COMPUTATIONAL DESIGN OF PROTEINS TARGETING THE REPLICATION MACHINERY OF MONONEGAVIRALES

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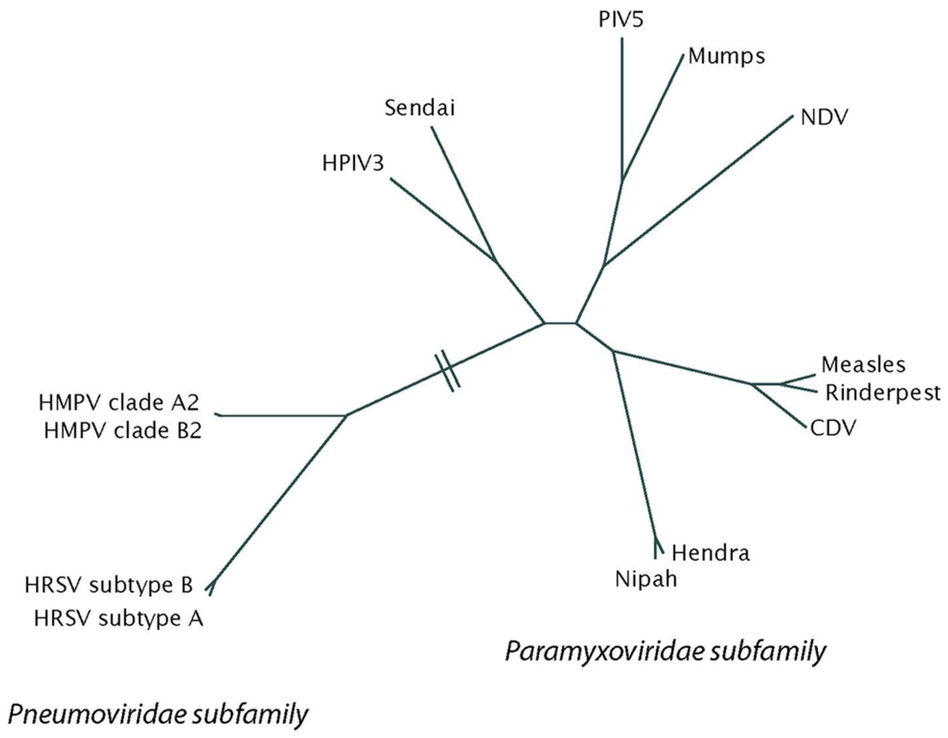
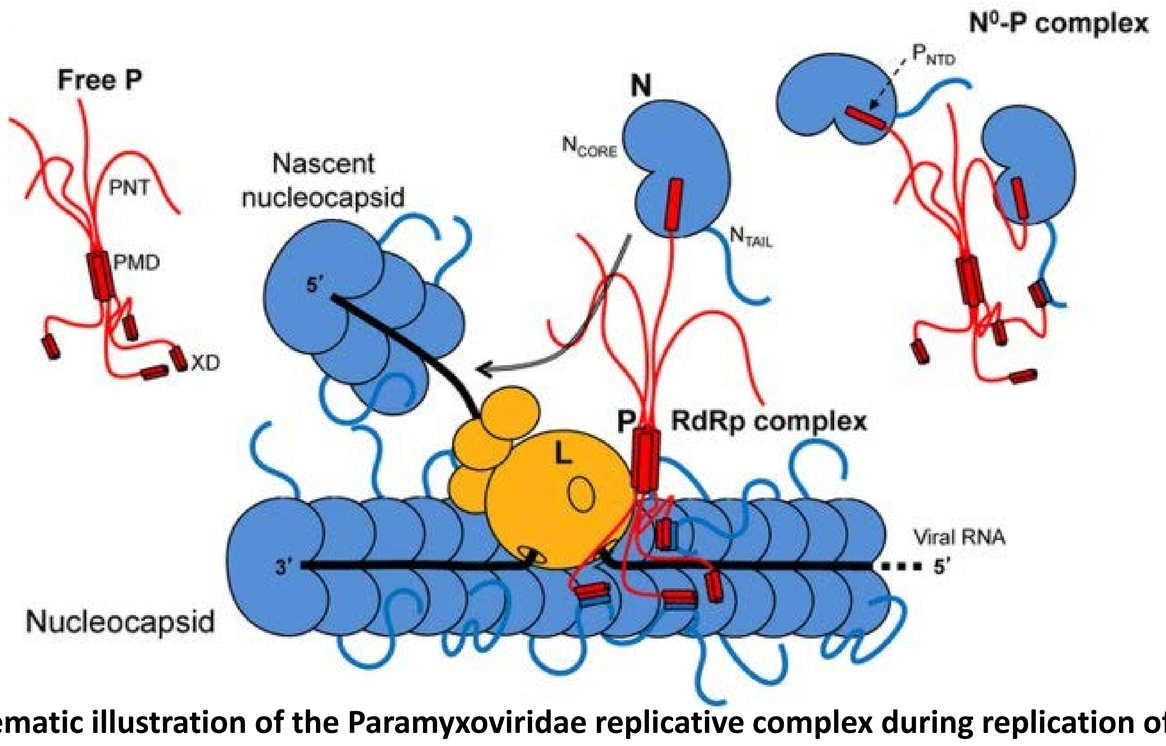
Background: Mononegavirales, comprising significant human and veterinary pathogens such as pneumoviruses and paramyxoviruses, rely on a multi-protein replication complex for viral RNA synthesis. In light of the urgent need for innovative antiviral strategies, our study employs an integrated computational framework to design protein binders that target critical components of this machinery, specifically the N (nucleoprotein), P (phosphoprotein), and L (large polymerase) proteins.

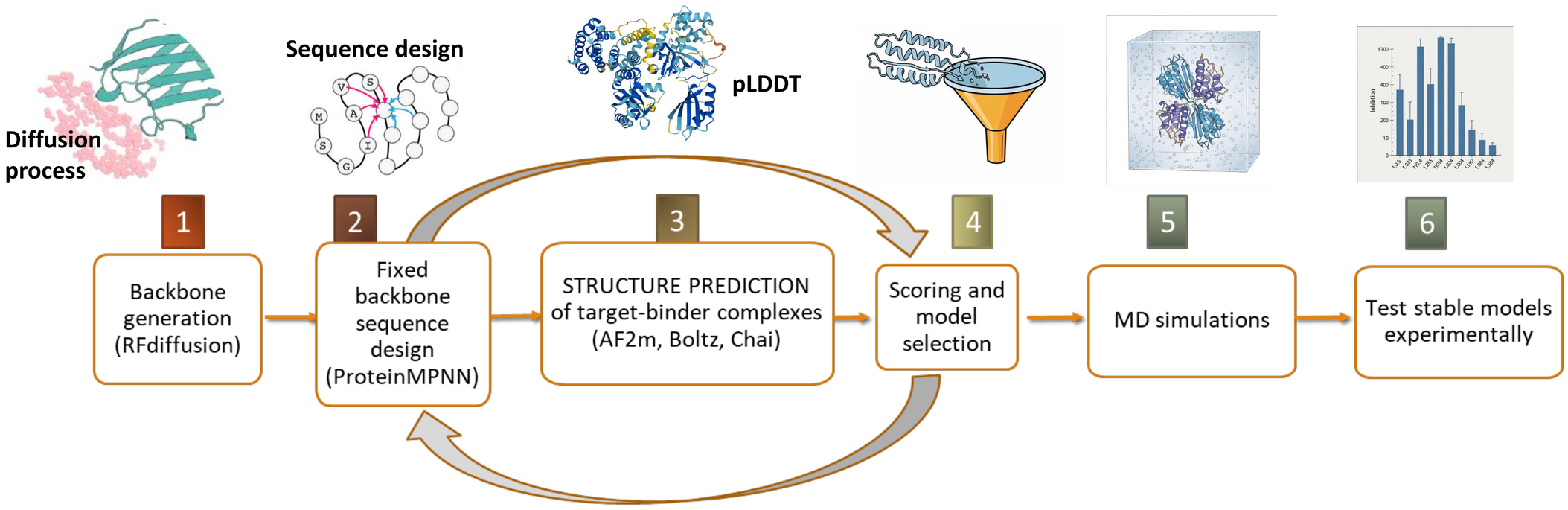


Phylogenic tree showing the major pathogenic viruses of the *pneumoviridae* & *paramyxoviridae* families

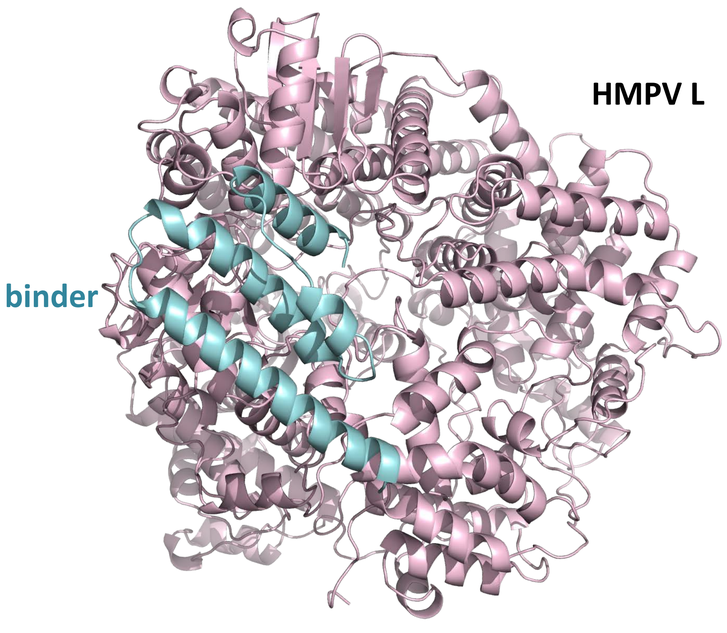


Schematic illustration of the Paramyxoviridae replicative complex during replication of the viral genome or anti-genome. N encapsidates both the viral RNA used as a template and the neo-synthetized RNA (adapted from Longhi et al., 2017, DOI:10.1007/s00018-017-2556-3**).**

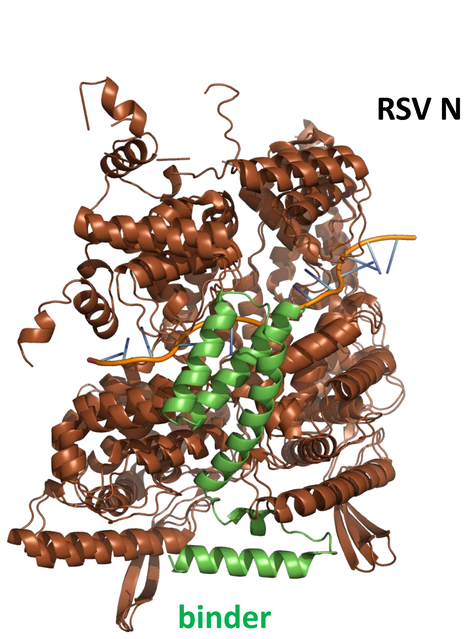
Methodology: Our design pipeline leverages state-of-the-art computational methods and is structured as follows: initial scaffold generation is achieved using RFdiffusion to sample potential binding conformations, followed by backbone sequence optimization with proteinMPNN. We generate complex models using AlphaFold2 to obtain interface pLDDT scores and then combine these with additional geometric and thermodynamic metrics to robustly rank candidates for optimal stability and target alignment. For selected leads, we optionally integrate molecular dynamics (MD) simulations to observe dynamic behavior in a near-physiological environment, with subsequent reranking based on the analysis of MD trajectories.



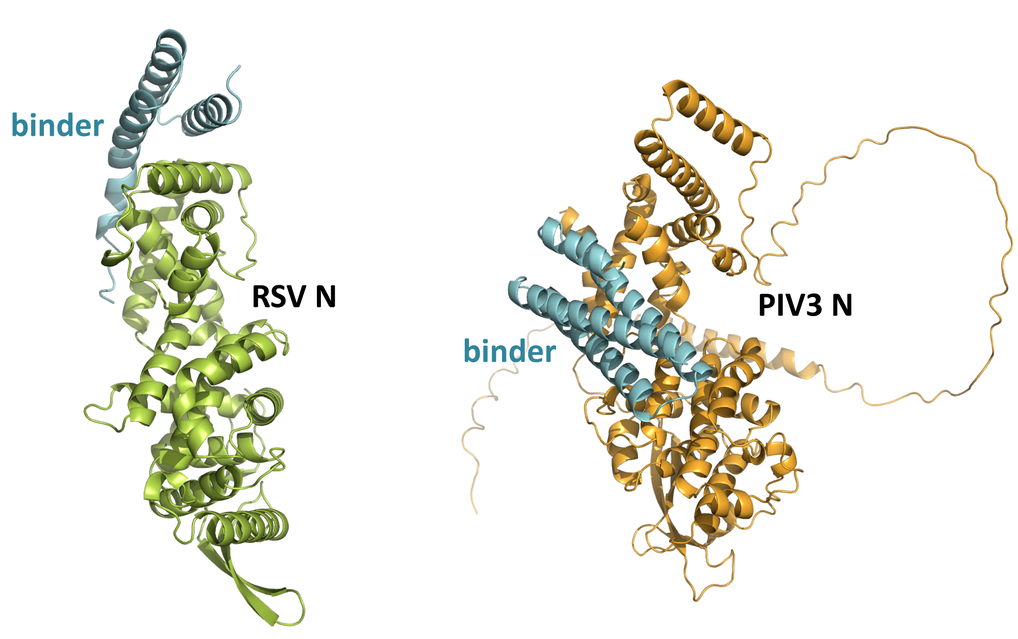
Results: Based on available structural data, we apply our computational pipeline to design novel proteins targeting critical steps in the transcription/replication cycle, such as RNA entry/exit from polymerase, RNA transcription and replication, RNA encapsidation, N oligomerization, N-P and P-L interactions.



HMPV L binders targeting RNA entry/exit sites



RSV NC binders preventing access to viral RNA



RSV/PIV3 N binders: inhibiting RNA encapsidation and N oligomerization

Conclusion: While specific experimental validation remains underway, preliminary results show that nanomolar binders can be obtained for most targets in a single round of experimental testing with less than 10 designs. In addition to engaging the replication machinery, these binders also offer powerful tools for mechanistic studies by enabling targeted stalling of the replication complex at defined interfaces. This dual utility underscores the potential of our computational approach—not only in accelerating the development of targeted antiviral therapeutics but also in advancing our understanding of the mechanistic underpinnings of viral replication.