Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2086-2092 | ISSN:2251-6727

Comparative Analysis of Ramachandran Plot Conformations in Spike Protein Sequences of SARS-CoV-2, SARS-CoV, and MERS-CoV

Uma Shekhawat[1], Anindita Roy Chowdhury (Chakravarty)[2*]

[1]Department of Physics, Pt. Jawaharlal Nehru Govt. College, Faridabad, Haryana, 121002, India
[2]School of Engineering and Sciences, G.D. Goenka University, Gurugram Haryana, 122103, India

(Received: 27 October 2025 Revised: 05 November 2025 Accepted: 04 December 2025)

KEYWORDS

Spike Protein, MERS-CoV, Ramachandran Plot, SARS-CoV, Torsion Angles, SARS-CoV-2

ABSTRACT:

Introduction: The spike protein of coronaviruses is crucial for host cell recognition and viral entry, making it a key target for therapeutic interventions and vaccine development. Understanding the structural dynamics and evolutionary relationships of spike proteins across different coronaviruses is crucial for elucidating their functional diversity and potential vulnerabilities.

Objectives: The objective of this study is to present a comprehensive analysis of spike protein sequences from three prominent coronaviruses: SARS-CoV-2, SARS-CoV and MERS-CoV, using the Ramachandran plot to assess protein backbone conformational quality. The study aims to identify the conformational space occupied by these spike proteins and highlight regions of structural stability and flexibility.

Methods: The spike protein sequences of SARS-CoV, MERS-CoV, and SARS-CoV-2 were retrieved from UniProt. The sequences were aligned using BLASTP and verified with ClustalW to identify conserved and variable regions. Ramachandran plots for each protein were generated through PDBsum to analyze backbone torsion angles and compare structural conformations.

Results: The analysis revealed distinct distribution of phi (ϕ) and psi (ψ) torsion angles across the spike proteins, elucidated the conformational space occupied by each spike proteins. The study identifies regions of structural stability and flexibility, contributing to the understanding of functional and evolutionary differences among the spike proteins.

Conclusions: The outcomes of this investigation provide valuable insights into the structural dynamics and evolutionary constraints shaping spike protein diversity across coronaviruses, offering new opportunities for the rational design of antiviral treatments and vaccines.

1. Introduction

The spike protein is essential for the infectivity and pathogenicity of coronaviruses, facilitating their entry into host cells [(1,2)]. Understanding the structural nuances of these proteins across different coronavirus strains is vital for developing therapeutic interventions and vaccines. The Ramachandran plot is a graphical depiction of the dihedral angles φ versus ψ of residues of amino acids in a protein structure, serves as a valuable tool for analyzing protein conformations and assessing structural integrity [(3,4)].

SARS-CoV-2, SARS-CoV and MERS-CoV are three highly pathogenic coronaviruses that have caused significant outbreaks in recent history, leading to substantial morbidity and death [(5–7)]. While the three viruses share structural similarities, their spike proteins exhibit distinct conformational features that influence their interaction with host receptors and subsequent immune evasion [(8,9)]. Comparative structural investigation of these proteins can reveal critical information into their functional processes and potential vulnerabilities [(8,10,11)].

In this work, the authors carried out an analysis of comparisons of the Ramachandran plot conformations in the spike protein sequences of SARS-CoV, MERS-CoV and SARS-CoV-2. By examining the variations and similarities in the backbone dihedral angles of these spike proteins, authors aim to uncover insights into their structural dynamics and functional implications. This comparative analysis not only enhances our

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(6), 2086-2092 | ISSN:2251-6727

understanding of the conformational landscapes of these crucial viral proteins but also provides foundational knowledge that could inform the design of broad-spectrum antiviral strategies and vaccines.

2. Methodology

2.1 Selection of protein sequences:

The spike protein sequence of three primary coronavirus sequences from UniProt Database (SARS-CoV, ID: P59594, MERS-CoV, ID: A0A7D5J875, and SARS-CoV-2, ID: P0DTC2) and their aligned protein sequences from the study by Shekhawat & Roy Chowdhury (Chakravarty) [12,13] were considered for this investigation. After the consideration of all protein sequences shown in Fig. 1, the authors used PDBsum software [14] for the Ramachandran plot of each protein sequences. This study utilized these sequences to perform a comprehensive comparative analysis of their structural conformations via Ramachandran plots.

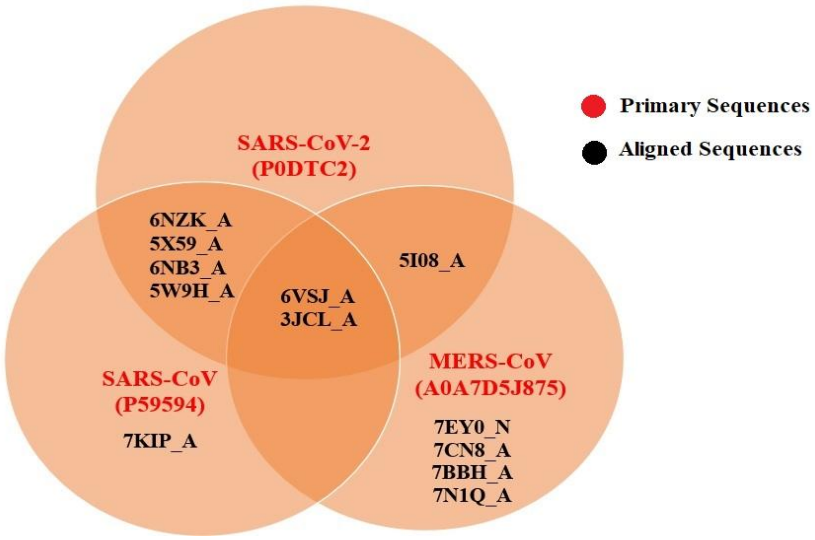

Fig. 1 Primary and aligned protein sequences related to SARS-CoV-2, SARS-CoV, and MERS-CoV [12]

2.2 Sequence Alignment:

To ensure an accurate comparative analysis, the protein sequences were first aligned using sequence alignment tool BLASTP [15]. This alignment facilitated the identification of conserved and variable regions across the spike proteins of the three coronaviruses. The alignment was visualized and verified using alignment visualization software BLASTP [15] and ClustalW [16].

2.3 Ramachandran Plot Generation:

For the generation of Ramachandran plots, the protein sequences were processed using the PDBsum software [14]. PDBsum is a well-established tool that provides detailed structural analysis, including the generation of Ramachandran plots. The steps involved in this process shown in Fig. 2.

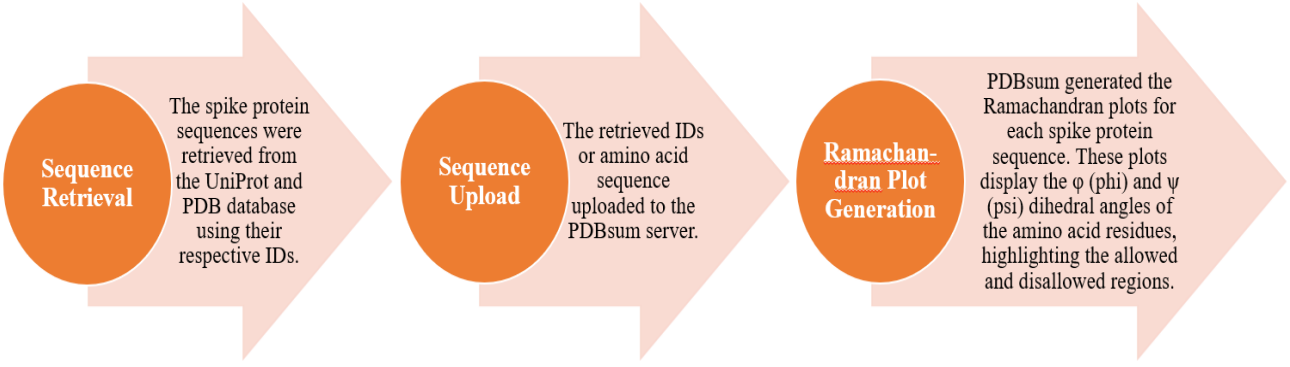

Fig. 2 Steps to generate Ramachandran Plot

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2086-2092 | ISSN:2251-6727

3. Results and Discussion

The provided methodology was employed to analyze and compare the Ramachandran plots to assess the conformational preferences and structural integrity of the selected protein sequences from the spike proteins of three coronavirus strains, SARS-CoV-2, SARS-CoV, and MERS-CoV, and their corresponding aligned sequences.

The Ramachandran plot is generally called the [φ, ψ] plot. It is a two-dimensional graph where all protein structures are displayed in terms of torsion angles between residues [10]. As glycine residues are not confined to the regions of the plot corresponding to the other sidechain types, they are denoted by triangles [17]. The authors employed PDBsum [14] software to develop the Ramachandran plot to calculate the torsion angles [φ, ψ] between amino acid residues of the all aligned protein sequences which were common with the primary spike protein sequences of SARS-CoV-2, SARS-CoV, and MERS-CoV (Fig. 1), respectively. However, the PDBsum software developed the Ramachandran plot for the combined chain of PDB IDs 5I08, 3JCL, 6VSJ, 6NZK, 5X59, 6NB3, 5W9H, 7EY0, 7CN8, 7BBH and 7N1Q [18], so the authors have considered the Ramachandran Plot (Fig. 3) for the whole protein sequence of these eleven PDB IDs.

The Ramachandran plot generated by PDBsum is based on 118 structures that have an R-factor of no more than 20% and a resolution of at least 2.0 Angstroms. Over 90% of the residues in the most allowed regions are usually indicative of a high-quality model [14]. This analysis revealed significant insights into their structural integrity, conformations and stability. The primary results, summarized in Table 1 and visualized in Fig. 3, showed varying distributions of residues of amino acids in the most allowed region, additionally allowed region, generously allowed region, and disallowed regions across different PDB IDs, where B, b, ~b representing beta sheet, L, l, ~l representing the left handed alpha helix and A, a, ~ a representing right handed alpha helix elements of secondary structure of protein [3,10,11]. The interaction between neighbouring or long-distance amino acid residues is represented by protein secondary structures which fundamentally include alpha helix, beta sheet, and coil ([19–21]).

The result in Table 3 indicated that three PDB IDs 6NZK, 5W9H, and 7CN8 exhibit over 90% of their amino acid residues in the most allowed regions of the Ramachandran plot. The PDB ID 6NZK belonged to the human coronavirus [2,22], it has a high percentage of residues (92.2%) in the most favored region, indicating a stable conformation and the PDB ID 5W9H associated with the MERS-CoV [2], also showed a high proportion of residues (90.1%) in the most favored region, and both the IDs best-aligned with the primary spike protein sequences of SARS-CoV as well as with SARS-CoV-2. PDB ID 7CN8 corresponded to the pangolin coronavirus and best-aligned with the primary spike protein sequence of MERS-CoV [22], displays a similarly high percentage (90.3%) of amino acid residues in the most allowed region. The remaining eight PDB IDs showed a range of 76.6% to 89.1% of residues in the most allowed region. These included structures from various coronaviruses, each with slightly differing conformational stability.

Among all PDB IDs, 7.6% to 23.1% of residues are in additionally allowed regions. This indicates some flexibility in the structure, which might be necessary for the protein's functional dynamics. A minor fraction of residues (0.1% to 2.7%) lie in generously allowed regions, suggesting areas of the protein that could adopt less common conformations. Interestingly, PDB IDs 6VSJ and 7BBH have 0% residues in disallowed regions, suggesting no steric clashes or unusual conformations. Other PDB IDs have a minimal percentage (0.1% to 1.3%) of residues in disallowed regions, indicating very few structural anomalies.

The results suggested that the spike proteins corresponding to PDB IDs 6NZK, 5W9H, and 7CN8 exhibit a higher degree of structural stability compared to the other proteins analyzed. This is based on the higher percentage of residues in the most favored regions, indicating a more stable and energetically favorable conformation. Being a human coronavirus structure, the high stability of PDB ID 6NZK suggests it might represent a well-adapted conformation that can efficiently interact with human host receptors. The stability observed in PDB ID 5W9H, MERS-CoV structure aligns with its known pathogenicity and ability to maintain a functional conformation under physiological conditions. Despite being from a pangolin coronavirus, the high stability of PDB ID 7CN8, when aligned with MERS-CoV primary spike protein sequence

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2086-2092 | ISSN:2251-6727

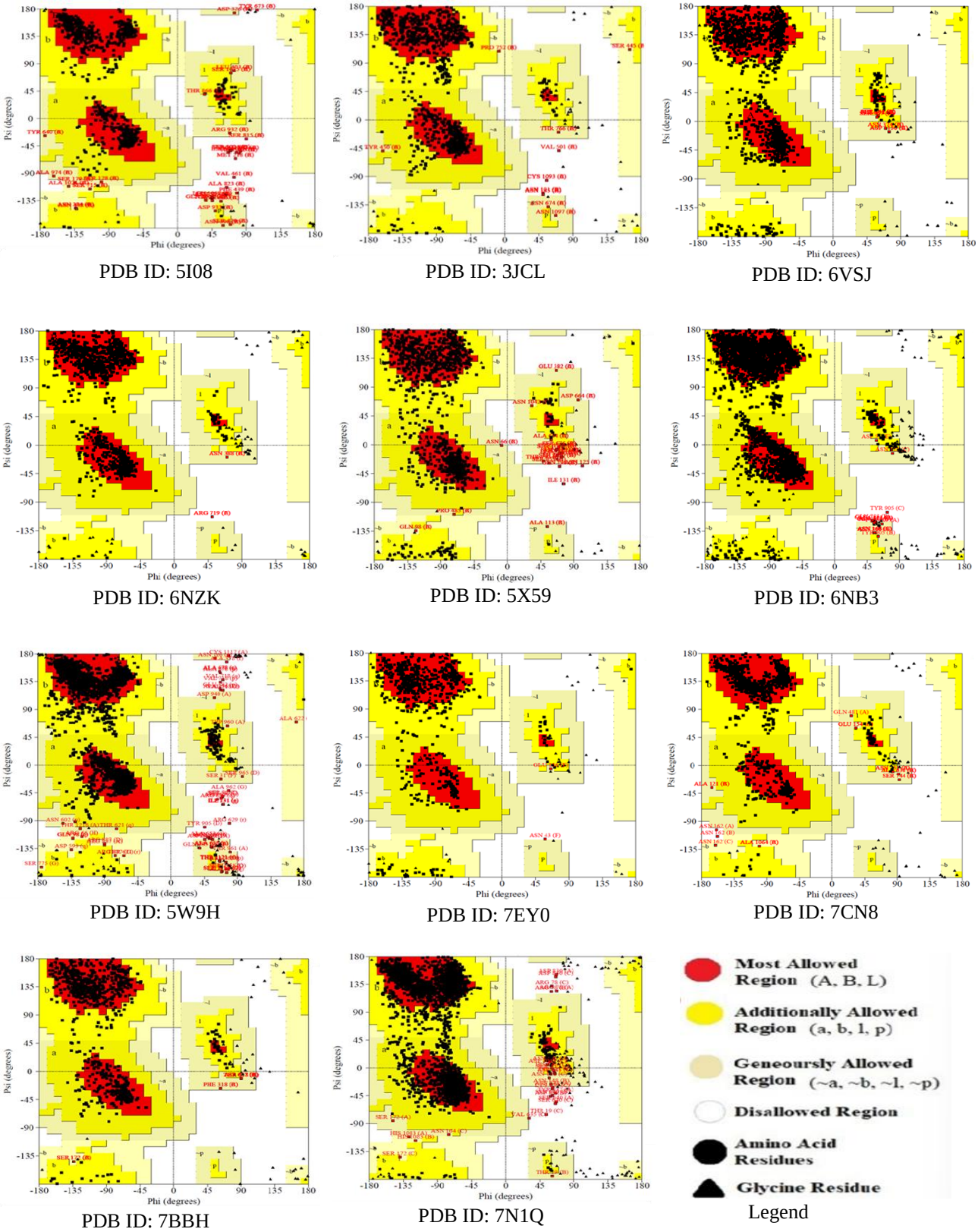

Fig. 3: Ramachandran Plots of Selected PDB IDs

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2086-2092 | ISSN:2251-6727

Table 1 Distribution of amino acid residues of aligned protein sequences

PDB ID / Region	Most Allowed Region	Additionally Allowed Region	Generously Allowed Region	Disallowed Region	Glycine Residues
5I08	85.7%	10.3%	2.7%	1.3%	153
3JCL	83.1%	15.9%	0.5%	0.4%	213
6VSJ	79.5%	20%	0.5%	0%	255
6NZK	92.2%	7.6%	0.1%	0.1%	231
5X59	77.7%	20.3%	1.7%	0.3%	234
6NB3	88.6%	10.9%	0.3%	0.1%	293
5W9H	90.1%	8.4%	0.9%	0.6%	306
7KIP	NA	NA	NA	NA	NA
7EY0	76.6%	23.1%	0.2%	0.2%	63
7CN8	90.3%	9%	0.6%	0.1%	231
7BBH	89.1%	10.4%	0.4%	0%	198
7N1Q	86%	13%	0.5%	0.5%	208

indicates structural similarities that may be leveraged in cross-species transmission studies.

The relatively lower stability of the remaining eight PDB IDs, indicated by a lower percentage of residues in the most favored regions, suggests these proteins may undergo conformational variations. Such variations could impact their functional efficiency or adaptability, potentially influencing viral infectivity and pathogenicity.

4. Conclusion

In this study, the Ramachandran plots have been compared which revealed that the spike proteins of three coronavirus strain SARS-CoV-2, SARS-CoV, and MERS-CoV exhibit varying degrees of structural stability. The higher stability observed in the spike proteins corresponding to PDB IDs 6NZK, 5W9H, and 7CN8 suggests a robust conformation, crucial for their role in viral infectivity. This stability is pivotal for the spike proteins to effectively function in viral entry and immune system interactions. The outcomes of this investigation provide critical insights into the structural dynamics of coronavirus spike proteins, aiding in the development of targeted antiviral strategies and vaccine designs. Such structural assessments support risk-based evaluations essential for chemical and biological health safety. Further investigations into the structural variations and their functional implications could enhance our understanding of coronavirus biology and pathogenesis.

Declarations

Funding: No funding was received to assist with the preparation of this manuscript.

Conflict of Interest: The authors have no competing interests to declare that are relevant to the content of this article.

Ethical Approval: This is a theoretical study. No ethical approval is required.

Informed Consent: Informed consent was not desirable as no living subject was involved in the study; moreover,

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2086-2092 | ISSN:2251-6727

open access databases were used and duly acknowledged.

Author Contributions: Uma Shekhawat wrote the first draft of the manuscript, Anindita Roy Chowdhury supervised the work and reviewed the manuscript.

References

[1] Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Krüger, N.; Herrler, T.; Erichsen, S.; Schiergens, T. S.; Herrler, G.; Wu, N.-H.; Nitsche, A.; Müller, M. A.; Drosten, C.; Pöhlmann, S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **2020**, *181* (2), 271-280.e8. https://doi.org/10.1016/j.cell.2020.02.052.

[2] Shekhawat, U.; Roy Chowdhury (Chakravarty), A. Computational and Comparative Investigation of Hydrophobic Profile of Spike Protein of SARS-CoV-2 and SARS-CoV. *J. Biol. Phys.* **2022**, *48* (4), 399–414. https://doi.org/10.1007/s10867-022-09615-x.

[3] DasGupta, D.; Kaushik, R.; Jayaram, B. From Ramachandran Maps to Tertiary Structures of Proteins. *J. Phys. Chem. B* **2015**, *119* (34), 11136–11145. https://doi.org/10.1021/acs.jpcb.5b02999.

[4] Ramachandran, G. N.; Ramakrishnan, C.; Sasisekharan, V. Stereochemistry of Polypeptide Chain Configurations. *J. Mol. Biol.* **1963**, *7* (1), 95–99. https://doi.org/10.1016/S0022-2836(63)80023-6.

[5] Campbell, F.; Archer, B.; Laurenson-Schafer, H.; Jinnai, Y.; Konings, F.; Batra, N.; Pavlin, B.; Vandemaele, K.; Van Kerkhove, M. D.; Jombart, T.; Morgan, O.; le Polain de Waroux, O. Increased Transmissibility and Global Spread of SARS-CoV-2 Variants of Concern as at June 2021. *Eurosurveillance* **2021**, *26* (24). https://doi.org/10.2807/1560-7917.ES.2021.26.24.2100509.

[6] de Wit, E.; van Doremalen, N.; Falzarano, D.; Munster, V. J. SARS and MERS: Recent Insights into Emerging Coronaviruses. *Nat. Rev. Microbiol.* **2016**, *14* (8), 523–534. https://doi.org/10.1038/nrmicro.2016.81.

[7] Zhu, N.; Zhang, D.; Wang, W.; Li, X.; Yang, B.; Song, J.; Zhao, X.; Huang, B.; Shi, W.; Lu, R.; Niu, P.; Zhan, F.; Ma, X.; Wang, D.; Xu, W.; Wu, G.; Gao, G. F.; Tan, W.; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N. Engl. J. Med.* **2020**, *382* (8), 727–733. https://doi.org/10.1056/NEJMoa2001017.

[8] Walls, A. C.; Park, Y.-J.; Tortorici, M. A.; Wall, A.; McGuire, A. T.; Veesler, D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* **2020**, *181* (2), 281-292.e6. https://doi.org/10.1016/j.cell.2020.02.058.

[9] Wrapp, D.; Wang, N.; Corbett, K. S.; Goldsmith, J. A.; Hsieh, C.-L.; Abiona, O.; Graham, B. S.; McLellan, J. S. Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation. *Science* **2020**, *367* (6483), 1260–1263. https://doi.org/10.1126/science.abb2507.

[10] Kumar, P.; Arya, A. Ramachandran Plot: A Simplified Approach. **2019**, 01–06.

[11] Park, S. W.; Lee, B. H.; Song, S. H.; Kim, M. K. Revisiting the Ramachandran Plot Based on Statistical Analysis of Static and Dynamic Characteristics of Protein Structures. *J. Struct. Biol.* **2023**, *215* (1), 107939. https://doi.org/10.1016/j.jsb.2023.107939.

[12] Shekhawat, U.; Chowdhury, A. R. In Silico Investigation and Correlation of Hydrophobic Stretches in Spike Proteins of SARS-CoV-2, SARS-CoV and MERS-CoV. *J. Adv. Res. Appl. Sci. Eng. Technol.* **2025**, *49* (1), 11–25. https://doi.org/10.37934/araset.49.1.1125.

[13] *UniProt*. https://www.uniprot.org/.

[14] *PDBsum home page*. http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html.

[15] *Protein BLAST: search protein databases using a protein query*. https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins.

[16] *Multiple Sequence Alignment - CLUSTALW*. https://www.genome.jp/tools-bin/clustalw.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2086-2092 | ISSN:2251-6727

[17] Morris, A. L.; MacArthur, M. W.; Hutchinson, E. G.; Thornton, J. M. Stereochemical Quality of Protein Structure Coordinates. *Proteins* **1992**, *12* (4), 345–364. https://doi.org/10.1002/prot.340120407.

[18] Protein Data Bank, *RCSB PDB: Homepage*. https://www.rcsb.org/.

[19] Chaudhary, N.; Saini, S. A Progress on Protein Structure Prediction Using Various Soft Computing Techniques. In *Computer Science and Information Technology Trends*; Academy and Industry Research Collaboration Center (AIRCC), 2022; 113–130. https://doi.org/10.5121/csit.2022.121410.

[20] Nguyen, T. T.; Pathirana, P. N.; Nguyen, T.; Nguyen, Q. V. H.; Bhatti, A.; Nguyen, D. C.; Nguyen, D. T.; Nguyen, N. D.; Creighton, D.; Abdelrazek, M. Genomic Mutations and Changes in Protein Secondary Structure and Solvent Accessibility of SARS-CoV-2 (COVID-19 Virus). *Sci. Rep.* **2021**, *11* (1), 3487. https://doi.org/10.1038/s41598-021-83105-3.

[21] Zhang, B.; Li, J.; Lü, Q. Prediction of 8-State Protein Secondary Structures by a Novel Deep Learning Architecture. *BMC Bioinformatics* **2018**, *19* (1), 293. https://doi.org/10.1186/s12859-018-2280-5.

[22] Shekhawat, U.; Roy Chowdhury (Chakravarty), A. Computational Investigation of Hydrophobic Profile of Spike Protein of MERS-CoV. *J. Xidian University*. **2022**, *16* (8), 389-398. https://drive.google.com/file/d/1nuJWAt6jIXEJ6OJhO0b42QWSwWG8B9nG/view.