Supplementary Material (ESI) for Lab On A Chip

An automated Teflon microfluidic peptide synthesizer

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1. Fabrication of whole Teflon microfluidic synthesizer

The fabrication of the chip included three parts: fabricating pattern on glass, converting pattern to PFA sheets and contructing the whole chip.

Fabricating pattern on glass was performed in following steps. SG2506 glass was used as the substrate, which is coated chromium and resists respectively (Resist, S-1805; Thickness of chrome, 145 nm; Thickness of resist, 570 nm). Ultraviolet lithography was applied on the substrate and positive pattern on resist was shown after developing resist. Wet etching of chrominum and glass were taken in turn to fabricate the glass mould which has positive pattern on glass. The glass mould was washed to remove residual resist and chrominum before nano-imprinting.

Patterns on glass were converted to PFA sheets by nano-imprinting. Different pressure were tested from 5 N/cm^2 to 50 N/cm^2, and the optimized pressure with best result was 20 N/cm^2. Imprinting temperature and time were also tested and optimized. The final protocal to pattern PFA sheets was curing PFA sheets in 285 ^{o}C with a pressure of 20 N/cm^2 for 10 minutes. It was especially important to cool the wafer slow enough, otherwise, PFA sheets would be obviously deformed because of internal stress.

The whole chip was constructed by thermo bonding of the three layers: fluidic layer, PFA film and control layer. The three layers were aligned carefully with the help of stereo microscope. Aligned PFA sheets were placed in clamped stainless steel fixture and bonded in 260 ^{o}C for 4 hours. Adhesive tape made of polyimide or PTFE was helpful to prevent sliding of PFA sheets because of low friction force on the surface of the material.

2. SPPS procedures on whole-Teflon microfluidic chip

The SPPS procedure on whole-Teflon microfluidic chip is shown as Fig. S1. First, Fmoc-AA-HMP resins (1a) was injected into the reaction chamber. In the deprotection step, Deprotection reagent of 20% piperidine in DMF was injected into the chip from main inlet at a flow rate of 1 μL min^{-1} for 5 minutes. After deprotection, a subsequent 6-minute DMF washing step was performed at a flow rate of 4 μL min^{-1} and the product 1b was obtained. Then, Fmoc-AA-OH and coupling reagent HBTU

re-dissolved in activating reagent (0.4 mol L^{-1} NMM in DMF solution) was injected into the chip from the comb-like inlet for 20 min at a flow rate of 1 μL min^{-1} and Fmoc protected dipeptide 2a was obtained. Those Amino acid solutions were stored in reservoirs and injected by a syringe pump. When one amino acid reagent was loading, only the corresponding microvalve was turned on (no pressure) and the target reagent will be injected into the channel while the other valves were turned off (pressure is 10 psi) to prevent other reagents into the reaction chamber. Solenoid valves were used to turn on microvalves (provide pressure) or turn off microvalves (remove pressure). After the reaction, the washing step was repeated. Then another deprotection procedure was carried out to initiate a new cycle. When the peptide elongation was completed, the system solution was replaced by DCM and then by methanol and product 3a was obtained. Both of the above process lasted for 5 min at a flow rate of 4 μL min^{-1}. On-chip peptide cleavage was carried out subsequently. Continuous-flow cleavage reagent (95% TFA, 2.5% TIS, 2.5% H_2O) was injected through the main inlets for 20 min at a flow rate of 2 μL min^{-1} and the peptide (c) solution was received from the outlet.”

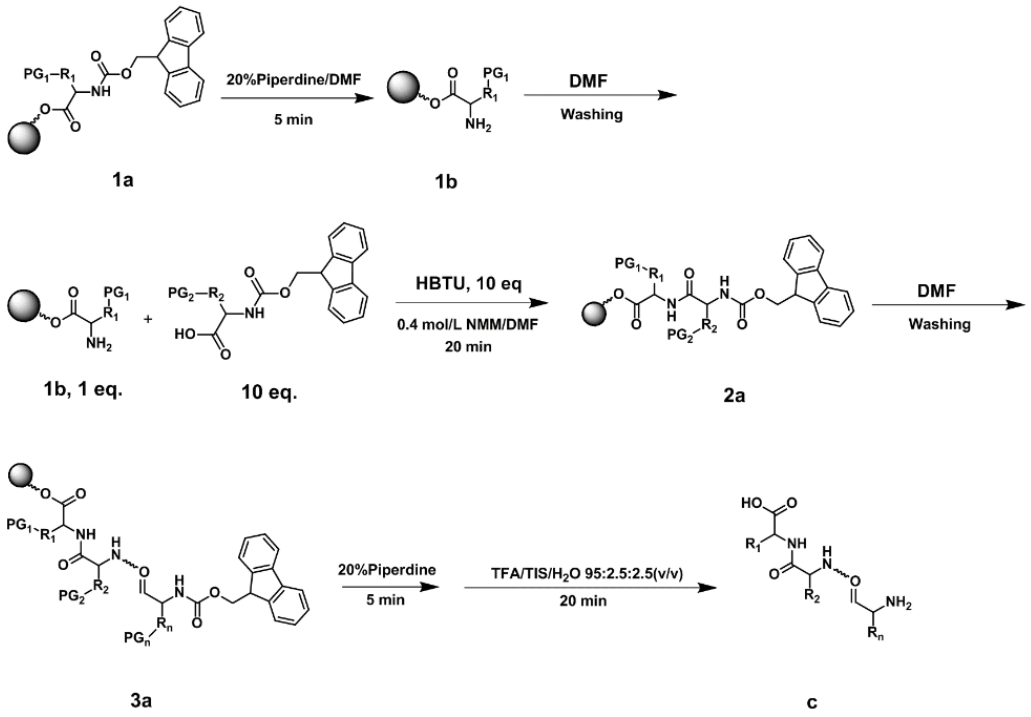

Fig. S1 Reaction procedures of the SPPS cycle

3. The preparation of the novel HMP solid support and the attachment of the flirt amino acid

One-step swelling polymerization method was introduced to prepare the solid phase supports for peptide synthesis in microfluidic chips. Polystyrene (PS) microspheres were firstly synthesized as seeds. [1]*4*-Vinylbenzyl chloride (2.5 mL) was used as monomer. DVB (2.5 mL) was employed as cross-linker. AIBN (0.1 g) was introduced as initiator. All reagents were emulsified and dropwise suspended in 120 mL of aqueous solution containing PS seeds (1 g), PVA (1.2 g, as stabilizer) and SDS (0.12 g,

as surfactant). Toluene (3.75 mL) and octanol (1.25 mL) were added into the mixture as porogens. Seeds were swollen at 30 ^{o}C for 24 hrs and then the polymerization was carried out at a temperature gradient of 55-60-65-70 ^{o}C for every 3 hrs. The obtained beads were extracted by acetone to remove the porogens. *4*-hydroxybenzyl alcohol (0.55 g) linker was attached to the microbeads in DMA (15 mL) solution with sodium methylate (0.24 g). The beads were washed with *1,4*-dioxane and water successively. HMP Resins were obtained. (Fig. R2)

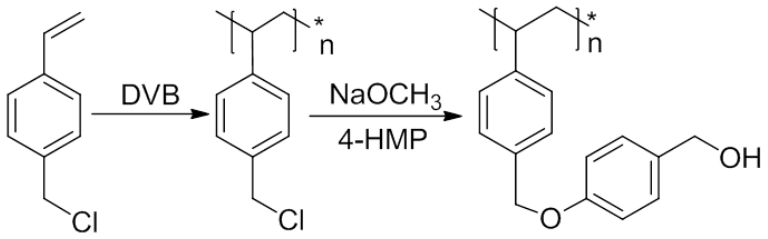

Fig. S2 Preparation of the solid and porous HMP Resin

0.8 g HMP resins were added into a 10 mL flask and suspended in DMF. 5 eq. Fmoc-amino acid (Fmoc-AA-OH) was dissolved in 5 mL dry DCM. 5 eq. DCC was added into the above mixture. The reaction was taken place at 0 ˚C for 20 min and remove DCM in rotary evaporator at room temperature. Resins with DMF mentioned in the fiast step were added in the flask following 1 eq. DAMP. The mixture was reacted for 1 hour at room temperature and the resin was washed with DMF and methanol, respectively. In accordance with the above-described method Fmoc-Lys-HMP resin and Fmoc-Ser-HMP resin were prepared. (Fig. R3). The loading of the amino acid is determined by deprotection and Fmoc absorption using an ultraviolet spectroscopy as the literature mentioned[2]. ”

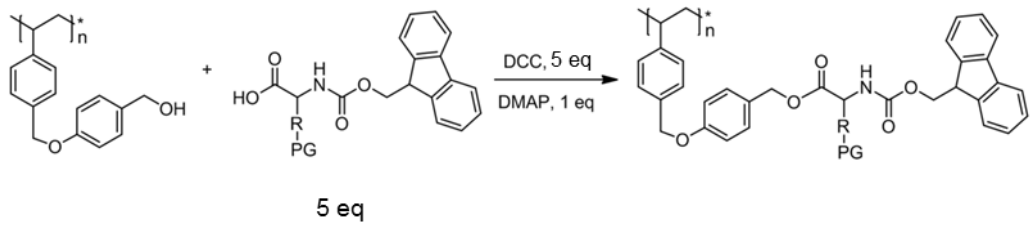

Fig. S3 Attachment of the first amino acid

4. Optimization of on-chip coupling and cleavage time

SPPS system was constructed on the whole-Teflon microfluidic chip aiming to obtain peptides integrately and automaticly. 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, five reaction times of 10, 15, 20, 25 and 30 min were tested, respectively. Parallel experiment were done and the final peptide product showed an average purity of 88.4% when the coupling time is 20 min with RSD=1.9%-3.2% (n=3). For the cleavage procedure, five reaction times of 10, 20, 30, 40 and 50 min were further tested. Parallel experiment were done and the final

peptide product showed an average purity of 89.3% when the cleavage time is 20 min with RSD=0.4%-2.4% (n=3). Coupling time of 20 min and cleavage time of 20 min gave good purities and were selected as the optimum. Fig. S1 shows the optimum selection of the coupling time and cleavage time.

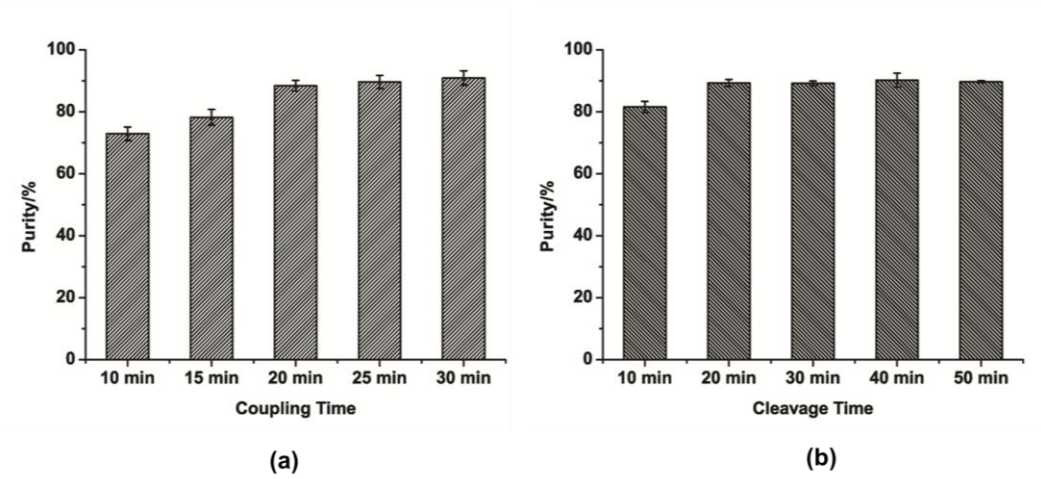

Fig. S4 Optimization of reaction time (a) Selection of coupling time (b) Selection of cleavage time

5. Synthesis of a model difficult sequence owns multi-aromatic amino acids

Another model peptide owns many aromatic amino acids was synthesized and the sequence is HYYYYYYYYY. This peptide has repetitive hydrophobic unit can be called as a difficult sequence in vessel peptide synthesis. However, in the microfluidic synthesis, the reaction is more efficiency. As shown in Fig 4, the crude product has a purity of 73.7% and a yield of 79.2% even the reaction condition is the same as that of the common peptide.

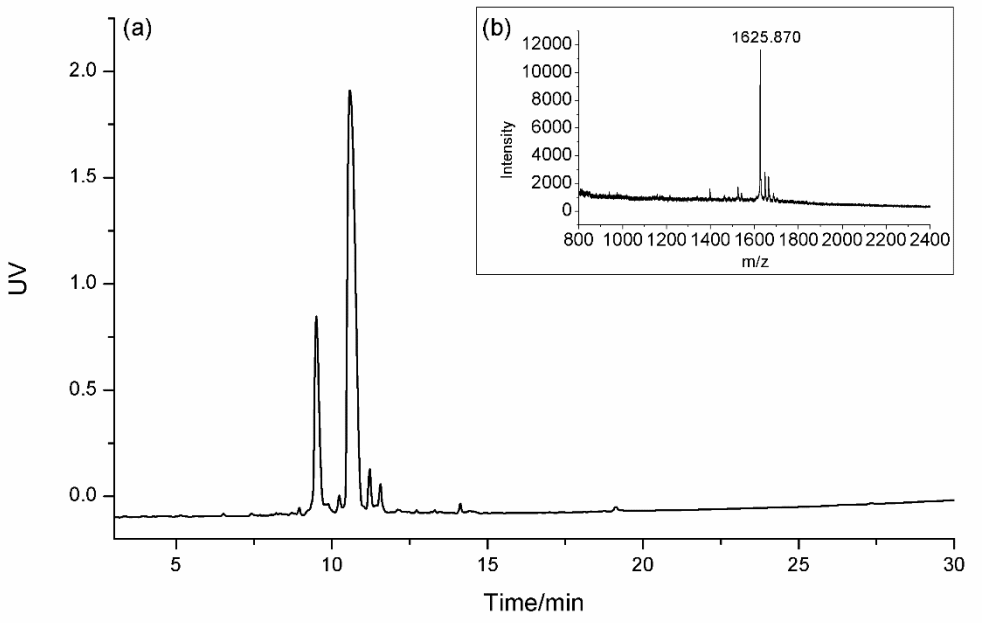

Fig.S5 Characterization of the on-chip synthesized decapeptide HYYYYYYYYY (a) RP-HPLC chromatograms of

on-chip synthesized HYYYYYYYYY (d) MALDI-TOF-MS of on-chip synthesized HYYYYYYYYY. Chromatography column: ODS-100V (3μm) HPLC column; Gradient: 0–30–35min, 5–80–80% aqueous acetonitrile containing 0.1%TFA; Flow rate: 1 mL/min, UV: 220 nm, AUFS: 0.01

References

1 A. J. Paine, W. Luymes and J. McNulty, *Macromolecules*, 1990, **23**, 3104-3109.

2 Solid phase peptide synthesis, Nova Biochem, Synthesis Note, S37.