Design And Evaluating The Putative Efficacy Of Novel Antibodies Enhancing Binding Affinity On CTLA-4 And PD-1 Immune Checkpoints

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Abstract. *Immune checkpoint proteins, including CTLA-4 and PD-1, have emerged as promising targets for cancer immunotherapy due to their vital role in immune regulation. In this comprehensive study, we conducted a thorough analysis of antibody-protein interactions by employing structural analysis, mutational studies, and antibody-antigen docking simulations. Our findings revealed that antibodies* **1** *and* **2** *exhibited strong binding affinities towards CTLA-4 and PD-1 proteins, respectively. Further analysis through alanine scanning identified specific residues, namely Tyr91 on antibody* **1**, *along with Asn31 and Asp96 on antibody* **2**, *as potential mutation sites. Introducing these mutations resulted in the generation of two novel antibodies,* **1a**, **2a** *and* **2b**, *which displayed enhanced binding affinity towards their respective target proteins. With their improved binding capabilities, these novel antibodies hold tremendous promise for enhancing the effectiveness of antibody-based therapies designed to target immune checkpoints in cancer treatment. The ultimate objective is to significantly improve the health outcomes of patients by leveraging the potential of these novel antibodies.*

Keywords: immune checkpoint proteins, protein - protein docking, alanine scanning.

1. Introduction

Cancer, an encompassing term for a collection of malignant pathologies characterized by uncontrolled cellular proliferation, presents a significant and growing global health challenge [1]. Reported epidemiological data indicates that the annual global burden of new cancer cases has been observed to increase to over fourteen million [2]. Prevalent types of cancer such as lung, breast, stomach, liver, and cervical cancer significantly contribute to the overall disease occurrence [3].

A diverse range of cancer treatment modalities, including surgical intervention, chemotherapy, radiation therapy, hormone therapy, and immunotherapy, has gained widespread clinical approval [4]. Each approach possesses distinct advantages and limitations, which is tailored to the specific conditions of individual patients and the capabilities of corresponding healthcare facilities. Notably, the evolving realm of immune

checkpoint inhibitors (ICIs) signifies a promising and swiftly advancing avenue in cancer therapeutics, experiencing a surge in both research and clinical development [5].

It is obvious that the immune system within the human body serves as a fundamental force in managing and eradicating cancer cells. However, through distinct mechanisms, these cells have evolved strategies to avoid immune surveillance and maintain their uncontrolled proliferation [6]. Among these pathways, immune checkpoint control has been demonstrated as a key tactic employed by cancer cells to evade immune scrutiny [7]. Structurally, immune checkpoints are inherently protein-based, and their function can be likened to "brakes" that restrain the activity of T cells [8]. Through the utilization of immune checkpoint inhibitors (ICIs), the blockade of immune checkpoint control enables the activation of T cells, empowering them to recognize, target, and eradicate cancer cells. Currently, three immune checkpoints, namely cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed death-ligand 1 (PD-L1), are targeted to 'unlock' T cells [9]. As a result, ICIs are classified into three main groups based on their respective targets, which are the aforementioned immune checkpoints. Among them, CTLA-4 and PD-1 are immune checkpoints located on the surface of T cells, whereas PD-L1 is expressed on the surface of cancer cells and, upon binding to PD-1, activates this protein [10].

In the ICIs group, the anti-CTLA-4 and anti-PD-1 antibodies have ushered in a revolution in the management of numerous cancer types. Specifically, in cases of longstanding malignant tumors or those exhibiting tumor regression, nearly 50% of patients have experienced recovery and control of the tumor mass, a remarkable increase from the previous rate of approximately 10% [11]. Furthermore, statistics reveal that nearly half of the metastatic cancer patients in high-income countries are being treated with these drugs above, and the proportion of patients showing favorable responses to ICIs is high [12].

However, current clinical reports also highlight that, despite their effectiveness and safety ICIs can induce significant side effects, including conditions like carditis, pneumonitis, pruritus, rash, dermatitis, erythema, diarrhea, and colitis [13,14]. Furthermore, recent research suggests that resistance to ICIs is becoming a concern. Several conditions are no longer exhibiting the favorable responses seen with previously effective ICI treatments [15–17]. This underscores the need for ongoing development of new drugs that employ immune checkpoint inhibition mechanisms. When it comes to CTLA-4, tremelimumab (Imjudo®) is a designated antibody used in cases of hepatocellular carcinoma and non-small cell lung cancer. It is known for its high tolerability and clinical effectiveness [18, 19]. Pembrolizumab (Keytruda®), a PD-1 antibody, is employed in non-small cell lung cancer treatment, showing a response rate of 25%. In the context of refractory malignancies, pembrolizumab demonstrates response rates of up to 34% [20]. Both antibodies hold great promise and efficacy in the field of cancer treatment. However, clinical resistance to tremelizumab treatment in non-small cell lung cancer patients has been linked to functional STK11 mutations, and the study underscores that this resistance can be effectively reversed through STAT3 knockdown [21]. Moreover, researchers have explored the effectiveness of OMTX705, a novel antibody-drug conjugate targeting FAP, in solid tumor models resistant to pembrolizumab [22]. Apart from the significant adverse reactions associated with antibodies in cancer treatment, the persistent resistance observed with tremelizumab and pembrolizumab presents an ongoing challenge in the quest for new monoclonal antibodies capable of adapting to the evolving landscape of cancer mutations.

Therefore, this study focuses on the development and identification of novel antibody structures targeting two immune checkpoints, CTLA-4 and PD-1, which are located on the surface of T cells. These new antibodies are constructed to optimize the binding affinity between antibodies and their target antigens, with the ultimate goal of enhancing treatment efficacy, minimizing side effects, and addressing drug resistance.

2. Materials and methods

2.1. Generating and preparing the structures

In this study, two antibody structures belonging to the group of immune checkpoint inhibitors, tremelimumab - a CTLA-4 antibody, and pembrolizumab - a PD-1 antibody, will be utilized to predict and design new antibodies with the variable fragment form (Fv).

The antibody and antigen structures, including tremelimumab, pembrolizumab, CTLA-4 protein, and PD-1 protein, were downloaded from the RCSB PDB (https://www.rcsb.org/) using the Maestro Bioluminate software. The obtained structures seuqntially underwent through automated preparation employing the Protein Preparation Wizard method. This process was conducted to add hydrogen atoms to the proteins, form bonds such as H-bonds and disulfide bridges, complete missing loop regions and other missing atoms, and remove water molecules [23, 24]. The Protein Preparation Wizard was designed to ensure structural accuracy from the project's beginning, providing a reliable structure suitable for a wide range of modeling applications.

Once the preparation was complete, the protein structures were validated using the Protein Reliability Report, ensuring that they met certain criteria. These criteria included a resolution of ≤ 2.0 Å, absence of missing loops or atoms, and minimal steric clashes if present [25]. This validation step is crucial to ensure the reliability and quality of the protein structures before proceeding with further calculations or analyses.

2.2. Antibody Structure Prediction

Utilizing the Antibody Structure Prediction tool, new antibody structures were proposed, drawing from the diverse array of existing antibodies available in the PDB database during the research period. Predictions were made for the variable light chain (VL) and variable heavy chain (VH) sequences of both antibodies, which together formed the variable fragment (Fv) in the immunoglobulin domains. These novel antibodies were required to meet certain criteria, including a resolution of ≤ 2.0 Å, high similarity to the original antibody, absence of antigen binding, and human origin [26]. This tool utilized computational algorithms and modeling techniques to generate these proposed antibody structures, ensuring their feasibility and adherence to the specified requirements.

2.3. Antibody-Antigen Docking

After the prediction, the newly designed antibodies underwent docking process with their corresponding antigens using the Protein-Protein Docking tool [27]. Protein-Protein Docking is a computational tool that allows the simulation of the binding between a protein and another protein, enabling the exploration of possible binding orientations between the antibody and the antigen. To assess the potential interactions between the proteins in the study, each antibody-antigen pair was predicted in 30 different binding poses.

Subsequently, the Interaction Fingerprints tool was employed to analyze the types of interactions and amino acids involved in the binding. This analysis helped identify crucial interactions and key amino acid residues that contributed to the binding process. By examining the interaction fingerprints, researchers were able to gain insights into the specific molecular interactions between the antibody and antigen, providing valuable information for further optimization and design of therapeutic antibodies.

Then, the interaction between antibodies and their corresponding antigens was analyzed using the Protein Interaction Analysis method. This tool supports the vizualization of the interactions in 2D and 3D formats, facilitating the analysis of crucial information such as inter-residue distances, hydrogen bonds, salt bridges, pi-pi stacking, and more. Protein Interaction Analysis enables researchers to gain a deeper understanding of the structure and interactions within the antibody-antigen system. The information derived from this analysis helps to identify significant interactions, assess the stability of the

interactions, and provide insights for optimizing the design of antibodies and antigens in biomedical research.

2.4. Computational Alanine Scanning and Affinity Maturation

The Alanine Scanning Mutagenesis method was employed to analyze the important amino acids of the antibody in its interaction with the antigen and identify potentially mutatable positions to optimize the antibody's affinity.

The principle of this method involves systematically mutating each amino acid within the antibody's complementarity-determining regions (CDRs) to alanine, a simple and physicochemically inert amino acid, and calculating the resulting change in energy or $\Delta\Delta E$ [28]. Once $\Delta\Delta E > 0$ kcal/mol, the amino acid before mutation plays a crucial role in the antibody-antigen interaction and mutating it to alanine increases the energy of the complex. Therefore, these amino acids should not be mutated. Conversely, when $\Delta\Delta E < 0$ kcal/mol, it suggests that the original amino acid decreases the affinity of the antibody. In such cases, these amino acids can be mutated into other potential amino acids to improve the affinity and energy between the antibody and the corresponding antigen. Subsequently, if residues were designated to change to other amino acids, and $\Delta\Delta E$ would be evaluated again to identify mutations that enhanced the antibody's affinity and created a more stable antigen-antibody complex. In this study, a mutation was considered significant if the $|\Delta\Delta E| \geq 3$ kcal/mol [29].

Following this principle, the research employed the Alanine Scanning method to analyze and introduce mutations into the predicted antibodies, aiming to identify mutations that optimize energy interactions with the target antigens. Additionally, Alanine Scanning was applied to tremelimumab and pembrolizumab to assess the feasibility of mutations on tremelimumab and pembrolizumab, considering whether any mutations of the two parent antibodies had significance with $\Delta\Delta E < -3$ kcal/mol.

3. Results

3.1. Protein structure preparation and evaluation of complex interactions

After screening the protein crystal structures in the RCSB PDB, two proteins were selected: the complex of CTLA-4 with tremelimumab (PDB ID: 5GGV) and the complex of PD-1 with pembrolizumab (PDB ID: 5GGS). Following the preparation using the Protein Preparation Wizard, the protein structures of both complexes were examined using the Protein Reliability Report.

The results indicated that both crystal structures of tremelimumab – CTLA-4 (PDB ID: 5GGV) and pembrolizumab – PD-1 (PDB ID: 5GGV) met the set requirements, with detailed outcomes presented in Table 1. From these structures, the binding capabilities of tremelimumab with CTLA-4 and pembrolizumab with PD-1 were evaluated using the Protein Interaction Analysis tool. Based on the Protein Interaction Analysis result shown in Figure 1, it can be observed that the main interactions facilitating the binding of tremelimumab and pembrolizumab to CTLA-4 and PD-1, respectively, were hydrogen bonds. This type of interaction contributed to 21 out of the total 24 analyzed interactions. Additionally, two other types of interactions, $\pi - \pi$ stacking, and salt bridges, were also present.

Tab. 1: Protein parameters after preparation.

	Tremelimumab and CTLA-4 (PDB: 5GGV)	Pembrolizumab and PD-1 (PDB : 5GGS)
Resolution (Å)	2.00	2.00
Steric Clashes	0 clashes	0 clashes
Missing loops	0 missing	0 missing
Missing atoms	0 missing	0 missing

The results of Protein Interaction Analysis only provided information about the interactions without estimating the energy aspects of the complexes. Therefore, it was unable to give a definitive confirmation whether the identified residues were crucial for the interaction between

antibodies and antigens. This would be further demonstrated through other methods. Nevertheless, these results had an impact on guiding further calculations and comparing them with the predicted binding abilities of newly designed antibodies.

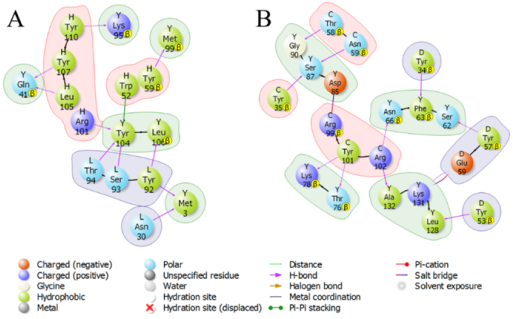

Fig. 1: 2D interaction mapping between tremelimumab and CTLA-4 (PDB ID: 5GGV) (A); pembrolizumab and PD-1 (PDB ID: 5GGS) (B). Antigen residues were covered in green regions, while heavy and light chain residues of the antibodies were covered in red and blue regions, respectively.

3.2. Alanine Scanning for tremelimumab and pembrolizumab

The Alanine Scanning method was applied to the tremelimumab – CTLA-4 and pembrolizumab – PD-1 complexes to evaluate the potential for mutations on these two antibodies to enhance their binding affinities to the respective antigens. Figure 2 showed that no changes in the amino acid composition of the parent antibodies resulted in $\Delta\Delta E$ values less than -3 kcal/mol. Based on the chart below and Supplementary Information 1, the most potential mutation site on tremelimumab and pembrolizumab were H:Asp54 with $\Delta\Delta E = -1.56$ kcal/mol and C:Asp104 with $\Delta\Delta E = -2.7$ kcal/mol, respectively. However, both values were greater than the standard set for a potential mutation site, which is $\Delta\Delta E \leq -3.0$ kcal/mol. Consequently, these mutations failed to significantly enhance affinity for the respective antigens. Given the limitations in optimizing binding based on tremelimumab and pembrolizumab, the study suggests the utilization of the Antibody Prediction tool to identify novel antibodies sharing characteristics with the parent antibodies. Subsequently, mutations were carried out to identify antibodies forming complexes with the lowest and most stable energy levels concerning the corresponding antigens.

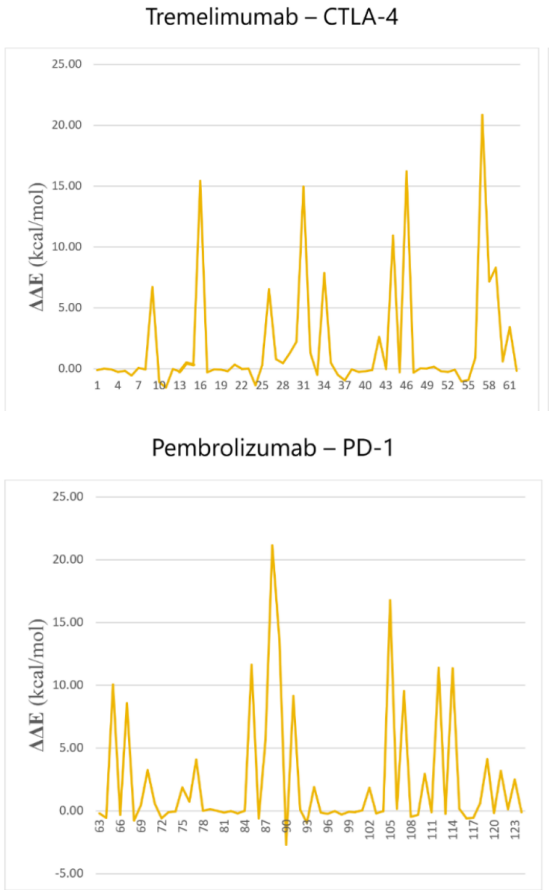

Fig. 2: Alanine Scanning results for tremelimumab – CTLA-4 (1-62) and pembrolizumab – PD-1 (63-124) complexes: Variants (horizontal axis), $\Delta\Delta E$ (vertical axis). Detailed results of the Alanine Scanning are presented in Supplementary Information 1.

3.3. Antibody Structure Prediction

Based on the original structure of tremelimumab and pembrolizumab, the Antibody Structure Prediction tool was utilized to investigate and

analyze structurally similar antibodies with potential affinity for the target receptors. Among the generated antibody structures, two particularly promising candidates, designated as **1** and **2**, were identified for targeting the antigens CTLA-4 and PD-1, respectively. Detailed information regarding the predicted antibodies is presented in *Supplementary Information 2 and 3*. Structures **1** and **2** were built based on the respective protein structures PDB 7K8O and 4JDV. Comprehensive information regarding these proteins is presented in Table 2.

Tab. 2: Information regarding antibodies **1** and **2** was predicted using the Antibody Structure Prediction.

Antibody	PDB ID	PDB resolution	Composite score*	Antigen Type	Species
1	7K8O	1.95	0.989	-	Homo sapiens
2	4JDV	1.65	0.941	-	Homo sapiens

* Calculated based on the corresponding original proteins, in this case, the original protein for **1** is tremelizumab with PDB ID 5GGV, and for **2** is pembrolizumab with PDB ID 5GGS.

From the information in Table 2, it can be seen that both antibodies **1** and **2** met all the specified criteria, including a resolution of $\leq$ 2.0 Å, high similarity to the original antibody – as indicated by a composite score with a maximum value of 1.0, the absence of antigen binding, and being of human origin. Therefore, these structures were deemed suitable for subsequent computational calculations. Detailed illustration of the 3D structures and amino acid sequences of **1** and **2** was presented in Figure 3.

3.4. Antibody-Antigen Docking

Antibodies **1** and **2** were docked with their respective targets, CTLA-4 and PD-1, using the Protein-Protein Docking tool, which resulted in thirty binding poses for each complex. The most stable binding pose for each complex was selected for subsequent investigation and evaluation. The docking results include 3D surface

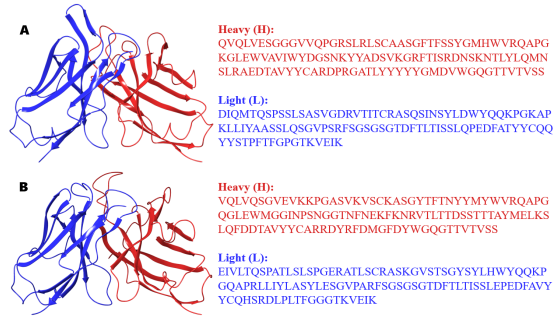

Fig. 3: Homology model of antibodies **1** (A) and **2** (B) with VH (red) and VL (blue) domains. The corresponding VH and VL sequences for each antibody are provided on the right-hand side.

modeling of the antibody-antigen complexes, 2D binding poses, and Interaction Fingerprints analysis of the formed complexes were repesented in Figures 4,5,6, and 7.

3.5. Computational Alanine Scanning and affinity maturation

Alanine Scanning

The results of the Alanine Scanning shown in Figure 8 identified three potential mutation positions with $\Delta\Delta E$ values below -3 kcal/mol. Detailed information about these three residues was provided in Table 3. These sites underwent mutation using various amino acids to identify mutations with the lowest energy values.

Tab. 3: Potential mutation residues at specific positions.

Variant	Antibody	Residue	Parent	$\Delta\Delta E$ (kcal/mol)
54	**1**	L:91	Tyr	-3.0
65	**2**	H:31	Asn	-12.6
89	**2**	H:96	Asp	-9.0

Affinity maturation of antibodies 1 and 2

After identifying the potential mutation positions on antibodies **1** and **2** to enhance their binding affinity with their respective antigens,

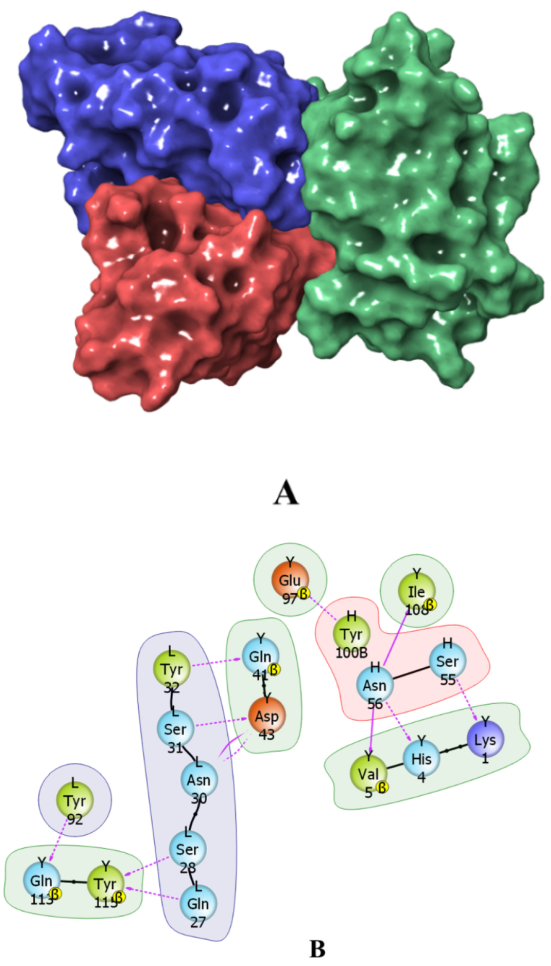

Fig. 4: Docking results of the most stable poses between antibody **1** and CTLA-4. (A) Visualization of the 3D surface structure of the antigen-antibody complex. Antibody **1** is depicted in red (VL) and blue (VH) surface, while CTLA-4 is depicted in green surface. (B) 2D interaction mapping between antibody **1** and CTLA-4, antigen residues were covered in green regions, while heavy and light chain residues of the antibody **1** were covered in red and blue regions, respectively.

the amino acids at these positions were designated to mutate into other amino acids. This mutation aimed to generate variants with the lowest energy complex formation. The results of the energy change analysis upon mutating the amino acids are present in Figure 9.

The results of the mutations performed at the potential sites generated multiple new antibod-

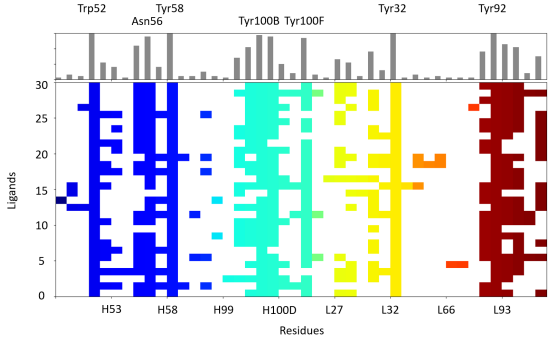

Fig. 5: Interaction Fingerprints analysis results of thirty complexes formed by antibody **1** and CTLA-4. The grey chart above illustrates the participation ratio of corresponding residues in the interactions. The colored chart below analyzes the occurrence of interactions by residues (x-axis) in each predicted pose (y-axis). Residue-specific column filling reflecting increased interaction participation across poses.

ies with an improved ability to form antigen-antibody complexes at lower energy levels. Specifically, the variants with the lowest energy values corresponding to each residue position are presented in Table 4.

Tab. 4: Variants with the lowest energy values correspond to each mutated residue.

Variant	Antibody	Residue	Parent	Mutation	$\Delta\Delta E$ (kcal/mol)
142	**1**	L:91	Tyr	Lys	-4.7
166	**2**	H:31	Asn	Ser	-14.61
189	**2**	H:96	Asp	Tyr	-21.08

4. Discussion

4.1. Antibody-Antigen Docking

Regarding antibody **1** and its interaction with CTLA-4, the docking results in Figure 4 demonstrated that in the best binding pose, antibody **1** formed twelve hydrogen bonds with the CTLA-4 target, which was notably higher compared to that of tremelimumab (PDB ID: 5GGV). This indicated the strong binding potential of antibody **1** to CTLA-4. Furthermore, the Interaction Fingerprint analysis in Figure 5 revealed that several amino acids in antibody **1** had the

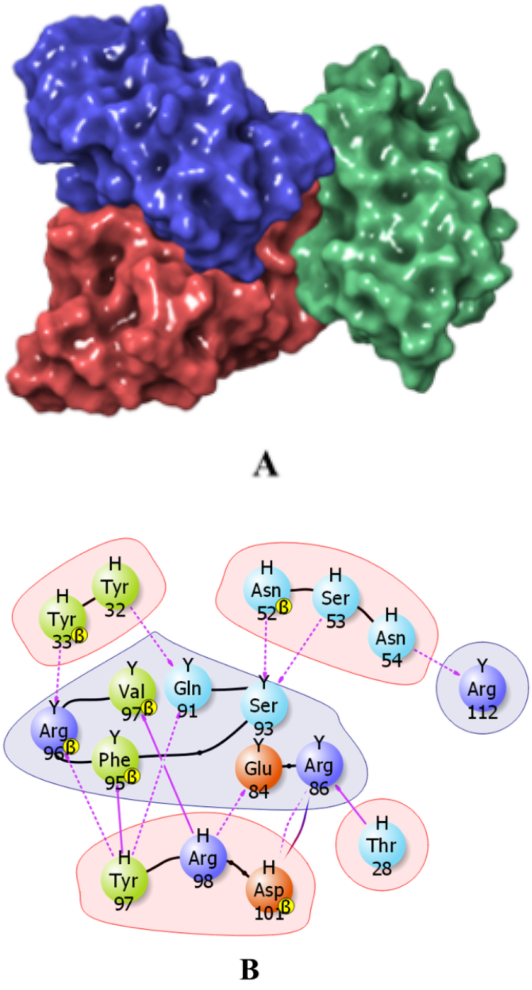

Fig. 6: Docking results of the most stable poses between antibody **2** and CTLA-4. (A) Visualization of the 3D surface structure of the antigen-antibody complex. Antibody **1** is depicted in red (VL) and blue (VH) surface, while CTLA-4 is depicted in green surface. (B) 2D interaction mapping between antibody **1** and CTLA-4, antigen residues were covered in blue regions, while heavy chain residues of the antibody **2** were covered in red regions.

potential to interact with CTLA-4. Remarkably, specific residues consistently appeared across thirty predicted poses, including Trp52, Asn56, Tyr58, Tyr100B, and Tyr100F from the heavy chain, as well as Tyr32 and Tyr92 from the light chain. These residues played a significant role in enhancing the binding affinity between antibody **1** and CTLA-4.

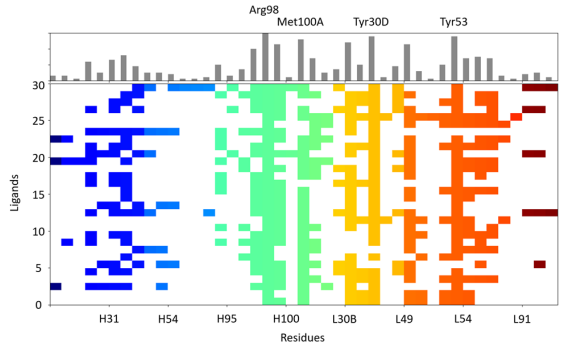

Fig. 7: Interaction Fingerprints analysis results of thirty complexes formed by antibody **2** and PD-1. The grey chart above illustrates the participation ratio of corresponding residues in the interactions. The colored chart below analyzes the occurrence of interactions by residues (x-axis) in each predicted pose (y-axis). Residue-specific column filling reflecting increased interaction participation across poses.

Concerning antibody **2** and its interaction with PD-1, the docking results in Figure 6 revealed that in the most stable configuration, antibody **2** engages with the PD-1 antigen through thirteen interactions. These interactions included twelve hydrogen bonds and one salt bridge. This count exceeded the number of interactions in the initial complex involving pembrolizumab found in protein PDB ID 5GGS, demonstrating the potential for a stronger binding affinity of antibody **2**. Furthermore, the Interaction Fingerprint analysis, presented in Figure 7, showcased the binding capacity of antibody **2** to the target through 43 distinct residues. Among these residues, certain ones exhibited high involvement across the thirty predicted poses. Notably, Arg98 and Met100A in the heavy chain, along with Tyr30D and Tyr53 in the light chain, played prominent roles in these interactions, contributing to the enhanced binding affinity between antibody **2** and PD-1.

In the most stable pose shown in Figure 6, it was noteworthy that, except for H:Arg98, the amino acids of antibody **2** involved in the interaction with PD-1 had a low occurrence rate in the remaining 29 poses, and all amino acids involved in the interaction initiated from the heavy chain. However, as can be seen in Figure 7, residues from the light chain possessed a higher participation rate in the interaction

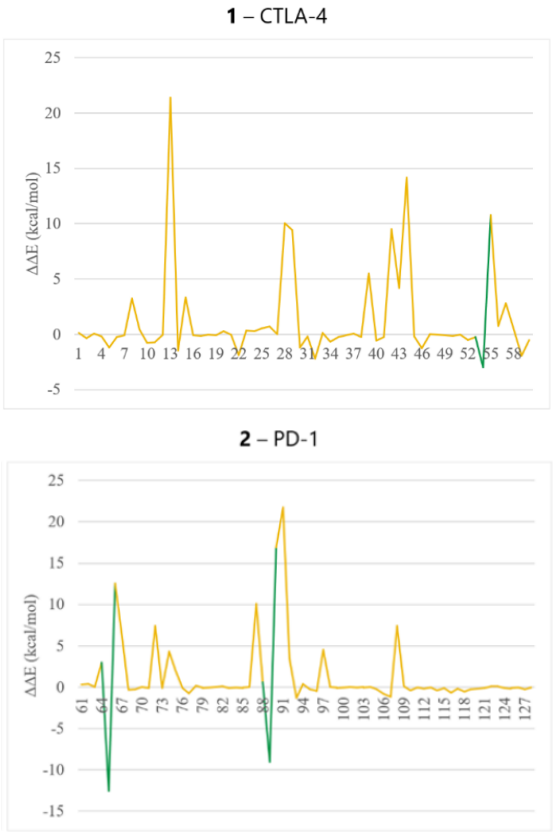

Fig. 8: Alanine Scanning results for the antibody **1** – CTLA-4 (1-60) and antibody **2** – PD-1 (61-128) complexes: Variants (horizontal axis), $\Delta\Delta E$ (vertical axis), green lines represent mutations with $\Delta\Delta E$ less than -3 kcal/mol (Detailed results of the Alanine Scanning are presented in *Supplementary Information 3)*.

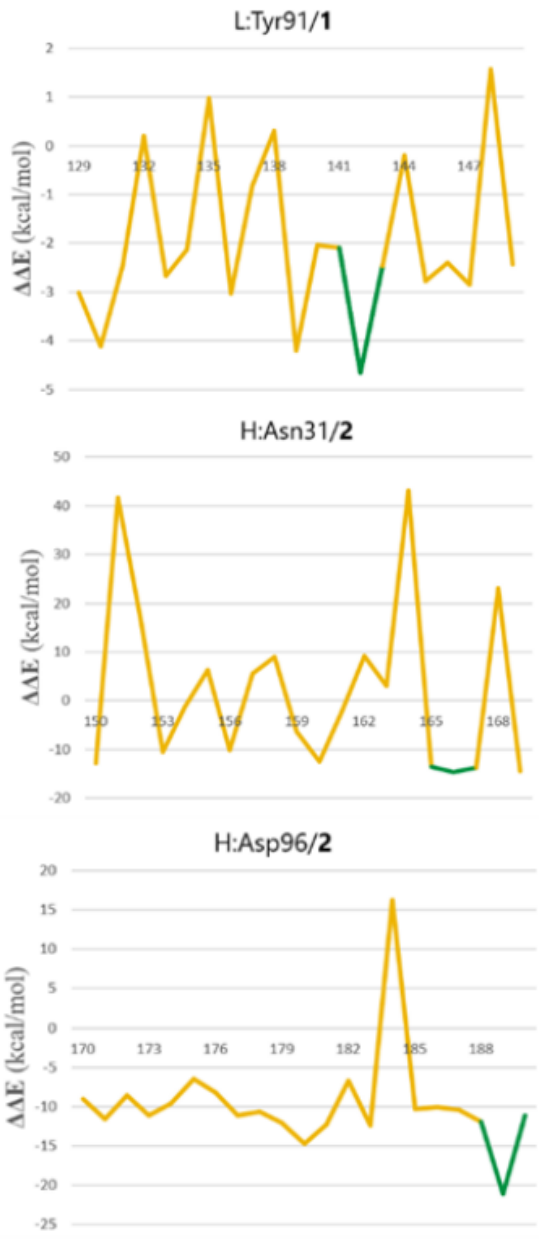

Fig. 9: The mutation results at potential residues L:Tyr91 (129-149), H:Asn31 (150-169), and H:Asp96 (170-190): Variants (horizontal axis), delta delta energy (vertical axis), green lines represent mutations with the lowest $\Delta\Delta E$ at each mutation potential residue (Detailed results of the mutation at three potential residues are presented in *Supplementary Information 4*).

with PD-1 compared to residues from the heavy chain. It could be explained that the light chain of antibody **2** exhibited various binding poses with the target, while the heavy chain contributed to stabilizing the interaction of this antibody with PD-1 protein.

The Protein-Protein Docking results demonstrated that both antibodies **1** and **2** exhibited strong binding affinity towards their respective antigens, CTLA-4 and PD-1. These antibodies effectively interacted with their target proteins similar to the original antibodies, with hydrogen bonds playing a crucial role in the antigen-antibody interaction. The observed exceptional binding affinity provided a foundation for conducting mutations aimed at identifying novel antibodies with enhanced potency.

4.2. Computational Alanine Scanning and Affinity Maturation

Computaional Alanine Scanning

In addition to identifying potential mutation sites, the results of the Alanine Scanning indicated the importance of the amino acids involved in the analyzed interactions mentioned in discussion of Antibody-Antigen docking. Based on the Comprehensive Alanine Scanning results on antibodies **1** and **2** (Supplementary Information 3), most of the amino acids involved in the most stable pose of both antibody**1** and antibody **2** contribute to the reduction of complex energy. For instance, considering two residues H:Asn56 and L:Tyr32 within antibody **1** – these two residues interact with the CTLA-4 antigen. The substitution of these two amino acids with alanine increased the energy of the complex by 21.38 kcal/mol and 12.52 kcal/mol, respectively, indicating a significant decrease in the stability of the antibody-antigen complex. Additionally, the amino acids that were frequently observed in the Interaction Fingerprints analysis also contributed to the stability of the antigen-antibody complexes.

Affinity Maturation

Variant 142, named antibody **1a** , introducing a mutation from Tyr to Lys at residue L:91 in antibody **1** resulted in a substantial decrease in the complex's energy by -4.7 kcal/mol, significantly augmenting the antibody's binding affinity to the CTLA-4 target. Regarding the PD-1 target, the two most promising antibodies associated with each mutated residue were denoted as **2a** and **2b** , corresponding to variant 166 and variant 189, respectively. Notably, antibody 2b demonstrated a notably lower free energy value on the PD-1 target (-21.08 kcal/mol compared to -14.61 kcal/mol), indicating its potential to enhance the binding to immune checkpoint proteins. It was evident that all mutated antibodies, **1a**, **2a** and **2b**, demonstrated the potential to enhance binding affinity on their respective immune checkpoint targets, with antibody 1a targeting the CTLA-4 antigen, antibodies **2a** and **2b** targeting the PD-1 antigen. Figure 10 presents the detailed presentations of the 3D structures and amino acid sequences of **1a**, **2a** and **2b** for further visualization.

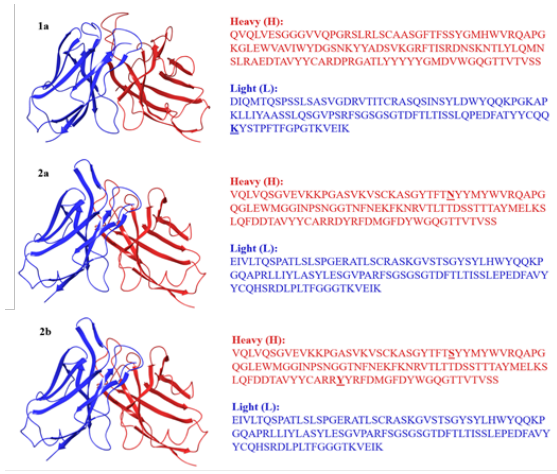

Fig. 10: Homology model of antibodies **1a**, **2a** and **2b** with VH (red) and VL (blue) domains. The corresponding VH and VL sequences for each antibody were provided on the right-hand side, the amino acids that undergo substitution mutations compared to the original antibodies are highlighted in bold and underlined.

5. Conclusions

The study aimed to optimize the binding affinity of monoclonal antibodies on immune checkpoints in cancer treatment. Three novel antibody structures, designated as **1a**, **2a** and **2b**, were proposed. These antibodies exhibited strong affinity towards CTLA-4 (**1a**) and PD-1 antigens (**2a**,**2b**), forming a durable antigen-antibody complex. By enhancing the binding capacity and durability toward their targets, the therapeutic activity of these antibodies in cancer treatment is expected to be improved. This can potentially enhance the patient's response to the therapy, ultimately leading to improved health and extended survival.

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