Supporting Information

A colorimetric and electrochemical dual-mode biosensor for thrombin using magnetic separation technique

*Wen-Jing Guo[a], Xiao-Yan Yang[a], Zhen Wu[b], Zhi-Ling Zhang[a, *]*

[a]Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, Wuhan University, Wuhan, 430072, P. R. China.

[b]Hubei Collaborative Innovation Center for Advanced Organic Chemical Materials, Ministry of Education Key Laboratory for the Synthesis and Application of Organic Functional Molecules, College of Chemistry and Chemical Engineering, Hubei University, Wuhan, 430062, P. R. China.

[*]Corresponding author: Zhi-Ling Zhang: Email: zlzhang@whu.edu.cn; Phone: 0086-27-68756759; Fax: 0086-27-68754067.

S1. Calculation of the numbers of TBA per magnetic nanobead (MB)

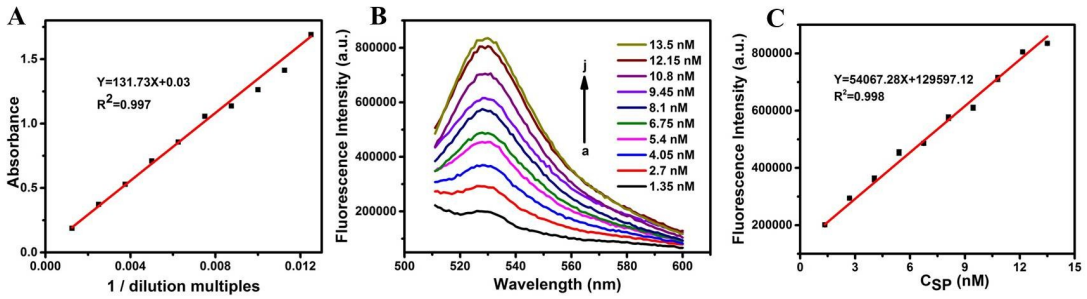

Fig. S1. (A) Standard curve of MBs (bright field). (B) Fluorescence spectra of alexa-488-SP at different concentration (1.35 nM, 2.7 nM, 4.05 nM, 5.4 nM, 6.75 nM, 8.1 nM, 9.45 nM, 10.8 nM, 12.15 nM, 13.5 nM). (C) Standard curve of alexa-488-SP..

S2. Optimization of detection conditions

Different experimental conditions were optimized for optimal performance. Firstly, according to the literature[1], 100 mV is taken as the operating voltage. From Fig S2A and S2B, the detection current is close to stable at 60 s, so 0.1 V and 60 s was taken as the working voltage and detection time, respectively. In addition, the amount of MBs-TBA-SP is also critical. In general, one parameter characterizing the affinity of the aptamer is the dissociation constant, and the formula is as follows:

$$Kd = \frac{[TBA][TB]}{[TBA-TB]}$$

Among them, TB represents thrombin; TBA represents thrombin aptamer; TBA-TB represents thrombin-thrombin aptamer complex. Under certain conditions, the dissociation constant of the thrombin aptamer is constant. Then, in theory, when the thrombin is fed, the more the aptamer is used, the more complexes of aptamers and thrombin are formed. Here, SP with the alexa-488 label at the 3' end were still used to optimize the amount of MBs-TBA-SP. The results showed that the more the amount of the complex injected, the stronger the fluorescence intensity of the replaced signal probe, when it was incubated with the same concentration of thrombin for a certain period of time. Taking into account the limitations of the experimental conditions, we

used 0.12 mg MBs-TBA-SP as the optimal input.

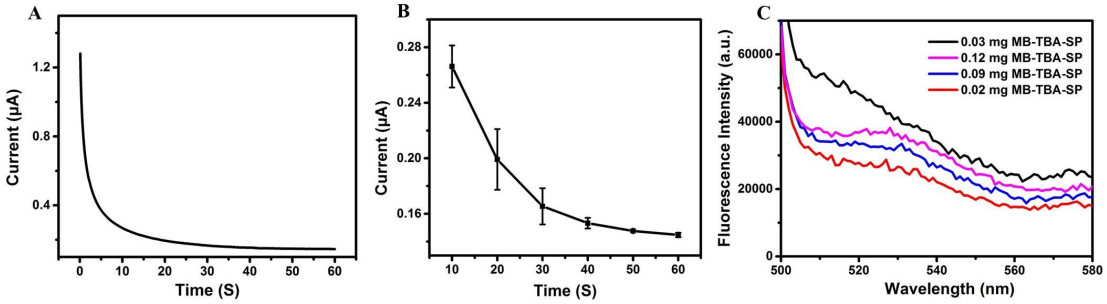

Fig. S2. (A) Colorimetric detection of HRP. (B) The effects of scan time on the detection signal. (C) The effects of different dosage of MB-TBA-SP on the fluorescence intensity.

S3. Comparison of this method and some other reported methods for thrombin assay.

Table S1. Comparison of this method and some other reported methods for thrombin assay.

Method	Signal output type	Linear range	LOD	Refs
Fluorescence	single	0.5 nM~10.5 nM	0.15 nM	2
Colorimetry	single	0.01nM~0.10 nM	4 pM	3
Electrochemiluminescence	single	0.01 nM~10 nM	6.3 pM	4
Electrochemistry	single	0.05 pM~60 nM	15 fM	5
Colorimetry	Dual (two kind of reporter probes)	0.5 pM~0.1 nM	0.12 pM	6
Electrochemistry		1 fM~1 μM	0.40 fM	
This work (Colorimetry	dual (one reporter probe)	10 nM~5 μM	10 nM	This work
Electrochemistry)		1 nM~10 μM	0.35 nM	

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