau regions at the concentration of 40 μM, suggesting a saturated state of glucose. Thus, 40 μM glucose solutions were the optimal concentration throughout the detection process.

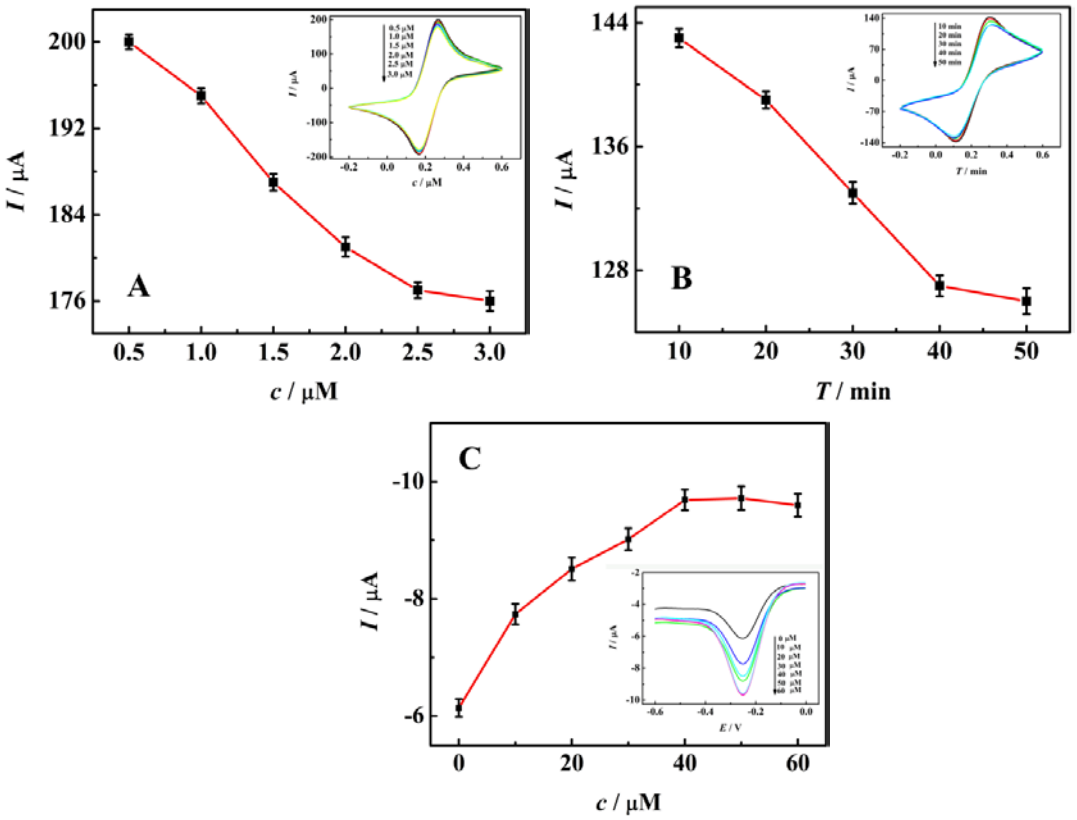

Fig. S1. The optimization of (A) NH_2-TBA I concentration and (B) TB incubation time in 1 mL $[Fe(CN)_6]^{3-/4-}$ (5.0 M, pH 7.4) and the optimization of (C) glucose concentration in 1 mL PBS (0.1 M, pH 7.0).

Table S1 Performance compared with other detection methodologies for TB detection.

Analytical methods[a]	Linear range	Detection limit	References
EIS	0.15-18 nM	0.02 nM	1
Flouresence	-	20 pM	2
CV	0.01-50 nM	6.3 pM	3
QCM	0.5-13 nM	0.1 nM	4
ECL	0.005-50 nM	1.7 pM	5
Colorimetric	1-100 nM	1 nM	6
DPV	0.0001-20 nM	0.013 pM	This work

[a] EIS: electrochemical impedance spectroscopy, CV: cyclic voltammetry, QCM: Quartz crystal microbalances, ECL: electrochemiluminescent, DPV: differential pulse voltammetry.

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