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Molecular docking of hiv-1 protease inhibitors and stabilization of the 4binding affinity monoclonal antibody through site-directed mutagenesis

Acoplamiento molecular de inhibidores de la proteasa del vih-1 y estabilización del anticuerpo monoclonal 4binding affinity mediante mutaciones dirigidas

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Abstract
The incorporation of protease inhibitors (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, and lopinavir) into antiretroviral therapy has significantly reduced the morbidity and mortality associated with AIDS. These non-peptidic compounds potently and selectively inhibit HIV-1 protease. Therefore, an affinity study was conducted between the protease enzyme and each of its inhibitors by their binding energy using molecular docking to determine which inhibitor exhibits the highest binding affinity. Additionally, hydrogen bond interactions between the receptor and each ligand were analyzed to evaluate their correlation. From another perspective, this study examines the synthesis of the monoclonal antibody 4BINDING AFFINITY (IgG2a, derived from a murine IgG3 subclass antibody. Several mutations were introduced into the protein structure, replacing residues identified as high-risk for deamidation reactions. The analysis aimed to compare structural modifications to identify those that minimize aggregation risk. A quality assessment of the mutated product was conducted, supporting the stability improvements observed.

Keywords**:** Potential energy, chemical instability, physical instability, mutation, isoelectric potential.

Resumen
La incorporación de los inhibidores de la proteasa (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir y lopinavir) en la terapia antirretroviral ha supuesto un importante descenso en la morbilidad y mortalidad provocada por el SIDA. Son compuestos no peptídicos que inhiben de forma potente y selectiva la proteasa del VIH-1. Es por esto por lo que se realiza un estudio de afinidad entre la enzima de la proteasa y cada uno de sus inhibidores por medio del cálculo de su energía de enlace usando Docking molecular para concluir cuál de ellos presenta mayor afinidad como fármaco. Adicionalmente se realiza un estudio del cálculo de puentes de hidrogeno entre el receptor y cada uno de los ligandos, evaluando los resultados frente a la relación con la energía de enlace. Desde otro punto de vista, este estudio considera la síntesis del anticuerpo monoclonal 4BINDING AFFINITY, (IgG2a, derivado de un anticuerpo de ratón 14,18 de la subclase IgG3), para lo cual se hicieron varias mutaciones en la estructura de la proteína, haciendo las sustituciones de los residuos catalogados como de alto riesgo de causar reacciones de desamidación, análisis y fotocopia, comprando la estructura del análisis para otros que más tarde arrendamiento y reducir el riesgo de agregación. Se realizó un estudio de la calidad del producto de las mutaciones, que apoyó los datos obtenidos de la minimización de la potencia antes y después de la determinación de las cosas mencionadas.

Palabras clave**:** Energía potencial, inestabilidad química, inestabilidad física, mutación, potencial isoeléctrico.

1. Introduction

The HIV-1 protease plays a crucial role in HIV replication and infectivity by cleaving polypeptide chains to generate mature enzymes and structural components of the virus (including itself). Research has shown that HIV viruses containing inactive protease cannot replicate or infect additional cells (Wlodawer et al., 1989). In the second half of the 1990s, protease inhibitors (PIs) were introduced as antiretroviral drugs for the treatment of human immunodeficiency virus type 1 (HIV-1) infection. This therapeutic breakthrough significantly reduced AIDS-related morbidity and mortality, leading to a decrease in the incidence of opportunistic infections and hospitalizations while improving the quality of life for HIV-positive patients [1]. Inhibitors such as saquinavir, ritonavir, indinavir, nelfinavir, lopinavir, and amprenavir share a common hepatic metabolism for elimination and a short elimination half-life, with differences in absorption and distribution. All, except for Indinavir, must be taken with food [2].

Monoclonal antibodies represent the fastest-growing class of pharmaceutical products. However, a significant challenge has always been the susceptibility of proteins to aggregation at the high concentrations used during manufacturing and storage. When the aggregation percentage increases, the product's efficacy is directly affected, leading to unwanted adverse effects, such as the body's immune response to the administered formulation [3]. Ensuring the stability of protein-based pharmaceuticals is a major challenge in the pharmaceutical industry to extend product shelf life. Optimizing drug formulation is one approach to enhancing stability, with one option being the addition of stabilizing excipients [4].

On the other hand, there is the option of modifying the protein sequence by introducing amino acid mutations that cause structural instability. This study focuses on replacing the amino acids that contribute the most to chemical instability and are also identified as the most exposed in terms of solvent-accessible surface area. Through quality analysis, the study aims to estimate the effectiveness of the selected models in carrying out these mutations.

1.1 Mechanism of Action

The synthesis of HIV protein occurs in the host cell. Once the translation of viral mRNA takes place, the components of the HIV assemble in the Golgi apparatus, and the virion begins to bud from the host cell. As budding occurs or shortly after the virion emerges, it matures and infects other host cells. It is during this maturation stage that the HIV-1 protease cleaves the gag and pol polyprotein precursors into mature functional proteins [5].

For HIV to infect a cell, it must bind to the CD4 antigen and a co-receptor [6,7], specifically a chemokine receptor. Chemokine receptors interact with chemokines and can block HIV from attaching to gp120. HIV assembly and budding from the host cell into an infectious virion depend on Pr55Gag, a precursor of Gag proteins. Pr55Gag is incorporated into a viral particle that buds from the cell while a maturation process occurs. During this process, the viral protease cleaves Pr55Gag into smaller functional proteins, including the immature capsid protein, matrix protein, nucleocapsid protein, and p6. The immature capsid protein (p25) is cleaved to form the mature capsid protein (p24). This maturation process involves structural rearrangements and reorganization, ultimately leading to the formation of a cone-shaped, electron-dense core within the mature virion [8].

1.1.1 Active site

HIV-1 protease is a homodimer consisting of 99 amino acids. It is an aspartyl protease with an active site that is C2-symmetric when unbound. The ligand inhibitor binds to the active site, preventing polyprotein cleavage. The protease contains two β-sheet flaps connected by glycine-rich loops. These flaps are part of the active site and play a crucial role in ligand binding [9].

The active site of HIV-1 protease consists of two loops containing the Asp-Thr-Gly sequence, known as the catalytic triad, which is conserved among proteases of this family. These active site loops are stabilized by a network of hydrogen bonds between the active site amino acids and the surrounding residues. The rigid structure of the active site is maintained by the "fireman's grip," which forms when each Thr 26 residue accepts a hydrogen bond from the amine group of the opposite Thr 26 and donates a hydrogen bond to the carbonyl oxygen of the adjacent Leu 24 residue, located next to each catalytic aspartate. Additionally, each Asp 25 residue is hydrogen-bonded to the backbone NH group of Gly 27 [10].

In Figure 1, the described active site can be observed.

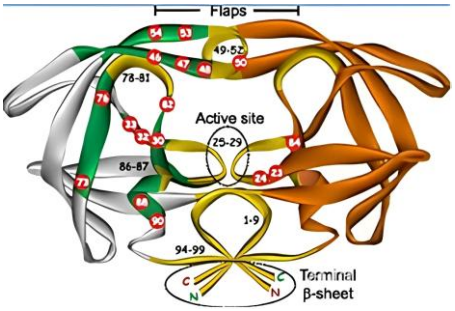


Figure 1. Identification of the active site of HIV-1 protease
Source [11]

1.1.2 Interactions

The interaction process was conducted using crystallized structures in the Protein Data Bank (PDB) [12]. When these proteins are crystallized alongside their inhibitors, they provide valuable insights into enzyme-substrate interactions.
By determining the spatial positions of the enzyme's amino acid residues, it becomes possible to propose new drug structures that, through desired interactions with the enzyme, may exhibit higher inhibitory potency or enhanced pharmacokinetic and pharmacodynamic properties [13].

Given the clinical relevance of HIV-1 protease inhibition and the challenges in developing stable antibody-based therapies, this study addresses the need for computational tools to guide both inhibitor selection and antibody stability optimization. Based on the available data, six inhibitors were analyzed, optimized, and simulated with the protease to identify the one with the highest affinity.

2. Methodology

For this study, six (6) molecular structures—indinavir, nelfinavir, saquinavir, ritonavir, amprenavir, and lopinavir—were obtained from the Protein Data Bank (PDB). Specialized computational tools, including AutoDockTools, Putty, Chimera, and WinSCP, were used to conduct the study.

The process began with the retrieval of molecular structures from the RCSB Protein Data Bank in PDB format. Using Chimera 1.11.2, energy minimization was performed in an aqueous environment with the Solvate and Minimize Structure tools. For molecular docking, ligands and the receptor were prepared in AutoDockTools by adding hydrogen atoms and charges to optimize interactions. The resulting files were saved in PDBQT format. The docking grid box was set based on the protease's active site to enhance affinity calculations.

A secure connection to the Universidad de los Andes cluster was established via WinSCP for efficient file transfers. Molecular docking simulations in AutoDock Vina calculated binding energies, with multiple runs identifying the highest-affinity inhibitor. Chimera analyzed hydrogen bonds to assess their impact on binding energy. For the monoclonal antibody 4BINDING AFFINITY, UCSF Chimera and Swiss PDB Viewer 4.1.0 identified high-risk residues, minimized energy, and introduced stabilizing mutations. The antibody structure, sourced from the RCSB Protein Data Bank, enabled a detailed structural analysis.

2.1 Residues prone to cause chemical destabilization, present in the structure.

Special attention was given to identifying Methionine, Tryptophan, Histidine, Cysteine, and Tyrosine to evaluate potential mutations that could mitigate their presence in the formulation. UCSF Chimera was used for this process. The specific amino acid was selected via *Select → Residue*, followed by *Actions → Label → Residue-name+specifier* to specify its position and name. To enhance visibility and facilitate structural identification, *Actions → Color* and *Actions → Atoms/Bonds → Show* were applied.

2.2 Mutations / Decisions to change

This study aims to (1) evaluate the binding affinity and hydrogen bonding interactions between HIV-1 protease and a set of clinical inhibitors using molecular docking, and (2) assess the impact of selected amino acid mutations on the structural stability of the monoclonal antibody 4BINDING AFFINITY to reduce its aggregation risk. However, the mutations introduced may gradually reduce the ability to interact with the GD2 antigen. Therefore, modifications in this section are intended to evaluate the level of interaction with the antigen while preventing strong deamidation reactions.

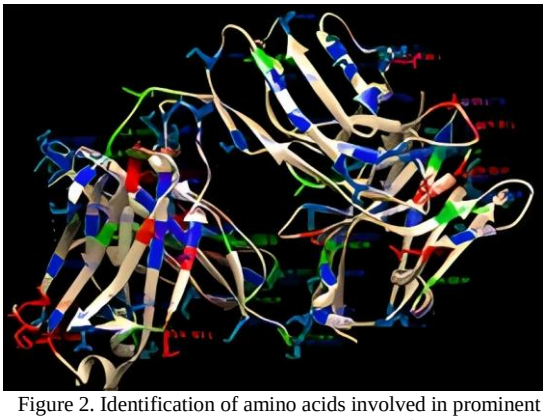


Figure 2. Identification of amino acids involved in prominent interactions using UCSF Chimera software.

Based on the study's objective and previous unsuccessful mutations affecting antigen interaction, Alanine residues (ALA 50. H and ALA 79.H) were replaced with Glycine. This substitution enhances antigen affinity due to Glycine's rapid interaction with free radicals while preventing prominent interactions in this region, thereby reducing the risk of deamidation.

Moreover, Glycine acts as a buffering agent, stabilizing pH and protecting samples during experimental techniques like electrophoresis. Its lack of a side chain—comprising only a hydrogen atom—allows it to adopt sterically hindered conformations that other amino acids cannot, providing greater local flexibility to the protein chain.

2.3 To avoid oxidation problems.

By identifying the amino acids responsible for destabilization in the formulation and those crucial for antibody functionality, targeted mutations were evaluated. Tryptophan, Methionine, and Histidine residues were modified, except for HIS 39.L, which forms hydrogen bonds. Tyrosine residues were also assessed, excluding TYR 32. H and TYR 37. L, as they contribute to weak hydrophobic interactions with the sugar ring.

Tryptophan (THR 78.H) was replaced with Lysine, as it is resistant to oxidation, hydrolysis, and deamidation. Lysine plays a key role in anion-binding proteins by interacting electrostatically with ligands. Additionally, unlike other amino acids, its side chain remains positively charged at physiological pH, improving stability.s, además de poseer una cadena lateral cargada a pH fisiológico a diferencia de otros aminoácidos.

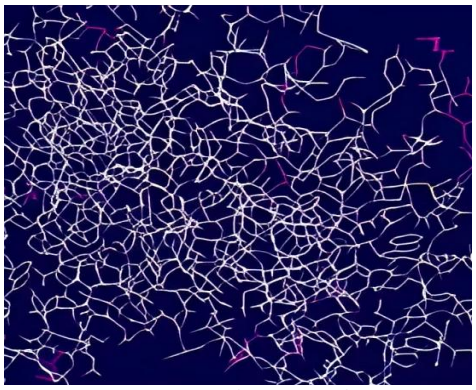


Figure 3: Mutation of THR 78.H for LYS 78.H

2.4 Avoid Hydrolysis problem

To mitigate hydrolysis-induced destabilization, we focused on the presence and positioning of Aspartic Acid (ASP) residues, key contributors to this issue. Chimera analysis identified 17 ASP residues across both antibody chains, with the Light (L) chain being more susceptible due to its higher ASP concentration. ASP residues in the Light chain were selected for replacement, except for ASP 52. H and ASP 1. L, which is essential for hydrogen bond formation.

To address this, ASP residues were replaced with Arginine (ARG) to prevent hydrolysis-related destabilization. ARG was also positioned near Lysine (LYS) to enhance antibody functionality, as interactions between these amino acids improve structural stability.

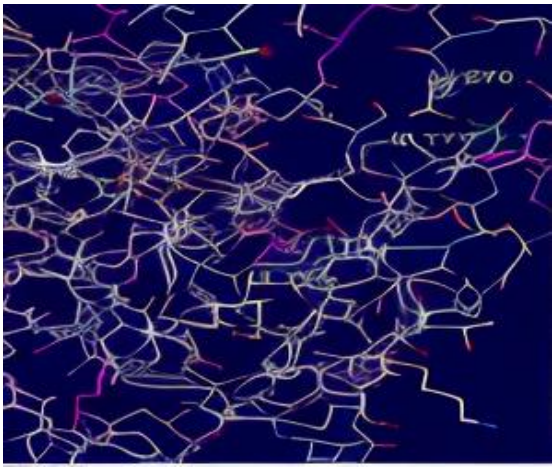


Figure 4: Mutation of ASP 73.H for ARG 73.H

3. Results and discussion

3.1 Box Positions

The three-dimensional structure of the monoclonal antibody 4BINDING AFFINITY, including the previously introduced mutations, is shown below.

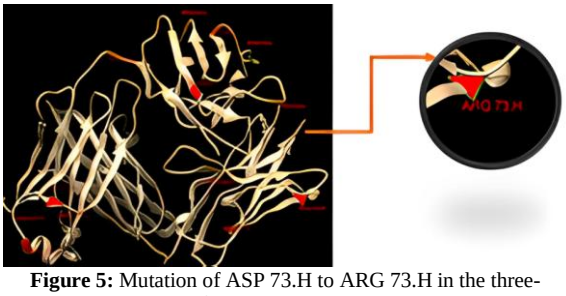


Figure 5: Mutation of ASP 73.H to ARG 73.H in the three-dimensional structure of the 4BINDING AFFINITY antibody.

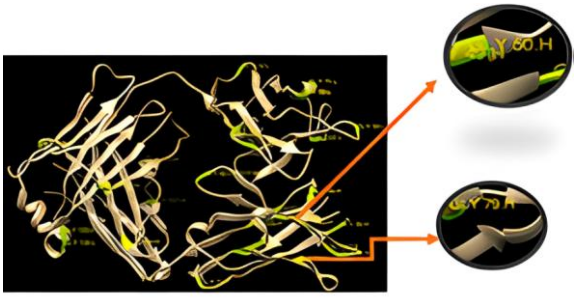


Figure 6: Mutation of ALA 50.H to GLY 50.H and ALA 79.H to GLY 79.H in the three-dimensional structure of the 4BINDING AFFINITY antibody.

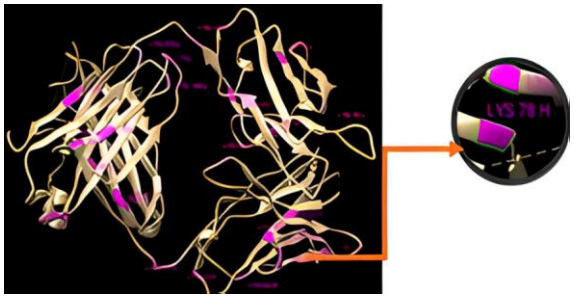


Figure 7: Mutation of THR 78.H to LYS 78.H in the three-dimensional structure of the 4BINDING AFFINITY antibody.

3.2 Calculation of Binding Energy – Box Positions

The binding energy for each inhibitor (saquinavir, amprenavir, nelfinavir, indinavir, lopinavir, and ritonavir) was calculated by initially evaluating interactions using three different box models covering the protease's active site. After determining the final measurements, definitive runs were conducted for each inhibitor. The boxes were generated using AutoDock Tools, with the data presented in Table 1.

Table 1. Measurement values and box configuration used.

Parameters	Box 1	Box 2	Box 3
X	42	42	42
Y	26	26	26
Z	50	50	50
X center	0.336	2.183	2.183
Y Center	-0.079	-1.034	-3.158
Z Center	-5.374	5.68	-5.68

The measurements of box 3 were used for all final runs.

Similarly, runs were conducted using the measurements from the class; however, most ligands yielded unsatisfactory results, as their binding energies were above -6.0. When defining the box dimensions, special consideration was given to covering the active site to enhance receptor-ligand interactions.

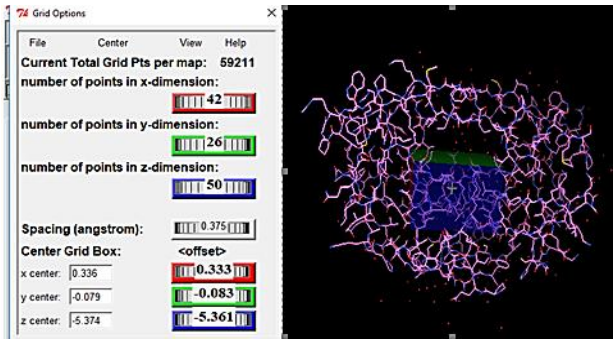


Figure 8. First box model of the active site

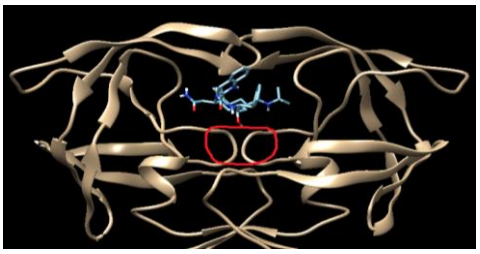


Figure 9. Ligand-Receptor interaction with the first box model

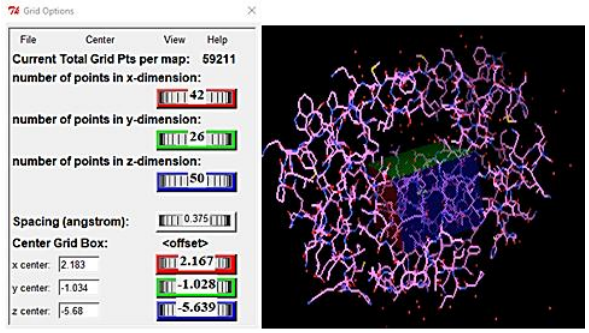


Figure 10. Second box model of the active site

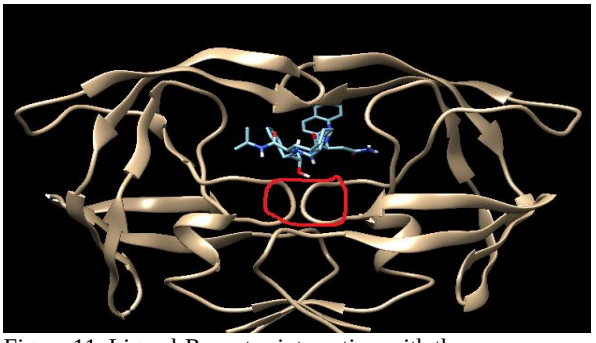


Figure 11. Ligand-Receptor interaction with the second box model.

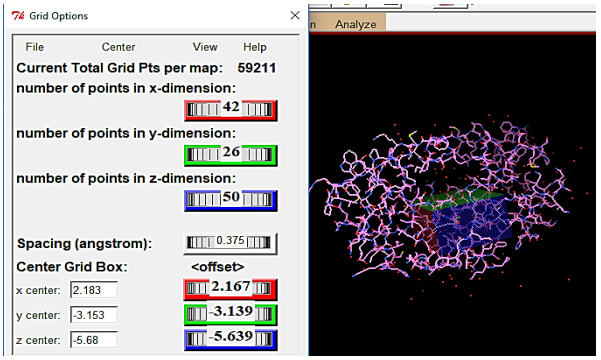


Figure 12. Third box model of the active site.

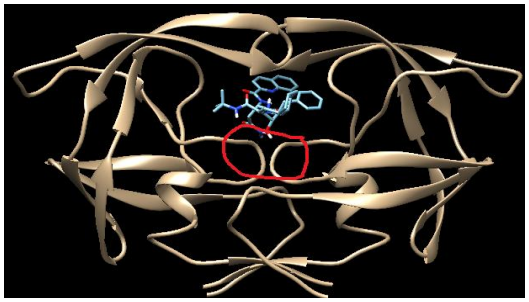
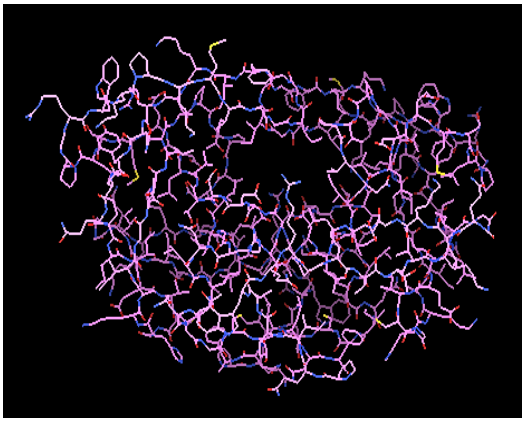


Figure 13. Ligand-Receptor interaction with the third box model.

Based on the analyzed interactions and proximity to the active site, the third box model was selected for further studies.

3.3 Ligand and Receptor Preparation for Docking

The following screenshots illustrate the preparation process of the ligand and receptor for docking.



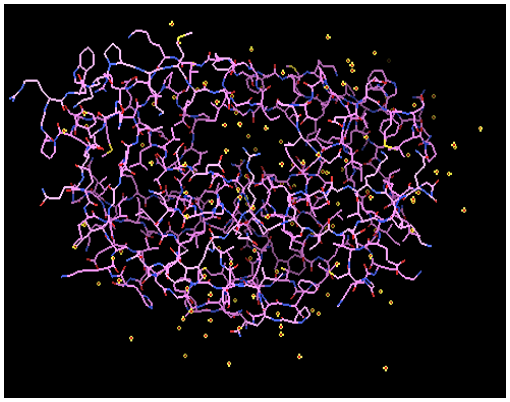


Figure 14. Disposal of solvent residues

3.3.1 Protease (1 HSG)

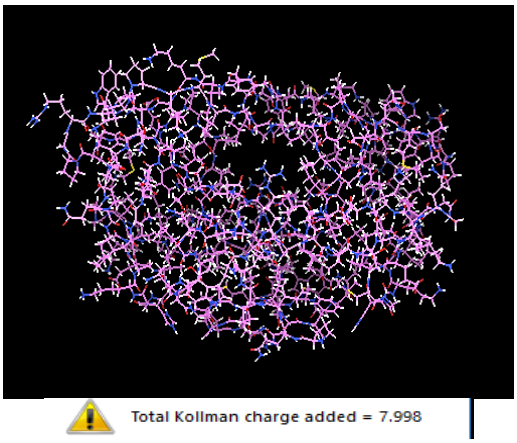


Figure 15. Addition of hydrogen

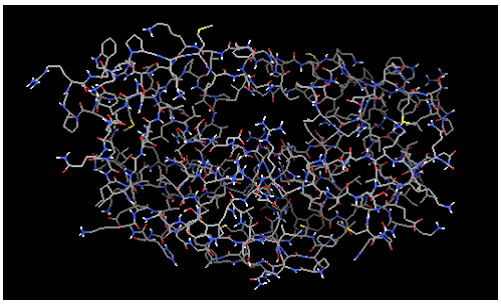


Figure 16. Receiver (1HSG) ready

3.4 Binding Energy.

Binding energy affinity results for each ligand were obtained using Putty. The results, categorized by inhibitor type, indicate that Indinavir exhibited the highest affinity for the receptor.

3.5 Drug with the Highest Affinity for the Receptor (Protease)

Due to the characteristics of the protease, the developed inhibitors generally follow two main approaches: inhibition of the catalytic activity at the active site and inhibition of enzyme dimerization. For active site inhibition, the best pose is determined by the lowest binding energy among all obtained poses. In this case, the Indinavir run reported the lowest energy at -11.5.

Regarding hydrogen bond interactions, these bonds are weaker than covalent bonds and require approximately 5 kcal/mol to break. This energy - presented in Table 2- is significantly higher than the binding affinity between the molecules, allowing for stable interactions in this type of bonding.

Table 2 . Resultados de Binding Energy y HBonds

Drug	Binding Energy (kcal/mol)	Number of hydrogen bridges
indinavir	-11.5	5
nelfinavir	-11.1	1
saquinavir	-10.4	3
ritonavir	-8.6	2
ampinavir	-8.0	1
lopinavir	-7.6	4

3.6 Calculation of the Number of Hydrogen Bonds

In Chimera, an analysis was conducted to determine the number of hydrogen bonds formed between each ligand and the HSG1 protease.

Based on the obtained results, Chimera was used to quantify the hydrogen bonds, allowing for a graphical representation of the inhibitor with the highest affinity using the same docking box (measurements around the active site). In this case, Indinavir and Nelfinavir showed the best positioning within the active site compared to inhibitors with higher binding energy values. The results are presented below:

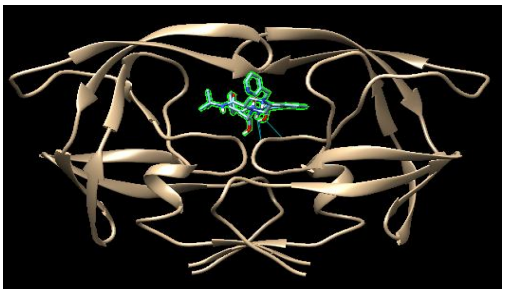


Figure 17. Indinavir

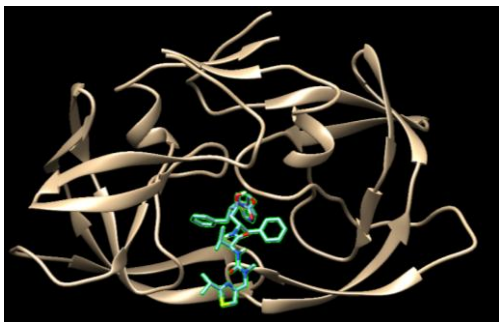


Figure 18. Ritonavir

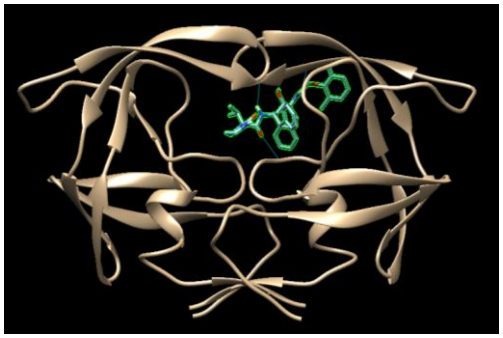


Figure 19. Lopinavir

The results show an increase in hydrogen bonds for the drugs with the highest affinity, while the others exhibited a decrease. This suggests that stronger molecular interactions promote hydrogen bond formation. Table 3 presents the detailed results.

Table 3. Identification of detected hydrogen bonds

Drug	Number of Hydrogen Bonds per-Run	Number of Hydrogen Bonds - Minimized
indinavir	5	1
nelfinavir	1	0
saquinavir	3	4
ritonavir	2	6
ampinavir	1	2
lopinavir	4	5

Under optimal conditions, a 10% covalent character is assumed. An important consequence is that the hydrogen atoms of a molecule can exchange with those of the solvent water.

3.7 Significant Changes in Aggregation

Considering the obtained results, the mutated antibody that showed the best performance was the one with the addition of the amino acid Arg 73. Based on the structural characteristics under study, the following formulation is proposed.

40 mg of protein
- 4.9 mg of NaCl
- 1.91 mg of dibasic potassium phosphate
- 0.69 mg of monobasic potassium phosphate
- 9.6 mg of mannitol
- 0.8 mg of polysorbate 80
- pH 6
- Store between 2-8°C. Do not freeze.

3.7 Isoelectric point:

The isoelectric point of 7.03 places the protein in the neutral range of the pH scale. For formulation, it is advisable to maintain the pH at a distance from this value, as at the isoelectric point, the protein lacks charge, preventing molecular interactions and leading to precipitation. To mitigate this effect and maintain an alkaline pH, it is recommended to increase the concentration of sodium chloride (NaCl) or monobasic potassium phosphate.

3.8 Discussion

3.8.1 HIV-1 Protease Inhibitors

The study identified *Indinavir* as the inhibitor with the highest binding affinity for the HIV-1 protease, with a binding energy of -11.5 kcal/mol and five hydrogen bonds. These results are consistent with prior research emphasizing Indinavir's efficacy in targeting the HIV-1 protease active site through potent interaction profiles [14].

In contrast, other inhibitors, such as *Lopinavir* and *Amprenavir*, showed lower affinity, which may be attributed to differences in docking geometry and hydrogen bond formation. This trend aligns with comparative pharmacodynamic analyses demonstrating variability in performance among first- and second-generation protease inhibitors [15].

3.8.2 Monoclonal Antibody Engineering

The mutations introduced into the 4TRP monoclonal antibody—such as replacing aspartic acid (ASP) with arginine (ARG) and threonine (THR) with lysine (LYS)—were effective in reducing susceptibility to deamidation, oxidation, and hydrolysis. These changes contribute to improved structural stability and agree with approaches discussed by Bayer (2019) [17], which advocate for replacing labile residues (e.g., Met, Trp, Cys) to enhance monoclonal antibody stability and shelf-life [18].

The proposed formulation, which includes mannitol, potassium phosphates, and polysorbate 80, is designed to improve solubility and reduce aggregation risk. Mannitol has been reported as a stabilizing agent that enhances the thermal and conformational stability of therapeutic proteins. Likewise, polysorbate 80 is widely used in antibody formulations due to its role in minimizing protein-protein interactions and surface adsorption.

The calculated isoelectric point of 7.03 further justifies maintaining a formulation pH away from this value to avoid precipitation, a phenomenon well-documented in the literature on protein formulation and buffer optimization.

3.8.3 Study Limitations

A major limitation of this study is the lack of vitro or vivo validation. All results were derived from silico molecular modeling and docking simulations. While computational methods are valuable for hypothesis generation and early-stage screening, they cannot fully replace empirical assays such as crystallography, enzymatic inhibition studies, or aggregation/stability testing. Therefore, additional experimental validation is essential to confirm the predicted binding affinities and structural impacts of antibody mutations.

4. Conclusions

Protease inhibitors have significantly reduced HIV-related mortality by blocking viral maturation. Despite their effectiveness, challenges such as resistance and treatment adherence drive ongoing improvements. To prevent monoclonal antibody degradation, excipients, and structural modifications have been tested. These advancements contribute to safer and more durable therapies.

Molecular modeling has enabled the design of more effective and stable inhibitors. This approach has also enhanced the resistance of monoclonal antibodies, supporting the development of more precise treatments. The study identified Indinavir as the inhibitor with the highest affinity for HIV-1 protease, exhibiting a binding energy of -11.5 kcal/mol and forming five hydrogen bonds. This finding aligns with previous research demonstrating Indinavir's efficacy in inhibiting HIV-1 protease. For instance, a study by Ghosh et al. (2022) [16] reported that Indinavir exhibits strong binding affinity to the active site of HIV-1 protease, contributing to its potent antiviral activity.

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