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From Disorder to Design: Ensemble-Based Computational Antibody Discovery for IDP Targets in Zika Virus

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INTRODUCTION & AIM

Zika virus (ZIKV), a mosquito-borne flavivirus, poses a major public health threat due to its association with neurological complications such as congenital microcephaly and Guillain–Barré syndrome. Despite repeated outbreaks, no licensed vaccines or specific antiviral therapies are currently available. Recent studies have revealed that several ZIKV proteins harbor intrinsically disordered regions (IDRs), which lack a fixed 3D structure and mediate dynamic interactions with host factors, facilitating viral replication and immune evasion. These IDR-rich segments, often referred to as part of the viral "dark proteome," are increasingly recognized as potential yet underexplored therapeutic targets.

Aim of This study was to Identify ZIKV proteins enriched in intrinsic disorder using consensus prediction tools, followed by Selection of disordered proteins with >30% disorder content (M, pr, and Capsid) for further analysis and Prediction of B-cell epitopes within these disordered regions to evaluate their potential as antibody targets. By integrating disorder profiling with epitope mapping, we establish a framework for computational antibody discovery against ZIKV disordered proteins, laying the groundwork for future antibody design and therapeutic intervention.

METHODS

The complete Zika virus (ZIKV) polyprotein sequence was retrieved from the UniProt database (ID: A0A024B7W1), which provides curated information on viral genomic organisation and protein features. The ZIKV polyprotein, like other flaviviruses, encodes both structural and non-structural proteins in a single continuous sequence. Based on UniProt feature mapping and residue boundaries, the polyprotein was segmented into its constituent proteins: the Capsid protein (C), the precursor membrane protein (prM), which further divides into the pr peptide and the M protein, the Envelope glycoprotein (E), and the seven non-structural proteins NS1 through NS5. This annotation ensured that each viral protein could be independently evaluated for disorder propensity and immunogenic potential.

To identify candidate proteins enriched in intrinsic disorder, multiple complementary predictors were applied, including DISPROT database annotations, IUPred3, flDPnn, ESpritz, and PredictProtein. These tools estimate disorder content by assessing sequence composition, structural flexibility, and absence of stable tertiary folding. Proteins exhibiting >30% disorder content were considered significantly disordered and were shortlisted for further antibody profiling. This consensus-based approach increased reliability and minimised predictor-specific bias.

Following target selection, linear B-cell epitope prediction was performed using BepiPred-3.0, which integrates protein language models and experimental datasets for improved epitope accuracy. The predicted epitope-rich regions were carefully compared with intrinsic disorder profiles to highlight overlapping stretches. Such overlap indicates epitopes that are not only accessible but also highly flexible, potentially enhancing their immunogenicity. The distribution of epitopes along protein sequences was further visualised as probability heat maps, enabling identification of hotspot regions within the M protein, pr peptide, and Capsid protein. These regions were prioritised as potential antibody-binding targets for downstream computational antibody design.

RESULTS & DISCUSSION

Disorder Profiling

The Zika virus polyprotein (UniProt ID: A0A024B7W1) was segmented into individual proteins and analyzed using consensus predictors (DISPROT, IUPred3, flDPnn, ESpritz, PredictProtein). Proteins with >30% disorder content were classified as intrinsically disordered proteins (IDPs). Proteins M, Pr, and Capsid exhibited the highest disorder propensity, with mean PPID values of 50.6, 46.9, and 45.2 respectively (Table 1). When NS2B also showed moderate disorder (60.7% by DISPROT) but fell below the mean PPID threshold and Other proteins (NS1, NS3, NS4B, NS5, NS2A, Envelope) displayed lower disorder content (<30%), suggesting more stable structured states.

Table 1. Disorder content of ZIKV proteins based on consensus profiling

Protein name	length	DISPROT based % disorder	NMR based % disorder	X-RAY based % disorder	Mean PPID
M	75	100	25.33	26.66	50.66
Pr	93	100	18.27	22.58	46.95
Capsid	121	86.77	22.31	26.44	45.17
NS2B	130	60.76	10	18.46	29.74
NS4A	127	29.92	18.11	18.89	22.30
NS1	352	4.545	38.06	14.2	18.93
NS5	903	6.644	35.32	7.419	16.46
NS4B	251	0	24.3	23.5	15.93
NS3	617	7.293	25.12	9.238	13.88
ENVELOPE	500	0	29.4	4.2	11.2
NS2A	226	0	10.61	9.292	6.63

Epitope profiling

BepiPred-3.0 linear B-cell epitope analysis revealed strong epitope signals across the M protein (residues 216–290), aligning with its fully disordered profile, followed by Pr protein showed high epitope density, particularly in residues 40–90, overlapping with disordered segments and Capsid protein displayed clustered epitopes in both N- and C-terminal regions, overlapping with disordered stretches. The overlap of predicted epitopes profiling with highly disordered regions supports the hypothesis that IDRs in viral proteins contribute to antigenicity and immune recognition. Disordered epitopes may enhance antibody accessibility due to structural flexibility. Among ZIKV proteins, M, Pr, and Capsid emerge as prime candidates for antibody targeting. These findings validate the initial steps of our pipeline and provide a foundation for computational antibody design and docking studies.

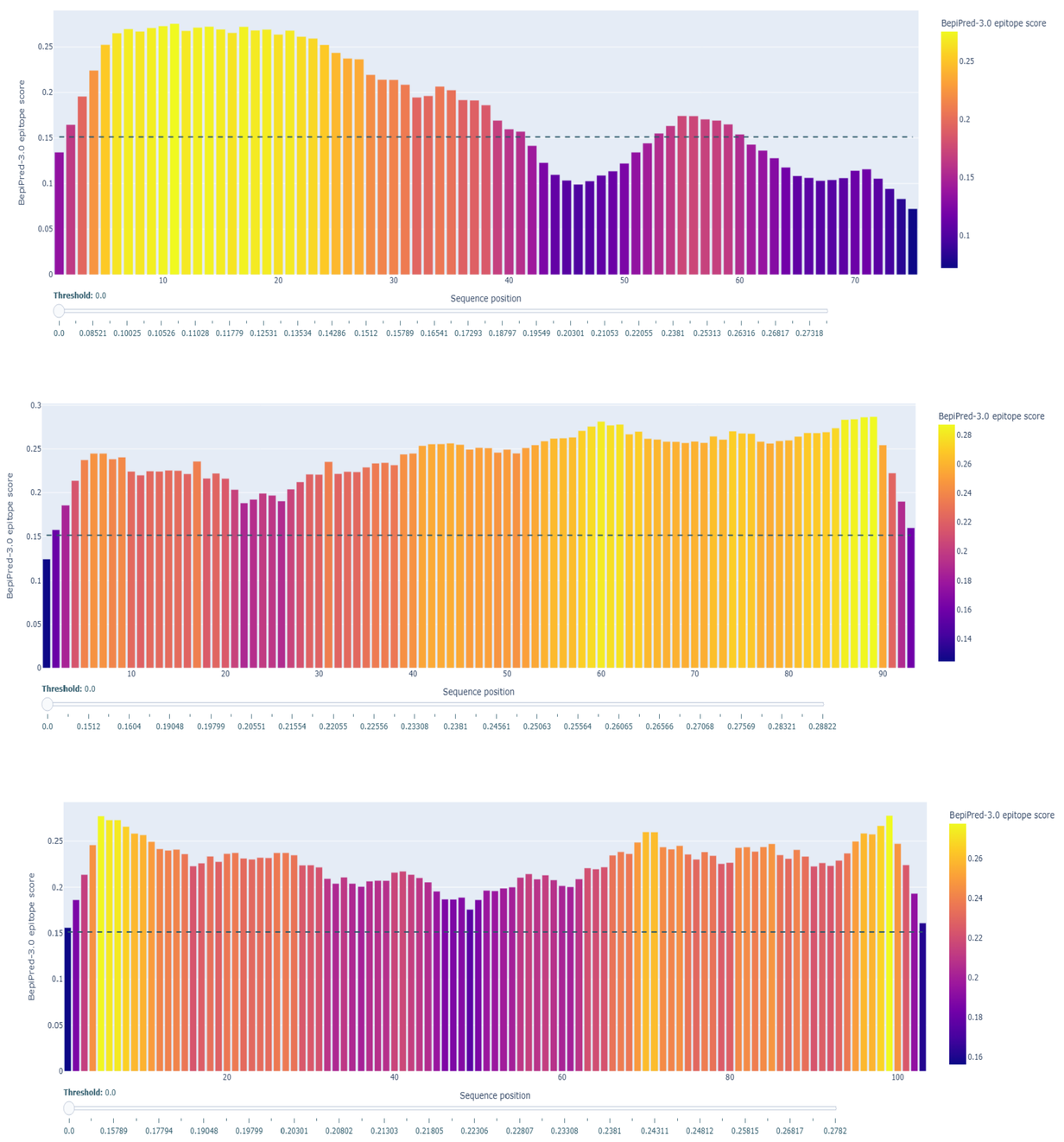

Figure 1. Epitope prediction for disordered ZIKV proteins (M, Pr, Capsid) using BepiPred-3.0. Predicted linear B-cell epitopes (yellow peaks) overlap with intrinsically disordered regions identified through consensus profiling. Strong epitope signals were observed across the M protein (residues 216–290), while the pr peptide showed high-density epitopes within residues 40–90. The Capsid protein displayed clustered epitope regions in both N- and C-terminal segments, highlighting the immunogenic potential of disordered viral proteins.

CONCLUSION

Comprehensive disorder profiling of the Zika virus polyprotein revealed that the M protein, pr peptide, and Capsid protein exhibit exceptionally high levels of intrinsic disorder, with disorder content exceeding 86%. These proteins were therefore designated as prime candidates for antibody targeting, as their structural flexibility may provide multiple accessible sites for immune recognition. Subsequent B-cell epitope prediction using BepiPred-3.0 confirmed this hypothesis, identifying strong clusters of predicted epitopes that directly overlap with intrinsically disordered regions. This overlap suggests that the conformational plasticity of these proteins enhances their immunogenic potential, enabling them to act as dynamic targets for neutralising antibodies. The findings from this study underscore the critical role of intrinsically disordered regions (IDRs) in shaping the antigenic landscape of Zika virus. Unlike well-structured viral proteins, IDRs provide adaptable and accessible epitopes that could be harnessed in the design of novel antibody therapeutics. By integrating disorder analysis with epitope prediction, this work lays the preliminary foundation for a computational pipeline in antibody discovery and engineering against the viral dark proteome. Such an approach not only advances our understanding of ZIKV immune evasion strategies but also opens a promising new direction for disorder-based drug design, specifically targeting flexible and unstructured protein regions that have traditionally been overlooked in classical antiviral strategies.

FUTURE WORK / REFERENCES

In the next phase, we aim to extend this study by performing antibody–antigen docking of predicted epitopes with human germline scaffolds using HADDOCK and ClusPro, followed by paratope optimisation through ABpredict2. Further, developpability and immunogenicity assessments (Thera-P, IEDB MHC-II binding) will be carried out to prioritize clinically viable candidates. Experimental validation using ELISA and neutralisation assays against ZIKV IDR-rich proteins will be critical to confirm computational predictions. Ultimately, the pipeline will be generalised for comparative analysis of other flaviviruses, enabling a systematic approach to design antibodies against viral dark proteomes.

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