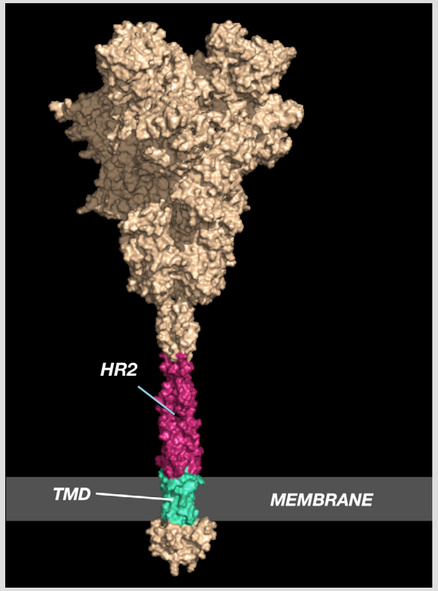
# A MODEL OF SARS-COV-2 SPIKE TRANSMEMBRANE DOMAIN LINKED TO THE HR2 REGION: STRUCTURAL ORGANISATION AND POSSIBLE ROLE IN MEMBRANE FUSION

*Elena T. Aliper, Anton A. Polyansky, Dmitry E. Nolde, Nikolay A. Krylov, Roman G. Efremov**
*Laboratory of Biomolecular Modelling at*
*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia*
**Correspondence: efremov@nmr.ru*

## THE OBJECTIVE

- Spike protein transmembrane domains (S-TMD) are likely to be crucial to viral fusion, while HR2 is also hypothesised to facilitate it.
- Little data on S-TMD structure, tools for the prediction of transmembrane (TM) trimers do not currently exist.
- How is S-TMD packed? How is HR2 involved in fusion?



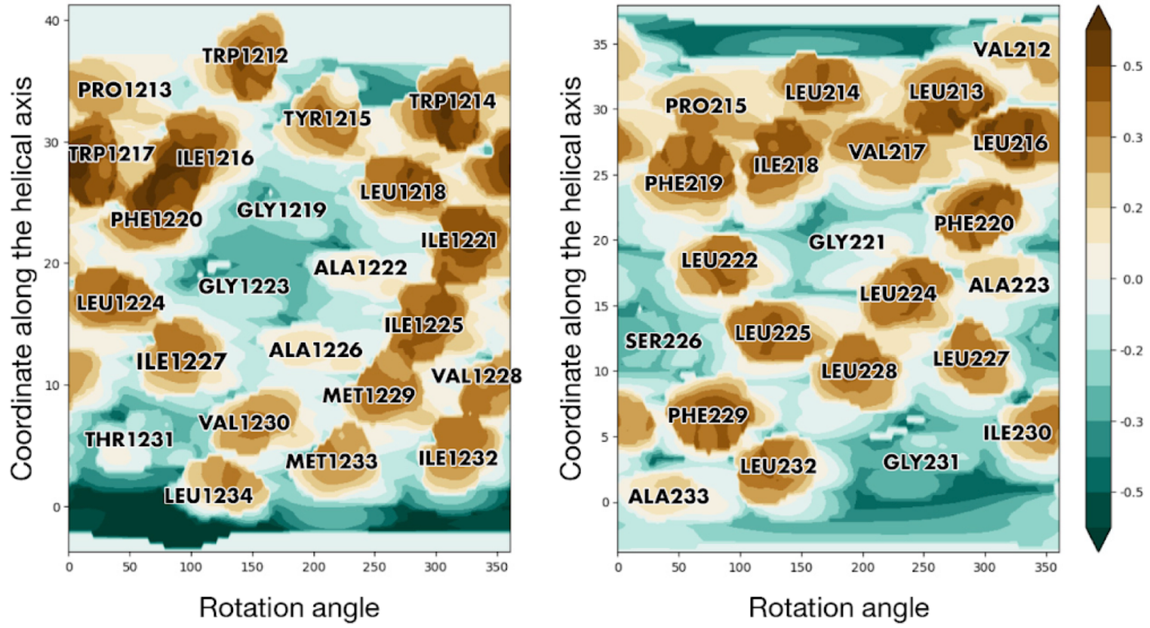

## METHODS

Comprehensive framework bringing together diverse computational tools designed:

- Molecular hydrophobicity potential (MHP) mapping [1] (https://model.nmr.ru/platinum)
- Template-based modelling (MODELLER 9.19)
- Monte Carlo (MC) conformational search
- Molecular dynamics (MD) simulations (GROMACS)
  explicit POPC bilayers
  tip3p water / counter-ions
  CHARMM36 FF, 325K

# TEMPLATE SELECTION

Pattern matching: amino acid sequence and MHP distribution on the helix surface: tumor necrosis receptor factor 1A (TNFR) selected for the template-based modelling

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spike    1212-WPWYIWLGFIAGLIAIVMVTIML-1234
TNFR-1    212-LPLVIFFGLALLSLLFIGLAYRY-234
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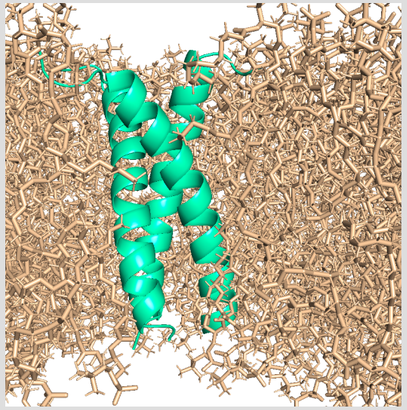
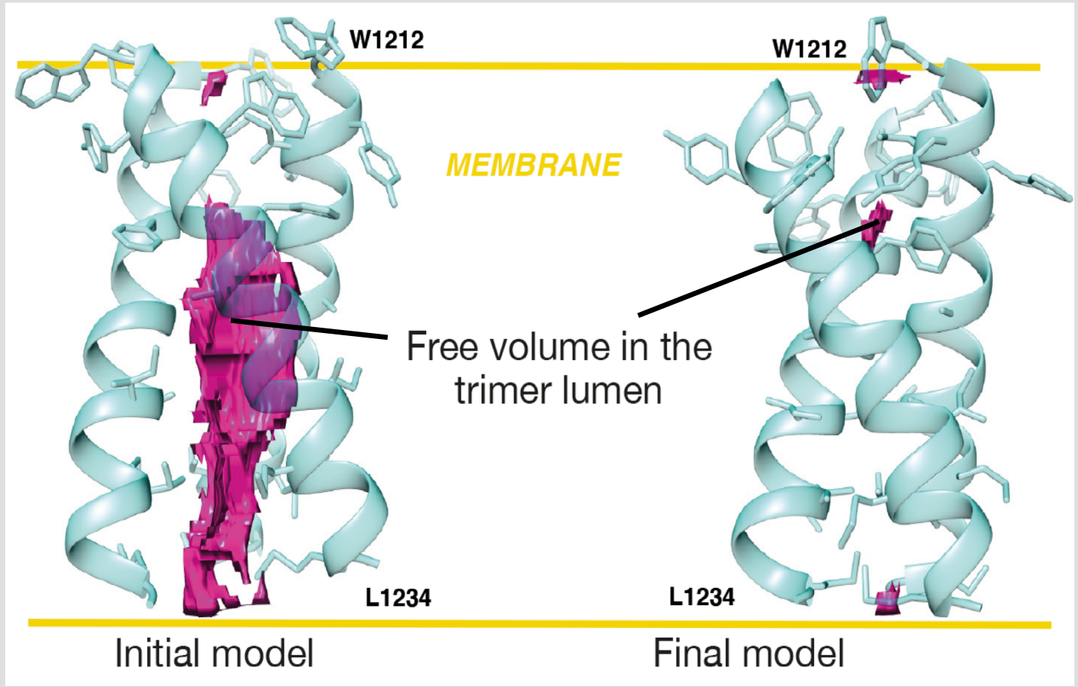
# ‘MHP DYNAMIC PORTRAIT’ ADJUSTMENT

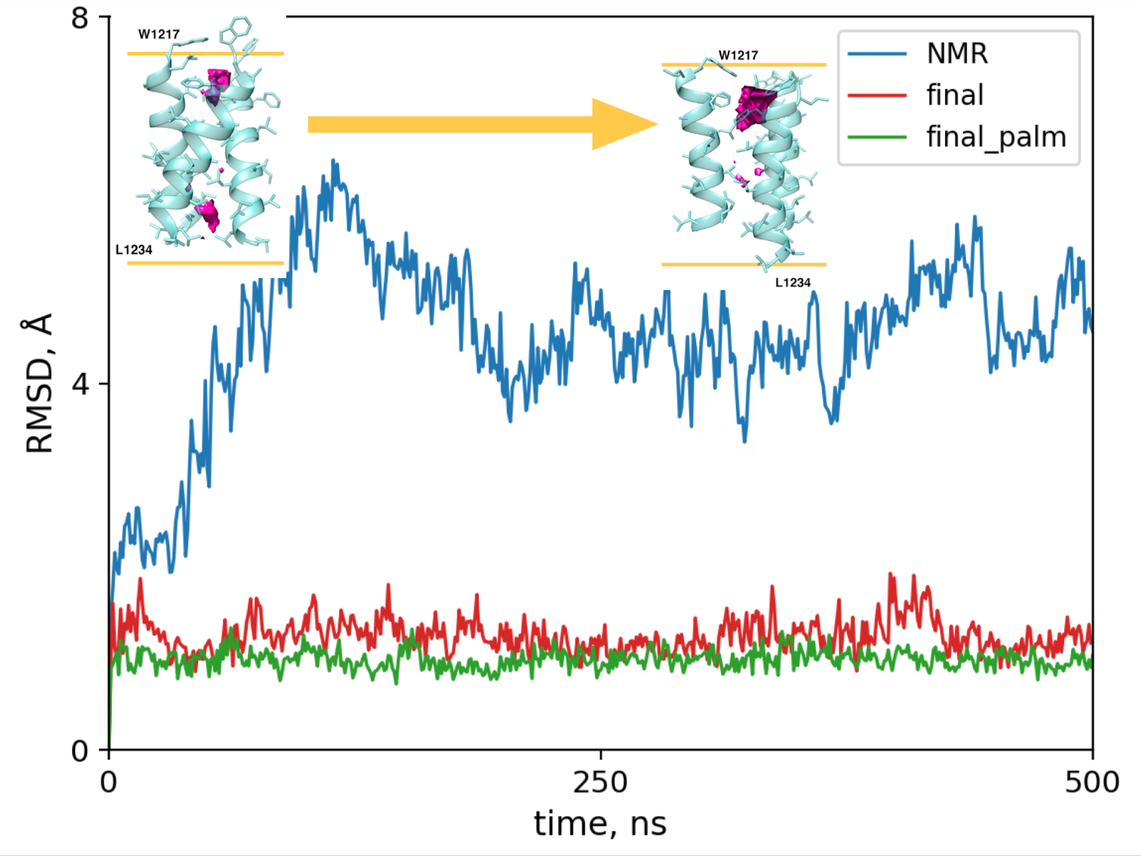
Model derived via template-based modelling tested via MD simulation in an explicit POPC bilayer. Changes observed used to fine-tune the model and put together a better one, tightly packed.





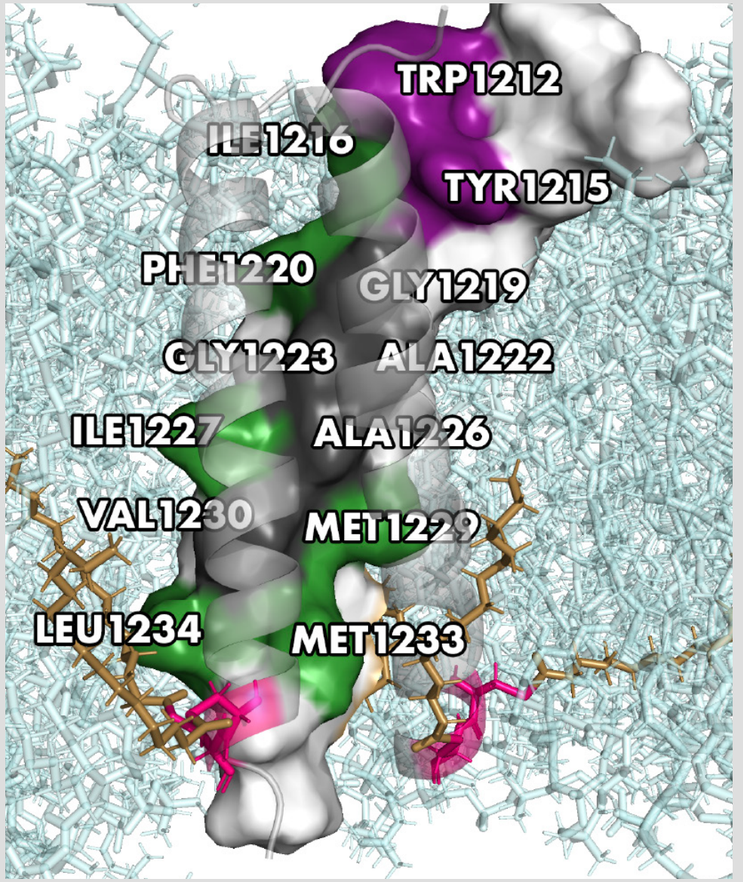
## STABILITY

The final model (red) comparess favourably to other models, including a recently published NMR structure (blue) of the proposed S-TMD. Retained stability when palmitoylated in accordance with experimental data (green).



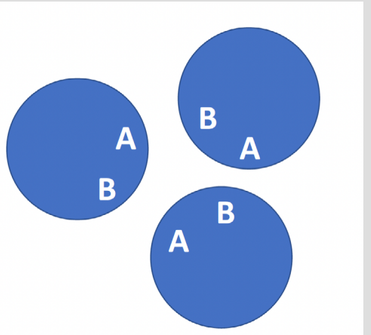

## HELIX/HELIX INTERFACES

Helix/helix interfaces included residues identical (purple) and (semi-) conservative (green) across genus *Betacoronavirus.* With palmitoyls (golden) appended at C1235 and C1236 (pink) the model remains stable.




Gly corresponding to G1219 & G1223 crucial to trimerization in SARS-CoV S-TMD [2].

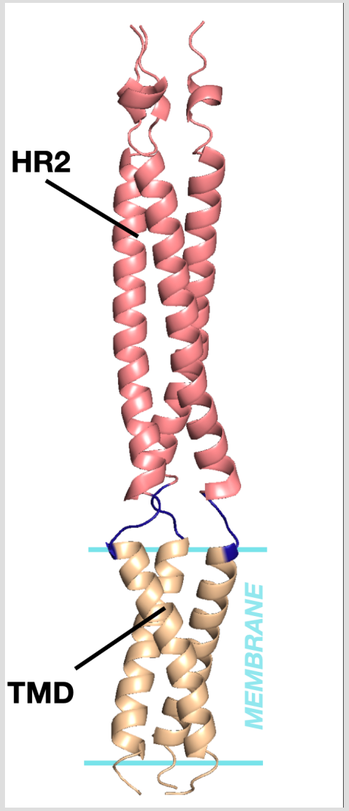
Two overlapping interfaces in each chain



# HR2 FACILITATES MEMBRANE BINDING? MD SIMULATIONS

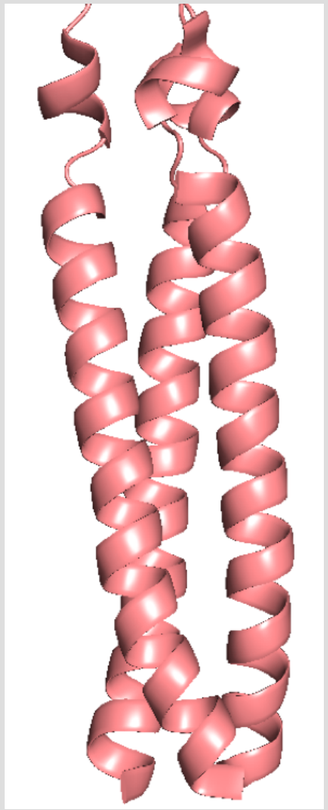
NMR structure of SARS-CoV spike HR2 used (PDB ID 2FXP), 100% identity to SARS-CoV-2's HR2

HR2 and TMD: different angles between helical axes, the former is a canonical water-soluble coiled-coil, while TMD is packed following the principles of TM packing. A hinge area was modelled.



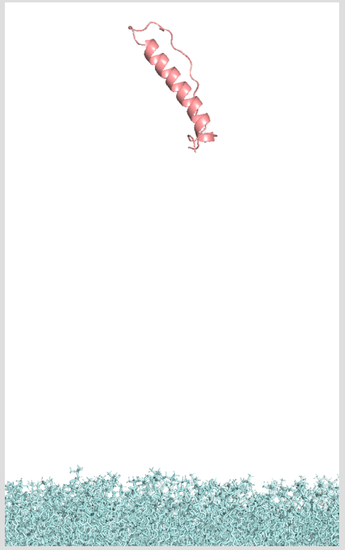

HR2+TMD anchored in a membrane: both domains stable during MD

HR2 in water: stable, remains trimeric, helix/helix interfaces stable



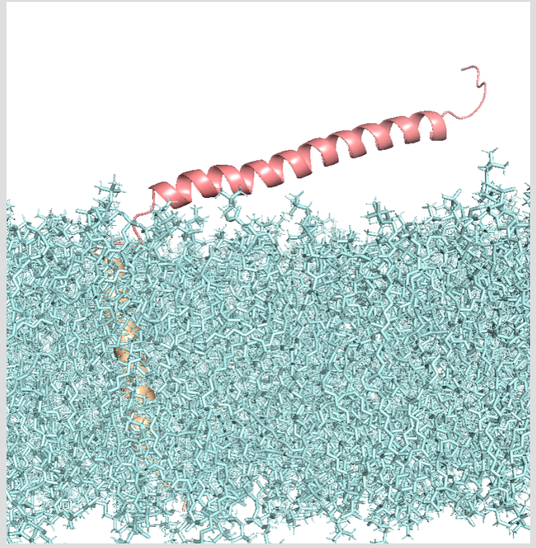
# HR2 AFFINITY FOR THE MEMBRANE

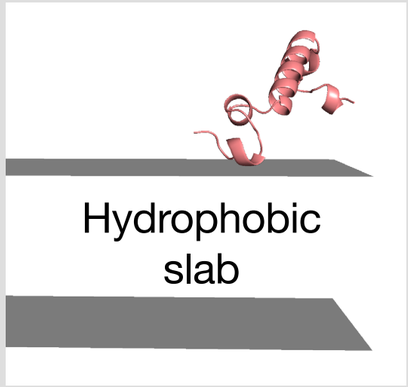
HR2 monomer: limited affinity for membrane



**MD**

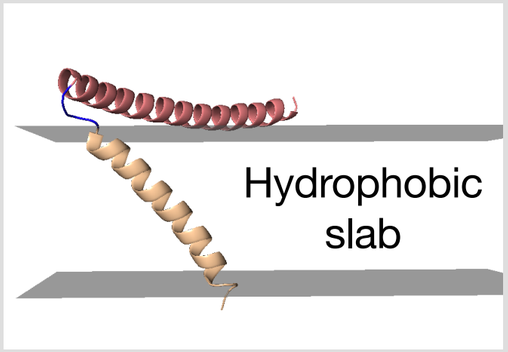
HR2 + TMD monomer: HR2 interacts with POPC membrane, but does not bind to it properly






**MC**

lowest energy states



## CONCLUSIONS

- A uniquely stable model of S-TMD was created conforming to basic principles of TM helix packing
- A comprehensive framework potentially applicable to TM trimer prediciton was designed.
- The model was used in the evaluation of HR2 domain's role in membrane fusion.
- While TMD acts to keep it in the vicinity of the membrane, additional factors might be required for HR2 to properly act as a force dragging the membrane into place during fusion (agrees with experimental data [3])

## REFERENCES


1. Efremov et al. (1992) J Protein Chem. 11:665-75.
2. Arbely et al. (2006) Biochemistry 45:11349-56.
3. Chiliveri et al. (2021) Sci Adv. 7:eabk2226.
4. Aliper et al. (2022) [preprint] https://doi.org/10.1101/2022.06.05.494856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