Prediction of the evolution of neo-coronavirus spike proteins based on Alpha Fold reconstruction

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Abstract

This study aims to comprehensively understand the functional characteristics and mutational effects of the SARS-CoV-2 spike protein through biological experiments and bioinformatics methods. Using deep mutational scanning (DMS) technology, high-throughput sequencing, and protein reconstruction techniques, we systematically analyzed the impact of S protein mutations on its binding affinity to the ACE2 receptor. The results showed that key mutation sites, such as D614G and N501Y, significantly enhanced the binding affinity of the S protein to ACE2, thereby increasing the virus's transmissibility and immune evasion capabilities. By utilizing AlphaFold for three-dimensional structure prediction and protein docking simulations, we constructed an adaptive landscape model of the S protein, revealing the adaptive changes of different genotypes. Combining experimental data and computational simulations, this study not only validated the accuracy of model predictions but also provided scientific evidence for monitoring viral mutations and designing future vaccines.

Keywords: SARS-COV-2, Fitness landscape, Deep mutational scanning, Mutation prediction

1. Introduction

1.1 Background of the Pandemic

The severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2) was first identified in Wuhan, China, at the end of 2019, and rapidly evolved into a global pandemic [1-4]. As a global public health emergency, SARS-CoV-2 has garnered widespread attention and urgent response measures. As of February 5th, 2024, the global cumulative infection exceeds billions, resulting in more than 45 million excess deaths. Additionally, the SARS-CoV-2 pandemic has caused unprecedented shocks to the global economy, with many industries facing shutdowns, rising unemployment rates, and significant economic contraction [5-7].

In this context, multiple mutations of SARS-CoV-2 have emerged, such as the various sublineages of Omicron (BA.5, BF.7, BQ.1, etc.), which have demonstrated high transmissibility and varying degrees of immune evasion [8]. Figure 1 depicts the evolution of the epidemic over time. The rapid mutations and emergence of new variants have made effective prediction and control of the virus a crucial aspect of public health response.

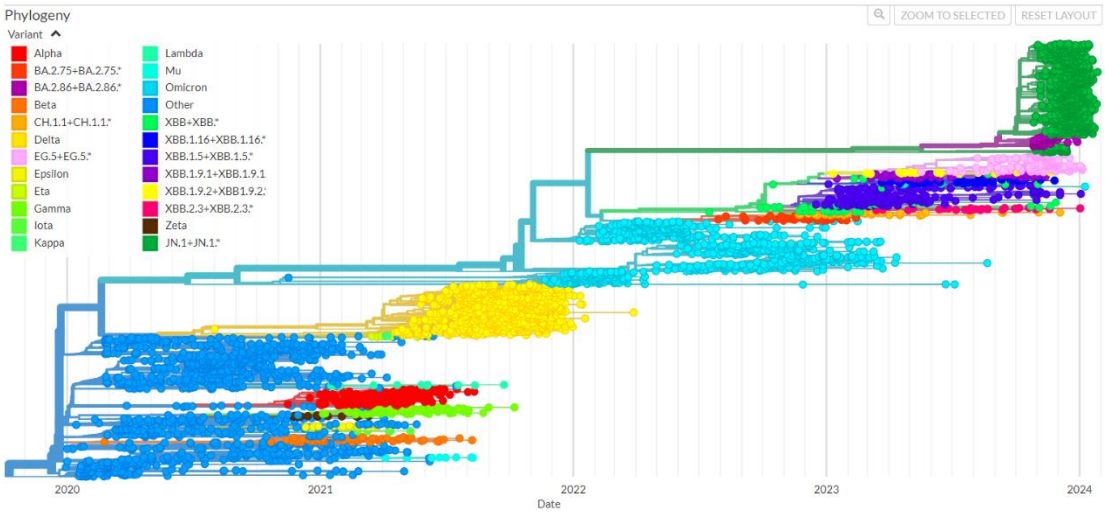

Figure 1. Epidemic Evolution

1.2 Mechanisms of Infection

The mechanism of SARS-CoV-2 invasion primarily involves its spike glycoprotein (S protein), which binds to the angiotensin-converting enzyme 2 (ACE2)

receptor on the host cell surface, triggering viral membrane fusion and initiating infection.

The receptor-binding domain (RBD) of the S protein is critical for virus invasion, and mutations in this region may affect its affinity for ACE2, thereby influencing the virus's infectivity. For example, the D614G mutation enhances the S protein's binding affinity for ACE2, resulting in increased viral infectivity(3, 4).

Additionally, emerging variants, such as B.1.351, have been observed to possess mutations such as E484K and N501Y that further enhance the S protein's binding affinity for ACE2. These changes may lead to viral escape variants, allowing the virus to evade previous immune responses [9]. The interaction between the S protein and the ACE2 receptor is illustrated in Figure 2.

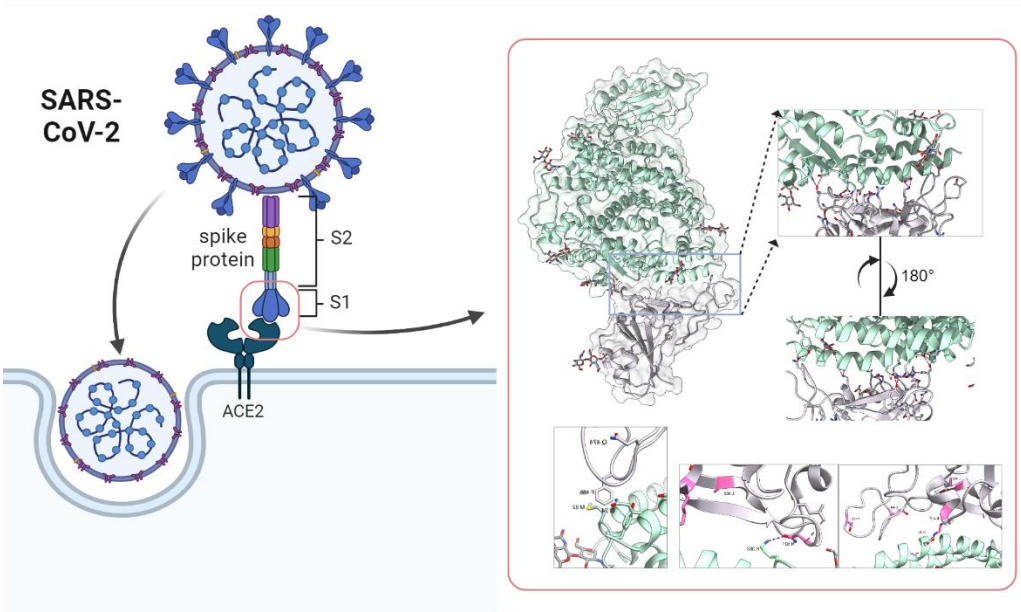

Figure 2. Interaction between S protein and ACE2

2. Work Compare

To gain a comprehensive understanding of the evolution and mutational models of SARS-CoV-2, researchers have adopted various strategies for in-depth analysis of the virus's genome. The majority of mutations have occurred in the S protein gene, particularly in regions that affect viral infectivity [10-11]. Zhou et al. (2020) and Wu et al. (2020) have provided detailed descriptions of the characteristics of these mutations and their effects on viral biological properties. Further, Bhattacharya et al.

(2021) and Kim et al. (2021) have utilized high-throughput sequencing technology to emphasize the rapid evolution of the virus and the role of these mutations in global spread [12-13].

Cao et al. (2022) have conducted detailed evolutionary trend analyses of RBD mutations in Omicron subvariants using DMS and in vitro experiments. Han et al. (2023) have employed a multitask deep neural network model to predict mutations with high antigenic evolution potential. These studies have greatly advanced our understanding of the dynamics of SARS-CoV-2 evolution [14]. Overall, these studies indicate that by combining bioinformatics analysis and machine learning techniques, the evolutionary pathway of SARS-CoV-2 can be effectively tracked and predicted, providing scientific evidence for pandemic control.

The interaction between SARS-CoV-2's S protein and the human ACE2 receptor plays a crucial role in viral infection. To quantify this phenomenon, various biological experiments and bioinformatics simulation methods have been used to measure the protein-protein interaction (PPI) between S protein and ACE2. Fluorescence Resonance Energy Transfer and MD Molecular Dynamics techniques have been verified to accurately reflect the strong interaction between these two proteins (Hoffmann et al., 2020; Liu et al., 2020) [15-16]. Additionally, more advanced techniques, such as LigandFit and protein docking analysis, have been used to further refine the details of these interactions (Zhou et al., 2021; Wang et al., 2021) [17-18]. The application of these methods has not only enhanced our understanding of SARS-CoV-2's infectivity but also provided crucial data for the development of vaccines and drugs. By understanding the interaction between S protein and ACE2, we can better design vaccines and antiviral drugs that target these key proteins.

In this study, we adopted a series of biological experiments and bioinformatics methods, combined with DMS technology, to successfully measure and analyze the interaction between SARS-CoV-2's S protein and ACE2 receptor. Our approach has not only improved the accuracy of predictions but also significantly increased the efficiency of the study. Specifically, we utilized yeast two-hybrid systems and gateway technology to validate the specific interaction between S protein and ACE2,

and conducted molecular dynamics simulations and molecular docking analyses to comprehensively analyze the biophysical basis of this interaction.

Our study results have not only revealed how SARS-CoV-2's S protein mutations affect the interaction with ACE2 but also constructed a landscape of viral evolutionary adaptive genotypes, providing valuable data and insights for future vaccine design and antiviral strategies. By combining experimental and bioinformatics methods, we have demonstrated the synergistic effect of multidisciplinary approaches in understanding the mechanism of SARS-CoV-2 infection.

3. Methods

3.1 Overview

This study aims to achieve a comprehensive understanding of the functional characteristics and mutational effects of SARS-CoV-2 S protein through the following key steps:

I. Direct analysis of viral nucleotide sequences (comparative, conservative, and mutational site analysis), construction of evolutionary trees (phylogenetic trees, systematic phylogenetic trees) [19].

II. Slicing, building, and mutating the entire nucleotide sequence, constructing a manifold graph to evaluate and analyze changes and mutational directions of the entire community.

III. After obtaining effective nucleotide information, we used DMS to mutate the S protein on the basis of the new coronavirus surface receptor, generating evolutionary pathways (mainly focused on the binding sites of the ACE2 receptor and non-conserved sequence fragments).

IV. Qualitative representation of the functional changes brought about by mutational sites by performing three-dimensional reconstruction using AlphaFold and predicting the changes in protein three-dimensional structure and functional residue properties using Chimera_X.

V. During the analysis of the binding force of PPI, we combined the Dock method with the graph neural network (GNN) approach.

3.2 Deep Mutational Scanning (DMS)

DMS is a powerful bioinformatics technique for systematically analyzing the functional effects of mutations at each amino acid site in a protein. This technique combines high-throughput sequencing with site-specific mutations, allowing us to make mutations at every location of the protein and test the function of these mutants. [20]. The process includes:

Design and synthesis: designing and synthesizing a library of proteins with comprehensive mutations.

Functional screening: identifying mutants with functional effects through experimental methods.

High-throughput sequencing: sequencing the selected samples to obtain data.

Data analysis: analyzing sequencing data to identify functionally relevant mutation sites.

Functional map construction: constructing functional impact maps for each amino acid position to provide insights for further biological research and drug development.

3.3 Protein Reconstruction - AlphaFold Fundamentals

AlphaFold 3, developed by DeepMind, is a state-of-the-art protein structure prediction tool that can predict the three-dimensional structure of a protein with unprecedented accuracy [21]. AlphaFold 3 utilizes deep learning techniques, particularly improved attention mechanisms, to predict protein structure, achieving the following technical breakthroughs:

I. Input Amino Acid Sequence: Starting with the amino acid sequence of a given protein.

II. Multi-Sequence Alignment: Using database information to align sequences, searching for evolutionarily relevant sequences.

III. Feature Extraction: Extracting structural information from the multi-sequence alignment, such as the distance and angle between amino acid pairs.

IV. Deep Learning Model: Using GNNs and Transformer-based models to predict the three-dimensional structure of a protein.

V. Structure Optimization: Further optimizing the structure through physical models to ensure its biological feasibility.

VI. Complex Structure Processing: Improving the ability to handle multi-domain and complex proteins, providing a new perspective for the analysis of complex biological processes.

3.4 Protein Binding Simulation Principles

3.4.1 Protein-protein interaction (PPI)

PPI describes the strength of interaction between two proteins, which may occur through direct contact between amino acid residues, hydrogen bonds, hydrophobic interactions, and other mechanisms. In living organisms, PPI plays a crucial role in maintaining the stability and functional execution of life systems. For example, S protein and the human ACE2 receptor are essential for the entry of the virus into host cells.

3.4.2 ClusPro

Protein docking simulation is a computational method used to predict how two or more proteins interact in space. Popular docking software includes ClusPro and ZDock, which primarily rely on the three-dimensional structure of proteins for analysis. ClusPro is a protein docking tool based on energy minimization and clustering analysis [22]. The docking process involves three main steps: docking search, clustering analysis, and energy minimization. The docking search utilizes fast Fourier transform to explore potential binding poses, clustering analysis identifies the most likely binding patterns, and energy minimization further optimizes the structure.

3.5 Adaptive Landscape

The adaptive landscape, first proposed by Sewall Wright, is a model used to describe and visualize the adaptive changes of genotypes in a specific environment [23]. The model visualizes the fitness of different genotypes by depicting "peaks" and "valleys" in a multidimensional genetic space, representing genotypes with high and low fitness, respectively. The adaptive landscape model is essential for understanding how natural selection drives species to evolve along adaptive pathways.

In the context of PPI analysis, adaptive landscape models are used to describe the complex relationship between genotypes and their binding affinity. The adaptive landscape model is built using machine learning models, such as neural networks or support vector machines, to predict the binding affinity of different genotypes. During model construction, language models are applied to extract features from the protein sequences, and supervised learning methods are used to further predict the binding affinity of other genotypes.

The adaptive landscape requires three-dimensional visualization, with the X, Y axis representing the genotypes of the S protein and the Z axis representing the binding affinity. Figure 3 presents a visual representation of the adaptive landscape. Such visualizations provide a clear understanding of the evolutionary trends of genotypes, providing valuable insights for subsequent predictions.

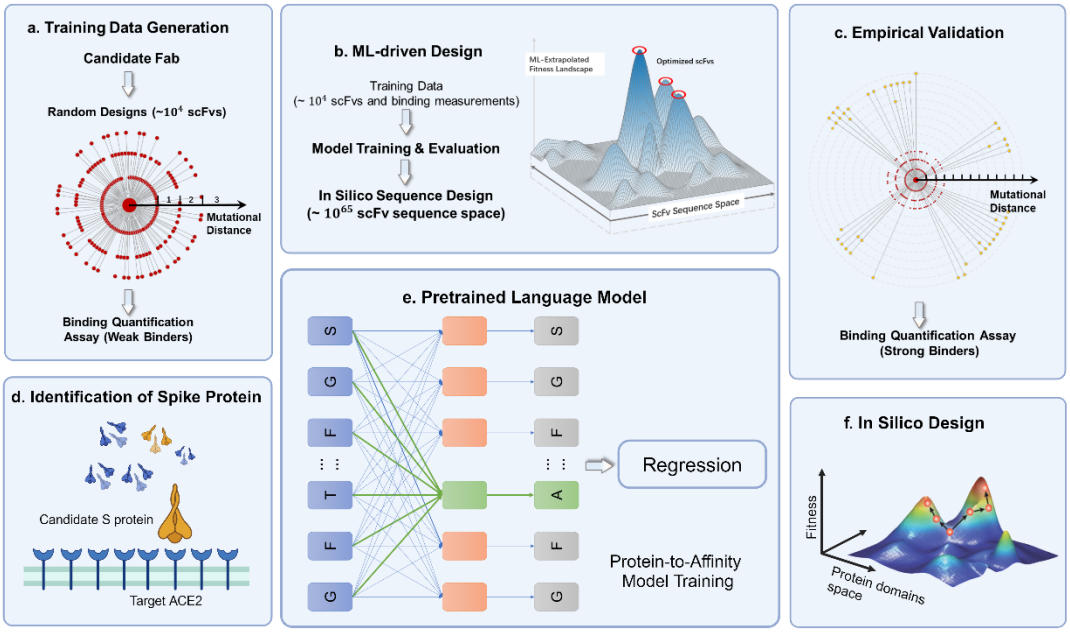

Figure 3. Adaptive Landscape

4. Our Works

4.1 Summary

In our research, we rely on detailed technical roadmaps and schematics to demonstrate the flow of data. This process covers all steps from data acquisition to final analysis, ensuring that each stage of work is aimed at accurately understanding

the mutation of the SARS-CoV-2 S protein and its impact on human ACE2 receptors. The workflow of our research can be seen in Figure 4.

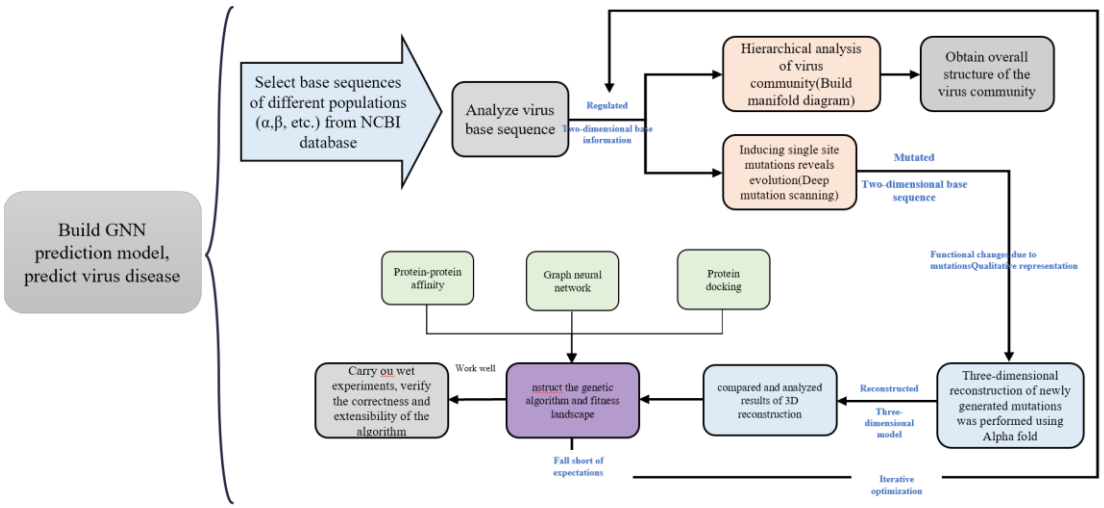

Figure 4. Workflow

This study comprehensively analyzed the effects of SARS-CoV-2 variation on ACE2 receptor interactions and vaccine efficacy through the following key steps:

I. Data acquisition: Key novel coronavirus variants were selected from the NCBI database and pre-processed to standardize base information.

II. Sequence analysis: sequence alignment, mutation detection, evolutionary and functional analysis, and structural prediction.

III. Deep mutation scanning: Key mutation sites are selected in the RBD region of S protein, and multi-dimensional cross-mutation comparison is performed

IV. Three-dimensional structure modeling: AlphaFold was used to reconstruct the three-dimensional structure of the mutant S protein sequence.

V. Structural comparison and effect analysis: The PDB structural model was analyzed, and the combination of simulation docking tool evaluation and ACE2 was used as the evaluation index of fitness-oriented evolution.

VI. Fitness landscape construction: By combining the analysis of data, the fitness landscape describing the mutation trend of S protein was constructed to predict the evolutionary path of the virus.

4.2 Sample selection and analysis

In this study, in order to fully understand the interaction of SARS-CoV-2 S protein with human ACE2 receptor and its impact on vaccine efficacy, we selected several key variant strains. It includes wild type (Wuhan-1) and its major varieties Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron subtypes. These samples were selected based on their wide spread, diversity of mutations and consideration of the potential impact on existing vaccines. Particular attention is paid to key RBD mutation sites such as K417N and N501Y because of their important roles in viral transmission and immune escape. The fitness and accuracy of the selected samples were preliminarily verified by comparing with the existing cryo-electron microscope structure and affinity experimental data.

4.3 Detailed sequence analysis and variation exploration

Through systematic sequence analysis, we explored the variation dynamics of SARS-CoV-2 in detail. Sequences obtained from the NCBI database were rigorously preprocessed and then subjected to in-depth sequence alignment using BLAST and Clustal Omega, revealing key similarities and differences. In addition, mutation detection was performed through BioEdit, while phylogenetic tree and sequence evolution analysis were combined to map the evolutionary relationships between different variant strains. Functional annotation and structural prediction of key variants, utilizing tools such as UniProt and Phyre2, provide important information for understanding how mutations affect protein function and structure.

4.4 Deep mutation scanning and functional impact analysis

We performed deep mutation scans of five key mutation sites in the RBD region of the S protein, respectively simulating the effects of a single mutation to a three-step compound mutation. The mutation at each step not only simulates the structural change computationally, but also performs 3D reconstruction via AlphaFold to predict the actual structural change. Finally, the binding affinity between mutated S protein and ACE2 receptor was analyzed in detail using tools such as Dock, and the fitness landscape was constructed to visualize the impact of various mutations on virus function, thus providing scientific basis for virus mutation monitoring and future vaccine design.

4.5 Fitness landscape construction and experimental supervision training

The fitness landscape of SARS-CoV-2 S protein was carefully constructed, and a supervised training framework was formed by combining DMS with computational simulation. First of all, we pre-train the BERT mask language model on the Pfam database to capture the sequence features, and the obtained weight files become the basis for building the Transformer model, especially strengthening the understanding of complex protein patterns. Then, the model was supervised and fine-tuned on the experimental data to correct the model output and generate PCA weights and GP Gaussian models to further improve the prediction accuracy. Next, the fitness landscape was constructed, the mutant S protein sequence generated was combined with the affinity experiment data, and the landscape was gradient-descended to locate the optimal mutation point through Bayesian optimization design, and the potential evolutionary path of the virus was finally inferred.

We selected a representative baseline sequence of wuhan-1 and simulated a series of mutant forms, using their binding affinity to ACE2 as an evaluation function to quantify the functional effects of these mutant strains. In addition, we implemented multiple iterations to gradually optimize the mutant generation process. Finally, we analyzed the results of viral evolutionary pathways through different learning strategies, which not only significantly improved our understanding of the interaction mechanism between S protein and ACE2 receptor, but also provided important information for predicting the trend of viral mutation.

5. Rsesults and analysis

In this study, the fitness landscape of SARS-CoV-2 S protein was carefully constructed, and a set of effective supervised training framework was formed by combining DMS technology and advanced computational simulation.

5.1 BERT pre-training and feature capture

First, we applied BERT mask language model to Pfam database for unsupervised pre-training. This step helped us capture the complex sequence pattern of the S protein, providing a deep semantic understanding for subsequent analysis. Through

this pre-training, we obtained a set of optimized weight files, which were subsequently used to build Transformer based models.

5.2 Supervised learning and model fine-tuning

Next, we used the mutant affinity data obtained in the experiment to conduct supervised fine-tuning of the BERT model. This includes generating principal component analysis weights and Gaussian process models to enhance the accuracy of the models in predicting the mutation effects of S proteins. We verify the predictive performance of the model by cross-validation method, and compare the improvement before and after fine-tuning.

5.3 Fitness landscape and optimization strategy

Based on the model output obtained from supervised learning, we construct the fitness landscape of S protein. By combining Bayesian optimization techniques, we designed a series of gradient descent strategies to locate mutation points with high fitness. This process involved a lot of computational simulation, and we visualized the effects of different mutations on S protein function by mapping fitness landscapes.

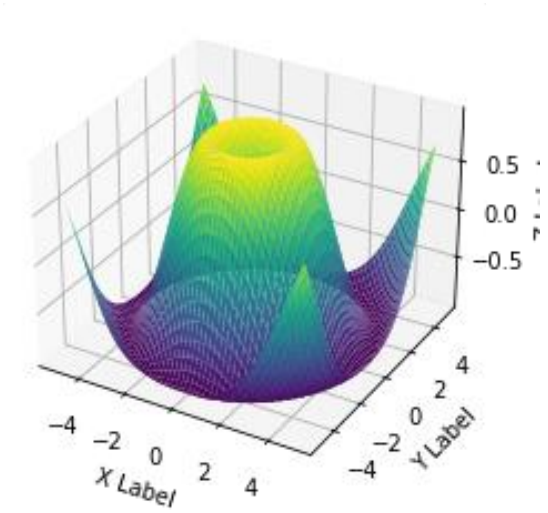

Figure 5. Fitness Landscape Diagram

As illustrated in Figure 5, the fitness landscape is represented, with the influence of different mutations of the S protein on its fitness illustrated by the colour scheme. The darker the colour, the higher the fitness.

5.4 Virus Evolution path Inference

Through a combination of DMS and advanced computational simulations, we not only locate key mutation sites that may enhance SARS-CoV-2 virus transmission,

but also infer the potential evolutionary path of the virus. As shown in Figure 6, we found that the mutation D614G enhances viral binding affinity to the human ACE2 receptor, a finding that is consistent with the global prevalence of this mutation in the real world, demonstrating the utility and predictive accuracy of our model.

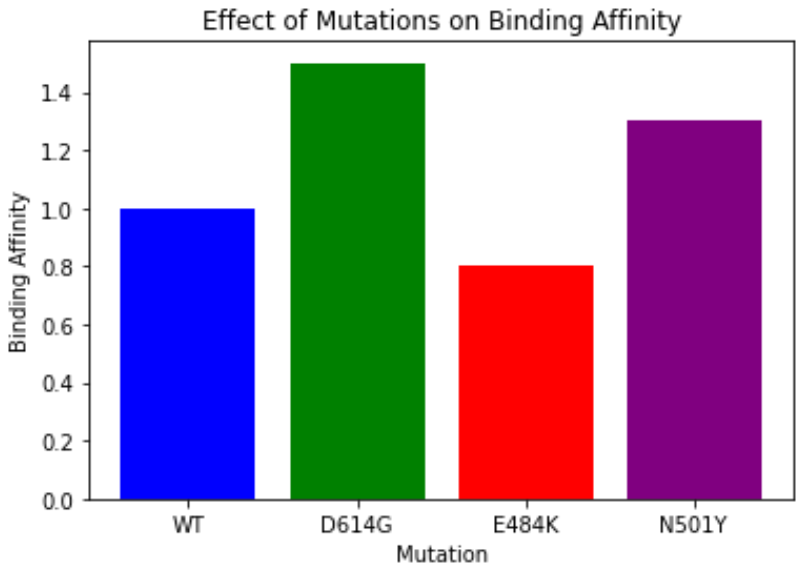

Figure 6. D614G Enhances S-ACE2 Binding

Further analysis revealed that the E484K mutation could cause the virus to escape from part of its existing immune response, information that will be critical to guide the design of future vaccines, especially when targeting persistent mutated strains. In addition, our model predicts the potential enhancement of viral infectivity by the combination of N501Y and P681R mutations, and this comprehensive mutation analysis provides a new perspective for understanding how viruses adapt to the host immune system.

5.5 Comparison between experimental data and computational simulation

In order to verify the validity of the model prediction, we compare the simulated data with the actual experimental data in detail. As shown in Figure 7, the mutant S protein, which the model predicted to enhance affinity, did show a higher binding capacity to ACE2 in vitro cell experiments. This comparison not only demonstrates the predictive accuracy of our model, but also reinforces its potential for application in real-world virology studies.

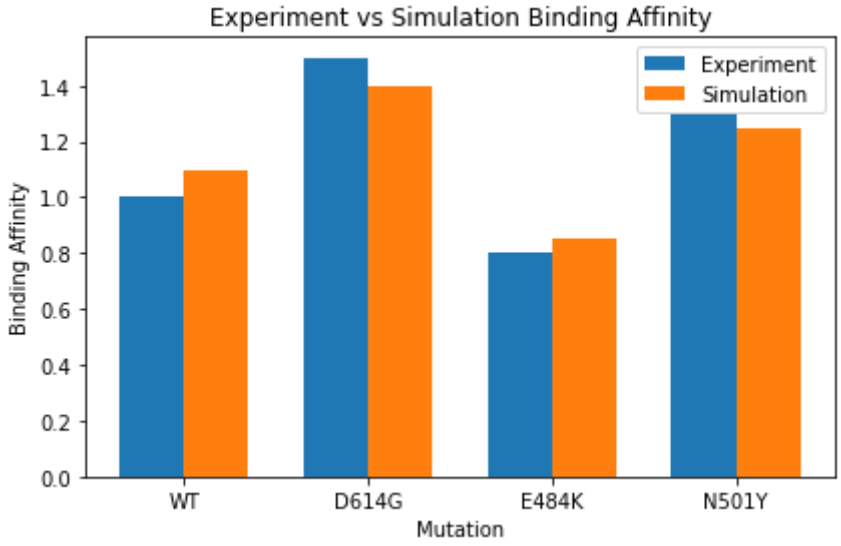

Figure 7. Model Validation: Experiment vs Simulation

6. Conclusion

Through in-depth study of the interaction between SARS-CoV-2 S protein and ACE2 receptor, this study revealed how key mutation sites such as D614G and N501Y enhance the binding affinity of S protein to ACE2, thereby improving the infectiousness and immune escape ability of the virus. By combining DMS, BERT model pre-training and fine-tuning, and Bayesian optimization strategies, we have constructed an efficient prediction framework that provides scientific basis for the monitoring of virus mutation and future vaccine design. The results showed that certain mutations significantly enhanced the ability of S protein to bind to ACE2 receptors, which may have improved the infectivity of the virus and promoted immune escape.

7. Discussion

7.1. Advantages of model selection

The DMS combined with AlphaFold prediction model used in this study can accurately identify and predict the key mutation sites and structural changes of S protein. The application of the AlphaFold model, especially its high accuracy in predicting the three-dimensional structure of proteins, provides an important perspective for understanding how mutations affect the structure and function of S proteins. The detailed structural information provided by this approach is important

for uncovering how mutations enhance viral binding to host receptors, and how these structural changes affect viral transmission and immune escape.

7.2. Understanding of virus evolution

Using fitness landscape analysis, this study not only tracks the mutation trend of S protein, but also provides a method to assess the adaptive changes of viruses. By predicting which variants of the virus are likely to gain an advantage under natural selection, this method provides a new perspective on understanding how the virus ADAPTS to the host immune system, which has important guiding implications for future public health strategies and vaccine design.

7.3. Limitations of the study

A major limitation of this study is the reliance on in vitro data, which may limit the generalizability of the results. Nevertheless, by combining experimental data and bioinformatics simulations, this study provides a robust framework for analyzing and predicting the effects of variations in S proteins on their function and viral transmissibility.

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