Supplementary Information

Atomistic insight into the essential binding event of ACE2-derived peptides to the SARS-CoV-2 spike protein

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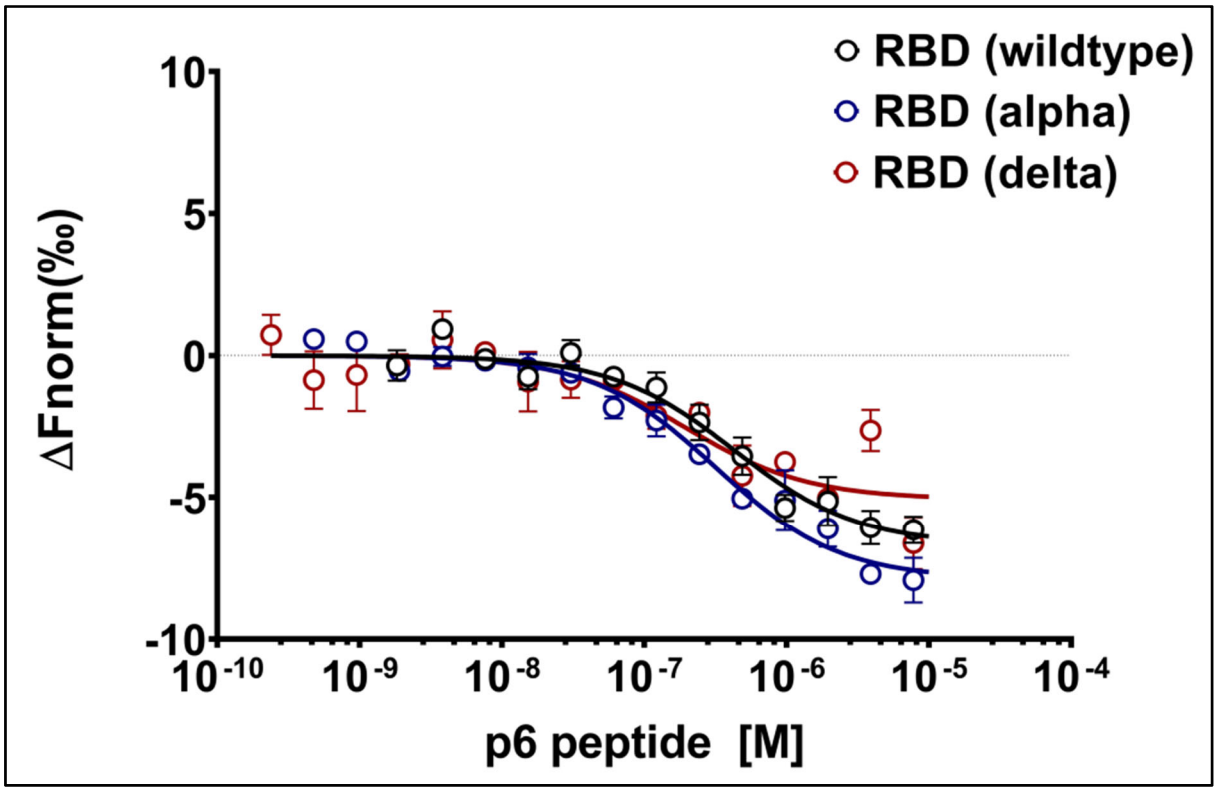
Experimental study of RBD variants alpha and delta



SI Figure 1. Affinity characterization of the p6 using MST against fluorescently labeled RBD from wildtype, alpha and delta strains for SARS-CoV-2. (N=3, error bars show SEM).

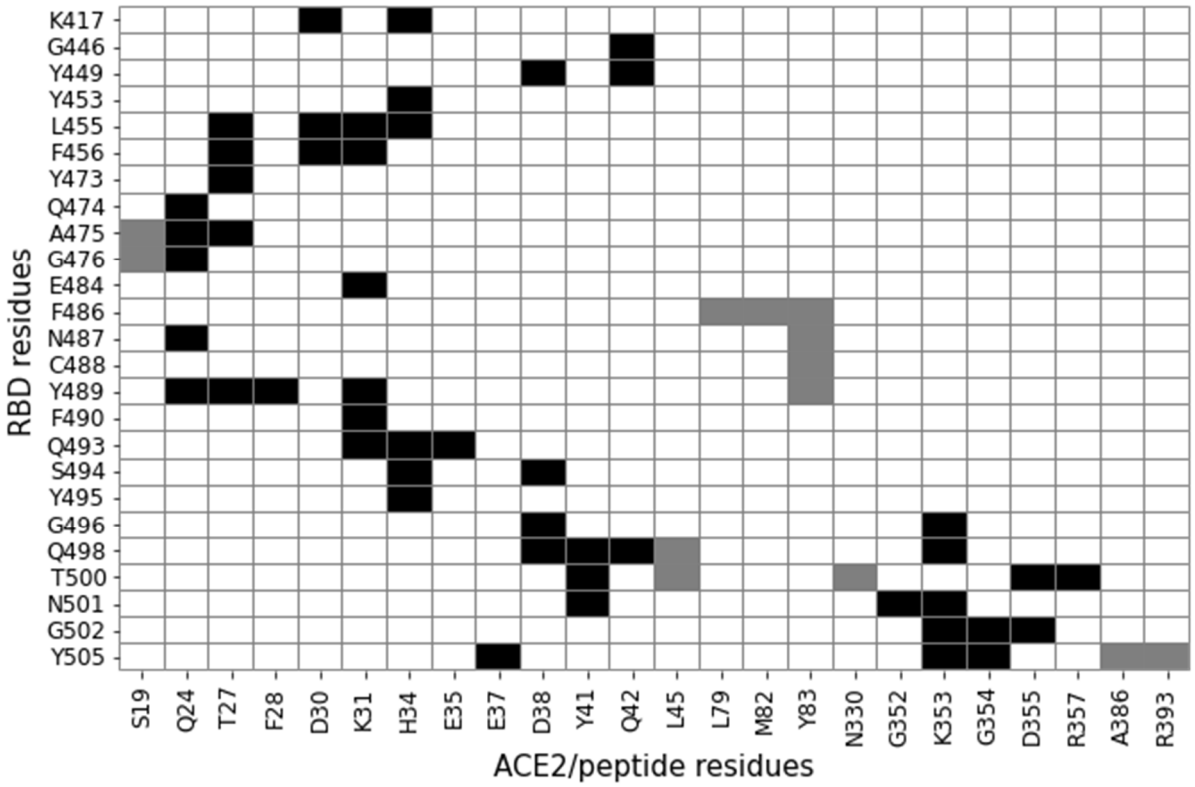
SI Table 1. Comparison of affinity values of p6 towards different RBD variants, as determined from MST experiments. For comparison, IC50 for p6 against SARS-CoV-1 is reported to be 0.1 μM (Han et al., 2006).

	wild-type	alpha	delta
Kd / nM	452.3 ± 96.5	319.3 ± 55.4	194.4 ± 88.5

Experimental study of p4, p6 and p6 variants

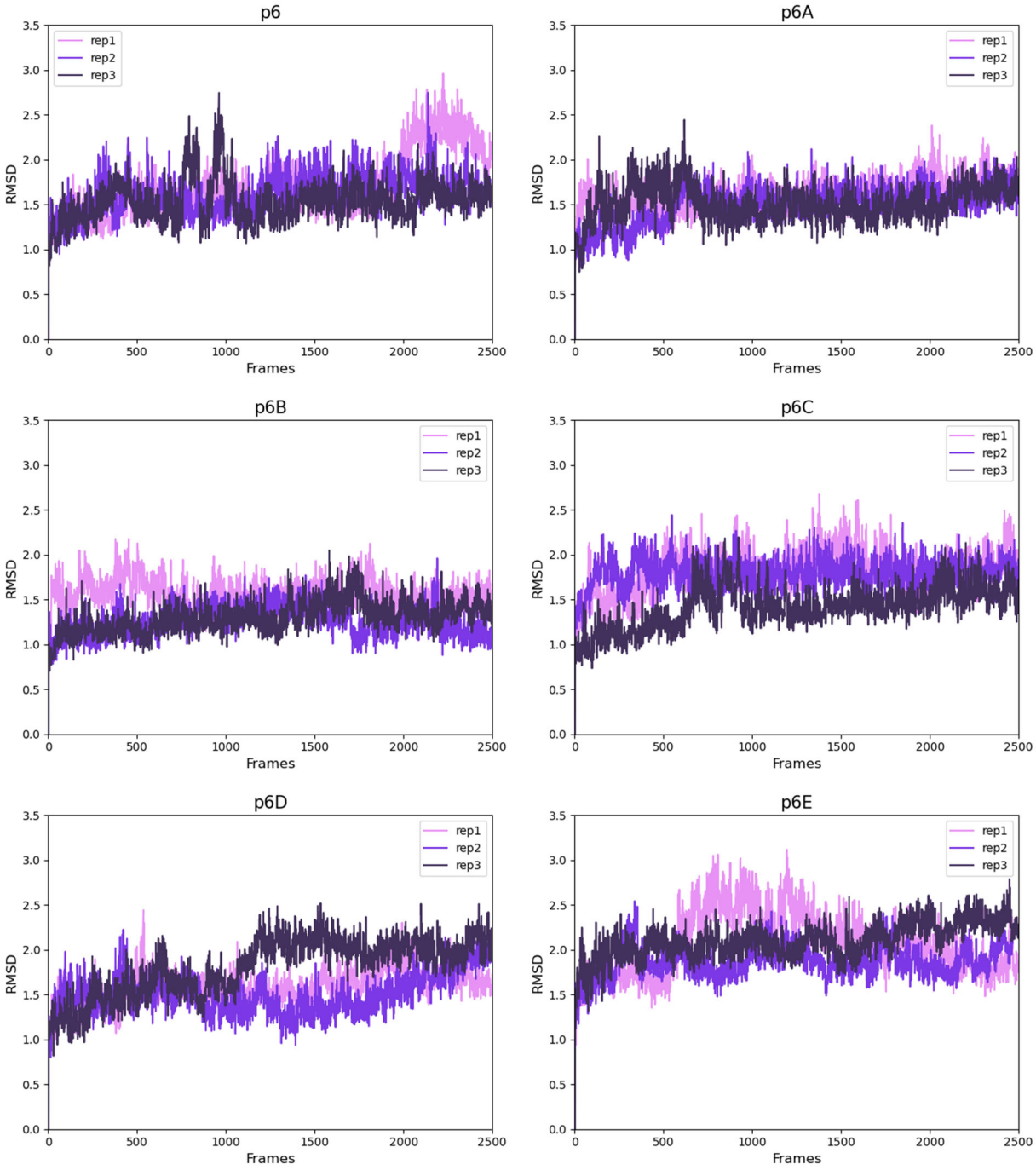
Key residues for the RBD-ACE2 interaction

A detailed analysis of the interactions observed between the ACE2 and the RBD is depicted in Figure 1. We compiled data already reported in the literature and divided ACE2 amino acids into two groups: p6 residues (black interactions in SI Table 2) and ACE2 residues which are not within p6 (grey interactions in SI Table 2). (Yan et al., 2020) (Lan et al., 2020) (Y. Wang et al., 2020) (Xu et al., 2021) (Q. Wang et al., 2020)

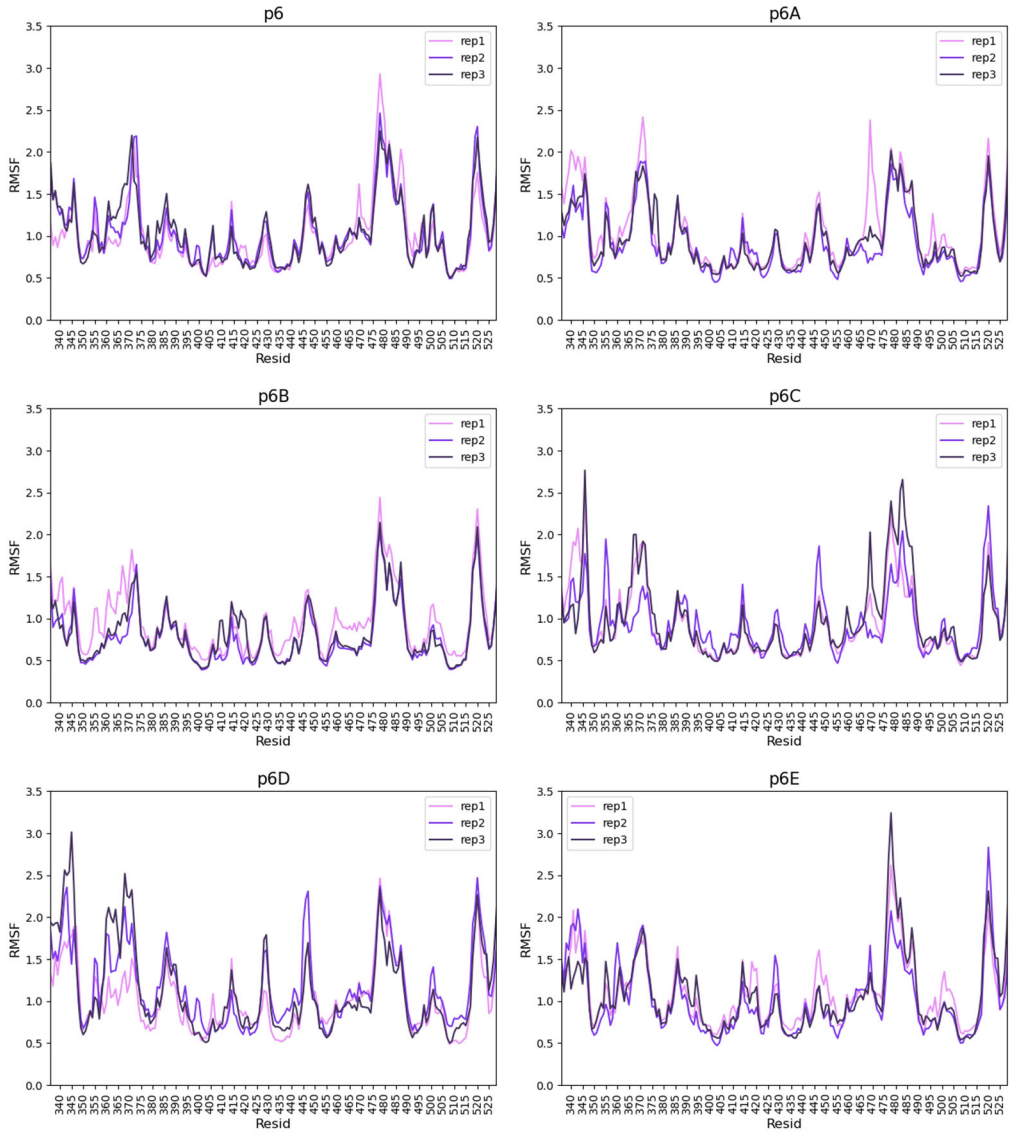


SI Table 2. Interactions reported between the ACE2 and the RBD. p6 residues are colored in black and the rest, in grey.

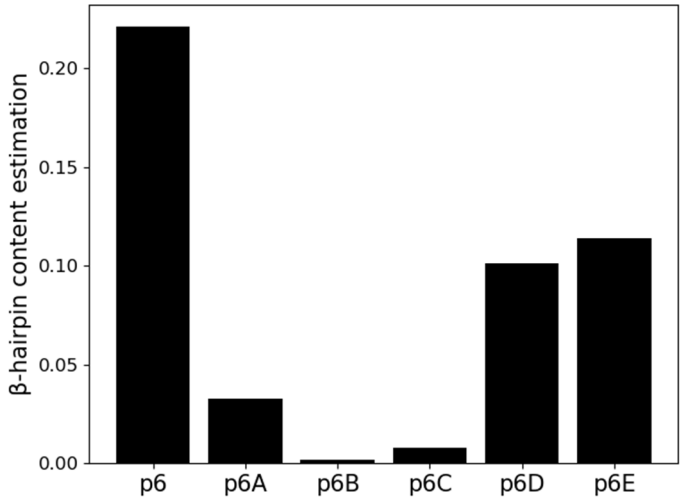
Molecular dynamics study of p6 and p6 variants



SI Figure 2a. RMSD of the RBD backbone atoms along the simulation timescale for the RBD-peptide complexes simulated.



SI Figure 2b. RMSF of the RBD residues along the simulation timescale for the RBD-peptide complexes simulated.

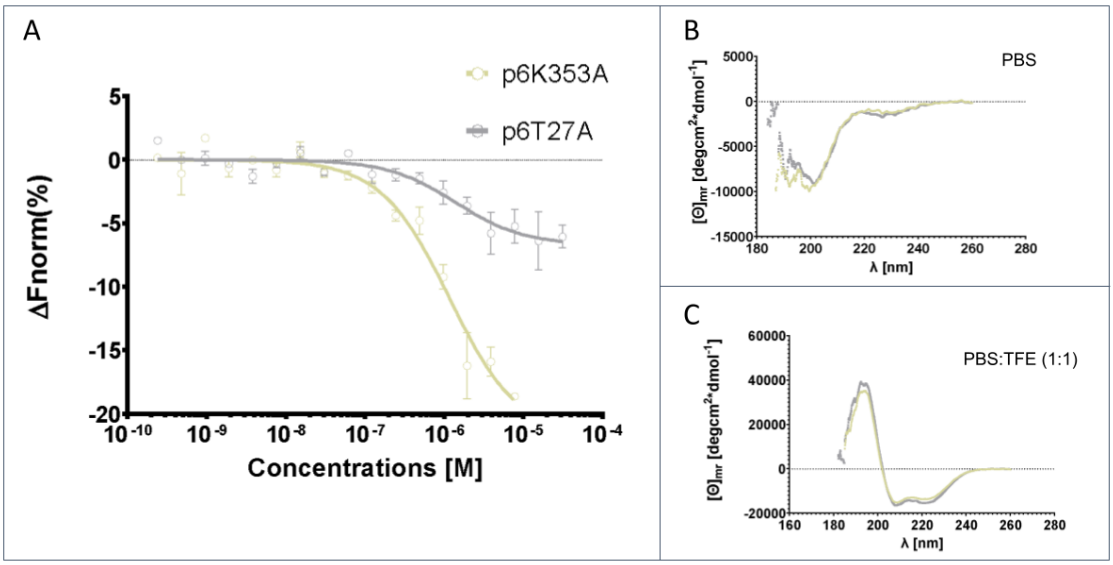


SI Figure 2c. Average of antiparallel β-strand structure of residues 351, 352, 355, 356, 357 along the simulation for the RBD-peptide complexes.

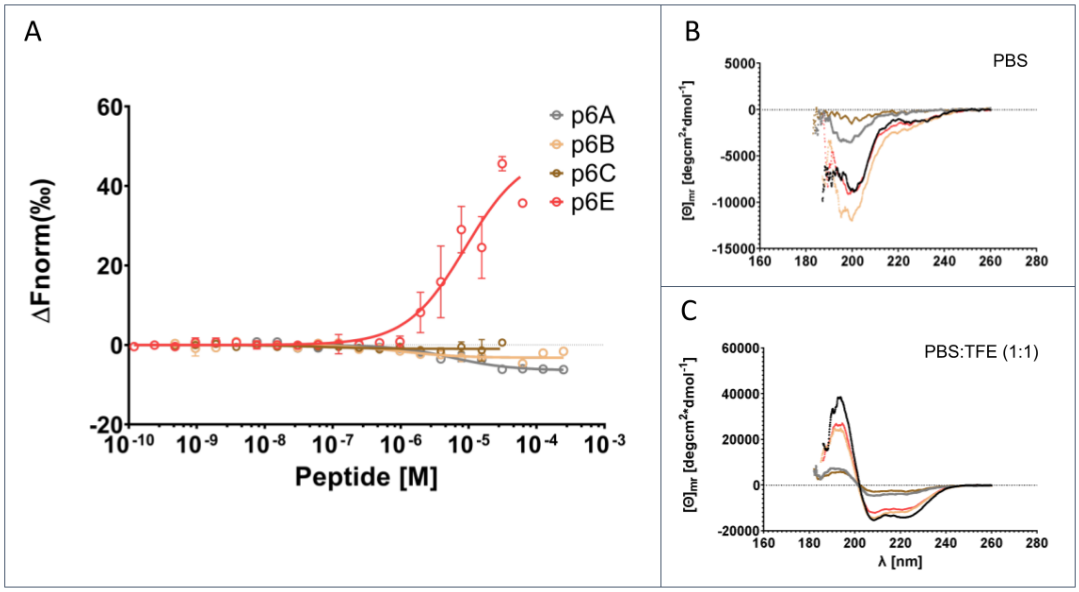
Experimental study of p4, p6 and p6 variants

SI Table 3. Affinity and CD-spectroscopy results from measurements with p6 and variants thereof. Experimental results of the analysis of all measured peptides.

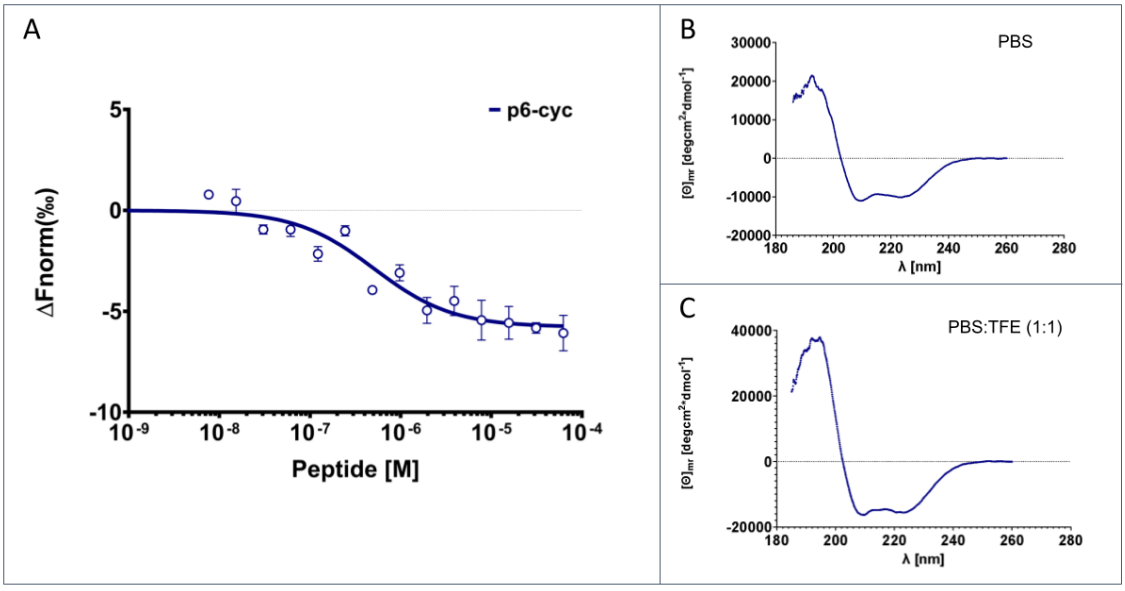
Code name	Sequence	Replicates on MST	Kd (µM)	Helicity in PBS	Helicity on PBS:TFE, 1:1
p4	EEQAKTFLDKFNHEAEDLFYQSS	3	0.93 ± 0.21	0	100
p6	EEQAKTFLDKFNHEAEDLFYQSSGLGKGDFR	6	0.45 ± 0.10	0	39
p6A	**Y**EQAKTF**A**DK**A**NHEAED**AY**YQSSGLGKGD**S**R	3	5.75 ± 1.55	0	5
p6B	**TI**EEQAKTF**D**DK**S**NHEAEDL**Y**YQSSGLGKGDFR	5	1.02 ± 0.46	0	31
p6C	LAQMYTIEEQAKTFLDKFDHEAEDLFYQSSGLGKGDFR	5	ND	0	2
p6E	LAQMYTI**T**EQA**A**TFLDKF**D**H**V**AEDLFYQSSGLGKGDFR	5	8.93 ± 2.76	0	27
K353A	EEQAKTFLDKFNHEAEDLFYQSSGLG**A**GDFR	4	1.17 ± 0.22	0	37
T27A	EEQAK**A**FLDKFNHEAEDLFYQSSGLGKGDFR	5	1.39 ± 0.55	0	43
p6-cyc	EEQAKTFLDKFNHE***X(cyc)***EDLFYQ***X(cyc)***SGLGKGDFR	3	0.27 ± 0.14	25	43



SI Figure 3. Affinity and structural characterization of the p6 single mutations p6(T27A) and p6 (K353A) using a) MST against fluorescently labeled wildtype RBD (N=3, error bars show SEM) and CD-spectroscopy in b) 20 mM phosphate buffer or c) 20 mM phosphate buffer with 50 % (v/v) TFE.



SI Figure 4. Affinity and structural characterization of p6 variants (see SI Table 3) using a) MST against fluorescently labeled wildtype RBD (N=3, error bars show SEM) and CD-spectroscopy in b) 20 mM phosphate buffer or c) 20 mM phosphate buffer with 50 % (v/v) TFE. In panels B and C p6 curves are shown in black.



SI Figure 5. Affinity and structural characterization of cyclized p6-cyc (see SI Table 3) using a) MST against fluorescently labeled wildtype RBD (N=3, error bars show SEM) and CD-spectroscopy in b) 20 mM phosphate buffer or c) 20 mM phosphate buffer with 50% (v/v) TFE.

Chemicals and solvents

Chemicals and solvents were purchased from Merck (Merck group, Germany), TCI (Tokyo chemical industry CO., LTD., Japan) and Acros Organics (Thermo Fisher scientific, USA) and

used without further purification. Dry solvents were purchased from Acros Organics (Thermo Fisher scientific, USA). Amino acids and resins for SPPS were purchased from Novabiochem (Merck, USA) or Iris Biotech GmbH (Germany).

Preparative HPLC

Preparative HPLC was performed on a Gilson PLC 2020 system (Gilson Inc, WI, Middleton, USA) using a VP 250/32 Macherey-Nagel Nucleodur C18 HTec Spum column (Macherey-Nagel GmbH & Co. Kg, Germany). The following gradient was used: A = H2O + 0.1% TFA (trifluoroacetic acid), B = MeCN (acetonitrile) + 0.1% TFA, flow rate 30 ml/min, 5% B 0-5 min, 5-90% B 5-60 min, 90% B 60-65 min

UPLC-UV/MS

UPLC-UV/MS traces were recorded on a Waters H-class instrument equipped with a quaternary solvent manager, a Waters autosampler, a Waters TUV detector and a Waters Acquity QDa detector with an Acquity UPLC BEH C18 1.7 µm, 2.1 x 50 mm RP column with a flow rate of 0.6 mL/min (Waters Corp., USA). The following gradient was used: A: 0.1% TFA in H2O; B: 0.1% TFA in MeCN. 5% B 0 - 0.5 min, 5- 95% B 0.5-3 min, 95% B 3-3.9 min, 5% B 3.9-5 min.

SPPS

SPPS was carried out on a Tribute-UV peptide synthesizer (Protein technologies, USA) via standard Fmoc-based protocols.

HR-MS

High resolution ESI-MS spectra were recorded on a Waters H-class instrument equipped with a quaternary solvent manager, a Waters sample manager-FTN, a Waters PDA detector and a Waters column manager with an Acquity UPLC protein BEH C18 column (1.7 µm, 2.1 mm x 50 mm). Samples were eluted with a flow rate of 0.3 mL/min. The following gradient was used: A: 0.01% FA in H2O; B: 0.01% FA in MeCN. 5% B: 0-1 min; 5 to 95% B: 1-7min; 95% B: 7 to 8.5 min. Mass analysis was conducted with a Waters XEVO G2-XS QTof analyzer.

CD-spectroscopy

CD-spectroscopy was measured on a Jasco J-720 spectropolarimeter at 22°C and parameters set to: measured wavelength range 190 – 260 nm; data pitch of 0.1 nm; continuous scanning mode; 100 nm/min scanning speed; 1sec. response; 1.0 nm band width; 0.1 cm cell length; 10 accumulations

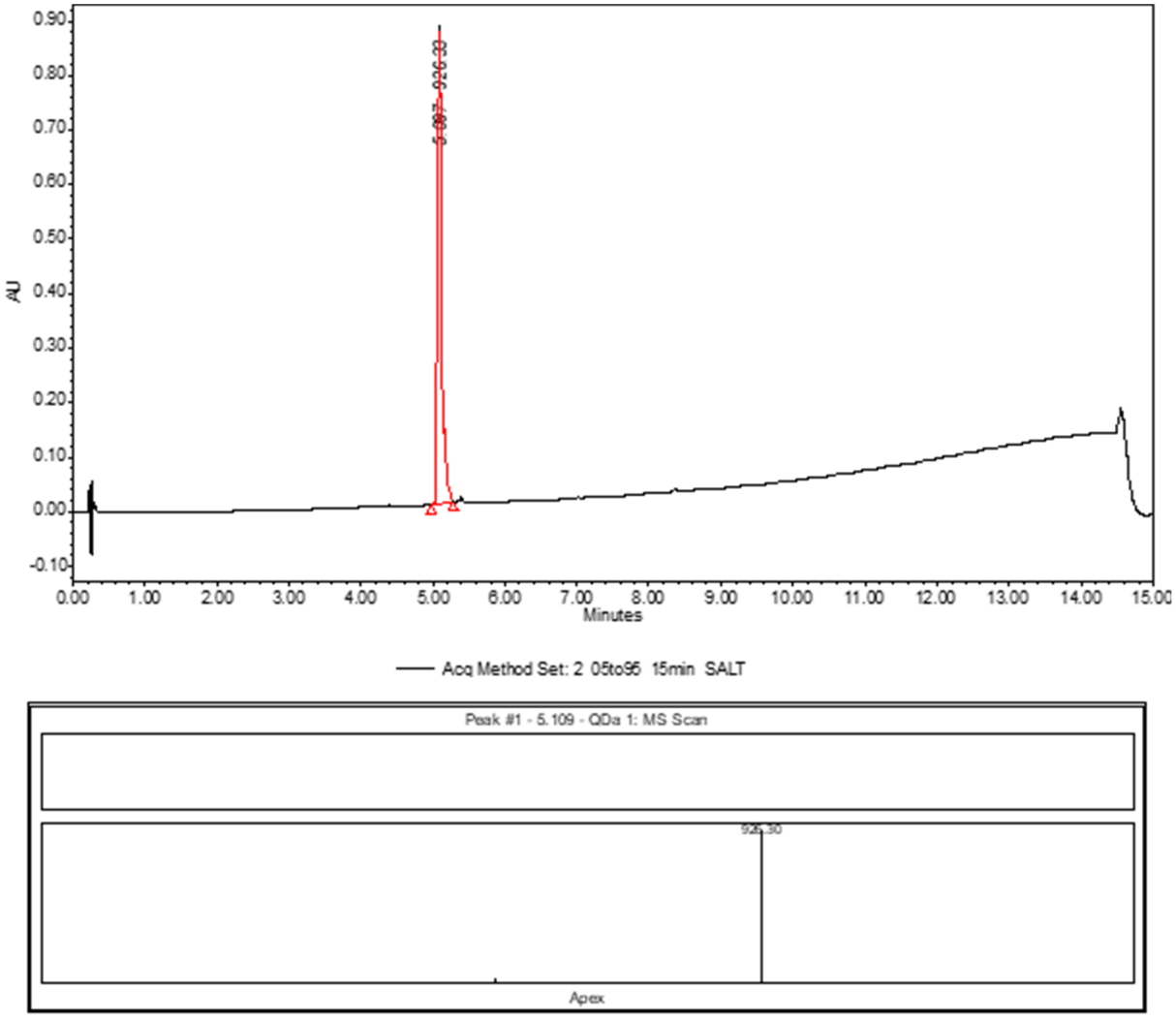
Peptides Characterization

P4

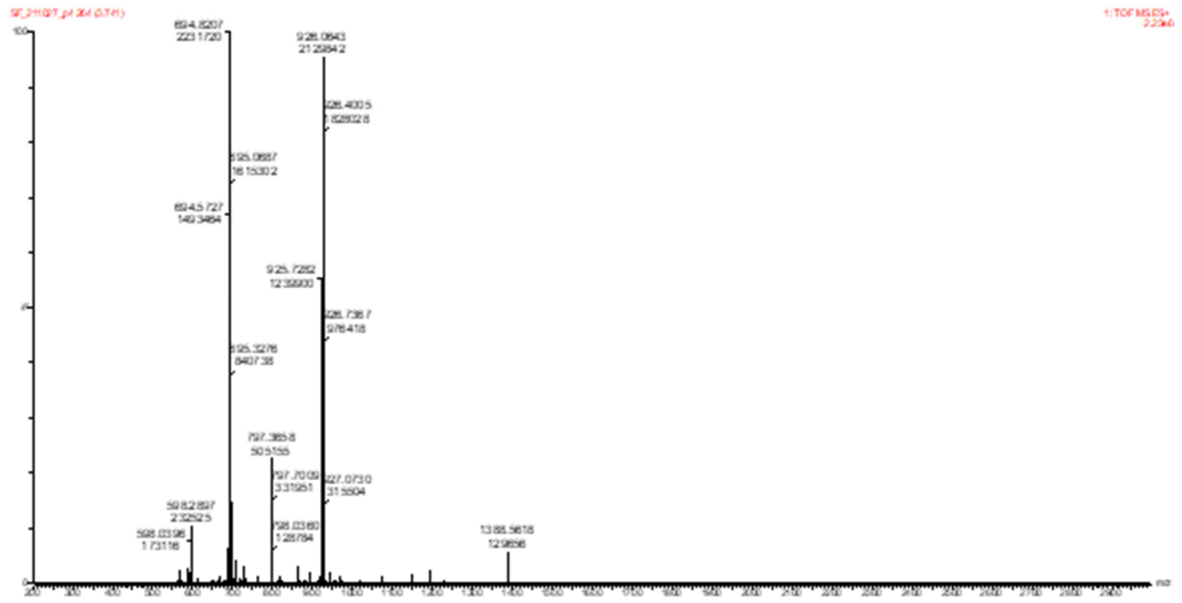
The peptide p4 was synthesized in a 0.05 mmol scale on a Rink Amide Resin with a loading of 0.78 mmol/g. Until the 15 amino acids the synthesis was carried out on a PTI synthesizer with single couplings (5 eq. amino acid for 40 minutes). All amino acids following number 15

were coupled in double coupling steps with five equivalents for 40 minutes. The final cleavage from resin was achieved by incubation with a mixture of TFA:TIS:H2O (95:2.5:2.5;v:v:v) for two hours followed by precipitation in cold diethylether. The crude peptide was purified by preparative reverse phase C18 HPLC and the product was obtained as white powder (64 mg, 19.8 µmol, 39.6 % yield).

HRMS: m/z: 1388,5618 $[M+2H]^{2+}$ (calc. 1388,98 m/z), 926,0643 $[M+3H]^{3+}$ (calc. 926,32 m/z), 694,8207 $[M+4H]^{4+}$ (calc. 694,99 m/z).



SI Figure 6. P4, UPLC-MS 15 minute run.

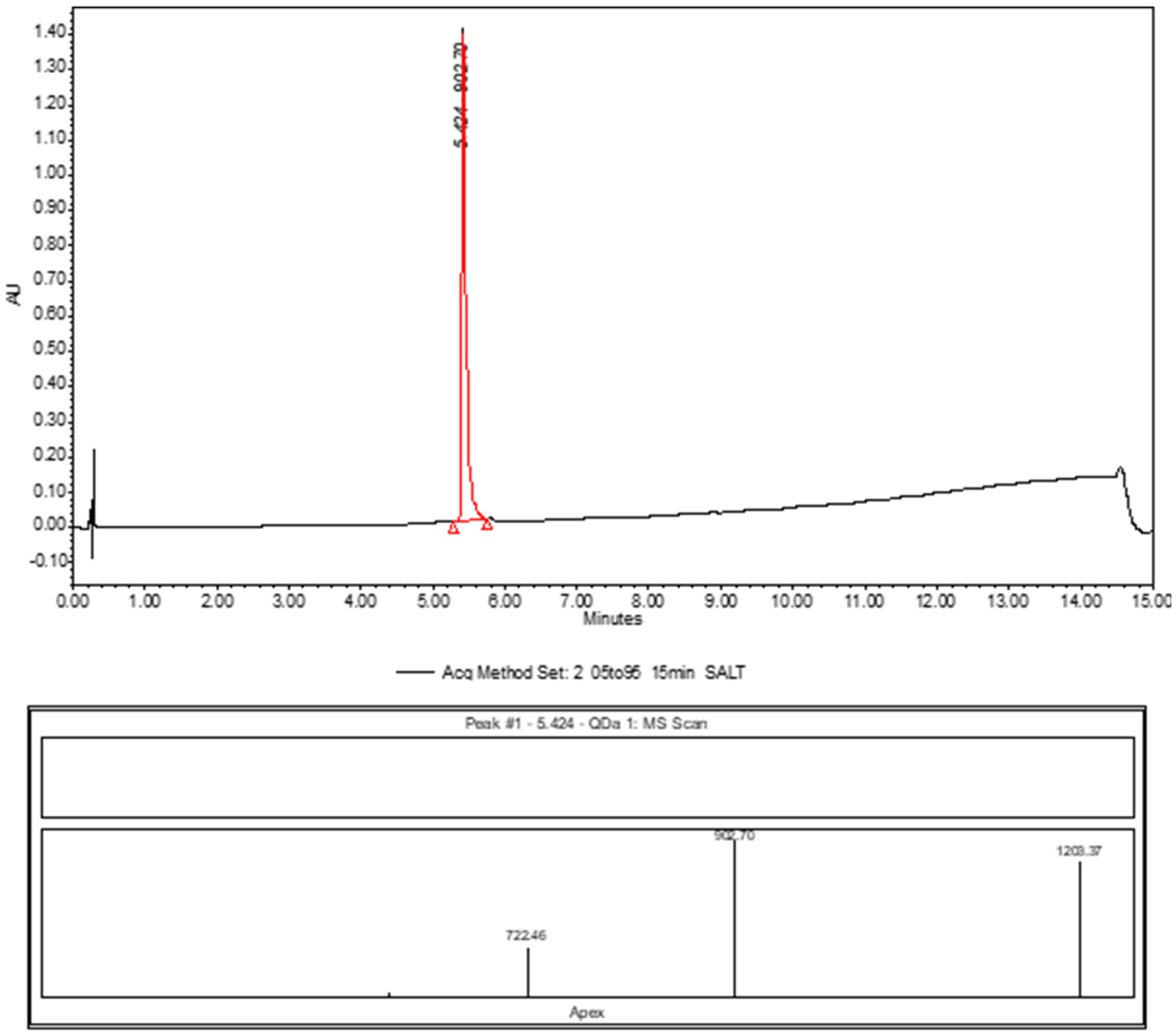


SI Figure 7. P4, HR-MS.

P6

The peptide p6 was synthesized in a 0.05 mmol scale on a Rink Amide Resin with a loading of 0.78 mmol/g. Until the 15 amino acids the synthesis was carried out on a PTI synthesizer with single couplings (5 eq. amino acid for 40 minutes). All amino acids following number 15 were coupled in double coupling steps with five equivalents for 40 minutes. The final cleavage from resin was achieved by incubation with a mixture of TFA:TIS:H2O (95:2.5:2.5;v:v:v) for two hours followed by precipitation in cold diethylether. The crude peptide was purified by preparative reverse phase C18 HPLC and the product was obtained as white powder (72 mg, 16.7 µmol, 33.5 % yield).

HRMS: m/z: 902,6592 $[M+4H]^{4+}$ (calc. 902,7278 m/z), 722,3497 $[M+5H]^{5+}$ (calc. 722,3822 m/z), 602,1285 $[M+6H]^{6+}$ (calc. 602,1519 m/z).



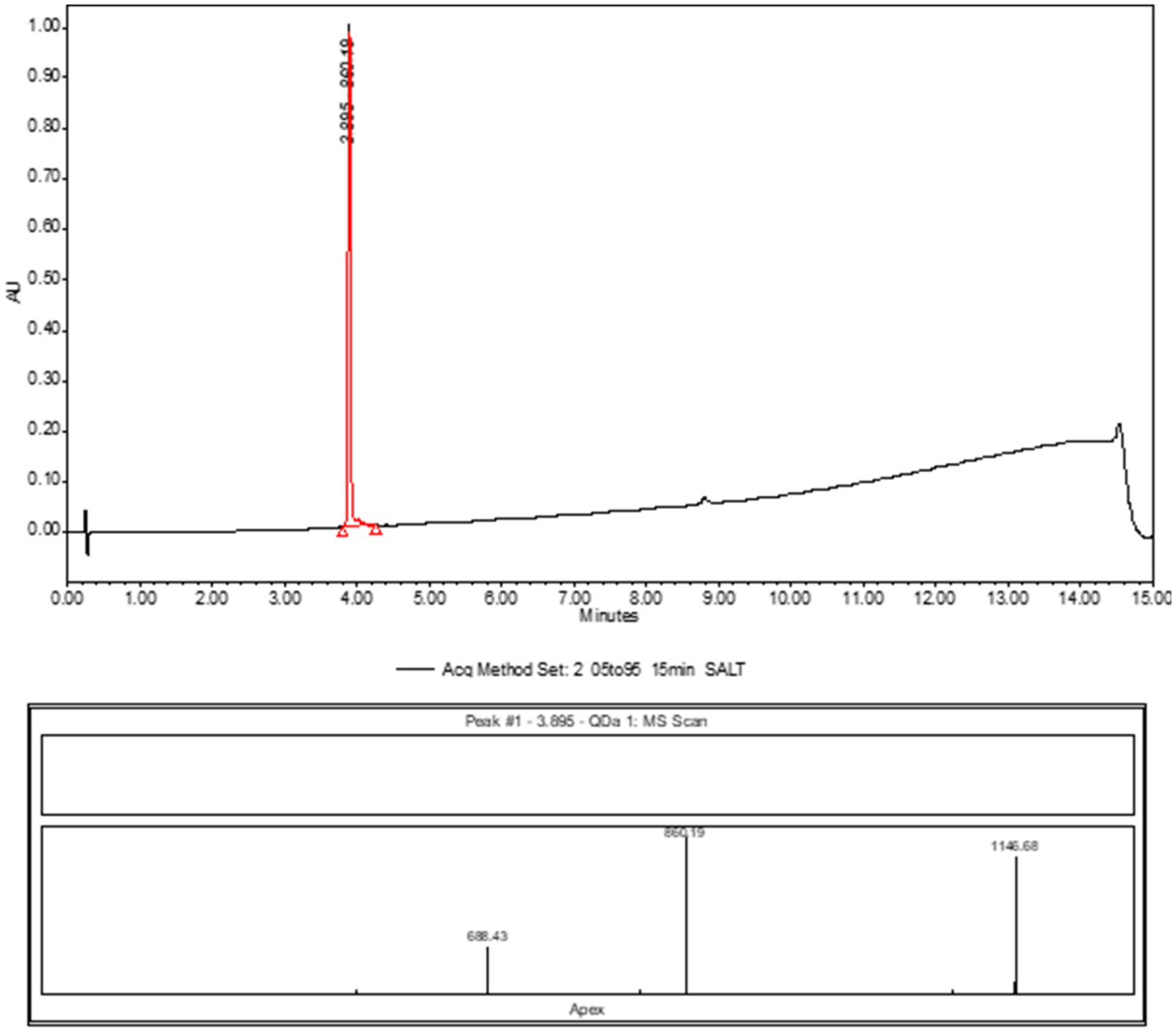
SI Figure 8. P6, UPLC-MS 15 minute run.

SI Figure 9. P6, HR-MS.

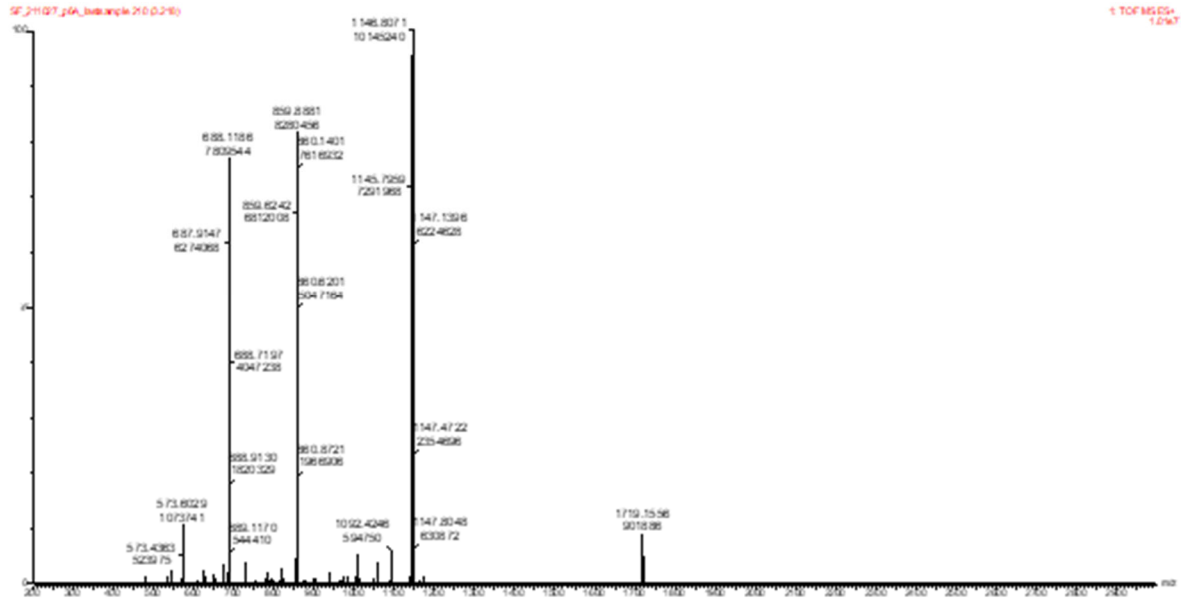
P6A

The p6A peptide was synthesized in a 0.05 mmol scale on a Rink Amide Resin with a loading of 0.78 mmol/g. Until the 15 amino acids the synthesis was carried out on a PTI synthesizer with single couplings (5 eq. amino acid for 40 minutes). All amino acids following number 15 were coupled in double coupling steps with five equivalents for 40 minutes. The final cleavage from resin was achieved by incubation with a mixture of TFA:TIS:H2O (95:2.5:2.5;v:v:v) for two hours followed by precipitation in cold diethylether. The crude peptide was purified by preparative reverse phase C18 HPLC and the product was obtained as white powder (33 mg, 8.0 µmol, 16.0 % yield).

HRMS: m/z: 1719,1556 $[M+2H]^{2+}$ (calc. 1719,31 m/z), 1146,8071 $[M+3H]^{3+}$ (calc. 1146,538 m/z), 859,8881 $[M+4H]^{4+}$ (calc. 860,1534 m/z), 688,1186 $[M+5H]^{5+}$ (calc. 688,3227 m/z), 573,6029 $[M+6H]^{6+}$ (calc. 573,7689 m/z).



SI Figure 10. P6A, UPLC-MS 15 minute run.

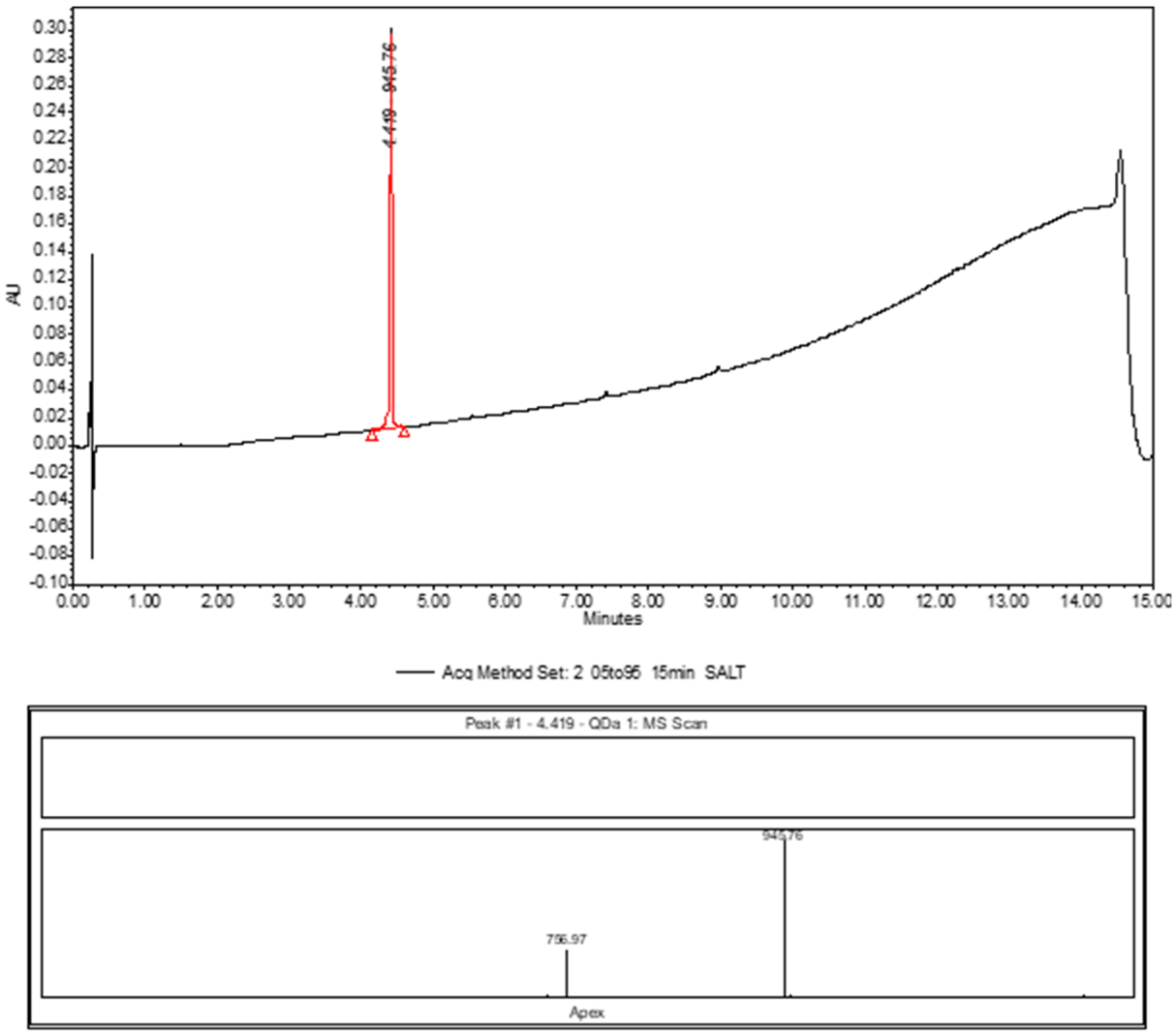


SI Figure 11. P6A, HR-MS.

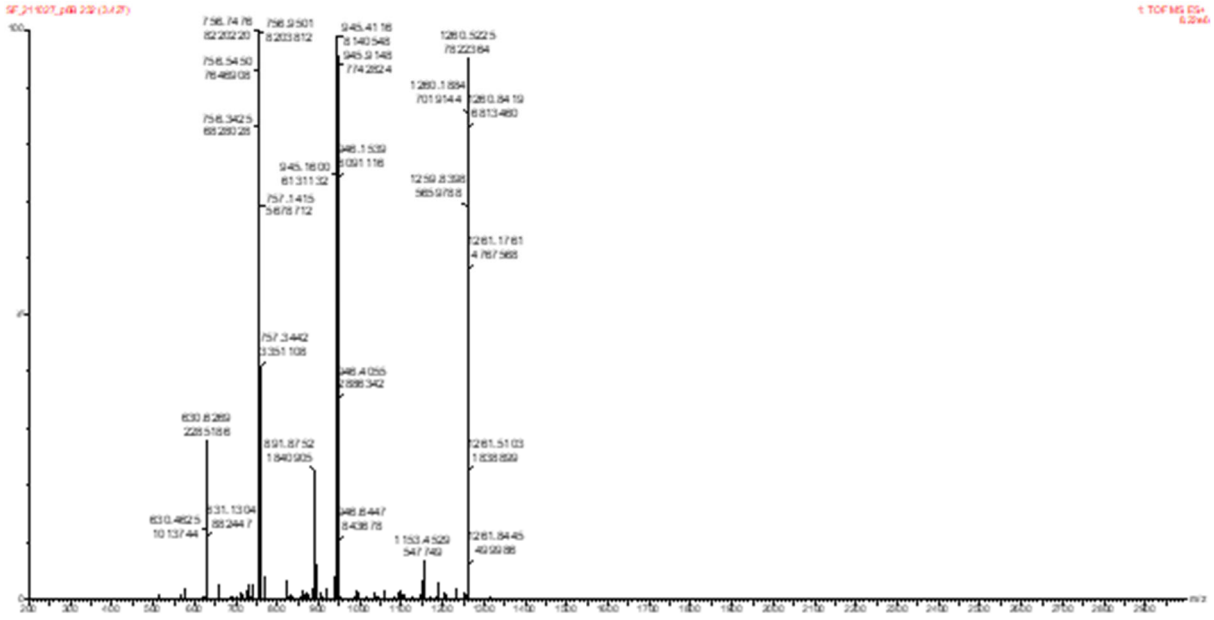
P6B

The p6B peptide was synthesized in a 0.05 mmol scale on a Rink Amide Resin with a loading of 0.78 mmol/g. Until the 15 amino acids the synthesis was carried out on a PTI synthesizer with single couplings (5 eq. amino acid for 40 minutes). All amino acids following number 15 were coupled in double coupling steps with five equivalents for 40 minutes. The final cleavage from resin was achieved by incubation with a mixture of TFA:TIS:H2O (95:2.5:2.5;v:v:v) for two hours followed by precipitation in cold diethylether. The crude peptide was purified by preparative reverse phase C18 HPLC and the product was obtained as white powder (37 mg, 8.3 µmol, 16.58 % yield).

HRMS: m/z: 1260,5225 $[M+3H]^{3+}$ (calc. 1260,669 m/z), 945,4116 $[M+4H]^{4+}$ (calc. 945,7515 m/z), 756,7476 $[M+5H]^{5+}$ (calc. 756,8012 m/z), 630,6269 $[M+6H]^{6+}$ (calc. 630,8343 m/z).



SI Figure 12. P6B, UPLC-MS 15 minute run.

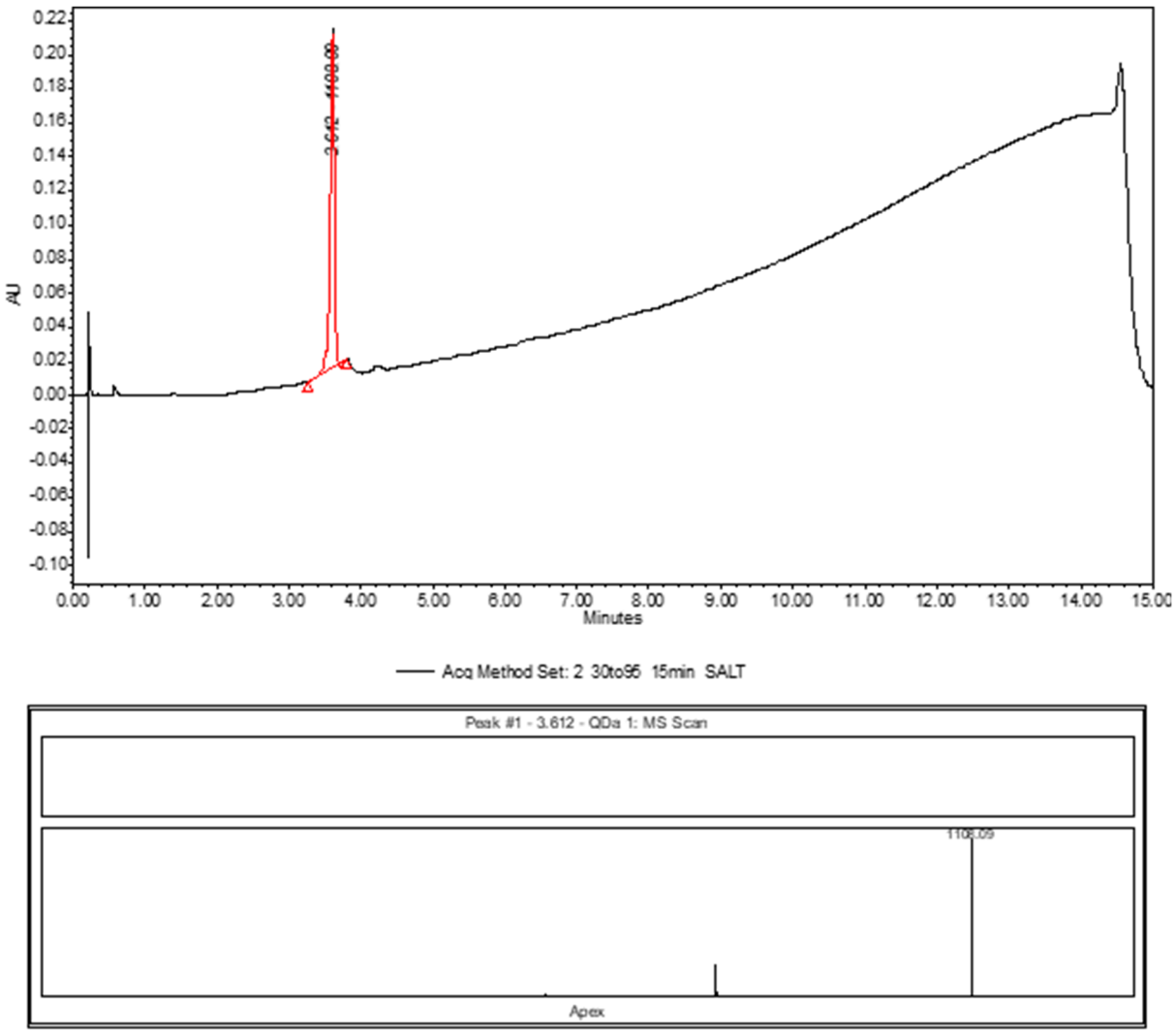


SI Figure 13. P6B, HR-MS.

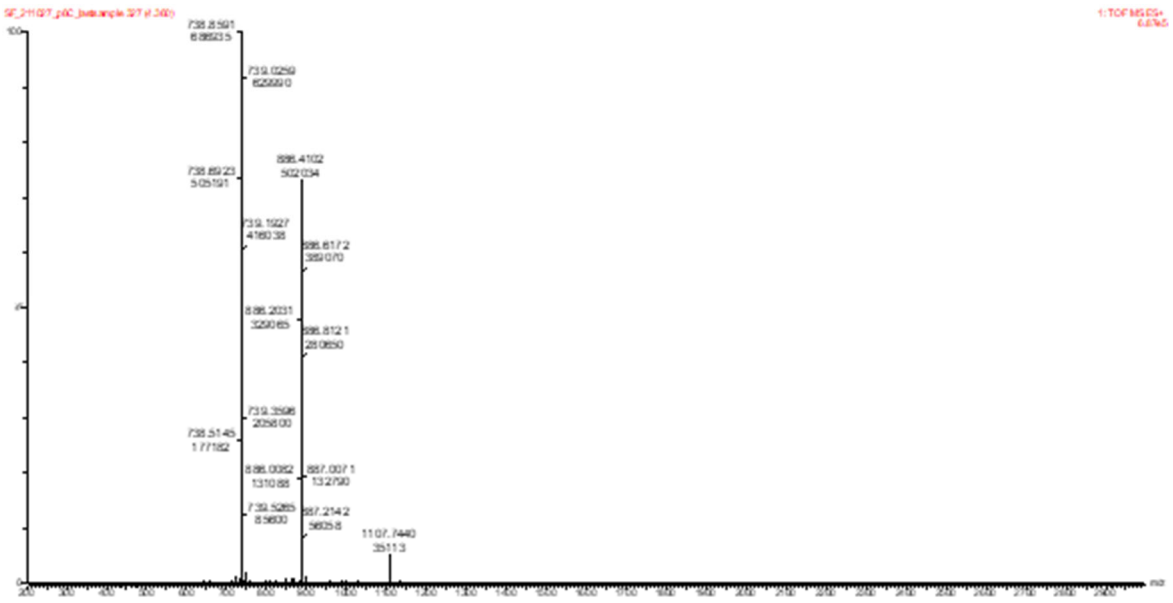
P6C

The p6C peptide was synthesized in a 0.05 mmol scale on a Rink Amide Resin with a loading of 0.78 mmol/g. Until the 15 amino acids the synthesis was carried out on a PTI synthesizer with single couplings (5 eq. amino acid for 40 minutes). All amino acids following number 15 were coupled in double coupling steps with five equivalents for 40 minutes. The final cleavage from resin was achieved by incubation with a mixture of TFA:TIS:H2O (95:2.5:2.5;v:v:v) for two hours followed by precipitation in cold diethylether. The crude peptide was purified by preparative reverse phase C18 HPLC and the product was obtained as white powder (15 mg, 2.93 µmol, 5.86 % yield).

HRMS: m/z: 1107,7440 $[M+4H]^{4+}$ (calc. 1108,226 m/z), 886,4102 $[M+5H]^{5+}$ (calc. 886,7808 m/z), 738,8591 $[M+6H]^{6+}$ (calc. 739,1507 m/z).



SI Figure 14. P6C, UPLC-MS 15 minute run.

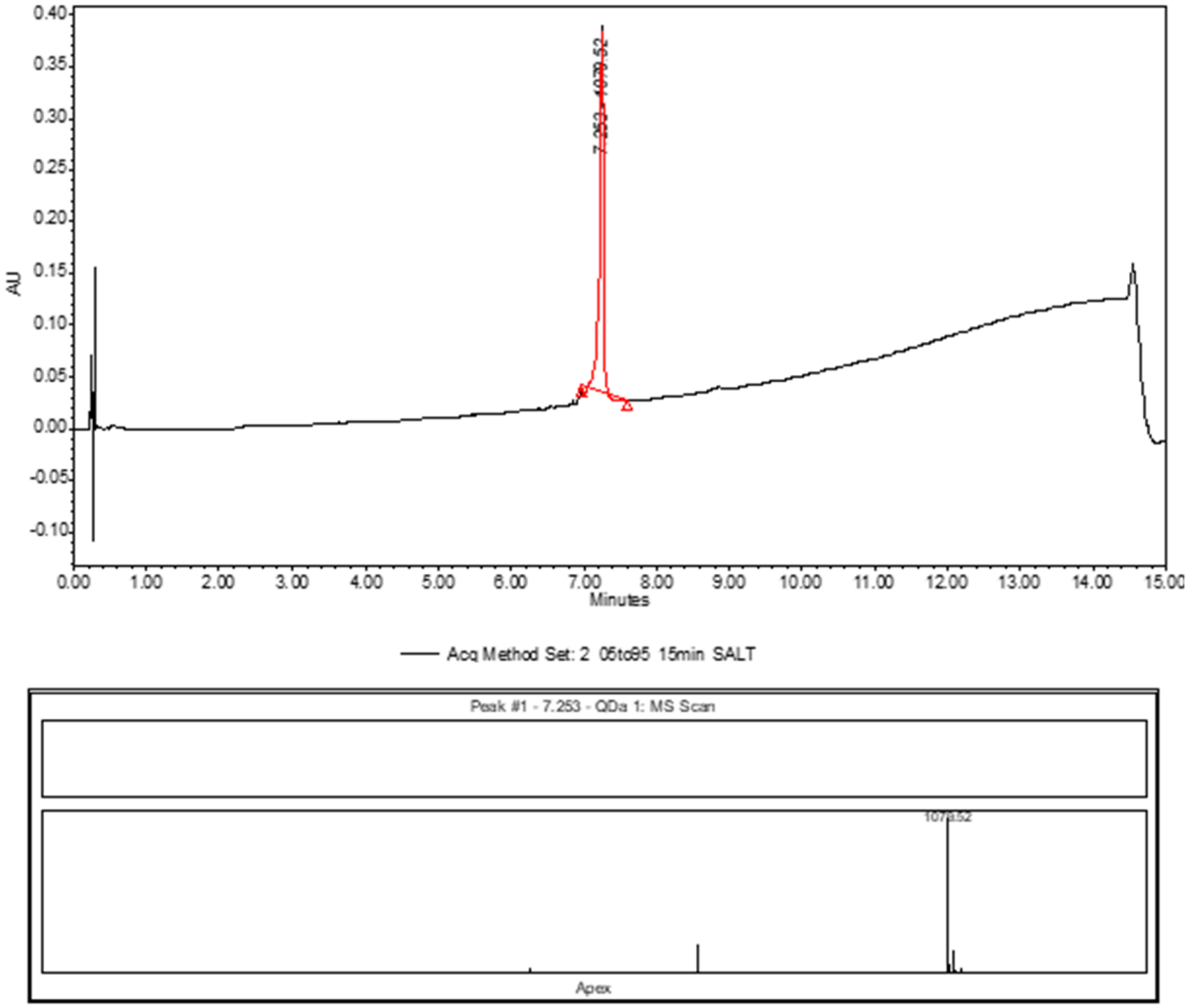


SI Figure 15 P6C, HR-MS.

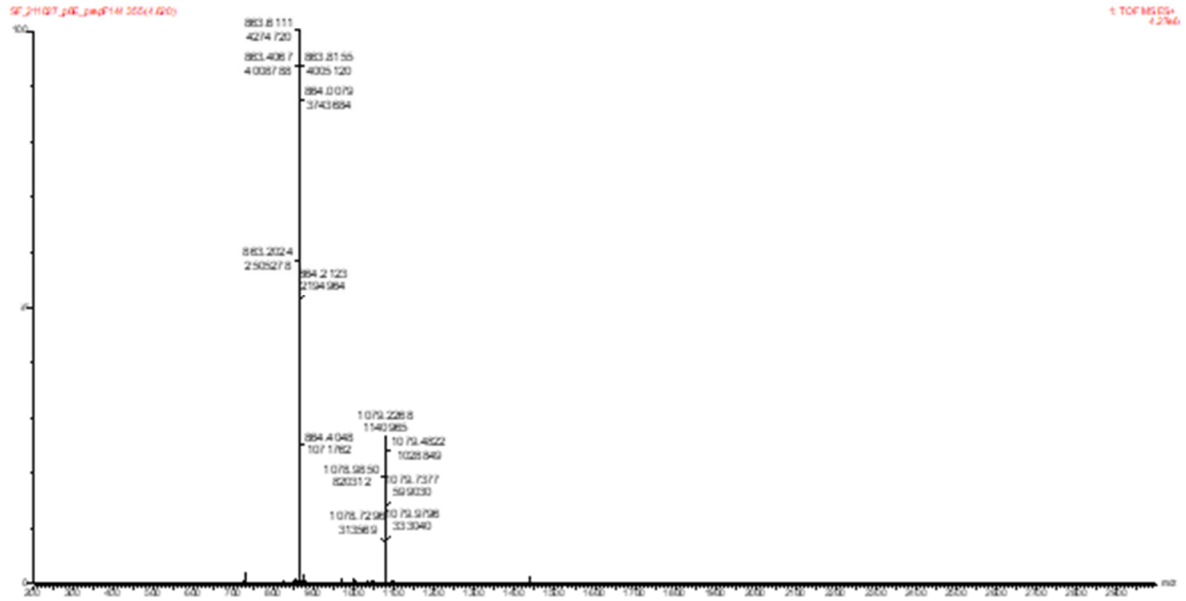
P6E

The p6E peptide was synthesized in a 0.05 mmol scale on a Rink Amide Resin with a loading of 0.78 mmol/g. Until the 15 amino acids the synthesis was carried out on a PTI synthesizer with single couplings (5 eq. amino acid for 40 minutes). All amino acids following number 15 were coupled in double coupling steps with five equivalents for 40 minutes. The final cleavage from resin was achieved by incubation with a mixture of TFA:TIS:H2O (95:2.5:2.5;v:v:v) for two hours followed by precipitation in cold diethylether. The crude peptide was purified by preparative reverse phase C18 HPLC and the product was obtained as white powder (19 mg, 3.9 µmol, 7.8 % yield).

HRMS: m/z: 1079,2268 $[M+4H]^{4+}$ (calc. 1079,454 m/z), 863,6111 $[M+5H]^{5+}$ (calc. 863,7631 m/z).



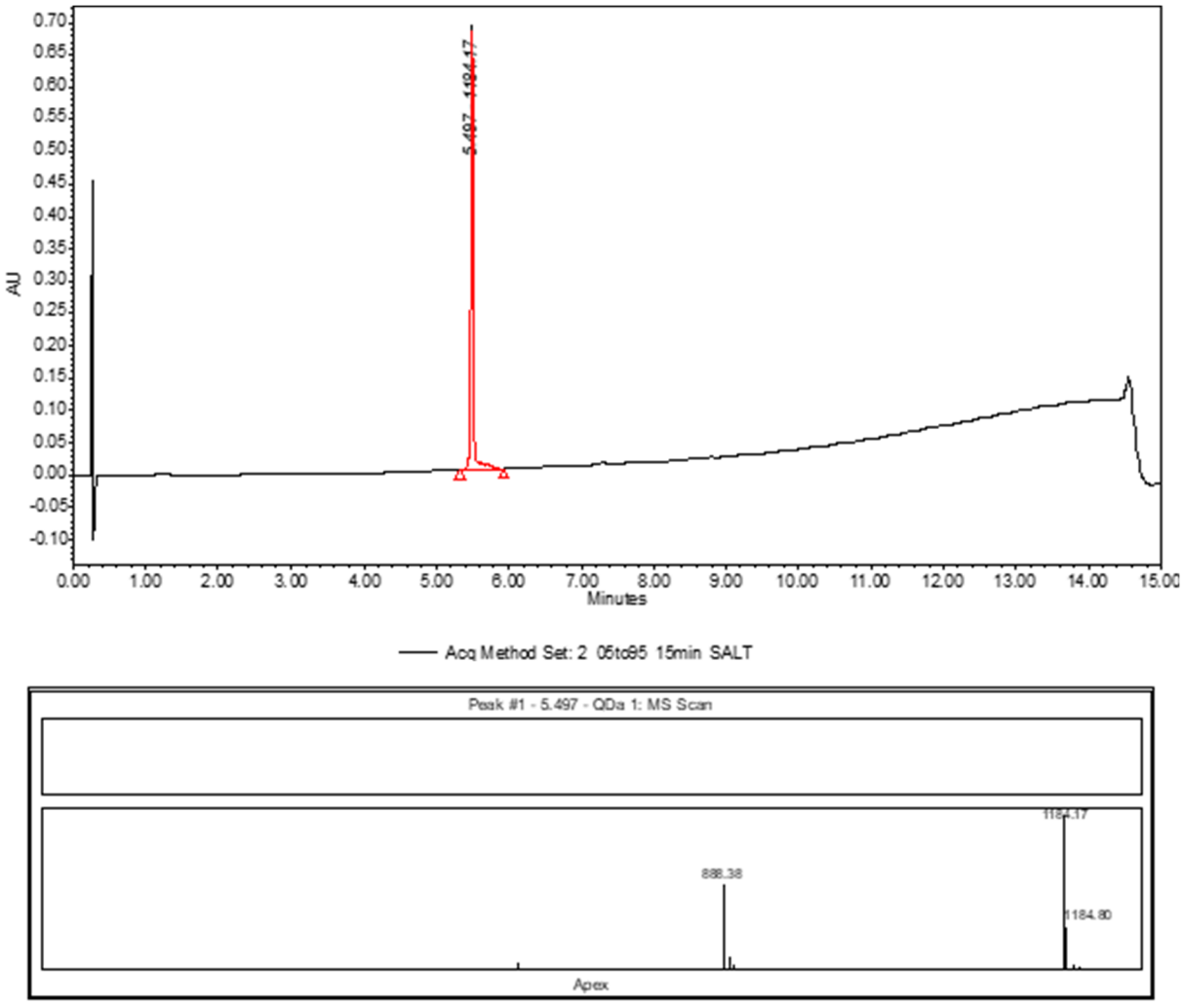
SI Figure 16. P6E, UPLC-MS 15 minute run.



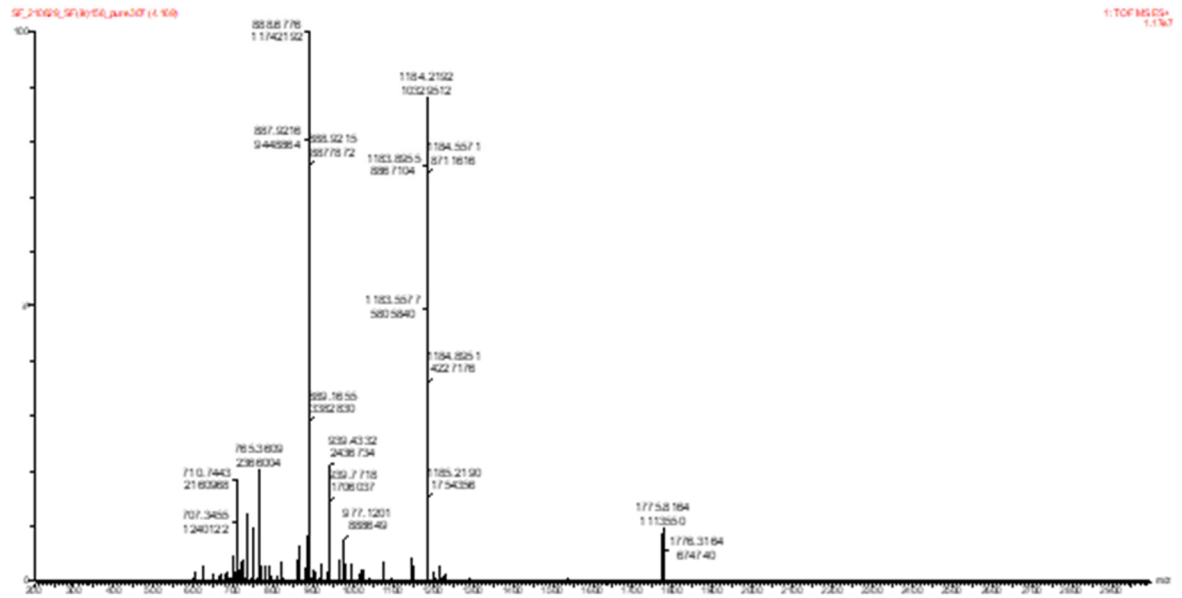
SI Figure 17. P6E, HR-MS.

P6(K353A).The p6(K353A) peptide was synthesized in a 0.05 mmol scale on a Rink Amide Resin with a loading of 0.78 mmol/g. Until the 15 amino acids the synthesis was carried out on a PTI synthesizer with single couplings (5 eq. amino acid for 40 minutes). All amino acids following number 15 were coupled in double coupling steps with five equivalents for 40 minutes. The final cleavage from resin was achieved by incubation with a mixture of TFA:TIS:H2O (95:2.5:2.5;v:v:v) for two hours followed by precipitation in cold diethylether. The crude peptide was purified by preparative reverse phase C18 HPLC and the product was obtained as white powder (36 mg, 8.73 µmol, 17.5 % yield).

HRMS: m/z: 1775,8164 $[M+2H]^{2+}$ (calc. 1775,91 m/z), 1184,2192 $[M+3H]^{3+}$ (calc. 1184,272 m/z), 888,6776 $[M+4H]^{4+}$ (calc. 888,454 m/z).



SI Figure 18. p6(K353A), UPLC-MS 15 minute run.

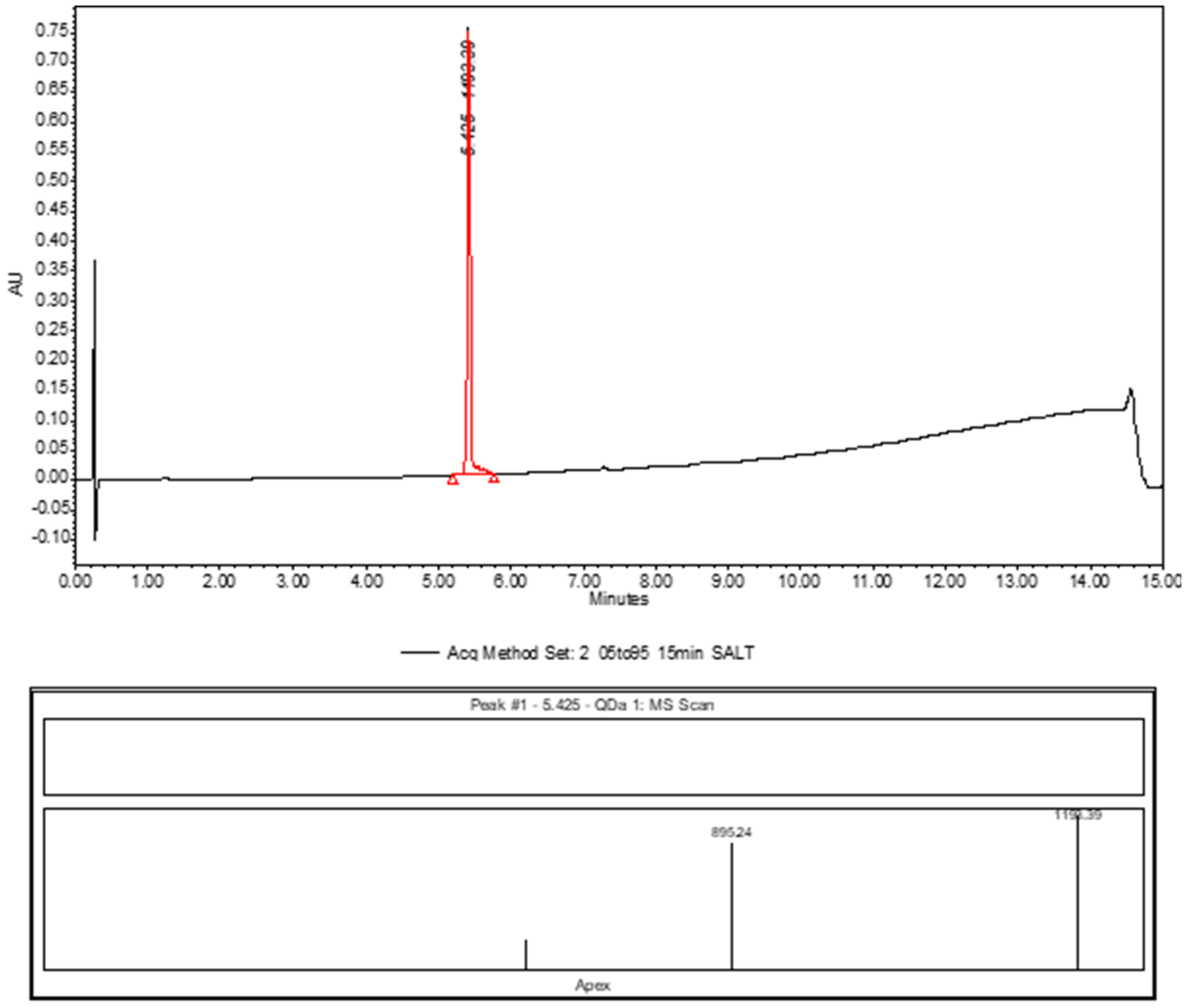


SI Figure 19. p6(K353A), HR-MS.

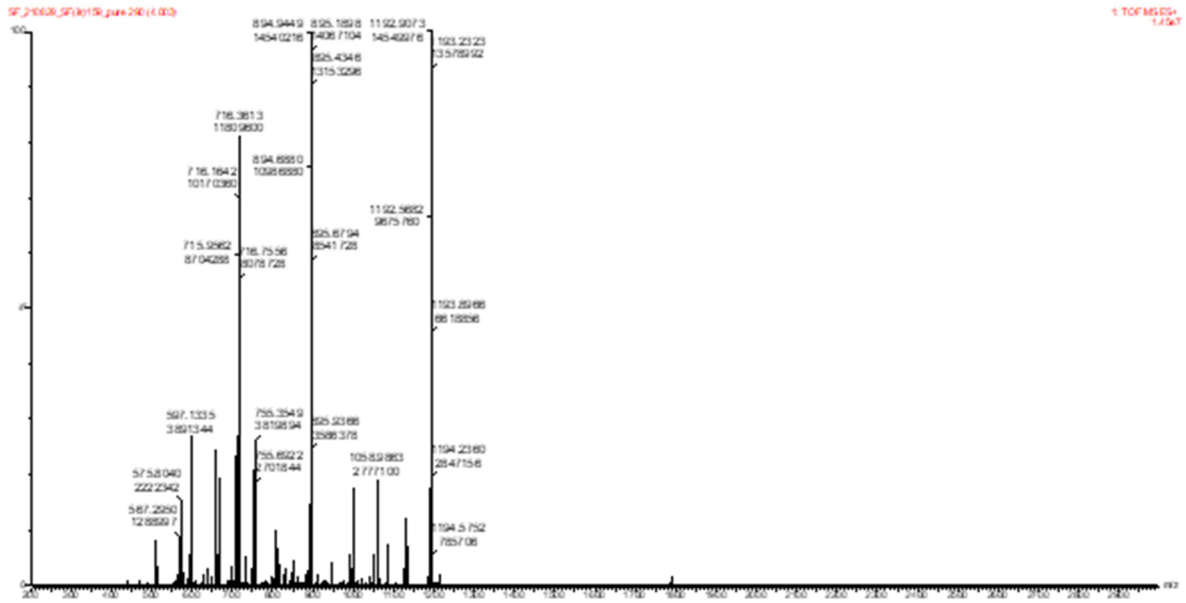
P6(T27A)

The p6(T27A) peptide was synthesized in a 0.05 mmol scale on a Rink Amide Resin with a loading of 0.78 mmol/g. Until the 15 amino acids the synthesis was carried out on a PTI synthesizer with single couplings (5 eq. amino acid for 40 minutes). All amino acids following number 15 were coupled in double coupling steps with five equivalents for 40 minutes. The final cleavage from resin was achieved by incubation with a mixture of TFA:TIS:H2O (95:2.5:2.5;v:v:v) for two hours followed by precipitation in cold diethylether. The crude peptide was purified by preparative reverse phase C18 HPLC and the product was obtained as white powder (29 mg, 6.8 µmol, 13.6 % yield).

HRMS: m/z: 1193,2323 $[M+3H]^{3+}$ (calc. 1193,295 m/z), 895,1898 $[M+4H]^{4+}$ (calc. 895,2212 m/z), 716,3613 $[M+5H]^{5+}$ (calc. 716,377 m/z).



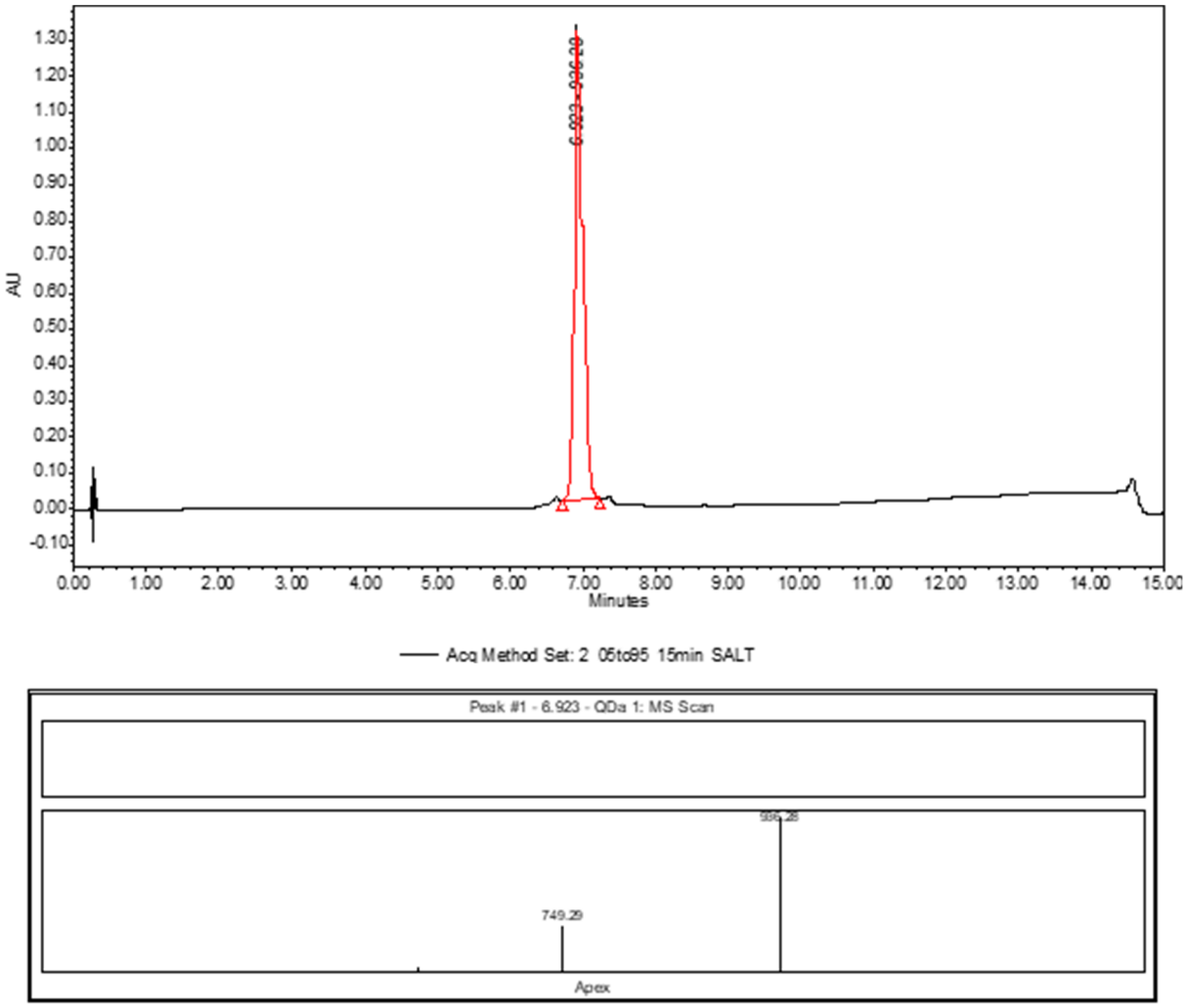
SI Figure 20. p6(T27A), UPLC-MS 15 minute run.



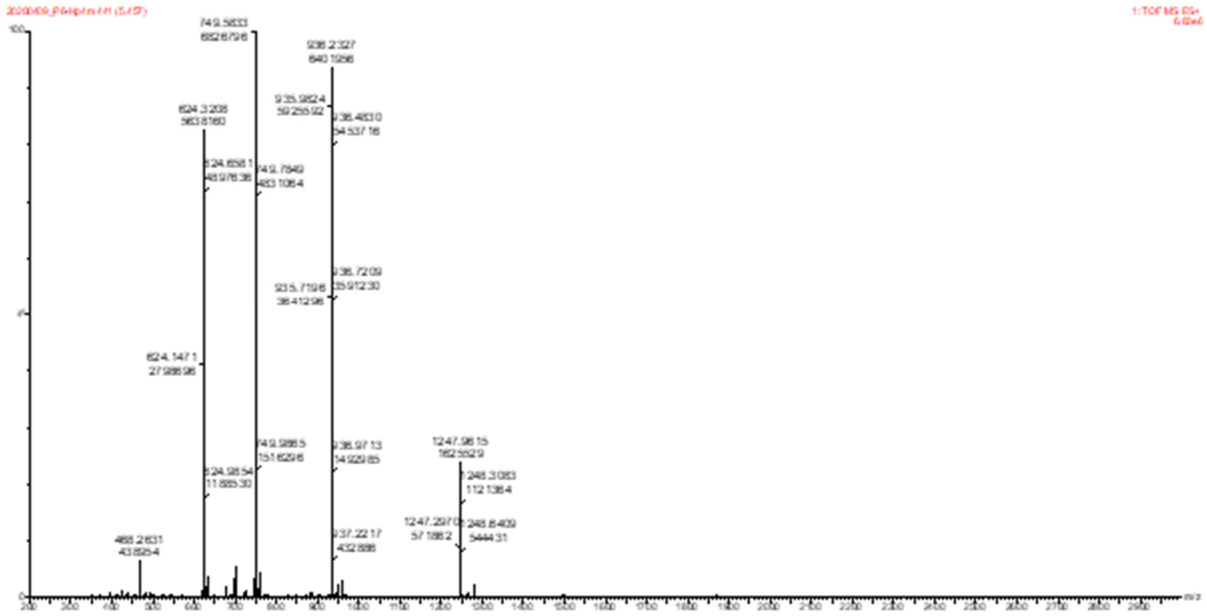
SI Figure 21. p6(T27A), HR-MS.

The cyclized p6 version p6-cyc was synthesized in a 0.05 mmol scale on a Rink Amide Resin with a loading of 0.78 mmol/g. Until the 15 amino acids the synthesis was carried out on a PTI synthesizer with single couplings (5 eq. amino acid for 40 minutes). All amino acids following number 15 were coupled in double coupling steps with five equivalents for 40 minutes. To generate the hydrocarbon staple the resin was incubated with a 10 mM solution of bis(tricyclohexylphosphine)-benzylidene ruthenium (IV) (1st generation Grubb's catalyst) in 1,2-dichloroethane for one hour twice. The final cleavage from resin was achieved by incubation with a mixture of TFA:TIS:H2O (95:2.5:2.5;v:v:v) for two hours followed by precipitation in cold diethylether. The crude peptide was purified by preparative reverse phase C18 HPLC and the product was obtained as white powder (12,3 mg, 2.8 µmol, 5,5% yield).

HRMS: m/z: 1247,9615 $[M+3H]^{3+}$ (calc. 1247,628 m/z), 935,9824 $[M+4H]^{4+}$ (calc. 935,9709 m/z), 749,5833 $[M+5H]^{5+}$ (calc. 748,9767 m/z), 624,3208 $[M+6H]^{6+}$ (calc. 624,3139 m/z).



SI Figure 22. P6-Cyc, RCM stapled peptide, UPLC-MS 15 minute run.



SI Figure 23. P6-Cyc, RCM stapled peptide, HR-MS.

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