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Original Research Article

Molecular Dynamics, Docking and Prediction of Absorption, Distribution, Metabolism and Excretion of Lycopene as Protein Inhibitor of Bcl2 and DNMT1

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ABSTRACT

Docking has been used currently in the early development of a drug candidate and a useful tool to predict the absorption, distribution, metabolism, and excretion (ADME) properties. Lycopene shows the strongest antioxidant activity among carotenoids, and has anticancer activity. This study predicts the ADME and simulates the docking of lycopene to predict the binding to the B-cell lymphoma 2 (Bcl2) and DNA (cytosine-5)-methyltransferase 1 (DNMT1) proteins in cancer cells. The computational method was conducted using pkCSM and SWISS ADME applications, and Gnina software to simulate lycopene docking to the proteins. Results show that lycopene has a molecular weight > 500, partition coefficient (log P) > 5, with a limited aqueous solubility, low skin permeability, however shows a good intestinal permeability and an active uptake through the blood brain barrier, the Convolutional Neural Network (CNN) Pose Score in protein Bcl2 of 0.7843, and DNMT1 score of 0.1279. Lycopene is predicted to have an interaction with Bcl2. There is a pi-alkyl interaction with amino acids PHE63 and TYR67, and a Pi-Sigma interaction with amino acid TYR161. ARG66, ALA108, LEU96, MET74, and VAL92 are among the amino acids that exhibit alkyl-alkyl interactions. Homology modelling was implemented due to the presence of a gap in the sequence within the chain, specifically between residues 31 and 49. The stability of lycopene-substrate interactions was evident during 20 ns molecular dynamics simulations, indicating its consistent behavior over the duration.

Keywords: Lycopene, Cancer, Docking, Molecular Dynamics, Bcl2, DNMT1

Introduction

Lycopene is a secondary metabolite of the carotenoid group, which shows the strongest antioxidant activity when compared to other carotenoids such as α-carotene, β-carotene, zeaxanthin and lutein.[1] The bioactivity of lycopene as an anticancer agent has been tested in vitro, in vivo, and in epidemiological studies. In addition to its radical scavenging activity, the moleculer mechanism of lycopene have been reported such as the inhibition of cell proliferation, the inhibition of cell cycle progression via modulating cell cycle regulatory proteins, the induction of apoptosis, and the regulation of several signal transduction pathways.[1] Previous studies have tested lycopene extract from tomato products against apoptosis, tumor protein 53 (TP53), Bcl-2 Associated X-protein (BAX), and Bcl2 expression in prostate cancer. The results showed that lycopene from four of tomato products inhibited cell viability, increased apoptosis and regulated transcriptional expression levels of TP53, BAX and Bcl2 prostate cancer cells at the dose used. [2] Studies on the effect of lycopene on cell viability in several human cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl**tetrazolium** bromide (MTT) assay have also been carried out, showing that the anticancer potential of lycopene on cell viability depends on time of exposure and dose.[3]

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Bcl2 and DNMT1 are proteins involved in cancer, Bcl2 has an important role in regulating apoptosis, the process of apoptosis through the mitochondrial pathway occurs due to caspase activation (caspase cascade). This activation is regulated by the Bcl2 group protein which plays a role in influencing mitochondrial permeability for the release of cytochrome C into the cytosol and binds to Apoptotic Protease Activating Factor-1 (APAF1) to form the apoptosome. This apoptosome activate pro-caspase-9 to caspase-9 which will inhibit cell death. DNMT1 is a protein that catalyzes DNA methylation which responsible for causing cervical cancer. It inhibits the transcription of tumor suppressor genes and facilitates tumorigenesis formation and finally develops into cervical cancer cells. From this explanation, these two proteins were selected for lycopene docking simulations to obtain the information about an interaction of lycopene with Bcl2 and DNMT1 as the basis for the in vitro test of lycopene as an anti-cancer drug, especially cervical cancer.[2, 3]

The use of computing in the field of chemical research is a new trend in the development of new drugs, such as using Artificial Intelligence (AI), databases and big data, thereby helping to understand the properties of a compound much more quickly and effectively in screening new drug candidates.[4, 5] Modelling the interaction between substances and biological targets provides an initial description of the physicochemical properties, toxicological effects and potential drug targets using computational methods (Machine Learning). [6] This study was conducted to predict the absorption, distribution, metabolism, excretion and to simulate docking of lycopene with proteins to predict its binding to Bcl2 and DNMT1 proteins.

Materials and Methods

Docking Simulation

The simulation in this study was conducted using a Graphics Processing Unit (GPU) 1660 Super with CUDA version 11.6. The target protein

Bcl2 (PDB 4EIH[7] and DNMT1 Chain B (PDB 4WXX) used in this study were downloaded on the website of protein data bank https://www.rcsb.org/. [8] Simulation of Docking was conducted using Software Gnina version 1.0.2 with blind docking. [9] This software is used as Deep Learning scoring function. The natural ligands from protein crystallography were compared with the docking results. This was done to see the Root Mean Square Deviation (RMSD) value. The RMSD score < 2 is the validation parameter employed, and it shows how closely the software can simulate molecular docking to crystallographic results. A rectangular prism will be automatically generated to find the maximum coordinates of x, y, and z using the CNN verbose software in Gnina. The simulation will use a grid box with exhaustiveness set at 64 and an autobox ligand. Tight bindings from xTB version 6.0.4 are used to perform geometry optimization on lycopene molecules. [10] Predictive analysis of docking interactions between protein and lycopene was analyzed using Discovery Studio version 2022.

Absorption, Distribution, Metabolism, and Excretion (ADME) predictions

The PKSCM website (http://structure.bioc.cam.ac.uk/pkcsm) was used to analyze lycopene's ADME. [6]

Homology Modeling

Homology modelling was built using the Bcl2 protein to solve the missing residue. It used SWISS-MODEL https://swissmodel.expasy.org/. [10, 11, 12] The search for templates was done first and followed by building the new model. The homology modelling pdb file is superposed using Chimera.

Molecular Dynamics

Gromacs version 2022.2 was used to simulate molecular dynamics. [13] The proteins preparation was carried out using pdb2gmx, employing AMBER14SB force field. [14] Parameter and topology file were prepared using ACPYPE with GAFF2 force field. [15, 16] The water model used was TIP3P. [17] A minimization process was carried out using 50,000 steps, with a maximum force threshold at < 1000.0 kJ/mol/nm. The equilibration stage used a canonical ensemble (NVT) with a verlet cutoff scheme and a coulomb type Particle Mesh Ewald (PME) with a step of 100 ps. [18] Then proceed with the isothermal-isobaric equilibration (NPT) process with Parinello-Rahman pressure coupling. [19] The production of simulation was carried out for 20 ns at a temperature of 310 K. [20]

Result and Discussion

Lipinski have analyzed more than 2000 drug compounds in clinical trials and concluded that a drug compound will have good absorption or permeation if the molecular weight is less than 500 Da, the log P value is less than 5, the number of hydrogen bond donors is less than 5, and the number of hydrogen bond acceptors is less than 10. [21] The Lipinski's rule analyzed the physicochemical properties of ligands to determine the hydrophobic-hydrophilic character of a compound and the passage through cell membranes by passive diffusion. [21] This rule was later called the "Lipinski Rule of Five". [22] The molecular weight of lycopene (Table 1) is more than 500 Da, thus it cannot diffuse across the cell membrane. Log P values greater than 5 indicates that the compound tends to have a very large volume of distribution leaving low concentration in blood therefore the selectivity of binding to the target enzyme is reduced. If Log P value is too low the molecule cannot pass through the lipid bilayer membrane due to its hydrophilic properties. [21] The number of hydrogen bond donors and acceptors describes the capacity for absorption, the higher the hydrogen bonding capacity, the higher the energy required for the absorption process to occur. Lycopene has neither a proton donor or acceptor in its chemical structure, indicating that it requires high energy to be absorbed, primarily via carrier mediated transport. [23] In general, Lipinski's rule describes a prediction of the ability of compounds to penetrate cell membranes by passive diffusion. [22]

Data in Table 2 suggest the ADME prediction of lycopene based on its physicochemical parameters obtained from the docking simulation. The solubility data confirms that lycopene has limited solubility in water, however it shows good intestinal permeability (log Paap > -5 cm/s). [21,24] The skin permeation coefficient (log Kp) value of -2.738 shows that lycopene has low skin permeability. The permeability surface area products (log PS) and the blood brain barrier permeability (log BB) values are high (> -4.5) indicating that lycopene is permeable to the brain through active uptake processes. [25,26] Lycopene does not metabolized by CYP1A2, CYP2C19, and CYP3A4 enzymes in the liver, however the data show overall clearance value of 1.949 ml/min/kg which means that it is anticipated to be eliminated quickly through metabolism of other enzymes or other route of excretion.

The role of Bcl2 in the process of apoptosis is through the intrinsic pathway by influencing mitochondrial permeability to release cytochrome C into the cytosol, which then binds to Apoptotic Protease Activating Factor-1 (APAF1) to form apoptosomes. The apoptosome will activate pro-caspase-9 to become caspase-9 which is the executioner of cell death. DNMT1 is essential in maintaining and regulating DNA methylation. Abnormal expression of DNMT1 leads to aberrant methylation of several CpG tumour suppressor genes, resulting in an inactivation of tumour suppressor genes and cell carcinogenesis. The interaction mechanism can be predicted with an in silico approach, especially with molecular docking. [27]

The target protein for Bcl2 and DNMT1 is accessible for downloading in the form of PDB. Bcl2 and DNMT1 were obtained from separating the proteins from water molecules and their ligand compounds. The protein's crystal structure is then saved in the PDB file. The validation stage by Gnina (Figure 1) involves re-docking the produced ligands with Bcl2 and DNMT1. Preparation of Gridbox uses an autobox from Gnina that will automatically provide Grid information in a form that corresponds to the natural ligand of the receptor.

Table 1: The physicochemical properties of lycopene

No	Physicochemical Properties	Value for lycopene	Lipinsky Rule
1.	Molecular weight	536.888	< 500 Da
2.	Log P	12.938	< 5
3.	Rotatable bond	16	na
4.	Proton acceptor	0	< 10
5.	Proton donor	0	< 5
6.	Surface area	248.007	na

*na = data are not available

Table 2: Prediction of the ADME of Lycopene

No	ADME	Value
1.	**Absorption**	
	Water solubility	-6.514 (log.mol/L)
	P-glycoprotein substrat	Yes
	Intestinal absorption	93.238 (Log Papp in 10^{-6} cm/s)
	Skin-Permeability	-2.738 (%)
2	**Distribution**	
	BBB Permeability	1.086 (Log BB)
	CNS Permeability	-0.267 (Log PS)
3	**Metabolism**	
	CYPI-A2	No
	CYP2C19	No
	CYP3A4	No
4	**Excretion**	
	Total Clearance	1.949 (Log ml/min/Kg)

Table 3: Bonding energy of lycopene with Bcl2 (4IEH) and DNMT1 (4WXX) proteins

PDB ID	CNN Pose Score	Affinity (kcal/mol)	CNN Affinity
4IEH	0.7843	-7.51	7.687
4WXX	0.1279	-7.40	6.503

Information: Convolutional neural network (CNN)

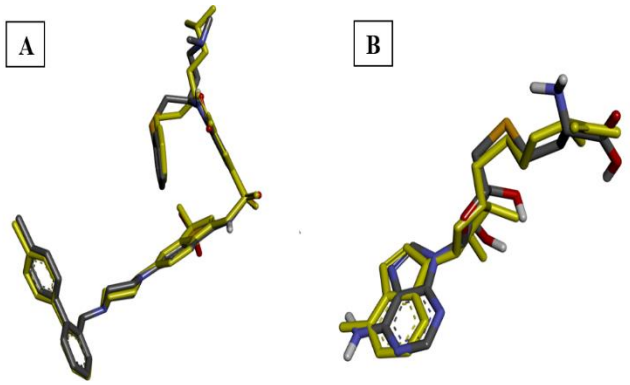

Figure 1. Validation of Protein of Bcl2 (PDB:4IEH) (A) and Protein of DNMT1, chain B pdb (PDB:4WXX) (B)

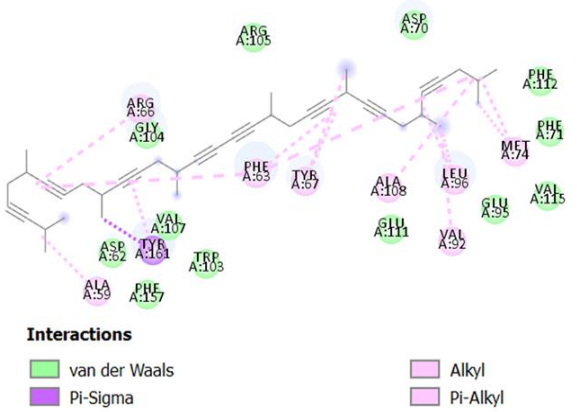

Figure 2: Visualization of Lycopene Interaction with Bcl2 Protein (PDB ID: 4IEH)

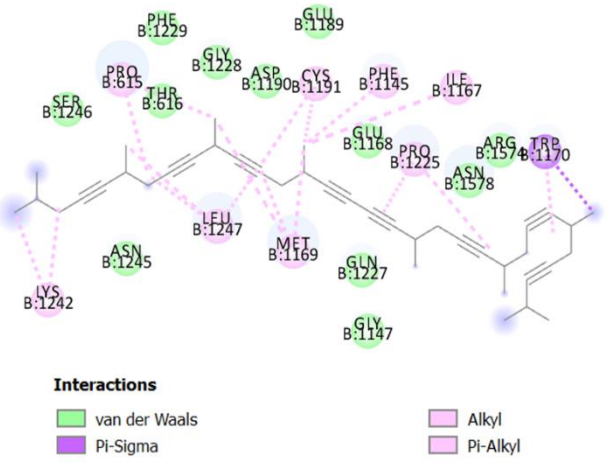

Figure 3: Visualization of Lycopene Interaction with DNMT1 Protein (PDB ID: 4WXX)

The RMSD (Root Mean Square Distance) value is used as the validation parameter. The protocol is acceptable if the RMSD value is less than 2, at which point docking is possible. [28] The minimal bond energy is an additional factor. In the lycopene docking simulation with Bcl2 protein, validation results were obtained with an RMSD value of 0.632 Å and DNMT1 1.01 Å.

The RMSD value indicates that the protocol has been accepted, allowing the docking process to proceed, The docking results reveal that lycopene has a negative binding energy with Bcl2 and DNMT1 protein suggesting the occurrence of spontaneous binding (Table 3). The docking process produces a bond energy value between the test compound (lycopene) and the target protein. The bond energy indicates the affinity of lycopene on the target protein, the more negative the bond energy obtained, the more stable the bond formed between lycopene and the target protein. The pose score that required for molecular dynamics simulation is a value close to one which indicates a similar action to its natural ligand. A potential enhancement in the treatment of cervical cancer could be achieved through the utilization of lycopene in combination with cisplatin chemotherapy. [28] The binding energy values of lycopene with DNMT1 and Bcl2 were -7.51 and -7.40 kcal/mol respectively. This shows that the prediction of binding lycopene with Bcl2 is a spontaneous reaction and is predicted to have a strong interaction when compared to DNMT1. Unlike previous studies, lycopene does not exhibit any interaction with Bcl2. [29]

Figure 2 shows the ligand - receptor interaction between lycopene with the amino acids PHE63 and TYR67. The interaction occurs through a pi-alkyl bond between the aromatic and alkyl groups. The Pi-Sigma interaction is TYR161. The alkyl-alkyl interactions are found in the amino acids ARG66, ALA108, LEU96, MET74 and VAL92. There is only one Pi-sigma interaction on the amino acid TYR161. In Figure 3, there is one Pi-Sigma interaction, TRP1170. The Pi-Alkyl interactions are the amino acids PHE1145, PRO1225 and PRO615. Alkyl-alkyl interactions are LEU1247, MET1169, and ILE1167. Homology modelling was carried out because of the sequence gap in the chain between residues 31 and 49. The following data (Figure 4) describes an arrangement of amino acids after Homology modelling. The modelling stage carried out is sequence alignment. It compares the target amino acid sequence to the template amino acid sequence. [30]

Matchmaking was carried out to see the comparison of proteins before and after homology modelling. Figure 5 shows the arrangement of the amino acids. Local Quality Estimate data with each residue was used to generate the model quality and local similarity targets using the QMEANDisCo Local values. Furthermore, QMEAN Z-Scores for QMEAN, C, Solvation, and Torsion are calculated. Figure 6 and Figure 7 show the outcomes of the experiment.

The identified similarity target in the image above, the blue bars, indicates that the prediction quality is closer to one, which shows a better value as a prediction. The residue between 20 and 80 in the image above has a low local similarity value. A value of -1.28 here indicates that the modelling is not yet at the ideal Score, where the QMEAN standard must be a value close to 1. The QMEAN Score in the image is a composite scoring function that can use a single model to produce absolute quality estimates for the structural system and each residue.

Molecular dynamics simulation was carried out to see the stability of Lycopene against Bcl2 protein with a total time of 20 ns, two data (the RMSD and RMSF) will be obtained. The stability of the complex formed between the natural ligand and Lycopene was analyzed based on the RMSD plot obtained during the simulation, which was for 20 ns (Figure 8). The RMSD plot analysis for the complex formed in the first anchorage model of each natural ligand and Lycopene tested showed that the complex formed was relatively stable. The stability of the two

tested complexes was achieved after the simulation ran for more than 20 ns with fluctuations of Lycopene ranging from 0,1-0,29 nm. As for the native ligand, the movement of the RMSD ranges from 0.1-0.3 nm. The results of the RMSD plot analysis above can be compared with the docking data, which describes the complex state between protein and Lycopene molecules during the time simulation process. [29,30]

Root Mean Square Fluctuation (RMSF) analysis was carried out in this study (Figure 9). The purpose of the RMSF analysis is to observe the flexibility during the simulation time. Results showed that RMSF between Native Ligand and Lycopene had relatively the same fluctuations in amino acid residues. The most fluctuating residue is the amino acid Histidine 53.

Conclusion

The physicochemical properties of lycopene do not comply with Lipinsky rules for drug candidates, but ADME predictions show that lycopene has good intestinal permeability and is permeable through the blood-brain barrier. The CNN Pose Score in Bcl2 protein is 0.7843, and the DNMT1 score is 0.1279. The binding of lycopene with Bcl2 is spontaneous and is stronger than the binding with DNMT1. Molecular Dynamics Simulation shows that lycopene is stable in the remaining 20 ns in the protein complex.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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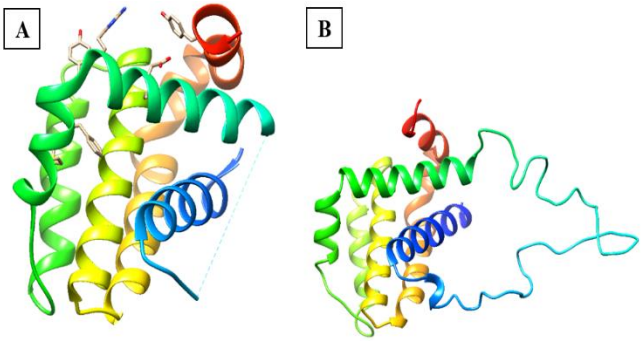

Figure 4: Visualization of Bcl2 protein (A) and Homology modeling protein (B)

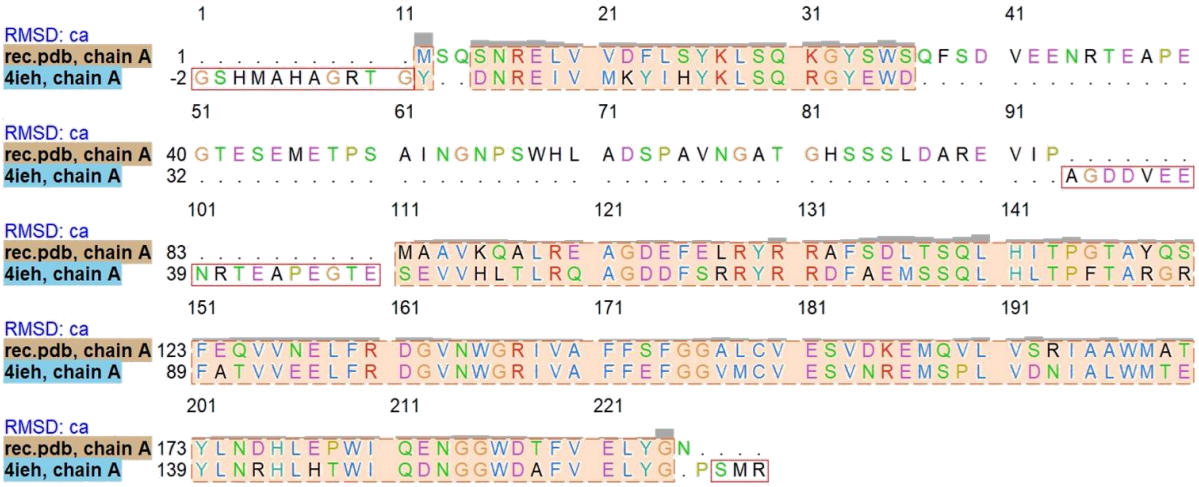

Figure 5: Superposed protein Bcl2 and the results of homology modelling (dotted lines indicate the presence of a sequence gap)

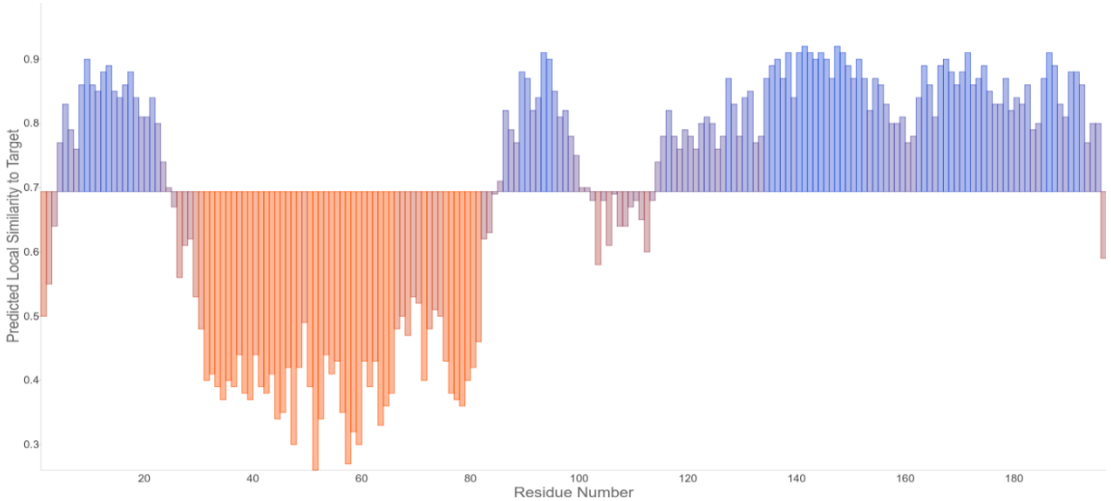

Figure 6: Local Quality Estimate

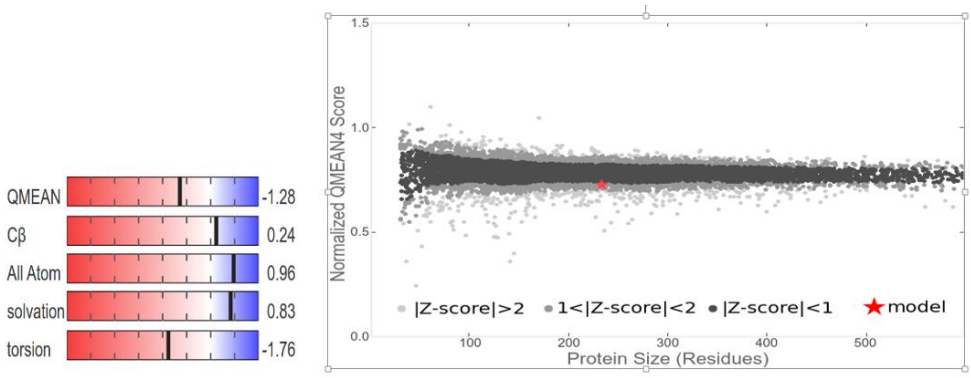

Figure 7: Local Quality Estimate and Comparison with non-redundant set of PDB structures

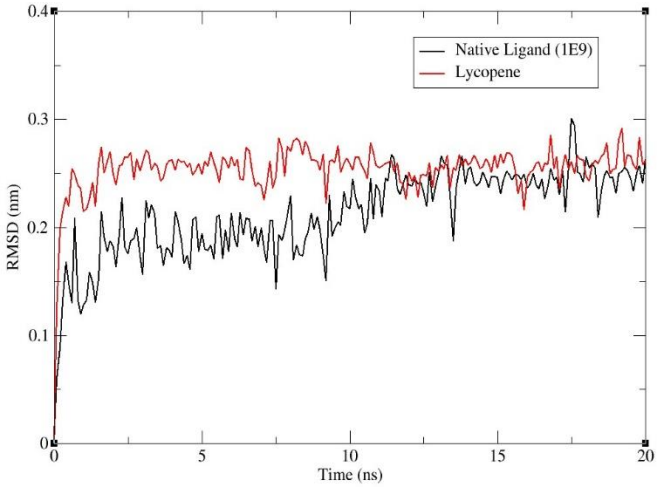

Figure 8: The RMSD protein results between native ligand and lycopene

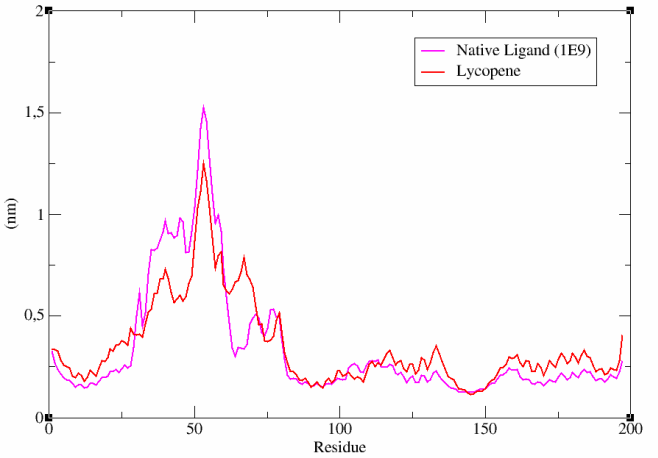

Figure 9: RMSF results between Bcl2 Protein with Native Ligand (Red) and Lycopene (Magenta)

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