Supporting Information

A novel β-cyclodextrin-assisted enhanced strategy for portable and sensitive detection of miR-21 in human serum

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1. Experimental Section

1.1 Sequences of the oligonucleotides used in the experiment

Table S1 DNA sequences and RNA sequences used in the experiment.

Name	Sequence (from 5′ to 3′)
Capture DNA (cDNA)	Biotin-TTTTTTTTTTTCAACATCAGT
Assist DNA (aDNA)	CTGATAAGCTACACGATCCGCTCATTC
H1	SH-TTTTTTTTTTCCTGGTTGCGGTGTC GTGGAATGAGCGGATCGTG
H2	CACGACACCGCAACCAGGTTCACGAT CCGCTCATTC
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-21 (DNA)	TAGCTTATCAGACTGATGTTGA
Single-base mismatch	UAGCUUAUCAGACUCAUGUUGA
Two-bases mismatch	UAGCUUAUCAGACUCAGGUUGA
non-complementary RNA	UUGUACUACACAAAAGUACUG
miR-155	UUAAUGCUAAUCGUGAUAGGGGU

1.2 Characterization of binding efficiency of cDNA to MNPs

The OD_{260} of pre, post, wash1 and wash2 were measured by ultra-micro spectrophotometer (Table S2), and their combined efficiency was calculated according to formula[1]:

$$Binding\ efficiency\ (\%) = \frac{OD_{260(pre)} - OD_{260(post)} - OD_{260(wash1)} - OD_{260(wash2)}}{OD_{260(pre)}} \times 100\%$$

(1). Finally, the average binding efficiency of the three reactions was 93.42%, indicating that the binding efficiency between MNPs and cDNA was high and stable.

Table S2 Binding efficiency of cDNA to MNPs.

Group	Pre	Post	Wash1	Wash2	Binding efficiency
1	0.104	0.004	0.001	0.000	95.19%
2	0.103	0.008	0.001	0.000	91.26%
3	0.113	0.004	0.002	0.001	93.81%

1.3 Evaluation of detection limits

Three low concentration miR-21 solutions below the linear range's lowest point and solvent blanks were detected, with three parallel samples for each concentration. The PGM signal of the detection limit was higher than the blank signal plus 3 times the standard deviation of the blank signal ($\bar{X}$+3SD). When the miR-21 concentration was 10 pmol/L, the PGM signal value was 3.83>3.58 ($\bar{X}$+3SD) (Table S3). The detection limit of this strategy was only at 10 pmol/L, but when β-CD was constructed to

assist the enhancement strategy the detection limit in this experiment was reduced to 5 pmol/L (3.91>3.78). Therefore, the detection limit of this method was 5 pmol/L.

Table S3 Comparison of detection limits before and after β-CD enhancement (n = 3).

C_{miR-21} (pmol/L)	5.0	10.0	20.0	Blank	SD_{blank}
Average PGM signal (before)	3.10	3.83	4.50	2.83	0.25
Average PGM signal (after)	3.91	4.22	5.13	2.73	0.35

1.4 Evaluation of spiked recovery and precision

The spiked recovery rate and precision after β-CD enhancement of this method was shown in Table S4. Three parallel repeated experiments had been performed. The spiked solution of miR-21 with three concentrations of low, medium and high was prepared by the binding buffer. The average value of the PGM signal obtained was put into the calibration curve to calculate the measured concentration. The 20 fmol target was added to the low concentration group (the final concentration was 100 pmol/L) and substituted into the standard curve of the low concentration. The 150 fmol target was added to the medium concentration group (the final concentration was 750 pmol/L), and the 400 fmol target was added to the high concentration group (the final concentration was 2000 pmol/L). The recoveries were 118.70%, 124.65% and 103.82%, and *RSD* were 6.43%, 3.09% and 2.59%, respectively.

Table S4 Precision and recovery of miR-21 detected after β-CD enhance (n = 3).

Added value of miR-21 (fmol)	Detected (fmol)	*SD*	*RSD* (%)	Recovery (%)
20.0	23.74	1.53	6.43	118.70
150.0	186.98	5.77	3.09	124.65
400.0	415.26	10.76	2.59	103.82

1.5 Preparation of H1-invertase

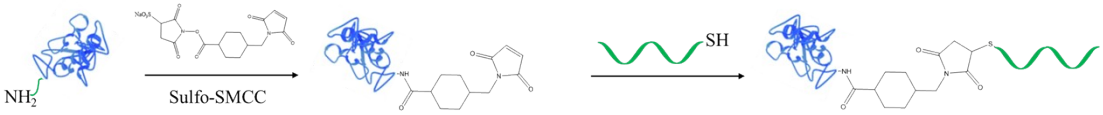

Fig. S1 The preparation principle of H1-invertase.

The preparation principle of H1-invertase was shown in Figure S1[2]. invertase and sulfhydrylated DNA were linked through 4-(N-Maleimidomethyl) cyclohexane-1-carboxylic acid sodium salt (sulfo-SMCC) as a cross-linking arm, in which the active NHS ester of sulfo-SMCC could form an amide bond with the amine group of invertase under the environment of pH 7~9. The maleimide at the other end of sulfo-SMCC and sulfhydryl group could form stable sulfur ether bonds in the buffer solution of pH 6.5~7.5, thus achieving the crosslinking of invertase and H1.

1.6 Optimum enzymatic reaction conditions

In order to explore the optimal conditions for the catalytic activity of invertase, the reaction solution, pH, temperature and substrate concentration of the catalytic reaction were optimized (Figure S2). We found that the invertase catalyzed reaction was more efficient in PBS than in water (Figure S2A). As the pH of the buffer solution increased from 3.5 to 4.5, the PGM signal value increased, but decreased from 4.5 to 7.5, indicating that the pH of 4.5 was the optimal pH for the invertase catalyzed reaction (Figure S2B). Similarly, the optimal temperature was 55℃ (Figure S2C), the optimal sucrose concentrate was 0.5 mol/L (Figure S2D).

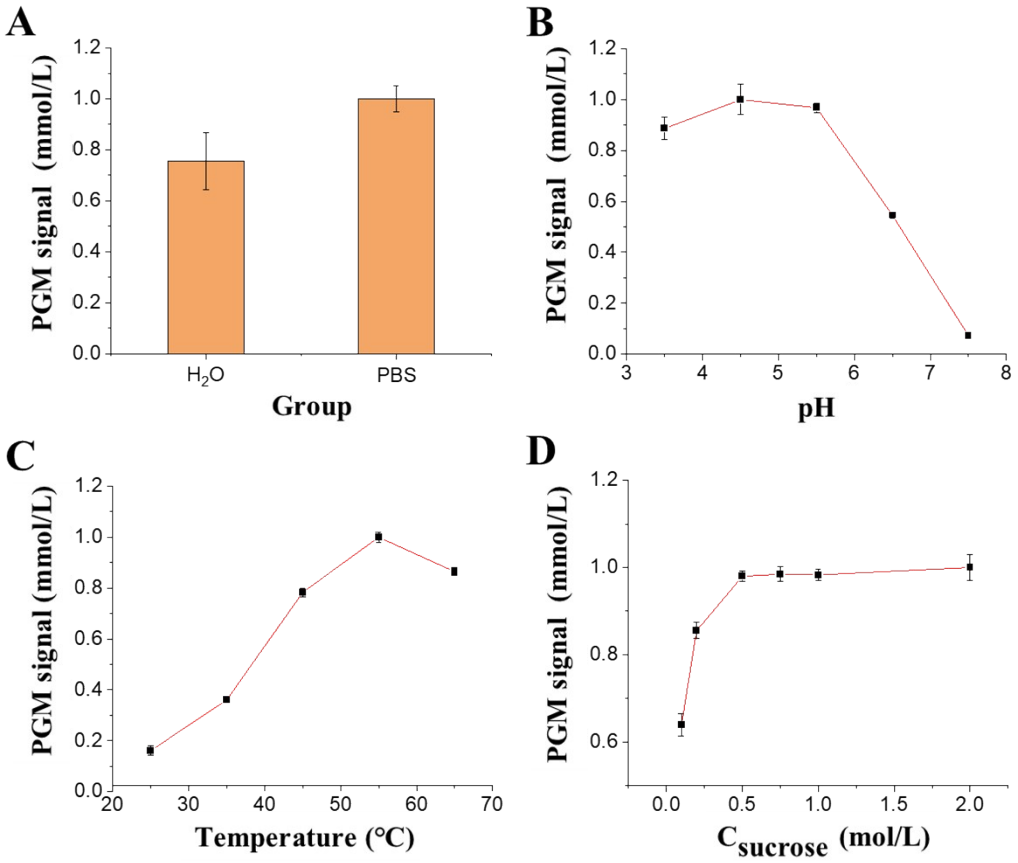

Fig. S2 Optimization of conditions for invertase catalyzed reaction. (A) Reaction solution; (B) pH; (C) Temperature; (D) Concentration of substrate sucrose.

1.7 Magnetic characterization of MNPs-cDNA

In order to explore whether the magnetic properties of MNPs after binding with cDNA were affected, the magnetic properties of MNPS-cDNA were characterized. As shown in Figure S3, in the absence of an external magnetic field, MNPs-cDNA was uniformly dark brown in solution (Figure S3A). Under the action of external magnetic field, MNPs-cDNA was rapidly aggregated and the supernatant was clarified and transparent (Figure S3B), indicating that MNPs-cDNA had strong magnetic separation performance and could be used for follow-up experiments.

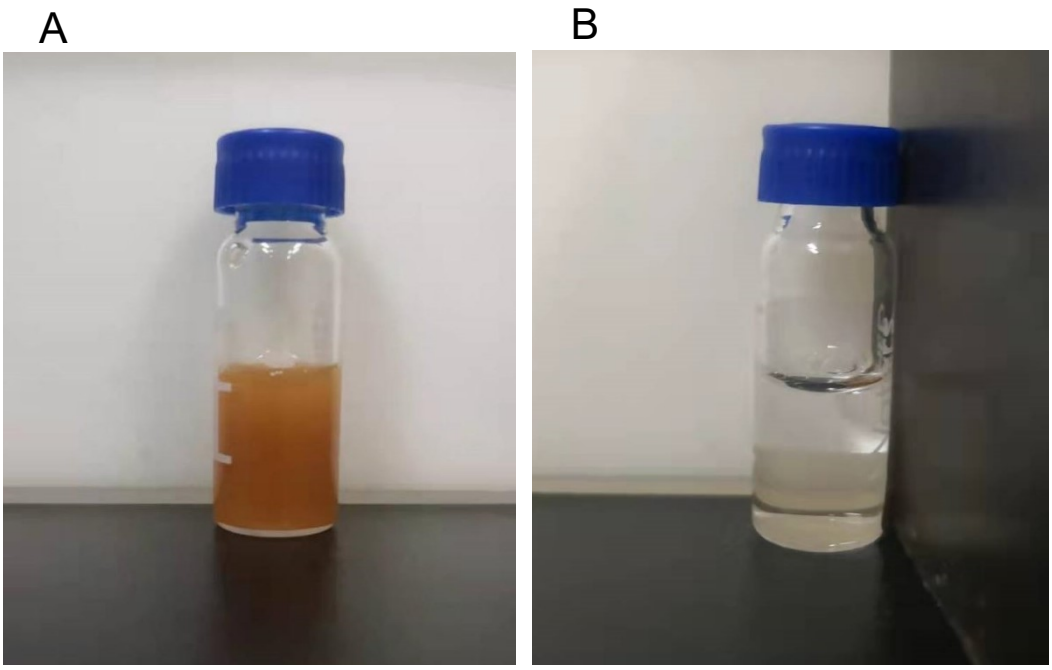

Fig. S3 Magnetic separation performance of MNPs-cDNA. Before magnetic separation (A) and after magnetic separation (B).

1.8 Kinetic curves of invertase activity.

The effect on the enzymatic reaction rate was investigated by fixing the invertase concentration and changing the sucrose concentration, based on which the data were fitted to the Michaelis-Menten equation to derive the kinetic curve of the enzymatic reaction and the maximum reaction rate (Vmax). As shown in the figure below, FIG. S4A is the control group (only sucrose and invertase exist), the curve is well fitted, and the Vmax is 0.448 mol/L/min. The four selected cyclodextrins all had enhanced effects on invertase activity, among which β-CD had the best enhanced effect on invertase activity, and the enhanced Vmax was 0.59 mol/L/min (Figure S4B-E).

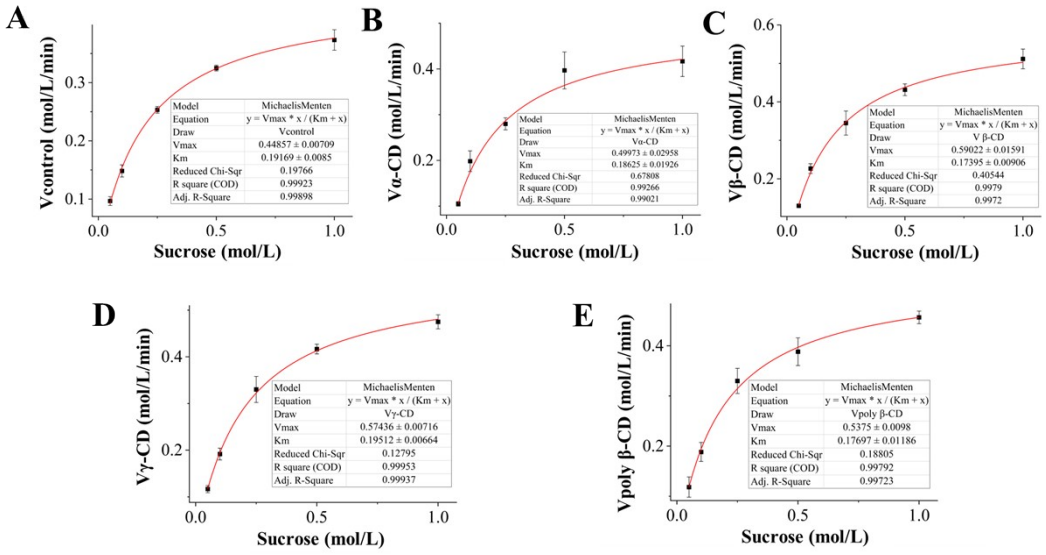

Fig. S4 Kinetic curves of different cyclodextrins on invertase activity. (A) Control group, only sucrose and invertase exist. (B) α-CD; (C) β-CD; (D) γ-CD; (E) poly β-CD.

On this basis, the effect of different concentrations of β-CD on the catalytic activity of invertase was further studied. As shown in Figure S5,

the Vmax of invertase gradually increased with the increase of β-CD concentration, indicating that the catalytic activity of invertase was gradually enhanced. However, when the concentration of β-CD exceeded 5 mM, the effect of β-CD in enhancing the catalytic activity of invertase was similar. These results indicated that β-CD could enhance the catalytic activity of invertase, and accordingly 10 mM of β-CD was used for subsequent experiments.

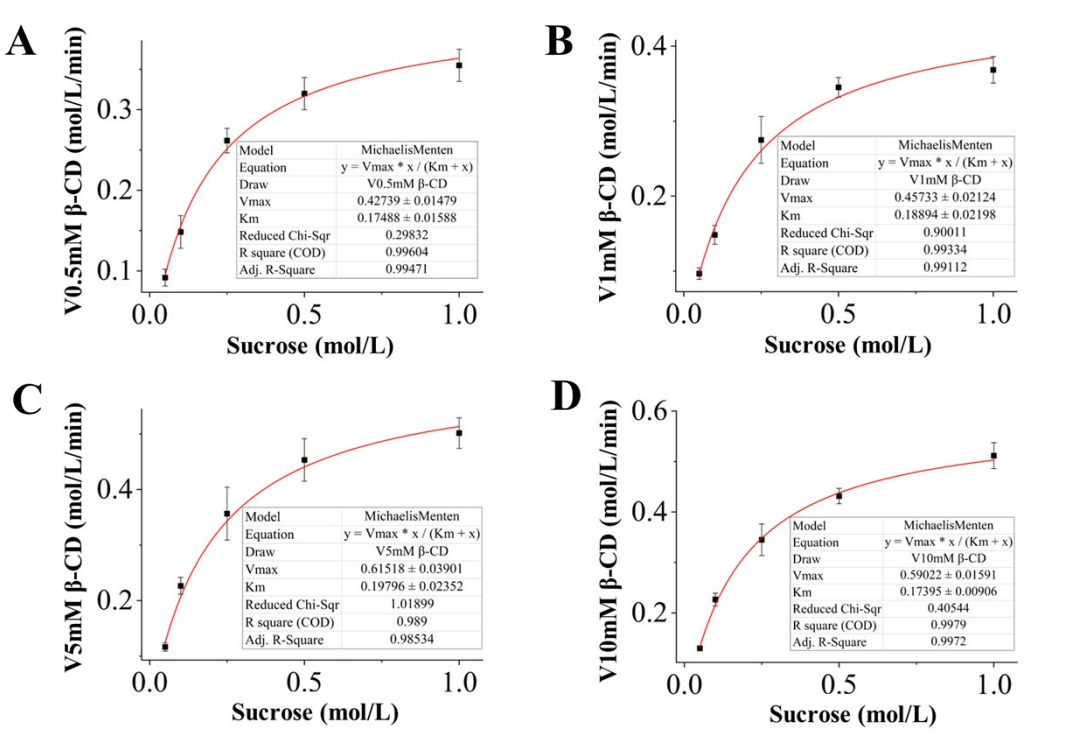

Fig. S5 Kinetic curves of different concentrations of β-CD on invertase activity. (A) 0.5mM β-CD; (B) 1mM β-CD; (C) 5mM β-CD; (D) 10mM β-CD.

References

1. L. He, L. Ding, S. Yu, F. Yu, Y. Tian, X. Xie, L. Qu, L. Liu and Y. Wu, *Sens. Actuators. B. Chem*, 2019, **279**, 440-446.
2. Y. Xiang and Y. Lu, *Nat. Chem*, 2011, **3**, 697-703.