



Exploring the role of oxidative defects in polyethylene binding and degradation by a *Rhodococcus opacus* R7 multicopper oxidase: a computational perspective

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Abstract

The enzymatic degradation of polyethylene (PE) by oxidative enzymes remains poorly understood at the molecular level, limiting the development of effective biotechnological strategies for plastic valorization. Here, we investigate the molecular basis of PE oxidation by the laccase-like multicopper oxidase LMCO2 from *Rhodococcus opacus* R7 with combined DFT and molecular docking calculations. LMCO2 is a particularly relevant model system because it has been experimentally shown to oxidatively modify low-density polyethylene without the need for mediators, despite its relatively low redox potential. DFT calculations indicate that direct oxidation of aliphatic C–H bonds in pristine PE is energetically prohibitive, and that hydroxylated defects do not significantly facilitate substrate activation. In contrast, oxidation at carbon atoms adjacent to carbonyl groups is associated with substantially lower barriers, owing to stabilization of the resulting radicals through conjugation and keto–enol tautomerism. Alternative pathways involving alkoxyl radicals and β -scission were also examined and found to be energetically unfavorable. Molecular docking calculations complement this picture by showing that both pristine and pre-oxidized PE segments can access the main cavity adjacent to the T1 copper site, although binding is weak and largely non-specific. Taken together, these results support a mechanism in which LMCO2 selectively acts on pre-oxidized, carbonyl-containing regions of polyethylene, promoting further radical chemistry that may ultimately lead to chain scission and the formation of low-molecular-weight oxygenated products.

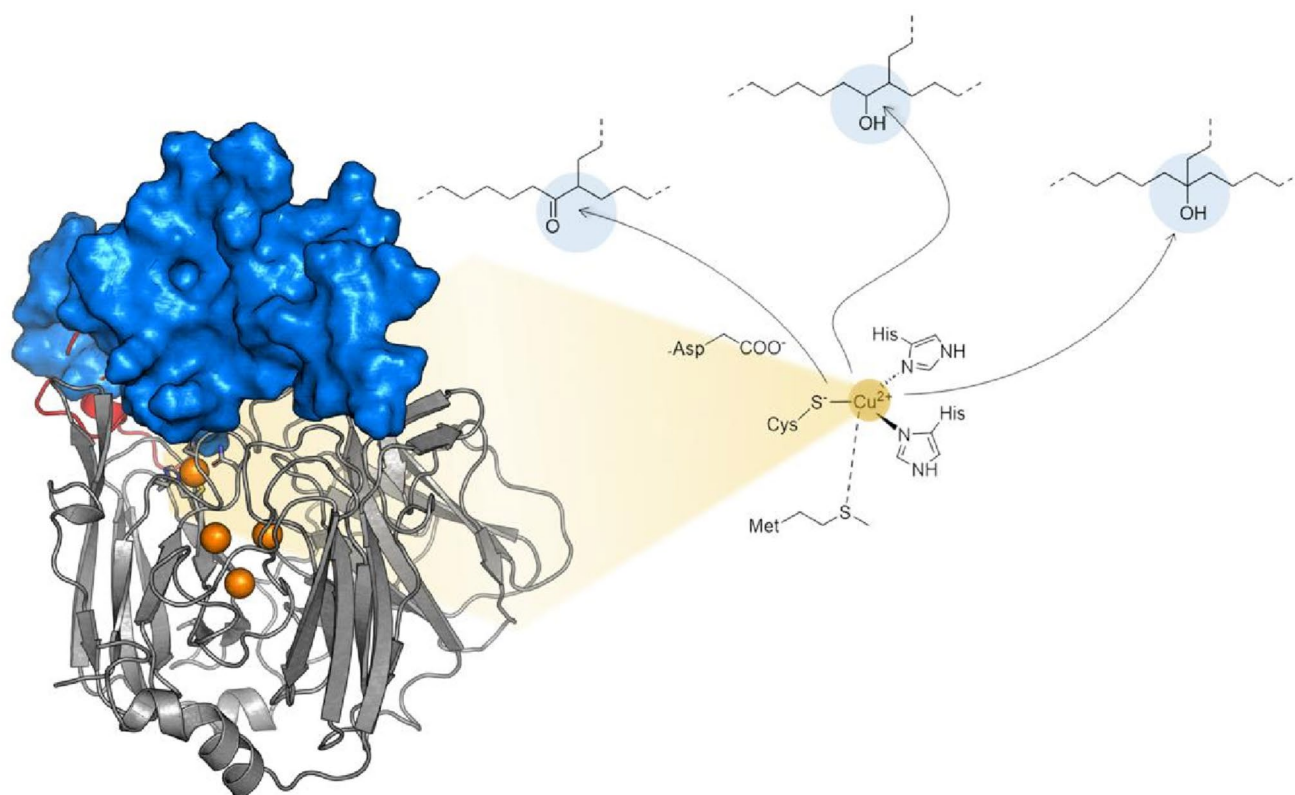
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Graphical Abstract



Keywords Bacterial Laccase · Polyethylene Degradation · Density Functional Theory · Molecular Docking

Abbreviations

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
COSMO	Conductor-like Screening Model
DFT	Density Functional Theory
FT-IR	Fourier-Transform Infrared spectroscopy
GC-MS	Gas Chromatography–Mass Spectrometry
GGA	Generalized Gradient Approximation
LDPE	Low-Density Polyethylene
LMCO2	Laccase-like Multicopper Oxidase 2
MCOs	Multicopper Oxidases
MD	Molecular Dynamics
NBO	Natural Bond Orbital
PBE	Perdew-Burke-Ernzerhof (functional)
PCET	Proton-Coupled Electron Transfer
PE	Polyethylene
PES	Potential Energy Surface
QM	Quantum Mechanical
RI	Resolution of Identity
TSs	Transition States

Introduction

The accumulation of PE in the environment has prompted significant efforts to develop biological strategies for its degradation [1, 2]. A growing body of literature reports that bacteria and fungi are capable of modifying or degrading LDPE, typically through complex enzymatic systems involving oxidative enzymes such as multicopper oxidases (MCOs), cytochrome P450s [2, 3], and peroxidases [4, 5].

As summarized in Table 1, numerous studies over the past decade have described the heterologous expression and characterization of bacterial and fungal laccases tested for polyethylene oxidation. In these works, enzymatic treatment is generally associated with the formation of low-molecular-weight oxygenated compounds (e.g., alcohols, ketones, and carboxylic acids) detected by GC-MS, along with increased carbonyl signals in FT-IR spectra [6–8]. While these observations are often interpreted as evidence of polymer degradation, they do not provide a clear molecular picture of the underlying reaction mechanisms.

Recent studies have raised important questions regarding the origin of these products and the actual role of enzymes in PE degradation. In particular, it has been suggested that

Table 1 List of publications (from 2013 onward) reporting the recombinant expression of bacterial or fungal laccases and their subsequent evaluation for polyethylene oxidation

Reference	organism	year	Organism
[31]	Bacterial	2013	<i>Rhodococcus ruber</i> C208
[32]	Fungal	2022	<i>Alternaria alternata</i> FB1 (marine fungal)
[33]	Bacterial	2022	Psychrophile <i>Psychrobacter</i> sp. NJ228
[16]	Bacterial	2022	<i>Bacillus subtilis</i>
[16]	Fungal	2022	<i>Botrytis aclada</i>
[34]	Bacterial	2023	<i>Lysinibacillus fusiformis</i>
[35]	Fungal	2023	<i>Trichoderma harzianum</i>
[36]	Fungal	2023	<i>Phanerochaete chrysosporium</i>
[37]	Bacterial	2023	<i>Klebsiella pneumoniae</i> Mk-1
[9, 10]	Bacterial	2023–2025	<i>Rhodococcus ruber</i>
[24]	Bacterial	2023	<i>Rhodococcus opacus</i> R7
[38]	Bacterial	2023	<i>Bacillus cereus</i> O2-B and <i>Pseudomonas aeruginosa</i> O1-P
[39]	Bacterial	2023	<i>Rhodococcus</i> strain A34
[40]	Bacterial	2024	Ancestral sequence reconstruction
[41]	Bacterial	2024	<i>Acinetobacter</i> and <i>Pseudomonas</i>
[42]	Bacterial	2024	<i>Pseudalkali bacillus</i> sp. MQ-1
[43]	Fungal	2024	<i>Pleurotus ostreatus</i>
[44]	Fungal	2024–2025	<i>Myceliophthora thermophila</i>
[45]	Bacterial	2025	<i>Morganella morganii</i> PQ533186
[46]	Bacterial	2025	<i>Acinetobacter dijkshoorniae</i> PE-9,
[47, 48]	Bacterial	2024–2025	Cold adapted laccase PsLAC
[49]	Bacterial	2025	<i>Amycolatopsis</i> sp. ATCC 39,116
[50]	Bacterial	2025	<i>Pantoea brenneri</i> CYD50–1 Ftsp
[51]	Fungal	2025	<i>Pichia pastoris</i>
[52]	Bacterial	2025	<i>Parageobacillus caldoxylosilyticus</i>
[53]	Bacterial	2025	<i>Klebsiella variicola</i>
[54]	Bacterial	2025	<i>Paenarthrobacter</i> sp. PE0901
[55]	Fungal	2025	<i>Kluyveromyces lactis</i>
[56, 57]	Fungal	2025	<i>Yarrowia lipolytica</i>
[58]	Bacterial	2025	<i>Gordonia</i> sp. C1 and <i>Bacillus</i> sp. C2
[59]	Bacterial	2025	<i>Ancylobacter</i> sp. PD-4
[60]	Bacterial	2025	<i>Micrococcus</i> sp. IITD107 (*)
[61]	Bacterial	2026	<i>Bacillus</i> sp. AS3 and <i>Sphingobacterium</i> sp. AS8
[62]	Bacterial	2026	<i>Acinetobacter</i> sp. PVC-6 A and <i>Bacillus</i> sp. PVC-6B (**)
[63, 64]	Fungal	2026	<i>Trametes versicolor</i>
[65]	Bacterial	2026	<i>Lysinibacillus fusiformis</i> Tm-07
[66]	Bacterial	2026	<i>Bacillus velezensis</i>
[67]	Bacterial	2026	<i>Paenibacillus polyethylenolyticus</i> JNU01

(*) tested on polypropylene (**) tested on polyvinyl chloride

microbial growth may result from the consumption of pre-existing medium- and long-chain alkanes or oxidized fragments, rather than from direct enzymatic attack on the polymer backbone [9]. Moreover, measured mineralization rates are low [10], and in some cases previously reported degradation activities could not be reproduced [11]. Nevertheless, in a very recently published study, ^{13}C -labelled PE tracing experiments demonstrated that a closely related *Rhodococcus* strain can assimilate PE-derived carbon into biomass and mineralize it to CO_2 , providing direct evidence that PE can serve as a carbon and energy source for bacteria of this genus [12]. These findings highlight a gap between experimental observations and mechanistic understanding.

A key factor that may help reconcile these discrepancies is the role of pre-oxidation. PE exposed to environmental conditions such as UV radiation, thermal stress, or mechanical aging [6, 13–15] is known to accumulate oxygen-containing functional groups, including alcohols and carbonyls [3, 16]. These defects are well established as reactive sites in abiotic degradation processes and are expected to significantly alter both the chemical reactivity and the physical properties of the polymer. However, their influence on enzyme–substrate recognition and catalytic activity in laccases remains largely unexplored. To date, no atomistic mechanistic framework has been proposed to explain how laccases may oxidize pristine or pre-oxidized polyethylene.

Laccases [17] are MCOs that catalyze the one-electron oxidation of substrates at a T1 copper site, coupled to the four-electron reduction of O_2 to water at a trinuclear T2/T3 copper cluster [18, 19]. In bacterial laccases, this T1 copper center is coordinated by two histidine (His) imidazoles, a cysteine (Cys), and a weak axial methionine (Met) ligand. Despite sharing very similar structural scaffolds, these enzymes exhibit a broad spectrum of redox potentials versus the normal hydrogen electrode (NHE), ranging from +400 to +800 mV. This variation is strongly influenced by the nature of the axial ligand of the T1 Cu site, with bacterial MCOs typically bearing a Met ligand and exhibiting lower redox potentials, whereas fungal high-redox-potential laccases generally contain Phe or Leu at this position.

Regarding the catalytic cycle, the mono-electronic oxidation of the substrate at the T1 site constitutes the rate-determining step of the overall enzymatic mechanism. While their catalytic mechanism is well understood for phenolic substrates [20–22], their activity toward non-phenolic and aliphatic systems such as polyethylene is far less clear. In particular, the relatively low redox potential of bacterial laccases raises doubts about their ability to directly activate strong aliphatic C–H bonds.

In this context, the bacterium *Rhodococcus opacus* R7 is particularly interesting because its genome encodes several distinct laccase-like multicopper oxidases (LMCOs) [23,

24]. Transcriptomic and subsequent time-resolved expression studies indicate that several LMCOs are induced when *R. opacus* R7 grows on PE as the sole carbon and energy source, with distinct temporal expression profiles [24]. Among these enzymes, LMCO2 has been experimentally shown to oxidatively modify LDPE in vitro without the need for redox mediators [24]. Previous molecular dynamics (MD) simulations [25] further showed that LMCO2 can form stable complexes with polyethylene through interactions involving a methionine-rich loop [26–28], suggesting a structural basis for substrate interaction despite the chemically inert nature of the polymer. In our previous study [25], the standard reduction potential of the Cu-T1 site of LMCO2 was determined to be $E^\circ = +413$ mV, placing this enzyme within the low-to-medium redox-potential range typically associated with bacterial laccases.

Notably, methionine-rich regions have also been implicated in copper recruitment and additional oxidative activity in other bacterial multicopper oxidases such as those found in lactic acid bacteria [29] and in EcCueO [30]. These precedents suggest that the methionine-rich loop mediating LMCO2–PE binding may not be a purely structural anchor, but could also contribute to copper recruitment or additional oxidative capacity relevant to substrate activation.

Building on these considerations, in this work we focus on two key questions: (i) through which energetically accessible molecular routes LMCO2 may promote polyethylene oxidation, particularly as a function of increasing degrees of pre-oxidation; and (ii) in what ways oxidative defects reshape substrate recognition and binding, in terms of binding affinity and preferred interaction modes along the polymer chain.

To answer the first question, we employ Density Functional Theory (DFT) calculations on representative cluster models of the catalytic site to explore possible reaction pathways and identify energetically viable mechanisms. To address the second, we extend previous modeling studies by performing molecular docking simulations on both pristine and oxidized polyethylene models of comparable size, combined with a detailed analysis of enzyme cavities and binding modes.

Materials and methods

Density functional theory computations

DFT calculations have been performed with the TURBO-MOLE 7.4 suite [68]. Geometry optimizations were performed using the pure GGA PBE [69] and hybrid PBE0 [70] functionals. The RI technique [71] was adopted to speed up calculations for PBE computations. Basis sets of triple- ζ

plus polarization split valence quality [72] (def-TZVP) were adopted for all atoms. Solvent effect is accounted for by using the Conductor-like Screening Model (COSMO) approach setting the dielectric constant set to 4. This computational setting provides groundstate geometry parameters in good agreement with experimental X-ray values [73]. Charge distribution was evaluated using Natural Bond Orbital (NBO) analysis. Geometry optimizations were carried out with convergence criteria fixed to 10^{-6} Hartree for the energy and 0.001 Hartree·bohr $^{-1}$ for the gradient norm vector. D3 Grimme empirical dispersion correction [74] was adopted. This computational protocol has been successfully applied in previous DFT investigations carried out in our laboratory on various bioinorganic copper systems [75–77] including benzo[a]pyrene and anthracene oxidation by *Trametes versicolor* laccase [78].

Molecular docking and cavity detection

The starting structure of the LMCO2–polyethylene (PE) complex was derived from previous molecular dynamics simulations [25], by selecting the most populated binding mode of a branched C₁₀₀H₂₀₂ alkane at the T1 copper site where the ligand relocates from the initial docking pose and adopts a stable Met-loop-anchored binding mode. Potential binding pockets were identified by cavity detection using Cavitomix plugin in PyMOL (see SI for details) on the apo enzyme using a grid-based approach, and the resulting cavities were compared with the MD-derived PE binding region. To investigate the effect of polymer oxidation, a series of oxidized PE models with different hydroxylation and carbonylation patterns were generated (namely mono- and penta-hydroxylated species C₁₀₀H₂₀₁OH and C₁₀₀H₁₉₇(OH)₅, alongside mono- and penta-carbonyl versions C₁₀₀H₂₀₀O and C₁₀₀H₁₉₂O₅, together with the redox mediator 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as a reference substrate.

Molecular docking calculations were performed using two receptor setups: a “dry” receptor and a “hydrated” receptor in which structural water molecules identified in MD simulations were retained. Ligands were treated as flexible, whereas the protein (and structural waters, when present) was kept rigid. Docking was carried out using AutoDock Vina [79] with a search space centred on the PE binding region. The best-scoring poses were analysed to assess whether all ligands bound in a similar region of the T1-proximal pocket, and characterize their main interactions. Further details on system preparation, cavity detection parameters, docking protocols and poses are provided in the Supplementary Materials.

Results and discussion

Mechanistic Insights from DFT Calculations

QM-cluster model

To investigate the molecular mechanism of polyethylene oxidation by LMCO2, we constructed a cluster model [80] derived from a representative frame of the MD simulation of the LMCO2/C₁₀₀H₂₀₂ complex reported in ref [25]. (Fig. 1A). The model comprises the T1 copper site coordinated by His⁵⁰², His⁴⁴⁹, Cys⁴⁹⁷, and Met⁴⁴⁷, together with a C₁₄ fragment of the substrate (6-ethyldodecane) positioned in proximity to the metal center. To allow proton transfer processes between protein and substrate [81], the side chain of

Asp²⁵⁶ was included, along with five explicit water molecules placed between the substrate and the aspartate residue. This water network is expected to mediate proton transfer and to actively participate in the oxidation mechanism, acting as a source of oxygen atoms for C–OH bond formation. This modeling choice is supported by previous DFT studies on laccase-catalyzed oxidation reactions, where structured water molecules enable nucleophilic attack on carbon-centered radicals, leading to oxygen incorporation [82]. The total charge of the model is zero. To probe the effect of substrate pre-oxidation, we considered a series of C₁₄ model compounds representative of partially oxidized polyethylene, namely 6-ethyldodecane, 7-ethyldodecan-6-ol, 6-ethyldodecan-6-ol and 7-ethyldodecan-6-one (Fig. 1B). The optimized geometries of the corresponding

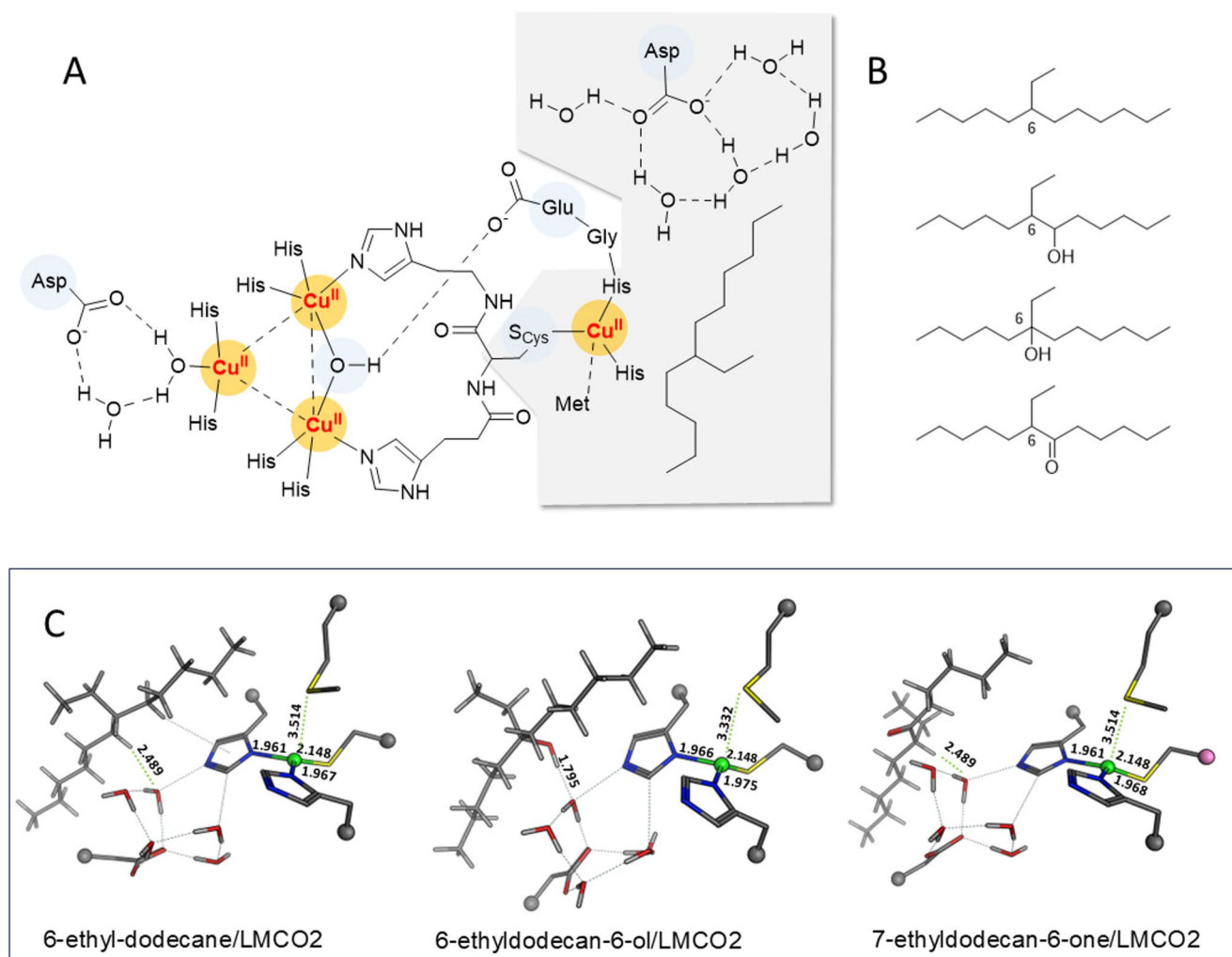


Fig. 1 (A) Structure of the LMCO2 catalytic site obtained from molecular dynamics simulations (Orlando et al., 2025), highlighting the four Cu²⁺ ions and their coordination environment. The region shown in gray represents the model employed in the DFT calculations, which includes the T1 Cu²⁺ site, a model ligand mimicking defect-free LDPE positioned near an aspartate side chain, and associated water molecules to account for proton transfer processes. Positive charges

are highlighted in light orange and negative charges in light blue. (B) Model compounds used to represent LDPE segments. (C) Optimized QM-cluster structures at the DFT/PBE0 level, with selected interatomic distances reported in Å. Spherical atoms denote the C α carbons of residues that were constrained to their initial positions from the molecular dynamics frame during geometry optimization

enzyme–substrate adducts (Fig. 1C and Table S2a) are very similar at both the PBE and PBE0 levels of theory. The main structural variability is associated with the axial methionine ligand, which displays moderate flexibility (Cu–S distance standard deviation ≈ 0.13 Å). Overall, the computed geometries are consistent with those reported for T1 copper sites in bacterial laccases.

C–H bond activation

The initial step in PE degradation is expected to involve oxidation of a C–H bond, leading to the formation of a carbon-centered radical capable of propagating radical-driven processes [83]. To evaluate the feasibility of this step, we explored the one-electron oxidation of C–H bonds in the different substrates by scanning the PES along the hydrogen abstraction coordinate, which proceeds from C–H bond cleavage toward a nearby water molecule, followed by proton transfer through the hydrogen-bonded water network until reaching the adjacent Asp²⁵⁶.

For each substrate, both secondary and tertiary radical formation pathways were considered, resulting in a total of six reaction channels. PES scans were carried out at the PBE level by progressively elongating the target C–H bond, with full geometry optimization at each point. Stationary points identified along the reaction pathways, including TSs and product-like structures, were subsequently refined at the PBE0 level (Figure S3 and Table S2b).

In all cases, the TSs are located late along the reaction coordinate and display a strong product-like character. The C–H bond is significantly elongated (≈ 1.7 Å), while the transferring hydrogen atom is positioned close to one of the water molecules bridging the substrate and Asp²⁵⁶. In the TS structures, Asp²⁵⁶ is protonated, consistent with a proton-coupled electron transfer (PCET) mechanism.

Spin density analysis (Table S3) reveals a progressive transfer of unpaired electron density from the Cu²⁺ center and the cysteine sulfur toward the substrate carbon undergoing oxidation. This effect becomes more pronounced at the PBE0 level, where the radical character is clearly localized on the carbon atom.

For the non-oxidized substrate (6-ethyldodecane), the computed activation barriers for both secondary and tertiary radical formation exceed 30 kcal·mol^{−1} (Table 2), indicating that direct C–H activation in an aliphatic environment is energetically prohibitive. The corresponding product states are only marginally more stable than the TSs, with the transferred proton localized on the water molecule and C–H distances exceeding 1.8 Å. These results are consistent with those reported in Reference 34; however, that study considered only the overall reaction energies and did not

evaluate the activation energies associated with the oxidation processes.

A similar scenario is observed for hydroxylated substrates. For both 7-ethyldodecan-6-ol and 6-ethyldodecan-6-ol radical formation at either secondary or tertiary positions leads to TS structures and final states closely resembling those of the non-functionalized alkane. In all cases, the activation barriers remain above 30 kcal·mol^{−1}, indicating that hydroxyl functionalities do not significantly facilitate C–H bond activation.

A markedly different behavior is observed when the reactive C–H bond is located α to a carbonyl group [84], as in 7-ethyldodecan-6-one. In this case, both the electronic structure and the energetics of the reaction are significantly altered. The TSs are shifted earlier along the reaction coordinate and are less product-like compared to the previous systems. The resulting radical structures exhibit a planar arrangement involving the carbonyl group and adjacent carbon atoms, indicating conjugation between the radical center and the carbonyl functionality.

Consistently, spin density is partially delocalized onto the carbonyl oxygen, (≈ 0.1 e[−]) and neighboring carbon atoms, reflecting stabilization of the radical intermediate through keto–enol tautomerism, (Fig. 3). As a consequence, the activation barriers for both secondary and tertiary radical formation decrease by approximately 10–15 kcal·mol^{−1}, falling below 30 kcal·mol^{−1}. These results highlight the key role of carbonyl functionalities in lowering the energetic cost of radical formation.

O–H bond activation and β -scission pathways

As an alternative mechanism, we examined oxidation at pre-existing alcohol functionalities via O–H bond cleavage, leading to the formation of alkoxy radicals [85]. These intermediates could, in principle, undergo β -scission of adjacent C–C bonds, generating carbonyl compounds and carbon-centered radicals (Fig. 4). PES scans along the O–H stretching coordinate were performed using the same protocol adopted for C–H activation.

In contrast to the previous case, no well-defined transition states leading to stable alkoxy radical intermediates were identified (Fig. 2). The endpoint structures obtained along the reaction coordinate do not correspond to local minima and, upon release of the constraints, the systems revert to the initial alcohol configurations (Figure S3 and Table S2b). Furthermore, the calculated energy required for the formation of these species averages 28 kcal·mol^{−1} at the PBE level and 35.5 kcal·mol^{−1} at the PBE0 level, making these values far too high to consider this a viable catalytic pathway.

At the PBE level, partial spin density is localized on the oxygen atom (≈ 0.24 – 0.30 e[−]), suggesting incipient radical

Table 2 Total activation energy values computed at the PBE and PBE0 levels for C–H and O–H bond activation in 6-ethyldodecane, 7-ethyldodecan-6-ol, 6-ethyldodecan-6-ol and 7-ethyldodecan-6-one

	$\Delta E(\text{pbe})$	$\Delta E(\text{pbe0})$
	40.5	39.1
	37.8	35.6
	41.4	41.7
	38.3	34.9
	25.3	29.4
	27.1	27.7
	27.4	35.0
	28.5	36.1

The C–H or C–OH bonds involved are highlighted in red. Relative energies are reported in kcal·mol^{−1} and are calculated relative to the starting points of the PES scans shown in Fig. 2

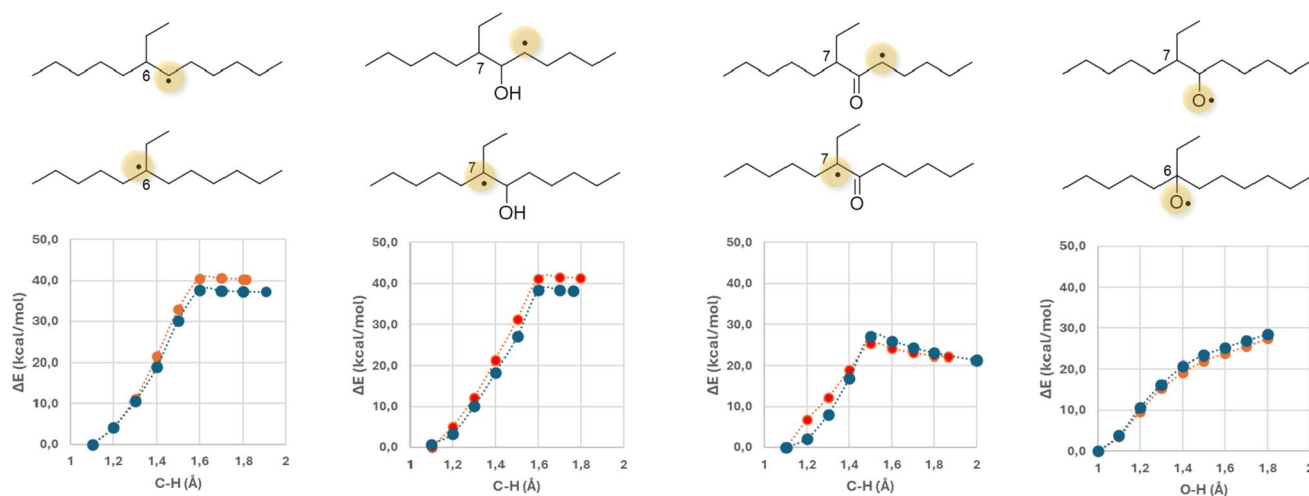


Fig. 2 PES scans at the PBE level for the LMCO2 T1-site model with the proposed substrates 6-ethyldodecane, 7-ethyldodecan-6-ol and 6-ethyldodecan-6-ol and 7-ethyldodecan-6-one. Relative energies are reported in kcal mol^{−1} relative to the starting point, and distances are

in Å. Dark blue and orange curves correspond to scans along the reaction coordinate leading to the formation of secondary (dark blue) and tertiary (orange) radicals, respectively

character. However, this feature is not retained at the PBE0 level, where the electronic structure instead converges toward an alkoxide anion (RO[−]). These results indicate that

the formation of an alkoxide radical is intrinsically unfeasible within the present catalytic environment (Table S3).

Fig. 3 Equilibrium scheme for the carbon-centered radical at the α -position relative to the ketone carbonyl group. Through keto–enol tautomerism, the initial electron deficiency localized on the α -carbon can delocalize either onto the carbonyl oxygen or onto the β -carbon relative to the carbonyl. The NBO spin populations (in electrons) for secondary (left) and tertiary (right) carbon-centered radical intermediates optimized at the PBE0 level are reported below

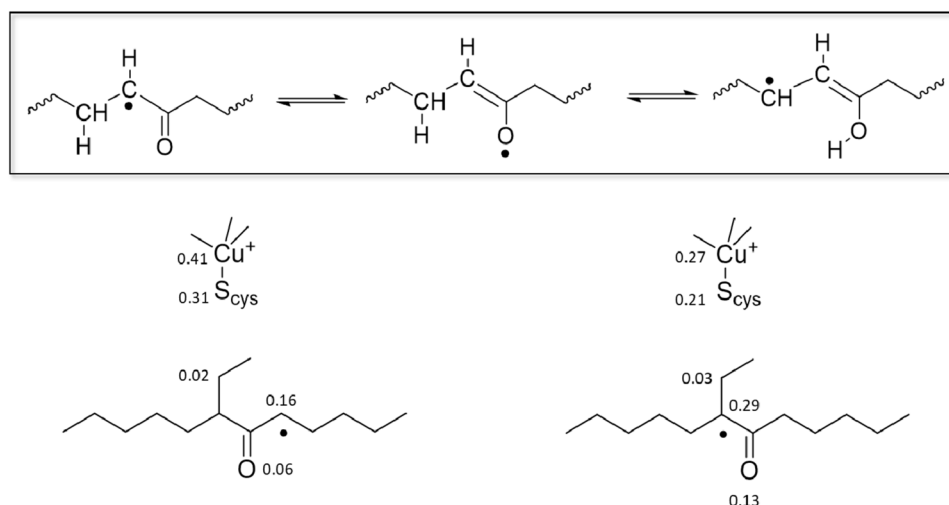
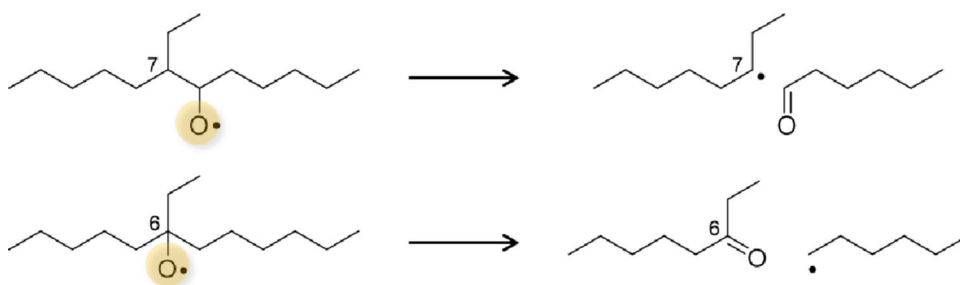


Fig. 4 Mechanism of β -scission of C–C bonds adjacent to secondary and tertiary alkoxy radicals generating carbonyl compounds and carbon-centered radicals



To further assess this pathway, we explored the possibility of β -scission from putative alkoxy radicals. For the secondary alcohol-derived system, C–C bond cleavage leads to formation of a primary radical and an aldehyde, but the process is highly endergonic (32.3 and 24.5 kcal·mol^{−1} at the PBE and PBE0 levels, respectively). When combined with the cost of radical formation, the overall energy requirement exceeds 40 kcal·mol^{−1}, rendering this pathway inaccessible.

A similar conclusion applies to tertiary alcohols. Although the intrinsic barrier for β -scission is lower (≈ 10 kcal·mol^{−1}), the instability of the precursor alkoxy radical results in a total energetic cost of approximately 40 kcal·mol^{−1}, making this mechanism unlikely under catalytic conditions.

Effect of copper redox state on radical stability

As shown above, when substrate oxidation is coupled to reduction of the T1 copper center ($\text{Cu}^{2+} \rightarrow \text{Cu}^+$), the resulting state (comprising a substrate radical and a reduced metal center) is generally unstable. A different scenario emerges when considering the one-electron oxidation of the T1 Cu^+ species with the oxidized substrate. This configuration represents the transient electronic configuration during which the electron initially abstracted via H-atom transfer (where the proton has protonated the aspartate residue) has migrated to the trinuclear copper cluster. In this case, the system is characterized by the presence of two unpaired electrons, one

localized on the substrate and one on the Cu^{2+} center. Within this framework, both carbon-centered and oxygen-centered (alkoxy) radical intermediates in triplet state are genuine local minima, with the latter showing a slightly higher relative stability. These findings suggest that the stability of radical intermediates may depend on efficient long-range electron transfer from the T1 site to the trinuclear copper cluster [86], preventing back-electron transfer and enabling stabilization of the oxidized substrate (Table S2). Stabilizing the carbon radicals may prolong their lifetime, allowing molecular oxygen to react more readily and trigger the polyethylene oxidative chain.

Characterization of Enzyme–Substrate Interactions

The apo form of LMCO2 was generated from the LMCO2–PE complex obtained from previous molecular dynamics simulations (Fig. 5, left panel) by removing both the PE ligand and structural water molecules. Subsequent cavity detection using Cavitomix identified 37 pockets, among which two main cavities are relevant for substrate binding (Fig. 5, central panel). Cavity 1 is the largest and most solvent-exposed (~ 1370 Å³), located beneath the methionine-rich loop and opening toward the T1 copper site, whereas cavity 2 is smaller (~ 409 Å³) and positioned between the T1 and T2 copper centers. To further assess the relative relevance of cavity 1 and cavity 2, exploratory docking

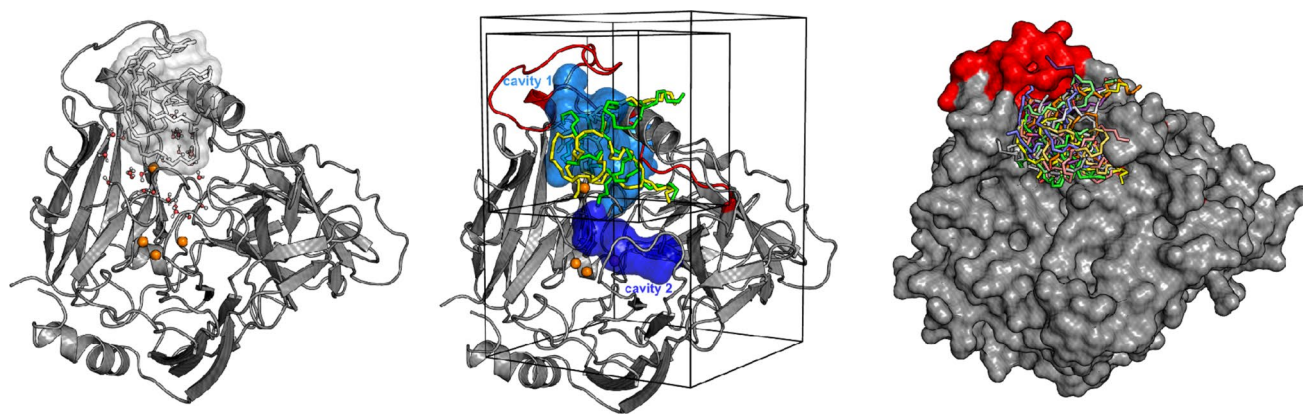


Fig. 5 Structural representation of LMCO2 cavities and ligand binding. *Left*: LMCO2-PE complex (from molecular dynamics simulations reported in Orlando et al., 2025) in grey with the copper ions of the T1, T2, and T3 sites shown as orange spheres. *Center*: The two main cavities identified by Cavitomix highlighted as surfaces (cavity 1 and cavity 2, respectively in cyan and blue); the best docking pose of the $C_{100}H_{202}$ polyethylene model, obtained using docking grids encom-

passing either both cavities (PE in green stick) or restricted to cavity 1 (PE in yellow stick); in both cases, the polymer binds within the T1-proximal pocket (cavity 1). The methionine-rich loop is highlighted in red. *Right*: Docking poses of LMCO2 (in grey surface with Met-loop highlighted in red) complexes including both ABTS and C_{100} ligands shown as coloured sticks

calculations were performed using two different grid definitions: (i) a large search box ($70 \times 100 \times 70 \text{ \AA}$) encompassing both cavities, and (ii) a smaller box ($50 \times 50 \times 50 \text{ \AA}$) centered on the MD-derived binding region and selectively targeting cavity 1 (Figure S2). Docking of $C_{100}H_{202}$ with the large grid confirms cavity 1 as the dominant binding site, with the best-scoring poses closely reproducing the MD-derived conformation. When the smaller grid is employed, the top-ranked pose overlaps almost perfectly with that obtained using the larger search space (Fig. 5, central panel). In contrast, cavity 2 is only marginally sampled, primarily by terminal segments of the polymer chain. These results unambiguously identify the T1-proximal cavity 1 as the main binding region for polyethylene in LMCO2.

Docking calculations were extended to include pre-oxidized polyethylene models, specifically hydroxyl- and carbonyl-functionalized PE analogues, as well as the reference laccase mediator ABTS, which was included as a well-established high-affinity oxidizable substrate commonly used to benchmark laccase active-site binding. The corresponding results are summarized in the Supplementary Information. In the “dry” receptor (i.e. in the complete absence of explicit water molecules), ABTS exhibits the most favorable docking score ($-6.8 \text{ kcal} \cdot \text{mol}^{-1}$), forming a H-bond with Trp⁴¹⁶ and a network of hydrophobic and polar contacts (Tyr⁵⁰¹, His⁵⁰², Arg²²⁰) (Figs. 6). By comparison, all PE-like ligands cluster around $-3.5 \pm 0.3 \text{ kcal} \cdot \text{mol}^{-1}$, indicating generally weak binding. In these conditions, $C_{100}H_{202}$ and its oxidized analogues uniformly occupy the Met-loop-lined hydrophobic pocket (Met²²⁴, Met²²⁶, Met²²⁹, Met²³⁹, Met⁴⁴¹) (Fig. 6, right panel), with the polymer backbone forming a network of hydrophobic contacts (e.g. Pro²¹², Asp²¹³, Gln²¹⁵, Arg²²⁰, Asp²⁵⁶, His⁵⁰², Trp⁴¹⁶)

(Figs. 6), and polar substituents largely exposed toward the cavity opening, consistent with their similar affinities. When the structural water layer is retained in the receptor, the trend of the ligands becomes polarity-dependent. ABTS score improved to $-7.3 \text{ kcal} \cdot \text{mol}^{-1}$, and the hydrated docking pose reveals additional water-mediated hydrogen bonds within the T1 pocket, between ABTS sulfonate oxygens and Trp⁴¹⁶, Tyr⁵⁰¹ and a nearby structural water molecule. Fully apolar $C_{100}H_{202}$ instead shows a less favourable score of $-3.2 \text{ kcal} \cdot \text{mol}^{-1}$. Hydroxylated and carbonylated PE analogues instead display similar average affinities in the hydrated receptor ($-3.4 \pm 0.2 \text{ kcal} \cdot \text{mol}^{-1}$), and largely retain the hydrophobic interaction network observed under dry docking conditions. Notably, the penta-hydroxyl analogue establishes a water-mediated bridge with His⁵⁰² whereas the penta-carbonyl forms different hydrogen bonds within the pocket, involving Trp⁴¹⁶ and a structural water molecule on one side, and Pro²¹² and Gln²¹⁴ on the opposite side (Figs. 6). Taken together, these observations suggest that the structural water layer is not optimal for stabilizing native, fully apolar PE, while it can provide additional hydrogen-bonding opportunities for oxidized chains.

To assess whether the mediator and the LDPE can be co-localized near the copper sites, we also performed simultaneous docking of ABTS and $C_{100}H_{202}$ into both dry and hydrated receptors. This approach allows both ligands to explore the binding landscape at the same time within the available cavities. Multiple-ligand docking shows that the LDPE chain remains predominantly bound in cavity 1, lining the Met-loop-associated hydrophobic pocket, as observed in the single ligand docking, whereas ABTS is relocated toward cavity 2 between the T1 and T2 copper sites (Figure S2). These results are consistent with previous

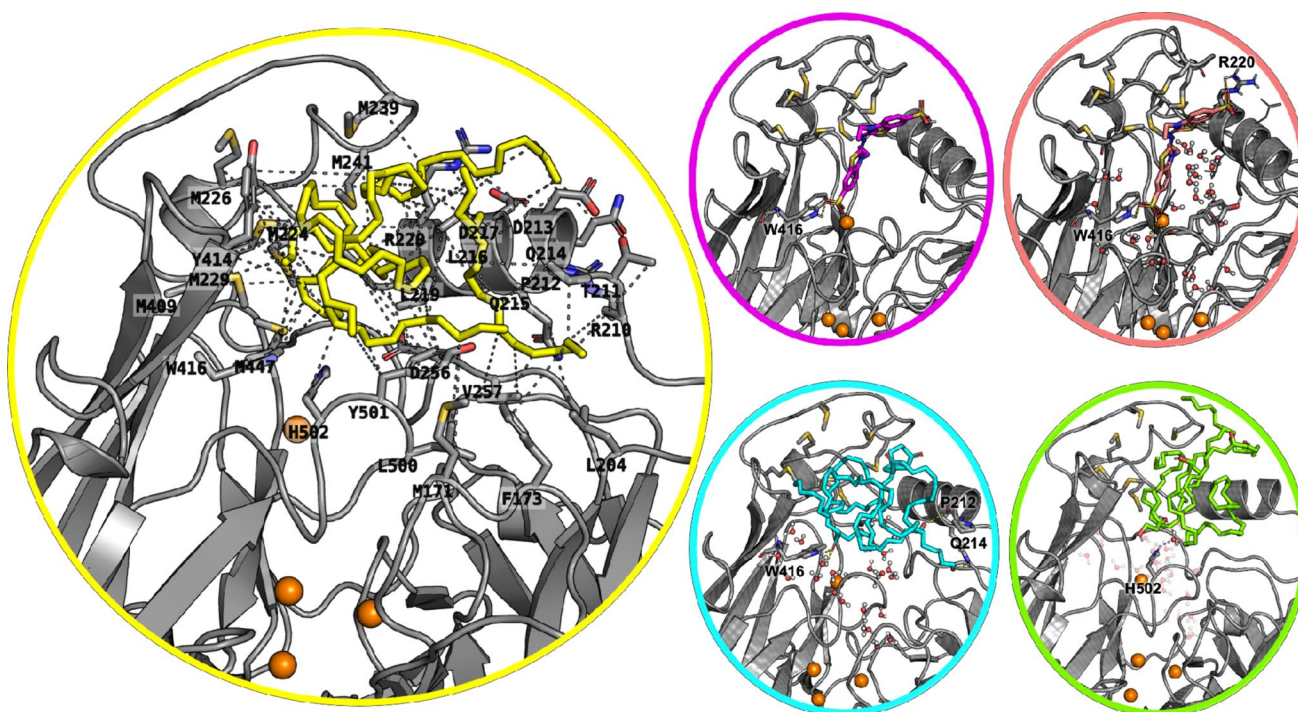


Fig. 6 Representative interaction for the best docking poses of LMCO2 with PE, ABTS, and oxidized PE models. LMCO2 is shown as a gray cartoon in all panels, while ligands are represented as sticks (PE in yellow, ABTS in magenta and pink, $C_{100}H_{197}(OH)_5$ in green and $C_{100}H_{192}O_5$ in cyan). Interacting residues are shown as gray sticks. The left panel displays the LMCO2-PE complex with hydrophobic

contacts indicated by light gray dashed lines. The upper right panels show the best LMCO2-ABTS poses obtained under dry and hydrated docking conditions, respectively, while the lower right panel shows the LMCO2- $C_{100}H_{197}(OH)_5$ and the LMCO2- $C_{100}H_{192}O_5$ complexes. Hydrogen-bond and water bridges interactions are shown in yellow and violet dashed lines respectively

multiscale studies on laccases and laccase-mediator systems, which have highlighted the ability of substrates and mediators to occupy multiple cavities around the T1 copper and the key role of structured water and loop dynamics [82, 87, 88]. Overall, multiple-ligand docking indicates that ABTS and PE can coexist within the same binding region without evidence of cooperative stabilization, in agreement with experimental observations indicating that ABTS does not enhance LDPE degradation by LMCO2.

Conclusions

The present computational analysis provides mechanistic insight into the early stages of LMCO2-mediated polyethylene oxidation. DFT calculations indicate that direct one-electron oxidation of aliphatic C-H bonds in pristine polyethylene is associated with high intrinsic barriers ($>30 \text{ kcal}\cdot\text{mol}^{-1}$), rendering this pathway energetically unfavorable within the present model. A similar scenario applies to hydroxylated substrates, for which neither the activation of the α -C-H bond adjacent to the OH group nor the oxidation of the O-H bond—followed by alkoxyl radical β -scission—appears to be accessible. In contrast, oxidation

at carbon atoms adjacent to carbonyl groups displays substantially lower activation barriers, reflecting stabilization of the resulting radical species through conjugation and keto-enol tautomerism. To facilitate this PCET process, the involvement of a nearby Asp side chain is suggested, in line with established literature on laccase reactivity. These results consistently identify carbonyl-containing defects as the most plausible reactive sites for LMCO2-mediated oxidation, in agreement with the relatively low redox potential of the enzyme. DFT calculations indicate that the oxidation process is energetically favored—albeit slightly—for tertiary C-H bonds, driving laccase activity more toward highly branched LDPE.

Interestingly, two very recently published studies on PE oxidation by bacterial multicopper oxidases have highlighted the potential importance of polymer heterogeneity in the initial oxidative attack. One study explicitly noted the presence of pre-existing oxidized features in the polymeric substrate, which therefore could not be regarded as ideal, defect-free pristine PE [89], whereas another considered the possibility that structurally disordered or otherwise chemically accessible regions of the polymer may represent preferential sites for the initial oxidative attack [67]. Together, these observations are particularly relevant to the present

mechanistic picture, in which structurally or chemically defective regions may provide more chemically plausible sites for oxidation than pristine methylene units.

Moreover, DFT calculations indicate that the oxidized state of T1 stabilizes the oxidized substrate intermediate. This behavior reflects the full catalytic mechanism, where the T1 site undergoes a reduction/re-oxidation sequence during electron transfer to the trinuclear cluster. This insight suggests that incorporating the complete four-copper catalytic site could be important in future mechanistic studies of bacterial laccases acting on PE.

Docking calculations further show that both pristine and pre-oxidized polyethylene models can access the T1-proximal binding cavity, with predominantly hydrophobic interactions and only minor dependence on the oxidation state. The explicit inclusion of structural water molecules does not significantly alter this behavior. Together, these observations indicate that oxygen-containing functionalities play a limited role in substrate binding, while strongly modulating chemical reactivity. Co-docking simulations with ABTS further suggest that mediator and polymer can coexist within the enzyme environment, providing a qualitative framework for their simultaneous presence near the protein surface, although competitive or cooperative effects and electron-transfer coupling remain outside the scope of the present model.

Taken together, DFT and molecular docking results support a coherent mechanistic picture of LDPE oxidation by LMCO2. DFT calculations indicate that only pre-oxidized regions bearing carbonyl functionalities display a sufficiently favorable intrinsic reactivity for further oxidation. Thus, within the present modeling framework, LMCO2 is not predicted to efficiently initiate oxidation of pristine polyethylene. Rather, the enzyme is more likely to act on oxidized polymer segments generated by abiotic oxidation or spontaneous aging, promoting radical propagation and potentially contributing to subsequent chain scission.

Although refinement or enlargement of the QM model may affect the absolute energetic values, we expect the qualitative trend identified here to remain unchanged, namely that carbonyl-containing defects represent the preferred entry point for LMCO2 reactivity. Future work will extend the model to include the trinuclear copper cluster, molecular oxygen, and explicit electron-transfer pathways to further refine the mechanistic hypothesis.

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Data availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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References

1. Yao Z, Seong HJ, Jang Y-S (2022) Environmental toxicity and decomposition of polyethylene. *Ecotoxicol Environ Saf* 242:113933
2. Ghatge S, Yang Y, Ahn J-H, Hur H-G (2020) Biodegradation of polyethylene: a brief review. *Appl Biol Chem* 63. <https://doi.org/10.1186/s13765-020-00511-3>
3. Jin J, Arciszewski J, Auclair K, Jia Z (2023) Enzymatic polyethylene biorecycling: Confronting challenges and shaping the future. *J Hazard Mater* 460:132449
4. Qiao J, Wu L, Ma X et al (2026) Advances in polyethylene biodegradation and bioconversion: Microbial, enzymatic, and biotechnological insights. *Eng Microbiol* 6:100255
5. Rovalletti A, De Gioia L, Fantucci P et al (2023) Recent Theoretical Insights into the Oxidative Degradation of Biopolymers and Plastics by Metalloenzymes. *Int J Mol Sci* 24. <https://doi.org/10.3390/ijms24076368>
6. Gardette M, Perthue A, Gardette J-L et al (2013) Photo- and thermal-oxidation of polyethylene: Comparison of mechanisms and influence of unsaturation content. *Polym Degrad Stab* 98:2383–2390
7. Corrales T, Catalina F, Peinado C et al (2002) Photooxidative and thermal degradation of polyethylenes: interrelationship by chemiluminescence, thermal gravimetric analysis and FTIR data. *J Photochem Photobiol Chem* 147:213–224
8. Chen L, Huan TD, Ramprasad R (2017) Electronic Structure of Polyethylene: Role of Chemical, Morphological and Interfacial Complexity. *Sci Rep* 7:6128
9. Sandholm RM, Boehlich GJ, Dahl Ø et al (2025) Microbial degradation of a widely used model polyethylene is restricted to medium- and long-chain alkanes and their oxidized derivatives. *ISME J*. <https://doi.org/10.1093/ismej/wraf276>. 19.

10. Goudriaan M, Morales VH, van der Meer MTJ et al (2023) A stable isotope assay with C-labeled polyethylene to investigate plastic mineralization mediated by *Rhodococcus ruber*. *Mar Pollut Bull* 186:114369
11. Stepnov AA, Lopez-Tavera E, Klauer R et al (2024) Revisiting the activity of two poly(vinyl chloride)- and polyethylene-degrading enzymes. *Nat Commun* 15:8501
12. Werz A, Brunner I, Qi W et al (2026) Polyethylene transformation by a psychrotolerant *Rhodococcus* strain assessed by transcriptomics and C-isotope tracing. *J Hazard Mater* 516:143358
13. Saikrishnan S, Jubinville D, Tzoganakis C, Mekonnen TH (2020) Thermo-mechanical degradation of polypropylene (PP) and low-density polyethylene (LDPE) blends exposed to simulated recycling. *Polym Degrad Stab* 182:109390
14. Oh S, Stache EE (2024) Recent advances in oxidative degradation of plastics. *Chem Soc Rev* 53:7309–7327
15. Oiffer T, Leipold F, Süß P et al (2024) Chemo-Enzymatic Depolymerization of Functionalized Low-Molecular-Weight Polyethylene. *Angew Chem Int Ed Engl* 63:e202415012
16. Yao C, Xia W, Dou M et al (2022) Oxidative degradation of UV-irradiated polyethylene by laccase-mediator system. *J Hazard Mater* 440:129709
17. Arregui L, Ayala M, Gómez-Gil X et al (2019) Laccases: structure, function, and potential application in water bioremediation. *Microb Cell Fact* 18:200
18. Christopher LP, Yao B, Ji Y (2014) Lignin Biodegradation with Laccase-Mediator Systems. *Front Energy Res* 2. <https://doi.org/10.3389/fenrg.2014.00012>
19. Yan L, Huertas-Alonso AJ, Liu H et al (2025) Lignin polymerization: towards high-performance materials. *Chem Soc Rev* 54:6634–6651
20. Roberts SA, Weichsel A, Grass G et al (2002) Crystal structure and electron transfer kinetics of CueO, a multicopper oxidase required for copper homeostasis in *Escherichia coli*. *Proc Natl Acad Sci U S A* 99:2766–2771
21. Enguita FJ, Marçal D, Martins LO et al (2004) Substrate and dioxygen binding to the endospore coat laccase from *Bacillus subtilis*. *J Biol Chem* 279:23472–23476
22. Chandra R, Chowdhary P (2015) Properties of bacterial laccases and their application in bioremediation of industrial wastes. *Environ Sci Process Impacts* 17:326–342
23. Zampolli J, Orro A, Manconi A et al (2021) Transcriptomic analysis of *Rhodococcus opacus* R7 grown on polyethylene by RNA-seq. *Sci Rep* 11:21311
24. Zampolli J, Mangiagalli M, Vezzini D et al (2023) Oxidative degradation of polyethylene by two novel laccase-like multicopper oxidases from *Rhodococcus opacus* R7. *Environ Technol Innov* 32:103273
25. Orlando C, Bellei M, Zampolli J et al (2025) Comparative analysis of Polyethylene-Degrading Laccases: Redox Properties and Enzyme-Polyethylene Interaction Mechanism. *Chemsuschem* 18:e202402253
26. Wernimont AK, Huffman DL, Finney LA et al (2003) Crystal structure and dimerization equilibria of PcoC, a methionine-rich copper resistance protein from *Escherichia coli*. *J Biol Inorg Chem* 8:185–194
27. Borges PT, Brissos V, Hernandez G et al (2020) Methionine-rich loop of multicopper oxidase McoA follows open-to-close transitions with a role in enzyme catalysis. *ACS Catal* 10:7162–7176
28. Contaldo U, Santucci P, Vergnes A et al (2025) How the Larger Methionine-Rich Domain of CueO from *Hafnia alvei* Enhances Cuprous Oxidation. *JACS Au* 5:1833–1844
29. Gascó R, Sendra R, Olmeda I et al (2026) Laccases from lactic acid bacteria show cuprous oxidase activity and capture Cu(II) and Ag(I) ions. *Protein Sci* 35:e70385
30. Contaldo U, Savant-Aira D, Vergnes A et al (2024) Methionine-rich domains emerge as facilitators of copper recruitment in detoxification systems. *Proc Natl Acad Sci U S A* 121:e2402862121
31. Santo M, Weitsman R, Sivan A (2013) The role of the copper-binding enzyme – laccase – in the biodegradation of polyethylene by the actinomycete *Rhodococcus ruber*. *Int Biodeterior Biodegradation* 84:204–210
32. Gao R, Liu R, Sun C (2022) A marine fungus *Alternaria alternata* FB1 efficiently degrades polyethylene. *J Hazard Mater* 431:128617
33. Zhang A, Hou Y, Wang Q, Wang Y (2022) Characteristics and polyethylene biodegradation function of a novel cold-adapted bacterial laccase from Antarctic sea ice psychrophile *Psychrobacter* sp. NJ228. *J Hazard Mater* 439:129656
34. Zhang Y, Plesner TJ, Ouyang Y et al (2023) Computer-aided discovery of a novel thermophilic laccase for low-density polyethylene degradation. *J Hazard Mater* 458:131986
35. Ruan Y, Hu F, Zhao J et al (2023) Degradation of polyethylene particles by *Trichoderma harzianum* and recombinant laccase cloned from the strain. *J Appl Polym Sci* 140. <https://doi.org/10.1002/app.54599>
36. Ray AS, Rajasekaran M, Uddin M, Kandasamy R (2023) Laccase driven biocatalytic oxidation to reduce polymeric surface hydrophobicity: An effective pre-treatment strategy to enhance biofilm mediated degradation of polyethylene and polycarbonate plastics. *Sci Total Environ* 904:166721
37. Zhang X, Feng X, Lin Y et al (2023) Degradation of polyethylene by *Klebsiella pneumoniae* Mk-1 isolated from soil. *Ecotoxicol Environ Saf* 258:114965
38. Shafana Farveen M, Madhavan T, Narayanan R (2023) Association of Laccase from *Bacillus cereus* O2-B and *Pseudomonas aeruginosa* O1-P with the bio-degradation of polymers: an in vitro to in silico approach. *Biodegradation* 34:383–403
39. Tao X, Ouyang H, Zhou A et al (2023) Polyethylene Degradation by a Strain Isolated from Naturally Weathered Plastic Waste Enrichment. *Environ Sci Technol* 57:13901–13911
40. Zeng B, Fu Y, Ye J et al (2024) Ancestral sequence reconstruction of the prokaryotic three-domain laccases for efficiently degrading polyethylene. *J Hazard Mater* 476:135012
41. Li X, Li G, Wang J et al (2024) Elucidating polyethylene microplastic degradation mechanisms and metabolic pathways via iron-enhanced microbiota dynamics in marine sediments. *J Hazard Mater* 466:133655
42. Meng Q, Yi X, Zhou H et al (2024) Isolation of marine polyethylene (PE)-degrading bacteria and its potential degradation mechanisms. *Mar Pollut Bull* 207:116875
43. Ramamurthy K, Thomas NP, Gopi S et al (2024) Is Laccase derived from *Pleurotus ostreatus* effective in microplastic degradation? A critical review of current progress, challenges, and future prospects. *Int J Biol Macromol* 276:133971
44. Diefenbach T, Sumetzberger-Hasinger M, Braunschmid V et al (2024) Laccase-mediated degradation of petroleum hydrocarbons in historically contaminated soil. *Chemosphere* 348:140733
45. Uddin M, Venkatesan SK, Pal SK et al (2025) Accelerating biodegradation efficiency of low-density polyethylene and its hazardous dissolved organic matter using unexplored polyolefin-respiring bacteria: New insights on degradation characterization, biomolecule influence and biotransformation pathways. *J Hazard Mater* 492:138144
46. Wu L, Wang X, Li A et al (2025) Discovery of a polyethylene biodegradable laccase from *Acinetobacter dijksboorniae*. *AIChE J* 71. <https://doi.org/10.1002/aic.70005>
47. Zhang A, Hou Y, Wang Y et al (2023) Highly efficient low-temperature biodegradation of polyethylene microplastics by using cold-active laccase cell-surface display system. *Bioresour Technol* 382:129164

48. Zhang A, Hou Y, Hou S et al (2025) Enhancement of microplastics degradation efficiency: Microbial laccase-driven radical chemical coupling catalysis. *Chem Eng J* 507:160579
49. Son HF, Hwang S, Kim Y et al (2025) Enzymatic depolymerization of polyethylene using a small laccase and its potential for bio-upcycling. *J Hazard Mater* 495:139021
50. Xu X, Liu S, Pang J et al (2025) Novel *Pantoea brenneri* CYD50–1 enables nitrogen-synergized enzymatic depolymerization of low-density polyethylene. *Chem Eng J* 524:169692
51. Zheng Y, Liu Y, Wang S et al (2025) A novel synergistic degradation of polyethylene microplastics by recombinant *Pichia pastoris* and purified laccase. *J Clean Prod* 534:147073
52. Abe MM, Moraes CE, Basaglia MV et al (2025) Evaluation of low-density polyethylene degradation using a new thermophilic bacterial isolate. *Sci Total Environ* 1004:180799
53. Hu J, Peng B, Liu Z et al (2025) Polyethylene degradation mediated by *Klebsiella variicola* isolated from the gut of insect larvae. *Int Biodeterior Biodegradation* 205:106182
54. Kang Z, Li N, Wang C et al (2025) Biodegradation of low-density polyethylene by a novel *Paenarthrobacter* sp. PE0901 from oilfield soil: Isolation, characterization, and degradation mechanism. *J Environ Chem Eng* 13:117418
55. Sabellico G, Baggetta A, Sandrucci E et al (2025) Oxidative deterioration of polypropylene by redox mediators and yeast expressing a fungal recombinant laccase. *Int Biodeterior Biodegradation* 196:105947
56. Buron-Moles G, Vandenbossche V, Gorret N et al (2025) Biodegradation of pre-treated low-density polyethylene (LDPE) by *Yarrowia lipolytica* determined by oxidation and molecular weight reduction. *Polym Degrad Stab* 236:111292
57. Liu F, Zhang N, Shang Y et al (2025) Construction of *Yarrowia lipolytica* for degradation of low-density polyethylene. *Process Saf Environ Prot* 195:106818
58. Chen X, Xue H, Jiang Z et al (2025) Biodegradation of polyethylene by *Gordonia* sp. C1 and *Bacillus* sp. C2 isolated from landfill. *J Environ Chem Eng* 13:116443
59. Bunsangiam S, Sakdapsiri C, Charoenpong C, Pinyakong O (2025) A versatile potential *Ancylobacter* sp. PD-4 capable of biodegrading polyethylene microplastics and di(2-ethylhexyl) phthalate. *J Hazard Mater* 501:140731
60. Kulshrestha P, Katiyar R, Zargar AN et al (2025) Multi copper oxidase from *Micrococcus* sp. IITD107, a promising candidate for polypropylene biodegradation. *Npj Mater Degrad*. <https://doi.org/10.1038/s41529-025-00720-5>
61. Khampratueng P, Anal AK (2026) Enhancing the biodegradation of low-density polyethylene (LDPE) using novel bacterial consortia: *Bacillus* sp. AS3 and *Sphingobacterium* sp. AS8. *J Environ Sci (China)* 159:263–270
62. Zhang H, Yin C-F, Song X et al (2026) Polyvinyl chloride depolymerization and dechlorination by *Tenebrio molitor* larva-derived bacteria. *J Hazard Mater* 503:141169
63. Ding R, Xu L, Shan X et al (2026) Enhanced degradation of microplastics by laccase under ambient conditions: Analysis of underlying molecular mechanisms. *J Hazard Mater* 503:141109
64. Ding R, Shan X, Ding L et al (2026) Coupled Reactive Regulation by Microplastic-Derived Dissolved Organic Matter Sustains Reactive Oxygen Species Cycling in Laccase-Metal Synergy for Continuous Microplastic Degradation. *Environ Sci Technol* 60:2101–2111
65. Qiu Q, Sun X, Li H et al (2026) Study on the dominant role and molecular mechanism of *Lysinibacillus fusiformis* Tm-07 derived from the gut of *Tenebrio molitor* in plastic degradation. *J Hazard Mater* 141325
66. Yang X, Chang H (2026) An advanced chemo-biooxidation process for the degradation of low-density polyethylene microplastics: role of redox mediators in toxicity of transformation products. *Polym Degrad Stab* 252:112263
67. Yun S-D, Kim S, An SJ et al (2026) A multicopper oxidase from *Paenibacillus polyethylenolyticus* JNU01 oxidizes polyethylene. *Chem Eng J* 547:180903
68. Ahlrichs R, Bär M, Häser M et al (1989) Electronic structure calculations on workstation computers: The program system turbo-mole. *Chem Phys Lett* 162:165–169
69. Perdew JP, Burke K, Wang Y (1996) Generalized gradient approximation for the exchange-correlation hole of a many-electron system. *Phys Rev B Condens Matter* 54:16533–16539
70. Adamo C, Cossi M, Barone V (1999) An accurate density functional method for the study of magnetic properties: the PBE0 model. *THEOCHEM* 493:145–157
71. Eichkorn K, Weigend F, Treutler O, Ahlrichs R (1997) Auxiliary basis sets for main row atoms and transition metals and their use to approximate Coulomb potentials. *Theor Chem Acc* 97:119–124
72. Schäfer A, Huber C, Ahlrichs R (1994) Fully optimized contracted Gaussian basis sets of triple zeta valence quality for atoms Li to Kr. *J Chem Phys* 100:5829–5835
73. Klamt A, Schürmann G (1993) COSMO: a new approach to dielectric screening in solvents with explicit expressions for the screening energy and its gradient. *J Chem Soc Perkin Trans* 2:799–805
74. Grimme S (2006) Semiempirical GGA-type density functional constructed with a long-range dispersion correction. *J Comput Chem* 27:1787–1799
75. Arrigoni F, Rizza F, Tisi R et al (2020) On the propagation of the OH radical produced by Cu-amyloid beta peptide model complexes. *Insight Mol modelling Metallomics* 12:1765–1780
76. Rovetta A, Carosella L, Arrigoni F et al (2023) Oxidation of phospholipids by OH radical coordinated to copper amyloid-β peptide—A Density Functional Theory modeling. *Inorganics* 11:227
77. Pirro F, La Gatta S, Arrigoni F et al (2023) A DE Novo-designed type 3 copper protein tunes catechol substrate recognition and reactivity. *Angew Chem Weinheim Bergstr Ger* 135. <https://doi.org/10.1002/ange.202211552>
78. Orlando C, Rizzo IC, Arrigoni F et al (2024) Mechanism of non-phenolic substrate oxidation by the fungal laccase Type 1 copper site from: the case of benzo[*a*]pyrene and anthracene. *Dalton Trans* 53:12152–12161
79. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 31:455–461
80. Sheng X, Himo F (2023) The Quantum Chemical Cluster Approach in Biocatalysis. *Acc Chem Res* 56:938–947
81. Li X, Xie Q, Luo M, Chen X (2024) Theoretical Insights into the Catalytic Oxidation of Phenols and Arylamines by Laccases via the Proton-Coupled Electron Transfer Mechanism. *J Phys Chem B* 128:8915–8926
82. Hilgers R, Vincken J-P, Gruppen H, Kabel MA (2018) Laccase/Mediator Systems: Their Reactivity toward Phenolic Lignin Structures. *ACS Sustain Chem Eng* 6:2037–2046
83. Ahn Y, Roma G, Colin X (2022) Elucidating the role of alkoxy radicals in polyethylene radio-oxidation kinetics. *Macromolecules* 55:8676–8684
84. Chamas A, Moon H, Zheng J et al (2020) Degradation rates of plastics in the environment. *ACS Sustain Chem Eng* 8:3494–3511
85. Gugumus F (2002) Re-examination of the thermal oxidation reactions of polymers 2. Thermal oxidation of polyethylene. *Polym Degrad Stab* 76:329–340
86. Xie Q, Luo M, Liu M et al (2025) Laccase Employing Water-Mediated Double-Proton-Coupled Electron Transfer To Achieve the Oxygen Reduction Reaction. *Inorg Chem* 64:21704–21717
87. Mehra R, Muschiol J, Meyer AS, Kepp KP (2018) A structural-chemical explanation of fungal laccase activity. *Sci Rep* 8:17285

88. Brissos V, Borges PT, Núñez-Franco R et al (2022) Distal Mutations Shape Substrate-Binding Sites during Evolution of a Metallo-Oxidase into a Laccase. *ACS Catal* 12:5022–5035
89. Zaman I, Moosa MM, Hossain MM (2026) Functional, genomic, and transcriptomic insights into linear low-density polyethylene (LLDPE) biodegradation by landfill-derived *Brucella* intermedia. *J Hazard Mater* 509:141999

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