Structural Analysis of Severe Acute Respiratory Syndrome Coronavirus-2 Proteins: Exploring Mutations of Nsp13

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INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), discovered in December 2019, caused the COVID-19 global pandemic. SARS-CoV-2 belongs to the family *Coronaviridae*, which is composed of positive-sense, single-stranded RNA viruses. These viruses are known to have one of the largest RNA viral genomes, which consists of approximately thirty-thousand basess. The SARS-CoV-2 genome encodes both non-structural proteins, which assist in replication of the virus upon infection, and various structural and accessory proteins.

The focus of this work is SARS-CoV-2 non-structural protein 13 (Nsp13), a 596-residue protein consisting of five domains. Nsp13 functions as a helicase unwinding double-stranded RNA. Helicase activity depends on NTP hydrolysis, catalyzed by six conserved active site residues. Nsp13 synergizes with the viral RNA-dependent RNA polymerase, a heterotetramer consisting of one copy of Nsp7, two copies of Nsp8, and one copy of Nsp12. Nsp13 represents a potential target for discovery and development for small molecules that combat SARS-CoV-2. Studying the structure of this protein will enhance our understanding of its mechanism of action and ways to inhibit the enzyme.

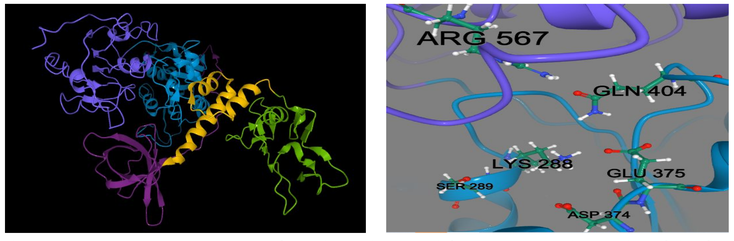

Fig. 1 Nsp13 structure and active site residues. Domains: Zinc-binding (green); Stalk (yellow); 1B (purple); 1A (light blue); 2A (dark blue)

METHODS

As part of a virtual summer research experience with the RCSB PDB, we studied how SARS-CoV-2 proteins evolved during the first six months of the COVID-19 pandemic by exploring amino acid sequence and 3D atomic-level structure changes using various structural bioinformatics tools, including Clustal Omega (www.ebi.ac.uk/Tools/msa/clustalo/) for sequence alignments and phylogenetic trees; Mol* (molstar.org) for 3D molecular visualization; and Foldit (fold.it) for structural/energetic effects of mutations.

SUMMARY OF RESULTS & IMPORTANT MUTATIONS

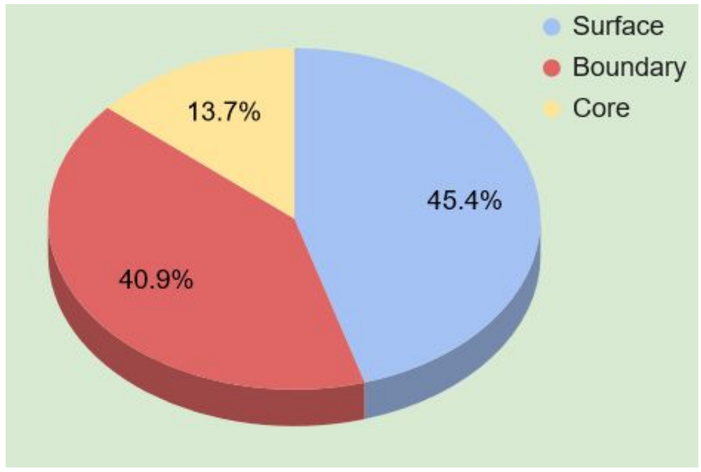

Fig. 2 Percentage of amino acid substitutions at each protein layer

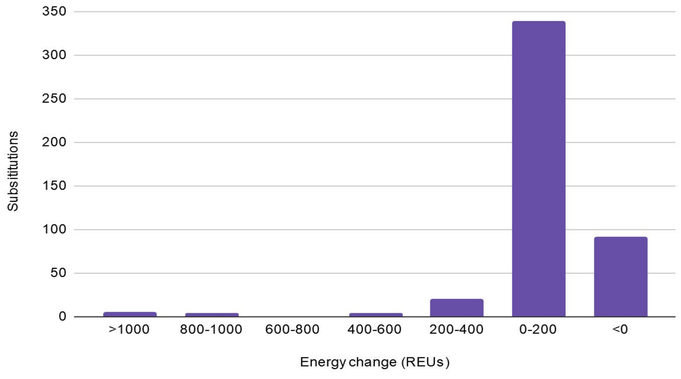

Fig. 3 Amino acid substitutions vs. Energy change

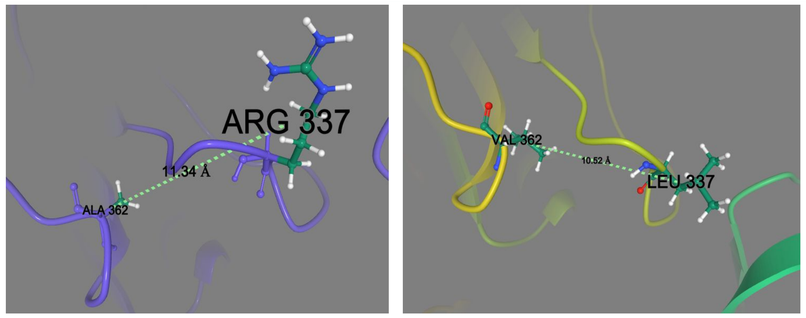

Fig. 4 Double mutant in Domain 1A: R337L; A362V

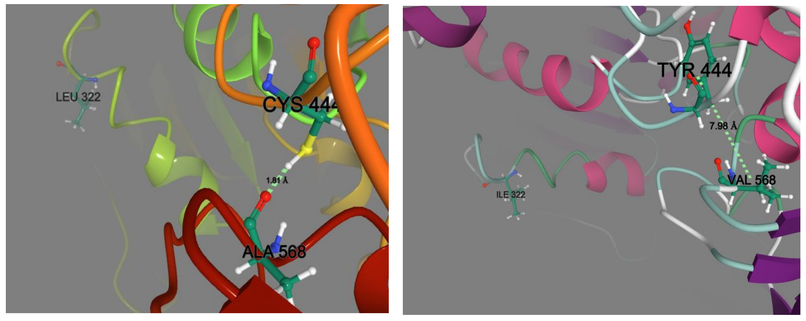

Fig. 5 Most energetically unfavorable mutant: L322I; C444Y; A568V

CONCLUSION

There is still a lot of unknown information about Nsp13 structure and helicase activity. In the future, there will be further structural and mutant analysis of Nsp13. The overall goal of the project is to use structural information about this protein to improve the likelihood of discovering and developing small molecules to inhibit this enzyme.

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