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Research Article

Identification and biophysical characterization of epitope atlas of Porcine Reproductive and Respiratory Syndrome Virus

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ABSTRACT

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) have been a critical threat to swine health since 1987 due to its high mutation rate and substantial economic loss over half a billion dollar in USA. The rapid mutation rate of PRRSV presents a significant challenge in developing an effective vaccine. Even though surveillance and intervention studies have recently (2019) unveiled utilization of PRRSV glycoprotein 5 (GP5; encoded by *ORF5* gene) to induce immunogenic reaction and production of neutralizing antibodies in porcine populations, the future viral generations can accrue escape mutations. In this study we identify 63 porcine-PRRSV protein-protein interactions which play primary or ancillary roles in viral entry and infection. Using genome–proteome annotation, protein structure prediction, multiple docking experiments, and binding energy calculations, we identified a list of 75 epitope locations on PRRSV proteins crucial for infection. Additionally, using machine learning-based diffusion model, we designed 56 stable immunogen peptides that contain one or more of these epitopes with their native tertiary structures stabilized through optimized N– and C–terminus flank sequences and interspersed with appropriate linker regions. Our workflow successfully identified numerous known interactions and predicted several novel PRRSV-porcine interactions. By leveraging the structural and sequence insights, this study paves the way for more effective, high-avidity, multi-valent PRRSV vaccines, and leveraging neural networks for immunogen design.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) has been known to be one of the most economically impactful swine infectious disease [1]. Annually, it is responsible for over billion-dollar losses to the worldwide pork industry [2]. PRRS is caused by an enveloped, positive-sense, and single-stranded RNA virus (PRRSV). PRRSV is of the Arterivirus genus within the order Nidovirales [2,3]. It was first discovered in 1987 in Iowa, Minnesota, and North Carolina and was called “swine mystery disease” or “swine infertility and respiratory syndrome” [4]. The PRRSV genome is a single-stranded, positive-sense RNA about 15 kb in length, with a 5′ cap and a 3′ polyadenylated tail. It contains two main open reading frames (ORF1a and ORF1b), which encode polyproteins that are processed into 14 non-structural proteins. Additionally, the genome includes structural genes in ORF2a, ORF2b, and ORFs 3 through 7, with unique transcription regulatory sequences facilitating the production of subgenomic mRNAs [5]. PRRSV infection causes severe reproductive failure in sows and respiratory diseases in piglets, which is further complicated by several secondary infections. This results in higher porcine livestock mortality and clinical manifestations [6–9].

PRRS is highly infectious and is spread through direct contact with contaminated sperm, feces, urine, and mammary or nasal secretions [10]. Therefore, prevention and the design of an effective vaccine is critical [11]. Modified live virus (MLV) vaccines cannot generate comprehensive protection. This is primarily due to the variability of PRRSV and the intricate nature of the interplay between PRRSV and the immune responses of the host. One of the many challenges facing its construction is the rapid evolution of the PRRS virus, which is estimated to have the fastest evolutionary rate of all known RNA viruses at 10^{-2} per site per year [12]. Overcoming these obstacles will require more exploration of how virus and hosts interact [13].

PRRSV has the ability to escape from the host immune response in various processes. Many novel strategies are being proposed to create

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more effective vaccines against this evolving virus [13]. The diversity of PRRSV is significantly influenced by high-frequency mutation and recombination between different lineages/sub-lineages. This phenomenon holds considerable importance in the acceleration of the evolution of PRRSV [14]. Antibody escape mutations influence the rates of viral reinfection and the duration of vaccine effectiveness. Therefore, the key to developing optimal vaccines and therapeutics depends on anticipating viral variants that can elude immune detection with enough lead time [15].

Ideally, experimental methods such as pseudovirus assay can help in anticipating viral immune assay, but that limits the impact for predicting immune escape early [16]. Therefore, it is of interest to develop computational methods that can predict viral escape. This information can be used to predict better vaccines for the virus. Moreover, an ideal model would be able to consider as-yet-unseen variation of antigen and antibody interaction throughout the full antigenic protein and would guide the formulations of specific experiment [17–19]. In this context, artificial intelligence (AI), and machine learning (ML), hold significant promise for expediting and enhancing the optimization of therapeutics [20]. AI has the capacity to rapidly design, and screen antibodies to zero in on lead molecules, leading to a reduction in the time and resources required for the development of therapeutic antibodies resources and/or immunogenic proteins for use in next generation vaccines. Overall, it has the potential to significantly enhance the speed, quality, and controllability of antibody design [21]. Rapid, multi-valent antibody design campaigns which target cross–neutralization of multiple strains of viruses are likely to be robust and add to the global biotechnological readiness for animal health.

Our understanding of the molecular mechanisms and interaction of PRRSV with swine proteins is expanding. Newer potential targets for antigens will help in the development of an improved vaccine. Normally, GP5 protein (encoded by the *ORF5* gene) is targeted by the antibodies (e.g., commercial PA5–111970) induced by PRRSV vaccination. As a result, it has largely been utilized as the main protective antigen [22]. Additionally, recent studies found that deletion of the E protein results in transmissible virus particles not being produced, as viral RNA synthesis is inhibited [23]. A recent strategy to potentially prevent PRRSV infection in pigs intervenes and outcompetes PRRSV and CD163 interactions [2]. Moreover, MYH-9 has been identified as an essential factor for PRRSV infection. C-terminal domain of MYH-9 physically interacts with the PRRSV GP5 protein, rendering cells susceptible to PRRSV infection [24]. Overall, GP2, GP4, GP5, M proteins, E proteins, and several non-structural proteins (*nsp*) have the potential to serve as more precise targets for vaccine development [25].

In our study, we aimed to first identify an atlas of possible protein-protein interaction between the virus and the host that likely facilitate viral entry. Next, we precisely identify the locations parts of the viral proteins (epitopes) bind to their target host receptor proteins (at specific locations; paratopes). This provided us with unique information about detailed binding information between residues in a three-dimensional (3D) space. PRRSV is known to have a very high evolutionary rate [26]. GP5 protein (corroborated from our analysis) is currently one of the main protective antigens which is used by PRRSV vaccination for protection. As GP5 is prone to escape mutations, its variability complicates vaccine development and makes the disease challenging to control [27,28]. Thus, targeting multiple epitopes for different PRRSV proteins for a potential vaccine is a strategic next step given the caveats of the current GP5 protein targeting approach [29]. Therefore, we constructed optimized short epitope-containing fragments of viral proteins which preserve their original structure (i.e., immunogens) derived from our epitope library and catalogued their 3D structures. We used the structural similarity (measured in Å of deviation) of the epitope's tertiary structures in immunogen peptides to those in their native PRRSV proteins as a measure of immunogen stability and utility. These immunogens represent a promising avenue for the development of an improved vaccine, potentially mitigating the challenges posed by the high evolutionary rate of the PRRSV.

2. Material and methods

Our workflow (see Fig. 1) for identifying epitope proteins of PRRSV infecting swine hosts through interactions with receptor proteins facilitates the design of immunogens – short protein fragments containing potent parts of viral proteins – as a basis for antiviral vaccine development. This is demonstrated using the PRSSV-pig virus-host pair, revealing key entry proteins like CD163 and identifying additional candidate proteins for vaccine synthesis. Our computational work comprises several modules designed to analyze virus-host interactions, identify epitope proteins, and synthesize immunogens for vaccine development.

For virus-host interaction, we used multiple state-of-the-art software tools in bioinformatics that ranges from ESMfold and Alphafold2 for structure prediction to HADDOCK 2.4, ClusPro 2.0, and GRAMMDOCK for information-driven modeling of biomolecular complexes and the structural interactions [30–34]. Epitope proteins were identified using our custom python scripts.

In addition, using PyRosetta, we performed relaxation on the final immunogen structures and obtained the RMSD values [35,36]. Additionally, ProteinMPNN (Protein Message Passing Neural Network) was used to predict other sequences that could fold into the same structure as our selected immunogens, while keeping the core unchanged. ProteinMPNN is a latest deep learning method with outstanding performance in both *in silico* and experimental tests for protein sequence design [37].

2.1. Annotation of the genome and 3D structure prediction

PRRSV genome was annotated using NCBI Refseq and RAST (Rapid Annotations Using Subsystem Technology). Various tools were employed to determine whether the proteins were transmembrane or not. These included BUSCA [38], machine learning-based Deep TMHMM [39], and protein language model-based DeepLoc 2.0 [40]. The 3D structure prediction of proteins containing transmembrane domains was performed using both ESMfold [34] and AlphaFold2 [33].

A comprehensive review of the relevant literature in PubMed has yielded insights into the swine proteins that demonstrate interactions with the PRRSV. Furthermore, same computational modeling techniques have been employed to predict the three-dimensional structures of these PRRSV-interacting host proteins.

2.2. Molecular docking, epitopes finding and scoring

HADDOCK 2.4, ClusPro 2.0, and GRAMM: Docking Web Server were utilized as part of the process to dock the PRRSV proteins against swine proteins. There were $9 \times 7 = 63$ pairs of proteins, and top 10 poses generated by each of the approaches were chosen.

We identified the key drivers of the interactions – the interacting interface residues between PRRSV and swine proteins in all possible poses using a threshold of 6.5 Å [41]. Additionally, we utilized *pdb_tools* and HADDOCK scoring to rigorously evaluate the quality of the poses [42]. Thus, the interacting complexes produced by all three tools underwent evaluation using the same scoring criteria. Furthermore, InterProSurf was employed to analyze the interface between PRRSV and porcine proteins, providing insights into the hydrophobicity and biochemistry of interaction surfaces [42]. ProtParam was utilized to analyze the amino acid composition and ProtScale to analyze their hydropathy, enhancing our understanding of the molecular characteristics and potential functional roles of interacting residues [42].

2.3. Design of stable immunogens and structural stability analysis

The epitopes within the top-scoring interaction complexes were

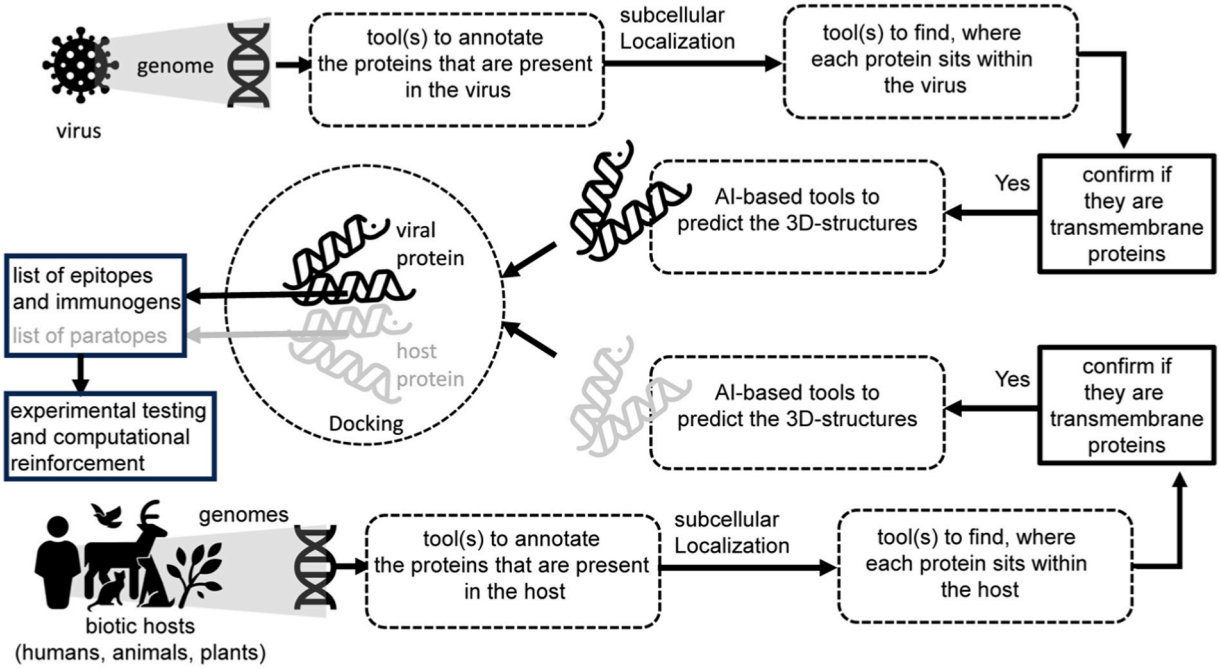

Fig. 1. Overall workflow demonstrating how genomes of virus and host are leveraged to first annotate the proteome and then pinpoint transmembrane proteins. Transmembrane proteins from virus and host are docked to identify potential list of epitope locations on antigentic viral proteins – which were subsequently stabilized in isolation (i.e., immunogens) through protein message passing neural networks. All steps delineated by dotted boxes utilize third-party open-source tools (and could leverage several different options from literature), while the steps within solid black boxes represent our custom developed methods and contributions.

delineated. We identified the top epitopes from the PRRSV-swine complexes that were of particular interest to our study. The selection of immunogens was based on PRRSV's robust binding affinity with swine proteins, as evidenced by the energy scores. Following the identification of the primary epitopes, we first designed immunogens naively by selecting a residue window (*immunogen window*) that includes five additional amino acids flanking the N– and C-termini around the chosen epitopes. In instances where the top epitopes overlapped within 2 residues of each other, we designed elongated immunogens that contained these proximate residues in the same manner.

Upon constructing the immunogens, we assessed their 3D structural integrity. First we isolated the *immunogen window* from the initially predicted in the original pose. Next, we performed computational energy minimization (*relaxation*) using *PyRosetta–fastrelax* iterations and rotamer repacking [43]. If the relaxed peptide deviated from its original secondary and tertiary structure (in the antigen) by > 2 Å, we deemed it unstable or unable to present the antigen for eliciting the identical immune response in the host. Consequently, we end up with a ranked list of the top, stable immunogens through naive flank design that are likely to induce an immune response in swine.

In addtion to the naive immunogen design, structure-aware machine learning was used to design an alternate set of synthetic immunogens. ProteinMPNN was used to generate variations of immunogen sequences with the biochemical objective of maintaining the epitope structure as seen in the native antigen [37,44,45]. This allows for an unrestricted choice of non-epitope residues in the designed immunogen, thereby offering a possibility to explore if there is an alternate set of N– and C–terminal flank sequences which is able to stabilize the epitope better. While this is a combinatorically explosive problem given that designing 10 residue positions simultaneously necessitates exploring 20^{10} sequences, a machine learning network which is trained to predict a library of sequences that all fold (as per AlphaFold2) to a *user-defined* template structure is useful to this end. As a demonstration, we leveraged ProteinMPNN-based immunogen design with GP5 variants from our top 56 immunogens. Additionally, a sequence alignment of the original immunogen and their variations were conducted using Color Align Conservation [46]. Utilizing PyRosetta, we calculated the RMSD values between the immunogens and their variant sequences generated by ProteinMPNN [35,36].

3. Results

3.1. Transmembrane viral proteins and swine proteins molecular docking

The genome annotation from PRRSV identified a total of 41 proteins. Out of these, predictions from at least two tools (BUSCA, Deep TMHMM, DeepLoc) indicated that 20 of the proteins have a transmembrane region. After removing isoforms (i.e., duplicates) and unstructured proteins, we compiled a list of 9 PRRSV proteins with the 3D structure (Table 1). Our literature review revealed 7 swine proteins that have been consistently found to interact with PRRSV in numerous instances (Table 1). These proteins play a crucial role in the interaction with the virus, contributing significantly to virus-protein interactions [28, 47–51]. Subsequently, we either retrieved or predicted the 3D conformation of these proteins after selection.

The combined results from HADDOCK 2.4, ClusPro 2.0, and GRAMM:Docking yielded a total of 1696 poses. It encompasses 436 poses from HADDOCK 2.4, 630 from ClusPro 2.0, and 630 from GRAMM:Docking, for all 63 potential docked complexes formed by the 9 PRRSV proteins and 7 swine ones.

3.2. Epitope – paratope interaction study

To enhance the reliability of our molecular docking results, we identified epitope-paratope interactions that were commonly predicted by at least two out of three docking tools, namely HADDOCK and any of the other tool(s). This approach yielded a total of 23,901 unique interactions between epitopes and paratopes (Supplementary Material 1). Additionally, instances where the same epitope-paratope pair exhibited different distances, within the threshold of 6.5 Å, across different docking tools were also considered unique interactions. Among these, 7685 were unique possible combinations of interactions by the epitope

Table 1
List of Interacting PRRSV and Swine Proteins with their residue numbers and RefSeq Accession.

PRRSV Proteins	Amino Acid Residues	NCBI RefSeq Accession	Swine Proteins	Amino Acid Residues	NCBI RefSeq Accession
E envelope protein	73	YP_009505550.1	CD209	240	NP_001123444.1
GP2	256	NP_047408.1	Vimentin	466	XP_005668163.1
GP3	265	A0MD32.1	CD151	253	XP_013845140.1
GP4	183	A0MD33.1	Sialoadhesin	1730	XP_020932962.1
GP5	200	NP_047411.1	Heparan Sulfate	882	XP_020940682.1
MembraneProtein M	174	NP_047412.1	CD163	1113	XP_020946779.1
nsp3	231	NP_740597.1	MYH−9	1981	XP_020947197.1
nsp5	170	NP_740599.1			
ORF5a	51	YP_006488613.1			

residues (Fig. 2, Supplementary Material 2). This comprehensive analysis allowed us to evaluate the interactions at both the residue and protein complex levels, enhancing our understanding of the molecular interactions studied. For example, investigating the porcine receptors' interactions with GP5 it is revealed that GP5 largely interacts with porcine proteins through its hydrophobic surface patches through packing interactions. These interactions are however supplemented by a wide variety of polar interactions. With CD163, MYH-9, and vimentin, it uses Asn-Arg, Pro-Gln, and Lys-Gln short-range electrostatic interactions, respectively, while with CD209 it shows a Thr-Thr hydrogen bonding much like Thr-zipper alpha-helical bundle proteins. With heparansulfate and sialoadhesin, it involves aromatic groups viz Tyr-Tyr pi-pi stacking and Arg-Trp cation-pi interactions, resepectively.

3.3. Energy score calculation and analysis

We calculated the score with HADDOCK 3.0 scoring function for all 1696 poses generated from three docking tools (Fig. 3, Supplementary Material 3). The computational binding score is a proxy for the affinity between viral and host proteins. Due to the well-established understanding on protein CD163, and pre-existing body of literature and industrial patents, our epitope discovery efforts deliberately excluded this protein from consideration. It is however noteworthy that our pipeline recognizes and corroborates that CD163 is a key porcine host receptor that is likely to facilitate PRRSV entry. We present the binding energy scores associated with CD163 and the viral epitopes to throw light onto the energetics and underlying biophysics of PRRSV-CD163 interactions. Through these analyses, we pinpointed and highlighted the top epitopes within the PRRSV-swine complexes. In total, we identified 75 epitopes from several PRRSV proteins that facilitate strongest interactions with its swine counterpart.

In our analysis of epitope-host interfaces using InterProSurf, it was observed that all interactions, except GP5-vimentin, exhibited a predominance of non-polar area/energy over polar interactions [62]. The Kyte & Doolittle hydrophobicity scoring further highlighted the hydropathy of these protein interfaces [63]. For instance, in the GP5-CD151 interaction, both proteins' interfaces were predominantly hydrophobic. This feature underpins our prediction that the epitope Leu2 and paratope Ile222 engage in hydrophobic interactions. Conversely, the CD163 interface displayed a combination of hydrophobic and hydrophilic residues, highly enriched in Leu and Thr. Similarly, the CD209-GP5 interface appeared mostly hydrophobic, while our predicted epitope residues, predominantly hydrophilic (Ala, Gly), interacted with Serine, Proline, and Glycine residues.

In interactions involving GP5 with heparansulfate, MYH-9, and sialoadhesin, the GP5 interface was predominantly hydrophobic. Conversely, the interface of porcine protein vimentin was largely hydrophilic but exhibited a high abundance of Leu residues (22.9 %), contributing to its hydrophobic character.

3.4. Biophysical characterization of known and predicted interactions

To discern the residue-level biophysical underpinnings of virus–host protein–protein interactions that drive binding affinity, we chose to explore one well reported, and one predicted interaction likely responsible for infection. CD163 represents the well-reported interaction which is mediated primarily by Glu534 in association with Arg561. They play primary role in viral infection as mutating them results in additional resistance against PRRSV [64]. Moreover, several studies have identified GP5 as a leading candidate for interaction, based on extensive wet-lab experiments. However, these studies have not pinpointed the exact locations where CD163-GP5 interactions might occur [60,65–67]. We characterized the interaction with the carboxylate group of Glu534 side chain as this is likely to form hydrogen bonds with the hydroxyl group of Ser32 side chain at a close distance of 1.9 Å. On the other hand, nsp3 and heparansulfate interacts between the positively charged imidazole ring of His4 and the carboxylate group of Asp536 at a distance of 5.7 (Fig. 4). Additional interactions can be analogously explored in detail within the molecular space using our epitope atlas, allowing for a comprehensive examination of all protein-protein interactions.

3.5. Selected top epitopes and corresponding conformational poses

Our top selected epitopes were linked to their corresponding three-dimensional conformations. This is crucial because the epitope list can be expanded to design immunogens, leveraging their 3D structures. Fig. 4 illustrates all the top epitopes alongside their specific associated poses. This visualization also highlights epitopes that are spatially proximal in positions, suggesting the potential for inclusion of multiple epitopes within a single immunogen structure.

3.6. Immunogen design

With the top epitopes and their associated poses (Fig. 4), we extracted an 11–residue long immunogen window containing the epitope residue flanked by five N– and C-terminus residues. For cases, where two epitopes were identified spaced by not more than five intermediate residues, we expanded the immunogen window by including five N-terminus residues upstream to the first epitope residue, followed by the intermediate linker, the second epitope residue, and a five amino acid long C–terminus flank (Fig. 4). Overall, we developed 61 immunogens of varying lengths by piecing them out from the original viral antigenic proteins. Our stability (in Ås of RMSD) analysis using PyRosetta energy minimization showed that 56 of the 61 immunogens were stable, with less than 2 Å root mean squared deviation from the original in-antigen structure of the immuogen window (Fig. 4 and Supplementary Material 4). Subsequent future endeavors will include assessment of immuogenicity of these immunogen peptides followed by in vitro binding characterization with their affiliated host proteins, and off-target characterization in other similar proteins in the host.

Fig. 4 displays the amino acid sequences and RMSD values of the original extended immunogens compared to those generated by the

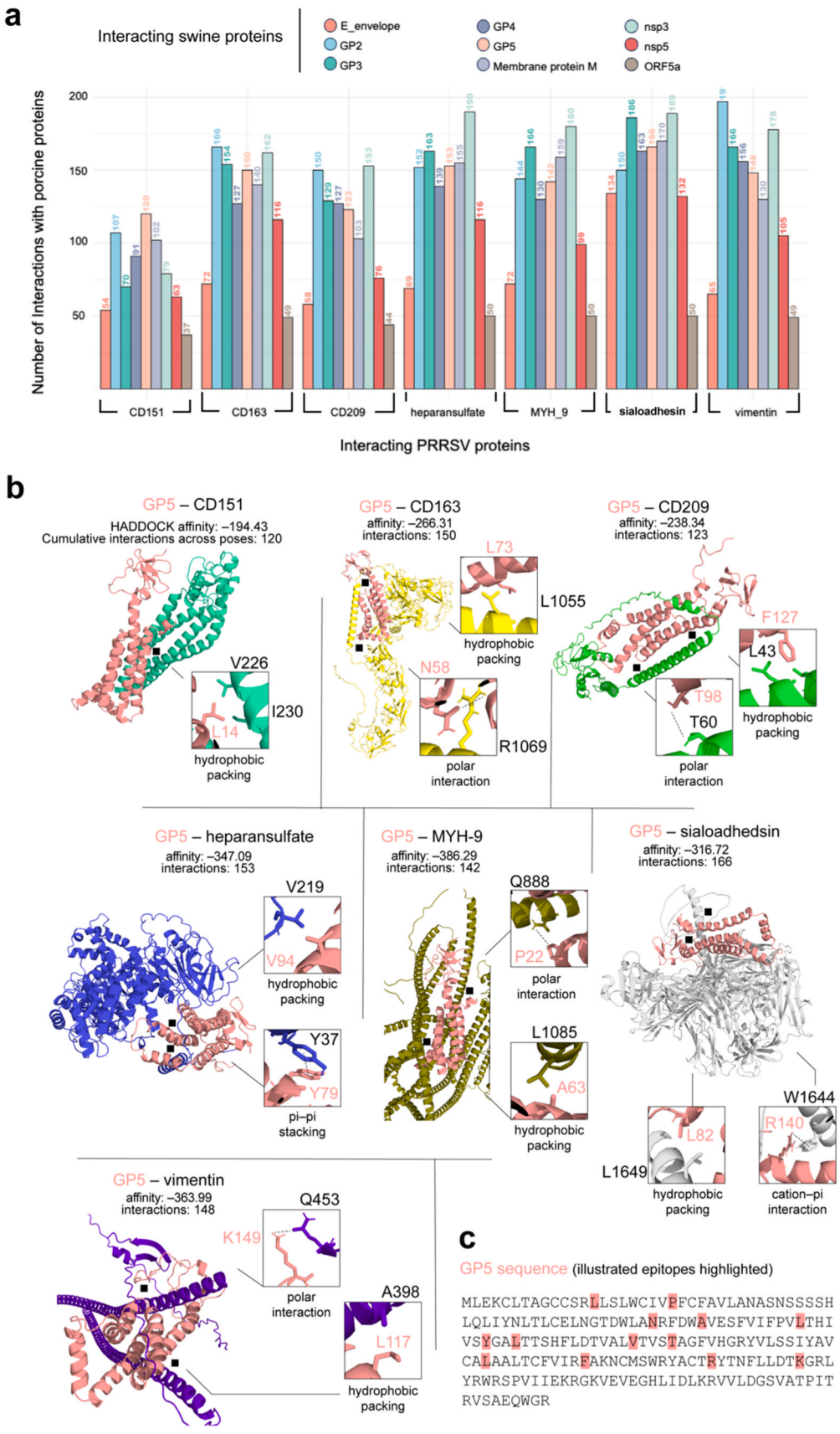

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Fig. 2. (a) 7685 unique interactions between epitopes and paratopes of PRRSV-swine complex. (b) Showing PRRSV GP5 protein's interaction with 7 Porcine proteins from our study. This figure represents a specific pose and includes the HADDOCK3 score and the number of all possible interactions between GP5 and its interacting partners. It also depicts specific interactions between the GP5 protein of PRRSV origin and various host cell proteins. Notably, hydrophobic packing interactions are observed between GP5 and CD151, vimentin, heparan sulfate, MYH-9, CD163, sialoadhesin, and CD209, highlighting regions of intimate contact crucial for molecular recognition. Polar interactions are also detailed, such as R1069 of GP5 with N58 of CD163, T98 of GP5 with T60 of CD209, and P22 and Q888 of GP5-MYH-9. Cation-pi and pi-pi stacking interactions are seen at the interfaces with heparansulfate and sialoadhesin. (c) GP5 residues involved in these interactions are highlighted.

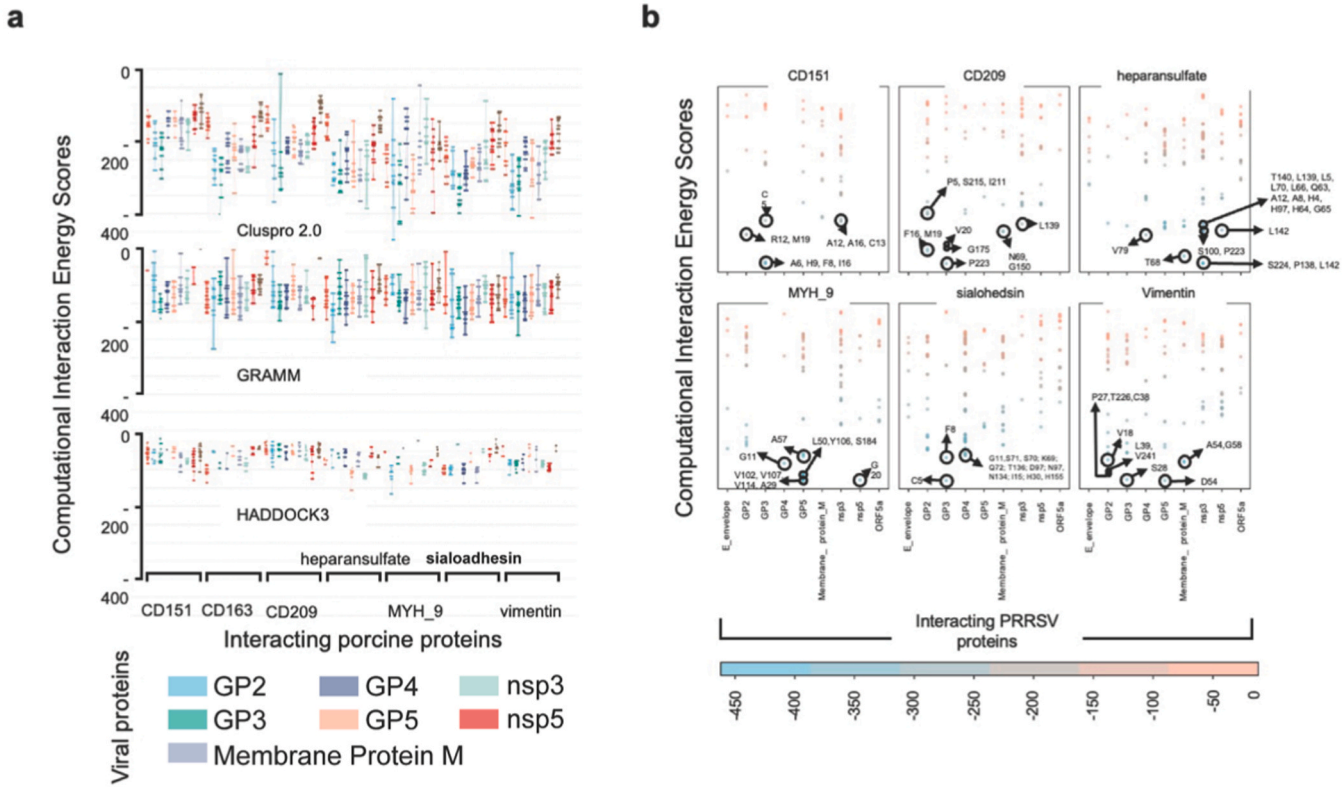

Fig. 3. (a) HADDOCK 3.0 score for all 1696 poses generated by 3 different methods as shown in the figure. (b) Top 75 epitopes from PRRSV-swine protein complex based on their computational interaction energy scores. Panel (a) illustrates the predicted energy scores generated by three docking tools for each interaction between PRRSV and porcine proteins. These computational energy scores were subsequently employed to select the most promising epitopes from each PRRSV protein, with the exception of CD163. In panel (b), a lower energy score indicates a more favorable pose, suggesting that the epitope is a better candidate for vaccine targeting.

structure-aware message passing neural network ProteinMPNN. The objective of the trained ProteinMPNN network is to identify a set of amino acid sequence combinations which are likely to fold into a user-defined target structural motif. One is able to constrain the choice of amino acid types at each position. For the task of stable immunogen design, we used the aforementioned native immunogen peptides in ProteinMPNN to identify amino acid combinations in all but the epitope loci that causes least structural deviation from the original antigenic conformation of the epitope. For the immunogens with single epitopes, the five the N– and C–terminal flanks were allowed to vary while for longer ones with multiple epitopes, the intermediate linker sequences were also allowed to vary. For instance, *de novo* sequences derived from V114–containing immunogen (from GP5 antigen; binding with MYH-9) showed the highest stability (best RMSD 1.49 Å) while Y109–containing *de novo* immunogens showed poorer stability (best RMSD 7.23 Å). This indicates that different epitopes show a wide variation in stability profile prompting the need to explore optimal flank (and linker) sequence and length engineering through predictions and experimental assessments to stabilize them.

4. Discussion

As a highly persistent and economically devastating pathogen in swine population, the significant impact of PRRSV underscores the urgent need for effective vaccine development [68,69]. Hence, the identification and characterization of epitopes for PRRSV vaccine development has the potential for a critical advancement in its control. In this context, deep learning and artificial intelligence approaches will be particularly beneficial. They have the potential to significantly enhance and reshape vaccine design, particularly in the areas of epitope detection and optimization [70]. Therefore, our comprehensive approach, integrating deep-learning based computational epitope prediction, sequence design, and structural validation, has yielded promising candidates for immunogen development.

Table 2 summarizes the critical interactions between the PRRSV GP5 protein and various host proteins, which are crucial for the virus's entry, internalization, and spread within host cells. The table also compiles specific experimental techniques used to confirm PRRSV-porcine interactions in different studies, such as immunofluorescence assays, co-immunoprecipitation, and CRISPR/Cas9. Our analysis also included other PRRSV proteins and their potential interactions with porcine proteins. These interactions encompass all 63 combinations of protein

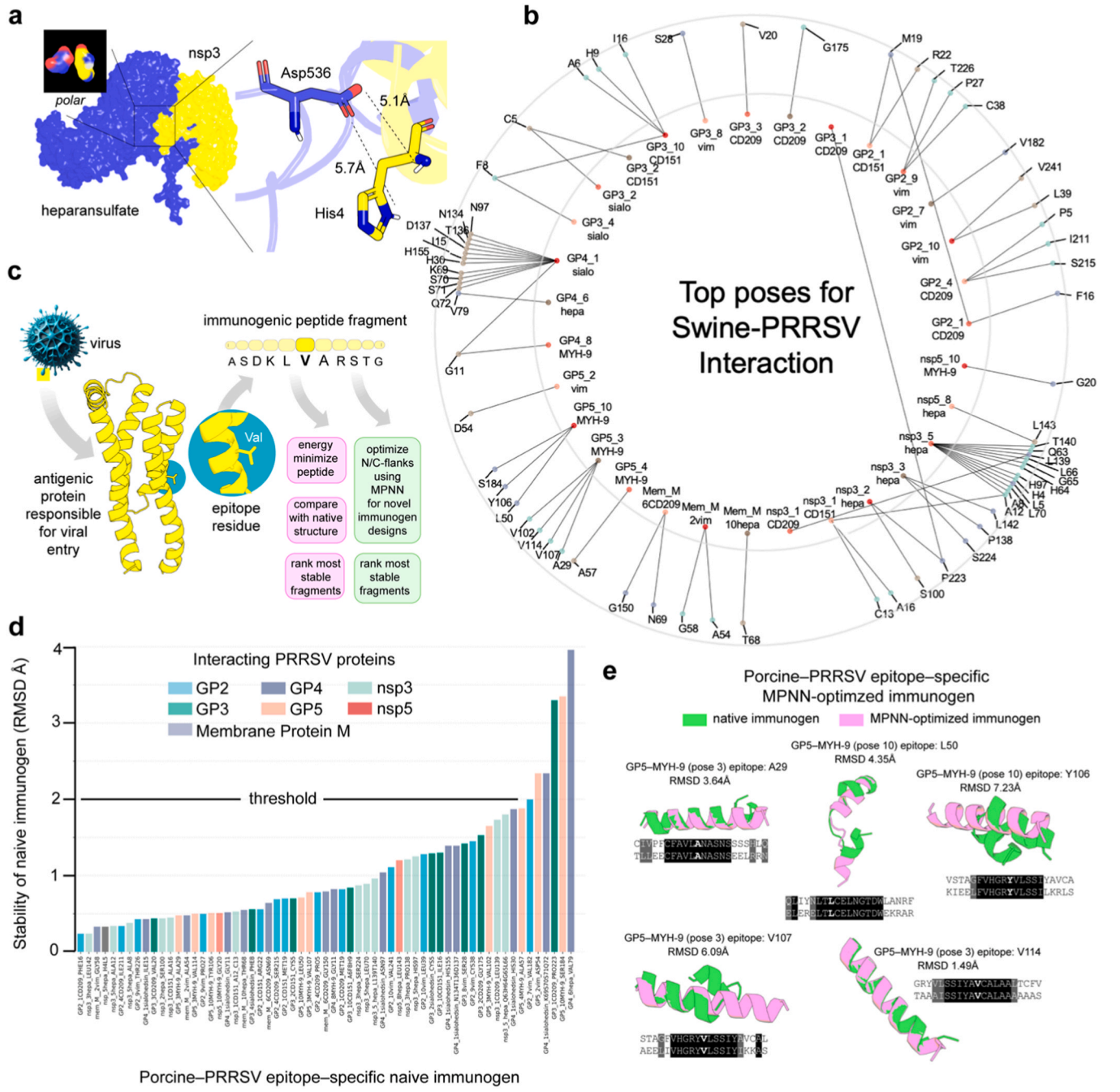

Fig. 4. (a) Interaction between Glu534 and Ser32 and predicted novel interaction between His4 and ASP536 of nsp3 and heparansulfate. (b) Top candidate epitopes based on energy scores and their associated poses determined by molecular docking. The inner circle contains the PRRSV protein name, specific source pose number of the epitope from our database, and the interacting porcine protein. (c) Overview of immunogen design from epitope engineering. This figure illustrates the process of immunogen creation from Ser32 epitope of GP5 protein. Initially, an epitope is identified and extracted from the antigen. Engineered flanking regions are added to stabilize the epitope's secondary structure. The stability of the resulting immunogen structure is then subjected to analysis. (d) Relaxing the immunogens structures in pyrosetta relax with 2 Å RMSD stability threshold. 92 % of the immunogens demonstrated stability in isolation retaining their original antigenic conformations. (e) Sequence variation and optimization of five selected GP5 immunogen's extended versions using ProteinMPNN. For each immunogen, the first sequence represents the original structure, while the second sequence illustrates one of the most stable variants. The RMSD values indicate the structural deviations from the original immunogen, reflecting the changes to the peptide structure after sequence modifications. The superimposed structures of the native and ProteinMPNN-optimized immunogens indicate parts of the peptides that contribute to loss of structural similarity in the designed sequences.

pairs outlined in Table 1. Understanding these interactions is vital for developing targeted therapies and interventions against PRRSV. This was done in all throughout the study, finding specific interactions and epitopes of all possible protein partners.

Our comprehensive approach reveals CD163 as a key gateway protein for PRRS virus entry into pigs. Clinical studies have already demonstrated that CD163 deletion in pigs confers immunity against PRRSV infection, validating the computational findings [64,71,72]. Furthermore, our analysis reveals that the Glu534 residue of CD163 interact with the Ser32 residue of GP5 (Fig. 4). The carboxylate group of glutamate forms hydrogen bonding with the hydroxyl group of serine. In one study it has been observed that mutating Arg561 and Glu534 of

Table 2
Summary of Key PRRSV-GP5 Interactions with Host Proteins, their Roles in Viral Entry, and Various Experimental Methods for Validation.

Host Protein	GP5-Host Interaction	Experimental Validation Methods	Citation
CD209	GP5 engages with CD209 to facilitate viral entry into host cells.	Immunofluorescence assay (IFA) and western blot	[52,53]
Vimentin	GP5 associates with vimentin; the specific role of polar interactions remains unclear.	Plaque reduction assay, MALDI-TOF MS, and 2-D gel electrophoresis	[54]
CD151	GP5 interaction with CD151 is limited but may involve polar interactions.	cDNA library screening, gel shift assays and in vivo RNA-protein interaction	[53,55]
Sialoadhesin	GP5 engages with sialoadhesin, promoting viral invasion.	precipitation assay followed by Western blot analysis	[53,56]
Heparan Sulfate	GP5 interacts with heparan sulfate, aiding in viral adhesion.	immunofluorescence assay (IFA)[57]	[28,58]
CD163	GP5 engages CD163, a key step in viral entry.	CRISPR/Cas9	[59,60]
MYH−9	GP5 associates with MYH−9, supporting internalization and viral spread.	Co-immunoprecipitation (Co-IP)	[50,59,61]

CD163 simultaneously provide additive resistance to PRRSV infection, and they play a significant role in the viral infection process [64,73]. Thus, we seem to capture one of the most significant interactions in PRRS disease. Arg561 is seen to establish a long-range electrostatic interaction with PRRSV. It is apparent that the positively charged guanidinium group of Arg561 interacts with the electron-rich aromatic ring of Trp18 (10.8 Å). As illustrated in Fig. 2(b) and further demonstrated by the interactions of GP5 with other host proteins in Table 2, polar interactions are likely to play a critical role in facilitating and stabilizing the binding between PRRSV GP5 and host proteins. Polar interactions, including hydrogen bonds and ionic interactions, are fundamental in stabilizing protein-protein complexes and enhancing binding affinity. In the context of PRRSV GP5 and host protein interactions, these polar interactions are likely significant, though not always explicitly detailed in studies. For instance, the interaction between GP5 and MYH9, crucial for viral internalization and intercellular spread, likely involves charged domains that stabilize the binding [50,74]. Similarly, the binding of GP5 to heparan sulfate for viral attachment is expected to involve electrostatic interactions between negatively charged sulfate groups and basic residues on GP5 [58]. Moreover, while specific polar interactions with CD163 are not explicitly detailed, they likely enhance binding and facilitate viral entry due to the nature of receptor-ligand interactions. CD163′s cysteine-rich domains, which include both acidic and basic residues, likely promote electrostatic interactions with viral glycoproteins [75].

Additionally, it is likely that many other epitopes contribute to viral entry and subsequent infection. Recent studies have found that several envelope glycoproteins eg., GP2, GP3, and GP4 can contribute to the virus infection [76,77]. Several nsps and the E protein have also been implicated in viral interaction and progression [47,78,79]. In fact, we characterized the interactions between nsp3 and heparansulfate, occurring between the glutamate and serine residues (Fig. 4). We predicted the 3D structures of 12 nsps in our study using AlphaFold2. However, the prediction quality was low, as indicated by the poor pLDDT scores, highlighting the complexity of accurately modeling these non-structural proteins. This is further detailed in the Supplementary Material 5, Figure. S1. Nevertheless, certain nsps—specifically nsp1α/β, nsp2, nsp4, and nsp11—are known to play critical roles in modulating the host's innate immune response during PRRSV infections [80]. Moreover, membrane proteins GP5 and membrane protein-M can form a disulfide-linked dimer together. They are major components of virions, which is required for virus budding [81]. Additionally, we visualized a potential interaction between each of these PRRSV and porcine proteins at the amino acid level (Fig. 2(b) and Supplementary Material 5, Figure. S2).

GP5 is targeted for vaccine development in most of the cases for its role in producing neutralizing antibodies [82]. GP5 is encoded by the PRRSV *ORF5* gene. Additionally, an alternative ORF of the sub genomic mRNA for GP5, has been identified to encode the ORF5a protein. This protein is expressed in infected cells, and swine infected with PRRSV also produce anti-ORF5a antibodies [82]. Our study integrates all relevant information and potential interactions into an epitope atlas (Supplementary Material 1). Targeting a single protein, such as GP5, for vaccine development can lead to viral resistance, and escape due to the protein's high mutation rate. Indeed, GP5 has substitutions, deletions, and insertions mutations in several sites like signal peptide region, and near T-cell antigenic sites [83]. By compiling a comprehensive list of all potential epitopes and ranking them, we open the possibility of developing a multiepitope/ multivalent vaccine and antibody design campaigns that can neutralize epitopes from several PRRSV proteins. This approach is anticipated to be more effective and less susceptible to mutations. Therefore, incorporating all possible PRRSV proteins into the epitope atlas significantly enhances the potential for successful vaccine development. Moreover, the use of advanced AI/ML ProteinMPNN allows for the flexible manipulation of sequences while preserving the 3D structure and core epitope integrity. This facilitates the exploration of amino acid sequence variations to achieve better adjustments while maintaining their functional integrity. We only conducted this with GP5 variant immunogens. It shows such a workflow can be used to generate similar *de novo* designed immunogens that fold into antigenic poses, ensuring stability and functionality [37,44].

The significance of our findings lies in their potential to address the ongoing challenges posed by PRRSV in the swine industry. This discovery is of significant economic importance to Iowa, the largest pork producer in the nation, as it offers a pathway to enhance disease resistance in pigs and safeguard the pork-based economy [84]. Due to PRRSV's high mutation rate, a vaccine incorporating epitopes from multiple PRRSV proteins is essential [68,85]. The integration of advanced computational tools and deep learning models has enabled the efficient identification and validation of PRRSV epitopes and immunogens, which holds a promise for the development of an effective PRRSV vaccine. However, our findings require validation through wet lab studies. Our approach primarily focuses on creating a comprehensive database and extending the interaction knowledge between epitopes and paratopes. Developing a multi-epitope vaccine based on this knowledge will require further efforts, including experimental validation of the candidates in vivo to assess their immunogenicity and protective efficacy in swine.

5. Conclusion

We identified top 75 epitopes interacting between PRRSV and swine and created an extended epitope atlas. Additionally, we used deep-learning and AI-based approach to design 56 immunogens from these epitopes. Possibility of sequential variations with structural similarity was also investigated. By providing a detailed roadmap for the development of PRRSV vaccines, this patent-pending work sets the stage for future research aimed at controlling this and other economically important swine pathogens.

Ethical approval statement

N/A.

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Author statement

R.C. conceived and designed the study. S.D. performed all the analyses. M.R. provided key literature and guided the study on PRRSV-porcine interactions for this work to be industrially relevant. J.B. and M.B. performed protein-protein docking and molecular analyses of interactions. All authors contributed to writing the manuscript and consents to the final version of the manuscript.

CRediT authorship contribution statement

Jennifer Bruner: Validation, Visualization, Writing – review & editing. **Ratul Chowdhury:** Writing – review & editing, Visualization, Validation, Supervision, Funding acquisition. **Maria Brown:** Validation, Visualization, Writing – review & editing. **Mike Roof:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Supantha Dey:** Writing – original draft, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2024.08.029.

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