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Supporting information

Three-Dimensional DNA Walking Machine for Ultrasensitive Dual-Modal Detection of MiRNA with Fluorimeter and Personal Glucose Meter

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Apparatus and reagents

The fluorescence intensity was recorded by a F-7000 fluorescence spectrometer (Hitachi, Tokyo, Japan). The glucose concentrations were measured by a personal glucose meter obtained from Johnson (OneTouch® SelectSimple, Beijing, China).

Streptavidin coated magnetic beads (SA-MBs) were obtained from Aladdin Sci. & Tech. Co., Ltd. (Shanghai, China). Invertase from baker's yeast (S. cerevisiae), sulfosuccinimidyl-4-(N-maleimidomethyl)-cyclohexane-2-carboxylate (sulfo-SMCC), tris(2-carboxyethyl)phosphine (TCEP), sucrose, phosphate buffered saline (PBS) buffer (pH = 7.4), sodium chloride-sodium citrate (SSC) buffer (pH = 7.0) were obtained from Sigma-Aldrich and used without any purification.

MiRNAs were obtained from Suzhou Gene-Pharma Co. Ltd. (Suzhou, China) and purified by high-performance liquid chromatography.

Target miRNA-21: 5'-UAG CUU AUC AGA CUG AUG UUG A-3'

One-base mismatched miRNA-21: 5’-UAG CUU AUC AGA CUG AUG U**A**G A-3’

Two-base mismatched miRNA-21: 5’-UAG CUU AUC AGA **A**UG AUG U**A**G A-3’

Three-base mismatched miRNA-21: 5’-UA**A** CUU AUC AGA **A**UG AUG U**A**G A-3’

The oligonucleotides were obtained from Sangon Biological Engineering Technology & Co. Ltd (Shanghai, China) and purified by high-performance liquid chromatography. The oligonucleotide sequences used in this study were as follows:

BHQ-H1-FAM: 5’-biotin/ TTT TTT TTT TTC AAC AT /BHQ/ CAG TCT GAT AAG CTA CCA TGT GTA GAT AGC TTA TCA GAC T/FAM-3’

H2: 5’-thio/ TTT TTT TTT TTA AGC TAT CTA CAC ATG GTA GCT TAT CAG ACT CCA TGT GTA GA-3’

Supporting Figures

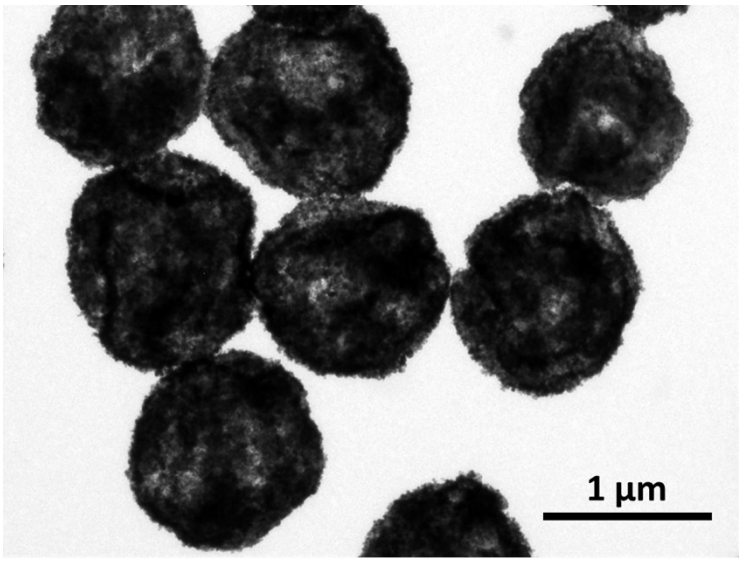

Fig. S1 TEM image of MBs.

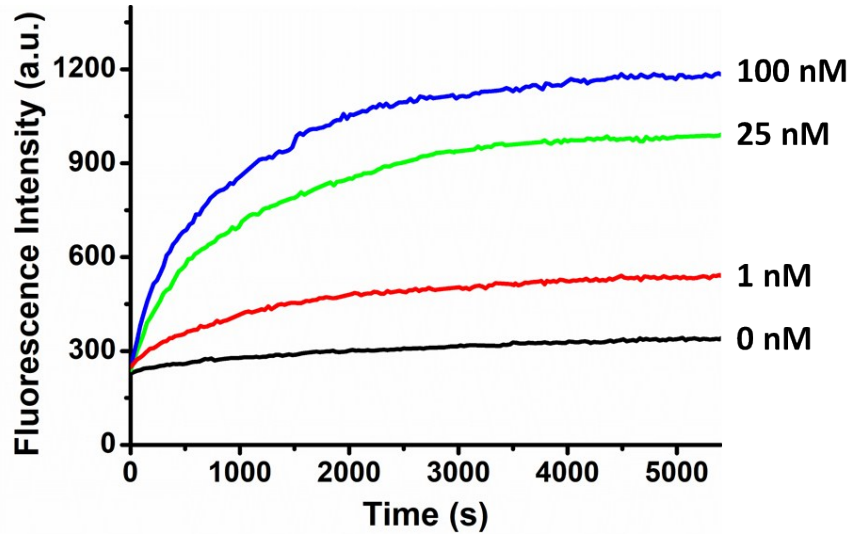

Fig. S2 Real-time monitoring of the fluorescence intensity of the 3D DNA walking machine over a period of 5400 s at different miRNA-21 concentrations (0 nM, 1 nM, 25 nM and 100 nM).

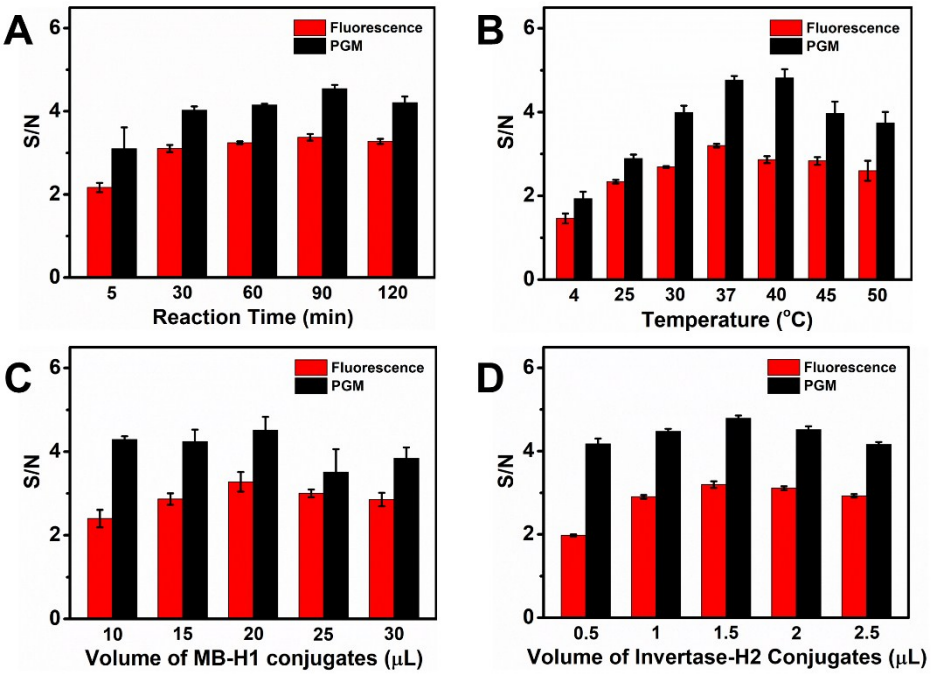

Fig. S3 Optimization of assay conditions. The signal-to-noise (S/N) ratio influenced by reaction time in the DNA walking stage (A), the reaction temperature in the DNA walking stage (B), the amount of MB-H1 conjugates (C) and the amount of invertase-H2 conjugates (D).

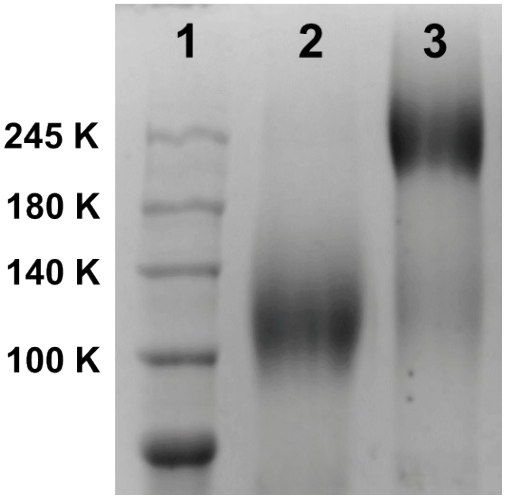

Fig. S4 Denaturing polyacrylamide gel (6%) electrophoresis image. Lane 1: marker; Lane 2: invertase; Lane 3: invertase-H2 conjugates.

Denaturing polyacrylamide gel (6%) was taken to demonstrate the successfully combination of H2 with invertase, as shown in Figure S1, Lane 1-3 represented marker, invertase, invertase-H2 conjugates, respectively. The band location of invertase was between 100K and 140K, which was consistent with the previous reports (135K).[1] After conjugation with H2, the increased molecular weight resulted the band location lagged behind, and the molecular weight was about 245 K. The molecular weight of H2 was 16K, thus each invertase was conjugated with about seven H2.

Considering the steric hindrance effect of the seven H2 for the toehold-mediated strand displacement reaction between H1 and H2, the gap between two H2 strands on the same invertase was estimated. We assumed that the invertase was a sphere with the diameter about 12 nm, and the perimeter was about 37.7 nm. The width of the duplex DNA was about 2 nm. Assuming the even distribution of seven H2 on the invertase, the gap between two H2 strands was calculated to be 3.38 nm, which had enough space for the toehold-mediated strand displacement reaction between H1 and H2.

References:

(1) Xiang, Y.; Lu, Y. Nat. Chem. 2011, **3**, 697-703.