Continuous Flow Microfluidic-MS System for Efficient OBOC Screening

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1. Fabrication process of the micro well array

From a functional point of view, the microwell array chip could be divided into two parts, the PDMS channel and the micro-well array chip with electrodes. As shown in Scheme S1, To fabrication the micro-well array chip, a 4-inch (100) silicon wafer bonded with a 4-inch Pyrex7740 (Corning Inc., USA) glass wafer was used as substrate. The silicon wafer acted as structure layer, while the glass wafer acted as strength layer. The thicknesses of both silicon and glass layer were 500 µm, and the total thickness of micro-well array chip was about 1 mm which fulfilled the requirement of Bruker ULTRAFLEXTREME mass spectrometer. The thickness of microwell array chip could be easily modified to fit the demands of different mass spectrometer. Using a sputtered aluminium layer (200 nm thick) as mask, micro-wells were formed by ICP (induced couple plasma) etching. The geometric parameters of micro-wells in Fig. 1 were 40 µm×40 µm, and size of micro-well could be varied by redesigning the photo-mask of lithography to fit the sizes of different micro-beads. After removing the aluminium layer by wet etching, a gold layer (300 nm) and a chrome layer (30 nm) were sequentially coated on the silicon wafer as the electrodes for mass detection. Gold was used because it has high conductivity and good chemical stability. Chrome was served as adhesive materials. To facilitate a silicon-PDMS bonding with good strength, the silicon surface of bonding area should be exposed. Therefore, another lithography process was employed to pattern the Au/Cr layer to cover the micro-well only. A thick photoresist (AZ4620, AZ Materials, Germany) was used to cover the side step of micro-wells. For the fabrication of PDMS channel, a silicon wafer was firstly wet etched as mold. In more detail, we used a 4-inch (100) silicon wafer. A 200 nm aluminium layer was coated by sputtering and patterned by lithography. Using aluminium layer as mask, the silicon wafer was etch by ICP. After cleaning the silicon wafer by acetone and alcohol, PDMS (polydimethylsiloxane) was cast into the mold and heated to 70 ºC for 1 h before it was peeled off and punched to create the inlet and outlet holes. Finally, both micro-well chip and PDMS channel were diced with the same size. After oxygen plasma treatment, the PDMS channel and glass substrate were bonded together to form the microfluidic chip.
Scheme S1. Fabrication process of the micro well array

2. Solid phase synthesis of the OBOC peptide library towards AHA
Fmoc strategy SPPS (solid phase peptide synthesis) was employed for synthesis of the three peptides. Tentagel Resin (Rapp Polymere, Germany, loading 0.26 mmol/g) is used as the solid phase support. Scheme S1 showed the detail synthesis process. Five kind of amino acid reagents: Fmoc-Tyr(tBu)-OH, Fmoc-Pro-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Val-OH, Fmoc-Ala-OH were employed in each synthesis cycle to construct a library with a capacity of $7 \times 10^4$. In scheme, ANP is the abbreviation for 3-Amino-3-(2-nitrophenyl) propionic acid, NMM is the abbreviation for N-Methyl morpholine, DMF is the abbreviation for N,N-dimethylformamide, TFA is the abbreviation for Trifluoroacetic acid and SP is stand for side chain protecting group.

3. Design and optimization of the peptide beads sorting flow rate

Optimization of the flow rate was also carried out. Five flow rates 30 µL/min, 40 µL/min, 50 µL/min, 60 µL/min and 70 µL/min were tested. It indicated that when the flow rated was set at 60 µL/min, the positive ratio is 70% (Fig. S1a) and the while there is not any false negative (Fig. S1b). So 60 µL/min was chosen as the optimized flow rate which is...
accord with the simulated result. When the negative beads were flow through, the sheath flow rate A was set as 10 µL/min and the sheath flow rate B was set as 60 µL/min so that the negative beads will get out through the negative outlet. On the contrary, the positive beads will flow through the microwell area in the downstream and trapped in the wells. The simulation of the velocity field was processed with the software COMSOL (Fig. S1c).

Figure S1. Simulation and optimization of the peptide beads sorting flow rate (a) Positive ratio optimization of the flow rates (b) Negative ratio optimization of the flow rates (c) Velocity field simulation by the software COMSOL

4. Design and optimization of the microwell sizes in the array

To prevent the exiting of the beads, we had tried microwells with different sizes (Fig. S2) The optimized size (40 µm×40 µm) fitted the peptide beads quite well. Under this condition, once the beads were dropped into the wells, they were hard to exit.
Figure S2. Optimization of the microwell sizes in the array (a) 30 µm×30 µm (b) 40 µm×40 µm (c) 50µm×50 µm (d) 60µm×60 µm

5. SPRi detection of the four affinity peptides towards APN

SPRi is a label-free, real-time method to detect molecular interactions on metal-coated microarray surface. SPRi analysis was performed on a Plexera PlexArray® HT system (Plexera LLC, Bothell, WA) using bare gold SPRi chips (Nanocapture® gold chips, with a gold layer of 47.5 nm thickness). Purified peptides were adsorbed onto the gold chip surface and then incubated in 4 °C overnight in a humid box. The SPRi chip was washed and blocked using 5% (m/v) non-fat milk in PBS overnight before use. The SPRi analysis procedure follows the following cycle of injections: running buffer (PBST, baseline stabilization); sample (four or five concentrations of the protein, binding); running buffer (PBST, washing); and 0.5% (vol/vol) H₃PO₄ in deionized water (regeneration). Protein (AHA or APN) was diluted with PBST to concentrations of 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL and 0.625 µg/mL (For protein AHA, this concentration have not been carried out.) Real-time binding signal were recorded and analyzed by PlexArray HT system.