Electronic Supplementary Information

An ultrasensitive electrochemical immunoassay based on proximity hybridization-triggered three-layer cascade signal amplification strategy

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(1) Sequences of oligonucleotides

Table S1 Sequences of oligonucleotides used in this work.

Oligonucleotides	Sequences (from 5' to 3')
DNA1	CTGACTGA AT TCG GAGTTTTTTTTTTTTTTT–SH
DNA2	SH–TTTTTTTTTTTTTTTCTC CGA CGACATCT AACCTAGC TTTTT
DNA3	SH–TTTTTTTTTTTTTTTGGC CGA CGACATCT AACCTAGC TTTTT
DNA4	SH–TTTTTTTTTTTTTAACTC CGA CGACATCT AACCTAGC TTTTT
DNA5	SH–TTTTTTTTTTTAAAACTC CGA CGACATCT AACCTAGC TTTTT
HP1	CGACATCT AACCTAGC TCACTGAC TTTT GCTAGGTT AGATGTCG TCAGTCAG
HP2	GTCAGTGA GCTAGGTT AGATGTCG CCATGTGTAGA CGACATCT AACCTAGC CCTTGTCA–$(CH_2)_6$–SH
MB-HP2	MB–$(CH_2)_6$–GTCAGTGA GCTAGGTT AGATGTCG CCATGTGTAGA CGACATCT AACCTAGC CCTTGTCA–$(CH_2)_6$–SH
HP3	AGATGTCG TCTACACATGG CGACATCT AACCTAGC CCATGTGTAGA AGTGC

Linear padlock	phosphate–TACACATGG CCT CTC CCT CTC CCA CAC
probe	CTC TCC CAC CCT GCACTTC
Primer probe	AGG CCATGTGTAGA AGTGC AGG GTG

(2) Determination of surface coverage

10 μL of 1 μM methylene blue labeled-thiolated HP2 (MB-HP2) was added onto the Au electrode surface and kept overnight at room temperature to obtain MB-HP2/Au electrode. After that, the resulting electrode was soaked in 2 mM MCH solution for 1 h to obtain MCH/MB-HP2/Au electrode. After that, the final electrode was measured via square wave voltammetry (SWV) in 10 mM PBS buffer (50 mM NaCl, 5 mM $MgCl_2$, pH 7.4). Thus, based on the integral voltammetric charge (Q) of SWV curve, the Q value of 2.39×10^{-9} C was obtained. Afterwards, based on the equation,

$$N = Q/n\cdot e\cdot N_A$$

The amount of substance (N) of MB was calculated as 1.24×10^{-14} mol. Where Q is the integral voltammetric charge, N is the amount of substance of MB, n is the number of electronic consumptions during MB redox process (n = 2), e is the electron charge (1.6×10^{-19} C), N_A is the Avogadro constant (6.02×10^{23} mol^{-1}). Therefore, the assembled density of the MB-HP2 on the Au electrode (2 mm in diameter) surface is 2.38×10^{11} molecules/cm^2.

(3) PAGE analysis

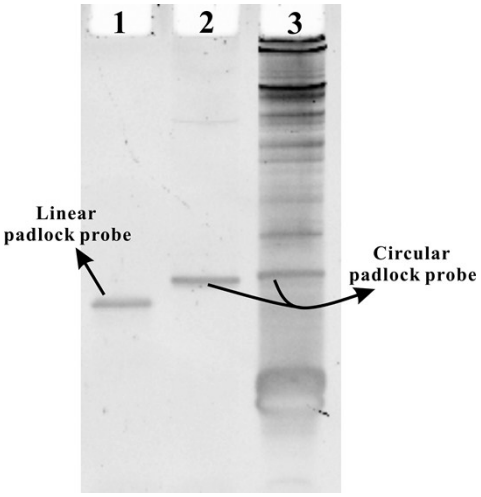

Fig. S1 PAGE analysis after T4 ligation and enzymes degradation: lane 1, linear padlock probe; lane 2, circular padlock probe after T4 ligation and enzymes degradation; lane 3, circular padlock probe after T4 ligation without enzymes degradation.

After T4 ligation and enzymes degradation, since the circular padlock probe could not be degraded by Exo I and Exo III, so it could be verified by 10% polyacrylamide gel electrophoresis (PAGE) operated in 1× TBE buffer at 200 V for 3 h and followed by staining in SYBR Gold dye solution. The gel was scanned using the ChemiDoc MP imaging system. As shown in Fig. S1, lane 1 showed the band of linear padlock probe. After T4 ligation and enzymes degradation, lane 2 showed a clear band of circular padlock, which ran a little slower than linear padlock probe maybe due to its circular structure. Compared with lane 2, lane 3 showed many different side-bands without enzymes degradation. These results revealed that clean circular padlock probe was obtained after T4 ligation and enzymes degradation.

(4) Effect of MB concentration

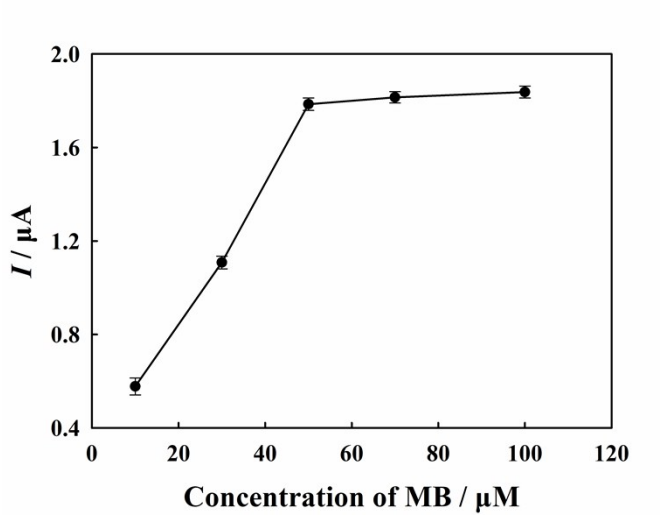

Fig. S2 Effect of MB concentration. Error bars represent standard deviations.

(5) Comparison of various analytical methods for CEA determination

Table S2 Comparison of various analytical methods for CEA determination.

Analytical Methods	Detection limit	Linear range	Reference
Colorimetry	1 ng mL^{-1}	1 ng mL^{-1}~50 ng mL^{-1}	1
Colorimetry	0.45 pg mL^{-1}	1 pg mL^{-1}~100 ng mL^{-1}	2
Fluorescence	10 pg mL^{-1}	20 pg mL^{-1}~200 pg mL^{-1}	3
Fluorescence	1.5 pg mL^{-1}	4.5 pg mL^{-1}~30 ng mL^{-1}	4
Photoelectrochemistry	1.4 pg mL^{-1}	5 pg mL^{-1}~20 ng mL^{-1}	5
Photoelectrochemistry	0.16 pg mL^{-1}	0.5 pg mL^{-1}~100 ng mL^{-1}	6
Electrochemiluminescence	1.67 pg mL^{-1}	5 pg mL^{-1}~500 ng mL^{-1}	7
Electrochemiluminescence	0.28 pg mL^{-1}	0.8 pg mL^{-1}~4 ng mL^{-1}	8
Electrochemistry	10 pg mL^{-1}	50 pg mL^{-1}~20 ng mL^{-1}	9
Electrochemistry	0.49 pg mL^{-1}	1 pg mL^{-1}~10 ng mL^{-1}	10
Electrochemistry	4.2 fg mL^{-1}	10 fg mL^{-1}~100 ng mL^{-1}	this work

REFERENCES

1. N. Shahbazi, S. Hosseinkhani and B. Ranjbar, *Sens. Actuators B: Chem.*, 2017, **253**, 794-803.
2. B. Li, G. S. Lai, B. Lin, A. M. Yu and N. J. Yang, *Sens. Actuators B: Chem.*, 2018, **262**, 789-795.
3. J. Zhu, J. F. Wang, J. J. Li and J. W. Zhao, *Sens. Actuators B: Chem.*, 2016, **233**, 214-222.
4. N. M. Danesh, R. Yazdian Robati, M. Ramezani, M. Alibolandi, K. Abnous and S. M. Taghdisi, *Sens. Actuators B: Chem.*, 2018, **256**, 408-412.
5. T. T. Wu, Y. R. Zhang, D. Wei, X. D. Wang, T. Yan, B. Du and Q. Wei, *Sens. Actuators B: Chem.*, 2018, **256**, 812-819.
6. G. C. Fan, H. Zhu, D. Du, J. R. Zhang, J. J. Zhu and Y. H. Lin, *Anal. Chem.*, 2016, **88**, 3392-3399.
7. N. L. Li, L. P. Jia, R. N. Ma, W. L. Jia, Y. Y. Lu, S. S. Shi and H. S. Wang, *Biosens. Bioelectron.*, 2017, **89**, 453-460.
8. J. J. Yang, J. T. Cao, Y. L. Wang, H. Wang, Y. M. Liu and S. H. Ma, *J. Electroanal. Chem.*, 2017, **787**, 88-94.
9. X. F. Gu, Z. She, T. X. Ma, S. Tian and H. B. Kraatz, *Biosens. Bioelectron.*, 2018, **102**, 610-616.
10. F. L. Gao, F. Y. Zhou, S. J. Chen, Y. Yao, J. Wu, D. Y. Yin, D. Q. Geng and P. Wang, *Analyst*, 2017, **142**, 4308-4316.