IN SILICO STUDY OF BIOREMEDIATION PROPERTY OF MICROBIAL LACCASE ENZYMES 3CG8 AND 1GYC

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Abstract: Microbial bioremediation is the process of removing environmental toxins by using microorganisms and/or their products (enzymes or wasted biomass). The current study sought to evaluate the potential of laccase enzymes of microbial origin (bacterial and fungal) as a bioremediating agent for scavenging pollutants such as pharmaceutical, microplastic, and paper mill effluents. Bacterial laccase enzyme with PDB ID 3CG8 and fungal laccase enzyme with PDB ID 1GYC were used; ligand structures were acquired from Pub Chem. UCSF Chimera were used for visualisation and preparation of the protein structures for docking. Achilles docking server was used for blind docking, while AutoDock 4.2.6 was utilised for site-specific docking. The active sites of target proteins were predicted using the Scf bio-online programme for site specific docking. Blind docking with target protein bacterial laccase 3CG8 exhibited prominent binding affinity with Clarithromycin (hydrogen bonding with Thr192 and with Leu78) and with fungal laccase PDB ID 1GYC with Bezafibrate (hydrophobic interactions only). In site specific docking, polycarbonate exhibited the lowest binding energy with both laccase enzyme due to four hydrogen bonding with Ala266, Ile262, His104 and His154 residue of protein 3CG8 and two hydrogen bonding with Ala80, Phe344 of protein 1GYC. The current study demonstrated through an *insilico* approach laccase enzyme does possess the property of binding with pollutants and might degrade them to fewer toxic by-products. The study also reflected that the binding affinity and stability of binding is more efficient with blind docking in comparison to site specific docking implicating flexibility of the enzyme does affect the binding ability. Further studies might be conducted in terms of in silico prediction of complex degradation products and confirmation of the findings in experimental studies by exposing the pollutants to laccase synthesising microorganisms.

Keywords: 3CG8; 1GYC; laccase; bioremediation; *in silico*

1. Introduction:

Ecosystem is suffering as a result of the substantial environmental pollution caused by industrialization. Globally, biotechnologists are researching, creating cutting-edge tools, and using non-polluting methods to counteract the effects of global pollution [1, 2]. Bioremediation is one of the popular biotechnological methods that reduces or eliminates pollution that employs organisms to remove or consume contaminants from contaminated areas.

One of the important groups of microbial enzymes that has an important role in bioremediation is laccase, a benzenediol oxygen reductases (EC 1.10.3.2), also known as urushiol oxidases and p-diphenol oxidases, and are members of the enzyme family multicopper oxidases (MCOs). Blue copper proteins are a varied category of proteins that contain 1 to 9 copper atoms per molecule and range in size from 100 to more than 1,000 amino acid residues. The blue colour is caused by type 1 copper, which has an extinction coefficient of around 5000 at 610 nm. It is known to be flexible in its substrate binding, hence considered versatile enzymes capable of oxidising a wide range of phenolic and non-phenolic molecules and releasing water as the harmless by product of its reaction. Laccases are found in a variety of fungi, plants, bacteria, lichens, and insects, with laccases from each species displaying unique catalytic properties and sequences [3, 4, 5].

About 150 laccases have been characterized completely. Laccase was discovered to be present in the bacterial species of *B. halodurans*, *B. subtilis* SF, *Bacillus sp.* HR03, *Azospirillum lipoferum*, *P. desmolyticum* NCIM 2112, *B. pumilus*, *B. subtilis* WP1, and *P. putida* [8]. Among the fungal species which have reported for laccase activity are *Pleurotus pulmonarius*, *Pleurotus ostreatus*, *Agaricus bisporus*, *Trametes versicolor*, etc [7]. Two disulfide linkages and four copper atoms are arranged in three copper centres in fungal laccases and there are three types of lacasses Type 1 (T1), Type 2 (T2), and Type 3 (T3). Type I (T1) is mononuclear and exhibits an absorption band at roughly 610 nm, which is responsible for the enzyme's distinctive blue hue. Type 2 (T2)/ Type (T3) constitute a trinuclear cluster. The substrate is oxidised at T1 via a His-Cys-His tripeptide sequence, and the

extracted electrons are transported to the T2/T3 site where the reduction of molecular oxygen to water occurs [9, 10, 11]. Because of this reaction mechanism, laccase is regarded as a "green instrument," since it can perform catalysis utilising molecular oxygen as the sole co-substrate rather than hydrogen peroxide like other oxidoreductases do (v.gr. lignin peroxidase and manganese peroxidase) [12]. Laccase and laccase-like enzymes are widely distributed and play a variety of biological tasks such as lignification, delignification, pathogenicity, detoxification, morphogenesis and sporulation, rhizomorph growth and development, polymerization of melanin precursors, and spore coat resistance. Laccase has been investigated for application in a variety of biotechnological processes due to its broad substrate range, utilisation of easily accessible oxygen as the final electron acceptor, and lack of a need for cofactors or peroxide. Laccases have recently piqued the interest of researchers because of their potential applications in pollution detoxification and phenolic chemical bioremediation

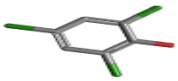
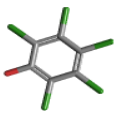
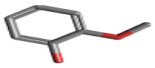
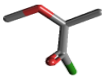
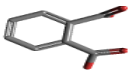
Understanding the mechanism of laccase enzyme, its properties etc, the present study was designed to understand the molecular interactions between bacterial laccase 3CG8 and fungal laccase 1GYC with various categories of pollutants namely- pharmaceutical pollutants, paper effluents and microplastics wastes.

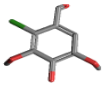
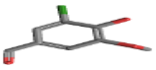
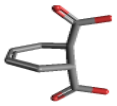
2 Materials and Methods

2.1 Preparation of target and pollutants as ligands

For target protein selection, crystal structures of the laccase enzyme (EC 1.10.3.2) were obtained from the protein data bank (PDB) at https://www.rcsb.org/ with PDB IDs 3CG8 and 1GYC. 3CG8 was a bacterial laccase, while 1GYC was a fungal laccase. The structures were downloaded from https://www.rcsb.org/ in.pdb format. UCSF Chimera was used to view the downloaded protein structures. The protein structures were then visualised in UCSF chimaera to remove any pre-existing ligands and water molecules, making the calculation easier and clearing the binding pocket.

Table 1: Paper Mill Effluent used as ligand

Sl. No	Paper Mill Effluent	Pub Chem Id	Structure
1	2-Chlorophenol	7245	
2	2,4,6- Trichlorophenol	6914	
3	Pentachlorophenol	992	
4	2- Methoxyphenol	460	
5	2-MethoPropanoyl chloride	92495	
6	Phthalic Acid	1017	
7	2,6- Dimethoxyphenol	7041	

8	2-Chlorosyringaldehyde	53479	
9	5- Chlorovanillin	29622	
10	Cis-Delta4-Tetrahydrophthalic acid	16823	

Based on the literature analysis, several ligands were chosen for ligand selection. The paper mill effluent was chosen as a ligand from the paper mentioned by Singh et al., 2020. Similarly, Duru et al., 2021 cited the use of microplastic as a ligand, while Singh et al., 2015 cited the use of pharmaceutical pollutants as a ligand. The ligand structures were obtained from the PubChem database, which is accessible at https://pubchem.ncbi.nlm.nih.gov/. Paper mill effluent, which contains 2-chlorophenol, microplastics such as Polyamide, and pharmaceutical pollutants such as Roxithromycin were among the pollutants utilised. The ligands used in ligand screening are listed in the table below. Additionally, energy reduction was performed to facilitate docking attempts on the structure and to minimise the total potential energy of the proteins and ligands.

Table 2: Microplastic used as ligand

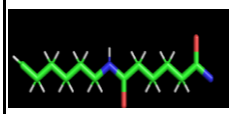
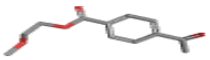
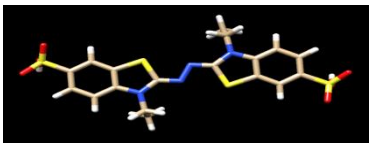
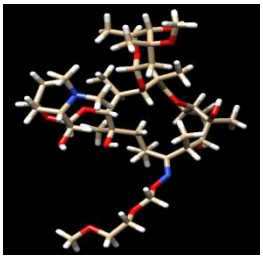
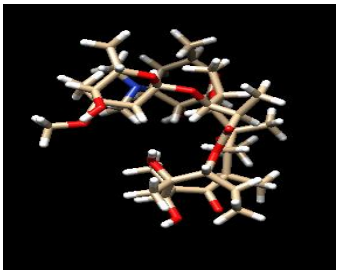
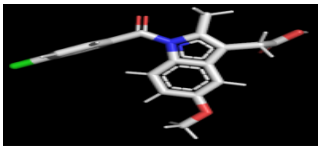
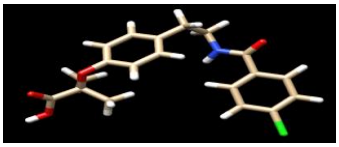
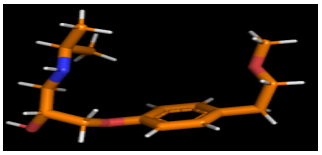
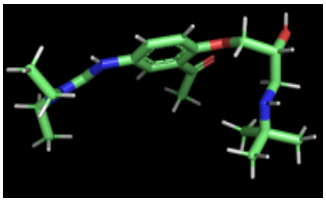
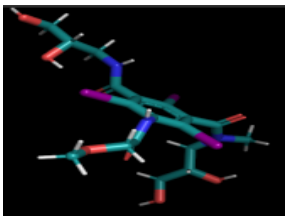
Sl. No	Microplastic	Pub Chem Id	Structure
1	Polyamide	36070	
2	Polyvinyl Chloride	6338	
3	Polycarbonate	6623	
4	Polyethylene terephthalate	18721140	
5	Polymethylene methacrylate	6658	
6	Polyurethane	12254	

Table 3: Pharmaceutical Pollutants used as ligand

Sl. No	Pharmaceutical Pollutant	Pub Chem Id	Structure
1	ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)]	9570474	
2	Roxithromycin	6915744	
3	Clarithromycin	84029	
4	Indomethacin	3715	
5	Bezafibrate	39042	
6	Metoprolol	4171	
7	Celiprolol	2663	

8	Iopromide	3736	

2.2 Molecular docking

Molecular docking is the study of how two or more molecular structures (for example, a drug and an enzyme or protein) fit together. Docking is a molecular modelling approach that predicts how a protein (enzyme) interacts with small molecules (ligands). The capacity of a protein (enzyme) or nucleic acid to build a supramolecular complex with small molecules has a significant influence on the protein's dynamics, which can aid in its biological activity. The behaviour of small molecules at target protein binding sites is described by molecular docking. The approach attempts to predict the affinity of a ligand for a protein by detecting accurate ligand poses in the binding pocket. The following two strategies were adopted for molecular docking.

2.2.1 Blind Docking

Blind docking occurs when a ligand is docked to the whole surface of a protein without knowing the target pocket. Blind docking necessitates multiple trials/runs and energy calculations before a good protein-ligand complex posture is achieved. For the blind docking, Achilles Blind Docking server, an online service was used to calculate protein-ligand interactions. It easily conducts blind docking calculations using a simple interface. No previous knowledge of the protein's binding sites is necessary; all that is required is structural data for both the ligand and the protein. The Achilles Blind Docking server is available at https://bio-hpc.ucam.edu/achilles/. Blind docking was accomplished using the binding energy was computed and documented.

2.2.2 Site specific docking

When a ligand is docked to particular site of a target protein while knowing the binding site of the protein it is called site- specific docking. In order to predict active sites, we utilized the Scf Bio Server http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp and noted the necessary X, Y, and Z coordinates for AutoDock. AutoDock 4.2.6 was used for site-specific docking. The AutoDock 4.2.6 application was docked using the Lamarckian Genetic Algorithm and the implemented empirical free energy function. AutoGrid was used to build the grid maps. Before doing molecular docking with AutoDock 4.2.6, hydrogen was given since it helped in establishing the ligand's binding affinity. Because the PDB structure lacks partial charges, additional charges were inserted before molecular docking. The grid map with X coordinate 24.481, Y coordinate 58.063, and Z coordinate 0.721 was used for molecular docking with protein PDB ID 3CG8. For molecular docking using protein PDB Id 1GYC, the grid box X coordinate was 27.752, Y coordinate was 21.985, and Z coordinate was 31.829. All dockings employed grid-point spacing of up to 1.000. The docking search produced the best conformation with the least amount of docked energy [13, 14]. The LIGPLOT programme was used to investigate the interactions of complex protein-ligand conformations, such as hydrogen bonds and bond lengths. LIGPLOT is a computer programme that uses Protein Data Bank information to generate schematic 2-D representations of protein-ligand interactions. Hydrogen bonds are shown as dashed lines linking the atoms, whereas hydrophobic interactions are shown as an arc with spokes extending towards the ligand atoms they contact.

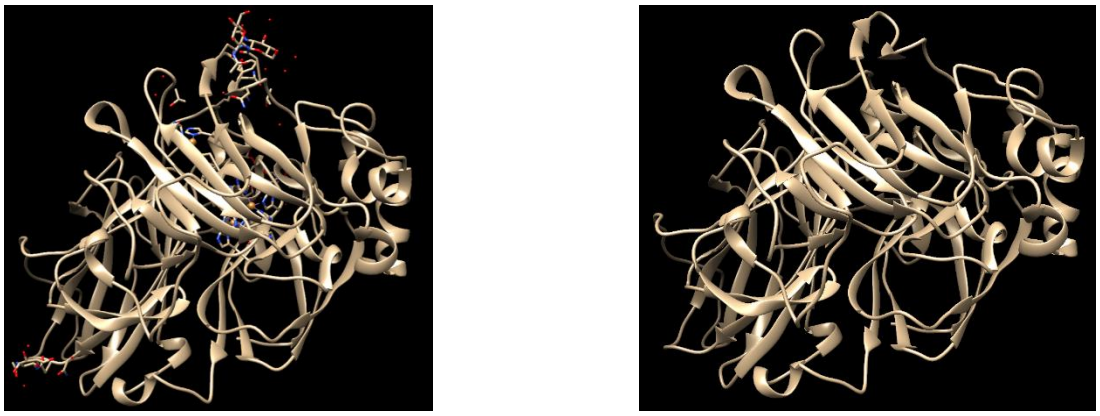
3. Results and Discussion

The structure of the laccase enzyme of bacterial and fungal origin PDB IDs 3CG8 and 1GYC was visualised by UCSF Chimera. It was used to eliminate any pre-existing ligands, including water molecules and the energy of the target protein was reduced, making docking tests on the target protein easier. The PDB ID 3CG8 protein contained three chains: A, B, and C, whereas the PDB ID 1GYC target protein has four chains: A, B, C, and D, with chain A serving as the active site. Chain A of target protein with PDB ID 3CG8 has four alpha helices and twenty-one beta sheets, whereas chain A of target protein with PDB ID 1GYC has twelve alpha helices and thirty beta sheets. The target proteins and ligands were prepared by getting 3-D structures of all of them and importing

them into the Chimera software. Using Chimera software, metals were removed from the ligand structure, and energy reduction was done for a viable docking research.

A B

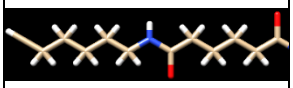
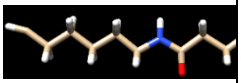
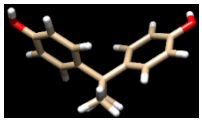
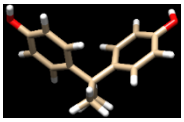
Figure1: Structure of protein PDB ID 3CG8 before preparation of target protein (A) and after preparation of target protein (B)

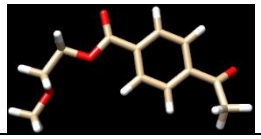
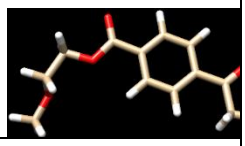
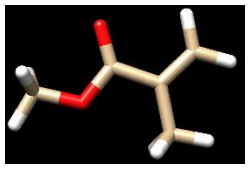
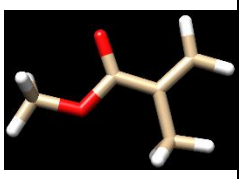
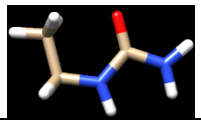
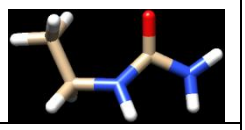
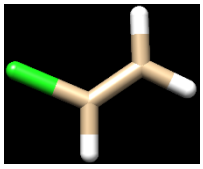
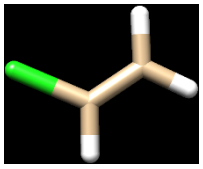
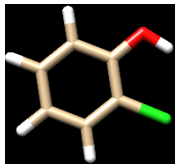
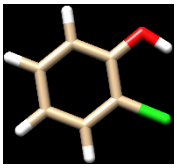
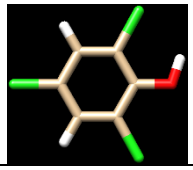
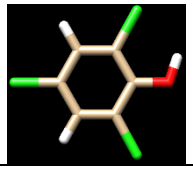
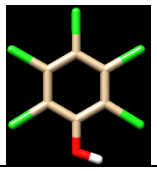
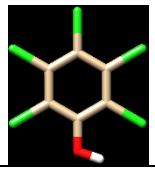
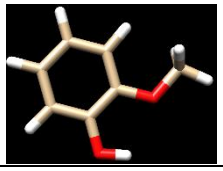
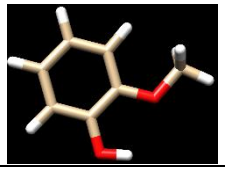
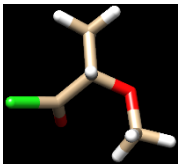
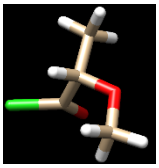


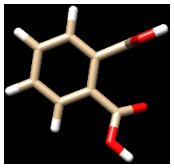
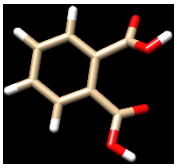
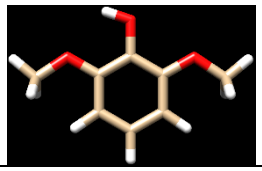
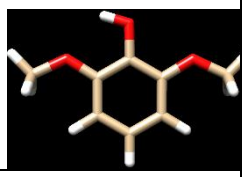
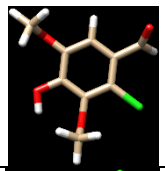
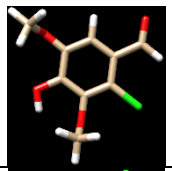
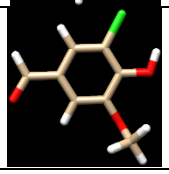
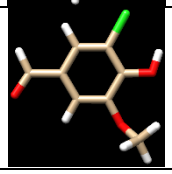
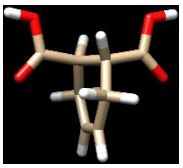
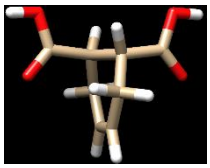
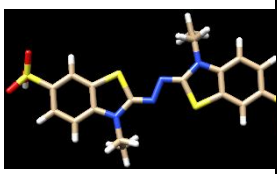
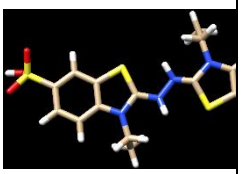
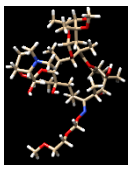
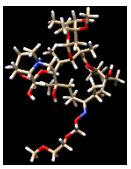
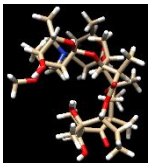
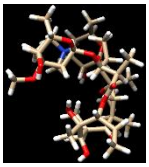
A B

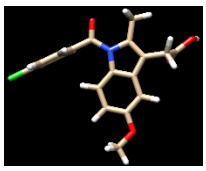
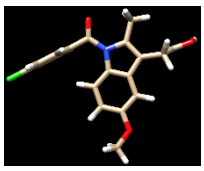
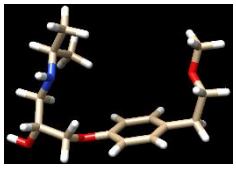
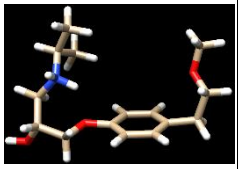
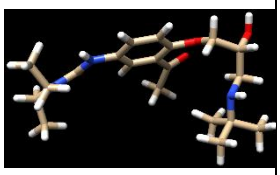
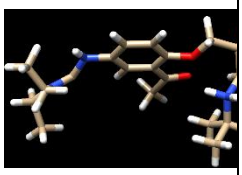
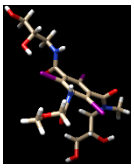
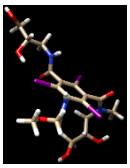
Figure 2: Structure of protein PDB ID 1GYC before preparation of target protein (A) and after preparation of target protein (B)

Table 4: Structure of ligands before and after energy minimization

Sl. No	Compounds	Structure of ligand before preparation	Structure of ligand after preparation
1	Polyamide [CH]CCCCCNC(=O)CCCCC(=O)[N]		
2	Polycarbonate CC(C)(C1=CC=C(C=C1)O)C2=CC=C(C=C2)O		

3	Polyethylene terephthalate CC(=O)C1=CC=C(C=C1)C(=O)OCCOC		
4	Polymethylene methacrylate CC(=C)C(=O)OC		
5	Polyurethane CCNC(=O)N		
6	Polyvinyl Chloride C=CCl		
7	2- Chlorophenol C1=CC=C(C(=C1)O)Cl		
8	2,4,6- Trichlorophenol C1=C(C=C(C(=C1Cl)O)Cl)Cl		
9	Pentachlorophenol C1(=C(C(=C(C(=C1Cl)Cl)Cl)Cl)Cl)O		
10	2- Methoxyphenol COC1=CC=CC=C1O		
11	2- Methoxy propanoyl chloride CC(C(=O)Cl)OC		

12	Phthalic Acid C1=CC=C(C(=C1)C(=O)O)C(=O)O		
13	2,6- Dimethoxyphenol COC1=C(C(=CC=C1)OC)O		
14	2- Chlorosyringaldehyde COC1=C(C(=C(C(=C1)C=O)Cl)OC)O		
15	5- Chlorovanillin COC1=C(C(=CC(=C1)C=O)Cl)O		
16	Cis- Delta4- Tetrahydrophthalic acid C1C=CCC(C1C(=O)O)C(=O)O		
17	ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] CCN1C2=C(C=C(C=C2) S(=O)(=O)[O])SC1=NN=C3N(C4=C(S3)C=C(C=C4)S(=O)(=O)[O])CC.[NH4+].[NH4+]		
18	Roxithromycin CCC1C(C(C(C(=NOCOCCOC)C(CC(C(C(C(C(C(=O) O1) C) OC2CC(C(C(O2) C) O) (C)OC) C) OC3C(C(CC(O3) C) N(C)C) O) (C)O) C) C) O) (C)O		
19	Clarithromycin CCC1C(C(C(C(=O)C(CC(C(C(C(C(C(=O)O1)C)OC2CC(C(C(O2)C)O)(C)OC)C)OC3C(C(CC(O3)C)N(C)C)O)(C)OC)C)C)O)(C)O		

20	Indomethacin CC1=C(C2=C(N1C(=O)C3=CC=C(C=C3)Cl)C=CC(=C2)OC)CC(=O)O		
21	Bezafibrate CC(C)(C(=O)O)OC1=CC=C(C=C1)CCNC(=O)C2=CC=C(C=C2)Cl		
22	Metoprolol CC(C)NCC(COC1=CC=C(C=C1)CCOC)O		
23	Celiprolol CCN(CC)C(=O)NC1=CC(=C(C=C1)OCC(CNC(C)(C)C)O)C(=O)C		
24	Iopromide CN(CC(CO)O)C(=O)C1=C(C(=C(C(=C1I)C(=O)NCC(CO)O)I)NC(=O)COC)I		

After the preparation of ligands and target proteins, blind docking was performed using Achilles server. 2-Chlorophenol with bacterial laccase showed the binding energy -4.30 kCal/mole which was due bonding between oxygen of Arg244 (A), Asn243 (A), and Gly270 (A) with oxygen atoms of the ligands and with fungal laccase the binding energy was -5.60 kCal/mole due to one hydrogen bond with the His residue at 111 positions with nitrogen atom of ligands. 2,4,6- Trichlorophenol showed binding energy of -4.50 kCal/mole with bacterial laccase due to the presence of two hydrogen bond with Oxygen of Arg244 (A) with oxygen and Gly at position 270 with nitrogen of the ligand. In comparison with fungal laccase, it showed the binding energy of -5.50 kCal/mole due to one hydrogen bond between Oxygen of His111 (A) with Oxygen. Pentachlorophenol binding energy with bacterial laccase was -4.80 kCal/mole due to the existence of hydrogen bonds oxygen of Arg244 (A) with Oxygen and bonding between Oxygen of Gly270 (A) with Nitrogen of ligand. With fungal laccase, the binding energy was -5.70 kCal/mole due to one hydrogen bond between oxygen of Glu142 (A) with oxygen.
2- Methoxypropanoyl chloride showed a binding energy of -3.90 kCal/mole with bacterial laccase due to the presence of hydrogen bonding between O1 (Oxygen at position 1) of Gly270 (A) with Nitrogen and bonding between O2 (Oxygen at position 2) of Tyr152 (A) with Nitrogen. In the case of fungal laccase binding energy of -3.90 kCal/mole was observed due to the presence of a hydrogen bond between O2 (Oxygen at position 2) of Ser113 (A) with Nitrogen and bonding between O1 (Oxygen at position 1) of His111 (A) with Nitrogen.

2- Methoxyphenol binding energy with bacterial laccase was -4.40 kCal/mole due to the bonding between O2 (Oxygen at position 2) of Gly270 (A) with Nitrogen and bonding between O2 (Oxygen at position 2) of Asn243 (A) with Oxygen and that with fungal laccase was -5.10 kCal/mole due to the hydrogen bond between Ser113 and His111. Phthalic acid binding energy with bacterial laccase was -5.50 kCal/mole due to one hydrogen bond with between O2 (Oxygen at position 2) of Lys204 (A) with Nitrogen. However, binding energy of the fungal laccase was -6.50 kCal/mole due to the hydrogen bonds between O2 (Oxygen at position 2) of Asp101(A) with Oxygen,

bonding between O2 (Oxygen at position 2) of Lys40 (A) with Nitrogen and bonding between O4 (Oxygen at position 4) of Asp128 (A) with Oxygen. 2,6- Dimethoxyphenol binding energy with bacterial laccase was -4.30 kCal/mole because of the existence of a hydrogen bond between Nitrogen of Tyr152 (A) with O3 (Oxygen at position 3), the bonding between O3 (Oxygen at position 3) of Arg244 (A) with Oxygen and the bonding between O2 (Oxygen at position 2) of Gly270 (A) with Nitrogen. The binding energy of the fungal laccase was -5.00 kCal/mole due to the existence of a bonding between O2 (Oxygen at position 2) of HIS402 (A) with Nitrogen and bonding between O2 (Oxygen at position 2) of Gln442 (A) with Nitrogen.

2- Chlorosyringaldehyde with bacterial laccase showed binding energy of -4.80 kCal/mole due to the existence of bonding between O2 (Oxygen at position 2) of Leu78 (A) with Nitrogen and bonding between O4 (Oxygen at position 4) Thr192 (A) with Nitrogen. Whereas in case of fungal laccase, the binding energy was -5.20 kCal/mole due to the bonding between O1 (Oxygen at position 1) of Leu35 (A) with Nitrogen and bonding between O2 (Oxygen at position 2) of Pro32 (A) with Oxygen. 5- Chlorovanillin binding energy with bacterial laccase was -4.80 kCal/mole due to the existence of a hydrogen bond between bonding between O2 (Oxygen at position 2) of Pro75 (A) with Oxygen, and bonding between O2 (Oxygen at position 2) of Ser73 (A) with Oxygen and the bonding between O3 (Oxygen at position 3) of Thr192(A) with Nitrogen. Fungal laccase demonstrated a binding energy of -5.50 kCal/mole due to hydrogen bonding between O1 (Oxygen at position 1) of Ala403 (A) with Nitrogen and bonding between O2 (Oxygen at position 2) of His402 (A) with Nitrogen.

Cis-Delta4- tetrahydrophthalic acid showed binding energy of -4.90 kCal/mole with bacterial laccase due to hydrogen bond bonding between O4 (Oxygen at position 4) of Pro137 (A) and the bonding between O2 (Oxygen at position 2) of Lys182 (A) with Nitrogen. The binding energy of fungal laccase was -6.30 kCal/mole due to hydrogen bonding between bonding between O1 (Oxygen at position 1) of Lys40 (A) with Nitrogen and bonding between O4 (Oxygen at position 4) of Asn227 (A) with Nitrogen. Polyamide showed binding energy of -5.00 kCal/mole with bacterial laccase due to the presence of hydrogen bond bonding between Nitrogen of Ser268 (A) with O2 (Oxygen at position 2) and the bonding between N1 (Nitrogen at position 1) of Tyr152 (A) with Oxygen. The bonding between O1 (Oxygen at position 1) of His402 (A) with Nitrogen, fungal laccase had a binding energy of -6.00 kCal/mole.

Polyvinyl Chloride with bacterial laccase has binding energy of -2.40 kCal/mole because of the presence of only hydrophobic interactions and absence of hydrogen bonding. Whereas in case of fungal laccase, the binding energy was -2.50 kCal/mole which was also because of the presence of hydrophobic interactions and absence of hydrogen bonding. Polycarbonate has binding energy of -6.30 kCal/mole in case of bacterial laccase due to the existence of hydrogen bonding bonding between Oxygen of Asn243 (A) with O2 (Oxygen at position 2), the bonding between O2 (Oxygen at position 2) of Gly270 (A) with Nitrogen and bonding between O1 (Oxygen at position 1) of Ala266 (A) with Oxygen. The binding energy of fungal laccase was -6.9 kCal/mole, which was owing to the existence of a hydrogen bond bonding between O1 (Oxygen at position 1) of Phe31 (A) with Oxygen, bonding between O2 (Oxygen at position 2) of Val145 (A) with oxygen and bonding between O2 (Oxygen at position 2) of Glu142(A) with oxygen. Polyethylene terephthalate with bacterial laccase showed binding energy of -5.10 kCal/mole due to the existence of bonding between O3 (Oxygen at position 3) of Leu78 (A) with Nitrogen and O4 (Oxygen at position 4) of Asn201 (A) with Nitrogen. The binding energy of fungal laccase was -5.9 kCal/mole due to the existence of bonding between O2 (Oxygen at position 2) of His402 (A) with Nitrogen and bonding between O1 (Oxygen at position 1) of Ala403 (A) with Nitrogen.

Polymethylene methacrylate showed binding energy of -3.90 kCal/mole with bacterial laccase due to the presence of only hydrophobic bond and absence of hydrogen bond. Whereas the binding energy of fungal laccase was -4.10 kCal/mole due to presence of hydrogen bond between O2 (Oxygen at position 2) of Ser113 (A) with Nitrogen and bonding between O1 (Oxygen at position 1) of His111 (A) with Nitrogen. Polyurethane has binding energy of -6.30 kCal/mole with bacterial laccase because of the existence of bonding between Oxygen of Tyr152 (A) with Nitrogen. Furthermore, fungal laccase showed the binding energy of -3.7 kCal/mole which is due to the presence of hydrogen bonding between Oxygen of Asp206 (A) with N1 (Nitrogen at position 1) and bonding between Oxygen of Asn264 (A) with N2 (Nitrogen at position 2).

ABTS [2,2'-azino-bis (3- bindinethylbenzothiazoline-6-sulfonic acid)] showed binding energy of -7.40 kCal/mole which is because of the presence of hydrogen bonding between Asn201 and Val74. Whether in case of fungal laccase, the binding energy was -6.8 kCal/mole due to the existence of bonding between O4 (Oxygen at position 4) of Asn201 (A) with Nitrogen and bonding between O6 (Oxygen at position 4) of Val74 (A) with Oxygen. Indomethacin with bacterial laccase showed binding energy of -6.60 kCal/mole due to the presence of one

hydrogen bond between Leu at position 78. In fungal laccase the binding energy was -6.90 kCal/mole due to the presence of hydrogen bonding between O5 (Oxygen at position 5) of Ala1 (A) with Nitrogen and bonding between O3 (Oxygen at position 3) of Val187 (A) with Oxygen.

Bezafibrate has binding energy of -6.30 kCal/mole with bacterial laccase because of the bonding between O3 (Oxygen at position 3) of Leu78 (A) with Nitrogen. Whereas the binding energy of fungal laccase was -7.90 kCal/mole because of the presence of bonding between O4 (Oxygen at position 4) of Ala103 (A) with Nitrogen and bonding between O2 (Oxygen at position 2) of Asn227 (A) with Nitrogen. Roxithromycin with bacterial laccase showed binding energy of –6.2 kCal/mole because of the presence of only hydrophobic interactions and absence of hydrogen bonding. The binding energy of fungal laccase was -6.8 kCal/mole due to the existence of hydrogen bonding O11 (Oxygen at position 11) of Lys40 (A) with Nitrogen and bonding between O13 (Oxygen at position 13) of Asn227 (A) with Nitrogen.

Clarithromycin showed binding energy of -7.6 kCal/mole with bacterial laccase due to the presence of bonding between O12 (Oxygen at position 12) of Thr192 (A) with Oxygen and bonding between O7 (Oxygen at position 7) of Leu78 (A) with Nitrogen. Whereas in case of fungal laccase, the binding energy was -7.3 kCal/mole because of the presence of only one hydrogen bond between Leu35. Metoprolol with bacterial laccase showed binding energy of -4.9 kCal/mole due to the presence of only one hydrogen bond between bonding between O2 (Oxygen at position 2) of Leu35 (A) with Nitrogen. Furthermore, the binding energy of fungal laccase was -5.6 kCal/mole due to the presence of Ala at position 103 and Asp at position 444.

Celiprolol has binding energy of -5.8 kCal/mole with bacterial laccase because of the presence of the hydrogen bonding bonding between O4 (Oxygen at position 4) of Asn201 (A) with Nitrogen and bonding between N1 (Nitrogen at position 1) of Thr190 with Oxygen. Whereas in case of fungal laccase, the binding energy was -6.9 kCal/mole because of the presence of hydrogen bonding between N2 (Nitrogen at position 2) of Ala403 (A) with Oxygen, bonding between O4 (Oxygen at position 4) of His402 (A) with Nitrogen, bonding between O4 (Oxygen at position 4) of Gln442 (A) with Nitrogen and bonding between N1 (Nitrogen at position 1) of Asp101 with Oxygen. Iopromide with bacterial laccase showed binding energy of -5.2 kCal/mole due to the existence of hydrogen bonding between bonding between O1 (Oxygen at position 1) of Leu78 (A) with Nitrogen and bonding between O4 (Oxygen at position 4) of Thr192 (A) with Nitrogen. The binding energy of fungal laccase was -5.5 kCal/mole due to the existence of bonding between O5 (Oxygen at position 5) of Asn172 (A) with Oxygen

Table 5: Binding Energy of PDB ID 3CG8 and 1GYC after docking via Achilles Blind Docking Server

Sl. No	Ligands	Binding Energy (kCal/mole) with Bacterial Laccase	Hydrogen Interaction	Binding Energy (kCal/mole) with Fungal Laccase	Hydrogen Interaction
1.	2-Chlorophenol	-4.30	Yes, The bonding between Oxygen of Arg244 (A) with Oxygen, bonding between Oxygen of Asn 243 (A) with Oxygen and bonding between Oxygen of Gly270 (A) with oxygen	-5.60	Yes, The bonding between Oxygen of His111 (A) with Nitrogen
2.	2,4,6-Trichlorophenol	-4.50	Yes, The bonding between Oxygen of Gly270 (A) with Nitrogen and Oxygen of Arg244 (A) with Oxygen	-5.50	Yes, The bonding between Oxygen of His111 (A) with Oxygen

3.	Penta Chlorophenol	-4.80	Yes, The bonding between Oxygen of Arg244 (A) with Oxygen and bonding between Oxygen of Gly270 (A) with Nitrogen	-5.70	Yes, The bonding between Oxygen of Glu142 (A) with Oxygen
4.	2- Methoxypropanoyl chloride	-3.90	Yes, The bonding between O1 (Oxygen at position 1) of Gly270 (A) with Nitrogen and bonding between O2 (Oxygen at position 2) of Tyr152 (A) with Nitrogen	-3.90	Yes, The bonding between O2 (Oxygen at position 2) of Ser113 (A) with Nitrogen and bonding between O1(Oxygen at position 1) of His111 (A) with Nitrogen
5.	2- Methoxyphenol	-4.40	Yes, The bonding between O2 (Oxygen at position 2) of Gly270 (A) with Nitrogen and bonding between O2 (Oxygen at position 2) of Asn243 (A) with Oxygen	-5.10	Yes, The bonding between O1 (Oxygen at position 1) of Ser113 (A) with Nitrogen and bonding between O2 (Oxygen at position 2) of His111 (A) with Oxygen
6.	Phthalic Acid	-5.50	Yes, The bonding between O2 (Oxygen at position 2) of Lys204 (A) with Nitrogen	-6.50	Yes, The bonding between O2 (Oxygen at position 2) of Asp101 (A) with Oxygen, bonding between O2 (Oxygen at position 2) of Lys40 (A) with Nitrogen and bonding between O4 (Oxygen at position 4) of Asp128 (A) with Oxygen
7.	2,6- dimethoxyphenol	-4.30	Yes, The bonding between Nitrogen of Tyr152(A) with O3 (Oxygen at position 3), the bonding between O3(Oxygen at position 3) of Arg244 (A) with Oxygen and the bonding between O2 (Oxygen at position 2) of Gly270 (A) with Nitrogen	-5.00	Yes, The bonding between O2 (Oxygen at position 2) of His402 (A) with Nitrogen and bonding between O2 (Oxygen at position 2) of Gln442 (A) with Nitrogen
8.	2-Chlorosyringaldehyde	-4.80	Yes, The bonding between O2 (Oxygen at position 2) of Leu78 (A) with Nitrogen and bonding between O4 (Oxygen at	-5.20	Yes, The bonding between O1 (Oxygen at position 1) of Leu35(A) with Nitrogen and bonding between O2

			position 4) Thr192 (A) with Nitrogen		(Oxygen at position 2) of Pro32 (A) with Oxygen
9.	5- Chlorovanillin	-4.80	Yes, The bonding between O2 (Oxygen at position 2) of Pro75 (A) with Oxygen, and bonding between O2 (Oxygen at position 2) of Ser73 (A) with Oxygen and the bonding between O3 (Oxygen at position 3) of Thr192(A) with Nitrogen	-5.50	Yes, The bonding between bonding between O1 (Oxygen at position 1) of Ala403 (A) with Nitrogen and bonding between O2 (Oxygen at position 2) of His402(A) with Nitrogen
10.	Cis-Delta4-tetrahydrophthalic acid	-4.90	Yes, The bonding between O4 (Oxygen at position 4) of Pro137 (A) and the bonding between O2 (Oxygen at position 2) of Lys182 (A) with Nitrogen	-6.30	Yes, The bonding between O1 (Oxygen at position 1) of Lys40 (A) with Nitrogen and bonding between O4 (Oxygen at position 4) of Asn227 (A) with Nitrogen
11.	Polyamide	-5.00	Yes, The bonding between the Nitrogen of Ser268 (A) with O2 (Oxygen at position 2) and the bonding between N1 (Nitrogen at position 1) of Tyr152 (A) with Oxygen	-6.00	Yes, The bonding between O1 (Oxygen at position 1) of His402 (A) with Nitrogen
12.	Polyvinyl Chloride	-2.40	Only hydrophobic interactions	-2.50	Only hydrophobic interactions
13.	Polycarbonate	-6.30	Yes, The bonding between Oxygen of Asn243 (A) with O2 (Oxygen at position 2), the bonding between O2 (Oxygen at position 2) of Gly270 (A) with Nitrogen, and bonding between O1 (Oxygen at position 1) of Ala266 (A) with Oxygen	-6.9	Yes, The bonding between O1 (Oxygen at position 1) of Phe31 (A) with Oxygen, bonding between O2 (Oxygen at position 2) of Val145 (A) with oxygen, and bonding between O2 (Oxygen at position 2) of Glu142 (A) with oxygen
14.	Polyethylene terephthalate	-5.10	Yes, The bonding between O3 (Oxygen at position 3) of Leu78 (A) with Nitrogen and O4 (Oxygen at position 4)	-5.9	Yes, The bonding between O2 (Oxygen at position 2) of His402 (A) with Nitrogen and bonding between O1

			of Asn201 (A) with Nitrogen		(Oxygen at position 1) of Ala403 (A) with Nitrogen
15.	Polymethylene methacrylate	-3.90	Only hydrophobic interactions	-4.10	Yes, The bonding between O2 (Oxygen at position 2) of Ser113 (A) with Nitrogen and bonding between O1 (Oxygen at position 1) of His111 (A) with Nitrogen
16.	Polyurethane	-4.20	Yes, The bonding between Oxygen of Tyr152 (A) with Nitrogen	-3.7	Yes, The bonding between Oxygen of Asp206 (A) with N1 (Nitrogen at position 1) and bonding between Oxygen of Asn264 (A) with N2 (Nitrogen at position 2)
17.	ABTS	-7.40	Yes, The bonding between O4 (Oxygen at position 4) of Asn201 (A) with Nitrogen and bonding between O6(Oxygen at position 4) of Val74 (A) with Oxygen	-6.8	Yes, The bonding between O5 (Oxygen at position 5) of Ala1(A) with Nitrogen and bonding between O3 (Oxygen at position 3) of Val187 (A) with Oxygen
18.	Indomethacin	-6.60	Yes, The bonding between O3 (Oxygen at position 3) of Leu78 (A) with Nitrogen	-6.90	Yes, The bonding between O4 (Oxygen at position 4) of Ala103 (A)with Nitrogen and bonding between O2 (Oxygen at position 2) of Asn227 (A) with Nitrogen
19.	Bezafibrate	-6.30	Yes, The bonding between O3 (Oxygen at position 3) of Asp210 (A) with Nitrogen, bonding between bonding between O4 (Oxygen at position 4) of Arg203 (A) with Nitrogen and bonding between O1 (Oxygen at position 1) of His191 (A) with Nitrogen	-7.90	Only hydrophobic interactions
20.	Roxithromycin	-6.2	Only hydrophobic interactions	-6.8	Yes, The bonding between O11 (Oxygen at position11) of Lys40 (A) with Nitrogen and

					bonding between O13 (Oxygen at position13) of Asn227 (A) with Nitrogen
21.	Clarithromycin	-7.6	Yes, The bonding between O12 (Oxygen at position 12) of Thr192 (A) with Oxygen and bonding between O7 (Oxygen at position 7) of Leu78 (A) with Nitrogen	-7.3	Yes, The bonding between O2 (Oxygen at position2) of Leu35 (A) with Nitrogen
22.	Metoprolol	-4.9	Yes, The bonding between O2 (Oxygen at position 2) of Thr190 (A) with Nitrogen	-5.6	Yes, The bonding between O3 (Oxygen at position3) of Ala103 (A) with Nitrogen and bonding between O2 (Oxygen at position2) of Asp444 (A)
23.	Celiprolol	-5.8	Yes, The bonding between O4 (Oxygen at position 4) of Asn201 (A) with Nitrogen and bonding between N1 (Nitrogen at position 1) of Thr190 with Oxygen	-6.9	Yes, The bonding between N2 (Nitrogen at position 2) of Ala403 (A) with Oxygen, bonding between O4 (Oxygen at position 4) of His402 (A) with Nitrogen, bonding between O4 (Oxygen at position 4) of Gln442 (A) with Nitrogen and bonding between N1 (Nitrogen at position1) of Asp101 with Oxygen
24.	Iopromide	-5.2	Yes, The bonding between O1 (Oxygen at position 1) of Leu78 (A) with Nitrogen and bonding between O4 (Oxygen at position 4) of Thr192 (A) with Nitrogen	-5.5	Yes, The bonding between O5 (Oxygen at position 5) of Asn172 (A) with Oxygen

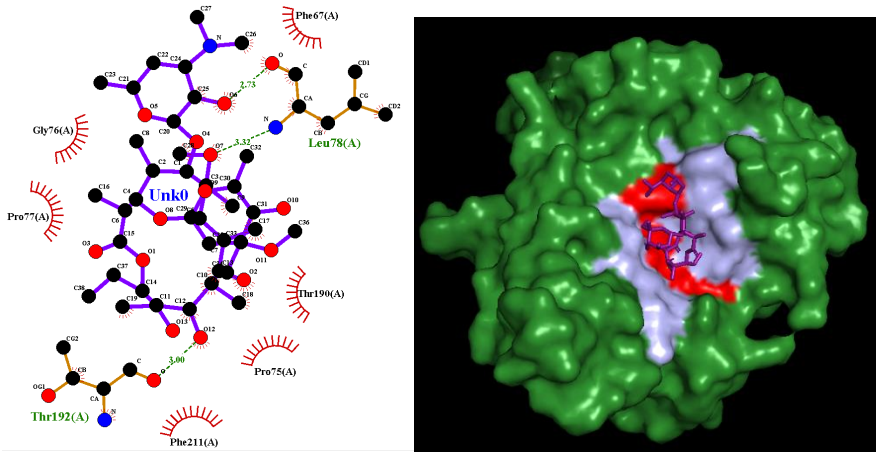


Figure 3: Interaction between Clarithromycin and PDB Id 3CG8 as visualized in Ligplot and Pymol (Binding Energy -7.60 kCal/mole)

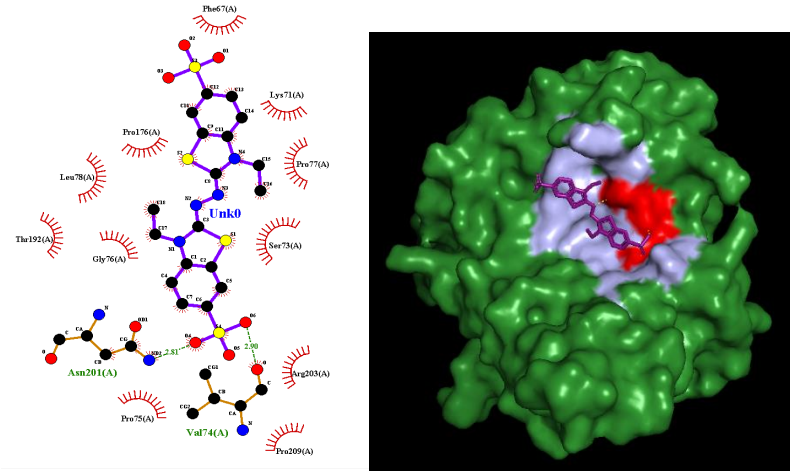


Figure 4: Interaction between ABTS and PDB Id 3CG8 as visualized in Ligplot and Pymol (Binding Energy -7.40 kCal/mole)

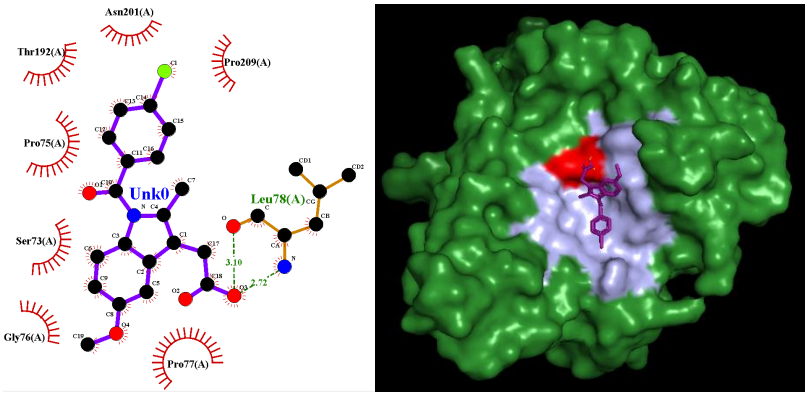


Figure 5: Interaction between Indomethacin and PDB Id 3CG8 as visualized in Ligplot and Pymol (Binding Energy -6.60 kCal/mole)

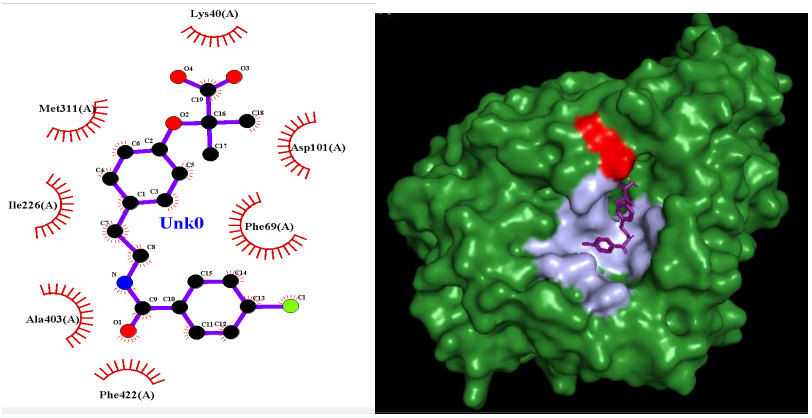


Figure 6: Interaction between Bezafibrate and PDB ID 1GYC as visualized in Ligplot and Pymol (Binding Energy -7.90 kCal/mole)

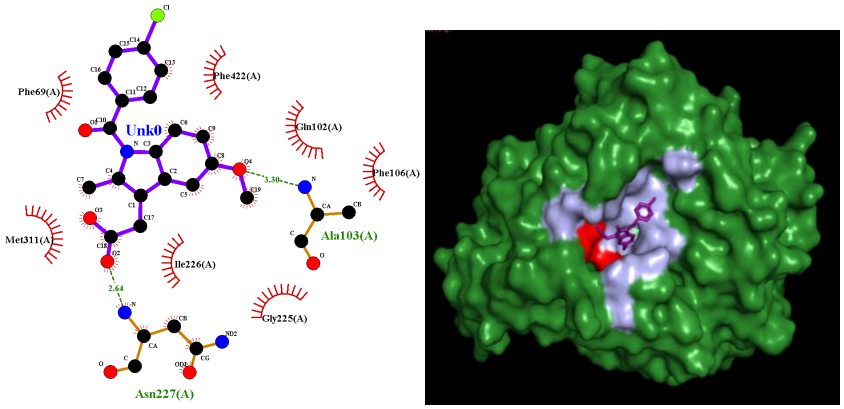


Figure 8: Interaction between Indomethacin and PDB ID 1GYC as visualized in Ligplot and Pymol (Binding Energy -6.9 kCal/mole)

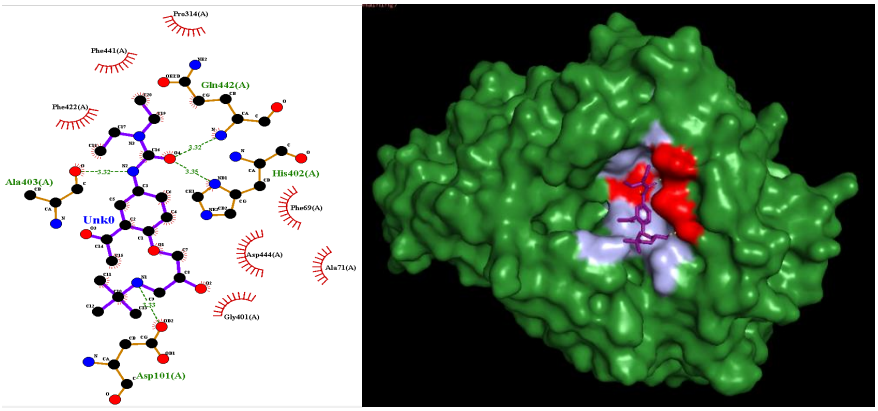


Figure 9: Interaction between Celiprolol and PDB ID 1GYC as visualized in Ligplot and Pymol (Binding Energy -6.9 kCal/mole)

On summarising the binding energies of the various ligands- protein complexes it was observe that with target protein PDB ID 3CG8, the compounds Clarithromycin , ABTS, and Indomethacin had the lowest binding energy of -7.6 kCal/mole (hydrogen bonding between Thr192 and Leu78) , -7.40 kCal/mole (Asn201 and Val74) , and -6.60 kCal/mole (Leu78) respectively The hydrogen bonding with leucine amino acid residue at leucine 78 of the target protein PDB ID 3CG8 is critical as in two of three complexes hydrogen bond with this residue is contributing to lower binding energy hence stable complex.

With the target protein with PDB ID 1GYC, compounds such as Bezafibrate, Indomethacin, and Celiprolol had the lowest binding energy of -7.90 kCal/mole (hydrophobic interactions only), -6.9 kCal/mole (Ala103 Asn227), and -6.9 kCal/mole (with Ala403 (A), His402 (A, Gln442 and Asp101) respectively. The four-hydrogen bonding in PDB ID 1GYC- Celiprolol complex is resulting in lower bonding energy in the complex. In PDB ID 1GYC Bezafibrate complex the hydrophobic interactions are only responsible the lower binding energy. It was also observed that the most of the ligands bound more conveniently with the fungal laccase as compared to the bacterial laccase which was reflected by the lower binding energy indicating the fungal laccase may exhibit more versatility and efficiency in bioremediating the pollutants.

In site-specific docking, hydrophobic areas inside a protein known as active sites were predicted and, in these pockets, there are the side chain atoms that may form the ligand-binding loci. The Scf bio web service was used to estimate the active site, and the X-, Y-, and Z-axis values for proteins with PDB IDs 3CG8 and 1GYC were obtained. The active site for bacterial laccase was estimated to be at X-axis 24.481 units, Y- axis 58.063 units, and Z- axis 0.712 units. The active site for fungal laccase was anticipated to be at X- axis 27.752-unit, Y- axis 21.985 units, and Z- axis 31.829 units.

Further, the site-specific docking was carried out by AutoDock 4,2.6 n determining the pollutant binding affinity with various categories of laccase enzymes. In order the confer flexibility to the structure the PDB structure extra charges were added prior to molecular docking. For molecular docking with protein PDB ID 3CG8, a grid map with X- axis 24.481, Y- axis 58.063, and Z- axis 0.721 was used as per the active site predicted by Scf bio web. The grid box X coordinate for molecular docking utilising protein PDB Id 1GYC was 27.752, the Y coordinate was 21.985, and the Z coordinate was 31.829. Grid-point spacings of up to 1.000 were used in all dockings. Furthermore, Pymol and LigPlot tools were used to examine protein-ligand conformation interactions, including hydrogen bonds and hydrophobic interactions.

2- Chlorophenol with bacterial laccase showed the binding energy -3.74 kCal/mole which was due to only one hydrogen bond between the Oxygen of Gly270 (A) with Nitrogen whereas with fungal laccase the binding energy was -4.31 kCal/mole due to one hydrogen bond between Oxygen of His111 (A) with Nitrogen. 2,4,6-Trichlorophenol showed binding energy of -1.2 kCal/mole with bacterial laccase due to the presence only hydrophobic interaction and absence of hydrogen bonding. Whereas in case of fungal laccase, it showed the binding energy of -4.51 kCal/mole due to one hydrogen bond between Oxygen of His111 (A) with Oxygen. Pentachlorophenol binding energy with bacterial laccase was -3.87 kCal/mole due to the existence of one hydrogen bond between the Oxygen of Ser292 (A) with oxygen of penatchlorophenol. In the case of fungal laccase, the binding energy was -4.97 kCal/mole due to the presence of only hydrophobic interactions and absence of hydrogen bonding. 2- Methoxypropanoyl chloride showed binding energy of -3.1 kCal/mole with bacterial laccase due to the presence of only hydrophobic interactions and absence of hydrogen bonding. Fungal laccase had a binding energy of -3.40 kCal/mole due to the presence of a hydrogen bond between O1(Oxygen at position 1) of SER 113 (A) with Nitrogen. 2- Methoxyphenol binding energy with bacterial laccase was -3.27 kCal/mole due to the hydrogen bond between O1 (Oxygen at position 1) of Tyr230 (A) with Nitrogen. The binding energy of fungal laccase was -3.8 kCal/mole due to the hydrogen bond between Ser113 and His111. Phthalic acid binding energy with bacterial laccase was -2.24 kCal/mole due to one hydrogen bond with bond between O4(Oxygen at position 4) of His293 (A) with Nitrogen and bond between O1 (Oxygen at position 1) of Tyr230 (A) with Nitrogen. The binding energy of the fungal laccase was -2.17 kCal/mole due to the presence of only hydrophobic interaction and absence of hydrogen bonding. 2,6- Dimethoxyphenol binding energy with bacterial laccase was –3.58 kCal/mole because of the existence of a hydrogen bond between O3 (Oxygen at position 3) of Met296 (A) with Oxygen. The binding energy of the fungal laccase was -3.6 kCal/mole due to the existence of a hydrogen bond between O1 (Oxygen at position 1) of Ser113 (A) with Nitrogen.

2- Chlorosyringaldehyde with bacterial laccase showed binding energy of -2.96 kCal/mole due to the existence of a hydrogen bond between O3(Oxygen at position 3) of Tyr230 (A) with Nitrogen and bond between O4 (Oxygen at position 4) of His293 (A) with Nitrogen. Whereas in case of fungal laccase, the binding energy was -3.7

kCal/mole due to the existence of a hydrogen bond between O2 (Oxygen at position 2) of Ala80 (A) with Oxygen. 5- Chlorovanillin binding energy with bacterial laccase was -4.03 kCal/mole due to the existence of a hydrogen bond between bond between O3 (Oxygen at position 3) of Met298 (A) with Nitrogen, bond between O1 (Oxygen at position 1) of Met296 (A) with Oxygen and bond between O2 (Oxygen at position 2) of Ile200 (A) with Nitrogen. Furthermore, the fungal laccase has a binding energy of -3.89 kCal/mole due to hydrogen bonding between bond between O3 (Oxygen at position 3) of His111(A) with Nitrogen. Cis-Delta4- tetrahydrophthalic acid showed binding energy of -2.7 kCal/mole with bacterial laccase due to hydrogen bond between O2 (Oxygen at position 2) of Tyr230(A) with Nitrogen and bond between O4(Oxygen at position 4) of His293 (A) with Nitrogen. The binding energy of fungal laccase was -2.3 kCal/mole due to hydrogen bonding bond between O1 (Oxygen at position 1) of Arg161 (A) with Nitrogen. Polyamide showed binding energy of –3.2 kCal/mole with bacterial laccase due to the presence of hydrogen bond between O1 (Oxygen at position 1) of His154 (A) with Nitrogen and bond between O2 (Oxygen at position 2) of Ser268 (A) with Nitrogen. Due to the existence of only one hydrogen bond between N1 (Nitrogen at position 1) of Gly227 (A) with Oxygen, fungal laccase had a binding energy of -2.86 kCal/mole. Polyvinyl Chloride with both bacterial laccase and fungal laccase showed binding energy of -2.2 kCal/mole and -2.64 kCal/mole respectively. Polycarbonate has binding energy of -4.84 kCal/mole in case of bacterial laccase due to the existence of hydrogen bonding between bond between O1 (Oxygen at position 1) of Ala266 (A) with Oxygen, bond between O2 (Oxygen at position 2) of Ile262 (A), bond between O1 (Oxygen at position 1) of His104 (A)with Nitrogen and bond between O1 (Oxygen at position 1) of His154 (A) with Nitrogen. The binding energy of fungal laccase was -5.7 kCal/mole, which was owing to the existence of a hydrogen bond between O1 (Oxygen at position 1) of Ala80 (A) with Oxygen, bond between O2 (Oxygen at position 2) of Phe344 (A) with Oxygen. Polyethylene terephthalate with bacterial laccase showed binding energy of -3.67 kCal/mole due to the existence bond between O1 (Oxygen at position 1) of Ser268 (A) with Nitrogen and bond between O4 (Oxygen at position 4) of Tyr152 (A) with Nitrogen. The binding energy of fungal laccase was -3.74 kCal/mole due to the existence of bond between O2 (Oxygen at position 2) of Arg157 (A) with Nitrogen. Polymethylene methacrylate showed binding energy of -2.99 kCal/mole with bacterial laccase due to the presence hydrogen bond bond between O2 (Oxygen at position 2) of Gly270 (A) with Nitrogen and bond between O1 (Oxygen at position 1) of Tyr152 (A) with Nitrogen. Whereas the binding energy of fungal laccase was -3.77 kCal/mole due to the presence of a hydrogen bond between O2 (Oxygen at position 2) of His111 (A) with Nitrogen and bond between O1 (Oxygen at position 1) of Ser113 (A) with Nitrogen. Polyurethane has a binding energy of -2.9 kCal/mole with bacterial laccase because of the existence of hydrogen bonding between N1 (Nitrogen at position 1) of Val179 (A) with Oxygen. Furthermore, fungal laccase showed a binding energy of -2.96 kCal/mole which is due to the presence of hydrogen bonding between N1 (Nitrogen at position 1) of His111 (A) with Nitrogen and Ser113 bond between Oxygen and Nitrogen. ABTS [2,2'-azino-bis (3- bindinethylbenzothiazoline-6-sulfonic acid)] showed binding energy of -4.6 kCal/mole which is because of the presence of hydrogen bonding between bonding between O6 (Oxygen at position 6) of Gly148 (A) with Oxygen, bonding between O1 (Oxygen at position 1) of Arg256 (A) with Nitrogen, bonding between O4 (Oxygen at position 4) of Arg181 (A) with Nitrogen and bonding between O3 (Oxygen at position 3) of Asp242 (A) with Oxygen. Whether in the case of fungal laccase, the binding energy was -3.3 kCal/mole due to the existence of bond between bonding between O6 (Oxygen at position 6) of Arg157 (A) with Oxygen and bonding between O1 (Oxygen at position 1) of Asn498 (A) with Nitrogen. Indomethacin with bacterial laccase showed binding energy of -4.24 kCal/mole due to the presence of one hydrogen bond between O4 (Oxygen at position 4) of ARG 181 (A) with Nitrogen. In fungal laccase, the binding energy was -4.5 kCal/mole due to the presence of hydrogen bonding between O3 (Oxygen at position 3) of ARG 161 (A) with Nitrogen. Bezafibrate has binding energy of -4.23 kCal/mole with bacterial laccase because of the presence of a hydrogen bond between Nitrogen of Ser268 (A) with Oxygen and bonding between O3 (Oxygen at position 3) of Arg181 (A) with Nitrogen. Whereas the binding energy of fungal laccase was -3.4 kCal/mole because of the presence of only hydrophobic interactions and the absence of hydrogen bonding. Roxithromycin with bacterial laccase showed binding energy of –0.37 kCal/mole because of the presence of hydrogen bonding between N1 (Nitrogen at position 1) of His104 (A) with Nitrogen, bonding between O7 (Oxygen at position 7) of Gly227 (A) with Nitrogen and bonding between O13 (Oxygen at position 13) of Pro265 (A) with Oxygen. The binding energy of fungal laccase was 0.8 kCal/mole due to the existence of only hydrophobic interaction. Clarithromycin showed binding energy of -7.6 kCal/mole with bacterial laccase due to the bond between O4 (Oxygen at position 4) of Lys261 (A) with Nitrogen. Whereas in the case of fungal laccase, the binding energy was -7.3 kCal/mole because of the presence of only one bond between O12 (Oxygen at position 12) of Thr345 (A) with Oxygen. Metoprolol with bacterial laccase showed binding energy of -3.1 kCal/mole due to the presence of hydrogen bond between O3 (Oxygen at position 3) of Tyr152 (A) with Nitrogen, bond between O2 (Oxygen at position 2) of Asp267 (A) with Oxygen and bond between Oxygen of Ile262 (A) with Nitrogen. Furthermore, the binding energy of fungal laccase was -4.0 kCal/mole due to the bond between O3 (Oxygen at position 3) of Ser113 (A) with Nitrogen, bond between O2 (Oxygen at position 2) of Glu460 (A) with Oxygen

and bond between O2 (Oxygen at position 2) of Arg161 (A) with Nitrogen. Celiprolol has binding energy of -3.08 kCal/mole with bacterial laccase because of the presence of the hydrogen bonding between Oxygen of Gly105 (A) with Nitrogen and bond between N3 (Nitrogen at position 3) of Tyr108 (A) with a hydroxyl group. For fungal laccase, the binding energy was -2.6 kCal/mole because of the presence of only hydrophobic interaction and the absence of hydrogen bonding. Iopromide with bacterial laccase showed binding energy of -1.2 kCal/mole due to the existence of a hydrogen bonding bond between O2 (Oxygen at position 2) of Ser268 (A) with Nitrogen. The binding energy of fungal laccase was -0.1 kCal/mole due to the existence of only one hydrogen bond between N3 (Nitrogen at position 3) of Gly334 (A) with Oxygen.

On summarising the results of site-specific docking with target protein PDB ID 3CG8, the compounds Polycarbonate, ABTS, and Indomethacin had the lowest binding energy of -4.84 kCal/mole (with Ala266, Ile262, His104, and His154) -4.6 kCal/mole (Gly148, Arg256, Arg181 and Asp242) and -4.24 kCal/mole (with ARG 181), respectively. The number of hydrogen bonds in the case of polycarbonate and ABTS might result in lower binding energy with stable interactions. In ABTS and Indomethacin, hydrogen bonds with Arg181 seemed to be critical in conferring stability to the complex. In the case of the target protein with PDB ID 1GYC, compounds such as Polycarbonate, Pentachlorophenol, and 2,4,6- Trichlorophenol had the lowest binding energy of -5.7 kCal/mole (with Ala80 (A), Phe344) -4.97 kCal/mole (hydrophobic interactions), and -4.51 kCal/mole (with His111), respectively. Like with bacterial laccase, polycarbonate interacts strongly with fungal laccase and as reflected by binding energy the interaction is better with fungal laccase. Hence the position of the hydrogen bond with fungal laccase which might be playing a critical role in the interactions.

Table 6: Binding Energy± Standard Error Value and Hydrogen Bond for Site-specific docking

Sl. No	Ligands	Binding Energy± Standard error value with bacterial laccase	Hydrogen Bond	Binding energy ± Standard error value with fungal laccase	Hydrogen Bond
1.	2-Chlorophenol	-3.74±0.045	Yes, The bond between Oxygen of Gly270 (A) with Nitrogen	-4.31 ± 0.02	Yes, The bond between Oxygen of His111 (A) with Nitrogen
2.	2,4,6-Trichlorophenol	-1.2±0.28	Only hydrophobic interaction	-4.51 ± 0	Yes, The bond between Oxygen of His111 (A) with Oxygen
3.	Penta Chlorophenol	-3.87±0.56	Yes, The bond between Oxygen of Ser292 (A) with Oxygen	-4.97 ± 0.05	Only Hydrophobic interaction
4.	2-Methoxypropanoyl chloride	-3.1±0.14	Only hydrophobic interaction	-3.40 ± 0.096	Yes, The bond between O1 (Oxygen at position 1) of Ser113 (A) with Nitrogen
5.	2-Methoxyphenol	-3.27±0.07	Yes, The bond between O1 (Oxygen at position1) of Tyr230 (A) with Nitrogen	-3.8 ± 0.02	Yes, The bond between O1 (Oxygen at position1) of Ser113 (A) with Nitrogen and bond between O2 (Oxygen at position 2) of His111 (A) with Oxygen

6.	Phthalic Acid	-2.24±0.15	Yes, The bond between O4 (Oxygen at position 4) of His293 (A) with Nitrogen and bond between O1 (Oxygen at position1) of Tyr230 (A) with Nitrogen	-2.17 ± 0.03	Only hydrophobic interaction
7.	2,6-dimethoxyphenol	-3.58±0.11	Yes, The bond between O3 (Oxygen at position 3) of Met296 (A) with Oxygen	-3.6 ± 0.09	Yes, The bond between O1 (Oxygen at position 1) of Ser113 (A) with Nitrogen
8.	2-Chlorosyringaldehyde	-2.96±0.38	Yes, The bond between O3 (Oxygen at position 3) of Tyr230 (A) with Nitrogen and bond between O4 (Oxygen at position 4) of His293 (A) with Nitrogen	-3.7 ± 0.08	Yes, The bond between O2 (Oxygen at position 2) of Ala80 (A) with Oxygen
9.	5-Chlorovanillin	-4.03±0.17	Yes The bond between O3 (Oxygen at position 3) of Met298 (A) with Nitrogen, bond between O1 (Oxygen at position 1) of Met296 (A) with Oxygen and bond between O2 (Oxygen at position 2) of Ile200 (A) with Nitrogen	-3.89 ± 0.19	Yes, The bond between O3 (Oxygen at position 3) of His111 (A) with Nitrogen
10.	Cis-Delta4-tetrahydrophthalic acid	-2.7±0.08	Yes, The bond between O2 (Oxygen at position 2) of Tyr230 (A) with Nitrogen and bond between O4 (Oxygen at position 4) of His293 (A) with Nitrogen	-2.3 ± 0.02	Yes, The bond between O1 (Oxygen at position 1) of Arg161 (A) with Nitrogen
11.	Polyamide	-3.2±0.44	Yes, The bond between O1 (Oxygen at position 1) of His154 (A) with Nitrogen and bond between O2 (Oxygen at position 2) of Ser268 (A) with Nitrogen	-2.86 ± 0.04	Yes, The bond between N1 (Nitrogen at position 1) of Gly227 (A) with Oxygen
12.	Polyvinyl Chloride	-2.2 ± 0.13	Only hydrophobic interaction	-2.64 ± 0	Only hydrophobic interaction
13.	Polycarbonate	-4.84±0.13	Yes, The bond between O1 (Oxygen at position 1) of Ala266 (A) with Oxygen, bond between O2 (Oxygen at position 2) of Ile262 (A), bond between O1(Oxygen at position 1) of His104 (A) with Nitrogen and bond	-5.7 ± 0.7	Yes, The bond between O1 (Oxygen at position 1) of Ala80 (A) with Oxygen, bond between O2 (Oxygen at position 2) of Phe344 (A) with Oxygen,

			between O1 (Oxygen at position 1) of His154 (A) with Nitrogen		
14.	Polyethylene terephthalate	-3.67±0.37	Yes, The bond between O1 (Oxygen at position 1) of Ser268 (A) with Nitrogen and the bond between O4 (Oxygen at position 4) of Tyr152 (A) with Nitrogen	-3.74 ± 0.05	Yes, The bond between O2 (Oxygen at position 2) of Arg157 (A) with Nitrogen
15.	Polymethylene methacrylate	-2.99±0.018	Yes, The bond between O2(Oxygen at position 2) of Gly270 (A) with Nitrogen and bond between O1 (Oxygen at position 1) of Tyr152 (A) with Nitrogen	-3.77 ± 0.03	Yes, The bond between O2(Oxygen at position 2) of His 111 (A) with Nitrogen and the bond between O1 (Oxygen at position 1) of Ser113 (A)with Nitrogen
16.	Polyurethane	-2.9±0.11	Yes, The bond between N1 (Nitrogen at position 1) of Val179 (A) with Oxygen	-2.96 ± 0.04	Yes, The bond between N1 (Nitrogen at position 1) of His 111 (A) with Nitrogen and Ser113 bond between Oxygen and Nitrogen
17.	ABTS	-4.6±0.18	Yes, The bonding between O6 (Oxygen at position 6) of Gly148 (A) with Oxygen, bonding between O1 (Oxygen at position 1) of Arg256 (A) with Nitrogen, bonding between O4 (Oxygen at position 4) of Arg181 (A) with Nitrogen and bonding between O3 (Oxygen at position 3) of Asp242 (A) with Oxygen	-3.3±0.09	Yes, The bond between bonding between O6 (Oxygen at position 6) of Arg157 (A) with Oxygen and bonding between O1 (Oxygen at position 1) of Asn498 (A) with Nitrogen
18.	Indomethacin	-4.24±0.1	Yes, The bond between O4 (Oxygen at position 4) of Arg181 (A) with Nitrogen	-4.5±0.26	Yes, The bond between O3 (Oxygen at position 3) of Arg161 (A) with Nitrogen
19.	Bezafibrate	-4.23±0.116	Yes, The bond between Nitrogen of Ser268 (A) with Oxygen and bonding between O3 (Oxygen at position 3) of Arg181 (A) with Nitrogen	-3.4±0.11	Only hydrophobic interaction

20.	Roxithromycin	-0.37±0.06	Yes The bond between N1 (Nitrogen at position 1) of His104 (A) with Nitrogen, bonding between O7 (Oxygen at position 7) of Gly227 (A) with Nitrogen and bonding between O13 (Oxygen at position 13) of Pro265 (A) with Oxygen	0.8±0.8	Only hydrophobic interactions
21.	Clarithromycin	-2.8±0.11	Yes, The bond between O4 (Oxygen at position 4) of Lys261 (A) with Nitrogen	−2.1 ± 0.09	Yes, The bond between O12 (Oxygen at position 12) of Thr345 (A) with Oxygen
22.	Metoprolol	-3.1±0.28	Yes, The bond between O3 (Oxygen at position 3) of Tyr152 (A) with Nitrogen, bond between O2 (Oxygen at position 2) of Asp267 (A) with Oxygen and bond between Oxygen of Ile262 (A) with Nitrogen	-4.0 ±1.04	Yes, The bond between O3 (Oxygen at position 3) of Ser113 (A) with Nitrogen, bond between O2 (Oxygen at position 2) of Glu460 (A) with Oxygen and bond between O2 (Oxygen at position 2) of Arg161 (A) with Nitrogen
23.	Celiprolol	-3.08±0.5	Yes, The bond between Oxygen of Gly105 (A) with Nitrogen and bond between N3 (Nitrogen at position 3) of Tyr108 (A) with OH	-2.6±0.1	Only hydrophobic interactions
24.	Iopromide	-1.2±0.03	Yes, The bond between O2 (Oxygen at position 2) of Ser268 (A) with Nitrogen	-0.1±0.13	Yes, The bond between N3 (Nitrogen at position 3) of Gly334 (A) with Oxygen

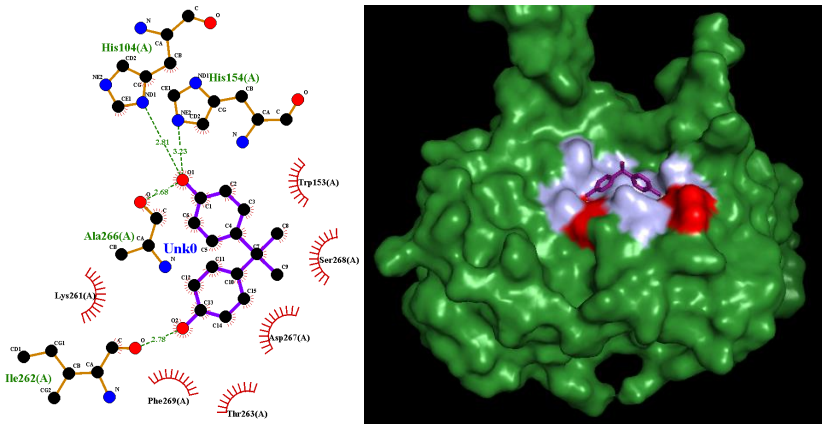


Figure 10: Interaction between Polycarbonate and PDB ID 3CG8 as visualized in Ligplot and Pymol (Binding Energy –4.84 kCal/mole)

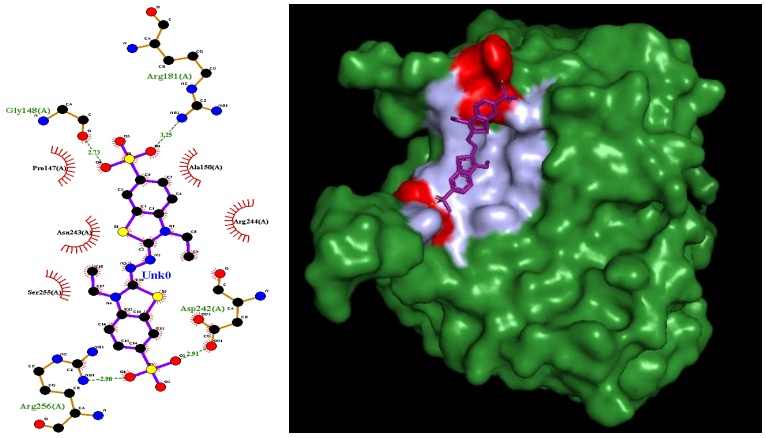


Figure 11: Interaction between ABTS and PDB ID 3CG8 as visualized in Ligplot and Pymol (Binding Energy –4.6 kCal/mole)

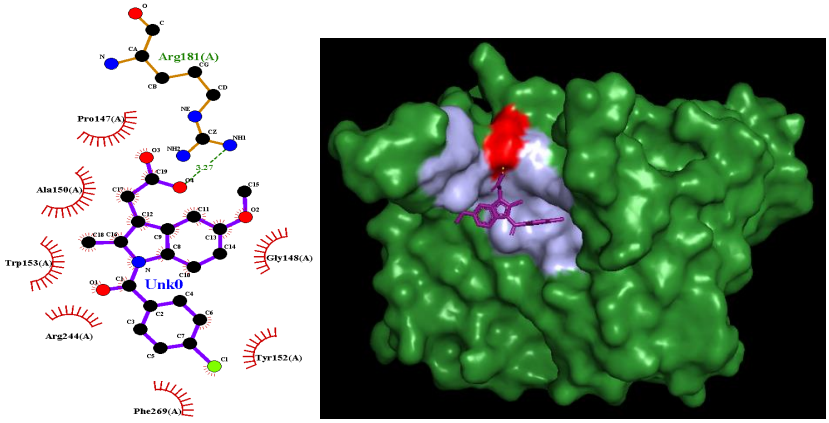


Figure 12: Interaction between Indomethacin and PDB ID 3CG8 as visualized in Ligplot and Pymol (Binding Energy –4.24 kCal/mole)

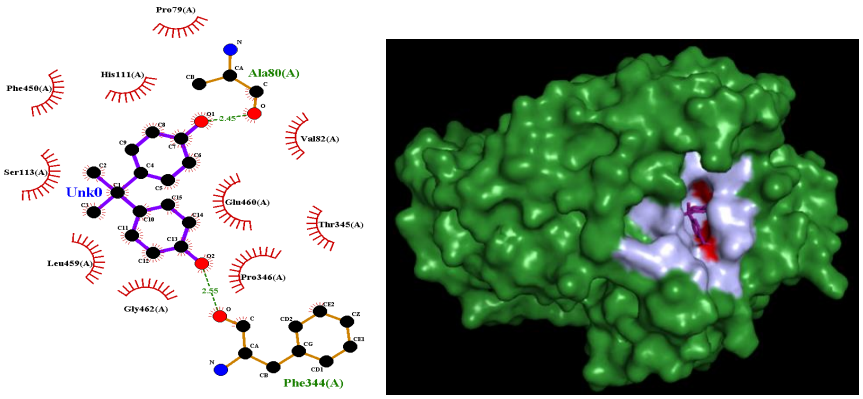


Figure 13: Interaction between Polycarbonate and PDB ID 1GYC as visualized in Ligplot and Pymol (Binding Energy –5.7 kCal/mole)

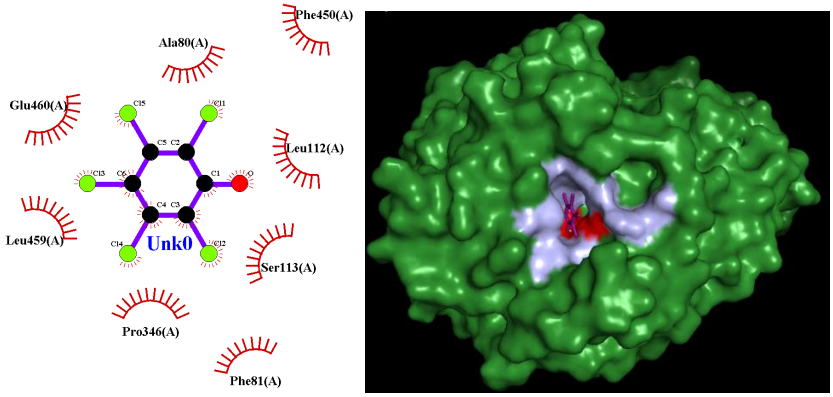


Figure 14: Interaction between Pentachlorophenol and PDB ID 1GYC as visualized in Ligplot and Pymol (Binding Energy –4.97 kCal/mole)

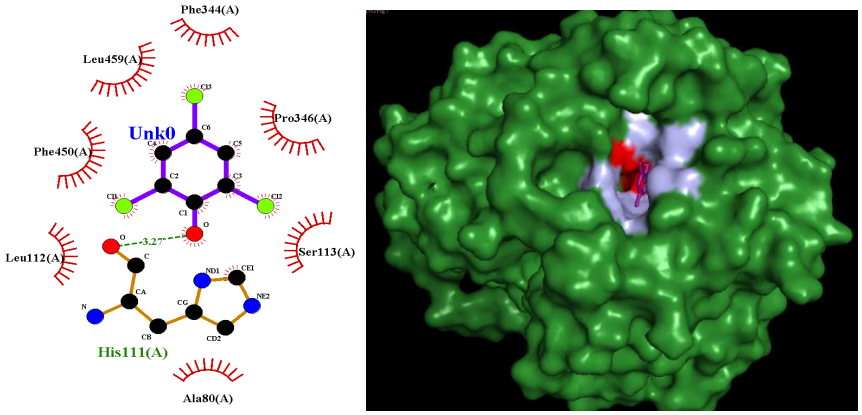


Figure 15: Interaction between 2,4,6- Trichlorophenol and PDB ID 1GYC as visualized in Ligplot and Pymol (Binding Energy –4.51 kCal/mole)

4. Conclusion

Bioremediation is the use of living organisms to remove pollutants, poisons, and contaminants from soil, water, and other environments. Pollutants of many types can be found in the environment, all of which have negative environmental implications and fall into several categories, such as pharmaceutical pollutants, paper mill effluent, and microplastic. Microbes have the ability to convert dangerous molecules into innocuous ones because they include enzymes with specific features, and laccase (EC 1.10.3.2) is one of these enzymes. The present study demonstrated that both bacterial and fungal laccase do exhibit affinity for the various pollutants suggesting these toxic ligands may be get bound to the laccase enzyme like substrate and get degraded into less harmful by products. However, the binding energy of the blind and site-specific docking exhibit the pollutants binds better in blind docking than in site specific one which could be explained by the fact the pollutants may have one or more binding pockets rather in restricted loci like in site specific docking. Further studies might be conducted in terms of in silico prediction of complex degradation products and confirmation of the findings in experimental studies by exposing the pollutants to laccase synthesising microorganisms.

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