

A structural/dynamic model of SARS-CoV-2 spike transmembrane domain in conjunction with the HR2 region: implications for membrane fusion

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Moscow, 25 May 2022

Acknowledgments

Research within the scope of the present study was conducted at the Laboratory of Biomolecular Modelling at Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow,

with the valuable participation of:

Anton A. Polyansky

Dmitry E. Nolde

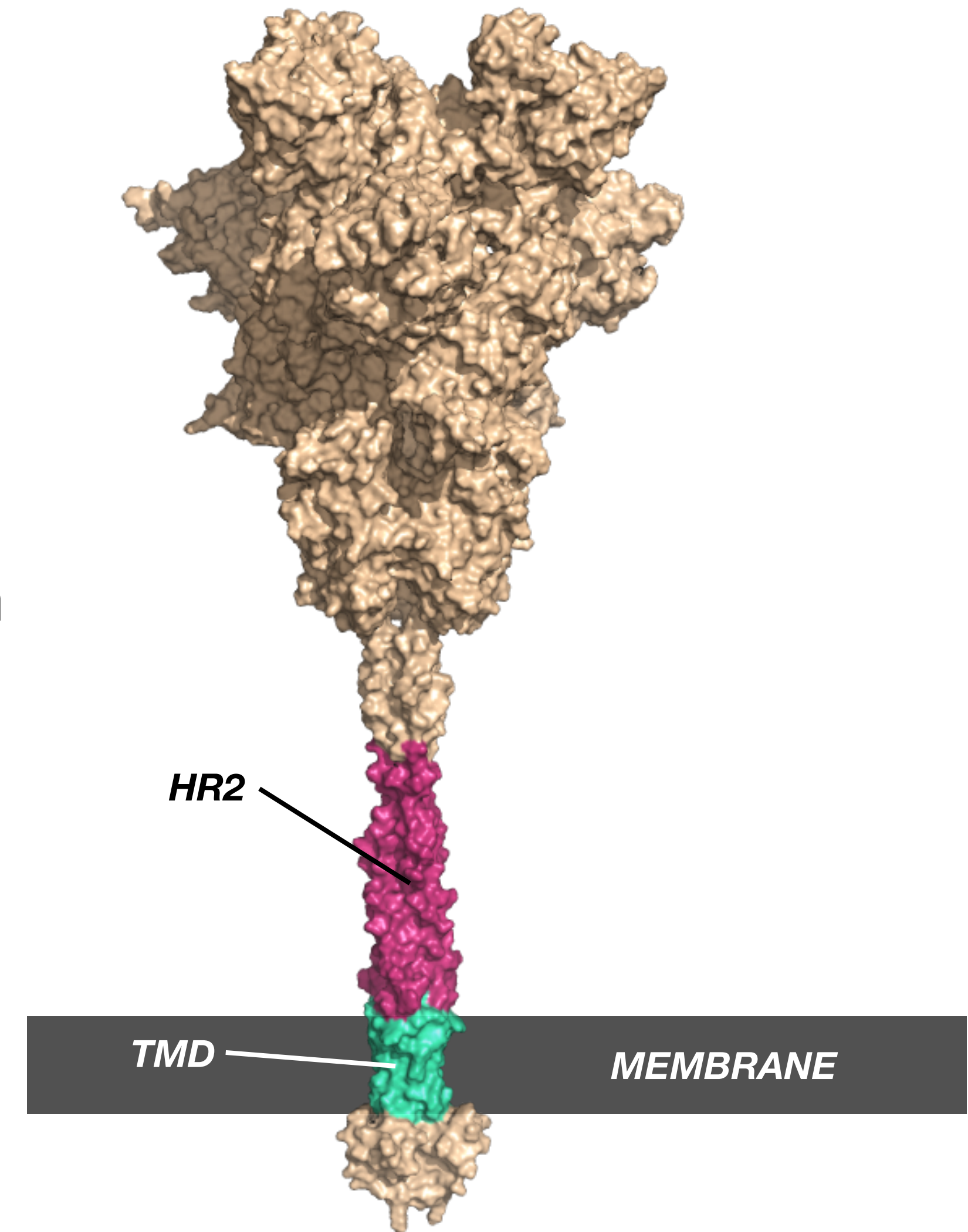
Nikolay A. Krylov

Roman G. Efremov

SARS-CoV-2 spike protein transmembrane domain (S-TMD)

Cryptic so far

- Class I fusion protein, TMD consists of 3 α -helices
- TMD is very likely to be crucial to membrane fusion
- HR2 hypothesised to interact with the membrane during fusion
- Data on S-TMD structure and functionality is scarce
- Tools for the prediction of TM trimer structure do not exist, models that exist gave the TMD brief consideration

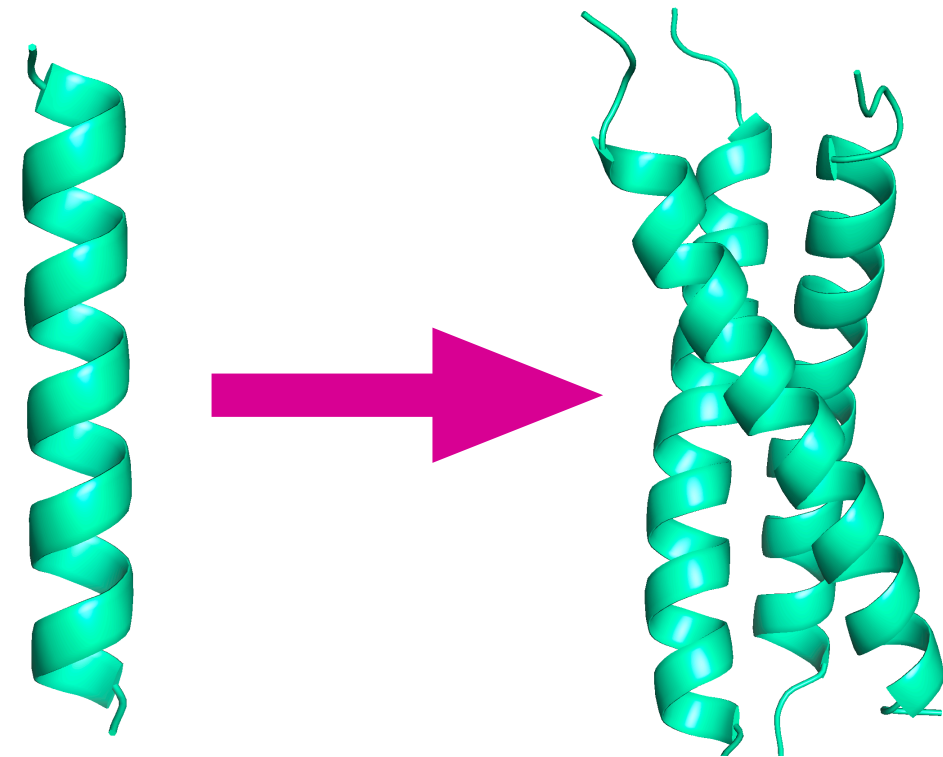


The purpose of the study

To predict the 3D organisation of SARS-CoV-2 spike TMD (S-TMD) and use the resulting model to explore the possible role of the HR2 region in membrane fusion.

Design of the study

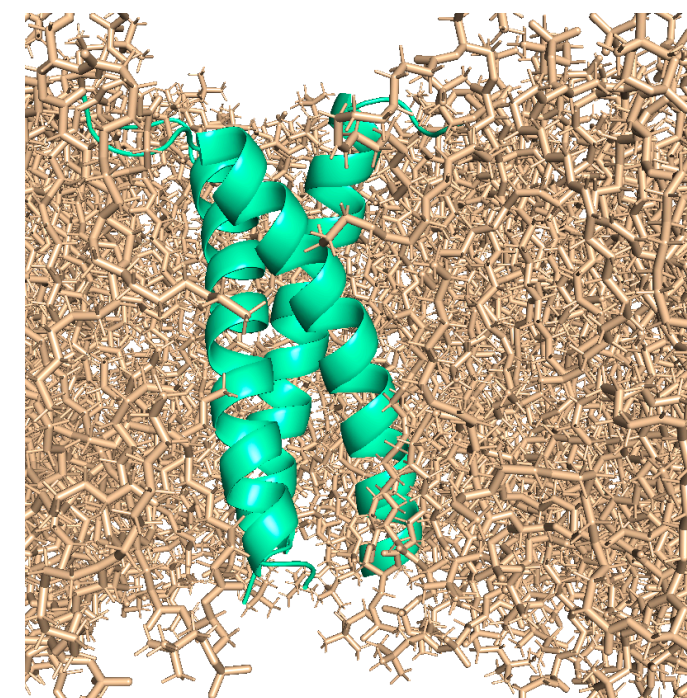
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how are helices packed?



*Primary structure analysis,
template-based modelling*

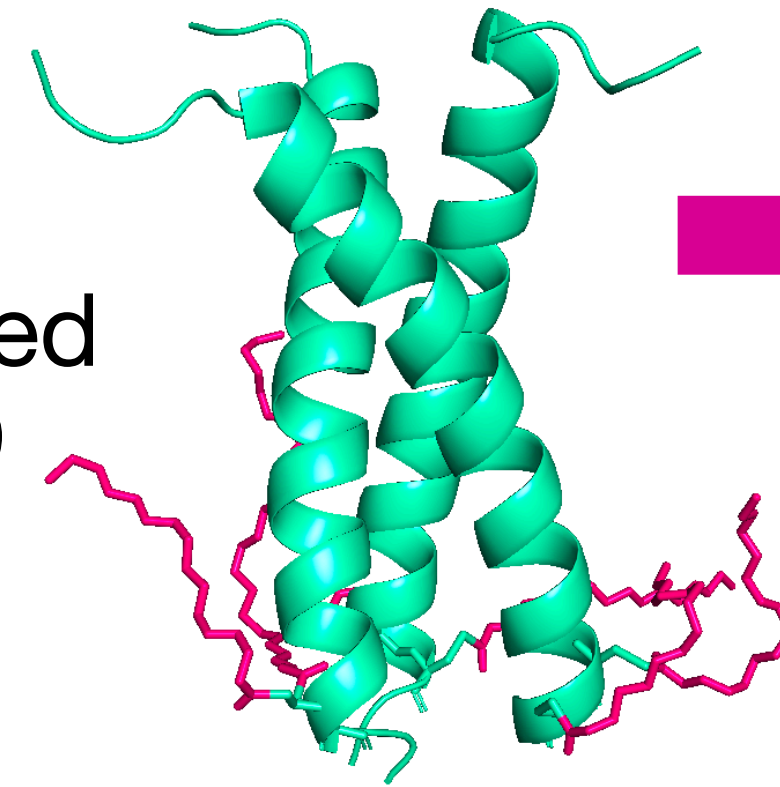
2. The model tested and fine-tuned in a lipid bilayer

*Molecular dynamics
simulations*



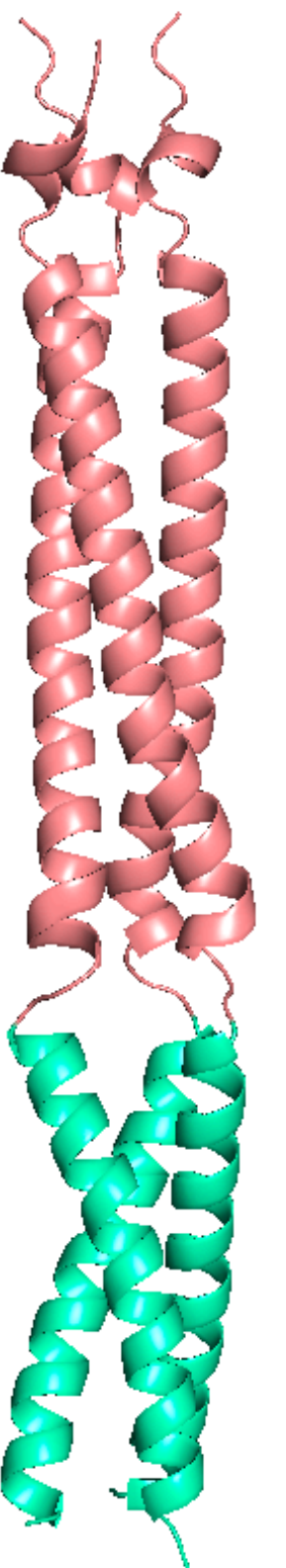
3. Palmitoyl modifications introduced downstream of S-TMD
impact on stability?

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5. To fit together the model S-TMD and the HR2 region, study the extended system in the presence of a lipid bilayer

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4. Compare to other available models and experimental structures

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Methods

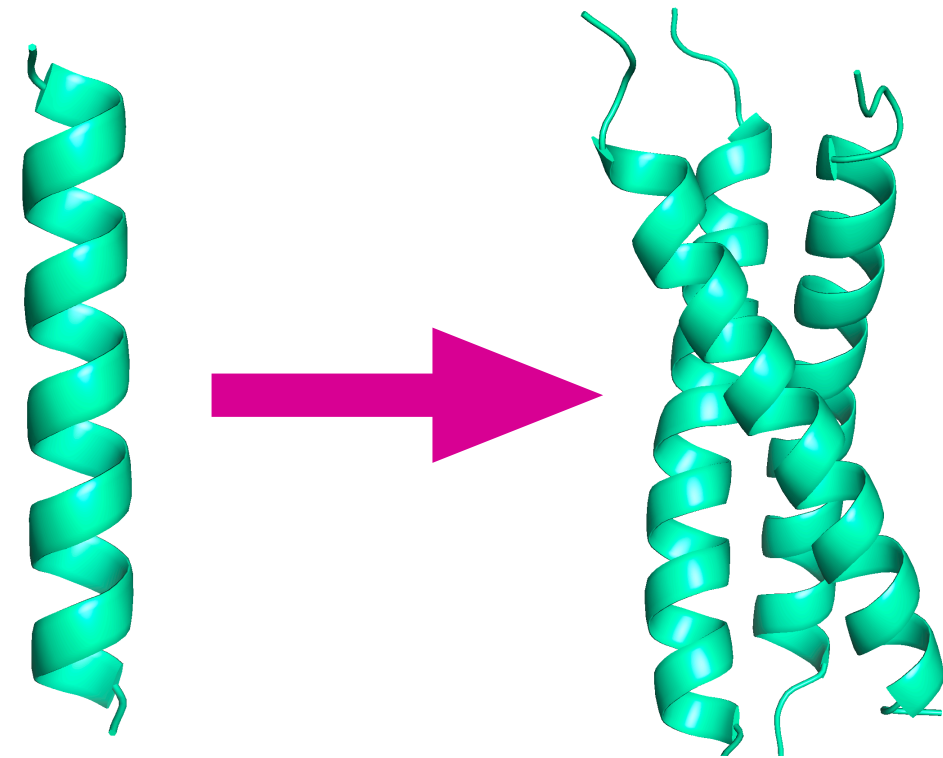
A comprehensive framework was designed in the course of the study, bringing together a variety of methods

- Molecular hydrophobicity potential (MHP) mapping* (<https://model.nmr.ru/platinum>)
- Template-based modelling (MODELLER 9.19)
- Molecular dynamics (MD) simulations (GROMACS)
 - explicit POPC bilayers
 - tip3p water / counter-ions
 - CHARMM36 FF
 - 325K

*:

Efremov RG et al. (1992) *J Protein Chem.* 11:665-75
Pyrkov TV et al. (2009) *Bioinformatics* 25:1201–1202.

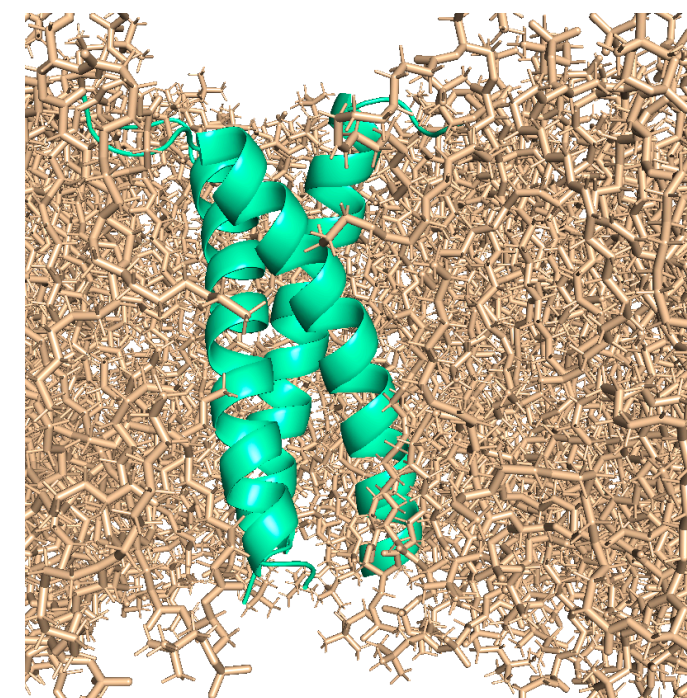
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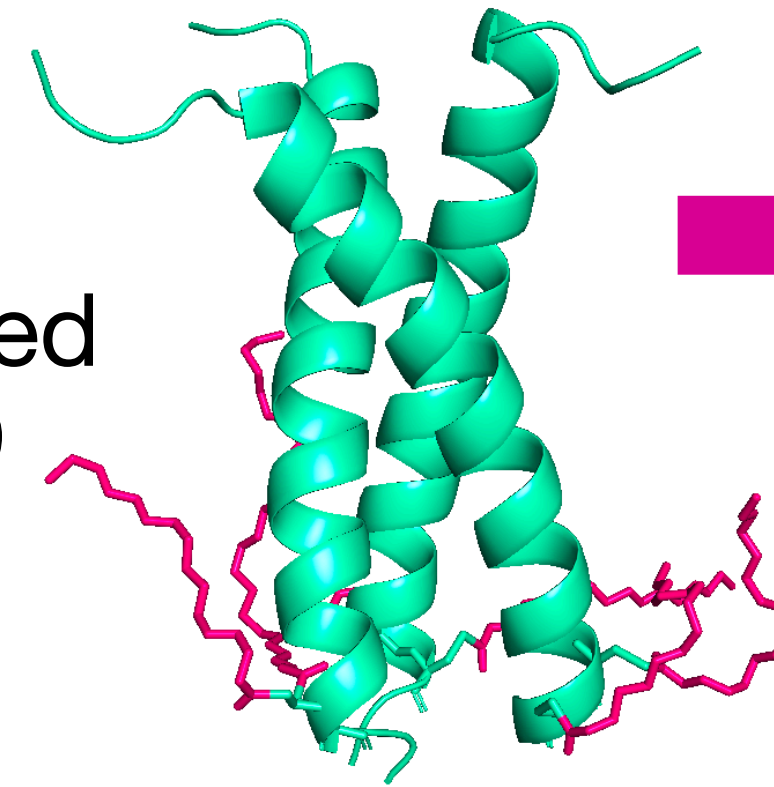
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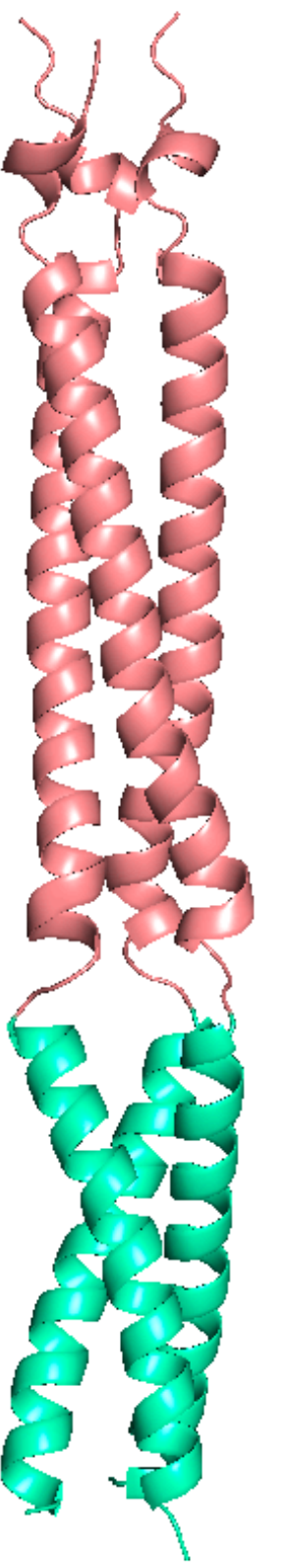
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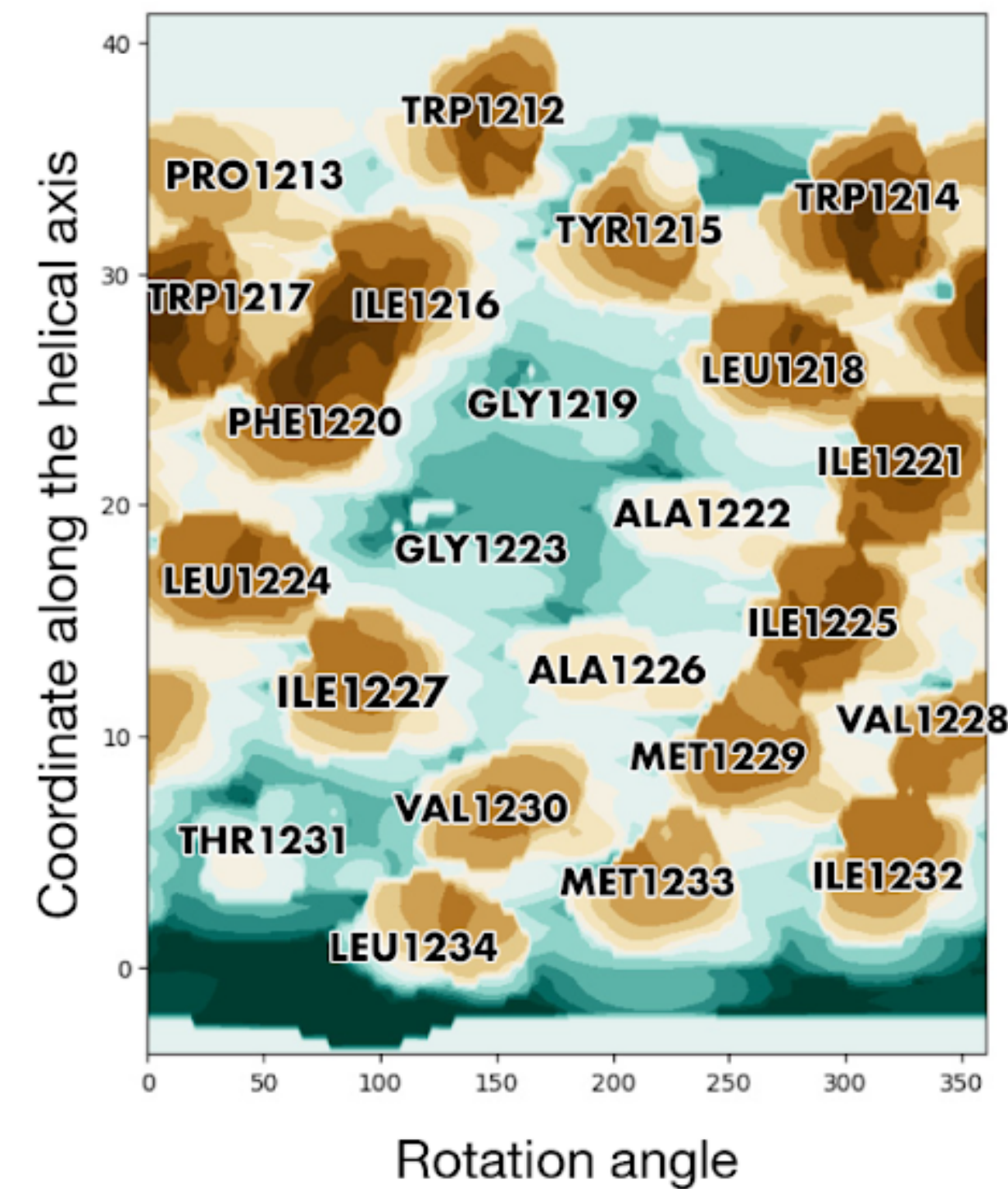
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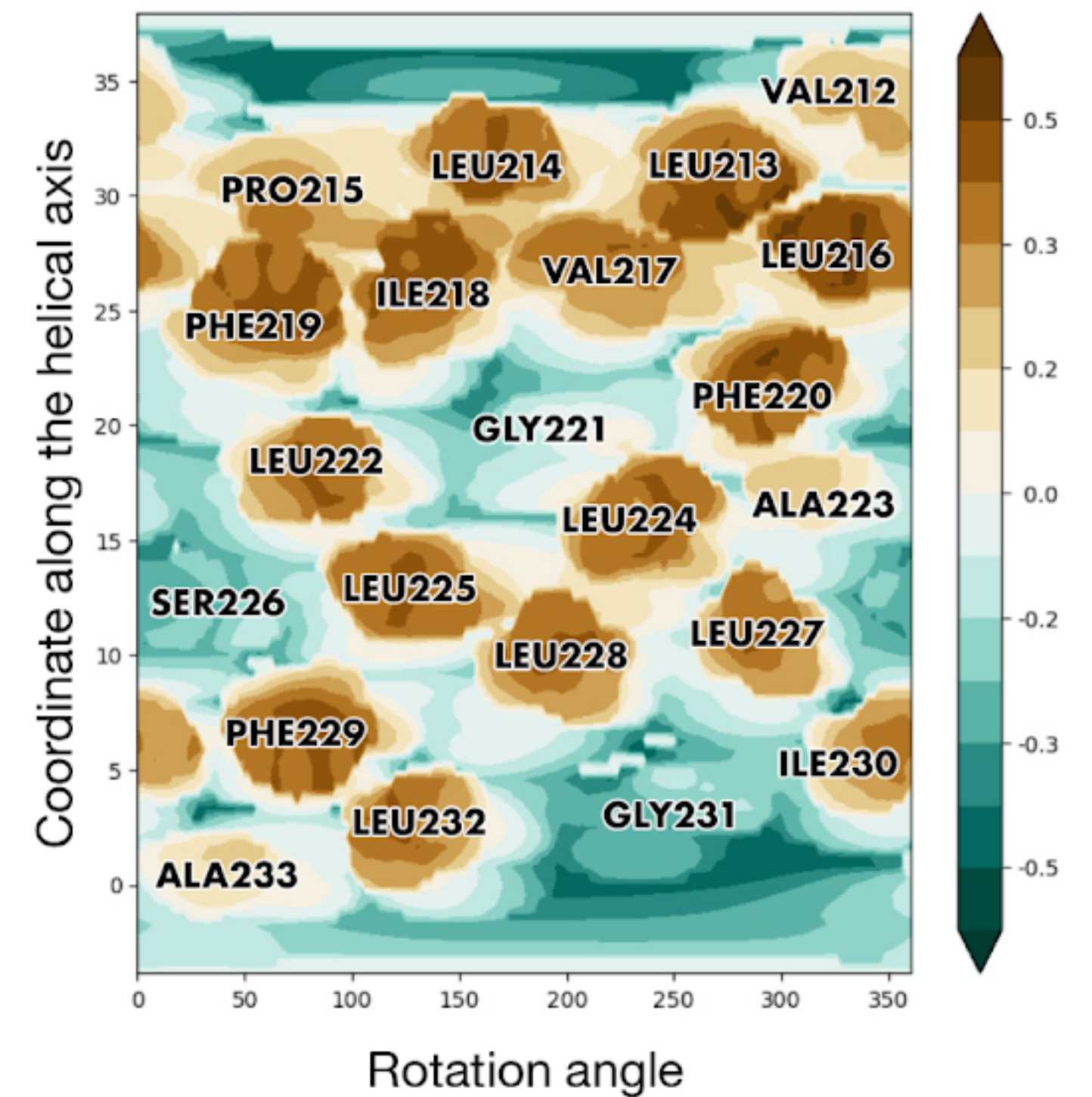
Molecular hydrophobicity potential (MHP) mapping

Residues 1212-1234 were considered

- Sequence patterns (small, Pro, charged, polar and hydrophobic AA) in S-TMD and candidate templates?
- The best match, the TMD of TNFR-1A, paid closer attention: translated into similar MHP patterns on the alpha-helix surface



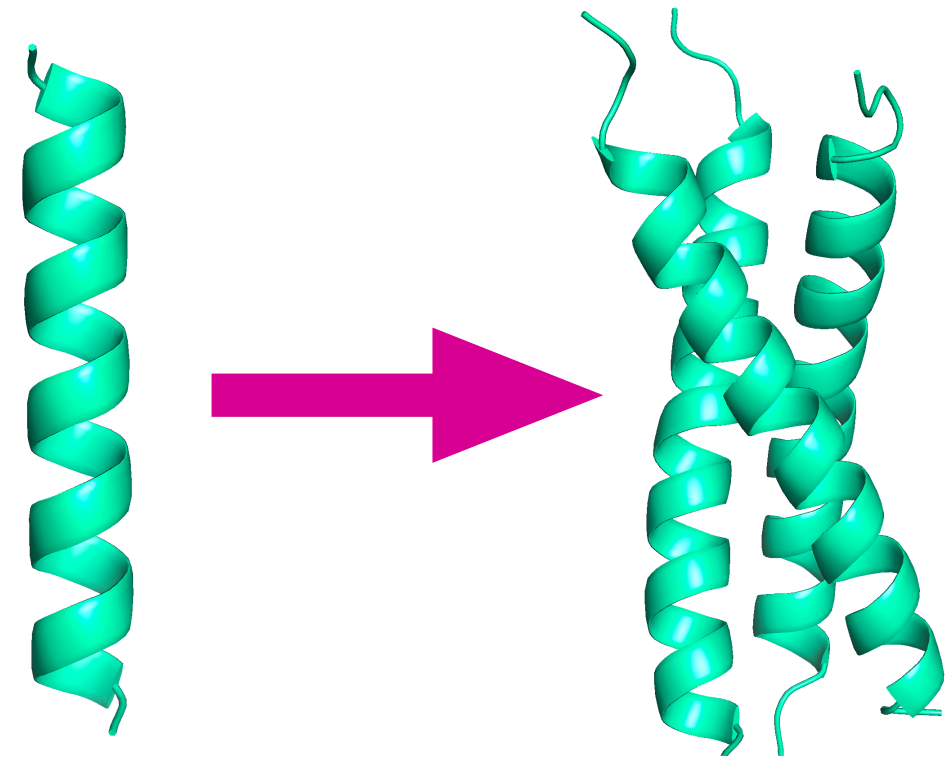
SPIKE



TNFR-1

spike	1212-W	P	W	Y	I	W	L	G	F	I	A	G	L	I	A	I	V	M	V	T	I	M	L	-----	1234
TNFR-1	214-L	P	L	V	I	F	F	G	L	A	L	L	S	L	L	F	I	G	L	A	Y	R	Y	-----	236

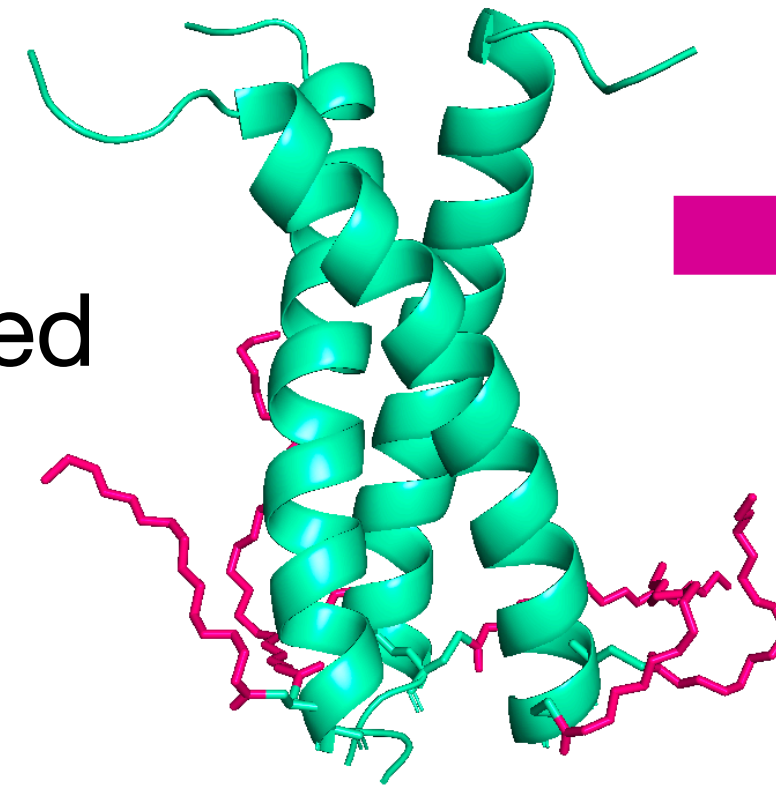
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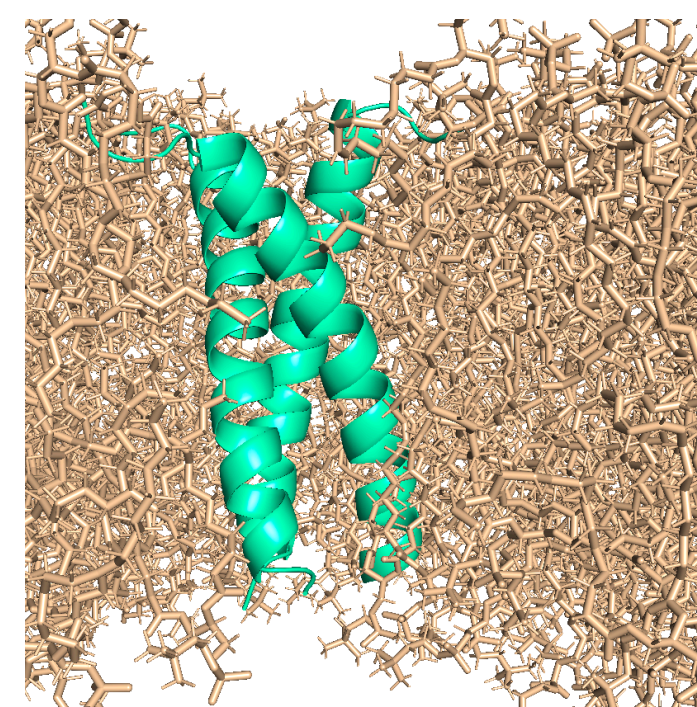


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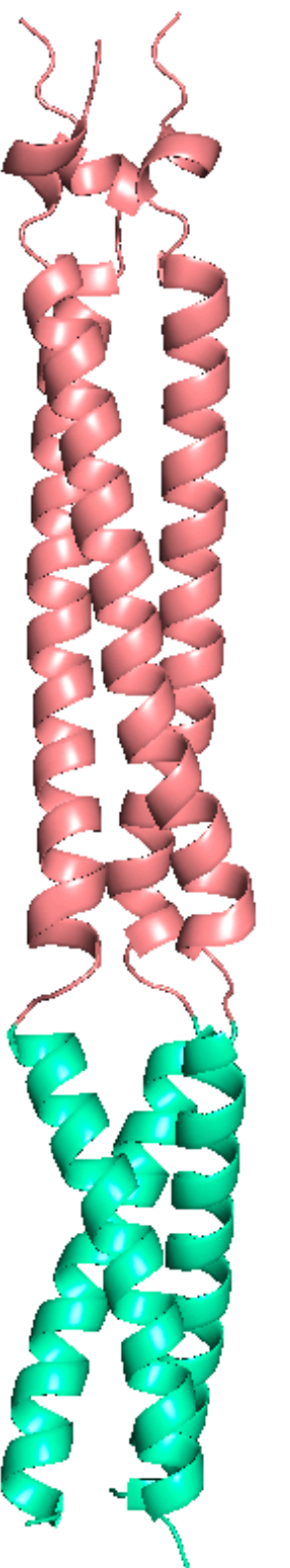
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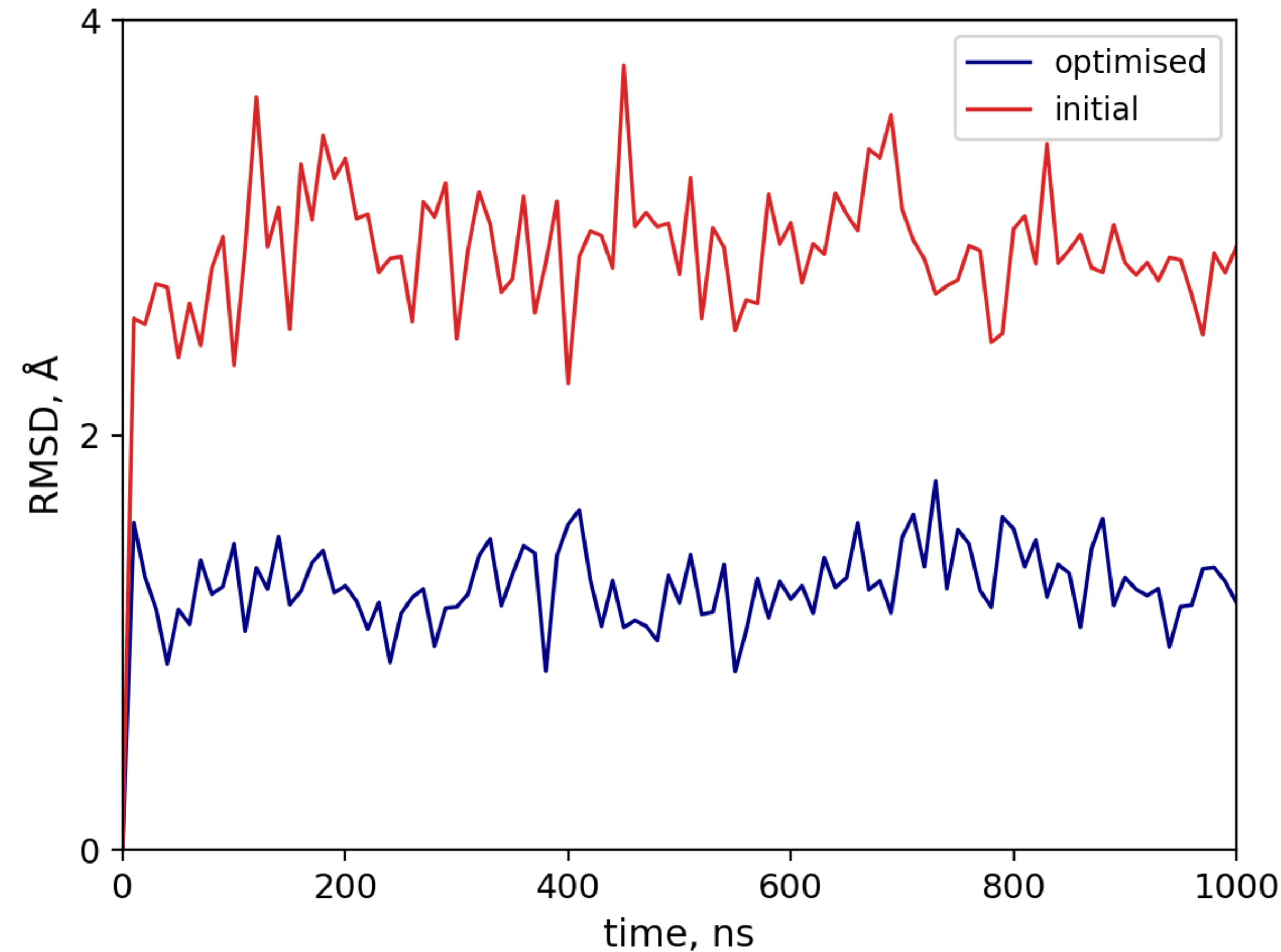
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‘MHP dynamic portrait’ adjustment

Fine-tuning of the trimer derived via template-based modelling

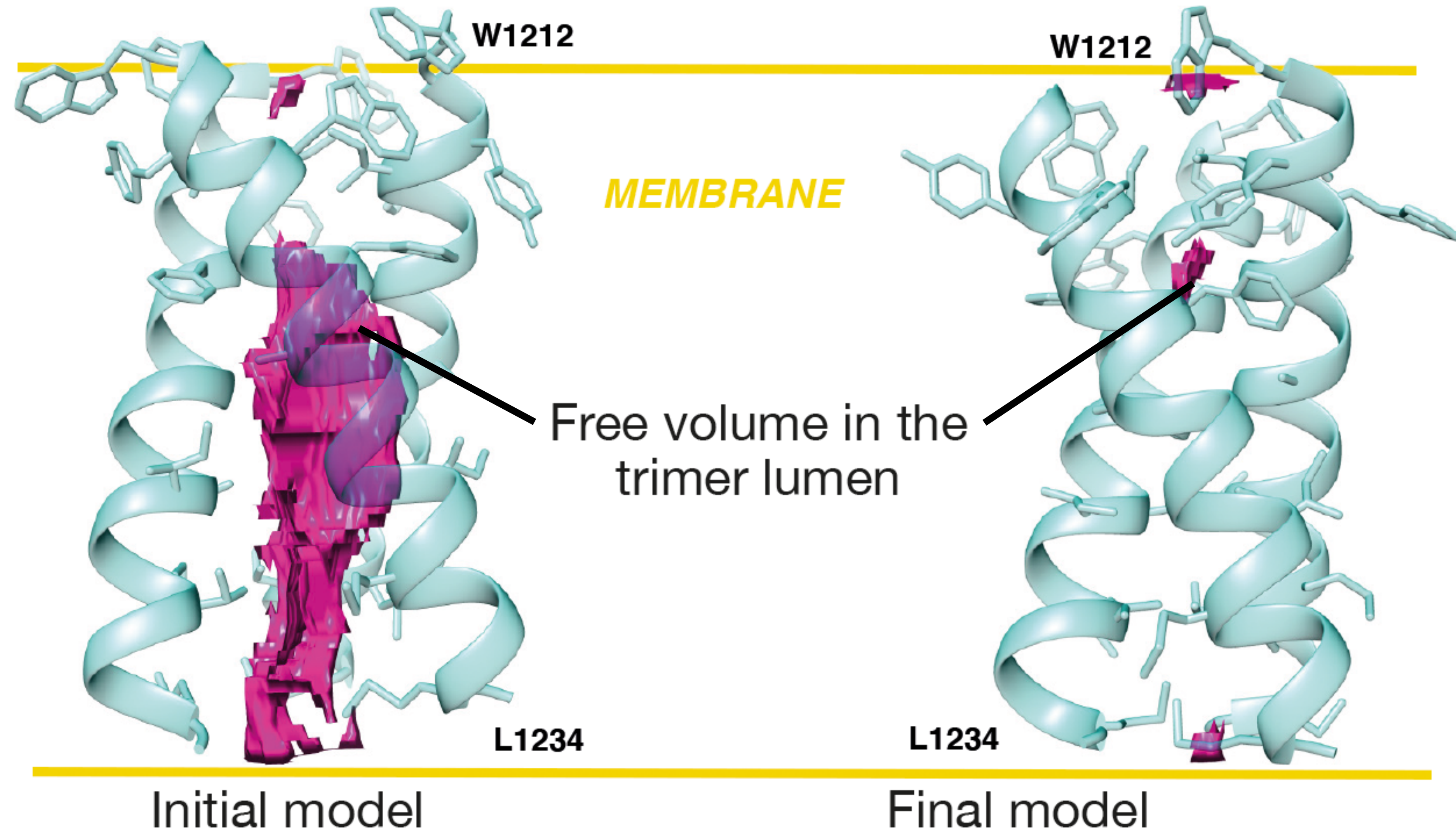
- The trimer derived via template-based modelling: not perfect
- Imperfections eliminated via MD simulations in an explicit POPC bilayer
- The final model much stabler than the initial one



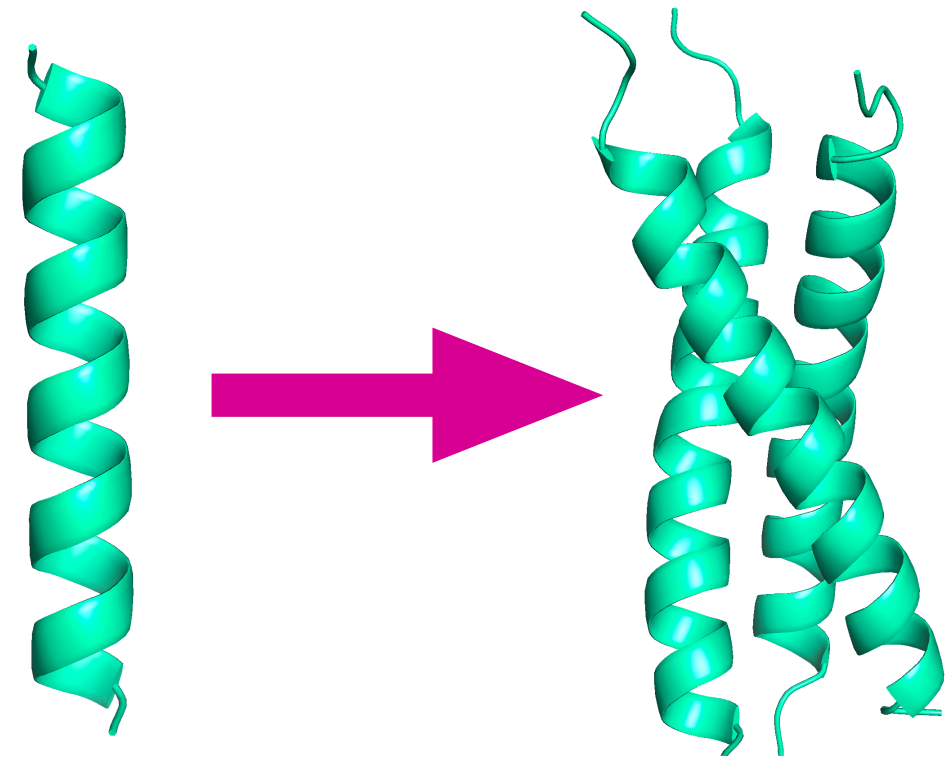
'MHP dynamic portrait' adjustment

Fine-tuning of the trimer derived via template-based modelling

- The resulting model sported a **nearly twofold** increase of contact area between helices in the trimer
- **~25-fold** decrease of **free volume** inside the trimer indicative of tight packing



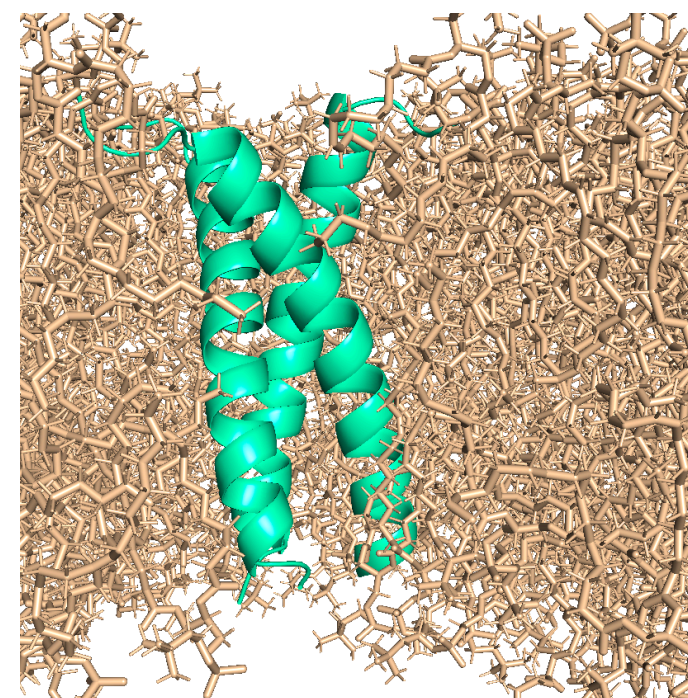
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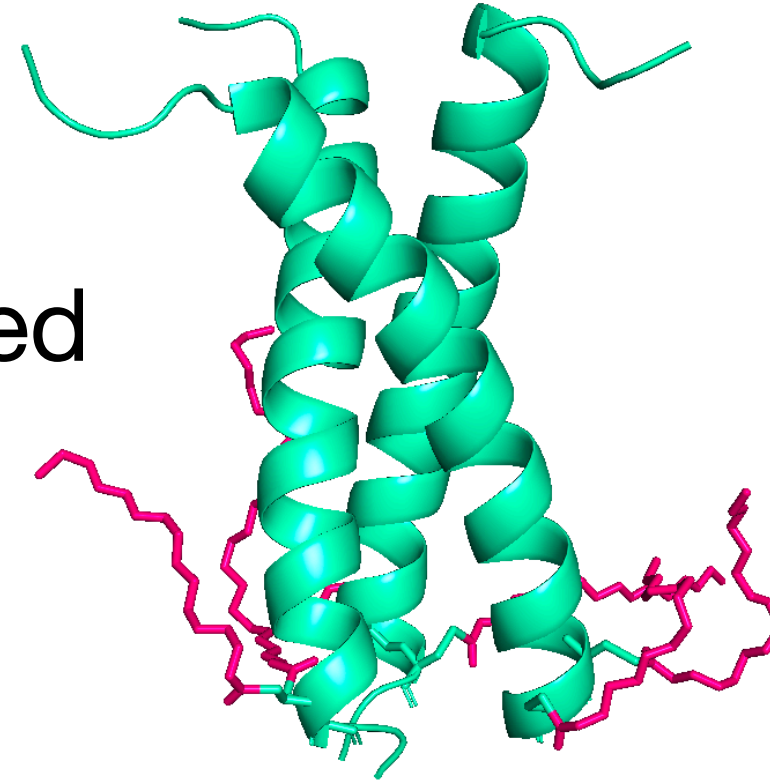
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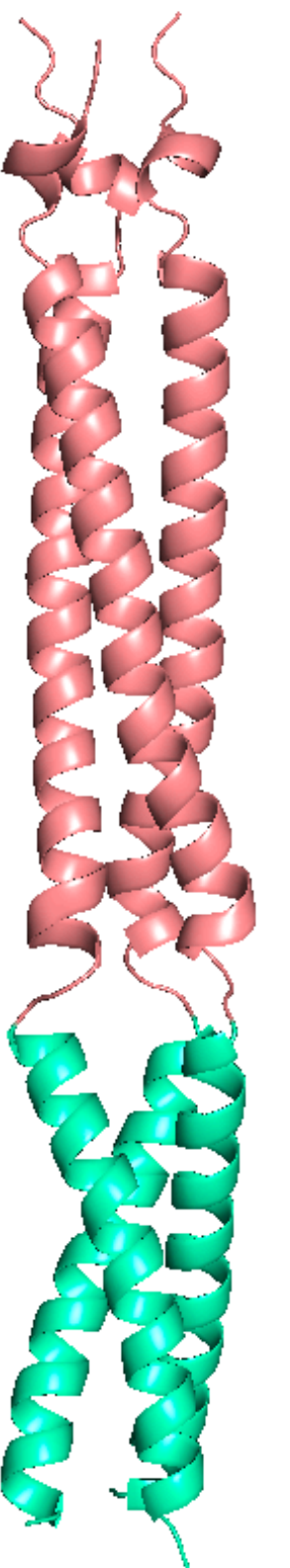
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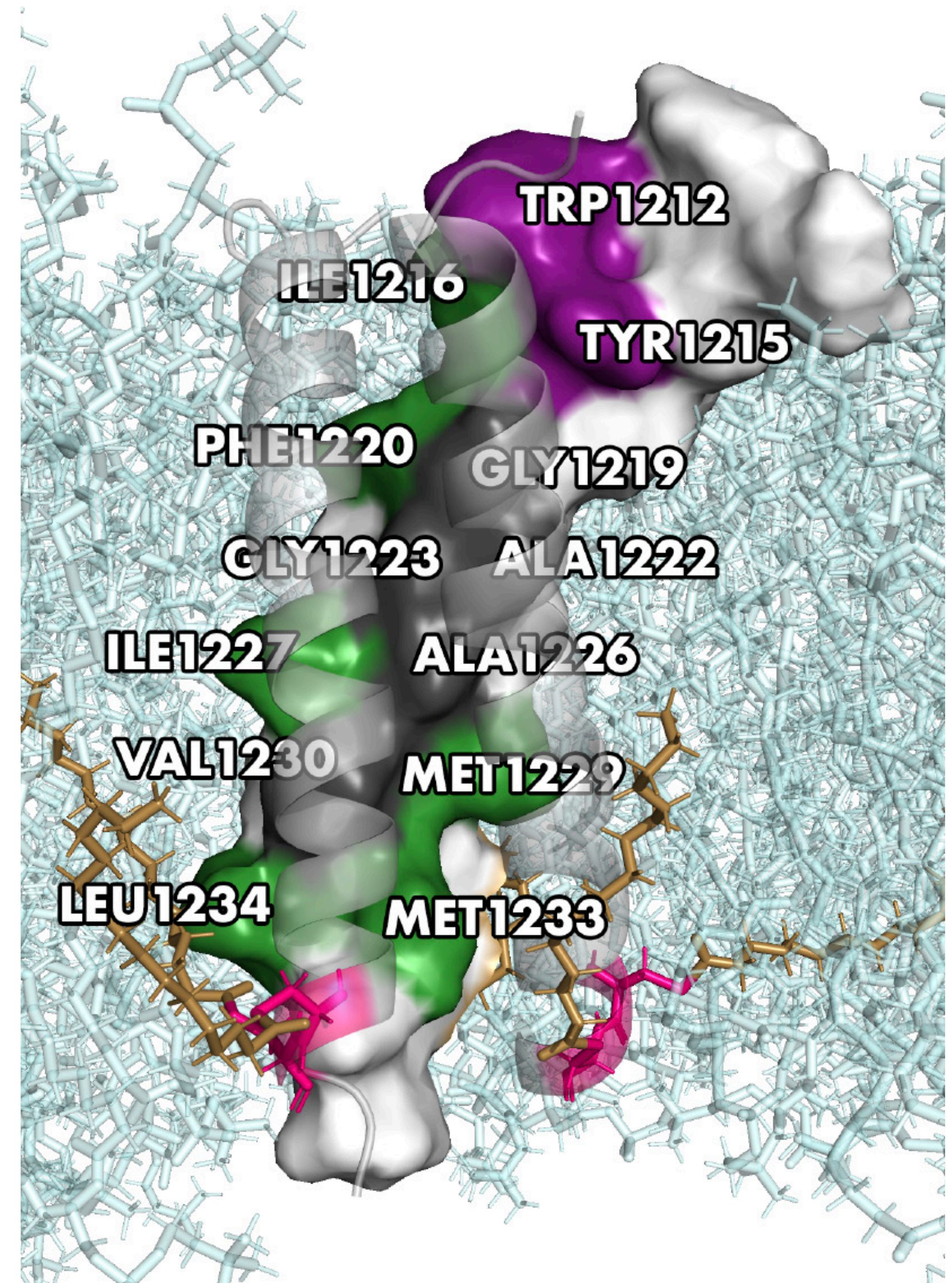
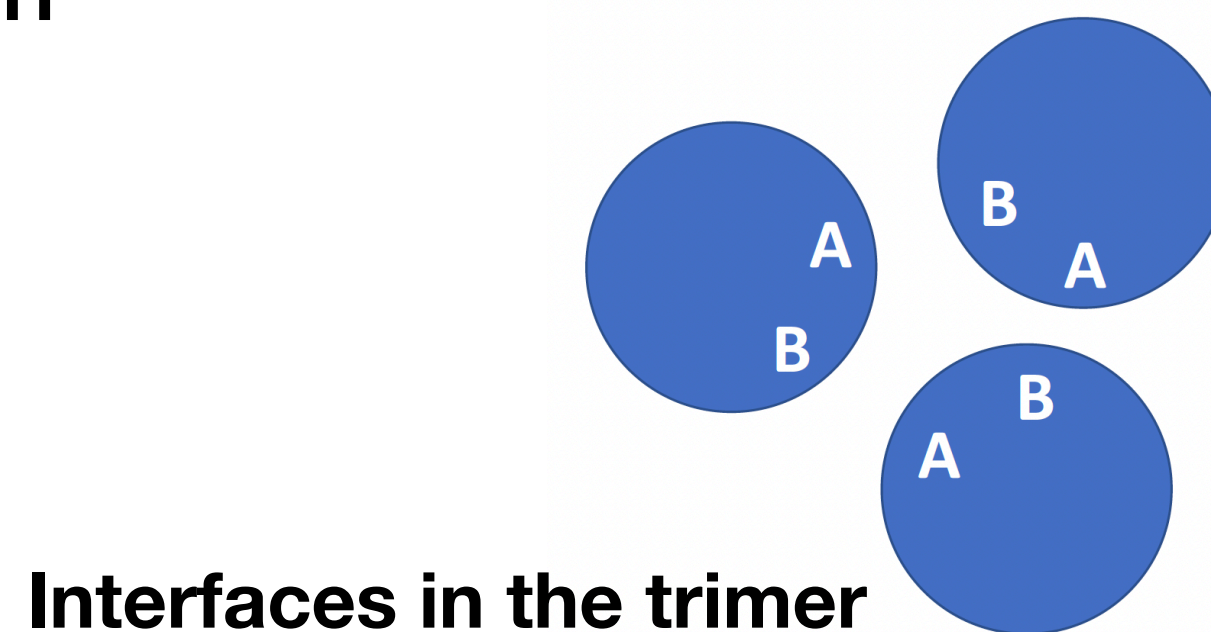
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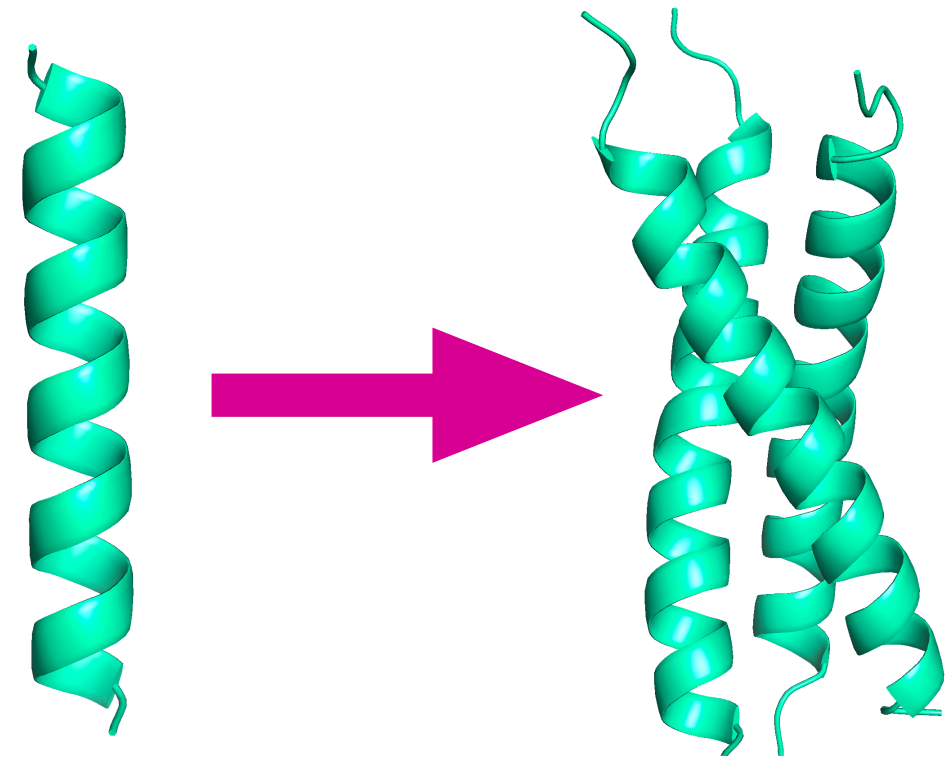
S-TMD model features

Helix/helix interfaces

- Helix/helix interfaces included residues identical (purple) and (semi-) conservative (green) across genus *Betacoronavirus*;
- Palmitoyls (golden) added at C1235 and C1236 (pink), the model retained its stability
- GxxxG motif



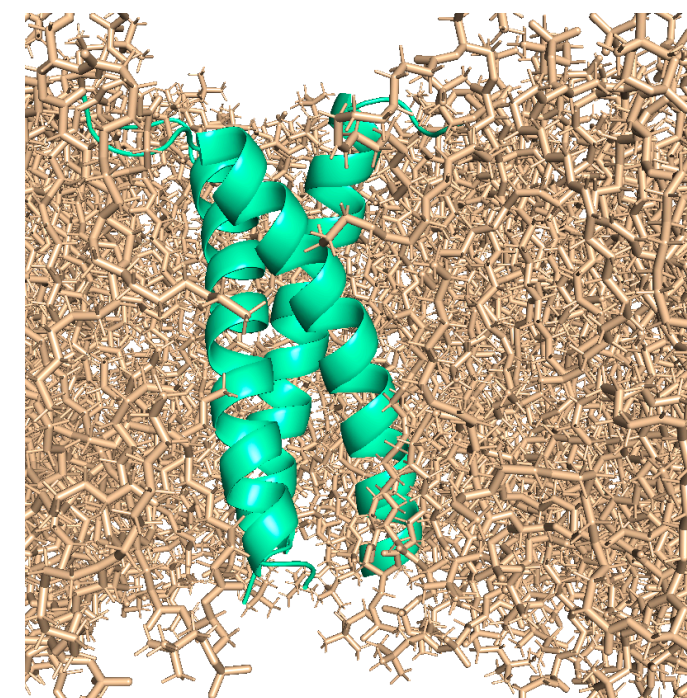
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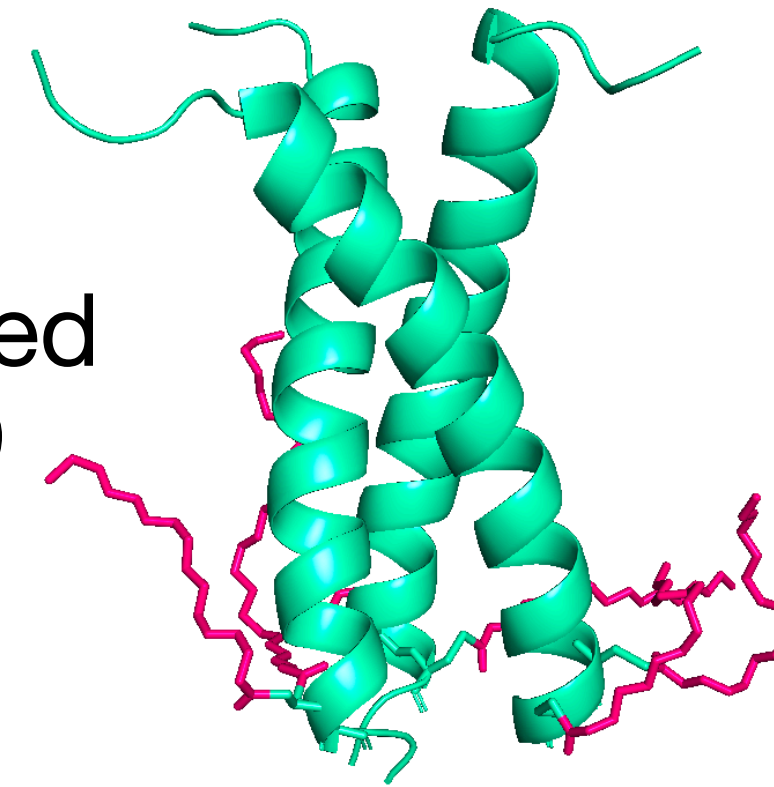
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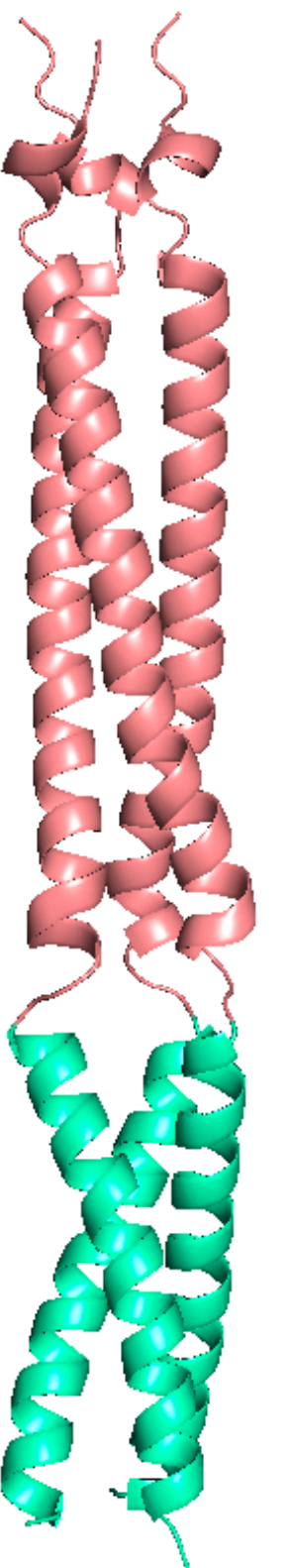
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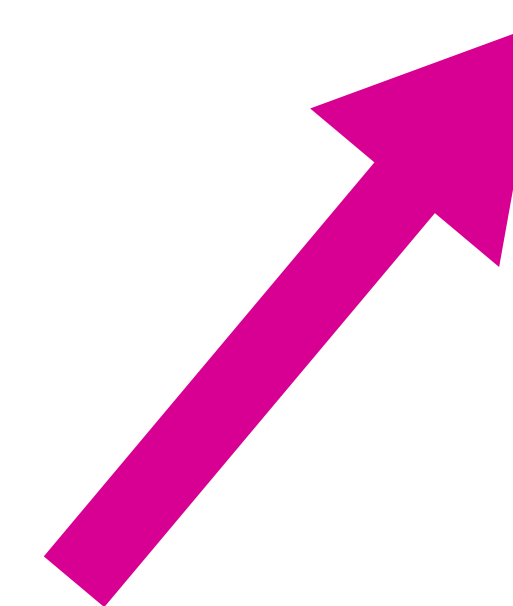
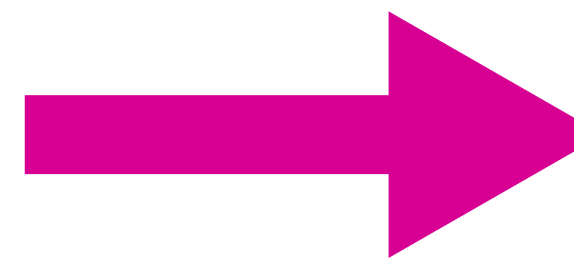
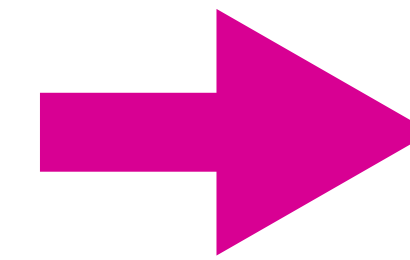
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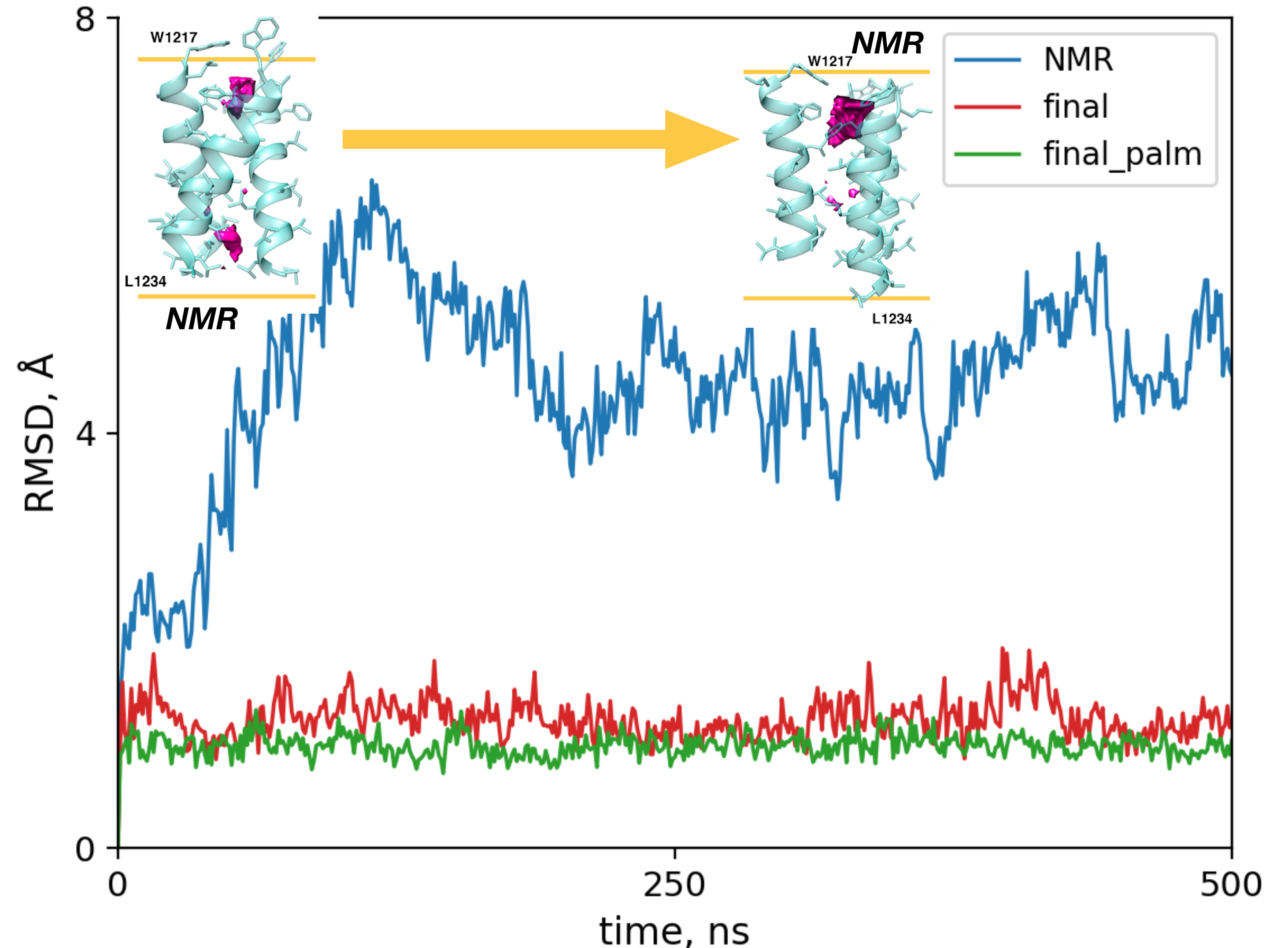
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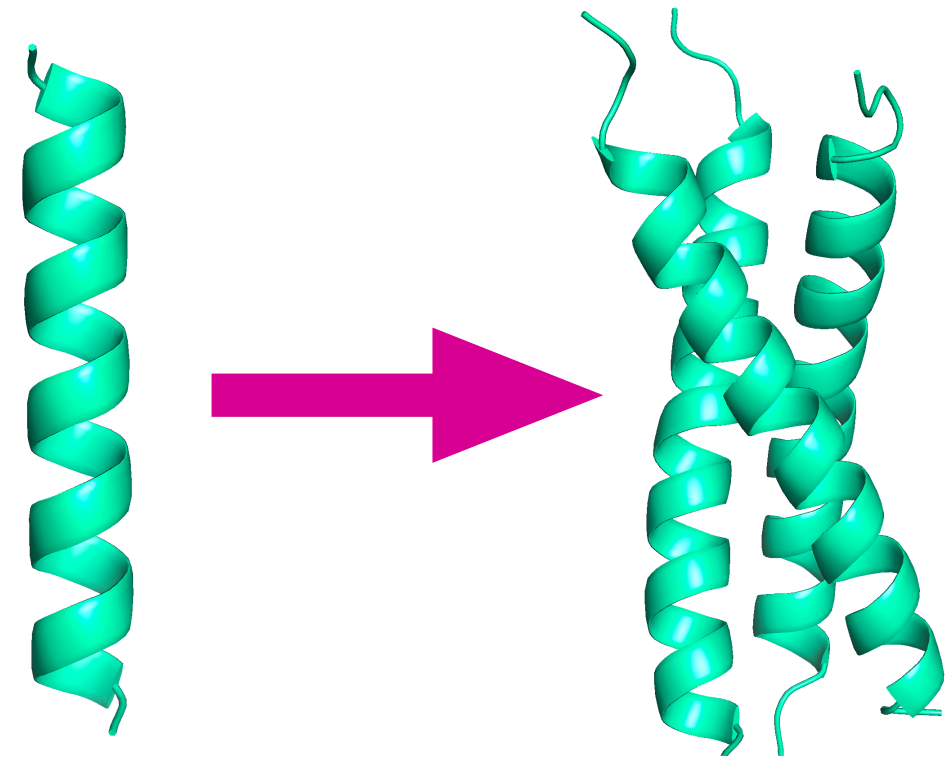
S-TMD model features

Comparison to other structures

- Our final model (red): stable in a POPC bilayer
- Palmitoylation (green) did not affect stability
- Other models and structures were tested
- Recent NMR structure (blue) of the proposed spike TMD performed poorly in a POPC bilayer (PDB ID 7LC8)



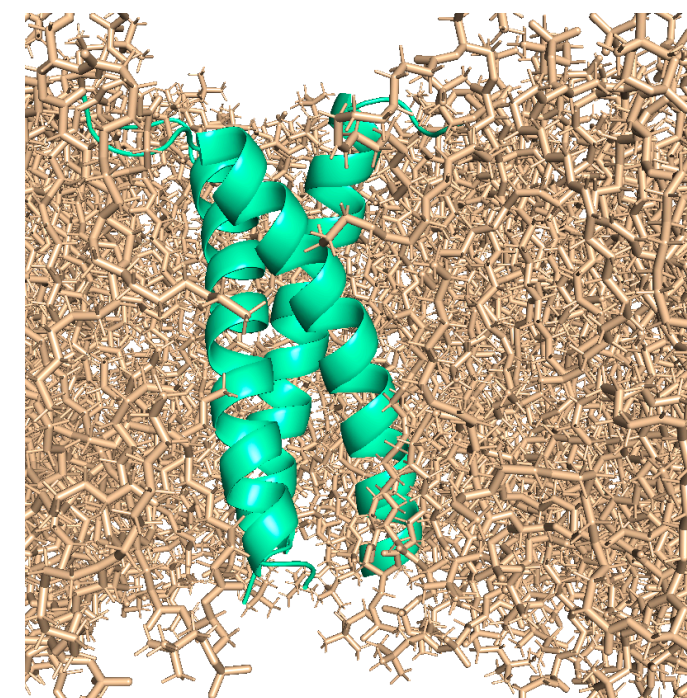
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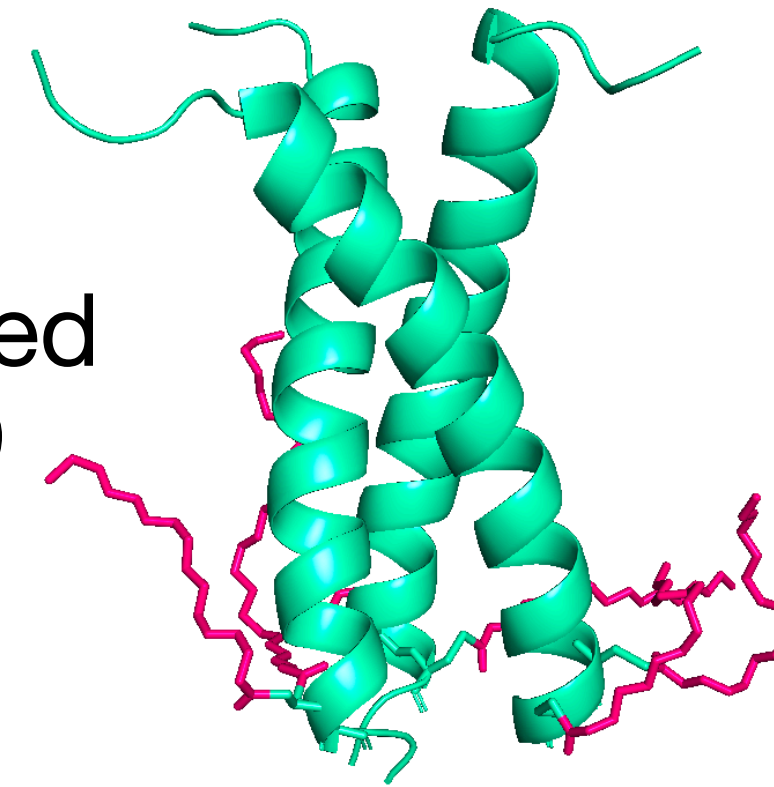
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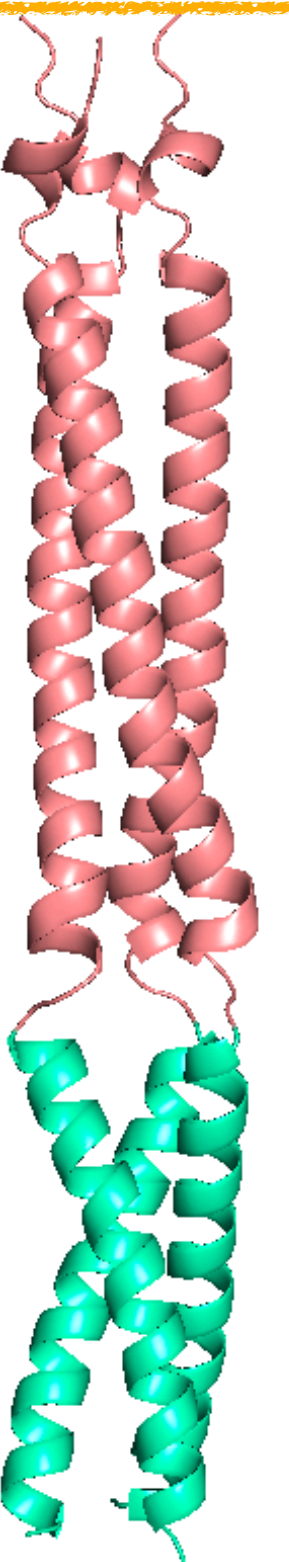
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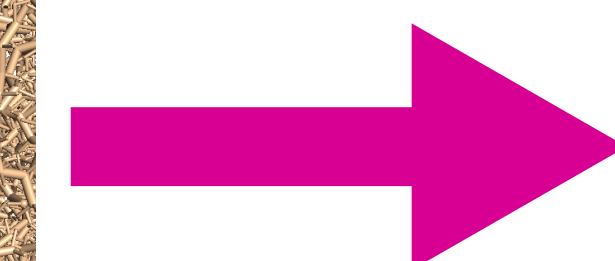
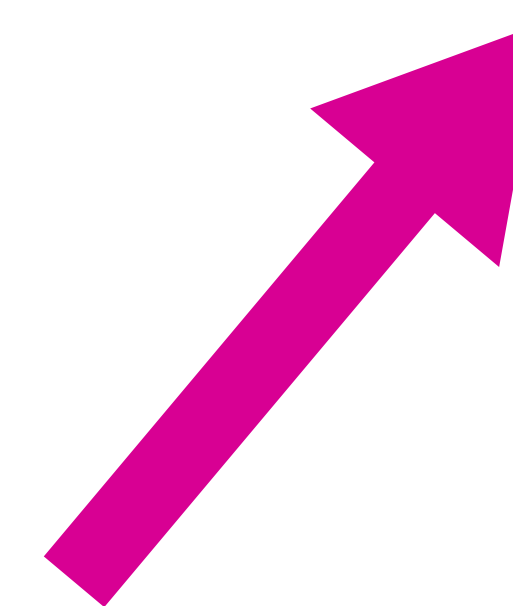
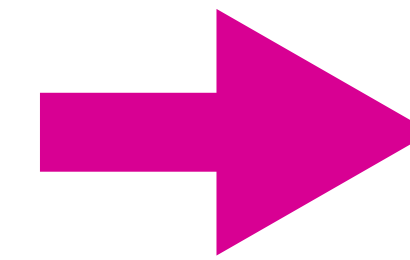
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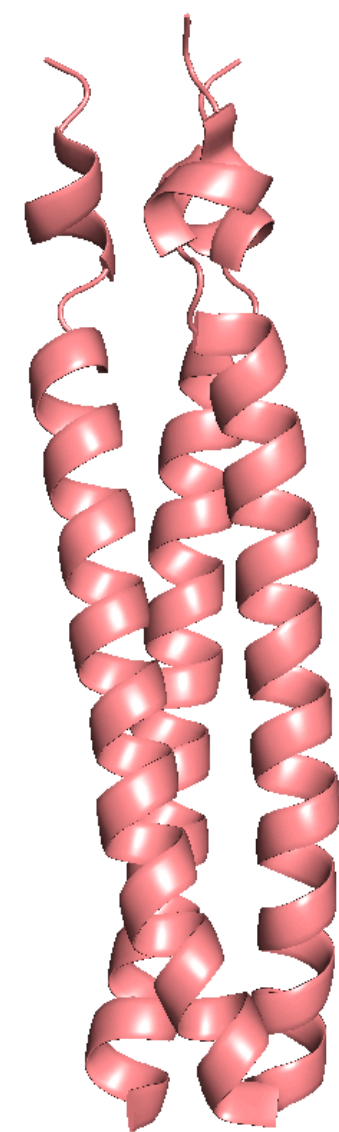
Molecular dynamics simulations



HR2: additional membrane adjuster during fusion?

A hinge area required, as HR2 and TMD don't appear to be part of one coiled coil

The systems studied were:



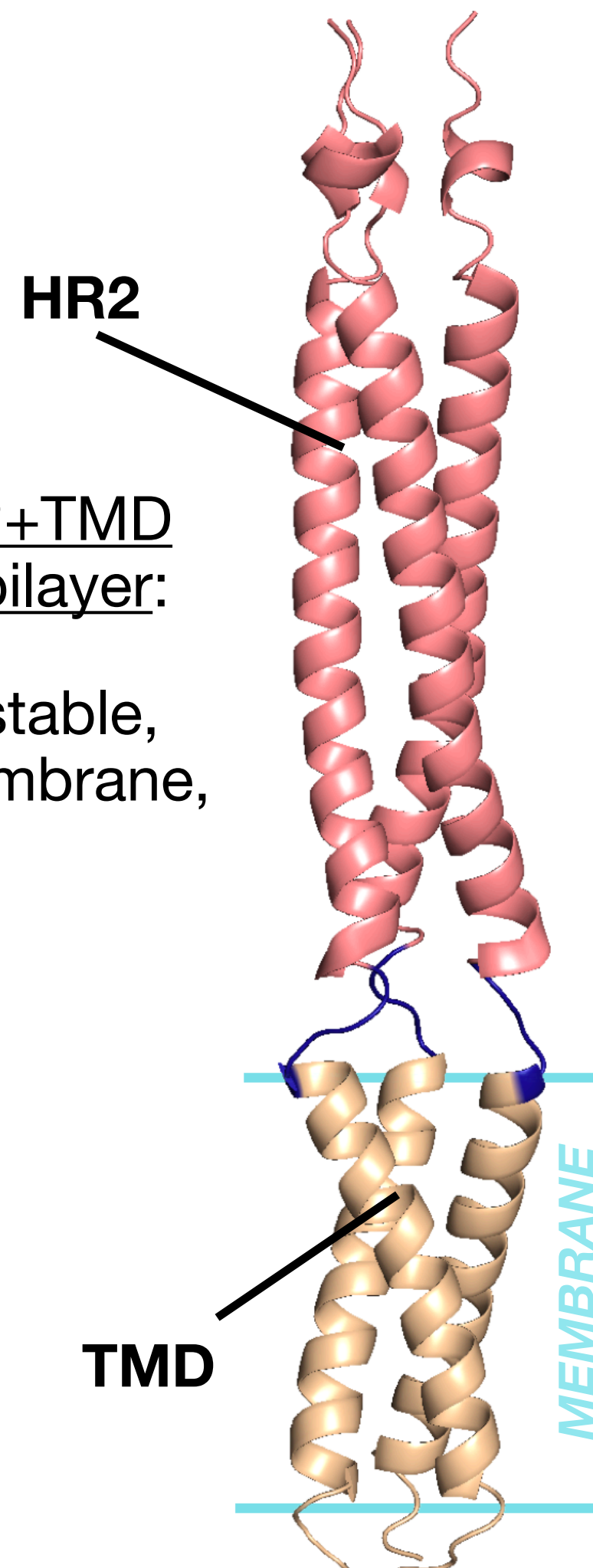
PDB ID 2FXP
(SARS-CoV)

1. Trimeric HR2 in water:

remained stable, helix/
helix interfaces preserved

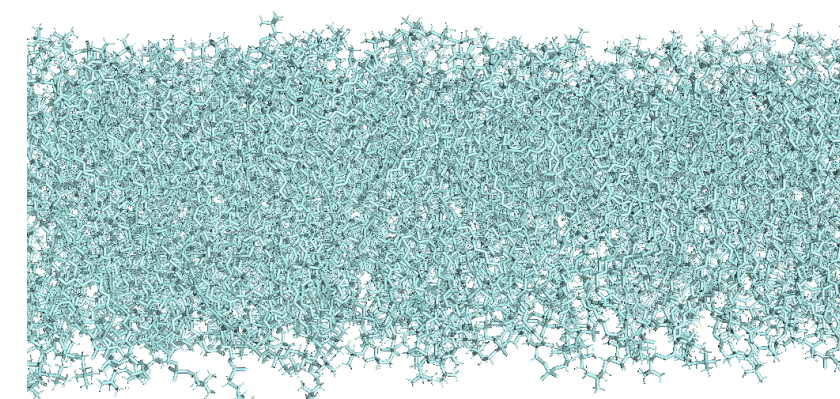
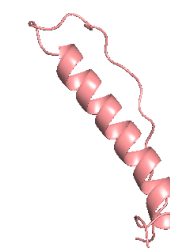
2. Trimeric HR2+TMD
anchored in a bilayer:

both domains stable,
TMD in the membrane,
HR2 in water



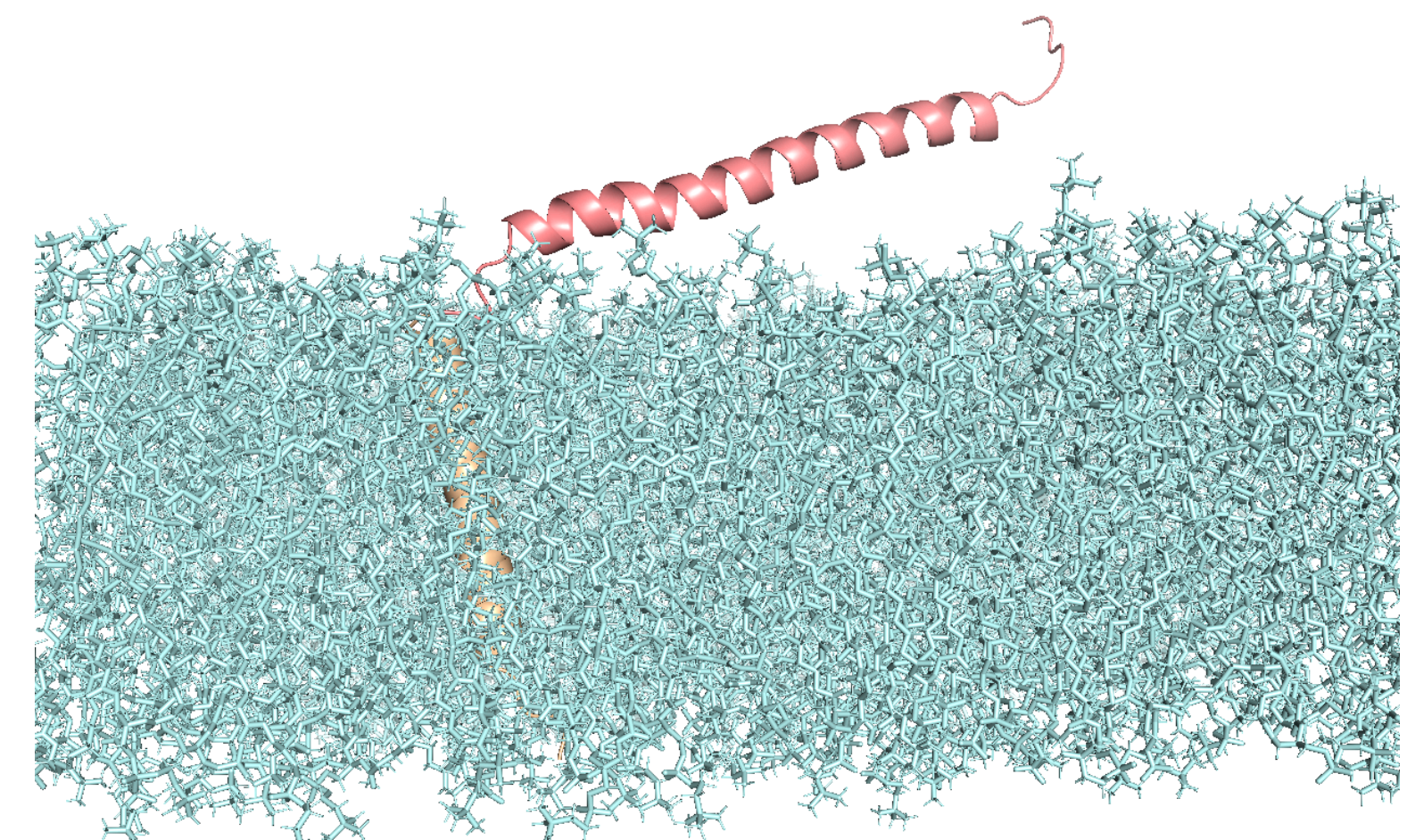
3. Monomeric HR2
placed above the
membrane:

Limited affinity to the
water/bilayer interface



4. Monomeric HR2+TMD anchored in a
bilayer:

HR2 interacted with the membrane, but
failed to firmly align itself on the water/
bilayer interface.



Agrees with experimental data:

Chiliveri SC et al. (2021) *Sci Adv.* 7:eabk2226.

Conclusions

- A highly stable model of SARS-CoV-2 spike protein transmembrane domain was created that conforms to the known principles of TM helix packing
- Diverse computational methods were employed to create a comprehensive strategy for TM trimer structure prediction that could be used to model other objects
- The model remained stable, either palmitoylated in accordance with experimental data or not, over the course of microsecond-range MD
- The model remained stable when extended to include the HR2 region
- The HR2 region was capable of interacting with a model bilayer when anchored via the TMD, but might require additional factors to properly serve as a force involved in membrane fusion