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Mechanistic investigation of repurposed photoenzymes with new-to-nature reactivity

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Biocatalysis is widely renowned for its remarkable efficiency, selectivity, and known for operating under mild conditions. While most enzymatic reactions progress without light irradiation, recent studies have identified light as a crucial factor in the activation of certain naturally occurring enzymes. These findings have spurred the rapid advancement of photoenzymatic catalysis in the past few years, where enzymes are not typically known for light activation perform excited-state chemistry with or without the presence of external photocatalysts to facilitate new-to-nature transformations that are challenging for traditional chemical synthesis. In this review, we summarize the experimental and computational methods used to investigate the catalytic mechanisms of repurposed photoenzymes with new-to-nature reactivity and discuss how these insights can inform the design of new photoenzymatic catalytic systems.

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Introduction

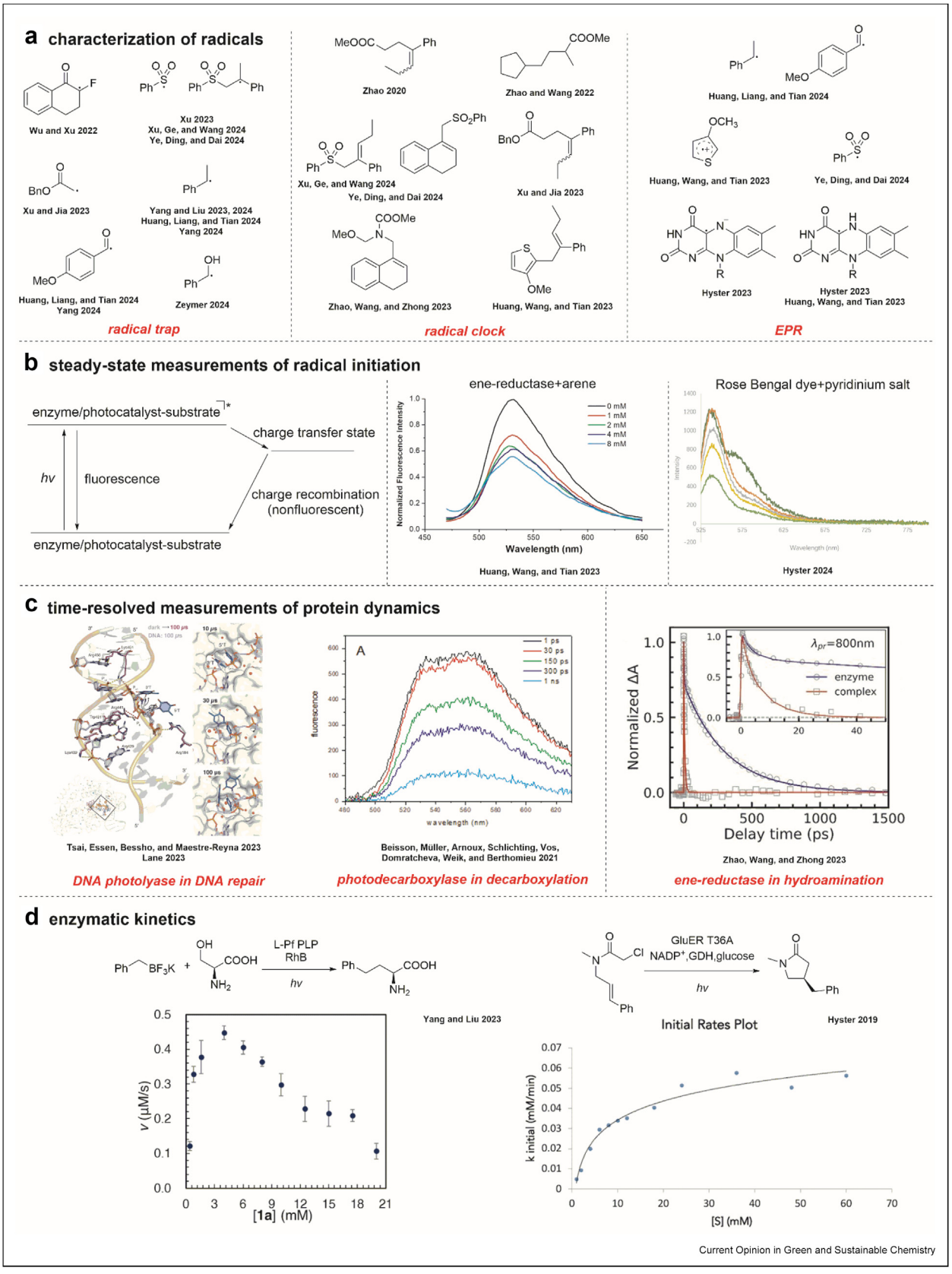
Biocatalysis holds significant promise for economical biomanufacturing and innovative biotechnologies, with many applications relying on novel enzyme reactivity and selectivity [1–3]. From a biology perspective, enzymes can be discovered through bioprospecting or genome mining, while the structure of an enzyme can be modified via mutagenesis or designed from scratch [4–7]. From a chemistry perspective, an enzyme specialized in one reaction could potentially catalyze reactions with similar mechanisms and can be chemically modified during or after protein expression for diverse catalytic purposes [8–10]. Unlike these traditional methods, photoenzymatic catalysis examines enzyme reactivity under light illumination, offering a novel approach to discovering new enzymatic reactions. Various natural and engineered oxidoreductases, transferases, and cyclases have been identified to produce radicals photocatalytically from unnatural precursors or facilitate the reaction between photochemically generated radicals and ground-state enzymatic intermediates [11–14]. This has enabled many challenging enantioselective transformations, including hydroalkylation, hydroamination, hydrosulfonation, [2 + 2] cyclization, acylation, reductive and oxidative couplings, and the synthesis of amino acid derivatives [15–28]. However, the full synthetic potential of photoenzymatic catalysis remains underexplored due to the limited types of reactions and enzymes studied. Uncovering enzymes with photoenzymatic behavior requires a multidisciplinary knowledge of synthetic methodologies, photochemistry, and enzymology.

In this review, we emphasize the experimental and computational methodologies employed to characterize repurposed photoenzymes, reaction intermediates, and essential catalytic processes, with a special focus on *in vitro* photoenzymatic systems. The resulting mechanistic insights will enable researchers across diverse disciplines to be more effectively design and enhance novel or existing photoenzymatic catalytic systems. This review intentionally steers away from detailing specific reaction developments, as these topics have been extensively covered in recent literature [11,29–31].

Experimental methods

Various experimental methods have been employed in the mechanistic studies of photoenzymatic catalysis. Characterization of radical intermediates confirms the involvement of radical mechanisms. Steady-state optical measurements of enzyme–substrate complexes aid in understanding the radical initiation step. Time-resolved measurements of photoenzymatic systems offer insights into detailed reaction pathways and their rates. Enzymatic kinetics sheds light on enzyme–substrate interactions, while isotope-related experiments can be conducted in parallel to the aforementioned experiments to further enhance the understanding of photoenzymatic reactions.

Figure 1


a characterization of radicals
Wu and Xu 2022
Xu 2023
Xu, Ge, and Wang 2024
Ye, Ding, and Dai 2024
Xu and Jia 2023
Yang and Liu 2023, 2024
Huang, Liang, and Tian 2024
Yang 2024
Huang, Liang, and Tian 2024
Yang 2024
Zeymer 2024
radical trap
Zhao 2020
Zhao and Wang 2022
Xu, Ge, and Wang 2024
Ye, Ding, and Dai 2024
Xu and Jia 2023
Zhao, Wang, and Zhong 2023
Huang, Wang, and Tian 2023
radical clock
Huang, Liang, and Tian 2024
Huang, Wang, and Tian 2023
Ye, Ding, and Dai 2024
Hyster 2023
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EPR
b steady-state measurements of radical initiation
enzyme/photocatalyst-substrate*
charge transfer state
hv
fluorescence
charge recombination
(nonfluorescent)
enzyme/photocatalyst-substrate
ene-reductase+arene
Normalized Fluorescence Intensity
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Huang, Wang, and Tian 2023
Rose Bengal dye+pyridinium salt
Hyster 2024
c time-resolved measurements of protein dynamics
Tsai, Essen, Bessho, and Maestre-Reyna 2023
Lane 2023
DNA photolyase in DNA repair
A
fluorescence
wavelength (nm)
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150 ps
300 ps
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Beisson, Müller, Arnoux, Schlichting, Vos,
Domratcheva, Weik, and Berthomieu 2021
photodecarboxylase in decarboxylation
Normalized ΔA
Delay time (ps)
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complex
Zhao, Wang, and Zhong 2023
ene-reductase in hydroamination
d enzymatic kinetics
L-Pf PLP
RhB
hv
Yang and Liu 2023
v (μM/s)
[1a] (mM)
GluER T36A
NADP+,GDH,glucose
hv
Hyster 2019
Initial Rates Plot
k initial (mM/min)
[S] (mM)
Current Opinion in Green and Sustainable Chemistry

Characterization of radicals

Photoenzymatic reactions often progress through radical mechanisms, which can be confirmed by identifying the radical intermediates using radical traps, radical clocks, and electron paramagnetic resonance (EPR). Radical traps such as 2,2,6,6-Tetramethylpiperidin-1-oxyl (TEMPO) can capture radicals after initiation and radical addition steps, with the trapped products being stable enough for subsequent analysis in mass spectrometry [20,23–25,27,28,32–35]. Radical clocks involve the reaction of radicals with unsaturated bonds, leading to cyclization or ring opening, and provide valuable information on reaction rates by comparing the reactivity of different types of radical clocks [16,18,20,34,36,37]. EPR measures electromagnetic absorption resulting from the splitting of electron spins in magnetic fields and is a useful technique for characterizing substances with unpaired electrons. The signal of radicals can be directly observed under low temperatures or with the presence of a spin trap such as 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) [25,27,37,38]. Both the type and environment of a radical significantly influence the EPR signal observed, thereby providing valuable information on the nature of the radical reaction. Figure 1a summarizes the types of radicals characterized by these methods in photoenzymatic catalysis. In addition, photoenzymatic catalysis can potentially benefit from many widely used techniques for the characterization of key intermediates in radical enzymes including but not limited to Mössbauer spectroscopy and electron nuclear double resonance (ENDOR) [39–43].

Steady-state measurements of radical initiation

Radicals in photoenzymatic reactions are typically produced by single-electron oxidation or reduction of substrates by direct excitation of cofactors, cofactor-substrate electron donor–acceptor (EDA) complexes, or external molecular photocatalysts. The thermodynamic energy barriers of these electron transfer events are determined by the relative differences in the energies of the corresponding molecular orbitals, which can also be studied by comparing the redox potentials of these molecules. The redox potential of small molecules can be routinely measured using cyclic voltammetry while the direct measurement of excited-state redox potential of enzymes can be complicated [44–46]. A more commonly employed protocol involves derivatizing an enzyme's ground-state reduction potential from the kinetic profile of its reduction/oxidation of a known molecule [47]. The excited-state reduction potential can then be estimated by incorporating the energy of an enzyme's absorption bands [37]. In parallel, Stern–Volmer studies characterize the emission spectrum of dyes (enzymes or photocatalysts) with the presence of different amounts of quenchers (substrates). A decrease in the emission intensity can provide indirect evidence for enzyme/photocatalyst–substrate interactions, as the extent of quenching can be influenced by many factors such as the diffusional process, the covalent or noncovalent interaction between an enzyme and a substrate, the efficiency of an electron or energy transfer event, and the change of the enzymatic environment of a cofactor. Figure 1b shows the mechanism for Stern–Volmer study and examples of fluorescent quenching of enzyme/photocatalyst with substrates [37,48].

Time-resolved measurements of protein dynamics

Time-resolved techniques, which involve the spectroscopic or structural characterization of a photoenzymatic system from picoseconds to microseconds after photoexcitation, have been applied to track the enzymatic intermediates, such as the cofactors' redox and protonation states, the interaction of cofactors with amino acid residues, and the formation of reaction intermediates. Analysis of this information provides evidence on the types and rates of the fundamental steps involved in photoenzymatic reactions. Exemplary works are highlighted in Figure 1c. Studies on protein dynamics of a DNA photolyase using time-resolved absorption and time-resolved X-ray crystallography allows better understanding of the DNA repairing process in nature [49–53]. Similar studies on photodecarboxylases and ene-reductases reveal the rates and sources of proton and electron transfer events, inspiring the design of new reactions with similar mechanisms [18,38,54–58]. In addition, coupling time-resolved techniques with isotope labeling and site-directed mutagenesis can provide useful information on the roles of specific amino acids, which can be further engineered for improved performances [56,59]. Certain light sources, such as blue light, can irreversibly damage proteins by causing amino acid crosslinking, generating reactive oxygen species (ROS), and forming unwanted by-products. These effects can interfere with the desired signals and compromise measurements. To mitigate these issues, alternative light sources (e.g. lower-intensity or near-infrared wavelengths) can be used to reduce photodamage. Adding ROS scavengers like ascorbate helps neutralize reactive species, while analyzing the signals of by-products can distinguish them from protein dynamics. These strategies enhance the accuracy and reliability of time-resolved measurements by minimizing light-induced artifacts.

Experimental methods for studying the mechanism of photoenzymatic catalysis. (a) Types of radicals from photoenzymatic reactions that have been characterized by radical trap, radical clock, and EPR. **(b)** Mechanism and examples of fluorescent quenching of enzyme/photocatalyst with substrates. **(c)** Time-resolved measurements of DNA photolyases in DNA repair and ene-reductases in hydroamination. **(d)** Enzymatic kinetics of photobiocatalysis with and without the presence of a molecular photocatalyst. Reproduced from ref. 15, 18, 23, 37, 48, 49, 58 with permission from American Chemical Society, Springer Nature, American Association for the Advancement of Science, copyright 2019, 2021, 2023, and 2024. EPR, electron paramagnetic resonance.

Beyond the already established time-resolved techniques used in photoenzymatic catalysis, many steady-state characterization techniques for photoenzymes such as cryogenic electron microscopy could potentially be adapted for time-resolved measurements to provide more mechanistic insights into photoenzymatic catalysis across multiple dimensions [60—62].

Enzymatic kinetics

Like ground-state enzymatic reactions, photoenzymatic reactions involve the formation of an enzyme—substrate complex, enzymatic reactions, and the release of products. Enzymatic kinetics is crucial in understanding the rates of these processes but remains less studied in photoenzymatic catalysis. As shown in Figure 1d, Hyster and coworkers investigated the kinetic profile of a photoenzymatic intramolecular hydroalkylation reaction catalyzed by ene-reductases and observed that the K_M of ~10 mM is similar to the ene-reductase-catalyzed reduction of unnatural α, β-unsaturated carbonyl compounds [15]. The k_{cat} of this reaction is light dependent, suggesting the entire process is photon limited. The identification of this limiting step inspires further engineering of proteins for larger quantum yields and red-shifted absorption features [63—65]. The authors proposed that the red-shifted absorption arises from a less favored conformation of substrate-cofactor donor—acceptor complexes. Excitation of these complexes bypasses the typical blue-light dependence observed in ene-reductase photocatalyzed reactions. In another study on the photobiocatalytic synthesis of amino acid derivatives catalyzed by pyridoxal phosphate (PLP)-dependent aminotransferases together with molecular photocatalysts, Yang, Liu, and coworkers observed an increase and a subsequent decrease in the initial rate as the substrate concentration increases, suggesting an enzyme inhibition with the substrate [23]. Addressing the incompatibility issues between molecular systems and enzymes may further enhance the application of photobiocatalysis. In another work, Huang, Zhang, Wang, and coworkers observed that adding a molecular sensitizer can improve the initial rate of an ene-reductase-photocatalyzed lactone synthesis reaction by 7.9-fold [66]. In addition, engineered photoenzymes with nonnatural amino acid residues for photoenzymatic [2 + 2] reactions showed greater k_{cat}/K_M values compared to many ground-state reactions catalyzed by engineered enzymes [21,26].

Isotope labeling and kinetic isotope effect

Isotope labeling and the kinetic isotope effect (KIE) are valuable tools for elucidating the mechanism of enzymatic reactions. In most photoenzymatic flavin-dependent ene-reductase-catalyzed hydroalkylation, hydroamination, hydrosulfonylation, and hydroarylation reactions, a higher percentage of deuterated product is observed when D-glucose-d7 is used compared to a deuterated buffer, suggesting that the hydrogen at the chiral center of the product primarily originates from flavin [17,18,20,37,67—69]. In the photoenzymatic C4-functionalization of indoles, the authors observed a KIE of 1.2 at the C4-H and proposed that the aromatization of key intermediates by flavin semiquinone is the rate-limiting step [38]. In another work, Huang, Wang, and coworkers observed that the KIE originated from deuterated glucose on the photoenzymatic hydroalkylation of protected enamine is 3.06, while that of glucose dehydrogenase-catalyzed NADPH regeneration is 1.51. The authors proposed that NADPH regeneration is the turnover-limiting step [68]. The KIE has also been investigated in time-resolved spectroscopic studies to identify key amino acid residues involved in proton and electron transfer events, as demonstrated in lactate monooxygenase-catalyzed photodecarboxylation reactions [56]. One intriguing application of the KIE is in investigating quantum tunneling, previously studied in ground-state enzymatic hydrogen transfer processes but not observed as much in photoenzymatic reactions [70].

Computational methods

Various computational methods model enzyme systems, providing insights difficult to obtain experimentally. Molecular dynamics (MD) simulations reveal enzyme—substrate interactions, crucial for understanding catalysis [71,72]. Quantum mechanics/molecular mechanics (QM/MM) combines precise quantum mechanics for the active site with molecular mechanics for the environment, creating a balanced enzyme model. Cluster and theozyme models select active site residues, optimize geometry using density functional theory (DFT), and perform energy calculations to study interactions and reaction pathways, aiding in catalyst design by offering mechanistic insights [27,34,73—76]. Together, these methods offer a comprehensive analysis of the movements and chemical changes in enzymatic reactions.

MD

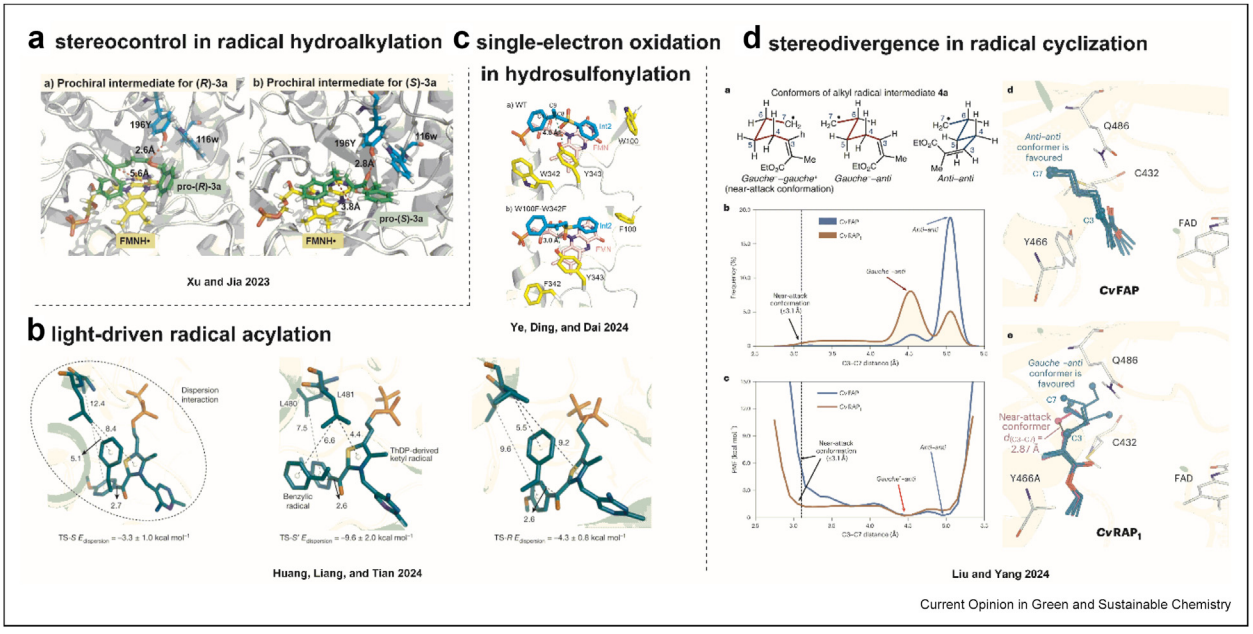
MD simulations have significantly advanced our understanding of enzyme-mediated photobiocatalytic processes, particularly by clarifying the dynamic interactions that influence enzyme reactivity and selectivity under light-driven conditions. For example, MD simulations were used to understand the origins of stereoselectivity in a photoenzymatic radical-mediated hydroalkylation of alkenes with diazo compounds [25]. The OYE1 catalyzed the transformation of benzyl 2-diazoacetate and α-methylstyrene. MD simulations revealed that the substrate's diazo group was stabilized by a hydrogen-bonding network involving His191 and Asn194, facilitating its entry into the enzyme's binding pocket. Additionally, the hydrogen bond between Tyr196 and the carbonyl moiety of the radical intermediate stabilized the intermediate. The simulations

showed that the distance between the benzylic radical in the pro-(*S*)-3a intermediate and FMNH* was shorter than in the disfavored pro-(*R*)-3a, which was crucial for the reaction's stereoselectivity (Figure 2a). In another example, MD simulations combined with DFT calculations were used to investigate the enantioselective radical acylation catalyzed by a ThDP-dependent lyase [27]. The enzyme-bound ThDP-derived ketyl radicals, generated through single-electron oxidation by a photoexcited organic dye, cross-coupled with prochiral alkyl radicals. MD simulations identified key interactions between the enzyme and transition states, especially the cationic π−π interaction between the radical parts and enzyme residues L480 and L481. These interactions were essential for stabilizing the transition state and achieving high enantioselectivity in the radical coupling process (Figure 2b). In addition, Ye, Ding, Dai, and coworkers used MD simulations to study the dynamics of radical intermediates in light-driven enzymatic enantioselective radical hydrosulfonylation [25]. The simulations highlighted the importance of specific enzyme−substrate interactions in stabilizing radical intermediates and transition states (Figure 2c). Further, Liu, Yang, and coworkers used MD simulations to explore the conformational space of alkyl radical intermediates in the active site of wild-type and engineered fatty acid photodecarboxylase (CvFAP) variants [77]. Focusing on stereodivergent photobiocatalytic radical cyclization, MD simulations revealed that the alkyl radical intermediate favored an anti−anti conformation in wild-type CvFAP, while the evolved $CvRAP_1$ allowed for a more flexible conformation with a shorter distance between the radical center and the β-carbon. This flexibility in $CvRAP_1$ was crucial for enhanced chemoselectivity and efficiency in catalyzing radical cyclization reactions (Figure 2d).

QM/MM

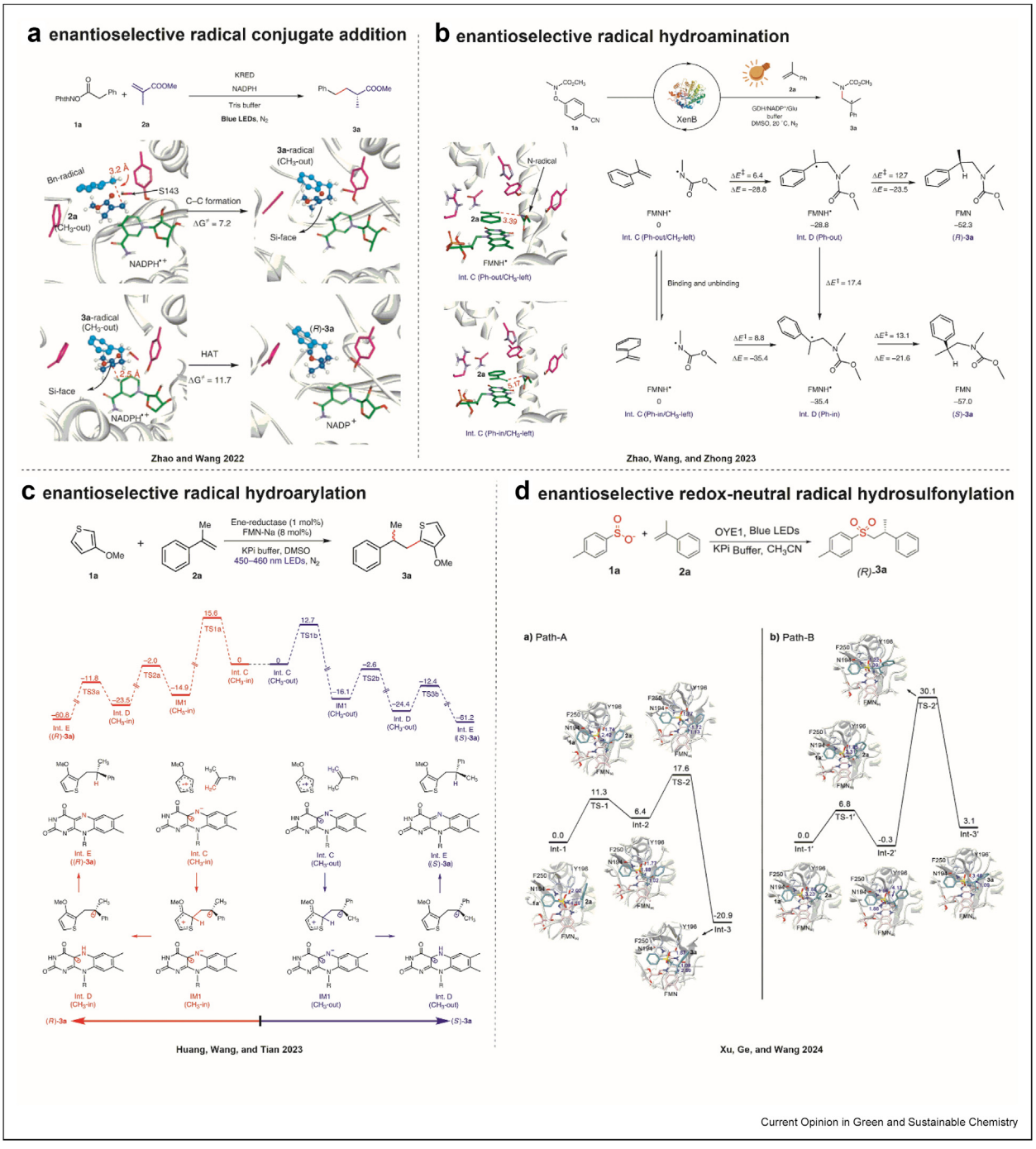
The application of QM/MM methods integrates quantum mechanical precision with molecular mechanical efficiency, enabling researchers to gain comprehensive insights into enzyme-catalyzed photoreactions. Zhao, Wang, and coworkers used QM/MM methods to investigate photoinduced chemomimetic biocatalysis for enantioselective intermolecular radical conjugate addition [36]. They combined nicotinamide-dependent ketoreductases (KREDs) with visible-light excitation. QM/MM simulations explored the in-enzyme cleavage of the N−O bond in the radical precursor, showing an energy barrier similar to that in the free state. Further QM/MM calculations provided insights into the radical conjugate addition to α,α-disubstituted terminal alkenes and the stereoselective hydrogen atom transfer (HAT) from $NADPH^{\bullet}$. These studies, supported by experimental observations, demonstrated how the enzyme's active site facilitated the desired reactions and suppressed racemic background reactions, achieving high enantioselectivity (Figure 3a). In a follow-up study,

Figure 2



Molecular dynamics insights into enzyme-mediated photobiocatalysis. (**a**). MD simulations for gaining insight into the origins of stereoselectivity (PDB 3TX9). a) Prochiral intermediate for (*R*)-3a, b) for (*S*)-3a. (**b**). The selected MD snapshots (t = 100 ns) and calculated dispersion energies ($E_{dispersion}$, kcal/mol) between residues (L480 and L481) and three transition states TS-*S* (left), TS-*S'* (middle) and TS-*R* (right), respectively. (**c**). MD snapshots of GluER-WT and GluER-W100F-W342F. (**d**). MD simulations on radical cyclization within the enzyme active site. Reproduced from ref. 25, 27, 34, 77 with permission from the American Chemical Society, Springer Nature, Wiley-VCH, copyright 2023 and 2024. MD, molecular dynamics.

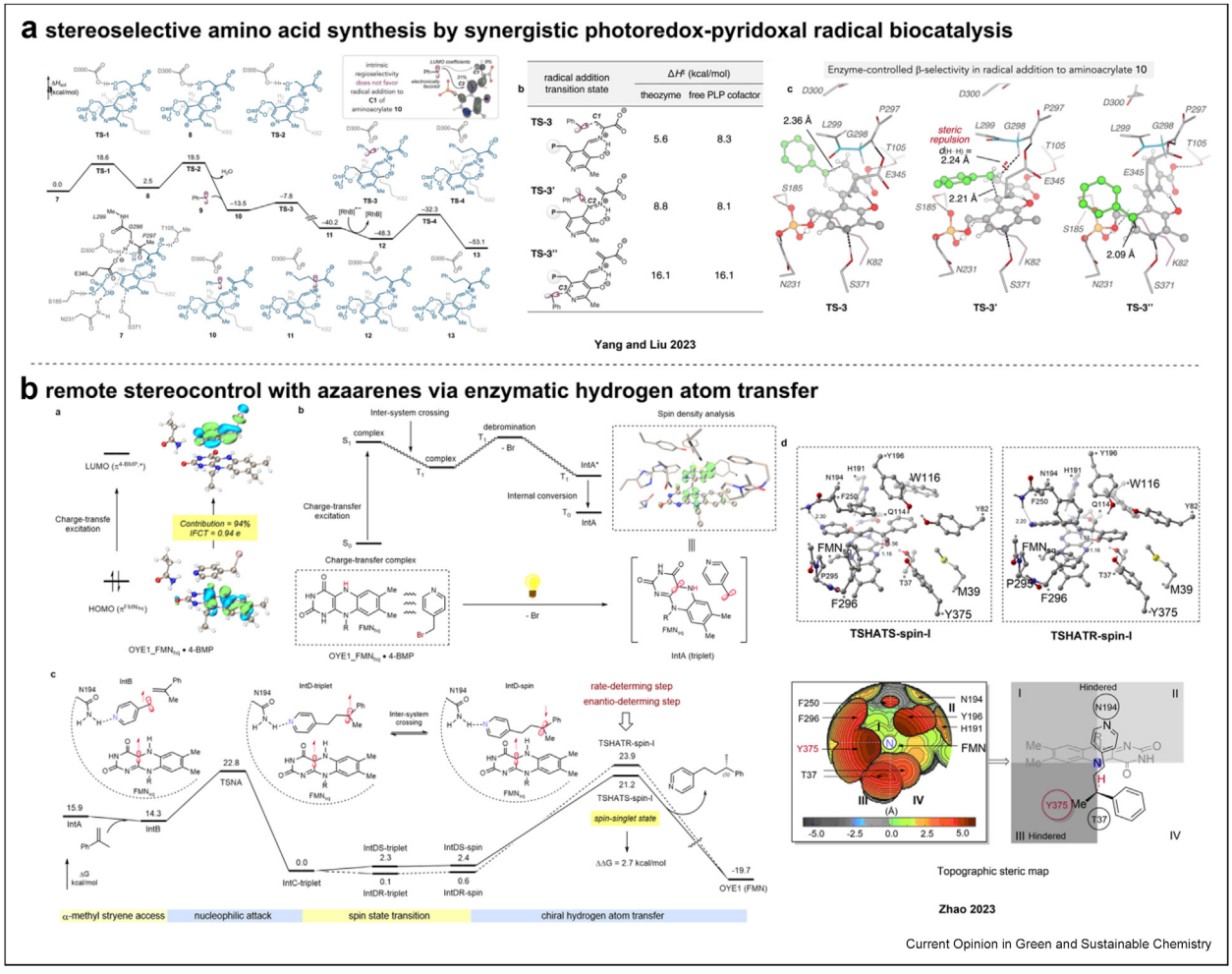
Figure 3



Mechanistic insights into enzyme-catalyzed photoreactions via QM/MM methods. (a) QM/MM simulations exploring radical conjugate addition and HAT in KREDs under visible-light excitation. (**b**) QM/MM simulations revealing complex formation between FMNH• and the substrate radical, focusing on nucleophilic attack and HAT steps. (**c**) QM/MM-calculated energy profiles of radical hydroarylation pathways affording either (*R*)- or (*S*)-**3a** in CH_3-in and -out conformations, respectively, within the active site of OYE1_F296G. The unit of energy is given in kcal/mol. (**d**) QM/MM studies. a) Path A: energy profile corresponding to the FMN_{sq}-mediated enantioselective HAT process. b) Path B: energy profile corresponding to the Y196-mediated enantioselective HAT process. Reproduced from ref. 18, 33, 36, 37 with permission from American Chemical Society, Springer Nature, copyright 2022, 2023, and 2024.

the authors explored the mechanism of enantioselective intermolecular radical hydroamination, catalyzed by an flavin mononucleotide (FMN)-dependent ene-reductase [18]. Their QM/MM simulations revealed the formation of a complex between $FMNH^-$ and the substrate, with the key step being the N−O bond cleavage to produce a nitrogen-centered radical. The reaction, involving carbamates and α-methyl styrene, achieved up to 96% yield and 97% enantiomeric excess (ee), highlighting the enzyme environment's role in stabilizing the radical intermediate and guiding its reactivity (Figure 3b). In the same year, Huang, Wang, Tian, and coworkers used QM/MM calculations to understand the photoenzymatic hydroarylation mechanism facilitated by the OYE1. The reaction focused on the hydroarylation of styrenes using visible light to activate the enzyme. QM/MM simulations detailed the steps of radical cation formation, proton transfer, and hydrogen atom transfer within the enzyme's active site. The study found that π−π stacking interactions between the substrate and FMN were crucial for the reaction's progression and enantioselectivity, optimizing reaction conditions to achieve high yields and selectivity (Figure 3c). Recently, Xu, Ge, Wang, and coworkers examined a redox-neutral radical hydrosulfonylation reaction using sulfinates or sulfonyl hydrazines as radical precursors with an FMN-dependent ene-reductase [33,37]. QM/MM calculations elucidated the

Figure 4



(**a**) **Computational studies on synergistic photoredox-pyridoxal radical biocatalysis via the theozyme model. a**, Computed energy profile using a theozyme model. **b**, Comparison of activation barriers from the theozyme and the free PLP cofactor models. **c**, Optimized structures of radical addition transition states from the theozyme model. (**b**) **Insights from computational analyses of enzyme mechanisms via the cluster model. a**, Illustration shows the dominant molecular orbital transitions contributing to charge-transfer excitation. **b**, Diagram details the photochemical process that results in the formation of an aza-benzylic radical and FMN_{sq}. **c**, Calculated Gibbs energy profile for the nucleophilic attack step and HAT step. **d**, Topographic steric maps for OYE1's catalytic pocket and DFT-optimized lowest−energy transition structures for *R* and *S* products. Reproduced from ref. 23 and 78 with permission from American Association for the Advancement of Science, Springer Nature, copyright 2023 and 2024, respectively. PLP, pyridoxal phosphate; DFT, density functional theory.

mechanism, where the excited FMN obtained an electron from the substrate, followed by hydrogen atom transfer to quench the reaction. The study included various alkenes and sulfonyl precursors, highlighting the potential of QM/MM methods to uncover detailed mechanistic insights that are experimentally challenging (Figure 3d).

Cluster model and theozyme model

Both the cluster model and the theozyme model use quantum chemical methods to focus on amino acid residues near enzyme active sites, offering higher precision than MD or QM/MM methods for studying enantioselectivity in asymmetric enzyme catalysis. Liu, Yang, and coworkers utilized the theozyme model to investigate stereoselective amino acid synthesis using engineered tryptophan synthase [23]. Key residues K82 and D300 facilitated the conversion from internal to external aldimine and deprotonation, forming a quinonoid intermediate that produced aminoacrylate species. Subsequently, a benzyl radical added to the β-carbon through a low-barrier transition state, creating an azaallyl radical intermediate. This underwent electron transfer to a reduced photocatalyst and a proton transfer, demonstrating the pathway's feasibility. The enzyme design ensured regioselectivity, favoring radical addition at the β-position due to steric hindrance from nearby residues (Figure 4a). They also applied the theozyme model to photobiocatalytic oxidative coupling for noncanonical amino acid (ncAA) synthesis, focusing on residues K199 and H83 from threonine aldolase [28]. DFT calculations revealed favorable deprotonation by H83-bound water, leading to a quinonoid intermediate. A photoredox-generated benzyl radical was added to the α-carbon, forming a nitrogen-centered radical with enhanced regio- and enantioselectivity, guided by the Fukui function and steric shielding from H83 and K199.

Zhao and coworkers used the cluster model to investigate enantioselective radical hydroalkylation in photobiocatalysis with FMN-dependent ene-reductases [78]. They docked 4-bromomethyl pyridine (4-BMP) into the X-ray structure of OYE1, isolating key residues and fixing their terminal atoms. Time-dependent density-functional theory (TDDFT) calculations explored the charge-transfer (CT) complex between FMN_{hq} and 4-BMP, revealing the charge transfer and debromination process. The analysis showed that the aza-benzylic radical undergoes nucleophilic attack followed by an enzyme-mediated hydrogen atom transfer (HAT) process, with the HAT step being both rate-determining and enantioselectivity-determining. Steric maps and mutagenesis studies highlighted residues like Y375 and T37 in stereochemical control (Figure 4b). The same approach was applied to study photoenzymatic radical hydrofluoroalkylation, identifying how trifluoroiodoethane binds within OYE1's active site and forms fluorinated radicals. Residues W116 and T37 were crucial for high enantioselectivity, with cluster model simulations revealing the interaction between the fluorinated unit and tyrosine residues [79].

Calculating the behavior and efficiency of biocatalysts is essential for advancing enzymatic reactions. While computational methods such as MD simulations and QM/MM calculations provide deep insights; they have limitations. MD simulations rely on force fields that may not capture all interactions, particularly complex electronic changes. QM/MM calculations are demanding and struggle to accurately separate the quantum and classical parts of a system. They often miss important interactions if the quantum region is too small or become too costly if it's too large. Additionally, these methods may not fully account for enzyme dynamics or changes in conditions like temperature and pH, which can lead to differences from actual experimental results. Both cluster models and theozyme models focus only on a small part of the enzyme's active site, possibly missing important long—range interactions and the broader context of the protein structure. Additionally, the choice of model size and boundary conditions can greatly affect the accuracy of these computational approaches.

Conclusions and future prospects

Bridging the gap between enzymology and photochemistry to unlock new-to-nature transformations, remarkable progress has been made in the field of photoenzymatic catalysis over recent years. In this review, we have highlighted the experimental and computational methods crucial for understanding the mechanistic underpinnings of these reactions. Despite significant advancements, several challenges, and opportunities remain that could shape directions of future research.

- *Enhancing enzyme efficiency and stability.* To overcome stability and efficiency challenges under light exposure, it is crucial to develop robust enzymes. This could involve enhancing quantum yields, optimizing absorption properties for visible light, or improving structural stability.
- *Mechanistic studies and kinetic analysis.* In-depth studies using time-resolved spectroscopy are essential to understand the fundamental steps of photoenzymatic reactions, such as enzyme—substrate interactions and radical formation. Insights from these studies can improve catalyst design and optimize reaction conditions.
- *Integration of advanced computational methods.* Incorporating artificial intelligence (AI) and machine learning with existing computational methods like MD, QM/MM, and cluster models can accelerate the

optimization of photoenzymatic processes, improving predictions and simulations of enzyme behavior.

- *Sustainable and scalable applications.* The goal is to make photoenzymatic catalysis viable for industrial use. This involves developing effective catalysis systems, optimizing conditions for scalability and partnering with industry to transit innovations from the laboratory to the market.

In conclusion, by expanding the variety of enzymes and reactions studied, enhancing enzyme performance, using advanced computational tools, and aiming for practical applications, photoenzymatic catalysis can continue to grow as a field, offering new opportunities for both basic and applied research.

Declaration of competing interest

None.

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Data availability

No data was used for the research described in the article.

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- • of special interest
- •• of outstanding interest

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