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Structure-based *in silico* evaluation of ribavirin targeting the RSV G protein central conserved domain (CCD)

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Abstract:

Respiratory syncytial virus (RSV) remains a leading cause of acute lower respiratory tract infections worldwide, with limited therapeutic options available beyond monoclonal antibody-based interventions. The central conserved domain (CCD) of the RSV attachment glycoprotein (G), containing the CX3C chemokine-like motif, represents an underexplored but an important target for small molecule drug discovery. Molecular docking revealed a binding affinity of -6.70 kcal/mol with six hydrogen bond interactions. One hundred nanosecond molecular dynamics simulations showed stable binding, favorable energetics and conserved binding-site integrity. This work advances knowledge by identifying ribavirin as a thermodynamically competent binder of the RSV G protein CCD and establishing a structure-guided framework for optimizing CCD-targeted antiviral agents.

Keywords: Respiratory syncytial virus (RSV), G protein, ribavirin, CX3C motif**Background:**

Respiratory syncytial virus (RSV) is a major cause of acute lower respiratory tract infections worldwide. It causes over 3.6 million hospitalizations and more than 100,000 pediatric deaths annually according to WHO latest report of 2023-2025 [1]. It can cause bronchiolitis, pneumonia or even complete respiratory failure in older persons and those with compromised immune systems [2]. Its infection in adults is just as damaging and contagious as seasonal flu [3]. Early-life RSV infection has been linked to long-term respiratory consequences including asthma and recurrent wheezing [4]. Palivizumab, a humanized monoclonal antibody that targets the RSV fusion (F) protein, has shown reductions in hospitalization rates among high-risk infants. However, palivizumab provides no therapeutic benefit after infection and remains costly [5]. But high needs in other populations due to known viral escape mutations and that it is only authorized for newborns and infants [6]. Clinical development of F protein inhibitors is limited by antiviral resistance and unfavorable pharmacokinetic profiles [7]. All of these drawbacks highlight the necessity for identifying alternative viral targets that are less susceptible to resistance mechanisms. The RSV attachment glycoprotein G has emerged as a promising target for the development of novel antiviral therapeutics [8]. The RSV G protein mediates viral attachment through heparan sulfate proteoglycans and CX3CR1 receptors. This interaction is essential for viral infectivity and pathogenesis [9]. One structural and functional characteristic of the G protein is the highly central conserved domain (CCD), which extends from residues 166-196 [10]. The CX3C motif binds the CX3CR1 receptor on natural killer cells, plasmacytoid dendritic cells and CD8⁺ T cells [11]. This promotes a pathological Th2-biased immune phenotype that increases disease severity and prolongs viral persistence, especially in pediatric patients [12]. Mutation of the CX3C motif significantly reduces RSV pathogenicity, it can act as a therapeutic target [13]. The structural conservation of the CCD between RSV subgroups A and B is an important feature from a drug research perspective. According to studies, CCD-directed inhibitors a broad-spectrum antiviral benefit that is noticeably absent which are available in the market [14, 15]. Most studies have focused on antibody neutralization rather than small-molecule targeting of the CCD [16]. This supports the idea that this domain may have translational importance as a preventative

and therapeutic target [17]. Computational drug discovery techniques that include virtual screening, molecular docking, molecular dynamics simulations and MM/GBSA, have inhibitor binding mechanisms at atomic-level [18]. These approaches are particularly useful for RSV, where antigenic variation and rapid evolution challenge conventional therapies [19]. Therefore, it is of interest to investigate the RSV G protein CCD which remains underexplored as a small-molecule therapeutic target and its binding landscape and dynamic behavior upon ligand interaction have not been investigated much.

Materials and Methods:**Protein structure preparation:**

The three-dimensional structural model of the ectodomain G protein (edG) used in this study was derived from our previous homology modeling research, where the model was developed and validated through recognized *in silico* structural modeling methodologies, as there is currently no experimentally determined crystal structure for the RSV G protein ectodomain in the Protein Data Bank [19]. The protein structure was prepared for docking using the Schrödinger Suite's Protein Preparation Wizard [20]. At a physiological pH of 7.4, protonation states were assigned using PROPKA. The OPLS4 force field was then used to do a restricted energy minimization in order to resolve steric conflicts and produce a geometrically optimized structure that was prepared for docking and simulation [20].

Ligand preparation:

The PubChem compound database provided us with the ribavirin (PubChem ID: 37542) two-dimensional structure in Structure Data File (SDF) format. Three-dimensional ligands were prepared using LigPrep (Schrödinger) [20]. It produced pertinent stereoisomers and tautomeric forms, listed potential ionization states at physiological pH (7.0 ± 0.5) and minimized energy under the OPLS4 force field to create geometrically optimized low-energy conformers appropriate for molecular docking and subsequent molecular dynamics analyses.

Molecular docking:

Using the Glide module (Schrödinger) in Standard Precision (SP) mode, we performed molecular docking calculations. In order to

increase the chances of finding best binding site, the most energetically advantageous binding orientation, a blind docking strategy was purposefully used to enable an unbiased search of the entire extended portion of central conserved domain for possible binding sites, free from assumptions about the location of the active site. The region of the RSV G protein ectodomain that carries the CX3C motif was included in the receptor grid. To identify and characterize the optimal binding position for ribavirin, we examined the docking scores and interaction profiles of this particular region.

Molecular dynamics simulation:

The complex was assembled for simulation using the Desmond System Builder [20]. Using a 10 Å solvent buffer, it created an orthorhombic periodic boundary simulation box that was the same size in all three dimensions. In particular, the TIP3P water model was used to solvate the system and Na⁺ and Cl⁻ counterions were added at body-normal amounts to neutralize it. Using the AMBER14 force field and maintaining the NPT ensemble at 300 K and 1.01325 bars, we conducted production molecular dynamics simulations for 100 ns. Systems underwent the typical Desmond multi-stage relaxing process prior to production, which includes energy minimization with constraints, NVT and NPT equilibration phases in that sequence. Snapshots of the

trajectory were captured at intervals of 100 ps. We performed post-simulation studies, including as RMSD, RMSF, protein-ligand interaction profiling, principal component analysis, free energy landscape generation and dynamic cross-correlation mapping, using the Simulation Interaction Diagram (SID) tool in the Maestro [20].

MM/GBSA binding free energy calculation:

In an Ubuntu 24.02 computer system, the thermal_mmgbsa.py script was executed to compute binding free energy across 1,001 uniformly spaced trajectory snapshots. And the solvent molecules and non-standard atomic species were eliminated prior to each frame calculation. Based on the number of rotatable bonds in the ligand structure, we calculated conformational entropy penalties. As a result, we were able to get entropy-corrected binding free energy estimates, which provide us with a more precise thermodynamic representation of the protein-ligand interaction throughout the whole simulation.

Table 1: Binding affinities of the ribavirin from molecular docking against RSV G protein CX3C motif-containing region.

S.No	Compound ID	Binding Affinity (kcal/mol)
1	Ribavirin	-6.70

Table 2: MM/GBSA binding free energy components (mean ± SD, kcal/mol) of the ribavirin, calculated over 1,001 trajectory frames.

Complex	Δ Gbind (kcal/mol)	Coulomb (kcal/mol)	Covalent (kcal/mol)	H-Bond (kcal/mol)	Lipophilic (kcal/mol)	VdW (kcal/mol)
Ribavirin	-75.11 ± 5.24	-20.17 ± 4.78	-4.99 ± 1.94	-1.93 ± 0.97	-30.05 ± 4.91	-50.21 ± 5.00

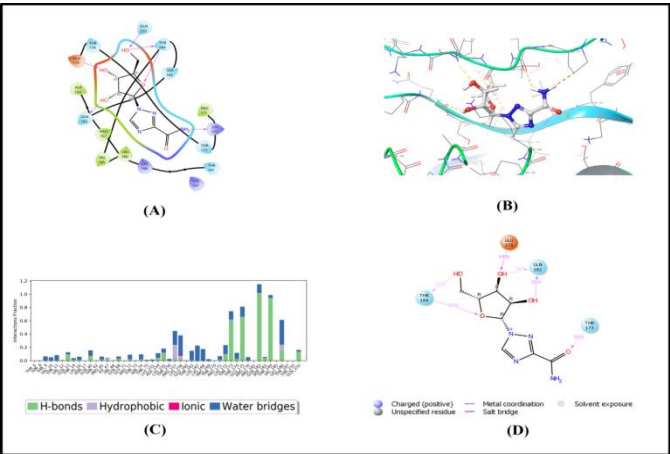


Figure 1: Molecular docking interaction profiles of ribavirin within the CCD domain of the RSV G protein ectodomain. (A) Two-dimensional (2D) ligand interaction map. (B) Three-dimensional (3D) ribbon representation of the docked ribavirin-CCD complex. (C) Time-averaged protein-ligand interaction (H-bonds, hydrophobic, ionic and water bridges), generated using the Simulation Interaction Diagram (SID) tool in Schrödinger Suite 2022-1. (D) 2D interaction diagram during the MD simulations, performed using Desmond and visualized using Simulation Interaction Diagram (SID) tool from Schrödinger Suite 2022-1.

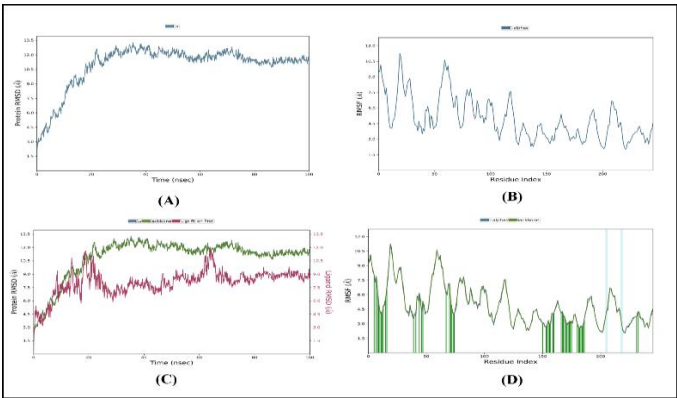


Figure 2: Representation of (A) RMSD and (B) RMSF graphs of the apoprotein throughout 100 ns MD simulation analysis. (C) RMSD graph (D) RMSF graph along with ligand i.e. Ribavirin.

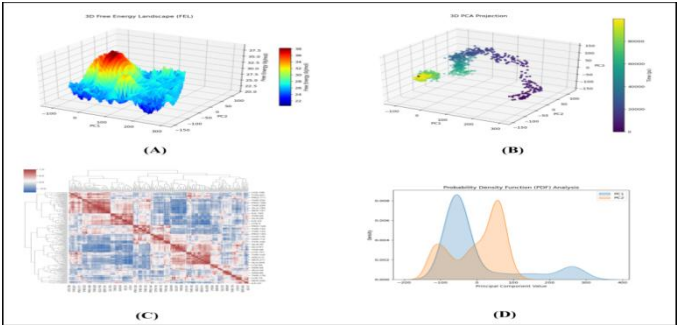


Figure 3: Conformational landscape and correlated motion analysis of the ribavirin-CCD domain of RSV G protein complex from the 100 ns MD simulation. **(A)** Gibbs free energy landscape generated by projecting the principal components, PC1 and PC2, during MD simulation with CCD binding domain of RSV G protein - Ribavirin. **(B)** Principal component analysis. 3D projections of trajectories showing different projections of ribavirin. **(C)** DCCM of Cα motions for the ribavirin-CCD domain of RSV G protein complex over 100 ns, showing correlated (red) and anti-correlated (blue) domain dynamics with preserved interactions at key binding residues (LYS151, LYS158). **(D)** Probability density function (PDF) analysis of PC1 (blue) and PC2 (orange), confirming a bimodal distribution for PC1 that is consistent with the two-state conformational transition identified in the free energy landscape.

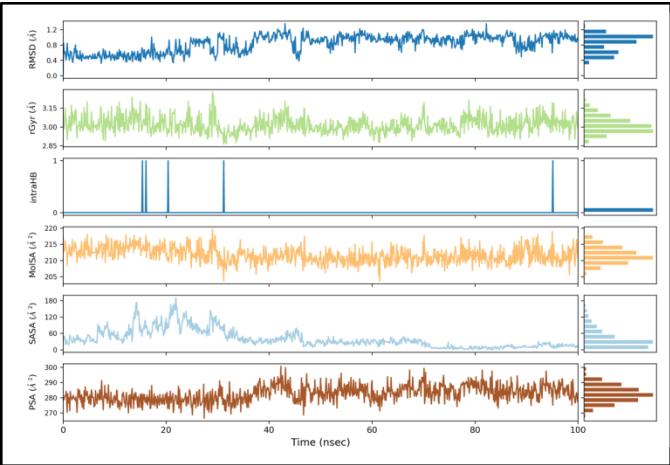


Figure 4: Time evaluation plot of ribavirin RMSD, Radius of gyration, intramolecular hydrogen bonds, molecular surface area, solvent accessible surface area (SASA) and polar surface area (PSA) showing system stability and equilibrium during the simulation.

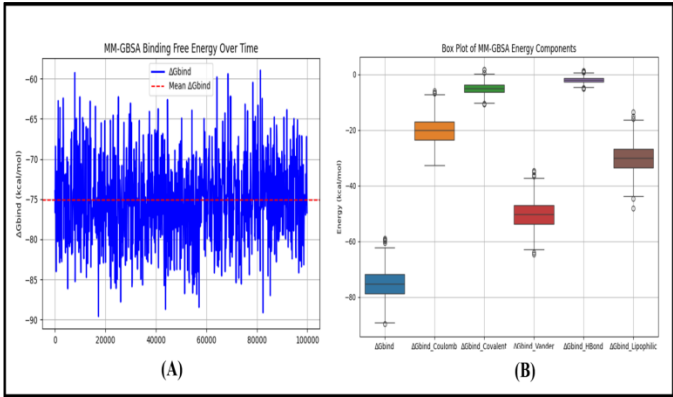


Figure 5: Illustration of MM/GBSA analysis conducted after MD simulations. **(A)** The influences of van der waal forces, hydrogen, lipophilic, covalent and coulomb bonds contributions to MM/GBSA binding free energy for CCD domain of RSV G

Protein-Ribavirin and **(B)** Time evolution changes of total binding free energy with moving average.

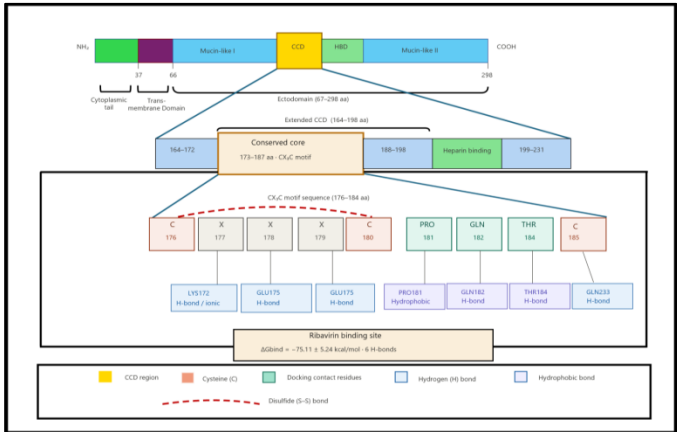


Figure 6: G protein gene of RSV showing Ribavirin site

Results and Discussion:

In this study, we identified and characterized the geometric and electrostatic properties of the CCD binding pocket to determine whether it possesses the structural features necessary for accommodating drug-like small molecules. Structure-based virtual screening was performed against the CCD containing the CX3C motif-containing region of the RSV G protein ectodomain. A docking binding affinity of -6.70 kcal/mol was observed (**Table 1**), indicating that the CCD pocket can accommodate a drug-like molecule with favorable binding energetics. This is consistent with reported binding affinities for inhibitors targeting comparable viral glycoprotein domains [21]. Six geometrically well-defined hydrogen bonds were identified at functionally significant CCD residues, GLN233, THR184, GLU175, GLN182 and LYS172 that establish a rich polar interaction landscape (**Figure 1A-D**). The 100 ns molecular dynamics simulation was subsequently performed to characterize the dynamic behavior of the ribavirin-CCD complex under physiological conditions. After equilibration, the protein backbone RMSD stabilized within a relatively narrow range of $0.85\text{--}1.10$ Å, indicating satisfactory structural convergence of the protein during the simulation (**Figure 2A**). The RMSF analysis showed that the binding-site residues Glu175 (1.53 Å), Gln182 (1.16 Å), Thr184 (1.14 Å) and Pro181 (1.33 Å) exhibited relatively low fluctuations compared with other residues, suggesting that the local binding region remained comparatively stable (**Figure 2B**). The RMSD profile of ribavirin further showed its conformational behavior throughout the 100 ns trajectory (**Figure 2C**), while the RMSF profile indicated residue-level fluctuations associated with the ligand during the simulation (**Figure 2D**). These contacts that involve the residues are functionally critical for CX3CR1 recognition that also support that CCD pocket geometry is suited for competitive small molecule inhibition. This shows that the CCD pocket, within an intrinsically disordered protein region, is capable of adopting a stable, well-defined conformation upon small molecule binding. The system underwent a full conformational state transition

during the simulation, with initial trajectory frames centered around PC1 values of +200 to +300 gradually shifting to -60 to -100 in the later simulation, crossing a free energy barrier of roughly 35–38 kJ/mol between two spatially distinct energy basins (**Figure 3A**). The 3D PCA trajectory projection, colour-coded by simulation time and it is captured by progressive directional shift in conformational space, with the trajectory migrating from one cluster to a second spatially distinct cluster over the course of the simulation (**Figure 3B**). Dynamic cross-correlation mapping confirmed that the binding relevant residues LYS151 and LYS158 moved steadily during the entire trajectory (**Figure 3C**). This shows that despite the proteins other shape changes, the binding interface remained consistent. The bimodal distribution observed in the probability density function (PDF) analysis of PC1 and PC2 values independently confirmed the presence of two well-separated conformational substates sampled during the trajectory (**Figure 3D**). Collectively, these analyses are consistent with the well-established intrinsic structural plasticity and disorder of the RSV G protein ectodomain [21]. This indicates that the CCD pocket exists in at least two distinct conformational states during simulation a property that is relevant to structure-based drug design, as both states can be targeted for ligand optimization. These results were supported by protein-ligand contact analysis, which shows that the stability of hydrogen bonds with GLU175, GLN182, THR184 and LYS172 throughout the trajectory (**Figure 4**). By forming hydrogen bonding networks in the solvent, water-bridged contacts may further contribute to the complex thermodynamic stability. During the late trajectory phase, ligand SASA gradually dropped from roughly 180 Å² to nearly zero by 60 ns, indicating that ribavirin was becoming further buried in the protein structure. Because ribavirin has seven rotatable bonds and a flexible ribose moiety, this could indicate that it is shifting to a secondary binding site rather than remaining in the initial one. An averaged binding free energy of -75.11 ± 5.24 kcal/mol was obtained from the MM/GBSA calculations across 1,001 trajectory frames. The most significant stabilizing factors were the van der Waals (-50.21 ± 5.00 kcal/mol) and lipophilic (-30.05 ± 4.91 kcal/mol) terms, which were followed by the hydrogen bonding (-1.93 ± 0.97 kcal/mol) and Coulombic (-20.17 ± 4.78 kcal/mol) interactions (**Table 2**). The modified ΔG_{bind} of -68 to -72 kcal/mol is still highly beneficial after accounting for the entropy penalty from seven rotatable bonds (**Figure 5**). This indicates that one of the RSV G proteins CCD is thermodynamically competent binders for ribavirin (**Figure 6**). Therefore, it may be a suitable active site for other inhibitors as well. Despite the fact that there are six docking-identified H-bonds, the dynamic average of interaction geometry across the 1,001 frames is responsible for the comparatively minor impact of explicit hydrogen bonding terms on the overall binding free energy. This is due to the fact that, in comparison to the static docked configuration, transient conformational changes reduce the time-averaged H-bond occupancy in the docked site.

Conclusion:

We show the computational characterization of the RSV G protein's central conserved domain (CCD) as a small molecule binding pocket. Over a 100 ns simulation, ribavirin maintained a thermodynamically favorable binding free energy of -68 to -72 kcal/mol and forming six hydrogen bonds at the CCD interface. Stable correlated dynamics at key residues and persistent interaction contacts support CCD as an efficient and durable pharmaceutical target.

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Author contribution:

Ufaq Razi has designed, performed, evaluated and written the research, Anwar Ahmed has performed data evaluation and manuscript preparation, Shama Parveen has designed, evaluated, written and proofread the manuscript.

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References:

- [1] [https://www.who.int/news-room/fact-sheets/detail/respiratory-syncytial-virus-\(rsv\)](https://www.who.int/news-room/fact-sheets/detail/respiratory-syncytial-virus-(rsv))
- [2] Choi JY *et al.* *Tuberc Respir Dis (Seoul)*. 2025 89:18. [PMID: 41255075]
- [3] Belongia EA *et al.* *Open Forum Infect Dis*. 2018 5:ofy316 [PMID: 30619907]
- [4] Sigurs N *et al.* *Thorax*. 2010 65:1045 [PMID: 20581410]
- [5] Piralla A *et al.* *Int Immunopharmacol*. 2025 166:115591 [PMID: 40997502]
- [6] Hammitt LL *et al.* *N Engl J Med*. 2022 386:837 [PMID: 35235726]
- [7] DeVincenzo JP *et al.* *N Engl J Med*. 2014 371:711 [PMID: 25140957]
- [8] Johnson SM *et al.* *PLoS Pathog*. 2015 11:e1005318 [PMID: 26658574]
- [9] Meineke R *et al.* *npj Viruses*. 2024 2:63 [PMID: 40295855]
- [10] Juarez MG *et al.* *Sci Rep*. 2025 15:8666 [PMID: 40082629]
- [11] Rivas-Fuentes S *et al.* *Int J Mol Sci*. 2024 25:9800 [PMID: 39337288]
- [12] Ferrier Esposito A *et al.* *Viruses*. 2026 18:150 [PMID: 41754493]
- [13] Jones HG *et al.* *PLoS Pathog*. 2018 14:e1006935 [PMID: 29509814]
- [14] Su C *et al.* *NPJ Vaccines*. 2022 7:168 [PMID: 36535957]
- [15] Boyoglu-Barnum S *et al.* *J Virol*. 2017 91:e02059-16 [PMID: 28275196]
- [16] Fuentes S *et al.* *J Virol*. 2007 81:8361 [PMID: 17494063]
- [17] Altharawi A & Alqahtani SM. *Pharmaceutics*. 2026 18:565 [DOI: 10.3390/pharmaceutics18050565]
- [18] Langedijk AC *et al.* *Nat Commun*. 2024 15:3083 [PMID: 38600104]

[19] Hamza A *et al.* *Molecules*. 2021 **26**:7398 [PMID: 34885979]

[20] <https://www.schrodinger.com/>

[21] Śledź P & Caflisch A. *Curr Opin Struct Biol*. 2018 **48**:93

[PMID: 29149726]

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