Dear anonymous referee,

we would like to thank you very much for your very careful reading of the manuscript and your interesting remarks. Please find below our replies.

A. Böckmann for all authors

The use of NMRtist in this manuscript adds very little scientifically as it is consistent with the reported and expected performance of the software and while it is noteworthy, its significance appears overstated.

-> We have no problem to tune down the significance statement

Figure 3 in particular appears to be some internal reporting from the software rather than novel analysis of the performance of that software (if it is a novel analysis it should be described in more detail). This figure may be suitable to deposit as supplementary data but does not add to the presented work in a meaningful way.

-> This is indeed not novel, but is the standard output from the software reporting on the assignments done by the program. It shows how the program has used the spectra and the result of the run. We had reported this type of output in a previous paper (Schmidt, Elena, Julia Gath, Birgit Habenstein, Francesco Ravotti, Kathrin Szekely, Matthias Huber, Lena Buchner, Anja Böckmann, Beat H Meier, et Peter Güntert. « Automated Solid-State NMR Resonance Assignment of Protein Microcrystals and Amyloids ». Journal of Biomolecular NMR 56, no 3 (mai 2013): 243-54. https://doi.org/10.1007/s10858-013-9742-x.), and we would have thought that this is good practice. If the referee has a strong opinion about this, we can certainly move it to the SI.

Similarly the abstract speak of remote access, but the manuscript does not detail how this is done or what is novel about the remote access that makes this of note in this publication (beyond perhaps a reference to NMRlib).

-> We can remove "remote" and insert NMRlib (the reference is actually present but was unfortunately not formatted in the reference list).

Major revision required:

1. The differences in chemical shift around residue 752 is a little concerning as this coordinates a Zn ion. I'm concerned that the Zn is not saturated. It is noted that a concentration of 0.1 mM of zinc sulfate is used, as was present in the sample used in the previously published work, but it is unclear if this is sufficient to saturate the Zn binding. The published work reported that a 1 mM of zincsulfate concentration was used during purification when the sample was dilute and the sample subsequently concentrated, with the final buffer containing zinc sulfate at a concentration of 0.1 mM. Thus, in the reported scenario the protein has been exposed to saturating

concentrations of Zn and likely maintains this throughout. But in this work it is unclear if the addition of 0.1 mM ZnSO4 during the processing is sufficient given that protein concentrations are much higher during CFPS. It would be important to perform a titration of ZnSO4 to the protein (monitored by 15N HSQC) to determine if the chemical shift difference remain when the sample is in the presence of saturating Zn concentrations.

-> In the literature, cell-free protein synthesis in dialysis mode was done in presence of 100 uM (Matsuda et al., 2006). We ourselves have worked before with a Zinc-binding protein produced in a very similar cell-free set-up, which yielded excellent solid-state NMR spectra of a well-folded protein (Jirasko et al., Angew. Chem. 2020). In the present report, we have worked throughout with buffers containing 100 uM Zn ions throughout all steps, and in absence of EDTA. The concentration of the protein in the cell-free reaction (here done in bilayer mode) was 46 uM, and thus not higher than during purification or in the final NMR sample.

-> Furthermore, the 15N HSQC spectrum shown in Estrada et al. 2011 does not actually show a peak at the chemical shifts given for His752 (coordinating ZF1). We therefore believe that this is rather an unfortunate misclassification. Indeed, the cysteine residues of ZF1 clearly show the chemical shifts described by Estrada et al. 2011, confirming the proper formation of the zinc finger.

2. It is not entirely clear why the deuterated sample was produced and when it was used. It is noted that the protonated sample was used to transfer assignments, but the sidechain assignments include proton detected hCCH experiments which suggests that the deuterated sample was not used for this. It would be helpful if the results section included a listing of which sample was used for which experiment. Ideally included a clear rationale why deuteration is required at all.

-> As is often the case, the reason is historical. Our first goal in this project was to confirm the assignments of Estrada et al. 2011, since we observed discrepancies with our solid-state NMR spectra. So we used a deuterated sample. We then realised that it would be interesting to also obtain side-chain assignments including protons, as we wanted to understand the proton line widths observed in the solid-state NMR spectra. So we made a protonated sample to do this. As we use spectrometer time on the National Access Programme, we found it exaggerated to ask to record all the sequential spectra on this sample again.

Minor:

It is noted that Europe is a non-endemic region for this virus but the endemic regions have not been defined. I appreciate this may be apparent from the name, but names of viruses are not always very descriptive of their origin or endemic regions.

-> CCHFV is endemic in most parts of Africa, in the Balkans, in the Middle East and in Asia (Shahhosseini et al., 2021)

Latin names such as Hyalomma should be italicised.

-> We can addressed this.

The note of an outbreak in France needs a reference or should be removed.

-> We did not refer to an outbreak, but to the first detection of the virus in France. We will insert the reference (Bernard, C., Joly Kukla, C., Rakotoarivony, I., Duhayon, M., Stachurski, F., Huber, K., Giupponi, C., Zortman, I., Holzmuller, P., Pollet, T., Jeanneau, M., Mercey, A., Vachiery, N., Lefrançois, T., Garros, C., Michaud, V., Comtet, L., Despois, L., Pourquier, P., Picard, C., Journeaux, A., Thomas, D., Godard, S., Moissonnier, E., Mely, S., Sega, M., Pannetier, D., Baize, S., and Vial, L.: Detection of Crimean–Congo haemorrhagic fever virus in Hyalomma marginatum ticks, southern France, May 2022 and April 2023, Eurosurveillance, 29, https://doi.org/10.2807/1560-7917.ES.2024.29.6.2400023, 2024).

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In the discussion, the statement that the high field provided the needed sensitivity and resolution is not supported by the data. Clearly it would help, but it is not shown that it is needed, and is unlikely to be the case for such a small protein. Perhaps replace needed with facilitated or similar.

-> We can do this.