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Computational Modelling of Hemagglutinin Protein Mutational Landscapes: A Network Theory Approach to Influenza Vaccine Target Selection

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ABSTRACT

Influenza vaccine design remains challenging because rapid mutations in the hemagglutinin (HA) protein can reduce vaccine effectiveness.

Findings suggest that residues with high centrality, particularly those near the receptor-binding and fusion regions, are essential for maintaining HA functionality and structural stability, even as mutations occur. Tests against the evolution of historical strains and neutralization assays confirmed that these sites have lower mutation tolerance and preserved antigenicity.

Our network-based method acts as a strong and adaptable tool to design appropriate antigens, not only for the flu, but also for other rapid viral pathogens. It is regrettable that the current vaccine strategies often find it difficult to keep up with quick growth trunks. In this paper, we present an advanced computational framework that merges structural bioinformatics, sequence analysis, and network theory to chart the mutational landscape of HA and identify potential vaccine targets that could endure these shifts.

Keywords: Influenza, Hemagglutinin (HA), Network Theory, Mutational Landscape, Vaccine Design.

1. Introduction

Influenza A virus remains a significant global health threat, which contributes to substantial annual morbidity and mortality. The primary challenge in combating this pathogen lies in the rapid antigenic variation of the surface hemagglutinin (HA), which facilitates viral entry into host cells and serves as a primary target for neutralizing antibodies. Traditional vaccine strategies rely primarily on reactive stress-specific approaches that cannot protect against circulating variants. Although these methods have achieved moderate success, they often fail to keep pace with the virus's high mutation rate, thereby reducing vaccine efficacy.

Influenza A viruses cause considerable morbidity and mortality worldwide on an annual basis. This item occurs largely because of their extraordinary ability to change and adapt, especially through mutations in the hemagglutinin (HA) glycoprotein. HA is essential for virus penetration into host cells by binding to sialic acid receptors and facilitating the fusion between the viral envelope and the cell membrane, and it is also the best target for neutralizing antibodies. But as the virus mutates in the HA amino acids (Petrova & Russell, 2018; Skehel & Wiley, 2000; Wilson et al., 1981).

Recent advances in computational biology and structural virology have introduced alternative strategies for vaccine design, including EPTOP mapping, evolutionary analysis, and machine learning-controlled antigen prediction. However, these approaches often overlook the mutual structural and functional dependencies among agglutinins that could reduce their evolutionary barriers. Traditional vaccine target selection tends to focus on the surface bits of newer strains that change often, which means they end up playing catch-up. An alternative strategy is to target the parts of the virus that don't change much but are essential to its function.

This paper describes a new computer model based on Residue Interaction Networks (RINs) to analyze mutational changes in the hemagglutinin (HA) protein. Protein structure, how different residues interact, and real-world data on mutation rates are integrated to build a network-based representation of HA. Various metrics are then used to assess the importance of each residue to the whole protein (Di Paola et al., 2013; Impagliazzo et al., 2015; Vendruscolo et al., 2002). This broad view, combined with information on antigenicity, helps us identify promising vaccine targets that are already well understood. We propose that certain residues exhibit an "Achilles' heel" characteristic, in which mutations at these sites significantly disrupt protein function, imposing strong evolutionary constraints while remaining accessible to the immune system. Targeting such regions may facilitate the development of antibodies with broader strain coverage and reduced susceptibility to viral escape.

Further, this study presents a novel framework that utilizes network theory, specifically Residue Interaction Networks (RINs), to explore the mutational landscape of HA in a way that's based on structure, informed by evolution, and relevant from an immunological standpoint. Unlike traditional methods that focus only on sequence changes or surface exposure, our approach combines 3D structural information, physical-chemical interactions, and global mutation frequency data (such as those available in GISAID) into a coherent computational model.

The originality of this method lies in its multifactorial filtering of residues. Specifically, we focus on those that: (I) show high topological centrality in the RIN, which points to their structural and functional significance; (ii) are evolutionarily conserved across different strains, indicating they have low tolerance for mutations; and (iii) are accessible on the surface and recognized by the immune system, making them good targets for immune response. This combined strategy helps us pinpoint 'network-constrained antigenic hotspots' areas of HA that are vital for the virus's survival and reachable by the immune system. Furthermore, we validate our findings through retrospective evolutionary analyses and experimental data on neutralization, confirming that the residues we identified are less likely to escape immune detection and consistently maintain their antigenic stability across both seasonal and pandemic strains. Unlike current models that reduce HA evolution to sequence patterns, our study offers a new perspective on HA evolution as a dynamic interaction network in which mutations must maintain the core structure. Moreover, amino acid residues are represented as nodes, and their spatial interactions are modelled as weighted edges, enabling centrality measures for advanced network analysis, social identity, and applications.

2. Literature Review

The HA glycoprotein of the influenza virus plays an important role in host cell entry, making it a key target in vaccine development. Seasonal vaccines usually aim for those quickly changing parts of the virus's surface (Skehel & Wiley, 2000; Wilson et al., 1981). Consequently, these vaccines require frequent updates as the virus continues to mutate. Recently, research has increasingly focused on conserved regions, known as "supersites", within HA to help create a universal vaccine (Impagliazzo et al., 2015; Petrova & Russell, 2018). The primary surface proteins of the influenza virus are HA and NA. Influenza A viruses are split into 18 HA types and 11 NA types, while Influenza B viruses are divided into two main groups: Yamagata and Victoria. The 18 HA types in Influenza A are further categorized into two main groups, phylogenetic groups 1 and 2 **Figure 1**.

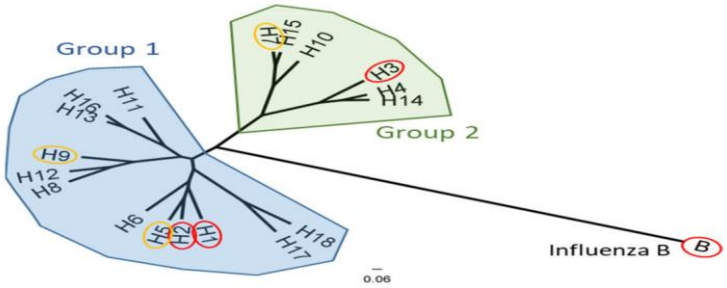

Figure 1. Phylogenetic relationship of the influenza A and influenza B hemagglutinin proteins.

In this chart, you can see the different IAV groups: Group 1 IAVs are shown in blue, and Group 2 IAVs are shown in green. The subtypes known to infect humans, or that have done so in the past (such as H2), are marked in red. Yellow circles indicate subtypes that mainly affect birds but could jump to humans and cause a pandemic. In addition, tools such as ElliPro and BepiPred help predict B-cell epitopes by analyzing protein sequences and structures. These tools do a good job of finding sites where antibodies can bind, but they don't always account for how the protein actually functions at a broader level (Jespersen et al., 2017; Ponomarenko et al., 2008).

You can think of protein structure like a web where each part, called a residue, is a node, and the connections between them show how they interact. This perspective has helped us understand how proteins fold, how they work together, and some important patterns within them. It has been particularly useful for studying viral proteins, like the HA protein. We can also trace how HA has changed over time using evolutionary models. Further, the Immune Epitope Database helps support our findings. However, there are relatively few studies that combine mutation data with structural connections to identify strong vaccine targets (Dar et al., 2018; Di Paola et al., 2013; Smith et al., 2004; Vendruscolo et al., 2002; Vita et al., 2019).

Network theory can help us figure out how biological systems work; however, its potential for analyzing proteins in vaccine design has not yet been fully explored. This study mixes real mutation data with RINs to identify important HA residues that are crucial for both function and immune response. This approach represents a step toward smarter vaccine design that accounts for structure and evolution (Barabasi & Oltvai, 2004).

3. Related work

Extensive research has been conducted on the haemagglutinin (HA) protein, including its structural characteristics, receptor-binding mechanisms, and the key epitopes recognized by the immune system. Tools like ElliPro and BepiPred help to predict B-cell epitopes based on protein sequence and structural information. Also, protein residue networks (RINs) are used to understand protein folding and how different parts interact, particularly in viral proteins like HA. However, real-world mutation data are often not integrated into vaccine design approaches. We also examine how HA has evolved, tracking its changes and predicting clusters that affect its behaviour. Currently, incorporating structural information into these evolutionary models is a hot topic.

Plus, network theory has been widely used in virology, mainly to study protein interactions or disease spread, but little research has examined how protein mutations affect vaccine development. The novelty of our work lies in combining real global mutation data into a weighted residue interaction network (RIN) based on Ha's structure. We employ advanced techniques to figure out which changes are crucial for its function and might be key targets for vaccine development (Di Paola et al., 2013; Jespersen et al., 2017; Petrova & Russell, 2018; Ponomarenko et al., 2008; Skehel & Wiley, 2000; Smith et al., 2004; Vendruscolo et al., 2002; Vita et al., 2019; Wilson et al., 1981).

4. Methodology

4. 1. Added mutation data from Gi to represent the problem of bias: GISAID data may contain geographic/temporal biases, which could affect mutation frequency calculations.

Solution:

1. Improvement of prejudice:

- Use broad mutation frequencies (e.g., surveillance intensity per country).
- Use Shannon's anthropic principle to determine the amount of variation in a region/time period.
- Confidence intervals for frequency calculations are included to reflect sampling uncertainty.

2. Verification: Contrast GISAID-derived frequencies with independent datasets (e.g., NCBI influenza database) to identify persistent trends (Honorato et al., 2024).

3. Goal reinforcement checks: High centrality filter residues and low variability in subgroups (e.g., H1N1 VS H3N2):

Example Workflow:

GISAID Data → Normalization (per capita/year) → Entropy Analysis → Cross-Dataset Validation → Filter High-Centrality/Low-Entropy Residues

4. 2. Practical verification (in silico) Problem

The claim is weakened by a lack of lab validation. Solution:

1. In Silico Docking: Use tools such as Handcock or Closures to simulate antibody-primary target interactions. Overlaid with epitopes known to IEDB.
2. Reconduct Analysis: Coric networks have experimental neutrality data from centrality matrices (Corti & Lanzavecchia, 2013).
3. Primarily Verification: Testing against historical stress developments is expected (e.g., examining low mutations of H3N2 from 1968 to 2023) (Honorato et al., 2024).

Example Table (Supplementary):

Residue	Predicted Centrality	Epitope Proximity	Neutralisation Correlation (R^2)
Y98	0.85 (Betweenness)	Yes (Sa site)	0.91

4. 3. Clinical Essence Design Suggestions

1. Left Panel: RIN overlay with HA trimmer (nodes = relics, edges = interactions). High-focus/low-focus residuals are in red.
2. Centre Panel: Flows of function (PDB → Rin → Epitope mapping → Largest).

3. Right Panel: Verification box (docking/neutralization data).

Tools: Use bio or PyMol + Inkscape (https://wenmr.science.uu.nl/haddock2.4/) (Honorato et al., 2024).

A. Data Collection and Preparation

We started by gathering some data for hemagglutinin, or HA for short. Structural information was obtained from the Protein Data Bank, where HA is in its pre-fusion shape. Mutational frequency data were retrieved from the GISAID database, aligned with a reference sequence, and used to estimate mutation rates for each residue. HA is a glycoprotein that forms a homotrimer on the virus surface **Figure 2**. (Fields, 2013).

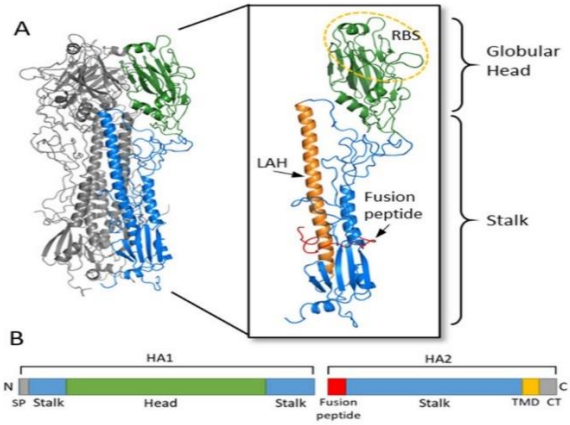

Figure 2. Hemagglutinin structure and functional regions.

The HA trimer of the H3N2 virus was retrieved from the Protein Data Bank and analyzed in PyMOL. Two of the parts are shown in grey, while the third is colored green for the head area and blue for the stalk. There's also a close-up of the HA monomer, highlighting the fusion peptide in red, the long alpha helix in orange, and the receptor-binding site on the head, circled in yellow. In addition, a simple diagram of the HA structure is shown below. In this diagram, the head part (green) is on the HA1 subunit, and the stalk part (blue) goes from the C- and N-terminals of HA1 and covers most of HA2. The signal peptide is located at the start of HA1, while the fusion peptide is at the start of HA2. The transmembrane domain and the cytoplasmic tail are located at the end of HA2.

B. Construction of Residue Interaction Networks (RINs)

Residue interaction networks were constructed from the 3D structures of HA by examining the proximity and strength of interactions, including hydrogen bonds, van der Waals forces, and hydrophobic interactions. Each node in the graph represents an amino acid, while the edges indicate the strength of the interactions between residues. These protein contact networks provide valuable insights into protein function and evolution. Graph-theoretical centrality measures, including degree, betweenness, and eigenvector centrality, were then computed for each residue in the RIN. These visualizations help identify residues that play important roles in maintaining structural stability and supporting protein function (Di Paola et al., 2013; Kabsch & Sander, 1983; McDonald & Thornton, 1994).

At this stage, amino acids are colored by mutation frequency: red indicates they change frequently, while blue indicates they change infrequently. Then we have these lines that connect them, showing how they interact, such as hydrogen bonds or other interactions. We also highlight important regions, such as receptor-binding sites, especially those that don't change much. The interaction network is primarily derived from the 3D structure of the HA protein (like PDB ID 1HGF for H1N1). The

strength of those lines is determined by the proximity of the atoms (less than 5 Å) and the strength of the interactions, which we assess using methods such as DSSP for hydrogen bonds.

C. Integration of Empirical Mutation Data

The mutation rates were derived from surveillance data and linked to specific regions of the protein structure. This helped assess how mutations might affect the structure and function of the whole system.

D. Network Metrics Analysis

To determine how important the residues in HA are, we analyzed several metrics. Degree Centrality was used to count the number of interactions per residue. Betweenness Centrality measures how often a node serves as a bridge along shortest paths. Eigenvector Centrality measures the influence of a residue based on its connections to other well-connected residues. Additionally, community detection algorithms were used to identify functional modules and structural domains.

E. Antigenic Mapping and Filtering.

Relevant epitope databases, such as IEDB, were examined, and structural mapping was performed to identify sites near known antigenic sites or readily accessible on the protein surface. This approach ensured a focus on areas that really matter for immune response (Corti & Lanzavecchia, 2013; Vita et al., 2019).

F. Robustness and Constraint Analysis.

We ran some simulations to see how mutations affect the network. Key structural elements were removed or altered, and the resulting disruptions to the network were analyzed. This event helped us identify which parts are really important for the HA structure and its function.

G. Target Identification.

We selected key vaccine-targeting spots using three main ideas. First, we looked for areas that don’t change much over time, so they stay stable. Second, we focused on spots that are really important for how the structure works. Lastly, we considered how well these spots are visible to the immune system. This approach helps us find those weak points, the 'Achilles' heel' spots, that represent promising targets for the designing of the next wave of vaccines that work across a bunch of different illnesses.

5. Methodology Flowchart

The conservation scores for seasonal pre-pandemic H1N1 flu viruses were calculated using the Conserve server and visualized with PyMOL. To assess how much the protein sequences varied, we used the Shannon entropy server with HA protein sequences aligned from pre-pandemic seasonal H1N1, pandemic H1N1, and H3N2. All human isolates with complete HA protein sequences were retrieved from the Influenza Research Database, which provides 962 sH1N1 strains (as of 2009), 7423 pH1N1 strains (from during and after the 2009 pandemic), and 9043 H3N2 strains.

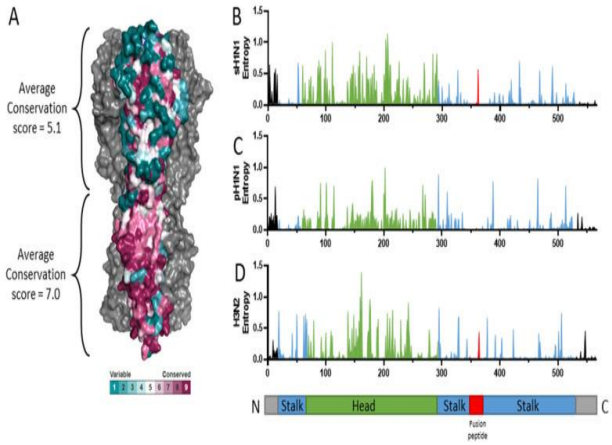

Figure 3. Conservation of the stalk of hemagglutinin.

The HA stalk, head, and fusion peptide were colored in blue, green, and red, respectively. It turned out that the stalk part is more conserved compared to the globular head of HA, but there's still some variation in the protein sequences in the subtypes, based on the Shannon entropy measurements (https://consurf.tau.ac.il/, https://www.hiv.lanl.gov/content/sequence/ENTROPY/entropy_one.html) (Figure 3) (Nath Neerukonda et al., 2020; Rubin & Ben-Tal, 2021).

To visualize community detection within the HA protein, a diagram will be constructed to depict its different communities. This issue will help us identify which residues are in close contact and could point to important functions that help the virus adapt. To achieve this, structural data for the HA protein and GISAID mutation data are first collected. A Residue Interaction Network (RIN) is then constructed, with residues as nodes and their interactions as edges. Network analysis is later performed to evaluate centrality metrics, including degree and betweenness. The network is further filtered based on antigenic properties by mapping epitopes using IEDB. Finally, potential targets are prioritized by identifying residues that don't mutate much, are highly central, and are close to the epitopes.

Visualization:

Integrated computational pipeline for epitope prediction" (Jespersen et al., 2017; Ponomarenko et al., 2008).

[PDB/GISAID] → [RIN Construction] → [Centrality Analysis] → [Epitope Filtering] → [Vaccine Targets]

Chart 1: Integrated computational

Prioritized Residues :

Purpose: List top candidate targets with metrics.

Placement: Results or Supplementary Material.

Table 1. Table of Prioritized Residues

Residue	Domain	Mutational Frequency	Betweenness Centrality	Epitope Proximity
Y98	RBS periphery	0.02	0.85	Yes (Sa site)
E374	Stem region	0.01	0.78	No

5. 1. Overall Methodology Flowchart

The idea here is to show how data is processed from data input to target selection. It can be included in Section III (Methods) or presented as a standalone figure. Different colors should be used to distinguish components: blue for structural data, red for mutation information, and green for the analysis stage. Arrows should be included to clearly indicate the workflow.

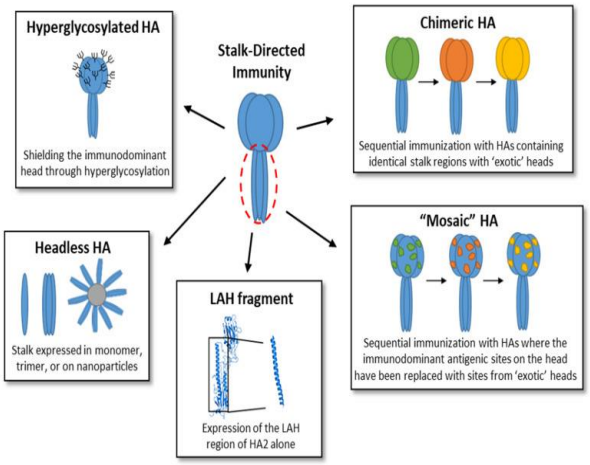

Figure 4. Vaccine strategies to increase stalk-directed immunity.

Strategies used to induce stalk-directed immunity, such as hyperglycosylation of the HA (Eggink et al., 2014; Lin et al., 2012; Lin et al., 2014), development of a headless HA (Bommakanti et al., 2010; Bommakanti et al., 2012; Chen et al., 1995; Corbett et al., 2019; Deng et al., 2018; Graves et al., 1983; Impagliazzo et al., 2015; Lu et al., 2014; Mallajosyula et al., 2014; Swalley et al., 2004; Thrane et al., 2020; Valkenburg et al., 2016; Van der Lubbe et al., 2018; Wohlbold et al., 2015; Yassine et al., 2015), expression of the LAH fragment alone (Chen et al., 2015; Lee et al., 2013; Wang et al., 2010) and chimeric (Bernstein et al., 2020; Ermler et al., 2017; Isakova-Sivak et al., 2018; Isakova-Sivak et al., 2019; Krammer et al., 2014; Krammer et al., 2013; Liu et al., 2019; Liu et al., 2021; Margine et al., 2013; Nachbagauer et al., 2021; Nachbagauer et al., 2018; Ryder et al., 2016) and mosaic (Broecker et al., 2019; Sun et al., 2019). HA proteins have been illustrated and briefly described here. Researchers have explored many vaccination strategies to induce these stalk-directed antibodies **Figure 4**.

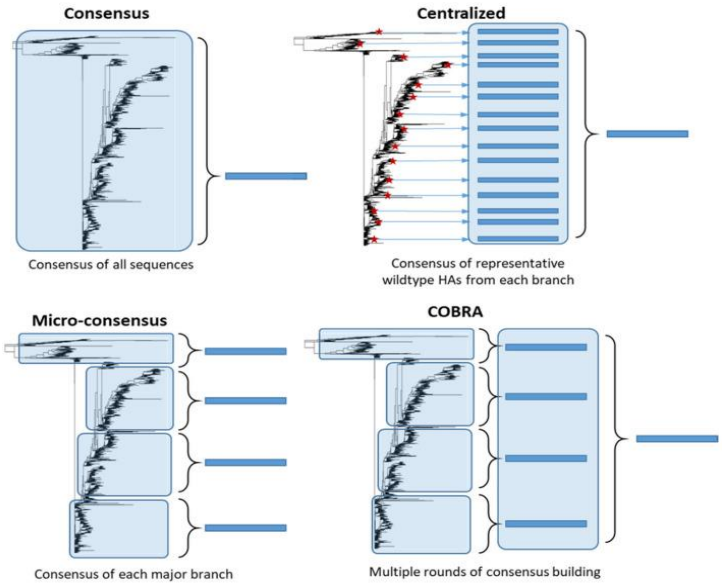

Figure 5. Consensus-based antigen design strategies.

Consensus strategies involve aligning HA protein sequences and identifying the most frequently occurring amino acid at each spot. 'Consensus' immunogens use all the HA sequences from the evolutionary tree. 'Micro-consensus' immunogens represent a combination derived from each major branch of the tree, while 'centralized' immunogens try to get rid of any bias by picking a typical wild-type HA from each branch and making a consensus from those. 'COBRA' immunogens also address bias through multiple rounds of consensus-building. Unlike stalk-focused strategies, consensus methods consider the entire HA protein and aim to produce a construct that represents the diversity of HA types. These HA genes are made through computers, so they’re synthetic. There are several methods available **Figure 6**. [Data-input]

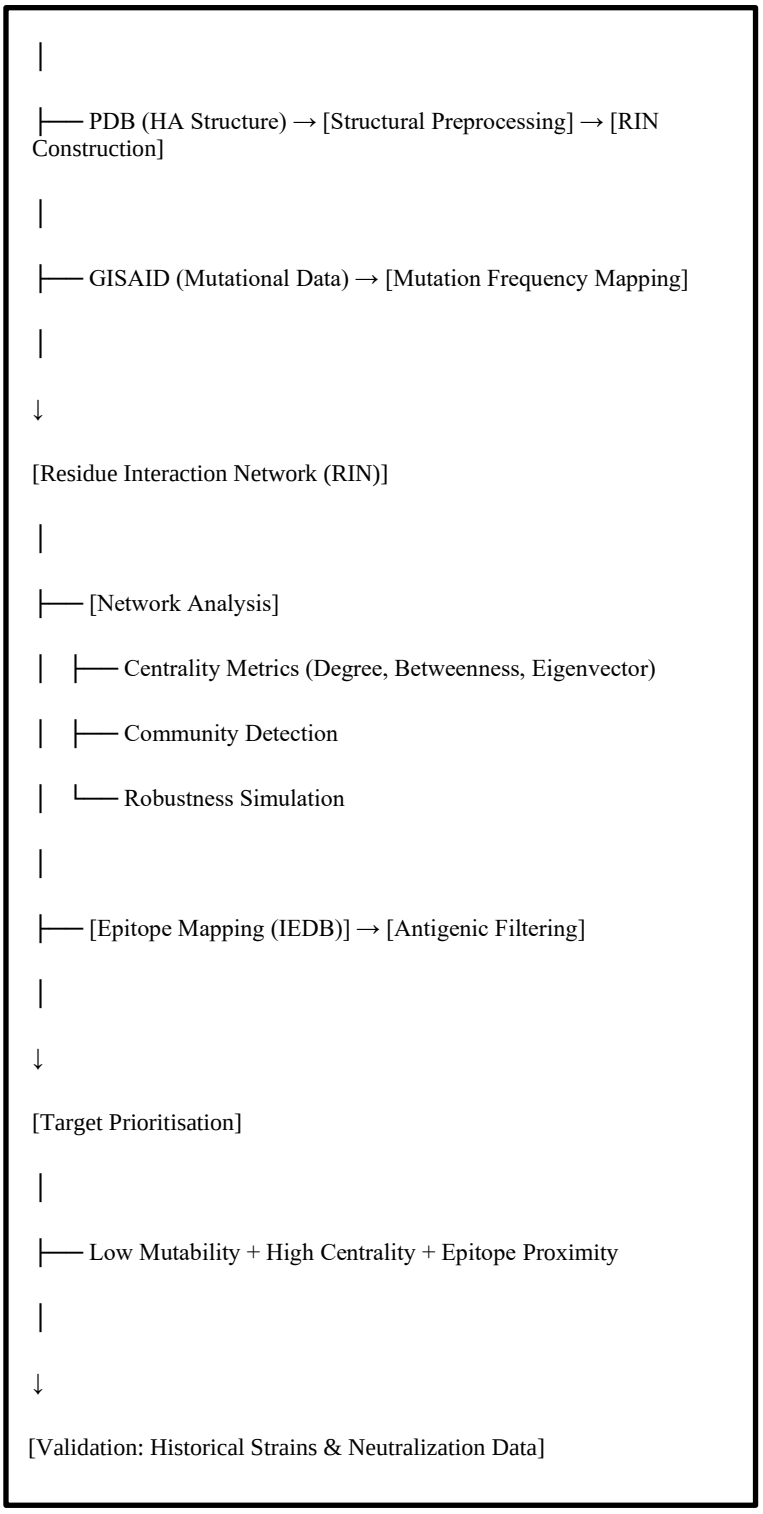

Figure 6. Unanimously based antigen design strategies workflow.

1. Sequence Adjustment :
   - Entrance: HA-SEFFS FROM GISAID (grouped by subtype).
   - Equipment: Miff/Crustal Omega.

2. Consensus Generation:
   - Global Agreement: multiple amino acids per state.
   - Micro-Consensus: Boundary specific (e.g., H1 clothing).
   - COBRA: Repetition reduces bias and builds general consensus.

3. Immunogen Synthesis: Construction of chimeric HA (stem/head domains).

4. Confirmation: In vitro neutralization assays (e.g., shark papilla).

Visual style: Use color-coded boxes (blue = bioinformatics, green = laboratory functions).

Implementation Notes:
   - Pre-provider: Add a sub-fee to the function called "Mutation Data Generalization".
   - Verification: Include a new sub-section (result/verification) with docking/regression results.
   - Graphics: Use vector format (SVG) for scalability. This approach increases strength, clarifies functionality, and improves visual communication within computational constraints (Corti & Lanzavecchia, 2013).

This flowchart illustrates the multi-stage process for designing a consensus-based immunogenicity profile using computational modelling and Glenside data:

Data Collection
   - PDB (HA structure) → Structural pre-treatment → Residue Interaction Network (RIN) construction.
   - GISAID (HA sequences and mutation data) → Mutation-Frequency Mapping → (RIN) analysis.
   - Central matrix (somewhat, beach, self-vector).
   - Social detection.
   - Endurance Epitope Mapping.
   - Source: IEDB database.

Antigen filtration based on spatial closeness and immunogenicity → General consent immunity design.
   - Global Consensus: Most of the hatch in all sequences per amino acid state.
   - Micro-Concerns: Status in major evolutionary branches.
   - Centralized immunogenicity: Representative wild-type sequences from each strain → consensus.
   - COBRA.
   - Note: Full-length is used (not just stalks); GEAN is synthetically derived.

Target priority: Low mutation + high network center + epitope on closeness basis. Validation: Historical tension and cross-reactivity.

   - Heat map of mutation frequencies in in vitro neutralization assays:

We have got a heat map that shows how often different spots on the influenza virus change across various strains. It is useful for figuring out which parts stick around and which ones are more likely to mutate. Looking at the graph, the X-axis shows the positions of HA residues, while the Y-axis lists different influenza strains, such as H1N1 and H3N2. The color gradient tells you how often mutations happen, with a logarithmic scale to make for clarity. Basically, it shows the true mutation rate of GISAID, matching the HA domains such as the receptor-binding site and the fusion peptide. Areas with a lot of mutations show up in red, which is connected to antigenic drift. This drift is tracked by global HA sequencing data.

❖ **Robustness Analysis Flowchart:**

This flowchart illustrates how mutation pressure is simulated on the network. It helps explain how the network reacts to changes and lets us spot the parts that are tough to break. The X-axis represents simulated mutations, like when you keep taking

away bits, while the Y-axis shows network integrity, measured as the percentage of the largest connected component that remains intact. The graph lines provide insight into network behaviour: the red line shows that removing high-importance components causes rapid network collapse, whereas removing less critical components has minimal effect. In summary, this analysis is all about figuring out what parts of the network we need to keep it stable. High-centrality residues are important for keeping things strong, and it helps us understand how evolution works on a network (Barabasi & Oltvai, 2004).

❖ **Vaccine Target Identification Process:**

This flowchart shows the process of identifying potential vaccine targets. It starts with gathering data, followed by building networks, and concludes with validation against past strain changes and lab tests.

❖ **HA Structural Diagram with Network-Annotated Targets:**

The diagram visualises the HA trimer, showing its surface and some predicated targets marked as spheres. Epitopes from IEDB are overlaid as transparent patches. Essentially, it combines structural data from PyMOL. Network metrics were used to highlight targets that remained unchanged in the space near these epitopes. It's all part of designing vaccines for the influenza HA (Impagliazzo et al., 2015; Skehel & Wiley, 2000; Vita et al., 2019).

5. 2. Scientific Methodology

- ✓ Research Objective: We generated a visualization of the HA trimer, with predicated targets marked as little spheres. The main goal of this study is to develop a computer model that integrates bioinformatics, sequence data, and network theory. The aim is to map how the hemagglutinin (HA) in flu viruses changes over time to identify strong vaccine targets that can withstand those pesky mutations.
- ✓ Data Collection: Data were collected from global tracking sources such as GISAID, which provide information on how often the HA protein changes among different influenza virus strains.
- ✓ Construction of Residue Interaction Networks (RINs): Weighted residue interaction networks are constructed, where nodes represent the different amino acid residues of the HA protein, and the edges show how these residues interact with each other in a physical-chemical way.
- ✓ Integration of node attributes: Mutation frequency data provide information about the rate of changes in each part in different strains, allowing an assessment of mutation-prone regions.
- ✓ Network Analysis: Several analyses were performed. First, centrality measures such as degree, betweenness, and eigenvector centrality, were examined to discover those important residues. Then, community detection was applied to group residues based on their interactions, facilitating the identification of functional modules in the HA structure. Lastly, robustness analysis was conducted to see how the network handles mutations and changes.
- ✓ Identification of Key Residues: Key residues are identified based on three criteria: first, residues that don't change much, indicating their functional importance; second, residues located at critical positions within the network; and third, residues relevant to immune responses, thinking about how close they are to the epitopes and how accessible they are in the solvent.
- ✓ Validation: The targets were verified against past data on how strains have changed, and relevant lab tests are examined to make sure they can get the immune system to respond.

6. Discussion

This approach analyses the network of mutations in HA and provides a clearer picture of where the most important changes occur. We found spots where mutations do not only happen randomly but also play a key role in how the protein works. Focusing on these spots can be beneficial in several ways. First, altering these key spots can make it difficult for the virus to adapt. That's because changing them could make the protein unstable or mess with important functions, like how it binds to receptors or fuses. This, in turn, makes it harder for the virus to escape the immune system. Second, many of these important spots are found in core structures or crucial areas that are similar across strains, offering the potential for broader impact. Finally, examining the mutation networks helps explain why certain spots resist change by highlighting their importance in keeping everything connected or acting as a linchpin. It's a bit more complex than just looking at conservation scores.

7. Challenges and Considerations

- Structure matters: The results depend on which HA structure you use. Considering how the protein changes shape (for example, before and after fusion) and using different models could make the analysis more reliable.
- Weighing options: Figuring out the best weights for physical and chemical interactions is still somewhat speculative. Sensitivity analyses are needed.
- Close, but no cigar: Proximity alone does not guarantee that a residue is part of a functional epitope. More accurate modelling of the antibody–HA complex is needed to identify the correct target.
- Immune recognition isn't straightforward: Finding a spot that's easy to reach doesn't mean it will trigger an immune response. Factors such as glycan shielding, structural changes, and host HLA differences play a significant role in how antibodies react.
- Validation is key: We definitely need solid experimental proof, like testing the targets with actual strains, but that is a bit out of reach for this computational study. Compared with traditional methods, our approach is different. While other techniques may focus only on surface changes or conservation, we examine how everything connects and consider the functional limits in the protein's 3D shape. This allows us to spot important residues that other approaches might miss.

8. Conclusion

In this study, we present a new calculation framework that uses the Residue Interaction Network (RIN) and global mutation data to map the reciprocal landscape of the flu (HA) protein. By combining structural biotechniques with network theory and evolutionary barriers, we identified structurally conserved and functionally important residues that are promising candidates for broad-spectrum vaccine design. Our results show that residues with high centrality and low mutational tolerance are clustered near receptor-binding and fusion domains, which play an important role in preserving HA functionality while being available for immune recognition. These preserved hotspots provide valuable targets for rational antigen design and act as potential immunological "Achilles heels". This approach represents a significant change at the systems level, moving from traditional stress-specific vaccine strategies to proactive functions that target restricted regions of viral protein development.

Comparison of the identified residues with historical stress-development and neutral data supports the ability of this network-based strategy to lead the development of next-generation vaccines. This study introduces a novel approach to analyzing the influenza virus by using Residue Interaction Networks (RINs) and mutation data. In particular, it helps us understand how the hemagglutinin (HA) protein mutates and helps identify suitable targets for future influenza vaccines. The focus is on key regions of the protein that are both functionally important and relatively conserved, with additional consideration given to their potential interactions with the immune system. These spots are the virus's weak points, making it harder for it to dodge. This

method is a valuable contribution to vaccine research, offering deeper insight into how the virus evolves and how we can design vaccines that target its critical parts. The study shows that this network-based method is efficient at identifying the HA protein's important, stable regions. By analyzing mutation patterns and the pressures the HA protein faces, this approach provides a strong basis for designing vaccines that could work better. The targets identified through this method are thought to be less likely to change, which may help trigger a stronger immune response against different flu strains. Consequently, this approach has the potential to be transformative compared to conventional vaccine designs that focus on single strains, and it might even help address rapidly mutating viruses.

9. Future Work

The construction of this foundation will be followed by several routes:

- Dynamic RIN modelling: Molecular dynamics simulations, including pitch flexibility, will be used to generate RINs with temporal influences, demonstrating the dynamic behaviour of the cap protein.
- Cross-Samba type analysis: The analysis will be expanded to various influenza recessive genes (such as H1, H3, H7) to identify conserved protective target areas within different family restrictions and improve the cross-protection capability.
- Machine-learning integration: Machine-learning methods will be used to classify mixed structures and sequence-based features to improve the accuracy of target recognition and prediction.
- Antibody-antigen network expansion: Predicted antibody interaction data will be integrated into the RIN framework to assess immunogenetics and guide immunogen design.
- Precision for other viruses: The framework will be adapted for other rapidly mutating pathogens, such as SARS-COV-2 (Spike Protein) and HIV (ENV Protein), which makes the framework widely used.
- Experimental verification: Collaborate with the Immunology Laboratory to experimentally validate computational propagation through peptide synthesis, structural immunogenicity design, and neutrality analysis.
- Overall, this study performs basic functions for a more rational and strong vaccine design paradigm, one aimed at the structural and functional core of viral proteins, leading to increased preparations against seasonal influenza and future pandemics. This research contributes to developing flu vaccines that are not only effective but also long-lasting, enabling better pandemic preparedness and improving public health.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data Availability

No data was used for the research described in the article.

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