Supplementary Information for

A Low-temperature Digital Microfluidic System Used for Protein-Protein Interaction Detection

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1. LTDMF-PPI-Box

LTDMF-PPI-Box is mainly composed of two parts, digital microfluidic system and temperature control system. The digital microfluidic system includes: DMF driven system, magnet, LTDMF-PPI chip and holder (Fig. S1). Table. S1 presents the performance parameters of TEC. Fig. S2 shows the structure and dimensions of LTDMF-PPI-Box.

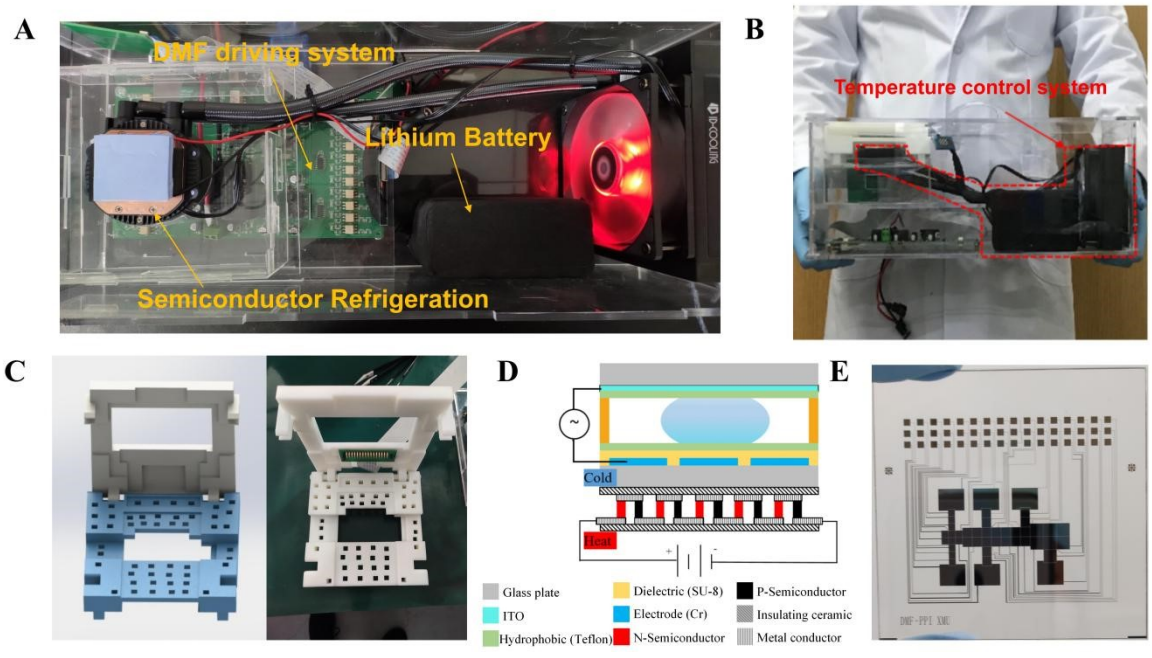

Fig. S1 (A) DMF driving system. (B) The overview of temperature control system. (C) The holder for LTDMF-PPI chip. (D) The structure diagram of the realization of LTDMF-PPI chip. (E) LTDMF-PPI chip.

Table S1 The performance parameters of the thermoelectric coolers

performance parameters	Values
Model number	TEC1-12706
Operating temperature range	-50 °C ~ 80 °C
Maximum working current /I_{max}	6.0 A
Maximum temperature difference/ ΔT_{max} ($Q_c = 0$)	68 °C
Maximum operating voltage /V_{max}	15.4 V
Maximum refrigerating power /Q_{max}	59.1 W
Internal resistance/R_i	1.0 ~ 1.1 Ω

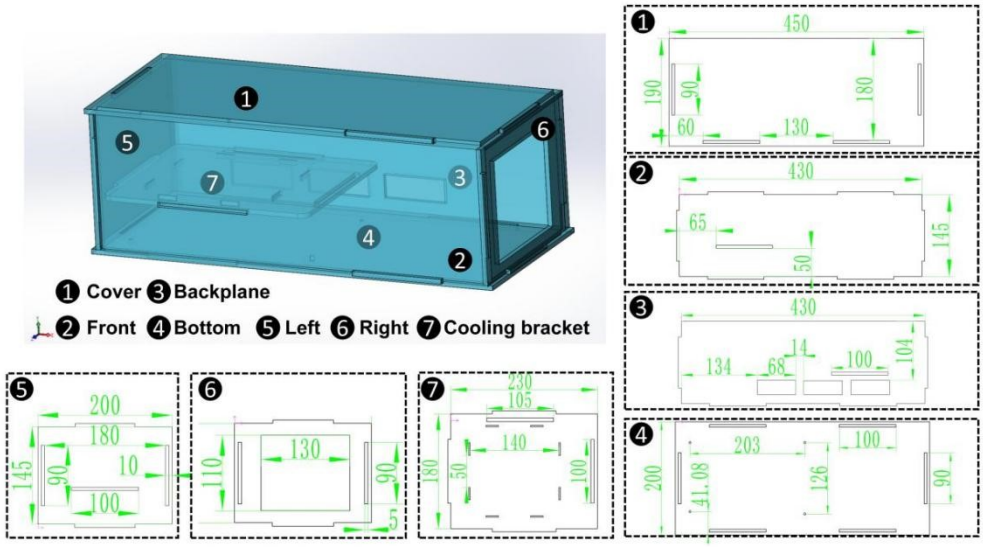

Fig. S2 The structure and dimensions of LTDMF-PPI-Box.

2. Fabrication of LTDMF-PPI chip

Fig. S3 shows the manufacturing flow chart for the bottom plate of LTDMF-PPI chip. The detailed process is described as follows:

a. A piranha solution (concentrated sulfuric acid:hydrogen peroxide = 3:1) was used to clean glass substrate.

b. A chromium layer (400 nm in depth) was grown on the glass substrate by magnetron sputtering.

c. A photoresist (AZ5214E) was coated on the chromium layer, exposed and developed.

d. The exposed chromium was etched by the etching solution.

e. After etching, the photoresist was removed by the acetone.

f. A photoresist (SU-8 2015) was coated on the substrate, exposed and post-baked.

g. The Teflon AF (~200 nm in depth) was coated on the substrate.

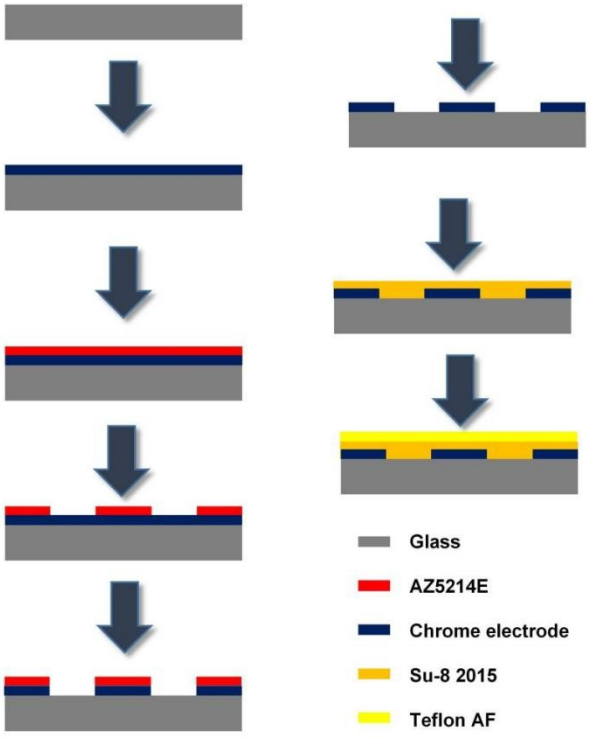

Fig. S3 Manufacturing flow chart for the bottom plate of LTDMF-PPI chip.

3. The low-temperature capability of LTDMF-PPI-Box

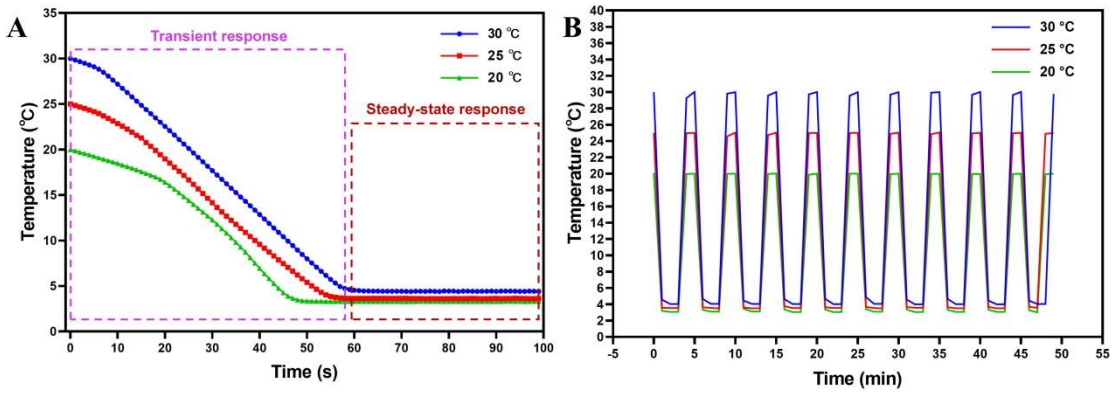

Fig. S4 The temperature curves of low-temperature control system. (A) Transient response and steady-state response, (B) Stability curve.

4. Cell transfection

Cell transfection experiments were carried out by using 293T human renal epithelial cells with high transfection efficiency. All operations were completed in a cell room. The process is described as follows:

(1) The condition of cells was observed under a microscope.

(2) 100 μL of cell culture medium without serum and 1 μg of plasmid vector were added to the centrifuge tube and shaken to mix.

(3) 100 μL of cell culture medium without serum and 5 μL of PEI transfection reagent were added to another centrifuge tube, and shaken to mix.

(4) After standing the above two centrifuge tubes for 5 min, added the PEI suspension into the plasmid carrier suspension and let it stand for 20 min.

(5) Replaced the medium of the transfected cells with a serum-free medium, and then added the transfection mixture into the cell culture dish.

(6) The cells were cultured in an incubator for 8 h and then replaced with the complete medium. Then confirmed the transfection efficiency of the cell under the fluorescence microscopy.

5. Optimization of cell lysis time for various cell types

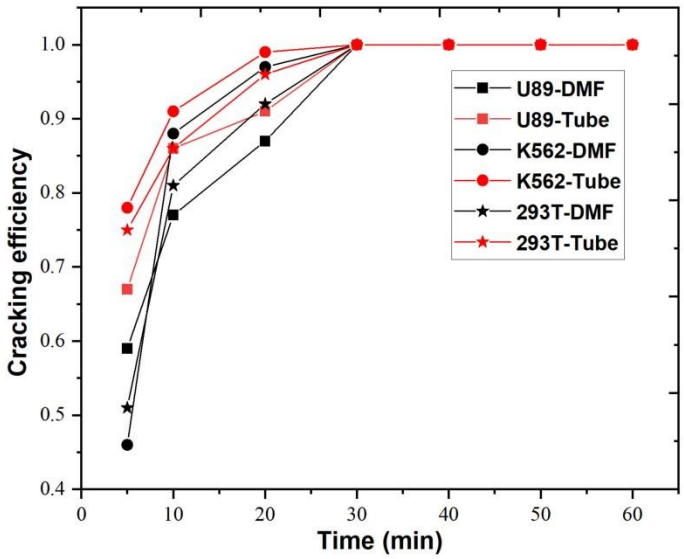

Fig. S5 The lysis efficiency of U89, K562 and 293T on LTDMF-PPI

6. Co-immunoprecipitation

Co-immunoprecipitation is a classic method to study PPI, which bases on the specific binding between antibodies and antigens. It is also the most convincing method in routine experiments. Beads used in Co-immunoprecipitation are usually protein A/G, which are not magnetic and cannot be used in LTDMF-PPI chips. The specific process of the improved immunoprecipitation method is described as follows:

(1) The fusion protein Myc-Rab26 and GFP-RILP were expressed in 293T cells by transfection experiment.

(2) The cells were lysed to obtain Myc-Rab26 protein and GFP-RILP protein, and then the 5% lysis mixture was used as control.

(3) The lysis mixture containing the fusion protein Myc-Rab26 and GFP-RILP were mixed with anti-Myc coated magnetic beads at 4 °C for 8 hours.

(4) The magnetic beads were washed 3 times with washing buffer.

(5) 20 μL SDS loading buffer were added in the above magnetic beads, and mixed for 5 minutes at 95 °C. Then western blot was carried to confirm whether there was an interaction between the two protein molecules.

7. Repeated experimental results

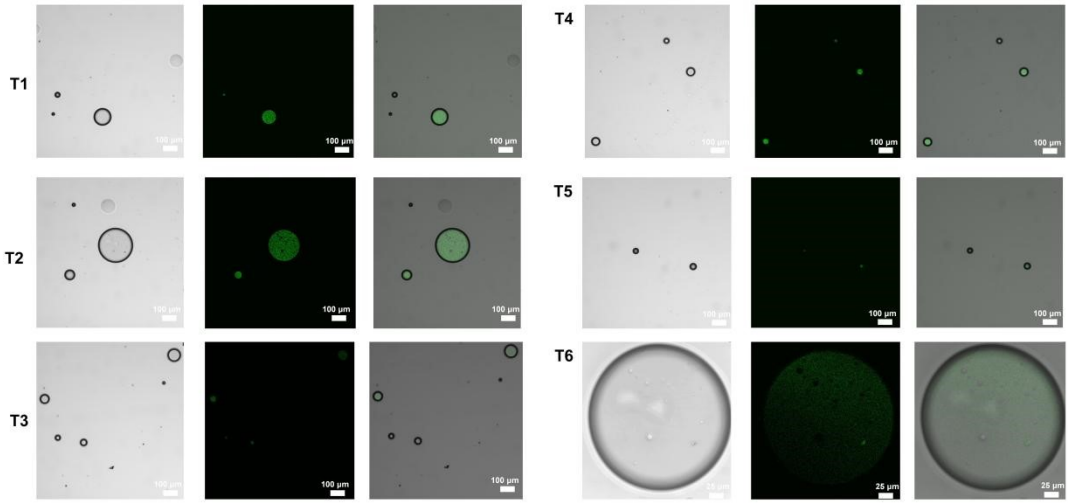

Fig. S6 Images on the interaction between Myc-Rab26 and GFP-RILP protein on LTDMF-PPI chip. (Left column: images from bright field, Middle column: fluorescent images, Right column: overlapped images)