Predicting Antibody and ACE2 Affinity for SARS-CoV-2 BA.2.86 and JN.1 with *In Silico* Protein Modeling and Docking

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The emergence of SARS-CoV-2 lineages derived from Omicron, including BA.2.86 (nicknamed "Pirola") and its relative, JN.1, has raised concerns about their potential impact on public and personal health due to numerous novel mutations. Despite this, predicting their implications based solely on mutation counts proves challenging. Empirical evidence of JN.1's increased immune evasion capacity in relation to previous variants is mixed. To improve predictions beyond what is possible based solely on mutation counts, we conducted extensive *in silico* analyses on the binding affinity between the RBD of different SARS-CoV-2 variants (Wuhan-Hu-1, BA.1/B.1.1.529, BA.2, XBB.1.5, BA.2.86, and JN.1) and neutralizing antibodies from vaccinated or infected individuals, as well as the human angiotensin converting enzyme 2 (ACE2) receptor. We observed no statistically significant difference in binding affinity between BA.2.86 or JN.1 and other variants. Therefore, we conclude that the new SARS-CoV-2 variants have no pronounced immune escape or infection capacity compared to previous variants. However, minor reductions in binding affinity for both the antibodies and ACE2 were noted for JN.1. We discuss the implications of the *in silico* 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 and JN.1 variants' 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.

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Introduction

The continual emergence of SARS-CoV-2 variants remains a formidable challenge for global public health initiatives. Notably, BA.2.86, which shares common ancestry with Omicron (BA.1), was initially identified in late July 2023 in Israel and Denmark (1, 2). Since then, it has disseminated globally (3–5). The initial worldwide spread of BA.2.86 sparked concerns regarding its potential impact on personal and public health. Acknowledging these concerns, the World Health Organization (WHO) designated BA.2.86 as a variant under monitoring on August 17th, 2023 (5).

The genetic makeup of BA.2.86 sets it apart from the original Omicron variant (B.1.1.529) and the XBB.1.5 variant (5), raising concerns due to its numerous mutations. These concerns are fueled by the observation of the rapid rise of previous variants with a high mutation count, exemplified by the surge in percent case counts of Omicron in late 2021 and early 2022, and XBB.1.5 in early 2023 (6, 7). BA.2.86 is distinguished by 35 mutations from the XBB.1.5 variant (8). Notably, the Spike gene (S) of BA.2.86 exhibits a distinctive profile with 33 mutations in comparison to the original Omicron variant, with 14 of these mutations occurring in the receptor binding domain (RBD) (8). Since antibodies primarily target the viral S protein's RBD (9), it is considered a region of particular interest in the viral genome. Moreover, mutations in this vital RBD region can affect the efficiency of crucial viral-cell binding events (10), impacting the virus' ability to bind to the human angiotensin converting enzyme 2 (ACE2) receptor. For instance, the emergence of novel SARS-CoV-2 variants, featuring RBD mutations, has been associated with an increased affinity for binding to the ACE2 receptor in XBB.1.5 (10). This heightened RBD and ACE2 affinity has been linked to the accelerated person-to-person transmissibility observed in viruses like Omicron B.1.1.529 and XBB.1.5, leading to their predominance within the population (11). RBD mutations can also facilitate cross-species infections and increase the virus' zoonotic potential (12). Furthermore, RBD mutations can hinder the efficacy of vaccines that target that region (13, 14).

Despite original concerns about the number of mutations in BA.2.86, and in a departure from past experiences, this variant has exhibited a notably low prevalence in the USA as of February 2024 (15). This indicates that relying solely on mutation counts does not suffice to gauge the severity of a new SARS-CoV-2 variant. More recently, the JN.1 variant, a relative of BA.2.86, emerged in 2023, once again sparking a concern owing to its increased mutation count. JN.1 is characterized by the Leu455Ser mutation in the RBD, which some authors have said makes it "one of the most immunologically evasive variants." (16). Preliminary evidence indicates that the Leu455Ser mutation diminishes the binding affinity of the ACE2 receptor with JN.1's RBD, potentially hindering viral entry into host cells while enhancing JN.1's capacity to

evade humoral immunity (17). Furthermore, Leu455Ser may confer greater evasion of JN.1 against the antibodies raised by a monovalent vaccine (18).In addition to JN.1's mutation within the RBD, it bears two additional distinct mutations lacking in BA.2.86, R3821K in ORF1a and F19L in ORF7b (19).

Despite preliminary indications that RBD mutations would make BA.2.86 or JN.1 more capable than its preceding variants of escaping current vaccines, treatments, or antibodies produced by natural infection, conclusive empirical evidence is lacking and may not be immediately available. Furthermore, recent studies indicate that these new variants may not present pronounced differences in immune escape or infective capabilities when compared to previous variants despite their accumulated mutations. For example, (20) attested that JN.1's serum neutralization escape did not increase over previously circulating strains despite its stronger increase in worldwide circulation when compared to BA.2.86 (16).

We build upon *in silico* methodologies established in our previous work (7, 21) to conduct extensive comparative analysis of the binding affinity of JN.1, BA.2.86, and other previous SARS-COV-2 variant RBDs (Wuhan-Hu-1, BA.1/B.1.1.529, BA.2, and XBB.1.5) to the human ACE2 receptor and neutralizing antibodies from infected individuals and patients vaccinated with different vaccines (including bivalent vaccines). Our findings hold significance for public health and contribute to ongoing endeavors to address the dynamic challenges posed by the evolving SARS-CoV-2 pandemic.

Methods

In short, we examined 31 different RBD structures (27 RBD structures were extracted from 26 PDB files; one RBD structure was taken from a previous publication; and three PDB structures were predicted) from six different SARS-CoV-2 variant RBDs (Wuhan-Hu-1, BA.1/B.1.1.529, BA.2, XBB.1.5, BA.2.86, and JN.1). Whenever more than one RBD structure was available for a variant, a single representative was selected based the presence of the interfacing residues and the results of docking validation experiments against the corresponding antibody. We cluster together BA.1 and B.1.1.529 in this study as they have identical RBD sequences. *In silico* docking experiments were used to calculate the binding affinity metrics between the six selected RBD structures and 27 different ligands, including 17 neutralizing antibody structures and ten ACE2 structures, resulting in 162 docking experiments total. All the analyzed structures are available on GitHub (see "Data Availability Statement"). The complete methodology is detailed below.

Viral Proteins. Given the high infectiousness of the Omicron subvariants and their predominance in the past two years, we selected BA.1/B.1.1.529, BA.2, XBB.1.5, BA.2.86, and JN.1 variants' RBDs as well as the original Wuhan-Hu-1 strain (referred to here as "wild type" or WT) for docking. Table 1 summarizes the sources of different RBD structures, as detailed below.

We retrieved a complete genome sequence of BA.2 from the GISAID's EpiCoV database (22). We annotated and translated the S gene following a similar method to Jacob Machado et al. 2021. We used the B.1.1.529 RBD sequence from our group's initial *in silico* antibody docking study as a template, following the methodology described in (21). Finally, we extracted a fragment of the corresponding RBD region (within amino acid residues 336 through 528 in relation to the Wuhan-Hu-1 reference) (24). We obtained the RBD sequences for JN.1 and BA.2.86 from Yang et al. 2023. These sequences were used for structural protein prediction using AlphaFold2 (25) via ColabFold using default parameters (26). We relaxed the side chains in the ColabFold generated structure with the Amber relaxation procedure for docking (27).

We obtained the XBB.1.5 structure from our group's previous *in silico* paper. The XBB.1.5 structure was generated using ColabFold (7). We downloaded other available SARS-CoV-2 Spike RBD crystal structures from the Protein Data Bank (PDB) (28). We derived the WT and BA.1/B.1.1.529 RBD structures from the Protein Data Bank as they represent empirically derived structures from an RBD-Antibody or RBD-ACE2 complex (29–38).

For every RBD-antibody and RBD-ACE2 complex we isolated on PDB, we extracted the RBD structure from that complex. We extracted the RBD structure from each complex and docked that structure against the corresponding Antibody or ACE2 structure to reproduce the initial complex for validation and ensure an accurate docking location.

Variant	Type	Citation	PDB	GISAID
WT	Empirical	(29–34, 38–41)	7X2H, 7XD2, 6XCN, 7KMG 7K8M, 8DW9, 6M0J, 7QEZ, 7A98, 8DWA, 7MMO, 7DF4	
BA.1/B.1.1.529	Empirical	(31, 42–44)	7ZF3, 7ZFB, 7YKJ, 7WLC 7WPB, 7WEE, 7WBL, 7WEF	
BA.2	Mixed	(35, 38, 42, 45, 46)	7YJ3, 7ZF7, 7XO9, 8DM6, 7ZF8	8002210
XBB.1.5	Mixed	(7, 36)	8SPI	
BA.2.86	Predicted	(17)	N/A	
JN.1	Predicted	(17)	N/A	

Table 1. Viral RBD structures. WT indicates "wild type," or Wuhan. Whenever more than one RBD structure was available for a variant, a single representative was selected based on sequence length, interfacing motif completeness, and the results of docking validation experiments against the corresponding antibody. The six RBD structures selected to represent each variant are available on GitHub (see "Data Availability Statement").

Antibody Selection. We expanded the antibody selection compared to previous studies that focused on therapeutic antibodies (7, 21).In this study, we selected 17 antibodies from the PDB including those that were derived from vaccinated patients, patients vaccinated with breakthrough SARS-CoV-2 infection, and patients who experienced SARS-CoV-2 infection without vaccination. We included two therapeutic antibodies. 13 of the 17 selected antibodies were derived from memory B-cells from human patients (30, 31, 37, 42, 47). The initial complexed RBD was docked to each antibody structure alongside the representative RBD structures we derived from PDB and ColabFold. Antibodies are listed in Table 2.

Human ACE2 Structures. We used PDB's ACE2 structures derived from studies analyzing the structure of the ACE2-

Condition	Vaccine	Antibody	PDB	Citation
Vaccinated	BBIBP-CorV	6-2C	7X2H	(30)
Vaccinated	BBIBP-CorV	10-5B	7XD2	(30)
Vaccinated but infected with BA.1 *Breakthrough infection*	BNT162b2	P3E6	7YKJ	(31)
Vaccinated but infected with BA.1 *Breakthrough infection*	BNT162b2	P2D9	8DW9	(31)
Vaccinated but infected with BA.1 *Breakthrough infection*	BNT162b2	P1D9	8DWA	(31)
Vaccinated but infected with BA.1 *Breakthrough infection*	BNT162b2	Omi-3	7ZF3	(42)
Vaccinated but infected with BA.1 *Breakthrough infection*	BNT162b2	Omi-31	7ZFB	(42)
Vaccinated but infected with BA.1 *Breakthrough infection*	BNT162b2	Omi-18	7ZFB	(42)
Infected (strain not specified)	N/A	COVOX-150	7ZF8	(42, 47)
Infected with WT	N/A	CV2.1169	7QEZ	(39)
Vaccinated	CoronaVac	XGv282	7WLC	(37)
Vaccinated	CoronaVac	XGv265	7WEE	(37)
Vaccinated	CoronaVac	XGv289	7WEF	(37)
Infected (strain not specified)	N/A	C105	6XCN	(33)
Infected (strain not specified)	N/A	C102	7K8M	(33)
Therapeutic	N/A	LY-COV1404	7MMO	(40)
Therapeutic	N/A	LY-COV555	7KMG	(41)

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. Sinopharm developed BBIBP-CorV and was the first whole inactivated virus SARS-CoV-2 vaccination to obtain an emergency use authorization by the World Health Organization (30). The entries without a vaccine are antibodies obtained from unvaccinated patients before mass SARS-CoV-2 vaccination was available in the USA. The first 13 entries within the table are derived from memory B-cells collected from patients, whereas the last 4 entries were antibody structures generated from antibody sequences obtained from convalescent donors before mass vaccination or therapeutic antibodies developed for clinical use.

RBD complex. The ACE2 structures were isolated for docking. We docked the initial complexed RBD to each ACE2 structure alongside the RBDs that we derived from PDB and ColabFold. We selected ACE2 structures with the PDB IDs 6M0J, 7A98, 7DF4, 7YJ3, 8SPI, 7WBl, 7WPB, 8DM6, 7ZF7, and 7X09 (29, 34–36, 38, 42–46). We chose ten different structures of ACE2 to have an increased sample size with a diversity of minor structural deviations that may affect docking.

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 (48, 49). We selected residues in the Fab structures' complementarity-determining regions (CDRs) as "active residues" for docking analysis to assess antibody neutralization. We selected residues in the ACE2 binding pocket forming polar contacts with the RBD in the crystallized structure as "active residues" for docking prediction and analysis of ACE2-RBD binding. We used the same active residues for each ACE2 and antibody structure for each dock to the six RBD structures. We selected residues in the S1 portion of the RBD as active 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. We docked each of the 17 antibody structures and 10 ACE2 structures against six RBD structures using HADDOCK, a biomolecular modeling software that provides docking predictions for provided protein structures (48, 49). Specifically, we used the HADDOCK Web Server (v2.4) for the docking simulations with the default parameters (48, 49).

The HADDOCK software produces multiple output PDB files of docking results and their subsequent docking metrics. We placed the top-scoring PDB output file, after qualitative analysis to ensure realistic binding pose, for each docking experiment with HADDOCK into PRODIGY (v2.1.3) for further analysis. PRODIGY is a web service collection focused on binding affinity predictions for biological complexes (50, 51).

This process resulted in 162 sets of docked structures. We selected the top-performing structure for each antibody-RBD or ACE2-RBD pair for quantitative analysis. Statistical tests were conducted in R (52), implementing the Kruskall-Wallis and the paired Wilcoxon-Mann-Whitney test to compare different predictions (53, 54). We also used this top structure to visually analyze the structural conformation of interfacing residues and docked proteins using PyMol (v2.5.5) (55).

Results

Docking Results for assessment of Antibody and ACE2 binding Affinity. We compared docking predictions of viral proteins to antibodies and ACE2 with Kruskall-Wallis and paired Wilcoxon-Mann-Whitney tests. The tested variables were HADDOCK score, van der Waals energy, electrostatic energy, desolvation energy, buried surface area, and PRODIGY's ΔG predictions. These tests return values that are not statistically significant between JN.1/BA.2.86 and previous Omicron variants at a 95% confidence level for every displayed metric, with a few exceptions, in Figures 1 and 2. The difference in Desolvation Energy is statistically significant, via the paired Wilcoxon-Mann-Whitney test, between XBB.1.5 and JN.1 in the antibody comparison, with JN.1 having a lower Desolvation Energy. The difference in desolvation energy between JN.1 and BA.1/B.1.1.529 is statistically significant in the ACE2 comparisons, with BA.1/B.1.1.529 having a lower desolvation energy. In the ACE2 analysis, the Kruskal-Wallis test yields statistically significant differences between all groups.

The Wuhan or WT variant exhibited a higher binding affinity, which is statistically significant, to ACE2 than the BA.2.86 and JN.1 variants when assessing ΔG value in Figure 2. The WT variant also exhibited a lower desolvation energy score when binding to ACE2, which is statistically significant, than the BA.2.86 and JN.1 variants. The WT variant exhibited a higher, statistically significant, electrostatic energy score than BA.2.86.

The Kruskal-Wallis test, which assesses statistical significance for group comparisons as a whole, returns a statistically significant p value at a 95% confidence interval in the ACE2 comparisons for Desolvation Energy in Figure 2.

We use the PRODIGY ΔG score as the primary metric to measure binding affinity. Thus, we conclude that all the Omicron subvariants' (including BA.2.86 and JN.1) performance for the ACE2 and antibody docking simulations were similar. Slight reductions in antibody-RBD and ACE2-RBD binding affinity were observed for JN.1 when compared to BA.2.86, but they are not statistically significant at a p-value of 0.81 and 0.22 respectively for a 95% confidence interval.

Figure 1 illustrates various metrics produced by HADDOCK and PRODIGY estimations of the protein-to-protein binding affinities between our aggregate antibody arsenal and RBD structures. It includes 17 antibody structures and six variant RBD structures (102 experiments in total). The results of ACE2 to RBD docking experiments are shown in Figure 2, including 10 ACE2 and six RBD structures (60 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-Mann-Whitney signed-rank test between each RBD, again with the exceptions mentioned above. These figures are derived from metrics obtained from the best PDB complex structure, determined by HADDOCK, for each experiment.

Structural Analysis. Figure 3 shows the structural analysis of the interfacing residues between the RBDs of JN.1 and BA.2.86 with antibodies. The only difference in the RBD in JN.1 and BA.2.86 is the Leu455Ser substitution in JN.1. The residue at position 455 does not form polar contacts in either the JN.1-RBD or BA.2.86-RBD complexes in Figure 3 (A) or (B), nor is the residue at position 455 near the docking interface for either complex. The Leucine at position 455, in the RBD of BA.2.86, may interact with residues within the RBD. The substitution of Leucine455 by Serine may alter intramolecular interactions of the RBD, inherently affecting its tertiary structure. This disparity in structure may affect the binding pose of the RBD in complex with the antibody, which is highly dependent on the antibody mechanism. In Figure 3(A), one can note that BA.2.86 generally forms polar contacts with residues that do not propagate steric hindrance. In contrast, Figure 3(B) shows that JN.1 forms two histidine polar contacts, a side chain that introduces more steric hindrance and rigidity. Histidine contacts may reduce proximal residue interdigitation, providing a lower binding affinity score. In Figure 3(C), the phenylalanine at position 489 of BA.2.86's RBD interdigitates well with antibody residues, potentially increasing nonpolar interactions and decreasing the effect of the steric hinderance from the bulky phenylalanine side chain. In Figure 3(D), one can notice that residues appear less interdigitated than in Figure 3(C). This may be due to the destabilizing effect of the Serine at position 445 in JN.1, in addition to the alteration of the RBD tertiary structure mentioned previously. Leucine455 is shown in yellow in Figure 3(C), close to Isoleucine100 of the antibody. Both of these residues may form nonpolar interactions, strengthening binding. In 3(D), this Serine substitution has caused a lack of attraction between the two residues, potentially causing the segment within the RBD to retract back in docking and decrease binding affinity.

Figure 4 shows the structural analysis of the interfacing residues between the RBDs of JN.1, BA.2.86, and XBB.1.5 with ACE2. When assessing Figure 4(A) and (B), one can notice that XBB.1.5 and JN.1 adopt a markedly different binding pose to each other, the angle of binding has approximately a 90-degree disparity. However, they do maintain similar interfacing residues. First, it is to note that at position 445, XBB.1.5 has a Proline residue while JN.1 has a Histidine residue. Proline offers enhanced rigidity to that of Histidine, potentially adding to the disparity in tertiary structure between XBB.1.5 and JN.1, affecting binding. Second, there is a deletion in the JN.1 RBD at position 483, where there is a Valine in position 483 in XBB.1.5. This Valine does not interface with ACE2. However, this deletion may lead to an alteration in tertiary structure that causes a variation in binding pose. Lastly, there is a Serine at position 490 in XBB.1.5, while there is a Phenyalanine in the corresponding position of 489 in JN.1. When assessing XBB.1.5, this Serine may form contacts in slight alterations of the docking pose with the Threonine27, Lysine31, and Glutamate35 in ACE2. These contacts could potentially increase stability. When assessing the corresponding structure of JN.1, the Phenylalanine is proximal to Histidine34 of ACE2. This proximity may increase pi-pi bonding. However, given the constraints of the Alpha Helix that Histidine34 is part of, there is limited flexibility, and steric hindrance between the two bulky side chains could cause detrimental effects on binding. When assessing Figure 4(C) and (D), one can notice a minimal difference in binding pose between BA.2.86 and JN.1. The only difference between the sequence of BA.2.86 and JN.1 is the residue at position 455. In JN.1, the residue at position 455 is a Leucine. This Leucine is close in proximity to Isoleucine100 and Phenylalanine72 in ACE2. This proximity could add to nonpolar interactions, increasing binding affinity. When assessing the Serine in position 455 in JN.1, it is positioned far away from the ACE2-RBD interface, leaving it unlikely to interact with any ACE2 residue to strengthen interaction significantly. There is a Lysine at position 68 in ACE2, which may form an interaction with the electronegative oxygen within Serine. However, Lysine68 is part of an alpha-helix of ACE2 and is angled away from Serine455 of the RBD, making the interaction less likely.

Broadly Neutralizing Antibody Performance.

P2D9. The antibody P2D9, identified in Luo et al. 2023, was obtained from the memory B cells of individuals vaccinated with the BNT162b2-vaccine (31). These individuals also had breakthrough BA.1 infection (31). P2D9 was found to neutralize all tested variants of concern and omicron sublineages (31). Luo et al. tested neutralization capabilities against variants such as the WT, Alpha, Delta, BA.1, and BA.2 variants (31). Pseudovirus neutralization profiles were determined for P2D9. Luo et al. found on the BA.1, BA.2. and the WT variant, that P2D9 had IC_{50} values of 0.0117, 0.1381, and 0.0075 µg/mL, respectively (31). When assessing the values in Supplementary Table 3, one can notice similar values for Van der Waals energy and HADDOCK score between the three variants. However, the WT variant has a significantly lower Prodigy ΔG value, correlating with the empirical values. It is noted that BA.2 records a lower Prodigy ΔG value than the BA.1 Prodigy ΔG value. However, BA.1 Electrostatic Energy value is substantially lower than that of BA.2 potentially explaining the discrepancy between their IC_{50} values despite their Prodigy ΔG values. The WT variant has an Electrostatic Energy value between BA.1 and BA.2. The metrics in Sup-

plementary Table 3 indicate P2D9 will perform similarly to previous Omicron variants on BA.2.86 and JN.1, maintaining its broadly neutralizing characteristic.

6-2C. The antibody 6-2C, identified in Liu et al. 2023, was obtained from the memory B-cells of individuals vaccinated with the BBIBP-CorV inactivated vaccine (30). Specifically, Liu et al. obtained samples from patients who had robust humoral immune responses after the second dose of the BBIBP-CorV vaccine (30). When assessing Supplementary Table 4, one can notice that the WT variant has lower values for Van der Waals energy, HADDOCK score, and Prodigy ΔG relative to the BA.1 and BA.2 variant. Electrostatic energy is slightly higher between 6-2C and the WT variant. Overall, these metrics indicate that 6-2C has a higher binding affinity to the WT variant relative to BA.1 and BA.2 variant. When assessing empirical data reported in Liu et al. 2023, the minimum amount of antibody quantity to achieve 50% reduction in viral infectivity for the WT, BA.1, and BA.2 variants is 37, 1149, and 948 ng/mL respectively (30). One can note that 6-2C is far more potent on the WT variant than BA.1 and BA.2 variant. The empirical values correlate with the obtained docking metrics and post-docking metrics. When assessing the data for BA.2.86 and JN.1 in Supplementary Table 4, the metrics indicate that 6-2C will maintain neutralization capabilities for BA.2.86 and JN.1.

CV2.1169. The antibody CV2.1169, identified in Planchais et al. 2022, was obtained from the memory B-cells of individuals infected with the WT strain. Planchais et al. specifically filtered for serum samples from individuals with high seroneutralization for single B-cell antibody cloning. Planchais et al. found that the antibody CV2.1169 potently neutralized SARS-CoV-2 variants of concern. Planchais et al. concluded that CV2.1169 was a prime candidate for the prevention and treatment of COVID-19. When analyzing the Planchais et al. 2022 study, IC_{50} values for BA.1 and BA.2 are 850 and 756 pM, respectively, corresponding with the Prodigy ΔG values displayed in Supplementary Table 5, with BA.2 demonstrating a lower Prodigy ΔG, lower electrostatic energy score, and lower HADDOCK score. The data in Supplementary Table 5 indicates CV2.1169 will maintain its neutralizing capabilities as the various docking and affinity metrics for BA.2.86 and JN.1 are similar to BA.1 and BA.2.

Discussion

The docking metrics we obtained tell a complex story when assessing them as a single component to form conlusions regarding the binding affinity of BA.2.86 and JN.1 to neutralizing antibodies and ACE2. Statistically significant Desolvation Energy differences are noted in the results. Desolvation Energy measures the energy change of protein atoms moving from interactions with a solvent to a non-solvent (56). Desolvation Energy can significantly impact binding affinity and is a component in the calculation of binding affinity (57). Taken alone, desolvation energy metrics may indicate lower or higher binding between two separate molecules within a solvent. However, we have the binding affinity metrics provided by PRODIGY, which do not indicate a statistically significant difference in binding affinity for neutralizing antibodies and ACE2 between JN.1 and BA.2.86 relative to previous Omicron variants.

Substantial changes in binding affinity are not observed between JN.1 and BA.2.86 to neutralizing antibodies and ACE2. BA.2.86 appears to have no significant evasion to RBD targeting antibodies and does not appear to bind with a significantly higher affinity to ACE2 than previous variants. Relative to BA.2.86, JN.1 does demonstrate a 3.9% increase in the median ΔG value to the RBD-targeting antibody arsenal in our study, indicating slightly increased antibody evasion. At the same time, JN.1 exhibits a lower ACE2 binding affinity than BA.2.86, with a 4.9% increase for the median ΔG value for JN.1 relative to BA.2.86.

BA.2.86 Empirical Evidence Corresponds with Predictions. Studies have been published regarding BA.2.86 and JN.1. In one study titled, "Sensitivity of BA.2.86 to prevailing neutralizing antibody responses," patients had blood sera collected before and after XBB.1.5 predominance (58). Sheward et al. found that blood samples collected before and after XBB.1.5 prevalence had moderately lower geometric mean neutralizing titers for BA.2.86 relative to XBB.1.5. Sheward et al. concluded that BA.2.86 did not appear to have the same level of relative immune escape as when the Omicron variant initially emerged in the shadow of the Delta variant in late 2021. The *in silico* results for BA.2.86 correspond with Sheward et al. 2023.

In an article published titled 'SARS-CoV-2 Omicron subvariant BA.2.86: limited potential for global spread', Wang et al. review numerous studies regarding BA.2.86 including Sheward et al. 2023. Wang et al. analyze multiple studies that show that recent infection or vaccination increases neutralization of BA.2.86. Wang et al. generally conclude that BA.2.86 may not possess the transmissibility that previous Omicron strains possessed. Wang et al.'s analysis corresponds with the *in silico* results for BA.2.86. Wang et al. do note that their conclusion does not apply to the JN.1 variant as they did not examine it. Wang et al.'s review corresponds with the results of our study.

Empirical Evidence Concerning Immune Evasion of JN.1 is Mixed. In an article titled 'Humoral immune escape

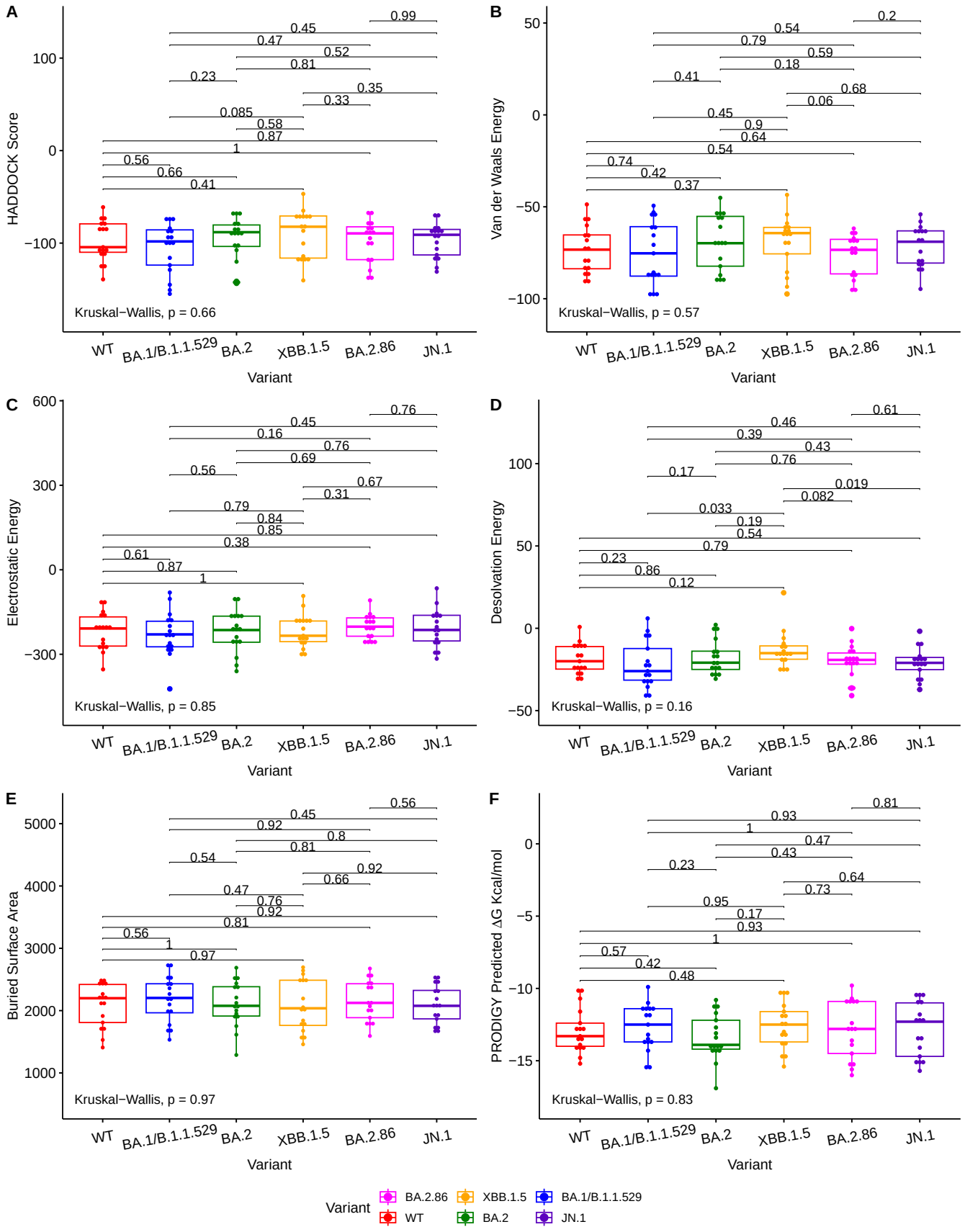

Fig. 1. Boxplots to illustrating the comparative docking scores of antibody and RBD structures generated by HADDOCK and PRODIGY. Each boxplot highlights the distribution of docking scores for different variants. Pairwise comparisons were performed using the Wilcoxon-Mann-Whitney signed-rank test, indicated by the horizontal lines. The Kruskal-Wallis test was used to compare the independent samples' medians, indicated in the bottom left.

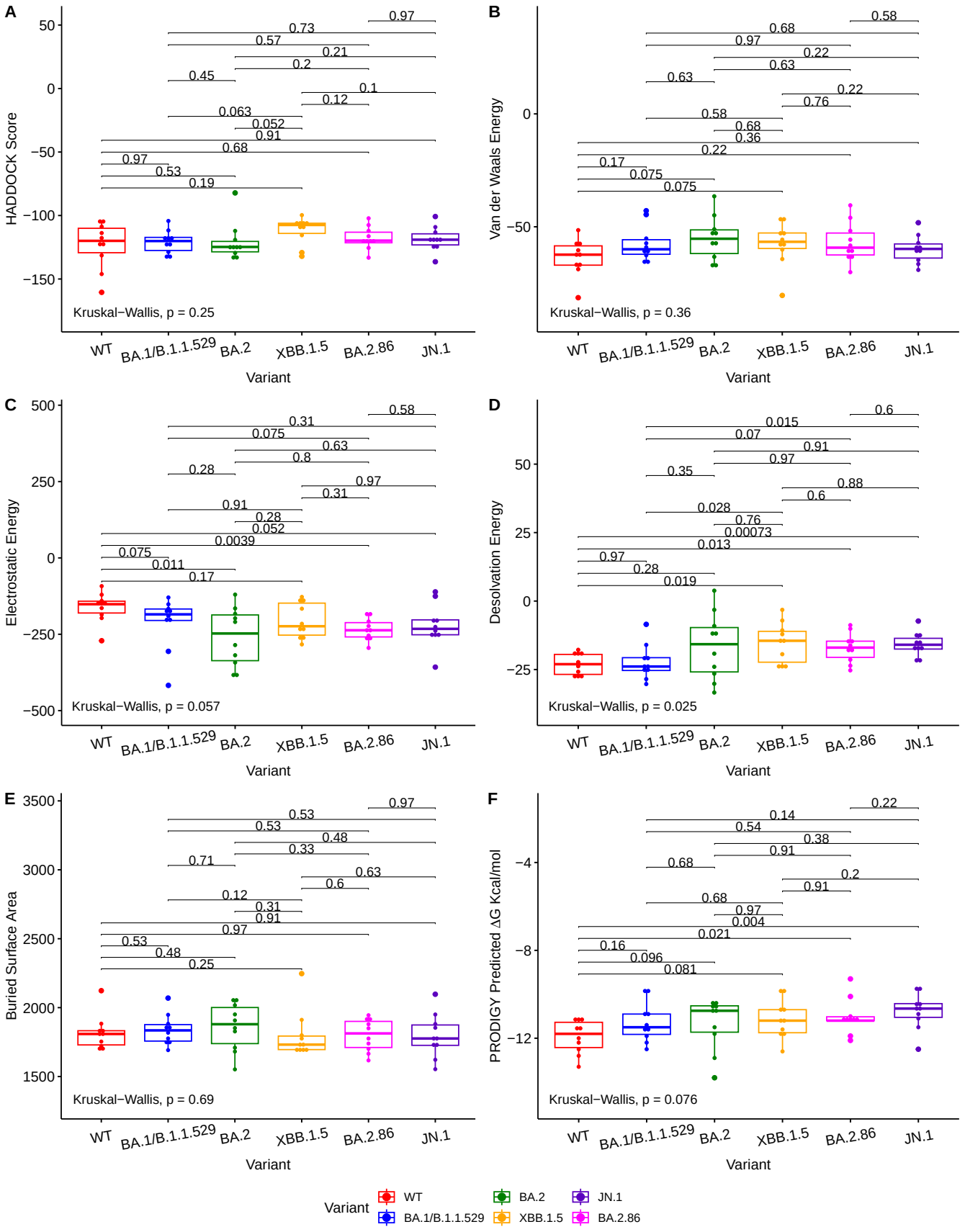

Fig. 2. Boxplots illustrating the comparative docking scores of ACE2 and RBD structures generated by HADDOCK and PRODIGY. Each boxplot highlights the distribution of docking scores for different variants. Pairwise comparisons were performed using the Wilcoxon-Mann-Whitney signed-rank test, indicated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

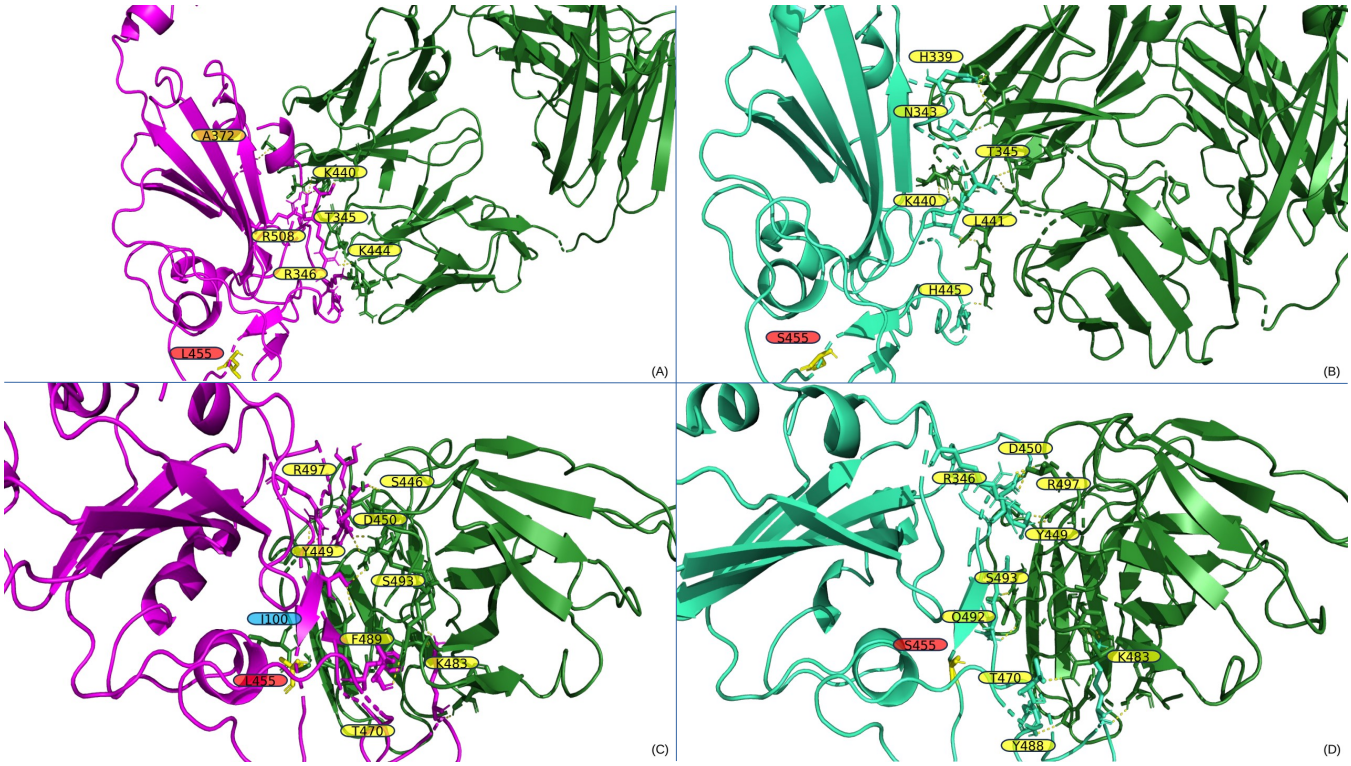

Fig. 3. A) and B) display the output PDB files from HADDOCK of the docking jobs of antibody P2D9 to BA.2.86 and JN.1, respectively. C) and D) display the output PDB files from HADDOCK of the docking jobs of antibody 10-5B to BA.2.86 and JN.1, respectively. The antibody structure in each is shown on the right in green, and the RBD is shown on the left in magenta for BA.2.86 or greencyan for JN.1. Interacting RBD residues are labeled in yellow. Residues dicussed in the results section on the antibody are labeled in blue. Important mutations referenced in the results section are highlighted in yellow and labeled in red.

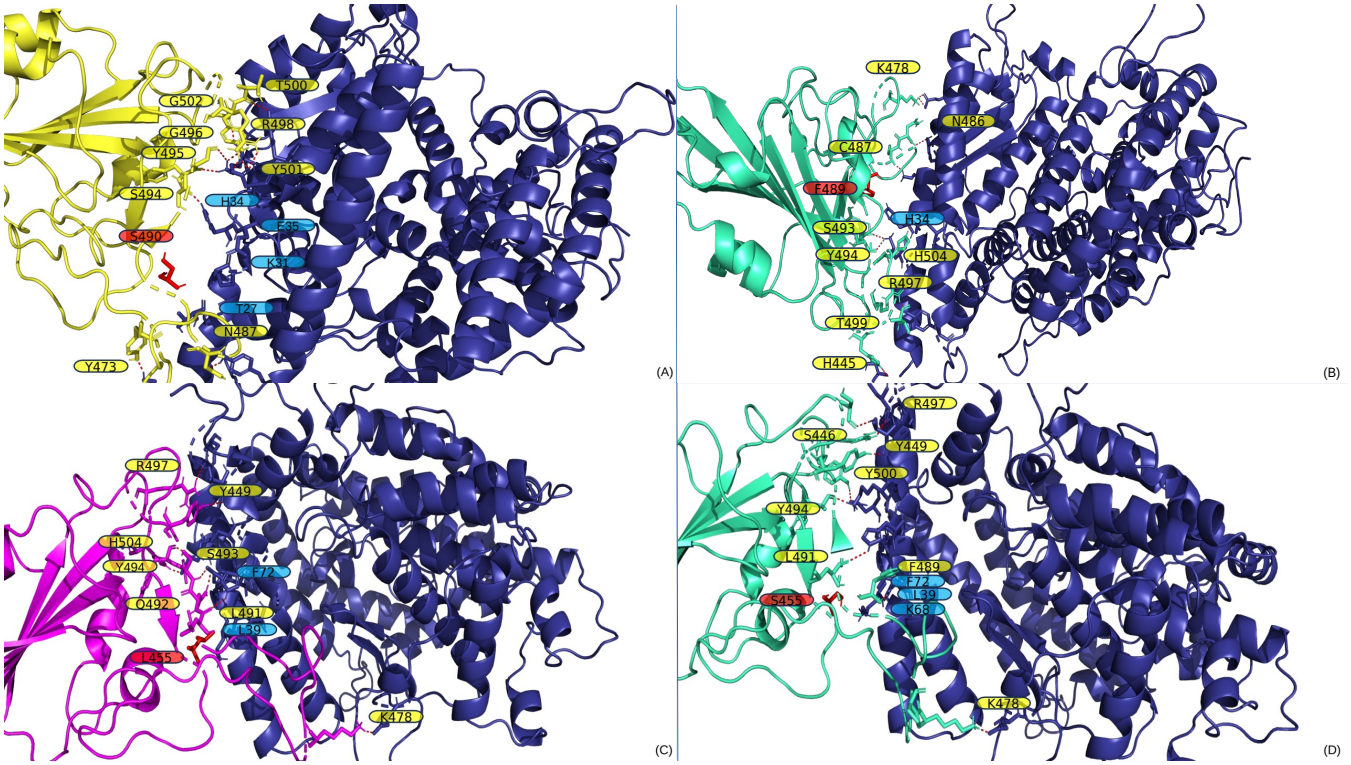

Fig. 4. A) and B) display the output PDB files from HADDOCK of the docking jobs of the ACE2 structure from PDB 7XO9 and XBB.1.5 and JN.1, respectively. C) and D) display the output PDB files from HADDOCK of the docking jobs between the ACE2 structure from PDB 7WPB and BA.2.86 and JN.1, respectively. In each, the images show the ACE2 structure on the right in blue and the RBD on the left, colored in yellow for XBB.1.5, magenta for BA.2.86, and greencyan for JN.1. Interacting ACE2 residues, referenced in the results section, are labeled in blue. Important mutations referenced in the results section are labeled in red and highlighted in red.

by current SARS-CoV-2 variants BA.2.86 and JN.1, December 2023', Jeworowski et al. collected sera from a group of individuals who had received three to four vaccine doses in September 2023, a majority having prior breakthrough infection (20). These individuals were initially vaccinated using mRNA or vector-based vaccinations (20). Jeworowski et al. found that JN.1 did not exhibit a significant reduction in serum neutralization titers relative to BA.2.86 (20). It is to be noted that all of the individuals in the study reported a SARS-CoV-2 infection during the period of Omicron prevalence (20). Jeworowski et al. concluded that antibody evasion did not appear to cause the increased prevalence of JN.1. Jeworowski et al. 2024's results correspond with the results of our study.

In another study on JN.1 titled "Fast evolution of SARS-CoV-2 BA.2.86 to JN.1 under heavy immune pressure', Yang et al. studied the humoral immune evasion and ACE2 binding affinity of JN.1 (17). Yang et al. collected blood samples from patients who were reinfected with XBB post BA.5 or BA.7 breakthrough infection (17). Yang et al. also collected samples from individuals who had received three doses of inactivated vaccines and subsequently contracted XBB (17). Yang et al. used a blood serum neutralization titer method to assess antibody neutralization. Yang et al. found that JN.1 had significantly higher antibody evasion than BA.2.86 but had a notably lower ACE2 binding affinity than BA.2.86 (17). Yang et al.'s trends generally correspond with our results. However, the magnitude to which we observe antibody evasion is distinct (17). We do not note a significant increase, which Yang et al. note, in antibody evasion relative to previous strains, there is a noted decrease in ACE2 binding, as there is a reduction in binding affinity for JN.1 relative to BA.2.86.

Evolution Outside of the RBD. We have correctly analyzed relative immune evasion for RBD targeting antibodies for the BA.1/B.1.1.529, XBB.1.5, and BA.2.86 variants with the *in silico* approach in the past (7, 21). It is not yet clear if JN.1 will correspond to the findings of our study. When assessing the CDC Data Tracker, JN.1 accounts for approximately 96.4% of current variants detected (15). Our approach in which we focus on RBD-antibody and RBD-ACE2 interaction does not explain the relative predominance of JN.1 to BA.2.86. This suggests the evolution of SARS-CoV-2 outside of the RBD to enhance transmissibility.

Immune evasion and transmissibility are not exclusively dictated by mutations in the RBD region. In the case of JN.1, several mutations of interest are present in three non-spike proteins, and even mutations in nonstructural proteins may play a role in immune evasion (13, 14, 60). For instance, mutations in nsP1 and nsP6 have been identified as capable of inhibiting the interferon type I (IFN-I) pathway (60). This inhibition leads to the absence of IFNA and IFNB production, crucial components in the defense against viral infections (61, 62). Unfortunately, a precise quantification of how much mutations outside the Spike protein contribute to JN.1's increased immune escape compared to other variants remains challenging (17).

Overall, these results have correspondence with previous articles and data on BA.2.86 and JN.1 (15, 17, 20, 58, 59, 63).

Limitations and Advantages. We acknowledge that we cannot, as of yet, assess the interaction of multiple antibodies with the spike protein in neutralization interactions. However, a significant benefit to this approach is the ability to study the neutralization capabilities of individual antibodies, not the aggregate capabilities of antibodies prevalent in the blood. The antibody selection significantly differs from the antibodies studied in existing serological studies. The studied antibody array primarily consists of memory B-cell derived antibodies that are studied to be broadly neutralizing against different variants (30, 31, 37, 39, 42, 47). Memory B-cell derived antibodies created in response to an antigen are not as easily observable when using blood serum neutralization titers. The *in silico* results support the empirical results that the selected antibody array, primarily consisting of broadly neutralizing antibodies, maintains its efficacy across different variants (30, 31, 37, 39, 42, 47). The presence of such broadly neutralizing antibodies also may indicate a level of prevalence of memory B-cells within vaccinated or infected individuals that differentiate to produce such antibodies (30, 37, 39, 42, 47, 64, 65). Serum antibody neutralization titers may be accurate in predicting the prevention of initial infection but is not as accurate in regards to the prediction of the prevention of serious disease. This is because serum antibody neutralization titers only account for antibodies prevalent in the blood at the time of collection. The secondary immune response, via the production of antibodies derived from memory B-cells, cannot easily 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. For example, in Yang et al. 2023, the authors specifically assess sera collected after XBB infection, biasing the proportions of antibodies within the blood that have neutralizing capabilities towards epitopes of XBB and structurally similar variants. When an immune response occurs to an introduced SARS-CoV-2 antigen in an individual with memory B-cells that can respond to that antigen, the proportions of neutralizing antibodies can and will change as the memory B-cells will differentiate to produce plasma cells to create neutralizing antibodies (64). In contrast to the limitation of a bias in the proportion of neutralizing antibodies provided in serum neutralization studies, this *in silico* method primarily uses antibodies that have been studied to be produced upon memory B-cell stimulation and neutralize SARS-CoV-2 variants, which may be more indicative of protection induced by prior infection or vaccination. A larger and broader study analyzing the presence of memory B-cells that differentiate to eventually produce broadly neutralizing antibodies is needed to support the notion that the general population can produce such broadly neutralizing and effective antibodies against current and future variants. In addition to the study of the natural production of broadly neutralizing antibodies, future work into developing therapeutic anti-

bodies based upon the highly capable antibodies produced by the memory B-cells used in the study could lead to improved treatment outcomes.

Conclusion

Our study indicates that ACE2 and antibody binding of the BA.2.86 and JN.1 variants is not profoundly different from previous variants. Moreover, it shows the ongoing efficacy of antibodies induced by various means in the global population to fight BA.2.86 and JN.1. Finally, the *in silico* results suggest that variation within the RBD has not led to the increased fitness JN.1 has demonstrated relative to BA.2.86. Mutations outside the RBD that may enhance fitness must be studied to enhance our ability to respond to the ongoing SARS-CoV-2 pandemic.

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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Supplementary Materials

Variant	HADDOCK Score	Van der Waals energy	Electrostatic energy	Prodigy ΔG Kcal/mol
WT	-85.5	-72.1	-248	-13.3
BA.1/B.1.1.529	-88.7	-65.5	-285.8	-11.5
BA.2	-88.2	-70.1	-204.9	-12.2
XBB.1.5	-70.7	-64.3	-185.9	-10.3
BA.2.86	-82.4	-71.8	-166.9	-12.4
JN.1	-82.7	-61.0	-215.6	-10.4

Table 3. Individual docking metrics for the antibody P2D9 against the different variants used in the study.

Variant	HADDOCK score	Van der Waals energy	Electrostatic energy	Prodigy ΔG Kcal/mol
WT	-106.4	-86.1	-205.8	-14.2
BA.1/B.1.1.529	-73.3	-54.9	-229.4	-9.9
BA.2	-90.7	-68.8	-216.4	-13.4
XBB.1.5	-82.3	-64.2	-240.6	-13.7
BA.2.86	-78.5	-74.1	-165.0	-10.7
JN.1	-88.8	-79.2	-118.2	-12.1

Table 4. Individual docking metrics for the antibody 6-2C against the different variants used in the study.

Variant	HADDOCK Score	Van der Waals energy	Electrostatic energy	Prodigy ΔG Kcal/mol
WT	-79.2	-60.1	-167.3	-12.7
BA.1/B.1.1.529	-74.6	-60.8	-182.7	-11.4
BA.2	-80.4	-60.9	-214	-13.1
XBB.1.5	-81.8	-65.4	-175.7	-13
BA.2.86	-94.7	-67.7	-251.9	-12.7
JN.1	-112.8	-80.6	-230	-14.1

Table 5. Individual docking metrics for the antibody CV2.1169 against the different variants used in the study.