

A Tetra-layer Microfluidic System for Peptide Affinity Screening through Integrated Sample Injection

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1. Solid phase synthesis and characterization of candidate peptides

Six candidate peptides, i.e. YGGFL, YGGFE, YGGFK, YGGFF, YGGFS and YGGFG were synthesized by solid-phase Fmoc strategy (Figure S-1). All the peptides were analyzed using RP-HPLC and MALDI-TOF-MS. As shown in the chromatogram in figure S-2, the purities of the six peptide are SP-L 91.4%, SP-E 93.2%, SP-K 92.3%, SP-F 90.6%, SP-S 91.7% and SP-G 94.1%, respectively. The six peptides synthesized were characterized by MALDI-TOF MS. Table S-1 shows the peaks of each peptide in the MS spectrogram.

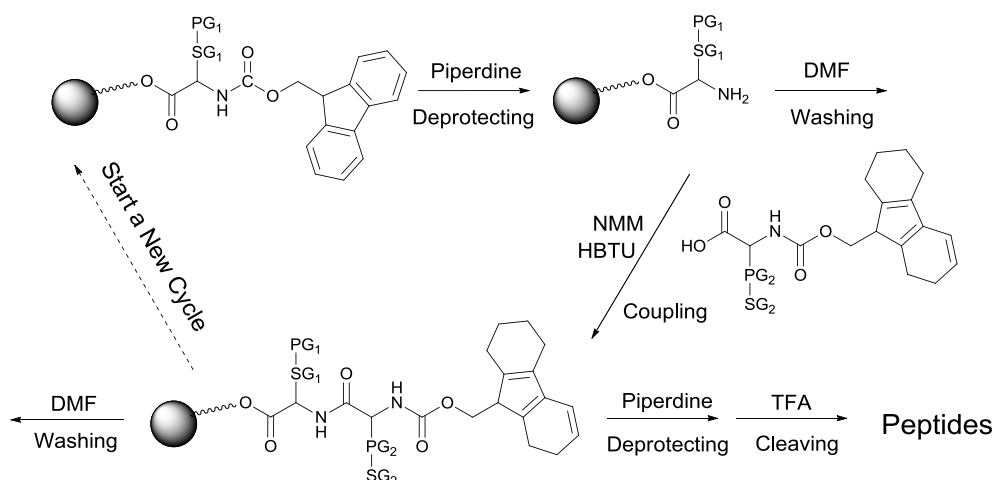


Figure. S-1 Scheme of Fmoc SPPS cycle

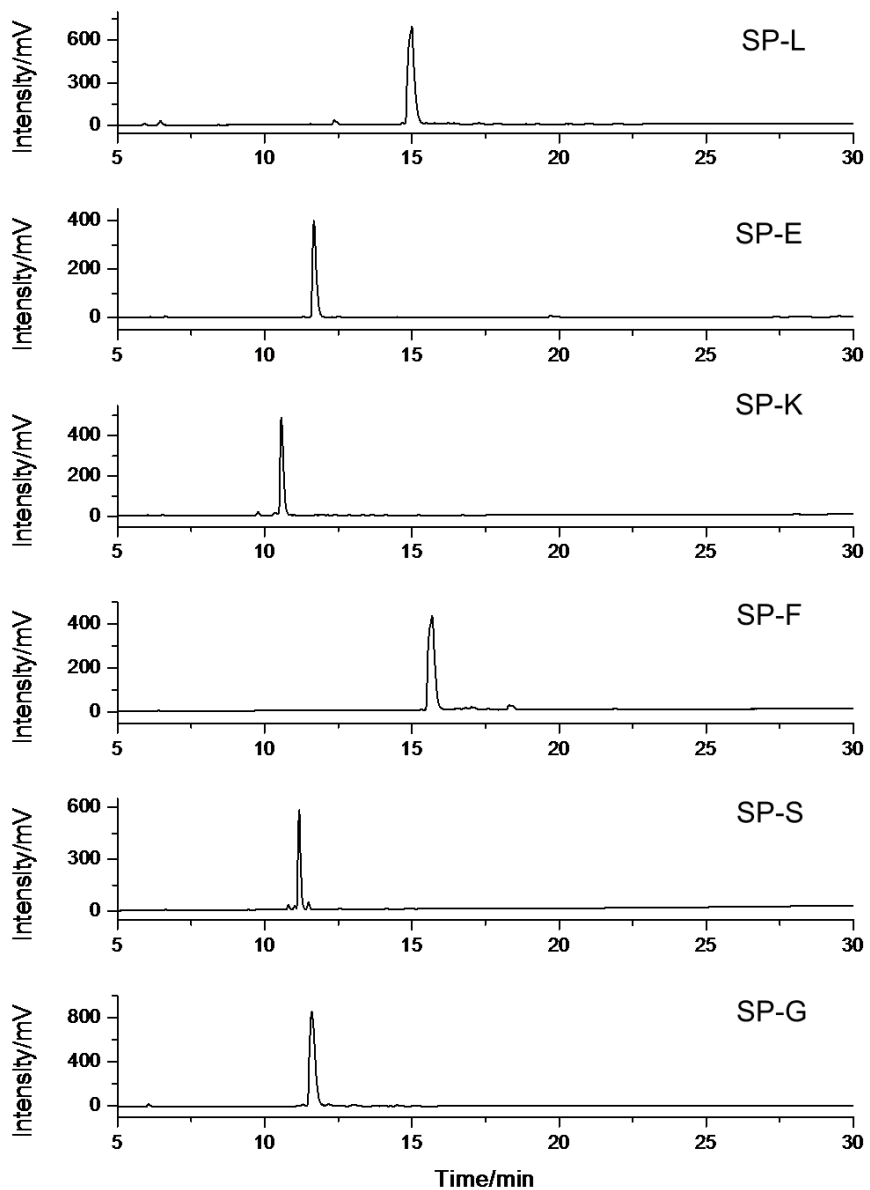


Figure. S-2 RP-HPLC chromatograms of Six candidate peptides, Column: TSK-gel ODS-100V (150 mm × 4.6 mm i.d.); Gradient: 0–30–35 min, 5–80–80% aqueous acetonitrile containing 0.1%TFA; Flow rate: 1 mL min⁻¹; UV: 220 nm; AUFS: 0.01

Table S-1 Peaks of each candidate peptide in the MS spectrogram

Peptide	Sequence	M.W	MALDI-TOF-MS detection m/z		
			M+H ⁺	M+Na ⁺	M+K ⁺
SP-L	YGGFL	555.3	556.4	578.4	594.4
SP-E	YGGFE	571.2	572.4	594.4	-
SP-K	YGGFK	570.3	571.4	-	-
SP-F	YGGFF	589.3	-	612.5	628.5
SP-S	YGGFS	529.2	530.3	552.4	568.3
SP-G	YGGFG	499.2	500.1	522.1	-

2. Peptide conjugation with the magnetic beads and detection of the peptides loadings

The six candidate peptides were further conjugated to the magnetic beads respectively through the NMM/HBTU method (Figure S-1). The loadings of the peptides on the magnetic beads were calculated by comparing the peptide concentration in the reaction solution before and after conjugation. The loadings of the magnetic beads were shown in table S-2.

Table S-2 Detection of the peptide loading on the magnetic beads

Candidate peptide	M.W.	Loading($\mu\text{mol/g}$)
SP-L	555.3	16
SP-E	571.2	16
SP-K	570.3	15
SP-F	589.3	20
SP-S	529.2	14
SP-G	499.2	18