Electronic supplementary material

Dual cascade isothermal amplification reaction based glucometer sensors for point-of-care diagnostics of cancer-related microRNAs

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Table of Contents

Additional Experimental Section

1. Apparatus and Measurements

PNA oligomers used in this work were manually prepared using Fmoc-solid phase peptide synthesis protocols according to the literature.[1] The crude PNA oligomers were purified by high-performance liquid chromatography (Agilent Technologies 1260 Infinity Ⅱ) on an Agilent Eclipse XDB-C18 column. The molecular weight of the pure PNA was characterized by an AB Sciex LC-Q-TOF 4600 Mass Spectrometer. All UV-vis results were determined by a DeNovix UV-Vis DS-11 Spectrophotometer. Glucose concentration was recorded on the Roche ACCU-CHEK performa nano personal glucose meter (PGM), and the PGM readings have been corrected by standard glucose solution (Fig. S5).

2. Synthesis of Inv-cDNA conjugate

The Inverase-cDNA1 (Inv-cDNA1), Inverase-cDNA2 (Inv-cDNA2) and Inverase-cDNA3 (Inv-cDNA3) conjugates were synthesized and purified according to the reported protocol with slight modifications.[2] First, invertase was conjugated to the sulfo-SMCC linker with amide bond by mixing yeast invertase (2.5 mg) and sulfo-SMCC linker (1 mg) in 1mL PBS buffer (10 mM Sodium Phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4) and shaking at 750 rpm for 2.5 h at room temperature. The mixture was then centrifuged at 6000 rpm for 1 min, and the insoluble excess sulfo-SMCC was removed. Subsequently, the clear product of invertase-SMCC conjugate was purified by filtration through an Amicon-10K filter six times using PBS. Meanwhile, SH-cDNA (120 μL, 125 μM) was activated for 1 h at room temperature by continuously mixing with 15 μL of 100 mM TCEP. Excess TCEP was removed by Amicon-10 K for six times using PBS. Activated SH-cDNA was linked to the invertase-SMCC conjugate by the maleimide end at pH 7.4 and then mixed and stirred overnight at 30 °C. Unreacted SH-cDNA was removed and the Inv-cDNA conjugate was purified by Amicon-50K at least six times using PBS. Finally, the resultant Inv-cDNA conjugate

was dispersed in PBS buffer at a concentration of 5 mg mL^{-1}. Before use, the Inv-cDNA conjugate was diluted to the concentration of 1 μM, while the invertase concentration was 0.15 mg/mL.

3. Optimization of the incubation time for enzymatic catalysis

The optimal temperature for sucrose to glucose conversion catalyzed by invertase from bakers' yeast is 55 °C according to the reports.[3] Then, 0.15 mg/ml invertase and 100 mM sucrose were used to assess the optimum reaction time of enzymatic catalysis.

4. Gel Electrophoresis Analysis

The CHA products were analyzed by native 10% (w/w) polyacrylamide gel electrophoresis (PAGE) in 1 × TBE buffer consisting of Tris (40 mM), acetic acid (20 mM), and EDTA (1 mM) (pH 8.3) at 100 V for 40 min. While, the CHA + HCR products were analyzed by native 6.8% (w/w) PAGE in 1 × TBE buffer at 100 V for 1 h. After washing, the gel was stained by gel-red at RT for 15 min and imaged via a Tanon 2500 Gel Imaging System.

Table S1. Sequences of DNA, RNA oligonucleotides and PNA probes used in this study[a].

Name	Sequence (5'-3' or N'-C')
H1	**CCCCT** **ATCACGATTAGCATTAA** CCTAGAGATGT TTAATGCTAATCGTGAT CCATAACTGTCCTG
H2	GCATTAA ACATCTCTAGG TTAATGCTAATCGTGAT CC***TAGAGATGT*** ***GAT***
H3	CAGGCTCGTGTGAAAAAA TGATCCATAACTGTCCTG TCAACTAAGT CAGGACAGTTATGGAT CACGATT
H4	ACTTAGTTGA CAGGACAGTTATGGATCA GCTACAATCG TGATCCATAACTGTCCTG AAAAAAGTCGTGTCCTCA
cDNA1	CACACGAGCCTGAAAAAA-SH
cDNA2	HS-AAAAAATGAGGACACGAC
cDNA3	HS-AAAAAACAGGACAGTTATGGATCACGA
H1'	TAATCGTGATCCATAACTGTCCTG
H2'	TAGAGATGTGAT
miRNA155	UUAAUGCUAAUCGUGAUAGGGGU
mismatched miRNA155	UUAAUUCUAAUCGUGAUAGGGGU
miRNA21	UAGCUUAUCAGACUGAUGUUGA
miRNAlet7a	UGAGGUAGUAGGUUGUAUAGUU
miRNA141	UAACACUGUCUGGUAAAGAUGG
PNA	ATCACATCTCTA
P-DNA	ATCACATCTCTA

[a] Self-complementary fragments of hairpin probes are marked in red.

Target binding sites of H1 are bold.

PNA binding sites of H2 are bold and italic.

Complementary fragments between H1 and H3 are underlined.

Complementary fragments of H3 or H4 to cDNA1 or cDNA2 are double underlined. The bases at the mutational position are highlighted in the box.

Table S2. Buffers used in this study.

Buffer name	Buffer formulation	pH
Immobilization buffer	100 mM Na_2CO_3	9.6
Capping solution (CAP)	25 mM Lys, 10 mM NaH_2PO_4, 100 mM NaCl, 0.1 mM EDTA	8.0
1 × PBS	9.0 g L^{-1} NaCl, 144 mg L^{-1} KH_2PO_4, 795 mg L^{-1} Na_2HPO_4	7.4
HCR reaction buffer	1 × PBS containing 140 mM $MgCl_2$	7.4
1 × PBST	1 × PBS with 0.05% Tween 20	7.4
Blocking buffer (BLB)	2% bovine serum albumin, 25 mM tris(hydroxymethyl) aminomethane (Tris), 150 mM NaCl, 0.05% TWEEN 20, 0.1 mM EDTA	7.4
BLBs	BLB with 0.1 mg mL^{-1} single-stranded salmon sperm DNA	7.4

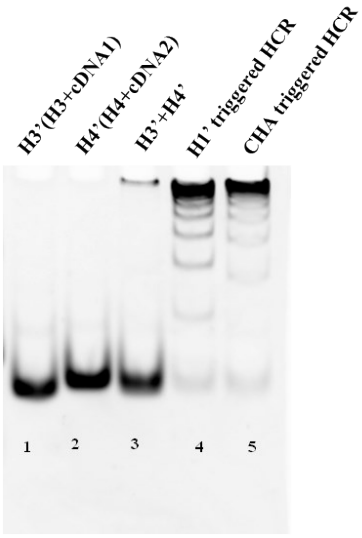

Fig. S1. 6.8% Native PAGE analysis of HCR reaction. The concentrations of H3', H4', and H1' are 1 μM, 1 μM and 100 nM, respectively. The concentrations of H3', H4', and PNA-CHA are 1 μM, 1 μM and 100 nM, respectively.

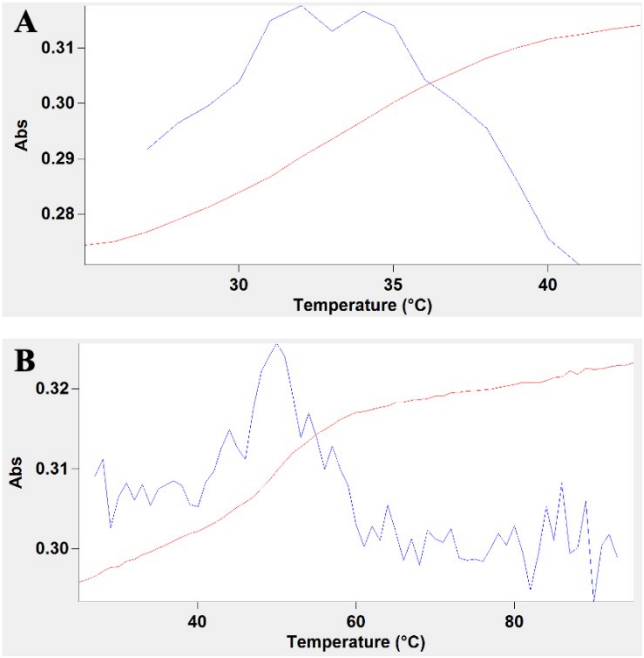

Fig. S2. Thermal melting profiles of (A) P-DNA-H2' homoduplex and (B) PNA-H2' heteroduplex under the same conditions.

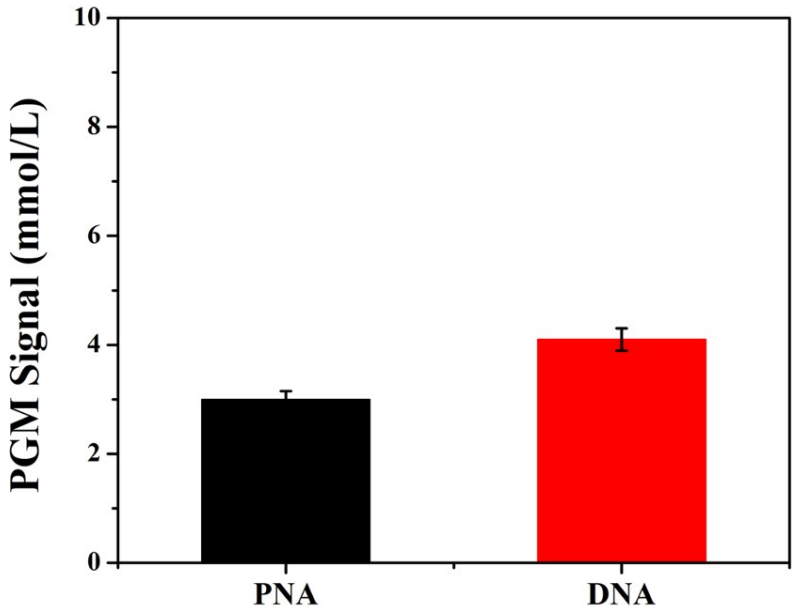

Fig. S3. PGM responses of the CHA-HCR system to different capture probes in the absence of miRNA155.

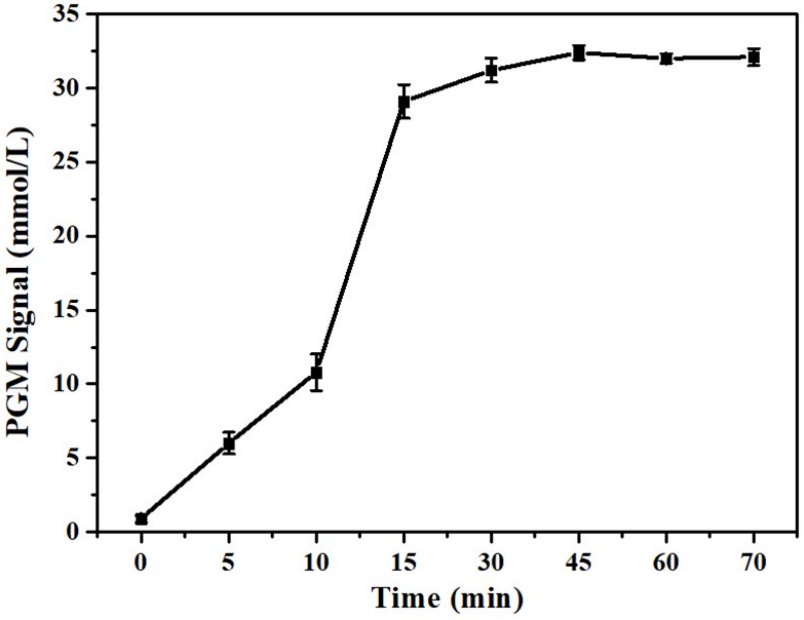

Fig. S4. PGM responses to sucrose at different times when incubating with 0.15 mg/mL invertase.

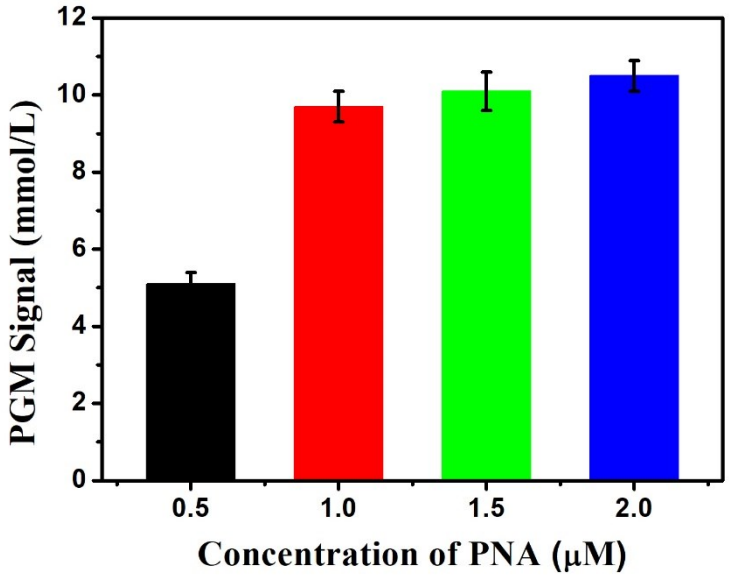

Fig. S5. PGM responses of the PNA-CHA system to different concentrations of PNA capture probe with the presence of 10 nM miRNA155.

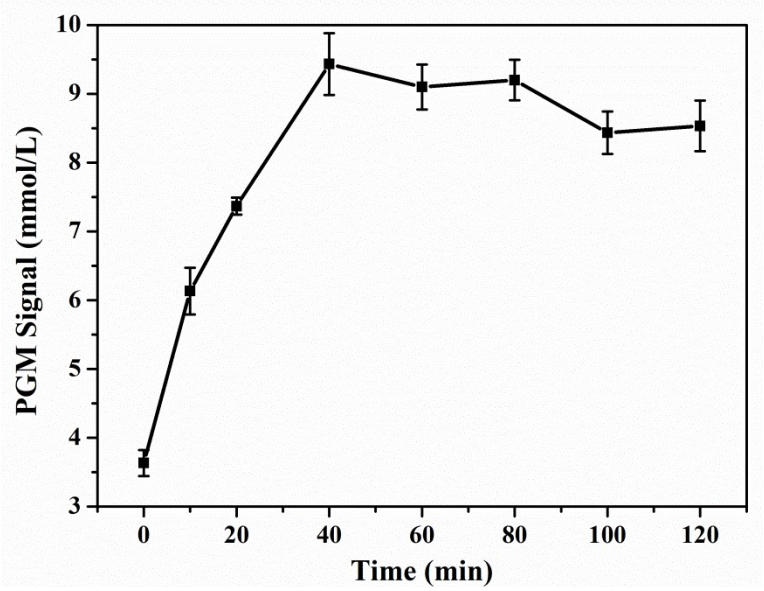

Fig. S6. Time-dependent PGM responses of the PNA-CHA system with the presence of 10 nM miRNA155.

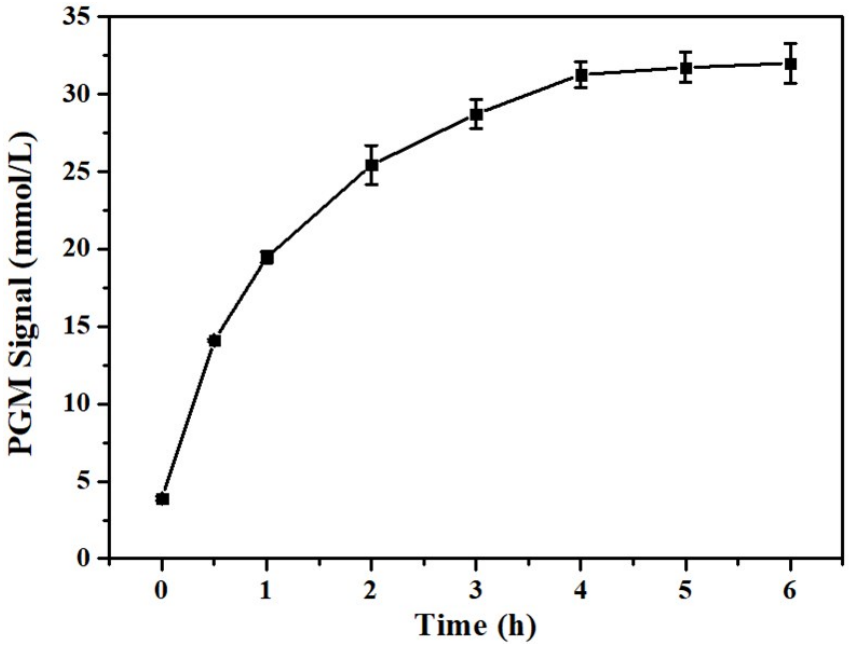

Fig. S7. Effect of the HCR reaction time on PGM responses with the presence of 10 nM miRNA155.

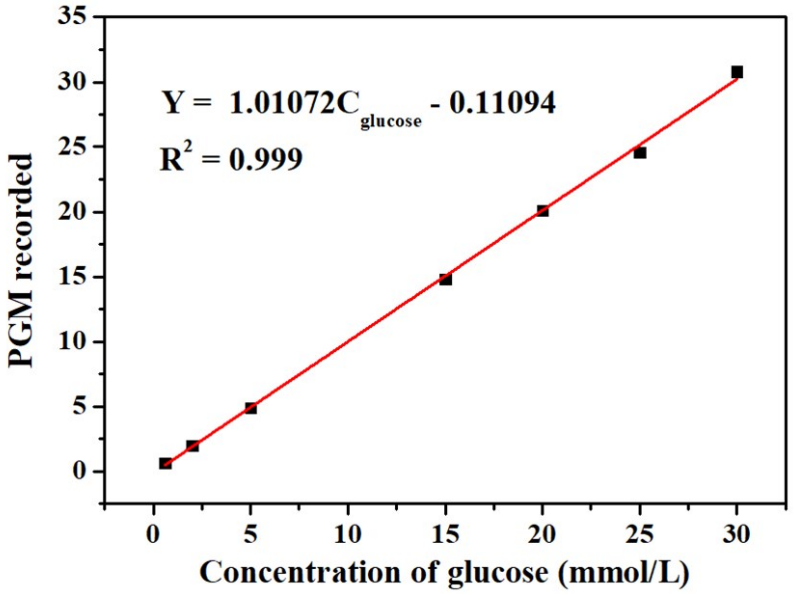

Fig. S8. PGM responses vs. the concentration of standard glucose solutions.

References

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2. Y. Du, R. A. Hughes, S. Bhadra, Y. S. Jiang, A. D. Ellington and B. Li, *Sci Rep*, 2015, **5**, 11039.

3. Y. Gu, T. T. Zhang, Z. F. Huang, S. W. Hu, W. Zhao, J. J. Xu and H. Y. Chen, *Chem Sci*, 2018, **9**, 3517-3522.