MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Nashiruddin *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 22 (2026) 422–433

RESEARCH ARTICLE

Immobilization of Laccase on Graphene-Support Material: A Molecular Dynamic Study

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Abstract Laccase is a versatile oxidative enzyme widely applied in the degradation of various environmental pollutants. However, its practical application is often limited by poor operational stability and reusability in free form. Immobilization onto suitable support materials has therefore emerged as an effective strategy to enhance enzyme stability and performance. Among carbon-based supports, graphene and its derivatives have attracted considerable attention due to their high surface area, abundant functional groups, and excellent physicochemical properties. This study investigates the intermolecular interactions between laccase and graphene-based supports to elucidate the structural stability, flexibility, and compactness of enzyme-support complexes at the molecular level. Molecular docking and molecular dynamics (MD) simulations were employed to evaluate graphene oxide (GO) and reduced graphene oxide (rGO) as immobilization supports. Docking results revealed that the laccase-GO (Lac–GO) complex exhibited the strongest binding affinity (-14.4 kcal/mol), forming three hydrogen bonds with bond lengths of 2.03 Å, 2.52 Å, and 3.13 Å, whereas weaker interactions were observed for laccase-rGO. MD simulations further demonstrated that free laccase exhibited the lowest root mean square deviation (RMSD), reflecting inherent structural stability, while the Lac-GO complex maintained lower RMSD values than Lac-rGO, indicating improved structural stability among immobilized systems. Root mean square fluctuation (RMSF) analysis showed moderate residue-level flexibility for Lac-GO compared to higher fluctuations in Lac-rGO, suggesting better conformational preservation upon GO binding. Additionally, the radius of gyration (Rg) analysis revealed that Lac-GO retained greater compactness than Lac-rGO while allowing slight structural expansion relative to free laccase, which may facilitate enhanced enzyme loading and reduced mass transfer limitations. Overall, the computational findings indicate that graphene oxide provides a superior immobilization platform for laccase compared to reduced graphene oxide, offering favourable interaction stability and structural characteristics.

Keywords: Molecular docking, Molecular Dynamic Simulation, Laccase, Graphene-oxide, Reduced graphene-oxide, Immobilization.

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Received: 6 Nov. 2025
Accepted: 29 Jan. 2026

Introduction

Recently, enzymes or 'biocatalysts' have been remarkably discovered in the bioprocess technologies field and widely accepted because of their exclusive properties; ease of production, substrate specificity and green chemistry in a large variety of industries [12,24]. Enzymes are proteins that catalyze chemical reactions by increasing the reaction rate to reduce the initial energy input. The minimal use of chemicals, the generation of non-toxic by-products and the reaction with high selectivity with mild parameters (temperature, pressure, and pH) make enzymes more attractive to scientists [25]. Enzymes are widely utilized in various fields such as food, pharmaceutical, biodiesel and biofuels because of their process relevance and environmental-friendly [12, 14]

Laccases have the capability to oxidize a broad range of environmental pollutants or substrates, for example, ortho and para-diphenols, aromatic amines, phenolic acids, and other electron-rich substrates, with concomitant reduction of the oxygen molecule (O_2) to a water molecule (H_2O) [9]. In environmental applications, laccase has been used widely, and extensive research has been done because of its capability to degrade and detoxify many environmental pollutants, including highly resistant dyes and highly toxic environmental pollutants [5]. However, there is limited application of free laccase in real set-up applications because of reusability issues that relatively implicate the enzyme cost [21]. Furthermore, there are many factors known to limit the full potential catalytic ability of free laccase enzymes, besides poor reusability, which is the stability issue. Therefore, the immobilization technique has been introduced and widely explored to eliminate these drawbacks and enable the laccase application [4].

Enzyme immobilization is the confinement of an enzyme to a phase (carrier/support) different from the substrate and product. This method is considered an ideal technique to enhance the potential of laccase in industrial applications by improving the laccase properties and reusability. Immobilization provides increased operational stability, reusability, thermal stability and tolerance to changes in ionic conditions during fermentation [13]. There are three categories of enzyme immobilization, which are support or carrier-binding, encapsulation or entrapment and cross-linking. On the other hand, the ideal support materials must be affordable, have characteristics like inertness, stability, and physical strength, can increase enzyme activity and reusability, and reduce product inhibition, nonspecific adsorption and microbial contamination [12].

Zdarta *et al*. 2018a [30] reported that ideal support for enzyme immobilization should protect both enzyme activity and structure under various parameters while keeping its own physical integrity. According to Zhang *et al*. 2021 [29], laccase immobilization over solid support could crucially increase stability and enable its reuse. On the other hand, Patel *et al*. 2019 [22] also reported the immobilized laccase properties, which are immobilization yield, kinetic parameters, residual activity and enzyme stability, depending upon the method of immobilization and support materials used. There are a few important characteristics of solid support that have been reported by Adamian *et al*. 2021 [1], which are particle size, pore size/specific area, functional groups and inertness and mechanical properties.

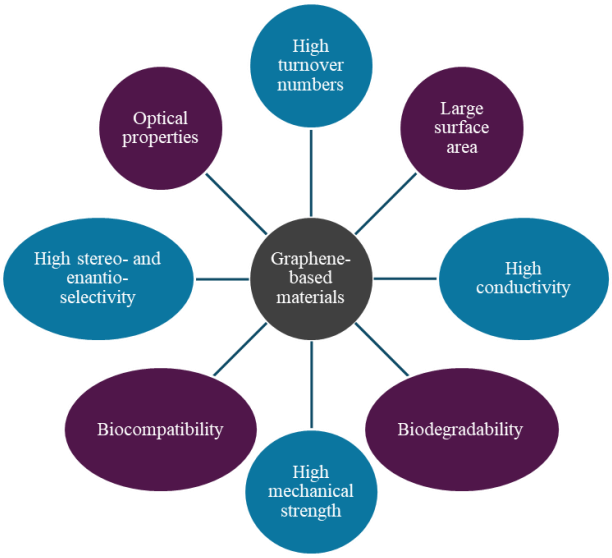


Figure 1. Novel characteristics of graphene-based material for enzyme immobilization modified from [2]

Nowadays, various carriers or support materials have been discovered for immobilized enzymes, either from natural biopolymers or synthetic polymers. The selection of appropriate support has always been one of the crucial steps since the support could broadly affect the properties of the resulting catalyst system [1]. There are a few properties of support that should take into consideration which are reusability, high affinity to enzyme, biocompatibility, mechanical and chemical stability, presence of functional groups and high affinity to the enzyme. Carbon-based material has been explored as a great candidate for laccase immobilization due to their properties such as high surface area, high porosity, a great number of functional groups and highly aromatic structure.

Graphene materials have been known as a promising carbon-based support for laccase immobilization due to their great properties, such as their high surface area (approximately 2630 $m^2\ g^{-1}$) and functional

groups such as carboxylic, hydroxyl and epoxide on their surface [11,18]. The immobilization of graphene materials is mostly through methods of covalent or adsorption. Adeel *et al*. 2018 [2] reported that graphene and its derivatives have unique properties such as optical, chemical, electrical, mechanical, multivalent functionalization and efficient surface loading with numerous biomolecules, making them a promising support for laccase immobilization in the enzyme engineering area. Figure 1 shows the novel characteristics of graphene-based material for enzyme immobilization.

Graphene oxide is an excellent derivative of one atom thick graphene layer with great physiochemical characteristics such as high intrinsic mobility (200,000 cm2 V^{-1} S^{-1}), large specific area (2630 m^2 g^{-1}), good optical transmittance (~97.7%), high Young's modulus (~1.0 TPa), and good thermal and electrical conductivity (~5000 W m^{-1} K^{-1}) [2,18]. GO can be prepared through many methods, such as Brodie, Hummer and Staudenmaier, by separating the graphite layers followed by an oxidation step with a strong oxidizing agent, while the distance between layers greatly depends on the extent of oxidation to produce oxygenated functional groups on GO [2.]. Zhou *et al*. (2022) [35] reported that, in a study on Lac-GO, laccase enzyme could attach to GO without need any further structure modification or cross-linking agent possibly due to enriched of abundant oxygen-containing functional groups in GO surface such as epoxide, hydroxyl, and carboxyl and could had a large adsorption rate. According to Catania *et al*. (2021) [10] and Olabi *et al*. (2021) [20], they also reported that as the extend reduction of GO increase, the support have better enzyme loading capability and stability due to high surface area.

On the other hand, reduced graphene oxide (rGO) is produced through removing oxygen functional groups from GO using few methods such as photoreduction, thermal reduction, microwave reduction, electrochemical reduction, and chemical reduction [1, 20]. Other than GO, rGO is also a rising star in the field of graphene research. The special properties like high surface area and stability makes rGO as the new generation of nanostructured carbon support for many catalytic applications [10]. Additionally, reduction of oxygen in GO makes rGO properties, hydrophobicity, improves compared to GO [3]. However, additional methods could increase cost of the production compared with GO which is way more less in production cost [10]. Figure 2 shows the structure of graphene, graphene oxide and reduced graphene oxide.

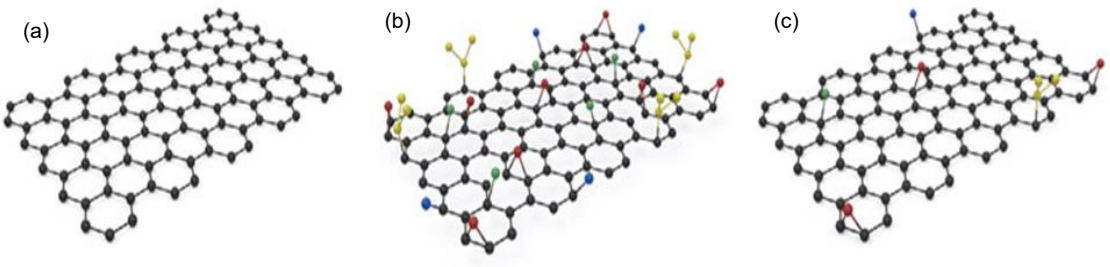


Figure 2. Structure of (a) graphene, (b) graphene oxide (GO), (c) reduced graphene oxide (rGO) [11]

In wastewater treatment technology, GO has been used as an absorbent to adsorb micro-pollutant for water purification, as it has a low operating cost, low energy consumption, easy to operate and fast speed [35]. Adsorption method for water purification posse few drawbacks including accumulation of micro-pollutant, lead to rapid deactivation of adsorbent, low micro-pollutant removal efficiency and high operational cost [26]. Therefore, material modification has been introduced as a promising strategy for adsorption saturation elimination and adsorption capacity promotion. "Green method" has been chosen as an ideal adsorbent modification where enzyme was immobilized to the adsorbent [35]. Zhou *et al*. (2022) [35] reported that immobilization of laccase on graphene oxide enhance the adsorption of micro-pollutant where micro-pollutant were timely degraded and adsorption sites are regenerated, promoting long-term continuous adsorption performances, and display the stable and sustainable micro-pollutant removal performance. Immobilized enzyme on adsorbent act as catalyst to catalyze the micro-pollutant degradation, showing the desirable characteristics for example mild reaction, substrate specificity, easy and green synthesis and environmentally friendly. Therefore, this can prove that GO has become an attractive alternative in materials science and biotechnology, especially in wastewater treatment due to their high stability to reaction conditions, low cost, and easy to produce [27].

Immobilization of enzyme has been used widely as standard experimental pipelines; however, the mechanism of immobilization has not been satisfactorily clarified at the microscopic level owing to the

complexity of the immobilizing agent and molecular nature of the protein-ligand surface interaction [8]. In immobilization of graphene oxide, studied by Zhao *et al*. (2018) [33] also reported that the studied of orientation of cytochrome c on immobilizing surface of graphene oxide is crucial for the electron transfer (ET), therefore, by using molecular dynamic (MD) simulation, they successfully investigated the conformational changes, the pathways of ET and dominant driving forces to understand the conformation, bioactivity and binding of cytochrome c. The understanding of conformation, binding and intermolecular interaction between GO and laccase is very limited. Furthermore, how the immobilization process can ultimately affect the efficiency of the enzyme is also a complex process which is poorly understood and difficult to rationalize. Therefore, in this study, two computational methods, which are molecular docking and MD simulation, will be used to compare the stability, flexibility and intermolecular interaction between laccase and graphene-support materials, graphene oxide (GO) and reduced graphene oxide (rGO).

Materials and Methods

Protein Preparation

The structure of laccase from *Trametes versicolor* was downloaded from RCSG Protein Data Bank with Protein ID 1GYC in PDB format. 1GYC in an oxidized form containing a full complement of copper ions are extracted at crystals room temperature. Figure 3 shows the laccase 3D structure from *Trametes versicolor.*

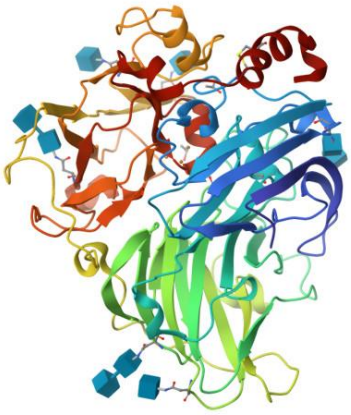


Figure 3. Laccase crystal structure determination at room temperature of a laccase from *Trametes versicolor* in its oxidised form containing a full complement of copper ions (1gyc) (Protein Data Bank)

Ligand Preparation

The structure of Graphene Oxide (GO) in SD file format was downloaded from Pubchem (Pubchem ID-163320950) while structure for reduced graphene oxide (rGO) were constructed using ChemDraw by partially removing selected oxygen-containing functional groups from the GO structure which adopted to represent a partially reduced grapheme oxide model. This model was performed and slightly modified method from [17]. Figure 4 shows the 2D structure of GO and rGO used in this study.

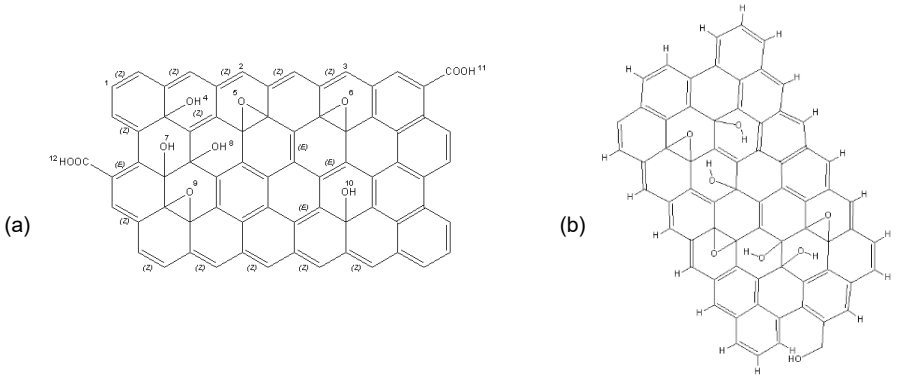


Figure 4. 2-Dimensional structure of (a) graphene oxide and (b) reduced graphene oxide

Molecular Docking

An automated molecular docking simulation of laccase and graphene materials were performed using AutoDock Tools and AutoDock Vina program. In AutoDock Tools, laccase (receptor) atom position was held fixed and torsion angle of graphene-material (ligand) bond were rotated to obtained the favorable docking position. The prepared receptor and ligand PDBQT files were combined with a configuration file specifying grid coordinates, search parameters, and output options. Docking simulations were performed using AutoDock Vina, which employs an empirical scoring function to predict binding affinity (ΔG, kcal/mol). Multiple docking runs were executed for each system to ensure reproducibility and to identify the most energetically favourable binding conformations of laccase-GO and laccase-rGO complexes. The lowest binding energy score was used to identify the stability and flexibility of the protein-ligand complex. The interaction of intermolecular bonds involve in molecular docking were visualized and analyzed using Discovery Studio program.

Molecular Dynamic Simulation

The molecular dynamics (MD) simulations were performed following and slightly modifying the protocol from the GROMACS tutorial website (https://www.mdtutorials.com/gmx/complex/index.html). The best binding mode of the protein–ligand complex obtained from molecular docking was used as the starting structure and simulated using GROMACS version 2021.4 (Ubuntu-2021.4-2) with the CHARMM36 force field. The simulation system was prepared in a dodecahedral box, solvated with Simple Point Charge (SPC) water molecules. Counter-ions (Na^+ and Cl^-) were then added to neutralize the overall charge of the system. The system underwent two stages of energy minimization (EM) to remove any steric clashes and to achieve a relaxed starting conformation. Subsequently, the equilibrated system was subjected to two consecutive equilibration phases. The first phase involved an isothermal–isochoric (NVT) ensemble for 100 ps to stabilize the temperature at 300 K, maintained using the V-rescale thermostat (modified Berendsen) with a coupling constant of 0.1 ps. The second phase was conducted under an isothermal-isobaric (NPT) ensemble for 100 ps to stabilize pressure and density at 1 bar, using the Parrinello-Rahman barostat with a coupling constant of 2.0 ps. During both equilibration steps, all bond lengths were constrained using the LINCS algorithm, and periodic boundary conditions were applied in all three dimensions. Following equilibration, the production MD simulation was carried out for 10 ns at pH 7.0, temperature 300 K, and pressure 1 bar, with a time step of 2 fs. The trajectory, energy, and structural coordinates were saved every 10 ps for subsequent analysis.

Trajectory Analysis

The structural stability and dynamic behaviour of the protein-ligand complex throughout the molecular dynamics simulation were analysed using standard GROMACS utilities. The root-mean-square deviation (RMSD) of the protein backbone atoms was calculated to evaluate the overall conformational stability of the system over the 10 ns simulation period. The root-mean-square fluctuation (RMSF) was computed to determine the flexibility of individual amino acid residues, providing insight into local conformational changes within the binding site. The radius of gyration (Rg) was analysed to assess the compactness of the protein structure during the simulation

Results and Discussion

Molecular Docking Analysis of Laccase Enzyme with Graphene-Support Material

In enzyme immobilization, selection for an ideal support is one of the crucial key factors to boost enzyme catalytic activity and stability of the immobilize enzyme [34]. In this study, the ideal support for laccase immobilization was investigated between graphene oxide (GO) and reduced graphene oxide (rGO). Therefore, in this study, two support materials, which are GO and rGO, were compared and analysed to determine the ideal support for laccase immobilization.

Compared with a traditional experimental laboratory, molecular docking techniques were used to determine the most favourable binding mode of ligand and protein before proceeding with the molecular dynamic simulation process. The 3D structure of laccase enzyme obtained from Protein Data Bank was used as the receptor molecule, while the 3D structure of ligand molecule, GO and rGO, were obtained from PubChem and ChemDraw, respectively. The molecular docking was performed using the AutoDock Vina program, and nine different conformations or orientations of the ligand binding mode were generated. The conformation with the lowest free binding energy (ΔG) was considered the most favourable, as determined by the scoring function n molecular docking, scoring functions are designed to estimate protein-ligand binding affinity by evaluating key intermolecular interactions, including van der Waals forces, electrostatic interactions, hydrogen bonding, and hydrophobic effects, thereby enabling the identification of energetically favourable and biologically relevant binding poses [23]. Determination of the most favourable orientation is important to form a stable complex with overall minimum energy. Table 1 shows the scoring of nine conformations of ligand binding mode for GO and rGO.

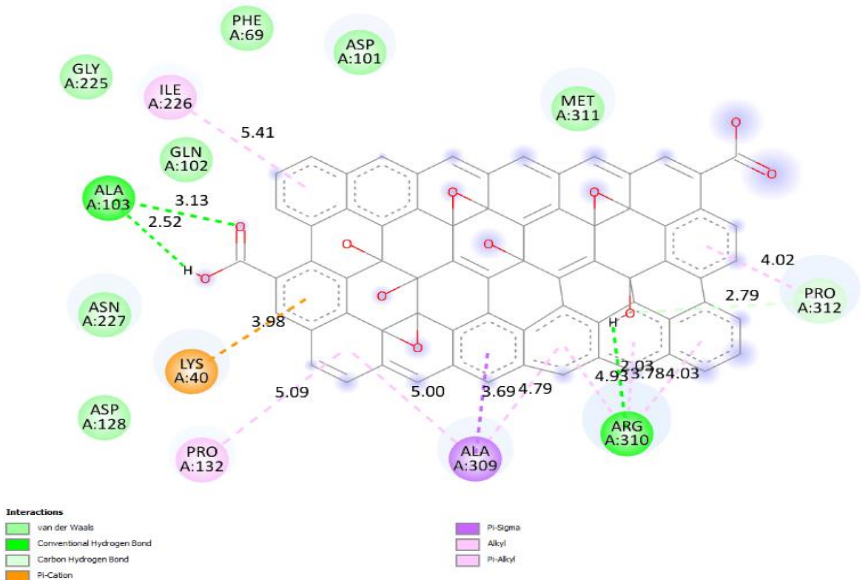
In molecular docking, protein-ligand binding affinity is one of the crucial factors to indicate the strength of the interaction and how tightly the molecule (ligand) and protein (receptor) bind together. Table 1 shows that based on the scoring function analysis from molecular docking, GO was found to have a strong binding affinity in the binding site of laccase enzyme based on the conformation, hydrogen bond interactions and lowest binding energy score, -14.4 kcal/mol compared with rGO, -12.3 kcal/mol. According to Banaganapalli *et al*. (2019) [6], strong binding affinity is associated with a low equilibrium dissociation constant value and with a low binding energy. AutoDock Vina employs an empirical scoring function to estimate protein-ligand binding affinity, which is calculated by summing up distance-dependent atom pair interactions such as steric interactions, combining of Gaussian function with a repulsive parabolic function, which reproduces the Lennard-Jones interaction's general shape [6]. Table 1 shows that GO was an ideal support material for the laccase enzyme compared with rGO. According to Jahan *et al*. (2022) [16] reported that GO had an excellent adsorption capacity of methylene blue (MB) compared to rGO due to contribution of electrostatic interactions, H-bonds, and pi-pi (π-π) interactions. Therefore, in this study, by using the best mode binding conformation, the bonding interactions between laccase-GO and laccase-rGO were determined using Discovery Studio Visualizer (Figure 5).

As shown in Figure 5, the hydrogen bonding interaction between laccase and support, GO and rGO were visualized, where GO showed the strongest binding affinity due to the existence of three hydrogen bond interactions between laccase and the functional group on the GO surface, compared to rGO, where there is no hydrogen bond interaction shown and only π-π interaction was shown. Hydrogen bonding was prioritized due to its strong directionality and critical role in defining binding specificity, orientation, and conformational stability of immobilized enzymes, whereas π–π stacking interactions, although relevant for graphitic surfaces such as rGO, are generally weaker and less specific in stabilizing protein conformations [32]. The docking interaction shown in Figure 5 (a) also reveals a strong hydrogen bond interaction between the -OH group of GO and laccase residue, ALA103, and ARG310, where two of them had bond lengths lower than 3.00Å, which were 2.52Å and 2.03Å, respectively, and another one bond length of 3.13Å, also with laccase residue ALA103. According to Pace *et al*. (2014), [36] hydrogen bonds in protein–ligand systems are commonly defined within donor-acceptor distances of approximately 2.5–3.5 Å, where interactions shorter than 3.0 Å are considered strong, while slightly longer distances can remain energetically meaningful, particularly when multiple hydrogen bonds act cooperatively to stabilise the complex. It was then proved by Zhang *et al*. (2020) [31] where the GO surface showed an abundance of oxygen-containing functional groups, such as epoxide, hydroxyl and carboxyl, through atomic force microscopy that attached to the laccase enzyme. Yu *et al*. (2022) [28] also reported the same finding where GO had an abundance of oxygen-containing functional groups that could enhance its excellent dispersion in water, organic solvents and various matrices. Compared with rGO, which is produced by removing oxygen functional groups [1]. Therefore, based on conformation, hydrogen bond interaction and had the lowest binding energy score, GO was found to be the best support material for laccase enzyme immobilization.

Table 1. Scoring function of nine conformation binding mode of GO and rGO on laccase enzyme

Graphene oxide				Reduced Graphene Oxide			
Mode	Affinity (kcal/mol)	Dist. From best mode		Mode	Affinity (kcal/mol)	Dist, from best mode	
		Rmsd l.b	Rmsd u.b			Rmsd l.b	Rmsd u.b
1	-14.4	0.000	0.000	1	-12.3	0.000	0.000
2	-14.0	1.726	6.260	2	-12.1	2.161	9.161
3	-14.0	28.225	31.498	3	-12.0	4.085	8.752
4	-13.4	1.563	6.060	4	-12.0	2.071	6.807
5	-13.4	4.271	6.800	5	-11.8	2.144	7.204
6	-13.4	34.948	38.594	6	-11.5	2.034	4.372
7	-12.8	6.037	9.619	7	-11.2	2.808	8.946
8	-12.8	1.505	10.853	8	-11.1	3.592	9.380
9	-12.8	2.071	9.135	9	-10.9	30.258	35.839

(a)



(b)

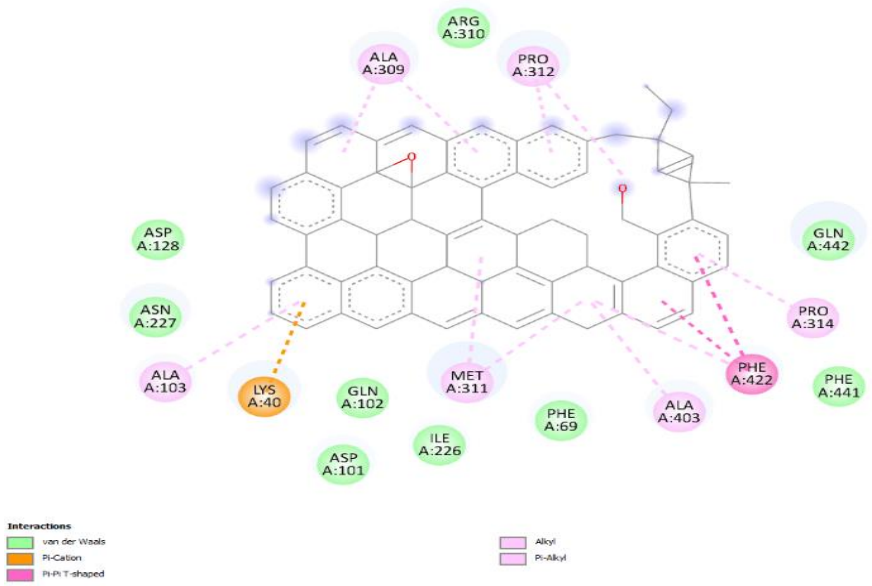


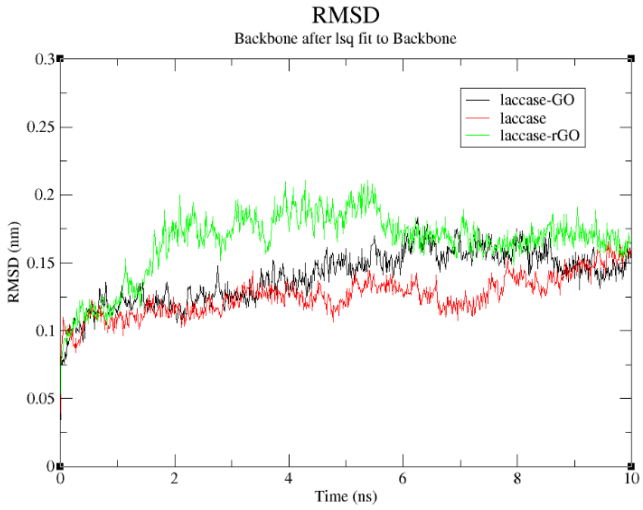
Figure 5. 2-Dimensional diagram of docking depicting the H-Bond interaction (a) laccase residue ALA103 (3.13Å), ALA103 (2.52Å) and ARG310 (2.03Å) on -OH group of GO (b) laccase and rGO

Stability of Docking Complex System During Molecular Dynamic Simulation

The best protein-ligand complex of Lac-GO and Lac-rGO and free Lac were then examined by Molecular dynamic simulation using Gromacs software to test the stability and flexibility of the structure. The MD simulations were run with operating conditions pH 7.0, temperature 27 °C, pressure 1.0 at 10 nano second (10 ns) time duration for all the support material for comparison. The laccase-material complex trajectory data for root mean square deviation (RMSD), root mean square fluctuation (RMSF) and radius of gyration (Rg) were analyzed with plotted data in Figure 6.

RMSD values were used to examine the degree of deviation for each structure and provide insight into the conformational stability of the complex, where a lower deviation value over time indicates higher stability [15]. As shown in Figure 6 (a), free laccase (red) displayed the lowest RMSD values (~0.10-0.15 nm) across the entire 10 ns simulation, reflecting minimal structural deviation and confirming its inherent stability. While the Lac-GO complex has a better stability complex with slightly higher RMSD deviation (~0.12-0.17 nm), suggesting some conformational adaptation upon GO binding, but still maintaining structural stability. On the other hand, the Lac–rGO complex consistently exhibited higher RMSD values (~0.18-0.22 nm), indicating larger backbone deviations and reduced stability compared to both free laccase and the Lac–GO complex. Zhang *et al*. (2023) [32] also reported that the RMSD values in Laccase-Magnetic Graphene Oxide (Lac-MGO) showed slightly higher values than free laccase. This shows that support materials in immobilization exert varying degrees of influence on the stability of laccase structure [19]. All systems reached equilibrium after approximately 2 ns, as evidenced by the plateauing of the RMSD curves. The findings found that free laccase has a higher stability compared immobilization with support due to minimal structure deviation, while Lac-GO had a better stability complex compared with Lac-rGO. These findings highlight that GO binding provides a stabilizing effect on the laccase backbone, whereas rGO binding introduces additional flexibility, leading to greater conformational deviation. Therefore, to confirm the data finding, root mean square fluctuation (RMSF) were determined to observe the flexibility of enzyme backbone of each complex.

(a)



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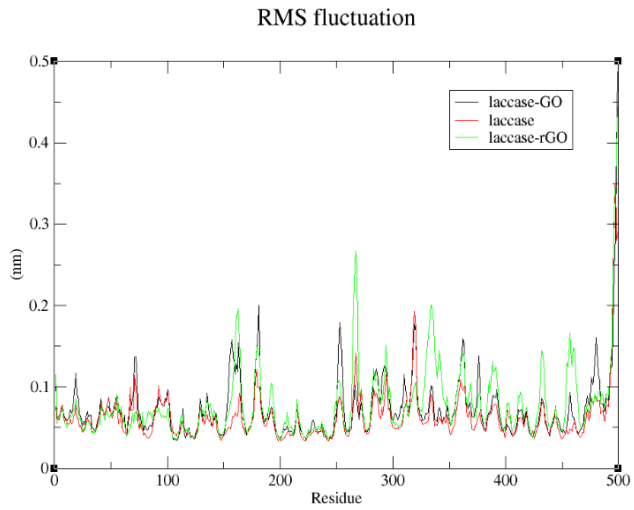

(c)

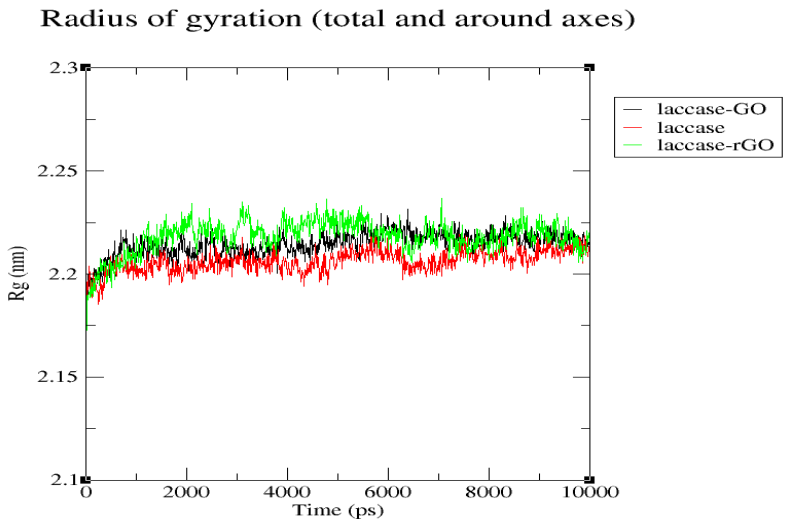

Figure 6. (a) Root mean square deviation (RMSD) plots of laccase-GO complex, laccase-rGO complex and laccase at 10 ns of molecular dynamic simulation (b) Root mean square fluctuation (RMSF) plots of the backbone heavy atoms of laccase-GO complex, laccase-rGO complex and laccase (c) Radius of gyration (Rg) plots of laccase-GO complex, laccase-rGO complex and laccase over the 10 ns simulation time

For root mean square fluctuation (RMSF) for the backbone atom of each complex was analyzed to observe the flexibility of the laccase backbone structure. RMSF can effectively capture structural changes in the enzyme before and after immobilization [32]. High RMSF values indicate higher flexibility, while lower RMSF values show rigidity of the complex. The RMSF analysis revealed distinct flexibility patterns for free laccase, Lac-GO and Lac-rGO complex. As shown in Figure 6 (b), free Lac exhibited the lowest RMSF value (~0.360 nm), indicating a relatively stable conformation throughout the simulation. For the Lac-GO complex, moderate fluctuation was observed with an RMSF value ~0.388 nm, suggesting that GO provided stabilizing interactions while allowing some conformational flexibility. However, the Lac-rGO complex showed higher fluctuations at several loop regions and surface residues, particularly around residues 150-200 and 300-400, with the highest peak at ~0.445 nm, indicating that rGO binding induces greater structural flexibility. This shows that, while GO contributes to stabilizing laccase dynamics, rGO binding promotes enhanced flexibility, which may influence the enzyme's structural integrity and functional performance. On the other hand, Zhang *et al*. (2023) [32] also reported that immobilization of Lac-MGO (57.01Å) and Lac-ILS-MGO (55.01Å) exhibited reduced overall structure changes compared to free laccase (73.96Å), showing that immobilization enhanced the laccase structure's rigidity. This result proved that the interaction of the enzyme and support materials could enhance the stability of enzymes and improve enzyme activity and reusability [7].

Further investigation was conducted on radius of gyration (Rg) profiles to support these stability observations by examining protein compactness. As shown in Figure 6 (c), free laccase maintained the lowest Rg values (~2.19-2.22 nm), indicating a compact and stable structure. In contrast, Lac–GO displayed slightly higher Rg values (~2.20-2.24 nm), suggesting moderate expansion but overall retention of compactness, while for Lac–rGO complex consistently exhibited the highest Rg values (~2.22-2.26 nm), implying reduced compactness and greater conformational looseness, which may influence the stability and enzyme performance. All systems stabilized after ~2 ns, with only small fluctuations (~0.02-0.04 nm), showing that none underwent large-scale unfolding

Conclusions

The present study employed molecular docking and molecular dynamics simulations to comparatively evaluate graphene oxide and reduced graphene oxide as support materials for laccase immobilisation at the molecular level. The results indicate that graphene oxide exhibits stronger and more stable interactions with laccase than reduced graphene oxide, as evidenced by lower binding energy, more favourable hydrogen bonding, and improved structural stability among the immobilised systems. Although free laccase remained the most structurally stable reference system, immobilisation on graphene oxide preserved backbone stability and overall compactness while allowing limited adaptive flexibility, which is considered advantageous for maintaining enzyme functionality under immobilised conditions. In contrast, laccase immobilised on reduced graphene oxide displayed increased flexibility and reduced compactness, suggesting a less stable immobilisation environment. These molecular dynamics findings provide mechanistic insights that are consistent with previously reported experimental studies demonstrating enhanced stability and operational performance of laccase immobilised on graphene oxide-based supports. Overall, the results support the potential of graphene oxide as a promising immobilisation platform for laccase, warranting further experimental validation for environmental and biotechnological applications.

Acknowledgement

The authors gratefully acknowledge the financial support from Ministry of Higher Education, Malaysia under Fundamental Research Grant Scheme (FRGS/1/2022/TK08/UTM/02/16) and Universiti Teknologi Malaysia under the UTM Fundamental Research Grant (Q.J130000.3809.22H00). The authors would also like to acknowledge Dr. Norfarhan Binti Mohd Assa'ad from Universiti Kebangsaan Malaysia (UKM) for her contributions in this project.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] Adamian, Y., Lonappan, L., Alokpa, K., Agathos, S. N., & Cabana, H. (2021). Recent developments in the immobilization of laccase on carbonaceous supports for environmental applications: A critical review. *Frontiers in Bioengineering and Biotechnology*, *9*, 1–29.

[2] Adeel, M., Bilal, M., Rasheed, T., Sharma, A., & Iqbal, H. M. N. (2018). Graphene and graphene oxide: Functionalization and nano-bio-catalytic system for enzyme immobilization and biotechnological perspective. *International Journal of Biological Macromolecules*, *120*, 1430–1440.

[3] Avornyo, A., & Chrysikopoulos, C. V. (2024). Application of graphene oxide (GO) in oily wastewater treatment: Recent developments, challenges, and opportunities. *Journal of Environmental Management*, *353*, 1–18.

[4] Ba, S., Arsenault, A., Hassani, T., Jones, J. P., & Cabana, H. (2012). *Critical Reviews in Biotechnology*, 1–15.

[5] Ba, S., & Vinoth Kumar, V. (2017). Recent developments in the use of tyrosinase and laccase in environmental applications. *Critical Reviews in Biotechnology*, 1–15.

[6] Banaganapalli, B., Morad, F. A., Khan, M., Kumar, C. S., Elango, R., Awan, Z., & Shaik, N. A. (2019). Molecular docking. In I. Vol (Ed.), *Essential bioinformatics* (pp. 335–353). Springer.

[7] Barbhuiya, N. H., Misra, U., & Singh, S. P. (2022). Biocatalytic membranes for combating the challenges of membrane fouling and micropollutants in water purification: A review. *Chemosphere*, *286*, 1–23.

[8] Bhattacharjee, N., Alonso-Cotchico, L., & Lucas, M. F. (2023). Enzyme immobilization studied through molecular dynamic simulation. *Frontiers in Bioengineering and Biotechnology*. https://doi.org/10.3389/fbioe.2023.1200293.

[9] Brugnari, T., Braga, D. M., Santos, C. S. A., Torres, B. H. C., Modkovski, T. A., Haminiuk, C. W. I., & Maciel, G. M. (2021). Laccase as green and versatile biocatalyst: From lab to enzyme market—An overview. *Bioresources and Bioprocessing*, *8*, 1–29.

[10] Catania, F., Marras, E., Giorcelli, M., Jagdale, P., Lavagna, L., Tagliaferro, A., & Bartoli, M. (2021). A review on recent advancement of graphene and graphene-related materials in biological applications. *Applied Sciences*, *614*, 1–21.

[11] Daneshmandi, L., Barajaa, M., Rad, A. T., Sydlik, S. A., & Laurencin, C. Y. (2020). Graphene-based biomaterials for bone regenerative engineering: A comprehensive review. *Advanced Healthcare Materials*, 1–25.

[12] Datta, S., Christena, L. R., & Rajaram, Y. R. S. (2013). Enzyme immobilization: An overview on technique and support materials. *3 Biotech*, *3*, 1–9.

[13] Datta, S., Veena, R., Samual, M. S., & Selvarajan, E. (2021). Immobilization of laccase and application for the detection and remediation of pollutants: A review. *Environmental Chemistry Letters*, *19*, 521–538.

[14] Hwang, E. T., & Gu, M. B. (2012). Enzyme stabilization by nano/microsized hybrid materials. *Enzyme and Life Science*, 1–13.

[15] Jailani, N., Jaafar, N. R., Suhaimi, S., Mohaman Mackeen, M. M., Abu Bakar, F. D., & Md Illias, R. (2022). *International Journal of Biological Macromolecules*, *213*, 516–533.

[16] Jahan, N., Roy, H., Reaz, A. H., Arshi, S., Rahman, E., Firoz, A. H., & Islam, M. S. (2022). A comparative study on sorption behavior of graphene oxide and reduced graphene oxide towards methylene blue. *Case Studies in Chemical and Environmental Engineering*, *6*, 1–10.

[17] Jayakumar, K., Zhong, Y., Camarada, M. B., Lu, X., Chen, T., Zhang, W., & Wen, Y. (2024). One-step electrochemical preparation of platinum nanoparticle decorated self-healing reduced graphene oxide nanoarray. *Journal of Environmental Chemical Engineering*, *12*, 1–11.

[18] Karthik, V., Senthil Kumar, P., Vo, D. V. N., Selvakumar, P., Gokulakrishnan, M., Keerthana, P., Audilakshmi, V., & Jeyanthi, J. (2021). Enzyme-loaded nanoparticles for wastewater contaminant degradation: A review. *Environmental Chemistry Letters*. https://doi.org/10.1007/s10311-020-01158-8.

[19] Liu, Y., Liu, Z., Zeng, G., Chen, M., Jiang, Y., Shao, B., Li, Z., & Liu, Y. (2018). Effect of surfactants on phenol interaction with laccase. *Journal of Hazardous Materials*, *357*, 10–18.

[20] Olabi, A. G., Abdelkareem, M. A., Wilberforce, T., & Sayed, E. T. (2021). Application of graphene in energy storage devices: A review. *Renewable and Sustainable Energy Reviews*, *135*, 1–20.

[21] Osma, J. F., Toca-Herrera, J. L., & Rodríguez-Couto, S. (2011). Cost analysis in laccase production. *Journal of Environmental Management*, *92*, 2907–2912.

[22] Patel, S. K. S., Choi, H., & Lee, J. K. (2019). Multimetal-based inorganic-protein hybrid system for enzyme immobilization. *ACS Sustainable Chemistry & Engineering*, *7*, 13633–13638.

[23] Pagadala, N. S., Syed, K., & Tuszynski, J. (2017). Software for molecular docking: A review. *Biophysical Reviews*, *9*, 91–102.

[24] Shokri, Z., Seidi, F., Karami, S., Li, C., Saeb, M. R., & Xiao, H. (2021). Laccase immobilization onto natural polysaccharide. *Carbohydrate Polymers*, *262*, 1–15.

[25] Sigg, S. J., Seidi, F., Renggli, K., Silva, T. B., Kali, G., & Bruns, N. (2012). ATRPases: Enzymes as catalyst for atom transfer radical polymerization. *Polymer and Colloid Highlights*, *66*(1), 66.

[26] Xiao, W., Jiang, X., Liu, X., Zhou, W., Garba, Z. N., Lawan, I., Wang, L., & Yuan, Z. (2021). *Journal of Cleaner Production*, *284*, 1–20.

[27] Yang, S., Yang, J., Wang, T., Li, L., Yu, S., Jia, R., & Chen, P. (2020). Combined enzyme system of graphene oxide and manganese peroxidase. *Nanoscale*. https://doi.org/10.1039/D0NR00408A

[28] Yu, D., Li, Z., Wang, W., Wang, L., Liu, T., & Du, J. (2022). Modification of magnetic graphene oxide and immobilized lipase. *International Journal of Biological Macromolecules*, *216*, 498–509.

[29] Zhang, Y., Hu, P., Muhammad, Y., Tang, Y., Shao, S., Gao, Z., Wang, J., Wang, R., Hu, Y., Kuang, L., Zhao, Z., & Zhao, Z. (2021). High-density immobilization of laccase on NH2-MIL88 (Fe). *Chemical Engineering Journal*, *405*, 1–14.

[30] Zdarta, J., Meyer, A. S., Jesionowski, T., & Pinelo, M. (2018a). Overview of support materials for enzyme immobilization. *Catalysts*, *8*, 1–27.

[31] Zhang, W., Yang, Q., Luo, Q., Shi, L., & Meng, S. (2020). Laccase-carbon nanotube nanocomposites. *Journal of Cleaner Production*, *242*, 1–11.
[32] Zhang, W., Zhang, Y., Lu, Z., Nian, B., Yang, S., & Hu, Y. (2023). Enhanced stability of laccase immobilized on graphene oxide. *Journal of Environmental Management*, *346*, 1–12.
[33] Zhao, D., Li, L., & Zhou, J. (2018). Cytochrome c adsorption on graphene surfaces. *Applied Surface Science*, *428*, 825–834.
[34] Zhou, W., Zhang, W., & Cai, Y. (2021). Laccase immobilization for water purification. *Chemical Engineering Journal*, *403*, 1–15.
[35] Zhou, W., Zhang, W., & Cai, Y. (2022). Enzyme-enhanced adsorption of laccase immobilized graphene oxide. *Separation and Purification Technology*, *294*, 1–10.
[36] Pace, C. N., Fu, H., Fryar, K. L., Landua, J., Trevino, S. R., Schell, D., Thurkill, R. L., Imura, S., Scholtz, J. M., Gajiwala, K., *et al*. (2014). Contribution of hydrogen bonds to protein stability. *Protein Science*, *23*, 652–661.