IOCAB 2025 Conference

The 1st International Online Conference by *Antibodies*. Therapeutic Antibodies: New Trends in Discovery, Developability and Characterization

13–14 October 2025 | Online

Chaired by Prof. Dr. Arne Skerra

antibodies MDPI

From Disorder to Defense: Intrinsically Disordered Region Based Antibody Engineering for Chandipura virus

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INTRODUCTION & AIM

Chandipura virus (CHPV), a neurotropic member of the *Rhabdoviridae* family, is an emerging pathogen in India associated with acute encephalitis and high fatality, yet no licensed vaccines or antivirals exist. Dark proteome analysis has shown that the CHPV phosphoprotein (P) contains the highest proportion of intrinsically disordered regions (IDRs, >30%), which are flexible, multifunctional, and enriched with molecular recognition features (MoRFs) critical for viral replication and host manipulation. Despite their biological importance, IDPs have been largely overlooked in vaccine design due to structural heterogeneity. In this study, I present the first IDP-guided antibody design pipeline for CHPV. Using consensus disorder prediction, MoRF and B-cell epitope mapping, AlphaFold2 structural modeling, and molecular dynamics simulations, I identified disordered yet immunogenic regions of P protein as potential antibody targets. This integrative strategy highlights the novelty of targeting conformationally adaptable epitopes and provides a rational framework for developing monoclonal antibodies against CHPV, with potential application to other RNA viruses rich in disorder.

METHODS

The complete proteome of Chandipura virus (CHPV), consisting of the N, P, M, G, and L proteins, was retrieved from the UniProt database, and the intrinsic disorder propensity of each protein was re-evaluated using a consensus approach integrating Espritz, IUPred2A, and DISOPRED3. A disorder score threshold of >0.5 was applied to classify intrinsically disordered regions (IDRs). Among all CHPV proteins, the phosphoprotein (P) showed the highest disorder content (>30%), further confirming earlier studies and establishing it as the primary candidate for further analysis. To explore its immunogenic potential, the disordered segments of P were screened using FusoDrop, and B-cell epitope prediction was carried out with BepiPred-3.0. Only epitopes overlapping with IDRs were prioritized, as these regions are conformationally flexible and highly immunogenic. Structural ensembles of the P protein were then generated using AlphaFold2, which allowed the modeling of both structured and disordered domains. Predicted models were evaluated by pLDDT confidence scores and cross-validated with disorder profiles to ensure reliable representation of flexible regions. The most representative model was subjected to molecular dynamics (MD) simulations on the WebGro platform under explicit solvent conditions for 100 ns using the GROMOS96 43a1 force field. Key parameters, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg), were analyzed to assess structural stability and flexibility of epitope-rich IDRs. This integrative pipeline, comprising disorder prediction, MoRF mapping, epitope identification, structural modeling, and MD simulation, enabled the identification of structurally adaptable, epitope-rich targets within the CHPV P protein, offering a rational basis for monoclonal antibody design.

RESULTS & DISCUSSION

All five proteins of Chandipura virus (CHPV) were assessed for intrinsic disorder. Mean disorder content varied significantly: Phosphoprotein (53.92%), Matrix protein (24.16%), Nucleoprotein (15.71%), Glycoprotein G (14.84%), and RNA-directed RNA polymerase L (10.73%). Among these, the phosphoprotein (P) clearly exhibited the highest disorder propensity (>30%), consistent across X-ray, DisProt, and NMR-based predictions. This established P protein as the most disordered viral component and the prime candidate for further immunoinformatics analysis. FuzDrop analysis indicated a high probability of spontaneous liquid–liquid phase separation (pLLPS = 0.91) in the P protein (Figure 1).

Several droplet-promoting regions (18–44, 56–80, 102–116, 171–218) overlapped with predicted aggregation hotspots and context-dependent interaction motifs, suggesting that the disordered nature of P may facilitate dynamic multivalent interactions during viral replication. BepiPred-3.0 analysis identified multiple linear B-cell epitope clusters across the phosphoprotein sequence, several of which overlapped with highly disordered and droplet-prone regions (Figure 2). This co-localization highlights the immunogenic potential of disordered epitopes, which may be more accessible to antibody recognition compared to structured domains. AlphaFold2 models of the P protein revealed low-confidence regions (low pLDDT scores) aligning with disorder-prone sequences in Figure 3. To capture dynamic flexibility, the best model was subjected to MD simulation (25 ns, GROMOS96 force field). RMSF analysis showed pronounced fluctuations in epitope-rich disordered segments, supporting their structural plasticity and functional adaptability. Together, these findings confirm that CHPV phosphoprotein harbors conformationally flexible, epitope-rich disordered regions with strong phase separation tendencies. Such features make them promising targets for IDP-guided antibody design, offering a novel immunotherapeutic avenue against CHPV. This approach also establishes a generalizable framework for targeting IDPs in other RNA viruses.

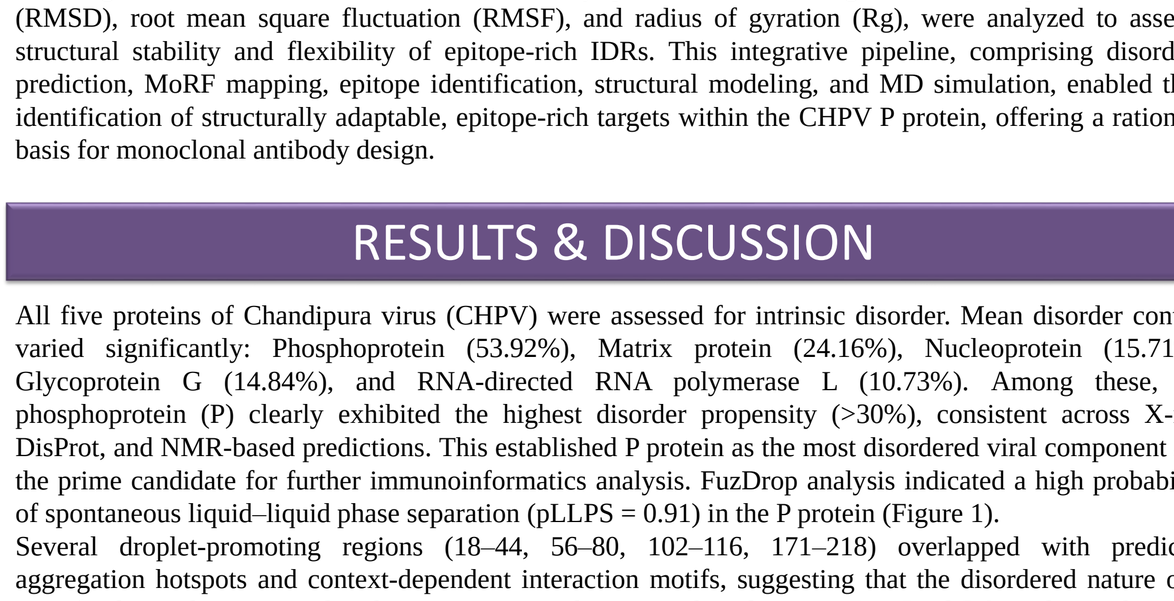

Figure 2. Predicted epitope distribution in CHPV phosphoprotein (P). BepiPred-3.0 linear epitope scores plotted against sequence position. Multiple immunogenic peaks are observed, particularly overlapping with disordered regions, highlighting their potential as antibody targets.

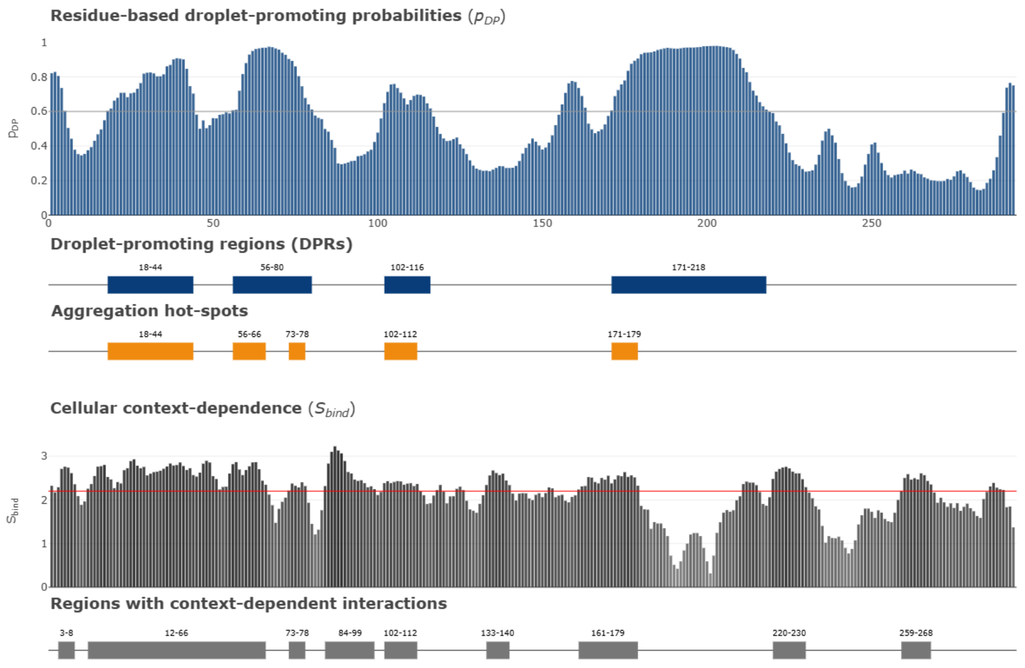

Figure 1. Phase separation and interaction propensity of P protein. FuzDrop predictions showing residue-level droplet-promoting probabilities (PDP), droplet-promoting regions (DPRs), aggregation hotspots, and context-dependent interaction regions. High pLLPS (0.91) suggests strong liquid–liquid phase separation potential in disordered domains.

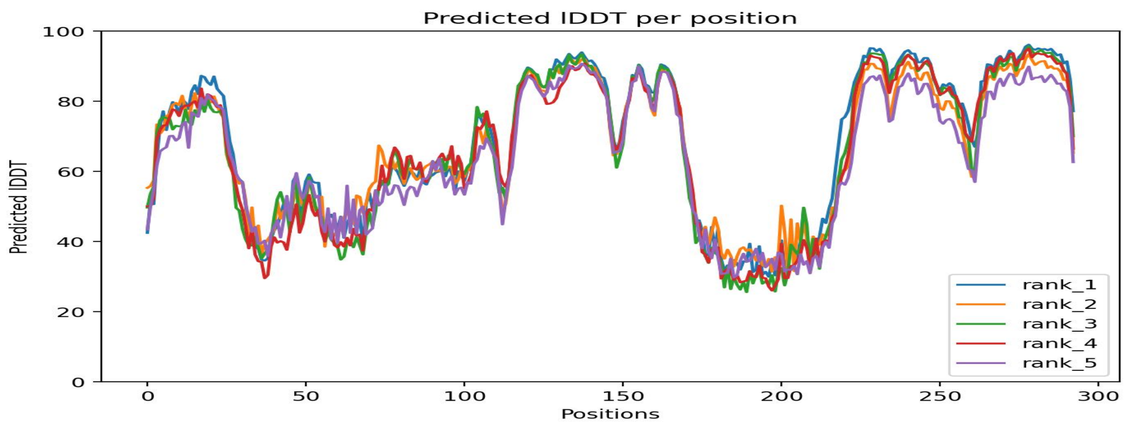

Figure 3. Structural modeling and confidence assessment of P protein. AlphaFold2 predicted models with IDDT scores per residue. Low-confidence regions coincide with disordered and epitope-rich segments, supporting their structural plasticity.

CONCLUSION

This study demonstrates that the CHPV phosphoprotein, with its high intrinsic disorder, strong phase separation propensity, and epitope-rich flexible regions, represents a promising target for antibody design. By integrating disorder profiling, epitope mapping, structural modeling, and molecular dynamics, I establish an IDP-guided pipeline that highlights disordered regions as novel, adaptable, and immunogenic targets. This approach not only offers a potential therapeutic strategy against CHPV but also provides a generalizable framework for antibody discovery in other RNA viruses rich in disorder.

FUTURE WORK / REFERENCES

Future efforts will focus on the experimental validation of the predicted B-cell epitopes through ELISA and peptide array assays, followed by the development of monoclonal antibodies specifically targeting disordered regions of the CHPV phosphoprotein. These antibodies will be evaluated for their efficacy in viral neutralization assays to establish therapeutic potential. In parallel, the proposed IDP-guided pipeline will be extended to other neurotropic RNA viruses, aiming to establish a broad-spectrum strategy for antibody discovery against intrinsically disordered viral proteins.

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