Electronic Supplementary Information

Universal point-of-care detection of protein based on proximity hybridization-mediated isothermal exponential amplification

Zibin Tang, ‡ [a] Wenyong Zhao, ‡ [b] Yuling Deng, ‡ [a] Yuanzhong Sun, [a] Cailing Qiu, [c] Binhua Wu, [d] Juan Bao, [a] Zhangquan Chen, * [a] and Luxin Yu *[a]

a. Medical Technology college, Guangdong Medical University, Dongguan 523808, PR China

b. Faculty of Forensic Medicine, School of Basic Medicine, Guangdong Medical University, Dongguan 523808, China

c. Department of Medical Laboratory, Dalang hospital of Dongguan, Dongguan 523770, China

d. Marine Biomedical Research Institute of Guangdong Medical University, 524023, Zhanjiang PR China

‡ These authors contributed equally to this work.

Fax: +86 76922896373; Tel: +86 76922896373;

E-mail: yuluxin2006@163.com, zqchen@gdmu.edu.cn

Preparation of anti-digoxin antibody-AuNP conjugates

Conjugation reactions were performed by adding 20 µL of 1 mg/ml anti-digoxin antibody to 2 mL of a tenfold–concentrated AuNP solution (pH 8.4), followed by incubation at room temperature with gentle shaking for 1 h. Then a 10% wt/vol BSA stock solution (pH 7.0) was added to the AuNP solution to a final concentration of 1% wt/vol. After incubation at room temperature for one hour, the resulting AuNP solution was concentrated by centrifugation for 30 min at 13 400 × g. The supernatant was discarded without disturbing the pellet. The pellet was resuspended with100 µL of suspension solution comprising 20 nM Na_3PO_4, 10% BSA, and 0.05% NaN_3 and stored at 4 °C

preparation of hairpin solution

Before DNA assay, three kinds of hairpin probes (10 µM in HEPES buffer) was heated to 95°C for 5 min, allowed to cool down at the rate of 1 °C s^{-1} to 65°C for 5 min, and then continued to cool down at the rate of 1 °C s^{-1} to room temperature (25°C) for 2 h to from the stem-loop hairpin structure. The obtained solution was stored at 4 °C for further use.

Gel electrophoresis

Gel electrophoresis of different reaction product was conducted with a
4% gel (3% NuSeive GTG agarose + 1% agrose) prepared in 1 × TBE buffer (89 mmol/L Tris; 89 mmol/L Boric acid; 2 mmol/L EDTA pH 8.3) at 65 V constant voltage for about 75 min. After the gel was stained for 30 min in a 4S GelRed solution, it was photographed by FluorChem FC3 Chemilumilescent imaging system. (ProteinSimple, USA).

Optimization of detection conditions

The hairpin probes used in this assay must be treated to form stem-loop structure.

AS shown in the Fig. S1, the untreated hairpin probe 2 (H2) formed self-dimmer (lane 3, upper band about 60 bp). All the treated hairpin probes have formed stem-loop structure without self-dimmers.

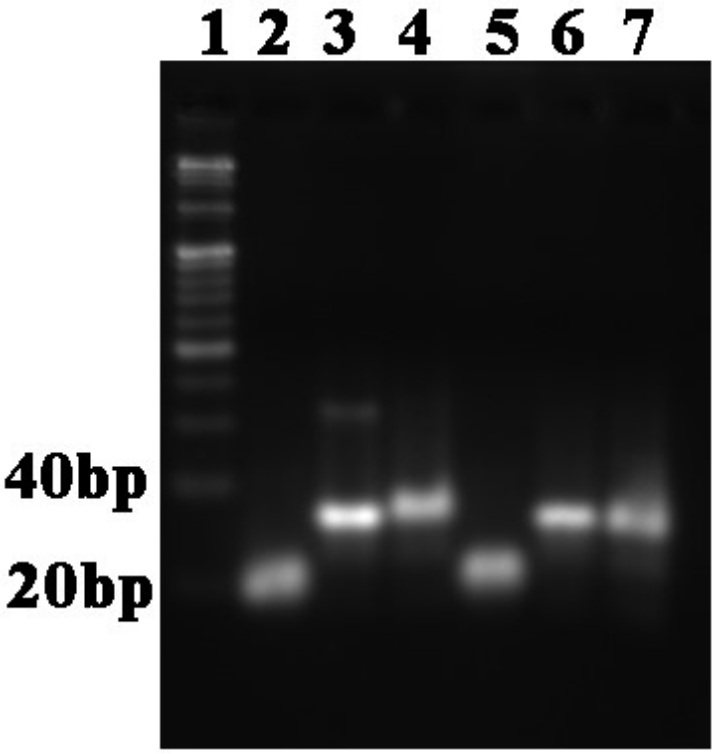

Fig. S1 Gel electrophoresis analysis of treated and untreated hairpins. 1: DNA ladder, 2: untreated H1, 3: untreated H2, 4: untreated H3, 5: treated H1, 6: treated H2, 7: treated H3.

Table S1 Comparison of our approach withother protein assay.

Methods	Strategy	LOD	Ref
Fluorescence	Dropcast Single Molecule Assays	19 aM	[1, 2]
Electrochemiluminescenc e	Dual-Polar Electrochemiluminescence from Au 25 Nanoclusters	0.43 pg mL^{-1}	[3, 4]
Electrochemical ELISA	Protein Biosensing in Undiluted Serum Using a Polypyrrole-Based Platform	78 pg mL^{-1}	[5]

Electrochemical	peptide inhibitor-based biosensing platform	0.03 ng mL^{-1}	[6, 7]

Table S2 Spiking test in human serum and saliva samples.

Sample	Spiked	Found	Recovery	RSD
serum	100 nM	98.2 nM	98.2%	5.2%
serum	10 pM	10.5 pM	105.5%	6.2%
serum	100 fM	96.2 fM	96.2%	5.5%
saliva	100 nM	99.3 nM	99.3%	4.3%
saliva	10 pM	9.53 pM	95.3%	4.9%
saliva	100 fM	102.5 fM	102.5%	6.0%

All the data in the table represents the average of three measurements

[1] S. Yue, Y. Li, Z. Qiao, W. Song, S. Bi, Rolling Circle Replication for Biosensing, Bioimaging, and Biomedicine, Trends in biotechnology, 39 (2021) 1160-1172.

[2] C. Wu, P.M. Garden, D.R. Walt, Ultrasensitive Detection of Attomolar Protein Concentrations by Dropcast Single Molecule Assays, Journal of the American Chemical Society, 142 (2020) 12314-12323.

[3] Y. Zhou, Y. Chai, R. Yuan, Highly Efficient Dual-Polar Electrochemiluminescence from Au(25) Nanoclusters: The Next Generation of Multibiomarker Detection in a Single Step, Analytical chemistry, 91 (2019) 14618-14623.

[4] Z. Qiao, J. Zhang, X. Hai, Y. Yan, W. Song, S. Bi, Recent advances in templated synthesis of metal nanoclusters and their applications in biosensing, bioimaging and theranostics, Biosensors & bioelectronics, 176 (2021) 112898.

[5] S.K. Arya, P. Estrela, Electrochemical ELISA Protein Biosensing in Undiluted Serum Using a Polypyrrole-Based Platform, Sensors (Basel, Switzerland), 20 (2020).

[6] F. Ma, Y. Zhu, Y. Chen, J. Liu, X. Zeng, Labeled and non-label electrochemical peptide inhibitor-based biosensing platform for determination of hemopexin domain of matrix metalloproteinase-14, Talanta, 194 (2019) 548-553.

[7] V. Vanova, K. Mitrevska, V. Milosavljevic, D. Hynek, L. Richtera, V. Adam, Peptide-based electrochemical biosensors utilized for protein detection, Biosensors & bioelectronics, 180 (2021) 113087.