Communications in Computational Chemistry
Commun. Comput. Chem., Vol. 7, No. 3, 190–201
doi: 10.4208/cicc.2025.133.01
Regular Article

Computational Investigation into the Enzyme-Catalyzed [4+2] Cycloaddition of Decatromicin

Wenhao Gu[1] and John Z. H. Zhang[1,2,3,4,*]

[1]*School of Chemistry and Molecular Engineering, East China Normal University at Shanghai, 200062, China*;

[2]*Faculty of Synthetic Biology, Shenzhen University of Advanced Technology, Shenzhen 518107, China*;

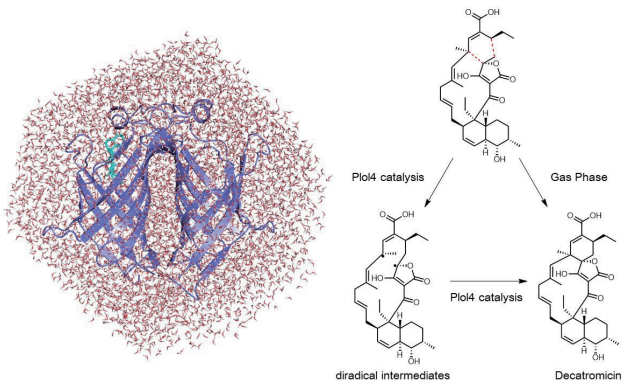
[3]*NYU-ECNU Center for Computational Chemistry and Shanghai Frontiers Science Center of AI and DL, NYU Shanghai, Shanghai 200126, China*;

[4]*Department of Chemistry, New York University, NY, NY10003, USA.*

[*] Corresponding author: John.zhang@nyu.edu

Received on 13 May 2025; Accepted on 3 June 2025

Abstract: This study presents a detailed examination of enzyme-catalyzed pericyclic reactions, specifically focusing on the synthesis of spirotetronate (decatromicin), catalyzed by the Plol4 enzyme. Utilizing the QM/MM method, we have discovered that the Plol4 enzyme significantly reduces the energy barrier in the Diels-Alder (DA) reaction, demonstrating its effectiveness in facilitating complex chemical processes. Our analysis suggests that this reduction is partly due to the interaction between the substrate and active site residues within the enzyme, which prearranges the substrate in a manner conducive to the [4+2] cycloaddition. Moreover, we observed a notable shift in the DA reaction mechanism from a concerted approach within the gas phase to an asynchronous approach within the enzymatic environment. This finding highlights the unique role of Plol4 in altering reaction pathways and underscores the enzyme's potential in synthetic applications. Overall, this research provides valuable insights into the mechanisms of enzyme-catalyzed reactions and their implications for the synthesis of polycyclic compounds.



Key words: Enzyme, Cycloaddition, Decatromicin, QM/MM, Diels-Alder, reaction pathways, synthesis.

1. Introduction

For almost a hundred years, the Diels-Alder reaction has been a key method for synthetic chemists in creating both important natural substances and their non-natural counterparts, which hold biological importance. This reaction is particularly useful for constructing cyclic products with excellent region and stereoselectivity, and thus is considered one of the most important reactions in organic synthesis. Over the past decade, numerous research groups have applied enzymes capable of catalyzing the Diels-Alder reaction to the biosynthesis of natural products [1-16]. Spirotetronate compounds, known for their anticancer and antimicrobial properties, hold potential as pharmaceutical agents. Consequently, many research groups have dedicated significant efforts in recent years to the synthesis and identification of these substances. One of the hottest areas of research is the biosynthesis of spirotetronates. In recent years, enzymes capable of catalyzing the synthesis of spirotetronates have been successively reported, marking a notable advancement in the field.

In 2020, the Liu Wen research group collaborated with the Houk research team to investigate the activation and stereoselective mechanism of the intramolecular Diels-Alder reaction catalyzed by the monofunctional D-A enzyme PyrI4 [17]. This enzyme plays a pivotal role in the biosynthesis of pyrroindomycins, an important natural product within the Spirotetronates family, particularly in the formation of its critical spiro-tetronate backbone. The key activation effects of PyrI4 stem primarily from acid catalysis, an induced-fit conformational adaptation mechanism, and its unique "lid-closing" feature that stabilizes the Diels-Alder reaction transition state. Additionally, PyrI4 enzyme enhances the intrinsic Diels-Alder stereoselectivity of the substrate, leading to the stereoselective synthesis of the product.

In the subsequent year of 2022, their investigations utilizing quantum mechanics and molecular dynamics demonstrated the distinct operational modes of the PyrE3 enzyme in catalyzing the biosynthesis of pyrroindomycins [18]. This enzyme adeptly arranges anionic polyene substrates into a high-energy reaction conformation. Within this conformation, an inverse electron demand Diels-Alder reaction can occur with a low energy barrier. The stereoselectivity is achieved through strong binding interactions within the endo stereochemical arrangement and local spatial constraints imposed on the endo-1, 3-diene unit. These findings elucidate the divergent mechanisms of PyrE3 and PyrI4, highlighting nature's evolutionary prowess in developing multiple methods to catalyze Diels-Alder reactions.

In 2023, Liu Wen, Pan Lifeng, and Ken N. Houk collaborated once again, and their research teams made a groundbreaking discovery in the biosynthetic pathway of pyrroindomycin: a novel cyclase enzyme responsible for the formation of chiral spiro-rings [19]. This enzyme, characterized by a β-barrel fold, operates independently of light activation. When used with pyrroindomycin intermediates as substrates, it not only produces exo and endo [4+2] addition products but also generates exo [2+2] addition products. Employing structural biology and computational chemistry, the authors elucidated the molecular mechanisms through which this enzyme catalyzes different cycloaddition reactions, achieving region- and stereoselectivity control. They proposed and validated a reaction process involving bi-radical intermediates, which thermodynamically favors [4+2] products and kinetically favors [2+2] products. Through directed evolution, the researchers precisely modulated the chemical and stereoselectivity of the enzyme, obtaining mutant proteins that exclusively catalyze single cycloaddition reactions of exo [4+2], endo [4+2], and exo [2+2].

However, another significant compound in the Spirotetronates family[20-30], decatromicin, has not been extensively reported in terms of its biosynthesis. Compared to pyrroindomycins,decatromicin possesses two additional carbon atoms in its main carbon chain. The Liu Wen research group hypothesized that the enzyme Plol4 might catalyze the synthesis of decatromicin-like substances [19]. In 2022, Yoganathan Kanagasundaram, Veronica W. Ng, Siew-Bee Ng, and others identified several previously unreported Spirotetronate compounds from extracts of Actimonadura sp [31]. They evaluated their antibacterial activities against Gram-negative bacteria, including Acinetobacter baumannii, and Gram-positive bacteria, such as Staphylococcus aureus. Additionally, they studied their cytotoxic effects on human cancer A549 cells. Among these, decatromicin D showed promising results in these aspects. Consequently, we decided to investigate whether the Plol4 enzyme catalyzes the synthesis of decatromicin D through advanced computational methods, including Density Functional Theory (DFT) calculations and Quantum Mechanics/Molecular Mechanics Molecular Dynamics (QM/MM MD) simulations. This approach reflects a sophisticated blend of experimental and computational strategies to unravel the intricate enzymatic pathways involved in the natural synthesis of complex molecular structures.

2. Computational details

2.1 DFT calculations

Gaussian16 was used for all density functional theory computations [32]. Geometry optimization and subsequent frequency calculations were conducted for each species at the B3LYP/6-31G(d) level of theory, incorporating Grimme's D3 empirical dispersion correction [33-34], in the gas phase and utilizing an ultrafine level integration grid. Analysis of normal vibrational modes ensured that the optimized structures were either minima or transition states. Zero-point vibrational energy (ZPE) and thermal corrections were determined using B3LYP/6-31G(d)-D3 frequencies. The potential energy of each structure was calculated at the B3LYP/6-31G(d)-D3 level. Gibbs free energies reported at 298K were the sum of these single-point electronic energies, ZPE, and thermal corrections, all determined using B3LYP/6-31G(d)-D3 frequencies and an ultrafine level integration grid.

2.2 Docking calculations

Autodock Vina[35] was employed for docking calculations. The docking target was Chain A of the crystal structure of Diels-Alderase Plol4 (PDB: 7X7Z). Any substrate present in the crystal structure was removed. We centered a 16Å×16Å×16Å grid box on the catalytic site. The DFT- optimized structure of each substrate, specifically the optimized transition state in the gas phase, was docked into the binding site. For docking the transition state, we used a rigid docking approach, meaning no bonds were allowed to rotate. The docking pose with the lowest energy was selected, reported, and utilized for further investigations..