

Dear Editor, dear Prof Otting,

we would like to thank you for the careful reading of the manuscript and the comments. Please find below our answers.

Morgane Callon on behalf of all authors

When reading through the revised manuscript to compare with the referees' comments, I came across a few points that deserve addressing.

Figure 3: A and B labels missing in figure.
Thank you, we added them.

Figure S2: please report the concentration of the protein.
The protein concentration in Figure S2 was 60 μ M. We added this information to the manuscript.

Line 57: please report the volume of the cell-free reaction.
The volume of the cell-free reaction is in total 36 mL (6 x 6 mL wells). We added this information to the manuscript.

Line 60: please report the (presumably commercial) source of amino acids.
The amino-acids are purchased from Cambridge Isotope Laboratories. We added this information to the manuscript.

Line 64: please specify the protease inhibitor used.
We used the cOmplete, EDTA-free protease inhibitor Cocktail (Roche). We added this information to the manuscript.

Line 68: please clarify, whether both spectrometers were equipped with a cryoprobe.
The two spectrometers are equipped with cryoprobe. We added this information to the manuscript.

Line 79: please clarify whether CFPS was used for one or two NMR samples.
All samples were produced using CFPS. We clarified this in the manuscript.

Line 84: Fig. S1 does not prove that zinc-finger formation is reversible (which would require the recovery of the CD spectrum following the addition of zinc after having extracted zinc with EDTA).
We now provide CD data showing that the zinc-finger formation is reversible, as already shown by Estrada et al. We could recover the CD spectrum after addition of zinc after its removal with EDTA. We added this Figure as Panel B to Figure S1.

Line 86: please spell atom names with small characters.

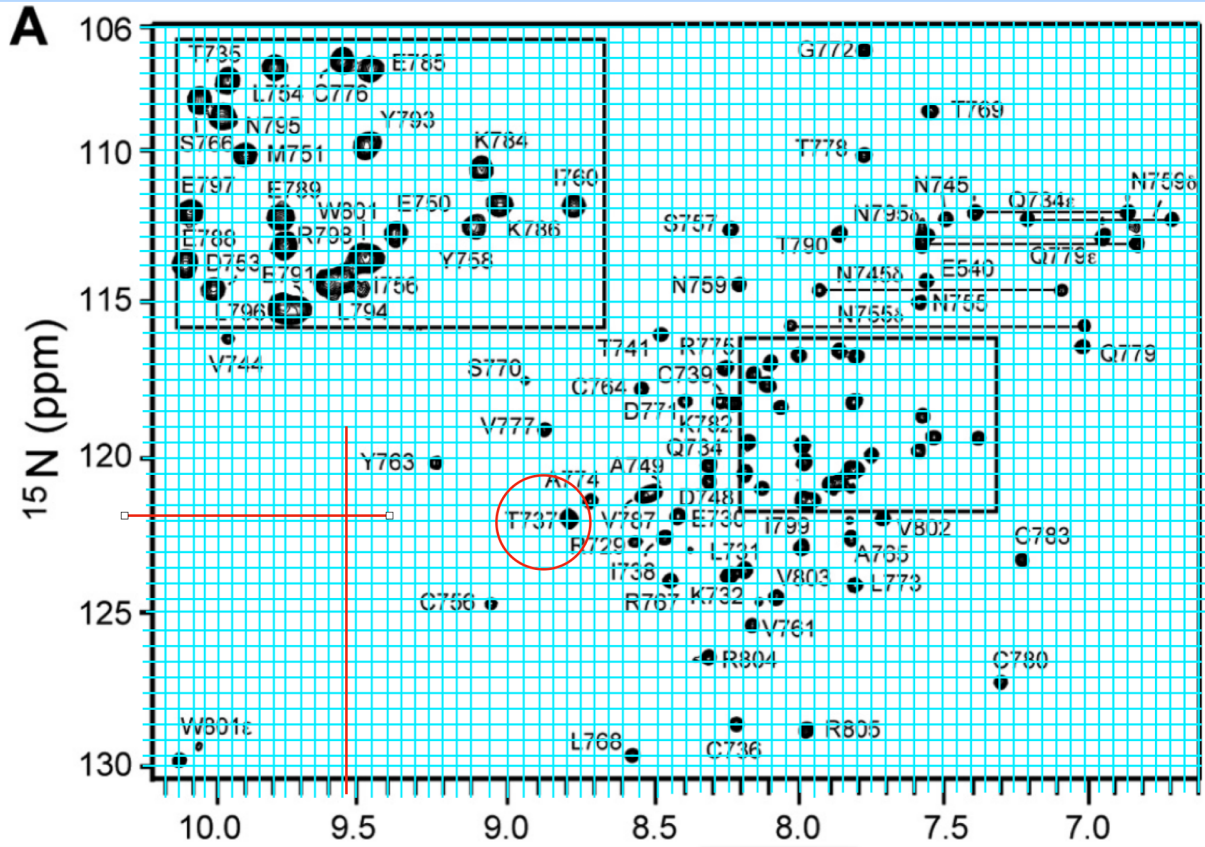
Thank you, we modified it in the manuscripts

Line 101: the spectral changes relative to the originally published ones are unexpectedly large. The explanation given that this is due to the use of different tags is not convincing. The structure 2L7X indicates that the C-terminal peptide is disordered and far from K732, T737 and A746, which show large changes in chemical shifts compared to the previous assignments (Fig. S4). The C-terminal peptide may well be very flexible (Fig. 2 indicates chemical shifts characteristic of random coil conformations and Fig. S6 shows CSPs characteristic of flexible C-termini). The C-terminal tag is unlikely to change chemical shifts on the opposite face of the folded protein domain. Arguably, the difference between different viral strains does not offer a plausible explanation either, as I779 is reported to be the only residue in the structured part of the protein core different between this construct and the one of the previous study. There is no evidence that this mutation changes the chemical shifts of the neighbouring residues.

One key factor in our decision to conduct a comprehensive assignment in-house were the discrepancies observed in the chemical shifts previously published by Estrada et al. Below we show some discrepancies between Figure 3A in their paper and their deposited BMRB files. Besides T737 chemical shift errors, neither A746 nor H752 are annotated anywhere in the 2D spectrum. Further annotation errors are shown in the last panel, and other issues were observed regarding carbon chemical shifts. We believe this data speaks for itself. Still, it is out of the scope of our manuscript to provide proof with respect to previous erroneous findings.

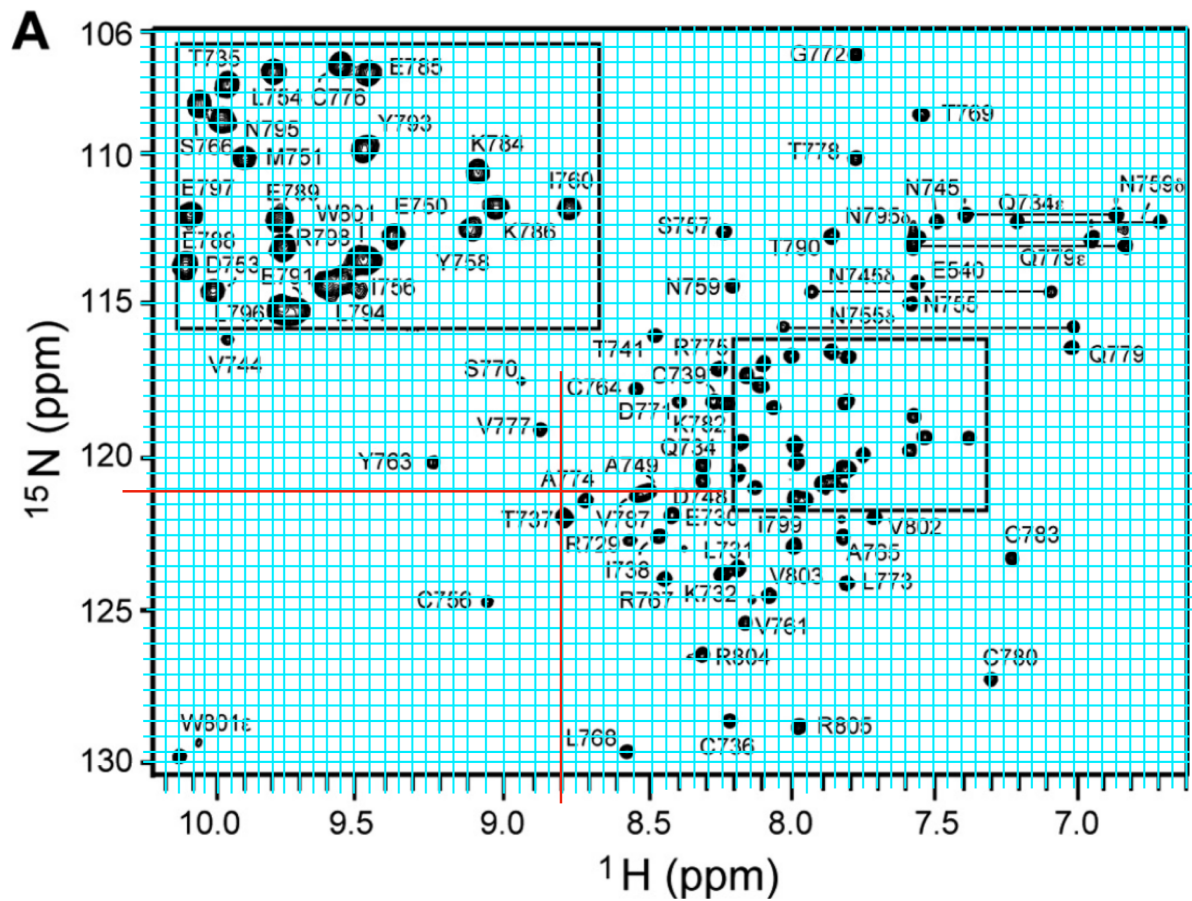
T737:

39	.	1	1	9	9	THR	H	H	1	9.553	.	.	1	.	.	.	37	THR	HN	.	17383	1
40	.	1	1	9	9	THR	HB	H	1	4.064	.	.	1	.	.	.	37	THR	HB	.	17383	1
41	.	1	1	9	9	THR	CA	C	13	63.839	.	.	1	.	.	.	37	THR	CA	.	17383	1
42	.	1	1	9	9	THR	CB	C	13	69.111	.	.	1	.	.	.	37	THR	CB	.	17383	1
43	.	1	1	9	9	THR	N	N	15	121.780	.	.	1	.	.	.	37	THR	N	.	17383	1



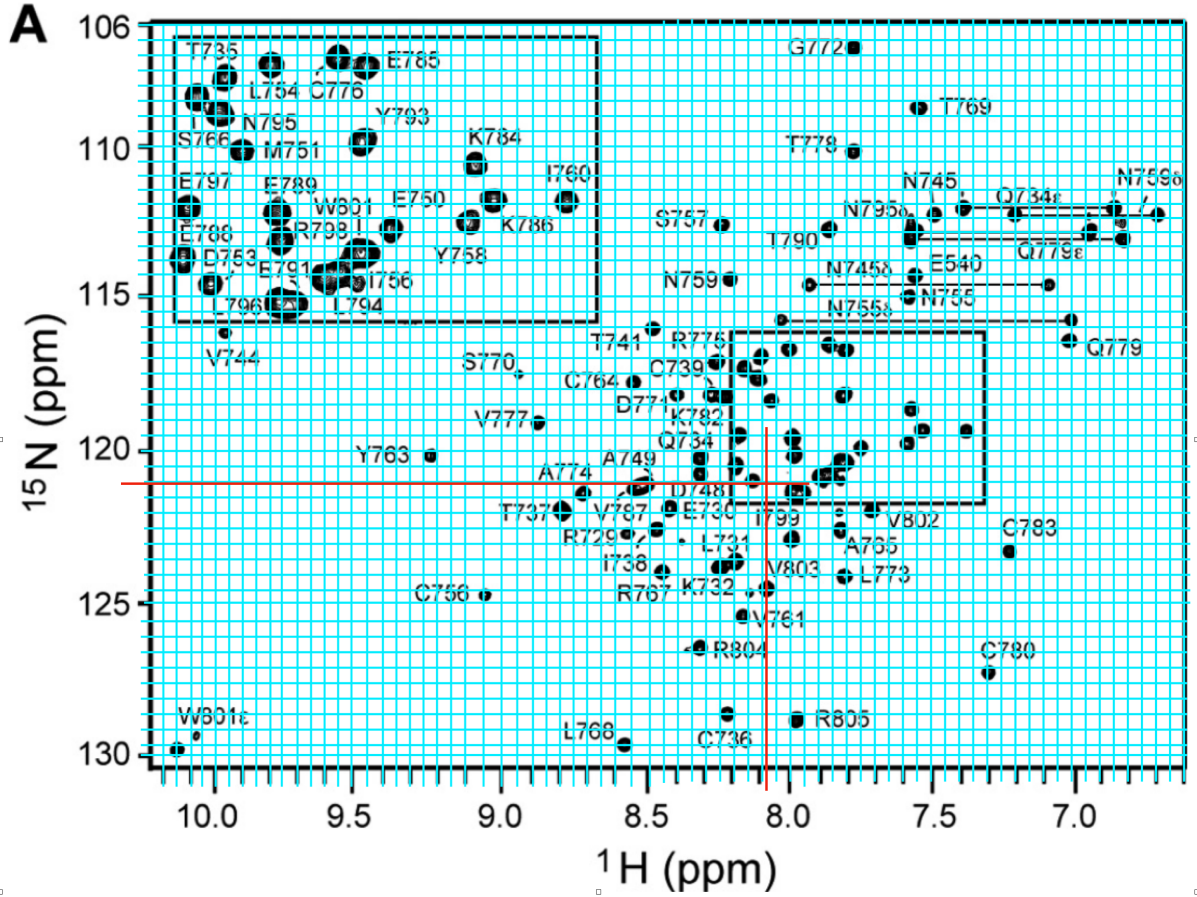
A746:

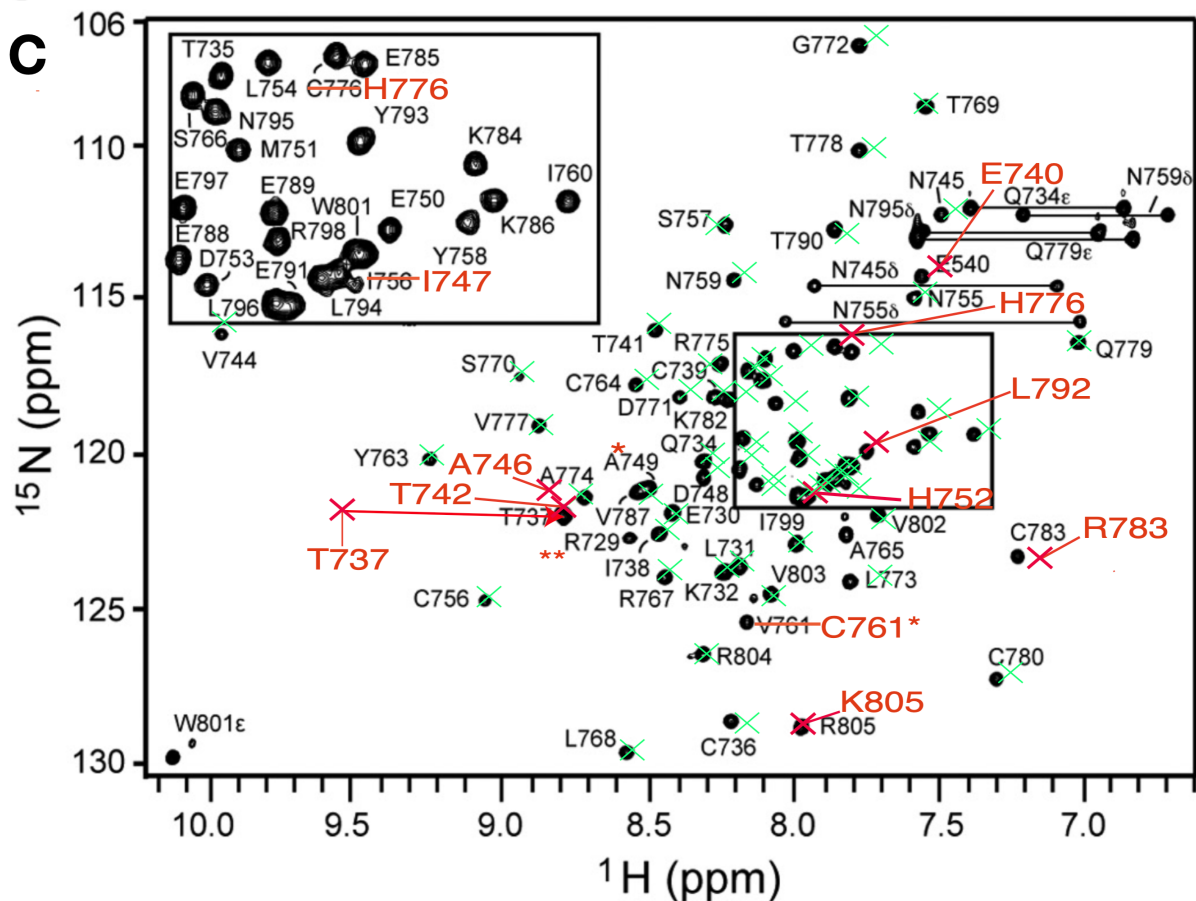
83	.	1	1	18	18	ALA	H	H	1	8.841	.	.	1	.	.	.	46	ALA	HN	.	17383	1
84	.	1	1	18	18	ALA	HA	H	1	4.176	.	.	1	.	.	.	46	ALA	HA	.	17383	1
85	.	1	1	18	18	ALA	CA	C	13	55.854	.	.	1	.	.	.	46	ALA	CA	.	17383	1
86	.	1	1	18	18	ALA	CB	C	13	18.389	.	.	1	.	.	.	46	ALA	CB	.	17383	1
87	.	1	1	18	18	ALA	N	N	15	121.144	.	.	1	.	.	.	46	ALA	N	.	17383	1



H752:

112	.	1	1	24	24	HIS	H	H	1	8.068	.	.	1	52	HIS	HN	.	17383	1
113	.	1	1	24	24	HIS	HA	H	1	4.739	.	.	1	52	HIS	HA	.	17383	1
114	.	1	1	24	24	HIS	CA	C	13	60.871	.	.	1	52	HIS	CA	.	17383	1
115	.	1	1	24	24	HIS	CB	C	13	29.995	.	.	1	52	HIS	CB	.	17383	1
116	.	1	1	24	24	HIS	N	N	15	120.999	.	.	1	52	HIS	N	.	17383	1





While these chemical shift differences are not the key message of this manuscript, the explanation for the chemical shift differences between old and new constructs remains unsatisfactory.

We hope that the above analysis provides a sufficient explanation.

Were the previously published assignments established using samples prepared without the addition of zinc during purification whereas the present assignment was of protein with zinc supplied during sample preparation? If so, the CD spectra of Fig. S1 seem to suggest that this could have a substantial impact on the structure.

In our experiments, the zinc is added during sample preparation, as also in the previously published assignment. The new CD curve showing that the zinc-finger formation is reversible, this would anyway not have an impact on the structure of the protein.

Please clarify whether the spectra of Fig. S2 were recorded in the presence of 50 mM phosphate – as referee 3 points out, zinc phosphate is insoluble (in fact, as Wikipedia tells, it is used as dental cement). The authors should either provide more convincing data (NMR spectra with different amounts of zinc recorded in the absence of phosphate) ...

The previously published spectrum in Figure 2S (Estrada et al., black spectrum) was recorded in 10 mM phosphate buffer while our spectrum (red spectrum) was

recorded with 50 mM phosphate buffer. However, in our CD experiments performed with 50 mM phosphate, we do not observe any precipitation.

... or give an acceptable explanation. An experimental resolution of the issue would significantly raise the significance of this manuscript.

Considering our above annotations of the published data, this manuscript could only become more relevant if we could show that the 3D structure described in the literature does not comply with the erroneous chemical shifts. As Figure S6 shows, the secondary structure elements are not much impacted by the differences. A quick check on NOEs used shows that mainly intra-residue, and $i, i+1, 2$ were used for 737 and 752. A comprehensive and solid verification of previous data is outside the scope of our manuscript.

Line 109: A more helpful sentence may read "All resonance assignments of backbone amides were verified by 3D NMR spectra recorded of the 2H-13C-15N labeled sample."

Thank you for the correction. We changed it in the manuscript.

Line 116: clearer wording would be 'amino acid sequence'.

Thank you, we corrected it in the manuscript.

Line 117: "assignment spectra" is unfortunate lab jargon.

We changed it.

Line 125: "completion of missing resonances" – please spell out more accurately.

We modified the sentence.

Line 133: "as is" appears superfluous.

We removed the end of the sentence.

Line 134: "15N-HSQC spectra". "increased errors": increased relative to what?

We are here referring to errors due to the different labeling (protonated vs deuterated) of the two samples, as deuteration leads to shifts of the CS. We clarified this in the manuscript.

Line 140: please report the correct DPB ID. Is it 2L7X?

Thanks - yes, it is 2L7X. We corrected in the manuscript.