Electronic Supplementary Information

In-situ growth of enzyme-inorganic hybrid nanoflowers on the paper strips for the visual detection of salivary glucose

Zifeng Zhang^{a#*}, Shiwen Wang^{c#}, Tingjun Chen^b, Hui Wang^{d*}, Qian Dou^{b,e*}

a Henan Provincial Key Laboratory of Nanocomposites and Applications, Institute of Nano-Structured Functional Materials, Huanghe Science and Technology College, Zhengzhou, 450000 China

b CAS Key Laboratory of Nanophotonic Materials and Devices, CAS Key Laboratory of Standardization and Measurement for Nanotechnology, National Center for Nanoscience and Technology, Beijing 100190, China

c School of Biomedical Sciences, Hunan University, Changsha 410082, China

d Laboratory of Theoretical and Computational Nanoscience, National Center for Nanoscience and Technology, Chinese Academy of Sciences, Beijing 100190, China

e Center of Materials Science and Optoelectronics Engineering, University of Chinese Academy of Sciences, Beijing 100049, China

[#]*These authors contributed equally to this work.*

*Corresponding authors.

E-mail: zhangzifeng@hhstu.edu.cn (Z. Zhang), wangh@nanoctr.cn (H. Wang), douq@nanoctr.cn (Q. Dou)



Fig. S1. Changes before and after picture processing.



Fig. S2. SEM images of (a) in-situ growth of $GO_x@HNF$ on the paper strip, (b) in-situ

growth of HRP@HNF on the paper strip.



Fig. S3. Color changes on the paper strip combined with $GO_x@HNF$ -based paper strip, HRP@HNF-based paper strip, HNF@paper strip, enzyme@gel@paper strip and HNF-based paper strip after adding 50 mg/L glucose, respectively.



Fig. S4. The effect of pH value on glucose detection assay.



Fig. S5. Linear equation of glucose detection.



Fig. S6. SEM images of the HNF-based paper strip after different days of storage: (a)

7 days, (b) 20 days, (c) 45 days, (d) 60 days.



Fig. S7. The specificity of HNF-based paper strip. The concentrations of interferences substance (glucose, mannose, galactose, lactose, xylose, ribose, maltose, sucrose, and fructose) are all 50 mg/L.



Fig. S8. The color change of dry paper strip after adding different glucose solution.



Fig. S9. The specificity of the HNF-based paper strip for 50 mg/L glucose solution

containing 10 mg/L fructose.



Fig. S10. The specificity of the HNF-based paper strip for 50 mg/L glucose solution containing 1.84×10³ mg/L Na⁺, 7.8×10² mg/L K⁺, 3.55×10³ mg/L Cl⁻, 1.06×10³ mg/L lysozyme and 2.25×10² mg/L lactate, respectively.



Fig. S11. The inter-batch of repeatability of HNF-based paper strip.

Table R1. Comparison of analytical properties of the HNF-based paper strip glucose

 sensor with other detection methods

Detection Glucose- Detection Limit of Response Specimen Reference

method	responsive material	range	detection	time		
Electrochemical	MnO ₂ /M WNTs	1.8 - 5040 mg/L		10 s	0.1 M NaOH	1
	Pt-Pb	0 - 3600 mg/L			7.4 PBS	2
	GOx	18 – 3240 mg/L	156.6 mg/L	15 min	PBS, 100% human serum	3
Optical	GOx	0 – 540 mg/L	36 mg/L		7.0 PBS, 20% human serum	4
	GOx	0 – 360 mg/L	0.09 mg/L	30 min	7.4 PBS, 10% serum	5
	PBA	0 – 18000 mg/L		90 min	7.4 PBS	6
	BA	0 – 180 mg/L		60 min	7.4 TES buffer, photosynt hetic organism	7
Paper strip	GOx-HPR	54 – 180 mg/L	38 mg/L	30 min	7.0 PBS, 100% human serum	8
	GOx-HPR	180 – 1980 mg/L	54 mg/L	6 min	7.0 PBS, 100% human serum	9
	GOx	18 – 3600 mg/L	18 mg/L	5 min	7.4 PBS, 100% human	10
	GOx-HPR	0 – 50 mg/L	10 mg/L	60 s	serum 100% human saliya	Our work

Table S2.	Recovery	of glucose	in	diabetic	human saliva	with	the proposed	sensor.
-----------	----------	------------	----	----------	--------------	------	--------------	---------

Saliva sample	Standard	HNF embedded strip	Recovery (%)

	(Ion chromatography)		
1	19.9 mg/L	17.9 mg/L	90
2	10.0 mg/L	10.03 mg/L	100
3	47.2 mg/L	46.8 mg/L	99
4	40.2 mg/L	37.8 mg/L	94
5	38.8 mg/L	34.9 mg/L	90

Reference:

1. J. Chen, W.D. Zhang, J.S. Ye. Nonenzymatic electrochemical glucose sensor based on MnO₂/MWNTs nanocomposite. *Electrochem. Commun.* 2008, **10**, 1268-1271.

2. J.P. Wang, D.F. Thomas, A. Chen. Nonenzymatic electrochemical glucose sensor based on nanoporous PtPb networks. *Anal. Chem.* 2008, **80**, 997-1004.

3. J. Xu, K.K. Xu, Y. Han, D. Wang, X. Li, T. Hu, H. Yi, Z.H. Ni. A 3D porous graphene aerogel@GOx based microfluidic biosensor for electrochemical glucose detection. *Analyst* 2020, **145**, 5141-5147.

4. X.D. Wang, H.X. Chen, T.Y. Zhou, Z.J. Lin, J.B. Zeng, Z.X. Xie, X. Chen, K.Y. Wong, G.N. Chen, X.R. Wang. Optical colorimetric sensor strip for direct readout glucose measurement. *Biosens. Bioelectron*. 2009, **24**, 3702-3705.

5. H.L. He, X.L. Xu, H.X. Wu, Y.D. Jin. Enzymatic plasmonic engineering of Ag/Au bimetallic nanoshells and their use for sensitive optical glucose sensing. *Adv. Mater.* 2012, **24**, 1736-1740.

 M. Elsherif, M.U. Hassan, Ali.K. Yetisen, H. Butt. Glucose sensing with phenylboronic acid functionalized hydrogel-based optical diffusers. *ACS Nano* 2018, 12, 2283-2291.

7. J.M. Li, H.H. Wu, I. Santana, M. Fahlgren, J.P. Giraldo. Standoff optical glucose sensing in photosynthetic organisms by a quantum dot fluorescent probe. *ACS Appl. Mater. Interfaces* 2018, **10**, 28279-28289.

8. W.J. Zhu, D.Q. Feng, M. Chen, Z.D. Chen, R. Zhu, H.L. Fang, W. Wang. Bienzyme colorimetric detection of glucose with self-calibration based on tree-shaped paper strip. *Sens. Actuators, B* 2014, **190**, 414-418.

9. A. Soni, S.K. Jha. A paper strip based non-invasive glucose biosensor for salivary analysis. *Biosens. Bioelectron.* 2015, **67**, 763-768.

10. W.Y. Li, S.Y. Lu, S.J. Bao, Z.Z. Shi, Z.S. Lu, C.M. Li, L. Yu. Efficient in situ growth of enzyme-inorganic hybrids on paper strips for the visual detection of glucose. *Biosens. Bioelectron.* 2018, **99**, 603-611.