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DECIPHERING THE MOLECULAR BASIS OF MUTATED BINDING SITE BROMODOMAIN-LIGAND COMPLEXES: INSIGHTS FROM MOLECULAR DYNAMICS SIMULATIONS AND DECODED INTERACTION FINGERPRINT ANALYSIS

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ABSTRACT

This study aims to predict structural stability changes and to identify the molecular determinants of ligand-bromodomain complex interactions that undergo mutations in the ligand binding site (LBS) using computational chemistry methods. The stability changes of the complexes were investigated using Molecular Dynamics (MD) simulations during a 25 ns production run. The identification of molecular interaction determinants was performed by decoding the interaction fingerprint, utilizing the output of trajectory data of the MD simulations, which were converted into a series of pdb files along the time step. The system preparations were done using CHARMM-GUI. The MD simulations were carried out using the GROMACS program. Protein-ligand interaction fingerprints (PLIF) were identified using the PyPLIF-HIPPOS program. This study successfully predicted the stability of both wild-type and mutated ligand-bromodomain complex structures, where the W81A mutation led to a decrease in complex stability. The key residues and non-hydrophobic interaction types responsible for the stabilities were identified as TRP81 aromatic edge-to-face, TYR139 aromatic edge-to-face, and TYR139 aromatic face-to-face.

Keywords: Bromodomain, Molecular Dynamics Simulations, Protein-Ligand Interaction Fingerprint, GROMACS, PyPLIF-HIPPOS

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INTRODUCTION

The ligand binding site (LBS) is an area on a protein structure where molecular interactions occur between the protein and a ligand. The LBS consists of a specific arrangement of amino acid residues in a particular geometry, typically in a pocket-like region (Zhao et al., 2020). Mutation in the LBS can affect the interaction between protein and ligand at that site. An analysis revealed that ligand-binding residues had a significantly higher mutation rate than other parts of the protein (Kim et al., 2017). Mutations can change the chemical properties of the LBS, such as the availability of residues that can form hydrogen bonds or hydrophobic interactions with ligands (Yu et al., 2015). Mutations in the LBS can also produce more complex effects on proteins and their biological activities. Some mutations in the LBS can cause changes in protein structure globally and affect enzymatic activity, cellular signal recognition ability, and protein-protein interactions. (Bosserman et al., 2013; Brand & Dehm, 2013; Sandy et al., 2005). These mutations can alter the structure and chemical properties of the LBS, potentially leading to changes in ligand binding affinity, specificity, or stability. Understanding the impact of these mutations on protein-ligand interactions is crucial for

studying the functional consequences and designing targeted interventions in drug discovery and protein engineering.

The Bromodomain and Extra-Terminal domain (BET) family of proteins is a group of proteins characterized by the presence of two tandem bromodomains and an extra-terminal domain. Bromodomain is a conserved protein modular domain found in various chromatin- and transcription-associated proteins (Fig. 1). The mammalian BET family includes BRD2, BRD3, BRD4, and BRDT, which are encoded by paralogous genes that may have been generated through repeated duplication of an ancestral gene during evolution (Taniguchi, 2016). They act as the primary reader for acetylated lysine residues, allowing bromodomain-containing proteins to participate in acetylation-mediated protein-protein interactions within the cell (Zaware & Zhou, 2019). The primary function of bromodomain proteins is to regulate gene transcription, DNA recombination, replication, and repair. They play a versatile role in the regulation of protein-protein interactions involved in these processes. Bromodomain proteins are critical for mediating chromatin-templated gene transcription by recognizing and binding to acetylated lysine residues on histones. This recognition leads to the recruitment of other factors involved in transcriptional regulation (Zaware & Zhou, 2019). Due to their involvement in chromatin and transcriptional regulation, bromodomain proteins have been implicated in the pathogenesis of various human diseases. Dysregulation of bromodomain-mediated processes can contribute to the development and progression of diseases such as cancer, cardiovascular disorders, inflammation, and neurological disorders. Therefore, bromodomains have become an attractive target for therapeutic interventions (Boyson et al., 2021; Cochran et al., 2019). The study of bromodomains and their potential as therapeutic targets has gained significant attention in drug discovery and development. Research has been actively developed on small molecule inhibitors that selectively bind to bromodomains, thereby modulating the activity of bromodomain-containing proteins. These inhibitors have shown promise in preclinical and clinical studies for the treatment of various diseases, particularly cancers (Cochran et al., 2019; Muller et al., 2011).

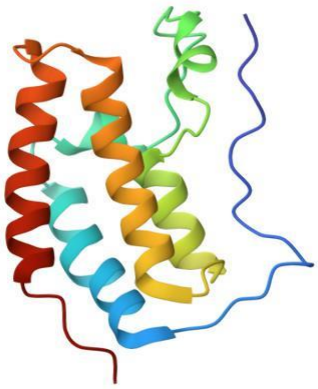

Figure 1. Ribbon diagram of bromodomain-containing Protein 4

Currently (as of June 2023) there are 34 mutations for bromodomain-containing protein 4 (BRD4) recorded and published by the Human Gene Mutation Database (https://www.hgmd.cf.ac.uk/ac/index.php) (Stenson et al., 2003). Specifically, for the LBS mutations, the PSnpBind database (https://psnpbind.org/) (Ammar et al., 2022) recorded and published 2 mutations for bromodomain, namely A89V, and A91R. The mutLBSgeneDB database (https://bioinfo.uth.edu/mutLBSgeneDB/) (Kim et al., 2017) recorded and published 6 LBS mutations, namely A89V, F426L, I394M, P375L, P95T, and D96N.

To understand the implications of a mutation in the ligand-binding site of a bromodomain, it is necessary to explore the role of bromodomain and their interaction with ligands. This research aims to study: the effect of mutations in the bromodomain LBS (1) on changes in the stability of complex structures and (2) to identify the molecular determinant responsible for the interaction between the ligand and the bromodomain. This study utilizes the in-silico technique by employing MD simulations and PLIF.

RESEARCH METHODS

The material used is the crystal structure of bromodomain-containing protein 4 (BRD4) with a pdb code of 4WIV (McKeown et al., 2014) accessed from the RCSB Protein Data Bank (Berman et al., 2000). The ligand used is the native ligand of the 4wiv structure, namely N-tert-butyl-2-[4-(3,5-dimethyl-1,2-oxazol-4-yl)phenyl]imidazo[1,2-a]pyrazin-3-amine with pdb code of 3p2 (Figure 2).

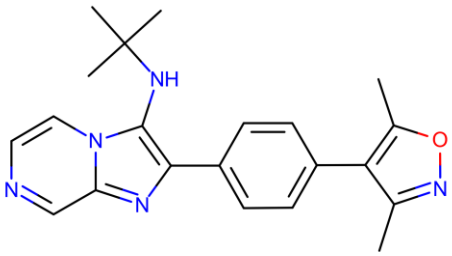

Figure 2. 2D structure of N-tert-butyl-2-[4-(3,5-dimethyl-1,2-oxazol-4-yl)phenyl]imidazo[1,2-a]pyrazin-3-amine

The hardware used is a set of personal computers with Intel(R) Core(TM) i5-10400 CPU @ 2.90GHz, GPU Intel(R) UHD Graphics 630, 16 GB DRAM. The operating systems used are Windows 11 and Ubuntu 22.04 LTS on Windows Subsystem for Linux (WSL 2.0). The main software used is GROMACS version 2021.4 (Abraham et al., 2015) for MD simulations, PyPLIF-HIPPOS (Istyastono et al., 2020) for PLIF analysis, CHARMM-GUI (Jo et al., 2008; Lee et al., 2016) for the preparation of protein structures and as the solution builder for MD simulations. The research procedure is summarized in Figure 3.

This research was conducted on three BRD4 structures, namely the wild-type (WT) structure and its two mutant structures namely: A89V (Ammar et al., 2022; Stenson et al., 2003) and W81A (Jung et al., 2014). Manipulation was carried out using CHARM-GUI software by replacing amino acid no. 89 in the WT structure from alanine to valine for mutant A89V and replacing amino acid no. 81 from tryptophan to alanine for the W81A mutant.

MD simulations were performed on each protein structure, both the wild type and the two mutants. The water box is set in a rectangular shape with an edge distance of 10 Å. Simple electrolytes for neutralization of Na and Cl were added using the Monte-Carlo method at a concentration of 0,15. The force field employed is AMBER (Lee et al., 2020), with the potential forces categorized as FF19SB for protein interaction models, TIP3P for water interaction models, and GAFF2 for ligand interaction models. Equilibration Input Generation using the NVT Ensemble and Dynamics Input Generation using the NPT Ensemble. The production temperature is set at 310 K. The simulation duration is set for 25 ns, with a timestep of 2 fs. Grid information settings for Periodic Boundary Conditions (PBC) are set automatically using the Particle-Mesh Ewald (PME)/Fast Fourier Transform (FFT) method.

Protein-ligand interaction fingerprinting was performed on the MD simulations output. Trajectory files in the form of *.xtc were converted using the *gmx trjconv* module to become *.pdb files for each frame throughout the MD simulations. The resulting *.pdb files were then used as input for PyPLIF-HIPPOS. To decode the outputs of PyPLIF-HIPPOS, which are in binary data, into qualitative data of the names of amino acids and the types of interactions formed, the *pdb2plif.sh* and *md2plif.sh* scripts (Istyastono & Riswanto, 2022) were utilized.

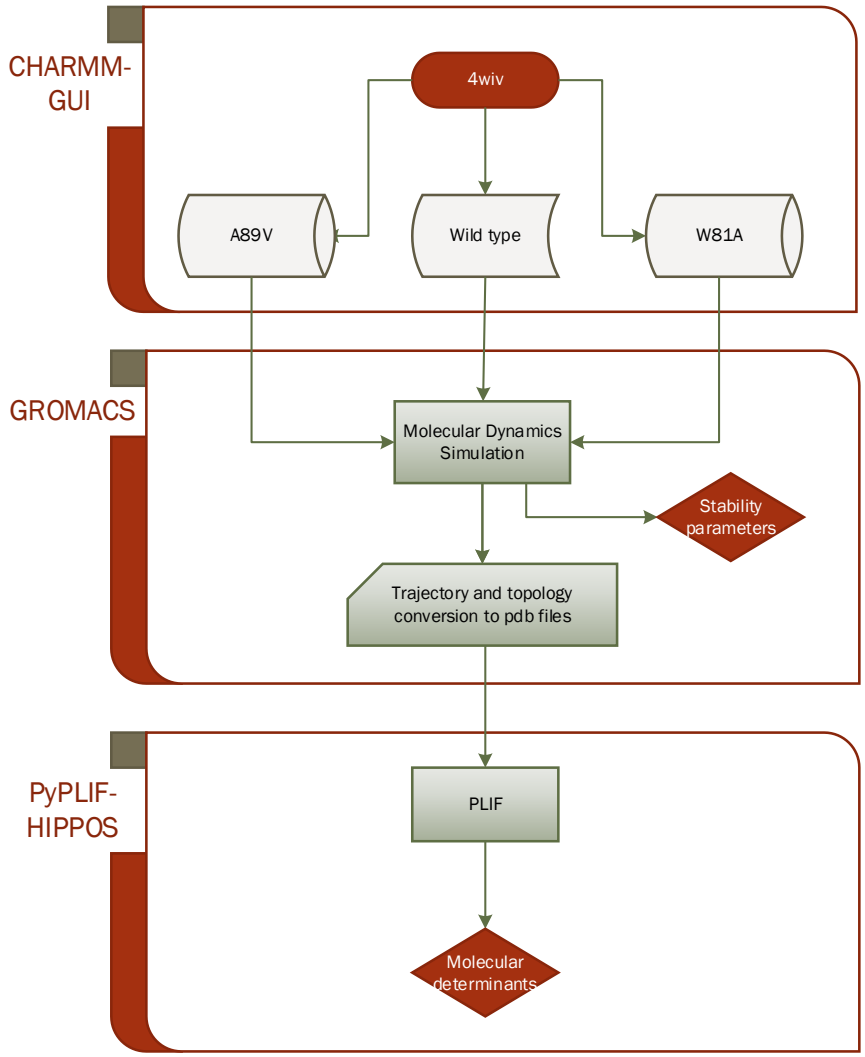

Figure 3. The flowchart of the experiment protocol

RESULTS AND DISCUSSION

This research was conducted by utilizing the capabilities of MD simulations and PLIF to decipher the molecular basis of LBS mutations in protein-ligand interactions, specifically in the case of bromodomain mutations. MD simulation is a computational technique that models the motion and behavior of atoms and molecules over time. It employs classical physics principles to simulate the movement of atoms in a system, allowing investigation of dynamic behavior and conformational changes of proteins and their interactions with ligands. MD simulations can provide insights into the structural and dynamic aspects of protein-ligand complexes, including the flexibility and stability of binding sites, the conformational changes induced by ligand binding, and the energetics of the interactions. By simulating the mutated protein-ligand complex and comparing it with the wild-type complex, MD simulations can help understand the impact of mutations on the stability of protein-ligand interactions. PLIF is a method used to characterize and compare protein-ligand interactions based on their structural features. PLIF generates a binary representation, or fingerprint, that summarizes the three-dimensional nature of interactions between a protein and a ligand. It captures information about various types of interactions, such as hydrogen bonds, van der Waals contacts, electrostatic interactions, and hydrophobic interactions. PLIF provides a quantitative measure of the similarities and differences between different protein-ligand complexes and can be used to analyze and compare the effects of mutations on ligand binding. By comparing the fingerprints of the wild-type and mutant complexes, it is possible to assess how mutations affect specific interactions and

binding modes, providing insight into the effects of mutations on protein-ligand interactions. One of the software tools for PLIF identification is PyPLIF-HIPPOS. While its primary application pertains to docking result analysis (Istyastono et al., 2020), it can also make significant contributions to MD simulations. Through the utilization of PyPLIF-HIPPOS, the ability to analyze molecular interaction fingerprints within the context of MD trajectories can be acquired. These tools facilitate the comprehension of how ligands interact with target receptors over time, aiding in the identification of essential interactions and stability patterns. The amalgamation of MD simulations with PyPLIF-HIPPOS provides profound insights into the stability and dynamic behavior of molecular complexes, which are critical aspects for both rational drug design and the investigation of ligand-receptor interactions. Several prior studies have leveraged the PyPLIF-HIPPOS combination alongside MD simulations for various purposes. This combination effectively elucidated the stability of the interleukin-17A complex with the small molecule STK630921 (Riandono & Istyastono, 2023). Subsequent research demonstrated the capability of this combination to discern interaction hotspots between dipeptidyl peptidase IV (DPP4) and its inhibitor, caffeic acid, through the course of MD simulations (Istyastono & Riswanto, 2022). Furthermore, this amalgamation proved instrumental in identifying protein-ligand interactions of ABT-341 with Dipeptidyl Peptidase IV during MD simulations (Perdana Istyastono & Gani, 2021). PyPLIF-HIPPOS is a valuable tool for assessing complex stability during MD simulations. Therefore, this study aims to harness the capabilities of this combined approach to investigate the effects of mutations on protein-ligand complex stability.

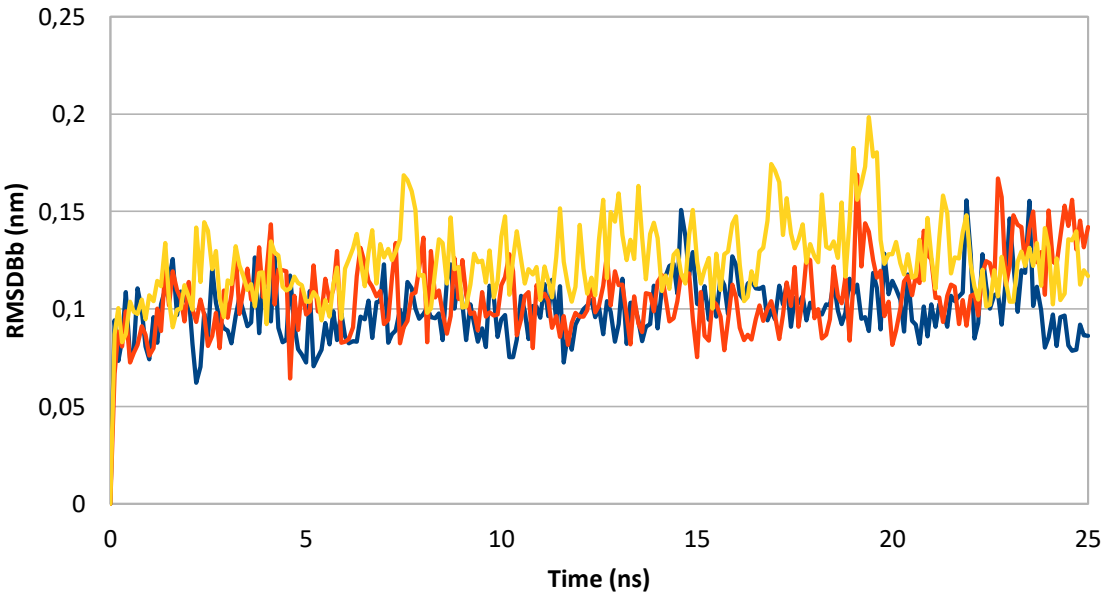

Figure 4. RMSDBb vs simulation time. The yellow color indicates the W81A ligand-mutant complex, the orange color indicates the A89V ligand-mutant complex, and the red color indicates the wild-type ligand complex.

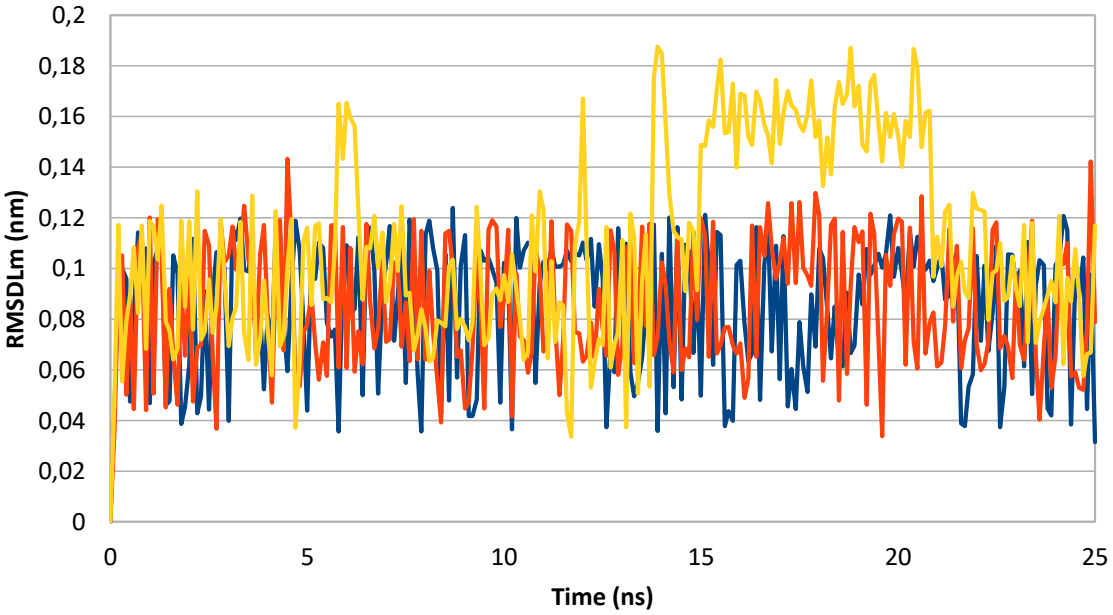

Figure 5. RMSDLm vs simulation time. The yellow color indicates the W81A ligand-mutant complex, the orange color indicates the A89V ligand-mutant complex, and the red color indicates the wild-type ligand complex.

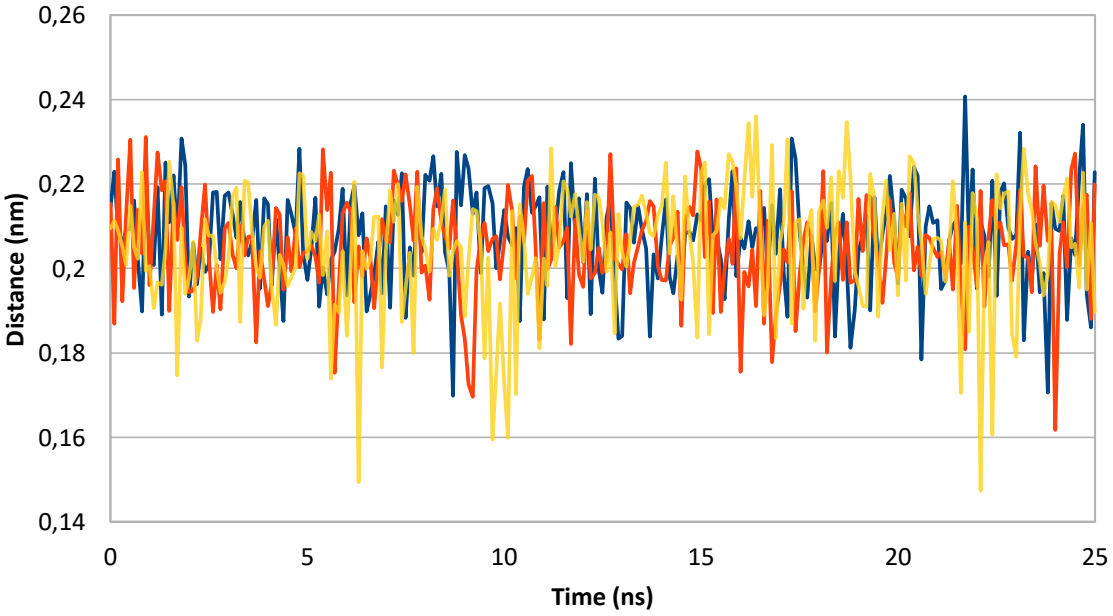

Figure 6. MINDIST vs simulation time. The yellow color indicates the W81A ligand-mutant complex, the orange color indicates the A89V ligand-mutant complex, and the red color indicates the wild-type ligand complex.

Mutations in the LBS region of the bromodomain appear to disrupt the stability of complexes formed to ligands. This can be seen in the RMSD of the backbone atoms (RMSDBb) value, which is a measure of how much the atoms in the backbone of the protein have moved from their original positions. A protein structure is considered more stable if there are smaller deviations, and less stable if there are larger deviations. Typically, to obtain useful information, the RMSD value for a macromolecule should be below 0,2 nm (Liu & Kokubo, 2020; Shukla & Tripathi, 2020). Figure 4 shows the RMSDBb values for a set of proteins with and without LBS mutations. While the

RMSDBb values for all three systems remained below the 0.2 nm threshold, it is important to highlight that the mutant protein structures display higher RMSDBb values compared to the native conformation. Notably, the W81A mutation shows the most significant deviation, indicating a decrease in stability. The mutant protein has a higher RMSDBb than the wild type, with the highest amplitude shown by the W81A mutation, indicating that they are less stable. RMSD Ligand movement (RMSDLm) throughout the simulations showed that protein mutation caused increased ligand movement towards the initial position, with the highest amplitude in mutation W81A (Figure 5). The dynamics of "the minimum distance of the ligand from the protein structure" (MINDIST) are directly proportional to RMSDLm, the W81A mutant has the largest MINDIST fluctuation than other complexes (Figure 6). The MD simulations stability parameters above show that the loss of Trp81 is responsible for the change in complex stability.

The vital role of the Trp81 residue is proven by the dynamics of the molecular determinants during the MD simulations through protein-ligand interaction fingerprinting using the PyPLIF-HIPPOS software (table 1). The distribution of molecular determinants underwent significant changes due to the W81A mutation.

Table I. The molecular determinants of the interactions in the respective protein-ligand complexes

Residue and interaction type	Wild Type	A89V	W81A
ILE146 hydrophobic	8,84%	8,43%	10,54%
LEU94 hydrophobic	8,73%	8,50%	10,06%
PHE83 hydrophobic	8,48%	8,50%	10,32%
LEU92 hydrophobic	8,31%	8,30%	10,40%
TYR139 hydrophobic	8,09%	7,58%	7,53%
TRP81 hydrophobic	7,99%	7,82%	**0,00%**
TRP81 aromatic edge to face	7,56%	6,49%	**0,00%**
ASN140 hydrophobic	7,02%	5,29%	5,83%
VAL87 hydrophobic	6,67%	6,11%	6,62%
TYR97 aromatic edge to face	5,88%	4,78%	3,27%
TYR97 hydrophobic	5,60%	6,28%	5,01%
MET149 hydrophobic	4,81%	2,80%	1,57%
PRO82 hydrophobic	4,53%	6,96%	9,40%
TYR139 aromatic edge to face	3,14%	3,65%	4,75%
TYR139 aromatic face to face	2,07%	1,60%	1,22%
LYS91 hydrophobic	0,96%	2,46%	1,13%
GLN85 hydrophobic	0,89%	3,58%	4,35%
ASP145 hydrophobic	0,25%	0,10%	0,26%
ASP145 ionic as the anion	0,14%	**0,00%**	**0,00%**
TRP81 aromatic face to face	0,04%	0,03%	0,00%
PHE79 hydrophobic	0,00%	0,00%	0,04%
ASP88 hydrophobic	0,00%	0,03%	0,44%
PRO86 hydrophobic	0,00%	0,68%	2,57%
ALA81 hydrophobic	0,00%	0,00%	4,70%

Tryptophan is an aromatic amino acid with a large, planar side chain. This side chain is well-suited for forming non-covalent interactions with ligands, such as hydrogen bonds, π-π stacking, and cation-π interactions. On the other hand, Alanine is a non-aromatic amino acid with a small, aliphatic side chain. This side chain is not as well-suited for forming non-covalent interactions with ligands. When a protein undergoes a tryptophan mutation to alanine, it can affect the protein's affinity for its ligand. Tryptophan is known to be a hydrophobic amino acid, which means it is not attracted to water and instead attracted to other hydrophobic molecules. Alanine, on the other hand, is a hydrophilic

amino acid, which means it is attracted to water and repelled by hydrophobic molecules. As a result, a tryptophan mutation to alanine can change the protein's specificity for its ligand. For instance, a protein that typically binds to hydrophobic ligands may not bind as effectively after a tryptophan mutation to alanine. Tryptophan also has an important role in stabilizing the protein's structure by forming non-covalent interactions with other residues in the protein. In contrast, alanine is not as effective in forming these interactions. Therefore, a tryptophan mutation to alanine can lead to problems such as reduced protein stability, altered protein function, and even toxicity. However, a valine mutation to alanine will have a minimal impact on protein-ligand interaction, as alanine and valine are both nonpolar amino acids with similar chemical properties. TRP81 plays a significant role within the binding site of the bromodomain for ligands. Previous studies have demonstrated its involvement in recognizing acetylated histones and its capability to bind the small molecule inhibitor JQ1 to BRD4 (Jung et al., 2014; Lori et al., 2016). Moreover, TRP81 may impact the *druggability* of the binding site, which is the ability to bind to small molecules that can modulate its activity (Vidler et al., 2012). The result of this study thus confirms TRP81 as a molecular determinant for the stability of the complex.

CONCLUSION

The protocol implemented in this study revealed the complexes' stability and the molecular determinants responsible for the interaction between the mutated bromodomain binding site and its ligand. The combination of MD simulations and PLIF analysis allows for a comprehensive understanding of the effects of mutations on protein-ligand interactions. MD simulations provide dynamic information and can reveal the structural changes induced by mutations, while PLIF analysis offers a quantitative description of the altered interaction patterns between the protein and ligand. By integrating these approaches, the molecular mechanisms underlying the effects of mutations on protein-ligand binding can be well elucidated, which is valuable for such as drug discovery, understanding genetic diseases, and predicting drug resistance. The LBS mutation in W81A causes a disturbance in the stability of the ligand-bromodomain complex. The residues and non-hydrophobic interaction types responsible for the stability were identified as TRP81 aromatic edge-to-face, TYR139 aromatic edge-to-face, and TYR139 aromatic face-to-face.

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