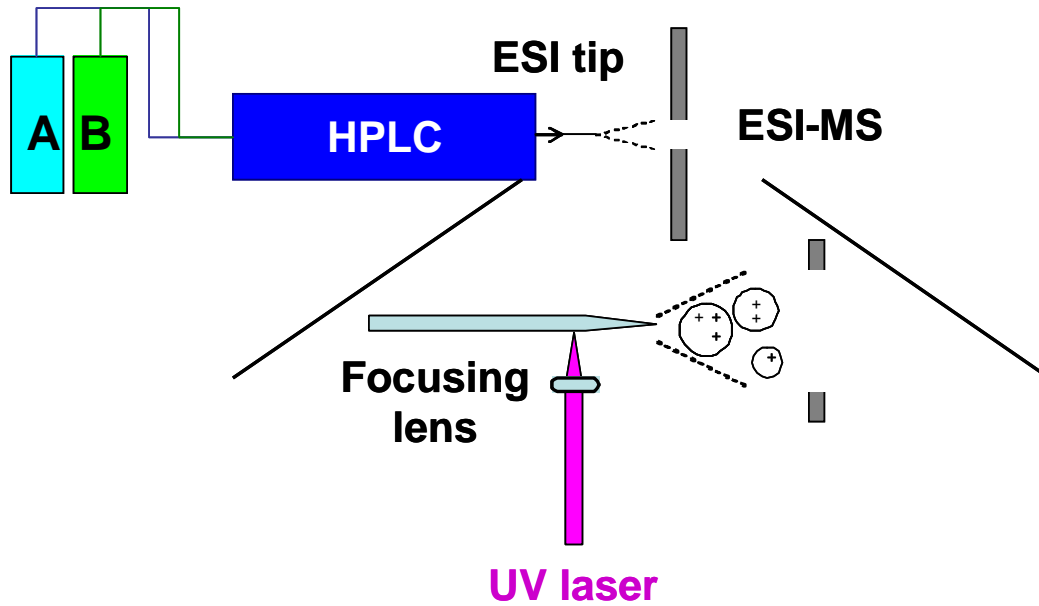
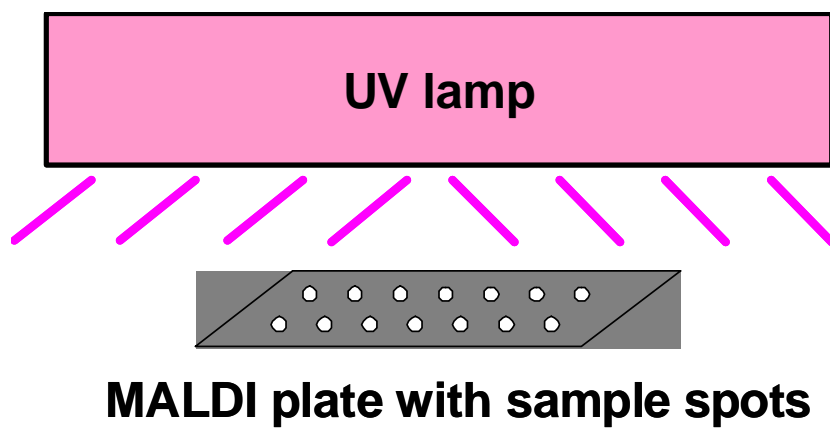


S. Figure 1. Instrument set up

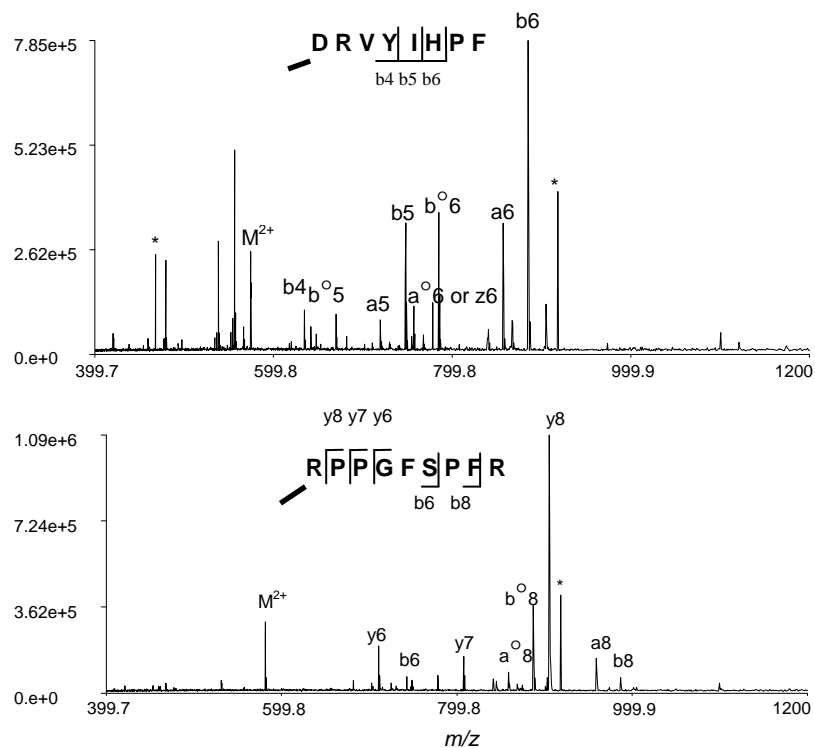


(a) On-line photocleavage with UV laser

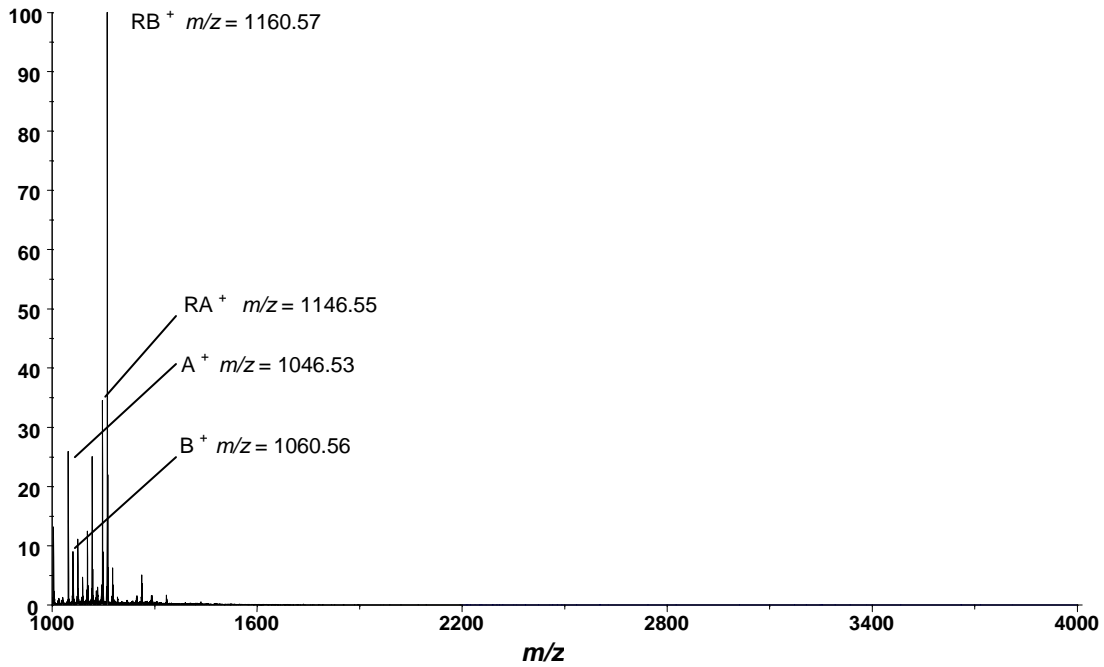
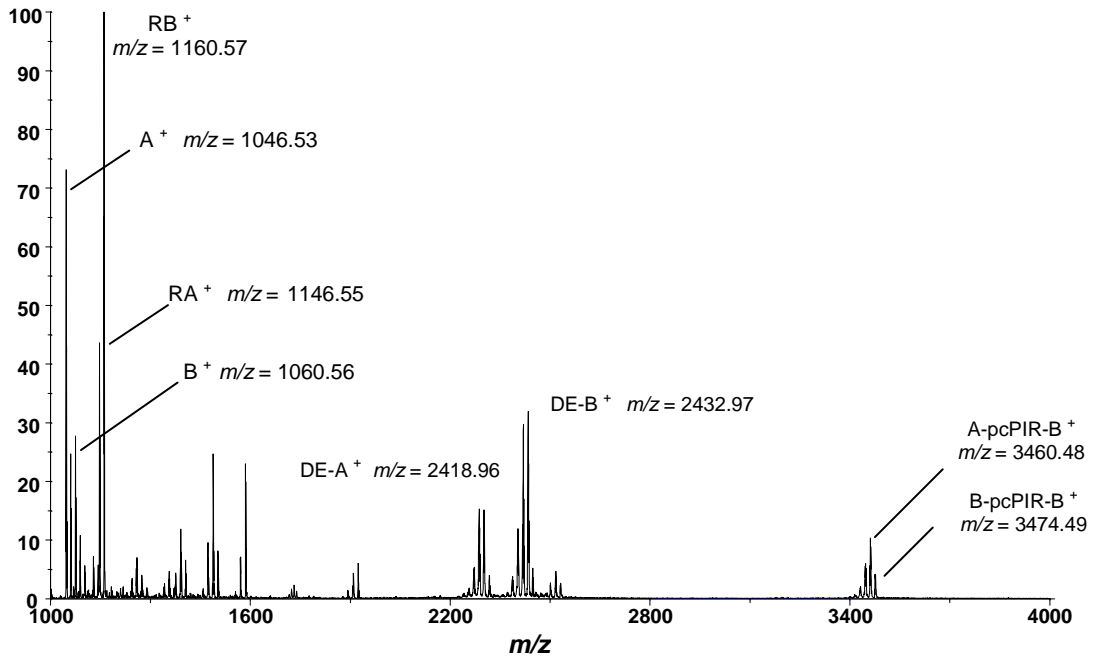


(b) Off-line photocleavage with UV lamp

S. Figure 2. MS/MS spectra of released Angiotensin II (upper) and released Bradykinin (lower) from on-line photocleavage-ESI experiment



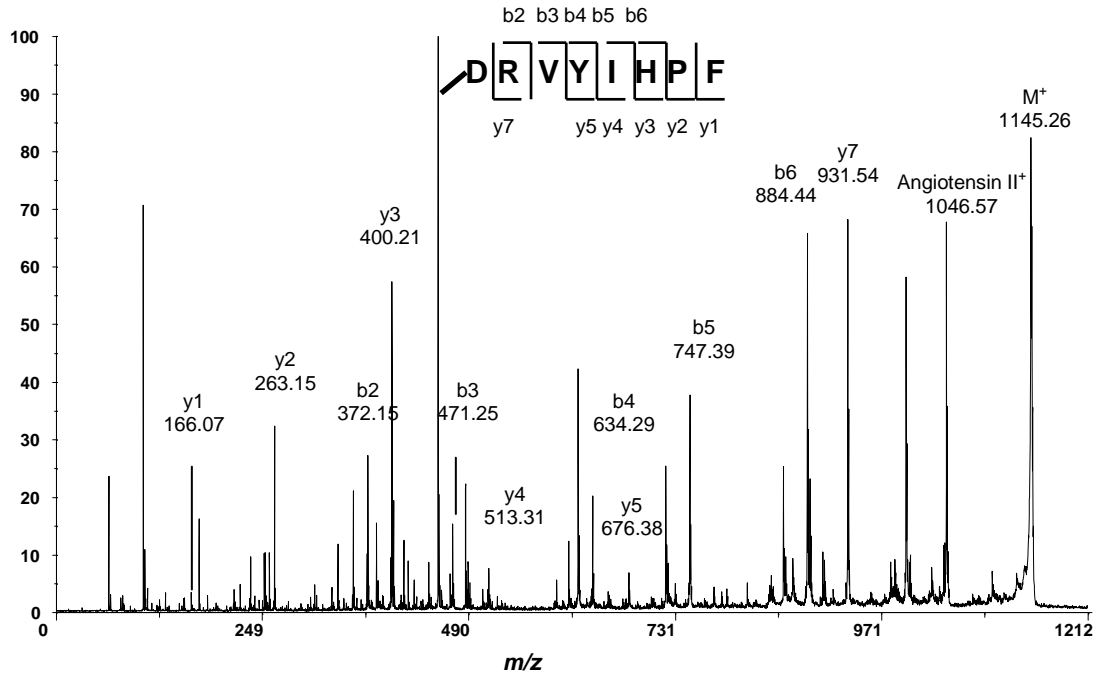
S. Figure 3. Off-line photocleavage of pcPIR cross-linked Angiotensin II (A) and Bradykinin (B).



(a) Upper: MS spectrum before UV irradiation; Lower: MS spectrum after UV irradiation.

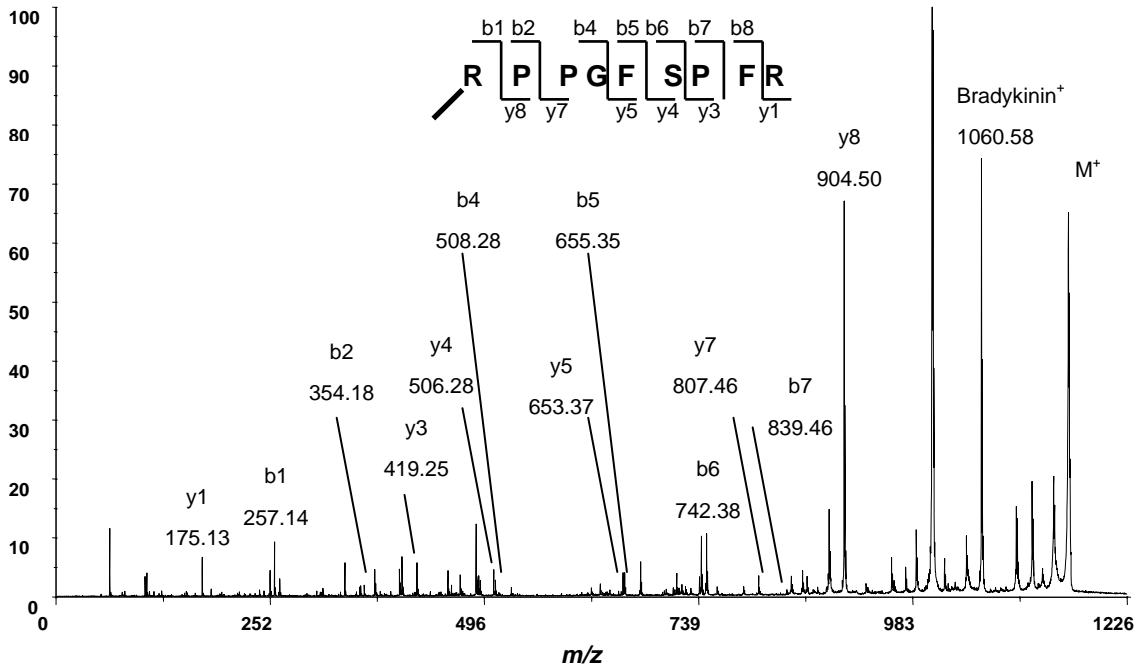
MS/MS spectrum of Released Angiotensin II

Precursor = 1146.5000



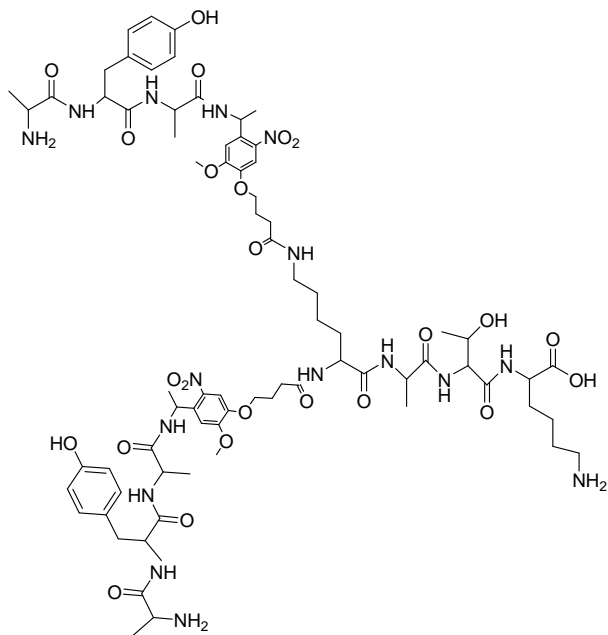
MS/MS spectrum of Released Bradykinin

Precursor = 1160.5000

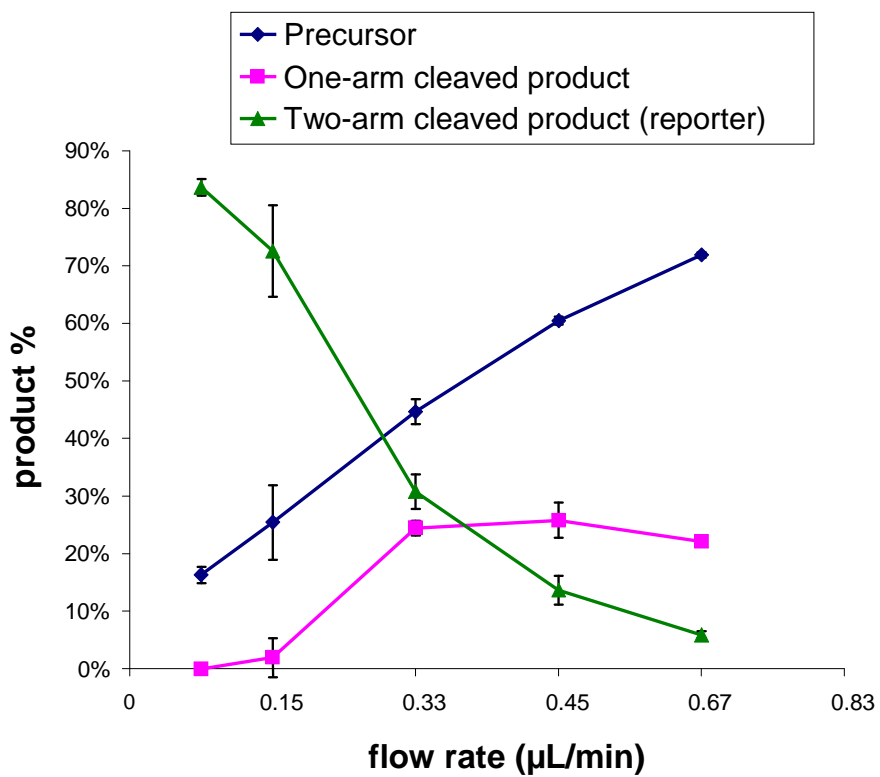


(b) MS/MS spectra of RA and RB from the off-line photocleavage-MALDI experiment

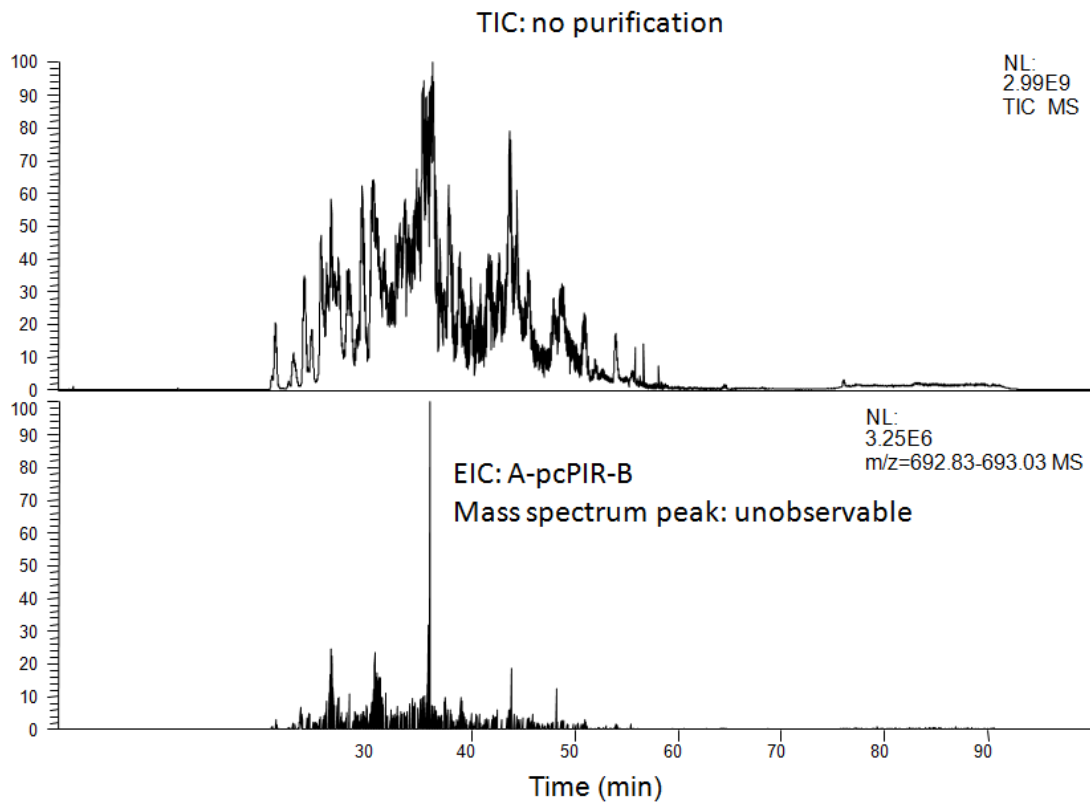
S. Figure 4. Structure of the photocleavable peptide



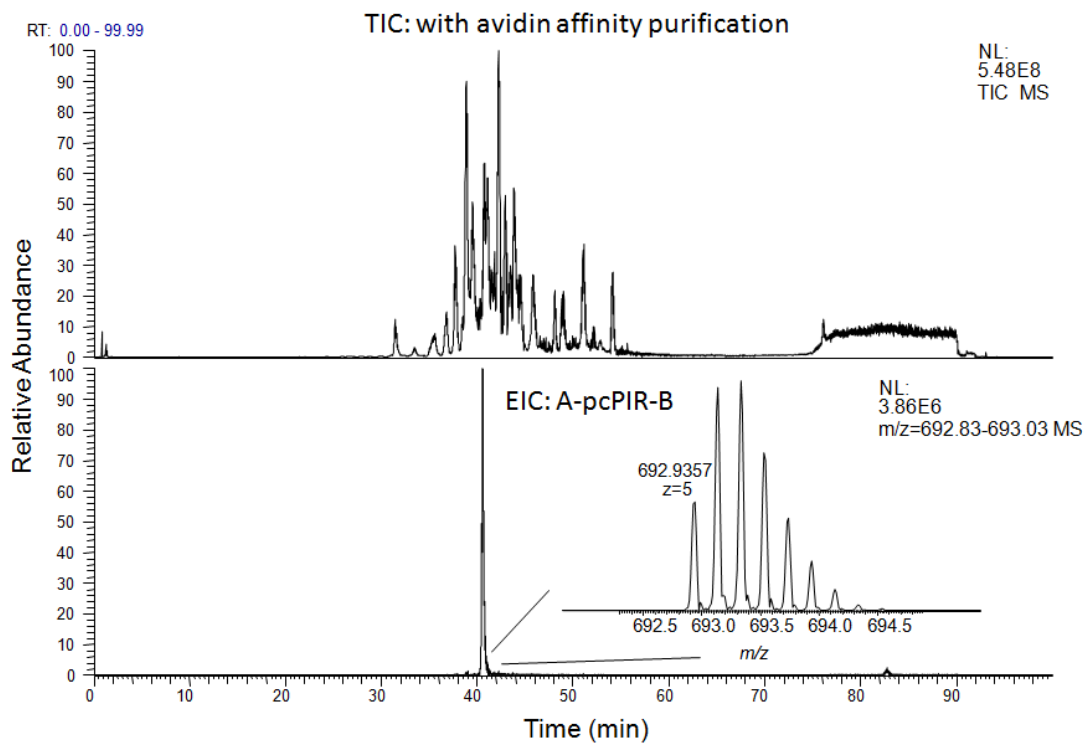
S. Figure 5. Photocleavage efficiency vs. sample flow rate in the ESI spray tip



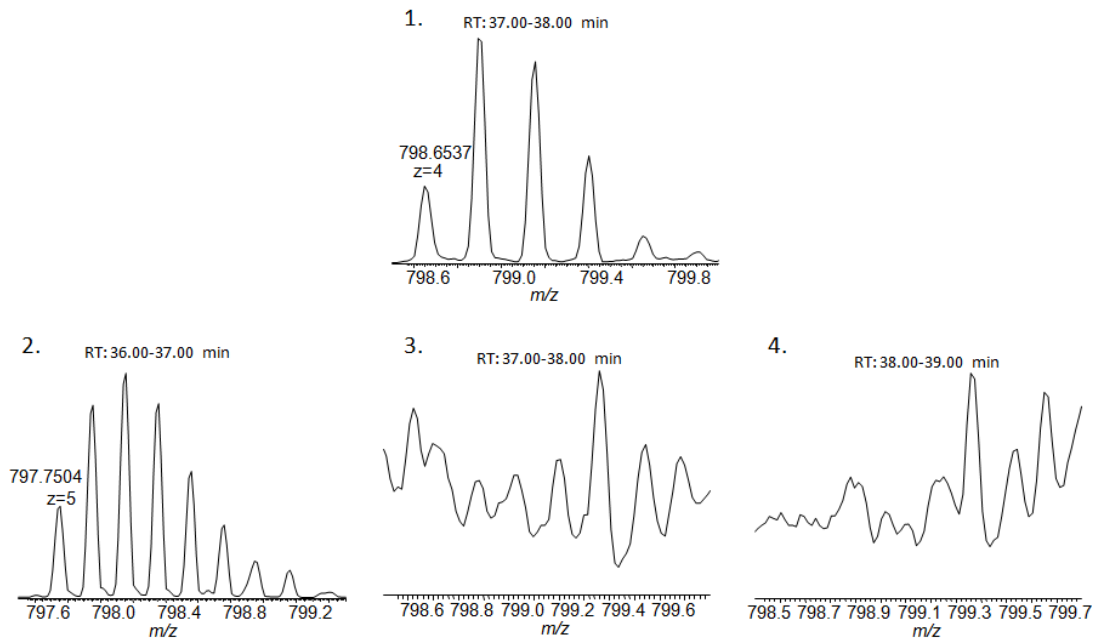
S. Figure 6. Avidin-biotin affinity purification



(a) A-pcPIR-B spiked into BSA tryptic digestion, without affinity purification



(b) With affinity purification. Inset: MS spectrum of A-pcPIR-B



(c) pcPIR cross-linked BSA

c1: inter-cross-linked precursor 798.65^{4+} observed after purification at retention time 37-38 min

c2: from the sample without purification, a similar m/z 797.75^{5+} was observed at retention time 36-37 min, which may suppress the desired product's ion

c3: from the sample without purification, at the same retention time (37-38 min), the inter-cross-link 798.65^{4+} was not observed

c4: from the sample without purification, at retention time 38-39 min, inter-cross-link was not observed

S. Table 1. Mascot identification results of pcPIR cross-linked peptides

Peptide name	Observed m/z	Observed mass (Da)	Calculated mass (Da)	Delta mass (Da)	Expect	Peptide sequence
Angiotensin II	1146.50	1145.49	1145.57	-0.07	0.011	-. DRVYIHPF. -
Bradykinin	1160.50	1159.49	1159.59	-0.10	0.012	-. RPPGFSPFR. -

S. Table 2. Comparison of pcPIR cross-linked BSA dead-end precursor with and without affinity purification

Component	m/z	EIC intensity		ratio(DE/BSA peptide)	
		with avidin	without avidin	with avidin	without avidin
Dead-end product	730.68 ³⁺	3.73 E6	1.83 E7	~4	~0.07
BSA tryptic peptide	582.32 ²⁺	9.86 E5	2.50 E8		

Avidin affinity purification enrichment factor = $4/0.07 \approx 60$ fold