Response to Reviewer 1 Comments

1. Summary

Thank you for reviewing our manuscript (ijms-3759454) entitled "Structural features of nucleoproteins from the recently discovered *Orthonairovirus songlingense* and *Norwavirus beijiense*" submitted for publication in IJMS.

We thank you for valuable suggestions that allowed us to make the manuscript more convincing and understandable. We accepted your suggestion and made corresponding change in the manuscript. We major revised our article. Below please find our detailed responses to your questions and comments. All modifications in the manuscript have been highlighted in red.

2.

Questions for General Evaluation	Reviewer's Evaluation	Response and Revisions
Does the introduction provide sufficient background and include all relevant references?	Can be improved	See point-by-point response.
Is the research design appropriate?	Can be improved	
Are the methods adequately described?	Yes	-
Are the results clearly presented?	Yes	-
Are the conclusions supported by the results?	Can be improved	See point-by-point response.
Are all figures and tables clear and well-presented?	Yes	-

3. Point-by-point response to Comments and Suggestions for Authors

Reviewer 1: The authors built 3D models of the nucleoproteins from *Orthonairovirus songlingense* (SGLV) and *Norwavirus beijiense* (BJNV) using AlphaFold 3. They verified the models by performing SAXS measurements. The authors also investigated the interaction of these proteins with genomic RNA. Since these viruses cause tick-borne diseases, the results are potentially interesting.

Comments 1: They propose several RNA-binding modes for these proteins. They should examine the structure of the complexes using light scattering, SAXS, or electron microscopic analyses. Simple estimation of molecular weight by light scattering can exclude certain models.

Response 1: We fully agree with the reviewer's insightful suggestion regarding the need for experimental validation of proposed RNA-binding modes through biophysical characterization. The reviewer's recommendation to employ light scattering, SAXS, or electron microscopy to examine nucleoprotein-RNA complexes is particularly valuable and aligns perfectly with our planned future research directions.

We appreciate this constructive suggestion and will incorporate these methods to provide a more comprehensive understanding of RNA recognition mechanisms in our future work on these

emerging viral nucleoproteins. These planned experiments will significantly strengthen the mechanistic conclusions of our structural models.

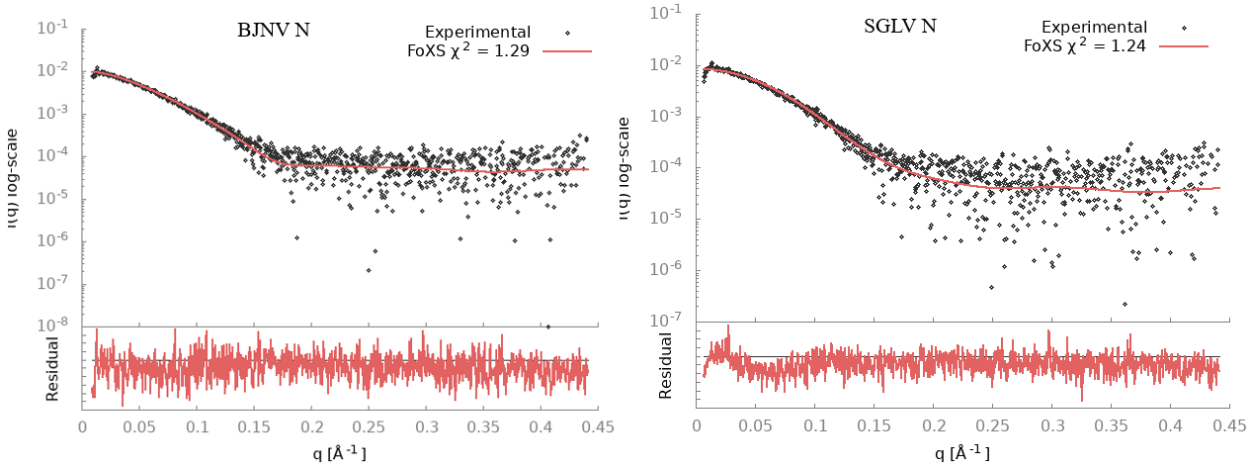
Comments 2: The authors mentioned that structural analyses of virus nucleoproteins are helpful for the development of medical treatments against these virus infections. They should provide examples of such development.

Response 2: We thank the reviewer for this valuable suggestion. We have now expanded our discussion to include concrete examples of how structural insights into viral nucleoproteins have directly contributed to antiviral development (see lines 351-366).

Comments 3: We can compute a SAXS profile from an atomistic 3D structure using FoXS software (Schneidman-Duhovny, D., Hammel, M., Tainer, J.A. and Sali, A., 2016. FoXS, FoXSDock and MultiFoXS: Single-state and multi-state structural modeling of proteins and their complexes based on SAXS profiles. Nucleic acids research, 44(W1), pp. W424-W429.) and compare directly with the experimental profiles. I wonder why they did not use this approach to validate the AlphaFold-proposed models by SAXS.

Response 3: We appreciate the reviewer's insightful suggestion regarding the use of FoXS for direct comparison between theoretical SAXS profiles derived from atomistic predicted 3D structures and experimental data. The utility of FoXS for such validation is well acknowledged in the field.

In our study, we opted to validate AlphaFold-predicted models against experimental SAXS data using the Crysol software (Table 2 in the manuscript), another industry-standard tool for calculating theoretical scattering profiles from atomic coordinates. The main advantage of choosing Crysol was that this instrument is an integral part of the ATSAS complex, which provides a full cycle of processing, modeling and validation of SAXS experiments. The small discrepancy in χ^2 values compared to Table 2 in the manuscript between FoXS and Crysol programs when comparing the same structure with the experimental SAXS profile is due to similar, but not identical, methods of fitting the theoretical SAXS profile.



This multi-software approach will further strengthen the robustness of our structural validations while providing more comprehensive assessment of model quality. We thank the reviewer for highlighting this important methodological consideration.

Sincerely,
Anastasia Gladysheva

