Large-Scale Computational Modeling of H5 Influenza Variants Against HA1-Neutralizing Antibodies

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In June 2024, the U.S. Department of Agriculture released a report that showed over 300 samples from 2022-2024 of highly pathogenic avian influenza have been detected in mammals (1). To date, the World Health Organization reports that there have been approximately 5 humans infected with H5N1 in 2024, but the broader potential impact to human health remains unclear. In this study, we computationally model 1,804 protein complexes consisting of various H5 isolates in 1959 to 2024 against 11 HA1-neutralizing antibodies. This study shows a trend of weakening binding affinity of existing antibodies against H5 isolates over time, indicating that the H5N1 virus is evolving immune escape of our medical defenses. This study also showcases the value of high-performance computing to rapidly model protein-protein interactions at-scale for functional insights into medical preparedness.

influenza | H5 | antibodies | protein modeling | docking

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Introduction

Wild aquatic birds (e.g., order Anseriformes) are fundamental hosts of influenza viruses, which are transmitted from domestic birds (e.g., Galliformes) and mammals (orders Artiodactyla, Carnivora, Cetacea, and Primates) (2, 3). In 1996, H5N1 influenza largely occurred in anseriforms and spread to humans and chickens in Hong Kong in 1997 (3, 4). As a response to H5N1 in chickens and occasional human infection chickens were culled in Hong Kong in the period 1997 to 2011 (5). In 2002, the most common hosts of H5N1 were anseriformes with occasional transmission to galliformes and humans throughout China and South East Asia (3, 6). Since 2003, various lineages of H5N1 have spread throughout China and Hong Kong, South East Asia, Russia, North Africa, the West Bank, Gaza strip and Israel, Pakistan, Bangladesh, India, Bhutan, Nepal, Europe, Japan and South Korea, using a wide variety of hosts (7, 8). Many avian taxa (Charadriiformes, Accipitriformes, Corvidae, Ardeidae, Columbidae, and Passeriformes) as well as primate, carnivore, artiodactyl, and arthropod hosts have been infected with H5N1 (3). H5N1 infections in humans have been reported by the World Health Organization (WHO) in: Hong Kong 1997–2003, in China and Hong Kong 2003–2014; in Thailand 2003–2007; in Indonesia 2005–2012; in Nigeria in 2007; in Bangladesh 2011-2013; in Azerbaijan, Turkey, Iraq, Myanmar, Pakistan, and Djibouti in 2006-2007; in Egypt 2003-2014; in Lao PDR 2007; in Vietnam 2003–2014; in Cambodia 2003–2014, and in Canada in 2014. The document produced by the WHO has not been updated since.

However, a comprehensive review from 2023 (9) illustrates the further spread of H5N1 in animals as follows:

- **Asia:** Hong Kong, India, Bhutan, Japan, Korea, Nepal, Philippines, Taiwan
- **Europe:** Austria, Belgium, Bulgaria, Czechia, Denmark, France, Germany, Hungary, Italy, Luxemburg, Moldova, Netherlands, Poland, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Croatia, Estonia, Finland, Latvia, Luxembourg, United Kingdom, Ireland, Lithuania, Norway, Serbia, Switzerland
- **The Middle East:** Israel, Turkey
- **Africa:** Gambia, Nigeria, Senegal, Bolivia
- **North and South America:** Canada, United States, Peru, Chile, Colombia, Panama, Brazil

And in 2024, H5N1 has been found in animals in Antarctica (10). Charostad et al. (2023) also report H5N1 human infections in 2022 and 2023 in Cambodia, Chile, China, Ecuador, Spain, United Kingdom, United States, and Vietnam. More specifically, In March 2024, an outbreak was reported among U.S dairy cattle which spread amongst herds, leading to fatal infections among cats, infection into poultry, and four reported infections for dairy workers (11). From 1997, through late April 2024, 909 human H5N1 cases were reported, with 52% of cases being fatal (12).

Continued transmission of avian strains to human populations and subsequent human-to-human transmission can have a devastating effect on public heath worldwide. As the human-animal interface increases due to shrinking of natural habitats, deforestation, and increased demand for animal products; animal-human disease transmission is becoming more common (13). Current human seasonal influenza vaccines do not confer immunity against H5N1 influenza or other animal influenza A viruses (14). Thus, it is of great public health interest to discover patterns and molecular insights into the effects of H5 mutations on existing human immunity (15). In this study, we present the results of a large computational corpus of molecular docking experiments between various H5 isolates against existing HA1-neutralizing antibodies and show their changes over time.

Results

From the study set of 18,693 H5 isolates, we show a breakdown of hosts similar to what has been reported in previous studies (2, 3). As shown in Table 1, approximately 88% of the isolates are from birds (class Aves). Also, note that all 666 isolates from Primates were collected from humans.

Class	Order	Isolates	% of Total
Aves	Galliformes	5,844	31.26%
	Anseriformes	4,469	23.91%
	Other Orders	934	5.00%
	Not Specified	5,287	28.28%
	Aves Total	*16,534*	*88.45%*
Mammalia	Primates	666	3.56%
	Carnivora	191	1.02%
	Artiodactyla	57	0.30%
	Other Orders	6	0.03%
	Not Specified	360	1.93%
	Mammalia Total	*1,280*	*6.85%*
Insecta	*Insecta Total*	*3*	*0.02%*
Other	Laboratory Derived	178	0.95%
	Other / Environmental	698	3.73%
	Other Total	*876*	*4.69%*
Grand Total		**18,693**	**100%**

Table 1. Taxonomic breakdown of the isolates used in this study.

As shown in Supplementary Figure 2, the proportion of H5 isolates collected from various continents has changed over time. To date, isolates collected in 2024 are predominately from Europe, driven by the increased sequencing of infected livestock.

Sequence Analyses. The clustering of the 18,693 HA1 sequences resulted in 250 distinct clusters at ≥97% identity. Further organization of the representative sequences of each cluster, shown in Supplementary Figure 3, indicates a continuous distribution of antibody binding performance.

Isolates EPI764389 and EPI597824 are outliers in that they have poor antibody binding performance across all of the antibodies tested (average van der Waals energies >-60. Conversely, isolates EPI2429052, EPI167665, and EPI275974 show the best antibody affinity, having average van der Waals energies <-100.

Of note, antibodies 12H5, 3C11, 65C6, AVFluIgG01, and H5M9 show the best performance overall, though there are exceptions of poor performance.

Phylogenetics. The alignment used in phylogenetics consisted of 18,508 taxa and 1,899 aligned positions. TNT searches at level 1 for 1000 replicates consistently produced 4 trees of 44,550 steps. Transmission network analyses of the best scoring tree of the H5 isolates in this study show a considerable pattern of transmission between various classes of organisms. Specifically, the most common being between avian-to-mammalian transmission, of which there are over 600 apomorphic transitions across the tree. See Figure 1a.

There is also a consistent transmission between many continents across the globe, especially between Europe and North America, as shown in Figure 1b.

Worsening Binding Affinity. With respect to collection date, there are significant correlations that show a worsening in antibody binding affinity in isolates collected from orders Anseriformes, Galliformes, and Primates. See Figure 2.

However, this worsening in binding affinity does not seem localized to specific antibodies. Though the antibodies' performances are from independent distributions, as shown by the Kruskal-Wallis test in Supplementary Figure 4, the overall trend suggests that more recent isolates have mutated to better evade existing antibodies in general.

Mutational Effects. As indicated in Shi et al. (2014), there are various mutations in the HA1 receptor binding domain that enable 'host jumping'. Our results show several statistically significant differences in binding affinity of antibodies given the presence of these mutations. Of note, N158S, T160A/S/V, E190N, and G225R all result in weakened antibody binding affinity across multiple metrics. Conversely, T160K and G228S are shown to increase binding affinity in some metrics. Significant changes based on van der Waals energies and HADDOCK Score are shown in Figure 4 and all other metrics are shown in Supplementary Figure 5.

In Figure 3, subfigures A and B represent the worst and best binding structures across the experimental results, isolates EPI168674 and EPI2429052, respectively. Though the epitope is different between isolates, note the variation in quantity of polar contacts within the respective complexes.

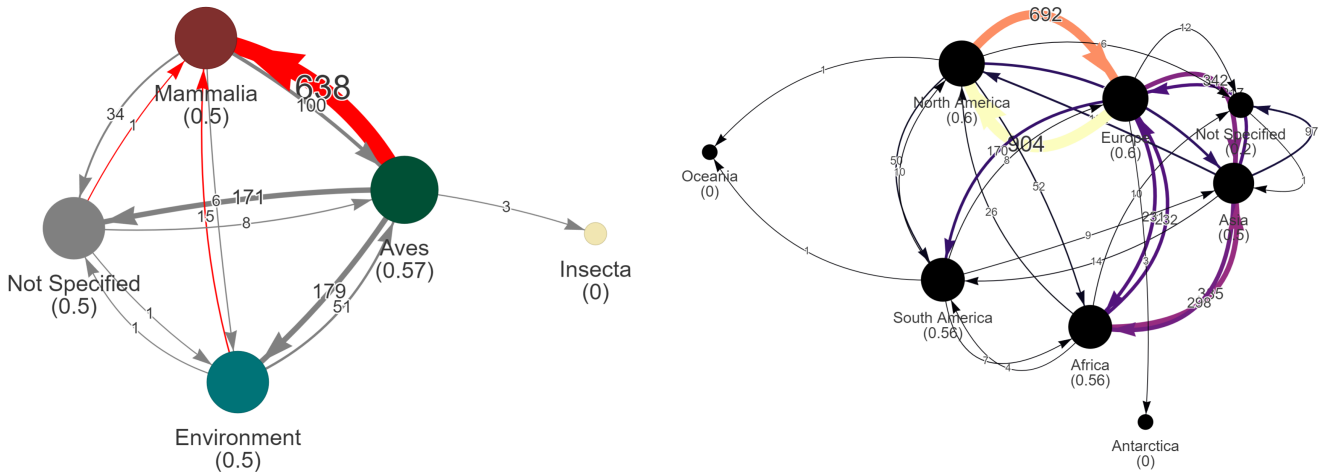
Figure 3D is an example of modest binding affinity between an isolate EPI658567 and antibody 12H5. However, this improved binding affinity compared to other isolates is not due to the G225R mutation as this residue is not in the epitope of the antigen.

Figure 3E shows the poor binding affinity of isolate EPI3178330 with antibody FLD194 due to the E190N mutation, though this residue is still forming a polar contact with the antibody CDR loop. The mutation from glutamic acid (E), a negatively charged side chain, to asparagine (N), a polar uncharged side chain.

Isolate EPI242227 is the oldest isolate in the dataset, collected in 1959. Note that this isolate contains the N158D mutation and its interaction with various antibodies results in a wide range of van der Waals energies from -52.81 (worst) to -92.84 (best, with antibody 65C3 as shown in Figure 3F).

Interfacing Residue Prevalence. An analysis of the interfacing residues in the best complexes from all 1,804 experiments shows patterns in particular residues forming polar contacts with the antibodies tested. Residues 156, 193, 222 are often interfaced (≥25% prevalence) in the antigen epitope. These are shown in red in Figure 5.

Many of the frequently interfaced residues shown in this study closely neighbor antigenic and receptor binding sites reported in Sriwilaijaroen and Suzuki (2012) Figure 2. Residues 130, 132, 158, and 159 are part of two glycosylation sites that, in 2012, were only found in seasonal H1 viruses (17).



(a) Host transmission network. **(b)** Geographic transmission network.

Fig. 1. Transmission networks, generated by StrainHub, showing apomorphic changes between (a) hosts and (b) continents. Node values in parentheses represent the source-hub ratio of that class/location. A source-hub ratio of 1 indicates that the state is always the source of the transmission. The numerical labels annotated on the edges of the graphs represent the number of apomorphic changes seen across the phylogenetic tree between metadata states.

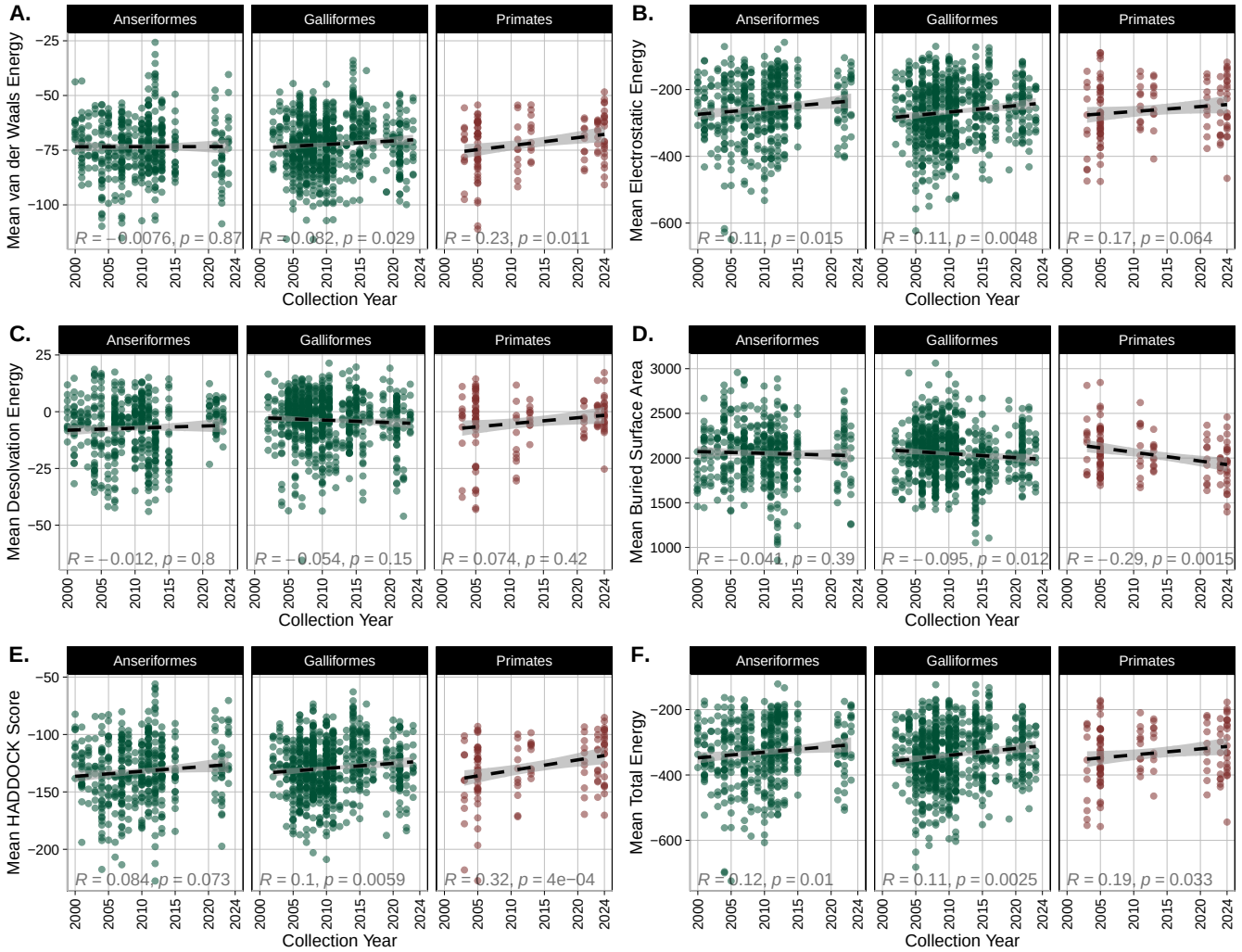


Fig. 2. Antibody binding performance metrics over time for isolates collected from Anseriformes (waterfowl), Galliformes (chickens, etc.), and Primates (humans). Note: Spearman correlations are shown based on all isolates in the data (from 1959-2024), but the plots are truncated to show collection years from 2000-2024.

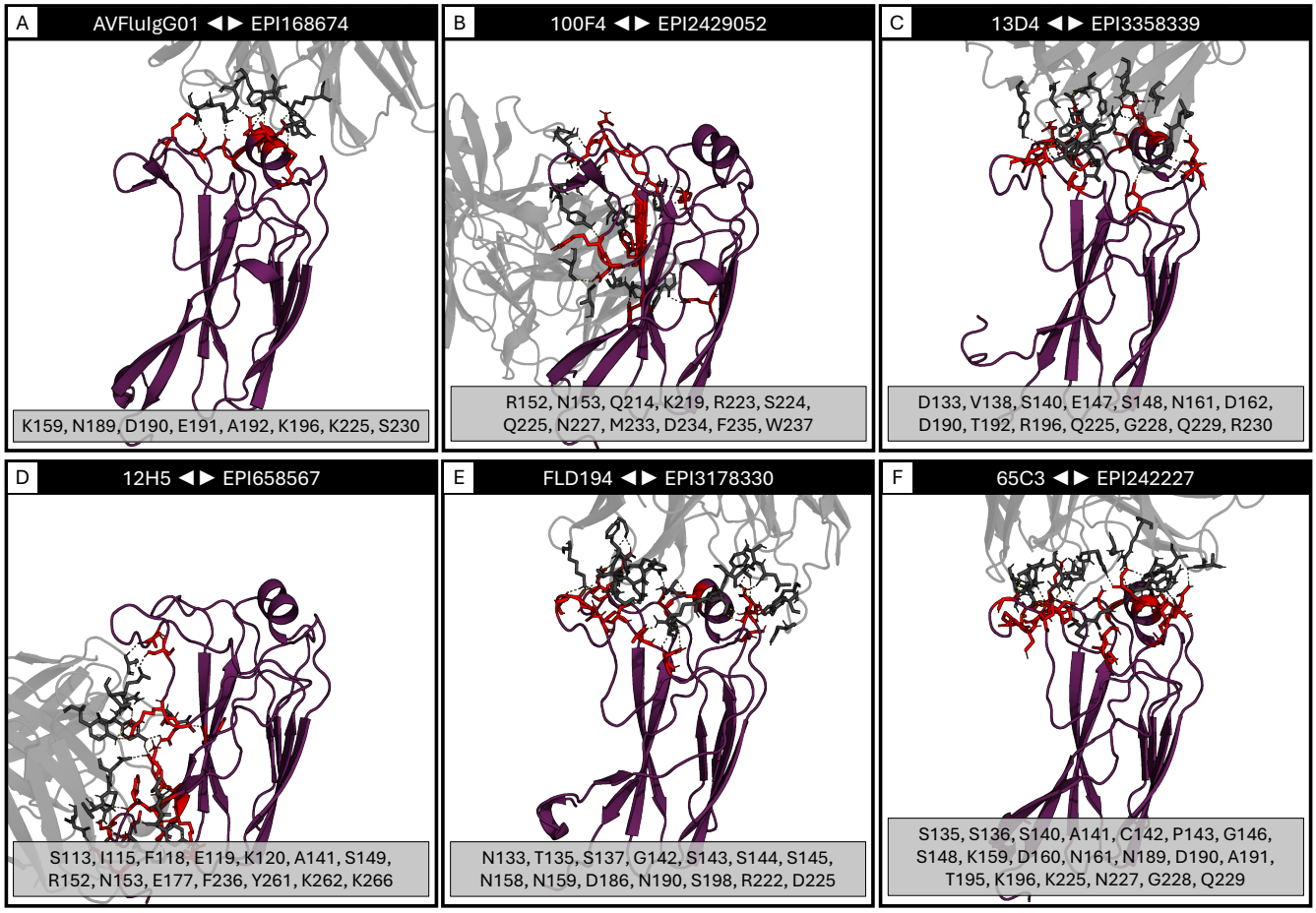


Fig. 3. Example interface renderings showing the diversity in epitopes, residues, and binding affinity. The grey cartoon structure is the Fab portion of the docked antibody and the purple structure is the HA1 antigen with sticks designating the polar contacts between them (shown as sticks). The list below each subfigure contains the interfacing residues on the antigen chain.

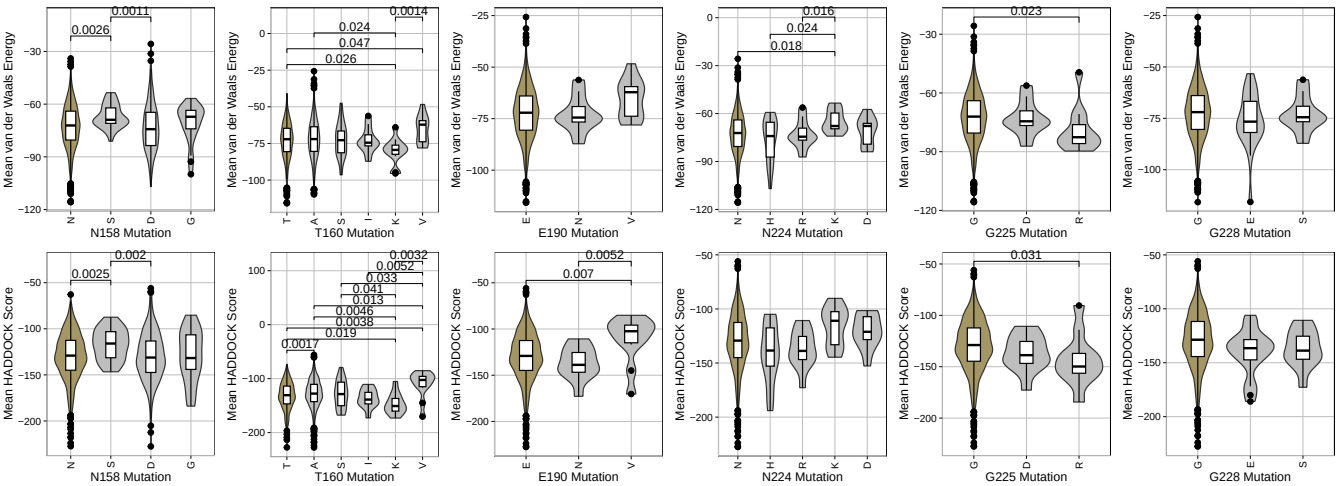


Fig. 4. The distribution of van der Waals energies and HADDOCK score docking metrics broken out by antigen mutations. The first amino acid shown on the left of each plot in gold represents the reference residue at that position as described in Shi et al. (2014). Statistical comparisons shown are significant Wilcoxon Rank Sum test p-values at the α<0.05 level.

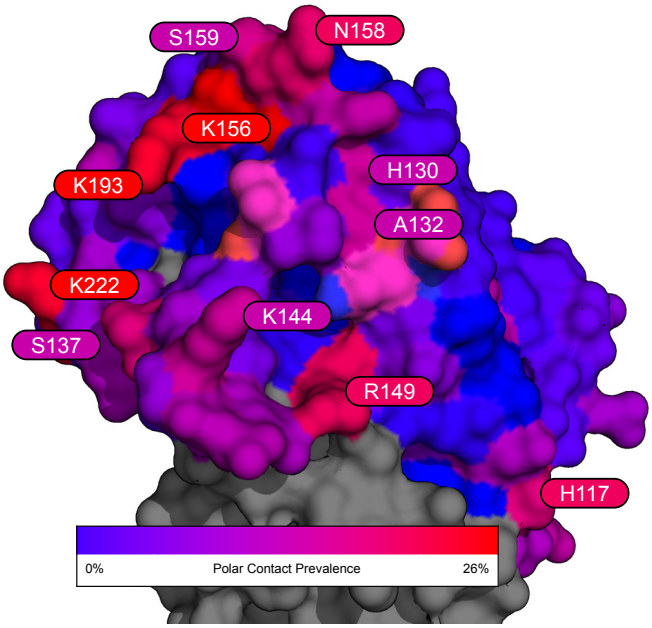


Fig. 5. Surface rendering of the HA1 globular head domain (reference PBD 2FK0) showing the prevalence of each residue to form polar contacts (within 3Å of antibody residues) across the experiments in this study. Annotated residues are those with $\geq$16% prevalence.

Discussion

Relation to Prior Studies. This *in silico* analysis yields the trend of a reduction in binding affinity for neutralizing antibodies against H5N1-designated influenza variants. This reduction in binding affinity reinforces previous studies that evolution has occurred to yield HA proteins that are elusive of antibody neutralization (18, 19). The trend observed is consistent with empirical studies and strengthens the relatively novel *in silico* approach taken within this study.

Diversity of H5N1 Interactions. As shown in Supplementary Figure 3, high disparities in antibody binding affinity exist between different sequences designated as H5N1. This disparity leads to concerns over the nomenclature of H5N1. For future clinical practice to effectively treat H5N1, clinicians must be able to more precisely identify sub-variants of H5N1 to identify antibodies that have specific neutralizing capabilities against the identified variant. For example, in Supplementary Figure 3, sequence EPI597824 has a large disparity in van der Waals energy between antibody 3C11 and AvFluIgG03. In addition, Supplementary Figure 5 demonstrates the effect of a single amino acid mutation on docking metrics. One change in an amino acid residue can lead to statistically significant differences in antibody binding affinity. Broadly categorizing a viral diversity as simply "H5N1" will lead to a lack of precision in the design of therapeutics and treatments, which may will have adverse health outcomes.

Zoonosis Analysis. In Figure 1a, a notable transition pattern is observed from avian species (class Aves) to mammals (class Mammalia), likely attributable to a mutagenic drift. Recent empirical studies have investigated the mutational dynamics of H5N1, revealing changes in the hemagglutinin (HA) protein. While Influenza prefers binding to the α2-3 sialic receptors in birds, this study demonstrated a binding affinity of H5N1 to α2-6 sialic acid receptors, predominant in mammals, at almost equal proportion (20). Concurrently, mutations in the HA protein also show reduced susceptibility to neutralization by antibodies.

Our *in silico* study aligns with these findings, indicating a progressive decrease in H5N1's binding affinity to antibodies in our isolates over time. As illustrated in Figure 2, while the binding affinity to antibodies remains relatively stable for avian isolates, there is a marked decline in affinity for primate isolates. This suggests a potential evolutionary trajectory for H5N1 towards increased infection in primate/mammal hosts, facilitated by decreased susceptibility to neutralizing antibodies.

Isolate EPI3358339. EPI3358339, a H5N2 subtype isolate, was added to this study as it is from the first ever human infection of H5N2 avian influenza seen in Mexico. Unfortunately, this strain was found in a person from Mexico who died of complications due to the infection. However, it is not yet known if the zoonotic "jump" of the isolate is cause for concern or if the individual had other comorbidities that played a role is his death. It is also not known if this case is related to recent poultry outbreaks in the area.

The experimental docking conformation between antibodies and this antigen (antibody 65C3 shown in Figure 3F) are predicted to have a relatively strong binding affinity (e.g., van der Waals energy: [-51.96, -95.45]) across all the tested antibodies.

Thus, our experiments using this isolate do not indicate that this isolate is highly mutated, though some mutations may have reduced the van der Waals and electrostatic energies of the interaction with this individual's existing antibodies, if any.

Structural Analysis. When comparing the predicted docking outputs to empirical structures, such as those listed in Table 2, we see similar binding epitopes in the the predicted docking complexes versus the empirical structures (21–28).

Furthermore, the binding conformations seen in the empirical structures often mimic the predicted complexes in the experiments in this study, though various mutations effect the binding angle, polar contacts, electrostatics, and overall affinity. In general, this supports the confidence in the predictive accuracy of the *in silico* experiments given their similarity to empirically derived structures. However, empirical studies are still needed to validate specific complexes.

Motifs of Interest and Future Research. H5N1 exists as an endemic in avian populations. This epidemic creates a pool containing vast numbers of host species in which the RNA virus evolves rapidly. It is an endemic that is difficult to diminish due to the nature of the HA protein's host receptors lacking homogeneity. Serological immunity from vaccination or prior infection in avian hosts may have yielded selective pressures in the evolution of specific mechanisms of

entry for H5N1 (29). Subsequently high infection rates lead to new mechanism of entry. Over time, serological immunity from original vaccination and/or infection is reduced, and the cycle of influenza transmission continues.

Highly conserved portions of HA are of high interest. The three primary conserved elements of the receptor-binding site (RBS) on the HA1 subunit are the 130 and 220-loops and the 190-helix (30–32). As shown in Figures 3 and 5, the antibodies docked to conserved motifs on the studied H5N1 strains, further supporting the empirical literature that initially identified their neutralizing capability. The inhibition of HA1-Host Receptor binding by RBS targeting antibodies may not however be the only mechanism of action of antibodies targeting HA to inhibit viral replication for future research. Inhibition of Neuraminidase activity via the steric hindrance provided by the antibody-HA1 complex has been demonstrated in the past (33). In the future, targeting of conserved portions of the HA1 protein whilst also inhibiting neuraminidase is a strategy that may yield substantial neutralization capacity to future therapeutics.

More recent development of multivalent mRNA-based vaccines has been successful in H5 influenza A clade 2.3.4.4b (from which there are 15 sequences used in this study) (34). The selection of high-quality sequences that elicit strong antibody responses is a complex process in mRNA vaccine development. However, the utility of *in silico* modeling as presented in this study will help to reduce the future lab workload by positing predicted candidate sequences from which vaccines can be developed.

Computational modeling of immunoprotein interactions as shown in this study and previous works (35–40) have proven to be highly effective in the understanding of the health impacts of pathogen variants. For H5 influenza, this study, along with recent preprints (41, 42), show an overall trend of worsening antibody binding and depicts the recent increase in avian-to-mammalian transmissions due to various mutations. This suggests that there is am impending danger to human health for highly pathogenic strains of H5 influenza that can infect avian and mammalian livestock and jump to humans.

Methods

This study closely follows the published methods in Tomezsko and Ford et al. (2024) and other protein modeling studies by this team (36–40). The specific workflow for this study is depicted in Figure 6.

HA1 Sequence Procurement. 18,693 influenza A H5 sequences were procured from the GISAID EpiFlu database (43, 44) on June 17, 2024. Isolate metadata, including collection date, country of origin, and host information, were also collected. We then derived taxonomic classifications from the provided host metadata along with continent of origin from the country information.

These amino acid sequences were clustered using CD-HIT v4.8.1 (45, 46). Ranging in size from 11 to 576 amino acids, the clustering resulted in 250 clusters, based on 97% identity. A representative sequence from each of these clusters was selected by the clustering program, which was then output in a FASTA file.

These representative sequences were then aligned using MUSCLE v3.8.425 (47) using default settings. Then, sequences that were of low quality, incomplete, or did not contain the desired HA1 domain were removed. This resulted in 164 amino acid sequences that were then used in the subsequent steps. These sequences were then trimmed to the HA1 receptor binding domain (approximately residues 111-269, depending on the presence of indels) (17).

Also, clusters consisting of only lab derived isolates ($n = 3$) were analyzed, but have been left out of the reported statistics and visualizations.

Structure Prediction. Structures for each of the 164 HA1 sequences were predicted using ColabFold v1.5.5 (48), a protein folding framework that uses AlphaFold2 (49) accelerated with MMseqs2 (50), with default settings. The side chains of these predicted structures were relaxed using the OpenMM/Amber method (51). The .PDB file output of the top structure (i.e., the one with the highest pLDDT[1] confidence) was selected and used for subsequent analyses.

Neutralizing Antibody Collection. Existing structures for 11 HA1-neutralizing antibodies were collected by a database search through Thera-SAbDab (52) and the Protein Data Bank (53). Each of these 11 antibody structures have an epitope on the target HA1 domain of the hemagglutinin protein, though not all share the same epitope. These antibodies and their respective PDB IDs are listed in Table 2.

Antibody ID	PDB ID	Reference	H/L Chain Subgroups	Source Information
100F4	5dur	(21)	II/I	Human Memory B-Cell, Recovered from H5N1 Infection
12H5	7fah	(22)	I/IV	Mouse, Immunized with three H1N1 strains, Humanized
13D4	6a0z	(23)	I/I	Mouse, Immunized with five H5N1 strains, Humanized
3C11	6iuv	(24)	I/II	Human Memory B-cell, Infected by H5N1 viruses
65C6	5dum	(21)	I/III	Human, Infected by H5N1 viruses
AVFluIgG01	6iut	(24)	II/I	Human, Infected by H5N1 viruses
AVFluIgG03	5dup	(21)	III/I	Human, Infected by H5N1 viruses
FLD194	5a3i	(25)	II/I	Human Memory B-cell, Recovered from H5N1 infection
FLD21.140	6a67	(26)	?/I	Human, Recovered from H5N1 Infection
H5M9	4mhh	(27)	I/IV	Mouse, Immunized with H5N1, Humanized
H5.3	4xrc	(28)	II/?	Human, Immunized with one H5N1 strain

Table 2. HA1-neutralizing antibodies.

Docking Analyses. Using HADDOCK3, a computational framework for the integrative modeling of biomolecular complexes, each antibody was docked to each antigen across the dataset. Given the 11 antibody structures and 164 HA1 structures, this resulted in 1,804 docking experiments to be performed.

Experiment Generation. HADDOCK3 inputs for each experiment were generated programmatically, defining the antibody and antigen .PDB file inputs on which to dock. Other experiment files were also copied or created programmatically that include the scripts to run the docking process and togenerate other configuration files.

[1]Predicted local distance difference test (pLDDT): An estimate of local confidence, scaled from [0, 100], where higher scores indicate higher confidence in the protein conformation.

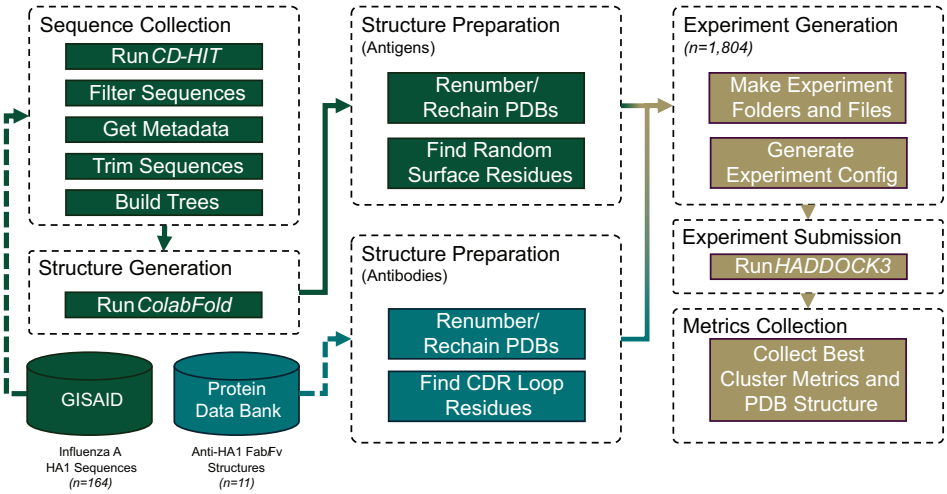


Fig. 6. Workflow diagram of the data procurement, data preparation, and analysis steps.

HADDOCK3 requires the definition of active/inactive residue restraints (AIRs) to help guide the protein docking process. To avoid biasing the docking placement of the antibody on the HA1 antigens, a random subset of surface residues were selected as "active" and were then included in the AIR file on which to dock. For the antibody structures, residues in the CDR loops were detected using ANARCI (54), a Python package for numbering antibody sequences.

Lastly, HADDOCK3 configuration files were generated programmatically, which define the input .PDB files, the output directory, and the steps of the docking process. The logic for this programmatic generation of HADDOCK3 configuration files is available in the supplementary GitHub repository.

Docking Process. HADDOCK3 provides a configurable interface for defining the individual steps of the docking process, including the rigid-body docking, flexible refinement, and solvent-based refinement along with any desired clustering and filtering steps.

For this study, we customized the published HADDOCK3 protocol for antibody-antigen modeling (55) to focus on generating the singular best cluster of docking results for each experiment and reducing excess work by the docking process. The specific steps of our HADDOCK3 configuration that was used for the experiments are shown in Table 3.

The HADDOCK3 system outputs multiple metrics for the predicted binding affinities and an output set of PDB files containing the antibody docked against the HA1 antigen. Some main metrics include:

- van der Waals intermolecular energy (*vdw*)
- Electrostatic intermolecular energy (*elec*)
- Desolvation energy (*desolv*)
- Restraints violation energy (*air*)
- Buried surface area (*bsa*)
- Total energy (*total*): $1.0vdw + 1.0elec$
- HADDOCK score: $1.0vdw + 0.2elec + 1.0desolv + 0.1air$

Note that the HADDOCK Score is a conglomerate metric used for assessing the best complexes (or best cluster of complexes) that get promoted through the various refinement iterations in the pipeline.

Computational Scalability. For this study, we utilized a Docker containerized version of HADDOCK3[2,3], which contains all of the software dependencies to allow HADDOCK3 to run more readily in a high-performance computing (HPC) environment.

HADDOCK3 was run in a Singularity container on the UNC Charlotte Orion HPC cluster on 14 nodes, each with dual 18-core Intel Xeon Gold 6154 3.00GHz CPUs (36 cores per node). The average walltime of the experiments was $14.6\bar{3}$ minutes, with the entire set of 1,804 experiments completing in under 2 days.

Post Processing. Once all experiments were completed, the metrics for each experiment were either retrieved from the CAPRI evaluation outputs (if the FCC clustering algorithm (56) reached convergence) or from the REMARK entries of the best cluster's .PDB files. These metrics were organized in a single aggregate table, representing each experiment's best cluster metrics, for subsequent visualization and statistical analyses. The full table of experiment results is available in the Supplementary Data.

Phylogenetic, Statistical, and Protein Structure Analyses. For phylogenetic analyses, laboratory derived isolates were filtered out ($n = 178$, shown in Table 1), resulting in a set of 18,515 isolates. An alignment of the input 18,515 sequences was generated using MAFFT v7.471 (57) and then a phylogenetic tree search was performed using this alignment with TNT v1.6 (58). One of the best scoring trees was used for downstream analyses. StrainHub v2.0.0 (59) was used to generate transmission networks of the phylogenetic tree by host class and continent in Figure 1.

Statistical analyses were performed using R v4.3.4 (60) and plots were generated using ggplot2 (61) and ggpubr (62). Any statistical significance reported in this study is based on a p-value threshold of $\alpha < 0.05$.

Visualizations and analyses of the protein complexes were generated using PyMOL v2.4.1 (63) and BioPandas v0.4.1 (64).

[2]Container GitHub Repository: https://github.com/colbyford/HADDOCKer
[3]Docker Hub Images: https://hub.docker.com/r/cford38/haddock

Step		Description	# Models
1	Topology Modeling	Creates CNS all-atom topology	-
2	Rigid Body Modeling $n = 200$	Rigid body energy minimization with CNS (*it0*). Samples n models.	200
3	Cluster with FCC	Calculates the contacts between chains and then calculates the FCC matrix for each model. Clusters the models based on the calculated contacts.	200
4	Select Top Clusters $n = 5, m = 10$	Selects top n clusters of m models based on HADDOCK score	50
5	Flexible Refinement	Semi-flexible refinement using a simulated annealing protocol. Uses molecular dynamics simulations in torsion angle space (*it1*)	50
6	Cluster with FCC	Calculates the contacts between chains and then calculates the FCC matrix for each model. Clusters the models based on the calculated contacts.	50
7	Select Top Cluster $n = 1, m = 10$	Selects top n clusters of m models based on HADDOCK score	10
8	Solvent Refinement	Refinement by a short molecular dynamics simulation in explicit solvent (*itw*)	10
9	Molecular Dynamics Scoring	Performs a short molecular dynamics simulation on the input models and scores them.	10
10	Cluster with FCC	Calculates the contacts between chains and then calculates the FCC matrix for each model. Clusters the models based on the calculated contacts.	$\sim$10
11	Calculate CAPRI Metrics	Calculates i-RMSD, I-RMSD, Fnat, DockQ of the top scoring models.	$\sim$10

Table 3. Descriptions of the steps of the HADDOCK3 docking configuration.

Author Contributions

Authors SY, KO, and SGM retrieved the H5 antigen sequences from GISAID and antibody structures from Protein Data Bank. SY performed the structure prediction of the antigens. Author RAW performed the clustering analysis. Authors KO and CTF curated the metadata of the H5 sequences. Author DJ performed the phylogenetic analyses. Authors SY and CTF performed the protein structure analyses. Author CTF performed the docking experiments and statistical analyses and generated all figures. All authors wrote and reviewed the manuscript.

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Data Availability Statement

All code, data, results, and additional analyses are openly available on GitHub at:
https://github.com/colbyford/Influenza_H5-Antibody_Predictions.
These data include all sequences and folded structures for the isolates and antibodies used in this study, analysis scripts, and docking metrics.

Conflict of Interest Disclosure

Author CTF is the owner of Tuple, LLC, a biotechnology consulting firm. All other authors declare no conflicts of interest.

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Supplementary Information

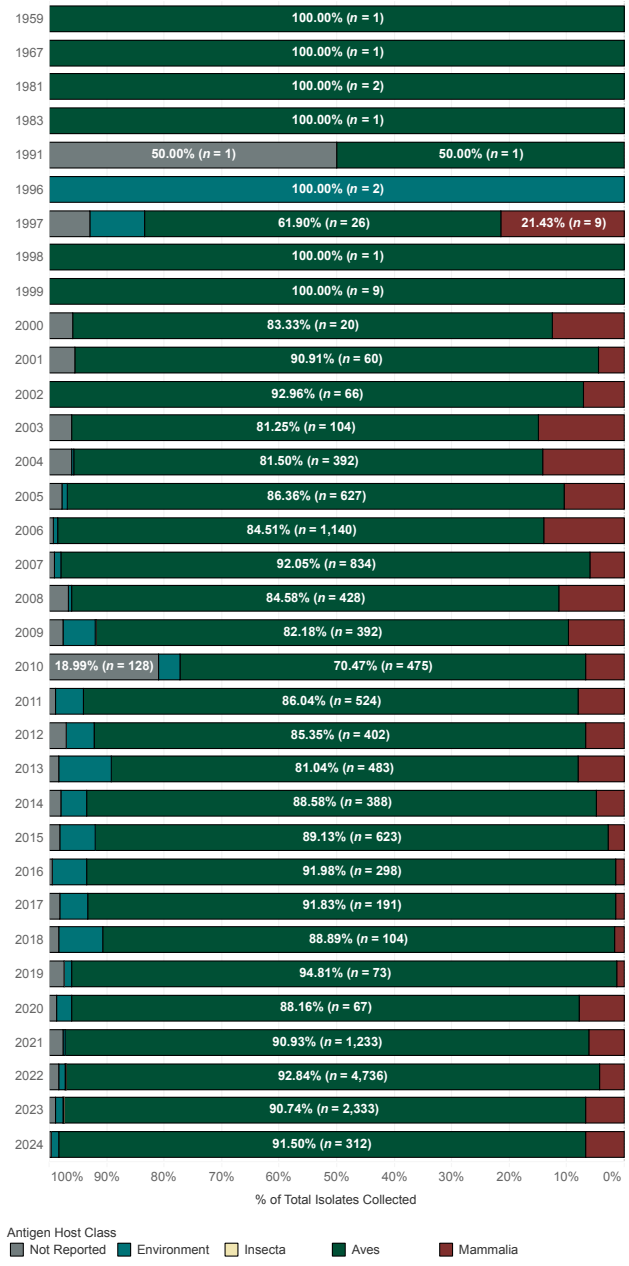

Fig. 1. Bar chart of the proportion of isolates collected from hosts of various taxonomic classes by year.

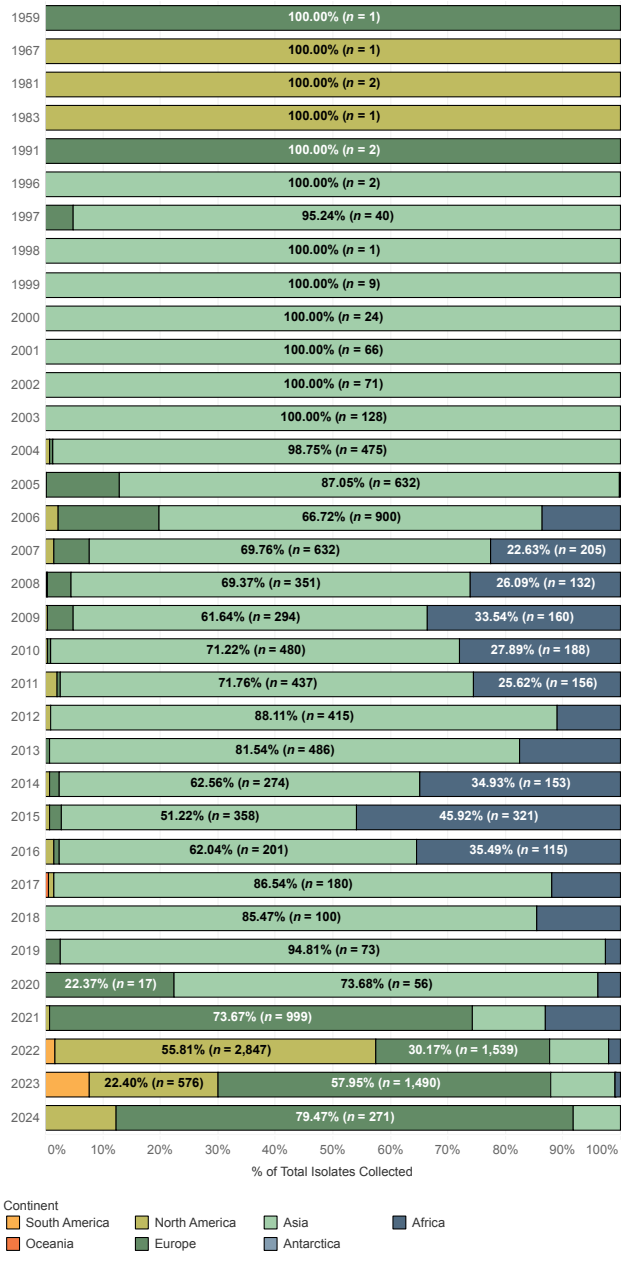

Fig. 2. Bar chart of the proportion of isolates collected from each continent by year.

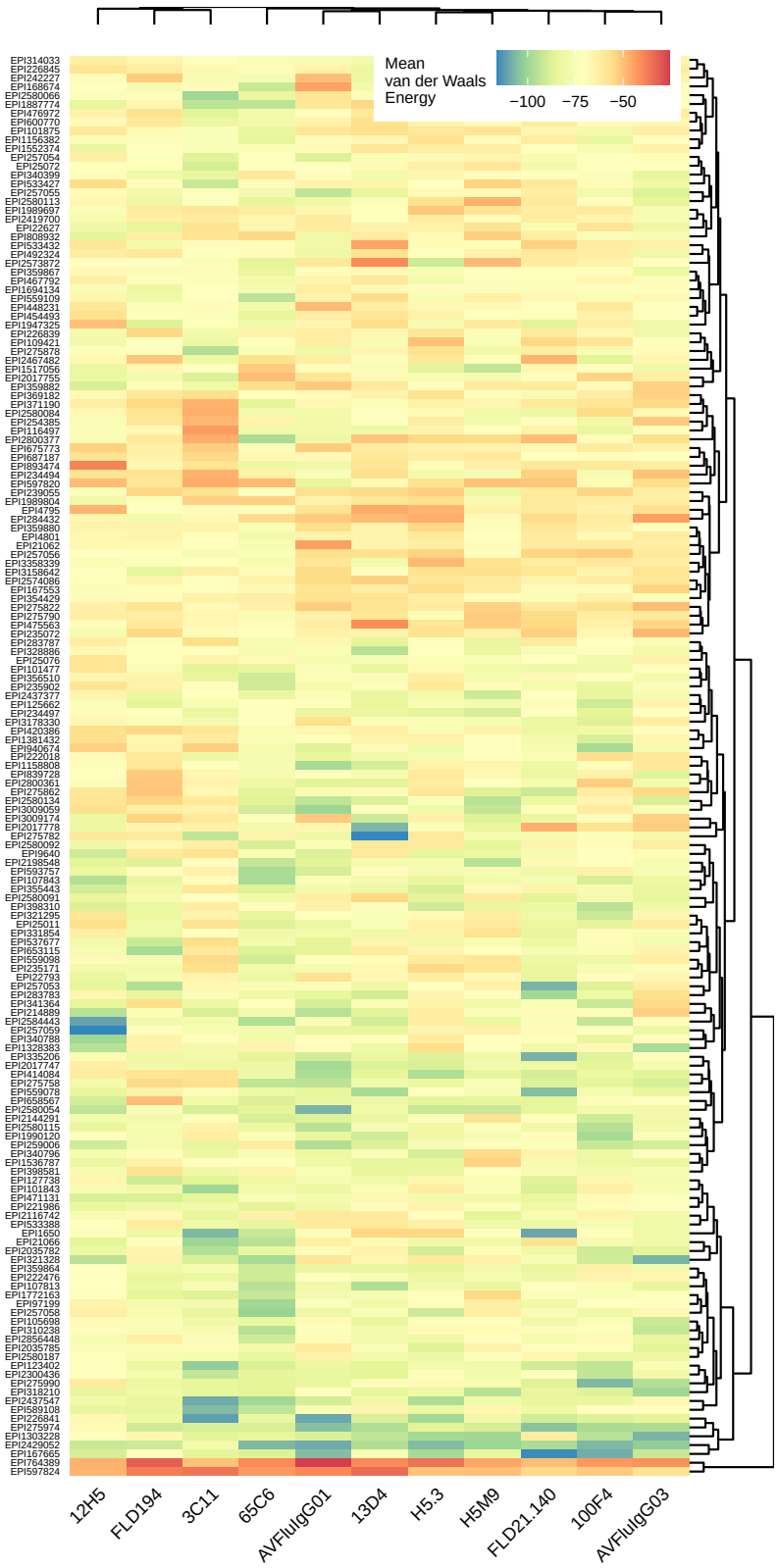

Fig. 3. Heatmap of van der Waals energies for each antibody-antigen experiment, shown with cluster dendrograms.

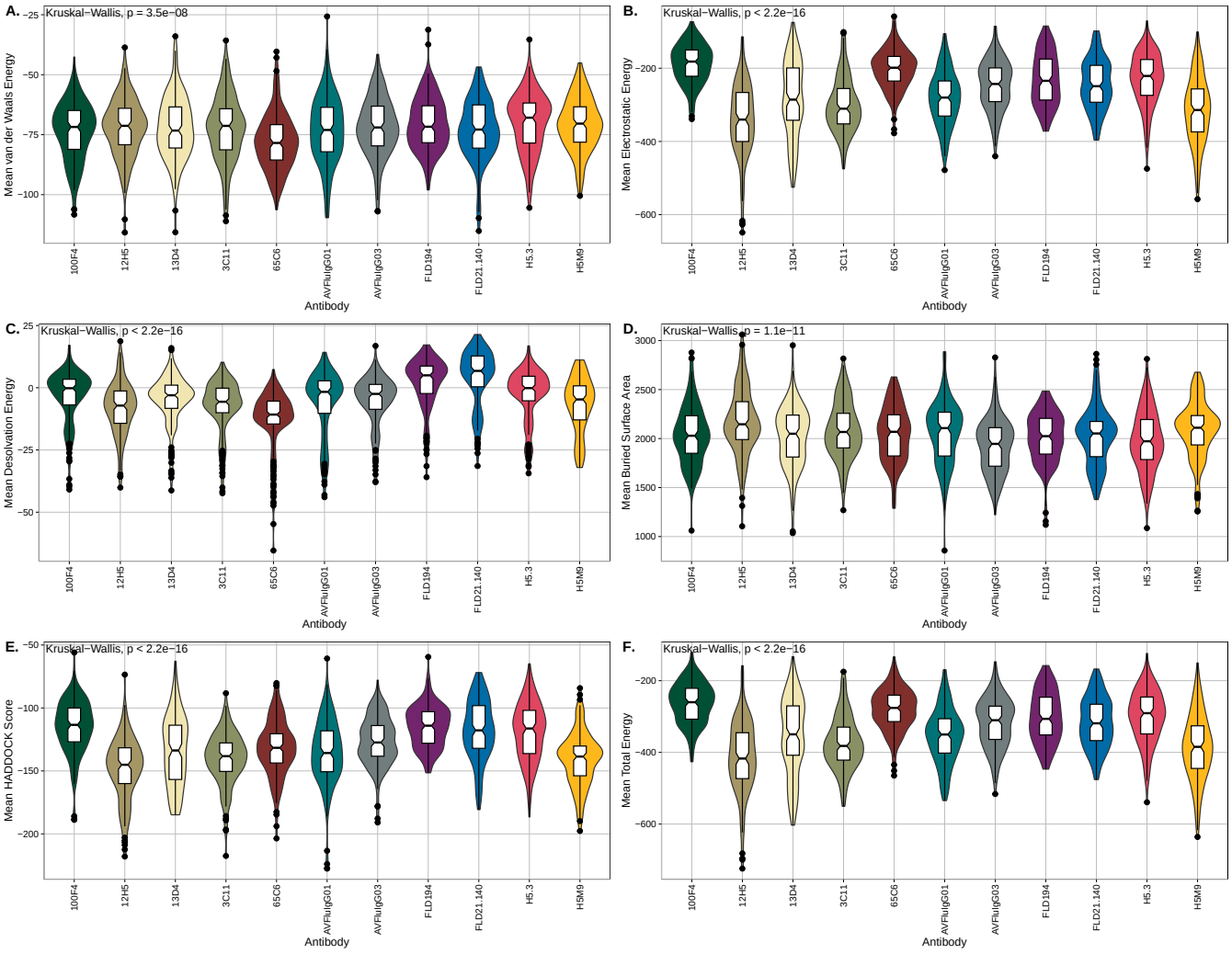

Fig. 4. The distribution of various docking metrics broken out by antibody. Statistics shown are Kruskal-Wallis test p-values.

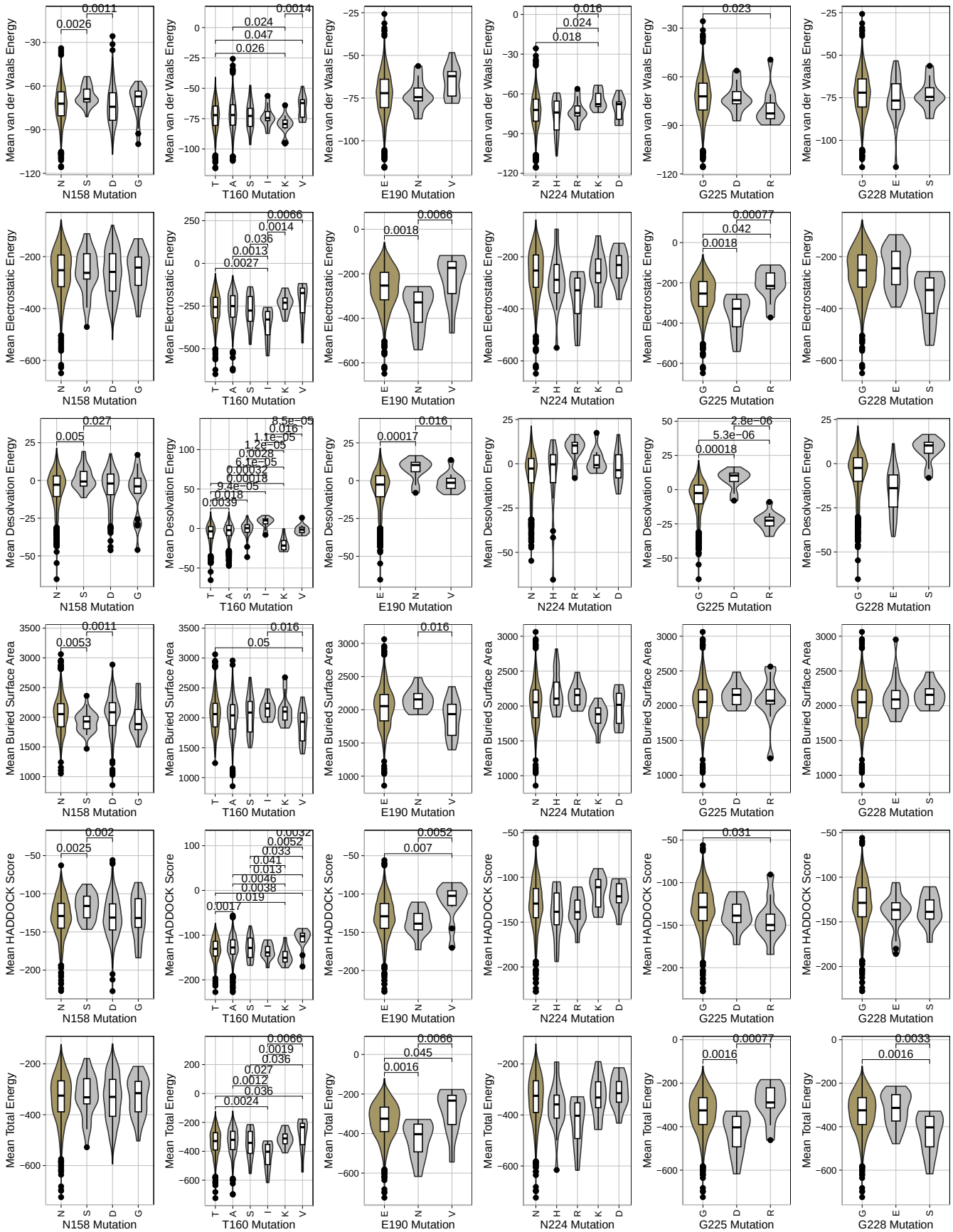

Fig. 5. The distribution of various docking metrics broken out by antigen mutations. The first amino acid shown on the left of each plot in gold represents the reference residue at that position as described in Shi et al. (2014). Statistical comparisons shown are significant Wilcoxon Rank Sum test p-values at the α<0.05 level.