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Research Article

Keywords: Cytochrome c oxidase, oxygen reduction reaction, Density functional theory, electronic structure

Posted Date: September 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10951683/v1>

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Additional Declarations: No competing interests reported.

Density Functional Theory Study of the Electronic Structure and Thermodynamics of Oxygen Reduction Intermediates in an Fe-Porphyrin Biomimetic Model of the Cytochrome c Oxidase Active Site

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ABSTRACT

Context:

The oxygen reduction reaction catalyzed by cytochrome c oxidase is a key step in biological energy conversion and remains an important reference for catalyst design. In this work, the catalytic behavior of an Fe-centered cytochrome c oxidase active-site model was examined through four representative configurations, namely the reduced iron center (Fe), the oxygen-bound state (Fe-OO), the protonated intermediate (Fe-O-OH), and the water-associated structure (Fe-O-H₂O). The calculations indicate that the Fe, Fe-O₂, and Fe-O-OH intermediates favor the triplet state, while the Fe-O-H₂O structure was calculated in the quintet state. Changes in the electronic structure are most pronounced upon oxygen coordination, where the Fe-OO intermediate exhibits the smallest HOMO-LUMO gap among the configurations considered. Thermochemical analysis shows that the reaction steps investigated are exothermic and thermodynamically favorable. The Sabatier activity analysis suggests that the Fe-porphyrin coordination environment maintains moderate interaction strengths during the oxygen reduction process. These results provide molecular-level insight into the electronic characteristics governing oxygen activation at the Fe-centered active site and may serve as a reference for the design of biomimetic Fe-based catalytic systems.

Methods:

Density functional theory (DFT) calculations were performed using the B3LYP functional with the 6-31G(d,p) basis set for the Fe-centered cytochrome c oxidase active-site models. Molecular oxygen was separately calculated at the B3LYP/6-31G(d) level. Geometry optimizations and vibrational frequency calculations were performed to obtain stable molecular structures and verify the absence

of imaginary frequencies. The spin-state energetics, HOMO and LUMO characteristics, HOMO-LUMO gaps, and thermochemical properties of the reaction intermediates were subsequently analyzed. All calculations were performed using Gaussian 09, with GaussView 5.0.8 used for molecular model construction and analysis of the calculated results.

Keywords: Cytochrome c oxidase; oxygen reduction reaction; Density functional theory; electronic structure

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1. Introduction

Energy use and conversion efficiency remain active topics of interest across many scientific and engineering disciplines [1, 2]. Rather than relying solely on conventional engineering strategies, increasing attention has been directed toward biological systems [3], which achieve energy conversion with notably high effectiveness that far exceeds those of most man-made energy systems [4]. From this perspective, biological energy conversion mechanisms can offer insights for the development of fuel cell technologies and other energy systems, providing an opportunity to solve environmental problems in the future.

A fuel cell is an electrochemical cell that converts the chemical energy of a fuel directly into electrical energy [5, 6]. From an electrochemical perspective, their operation can be viewed as the reverse process of water electrolysis [7]. Of particular interest is the cathodic oxygen reduction reaction (ORR), which proceeds through the reduction of molecular oxygen to water. Notably, an analogous ORR occurs at cytochrome c oxidase within the mitochondrial inner membrane electron transport chain. Although modern electrochemical energy-conversion devices, including proton exchange membrane fuel cells, have reached practical maturity, their overall efficiencies remain constrained, typically falling within the range of 40-60% under realistic operating conditions [8, 9]. Such limitations stand in noticeable contrast to biological energy conversion in mitochondria, where oxidative phosphorylation has been reported to operate with substantially higher efficiency, approaching 90% in optimized physiological environments. This exceptional performance has led researchers to examine biological redox enzymes for alternative strategies for coupling electron transfer, proton transport, and chemical reactions at the molecular scale. If principles underlying mitochondrial energy conversion can be incorporated into the design of fuel cell catalysts [10], this approach may help reduce reliance on fossil-based energy sources. Such developments could, in turn, contribute to lower carbon dioxide emissions and provide a potential pathway toward mitigating the environmental impact associated with greenhouse gas accumulation.

Li et al. [11] studied the ORR mechanisms, catalyst design, and practical applications in

renewable energy devices. The analysis included activity trends related to the adsorption free energies of key intermediates, demonstrating how scaling relationships and volcano plots serve as critical thermodynamic indicators. Chang et al. [12] applied an ORR volcano framework to examine the connection between adsorption energetics and theoretical limiting potentials, illustrating how moderate adsorption strength is linked to improved ORR performance. More recently, Zhang et al. [13] summarized progress in Fe-based dual-atom catalysts for oxygen reduction, with particular attention to the role of neighboring metal sites in tuning adsorption behavior and mitigating the intrinsic limitations observed in single-atom catalyst systems.

Energy conversion in living cells is achieved through organelle-specific processes that transform available energy into chemically usable forms. In photosynthetic organisms, this role is associated with chloroplast activity, whereas in eukaryotic cells, mitochondria are responsible for respiratory energy production [14, 15]. Although these processes operate through different pathways, photosynthesis and mitochondrial respiration are functionally linked within cellular energy metabolism [16]. Notably, mitochondrial respiration proceeds with high efficiency under mild physiological conditions, a feature rarely achieved in artificial energy conversion systems. Through the mitochondrial electron transport chain, coupled with proton pump mechanisms [17], the chemical energy derived from ingested nutrients is transformed into usable energy, leading to the synthesis of adenosine triphosphate (ATP) [18], which supplies the energy required for normal biological function. For this reason, mitochondria have long been regarded as a valuable biological model for investigating fundamental mechanisms of energy transduction [19]. This mechanism enables highly efficient energy utilization under mild physiological conditions, distinguishing mitochondrial respiration from most artificial energy conversion systems. Owing to this efficiency, mitochondria have long served as an important model for understanding biological energy transduction [20].

Cytochrome c oxidase (CcO) is the terminal enzyme in the electron transport chain, which belongs to a kind of membrane-bound enzyme of mitochondria [21]. To generate the electrochemical proton gradient across the membrane for ATP synthesis, CcO catalyzes the reduction of molecular

oxygen to water, which takes place at the binuclear center of the CcO [22]. Owing to the involvement of multiple redox-active metal centers and coupled electron-proton transfer steps, the catalytic performance of CcO is ultimately governed by its local electronic structure. To elucidate these electronic-level features, density functional theory (DFT) has been extensively applied to investigate the molecular-scale properties and catalytic behavior of CcO [23]. In particular, analyses of frontier molecular orbitals, including the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), offer valuable insight into electronic excitation characteristics and chemical reactivity at the active site [24, 25].

Within this framework, extensive experimental and theoretical efforts have been devoted to establishing the structural and electronic foundations of oxygen reduction in CcO. Han Du et al. [26] employed density functional theory using the OLYP-D3(BJ) functional to investigate the electronic structure of the cytochrome *c* oxidase active center, showing that variations in the local electronic environment influence oxygen reduction behavior. Ovalle and Malardier-Jugroot [27] compared the performance of different DFT methods in predicting the geometries, spin states, and binding energies of Fe-porphyrin oxygen-binding systems. Their results showed that the combination of the 6-311+G(2df) basis set for Fe atoms, 6-311+G(d) for N/O atoms, and 6-31G for C/H atoms provided satisfactory agreement for describing the electronic and structural characteristics of these systems. Chen et al. [28] further investigated the influence of different N/O coordination environments on the ORR/OER performance of single-atom catalysts using DFT calculations, while Zeng et al. [29] examined how axial ligands regulate the electronic structure and C-H bond activation selectivity of cytochrome P450 systems. In addition, Kajikawa and Muraoka [30] evaluated the performance of different functionals for describing spin-state energetics, structural variations, and vibrational properties in Fe(II) spin-crossover complexes, where the B3LYP/6-31G(d) level of theory was also studied.

Although previous computational studies have investigated the structural and catalytic characteristics of cytochrome *c* oxidase and related Fe-porphyrin systems, the molecular-level

relationship between electronic structure evolution and oxygen reduction activity has not yet been fully understood. In particular, the manner in which spin-state stability, charge redistribution, frontier orbital evolution, and reaction energetics collectively regulate oxygen activation during the catalytic cycle remains to be systematically clarified. In the present study, density functional theory (DFT) calculations were performed to investigate an Fe-centered cytochrome c oxidase active-site model through a sequential oxygen reduction pathway consisting of the Fe, Fe-OO, Fe-O~OH and Fe-O~OH₂ intermediates. Unlike previous studies that primarily focused on optimized structures or reaction energetics, the present work integrates structural evolution, electronic properties, charge-transfer behavior, and thermodynamic analysis to elucidate the origin of the highly efficient oxygen activation at the biological iron center. The results provide molecular-level insight into the electronic factors governing oxygen activation and proton-coupled reduction in cytochrome c oxidase and establish a theoretical basis for the design of biomimetic Fe-based catalytic systems.

2. Methodology

This section describes the computational methodology employed to investigate the electron-transfer processes and the oxygen reduction reaction (ORR) mechanism at the active site of cytochrome c oxidase (CcO). To gain molecular-level insight into the quantum-mechanical origin of the high efficiency of energy conversion in CcO, first-principles density functional theory (DFT) calculations were adopted. The computational approach was designed to evaluate the geometric stability of reaction intermediates, variations in electronic band gaps, and frontier molecular orbital characteristics, as well as to estimate thermochemical parameters relevant to catalytic activity and reaction kinetics.

Molecular models of the Fe-porphyrin biomimetic model were constructed using GaussView 5.0.8, including an Fe-centered heme structure, an Fe-O adduct representing the oxygen-bound intermediate, and hydrogen-incorporated configurations simulating proton-coupled steps associated with water formation and desorption. All electronic structure calculations were performed using the Gaussian 09 software package. The computational setup utilized the 6-31G(d,p) basis set in conjunction with DFT to perform geometry optimizations and vibrational frequency calculations. Using Gaussian 09, we conducted post-simulation analyses to extract optimized geometries, electronic band gaps, frontier molecular orbitals, absorption spectra, and thermochemical properties for all systems investigated.

2.1 Computational Details

The molecular structure was generated using GaussView 5.0.8. The Builder interface defined each atom and its bonding configuration, and then the atom positions were adjusted using the *Atom List* editor, specifying bond lengths, bond angles, and dihedral angles. Because CcO represents a relatively large molecular system, manual pre-optimization of the structure was performed to account for interatomic interactions and to improve the likelihood of successful convergence during geometry optimization with Gaussian 09. The atom types and their corresponding color representations employed for molecular visualization in this study are summarized in Table 1.

We modeled the active site of cytochrome c oxidase using a planar porphyrin ring, with the central Fe^{2+} ion coordinated by four nitrogen atoms. The structure has 87 atoms, including 36 C, 40 H, 4 N, 4 O, and 2 S atoms to represent the local biological environment surrounding the metal center. We examine the electron-transfer mechanism involved in the oxygen reduction reaction by considering four key reaction states: Fe, Fe-OO, Fe-O~OH, and Fe-O~OH₂.

Table 1: Atomic symbols and corresponding color representations used in molecular modeling.

Element	Carbon	Hydrogen	Oxygen	Nitrogen	Sulfur	Iron
Symbol	C	H	O	N	S	Fe
Color						

Geometry optimizations and vibrational frequency calculations were carried out within the framework of DFT. Vibrational frequency analyses were employed not only to obtain infrared vibrational spectra but also to verify the nature of the optimized structures. The absence of imaginary (negative) frequencies confirms that the optimized geometries correspond to stable minima on the potential energy surface, whereas the presence of imaginary frequencies indicates transition-state structures. All computations were based on density functional theory using the B3LYP exchange-correlation functional. For the cytochrome c oxidase active-site models, a 6-31G(d,p) basis set was employed to describe the electronic structure of the metal-ligand framework with sufficient accuracy. The oxygen molecule, treated separately as an isolated diatomic species, was described using the smaller 6-31G(d) basis set, which is commonly adopted for this purpose and provides reliable results without high computational cost.

During the electron transfer process, the oxidation of ferrous iron involves molecular oxygen. For this reason, molecular oxygen was also examined in the present analysis. Gaussian and GaussView were used to perform quantum chemical simulations, with the calculation parameters set

at the B3LYP/6-31G(d) level of theory. After geometry optimization, the O-O bond length was obtained as 1.215 Å. The electronic configuration of molecular oxygen is $(\sigma_{1s})^2(\sigma_{1s}^*)^2(\sigma_{2s})^2(\sigma_{2s}^*)^2(\sigma_{2p})^2(\pi_{2p})^4(\pi_{2p}^*)^2$. The formation of the adsorption bond is inferred to arise mainly from the interaction between the metal d orbitals and the π^* orbitals of molecular oxygen. The π^* orbitals of oxygen are not fully occupied, whereas the metal d orbitals contain a relatively high electron density. Under these conditions, electron sharing from the metal center to oxygen becomes possible.

The calculations were carried out using the B3LYP/6-31G(d,p) level of theory. This computational setup was chosen to follow the electron transfer process step by step, allowing the evaluation of energy gaps between intermediates, optimized geometries, vibrational frequencies, and the corresponding HOMO and LUMO characteristics at each stage. The 6-31G(d,p) basis set was employed as a split-valence scheme, where atomic orbitals are represented by Gaussian-type functions with different levels of contraction. The core, inner-shell, and valence orbitals are described using six, three, and one Gaussian functions, respectively. In practice, this provides sufficient flexibility to describe changes in electron distribution during bond formation and charge transfer. For systems with noticeable polarity, polarization functions are included to account for orbital distortion. In the present work, the addition of d- and p-type polarization functions improves orbital overlap for heavier atoms and leads to a more reliable representation of electronic interactions.

2.2 Reaction kinetics

In this study, total electronic energies obtained from quantum chemical simulations are integrated with statistical mechanics to evaluate the thermodynamic spontaneity and kinetic rates of the catalytic process.

2.2.1 Gibbs free energy change (ΔG)

To evaluate the thermodynamic landscape of the simulated catalytic cycles, the Gibbs free energy change (ΔG) is employed as the definitive criterion for determining the feasibility of each reaction step. It represents the maximum amount of non-expansion work that can be extracted from a closed system at constant temperature and pressure. To derive this value from quantum chemical

simulations, the raw ground-state electronic energy (ϵ_0) obtained from Gaussian 09, which reflects a molecule's potential energy at absolute zero, must be adjusted through thermochemical corrections. These corrections account for the zero-point energy (ϵ_{ZPE}), representing residual vibrations at 0 K, as well as the total internal thermal energy (E_{tot}) and entropy (S_{tot}). By applying this framework, we systematically quantified the thermodynamic feasibility of each catalytic step at the Fe-porphyrin biomimetic model and compared their relative energetic favorability along the reaction coordinate. The reaction enthalpy change (ΔH) is integrated with these entropy shifts to determine the free energy flux via the fundamental relationship:

$$\Delta G = \Delta H - T\Delta S \quad (1)$$

where T is the absolute temperature, and ΔS represents the entropy change.

2.2.2 Reaction Rate (k)

To quantitatively characterize the catalytic efficiency of the Fe-porphyrin biomimetic model, the calculated Gibbs free energy change is substituted into equations derived from transition state theory to estimate the reaction rate constant (k). The calculation incorporates the Boltzmann constant (k_B), Planck's constant (h), and the absolute temperature (T) [31]:

$$k(T) = \frac{k_B T}{h} e^{\frac{-\Delta G}{RT}} \quad (2)$$

This formula bridges microscopic quantum energies with the macroscopic reaction rate, enabling a kinetic evaluation of critical steps such as oxygen adsorption, O-O bond dissociation, and water desorption.

2.3 Sabatier activity analysis

To further evaluate the catalytic performance of the CcO active center in comparison with conventional inorganic catalysts (e.g., Pt), we applied Sabatier activity analysis. This approach is based on the Sabatier principle, which states that optimal catalytic performance requires an intermediate interaction strength between the catalyst and reactants. In the present quantitative analysis, the catalytic activity (A) of each elementary reaction step was evaluated using the reaction

rate constants from Eq. (2) and defined as [32, 33]:

$$A = k_B T \min_i \left(\log \left(\frac{k_i}{k_o} \right) \right) \quad (3)$$

where k_i denotes the rate constant of a specific elementary step. The parameter k_o represents a normalized reference rate constant introduced to establish a unified comparison scale across different catalytic systems. The physical implication is that the overall catalytic activity is governed by the least active (*i.e.*, slowest) elementary step, which typically corresponds to the rate-determining step of the catalytic cycle. By correlating the calculated activity values with the corresponding reaction energies, we constructed a comparative framework for evaluating catalytic performance.

3. Results and discussion

This section presents and discusses the computational findings regarding the catalytic activity of the CcO active center during the ORR. Through a detailed investigation of four primary intermediate configurations, namely the reduced state (Fe), the oxygen-adsorbed state (Fe-OO), the protonated state (Fe-O~OH), and the water-desorbing state (Fe-O~OH₂), the underlying high-efficiency catalytic mechanisms of this terminal respiratory enzyme are elucidated. Our analysis integrates multiple physical dimensions, including geometric stability, electronic structure characteristics (HOMO/LUMO), bandgap transitions, and thermochemical kinetics.

3.1 Molecular structure optimization

To examine how the choice of molecular center influences structural stability, a series of Fe-centered active-site models was investigated using geometry optimization and vibrational frequency analysis. In this work, the ferrous ion (Fe²⁺) was adopted as the central atom of the cytochrome c oxidase active-site model. Spin-state dependence was assessed by independently optimizing several electronic configurations of the Fe-centered model. We optimized the molecular structures in the singlet, triplet, and quintet spin states and assessed their relative stability by comparing the total electronic energies. The corresponding HOMO-LUMO energies, band gaps, and total energies are summarized in Table 2. Inspection of the optimized results shows that the triplet configuration lies lower in energy than the other two spin states. While no substantial differences are observed in the overall electronic features of the three configurations, the energy ordering clearly favors the triplet state within the present calculations. For this reason, the triplet configuration was selected as the reference electronic state for subsequent analysis of the Fe-centered model. Differences in the calculated band gaps further point to changes in the electronic structure associated with spin multiplicity, with the triplet state lying between the singlet and quintet cases.

Table 2: Total energies of optimized structures with different electronic configurations.

Energy parameter	Fe (01, singlet)	Fe (03, triplet)	Fe (05, quintet)
HOMO	-0.18122	-0.1827	-0.1595
LUMO	-0.08817	-0.07382	-0.07491
Bandgap (Ha)	0.09403	0.10888	0.08459
Bandgap (eV)	2.55865	2.96273	2.30178
Energy (Ha)	-3976.006	-3976.065	-3976.047
Energy (eV)	-108191.099	-108192.705	-108192.215

When cytochrome c oxidase is described using a ferrous ion as the central species, the optimized structures obtained from the present calculations show a clear dependence on spin multiplicity. The triplet configuration consistently converged to a lower energy than the other two states. While the broad electronic profiles of these optimized structures appear similar, the calculated energy variance indicates that the triplet state is the most stable configuration within our current DFT framework. This behavior is closely related to the electronic configuration of $1s^2 2s^2 2p^6 3s^2 3p^6 3d^6$, where the partially filled $3d^6$ shell allows several competing electron arrangements. Ultimately, the system's ground state is determined by a delicate trade-off between exchange stabilization and the energy cost of electron pairing. In the present case, this balance favors a triplet configuration rather than a fully paired singlet state.

Figure 1 presents the optimized geometries of the Fe-centered CcO active-site model at different stages of the oxygen reduction process, as obtained from density functional theory calculations at the B3LYP/6-31G(d,p) level. The optimized structure shown in Fig. 1(a) corresponds to the bare Fe-centered active site in the triplet spin state. Based on these findings, the triplet spin configuration was adopted in all subsequent simulations to represent the ground-state electronic structure of the Fe-centered cytochrome c oxidase active-site model. Upon the introduction of molecular oxygen, the optimized structure shown in Fig. 1(b) exhibits a clear geometric response of the Fe active center to O_2 coordination. The oxygen molecule is found to bind directly to the central Fe atom, forming an Fe-OO intermediate, as confirmed from the front-view configuration. This adsorption step indicates

that the Fe center can activate molecular oxygen via direct metal-oxygen interaction and the charge redistribution occurs between the metal center and the adsorbed oxygen species, yielding a negatively charged complex characteristic. After molecular oxygen binds to the Fe center, the optimized Fe-OO structure uses an oxygen-bound configuration as a dianionic complex since an electron transfer from the metal center to the coordinated O₂ species. Upon proton addition, the system evolves into an Fe-OOH intermediate, where the hydrogen atom associates with the oxygen moiety bonded to Fe. During this process, a hydroxide ion is released, and the overall negative charge of the system is correspondingly reduced. The resulting optimized geometry of the Fe-O~OH-centered configuration is presented in Fig. 1(c). At the final stage, Figure 1(d) shows the optimized geometry obtained after further protonation of the oxygen-bound intermediate. At this stage, the O-O bond is no longer preserved, and the structure relaxes into a configuration consistent with water formation at the Fe center. The additional protonation step drives the cleavage of the O-O linkage, and the resulting complex no longer carries a net charge. Checking the preceding structures in Fig. 1(a)-(d), this geometry reflects the final structural outcome of the oxygen reduction sequence at the cytochrome c oxidase active site.

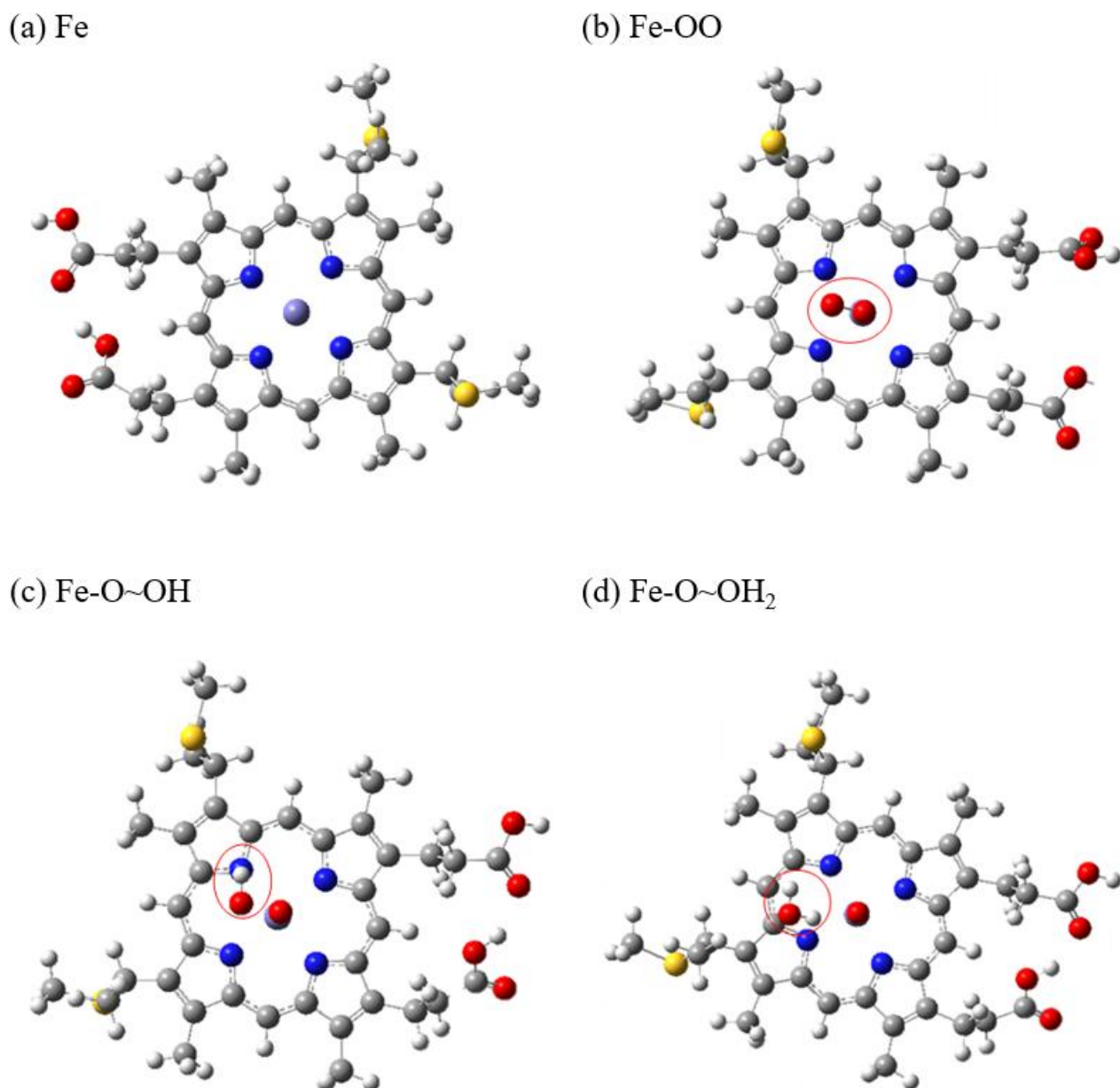


Figure 1: Optimized geometry of the Fe-centered cytochrome *c* oxidase active-site model: (a) bare Fe center, (b) Fe-OO intermediate, (c) Fe-O~OH intermediate, and (d) Fe-OOH₂ configuration.

Figure 2 displays the simulated infrared (IR) absorption spectrum of the Fe-centered cytochrome *c* oxidase model, calculated using DFT. The spectrum plots vibrational frequency on the horizontal axis against both the molar absorption coefficient and the dipole strength on the vertical axis. The complex bonding environment of the catalytic center is reflected in the overall spectral distribution. The IR spectrum confirms that no imaginary frequencies are present after geometry optimization, verifying that the computed structure represents a stable energy minimum. Thus, the simulated

vibrational profile supports the reliability of the optimized model and the consistency of its bonding configuration.

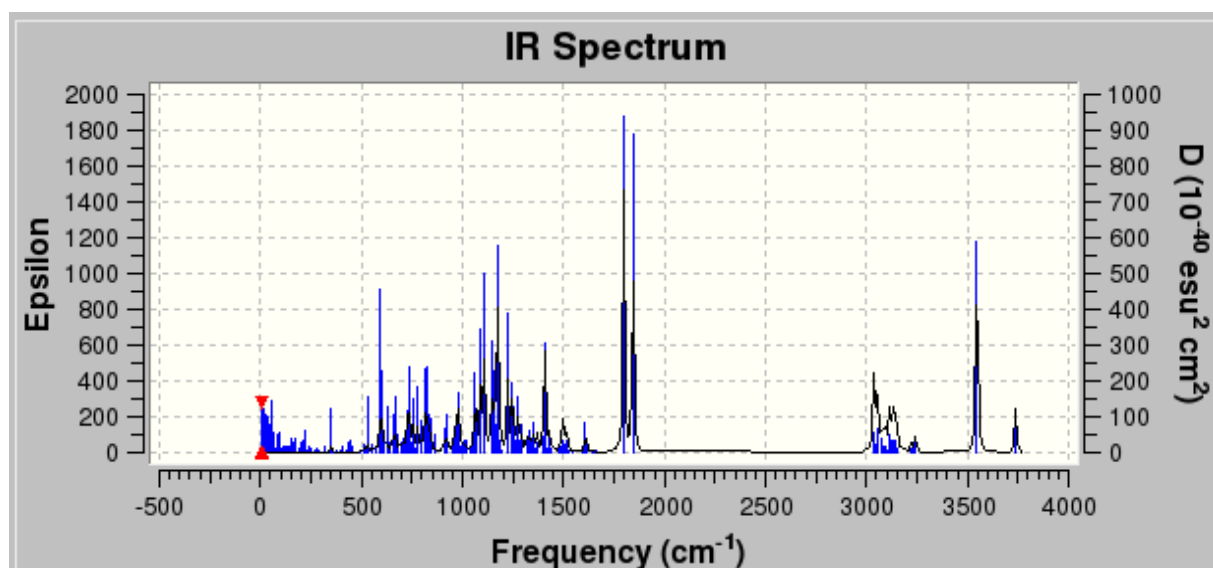


Figure 2: Simulated IR absorption spectrum of the Fe-centered cytochrome c oxidase model.

As shown in Fig. 2, distinct vibrational features can be identified for different reaction intermediates in the simulated IR spectra. The absorption bands observed in the low-frequency region (400 to 600 cm^{-1}) are mainly attributed to the Fe-N and Fe-O stretching vibrations, indicating that the coordination structure between the iron center and the porphyrin ring remains stable throughout the reaction. These bands also confirm the successful coordination of molecular oxygen to the Fe active site. Upon oxygen adsorption, a pronounced O-O stretching band appears in the range of 900-1200 cm^{-1} . Compared with free molecular oxygen, this vibrational band exhibits an obvious red shift, indicating electron back-donation from the Fe center to the antibonding orbitals of the oxygen molecule. As a result, the O-O bond is elongated and weakened, suggesting that molecular oxygen has been effectively activated for the subsequent oxygen reduction reaction. In the region between 1500 and 1700 cm^{-1} , characteristic C=C and C=N skeletal vibrations of the porphyrin ring are observed, indicating that the conjugated π -electron system remains structurally stable during the reaction process. This stable conjugated framework facilitates electron transport and promotes the electron-transfer process associated with oxygen reduction. In addition, pronounced O-H stretching bands are observed in the region of 3200-3700 cm^{-1} for the Fe-OOH and Fe-OOH₂ intermediates,

indicating the incorporation of protons and the formation of hydrogen bonds during the reaction process. These spectral features are consistent with the proton-coupled electron transfer mechanism and further demonstrate the essential role of water formation in the oxygen reduction reaction. The characteristic vibrational features identified for the different reaction intermediates are summarized in Table 3.

Table 3: Characteristic vibrational features of different reaction intermediates identified from the simulated IR spectra.

Wavenumber (cm ⁻¹)	Vibrational mode	Intermediate	Physical significance
400-600	Fe-N / Fe-O stretching	Fe, Fe-OO	Indicates the stable coordination structure between the Fe center and the porphyrin ring, confirming the formation of the Fe-O coordination bond upon oxygen adsorption.
900-1200	O-O stretching	Fe-OO	The red shift relative to free O ₂ indicates electron back-donation from the Fe center to the antibonding orbitals of oxygen, resulting in O-O bond weakening and activation.
1100-1400	C-N / C-C skeletal vibrations	All intermediates	Reflects the structural stability of the conjugated porphyrin framework, facilitating electron transport during the oxygen reduction reaction.
1500-1700	Porphyrin C=C / C=N skeletal vibrations	Fe, Fe-OO, Fe-OOH	Indicates that the conjugated π -electron system remains stable during oxygen adsorption and protonation.
1800-1900	Polarized Fe-O coordination vibration	Fe-OO, Fe-OOH	Suggests local charge redistribution after oxygen adsorption, leading to a highly polarized Fe-O coordination environment.
3200-3600	O-H stretching	Fe-OOH, Fe-OOH ₂	Indicates proton incorporation and hydrogen-bond formation, consistent with the proton-coupled electron transfer (PCET) mechanism.
3400-3700	H ₂ O-related O-H vibration	Fe-OOH ₂	Corresponds to water formation and desorption, representing the final product of the oxygen reduction reaction.

3.2 Spin-state analysis of the oxygen activation pathway at the Fe center

To further investigate the electronic structure evolution during oxygen activation, density functional theory (DFT) calculations were performed for the Fe-centered active site of cytochrome c oxidase (CcO). By comparing the relative energies of different spin states, the most stable electronic configurations of the reaction intermediates were identified, allowing the oxygen activation pathway involving the Fe-O₂, Fe-O-OH, and Fe-O-H₂O intermediates to be established. The calculated spin-state energetics and relative stabilities of these intermediates are summarized in Table 4.

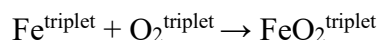
Table 4: Spin-state energetics and oxygen activation pathway of the Fe-centered cytochrome c oxidase active-site model calculated by DFT.

Intermediate	Spin state	Charge	Multiplicity	SCF Energy (Hartree)	Relative Energy (kcal/mol)	Stability
O ₂	Triplet	0	3	-150.320042	—	Ground state O ₂
Fe center	Singlet	0	1	-3976.006253	+36.9	Higher
Fe center	Triplet	0	3	-3976.065049	0	Lowest
FeO ₂	Singlet	-2	1	-4126.344908	+12.3	Higher
FeO ₂	Triplet	-2	3	-4126.364550	0	Lowest
Fe-O-OH	Singlet	-1	1	-4127.052923	+7.9	Higher
Fe-O-OH	Triplet	-1	3	-4127.065439	0	Lowest
Fe-O-H ₂ O	Quintet	0	5	-4127.666491	—	Calculated state

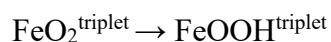
The spin-state energetics summarized in Table 4 indicate that the triplet configuration is energetically more favorable than the singlet configuration for the isolated Fe center by approximately 36.9 kcal/mol. This result suggests that the Fe active site preferentially adopts a high-spin triplet ground state with two unpaired electrons, which is expected to facilitate the subsequent adsorption and activation of molecular oxygen.

After oxygen adsorption, the Fe-O₂ intermediate also favors the triplet configuration, which is 12.3 kcal/mol lower in energy than the corresponding singlet state. The preservation of the triplet

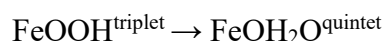
state indicates that the interaction between molecular oxygen and the Fe center is stabilized through metal-oxygen electronic coupling without changing the overall spin multiplicity. Accordingly, the oxygen adsorption process can be described as



Following protonation, the Fe-O-OH intermediate remains energetically more stable in the triplet state, with an energy difference of 7.9 kcal/mol relative to the singlet configuration. This result suggests that oxygen activation and protonation proceed while maintaining the triplet electronic configuration. The corresponding reaction step can therefore be expressed as



When the oxygen species is further converted into the hydrated Fe-O-H₂O intermediate, the preferred spin state changes from triplet to quintet, indicating that electronic rearrangement occurs during the final stage of oxygen reduction. This spin-state transition can be represented as



Based on the calculated spin-state energetics of all reaction intermediates, the proposed spin-state-dependent oxygen activation pathway is summarized in Fig. 3.

Spin-State-Driven Oxygen Activation Pathway at the Fe Active Site

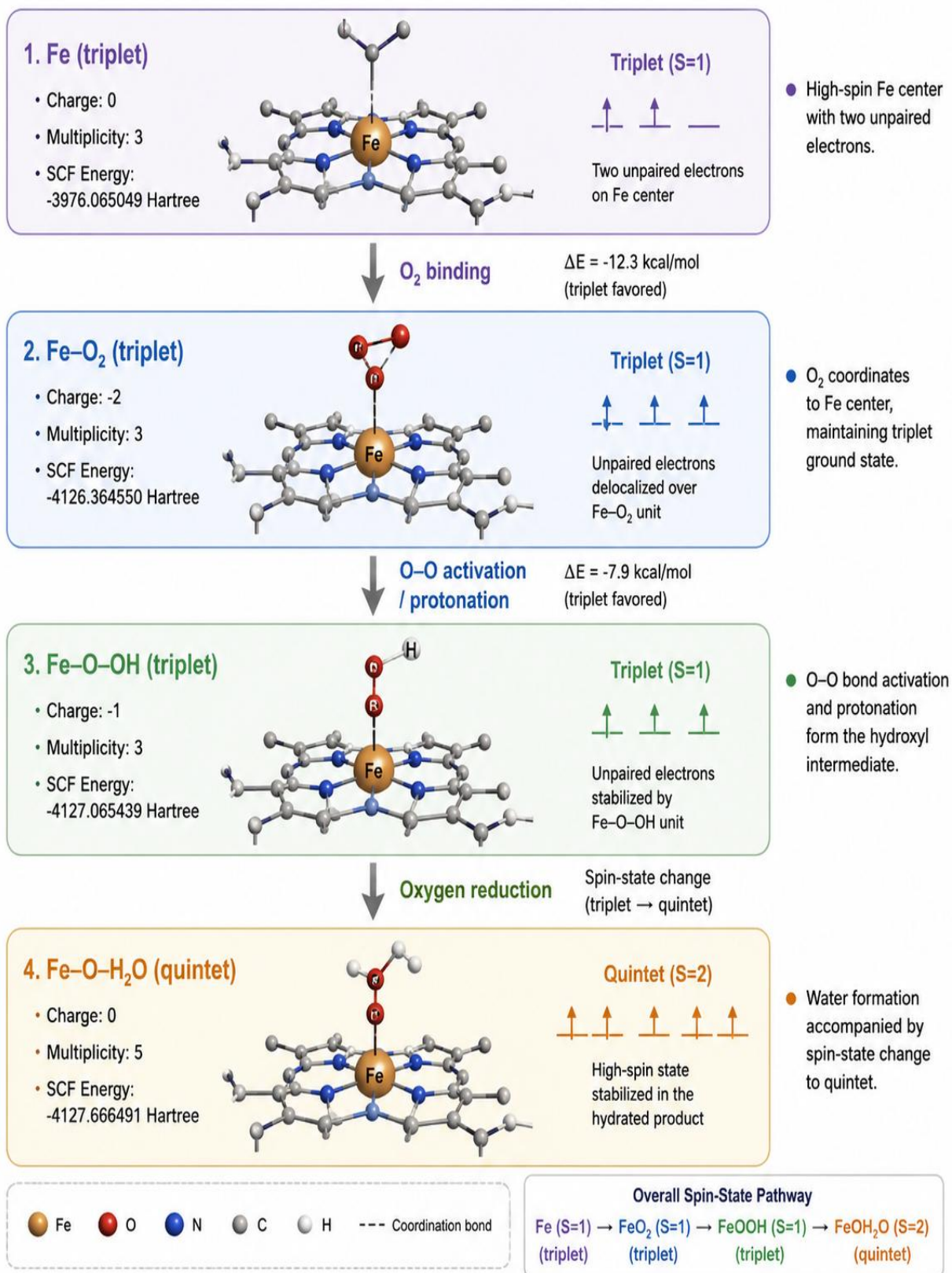


Figure 3: Proposed spin-state-dependent oxygen activation pathway at the Fe-centered cytochrome c oxidase active site.

3.3 Molecular orbital characteristics

For deeper insights into the electronic behavior and chemical reactivity of the CcO active center, the frontier molecular orbitals (FMOs), specifically the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), were analyzed for each catalytic intermediate. According to FMOs theory, the spatial distribution of these orbitals reveals the primary sites for electron donation and acceptance, while the energy gap between them serves as a critical indicator of the molecule's kinetic stability and chemical reactivity.

The electronic structures of the Fe-centered CcO intermediates (Fe, Fe-OO, Fe-O~OH, and Fe-O~OH₂) were analyzed by examining the energies of HOMO and LUMO and the corresponding HOMO-LUMO gaps. The calculated orbital energies and total electronic energies for these species are compiled in Table 5, while the relative alignment of the HOMO and LUMO levels is shown in Figure 4. Inspecting the results reveals that the separation between the frontier orbitals is not uniform across the intermediates. The Fe-OO structure, in particular, converges to a noticeably smaller HOMO-LUMO gap than the other configurations examined. This feature points to a higher degree of electronic adaptability following O₂ coordination at the Fe center. This minimal energy gap identifies the oxygen-bound complex as the most chemically active intermediate among the four structures, facilitating the rapid activation and subsequent cleavage of the O-O bond. In contrast, the remaining intermediates retain wider gaps, suggesting that their electronic structures are less readily perturbed.

Table 5: Energy output results for the CcO active center across different molecular intermediates.

Energy parameter	Fe	Fe-OO	Fe-O~OH	Fe-O~OH ₂
HOMO	-0.18266	0.10687	-0.05012	-0.19228
LUMO	-0.07382	0.14299	0.03045	-0.12146
Bandgap(Ha)	0.10888	0.03612	0.08057	0.07082
Energy (Ha)	-3976.065	-4126.344	-4127.073	-4127.666

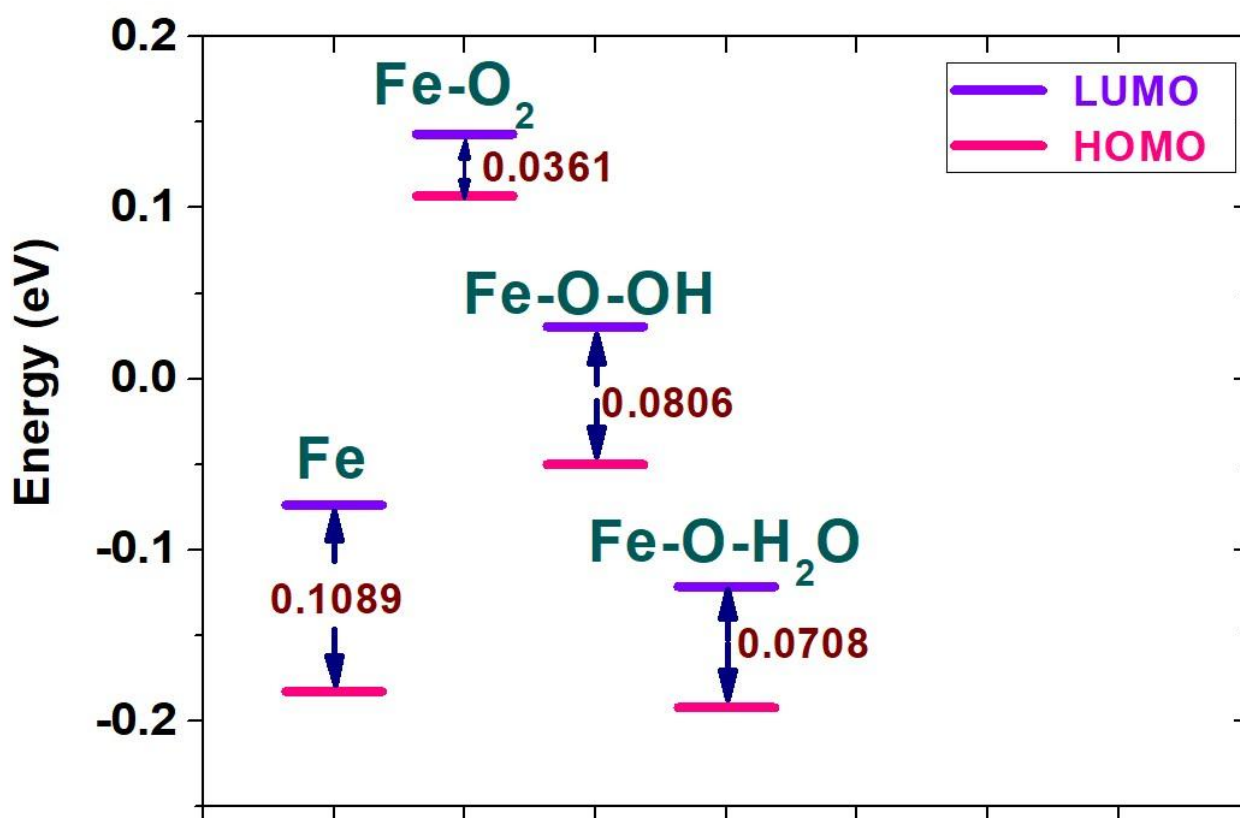


Figure 4: Graphical representation of the HOMO and LUMO energy levels for the CcO active center across different molecular intermediates.

To provide a clearer view of the electronic characteristics along the reaction pathway, the spatial distributions of the HOMO and LUMO were examined for each intermediate. The corresponding frontier orbital plots are summarized in Figure 5. For the reduced state (Fe), the electron density is predominantly distributed across the porphyrin ring, with the LUMO showing significant concentration at the metallic center, designating it as the primary site for electron acceptance. Upon the formation of the oxygen-adsorbed state (Fe-OO), the HOMO density becomes highly localized at the coordination interface between the superoxo species and the central iron ion, as well as within the porphyrin ring. This uniform distribution confirms that this region serves as the functional chemical reaction center for oxygen activation. At the same time, the LUMO distribution appears more spatially extended and less uniform than in the reduced state, suggesting an increase in electronic delocalization associated with oxygen binding. In the subsequent protonated intermediate (Fe-O~OH), the HOMO density shifts significantly toward the oxygen-bonding region and the hydroxyl group. Interestingly, during the transition to the LUMO energy level, the electron density around the hydroxyl group becomes nearly negligible, with the density relocating back to the CcO porphyrin ring. Finally, for the water-desorbing state (Fe-O~OH₂), the redox transformations are primarily localized within two specific functional regions: the macrocyclic porphyrin ring and the coordination interface between the central iron ion and its axial ligands.

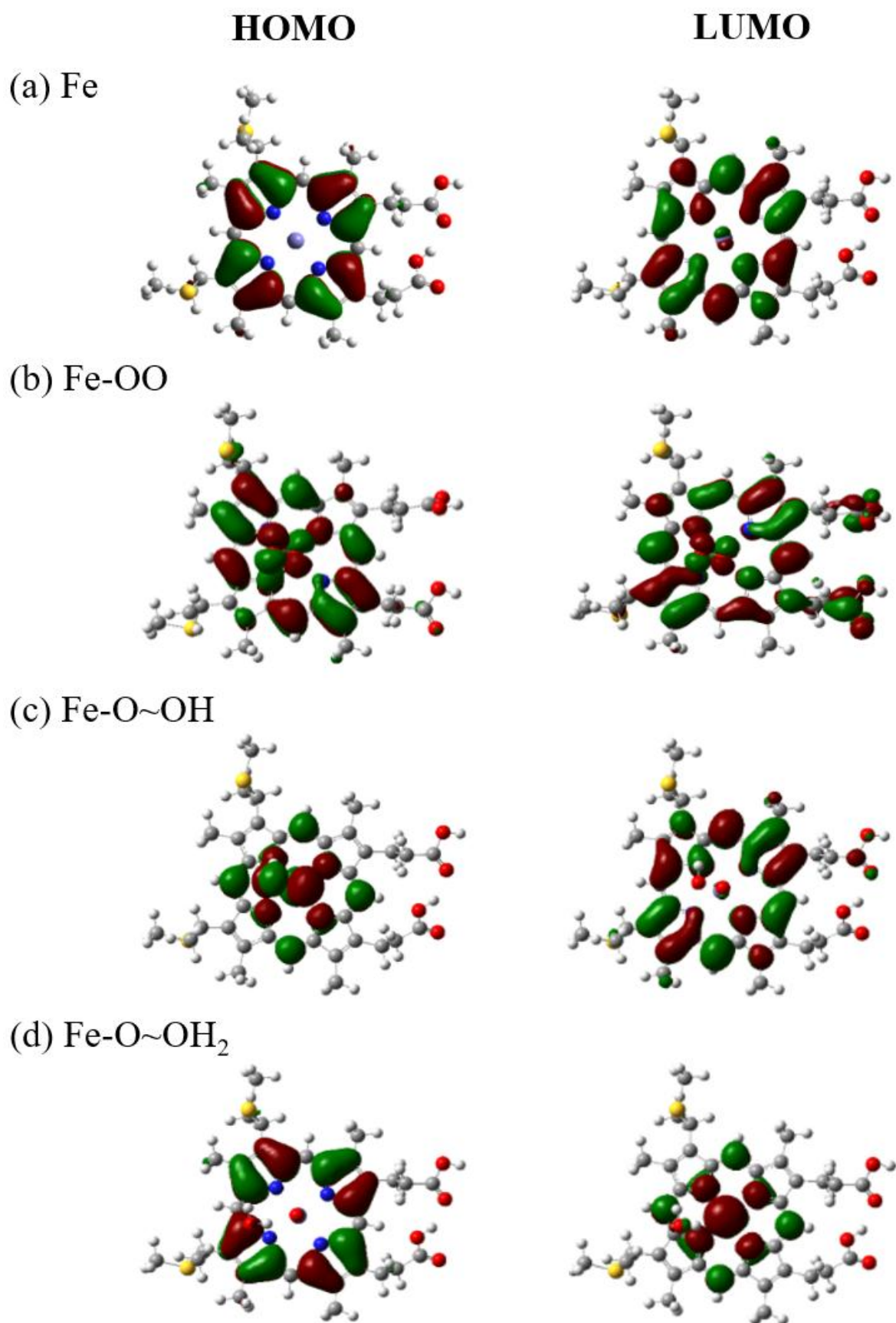


Figure 5: Spatial distributions of the HOMO and LUMO for the Fe-centered CcO intermediates: (a) Fe, (b) Fe-OO, (c) Fe-O~OH, and (d) Fe-O~OH₂.

3.4 Thermochemical energy and kinetics

The quantitative evaluation of the catalytic efficiency of the CcO active center is based on the thermochemical parameters and reaction kinetic constants calculated for each stage. The fundamental thermochemical values required for this study were obtained from the GaussView output files following the completion of DFT simulations, as summarized in Table 6. These values include the total electronic energy (ϵ_0), zero-point energy corrections (ϵ_{ZPE}), and thermal corrections to enthalpy and Gibbs free energy. By utilizing the data from Table 5, we determined the transition energies for oxygen adsorption, dissociation, and water desorption. The resulting enthalpy of reaction (ΔH), Gibbs free energy (ΔG), and reaction rate constants (k) are presented in Table 7. Our simulation results reveal that the ΔH for all investigated steps is negative, which confirms that the entire reduction process at the Fe-centered active site is highly exothermic. An examination of the entire reaction coordinate indicates that the ΔG for each catalytic phase consistently resides in the negative regime. This sustained exergonic characteristic substantiates that every stage possesses a significant degree of thermodynamic spontaneity under the simulated physiological conditions. From a kinetic perspective, the theoretical reaction rate constants suggest high turnover frequencies during the catalytic cycle. Despite the thermodynamic spontaneity of all steps, the oxygen adsorption process is identified as the critical energy threshold and serves as the rate-determining step for the entire reaction. The efficiency with which the CcO active center overcomes this barrier is a cornerstone of its biological function and high energy conversion efficiency.

Table 6: Calculated electronic energies and thermochemical corrections (in Hartree) for the Fe-centered active site intermediates of CcO.

	Fe	Fe-OO	Fe-O~OH	Fe-O~H ₂ O
ϵ_0	-3955.674	-4105.139	-4105.877	-4106.459
ϵ_{ZPE}	0.706414	0.702605	0.718291	0.729454
E_{tot}	0.753711	0.754656	0.769761	0.781547
H_{corr}	0.754656	0.755601	0.770705	0.782491
G_{corr}	0.622179	0.609846	0.628672	0.638968
$\epsilon_0 + \epsilon_{\text{ZPE}}$	-3954.968	-4104.437	-4105.158965	-4105.730
$\epsilon_0 + E_{\text{tot}}$	-3954.920	-4104.385	-4105.107	-4105.678
$\epsilon_0 + H_{\text{corr}}$	-3954.919	-4104.384	-4105.10	-4105.677
$\epsilon_0 + G_{\text{corr}}$	-3955.052	-4104.530	-4105.248	-4105.820

Table 7: Calculated ΔH , ΔG , and K for the key stages.

	ΔH (Hartree)	ΔH (eV)	ΔG (Hartree)	ΔG (eV)	k (1/s)
O ₂ absorption	-0.19	-5.17	-0.172	-4.702E-06	8.211E91
O ₂ dissociation	-0.24	-6.58	-0.23	-6.47	3.951E118
H ₂ O desorption	-0.1058	-2.89	-0.094	-2.564E-06	8.463E55

3.5 Comparative analysis of catalytic performance

To place the catalytic behavior of the Fe-centered CcO model in a broader context, its performance was compared with representative inorganic catalyst systems. While traditional fuel cell technologies typically achieve only 40% to 60% energy conversion efficiency, biological systems like the electron transport chain demonstrate an extraordinary efficiency. This performance gap underscores the potential of biomimetic structures to overcome the kinetic limitations of conventional noble metal materials. Recent DFT studies on Pt-based catalysts have shown that the reaction activity is highly sensitive to the carbon substrate's morphology and Pt loading. Specifically, Liu et al. [34] identified that 18.48 wt% Pt on single-wall carbon nanotubes and 85.27 wt% Pt on graphene represent the optimal configurations for maximizing reaction area and maintaining activity. Their findings highlight that substrate curvature significantly alters the oxygen binding energy, where the most effective catalysts must balance the force of reactant adsorption with product desorption near a zero activation energy.

In contrast, the thermochemical analysis of the Fe-centered CcO model reveals a more favorable energetic profile across the entire reaction coordinate. As documented in Table 7, every stage, encompassing oxygen adsorption, dissociation, and water desorption, is thermodynamically spontaneous ($\Delta G < 0$) and highly exothermic, with negative reaction enthalpies (ΔH). Sabatier analysis (Fig. 6) further demonstrates that the biological iron-porphyrin environment provides a superior electronic configuration that achieves a more optimal catalytic balance than the advanced platinum-carbon catalyst systems. In this representation, the horizontal axis denotes the reaction energy (ΔE_0), while the vertical axis corresponds to the calculated catalytic activity. The black diagonal reference lines and the associated data points are taken from the literature [33], serving as benchmarks for established metallic catalysts. The line with a positive slope represents the water desorption-limited regime, whereas the line with a negative slope corresponds to the oxygen dissociation-limited regime, reflecting the classical volcano-type relationship predicted by Sabatier theory. To validate the reliability of the computational methodology employed in the present study,

additional reference data were calculated for graphene-based and Pt(111) systems and are shown as light-blue and brown markers. These results exhibit good agreement with previously reported values, confirming the consistency of the adopted computational approach. The red and blue symbols correspond to Pt-doped single-walled carbon nanotubes and Pt-doped graphene systems, respectively [34]. The green symbols represent the results obtained in this work for the Fe-centered cytochrome c oxidase active site. Different reaction steps are identified using distinct marker shapes, with circles representing oxygen dissociation, diamonds indicating oxygen adsorption, and triangles corresponding to water desorption. The data associated with the Fe-centered biomimetic active site exhibit intrinsic catalytic features that are competitive with those of conventional Pt-based systems. These comparative results indicate that bio-inspired structures may serve as effective design models for developing alternative non-noble-metal catalysts with improved efficiency for fuel cell technologies.

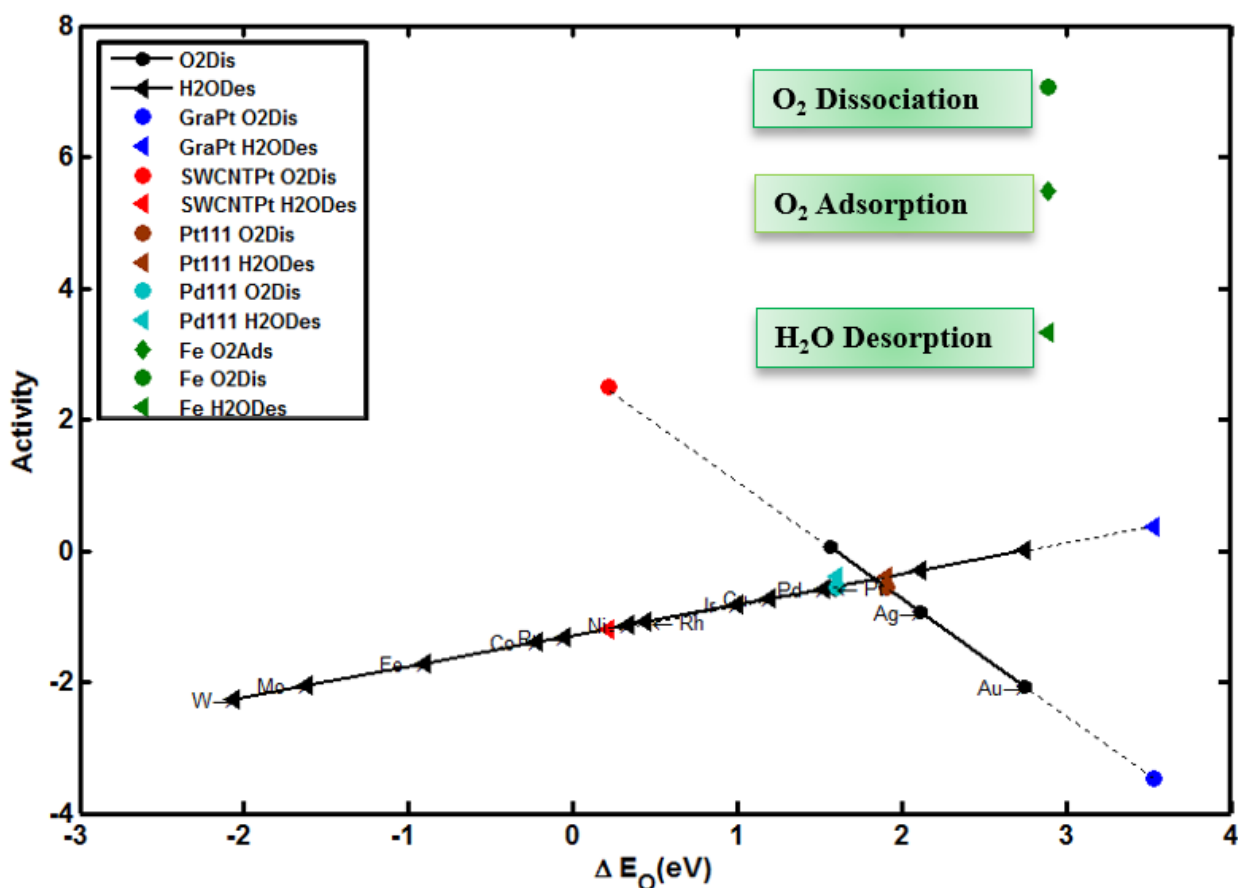


Figure 6: Sabatier-type activity plot comparing the Fe-centered CcO active site with inorganic catalysts.

4. Conclusions

In this study, density functional theory calculations were performed to investigate the oxygen reduction reaction at an Fe-centered cytochrome c oxidase active site model. To characterize the catalytic behavior of the biologically active center, we carried out geometric optimization together with electronic structure, thermochemical, and kinetic analyses. Our findings lead to the following key insights:

- The triplet configuration consistently converged to the lowest total energy, providing the most reasonable electronic description of the Fe-centered active site within the current model.
- Oxygen coordination was found to markedly alter the frontier orbital structure. The Fe-OO intermediate exhibits a reduced HOMO-LUMO separation, indicating enhanced electronic flexibility during the oxygen activation stage.
- Through Sabatier's activity analysis, it becomes evident that the Fe-porphyrin coordination environment is uniquely tuned for catalysis. It manages to strike a "perfect" balance: the interaction is strong enough to capture reactants yet weak enough to facilitate product desorption, maintaining high activity across the entire reaction coordinate.
- Compared with conventional Pt-based catalysts, the Fe-centered CcO model exhibits a more uniformly favorable energetic profile, highlighting the intrinsic efficiency of biologically active sites.

These results support the potential of bio-inspired iron-based architectures as effective design models for catalysts in fuel cell applications. By comparing the energies of different spin states, the oxygen activation pathway at the Fe center can be further clarified. The calculated results indicate that the Fe, Fe-O₂, and Fe-O-OH intermediates preferentially adopt the triplet state, suggesting that unpaired electrons play an important role in molecular oxygen activation. When the reaction proceeds to the Fe-O-H₂O structure, the electronic configuration is rearranged and shifts toward the quintet state. Therefore, spin-state variation can be regarded as an important factor for describing the oxygen reduction mechanism at the active site of cytochrome c oxidase. These findings reveal that the

intrinsic electronic regulation provided by the Fe-porphyrin environment plays a key role in oxygen activation and provides mechanistic guidance for the design of biomimetic non-precious-metal catalysts.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding Declaration

The authors received no financial support for the research and publication of this article.

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