

1. Appendix

1.1. Chapter 1 and 2: HPLC and LC-MS Characterisation

Peptide 77: Wt-Type (WSW): FITC-Ahx-SYPGWSW

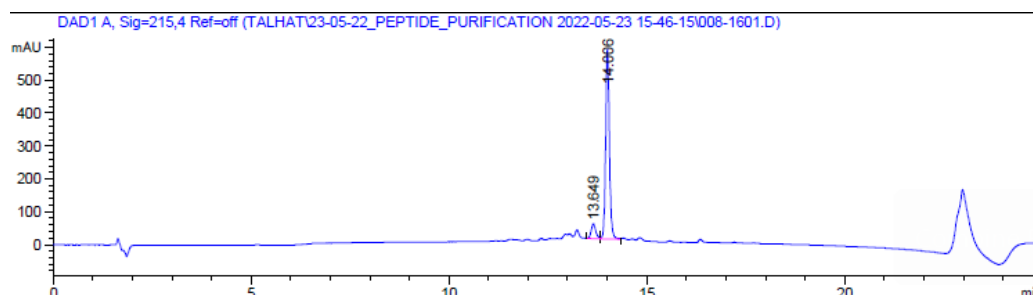


Figure S 1.1: Peptide 77. WSW-Wild Type (FITC-Ahx-SYPGWSW). Peptide purity > 95%. Top HPLC spectra at 215 nm retention time 14.006 minutes.

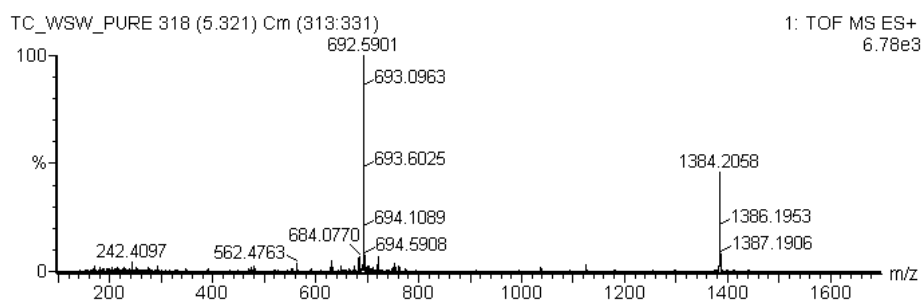


Figure S 1.2: Peptide 77. LC-MS data for WSW-Wild Type (FITC-Ahx-SYPGWSW). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1383 [M+H]⁺ and 692 [M+2H]⁺ .

Peptide 78. WSW-Cntrl: FITC-Ahx-SWYGPSW

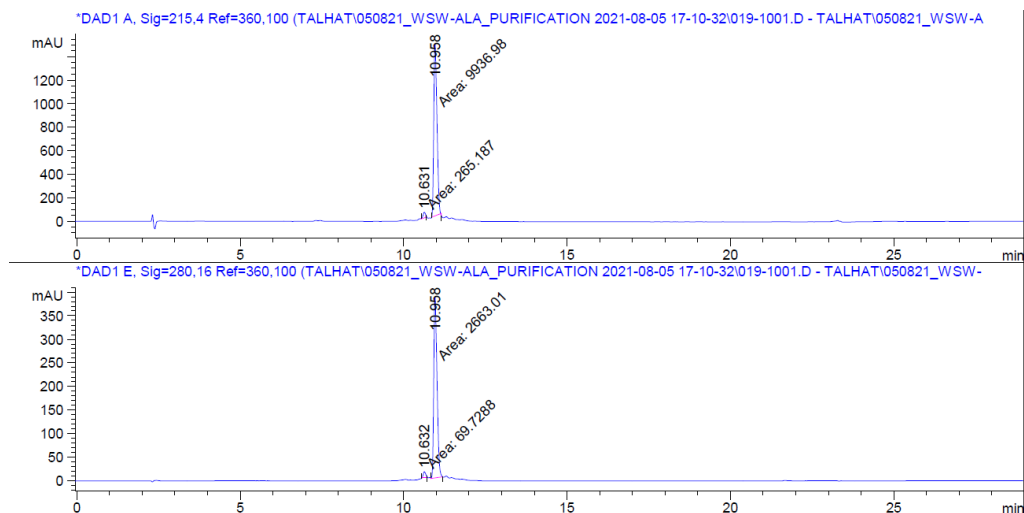


Figure S 1.3: Peptide 78. WSW-Cntrl (FITC-Ahx-SWYGPSW). Peptide purity > 95%. Top HPLC spectra at 215 nm retention time 10.958 minutes. Bottom HPLC spectra at 280 nm retention time 10.958 minutes.

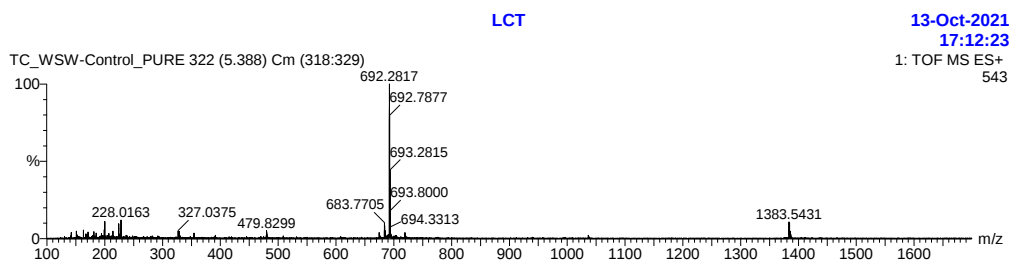


Figure S 1.4: Peptide 78. LC-MS data for WSW-Cntrl (FITC-Ahx-SWYGPSW). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1383 $[M+H]^+$ and 692 $[M+2H]^+$.

Peptide 79. Y2A: FITC-Ahx-SAPGWSW

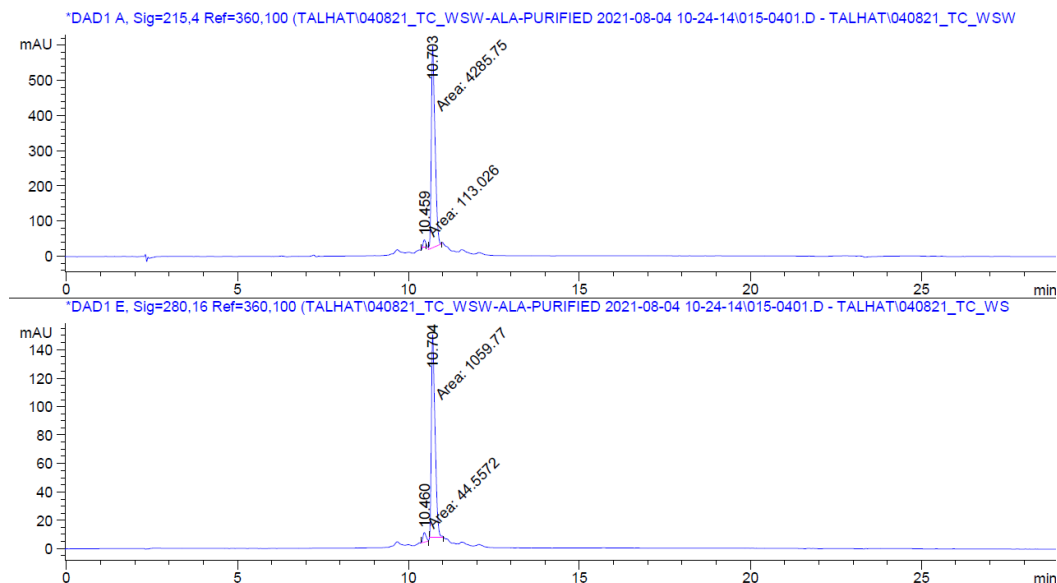


Figure S 1.5: Peptide 79. Y2A (FITC-Ahx-SAPGWSW). Peptide purity > 95%. Top HPLC spectra at 215 nm retention time 10.703 minutes. Bottom HPLC spectra at 280 nm retention time 10.704 minutes.

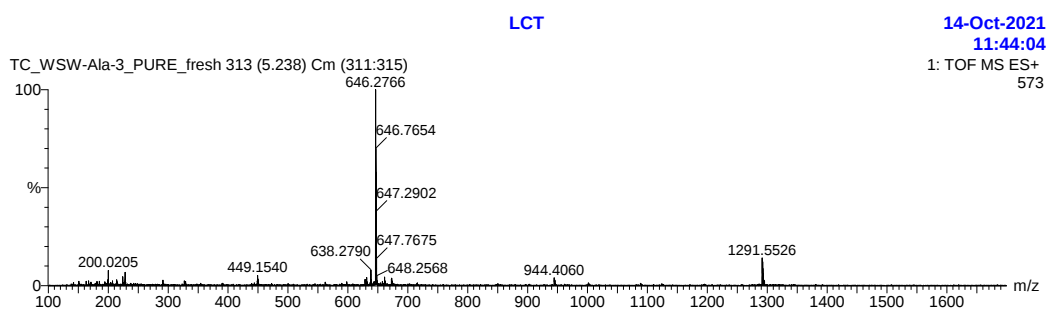


Figure S 1.6: Peptide 79. LC-MS data for Y2A (FITC-Ahx-SAPGWSW). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1291 [M+H]⁺ and 646 [M+2H]⁺.

Peptide 80. P3A: FITC-Ahx-SYAGWSW

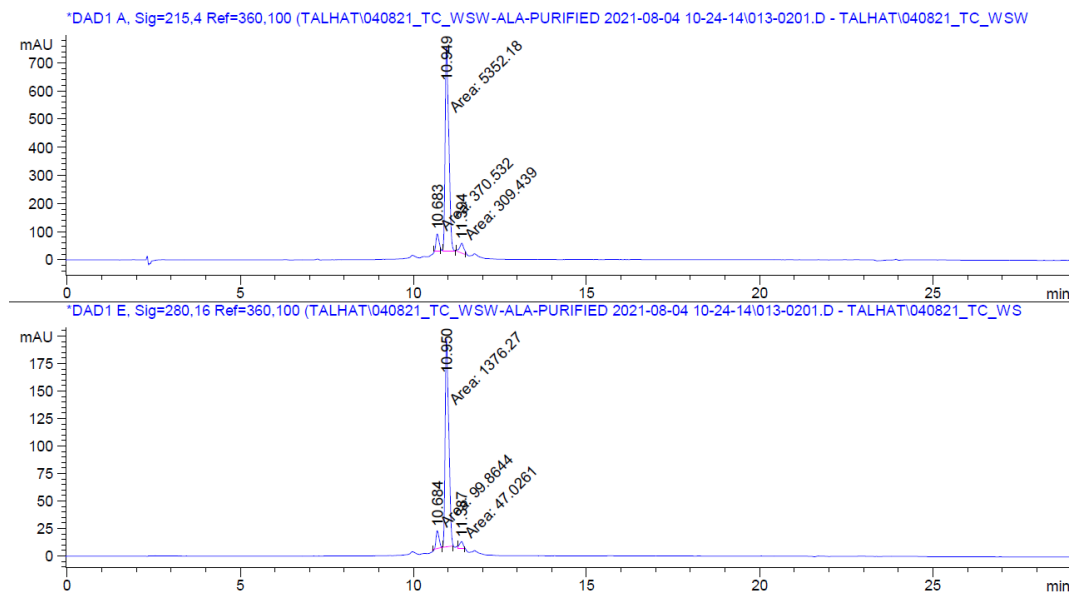


Figure S 1.7: Peptide 80. P3A (FITC-Ahx-SYAGWSW). Peptide purity > 95%. Top HPLC spectra at 215 nm retention time 10.949 minutes. Bottom HPLC spectra at 280 nm retention time 10.950 minutes.

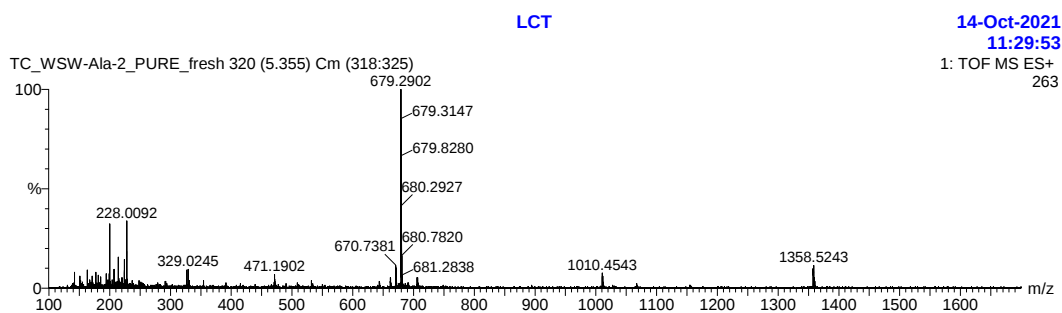


Figure S 1.8: Peptide 80. LC-MS data for P3A (FITC-Ahx-SYAGWSW). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1358 $[M+H]^+$ and 679 $[M+2H]^+$.

Peptide 81. W5A: FITC-Ahx-SYPGASW

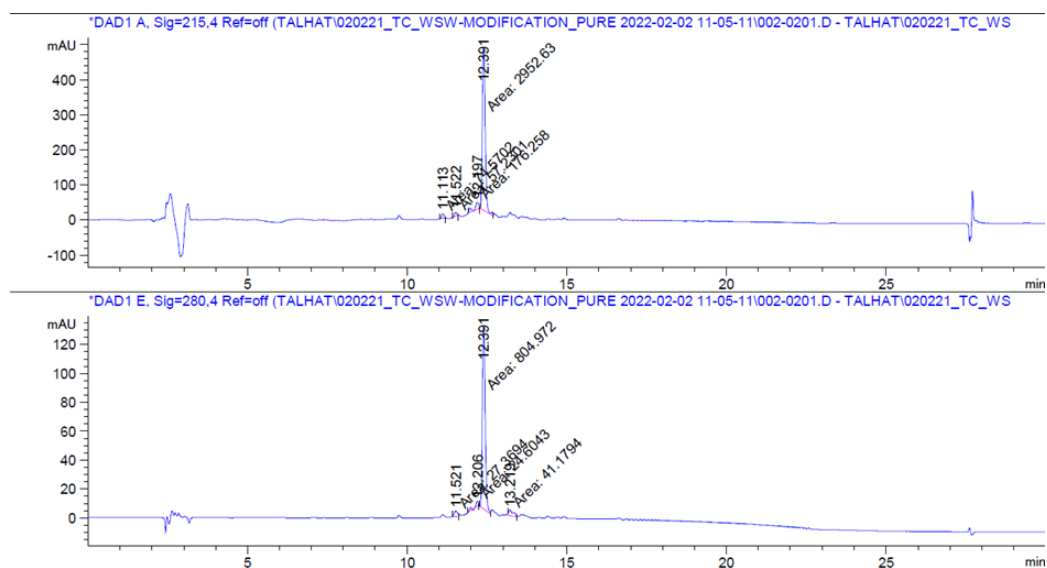


Figure S 1.9: Peptide 81. W5A (FITC-Ahx-SYPGASW). Peptide purity > 95%. Top HPLC spectra at 215 nm retention time 12.391 minutes. Bottom HPLC spectra at 280 nm retention time 12.391 minutes.

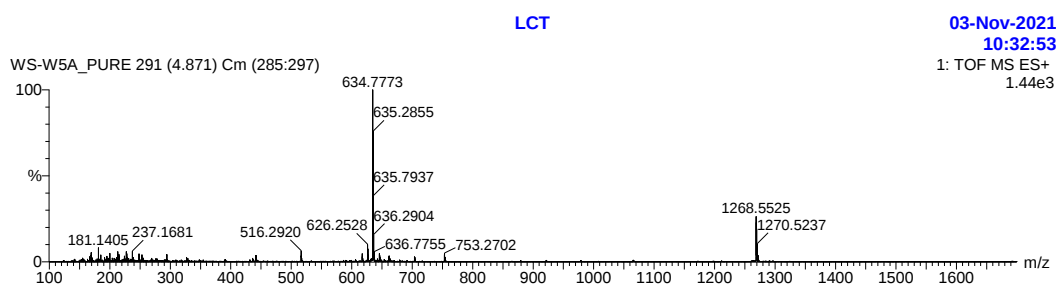


Figure S 1.10: Peptide 81. LC-MS data for W5A (FITC-Ahx-SYPGASW). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1268 $[M+H]^+$ and 634 $[M+2H]^+$ (bottom).

Peptide 82. W7A: FITC-Ahx-SYPGWSA

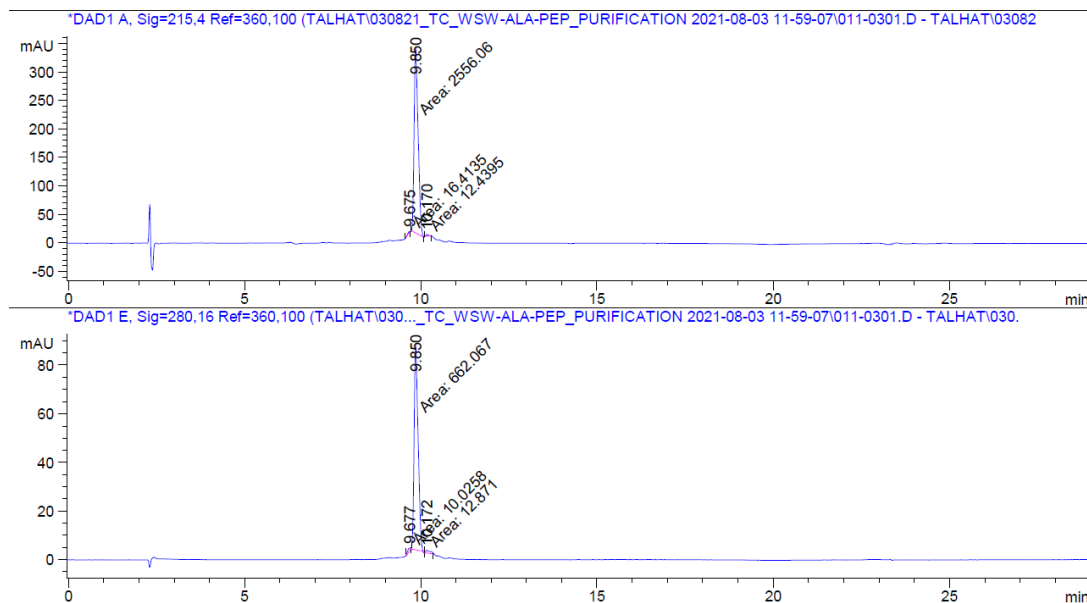


Figure S 1.11: Peptide 82. W7A (FITC-Ahx-SYPGWSA). Peptide purity > 95%. Top HPLC spectra at 215 nm retention time 9.850 minutes. Bottom HPLC spectra at 280 nm retention time 9.850 minutes.

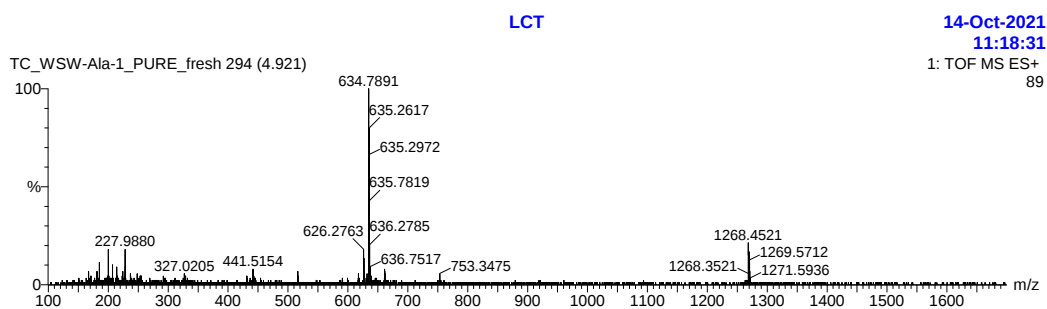


Figure S 1.12: Peptide 82. LC-MS data for W7A (FITC-Ahx-SYPGWSA). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1268 [M+H]⁺ and 634 [M+2H]⁺.

Peptide 83. Y2F: FITC-Ahx-SFPGWSW

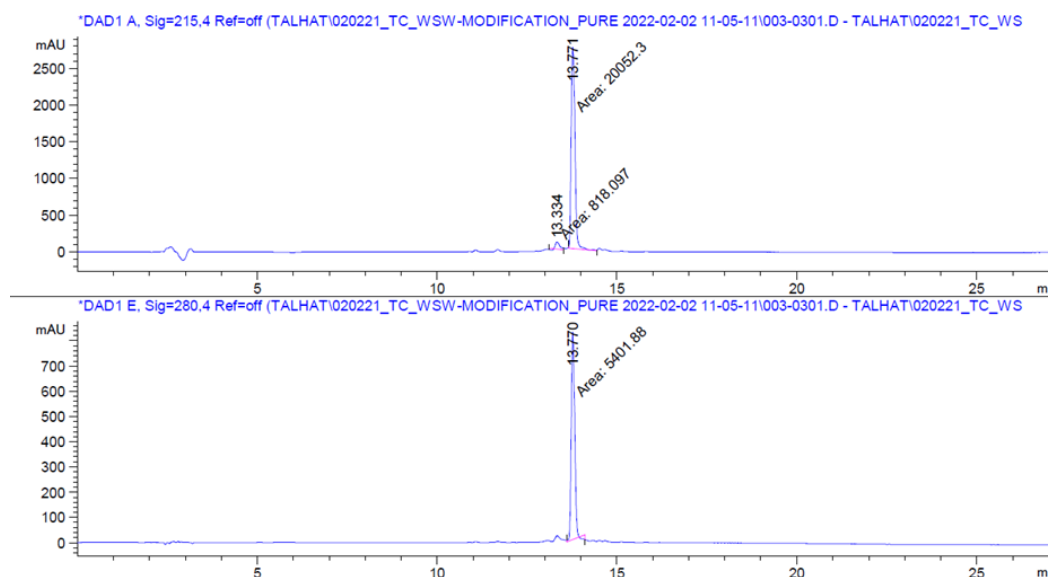


Figure S 1.13: Peptide 83. Y2F (FITC-Ahx-SFPGWSW). Peptide purity > 95%. Top HPLC spectra at 215 nm retention time 13.771 minutes. Bottom HPLC spectra at 280 nm retention time 13.770 minutes.

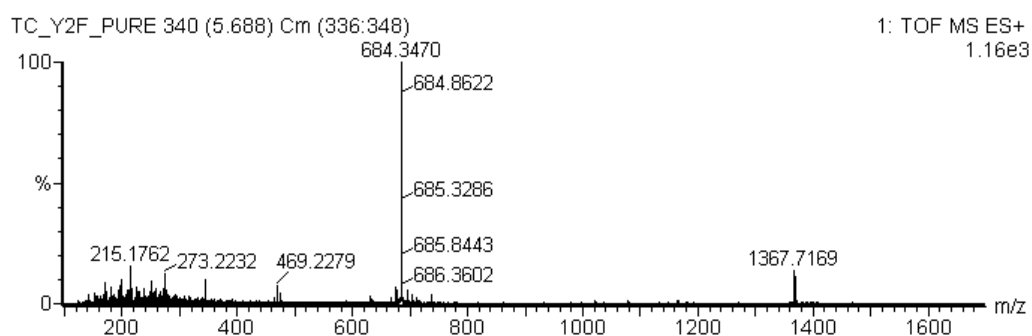


Figure S 1.14: Peptide 83. LC-MS data for Y2F (FITC-Ahx-SFPGWSW). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1367 $[M+H]^+$ and 684 $[M+2H]^+$.

Peptide 84. W5F: FITC-Ahx-SYPGFSW

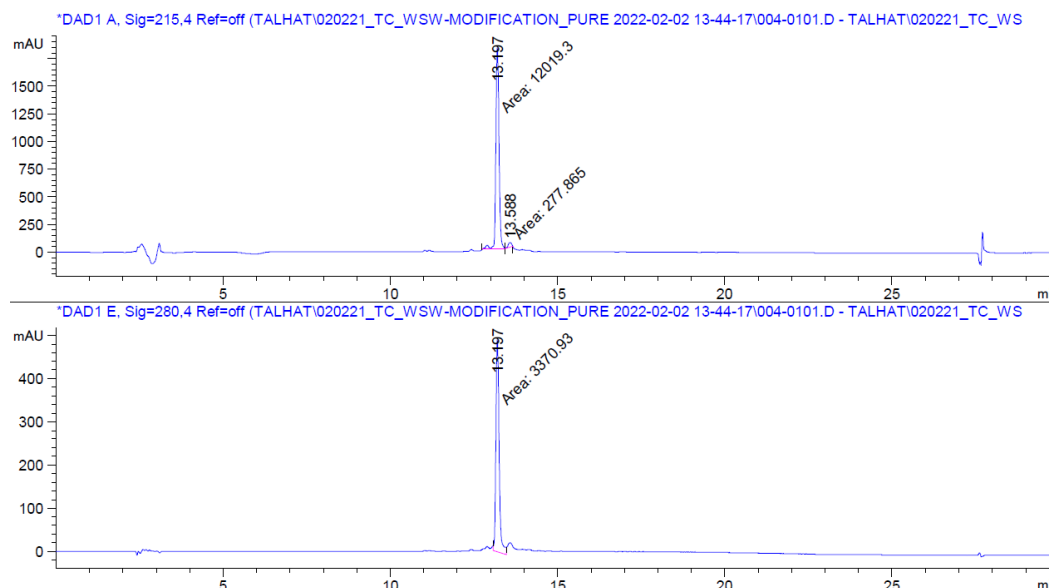


Figure S 1.15: Peptide 84. W5F (FITC-Ahx-SYPGFSW). Peptide purity > 95%. Top HPLC spectra at 215 nm retention time 13.197 minutes. Bottom HPLC spectra at 280 nm retention time 13.197 minutes.

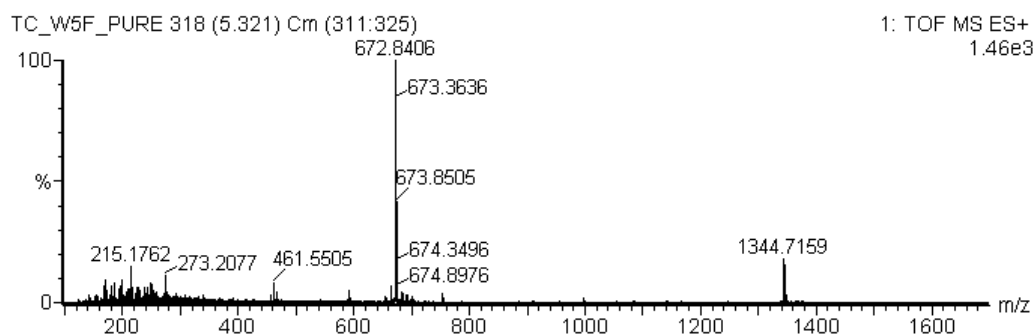


Figure S 1.16: Peptide 84. LC-MS data for W5F (FITC-Ahx-SYPGFSW). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1344 [M+H]⁺ and 672 [M+2H]⁺.

Peptide 85. W7F: FITC-Ahx-SYPGWSF

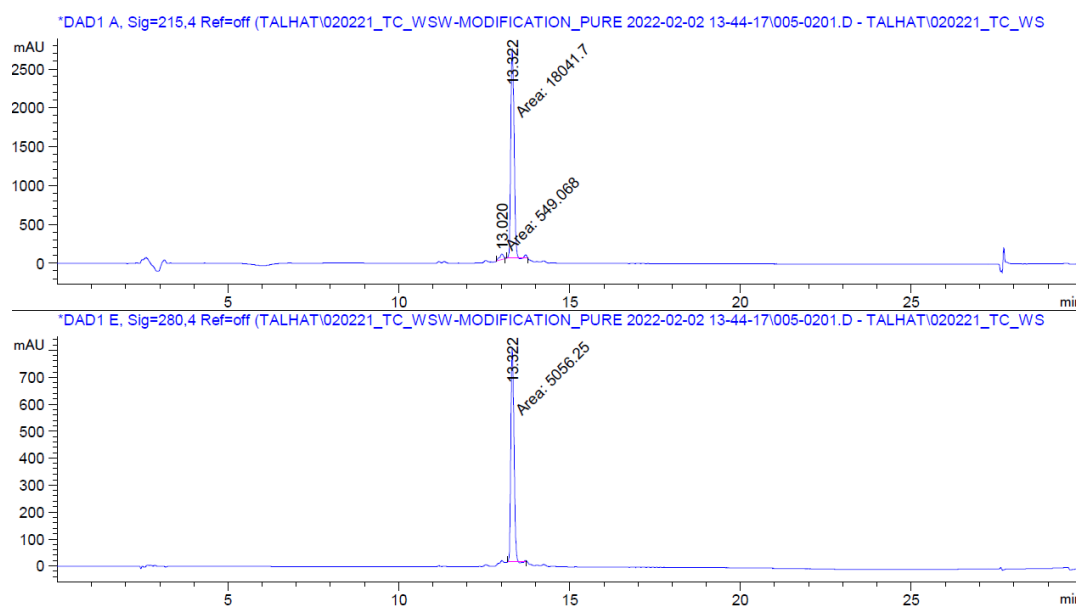


Figure S 1.17: Peptide 85. W7F (FITC-Ahx- SYPGWSF), peptide purity > 95%. Top HPLC spectra at 215 nm retention time 13.322 minutes. Bottom HPLC spectra at 280 nm retention time 13.322 minutes.

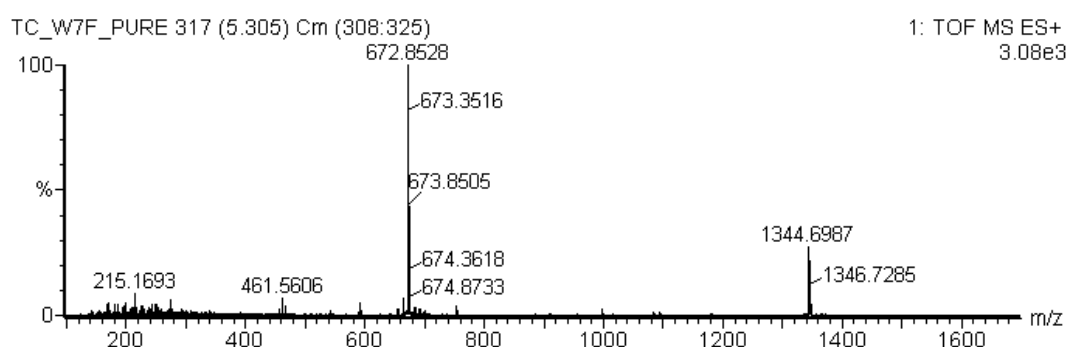


Figure S 1.18: Peptide 85. LC-MS data for W7F (FITC-Ahx-SYPGWSF). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1344 [M+H]⁺ and 672 [M+2H]⁺.

Peptide 86. W5F-W7F: FITC-Ahx-SYPGFSF

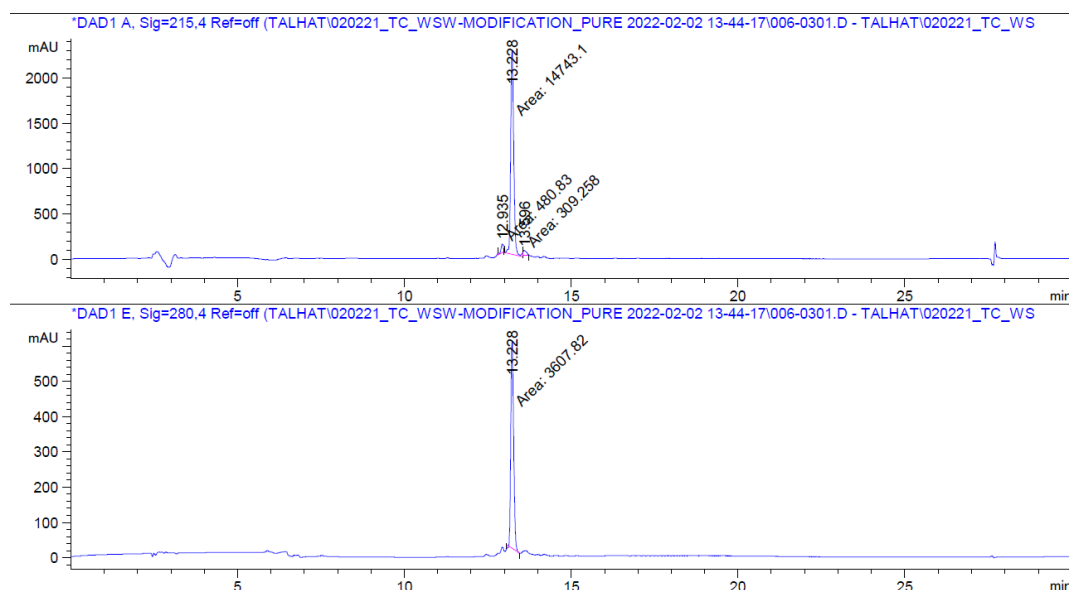


Figure S 1.19: Peptide 86. W5F-W7F (FITC-Ahx- SYPGFSF), peptide purity > 95%. Top HPLC spectra at 215 nm retention time 13.228 minutes. Bottom HPLC spectra at 280 nm retention time 13.228 minutes.

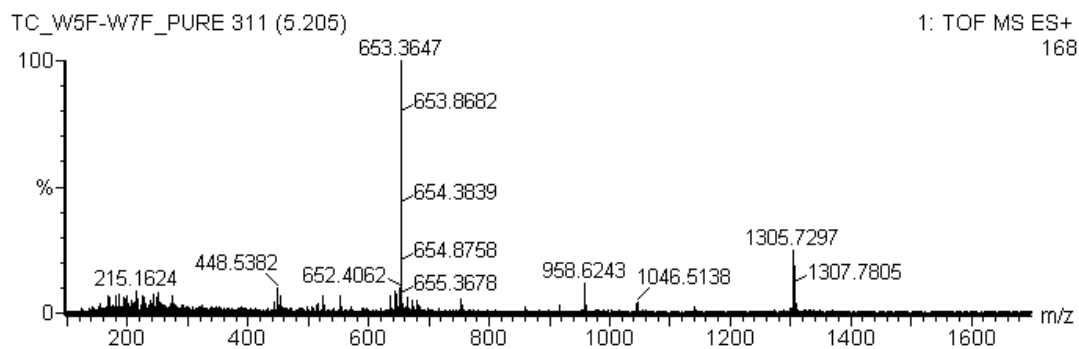


Figure S 1.20: Peptide 86. LC-MS data for W5F-W7F (FITC-Ahx-SYPGFSF). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1305 [M+H]⁺ and 653 [M+2H]⁺.

Peptide 87. Y2Cha: FITC-Ahx-S(Cha)PGWSW

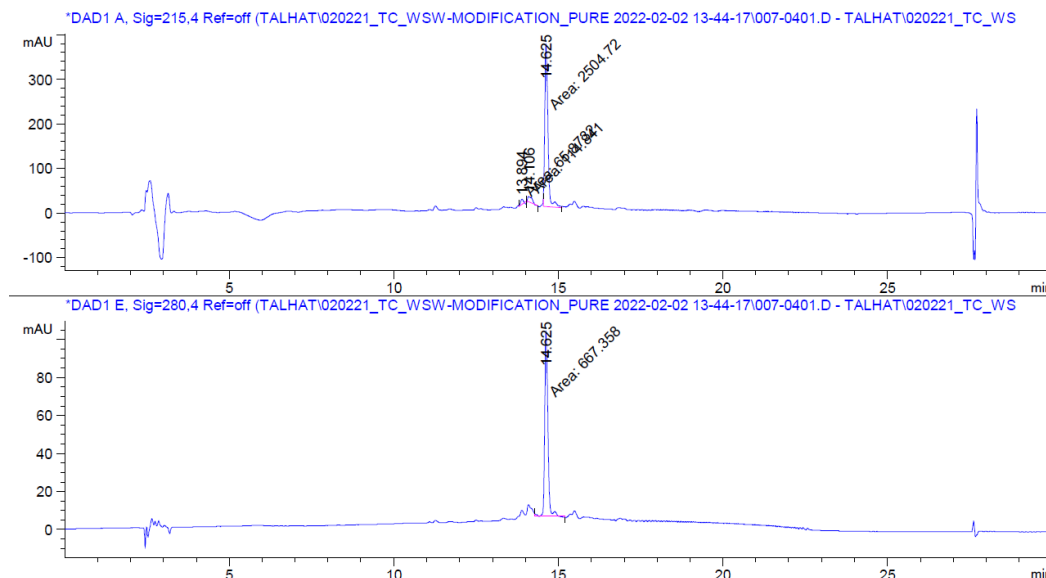


Figure S 1.21: Peptide 87. Y2Cha (FITC-Ahx- S(Cha)PGWSW), peptide purity 93%. Top HPLC spectra at 215 nm retention time 14.625 minutes. Bottom HPLC spectra at 280 nm retention time 14.625 minutes.

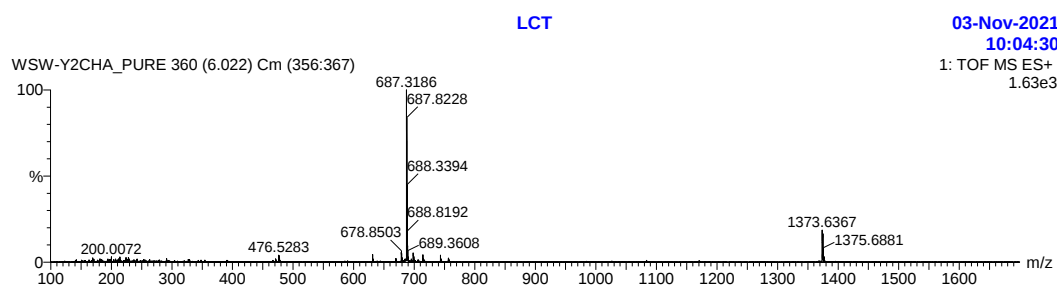


Figure S 1.22: Peptide 87. LC-MS data for Y2Cha (FITC-Ahx-S(Cha)PGWSW). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1373 $[M+H]^+$ and 687 $[M+2H]^+$.

Peptide 88. W5Cha: FITC-Ahx-SYPG(Cha)SW

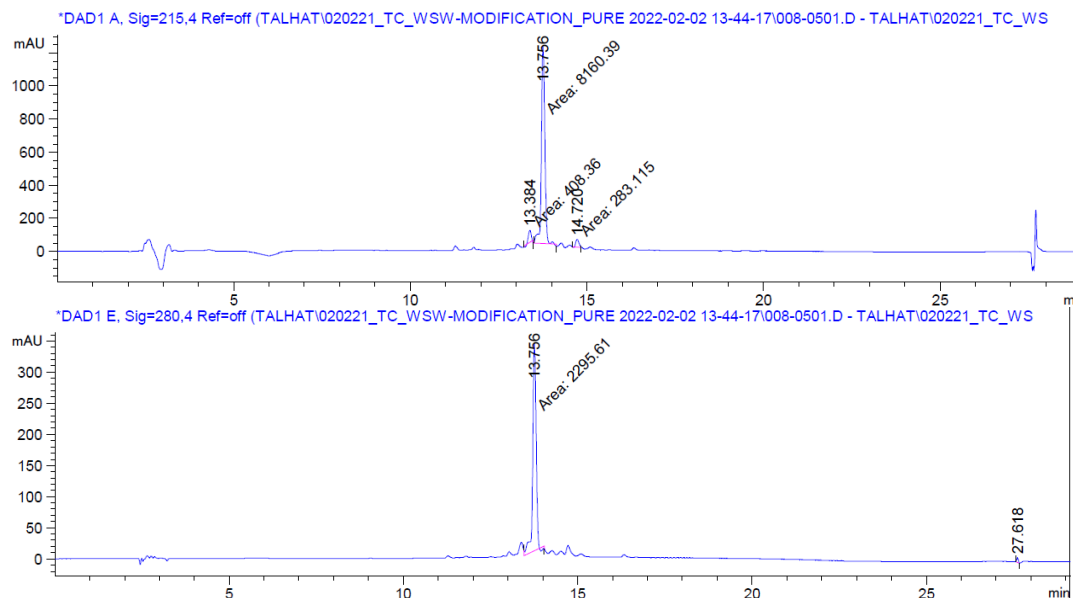


Figure S 1.23: Peptide 88. W5Cha (FITC-Ahx- SYPG(Cha)SW), peptide purity 92%. Top HPLC spectra at 215 nm retention time 13.756 minutes. Bottom HPLC spectra at 280 nm retention time 13.756 minutes.

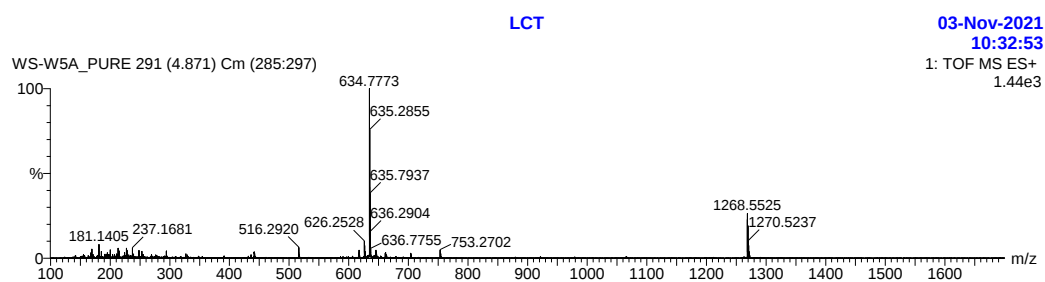


Figure S 1.24: Peptide 88. LC-MS data for W5Cha (FITC-Ahx-SYPG(Cha)SW). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1268 $[M+H]^+$ and 634 $[M+2H]^+$.

Peptide 89. W7Cha: FITC-Ahx-SYPGWS(Cha)

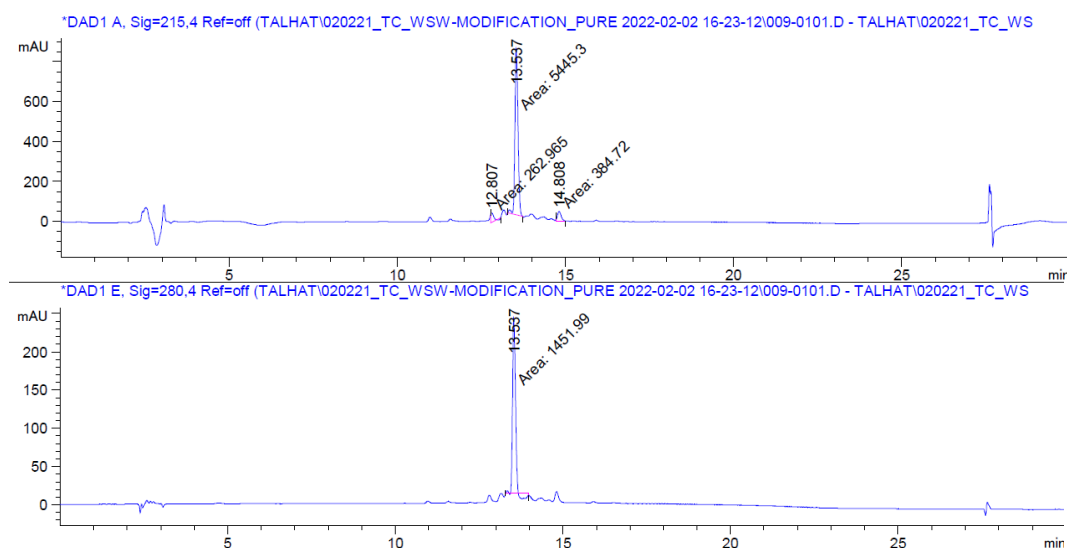


Figure S 1.25: Peptide 89. W7Cha (FITC-Ahx- SYPGWS(Cha)), peptide purity 90%. Top HPLC spectra at 215 nm retention time 13.756 minutes. Bottom HPLC spectra at 280 nm retention time 13.756 minutes.

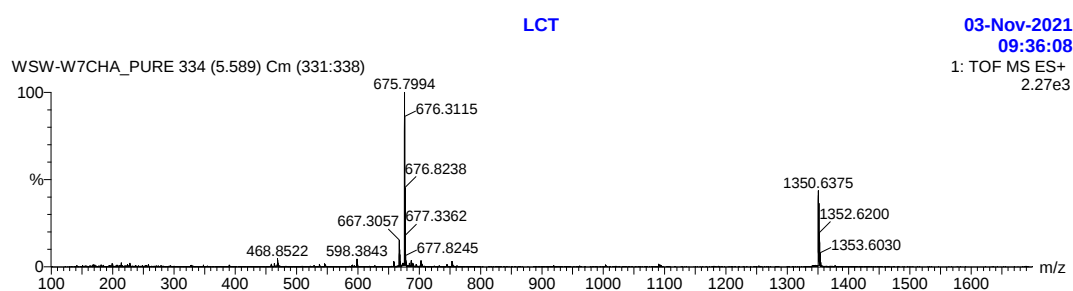


Figure S 1.26: Peptide 89. LC-MS data for W7Cha (FITC-Ahx-SYPGWS(Cha)). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1350 $[M+H]^+$ and 675 $[M+2H]^+$.

Peptide 90. W5Cha-W7Cha: FITC-Ahx-SYPG(Cha)S(Cha)

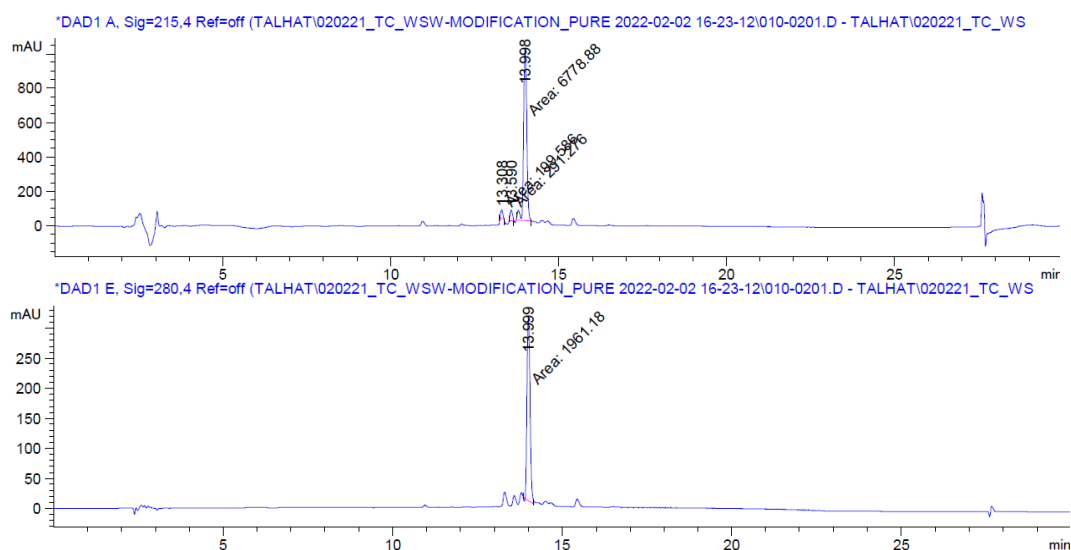


Figure S 1.27: Peptide 90. W5Cha-W7Cha (FITC-Ahx-SYPG(Cha)S(Cha)), peptide purity 93%. Top HPLC spectra at 215 nm retention time 13.998 minutes. Bottom HPLC spectra at 280 nm retention time 13.998 minutes.

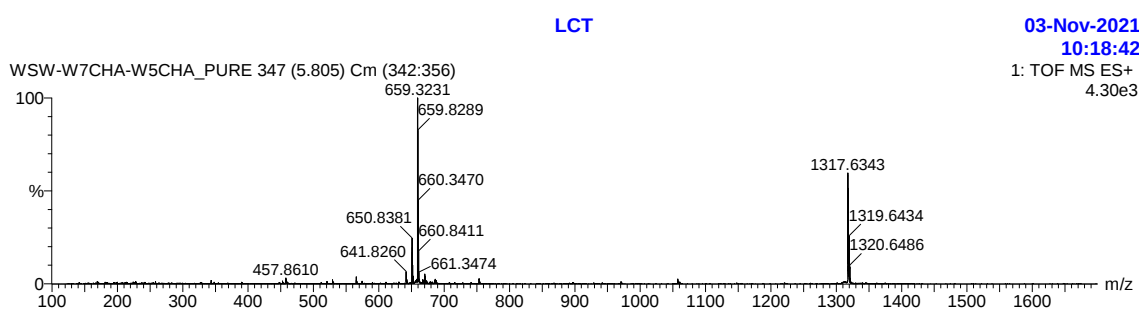


Figure S 1.28: Peptide 90. LC-MS data for W5Cha-W7Cha (FITC-Ahx-SYPG(Cha)S(Cha)). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1317 [M+H]⁺ and 659 [M+2H]⁺.

Peptide 91. Y2Nal: FITC-Ahx-S(Nal)PGWSW

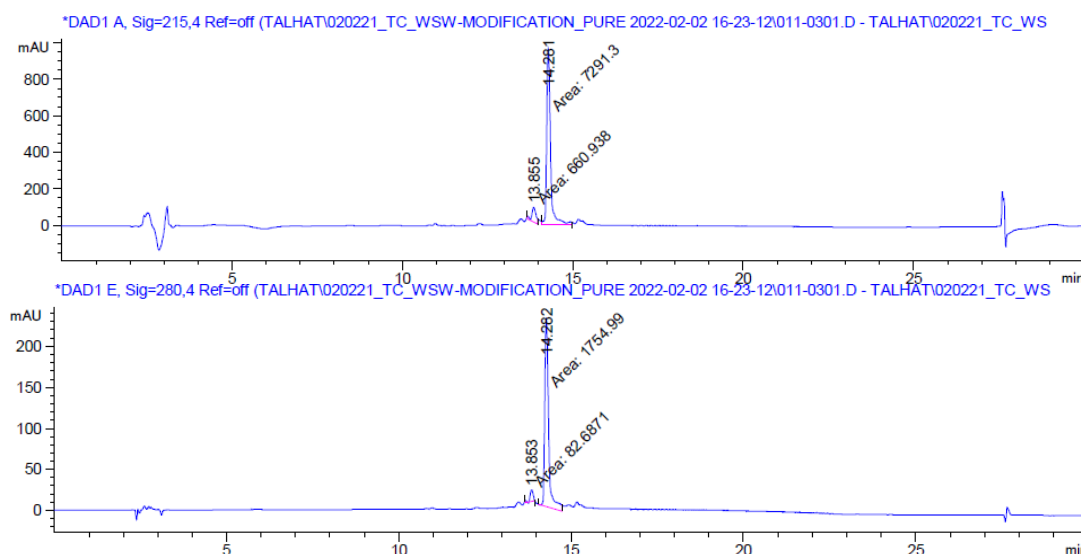


Figure S 1.29: Peptide 91. Y2Nal (FITC-Ahx- S(Nal)PGWSW), peptide purity 92%.
Top HPLC spectra at 215 nm retention time 14.282 minutes. Bottom HPLC spectra at 280 nm retention time 14.282 minutes.

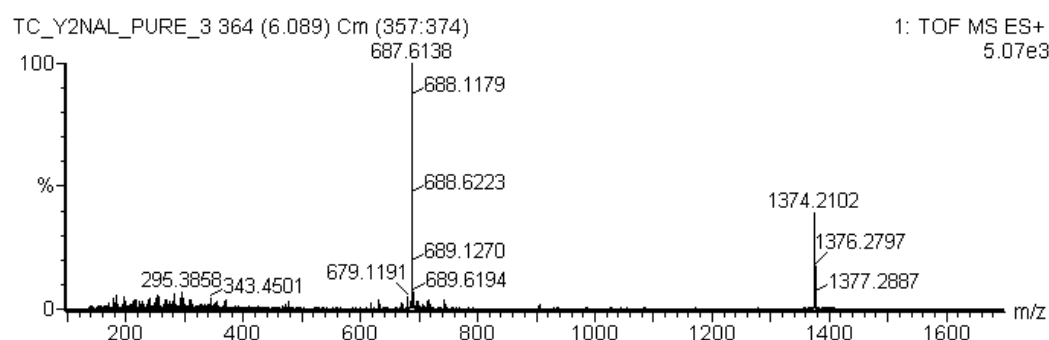


Figure S 1.30: Peptide 91. LC-MS data for Y2Cha (FITC-Ahx-S(Nal)PGWSW).
Extracted mass spectra showed expected mass-to-charge ratios: m/z 1374 [M+H]⁺ and 687 [M+2H]⁺.

Peptide 92. W5Nal: FITC-Ahx-SYPG(Nal)SW

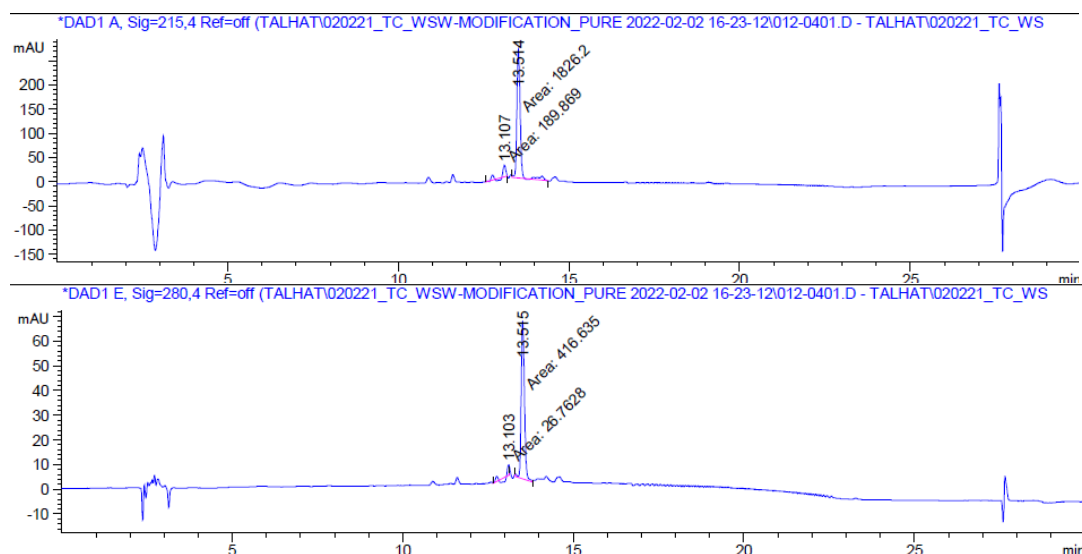


Figure S 1.31: Peptide 92. W5Nal (FITC-Ahx- SYPG(Nal)SW), peptide purity 91%. Top HPLC spectra at 215 nm retention time 13.314 minutes. Bottom HPLC spectra at 280 nm retention time 13.315 minutes.

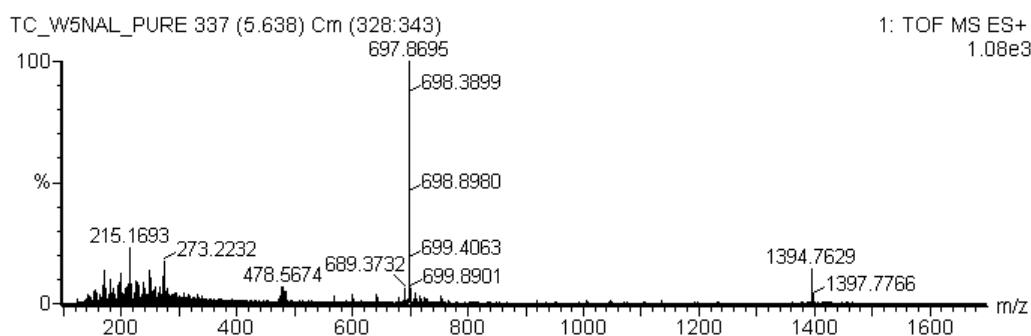


Figure S 1.32: Peptide 92. LC-MS data for W5Cha (FITC-Ahx-SYPG(Nal)SW). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1394 [M+H]⁺ and 697 [M+2H]⁺ (bottom).

Peptide 93. W7Cha: FITC-Ahx-SYPGWS(Cha)

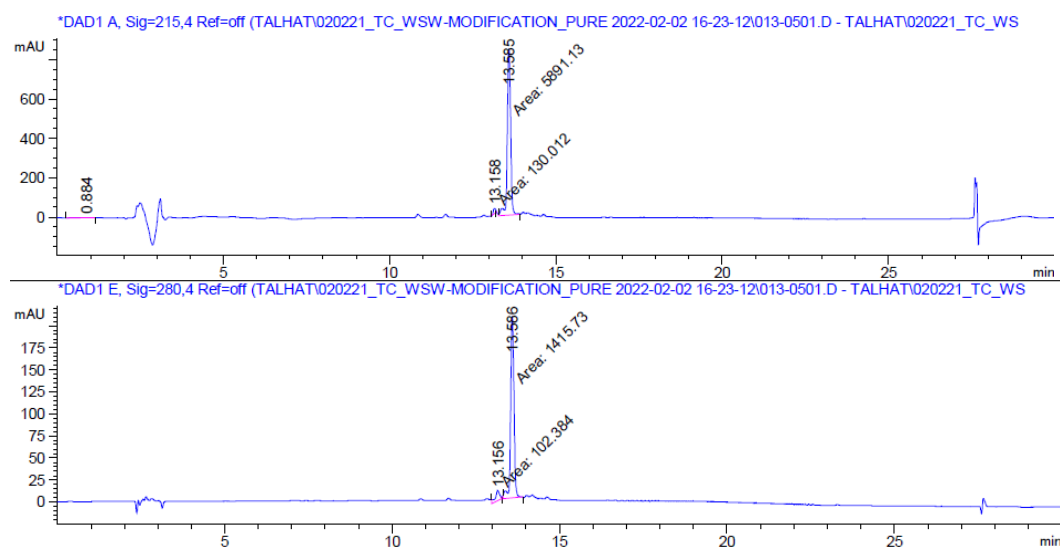


Figure S 1.33: Peptide 93. W7Cha (FITC-Ahx- SYPGWS(Cha), peptide purity > 95%. Top HPLC spectra at 215 nm retention time 13.585 minutes. Bottom HPLC spectra at 280 nm retention time 13.586 minutes.

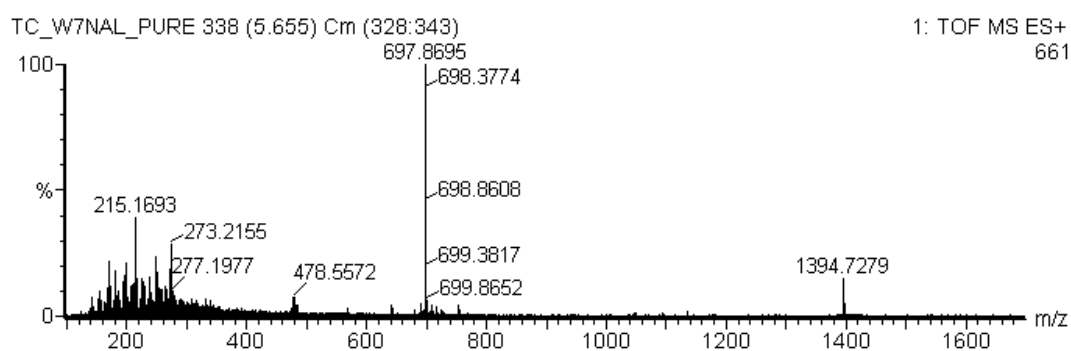


Figure S 1.34: Peptide 93. LC-MS data for W7NaI (FITC-Ahx-SYPGWS(NaI) HPLC spectra (top); Extracted mass spectra showed expected mass-to-charge ratios: m/z 1394 [M+H]⁺ and 697 [M+2H]⁺ (bottom).

Peptide 94. W5Nal-W7Nal: FITC-Ahx-SYPG(Nal)S(Nal)

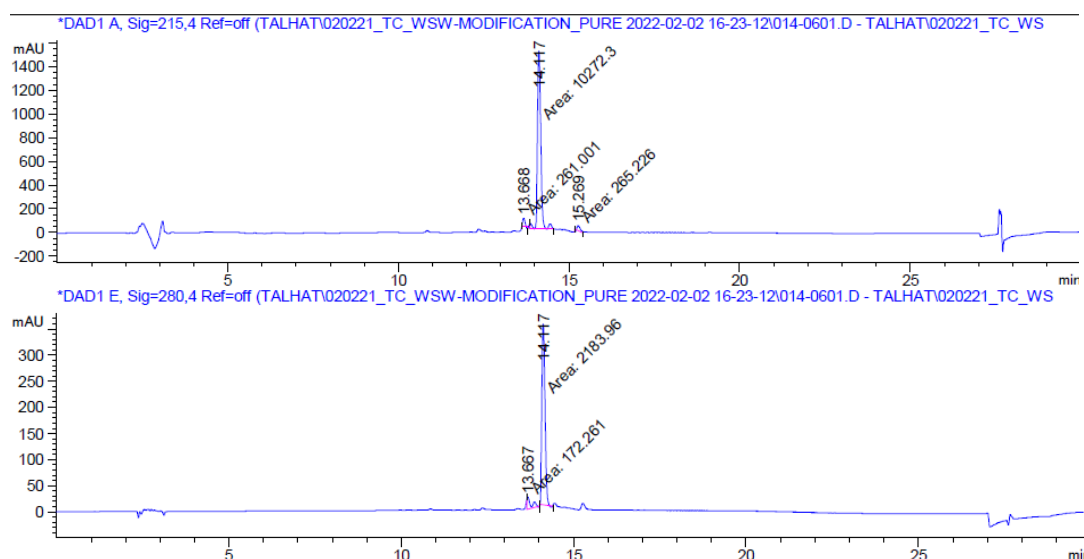


Figure S 1.35: Peptide 94. W5Nal-W7Nal (FITC-Ahx-SYPG(Nal)S(Nal)), peptide purity > 95%. Top HPLC spectra at 215 nm retention time 14.117 minutes. Bottom HPLC spectra at 280 nm retention time 14.117 minutes.

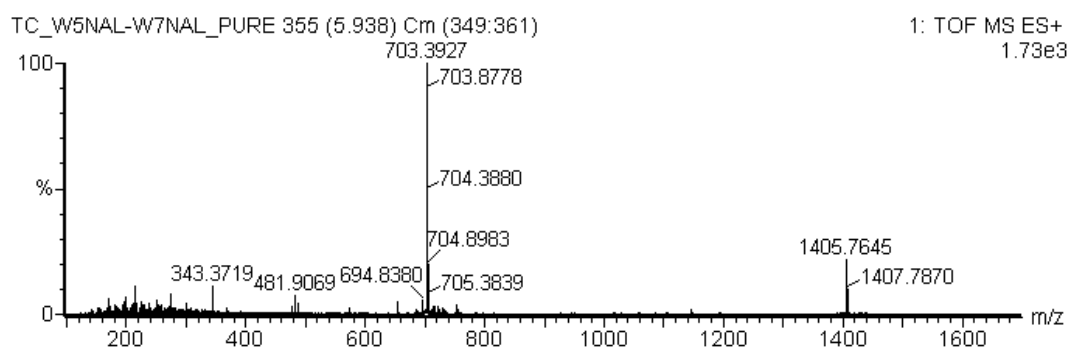


Figure S 1.36: Peptide 94. LC-MS data for W5Cha-W7Cha (FITC-Ahx-SYPG(Nal)S(Nal)). Extracted mass spectra showed expected mass-to-charge ratios: m/z 1405 [M+H]⁺ and 703 [M+2H]⁺.

1.2. Chapter 3 HPLC and LC-MS Characterisation

Peptide 49. PIMP21-01: ALWPPNLHAWVP

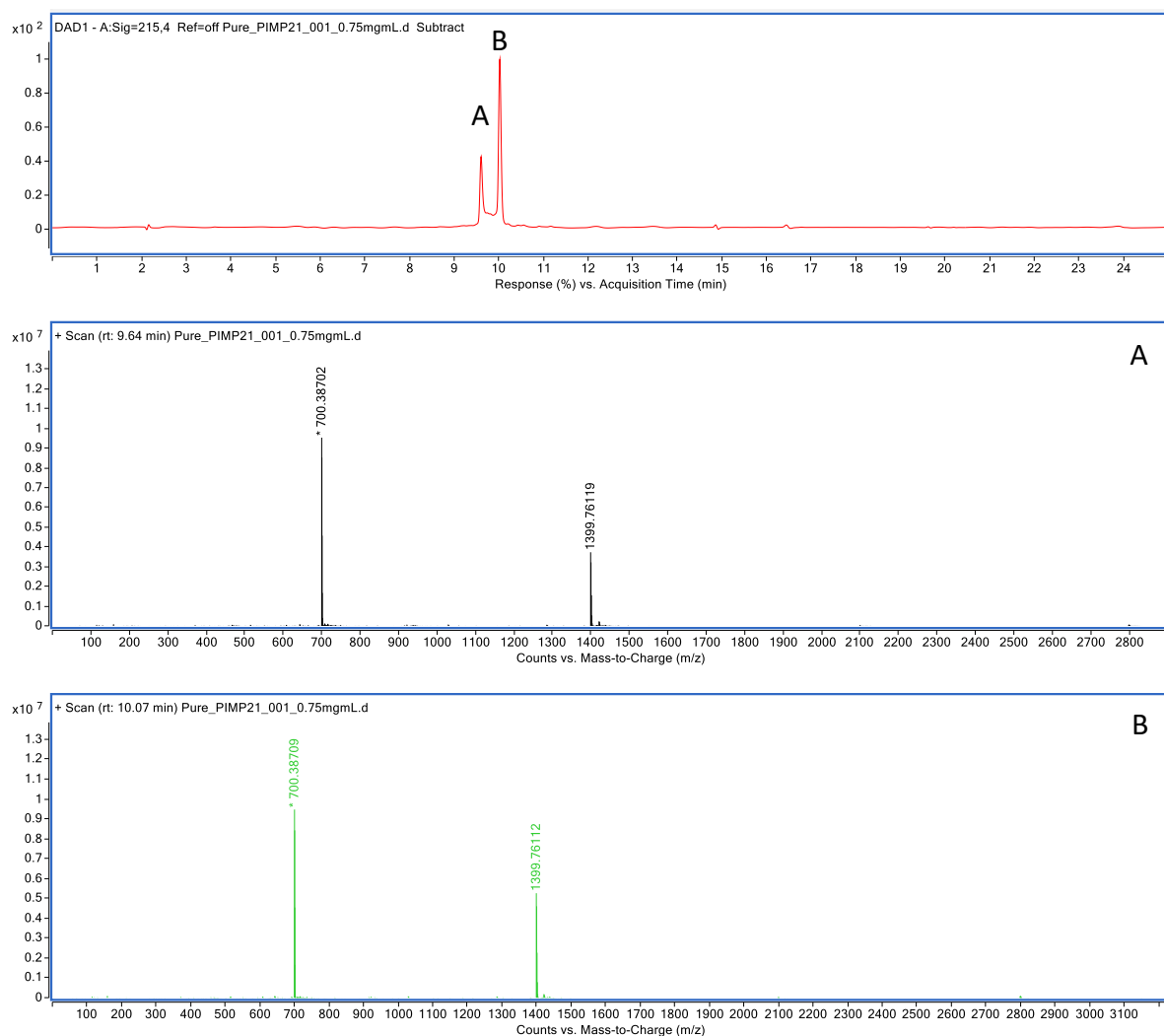


Figure S 1.37: The analysis of prep-HPLC purified PIMP21-01. HPLC (top) and extracted mass spectra for peaks **A** and **B** (bottom). Expected m/z of PIMP21-01 was 1399 $[M+H]^+$ or 700 $[M+2H]^+$.

Peptide 95. Scrambled PIMP21: LPPAPWNWAHVL

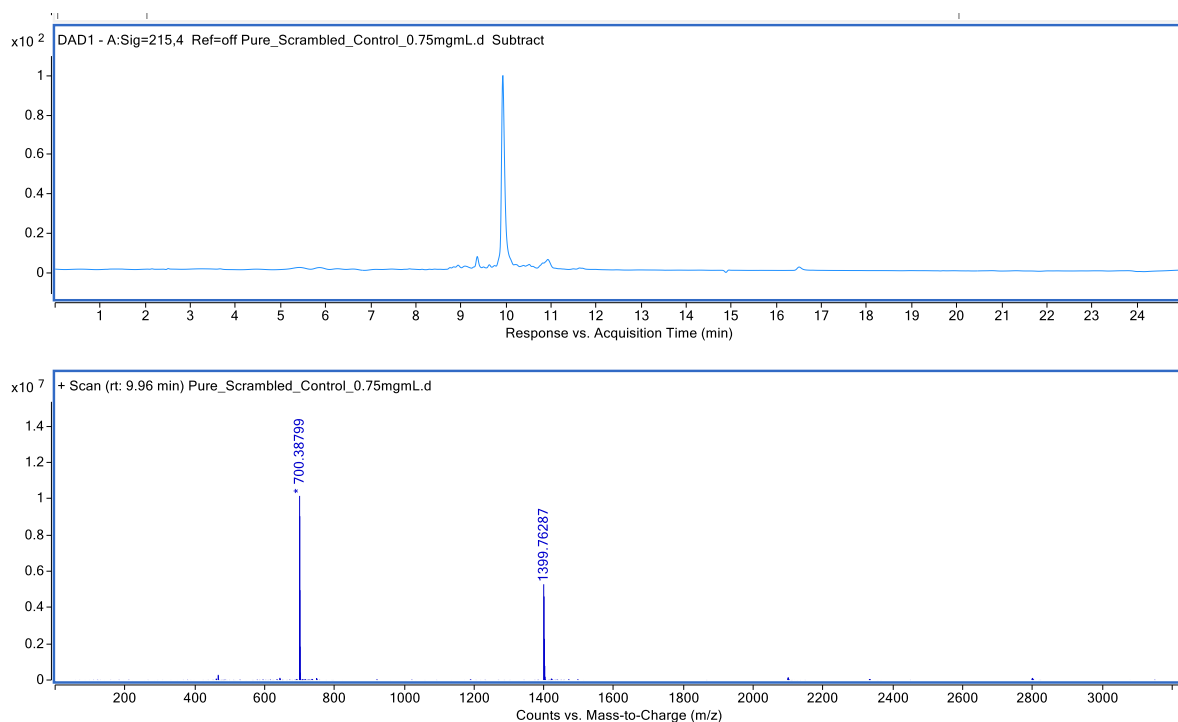


Figure S 1.38: The analysis of the purified scrambled peptide. HPLC (top) and extracted mass spectra (bottom), of the purified scrambled control, expected m/z 1399 $[M+H]^+$ or 700 $[M+2H]^+$.

Peptide 96. PIMP21-02: AAWPPNLHAWVP

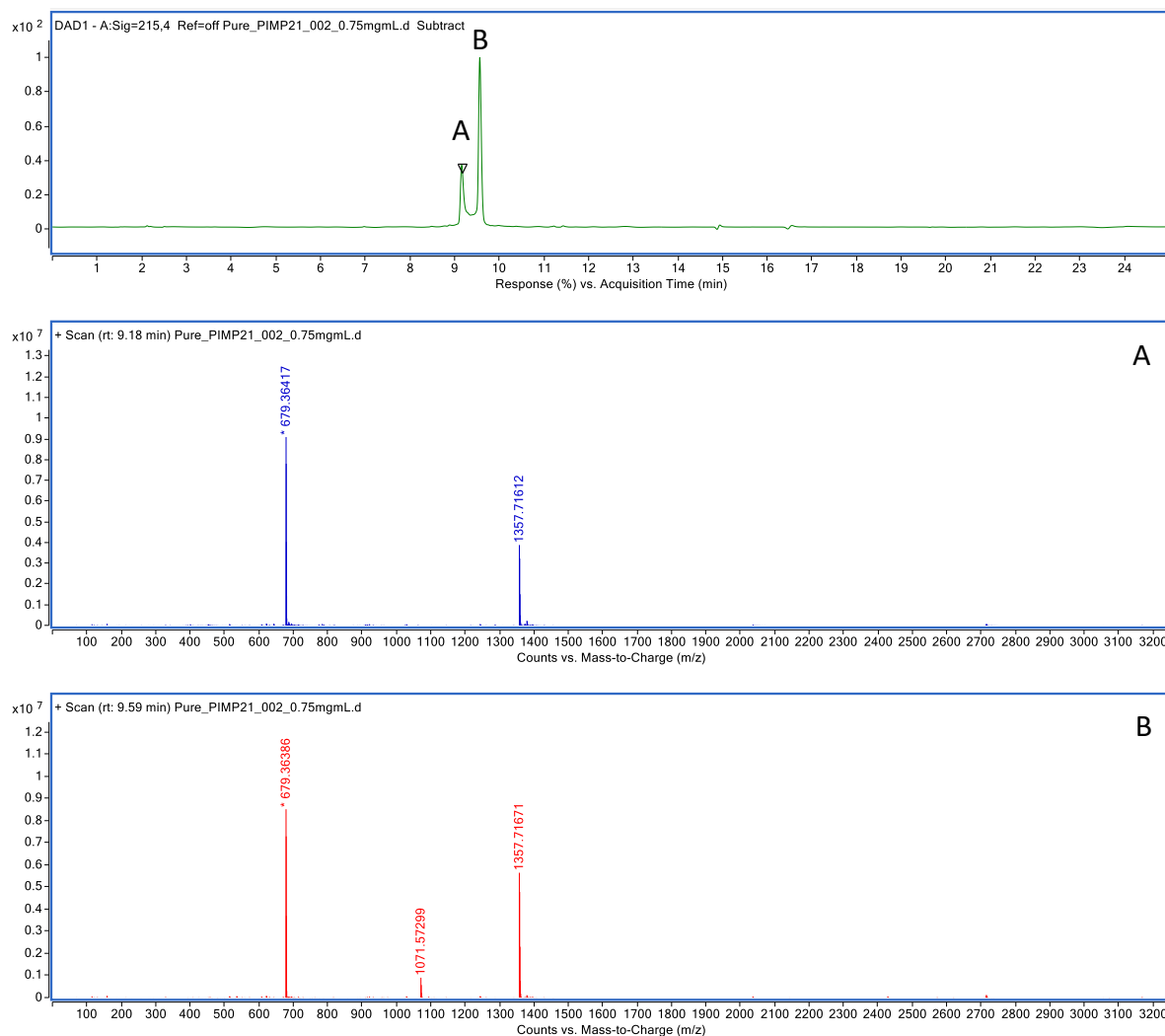


Figure S 1.39: The analysis of prep-HPLC purified PIMP21-02. HPLC (top) and extracted mass spectra for peaks **A** and **B** (bottom). Expected m/z of PIMP21 was 1357 [M+H]⁺ or 679 [M+2H]⁺.

Peptide 97. PIMP21-03: ALAP**PNLHAWVP**

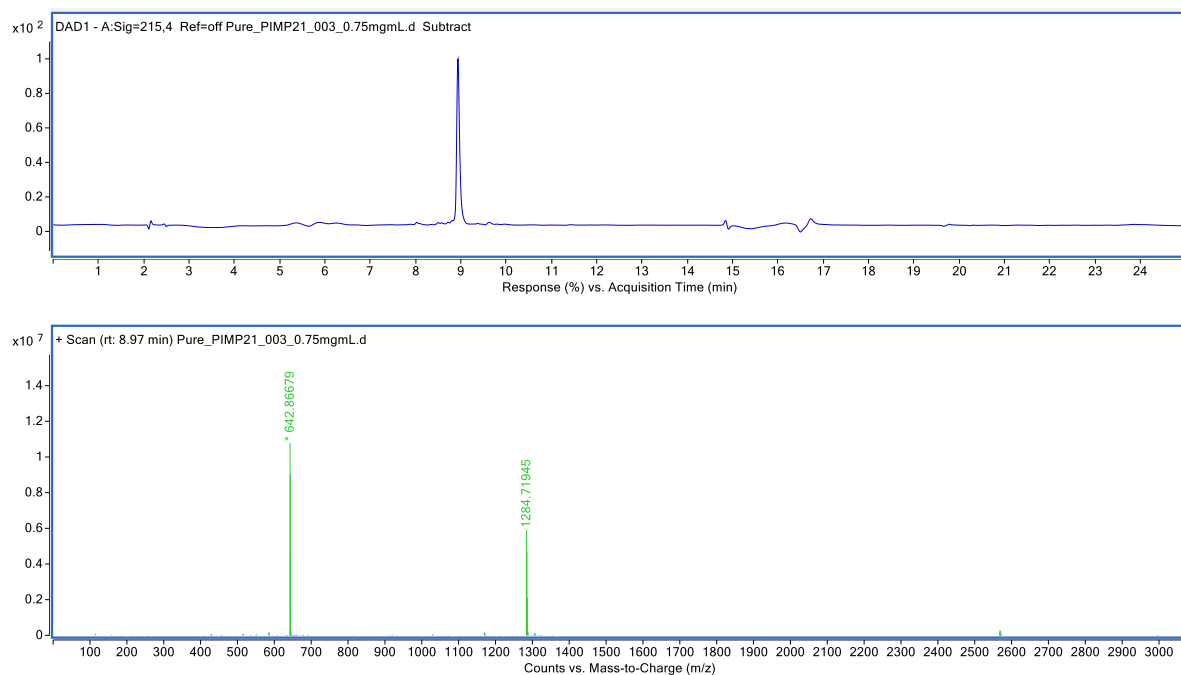


Figure S 1.40: The analysis of purified PIMP21-02. HPLC (top) and extracted mass spectra (bottom), of the purified PIMP21-03, expected m/z 1284 $[M+H]^+$ or 624 $[M+2H]^+$.

Peptide 98. PIMP21-04: ALWAPNLHAWVP

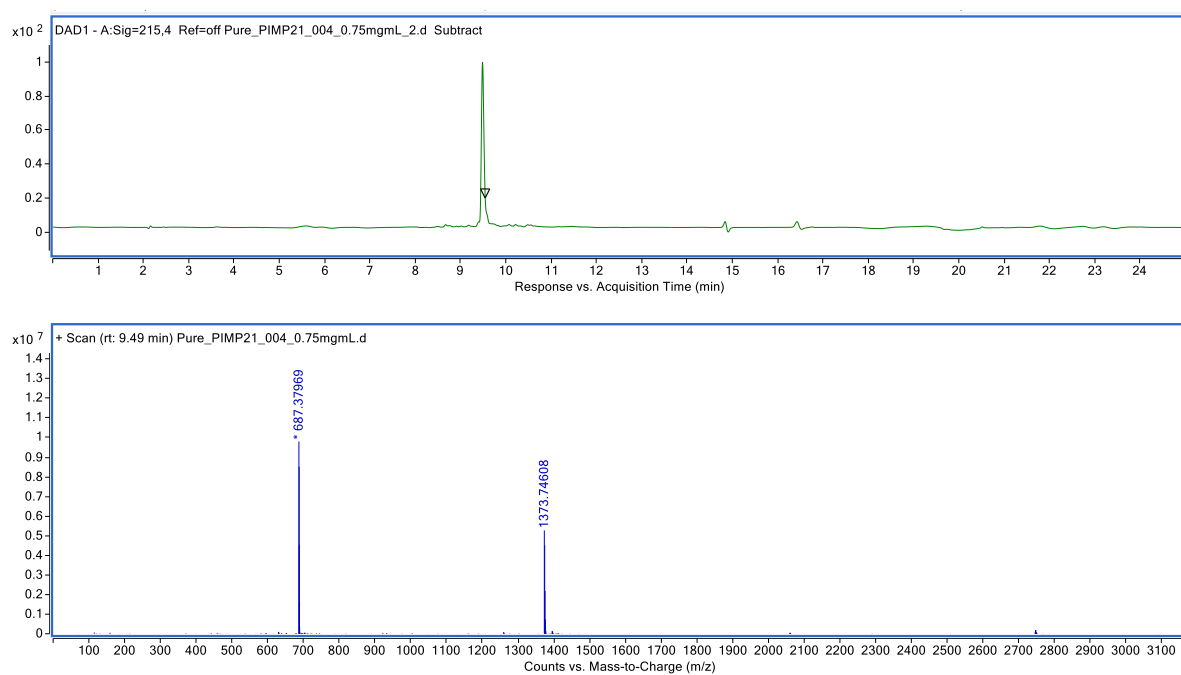


Figure S 1.41: The analysis of purified peptide PIMP21-04. HPLC (top) and extracted mass spectra (bottom), of the purified PIMP21-04, expected m/z 1373 $[M+H]^+$ or 687 $[M+2H]^+$.

Peptide 99. PIMP21-05: ALWPA^WNLHAWVP

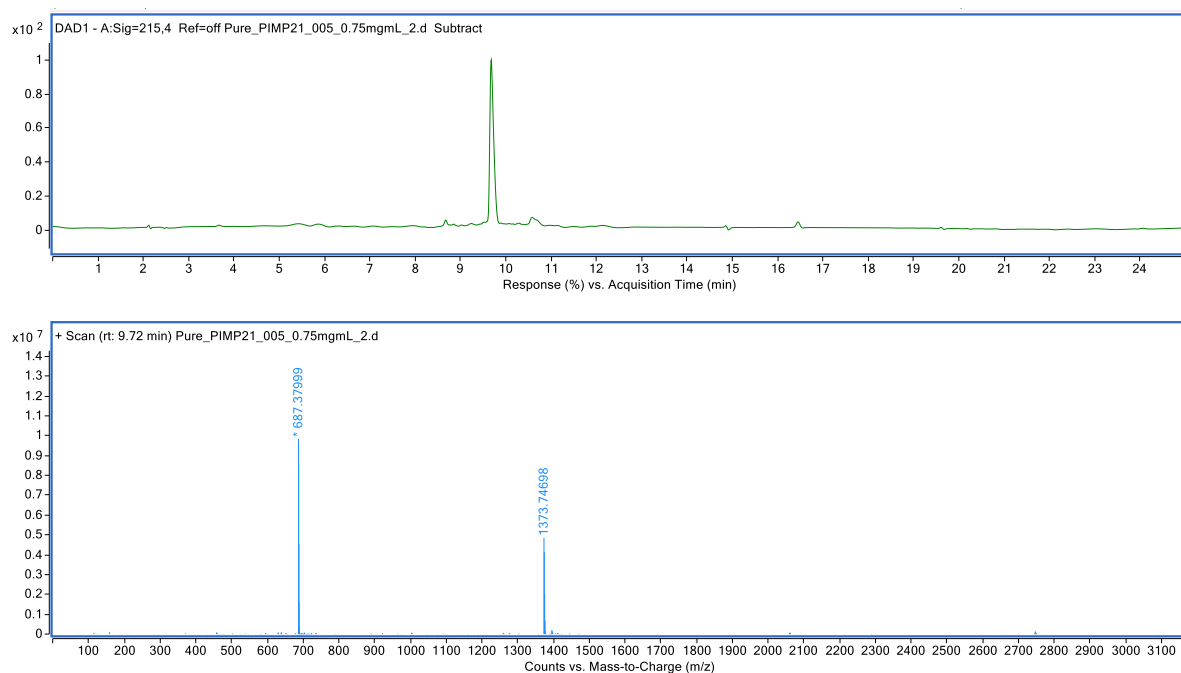


Figure S 1.42: The analysis of purified PIMP21-05 peptide. HPLC (top) and extracted mass spectra (bottom), of the purified PIMP21-05, expected m/z 1373 $[M+H]^+$ or 687 $[M+2H]^+$.

Peptide 100. PIMP21-06: ALWPPALHAWVP

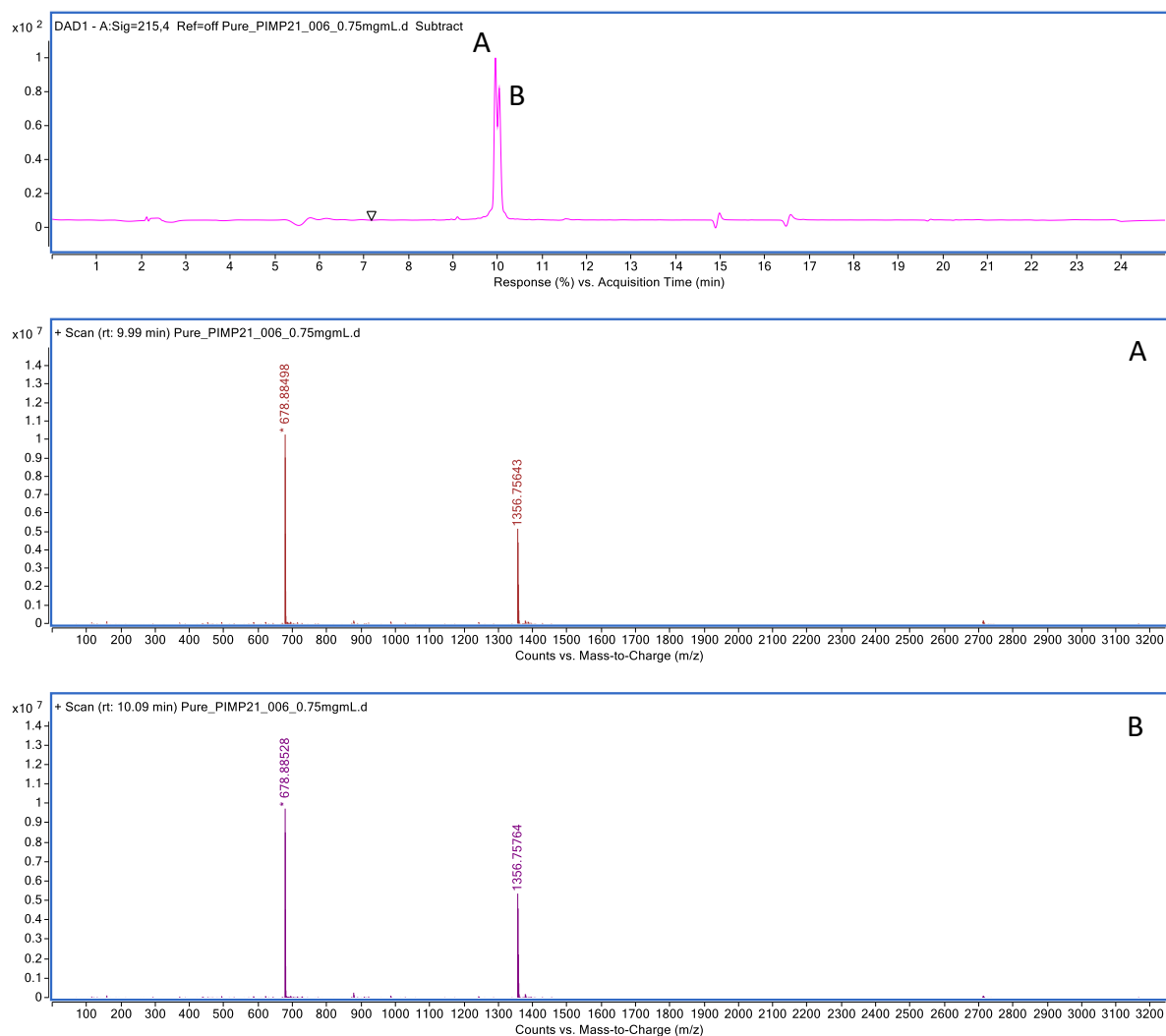


Figure S 1.43: The analysis of prep-HPLC purified peptide PIMP21-06. HPLC (top) and extracted mass spectra for peaks A and B (bottom). Expected m/z of PIMP21 was 1356 $[M+H]^+$ or 678 $[M+2H]^+$.

Peptide 101. PIMP21-07: ALWPPNA**HAWVP**

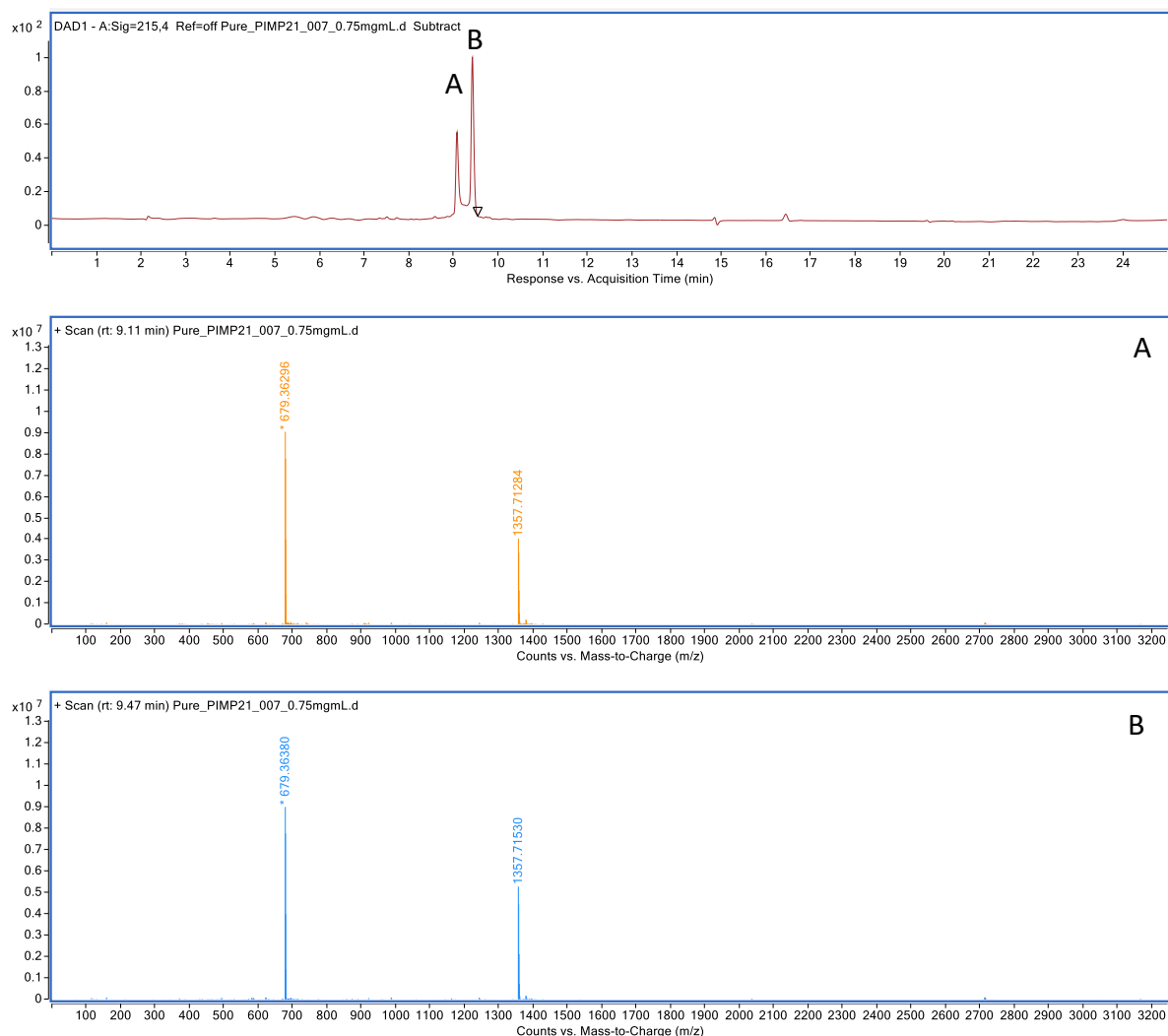


Figure S 1.44: The analysis of prep-HPLC purified peptide PIMP21-07. HPLC (top) and extracted mass spectra for peaks A and B (bottom). Expected m/z of PIMP21 was 1357 [M+H]⁺ or 679 [M+2H]⁺.

Peptide 102. PIMP21-08: ALWPPNLA^WAWVP

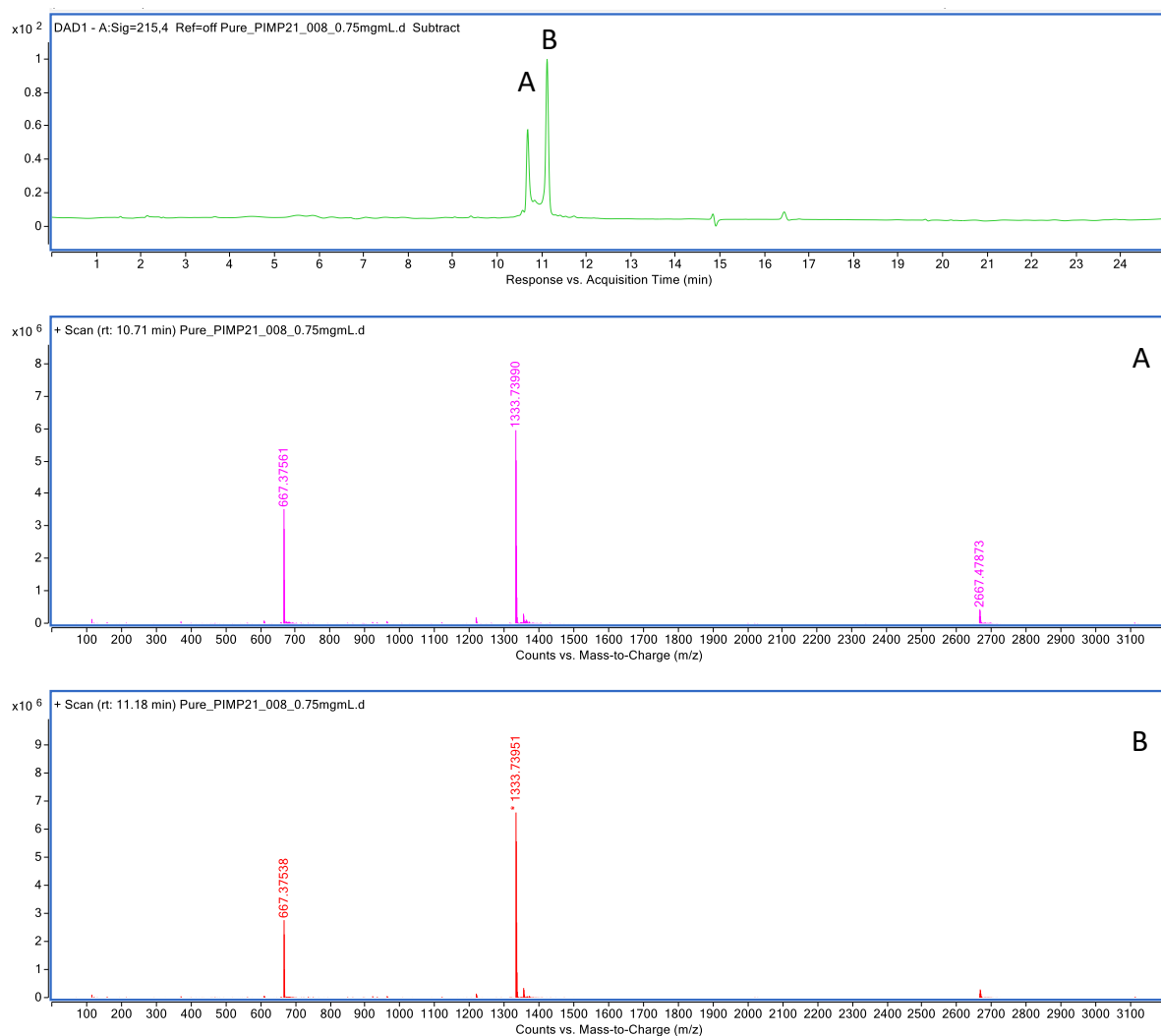


Figure S 1.45: The analysis of prep-HPLC purified PIMP21-08. HPLC (top) and extracted mass spectra for peaks A and B (bottom). Expected m/z of PIMP21 was 1333 [M+H]⁺ or 678 [M+2H]⁺.

Peptide 103. PIMP21-09: ALWPPNLHA^{VP}

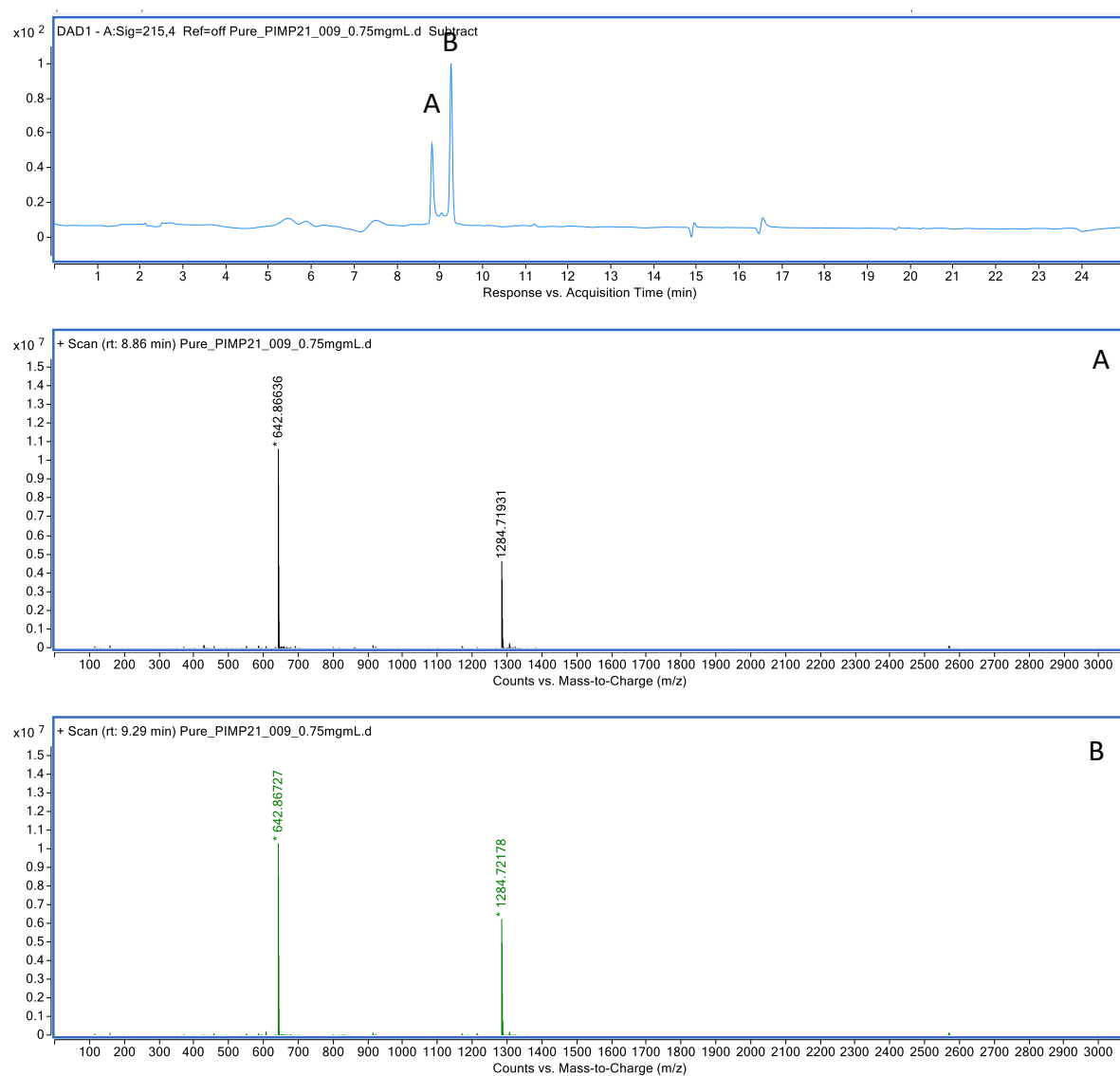


Figure S 1.46: The analysis of prep-HPLC purified peptide PIMP21-09. HPLC (top) and extracted mass spectra for peaks A and B (bottom). Expected m/z of PIMP21 was 1284 [M+H]⁺ or 642 [M+2H]⁺.

Peptide 104. PIMP21-10: ALWPPNLHAWAP

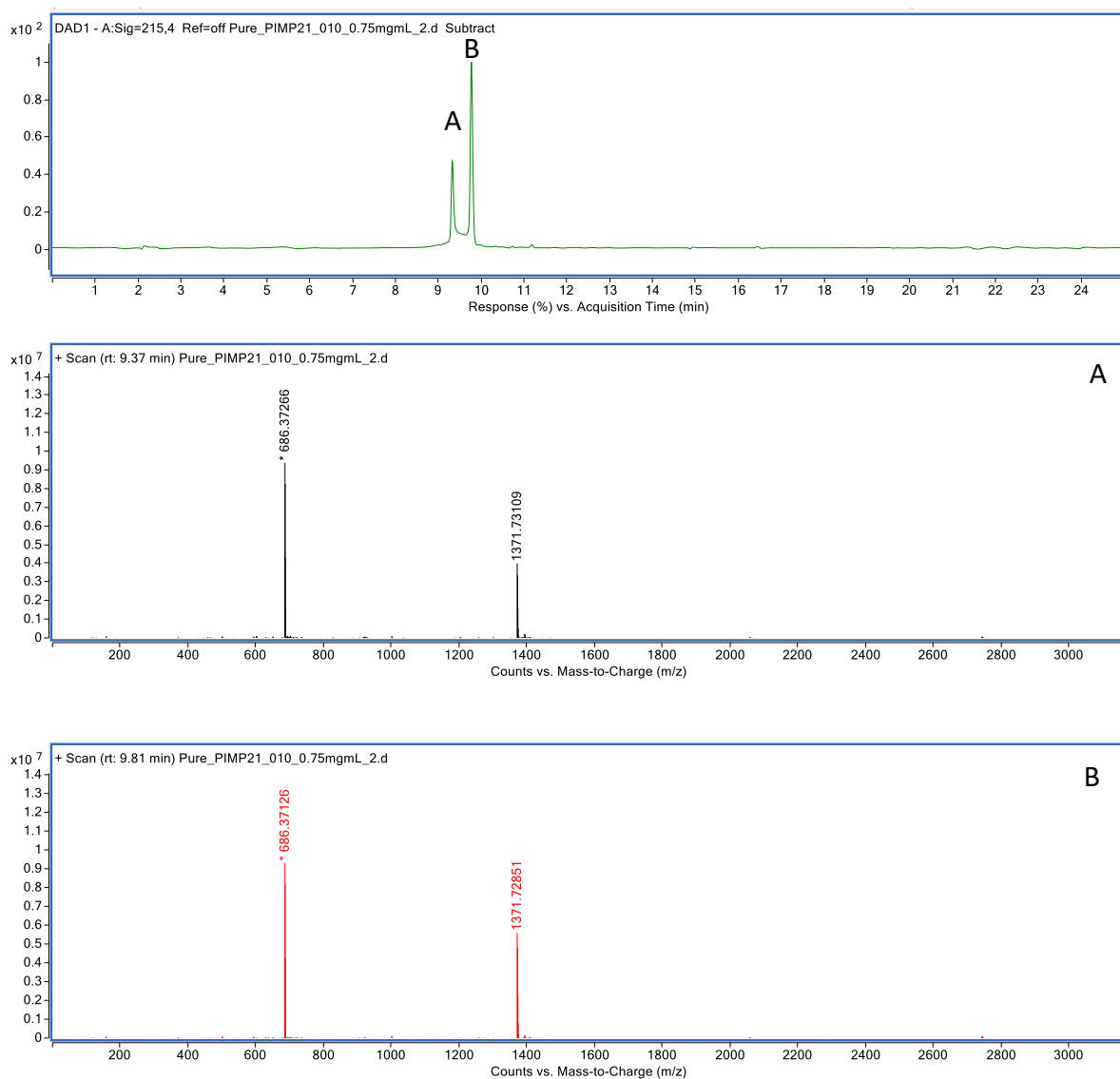


Figure S 1.47: The analysis of prep-HPLC purified peptide PIMP21-10. HPLC (top) and extracted mass spectra for peaks A and B (bottom). Expected m/z of PIMP21 was 1371 [M+H]⁺ or 686 [M+2H]⁺.

Peptide 105. PIMP21-11: ALWPPNLHAWVA

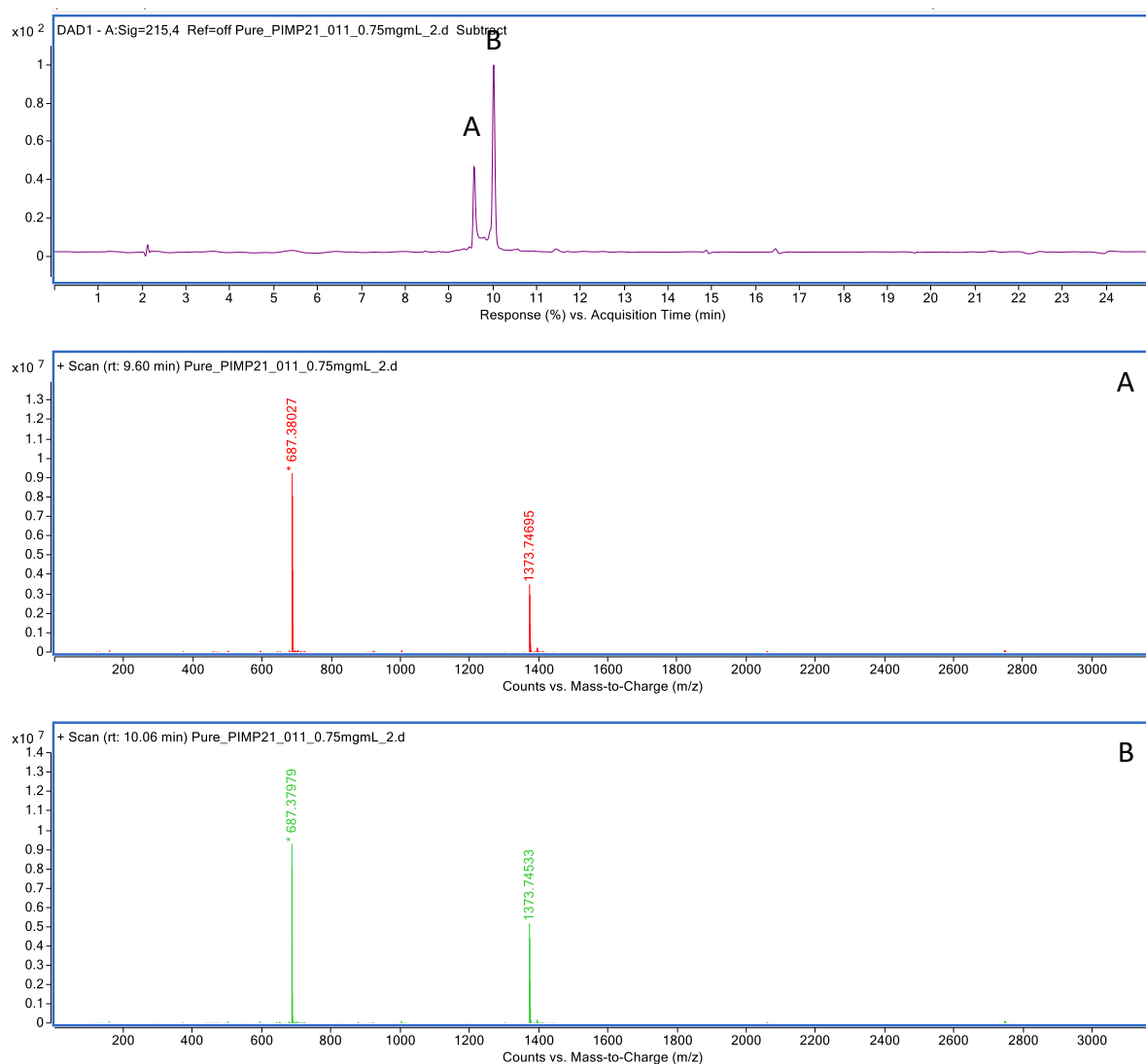


Figure S 1.48: The analysis of prep-HPLC purified peptide PIMP21-11. HPLC (top) and extracted mass spectra for peaks A and B (bottom). Expected m/z of PIMP21 was 1373 [M+H]⁺ or 687 [M+2H]⁺.

Peptide 106. PIMP21-FITC: FITC-AHX-ALWPPNLHAWVP

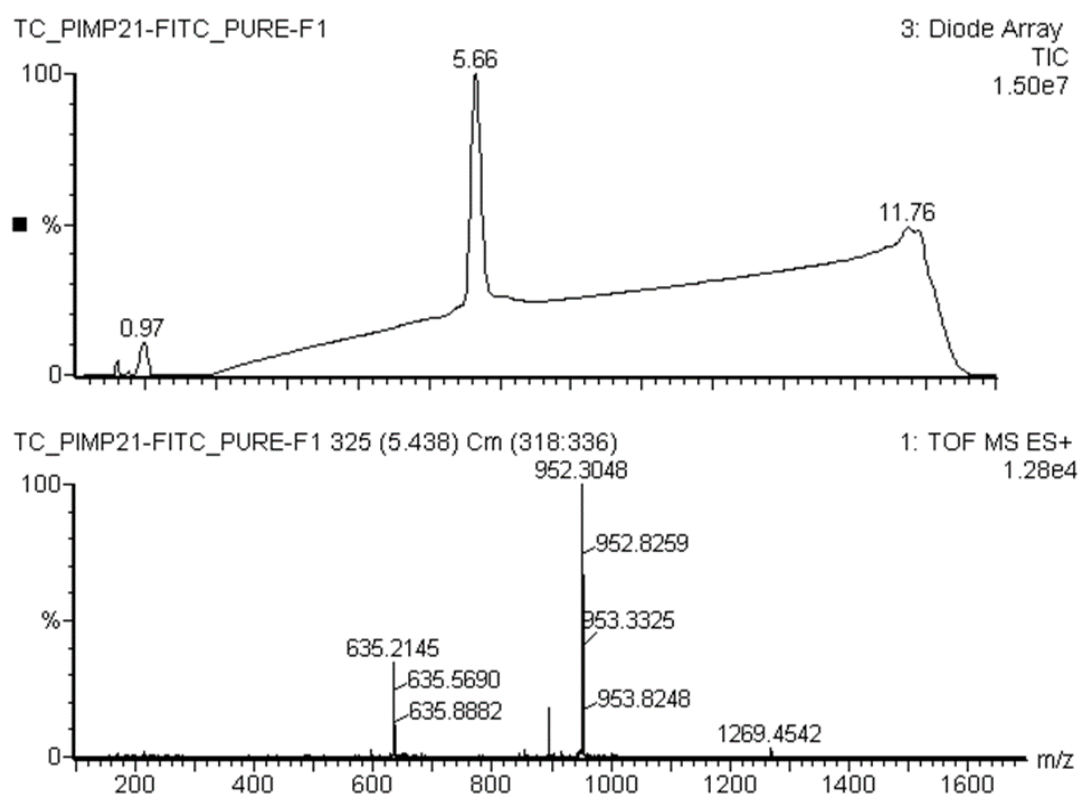


Figure S 1.49: The analysis of prep-HPLC purified peptide 106. HPLC (top) and extracted mass spectra for peaks A and B (bottom). Expected m/z of the peptide was 1269.4542 $[M+H]^+$ or 952.3048 $[M+2H]^+$.

Peptide 107. 4-Fluoro Benzoyl-PIMP21: 4-Fluoro Benzoyl-ALWPPNLHAWVP

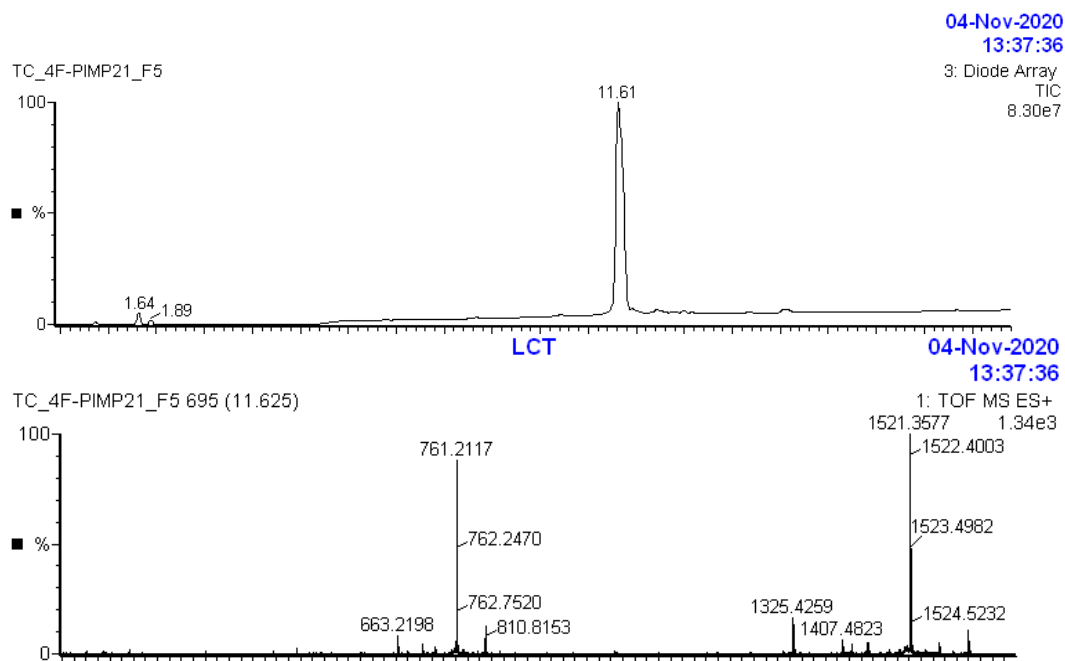


Figure S 1.50: The analysis of purified peptide 107, 4-Fluoro Benzoyl-PIMP21. HPLC (top) and extracted mass spectra (bottom). Expected m/z of PIMP21 was 1521 $[M+H]^+$ or 761 $[M+2H]^+$.

Peptide 108. 4-Fluoro Benzoyl-PIMP21 Scrambled: 4-Fluoro Benzoyl-LPPAPWNWAHVL

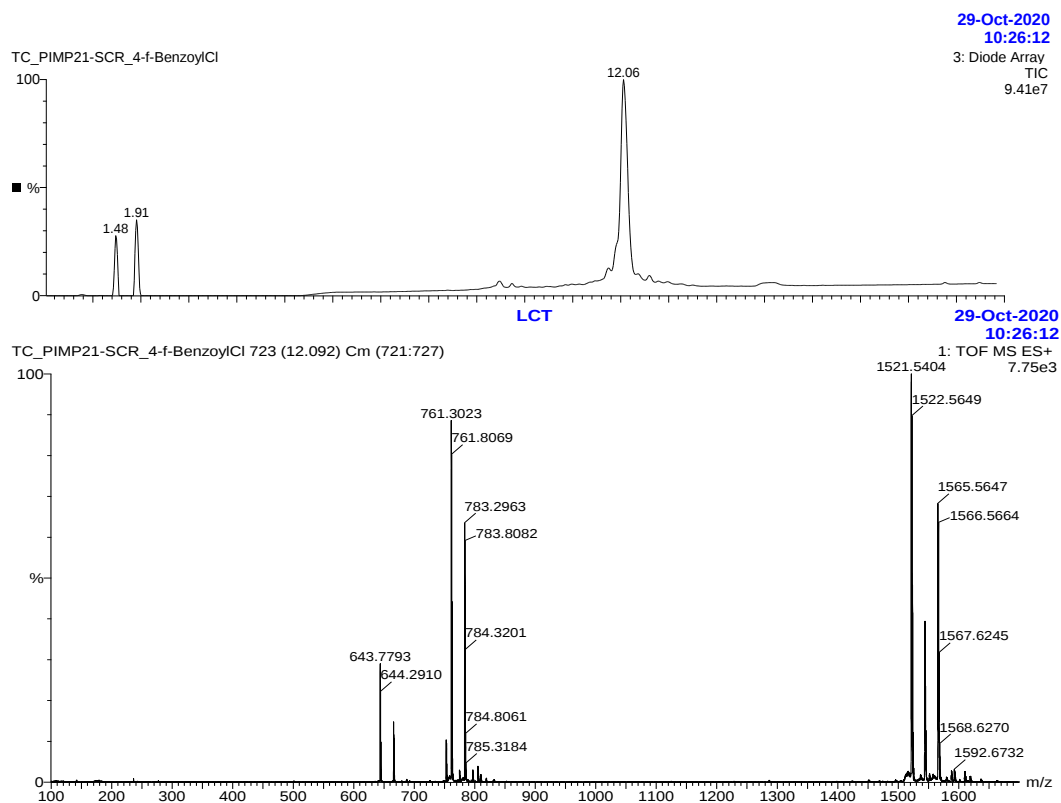


Figure S 1.51: The analysis of purified peptide 108, 4-Fluoro Benzoyl-PIMP21 Scrambled. HPLC (top) and extracted mass spectra (bottom). Expected m/z of PIMP21 was 1521 $[M+H]^+$ or 761 $[M+2H]^+$.

Peptide 109. WSW-PIMP21: SYPGWSW-Ahx-K-Ahx-ALWPPNLHAWVP

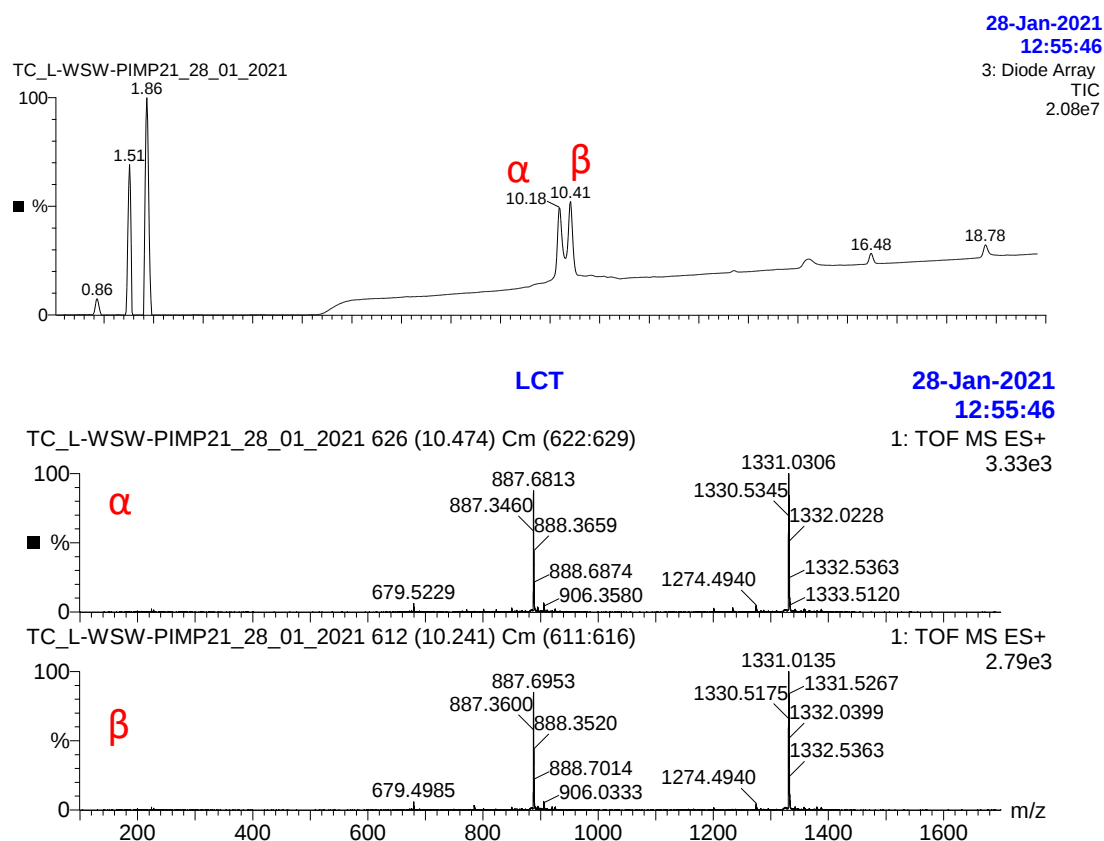


Figure S 1.52: Analytical data for L-WSW-PIMP21, peptide 109. (A) HPLC spectra for L-WSW-PIMP21; (B) Analysis of peaks (α,β) showed expected mass-to-charge ratios: m/z 1331 $[M+2H]^+$ and 887 $[M+3H]^+$ for both peaks. Indicating the potential presence of conformers.

Peptide 110. WSW-PIMP21 Scrambled: SYPGWSW-Ahx-K-Ahx-LPPAPWNWAHVL

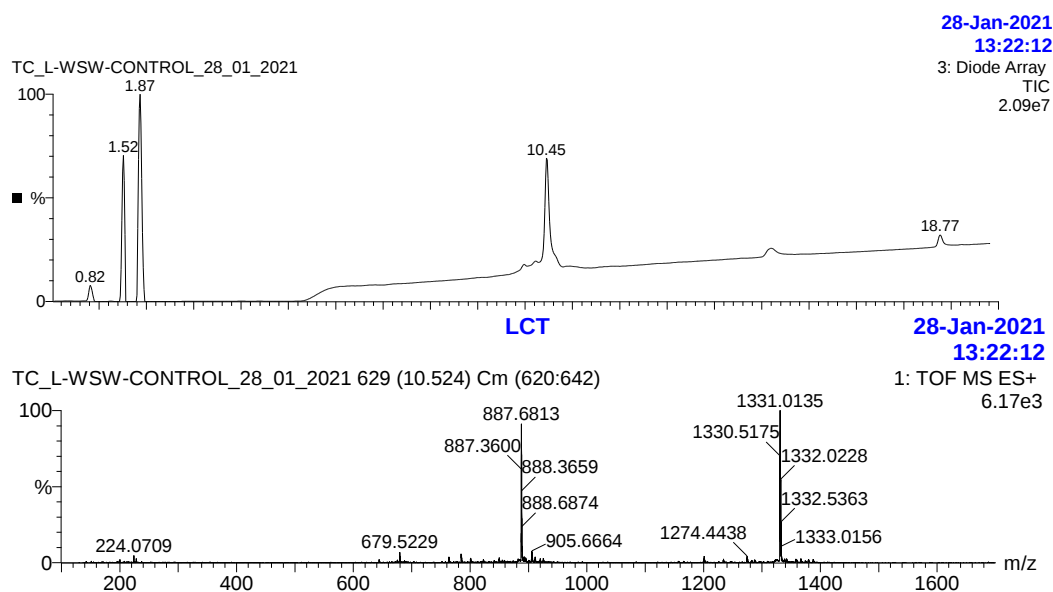


Figure S 1.53: Analytical data for L-WSW-Scrambled PIMP21, peptide 110. (A) HPLC spectra for L-WSW-Scrambled control; (B) Analysis of peaks (α , β) showed expected mass-to-charge ratios: m/z 1331 $[M+2H]^+$ and 887 $[M+3H]^+$.

Peptide 111. WSW-PIMP21 (FITC): SYPGWSW-Ahx-K(FITC)-Ahx-ALWPPNLHAWVP

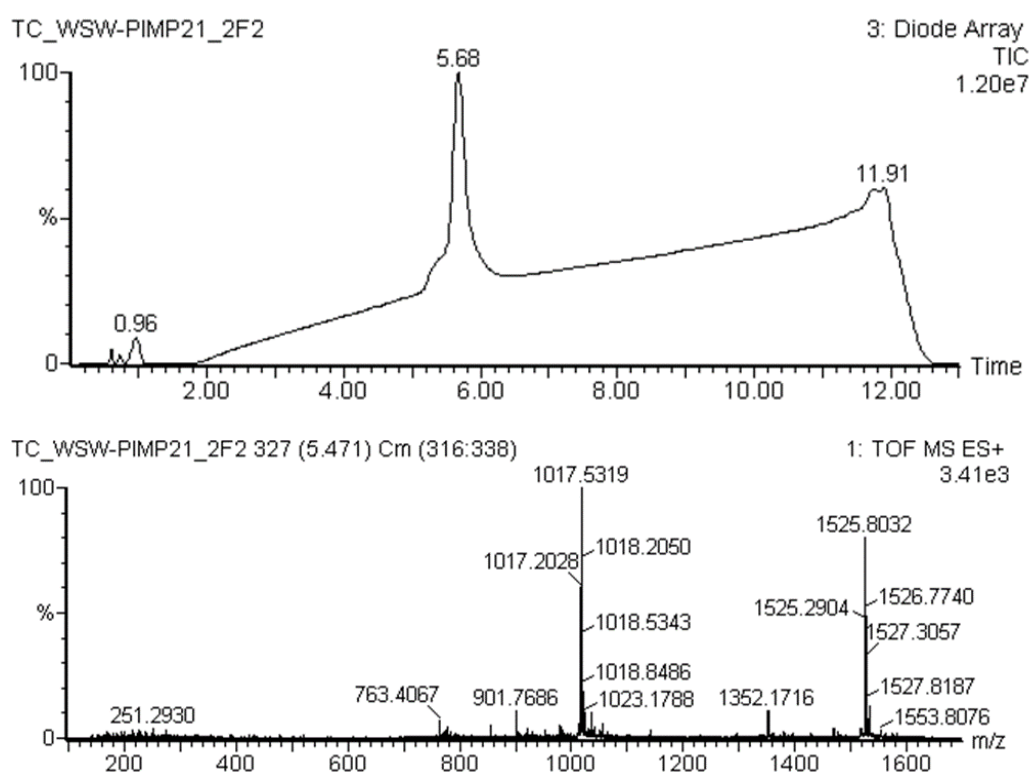


Figure S 1.54: Analytical data for L-WSW-Scrambled PIMP21, peptide 111. (A) HPLC spectra for FITC labelled L-WSW-PIMP21; (B) Analysis of peaks (α , β) showed expected mass-to-charge ratios: m/z 1525.8032 $[M+2H]^+$ and 1017.5319 $[M+3H]^+$.

Peptide 112. WSW-Scrambled PIMP21 (FITC): SYPGWSW-Ahx-K(FITC)-Ahx-LPPAPWNWAHVL

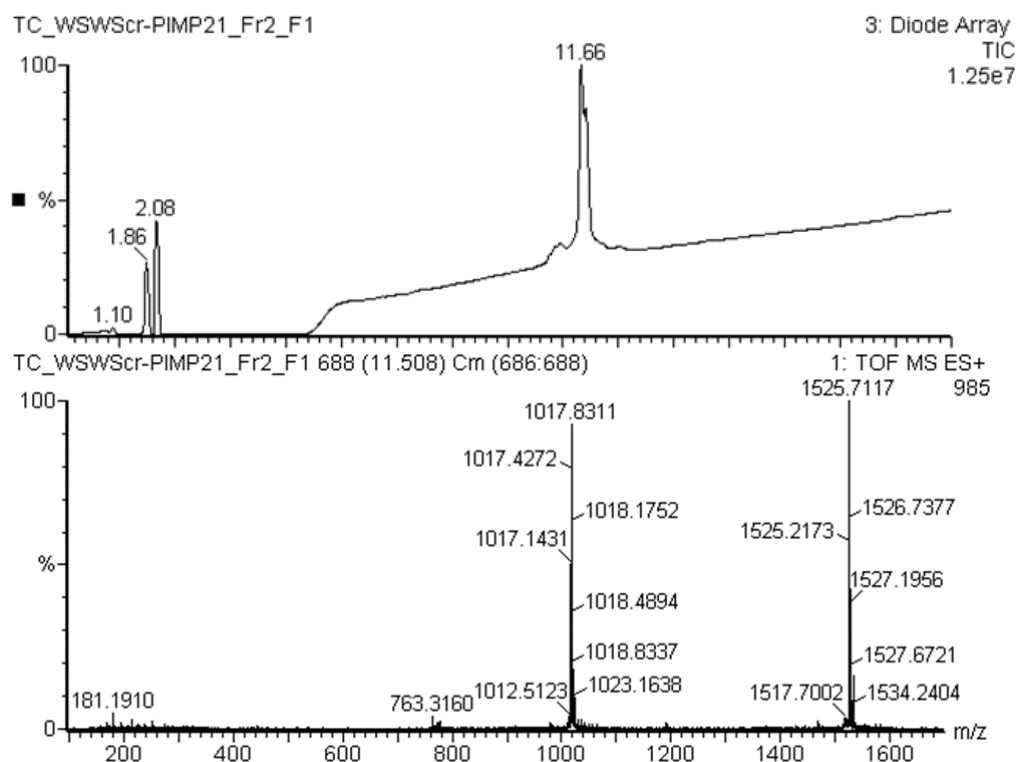


Figure S 1.55: Analytical data for FITC labelled L-WSW-Scrambled PIMP21, peptide 112. (A) HPLC spectra for L-WSW-Scrambled control; (B) Analysis of peaks (α, β) showed expected mass-to-charge ratios: m/z 1525.7117 $[M+2H]^+$ and 1017.8311 $[M+3H]^+$.