RpoN-based stapled peptides with improved DNA binding supress *Pseudomonas aeruginosa* virulence

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Supporting Information

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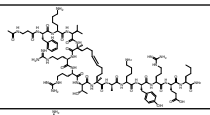
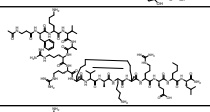
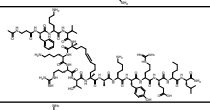
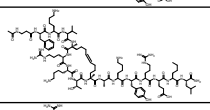
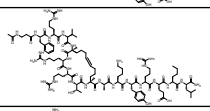
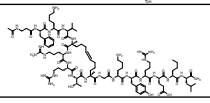
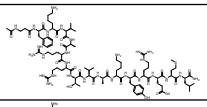
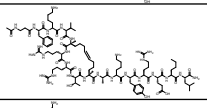
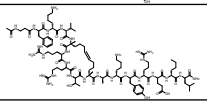
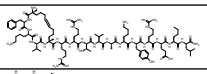
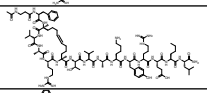
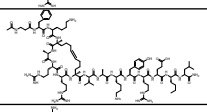
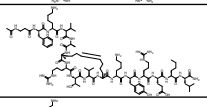
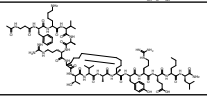
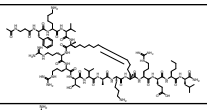
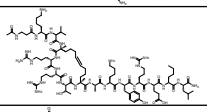
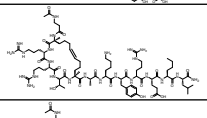
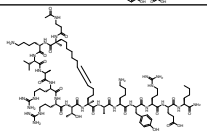
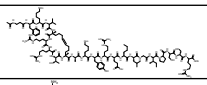
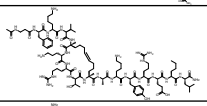
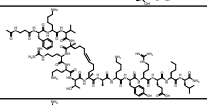
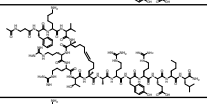
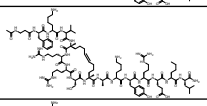
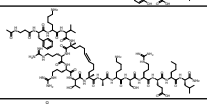
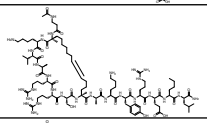
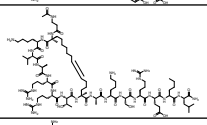
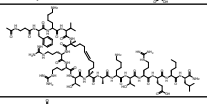
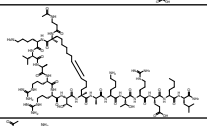
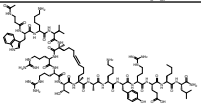
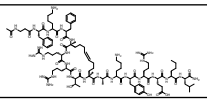
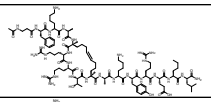
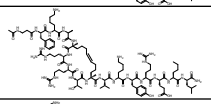
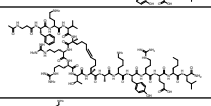
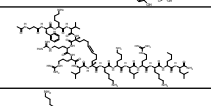
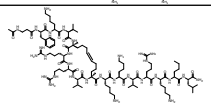
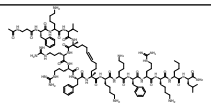
Table S1. Lead peptide purity and peptide library. Peptide sequence, structure, molecular weight (MW), calculated mass $[M+3H]^{3+}$ and observed masses $[M+3H]^{3+}$ are reported. All peptides are N-terminally acetylated and contain C-terminus amide. B: β-alanine, X: (S)-2-4-(pentenyl)alanine, Z: (R)-2-(7-octenyl)alanine, Nle: Norleucine, O: Ornithine

	New	Purity (%)	Sequence	Structure	Exact mass	Mass calc.	Mass obs.
σ54-10	**14**	99.61	BFKVXRRTXAKYRENle		1928.15	643.72	643.90
σ54-40	**10**	95.13	BFKVARRXVAKXRENleL		1947.23	650.08	650.60
σ54-20	**17**	96.81	BFKVXKRTXAKYRENleL		2013.13	672.04	672.50
σ54-21	**18**	95.75	BFKVXRKTXAKYRENleL		2013.13	672.04	672.55
σ54-23	**33**	95.03	BFRVXRRTXAKYRENleL		2070.53	691.84	691.25
σ54-27	**31**	97.03	BFKVXRRTXGKYRENleL		2027.22	676.74	677.20

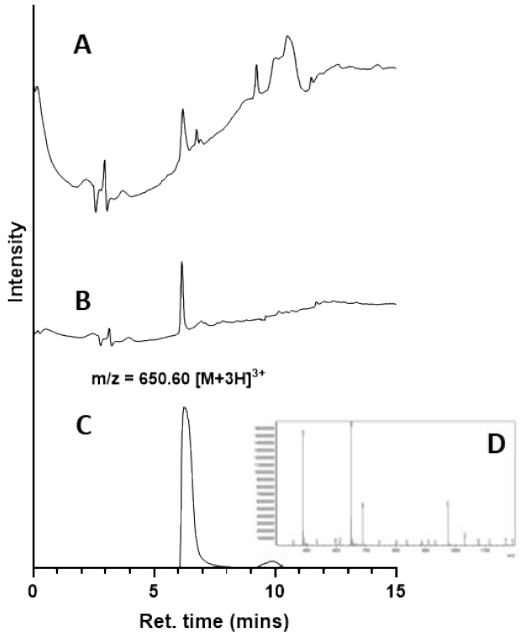
		Sequence	Structure	Exact mass	Mass calc.	Mass obs.
σ54-WT	**WT**	BFKVARRTVAKYREML		1979.13	660.71	661.20
σ54-2	**2**	BFKVXRRTXAKYRENleL		2041.23	681.41	682.00
	D-**2**	D-(BFKVXRRTXAKYRENleL)		2041.23	681.41	681.80
	3*	BZKVARRTXAKYRENleL				
σ54-35	**5**	XFKVXRRTVAKYRENleL		2027.25	676.75	676.75
σ54-36	**6**	BFXVARXTVAKYRENleL		1927.14	643.38	643.90
σ54-37	**7**	BFKXARRXVAKYRENleL		2011.22	671.41	671.85
σ54-38	**8**	BFKVAXRTVXKYRENleL		1984.20	662.40	662.85
σ54-39	**9**	BFKVARXTVAXYRENleL		1927.14	643.38	643.90

σ54-48	**11**	BFKVZRRTVAKXRENleL		2019.28	674.09	674.55
σ54-7	**12**	BKVXRRTXAKYRENleL		1894.16	632.39	633.00
σ54-9	**13**	BXRRTXAKYRENleL		1667.00	556.67	557.00
σ54-15	**15**	BZKVARRTXAKYRENle		1894.16	632.39	632.90
σ54-91	**16**	BFKVXRRTXAKYRENleLGIPSSR		2638.55	880.52	$[M+4H]^{4+}$ 661.05
σ54-79	**19**	BFKVXORTXAKYRENleL		1999.21	667.40	667.85
σ54-80	**20**	BFKVXROTXAKYRENleL		1999.21	667.40	667.90
σ54-24	**21**	BFKVXRRTXARYRENleL		2069.24	691.18	691.20
σ54-53	**22**	BFKVXRRSXAKYRENleL		2027.22	676.74	676.90
σ54-54	**23**	BFKVXRRTXAKSRENleL		1965.20	656.07	656.50
σ54-55	**24**	BZKVARRSXAKYRENleL		1993.23	660.41	660.80
σ54-56	**25**	BZKVARRTXAKSRENleL		1931.22	644.74	645.10
σ54-57	**26**	BFKVXRRTXAKTRENleL		1979.22	660.74	661.15
σ54-58	**27**	BZKVARRTXAKTRENleL		1945.23	649.41	649.80
σ54-25	**28**	BWKVXRRTXAKYRENleL		2080.24	694.41	695.00
σ54-26	**29**	BFKFXRRTXAKYRENleL		2089.23	697.41	697.95

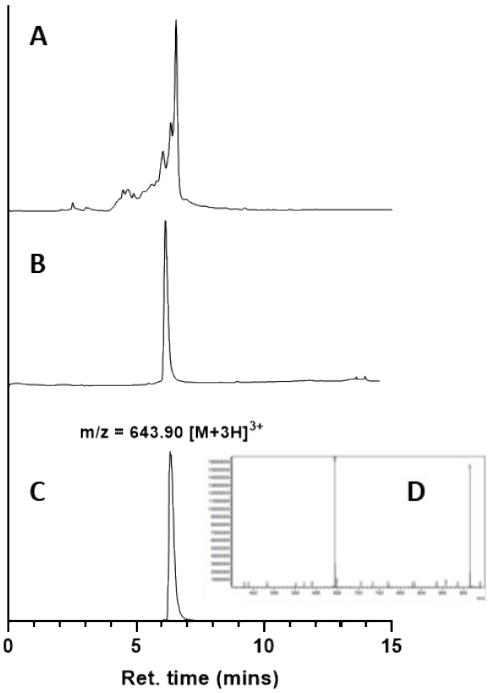
σ54-99	**30**	BFKAXRRTXAKYRENleL		2013.2	672.07	672.50
σ54-98	**32**	BFKAXRRTXVKYRENleL		2041.23	681.41	681.00
σ54-78	**34**	BFOVXRRTXAKYRENleL		2027.22	676.74	677.20
σ54-100	**35**	BFKVXRRLXKKLRKNleL		2045.42	682.81	681.85
σ54-102	**36**	BFKVXRRVXKKVRKNleL		2031.37	678.12	678.55
σ54-103	**37**	BFKVXRRFXKKFRKNleL		2127.37	710.12	711.2

*Peptide 3 was used as a reference sequence for *i*,*i*+7 constructs[1].

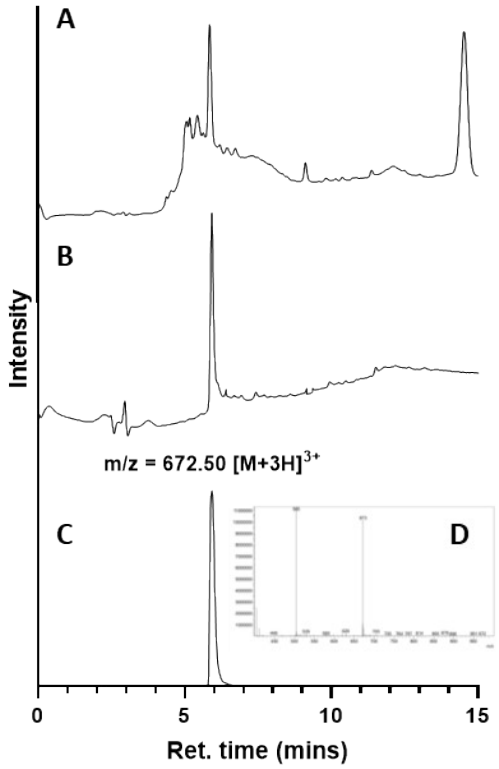
Figures S1. HPLC purified peptides 10, 14, 17, 18, 31, 33, WT, 2 and D-2 and LCMS traces. A. Crude chromatogram traces (absorbance at 274 nm). **B.** Extracted ion chromatogram (EIC). **C.** Purified chromatogram trace (absorbance at 274 nm). **D.** ESI+/MS spectra. Data were collected using a 2020-LCMS Shimadzu instrument (ESI+ and UV-Vis detection at 274 nm or 210 nm) with a Prontosil C18 analytical column. A linear gradient of 5-95% (0.05% formic acid H_2O- 0.0.5% formic acid MeOH) over 20 minutes was used for analytical runs. Purification was performed on a 1100 Series Agilent HPLC with fraction collector and DAD at 274 nm on a Thermo C18 column. A linear gradient of 5-95% (0.1% TFA H_2O- 0.1% TFA MeOH) over 20 minutes was used.



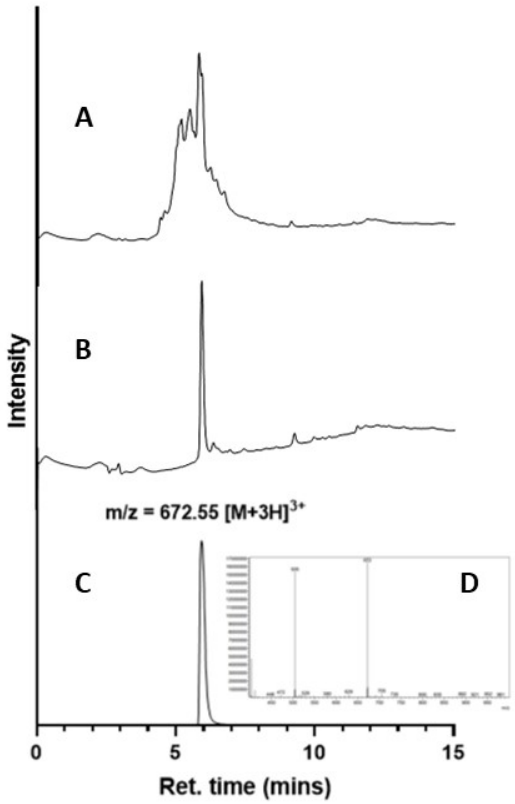
Analysis of peptide 10 – A. Crude LCMS trace detected at 274 nm B. Purified LCMS trace detected at 274 nm C. Purified LCMS trace with extracted ion chromatogram, m/z = 650.60 D. ESI/MS of peptide **10**



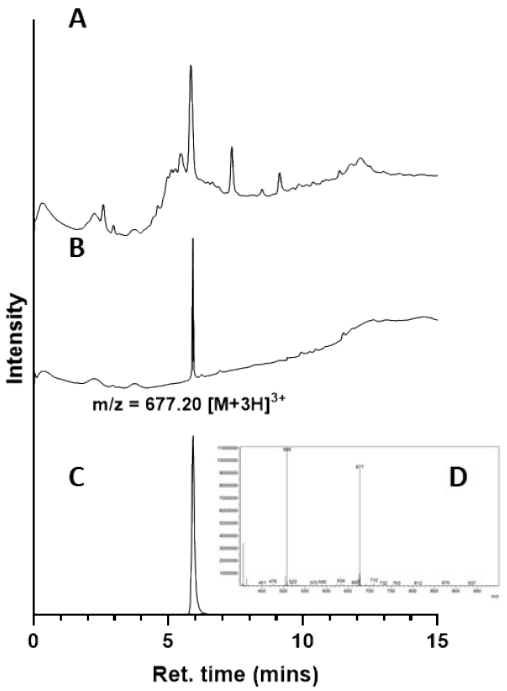
Analysis of peptide 14 – A. Crude LCMS trace detected at 274 nm B. Purified LCMS trace detected at 274 nm C. Purified LCMS trace with extracted ion chromatogram, m/z = 643.90 D. ESI/MS of peptide **14**



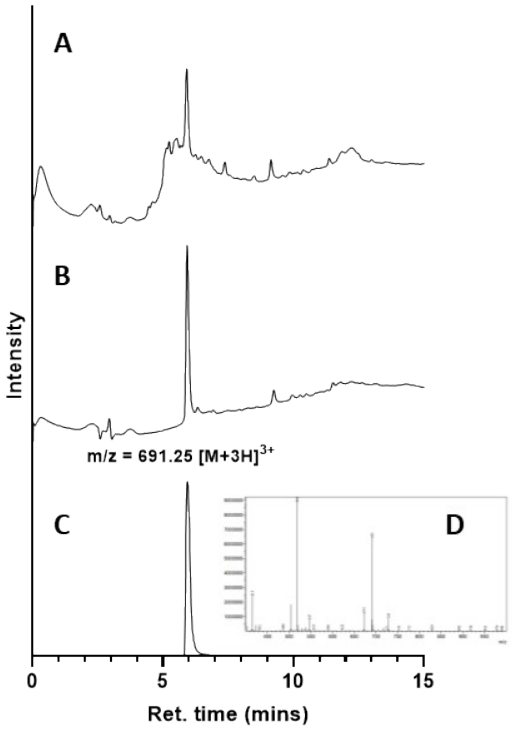
Analysis of peptide 17 – A. Crude LCMS trace detected at 274 nm B. Purified LCMS trace detected at 274 nm C. Purified LCMS trace with extracted ion chromatogram, m/z = 672.50 D. ESI/MS of peptide **17**



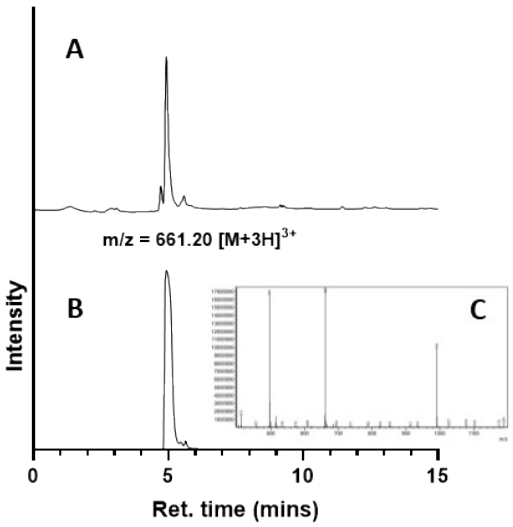
Analysis of peptide 18 – A. Crude LCMS trace detected at 274 nm B. Purified LCMS trace detected at 274 nm C. Purified LCMS trace with extracted ion chromatogram, m/z = 672.55 D. ESI/MS of peptide **18**



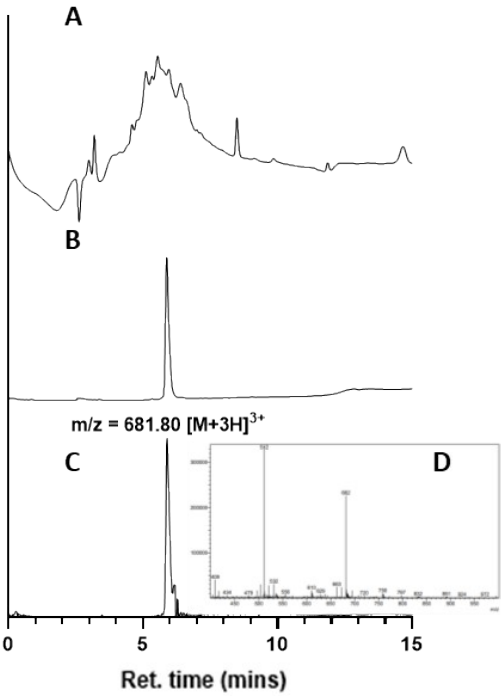
Analysis of peptide 31 – A. Crude LCMS trace detected at 274 nm B. Purified LCMS trace detected at 274 nm C. Purified LCMS trace with extracted ion chromatogram, m/z = 677.20 D. ESI/MS of peptide **31**



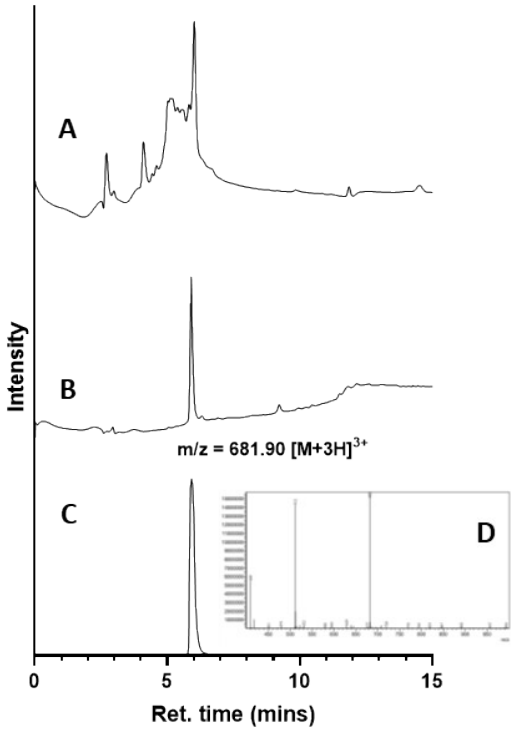
Analysis of peptide 33 – A. Crude LCMS trace detected at 274 nm B. Purified LCMS trace detected at 274 nm C. Purified LCMS trace with extracted ion chromatogram, m/z = 691.25 D. ESI/MS of peptide **33**



Analysis of WT peptide – A. Pure LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 661.20 D. ESI/MS of WT peptide

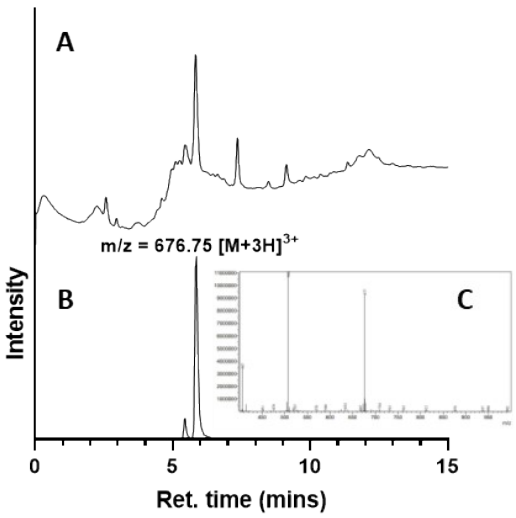


Analysis of peptide D-2 – A. Crude LCMS trace detected at 274 nm B. Purified LCMS trace detected at 274 nm C. Purified LCMS trace with extracted ion chromatogram, m/z = 681.80 D. ESI/MS of peptide D-2

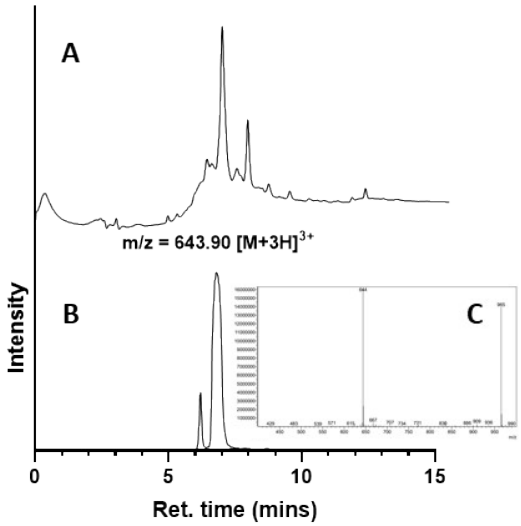


Analysis of peptide 2 – A. Crude LCMS trace detected at 274 nm B. Purified LCMS trace detected at 274 nm C. Purified LCMS trace with extracted ion chromatogram, m/z = 682.00 D. ESI/MS of peptide **2**

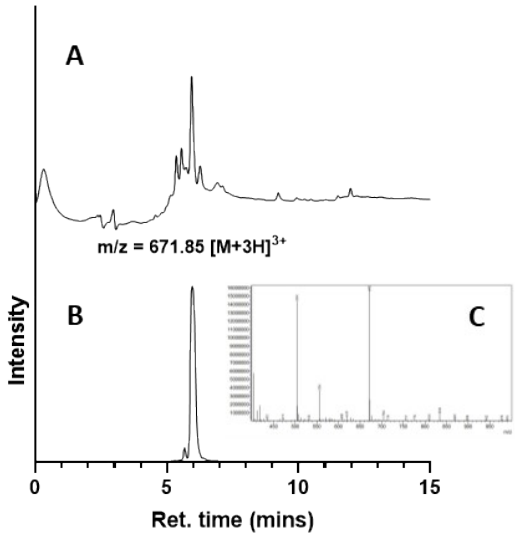
Figures S2. Crude peptides LCMS traces. A. Crude chromatogram traces (Absorbance at 274 nm). **B.** Extracted ion chromatogram (EIC). **C.** ESI+/MS spectra. Spectra were obtained on a 2020-LCMS Shimadzu instrument (ESI+ and 274 nm detection) with a Prontosil C18 analytical column. A linear gradient of 5-95% (0.05% formic acid H_2O- 0.05% formic acid MeCN) over 20 minutes was used.



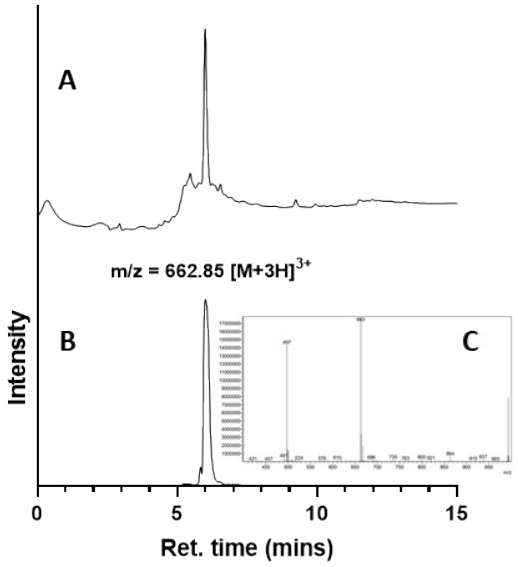
Analysis of peptide 5 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 676.75 D. ESI/MS of peptide **5**



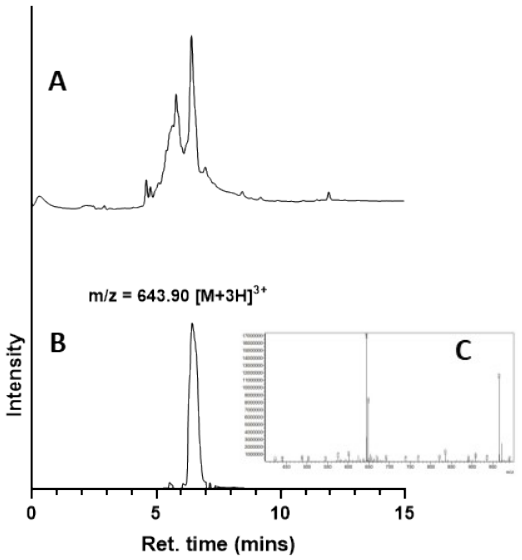
Analysis of peptide 6 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 643.90 D. ESI/MS of peptide **6**



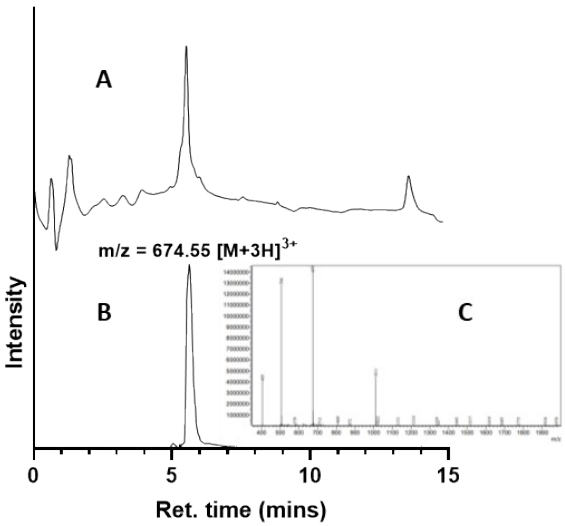
Analysis of peptide 7 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 671.85 D. ESI/MS of peptide **7**



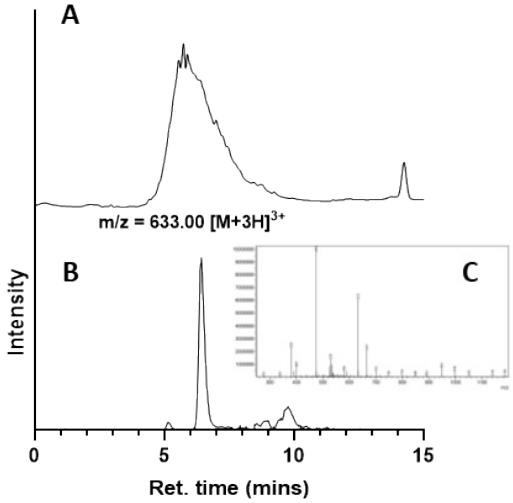
Analysis of peptide 8 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 662.85 D. ESI/MS of peptide **8**



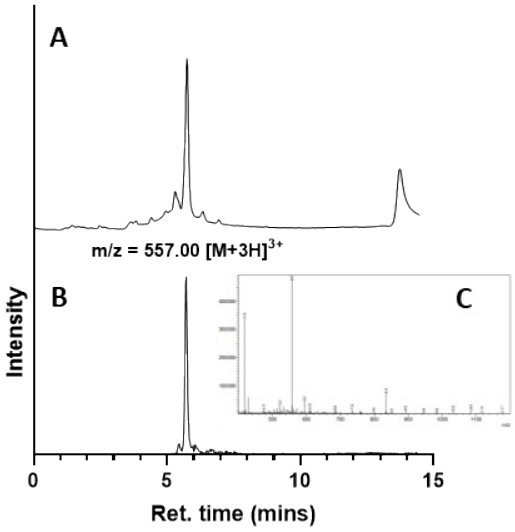
Analysis of peptide 9 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 643.90 D. ESI/MS of peptide **9**



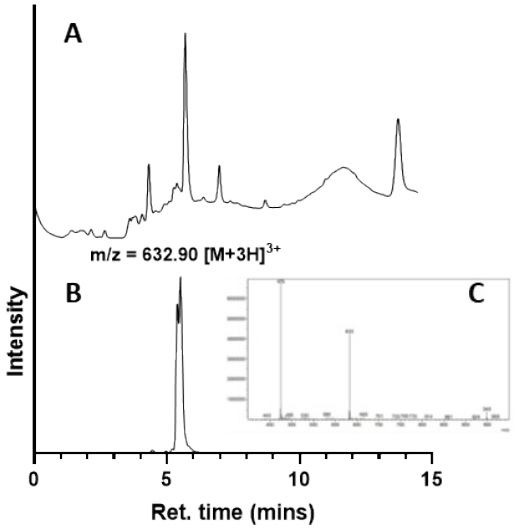
Analysis of peptide 11 – A. Crude LCMS trace detected at 210 nm B. LCMS trace with extracted ion chromatogram, m/z = 674.55 D. ESI/MS of peptide **11**



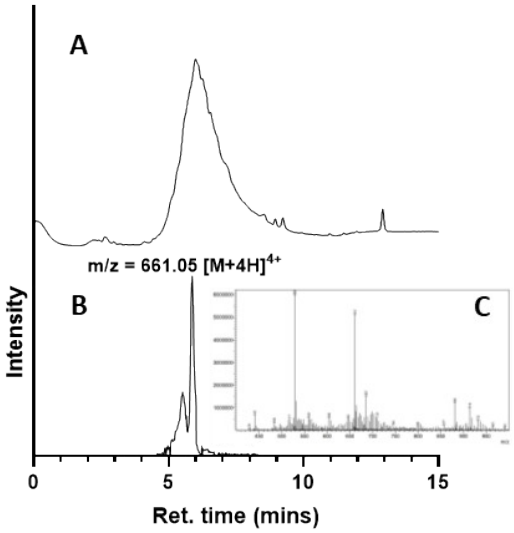
Analysis of peptide 12 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 633.00 D. ESI/MS of peptide **12**



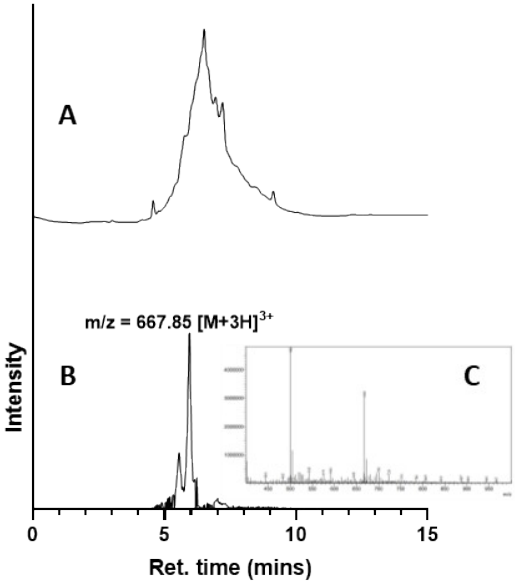
Analysis of peptide 13 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 557.00 D. ESI/MS of peptide **13**



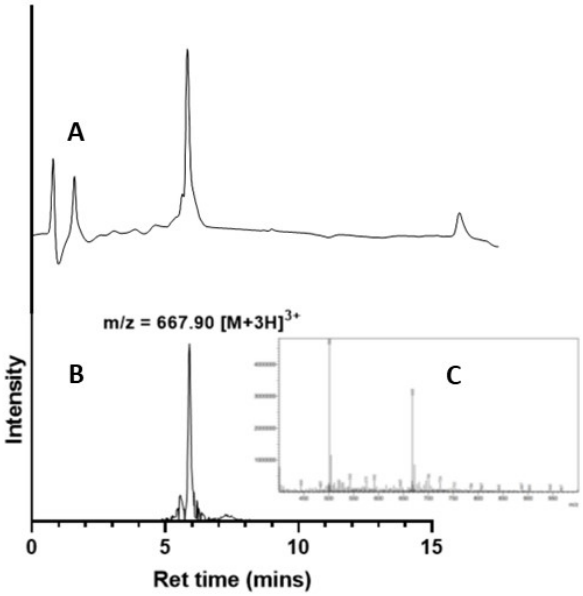
Analysis of peptide 15 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 632.90 D. ESI/MS of peptide **15**



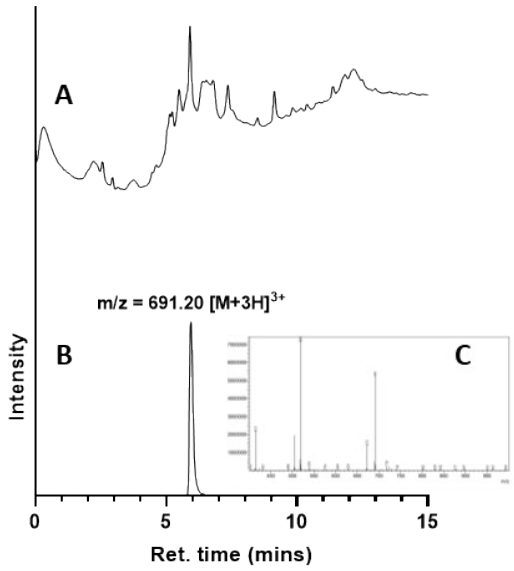
Analysis of peptide 16 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 661.05 D. ESI/MS of peptide **16**



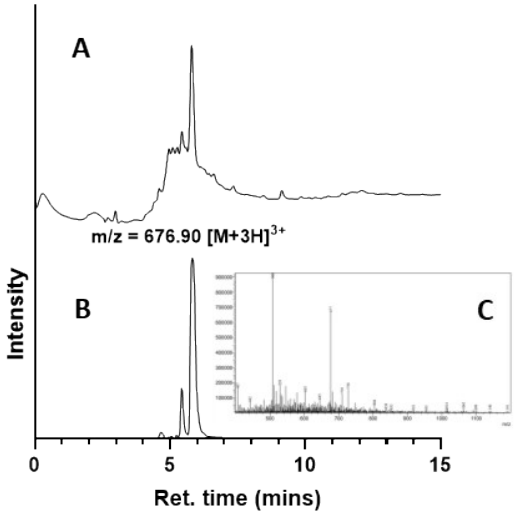
Analysis of peptide 19 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 667.85 D. ESI/MS of peptide **19**



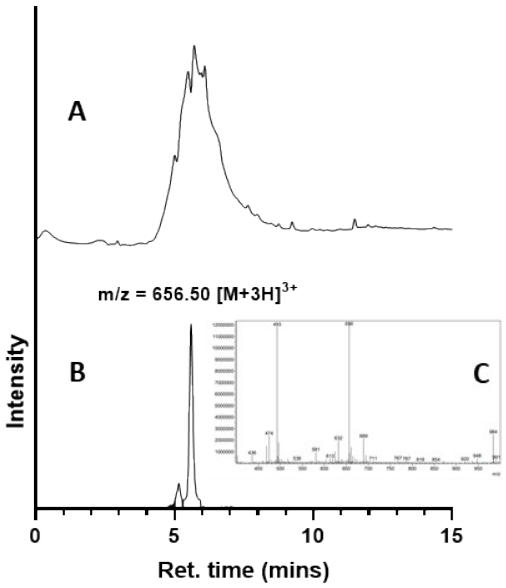
Analysis of peptide 20 – A. Crude LCMS trace detected at 210 nm B. LCMS trace with extracted ion chromatogram, m/z = 667.90 D. ESI/MS of peptide **20**



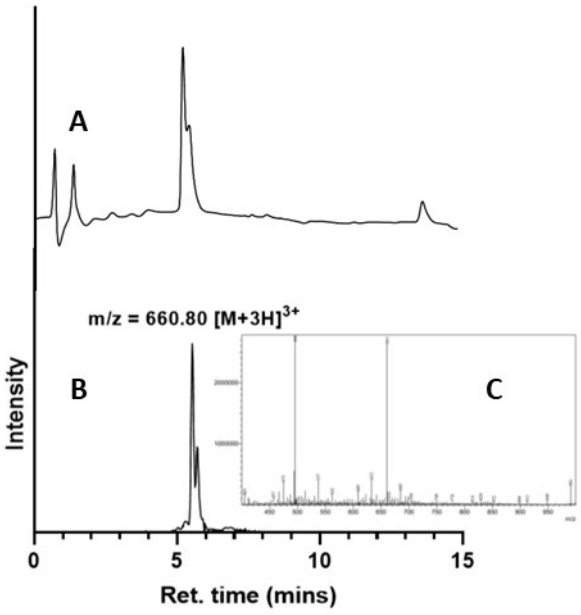
Analysis of peptide 21 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 691.20 D. ESI/MS of peptide **21**



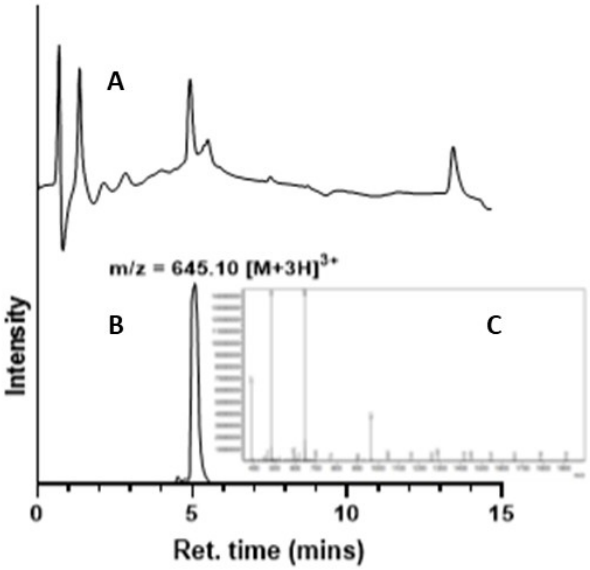
Analysis of peptide 22 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 676.90 D. ESI/MS of peptide **22**



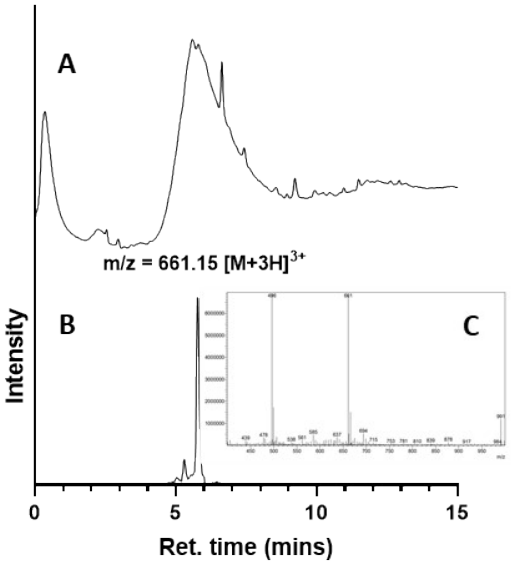
Analysis of peptide 23 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 656.50 D. ESI/MS of peptide **23**



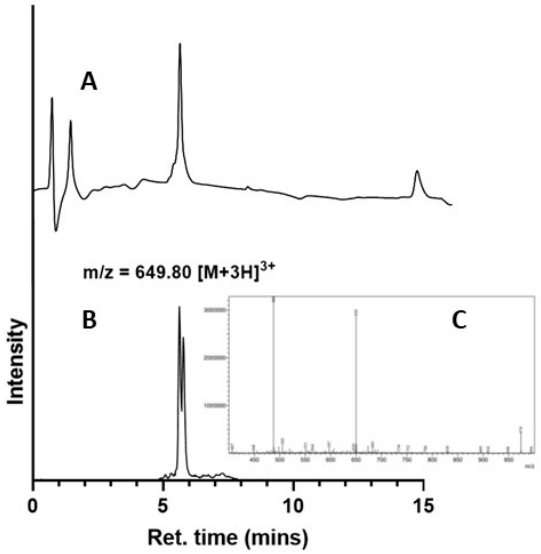
Analysis of peptide 24 – A. Crude LCMS trace detected at 210 nm B. LCMS trace with extracted ion chromatogram, m/z = 660.80 D. ESI/MS of peptide **24**



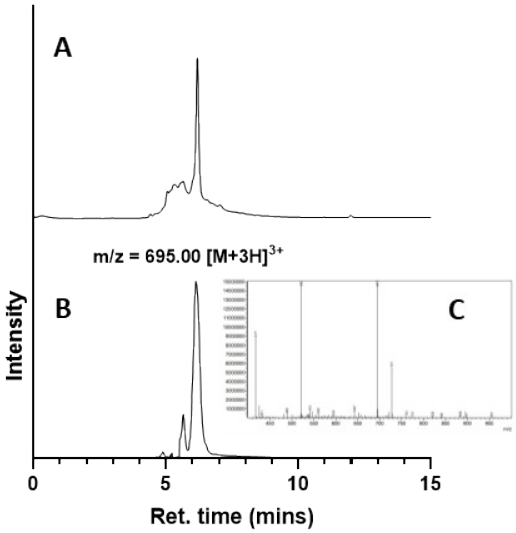
Analysis of peptide 25 – A. Crude LCMS trace detected at 210 nm B. LCMS trace with extracted ion chromatogram, m/z = 645.10 D. ESI/MS of peptide **25**



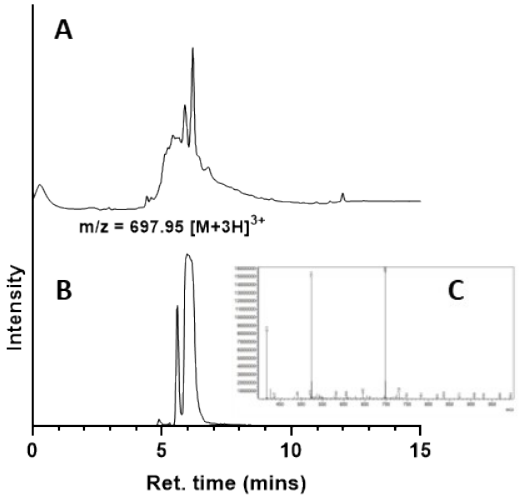
Analysis of peptide 26 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 661.15 D. ESI/MS of peptide **26**



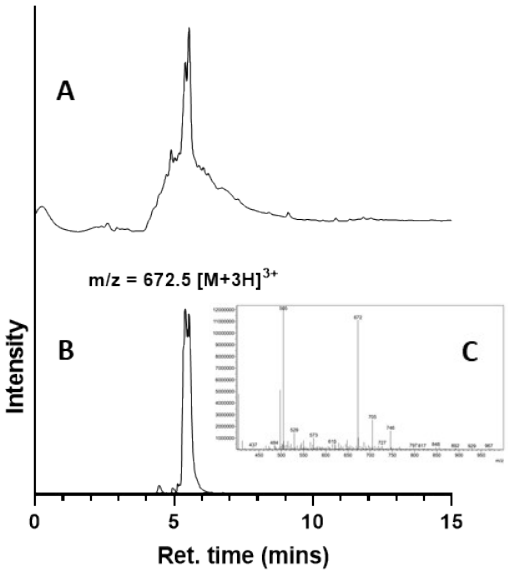
Analysis of peptide 27 – A. Crude LCMS trace detected at 210 nm B. LCMS trace with extracted ion chromatogram, m/z = 649.80 D. ESI/MS of peptide **27**



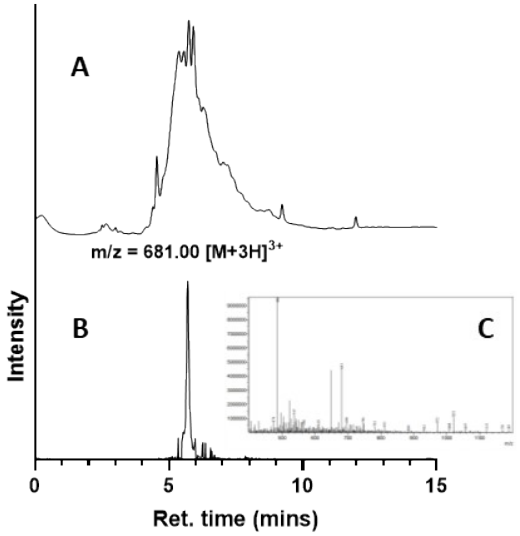
Analysis of peptide 28 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 695.00 D. ESI/MS of peptide **28**



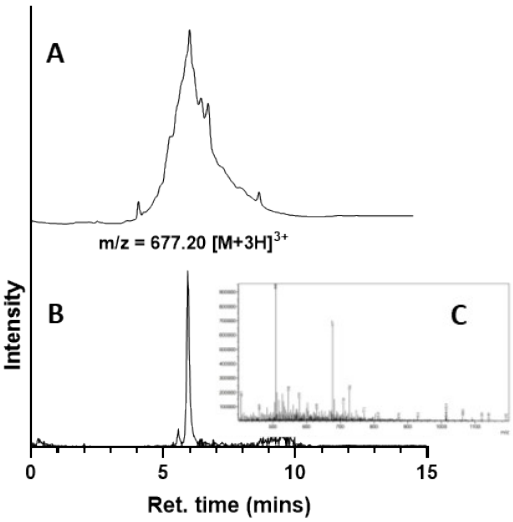
Analysis of peptide 29 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 697.95 D. ESI/MS of peptide 29



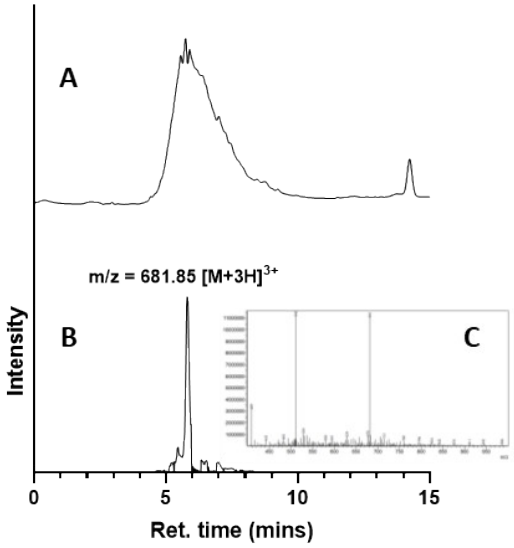
Analysis of peptide 30 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 672.50 D. ESI/MS of peptide **30**



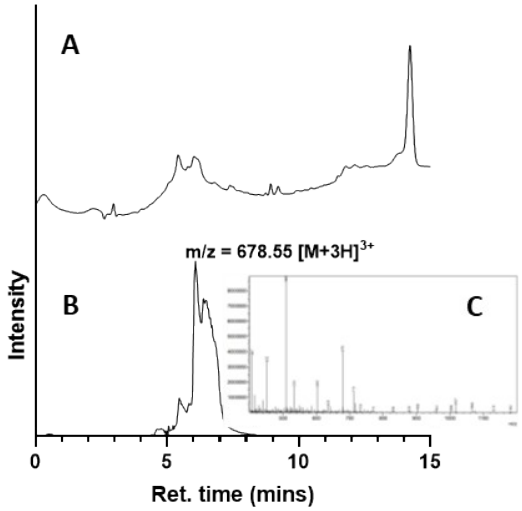
Analysis of peptide 32 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 681.00 D. ESI/MS of peptide **33**



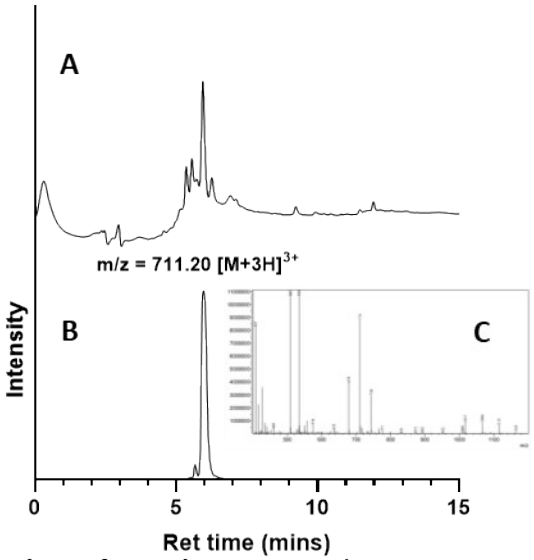
Analysis of peptide 34 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 677.20 D. ESI/MS of peptide **34**



Analysis of peptide 35 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 681.85 D. ESI/MS of peptide **35**



Analysis of peptide 36 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 678.55 D. ESI/MS of peptide **36**



Analysis of peptide 37 – A. Crude LCMS trace detected at 274 nm B. LCMS trace with extracted ion chromatogram, m/z = 711.20 D. ESI/MS of peptide **37**

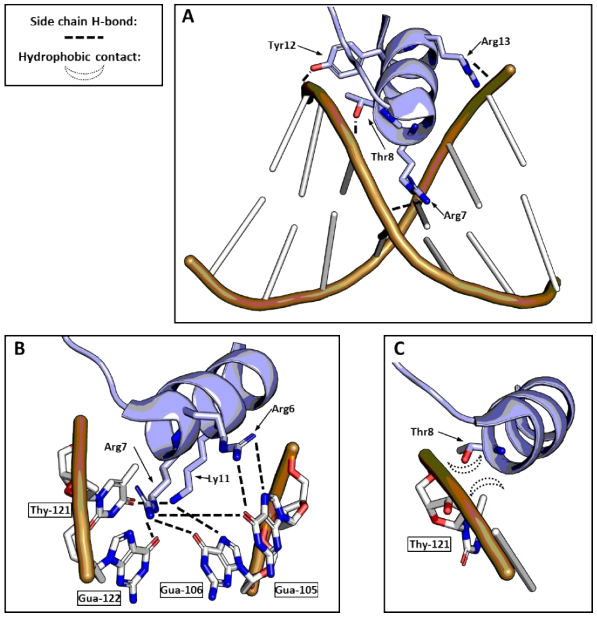


Figure S3. σ54 -24 promoter recognition model. A. RpoN helix with DNA phosphate binding residues Arg7, Thr8, Tyr12 and Arg13. **B.** RpoN helix with nucleobase H-bonding residues Arg6, Arg7 and Lys11. **C.** RpoN helix with hydrophobic interaction of Thr 8. Based on PDB: 2O8K

Table S2. Minimal Inhibitory concentration (MIC). Peptide **WT**, **2**, **10**, **14**, **17**, **18**, **31** and **33** MIC against *Escherichia coli* (XL1Blue and BW25113) and *Pseudomonas aeruginosa* (PA01) starting from 32 µg/mL**.**

	MIC (µg/mL)							
Bacterial species	**WT**	**2**	**10**	**14**	**17**	**18**	**31**	**33**
XL1Blu	>32	>32	32	>32	>32	>32	16	32
BW25113	>32	>32	32	>32	>32	>32	32	32
PA01	>32	>32	>32	>32	>32	>32	>32	>32

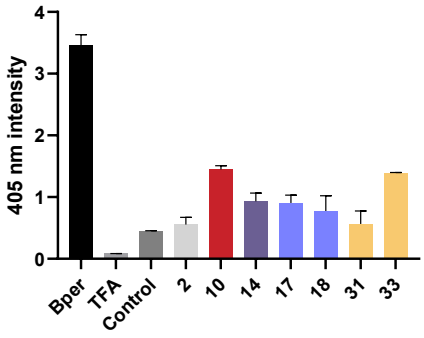


Figure S4. Inner membrane permeability assay. Peptide **WT**, **2**, **10**, **14**, **17**, **18**, **31** and **33** permeability. 0.1% TFA was used as positive control, control conditions is 1.5 mM ONPG and BPer treatment as negative control

>PglnAP2-GFP

CGGAATTCGCGGCCGCTTCTAGAGACATCCTCCGCAAACAAGTATTGCAGAGTCCCTTTGTGATCGCTTTCACGGA
GCATAAAAAGGGTTATCCATCATGAGATGCACCAACATGGTGCTTAATGTTTCCATTGAAGCACTATATTGGTGCA
ACATTCACATCGTGGTGCAGCCCTTTTGCACGATGGTGCGCATGATAACGCCTTTTAGGGGCAATTTAAAAGT*TGG*
*CAC**AGATTT**CGCTT*TATCTTTTTAAATACTAGGATTAAAGAGGAGAAATACTAG***ATG***CGTAAAGGAGAAGAACTTT
TCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGG
TGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAA
CACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATACCCAGATCATATGAAACAGCATGACTTTTTC
AAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGT
GCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGA
AACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATG
GAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAA
TACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATC
CCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAAC
TATACAAA***TAA***TAATACTAGAGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTA
TCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATA
CTAGTAGCGGCCGCTGCAGGGTACCCC

Figure S5. PglnAP2-30 GFP construct sequence. Fasta format sequence of the synthetic construct used for the whole cell assay to evaluate stapled peptide inhibition of RpoN-mediated transcription. The construct encodes GFP (start and stop codons in bold italics) under the control of the *glnAP2* promoter (italicized with the -24 and -12 sites underlined).

Table S3. Primer sequences used for GFP cloning. Oligos were designed using standard primer design guidelines and ordered from Integrated DNA Technologies with standard desalting. All oligos are shown going 5' to 3' direction. Restriction sites are underlined in the appropriate primers.

Oligo name	Sequence
GFP_fwd	TCATGAATTCAATGCGTAAAGGAGAAGAACTTTTC
GFP_rev	GATCGTCTAGATTATTTGTATAGTTCATCCATGCCATG
pUC18_seq_fwd	GCACGACAGGTTTCCCGACTG
pUC18_seq_rev	CGGGCCTCTTCGCTATTACGC

Table S4. GFP assay fluorescence results for all peptides. Peptides were tested at 10 µM and 2.5 µM in M9 40 µM NH_4Cl content. Data are represented as n = 3 (except for PBS and **2** where n = 12), means ± STD. Fluorescence is normalized to untreated samples.

Peptide	Sequence	Net Fluorescence Change (GFP)	
		2.5 µM	10 µM
2	**BFKVXRRTXAKYRENleL**	0.935 ± 0.0656	0.246 ± 0.160
WT	**BFKVARRTVAKYREML**	0.888 ± 0.0479	0.892 ± 0.0468
D-**2**	D-**(BFKVXRRTXAKYRENleL)**	0.916 ± 0.0415	0.677 ± 0.0178
5	**XFKVXRRTVAKYRENleL**	1.19 ± 0.0758	1.009 ± 0.122
6	**BFXVARXTVAKYRENleL**	1.13 ± 0.0403	0.831 ± 0.115
7	**BFKXARRXVAKYRENleL**	0.970 ± 0.0386	0.854 ± 0.136
8	**BFKVAXRTVXKYRENleL**	1.09 ± 0.219	0.888 ± 0.503
9	**BFKVARXTVAXYRENleL**	1.10 ± 0.141	0.951 ± 0.117
10	**BFKVARRXVAKXRENleL**	0.577 ± 0.0908	0.0298 ± 0.0768
11	**BFKVZRRTVAKXRENleL**	0.4901 ± 0.0406	0.505 ± 0.0330
12	**BKVXRRTXAKYRENleL**	0.962 ± 0.0819	0.947 ± 0.0883
13	**BXRRTXAKYRENleL**	0.919 ± 0.888	0.843 ± 0.0902
14	**BFKVXRRTXAKYRENle**	0.885 ± 0.114	0.0288 ± 0.0343
15	**BZKVARRTXAKYRENle**	0.963 ± 0.0652	0.757 ± 0.113
16	**BFKVXRRTXAKYRENleLGIPSSR**	0.894 ± 0.0628	0.427 ± 0.0278
17	**BFKVXKRTXAKYRENleL**	0.943 ± 0.0699	0.0801 ± 0.0308
18	**BFKVXRKTXAKYRENleL**	1.008 ± 0.00746	0.0374 ± 0.0129
19	**BFKVXORTXAKYRENleL**	0.519 ± 0.0375	0.523 ± 0.0224
20	**BFKVXROTXAKYRENleL**	0.909 ± 0.155	0.773 ± 0.0516
21	**BFKVXRRTXARYRENleL**	0.935 ± 0.0113	0.4493 ± 0.0446
22	**BFKVXRRSXAKYRENleL**	1.02 ± 0.0916	0.475 ± 0.0976
23	**BFKVXRRTXAKSRENleL**	0.801 ± 0.0990	0.818 ± 0.0371
24	**BZKVARRSXAKYRENleL**	0.850 ± 0.476	0.733 ± 0.0815
25	**BZKVARRTXAKSRENleL**	0.529 ± 0.0398	0.471 ± 0.0321
26	**BFKVXRRTXAKTRENleL**	0.999 ± 0.0911	0.675 ± 0.124
27	**BZKVARRTXAKTRENleL**	0.883 ± 0.113	0.813 ± 0.170
28	**BWKVXRRTXAKYRENleL**	0.9201 ± 0.0142	0.179 ± 0.262
29	**BFKFXRRTXAKYRENleL**	1.006 ± 0.0646	0.739 ± 0.330
30	**BFKAXRRTXAKYRENleL**	0.520 ± 0.0126	0.454 ± 0.0508
31	**BFKVXRRTXGKYRENleL**	0.451 ± 0.0839	0.0358 ± 0.0292
32	**BFKAXRRTXVKYRENleL**	0.919 ± 0.0740	0.477 ± 0.0615
33	**BFRVXRRTXAKYRENleL**	0.700 ± 0.657	0.0247 ± 0.0152
34	**BFOVXRRTXAKYRENleL**	1.11 ± 0.140	0.872 ± 0.0590
35	**BFKVXRRLXKKLRKNleL**	0.926 ± 0.0676	0.346 ± 0.156
36	**BFKVXRRVXKKVRKNleL**	0.998 ± 0.0802	0.800 ± 0.0587
37	**BFKVXRRFXKKFRKNleL**	0.743 ± 0.00807	0.623 ± 0.212

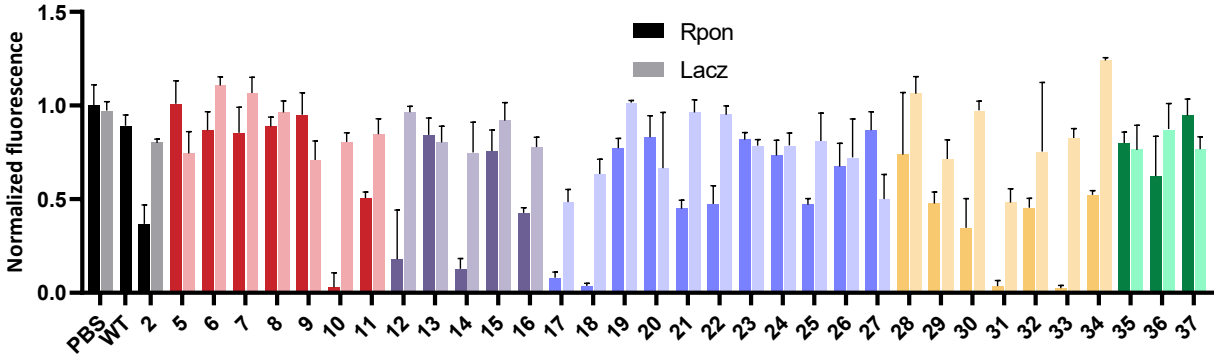


Figure S6. Constitutive promoter GFP and RpoN-GFP results. Library screen of RpoN-*glnAp2* promoter (black or dark colored) and constitutive *lacUV5* promoter (grey or light colored) GFP screen. Means ± STD, normalized to vehicle control

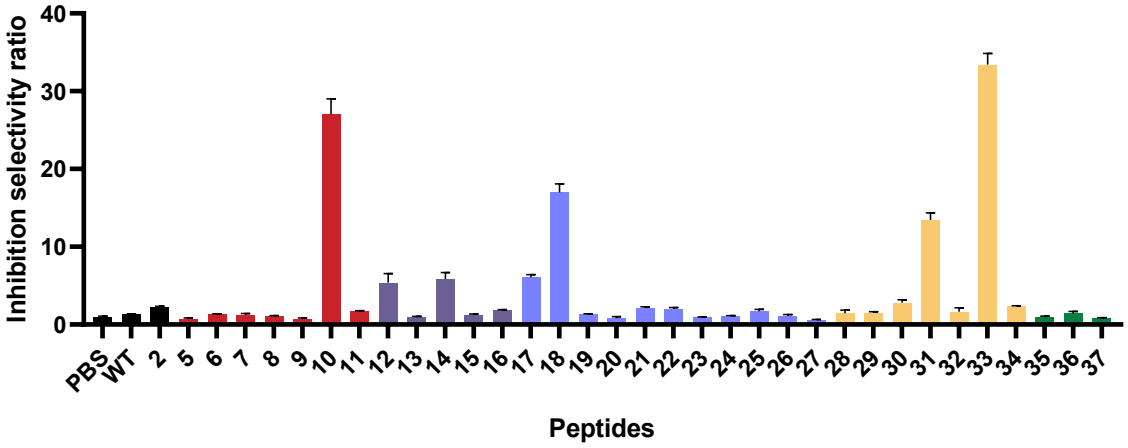


Figure S7. Inhibitory selectivity ratio. Ratio of GFP expression results of peptides **2**-**37** of RpoN-*glnAp2* promoter and counter-screen *lacUV5* promoter GFP screen. Means ± STD, normalized to vehicle control

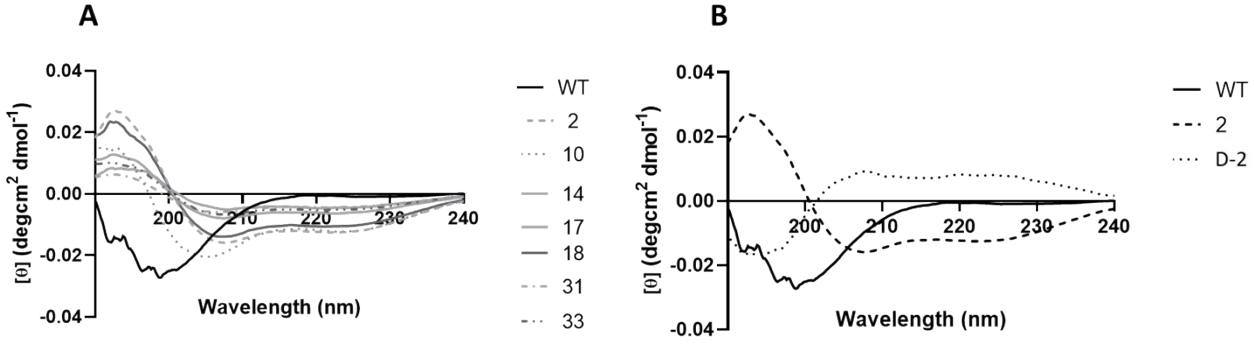


Figure S8. Circular Dichroism (CD). A. CD of purified peptides **10**, **14**, **17**, **18**, **31 and 33**. **B.** CD of controls **WT**, **2**, D-**2**.

A

Name	Sequence (5’ -> 3’)
FITC *glnAP2* 30mer Forward	/5FluorT/AGTTGGCACAGATTTCGCTTTATCTTTTTT
FITC *glnAP2* 30mer Reverse	/5FluorT/AAAAAAGATAAAGCGAAATCTGTGCCAACT
FITC *glnAP2* 30mer-scrambled Forward	/5FluorT/GTGCTATTATTCCGTTATCGTTGTTACTAT
FITC *glnAP2* 30mer-scrambled Reverse	/5FluorT/ATAGTAACAACGATAACGGAATAATAGCAC

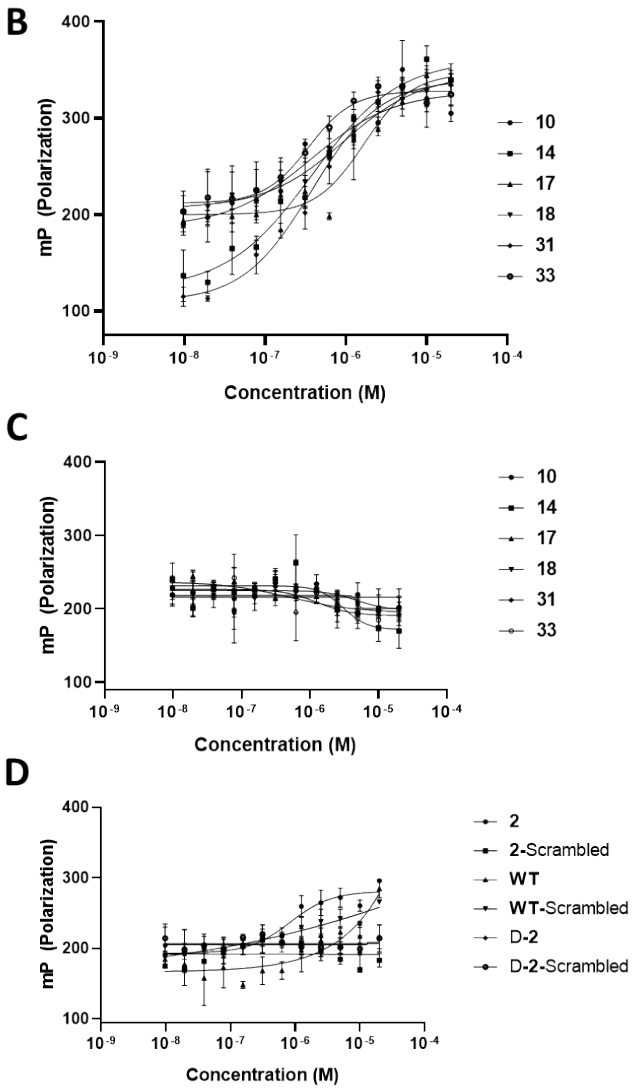


Figure S9. Fluorescence polarization assay (FPA) binding of purified peptides. Binding of serially diluted (from 20 mM) peptides incubated with DNA (5.625 nM), reporting binding affinity (K_d)**A.** *glnAP2* and scrambled forward and reverse fluorescein labelled DNA oligos name and sequence. **B.** Binding curves of peptides with *glnAP2* promoter. **C.** Binding curves of peptides with scrambled DNA oligo. **D.** Controls **WT**, **2** and D-**2** binding to *glnAP2* promoter and scrambled DNA sequence.

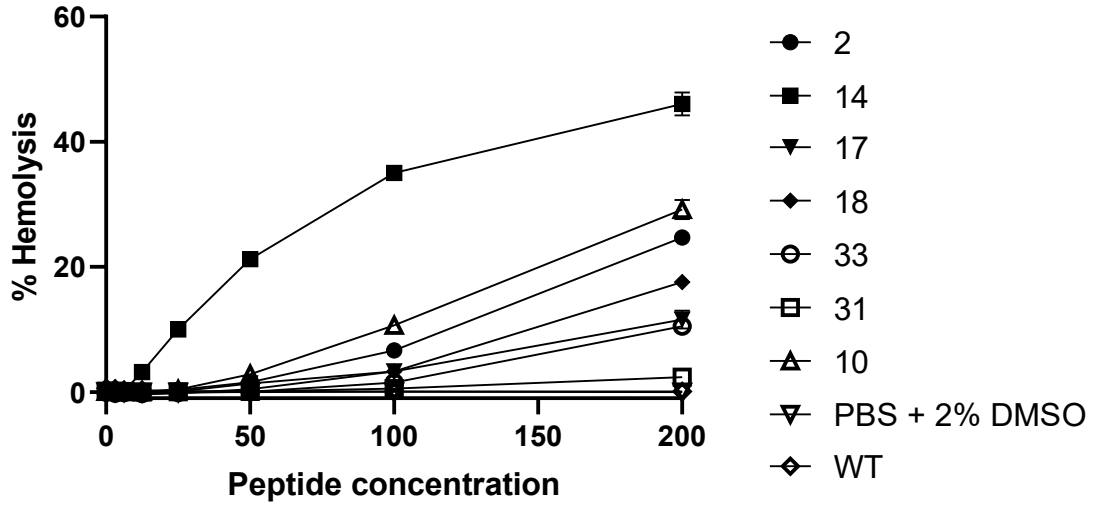


Figure S10. Hemolysis data up to 200 µM in human red blood cells. Purified peptides, **2** and **WT** % hemolysis.

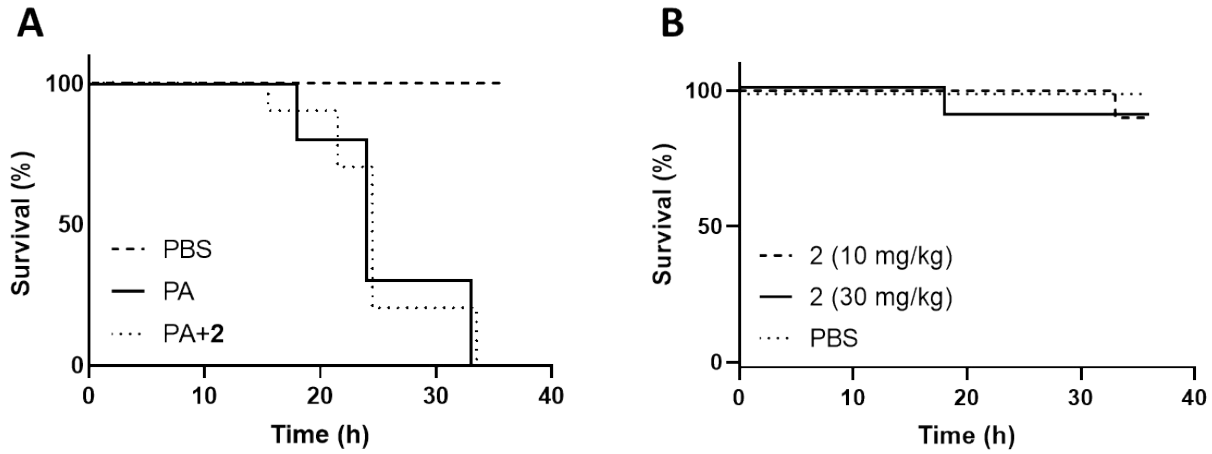


Figure S11. *In vivo G. mellonella* supplemental data. A. PBS vehicle control, *Pseudomonas aeruginosa* (PA) and PA + **2**. B. **2** (10 mg/kg), **2** (30 mg/kg) and PBS vehicle control.

References

1. Payne SR, Pau DI, Whiting AL, et al. Inhibition of Bacterial Gene Transcription with an RpoN-Based Stapled Peptide. *Cell Chem Biol*. 2018;25(9):1059-1066.e4. doi:10.1016/j.chembiol.2018.05.007