# Anti-acne Property of Octopus Skin Pigment, Xanthommatin An Insilico Evaluation

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**Short Report**

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# Abstract

Acne vulgaris and other diseases such as endocarditis, endophthalmitis and prosthetic joint infections are caused by the pathogenic bacterium, Propionibacterium acnes. In particular, Acne vulgaris affects people of all ages. Research prospects in the field of skin health has benefitted tremendously by the use of computational systems employing sophisticated docking algorithms and simulation studies to help identify right chemical compounds to target opportunistic pathogenic bacteria. Xanthommatin, an antioxidant skin pigment molecule of the marine organism, Octopus, or in general, Cephalopods, has been identified, for the first time in the world, as a potential candidate for topical application to target the acne bacterium through bioinformatics studies. Xanthommatin is shown to interact with the surface protein, Sialidase enzyme, of the P. acnes bacterium through docking studies followed by the MD simulation studies for 100 nanoseconds.


# Introduction

The gram-positive bacterium known as P. acnes has a strong connection to the condition known as acne vulgaris. Isotretinoin, also known as 13-cis-retinoic acid, is a retinoid that is derived from vitamin A and has been used to treat severe acne since its introduction though its usage is tightly controlled. Other treatments, such as systemic antibiotic therapy have the potential to kill bacteria on the skin in a non-specific manner. There is also the possibility that oral antibiotics will cause damage to the microflora that lives in the intestines. Furthermore, it has been reported that P. acnes can transfer anti-bacterial resistance to other bacteria that are present in the resident skin microflora when systemic antibiotic therapy is administered. Recent research has shown that vaccines that decrease the inflammation and pathogenesis caused by P. acnes can be therapeutically useful but it will take another five to ten years for them to hit the market(1). Until then, topical treatments will provide some level of comfort. So far, plant based chemical compounds have been used in the ointments but the disadvantage is the use of suspension media due to very low solubility in safe solvents such as water. Octopus, or in general, Cephalopods have been around for more than 300 million years but the secret that their skin pigment, Xanthommatin possess anti-acne property wasn't known until now. Synthetic versions of Xanthommatin and its salts are highly water soluble(2) and are currently available and they target the surface enzyme, Sialidase, of the bacterium P. acnes. Xanthommatin is able to ineract with the key Arginine triad, Arg 121, Arg 329 and Arg 395 and the stability of the interaction provides sufficient evidence to do further studies on its effectiveness as a topical anti-acne solution(3).

# Materials and methods

# Software and Modules

Schrodinger and Desmond simulation software (4) were used to obtain the necessary knowledge about the interactions at the atomic level. The same set of softwares are used to understand the stability of the complexes formed. The software modules, namely, protein preparation, Grid generation, Site map

prediction, Ligprep, and glide docking (5) are used to study molecular mechanical interactions of the complex between xanthommatin salt and the sialidase protein of the P. acnes bacterium. Quantum Mechanical procedural software consists of the modules that monitors simulation's real time dynamism, interactions and its quality (6,7). The QM software was used to study the stability of the xanthommatin – sialidase enzyme complex in a better way.

## Protocol for preparing the Ligand and the Protein

To promote interaction with the Sialidase protein (7LBV pdb), the energy of the xanthommatin molecule must be reduced to a local energy level that ensures its stability. The protein was preprocessed by setting on the Assign bond order using the CCD database along with hydrogens substituted (8). In the preparation method, the PDB file 7LBV was used as an entry for the source. The metal and disulfide linkages are made using zero-order. The pH level of 7.0 +/- 2.0 was generated using the Epik module.

The protein after preprocessing was assigned an optimised H-Bond using the PROPKA optimisation technique (9) and sample water orientation. Finally, the protein was minimised using the OPLS4 force field computation, RMSD coverage of heavy atom set as 0.30 A. Any water molecule beyond 5 A from the PDB ligand was removed. The Xanthommatin molecule was prepared for docking by the LigPrep technique. By enabling parameters of OPLS4 force field, the Epik algorithm was utilised to desalt the system. (In total, 64 stereoisomers of the ligand molecule were created.)

## Analysis of the binding pocket by the Grid generation method

The receptor grid-generating procedure used the ligand position in the pdb protein 7LBV as a suitable site for binding xanthommatin. The VdW radius scaling factor is set to 1.0, with a cutoff partial charge of 0.25. The enclosing box was sized to fit the workspace ligand's centroid, and the dock ligands were set to be 12 inches long.

## SET UP FOR DOCKING LIGANDS

Scaling factor approach was set to 0.80 for ligand docking, and partial charge cutoff for the van der Waals radii was set to 0.15. Using the extra precision technique, the flexible ligand sampling setup was used in conjunction with the imported grid (10). For bias sampling of torsions, all predefined functional groups were used along with ring conformations and sample nitrogen inversion. Finally, Epik state penalties raised the docking score. The energy for the ring sampling window was set at 2.5 kcal/mol for the generation of conformers, and the dielectric constant value as a function of distance was chosen at 2.0 for minimization (11). The Prime-MMGBSA approach was also used to investigate binding energies with 7LBV protein for better poses of xanthommatin. The solvated model was set as VSGB and force field protocols was set as OPLS4(12).

## Stability studies of Dynamic Simulation

The stability of best-docked pose of the Xanthommatin was analysed using the system builder protocol in Desmond software. The xanthommatin – 7LBV system was initially solvated for molecular dynamic simulation using the SPC solvent model with orthorhombic boxes as boundary conditions, and the size of buffer box was estimated at a distance of 10.0 A.

Furthermore, the system was neutralised with sodium and chloride ions, and it's final volume was minimised based on the system's surface occupation. All the settings for trajectory recording like the energy gap, simulation length, time interval was set suitably(13). For the NPT ensemble class, 1000 frames were created at normal temperature and pressure after relaxing the model before the simulation. The completed simulation was loaded, and the average length of the block was set to 10.0 ps for the trajectory quality analysis. The simulation quality analysis technique is used to generate graphs using the parameters Potential energy (kcal/mol), Total energy (kcal/mol), pressure (bar), temperature (k), and volume (A3). The simulation interaction diagram module calculated the RMSD, RMSF, and Torsion values of a complex across a time scale of 100 ns.

## Results and Discussion

Docking Analysis of Xanthommatin with the Sialidase Protein

Glide docking procedures revealed that xanthommatin formed an excellent pose in the location for binding. The Xanthommatin's binding site energy was found to be -2.185 kcal/mol and the residues with which it binds are shown in Fig. 1.

The carboxylate ion is anchored by the Arginine triad of the catalytic center, Arg 121, Arg 329, Arg 395, along with the hydrophobic contacts Tyr 423, Ala 147, Pro 148, Phe 209 among others(2). An Mmgbsa value of -32.54 kcal/mol for the binding of Xanthommatin with the active site confirms the strength of the binding.

## Simulation Dynamics Stability Analysis

Interaction studies alone cannot establish the Xanthommatin's potential efficacy and bond formation. It should be dynamically tested utilising MD simulation experiments (14). The Xanthommatin - enzyme complex was dissolved in water and neutralised with Na + and Cl- ions. The system builder protocol included 5602 water molecules for the 100ns simulation trials for the Curcumin derivative active site binding complex (Fig. 5).

The quality of the molecular dynamic simulation run was investigated, and the parameter values were discovered to be within a suitable range. As demonstrated in Fig. 6, the complicated simulation quality has trustworthy values for volume (1984368.644), pressure (1.183 bar), temperature (298.707), and

potential energy (-646278.828). The overall energy values of (-527580.359 kcal/mol) demonstrated that the complex of Xanthommatin with the Sialidase protein remained stable across the 100 ns simulation.

**Root Mean Squared Deviation( RMSD) and Fluctuation (RMSF) of the Protein-ligand Interactions in the active site**

The 7LBV-xanthommatin complex was stable from 20 to 60 nanoseconds and further from 70 to 100 nanoseconds, according to the RMSD graph, with an RMSD range of C-α 1.75–2.2 (Fig. 7). Based on the RMSD the complex was later confirmed to be completely stabilised.

The interactions between Xanthommatin and 7LBV protein shown in Fig. 5, favored the stablity for 100ns. During the simulation, the carboxylate ions of the ligand was able to favour considerable interactions with Arg 121, Arg 329, and Arg 395 which forms an active triad of the catakytic centre of the sialidase protein of the P. acnes bacterium. These interactions and amino acid combinations help to stabilise the complex and decrease oscillations.

The graph in Fig. 6 depicts the Root Mean Square Fluctuation (RMSF) of the Cα atoms across the residue indices of a protein, highlighting its structural dynamics, with residues establishing bonds with the ligand shown as green bars. Peaks in the RMSF values, such as the prominent one near residue 150 and those at the termini, indicate highly flexible regions, often corresponding to loops or unstructured segments, while troughs suggest more rigid regions, typically associated with secondary structure elements. Ligand contacts are observed at 17 distinct residue indices, with fluctuations reaching up to 1.8 Å, and an average RMSF of 1.8 Å was calculated for these interactions.

The Xanthommatin ion binds very strongly in the active site of the sialidase protein. The MD tests confirmed the stability of the sialidase protein-xanthommatin complex while binding.

# Conclusion

The results of this study's interaction analysis and stability examinations reveals that Xanthommatin is capable of specifically altering or inhibiting the sialidase protein of the acne causing bacterium. The MD stability analysis encourages the formation of unbreakable non-bonded links, which keep the molecule stable within the protein.

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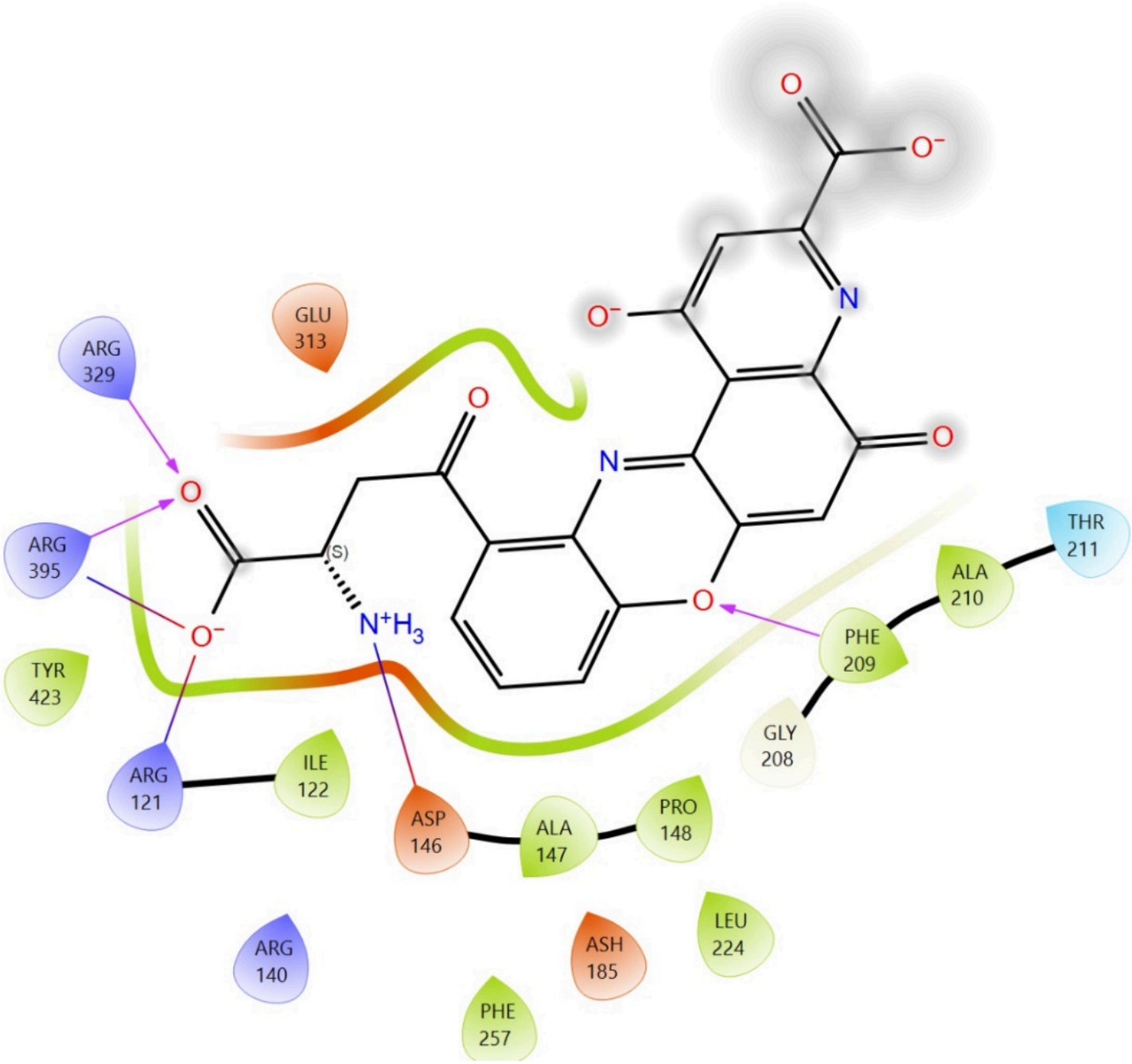
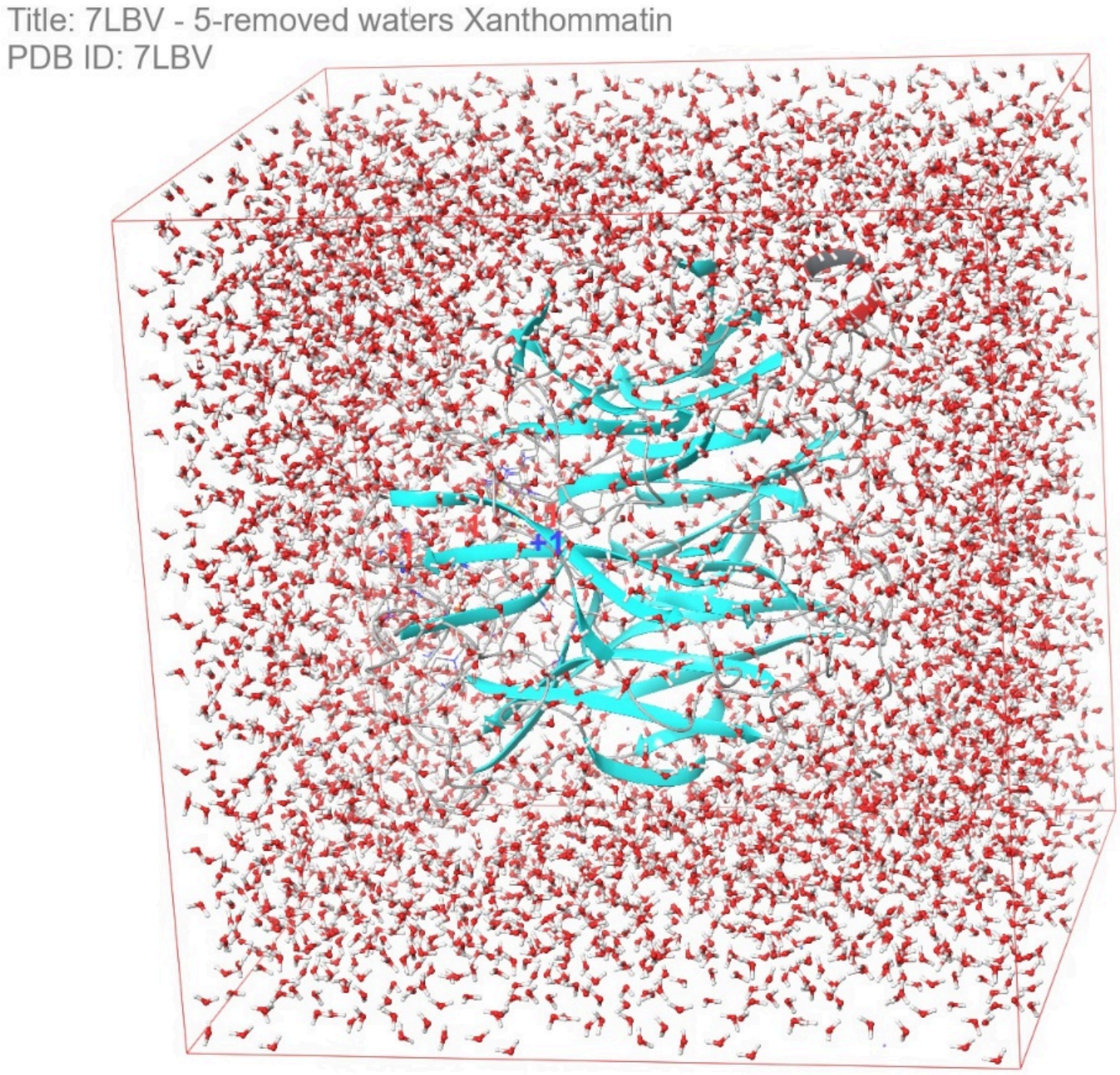
# Figures




## Figure 1

2-Dimensional representation of the interaction between amino acids and Xanthommatin ion in the 7LBV PDB's active site




## Figure 2

Xanthommatin binding to the active site in a solvated model.

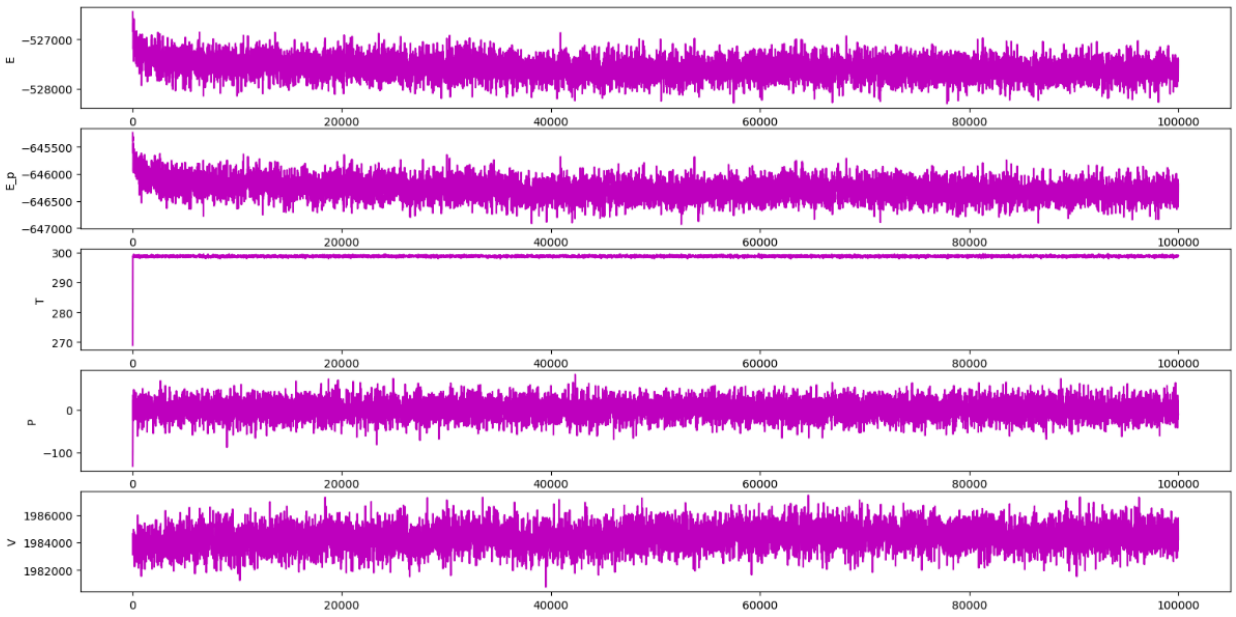



**Figure 3**

Xanthommatin-7LBV protein simulation run quality analysis report for 100 ns

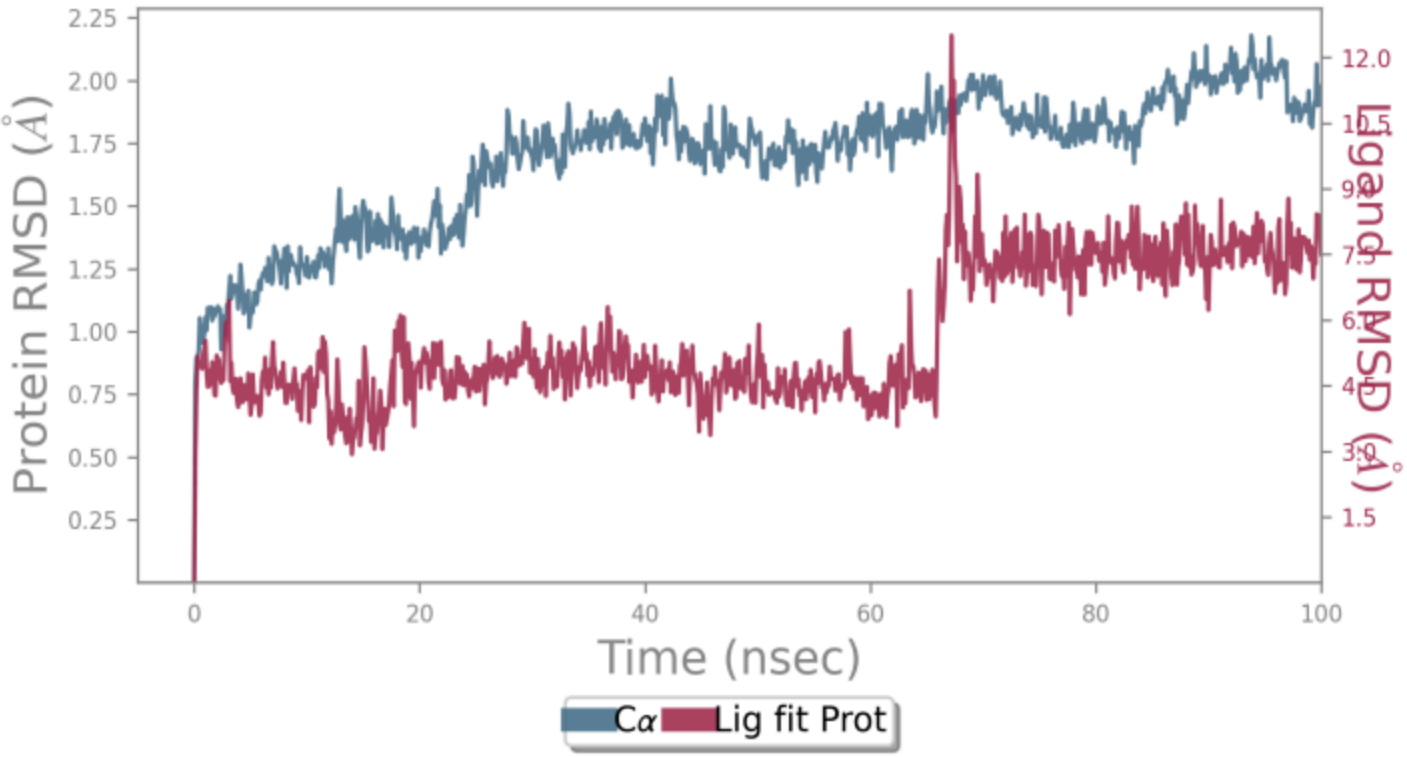



**Figure 4**

RMSD of different conformations of 7LBV-xanthommatin  for the duration of 100ns.

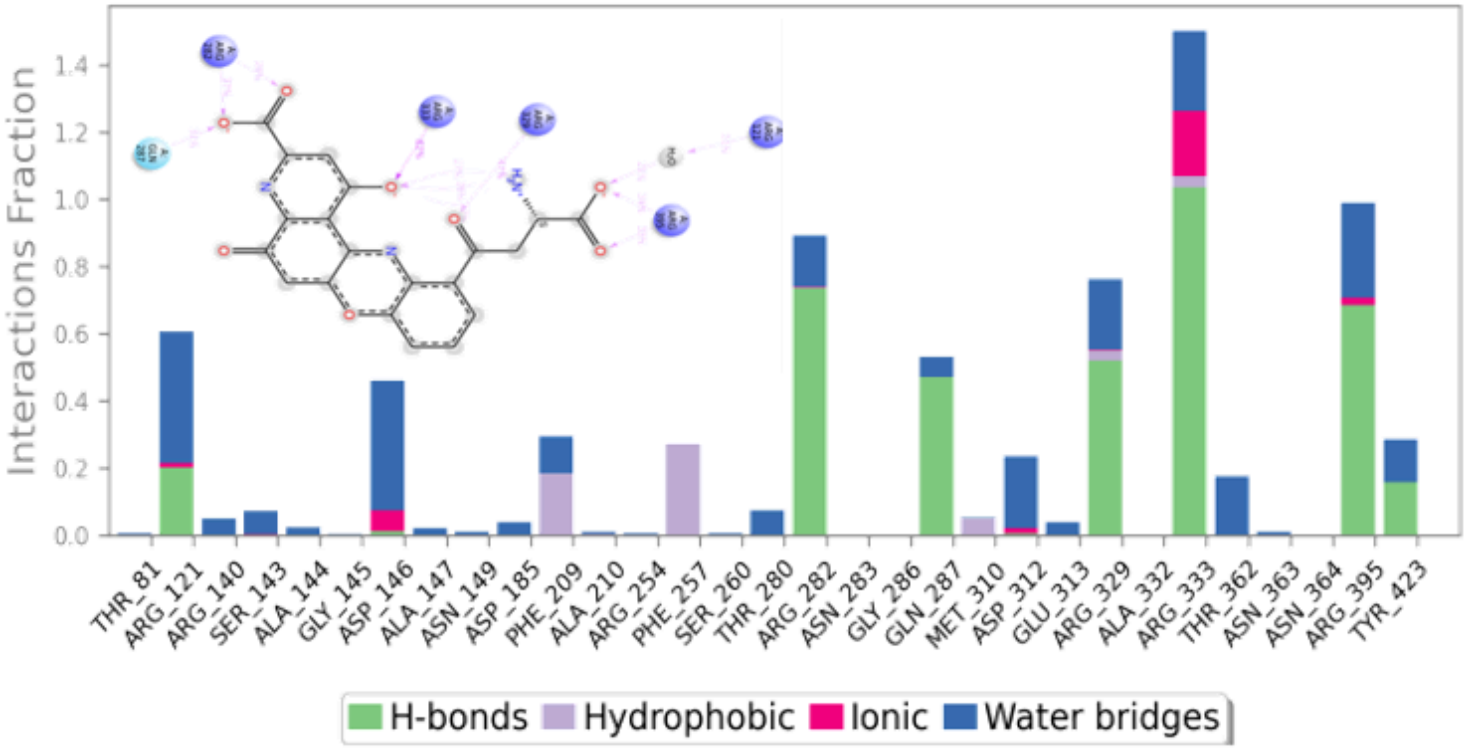



**Figure 5**

Interaction chart of the curcumin derivative during the 100ns simulation

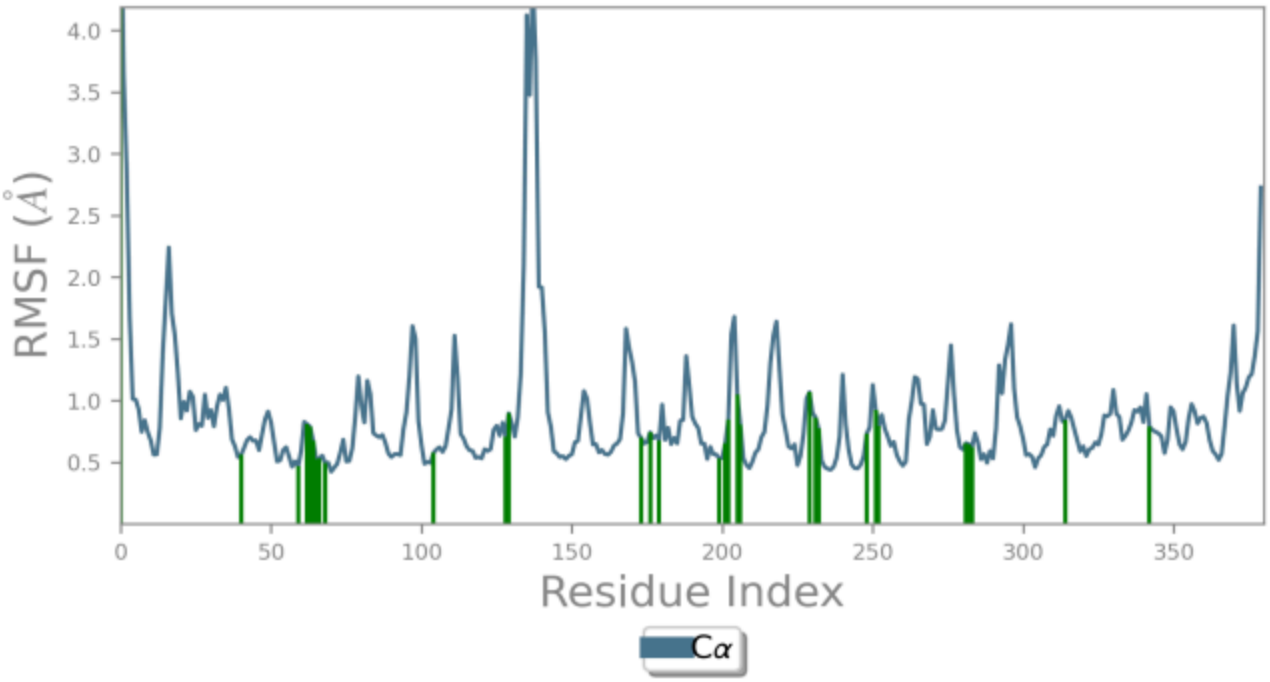



**Figure 6**

Xanthommatin-7LBV protein's RMSF.