

## Immune profile score of modified Tremelimumab with CTLA-4 could enhance immunotherapy using molecular docking

Najla A. Ieban <sup>1</sup>  , Halima A. Gbaj <sup>2</sup>  , Mohamed A. Gbaj <sup>3</sup>    
Anton Hermann <sup>4</sup>  , and Abdul M. Gbaj <sup>1\*</sup>  

<sup>1</sup> Department of Medicinal Chemistry, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya

<sup>2</sup> Department of Microbiology and Immunology, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya

<sup>3</sup> Department of Chemical Engineering, Faculty of Engineering, University of Tripoli, Tripoli, Libya

<sup>4</sup> Department of Biosciences, University of Salzburg, Salzburg, Austria

\* Author to whom correspondence should be addressed

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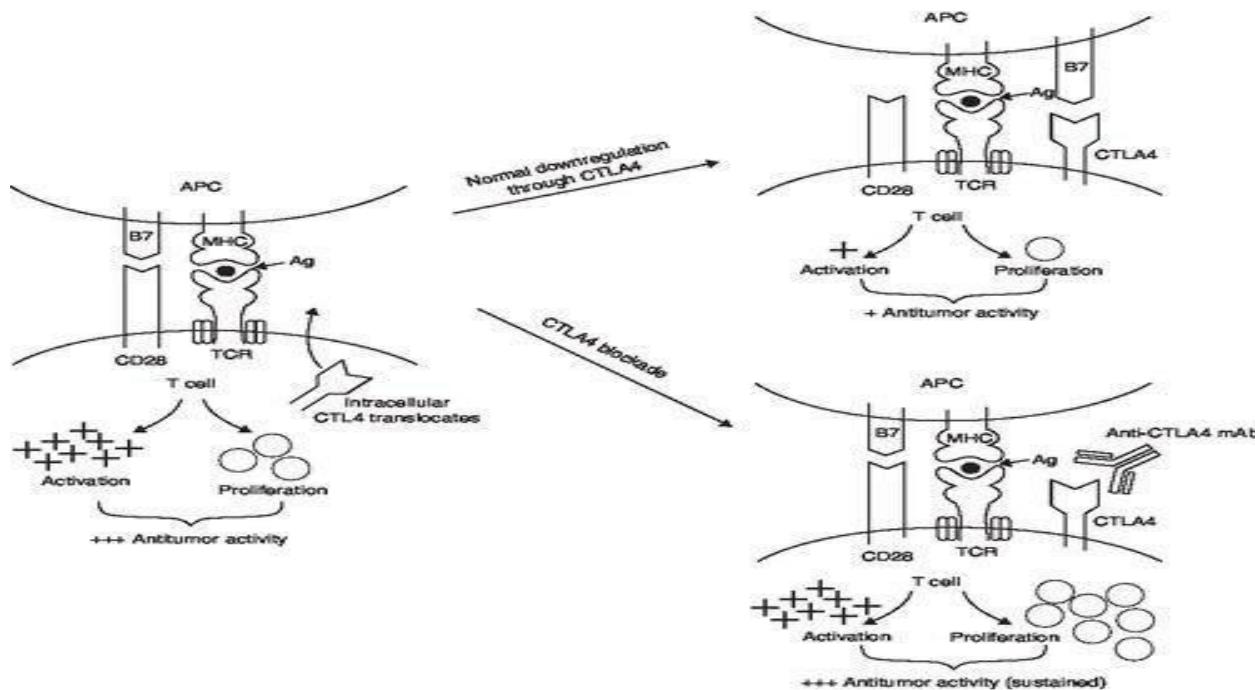
**Abstract:** Cancer remains a leading cause of death worldwide and arises from genetic and epigenetic alterations that disrupt normal cellular regulation. Immune checkpoint pathways, particularly those involving CTLA-4, play a crucial role in suppressing antitumor immune responses. Immune checkpoint inhibitors such as Tremelimumab have revolutionized cancer therapy by restoring T-cell activity. Improving antibody-receptor interactions through molecular modification may further enhance therapeutic outcomes. Computational approaches such as molecular docking offer valuable tools for studying protein-protein interactions and guiding antibody optimization. The three-dimensional structure of the CTLA-4 receptor was obtained from the Protein Data Bank and prepared through energy minimization. The Tremelimumab antibody was modeled and modified at selected binding regions. Molecular docking simulations were performed to evaluate the interactions between the antibody and receptor. Docking complexes were analyzed for binding affinity, hydrogen bonding, and hydrophobic interactions using molecular visualization tools. Docking results demonstrated stable binding between modified Tremelimumab and CTLA-4. Certain modifications resulted in improved binding affinity, indicated by lower docking energy scores and enhanced interaction networks. Other modifications negatively affected binding due to steric hindrance or loss of key interactions. The study highlights the importance of antibody structure in determining immune checkpoint binding efficiency. Enhanced binding affinity may improve CTLA-4 blockade and strengthen antitumor immune responses. Molecular docking proved effective in predicting interaction patterns, though experimental validation is necessary to confirm biological relevance. This study aims to demonstrate that molecular modification of Tremelimumab can influence its interaction with CTLA-4.

### Introduction

Cancer is considered as one of the major global health problems due to its notable social and economic burden [1]. Cancer is defined as a wide range of diseases characterized by the loss of normal cellular regulation, leading to uncontrolled cell proliferation, evasion of programmed cell death, and the ability to invade surrounding tissues

and metastasize to distant organs [2]. Immunotherapy for cancer, often referred to as biological cancer treatment, involves the strategic modulation of the patient's immune responses to identify and eliminate malignant cells. Rather than relying solely on external therapeutic interventions, this approach emphasizes the development of agents that stimulate or amplify the immune system's ability to detect and eradicate neoplastic cells [3, 4]. The objective of the method is to enhance immunological responses, enabling the body to generate a more robust and targeted defense against tumor progression [5]. One of these approaches targets immune checkpoints directly, specifically by blocking the interactions between cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or programmed cell death protein 1 and their respective ligands, B7 or PD-L1 [5]. CTLA4 (CD152) is a receptor found on the surface of activated T cells [6]. For a T cell to be fully activated, it needs two signals: First, a T cell receptor recognizing an antigen presented by the major histocompatibility complex on antigen-presenting cells (APCs), and second, the interaction between CD28 on the T cell and B7 family proteins (like B7.1/CD80, B7.2/CD86, B7-H3, and B7-H4) on the APCs. After the T cell is activated, it starts to express more CTLA4 on its surface. CTLA4 is similar to CD28, but it binds more strongly to B7 molecules. Because of this stronger binding, CTLA4 takes over and blocks CD28 from connecting with B7, which stops the second activation signal and reduces T cell activity [7].

Tremelimumab (previously known as ticilimumab) is a fully human monoclonal antibody of the IgG type [6], composed of two identical heavy chains and two identical light chains, connected by disulfide bonds, targeting CTLA-4 receptors, proteins found on T cells, also helping in boosting interleukin-2 (IL-2) activity. Pfizer company developed this antibody as a cancer treatment, originally created using XenoMouse technology from Abgenix (now part of Amgen) [6]. Tremelimumab is used intravenously with durvalumab, in a STRIDE regimen (Single Tremelimumab Regular Interval Durvalumab) [8] when Tremelimumab is combined with durvalumab platinum-based chemotherapy is considered as first-line treatment for metastatic non-small lung cancer (mNSCLC) [9]. Monoclonal antibodies (mAbs) that target CTLA4 can block it from binding with B7 molecules on antigen-presenting cells (APCs). This stops the usual downregulation of T cell activity (**Figure 1**).



**Figure 1:** Blockade of cytotoxic T lymphocyte-associated antigen 4 (CTLA4) can prolong T-cell activation

Ag: Antigen; APC: Antigen-presenting cell; CTLA4: Cytotoxic T lymphocyte-associated antigen 4; mAb: Monoclonal antibody; MHC: Major histocompatibility complex; TCR: T-cell receptor [7]

When the CTLA4-B7 signal is blocked, T cells stay active for longer and work better. This can be observed through higher levels of cytokines like IL-2, IFN- $\gamma$ , IL-3, IL-4, IL-5, and IL-10. Researchers have tested this idea in mouse models using antibodies against mouse CTLA4, and the results showed stronger T cell responses and more efficient elimination of solid tumors like fibrosarcoma, colon cancer, and prostate cancer [7]. The aim of this study is to examine the binding interaction between Tremelimumab and the CTLA4 receptor through molecular docking methods. In addition, this research explores how specific modifications in the amino acid sequence of Tremelimumab may alter its binding affinity. By analyzing both the original and altered sequences of Tremelimumab, this study attempts to better understand how minor adjustments at the molecular level can influence antibody function. It is hoped that the results will offer valuable information for future improvements in immune-based therapies for cancer.

## Materials and methods

*Molecular modeling tools:* Tremelimumab and the CTLA4 receptor were obtained from PDB [10]. Visualized and modified via PyMOL [11], and PDBsum were used to illustrate the residues responsible for interaction between the receptor and ligand [12, 13].

*Molecular docking:* The receptor and the ligand were excluded from water molecules, the identical chains from the receptor and the ligand, leaving a single chain for the receptor and a single arm for the ligand. The hydrogen atoms were added, the ligand was modified at residues crucial for binding to the receptor, and at the end, the file was saved in PDB form. The docking process is done by Cluspro 2.0 server [14, 15].

*Antibody developability:* The allergenicity is predicted based on the amino acid sequence of protein via AlgPred [16]. Antigenic Peptide Prediction Tool - Immunomedicine Group is a tool designed to identify potential antigenic regions within protein sequences [17]. While the protparam tool is used to estimate some of the physicochemical properties like the half-life and instability index [18]. And finally, the TAP -Therapeutic Antibody Profiler - is an interactive molecular viewer allowing users to explore surface features such as hydrophobicity, charge distribution, and potential sequence liabilities. Additionally, it offers an estimate of the model's quality to support result interpretation, and it automatically identifies the canonical forms of all non-CDRH3 loops [19].

## Results and discussion

A comprehensive analysis was conducted to evaluate docking results, binding patterns, including hydrogen bonds, non-bonded contacts and salt bridges. This analysis was extended to assess some crucial aspects of antibody developability, i.e. its predicted allergenicity, immunogenicity/antigenicity, stability, half-life, and its therapeutic profile. **Table 1** shows molecular docking results conducted via ClusPro, to investigate the intricate effects of sequential and cumulative alteration on the binding affinity of a protein complex. The analysis exclusively concentrated on Cluster 0 for all analyses, a decision driven by its consistent representation of the highest number of members across all docking results. This study involves a direct comparison between the binding affinity of the wild-type protein (Non-Modified (STD)) and a set of modifications that were introduced step by step. The binding affinity was measured in kcal/mol, where more negative values indicate a stronger and more stable interaction between molecules. The analysis was started by the wild-type protein STD with a binding affinity of (-766.5 kcal/mol). The first pair of modifications were tyrosine (Tyr) in position 53, replaced with arginine (Arg) in chain H, and serine (Ser) in position 93 replaced with Isoleucine (Ile) indicating a marked improvement in the binding affinity of -944.3 kcal/mol. However, the next two pairs of modifications show notable deteriorations in their binding affinity. The glycine (Gly102) changed to cystine (Cys) in chain H and tyrosine (Tyr92) changed to phenylalanine (Phe) in chain L reducing the binding affinity to (701.2 kcal/mol). Tyrosine (Tyr107) replaced with

tryptophan (Trp) in chain H and tyrosine (Tyr32) replaced with arginine (Arg) in chain L resulted in a binding affinity of -764.7 kcal/mol. In contrast to the previous two pairs of modifications, the remaining substitutions improved the binding affinity; however, the last set of substitutions shows the best binding affinity of (-945.9 kcal/mol) among all sets.

**Table 1:** Molecular docking results obtained using ClusPro, showing the effect of sequential amino-acid substitutions on protein-protein binding affinity.

Cluster	Members	Modification		Center score kcal/mol
		Chain H	Chain L	
0	118	No Modifications (STD)		- 766.5
0	170	Tyr53 to Arg	Ser93 to Ile	- 944.3
0	116	Gly102 to Cys	Tyr92 to Phe	- 701.2
0	107	Tyr107 to Trp	Tyr32 to Arg	- 764.7
0	099	Asn57 to Met	Thr94 to Arg	- 816.4
0	109	Tyr106 to Lys	Asn30 to Gln	- 825.2
0	105	Lue105 to His	Tyr91 to Phe	- 862.8
0	125	Tyr110 to Phe	Ser31 to Gln	- 867.8
0	123	Tyr108 to Phe	Gln30 to Ile	- 903.9
0	142	Trp52 to Phe	Arg32 to His	- 815.5
0	116	Cys101to val50	Ala50 to Val	- 945.9

Analysis was limited to Cluster 0, which consistently contained the highest number of members. Binding affinities are reported in kcal/mol, with more negative values indicating stronger interactions. Compared with the wild-type protein (STD; - 766.5 kcal/mol), initial substitutions enhanced binding affinity, whereas intermediate modifications reduced it. Subsequent substitutions improved binding, with the final modification set exhibiting the strongest affinity (- 945.9 kcal/mol)

*Positive impacts (improved affinity):* A study introducing mutations across CDRs of 21 monoclonal antibodies (mAbs) found that 38 substitutions in 21 CDR positions improved binding affinity by up to 870-fold. For example, replacing Gly102 with Cys in CDR-H3 optimized hydrophobic interactions, leading to stronger antigen binding [20, 21]. Introducing a YSLLL-motif in the CDR3 loop of shark IgNAR antibodies improved affinity by 10-fold due to slower off-rates [22].

*Negative impact (reduced affinity):* In cetuximab Fab complexes, replacing hydrophobic residues (Phe3→His) reduced affinity by disrupting hydrophobic packing [23]. Substituting arginine with citrulline (neutral) at positions 8 and 9 in meditope-Fab complexes abolished salt bridges, reducing affinity by >10-fold [23]. The academic studies confirm that amino acids substitution in the Fab especially in the CDR region have a significant impact on modulating binding affinity. The results are also provided in the **Table 1** aligned with these principles, like substitution of tyrosine in position 59 in chain H with arginine and serine in chain L position 93 with isoleucine improved the center score (-944.3 vs -799.5) by adding charged and hydrophobic residues.

**Table 2** elucidates the summary of the amino acid residues involved in the interaction between chain B, representing the CTLA4 receptor, and modified drug chains H and L. Compared with the wild-type (STD in the wild-type) complex, the interaction between chain B and chain H involved 15: 14 amino acid residues, with an interface area of 608: 645 Å<sup>2</sup>. This interaction was stabilized with one salt bridge, ten hydrogen bonds, and 103 non-bonded contacts (hydrophobic interactions, *van der Waals* forces). On the other hand, chain B was engaged with chain L via 8: 4 amino acid residues respectively, covering an interface area of 334: 360 Å<sup>2</sup>, supported by 3 hydrogen bonds, and 55 non-bonded contacts. In the first set of substitutions (Arg53 and Ile93) the number of interacting residues between chain B and chain H increased to 18, respectively. This incremented to an expansion

of the interface area to 638: 710 Å<sup>2</sup>, and improved the complex stability through the formation of 3 salt bridges, 9 hydrogen bond, and 122 non-bonded contacts. The impact of this pair of substitutions, also led to an increment of the number of interacting residues to 8: 7. At a decrease in the interface area to 311: 327 Å<sup>2</sup>, the complex stabilized with 3 hydrogen bonds and 50 non-bonded contacts between chain B and chain L. The other sets of substitutions showed a variety of results and effects on the interacted residues, interface area (Å<sup>2</sup>), Salt Bridges and Hydrogen bonds. However, the last set of substitution showed a decline in the interacted residues to 9: 9, the interface Area to 481: 528, and a notable decline in the hydrogen bonds, non-bonded contacts 5 and 57, for chain B and H, respectively. In contrast, the impact of this set showed an increment of the interacted residues to 11: 10, an expansion of the interface area to 458: 506 and improvement in complex stability, which was stabilized by 7 hydrogen bonds, 63 non-bonded contacts, for chain B and L of the last set. In SARS-CoV-2 antibodies, computational redesign of Fab CC12.3 increased salt bridges in CDR-L1/L3, improving predicted binding affinity beyond ACE2 [24]. Crystal structures show native antibody-antigen complexes have 7.7 ± 3.3 hydrogen bonds, while engineered models average only 3.0 ± 1.5, explaining weaker binding [25]. Hydrophobic interactions account for 50.0%-70.0% of binding energy in antibody-antigen complexes [26].

**Table 2:** Amino-acid interactions between chain B (CTLA4) and drug chains H and L for the wild-type (STD) and modified complexes

No.	Receptor chain B: Drug chain H						Receptor chain B: Drug chain L					
	Interacted residues	Interface Area (Å <sup>2</sup> )	Salt Bridges	Disulfide bonds	Hydrogen bonds	Non-bonded Contacts	Interacted residues	Interface area (Å <sup>2</sup> )	Salt Bridges	Disulfide Bonds	Hydrogen Bonds	Non-bonded contacts
<b>STD</b>	15: 14	608: 645	1	-	10	103	8: 4	334: 360	-	-	3	55
Arg53 Ile93	18: 13	638: 710	3	-	9	122	8: 7	311: 327	-	-	4	50
Cys102 Phe92	12: 9	538: 571	2	-	4	76	6: 4	289: 304	-	-	4	42
Trp107 Arg32	16: 11	588: 663	3	-	8	106	6: 4	330: 363	-	-	6	37
Met57 Arg94	16: 12	594: 651	3	-	9	97	10: 8	382: 416	-	-	3	64
Lys106 Gln30	16: 13	638: 685	3	-	10	94	10: 8	396: 433	-	-	3	68
His105 Phe91	20: 14	666: 738	3	-	12	139	7: 6	356: 381	-	-	4	48
Phe110 Gln31	19: 14	666: 750	3	-	12	130	7: 6	351: 357	-	-	4	47
Phe108 Ile30	17: 12	621: 699	2	-	9	105	7: 5	358: 377	-	-	4	46
Phe52 His32	10: 8	488: 516	1	-	4	60	12: 7	422: 470	-	-	7	60
Cys101 Val50	9: 9	481: 528	1	-	5	57	11: 10	458: 506	-	-	7	63

This presents interacting residues, interface area (Å<sup>2</sup>), and stabilizing interactions (salt bridges, hydrogen bonds, and non-bonded contacts). Amino-acid substitutions produced variable effects, with early modifications enhancing chain B-H interactions, while the final substitution reduced chain B-H contacts but strengthened chain B-L interactions

**Table 3** provides the allergenic potential of antibody chains and the immunologic response of the wild-type and the 10 consecutive pairs of modifications. To evaluate the allergenic potential of the antibody chains, the heavy (H) and light (L) chains were analyzed in their standard and modified forms using the AlgPred server. This tool applies to several approaches, including machine learning (ML), motif-based detection via MERCI, sequence alignment through BLAST, and an overall hybrid score that combines all outputs. The final result - either Allergen or Non-allergen - is based on the integration of these scores as shown the ST chains labeled as allergen where they scored via ML score (0.48) (0.36), MERCI score (0.5) (0.0), BLAST score (0.5) (0.0), and hybrid score

(1.48) (0.36) for chain H and L, respectively. As noted, the chain H was labeled as an allergen with varied values, where the ninth set (Phe 52, His 32) gave the lowest value among all sets. In contrast the chain L revealed that the first (Arg53, Ile93), second (Cys102, Phe92), seventh (Phe110, Gln31), eighth (Phe108, Ile30), and the ninth (Phe52, His32) sets labeled as allergen, while the third (Trp107, Arg32), fourth (Met57, Arg94), fifth (Lys106, Gln31), sixth (His105, Phe91), and the tenth (Cys101, Val50) sets labeled as non-allergen. The sets were found to be scored less than the STD, were the third (Trp107, Arg32) chains H and L scores were ML (0.45) (0.36), MERCI (0.5) (0.0), BLAST (0.5) (-0.5), and Hybrid (1.45) (-0.16). For the fourth set (Met57, Arg94) chains H and L scores were ML (0.42) (0.31), MERCI (0.5) (0.0), BLAST (0.5) (-0.5), and Hybrid (1.42) (-0.19), fifth modified set (Lys106, Gln30) chains scored as followed, ML (0.44) (0.31), MERCI (0.5) (0.0), BLAST (0.5) (-0.5), and Hybrid (1.44) (-0.19), for sixth set (His105, phe91) the scoring function were: ML (0.44) (0.31), MERCI (0.5) (0.0), BLAST (0.5) (-0.5), and Hybrid (1.43) (-0.18). The tenth (Cys101, Val50) set was found to have a higher result compared to the STD regarding the chain H, To the scoring function were as following: ML (0.51) (0.31), MERCI (0.5) (0.0), BLAST (0.5) (-0.5), hybrid (1.51) (-0.19) and the ninth set gave the best scores among all the sets. The prediction of allergenic potential is crucial to ensure safety, due to the rapid increase in protein application. They are used in therapeutics, food, household products, and pharmaceuticals [27]. Innovations in protein engineering can help redesign allergenic proteins to reduce adverse reactions in sensitive individuals. To accomplish this, a comprehensive understanding of the molecular properties and features that confer allergenicity to proteins is essential [28]. **Table 3** shows the average antigenic propensity; the STD score was 1.0413. Scores were found to give a slight variation from the STD. The second and the tenth had higher scores than the STD, while the other sets showed values less than STD, except for the ninth which had exact the same value as STD. Notably, set no.7 gave 1.0401 which is considered as the best among all. Protein-based therapeutics may exhibit undesired immune responses in a subset of patients, leading to the production of antidrug antibodies. In some cases, antidrug antibodies have been reported to affect the pharmacokinetics, efficacy, and/or safety of the drug [29]. As a result, numerous approaches have been developed to assess the immunogenicity risk of biologics using in silico and in vitro methods [30].

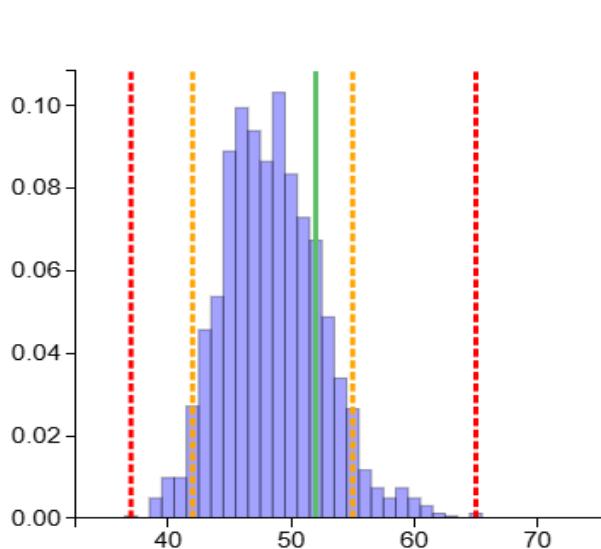
**Table 1:** In silico evaluation of allergenic potential and immunogenicity of the wild-type (STD) and consecutively modified antibody heavy (H) and light (L) chains

	AlgPred										Immunomedicine group	
	ML score		MERCI score		BLAST score		Hybrid score		Prediction score			
	H	L	H	L	H	L	H	L	H	L		
STD	0.48	0.36	0.5	0.0	0.5	0.0	1.48	0.36	Allergen	Allergen	1.0413	
Arg53 Ile93	0.44	0.37	0.5	0.0	0.5	0.0	1.44	0.37	Allergen	Allergen	1.0409	
Cys102 Phe92	0.45	0.36	0.5	0.0	0.5	0.0	1.45	0.36	Allergen	Allergen	1.0420	
Trp107 Arg32	0.45	0.36	0.5	0.0	0.5	- 0.5	1.45	- 0.16	Allergen	Non-allergen	1.0407	
Met57 Arg94	0.42	0.31	0.5	0.0	0.5	- 0.5	1.42	- 0.19	Allergen	Non-allergen	1.0408	
Lys106 Gln30	0.44	0.31	0.5	0.0	0.5	- 0.5	1.44	- 0.19	Allergen	Non-allergen	1.0408	
His105 Phe91	0.43	0.32	0.5	0.0	0.5	- 0.5	1.43	- 0.18	Allergen	Non-allergen	1.0403	
Phe110 Gln31	0.44	0.32	0.5	0.0	0.5	0.0	1.42	0.32	Allergen	Allergen	1.0401	
Phe108 Ile30	0.42	0.32	0.5	0.0	0.5	0.0	1.42	0.32	Allergen	Allergen	1.0403	
Phe52 His32	0.41	0.32	0.5	0.0	0.5	0.0	1.41	0.32	Allergen	Allergen	1.0413	
Cys101 Val50	0.51	0.31	0.5	0.0	0.5	- 0.5	1.51	- 0.19	Allergen	Non-allergen	1.0433	

Allergenicity was predicted using the AlgPred server based on machine learning, MERCI motifs, BLAST alignment, and hybrid scores, while average antigenic propensity was also reported. The modifications produced variable effects on allergenicity and immunogenicity, with several substitution sets reducing scores compared with the STD. Overall, selected variants showed improved safety-related profiles relative to the wild-type antibody

**Table 4** showed that STD and all the modified sets exhibit identical half-lives, assessed in three different biological systems: mammalian reticulocytes in vitro, yeast in vivo and *E. coli* in vivo; the results were elicited at 0.8 hr., 10 min and 10 hr., respectively. On the other hand, the instability index for the STD and all sets of substitutions exhibited as 94.06, 48.52, 48.62, 48.26, 48.15, 49.22, 49.96, 48.85, 48.88, 48.88, 48.33, respectively, which are all classified as unstable, despite the slight improvement shown in some of these modified sets. The estimated half-life is determined by the N-terminal amino acid sequence under investigation. The half-life is the prediction of the time it takes for half of the amount of protein in a cell to disappear after its synthesis in the cell. The prediction is given for three organisms (human, yeast, and *E. coli*), but it is possible to extrapolate the result to similar organisms. A stability index value over 40 indicates that the investigated protein may be unstable, while the a value less than 40 is stable [16]. For the total CDR length, the STD and all modified versions exhibit identical values of 52. Regarding the CDR vicinity PSH score, the STD and modified sets yield values of 121.53, 129.3421, 153.3187, 158.023, 153.7158, 153.7603, 156.3226, 146.0911, 159.2098, 157.5302, 150.1099, respectively, for the CDR vicinity PPC score, the (STD) exhibit a value of 0.0434, while all of the modified versions show values of 0.0886, 0.0999, 0.1173, 0.3434, 0.2877, 0.4968, 0.3841, 0.4634, 0.4424, 0.1873, respectively. Furthermore, for the CDR vicinity PNC and SFvCSP scores, the results of the STD and modified sets presents values of 0.1104 and 15.0 for PNC and SFvCSP, respectively, while the modified sets display values of (0.0962, 18.0), (0.0825, 15.0), (0.094, 20.0), (0.0932, 25), (0.1637, 25.0), 0.1588, 25.5), (0.1774, 25.5), (0.0608, 30.5), (0.0611, 25.01), (0.0938, 20.91) for each, respectively. Based on the acceptable ranges for these properties [17], all obtained results fall in acceptable criteria. However, a detailed comparison reveals that the STD generally demonstrates more favorable outcomes across most parameters, compared with all sets, which makes all the sets have favorable developability. Therapeutic Antibody Profiler is designed to identify antibodies that possess characteristics that are rare/unseen in clinical-stage mAb therapeutics. This is particularly relevant given that hydrophobicity within the Complementarity Determining Regions (CDRs) has been consistently linked to aggregation propensity in monoclonal antibodies (mAbs). Furthermore, surface patches of both positive and negative charge have also been implicated in undesirable biophysical characteristics. Specifically, mAbs exhibiting oppositely charged heavy (VH) and light (VL) chains typically demonstrate elevated in vitro viscosity values, higher rates of clearance, and suboptimal expression levels. Similarly, asymmetry in the net charge of the heavy- and light-chain variable domains correlates with increased self-association and viscosity at high concentrations [17].

Total CDR Length



Total CDR Length

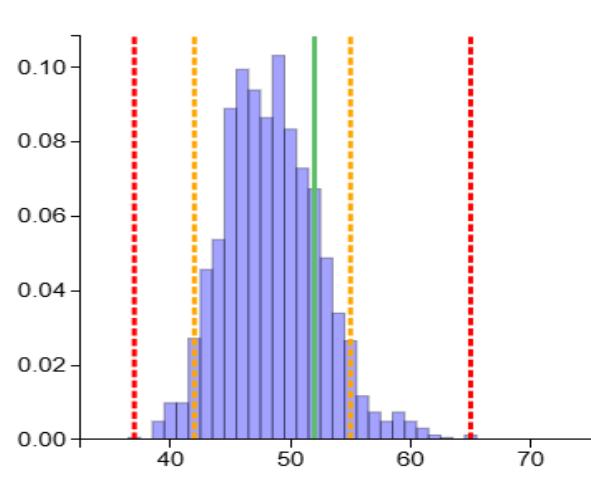
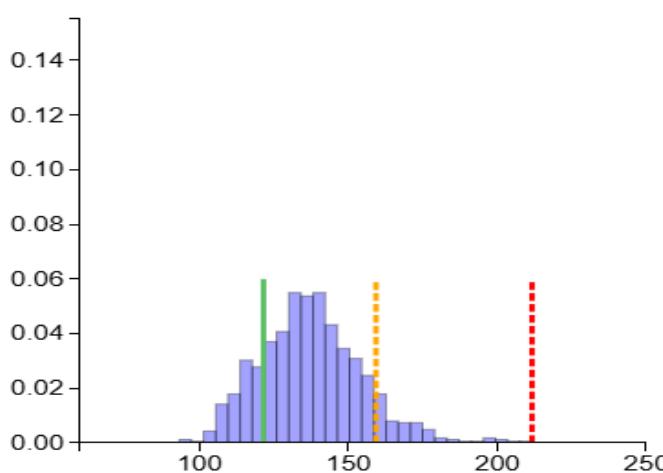


Figure 2: Total CDR length of STD and modified version -last set: contain all sets -

PSH Score



PSH Score

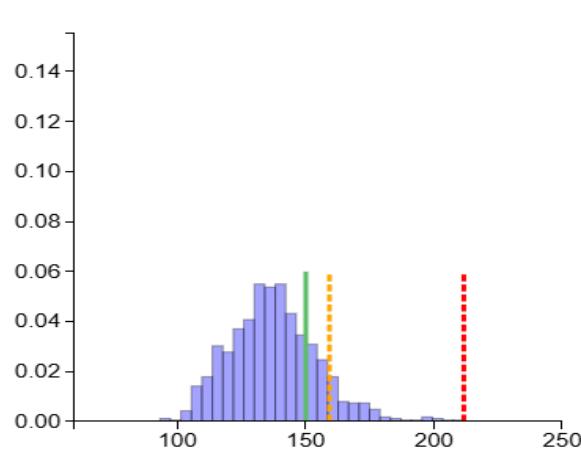
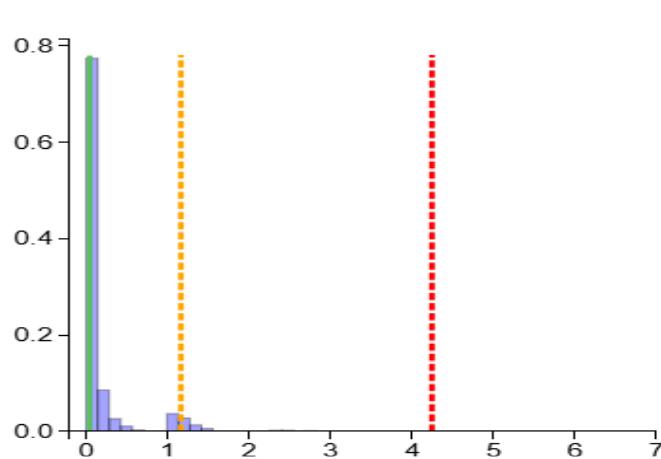


Figure 3: PSH score for STD and modified version -last set: contain all sets -

PPC Score



PPC Score

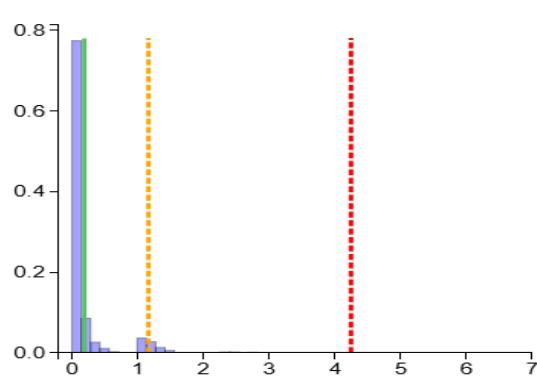
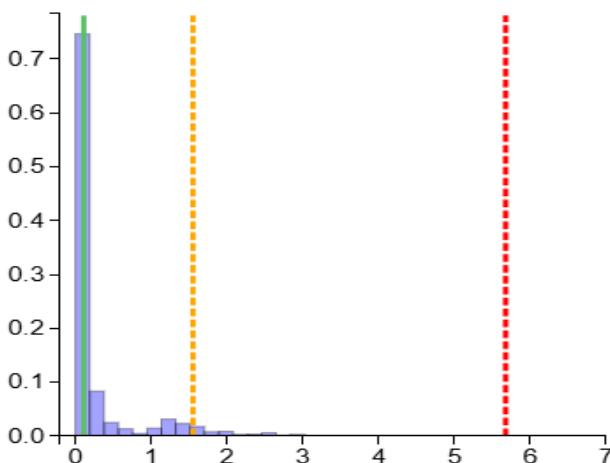


Figure 4: PPC score for STD and modified version -last set: contain all sets -

PNC Score



PNC Score

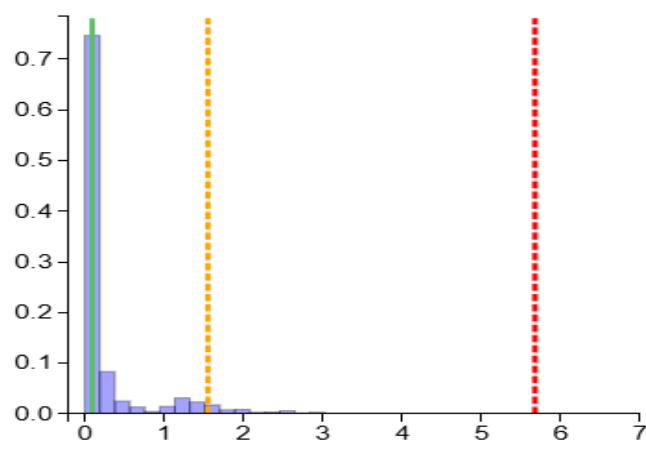
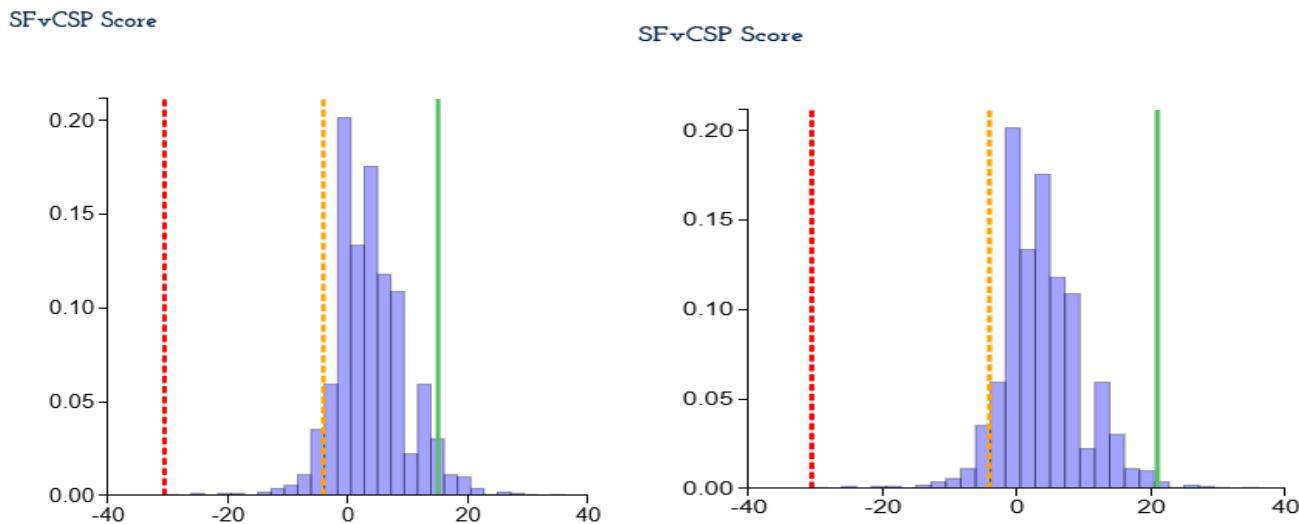


Figure 5: PPC Score for STD and modified version -last set: contain all sets -



**Figure 6:** SFvCSP score for STD and (modified version-last set: contain all sets -

These figures show the properties of the STD and the final version of medications- which contain all the previous modification sets, as mentioned before-, where estimated from TAP. This is because the study focused mainly on improving the binding affinity, which this condition complies with this version.

**Table 4:** Developability assessment of the wild-type (STD) and modified antibody variants

	Protparm			TAP					
	Half-life			Instability index	Total CDR length	CDR vicinity PSH score (kyte and doolittle)	CDR vicinity PPC score	CDR vicinity PNC score	
STD	Mammalian reticulocytes <i>in-vitro</i>	Yeast <i>in-vivo</i>	<i>E. coli</i> <i>in-vivo</i>						
	0.8 hr.	10 min	10 hr.	49.06 unstable	52	121.53	0.0434	0.1104	15.0
Arg53 Ile93	0.8 hr.	10 min	10 hr.	48.52 unstable	52	129.3421	0.0886	0.0962	18.0
Cys102 Phe92	0.8 hr.	10 min	10 hr.	48.62 unstable	52	153.3187	0.0999	0.0825	15.0
Trp107 Arg32	0.8 hr.	10 min	10 hr.	48.26 unstable	52	158.023	0.1173	0.094	20.0
Met57 Arg94	0.8 hr.	10 min	10 hr.	48.15 unstable	52	153.7158	0.3434	0.0932	25.0
Lys106 Gln30	0.8 hr.	10 min	10 hr.	49.22 unstable	52	153.7603	0.2877	0.1637	25.0
His105 Phe91	0.8 hr.	10 min	10 hr.	49.96 unstable	52	156.3226	0.4968	0.1588	25.5
Phe110 Gln31	0.8 hr.	10 min	10 hr.	48.85 unstable	52	146.0911	0.3841	0.1774	25.5
Phe108 Ile30	0.8 hr.	10 min	10 hr.	48.88 unstable	52	159.2098	0.4634	0.0608	30.5
Phe52 His32	0.8 hr.	10 min	10 hr.	48.88 unstable	52	157.5302	0.4424	0.0611	25.01
Cys101 Val50	0.8 hr.	10 min	10 hr.	48.33 unstable	52	150.1099	0.1873	0.0938	20.91

All variants exhibited identical predicted half-lives in mammalian reticulocytes, yeast, and *E. coli*. Instability indices classified the STD and all modified sets as unstable, with minor variations among substitutions. CDR length, CDR vicinity scores, and therapeutic antibody profiler parameters were within acceptable ranges for all variants. Overall, while the STD showed slightly more favorable values across several metrics, all modified sets met the developability criteria

**Conclusion:** This study investigates the impact of specific modifications on the binding interaction between CTLA4 and Teremelimumab, by employing molecular docking. On the other hand, this could improve the binding affinity and increase complex stability, as shown in the cumulative alteration number 10 (Arg101 to Cys/ Ala50 to Val, where binding affinity is equal to -945.9 Kcal/mole). These divergent outcomes highlight the critical sensitivity of the antibody-antigen interface to amino acid changes, underscoring the delicate balance required for optimal therapeutic binding. The ability to identify specific modifications that either enhance or diminish binding affinity provides invaluable molecular-level insights into the determinants of Tremelimumab's efficacy. Such findings are paramount for guiding rational drug design, allowing for the targeted optimization of antibody characteristics to achieve desired pharmacological profiles. The Tremelimumab demonstrated susceptibility to further optimization in its binding affinity through targeted modifications, with some improvement in its developability properties, and some regression in other properties.

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