Predicting Antibody and ACE2 Affinity for SARS-CoV-2 BA.2.86 with *In Silico* Protein Modeling and Docking

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The emergence of the Omicron sublineage of SARS-CoV-2 virus BA.2.86 (nicknamed "Pirola") has raised concerns about its potential impact on public health and personal health as it has many mutations with respect to previous variants. We conducted an *in silico* analysis of neutralizing antibody binding to BA.2.86. Selected antibodies came from patients who were vaccinated and/or infected. We predicted binding affinity between BA.2.86 and antibodies. We also predicted the binding affinity between the same antibodies and several previous SARS-CoV-2 variants (Wuhan and Omicron descendants BA.1, BA.2, and XBB.1.5). Additionally, we examined binding affinity between BA.2.86 and human angiotensin converting enzyme 2 (ACE2) receptor, a cell surface protein crucial for viral entry. We found no statistically significant difference in binding affinity between BA.2.86 and other variants, indicating a similar immune response. These findings contradict media reports of BA.2.86's high immune evasion potential based on its mutations. We discuss the implications of our findings and highlight the need for modeling and docking studies to go above and beyond mutation and basic serological neutralization analysis. Future research in this area will benefit from increased structural analyses of memory B-cell derived antibodies and should emphasize the importance of choosing appropriate samples for *in silico* studies to assess protection provided by vaccination and infection. This research contributes to understanding the BA.2.86 variant's potential impact on public health. Moreover, we introduce new methodologies for predictive medicine in ongoing efforts to combat the evolving SARS-CoV-2 pandemic and prepare for other hazards.

SARS-CoV-2 | coronavirus | antibodies | ACE2 | protein docking
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Introduction

The emergence of SARS-CoV-2 variants continues to pose a significant challenge to global public health efforts. Here, we present an analysis of the recently discovered SARS-CoV-2 variant, BA.2.86, colloquially known as "Pirola" (1). This sub-lineage of the Omicron variant (BA.2) was first detected in late July 2023 in Israel and Denmark (2, 3) and has now spread worldwide (1, 4, 5). The international dissemination of BA.2.86 has raised concerns about its potential impact on personal and public health, and the World Health Organization (WHO) officially classified it as a variant under monitoring on August 17th, 2023 (5).

The genetic sequence of BA.2.86 distinguishes it from the original Omicron variant (B.1.1.529) and the XBB.1.5 variant (5). The most interesting difference between BA.2.86 and its ancestors is in BA.2.86's Spike gene (S), which displays an array of 33 mutations in relation to the original Omicron variant. Of those, 14 are in the receptor binding domain (RBD) (6). Furthermore, BA.2.86 is separated by 35 mutations from XBB.1.5 (6). These notable divergences prompt concern regarding BA.2.86's transmissibility and potential to escape current treatments and the protection provided by prior infection or vaccination.

Since antibodies primarily target the viral S protein's RBD, these mutations have raised concerns about the efficacy of current antibodies against BA.2.86 (7). This concern was derived from the rapid ascent of previous variants that were also rich in mutations, such as the increase in percent case counts of Omicron (in late 2021 and early 2022) and XBB.1.5 (in early 2023) (8, 9). However, in contrast to this previous experience, BA.2.86 has become a very poorly represented strain in the USA as of the end of October 2023 (10). Thus, counting mutations is not a way to assess the severity of a variant. Herein, we present computational protein modeling and antibody docking methods to assess the potential for immune evasion and transmissibility of new variants.

In this study, we employ methodologies, established in our previous work, to assess the binding potential of antibodies derived from infected patients, vaccinated patients, and patients with breakthrough infections (9, 11). We used *in silico* docking and modeling to quantify the binding potential of existing antibodies to BA.2.86's Spike protein and compare these results with earlier variants (9, 11). We also investigate BA.2.86's binding affinity to the human angiotensin converting enzyme 2 (ACE2) receptor.

The ACE2 receptor serves as the primary point of interaction between human cells and SARS-CoV-2. A mutation in the vital RBD region can affect the efficiency of this crucial viral-cell binding event (12). With the emergence of novel SARS-CoV-2 variants featuring mutations within the RBD domain, we have observed an increased affinity for binding to the ACE2 receptor in XBB.1.5 (12). This enhanced affinity of RBD and ACE2 has contributed to the rapid person-to-person transmissibility of viruses such as Omicron B.1.1.529

and XBB.1.5, which became predominant within the population (13). Alternatively, RBD mutations can also lead to cross-species infections (14). Understanding the effects of these mutations on a variant's ability to bind with the ACE2 receptor can be instrumental in predicting its transmissibility and zoonotic potential (14).

Thus, by comparing the binding affinity of BA.2.86 and predecessor variants to neutralizing antibodies and the human ACE2 receptor, we provide valuable insights into the transmissibility of BA.2.86 and its interaction with human cells. This information has implications for public health and informs ongoing efforts to combat the evolving SARS-CoV-2 pandemic.

Methods

In short, our *in silico* approach includes curating or generating the RBD structures for five SARS-CoV-2 variants, seven neutralizing antibody structures, and five ACE2 structures. Each antibody or ACE2 structure was docked against the viral RBD structures, and binding affinity metrics were collected for comparison. We performed a total of 60 docking experiments. These materials and methods are detailed below.

A. Viral Proteins. Given the high infectiousness of the Omicron subvariants and their predominance in the past two years, we selected BA.1, BA.2, and XBB.1.5 variants' RBDs as well as the original Wuhan strain (referred to here as "wild type" or WT) for docking.

Complete genome sequences of BA.2.86 and BA.2 were retrieved from the GISAID's EpiCoV database (accession numbers 8002210 and 18097315, respectively) (15). We annotated and translated each S gene following Jacob Machado et al. 2021. Finally, we extracted the corresponding RBD regions (residues 338 through 525 for BA.2.86 and 338 through 528 for BA.2.) (17). These sequences were used for structural protein prediction using AlphaFold2 (18) via ColabFold (19). We relaxed the side chains in the ColabFold generated structure with the Amber relaxation procedure for docking (20).

The XBB.1.5 structure is from our previous paper, in which we generated the structure with ColabFold (9). Other available SARS-CoV-2 Spike RBD crystal structures were downloaded from the Protein Data Bank (PDB) (21). We derived the WT and BA.1 RBD structures from the Protein Data Bank as they represent empirically derived structures from an RBD-Antibody or RBD-ACE2 complex (22–31). Table 1 summarizes the sources of different RBD structures.

Variant	Type	Citation	PDB	GISAID
WT	Empirical	(22–27, 31)	7X2H, 7XD2, 6XCN, 7K8M, 8DW9, 6M0J, 7A98, and 7DF4	
BA.1	Empirical	(24)	7YKJ, 7WLC	
BA.2	Mixed	(28, 31)	7YJ3	8002210
XBB.1.5	Mixed	(9, 29)	8SPI	
BA.2.86	Predicted	N/A	N/A	18097315

Table 1. Selected RBD structures. WT indicates "wild type," or Wuhan.

B. Antibody Selection. We diverge from our previous studies focusing on therapeutic antibodies (9, 11). In this study, we selected seven antibodies from the Protein Data Bank that were derived from vaccinated patients, vaccinated with breakthrough infection and had infection without vaccination. Five of the seven selected antibodies were derived from memory B-cells from human patients. (23, 24, 30). Antibodies are listed in Table 2.

Condition	Vaccine	Antibody	PDB	Citation
Vaccinated	BBIBP-CorV	6-2C	7X2H	(23)
Vaccinated	BBIBP-CorV	10-5B	7XD2	(23)
Vaccinated with BA.1 breakthrough infection	BNT162b2	P3E6	7YKJ	(24)
Vaccinated with BA.1 breakthrough infection	BNT162b2	P2D9	8DW9	(24)
Vaccinated	CoronaVac	XGv282	7WLC	(30)
Infected (strain not specified)	N/A	C105	6XCN	(25)
Infected (strain not specified)	N/A	C102	7K8M	(25)

Table 2. Selected antibodies from different conditions and their PDB structures. Note that the BNT162b2-CorV vaccine is commonly known as the "Pfizer-BioNtech" vaccine. BBIBP-CorV was developed by Sinopharm and was the first whole inactivated virus SARS-CoV-2 vaccination to obtain an emergency use authorization by the World Health Organization (23). The entries without a vaccine are antibodies obtained from unvaccinated patients before mass SARS-CoV-2 vaccination was available in the USA. The first five entries within the table are derived from memory B-cells collected from patients whereas the last 2 entries were antibody structures generated from antibody sequences obtained from convalescent donors before mass vaccination.

C. ACE2 Structures. We used ACE2 structures found on the Protein Data Bank derived from studies analyzing the structure of the ACE2-RBD complex. The ACE2 structures were isolated for docking. The initial complexed RBD was docked to each ACE2 structure alongside the RBDs we derived from PDB and ColabFold. The selected ACE2 instructions are listed in Table 3.

PDB	RBD in Complex	Citation
6M0J	WT	(22)
7A98	WT	(27)
7DF4	WT	(31)
7YJ3	BA.2	(28)
8SPI	XBB.1.5	(29)

Table 3. Selected ACE2 crystal structures. WT indicates "wild type," or Wuhan.

D. Protein-to-Protein Docking. To prepare the Fab structures for docking, we renumbered the residues according to HADDOCK's (v2.4) requirements such that there were no overlapping residue IDs between the heavy and light chains (32, 33). Residues in the Fab structures' complementarity-determining regions (CDRs) were selected as "active residues" for docking analysis to assess antibody neutralization. Residues in the ACE2 binding pocket forming polar contacts with the RBD in the crystallized structure were selected as "active residues" for docking prediction and analysis of ACE2-RBD binding. The same active residues for each ACE2 and antibody structure are used for each dock to the five RBD structures. Residues in the S1

portion of the RBD were selected as RBD residues. Each RBD has similar active residues when docking against an antibody. However, there are variations in active residue selection to account for differences in amino acid composition between variants. Each of the seven antibody structures and five ACE2 structures were docked against five RBD structures using HADDOCK, a biomolecular modeling software that provides docking predictions for provided protein structures (32, 33).

The HADDOCK software produces multiple output PDB files of docking results and their subsequent docking metrics. A PDB output file for each docking experiment with HADDOCK was placed into PRODIGY (v2.1.3) for further analysis. PRODIGY is a web service collection focused on binding affinity predictions for biological complexes (34, 35). This process resulted in 60 sets of docked structures. For quantitative analysis, we selected the top-performing structure for each antibody-RBD or ACE2-RBD pair. Statistical tests were conducted in R (36), implementing the Kruskall-Wallis and the paired Wilcoxon test to compare different predictions (37, 38). We also used this top structure to visually analyze the structural conformation of interfacing residues and docked proteins using PyMol (v2.5.5) (39).

Results

We compared docking predictions of viral proteins to ACE2 receptors or antibodies with Kruskall-Wallis and paired Wilcoxon tests. These tests return values that are not statistically significant at a 95% confidence level for different tested variables: HADDOCK score, van der Waals energy, electrostatic energy, desolvation energy, buried surface area, and PRODIGY's ΔG predictions. Thus, we conclude that all the Omicron subvariants' (including BA.2.86) performance for the ACE2 and antibody docking simulations were similar.

Figure 1 illustrates various metrics produced by HADDOCK and PRODIGY estimations of the protein-to-protein binding affinities between antibodies and RBD structures. It includes seven antibody structures and five variant RBD structures (35 experiments in total). The results of ACE2 to RBD docking experiments are shown in Figure 2, including five ACE2 and five RBD structures (25 docking experiments in total). Figures 1 and 2 also show the non-significative p values of the Kruskal-Wallis statistical test in the bottom left of each plot and the Wilcoxon signed-rank test between each RBD. These figures are derived from metrics obtained from the best PDB complex structure, determined by HADDOCK, for each experiment.

Figure 3 shows the analysis of the interfacing residues between an antibody and the RBD of XBB.1.5 and BA.2.86. Differences in tertiary structure between the RBD of BA.2.86 and XBB.1.5 lead to different binding poses for the two variant RBDs on both antibodies. These differences, in part, derive from the three deletions contained in the sequence of BA.2.86. Another reason for differences in tertiary structure may be due to the proline at position 445 in the RBD of XBB.1.5. In the RBD of BA.2.86 the corresponding residue is histidine at position 444, due to the deletions in BA.2.86. The proline may increase the rigidity of the nearby loop residues within the RBD in BA.2.86 adding to a disparity in binding between BA.2.86 and XBB.1.5 to neutralizing antibodies targeting the RBD. The histidine in place of that proline in BA.2.86 reduces rigidity and additionally increases steric hindrance from the more bulky side chain of histidine, thus adding to the disparity in binding pose and docking metrics between the two variant RBDs.

Figure 4 shows the analyses of the interfacing residues between ACE2 and the RBD of XBB.1.5 and BA.2.86. Differences in binding pose may derive from similar reasons to the antibody analysis above. The deletions within BA.2.86 create alterations in the overall tertiary structure of the binding motif within the RBD. The proline at position 445 in XBB.1.5 stabilizes nearby residues to allow for increased binding around residue 445, thus creating a slightly different binding pose and different stability than that of BA.2.86 to ACE2. In addition to the proline at position 445 in XBB.1.5, the F486P mutation within XBB.1.5 adds additional stability to XBB.1.5 that BA.2.86 lacks near the binding motif within the RBD. The stability is displayed by the polar contact formed by the asparagine at position 487 and the serine at position 446 shown in 4.B. These polar contacts are lacking in the corresponding BA.2.86 dock shown in 4.A. In the docking to ACE2 in 4.C and 4.D, there are noticeable yet slight differences in binding residues. However, there is a significant difference in binding angle between the RBD of BA.2.86 and XBB.1.5 to the ACE2 Structure retrieved from PDB file 7A98. This disparity in binding angle may be due to the lack of stability provided by the histidine at position 444 in BA.2.86, whereas in XBB.1.5 the corresponding residue is a proline at position 445, this inherently creates a more stable complex for XBB.1.5, creating a greater disparity in sampling and binding prediction from HADDOCK.

However, as stated above, these variations do not appear to cause significant changes in binding affinity. Again, these figures represent the best HADDOCK output for each experiment.

Discussion

Although there are variations in the ACE2 docking metrics when comparing previous variants and BA.2.86, no overall pattern is observed. The lack of such a pattern indicates that BA.2.86 does not significantly differ from previous variants in its capability to evade antibodies or bind to the ACE2 receptors.

Early studies have been published regarding BA.2.86. In one study titled, "Sensitivity of BA.2.86 to prevailing neutralizing antibody responses," patients had blood sera collected before XBB.1.5 predominance (40). In the referenced study samples were then analyzed in a pseudovirus neutralization assay which found the highest blood serum neutralization titers for BA.2, XBB.1.5, and BA.286 in descending order (40).

The results found in Sheward et al. 2023 are consistent with our Gibbs Free Energy calculation provided by PRODIGY results regarding the three variants. The median Gibbs Free

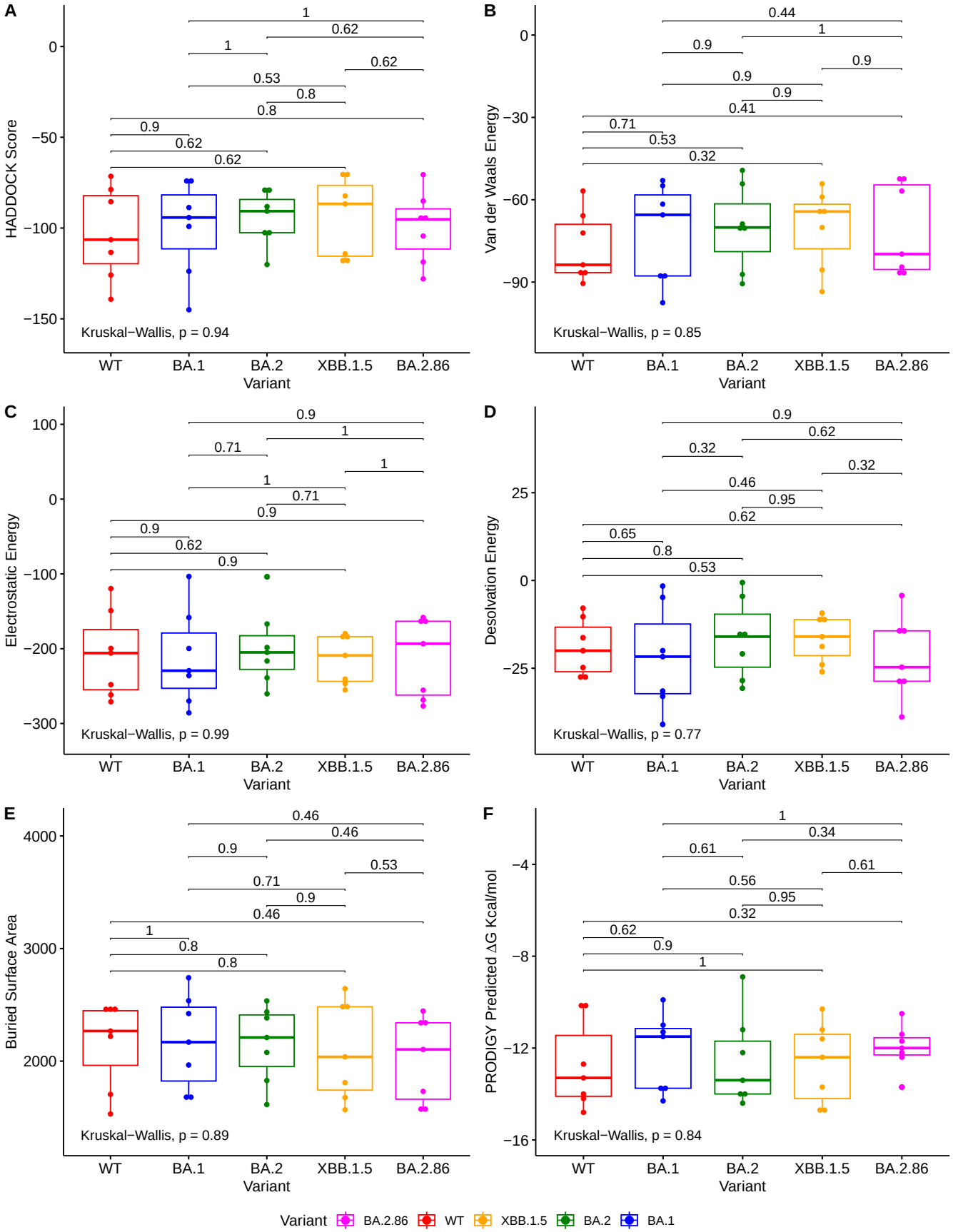

Fig. 1. In this figure, we use boxplots to illustrate the comparative docking scores of antibody and RBD structures generated by HADDOCK and PRODIGY. Each boxplot highlights the distribution of docking scores for different variants. Pairwise comparisons were performed using the Wilcoxon signed-rank test, indicated by the horizontal lines. The Kruskal-Wallis test was used to compare the medians of all the independent samples, indicated in the bottom left.

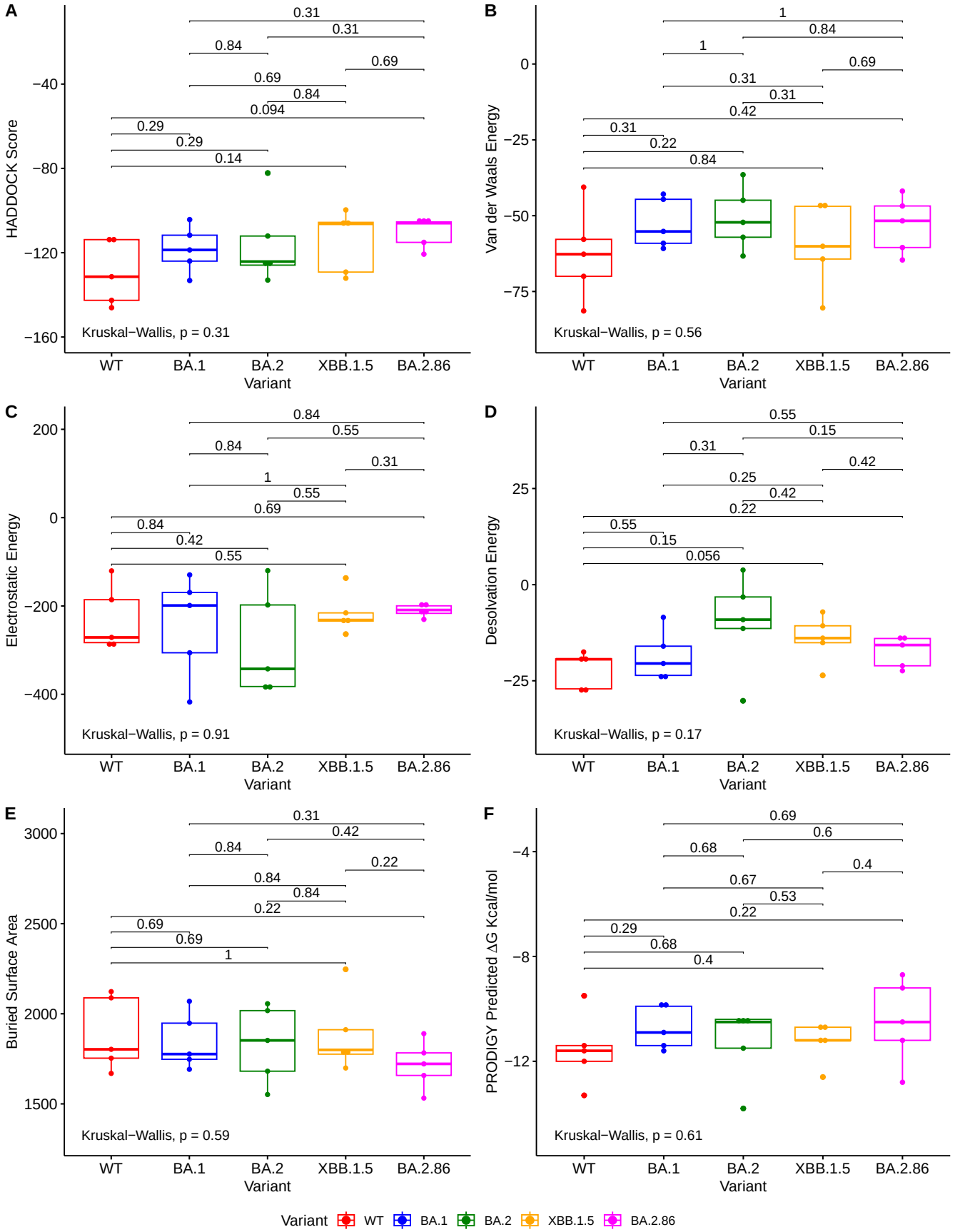

Fig. 2. Boxplots illustrating the comparative docking scores of ACE2 and RBD structures generated by HADDOCK and PRODIGY. Each boxplot highlights the distribution of docking scores for different variants. Pairwise comparisons were performed using the Wilcoxon signed-rank test, indicated by the horizontal lines. The Kruskal-Wallis test was used to compare the medians of all the independent samples, indicated in the bottom left.

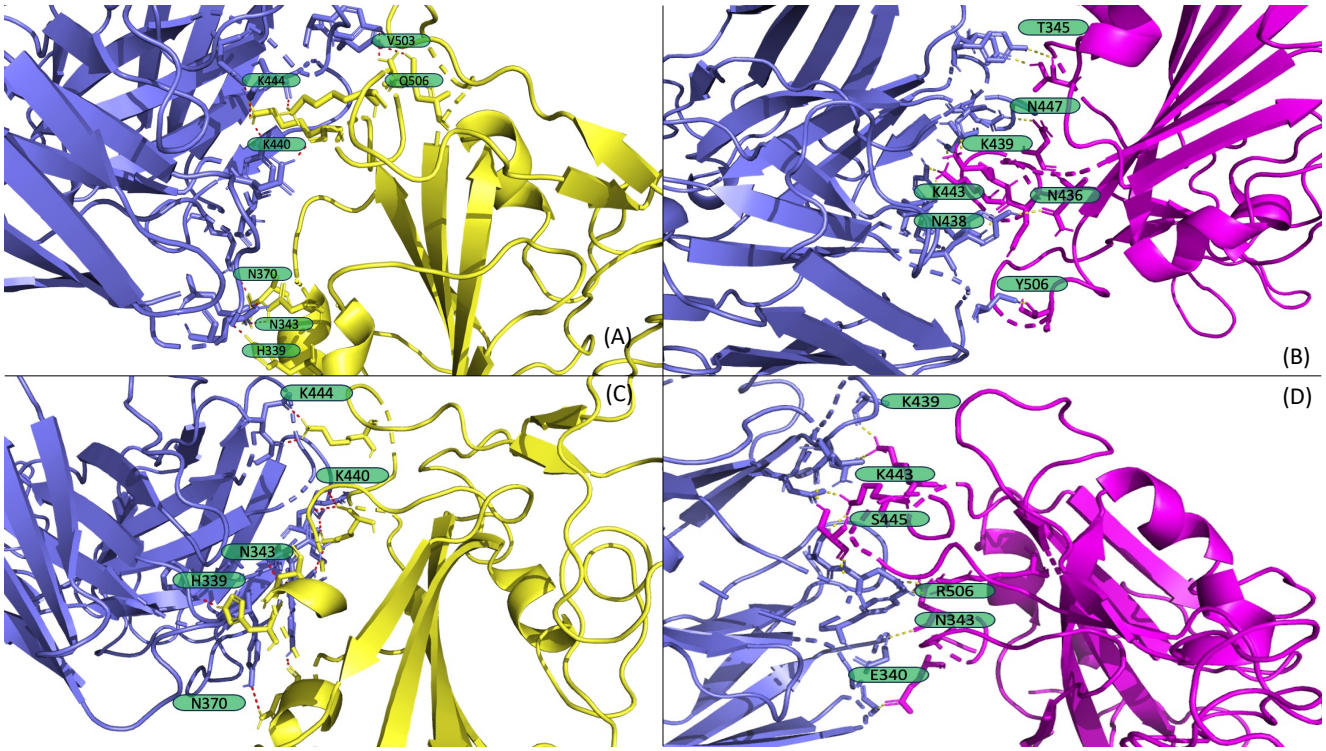

Fig. 3. A) and B) display the output PDB files from HADDOCK of the docking jobs of antibody 6-2C to XBB.1.5 and BA.2.86 respectively. C) and D) display the output PDB files from HADDOCK of the docking jobs of antibody P2D9 to XBB.1.5 and BA.2.86 respectively. The antibody structure in each is shown on the left in blue and the RBD is shown on the right in yellow for XBB.1.5 or magenta for BA.2.86. Interacting RBD residues are highlighted in green labels.

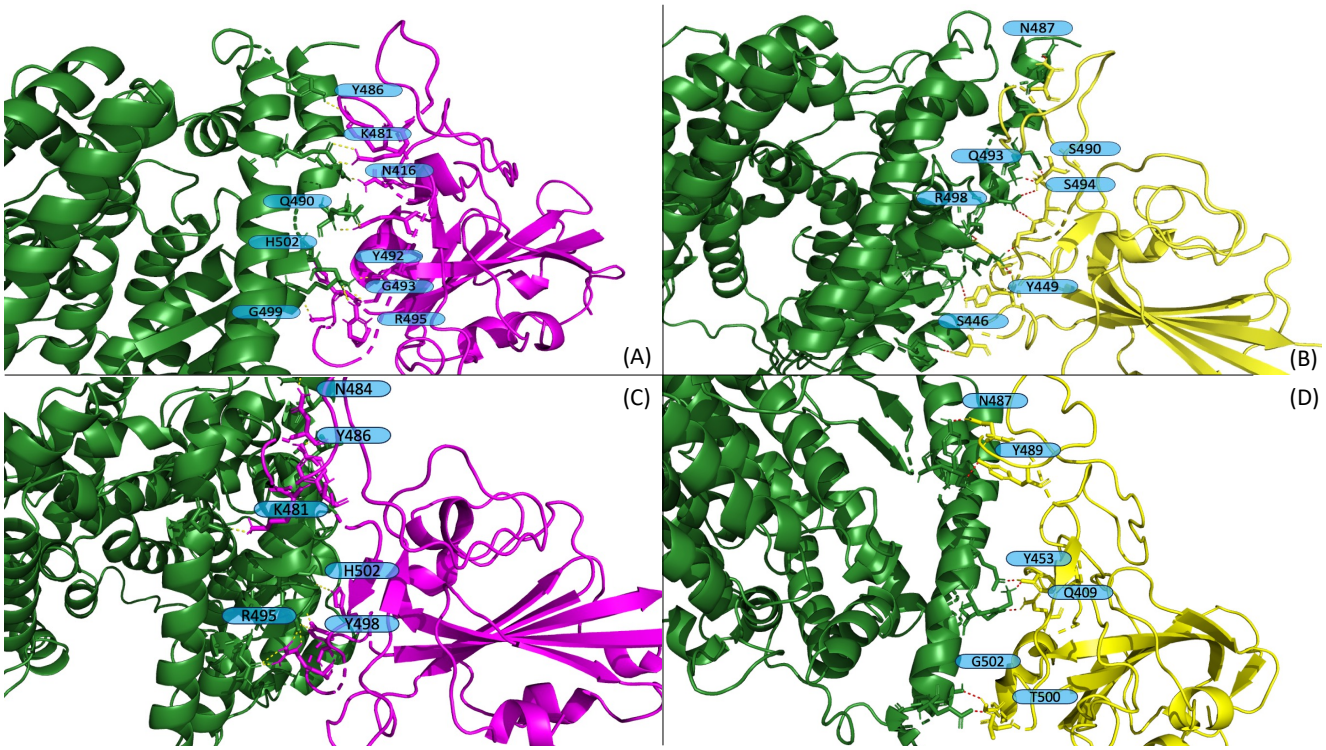

Fig. 4. In A) and B) we display the output PDB files from HADDOCK of the docking jobs of the ACE2 structure from PDB 6M0J and BA.2.86 and XBB.1.5 respectively. In C) and D) we display the output PDB files from HADDOCK of the docking jobs between the ACE2 structure from PDB 7A98 and BA.2.86 and XBB.1.5 respectively. We show the ACE2 structure, in each, on the left in green and the RBD on the right in yellow for XBB.1.5 or magenta for BA.2.86. Interacting RBD residues are highlighted in blue labels.

Energy calculation for each of the three variants ascends from BA.2, XBB.1.5, and BA.2.86, indicating weaker binding and thus weaker neutralization efficacy of the antibody. The results demonstrated that blood sera against BA.2.86 have moderately lower geometric mean lower neutralizing titers than against XBB.1.5 (40). The array of antibodies we selected derived from natural infection, breakthrough infection, and vaccination without known infection. The array of antibod-

ies is likely to simulate the samples in this study as the blood sera that were collected before XBB.1.5 prevalence (40).

In Uriu et al., published in September 2023, researchers obtained blood samples from individuals vaccinated with third-dose monovalent, fourth-dose monovalent, BA.1 bivalent, and BA.5 bivalent mRNA vaccines (41). They found that the sera exhibited very little or no antiviral effects against BA.2.86 in a neutralization assay assessing neutralization titers. This study also found that monoclonal antibodies bebtelovimab, sotromivab, and cilgavimab did not exhibit antiviral effects against BA.2.86, however, these monoclonal antibodies exhibited antiviral effects against BA.2 (41, 42).Uriu et al. suggested that BA.2.86 is one of the most evasive SARS-CoV-2 variants ever, from results within the study.

There was no statistically significant difference in any metric, including Gibbs Free Energy calculation, Electrostatic Energy, van der Waals Interaction, and HADDOCK score for ACE2 or antibody binding between any two of the selected variants. Our results do not correspond with previous reports in which computational and empirical methods were used to determine ACE2 binding for XBB.1.5 (43, 44). However, our method is significantly different.

Sugano et al. 2023 performed an *in silico* study, in which they used molecular docking software to determine SARS-CoV-2 and ACE2 binding affinity. Sugano et al. showed that ACE2 binding affinity has increased in BA.1 to 1.55 times the binding affinity compared to the WT strain and in XBB.1.5 the binding affinity increased three times the binding affinity compared to the WT strain.

Our results do not agree with this (43). We did not use ZDock, which was the docking software utilized in their study (43). Our use of HADDOCK and PRODIGY provides dissimilar results. Our assessment shows that ACE2 binding affinity is not statistically significant in its difference between the WT, BA.1, BA.2, and XBB.1.5 strain. Sugano et al. utilize a ratio method of comparing ACE2 binding affinity, whereas we utilize a statistical test to compare binding affinity. This disparity in results caused by different docking software and methods is a area for future research.

Yue et al. 2023 analyzed XBB.1.5 antibody evasion and ACE2 binding. Yue et al. used blood serum samples from patients who had received three doses of CoronaVac before a BA.1 or BF.7 breakthrough infection. Yue et al. also used serum from patients who had a BA.5 breakthrough infection after at least two doses of BNT162b2 or mRNA-1273 vaccine. The serum samples in the study demonstrated a substantial decrease in neutralization against XBB.1.5 in comparison with the B.1 variant, which shares a recent common ancestry with BA.1 and BA.2 (44). Yue et al. 2023 also demonstrated that XBB.1.5 has a similar ACE2 binding affinity to BA.2.75, which is a variant that has been demonstrated to have higher ACE2 binding than BA.2 (44, 45).

Tamura et al. 2023 performed a study which they analyzed ACE2 binding for the BA.2.86 variant. Tamura et al. demonstrate that the binding affinity of the BA.2.86 spike protein to ACE2 was similar to that of the XBB.1.5 spike protein, corresponding to our results analyzing RBD-ACE2 affinity. However, Tamura et al. found that XBB.1.5 and BA.2.86 has significantly higher ACE2 binding affinity than BA.2, disagreeing with our results.

Wang et al. 2023 performed a study in which they analyzed ACE2 binding affinity. Wang et al. found that the XBB.1.5 spike protein exbibited a modest increase in ACE2 afffinity compared to BA.2, which corresponds to a greater extent with our results regarding XBB.1.5 than previously mentioned studies. In addition, (47) found that that BA.2.86 had a greater than two fold increase in binding affinity in comparison to BA.2, which contrasts with our results regarding ACE2.

At the time of writing this in November 2023, BA.2.86 does not appear to display the increased evasion indicated by Uriu et al. 2023. The Center for Disease Control and Prevention (CDC) COVID Data tracker has not shown BA.2.86 to be a prevalent strain within the past month, with XBB.1.5 being in higher prevalence (10). The CDC data corresponds with our results that vaccine and infection induced antibodies maintain neutralizing capabilities against BA.2.86 (10). The CDC data also corresponds with our results of BA.2.86 not having a signficant difference in ACE2 binding to previous omicron variants (10).

Our results contradict media reports about the potential severity of BA.2.86 (48) and are consistent with the United States Center for Disease Control and Prevention's more muted warnings (49). Throughout the pandemic it has become clear that the media narrative can go one direction and the science narrative can be dynamic and take on a separate narrative.

Our results have mixed correspondence with previous studies and data on XBB.1.5 and BA.2.86 (10, 40, 41, 44, 46, 47). There are many potential explanations for our lack of statistically significant differences. First, molecular docking analysis focuses on one antibody structure or ACE2 structure docking to an RBD structure. Molecular docking does not account for multiple interactions that can be assessed empirically with a neutralization titer study using blood samples. Molecular docking analysis does not consider the quantity of different types of neutralizing antibodies in the bloodstream. Molecular docking only considers individual antibodies. These differences create a smaller analytical space that focuses on the neutralization capabilities of individual antibodies, not the aggregate capabilities of a group of varied antibodies as demonstrated in serum neutralization titer assays. It is to be noted that the antibodies used in our molecular docking work were derived from breakthrough Omicron patients, unvaccinated infected patients, and vaccinated patients but analyzed in single antibody/strain pairs. The chosen antibody array consists of antibodies that protect against Omicron strains to a greater extent alongside the WT strain.

Our study size was limited by the number of eligible infection and vaccine-derived antibody-RBD complexes, and ACE2-RBD complexed PDB files available in the Protein Data Bank.

One biological explanation as to why we lack statistically significant differences in antibody binding between the vari-

ants is the composition of our antibody array. Our antibody selection is significantly different than the antibodies studied in existing serological studies. Our antibody array primarily consists of memory B-cell derived antibodies that are studied to be broadly neutralizing against different variants (23, 24, 30). Our results support the empirical results that our antibody array, primarily consisting of broadly neutralizing antibodies, maintains their efficacy across different variants (23, 24, 30). The presence of such broadly neutralizing antibodies also may indicate a level of prevalence of memory B-cells within vaccinated individuals that generate such antibodies (23, 30, 50, 51). A larger and broader study analyzing the presence of memory B-cells that produce broadly neutralizing antibodies is needed to support the notion that the population in general contains memory B-cells that can produce such broadly neutralizing and effective antibodies. Such memory B-cells differentiate, and broadly neutralizing antibodies are produced upon a secondary immune response to new variant antigens (29, 50).

The utilization of serum antibody neutralization titers can be accurate in predicting the prevention of initial infection but are not as accurate in regards to the prediction of the prevention of serious disease as they only account for antibodies prevalent in the blood at the time of collection, the secondary immune response cannot be measured via this method. Neutralization titers, using blood samples, is a biased measurement method as the proportions of antibodies within the bloodstream will reflect specific neutralizing capabilities against recently introduced antigens from infection or vaccination.

In contrast to this limitation, in our *in silico* method we use antibodies that have been studied to be produced upon memory B-cell stimulation and neutralize SARS-CoV-2 variants (23, 24, 30). Given a larger sample size of memory B-cell derived antibodies, we believe that this approach will be a more accurate and comprehensive approach to assess how well prior vaccination and/or infection will protect against infection and severe disease against future SARS-CoV-2 variants and other threats to public and personal health.

Conclusions

Overall, our study indicates ACE2 binding and antibody neutralization of the BA.2.86 variant has not increased over previous variants. Moreover, we show the ongoing efficacy of antibodies induced by a variety of means in the global population to fight the BA.2.86 variant. The metrics and methods we used provide different results from previous empirical sudies and the media narrative. With increased sample sizes our metrics and methods will provide improved insight into the secondary immune response provided by memory B-cells over traditional blood serum neutralization assays.

Data availability statement

All code, data, results, and additional analyses are openly available on GitHub at:

https://github.com/colbyford/SARS-CoV-2_BA.2.86_Spike-RBD_Predictions.

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