Photobiocatalysis

More than just an interesting lab curiosity?

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Review

Photobiocatalysis: More than just an interesting lab curiosity?

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THE BIGGER PICTURE Photobiocatalysis is a rapidly evolving field of research. New photobiocatalytic reactions are published almost weekly, presenting fascinating opportunities that enrich chemical synthesis. However, it is also the time to question the feasibility and practicality of these novel reactions. Only if the reported approaches can be economically viable can photobiocatalysis transition from "scientifically interesting" concepts to practical applications. In this review, we provide a critical analysis of the current state of the art, highlighting performance indicators such as turnover number and the environmental footprint of the reactions. We hope to spark a critical and lively discussion, thereby advancing the exciting field of photobiocatalysis.

SUMMARY

Photobiocatalysis is currently in vogue. The number of reports combining the disciplines of biocatalysis and photocatalysis is rapidly increasing. While the synthetic possibilities enabled by photobiocatalysis are fascinating, the economic feasibility and environmental impact are largely neglected in the current literature. In this contribution, we present a range of key indicators for economic feasibility and environmental impact that may be useful for readers to assess their own work and thereby avoid unrealistic exaggerations. We also critically review the current state of the art in photobiocatalysis and question its synthetic practicability beyond the laboratory. With this contribution, we aim to provoke an open discussion.

INTRODUCTION

Photobiocatalysis is an emergent field at the intersection of photocatalysis and biocatalysis. By integrating the specificity of biocatalysts with the energy efficiency of photocatalysis, it ushers in an era of interdisciplinary innovation, offering new insights into reaction mechanisms and enabling novel reaction pathways previously unattainable through conventional methods. This synergy not only advances our understanding of light-driven and enzymatic processes but also aligns with the principles of green chemistry, advocating for reduced energy consumption and minimal waste production. Leveraging solar energy to drive chemical reactions, photobiocatalysis paves the way for reducing reliance on fossil fuels, contributing to a sustainable chemical industry with a lower carbon footprint and enhanced resource efficiency. As such, it holds promise for delivering sustainable solutions in chemical synthesis, underpinning the development of renewable energy technologies and fostering environmental stewardship.

Yet, these assertions are frequently made without robust evidence. Undoubtedly, a comprehensive life cycle assessment (LCA) and a techno-economic analysis of an established process provide concrete, quantitative metrics to evaluate these aspects. However, even in the initial stages of development—common in academic settings—a semi-quantitative assessment can offer valuable insights, highlight constraints, and steer subsequent progress. In the ensuing section, we present straightforward concepts and metrics designed to furnish the academic researcher with an accessible and substantive preliminary evaluation.

Overall, we aspire to enhance academic researchers' awareness of the intricate factors influencing environmental and economic impacts. We hope that the tools presented for discussion here will help to avoid overly simplified and unsubstantiated claims regarding sustainability and industrial applicability.

Economic considerations

A common misconception about biocatalysis is its perceived high cost, primarily due to the expensive enzymes sourced from specialty chemical suppliers. Yet, it is crucial to recognize that enzyme production costs are linked to the scale of fermentation (Figure 1A). Tufvesson et al. projected that the costs for

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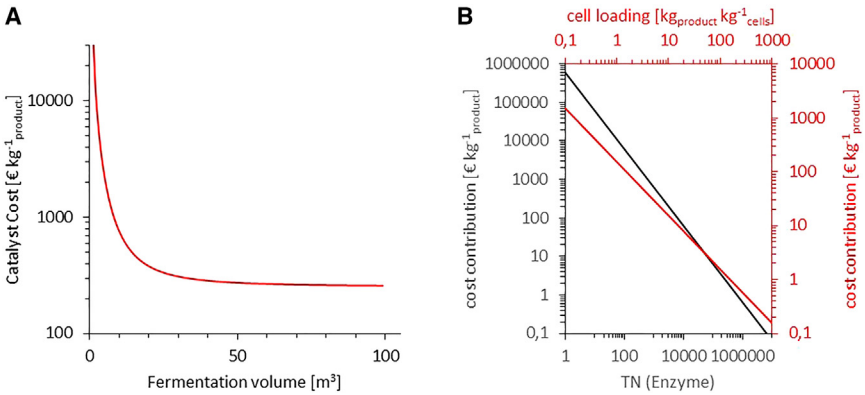

Figure 1. Factors influencing the cost contribution of a biocatalyst to the final product

(A) Dependency of the catalyst (cells) production cost on the scale of the fermentation.

(B) Cost contribution of the catalyst (enzyme as crude cell extract or whole cells) depending on the turnover number (TN) or the crude enzyme and the cell productivity ($kg_{product}$ kg^{-1}_{cells}). Assumptions made are as follows. Production costs: whole cells = 150 € kg^{-1}, crude cell extract = 250 € kg^{-1}, molecular weight $(MW)_{product}$ = 200 g mol^{-1}, and MW_{enzyme} = 50 kDa.

whole cells and crude cell extracts could be as low as 150 € per kg_{cells} and 250 € per kg_{Enzyme}s, respectively, when manufactured on a large scale.[1] These numbers should be taken as rough guidelines. Naturally, additional factors, such as expression level, cell density, cultivation time, and the complexity of downstream processing, have a significant impact on the production costs of an enzyme. A notable point is that typical laundry detergents include enzymes at concentrations of 1%–5% (w/w).[2] Furthermore, the formulation of the biocatalyst significantly impacts its production costs. Utilizing whole cells, which require minimal further processing, proves to be the most cost-effective approach. Producing crude cell extracts entails additional steps, thus slightly increasing the costs. Enzyme purification, involving more extensive procedures, can result in a substantial increase (approximately 5-fold) in production costs, although recent advancements in purification techniques may reduce these expenses. However, it is essential for practitioners to assess whether purification is necessary; for instance, due to the presence of unwanted activities from the host organism or issues with stability.

Besides production costs, the efficacy of a (bio)catalyst plays a critical role in determining its contribution to the overall cost of producing a specific product (Figure 1B). Evidently, the greater the amount of product generated per unit of catalyst, the lower the cost contribution of the (bio)catalyst to the final product.

Table 1. Allowable cost contribution of biocatalysts (whole cells or crude enzyme extracts) and minimal performance parameters for economically feasible use in various industrial sectors

Sector	Typical product cost (€ $kg_{product}^{-1}$)	Allowable catalyst contribution (€ $kg_{product}^{-1}$)	Minimal catalyst performance (TN and $kg_{product}$ kg^{-1}_{cells})
Pharma	100	10	15,000 15
Fine/speciality chemicals	10	0.8	150,000 200
Bulk chemicals	1	0.05	2,000,000 3,000

TN = $mol_{product}$ × $mol_{catalyst}^{-1}$.

Tufvesson, Woodley, and their colleagues delineated industrial sectors—pharmaceuticals, commodity and fine chemicals, and bulk chemicals—each characterized by distinct product cost structures. Table 1 condenses the typical product costs, permissible catalyst cost contributions, and the ensuing minimum performance criteria for catalysts within each sector.

While these figures offer a preliminary understanding of the commercial viability of a particular biocatalytic transformation, it is important to consider that other, less apparent factors can significantly influence the process. For instance, Evonik's replacement of the traditional Lewis acid-catalyzed esterification of fatty acids with fatty alcohols using an enzymatic method reduced the reaction temperature from over 180°C to just 60°C. This change eliminated unwanted thermal side reactions, markedly simplifying and abbreviating both the synthesis and downstream processing of the product (Figure 2).[3,4] This adjustment led not only to a more cost-effective procedure but also to a considerable reduction in environmental impact.

Environmental impact

Another pervasive *a priori* assumption is that both bio- and photocatalysis are inherently benign technologies.[5,6] In the case of biocatalysis, the mild reaction conditions and the renewable nature of the catalyst are often highlighted. For photocatalysis, the potential use of sunlight is presented as evidence of environmental friendliness. While we largely concur with the promise of both technologies for more sustainable chemical synthesis, it is imperative to acknowledge that here too, rigorous scrutiny is essential.

The synthesis history of a given catalyst of course has an influence on its environmental impact. Complex resource- and energy-consuming syntheses increase the environmental footprint of catalysts (and essentially all products). A complete $E^{(+)}$ analysis of all steps of a regent's history obviously rapidly becomes very time and data intensive. As a doable but still meaningful alternative, Jessop proposed the qualitative comparison of the individual synthesis trees.[7] Particularly, comparing the number of synthesis steps already provides a realistic, qualitative handle to compare the resource intensity of different compounds.

Figure 3 compares some synthesis trees of some biocatalysts and some representative transition-metal catalysts, organocatalysts, and inorganic catalysts. Based on the "number of steps" comparison, mesoporous silicates appear to leave behind the

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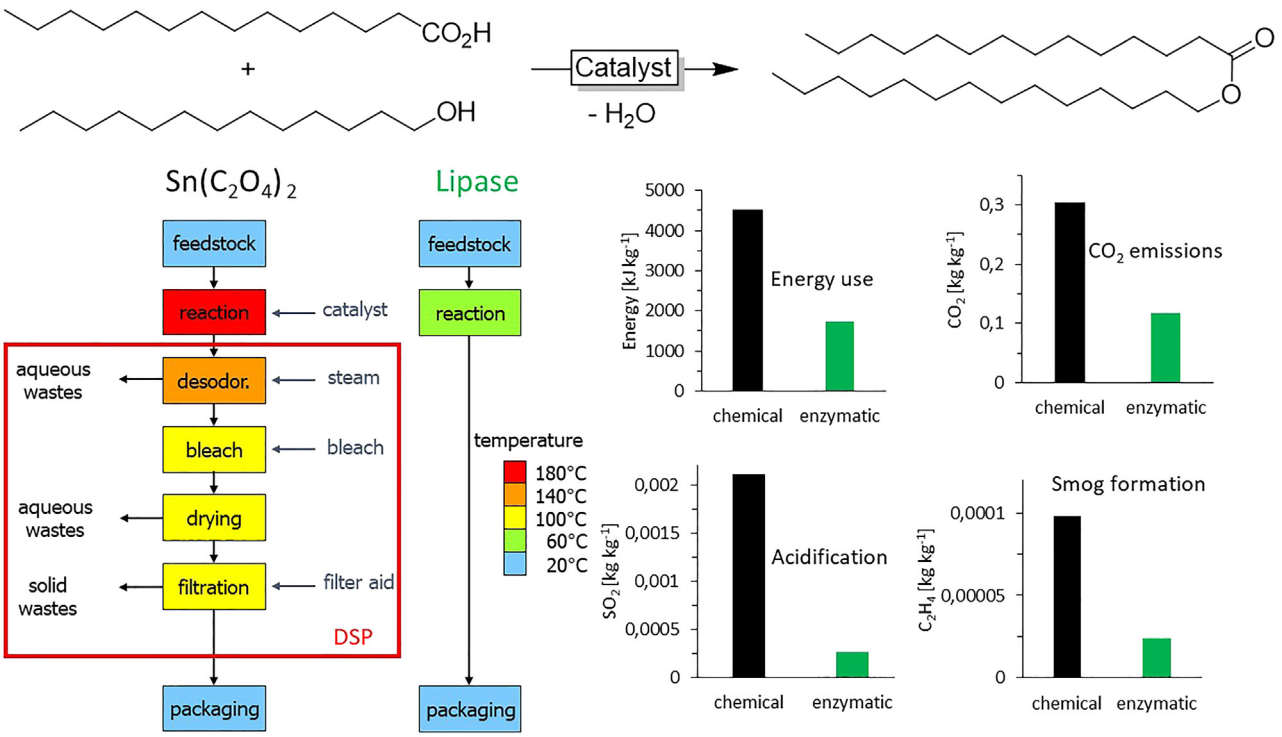

Figure 2. Industrial synthesis of the emollient ester myristyl myristate

(Left) The original process utilized tin oxalate as catalyst and consequently had to be operated at least 180°C. As a result, excessive downstream processing (DSP) was inevitable. The enzymatic process operates at more ambient temperatures, making additional DSP superfluous, thereby considerably shortening the production time.

(Right) Comparative LCA of the former Sn-oxalate (black) and the new, lipase-catalyzed reaction (green).

smallest environmental footprint, followed by enzymes, while organocatalysts and transition-metal-based homogeneous catalysts appear to have the largest footprint because of the complex synthesis of organic ligands/substituents. The synthesis tree analysis also visualizes that the "100% renewable" character of organo- and biocatalysts is somewhat questionable. In both cases, some fossil, non-renewable base chemicals can be found in the synthesis trees. Of course, this analysis is only a snapshot of the current state of the-art, and the synthesis trees will be simplified if, e.g., H_2 will be obtained from renewable energies.

In any case, we believe that this synthesis tree analysis should foster a more critical view of catalyst preparation and relativize the "complete renewability" of bio- and organocatalysts.

It is also crucial to consider that the fermentation process is resource intensive and consumes a significant amount of energy.[8] We delved deeper into this aspect by applying Sheldon's E factor[9,10] to the synthesis of two enzymes via 10 L-scale fermentation (Figure 4).[8]

The classical E-factor is largely contingent on the enzyme yield from the fermentation process. In this particular case study, the E factor was a minimum of 2,800 kg of waste per 1 kg of enzyme. While it may be contended that the by-products of fermentation could be repurposed, for example, as fertilizers, thereby potentially not qualifying as waste, this perspective still provides insight into the resource expenditure as raw material for enzyme production. Moreover, it is debatable whether water, as a "green solvent," should be included in these calculations. More critical, in our view, is the non-obvious, "hidden" waste generated in the form of energy consumption. Given that energy production today still largely relies on the combustion of fossil fuels (Table 2), the resultant CO_2 emissions should not be overlooked. Table 2 gives an overview of the typical CO_2 footprints of various energy sources.[11] The local, individual CO_2 footprint of the energy used obviously depends on the local mix of the different energy sources.

At initial glance, the figures for the $E^{(+)}$ factor appear alarmingly high, particularly when considering emissions related to energy consumption. However, it is important to note that biocatalysts are typically not the end product but a tool to synthesize the desired final product. Thus, if the (bio)catalyst is utilized efficiently (for instance, achieving high turnover numbers), its overall impact on the final product's footprint could be considered negligible (Figure 4B).

It is also worth mentioning that major enzyme suppliers have performed LCA analyses for their products.[12] At first sight, the CO_2 emissions reported there are approximately 4 orders of magnitude lower than the numbers shown in Figure 4A. However, it should be noted that, in case of industrial enzymes, the liquid formulation is taken as basis for the CO_2 calculations, whereas in Figure 4A, the basis is the amount of biocatalyst. Thus, taking an average enzyme concentration of 1–50 g_{Enzyme} $L_{Formulation}^{-1}$, similar values can be calculated. Also, some

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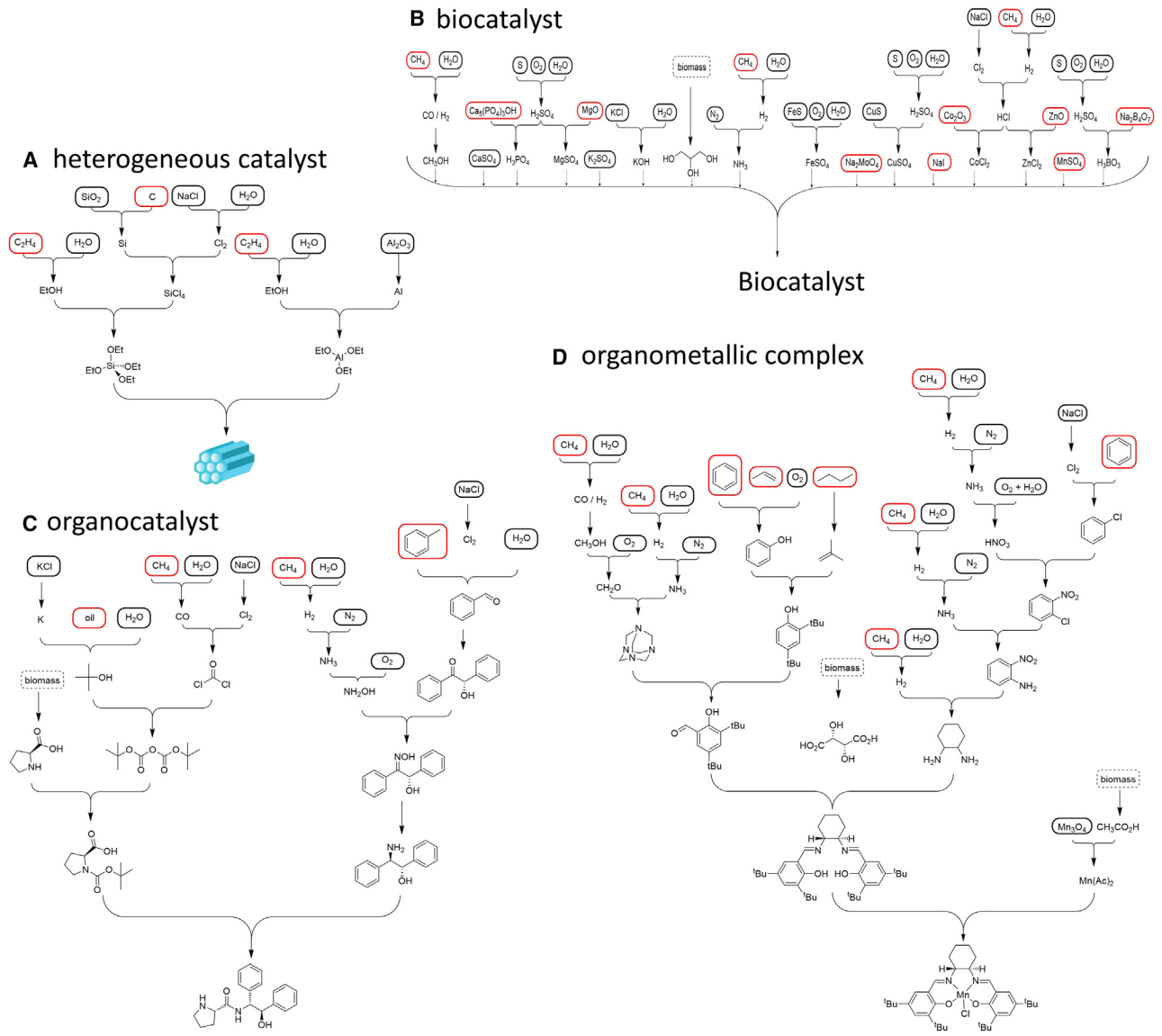

Figure 3. Synthesis trees of some representative catalysts

Encircled compounds are "base chemicals." Those marked in red are running scarce.

(A) Alumosilicate.

(B) Enzyme.

(C) Proline-derived organocatalyst.

(D) Organometallic catalyst.

economy-of-scale effects in terms of energy efficiency of enzyme production (10 L vs. up to 500 m^3) may be expected.

Regarding photocatalysis, it is essential to acknowledge that its primary environmental advantage is purported to stem from utilizing sunlight to hasten chemical transformations or provide the thermodynamic impetus for otherwise energetically prohibitive reactions. Nevertheless, the majority of documented photocatalysis instances employ artificial lighting instead of sunlight. Here, we assert that the energy consumed for illumination (and the consequent CO_2 emissions elsewhere) should be factored in when evaluating the environmental credentials of photocatalytic processes. To exemplify this, we have assessed the electricity usage for a photocatalytic reaction and calculated an E^+ value for the total reaction (Figure 5).[13]

In this particular example, the CO_2 emissions caused by the LED (light emitting diode) illumination accounted for more than 50% of the overall E^+ factor of the reaction. The high absolute values are mainly due to the very dilute reaction mixtures with maximally 10 mM of product. "Raising the product titers to industrially demanded 50–200 g L^{-1} will reduce the E-factor … ."[13] Though we still stand with this statement, we note that the demonstration is still elusive. It should also be noted that 10 mM is not an

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A

	E-factor [kg kg^{-1}]	E$^+$-factor (including CO_2 from energy) [kg kg^{-1}]
crude r*Aae*UPO	4.300 (15.700)	110.900
purified r*Aae*UPO	18.500 (209.000)	566.800
crude *Ao*FOx	2.800 (49.800)	99.700
purified *Ao*FOx	4.300 (106.100)	157.800

excluding water / including water

B

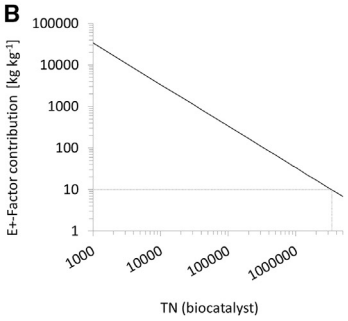

Figure 4. $E^{(+)}$ factor calculation of two enzymes produced at 10-L scale, taking into account water and energy consumption-related CO_2 emissions

(A) Summary of $E^{(+)}$ factors determined for the production of two enzymes.

(B) E factor contribution of an enzyme (own E factor = 500,000) to the E factor of the final product depending on its performance (in terms of TNs).

*Aae*UPO, peroxygenase from *Agrocybe aegerita* (expressed in *Pichia pastoris*); *Ao*FOx, formate oxidase from *Aspergillus oryzae* (expressed in *Escherichia coli*). The CO_2 intensity of the fermentation was determined by measuring the electricity used for the single operation steps (e.g., stirring, pumping, heating, cooling etc.) and multiplying these values with the local CO_2 intensity of electricity.

exceptionally low substrate/product concentration (*vide infra*). Figure 6 visualizes the impact of product concentration and wastewater formation. Still today, the majority of biocatalytic reactions are performed in dilute aqueous reaction media,[14–16] resulting in several hundred liters of wastewater amounts per kilogram of product.

In summary, we ascertain that economic and environmental advantages are not inherently guaranteed for either photocatalysis or biocatalysis. Several factors must be thoroughly evaluated before asserting such benefits. In the subsequent sections, we will critically examine a selection of photobiocatalytic examples, endeavor to identify the primary limitations, and propose potential enhancements.

TRUE PHOTOBIOCATALYTIC TRANSFORMATIONS

Today, three naturally occurring classes of photoenzymes (i.e., wild-type enzymes relying on illumination for activity) are known: being part of the chlorophyl biosynthetic pathway, protochlorophyllide oxidoreductases (LPORs)[17] catalyze the light-dependent reduction of the C_{17}=C_{18} double bond in protochlorophyllide (Figure 7). It is interesting to note that, in this case, the substrate itself serves as photosensitizer. From a biocatalytic perspective, LPORs are largely unchartered terrain, which may be largely due to the highly specific substrate range.

Like LPORs, photolyases (PLs) have also been receiving intensive scientific interest for decades, albeit not from the biocatalysis community.[18] PLs catalyze the repair of photodamaged DNA (thymine dimers). In PLs, a methenyltetrahydrofolate photosensitizer activates a photoinactive, reduced flavin via fluorescence resonance energy transfer (FRET) followed by single-electron transfer (SET) to the thymidine dimer, ring cleavage, and back-SET (Figure 8).[18]

The youngest known members of the "true" photoenzymes are the fatty acid photodecarboxylases (FAPs). Since the first report by Beisson and coworkers in 2017,[19,20] the FAP from *Chlorella variabilis* (*Cv*FAP) has been attracting a lot of attention.[21–29] The catalytic mechanism of this (and related decarboxylases) comprises photoexcitation of the enzyme-bound flavin cofactor by blue light. The activated flavin then mediates a SET step from the enzyme-bound, deprotonated carboxylic acid, thereby initiating the almost instantaneous decarboxylation step and back-electron transfer from the semi-reduced flavin. Active-site-residing Arg and Cys also play an important role, presumably in proton transfer (Figure 9).[19,30]

Applications of FAPs

*Cv*FAP and its homologs from other organisms[31–33] have received considerable attention from biotechnologists, particularly for the

Table 2. CO_2 footprint of some common energy sources

Energy source	Specific energy content (kW h^{-1} × kg^{-1})	Theoretical CO_2 emissions (g(CO_2) × kW h^{-1})	Realistic CO_2 emissions (g(CO_2) × k Wh^{-1})[a]
Methane	55.2	50	90
Gasoline	46.4	67.2	240
Coal (black)	26–33	77–93	200
Coal (brown)	10–20	80–160	300–400
Nuclear power	2.4 × 10^7	0	2–30
Solar energy	–	0	20–75
Wind energy	–	0	3–14
Hydroelectric power	–	0	1–30

[a]The discrepancy between theoretical and realistic CO_2 emissions stems from several factors, such as often low efficiency in energy conversion at power plants, etc.; the numbers presented for the non-fossil energy sources also contain indirect emissions during mining and manufacturing of the power plants.

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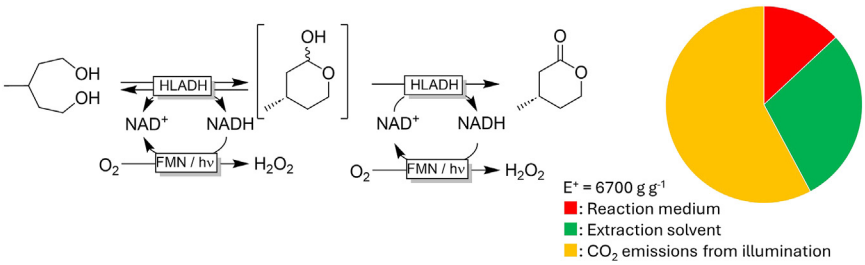

Figure 5. Estimation of the E⁺ factor of a photobiocatalytic oxidative lactonization reaction

valorization of (waste) fatty acids and oils into a new generation of biodiesel (Figure 10).[34–46] Traditional biodiesels (i.e., fatty acid methyl esters [FAMEs]) are obtained from the reversible (*trans*) esterification of fatty acids or their glycerides. This results in the need for extensive pretreatment (drying) of the feedstock and necessitates molar surpluses of methanol to maximize the FAME yield. Despite these apparent shortcomings, biocatalytic FAME production is also well established on the industrial scale.[47–50] The lipase/FAP route appears to be simpler and more efficient, as the aforementioned disadvantages do not apply here, and, furthermore, the caloric value of the alkane product is higher than those of the corresponding FAMEs.

Next to biofuel applications, some very creative approaches to embed FAPs into synthesis routes for more value-added products have been reported, some of which will be discussed in more detail. Very early on, several groups started engineering *Cv*FAP for increased selectivity on various compounds, mainly in light-driven kinetic resolution reactions (Figure 11). For example, Wu et al. engineered *Cv*FAP for the kinetic resolution of α-substituted carboxylic acids,[51] for the discrimination between *cis*- and *trans*-unsaturated fatty acids,[52] or for the deuterative, kinetic resolution of racemic glycolic acid ethers.[53] Also, the kinetic resolution of racemic phosphinothricin using engineered *Cv*FAP has been reported.

Very recently, Yang and coworkers further engineered FAPs into so-called radical photocyclases (Figure 12), thereby expanding the reaction scope of FAPs.[55]

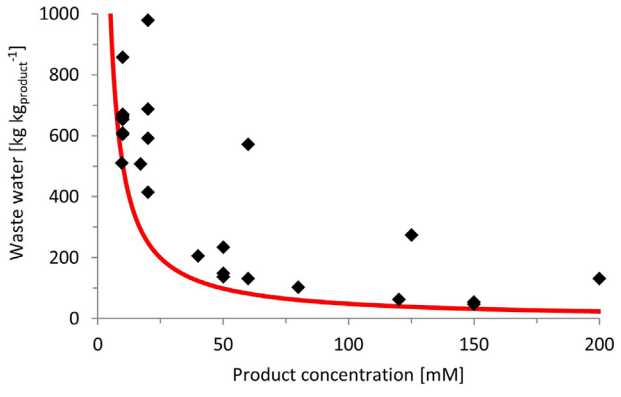

Figure 6. Correlation between product concentration and wastewater formation in aqueous biotransformations

Black triangles represent values calculated from representative literature examples.[14] The red line indicates the theoretical wastewater generated for a product with an MW of 200 g mol^{-1}.

*Cv*FAP has also been used in a range of cascade transformations, particularly for the conversion of unsaturated fatty acids into value-added products.[56–59] An interesting newer development comprises the application of FAPs in whole-cell biotransformations, such as the conversion of carbohydrates to alkanes[60,61] or base chemicals (Figure 13).[62]

A critical evaluation of FAP performance

One possible application of FAPs mentioned frequently in the academic contributions around FAPs is the valorization of renewable (waste) fatty acids into combustible fuels (*vide infra*). In this light, of course the production costs will play a major role for the commercial success of this application. We therefore analyzed the current FAP literature with respect to catalyst turnover numbers (Figure 14).

This analysis reveals that the current state of the art in FAP catalysis in terms of enzyme utilization is still several orders of magnitude away from economic feasibility (Table 1). Rarely, turnover numbers higher than 10,000 and/or product-to-catalyst ratios of higher than 1 have been reported. Obviously, this stands in stark contrast to the envisioned preparation of fuel alkanes.

Addressing the issues of FAP catalysis

Intensification of FAP-catalyzed fatty acid decarboxylations has been a topic early on. One focus has been to increase the quantum yield of the reaction. The poor penetration depth of (visible) light into the reaction mixtures of classical stirred tank reactors renders dissolved, bulk photoenzymes largely inactive. To address this, flow chemistry reaction setups to increase the surface-to-volume ratio have been established.[72,73] Also, parallel illumination[68] and internal illumination[70] have been evaluated. Generally speaking, improving the light penetration indeed results in higher volumetric productivity but does not positively influence the total turnover number of the FAPs (both in whole cells and with isolated enzymes).

In case of whole-cell biotransformation, the surface display of the FAP on the well membrane of the expression host has been shown to increase activity, presumably by reducing diffusion limitations over the cell membrane.[66,67]

Most wild-type FAPs exhibit a clear preference for fatty acids, i.e., acids with alky chain lengths larger than 10. This can either be addressed using the decoy molecule approach[43,74] or via enzyme engineering.[75]

As shown in Figure 14, the major limitation of FAPs in view of economic feasibility is their low stability. Early on, Scrutton and coworkers raised the issue of poor photostability of FAPs,[29,76] and further evidence underlined the importance of a convertible

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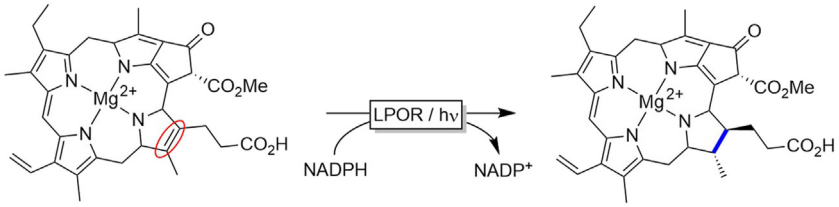

Figure 7. Regio- and stereoselective reduction of the C_{17}=C_{18} double bond in protochlorophyllide catalyzed by LPOR

substrate (carboxylic acid) being present upon illumination.[69] It was hypothesized that, in the absence of a substrate, the photoexcited, enzyme-bound flavin may oxidize nearby active-site residues. However, further experimental evidence is so far lacking. Additionally, flavins function as photosensitizers for molecular oxygen. Indeed, the photostability of *Cv*FAP was somewhat higher in the absence of O_2 compared to the ambient atmosphere.[64]

The influence of the illumination wavelength on *Cv*FAP has been investigated by various groups.[63,77,78] A direct correlation of both *Cv*FAP activity and stability with the spectral overlap with the flavin UV-visible spectrum was observed (Figure 15). The strongest inactivating influence was observed with blue LED light, almost entirely overlapping with the *Cv*FAP-absorption spectrum, whereas red light had only a minor inactivating effect. However, with decreasing overlap, the decarboxylation activity also decreased. Hence, the stabilizing effect appears to be largely compensated by the reduced activity.[63] Recent reports on increased activity and stability upon illumination with violet light[78] are difficult to rationalize and necessitate further investigation.

Immobilization of enzymes typically brings about stabilization against hostile reaction conditions.[79] Primarily, immobilization increases the rigidity of an enzyme and thus counteracts opposing effects that weaken the tertiary and quaternary structure of an enzyme, such as elevated temperatures, coordinating and solvating medium components (solvents, salts, and pH values) or reactive functional groups like aldehydes, and so forth. In the case of photodecarboxylases, however, the main reason for inactivation lies with the prosthetic group and/or amino acids in the active site. The latter are not affected by immobilization. Hence, it is not very astonishing that immobilization hardly influences the photostability of FAPs.[73,80,81]

Future directions

It is clear that FAP catalysis needs dramatic improvements to reach economic feasibility. Particularly, the photoinactivation process so far is poorly understood. Possibly, enzyme engineering can solve this issue.[75] To base any engineering efforts on experimental evidence, it would be very interesting to identify the amino acid residues involved in the photoinactivation; e.g., by tryptic digestion and high-performance liquid chromatography-mass spectrometry characterization. Also, the photobleached flavin cofactor should be characterized in more detail. Another interesting approach may be to generate FAP mutants with increased affinity to the carboxylate starting material in order to minimize illumination of "empty" FAPs leading to inactivation.

Finally, as suggested by Scrutton and coworkers, embedding FAP catalysis into metabolically active whole-cell systems where the photoinactivated FAPs may be replaced certainly is an interesting route to explore further.

TEACHING OLD DOGS NEW TRICKS: PHOTOBIOCATALYSIS FOR NEW-TO-NATURE REACTIONS

Studies of the photochemical activation of enzyme cofactors, such as nicotinamide cofactors (NADH, NADPH) have been documented since the mid-1970s.[82–85] The enhanced reduction

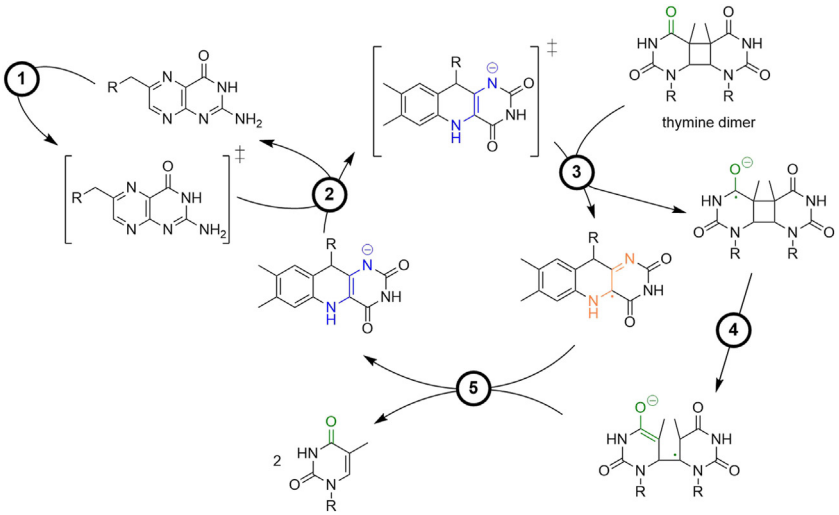

Figure 8. PL mechanism

(1) Blue-light photoexcitation of the methyltetrahydrofolate cofactor. (2) FRET from the excited folate to the reduced flavin cofactor. (3) SET from $FADH_2^{\ddagger}$ to the thymidine dimer, forming a ketyl radical. (4) Rapid cyclobutane ring opening. (5) Back-electron transfer.

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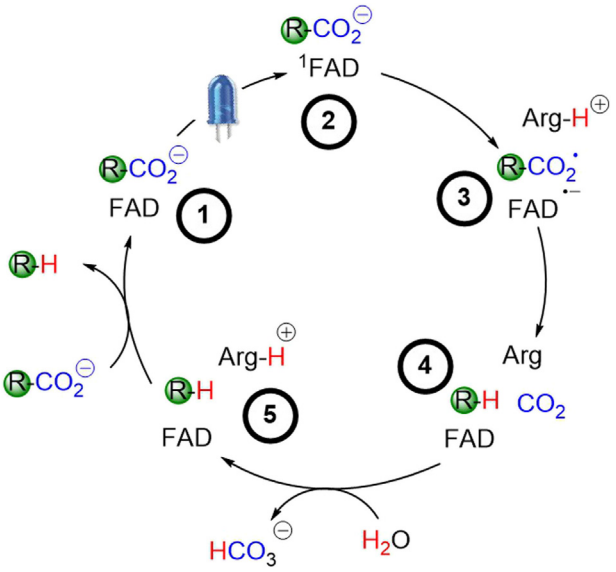

Figure 9. Simplified mechanism of *Cv*FAP-catalyzed decarboxylation of carboxylic acids

The catalytic cycle starts from the substrate-bound resting state (1) with the absorption of blue light (~400–550 nm), resulting in the photoexcited (singlet) state (2). Fast SET from the acid substrate to the flavin (2 > 3), followed by fast protonation of the developing carbon-centered radical and electron back transfer to the carbon radical (3 > 4) finally results in the product-bound resting state (5), which, upon substitution of the product by another acid molecule, enters a new catalytic cycle.

potential of the photoactivated, reduced nicotinamide moiety has been demonstrated to facilitate the radical reduction of inactive precursors. However, it was only recently that Hyster and colleagues recognized the synthetic potential for stereoselective radical coupling when cofactors are encapsulated within the chiral milieu of an enzyme's active site.[86] In an initial study, they illustrated that a photoexcited charge-transfer complex is formed between NAD(P)H and α-brominated esters (Figure 16A).[87] In a similar vein, Zhao and colleagues expanded this approach to include photocatalytic radical formation in C–C bond-forming reactions (Figure 16B).[88]

Recently, attention has shifted toward photoinduced abiological reactions utilizing flavin-dependent enzymes, likely due to the superior radical stabilization capacity of the alloxazine moiety compared to the nicotinamide ring. The overarching mechanism consistently relies on the photoactivation of the reduced flavin cofactor, followed by SET to the enzyme-bound substrate and the elimination of a leaving group. The resultant radical subsequently engages with appropriate reaction partners, such as C=C double bonds (both inter- and intramolecular), aromatic compounds, or C-H acidic nitro compounds (Figure 17).

Especially when combined with enzyme engineering to adjust the selectivity, photobiocatalytic radical reactions are potentially very powerful methods for the selective generation of previously inaccessible new C–C bonds.[26,86,96,97]

Another exciting development in artificial photoenzymes is the incorporation of non-natural amino acids exhibiting photocatalytic properties.[98] One particularly interesting approach was reported by Green and coworkers[99]: installation of a genetically encoded benzophenone photosensitizer into the active site of tyrosyl-tRNA synthetase from *Methanococcus jannaschii.* The resulting non-natural Diels Alderase was capable of [2+2] cycloaddition reactions (Figure 18). Up to 300 turnovers have been reported for the new-to-nature photoenzyme.

ECONOMIC FEASIBILITY AND ENVIRONMENTAL IMPACT OF THE "NEW-TO-NATURE" PHOTOBIOCATALYTIC REACTIONS

Today, the aforementioned photobiocatalytic transformations are still in their infancy, and little effort has been made to increase the catalytic performance of these transformations. A very typical catalyst loading is 1 mol % (i.e., turnover number [TN] < 100), therefore corresponding more to the performance of a typical organocatalyst than to an enzyme. The reagent loadings of, typically, <10 mM, however, resemble those generally use in biocatalytic transformations. Regardless of the scientific merits, when applying the economic and environmental considerations made above, it becomes clear that an economically feasible application beyond pharmaceutically relevant products is rather unrealistic. Also the environmental benefits, based on the current state of the art, are questionable, considering the high catalyst loadings and the low product titers.

To obtain a clearer picture of the practical feasibility of these approaches, further studies focusing on exploring the limits of catalyst usage are highly desirable. Our concern, however, is that, similar to the case of photodecarboxylases, photoinactivation of the biocatalysts will represent a major hurdle.

PHOTOBIOCATALYTIC REACTIONS

In this section we will discuss cases where photocatalytic reactions are combined with enzymatic steps. Classic examples comprise photocatalytic cofactor regeneration to promote biocatalytic redox reactions. More recently, the portfolio of photobiocatalytic reactions was enlarged by sequential cascade, where either the enzyme substrate is generated photochemically, or the enzyme product is converted further photocatalytically.

Photocatalytic regeneration of redox enzymes

Reductive regeneration of oxidoreductases

NAD(P)H regeneration systems. Formally, simple reductants can be employed to convert $NAD(P)^+$ into NAD(P)H. However, photochemical methods in particular are plagued by substantial formation of enzymatically inactive NAD(P) dimers and NAD(P)H isomers. This issue can be largely attributed to the radical nature of the photochemically induced reduction of NAD^+ (Figure 19).

To avoid the undesirable side reactions stemming from the radical nature of two non-catalyzed SET, a relay system is typically employed to convert the two SETs (and a protonation step) into a single, selective hydride transfer. Notably, enzymes and the organometallic complex $[Cp^*Rh(bpy)(H_2O)]^{2+}$ are widely utilized for this purpose.

Initial efforts to regenerate NAD(P)H from $NAD(P)^+$ date back to the 1980s,[100] using tris(bipyridine)ruthenium(II) ($[Ru(bpy)_3]^{2+}$) as a photosensitizer to mediate electron transfer from

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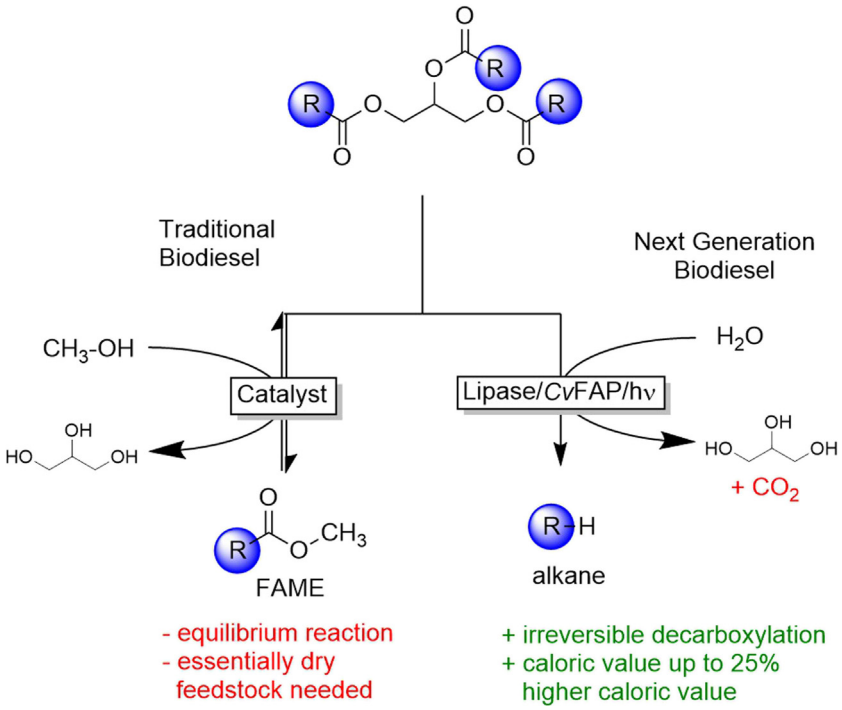

Figure 10. Qualitative comparison of traditional biodiesel and bioalkanes as obtained via decarboxylation

triethylamine to NAD(P)$^+$. The SET-protonation-electron transfer nature of this approach, however, also leads to considerable formation of enzymatically inactive NAD(P) dimers and NAD(P)H isomers. A switch in the NAD(P)$^+$ reduction catalyst to [Cp*Rh(bpy)(H$_2$O)]$^{2+}$, by Ruppert and Steckhan and Wienkamp and Steckhan, resolved this issue by enabling highly selective formation of the desired 1,4-NAD(P)H isomers,[101,102] a finding later rationalized by Fish and co-workers (Figure 20).[103]

Ever since then, [Cp*Rh(bpy)(H$_2$O)]$^{2+}$ has been the preferred non-enzymatic NAD(P)H regeneration catalyst for chemical,

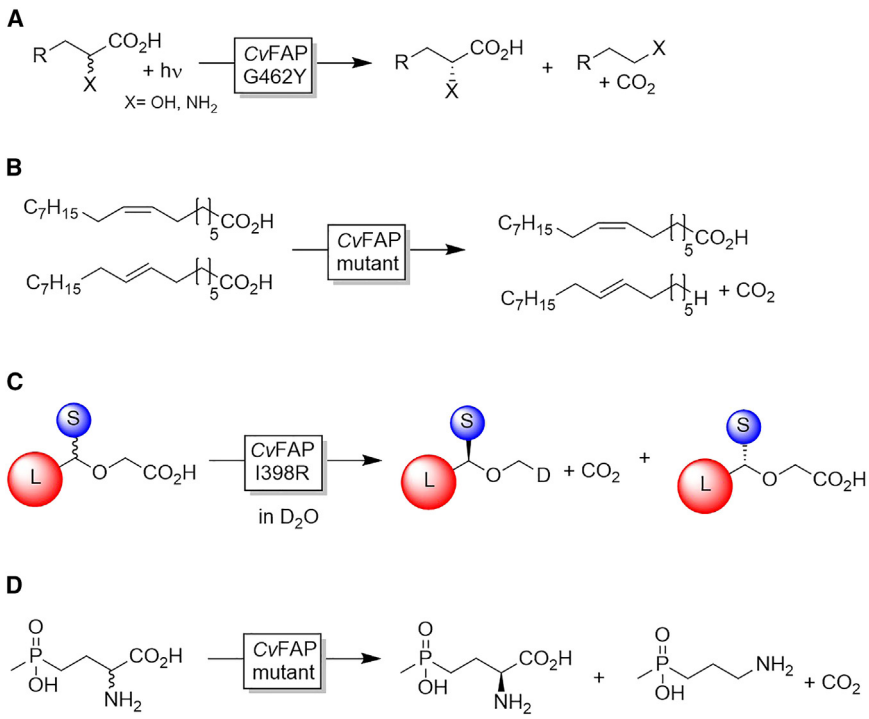

Figure 11. Selected examples of engineered *Cv*FAP for kinetic resolution reactions
(A) Xu et al.[51]
(B) Li et al.[52]
(C) Xu et al.[53]
(D) Cheng et al.[54]

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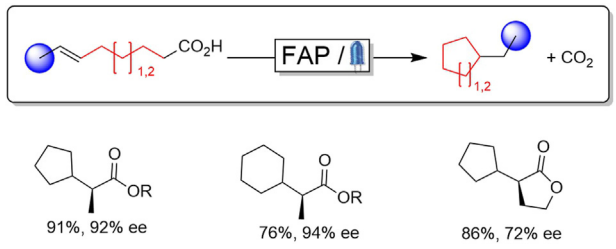

Figure 12. Engineered FAPs to function as radical cyclases of ε-unsaturated carboxylic acids and selected examples

electrochemical, and photochemical[104–107] NAD(P)H regeneration. As of today, however, TNs of $[Cp^*Rh(bpy)(H_2O)]^{2+}$ rarely exceed dozens to a few hundred, thereby reducing the economic attractiveness of this approach. Also, the mutual inactivation of $[Cp^*Rh(bpy)(H_2O)]^{2+}$ and enzymes will necessitate further development.[108]

Among the photobiocatalytic methods to regenerate NAD(P)H, hydrogenase,[109] liponamide dehydrogenases, and ferredoxin-$NADP^+$ reductases have been used.[110] However, none of these systems have been evaluated so far, even at small-scale preparative transformations.

The employment of phototrophic organisms also constitutes a form of photobiocatalysis, though a discussion of this exceeds the scope of this review. In recent years, there has been significant progress in the development of recombinant *Synechocystis* spp. for various redox transformations.[111–116] To bypass the sometimes laborious creation of recombinant phototrophic organisms, an alcohol/ketone-redox shuttle system has been introduced (Figure 21).[117] Although still in its nascent stages, this system displays several highly promising features; it is one of the few photobiocatalytic reaction systems genuinely utilizing water as an electron donor, it is broadly applicable to both NADH and NADPH-dependent reactions, and it potentially mitigates issues of photoinactivation through the use of metabolically active cells. Only time will determine whether this system can fulfill its potential.

NAD(P)H-independent, direct reductive regeneration. A variety of enzymes indirectly rely on reduced nicotinamide cofactors, notably Fe-containing monooxygenases and certain flavin-dependent monooxygenases and reductases. Therefore, with such biocatalysts, direct electron transfer to the enzyme-bound prosthetic group is principally feasible, theoretically simplifying the reaction scheme (Figure 22).

Therefore, it is hardly surprising that the widely studied heme-containing P450 monooxygenases have been targeted for protochemically driven, direct reductive regeneration, replacing natural single-electron donors such as ferredoxin. The catalytic mechanism of P450 monooxygenases involves two distinct SET steps (Figure 23; steps 1 and 3). These electrons, typically sourced via an electron transport chain from NAD(P)H, can be substituted by artificial single-electron donors such as ruthenium complexes[118–121] or organic photosensitizers like flavins[122–124] or eosin Y.[125]

Also, the direct photochemical regeneration of non-heme iron monooxygenases[126,127] and of some flavin-dependent monooxygenases has been reported.[128–131]

Unfortunately, however, photochemical, direct regeneration of monooxygenases has yet not delivered robust reaction schemes able to compete with the natural regeneration systems.

A potential explanation for this may be found in what is known as the "oxygen dilemma."[132] Essentially, the majority of mediators used for the photochemical, direct regeneration of monooxygenases are single-electron mediators, which the P450 monooxygenase mechanism also requires (Figure 23). Radicals formed in these processes react very rapidly with molecular oxygen (itself a diradical), whereas the reactions involving two-electron or hydride donors with triplet oxygen ($^3O_2$) are spin forbidden, making them kinetically very slow.

Flavin-containing ene-reductases from the old yellow enzyme (OYE) class are a group of O_2-independent oxidoreductases that only indirectly rely on the nicotinamide cofactor for reduction. The photochemical reduction of flavoproteins has been recognized since the 1970s,[133] but for a long time, this was not considered viable for promoting catalytic turnover in preparative biocatalysis, such as with OYEs. However, over the past decade, there has been a shift, with a variety of NAD(P)H-independent, light-driven reductions being evaluated (Figure 24).[134–140]

Indeed, the productivities observed for photobiocatalytic reduction reactions surpass those of corresponding oxyfunctionalization reactions discussed previously. However, the enzyme TNs still do not exceed several tens of thousands, posing a challenge to their economic viability. A potential limiting factor could be the known photoinactivation of flavins and flavoproteins, which may affect the robustness of the enzymes.[139]

Another intriguing example of photocatalytic activation of flavoenzymes is seen in fatty acid hydratases. Many of these enzymes contain a flavin molecule in their active site, which needs to be in its reduced form to facilitate efficient C=C double-bond hydration (even though the addition of water to a double bond is not a redox reaction).[141] Park and colleagues elegantly demonstrated the impact of *in situ* photoreduction of the active-site flavin on the activity of the oleate hydratase from *Lacticaseibacillus rhamnosus*.[142]

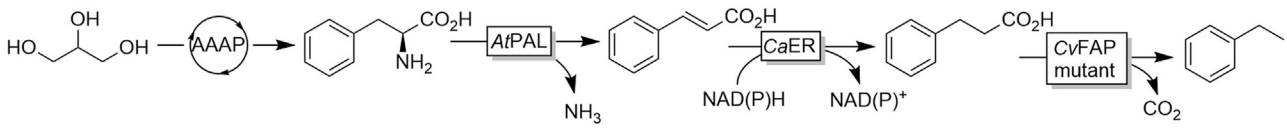

Figure 13. Whole-cell artificial cascade transforming glycerol into ethyl benzene

AAAP, aromatic amino acid pathway; *At*PAL, phenylalanine ammonia lyase from *Arabidopsis thaliana*; *Ca*ER, enoate reductase from *Clostridium acetobutylicum*.[62]

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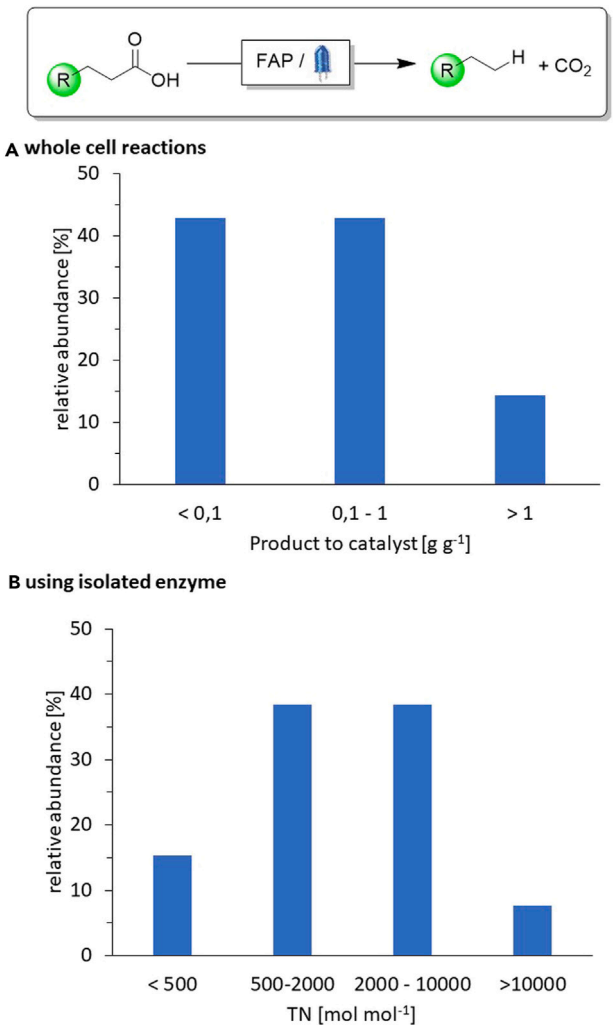

Figure 14. Catalyst performance of some representative FAP-catalyzed decarboxylation reactions

This is based on (A) whole-cells reactions[34,37,41,45,53,58,59,63–67] and (B) reactions using free enzymes.[32,36,39,40,43,44,46,51,52,56,68–71]

Oxidative regeneration of oxidoreductases

NAD(P)$^+$ regeneration systems. Like the corresponding NAD(P)$^+$ reductions approaches, also photocatalytic NAD(P)$^+$ regeneration systems date back to the 1980s[110] Particularly, quinoid systems, such as methylene blue[143,144] or flavins,[13,145,146] have gained some popularity, but single-electron acceptors, such as $[Ru(bpy)_3]^{3+}$,[147] can also be applied as in oxidative-direction, regioselectivity issues, as in the case of reductive NAD(P)H regeneration.

All in all, however, photochemical NAD(P)$^+$ regeneration reactions, like the corresponding NAD(P)$^+$-dependent oxidation reactions, play only a minor role in contemporary photobiocatalysis.

H_2O_2-driven biocatalytic oxyfunctionalizations. The aforementioned oxygen dilemma does not necessarily pose a challenge to redox biocatalysis. Since H_2O_2 is generally the end product of this dilemma, it can be leveraged to promote H_2O_2-dependent oxidation and oxyfunctionalization reactions (Table 3).[148] Peroxygenases[149–152] are exemplary enzymes that benefit from *in situ* H_2O_2 generation, as they depend on H_2O_2 as a stoichiometric oxidant and are irreversibly inactivated by high concentrations of it. Thus, maintaining a balance between the photochemically generated H_2O_2 rate and the H_2O_2 consumption catalyzed by peroxygenases is suspected to yield robust reaction schemes.

Early instances of photochemically driven peroxygenase reactions utilized flavin photosensitizers and EDTA as a sacrificial electron donor.[153,154,160–163] Respectable TNs were achieved for both the peroxygenase and flavin, yet the use of EDTA as a sacrificial electron donor resulted in significant waste production, including formaldehyde and primary amines. To address this, alternative electron donors have been assessed. The cathode emerges as an appealing reductant, with its relatively high O_2 reduction overpotential significantly reduced through photoactivation.[157,164–167] Methanol is another promising sacrificial electron donor, as, ideally, three equivalents of H_2O_2 can be obtained from one equivalent of MeOH.[155–157] Additionally, polyethylene terephthalate-based microplastic has been employed as a reductant.[168]

Ideally, water would serve as the sacrificial electron donor. Using Au-doped TiO_2, Zhang et al. demonstrated a proof-of-concept for this approach, albeit with very low productivities so far.[158] Reactive oxygen species, such as hydroxyl radicals, originating from photocatalytic water oxidation, pose a major challenge to the robustness of the biocatalyst.[169] Physical separation of the bio- and photocatalysts may resolve this issue upon scaling up. Furthermore, waste lignin has been shown to minimize the occurrence of reactive oxygen species (ROS) other than H_2O_2.[170]

Finally, broadening the spectrum of usable wavelengths also merits brief discussion. To cover a broader spectrum of visible light, Willot et al. employed complementary soluble photocatalysts, demonstrating an additive effect on productivity.[171] Moving to longer wavelengths, Park et al. utilized thermal irradiation to promote peroxygenase reactions by exploiting the Seebeck effect of Bi_2Te_3.[159] At the other extreme of the light spectrum, γ radiation is known to induce water splitting into H_2O_2 and H_2. By exposing a reaction mixture to γ radiation, such as that emitted by used nuclear fuel elements, catalytic turnover of a peroxygenase has been observed.[172] Once again, *in situ*-generated ROS posed the biggest issue in terms of robustness, and physical separation may represent a viable solution.

Overall, compared to other photobiocatalytic approaches, the *in situ* generation of H_2O_2 enables higher TNs. However, the preparative potential, particularly in terms of scaling up, remains to be demonstrated.

Photocatalysis/biocatalysis cascades

Finally, reaction schemes in which a biocatalytic and a photocatalytic step are also worth discussing.

Particularly, photobiocatalytic deracemization reactions have been reported. Either photochemical *in situ* racemization of C centers combined with a stereoselective enzymatic step or non-stereoselective, photocatalytic reductions of prochiral C=X

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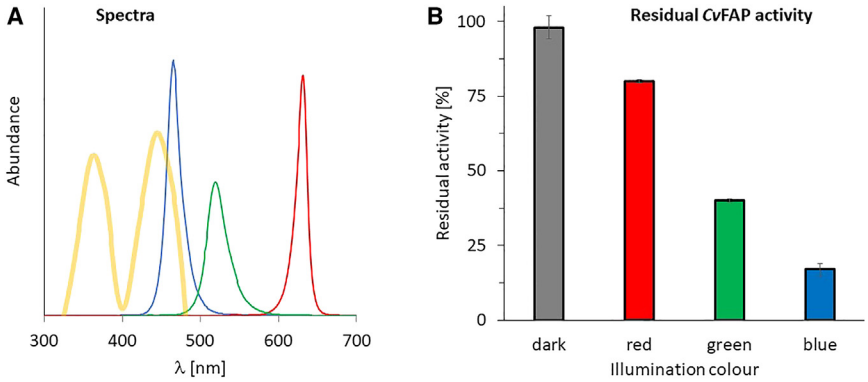

Figure 15. Photochemical properties of *Cv*FAP

(A) *Cv*FAP absorption and LED emission spectra.[13]

(B) Residual *Cv*FAP activity after preincubation under illumination.[69]

bonds combined with enzymatic, stereoselective oxidation (or vice versa) have been reported. An early example of the first approach stems from demonstrating the photocatalytic *in situ* racemization of chiral amines combined with a hydrolase-catalyzed kinetic resolution reaction (Figure 25).[173–175]

Also, non-activated β-C–H bonds in carbonyl compounds can be racemized photochemically by turning the carbonyl group into the corresponding enamine (Figure 26).[176]

Guo et al. established a deracemization of secondary amines by combining the non-stereoselective photo-induced reduction of imines with the oxidative kinetic resolution mediated by a monoamine oxidase (Figure 27).[177]

In the reverse direction, a photochemical oxidation of sulfides to racemic sulfoxides can be coupled to an enantioselective reduction mediated by sulfoxide reductases (Figure 28).

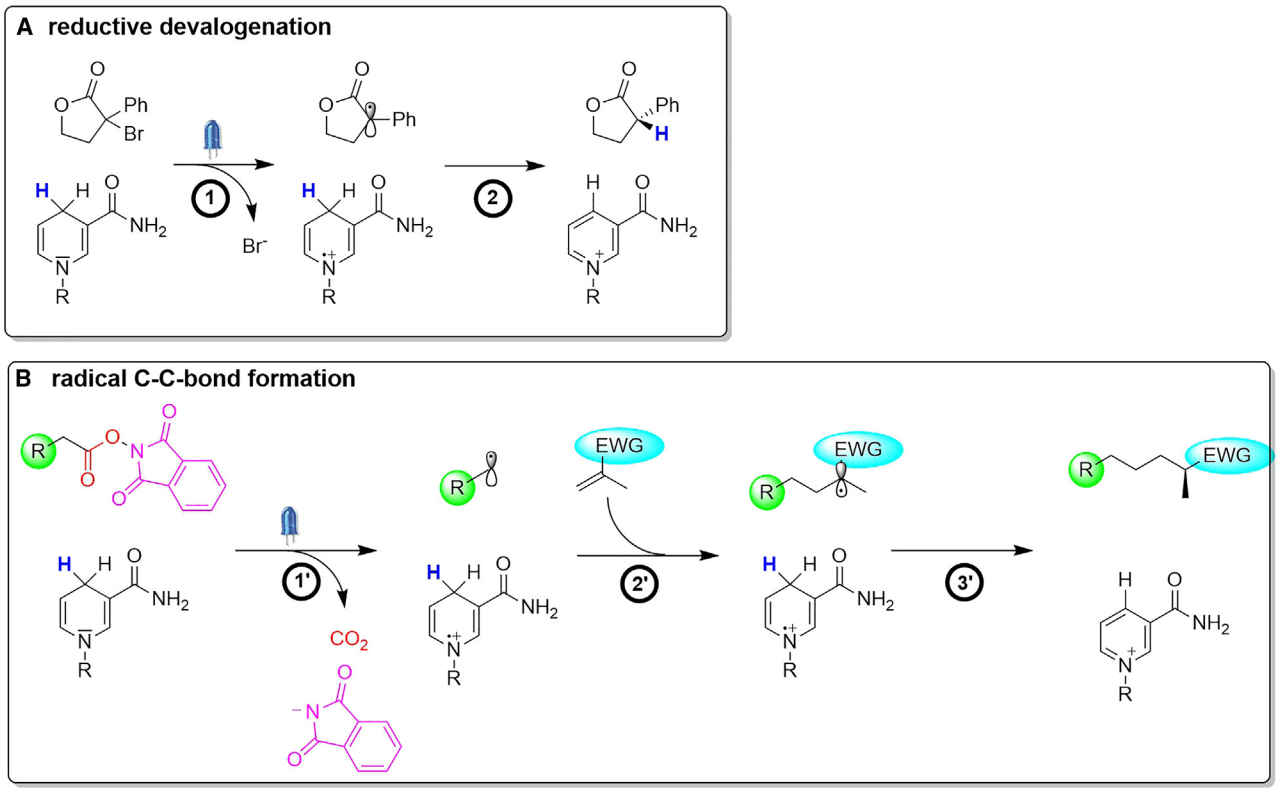

Figure 16. New-to-nature reactions of alcohol dehydrogenases enabled by photoexcitation of the enzyme-bound reduced nicotinamide cofactor

(A) Reductive dehalogenation of α-bromo esters. (1) Photoexcitation of the CT complex between enzyme-bound NADH and substrate enables SET from NADH to the substrate, followed by elimination of Br^-. (2) The mesomerically stabilized radical is reprotonated stereoselectively in an H-abstraction reaction, resulting in NAD^+ and the dehalogenated product.

(B) Radical coupling based on photocatalytic radical formation in N-(acyloxy)phthalimides and reaction with conjugated C=C double bonds. (1′) Similar to (1). (2′) C–C-bond formation by conjugate addition of the carbon-centered radical to a conjugated C=C double bond. (3′) Similar to (2).

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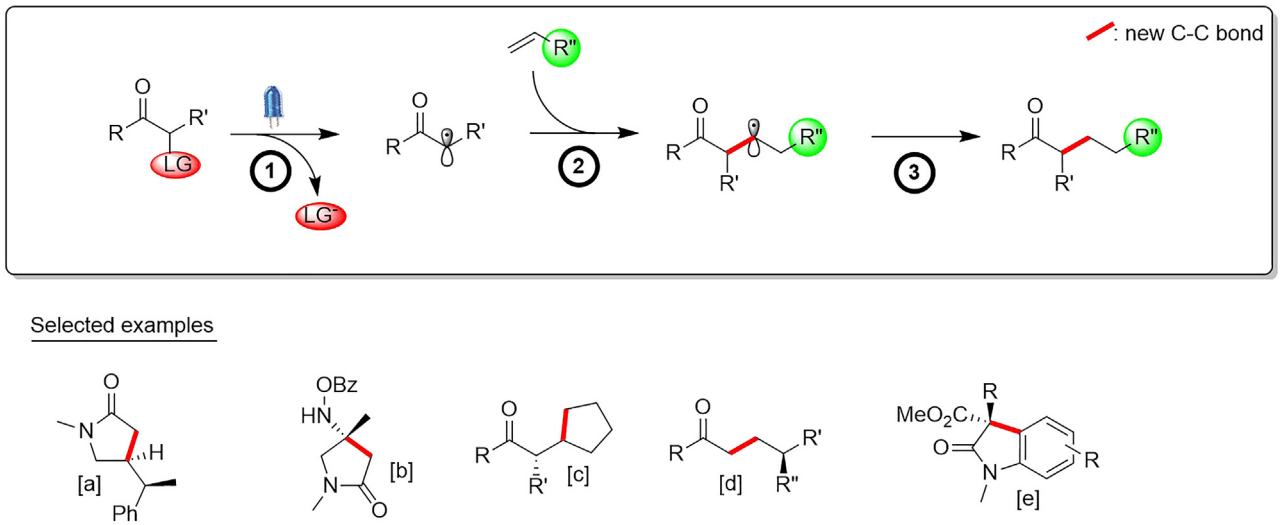

Figure 17. Flavoenzyme-catalyzed photoinitiated radical C–C-bond formation

[a] Sandoval et al.[89] and Cesana et al.[90]

[b] Gao et al.[91]

[c] Starting from α,β-unsaturated ketones.[92]

[d] Page et al.[93] and Huang et al.[94]

[e] Black et al.[95]

Finally, photo-induced E/Z-isomerization of conjugated C=C double bonds has been coupled to ene reductase (ER)-catalyzed C=C-bond reduction (Figure 29).[178] The thermodynamically favored E isomer is not accepted by the ER, whereas the unfavored Z isomer is readily converted. Using Ir-based or flavin photosensitizers, the equilibrium can be shifted to the Z isomer, thereby enabling the ER-catalyzed reduction.

The photocatalytic oxyfunctionalization of C–H bonds to prochiral carbonyl groups followed by selective, enzymatic reactions has been reported by Schmid and coworkers.[179] Starting materials containing activated C–H bonds, such as benzylic- or allylic C–H bonds or alcohols, were selectively oxidized to the carbonyl product using sodium anthraquinone sulfate as the photocatalyst (Figure 30). A range of diverse products is accessible through this combination; one-pot, one-step concurrent reactions are particularly feasible with cofactor-independent enzymes.

Overall, combining "the best of both worlds" from photo- and biocatalysis enables new possibilities for selective organic chemistry. This research area is relatively young and currently focuses on proof-of-concept studies. Hopefully, in the nearer future, interest in the catalytic performance of photo- and enzyme catalysts will increase and identify/address challenges, such as compatibility issues and productivity.

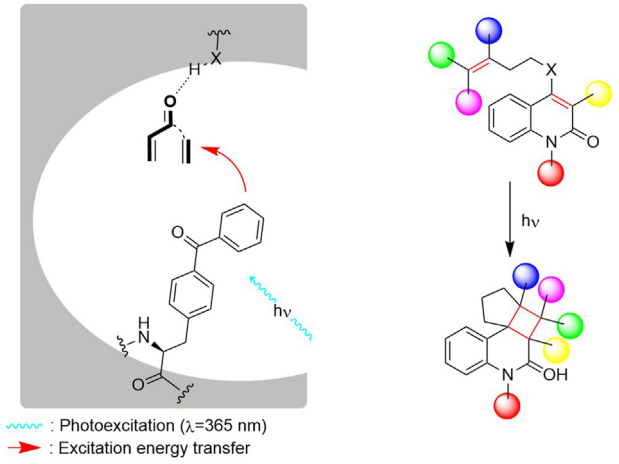

Figure 18. Creation of an artificial photoenzyme by incorporating a non-natural benzophenone amino acid and use of this for the stereoselective intramolecular Diels-Alder reaction

CONCLUSIONS AND OUTLOOK

In summary, photobiocatalysis is an exciting, dynamic, and rapidly growing field of research. Novel "new to nature" reactions are enabled by combining photocatalysis with biocatalysis, opening up a multitude of new synthetic possibilities.

It is entirely understandable that there is currently a "gold rush" atmosphere, with many research groups eager to explore the synthetic potential. This is good and proper. However, we must not forget that economically inefficient synthesis routes will ultimately remain laboratory curiosities. If our intention is to contribute to a better future through new chemistry, then we must not lose sight of this aspect.

To date, many, if not most, examples of photobiocatalytic reactions exhibit very low catalyst efficiencies in terms of TNs. Specifically, in the case of photoactivated flavoproteins, the

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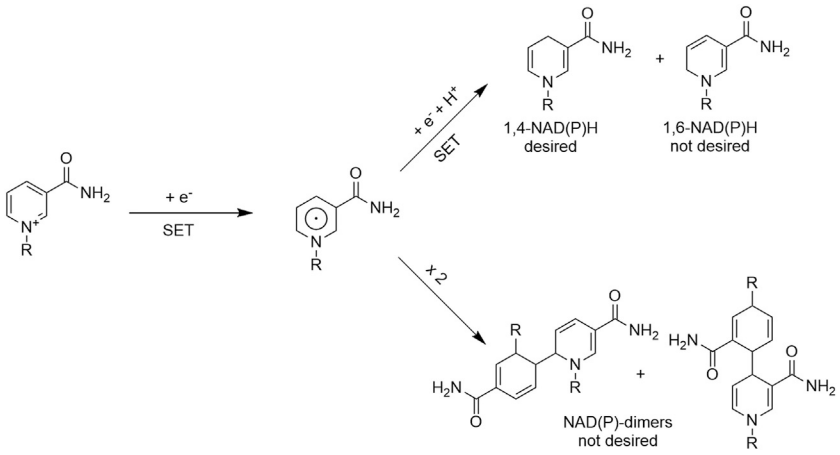

Figure 19. Direct reduction of NAD(P)$^+$ leads to significant amounts of undesired side products

quantities of biocatalysts used are in the mol % range (equivalent to only a few hundred turnovers). Even for photodecarboxylases, a maximum of 10,000 turnovers has been achieved so far. In this case, the hypothetical cost contribution of the enzyme to product synthesis would be in the range of at least several hundred Euros per kilogram. It is obvious that this excludes any application in the field of new fuels. We have seen that many researchers are aware of this and are working to increase the efficiency of these reactions. However, we are not aware of any work to date that addresses the photobleaching of the flavin group and/or the effects of photoactivated flavins on the amino acids of the active site. We believe that enzyme engineering can at least mitigate photoinactivation. Since classical high-throughput screening assays fail in the case of unreactive alkanes, small mutant libraries are necessary. These require an intelligent selection of amino acid positions. Structure-guided approaches[75] as well as AI-based approaches[180,181] may play a significant role here. Similar considerations may apply to other flavin-based photobiocatalytic systems.

Also, in the field of photocatalytic regeneration methods, the efficiencies achieved so far do not match those of purely biocatalytic counterparts. Here, too, it is important to clearly identify and eliminate the limiting factors. Presumably, photobleaching and enzyme inactivation by reactive, excited catalysts and/or ROS are significant here as

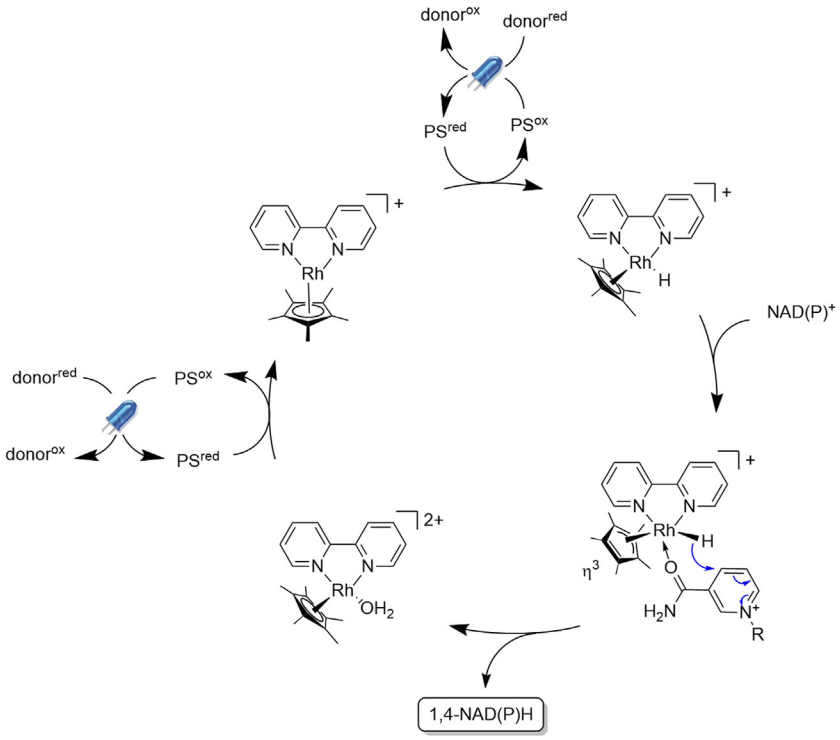

Figure 20. Using [Cp*Rh(bpy)(H$_2$O)]$^{2+}$ as catalyst for the selective reduction of NAD(P)$^+$ into enzyme-active 1,4-NAD(P)H

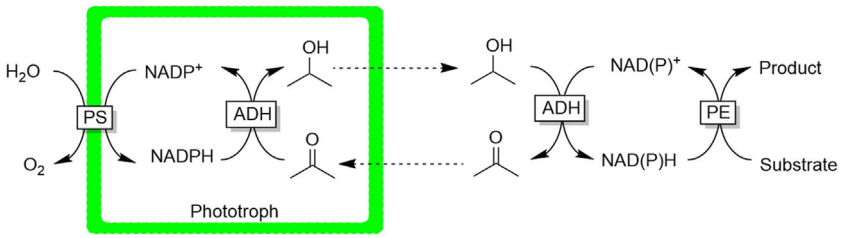

Figure 21. Photobiocatalytic regeneration of NAD(P)H using a phototropic cyanobacterium
The cell redox metabolism is coupled to an *in vitro* enzymatic productions system through an alcohol/ketone shuttle. PS, photosystem; PE, production enzyme.

well. A practical solution may be compartmentalization using flow chemistry.[182] The same applies to cascade reactions, where photocatalytic steps are combined with enzymatic steps.

Numerous challenges remain before photobiocatalysis can transition from a laboratory curiosity to the method of choice for chemical synthesis on an industrial scale. It remains unclear whether this transition will succeed, but this very uncertainty makes this field so exciting to study!

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AUTHOR CONTRIBUTIONS

Y.M., Yutong Wang, B.W., J.Z., S.Y., F.Z., and K.L. performed the literature search, analyzed literature data, and provided quantitative analyses. Y.M., Yonghua Wang, and F.H. conceived the study and wrote the manuscript. All authors have approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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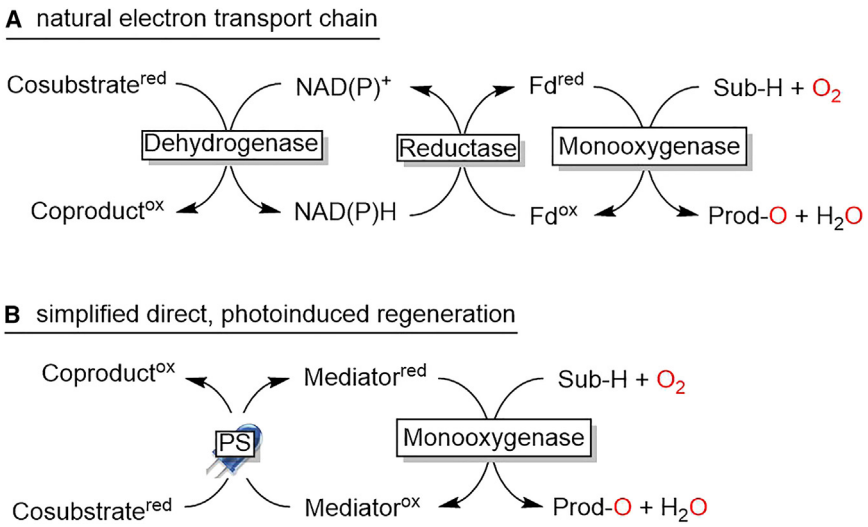

Figure 22. The promise of simplified reaction schemes simplifying monooxygenase catalysis
(A) The natural electron transport chain comprising two additional regeneration enzymes and two cofactors.
(B) Simplified photocatalytic regeneration.

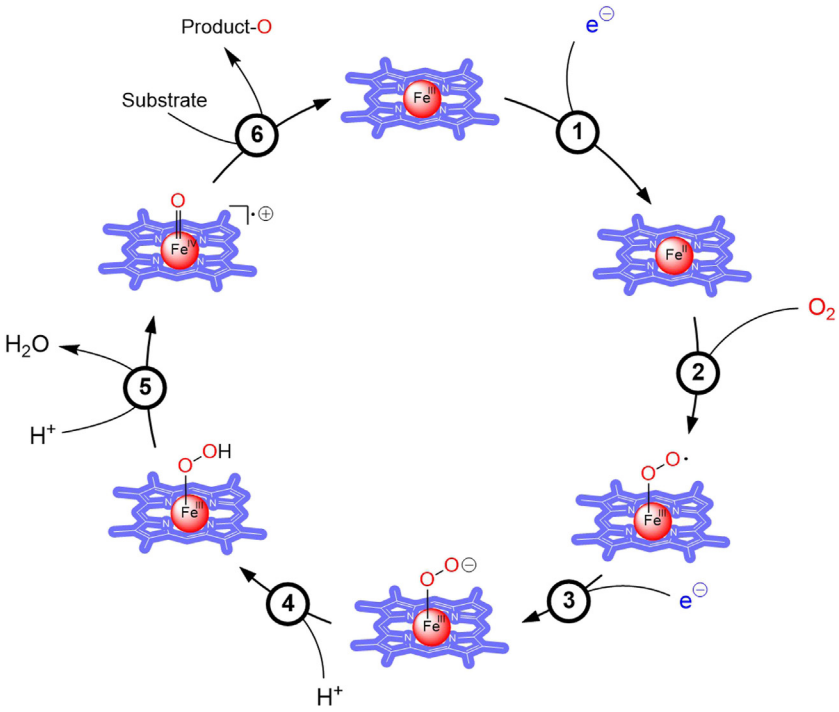

Figure 23. Simplified mechanism of P450-catalyzed oxyfunctionalization reactions

The mechanism comprises two SET steps (1 and 3) with an intermediate binding of molecular oxygen (2); the catalytically active Compound I is then formed in a sequence of protonation and water elimination steps (4 and 5). Finally, compound I transfers the highly activated oxygen atom to the substrate (6, highly simplified).

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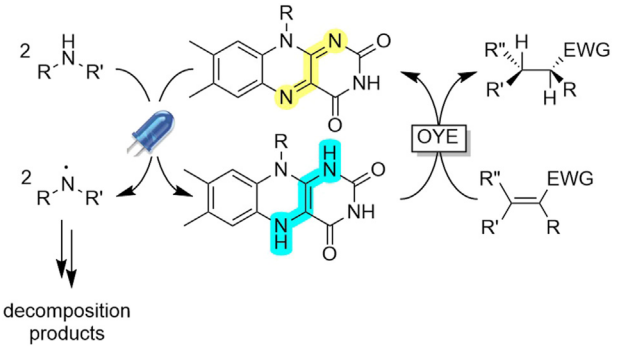

Figure 24. Photochemical reductive regeneration of OYEs for enantioselective reduction of conjugated C=C double bonds

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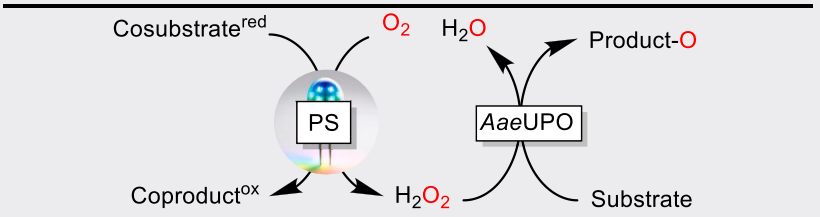
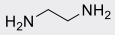
Table 3. Selected examples of photocatalytic H_2O_2 generation to promote peroxygenase-catalyzed oxyfunctionalization reactions



Cosubstrate	Coproduct	PS	Waste [g mol$^{-1}_{H_2O_2}$]	turnover numbers PS	turnover numbers *Aae*UPO
EDTA	4 CH_2O + 4 CO_2 	FMN	81.5	800	126.000 [a]
				20	30.000 [b]
CH_3OH	CO_2	Au-TiO_2	14.7	n.d.	80.000 [c]
		g-C_3N_4		n.d.	60.000 [d]
		SAS		74	318.000 [e]
H_2O	O_2	Au-TiO_2	-	n.d.	39.000 [f]
		Bi_2Te_3		n.d.	32.000 [g]

a,[153] b,[154] c,[155] d,[156] e,[157] f,[158] g.[159]

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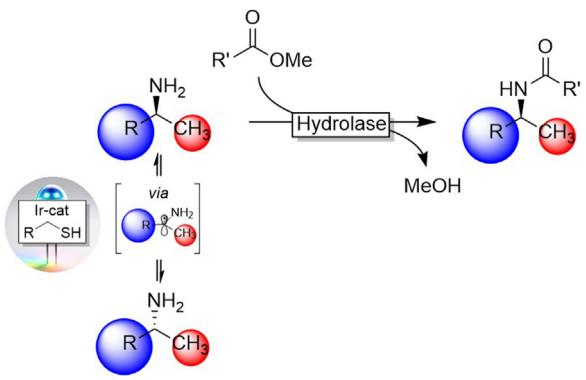

Figure 25. Dynamic kinetic resolution of racemic amines
The *in situ* racemization is achieved by a thiyl radical formed by hypoxanthine aminopterin-thymidine medium to a photoexcited Ir catalysts.

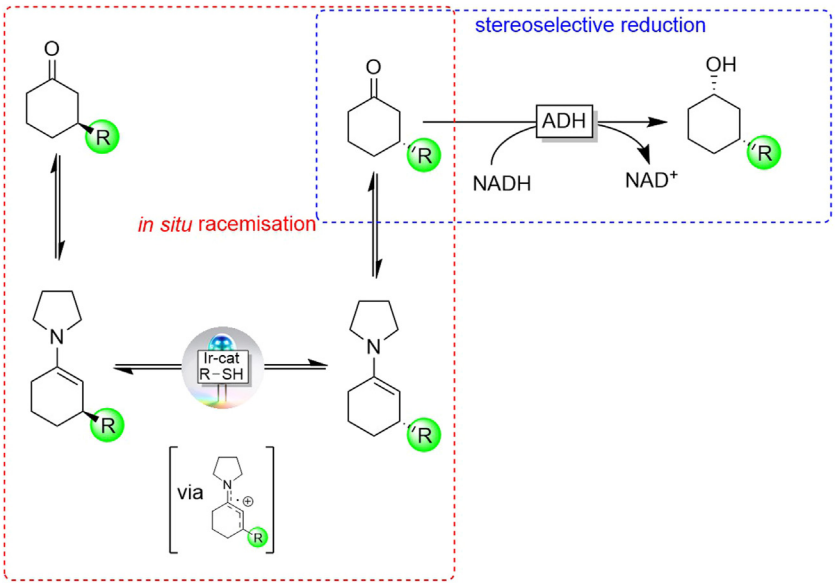

Figure 26. Photochemical *in situ* racemization of β-substituted carbonyl compounds followed by enantioselective, alcohol dehydrogenase-catalyzed carbonyl reduction

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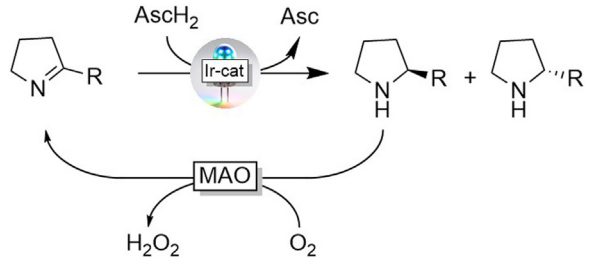

Figure 27. Deracemization of amines using stereoselective, monoamine oxidase-catalyzed, stereoselective oxidation and non-selective, photocatalytic imine reduction

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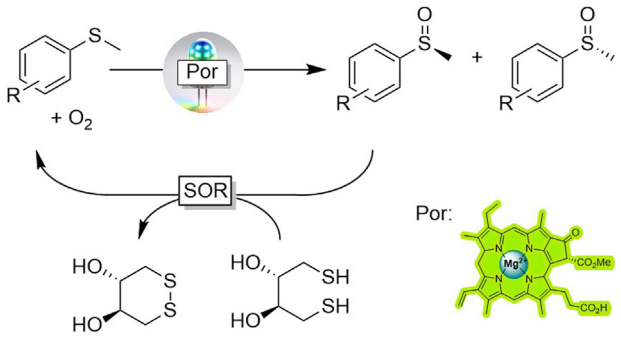

Figure 28. Enantioselective sulfoxide reduction combined with photochemical, non-enantioselective sulfoxidation

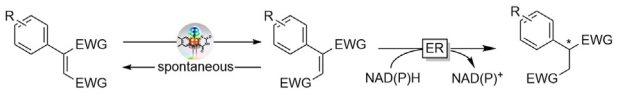

Figure 29. Photoinduced E/Z isomerization to generate the thermodynamically unfavored substrate for an ene-reductase-catalyzed C=C reduction

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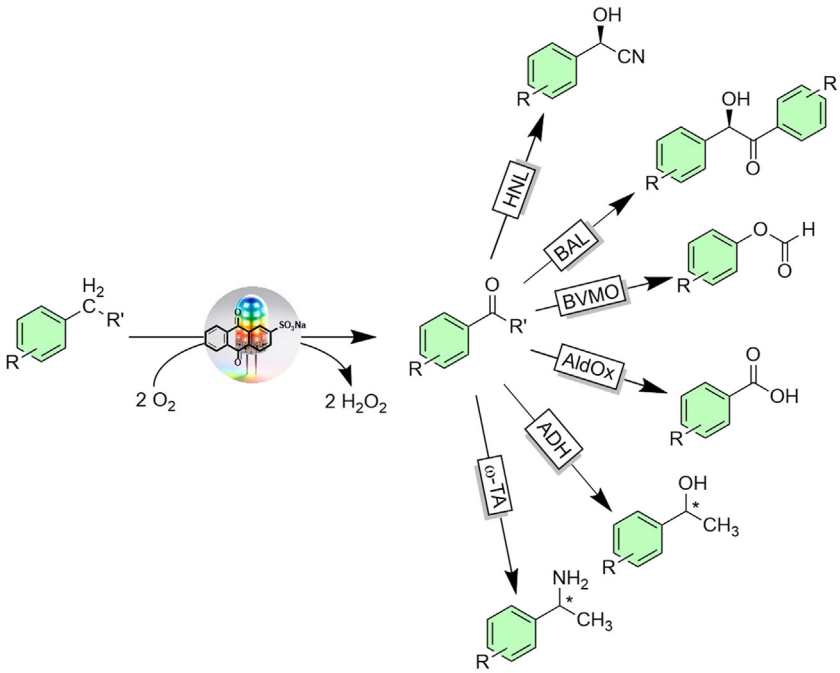

Figure 30. Photocatalytic oxyfunctionalization of activated benzylic C–H bonds coupled with biocatalytic functional group conversion

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