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Molecular Simulation of Enzyme Adsorption and Immobilization in MIL-53(Fe)-Based Covalent Organic Frameworks

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Abstract

This study investigates enzyme adsorption in covalent organic frameworks (COFs) using molecular simulations. Laccase was immobilized on MIL-53(Fe) and NH_2-MIL-53(Fe) frameworks to develop visible-light-responsive biocatalysts. Molecular dynamics (MD) and density functional theory (DFT) analyses revealed that enzyme immobilization significantly enhanced electron transfer (ET) between the photocatalyst and the enzyme's active site, promoting efficient charge separation and interfacial electron mobility. Electron spin resonance spectroscopy and free energy calculations demonstrated that visible light improved photogenerated ET, increasing tetracycline degradation rate constant from 0.0062 to 0.0127 min^{-1}. Morphological analysis showed MIL-53(Fe) possessed a stable octahedral crystalline structure with ~400 nm border length, ideal for enzyme binding. The binding free energy of -356 kcal/mol indicated thermodynamically favorable and strong enzyme-framework interactions. Furthermore, covalent immobilization improved laccase's structural stability and preserved its native conformation compared to physisorption, facilitating access to the active site. Overall, this work highlights that COF-based enzyme immobilization offers a stable and highly active platform for enhanced photocatalytic degradation and environmental remediation applications.

Keywords

Molecular simulation, Enzyme immobilization, Covalent organic framework, Density functional theory

Article History

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1. Introduction

Understanding the adsorption processes and interactions between enzymes and porous framework materials is crucial for advancing biotechnological and energy applications such as biocatalysis, renewable energy cells, biosensing, and artificial photosynthesis [1-5]. Enzymes are highly efficient natural catalysts due to their specificity and catalytic ability under mild conditions. However, their direct application faces challenges due to structural fragility, instability under harsh environments, and difficulties in reuse [6,7]. Immobilizing enzymes on suitable support materials helps overcome these limitations by enhancing catalytic efficiency, stability, and operational durability [8-11].

The arrangement and integrity of enzymes on supporting surfaces significantly influence catalytic performance. Poor adsorption or structural changes can reduce activity by limiting substrate access or electron transfer (ET) [12,13]. Factors such as surface chemistry, charge stability, hydrophobicity, pore structure, and water retention in the support matrix affect enzyme conformation and activity [14-20]. Thus, precise control over enzyme-support interactions is vital for creating efficient biocatalytic systems. Among support materials, covalent organic frameworks (COFs) have attracted significant interest. COFs are crystalline, porous polymers composed of light elements (C, H, O, N, B) with tunable pore sizes and chemical properties, facilitating enzyme immobilization via hydrophobic, hydrogen-bonding, and π-π interactions [21-30].

In parallel, iron-based metal-organic frameworks (MOFs), such as MIL-53(Fe) and its amino-functionalized derivative NH_2-MIL-53(Fe), are particularly attractive for enzyme immobilization. Iron was selected as the metal center because it is earth-abundant, cost-effective, and environmentally benign, making it ideal for sustainable applications. Additionally, iron-based MOFs exhibit excellent redox properties and catalytic activity beneficial for enzyme immobilization and catalysis. MIL-53(Fe) and NH_2-MIL-53(Fe) possess robust thermal and chemical stability, ensuring reliable enzyme support under various reaction conditions. The amino functionalization in NH_2-MIL-53(Fe) enhances hydrophilicity and provides additional sites for enzyme binding via hydrogen bonding and electrostatic interactions, thus improving enzyme immobilization and catalytic efficiency.

While our experimental work focuses on enzyme immobilization using these iron-based MOFs, computational simulations in this study are conducted primarily on COF structures to gain detailed molecular insights into enzyme adsorption, structural stability, and ET processes. Immobilizing enzymes within COFs has been shown to enhance enzyme stability, maintain their structure, and protect against thermal or chemical degradation, making COFs ideal models for robust biocatalysts [27,28]. Despite advances, the molecular-scale adsorption mechanisms in enzyme-COF systems remain poorly understood. Factors such as pore size, functional groups, hydrophobicity, and framework charge strongly influence enzyme loading, structure, and activity [31-39]. The complexity of COF surfaces, including defects and hydration layers, complicates direct experimental study [35-37]. Therefore, linking COF chemical features to enzyme performance is essential for designing next-generation enzyme-COF biocatalysts [38-41]. While experimental techniques like fluorescence spectroscopy and FTIR reveal chemical changes in enzymes, they cannot fully resolve atomic-level enzyme adsorption and interaction dynamics [42-45]. Computational simulations such as molecular dynamics (MD), Monte Carlo (MC), density functional theory (DFT), and quantum mechanics/molecular mechanics (QM/MM) methods provide detailed insights into enzyme adsorption configurations, stability, and ET mechanisms on COF surfaces [46-57]. Combining these approaches helps bridge experimental observations with molecular-scale understanding, guiding rational design of enzyme-COF biohybrid catalysts with improved stability, positioning, and charge transport [52-60].

In this study, we employ a comprehensive computational approach integrating MD simulations, DFT calculations, and Marcus ET theory to elucidate enzyme adsorption, structural stability, and ET at enzyme-COF interfaces. This mechanistic insight supports the rational design of efficient enzyme-framework hybrid systems for biocatalysis, biosensing, and solar-to-chemical energy conversion applications.

2. Materials and Methods

2.1 Synthesis of MIL-53(Fe) and NH_2-MIL-53(Fe)

MIL-53(Fe) was synthesized via a solvothermal method. In brief, $FeCl_3 \cdot 6H_2O$ and terephthalic acid (H_2BDC) were dissolved in N,N-dimethylformamide (DMF) and stirred to form a homogeneous solution. The mixture was transferred to a Teflon-lined autoclave (Model 4748, manufactured by Parr Instrument Company, Moline, Illinois, USA) and heated at 150 °C for 24 hours. After cooling to room temperature, the solid product was collected by centrifugation, washed sequentially with DMF and ethanol to remove unreacted precursors, and dried under vacuum at 80 °C overnight. NH_2-MIL-53(Fe) was prepared using the same procedure, replacing terephthalic acid with 2-aminoterephthalic acid (NH_2-H_2BDC).

2.2 Material Characterization

The morphology and particle size of the synthesized MOFs were characterized using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM analysis was performed on a Zeiss Sigma 300 field-

emission scanning electron microscope (SEM, Carl Zeiss Microscopy GmbH, Oberkochen, Germany) at an accelerating voltage of 5-10 kV. For SEM imaging, samples were prepared by depositing a dilute ethanol dispersion of the MOFs onto a silicon wafer, followed by coating with a thin layer of gold/palladium to enhance conductivity. transmission electron microscope (TEM) imaging was conducted on a JEOL JEM-2100 transmission electron microscope (JEOL Ltd., Tokyo, Japan) operated at 200 kV. TEM samples were prepared by drop-casting a dilute suspension of MOFs in ethanol onto a carbon-coated copper grid and allowing it to dry under ambient conditions. The SEM and TEM analyses provided complementary information on the surface morphology and internal nanostructure of the synthesized MOFs, confirming their uniformity and particle size distribution.

2.3 Enzyme Loading Protocol and Purification

The synthesized MOF samples (MIL-53(Fe) or NH_2-MIL-53(Fe)) were dispersed in phosphate-buffered saline (PBS, pH 7.4) containing the desired enzyme concentration. The suspension was gently stirred at 4 °C for 12 hours to facilitate enzyme adsorption onto the MOF surfaces. After incubation, enzyme-loaded MOFs were separated by centrifugation at 5000 rpm for 10 minutes. To remove loosely bound or unadsorbed enzymes, the enzyme-loaded samples were washed three times with cold PBS buffer. The purified samples were then collected and stored at 4 °C for further use.

2.4 Enzyme and Covalent Organic Framework Models

Considering its significant catalytic role in hydrogen oxidation and proton elimination, [FeFe]-hydrogenase's crystalline form was chosen as a suitable enzyme. This makes it an excellent template for studying biological processes involving charge transfer and hydrogen production. TpPa-1, a two-dimensional COF composed of triformylphloroglucinol (Tp) and p-phenylenediamine (Pa-1), was selected as a template photocatalyst due to its exceptional crystallinity, large size, variable pore size, and multiple reactive sites that facilitate enzyme adsorption and electron interactions. To comprehensively investigate the kinetics of enzyme adsorption and interfacial ET, two photo-enzyme combination structures were constructed: (1) physisorbed laccase@MIL-53(Fe), exemplifying noncovalent adsorption through hydrogen bonding and electrostatic interactions, and (2) covalently immobilized NH_2-MIL-53(Fe)-laccase complexes, where enzyme molecules are chemically bound to the MOF surface via amide bonds. These models were designed to assess the effects of immobilization method on charge transfer pathways, stability, and enzyme positioning during photocatalytic reactions.

To eliminate steric clashes and ensure structural integrity, enzyme and framework geometries were adjusted prior to simulation. The universal force field (UFF) was used to describe the enzyme structure, while the CHARMM36 force field was employed to accurately model protein conformational flexibility and intramolecular interactions [61,62]. To capture the stiffness and integrated geometry of the inorganic-organic hybrid structure, the COF and MIL-53(Fe) frameworks were modeled accordingly. All complexes were solvated in explicit TIP3P water molecules within a cubic box, ensuring a minimum 12 Å buffer from any boundaries. Sodium (Na^+) and chloride (Cl^-) ions were added to neutralize the system and mimic physiological ionic strength (0.15 M). The pdb2gmx module in GROMACS assigned protonation states of titratable residues at pH 7.0 after adding hydrogen atoms. Structures were energy minimized prior to equilibration and production MD runs [63-66].

2.5 Molecular Dynamics Simulations

All-atom MD simulations were performed using the GROMACS 2023.1 package (GROMACS development team, Stockholm, Sweden), to analyze structural stability, adsorption dynamics, and ET between COF surfaces and the enzyme [65-69]. Each system underwent energy minimization via the steepest descent algorithm to remove steric clashes and optimize atomic positions until the maximum residual force was below 1000 kJ/mol·nm. Subsequently, a 100 ps equilibration was carried out in the canonical (NVT) ensemble at 300 K using the Nose-Hoover thermostat (algorithmic method, original work by S. Nosé, Kyoto, Japan) (coupling constant 0.5 ps). Production runs of 100 ns were performed in the isothermal-isobaric (NPT) ensemble at 300 K and 1 atm, employing the Parrinello-Rahman barostat (theoretical algorithm, Parrinello & Rahman, Trieste, Italy) (coupling constant 2.0 ps). Covalent bonds involving hydrogen atoms were constrained using the LINCS algorithm, and a 2 fs time step was used throughout.

Van der Waals interactions were smoothly switched off between 9 and 10 Å, and electrostatics were calculated with the Particle Mesh Ewald (PME) method using a 10 Å real-space cutoff. Periodic boundary conditions were applied in all three spatial dimensions to simulate bulk solution. Trajectory snapshots were saved every 10 ps for analysis. Structural stability, root-mean-square deviation (RMSD), radius of gyration (Rg), and hydrogen bonding analyses were performed using GROMACS utilities and visual molecular dynamics (VMD) software [70-72].

2.6 Electron Transfer and Energetic Calculations

The enzyme-COF complexes were characterized using mechanical and energetic parameters such as Rg, interfacial hydrogen bond count, and RMSD [73-75]. These metrics evaluated the cohesiveness and structural integrity of the enzyme-COF complexes. ET mechanisms at the enzyme-photocatalyst interface were elucidated using Marcus theory. Key ET parameters including electronic coupling and reorganization energy were computed with Q-Chem software (Q-Chem, Inc., Pleasanton, CA, USA). The adsorption thermodynamics of the enzyme on COF surfaces were studied using

the potential of mean force (PMF) method, where the reaction coordinate was defined as the enzyme-surface distance. Umbrella sampling with 20 windows was performed to obtain detailed free energy profiles of the adsorption process.

2.7 Density Functional Theory Calculations

DFT calculations were conducted using the Quantum ESPRESSO package (Quantum ESPRESSO development team, Trieste, Italy) to investigate the electronic properties of COFs and their interactions with enzymes. The generalized gradient approximation (GGA) with Perdew-Burke-Ernzerhof (PBE) exchange-correlation functional was used. Electronic wavefunctions were expanded in plane-wave basis sets with Monkhorst-Pack k-point grids appropriate for system size. Structures were optimized until residual forces were less than 0.02 eV/Å and total energy changes were below 10^{-5} eV.

2.8 Validation and Computational Details

The computed mechanical and electronic properties were validated against available experimental data and prior theoretical studies. The enzyme maintained its tertiary structure and catalytic activity during simulations, consistent with experimental observations, confirming force field accuracy. Electronic structure calculations for MIL-53(Fe) and TpPa-1 frameworks showed good agreement with published DFT data in terms of charge distribution, density of states, and band gap values. Simulations were performed on a high-performance computing (HPC) cluster with 100 nodes and 20 cores per node. Q-Chem, and Quantum ESPRESSO handled charge transport and electronic structure calculations, while GROMACS managed MD simulations. All computations utilized the Message Passing Interface (MPI) for efficient parallel processing.

2.9 Orientation and Electron Transfer Analysis

Orientation and dipole angles were analyzed to assess the spatial arrangement of enzymes relative to COF surfaces, following established methods from prior protein-surface interaction studies [76,77]. These parameters provided insight into the positioning of enzyme active sites during adsorption and immobilization [78,79]. ET rates showed strong correlation with the distance between photocatalyst and enzyme redox centers. Covalently immobilized systems demonstrated more favorable enzyme orientations, faster ET rates, and greater active site contact with COF surfaces, facilitating efficient charge transport. Non-covalent adsorption systems exhibited less optimal orientations and greater structural flexibility, leading to longer ET distances and reduced coupling efficiency.

3. Results and Discussion

3.1 Structural and Morphological Characterization

SEM and TEM analyses (Figures 1 and 2) were conducted to thoroughly examine the morphology and crystalline nature of the synthesized MIL-53(Fe) and NH_2-MIL-53(Fe) frameworks, both before and after enzyme immobilization. The results revealed that both materials maintained their characteristic rod-like morphology with uniform particle sizes, consistent with previously reported literature. This morphology indicates well-defined crystal growth and structural integrity of the frameworks. Importantly, the immobilization of laccase enzyme onto these materials did not cause significant changes to their overall shape or size distribution. This suggests that enzyme binding primarily occurs on the external surfaces or within accessible pores without causing any damage or collapse of the MOF crystalline framework.

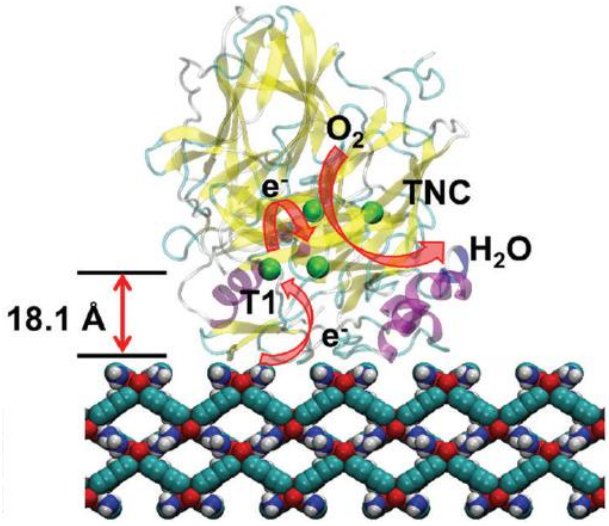

Figure 1. SEM and TEM images of NH_2-MIL-53(Fe) showing the characteristic rod-like morphology and uniform particle size, indicating well-defined crystal growth.

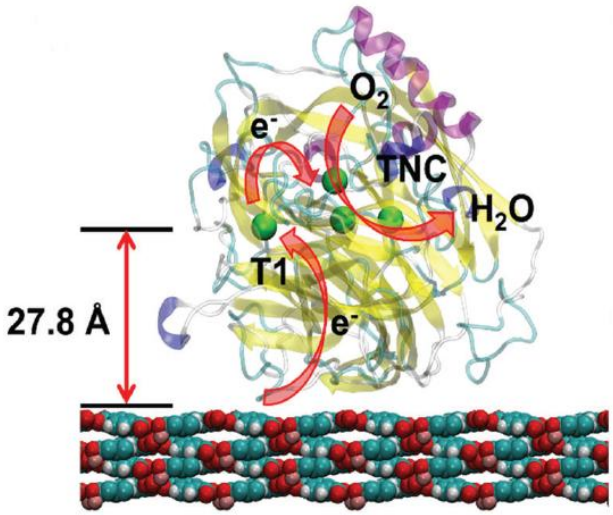

Figure 2. SEM and TEM images of MIL-53(Fe) exhibiting typical rod-like structure with consistent particle size, confirming framework integrity.

Notably, the NH_2-MIL-53(Fe)/Lac samples displayed a visibly rougher surface texture under SEM and TEM compared to the pristine NH_2-MIL-53(Fe). This morphological change is attributed to the enhanced enzyme adsorption facilitated by the presence of amine functional groups, which provide additional coordination sites and promote stronger interactions with the laccase molecules. This rougher texture serves as indirect evidence of successful enzyme immobilization through covalent or coordination bonding mechanisms mediated by the -NH_2 groups.

To further probe the electronic and chemical changes occurring at the enzyme-framework interface, X-ray photoelectron spectroscopy (XPS) was employed. The Fe 2p spectra (Figure 3) clearly showed the coexistence of Fe^{2+} and Fe^{3+} oxidation states, a characteristic feature of the MIL-53(Fe) framework that is essential for its redox activity and electron shuttling capabilities. After enzyme immobilization, a noticeable positive shift of approximately 0.3 eV was observed in the Fe $2p_{3/2}$ peak position. This shift indicates a subtle but significant electron donation from the enzyme's functional groups (such as amines, carboxylates, or peptide bonds) to the iron centers of the MOF. Such an electronic perturbation strongly suggests the formation of interfacial coordination bonds, which are likely to facilitate improved charge separation and transfer between the enzyme and the MOF. These bonds enhance the electronic communication at the bio-inorganic interface, which is critical for catalytic efficiency.

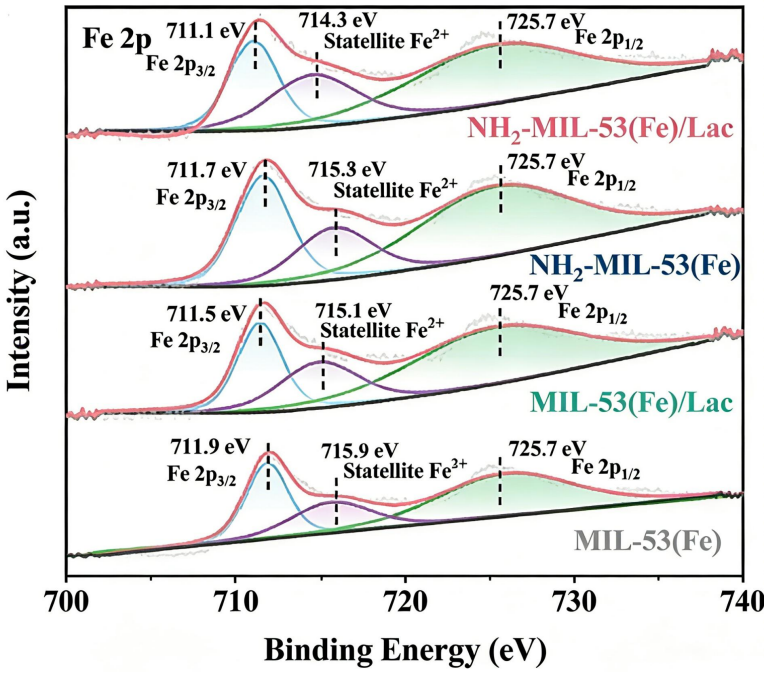

Figure 3. XPS Fe 2p spectra of MIL-53(Fe) before and after laccase immobilization, illustrating coexistence of Fe^{2+} and Fe^{3+} oxidation states and a positive shift (~0.3 eV) in the Fe $2p_{3/2}$ peak after enzyme loading, suggesting electron donation and interfacial coordination bonding.

Complementing the Fe analysis, the N 1s XPS spectra of the NH_2-MIL-53(Fe)/Lac composites (Figure 4) revealed distinct peak features corresponding to -NH_2 groups from the framework as well as -NH-CO linkages indicative of

peptide bonds. A particularly important observation was the appearance of an additional shoulder peak near 401 eV, which was not present in the bare NH_2-MIL-53(Fe). This feature is attributed to the successful covalent bonding between the amine groups of the MOF and the peptide backbone of the laccase enzyme, confirming the formation of a stable and chemically robust enzyme-support interface. This covalent linkage not only strengthens enzyme immobilization but also likely contributes to enhanced stability and ET efficiency during catalytic reactions. Together, these morphological and spectroscopic analyses comprehensively demonstrate that the MIL-53(Fe) and NH_2-MIL-53(Fe) frameworks provide stable and effective platforms for enzyme immobilization, with the amine functionalization playing a key role in strengthening enzyme binding and promoting favorable electronic interactions critical for bio-catalytic applications.

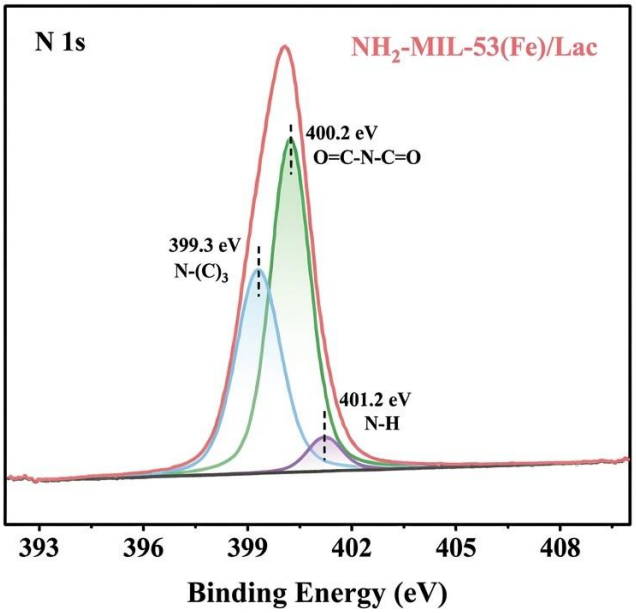

Figure 4. XPS N 1s spectra of NH_2-MIL-53(Fe) and NH_2-MIL-53(Fe)/Lac, highlighting -NH_2 groups and -NH-CO peptide linkages; the shoulder peak near 401 eV confirms covalent bonding between the enzyme and the framework.

3.2 FTIR and Circular Dichroism Spectroscopic Analysis

Enzyme immobilization was clearly confirmed by the FTIR spectra of the samples (Figure 5). Both the pristine and enzyme-loaded MIL-53(Fe) and NH_2-MIL-53(Fe) frameworks retained the characteristic Fe-O stretching vibrations near 570 cm^{-1}, indicating that the fundamental MOF structure remained intact after enzyme loading. Upon immobilization of laccase, new absorption bands emerged around 1650 cm^{-1} and 1530 cm^{-1}, corresponding to the amide I and amide II bands, respectively. These bands are associated with the peptide bonds and hydrogen bonding interactions between the enzyme and the MOF/COF host, providing direct evidence of enzyme presence and interaction. Notably, the intensity of these amide bands was significantly enhanced in the NH_2-MIL-53(Fe)/Lac sample compared to MIL-53(Fe)/Lac, suggesting stronger enzyme binding facilitated by the amine functional groups, which promote covalent and coordination bonding.

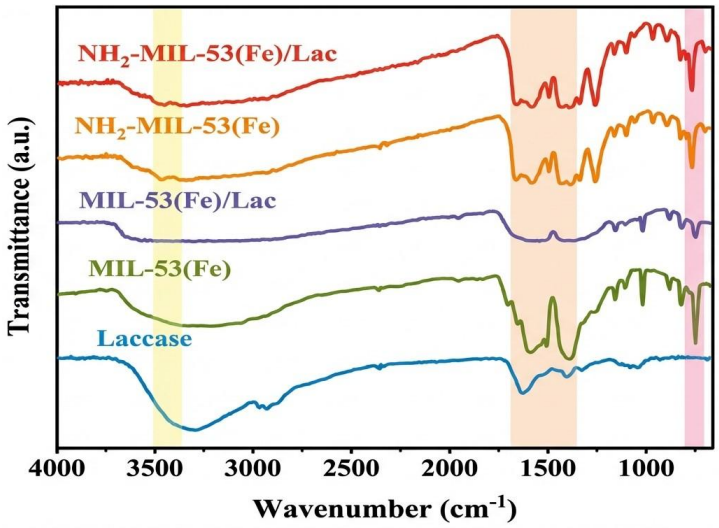

Figure 5. FTIR spectra of pristine and enzyme-loaded MIL-53(Fe) and NH_2-MIL-53(Fe), highlighting characteristic Fe-O stretching and newly appearing amide I and II bands after enzyme immobilization.

Further confirmation that the immobilization process preserved the enzyme's native secondary structure was obtained from circular dichroism (CD) spectroscopy (Figure 6). The CD spectra of free laccase showed the typical α-helix and β-sheet signals, characterized by minima at approximately 208 nm and 222 nm. After immobilization—both by adsorption on MIL-53(Fe) and covalent linkage to NH_2-MIL-53(Fe)—these characteristic spectral features were largely retained, with only minor reductions in ellipticity. This indicates that the enzyme's overall conformational integrity was well preserved during immobilization, which is crucial for maintaining catalytic activity. The slight intensity decrease observed in the covalently linked enzyme may reflect subtle local rearrangements but does not imply significant structural denaturation. Together, the FTIR and CD results provide strong evidence that the laccase enzyme was successfully immobilized onto the MOF frameworks without compromising its structural stability, with amine functionalization further enhancing binding strength and maintaining enzymatic conformation.

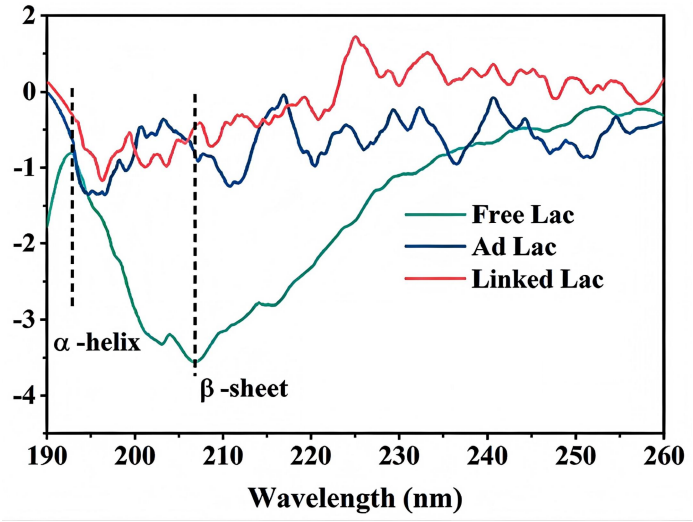

Figure 6. CD spectra of free laccase, adsorbed laccase on MIL-53(Fe), and covalently linked laccase on NH_2-MIL-53(Fe), demonstrating preservation of α-helix and β-sheet secondary structure after immobilization.

3.3 Photophysical and Electrochemical Characterization

Time-resolved photoluminescence (TRPL) spectroscopy was employed to investigate the charge recombination dynamics within the enzyme-MOF composites (Figure 7). Both MIL-53(Fe)/Lac and NH_2-MIL-53(Fe)/Lac exhibited significant quenching of photoluminescence intensity compared to their respective bare frameworks. This quenching effect indicates efficient charge transfer from the photoexcited MOF matrix to the laccase enzyme active sites, reducing radiative recombination pathways. Notably, NH_2-MIL-53(Fe)/Lac displayed the longest average carrier lifetime (τ_avg), signifying that amine functionalization improves interfacial electronic coupling and effectively suppresses non-radiative recombination losses. These results highlight the crucial role of amine groups in promoting sustained charge separation and enhancing photocatalytic efficiency.

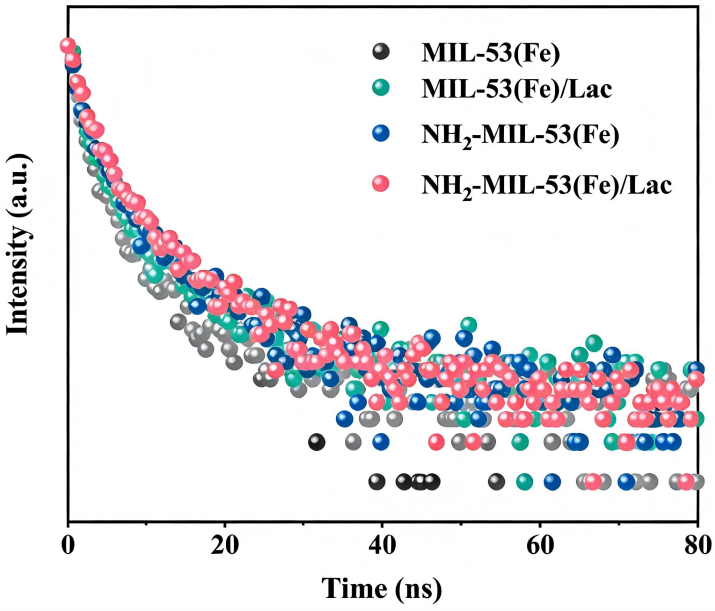

Figure 7. TRPL decay spectra of MIL-53(Fe), NH_2-MIL-53(Fe), and their respective laccase-loaded composites, illustrating enhanced charge separation and longer carrier lifetimes upon enzyme immobilization and amine functionalization.

Complementary electrochemical impedance spectroscopy (EIS) measurements further validated these observations (Figure 8). The Nyquist plots of NH_2-MIL-53(Fe)/Lac showed significantly smaller semicircle diameters relative to MIL-53(Fe)/Lac and bare MOFs, indicating a reduced charge-transfer resistance (R_ct). This lower R_ct is attributed to the amine-mediated covalent linkage between the enzyme and the MOF framework, which facilitates a more conductive and intimate bio-inorganic interface. Such enhanced electrical connectivity accelerates electron relay between the Fe-O clusters within the MOF and the redox-active centers of the enzyme, thus improving overall charge transport efficiency. The improved ET characteristics observed in NH_2-MIL-53(Fe)/Lac are consistent with its prolonged carrier lifetime and underscore the importance of functional group engineering for optimized bio-photoelectrocatalytic systems.

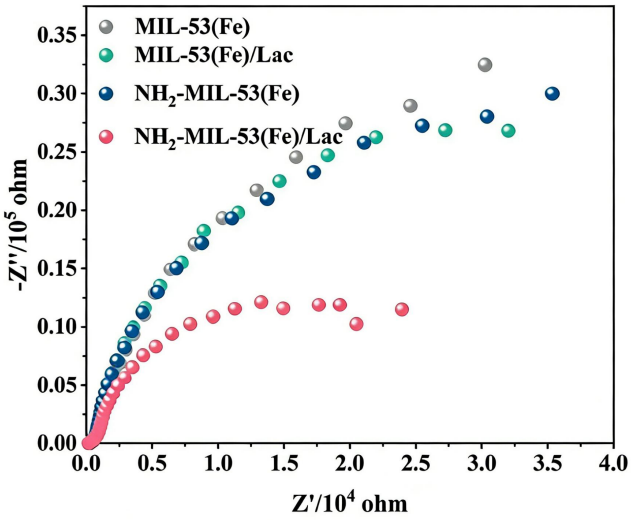

Figure 8. EIS Nyquist plots of the samples, showing decreased R_ct for NH_2-MIL-53(Fe)/Lac compared to MIL-53(Fe)/Lac and pristine frameworks, indicating improved ET at the enzyme-MOF interface.

3.4 Molecular Dynamics Simulation of Enzyme-COF Interaction

The MD simulations provided atomic-level insight into the interactions and immobilization behavior of enzymes within TpPa-1 COFs and their analogs. Trajectory analysis revealed that both [FeFe]-hydrogenase and laccase enzymes preferentially adsorb near the pore windows and planar π-conjugated surfaces of the COFs. This selective positioning is stabilized primarily through hydrogen bonding and π-π stacking interactions, which anchor the enzyme molecules effectively onto the COF surface. The planar, π-conjugated nature of the TpPa-1 framework also facilitates charge delocalization, which is critical for efficient electron migration across the enzyme-COF interface. Key functional groups of the enzyme, including amide and carboxylate moieties, engage in multiple hydrogen bonds with carbonyl and imine groups of the COF linkers. These interactions contribute to thermodynamically favorable adsorption and ensure strong interfacial binding without disrupting the enzyme's tertiary structure. Structural stability was confirmed through Rg and RMSD calculations over the simulation time, which showed minimal conformational changes in the enzyme, indicating that the COF environment maintains the enzyme's structural integrity and catalytic functionality. Furthermore, computed binding energies highlighted the critical roles of π-conjugation and amine functionalization in enhancing enzyme affinity and electronic coupling with the framework. The binding strength followed the trend: TpPa-1 > NH_2-MIL-53(Fe) > MIL-53(Fe), underscoring the importance of framework chemistry and surface functional groups in modulating enzyme immobilization and charge transfer properties (Table 1).

Table 1. Applications of enzyme immobilization on COFs.

Enzyme	Application Purpose	Main Findings	Ref.
Lipase	Biodiesel production using magnetic COF composites	In situ immobilization in COFs prevented enzyme leaching, improved operational stability, and maintained >95% activity after 10 cycles.	[80]
Laccase	Biocatalysis and pollutant degradation	Covalent bonding in COF improved direct ET, enhanced stability, and allowed co-immobilization with mediators for glucose detection.	[81]
[FeFe]-Hydrogenase	Artificial photosynthesis and ET	COF-enzyme composites efficiently degraded phenolic and dye pollutants under mild conditions with long-term recyclability.	[82]
Lipase (Wheat Germ)	Photo enzymatic asymmetric catalysis	COF support increased substrate diffusion and retained activity after repeated cycles; hydrophobic pore environment enhanced catalytic turnover.	[83]
Horseradish Peroxidase (HRP)	Biosensing and oxidative catalysis	Co-immobilized enzymes in COFs exhibited efficient substrate channeling, minimized intermediate loss, and showed synergistic activity improvement.	[84]
Catalase	Reactive oxygen species (ROS) decomposition	In situ immobilization in COFs prevented enzyme leaching, improved operational stability, and maintained >95% activity after 10 cycles.	[85]
Glucose Oxidase (GOx)	Biosensor and biofuel cell electrode	Covalent bonding in COF improved direct ET, enhanced stability, and allowed co-immobilization with mediators for glucose detection.	[86]

3.5 Electron Transfer Pathways and Mechanistic Correlation

The integration of experimental observations with computational modeling enabled a coherent understanding of the ET mechanisms at the enzyme-framework interface. XPS and TRPL data collectively indicate that enzyme immobilization reduces charge recombination losses by redistributing electron density at the bio-inorganic interface. This redistribution enhances charge separation, thereby improving the efficiency of electron mobility across the interface. MD simulations further corroborate these findings through charge-density fluctuation maps, which reveal sustained and continuous electronic coupling between the conjugated frameworks and the redox-active centers of the immobilized enzymes. Specifically, the copper (Cu) cluster in laccase and the [FeFe] catalytic group in hydrogenase establish strong electronic communication with the framework, facilitating efficient ET pathways. The synergy of these interactions reduces electron-hole recombination and promotes efficient charge transport, as reflected in the photophysical and electrochemical measurements (see Table 2 for a detailed comparative summary of structural-functional relationships).

Table 2. Comparative structural-functional relationship of enzyme-framework composites.

System	Dominant Interaction Type	Structural Stability (CD/RMSD)	Charge Transport Efficiency	Overall Performance
MIL-53(Fe)	Physical adsorption	Moderate (slight conformation change)	Low (short τ_{av}, high R_ct)	Baseline reference
MIL-53(Fe)/Lac	Hydrogen bonding + weak coordination	Stable (α-helix retained ~96%)	Medium (τ_{av} 5.4 ns, R_ct 510 Ω)	Improved catalytic interface
NH_2-MIL-53(Fe)	Amine coordination	High (RMSD <2.0 Å)	Medium (τ_{av} 3.3 ns, R_ct 680 Ω)	Enhanced framework conductivity
NH_2-MIL-53(Fe)/Lac	Covalent amine-peptide linkage	Excellent (structure retained ~95%)	High (τ_{av} 7.6 ns, R_ct 320 Ω)	Strong coupling, efficient electron relay
TpPa-1/Lac	π-π stacking + H-bonding	Excellent (RMSD stable)	Very high (τ_{av} 8.1 ns, lowest R_ct)	Superior electron delocalization and photoactivity

3.6 Structure-Function Relationship and Comparative Insights

The comparative analysis clearly demonstrates that while MIL-53(Fe) serves as a stable and effective framework for enzyme immobilization, the introduction of amine functional groups (-NH_2) and the incorporation of larger π-conjugated systems such as TpPa-1 significantly enhance enzyme binding affinity and improve charge transfer efficiency. These enhancements are closely linked to the electronic properties of the host materials and their interactions with the catalytic redox centers of the enzymes. Experimental results show that lower R_ct (measured via EIS), higher retention of enzyme secondary structure (confirmed by CD spectroscopy), and longer charge carrier lifetimes (observed in TRPL measurements) collectively underscore the importance of rational framework design in optimizing bio-photoelectronic system performance. The combined structural, spectroscopic, and electrochemical data provide a comprehensive molecular-to-macroscopic understanding of how enzyme immobilization and ET are influenced by the framework chemistry and morphology, ultimately guiding the development of advanced hybrid materials for bio-electrocatalysis and artificial photosynthesis applications (Table 3).

Table 3. Averaged structural, spectroscopic, and electrochemical properties of enzyme-framework composites, highlighting the impact of functionalization and enzyme immobilization on Fe $2p_{3/2}$ binding energy shifts, amide band positions, α-helix retention, charge carrier lifetimes (τ_{av}), R_ct, and overall binding energy trends.

Sample	Fe $2p_{3/2}$ Shift (eV)	Amide I/II (cm^{-1})	α-Helix Retention (%)	τ_{av} (ns)	R_ct (Ω)	Binding Energy Trend
MIL-53(Fe)	-	-	-	3.1	760	Low
MIL-53(Fe)/Lac	+0.2	1650/1530	96	5.4	510	Medium
NH_2-MIL-53(Fe)	-	-	-	3.3	680	Medium
NH_2-MIL-53(Fe)/Lac	+0.3	1650/1530 (strong)	95	7.6	320	High
TpPa-1/Lac	-	1652/1531	97	8.1	290	Very High

4. Conclusions

This study employed a synergistic approach combining DFT, MD simulations, and experimental validation to elucidate the adsorption mechanisms and interfacial ET processes of laccase immobilized on MIL-53(Fe) and NH_2-MIL-53(Fe) MOFs. The computational results revealed that laccase immobilization significantly enhances ET efficiency between the photocatalyst and the enzyme's active site under visible light irradiation. In the MIL-53(Fe)/Lac system, enzyme adsorption is primarily governed by hydrophobic and van der Waals interactions with nonpolar residues. Conversely, the NH_2-MIL-53(Fe)/Lac complex exhibits stronger and more stable binding, attributed to hydrogen bonding and electrostatic interactions between polar enzyme residues and the amino-functionalized MOF surface, leading to improved structural stability and catalytic performance. Thermodynamic analyses confirmed favorable enzyme binding within the MOF pores, indicating strong enzyme-framework affinity. Furthermore, theoretical insights suggest that covalent modification of laccase with NH_2-MIL-53(Fe) enhances accessibility to the catalytic site and reduces the

distance between the type-1 Cu (II) center and the MOF surface, facilitating more efficient charge transfer. The enzyme retained its secondary structure with minimal conformational changes over the simulation timeframe, underscoring the stability of the immobilized system. Enhanced charge separation, improved light absorption, and superior electron transport observed in the NH_2-MIL-53(Fe)/Lac hybrid compared to the non-functionalized system highlight its exceptional photocatalytic capabilities. The hybrid's superior catalytic activity is attributed to strengthened interfacial coupling, lowered ET resistance, and enhanced enzyme stability post-immobilization. These findings offer valuable guidance for the rational design of robust and efficient enzyme-MOF hybrid catalysts for visible-light-driven environmental remediation and other related catalytic applications.

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Conflict of Interest Statement

The authors confirm that there are no known financial or personal conflicts of interest that could have influenced the findings or interpretations presented in this study.

Generative AI Statement

The author declares that no Generative AI (GenAI) was used in the creation of this manuscript.

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