

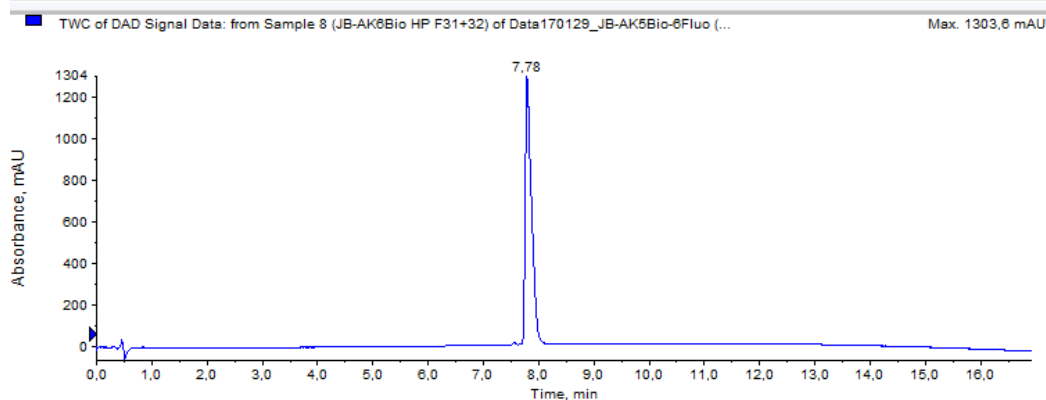
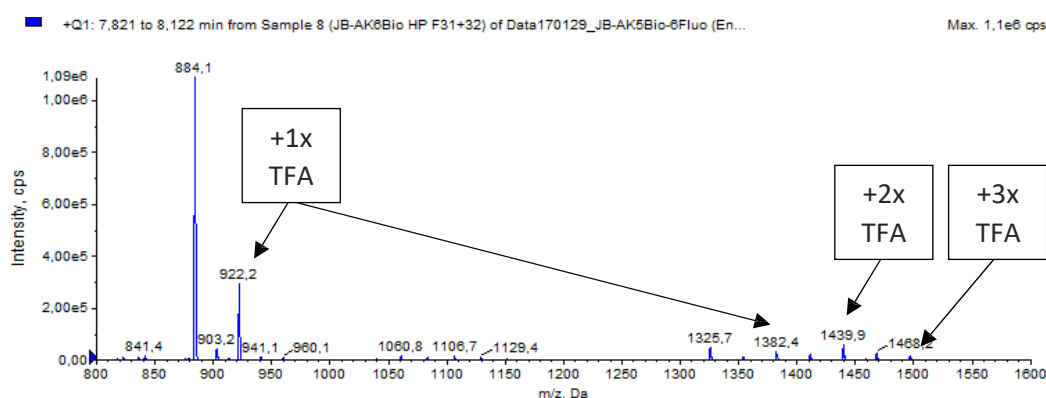
Electronic Supplementary Information

Bind&Bite: Covalently stabilized heterodimeric coiled-coil peptides for the stable, site-selective, cysteine-free chemical modification of proteins

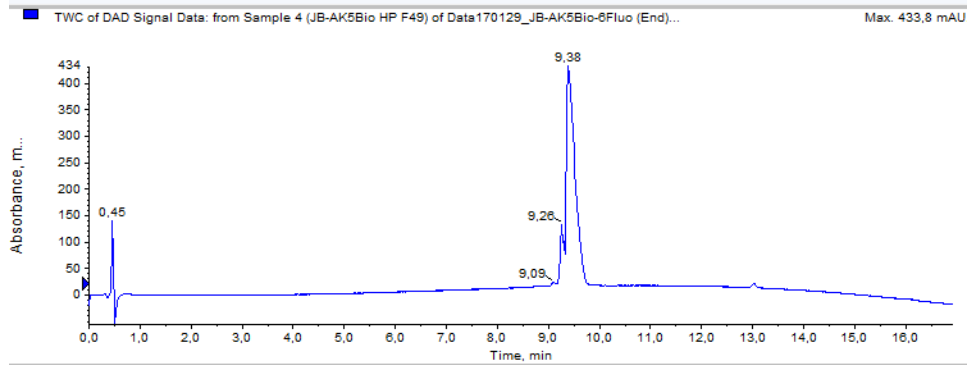
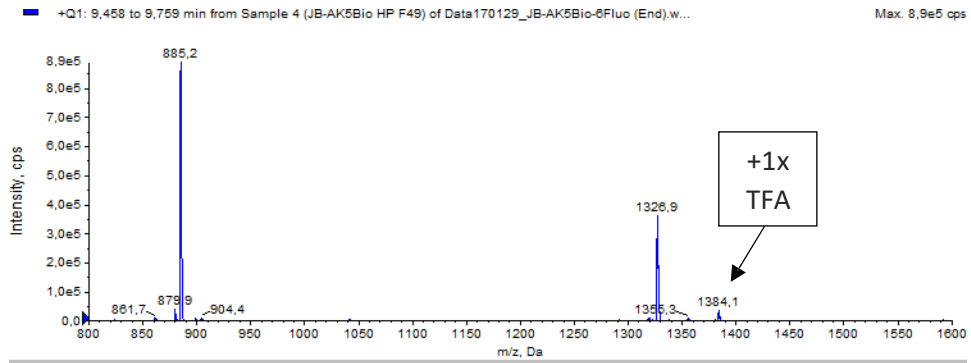
Jannis Beutel, Pierre Tannig, Riccardo Di Vincenzo, Thomas Schumacher, Klaus Überla and Jutta Eichler

Analytical data of peptides

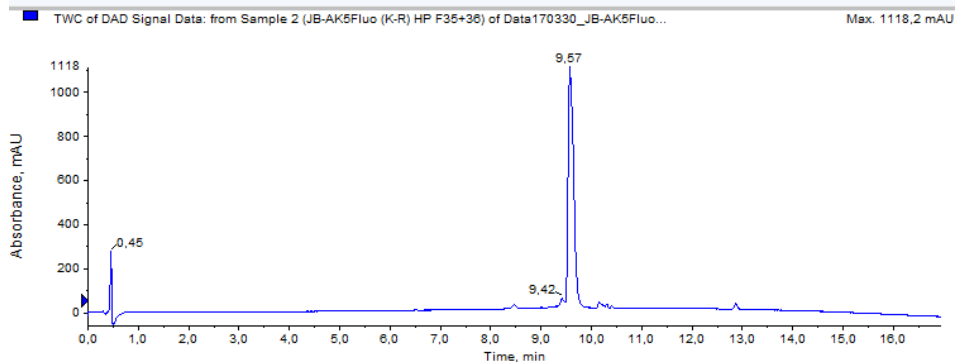
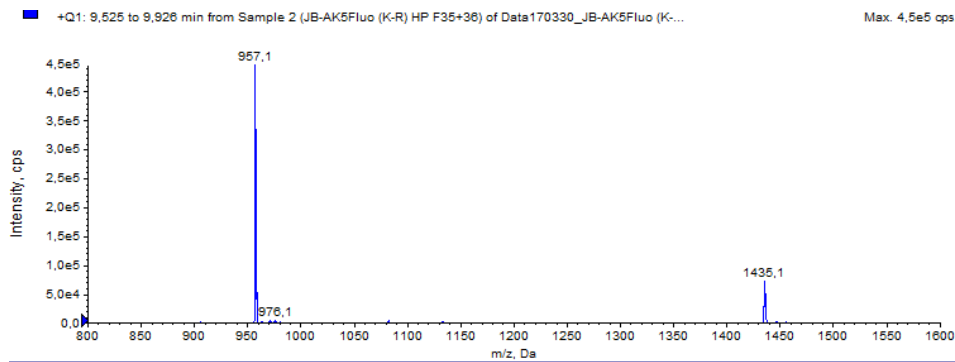
Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺
Bio-PepK	Bio-Aoa-KIAALKEKIAALKEKIAALKE-NH ₂	2650.3	2651.3	1326.2	884.4



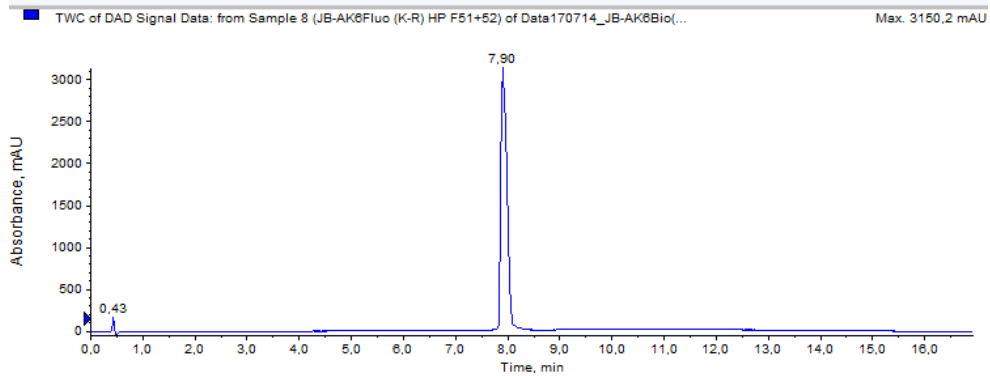
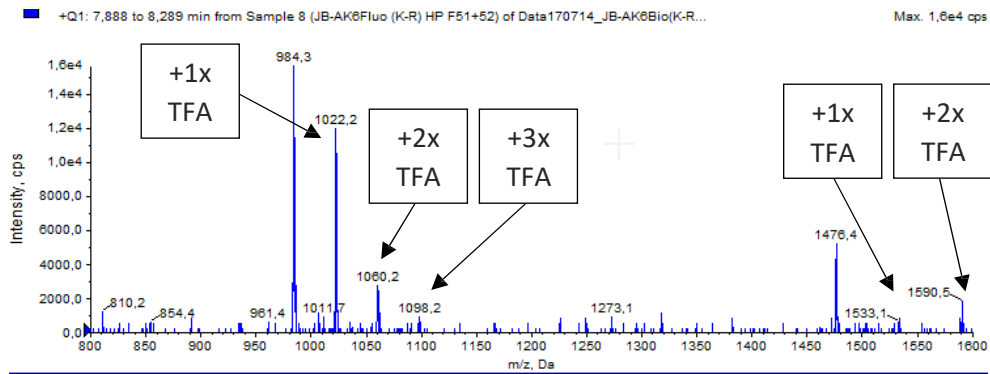
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Bio-PepE	Bio-Aoa-EIAALEKEIAALEKEIAALEK-NH ₂	2653.1	2654.1	1327.6	885.4



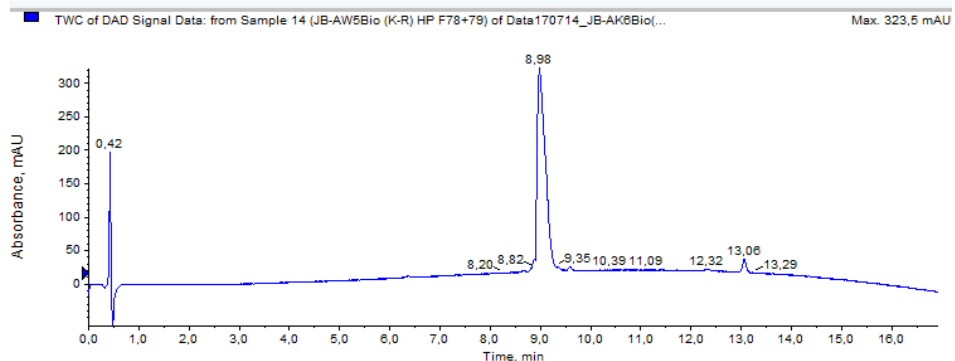
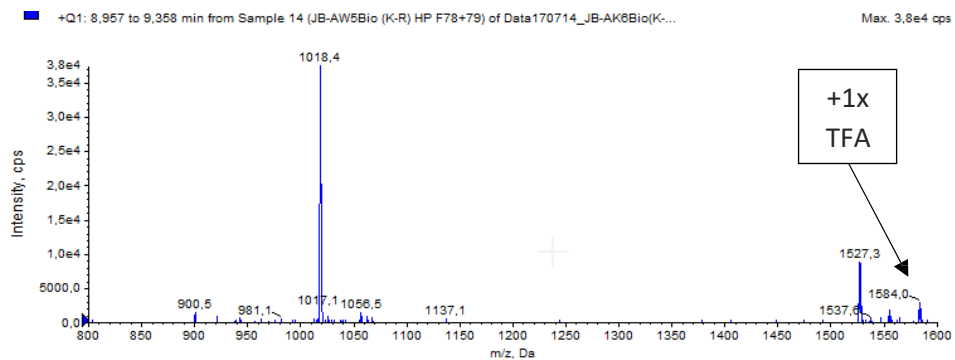
Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺
Fluo-PepE	Fluo-Aoa-EIAALEREIAALEREIAALER-NH ₂	2869.2	2870.2	1435.6	957.4



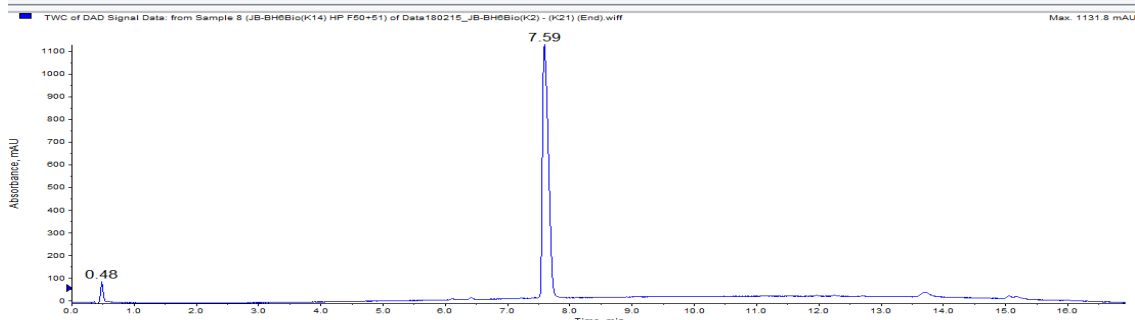
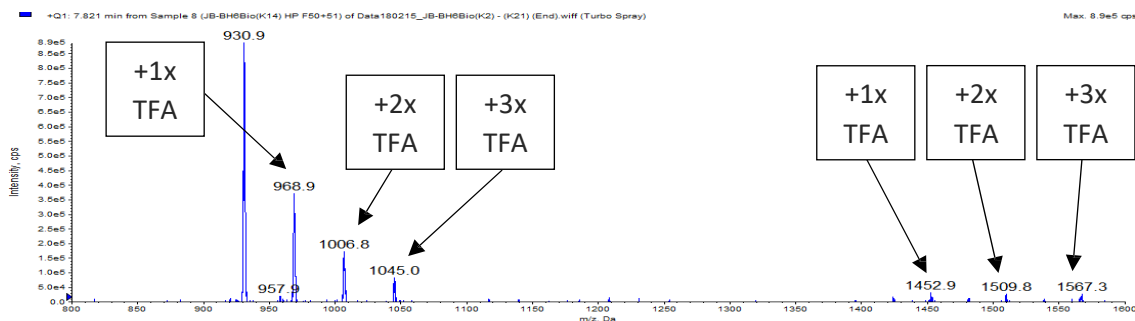
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Fluo-PepK	Fluo-Aoa-RIAALRERIAALRERIAALRE-NH ₂	2953.3	2954.3	1477.7	985.4



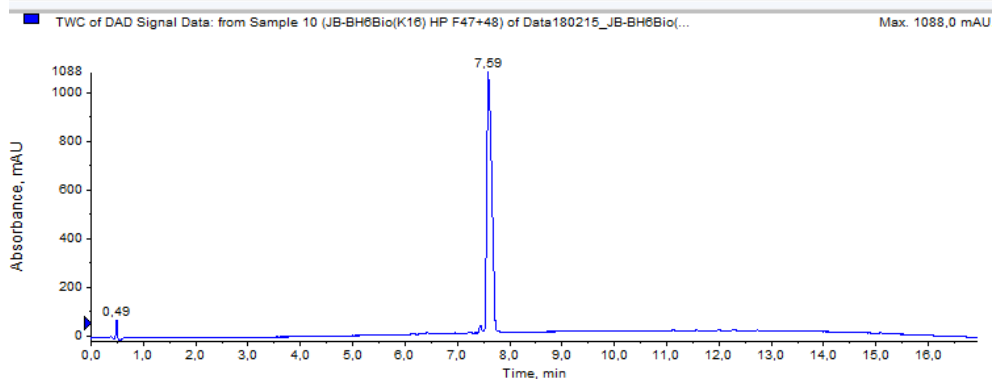
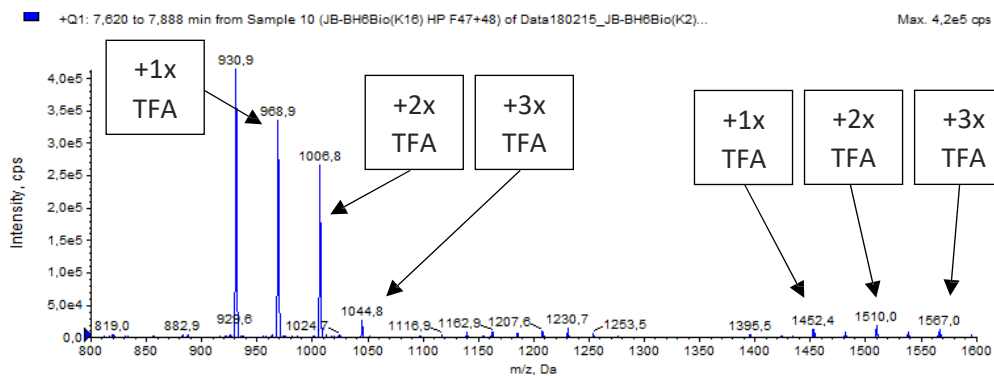
Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺
PepE-Bio	Ac-EIAALEREIAALER-Aoa-Aoa-Lys(Bio)-NH ₂	3055.4	3056.4	1528.7	1019.5



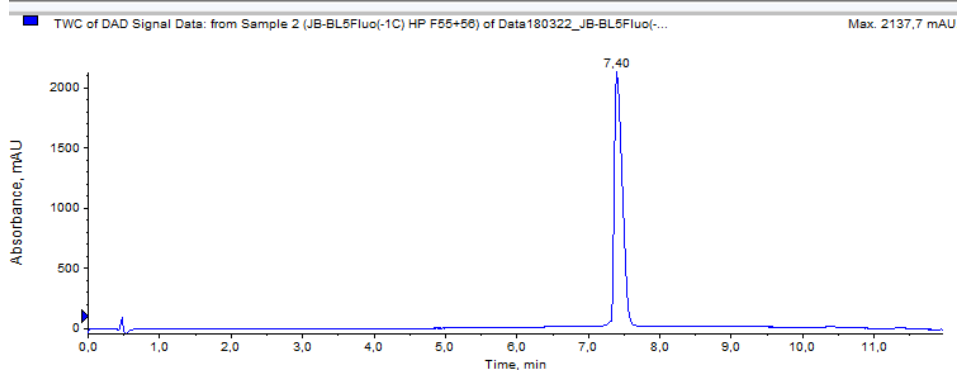
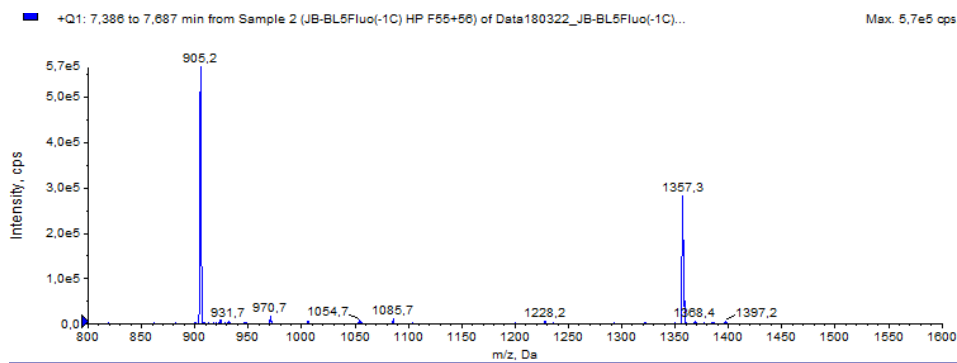
Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺
PepK(K13)	Bio-Aoa-RIAALRERIAALKERIAALRE-NH ₂	2791.3	2792.3	1396.7	931.4



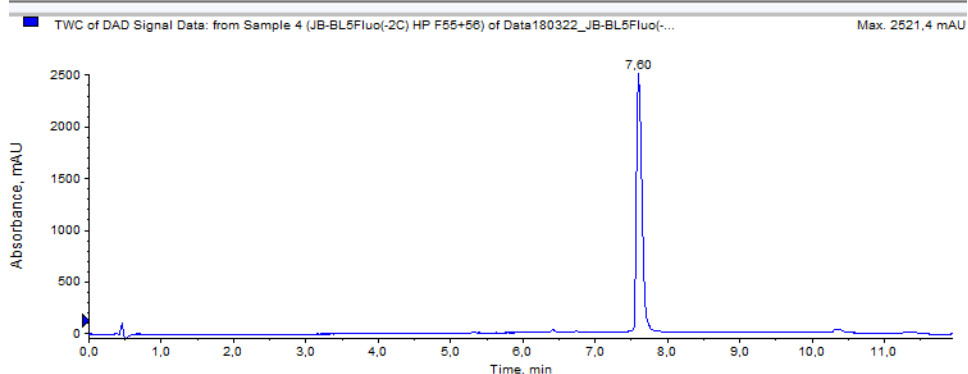
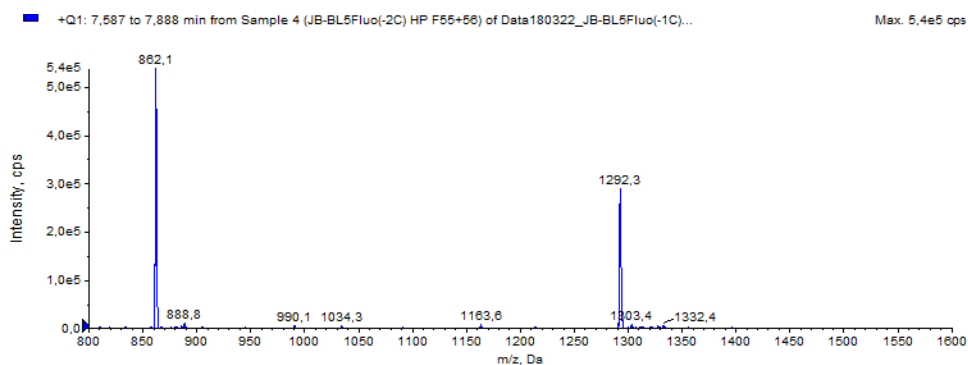
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PepK(K6)	Bio-Aoa-RIAALKERIAALRERIAALRE-NH ₂	2791.3	2792.3	1396.7	931.4



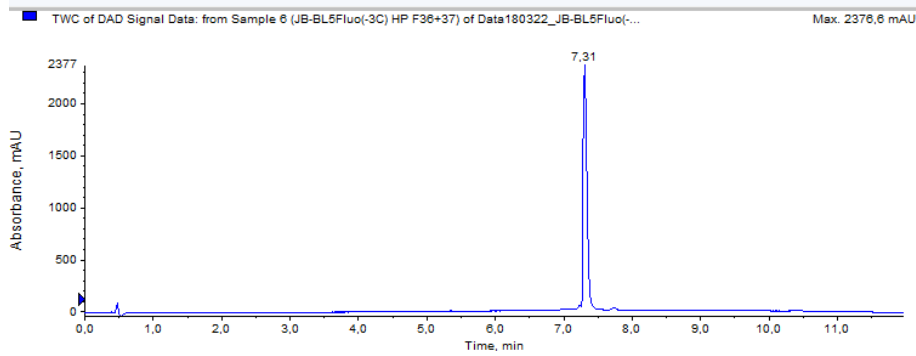
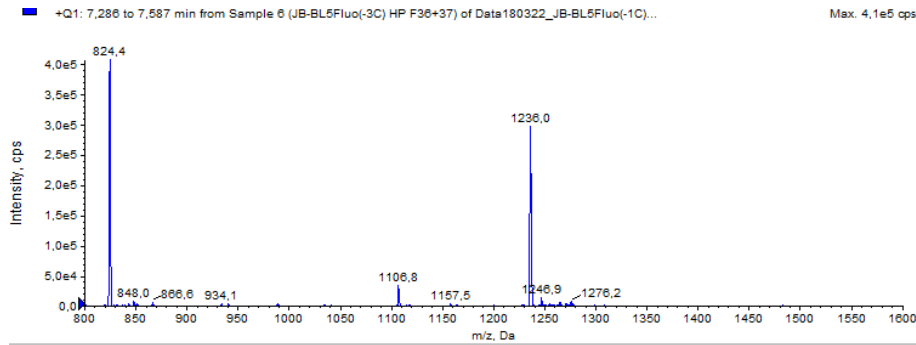
Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺
PepE(-1C)	Fluo-Aoa-EIAALEREIAALEREIAALE-NH ₂	2712.8	2713.8	1357.4	905.3



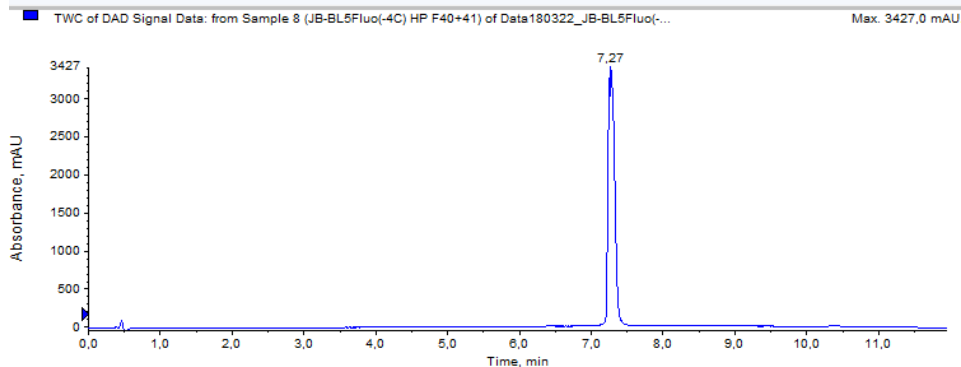
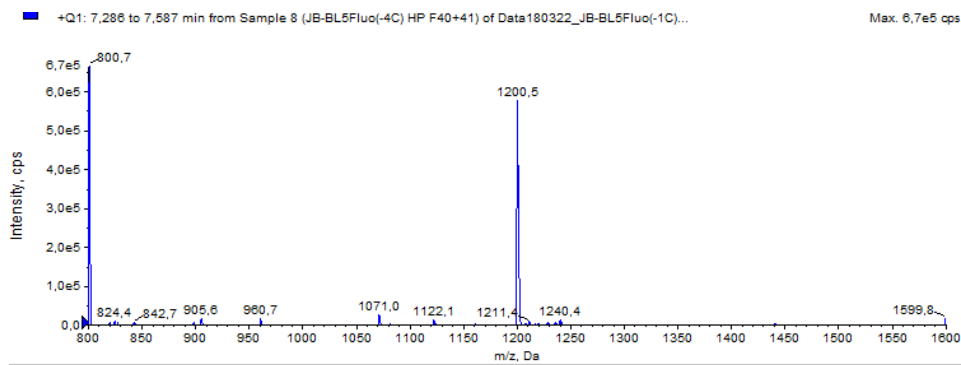
Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺
PepE(-2C)	Fluo-Aoa-EIAALEREIAALEREIAAL-NH ₂	2583.7	2584.7	1292.9	862.2



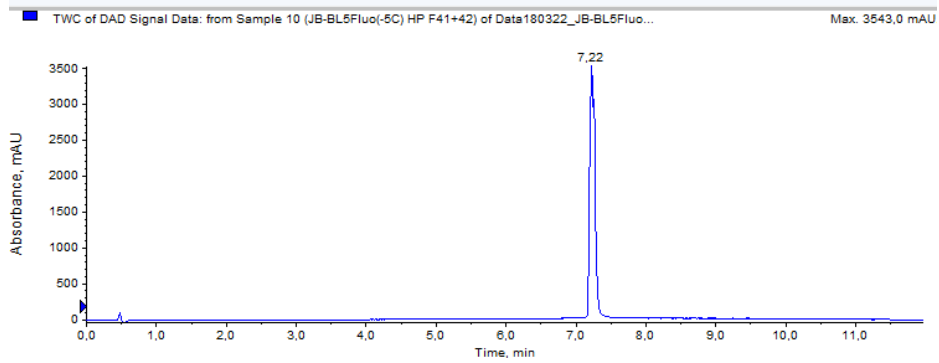
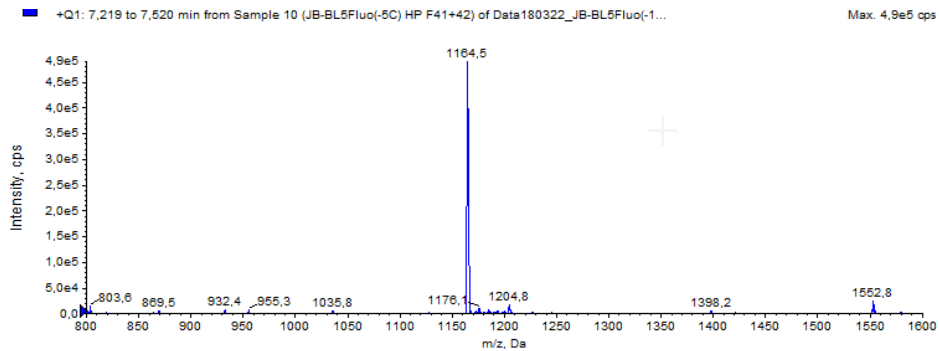
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PepE(-3C)	Fluo-Aoa-EIAALEREIAALEREIAA-NH ₂	2470.5	2471.5	1236.3	824.5



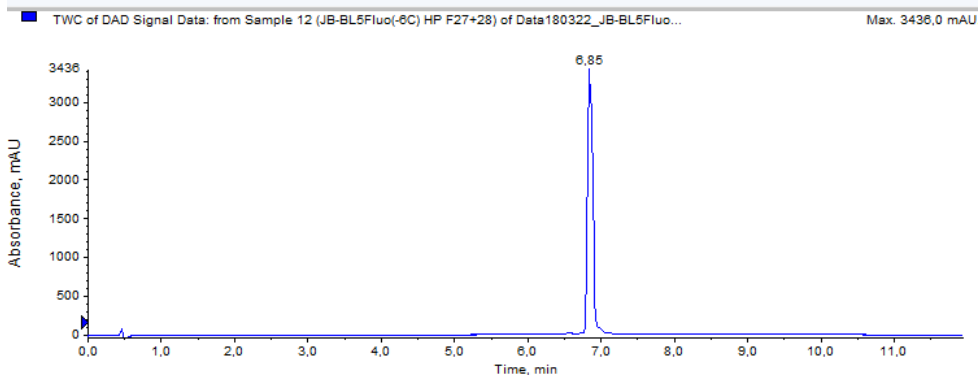
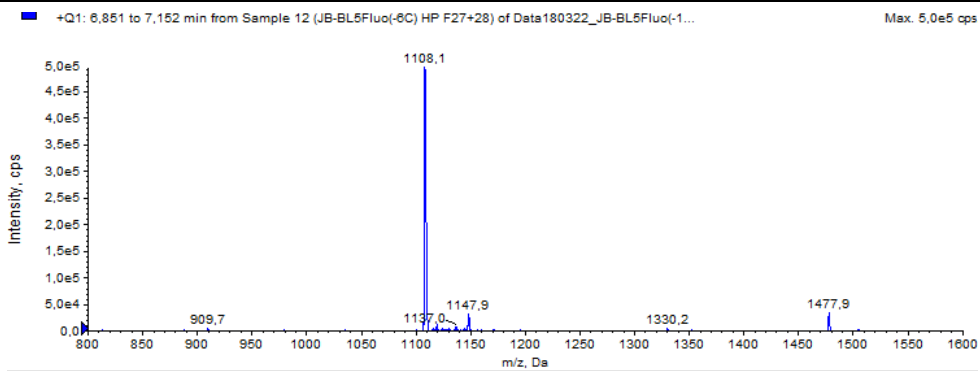
Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺
PepE(-4C)	Fluo-Aoa-EIAALEREIAALEREIA-NH ₂	2399.5	2400.5	1200.8	800.8



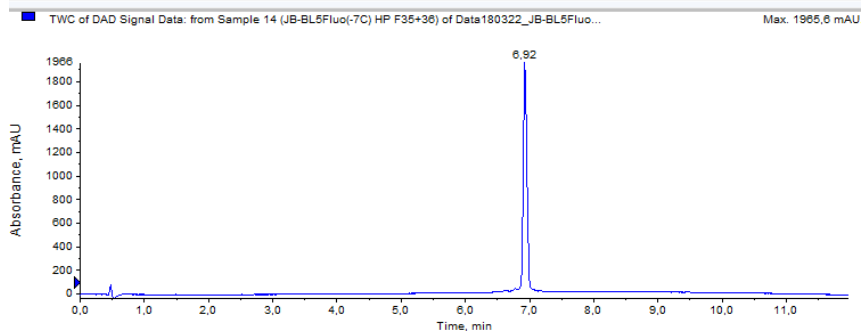
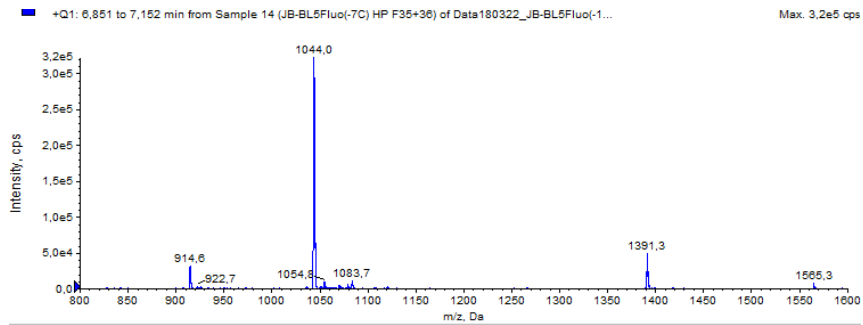
Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺
PepE(-5C)	Fluo-Aoa-EIAALEREIAALEREI-NH ₂	2328.4	2329.4	1165.2	777.1



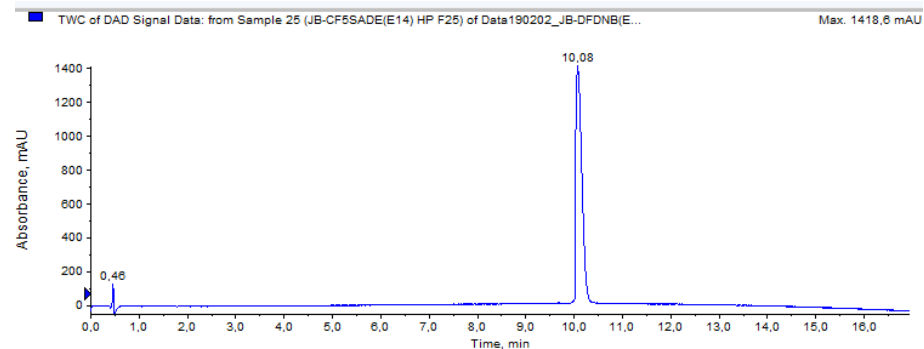
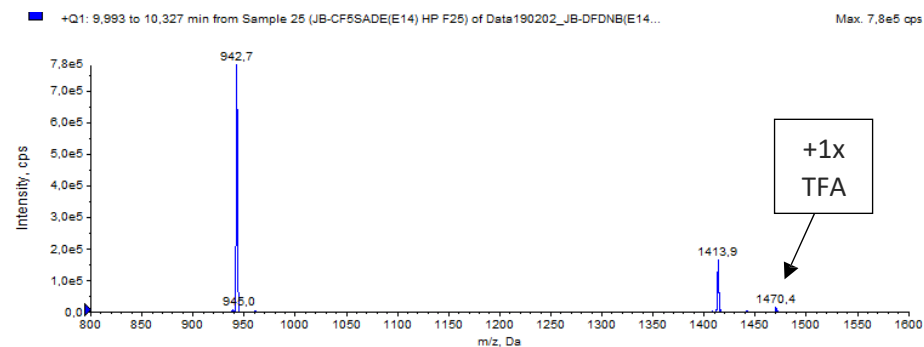
Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺
PepE(-6C)	Fluo-Aoa-EIAALEREIAALEREI-NH ₂	2215.2	2216.2	1108.6	739.4



Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺
PepE(-7C)	Fluo-Aoa-EIAALEREIAALER-NH ₂	2086.1	2087.1	1044.1	696.4



Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺
PepE(ARS8)	NBD-Sarc-Aoa-EIAALER-Dap(EDCB)-IAALEREIAALER-NH ₂	2825.1	2826.1	1413.5	942.7



Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺
PepE(ARS15)	NBD-Sarc-Aoa-EIAALERIAALER-Dap(EDCB)-IAALER-NH ₂	2825.1	2826.1	1413.5	942.7

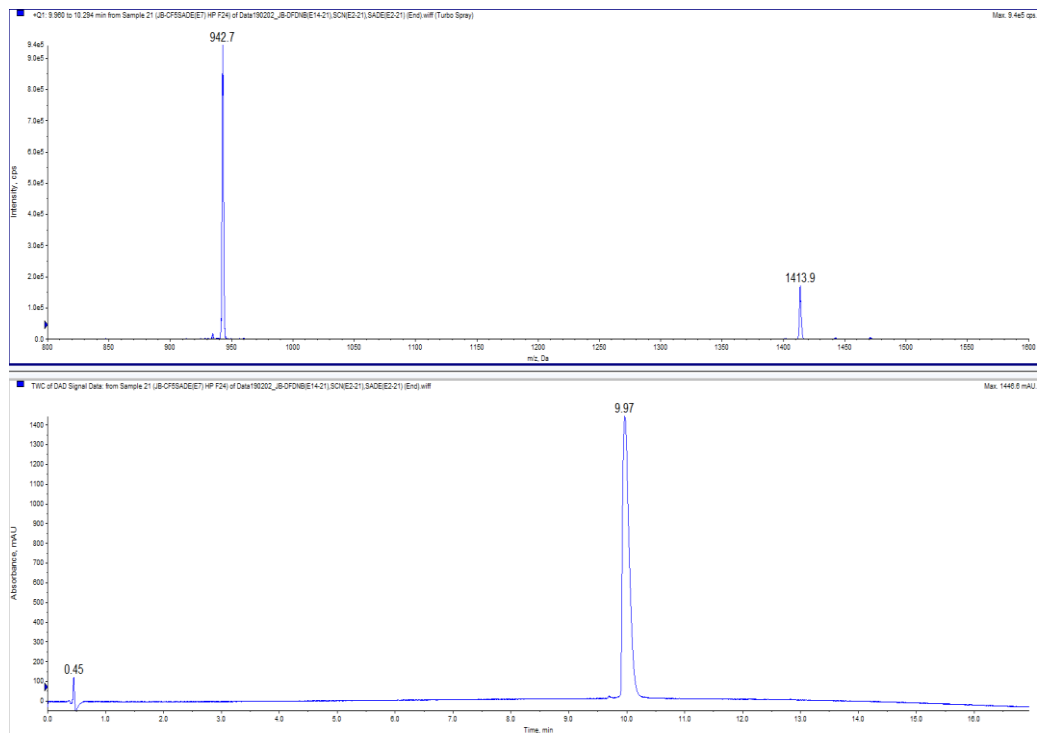
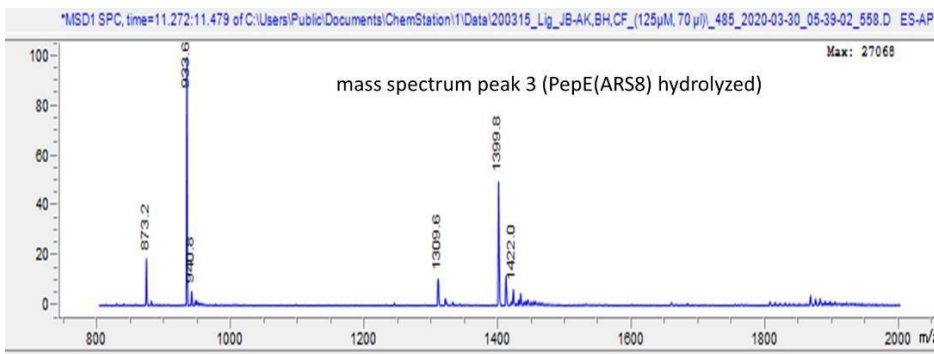
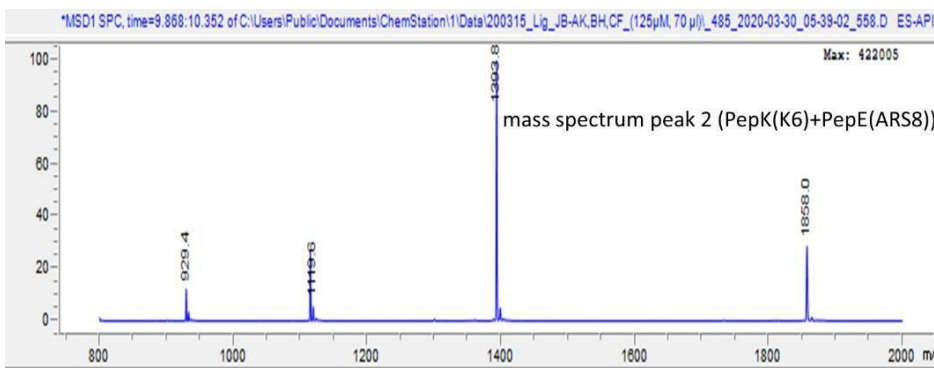
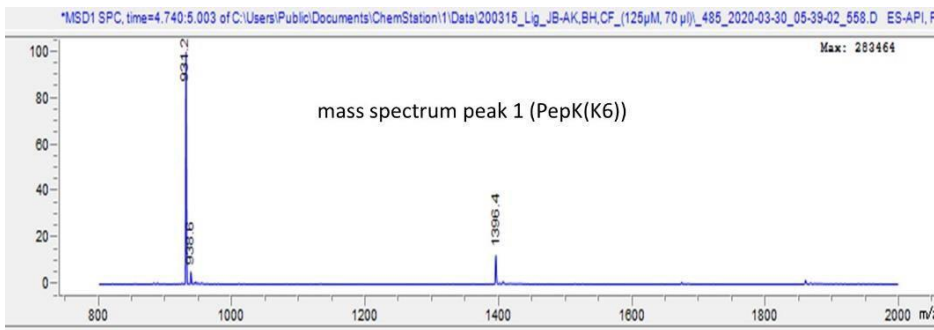
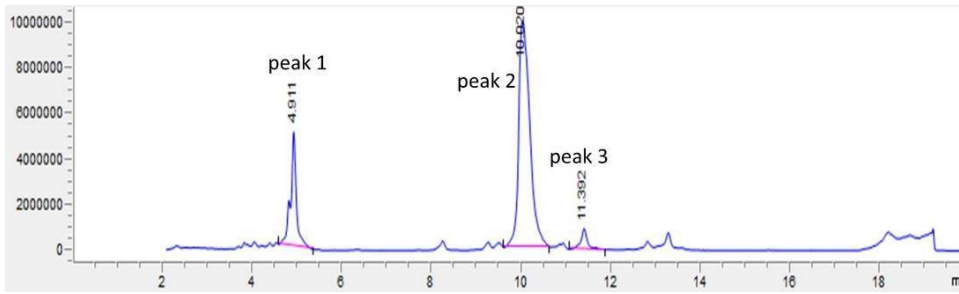


Figure S1. LC-MS data of peptides. Tables: ESI mass spectrometry data. Graphs: top: mass spectra, bottom: UV chromatograms (combined absorption at 220 and 280 nm). Masses of single, double and triple TFA adducts, which have been reported for arginine-rich peptides (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3397831/>), are marked accordingly.

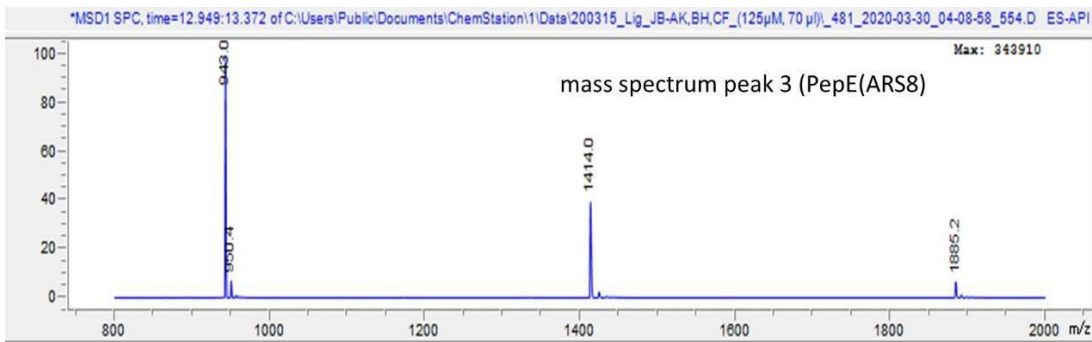
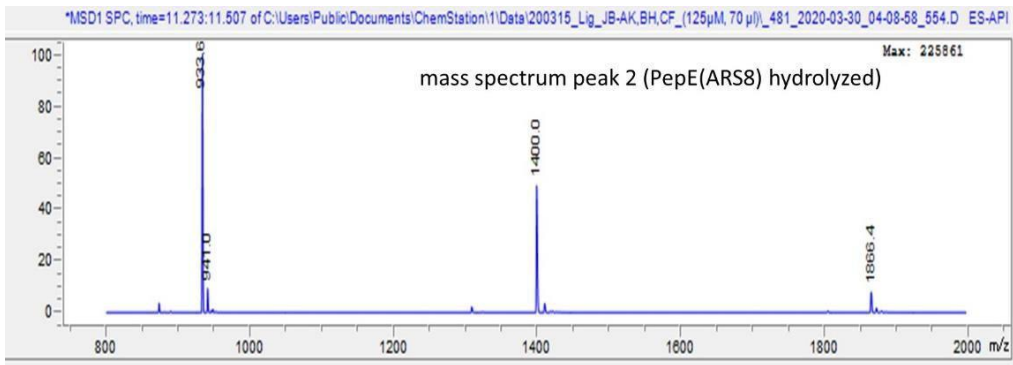
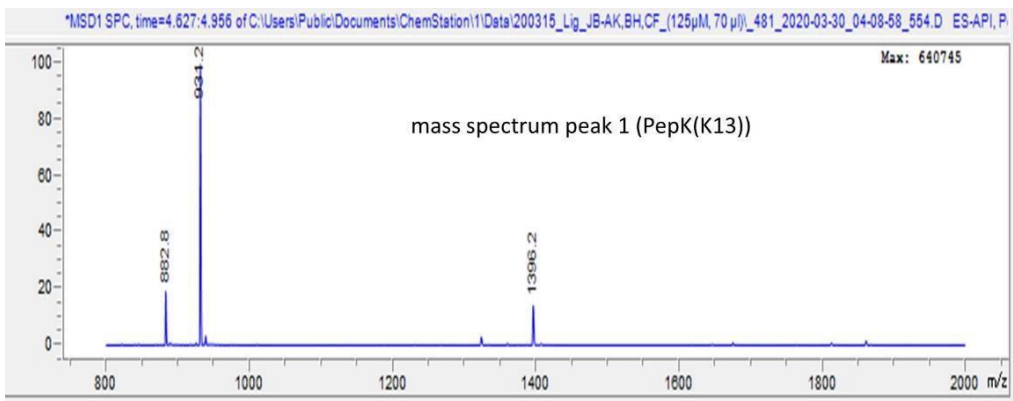
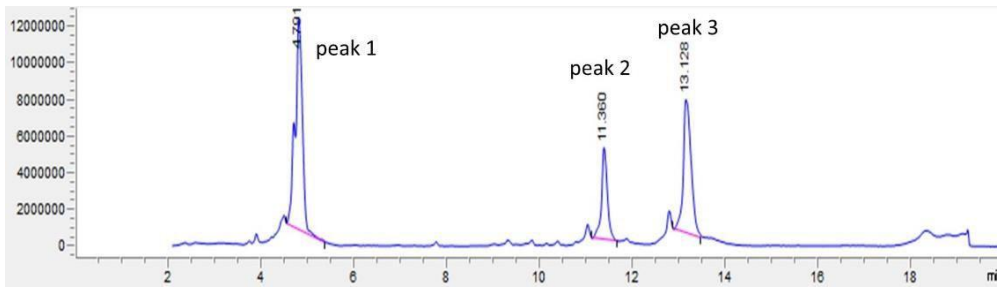
match 1: PepK(K6)+PepE(ARS8)

Chromatogram:



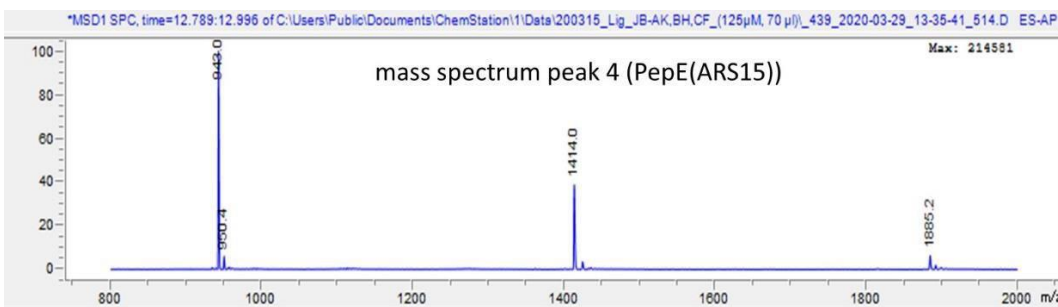
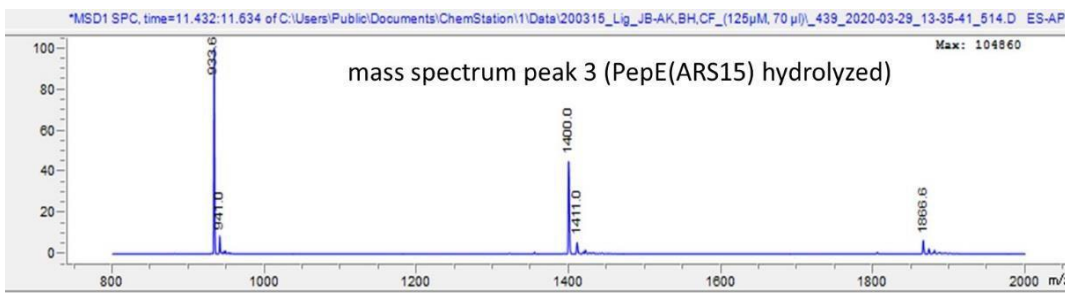
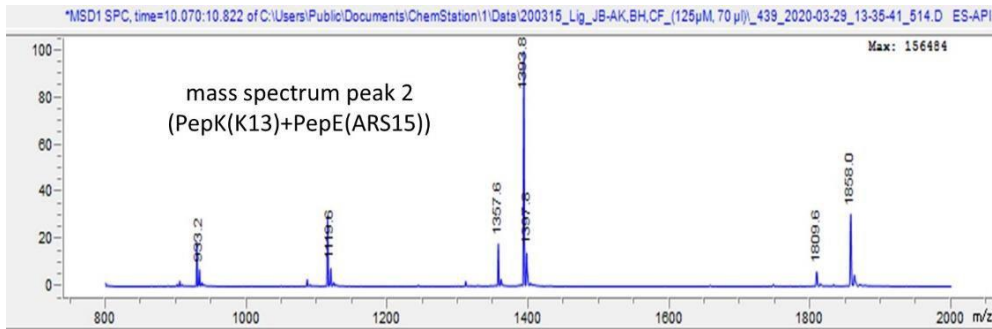
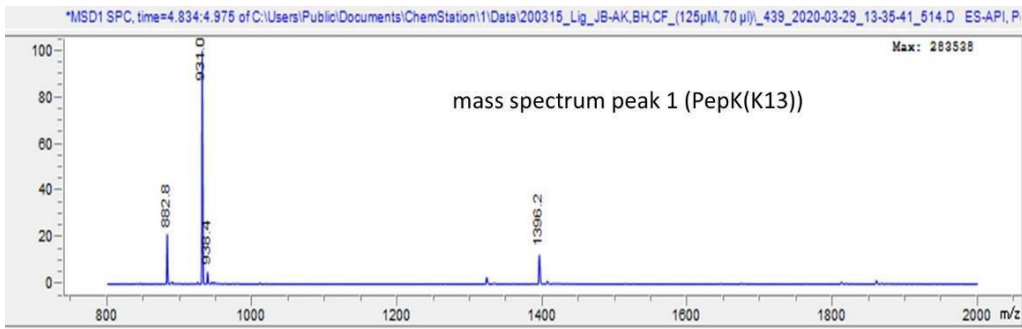
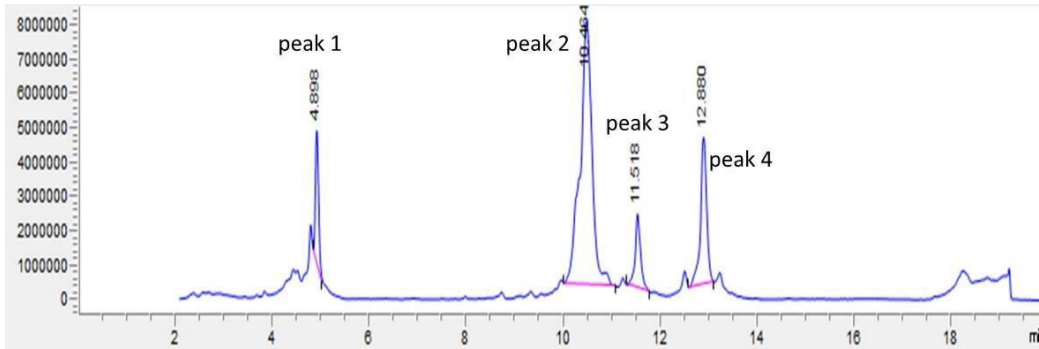
mismatch 1: + PepK(K13)+PepE(ARS8)

Chromatogram:



match 2: PepK(K13)+PepE(ARS15)

Chromatogram:



mismatch 2: PepK(K6)+PepE(ARS15)

Chromatogram:

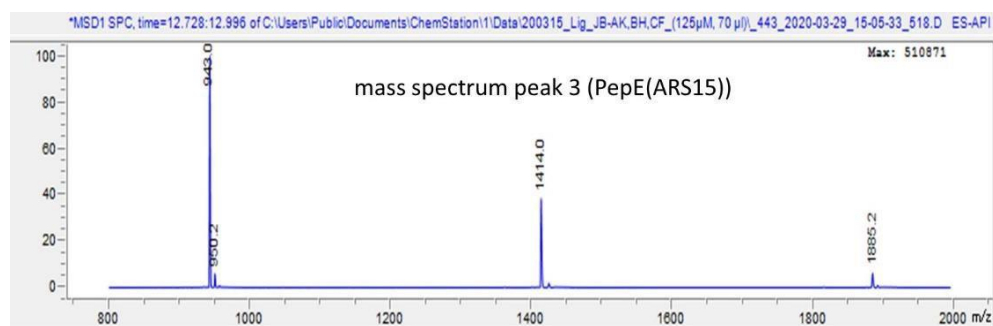
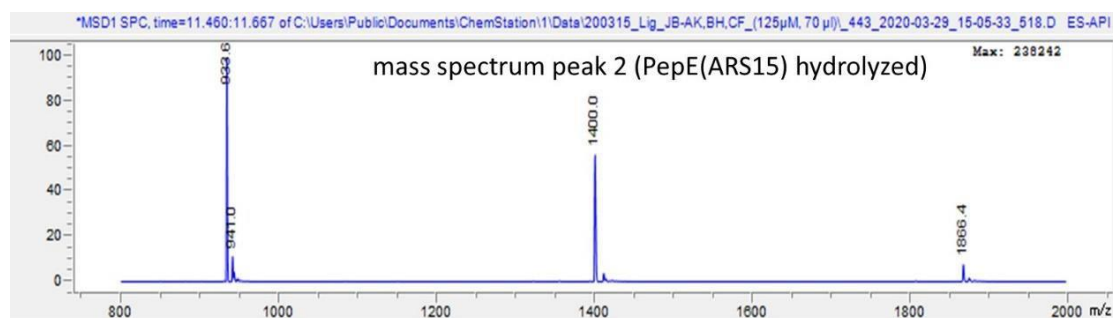
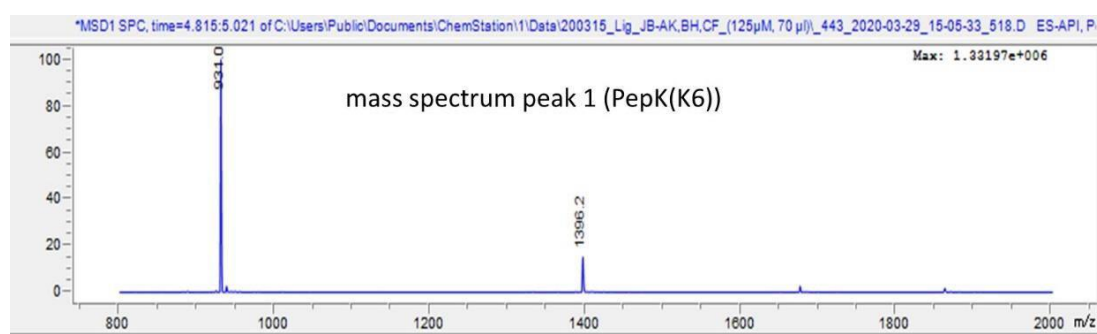
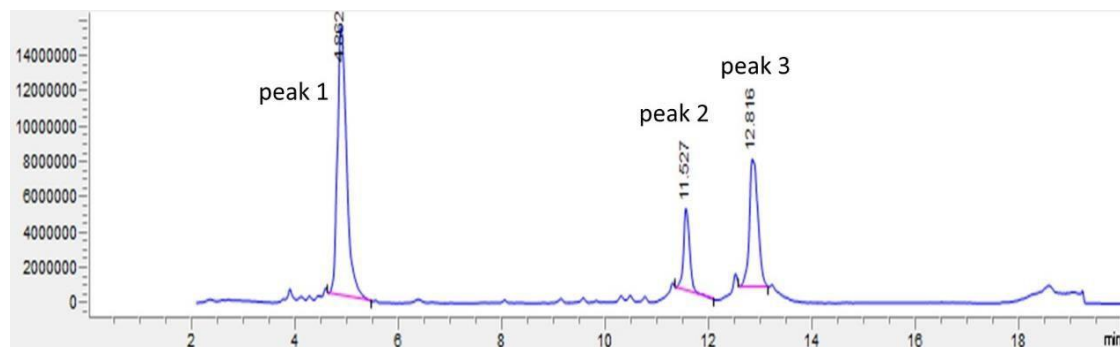


Figure S2. LC-MS data (TIC chromatograms and mass spectra of individual peaks of all match and mismatch reaction products of orthogonal pairs of coiled-coil peptides.

Peptide	Sequence	M(calc.)	[M+H] ⁺	[M+2H] ²⁺	[M+3H] ³⁺	[M+4H] ⁴⁺	[M+5H] ⁵⁺
PepE-Bio + Bio-PepK (1 isopeptide bond)	Ac-EIAALEREIAALEREIAALER-Aoa-Aoa- Lys(Bio)-NH2 + Bio-Aoa-KIAALKEKIAALKEKIAALKE-NH2	5685.2		1895.7	1422.1	1137.8	948.4
PepE-Bio + Bio-PepK (2 isopeptide bonds)	Ac-EIAALEREIAALEREIAALER-Aoa-Aoa- Lys(Bio)-NH2 + Bio-Aoa-KIAALKEKIAALKEKIAALKE-NH2	5667.2		1889.7	1417.6	1134.2	945.4

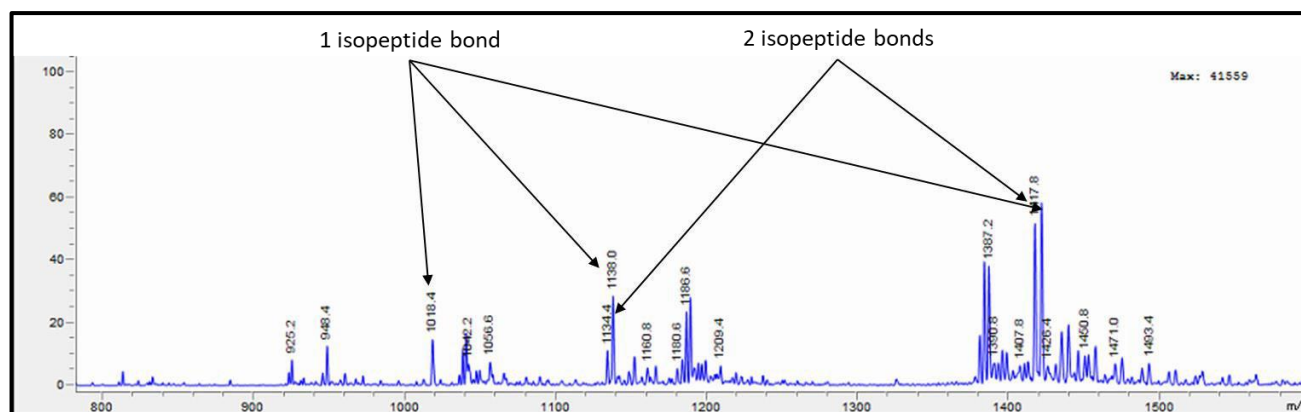


Figure S3. Mass spectrum of the product of the reaction of pre-activated PepE-Bio with Bio-PepK.