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Comparative Structural Analysis of HMPV Proteins Using Swiss-Modeler and Alpha Fold

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Abstract:

Background and Objectives
Human metapneumovirus (HMPV) is a significant pathogen causing respiratory infections, particularly in vulnerable populations such as infants, the elderly, and immunocompromised individuals. It shares clinical symptoms with respiratory syncytial virus (RSV), including coughing, wheezing, and pneumonia. Discovered in 2001, HMPV is genetically related to RSV and avian pneumovirus, and its fusion (F) and attachment (G) proteins play crucial roles in viral entry. The F protein is involved in both attachment and fusion, which is different from other paramyxoviruses where the attachment protein is typically essential for entry. Despite its clinical relevance, the structural biology of HMPV proteins is not well understood. Advances in computational biology, especially using tools like AlphaFold (for ab initio structure prediction) and Swiss-Modeler (for homology modeling), offer new opportunities to predict the three-dimensional structures of HMPV proteins. This research aims to conduct a comparative structural analysis of HMPV using these methods to gain insights into the virus's molecular mechanisms and potential therapeutic targets. By enhancing our understanding of HMPV's biology, this study may contribute to developing new antiviral strategies to mitigate the virus's impact, particularly in high-risk groups.

Methods
HMPV's conserved RNA sequences were converted into protein sequences using Biopython. The protein sequences were input in FASTA format for structure prediction using AlphaFold2 on Google Colab. The default MSA pipeline was used for multiple sequence alignments (MSA). The selected sequences were analyzed with a BLAST search using the non-redundant protein sequence (nr) database and blastp algorithm. Swiss-Model and AlphaFold were used for 3D structure prediction. For the 5 protein sequences with matches from BLAST, comparisons were made between experimentally determined structures (EM or X-ray) and the predicted models. For the 13 sequences without BLAST matches, comparisons between Swiss-Model and AlphaFold predictions were conducted. ChimeraX was used for structure visualization, and R was employed for data analysis, with all code implemented in RStudio.

Results
While AlphaFold offers superior coverage and confidence, especially for novel insights and structural regions lacking templates, Swiss-Model remains valuable for validating conserved domains. Together, these approaches

provide a comprehensive framework for advancing HMPV research, aiding in pandemic response and vaccine development efforts.

Conclusion

By combining the strengths of both Swiss-Modeler and AlphaFold, this study presents a robust approach to HMPV structural biology, which could lead to new therapeutic strategies, vaccine candidates, and drug discovery efforts. Future integration of dynamic simulations and experimental validation will enhance the predictive power of these models and deepen the understanding of HMPV's molecular mechanisms.

Keywords: Human metapneumovirus, Swiss-Model, avian pneumovirus, AlphaFold

Introduction

The human metapneumovirus (HMPV) is a significant pathogen responsible for respiratory infections, particularly affecting vulnerable populations such as children, the elderly, and immunocompromised individuals. Epidemiological studies indicate that HMPV accounts for 5% to 15% of respiratory diseases among hospitalized infants, with clinical presentations often resembling those of respiratory syncytial virus (RSV) infections [1,11].

Human metapneumovirus (HMPV), discovered in 2001, is a global cause of respiratory tract illness, especially in children under five, where it accounts for 6–12% of acute infections. Symptoms are similar to those of respiratory syncytial virus (RSV), including coughing, wheezing, bronchiolitis, and pneumonia. HMPV has likely circulated in humans since at least 1958. Its cytopathic effects vary by strain, with some causing syncytium formation like RSV. Genetically, HMPV belongs to the Pneumovirinae subfamily within the Paramyxoviridae family and is closely related to both avian pneumovirus and RSV [2,4].

Human metapneumovirus (HMPV), like other enveloped paramyxoviruses, enters host cells by fusing its membrane with the host's, a process typically mediated by two viral glycoproteins: fusion (F) and attachment (G, H, or HN) proteins. Fusion generally occurs at neutral pH, although some exceptions exist. While early studies suggested that SER virus requires low pH for fusion, recent findings show its F protein can mediate fusion at neutral pH. Paramyxovirus glycoproteins also cause cell-to-cell fusion, forming multinucleated syncytia—a feature seen in infections like RSV. Although syncytia are prominent in cultured cells, they may be less common in vivo. Still, studying syncytium formation in lab settings is useful for analyzing viral fusion protein function independently of other viral components [3,5,6,7,8,9].

The HMPV fusion (F) protein shares 33% sequence identity with the RSV F protein and contains features typical of type I viral fusion proteins. However, it is only 10–18% homologous to F proteins from paramyxoviruses outside the Pneumovirinae subfamily. The HMPV attachment (G) protein lacks significant sequence similarity with other paramyxovirus attachment proteins but shares structural features—like high serine, threonine, and proline content—with the RSV G protein. Unlike in other paramyxoviruses where the attachment protein is essential for entry, in HMPV and related viruses, the F protein alone can mediate both attachment and membrane fusion, indicating a different functional role for the G protein within the Pneumovirinae subfamily [8,9].

Despite its clinical importance, the structural biology of HMPV proteins remains inadequately characterized, limiting our understanding of their functions and interactions within host cells. Recent advancements in computational biology, particularly in protein structure prediction methods like AlphaFold (ab initio approach) and Swiss-Modeler (comparative homology modeling), provide unprecedented opportunities to elucidate the three-dimensional structures of these viral proteins.

The ab initio approach in protein structure prediction refers to methods that aim to predict the three-dimensional structure of a protein solely from its amino acid sequence, without relying on homologous templates. This method is particularly valuable for proteins that lack known structural relatives in databases. Ab initio modeling typically involves a conformational search guided by energy functions that evaluate the stability of various structural conformations. The success of this approach hinges on accurately modeling the energy landscape of the protein, allowing for the identification of the lowest energy conformation, which is presumed to correspond to the native structure. Despite its potential, ab initio methods face challenges such as the complexity of accurately defining scoring functions and efficiently sampling conformational space, which can limit their effectiveness compared to template-based methods [10].

In contrast, homology modeling (or comparative modeling) leverages known structures of homologous proteins to predict the structure of a target protein with an unknown structure. This approach assumes that evolutionarily related proteins will share similar structures due to conserved folding patterns [11]. Homology modeling typically involves aligning the target sequence with one or more templates, followed by building a model based on these alignments. The accuracy of homology models is highly dependent on the quality and closeness of the templates used; thus, it is most effective when high-quality templates are available. While homology modeling can yield reliable structural predictions when suitable templates exist, it

is limited in cases where no close homologs are available, necessitating the use of ab initio methods for accurate predictions in such scenarios[12].

This research paper aims to conduct a comparative structural analysis of HMPV proteins using both AlphaFold and Swiss-Modeler. The integration of these advanced modeling techniques allows for a comprehensive examination of protein structures, even in cases where experimental data is lacking. By leveraging the strengths of AlphaFold's deep learning capabilities and Swiss-Modeler's template-based approaches, we can create reliable structural models that not only enhance our understanding of HMPV biology but also facilitate the identification of potential therapeutic targets.

The significance of this study lies in its potential to bridge the gap between sequence data and functional insights into HMPV proteins. As the field of structural biology evolves with computational tools, this research will contribute to a deeper understanding of HMPV's molecular mechanisms and its interactions with host factors. Given that HMPV infections have been associated with severe respiratory illnesses requiring hospitalization—especially in high-risk groups—the findings from this study could pave the way for novel antiviral strategies aimed at mitigating the impact of this pathogen on public health[13].

Methods

Protein Sequence Retrieval:
hMPV conserved RNA sequences were converted to protein sequences using Biopython.
To predict the three-dimensional structure of proteins, the **AlphaFold2_advanced_v2** notebook on Google Colab was utilized [14]. The target protein sequences were input in FASTA format, and the following parameters were configured as default: **max_recycles (3)**, defining the number of structure refinement cycles; **tol (0.1)**, indicating the tolerance for convergence during optimization; **is_training (false)**, specifying the use of inference mode rather than training; **model_preset ("monomer")**, suitable for single-chain predictions; and **num_relax (3)**, indicating the number of relaxation steps applied to the predicted models. Additionally, **use_amber (true)** enabled the application of the AMBER force field for energy minimization. The default MSA generation pipeline was used without additional customization. This setup facilitated the generation of high-quality predicted 3D structures of proteins for further scientific analysis. Among the many sequence generated from the conserved sequences only 18 sequences were selected from 563 upto the length of 30 amino acids (AA), represented in Table 1.

Protein BLAST Analysis:
Selected sequences were subjected to a BLAST search, using the non-redundant protein sequence (nr) database. Algorithm blastp was utilized keeping rest of the settings default. Four proteins viz. Fusion protein (F), Nucleoprotein (N), Small hydrophobic protein (SH), and matrix protein 2-2 (M2-2) were identified. Fourteen proteins sequences have not found with any corresponding entries or results.

Structural Prediction:
Swiss-Model (swissmodel.expasy.org) and AlphaFold (colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb) were used to predict the 3D structures of all 18 sequences. For Swiss-Model structure prediction templates were selected based on GMQE and identity scores. Only EM and X-ray determined templates were selected for structure prediction. Only monomer structures were predicted using Swiss-Model, multiple monomer models were predicted based on the corresponding sequence search and best model was selected based on QMEANDisCo Global score. For AlphaFold model prediction, AlphaFold2 (with MMseqs2) in google colab (ColabFold 1.5.5) platform was used with default settings. From all the predictions of AlphaFold for a corresponding sequence, best models were selected based on the Predicted Local Distance Difference Test (pLDDT) and Predicted Aligned Error (PAE) matrix results.

Comparative Analysis and Data Analysis:
For the 5 protein sequences with matches found in BLAST analysis, comparisons were made between the experimentally determined structures (EM or X-ray) and the predicted models (Swiss Model and AlphaFold). For the 13 protein sequences with no matches found in BLAST analysis, comparisons were conducted between the Swiss-Modeler and AlphaFold predictions. Structure visualization and analysis was conducted on chimeraX and rest of all the exploratory data analysis was conducted in R and codes were implemented in Rstudio.

Results

Selected protein sequences:

Table 1: Summarized table with selected protein sequences along with name and length.

Sample	Name	Protein Sequence	Length
P1	Fusion protein	SHHSSYIKIKLEQELNQSRTGQIKMSWKVVIIFSLLITPQHGLKES YLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLI KTELDLTKSALRELRTVSADQLAREEQIENPRQSRFVLGAIALGV ATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVL ATAVRELKDFVSKNLTRAINKNKCDIADLKMAVSFSQFNRRFLN VVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLE NRAMVRRKGFGFLIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPS CSGKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDH VFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSP LGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDN TVYQLSKVEGEQHVIKGRPVSSSFDPVKFPEDQFNVALDQVFE SIENSQALVDQSNRILSSAEKGNTGFIIVIILIAVLGSTMILVSVFIIIK KTKKPTGAPPELSGVTNNGFIPHN	563
P2	Nucleoprotein	IRFQKNMGQVKMSLQGIHLSDLSYKHAILKESQYTIKRDVGTTTA VTPSSLQQEITLLCGEILYAKHADYKYAAEIGIQYISTALGSERVQ QILRNSGSEVQVVLTRTYSLGKIKNNKGEDLQMLDIHGVEKSWV EEIDKEARKTMATLLKESSGNIPQNQRPSAPDTPIILLCVGALIFT KLASTIEVGLETTVRRANRVLSDALKRYPRMDIPKIARSFYDLFE QKVYHRSLFIEYGKALGSSSTGSKAESLFVNIFMQAYGAGQTML RWGVIARSSNNIMLGHVSVQAELKQVTEVYDLVREMGPESGLL HLRQSPKAGLLSLANCPNFASVVLGNASGLGIIGMYRGRVPNTE LFSAAESYAKSLKESNKINFSSLGLTDEEKEAAEHFLNVSDDSQ NDYE	405
P3	Attachment glycoprotein	KVKNNNMGQVVMEVKVENIRTIDMLKARVKNRVARSKCFKNAS LVLIGITTLSIALNIYLIINYKMQKNTSESEHHTSSSPMESSRETPT VPTDNSDTNSSPQHPTQQSTEGSTLYFAASASSPETEPTSTPD TTNRPPFVDTHTTPPSASRTKTSPAVHTKNNPRTSSRTHSPPR ATTRTARRTTTLRTSSTRKRPSTASVQPDISATTHKNEEASPAS PQTSASTTRIQRKSVEANTSTTYNQTS	247
P4	Short hydrophobic protein	MTMITLDVIKSDGSSKTCTHLKKIIKDHSGKVLIVLKLILALLTFLTV TITINYIKVENNLQICQSKTESDKKDSSSNTTSVTTKTTLNHDITQ YFKSLIQRYTNSAINSDTCWKINRNQCTNITTYKFLCFKSEDTKT NNCDKLTDLCRNKPKPAVGVYHIVECHCIYTVKWKCYHYPTDE TQS	185
P5	Matrix protein 2-2	LTYQQVLKMTLHMPCKTVKALIKCSEHGPVFITIEVDDMIWTHKD LKEALSDGIVKSHTNIYNCYLENIEIIYVKAYLS	79
P6	Not found	QKIQNNSKINHADTNNGEAKRQFTISPKRQQHHISSAQISLEKTL AHIPKIPQPPQEKNWAKQHPRDK	68
P7	Not found	SNRKGPVTCKPNNMVPFVSGQHTTSSAARSAKNPDNNHSVCC ITKWSNTQSECISPRCSNVCTSQKI	67
P8	Not found	SRPSSNTGQNCTLCGINYDHDYEQSQRHIQKAWSWDSSHSRT RSICPG	48
P9	Not found	KIIFSRKTGVTGSYRIMLLWTSKYSYVSLKLNFYVVGGPKGKTLK MKI	48
P10	Not found	QKKQKSSTKLLKMKAQVENPKKKKNQKTHKTIVKKMTFTS	40
P11	Not found	KHKQNMQDLEPSRDKICLEVQITTKHLGQELLTLSHRS	38
P12	Not found	ISYTGYLSVVDLWNSQHQFQLIEQQITILTLVLLIKH	37
P13	Not found	CLARLRANMKCGANAIEEVSASLTTITGVGQIDTY	35
P14	Not found	SKQSKSWIPLKKKSLPRRRCYPPVMGKPLQKRN	33
P15	Not found	LCSLIKINNGTSKNGVLPSRHLSRHSLHSSCSS	33
P16	Not found	TLKFCTLLEKGQEIGWPEQHVNILTSNLYTEV	32
P17	Not found	FYGGNMYYHAEFALLMGQTSIYSQSIMLKTAM	32
P18	Not found	SKNSLLNNHETIRDGIKICELSQISWQKNT	30

Ramachandran Plot analysis of Fusion Protein:

For this purpose of getting the Ramachandran Plot of Fusion Protein found in Human Metapneumovirus having 563 amino acids given in Table No. 1 is given below. That structure and further clarification is obtained from "SWISSMODEL.EXPASY" software (https://swissmodel.expasy.org/assess/CAXSpp/01).

General Assessment:

Ramachandran favored region: 92.71% of residues fall into the favored region, indicating a largely stable protein structure.
Ramachandran outliers: 1.73% residues (e.g., B115 ARG, A410 LYS, C128 VAL) are outliers. This suggests potential structural or modelling errors.
Additional Observations:
Mol Probity Score: 1.55 — indicates a good-quality model.
Clash Score: 3.00, involving specific residues like A112 GLN and A117 GLU.
Rotamer Outliers: 0.68% residues such as A280 PHE and C126 ARG exhibit unusual side-chain conformations.
C-Beta Deviations: 13 deviations were observed, which may point to issues like backbone strain or geometry deviations.

QMEAN Analysis:

QMEANDisCo Global Score: 0.75 ± 0.05, suggesting the structure is moderately reliable compared to a non-redundant set of PDB structures.
Local quality estimates indicate varying levels of agreement across the structure.
Potential Research Avenues
Investigate the causes of the Ramachandran outliers and C-beta deviations. These could result from errors during structure prediction or crystallographic refinement.
Focus on improving the refinement of residues flagged in outlier regions (like B115 ARG and C128 VAL).2-acetamido-2-deoxy-beta-D-glucopyranose, also known as N-acetylglucosamine, is a small molecule drug and aminoglycan. It has the molecular formula {C_8H_{15}NO_6}. [15]

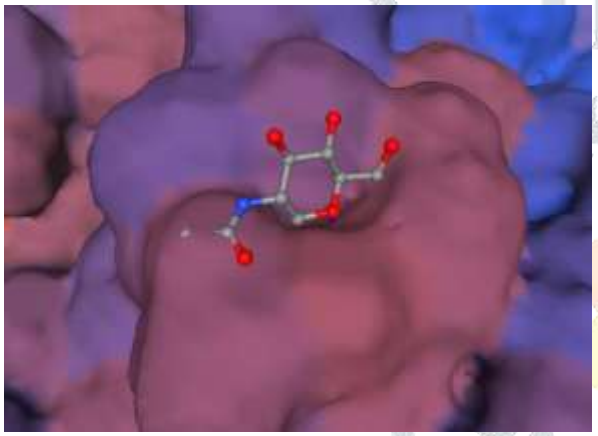

Fig: Hydrogen bonds: C:N.377

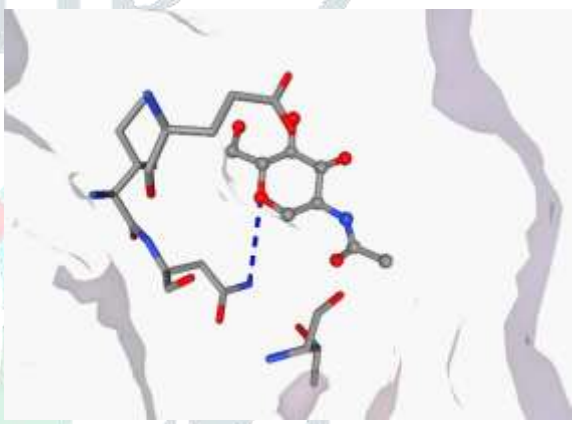

Fig: Binding Sites. Chain C: E.373, I.376, N.377,T.380

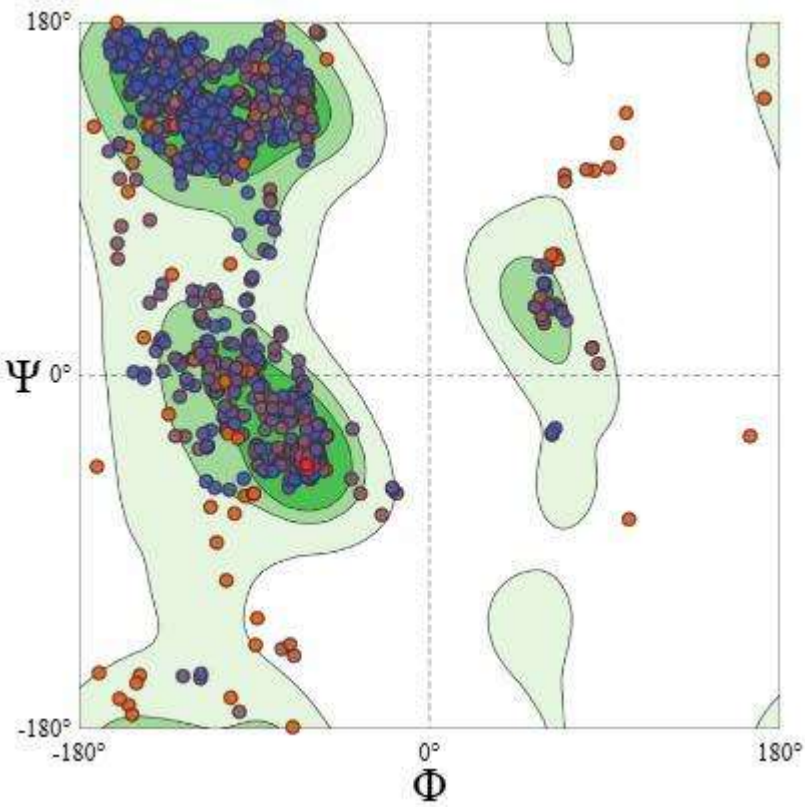

Fig: Ramachandran Plot diagram of Fusion Protein Having 563 amino acid using SWISSMODEL.

That has been used in biotransformations to generate a selectively deprotected substrate for SN2 inversion [16]. There are several related compounds to that. However, there are two most prominent compounds. These are as follows:

- 2-acetamido-2-deoxy-beta-D-glucopyranosyl azide, which is suitable for click chemistry reactions [17]
- 2-acetamido-2-deoxy-3-O-beta-D-glucopyranuronosyl-beta-D-glucopyranose, which is an amino disaccharide and glucosamine oligosaccharide [18]

Comparison of PDB-available Proteins:

Structural alignments showed varying degrees of similarity between PDB structures and predicted models.

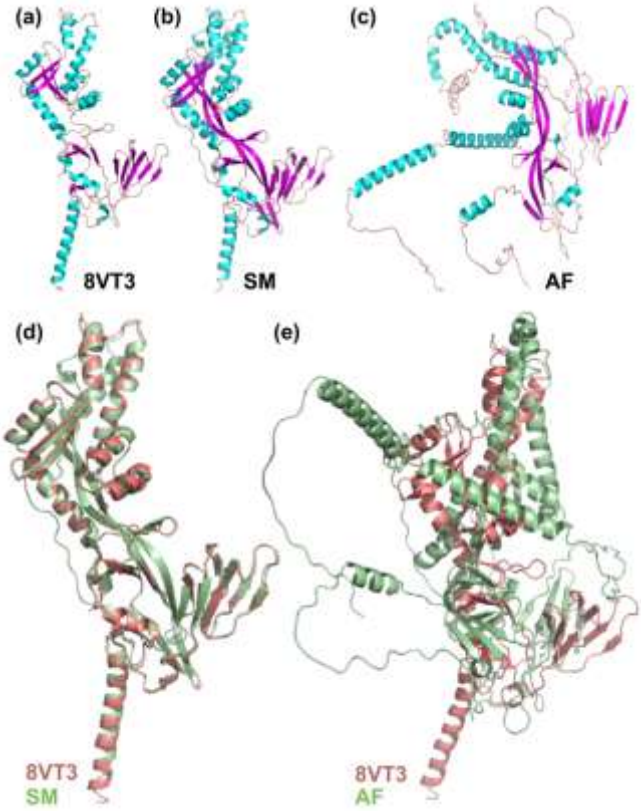

Figure : Figure showing Fusion protein from experimental data (a), predictions from swiss-model (b) & alphafold (c) and comparisons between experimental model & swiss-model (d) and experimental model alphafold prediction (e).

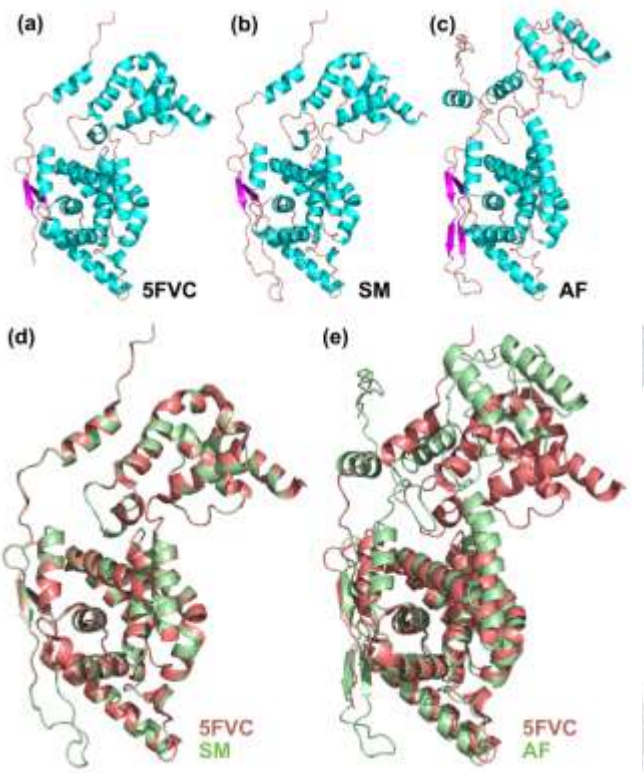

Figure: Figure showing nucleoprotein protein from experimental data (a), predictions from swiss-model (b) & alphafold (c) and comparisons between experimental model & swiss-model (d) and experimental model alphafold prediction (e).

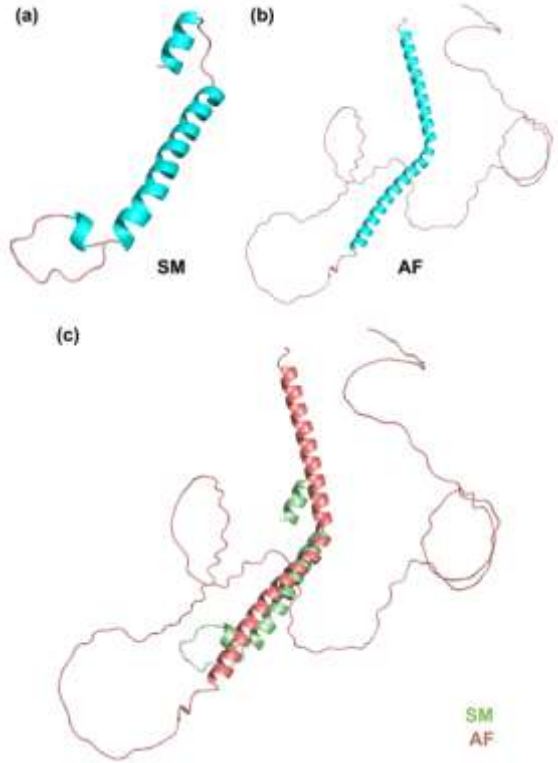

Figure: Figure showing attachment glycoprotein predictions from swiss-model (a) & alphafold (b) and comparisons between swiss-model prediction and alphafold prediction (c).

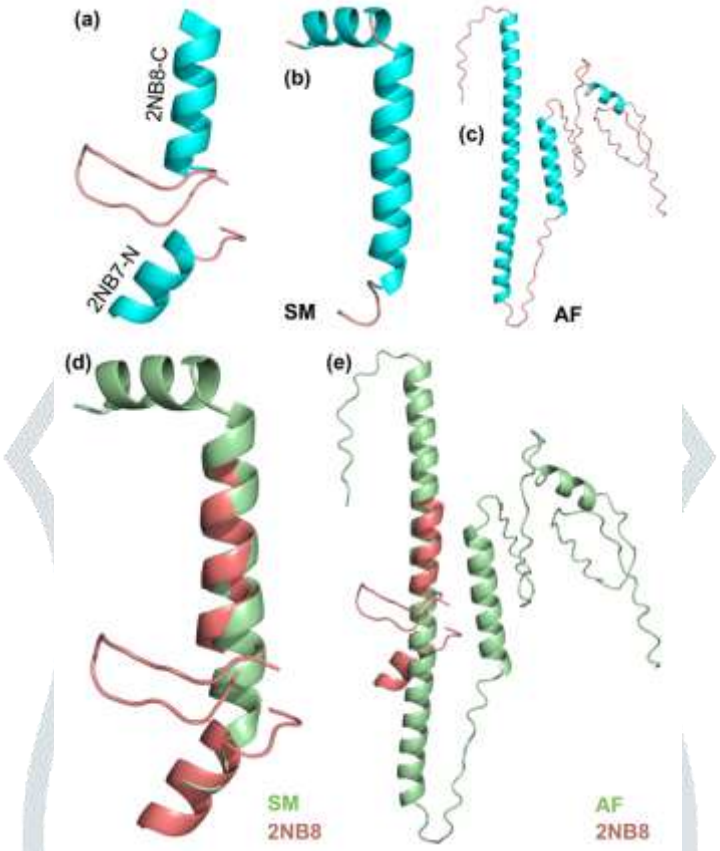

Figure: Figure showing short hydrophobic protein from experimental data (a), predictions from swiss-model (b) & alphafold (c) and comparisons between experimental model & swiss-model (d) and experimental model alphafold prediction (e).

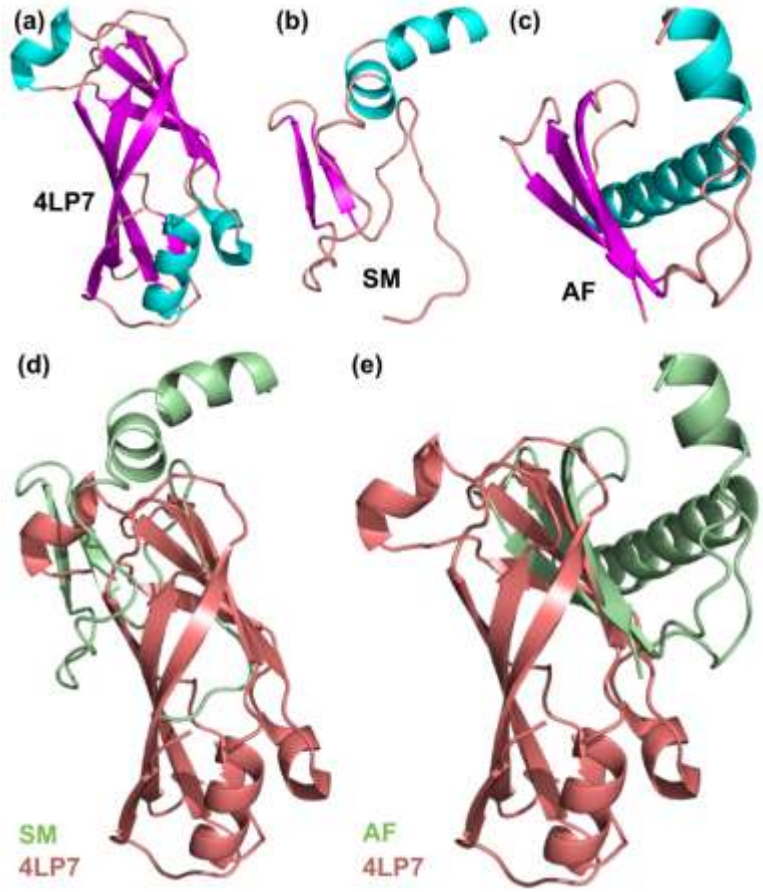

Figure: Figure showing matrix protein M2-2 from experimental data (a), predictions from swiss-model (b) & alphafold (c) and comparisons between experimental model & swiss-model (d) and experimental model alphafold prediction (e).

AlphaFold models demonstrated higher overall structural accuracy compared to Swiss-Modeler.

Comparison of Predicted Structures for Non-PDB Proteins:

Structural discrepancies were observed between Swiss-Modeler and AlphaFold predictions, with AlphaFold generally producing more detailed secondary structure elements.

RMSD values indicated closer alignment in conserved regions but variations in flexible domains.

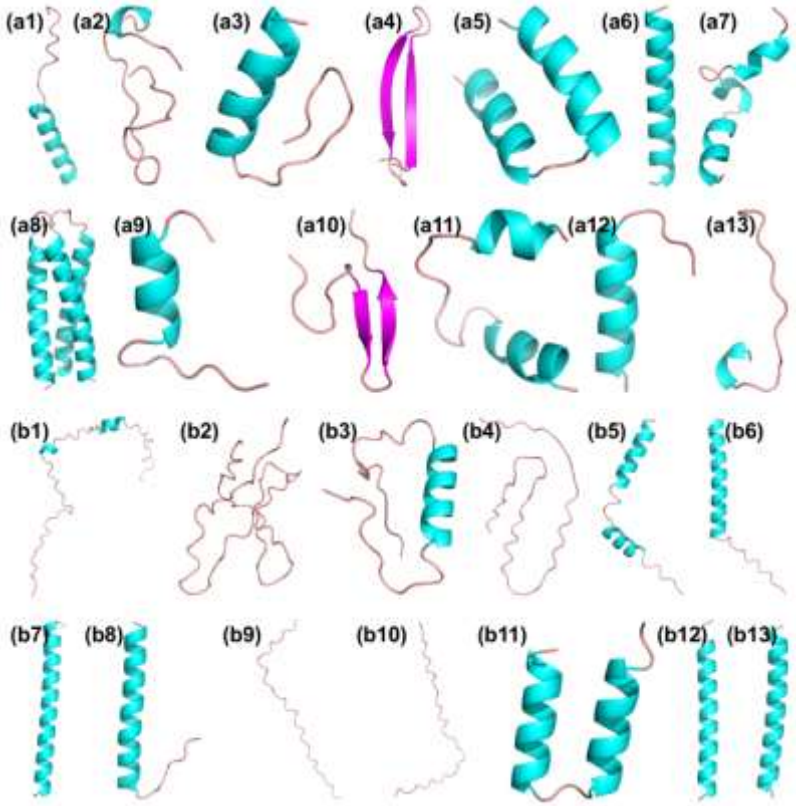

Figure showing unreported protein predictions from swiss-model (b) & alphafold (c) and comparisons between experimental model & swiss-model (d) and experimental model alphafold prediction (e).

Discussion

The Swiss-Model and AlphaFold comparative structural analysIs of Human Metapneumovirus (HMPV) proteins reveal important differences in the precision of structural predictions and their applicability to virological research. Swiss-Model is a template-based homology modeling program that generates accurate models for conserved regions by using experimentally resolved structures with high sequence similarity [19]. The deep learning-based predictor AlphaFold, on the other hand, is better at modeling proteins with few or no homologous templates. It provides high-confidence predictions for complete proteins, including parts that are inherently flexible or disordered. F (fusion), G (glycoprotein), N (nucleocapsid), and M2-1 (transcription processivity factor) are important HMPV proteins. Structural alignment of these proteins showed that AlphaFold-predicted models consistently have higher completeness and confidence scores, especially for regions in PDB that lack crystallographic data [20]. Interestingly, both techniques demonstrated strong structural agreement in the prefusion core of the F protein, which is crucial for viral entry; however, AlphaFold was more accurate in extending into flexible heptad repeats and transmembrane regions. Whereas Swiss-Model was unable to resolve non-conserved extracellular domains, AlphaFold's end-to-end modeling greatly improved the G protein, which is marked by considerable variability and glycosylation. Per-residue confidence metrics (pLDDT for AlphaFold and GMQE/QMEAN for Swiss-Model) and root-mean-square deviation (RMSD) research confirmed AlphaFold's resilience in simulating HMPV proteins outside of conserved motifs. Only AlphaFold was able to fully model the M2-1 protein, which lacks a wealth of structural information, with reliable secondary structure elements that support its RNA-binding function [20][21]. Our knowledge of Human Metapneumovirus (HMPV) has significantly improved as a result of recent developments in structural bioinformatics, especially with the use of Swiss-Model and AlphaFold. Even though both approaches provide fundamental insights, comparing how well they function becomes particularly pertinent when looking at current outbreaks and the growing demand for pandemic vaccine targets [22].

In terms of prediction coverage and confidence for HMPV proteins, AlphaFold outperforms Swiss-Model overall, particularly in cases where template restrictions are present. Nevertheless, Swiss-Model is still useful for using template comparison to validate conserved domains. A comprehensive perspective is obtained by combining the two approaches, using AlphaFold for new insights into protein dynamics, possible epitope mapping, and antiviral medication targeting and Swiss-Model for benchmarking. This two-pronged strategy speeds up functional annotation in poorly understood pathogens like HMPV and improves structural virology workflows.

Conclusion

The complimentary advantages of homology-based and AI-driven predictive modeling techniques are demonstrated by this comparative structural analysis of human metapneumovirus (HMPV) proteins using Swiss-Modeler and AlphaFold. Even in low-homology regions, AlphaFold consistently yields high-confidence predictions, revealing potentially functional motifs and structural characteristics that had not been previously resolved, whereas Swiss-Modeler gives dependable structures when high-identity templates are available. By identifying structurally conserved and functionally significant sections, the analysis's findings not only improve our understanding of the structure of the HMPV protein but also aid in the development of future antiviral medications and vaccines. Viral structural biology finds that integrating various modeling platforms is a potent tactic. A strong foundation for structural virology is provided by this study by combining the advantages of both modeling tools. It also offers useful templates for other applications such as epitope mapping, logical vaccine design, and structure-based drug discovery against HMPV. These models will be further refined in future research by integrating dynamic simulations and experimental validation, which will advance our molecular understanding of HMPV pathogenesis and inform more comprehensive antiviral therapies.

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