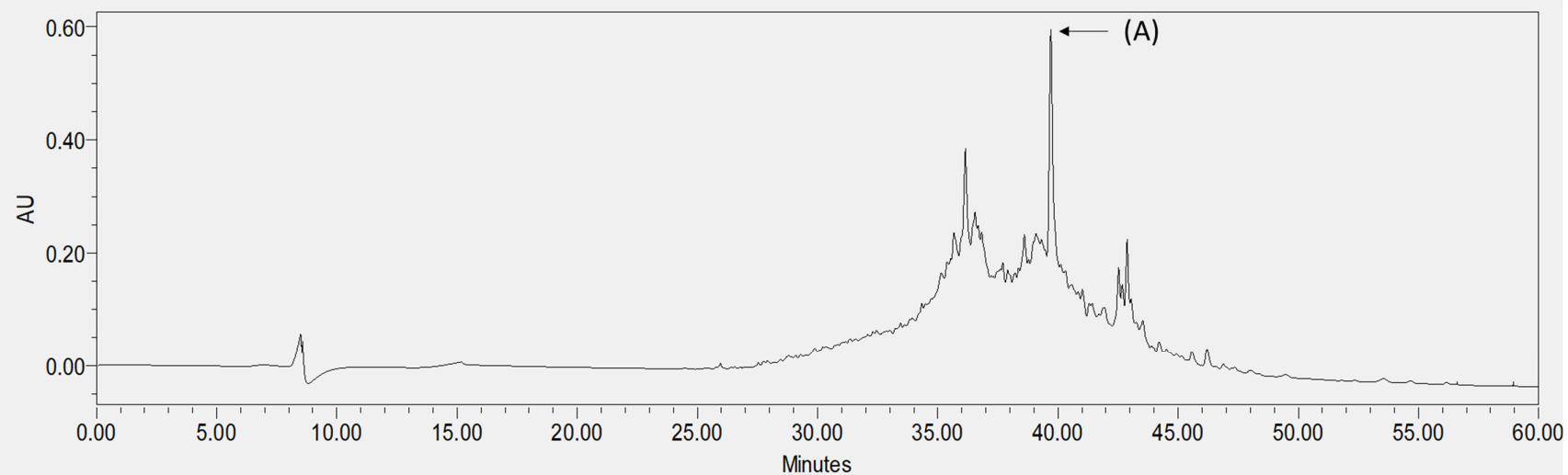
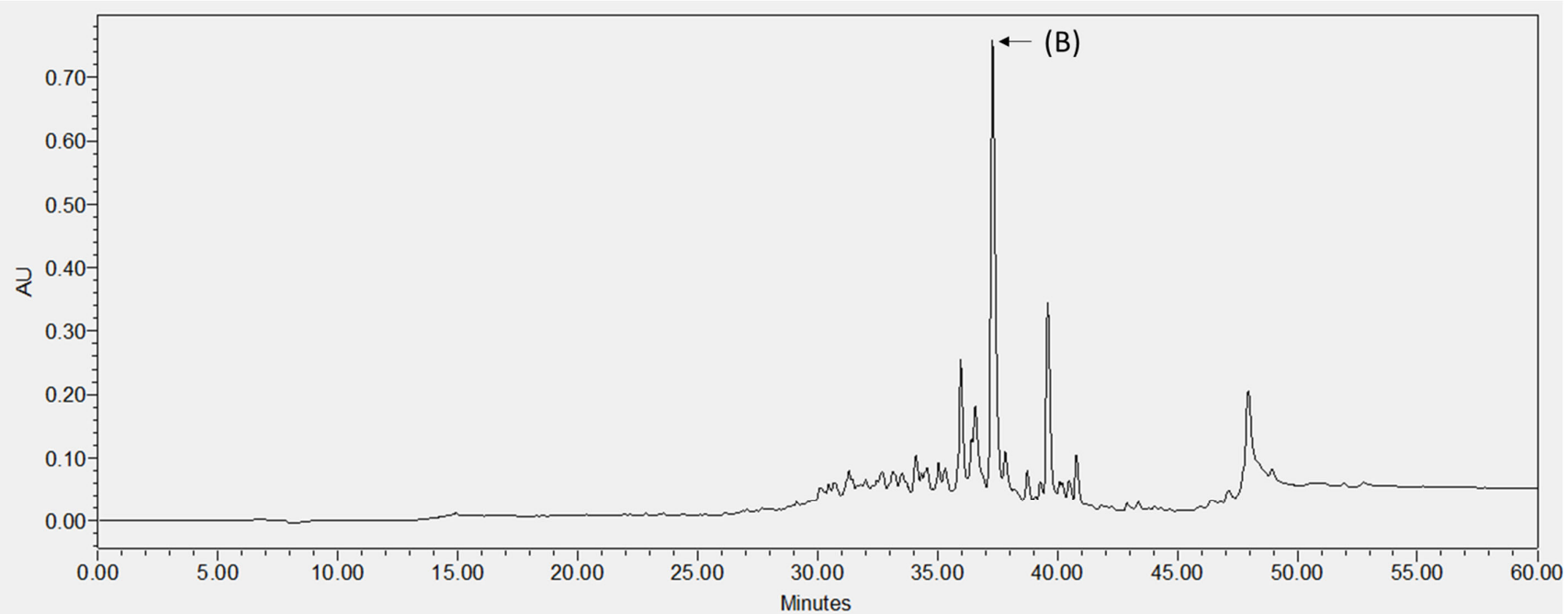
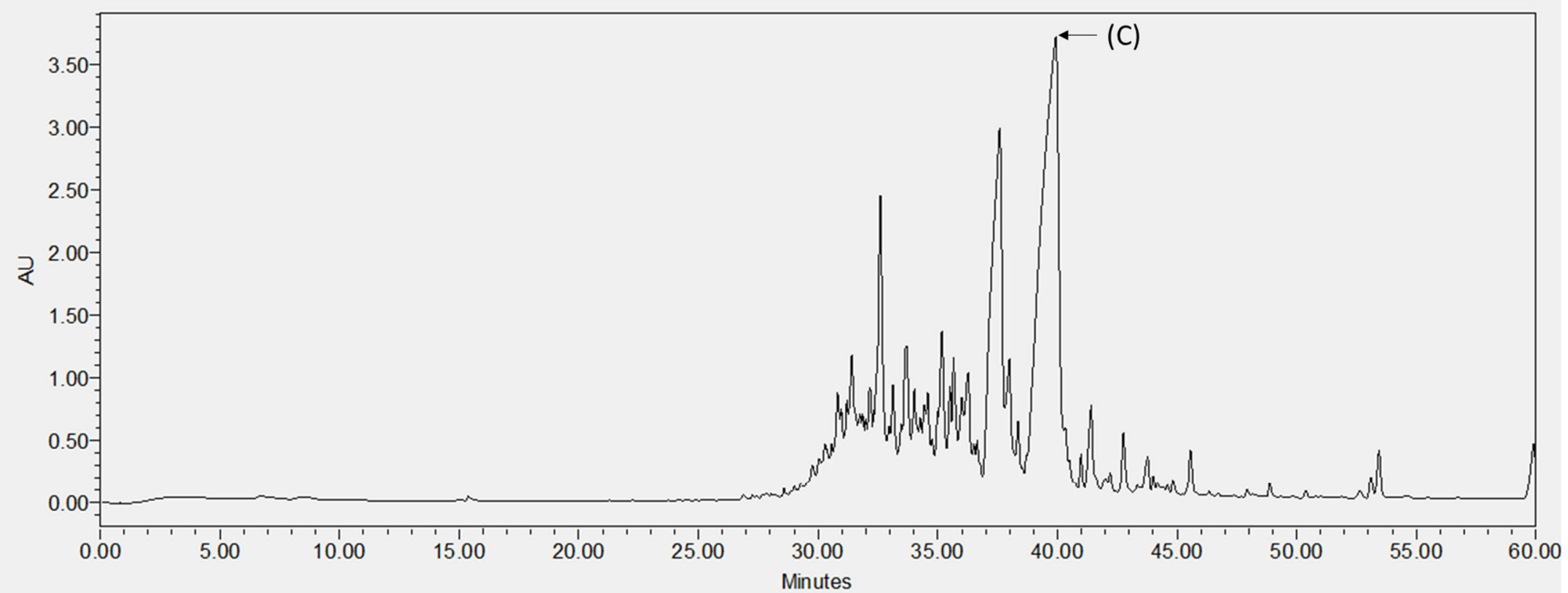


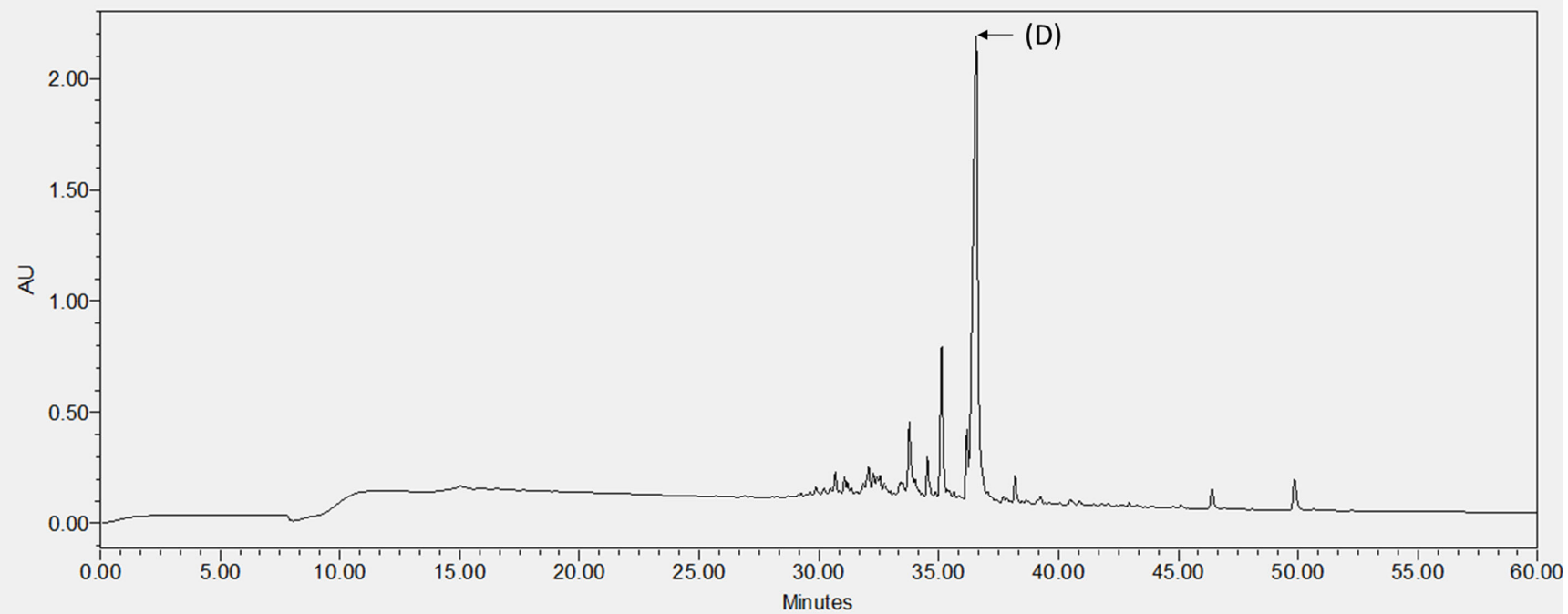
Supplementary Materials

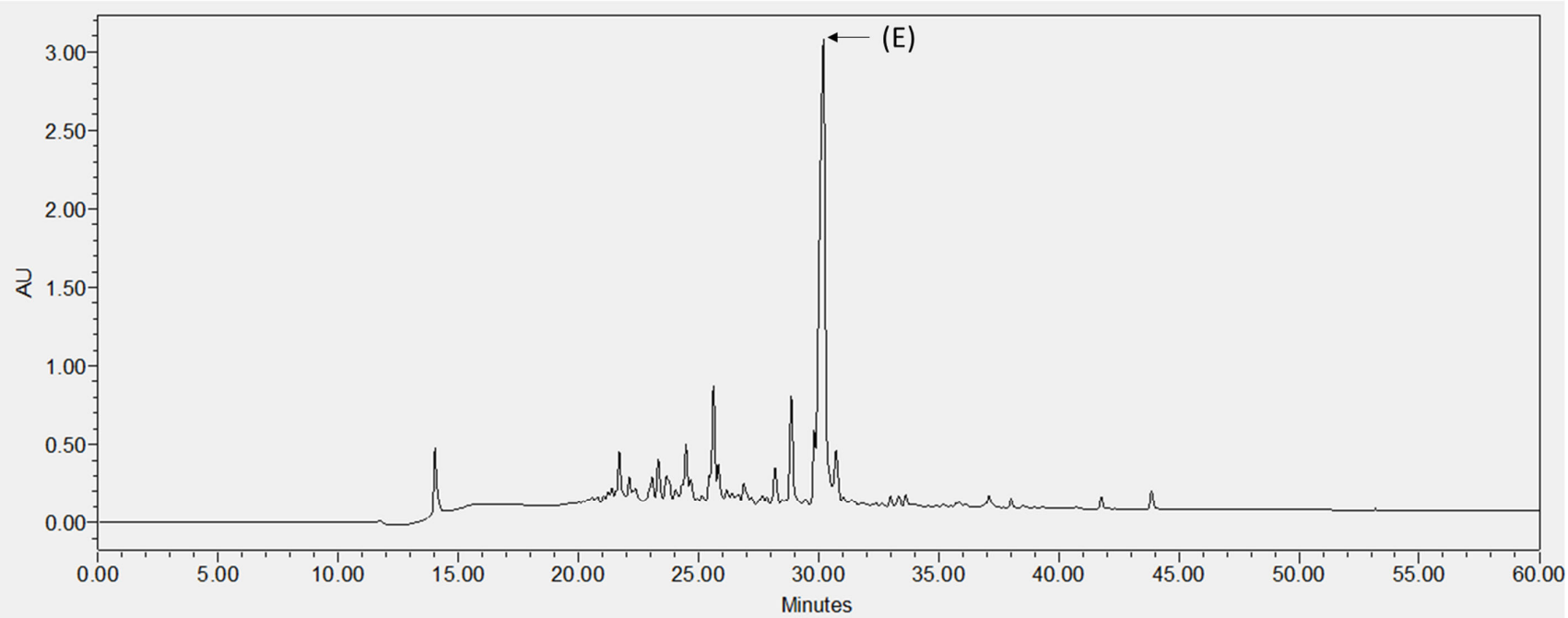
Study on the Structure-activity Relationship of an Antimicrobial Peptide, Brevinin-2GUb, from the Skin Secretion of *Hylarana guntheri*

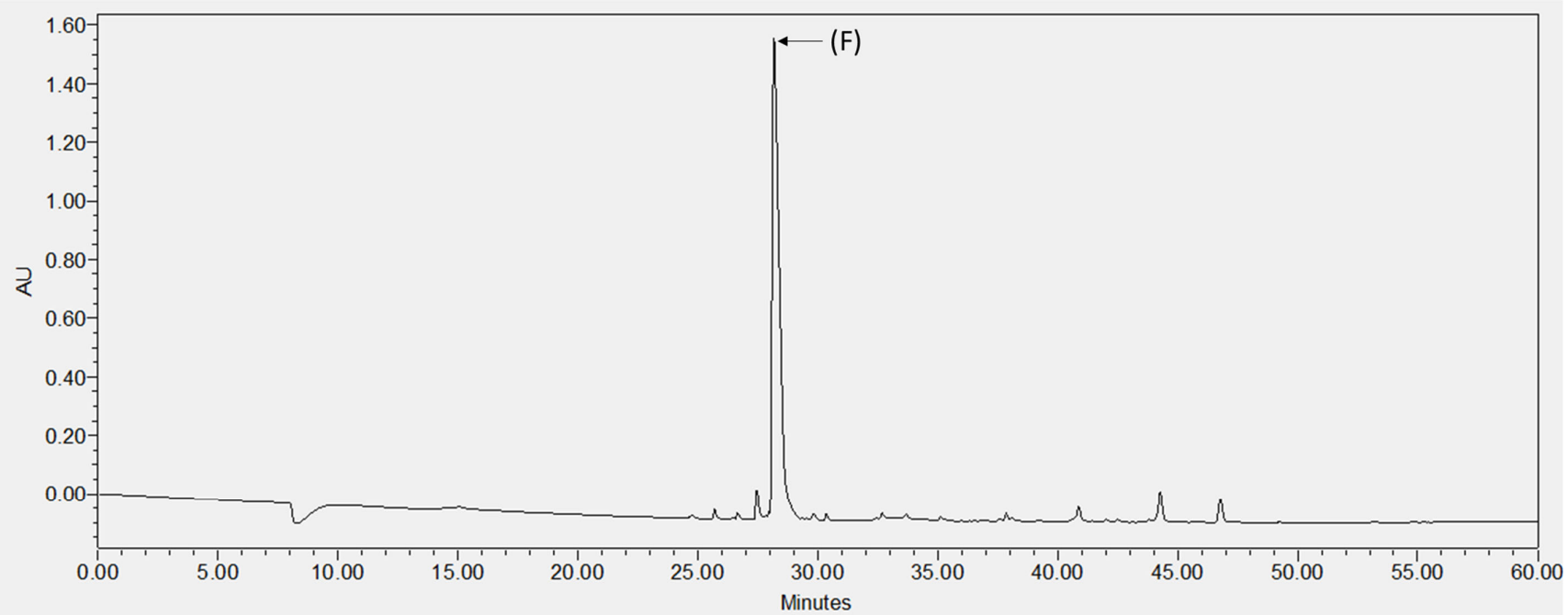












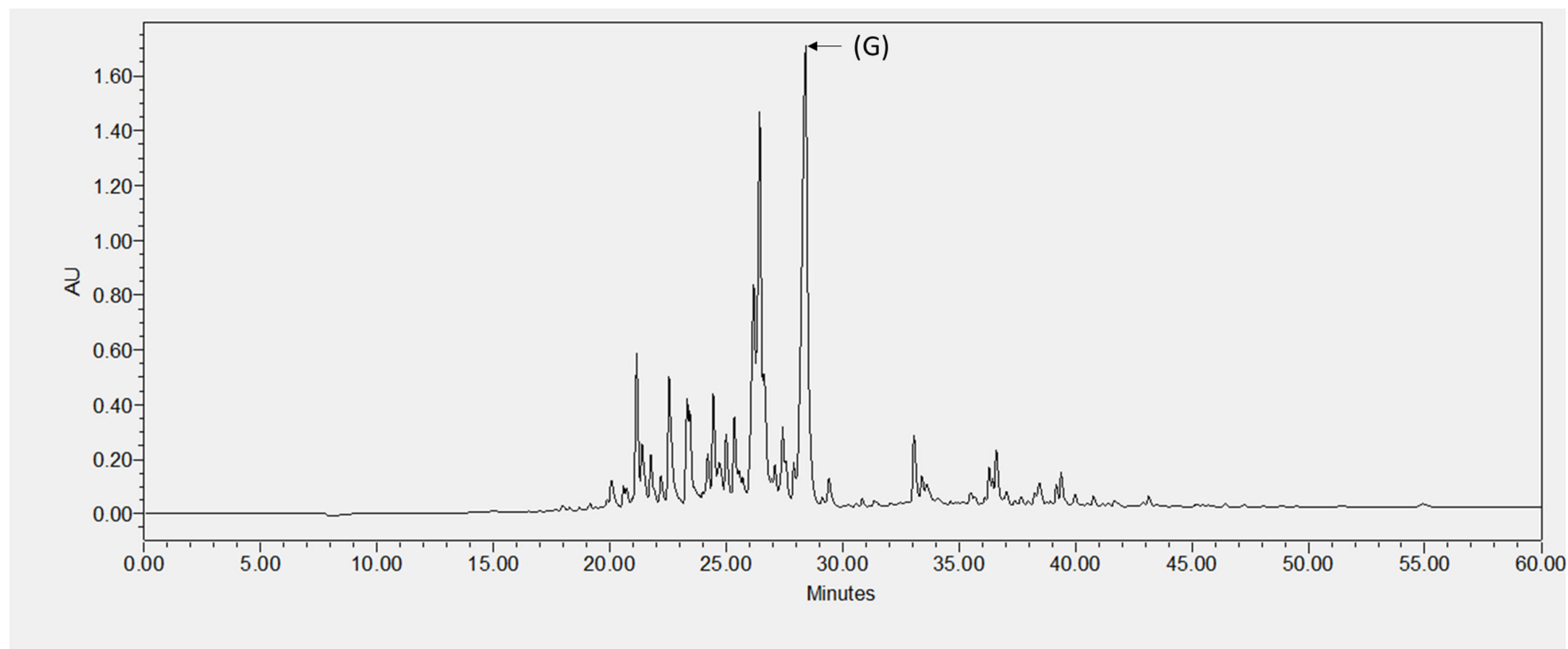
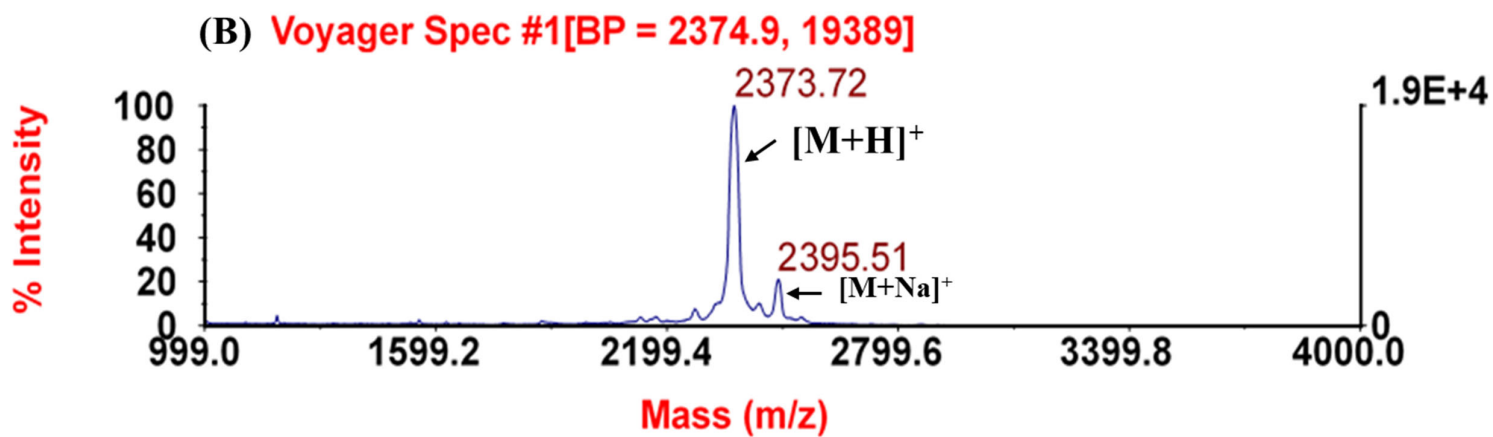
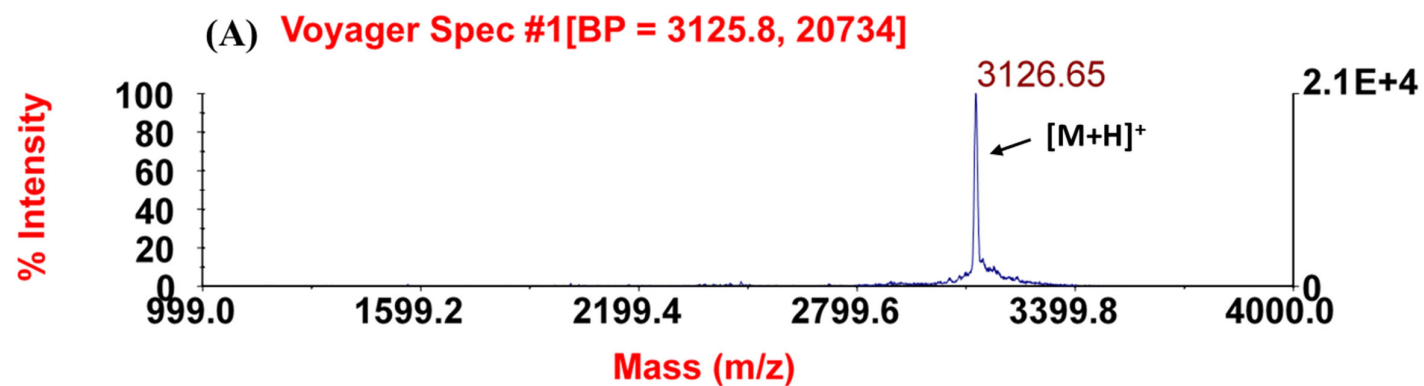
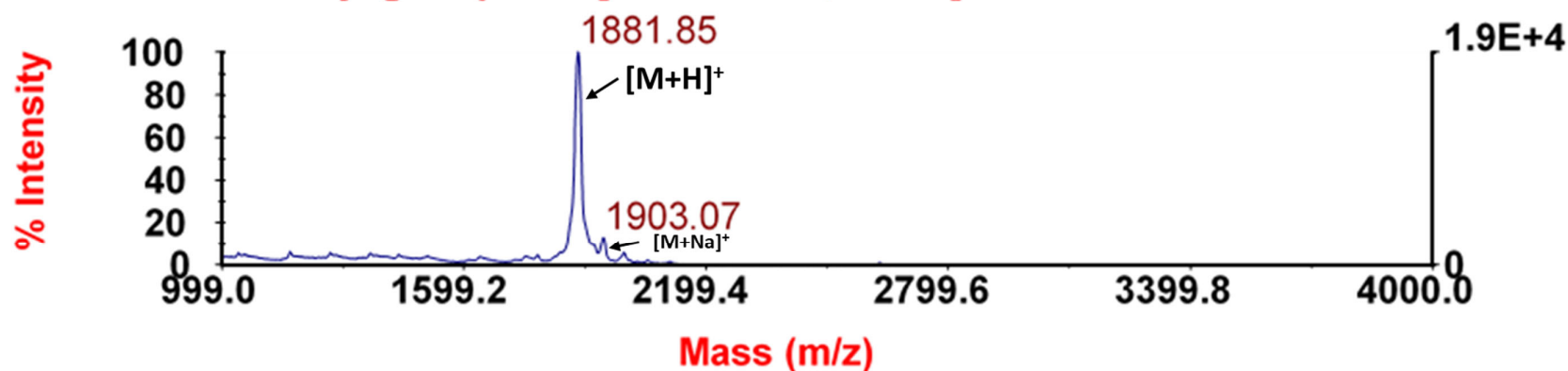


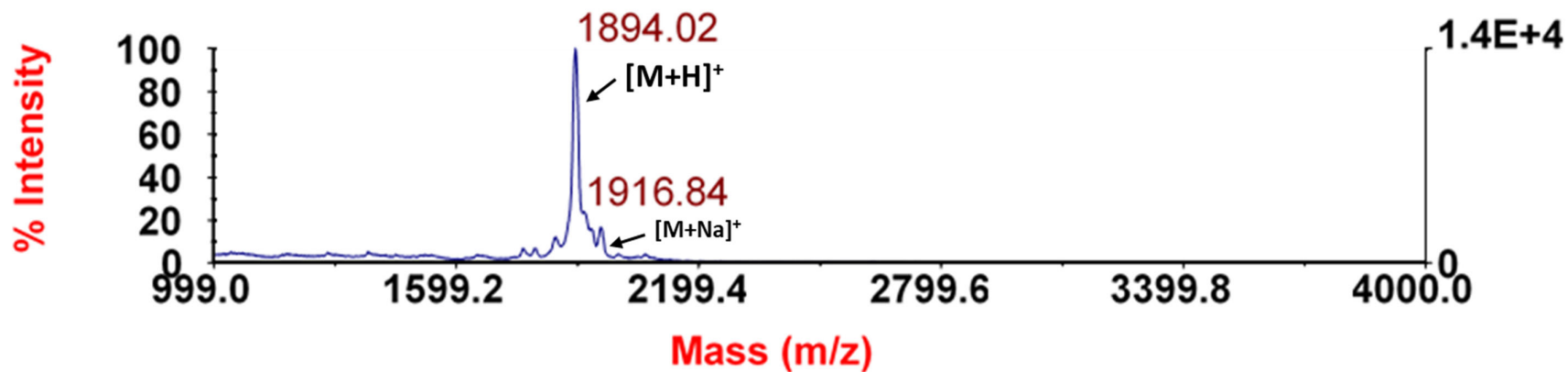
Figure S1. The RP-HPLC chromatograms of (A) Brevinin-2GUb, (B) tB2U, (C) tB2U- α , (D) tB2U-K, (E) tB2U-6K, (F) 7-tB2U-K and (G) 14-tB2U-K. The elution peak of each peptide is indicated by the arrow.



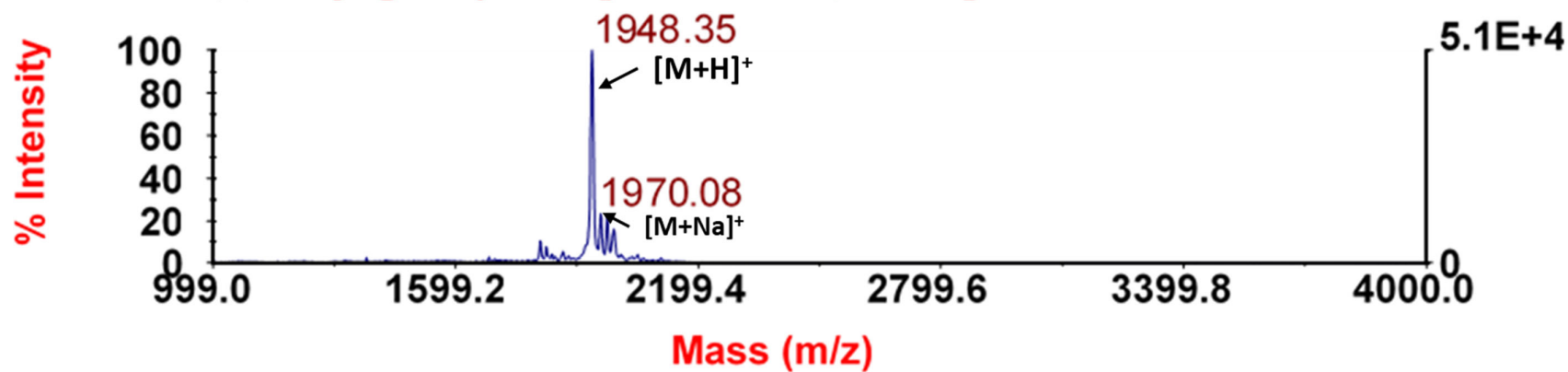
(C) Voyager Spec #1[BP = 1882.5, 18939]



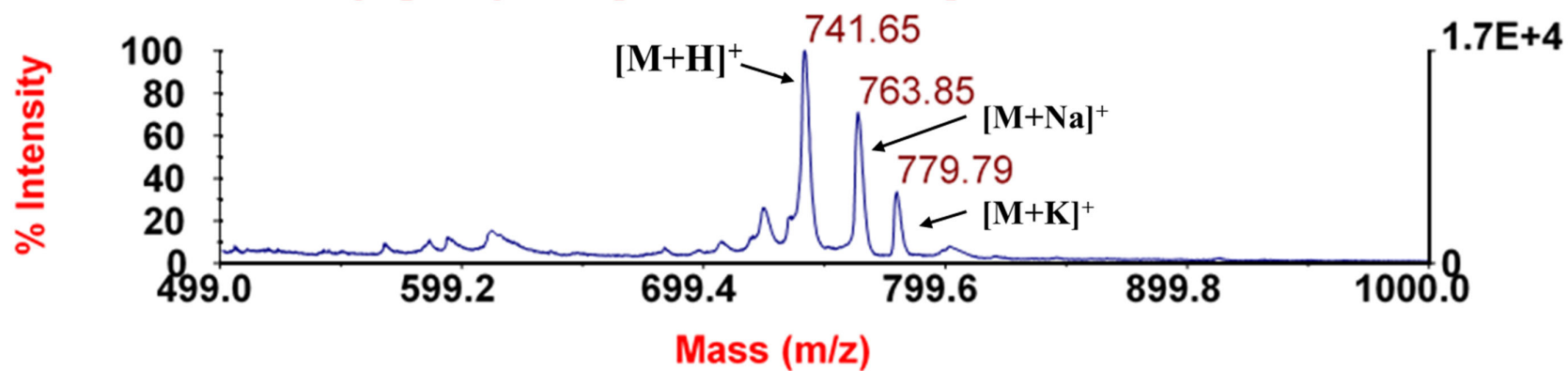
(D) Voyager Spec #1[BP = 1893.9, 14413]



(E) Voyager Spec #1[BP = 1948.6, 50836]



(F) Voyager Spec #1[BP = 741.2, 16860]



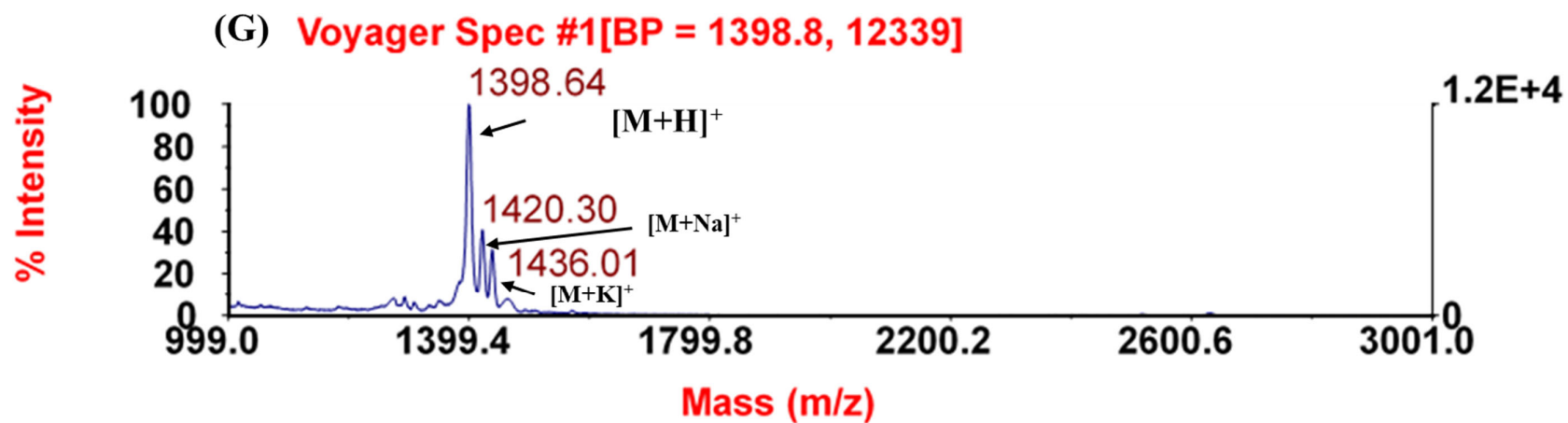
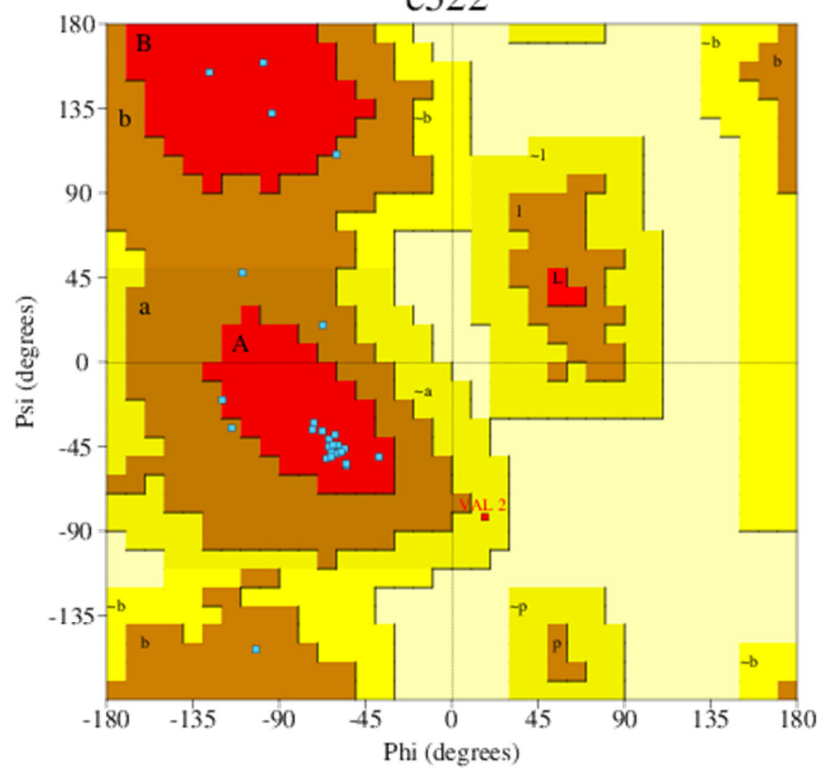


Figure S2. The mass spectra of (A) Brevinin-2GUb, (B) tB2U, (C) tB2U- α , (D) tB2U-K, (E) tB2U-6K, (F) 7-tB2U-K and (G) 14-tB2U-K obtained from MALDI-TOF MS. The observed $[M+H]^+$ ion, the sodium and potassium adduct ions are indicated by arrows.

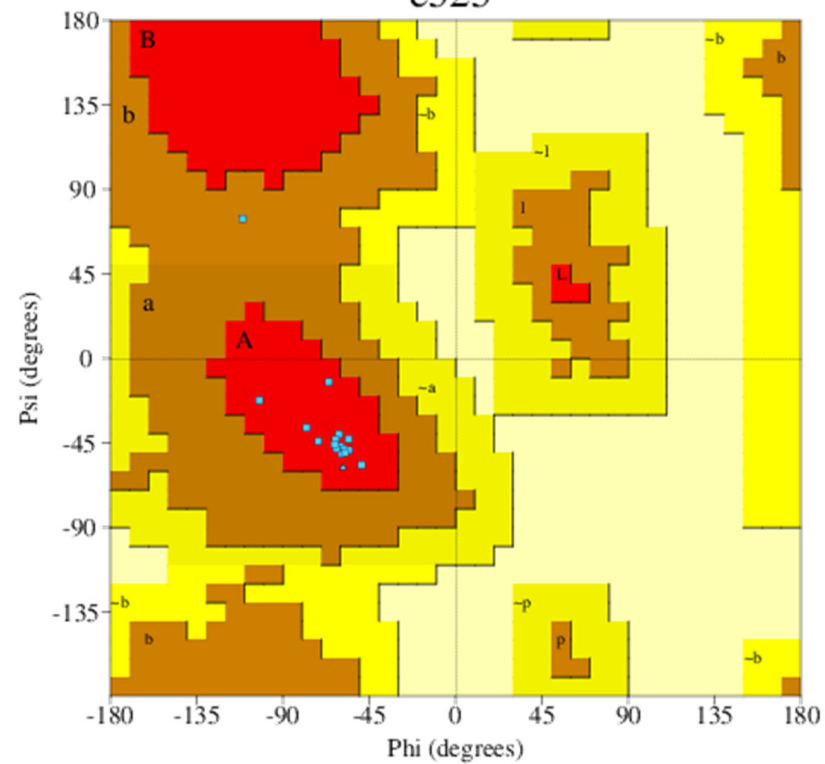
(A)

e522



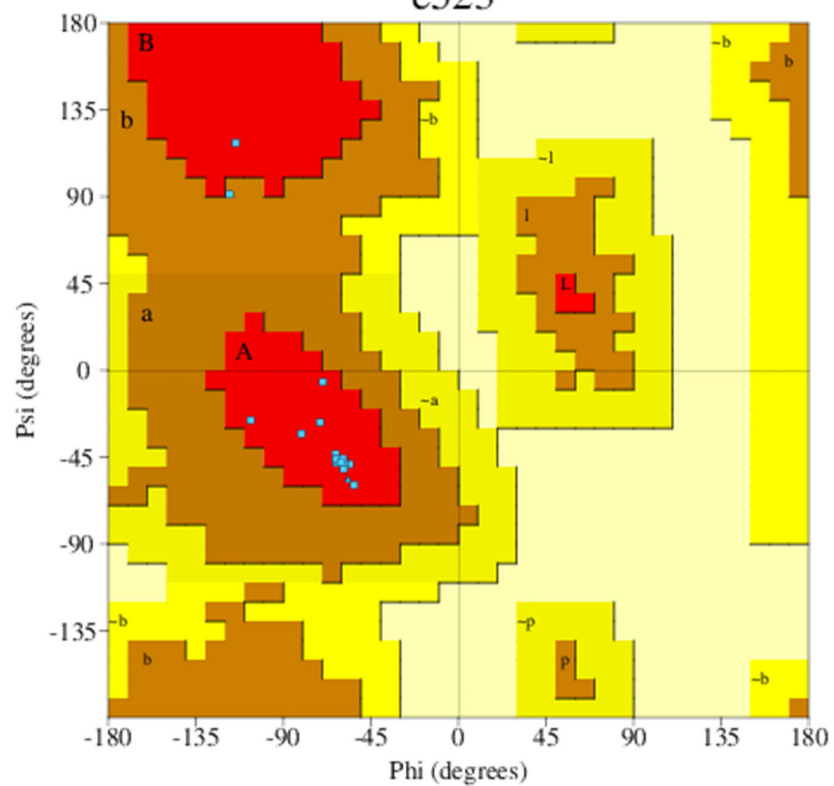
(B)

e525



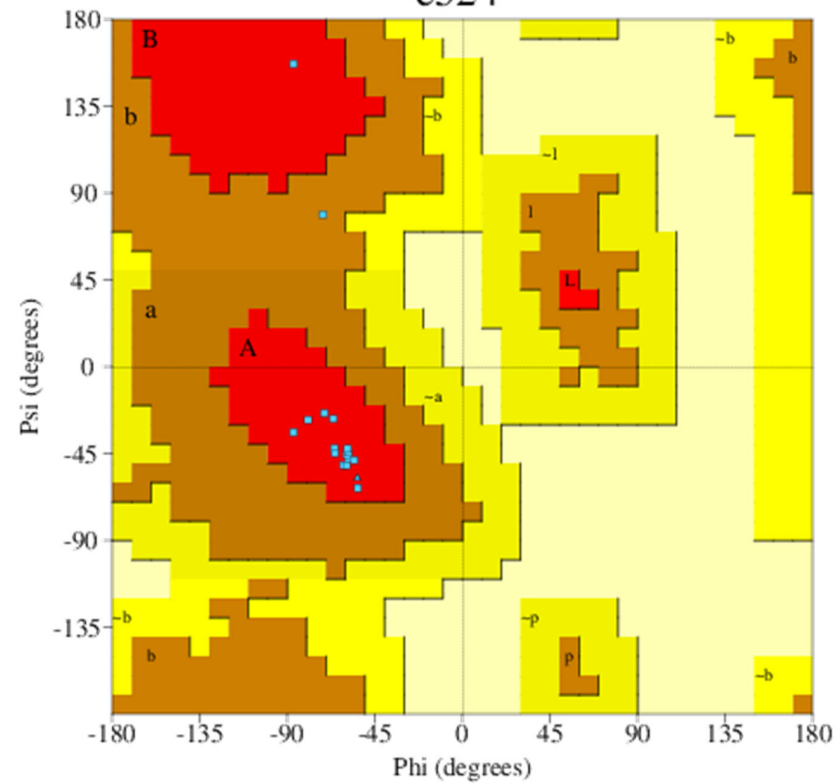
(C)

e523



(D)

e524



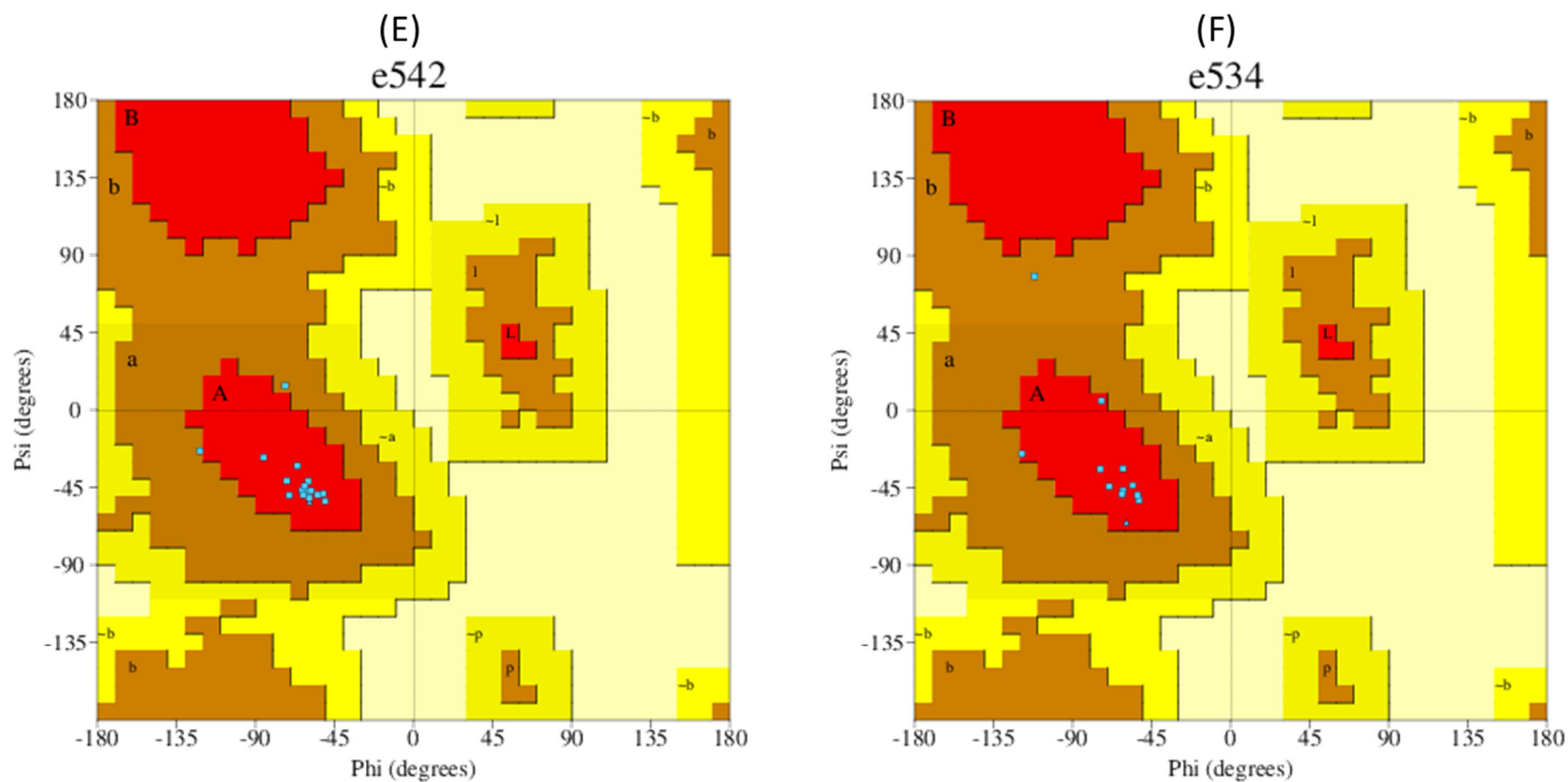


Figure S3. The Ramachandran plots of (A) Brevinin-2GUb, (B) tB2U, (C) tB2U- α , (D) tB2U-K, (E) tB2U-6K and (F) 14-tB2U-K predicted by PROCHECK (<https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/>). The X and Y axis is the torsional angles-Psi (Ψ) and Phi (Φ),

respectively. The Glycine residues are separately represented by triangles and the red regions are the core areas which represent the most favourable combinations of phi-psi values.

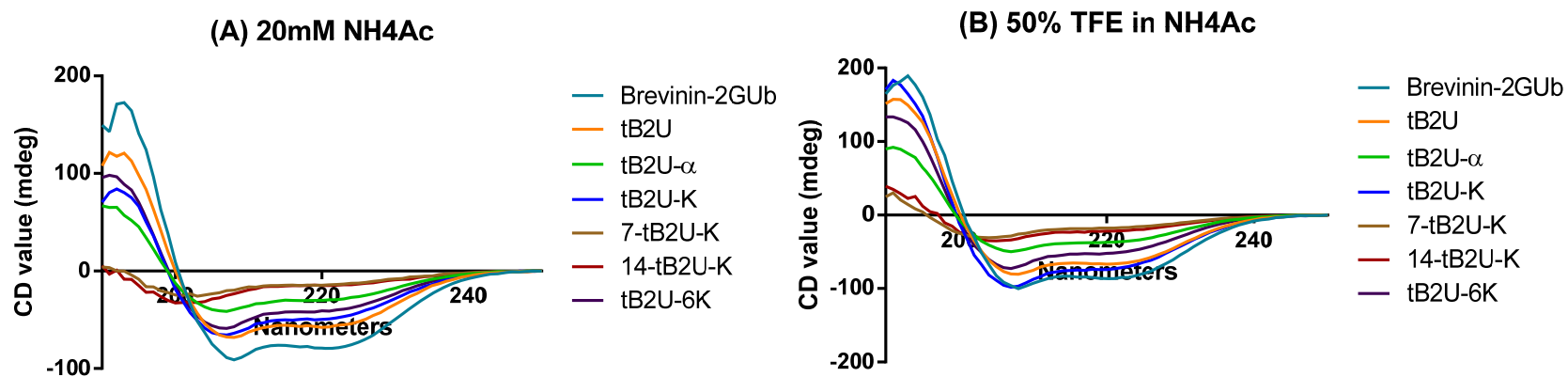
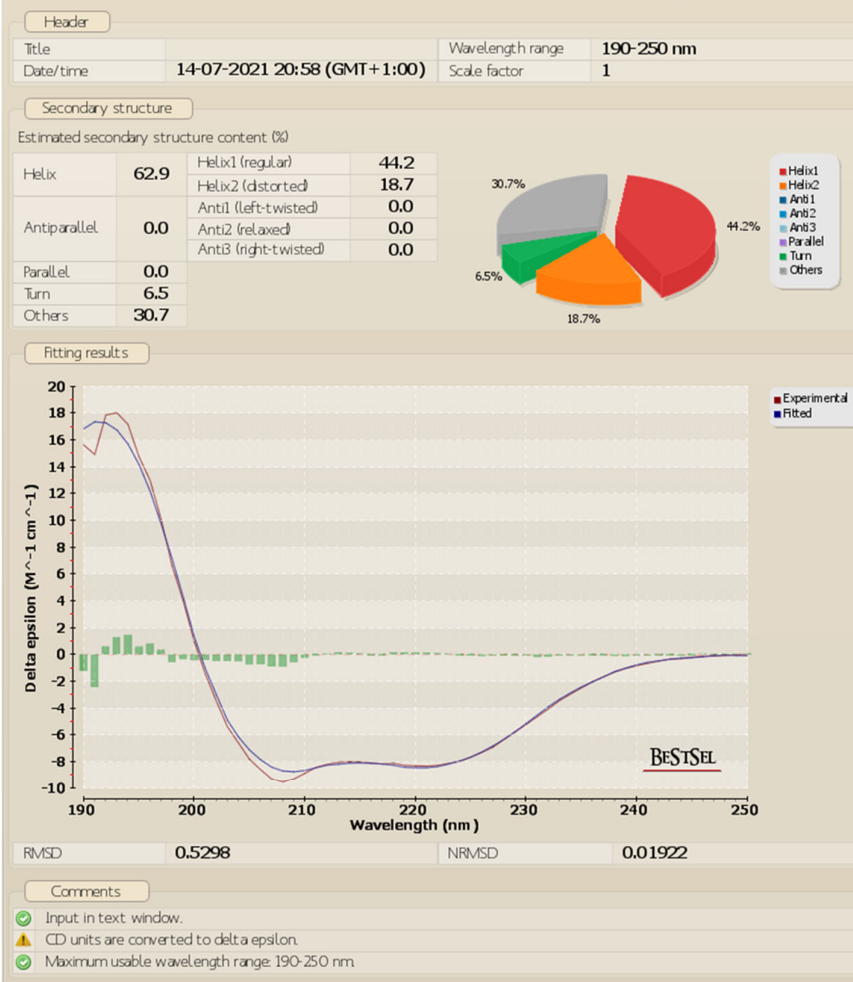


Figure S4. The CD spectra of Brevinin-2GUb and its analogues in (A) aqueous solution (20mM NH₄Ac) and (B) membrane-mimicking solution [50% (v/v) trifluoroethanol (TFE) in NH₄Ac].

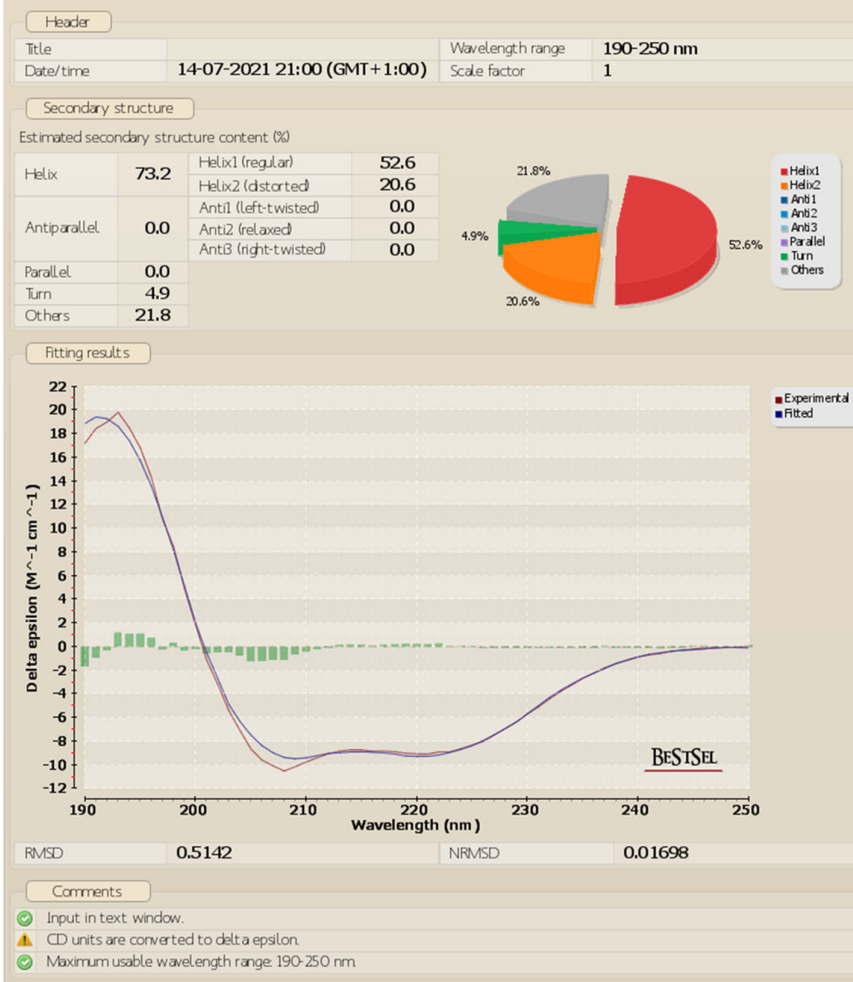
(A1)

References: Micsonai et al. Nucleic Acids Res. 46:W315-22 (2018), Miksonai et al. PNAS 11:E3095-103 (2015)



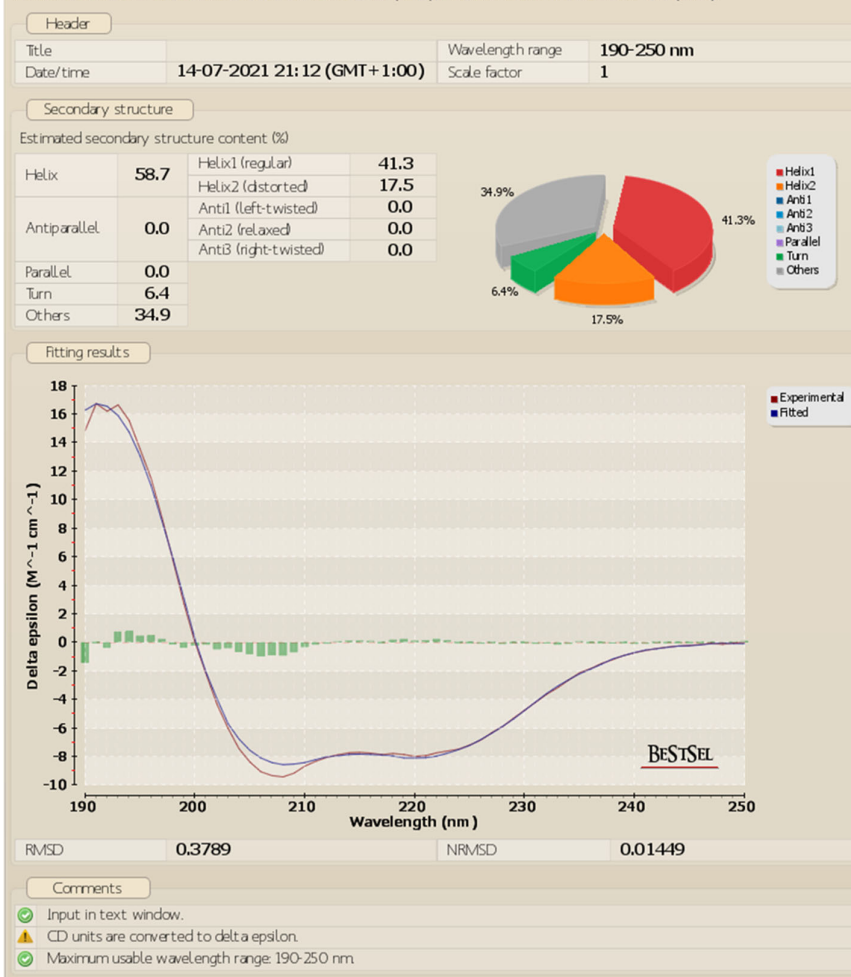
(A2)

References: Micsonai et al. Nucleic Acids Res. 46:W315-22 (2018), Miksonai et al. PNAS 11:E3095-103 (2015)



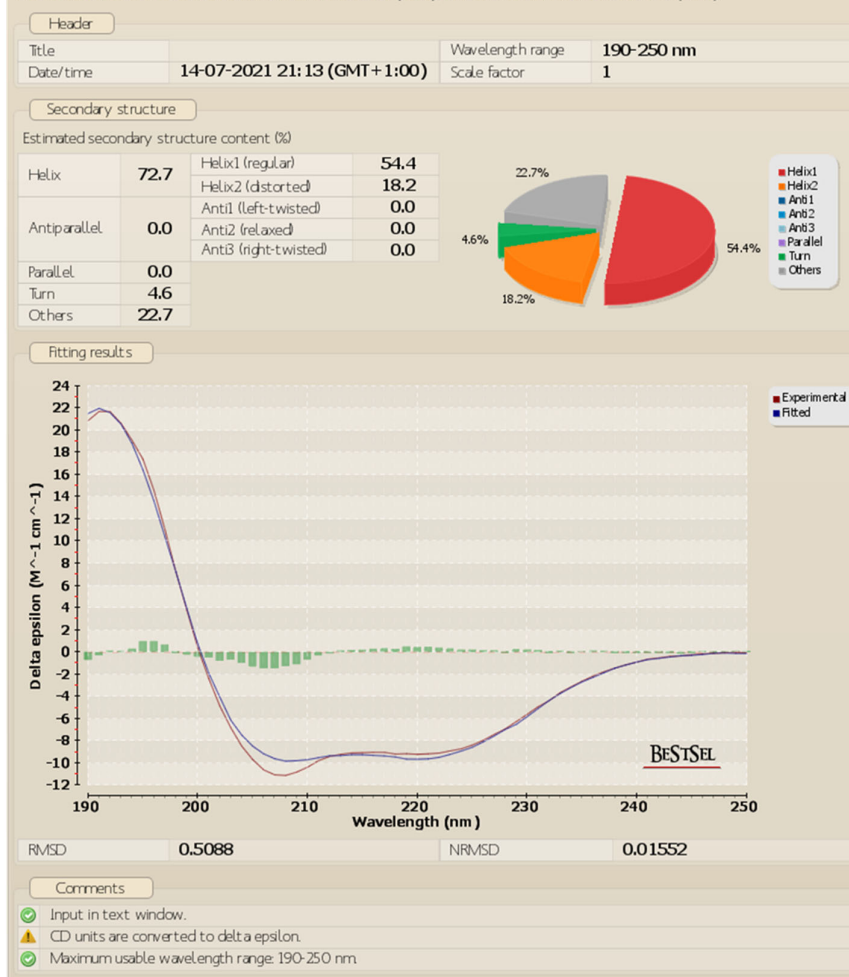
(B1)

References: Micsonai et al. Nucleic Acids Res. 46:W315-22 (2018), Micsonai et al. PNAS 11:E3095-103 (2015)

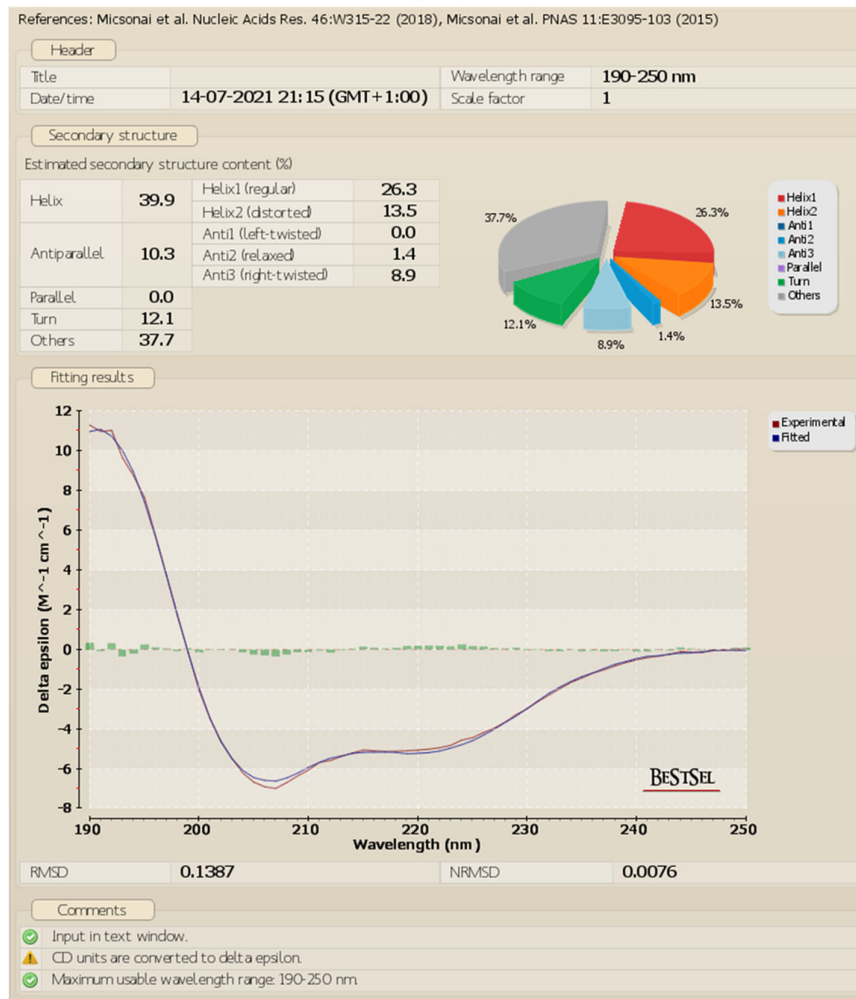


(B2)

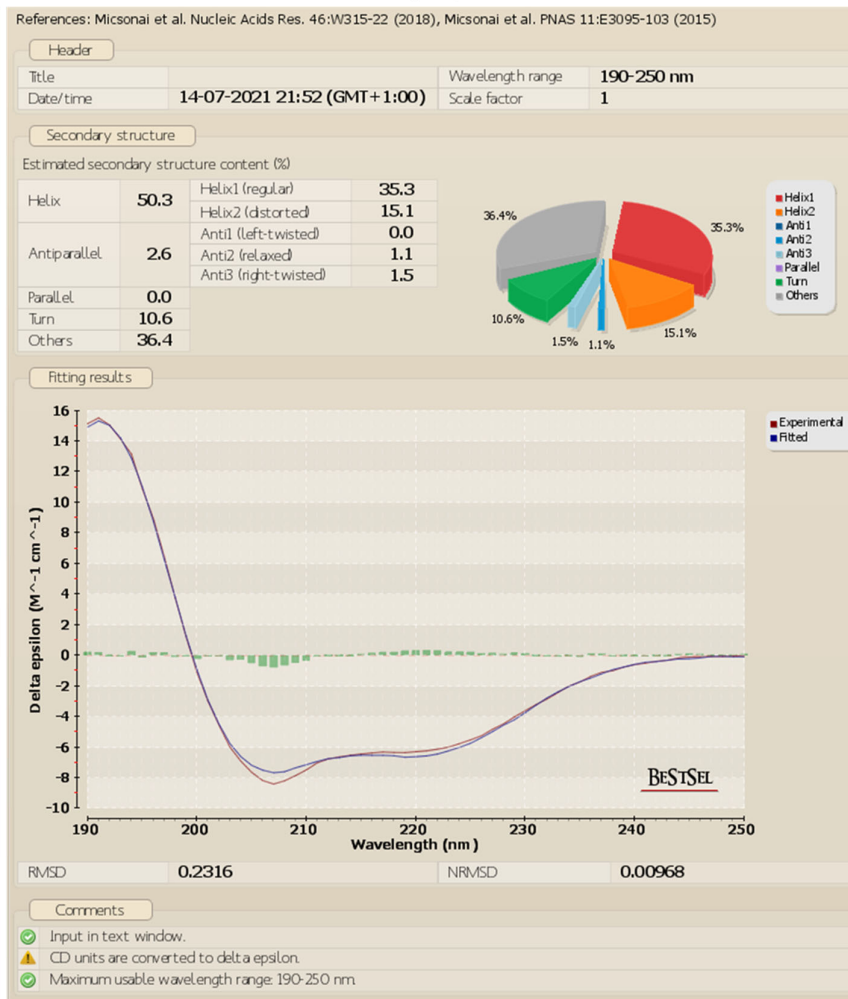
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(C1)

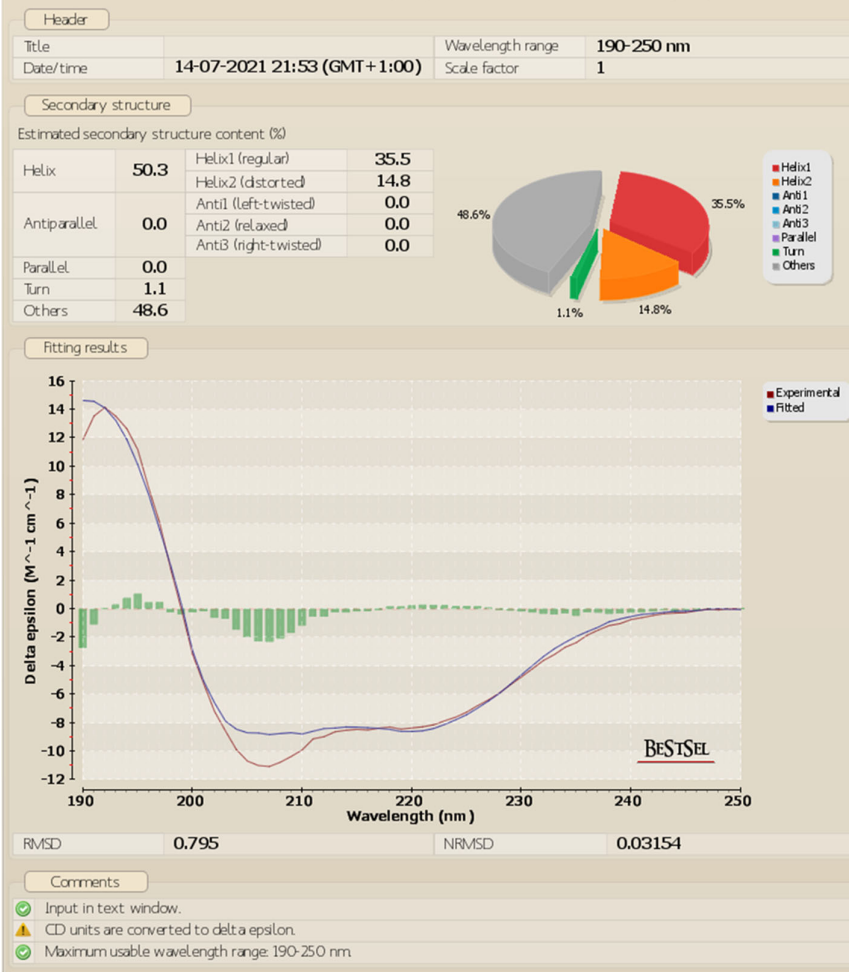


(C2)



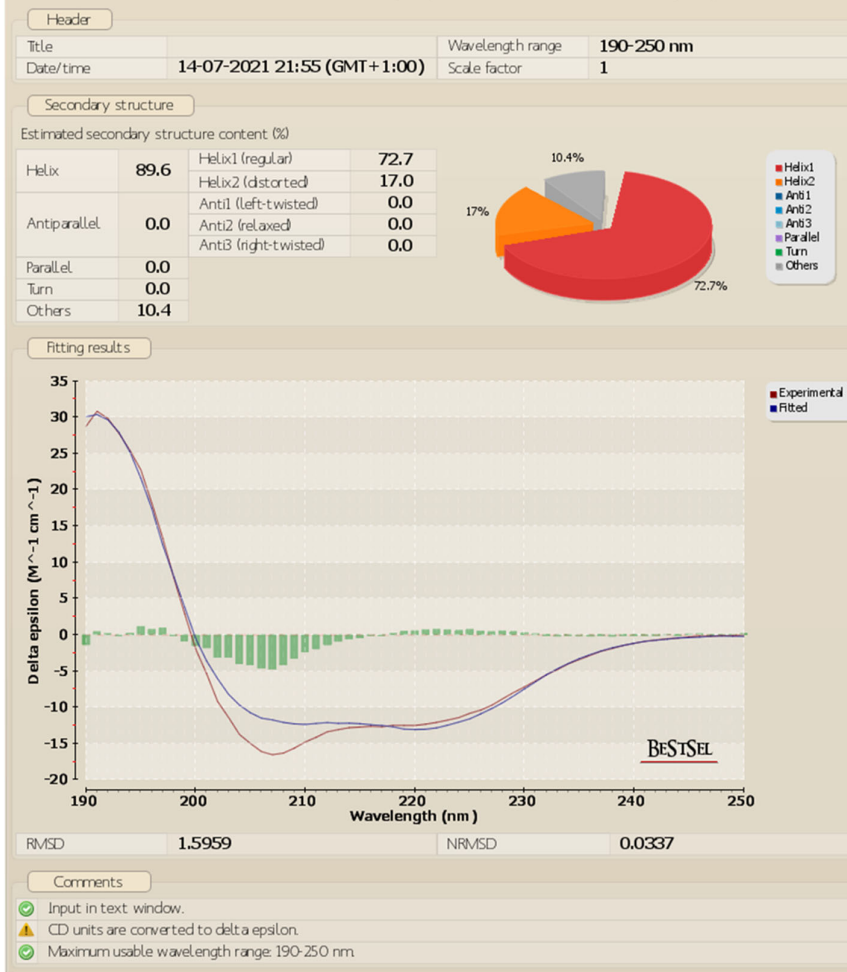
(D1)

References: Micsonai et al. Nucleic Acids Res. 46:W315-22 (2018), Micsonai et al. PNAS 11:E3095-103 (2015)



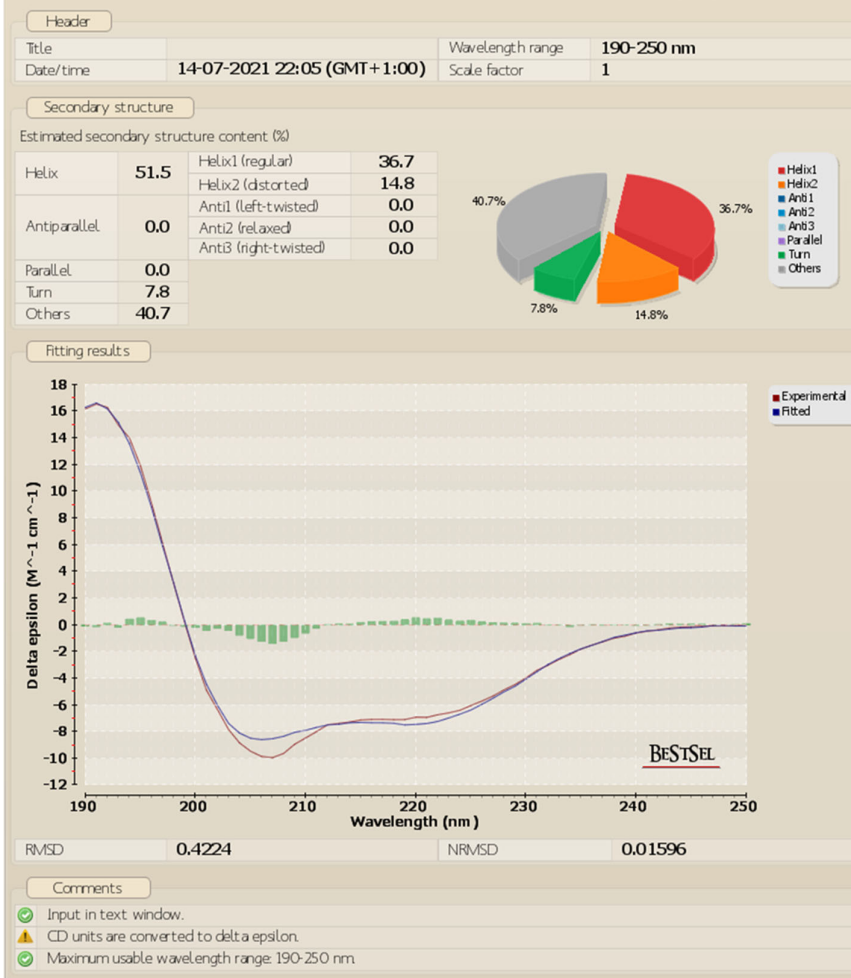
(D2)

References: Micsonai et al. Nucleic Acids Res. 46:W315-22 (2018), Micsonai et al. PNAS 11:E3095-103 (2015)



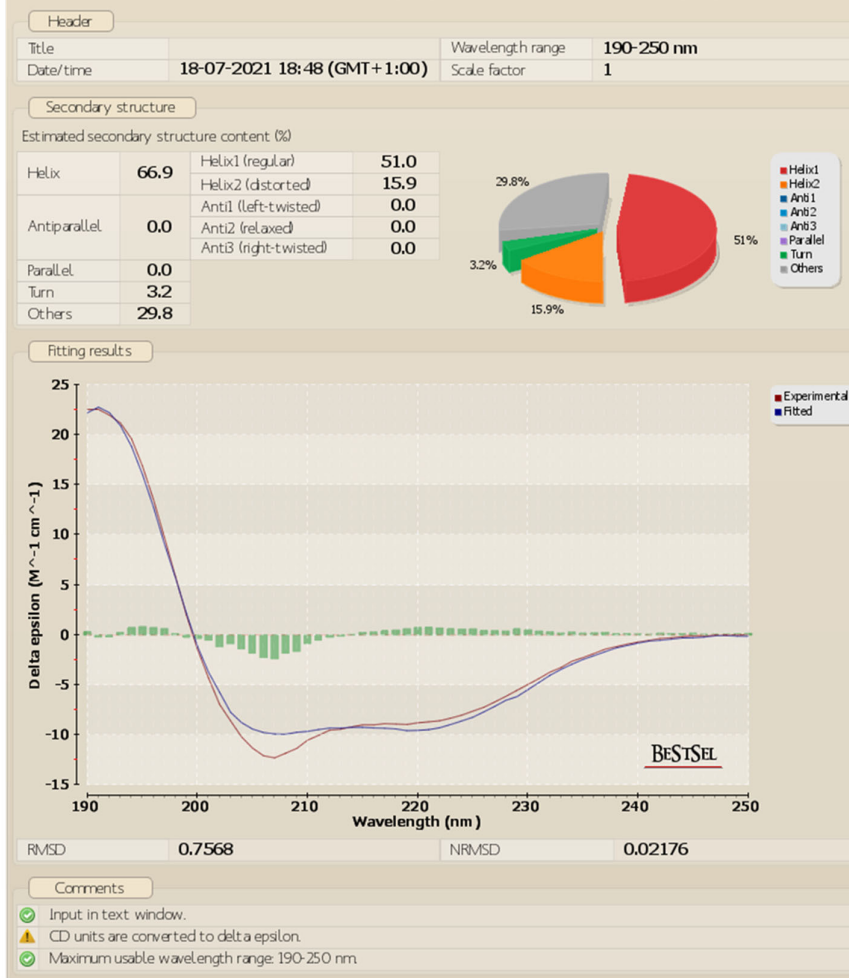
(E1)

References: Micsonai et al. Nucleic Acids Res. 46:W315-22 (2018), Micsonai et al. PNAS 11:E3095-103 (2015)

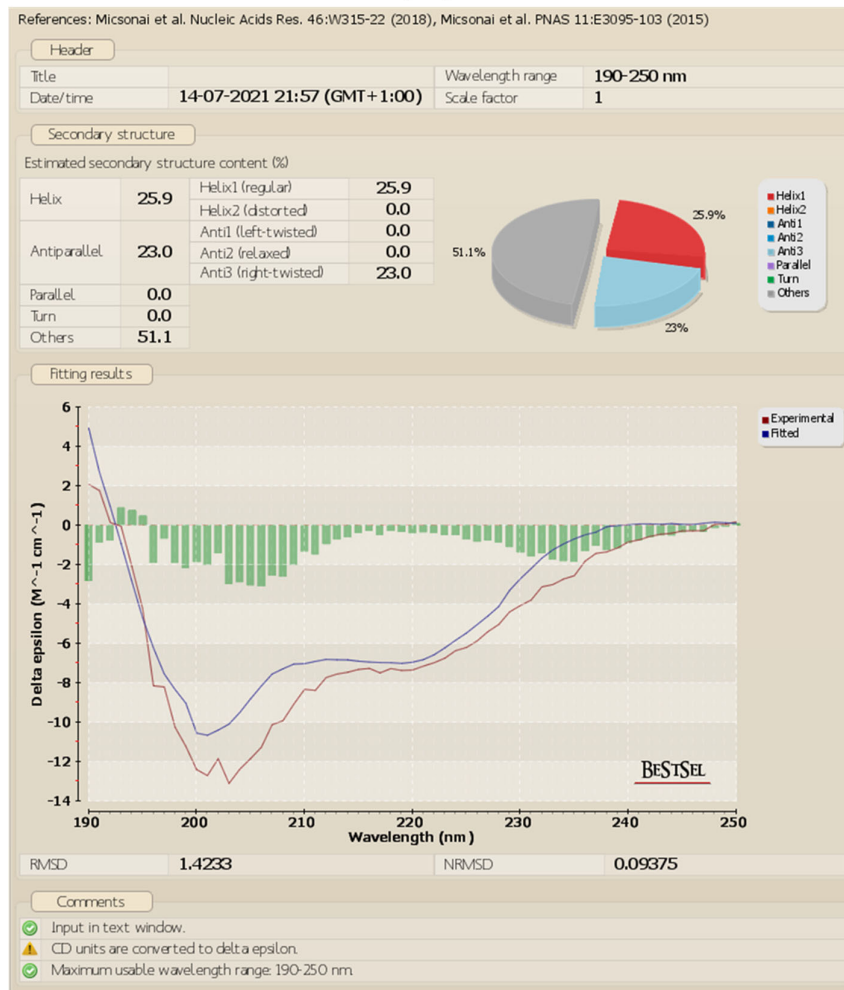


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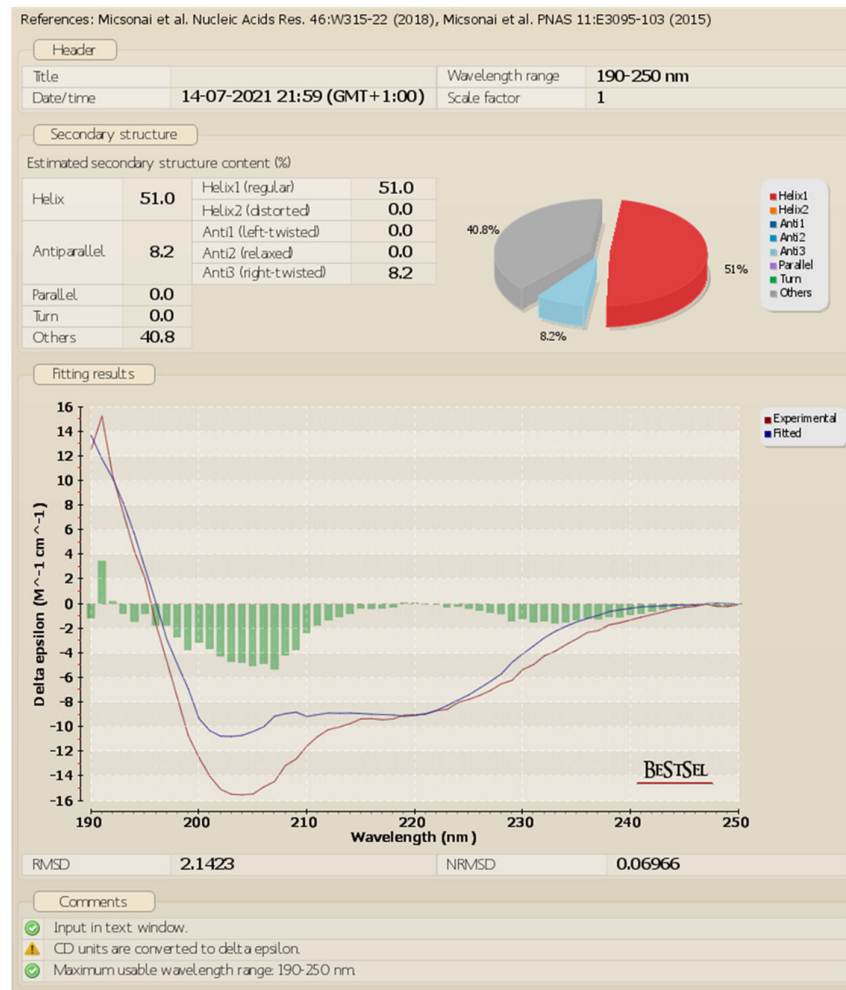
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(F1)

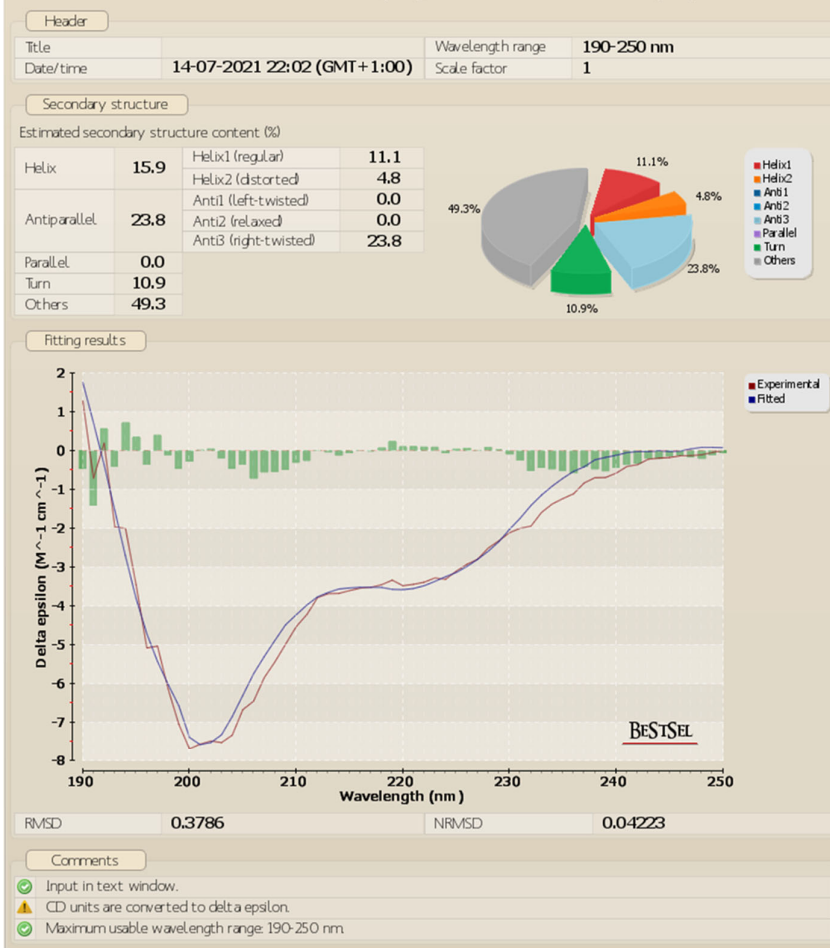


(F2)



(G1)

References: Micsonai et al. Nucleic Acids Res. 46:W315-22 (2018), Micsonai et al. PNAS 11:E3095-103 (2015)



(G2)

References: Micsonai et al. Nucleic Acids Res. 46:W315-22 (2018), Micsonai et al. PNAS 11:E3095-103 (2015)

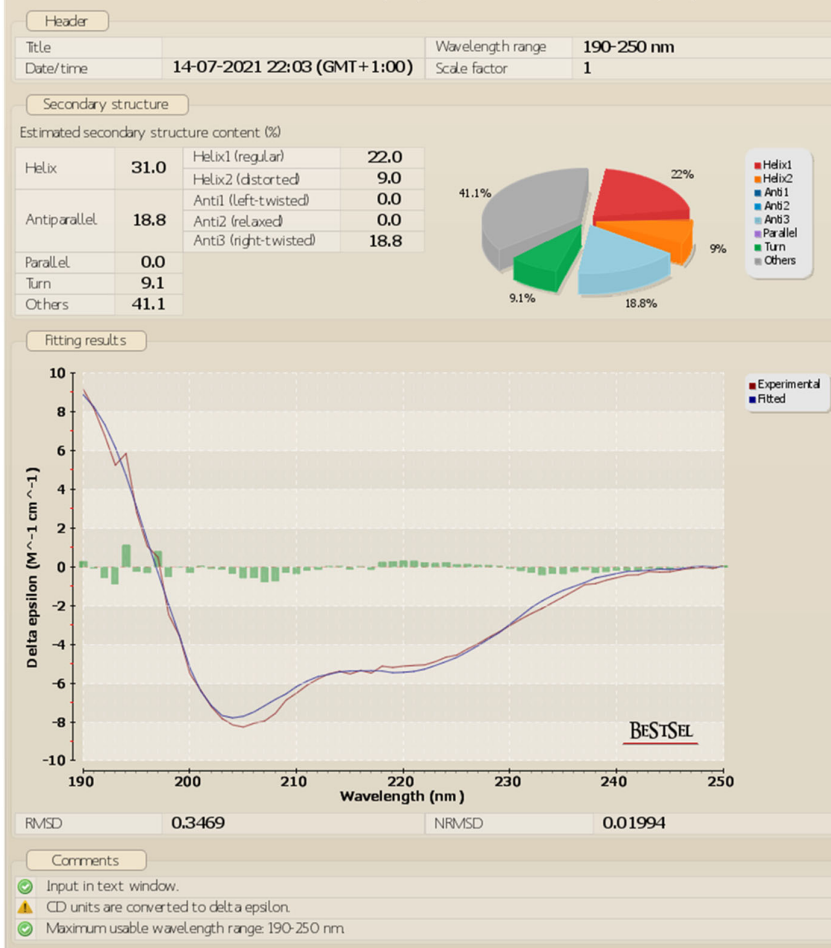


Figure S5. The estimated secondary structure contents analysed by Bestsel. The (A1) Brevinin-2GUb, (B1) tB2U, (C1) tB2U- α , (D1) tB2U-K, (E1) tB2U-6K, (F1) 7-tB2U-K and (G1) 14-tB2U-K was in the environment of 20mM NH₄Ac. The (A2) Brevinin-2GUb, (B2) tB2U, (C2) tB2U- α , (D2) tB2U-K, (E2) tB2U-6K, (F2) 7-tB2U-K and (G2) 14-tB2U-K was in the environment of 50% TFE in 20mM NH₄Ac.

Table S1. Two-way ANOVA analysis of haemolysis activity of Brevinin-2GUb and its analogues. The significance was presented by the symbol **** (P<0.0001).

Dunnett's multiple comparison test				
Peptide name	Groups	Mean Diff.	95% CI of diff.	Significance
	N vs. P	-100.7	-102.8 to -98.56	****
Brevinin-2GUb	N vs. 512 μ M	-21.77	-24.01 to -19.53	****
	N vs. 256 μ M	-8.063	-10.30 to -5.825	****
	N vs. 128 μ M	-5.500	-7.739 to -3.261	****
tB2U	N vs. 512 μ M	-3.363	-5.241 to -1.486	****
tB2U- α	N vs. 512 μ M	-4.657	-6.534 to -2.779	****

tB2U-K	N vs. 512 μ M	-11.94	-13.47 to -10.41	****
	N vs. 256 μ M	-7.883	-9.328 to -6.438	****
	N vs. 128 μ M	-6.718	-8.163 to -5.273	****
7-tB2U-K	N vs. 512 μ M	-3.697	-5.864 to -1.529	****
14-tB2U-K	N vs. 512 μ M	-4.780	-6.948 to -2.612	****
tB2U-6K	N vs. 512 μ M	-6.963	-9.202 to -4.725	****
	N vs. 256 μ M	-5.481	-6.926 to -4.036	****
	N vs. 128 μ M	-4.051	-5.496 to -2.606	****

Table S2. Two-way ANOVA analysis of MTT assay of Brevinin-2GUb and its analogues against HaCaT. The significance was presented by the symbol **** (P<0.0001).

Dunnett's multiple comparison test				
Peptide name	Groups	Mean Diff.	95% CI of diff.	Significance
	N vs. P	99.69	97.42 to 102.0	****
Brevinin-2GUb	N vs. 10 ⁻⁴ M	54.35	52.08 to 56.63	****

Table S3. The survival analysis of the antimicrobial activities in vivo of Brevinin-2GUb and its analogues. The significance was presented by the symbol * (P<0.05), ** (P<0.01), *** (P<0.001) and **** (P<0.0001).

Log-rank (Mantel-Cox) Test				
Peptide	Groups	Chi Square	P value	P value summary
	N vs. P	19.26	<0.0001	****

Brevinin-2GUb	N vs. 30 mg/kg	15.01	0.0001	***
	N vs. 15 mg/kg	6.565	0.0104	*
	N vs. 7.5 mg/kg	6.350	0.0117	*
tB2U-K	N vs. 30 mg/kg	15.01	0.0001	***
	N vs. 15 mg/kg	8.308	0.0039	**
	N vs. 7.5 mg/kg	5.880	0.153	*
tB2U-6K	N vs. 30 mg/kg	19.26	<0.0001	****
	N vs. 15 mg/kg	13.95	0.0002	***
	N vs. 7.5 mg/kg	5.026	0.0250	*