Comparative Molecular docking analysis of Target fruit ripening enzyme *Tomato Beta galactosidase (TBG-4)*

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Abstract

Tomatoes comprise a high level of TBG4 (Tomato Beta galactosidase-4) enzyme activity that plays a key role in fruit softening by significant changes in the galactosyl content in the pericarp cell wall. In the present work, in silico docking studies of beta galactosidase with specific elucidated ligands were carried out. For the better understanding of protein ligand interactions, a set of 16 ligands were used for docking studies.

In the present study, two different comparative docking softwares, Autodock4.0 and iGEMDOCK were used to study the protein–ligand interactions and performed to get the best docking scores. PLIP software was used for visualization of protein ligand complex and their interactions. Binding energies of 16 ligands were predicted among which 5 ligands 151, 2FL, B2G, EPE and LAT were analysed and confirmed as best ligands. Among them 151(2S)-3-Methyl-2-((2R,3S)-3-[(Mehtylsulfonyl)amino]-1-[2-(Pyrolidin-1-ylmethyl)-1,3-Oxazol-4yl} Butanoic acid is the best inhibitor of TBG4 enzyme activity leading to significant enhancement in fruit shelf life.

Keywords: Beta-galactosidase, docking, PLIP, iGEMDOCK.

Introduction

Several plant beta-galactosidases (βgals) (EC 3.2.1.23) have been identified with the ability to hydrolyse cell wall β-(1,4)-galactans residues from β-D-galactosidase. The role of enzyme activity has been involved in many biological processes, especially in plant development and fruit ripening. Plant β-galactosidases play important roles in cell wall degradation during ripening.[3,4,15] Tomato β-galactosidase 4 (TBG4) activity leads to fruit ripening in many fruit crops.[13] Beta galactosidases are existent in many organisms and grouped into families. Tomato BGals grouped under GH35 family includes 7 genes expressed during ripening stage.[17]

In *Solanum lycopersicum* (tomato), fruit softening is greatly affected by significant changes in β-galactosyl residues of the pericarp cell wall.[6] Docking is a major computational method used to analyse protein – ligand interactions. Accuracy and reliability are two important features of any docking software. There are several docking softwares available online. Autodock4 and iGEMDOCK softwares are freely available and accessible. These tools take very less time for analysis and provide accurate docking scores.

Material and Methods

Sequence Information: *Solanum lycopersicum* (Tomato) gene TBG4 sequences are available in Gene bank with ACC No. LOC101250282. The 3W5G sequence retrieved from NCBI and BLAST search tomato fruit ripening proteins were obtained. These sequences were translated into the amino acid sequences.

Molecular docking studies: The Auto dock tool was used for screening to select the best pose and binding efficiency between the protein and ligand. In this study, AUTODOCK and IGEM dock tools were used for comparative analysis and selection of best docking scores among them. Protein docking analysis majorly includes 2 steps i.e. identification of active sites in protein pdb structure followed by identification of specified region of proteins docked with ligands.

AUTODOCK4.0 tool: All steps related to docking procedure were performed starting with preparation of ligands and proteins followed by simulation and binding sites of target protein. Further, the prediction of binding modes of the ligand was confirmed.[14] The free energy force field allows incorporation of intramolecular energies into the predicted free energy binding in most of the grid based docking methods.[8] The steps involved in Autodock are shown in fig. 1.

Calculation of biding energy

$$Energy_{Binding} = E_{Complex} - E_{Ligand} - E_{Receptor}$$

Protein Entropy energy- (Kcal/mol); Ligand Entropy energy- (Kcal/mol)
Complex Entropy energy- (Kcal/mol)

Preparation of Protein: The macromolecule 3W5G (beta Galactosidase) is essentially a fruit softening related enzyme. Its 3D structure is downloaded from PDB. To prepare the protein molecule, ADT computation tool was used. In the macromolecule, water molecules were removed and polar hydrogen was added. Already existed ligands and head atoms were removed.

In the next step Kollaman charges and Gasteiger-Marsili empirical atomic partial charges were added to the protein molecule. AD4 type force fields were assigned for protein preparation and arranged in the form of pdbqt file format.[11]

Ligand Preparation: A set of 16 ligands was used for the docking studies of TBG4 protein. The organic structure of molecules and their chemical names is represented in table 1. Auto dock ligands are written in the form of root and end root that has a torsion tree and branches. TORDOF is the number of torsional degrees of ligand useful to calculate the free energy changes. Finally, the prepared ligands were saved in the form of pdbqt file by adding hydrogen atoms.

Active site identification: Active sites of particular protein were identified through the reference ligands from pdb. Castp server was mostly used for binding sites of ligands on macromolecule.[1]

Preparation of grid parameter file*:* Grid parameter files are useful to compute the auto grid maps. The location and extent of those maps specify pair-wise potential energy parameters. The grid options widgets display thumbwheel containing x, y and z dimensions. The thumbwheel is useful for spacing between the grid points. Adjust the points (x, y and z) 60 dimensions, map will show 226981points. Save the file in the gpf file format to start the auto grid. 'AD4.1-boud.dat' auto dock tool is used for this purpose to get 'glg file' in result. Command to start the auto grid job.

% autogrid4 –p hsgl.gpf -l hsgl.glg &

Preparation of docking parameter file: Docking parameter files are maintained in the form of dpf file. They are useful for the ligand specific position and using different algorithm. Generally, Lamarckian genetic algorithm (LGA) is used for dpf. 'AD4.parameters.dat'ADT tool is used to create the 'dlg file'.

% autogrid4 –p ind.dpf -l ind.dlg &

These dlg files generate the results of auto dock for particular enzyme and ligand. Results are represented in the docking log as conformations. The conformations reveal binding affinities: torsional energy and docking energy and analysis of results for enzyme and ligand complex as follows

Binding Energy = Intermolecular Energy + Torsional Energy
Docking Energy = Internal Energy + Intermolecular Energy

This conformational analysis is a combination of translation, quaternion and torsion angles. It is categorized by intermolecular energy, internal energy and torsional energy. The combination of internal energy and torsional energy gives the binding energy and the combination of internal energy and intermolecular energy provides the docking energy. The total energy is also considered as Vander Waal energy and Electrostatic energy in Auto dock for each atom.

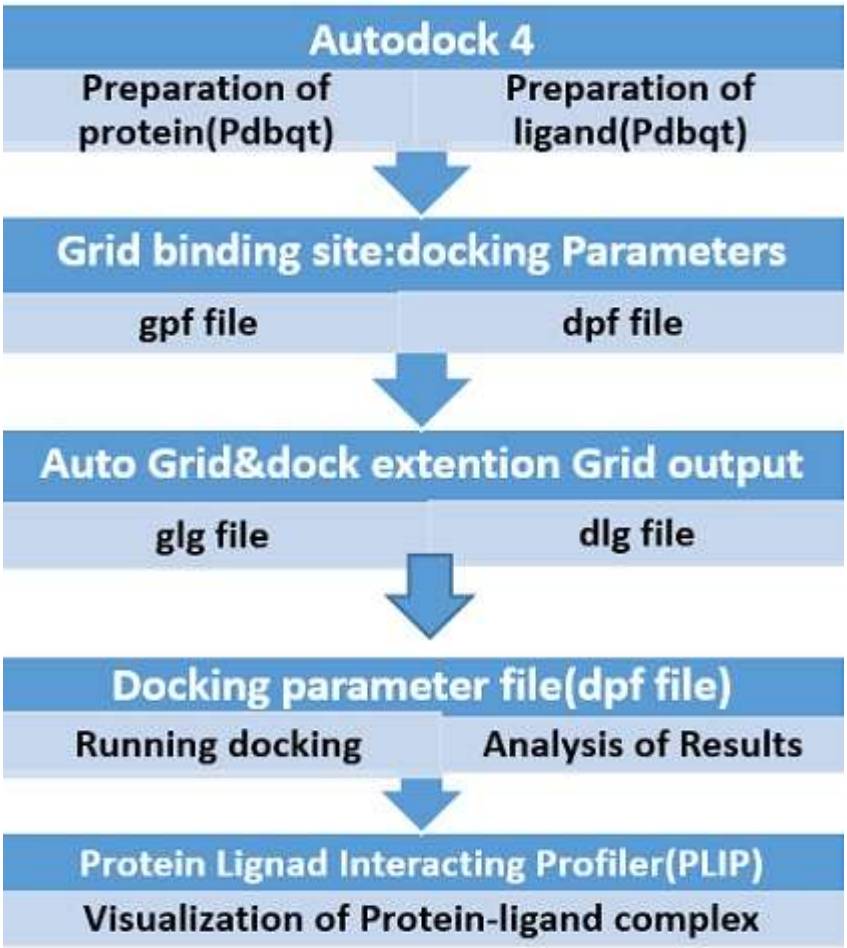
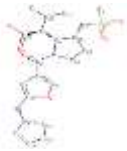
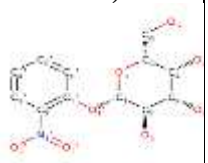
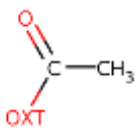
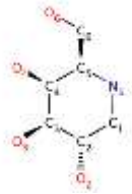
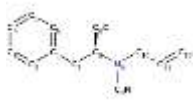
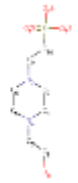
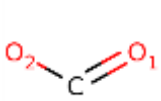
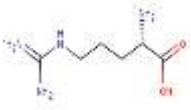


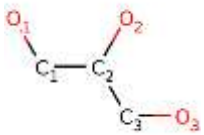
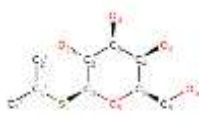
Fig. 1: Steps involved in AUTODOCK 4

Table 1
Docking studies between enzymeTBG4 and set of 16 ligand molecules

S.N.	Ligand	Interactions	Binding Energies	Ligand efficiency	Docking energy	H bond	Intermolecular energy	No. of conformations clusters	Active Sites
1	151(2S)-3-METHYL-2-((2R,3S)-3-[(METHYLSULFONYL)AMINO]-1-{[2-(PYRROLIDIN-1-YLMETHYL)-1,3-OXAZOL-4-YL] CARBONYL} PYRROLIDIN-2-YL) BUTANOIC ACID 	3w5g: A: TYR256: 151:01S 3w5g: A: ALA119: 151:08	-6.34	-0.21	-11.37	2	-9.03		ALA119,GLU 120,CYS118,V AL117,ASN54 7,ASN230,TR P252,TYR289
2	145 (1-O-[O-NITROPHENYL]-BETA-D-GALACTOPYRA NOS) 	3w5g: A: LYS217:145:02 3w5g: A: LYS217:145:03 3w5g: A: GLN218: 145:03 3w5g: A:ASP227: 145:03 3w5g: A: ASN230 145:03	-5.57	-0.27	-10.8	5	-7.96	8	ALA119,GLU 120,CYS118,V AL117,ASN54 7,ASN230,TR P252,TYR289
3	2FL (2-FLUORO-2-DEOXY-LACTOSE) 	3w5g: A: ALA119:2FL:0 2 3w5g: A: ASN230:2FL:0 3 3w5g: A: ASN180: 2FL:03 3w5g: A: TYR74:2FL:03 3w5g: A: GLU250: 2FL:03	-4.03	-0.18	-15.27	4	-7.31		ALA119,GLU 120,CYS118,V AL117,ASN54 7,ASN230,TR P252,TYR289
4	ACY (ACETIC ACID) 	3w5g: A: GLN679: ACY:02. co2	-3.85	-0.96	-4.23	3	-4.15	1	ALA119,GLU 120,CYS118,V AL117,ASN54 7,ASN230,TR P252,TYR289

5	ARG (ARGININE $C_6 H_{15} N_4 O_2$ 	3w5g: A: GLU181: ARG:02 3w5g: A: TYR312: ARG:02	-3.45	-0.29	-9.04	2	--5.53	9	ALA119,GLU 120,CYS118,V AL117,ASN54 7,ASN230,TR P252,TYR289
6	B2G GalactobioseC$_{12}$ H$_{22}$ O$_{11}$ 	3w5g: A: GLU181:B2G:0 3 3w5g: A: GLU181: B2G:03 3w5g: A: LYS217:B2G :03 3w5g: A: VAL550: B2G:03	-.046	-0.02	-13.3	4	-4.04	10	ALA119,GLU 120,CYS118,V AL117,ASN54 7,ASN230,TR P252,TYR289
7	DGJ (2R,3S,4R,5S)-2-(hydroxyethyl) piperidine-3,4,5-triol 	3w5g: A: ASN180: DGJ:03 3w5g: A: ASN230: DGJ:03 3w5g: A: TYR289: DGJ :03 3w5g: A: TYR312: DGJ:03 3w5g: A: TYR289: DGJ:03 3w5g: A: TYR312: V:03 3w5g: A: GLU120119: DGJ:03 3w5g: A: GLU250: DGJ:03 3w5g: A: GLU250: DGJ:03 3w5g: A: GLU250: DGJ:02	-3.46	-0.31	-8.71	10	-4.96	4	ALA119,GLU 120,CYS118,V AL117,ASN54 7,ASN230,TR P252,TYR289
8	DPK (2R)-N-methyl-1-Phenyl-N-prop-2-enyl-propan-2-amine (DEPRENYL) 	3w5g: A: TYR116: DPK:03 3w5g: A: GLY549: DPK: 0.co2	-4.19	-0.34	-	2	-7.2	7	ALA119,GLU 120,CYS118,V AL117,ASN54 7,ASN230,TR P252,TYR289

9	EPE 4-(2-HYDROXYETHYL)-1-PIPERAZINE ETHANESULFONIC ACID 	3w5g: A: TYR256: EPE: 01S 3w5g: A: ala119: EPE: 08	-4.19	-1.25	-64.09	2	-4.04	2	ALA119,GLU120,CYS118,VAL117,ASN547,ASN230,TRP252,TYR289
10	FMT FORMIC ACID 	3w5g: A: LYS676: FMT:02 3w5g: A: GLN679: FMT:02	-4.02	-0.37	-4.14	5	-5.21		ALA119,GLU120,CYS118,VAL117,ASN547,ASN230,TRP252,TYR289 ALA119,GLU120,CYS118,VAL117,ASN547,ASN230,TRP252,TYR289
11	FUC ALPHA-L-FUCOSE 	3w5g: A: SER309: FUC:02 3w5g: A: GLU318: FUC:03 3w5g: A: GLU318: FUC:03 3w5g: A: GLN687: FUC:03 3w5g: A: TRP689: FUC:03 3w5g: A: ARG722: FUC:03	-3.73	-0.31	-7.54	4	-5.52	5	
12	GLA ALPHA D-GALACTOSE 	3w5g: A: TYR74: GLA:03 3w5g: A: TYR74: GLA:03 3w5g: A: ALA119: GLA:03 3w5g: A: GLU120: GLA:03 3w5g: A: GLU120: GLA:03 3w5g: A: ASN180: GLA:03 3w5g: A: TYR312: GLA:03 3w5g: A: ASN230: GLA:03	-5.79	-0.48	-9.3	4	-7.58		ALA119,GLU120,CYS118,VAL117,ASN547,ASN230,TRP252,TYR289

13	GLC ALPHA-D-GLUCOSE 	3w5g: A: TYR74: GLC:03 3w5g: A: ALA119: GLC:03 3w5g: A: GLU120: GLC:02 3w5g: A: ASN180: GLC:02 3w5g: A: ASN230: GLC :03 3w5g: A: GLU250: GLC:02 3w5g: A: GLU250: GLC:03 3w5g: A: TRP252: GLC:03 3w5g: A: TYR310: GLC:03 3w5g: A: TYR312: GLC:03	-2.8	-0.47	-10.57	5	-4.29		ALA119,GLU 120,CYS118,V AL117,ASN54 7,ASN230,TR P252,TYR289
14	GOL GLYCEROL 	3w5g: A ARG139256: GOL: 03 3w5g: A: ARG139: GOL: 03 3w5g: A: GLU181: GOL:03 3w5g: A: GLU181: GOL:03 3w5g: A: GLU181: GOL:03 3w5g: A: GLU181: GOL:03	-5.79	-0.48	-7.25	3	-7.58	2	ALA119,GLU 120,CYS118,V AL117,ASN54 7,ASN230,TR P252,TYR289
15	IPT ISOPROPYL-1-BETA-D-THIOGALACTOSIDE 	3w5g: A: GLU120: IPT:03 3w5g: A: ASN180: IPT:03 3w5g: A: GLU250: IPT:02 3w5g: A: TRP252: IPT:03 3w5g: A: TYR256: IPT :03 3w5g: A: TYR312: IPT:03	-3.78	-0.25	-8.75	3	-5.87	10	ALA119,GLU 120,CYS118,V AL117,ASN54 7,ASN230,TR P252,TYR289

Analysing Auto dock results: Generally, the auto dock output results are saved in the form of Pdb files as protein – ligand complexes. PLIP (Protein ligand interaction profiler) is a python based command line application which can read the pdb interaction complexes.[16] It will identify the noncovalent bonds between the protein and ligands. It gives details of binding sites of ligands and their interactions between ligand and protein in 3d viewer.

iGEMDOCK: iGEMDOCK is a robust graphical and automatic drug design system for protein docking, screening and scoring in post analysis. Using iGEMDOCK, docking process can be visualized and analysed by k-means and hierarchical clustering methods.[19]

Before starting the iGEMDOCK, the input ligand file has to be loaded in the form of MDl, MOl or PDB format. For running docking process, set up output path and set up the GA (Generic evolutionary algorithm) parameters. The set of proteins, ligands, output and parameters need to be sequentially prepared to start docking. The number of docked compounds will be show on the screen.

Results

Around 16 ligands were used in this study in which only 5 complexes showed different binding energies (Table1). Sixteen ligands were docked with 3w5g fruit softening protein. All the ligands were docked in the respective binding sites of protein TBG-4 successfully. The results revealed different binding energies and docking scores of autodock4. The docking tool iGEMDOCK was also used for docking purpose with same set of 16 ligands and 3w5g protein. All the ligands docked and located in the binding site regions are shown in fig. 2. The comparative results of Auto dock and iGEMDOCK are shown in fig. 3.

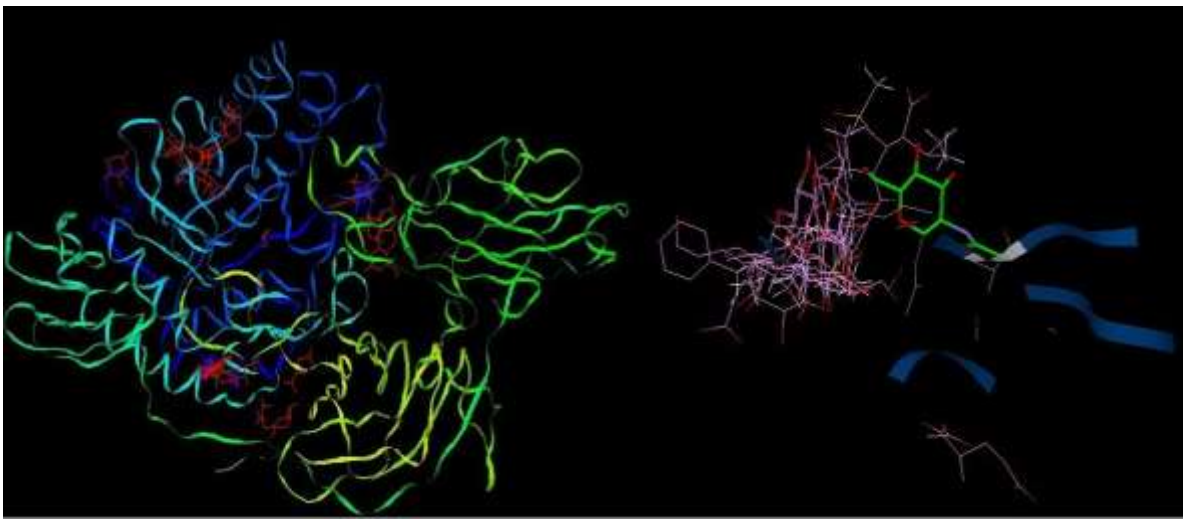


Fig. 2: Docking of all 16 ligands and 3w5g using iGEMDOCK

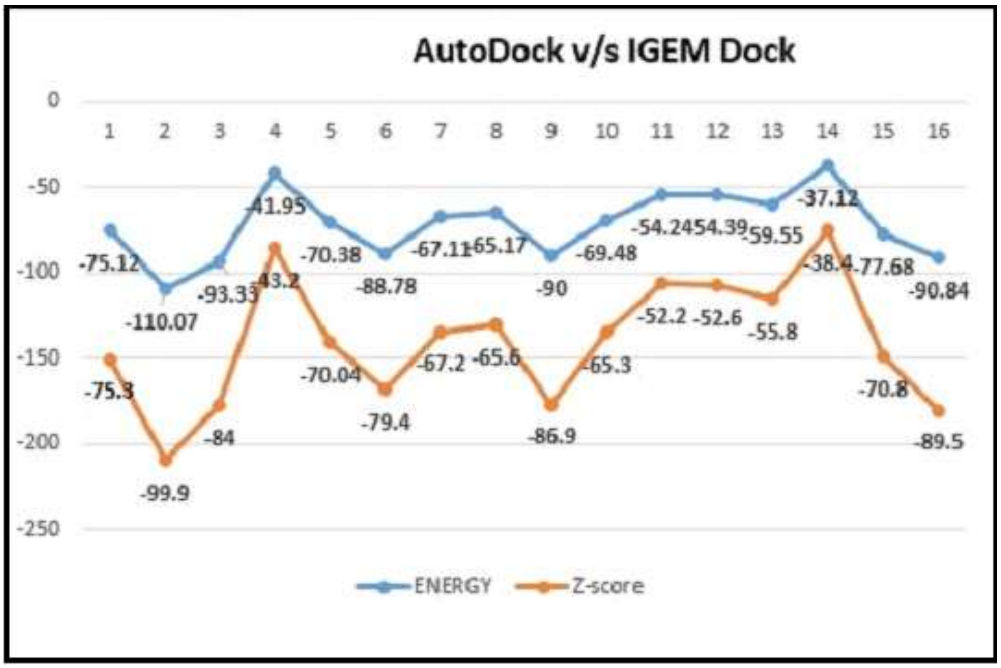


Fig. 3: Comparison of results of Auto dock and iGEMDOCK

The Binding energies of docking molecules were represented in the table 1. 151(2S)-3-Methyl-2-((2-R,3S)-3-[(Methylsulfonyl)amino]-1-2-(pyrrolidin-1-ylmethyl)-1,3-Oxzol-4-yl] carbonyl} pyrrolidin-1-ylmethyl)-1,3ylmethyl-1,3-oxazol-4-yl] Carbonyl} pyrrolidin-2-yl) botanic acid showed binding energy (-6.34Kcal/mol) for 3w5g. 151 showed lowest binding energy followed by 2FL(2-FLUORO-2-DEOXY-LACTOSE), B2G (Galactobiose), EPE(4-(2-Hydroxyethyl)-1-piperazine Ethane sulfonic acid), LAT(Lactose) and their binding energies -4.03Kcal/mol, -0.046Kcal/mol, -4.19Kcal/mol,-1.88Kcal/mol. The results of iGEMDOCK for docking all the16 ligands with 3w5g are given in table 2. However, pose with lowest binding scores and Z-Scores indicates highest enzyme affinity, thus confirmed as significant and selected for further docking studies. Further the protein ligand complex format was visualized using PLIP (Protein ligand interaction profiler). The 5 best complexes were predicted and have been shown in fig. 4.

The analysis revealed the type of interacting amino acid residues involved in the interactions between the ligand and the enzyme. Hydrogen bonds, halogen bonds, hydrophobic interactions, salt and water bridges, metal complexation, π stacking and π-cation interactions are shown in fig. 5.

Table 2
IGEMDOCK: Docking Score Analysis

S.N.	COMPOUND	ENERGY	VDW	Hbond	ELEC	Int. Cluster ID	Com. Cluster ID	Z-score	Energy
1	3w5g-145	-75.12	-75.12	0	0	4	4	-75.3	-75.3
2	3w5g-151	-110.07	-110.07	0	0	2	4	-99.9	-84
3	3w5g-2FL	-93.33	-93.33	0	0	3	4	-84	-55.8
4	3w5g-ACY	-41.95	-41.95	-12.97	-1.35	2	1	-43.2	-43.2
5	3w5g-ARG	-70.38	-70.38	0	0	2	3	-70.04	-70.4
6	3w5g-B2G	-88.78	-88.78	-28.29	0	3	4	-79.4	-79.4
7	3w5g-DGL	-67.11	-67.11	0	-0.28	2	4	-67.2	-67.2
8	3w5g-DKA	-65.17	-65.17	-22.84	0	2	3	-65.6	-65.6
9	3w5g-DPK	-90	-90	0	0	2	4	-86.9	-86.9
10	3w5g-EPE	-69.48	-69.48	0	0	3	4	-65.3	-65.3
11	3w5g-FUC	-54.24	-54.24	0	0	1	2	-52.2	-52.2
12	3w5g-GLA	-54.39	-54.39	0	0	2	4	-52.6	-52.6
13	3w5g-GLC	-59.55	-59.55	0	0	2	4	-55.8	-38.4
14	3w5g-GOL	-37.12	-37.12	0	0	2	4	-38.4	-99.9
15	3w5g-IPT	-77.68	-77.68	0	0	2	4	-70.8	-70.8
16	3w5g-LAT	-90.84	-90.84	0	0	4	4	-89.5	-89.5

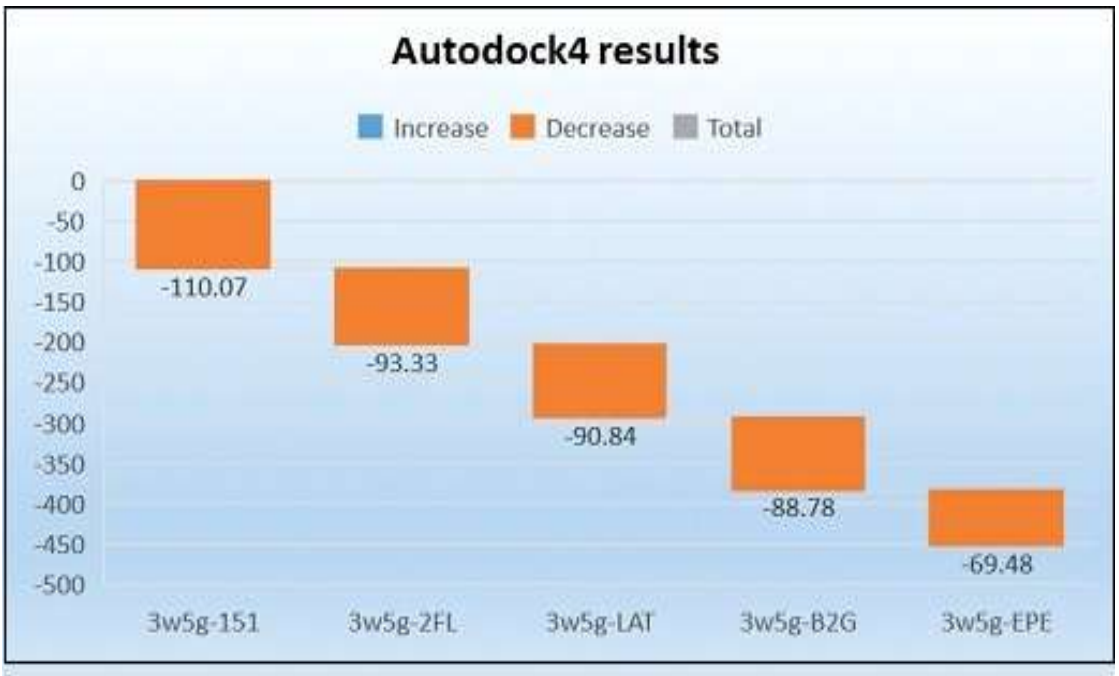


Fig. 4: The 5 best complexes predicted through AUTODOCK4

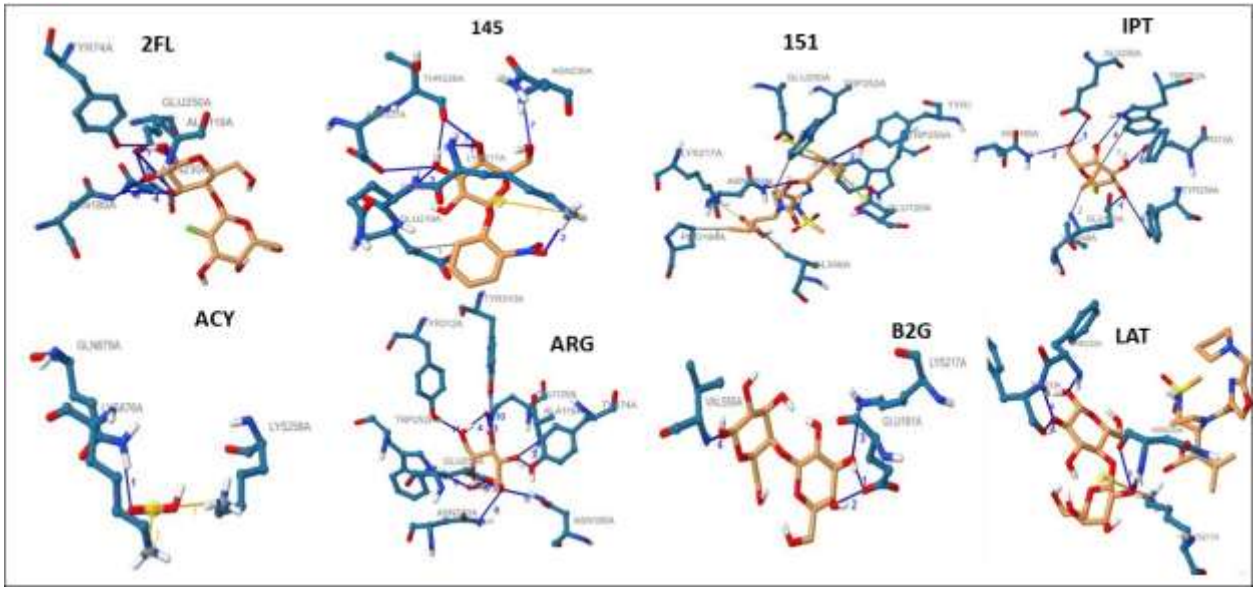


Fig. 5: Enzyme ligand complexes and their amino acid interactions

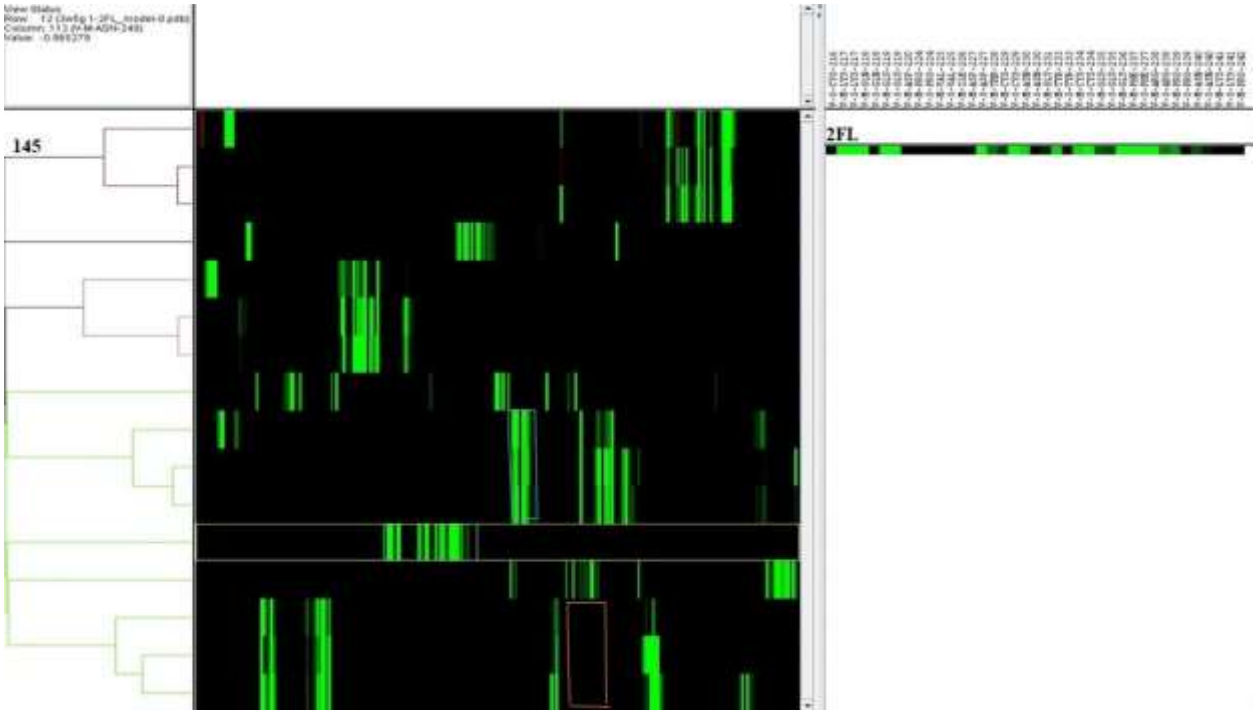


Fig. 6: The interaction profiles of the compounds

Validation of docked protein-ligand interactions: The present study involves comparison of results in Auto dock and iGEMDOCK based on binding energy and docking scoring values between 3w5g protein and set of 16 ligand molecules. Hierarchical clustering of the interaction profile for the active compounds and top ranked compounds selected by energy based scoring function have been done. The interaction profiles of the compounds belonged to the compound cluster (Blue block); the molecule in the lowest energy cluster orange block are shown in fig. 6.

Discussion

Beta galactosidases are widely distributed in plant tissues and organs. Bgals are reported to be involved in many physiological processes including plant growth and fruit softening.[2] The plant Bgals are usually dimeric.[12] The enzyme activity levels are stable throughout the pre ripening stages of fruit development and rapidly enhanced during ripening.

In this study, active sites for enzyme 3w5g were analysed using computer atlas of surface topography of proteins (CASTp).[18] Most of the active sites for Bgals were found in motif 3.[7] The active sites for specific for all 16 ligand binding sites amino acids ALA119, GLU120, CYS1, VAL117, ASN547, ASN230, TRP252, TYR289 were predicted for docking the molecules by using Auto dock 4. Mostly aromatic hydrophobic residues are located at the

active sites of protein and are useful for ligand recognition by Van Der Waals interactions. The best ligand was screened and scored based on the binding free energy between receptor and ligand complex.[9]

Enzyme and ligand complex models produced after docking were obtained based on the parameters such as hydrogen bonds, binding energies, docking scores, active site amino acid residues and location of the docked compound within the catalytic site region.[17] In the present study, five ligands 151,2FL, B2G, EPE and LAT showed best poses and lowest binding energies. The earlier docking studies showed that various substrate specificity and ligand protein interactions were related to fruit softening related enzyme Plant beta galactosidase-4.[9] p-nitro phenyl-β-D-galacto pyranoside and 2-[4-(2-hydroxyethyl) piperazin-1-yl) ethane sulfonic acid were used as substrates for both tomato and mango Beta galactosidases for their retaining mechanism. In another study, identification of V548 amino acid residue of TBG4 confer the substrate specificity in β-galactans using docking studies.[5]

Further AUTODOCK4 and iGEMDOCK were performed for comparison of docking parameters. The results were analysed using PLIP and visualised by RasMol server. The results revealed that ligand binding sites with enzyme 3w5g and their binding energies are almost similar. This has been an added advantage for studying the interaction and the confirmation of enzyme ligand complexes.

Conclusion

The fruit ripening is plant development process triggered by β-gals. The increased pectin solubility leads to fruit softening. *In silco* docking studies provide valuable insights in enzyme ligand interactions of TBG4 with 5 ligands 151,2FL, B2G, EPE, LAT. These ligands are useful to control the activity of fruit softening enzyme. These analyses were useful for further studies on controlling the fruit softening and generate genetically modified fruit crops with increased shelf life. Two *in silco* approaches were used for interpreting the accurate docking results.

Further the comparative analysis of Auto dock4 and iGEMDOCK tools could lead to extensive studies for structural research on tomato beta galactosidase enzyme-4 as potential inhibitor for controlling the fruit softening.

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