Supplementary Material (ESI) for Lab on a Chip

Integrated SPPS on Continuous-flow Radial Microfluidic Chip

Weizhi Wang, Yanyan Huang, Jizhong Liu, Rui Zhao*, Shaoxiang Xiong, Guoquan Liu, Yi Chen and Huimin Ma

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Zhongguancun North First Street 2,100190 Beijing, PR China. Tel: 86 10 62557910; Fax: 86 10 62559373; E-mail: zhaorui@iccas.ac.cn

1. Design and fabrication of the tri-row cofferdam-fence structure

In the first place, the whole glass chip was protected by adhesive tapes except the area of the reaction chamber. After the 160-min etch of the chamber area, the adhesive tapes were torn off. Then, the tri-row fence area was locally protected and the rest of the chip figure was etched. After the 100-min etch, the chip was uncovered and an additional 15-min etch of the whole chip was carried out. Stepwise etch led to different etching times on different areas, a glass substrate with a reagent channel depth of 60 μm, a reaction chamber depth of 150 μm and a fence area depth of 8 μm was eventually achieved. Because of the different etching depths on and off the fence area, a cofferdam-fence structure was shaped.

2. Flow rate selection based on Reynolds number

In the microfluidic condition, Reynolds number is an important character for evaluating of the stably stratified flow. When Reynolds number is smaller than 1, viscosity flow was fully developed and mixed flow was avoided as far as possible. The channel shape in glass-based microfluidic devices is trapezoidal. Usually, the trapezium channel can be approximated to a rectangular slot and the flow rate in microchannel can be expressed by

$$\mathrm{Re} = \frac{\rho(4(A/P))v}{\mu} \tag{1}$$

where ρ is fluid density, A is device cross-sectional area, P is wetted perimeter, v is average velocity, and μ is absolute viscosity. After the parameters of the fabricated continuous flow channels were substituted into the equation (1), the flow rate of less than 2.8 μL min^{-1} was obtained when Reynolds number was smaller than 1. On the other hand, the reaction chamber of the chip owned a volume of approximate 2 μL. Considering the fluid status and the chamber volume, a reaction flow rate of 2 μL min^{-1} was selected in the continuous-flow microfluidic SPPS to make the reagents contact and react with the functional groups on the polymer beads throughly and efficiently.

3. Optimization of on-chip coupling and cleavage time

SPPS system was constructed on the microfluidic chip aiming to obtain peptides with high qualities. Since the products amount was sufficient for the follow-up research, we concerned over good purity rather than absolute yield of the peptide products achieved within the shortest possible time. For the coupling procedure, four reaction times of 10, 20, 30 and 40 min were tested, respectively. For the cleavage procedure, four reaction times of 20, 30, 60 and 120 min were further tested. Coupling time of 20 min and cleavage time of 30 min gave good purities and were selected as the optimum. Fig. S1 shows the optimum selection of the coupling time and cleavage time.

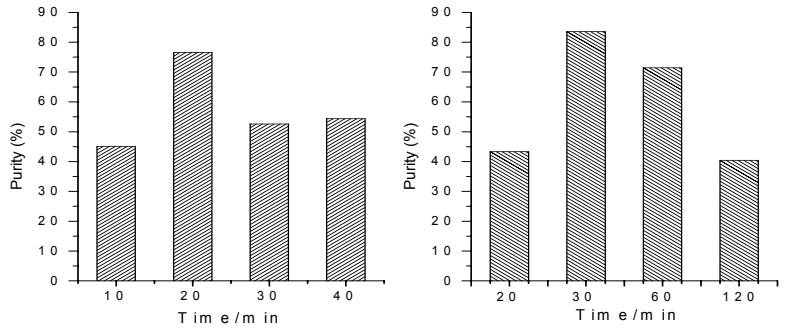

Fig. S1 Optimization of reaction time (a) Selection of coupling time (b) Selection of cleavage time

4. HPLC chromatograms of the radial microfluidic synthesized peptides

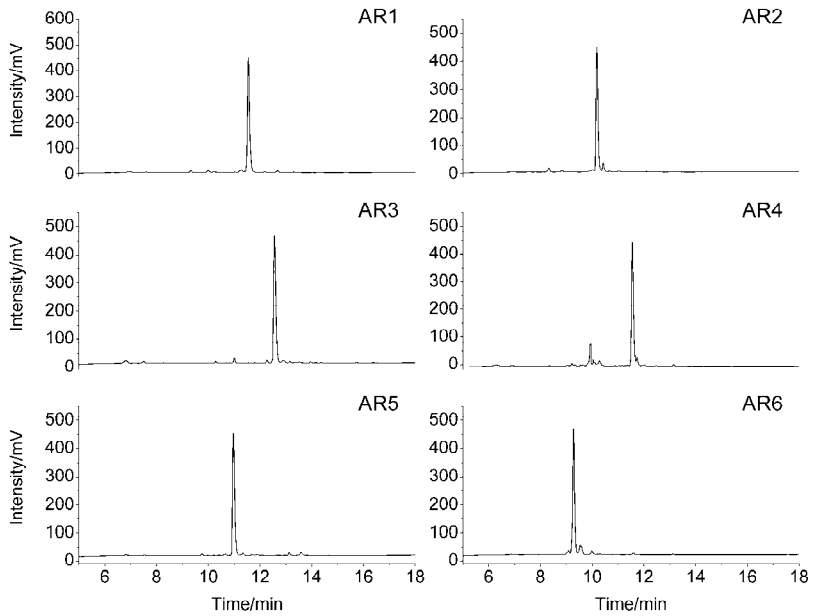

Fig. S2 RP-HPLC chromatograms of hexa-channel radial chip synthesized peptides, Column: TSK-gel ODS-100V (150 mm × 4.6 mm i.d.); Gradient: 0–20–25 min, 5–80–80% aqueous acetonitrile containing 0.1%TFA; Flow rate: 1 mL min^{-1}; UV: 220 nm; AUFS: 0.01

5. MALDI-TOF mass spectrogram of the chip synthesized peptides

5.1 Mass spectrogram of the monochnnel chip synthesized peptides

Leucine-enkephalin synthesized on the mono-channelchip was characterized. As shown in Fig. S3, signals were observed in the spectrum at m/z = 556.4，578.4 and 594.3 which matched $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ of YGGFL respectively.

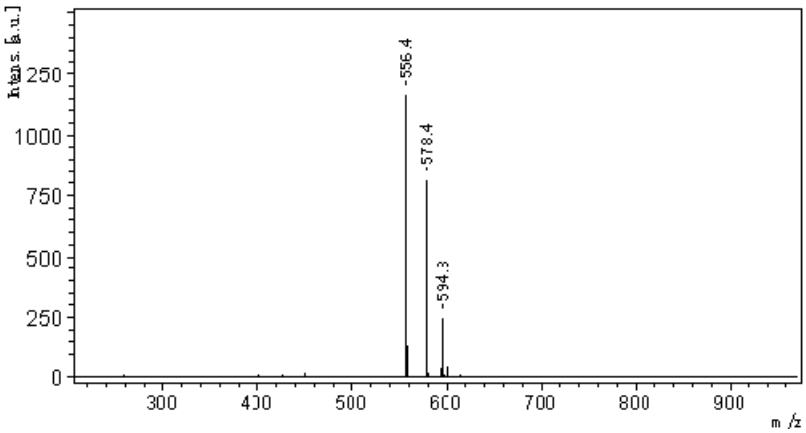

Fig. S3 MALDI-TOF mass spectrogram of monochnnel-chip-synthesized leucine-enkephalin

5.2 Mass spectrograms of the radial chip-synthesized peptides

The six peptides synthesized on the hexa-channel radial chip were characterized by MALDI-TOF MS. As the MS spectrogram shown in Fig. S4, for product AR-1, peaks were m/z =657.3, 679.3 and 695.3 which matched $[M+H]^+$, $[M+Na]^+$and $[M+K]^+$ of YGAFLS. For product AR-2, peaks were m/z =544.2, 566.2 and 582.1 which matched $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ of YGAFS; For product AR-3, peaks were m/z =570.4 and 608.1 which matched $[M+H]^+$ and $[M+K]^+$ of YGAFL; For product AR-4, peaks were m/z =643.3, 665.3 and 681.3 which matched $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ of YGGFLS; For product AR-5, peaks were m/z =457.1, 479.1 and 495.1 which matched $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ of YGAF; For product AR-6, peaks were m/z =510.2, 532.2 and 548.2 which matched $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ of YGALS. These results clearly testified the success of the on chip SPPS synthesis in the hexa-channel radial chip system.

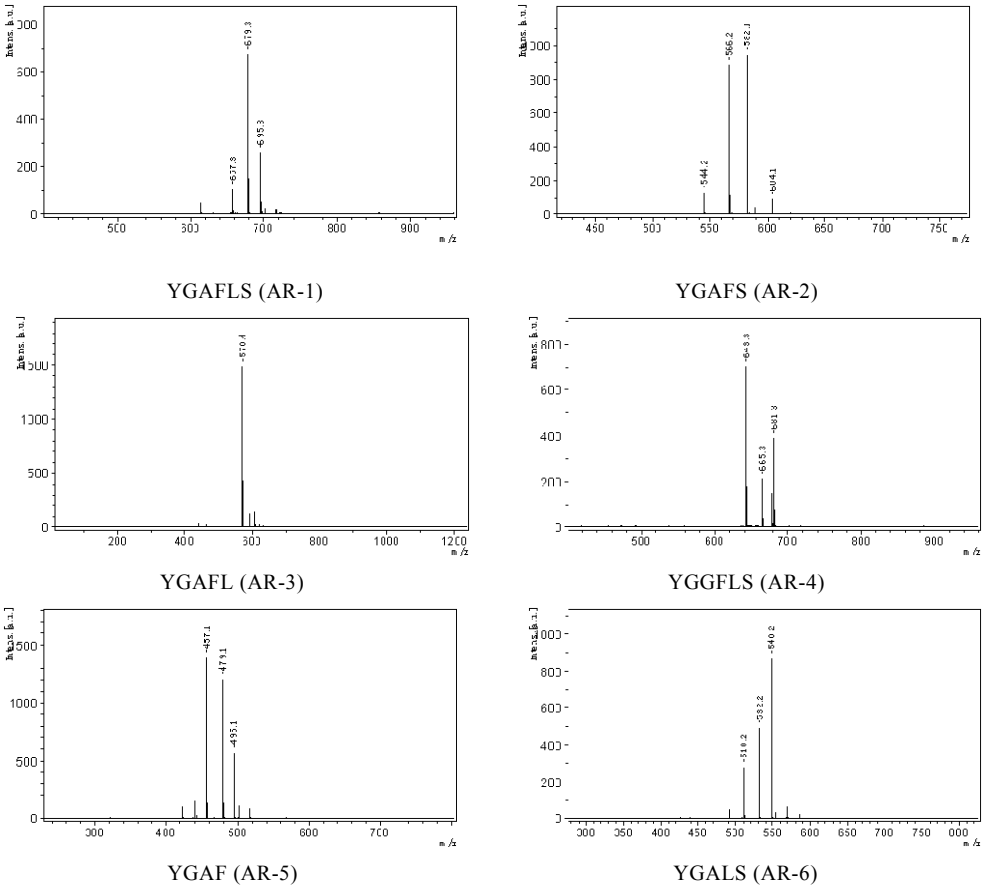

Fig. S4 MALDI-TOF mass spectrograms of hexa-channel radial chip synthesiaed peptides