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Binding interaction of laccases and Peroxidases from *Bacillus Subtilis* after Industrial dyes exposure: Molecular docking and Molecular dynamics simulation studies

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Wastewater treatment in textile and dye industry mainly involves treatment of highly colored water containing variety of dyes in different concentrations. The wastewater needs to be treated prior to discharge by effectively removing dye color in order to protect environment and as per the statutory guidelines. Laccases and peroxidase are key enzymes that help microbes to degrade dyes as well as their intermediate metabolites. Various dyes have been reported to be degraded by bacteria, but it is still unclear how these enzymes function during dye degradation, for complete removal of these toxic dyes from the system, it is essential to understand the molecular function of enzymes. The interaction of laccase and peroxidase with different toxic dyes was investigated using molecular docking. Based on the highest binding energy five dyes were screened showing high binding interaction with laccase and peroxidase. Molecular docking results indicate that out of the five dyes two were found to be more stable as a target for degradation through *Bacillus subtilis* laccase and peroxidase, which is yellow 2g and reactive blue respectively. As a result, subsequent research focused solely on the results of two substrates: yellow 2g and reactive blue. Analysis of Molecular Dynamics simulation revealed that yellow 2g and reactive blue form hydrogen and hydrophobic bond with the active site of laccase and peroxidase to keep it stable in aqueous solution. The conformation of laccase and peroxidase is greatly altered by the inclusion of all two substrates in the active site. The Molecular Dynamics simulation findings show that laccase and peroxidase complexes remain stable throughout the catalytic reaction. Therefore, this research provides a molecular understanding of laccase and peroxidase expression and its role in the bioremediation of the yellow 2g and reactive blue.

Keyword: Laccase, Molecular docking, Molecular dynamics, Peroxidase, Reactive blue, Yellow 2g

The significance of water in our lives is self-evident. It is an essential part of our daily routine, particularly in quenching our thirst. The purity of water is equally important; as contaminated water can lead to waterborne illnesses that can be deadly[1,3]. The wastewater generated by the textile industry contains high levels of dyes, as well as elevated levels of COD, BOD, pH, color, and heavy metals[2,3]. Many industries use hazardous materials, like synthetic dyes, in applications such as textile production, rubber, paper, food, leather, cosmetics, and drug production. These dyes can have a significant environmental impact due to their strong toxicity and tendency to bio-accumulate and persist in the environment. As such, prior treatment is necessary before their discharge into the environment. Currently, various processes have been investigated for the removal of dyes from polluted waters[4]. The removal of toxic chemicals is mainly mediated by enzymes such as laccases, peroxidases, dehydrogenases, and oxygenases [5,37]. Peroxidases are oxido reductases that can catalyze the oxidation of a wide variety of organic and inorganic compounds, as well as the reduction of hydrogen peroxide (H_2O_2). These enzymes are also able to use lipid peroxides as both electron acceptors and donors[6,7]. Laccases, on the other hand, are multicopper oxidases (EC 1.10.3.2) present in bacteria, fungi, and plants. They can catalyze phenolic compounds and help facilitate their removal from the environment. Aromatic phenolic compounds are first converted into phenoxy radicals by laccase, which are then involved in radical rearrangement or coupling-based polymerization[8,37]. The initial acceptor for laccase-catalyzed reactions is copper T1, which is present in the cavity of the enzyme near the surface. Aromatic phenolic compounds are catalyzed by

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Abbreviations: MD, Molecular dynamics; MolDock, Molecular docking; Rg, Radius of gyrations; RMSD, Root mean square deviation; RMSF, Root mean square fluctuation; SASA, Surface accessible to solvents

laccase into phenoxy radicals, which are then further involved in radical rearrangement or coupling-based polymerization. Several dyes, including Red 40, Yellow 2g, Methyl yellow, Nile blue, and Reactive Blue (with the molecular formulas $C_{18}H_{14}N_2Na_2O_8S_2$, $C_{16}H_{10}C_{l2}N_4Na_2O_7S_2$, $C_{14}H_{15}N_3$, $C_{40}H_{40}N_6O_6S$, and $C_{29}H_{20}C_lN_7O_{11}S_3$, respectively) are widely used in beverages, bakery products, meat, powder desserts, candies, cereals, medicines, cosmetics, and tattoo inks. Due to their toxicity and environmental impact, their use has been restricted or eliminated in countries of the European Union, such as Denmark, Belgium, France, Germany, Switzerland, Sweden, Austria, and Norway. Laccases and peroxidases, two important oxidoreductases, have the potential to decolorize chromophore compounds, such as azo and triphenylmethane dyes. Therefore, they may be employed in industrial dyes decoloration processes[9,10]. To treat hazardous effluents, biological approaches such as microbial bioremediation are often more effective. Degradation and decoloration of toxic dyes through the help of bacteria is mostly facilitated by bacterial enzymes belonging to the class of oxidoreductases[11].

The application of bioinformatics and molecular docking (MolDock) techniques, can offer valuable insights into dye degradation vulnerability[12]. In addition, Molecular Dynamics (MD) Simulation offers a more comprehensive understanding of interactions between enzymes and dyes, as well as the stability of enzyme-dye complexes. Moreover, it allows for valuable insights into the mechanism of dye degradation.

Studies have also been conducted to explore the potential use of MolDock and MD simulation of protein-ligand complex stability in bioremediation and treatment of various diseases[13,11]. The action of laccase and peroxidases on dye degradation is not well understood. In this study, molecular modeling of laccase and peroxidases was used to investigate the binding of five industrial dyes (methyl yellow, nile blue, yellow 2g, reactive blue, and red40) belonging to the molecular classes of azo, anthraquinones, and triphenylmethane. The study included docking studies, MD simulations and hydrogen-bond interaction analysis. The MD simulation studies of laccase–yellow 2g dye and peroxidase–reactive blue binding mechanisms led to several useful conclusions. Based on these results, it is possible to improve our understanding of yellow 2g dye and reactive blue biodegradation as a valuable biological treatment method.

Materials and Methods

Dyes

The study used five different industrial dyes commonly employed in the textile and food industries: Yellow 2g, Red 40, Methyl yellow, Reactive Blue, and Nile Blue. The spatial data files (SDFs) of these dyes were obtained from the NCBI PubChem database and are shown in (Fig. 1).

Homology modelling and validation

The three-dimensional structure of *B. subtilis* peroxidases is available in the Protein Data Bank (PDB) under the ID 6KMN (Resolution -2.44 Å), but the structure of *B. subtilis* laccase was unavailable; hence, homology modelling was employed to generate the protein structure of the enzyme. The NCBI Protein database was used to find the *B. subtilis* laccase protein sequence (accession number ARO72333) in order to predict its secondary structure and physicochemical properties[14]. BLASTp was then used to identify the template structure, and based on its findings, 1GSK (Resolution- 1.70 Å, Chain-A endospore coat protein from *B. subtilis*) was selected as the laccase template. The structures of the protein were retrieved from the Protein Data Bank (PDB) and applied for homology modelling. Modeller 10.2 (https://salilab.org/modeller/)[15] was used to model the

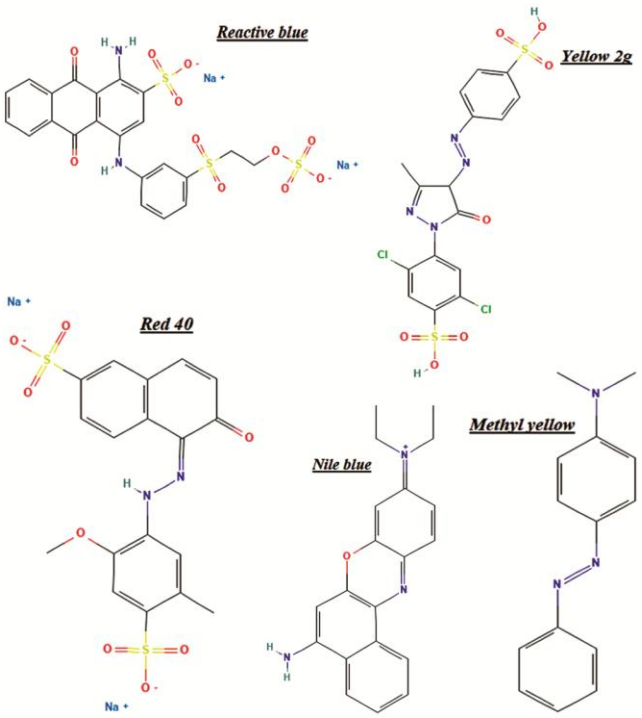

Fig. 1 — Ligands structure used in this study (Source: ChemSpider web site)

homology of selected target sequences, On the basis of dope score predicted protein structure are selected further validation of predicted protein models was done by using UCLA-DOE LAB-SAVES v6.0 server (https://saves.mbi.ucla.edu/) to run ERRAT (program for verifying protein structures determined by crystallography)[16], VERIFY 3D (Determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar) and comparing the results to good structures.), PROCHECK (checks the stereo-chemical quality of a protein structure)[17], ProSA server (web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids)[18].

Binding Sites Identification:

In this study, CASTp (CASTp 3.0: Computed Atlas of Surface Topography of proteins (uic.edu)[19] and P2Rank is a new open-source software package for ligand binding site prediction from protein structure. It is available as a user-friendly stand-alone command line program and a Java library. P2Rank has a lightweight installation and does not depend on other bioinformatics tools or large structural or sequence databases[20]. Both servers used to locate, delineate, and calculate topological geometric and properties of protein structures where the common active sites were selected.

Molecular Docking

Docking was done with PyRx[21] open-source software and confirmed using Autodock Vina[22] to determine the ligand's potential orientations and conformations at the binding location. The docking study's grid centre was set to X = 101.342, Y= 34.0437, and Z= -18.0587 for Laccase and X =-6.336, Y=18.492, and Z=45.262 for Peroxidase with grid box dimensions of X= 40, Y= 40, Z= 40 for Laccase and X=40, Y=40, Z=40 for Peroxidase coordinates, respectively.

Dye-degrading amino acids Visualization

BIOVIA Discovery Studio Visualizer (free, feature-rich molecular modeling application for viewing, sharing, and analyzing protein and small molecule data)[23] was used to analyze the two-dimensional interactions of enzyme-substrate structure, as well as hydrogen bonds, bond lengths and PyMol (https://pymol.org/2/) a molecular visualization programme, was used to examine the three-dimensional enzyme-ligand structure[24].

Molecular dynamics simulation

The most efficient protein-ligand combination with the lowest binding free energy (obtained after docking) was then applied to 100 ns MD simulation using Gromacs 2021 (https://ftp.gromacs.org/gromacs/gromacs-2021)[25]. At the initial point, it is started by separating the protein and ligand from their respective complex form, to generate the topology file for an individual; protein and ligand. Here we constructed the protein topology with the help of the parameters implemented in CHARMM-36–feb2021[26]. The protein topology is constructed with "charmm36-feb2021.ff" and the water model “TIP3-Point, TIP3-point, recommended, by standard uses CHARMM TIP3”. While the topologies for ligand were constructed using the CHARMM-based online server - CGenFF server[27]. The protein-ligand complex is regenerated by manually fitting the residues from complex_processed.gro and unk.gro file in a new file complex.gro. The next step is followed by the solvation of a complex in dodecahedron form and is carried out by using the water model. The system is neutralized with appropriate positive (Na) and negative (Cl) ions. The generated complex with the solvent system was neutralized by the addition of 14 Na (+ve) ions residues.

To minimize the energy of the generated complex-solvent system, the steepest descent minimization was used; the energy minimization was processed for 50000 numbers of maximum steps and potential energy was recorded with -7.00869e+05 kj/mol. The ligand position restrained index file is generated and generated.itp file was incorporated into the main topology file. By using the leap-frog integrator algorithm, the NVT (ensemble maintaining the constant number of particles, volume, and temperature) and NPT (ensemble maintaining the constant number of particles, pressure, and temperature) are subjected to 50000 steps, equal to 100 picoseconds, and temperature 300-K and the analysis exhibited total energy of -5.54128e+05 kj/mol and -5.61911e+05 k/mol.

The generated equilibrated system was finally subjected to MD simulation for 50000000; 100000 ps (100 ns). Particle Mesh Ewald (PME)[28] was used to retrain the system with the LINCS algorithm[29] for long-range electrostatics, like Coulomb and Lennard Jones. For covalent bonds, the cut-off value was set to 12 Å. In the post-MD simulation analysis, deviations in the protein and ligand atoms were measured as RMSD. The graphs were generated using the XM grace Linux-based tool.

Results and Discussion

Homology modeling and validation

Based on the dope score, Modeler10.2 generates five distinct models for each enzyme. To determine the structure for further research, the lowest Dope score was used. For a protein or enzyme to perform its function, its structure is critical.3D structure of the protein (Laccase) Visualization with PyMOL (Fig. 2A). The modelled protein was validated by UCLA-DOE LAB — SAVES v6.0 server having molecular weight of 58.51 kDa, the residuals from the developed model were found to be in a favorable area, and the overall structural quality was good. (Table 1), Ramachandran Plot, (Fig. 2B).

Binding Sites Identification

P2Rank was used to predict the possible binding sites of Laccase's and Peroxidase's 3D structures. P2Rank is able to automatically produce predictions for any PDB file (single or multi chained) by running a single command (prank predict -f protein.pdb). No preprocessing steps on part of the user are needed. For each input protein, P2Rank produces an output CSV file which contains an ordered list of predicted pockets and their scores. Pockets are characterized by coordinates of their centers, by a list of solvent exposed protein atoms and by a list of amino acid residues that constitute the binding site. PDB file with labeled SAS points (which form a primary internal representation of predicted pockets) can be also produced[20] as shown in the (Table 2 and Fig. 3A).

Molecular Docking and Visualization

PyRx Software followed by Autodock Vina tools was used to investigate dye interactions with laccase and Peroxidase. Dye structures were obtained from the NCBI PubChem database in the form of spatial data files (SDFs). The ligands energy minimization using Open Babel program tools under uff force field, Conjugate gradient optimization algorithms method with 200 steps. Before docking, the Open Babel programme tools were used to convert the data to PDBQT format. For protein minimization, all water molecules were eliminated and hydrogen atoms were affixed in BIOVIA Discovery Studio Visualizer2021.

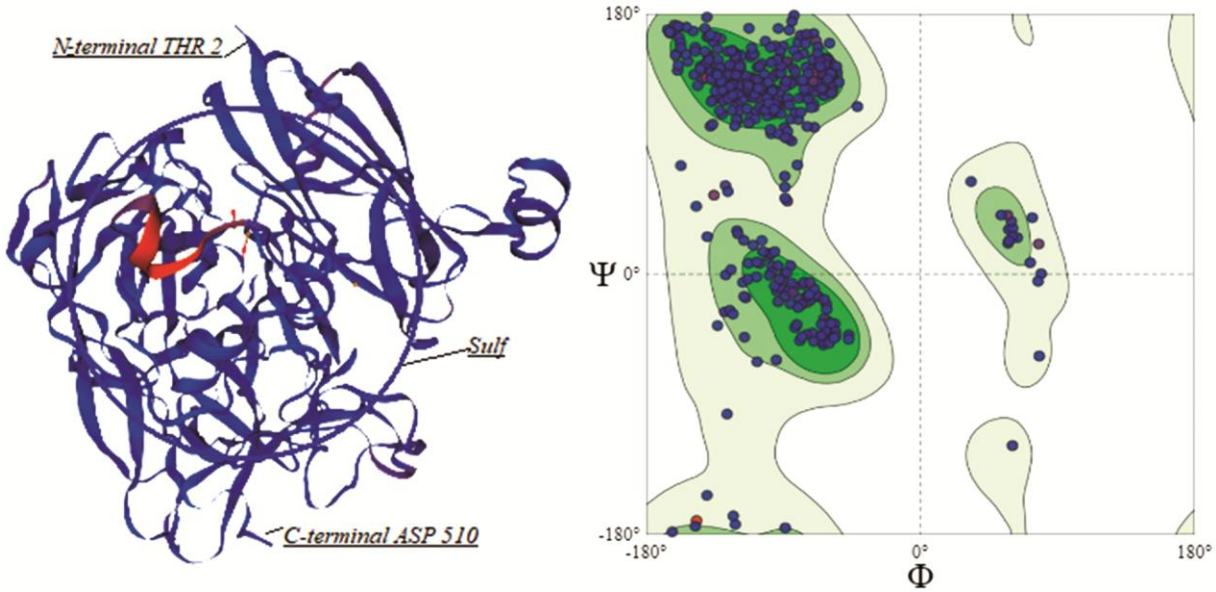

Fig. 2 — Three-dimensional structure of the protein (Laccase) showing N terminal, C terminal and (A) one conserved domain (Sufl); and (B) Ramachandran plot

Table 1 — Parameters for evaluating predicted three-dimensional structures of laccase enzyme using UCLA. -DOE LAB— SAVES v6.0 server to run ERRAT and verify 3D, PROCHECK and by ProSA server

Enzyme	PROCHECK Analysis	ERRAT analysis	VERIFY 3D	ProSA analysis
	RMFR, RAR, RDR	Overall quality factor		
Laccase	89.9%, 9%, 0.2%	71.82	Pass (92.79%)	-8.22

RMFR: Number of residues in most favored region, RAR: Number of residues in allowed region, RDR: Number of residues in disallowed region

Table 2 — Interaction sites for PDB IDs with the surface accessible solvents (sas_point), surface atoms (surf_atoms), residue identities (residue_ids) and surface atom identities (surf_atom_ids)

	1GSK	6KMN
sas_points	76	184
surf_atoms	37	85
residue_ids	A_226 A_227 A_260 A_262 A_321 A_322 A_323 A_384 A_386 A_415 A_416 A_417 A_418 A_419 A_494 A_497	C_120 C_121 C_122 C_181 C_183 C_185 C_186 C_187 C_188 C_189 C_190 C_223 C_225 C_227 C_244 C_246 C_263 C_273 C_274 C_277 C_278 C_282 C_283 C_284 C_286 C_306 C_308 C_319 C_322 C_323 C_326 C_327 C_330 C_332 C_333 C_336 C_338
surf_atom_ids	1775 1776 1782 2054 2057 2069 2073 2074 2075 2518 2520 2524 2525 2526 3018 3019 3033 3034 3035 3270 3271 3272 3273 3277 3284 3285 3286 3288 3291 3292 3293 3294 3303 3304 3907 3933 3934	6543 6549 6550 6551 6555 6561 7003 7004 7005 7021 7033 7036 7043 7050 7054 7055 7056 7057 7061 7064 7065 7068 7069 7071 7329 7348 7349 7366 7510 7512 7514 7516 7525 7526 7527 7528 7529 7659 7736 7737 7738 7740 7742 7744 7745 7762 7764 7765 7766 7769 7770 7772 7773 7794 7807 7809 7812 7814 7816 7831 7832 7833 7988 8003 8004 8005 8006 8007 8099 8121 8123 8124 8131 8151 8153 8156 8160 8182 8195 8203 8230 8231 8241 8245 8246

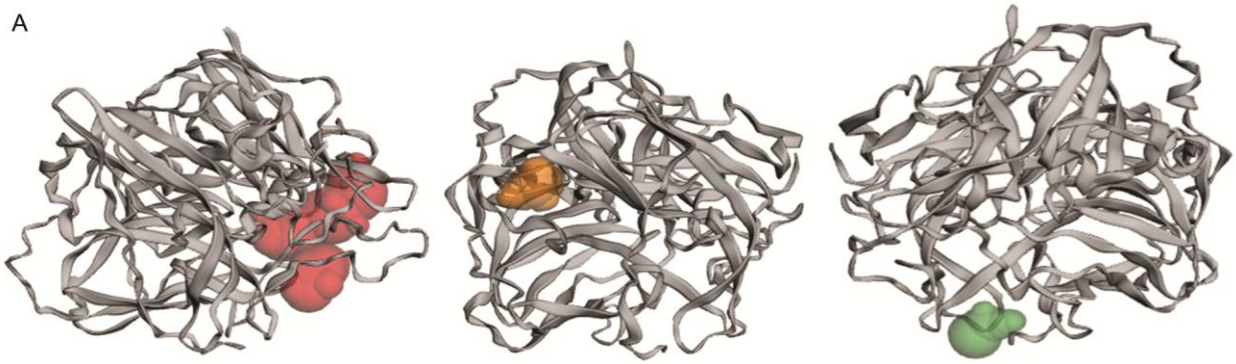

Fig. 3A — Active binding pockets into modelled protein (laccase -*B. subtilis*)

Based on data gathered by surrounding pocket active site residue, the docking grid box was chosen by the web server, P2 Rank and Computed atlas of surface topography of proteins (CASTp) calculation produced pockets of the modelled protein. The lowest Vina score, binding affinity (kcal/mol) and maximum number of hydrogen bond interacting complex were elect for farther evaluation of the interactions of dyes and enzyme. Which is shown in Tables 3 and 4, where laccase-yellow 2g and Peroxidase-reactive blue complex show lower binding affinity and maximum number of hydrogen bond formation than other tow binding pocket. So laccase-yellow 2g and peroxidase-reactive blue complex was selected for further analysis of the interaction. The two-dimensional enzyme-substrate complex interactions, along with hydrogen bonds and bond lengths, had been studied applying the Biovia discovery studio (Table 5). Further it was observed that SER 293, THR 262, GLY 321 are important hydrogen bonds stabilizing Laccase-yellow 2g complex and Peroxidase-Reactive Blue stabilized by SER 327, ARG 223, ASN 333, ARG 246 as shown in (Fig. 3B).

Molecular dynamics simulation

The MD simulation provides greater observation into the binding and interactions of ligands and understanding pharmacophoric based interaction within the binding site of the protein including the ligand dynamicity behavior. The conformational space acquired by the ligand in the binding site region over the period specifies the stability of the protein-ligand complex[30]. The generated complex.gro file with all topology and constraints, after the neutralization and solvation, the laccase-yellow 2g complex consists of 513-protein residues and similarly peroxidase-reactive blue consists 592-protein residues, 1- other residues (ligand), 16178-solvent (water residues). After the MD simulation of a subjected protein-ligand complex, the measurement of protein and ligand RMSD provides a good estimate of the conformational stability of the system.

RMSD

RMSD was calculated for the backbone of the laccase, laccase- yellow 2g complex, Peroxidase, and peroxidase-reactive blue complex (Fig. 4). Both the

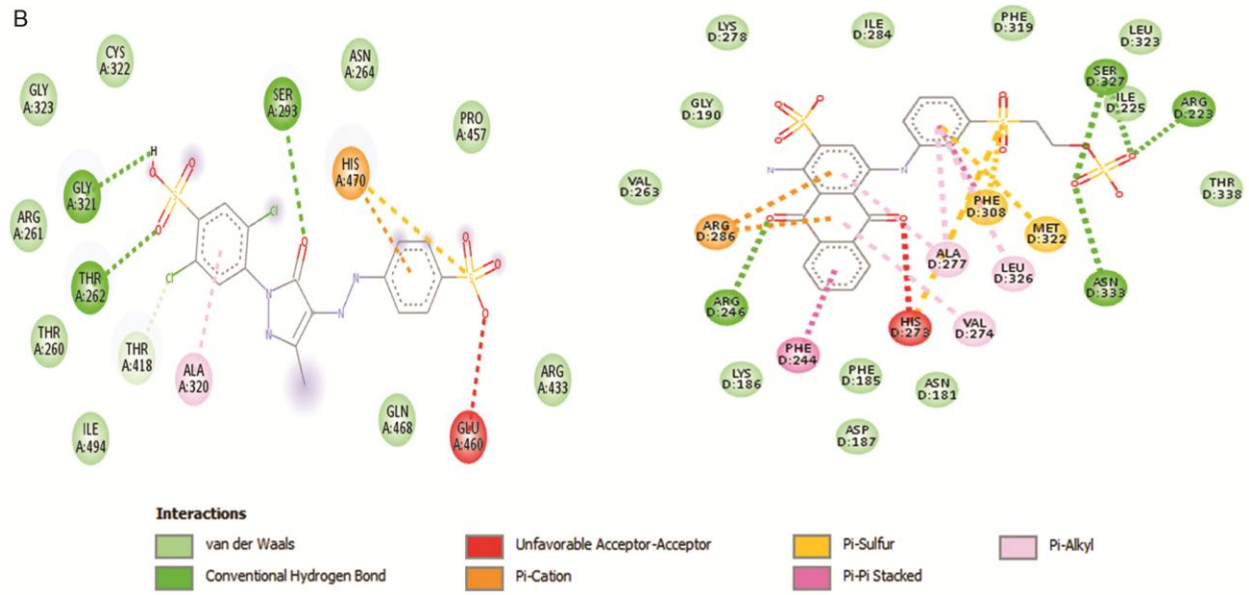

Fig. 3B — 2D Molecular interaction plot between top hit ligands and H-bonds for laccase-yellow 2g and peroxidase-reactive blue, respectively

Table 3 — Molecular docking scores and Number of Hydrogen bond of several screened dyes along with laccase

S. No	Enzyme-ligand complex	Binding energy Kcal mol^{-1}	Number of Hydrogen bond
1.	Laccase – Yellow 2g	-7.4	3
2.	Laccase – Methyl Yellow	-5.6	1
3.	Laccase – Nile Blue	-6.9	1
4.	Laccase - Reactive blue	-7.1	3
5.	Laccase – Red 40	-7.2	2

Table 4 — Molecular docking scores and Number of Hydrogen bond of several screened dyes along with Peroxidase

S. No	Enzyme-ligand complex	Binding energy Kcal mol^{-1}	Number of Hydrogen bond
1.	Peroxidase – Yellow 2g	-10.4	5
2.	Peroxidase – Methyl Yellow	-7.4	0
3.	Peroxidase – Nile Blue	-9.1	0
4.	Peroxidase - Reactive blue	-11.4	5
5.	Peroxidase – Red 40	-9.7	4

Table 5 — The residues involved in the enzyme-substrate complex interaction

Enzyme-substrate complex	No. of Hydrogen-bond	Involved residues
Laccase-Yellow 2g	03	SER 293, THR 262, GLY 321
Peroxidase-Reactive Blue	05	SER 327, ARG 223, ASN 333, ARG 246

system *i.e.*, laccase and laccase - yellow 2g complex were stable as observed in RMSD graph. There is not much difference lying between laccase and laccase-yellow 2g complex MD trajectories. In case of peroxidase and peroxidase-reactive blue complex both the systems achived stability during MD and simulation. Though peroxidase-reactive blue complex is more stable than peroxidase enzyme only and having an average RMSD of 0.2 nm. Based on the principle that globular proteins fold by minimizing the non polar surface that is exposed to water and known that hydrophobic interactions play a crucial role in determining the conformational stability of proteins.

The enzyme-ligand complexes are stable for reactive blue and yellow 2g biodegradation, as evidenced by minor fluctuations and a lower RMSD. Several research has evaluated the RMSD for enzyme-substrate complexes in order to better understand the variability within the complex all over biological processes[30-33].

RMSF

Furthermore, Salicylaldehyde dehydrogenases have been evaluated using the RMSF method, that

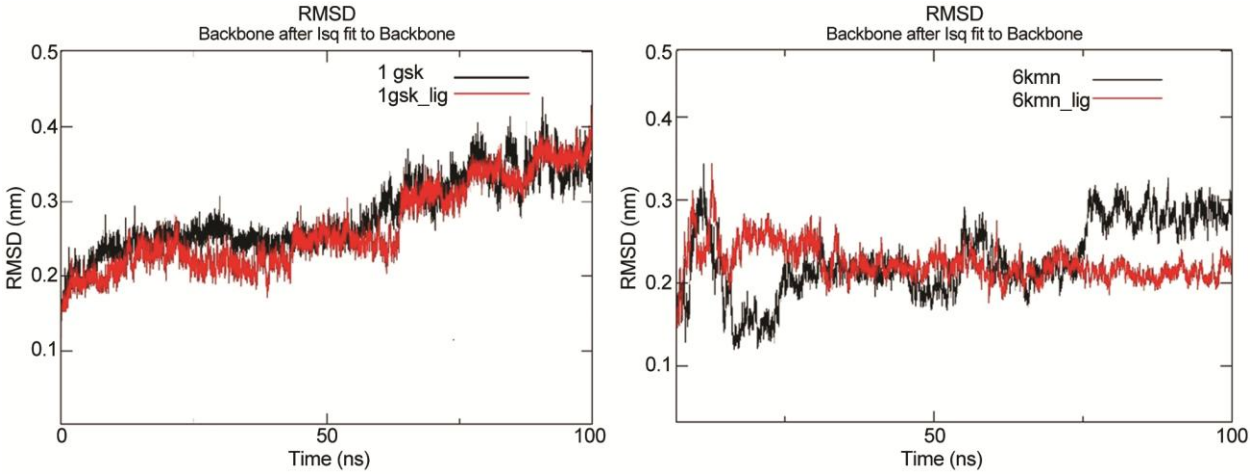

Fig. 4 — Graphic depiction of RMSD for laccase-yellow 2g (1gsk with black, 1gsk_lig with red) (A), peroxidase-reactive blue (6kmn with black, 6kmn_lig with red) (B) profile of the enzyme-substrate complex for 100 ns MD simulation

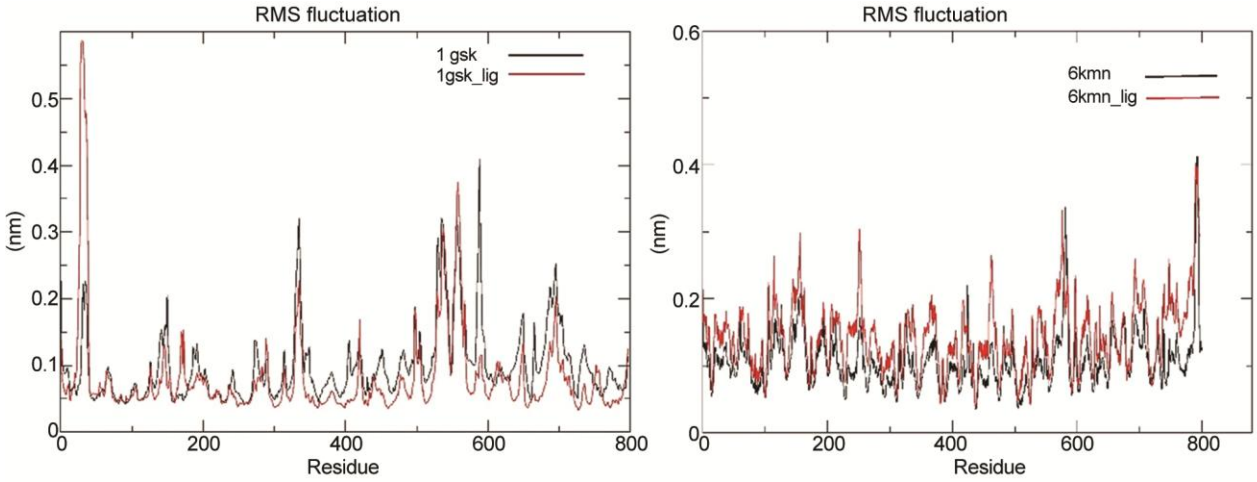

Fig. 5 — Graphical representation of RMSF for laccase, laccase -yellow 2g (1gsk in black, 1gsk_lig in red)and peroxidase and peroxidase-reactive blue (6kmn in black, 6kmn_lig red) complexes

exhibited RMSF high scores, indicating moldable protein residues. The RMSF was already correlated to the bounden of co-factors and substrates[32]. These studies demonstrate as an enzyme's flexibility declines; it experiences conformational changes that alter substrate binding during catalysis. The effects of laccase-based phenol remediation additionally confirmed[34]. Thus, here we determined that the pockets had formed on the laccase and peroxidase surface. Based on a study, the intrusion of enzyme-catalyzed reactions is triggered using adjustments in the cavity. At a given temperature and pressure, the location of an atom was determined by RMSF analysis. The RMSF analysis shown the protein's flexibility sections and calculated the net variations of the protein during the MD simulation. A lower RMSF (Root mean square fluctuation) value showed a more stable enzyme-substrate combination, whereas a higher value showed greater MD flexibility. During the MD simulations, the reactive blue complex and yellow 2g complex was stable, with less fluctuation and higher consistency according to the RMSF findings of the study.

For the enzyme-substrate complexes, fluctuations in the residue content were observed during the 100 ns trajectory time frame (Fig. 5). The study's RMSF data demonstrated that Laccase-yellow 2g complex and peroxidase- reactive blue complex were remained steady throughout the MD simulations, with less fluctuation and higher consistency.

Radius of gyration

Radius of gyration (Rg) is a unit of parameters for differences in enzyme-ligand complex closeness. It

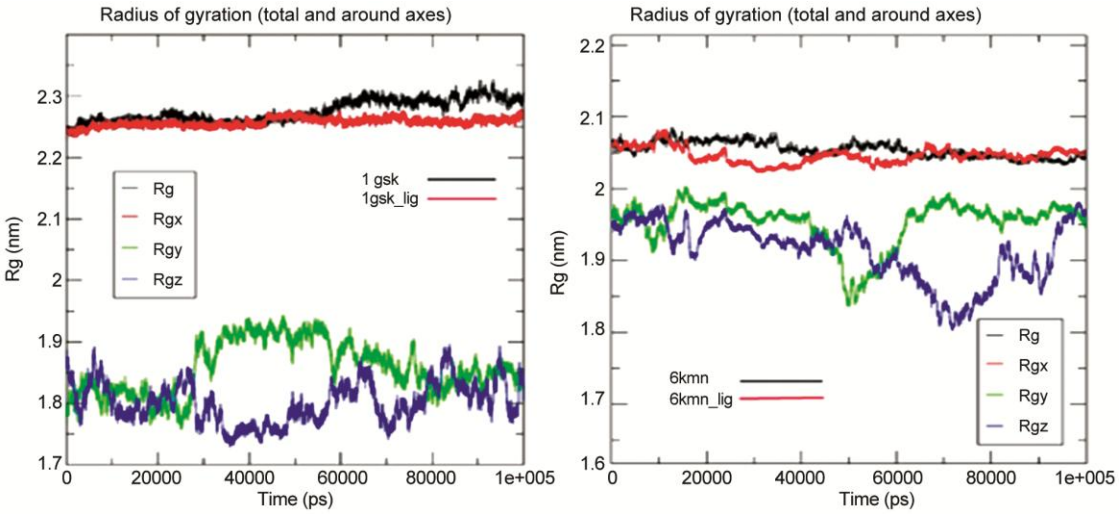

Fig. 6 — Radius of gyration profile of the laccase-yellow 2g and peroxidase-reactive blue complex for 100 ns MD simulation

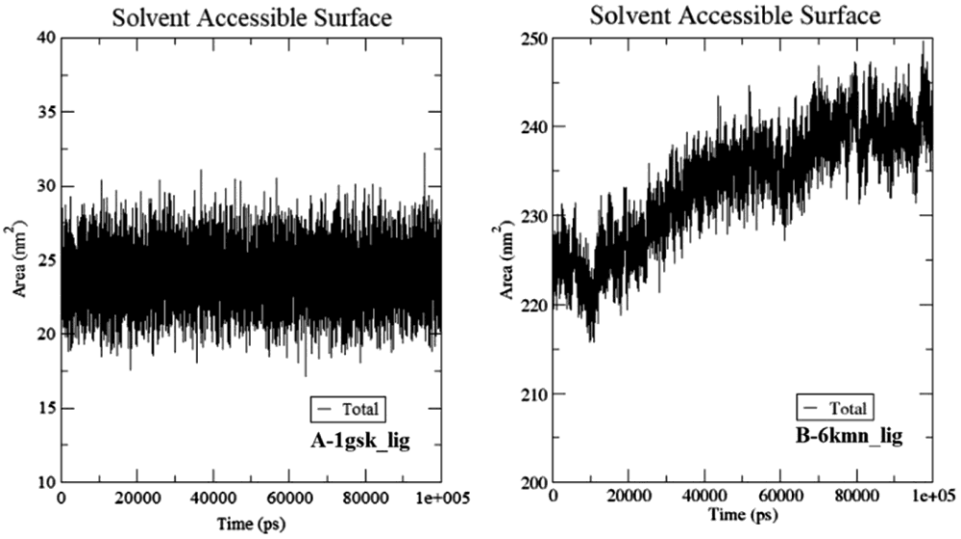

Fig. 7 — Solvent accessible surface profile of the laccase-yellow 2g peroxidase-reactive blue complex for 100 ns MD simulation

simply links to protein folding and unfolding. For this analysis, the last 100 ns of trajectory data were used to calculate the Rg. The overall Rg score for the laccase-yellow 2g was ± 2.8 nm whereas the overall Rg score for the peroxidase-reactive blue was ± 2.33. A stable Rg occurs when the enzyme is folded, and a fluctuating Rg indicates the unfolding of the enzyme[33]. The overall Rg score demonstrated that all enzyme-substrate combination had a similar and consistent Rg value (Fig. 6). This means they are perfectly overlaid over each other, resulting in the substrate-enzyme complex being compacted and stabilized.

Solvent accessible surface area

The surface accessible to solvents (SASA) using the MD simulation, we can determine how much of the protein surface is exposed to the water solvent and investigate contacts between the complex and the solvent. The overall SASA of was calculated for laccase-yellow 2g and peroxidase-reactive blue respectively. As a result, the SASA values for laccase-yellow 2g and peroxidase-reactive blue compounds throughout 100 ns of MD simulation are impressively stable, indicating that the shape of the protein has not altered significantly (Fig. 7).

Pre MD and Post MD simulation analysis of laccase- yellow 2g complex

The molecular dynamics simulations of Laccase (only protein), laccase -yellow 2g complex, peroxidase (only protein) and peroxidase-reactive blue complex, were done and observed valuable insights into

the stable complexes formed between the proteins and dyes over time[38,39]. Analysis of the trajectory of the laccase-yellow 2g complex depicts an increase in binding interactions occurring from 0 to 100 nanoseconds, effectively explaining the movement and interaction of atoms within the protein-ligand system during the simulation period. From these results, we can reasonably conclude that ligand binding does not negatively impact or compromise the structural integrity of either protein complex.

Examining the pre-simulation and post-simulation molecular structures provides critical insight into the initial hydrogen bond formation observed between Glu 460 of laccase with yellow 2g. Upon closer inspection, this initial bond involves the OE2 oxygen atom of a glutamate residue interacting with the O7 oxygen atom of yellow 2g, as clearly depicted in (Fig. 8A). The protein-ligand structure obtained after 100 nanoseconds of simulation further depicts additional hydrogen bonds formed with residues THR

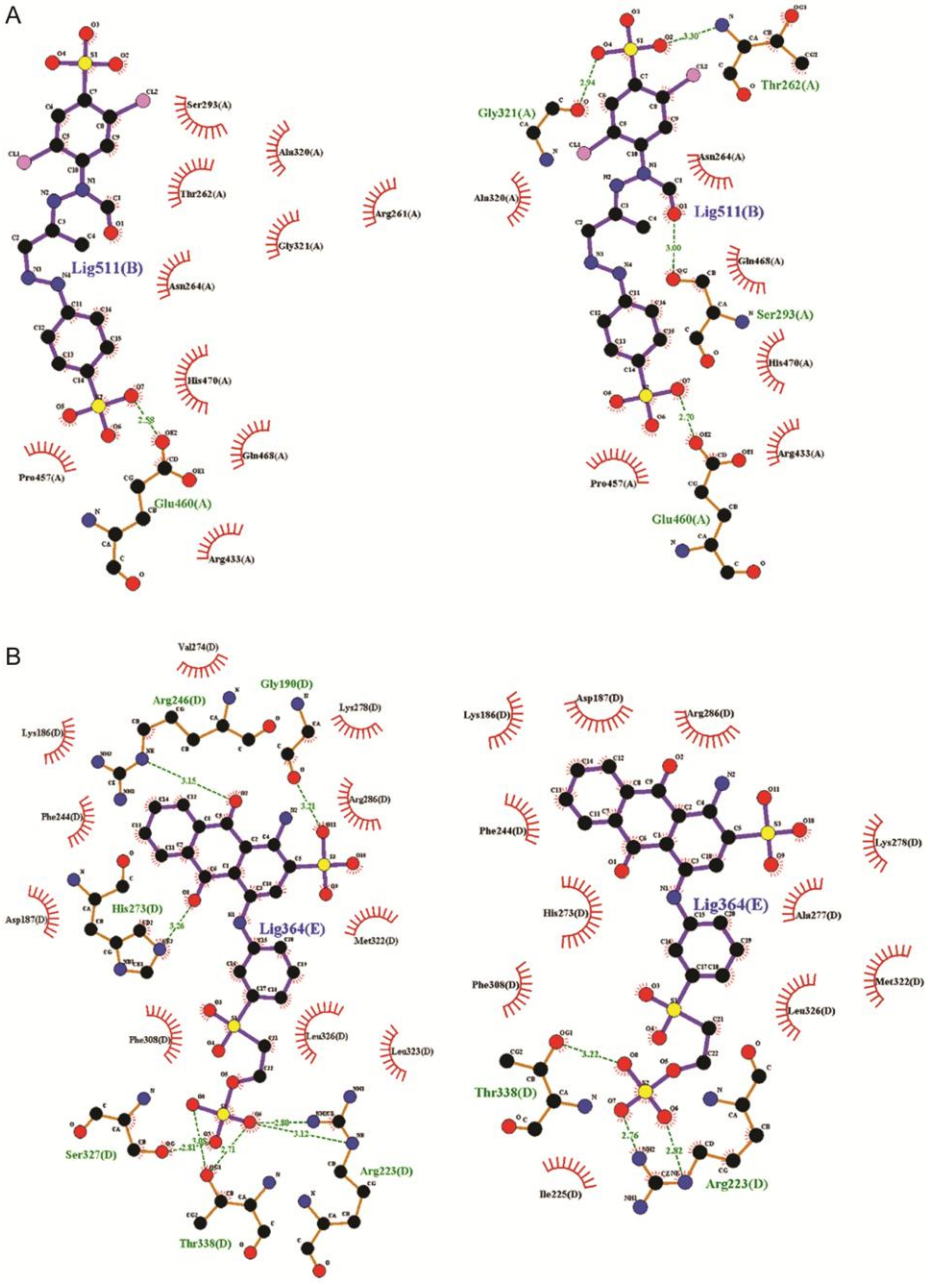

Fig. 8 — (A) pre MD interaction between laccase and yellow 2g, and post MD interaction between laccase and yellow 2g; and (B) pre-MD interaction between peroxidase and reactive blue, and post MD interaction between peroxidase and reactive blue

262, SER 293, GLY 321, and GLU 340 (Fig. 8B). This suggests that interactions arise from the electronegative oxygen atoms on the ligand interacting favorably through space with the lone electron pairs on electronegative atoms of interacting protein residues via hydrogen bonding.

Overall, the molecular dynamics simulations have helped elucidate the stable binding interactions that develop between the proteins and dyes over an extended simulation period. Careful analysis of structural changes pre- and post-simulation has revealed key insight into initial and developing hydrogen bonding patterns between residues and ligands.

Pre MD and Post MD simulation Analysis of Peroxidase-reactive blue complex

A structural analysis of the binding interaction between peroxidase and a reactive blue were able to identify six amino acid residues within the enzyme's binding pocket, forming a network of nine hydrogen bonds with the ligand. Specifically, glycine 190 contributed one hydrogen bond via its main chain nitrogen to the O11 atom of the ligand. Arginine 223 exhibited two distinct hydrogen bonds, one between each of its guanidinium nitrogen and the O6 atom. Arginine 246 displayed a single hydrogen bond between its guanidinium nitrogen and the O2 atom. Histidine 273 demonstrated one interaction between its main chain nitrogen and the O1 atom. Serine 327 formed a hydrogen bond via its main chain nitrogen and the O7 atom. Threonine 338 exhibited the most interactions, forming three separate hydrogen bonds: one between its main chain nitrogen and the O6 atom, and two between its side chain hydroxyl group and the O8 atom. This complex network of hydrogen bonds provided insight into the strong binding affinity observed between the enzyme and dye ligand. The binding pocket region of the peroxidase features multiple complementary electrostatic and hydrogen bonding interactions with the electronegative oxygen atoms within the ligand structure. Following molecular dynamics simulation to study the complex over time, only two of the original nine interactions remained: between the guanidinium nitrogen of Arginine 223 and the O6 and O7 atoms. Threonine 338 now shows a single remaining hydrogen bond via its side chain to the O8 atom. These post-simulation observations further elucidated the role of electrostatic interactions between electronegative atoms in contributing to binding affinity as well as provided insight into the degradation of the dye ligand over the simulation period.

Conclusion

In this study the modelled protein (laccase) was validated by UCLA-DOE LAB — SAVES v6.0 server, the results showed that the residues from the built model fell in favorable regions and the overall structure quality was good. The docking results illustrate the interactions between laccase-yellow 2g complex and peroxidase -reactive blue complex respectively. The mainly polar amino acids of laccases and peroxidases had been interacting with textile dyes. The MD simulation was used to learn more about how yellow 2g and reactive blue dyes interacts with laccase and peroxidase respectively. MD and Simulation was already applied to investigate enzyme-substrate complex in the past. The laccase-phenol complex has been investigated and found to be useful in remediation[31].The laccases derived from *B. subtilis* have been identified as the most optimal catalysts for the breakdown of methyl-orange dye[3]. The findings of a study conducted by Bhatt *et al*. (2023) indicate that the laccase enzyme exhibits strong interactions with various pollutants, including glyphosate, lignin polymer, isoproturon, and parathion. The binding energies observed in these interactions range from -2.5 to -8.7 kcal/mol[35,36].

Based on the results from the overall MD simulation (including RMSD, RMSF, and Rg analyses), the post-MD simulation (containing SASA analyses), and the binding free energy analysis, determined that yellow 2g and reactive blue is a fairly stable compound with excellent binding affinities with laccase and peroxidase respectively. As a result, it was concluded that laccases and peroxidase from *B. subtilis* are found to be the most favorable in yellow 2g and reactive blue dyes degradation and need further experimental characterization for their potential large-scale bio-remedial applications. This study also presents a pipeline for the screening of microbial enzymes for azo dye degrading abilities before the wet lab affirmation. Based on binding energies and H-bonding results, we propose that laccase, peroxidase from *B. subtilis* can efficiently bind with the yellow 2g, reactive blue respectively and in addition, induce its degradation. It was anticipated that this model can serve as a reliable *in silico* predictor for the potential of microbial enzymes for azo dye degradation[40,41]. Further wet lab experiments are needed to enhance our understanding of the mechanisms underlying the degradation of dyes by

microbial enzymes. This research serves as a basis for further exploration of laccases and peroxidases and other microbial enzymes responsible for dye degradation.

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Conflicts of interest

All authors declare no conflicts of interest.

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