

## Electronic Supplementary Information

### Multiplex detection of nucleic acids with low cost microfluidic chip and personal glucose meter at the point-of-care

Qing Wang, Hui Wang, Xiaohai Yang, Kemin Wang,\* Fang Liu, Qing Zhao, Pei Liu, Rongjuan Liu  
*State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering,  
Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, Hunan University,  
Changsha 410082, China.*

*E-mail: kmwang@hnu.edu.cn; Tex/Fax: +86 731 88821566.*

### Experimental details

1. Materials and reagents
2. Surface modification and characterization
3. Synthesis and characterization of report probe
4. Procedures of multiplex target detection
5. Optimized procedure for HBV detection

## Experimental details

### 1. Materials and reagents

Invertase (300U/mg), avidin, tris (2-carboxyethyl) phosphine hydrochloride (TCEP), sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), biotinylated bovine serum albumin were purchased from Sigma-Aldrich Corp. (USA). Amicon ultra centrifugal filters (3K, 30K) were purchased from Millipore Corporation (Billerica, MA, USA). The DNA oligonucleotides used were synthesized by Sangon Biotech. (Shanghai) Co., Ltd. The sequences of the DNA were list in Table S1. Personal glucose meter with a dynamic range of 2.2-27.8 mM was a product from Sinocare Inc. (China). High-stringency washing buffer contained 1× SSC (pH 7.4), 0.1% SDS, and 50% deionized formamide (Beijing Dingguo Changsheng Biotech. Co., Ltd.).

**Table S1** Sequences of oligonucleotides used

Name	Sequences (from 5' to 3')
Capture-B	GTAACAGCGGCATAAAGGGACTCAAGATGTTGTTTTTTTTT-biotin
Capture-C	GTAATAGAGGTAAAAAGGGACTCAAGATGTTGTTTTTTTTT-biotin
Capture-D	GTAACAGCGGTAAAAAGGGACTCAAGATGCTGTTTTTTTTT-biotin
Capture-R	CCCTCCTTCCTTCGACGTAGATCTGCTGCGTTGTTCCGA-biotin
HBV-B	CAACATCTTGAGTCCCTTTATGCCGCTGTTACCAATTTTCTTTTGT CTTTGGGTATACAT
HBV-C	CAACATCTTGAGTCCCTTTTACCTCTATTACCAATTTTCTTTTGT CTTTGGGTATACAT
HBV-D	CAGCATCTTGAGTCCCTTTTACCGCTGTTACCAATTTTCTTTTGT CTTTGGGTATACAT
Assistant DNA	HS-(T) <sub>10</sub> ATGTATACCCAAAGACAAAAGAAAATTG

## **2. Surface modification and characterization**

Four types of capture probe, including three capture probes (i.e., Capture-B, Capture-C and Capture-D) corresponding to HBV-B, HBV-C, HBV-D sequences, and a random probe (Capture-R) serving as a negative control, were immobilized respectively on different branch channels using biotin-avidin system. Since the polystyrene microfluidic chip had hydrophobic nature, it was necessary to alter the hydrophilic-hydrophobic property of the microfluidic chip. Briefly, biotin-BSA (1 mg/mL) was injected into the microfluidic chip and incubated for 12 h at 4 °C. The biotin-BSA could be firmly adhered on the surface of microfluidic channels in a way of physical adsorption and hydrophobic interaction. The microfluidic chip surface was characterized by angel contact measurement before and after the biotin-BSA treated. Drop Shape Analyzer (KRUSS GmbH, Hamburg, Germany) was used to measure the contact angles of deionized water, on both a native and a modified polystyrene film.

Then the biotin-adhered surface was incubated with avidin (1 mg/mL) over 1 h at room temperature. Next, four types of biotinylated capture probe (1  $\mu$ M) were added into four branch channels respectively and incubated for 1 h. The capture probe immobilized chip was washed repeatedly with 10 mM PBS buffer and then stored at 4 °C for the following experiments.

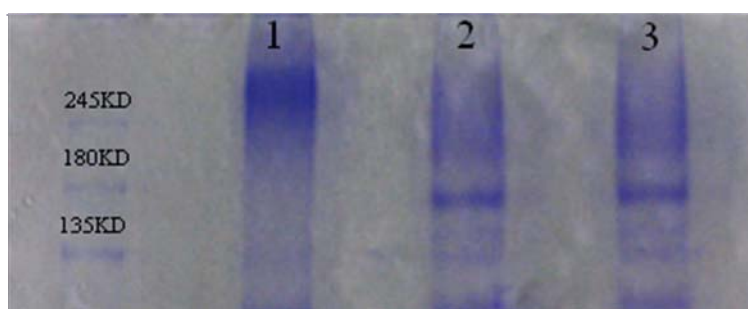
The change of hydrophilic-hydrophobic property of the polystyrene microfluidic chip surface was characterized after biotin-BSA was modified on chip. The contact angels of deionized water on native polystyrene film and biotin-BSA treated polystyrene film were about 76.5° and 180°, respectively. The results indicated that

the hydrophilicity of the modified polystyrene surface increased significantly, thus demonstrating that the biotin-BSA was successfully modified on the surface of microfluidic chip.

### 3.Synthesis and characterization of report probe

The synthesis of report probe (invertase-DNA conjugate) was similar to the previous work (Y. Xiang and Y. Lu, *Nat. Chem.*, 2011, **3**, 697-703). The assistant DNA was first modified with sulfhydryl at the 5' end. Then sulfo-SMCC was used as a linker to conjugate invertase and assistant DNA. Next, the invertase-DNA conjugate was characterized using an SDS-PAGE experiment. This gel was stained by Coomassie brilliant blue.

As shown in Fig. S1, the band of invertase in the lane 3 was located between 135 KDa and 180 KDa, which was in agreement with the molecular weight of invertase (ca. 135~270 KDa). Upon conjugation with assistant DNA, the migration of the invertase-DNA conjugate band in the lane 1 was less than that of invertase, since assistant DNA could conjugate with invertase and increase the molecular weight of invertase. In addition, since assistant DNA was not conjugated with invertase in the mixture of assistant DNA and invertase, little difference was observed between lane 2 and lane 3. The results demonstrated that the invertase-DNA conjugate was formed.



**Fig. S1.** 8% SDS-PAGE image for invertase-DNA conjugate.

lane 1: invertase-DNA conjugate; lane 2: mixture of 0.5 mM assistant DNA and 5 mg/mL invertase; lane 3: 5 mg/mL invertase.

#### **4. Procedures of multiplex target detection**

For multiplex target detection, samples and reagents were manually pipetted into the modified chip. The following testing procedures were performed:

(1) Sample solution was injected into the chip with a pipette from the central channel, and all channels were full of sample within seconds. Then, a filter paper was used to quickly blot the sample in central channel, and the sample was separated into four branch channels. After the sample was incubated at room temperature for one hour, a filter paper was used to blot the sample solution in all branch channels.

(2) 200  $\mu\text{L}$  high-stringency washing buffer was introduced into the chip from the central channel. 15 minutes later, a filter paper was used to blot the high-stringency washing buffer in all channels. Then the chip was washed repeatedly with 10 mM PBS buffer.

(3) Report probe was added into the chip from the central channel, and the report probe in central channel was subsequently removed with filter paper. The report probe in branch channels was incubated at room temperature for one hour. Then the chip was washed repeatedly with 10 mM PBS buffer to remove the unbonded report probe.

(4) 500 mM sucrose was introduced from the central channel, and the sucrose solution in central channel was subsequently removed with filter paper. The sucrose solution in branch channels was incubated at room temperature for 50 minutes.

(5) The solution in different branch channels spontaneously entered into the test strips of PGM through capillary action. By analysing the PGM signal values obtained from different branch channels, the concentration of targets can be determined.

## 5. Optimized procedure for HBV detection

Some factors, such as concentration of deionized formamide, concentration of sucrose, catalytic reaction time and reaction temperature, would affect the performance of the detection system, thus the effects of these factors were investigated. All the optimized procedure utilized HBV-C as the target DNA. In this assay, a random probe (Capture-R) was served as a negative control and immobilized on one of branch channels (i.e. control channel). The background value obtained from control channel was deducted from each measured values using PGM.

### (1) Effect of deionized formamide concentration

Based on the melting temperature ( $T_m$ ) differentiation between the matched hybrids and the mismatched hybrids, the deionized formamide was chosen as elution buffer and the concentration of deionized formamide was optimized. As shown in Fig. S2a, the PGM signal of the Capture-C modified channel (completely matched hybrids) was found to be remarkably higher than the signal values of Capture-B and Capture-D modified channels (mismatched hybrids). When the concentration of deionized formamide was less than 40%, Capture-B and Capture-D modified channels showed higher signal values due to the insufficient elution. With increasing deionized formamide concentration, the dehybridization of mismatched hybrid process was gradually obvious and tended to stable values. Thus, 50% deionized formamide concentration was used in the following experiment.

## (2) Effect of the concentration of sucrose

The initial amount of sucrose played an important role in the performance of the sensor, so the concentration of sucrose was then optimized. As shown in Fig.S2b, the signal of PGM shifted obviously as the concentration of sucrose increased, and gave the highest signal as the sucrose concentration was up to 500 mM. Therefore, 500 mM final sucrose concentration was used in the following experiment.

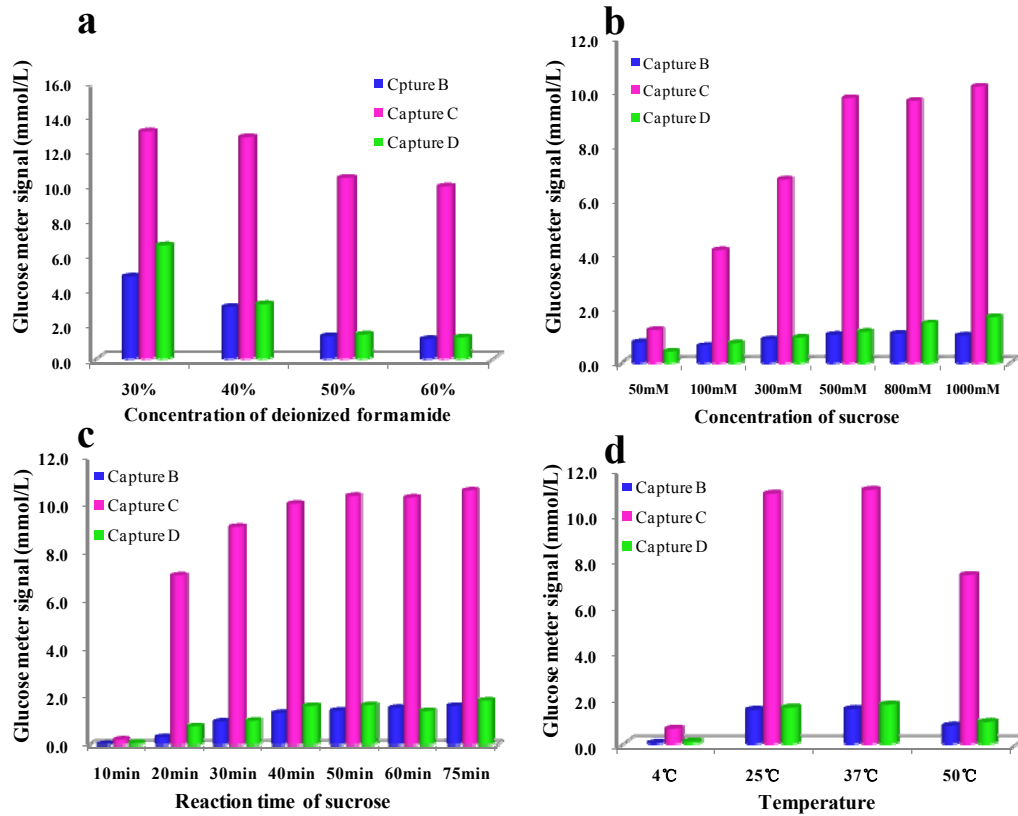
## (3) Effect of reaction time of sucrose

The kinetics of invertase-catalyzed production of glucose from sucrose was also studied. As shown in Fig.S2c, with increasing reaction time with sucrose, the amount of glucose produced was increased and tended to be saturated when the reaction time was up to 50 min. Thus the reaction time of 50 min was chosen in the following experiments.

## (4) Effect of operation temperature

Since DNA hybridization and enzyme reaction are all influenced by the reaction temperature, the operation temperature was also investigated. As shown in Fig. S2d, when the temperature was too low (4 °C), the signal was low due to the low activity of invertase; when the temperature was too high (50 °C), the DNA hybridization was not strong and the sensor system became unstable compared to that at room temperature, so the signal decreased. Thus, 25 °C of reaction temperature was chosen in the following experiment.





**Fig. S2.** The effect of different conditions for HBV-C detection. The background value obtained from control channel (i.e. Capture-R modified branch channel) was deducted from each measured values using PGM.

(a) Different concentration of deionized formamide. Incubation time with HBV-C: 60 min, concentration of sucrose: 0.5M, reaction time with sucrose: 60 min, operation temperature: 25 °C, concentration of HBV-C: 50 nm;

(b) Different concentration of sucrose. Incubation time with HBV-C: 60 min; reaction time with sucrose: 60 min, operation temperature: 25 °C, concentration of HBV-C: 50 nM;

(c) Different reaction time with sucrose. Incubation time with HBV-C: 60 min, concentration of sucrose: 0.5 M, operation temperature: 25 °C, concentration of HBV-C: 50 nM;

(d) Different operation temperature. Incubation time with HBV-C: 60 min, concentration of sucrose: 0.5M, reaction time with sucrose: 50 min.