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CAMPUS DE EXCELENCIA INTERNACIONAL

INTERSYSTEM CROSSING IN SYSTEMS LACKING METAL
COFACTORS

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Tesis doctoral

Salamanca, 2023

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La presente Tesis Doctoral se ha elaborado mediante un compendio de los siguientes trabajos publicados:

1. **DpgC-catalyzed peroxidation of 3,5-dihydroxyphenylacetyl-CoA (DPA-CoA): Insights into de spin-forbidden transition and charge transfer mechanisms.**

Pablo Ortega, Alexander Zanchet, Cristina Sanz-Sanz, Susana Gómez-Carrasco, Lola González-Sánchez, Pablo G. Jambrina

Chemistry: An European Journal (2020), 27, 1700-1712

<https://doi.org/10.1002/chem.202002993>

Factor de impacto (2020): 5.236

Posición JCR: Química: Multidisciplinar (52/178) Q2

2. **Multi- and single-reference methods for the analysis of multi-state peroxidation of enolates.**

Pablo Ortega, Sara Gil-Guerrero, Anzhela Veselinova, Alexander Zanchet, Lola González-Sánchez, Pablo G. Jambrina, Cristina Sanz-Sanz

Journal of Chemical Physics (2021), 154, 144303

<https://doi.org/10.1063/5.0046906>

Factor de impacto (2021): 4.304

Posición JCR: Física atómica y molecular (8/36) Q1

3. **Spin-forbidden addition of molecular oxygen to stable enol intermediates. Decarboxylation of 2-methyl-1-tetralone-2-carboxylic acid.**

Pablo Ortega, Sara Gil-Guerrero, Cristina Sanz-Sanz, Lola González-Sánchez, Pablo G. Jambrina

International Journal of Molecular Sciences (2023), 24(8), 7424

<https://doi.org/10.3390/ijms24087424>

Factor de impacto (2021): 6.208 (2023 aún no disponible)

Posición JCR: Bioquímica y biología molecular (69/297) Q1

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Acknowledgements

This thesis is the collaborative work of a team of people (and parts of others), and each piece is indispensable to the successful completion of the research work. For this, I thank my thesis directors and all the co-authors present in the publications achieved.

Thanks are also due to the funding obtained from the Salamanca City Council and the Salamanca Cultura y Saberes Foundation, which started this project, the subsequent pre-doctoral contract from the Junta de Castilla y León (EDU/601/2020), and the project funding from Ministerio de Ciencia e Innovación (PID2020-113147GA-I00 funded by MCIN/AEI/10.13039/501100011033).

List of publications

Related to this thesis

- P. Ortega, A. Zanchet, C. Sanz-Sanz, S. Gómez-Carrasco, L. González-Sánchez, P. G. Jambrina, *Chemistry*, (2021), **27**, 1700–1712.
- P. Ortega, S. Gil-Guerrero, A. Veselinova, A. Zanchet, L. González-Sánchez, P. G. Jambrina, C. Sanz-Sanz, *J. Chem. Phys.*, (2021), **154**, 144303.
- P. Ortega, S. Gil-Guerrero, C. Sanz-Sanz, L. González-Sánchez, P. G. Jambrina, *Int. J. Mol. Sci.*, (2023), **24(8)**, 7424

Other collaborations

- A. Esteban, I. Izquierdo, N. García, M. J. Sexmero, N. M. Garrido, I. S. Marcos, F. Sanz, P. G. Jambrina, P. Ortega, D. Díez, *Tetrahedron*, (2020), **76**, 130764.
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- M. Sanz-Novo, P. Ortega, P. Redondo, A. Largo, J. L. Alonso, C. Barrientos, *ApJ*, (2022), **941**, 40

List of acronyms used in this text

ASCI: Adaptively Selected Configuration Interaction

BS-DFT: Broken-symmetry Density Functional Theory

CASPT2: Complete Active Space Perturbative Theory 2

CASSCF: Complete Active Space Self-Consistent Field

CC: Coupled Cluster

CCSD(T): Coupled Cluster with Singles, Doubles and perturbative Triples

CI (S,D,Q...): Configuration Interaction (with Singles, Doubles, Quadruples, etc)

CPO: Coproporphyrin oxidase

CSF: Configuration state function

DFT: Density Functional Theory

DPA-CoA: 3,5-dihydroxyphenylacetyl-CoA

DpgC: 3,5-dihydroxyphenylacetyl-CoA 1,2-dioxygenase

FAD: Flavin adenine dinucleotide

FCI: Full Configuration Interaction

FMN: Flavin mononucleotide

HF: Hartree-Fock

HOD: Hydroxyoxoquinaldine dioxygenase

IBO: Intrinsic Bond Orbitals

ISC: Intersystem Crossing

MECP: Minimum Energy Crossing Point

MEP: Minimum Energy Path

MRCI: Multireference Configuration Interaction

MRPT: Multireference Perturbation Theory

NA-TST: Non-adiabatic Transition State Theory

NEVPT: N-Electron Valence Perturbation Theory

NMO: Nogalamycin monooxygenase

ONIOM: Our own N-layered Integrated molecular Orbital and molecular Mechanics

PDT: Photodynamic therapy

PES: Potential Energy Surface

PNO-LCCSD(T): Pair Natural Orbital Local CCSD(T)

QDPT: Quasi-degenerate Perturbation Theory

QM/MM: Quantum Mechanics/Molecular Mechanics

RASCI: Restricted Active Space Configuration Interaction

ROS: Reactive oxygen species

SCF: Self-Consistent Field

SCI: Selected Configuration Interaction

SHCI: Semi Heat-Bath Configuration Interaction

SMD: Solvation Model Density

SOC: Spin-Orbit Coupling

UOX: Urate oxidase

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1 Resumen, objetivos, relación entre artículos y conclusiones

1.1 Resumen y antecedentes

Pese a considerarse procesos impedidos según la Mecánica Cuántica convencional, las reacciones con cambio de espín (o cruces intersistema) se producen gracias al desarrollo relativista del acoplamiento espín-órbita. La molécula de oxígeno, una de las moléculas más conocidas con un triplete como estado fundamental, participa en multitud de reacciones con sustratos orgánicos, mayoritariamente en su estado fundamental singlete. En este trabajo se estudian los mecanismos teóricos que gobiernan este tipo de reacciones, entrando en el máximo detalle posible sobre sus características, sus particularidades y considerando especialmente la propiedad de acoplamiento espín-órbita para dar respuesta a las incógnitas que rodean estos mecanismos, principalmente en reacciones biológicas catalizadas sin ningún metal ni cofactor.

El cruce intersistema (ISC, por sus siglas en inglés) es un fenómeno que implica la transición de un estado electrónico a otro de diferente multiplicidad de espín, generalmente, a lo largo de este texto, de un estado singlete a uno triplete, o viceversa, sin emisión o absorción de radiación. En condiciones normales, estas transiciones están prohibidas por las reglas de selección de espín, sin embargo, pueden ocurrir a través de la interacción entre el espín y el momento angular orbital, conocido como acoplamiento espín-órbita (SOC, por sus siglas en inglés).

La eficiencia de los ISC es directamente proporcional a la magnitud del SOC, que a su vez depende del número atómico del átomo implicado. En el caso de átomos pesados, como los metales de transición, el SOC es mayor y puede facilitar el ISC. Por

esta razón, entre otras, los metales pesados son catalizadores comunes para diversas reacciones, que abarcan desde la síntesis bioquímica hasta la orgánica/inorgánica y los procesos industriales. Sin embargo, en los sistemas que carecen de átomos metálicos, el SOC es relativamente débil y el ISC es generalmente lento.

A pesar de estas limitaciones, se ha observado que ciertos sistemas orgánicos pueden experimentar ISC eficientes en ausencia de átomos metálicos pesados. Este fenómeno intrigante sugiere la existencia de mecanismos adicionales que pueden facilitar la transición entre estados de diferente multiplicidad de espín.

Inicialmente se asumía que todas las reacciones catalizadas por oxidasas y oxigenasas necesitaban la presencia de metales pesados, tales como los que intervienen en la oxidación de la hemoglobina, o la incorporación de otros cofactores. Tomemos como ejemplo la oxidación de la hemoglobina, una reacción catalizada por metal donde los grupos hemo desempeñan un papel protagónico. Los grupos hemo son estructuras orgánicas que integran en su composición un átomo de hierro que se encuentra coordinado dentro de una extensa estructura orgánica heterocíclica conocida como porfirina. Estas estructuras tienen relevancia significativa en varios procesos biológicos, siendo el transporte de oxígeno uno de los más destacados. En este contexto, es el átomo de hierro ubicado en el núcleo del grupo hemo el que aporta el suficiente SOC para que se lleve a cabo la reacción.

En cuanto a los cofactores no metálicos, la flavina es la más común, utiliza el mecanismo de transferencia de carga para facilitar el cambio de espín en el oxígeno. Las especies de flavina están entre los cofactores más comunes en procesos que involucran oxígeno. Su sistema π deslocalizado permite que una amplia gama de eventos metabólicos ocurra en el entorno celular, incluyendo incluso otros mecanismos alternativos a la transferencia de carga, como la protonación o desprotonación de los sustratos.

En los últimos 20 años, se han descubierto algunas enzimas que catalizan reacciones con O_2 en ausencia de cualquier metal o cofactor. El mecanismo exacto por el cual estas enzimas únicas catalizan estos procesos y facilitan la transición del estado triplete al singlete aún no está claro. A pesar de la diversidad de reacciones, hay algunas hipótesis generales para estos tipos de reacciones enzimáticas. Una idea propuesta es que estas reacciones se han adaptado a la falta de metales o flavinas empleando un mecanismo similar al de la flavina, que implique una transferencia de carga. La enzima facilita la desprotonación del sustrato, lo que lleva a la formación de un anión que aumenta la transferencia de carga al oxígeno, simulando así el papel de la flavina. Sin embargo, también se sugiere que algunas enzimas pueden seguir una ruta diferente, como la formación de un intermediario de peróxido. En última instancia, estas enzimas podrían describirse como “asistidas por sustrato” debido a su dependencia de las propiedades del sustrato.

Esta tesis está centrada en la investigación de los ISC en sistemas que carecen de cofactores metálicos. Para ello, se ha aplicado una combinación de técnicas en Química Teórica, incluyendo cálculos de estructura electrónica, para explorar los mecanismos subyacentes y proporcionar una comprensión detallada de este fenómeno. En este trabajo se han estudiado las reacciones de peroxidación de la molécula de DPA-CoA (Refs. 1 y 2) y un intermedio enólico en la descarboxilación del ácido 2-metil-1-tetralona-2-carboxílico (Ref. 3). En el primer caso, se trata de un sistema que aparece en la biosíntesis de antibióticos como la vancomicina, y su oxigenación se lleva a cabo en el entorno enzimático del DpgC. La enzima desprotona al sustrato en primer lugar, y el grupo enolato resultante es atacado por la molécula de oxígeno triplete, produciendo un producto singlete. Sin embargo, el sistema de la tetralona fue escogido porque el sustrato inicial es electrónicamente neutro (grupo enol en lugar de enolato), y aporta

un punto de vista diferente al estudio.

El estudio de estas reacciones se ha abordado desde un grupo bastante heterogéneo de métodos teóricos, que se pueden dividir entre monorreferenciales o multirreferenciales. En el primer grupo, se ha trabajado principalmente con la teoría del funcional de la densidad (DFT) y con los métodos *coupled cluster* (en particular con el método PNO-LCCSD(T)). En el segundo grupo, se han utilizado principalmente métodos de espacio activo, desde el desarrollo más básico que supone el CASSCF hasta correcciones posteriores como MRCI, CASPT2, o incluso métodos más modernos como SHCI. También se han llevado a cabo cálculos de mecánica cuántica/mecánica molecular (QM/MM) para sistemas más grandes. Todos estos métodos se describen más detalladamente en el capítulo "Fundamentals".

1.2 Objetivos

1. **El objetivo principal es entender el mecanismo que utilizan las enzimas para catalizar reacciones prohibidas por espín en las que participa la molécula de O_2 y que no involucran cofactores metálicos.**
2. Para lograr el objetivo principal, utilizaremos la peroxidación del grupo enolato como sistema modelo. Buscamos extender estas observaciones a otras reacciones bioquímicas y establecer un marco teórico para este tipo de reacciones no adiabáticas utilizando métodos monorreferenciales y multirreferenciales.
3. Investigar la transición prohibida por espín y los mecanismos de transferencia de carga en la peroxidación de 3,5-dihidroxifenilacetil-CoA (DPA-CoA) catalizada por DpgC.

4. Explorar las similitudes y diferencias entre las reacciones del grupo enolato con el oxígeno y las reacciones de un grupo enol neutro, utilizando como caso de estudio la peroxidación de un intermedio de reacción en la descarboxilación del ácido 2-metil-1-tetralona-2-carboxílico.
5. Examinar el papel del SOC en estas reacciones no adiabáticas, utilizando métodos de cálculo multirreferenciales.
6. Profundizar en el estudio de las implicaciones de la solvatación o del entorno enzimático en el cruce intersistema, y cómo estas interacciones alteran el SOC.
7. Evaluar la eficacia de diferentes modelos teóricos para predecir el comportamiento de sistemas que experimentan ISC.
8. Nuestro objetivo final es contribuir a una comprensión más amplia de este tipo de procesos, describiendo el mecanismo de estas dos reacciones en la búsqueda de un mecanismo general para las reacciones de adición de oxígeno molecular a sustratos orgánicos.

1.3 Relación entre artículos

El núcleo central de esta tesis doctoral es la exploración de las reacciones de peroxidación en distintos sistemas bioquímicos y orgánicos, con especial interés en la transición de estados prohibidos por espín y los mecanismos de transferencia de carga. Aunque cada uno de los tres artículos que componen esta tesis por compendio aborda un sistema químico distinto, todos ellos están unidos por esta temática común y por el enfoque metodológico. A continuación, se proporciona una visión de cómo cada artículo se integra en esta narrativa general.

El primer artículo, "Multi- and single-reference methods for the analysis of multi-state peroxidation of enolates", establece las bases metodológicas y temáticas para el trabajo posterior. En este estudio, se analizan las reacciones de peroxidación en enolatos, que son intermediarios comunes en varias reacciones bioquímicas, y para ello se emplean tres sistemas químicos modelo, basados en el DPA-CoA, pero de menor tamaño, para aumentar progresivamente la complejidad del sistema. Se utilizan tanto métodos monorreferenciales como multirreferenciales para comparar su precisión sobre estos sistemas, obteniendo de esta forma un marco de referencia sobre el que basar la investigación posterior.

El segundo artículo, "DpgC-catalyzed peroxidation of 3,5-dihydroxyphenylacetyl-CoA (DPA-CoA): Insights into the spin-forbidden transition and charge transfer mechanisms", profundiza en la temática de las reacciones de peroxidación, pero ahora en un contexto bioquímico más específico. Se estudia la peroxidación catalizada por DpgC de 3,5-dihydroxyphenylacetyl-CoA (DPA-CoA), una etapa relevante en la biosíntesis de antibióticos. La transición de estados prohibidos por espín y los mecanismos de transferencia de carga juegan un papel central en este proceso, por lo que se utilizan métodos de referencia múltiple y cálculos de acoplamiento de espín-órbita para caracterizarlos. Aunque es cierto que este trabajo se realizó el primero cronológicamente, su temática encaja mejor como enlace entre las tres publicaciones, y además los resultados obtenidos justificaron en su momento una revisión posterior del marco teórico, realizado en el primer artículo. Junto a los cálculos de estructura electrónica, también se llevaron a cabo cálculos de dinámica molecular en el entorno enzimático, muy relevantes para establecer los mecanismos de reacción desde el punto de vista bioquímico.

Finalmente, en el tercer artículo, "Spin-forbidden addition of molecular oxygen to stable enol intermediates. Decarboxylation of 2-methyl-1-tetralone-2-carboxylic acid",

se examina la adición prohibida por espín de oxígeno molecular a intermedios de síntesis orgánica estables, que reaccionan con el oxígeno atmosférico. Esta reacción supone un complemento al estudio de las reacciones bioquímicas, entrando también en el ámbito de la síntesis orgánica, y además amplía los resultados obtenidos en las otras publicaciones, ya que en este caso el sustrato orgánico es un enol sin carga electrónica.

En resumen, la temática de la investigación llevada a cabo a lo largo de la tesis, sigue un hilo conductor claro, y sin perjuicio de otros trabajos de colaboración desarrollados en paralelo, este ha consistido en todo momento el tema principal del proyecto de investigación. Esta coherencia temática y metodológica justifica la presentación de estos trabajos como una tesis por compendio de publicaciones.

1.4 Conclusiones

- Hemos utilizado métodos computacionales monorreferenciales y multirreferenciales para estudiar la reacción entre el O_2 y dos tipos de moléculas: un enolato (tanto en fase gaseosa como en un entorno proteico) y un enol neutro.
- En relación con los objetivos planteados, se ha dilucidado un mecanismo para la oxigenación prohibida por espín con una enzima sin cofactor (DpgC). Se ha considerado el papel del SOC en esta reacción, identificando su influencia en el incremento de la barrera energética para estas reacciones. En Ref. 2, se han estudiado en detalle los posibles estados electrónicos involucrados en la molécula de oxígeno, desde el triplete fundamental hasta otros estados singletes excitados. Los resultados muestran que el estado singlete del producto proviene de un hipotético ion O_2^{2-} , relacionado con una transferencia de carga.
- Se han analizado a fondo los conceptos de transferencia de carga en las reacciones

de oxigenación estudiadas. Esta investigación ha destacado su papel crítico en la facilitación de la reacción, y ha llevado a seleccionar la hipótesis de transferencia de carga como la descripción más adecuada para estas reacciones.

- El entorno de la enzima DpgC ha sido estudiado utilizando métodos QM/MM, ofreciendo nuevas perspectivas sobre la función de la enzima. Estos estudios revelaron que no había aminoácidos disponibles para protonar O_2 . Los resultados sugieren que la enzima juega un papel significativo al sustraer un protón para generar un anión capaz de reducir el oxígeno a través de la transferencia de carga.
- Se han utilizado nuevos algoritmos de optimización geométrica, utilizando energías y gradientes de diversos métodos para optimizar MECPs a nivel DFT (incluyendo BS-DFT). Es importante mencionar que en el área cercana al MECP, pueden presentarse diferencias entre el DFT estándar y BS-DFT, por lo que es más seguro utilizar BS-DFT, al menos con enoles neutros. En cualquier caso, el método BS-DFT representa con mayor precisión la energía de excitación triplete-singlete para la molécula de oxígeno.
- La metodología adquirida del estudio de las reacciones enzimáticas ha sido aplicada a una reacción orgánica de características similares. En este caso, el sustrato tenía una carga neutra (grupo enol). El mecanismo de reacción presentó resultados similares para el caso de carga neutral, también se observó una transferencia de carga hacia el oxígeno, facilitada por un doble enlace en el sustrato. Además, en este caso se produjo una protonación del dióxígeno simultáneamente con la transferencia de carga.
- Se ha profundizado en conocimiento de los métodos multi-configuracionales/multi-referenciales, aplicándose a sistemas complejos. Además, se ha observado que a

pesar de los avances recientes en este tipo de métodos, todavía queda mucho trabajo por hacer en términos de reproducibilidad de resultados. Los algoritmos deben ser más estables numéricamente e incluir más funcionalidades (por ejemplo, un tratamiento efectivo de los estados excitados, perturbaciones posteriores en el caso de SHCI, o en general mayor facilidad de identificación de orbitales).

OBJECTIVES

2 Objectives

1. **The aim of this thesis is to understand the mechanism used by enzymes to catalyse spin-prohibited reactions involving the O_2 molecule and not involving metal cofactors.**
2. To achieve this aim, we will use the peroxidation of the enolate group as a model system. We seek to extend these observations to other biochemical reactions and establish a theoretical framework for such non-adiabatic reactions using single-reference and multireference methods.
3. We will investigate the spin forbidden transition and charge transfer mechanisms in enzymatic reactions by studying the peroxidation of 3,5-dihydroxyphenylacetyl-CoA (DPA-CoA) catalysed by DpgC.
4. To explore the similarities and differences between reactions of the enolate group with oxygen and reactions of a neutral enol group, using as a case study the peroxidation of a reaction intermediate in the decarboxylation of 2-methyl-1-tetralone-2-carboxylic acid.
5. We will examine the role of SOC in these non-adiabatic reactions, using multi-reference calculation methods.
6. Further study the implications of solvation or enzyme environment on intersystem crosstalk, and how these interactions alter SOC.
7. We will evaluate the effectiveness of different theoretical models for predicting the behaviour of systems undergoing ISC.
8. Our final aim is to contribute to a broader understanding of these processes

by describing the mechanism of these two reactions in the search for a general mechanism for molecular oxygen addition reactions to organic substrates.

FVNDAMENTALS

3 Fundamentals

This doctoral thesis studies the theoretical reaction mechanism between the triplet dioxygen and two organic systems with different characteristics, DPA-CoA and 2-methyl-3,4-dihydro-1-naphthol. In order to understand the theoretical physical framework, we start with a general description of the main theoretical and computational methods used in the research.

We start by outlining the electronic structure theory, which provides the theoretical framework for understanding and predicting the behaviour of electrons in molecules and materials. The electronic structure methods we discuss are based on various approximations and levels of theory, ranging from mean-field approaches to more sophisticated multireference and quantum-chemical methods.

The chapter also covers the inclusion of spin-orbit coupling effects, which are particularly important for intersystem crossing reactions, and the search for minimum energy crossing points (MECPs) in the context of geometric optimization.

3.1 Electronic structure theory

Electronic structure theory is a branch of quantum chemistry that seeks to describe the behaviour of behavior and properties of atoms, molecules, and solids at a microscopic level.⁴ At its core, it aims to solve the time-independent Schrödinger equation. A key simplification to this problem would be the Born-Oppenheimer approximation.⁵ This approximation considers the nuclear and electronic motions separately due to their significant difference in mass and consequently, in speed. Effectively, it treats the nuclei as stationary while the electrons move, adapting instantaneously to the nuclear

configuration. The validity of this approximation lies in the fact that the nuclear motion is typically much slower compared to the electronic motion.

Under the Born-Oppenheimer approximation, the total wavefunction of a molecule can be expressed as a product of its electronic and nuclear wavefunctions. Consequently, we can separate the total Hamiltonian into electronic and nuclear parts and the Schrödinger equation can be simplified. Therefore, we primarily focus on solving the electronic Schrödinger equation:⁶

$$\hat{H}\Psi(\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_N) = E\Psi(\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_N), \quad (1)$$

where $\hat{H}$ is the electronic Hamiltonian operator, $\Psi(\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_N)$ is the electronic wavefunction, $\mathbf{r}_i$ denotes the position of the i -th electron, and E is the energy eigenvalue. Solving this equation for many-electron systems is a challenge due to the high dimensionality of the problem and the presence of electron-electron Coulombic interactions, an effect known as electronic correlation. The electron correlation can be divided into two types: static correlation, which is related to near-degeneracy effects, and dynamic correlation, which arises from the simultaneous interaction of one electron with many others.⁷

To tackle this problem, various approximations and theoretical frameworks have been developed, which can be broadly classified into wavefunction-based methods and density-based methods. Wavefunction-based methods, such as Hartree-Fock theory,⁸ Configuration Interaction,⁹ and Coupled Cluster,¹⁰ seek to approximate the many-electron wavefunction directly.^{4,11} In contrast, density-based methods, such as density functional theory (DFT), aim to describe the electronic structure in terms of the electron density, a three-dimensional function that captures the spatial distribution of

electrons in the system.^{12,13} Wavefunction-based methods can be further classified into single-reference and multireference methods, depending on their ability to account for static correlation.

Single-reference methods, such as Møller-Plesset perturbative method (MP2)¹⁴ and Coupled Cluster (CC),^{10,15} are based on a single determinant reference wavefunction and are well-suited for treating dynamic correlation. In contrast, multireference methods, such as complete active space self-consistent field (CASSCF)^{16,17}, multireference perturbation theory (MRPT),¹⁸ and multireference Configuration Interaction (MRCI),^{19,20} are based on multiple determinants and can account for both static and dynamic correlation.

Throughout this subsection, we will explore the foundations of these various methods, including their mathematical formulations, key approximations, and range of applicability.

3.1.1 Hartree-Fock Theory

Hartree-Fock (HF) theory is a widely-used method in electronic structure calculations that provides an approximate solution to the Schrödinger equation for many-electron systems.^{11,21,22} The main idea behind HF theory is to represent the many-electron wavefunction as a single Slater determinant, which is constructed from a set of orthonormal one-electron spin-orbitals. By using the variational principle,^{8,11} HF theory minimizes the energy of the system with respect to these spin-orbitals, subject to the constraint of orthonormality.

The HF energy expression for a many-electron system can be written as:

$$E_{\text{HF}} = \sum_i \langle i | \hat{h} | i \rangle + \frac{1}{2} \sum_{ij} (\langle ij | \hat{J} | ij \rangle - \langle ij | \hat{K} | ji \rangle), \quad (2)$$

where $\hat{h}$ is the one-electron Hamiltonian operator (including the kinetic energy terms for individual electrons and electron-nucleus attraction), $\hat{J}$ and $\hat{K}$ are the Coulomb and exchange operators, respectively, and i, j are indices of the occupied spin-orbitals. The first term in the energy expression accounts for the kinetic energy and electron-nucleus attraction, while the second term represents electron-electron repulsion (Coulomb interaction) and exchange interactions.

To minimize the HF energy functional, the Fock operator $\hat{F}$ is formed from these terms:

$$\hat{F} = \hat{h} + \sum_j \hat{J}_j - \hat{K}_j, \quad (3)$$

and then applied to the spin-orbitals, resulting in the Roothaan-Hall equations:^{21,23}

$$\hat{F}\phi_i = \epsilon_i\phi_i, \quad (4)$$

where ϕ_i are the HF spin-orbitals and ϵ_i are the corresponding orbital energies. To solve these equations, they are transformed into the matrix form using a basis set of atomic orbitals:

$$\mathbf{FC} = \mathbf{SC}\epsilon, \quad (5)$$

where $\mathbf{F}$ is the Fock matrix, $\mathbf{C}$ is the coefficient matrix containing the linear combinations of atomic orbitals that form the spin-orbitals, $\mathbf{S}$ is the overlap matrix, and ϵ is

the diagonal matrix of orbital energies.

The HF procedure is an iterative process, where the Fock matrix is constructed and diagonalized at each iteration, and the resulting orbitals are used to update the Fock matrix until convergence is reached. Once the self-consistent field (SCF) procedure converges, the HF energy and orbitals are obtained, which can be used as a starting point for more advanced electronic structure methods that include electron correlation effects (generally denoted as post-Hartree-Fock methods).

It is important to note that HF theory has its limitations, such as the inability to describe static electron correlation and the underestimation of the dispersion interaction.²⁴ Nevertheless, it remains a fundamental method in electronic structure calculations and provides a relatively accurate and computationally efficient starting point for more sophisticated methods.¹¹

3.1.2 Configuration Interaction

Configuration Interaction (CI) is a post-Hartree-Fock electronic structure method that provides a systematic way to include electron correlation effects in the many-electron wavefunction.⁹ In CI, the many-electron wavefunction is expressed as a linear combination of Slater determinants, with each determinant corresponding to a specific electron configuration. The CI wavefunction can be written as:¹¹

$$|\Psi_{\text{CI}}\rangle = c_0|\Phi_0\rangle + \sum_{ia} c_{ia}|\Phi_{ia}\rangle + \sum_{ijab} c_{ijab}|\Phi_{ijab}\rangle + \cdots, \quad (6)$$

where $|\Phi_0\rangle$ is the Hartree-Fock reference determinant (ground-state configuration), $|\Phi_{ia}\rangle$ represents single excitations, $|\Phi_{ijab}\rangle$ represents double excitations, and so on.

The coefficients $c_0, c_{ia}, c_{ijab}, \dots$ are determined by solving the CI eigenvalue problem:

$$\mathbf{H}_{\text{CI}}\mathbf{c} = E_{\text{CI}}\mathbf{c}, \quad (7)$$

where $\mathbf{H}_{\text{CI}}$ is the CI Hamiltonian matrix, $\mathbf{c}$ is the vector of CI coefficients, and E_{CI} is the CI energy. The CI Hamiltonian matrix elements are given by the following expression:

$$H_{\text{CI},IJ} = \langle \Phi_I | \hat{H} | \Phi_J \rangle, \quad (8)$$

where Φ_I and Φ_J are Slater determinants, and $\hat{H}$ is the electronic Hamiltonian.

The accuracy of the CI method depends on the number of determinants included in the expansion. The simplest CI approach, known as the CI Singles (CIS) method, includes only single excitations. CI Doubles (CID) considers both single and double excitations, while CI Singles and Doubles (CISD) includes single, double, and their interactions.^{25,26} The most accurate CI method, called Full Configuration Interaction (FCI), includes all possible excitations, and its solution is the exact solution of the Schrödinger equation within the given basis set. However, the computational cost of FCI scales factorially with the system size, which makes it prohibitive for all but the smallest molecular systems.²⁷

To overcome the computational limitations of FCI, various truncated CI methods and selected CI approaches have been developed. These methods aim to include only the most important determinants in the CI expansion, based on various criteria such as perturbation theory or determinant contributions to the total energy. Examples include the Multireference CI (MRCI)^{19,20} and Selected CI (SCI)²⁷ methods, which will be discussed in subsequent sections.

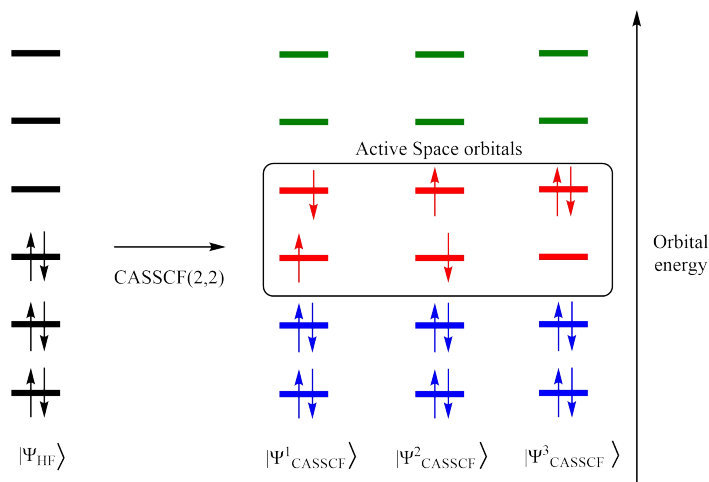


Figure 1: In active space methods, we divide the set of molecular orbitals into three subspaces: inactive (blue), active (red), and virtual (i.e. unoccupied) (green). The diagram on the left corresponds to the ground-state wavefunction used in the Hartree-Fock calculation. The states generated from excitations within an active space consisting of two orbitals and two electrons, $\text{CASSCF}(2,2)$, are shown to the right of the arrow.

Despite the potential accuracy of CI methods, they suffer from two important limitations: the lack of size consistency and size extensivity.¹¹ Size consistency refers to the property that the calculated energy of a non-interacting collection of molecules should be equal to the sum of the energies of the individual molecules. Size extensivity means that the calculated energy should scale linearly with the size of the system, such as when adding an identical molecular unit to an existing system.^{8,11} The absence of these properties in CI methods can lead to erroneous results for large systems or those involving bond dissociation.²⁸ Nevertheless, CI remains an essential building block in the development of more sophisticated and accurate electronic structure methods.

3.1.3 Active space methods

Active space methods are a family of multireference electronic structure approaches designed to address the limitations of single-reference methods, such as Hartree-Fock and

Configuration Interaction, when applied to systems with strong static or dynamic electron correlation effects.^{16,17} These methods are particularly useful for studying molecular systems with near-degenerate electronic states, bond dissociation processes, and transition metal complexes.

The key idea behind active space methods is to partition the molecular orbitals into three subspaces: the inactive orbitals, the active orbitals, and the virtual orbitals. The inactive orbitals are fully occupied and kept fixed during the calculations, while the active orbitals are partially occupied and allowed to mix with the virtual orbitals to form the many-electron wavefunction. The wavefunction in active space methods is expressed as a linear combination of determinants obtained by distributing the active electrons among the active orbitals:

$$|\Psi_{\text{CAS}}\rangle = \sum_I c_I |\Phi_I\rangle, \quad (9)$$

where $|\Psi_{\text{CAS}}\rangle$ is the active space wavefunction, $|\Phi_I\rangle$ are the determinants formed by distributing the active electrons in the active orbitals, and c_I are the corresponding expansion coefficients.

One of the most widely used active space methods is the complete active space self-consistent field (CASSCF) method. In CASSCF, the active space is defined by specifying the number of active electrons (n_e) and the number of active orbitals (n_o), using the notation CASSCF(n_e, n_o) (Figure 1). The CASSCF wavefunction is obtained by optimizing both the molecular orbitals and the CI coefficients simultaneously:

$$|\Psi_{\text{CASSCF}}\rangle = \sum_I c_I |\Phi_I^{\text{CASSCF}}\rangle, \quad (10)$$

where $|\Phi_I^{\text{CASSCF}}\rangle$ are the determinants formed by distributing the active electrons in the CASSCF active orbitals. The CASSCF energy is calculated as the expectation value of the electronic Hamiltonian:

$$E_{\text{CASSCF}} = \frac{\langle \Psi_{\text{CASSCF}} | \hat{H} | \Psi_{\text{CASSCF}} \rangle}{\langle \Psi_{\text{CASSCF}} | \Psi_{\text{CASSCF}} \rangle}. \quad (11)$$

The selection of the active space is crucial to the accuracy and efficiency of the CASSCF method. Ideally, the active space should be large enough to capture the essential static correlation effects but small enough to keep the computational cost manageable. There are some rules of thumb about active space selection, explained in the next section.

While CASSCF captures the static correlation effects, it often provides a poor description of dynamic correlation.¹⁶ Therefore, it is common to use CASSCF as a starting point for more advanced multireference methods, such as multireference perturbation theory (MRPT)¹⁸ and multireference Configuration Interaction (MRCI),^{19,20} which will be discussed in subsequent sections.

Selection of orbitals and active space

Once the active space methods have been described, the most critical issue remains to be addressed in order to use them correctly. Unlike black-box methods (which use very little input from user), the success of these methods depends on the selection of the orbitals used to generate the vertical excitations, so it is important that the user has some knowledge and experience with the system under study.

The following is the standard procedure for choosing an active space:²⁹ All the molecular orbitals are computed using a straightforward electronic structure calculation,

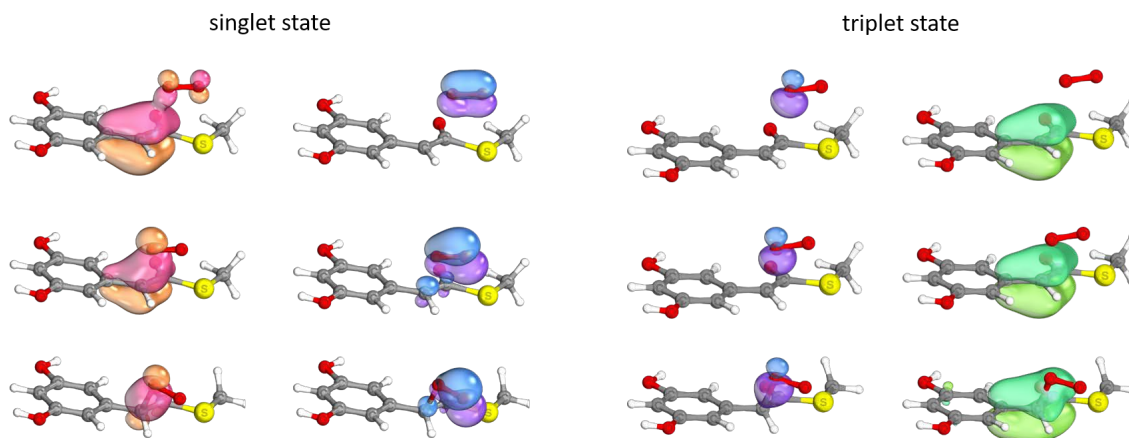


Figure 2: Sample IBO orbitals generated for the DPA-CoA molecule in its reaction with dioxygen. These orbitals are clearly localised and their identification (both visually and numerically) is easier, allowing an energetic order of orbitals to be maintained across different geometries of a reaction. They represent the $O-C_{\alpha}$ bond formation in the DPA-CoA reaction with dioxygen, studied in this thesis.

such as Hartree-Fock. Then, the orbitals thought to be chemically most important are chosen into the active space. The standard rule of thumb to choose this set of active orbitals is to include any d-orbital for metals, any delocalised π cloud or any bond which is formed or broken along a reaction.³⁰ Even with these hints for choosing a suitable set of starting orbitals, this approach has several limitations. First, the chemical orbitals for the active space are still chosen manually, frequently using prior knowledge. Therefore, this multireference procedure is challenging to use and provides findings difficult to replicate. Second, different types of valence orbitals combine to form molecular orbitals. For instance, the d-atom valence orbitals of a metal typically contribute to a large number of molecular orbitals, and it is not easy to combine these into a small active subset while maintaining the ability to accurately describe all required physical processes in complex systems. Large complexes, especially those with numerous metal ions, often require a careful study with many tests for different active spaces.³¹

This problem is further aggravated when one tries to study molecular systems through different geometrical structures, following for example a chemical reaction or a molecular dynamics simulation. Early CASSCF studies simply took one geometry as a reference, but it must be taken into account that the energetic order of the orbitals changes considerably across different geometries, which makes it difficult to have a simple active space throughout the study. In this thesis, where we work across different sets of geometries following a chemical reaction process, this problem has turned out to be central. While it was not taken into account at the beginning, the subsequent results showed the need to find a solution to the conservation of the active space along all the geometries considered. Although we finally worked with the "brute-force" solution shown in the results, some of the solutions proposed:

1. Cárdenas and Nogueira seek a solution applicable to molecular dynamics simulations, and their identification of orbitals is based on the electronic overlap between different frames of the simulation, calculated quantitatively by means of the overlap integrals.³² Although this method has a success rate of around 80% when identifying the orbitals, it is a reasonable starting point.
2. Knizia et al. propose to generate a new set of orbitals that can be followed more easily throughout a reaction, called Intrinsic Bonding Orbitals (IBO) (Figure 2).^{33,34} These orbitals are easily identifiable, as they are fairly localised around the two atoms they bond, and furthermore, from this molecular description, the Lewis structure and the curly arrow electron transfer model of Robinson and Ingold emerge naturally.³⁵ This method provides consistent results, although the question of virtual orbitals remains, as this method only works with occupied orbitals. Since the active space includes both occupied orbitals and virtual orbitals, further progress is still needed in this aspect.

3. Finally, the method proposed by Sayfutyarova et al.,²⁹ as a further development of Knizia’s IBO theory, uses this set of orbitals to generate by overlapping the whole set of orbitals required to obtain a robust CASSCF calculation, starting from a basic input provided by the user, which can be generalised by following the list of general hints for choosing the active space. For example, if a system contains a transition metal, the user would indicate its d orbitals in the input and the program would generate a set of orbitals correlated with them to be included in the active space. The issue with this method for the problem at hand is that the size of the active space varies across different geometries, so it is not suitable for solving our question.

Several other suggestions and advancements of diverse nature have been proposed in recent studies.³⁶⁻³⁹ The late publication dates of most research in this field suggest that this is a newly identified challenge. There is a rising interest in incorporating active space methods into the study of reaction trajectories, and this development is expected to shape future work significantly.

Selected CI

Selected Configuration Interaction (SCI) is an extension of the traditional CI approach that seeks to lighten the computational cost by considering only a subset of the most important configurations. This selective treatment of the configuration space allows for more accurate calculations with a significantly reduced computational effort.²⁷

In the last decade, great progress has been made in solving this scaling problem, working on different techniques to reduce the scaling pre-exponential factor and allow calculations with a larger number of orbitals.⁴⁰ One of the first solutions proposed consists of

increasing the number of CSFs included in the calculation stochastically until convergence is achieved. In this way, convergence can be achieved with a very small number of CSFs and a much lower computational cost.⁴¹

Some later developments select the CSFs from the initial Hartree-Fock determinant,^{42–44} depending on the interaction they have with it, generally known as Selected CI. The key challenge in the SCI method is to identify the most important configurations to include in the calculation. Several strategies have been proposed to achieve this goal, including:

Importance sampling: Importance sampling techniques, such as the Adaptive Sampling CI (ASCI)⁴² and Semistochastic Heat-bath CI (SHCI) methods,^{40,43,45,46} use stochastic algorithms to iteratively generate and include configurations that are expected to have the largest contributions to the wavefunction. The importance of a configuration can be estimated from the CI coefficients obtained in previous iterations.

Perturbative selection: Perturbative selection methods, such as the CI using perturbative selected iterations (CIPSI) approach,⁴⁷ use perturbation theory to estimate the importance of each configuration. By including only those configurations whose perturbative contributions exceed a predefined threshold, one can significantly reduce the size of the CI space while maintaining the accuracy of the calculation.

Excitation level truncation: The restricted active space CI (RASCI) approaches limit the number of excitations allowed within the active space. By considering only single and double excitations or a restricted number of higher excitations, the CI space can be dramatically reduced without sacrificing the essential features of the wavefunction.⁴⁴

In our case, the most used method was the Selected Heat-bath Configuration Interac-

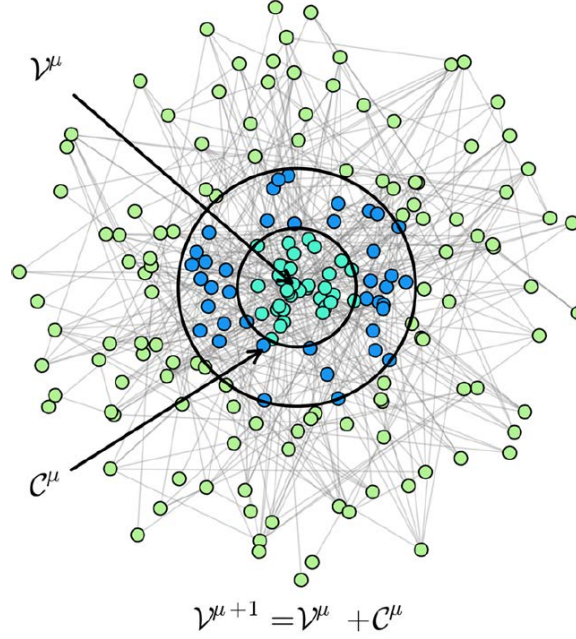


Figure 3: In the SHCI method, the Hartree-Fock ground state wavefunction is used as a starting point. By searching for other correlated determinants and generating vertical excitations from them, the wavefunction is iteratively increased in an efficient way, as all determinants below a threshold energy value are ignored and discarded. $\mathcal{V}^{\mu}$ represents the current variational space, and the next iteration $\mathcal{V}^{\mu+1}$ will also include the determinants in $\mathcal{C}^{\mu}$. In cyan are shown the determinants included in a certain iteration, and in blue are the new determinants. All determinants in green are excluded because they are below the threshold, thus saving a lot of computational resources. Figure extracted from Ref. 40

tion (SHCI).^{40,46} The SHCI method consists of two main steps:

- In the first step, a collection of determinants is iteratively selected to construct a variational wavefunction (Figure 3). These determinants are obtained from the full space of possible Slater determinants by stochastically sampling and filtering based on their contribution to the wavefunction, specifically when their contribution falls below a particular energy threshold (ϵ_1). This iterative selection process continues until the energy of the wavefunction becomes stable and converges.
- In the second step, Epstein-Nesbet perturbation theory with multiple reference

determinants is employed to determine the second-order energy correction⁴⁸. This correction accounts for the dynamic correlation not captured in the first step, providing a more accurate description of the electronic structure.

This method could include more than 100-200 orbitals in the active space, and indeed, we used it in our work to include the whole valence space for systems with 10-30 atoms. In Ref. 1, we studied three molecular systems, for the smallest one we use a CAS(40,33) active space, while for the largest one we use a CAS(82,69), unthinkable for any traditional CASSCF development. This active space calculation includes around 10^{38} Slater determinants, a record number at the time of publication.

3.1.4 Multireference perturbation theory

Multireference perturbation theory (MRPT) is a class of methods designed to address the limitations of single-reference perturbation theory, such as Møller-Plesset (MP) perturbation theory, when dealing with systems exhibiting strong static correlation effects.¹⁸ In MRPT, the wavefunction is expressed as a linear combination of multiple reference Slater determinants, which allows for a more accurate description of the electronic structure of these systems.

The general form of a multireference wavefunction is given by:

$$|\Psi_{\text{MR}}\rangle = \sum_I c_I |\Phi_I\rangle, \quad (12)$$

where $|\Psi_{\text{MR}}\rangle$ is the multireference wavefunction, c_I are the coefficients, and $|\Phi_I\rangle$ are the Slater determinants formed from the reference configurations.

A commonly used MRPT method is the N-electron Valence State Perturbation Theory (NEVPT),⁴⁹ which can be applied in conjunction with the CASSCF method. The NEVPT wavefunction is written as:

$$|\Psi_{\text{NEVPT}}\rangle = \sum_{I,J} c_{IJ} |\Phi_I\rangle |\Phi_J\rangle, \quad (13)$$

where $|\Phi_I\rangle$ and $|\Phi_J\rangle$ are the Slater determinants of the CASSCF wavefunction (Eq. 10), and c_{IJ} are the coefficients of the perturbed wavefunction.

The MRPT energy expression can be written in terms of the zeroth-, first-, and second-order energies:

$$E_{\text{MRPT}} = E^{(0)} + E^{(1)} + E^{(2)} + \dots, \quad (14)$$

where $E^{(0)}$ is the energy of the reference wavefunction, and $E^{(1)}$ and $E^{(2)}$ are the first- and second-order perturbation corrections, respectively.

A major challenge in MRPT is to avoid intruder states, which are energetically close to the reference states and can lead to numerical instabilities. Various MRPT methods, such as the quasi-degenerate perturbation theory (QDPT)⁵⁰ and the multi-state complete active space second-order perturbation theory (MS-CASPT2),^{51,52} have been developed to address this issue by introducing level shifts or modified denominators in the perturbation expansion.

Finally, by combining some of the advantages of multi-configurational methods and perturbative methods, we arrive at the MRCI method in the next section.

3.1.5 Multireference Configuration Interaction (MRCI)

Multireference Configuration Interaction (MRCI) is a wavefunction-based method designed to treat systems with strong static correlation effects that cannot be accurately described by single-reference methods, but it takes a step further than CASSCF, including perturbation terms (dynamic correlation).^{19,20} MRCI takes into account multiple reference configurations, providing a more accurate description of the electronic structure in such systems.

The MRCI wavefunction is expressed as a linear combination of reference and excited configurations:

$$|\Psi_{\text{MRCI}}\rangle = \sum_I c_I |\Phi_I\rangle + \sum_{I, a, r} c_{Ia}^r |\Phi_{Ia}^r\rangle + \sum_{I, a < b, r < s} c_{Iab}^{rs} |\Phi_{Iab}^{rs}\rangle + \dots, \quad (15)$$

where $|\Phi_I\rangle$ are the reference Slater determinants, $|\Phi_{Ia}^r\rangle$ and $|\Phi_{Iab}^{rs}\rangle$ represent singly and doubly excited determinants, respectively, and c_I , c_{Ia}^r , and c_{Iab}^{rs} are the corresponding coefficients.

The coefficients in the MRCI wavefunction are determined by diagonalizing the CI matrix, which contains the Hamiltonian matrix elements between all pairs of configurations. This process involves solving the following eigenvalue equation:¹¹

$$\mathbf{H}\mathbf{C} = \mathbf{C}\mathbf{\Lambda}, \quad (16)$$

where $\mathbf{H}$ is the CI matrix, $\mathbf{C}$ is the matrix of coefficients, and $\mathbf{\Lambda}$ is the diagonal matrix of energies.

The most common approach to perform MRCI calculations is to start from a CASSCF wavefunction, which provides a balanced description of the most important reference configurations. The active space is defined by a set of active orbitals and electrons, while the remaining orbitals are treated as inactive or virtual.¹¹

The MRCI method has several advantages over single-reference methods, including the ability to describe multi-configurational wavefunctions, greater accuracy for systems with static correlation effects, and the possibility to treat states with different symmetries and multiplicities in a single calculation.^{53,54} However, MRCI is computationally more expensive due to the large number of configurations involved, and the size of the CI matrix can become a limiting factor for larger systems.²⁷

3.1.6 Broken-symmetry DFT

Density functional theory (DFT) methods are currently the most widely used family of theoretical methods in the chemical community, their black-box characteristics allow them to be used without the need for a great deal of numerical detail and they offer reasonably high accuracy for a wide variety of chemical problems at a low computational cost.^{55,56} However, it has rather severe limitations for certain types of systems, and cannot be employed lightly for any chemical system. It works especially well in describing monoreferential systems, transition states of organic reactions, and even in ab-initio or QM/MM molecular dynamics.⁵⁷⁻⁵⁹ In addition, in recent years there have been advances in the problem of dispersion or long-distance interactions, introducing the Grimme dispersion term to some functionals and considerably reducing their error compared to functionals such as M06-2X or wB97XD, specifically tuned to reproduce these interactions.^{60,61} It is also often used in conjunction with implicit solvent models, such as SMD,⁶² which allow the experimental conditions of solvent-phase reactions

to be reproduced. These models generate a cavity and an electric field over the system under study, so that they modify its Hamiltonian perturbatively and change its properties as a function of the dielectric constant of the solvent.⁶²

For those systems in which a multiconfigurational description of the chemical system is required, the standard DFT method is not the most suitable and generates too large errors (for example, as we will see in Chapter 5, a simple chemical system such as the oxygen molecule has an error in the singlet-triplet energy gap of more than 10 kcal/mol, even using good quality basis sets). A common solution to this type of problem is to use the correction known as broken-symmetry DFT. An intermediate solution between employing multiconfigurational systems and the purely monoreferential DFT, the wavefunction generated by the broken-symmetry DFT introduces an approximation for which it considers two orbitals occupied with one electron each, and by means of a CSF carries out a description similar to the H_2 model, in terms of separating an ionic part, a covalent part and taking both into account in the description. Some recent studies using this methodology are given in the literature.^{63–66}

Despite providing an accurate electron density description, this method also has shortcomings. For instance, it fails to accurately account for spin density, leading to an incorrect spin multiplicity.⁶⁷ As a result, there is artificial spin contamination, which can cause the calculated energy to be lower than it should be. To mitigate this, one can employ Yamaguchi’s spin projection method, which corrects the energy term with a simple operation, taking also into account the associated triplet state.^{68–70} The spin-projected singlet energy ($E_{\text{SP}}^{\text{singlet}}$) can be computed using the singlet and triplet energies (E_{singlet} and E_{triplet} , respectively) as well as the spin contamination coefficient (C_{SC}):

$$E_{\text{SP}}^{\text{singlet}} = E_{\text{singlet}} + C_{\text{SC}} [E_{\text{singlet}} - E_{\text{triplet}}] \quad (17)$$

The spin contamination coefficient (C_{SC}) is defined as:

$$C_{\text{SC}} = \frac{\langle S^2 \rangle_{\text{singlet}}}{\langle S^2 \rangle_{\text{triplet}} - \langle S^2 \rangle_{\text{singlet}}} \quad (18)$$

Here, $\langle S^2 \rangle_{\text{singlet}}$ and $\langle S^2 \rangle_{\text{triplet}}$ are the expectation values of the squared total spin operator S^2 for the singlet and triplet states. The spin contamination coefficient is used to correct the singlet energy by considering the difference between the singlet and triplet energies, scaled by the spin contamination coefficient, providing a more accurate description of the system’s energy and accounting for the spin density issues associated with broken-symmetry DFT calculations. Other solutions, not used in this work, include spin-flip DFT⁷¹ or spin-projected unrestricted DFT.⁷²

To reduce the amount of spin contamination, Gaussian performs an annihilation step during the SCF and provides two values of S^2 , before and after annihilation. In Ref. 1, we used the after annihilation (corrected) values, and the changes observed compared to the before annihilation version were almost imperceptible. However, we found that using the before annihilation S^2 resulted in results that were more in line with other literature values,^{69,70} and the triplet-singlet excitation energy of oxygen was closer to the experimental value. Based on these findings, we used S^2 before annihilation in Ref. 3.

3.1.7 Pair Natural Orbital Coupled Cluster

The Pair Natural Orbital (PNO) Local Coupled Cluster with single and double excitations and perturbative triples correction (PNO-LCCSD(T)) is a highly efficient and accurate method for calculating the correlation energies of molecular systems.^{73,74} The

PNO-LCCSD(T) method is based on the Coupled Cluster (CC) theory,^{10,15} which is a highly accurate approach for including electron correlation effects in quantum chemical calculations. The CC theory expresses the correlated wavefunction as an exponential ansatz of the reference wavefunction:

$$|\Psi_{\text{CC}}\rangle = e^{\hat{T}}|\Psi_0\rangle, \quad (19)$$

where $|\Psi_0\rangle$ is the reference wavefunction, typically the Hartree-Fock wavefunction, and $\hat{T}$ is the cluster operator, which is a sum of the single, double, and higher excitations operators:

$$\hat{T} = \hat{T}_1 + \hat{T}_2 + \hat{T}_3 + \cdots. \quad (20)$$

In the LCCSD(T) method, the triple excitations are treated perturbatively, which significantly reduces the computational cost compared to the full CCSDT method.⁷⁵

The PNO-LCCSD(T) approach introduces two key approximations to further reduce the computational cost of the LCCSD(T) method without sacrificing much of its accuracy. The first approximation is the localization of the molecular orbitals, which allows the CC amplitudes to be represented in a compact form. The localized molecular orbitals are obtained by applying a unitary transformation to the canonical molecular orbitals that preserves their orthonormality. Various localization schemes, such as Pipek-Mezey or Boys localization, can be employed for this purpose.⁷⁶⁻⁷⁸

The second approximation is the use of PNOs to represent the virtual space in the CC calculations. PNOs are linear combinations of virtual orbitals that are optimized to describe the electron correlation within a pair of occupied orbitals.⁷⁹ The PNOs are

constructed by diagonalizing the pair density matrix:

$$\mathbf{D}_{ij}^{\text{PNO}} = \mathbf{C}_{\text{virt}}^\dagger \mathbf{D}_{ij}^{\text{virt}} \mathbf{C}_{\text{virt}}, \quad (21)$$

where $\mathbf{D}_{ij}^{\text{virt}}$ is the virtual-virtual block of the pair density matrix for the occupied orbital pair i and j , and $\mathbf{C}_{\text{virt}}$ is the coefficient matrix of the virtual orbitals. By truncating the PNO space based on a user-defined threshold, the computational cost of the CC calculations can be significantly reduced.

The PNO-LCCSD(T) method has been shown to provide highly accurate results for a wide range of molecular systems with a substantially reduced computational cost compared to the canonical LCCSD(T) method.⁷³ The combination of the localized molecular orbitals and the PNO approximation allows for an efficient treatment of electron correlation effects, making the PNO-LCCSD(T) method an attractive choice for large-scale quantum chemical calculations.

3.1.8 QM/MM calculations

Quantum Mechanics/Molecular Mechanics (QM/MM) is a hybrid computational approach that combines the accuracy of quantum mechanical (QM) methods with the efficiency of molecular mechanics (MM) methods to model large molecular systems.⁵⁷ In QM/MM calculations, the system is partitioned into two regions: the QM region, which is treated using quantum mechanical methods, and the MM region, which is described using classical force fields. The QM region typically consists of the chemically active part of the system, where electronic structure changes occur, while the MM region includes the surrounding environment, such as solvent molecules or protein

residues, that provide an important context for the QM region.

The total energy of a QM/MM system can be expressed as the sum of the QM energy, the MM energy, and the QM-MM interaction energy:

$$E_{\text{total}} = E_{\text{QM}} + E_{\text{MM}} + E_{\text{QM-MM}}. \quad (22)$$

The QM energy E_{QM} is computed using a suitable quantum mechanical method, such as Hartree-Fock, DFT, or PNO-LCCSD(T), for the QM region. The MM energy E_{MM} is calculated using a classical force field, such as AMBER⁸⁰ or CHARMM,⁸¹ for the MM region. The QM-MM interaction energy $E_{\text{QM-MM}}$ accounts for the coupling between the QM and MM regions and is typically described by electrostatic and van der Waals interactions.⁵⁷

There are several approaches to treat the QM-MM interaction energy, such as the mechanical embedding (ME) scheme, the electrostatic embedding (EE) scheme, and the polarizable embedding (PE) scheme.^{83,84} In the ME scheme, the QM and MM regions interact only through non-bonded interactions, and the QM region is not affected by the electrostatic environment provided by the MM region. In the EE scheme, the QM region is embedded in the electrostatic field generated by the MM region, allowing for a more realistic description of the electronic structure of the QM region. In the PE scheme, both the QM and MM regions are allowed to polarize each other, which provides an even more accurate description of the QM-MM interactions.

A popular multi-layered strategy that builds on these principles is the ONIOM (Our own N-layered Integrated molecular Orbital and molecular Mechanics) method.⁸⁵ This approach can even include more than two layers, and for most applications, there will

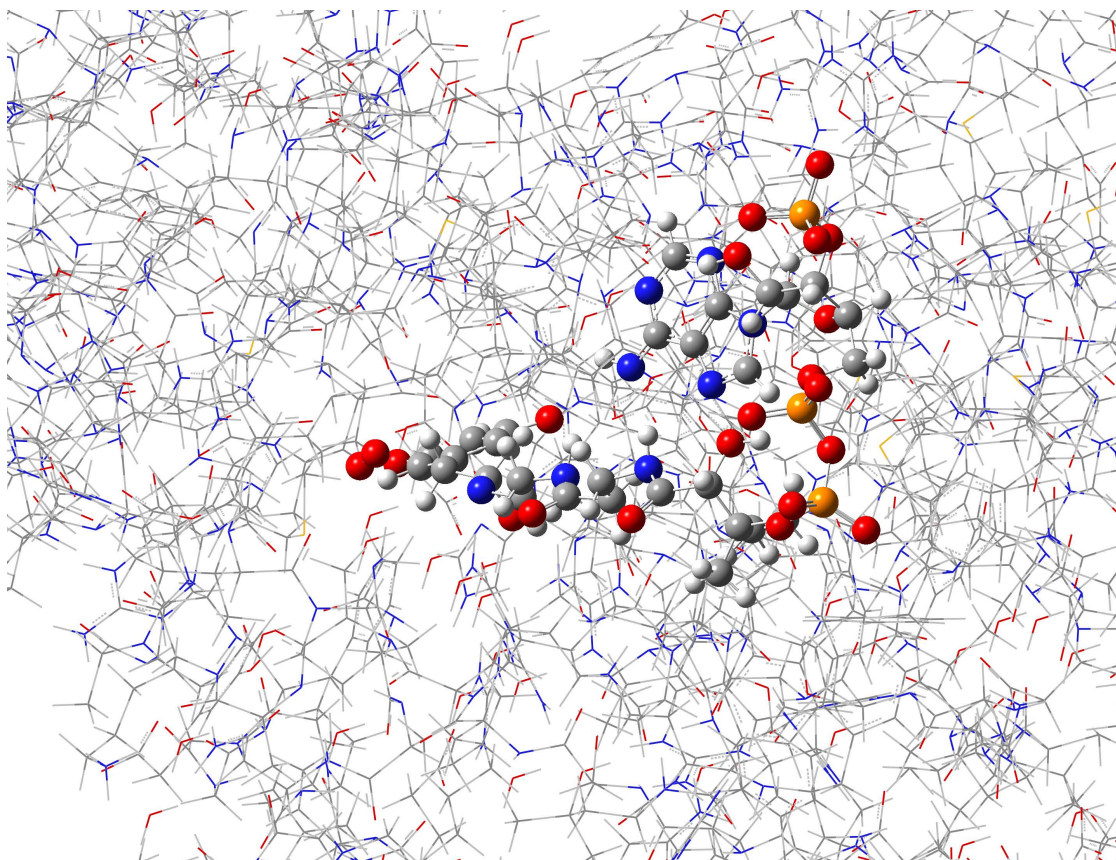


Figure 4: ONIOM scheme visualised with GaussView6.⁸² This image shows the main system studied in this thesis, DPA-CoA, with the dioxygen molecule as the two red balls on the far left and the rest of the molecule shown as balls and sticks representation, with the phosphate groups of CoA on the right in orange-yellow. The protein environment, which is treated with a low-level computation method (molecular mechanics) is shown as a thin sticks representation.

be three layers (a QM high-quality region, an intermediate low quality QM region and then the MM region). Figure 4 shows a chemical system (the enzymatic environment of DPA-CoA oxygenation, studied in Ref. 2), divided into several regions. The QM part is usually the active centre of the enzyme and the reactants, while the MM part is the rest of the enzyme.

QM/MM calculations can be performed using various levels of QM theory and force fields, depending on the desired balance between accuracy and computational cost.

The choice of the QM method and the partitioning of the system into QM and MM regions play crucial roles in determining the accuracy and reliability of the QM/MM results. QM/MM methods have been widely used to study complex molecular systems, such as enzymatic reactions, solvation effects, and electronic excited states, providing valuable insights into the underlying chemistry and physics of these systems.^{86,87}

3.2 Non-adiabatic processes and intersystem crossing

A non-adiabatic transition refers to a phenomenon that occurs in quantum mechanics and chemical dynamics when a system undergoes a change in its electronic state while not obeying the adiabatic approximation.⁸⁸ Here, "adiabatic" refers to a process that is slow enough for the system to remain in its ground state throughout the entire transition. In other words, during an adiabatic transition, the system has enough time to adjust to the changes and maintain equilibrium. Non-adiabatic transitions play a crucial role in various chemical and physical processes, such as photochemical reactions, energy transfer, and the generation of reactive species.^{89,90} In order to explain non-adiabatic processes and specifically intersystem crossings, the central concept of this thesis, we move from electronic structure methods to potential surfaces. In this way, nuclear motion, neglected by the Born-Oppenheimer approach, is reintroduced. Chemical processes usually involve a change in the geometry of the system, so it is important to follow the motion of the atomic nuclei in order to study the process.

3.2.1 Potential energy surfaces and minimum energy crossing points (MECP)

With the nuclear coordinates fixed, the separation of the electronic and nuclear Hamiltonian allows us to obtain an electronic energy for each set of coordinates (generally denoted as 'single-point calculation'). The whole domain of coordinate values with their respective electronic energies is defined as the potential energy surface (PES).^{11,91}

This PES can be obtained with any of the methods described in the previous section, and the quality of these methods will determine how closely they resemble the PES of the real system. In addition, each electronic state will have a corresponding PES, which may intersect or interact with others, and the motion of the nuclei is governed by the shape of the surfaces. Therefore, any study of the theoretical mechanism of a chemical reaction must focus on the calculation of the potential surfaces, and the geometrical optimization of its critical points. In general, geometric optimization seeks to find the minimum energy configurations of molecular systems, which can correspond to reactants, products, transition states, or intermediate species⁹¹.

One type of non-adiabatic transition is intersystem crossing (ISC). ISC occurs when the transition happens between two potential energy surfaces with different spin multiplicity and without the emission or absorption of a photon.⁹² ISC is particularly relevant in the context of spin-forbidden reactions, which involve a change in the total spin state of a system during a chemical reaction. The "forbidden" term originates from quantum mechanical selection rules, which predict that transitions between states of different multiplicities will be spectroscopically forbidden, and thus, they would proceed at a slower rate.⁴

ISC processes can be represented using a Jablonski diagram, which is a graphical

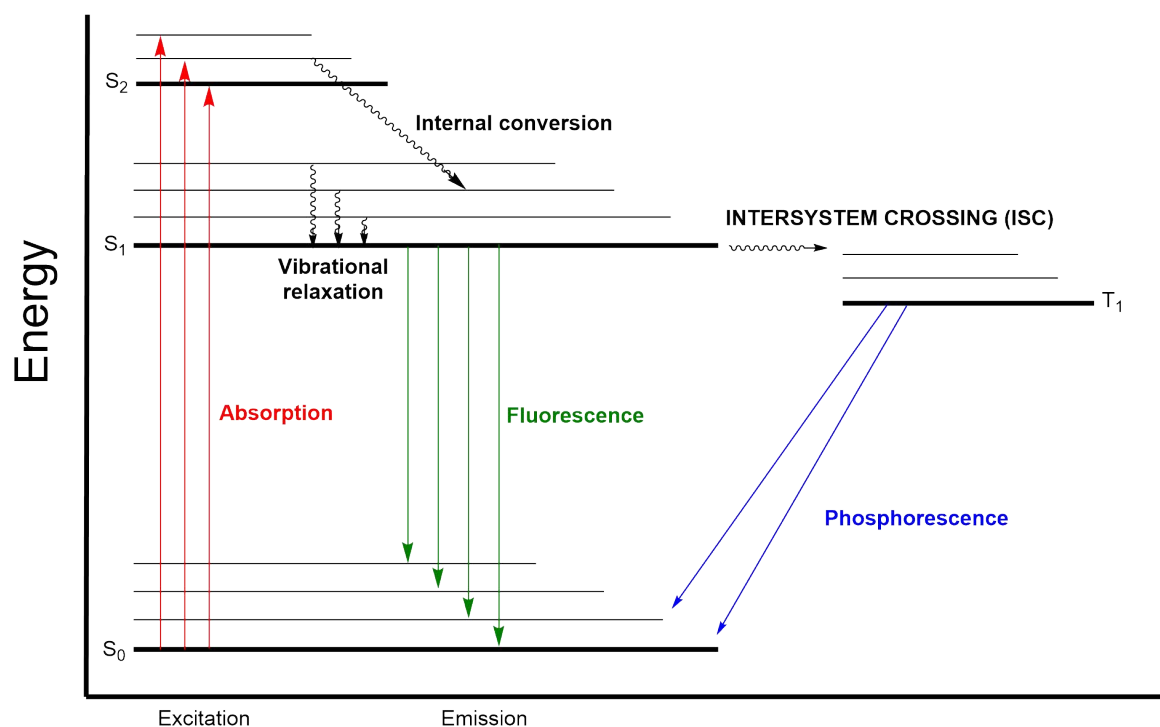


Figure 5: Jablonski diagram.⁹² This figure shows some of the available transitions between electronic states of a chemical system, among them, the most related to this work are the intersystem crossings (ISC), produced when the system changes its spin number without radiation. The wavy lines imply there is no radiation absorbed or emitted.

scheme of the energy levels of electronic states and the transitions between them (Figure 5)⁹². In a Jablonski diagram, the horizontal lines represent the energy levels of different electronic states, while the vertical lines connecting the energy levels correspond to various types of transitions, such as absorption, emission, internal conversion, and ISC.

At the crossing points between the two state surfaces, the energy difference between the states is zero, and the coupling between the states should be high enough (spin-orbit coupling, SOC, explained in the next section), thus enhancing the probability of the transition. These points are known as minimum energy crossing points (MECPs) and play a central role in the study of non-adiabatic processes^{93,94}.

To locate the MECP between two electronic states, a special optimization technique must be employed, such as the algorithm proposed by Harvey and collaborators⁹³. This algorithm defines two effective orthogonal gradients, f and g , in terms of the gradients of the two crossing PESs, G_1 and G_2 (Figure 6):

$$f = (E_1 - E_2)(G_1 - G_2) = (E_1 - E_2)\Delta G \quad (23)$$

$$g = G_1 - \frac{G_1 \cdot \Delta G}{|\Delta G|} \frac{\Delta G}{|\Delta G|} \quad (24)$$

Here, E_1 and E_2 are the energies of the two electronic states, $\Delta G = G_1 - G_2$ and $|\Delta G|$ is the module of this vector. The gradient f is orthogonal to the seam, and the seam gradient g approaches zero at the MECP. The MECP structure can be located by minimizing the sum of the effective gradients, $f + g$. This MECP search algorithm has been successfully used in several works related to ISCs^{93,95-98}.

In Ref. 2, we used a different MECP optimization technique, which uses a harmonic potential to find the MECP. This biased potential is defined by:

$$V_{bias} = \frac{1}{2}k \left(\Delta E^{sing/trip} \right)^2 \quad (25)$$

where $\Delta E^{sing/trip}$ is the energy difference between triplet and singlet states. At MECP, the potential will vanish and the total energy will be minimum.⁹⁹

Upon determining the MECP structure, it is necessary to obtain certain parameters that describe the relationship between the two electronic surfaces, such as the SOC, the difference in their gradients or the reduced mass associated with the vibration of

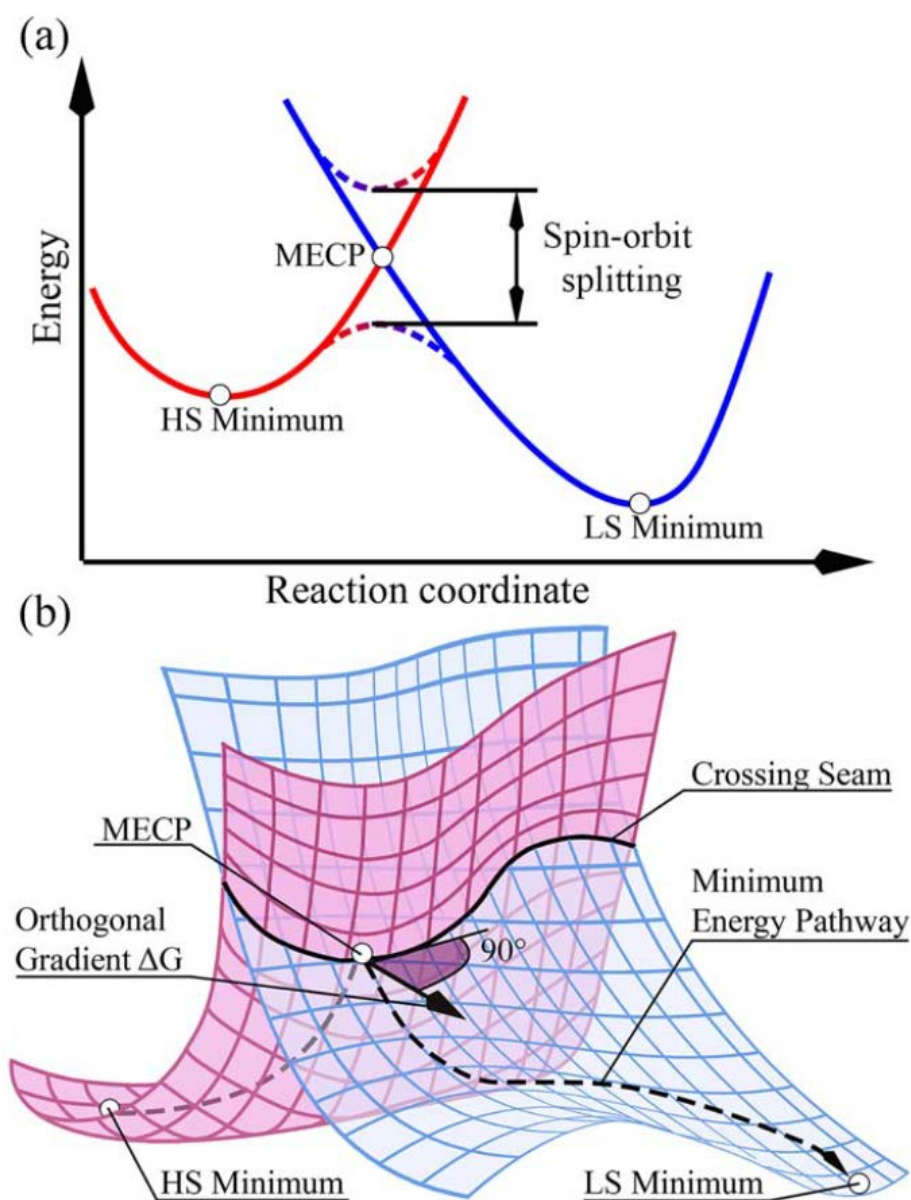


Figure 6: Intersystem crossing process (ISC). An ISC is defined as any chemical process in which the system undergoes a change in its spin number, either from reactants to products or at some point in the trajectory. ΔG is the difference between the gradients of both surfaces and f gradient is parallel to the crossing seam (Eq. 23). The HS and LS surfaces represent the high-spin and low-spin states (triplet and singlet for this whole thesis). Figure extracted from Ref. 95.

the ISC.¹⁰⁰.

3.2.2 Spin-orbit coupling and its role in intersystem crossing

Spin-orbit coupling (SOC) is an effect that occurs due to the interaction between an electron’s intrinsic spin and its orbital angular momentum around an atomic nucleus^{101,102}. It leads to the splitting of energy levels and mixing of electronic states, which can significantly influence the properties and behaviour of molecules and materials^{103,104}. The magnitude of SOC is generally proportional to the atomic number (Z) and is more pronounced in heavier elements.¹⁰⁵

ISC processes mediated by spin-orbit coupling are particularly relevant for transition metals, which can easily change the spin of their electrons due to this coupling property^{102,106}. A common example of this type of reaction is the oxidation of haemoglobin, a spin-forbidden reaction that occurs rapidly due to the high spin-orbit coupling of the system with an iron atom at the center¹⁰⁷. Moreover, the solvation or enzyme environment is crucial to understand it, as interactions with nearby molecules could alter the spin-orbit coupling¹⁰⁸.

The total angular momentum, denoted by J , is the sum of the electron’s spin angular momentum, S , and its orbital angular momentum, L :

$$\mathbf{J} = \mathbf{L} + \mathbf{S} \tag{26}$$

The Hamiltonian for a system with spin-orbit coupling can be written as:¹⁰²

$$\hat{H} = \hat{H}_0 + \hat{H}_{\text{SOC}}, \tag{27}$$

Here, $\hat{H}_0$ represents the non-relativistic electronic Hamiltonian, and $\hat{H}_{\text{SOC}}$ symbolizes the spin-orbit contribution to the Hamiltonian. There are several different ways to represent the SOC Hamiltonian, but here we have used the Breit-Pauli Hamiltonian, $\hat{H}_{\text{BP}}$. It can be written as¹⁰⁹:

$$\hat{H}_{\text{BP}} = \hat{H}_0 + \hat{H}_{\text{SOC}} + \hat{H}_{\text{SRC}} + \hat{H}_{\text{SS}} + \hat{H}_{\text{OO}} \quad (28)$$

where $\hat{H}_{\text{SRC}}$ is the scalar-relativistic correction and the last two terms, $\hat{H}_{\text{SS}}$ and $\hat{H}_{\text{OO}}$ represent the spin-spin and orbit-orbit interactions, respectively. The SOC term of this Hamiltonian reads:

$$\hat{H}_{\text{SOC}} = \frac{p^4}{8m^3c^2} - \frac{p^2}{2mc^2} \left(\frac{Ze^2}{r} \right) - \frac{Ze^2}{2m^2c^2r} \mathbf{L} \cdot \mathbf{S}, \quad (29)$$

where p is the electron momentum, m is the electron mass, c is the speed of light, Z is the atomic number, e is the elementary charge, r is the radial distance between the electron and nucleus, and $\mathbf{L} \cdot \mathbf{S}$ is the dot product of the electron's orbital and spin angular momenta. The last term in this Hamiltonian represents the spin-orbit interaction. This operator has to be applied to all the components of the electronic states, so it must be represented with a matrix. The effective spin-orbit coupling matrix element, used to calculate the transition probability between a singlet and a triplet surface, can be obtained from the Hamiltonian using the expression:⁹⁵

$$H_{\text{SOC}}^2 = \sum_{M_S=-1}^1 \left| \langle SM_S | \hat{H}_{\text{SOC}} | S' M'_S \rangle \right|^2 \quad (30)$$

where $S(S')$ and $M_S(M'_S)$ are the quantum numbers of the spin angular momentum

and the spin (prime for the final state). Taking square roots, this equation follows:

$$H_{SOC} = \sqrt{S_{-1}^2 + S_0^2 + S_1^2} \quad (31)$$

Here, S_M is the coupling between the singlet state and each of the components of the triplet state (with quantum numbers $M_S=\{-1,0,1\}$). This spin-orbit coupling will be used in the next section to finally obtain the kinetics of the non-adiabatic reaction, connecting the theoretical framework developed in this chapter with reliable experimental results.

3.2.3 Non-adiabatic transition state theory

Non-adiabatic transition state theory (NA-TST) is an extension of traditional transition state theory (TST) that takes into account the effects of electronic transitions in addition to nuclear motion.¹¹⁰ Briefly, TST is a theoretical framework that provides a method to predict reaction rates by focusing on the highest energy point along the reaction path – the transition state.¹¹¹ In NA-TST, the reaction rate constant is calculated by considering the probability of non-adiabatic transitions between potential energy surfaces of different electronic states, which is a crucial aspect for understanding the kinetics of reactions involving intersystem crossings.

One key aspect of NA-TST is the use of minimum energy crossing points (MECPs) as reference structures for calculating rate constants. In contrast to traditional TST, which relies on the identification of transition state structures along the reaction coordinate, NA-TST considers the MECPs as the critical points governing the probability of non-adiabatic transitions⁹⁵.

The Eyring equation,¹¹² which is commonly used in traditional TST to calculate the reaction rate constant $k_{adiabatic}(T)$, can be extended to incorporate the effects of electronic transitions in NA-TST:

$$k_{\text{NA-TST}}(T) = k_{adiabatic}(T)P_{trans} \quad (32)$$

with $k_{adiabatic}(T)$ being:

$$k_{adiabatic}(T) = \frac{k_B T}{h} \exp\left(\frac{-\Delta G^\ddagger}{RT}\right) \quad (33)$$

Here, k_B is the Boltzmann constant, h is the Planck constant, $\Delta G^\ddagger(T)$ is the Gibbs free energy of activation, R is the gas constant, T is the temperature, and P_{trans} is the probability of the non-adiabatic transition between electronic states. However, it represents an average probability over all possible energies. This requires to compute the integral for the whole ensemble, taking into account the Maxwell-Boltzmann statistical distribution of energies, $Q_{MB}(\varepsilon)$. This was evaluated numerically, following the procedure described by Lykhin et al.⁹⁵

$$P_{trans} = \frac{\int_{E_{MECP}}^{\infty} P_{trans}(\varepsilon) Q_{MB}(\varepsilon) d\varepsilon}{\int_{E_{MECP}}^{\infty} Q_{MB}(\varepsilon) d\varepsilon} \quad (34)$$

where $Q_{MB}(\varepsilon)$ comes from:

$$Q_{MB}(\varepsilon) = 2\sqrt{\frac{\varepsilon}{\pi}} \left(\frac{1}{RT}\right)^{\frac{3}{2}} \exp\left(\frac{-\varepsilon}{RT}\right) \quad (35)$$

where R is the gas constant. The term $P_{trans}(\varepsilon)$ can be obtained from the Landau-

Zenner probability expression, in its double-passage version, given by:¹¹³

$$P_{trans}(\varepsilon) = (1 - p^{LZ}(\varepsilon)) + p^{LZ}(\varepsilon)(1 - p^{LZ}(\varepsilon)) \quad (36)$$

with $p^{LZ}(\varepsilon)$ being:

$$p^{LZ}(\varepsilon) = \exp \left(-\frac{2\pi H_{SOC}^2}{\hbar |\Delta G|} \sqrt{\frac{\mu}{2(\varepsilon - E_{MECP})}} \right) \quad (37)$$

with μ being the reduced mass associated with the ISC, ε is the internal energy, represented as a sum of the component orthogonal to the crossing seam, and $|\Delta G|$ is the norm of the difference between the gradients in the singlet and triplet states (Eq. 23).⁹⁵ H_{SOC} is the effective spin-orbit coupling, taken from Eq. 31.

By combining Eqs. 32 and 33 and taking logarithms, we can determine the free-energy barrier increase caused by the spin-forbidden nature of the reaction, represented as $\Delta\Delta G^\ddagger$.

$$\Delta\Delta G^\ddagger = \Delta G_{NA-TST} - \Delta G_{adiabatic} = -RT \ln \left(\frac{k_B T P_{trans}}{h} \right) + RT \ln \left(\frac{k_B T}{h} \right) \quad (38)$$

$$\Delta\Delta G^\ddagger = RT \left[\ln \left(\frac{k_B T}{h} \right) - \ln \left(\frac{k_B T}{h} \right) - \ln(P_{trans}) \right] \quad (39)$$

$$\Delta\Delta G^\ddagger = -\ln(P_{trans})RT \quad (40)$$

Adding $\Delta\Delta G^\ddagger$ to $\Delta G^\ddagger$ yields the free-energy barrier for a corresponding spin-allowed reaction. For hopping probabilities ranging from 10^{-4} to 0.1, the typical values of $\Delta\Delta G^\ddagger$ lie between 1 and 4 kcal/mol.¹⁰⁶

RESEARCH CONTEXT AND BACKGROUND

4 Research context and background

Building upon the foundational physical concepts introduced in Chapter 3, in this chapter we discuss the properties of molecular oxygen and how they determine the reactivity with organic molecules.

Oxygen is a fundamental element that plays a vital role in various biological and chemical processes.¹¹⁴ In the atmosphere, it is present as a dimer, O₂, named dioxygen or molecular oxygen. In this thesis we studied reactions between dioxygen and organic substrates, focusing on intersystem crossings (ISC). These spin-flip processes are particularly intriguing and complex when they occur in enzymatic systems without the presence of metal cations.

4.1 Oxygen systems & electronic structure

The oxygen molecule has two oxygen nuclei, each of which has eight protons, and sixteen electrons. The most abundant isotope is ¹⁶O, with almost a 100% natural abundance, followed by ¹⁸O, accounting for 0.2%.¹¹⁵ It possesses the point-group symmetry D_{∞h} and the three electronic states lying lower in energy are as follows (Figure 7): The ground state, ³Σ_g⁻, contains two unpaired electrons, the first excited state, ¹Δ_g, has double spatial degeneracy, and the following excited state, ¹Σ_g⁺, would couple electrons in different orbitals. The energy difference between the ¹Δ_g singlet state and the triplet ³Σ_g⁻ ground state is 0.9773 eV (experimental values, see Ref. [116, 117]), whereas the energy difference between the ¹Σ_g⁺ singlet state and the ground state is 1.6268 eV.^{116,117} The ¹Δ_g singlet state, which is doubly degenerate in the absence of interaction with a partner molecule, transitions to a non-degenerate state upon such interaction. This is a consequence of the spatial distribution of the electron wave functions in the molecu-

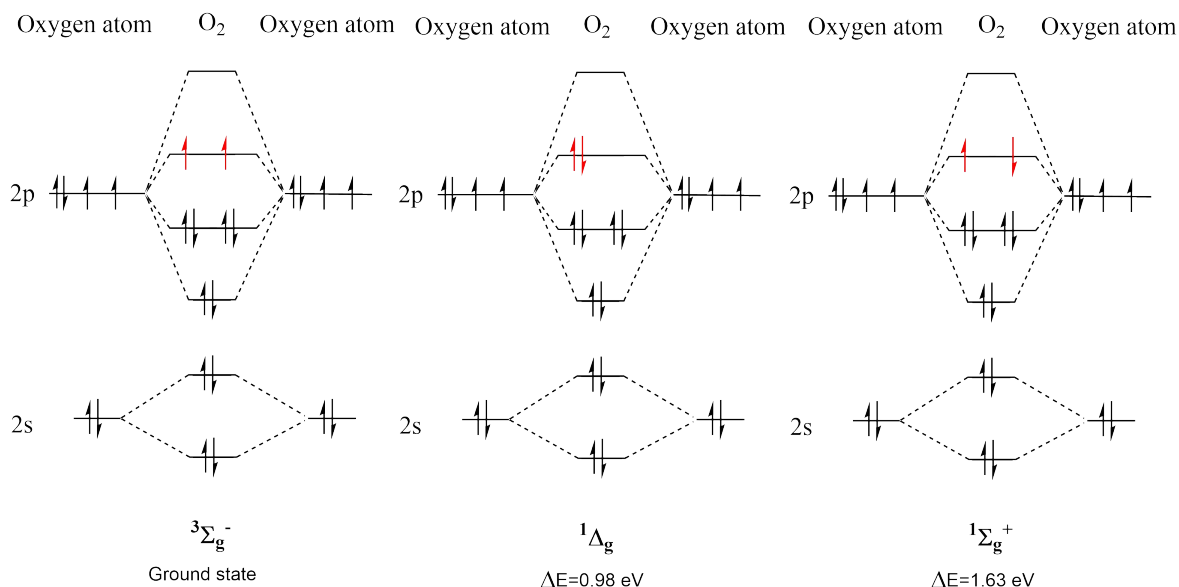


Figure 7: Molecular orbital diagram of two singlet excited states as well as the triplet ground state of molecular dioxygen. From left to right, the diagrams are for: $^3\Sigma_g^-$ triplet oxygen (ground state), $^1\Delta_g$ singlet oxygen (first excited state), and $^1\Sigma_g^+$ singlet oxygen (second excited state). The $^1\Delta_g$ state is doubly degenerate, losing this degeneracy upon interaction with a partner molecule. In all three cases, the oxygen atoms that make up the molecule come from the $O(^3P)$ states. Experimental values from Ref. 116, 117.

lar orbitals. In addition, dioxygen possesses paramagnetic properties, derived from its unpaired electrons in the ground state.

We calculated the CASSCF(12e,8o)/aug-cc-pVTZ level dioxygen dissociation curves versus O-O internuclear distance (Figure 8). The notation means that we have included 12 electrons and 8 orbitals in the active space, in this case, all valence electrons and valence orbitals. These curves show the enormous complexity of the electronic states formed from two oxygen atoms. The three most relevant states, described in the previous paragraph, are shown in more detail in the bottom panel.

The most significant kinetic obstacle to oxygen's direct interaction with an organic substrate is the fact that the oxygen molecule is a triplet with two unpaired electrons in its ground state. On the other hand, common organic compounds that naturally

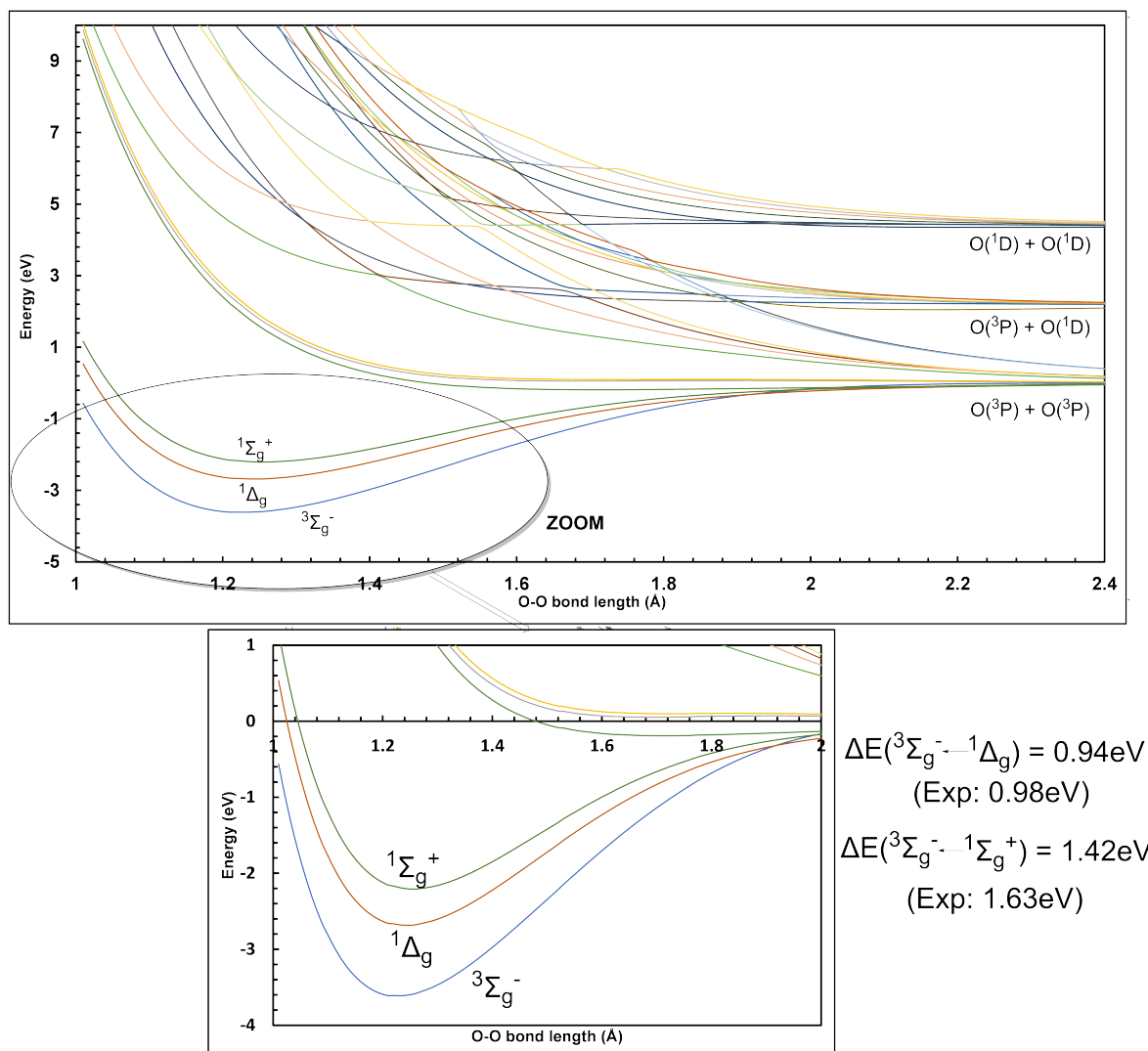


Figure 8: Upper box: CASSCF(12e,8o)/aug-cc-pVTZ curves for the first 20 singlet and 20 triplet electronic states arising from the mix of several atomic oxygen combinations. For long distances between oxygen atoms, they will degenerate, and they will spread as the molecule approaches the equilibrium bond distance. Lower box: Zoom in for the most relevant states. The triplet ground state $^3\Sigma_g^-$ is shown in blue, the degenerate singlet $^1\Delta_g$ in red and the upper singlet $^1\Sigma_g^+$ in green. Other states are not relevant to this work.

exist in biological substrates often have a singlet ground state, which means they do not have unpaired electrons, and their oxygenation products typically have a singlet ground state as well. The spin change process (triplet to singlet) is spin-forbidden according to “selection rules”, a set of rules derived from Group Theory that restrict transitions between quantum states. So, typically, the spin-change step will be the rate-limiting step and the reaction will not be able to proceed in a single, quick step.¹¹⁸ This prohibition will not be totally restrictive, as we have seen in Chapter 3 that there is a property called spin-orbit coupling (SOC) which allows spin change, and whose probability will be directly proportional to the SOC value.¹⁰² This coupling is, in general, greater for heavier atoms, so oxygen, being a light atom, will not have high SOC values under normal circumstances. However, the SOC can be amplified under certain conditions, increasing the rate of the singlet-triplet transition. These conditions are explained in the next subsection.

4.1.1 Analysis of spin-orbit coupling and spin-forbidden transitions for dioxygen molecule

The SOC parameter will be crucial in the transitions between electronic states related to the dioxygen molecule. This is illustrated by the difference in lifetimes of the two dioxygen singlets seen so far, $^1\Delta_g$ and $^1\Sigma_g^+$. The SOC for the transition $^3\Sigma_g^- - ^1\Delta_g$ is zero in the gas phase, while for the $^3\Sigma_g^- - ^1\Sigma_g^+$ transition it ranges from 150-180 cm^{-1} .¹¹⁹ As a result, the lifetime for isolated $^1\Delta_g$ in the upper layers of the atmosphere is about one hour, while for the $^1\Sigma_g^+$ it is 6 minutes. Moreover, in solution, the SOC increases for both transitions, so the lifetimes are reduced to 1 second for $^1\Delta_g$ and even picoseconds for $^1\Sigma_g^+$.¹²⁰

In parallel to the work of this thesis, Ogilby’s group has published several papers related

to this topic.^{119,121,122} Particularly enlightening is the calculation of spin-orbit couplings for different interacting geometries, where oxygen transfers charge asymmetrically between its π -orbitals by interaction with ligands, such as metal atoms or organic solvent molecules. It has been shown that the SOC value is directly proportional to the difference in energies of the π -orbitals of the dioxygen, making it clear that the singlet-triplet transition can take place as long as there is something around to break the original orbital symmetry of the dioxygen molecule.¹¹⁹ The spin-orbit coupling values for the $^3\Sigma_g^- \rightarrow ^1\Delta_g$ transition obtained in their publication (at CASSCF(12o,8e)/aug-cc-pVDZ level of theory) are of the same order as those obtained in the present thesis, in the range 0-90 cm^{-1} .

This phenomenon, although quantitatively confirmed in recent years, agrees with the qualitative description of the spin-orbit coupling proposed in the 1960s, known as the El-Sayed rule.^{123,124} According to this rule, the strength of the intersystem transition (singlet-triplet in this case) is stronger when the change of electronic structure is associated with a change in orbital angular momentum. By breaking the degeneracy between the two π -orbitals of dioxygen, any electronic transition between these orbitals will produce a change in orbital angular momentum that is more likely to occur than in isolated dioxygen.

4.1.2 Reaction mechanisms between dioxygen molecule and organic substrates

With these elements, oxygen in its triplet ground state and the substrate as a singlet, plus singlet products, we could consider three possible mechanisms for an oxygenation reaction to take place, as illustrated in Figure 9.

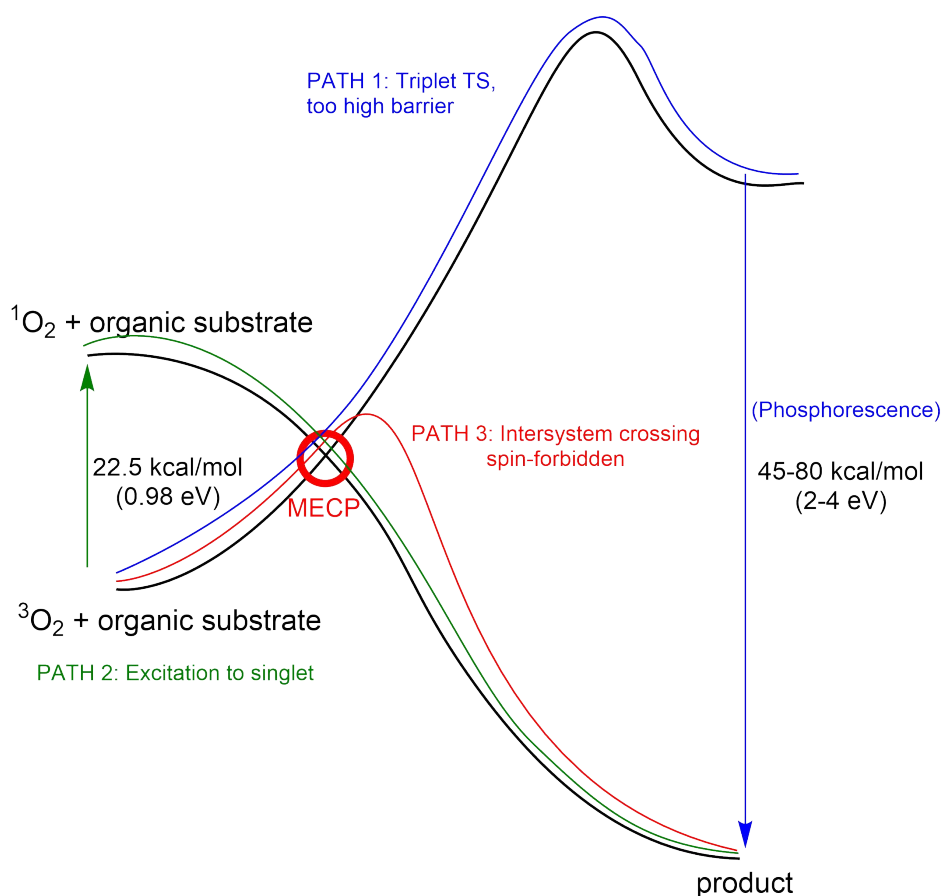


Figure 9: Illustrative reaction between $^3\Sigma_g^-$ oxygen and an organic singlet substrate. The reactants are initially on a triplet surface, due to the electronic configuration of the oxygen molecule. They may overcome the high barrier on this surface (blue line), get photoactivated to singlet (green line) or undergo a spin-crossing process through the MECP (red line). The activation barriers for the triplet state are usually too high. In principle, neither of these paths would be easy to overcome and it is necessary to analyse the spin-orbit coupling for this system.

The first path (Path 1 in Fig. 9) would follow the minimum energy path (MEP) along the triplet state surface, leading to the products in a triplet (excited) state. However, if this MEP is followed, there will usually involve high reaction barriers, moreover, the product will be highly endothermic. For example, it could be the case that an oxygen molecule attacks an organic substrate and overcomes the triplet transition state, however, this barrier is likely to exceed 50 kcal/mol (45-80 kcal/mol for unsaturated

molecules, even higher for saturated substrates)¹²⁴, excessively slow at room temperature. From this triplet unstable product, the singlet product could be reached by a phosphorescence process. The rate coefficient for phosphorescence, k_{ph} , can be given by Fermi's Golden Rule as follows:¹²⁵

$$k_{\text{ph}} = \frac{2\pi}{\hbar} |\langle f | H' | i \rangle|^2 \rho(E), \quad (41)$$

where H' is the interaction Hamiltonian (which includes the Born-Oppenheimer Hamiltonian and a spin-orbit coupling Hamiltonian), $|i\rangle$ and $|f\rangle$ are the initial and final states of the transition, and $\rho(E)$ is the density of the final states. This equation implies that phosphorescence is not only spin-forbidden, but also statistically improbable, contributing to its overall slowness. Therefore, phosphorescence usually results in a long-lived excited state, which is not favorable for many biochemical reactions that require efficient energy transfer. All in all, this reaction path has too many setbacks.

The second option (Path 2 in Fig. 9) would be to access the singlet excited state of oxygen first and then proceed with the reaction. This can be achieved by photochemical activation. In photoactivation, it is necessary to use other compounds known as photosensitisers, which will first perceive the electronic excitation and in turn, through charge transfer, allow the triplet-singlet transition in the oxygen molecule.^{117,126} Direct excitation of oxygen is not feasible because its isolated electronic transition is weak. Photosensitisers range from organometallic compounds, common light receptors such as porphyrins or chlorophylls, metal cations and others.^{127,128} Although this method is common in organic synthesis, or even in some anti-cancer therapies known as photodynamic therapies (PDT),^{129,130} photosensitisers are not naturally present in most environments. Additionally, the products of ordinary singlet oxygen reactions on or-

ganic substrates differ greatly from those of oxygen reactions on organic substrates catalysed by oxygenase enzymes. Singlet oxygen reactions can generate reactive oxygen species (ROS), which are responsible for the therapeutic effects in photodynamic therapy, as they can cause oxidative stress and cell damage.¹³¹

The last option (Path 3 in Fig. 9) happens through an intersystem crossing (ISC) from the triplet to the singlet surface along the reaction path. Since both singlet and triplet surfaces intersect along a crossing seam, the equivalent of the transition state in the context of the NA-TST will be the minimum energy crossing point (MECP) (expecting to have a significant value of SOC at that point). This option has a higher probability compared to the first mechanism and is more controlled than the second one, which may lead to other products and requires photosensitizers. However, the ISC process is not barrierless. The hopping between electronic states is associated with a probability, which could increase the overall reaction barrier. The increased energy barrier due to the spin-forbidden nature of the transition is often in the range of 1-4 kcal/mol, affecting the overall reaction rate.⁹⁴

For this mechanism, nature has adapted different strategies with great diversity, making use of available resources such as metal cations or synthesizing specific enzymes.¹³² Metal cations can facilitate ISC by increasing spin-orbit coupling, while enzymes provide the appropriate environment for the reaction to occur.¹³³ In this text, we will refer to the reaction path involving ISC as a direct path or a spin-forbidden reaction path.

The viability of this reaction pathway will depend mainly on the charge transfer behaviour of the functional groups, such as double bonds, or the assistance of groups that increase the SOC of the system, such as metal atoms or some organic molecules such as flavin.

For some organic substrates may be easier to react through an intersystem crossing, mainly due to their electronic structure, with delocalized π clouds functional groups, which facilitate charge transfer processes and increase their reducing potential.³ When these organic compounds are required for an enzymatic biochemical reaction, they are collectively known as cofactors, although they form a very heterogeneous group.¹³⁴ It has been demonstrated that such a process is involved in the reaction of dioxygen with reduced flavins, a paramount example of an organic cofactor.^{135,136} This mechanism is shown in Figure 10.

In the case of molecules with lower charge transfer to oxygen, the reaction becomes much more challenging. Without metal atoms to increase the SOC, some reactions may not occur, such as in methane, which requires very high activation energies and extreme combustion temperatures to initiate a reaction with oxygen.¹³⁷ The primary focus of this work is on reactions involving organic substrates that are not assisted by metal atoms or organic cofactors.

4.2 Biochemical systems: oxidases and oxygenases

In this section, we will focus on the reactions between dioxygen and organic substrate that are catalyzed by enzymes. These biochemical reactions can be broadly classified into two types: redox (electron transport) reactions and oxygen incorporation reactions. The enzymes catalyzing redox reactions are generally known as oxidases, which encompass a diverse range of enzyme types.¹³⁸ Cytochrome C oxidase is a well-known example of an oxidase enzyme that uses metal atoms, such as copper and iron, to facilitate spin change during the redox process.^{139,140}

On the other hand, oxygen incorporation reactions involve the integration of oxygen

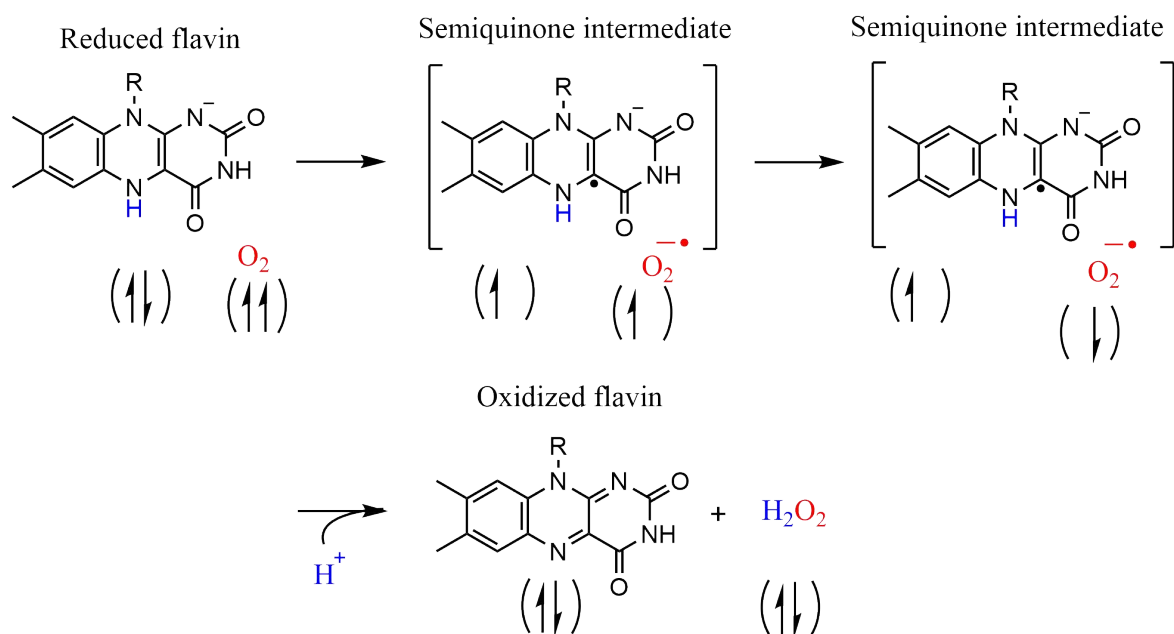
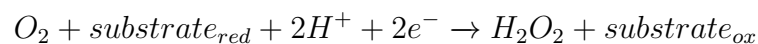


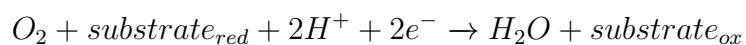
Figure 10: Proposed mechanism for the flavin oxidation via charge transfer between O_2 and the substrate.¹³⁶ The dioxygen molecule captures electronic charge in the first step. This charge transfer usually facilitates the spin change in the diradical pair, which then forms a final stable singlet product.

atoms into organic molecules as part of a synthetic route. The enzymes catalyzing these reactions are classified as monooxygenases or dioxygenases, depending on whether the final product incorporates a single oxygen atom or both oxygen atoms from the molecular oxygen.^{114,141}

Oxidases mechanism:



Monooxygenases mechanism:



Dioxygenases mechanism:



For some time, it was assumed that all oxidase and oxygenase reactions required either heavy metals, such as those involved in hemoglobin oxidation, or cofactors, which were briefly introduced in the previous section. Heavy metals, including transition metals, are common catalysts for various reactions, spanning from biochemical to organic/inorganic synthesis and industrial processes. The use of metal cations in these reactions is advantageous because they can facilitate intersystem crossing by providing strong spin-orbit coupling, which helps overcome the change in spin. Extensive experimental and theoretical studies have been conducted on metal-catalyzed reactions involving dioxygen, which highlight their importance in various contexts.^{139,140,142–144}

Heme groups are complex organic moieties that contain an iron atom coordinated within a large heterocyclic organic ring called a porphyrin. Notably, heme groups play a crucial role in biological systems, and their interactions with metals significantly influence biochemical reactions.¹⁴⁵ The oxidation of hemoglobin is a prime example of a metal-catalyzed reaction involving heme groups. An iron atom (Fe^{2+}) at the center of the heme group mediates the process. Here, the intersystem crossing, facilitated by the spin-orbit coupling of the iron atom, enables the rapid oxidation of hemoglobin, a reaction otherwise considered spin-forbidden.¹⁰⁷ Another example of oxygenase reactions involving heme groups is the cytochrome P450 enzymes. These are a class of heme-containing proteins that catalyze the insertion of an oxygen atom into organic substrates, an important reaction for drug metabolism and the biosynthesis of steroids and other lipids.¹⁴⁶

Transition metals can also be part of complex enzymes without heme groups, facilitating essential biological reactions. For instance, copper plays a significant role in various oxidation reactions. This includes the conversion of dopamine to norepinephrine by the enzyme dopamine β -hydroxylase, and the oxidation of water to oxygen in photosystem II during photosynthesis.^{147,148}

Manganese (Mn^{3+} and Mn^{4+}) is also an important metal in oxidation and oxygenation reactions, for example in the cytochrome C oxidase reaction, where it catalyses the respiratory reduction of dioxygen.¹⁴⁰ Cobalt, another transition metal, forms as Co^{2+} a critical component of vitamin B12-dependent enzymes such as methylmalonyl-CoA mutase and methionine synthase. These enzymes catalyze essential reactions involving the transfer of methyl groups and the rearrangement of carbon skeletons.¹⁴⁹

Flavin species are among the most prevalent cofactors in processes involving oxygen (see Figure. 10).^{134,150–152} Their delocalized π system enables a wide range of metabolic events to occur within various species. Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) are two significant flavin derivatives that participate in numerous reactions involving oxygen, including redox reactions and other essential cellular activities such as cellular respiration and fatty acid oxidation.^{135,153,154} Both FAD and FMN act as electron donor-acceptor cofactors in these processes. Flavoproteins, which are proteins containing a flavin prosthetic group (either FAD or FMN), play a vital role in various biological systems, from bacterial to mammalian cells.¹⁵⁵

A diverse range of reactions involving flavin and oxygen has been reported in the literature.^{134–136,150–152,156} Some examples include flavin-catalyzed monooxygenase reactions, where one atom of molecular oxygen is inserted into a substrate, while the other is reduced to water.¹³⁶ Additionally, there are flavin-dependent hydroxylases that catalyze the hydroxylation of substrates using FAD or FMN as cofactors.¹⁵⁶ These

flavin-mediated reactions are of great importance in various biological processes, and understanding their mechanisms is crucial for advancing our knowledge of biochemistry and enzymology.¹⁵⁷

In recent years, enzymes that catalyze reactions with O_2 in the absence of any metal or cofactor have been reported.¹⁵⁸ The exact mechanism by which these unique enzymes catalyze these processes and facilitate the transition from triplet to singlet PES remains unclear.

One of the earliest research studies on a cofactor-independent oxidase enzyme can be traced back to 1997.¹⁵⁹ Urate oxidase (UOX) has been extensively studied, and its mechanism has served as a model for other cofactor-independent oxidases. For example, in Refs. [160, 161, 162, 163], its mechanism is investigated using experimental techniques such as X-ray crystallography, site-directed mutagenesis, and steady-state kinetics. Another well-known example of cofactor-independent oxidation is the reaction catalyzed by coproporphyrin oxidase (CPO), which is involved in the metabolic pathway of porphyrins and regulates their concentration in the body.¹⁶⁴ Figure 11 illustrates the proposed charge transfer mechanism for both oxidases.

In this work, we will also examine cofactor-independent monooxygenases and dioxygenases. In a recent review on this subject by Bui and Steiner, the authors presented various experimental and computational approaches that could be used to explain the process of oxygen incorporation in cofactor-independent enzymes.¹⁶⁵ This review presents some of the discovered systems to date, including both monooxygenases and dioxygenases, along with their proposed oxygenation mechanisms. Despite their differences, they all share certain common features. For example, in all cases, a proton is removed prior to the oxygenation reaction, leaving the substrate with an anionic charge. This characteristic could be crucial for the charge transfer processes expected

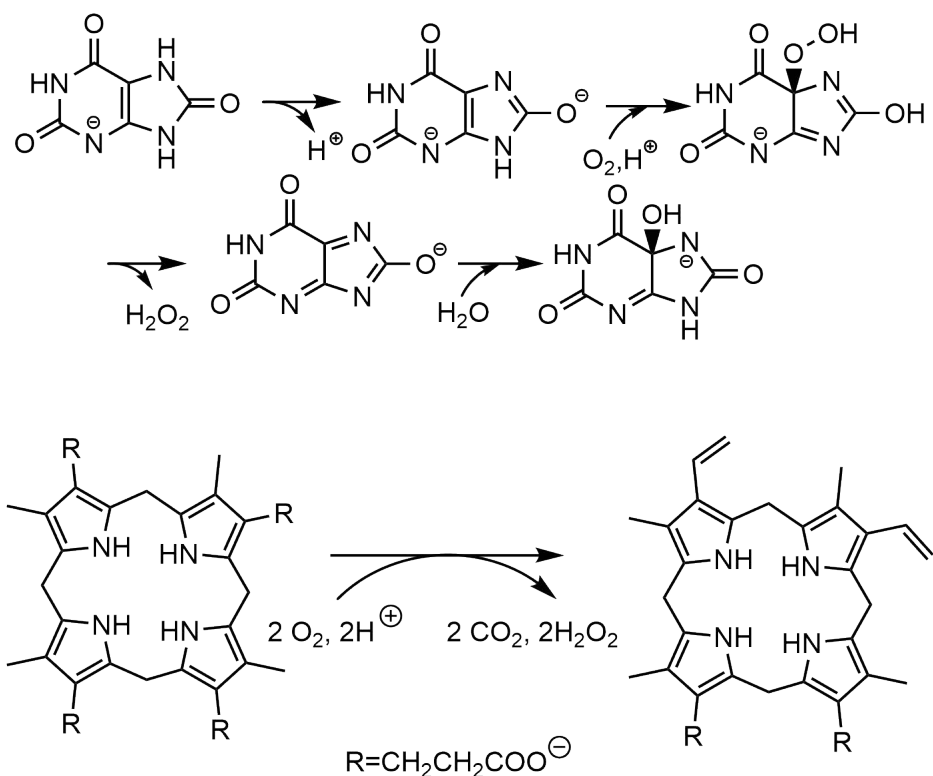


Figure 11: Top panel: Proposed mechanism for the uric acid oxidation reaction, catalysed by the enzyme urate oxidase. In the first step, the enzyme deprotonates the substrate, so that the charge transfer to oxygen is promoted. After an oxidation step in which the oxygen is converted into hydrogen peroxide, a water molecule is finally incorporated into the substrate. Bottom panel: Porphyrin oxidation reaction, catalysed by CPO.

to facilitate the reaction. Moreover, these systems often have delocalized bonds, which further enhance charge transfer (e.g., enolates are of great relevance, as we will see below) and there is always a peroxide reaction intermediate. Figure 12 illustrates the proposed mechanisms.

Among these systems, we chose the oxygenation of 3,5-dihydroxyphenylacetyl-CoA (DPA-CoA), for several reasons (see Figure 13). First, the lack of basic residues in the vicinity of the active site enables us to assess the likelihood that the peroxide will develop in the absence of amino acids that may protonate it. Second, the idea

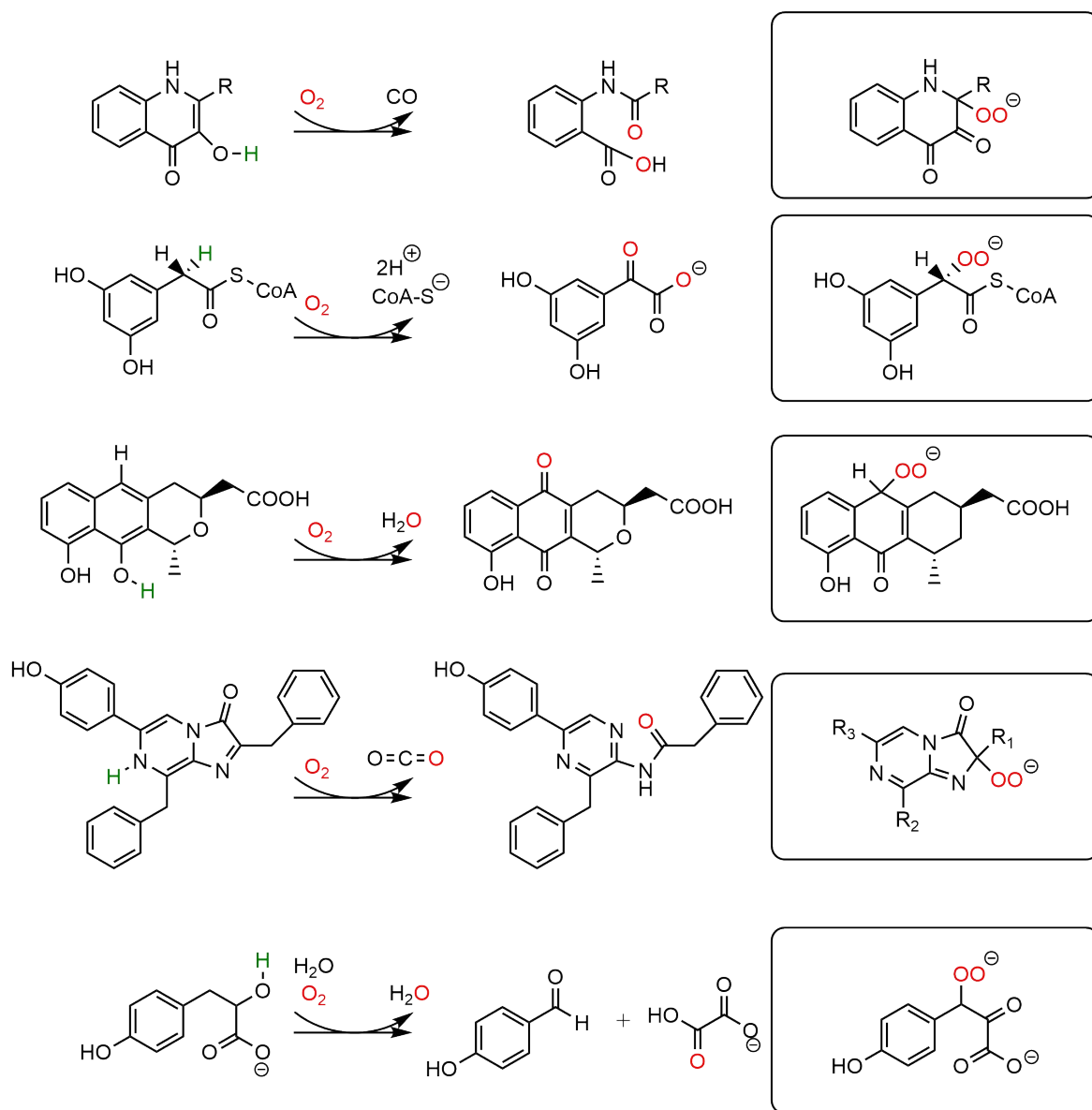


Figure 12: Some examples of cofactorless biochemical substrates and their reactions with molecular oxygen. All of these systems have some common features, they have been subtracted a proton (in green) and thus they have a negative electronic charge. The right panels show the peroxide intermediate for each reaction. Figure adapted from Ref. 165.

of a “spin well” may be dismissed since there are no aromatic residues that could be implicated in π -stacking interactions. Third, because the peroxidation occurs outside the aromatic ring, it is easier for the multireference computations to converge because,

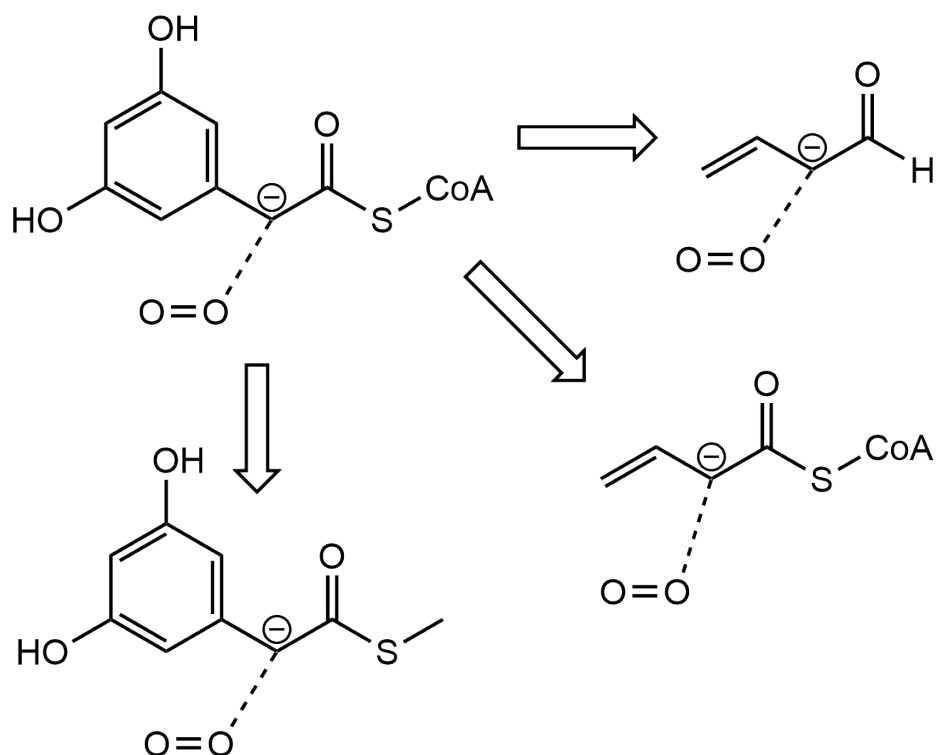


Figure 13: Reaction of DPA-CoA with triplet dioxygen and the three model systems used to study it in our work. In the top left panel, O_2 and the DPA moiety of DPA-CoA are depicted. These smaller systems have been used to reduce the computational cost of the calculations and to be able to extract information about charge transfers or the set of reaction orbitals more easily.

roughly speaking, the orbitals of the ring do not need to be included in the active space.

This reaction is catalyzed by the enzyme DpgC dioxygenase. DpgC is primarily involved in the biosynthesis route for the vancomycin and teicoplanin family of antibiotics.¹⁶⁶ Widboom was able to determine the structure of this enzyme through the use of X-ray diffraction, which also verified that there are no cofactors present.¹⁶⁷ In the Chapter 5, the first two papers provide a thorough and extensive description of the electronic structure and energy features of this reaction, along with its spin-orbit coupling property.

Other examples include the reaction catalyzed by hydroxyoxoquinaldine dioxygenase (HOD), which participates in the degradation of quinolones to form carbon monoxide and has been studied through experimental and computational methods,^{168–170} the mechanism of glutamate carboxylase that involves the carboxylation of glutamate residues,¹⁷¹ and the recently discovered nogalamycin monooxygenase (NMO) that oxidizes nogalamycin A to nogalamycin B.¹⁷² Despite the diversity of reactions, there are a few general hypotheses for these types of enzymatic reactions. One proposed idea is that these reactions have adapted to the lack of metals or flavin by employing a mechanism similar to that of flavin, which involves charge transfer. The enzyme deprotonates the substrate, leading to the formation of an anion that increases charge transfer to oxygen, thus simulating the role of flavin. Irrespective of the specific mechanism employed, these enzymes could be fittingly described as "substrate-assisted" due to their reliance on the substrate's properties for facilitating the reaction.

In general terms, there are several hypotheses regarding the mechanisms employed by these cofactorless enzymatic reactions. Some of the proposed mechanisms include charge transfer similar to that of flavin, the formation of a peroxide intermediate, or other substrate-assisted pathways. However, none of these hypotheses have been definitively confirmed, and experimental data suggest similarities among the mechanisms of all cofactorless enzymes. Throughout this work, we will address these questions by investigating some conceptual features that have been overlooked in previous research, such as spin-forbidden transition probabilities or MECP geometry optimization. By doing so, we aim to propose mechanisms based on theoretical calculations that may support some of these hypotheses and ultimately confirm the mechanism of cofactorless enzymes.

4.3 Triplet oxygen in organic synthesis

The increasing focus on green chemistry has driven the search for sustainable and environmentally friendly reagents, which is where the use of triplet oxygen stands out. As an abundant and non-toxic reagent, it produces far less polluting waste compared to other oxidants.^{173,174}

The reaction mechanisms and catalysts used in organic synthesis with triplet oxygen show parallels with the topics described so far.^{175–179} The role of metal catalysts in facilitating reactions involving triplet oxygen is well-documented. These catalysts often serve to lower the activation energy or facilitate the formation of intermediates, thereby enhancing the reaction rate and selectivity. Various metals, such as palladium, copper, and iron, have been utilized in this context.^{178,180–182}

In addition to metal catalysts, radical activation is another common approach in reactions involving triplet oxygen. This method relies on the generation of radical species, which can readily react with triplet oxygen. The use of light or heat often initiates these radical reactions. A myriad of organic compounds, including alkanes, alkenes, and alkynes, have been successfully oxidized using this approach.^{178,183}

Electron transfer processes also play a vital role in the incorporation of triplet oxygen into synthetic routes. These reactions typically involve the transfer of one or more electrons from the organic substrate to the oxygen molecule, facilitating the formation of reactive oxygen species that can undergo further reaction steps. Notable examples include the oxidation of alcohols to carbonyl compounds and the conversion of amines to nitroso compounds.^{179,184}

As a complement to the enzymatic reactions studied in the first two papers in Chapter 5, we also studied a side reaction from an organic synthesis, which shares many

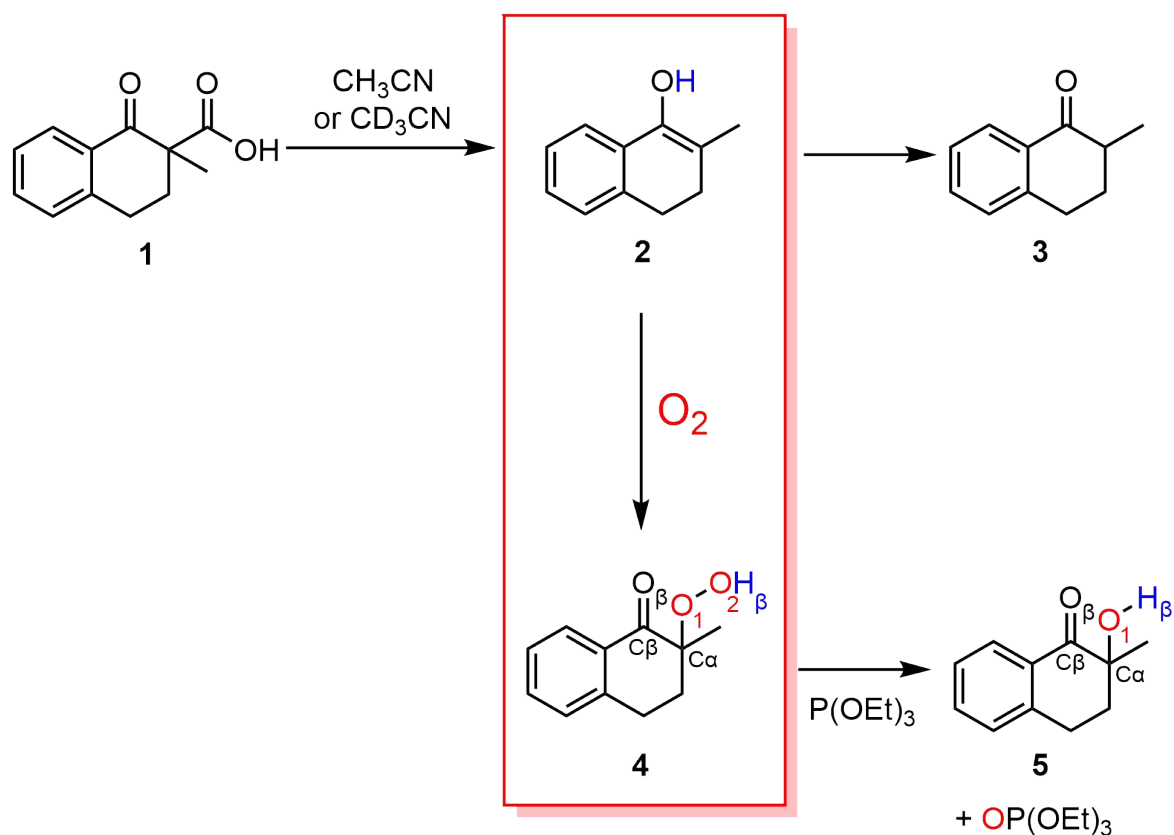


Figure 14: Decarboxylation of 2-methyl-1-tetralone-2-carboxylic acid (**1**) involves the formation of enol (**2**).¹⁷⁵ This enol is affected by a side reaction with atmospheric oxygen to obtain the hydroperoxide tetralone-derivative (**4**), whose theoretical mechanism is studied in this text. The transferred proton is coloured blue.

similarities with the enolate reactions of cofactorless enzymes. This reaction is depicted in Figure 14. The main reaction is the synthesis of 2-methyl-1-tetralone from 2-methyl-1-tetralone-2-carboxylic acid, whose intermediate produces a peroxide by direct oxygenation with atmospheric molecular oxygen.¹⁷⁵ In Ref. 3, we evaluate the calculations provided by Riahi et al. (Ref. 175) in order to predict if, due to the spin-orbit coupling, the mechanism of the reaction could be different from the one proposed by them. This reaction only takes place when the flask is not completely isolated from the air, and experimentally it is completely described and its structure confirmed. Moreover, it has been found that the reaction takes place in the absence of light, which

means that no singlet oxygen is involved by photoactivation. Unlike the other mechanisms studied in this work, the starting reagent has no anionic charge, which makes it even more interesting for evaluating mechanisms other than biochemical pathways. Neither is it in an enzymatic environment nor does it require any type of catalysis by acids, bases or solvents, for example in the oxygenation of DPA-CoA. All these characteristics generate great interest in this reaction and it represents a complementary contribution of great value to the rest of the mechanisms studied in the thesis.

RESULTS AND DISCUSSION

5 Results and discussion

5.1 Multireference analysis of peroxidation of the enolate group

This paper establishes a theoretical framework for the study of enolate group oxygenation reactions in biochemical reactions. Despite being in chronological order after the following paper, it has been considered that its relevance as a starting point could be greater for understanding the general mechanism of this type of reactions.

The focus of this work is on studying the phenomenon of spin change in the reaction between dioxygen and a enolate group, so that starting from a triplet state surface, a singlet final product is obtained (or even subsequent products, beyond the scope of this study, but singlets in any case).

Using the three model systems shown in Figure 15, we started from the anion corresponding to the deprotonation of α carbon atom, so that the negatively charged ion enhances the electronic charge transfer to the dioxygen molecule, facilitating the spin change of the process. The initial substrate is considered to be negatively charged because there is experimental evidence that the enzyme deprotonates the substrate in

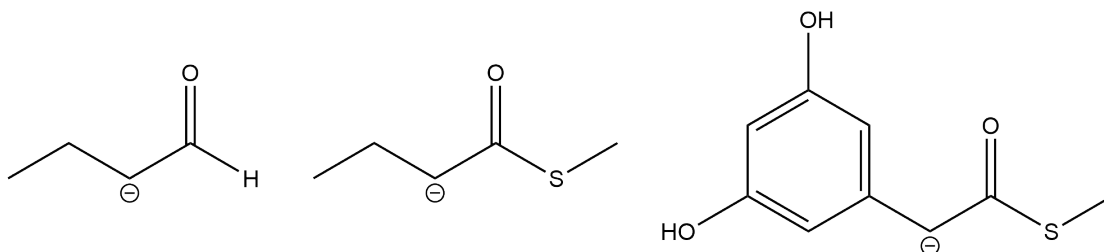


Figure 15: Model systems used to study the reaction with dioxygen. Starting with a smaller molecule, the size of the system is progressively enlarged up to DPA, which is of particular biochemical relevance.

a step prior to oxygenation.^{165,167} The larger system (3,5-dihydroxyphenylthioacetate, DPA, on the right) is identical to the actual DpgC substrate, except for the CoA fragment. From this, we have made two progressive reductions, removing the aromatic ring (to S-methyl-but-3-enethioate, in the middle) and the thioacetyl group (to 1-oxobut-3-en-2-ide, on the left).

Our research revealed a concerted mechanism in which transition from the triplet to the singlet potential energy surface is coupled with an electron density transfer from the O-O to the C-O bond. The results also confirm that intersystem crossing is promoted by stabilization of the singlet state along the reaction path. In this paper, spin-orbit coupling values have not been obtained, for two reasons. Firstly, they were already known previously from Ref. 2, and secondly, the interest of this publication is to verify that the theoretical methods used correctly describe the oxygenation process in gas phase for all the models used.

The main conclusion of this work is that the three theoretical methods used (SHCI, broken-symmetry DFT and PNO-LCCSD(T)) are valid to study the oxidation process, although they all show some important disadvantages (such as the low accuracy of the PNO-LCCSD(T) method in the regions of the PES with higher multireference character, the difficulty to represent the triplet-singlet excitation of dioxygen due to charge transfers between fragments, or the difficulty to replicate SHCI results depending on the active space of choice). When multireference methods are used, large active spaces are required, and in this situation, semistochastic heat-bath configuration interaction emerges as a powerful method to study these multi-configurational systems and is in good agreement with PNO-LCCSD(T) when the system is well-represented by a single-configuration. Our findings indicate that employing SHCI proves advantageous when addressing multisurface issues. This approach enables the incorporation of larger ac-

tive spaces, simplifying the selection of a robust active space throughout the reaction trajectory, while also recovering a substantial amount of correlation energy, thereby enhancing the accuracy of the results.

5.1.1 Resumen en español

Este trabajo establece un marco teórico para el estudio de las reacciones de oxigenación de grupos enolato en reacciones bioquímicas. A pesar de ser posterior al siguiente trabajo, se ha considerado que su relevancia como punto de partida podría ser mayor para la comprensión del mecanismo general de este tipo de reacciones.

Este trabajo se centra en el estudio del fenómeno de cambio de espín en la reacción entre el dioxígeno y un grupo enolato, de forma que partiendo de una superficie en estado triplete se obtiene un producto final singlete (o incluso productos posteriores, fuera del alcance de este trabajo, pero singletes en cualquier caso).

Utilizando los tres sistemas modelo de la Figura 15, partimos del anión correspondiente a la desprotonación del átomo de carbono α , de modo que el ion cargado negativamente potencia la transferencia de carga electrónica a la molécula de dioxígeno, facilitando el cambio de espín del proceso. El sustrato inicial se consideró como cargado negativamente porque existen pruebas experimentales de que la enzima desprotona al sustrato en un paso previo a la oxigenación.^{165,167} El sistema más grande (3,5-dihidroxifeniltioacetato, DPA) es idéntico al sustrato real del DpgC, pero sin el fragmento CoA. A partir de este, hemos hecho dos reducciones progresivas, eliminando el anillo aromático (al S-metil-but-3-entioato) y el grupo tioacetilo (al 1-oxobut-3-en-2-uro).

Nuestra investigación reveló un mecanismo concertado en el que la transición desde la

superficie de energía potencial triplete a la singlete se acopla con una transferencia de densidad electrónica desde el enlace O-O al C-O. Los resultados también confirman que el cruce intersistema se ve favorecido por la estabilización del estado singlete a lo largo de la ruta de reacción. En este trabajo no se han obtenido valores de acoplamiento espín-órbita por dos razones. En primer lugar, ya se conocían previamente por la Ref. 2, y en segundo lugar, el interés de esta publicación es verificar que los métodos teóricos utilizados describen correctamente el proceso de oxigenación en fase gas para todos los modelos utilizados.

La principal conclusión de este trabajo es que los tres métodos teóricos utilizados (SHCI, DFT de simetría rota y PNO-LCCSD(T)) son válidos para estudiar el proceso de oxidación, aunque todos ellos muestran algunas desventajas importantes (como la baja precisión del método PNO-LCCSD(T) en las regiones del PES con mayor carácter multirreferencial, la dificultad para representar la excitación triplete-singlete del dióxígeno debido a las transferencias de carga entre fragmentos, o la dificultad para replicar los resultados de SHCI dependiendo del espacio activo de elección). Cuando se utilizan métodos multireferencia, se requieren grandes espacios activos, y en esta situación, el SHCI emerge como un método potente para estudiar estos sistemas multiconfiguracionales y está en buen acuerdo con PNO-LCCSD(T) cuando el sistema está bien representado por una única configuración. Nuestros resultados indican que el empleo de SHCI resulta ventajoso cuando se abordan problemas de multisuperficie. Este enfoque permite incorporar espacios activos más grandes, simplificando la selección de un espacio activo robusto a lo largo de la trayectoria de reacción, al tiempo que recupera una cantidad sustancial de energía de correlación, mejorando así la precisión de los resultados.

Multi- and single-reference methods for the analysis of multi-state peroxidation of enolates

Cite as: J. Chem. Phys. **154**, 144303 (2021); <https://doi.org/10.1063/5.0046906>

Submitted: 08 February 2021 . Accepted: 19 March 2021 . Published Online: 08 April 2021

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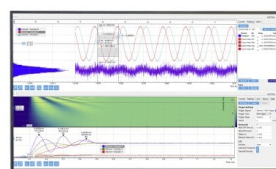
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Submitted: 8 February 2021 • Accepted: 19 March 2021 •

Published Online: 8 April 2021



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ABSTRACT

In spite of being spin-forbidden, some enzymes are capable of catalyzing the incorporation of $O_2(^3\Sigma_g^-)$ to organic substrates without needing any cofactor. It has been established that the process followed by these enzymes starts with the deprotonation of the substrate forming an enolate. In a second stage, the peroxidation of the enolate formation occurs, a process in which the system changes its spin multiplicity from a triplet state to a singlet state. In this article, we study the addition of O_2 to enolates using state-of-the-art multi-reference and single-reference methods. Our results confirm that intersystem crossing is promoted by stabilization of the singlet state along the reaction path. When multi-reference methods are used, large active spaces are required, and in this situation, semistochastic heat-bath configuration interaction emerges as a powerful method to study these multi-configurational systems and is in good agreement with PNO-LCCSD(T) when the system is well-represented by a single-configuration.

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I. INTRODUCTION

Every computational chemistry study starts with the selection of a suitable electronic structure method. This selection is made based on a trade-off between accuracy and computational cost, and the method is finally chosen depending on the size of the system under study. If the system can be reasonably described by one configuration, single-reference methods, such as MP2¹ and especially density functional theory (DFT),² are often used. Coupled-cluster single double triple [CCSD(T)],³ considered as the “gold standard” in quantum chemistry, is the best choice for small systems for which the calculation is affordable. However, when the ground state is strongly coupled to excited states, the aforementioned are not reliable, and an accurate description of the physics of these systems requires the use of multi-reference methods.

The most widely used multi-configuration method is the complete active space self-consistent field (CASSCF) method, developed more than 30 years ago.⁴ In CASSCF, the total orbital space is divided into three sets: inactive orbitals that are doubly occupied in all configurations, virtual orbitals that are always unoccupied, and the active orbitals where all possible occupations are considered. Based on these configurations, the multi-reference wavefunction is built, and subsequently, the coefficients of all configurations and orbitals are optimized. This allows us to account for the static electronic correlation and describe properly electronic states with a strongly mixed character. Dynamic correlation can be introduced via perturbation theory (CASPT2) or the variational multi-reference configuration interaction (MRCI) method. The number of configurations (and the computational cost) rises exponentially with the number of active orbitals, which usually limits the number of active space orbitals to 18 electrons in 18 orbitals [CAS(18,18)]

considering current computational resources, with the most recent and efficient implementations,⁵ which could be increased up to 20 electrons for 20 orbitals in large parallel machines.⁶ Larger active spaces can be used using restricted active space (RAS) methods, which limits the number of possible occupations arising from the active space orbitals.⁷ Not only the limitation in the number of active space orbitals hampers the use of CASSCF-like methods. The active space should be robust throughout the reaction path and include all the configurations of interest along this path. Although some strategies have been developed to automatically detect rotations in the active space orbitals,^{8–10} the selection of a robust and coherent active space can still be very cumbersome.

In the last decade, we have witnessed the development of a plethora of new multi-configuration methods,¹¹ on the one hand, single-reference approaches such as the spin-flip family of methods (see Ref. 12 for a recent review) and, on the other hand, stochastic multi-reference methods using Monte Carlo methods,^{13,14} which allows extending the number of active space to around 100 orbitals.^{15–17} Among these methods, the density matrix renormalization group (DMRG) method^{18,19} and the Semistochastic Heat-Bath Configuration Interaction (SHCI) have been widely used.^{20–22} These methods alleviate the problems arising from the selection of the active space, and using SHCI, active spaces of (30,108)²³ and (32,204)²⁴ have been reported. In this article, we use the SHCI method to describe the addition of molecular oxygen to enolates, intrinsically multi-configuration systems.

From an electronic structure perspective, reactions between O_2 and most organic molecules are, in principle, spin-forbidden. This is because O_2 in its ground state is a triplet ($^3\Sigma_g^-$), while most of the organic molecules, including the peroxide formed by the addition of O_2 to double bonds, are singlets. At very high temperatures, radicals are formed, which react with O_2 via a spin-allowed process. Since working at such temperatures is not an option for living organisms, enzymes devised other strategies to catalyze a reaction with O_2 . Most enzymes rely on transition metals, particularly Fe with or without a heme group.^{25–31} Crystallographic structures have shown unequivocally that few enzymes use organic cofactors, such as flavin,^{32,33} while others carry out their catalytic activity without the presence of any cofactors.^{34–36} The mechanism followed by these enzymes has been thoroughly studied (see, for example, Refs. 37–42), and it has been established that the first stage is deprotonation of the substrate prior to the diffusion of O_2 to the active site,⁴³ forming an enolate that will subsequently react with O_2 forming a peroxide.

3,5-dihydroxyphenylacetyl-CoA 1,2-dioxygenase (DpgC) is a protein that plays a key role in the biosynthesis of antibiotics of

last resort such as vancomycin or teicoplanin and was the first protein for which a crystal structure showing a substrate analog and O_2 bound to the enzyme was resolved.^{44,45} DpgC catalyzes the cofactor-independent oxidation of 3,5-dihydroxyphenylacetyl-CoA (DPA-CoA) to 3-5-dihydroxyphenyl-glyoxylate (DPGX), and a scheme of the proposed mechanism is depicted in Fig. 1. Previous calculations in our group⁴² combining quantum mechanics/molecular mechanics (QM/MM) (DFT), MRCI calculations on a model system with a reduced active space, have shown that once the enolate is formed, the reaction proceeds via transference of two electrons from the enolate to O_2 and without requiring activation of O_2 . The inter-system crossing was found to be concomitant with the first electron transfer, and the minimum energy crossing point (MECP), which plays the role of an effective barrier,⁴⁶ is stabilized by the formation of a stable intermolecular complex between DPA-CoA and O_2 .

The fact that at least two electronic states of different spin multiplicities are necessary to describe the system implies that multi-configuration methods should be used to understand the physics of these systems. Ideally, to treat this system using a CAS-like method, the active space should include all the π orbitals of the aromatic system and O_2 . However, since the hybridization of C_α changes from sp^2 to sp^3 , the aromaticity is broken along the reaction path and the related orbitals are generally expelled from the active space, leading to an artificial discontinuity in the electronic wavefunction. In Ref. 42, the problem of the stability was circumvented by using a small active space including only the frontier orbitals. Our results allowed us to demonstrate the multi-surface nature of the peroxidation reaction. The drawback of this approach is that only a small amount of correlation was gathered. The use of stochastic multi-reference methods permits to use a different approach, including all valence orbitals and electrons in the active space.

In this work, we will calculate the reaction path for this reaction using SHCI on three models of different size, and we will compare these reaction paths with those obtained using single-reference methods such as DFT and CCSD(T), the latter in the form of the explicitly correlated open-shell pair natural orbital local coupled-cluster methods, PNO-LCCSD(T)-F12.⁴⁷ Using these calculations, we will shed light on the mechanism of the peroxidation reaction of enolates and also assess if single-reference methods such as CCSD(T) and stochastic multi-reference methods (SHCI) are capable of describing the process. Moreover, we will evaluate the performance of the DFT method, which is widely used for the study of these reactions in biological conditions.

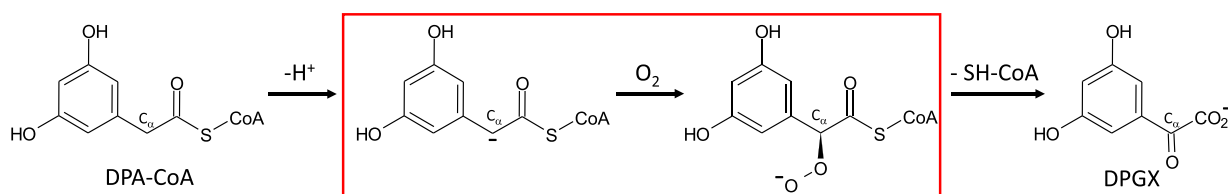


FIG. 1. Scheme of the proposed mechanism for the oxidation of DPA-CoA and synthesis of DPGX. The step highlighted in red is that studied in the present manuscript (reaction between O_2 and the enolate).

This manuscript is organized as follows: In Sec. II, we present the three models used to describe this process, along with a short description of the main features of the electronic structure methods used. In Sec. III, we will show the energy profiles for the peroxidation of the enolates. Finally, a summary and the conclusions of this work are presented.

II. METHODS

There are two reasons why the reaction between DPA-CoA and O_2 is an excellent system for the study of cofactorless addition of O_2 to enolates. On the one hand, no basic residues are present in the active site, and the protein plays a passive role in promoting intersystem crossing⁴² by restraining the carbon atom's backbone close to a planar conformation and guiding the oxygen to the active site. On the other hand, peroxidation takes place out from the aromatic ring, making possible to propose smaller systems that may reproduce the main features of the overall system. Following this idea, in this manuscript, we built three models to study the reaction between DPA and O_2 . The three models, and their analogy with DPA-CoA + O_2 system, are depicted in Fig. 2. In the largest system, the CoA moiety is replaced by a methyl group. For the middle size system, the DPA moiety is also simplified to S-methyl-but-3-enethioate (hereinafter S-methyl-but-3-enethioate) and in the smallest system to 1-oxobut-3-en-2-ide (hereinafter oxobutenide). To maintain the effect of the chemical bond, hydrogen atoms were added along the “broken” bonds.

For each of the models, the minimum energy path (MEP) along the distance between C_α and O_2 ($r_{C_\alpha O}$) was obtained

at a B3LYP/aug-cc-pVDZ calculation level of theory, as implemented in Gaussian16.⁴⁸ Following the same procedure of Ref. 42, constraints were applied to keep the planarity of all the C atoms and better reproduce the effect of the protein scaffold on the reaction. These constraints are only important at small $r_{C_\alpha O}$ distances, as for larger $r_{C_\alpha O}$, the molecule is intrinsically planar. No constraints were applied to the H atom bonded to C_α . Starting from the optimized geometries, the following methods were used to characterize the reaction paths: broken-symmetry DFT (BS-DFT), SHCI, and PNO-LCCSD(T)-F12.

A. Broken-symmetry DFT

The Broken-Symmetry DFT (BS-DFT) method^{49–52} is an affordable method to describe open-shell singlets within a monodeterminantal scenario. This method, widely used for the treatment of diradical systems,^{39,53–58} introduces the breakdown of the spatial and spin symmetry in decoupled alpha and beta spin-orbital contributions. Then, the splitting of the spin contribution and the frontier orbital mixing introduces the correction of the static correlation. The main advantage of this method against multi-reference methods is that it does not require the selection of any active space, and it is considerably less expensive, making the study of significantly larger systems possible.

BS-DFT methodology may suffer from the spin contamination that arises intrinsically from the method splitting procedure. It is possible, however, to correct it using Yamaguchi's formula.^{59–61} It is based on a spin projection (SP) method in which the spin symmetry for the open-shell singlet is recovered. In a recent paper, Visitsatthawong *et al.*³⁹ introduced this correction for the study of the oxygen

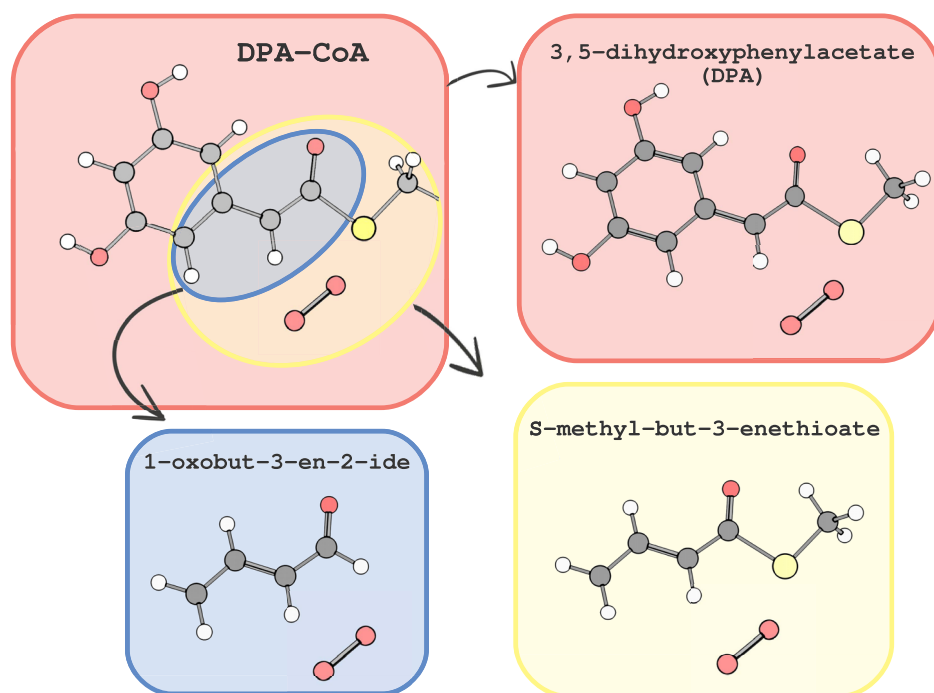


FIG. 2. Scheme of the three model systems used to describe the reaction between DPA-CoA and O_2 . In the top-left panel, O_2 and the DPA moiety of DPA-CoA are depicted. The three model systems are depicted in the bottom-left, bottom-right, and top-right panels, where the colors show the relationship between the models and the system.

activation in a flavin-dependent monooxygenase by means of the following equations:⁵⁹

$${}^{\text{singlet}}_{\text{SP}}E = {}^{\text{singlet}}E + C_{\text{SC}}[{}^{\text{singlet}}E - {}^{\text{triplet}}E], \quad (1)$$

$$C_{\text{SC}} = \frac{{}^{\text{singlet}}\langle S^2 \rangle}{{}^{\text{triplet}}\langle S^2 \rangle - {}^{\text{singlet}}\langle S^2 \rangle}, \quad (2)$$

where ${}^{\text{singlet}}_{\text{SP}}E$ is the corrected open-shell singlet energy, ${}^{\text{singlet}}E$ is the open-shell singlet electronic energy obtained with BS-DFT, and ${}^{\text{triplet}}E$ is the triplet electronic energy.

B. Semistochastic heat-bath configuration interaction

SHCI method involves two stages. First, the wavefunction is optimized by selecting iteratively a set of determinants until meeting convergence. These determinants, which are pre-sorted according to their energy, are filtered out below a threshold (ϵ_1). Then, the Hamiltonian is diagonalized only in the subspace of these determinants, and the total energy is calculated. In the second stage, the second-order correction to the variational energy, E_p , is computed using multi-reference Epstein-Nesbet perturbation theory. The value of ϵ_1 determines the accuracy and the computational cost of the calculations. The values of ϵ_1 below $10^{-8} E_{\text{H}}$ could yield highly accurate results, while the results for $\epsilon_1 > 10^{-2} E_{\text{H}}$ are probably meaningless. Following the procedure described in Ref. 24, we carried out calculations for different values of ϵ_1 and finally extrapolated to $\epsilon_1 \rightarrow 0$. The lowest value of ϵ_1 in this work is $5.0 \cdot 10^{-3} E_{\text{H}}$ for orbital optimization (only variational energy) and $5.0 \cdot 10^{-4} E_{\text{H}}$ for functional configuration identification (FCI) extrapolation.

SHCI calculations have been carried out in PySCF software,⁶² interfaced to Dice and Arrow.^{21,63} The active space selected included all the valence orbitals, i.e., the 2s and 2p orbitals for the C and O, the 1s of the H, and the 3s and 3p of the S. For the smallest system, oxobutenide + O₂, this active space spans 40 electrons in 33 orbitals, (40,33). For the middle-size system, S-methyl-butenthioate + O₂, it results in a (52,43) active space. For the largest system, DPA + O₂, it involves an (82,69) active space, which is, to the best of our knowledge, one of the largest active space calculations up to date, spanning a 10^{38} Slater determinants space. To better achieve convergence, calculations were carried out in three steps: a first step in which only active space orbitals were optimized using $\epsilon_1 = 5 \cdot 10^{-3} E_{\text{H}}$, a second step where the full set of orbitals were reoptimized at the same value of ϵ_1 (vHCISCF), and a third step in which starting from the fully optimized set of orbitals, calculations are repeated for $\epsilon_1 = 1 \cdot 10^{-3}$ and $5 \cdot 10^{-4} E_{\text{H}}$ and finally extrapolated toward $\epsilon_1 \rightarrow 0$.

C. PNO-LCCSD(T)-F12

Coupled cluster methods, and in particular CCSD(T), are among the most accurate single-reference methods.^{47,64,65} However, its computational cost impairs its use for medium-size systems. To reduce the computational burden of the calculations, some approximations have been proposed. One of these approximations consists in restricting the electron correlation within the whole system to local contributions. This is performed by means of the Pair Natural Orbitals (PNOs),^{47,66,67} which allow the reduction of the virtual space to an independent set of virtual orbitals for each occupied orbital pair. For systems for which CCSD(T) calculations are

feasible, both methods are in very good agreement, with relative energies differing in around 0.2 kcal/mol. In this manuscript, we will use the PNO-LCCSD(T) method as the reference single-reference method. PNO-LCCSD(T) calculations were carried out using Molpro 2020⁶⁸ with a cc-pVDZ-F12 basis set.

D. Electron-density descriptors

Electron density descriptors are a useful tool to understand how electron density rearranges along a reaction pathway. In these methods, the total electron density of a system splits into atomic contributions by means of several fragmentation methods.^{69,70} In this frame, the electron sharing between a set of atomic centers can be calculated with the so-called electron delocalization indices (DIs).^{71–73} These indices can be tightly related with some chemical concepts such as bond orders, atomic charges, and aromaticity. Herein, DIs were calculated as implemented in the NDELOC code.⁷⁴

Here, the fragmentation procedure chosen according to the size of the system was the Mulliken scheme.⁷⁰ To avoid a wrong definition of the Hilbert space, calculations were performed without a diffuse basis set. In addition, during this work, we will focus on the analysis of two-order delocalization indices, which measure the number of electrons between two regions as follows:

$$\delta_{\text{AB}} = \sum_{\alpha \in A} \sum_{\beta \in B} (PS)_{\alpha\beta} (PS)_{\beta\alpha}, \quad (3)$$

where A and B are the atomic centers, α and β the basis functions, and S and P the overlap and density matrices, respectively.

III. RESULTS

We will start by analyzing the results obtained for the smallest system, in which DPA-CoA is replaced by oxobutenide (see Fig. 2). The reaction paths for the peroxidation of oxobutenide as a function of $r_{\text{C}_{\alpha}\text{O}}$ are depicted in Fig. 3 for the three different methods used: SHCI, DFT (using the B3LYP functional), and PNO-LCCSD(T).

Regardless of the method considered, all the sets of potential energy curves (PECs) share the same main features. At large $r_{\text{C}_{\alpha}\text{O}}$ distances (where O₂ and oxobutenide are far from each other), the ground state is a triplet, while at short $r_{\text{C}_{\alpha}\text{O}}$ distances, where the peroxide is formed, the triplet is a repulsive state, and the system is only stable as a singlet. With decreasing $r_{\text{C}_{\alpha}\text{O}}$, the energy of the triplet PEC increases and, simultaneously, there is a stabilization of the singlet state.

The PEC for the triplet state at the SHCI level of theory is more repulsive than for the single-reference methods, causing that the distance at which the singlet and triplet curves cross is significantly larger at the SHCI level ($r_{\text{C}_{\alpha}\text{O}} = 2.4 \text{ \AA}$) than for PNO-LCCSD(T) and DFT (2.2 and 2.0 Å, respectively). The energy of the crossing point is significantly larger for DFT (0.69 eV) than for PNO-LCCSD(T) (0.43 eV) and SHCI (0.57 eV). We should remark that due to the lack of analytical gradients for the SHCI method, we did not attempt to optimize the MECF, which in any case should be lower than the crossing points of Fig. 2. In Ref. 42, the MECF was calculated for the reaction between O₂ and S-methyl-but-3-enethioate and it was located at $r_{\text{C}_{\alpha}\text{O}} = 2.1 \text{ \AA}$, and its energy was 0.7 eV above the triplet asymptote.

Regarding the singlet PECs, SHCI and PNO-LCCSD(T) singlet curves are similar and mainly differ on the depth of the peroxide

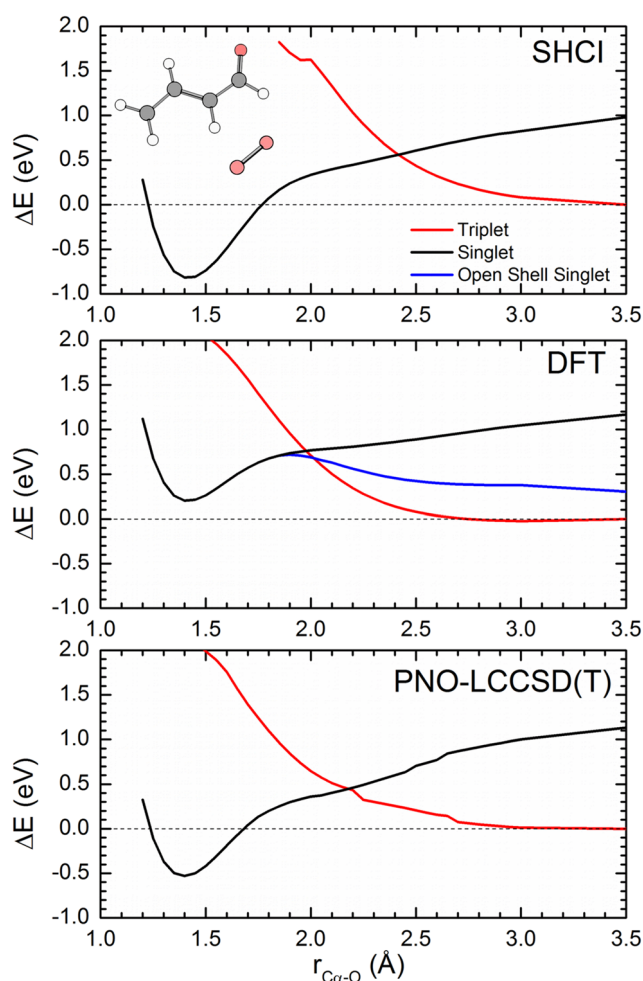


FIG. 3. Potential energy curves for the addition of O₂ to the oxobutenide anion at SHCI/aug-cc-pVDZ (top panel), DFT B3LYP/aug-cc-pVDZ (middle panel), and PNO-LCCSD(T)-F12/cc-pVDZ-F12 level (bottom panel). The energy of the triplet state for the O₂ + oxobutenide asymptote is taken as zero reference energy.

well, being the SHCI singlet the most stable. For both methods, the energy of the singlet states monotonically decreases with decreasing $r_{C\alpha O}$. As it was noted in Sec. II, to account for the possible effect of the protein on the enolate, constraints were applied to keep the planarity of the C scaffold of the molecule,⁴² so, if relaxed, the singlet state would be significantly more stable regardless of the method. As no constraints are applied to the H bonded to C_α, it moves outside the plane of the molecule for $r_{C\alpha O} < 2.2$ Å. It is a consequence of the change of hybridization from a sp^2 to a sp^3 .

We calculated two different singlet states using DFT: a restricted singlet state and the open-shell singlet using the broken-symmetry framework. At close $r_{C\alpha O}$ distances, where the peroxide is stable, the wavefunction is well approximated by just one configuration, and accordingly, the restricted and open-shell singlet states converge to the same solution. The peroxide minimum is less stable for DFT than for SHCI or PNO-LCCSD(T). For $r_{C\alpha O} > 1.9$ Å, the broken-symmetry solution does not longer overlap

the restricted singlet PEC, and at large $r_{C\alpha O}$, the difference between these states is about 0.8 eV. The open-shell character of O₂ (π_g^*)² of the first singlet state is the actual cause of this divergence, as the restricted solution does not longer represents correctly the system. In the broken-symmetry framework, an unrestricted wavefunction is constructed in which the mixture of the frontier orbitals is allowed (an analog to a multi-reference wavefunction with two electrons in two orbitals). According to this, one would expect that the open-singlet PEC would reproduce the behavior of the SHCI or PNO-LCCSD(T) singlet PECs at large $r_{C\alpha O}$ better than its restricted counterpart. However, that is not the case, and the energy gap between the open-singlet and triplet PEC at large $r_{C\alpha O}$ is significantly smaller than for any other method. Incidentally, it creates a barrier in the singlet PEC, which is not present in any other method. For the three model systems studied, curves obtained using MP2 showed discontinuities and were excluded from the comparison. Similar errors were obtained when we attempt to converge CASPT2 calculations. We believe that this may be caused by the appearance of intruder states, even in MP2.

In the absence of a barrier for the singlet state, the energy gap between singlet and triplet states may have a strong influence on the reactivity. At very large $r_{C\alpha O}$ distances, we can assume that there is no interaction between oxobutenide and O₂. Under these circumstances, the negative charge should be located in the enolate, and the difference between the singlet and triplet PECs should correspond to that for isolated O₂. Experimentally, the energy difference between the first triplet ($^3\Sigma_g^-$) and singlet ($^1\Delta_g$) states of O₂ is 0.981 eV.⁷⁵ The energy gap obtained with SHCI (1.20 eV), PNO-LCCSD(T) (1.19 eV), and standard DFT (1.19 eV) is in a reasonable agreement with the experimental datum, but for broken-symmetry DFT, the energy difference is just 0.36 eV, clearly underestimating the experimental energy gap. It is worth mentioning that spin-contamination was found to be very small, and correction using Yamaguchi's formula (see Sec. II) barely affects the result.

To discard the hypothesis that the differences in the energy gap were not intrinsic of the electronic structure method but caused by some peculiarities of the system, we calculated the energy difference between triplet and singlet O₂ with the methods employed in this study by applying a small set of basis sets, and additionally other widely used DFT functionals. The energy gaps, collected in Tables I and II, are somewhat insensitive of the basis set but strongly depend on the electronic structure method used. Regardless of the DFT functional used (even for M06-HF that includes a 100% Hartree-Fock exchange), there is a clear underestimation of the energy gap when the broken-symmetry framework is used and a clear overestimation of the energy gap when it is not used. Our results agree with those by Kitagawa and co-workers⁷⁶ who reported that the energy-gap for carbene (CH₂) calculated using broken symmetry methods along with hybrid functionals is clearly underestimated. It should be noted, however, that the broken-symmetry in combination with HF yields values of the energy-gap close to the experimental ones.

According to Tables I and II, the triplet-singlet energy gap is best reproduced by SHCI. However, CCSD(T) and PNO-LCCSD(T) also provide reasonable values for the energy gap. In Ref. 42, the energy gap was also calculated using MRCI and a small active space (four electrons in three orbitals), and the value obtained was 1.13 eV. It should be mentioned that for DFT calculations, there are some

TABLE I. Energy gap between (closed-shell) singlet and triplet O₂ for a series of methods and basis sets. Energies are given in eV. For PNO-LCCSD(T), the basis set used was cc-pVDZ-F12.

ΔE_{ST}	B3LYP	B3PW91	M062X	M06HF	CAM-B3LYP	HF	MP2	CCSD(T)	PNO-LCCSD(T)
3-21G	1.712	1.759	1.638	1.172	1.738	2.229	1.257	1.167	*1.259
6-31+G(d,p)	1.674	1.729	1.614	1.405	1.698	2.316	1.534	1.335	
aug-cc-pVDZ	1.675	1.728	1.613	1.405	1.699	2.317	1.540	1.336	
aug-cc-p-VTZ	1.667	1.723	1.608	1.401	1.689	2.321	1.477	1.295	

TABLE II. Energy gap between (open-shell) singlet and triplet O₂. Energies in eV. For DFT, HF, and MP2 methods, open-shell singlet was calculated using the broken-symmetry framework, and the energy was corrected using Yamaguchi's formula. For SHCI, we used a (12,8) active space including the 2s and 2p electrons and orbitals.

ΔE_{ST}	B3LYP	B3PW91	M062X	M06HF	CAM-B3LYP	HF	MP2	SHCI
3-21G	0.469	0.472	0.595	0.264	0.487	0.858	0.827	0.987
6-31+G(d,p)	0.459	0.463	0.619	0.558	0.476	0.865	0.526	0.824
aug-cc-pVDZ	0.454	0.458	0.623	0.557	0.471	0.850	0.464	0.966
aug-cc-pVTZ	0.445	0.450	0.637	0.571	0.463	0.836	0.341	0.943

discrepancies between the energy-gap obtained for O₂ and for our model at large r_{Ca-O} . As we will discuss later, this discrepancy arises from the charge of the system, which in DFT is not fully located on the enolate.

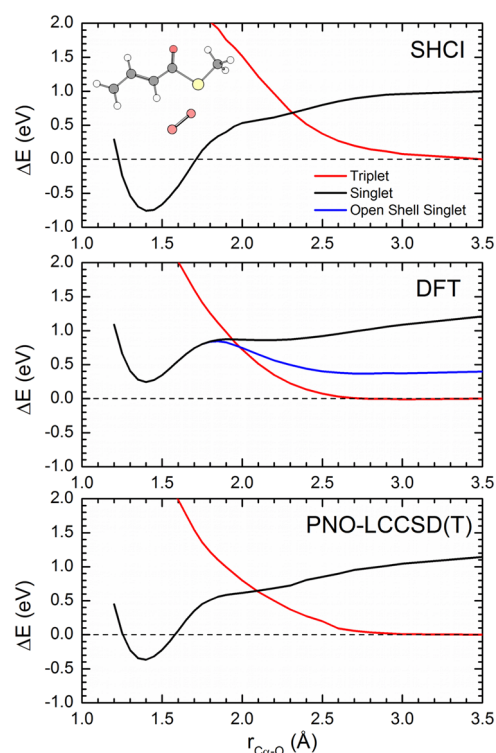
For strong-correlated systems, single-configuration methods may fail due to the lack of static correlation. To assess the reliability of the PNO-LCCSD(T) calculations, we calculated the diagnostic T_1 .⁷⁷ T_1 is the most widely used diagnostic tool to verify if non-dynamical correlation effects are important enough to compromise CCSD calculations. It is assumed that calculations for which $T_1 < 0.02$ are reasonably described using a CCSD approach. For open-shell systems, it has been suggested that this threshold value may be closer to 0.044.⁷⁸ When we calculated the T_1 along the reaction path, we observed that both the singlet minimum and the triplet asymptote are reasonably described with the PNO-LCCSD(T) method as they have associated values under the 0.02 threshold ($T_1 \approx 0.015$ – 0.017). However, T_1 values rise in the crossing region, especially for the triplet PEC whose typical T_1 is larger than 0.03.

In Fig. 4, we show the PECs corresponding to the addition of O₂ to S-methyl-butenthioate (see Fig. 2). S-methyl-butenthioate was the system used in Ref. 42 to simulate the addition of O₂ to DPA-CoA using MRCI calculations with a small active-space (four electrons in three orbitals). Using this small active-space, it was possible to determine the reaction paths corresponding to all the nine states that arise from that active space, showing that the peroxide well correlates with the O₂²⁻ asymptote, thus requiring a double electron transfer. In Ref. 42, SHCI calculations were carried out using an active space of (18,23), significantly smaller than the active space used here (52,43). Although the curves using (18,23) and (52,43) are similar, the peroxide well is three times deeper for the larger active space.

The curves calculated for peroxidation of S-methyl-butenthioate using SHCI, DFT, and PNO-LCCSD(T) are in a good qualitative agreement and are also similar to those obtained for the smallest system. The singlet PECs are less attractive for peroxidation of S-methyl-butenthioate than for peroxidation of oxobutenide, and as

a consequence, the energies of the crossing points are slightly larger: 0.73 eV for SHCI, 0.77 eV for DFT, and 0.60 eV at PNO-LCCSD(T).

The results for the largest model (DPA, see Fig. 2) are shown in Fig. 5. Again there is a qualitative agreement between all

**FIG. 4.** Potential energy curves of the S-methyl-butenthioate anion reaction with O₂ at SHCI/aug-cc-pVDZ (top panel), B3LYP/aug-cc-pVDZ (middle panel), and PNO-LCCSD(T)-F12/cc-pVDZ-F12 level (bottom panel). The energy of the triplet state for O₂ + S-methyl-butenthioate is taken as zero reference energy.

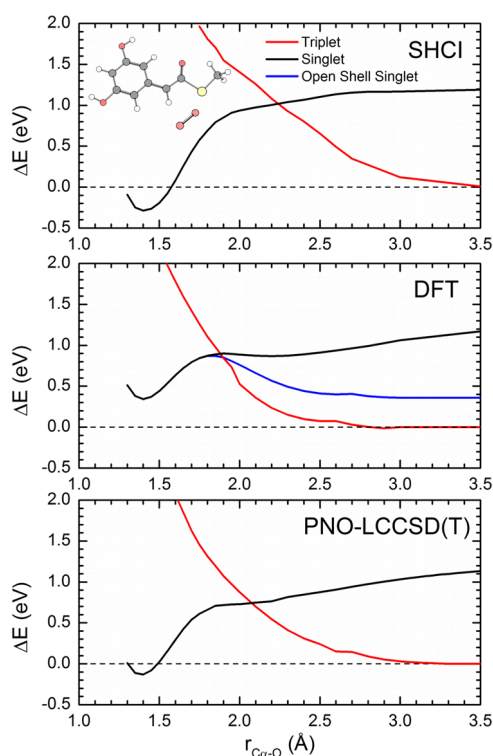


FIG. 5. Potential energy curves for peroxidation of DPA⁻. Calculations are carried out at the SHCI/aug-cc-pVDZ B3LYP/aug-cc-pVDZ and PNO-LCCSD(T)-F12/cc-pVDZ-F12 level. The energy of the triplet state for the O₂ + DPA⁻ is taken as zero reference energy.

methods, and they are also similar to those obtained for smaller systems. For this system, the singlet PEC is less attractive, leading to a shallower peroxide minimum, in particular, using DFT. The crossing points are also higher in energy than for the smaller systems, in particular, for the SHCI method, as the energy gap between the triplet and singlet PEC is larger than that obtained for O₂ (see Table II). The discrepancy in the singlet-triplet energy gap comes from the selection of the active space, which is selected at the smallest $r_{C_\alpha O}$, and then, the wavefunction is propagated to larger $r_{C_\alpha O}$. Using this procedure, the active space is robust, but it originates a larger energy gap at the largest $r_{C_\alpha O}$.

Regarding the PNO-LCCSD(T) calculations, the values of T_1 diagnosis are similar for the three systems at large $r_{C_\alpha O}$ ($T_1 \approx 0.015$ – 0.017), while around the crossing region, although presenting larger values, they decrease with the size of the system. For DPA⁻ + O₂, on the singlet PEC, they are typically below 0.02, and only around $r_{C_\alpha O} = 1.8$ Å, they go up to 0.022. On the triplet PEC, T_1 values are also smaller than for the smallest models and are always below 0.03. These results suggest that even for this intrinsically multi-surface reaction, it is possible to rely on the CCSD(T) treatment. This conclusion is reinforced by the good agreement between the PNO-LCCSD(T) and SHCI PECs.

To get more insight about the reaction mechanism, in Fig. 6, we show the two-center electron delocalization indices along the

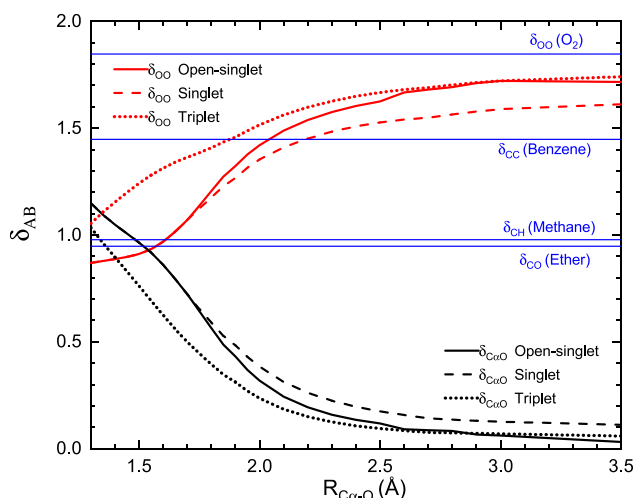


FIG. 6. Evolution of delocalization indices along the addition of O₂ to DPA⁻ as a function of the C_α-O distance calculated at the B3LYP/cc-pVDZ (top panel) level of theory. Reference values are shown as horizontal solid blue lines.

reaction path for the DFT singlet and triplet PECs of the DPA⁻ + O₂ reaction. They represent the bond-order, and we calculated these indexes for the two covalent bonds that are expected to change throughout the reaction: (i) that between the two O atoms (δ_{OO}) and (ii) that between C_α and O (δ_{CO}). At large $r_{C_\alpha O}$, the values of δ_{OO} and δ_{CO} are almost constant, the latter being close to zero, showing that there is almost no interaction between the enolate and O₂. When the two reactants approach, in particular, for $r_{C_\alpha O} < 2.2$ Å, δ_{OO} shrinks, while δ_{CO} rises. This indicates that the peroxidation occurs via a concerted mechanism in which electron density is transferred from the O–O to the C_α–O bond. At $r_{C_\alpha O} = 1.4$ Å, at the equilibrium distance of the peroxide, both indices are similar, showing “bond-orders” that are slightly below those for standard single-bonds (such as the C–O bond in ether). There are two aspects that deserve further discussion: first, regardless of $r_{C_\alpha O}$, δ_{OO} is larger for the triplet than for the singlet PEC, while the opposite occurs for δ_{CO} . Hence, intersystem crossing intrinsically involves electron transfer from the O–O to the C_α–O bond. This result agrees with predictions based on the Mulliken charges, which showed that intersystem crossing was associated with some electron density transfer from O₂ to the enolate.⁴² Second, it is also interesting that δ_{OO} never approaches the value obtained for isolated O₂. This discrepancy arises from an excess of electronic charge on O₂, which is not zero even at very large $r_{C_\alpha O}$. This is the reason why the triplet-singlet energy gap for DFT calculations is lower for our systems than for isolated O₂. To ascertain this statement, the singlet-triplet energy gap and delocalization indices were recalculated for an uncharged model system, in which the charge was removed by adding a second hydrogen to C_α, which now shows an sp³ hybridization. On this system, the triplet-singlet energy gap is coincident with that obtained for O₂, and this is also the case of δ_{OO} .

Using the IBO/IAO formalism,^{10,79} we represented the orbitals whose nature changed significantly along the addition of O₂ to DPA⁻. The results (shown in Fig. S1) reveal that for both singlet and triplet states, they are related to the formation of the bond between O

and C_α . On the singlet state, they correspond to the mixing between one π and one π^* of O_2 and p_z orbital of C_α . On the triplet state, however, mixing between those orbitals is not significant, explaining the lack of stability of the triplet peroxide.

IV. CONCLUSIONS

Throughout this article, we have studied the addition of O_2 to enolates using two recently developed theoretical approaches such as SHCI and PNO-LCCSD(T) and compared them to DFT methods, widely used to study these reactions on biological media. On top of the fundamental importance of these intrinsically multi-configuration systems, the addition of O_2 to enolates is the key step for the enzymatically catalyzed cofactorless incorporation of O_2 into organic substrates. To shed light on the reaction mechanism, we calculated the delocalization indices along the reaction path. Our results show that the reaction follows a concerted mechanism in which hopping from the triplet to the singlet potential energy surface is associated with an electron density transfer from the O–O to the C–O bond.

To study this reaction, we used three different model systems of different sizes. The potential energy curves (PECs) obtained using these three methodologies and the three models are qualitatively similar. Regardless of the model used, the singlet PEC is less attractive for the largest systems, leading to crossing points of larger energies. DFT calculations cast some doubts due to inaccuracies in the singlet–triplet gap at large $r_{C_\alpha O}$ where O_2 and enolates are not interacting, and a comparison with experimental values are possible. When the energy of the singlet state is corrected using the broken-symmetry framework, the energy gap is, in turn, too small, causing a barrier on the singlet PEC, which is not predicted for any other method.

The singlet PECs obtained using SHCI and PNO-LCCSD(T) are very similar to each other, while the triplet PEC is more repulsive for SHCI. The T_1 diagnosis was evaluated showing that PNO-LCCSD(T) calculations are reliable both when the peroxide is formed (small $r_{C_\alpha O}$) and in the triplet asymptote. In the crossing region, T_1 diagnosis is larger, especially for the smallest enolate, while for the largest one, its highest value is 0.022 for the singlet and 0.028 for the triplet, slightly above the usually accepted empirical threshold value of 0.02 for closed-shell systems and well below the proposed threshold of 0.044 for open-shell systems. It suggests that PNO-LCCSD(T) results may be accurate for these systems, opening the gate to use this method for the study of this kind of reactions.

The study of these reactions using multi-reference methods is challenging, requiring a very large active space. Using SHCI, we could carry out calculations using an active space that includes all the valence orbitals and electrons, 82 electrons in 69 orbitals, far beyond the possibility of the classical CAS-like methods. SHCI PECs are in a reasonable agreement with PNO-LCCSD(T) in the regions where PNO-LCCSD(T) is expected to reproduce faithfully the behavior of the system and shows energy crossing points of higher energy. Altogether, our results suggest that SHCI is a good approach to deal with intrinsic multisurface problems, as it permits to include oversized active spaces, which makes easier to select a robust active space along the reaction path and also to recover most of the correlation energy, which makes the results more accurate.

SUPPLEMENTARY MATERIAL

See the [supplementary material](#) for the geometries of oxobutenide, S-methyl-butenthioate, and DPA anions + O_2 , the raw data for the energy profiles of oxobutenide, S-methyl-butenthioate, and DPA anions, and the SHCI extrapolation raw data for the three mentioned anions in their singlet and triplet states. A representation of the orbitals whose nature changes more significantly along the addition of O_2 to DPA^- using IBO/IAO formalism is included as well.

DEDICATION

Throughout our scientific careers, we have worked along with many outstanding female physical chemists (and learn from them). We would like to dedicate this article to Professor Carmen Barrientos, Dr. Béatrice Bussery-Honvault, Dr. Nadja Doslic, Dr. Shirin Faraji, Dr. Susana Gómez-Carrasco, Dr. Mari-Luz Hernández, Dr. Marta Menéndez, Dr. Ángeles Peña-Gallego, Dr. Edina Rosta, and Professor Carmela Valdemoro.

ACKNOWLEDGMENTS

Funding by the Spanish Ministry of Science and Innovation (Grant Nos. FIS2017-83473-C2-P, PID2019-107115GB-C21, and PGC2018-096444-B-I00) is acknowledged. P.O. acknowledges Grant No. EDU/601/2020 (Junta de Castilla y León and European Social Fund) and thank James E. T. Smith for his practical hints and helpful comments on the PySCF code. The authors acknowledge funding by the Fundación Salamanca City of Culture and Knowledge (program for attracting scientific talent to Salamanca). The authors thank Dr. M. Mandado from the University of Vigo for kindly providing the NDELOC code used to calculate the electron density descriptors.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Multi and single-reference methods for the analysis of multi-state peroxidation of enolates

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r(C-O) / Å	RB3LYP Singlet	BS-B3LYP Singlet	UB3LYP Triplet	PNO-CCSD(T) Singlet	PNO-CCSD(T) Triplet	T1 Singlet	T1 Triplet
1.20	-381.0157	-381.0157	-380.9307	-380.5064		0.0172	
1.25	-381.0319	-381.0319	-380.9484	-380.5223	-380.4112	0.0172	0.0417
1.30	-381.0419	-381.0419	-380.9606	-380.5319	-380.4245	0.0172	0.0319
1.35	-381.0473	-381.0473	-380.9689	-380.5366	-380.4319	0.0173	0.0363
1.40	-381.0493	-381.0493	-380.9744	-380.5379	-380.4370	0.0174	0.0397
1.45	-381.0490	-381.0490	-380.9782	-380.5367	-380.4416	0.0177	0.0399
1.50	-381.0471	-381.0471	-380.9814	-380.5337	-380.4452	0.0182	0.0404
1.55	-381.0444	-381.0444	-380.9848	-380.5297	-380.4489	0.0190	0.0413
1.60	-381.0414	-381.0414	-380.9889	-380.5252	-380.4537	0.0201	0.0418
1.65	-381.0384	-381.0384	-380.9939	-380.5208	-380.4608	0.0216	0.0391
1.70	-381.0357	-381.0357	-380.9994	-380.5168	-380.4671	0.0230	0.0348
1.75	-381.0336	-381.0336	-381.0051	-380.5135	-380.4727	0.0245	0.0338
1.80	-381.0319	-381.0319	-381.0108	-380.5110	-380.4780	0.0254	0.0329
1.85	-381.0307	-381.0308	-381.0164	-380.5091	-380.4829	0.0256	0.0320
1.90	-381.0298	-381.0304	-381.0216	-380.5074	-380.4874	0.0249	0.0312
1.95	-381.0291	-381.0307	-381.0263	-380.5062	-380.4912	0.0240	0.0309
2.00	-381.0286	-381.0315	-381.0307	-380.5051	-380.4945	0.0230	0.0310
2.05	-381.0282	-381.0326	-381.0347	-380.5046	-380.4973	0.0223	0.0315
2.10	-381.0278	-381.0337	-381.0383	-380.5035	-380.4995	0.0218	0.0326
2.15	-381.0274	-381.0350	-381.0414	-380.5025	-380.5011	0.0216	0.0342
2.20	-381.0271	-381.0363	-381.0441	-380.5015	-380.5024	0.0216	0.0365
2.25	-381.0266	-381.0375	-381.0465	-380.5003	-380.5064	0.0219	0.0277
2.30	-381.0262	-381.0385	-381.0485	-380.4990	-380.5073	0.0222	0.0282
2.35	-381.0257	-381.0395	-381.0502	-380.4976	-380.5081	0.0224	0.0291
2.40	-381.0252	-381.0403	-381.0517	-380.4962		0.0227	
2.45	-381.0246	-381.0410	-381.0529	-380.4950	-380.5098	0.0230	0.0386
2.50	-381.0240	-381.0415	-381.0539	-380.4924	-380.5108	0.0219	0.0388
2.55	-381.0235	-381.0420	-381.0547	-380.4912	-380.5117	0.0218	0.0389
2.60	-381.0229	-381.0424	-381.0554	-380.4900	-380.5126	0.0218	0.0389
2.65	-381.0222	-381.0427	-381.0560	-380.4873	-380.5130	0.0201	0.0386
2.70	-381.0216	-381.0429	-381.0564	-380.4864	-380.5155	0.0201	0.0224
2.75	-381.0210	-381.0430	-381.0568	-380.4856	-380.5161	0.0200	0.0212
2.80	-381.0204	-381.0431	-381.0570	-380.4847	-380.5165	0.0198	0.0201
2.85	-381.0199	-381.0432	-381.0572	-380.4838	-380.5170	0.0197	0.0193
2.90	-381.0193	-381.0432	-381.0574	-380.4831	-380.5173	0.0195	0.0186
2.95	-381.0188	-381.0432	-381.0575	-380.4823	-380.5176	0.0193	0.0181
3.00	-381.0183	-381.0432	-381.0576	-380.4815	-380.5178	0.0191	0.0176
3.50	-381.0138	-381.0461	-381.0568	-380.4768	-380.5183	0.0172	0.0159
4.00		-381.0446	-381.0562	-380.4748	-380.5179	0.0169	0.0154
5.00	-381.0063	-381.0415	-381.0568	-380.4735	-380.5173	0.0170	0.0151

Table S4. Raw energies for oxobutenide anion. DFT calculations use the aug-cc-pVDZ basis set. PNO-CCSD(T) calculations use the cc-pVDZ-F12 basis set. Last two columns show the raw results for the T1 diagnosis test of CC calculations.

r(C-O) / Å	RB3LYP Singlet	BS-B3LYP Singlet	UB3LYP Triplet	PNO-CCSD(T) Singlet	PNO-CCSD(T) Triplet	T1 Singlet	T1 Triplet
1.20	-818.5647	-818.5647	-818.4644	-817.5352		0.0174	
1.25	-818.5803	-818.5803	-818.4826	-817.5507		0.0175	
1.30	-818.5897	-818.5897	-818.4955	-817.5599		0.0175	
1.35	-818.5945	-818.5945	-818.5043	-817.5643		0.0175	
1.40	-818.5958	-818.5958	-818.5104	-817.5651	-817.4607	0.0176	0.0333
1.45	-818.5947	-818.5947	-818.5149	-817.5633	-817.4654	0.0177	0.0349
1.50	-818.5920	-818.5920	-818.5193	-817.5598	-817.4697	0.0180	0.0373
1.55	-818.5883	-818.5883	-818.5243	-817.5550	-817.4721	0.0184	0.0347
1.60	-818.5843	-818.5843	-818.5307	-817.5494	-817.4786	0.0191	0.0353
1.65	-818.5805	-818.5805	-818.5383	-817.5436	-817.4869	0.0204	0.0351
1.70	-818.5774	-818.5774	-818.5459	-817.5387	-817.4950	0.0221	0.0325
1.75	-818.5752	-818.5752	-818.5527	-817.5351	-817.5016	0.0237	0.0303
1.80	-818.5738	-818.5739	-818.5585	-817.5328	-817.5068	0.0244	0.0293
1.85	-818.5730	-818.5736	-818.5638	-817.5311	-817.5111	0.0239	0.0287
1.90	-818.5726	-818.5743	-818.5687	-817.5301	-817.5151	0.0229	0.0283
1.95	-818.5728	-818.5758	-818.5738	-817.5295	-817.5186	0.0218	0.0280
2.00	-818.5729	-818.5774	-818.5782	-817.5290	-817.5222	0.0208	0.0273
2.10	-818.5731	-818.5809	-818.5858	-817.5278	-817.5284	0.0196	0.0311
2.20	-818.5731	-818.5842	-818.5919	-817.5265	-817.5333	0.0192	0.0328
2.30	-818.5727	-818.5867	-818.5965	-817.5250	-817.5379	0.0192	0.0335
2.40	-818.5719	-818.5888	-818.5998	-817.5221	-817.5415	0.0184	0.0338
2.50	-818.5709	-818.5901	-818.6021	-817.5203	-817.5443	0.0180	0.0338
2.60	-818.5697	-818.5910	-818.6036	-817.5186	-817.5482	0.0177	0.0189
2.70	-818.5684	-818.5914	-818.6044	-817.5166	-817.5496	0.0171	0.0174
2.80	-818.5671	-818.5915	-818.6048	-817.5155	-817.5504	0.0169	0.0165
2.90	-818.5659	-818.5913	-818.6048	-817.5144	-817.5509	0.0167	0.0158
3.00	-818.5647	-818.5913	-818.6050	-817.5132	-817.5514	0.0165	0.0155
3.50	-818.5603	-818.5904	-818.6047	-817.5096	-817.5516	0.0162	0.0150
4.00	-818.5460	-818.5918	-818.6044	-817.5077	-817.5509	0.0162	0.0149
5.00	-818.5570	-818.5931	-818.6038	-817.5060	-817.5501	0.0162	0.0147

Table S5. Raw energies for S-methyl-butenthioate anion. DFT calculations use the aug-cc-pVDZ basis set. PNO-CCSD(T) calculations use the cc-pVDZ-F12 basis set. Last two columns show the raw results for the T1 diagnosis test of CC calculations.

r(C-O) / Å	RB3LYP Singlet	BS-B3LYP Singlet	UB3LYP Triplet	PNO-CCSD(T) Singlet	PNO-CCSD(T) Triplet	T1 Singlet	T1 Triplet
1.30	-1122.7426	-1122.7426	-1122.6501	-1121.2976		0.0162	
1.35	-1122.7475	-1122.7475	-1122.6590	-1121.3020		0.0163	
1.40	-1122.7489	-1122.7489	-1122.6653	-1121.3027		0.0163	
1.45	-1122.7479	-1122.7479	-1122.6751	-1121.3009		0.0165	
1.50	-1122.7453	-1122.7453	-1122.6813	-1121.2971		0.0166	
1.55	-1122.7418	-1122.7418	-1122.6886	-1121.2923	-1121.2145	0.0170	0.0280
1.60	-1122.7381	-1122.7381	-1122.6960	-1121.2871	-1121.2221	0.0178	0.0287
1.65	-1122.7348	-1122.7348	-1122.7030	-1121.2822		0.0190	
1.70	-1122.7323	-1122.7323	-1122.7092	-1121.2781	-1121.2382	0.0201	0.0263
1.75	-1122.7305	-1122.7305	-1122.7150	-1121.2755	-1121.2444	0.0211	0.0249
1.80	-1122.7293	-1122.7294	-1122.7208	-1121.2737	-1121.2496	0.0214	0.0244
1.85	-1122.7287	-1122.7294	-1122.7255	-1121.2718	-1121.2539	0.0209	0.0241
1.90	-1122.7283	-1122.7301	-1122.7304	-1121.2715	-1121.2585	0.0200	0.0238
1.95	-1122.7286	-1122.7318	-1122.7345	-1121.2712		0.0191	
2.00	-1122.7288	-1122.7335	-1122.7421	-1121.2711	-1121.2657	0.0182	0.0242
2.10	-1122.7294	-1122.7373	-1122.7483	-1121.2704	-1121.2722	0.0173	0.0265
2.20	-1122.7296	-1122.7408	-1122.7528	-1121.2698	-1121.2779	0.0170	0.0275
2.30	-1122.7294	-1122.7435	-1122.7559	-1121.2679	-1121.2828	0.0165	0.0279
2.40	-1122.7288	-1122.7454	-1122.7578	-1121.2668	-1121.2865	0.0164	0.0281
2.50	-1122.7279	-1122.7466	-1122.7587	-1121.2657	-1121.2890	0.0162	0.0282
2.60	-1122.7270	-1122.7470	-1122.7587	-1121.2646	-1121.2924	0.0160	0.0156
2.70	-1122.7260	-1122.7468	-1122.7604	-1121.2633	-1121.2926	0.0158	0.0149
2.80	-1122.7250	-1122.7478	-1122.7614	-1121.2621	-1121.2946	0.0157	0.0147
2.90	-1122.7237	-1122.7483	-1122.7619	-1121.2610	-1121.2960	0.0156	0.0146
3.00	-1122.7225	-1122.7485	-1122.7614	-1121.2600	-1121.2968	0.0155	0.0146
4.00	-1122.7143		-1122.7614	-1121.2543	-1121.2974	0.0153	0.0144

Table S6. Raw energies for DpA anion. DFT calculations use the aug-cc-pVDZ basis set. PNO-CCSD(T) calculations use the cc-pVDZ-F12 basis set. Last two columns show the raw results for the T1 diagnosis test of CC calculations.

r(C-O) / Å	vHCISCF	vSHCI (ε ₁ =5e-3)	SHCI (ε ₁ =5e-3)	vSHCI (ε ₁ =1e-3)	SHCI (ε ₁ =1e-3)	vSHCI (ε ₁ =5e-4)	SHCI (ε ₁ =5e-4)	FCI extrap.
1.20	-0.241734	-0.207020	-0.259114	-0.241764	-0.260240	-0.248294	-0.260890	-0.261219
1.25	-0.265596	-0.236203	-0.277645	-0.265660	-0.279429	-0.270318	-0.279877	-0.280452
1.30	-0.277263	-0.248545	-0.289299	-0.277279	-0.291104	-0.282173	-0.291575	-0.292165
1.35	-0.283903	-0.255810	-0.296345	-0.283916	-0.297864	-0.288954	-0.298314	-0.298808
1.40	-0.286278	-0.258349	-0.298696	-0.286320	-0.300395	-0.291488	-0.300873	-0.301443
1.45	-0.285652	-0.257641	-0.297704	-0.285695	-0.299831	-0.291006	-0.300378	-0.301114
1.50	-0.282763	-0.254146	-0.295489	-0.282780	-0.297385	-0.288248	-0.297910	-0.298559
1.55	-0.278266	-0.249204	-0.291350	-0.278281	-0.293280	-0.283907	-0.293828	-0.294495
1.60	-0.272650	-0.243198	-0.285892	-0.272654	-0.287975	-0.278459	-0.288621	-0.289344
1.65	-0.266473	-0.236233	-0.280112	-0.266523	-0.282276	-0.272411	-0.282954	-0.283710
1.70	-0.260274	-0.228774	-0.272909	-0.260286	-0.276222	-0.266319	-0.277018	-0.278239
1.75	-0.255412	-0.225957	-0.268631	-0.255436	-0.271321	-0.261548	-0.272043	-0.273064
1.80	-0.250650	-0.220305	-0.264248	-0.250713	-0.267051	-0.256982	-0.267743	-0.268813
1.85	-0.246597	-0.215006	-0.260282	-0.246632	-0.263340	-0.253160	-0.264128	-0.265270
1.90	-0.243491	-0.211213	-0.257515	-0.243535	-0.260710	-0.250213	-0.261496	-0.262707
1.95	-0.241134	-0.207810	-0.255577	-0.241206	-0.258736	-0.248132	-0.259559	-0.260717
2.00	-0.239341	-0.206106	-0.254185	-0.239421	-0.257248	-0.246515	-0.258084	-0.259218
2.05	-0.237791	-0.204584	-0.252899	-0.237903	-0.255935	-0.245186	-0.256806	-0.257930
2.10	-0.236572	-0.203320	-0.251416	-0.236679	-0.254750	-0.243996	-0.255663	-0.256923
2.15	-0.235422	-0.201903	-0.250365	-0.235558	-0.253736	-0.242943	-0.254672	-0.255939
2.20	-0.234687	-0.201051	-0.249421	-0.234787	-0.252856	-0.242018	-0.253766	-0.255063
2.25	-0.234711	-0.202171	-0.249604	-0.234862	-0.252330	-0.241636	-0.253108	-0.254108
2.30	-0.233853	-0.201257	-0.248852	-0.234048	-0.251438	-0.240746	-0.252206	-0.253137
2.35	-0.232939	-0.199743	-0.247988	-0.233119	-0.250505	-0.239775	-0.251264	-0.252146
2.40	-0.231991	-0.198542	-0.247356	-0.232196	-0.249645	-0.238812	-0.250368	-0.251153
2.45	-0.230968	-0.197427	-0.246071	-0.231206	-0.248580	-0.237824	-0.249334	-0.250201
2.50	-0.230048	-0.196143	-0.245157	-0.230325	-0.247618	-0.236848	-0.248364	-0.249196
2.55	-0.229106	-0.195394	-0.244167	-0.229445	-0.246612	-0.235888	-0.247368	-0.248190
2.60	-0.228218	-0.194438	-0.243656	-0.228576	-0.245802	-0.235000	-0.246515	-0.247217
2.65	-0.227320	-0.193534	-0.242539	-0.227706	-0.244782	-0.234130	-0.245527	-0.246254
2.70	-0.226534	-0.192716	-0.241405	-0.226981	-0.243840	-0.233294	-0.244600	-0.245392
2.75	-0.225806	-0.191713	-0.241061	-0.226289	-0.243156	-0.232537	-0.243871	-0.244523
2.80	-0.225112	-0.190902	-0.239741	-0.225618	-0.242191	-0.231820	-0.242955	-0.243722
2.85	-0.224541	-0.190194	-0.239467	-0.225032	-0.241631	-0.231172	-0.242335	-0.242996
2.90	-0.223929	-0.189636	-0.238461	-0.224466	-0.240837	-0.230591	-0.241590	-0.242313
2.95	-0.223401	-0.189224	-0.238377	-0.223971	-0.240426	-0.230097	-0.241139	-0.241745
3.00	-0.222933	-0.188735	-0.237235	-0.223541	-0.239710	-0.229641	-0.240482	-0.241227
3.50	-0.217466	-0.183992	-0.231911	-0.218219	-0.234076	-0.224149	-0.234762	-0.235401
4.00	-0.213720	-0.180836	-0.227566	-0.214476	-0.230007	-0.220316	-0.230718	-0.231460
5.00	-0.209538	-0.176785	-0.223374	-0.210351	-0.225763	-0.216122	-0.226469	-0.227187

Table S7. Raw SHCI extrapolated absolute energies for oxobutenide anion, singlet state. vHCISCF stands for orbital optimization, vSHCI for CI with no perturbation, SHCI includes full perturbation and FCI is the final extrapolated value, following the reference method in text. ε₁ parameters are in parenthesis. Energies are given in E_h and shifted 379 E_h.

$r(\text{C-O}) / \text{\AA}$	vHCISCF	vSHCI ($\epsilon_1=5e-3$)	SHCI ($\epsilon_1=5e-3$)	vSHCI ($\epsilon_1=1e-3$)	SHCI ($\epsilon_1=1e-3$)	vSHCI ($\epsilon_1=5e-4$)	SHCI ($\epsilon_1=5e-4$)	FCI extrap.
1.20	-0.141518	-0.130984	-0.146643	-0.136789	-0.147232	-0.144374	-0.148044	-0.148468
1.30	-0.162828	-0.170181	-0.185325	-0.175976	-0.186019	-0.183220	-0.186845	-0.187329
1.40	-0.186945	-0.186680	-0.201995	-0.192523	-0.202624	-0.199800	-0.203381	-0.203808
1.50	-0.144484	-0.169486	-0.191412	-0.178149	-0.192882	-0.189060	-0.194604	-0.195703
1.60	-0.151372	-0.141881	-0.164044	-0.149561	-0.164989	-0.160578	-0.166341	-0.167149
1.70	-0.131823	-0.165215	-0.184796	-0.172249	-0.185661	-0.181845	-0.186785	-0.187463
1.80	-0.144407	-0.177997	-0.197833	-0.185089	-0.198789	-0.195006	-0.200052	-0.200817
1.90	-0.188159	-0.185089	-0.206311	-0.192863	-0.207676	-0.203989	-0.209367	-0.210434
2.00	-0.191390	-0.190922	-0.210132	-0.197885	-0.211340	-0.207766	-0.212766	-0.213725
2.10	-0.202929	-0.203673	-0.221593	-0.210192	-0.222578	-0.219343	-0.223792	-0.224537
2.20	-0.214936	-0.215428	-0.232648	-0.221737	-0.233523	-0.230566	-0.234625	-0.235249
2.30	-0.224723	-0.225212	-0.241850	-0.231297	-0.242625	-0.239699	-0.243635	-0.244195
2.40	-0.232585	-0.232628	-0.249053	-0.238615	-0.249801	-0.246951	-0.250794	-0.251331
2.50	-0.238525	-0.238598	-0.254652	-0.244466	-0.255366	-0.252634	-0.256314	-0.256814
2.60	-0.243216	-0.243166	-0.258946	-0.248919	-0.259619	-0.256939	-0.260529	-0.260998
2.70	-0.246609	-0.246535	-0.262172	-0.252251	-0.262825	-0.260191	-0.263714	-0.264165
2.80	-0.249105	-0.249101	-0.264594	-0.254792	-0.265241	-0.262638	-0.266104	-0.266543
2.90	-0.251138	-0.251037	-0.266442	-0.256704	-0.267070	-0.264489	-0.267915	-0.268338
3.00	-0.252555	-0.252031	-0.267698	-0.257752	-0.268344	-0.265711	-0.269234	-0.269681

Table S8. Raw SHCI extrapolated absolute energies for oxobutenide anion, triplet state. vHCISCF stands for orbital optimization, vSHCI for CI with no perturbation, SHCI includes full perturbation and FCI is the final extrapolated value, following the reference method in text. ϵ_1 parameters are in parenthesis. Energies are given in E_h and shifted 379 E_h .

r(C-O) / Å	vHCISCF	vSHCI ($\epsilon_1=5e-3$)	SHCI ($\epsilon_1=5e-3$)	vSHCI ($\epsilon_1=1e-3$)	SHCI ($\epsilon_1=1e-3$)	vSHCI ($\epsilon_1=5e-4$)	SHCI ($\epsilon_1=5e-4$)	FCI extrap.
1.20	-0.897990	-0.850143	-0.914997	-0.898085	-0.925886	-0.909255	-0.929053	-0.934795
1.25	-0.926677	-0.884868	-0.937633	-0.926727	-0.947509	-0.935047	-0.949689	-0.954168
1.30	-0.937511	-0.895130	-0.948526	-0.937636	-0.958517	-0.946073	-0.960769	-0.965231
1.35	-0.942712	-0.899974	-0.954358	-0.942857	-0.964286	-0.951379	-0.966541	-0.971054
1.40	-0.944686	-0.902623	-0.959710	-0.944985	-0.967695	-0.953652	-0.969657	-0.973316
1.45	-0.944743	-0.904739	-0.963915	-0.944961	-0.969010	-0.953574	-0.970404	-0.972799
1.50	-0.941606	-0.900674	-0.960544	-0.941773	-0.965940	-0.950491	-0.967382	-0.969889
1.55	-0.935933	-0.894519	-0.955763	-0.936120	-0.961258	-0.945036	-0.962693	-0.965343
1.60	-0.931265	-0.890018	-0.949968	-0.931399	-0.955768	-0.940245	-0.957298	-0.960034
1.65	-0.923864	-0.881794	-0.943230	-0.923958	-0.949182	-0.933095	-0.950768	-0.953630
1.70	-0.916321	-0.873864	-0.936183	-0.916421	-0.942273	-0.925735	-0.943928	-0.946923
1.75	-0.909421	-0.865751	-0.929744	-0.909519	-0.936014	-0.919101	-0.937771	-0.940841
1.80	-0.903885	-0.858915	-0.924404	-0.903972	-0.931078	-0.913871	-0.932952	-0.936213
1.85	-0.899764	-0.853743	-0.920721	-0.899863	-0.927606	-0.910083	-0.929577	-0.932937
1.90	-0.896922	-0.850142	-0.918181	-0.897052	-0.925218	-0.907430	-0.927215	-0.930646
1.95	-0.892139	-0.844030	-0.914461	-0.892266	-0.921855	-0.903043	-0.923933	-0.927661
2.00	-0.890938	-0.843270	-0.913013	-0.891084	-0.920206	-0.901836	-0.922328	-0.925890
2.10	-0.889153	-0.841786	-0.911668	-0.889293	-0.918704	-0.900207	-0.920830	-0.924353
2.20	-0.887732	-0.840978	-0.911217	-0.887882	-0.917609	-0.898865	-0.919607	-0.922820
2.30	-0.886228	-0.840112	-0.909870	-0.886392	-0.915791	-0.897273	-0.917729	-0.920665
2.40	-0.884827	-0.839689	-0.908479	-0.884950	-0.913897	-0.895677	-0.915722	-0.918381
2.50	-0.883402	-0.839557	-0.906705	-0.883539	-0.911845	-0.894065	-0.913600	-0.916123
2.60	-0.883952	-0.842938	-0.905158	-0.884039	-0.910113	-0.893775	-0.911713	-0.914118
2.70	-0.884510	-0.845965	-0.904122	-0.884613	-0.908851	-0.893856	-0.910346	-0.912598
2.80	-0.884568	-0.847412	-0.903175	-0.884644	-0.907845	-0.893585	-0.909240	-0.911450
2.90	-0.884478	-0.848226	-0.902438	-0.884574	-0.907045	-0.893198	-0.908336	-0.910507
3.00	-0.884805	-0.848802	-0.902159	-0.884904	-0.906752	-0.893314	-0.908004	-0.910119
3.50	-0.884862	-0.849182	-0.901201	-0.884965	-0.905674	-0.892974	-0.906814	-0.908769
4.00	-0.884438	-0.849139	-0.900298	-0.884554	-0.904768	-0.892448	-0.905865	-0.907786
5.00	-0.883565	-0.848483	-0.899182	-0.883685	-0.903676	-0.891546	-0.904765	-0.906685

Table S9. Raw SHCI extrapolated absolute energies for S-methyl-butenthioate anion, singlet state. vHCISCF stands for orbital optimization, vSHCI for CI with no perturbation, SHCI includes full perturbation and FCI is the final extrapolated value, following the reference method in text. ϵ_1 parameters are in parenthesis. Energies are given in E_h and shifted 815 E_h .

$r(\text{C-O}) / \text{\AA}$	vHCISCF	vSHCI ($\epsilon_1=5e-3$)	SHCI ($\epsilon_1=5e-3$)	vSHCI ($\epsilon_1=1e-3$)	SHCI ($\epsilon_1=1e-3$)	vSHCI ($\epsilon_1=5e-4$)	SHCI ($\epsilon_1=5e-4$)	FCI extrap.
1.65	-0.820915	-0.821285	-0.849348	-0.829701	-0.850346	-0.844329	-0.852968	-0.854509
1.75	-0.835759	-0.835900	-0.863860	-0.845335	-0.865270	-0.859167	-0.867226	-0.868600
1.85	-0.847203	-0.872102	-0.855796	-0.873353	-0.868041	-0.874954	-0.876069	-0.873408
1.90	-0.848394	-0.851509	-0.877685	-0.860592	-0.879061	-0.873565	-0.880934	-0.882217
1.95	-0.856273	-0.856455	-0.881843	-0.865209	-0.883165	-0.877829	-0.884931	-0.886145
2.00	-0.861500	-0.822699	-0.882124	-0.862322	-0.886895	-0.870794	-0.888119	-0.890464
2.10	-0.868121	-0.819590	-0.890731	-0.868169	-0.897160	-0.878369	-0.898729	-0.901802
2.30	-0.891513	-0.894255	-0.916204	-0.902038	-0.917478	-0.913540	-0.919398	-0.920557
2.40	-0.901159	-0.901658	-0.923326	-0.909373	-0.924577	-0.920723	-0.926434	-0.927544
2.50	-0.906762	-0.907253	-0.928634	-0.914885	-0.929849	-0.926086	-0.931655	-0.932717
2.60	-0.911252	-0.911390	-0.932533	-0.918909	-0.933709	-0.929979	-0.935469	-0.936495
2.70	-0.914014	-0.914065	-0.935267	-0.921564	-0.936433	-0.932689	-0.938212	-0.939244
2.80	-0.916444	-0.730710	-0.754054	-0.923907	-0.938468	-0.934800	-0.940165	-0.940165
2.90	-0.917640	-0.729315	-0.751575	-0.925040	-0.939537	-0.935896	-0.941216	-0.941216
3.00	-0.919254	-0.728840	-0.750496	-0.926587	-0.940995	-0.937372	-0.942639	-0.942639
3.50	-0.921509	-0.713650	-0.741706	-0.929055	-0.943862	-0.940033	-0.945447	-0.945447
5.00	-0.720250	-0.631211	-0.652187	-0.913950	-0.928101	-0.924051	-0.929296	-0.946566

Table S10. Raw SHCI extrapolated absolute energies for S-methyl-butenthioate anion, triplet state. vHCISCF stands for orbital optimization, vSHCI for CI with no perturbation, SHCI includes full perturbation and FCI is the final extrapolated value, following the reference method in text. ϵ_1 parameters are in parenthesis. Energies are given in E_h and shifted 815 E_h .

r(C-O) / Å	vSHCI ($\epsilon_1=5e-3$)	SHCI ($\epsilon_1=5e-3$)	vSHCI ($\epsilon_1=1e-3$)	SHCI ($\epsilon_1=1e-3$)	vSHCI ($\epsilon_1=5e-4$)	SHCI ($\epsilon_1=5e-4$)	FCI extrap.
1.30	-0.532912	-0.677809	-0.627215	-0.697204	-0.647215	-0.702357	-0.716648
1.35	-0.538301	-0.683159	-0.632359	-0.702667	-0.652564	-0.707852	-0.722340
1.40	-0.538695	-0.684669	-0.633619	-0.704296	-0.653887	-0.709501	-0.724015
1.45	-0.537855	-0.684203	-0.632261	-0.703460	-0.652653	-0.708631	-0.723000
1.50	-0.538724	-0.682696	-0.629989	-0.701152	-0.650599	-0.706222	-0.720362
1.55	-0.536425	-0.679267	-0.626449	-0.697318	-0.647113	-0.702298	-0.716180
1.60	-0.529802	-0.673135	-0.619829	-0.691180	-0.640731	-0.696272	-0.710245
1.65	-0.522137	-0.666051	-0.612232	-0.684158	-0.633386	-0.689397	-0.703545
1.70	-0.514223	-0.658914	-0.604721	-0.677368	-0.626161	-0.682763	-0.697330
1.75	-0.507002	-0.652923	-0.598099	-0.671651	-0.619930	-0.677217	-0.692120
1.80	-0.500630	-0.648343	-0.592783	-0.667214	-0.614927	-0.672898	-0.687909
1.85	-0.491070	-0.641945	-0.584872	-0.661239	-0.607399	-0.667098	-0.682654
1.90	-0.494619	-0.643766	-0.586567	-0.662305	-0.609036	-0.668077	-0.683090
1.95	-0.490895	-0.640475	-0.582761	-0.658947	-0.605386	-0.664774	-0.679832
2.00	-0.491373	-0.640367	-0.582630	-0.658370	-0.605306	-0.664223	-0.678819
2.10	-0.489953	-0.639176	-0.581336	-0.657097	-0.604072	-0.662955	-0.677435
2.20	-0.489634	-0.638684	-0.581113	-0.656388	-0.603779	-0.662161	-0.676275
2.30	-0.492345	-0.638530	-0.581823	-0.655681	-0.604259	-0.661379	-0.675029
2.40	-0.499052	-0.638671	-0.584806	-0.655384	-0.606650	-0.660791	-0.673985
2.50	-0.501493	-0.637827	-0.585759	-0.654489	-0.607126	-0.659697	-0.672724
2.60	-0.503125	-0.637041	-0.586206	-0.653523	-0.607183	-0.658643	-0.671444
2.70	-0.503762	-0.636155	-0.586193	-0.652573	-0.606816	-0.657550	-0.670223
2.80	-0.505701	-0.636808	-0.587724	-0.653133	-0.608025	-0.657966	-0.670422
2.90	-0.507232	-0.637121	-0.588712	-0.653335	-0.608866	-0.658120	-0.670398
3.00	-0.507952	-0.637246	-0.589361	-0.653421	-0.609447	-0.658199	-0.670324
4.00	-0.508705	-0.636138	-0.589822	-0.652274	-0.609514	-0.656936	-0.668735
5.00	-0.508377	-0.635168	-0.589249	-0.651299	-0.608896	-0.655947	-0.667692

Table S11. Raw SHCI extrapolated absolute energies for DPA anion, singlet state. vHCISCF stands for orbital optimization, vSHCI for CI with no perturbation, SHCI includes full perturbation and FCI is the final extrapolated value, following the reference method in text. ϵ_1 parameters are in parenthesis. Energies are given in E_h and shifted 1118 E_h .

r(C-O) / Å	vSHCI ($\epsilon_1=5e-3$)	SHCI ($\epsilon_1=5e-3$)	vSHCI ($\epsilon_1=1e-3$)	SHCI ($\epsilon_1=1e-3$)	vSHCI ($\epsilon_1=5e-4$)	SHCI ($\epsilon_1=5e-4$)	FCI extrap.
1.75	-0.543244	-0.615605	-0.564915	-0.621397	-0.601799	-0.630794	-0.640978
1.80	-0.548344	-0.621088	-0.570164	-0.626911	-0.607223	-0.636389	-0.646655
1.85	-0.552368	-0.625010	-0.574027	-0.630827	-0.611099	-0.640288	-0.650581
1.90	-0.558275	-0.630881	-0.580079	-0.636666	-0.617043	-0.646123	-0.656328
2.10	-0.572365	-0.642960	-0.593486	-0.648269	-0.629479	-0.657438	-0.666923
2.20	-0.581747	-0.650693	-0.602609	-0.655858	-0.637789	-0.664627	-0.673505
2.30	-0.590010	-0.657426	-0.610331	-0.662353	-0.644838	-0.670793	-0.679156
2.40	-0.596144	-0.662744	-0.616372	-0.667580	-0.650353	-0.675726	-0.683714
2.50	-0.600858	-0.666782	-0.620689	-0.671436	-0.654377	-0.679459	-0.687236
2.70	-0.612826	-0.679767	-0.633169	-0.684505	-0.667453	-0.692658	-0.700430
3.00	-0.619650	-0.687789	-0.640418	-0.692654	-0.675040	-0.700858	-0.708824
4.00	-0.621053	-0.691471	-0.641846	-0.696430	-0.677812	-0.705126	-0.713772
5.00	-0.619731	-0.690577	-0.640265	-0.695548	-0.676592	-0.704354	-0.713230

Table S12. Raw SHCI extrapolated absolute energies for DPA anion, triplet state. vHCISCF stands for orbital optimization, vSHCI for CI with no perturbation, SHCI includes full perturbation and FCI is the final extrapolated value, following the reference method in text. ϵ_1 parameters are in parenthesis. Energies are given in E_h and shifted 1118 E_h .

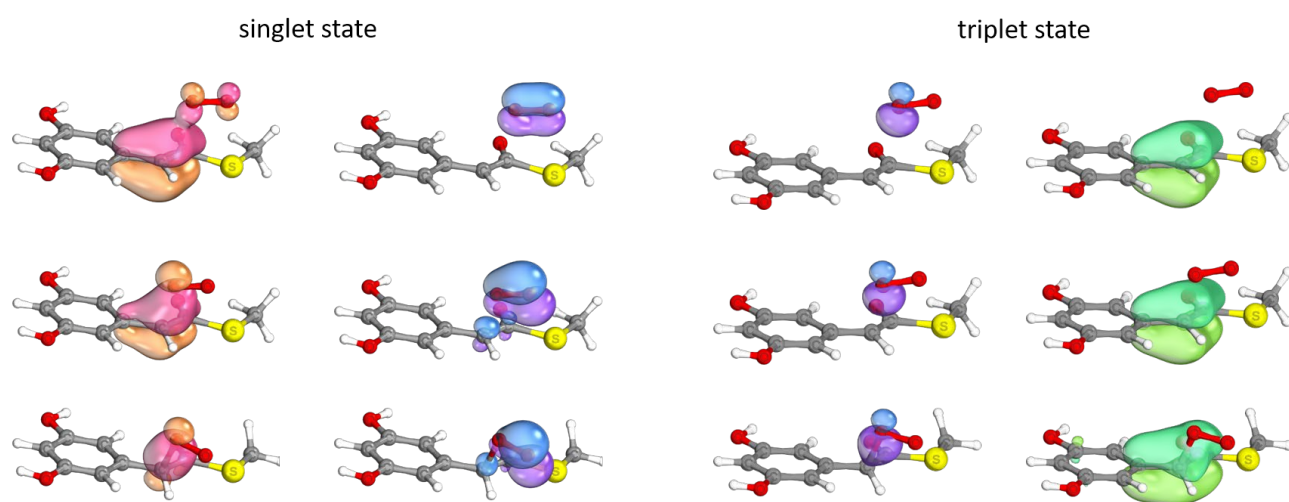


Fig S1: IBO/IAO orbitals whose nature change the most along the addition of O_2 to DPA^- for the singlet and triplet states. Top panel: large $r_{C\alpha O}$ distances. Middle panels: Crossing region. Bottom panels: short $r_{C\alpha O}$ distance, where the peroxide is formed.

5.2 DpgC-catalyzed peroxidation of 3,5-dihydroxyphenylacetyl-CoA (DPA-CoA)

This work is chronologically the first paper of this project, published in 2020. The DpgC system (shown in Figure 16) was chosen to study the oxygenation reaction, for the reasons already stated in the Chapter 4 (size of the molecule, more localised electronic charge, etc.). After studying the system with small sets of orbitals in Active Space, it was concluded that it was necessary to introduce more orbitals in the calculation, so the new methods such as SHCI were introduced. This newly developed method makes it possible to include a set of up to 200 orbitals in the active space, so that it is possible to follow the reaction including all orbitals involved.

The main novelty with respect to previous studies with this type of systems, related to dioxygenases, comes from the type of theoretical methodology employed, so that for the first time aspects relevant to spin-changing reactions, such as spin-orbital coupling or the multireference character of the electronic structure calculations, are considered.

We used MRCI calculations to produce the potential energy surfaces of the 9 concurrent electronic states, and assign an electronic configuration to each. Our findings indicated that once the enolate is formed, the reaction can happen without prior activation of O_2 , primarily through two electron transfer processes. The minimum energy crossing point

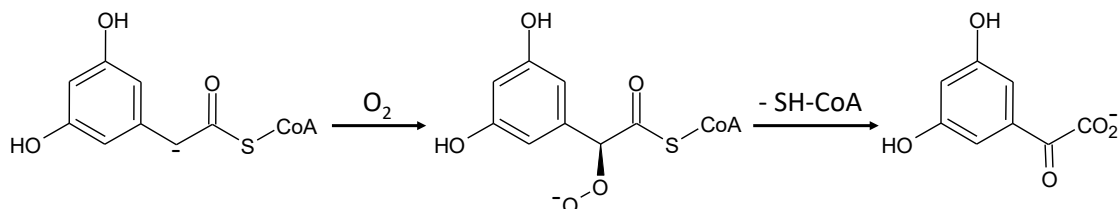


Figure 16: DPA-CoA dioxygenation reaction. DPA-CoA has already been deprotonated in a previous step. Then, in this figure, it is attacked by dioxygen.

(MECP) was found to be only 16.7 kcal/mol above the DPA-CoA + $^3\text{O}_2$ asymptote, aligning with experimental values of kcat. Our results showed that intersystem crossing takes place between the triplet state associated with $\text{DPA}^{\cdot-}\text{-O}_2$ and the singlet state associated with DPA-O_2^- . This involves transferring a charge from DPA to O_2 to form a superoxide anion. The strength of the spin-orbit coupling was more than 70 cm^{-1} , a high value for a system that does not include heavy atoms.

While we also conducted gas-phase electronic structure calculations, we complemented our understanding of the enzymatic reaction mechanism by employing QM/MM simulations. Our QM/MM results disclosed that the MECP is the principal reaction bottleneck. They also suggested that the protonation of O_2 occurs post-peroxide formation, which explains the absence of basic residues in the active site. This later protonation would probably happen through a water wire connected to the bulk solvent. The QM/MM simulations also underscored the passive role of the protein environment in promoting the spin-forbidden reaction, although it is crucial for facilitating O_2 diffusion towards the active site and the formation and stabilization of the enolate.

In conclusion, this study has given us a clearer understanding of the DpgC-catalyzed oxygenation of DPA-CoA and has provided insight into similar reactions in cofactorless spin-forbidden reactions that proceed via the formation of a peroxide.

5.2.1 Resumen en español

Este trabajo es cronológicamente el primer paper de este proyecto, publicado en 2020. Se eligió el sistema DpgC (mostrado en la Figura 16) para estudiar la reacción de oxigenación, por las razones ya expuestas en el Capítulo 4 (tamaño de la molécula, carga electrónica más localizada, etc.). Tras estudiar el sistema con conjuntos más reducidos

de orbitales en el espacio activo, se llegó a la conclusión de que era necesario introducir más orbitales en el cálculo, por lo que se utilizaron nuevos métodos como el SHCI. Este método permite incluir un conjunto de hasta 200 orbitales en el espacio activo, de forma que es posible seguir la reacción incluyendo todos los orbitales implicados.

La principal novedad respecto a estudios previos con este tipo de sistemas, relacionados con dioxigenasas, proviene del tipo de metodología teórica empleada, de forma que por primera vez se consideran aspectos relevantes para las reacciones de cambio de espín, como el acoplamiento espín-orbital o el carácter multirreferencia de los cálculos de estructura electrónica.

Utilizamos cálculos MRCI para producir las superficies de energía potencial de los 9 estados electrónicos concurrentes, y asignar una configuración electrónica a cada uno. Nuestros resultados indicaron que, una vez formado el enolato, la reacción puede producirse sin necesidad de activar primero el O_2 , principalmente a través de dos procesos de transferencia de electrones. El punto de cruce de energía mínima (MECP) se encontró a sólo 16,7 kcal/mol por encima de la asíntota $DPA-CoA + {}^3O_2$, alineándose con los valores experimentales de k_{cat} . Nuestros resultados mostraron que el cruce intersistema tiene lugar entre el estado triplete asociado con DPA^--O_2 y el estado singlete asociado con $DPA-O_2^-$. Esto implica la transferencia de una carga de DPA a O_2 para formar un anión superóxido. La fuerza del acoplamiento espín-órbita fue superior a 70 cm^{-1} , un valor elevado para un sistema que no incluye átomos pesados.

Aunque también realizamos cálculos de estructura electrónica en fase gaseosa, complementamos nuestra comprensión del mecanismo de reacción enzimática empleando simulaciones QM/MM. Nuestros resultados QM/MM revelaron que el MECP es el principal cuello de botella de la reacción. También sugirieron que la protonación del O_2 se produce tras la formación del peróxido, lo que explica la ausencia de residuos

básicos en el sitio activo. Esta protonación posterior se produciría probablemente a través de un hilo de agua conectado al disolvente a granel. Las simulaciones QM/MM también subrayaron el papel pasivo del entorno de la proteína en la promoción de la reacción de espín prohibido, aunque es crucial para facilitar la difusión del O_2 hacia el sitio activo y la formación y estabilización del enolato.

En conclusión, este estudio nos ha proporcionado una comprensión más clara de la oxigenación de DPA-CoA catalizada por DpgC y ha aportado información sobre reacciones similares en reacciones de espín prohibido sin cofactor que proceden a través de la formación de un peróxido.

Oxygenases

DpgC-Catalyzed Peroxidation of 3,5-Dihydroxyphenylacetyl-CoA (DPA-CoA): Insights into the Spin-Forbidden Transition and Charge Transfer Mechanisms**

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Abstract: Despite being a very strong oxidizing agent, most organic molecules are not oxidized in the presence of O₂ at room temperature because O₂ is a diradical whereas most organic molecules are closed-shell. Oxidation then requires a change in the spin state of the system, which is forbidden according to non-relativistic quantum theory. To overcome this limitation, oxygenases usually rely on metal or redox cofactors to catalyze the incorporation of, at least, one oxygen atom into an organic substrate. However, some oxygenases do not require any cofactor, and the detailed mechanism followed by these enzymes remains elusive. To fill this gap, here the mechanism for the enzymatic cofactor-independent oxidation of 3,5-dihydroxyphenylacetyl-CoA (DPA-CoA) is studied by combining multireference calculations on a

model system with QM/MM calculations. Our results reveal that intersystem crossing takes place without requiring the previous protonation of molecular oxygen. The characterization of the electronic states reveals that electron transfer is concomitant with the triplet-singlet transition. The enzyme plays a passive role in promoting the intersystem crossing, although spontaneous reorganization of the water wire connecting the active site with the bulk presets the substrate for subsequent chemical transformations. The results show that the stabilization of the singlet radical-pair between dioxygen and enolate is enough to promote spin-forbidden reaction without the need for neither metal cofactors nor basic residues in the active site.

1. Introduction

The great oxygenation event, approximately 2.4 billion years ago, was one of the most important episodes in the history of life on Earth.^[1] The accumulation of molecular oxygen (O₂) in the atmosphere eventually caused the extinction of a myriad of life forms.^[2] Those that survived evolved to exploit the presence of O₂, using it to release the energy that cells will use for their maintenance and growth. The oxygen reduction reaction

is also crucial for emerging energy technologies, such as solar fuels production.^[3–5]

From a chemical point of view, reactions between O₂ and organic matter are conceptually complicated. The majority of the stable molecules involved in a biological process are singlets (all the electrons are paired). O₂ is an exception to this rule, and in its ground electronic state it has two unpaired electrons: it is a triplet. Hence, reactions between O₂ and organic molecules should involve the change of the spin state (intersystem crossing), a process that is forbidden in non-relativistic quantum chemistry, and accordingly should be very slow at room temperature. This explains why organic matter exists in our oxidizing atmosphere.

To circumvent this limitation, enzymes typically use transition metals, mostly iron, to catalyze reactions with O₂.^[6–15] Transition metals also contain unpaired electrons so may react with O₂ via a spin-allowed reaction. Moreover, metals can be used to preactivate the substrate, forming a radical that can react with O₂.^[6,14,16] Last but not least, spin-orbit coupling (SOC),^[17,18] the relativist effect that permits intersystem crossing, is expected to be significantly strong for systems that include transition metals. Other proteins use redox organic cofactors to catalyze spin-forbidden reactions, especially flavin.^[19–21]

Intriguingly, some oxygenases are capable of catalyzing the incorporation of at least one oxygen atom from O₂ to an organic substrate without needing any cofactor.^[22–24] The catalyt-

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[**] A previous version of this manuscript has been deposited on a preprint server (<https://doi.org/10.1002/chem.202002993>).

 Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under:
<https://doi.org/10.1002/chem.202002993>.

ic mechanism followed by these enzymes has been studied both computational and experimentally (see for example Refs. [25]–[38]) although the procedure used to facilitate inter-system crossing is still controversial.^[29,35]

From among these proteins, we will focus on DpgC, the first protein for which a crystal structure showing a substrate analog and O₂ bound to the enzyme was resolved.^[36–38] DpgC is a hexameric crotonase oxygenase^[39,40] that plays a key role in the biosynthesis of dihydroxyphenylglycine (DPG), a non-natural amino acid found in “antibiotics of last resort” such as vancomycin or teicoplanin.^[41] In particular, DpgC catalyzes the cofactor-independent oxidation of 3,5-dihydroxyphenylacetyl-CoA (DPA-CoA) to 3,5-dihydroxyphenyl-glyoxylate (DPGX; Figure 1). When DPA-CoA binds to the active site, it is deprotonated at C_α. Deuterium atom exchange was observed even at anaerobic conditions, confirming that this first step is O₂ independent and that DpgC stabilizes the enolate before O₂ accesses the active site.^[42] Following deprotonation of C_α, O₂ diffuses into the active site. Molecular dynamics (MD) calculations predict that there are three main pathways for O₂ diffusion,^[43] all of them guiding O₂ to the hydrophobic pocket in which O₂ was resolved in the crystal structure. These simulations also suggested that the binding of O₂ to the active site is rather weak, which is compensated by a higher frequency of O₂ entry into the active site.^[43]

The reaction between O₂ and DPA-CoA is expected to proceed via a peroxide intermediate.^[36,38] This is a spin-forbidden process, and it is likely the rate-limiting stage of the overall process. Contrary to what was observed for other proteins such as glucose oxidase^[33] where a His516 residue acts as a proton donor and protonates O₂ throughout the reaction, in the O₂ pocket of DpgC there are no amino acids that could play that role. In a subsequent step, the peroxide breaks leading to the formation of DPGX via either a Criegee rearrangement or following a dioxetane intermediate.^[42]

There are three reasons why we believe that DpgC is an excellent system for the characterization of the general mechanism of a spin-forbidden cofactorless addition of O₂ to an organic enolate. First, the lack of basic residues around the active site permits us to analyze to what extent the formation of the peroxide is possible in the absence of amino acids, which could protonate the peroxide. Second, there are no aromatic residues that could be involved in π – π stacking interactions, so the hypothesis of a “spin-well” could be ruled out.^[25] Third, the peroxidation occurs outside the aromatic ring, so in a first approximation the π orbitals of the ring do not have to be included in the active space, which facilitates convergence in the multireference calculations.

From a fundamental point of view, the study of spin-forbidden reactions is very challenging. It requires the characterization of all the concurrent potential energy surfaces (PESs), for at least two different spin multiplicities. For these reactions, the minimum energy crossing point (MECP) between the two PESs of different spin multiplicities plays the role of the effective barrier.^[44–49] To determine the rate coefficient of a spin-forbidden reaction, it is also necessary to calculate the SOC. In the non-adiabatic transition state theory, the transmission coefficient is approximated by the hopping probability, which depends on the magnitude of the SOC, and also on the difference in slope of the PESs along with the reaction coordinate in the crossing point. In its simplest case, the hopping probability is calculated by using the celebrated Landau–Zener formula.^[50] For typical values of SOC, the hopping probability lies between 0.001 and 0.1, which is equivalent to an increase in the activation energy of 1–4 kcal mol^{–1} at room temperature.^[47,49] Similar methodologies have been successfully applied to the study of some enzymatic reactions,^[15,28–33,44,52,53] including glucose oxidase^[32,33] and *p*-hydroxyphenylacetate hydroxylase,^[28] for which O₂ reacts with flavin, as well as the cofactorless oxygenases (1*H*)-3-hydroxy-4-oxoquinoline 2,4-dioxygenase (HOD)^[29,31] and nogalamycin monooxygenase,^[30] some of them using small active site models.

Regarding the available ab initio methods that could be used to describe spin-forbidden reactions, multireference methods are advised as they account for several electronic states on an equal footing, and estimate the magnitude of the SOC. Among these methods, the multiconfiguration reference internally contracted configuration interaction (MRCI) method is one of the most accurate, and it is considered the golden standard for multireference systems. MRCI is routinely applied for the calculation of ground and excited PESs for systems involving up to three or four atoms in several electronic states (see for example Refs. [54]–[59]). For larger systems, the use of MRCI is not common owing to its computational cost, and convergence issues. However, if it is possible to select a stable active space that properly describes the process under study, it becomes a powerful tool for the understanding of chemical reactivity.

The goal of this article is to understand the detailed mechanism of the spin-forbidden reactions between O₂ and an organic substrate that does not require the presence of any cofactor. To address this question, we studied the reaction between DPA-CoA and O₂ by using two sets of quantum calculations: high level multireference ab initio methods on a model system, and a DFT-based QM/MM approach.

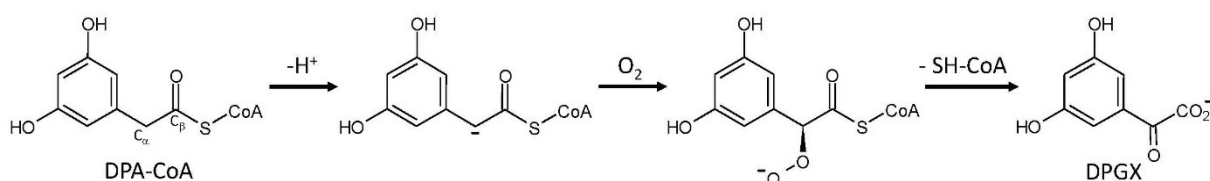


Figure 1. Proposed mechanisms for the conversion of 3,5-dihydroxyphenylacetyl-CoA (DPA-CoA) to 3,5-dihydroxyphenyl-glyoxylate (DPGX) by DpgC.

2. Results and Discussion

As stated above, we are going to combine the results obtained by using two kinds of calculations. The first group consisted of MRCI calculations describing the nine electronic states that are relevant for O_2 addition to the enolate. Owing to the large computational cost of MRCI calculations, we used the model system depicted in the top panel of Figure 2, where DPA-CoA has been replaced by *S*-methyl-but-3-enethioate (hereinafter butenethioate). The second group of calculations consisted of a QM/MM description of the enzymatic reaction, relying on DFT methods. The atoms included in the QM region (shown in the bottom panel of Figure 2) are O_2 , the DPA moiety of the substrate, and the sidechain of three residues: Ala319 and Ile324 in the hydrophobic pocket of O_2 , and Gln299. We also added to the QM region four water molecules nearby, which could be involved either in the protonation of the peroxide formed or in the stabilization of DPA-CoA. The QM/MM calculations will provide clues to the role played by the protein scaffold to promote the process, and in particular, will serve to discuss the relevance of the possible proton transfer steps.

2.1. Reaction between O_2 and *S*-methyl-but-3-enethioate

In its ground state, the electronic configuration of O_2 is $(1\sigma_g)^2(1\sigma_u^*)^2(2\sigma_g)^2(2\sigma_u^*)^2(3\sigma_g)^2(1\pi_u)^4(1\pi_g^*)^2$. The distribution of two electrons in two degenerated π orbitals leads to three electronic states relatively close in energy: the ground $^3\Sigma_g^-$ state, the $^1\Delta_g$ (doubly degenerate, which splits into two states in the presence of any colliding partner), and the $^1\Sigma_g^+$. The energy difference between $^1\Sigma_g^+$ and $^3\Sigma_g^-$ is only of $37.7 \text{ kcal mol}^{-1}$,^[60] with $^1\Delta_g$ lying between them ($22.64 \text{ kcal mol}^{-1}$ above $^3\Sigma_g^-$). Further excited states involve excitation of one electron from the $(1\pi_g^*)$ to $(3\sigma_u^*)$ orbital, but they appear much higher in energy and are unlikely to play any role in the intersystem crossing. On the other hand, butenethioate is a closed-shell molecule that does not present any excited state close in energy.

For the MRCI calculations, we selected an active space that includes four electrons in three orbitals: the HOMO orbital of butenethioate (hereinafter p_{car} although it is delocalized over the three sp^2 C atoms) and the two $1\pi_g^*$ orbitals of O_2 , which are singly occupied in the ground state of O_2 and we will denote as $\pi_{O_2}^*$. With this active space we can describe up to nine electronic states: three triplets and six singlet states, denoted as $1-3^3[\text{DPA-O}_2]$ and $1-6^1[\text{DPA-O}_2]$, respectively.

Figure 3 displays the energy profiles of the considered electronic states during the addition of O_2 to butenethioate as a function of $r_{C_\alpha O}$, the distance between C_α and O_2 (the two atoms between which the new bond will be formed, as shown in Figure 2). To build these energy profiles, optimized geometries were considered for each value of $r_{C_\alpha O}$, following the procedure described in the Experimental Section. It is worth noticing that, based on QM/MM optimizations of the peroxide, we applied constraints to keep all the C atoms of butenethioate in the same plane. This constraint is only relevant at small $r_{C_\alpha O}$, where the peroxide is formed, and was chosen to better mimic

S-Methyl-but-3-enethioate model

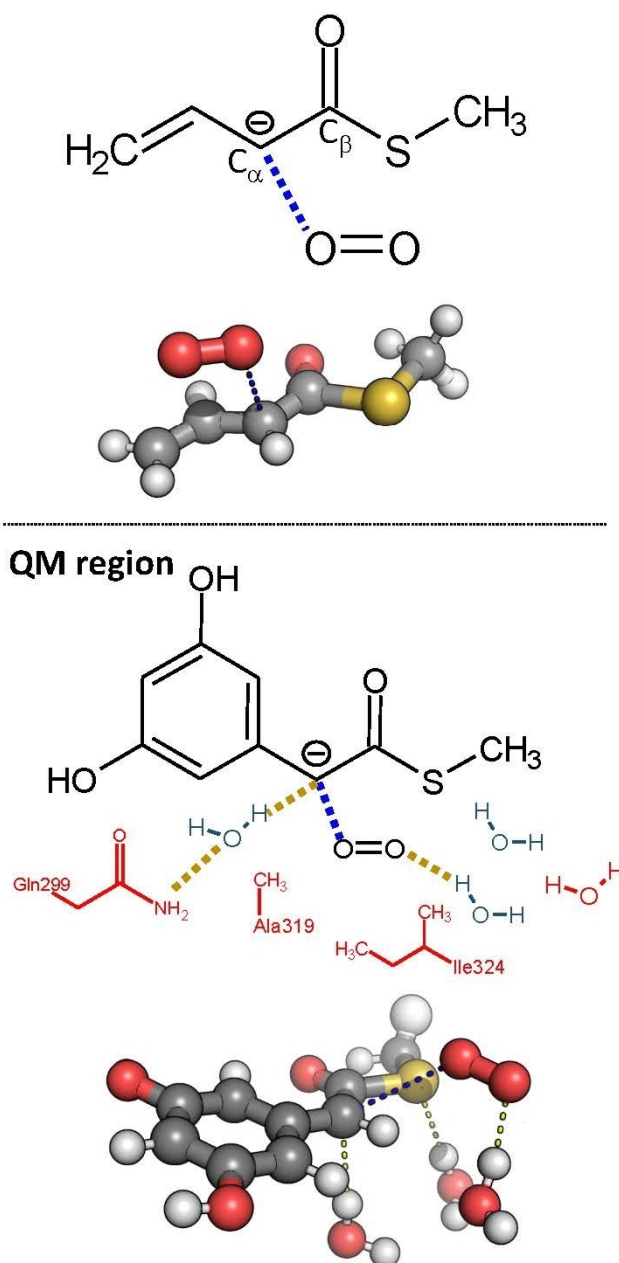


Figure 2. Schematic representation of the atoms included in the *S*-methyl-but-3-enethioate (top panel), and in the QM region for the QM/MM calculations (bottom panel). Two different QM regions were used in the later calculations. A larger one including all residues highlighted, and a smaller one in which the residues highlighted in red were included in the MM region. The hydrogen bonds between DPA and the water molecules nearby are highlighted in yellow, whereas the distance between C_α and O_2 is highlighted in blue. For the sake of clarity, we only show in the 3D representation the atoms included in the small QM region.

the behavior of DPA-CoA in the protein environment (see the Experimental Section).

At large $r_{C_\alpha O}$ distances, it is safe to assume that there is almost no interaction between butenethioate and O_2 , and the active orbitals do not mix. Under these circumstances, it is pos-

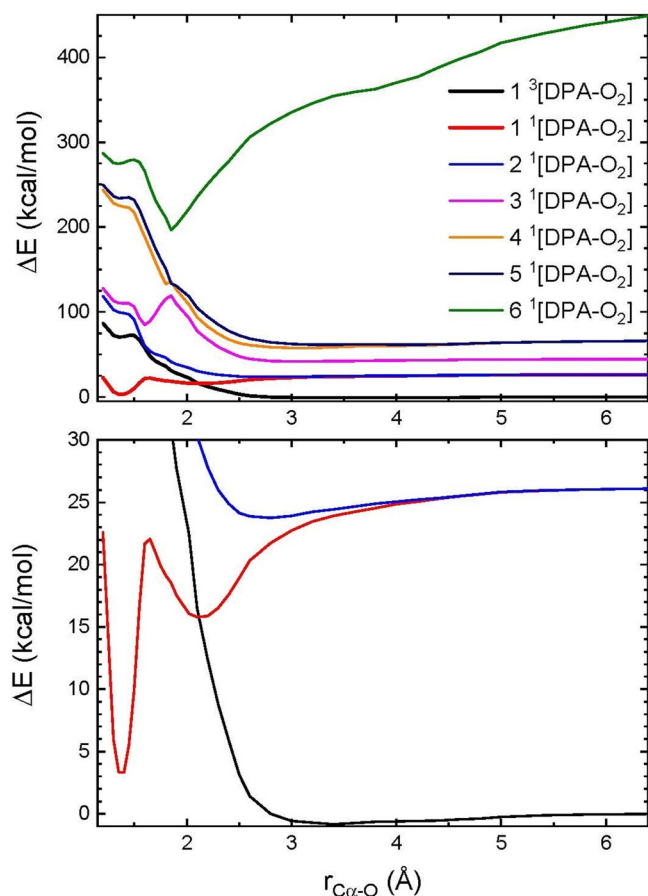


Figure 3. Top panel: Energy profiles for the addition of O₂ to S-methyl-but-3-enethioate as a function of the C_α–O distance. The ground triplet state and the six singlets involved are shown. A zoom of the lower energy region is shown in the bottom panel. The two excited triplet states are not depicted for the sake of clarity. Calculations carried out at the MRCI(4,3)/6-31 + G(d,p) level of theory.

sible to correlate the adiabatic states with the electronic configurations of the two colliding partners. The first four states (the ground triplet state, $1^3[\text{DPA-O}_2]$, and the three first singlet states, $1\text{--}3^1[\text{DPA-O}_2]$) are well described with a $(p_{\text{Ca}})^2(\pi_{\text{O}_2}^*)^2$ electronic configuration, namely DPA^-O_2^- , where the negative charge is located on the butenthioate. In this conformation, the four states correlate to the four lowest energy states of O₂: the ground $^3\Sigma_g^-$ state, the $^1\Delta_g$ (doubly degenerate), and the $^1\Sigma_g^+$. In our calculations, the energy difference between $^1\Delta_g$ and $^3\Sigma_g^-$ is 26.1 kcal mol^{−1}, just a 15% larger than that observed for gas-phase isolated O₂,^[60] and the same trend is observed for the difference between $^1\Sigma_g^+$ and $^3\Sigma_g^-$, which confirms the validity of the applied theoretical methods.

The following four states, $4\text{--}5^1[\text{DPA-O}_2]$ and $2\text{--}3^1[\text{DPA-O}_2]$ can be described by using a $(p_{\text{Ca}})^1(\pi_{\text{O}_2}^*)^3$ electronic configuration, namely DPA^-O_2^- , which corresponds to a radical-pair between the superoxide anion O₂[−] and the butenthionyl radical. As there is no interaction between butenthioate and O₂, all of them are degenerate at large $r_{\text{C}_\alpha\text{O}}$. The energy difference between $1^3[\text{DPA-O}_2]$ and these states is 66.1 kcal mol^{−1}, and corresponds to the energy required to transfer one electron from

butenthioate to O₂ in the gas phase. The presence of the protein environment stabilizes the charge on the O₂[−], shifting this energy to 36.6 kcal mol^{−1}. This value, however, is significantly larger than that calculated for the reaction between O₂ and flavin, 2.8 kcal mol^{−1} at a DFT level (and implicit water solvent).^[30] This difference may explain why enzymes use flavin as a redox catalyzer.

The electronic configuration of the last state, $6^1[\text{DPA-O}_2]$, is $(p_{\text{Ca}})^0(\pi_{\text{O}_2}^*)^4$ or $\text{DPA}^+\text{O}_2^{2-}$. It corresponds to the interaction between the peroxide anion (O₂^{2−}) and the butenthionyl cation. The energy of this state is very high (more than 450 kcal mol^{−1}), as it requires the transference of two electrons from butenthioate to O₂. Its energy, however, decreases quickly with $r_{\text{C}_\alpha\text{O}}$ distance, as expected for an ion–ion interaction ($E \propto r^{-1}$). If we had used a larger active space, we would have obtained several states between $5^1[\text{DPA-O}_2]$ and $6^1[\text{DPA-O}_2]$, and this would have impaired the description of the $6^1[\text{DPA-O}_2]$. Despite the very large energy difference between $6^1[\text{DPA-O}_2]$ and the ground $1^3[\text{DPA-O}_2]$ state, the former state will be important to describe the formation of the peroxide, as we will discuss below.

When O₂ and butenthioate approach each other, the orbitals mix and the correspondence between our adiabatic states and the electronic configuration of the two independent molecules is no longer valid. This motion is associated with a change in the relative orientation of O₂ with respect to butenthioate (see Figure S1 in the Supporting Information). At $r_{\text{C}_\alpha\text{O}} < 4$ Å, the interaction between O₂ and butenthioate is already strong enough to break the degeneracy of $2\text{--}3^1[\text{DPA-O}_2]$ (former $^1\Delta_g$ states). The inspection of the active space orbitals reveals that p_{Ca} starts to overlap with one of the two no longer equivalent $\pi_{\text{O}_2}^*$. The same effect is observed for the four DPA-O_2^- states, also no longer degenerate. The interaction of butenthioate and O₂ means that for $2 \text{ Å} < r_{\text{C}_\alpha\text{O}} < 3 \text{ Å}$ only $1^1[\text{DPA-O}_2]$ and $6^1[\text{DPA-O}_2]$ are not repulsive. For $r_{\text{C}_\alpha\text{O}} < 2$ Å, the ground singlet state becomes more stable than the triplet state, showing a minimum for $r_{\text{C}_\alpha\text{O}} = 1.4$ Å. $6^1[\text{DPA-O}_2]$ reaches a minimum at 1.85 Å, after which its energy rises. This behavior is not compatible with the description of an ion–ion interaction, suggesting that this state has changed its character owing to an avoided crossing caused by strong non-adiabatic couplings with other singlet states.

The bottom panel of Figure 3 shows an expanded view that allows us to appreciate the main features of the PESs for the three lowest energy states, $1^3[\text{DPA-O}_2]$ and $1\text{--}2^1[\text{DPA-O}_2]$. The $1^3[\text{DPA-O}_2]$ state is purely repulsive, and its energy rises quickly at $r_{\text{C}_\alpha\text{O}} < 2.5$ Å. That behavior is shared with the $2^1[\text{DPA-O}_2]$ state. On the contrary, $1^1[\text{DPA-O}_2]$ is attractive and displays a double-well structure. The energy of the minimum of the first well is 15.9 kcal mol^{−1} at $r_{\text{C}_\alpha\text{O}} = 2.1$ Å, a distance large enough so the C_αO covalent bond is still not formed. Inside the well, the electronic wavefunction exhibits a strong superoxide (O₂[−]) character, and it seems that the well is stabilized by a strong charge-dipole interaction. The singlet and the triplet states cross at the bottom of the well, which coincides with the MECP. The second minimum is significantly deeper (just 3.4 kcal mol^{−1} higher than the minimum of the triplet state)

and is located at $r_{\text{C}\alpha\text{O}} = 1.4 \text{ \AA}$. This minimum is associated with the peroxide formation.

The two minima are separated by a maximum on the one-dimension curve, which corresponds to a saddle-point. It lies slightly below the asymptotic energy of the $1^1[\text{DPA-O}_2]$ state. This saddle-point is associated with the barrier of the superoxide to peroxide transformation and arises from an avoided crossing. The huge gap in energy between states at the saddle-point indicates that the non-adiabatic couplings are very strong in this region, suggesting that the charge transfer may occur adiabatically. We should point out that the barrier height predicted by our method is probably overestimated, partially because of the constraints imposed to keep the structure planar and also, as we will discuss later, owing to the small size of the active space. The planar restraint imposed on butenthioate also affects the relative energy of the minimum of the first singlet and triplet states and causes the $1^1[\text{DPA-O}_2]$ minimum to lie above the minimum energy $1^3[\text{DPA-O}_2]$ state. It should be noticed that this does not affect the energy profile for $r_{\text{C}\alpha\text{O}} > 2 \text{ \AA}$, where the peroxide bond is not formed, and the

molecule is intrinsically planar. Thus, the MECP region should not be affected by the planar restraint.

To get more insight into the nature of the states that are involved in the intersystem crossing, we generated the quasi-diabatic states for the addition of O_2 to butenthioate. Diabatic curves are not appropriate to track the position of MECP, as diabatic energies do not include the couplings between the states, which, in the diabatic representation, are included in the non-diagonal elements of the electronic Hamiltonian matrix. However, quasi-diabatic states retain the character displayed at asymptotic distances, making it possible to extend the assignment of an electronic configuration for independent O_2 and butenthioate to small $\text{C}\alpha\text{-O}$ distances. Diabatic states are calculated as a function of $r_{\text{C}\alpha\text{O}}$ for three different $\text{C}\alpha\text{-O-O}$ angles: 110° , which is close to the value obtained for the fully relaxed geometries shown in Figure 3; 180° for which O_2 inserts perpendicular to the butenthioate plane and minimizes the overlap between ($p_{\text{C}\alpha}$) and ($\pi_{\text{O}_2}^*$) orbitals; and 150° , an intermediate value in order to see the evolution. In the left panels of Figure 4 we show the adiabatic curves of the ap-

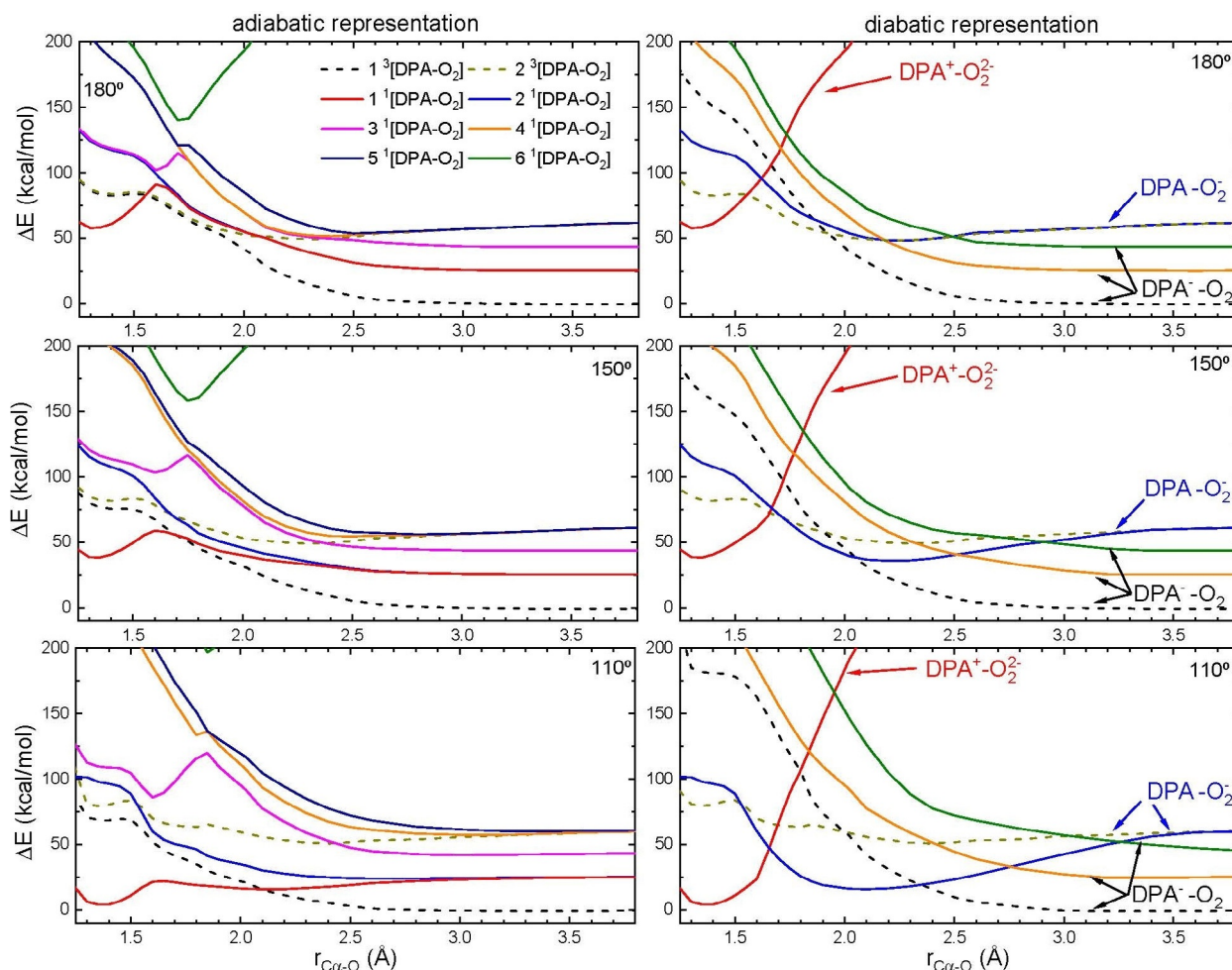


Figure 4. Energy profiles for the addition of O_2 to 5-methyl-but-3-enethioate as a function of the $\text{C}\alpha\text{-O}$ distance and the $\text{C}\alpha\text{-O-O}$ angle. In the left panels, the adiabatic curves are shown and their corresponding diabatic curves are shown in the right panels. $\text{C}\alpha\text{-O-O} = 110^\circ$ corresponds to the minimum energy at the MECP shown in Figure 3. The curves for the singlet (triplet) states are shown in solid (dashed) lines. Calculations carried out at the MRCI(4,3)/6-31 + G(d,p) level of theory.

proaching of O₂ to butenthioate, whereas the diabatic states are shown in the right panels.

In the absence of overlap between ($p_{C\alpha}$) and ($\pi_{O_2}^*$) orbitals, $C_{\alpha}-O-O=180^\circ$, only the $6^1[\text{DPA}-\text{O}_2]$ adiabatic state is not repulsive for $r_{C_{\alpha}O} > 2 \text{ \AA}$. As non-adiabatic couplings are small for this orientation, diabatic and adiabatic representations are very similar. At $C_{\alpha}-O-O=180^\circ$, $1-2^1[\text{DPA}-\text{O}_2]$ as well as $4-5^1[\text{DPA}-\text{O}_2]$ adiabatic states are nearly degenerate for distances down to $1.7 \text{ \AA}$. For this orientation and $r_{C_{\alpha}O}=1.4 \text{ \AA}$, we observe a shallow minimum on the first singlet state. The quasi-diabatization procedure reveals unambiguously that this minimum correlates to the $6^1[\text{DPA}-\text{O}_2]$ ionic state, and it is associated with a $\text{DPA}^+-\text{O}_2^{2-}$ (peroxide) conformation.

When the orientation deviates from $C_{\alpha}-O-O=180^\circ$, ($p_{C\alpha}$) and ($\pi_{O_2}^*$) orbitals mix, non-adiabatic couplings get stronger, and the degeneracy between $1-2^1[\text{DPA}-\text{O}_2]$ and $4-5^1[\text{DPA}-\text{O}_2]$ breaks again. It is worth noticing that with the increasing strength of the non-adiabatic couplings, the adiabatic picture deviates progressively from the diabatic view, implying that couplings affect several states simultaneously.

Using the diabatic representation, we can establish that the peroxide minimum is associated with $\text{DPA}^+-\text{O}_2^{2-}$, as expected. The diabaticization also permits us to assign the first minimum of the first singlet state, around the MECP, to the well associated with a $\text{DPA}-\text{O}_2^-$ state. This finding is confirmed by the coefficients of the CI vector displayed in Figure S2 (in the Supporting Information).

The mechanism that emerges from these results is the following: O₂ addition starts with a first charge transfer between butenthioate and O₂, leading to the formation of the superoxide anion. This charge transfer is concomitant with intersystem crossing and is therefore mediated by the strength of SOC in this region. Following this process, a second charge transfer step takes place, between two singlet states, and leads to the formation of the peroxide.

In the above discussion we have assumed that the electron is transferred “at once”. However, this classical picture is not fully correct because, although the dominant character in the well is the one of the superoxide, both $\text{DPA}-\text{O}_2^-$ and DPA^+-O_2 states are coupled and strongly mixed. It is therefore more accurate to discuss about the transference of electronic density. To account for this effect, Figure S3 (in the Supporting Information) shows the evolution of the partial charge, which is accumulated on the O₂ moiety as a function of $r_{C_{\alpha}O}$. As can be observed, spin flipping is associated with an appreciable electron density transfer from butenthioate to O₂, although part of the electronic density had been already transferred to O₂ before the intersystem crossing.

The nature of the two states involved in the spin-change process has important implications for the feasibility of the process. According to El-Sayed rules, if the change of spin is associated with a change in the orbital angular momentum (or electronic configuration), the spin-orbit coupling would be strong, and the rate coefficient of the spin-forbidden process would be large,^[61] even if there is only a small change in electronic density.^[62] The values of the SOC between the ground triplet state and the six singlet states are shown in Figure 5. At

large $r_{C_{\alpha}O}$ distances, only the SOC with the $3^1[\text{DPA}-\text{O}_2]$ ($^1\Sigma_g^+$ of O₂) is large, as was observed for isolated O₂.^[63,64] When O₂ and butenthioate approach, the value of the SOC between the first singlet and triplet states increases smoothly with the energy, which could be explained by the transfer of electronic density from butenthioate to O₂ (and the higher weight of the O₂ states in the CI vector of the first singlet state, as shown in Figure S2 in the Supporting Information). The SOC reaches a plateau value of 75 cm^{-1} in the region of the MECP, in accordance with the estimated value of 76.5 cm^{-1} based on O₂ spectroscopic assignments.^[31,33] This value of SOC is strong enough to promote intersystem crossing, even in the absence of any metal cofactor.

Larger SOC values are typically associated with the presence of heavy atoms. To check the role of the S-CH₃ group, SOC values were calculated by replacing S with O. As a result, similar values of SOC were obtained, concluding that S does not influence the SOC, and that the values of SOC obtained for this system should be similar for other cofactorless spin-forbidden reactions between O₂ and enolates.

To get more insight into the geometry of the crossing seam (the region in which singlet and triplet states are degenerate), Figure 6 displays energy contour plots for the approach of O₂ to butenthioate as a function of both $r_{C_{\alpha}O}$ and $\varphi(C_{\beta}-C_{\alpha}-O-O)$, the dihedral angle between C_β-C_α-O-O for the first singlet and triplet states (C_β is defined in Figure 2). For the triplet state, the energy barely depends on $\varphi(C_{\beta}-C_{\alpha}-O-O)$, and the curve is purely repulsive (as expected from Figure 3). On the other hand, the PES of the first singlet state is considerably anisotropic. The first well, that associated to $\text{DPA}-\text{O}_2^-$, is located at $r_{C_{\alpha}O}=2.1 \text{ \AA}$, and $\varphi(C_{\beta}-C_{\alpha}-O-O)\approx 210^\circ$. A second shallower minimum is found at $\varphi(C_{\beta}-C_{\alpha}-O-O)\approx 60^\circ$, when the O₂ is closer to S than to the C=C double bond. The absolute minimum is associated with the $\text{DPA}^+-\text{O}_2^{2-}$ configuration, and is reached at

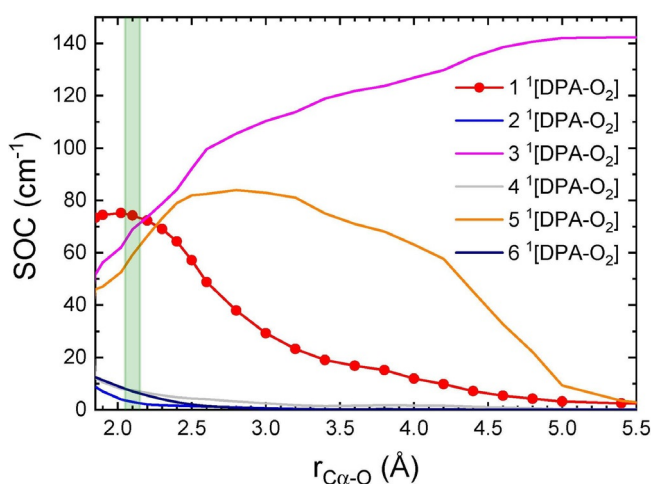


Figure 5. Values of the Spin-Orbit Coupling (SOC) between the ground triplet state and the six singlets involved in the oxidation of DPA-CoA as a function of the $C_{\alpha}-O$ distance. The values of the SOC between the ground singlet and triplet states are shown as red, closed circles. The region in which the MECP was found is shaded in green. Calculations carried out at the MRCI(4,3)/6-31+G(d,p) level of theory.

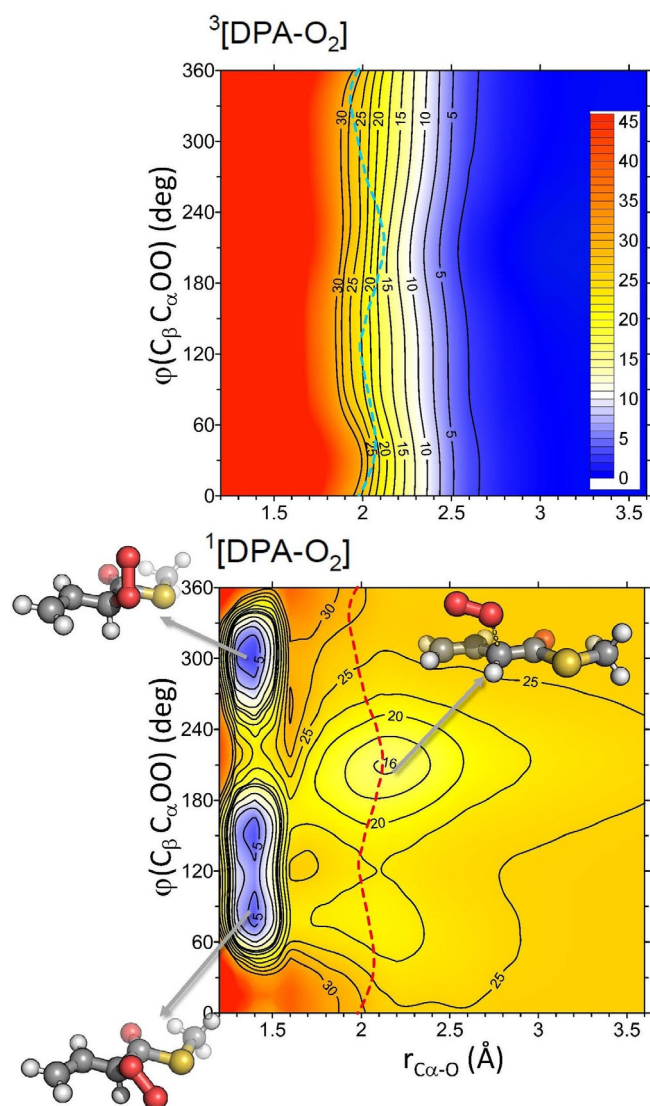


Figure 6. 3D contour map of the energy profile for the addition of O₂ to S-methyl-but-3-enethioate as a function of C_α-O distance and the C_β-C_α-O-O dihedral angle. Top panel: Lowest triplet state. Bottom panel: Lowest singlet state. The crossing seam is shown as a dashed line on top of the contour maps. The geometry of the MECP and two of the peroxide minima are shown. Energies are in kcal mol⁻¹. Calculations carried out at the MRCI(4,3)/6-31 + G(d,p) level of theory.

$r_{C_{\alpha}O} = 1.4 \text{ \AA}$ for three different values of $\varphi(C_{\beta}-C_{\alpha}-O-O)$. The crossing seam is represented by the dashed line on top of the contour maps. Regardless of the value of $\varphi(C_{\beta}-C_{\alpha}-O-O)$, the seam is found for $2 \text{ \AA} < r_{C_{\alpha}O} < 2.1 \text{ \AA}$, where the triplet PES crosses the singlet PES at the DPA-O₂⁻ minimum.

The geometries of the MECP and two of the absolute minima are also depicted in Figure 6. The carbon scaffold of butenethioate was restrained to be planar and, at the MECP, the bond between H and C_α nearly lies in the butenethioate plane, suggesting that C_α still presents a sp² hybridization, and that the aromatic cloud is not yet perturbed by the O₂ approach. The O-O distance is 1.29 Å, closer to that observed for O₂⁻ (1.345 Å) than for O₂ (1.215 Å).^[65] At smaller values of $r_{C_{\alpha}O}$, the

hydrogen progressively folds out of the plane while the C_αO bond is formed, as expected for a sp³ hybridization of C_α.

To study the effect of increasing considerably the number of active orbitals, we recalculated the energy profile for the addition of O₂ to butenethioate by using the Semistochastic Heat-Bath Configuration Interaction (SHCI) method.^[66–68] SHCI is a recently developed semistochastic method, which permits us to circumvent the limitation in the number of orbitals of the active space, and can account for dynamic correlations by perturbation theory. For the SHCI calculations, the active space was formed by 23 orbitals, including the p_z orbitals of the sp² C and the 2p and 3d orbitals of O₂ (the 23 orbitals are shown in Figure S4 in the Supporting Information). The results using SHCI are shown in Figure 7 and are in qualitative agreement with the MRCI calculations, with the main differences being the much lower barrier between the superoxide and peroxide region, and the larger stability of the peroxide. The energy height of the MECP, however, is very similar to that obtained by using MRCI. In Figure 7, we also show the SHCI results obtained when the 3d orbitals of O₂ were not included. In this case, there is a huge barrier that prevents intersystem crossing. It clearly shows that the SHCI results strongly depend on the number of orbitals of the active space, which let us believe that the MRCI barrier between peroxide and superoxide would be significantly smaller if calculations could be carried out including a very large active space that also includes the 3d orbitals of O₂.

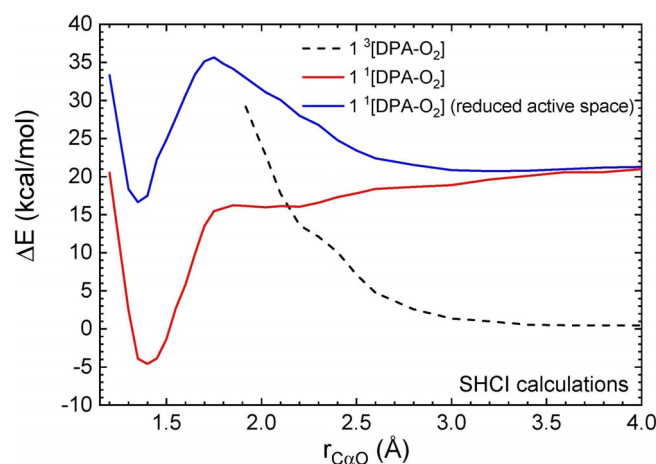


Figure 7. Energy profile for the addition of O₂ to S-methyl-but-3-enethioate as a function of the C_α-O distance by using SHCI method, a 6-31 + G(d,p) basis set, and a (18,23) active space. The solid blue curve shows the results obtained for the singlet state when the 3d orbitals of O₂ have been excluded from the active site.

2.2. Reaction between O₂ and DPA-CoA in DpgC

Once we have described the nature of the electronic states involved in the process, and how intersystem crossing takes place in our model system, we studied the addition of O₂ to DPA-CoA catalyzed by DpgC, which is likely to be the rate-limiting step for the formation of DPGX (Figure 1) using QM/MM calculations. Owing to the larger system size of the QM region

(see bottom panel of Figure 2), we cannot go beyond DFT calculations. This impedes the calculation of all the relevant electronic states, but allows us to determine the role of the protein environment and to study the possible proton transfer mechanisms.

To analyze the mechanism of this reaction, we carried out restrained geometry optimizations by using $\Delta E^{\text{Sing/Trip}}$, the energy difference between the first singlet and triplet states, as a reaction coordinate. This selection has two important advantages: i) the MECP is localized exactly at $\Delta E^{\text{Sing/Trip}} = 0$, and ii) the evolution from the triplet to the singlet state is smoother.

The QM/MM energy profile for the O_2 addition to DPA-CoA is shown in Figure 8. QM/MM results show that both the triplet and the singlet states show only one minimum, which is very broad for the case of the singlet state. Interestingly, we observe that the MECP is the only barrier for the reaction, and accordingly should act as the dynamic bottleneck. MECP lies $16.7 \text{ kcal mol}^{-1}$ above the minimum for the triplet state, in very good agreement with the value obtained for the O_2 -butenthioate model. The formation of the peroxide is driven by an exothermicity of $9.9 \text{ kcal mol}^{-1}$.

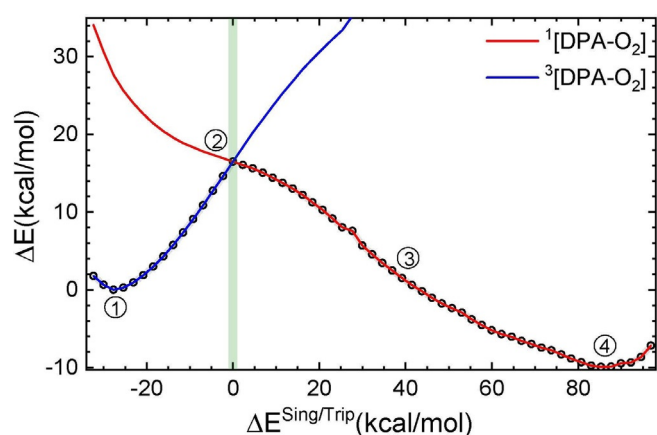


Figure 8. QM/MM energy profile for the addition of O_2 to the DPA model as a function of the energy difference between the ground singlet and triplet states. $\Delta E^{\text{Sing/Trip}} = 0$ corresponds to the MECP, and it is shaded in green. The framed numbers highlight the values of $\Delta E^{\text{Sing/Trip}}$ corresponding to the structures shown in Figure 9. Calculations carried out at the B3LYP/6-31 + G(d,p) level of theory.

To investigate the effect of the protein in the QM/MM energy profile, we repeated the calculations using a reduced QM region, in which Ile324, Gln299, Ala319, and one of the water molecules were excluded from the QM region. The obtained results were pretty similar, except for the exothermicity, which is somewhat smaller ($5.6 \text{ kcal mol}^{-1}$), evidencing that the protein scaffold plays only a minor role in the promotion of the intersystem crossing beside the stabilization of the enolate (results are not shown).

The structures of the minimum of the triplet state, the MECP, the point at $\Delta E^{\text{Sing/Trip}} = 39 \text{ kcal mol}^{-1}$, and the minimum of the singlet state are depicted in Figure 9. The structure obtained for the triplet state minimum is very similar to the crys-

tal structure, although in this case O_2 has moved slightly closer to C_α . Indeed, the position of the two water molecules that are close to O_2 and C_α is very similar in the crystal. Regarding these water molecules, it could have been expected that one of the water molecules could protonate the triplet O_2 as was predicted for the reaction between O_2 and flavin.^[28,30,33] However, at the MECP, the proton is still on the water. In fact, the main differences between the minimum associated with the triplet and the MECP are that the hydrogen bound to C_α has moved slightly below the plane formed by the π system of DPA, and that O_2 is significantly closer to C_α . This is shown in Figure 10, where we display the evolution of the $\text{C}_\alpha\text{--O}$, and the O--O distances along the reaction path. In the figure, we also show the progress of the proton transfer, which is defined as the difference between the $r_{\text{H--OH}}$ and the $r_{\text{O--H}_2\text{O}}$ (as defined in Figure 9).

Between the triplet minimum and the MECP, $r_{\text{C}_\alpha\text{O}}$ has changed from 3.2 to 2 Å, in excellent agreement with the results obtained for the butenthioate model. Based on these results, the first triplet state is purely repulsive, so we can predict that systems for which $r_{\text{C}_\alpha\text{O}}$ is larger (smaller) at the MECP will be more (less) reactive.

After the MECP, $r_{\text{C}_\alpha\text{O}}$ decreases slowly, and only reaches its equilibrium value of 1.4 Å for $\Delta E^{\text{Sing/Trip}} > 45 \text{ kcal mol}^{-1}$. Interestingly, at $\Delta E^{\text{Sing/Trip}} = 39 \text{ kcal mol}^{-1}$, the structure resembles that obtained for the MECP with the sole exception that the $\text{C}_\alpha\text{--O}$ bond is formed. Still, the proton has not been transferred to the peroxide. This means that proton transfer occurs significantly after the peroxide is formed. Probably, that is the reason why there are not basic residues around the active site that could donate a proton to O_2 , as was observed for other cofactorless reactions.^[28,30,33] Proton transfer drifts the system away from the MECP and, even in the minimum associated with the singlet state, the proton is only shared between the peroxide and the water.

In the minimum of the singlet state, the water wire that connects the bulk to the active site has reorganized. In particular, the water molecule that connects Gln299 and C_α is reoriented to a position suitable for the extraction of the C_α proton, which would facilitate the following step in DPGX formation. This water molecule was resolved in the crystal structure and was not exchanged with the solvent throughout the MD simulations.

Combining the height of the MECP obtained in the QM/MM calculations, with the SOC obtained using our model, and plugging them in the Landau–Zener equation,^[45,50] we could estimate k_{cat} . We obtained a hopping probability of 0.06, which is equivalent to an increase of $1.6 \text{ kcal mol}^{-1}$ in the activation energy at 300 K. By using these values, we obtain a $k_{\text{cat}} = 0.23 \text{ s}^{-1}$, very close to the experimental value of 0.15 s^{-1} .^[43] The quantitative agreement between the experiment and the simulation is coincidental, considering the approximations used. However, it supports the validity of the mechanism proposed in this article for intersystem crossing and subsequent formation of the peroxide.

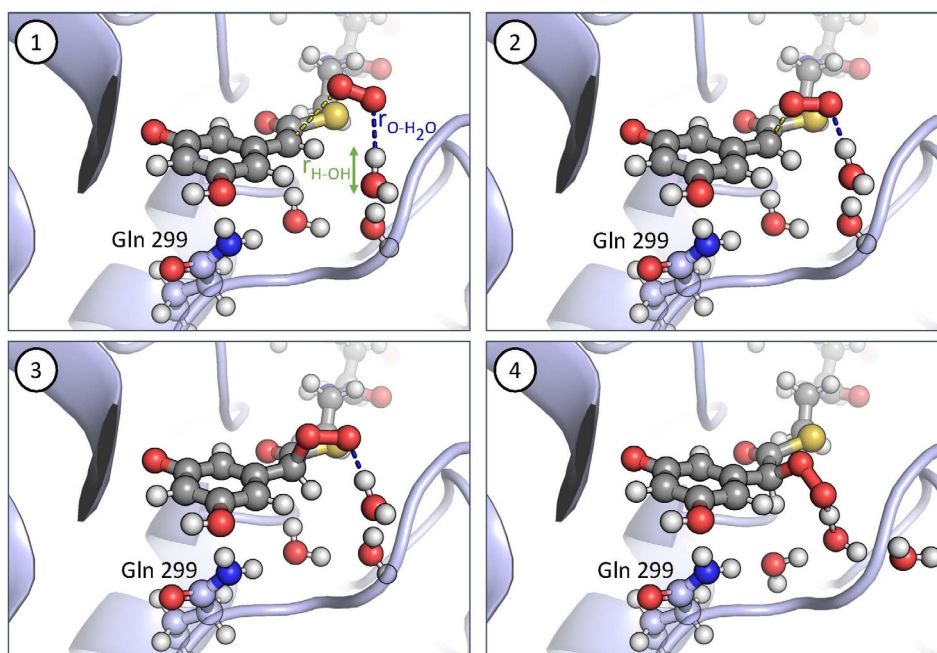


Figure 9. QM/MM structures along the $\Delta E^{\text{Sing/Trip}}$ path. 1) Structure corresponding to the optimized triplet geometry. 2) Structure of the system at the MECP. 3) Structure of the system for $\Delta E^{\text{Sing/Trip}} \approx 40 \text{ kcal mol}^{-1}$. 4) Structure of the optimized singlet structure. $C_{\alpha}-O$, $H-OH$, and $O-H_2O$ distances are shown in yellow, green, and blue. Gln299, three water molecules that are included in the QM region, DPA-CoA, and O_2 are shown atomistically.

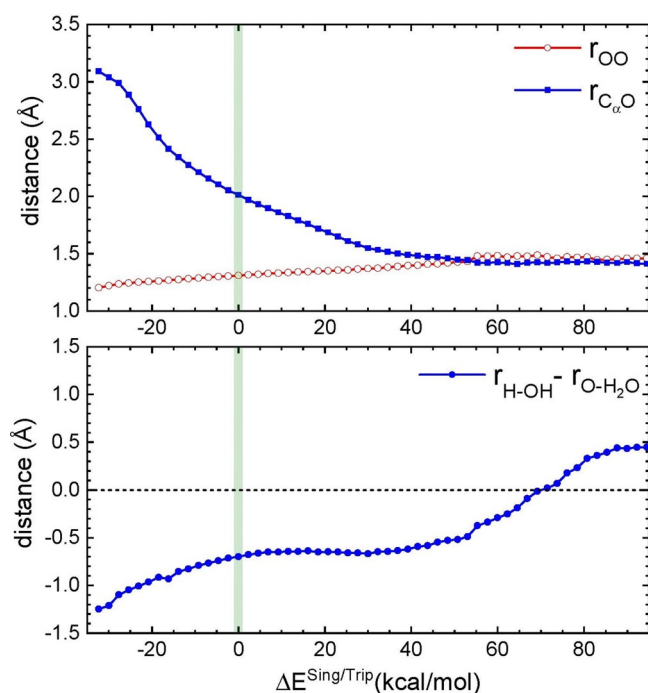


Figure 10. Evolution of the $O-O$, $C_{\alpha}-O$, and proton transfer coordinate (defined as $r_{H-OH} - r_{O-H_2O}$) along the $\Delta E^{\text{Sing/Trip}}$ path. $\Delta E^{\text{Sing/Trip}} = 0$ corresponds to the MECP, and it is shaded in green. Calculations carried out at the B3LYP/6-31+G(d,p) level of theory.

3. Conclusion

Incorporation of molecular oxygen into an organic substrate involves a change in the spin state of the system, that is, proceeds through intersystem crossing. To catalyze these reactions, enzymes typically require metal cofactors, generally iron. Intriguingly, a subgroup of oxygenases performs these reactions without the presence of any cofactor. Here, we have investigated the mechanism of this spin-forbidden process by using MRCI quantum calculations and QM/MM simulations on the reaction between DPA-CoA and O_2 catalyzed by DpgC. By using MRCI calculations, we calculated the potential energy surfaces of the nine concurrent electronic states on an equal footing, and assigned an electronic configuration to each of them through a diabaticization procedure. Our results provide evidence that, once the enolate is formed, the reaction can occur without any previous activation of O_2 and via two successive electron transfer processes. The minimum energy crossing point (MECP), which plays the role of an effective barrier for spin-forbidden processes, lies only $16.7 \text{ kcal mol}^{-1}$ above the $DPA-CoA + {}^3O_2$ asymptote, which is compatible with the experimental k_{cat} value.

Intersystem crossing occurs between the triplet state that correlates to DPA^-O_2 and the singlet state that correlates diabatically with a $DPA-O_2^-$ state, that is, it is associated to the charge transfer from DPA to O_2 to form a superoxide anion. As this change of spin is associated with a change in the electronic configuration, it complies with the El-Sayed rules, and the strength of the spin-orbit coupling is above 70 cm^{-1} , a relatively large value for a system that does not include heavy atoms.

For other spin-forbidden processes, it has been postulated that the rate for the spin-forbidden reaction is enhanced by the presence of a broad crossing region, which maximizes the cross-over probability. That is not the case for the reaction between DPA-CoA and O₂, for which this probability is maximized by the presence of a stable intermolecular complex arising from the charge-dipole interaction, which stabilizes the superoxide singlet state, lowering the energy of the MECP. For the formation of peroxide, a second charge transfer from DPA to O₂ is required.

Our QM/MM calculations reveal that the minimum energy crossing point is the main reaction bottleneck. They also suggest that protonation of O₂ takes place after the formation of the peroxide, which is already stable in its deprotonated form. This could explain the absence of basic residues in the active site that could protonate O₂ during its addition to the enolate. It is also relevant that the first molecule of the water wire that connects the active site to the bulk changes its orientation following the formation of the peroxide. In their final configuration, it could easily capture the proton, which could initiate the following step in the formation of DPGX. Finally, the comparison between the energy profiles calculated by using different QM regions suggests that although the protein environment plays an important role in the diffusion of O₂ towards the active site, and in the formation and stabilization of the enolate, it plays a passive role in the promotion of the spin-forbidden reaction. We believe that these results are general and that similar behavior should be observed for other cofactorless spin-forbidden reactions that proceed via the formation of a peroxide.

4. Experimental Section

4.1. Initial coordinates and molecular dynamics simulations

The initial structure adopted in this work is based on the crystal structure of the DpgC/DPA-NH-CoA/O₂ complex (PDB: 5KAG, 2.68 Å resolution).^[38] That crystal structure was obtained by replacing the original substrate (DPA-CoA, see Figure 1) by the stable substrate analog (DPA-NH-CoA) to prevent the reaction even in aerobic conditions. This structure corrected the crystallographic pathologies of the original crystal structure,^[36] (PDB: 2NP9) caused by a wrong assignment of the crystal lattice. In our simulations, we exchanged DPA-NH-CoA with DPA-CoA while keeping O₂ in its crystallographic position, a hydrophobic pocket less than 4 Å away from C_α of DPA, and the initial geometry was prepared by using CHARMM-GUI.^[69,70] Hydrogen coordinates were generated with standard protonation states for all titrable residues by using CHARMM,^[71] and the protein complex was placed in the center of a cubic TIP3 water box large enough to include the protein and at least 10 Å of solvent on all sides. Additionally, water molecules were randomly replaced by K⁺ and Cl[−] ions to ensure the neutralization of the system, and provide an additional concentration of 0.15 M KCl. The system was subject to a classical MD equilibration of 10 ns at constant temperature (*T* = 303.15 K) and pressure (1 atm) by using NAMD (<http://www.ks.uiuc.edu/Research/namd/>),^[72] and CHARMM-36m forcefield.^[73,74] The Particle Mesh Ewald method was used for the electrostatics of the periodic boundary conditions.^[75] A time step of 2 fs was used with the ShakeH algorithm.^[76] To avoid O₂ diffusion outside the active site, the positions

of O₂ and the non-hydrogen atoms of DpgC were fixed throughout the equilibration. The root-mean-square deviation (RMSD) with respect to the initial conformation was calculated for all the non-hydrogen protein atoms, which were not located in any turn or coil (see Figure S5 in the Supporting Information) showing that the structure was well converged after 4 ns, with an average RMSD of 1.56 Å.

To select an initial frame for the subsequent QM/MM calculations, we calculated the occupancy of water molecules in the active site by following the same procedure used in ref. [77]. Considering the last 5 ns of the MD trajectory, we calculated the averaged position of the water molecules within 7 Å of the O₂ molecule, and selected a frame for which the position of the waters was close to their average position. Compared with the initial structure, the two water molecules crystallized around the active site kept their positions (although one of them exchanged with the solvent during the simulation time), whereas the active site recruited a third water molecule and a water wire connecting the active site and the bulk was formed. A representation of the initial structure for QM/MM calculations is shown in Figure S6 (in the Supporting Information).

4.2. QM/MM calculations

Once the system was classically equilibrated and the initial frame was selected, it was trimmed to a sphere of 26 Å centered at the S atom of DPA-CoA. Atoms further away than 18 Å from the S atom of DPA-CoA were kept frozen during all the QM/MM calculations. A full electrostatic embedding^[78] was adopted in all the calculations, using hydrogen link atoms to treat the QM/MM boundaries, maintaining the effect of the broken chemical bond between the QM and the MM region. QM/MM calculations were run coupling Q-Chem v5.2^[79] and CHARMM.^[71] QM calculations were carried out at the B3LYP/6-31G + (d,p) DFT level of theory for the ground singlet and triplet states. The D3 version of Grimme's dispersion correction was used.^[80,81]

Initial optimization of the reactants (DPA[−] + O₂) and products (DPA-OO[−]) confirmed that the reactants were only stable in their triplet state, whereas the peroxide was only stable as a singlet. It also revealed that the protein prevents the bending of the DPA-OO[−] peroxide, which remains almost planar. The reaction path was scanned by performing restrained geometry optimizations along a reaction coordinate, which was defined as the energy difference between the ground triplet and singlet state, $\Delta E^{\text{Sing/Trip}} = E^{\text{triplet}} - E^{\text{singlet}}$, so the crossing seam is found for $\Delta E^{\text{Sing/Trip}} = 0$. To impose that restraint, we added the following harmonic term to the potential:

$$V = V_0 + \frac{1}{2} K \left(\Delta E^{\text{Sing/Trip}} - \Delta E_i^{\text{Sing/Trip}} \right)^2 \quad (1)$$

where V_0 is the minimum value between E^{triplet} and E^{singlet} , K is the spring constant (the value of which was set to 500 eV^{−1}), $\Delta E_i^{\text{Sing/Trip}}$ is the reference value of $\Delta E^{\text{Sing/Trip}}$ to which the potential is biased, and V is the resulting biased potential energy. More details for the MECP search algorithm for QM/MM calculations are given in the extended methods section in the Supporting Information.

4.3. Multireference calculations

4.3.1. MRCI calculations

The reaction between S-methyl-but-3-enethioate and O₂ was simulated by using the multiconfiguration reference internally contracted configuration interaction method (MRCI), which captures the static correlation by the selection of all the electronic configura-

tions that can be generated by the distribution of the electrons considered in the orbitals that belong to the active space. The dynamic correlation is then captured by optimizing variationally the best solution of a linear combination of all the electronic configurations generated by simple and double electronic excitations from all the reference configurations generated by the active space towards the orbitals of the virtual space. To account for the quadruple excitations and reduce the size-consistency error inherent to the method, Davidson correction has been applied.

Calculations were carried out by using the MOLPRO package,^[82] and a 6-31G+(d,p) basis set. For efficiency reasons, the geometries were first optimized at a DFT level using the B3LYP functional, and the D3 version of Grimme's dispersion correction, following by single-point MRCI calculations. This procedure is similar to the approach employed to shed light on the reactivity of metallic clusters.^[83,84] The only difference is that here, the MECF was also optimized.

The procedure applied here is the following: first, the MECF between triplet and singlet was optimized at the DFT level. Then, starting from that structure, the geometry was relaxed for different values of the reaction coordinate, which we assimilated to $C_{\alpha}-O$ distance ($r_{C_{\alpha}O}$). $r_{C_{\alpha}O}$ was sampled from 1.2 to 7 Å, and taking as a reference value the $r_{C_{\alpha}O}$ distance at the MECF, for larger (smaller) $r_{C_{\alpha}O}$ values the geometry was optimized in its triplet (singlet) state. Furthermore, it allows a double-check to confirm that the electronic wavefunctions converge to the correct state, which is particularly tedious in the case of O_2 .

The energy of the geometries obtained at DFT level was recalculated by using the internally contracted version of MRCI available in MOLPRO.^[85,86] Within this method, SOC can be calculated rigorously by using the Breit–Pauli spin-orbit operator. The first step required before running MRCI calculations is the definition of the initial wavefunction. In this work, the guess wavefunction was generated through a state-average CASSCF calculation^[87,88] including in the active space four electrons in three orbitals: the two π_g^* orbitals of O_2 and the HOMO of *S*-methyl-but-3-enethioate. In the state-average CASSCF wavefunction, one triplet and five singlet states were treated on an equal footing. This approach leads to stable optimized orbitals and configurations along the reaction path, which is required to obtain accurate and meaningful potential energy curves at a multireference level, which is hard to achieve for relatively large polyatomic systems. For the subsequent MRCI calculation, we computed the nine states (three triplet and six singlet states) and the SOC between them. Under these conditions, the electronic wavefunctions of the triplet (singlets) states are constructed over a linear combination of about 6×10^6 (30×10^6) contracted configurations.

To check how the results change when a larger active space is used, we compared the calculations using the (4,3) active space with those including ten electrons in seven orbitals. The relative energies for the two active spaces are similar (see Figure S7 in the Supporting Information) with MRCI(10,7) predicting a shallower peroxide well for the first singlet state. In fact, PESs are more sensitive to the number of states included in the MRCI treatment.

Owing to the relatively large number of coupled electronic states, avoided crossings in the adiabatic potential energy curves may impair their interpretation. In these cases, it may be useful to calculate the states in the diabatic representation, which also sheds light on the nature of the electronic states involved. As we are considering singlet and triplet states simultaneously, an automatic diabaticization algorithm cannot be employed, so we applied the diabaticization model based on the geometrically based approach,^[89] which has been successfully applied to diabaticize highly entangled

excited states of alkyl radicals.^[90–92] It consists of a geometric approach where successive 2×2 diabaticizations are performed between all coupled states iteratively. The validity of the approach was confirmed by the comparison between our diabatic curves and those obtained by using the default MOLPRO algorithm for the three triplet states (see Figure S8 in the Supporting Information). More details about the diabaticization method can be found in the extended methods section in the Supporting Information.

4.3.2. Semistochastic heat-bath configuration interaction

The reaction between *S*-methyl-but-3-enethioate and O_2 was also simulated by using the recent Semistochastic Heat-Bath Configuration Interaction (SHCI) method,^[66–68] which permits us to overcome the critical limitation to the maximum number of orbitals that can be included in a multiconfigurational self-consistent field (MCSCF) calculation (depending on the system, up to 14–18 orbitals).

The SHCI algorithm consists of two stages. First, a variational wavefunction is computed by using a set of iteratively selected determinants. These determinants are stochastically sampled, and they are filtered under a chosen energy value threshold (ϵ_1). The algorithm increases the number of determinants included in the set until the wavefunction has constant and stable energy. In the second stage, the second-order correction to the variational energy is computed by using the multireference Epstein–Nesbet perturbation theory.

The resulting energy is considered to be Full-Configuration Interaction (FCI) for a given active space and basis set. To obtain the FCI energy, several calculations are computed for different values of ϵ_1 and they are extrapolated to $\epsilon_1 \rightarrow 0$. All the SHCI calculations were done in the open-source Python software package PySCF,^[93] interfaced with the fast version of the algorithm, Arrow.^[94]

Acknowledgements

The authors thank Prof. Enrique Vardasco for his support and help with the calculations. Funding by the Spanish Ministry of Science and Innovation (grants PGC2018-096444-B-I00, and FIS2017-83473-C2-2-P) is also acknowledged. The authors acknowledge funding by Fundación Salamanca City of Culture and Knowledge (Programme for Attracting Scientific Talent to Salamanca).

Conflict of interest

The authors declare no conflict of interest.

Keywords: charge transfer mechanism · cofactor-independent enzymes · oxidation · oxygenases · spin-forbidden transitions

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Manuscript received: June 22, 2020

Accepted manuscript online: September 25, 2020

Version of record online: December 14, 2020

Chemistry–A European Journal

Supporting Information

DpgC-Catalyzed Peroxidation of 3,5-Dihydroxyphenylacetyl-CoA (DPA-CoA): Insights into the Spin-Forbidden Transition and Charge Transfer Mechanisms**

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I. ADDITIONAL FIGURES

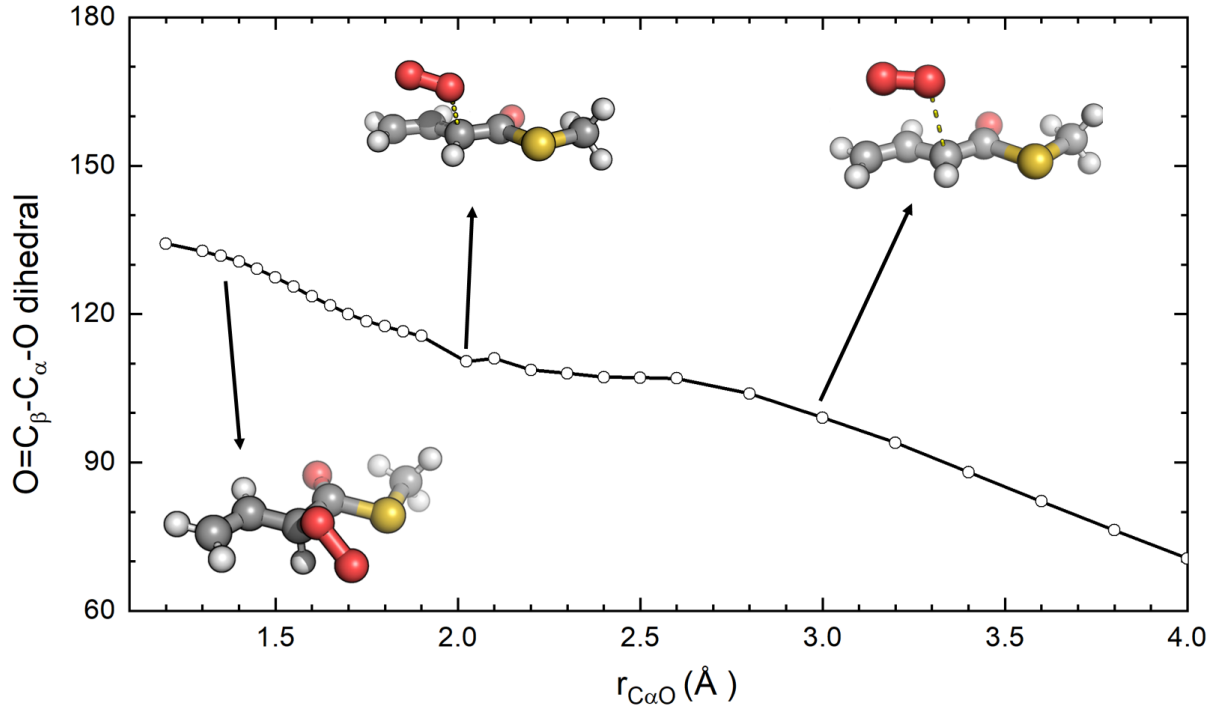


Fig. S 1. Evolution of the O=C β -C α -O dihedral angle as a function of $r_{C\alpha O}$. Since all the C atoms are constrained to be kept in the same plane, for a given $r_{C\alpha O}$, O=C β -C α -O dihedral angle does not change with C α -O-O angle for the geometries shown in Fig. 4.

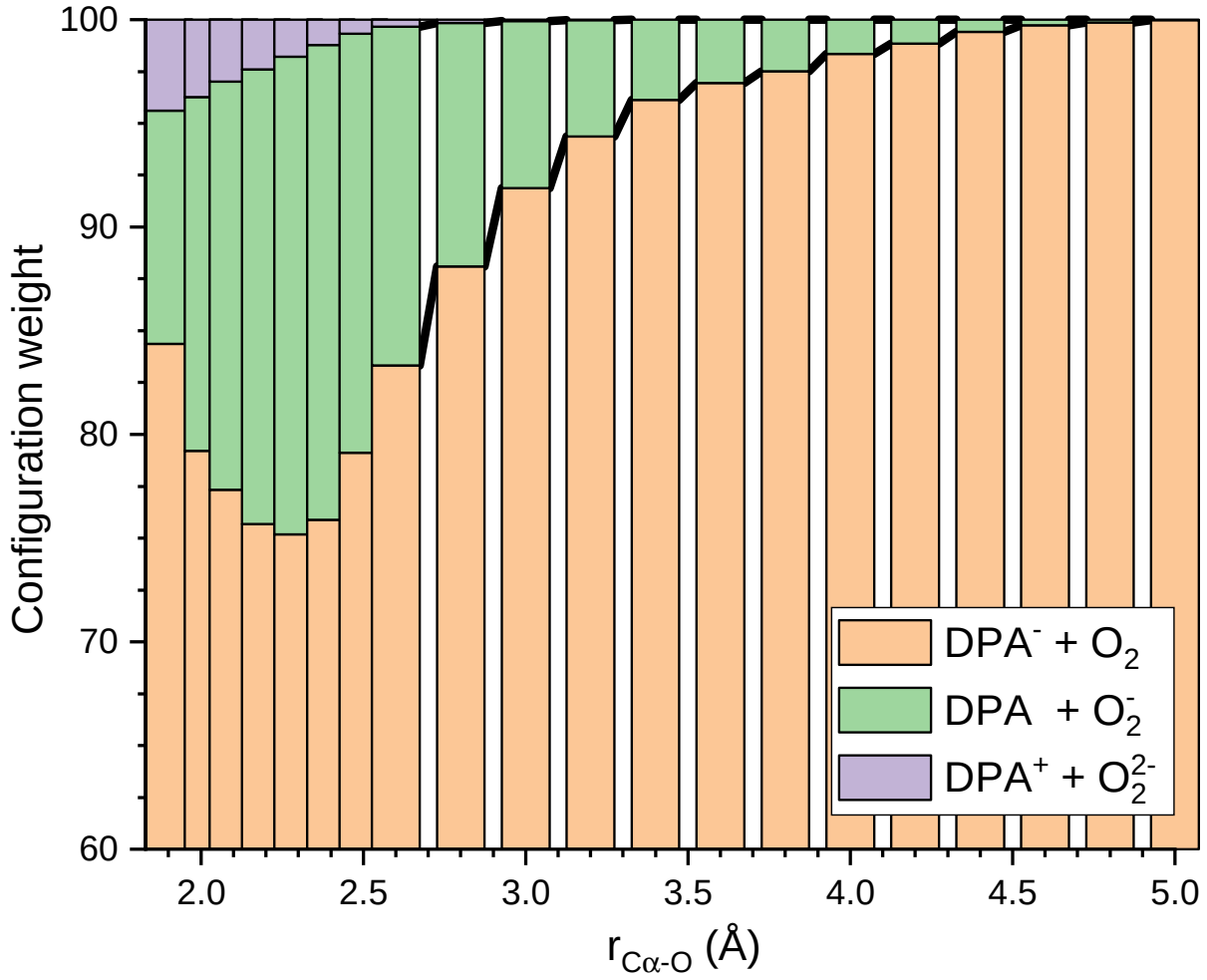


Fig. S 2. Coefficients of the CI-vector for the ground singlet state as a function of the C_{α} -O distance. Unlike the diabaticization procedure, the coefficients of the CI vector do not account for the change of the orbitals throughout the O_2 addition to the enolate, and do not provide accurate results for small $r_{C_{\alpha}O}$. Altogether, the coefficients of the CI vector and the diabaticization confirm that the first well observed for the ground singlet state is associated to a DPA- O_2^- conformation. Calculations carried out at the MRCI(4,3)/6-31+G(d,p) level of theory.

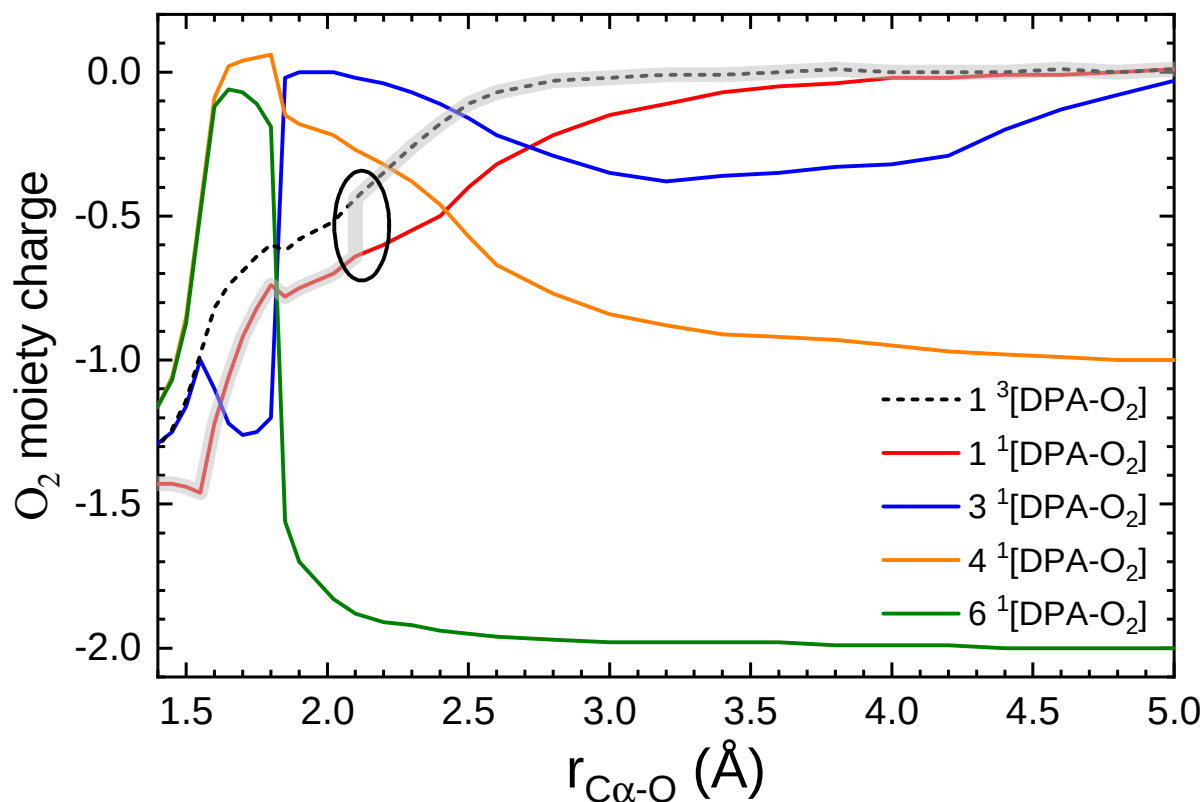


Fig. S 3. Mulliken partial charges on the O₂ moiety as a function of the C_α-O distance for the adiabatic states considered. Results for the second and fifth singlet have not been included for the sake of clarity. Results for the most stable state (singlet or triplet) are shaded. Calculations carried out at the MRCI(4,3)/6-31+G(d,p) level of theory.

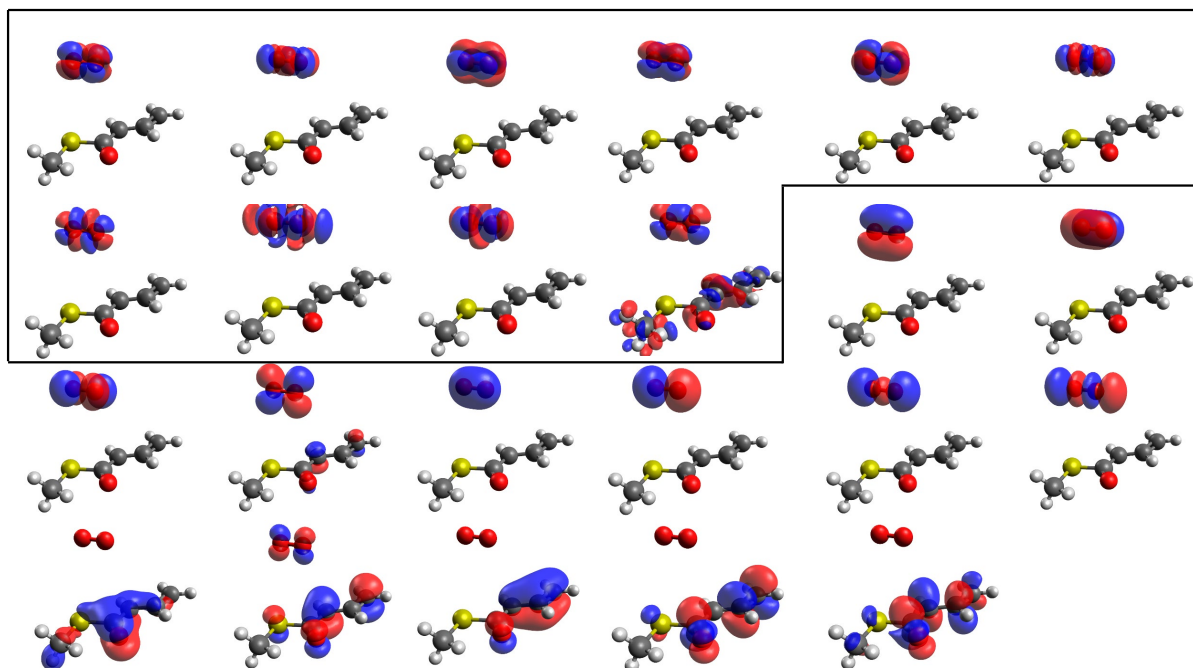


Fig. S 4. Representation of the 23 orbitals that were included in the SHCI calculation. The 10 d orbitals for O_2 are surrounded by a black frame.

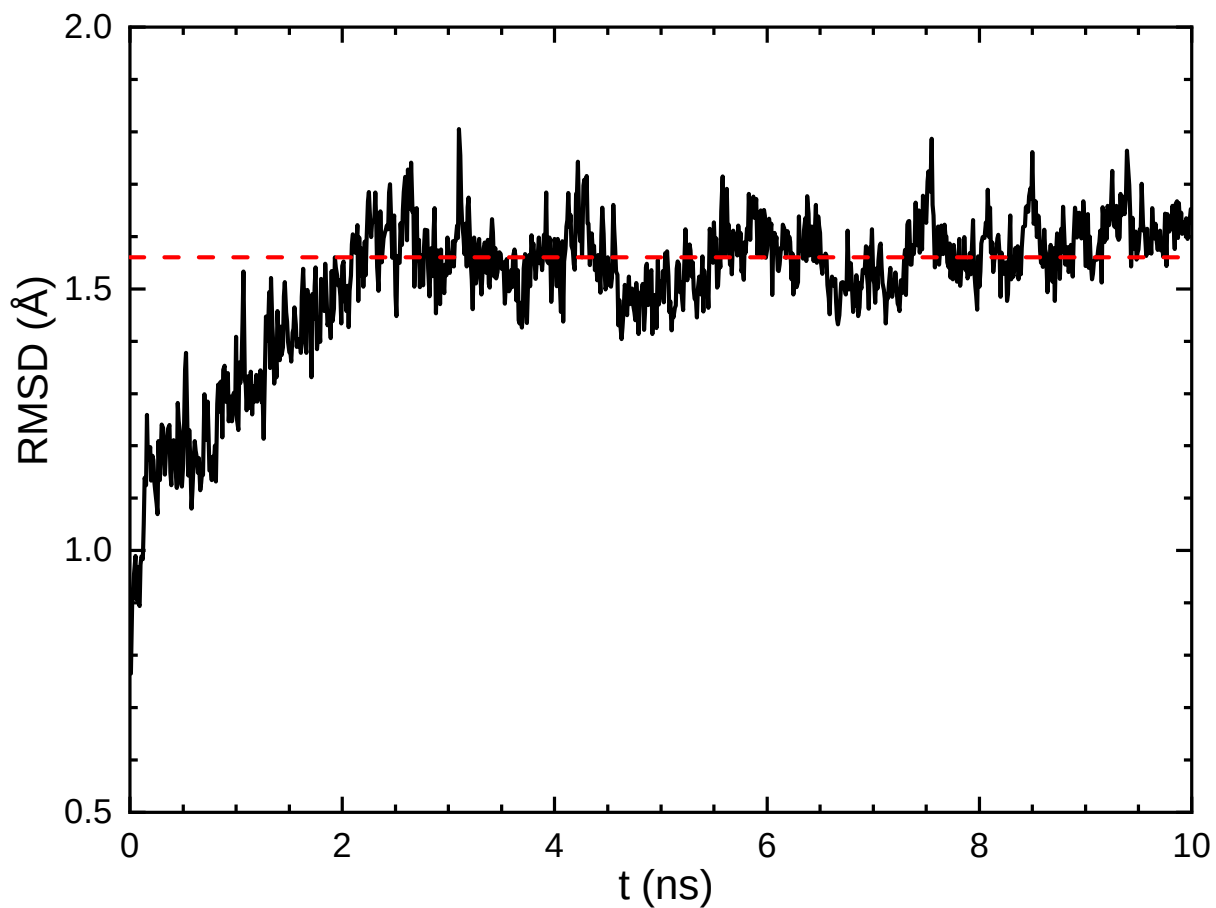


Fig. S 5. Root-mean-square deviation (RMSD) values as a function of the MD equilibration simulation with respect to the initial conformation based on PDB structure 5KAG. For the calculation of the RMSD we consider all the non-hydrogen atoms of the protein that were not located in any turn or coil. The average RMSD is shown as a dashed red line.

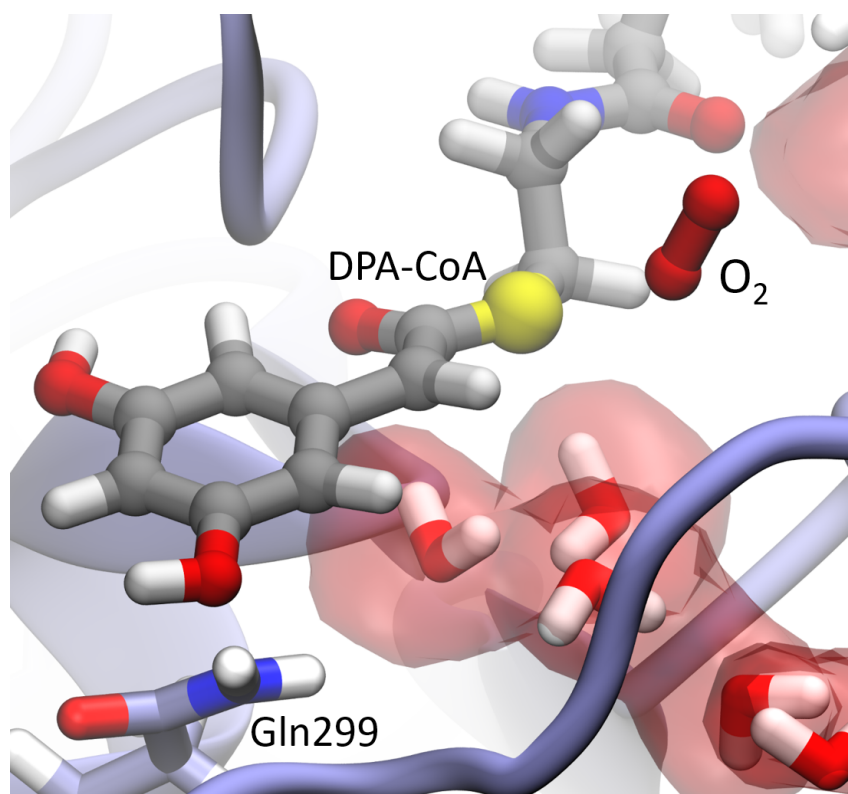


Fig. S 6. Occupancy of water molecules in the neighborhood of DPA-CoA from MD simulation. The occupancy is displayed as a red isosurface with an isovalue of 0.3. Instantaneous positions of water molecules in the frame that was considered as initial state for the further QM calculations are shown atomistically. DPA, O₂, and Gln299 are also shown atomistically.

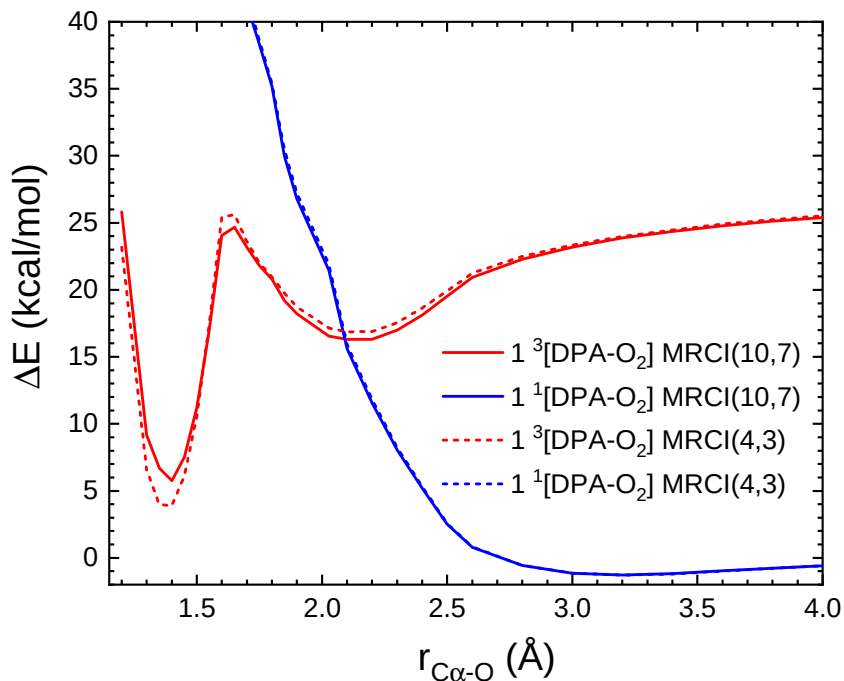


Fig. S 7. MRCI Energy profiles for the addition of O_2 to S-Methyl-but-3-enethioate as a function of the $\text{C}_\alpha\text{-O}$ distance calculated using two different active spaces (4,3) and (10,7). The results are similar, with MRCI(10,7) predicting a shallower minimum for the singlet state. These energy profiles, however, are very sensitive to the number of states included in the calculations. Those shown here have been calculated including only one singlet and one triplet state in the wavefunction, resulting in higher barriers. MRCI results shown in Figs. 3-6 include all the 9 states of the MRCI(4,3), and are accordingly more accurate.

II. EXTENDED METHODS

A. Diabatization procedure

Throughout this work we have used the diabatization model proposed in Ref. 1. The algorithm is based on a geometric approach where successive 2x2 diabatizations are performed iteratively until all the states are uncoupled.

Briefly, each 2x2 diabatization is done by defining a diabatization coordinate range, where the coupled states are allowed to cross. Outside of this interval, diabatic and adiabatic curves are assumed to be identical. The 2x2 diabatization is performed in three stages. In a first stage, one of the diabatic curves is generated by interpolation between the two adiabatic curves, while the energy of the second diabatic curve is assigned so the total energy is conserved. In a second stage, the optimized mixing angle is calculated based on the preliminary diabatic curves calculated in the first stage. In the third stage, the diabatic curves are calculated from the optimized mixing angle.

To calculate the diabatic curves shown in the right panels of Fig.4 of the main text, the procedure to obtain the diabatic states D1-D6 was the following (where A1-A6 correspond to the six singlet adiabatic states, and D1-D6 to the corresponding singlet diabatic states):

- A5 and A6 were diabatized in the $r_{C_\alpha O} = 1.85\text{-}2.4 \text{ \AA}$ range to obtain D5 and D6
- D5 and A4 were diabatized in the $r_{C_\alpha O} = 1.8\text{-}1.85 \text{ \AA}$ range to obtain D4 and D5
- D4 and A3 were diabatized in the $r_{C_\alpha O} = 1.6\text{-}1.9 \text{ \AA}$ range to obtain D3 and D4
- D3 and A2 were diabatized in the $r_{C_\alpha O} = 1.55\text{-}2 \text{ \AA}$ range to obtain D2 and D3
- D2 and A1 were diabatized in the $r_{C_\alpha O} = 1.55\text{-}2 \text{ \AA}$ range to obtain D1 and D2

At this stage, D1 corresponds to the definitive diabatic state associated to the peroxide potential energy curve. The remaining diabatic states D2 to D6 are still coupled and need to be untangled. This was done through the following sequence:

- D4 and D6 were diabatized in the $r_{C_\alpha O} = 2.6\text{-}5.2 \text{ \AA}$ range to obtain the updated D4 and D6
- D2 and D5 were diabatized in the $r_{C_\alpha O} = 2.1\text{-}3.6 \text{ \AA}$ range to obtain the updated D2 and D5

- D3 and D4 were diabaticized in the $r_{C\alpha O} = 1.9\text{-}3.2$ Å range to obtain the updated D3 and D4

For the three triplet, only two states are coupled since one component of the Δ state should not couple with either its counterpart or the ground state. The diabaticization was performed in the $ir_{C\alpha O} = 1.7\text{-}3$ Å range. To show the reliability of the applied procedure, in Fig. S8 we compare the diabatic curves obtained using this method, and the quasidiabatization method implemented in MOLPRO². As can be observed, both set of diabatic curves are very similar.

B. MECP calculation for QM/MM calculations

To calculate the reaction path along the energy difference between the singlet and the triplet states ($\Delta E^{\text{Sing/Trip}} = E^{\text{triplet}} - E^{\text{singlet}}$) we coupled Q-Chem v5.2³ and CHARMM⁴ using the standard CHARMM-QChem interface.⁵ To drive the minimizations, we used the standard minimization algorithms implemented in CHARMM.

To restrain the system in a configuration given by a defined value of $\Delta E_i^{\text{Sing/Trip}}$ we added the following harmonic term to the potential,

$$V_{\text{bias}} = \frac{1}{2}K \left(\Delta E^{\text{Sing/Trip}} - \Delta E_i^{\text{Sing/Trip}} \right)^2 \quad (1)$$

where K is the spring constant. The use of a harmonic biased term is common in molecular dynamics (MD) and QM/MM calculations for constrained optimizations and for the calculation of reaction profiles. In the present case, instead of using a geometrical restrain, the system is pulled towards a target value of the energy difference between singlet and triplet states. The value of K was set to 500 eV^{-1} . Test results using different values of K in the $100 \text{ eV}^{-1}\text{-}1000 \text{ eV}^{-1}$ range provided similar results. In eq. 1 $\Delta E^{\text{Sing/Trip}}$ is the energy difference between the first singlet and triplet states for the actual geometry, and $\Delta E_i^{\text{Sing/Trip}}$ is the value to which the system is restrained.

For MECP search, $\Delta E_i^{\text{Sing/Trip}} = 0$, and eq. 1 reduces to:

$$V_{\text{bias}} = \frac{1}{2}K \left| \Delta E^{\text{Sing/Trip}} \right|^2 \quad (2)$$

and the minimum of the biased potential is located in the crossing seam. The energy gradient of the biased potential, $\mathbf{G}_{\text{bias}}$, is then given by:

$$\mathbf{G}_{\text{bias}} = K \Delta E^{\text{Sing/Trip}} (\mathbf{G}_{\text{s}} - \mathbf{G}_{\text{t}}) \quad (3)$$

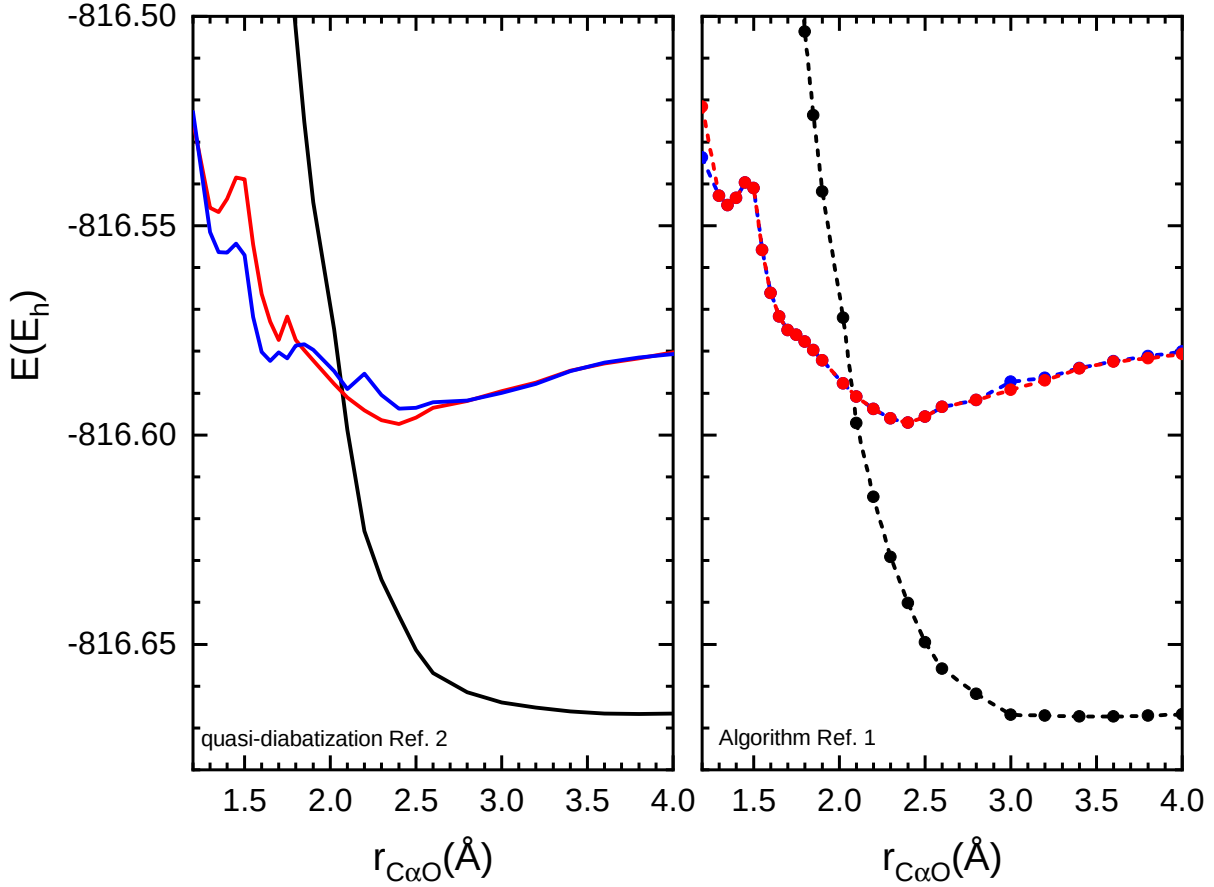


Fig. S 8. Diabatic curves for the three triplet states for the addition of O_2 to S-Methyl-but-3-enethioate as a function of the C_α -O distance and C_α -O-O=110°. Calculations carried out at the MRCI(4,3)/6-31+G(d,p) level of theory. The left panel corresponds to the diabatic curves obtained using the quasi-diabatization procedure implemented in MOLPRO and described in Ref. 2. The diabatic curves shown in the right panel correspond to those calculated using the algorithm described in Ref. 1.

where $\mathbf{G}_s$ and $\mathbf{G}_t$ are the gradients for the singlet and triplet states, respectively. Hence, the effective gradient ($\mathbf{G}_{\text{eff}}$) is given by:

$$\mathbf{G}_{\text{eff}} = \mathbf{G}_{\text{ground}} + \mathbf{G}_{\text{bias}} \quad (4)$$

where $\mathbf{G}_{\text{ground}} = \mathbf{G}_s$ if at the present geometry the singlet state is lower in energy, and $\mathbf{G}_{\text{ground}} = \mathbf{G}_t$ if the triplet state is lower in energy. The present algorithm for MECP location differs from those used in Ref. 6,7, where two gradients, one perpendicular (such as $\mathbf{G}_{\text{bias}}$) and another parallel to the seam hypersurface, combine to form $\mathbf{G}_{\text{eff}}$.

Due to the high dimensionality of the system, the Potential Energy Surface may have many local MECP. To ensure that we obtained the absolute MECP that couples reactants with products (instead of a local MECP), we started the optimization from several initial structures. Furthermore, we carried out restrained geometry optimizations along a small range of $\Delta E^{\text{Sing/Trip}}$, allowing further relaxation of the MECP structure.

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5.3 Mechanism of peroxidation of 2-methyl-3,4-dihydro-1-naphthol

In the final phase of this research project, we delved into the spin-forbidden oxygenation mechanisms of a neutral molecule, namely an enol instead of an enolate. To do this, we picked an organic synthesis paper that explained the experimental process of adding atmospheric oxygen to an organic substrate (Ref. 175). While Riahi et al. provided electronic structure calculations, they lacked an appropriate methodology for studying multireferential systems. They did not account for spin-orbit coupling, and more crucially, they overlooked a second potential reaction path. Besides, the MECP structures are not obtained.

Our calculations led us to a different conclusion from the original paper.¹⁷⁵ The most favourable reaction mechanism was not the direct attack of oxygen on the organic substrate, as initially proposed. Instead, an overlooked alternative mechanism emerged: oxygen first protonates, leading to a charge transfer and subsequent oxygenation. This

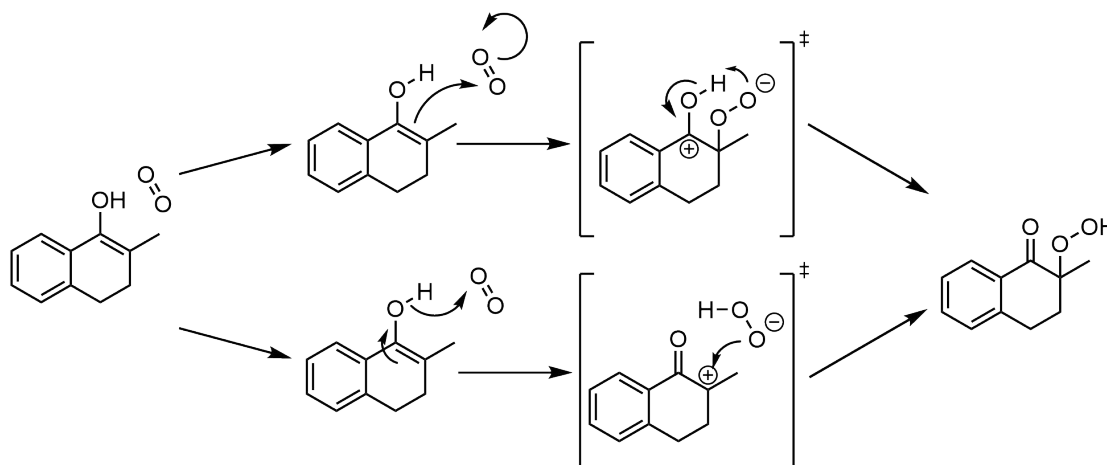


Figure 17: Proposed mechanisms for tetralone oxygenation. Top path, the mechanism previously proposed by our reference publication. Bottom path, the alternative mechanism proposed by us, which in the end is the most energetically favoured.

mechanism also resonates with enzymatic reactions, where we often see substrates deprotonated prior to the reaction. This strategy reduces free energy barriers, enabling barrierless approach of O_2 to the substrate and enhancing the value of spin-orbit coupling.

In addition to the electronic structure calculations in the gas-phase, we have also provided the spin-orbit coupling values and solvation free energy calculations. We also explored the subsequent reduction reaction where the newly formed peroxide is treated with triethyl phosphite, leading to the production of the final product, alcohol.

The study of this neutral molecule provides a broader perspective on the role of protonation in spin-forbidden oxygenation reactions. In the enzymatic cofactorless spin-forbidden addition of O_2 to an organic substrate, it is commonly observed that the substrate undergoes deprotonation prior to the reaction. Our findings indicate that substrate deprotonation serves as an effective strategy, resulting in substantially reduced free energy barriers. Furthermore, it facilitates the barrierless approach of O_2 to the substrate and leads to considerably higher values of spin-orbit coupling. We believe that these findings contribute significantly to our understanding of oxygenation mechanisms with spin change and can be generalized to other spin-forbidden additions of O_2 to neutral double bonds.

5.3.1 Resumen en español

En la fase final de este proyecto de investigación, nos adentramos en los mecanismos de oxigenación prohibidos por espín de una molécula neutra, concretamente un enol en lugar de un enolato. Para ello, escogimos un trabajo de síntesis orgánica que explicaba el proceso experimental de adición de oxígeno atmosférico a un sustrato orgánico (Ref.

175). Aunque el artículo de Riahi et al. proporcionaba cálculos de estructura electrónica, carecían de una metodología adecuada para estudiar sistemas multirreferenciales. No tuvieron en cuenta el acoplamiento espín-órbita y, lo que es más importante, pasaron por alto una segunda posible vía de reacción. Tampoco se optimizó ningún MECP.

Nuestros cálculos nos llevaron a una conclusión distinta de la del artículo original.¹⁷⁵ El mecanismo de reacción más favorable no era el ataque directo del oxígeno al sustrato orgánico, como se había propuesto inicialmente. En su lugar, surgió un mecanismo alternativo que se había pasado por alto: el oxígeno primero se protona, lo que conduce a una transferencia de carga y a la posterior oxigenación. Este mecanismo también coincide con el de las reacciones enzimáticas, en las que los sustratos suelen desprotonarse antes de la reacción. Esta estrategia reduce las barreras de energía libre, lo que permite la aproximación sin barreras del O_2 al sustrato y aumenta el valor del acoplamiento espín-órbita.

Además de los cálculos de la estructura electrónica en fase gaseosa, también hemos proporcionado los valores del acoplamiento espín-órbita y los cálculos de la energía libre de solvatación. También exploramos la reacción de reducción posterior en la que el peróxido recién formado se trata con fosfito de trietilo, lo que conduce a la producción del producto final, el alcohol.

El estudio de esta molécula neutra proporciona una perspectiva más amplia sobre el papel de la protonación en las reacciones de oxigenación con espín prohibido. En la adición enzimática sin cofactor de O_2 a un sustrato orgánico, suele observarse que el sustrato sufre una desprotonación antes de la reacción. Nuestros hallazgos indican que la desprotonación del sustrato es una estrategia eficaz que reduce sustancialmente las barreras de energía libre. Además, facilita la aproximación sin barreras del O_2 al

sustrato y conduce a valores considerablemente más altos del acoplamiento espín-órbita. Creemos que estos hallazgos contribuyen significativamente a nuestra comprensión de los mecanismos de oxigenación con cambio de espín y pueden generalizarse a otras adiciones de O_2 con espín prohibido a dobles enlaces neutros.



Article

Spin-Forbidden Addition of Molecular Oxygen to Stable Enol Intermediates—Decarboxylation of 2-Methyl-1-tetralone-2-carboxylic Acid

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Abstract: The deprotonation of an organic substrate is a common preactivation step for the enzymatic cofactorless addition of O₂ to this substrate, as it promotes charge-transfer between the two partners, inducing intersystem crossing between the triplet and singlet states involved in the process. Nevertheless, the spin-forbidden addition of O₂ to uncharged ligands has also been observed in the laboratory, and the detailed mechanism of how the system circumvents the spin-forbiddenness of the reaction is still unknown. One of these examples is the cofactorless peroxidation of 2-methyl-3,4-dihydro-1-naphthol, which will be studied computationally using single and multi-reference electronic structure calculations. Our results show that the preferred mechanism is that in which O₂ picks a proton from the substrate in the triplet state, and subsequently hops to the singlet state in which the product is stable. For this reaction, the formation of the radical pair is associated with a higher barrier than that associated with the intersystem crossing, even though the absence of the negative charge leads to relatively small values of the spin-orbit coupling.

Keywords: intersystem crossing; peroxidation; minimum-energy-crossing-point



Citation: Ortega, P.; Gil-Guerrero, S.; González-Sánchez, L.; Sanz-Sanz, C.; Jambrina, P.G. Spin-Forbidden Addition of Molecular Oxygen to Stable Enol Intermediates—Decarboxylation of 2-Methyl-1-tetralone-2-carboxylic Acid. *Int. J. Mol. Sci.* **2023**, *24*, 7424. <https://doi.org/10.3390/ijms24087424>

Academic Editor: Dongho Kim

Received: 17 February 2023

Revised: 12 April 2023

Accepted: 13 April 2023

Published: 18 April 2023



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1. Introduction

Photodynamic therapy (PDT) is a clinically approved technique that has shown great potential for the treatment of certain kinds of tumours [1–3]. In PDT, a photosensitizer is introduced into the organism, where it is photo-excited to a singlet excited electronic state, followed by its relaxation via intersystem crossing to a triplet state. Once in the triplet state, the photosensitizer interacts with molecular oxygen, leading to the formation of singlet oxygen (or another reactive oxygen species), a cytotoxic molecule [4–6] that can induce the apoptosis and necrosis of tumour cells, damage the tumour-associated vasculature, or induce an immune response [7].

The production of singlet O₂ from its triplet ground state is not efficient, as this transition is forbidden by the spin, parity, and angular momentum selection rules for the electric dipole process [8], as is evinced by the long lifetime of singlet O₂ in the gas phase (~1 h) [9]. The drastic difference in reactivity between the ground state (triplet) O₂ and the singlet O₂ is caused by the nature of the electronic ground state. While most organic molecules are closed-shell (singlet) molecules, O₂ is a diradical, and so reactions between them are spin-forbidden.

To catalyze spin-forbidden reactions with O₂, enzymes typically rely on metal cofactors, which can easily switch the total spin of the system [10–17]. Others, particularly flavoenzymes, use a non-metal cofactor, or require no cofactor at all [18–25], and although there are some common features, such as the activation of the organic substrate via deprotonation [20], it is not clear regarding the role played by enzymes to catalyze spin-forbidden

reactions. Electronic structure calculations have been used to unravel the mechanisms of these reactions (see, for example, Refs. [26–35]), but there is no consensus mechanism. For example, the reaction catalyzed by glucose oxidase needs a proton donor (His516) [31], whereas for the DpgC-catalyzed reaction, peroxidation occurs without charged residues or water molecules that could act as a base [27,36].

To investigate spin-forbidden processes using computational methods, non-adiabatic Transition State Theory (NA-TST) is one of the most powerful frameworks [37–39]. In NA-TST, the minimum energy crossing point (MECP) between the two electronic states with different multiplicities plays the same role as the transition state in “adiabatic TST”, so that its energy has to be determined. However, overcoming the MECP is not sufficient for the reaction to proceed. In NA-TST, the transmission coefficient depends on the hopping probability, which ultimately depends on the value of the spin-orbit coupling (SOC) [40], the non-relativistic quantum effect that permits transitions between PESs of different multiplicities. Hence, the spin-forbidden character of the reaction acts as an additional barrier [38], and only those processes for which the value of SOC is not negligible could proceed at a reasonable speed. SOC strength depends strongly on the nature of the two states involved in the spin-change process. According to El-Sayed’s rules [41], SOC is expected to be larger when the change of spin is associated with a change in the orbital angular momentum (or electronic configuration). For collisions involving O₂, it implies that the ability of the solvent or organic molecules to promote O₂ electronic transitions is associated with the perturbation of the symmetry of O₂, even if they only interact via weak Van der Waals forces [8,42,43]. Recent studies have suggested a correlation between the value of SOC and the energy difference between the two π orbitals for complexes involving O₂ [43]. On top of the importance of the SOC value, according to the Landau-Zener equation [39,44], the hopping probability also depends on the reduced mass associated with the vibrational coordinate parallel to the crossing seam, and the difference of the gradient between the two PESs involved [45].

Ground state (triplet) O₂ is also used as an oxidant for many synthetic pathways (see, for example, Refs. [46–57] and references therein) as it is a very abundant and environmentally friendly reagent. To circumvent the limitation imposed by the spin-forbidden characters of these reactions, most of them use metal centers as catalyzers. One example of the use of triplet O₂ in the absence of metal cofactors is that proposed by Riahi et al. [51], who reported on the formation of a hydroperoxide (2-hydroperoxy-2-methyl-1-tetralone (4)) in the decarboxylation of 2-methyl-1-tetralone-2-carboxylic acid (1) in acetonitrile, as depicted in Figure 1. Under aerobic (and dark) conditions, O₂ reacts with a stable enol intermediate (2), leading to the formation of the hydroperoxide that was isolated and subsequently reduced with P(OEt)₃, leading to the formation of an α -ketol (5). Based on unrestricted DFT calculations, Riahi et al. [51] postulated a mechanism in which the system first overcomes a barrier in the triplet state, after which it undergoes a transition from the triplet to the singlet state, which will evolve to the formation of the hydroperoxide. There are some reasons for why the study of the mechanism of this reaction is particularly interesting. First, the addition of O₂ to the double bond resembles the kind of processes that occur in the enzymatic additions of O₂. Second, unlike most cofactorless enzymatic additions of O₂, the process does not require prior deprotonation of the substrate. Then, this study might shed light on why prior deprotonation occurs in biological media. Three, the process involves both the formation of the peroxide bond and hydrogen (or proton) transfer from the substrate to the O₂ moiety. Deciphering the sequence of events could help us to advance toward a consensus mechanism for the cofactorless addition of molecular oxygen to organic molecules. Finally, from a computational point of view, this is a somewhat small system, and so higher-level electronic structure methods such as CASPT2 (multi-reference) or Coupled-Cluster (single-reference) methods could be applied.

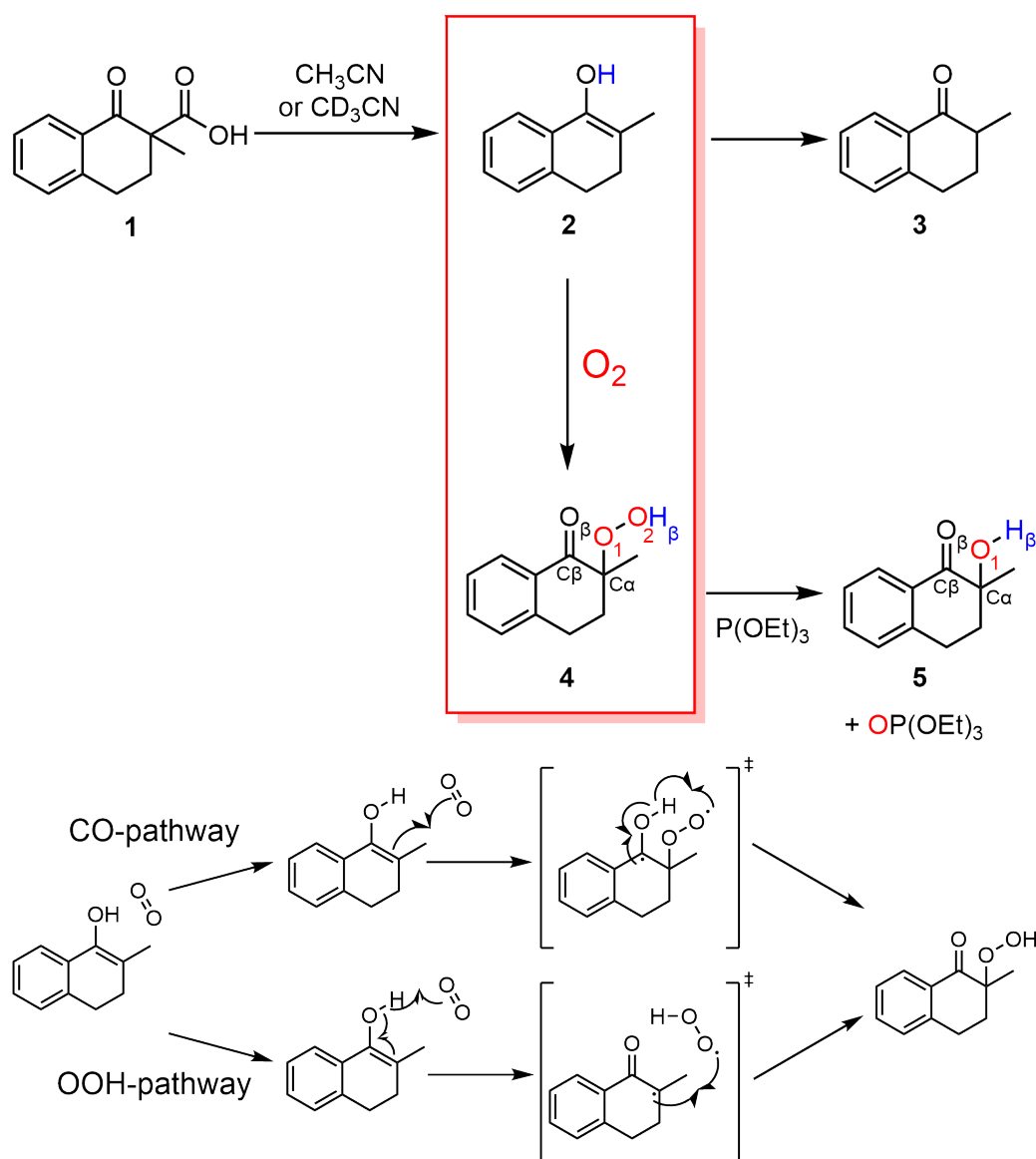


Figure 1. Top panel: Decarboxylation of 2-methyl-1-tetralone-2-carboxylic acid (1) involves the formation of an enol intermediate (2) [51]. This enol is affected by a side reaction with atmospheric oxygen to obtain the hydroperoxide tetralone-derivative (4). The reduction of (4) using $\text{P}(\text{OEt})_3$ leads to the α -ketol (5). The transferred proton is colored in blue, and atoms are labeled according to the notation used in the article. Bottom panel: Possible reaction pathways for the peroxidation of the 2-methyl-3,4-dihydro-1-naphthol molecule. CO-pathway (upper path), where the $\text{C}_\alpha\text{-O1}$ bond is formed first, and then the peroxide is protonated, and the OOH-pathway, in which the protonation of O_2 occurs prior to the formation of the $\text{C}_\alpha\text{O1}$ bond.

In this manuscript, we have carried out a comprehensive computational study of the formation of 2-hydroperoxy-2-methyl-1-tetralone using DFT, CASPT2, and Coupled-Cluster calculations. Two different pathways will be evaluated (see the bottom panel of Figure 1), that proposed by Riahi et al. [51], hereinafter, the CO-pathway, for which the peroxide bond is formed prior to ISC, and a second mechanism, the OOH-pathway, in which the first step is the protonation of the O_2 moiety. Our results show that the OOH-pathway is preferred, although the barrier for both mechanisms is similar, suggesting that both mechanisms are feasible for similar reactions.

2. Results and Discussion

Figure 2 displays the energy contour plots, calculated at the M062X-D3/maug-cc-pVDZ level of theory, for the approach of O₂ to the enol on the singlet (restricted and open-singlet) and triplet PESs as a function of r_{CO} , the distance between C_{vx} and the closest O atom of O₂, and r_{HT} , the reaction coordinate for the proton transfer from the enol to O₂ (see Methods section for details). On the triplet state (top panel), we observed the minimum corresponding to the reactants' asymptote at $r_{CO} = 3.2$ Å, and with H_β bonded to C_β ($r_{HT} > 0$) (Structure #1). We also observe two local minima that are considerably higher in energy (17–19 kcal/mol above the reactants asymptote, see Table 1), corresponding to the deprotonated peroxide (Structure #2) and with the protonated ·O₂-H radical (Structure #3). To reach any of these secondary minima, the system has to surmount electronic barriers of about 22 kcal/mol (see Table 1). It is worth noticing that there are no minima corresponding to the protonated peroxide, which would appear at the bottom-left corner of the figure (negative values of r_{HT} and small values of r_{CO}).

Table 1. Electronic energies of critical points along the reaction paths for the different methods used: M062X-D3 (with broken symmetries in parentheses), CASPT2(8,5), and DLPNO-CCSD(T), using the basis set maug-cc-pVDZ. The energies (in kcal/mol) refer to the reactants complex. The MECP energies for the DLPNO-CCSD(T) method correspond to regions where the singlet is not accurately represented by a monoreference method according to the T1 diagnosis, and are not completely reliable. ΔE_S^T O₂ refers to the energy difference between the singlet and triplet reactant complex (that is associated with the ³O₂ → ¹O₂ excitation). The reactants and products are the same for the two pathways.

ΔE CO-pathway	M062X-D3	DLPNO-CCSD(T)	CASPT2(8,5)
Reactant complex (triplet)	0.00	0.00	0.00
ΔE_S^T O ₂	36.9 (27.9)	31.8	23.2
TS (triplet)	22.5	25.7	28.6
Intermediate (triplet)	17.1	20.8	26.1
MECP	22.6 (17.2)	26.2	27.0
Product (singlet)	−27.8	−25.5	−20.5
ΔE OOH-pathway	M062X-D3	DLPNO-CCSD(T)	CASPT2(8,5)
Reactant complex (triplet)	0.00	0.00	0.00
ΔE_S^T O ₂	37.0 (27.9)	31.8	23.2
TS (triplet)	22.7	30.4	32.9
Intermediate (triplet)	19.6	22.5	26.6
MECP	30.5 (20.0)	26.9	27.7
Product (singlet)	−27.8	−25.5	−20.5

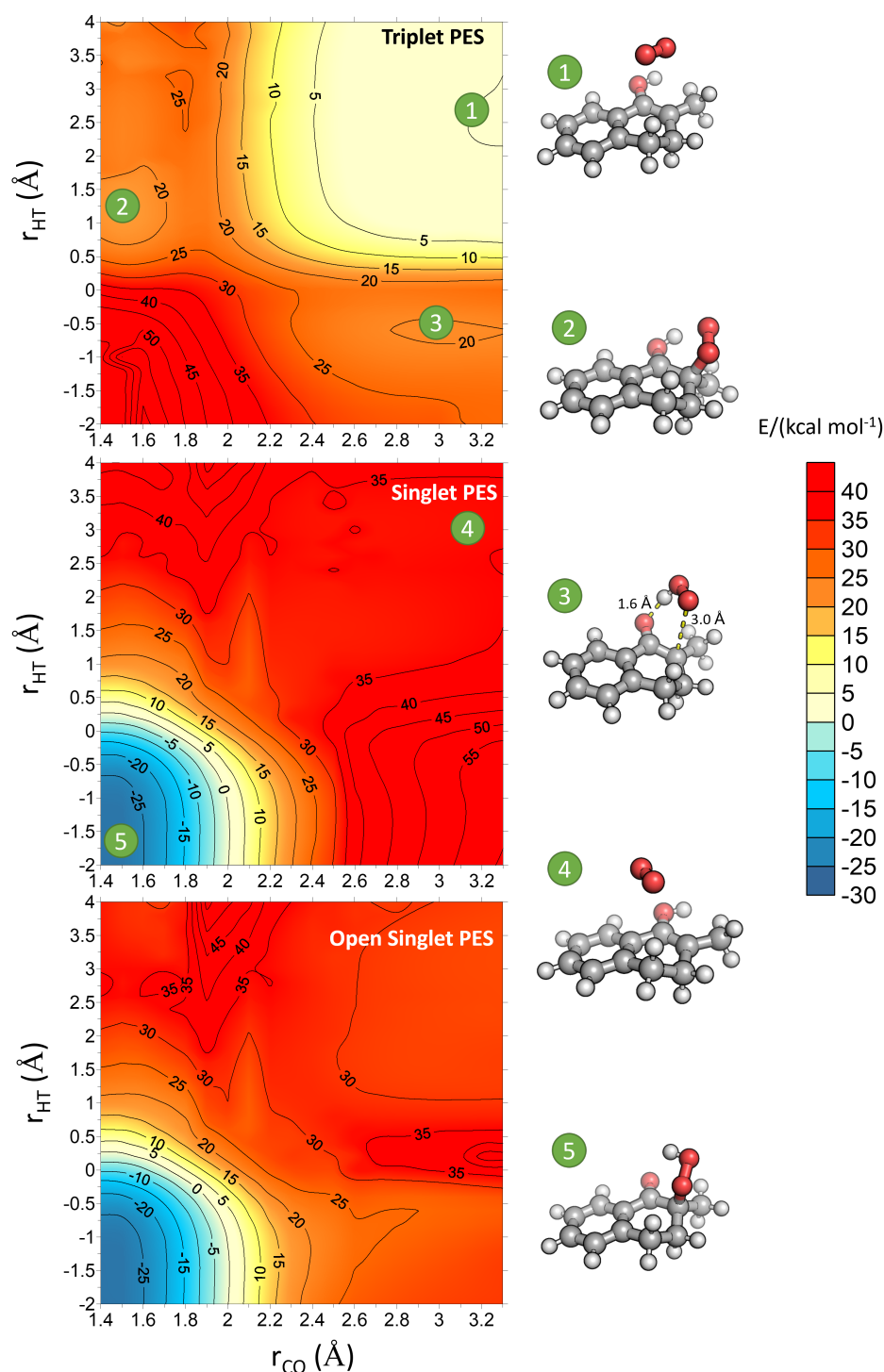


Figure 2. Three-dimensional contour maps of the energy profile for the addition of O_2 to 2-methyl-3,4-dihydro-1-naphthol as a function of the $C\alpha-O1$ distance and $r_{HT} = r_{O2-H\beta} - r_{O\beta-H\beta}$. Geometries were optimized for their corresponding spin state. Top panel: Lowest triplet state. Middle panel: Lowest closed-shell singlet state. Bottom panel: Lowest open-shell singlet state. The relevant geometries are depicted on the right and labeled over the contour map (see main text). Energies are expressed in kcal/mol, following the color scheme shown in the scale, and distances are expressed in Å. Calculations were carried out at the M062X-D3/maug-cc-pVDZ level of theory. Cartesian coordinates corresponding to structures #1–#5 are shown in Table S1. The zero of the energy scale corresponds to the reactants complex.

On the contrary, on the singlet PES (middle panel of Figure 2), we observed one deep minimum (Structure #5) that corresponds to the protonated peroxide, 27.8 kcal/mol below the reactants asymptote. No minima associated with either the deprotonated peroxide or the $\cdot\text{O}_2\text{-H}$ radical appear. The energy difference between the singlet and the triplet PES at the reactants asymptote (Structure #1 and #4) is around 37 kcal/mol, well above that corresponding to isolated O_2 (22.6 kcal/mol) [58]. On the bottom panel, the open-shell singlet energies were calculated using the broken-symmetry DFT approach. Around the singlet minimum, singlet and open-shell singlet energies are equal, as expected. However, for large r_{CO} and/or r_{HT} the open-shell singlet energies are significantly smaller. In particular, at the geometries corresponding to the reactants asymptote, the energy difference between the singlet and the triplet state is significantly smaller (27 kcal/mol), in better agreement with the experimental energy difference for the $^3\text{O}_2 \rightarrow ^1\text{O}_2$ transition.

The cuts of the PESs shown in Figure 2 confirm that the reaction proceeds via a transition from the triplet state (only PES where the reactants are stable) to the singlet state (only PES where the peroxide is stable). For each point of the contour plot, the minimum energy between the singlet and triplet states is shown in Figure 3, where the dashed cyan line divides the plot according to the more stable state, with the singlet state being more stable in the bottom-left region, and the triplet state in the upper-right region of the plot. It should be noticed that the dashed line does not correspond to the crossing seam, as the geometries were optimized separately on the triplet and singlet PESs. Starting from different geometries of the contour map, we calculated the MECPs, obtaining two MECPs whose structures are displayed as #1 and #2 in Figure 3, and whose energies are 17.2 kcal/mol and 20.0 kcal/mol, respectively, above the reactants asymptote.

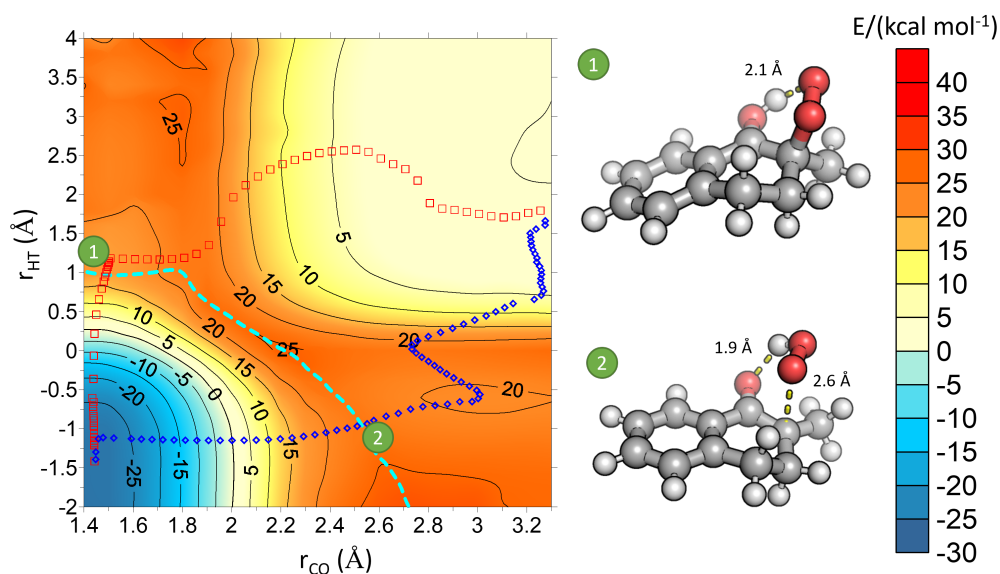


Figure 3. Representation of reaction paths over the 3D potential map, as a function of the energy minimum between the singlet and triplet states. Structures #1 and #2 correspond to the two MECPs obtained, and the red squares represent the geometries of the CO pathway, with the blue diamond showing the geometries of the OOH pathway. The cyan dashed line separates the region in which the singlet and the triplet are more stable. Energies are expressed in kcal/mol, following the color scheme shown in the scale, and distances are expressed in Å. Calculations were carried out at the M062X-D3/maug-cc-pVDZ level of theory. Cartesian coordinates corresponding to the two MECPs are shown in Table S1.

The presence of two TSs on the triplet PES and the two MECPs suggested that, at least, two different mechanisms coexist for this reaction. To characterize them, we calculated the minimum energy paths (MEPs), combining the IRC calculations, starting from the two saddle points, with downhill optimizations from the two MECPs on the triplet and

singlet states. An analysis of the MEPs confirmed that each of the TSs on the triplet states was connected with one MECP, which allowed us to define two different mechanisms (see the bottom panel of Figure 1): (i) the CO-pathway, in which the $C_{\alpha}O$ bond is formed before the proton is transferred, and in which the singlet-triplet hop occurs with the $C_{\alpha}O1$ bond already formed (geometries are shown as red squares in Figure 3); and (ii) the OOH-pathway, in which O_2 first picks up the proton of 2 and ISC occurs once the OOH moiety is formed (geometries are shown as blue diamonds in Figure 3). As for both pathways we have a two-step process, we used as the reaction coordinate first, the $C_{\alpha}O1$ distance and then the r_{HT} distance for the CO-pathway, and vice versa for the OOH-pathway. Interestingly, we did not find a mechanism in which CO formation and H-transfer occurs simultaneously.

To obtain a quantitative insight into the order in which the bonds are formed/broken throughout the two mechanisms, we calculated the Delocalization Indices (DIs) along the two MEPs. Delocalization indices represent the electron density that is shared between two partners, and it is intimately related to the concept of “bond order”. Unlike internuclear distances, whose evolution can also be used to obtain some insight into the reaction mechanism, DIs depend on the electronic state, and hence, at each point of the MEPs, we represented that corresponding to the ground state. DIs also permit to discern between proton transfer or hydrogen atom (proton+electron) transfer mechanisms. In Figure 4, we display the DIs (δ) for all the bonds that are formed/broken throughout the reaction. At the reactants asymptote, δ_{O1-O2} is 1.83, which is very close to the value obtained for isolated O_2 (1.84), which indicates that the $O=O$ bond is not perturbed by the substrate. This is different from what was observed for the interactions between O_2 and the negatively charged enolates, for which δ_{O1-O2} was somewhat smaller, even at large internuclear distances [36]. Also at the reactants asymptote, $\delta_{C_{\alpha}-C_{\beta}}$ is compatible with a double bond, while $\delta_{C_{\beta}-O_{\beta}}$ and $\delta_{O_{\beta}-H_{\beta}}$ are close to the expected values for single bonds ($\delta \sim 1$).

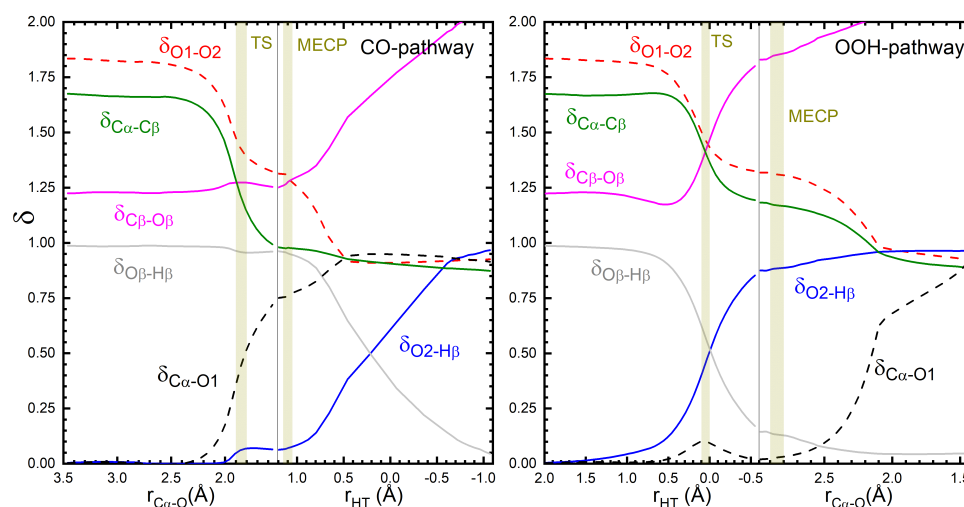


Figure 4. Evolution of delocalization indices along the addition of O_2 to 2-methyl-3,4-dihydro-1-naphthol along the reaction path calculated at the M062X-D3/maug-cc-pVDZ level of theory. Results for the CO-pathway and the OOH-pathway are shown in the left and right panels, respectively. The locations of the two TSs and MECs are shown as vertical shaded lines.

For the CO-pathway, the DIs barely change until the two reactants are significantly close to each other ($r_{C_{\alpha}-O} = 2.2$ Å). When the reactants are closer, electron density starts to be transferred from the $O1-O2$ bond to $C_{\alpha}-O$ bond, and as a consequence of that, $\delta_{C_{\alpha}-C_{\beta}}$ also decreases. At the TS, δ_{O1-O2} is between the expected value for a single and a double bond, while $\delta_{C_{\alpha}-O1}$ is half of the expected value for a single bond. At the MECP, the latter has raised so we could consider that the $C_{\alpha}-O$ bond is almost completely formed, while the H atom remains on the substrate. After MECP, the H atom is completely

transferred, as evinced for the increase in $\delta_{O2-H\beta}$, which is also associated with the $C\alpha - C\beta$ double bond and the lack of electron density shared between $O\beta$ and $H\beta$.

The mechanism for the OOH-pathway is significantly different. First, the H is transferred, and at the TS, the electron density between $\delta_{O2-H\beta}$ is similar to that between $\delta_{O\beta-H\beta}$. It leads to a smaller density shared between $O1$ and $O2$, and $C\alpha$ and $C\beta$. DIs are compatible with a hydrogen atom transfer instead of a proton transfer, and the formation of a radical pair. To confirm this finding, spin populations are displayed in Figure S2 of the Supplementary information for the two fragments. After the TS, the proton is transferred to $O2$, and δ_{O1-O2} and $\delta_{C\alpha-C\beta}$ are between the values expected for a single and a double bond. Once H is transferred, the electron density barely changes with the decrease in $C\alpha - O$ distance until the MECP is reached and the system swaps to the singlet state. In fact, hopping from the triplet to the singlet state is associated with a significant increase in the electron density shared between $\delta_{C\alpha-O1}$, which is the last bond to be formed in the OOH pathway.

Once the main features of the two possible pathways were described, their corresponding reaction energy profiles were calculated at the DFT level of theory and are compared with those obtained using a higher level of theory methods such as DLPNO-CCSD(T), and CASPT2. We selected these two methods because CASPT2 includes both static and dynamic correlations, and is among the most accurate methods for multiconfigurational problems, while Coupled-Cluster methods are considered as the gold standard for the study of single-reference systems. Regardless of the method used and the pathway, the energy profiles displayed in Figure 5 showed similar features, with DFT predicting somewhat smaller barriers. Within the DFT framework, we calculated the energy of the singlet state both using restricted DFT and a broken symmetry approach. Our results show that the restricted DFT energy profile for the singlet state resembles that obtained using DLPNO-CCSD(T) calculations, while the broken symmetry solution is more similar to that obtained using CASPT2.

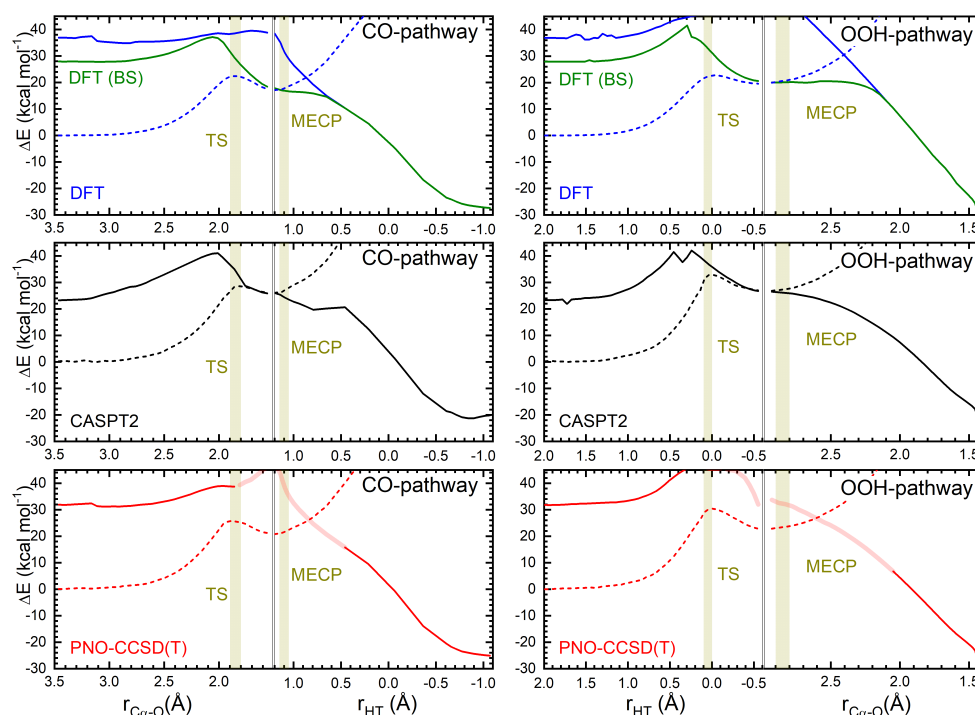


Figure 5. Energy profile for the addition of O_2 to 2-methyl-3,4-dihydro-1-naphthol along the two possible reaction paths at M062X-D3 (top panels), CASPT2(8,5) (middle panels), and DLPNO-CCSD(T) (bottom panels). Dashed and solid lines denote triplet and singlet states, respectively. For DLPNO-CCSD(T) calculations, the regions where T1 diagnosis predicts a significant non-monoreference character are shown in brighter red. The basis set employed is maug-cc-pVDZ for all the panels.

For the CO-pathway, the system first has to overcome a barrier of around 22–29 kcal/mol (depending on the level of theory, see Table 1) on the triplet state. As commented above, the singlet state was significantly higher in energy, but contrary to what was observed for spin-forbidden reactions between a negatively charged enolate and molecular oxygen [36], the energy of the singlet state does not decrease with the approach of molecular oxygen and the substrate, and we observe either an energy plateau or even a barrier. For those systems, it was proposed that the reaction could also proceed via direct electron transfer, after which the reaction proceeds barrierless [30]. The presence of an electronic barrier on the singlet state makes direct electron transfer unlikely for this system. Regarding the energy difference between the singlet and the triplet state, an analysis of the asymptotic delocalization indices (Figure 4) revealed that the molecular oxygen molecule was unperturbed at the reactants asymptote, so the energy difference should resemble that for isolated O₂. That is the case when the CASPT2 method is used, while monoreference methods such as DFT or DLPNO-CCSD(T) predict a higher energy difference between the singlet and the triplet state.

After the TS, the energy of the triplet state slightly decreases, and between the TS and the MECF it reaches a plateau. At this region, differences between the three methods arise. For CASPT2, the singlet and the triplet states are approximately degenerate in this region, and this degeneracy is only broken when the H is being transferred to the peroxide. This behavior is well accounted for broken symmetry DFT. The main difference between the CASPT2 and DLPNO-CCSD(T) energy profiles is that for the latter, the singlet and triplet states are not approximately degenerate between the TS and the MECF, due to a significant destabilization of the singlet state, and it only crosses at one point, $r_{\text{HT}} = 0.7 \text{ \AA}$, close to the MECF predicted from restricted DFT calculations. As commented above, CCSD(T) methods are the gold standard for single-reference methods but they may not be fully reliable for strong-correlated systems. To assess the reliability of the DLPNO-CCSD(T) calculations, we calculated the diagnostic T1 along the reaction path. Values of T1 below 0.02 represent regions that are reasonably described using Coupled-Cluster methods (0.044 for open-shell systems) [59,60]. Regions that are not well described using DLPNO-CCSD(T), according to its T1, are shown as shaded in Figure 5, and for the CO-pathway, DLPNO-CCSD(T) is not fully reliable only for the singlet state and between the TS and the crossing point. As a consequence of that, CCSD(T) calculations around the MECF are not accurate. Regardless of the method used, after reaching the MECF, the singlet state becomes stabilized as expected, and the reaction proceeds barrierless.

Qualitatively, the energy profiles are similar for the OOH pathway, with the difference being that the T1 diagnosis predicts that DLPNO-CCSD(T) not only is not reliable for the singlet state between the TS and the crossing point, but also beyond the MECF. This is not surprising, since DIs indicate that a radical pair was formed in that region.

To obtain a deeper understanding of the nature of the lowest energy states involved in the reaction, energy profiles were also calculated using MRCI based on a state average CASSCF wave-function that treats all the states on equal footing (Figure S3 in the Supplementary Material). Qualitatively, energy profiles for the lowest singlet and triplet states (S1 and T1) are similar to those obtained for CASPT2, although the barriers obtained are significantly larger, as expected due to the limited dynamic correlation included. The reference active space was small, with the dioxygen π orbitals and the p_z orbital for the bonding carbon atom, forming a CAS(4,3) space [27]. These calculations were carried out with Molpro2020 [61]. At the reactants asymptote, the order of the states involved mimics that for molecular oxygen: the ground triplet state (that correlates to the $^3\Sigma_g^-$ of O₂), two degenerate singlet states (that originate from the $^1\Delta_g$), and a higher energy singlet state (that comes from the $^1\Sigma_g^+$ of O₂). At higher energies, we obtain two degenerate singlet states that come from charge-transfer ionic pairs between tetralone and O₂. Between TS and the MECF, the ground singlet and triplet states are degenerate, as we found for CASPT2 and BS-DFT.

According to NA-TST, the determination of the MECF is not enough to characterize the dynamical bottlenecks for spin-forbidden processes, as the hopping probability

depends on other factors, such as the SOC. In Figure 6, we calculated the SOC between the lowest energy singlet and triplet states along the reaction path using the CASPT2 method. As SOC depends strongly on the nature of the two states involved, it is expected to be very different for the two pathways in the “hopping” region, where the singlet and triplet states show similar energies. For the CO-pathway, the largest SOC (about 90 cm^{-1}) is found before the TS, when $r_{\text{C}\alpha\text{-O}}$ is around $2\text{ \AA}$. As a reference, the spin-orbit coupling value for systems with metal atoms can be between 300 and 1000 cm^{-1} [38], and the obtained values for other systems involving the cofactorless addition of O_2 to the organic substrate are about $70\text{--}80\text{ cm}^{-1}$ [27,32,43]. The SOC value decreases with the approach of the two partners, leading to values even close to zero and rising again at the end of the interaction region, when the peroxide is being protonated. In the region where the singlet and triplet are approximately degenerate, SOC goes from almost null to around 10 cm^{-1} , and together with the difference of the gradients variations, it leads to an additional hindrance of $2.5\text{--}4.0\text{ kcal/mol}$ in that region (Figure S4 of the Supplementary Information). In particular, at the MECP, the SOC is $\sim 10\text{ cm}^{-1}$, leading to $\Delta\Delta G^\ddagger = 3.1\text{ kcal/mol}$. The barrier obtained is in any case lower than the previous triplet TS, so the MECP step would not be the limiting step of this reaction.

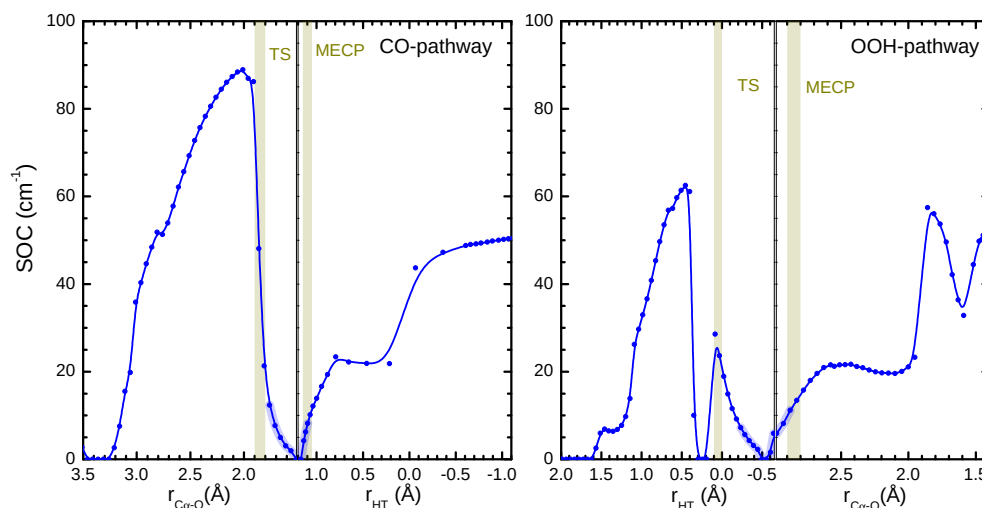


Figure 6. Spin-orbit coupling (SOC) between the ground triplet state and the singlet state involved in the oxidation of 2-methyl-3,4-dihydro-1-naphthol. Calculations carried out at the CASPT2(8,5)/maug-cc-pVDZ theory level. Dots represent the actual values, while the solid lines are displayed to guide the eye. The regions for which singlet and triplet states are almost degenerate are highlighted, as they represent the regions for where hopping is more likely to occur.

For the OOH-pathway, SOC reaches a maximum at around 60 cm^{-1} at the beginning of the interaction region, after which it drops where the proton is being transferred to the O_2 moiety, and a radical pair is formed. As predicted by the El-Sayed rules, this is caused by the similar character of the two involving states, as hopping between singlet and triplet only involves the change of spin of one electron. Once the proton has been fully transferred and the two partners approach, SOC increases, and it obtains a value of about 15 cm^{-1} at the end of the “hopping” region, where $r_{\text{C}\alpha\text{-O}}$ is around $2.4\text{--}2.5\text{ \AA}$. Even higher values are obtained at lower distances, although they are not relevant, as singlet and triplet states have considerably different energies in that region. Considering the region where singlet and triplet are almost degenerate, the SOC ranges between 0 and 20 cm^{-1} . However, the lower difference of gradients favors this path, and $\Delta\Delta G^\ddagger$ is only 2.0 kcal/mol at MECP. Again, this step does not seem to be limiting to the reaction, and the final kinetics will be determined from the first step, at the triplet TSs.

In Figure 7, we show the free energies calculated for both paths using an improved basis set and also considering the effect of the solvent (using the SMD implicit solvent

model with the dielectric constant of acetonitrile). The free energies of TSs and MECPs are considerably higher than the electronic energies due to entropy penalty [62]. According to Figure 7, the OOH pathway is preferred over the CO-pathway for DFT and CASPT2, while for Coupled-Cluster, the CO-pathway is preferred. Regarding the rate-limiting step, the barriers associated with the TS on the triplet state and the ISC are similar, with the former being the rate-limiting step for the OOH pathway for DFT and CASPT2. The barrier associated with ISC is larger using DLPNO-CCSD(T), and in particular, for the OOH pathway, albeit results should be taken with care due to the high value of the T1 diagnosis. According to our free energy barriers, the reaction should be slow at 298 K, happening over a few days (as described in the experiment, 129 h) [51]). At this point, it is possible to compare the results obtained for this reaction with those obtained for the enzymatic cofactorless of O₂ to organic substrates, for which the deprotonation of the substrate is a common preactivation step. When the substrate is deprotonated, there is no barrier on the triplet potential energy surface, and the rate constant of the process depends on the energy of the MECP and the hopping probability, the latter being increased by the negative charge of the system [27,36]. If O₂ is added to a neutral substrate, there is a higher barrier on the triplet state, similar to or even higher than that associated with ISC. Hence, a lower hopping probability has a small effect on the rate of the process.

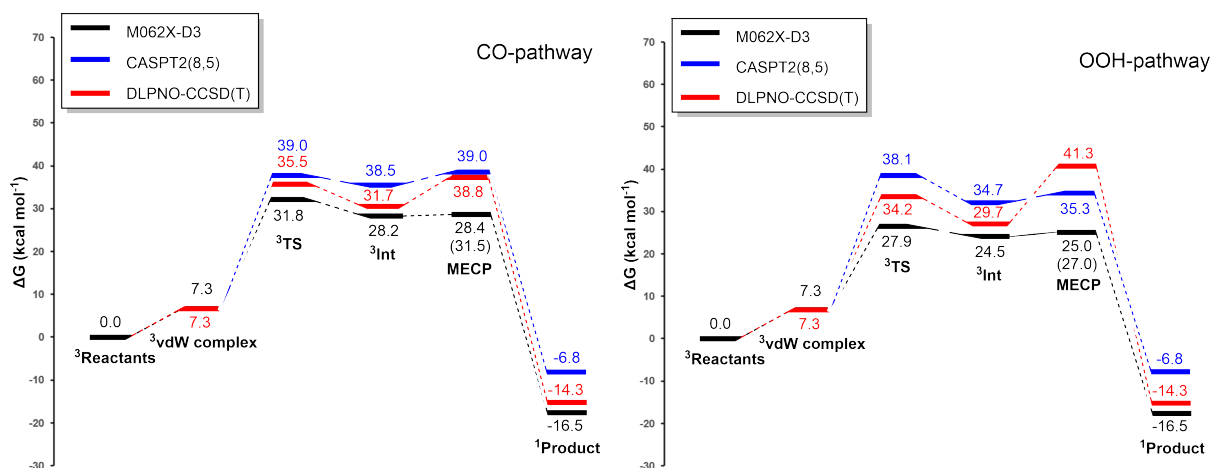


Figure 7. Free energy profile for the addition of O₂ to 2-methyl-3,4-dihydro-1-naphthol along the two possible reaction paths for M062X-D3, CASPT2(8,5), and DLPNO-CCSD(T). M062X-D3 and DLPNO-CCSD(T) single-points with maug-cc-pVTZ basis set over maug-cc-pVDZ geometries and vibrational analysis in acetonitrile implicit solvent (SMD) level of theory. CASPT2 single-points with maug-cc-pVDZ basis set. Each structure was optimized for the lowest spin state. At MECP, the increase in free energy caused by the limited hopping probability is shown in parentheses. Left panel: CO-pathway. Right panel: OOH-pathway. Energies (in kcal/mol) refer to the reactants' asymptote where the two reactants are separated. Energies correspond to the ground state for each geometry. Energies for triplet and singlet states are shown in Figure S5.

Phosphite Reduction Mechanism

Experimentally, the reduction of the hydroperoxide with P(OEt)₃ was very fast, leading to formation of the α -ketol in a few minutes [51]. Our calculations predict that the free energy barrier for the process is just 14.5 kcal/mol at room temperature (Figure 8), considerably smaller than the one obtained for the formation of the peroxide. This free energy barrier is mostly associated with the entropy burden (the electronic barrier is just 2.3 kcal/mol). According to our calculations, the reaction occurs in a single step, in which the phosphorous attacks the hydroxyl oxygen of the hydroperoxide, leading to the breaking of the peroxide bond, which also triggers proton transfer, forming the α -ketol. Proton transfer is favored by interaction with the carbonyl oxygen in α to the peroxide. This mechanism is in good agreement with the proposal of Denney et al. [63] in the 1960s, which

suggested a simultaneous breaking of the peroxide and proton transfer. As the reaction was carried out in EtOH, we also carried out calculations with one explicit EtOH molecule that may stabilize the transition state, and observed a small effect. This is not surprising since phosphites can also reduce hydroperoxide in pentane at low temperatures [63].

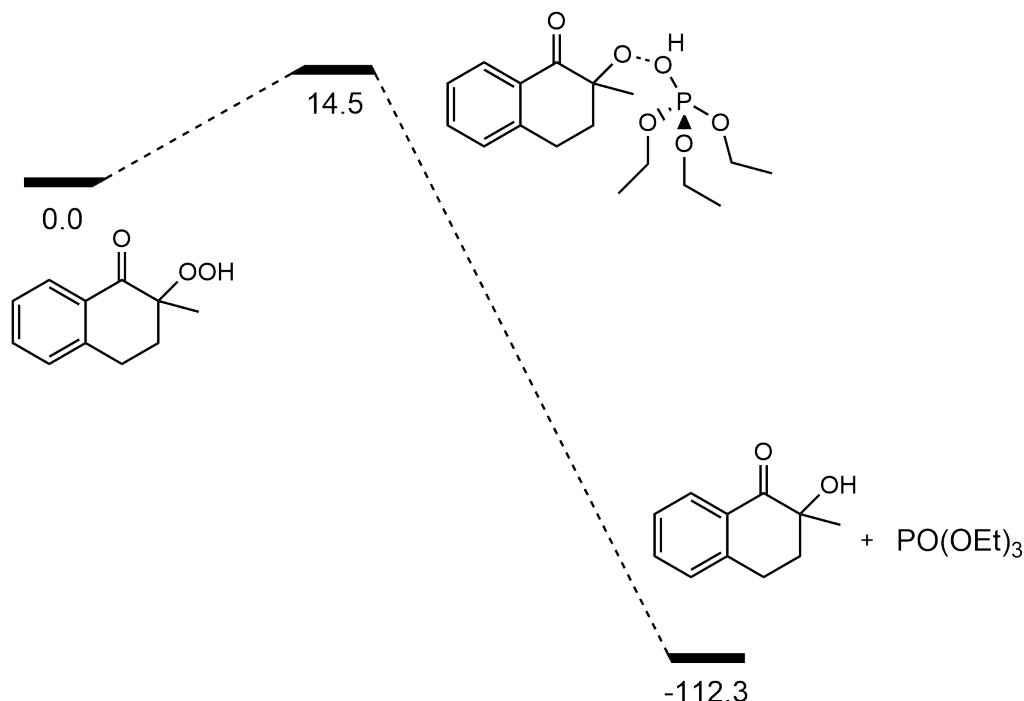


Figure 8. Free energy profile for the reduction of the hydroperoxide to the α -ketol at DLPNO-CCSD(T)/maug-cc-pVTZ//M062X-D3/maug-cc-pVDZ in the ethanol implicit solvent (SMD) level of theory. Energies are given in kcal/mol, and the zero of the energy scale corresponds to the energy of the isolated reactants. Cartesian coordinates of reactants and the transition state are shown in Table S1.

Although we are not aware of modern mechanistic studies for the reduction of hydroperoxides by phosphites, the oxidation of aromatic substrates by C4a-hydroperoxy-FADH has been widely studied over the last few years. Very recently [64], it has been proposed that the breaking of the C4a-hydroperoxy-FADH peroxide may follow a hydroxyl radical-coupled electron-transfer mechanism with the participation of singlet and triplet states. We also explored this possibility, but in our case, the triplet state was high enough in energy to disregard any role in the reaction.

3. Materials and Methods

3.1. Calculations for the Peroxidation of 2-Methyl-3,4-dihydro-1-naphthol (2)

To investigate the peroxidation mechanism of the enol (2), we carried out geometry-restrained optimizations at an M062X-D3/maug-cc-pVDZ [65] level of theory using two reactions coordinates, which were defined as: (a) r_{CO} , the distance between C α of the enol (see Figure 1) and the closest oxygen atom of O $_2$, and (b) the proton transfer coordinate from C β of the enol to molecular oxygen, which was defined as $r_{HT} = r_{O2-H\beta} - r_{O\beta-H\beta}$. The values of r_{CO} range from 1.4 Å to 3.2 Å in steps of 0.1 Å, and the values of r_{HT} range from −2 Å to 4 Å in steps of 0.2 Å. Further restraints were added to impede the addition of O $_2$ to C β and the formation of an endothermic product (Figure S1 in the Supplementary Material), associated with a higher barrier and which otherwise appears in the contour maps of the singlet PES around ($r_{HT} = 1$ Å, $r_{CO} = 2.1$ Å). Calculations were carried out using Gaussian16 [66] for the two possible multiplicities, singlet and triplet. Stationary points (minima and saddle points) were optimized again without any restraint using

the M062X-D3/maug-cc-pVDZ level of theory using Gaussian16 [66], starting from the different geometries extracted from the 3D energy profile. Frequencies were calculated to ensure convergence.

Calculations for the singlet state were repeated using broken symmetry DFT (BS-DFT) [67,68], as implemented in Gaussian16 [66], which introduces a breakdown of the spatial and spin symmetry in decoupled alpha and beta spin-orbital contributions. To correct spin contamination, we used Yamaguchi's spin projection [69,70] to describe the energies of the open-singlet states:

$$E_{\text{SP}}^{\text{singlet}} = E^{\text{singlet}} + C_{\text{SC}} [E^{\text{singlet}} - E^{\text{triplet}}] \quad (1)$$

where $E_{\text{SP}}^{\text{singlet}}$ is the corrected open-shell singlet energy, E^{singlet} , the open-shell singlet electronic energy obtained with BS-DFT, and E^{triplet} , the triplet electronic energy. The coefficient C_{SC} is calculated as follows:

$$C_{\text{SC}} = \frac{\langle S^2 \rangle^{\text{singlet}}}{\langle S^2 \rangle^{\text{triplet}} - \langle S^2 \rangle^{\text{singlet}}} \quad (2)$$

where $\langle S^2 \rangle^{\text{singlet}}$ and $\langle S^2 \rangle^{\text{triplet}}$ are the uncorrected expectation values for the total spin angular momentum of the open-shell singlet and triplet, respectively. This methodology should yield similar qualitative results as the more expensive methods employed here [36,71].

The minimum energy crossing points (MECPs) between the singlet and triplet states were calculated using the method developed by Harvey et al. [72]. In this method, an MECP search is performed by following the effective gradient given by the combination of f and g , which are defined as:

$$f = (E_{\text{SP}}^{\text{singlet}} - E^{\text{triplet}}) x_1 \quad (3)$$

$$g = \left(\frac{\partial E^{\text{singlet}}}{\partial q} - \frac{x_1}{|x_1|} \left[\left(\frac{\partial E^{\text{singlet}}}{\partial q} \cdot \frac{x_1}{|x_1|} \right) \right] \right) \quad (4)$$

where q represents the cartesian coordinates, and x_1 is defined as:

$$x_1 = \left(\frac{\partial E^{\text{singlet}}}{\partial q} - \frac{\partial E^{\text{triplet}}}{\partial q} \right) \quad (5)$$

The reaction paths connecting stationary points (saddle points and minima) and MECPs were calculated at a M062X-D3/maug-cc-pVDZ level of theory using intrinsic reaction coordinate (IRC) calculations. When the MECPs were used as starting coordinates, calculations were made independently for the singlet and triplet states. Calculations were repeated at the CASSCF/CASPT2 [73–77], and DLPNO-CCSD(T) [78] levels of theory, using a subset of the geometries generated in the IRC calculations. Finally, Gibbs free energies were calculated at the M062X-D3/maug-cc-pVDZ, and energies were refined at M062X-D3/maug-cc-pVTZ in implicit SMD solvent using an acetonitrile dielectric constant [79–82].

DLPNO-CCSD(T) calculations were performed using Orca5 [83] and a maug-cc-pVDZ basis set. Final single-points were refined with the maug-cc-pVTZ basis set. As the accuracy of the Coupled-Cluster methods is limited to mono-referential regions, a T1 diagnosis was carried out as a threshold for reliability. For mono-referential systems, the accuracy of DLPNO-CCSD(T) is comparable to that of CCSD(T), with differences in energies of less than 0.2 kcal/mol [84]. CASPT2 calculations were carried out using OpenMolcas [85]. We selected an (8,5) active space, including the π and π^* O₂ orbitals and the p orbital of C α perpendicular to the molecular plane.

To shed more light on how the electronic density is rearranged along the pathways, delocalization indices (DIs) [86–88] were calculated as implemented in the NDELOC code [89] with the Mulliken partition scheme [90]. For DIs calculations, diffuse functions were not included, to avoid an erroneous definition of the Hilbert space.

3.2. Spin-Orbit Coupling and Hopping Probabilities

The spin-orbit couplings (SOCs) were calculated at the CASPT2 level of theory, and the effective SOC was obtained using the expression [39]:

$$H_{SO}^{total} = \sqrt{S_{-1}^2 + S_0^2 + S_1^2} \quad (6)$$

where S_M is the coupling between the singlet state and each of the individual M_S components of the triplet state. H_{SO}^{total} was used to calculate the hopping probability between surfaces, using the Landau-Zener formula [37,39]:

$$p^{LZ}(\varepsilon) = \exp\left(-\frac{2\pi H_{SO}^2}{\hbar|\Delta F|} \sqrt{\frac{\mu}{2(\varepsilon - E_{MECP})}}\right) \quad (7)$$

with μ being the reduced mass associated with the ISC. $|\Delta F|$ is the norm of the difference between the gradients in the singlet and triplet states. The latter two variables were computed using the Glowfreq software [91]. The double passage version was used:

$$P_{trans}(\varepsilon) = (1 - p^{LZ}) + p^{LZ}(1 - p^{LZ}) \quad (8)$$

and the hopping probability, p^{LZ} , was calculated upon integration over the energy component perpendicular to the crossing seam, after accounting for the energy distribution using a Maxwell-Boltzmann distribution.

For spin-forbidden reactions, non-adiabatic Transition State Theory (NA-TST) provides the following rate coefficient [37]:

$$k_{NA}(T) = p^{LZ} k_{adiabatic}(T) \quad (9)$$

where $k_{NA}(T)$ and $k_{adiabatic}(T)$ are the non-adiabatic and the hypothetical adiabatic rate coefficients for an analogous spin-allowed reaction, with the latter being given by:

$$k_{adiabatic}(T) = \frac{k_B T}{h} e^{\frac{-\Delta G^\ddagger}{RT}} \quad (10)$$

where k_B is the Boltzmann constant and T is the temperature. Through the combination of Equations (9) and (10), it is possible to calculate the hindrance to the reaction caused by the spin-forbiddance of the process, $\Delta\Delta G^\ddagger$,

$$\Delta\Delta G^\ddagger = -\ln(p^{LZ})RT \quad (11)$$

which, once added to $\Delta G^\ddagger$, provides the free-energy barrier for an analogous spin-allowed reaction. For hopping probabilities of between 10^{-4} –0.1, typical values of $\Delta\Delta G^\ddagger$ are between 1–4 kcal/mol [38].

3.3. Calculations for Reduction with Triethylphosphine

To elucidate the mechanism for the reduction of the peroxide (3) with triethylphosphine, stationary points (minima and saddle points) were optimized at the M062X-D3/maug-cc-pVDZ level, and then single-point calculations were obtained using Orca5 [83] at the DLPNO-CCSD(T)/maug-cc-pVTZ level [65]. Gibbs free energies were calculated by combining electronic energies at a DLPNO-CCSD(T)/maug-cc-pVTZ level of theory, with enthalpy and entropy corrections calculated at the M062X-D3/maug-cc-pVDZ level.

4. Conclusions

In this work, we have studied the mechanism of the spin-forbidden peroxidation of a naphthol by molecular oxygen using different single-reference and multi-reference computational methods. According to our calculations, the reaction follows a two-step process, and the most likely mechanism is that in which the first molecular oxygen abstracts the O-H proton, forming a radical pair, and second, it swaps its spin state, leading to the barrierless formation of the peroxide. Contrary to what was found for reactions between O₂ and the enolates, the two steps of the process show similar barrier heights: one on the triplet PES, associated with O₂ protonation, and that associated with the MECF, which provides the effective barrier for the second step. Once in the singlet state, the protonated peroxide is formed with no barrier. ISC between these states is not favorable, as predicted by the El-Sayed rules, leading to an additional hindrance of 2–3 kcal/mol for the intersystem crossing step, which is not high enough to prevent this reaction.

For the enzymatic cofactorless spin-forbidden addition of O₂ to an organic substrate, it is typically found that the organic substrate is deprotonated before the reaction. Our results suggest that the deprotonation of the substrate is a successful strategy as it leads to significantly smaller free energy barriers, and even the approach of O₂ to the substrate becomes barrierless. It also leads to significantly larger values of the spin-orbit coupling. We believe that our results are general and can be applied to other spin-forbidden additions of O₂ to neutral double bonds.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms24087424/s1>.

Author Contributions: Calculations: P.O., S.G.-G., L.G.-S. and C.S.-S.; supervision and project administration: P.G.J.; P.O. and P.G.J. write the manuscript with contributions of all the authors. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by MCIN/AEI/10.13039/501100011033 (Ministerio de Ciencia e Innovación) grant number PID2020-113147GA-I00, and by the Fundación Salamanca City of Culture and Knowledge (a programme for attracting scientific talent to Salamanca). The PhD scholarship of P.O. was funded by Junta de Castilla y Leon and European Social Fund grant number EDU/601/2020. C.S.-S. acknowledges Ministerio de Ciencia e Innovación for the funding with grant number PID2021-122549NB-C22.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Cartesian coordinates of all relevant structures are deposited in Table S1 of the Supplementary Material. Other data is available upon reasonable request.

Acknowledgments: The authors thank M. Mandado from University of Vigo for kindly providing the NDELOC code used to calculate the electron density descriptors.

Conflicts of Interest: The authors declare no conflicts of interest.

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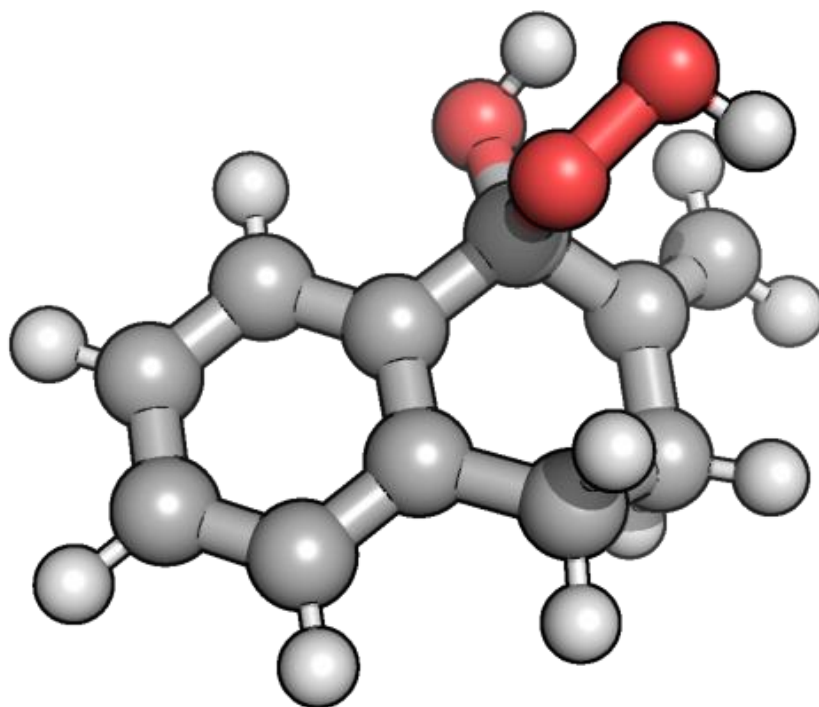
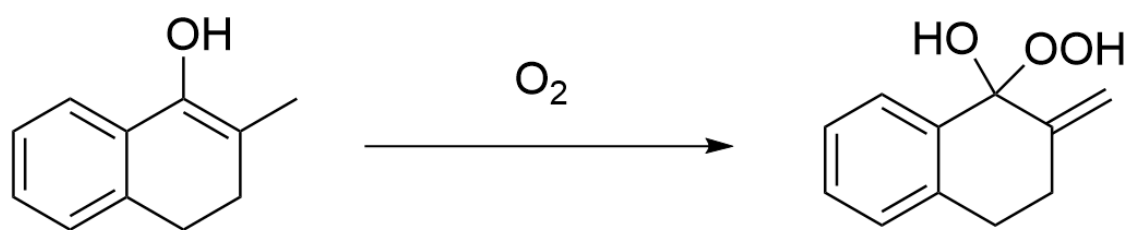


Figure S1. Top panel: Scheme of the side-reaction leading to a non-productive product. Formation of this product is associated with a higher barrier and its electronic energy is 12.9 kcal/mol above that of the products. Bottom panel: Optimized geometry. Cartesian coordinates of the side-product obtained are displayed in the cartesian coordinates section of this supplementary material.

OOH-pathway

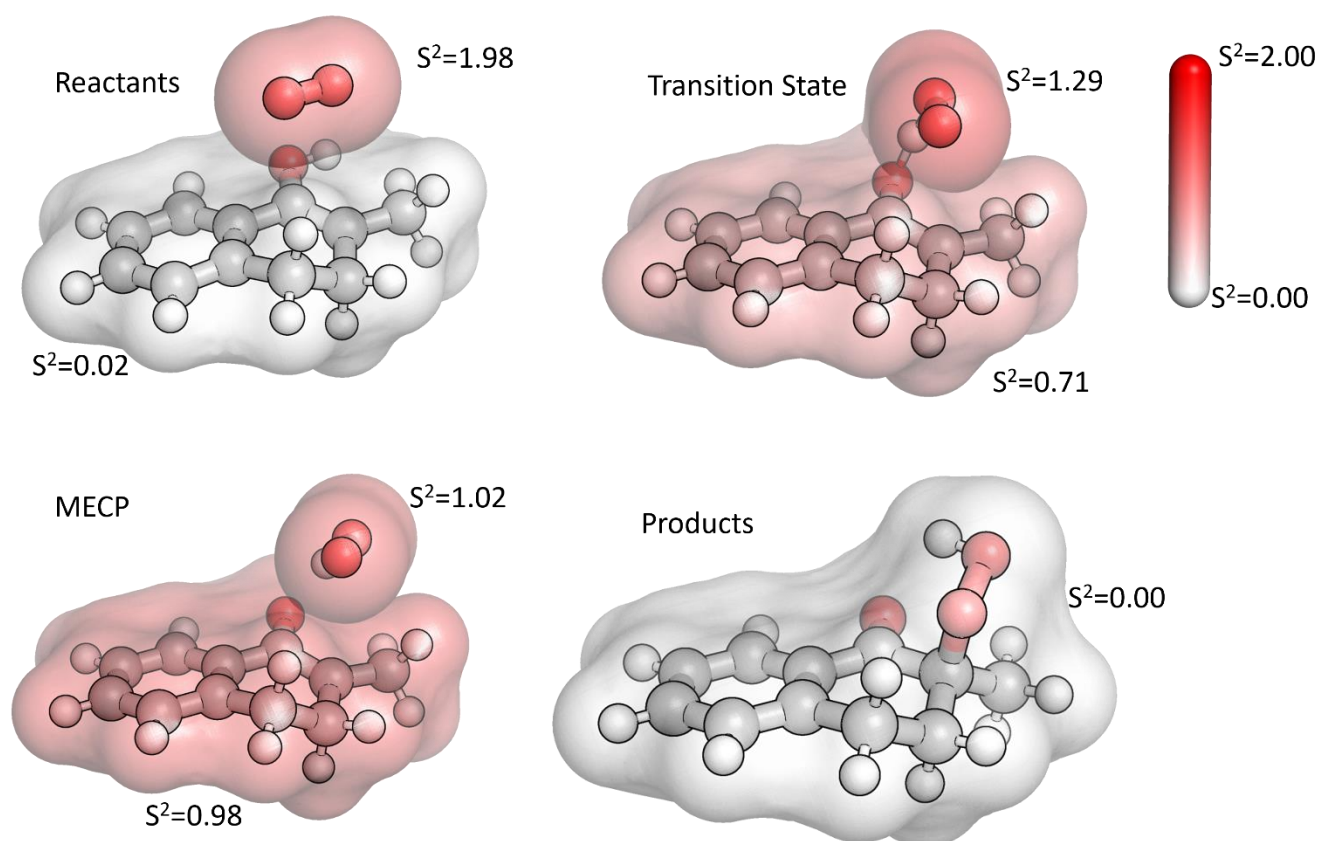


Figure S2: Mulliken Spin populations calculated at the M062X-D3/maug-cc-pVDZ level of theory for the OOH-pathway. Calculations are carried out for the triplet state, except for the products which are optimized at the singlet state. The atoms are displayed in a ball-and-stick representation surrounded by a surface which is coloured using a white-red scheme based on the spin population for each group of atoms. The groups of atoms are defined according to the internuclear distances and at the transition state and MECP one group correspond to the OOH moiety. Mulliken spin populations agree with the DIs calculations supporting that the Hydrogen atom transfer and formation of a radical pair.

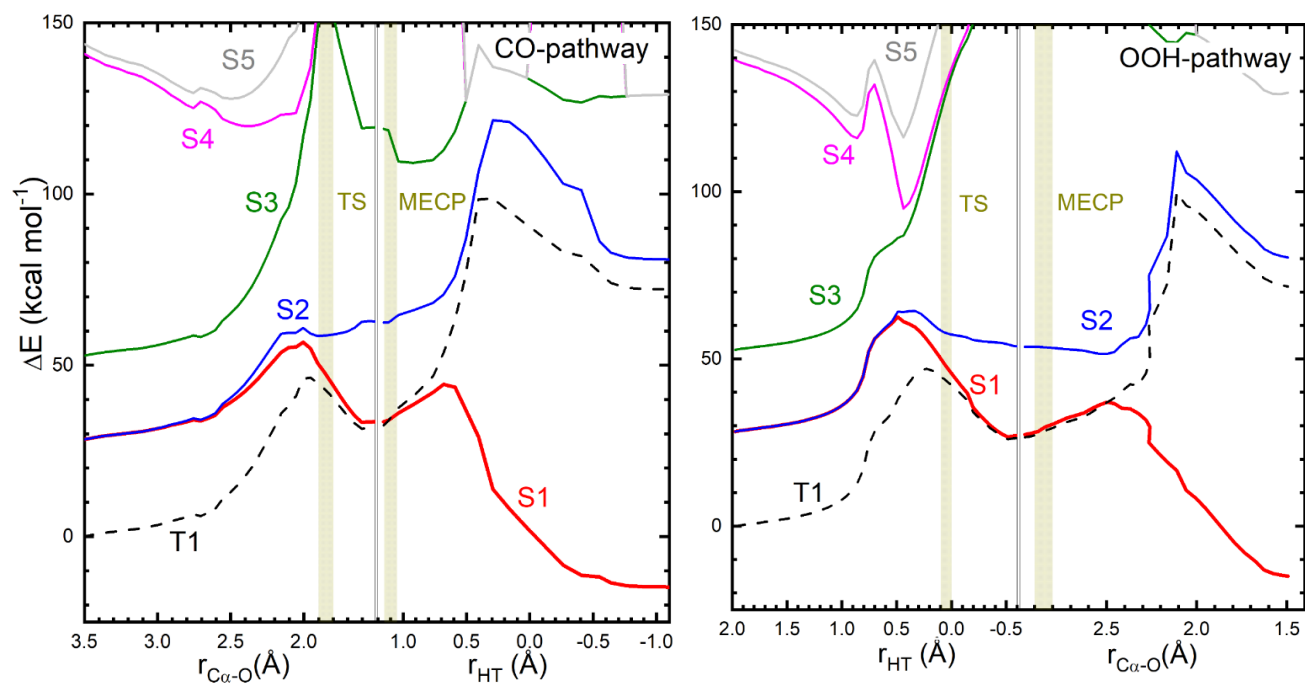


Figure S3. Energy profile for the addition of O₂ to 2-methyl-3,4-dihydro-1-naphthol along the two possible reaction paths for MRCI/6-31+G(d,p) level of theory. Results for the CO-pathway are shown in the top panel and those for the OOH pathway in the bottom panel. Triplet state is shown as dashed lines while the five singlet states are shown as solid lines. The active space is formed by 2 π^* antibonding orbitals from O₂ and the p-orbital from C α (CAS(4,3)).

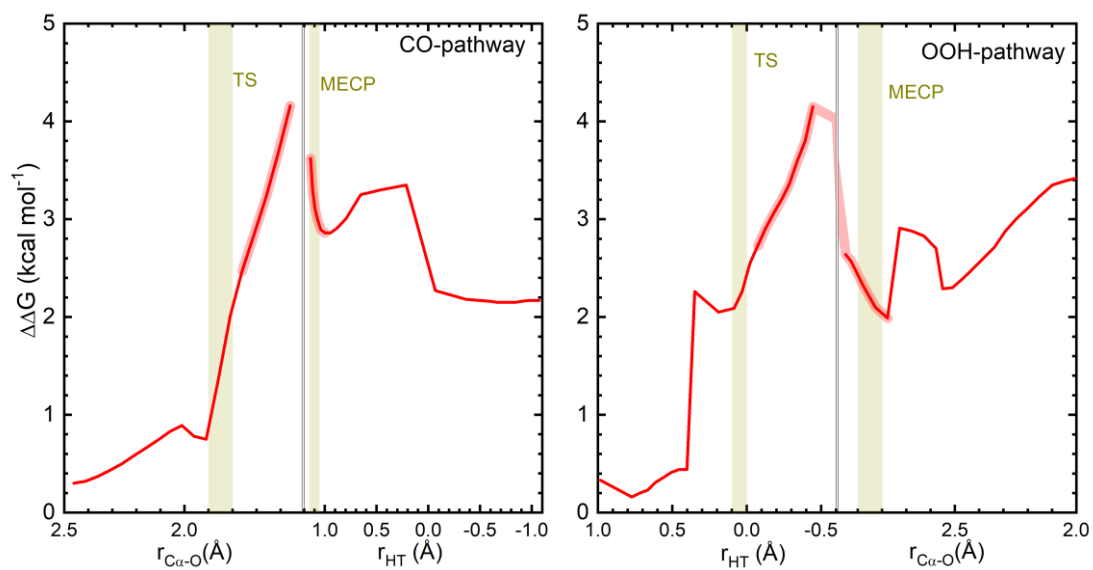


Figure S4. Calculated increase in the free energy profile for the intersystem crossing (ISC) between triplet and singlet, calculating according to Eq. 11 of the main text. Regions in which singlet and triplet states are almost degenerate are red-shaded.

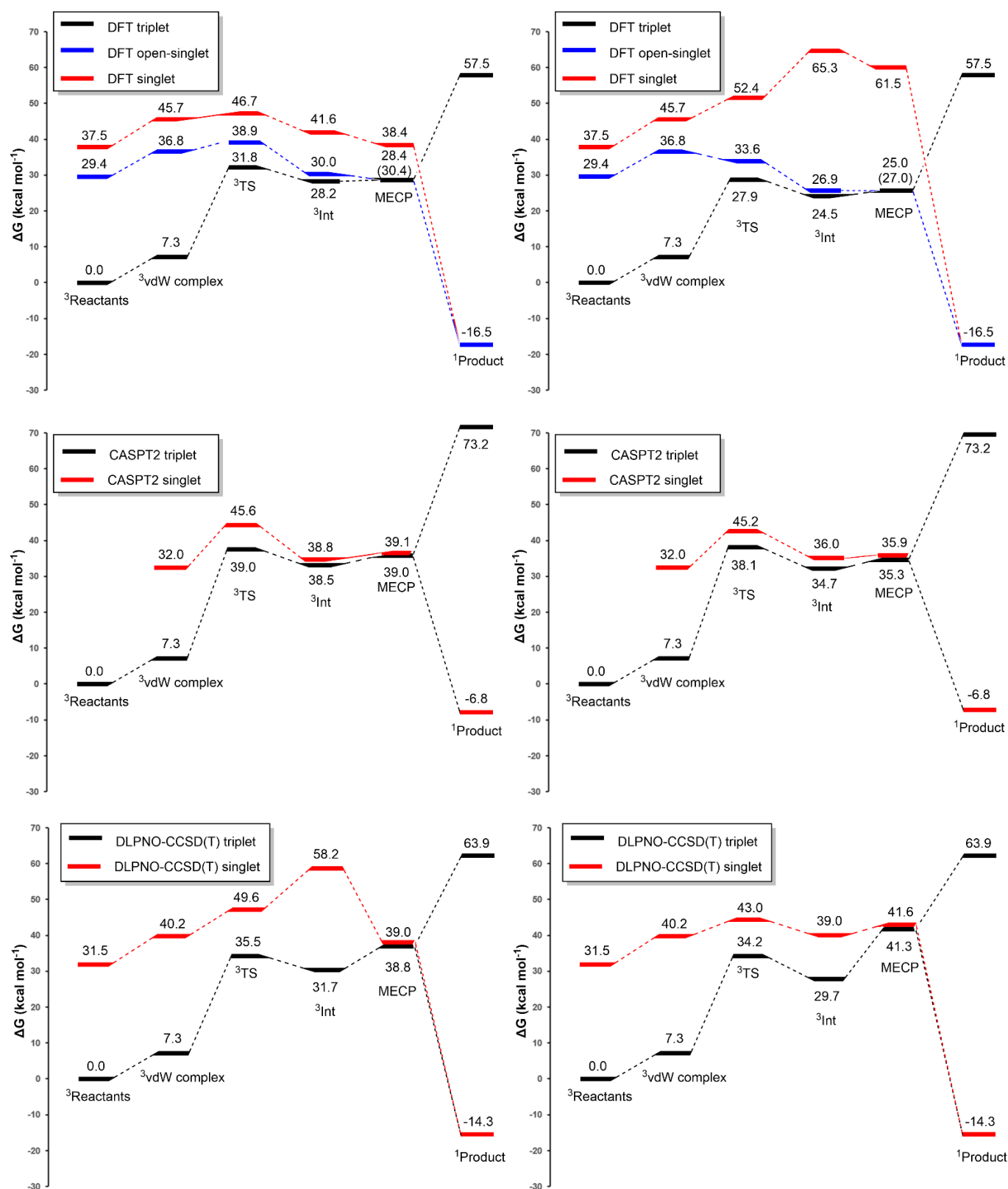


Figure S5. Free energy profile for the addition of O₂ to 2-methyl-3,4-dihydro-1-naphthol along the two possible reaction paths for M062X-D3, CASPT2(8,5) and DLPNO-CCSD(T). Single-points with maug-cc-pVTZ basis sets over maug-cc-pVDZ geometries and vibrational analysis in acetonitrile implicit solvent (SMD) level of theory. Each structure was optimized for the lowest spin state. Increase in free energies due to hopping probability in parentheses for MECP structures. Left panel: CO-pathway. Right panel: OOH-pathway.

CONCLUSIONS

6 Conclusions

- We have used multireference and single-reference computational methods to study the reaction between O_2 and two kind of molecules: an enolate (both in gas phase and in a protein environment) and a neutral enol.
- In connection with our objectives, we have elucidated a mechanism for the spin-forbidden oxygenation with a cofactorless enzyme (DpgC). Notably, we have considered the role of spin-orbit coupling in this reaction, identifying its influence on raising the energy barrier for these reactions. In Ref. 2, we have studied in detail the possible electronic states involved in the oxygen molecule, from the ground triplet to other excited singlet states. The results show that the singlet state of the product comes from a hypothetical O_2^{2-} ion, related to a charge transfer.
- We have further analyzed the concepts of charge transfer in the studied oxygenation reactions. Our investigation has highlighted their critical role in facilitating the reaction, and led us to favor the charge transfer hypothesis as the most accurate description of these reactions. Further development on these cofactorless oxidation/oxygenation processes is required to extend this charge transfer hypothesis to other cofactorless enzymes.
- The DpgC enzyme environment has been studied using QM/MM methods, offering novel insights into the function of the enzyme. Our studies of DpgC enzyme revealed that no amino acids were available to protonate O_2 . Our results suggest that the enzyme plays a passive role in promoting the intersystem crossing, subtracting a proton to generate an anion capable of reducing oxygen via charge transfer. The proton is eventually captured by a water wire that connects the

active site and the bulk.

- We have developed new geometric optimization algorithms, employing energies and gradients from diverse methods to optimize MECPs at the DFT level (including BS-DFT). It is important to note that in the area close to the MECP, differences between the standard DFT and BS-DFT may occur, so it is safer to use BS-DFT, at least in the case of reactions between O_2 and neutral enol. In any case, the BS-DFT method represents more accurately the triplet-singlet excitation energy for the oxygen molecule.
- The insights acquired from our study of enzymatic reactions have been applied to an organic reaction of similar characteristics. In this case, the substrate had no charge (enol group). In this case, there was also some amount of charge transfer towards oxygen, facilitated by a double bond in the substrate. Dioxygen protonation occurred simultaneously with the charge transfer in this case.
- The knowledge of multi-configurational/multi-referential methods has been deepened, applying them to complex systems. Furthermore, it has been observed that despite recent advances in this type of methods, there is still a lot of work to be done in terms of stability and reproducibility of results, the algorithms must be more stable numerically and must include more functionalities (for example, an effective treatment of excited states, subsequent perturbations in the case of SHCI, or in general greater ease of identification of orbitals).

7 Appendix: Software technical details

High-performance computing and parallel programming

In the context of Theoretical Chemistry, the importance of computational techniques and resources cannot be understated. This thesis extensively utilizes such methodologies for precision-based simulations and analyses, using Quantum Mechanics-based programs for numerical computations and molecular visualizations.

The evolution of computer science, deeply rooted in mathematics and spurred by the electronics explosion during the 19th and 20th centuries, has influenced the development of advanced computational techniques. The manifestation of Moore’s Law¹⁸⁵ over the decades has seen processor power grow exponentially with respect to size, hitting now potential physical limits. Consequently, the focus has shifted towards parallelisation in hardware and software, leading to more processors in mainstream computers.

Parallelisation is of great importance in scientific computing, since much of the research work on new computational methods focuses on the theoretical possibility of parallelising certain types of computation (e.g., electronic integrals, diagonalisation of matrices, etc.). Some of the commercial codes that have been used, described below, obtain parallelisation efficiencies close to 100%, so that the computational speed can be increased as many times as desired, simply by including more processors in the computation. Most modern computing servers include extensive compute nodes with hundreds or thousands of processors in parallel. A simple scheme is shown in Figure 18. To work with parallel computing, one of the most common libraries is OpenMPI,¹⁸⁶ or in the case of Python, its adaptation as MPI4Py.¹⁸⁷

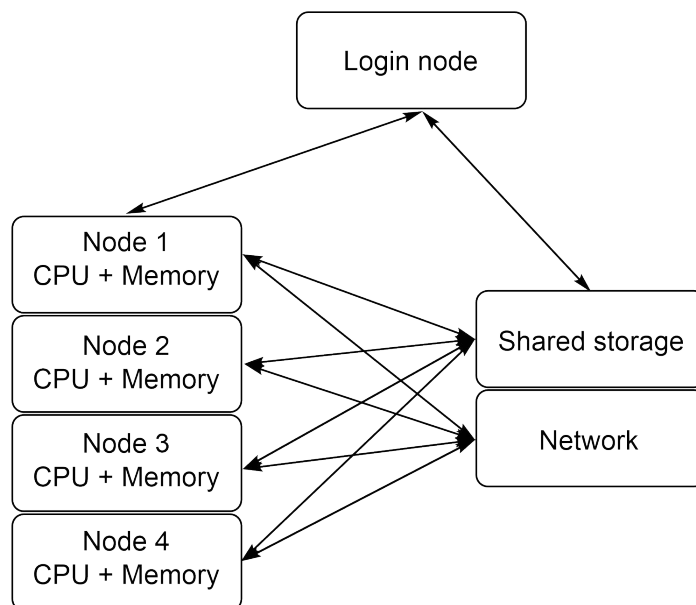


Figure 18: Generic diagram of a computing cluster. The login node is the only node with access to the external internet network, all other connections are internal. In general, these clusters have shared disk, and RAM memory for each node separately.

Commercial software

To carry out all the calculations published throughout the thesis, a number of programmes have been used, both commercial and open source. Commercial software is licensed, while open-source software is freely available to everyone, at least for research purposes.

The most widely used commercial software in this thesis has been Gaussian,¹⁸⁸ one of the best-known programs in the field of Computational Chemistry and a benchmark in its sector. This program allows a large number of calculations to be carried out with different theoretical methods, including many of the methods described in the previous sections. It is mainly used in this work to carry out geometrical optimisations and to perform DFT calculations on a routine basis. Q-Chem¹⁸⁹ has also been used for the same purposes, sometimes for purely comparative purposes and to improve the

robustness of the studies.¹⁹⁰

Two other programs used in some calculations have been Molpro¹⁹¹ and Molcas¹⁹², both focused on Active Space methods. Single-point calculations have been done with CASSCF and CASPT2 with different active spaces.

Finally, Orca is a commercial program,¹⁹³ although licensed free of charge for research purposes, which has been used for various DFT calculations, geometric search of MECP and coupled-cluster calculations.

Regarding molecular dynamics, there are mainly two programs that are the most used in general to solve this kind of problem. CHARMM⁸¹ and NAMD¹⁹⁴, both of which are mainly used to carry out simulations with macromolecules such as proteins or DNA chains. For the treatment of molecular mechanics it is necessary to use data files known as force fields, which consist of lists of interactions between different types of atoms, angles, different types of amino acids and other biomolecules, optimised using empirical information.^{195,196} The CHARMM-GUI graphical interface has also been used to generate some of the models used as initial geometries in molecular dynamics.^{197,198} These initial geometries are obtained from an X-ray crystal structure to which hydrogens are added according to empirical data and metal ions are introduced to reproduce the cellular environment.

There are three graphical software programs which have been employed in this thesis to visualize molecules in different states or representations. Namely, these are GaussView6,⁸² Avogadro¹⁹⁹ and PyMol.²⁰⁰ GaussView6 has the same origins as Gaussian and its license is mostly the same, Avogadro is an open-source visualization software and PyMol is developed under a commercial license, although free versions are available to researchers.

In addition to these commercial programs, the following section describes the most widely used open-source software used throughout the thesis, for various specialised computational purposes and methods.

Python and PySCF

In scientific computing, the Python programming language is becoming more and more significant. Python provides quick development processes and simple program maintenance as a high-level language. Although it might be challenging to quantify programming productivity, it is generally accepted that Python is more effective at prototyping novel concepts than conventional compiled low-level languages like Fortran or C/C++. Using the excellent numerical libraries in Python, like NumPy, SciPy, and MPI4Py, applications written in Python may compete with those written in optimised Fortran and C/C++, including those that use complex computational architectures.

Several attempts to include Python in applications for electrical structures have been done in the past. The quantum chemistry program Psi4 utilises its own "Psithon" dialect to control the underlying C++ implementation,²⁰¹ while broader simulation environments like ASE and PyMatGen offer Python front-ends for a number of quantum chemistry and electron structure packages to organise complicated processes. The Tensor Contraction Engine and the SecondQuantizationAlgebra module are two examples of symbolic code-generating tools that have been implemented in Python.

The open-source collection of electronic structure modules known as PySCF (Python-based Simulations of Chemistry Framework) is based on Python.^{202–204} The program offers a straightforward, portable, and effective framework for developing quantum chemistry computations and methods. Mean-field and post-mean-field techniques may

be employed with PySCF to replicate the characteristics of molecules, crystals, and modified Hamiltonians. Nearly all functions in PySCF are built in Python to facilitate expansion, while computationally essential components are developed and optimised in C. The package is as effective as the top C or Fortran-based quantum chemistry applications thanks to this combined Python/C implementation.

A vast ecosystem of extension modules is supported by PySCF in addition to the core libraries. CASSCF, coupled cluster theory, DFT, and pertinent modules for molecular characteristics have all been added to the program over time. The first stable version, PySCF 1.0, which focuses on efficiency, universality, and simplicity, was published in 2015. This decision led to the majority of PySCF’s functions being written entirely in Python, with very little C code other than for the most time-sensitive sections. Users may quickly customise the code to their own scientific needs because the numerous functions and application programming interfaces (API) have been developed and built to be as basic and easy as feasible. The SHCI used in this thesis is connected to PySCF this way, through an external interface. There are currently two implementations of this method: a more general one, called Dice⁴³, and an accelerated version, called Arrow⁴⁵, still under development.

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