An Entropy-Driven DNA Nanomachine for MicroRNA Detection Using Personal Glucose Meter

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Experimental Section

Materials

Streptavidin-coated magnetic beads (MBs, 1 μM in average diameter, 10 mg/mL) and sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) were purchased from Thermo Fisher Scientific. Amicon-3K/10K centrifugal filters were purchased from Merck Millipore Ltd. Bovine serum albumin (BSA) was purchased from Dingguo Biotech Co., Ltd. (Shanghai, China). Fetal bovine serum (FBS) was obtained from Thermo Fisher. Grade VII invertase from baker's yeast (S. cerevisiae), Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and other chemicals for buffers and solvents were purchased from Sigma-Aldrich Co., Ltd. All chemicals were used as received from the suppliers without further purification. Ultrapure water (≥18.2 MΩ·cm, Millipore, Bedford, MA) was used throughout the study. All oligonucleotides used in this study were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) (Table S1).

Table S1. Synthesized oligonucleotides used in the experiment.

No.	**Sequence (5'→3')**
S_1	Biotin-AAAAAAAAAAAATCAACATCAGTCTGATAAGCTATAGGTAC
S_2	ACCTATAGCTTATCAGACTGA
Fuel	SH-AAAAAAAAAAAAGTACCTATAGCTTATCAGACTGA
FAM-c-Fuel	FAM-TCAGTCTGATAAGCTATAGGTAC
Target (T)	TAGCTTATCAGACTGATGTTGA
miR-21	UAGCUUAUCAGACUGAUGUUGA
let-7a	UGAGGUAGUAGGUUGUAUAGUU
miR-155	UUAAUGCUAAUCGUGAUAGGGGU
Mismatch-1	TAGCTTATCACACTGATGTTGA
Mismatch-2	TAGCTTATCACTCTGATGTTGA
SH-S_2	SH-AAAAAAAAAAAAACCTATAGCTTATCAGACTGA
S_3	CTGATAAGCTAAAAAAAAAAAAA-Biotin
S_4	SH-AAAAAAAAAAAATCAACATCAGT

Note: the mismatched bases are given in red.

The nucleic acid (dissolve in DNase-free water) and enzyme stock solution were stored at -20 °C before use.

Buffer used in this work:

Buffer A: 0.1 M sodium phosphate buffer, 0.1 M NaCl, pH 7.3

Buffer B: 0.1 M sodium phosphate buffer, 0.1 M NaCl, pH 6.2

Buffer C: 20 mM Tris, 1 M NaCl, 1 mM EDTA, 0.0005% Triton X-100, pH 7.5

Buffer D: 20 mM Tris, 300 mM NaCl, 5 mM KCl, 0.01% Triton X-100, pH 7.4

Measurements and Characterizations

The glucose meter, ACCU-CHEK Active, was purchased from Roche Diabetes Care GmbH (Mannheim, Germany). Ultraviolet-visible (UV-vis) spectroscopy spectra were recorded with a Cary Series spectrometer (Agilent Technologies). The gel was stained with Coomassie brilliant blue and the fluorescence image was acquired by FluorChem FC3 (ProteinSimple, USA) under 365 nm irradiation. Dynamic light scattering (DLS) analysis was carried out on Zetasizer Nano-ZS90 (Malvern, UK).

Fuel-Invertase (F) Conjugation

The conjugates of thiol-modified Fuel and invertase were prepared according to the literature with some modifications.[1] Briefly, 30 μL of 130 μM thiol-modified Fuel was mixed with 2 μL of 1 M sodium phosphate buffer (pH 5.0) and 2 μL of 7 mM TCEP solution to activate the thiol-modified Fuel. The mixture was kept at room temperature for 2 h and then the excess TCEP was removed by 3K ultrafiltration tube for 8 times using Buffer A. Simultaneously, 20 μL of 9 mM sulfo-SMCC was added to 90 μL of 5 mg/mL invertase in Buffer A, and mixed well for vortexing 5 minutes, the mixture was then plated on a shaker for 2 h at 30 °C. The solution was subsequently purified in a 10K ultrafiltration tube for 8 times using Buffer A. The purified solution of sulfo-SMCC-activate invertase and TCEP-activate thiol-Fuel was mixed, and shaken at room temperature for 48 h. To remove the excess thiol-Fuel, the solution was purified in a 10K ultrafiltration tube 8 times using Buffer A, and stored at -20 °C for further use. The preparation can easily be scaled up by increasing the amount of materials but maintaining the same molar ratio.

MB-S Preparation

100 μL Streptavidin-coated magnetic beads (MBs) from stock solution were washed three times with Buffer C and then dispersed in 1 mL Buffer C. 20 μL of 100 μM S_1 was added and well mixed on a roller for 1 h at room temperature. After that, the MBs were washed five times using Buffer D to remove excess S_1 and then resuspended in 1 mL Buffer

D. Later, 15 μL of 100 μM S_2 was added to the above solution and mixed on a roller for 1 h to prepare MB-S probes. After five times washing by Buffer D, the MB-S were dispersed in 1 mL Buffer D with 2 mg/mL BSA at room temperature to block nonspecific binding sites, then washed five times to remove excess BSA, and finally stored at 4 °C for further use.

Procedures for Target miRNA Detection Using PGM

Each 50 μL aliquot of the above MB-S contained solution was used to analyze one sample. Various concentrations of target miRNA and F in Buffer A (about 10 mg/mL) were added to the solution and well mixed at room temperature for 1 h. Excess F was washed off by Buffer D and recycled for further use by condensing the solutions using a 10K ultrafiltration tube. After washing the MBs residue seven times by Buffer D (containing 2 mg/mL BSA), 10 μL of 0.8 M sucrose was added to the MBs residue and shaken for 1 h at 37 °C. A portion of 2 μL of the above solution was tested by a commercially available PGM. Note that for lower target detection, the amount of the MB-S should be correspondingly increased.

The ΔGlucose Meter Signal is defined as:

$$\Delta\text{Glucose Meter Signal}=G-G_0$$

in which G and G_0 refer to the PGM signal of the sample with and without target, respectively.

Scheme of the toehold-mediated strand displacement reactions (TDR)

S_1 contains a target miRNA-identified toehold 1 at the 5' end (domain a), and a F-hybridized toehold 2 at the 3' end (domain d), respectively. Toehold 2 is initially sequestered by S_2 to inhibit the interaction between the F and the S_1. In the presence of target (T), S_2 is displaced by T via TDR1, exposing the toehold 2 for F hybridization. Subsequently, T is superseded by F via TDR2 to form duplex S_1/F. The released T drives the nanomachine to operate in a catalytic fashion.

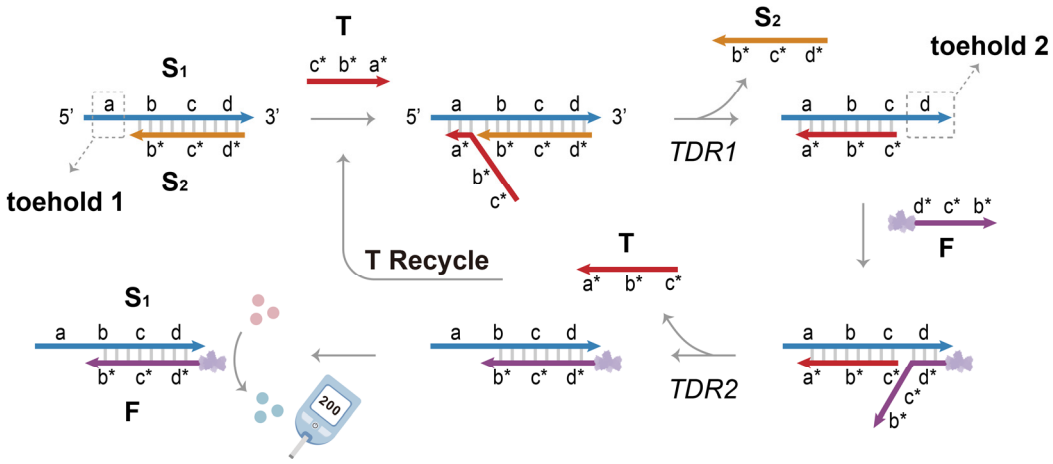

Fig. S1 Scheme of the toehold-mediated strand displacement reactions (TDR).

Scheme of the synthesis of Fuel-invertase conjugate (F)

Procedure for the synthesis of F was similar to the reported work[1]. Sulfo-SMCC was used as the linker to conjugate thiol-modified Fuel and invertase (Fig. S2). The conjugates F were verified using an 8% SDS-PAGE experiment. 25 mM Tris (0.25 M glycine, 0.1% SDS, pH 8.3) was used as the running buffer. This gel was stained by Coomassie brilliant blue, and the fluorescence image of the gel was acquired with FluorChem FC3 (ProteinSimple, USA).

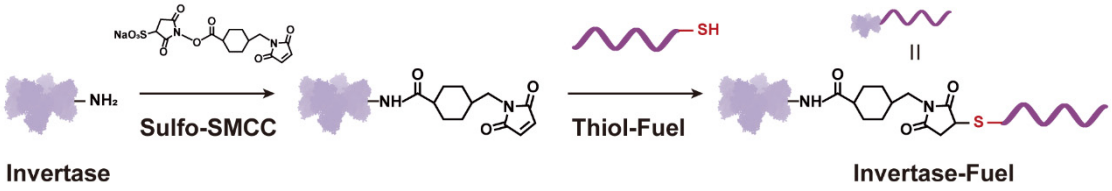

Fig. S2 The conjugation of Fuel and invertase by the heterobifunctional linker (sulfo-SMCC).

Relationship between PGM signals and actual glucose concentration

The glucose signal obtained from the PGM and the actual concentration of glucose added into the sample were compared to confirm the accuracy of the PGM detection at different temperatures. Fig. S3 showed that the signal obtained from the PGM was proportional to the actual glucose concentration in the samples at 4 °C and 37 °C.

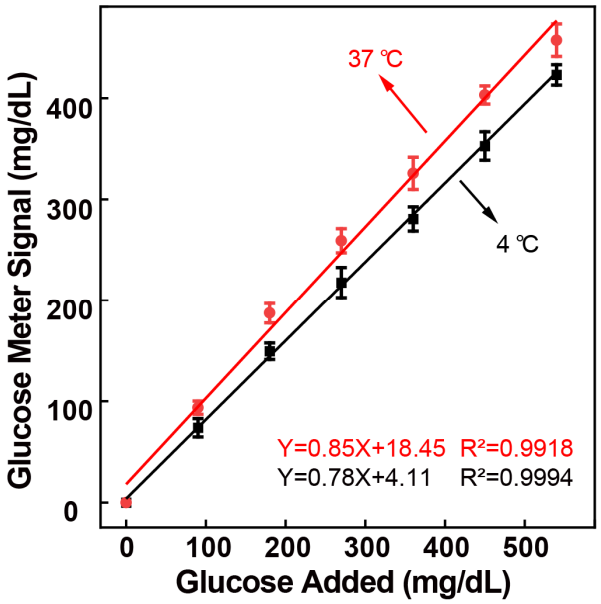

Fig. S3 Correlation between the actual glucose concentration and that detected by a PGM at 4 °C and 37 °C. The error bars represent the standard deviation of three measurements.

Some factors, such as catalytic reaction time and temperature, concentration of sucrose and F, sucrose hydrolysis time, and the pH of the buffer for the catalytic performance of invertase, would affect the performance of the TDR-PGM machine, thus the effects of these factors were investigated. All the optimized procedures utilized 3 nM and 10 nM of miR-21.

Optimization of the reaction time with target miR-21 and F

The kinetics of F hybridize to S_1 upon the addition of miR-21 was studied. After mixing the prepared MB-S with miR-21 and F, the mixture was placed close to a magnetic rack for 2 min and the supernate was removed at different time intervals. Then the sucrose was added into the MBs residue and the glucose product was measured by a PGM. As shown in Fig. S4, the PGM signal was increased rapidly and leveled off after 30 min, therefore 30 min was mainly enough for F binding to the MBs by the miR-21. In order to ensure the effective binding in the presence of low concentration target, a fixed time of 60 min was then chosen as the optimized reaction time for the subsequent experiments.

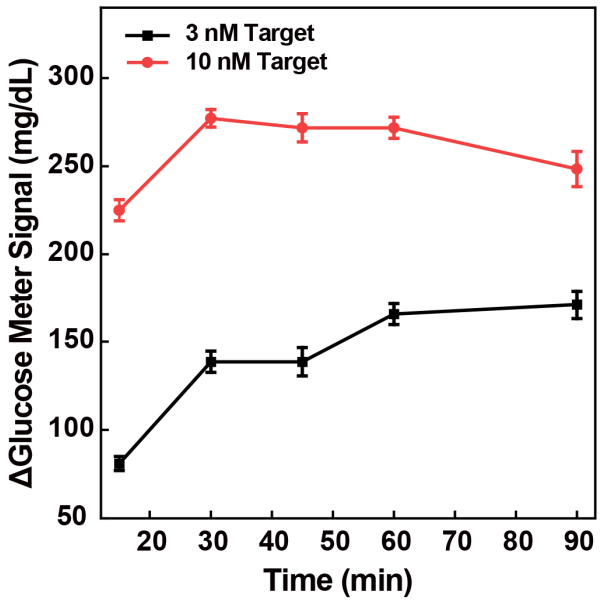

Fig. S4 Investigation of the reaction times of the MB-S with miR-21 and F. The error bars represent the standard deviation of three measurements.

Optimization of F concentration

Because invertase could nonspecifically adsorb to the surface of MBs and superabundant may also cause relatively high background, therefore the effect of F concentration was evaluated. Fig. S5 showed that 0.1 mg/mL of F was almost sufficient, on account of the recycling of F throughout the study, thus 0.4 mg/mL of F was used in the subsequent experiments.

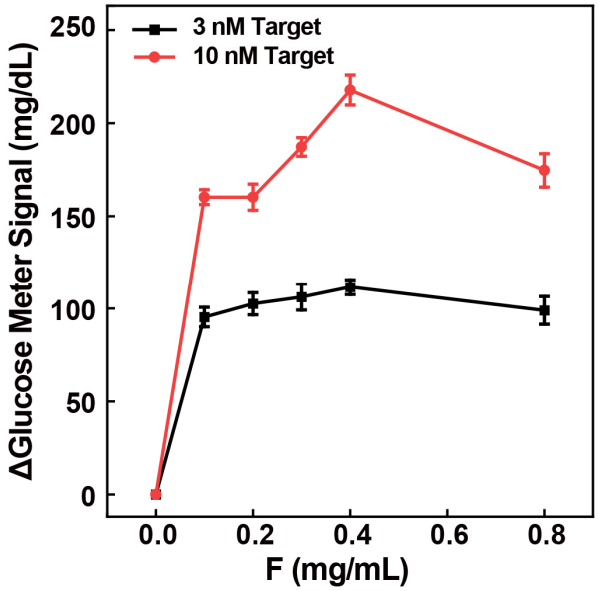

Fig. S5 The influence of different concentrations of F on the detection of target by TDR-PGM sensor. The error bars represent the standard deviation of three measurements.

Optimization of sucrose concentration and incubation time

The concentration and hydrolyzation time of sucrose used for the production of glucose also played vital roles in the final signal obtained by a PGM. As shown in Fig. S6, the absolute PGM signal (ΔGlucose meter signal) was observed to increase with the addition of sucrose. In addition, the incubation time was also examined. The absolute PGM signal showed an increasing trend with the increase of time. To improve the target detection performance of the TDR-PGM sensor, we chose 800 mM as the ultimate sucrose concentration and 60 min as the incubation time for the subsequent experiments.

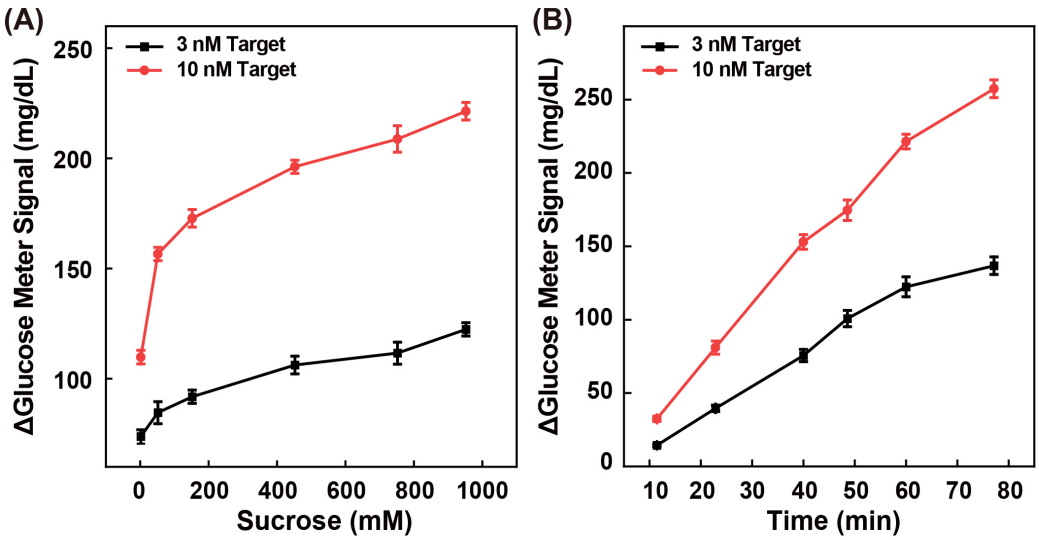

Fig. S6 The influence of sucrose concentration (A) and incubation time (B) on the detection of target by TDR-PGM sensor. The error bars represent the standard deviation of three measurements.

Optimization of operation temperature

Because the immobilization of F to the MB-S is based on DNA hybridization and displacement, which are temperature-dependent, thus the operation temperature was also investigated. We carried out the target detection under different temperatures (Fig. S7). When the temperature was neither too low (4 °C) nor too high (40 °C), the signal was obviously low, because the reaction was relatively slow and the fewer F was attached to the MB-S in a certain period of time at 4 °C; while the DNA hybridization was not strong and the F was partially released at 40 °C. Fig. S7 indicated that the appropriate temperature is in the range of 25-28 °C.

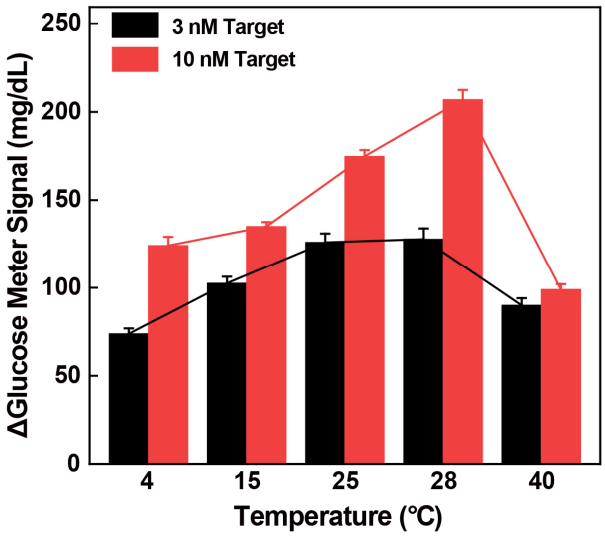

Fig. S7 The influence of different temperatures on the detection of target by TDR-PGM sensor. The error bars represent the standard deviation of three measurements.

Optimization of the pH on the catalytic hydrolysis of invertase

The optimal pH is responsible for the hydrolysis of sucrose to glucose, thus the pH of the hydrolysis buffer was evaluated. As shown in Fig. S8, the highest catalytic hydrolysis performance of invertase was reached at pH = 6.2, thus pH 6.2 was applied in the subsequent experiments.

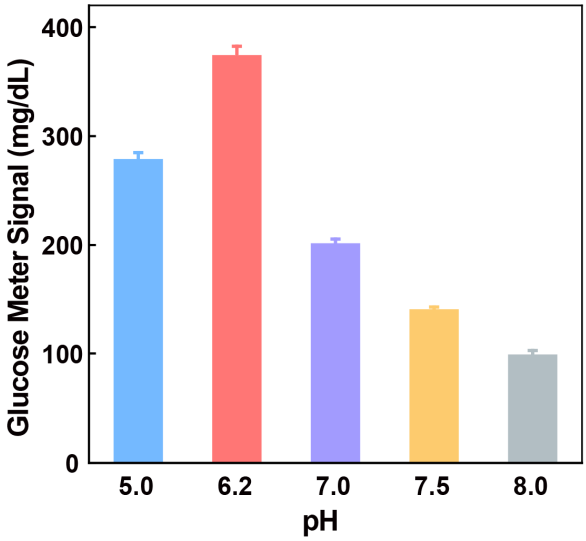

Fig. S8 The influence of pH on the catalytic performance of invertase for sucrose hydrolysis to glucose. The detection was conducted in 0.1 mg/mL invertase incubated with 800 mM sucrose for 10 min at 25 °C. The error bars represent the standard deviation of three measurements.

The kinetics of glucose signal with various concentrations of target

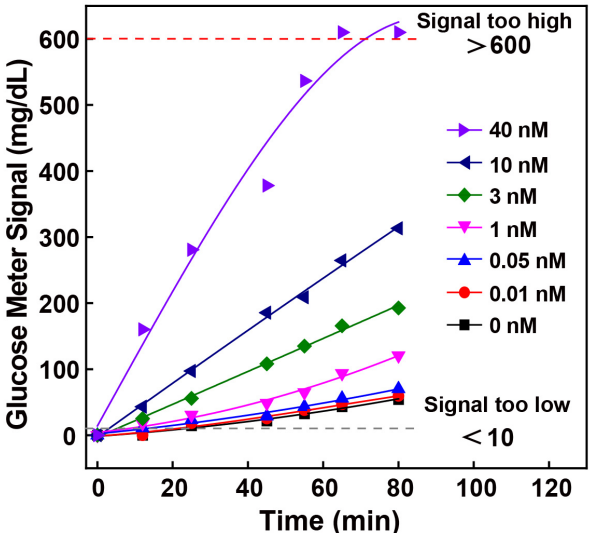

Fig. S9 The kinetics of glucose signal for the samples containing various concentrations of target. When the glucose concentration is higher than 600 or lower than 10, it can't be measured by PGM.

The proposal for improving the sensitivity of the TDR-PGM sensor

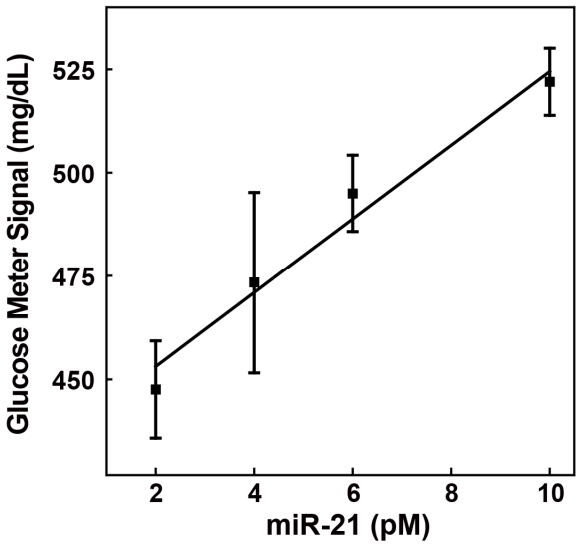

Fig. S10 Calibration curves of the TDR-PGM sensor-mediated miR-21 assay using 200 μL of MB-S (1 mg/mL) per sample. The error bars represent the standard deviation of three measurements.

The design of the sensor using traditional DNA sandwich

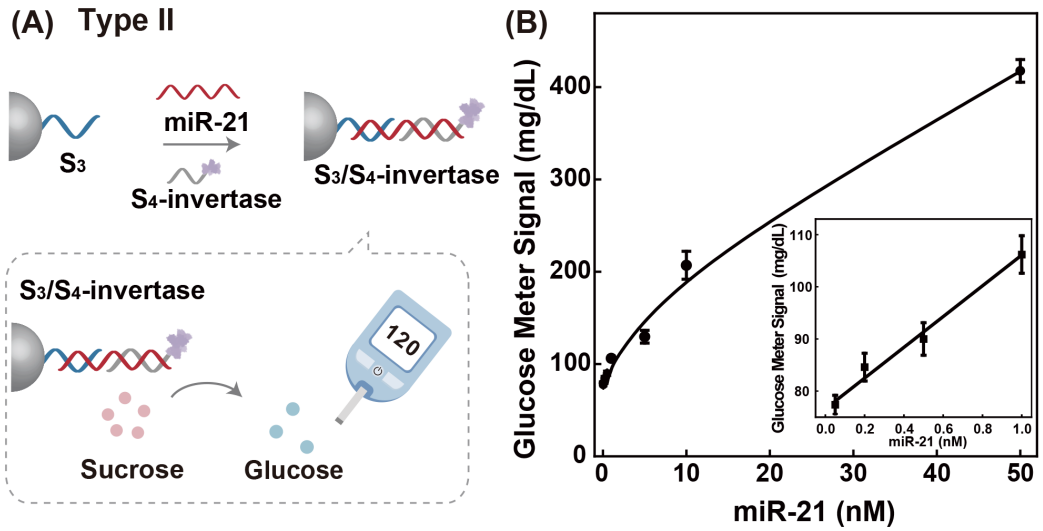

Fig. S11 The design employs the traditional DNA sandwich assay for the detection of miR-21 by PGM. The incubation time of invertase with sucrose was prolonged to 2 h. The error bars represent the standard deviation of three measurements.

Investigation of the influence of glucose on the TDR-PGM sensor

To investigate the influence of serum glucose on the performance of our TDR-PGM sensor on miRNA detection, 50 mg/dL and 200 mg/dL of glucose concentrations were chosen for the experiment. We pre-added glucose to Buffer A to mimic a serum sample containing glucose. As shown in **Fig. S12**, the TDR-PGM sensor exhibited a good linear relationship (with a good correlation coefficient $R^2 = 0.996$) for miR-21 analysis even in the presence of 200 mg/dL glucose concentration. Therefore, these results demonstrated that serum glucose has no influence on the detection performance of TDR-PGM sensor.

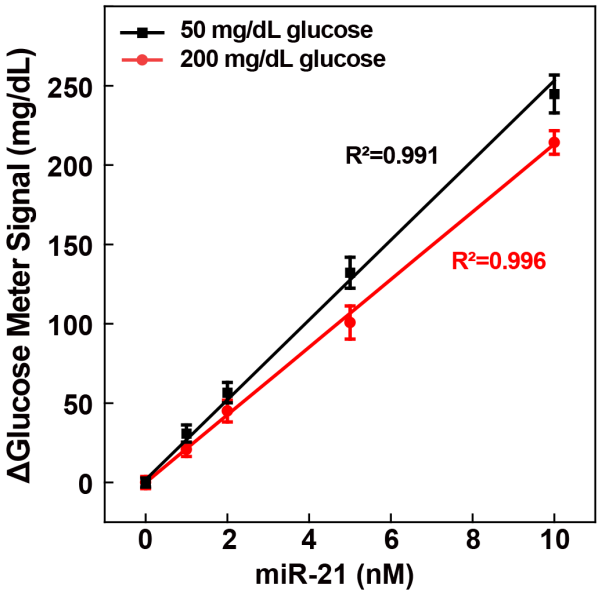

Fig. S12 Investigation of the influence of glucose on the performance of TDR-PGM sensor for miR-21 detection. The error bars represent the standard deviation of three measurements.

Investigation of the stability of the MB-S

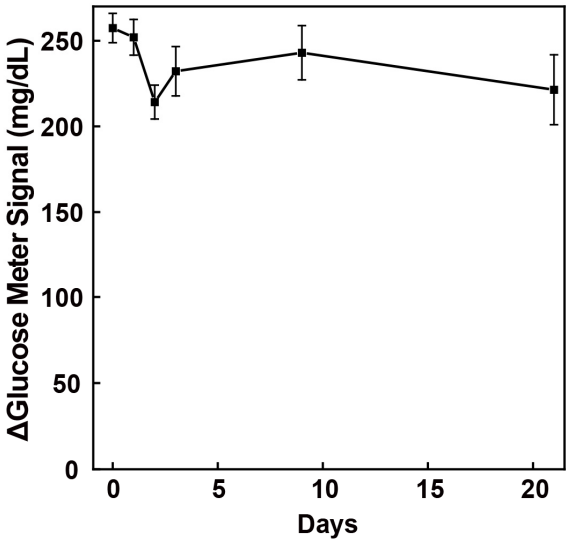

Fig. S13 Investigation of the stability of the MB-S stored at 4 °C. The error bars represent the standard deviation of three measurements.

Investigation of the activity of the invertase under different treatments

0.1 mg/mL of fresh invertase, invertase-sulfo-SMCC and conjugates F were prepared and separately incubated with 0.5 mM sucrose (the substrate of invertase) at 37 °C for 10 min. Then we measured the glucose signal by a PGM.

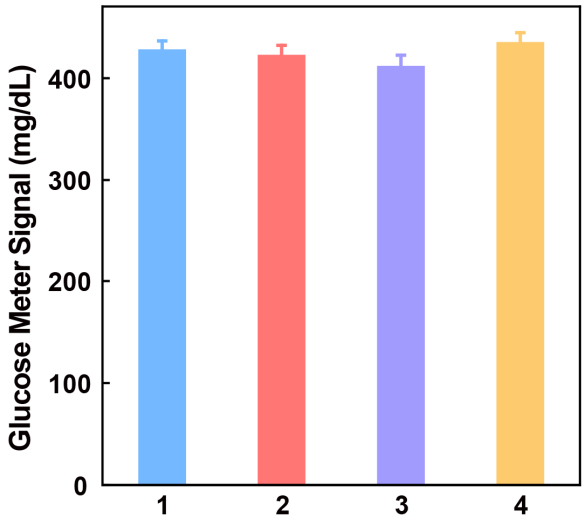

Fig. S14 The activity of invertase after different treatments: 1. the fresh stock solution of invertase at -20 °C; 2. invertase was treated with sulfo-SMCC at 30 °C for 1 h; 3. conjugates F at room temperature for 48 h; 4. conjugates F stored at -20 °C for 40 days. The error bars represent the standard deviation of three measurements.

REFERENCES

1 Y. Xiang, Y. Lu, *Nature Chem.*, 2011, **3**, 697-703.