# Colorimetric immunosensor constructed by 2D metal-organic

# frameworks nanosheets as enzyme mimics for the detection of

## protein biomarker

Yujing Zeng,<sup>a</sup> Minghui Wang,<sup>a</sup> Zhaowei Sun,<sup>\*b</sup> Lingjun Sha,<sup>c</sup> Jie Yang<sup>a</sup> and Genxi Li<sup>\*ac</sup>

<sup>a</sup> State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210023, P. R. China. E-mail: genxili@nju.edu.cn
<sup>b</sup> School of Life Sciences, Zhengzhou University, Zhengzhou, 450001, P. R. China. E-mail: szw@zzu.edu.cn

<sup>c</sup> Center for Molecular Recognition and Biosensing, School of Life Sciences, Shanghai University, Shanghai 200444, P. R. China

### **Table of Contents**

Figure S1	S-2
Figure S2	
Figure S3	
Figure S4	
Figure S5	
Figure S6	
Figure S7	S-5
Figure S8	
Figure S9	S-6
Table S1	
References	S-7



Figure S1 Au 4f XPS spectrum of Au NPs/Cu-TCPP hybrid nanosheets.



**Figure S2** Variance of initial velocity of (A) Cu-TCPP nanosheets and (B) AuNP/Cu-TCPP hybrid nanosheets in response to various concentrations of TMB substrate. The material concentration is 2.5  $\mu$ g/mL. Error bars represent the standard derivation of three independent experiments.



Figure S3 Photographs of colorimetric reaction in different cases. A: (a) Tetrakis  $Cu(NO_3)_2 \cdot 3H_2O + TMB + H_2O_2$ , (4-carboxyphenyl) porphyrin (b) (Fe)+TMB+H<sub>2</sub>O<sub>2</sub>; B: TMB, TMB+H<sub>2</sub>O<sub>2</sub>, (c) Cu-TCPP (a) (b) nanosheets+TMB+H2O2, (d) AuNPs+TMB+H2O2, and (e) AuNPs/Cu-TCPP hybrid nanomaterials+TMB+H<sub>2</sub>O<sub>2</sub>.



**Figure S4** The enzyme activities of HRP and AuNPs/Cu-TCPP after treated at 40°C for different time. Error bars represent the standard derivation of three independent experiments.



Figure S5 The stability of the AuNPs/Cu-TCPP materials. Error bars represent the standard derivation of three independent experiments.



**Figure S6** Activity of HRP and AuNPs/Cu-TCPP after treatment under different harsh conditions for 1h. Error bars represent the standard derivation of three independent experiments.



**Figure S7** The supernatant of DNA is determined by UV/Vis spectroscopy after incubation without (a) and with (b) Cu-TCPP nanosheets for 1 h, the values of absorbance at the 260 nm are labeled (Cu-TCPP: 25  $\mu$ L with100  $\mu$ g/mL; DNA:25  $\mu$ L with 10 uM).



**Figure S8** Optimization of (A) the incubation time of the AuNPs/Cu-TCPP materials and (B) the temperature of colorimetric reaction. Error bars represent the standard derivation of three independent experiments.



**Figure S9** Peak currents obtained by UV/Vis spectroscopy response to different concentrations of CEA diluted in PBS and 10% FBS. Error bars represent the standard deviations of three independent experiments.

Method	Linear range (ng/mL)	LOD (pg/mL)
Electrochemical immunosensor <sup>1</sup>	0.0005 - 50	0.167
Colorimetric Immunoassay <sup>2</sup>	0.05 - 100	21.1
Nanopore-based strategy <sup>3</sup>	2 - 200	0.6
Fluorescence resonance energy	0.004 - 0.1	1.7
transfer biosensor <sup>4</sup>		
Microfluidic paper-based	0.05 - 500	10
immunosensor <sup>5</sup>		
This proposed method	0.001 - 1000	0.742

Table S1 Comparisons of thisimmunosensor with previous methods.

### References

- 1. H. Lv, Y. Li, X. Zhang, Z. Gao, C. Zhang, S. Zhang and Y. Dong, *Biosensors* and *Bioelectronics*, 2018, **112**, 1-7.
- 2. S. Wu, H. Tan, C. Wang, J. Wang and S. Sheng, *ACS Appl Mater Interfaces*, 2019, **11**, 43031-43038.
- 3. H. Tang, H. Wang, C. Yang, D. Zhao, Y. Qian and Y. Li, *Anal. Chem.*, 2020, **92**, 3042-3049.
- 4. H. Li, L. Shi, D. E. Sun, P. Li and Z. Liu, *Biosens Bioelectron*, 2016, **86**, 791-798.
- 5. Y. Wang, H. Xu, J. Luo, J. Liu, L. Wang, Y. Fan, S. Yan, Y. Yang and X. Cai, *Biosens Bioelectron*, 2016, **83**, 319-326.