Research Square

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# Finding inhibitor from phytochemicals for novel target Glycosyltransferase family 62 protein in Trichophyton rubrum using insilico study

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## Abstract

The dermatophyte *Trichophyton rubrum* is producing more than 70% of dermatophytosis in human and animals. Glycosyltransferase family 62 protein in *T.rubrum* is potential and novel drug target which is non-homologous to human, human gut microbiota and it is not targeted by any drug. It is very essential for priming mannosyltransferase activity and different types of N-glucan biosynthesis. Various parts of medicinal plant *Balanites aegyptiaca* are used in treating many diseases in human especially skin diseases. Aim of this study is to find potential inhibitor from phytochemicals of various medicinal plant sources against the novel drug target. 3D structures of Glycosyltransferase family 62 protein was obtained by homology modeling and docked with the compounds from phytochemicals of various plant species using GLIDE and best pose of docked complex free energy was calculated by MM-GBSA analysis using PRIME. The stability of the best docked complex was evaluated by molecular dynamics simulation studies using Desmond module of Schrödinger. Cyanidin 3-O-rhamnoside had better result with novel target Glycosyltransferase family 62 protein of *T.rubrum* which has to be further assessed *in vitro* and *in vivo*.


## Introduction

Dermatophytosis is a superficial infection caused by dermatophytes that invade the skin and nail through hydrolysis of keratin by producing proteolytic enzyme keratinase and grow on the keratinized tissues [1]. *T.rubrum* is one of the important species among dermatophytes to cause more number of superficial infections [2]. Most commonly it is causing tinea pedis, tinea cruris and tinea corporis throughout the world [3].

Currently available drugs are mainly targeting ergosterol biosynthesis pathway and these drugs are not only interacting with fungal proteins but also with human proteins thereby causing side effects in human [4]. For avoiding these interactions, this study focuses on the putative drug target Glycosyltransferase family 62 protein (F2SC98) which is present only in *T.rubrum* not in human, human gut microbiota and also it is non-druggable target [5].

Glycosyltransferase family 62 protein is conserved protein in fungal pathogens. It is essential for the biological process of added of carbohydrate or carbohydrate derivative to a protein trough the Nitrogen atom of asparagine called N-linked glycosylation [6]. Also, molecular function of this enzyme that catalyzes the reaction in which transfer of mannose residue to an oligosaccharide, forming alpha-(1->6) linkage. Glycosyltransferases are important enzymes that transfer monosaccharide molecules to biological products like proteins, lipids, and carbohydrates [7]. Also it plays key roles in cell wall biosynthesis and cell wall aggregation in fungus [8].

*Balanites aegyptiaca* is an important medicinal plant, belonging to the family Balanitaceae, commonly known as “Desert date”. It is a very rich source of secondary metabolites and useful in treating many diseases [9]. The edible fruit pulp of *Balanites aegyptiaca* is used to treat jaundice, purgative, diabetes, whooping cough, dysentery, leukoderma, constipation and liver diseases [10] and is the main source of saponins which have wide range of medicinal and industrial uses including antimicrobial properties [11].

This study is to find potential compounds from the phytochemicals of various medicinal plants that can inhibit the novel target Glycosyltransferase family 62 protein of *T.rubrum* through molecular docking, binding free energy calculation and molecular dynamics simulation studies.

## Materials And Methods

# Finding importance of protein

The similar proteins were found for the novel target Glycosyltransferase family 62 protein using NCBI-BLAST search [12] and domain search analysis using Pfam [13] for finding the function and importance of protein. The species of *Trichophyton, Microsporum, Candida, Aspergillus and Saccharomyces* were used for this analysis.

# Structure prediction and Validation

The 3D structure was modeled for Glycosyltransferase family 62 protein target by Homology modeling using Prime module of Schrödinger. Templates were identified through BLASTp search, aligned with sequences of drug targets and modeled. It was further refined using PREFMD (Protein REFinement via Molecular Dynamics) and locPREFMD offered by Feig Computational and Biophysics lab, Michigan State University [14, 15]. Further the modeled proteins were validated using PROCHECK server for its quality [16].

# Active site and Conserved residues prediction

Protein active site was predicted by Uniprot which is having the experimental data of proteins and also the data derived from Interproscan [17]. Also found active site of drug target using existing available protein domains by sequence alignment server Clustal Omega [18]. The conserved residues were identified by ConSurf server [19] which is used for finding functionally and structurally conserved residues in proteins.

# Preparation of Ligands

Various phytochemical compounds from different medicinal plant species were used for this study. The 2D structures of the compounds were retrieved from Pubchem and ADMEToxicity of the compounds was analyzed using QikProp module of Schrödinger [20].

# Molecular Docking

The plant derived compounds were docked with putative drug target Glycosyltransferase family 62 protein of *T.rubrum* using Glide module, Schördinger [21, 22]. The XP docking was processed through 4 steps – 1.Protein Preparation, 2.Receptor Grid generation, 3.Ligand preparation and 4.Ligand docking.

Protein preparation started with the preprocessing of protein molecule, added missing hydrogen atoms, assigned the bond orders and creating disulfide bonds. Following this, optimization of amino acid orientation of hydroxyl groups, amide groups of Asn and Gln was carried out. All amino acid flips were assigned and H-bonds were optimized. OPLS3e force field was fixed for energy minimization, non-hydrogen atoms were minimized until the average root mean square deviation (RMSD) reached default value of 0.3 Å [23].

Grid was generated covering the active site residues which are derived from the active site analysis using Uniprot, sequence alignment and Consurf server. For optimizing ligand molecules, the force field OPLS3e was fixed and Epik was used instead of ionizer to generate the probable ionized and tautomerized structures at the pH range of 7.0 ± 2.0.

Ligand docking was performed using selected ligands with identified targets of pathogen through XP (extra precision) docking with flexible approach [24].

# Induced Fit Docking (IFD)

Three top scored compounds from XP docking analysis were further analysed by using Induced Fit Docking (IFD), Schördinger [25, 26]. The process of IFD begins with protein preparation and extends to protein grid generation by selecting active site residues and fixing the ligand molecules with length of < 20Å for binding. The residues which are within 5.0 Å of ligand poses were refined and side chains of proteins were optimized. The selected ligand molecules

were prepared again and these ligands were docked with each protein. IFD generated a diverse ensemble of ligand poses and the best pose was selected after analyzing all poses.

# MM-GBSA analysis

Molecular Mechanics with Generalised Born Surface Area (MM-GBSA) analysis was carried out using Prime module of Schrödinger for relative binding free energy calculation of protein-ligand complex. The relative binding free energy $\Delta G_{bind}$ was calculated by the following equation [27],

$\Delta G_{bind} = E_{complex}$ (minimized) – [ $E_{ligand}$ (unbound, minimized) + $E_{receptor}$ (unbound, minimized)

$E_{complex}$ (minimized) is the energy of minimized complex structure in MM-GBSA, $E_{ligand}$ (unbound, minimized) is the MM-GBSA energy of minimized ligand after taking out it from the complex sturucture and $E_{receptor}$ (unbound, minimized) is the MM-GBSA energy of minimized protein after eliminating from the complex [28]. The binding free energy could be disassociated into gas phase energy $\Delta G_{gas}$ and salvation energy $\Delta G_{sol}$. The salvation energy is distributed into polar and non-polar energy terms, polar energy $\Delta G_{GB}$ is calculated by SGB salvation and non-polar energy is estimated with solvent accessible surface area (SAS) and Van der Waals interactions [29]. The best complex pose from IFD is used for calculating binding free energy using GB model as VSGB 2.1 and OPLS3 force field [30].

# MD simulation

The selected complexes from IFD pose analysis were further subjected to Molecular Dynamics Simulation to check the stability of the complexes by using Desmond module of Schrödinger [31]. Molecular system was solvated using TIP3P water model, force field OPLS 2005 was selected for simulation. Orthorhombic box with distances of 10Å was fixed as boundary condition [32].

Before starting the simulation process, the steepest descent minimization which is default relaxation/equilibration protocol in Desmond module was performed for refining and minimizing the energy of complex structures. After the equilibration, the molecular system was simulated for 100ns using OPLS 2005 force field and NPT ensemble class. Temperature was set at 300K and pressure at 1 bar processed by Nose-Hoover chain and Martyna-Tobias-Klein methods, respectively [33]. Cut-off radius was kept as 9Å for calculating the columbic interactions of short range. REPSA integrator was used with 2.0 fs time for short bonded interactions and 6.0 fs time for long bonded interactions. In between the simulations, the energy was recorded at every 1.2ps interval [34].

The data of simulation was analysed using simulation event analysis by assessing the RMSD (Root mean square deviation) of complex, RMSF (Root mean square fluctuation) of protein and ligand, protein- ligand interactions and ligand torsion study.

## Result

# Finding importance of protein

For finding the function and essentiality of novel Glycosyltransferase family 62 protein, the protein was analysed using BLAST search with other fungal pathogens like *Aspergillus, Candida, Trichophyton, Microsporum, and Saccharomyces* species. The result indicates that the Glycosyltransferase family 62 protein was present in all fungal pathogens. The Anp1 domain present in Glycosyltransferase family 62 protein of *T.rubrum* from region of 74–340, the same Anp1 domain was present in all fungal species of *Trichophyton, Microsporum, Aspergillus, Candida and*

*Saccharomyces* (Table 1). Glycosyltransferase family 62 protein was present in all fungal pathogen as mannan protein and it is important novel target for fungal species (Fig. 1).

Table 2. showed that the Glycosyltransferase family 62 protein was highly similar with the Mannan polymerase complex subunit protein of *T.mentagrophytes* (99.46%), MNN9 protein of *M.canis* (92.95%), Alpha-1,6 mannosyltransferase subunit of *A.niger* (65.71%), Alpha-1,6 mannosyltransferase subunit (Mnn9) of *A.fumigatus* (65.97%), Mannan polymerase complex subunit Mnn9 of *A.flavus* (68.60%), Mannosyltransferase complex subunit of *C.albicans* (57.33%), Mnn9 of *S.cerevisiae* (47.95%). The result revealed that the Glycosyltransferase family 62 protein was highly conserved protein among the fungal pathogens.

**Table 1. Domain analysis of similar proteins of Glycosyltransferase family 62 protein**

| S.No | Matched protein | Organism Name | Domain Name | Domain Region |
|---|---|---|---|---|
| 1. | **Glycosyltransferase family 62 protein** | ***Trichophyton rubrum*** | **Anp1** | **74-340** |
| 2. | Mnn9 | *Trichophyton mentagrophytes* | Anp1 | 74-340 |
| 3. | Mnn9 | *Microsporum canis* | Anp1 | 75-341 |
| 4. | Alpha-1,6 Mannosyltransferase subunit | *Aspergillus niger* | Anp1 | 474-738 |
| 5. | Alpha-1,6 Mannosyltransferase subunit | *Aspergillus fumigatus* | Anp1 | 88-352 |
| 6. | Mannan polymerase complex subunit Mnn9 | *Aspergillus flavus* | Anp1 | 79-343 |
| 7. | Mannosyltransferase complex subunit | *Candida albicans* | Anp1 | 72-334 |
| 8. | Mnn9 | *Saccharomyces cerevisiae* | Anp1 | 95-358 |

Table 2
Similar proteins were identified for Glycosyltransferase family 62 protein using NCBI-BLAST search

| S.No | Matched protein | Organism Name | Similarity (%) |
|---|---|---|---|
| 1. | Mannan polymerase complex subunit | *Trichophyton mentagrophytes* | 99.46 |
| 2. | Mnn9 protein | *Microsporum canis* | 92.95 |
| 3. | CAZyme family GT62 | *Aspergillus niger* | 65.97 |
| 4. | Alpha-1,6 Mannosyltransferase subunit (Mnn9) | *Aspergillus fumigatus* | 65.71 |
| 5. | Mannan polymerase complex subunit Mnn9 | *Aspergillus flavus* | 68.87 |
| 6. | Mannosyltransferase complex subunit | *Candida albicans* | 57.33 |
| 7. | Mnn9 | *Saccharomyces cerevisiae* | 47.95 |

# Structure Analysis

The 3D structures of identified novel target Glycosyltransferase family 62 protein is not available in PDB, hence the structures were built by Homology modeling in the Prime suite of Schrodinger using the 3ZF8 with the identity of

55.02%, further refined and validated which resulted with modeled proteins having good quality with 93.2% of residues in the most favored region (Fig. 2).

# Protein active site prediction

Anp1 domain which is present in Glycosyltransferase family 62 protein is also present in Mnn9 protein of *Saccharomyces cerevisiae* and interacting residues of *Sc*Mnn9 was obtained from the PDB id 3ZF8. Hence both protein sequences were aligned and active site residues matched of Mnn9 in *S.cerevisiae* with Glycosyltransferase family 62 protein in *T.rubrum* as shown in Fig. 3.

Residues Pro 100, Ile 127, Pro 129, Gln 165, Arg 188, Arg 189, Met 192, Ala 195, Arg 196, Asp 215, Ala 216, Asp 217, Tyr 258, Asp 259, Glu 284, Glu 345 and His 370 are in the active site of Glycosyltransferase family 62 protein and these residues were within Anp1 domain region. Using Consurf server the conserved residues and region of protein was identified and presented in Fig. 4. It also showed the functionally and structurally important residues of Glycosyltransferase family 62 protein by comparing with more than 150 proteins of different species.

# ADMETox analysis

Various phytochemical compounds were selected and checked their ADMEtox properties, the result showed that all the selected phytocompounds were obey the rule of Lipinski (Rule of five) except the compound Flavonoid LP because it has more than 2 violations (Table 3).

Table 3
ADMETox properties of selected phytochemical compounds

| Compound Name | MW | HD | HA | QPlogPo/w | QPlogBB | CNS | HOA | QPlogS | RO5 | rtvFG |
|---|---|---|---|---|---|---|---|---|---|---|
| Andrographolide | 350.454 | 3 | 8.1 | 1.487 | -1.278 | -2 | 3 | -2.949 | 0 | 0 |
| Artocarpin | 436.504 | 2 | 4.5 | 5.166 | -1.39 | -2 | 1 | -6.915 | 1 | 0 |
| Berberine | 336.36 | 1 | 4 | 3.62 | -2.124 | -2 | 3 | -4.55 | 0 | 0 |
| Caffeic acid | 180.16 | 3 | 3.5 | 0.545 | -1.546 | -2 | 2 | -1.293 | 0 | 1 |
| Carnosic acid | 332.439 | 3 | 3.5 | 3.755 | -0.699 | -1 | 3 | -4.423 | 0 | 0 |
| Cefaclor | 367.806 | 3.25 | 7.25 | -1.333 | -1.187 | -2 | 2 | -0.446 | 0 | 0 |
| Cyanidin-3-O-rhamnoside | 433.39 | 7 | 10 | -2.27 | -1.147 | -2 | 1 | -2.38 | 2 | 2 |
| Epigallo catechin gallate | 458.378 | 8 | 8.75 | -0.287 | -4.346 | -2 | 1 | -3.53 | 2 | 1 |
| Eupatin | 360.32 | 2 | 6 | 2.239 | -1.468 | -2 | 3 | -4.098 | 0 | 0 |
| Eupatoretin | 374.346 | 2 | 7 | 2.413 | -1.035 | -2 | 3 | -4.007 | 0 | 0 |
| Flavonoid LP | 594.52 | 9 | 15 | -2.651 | -4.73 | -2 | 1 | -2.431 | 3 | 1 |
| Galangin | 270.241 | 2 | 3.75 | 1.777 | -1.309 | -2 | 3 | -3.412 | 0 | 0 |
| Hispidone | 346.336 | 1 | 5.5 | 2.766 | -0.974 | -1 | 3 | -4.285 | 0 | 0 |
| Kaempferol | 286.24 | 3 | 4.5 | 1.041 | -1.893 | -2 | 3 | -3.157 | 0 | 0 |
| Lanceoletin A | 336.387 | 1 | 4 | 4.45 | -0.51 | 0 | 3 | -5.741 | 0 | 0 |
| Laurifolin | 356.374 | 2 | 5.7 | 2.556 | -1.383 | -2 | 3 | -4.917 | 0 | 0 |
| Licocoumarone | 340.375 | 3 | 3.5 | 3.697 | -1.097 | -2 | 3 | -5.229 | 0 | 0 |
| Mebeverine | 429.555 | 0 | 6.25 | 5.345 | -0.497 | 1 | 3 | -4.065 | 1 | 1 |
| Oridonin | 364.438 | 3 | 7.6 | 1.51 | -0.899 | -1 | 3 | -2.87 | 0 | 1 |
| Papaverine | 339.39 | 0 | 4 | 4.813 | 0.131 | 1 | 3 | -0.54 | 0 | 0 |
| Phloretin | 274.273 | 2 | 3 | 2.066 | -1.683 | -2 | 3 | -2.81 | 0 | 0 |
| Piperine | 285.342 | 0 | 4.5 | 3.261 | -0.106 | 0 | 3 | -3.511 | 0 | 2 |
| Propolin D | 424.493 | 3 | 4.75 | 4.164 | -1.897 | -2 | 1 | -5.729 | 0 | 0 |
| Protopine | 353.374 | 0 | 7 | 1.64 | 0.728 | 2 | 3 | -5.14 | 0 | 0 |
| Quercetin | 302.24 | 4 | 5.25 | 0.367 | -2.419 | -2 | 2 | -2.909 | 0 | 0 |
| Reserpine | 608.687 | 1 | 10.7 | 4.942 | -0.141 | 1 | 2 | -5.55 | 2 | 2 |
| Rottlerin | 516.546 | 2 | 5.5 | 5.552 | -2.213 | -2 | 2 | -8.624 | 2 | 1 |
| Ternatin | 374.346 | 1 | 6 | 2.801 | -1.001 | -2 | 3 | -3.92 | 0 | 0 |
| Taurocholic acid | 515.704 | 4 | 10.6 | 2.216 | -2.498 | -2 | 2 | -4.964 | 1 | 0 |

| Compound Name | MW | HD | HA | QPlogPo/w | QPlogBB | CNS | HOA | QPlogS | RO5 | rtvFG |
|---|---|---|---|---|---|---|---|---|---|---|
| Taurocholic acid | 515.704 | 4 | 10.6 | 2.216 | -2.498 | -2 | 2 | -4.964 | 1 | 0 |
| Ursolic acid | 456.707 | 2 | 3.7 | 6.204 | -0.375 | -1 | 1 | -6.929 | 1 | 0 |
| Xanthurenic acid | 205.17 | 3 | 5.25 | 0.138 | -1.392 | -2 | 2 | -4.2 | 0 | 0 |

**MW-**Molecular weight, **HD-**Hydrogen bond Donor, **HA-**Hydrogen bond Acceptor, **QPlogPO/w-**Octonal/water partition coefficient, **QPlogBB-**Blood Brain Barrier, **CNS-**Central Nervous system, **HOA –** Human Oral Absorption, **QPlogS-**Water solubility, **RO5** - Rule of Five, **rtvFG – Reactive functional groups.**

**Docking Studies**

The result of XP docking study showed that Cyanidin 3-O-rhamnoside had better result with dock score − 8.3 Kcal/mol and interacted with the active site residues Arg 188, Tyr 258, also interacted with Asp 217, Glu 345 and Asp 215, Gly 319 which are functionally and structurally important residues, respectively. EGCG and Flavonoid LP were other top ranked compounds with dock score − 7.0 and − 8.0 Kcal/mol, respectively (Table 4).

From the analysis of IFD, Cyanidin 3-O-rhamnoside had interactions with the active site residues Arg 188, Tyr 258, Glu 284 and also interacted with functionally important residues Asp 217, Glu 345, Ser 346 and structurally important residues Asp 215 and Gly 319 with dock score − 11.3 Kcal/mol (Fig. 5). EGCG and Flavonoid LP had dock score − 10.9 and − 10.4 Kcal/mol, respectively (Table 5).

Table 4
Docking result of phytocompounds with Glycosyltransferase family 62 protein Using XP method

| S.No | Compound Name | Dock score (Kcal/mol) | Interacting residues | Bond Length (Å) |
|---|---|---|---|---|
| 1. | Cyanidin-3-o-rhamnoside | -8.3 | Arg 188, Asp 215, Asp 217(3), Tyr 258, Gly 319(2), Glu 345 | 2.21, 1.73, 1.61, 2.10, 2.14, 2.14, 2.40, 2.08 |
| 2. | Flavonoid LP | -8.0 | Asp 175, Gly 319, Thr 320, Ser 346 | 2.17, 2.16, 1.72, 2.51 |
| 3. | Epigallo catechin gallate | -7.0 | Pro 100, Arg 188, Arg 196(2), Gly 319, Glu 345(2) | 1.91, 2.15, 5.70, 3.94, 2.67, 1.86, 2.16 |
| 4. | Quercetin | -6.5 | Pro 100, Ser 102, Arg 196, Gly 319, Glu 345 | 2.04, 2.18, 3.73, 1.84, 2.23 |
| 5. | Rottlerin | -6.2 | Ser 102, Tyr 258, Asp 259, Gly 319 | 1.83, 2.04, 2.20, 2.01 |
| 6. | Phloretin | -5.7 | Pro 100, Ser 102, Arg 188, Gly 319, Thr 320, Glu 345 | 1.93, 2.37, 2.87, 1.77, 2.08, 2.15 |
| 7. | Eupatin | -5.5 | Pro 100, Arg 188, Asp 217, Tyr 258 | 1.76, 3.27, 1.91, 2.24 |
| 8. | Cefaclor | -5.3 | Arg 188, Asp 215, Tyr 258 | 1.76, 1.54, 1.80 |
| 9. | Kaempferol | -5.2 | Pro 100, Tyr 258 | 1.91, 1.99 |
| 10. | Caffeic acid | -5.0 | Pro 100, Ser 102(2), Arg 188 | 1.78, 1.92, 2.18, 2.87 |
| 11. | Carsonic acid | -4.9 | Arg 188(2), Asp 215(2) | 2.05, 4.53, 1.74, 1.95 |
| 12. | Mebeverine | -4.8 | Tyr 258, Gly 319 | 2.10, 1.76 |
| 13. | Propolin D | -4.7 | Ser 102, Arg 188, Arg 196 | 1.96, 3.37, 2.47 |
| 14. | Eupatoretin | -4.6 | Pro 100, Ser 102, Asp 217 | 1.86, 2.17, 2.32 |
| 15. | Artocarpin | -4.5 | Ser 102, Arg 188, Arg 196, Gly 319, Thr 320, Glu 345 | 2.23, 5.43, 3.95, 2.01, 2.10, 1.94 |
| 16. | Oridonin | -4.5 | Arg 188 | 2.13 |
| 17. | Andrographolide | -4.4 | Ser 102 | 1.96 |
| 18. | Laurifolin | -4.2 | Pro 100, Ser 102, Asp 217 | 2.44, 2.33, 1.65 |
| 19. | Galangin | -4.2 | Arg 188(2), Asp 217, Gly 319, Glu 345 | 5.62, 5.80, 1.86, 2.15, 1.91 |
| 20. | Hispidone | -4.1 | Arg 196, Tyr 258, Thr 320 | 5.97, 5.30, 2.15 |
| 21. | Lanceolatin A | -4.1 | Pro 100, Ser 102, His 370(2) | 1.72, 2.31, 5.41, 6.49 |
| 22. | Taurocholic acid | -3.9 | Thr 131, Glu 133, Asp 215, Tyr 258 | 2.30, 1.76. 2.36, 1.85 |
| 23. | Xanthurenic acid | -3.9 | Arg 188(2), Arg 196, Asn 261, Glu 284 | 1.90, 2.41, 1.76, 2.40, 1.65 |
| 24. | Protopine | -3.9 | Arg 196, Tyr 258, Gly 319 | 2.38, 1.71, 1.88 |
| 25. | Ternatin | -3.8 | Pro 100 | 2.18 |
| 26. | Piperine | -3.6 | Ser 102, Arg 103, His 370(3) | 2.09, 1.71, 5.44, 5.46, 5.75 |

| S.No | Compound Name | Dock score (Kcal/mol) | Interacting residues | Bond Length (Å) |
|---|---|---|---|---|
| 27. | Berberine | -3.3 | Arg 196(2), Asp 215, Asp 217 | 4.23, 4.32, 3.53, 4.35 |
| 28. | Reserpine | -3.3 | - | - |
| 29. | Licocoumarone | -3.2 | Arg 188, Thr 320, His 370 | 2.09, 2.03, 5.12 |
| 30. | Papaverine | -3.2 | Ser 102, Tyr 258, Gly 319 | 1.83, 2.11, 2.16 |
| 31. | Ursolic acid | -2.2 | Pro 100 | 1.87 |

Table 5

**Docking result of top 3 compounds selected from the XP method with Glycosyltransferase family 62 protein using IFD method**

| Compound Name | Dock Score (Kcal/mol) | Interacting Residues | Active Site residues | Bond Length (Å) |
|---|---|---|---|---|
| Cyanidin 3-O-rhamnoside | -11.3 | Ser 102, Arg 196, Tyr 258, Asp 259, Tyr 260, Asn 261(2), Glu 284(2), Gly 319, Thr 320(2), Glu 345 | Arg 196, Tyr 258, Asp 259, Glu 284, Glu 345 | 2.00, 2.20, 1.97, 1.97, 1.96, 2.20, 2.24, 1.83, 2.12, 2.40, 2.20, 2.48, 1.77 |
| EGCG | -10.9 | Pro 100(2), Ser 102, Tyr 105, Arg 188(2), Arg 196(2), Tyr 260, Glu 284, Gly 319, Glu 345(2), Ser 346 | Pro 100, Arg 188, Arg 196,Glu 284, Glu 345 | 1.66, 1.85, 1.88, 2.00, 2.13, 2.15, 2.24, 2.31, 1.93, 1.85, 1.93, 1.79, 2.01, 1.96 |
| Flavonoid LP | -10.4 | Ser 102, Arg 103, Ile 127, Gln 165, Tyr 258, Tyr 260, Glu 284 | Ile 127, Gln 165, Tyr 258, Glu 284 | 2.07, 2.42, 2.80, 2.15, 1.61, 2.05, 2.22 |

# Mm-gbsa

From the analysis of MM-GBSA, docked complexes were reranked which are in the 2nd and 3rd position of the target based on docking result. The docked complexes of compound Cyanidin 3-O-rhamnoside with Glycosyltransferase family 62 protein was − 75.09 Kcal/mol which suggests that the compound Cyanidin-3-O-rhmanoside has more binding efficiency with the target Glycosyltransferase family 62 protein. The compounds EGCG and Flavonoid LP has − 80.58 and − 69.22 kcal/mol, respectively (Table 6).

Table 6
Binding free energy calculation of selected compounds with Glycosyltransferase family 62 protein

| S.No | Compound Name | MMGBSA dG Bind |
|---|---|---|
| 1. | Cyanidin 3-O-rhamnoside | -85.09 |
| 2. | EGCG | -80.58 |
| 3. | Flavonoid LP | -69.22 |

# Molecular Dynamics Simulation

The molecular dynamics simulation of complex structure of Glycosyltransferase family 62 protein was carried out for 100 ns. The complex structure of Cyanidin-3-O-rhmanoside with Glycosyltransferase family 62 protein protein, an RMSD value exhibited that the C-α backbone of protein and heavy atoms of ligand were arranged within an acceptable range. The complex was relatively stable throughout the simulation time and peaks at the range of 2.8 to 3.0 Å (Fig. 6) hence the complex was more stable.

RMSF (Root Mean Square Fluctuation) is used to characterize conformational and structural changes in protein during simulation. In the case of Glycosyltransferase family 62 protein, some of backbone amino acid residues had fluctuations at 4 Å and the active site residues Pro 100, Ile 127, Gln 165, Arg 188, Arg 189, Ala 216, Tyr 258, Asp 259, Glu 284 and His 370 had lower fluctuations than other residues and they are within the acceptable range (0.5 to 2.0 Å) (Fig. 7).

# Interactions of complexes throughout simulation

Protein-ligand interactions throughout the simulation have been shown in the timeline representation and histogram of protein-ligand contacts. From the analysis of complex structure of Cyanidin-3-O-rhmanoside with Glycosyltransferase family 62 protein, timeline representation and histogram of protein-ligand interaction showed Arg 188, Asp 215, Asp 217, Tyr 258, Glu 284, Gly 319 and Glu 345 were involved in the H-bond interactions, hydrophobic interactions and water bridges from starting to end of the simulation period (Figs. 8 and 9). From the interaction analysis, Arg 188 (51%), Asp 215 (> 100%), Asp 217 (> 100%), Tyr 258 (82%), Asp 259 (51%), Glu 284 (75%), Gly 319 (91%) and Glu 345 (> 100%) had different interactions with the ligand and these interactions were maintained for more than 50% of the simulation period and it is shown in Fig. 10.

MD simulation study confirmed that the complex structure of Cyanidin 3-O-rhamnoside with Glycosyltransferase family 62 protein was stable and interactions found in the XP and IFD molecular docking were retained in MD simulation also.

## Discussion

Dermatophytosis is also called “Tinea” and “ringworm” because they are growing outwards on skin and producing ring like pattern [35]. *Trichophyton rubrum* is anthropophylic in nature, main host of this species is human being but it may affect animals too and accounts for 69.5% of the diseases caused by *Trichophyton* [36]. Currently available drugs such as ketoconazole, fluconazole, itraconazole, amphotericin, nystatin, micafungin, micafungin, etc, were not fully effective and most of them are producing side effects in human [37].

Modeled structure of alpha-1,6 mannosyltransferase subunit Mnn9 have 93.2% by homology modeling. According to Procheck, ideally the modeled protein must have more than 90% of residues in the favored region [38]. Hence, modeled

structure of Glycosyltransferase family 62 protein is of good quality.

Glycosyltransferase family 62 protein, is present in *T.rubrum* and other fungal species and it is not in human and human gut microbiome, also it is non-druggable target. Since many glycosyltransferases in fungi are play important roles in the synthesis of various compounds, they provide for promising targets for antifungal therapy [7]. Mnn9 protein is a very potential target as it is mainly involved in the metabolic pathway and various types of N-glucan biosynthesis pathway [5]. Metabolic pathways are very important for the production of many biological materials such as carbohydrates, lipids, proteins, lipoproteins, nucleic acids, amino acids, proteins and secondary metabolites which is essential for the growth and reproduction of species [39]. Also, this protein can be considered as broad spectrum target as they are present in various species of dermatophytes, *Aspergillus*, *Candida* and *Saccharomyces*.

Compounds Cyanidin 3-O-rhamnoside, EGCG and Flavonoid LP had better dock score and interacted with active site residues of Glycosyltransferase family 62 protein. Cyanidin 3-O-rhamnoside, EGCG and Flavonoid LP were top ranked compounds and they are from the class of flavonoids. Flavonoids are mostly present in plants also present in food supplements such as onion, citrus fruits, bananas, black tea, red wine and green tea [40]. Flavonoids are highly potential as antifungal, antibacterial and antioxidant agents [41]. Since olden days, flavonoids have been widely employed to treat a multiplicity of human ailments. A diversity of mechanisms, including disruption of the plasma membrane, the induction of mitochondrial dysfunction, and inhibition of the production of cell walls, cell division, RNA and protein synthesis, as well as the efflux mediated pumping system, are used by flavonoids to inhibit the growth of fungi [42].

The results of the docking study was reinforced by MM-GBSA analysis where greater binding affinity of compound Cyanidin 3-O-rhamnoside with the target Glycosyltransferase family 62 protein was observed. Previous studies proved that the MM-GBSA based rescoring docked complexes are good resulted with experimental binding affinities [43]. The structural stability of the docked complexes was studied by analyzing the RMSD (Root Mean Square Deviation) values based on their deviations, smaller deviation indicates the structure was more stable [44] and protein-ligand interactions which are classified into 4 types, i) H-bond, ii) Hydrophobic, iii) Ionic bond, iv) Water bridges. Among them, H-bond interaction is important of its influence on the drug specificity [45]. From the docking and simulation studies, the docked complexed structure of Glycosyltransferase family 62 protein with Cyanidin 3-O-rhamnoside exhibited the interactions with same residues which are involved in protein active site in XP, IFD methods and also in the MD simulation. Mainly Arg 188, Asp 215, Asp 217, Glu 284, Gly 319 and Glu 345 were involved in the interactions.

The importance of the residues Arg 209 and Asp 236 present in mannose binding site and catalytic motif, respectively in *S.cerevisiae* Mnn9 was reported by mutating them *in vitro* which leads to the loss of the protein's activity [46]. In the present docking and simulation of Cyanidin 3-O-rhamnoside and Glycosyltransferase family 62 protein of *T.rubrum*, the compound interacted with Arg 188 and Asp 215, the equivalent residues in *T.rubrum* which suggests that the activity of the protein may be lost due to the docking and the docked complex is stable.

# Conclusion

Glycosyltransferase family 62 protein (F2SC98) is very potential novel drug target because it is present only in *T.rubrum* and not in human, human gut flora and non-druggable target. Also, this protein is not only present in *T.rubrum* but also in other species of *Trichophyton, Microsporum, Epidermophyton, Aspergillus, Candida, and Saccharomyces*. Hence targeting this protein by compounds may be for not only dermatophytosis also treating many other diseases. Compound Cyanidin 3-O-rhamnoside exhibited better *in silico* result with the drug target Glycosyltransferase family 62 protein of *T.rubrum* which can be further validated as anti-dermatophytic agent using *in*

*vitro* and *in vivo* experiments. Moreover, this drug target could be of importance in the field of drug therapeutics as they may lead to the discovery of new drug molecules without side effects in human.

# Declarations

### Conflict of Interest

No conflict

### Ethical Statement

This study did not used human and animal sample

### Data Availability

Some data were attached as supplementary files

### Authors Contribution

MJ - Planned and guided the work, SAMH - Execute and written all the works, NDE - Contributed in data analysis and writing portions, AM – Manuscript writing and financial contribution, MK – Data analysis and Financial support.

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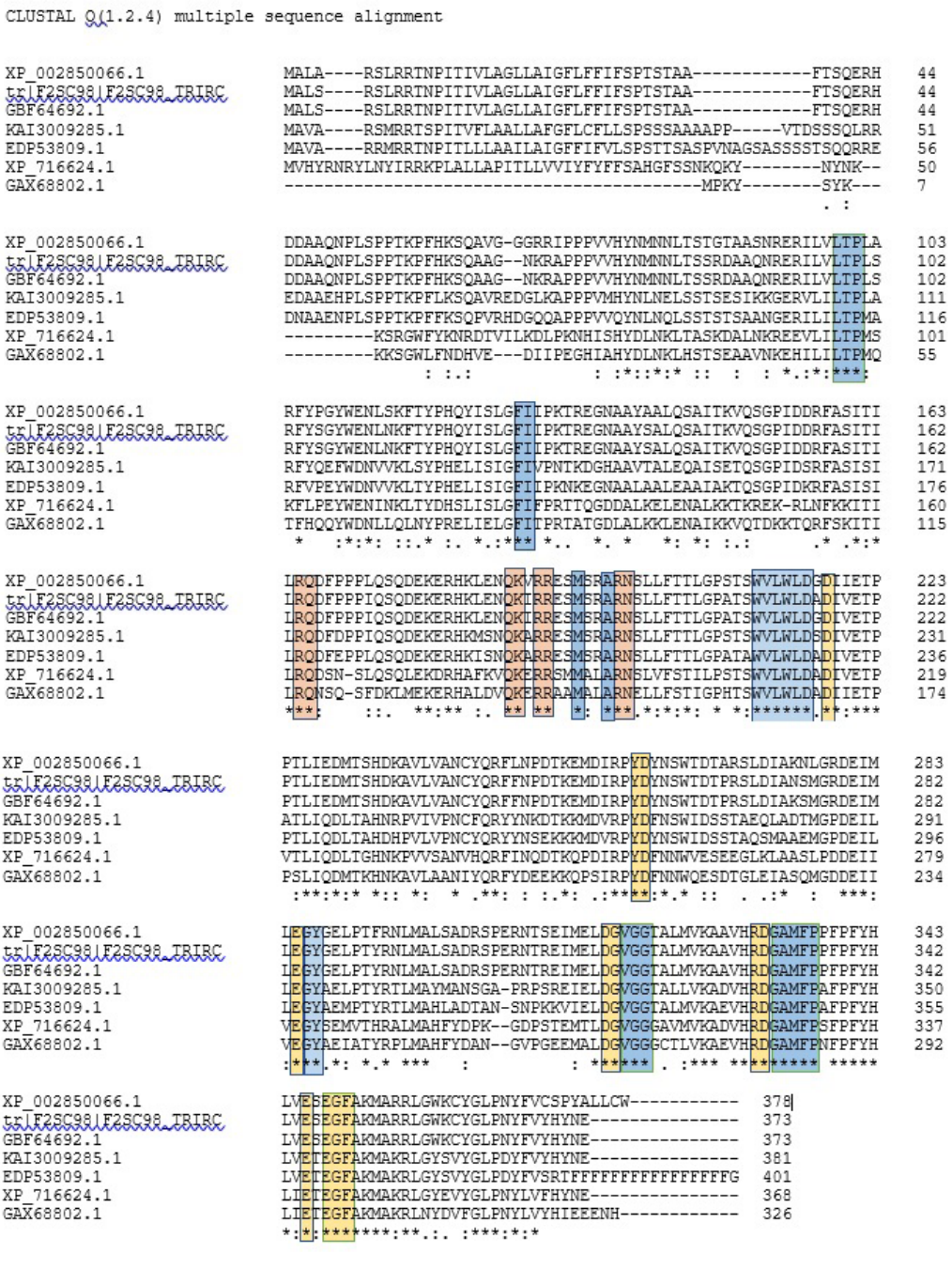
# Figures




## Figure 1

**Sequence alignment of similar sequences with Glycosyltransferase family 62 protein from various fungal species and structurally (blue), functionally conserved residues (Orange) highlighted**

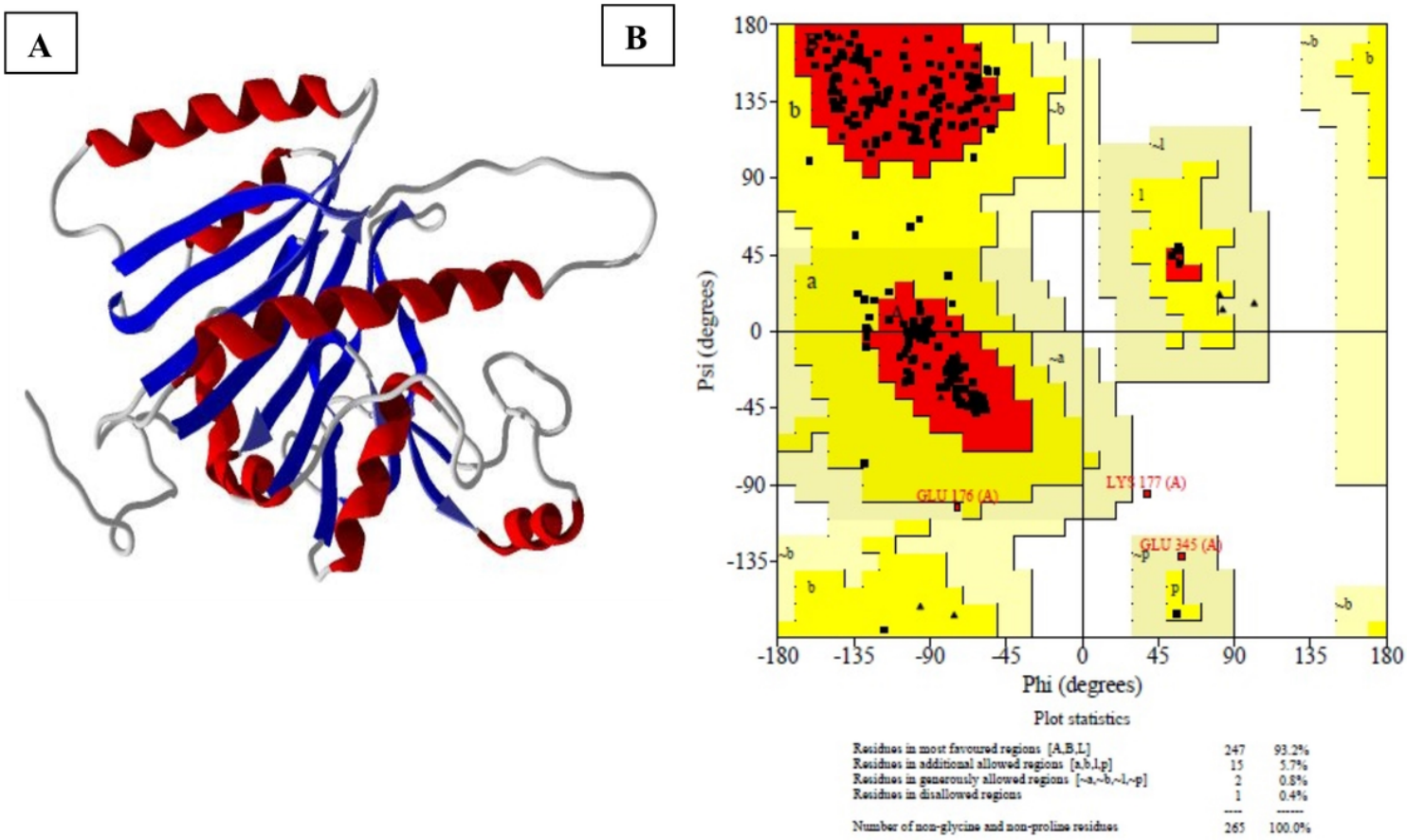



Figure 2

Novel drug target Glycosyltransferase family 62 protein A) Modeled 3D structure B) Ramachandran plot analysis

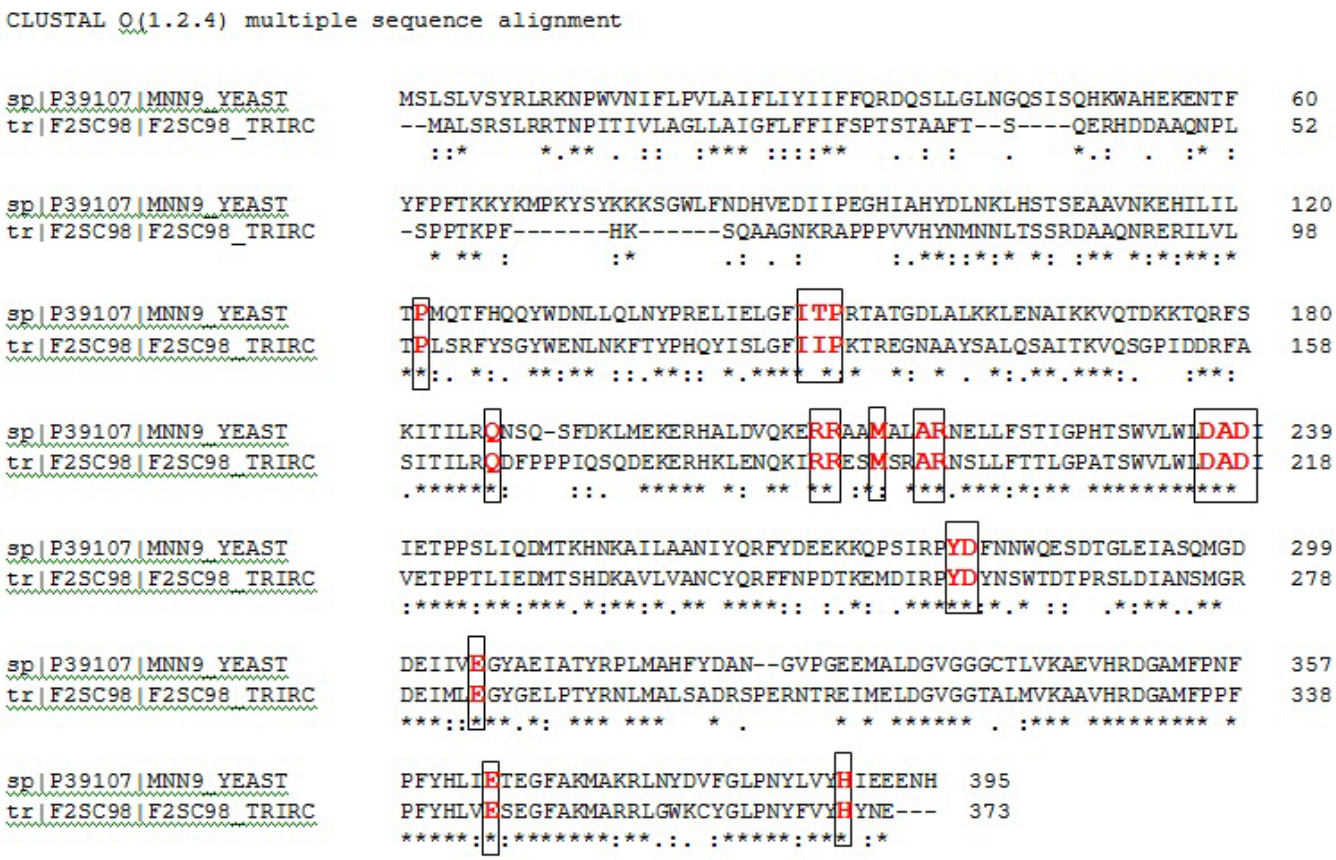



Figure 3

Sequence alignment of Mnn9 protein of *S.cerevisiae* with Glycosyltransferase family 62 protein in *T.rubrum* showing the position of active site residues

Figure 4

Predicted structurally (s in blue colour) and functionally (f in red colour) conserved residues of Glycosyltransferase family 62 protein *T.rubrum*

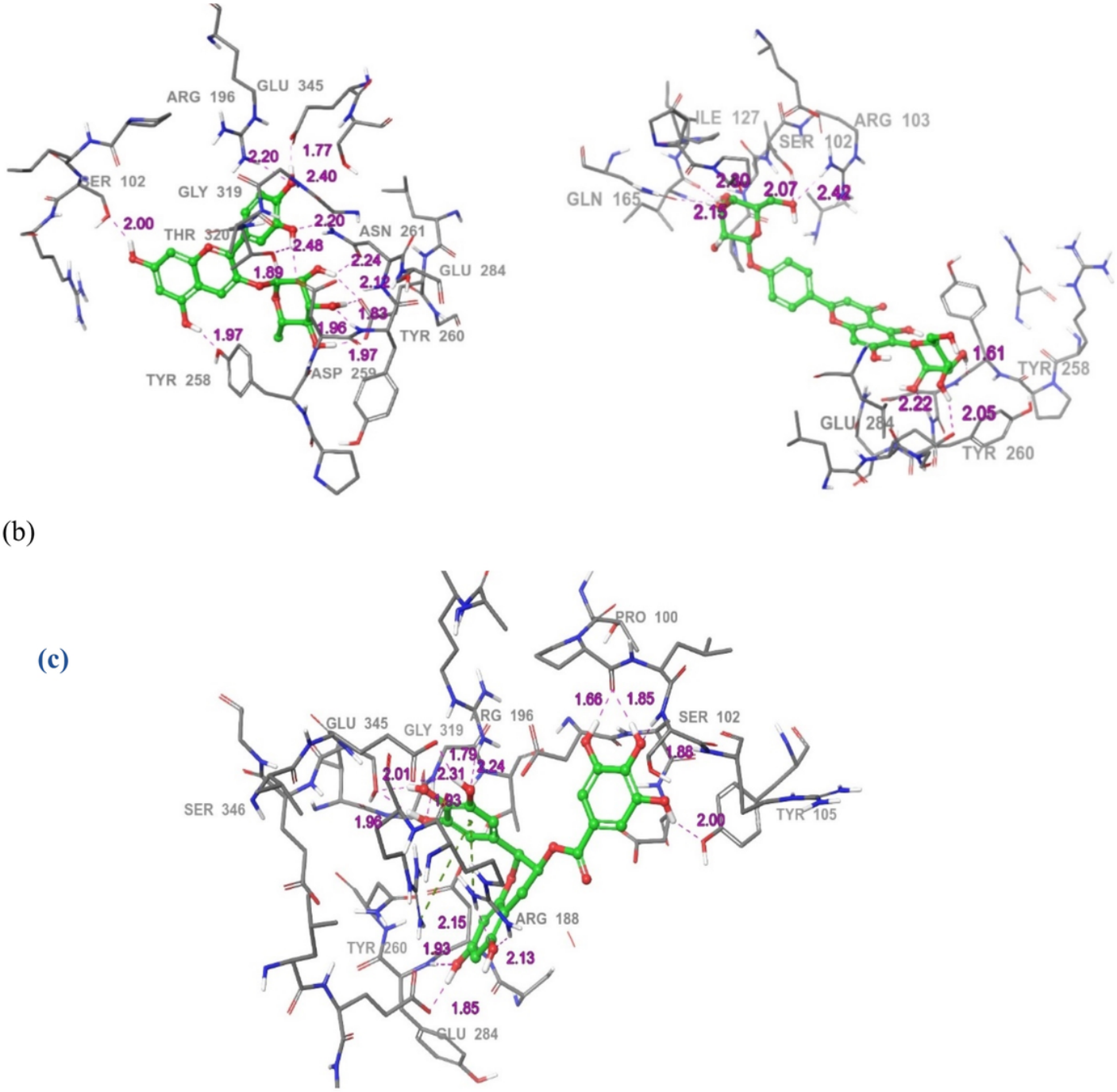



Figure 5

Docked complex structure of Glycosyltransferase family 62 protein with (a) Cyanidin-3-O-rhamnoside, (b) Flavonoid LP, (c) EGCG

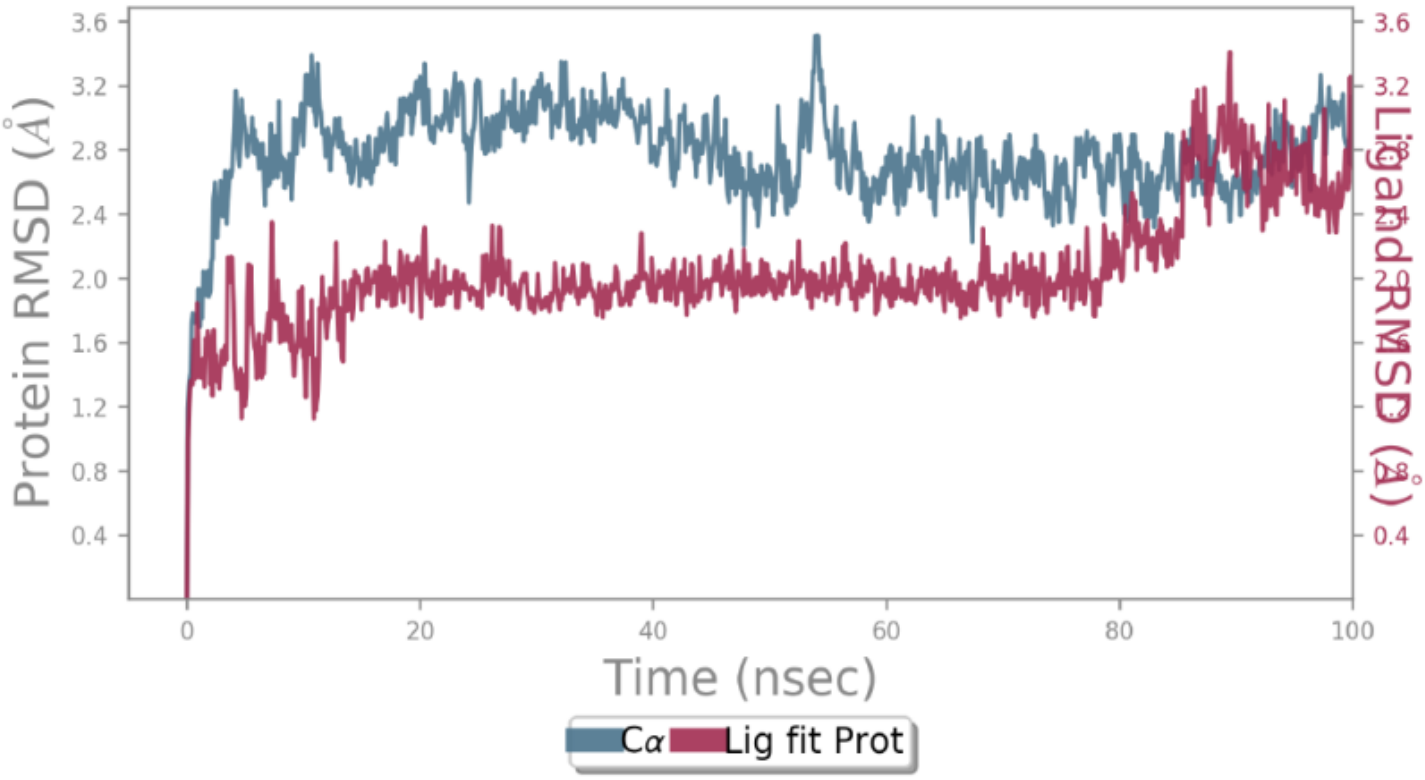



Figure 6

Protein-Ligand RMSD plot of docked complex structure of Cyanidin 3-O-rhamnoside with Glycosyltransferase family 62 protein

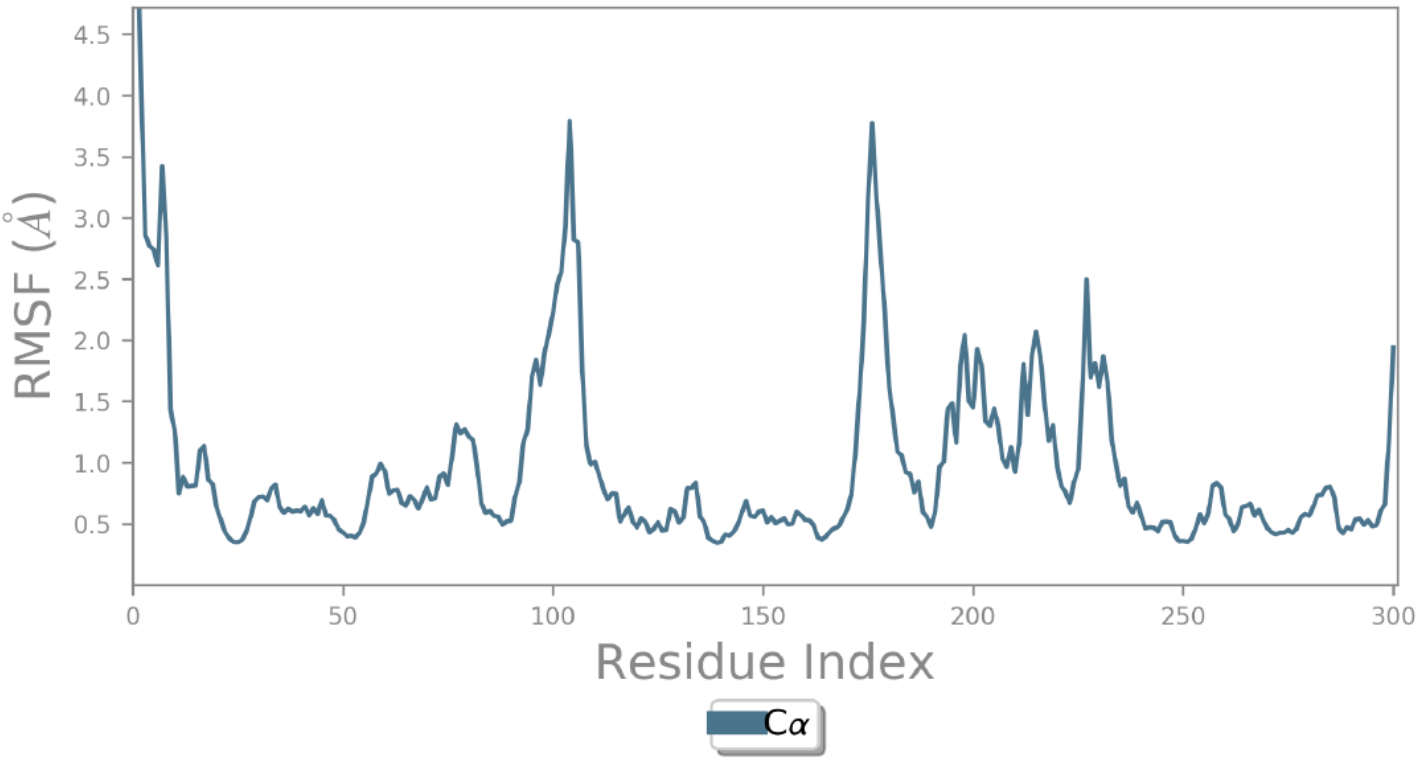


Figure 7

RMSF plot of Glycosyltransferase family 62 protein

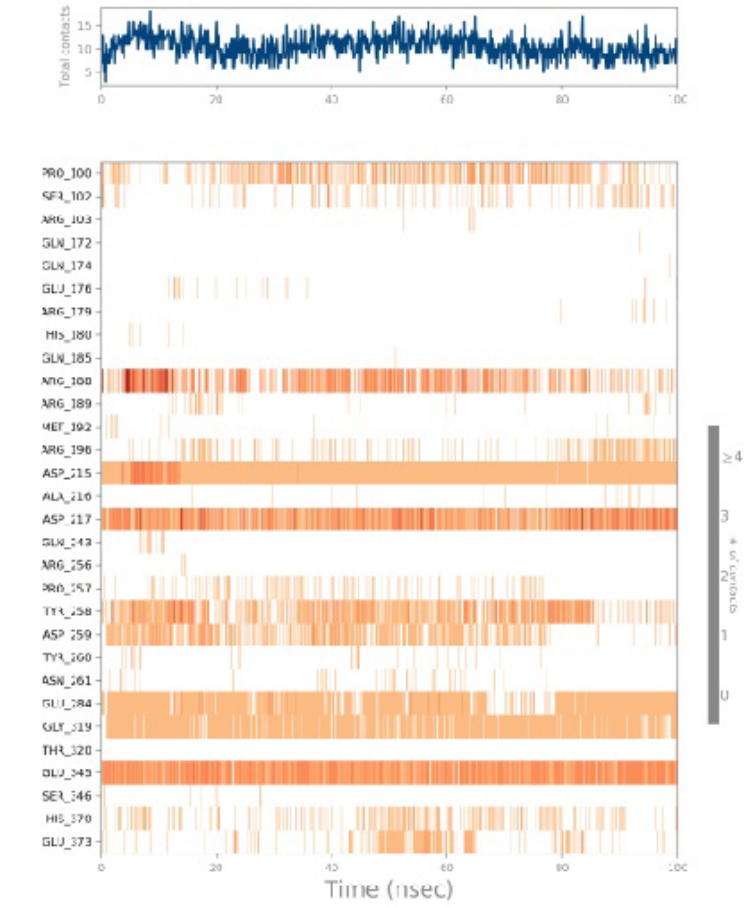



Figure 8

Timeline representation of protein-ligand contacts of Cyanidin 3-O-rhamnoside with Glycosyltransferase family 62 protein

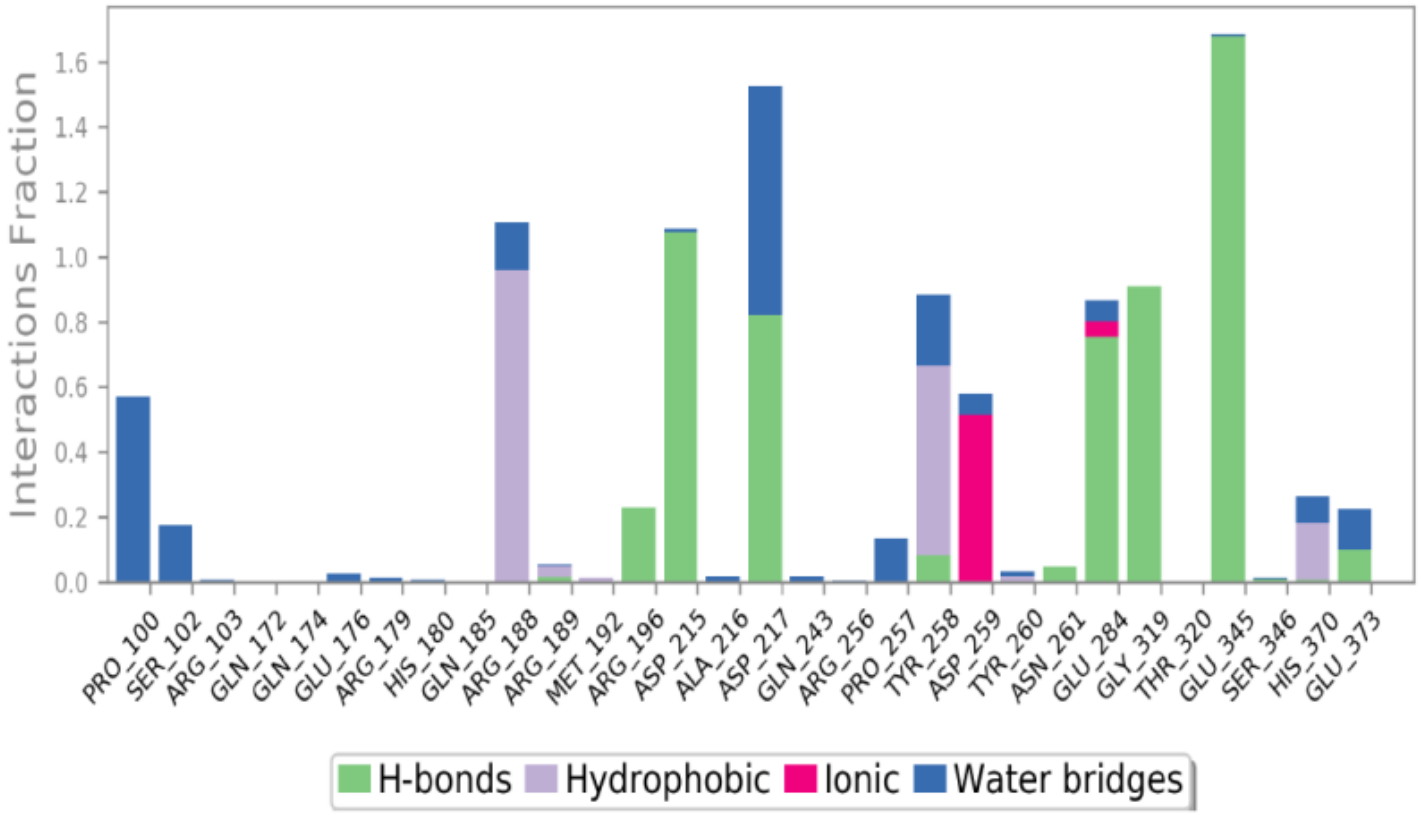



Figure 9

Histogram of protein-ligand contacts of docked complex structure of Cyanidin 3-O-rhamnoside with Glycosyltransferase family 62 protein

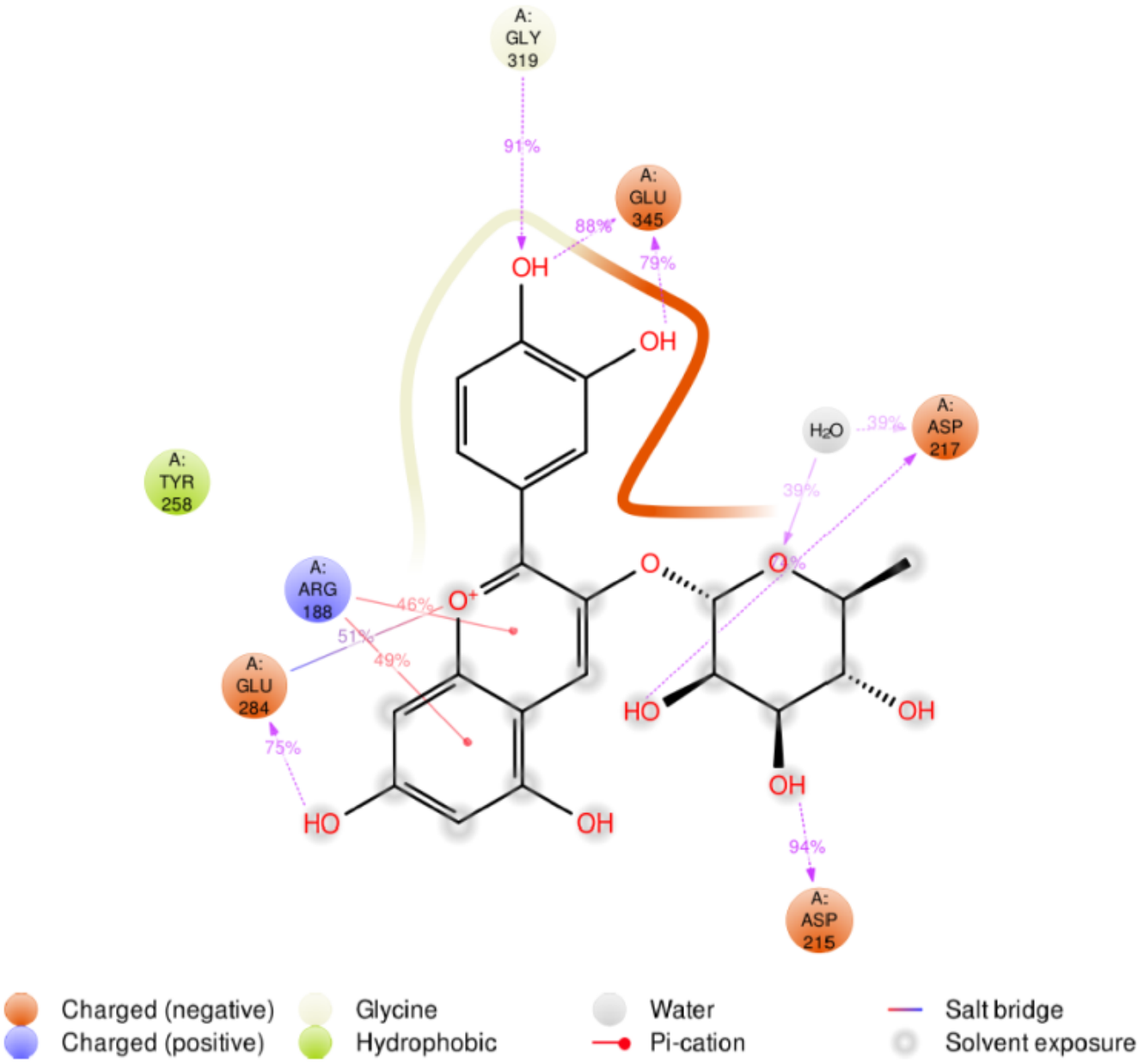



Figure 10

2D structure of interactions of Cyanidin 3-O-rhamnoside with Glycosyltransferase family 62 proteinat 100 ns during MD Simulation