

## Bioinformatics Analysis of SARS Proteins and Molecular Dynamics Simulated Structure of an Alpha-helix Motif

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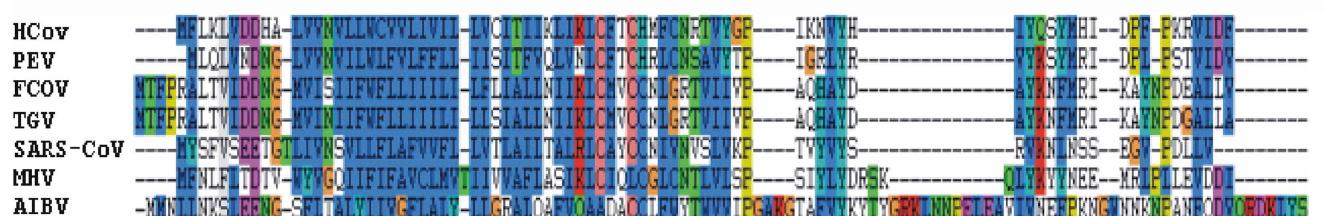
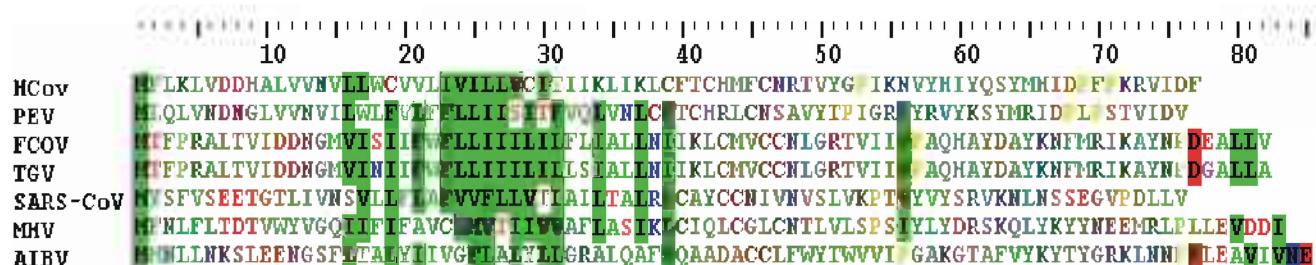
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The complete sequencing of the severe acute respiratory syndrome (SARS) coronavirus (CoV) within a short span of time is a remarkable achievement, which has given the scope for extensive research on the virus.<sup>1</sup> Sequence analysis reveals the phylogeny of SARS-CoV that the genome has most of the characteristic features of a coronavirus, but it belongs to a new group which is sufficiently different from

all known coronaviruses.

All the coronaviruses consist of four important structural proteins: spike glycoprotein (S), small envelope protein (E), matrix glycoprotein (M) and nucleocapsid protein (N). The E protein comprised of at least 76 amino acids (aa) is a pivotal player in the morphogenesis of the virion envelope by interacting with the M protein.

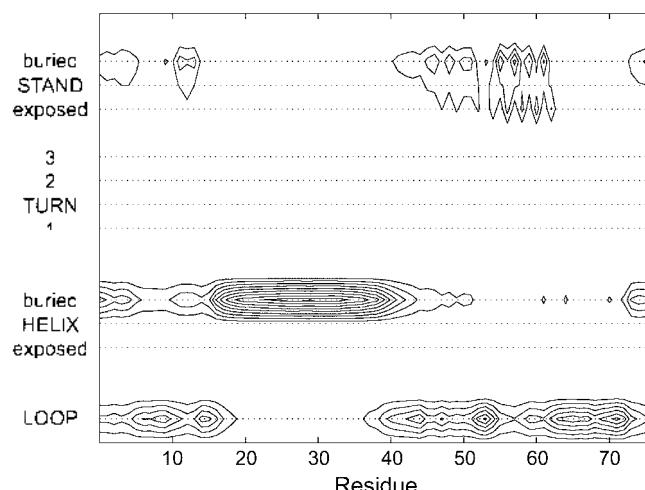


**Figure 1.** Sequence alignment of all the E proteins among coronaviruses using Bioedit (top) and ClustalX (below); SARS-CoV [NP\_828854], HCoV [Human CoV 229E, NP\_073554], FCoV [Feline CoV CAA74228.1], TGV [Transmissible gastroenteritis virus, NP\_058426], PEDV [Porcine epidemic diarrhea virus, NP\_598312], MHV [Murine hepatitis virus, NP\_068673], AIBV [Avian infectious bronchitis virus, AAO33465].



**Figure 2.** Secondary structure prediction of the SARS-CoV E protein by PredictProtein (PP).

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**Figure 3.** Secondary structure probabilities for SARS-CoV E protein from PSA Protein structure prediction.

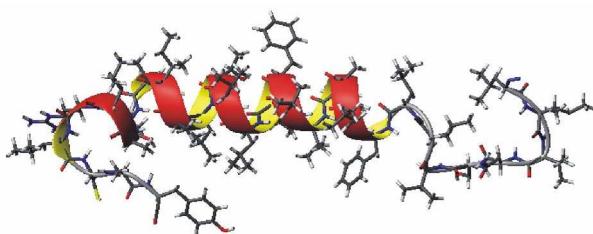
**Table 1.** Sequence-structure homology recognition based on FUGUE

Profile Hit	#aa	Zscore	ZSCORE:
hs1jdma	31	3.85	>6.0 (CERTAIN 99% confidence)
hs1khvaa	493	3.60	>4.0 (LIKELY 95% confidence)
hs1ce4a	100	2.78	>3.5 (MARGINAL 90% confidence)
hsd2occm1	43	2.69	>2.0 (GUESS 50%)
hsd1ewva5	100	2.63	<2.0 (UNCERTAIN)

When the E protein is compared with corona-viruses, the pairwise sequence identity is less than 40%. The sequence alignment of all the E proteins among coronaviruses shows a clear feature that the aa are well conserved. This is more significant in the region of residues 12-39 (based on Bioedit) or 12-52 (based on ClustalX) (Figure 1).

The bioinformatics analysis of SARS-CoV E protein was done using public databases and the bioinformatics tools.<sup>2</sup> The BLAST and FASTA comparisons show significant matching. The 3D PSSM prediction for the protein structure reveals that E protein belongs to a class of membrane protein with high reliability of helix structure in the conserved region. The 3D PSSM results indicate that the region between 17 and 42 is a hydrophobic region of alpha helix type buried in the membrane. TMHMM predicts that the protein is a transmembrane type (aa: 12-34) with most of the hydrophilic domain consisting of the carboxy terminal tail inside the cell and a short hydrophilic amino terminus (aa: 1-11).

As for the secondary structure, PredictProtein (PP) predicts an  $\alpha$ -helix for aa: 14-43 and two extended  $\beta$ -sheets



**Figure 4.** Simulated structure of  $\alpha$ -helix (aa: 12-40) in SARS. Note that aa: 19-38 is an  $\alpha$ -helix.

for aa: 47-52 and 56-63 (Figure 2). The secondary structure probabilities obtained from PSA Protein structure prediction (Figure 3) are  $\alpha$ -helix (aa: 19-38) and  $\beta$ -sheets (aa: 45-52 and 55-63) (Figure 3). Other programs predict these  $\beta$ -sheets as  $\alpha$ -helices or loops. Thus, this assignment is not clear yet, so it is being studied using simulation in our laboratory.

The part of a structure that showed high conservation and transmembrane  $\alpha$ -helix type is taken and more systematically exploited by the homology recognition program FUGUE (Table 1). This part is taken to model the protein motif.

The molecular modeling of the  $\alpha$ -helix region has been investigated using molecular dynamics (MD) simulation.<sup>3</sup> The initial geometry was used with the NMR structure of hs1jdma which shows the highest Zscore. Taking aa: 11-42 as the starting structure, we have performed simulated annealing optimization<sup>4</sup> (Figure 4). The aa: 19-32 was calculated to be an  $\alpha$ -helix.

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## References and Notes

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