RC3-1 - Figure 2 shows the template for the description of ncAAs. What happens if the nitrogen atom of the peptide bond is not bound to an hydrogen but to a carbon such as in methylated AA or di-amino butyric acid found in some bacterial siderophores or a modified proline ?

1. The template recognition matches the ends of the chain and works inwards, so the amino acid itself only has to start with a nitrogen and end with a carbonyl carbon. In principle, it will also recognise beta amino acids in this way. We have tested the procedure with amino acids such as proline and its derivatives and it works as intended (see also the link to the amino acid tab of the ATB below).

RC3-2- The statement in the first result paragraph is rather odd:

" In general the recalculated structures are very similar to those previously calculated …."

I disagree with the statement that it is beyond the scope of the work to compare in detail the results of both procedure. The demonstration that the automated approach delivers the same results as the manual one should be provided in a quantitative way and the origin of possible "subtle" differences should be carefully analysed and addressed. The results of structure calculations should comply with accepted standards showing the tables with structural statistics.

2. The statement refers to the observation that the differences are within the error/precision of the structure calculation by CYANA. The below table demonstrates this:

Table. Torsion angles of disulfide bonds in venom peptide (Ta1a, PDBID: 2KSL) using either the CYSS template (traditional method) or CYSX template (new) method of joining the sidechain of amino acids in CYANA. The average value is that calculated over 20 structures in the ensemble. All details of the structure calculation are identical to those presented previously (2KSL).[1]

	CYSS		CYSX	
	Average	(+/-)	Average	(+/-)
DSB-1				
χ_2 [7]	72	113	87	88
χ_3 [7-37]	86	120	77	82
χ_2 [37]	-45	84	-16	97
DSB-2				
χ_2 [23]	-52	137	9	154

χ_3 [23-33]	-8	45	13	53
χ_2 [33]	-40	151	17	151
DSB-3				
χ_2 [26]	-101	113	-83	109
χ_3 [26-46]	-47	48	-38	57
χ_2 [46]	-65	97	-46	100

1. Undheim EA*, et al.* Weaponization of a Hormone: Convergent Recruitment of Hyperglycemic Hormone into the Venom of Arthropod Predators. *Structure* **23**, 1283-1292 (2015).

The differences in torsion angles obtained using either of the two methods were within the spread of the other. We have edited the text in our revision to say that the structures are comparable and removed references to "subtle" differences.

RC3-3- As already mentioned, comparative structural calculations should be provided also for CNS or XPLOR-NIH. It would be very helpful to have the example of a topology entry for a modified amino-3. acid in one of the routinely used force field of CNS.

3. This already exists on the amino acids tab of the ATB (https://atb.uq.edu.au/index.py?tab=amino_acids), we also included the files used for the calculation of the stapled peptide on github: https://github.com/ATB-UQ/APP-RCM-CNS-Files. We will include the above links in the revised manuscript.

RC3-4- In section 3.3, the authors present a practical application on a stapled peptide. Details that are provided should be displaced in the method section rather. As for other examples, a table recapitulating structural statistics should be provided. It would also be interesting to detail how the cis-trans isomery of the double bond is defined from the input structure.

4. This an oversight on our behalf, and the below table will be added to the results section (based on CNS calculation in water) in the revised manuscript.

Table: Structural statistics from CNS calculations	
Energies (kcal mol^{-1})	

Overall	-375.20 ± 14.91
Bonds	11.05 ± 1.48
Angles	45.68 ± 4.92
Improper	8.00 ± 1.45
Dihedral	35.48 ± 1.03
van der Waals	-64.21 ± 5.85
Electrostatic	-411.82 ± 22.23
NOE	0.22 ± 0.03
cDih	0.40 ± 0.25
Ramachandran statistics	
Ramachandran favoured (%)	90.0 ± 12.57
Ramachandran outliers	0
Atomic RMSD residues 4-12 (Å)	
Mean global backbone	0.32 ± 0.13
Mean global heavy	1.15 ± 0.23
Experimental restraints	
Distance restraints	
Short range (i–j < 2)	158
Medium range (i–j < 5)	71
Long range (i–j ≥ 5)	0
Hydrogen bond restraints	0
Total	229
Dihedral angle restraints	
ϕ	8
ψ	7
χ1	4
Total	19
Violations from experimental restraints	
NOE violations exceeding 0.2 Å	0
Dihedral violations exceeding 2.0°	0

The trans isomer was determined by analysis of the J-coupling and the NOE patterns. Further details of this are provided in a related manuscript where the initial structural characterisation of this series of compounds is included (Bartling et al. J Med Chem). The manuscript has been accepted pending minor changes and we will update the reference upon revision as we expect the following DOI (https://doi.org/10.1021/acs.jmedchem.2c02017) to be available shortly.

RC3-5- It would be very interesting to provide an example where fluorinated amino-acids are incorporated in a peptide or a protein.

5. There are many fluorinated building blocks in the amino acid page, for example, pentafluoro-phenylalanine: https://atb.uq.edu.au/molecule.py?molid=1210306#panel-nmr_refinement

There are already examples of chlorine containing peptides in the manuscript and replacing this with a fluorine would follow the exact same procedure, there is no difference as far as our pipeline is concerned. We encourage users to try the many different functional groups (F, I, Br, NO2, CHO etc.) that we do not cover in the manuscript.