Conformational analysis between 6M71 (SARS COV2 RNA-dependent RNA polymerase) and CHEMBL3120791 using GROMACS Molecular Dynamic simulation.

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Repository: https://github.com/DSPavan/covid19Research

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ABSTRACT:

We analyzed molecular dynamic simulation using GROMACS to study the interaction between SARS-Cov-2 cryo-EM structure of RNA-dependent RNA polymerase (PDB ID: 6M71) and compound CHEMBL3120791 with 20ns simulation using NVIDIA GPU for high performance. SARS Cov2 RNA-dependent RNA polymerase (RdRp) is an enzyme that catalyzes the synthesis and replication of viral RNA from an RNA template. CHEMBL3120791 is in clinical trial for the treatment of HCV (Hepatitis C Virus) infections, and HCV Viruses are like SARS viruses. In our study, we found amino acids LYS47, ASN138, ASN781, THR141, THR710, SER709, SER778, SER784, TYR129 can potentially form hydrogen bonds with the drug molecule. Among all the amino acids mentioned in the list Asparagine 138 and Serine 709 may form hydrogen bonds with CHEMBL3120791 and this interaction can cause changes in conformation between coil and Helix. These amino acids are located around the active site of the enzyme and can be utilized for protein ligand interaction. ASN138, SER709 will actively play and change in conformation during Protein-ligand bond interactions

INTRODUCTION:

SARS -Cov2 has immerged as one of the deadly viruses of the 21st century and starting late 2019 and beginning of 2020 , COVID-19 has been declared a pandemic effecting not only health of the existing population but also causing economic disasters in several places around the world [1]. SARS -Cov2 is a positive strand RNA virus and replication of its genetic material is crucial to its survival and spread among human host [2]. RNA dependent RNA polymerase is utilized by SARS-COV2 to propagate and spread among human population and is one of the drug targets in treating COVID-19 [3,4,5] .

RNA dependent RNA polymerase or RdRp has been used extensively as a drug target in treatment of COVID-19 [3]. Recently one such drug Remdesivir, which is a nucleotide analogue has proved effective in treatment of COVID-19 in some clinical trials. Remdesivir binds to RdRp and stops viral replication thereby preventing replication and multiplication of virus inside host cells [6, 7, 8] . RdRp of SARS CoV2 has several non-structured proteins like NSP12, NSP 7 and NSP8 [9, 16]. Remdesivir binds to active site residues located in NSP12 molecule of RdRp [10] . Earlier by docking studies we investigated several Remdesivir analogues that can be used as potential drug target in treatment of COVID-19 [11].

In this study, we analyzed CHEMBL3120791 [12] and performed Molecular dynamic simulations of protein drug complex using GROMACS [13] . The structure we used here is Cryo EM structure of RdRp from SARS CoV2(PDB ID 6m71) [14,15] . This drug molecule has been effective against HCV virus and is under clinical trial [17]. The present Molecular Dynamic (MD) simulation studies will provide insights into protein drug interaction and a closer view of the interacting residues present in the active site of RdRp. The dynamics of binding site will be an important step in structure-based drug design of SARS CoV2.

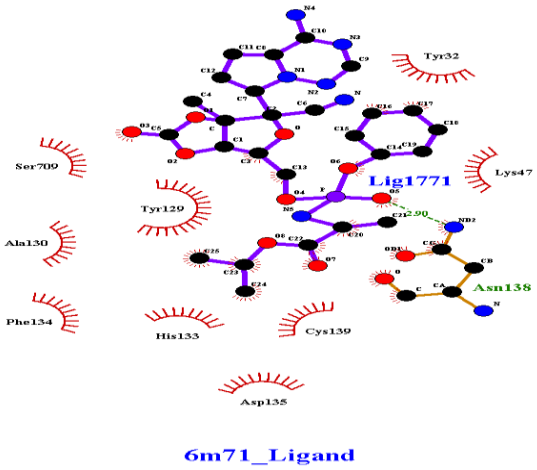
MATERIAL & METHODS:

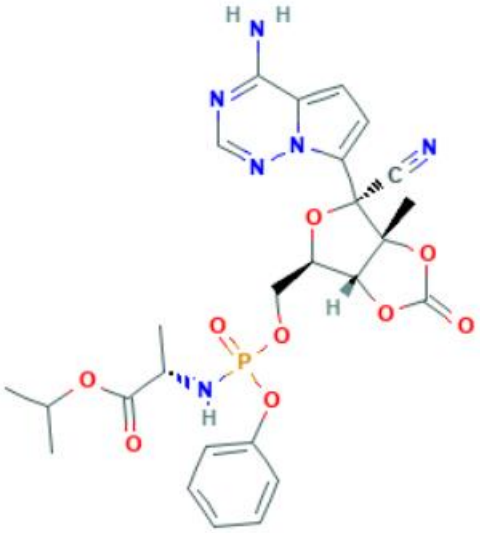
Based on our docking studies [11], we identified CHEMBL3120791 which is analogues to Remdesivir, is potential binding ligand molecule on (PDB ID 6m71) SARS-Cov-2 cryo-EM structure of RNA-dependent RNA polymerase (RdRp). We have used GROMACS for molecular dynamic simulation. We have used NVIDIA NGC GROMACS 2020.2 and with of NVIDIA 1080 Ti GPU.

We have prepared protein-ligand complex using outputs of Autodock Vina [18] and later PyMol [19] is used to save as PDB file. To fix missing hydrogen or residues of this complex, we used Swiss PDB Viewer [20]. CHARMM force field was added on ligand using swissparam.ch website [21]. For GROMACS commands, you can visit my GitHub link (https://github.com/DSPavan/covid19Research). For our ligand-protein complex of 851 amino acids, for 20ns MD simulation, on 2 GPU, 22 GB RAM, NVIDIA 1080 Ti, it took 26 hour time (more than 1 day).

RESULTS AND DISCUSSION:

We analyzed for active site and interaction between protein-ligand, after 20ns Molecular Dynamic simulation. Using Ligplot+ [22], we found that active site is at ASN138 (Fig. 1). We analyzed further for Hydrogen Bond interactions between Protein and Ligand, Results are summarized in Table 1. Amino acids LYS47, ASN138, ASN781, THR141, THR710, SER709, SER778, SER784, TYR129 which are forming Hydrogen bonds with Ligand.





Ligand: CHEMBL3120791

Fig 1: Protein: 6M71, Ligand = CHEMBL3120791, Active site amino acids is ASN138, using Ligplot+

Table 1: Amino acids, which are interacting in Hydrogen Bonds between 6M71 (RdRP) and CHEMBL3120791

Amino Acid	Amino Acid Full Name	Properties of Amino acid	Amino acid number in a Protein
LYS	Lysine	Positive, Polar, Hydrophilic	47
ASN	Asparagine	Non Charge, Polar, Hydrophilic	138, 781
THR	Threonine	Non Charge, Polar, Hydrophilic	141, 710,
SER	Serine	Non Charge, Polar, Hydrophilic	709,778, 784
TYR	Tyrosine	Polar, Aromatic, hydrophobic	129

We analyzed further, for conformation changes during simulation on above amino acids at active sites. From these amino acids, we observed, ASN138 and SER709 only changed during this simulation. This indicates ASN138, SER709 will actively play and change in conformation during Protein-ligand bond interactions.

ASN138 showing changes of conformations from Coil and Helix during simulation as in Fig 2 and Fig 3. Similarly SER709 also changing between Coil and Helix as in Fig 4 and Fig 5

Fig 2: Changes in Aspargine 138 during the simulation. X axis: Time Frame 0 to 2000 (for 20ns simulation). This shows there is change of Coil to Helix during this period and it is moving more towards Helix.

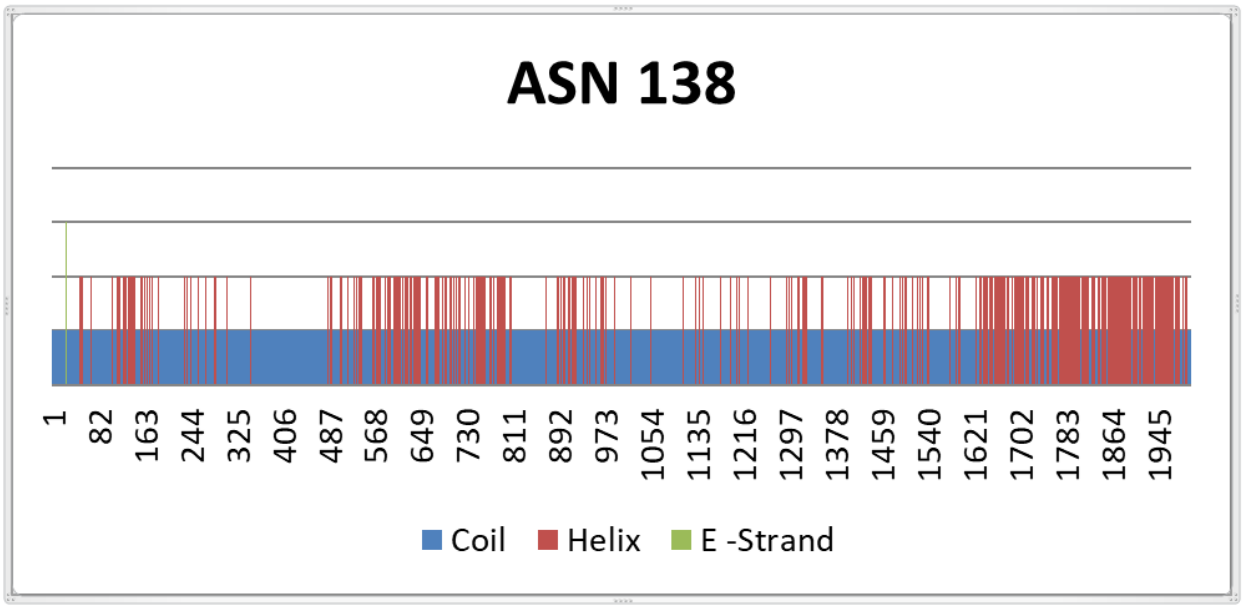
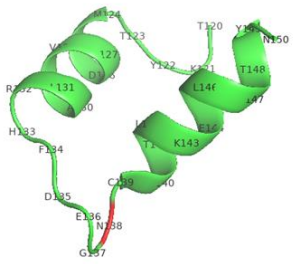
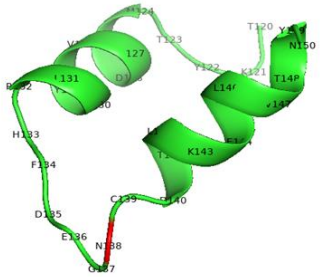


Fig 3: Changes in ASN138 during the simulation, we observed that It is changing Coil, Helix, Strand (E). Conformational changes are shown using PyMol for visualization (ASN138 in Red color)

As a Coil



As a Strand



As Helix

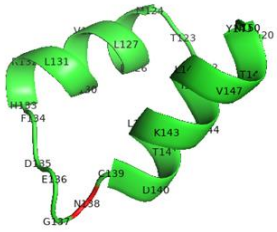


Fig 4: Changes in SER 709 during the simulation, we observed that It is changing Coil, Helix. (SER 709 – Red Color). Most dominantly it is Helix form. Fig , Fig

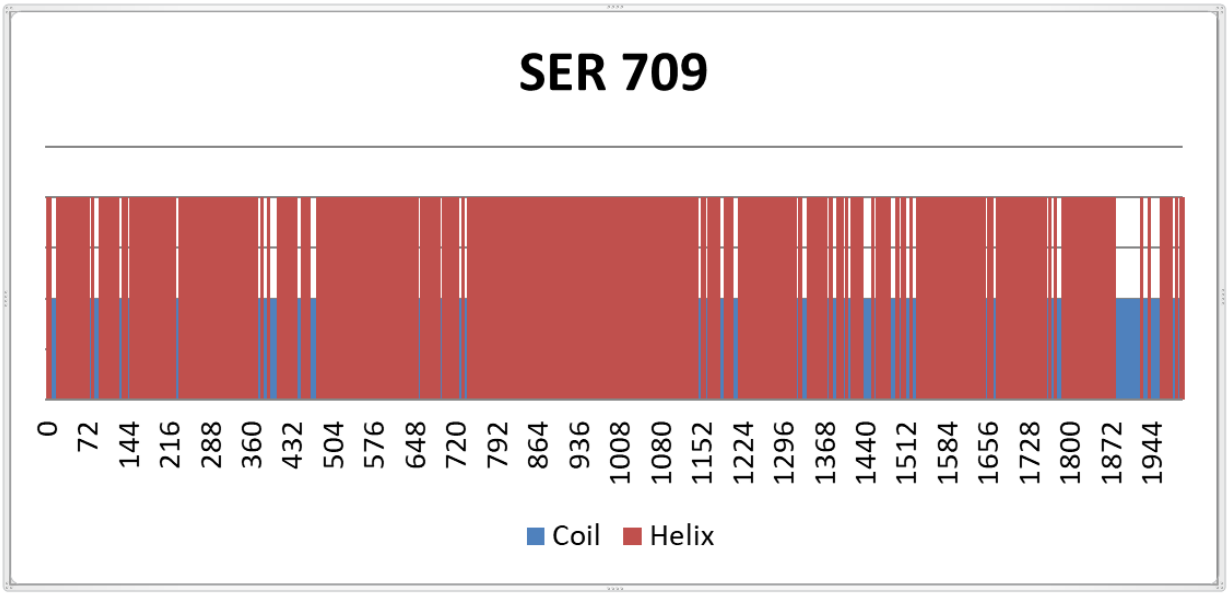
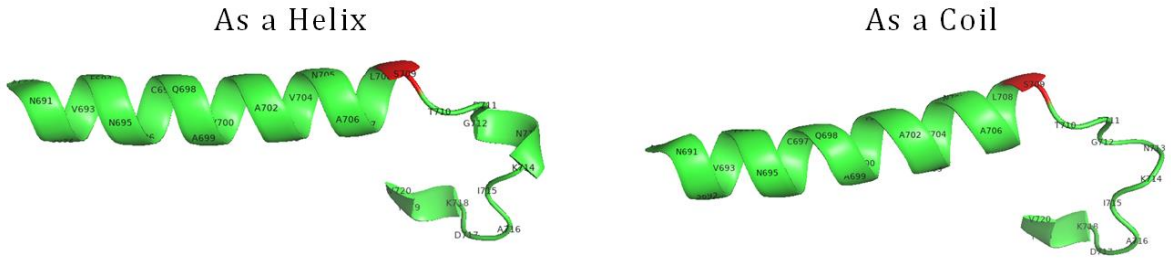


Fig 5: Changes in SER709 during the simulation. We observed Coil and Helix. Conformational changes are shown using PyMol for visulaizatio



CONCLUSION:

Molecular dynamics using GROMACS with 20ns simulation indicates, Amino acids LYS47, ASN138, ASN781, THR141, THR710, SER709, SER778, SER784, TYR129 which are forming Hydrogen bonds with Ligand. And in this Asparagine 138 and Serine SER709 of PDB ID: 6M71 (SARS COV2 RNA-dependent RNA polymerase) are forming Hydrogen bonds with CHEMBL3120791 and present in active site location clearly there is a change in conformation between coil and Helix. This indicates CHEMBL3120791 inducing and changing the conformation of a

protein RNA-dependent RNA polymerase CHEMBL3120791 which is in clinical trial for HCV can be potentially used for SARS Coronavirus 2. This work can be extended for Free Energy Perturbations (FEP) with 100ns and above for further analysis.

REFERENCES:

1). https://covid19.who.int/

2). SARS-CoV-2: March toward adaptation Francesca Benedetti, Maria Pachetti, Bruna Marini, Rudy Ippodrino, Massimo Ciccozzi, Davide Zella

J Med Virol. 2020 Jul 11 : 10.1002/jmv.26233. doi: 10.1002/jmv.26233 [Epub ahead of print] PMCID: PMC7361333

3). Elfiky, Abdo. (2020). SARS-CoV-2 RNA dependent RNA polymerase (RdRp) targeting: an in silico perspective. Journal of Biomolecular Structure and Dynamics. 1-15. 10.1080/07391102.2020.1761882.

4). Ahmad, Jamshaid & Ikram, Saima & Ahmad, Fawad & Rehman, Irshad & Mushtaq, Maryam. (2020). SARS-CoV-2 RNA Dependent RNA polymerase (RdRp) – A drug repurposing study. Heliyon. 6. e04502. 10.1016/j.heliyon.2020.e04502.

5). Park, Minah & Cook, Alex & Lim, Jue & Sun, Yinxiaohe & Dickens, Borame. (2020). A Systematic Review of COVID-19 Epidemiology Based on Current Evidence. Journal of Clinical Medicine. 9. 967. 10.3390/jcm9040967.

6). Zhang, Leili & Ruhong, Zhou. (2020). Binding Mechanism of Remdesivir to SARS-CoV-2 RNA Dependent RNA Polymerase. 10.20944/preprints202003.0267.v1.

7). Eastman, R. T., Roth, J. S., Brimacombe, K. R., Simeonov, A., Shen, M., Patnaik, S., & Hall, M. D. (2020). Remdesivir: A Review of Its Discovery and Development Leading to Emergency Use Authorization for Treatment of COVID-19. ACS central science, 6(5), 672–683.

8). Green, N., Ott, R. D., Isaacs, R. J., & Fang, H. (2008). Cell-based Assays to Identify Inhibitors of Viral Disease. Expert opinion on drug discovery, 3(6), 671–676. https://doi.org/10.1517/17460441.3.6.671

9). Mittal, L., Kumari, A., Suri, C., Bhattacharya, S., & Asthana, S. (2020). Insights into structural dynamics of allosteric binding sites in HCV RNA-dependent RNA polymerase. Journal of biomolecular structure & dynamics, 38(6), 1612–1625. https://doi.org/10.1080/07391102.2019.1614480

10). Yin, W., Mao, C., Luan, X., Shen, D. D., Shen, Q., Su, H.,Wang, X., Zhou, F., Zhao, W., Gao, M., Chang, S., Xie, Y. C., Tian, G., Jiang, H. W., Tao, S. C., Shen, J., Jiang, Y., Jiang, H., Xu, Y., Zhang, S., … Xu, H. E. (2020). Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. Science (New York, N.Y.), 368(6498), 1499–1504. https://doi.org/10.1126/science.abc1560

11). . Gudipati, Pavan & Biswas, Shyamasri & goyal, pankaj. (2020). Anti Hepatitis C Viral Drugs Remdesivir and Uprifosbuvir Derivatives Are Better Inhibitors of SARS Cov2 RNA-Dependent RNA Polymerase Determined by Docking Studies. International Journal of Engineering Applied Sciences and Technology, 2020 , Vol. 5, Issue 3, ISSN No. 2455-2143, Pages 474-481 Published Online July 2020 in IJEAST (http://www.ijeast.com)

12). National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 76325303.

Retrieved August 2, 2020 from
https://pubchem.ncbi.nlm.nih.gov/compound/76325303

13). M.J. Abraham, D. van der Spoel, E. Lindahl, B. Hess, and the GROMACS development team, GROMACS User Manual version 2019, http://www.gromacs.org

14). Gao, Y., Yan, L., Huang, Y., Liu, F., Zhao, Y., Cao, L., Wang, T., Sun, Q., Ming, Z., Zhang, L., Ge, J., Zheng, L., Zhang, Y., Wang, H., Zhu, Y., Zhu, C., Hu, T., Hua, T., Zhang, B., Yang, X., … Rao, Z. (2020). Structure of the RNAdependent RNA polymerase from COVID-19 virus. Science (New York, N.Y.), 368(6492), 779–782. https://doi.org/10.1126/science.abb7498

15). PDB ID: 6m71, Available: https://www.rcsb.org/structure/6M71

16). Kirchdoerfer, R. N., & Ward, A. B. (2019). Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 cofactors. Nature communications, 10(1), 2342. https://doi.org/10.1038/s41467-019-10280-3

17). Cho, A., Zhang, L., Xu, J., Lee, R., Butler, T., Metobo, S., Aktoudianakis, V., Lew, W., Ye, H., Clarke, M., Doerffler, E., Byun, D., Wang, T., Babusis, D., Carey, A. C., German, P., Sauer, D., Zhong, W., Rossi, S., Fenaux, M., … Kim, C. U. (2014). Discovery of the first C-nucleoside HCV polymerase inhibitor (GS-6620) with demonstrated antiviral response in HCV infected patients. Journal of medicinal chemistry, 57(5), 1812–1825. https://doi.org/10.1021/jm400201a

18). O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, Journal of Computational Chemistry 31 (2010) 455-461

19). Schrodinger, LLC. 2010. The PyMOL Molecular Graphics System, Version 2.X.

20). Guex, N. and Peitsch, M.C. (1997) SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. Electrophoresis 18, 2714-2723.

21). V. Zoete, M. A. Cuendet, A. Grosdidier, O. Michielin, SwissParam, a Fast Force Field Generation Tool For Small Organic Molecules, J. Comput. Chem, 2011, 32(11), 2359-68. PMID: 21541964, DOI: 10.1002/jcc.21816. https://www.swissparam.ch/

22). Laskowski R A, Swindells M B (2011). LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J. Chem. Inf. Model., 51, 2778-2786. [PubMed id: 21919503]