

# **Label-free microfluidic paper-based electrochemical aptasensor for ultrasensitive and simultaneous multiplexed detection of cancer biomarkers**

Yang Wang<sup>a,b</sup>, Jinping Luo<sup>a,b</sup>, Juntao Liu<sup>a,b</sup>, Shuai Sun<sup>a,b</sup>, Ying Xiong<sup>c</sup>, Yuanyuan Ma<sup>c</sup>, Shi Yan<sup>c</sup>, Yue Yang<sup>c</sup>, Huabing Yin<sup>d</sup>, Xinxia Cai<sup>a,b\*</sup>

<sup>a</sup> State Key Laboratory of Transducer Technology, Institute of Electronics, Chinese Academy of Science, Beijing 100190, China.

<sup>b</sup> University of Chinese Academy of Sciences, Beijing 10090, China

<sup>c</sup> Key laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Thoracic Surgery II, Peking University Cancer Hospital & Institute, Beijing 100142, China

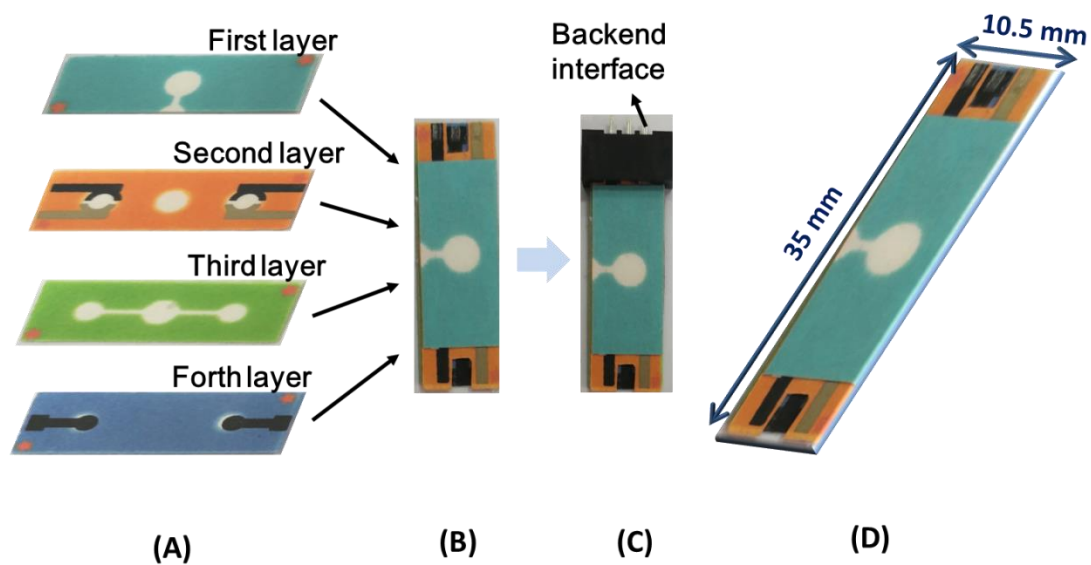
<sup>d</sup> Division of Biomedical Engineering, School of Engineering, University of Glasgow, Oakfield Avenue, Glasgow G12 8LT, United Kingdom

\* Corresponding Email: xxcai@mail.ie.ac.cn Tel: +86 10 58887193

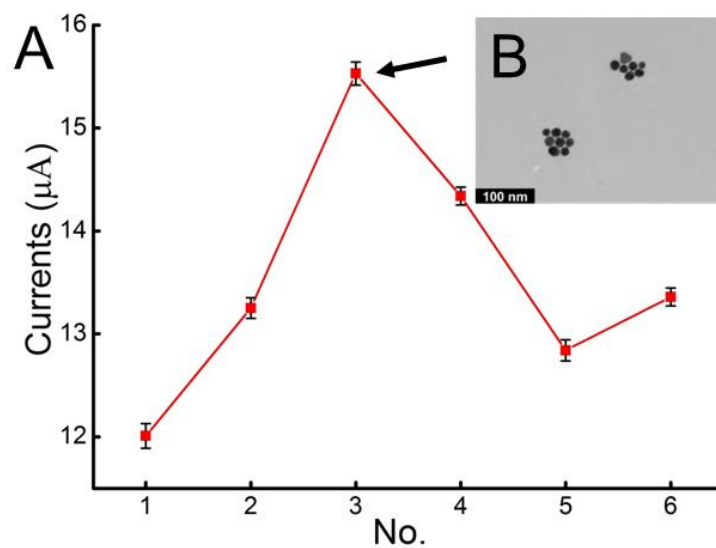
## **Experimental**

### **Preparation of AuNPs**

A solution of 15 nm AuNPs was prepared according to a literature (Yang et al. 2009). Briefly, an aqueous solution of HAuCl<sub>4</sub> (0.01%, 50 mL) was heated and refluxed for 10min. After boiling, a solution of trisodium citrate (1%, 2.5 mL) was rapidly added to the refluxed HAuCl<sub>4</sub> solution. The solution was refluxed and stirred for an additional 15min. After being slowly cooled down to room temperature, 10 mL of the solutions were centrifuged at 8000 r/min for 10 min to remove surplus water and ions. Finally, 9.5 mL of supernatants were removed and diluted with 500  $\mu$ L ethanol. The absorption peak of AuNPs was at 520 nm.



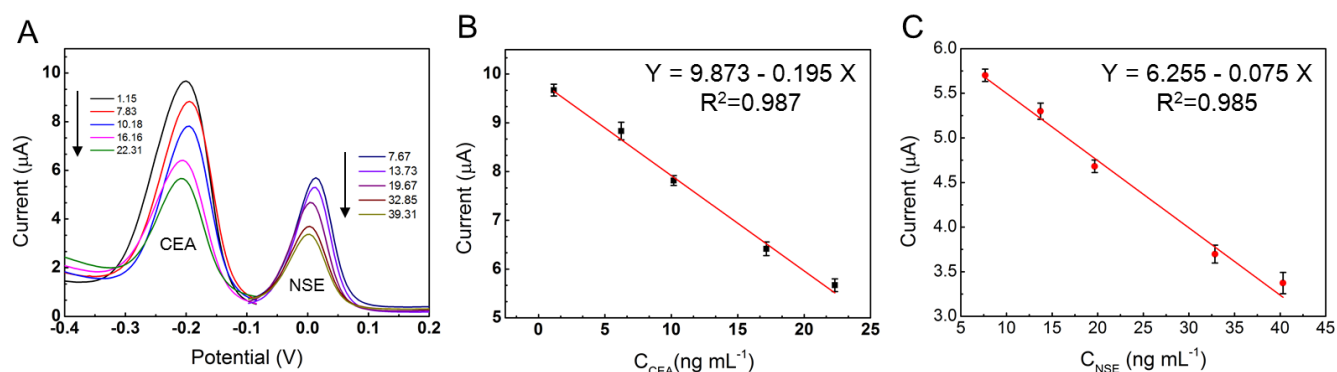
**Fig.S1** Pictures of the two-parameter electrochemical paper-based device: (A) pictures of each layer of the device; (B) picture of the integrated paper-based device; (C) the paper-based device with backend interface; (D) the real size of the paper-based device.



**Fig.S2** (A) Effects of the size of AuNPs on DPV responses and (B) the TEM image of AuNPs with best DPV response.

**Table.S1** Comparisons of different kinds of biosensors and their analytical properties towards CEA and NSE.

No.	Method	Linear Range (ng mL <sup>-1</sup> )		LOD (ng mL <sup>-1</sup> )		References
		CEA	NSE	CEA	NSE	
1	Fluorescence Immunoassay based on quantum dots	3~100	3~100	1	1	Li et al. 2011
2	Electrochemical immunosensors based on PANI derivatives	0.01~100	0.01~100	0.0063	0.0079	Wang et al. 2015
3	Fluoroimmunoassay based on dual-color quantum dots	1.25~80	1.25~80	0.625	0.625	Cao et al. 2011
4	Nanobeads-Based Lateral Flow Test Strip	1~50	1~50	0.094	0.045	Lu et al. 2017
5	SERS-based sandwich immunoassay	0.01~100	0.01~100	0.00148	0.00204	Song et al. 2016
6	Microfluidic paper-based electrochemical aptasensor	0.01~500	0.05~500	0.002	0.01	This work



**Fig.S3** Assay results of simultaneous multiplexed detection of CEA and NSE in clinical serum samples. (A) DPV responses to different concentrations of CEA and NSE antigens in clinical serum samples; (B) The calibration curve between the peak current and concentration of CEA; (C) The calibration curve between the peak current and concentration of NSE.

## References

- Cao, Z., Li, H., Lau, C., Zhang, Y., 2011. *Anal. Chim. Acta.* 698(1-2), 44-50.
- Li, H., Cao, Z., Zhang, Y., Lau, C., Lu, J., 2011. *Analyst* 136(7), 1399-1405.
- Lu, W., Wang, K., Xiao, K., Qin, W., Hou, Y., Xu, H., Yan, X., Chen, Y., Cui, D., He, J., 2017. *Sci. Rep.* 7, 42414.
- Song, C., Yang, Y., Yang, B., Min, L., Wang, L., 2016. *J. Mater. Chem. B.* 4(10), 1811-1817.
- Wang, L., Liu, N., Ma, Z., 2015. *J. Mater. Chem. B.* 3(14), 2867-2872.
- Yang, L., Tang, F., Zhang, L., 2009. *Biosens. Bioelectron.* 25, 889-895.