Origami-Based "Book" Shaped Three-Dimensional Electrochemical Paper Microdevice for Sample-to-Answer Detection of Pathogens

Tao He[a], Jingwen Li[a,b], Lisheng Liu[c,d], Shenguang Ge[a], Mei Yan[a,e], Haiyun Liu[a,f,*], Jinghua Yu[a,e]

[a] *Collaborative Innovation Center of Technology and Equipment for Biological Diagnosis and Therapy in Universities of Shandong, Institute for Advanced Interdisciplinary Research (IAIR), University of Jinan, Jinan 250022, P.R. China*

[b] *College of Biological Sciences and Technology, University of Jinan, Jinan 250022, P.R. China*

[c] *Key Laboratory of Animal Resistance Research, College of Life Science, Shandong Normal University, Jinan, 250014, P. R. China*

[d] *Clinical Laboratory，Shandong Cancer Hospital Affiliated to Shandong University，Shandong Academy of Medicine Science, Jinan, 250117, P. R. China.*

[e] *School of Chemistry and Chemical Engineering, University of Jinan, Jinan, 250022, P. R. China*

[f] *College of Chemistry, Chemical Engineering and Materials Science, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Provincial Key Laboratory of Clean Production of Fine Chemicals, Shandong Normal University, Jinan, 250014, P. R. China*

** Corresponding author.Tel.: +86-531-82767161; Fax: +86-531-82765956.*

E-mail address: chm_liuhy@ujn.edu.cn

Table S1: LAMP primers set

Primer	Sequence (5′ ～ 3′)
FIP:	GACGACTGGTACTGATCGATAGTTTTTCAACGTTTCCTGCGG
BIP:	CCGGTGAAATTATCGCCACACAAAACGCCACCGCCAGG
F3:	GGCGATATTGGTGTTTATGGGG
B3:	AACGATAAACTGGACCACGG
LF:	GACGAAAGAGCGTGGTAATTAAC
LB:	GGGCAATTCGTTATTGGCGATAG

Manufacturing procedures of wax-printing paper electrode

The paper was first printed with wax using an office printer, then heated at 120 °C for 1 min on the hotplate to melt the printed wax, which diffused through the paper to form the same hydrophobic circle. The circle hydrophilic zones were designed for screen-printing carbon counter electrode and Ag/AgCl reference electrode. Then, the as-prepared sheet was ready for printing electrode after cooling to room temperature. A disk-like carbon electrode (6.0 mm in diameter) was screen-printed onto the circle hydrophilic zones and arcuate carbon counter electrode and Ag/AgCl reference electrode were screen-printed onto other hydrophilic zones. Gold nanocomposite modified paper cathode electrode (Au-PCE) were fabricated through growth of an Au layer on the surfaces of cellulose fibers in the hydrophilic circle that was screen-printed a disk-like carbon electrode. The Au layer enhanced the conductivity and enlarged the effective surface area of bare PCE. Firstly, the suspension of Au seeds

were prepared by using $NaBH_4$ as the reductant and stabilized with sodium citrate. Then, 20.0 mL as-prepared Au seeds solution were dropped into the paper sample zone of bare PCE. Then the origami device was equilibrated at room temperature for 1 h to optimize the surface immobilization of Au seeds on cellulose fibers. After rinsing with water thoroughly to remove loosely bound Au seeds, 20 μL freshly prepared growth aqueous solution of PBS (10.0 mM, pH 7.0) containing 1.2 mM $HAuCl_4$, 2.0 mM cetyltrimethylammonium chloride and 7.2 mM H_2O_2 for seeds growth were applied into the Au seeded PCE, and incubated at room temperature for 15 min. Subsequently, the resulting Au- PCE was washed with water thoroughly. Thus a layer of interconnected Au on cellulose fibers with good conductivity were obtained, which were dried at room temperature for 20 min. The SEM images of bare paper and Au-PCE was showed in Fig.S1.

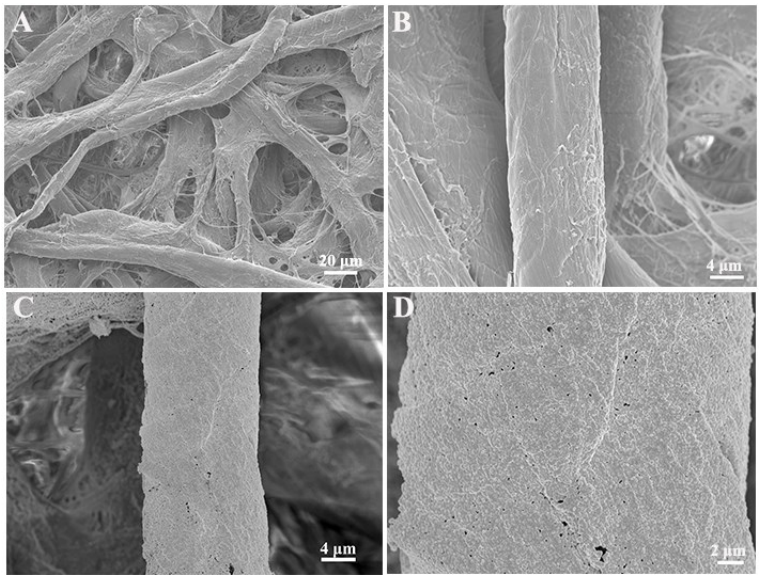

Fig. S1 SEM images of bare paper (A), magnification SEM images of bare paper (B) and Au-PWE (C, D).

Temperature stability of the hotplate

The temperature of the hotplate was first calibrated using a hand-held thermometer. The temperature of the hotplate was set and measured at 57.5, 60, 62.5, 65, 67.5 and 70°C. Three measurements were taken for each temperature, with 10, 20, and 30 min after the temperature displayed by the hotplate has reached the set temperature. We also verified the temperature of the reaction chamber during the amplification process. The average temperature measured during the amplification process was 64.6 ± 0.3 °C (n = 10), and a stable temperature was maintained over 45 min, which was enough for running one complete reaction.

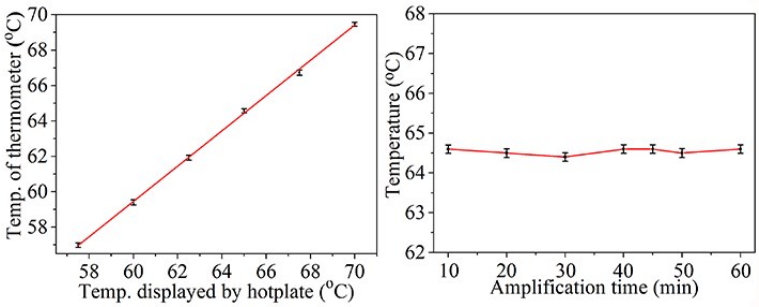

Fig. S2 (a) Temperature calibration curve for the hotplate. Each data point was measured three times with 5 min in between. (b) Temperature of the amplification chamber during the amplification reaction.

The optimum concentration of MB

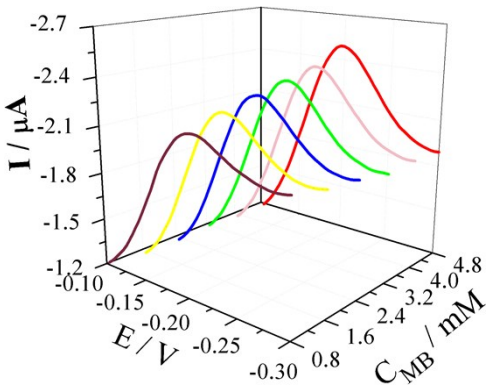

Fig. S3 The optimum concentration of MB. The dependence of peak currents on each concentration of MB

Optimization the amplification temperature of on-chip LAMP

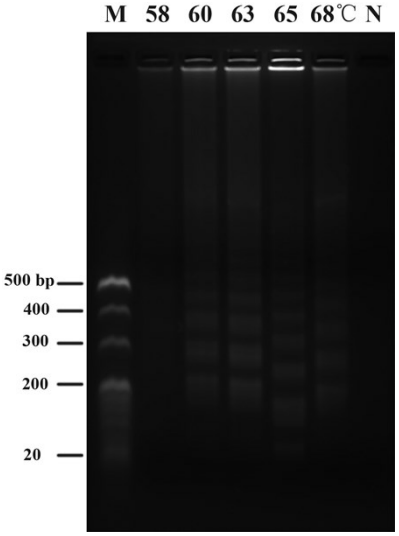

Fig. S4 Optimization the amplification temperature of on-chip LAMP. A range of amplification temperature (58, 60, 63, 65 and 68 °C) was evaluated at the optimum amplification time (45min). With SYBR Green I fluorescence staining, agarose gel electrophoresis was used to verify the amplification efficiency of LAMP amplification at different temperatures. We found that the bands of amplification at 65 °C produce the most clearly visible light among all temperatures tested, which indicated that 65 °C was the highest efficiency of amplification temperature.

Integrated assay with spiked drinking water and milk sample

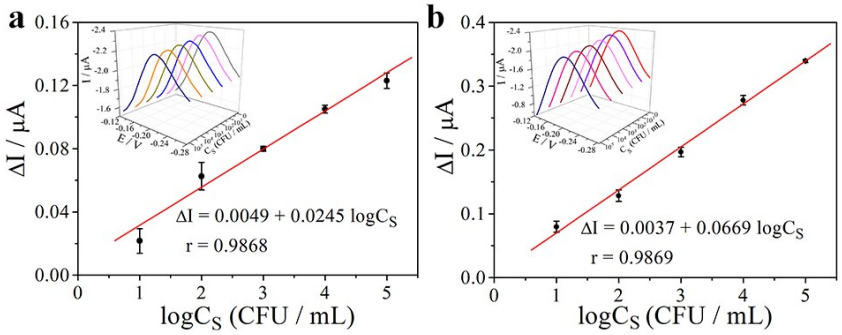

Fig. S5 Assay integrated with electrochemical paper microdevice using spiked drinking water and milk sample. The device could effectively detect *S. typhimurium* from drinking water (A) and milk (B) with a detection limit of 1.57, 5.51 CFU ml^{-1}, respectively, showing great potential for future food and water safety analyses. The insets are DPVs of detection *S. typhimurium* at different concentrations: 10^0～10^5 CFU mL^{-1} in drinking water (A) and milk (B), respectively.