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Höfler, Georg; Hollmann, Frank; Paul, Caroline E.; Rauch, Marine; van Schie, Morten; Willot, Sebastien

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Georg Höfler, Frank Hollmann, Caroline E. Paul, Marine Rauch,
Morten van Schie, Sebastien Willot

Chapter 9
Photocatalysis to promote cell-free biocatalytic reactions

Abstract: Cofactors assist enzymes to catalyze reactions and are indispensable and ubiquitous in nature, playing a central role in metabolic pathways. In biocatalysis, common redox cofactors such as nicotinamide, flavin and heme can be activated by light or synthetized to vary redox potentials, leading to different types of reactions for the formation of interesting chiral products, unattainable through classical chemical methods. This chapter will focus on light-driven cell-free biocatalytic reactions activated via their redox cofactors.

Keywords: redox reactions, nicotinamide cofactor, flavin, oxidoreductases, photobiocatalysis

9.1 Introduction

Organic synthesis using enzymes is usually called biocatalysis. During the past decades, biocatalysis has been enjoying an ever-increasing popularity among synthetic organic chemists. Especially, the mild reaction conditions and the usually high selectivity of enzyme-catalyzed reactions are valued on lab and industrial scale [1–5].

While industrial biocatalysis mostly relies on one-step transformations the trend in academic research more and more is shifting toward multistep syntheses, transforming simple starting materials into significantly more complex (and value-added) products [6, 7]. Such cascade reactions are particularly attractive if intermediate product isolation and purification can be omitted leading to significant savings in solvent use and reduced environmental footprints [8]. Cascades comprising several enzymatic steps or combining transition metal catalysis, organo catalysis or heterogeneous catalysis are frequently reported nowadays [6, 7]. Following them, autotrophic organisms such as cyanobacteria are starting to be increasingly used for applications in biocatalysis (see Chapters 5–8), and cell-free photoenzymatic reactions (combining photocatalytic reactions with biocatalytic ones) are catching up [9–12].

Photobiocatalysis using isolated enzymes can be divided into (1) photocatalytic regeneration cascades, (2) “true” photoenzymatic cascades and (3) photoenzymatic reactions. In photoenzymatic cascades, redox enzymes are supplied with redox equivalents needed for their catalytic cycles, that is, photocatalytic regeneration of redox enzymes. “True” photoenzymatic cascades combine a biocatalytic transformation with

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a photocatalytic generation of the enzyme's starting material or a follow-up step of the enzymatic product. "Photoenzymes" need light to perform their catalytic reaction.

In this contribution we critically review the current state-of-the-art of all types of photoenzymatic cascades.

9.2 Photocatalysis to regenerate redox enzymes

Cofactors (Box 9.1) can refer to inorganic metal ions such as Zn or Fe, or organic molecules called coenzymes, that assist enzymes to catalyze reactions (see info Box 9.1). In particular, nicotinamide adenine dinucleotide (NAD(P))- and flavin-dependent oxidoreductases (see info Box 9.2) play a central role in the energy metabolism of heterotrophic and autotrophic organisms, which place their redox cofactors at the center of metabolic pathways. Understanding the role these cofactors play and how to use them is necessary for the development of photobiocatalysis processes via the regeneration of their cofactor.

Box 9.1: Cofactors and coenzymes in biotechnology

Cofactors are non-protein organic molecules (also known as coenzymes) or inorganic metal ions required by an enzyme to assist during a biocatalytic reaction. Typical cofactors for oxidoreductases are redox coenzymes: nicotinamide adenine dinucleotide NAD(P), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and heme. Coenzymes can be found to be covalently or tightly bound as a prosthetic group to a protein, or only transiently bound and used as co-substrates. Inorganic cofactors, such as Mg, Zn, Co, Mo and iron-sulfur (Fe-S) clusters, can play both functional and structural roles. A cofactor-bound enzyme is a holoenzyme, whereas an enzyme without its cofactor is an inactive apoenzyme. As enzymes can display high cofactor specificity, coenzymes require an appropriate and efficient regeneration system in whole-cells and cell-free biocatalytic systems. Protein engineering can be used to switch cofactor preference. New synthetic (biomimetics) and natural coenzyme derivatives (F_{420}) are continuously being discovered for improved and diverse types of reactions.

9.2.1 Reductive regeneration

A broad range of biocatalytic redox reactions require reductive regeneration, that is, provision of the production enzyme with reducing equivalents. First, reduction reactions catalyzed by reductases obviously require reducing equivalents. However, a wide variety of oxidation reactions involve reduction of the production enzymes (monooxygenases). This seeming contradiction can be explained by the catalytic mechanism of monooxygenases: molecular oxygen is reductively activated at the enzymes' active sites to be incorporated into the substrates.

Principally, reductive regeneration of redox enzymes (Box 9.2) can be achieved either directly, that is, by direct reduction of the enzymes' active sites or indirectly,

that is, involving the nicotinamide cofactors. Both approaches will be outlined in the following sections.

9.2.1.1 Via regeneration of reduced nicotinamide cofactors

The reduced nicotinamide cofactors NADH and NADPH play a pivotal role as electron donors in many biocatalytic redox reactions (Scheme 9.1).

The basic electrochemical features of the nicotinamide cofactors are shown in Scheme 9.2. In essence, NAD(P)H serves as biological hydride donor while its oxidized pendants ($NAD(P)^+$) serve as hydride acceptors.

Box 9.2: Oxidoreductases and cofactors

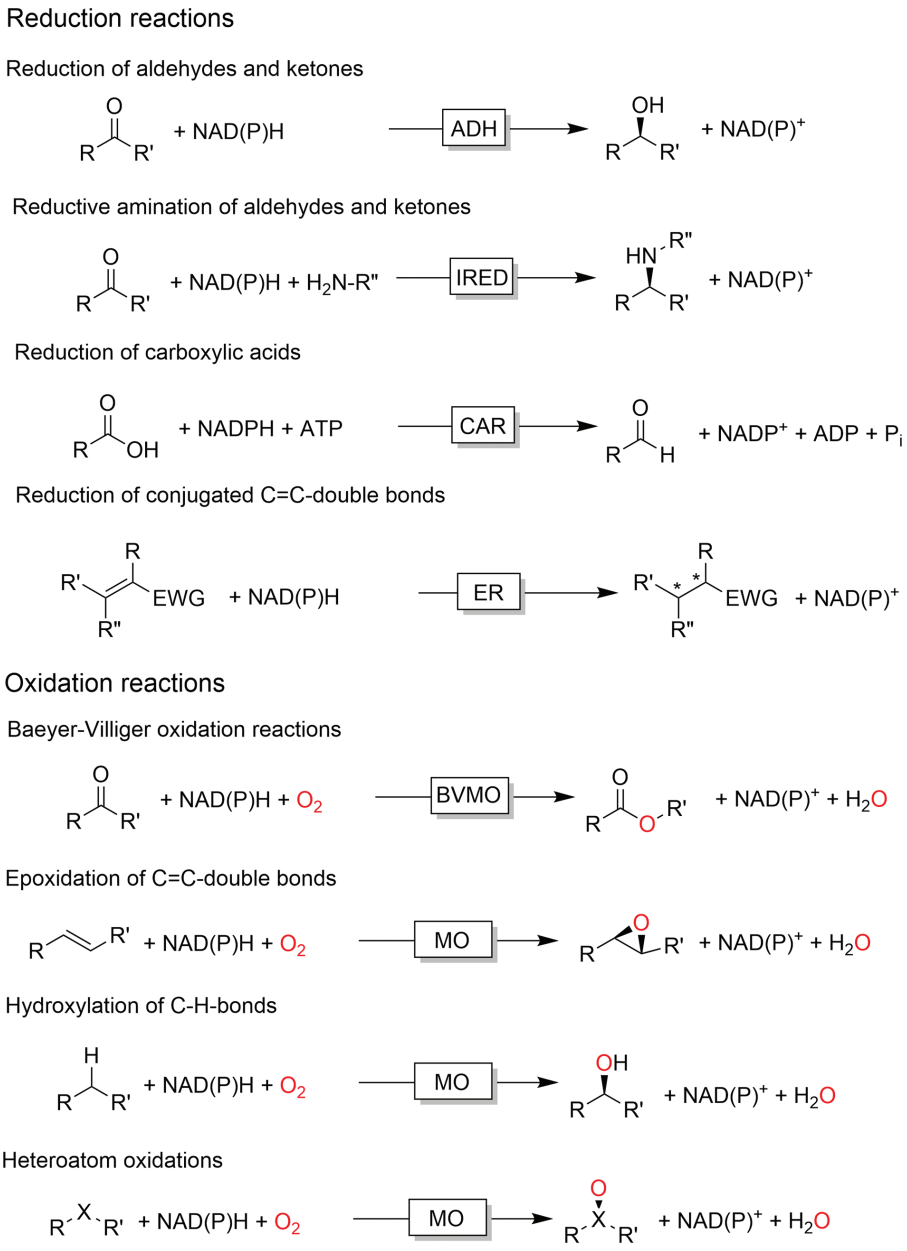
Oxidoreductases are one of the seven classes of enzymes (EC 1), and these enzymes catalyze reduction (gain of electrons), such as carbonyl reduction, and oxidation (loss of electrons) reactions, such as hydroxylation, *via* their redox cofactor. Approximately 80% of known oxidoreductases require the nicotinamide adenine dinucleotide cofactor, which plays a central role in metabolic pathways (see info Box 9.1). Several oxidoreductases, such as alcohol dehydrogenases (ADHs) and medium chain reductases, can also depend on Zn for structural and catalytic activity. Coenzymes needed in cell-free biocatalytic reactions, for example, NAD(P)H, tend to be expensive and thus are used in catalytic amounts coupled to a regeneration system.

The central role of NAD(P) as electron donor and acceptor in biocatalytic redox reactions has motivated researchers to develop *in situ* regeneration systems to allow for the use of these costly cofactors in catalytic amounts and thereby reduce their cost contribution to the desired product [13].

Today, enzymatic regeneration systems prevail in preparative application, mostly due to their inherent compatibility with the enzymatic production systems but also due to the ease of application. The most common systems are shown in Table 9.1.

Another reason for the dominance of enzymatic regeneration systems lies in their intrinsic regioselectivity. The reduction of $NAD(P)^+$ to NAD(P)H can principally lead to three different regioisomers of NAD(P)H while only the 1,4-NAD(P)H can be used by the production enzyme. Hence, a successful NAD(P)H regeneration system must be highly selective otherwise, losses in the costly nicotinamide cofactor due to formation of inactive regioisomers will make the approach economically unattractive [14].

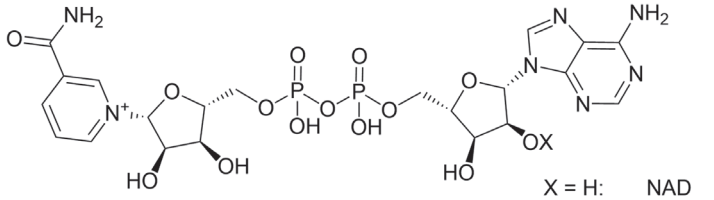
Unfortunately, the majority of photocatalysts follow a so-called ECE (electron transfer – chemical – electron transfer) mechanism resulting in two major issues for the selective formation of 1,4-NAD(P)H. First, the intermediate NAD-radical can dimerize (comprising yet another pathway to inactivate the nicotinamide cofactor). Second, the chemical protonation step seldom is regioselective leading to the formation of the undesired NAD(P)H isomers (Scheme 9.3) [15].



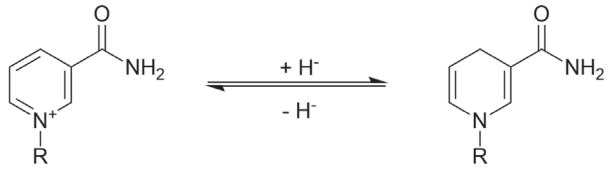
Scheme 9.1: Selection of preparatively relevant NAD(P)H-dependent redox reactions. ADH: alcohol dehydrogenase, IRED: imine reductase, CAR: carboxylic acid reductase, ER: ene reductase, BVMO: Baeyer-Villiger monooxygenase; MO: monooxygenase (general).

To circumvent (or at least alleviate) the loss of enzyme-active 1,4-NAD(P)H due to direct single electron reduction by the reduced photocatalyst, generally a relay system is applied to convert the ECE-steps into a regioselective hydride transfer step. The organometallic complex $[Cp^*Rh(bpy)(H_2O)]^{2+}$ proposed by Steckhan[[16–19]] or NAD(P)H:flavin oxidoreductases[[20–23]] are the most frequently used for this purpose.

chemical structure of NAD and NADP

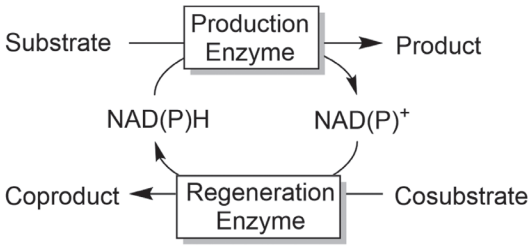


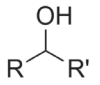
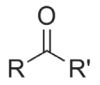
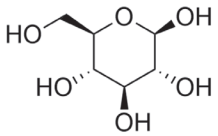
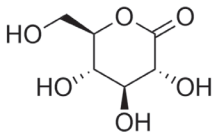
basic redox chemistry of NAD(P)

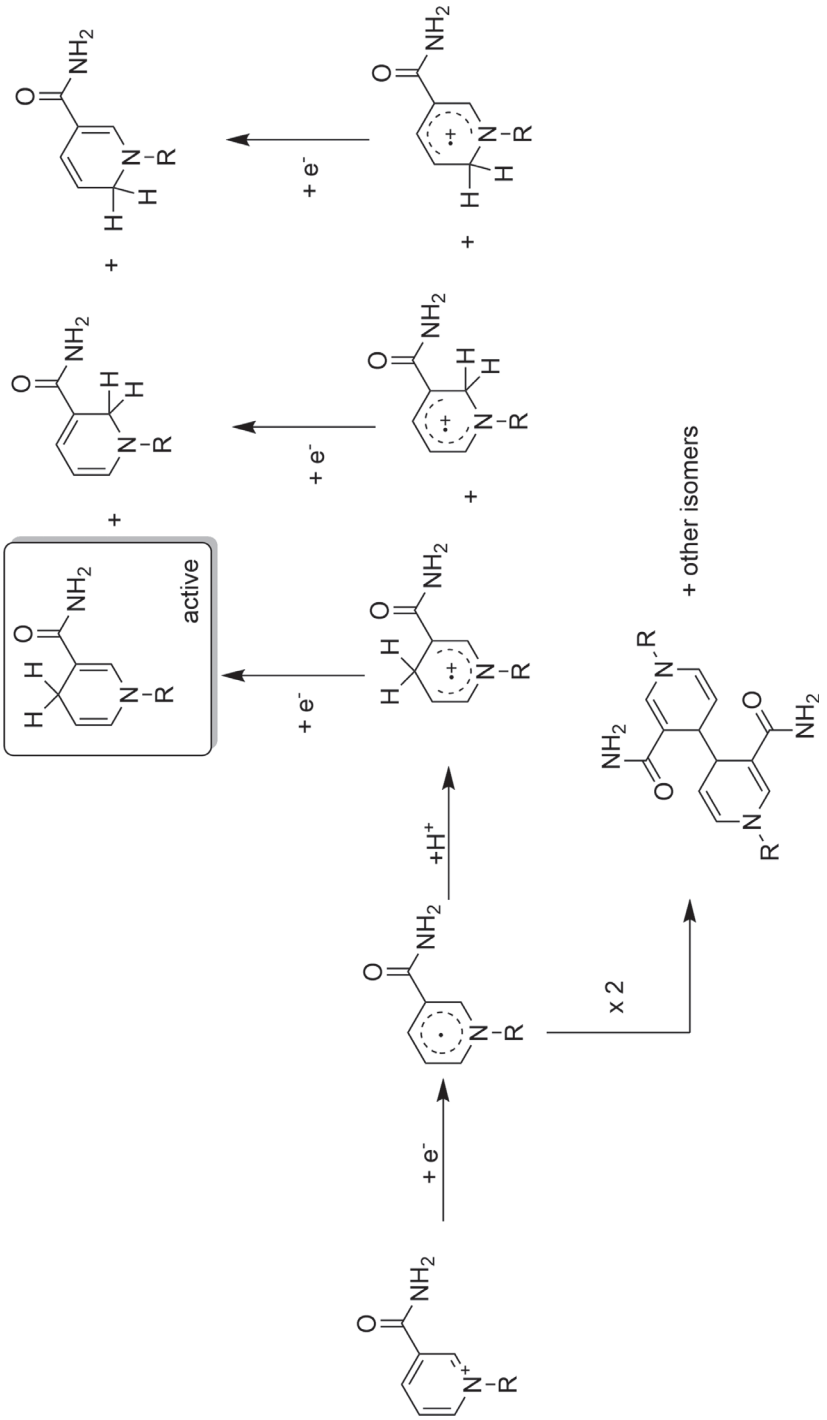


Scheme 9.2: Structure and basic electrochemistry of the nicotinamide cofactors.

Table 9.1: Selection of common enzymatic NAD(P)H regeneration systems.



Regeneration Enzyme	Cosubstrate	Coproduct
Formate dehydrogenase (FDH)	HCO_2H	CO_2
Alcohol dehydrogenase (ADH)		
Glucose dehydrogenase (GDH)		
Phosphite dehydrogenase (PDH)	H_3PO_3	H_3PO_4
Hydrogenase (HAse)	H_2	-

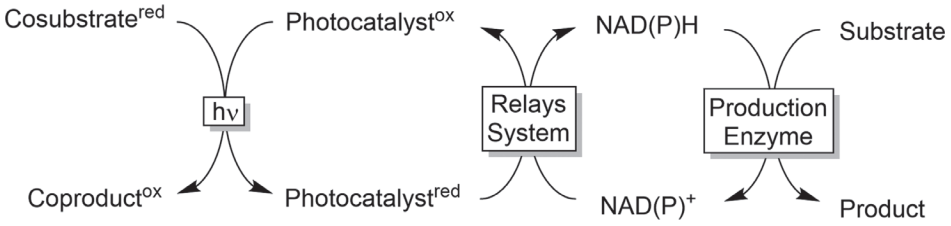


Scheme 9.3: ECE mechanism of NAD(P)$^+$ reduction and its consequences for the formation of NAD(P)-dimers and NAD(P)H isomers.

A selection of photochemical NAD(P)H regeneration systems used to promote biocatalytic reduction reactions are summarized in Table 9.2. Although various photocatalysts and relay systems have been reported in the past ten years, the overall NAD(P) turnover numbers and the product concentrations achieved so far are disillusioning. Compared to the multiple thousands (even millions) reported for enzymatic regeneration systems the current performance falls back by orders of magnitude.

Significant improvements will be necessary in the nearer future to make photochemical NAD(P)H regeneration systems a viable alternative (rather than a lab curiosity) to existing enzymatic systems.

Table 9.2: Selection of indirect photochemical NAD(P)H regeneration systems.



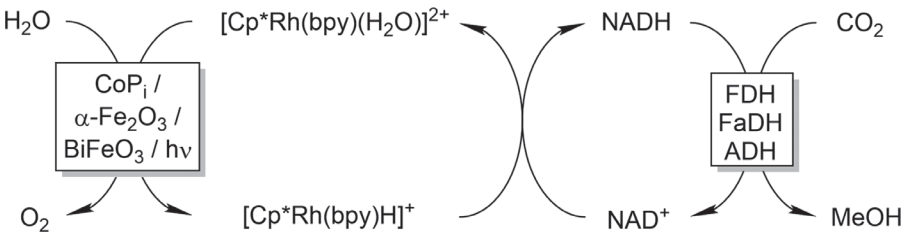
Cosubstrate	Photocatalyst	Production enzyme / Product (final con. [mM])	TN (NAD(P)	TN (Catalysts)	Ref
$[Cp^*Rh(bpy)(H_2O)]^{2+}$ as a relay system[24]					
TEOA	CNR	GluDH / Glutamate (10)	10	Rh: 20 CNR: n.d. GluDH: n.d.	[25]
TEOA	mCNS	LacDH / Lactate (5)	5	Rh: 20 mCNS: n.d. LacDH: n.d.	[25]
TEOA	Eosin Y	GluDH / Glutamate (10)	200	Rh: 40 Eosin Y: 500 GluDH: n.d.	[26, 27]
TEOA	$[Ru(bpy)_3]^{2+}$	GluDH / Glutamate (5)	5		[28]
H_2O	$[Co_4(H_2O)_2(PW_9O_{34})_2]^{10-}$	GluDH / Glutamate (5)	1.5		[28]
TEOA	Chemically converted graphene	*Lb*ADH / Various alcohols (<10 mM)	15	Rh: 30	[29, 30]

Table 9.2 (continued)

TEOA	Chemically converted graphene	FDH / HCO_2H	116	Rh: 232	[31–33]
	Hydrogen-Terminated Silicon Nanowires	GluDH / Glutamate (5)	4	Rh: 20	[34]
NAD(P)H:flavin oxidoreductases as relay system					
Ascorbic acid	Quantum dots	*Tb*ADH / Isobutanol	8	FNR: 3167	[21]
EDTA	DRf	ADH-A / Chiral alcohols (<5 mM)	21	PDR: 870 DRf: 72 MV: 17	[35]

TEOA: triethanolamine; CNR: graphitic carbonitride nanorods; mCNS: mesoporous carbonitride spheres; LacDH: lactate dehydrogenase; GluDH: glutamate dehydrogenase; FDH: formate dehydrogenase; DRf: 5-deazariboflavin; MV: methyl viologen.

An interesting cascade for the complete reduction of CO_2 to methanol using solar-powered regeneration of NADH to promote dehydrogenase-catalysis was reported by Park and coworkers (Scheme 9.5) [36]. Though also here methanol yields and efficiency of the complex cascade still leave room for improvement, a convincing proof-of-concept was provided.



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Scheme 9.4: Coupling photochemical water oxidation to enzymatic CO_2 reduction. The photocatalytic cascade comprises $BiFeO_3$ as photoactive catalyst transferring electrons to [Cp*Rh(bpy)(H^2O)]$^{2+}$. The electrons are obtained from water by co-catalysis of cobalt phosphate (CoPi) and α-Fe_2O_3. The reduced Rh-complex specifically transfers NAD^+ into NADH, which drives the reduction of CO_2 to MeOH through a cascade of formate dehydrogenase (FDH), formaldehyde dehydrogenase (FaDH) and alcohol dehydrogenase (ADH).

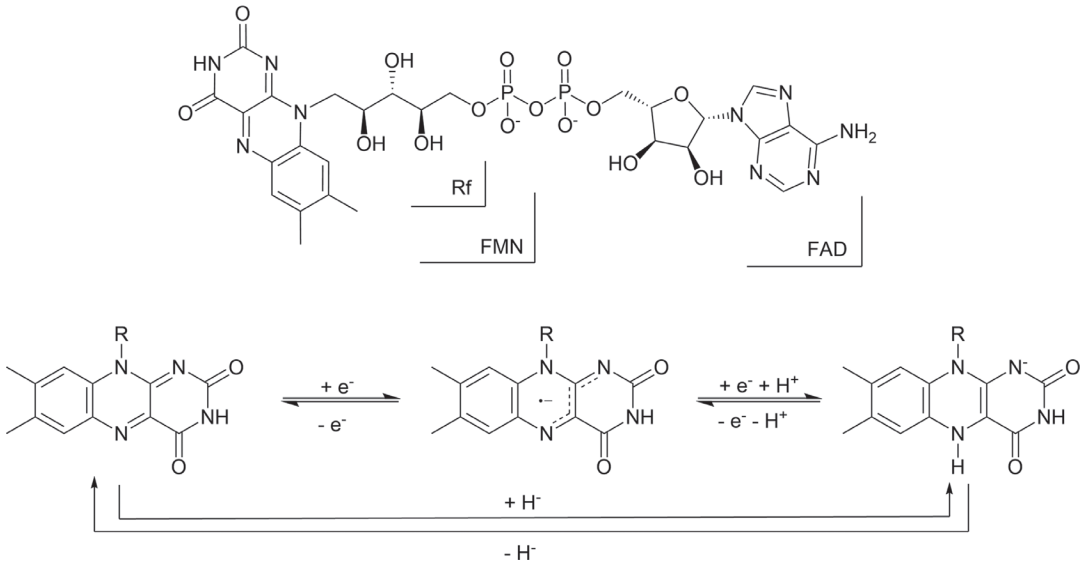
Conspicuously, most reduction reactions have been reported so far with few exceptions on monooxygenases.[37,38] A plausible explanation for this is the so-called *oxygen dilemma* [39]. Since most photochemical redox reactions follow single electron transfer mechanisms, radicals are involved in the NAD(P)H regeneration step. Radicals, however, react very fast (diffusion-controlled) with molecular oxygen, thereby diverging the electron flow away from $NAD(P)^+$ (or the relay catalysts) to O_2 [40].

9.2.1.2 Via direct regeneration (NAD(P)H-independent approaches)

Although NAD(P)H serves as a universal reductant in biocatalytic systems, it is not necessarily involved in the enzymes' catalytic mechanisms. In these cases, other reductants can take over from NAD(P)H, thereby significantly simplifying the overall regeneration scheme.

Flavin-dependent reductases

Flavin-dependent ene reductases Old Yellow Enzymes (OYEs), for example, have been in focus of direct photochemical regeneration for some time now. Flavins (Scheme 9.5) exhibit a more flexible redox chemistry especially if compared to the aforementioned nicotinamide cofactors. Therefore, flavoenzymes appear more suitable for direct (not including NAD(P)H) regeneration, for example, by reduced photosensitizers.

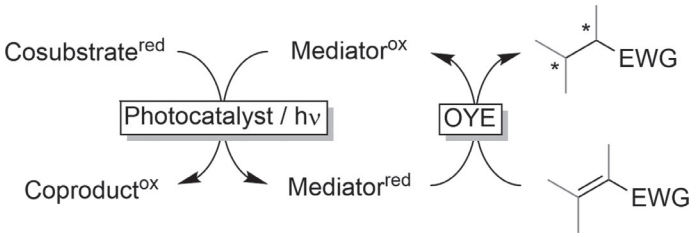


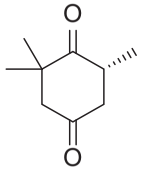
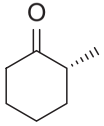
Scheme 9.5: Structural features and basic redox chemistry of flavins.

A selection of recent examples comprising photochemical regeneration of OYEs is listed in table 9.2.

One advantage of the NAD(P)H-independent, direct regeneration of OYE is that the costly and instable nicotinamide cofactor (together with an enzymatic regeneration system) can be omitted from the reaction scheme. Furthermore, photochemical OYE-regeneration systems do not regenerate the nicotinamide cofactor. Thus, NAD(P)H-dependent enzymes are not regenerated and possible side-reactions such as ketoreduction is avoided. To achieve this chemoselectivity with traditional regeneration schemes, highly purified enzyme preparations (devoid of any ADHs) are required.

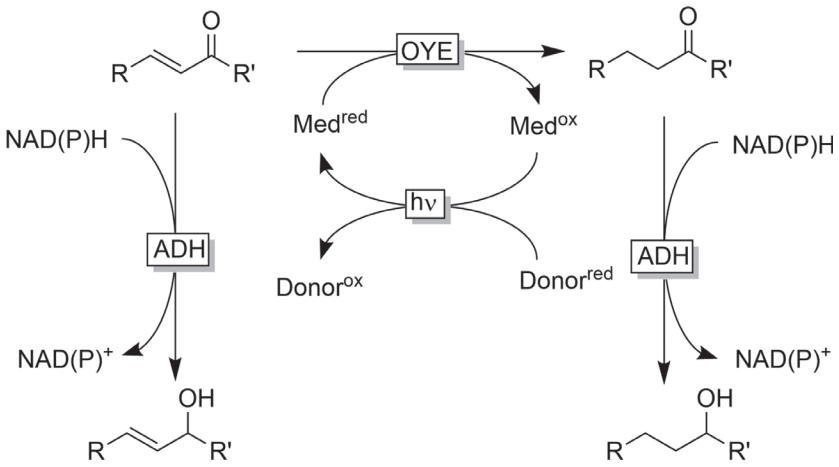
Table 9.3: Selected example of C = C-bond reductions using photochemically regenerated OYEs.



Product	Cosubstrate	OYE	Photocatalyst	Mediator	TN (OYE / Photocatalyst / Mediator)	ref
	EDTA	YqjM	FMN	FMN	10,900 / 1,000	[41, 42]
	TEOA	YqjM	CdSe	MV^{2+}	n.d.	[43]
	MOPS/ H_2O	YqjM	Au-TiO_2	FMN	650 / n.d. / 50	[44]
	Cathode	*Ts*OYE	Flavin-modified CNT-cathode	–	230 / 2	[45]
		*Ts*OYE	Rose bengal	–	250 / 40	[46]
	EDTA	*Dr*ER & *Rm*ER	FMN	–	2080 / 16	[47]
	H_2O	Flavocytochrome c (fcc_3)	TiO_2-modified FTO anode for water oxidation	–	n.d.	[48]

YqjM: OYE from *Bacillus subtilis*; *Ts*OYE: OYE from *Thermus scotoductus*; FTO: fluorine-doped tin oxide; MV: methyl viologen; FMN: flavin mononucleotide.

Hence, photochemical, direct regeneration of OYE offers not only the opportunity of saving costs by omitting the nicotinamide cofactor (and its regeneration system) but also products of higher purity due to the high chemoselectivity of the reaction (Scheme 9.6).



Scheme 9.6: Increased chemoselectivity of OYE-catalyzed reduction of conjugated C = C-double bonds *via* direct, NAD(P)H-independent regeneration of the flavin-prosthetic group. "Contaminating" alcohol dehydrogenases (ADHs) catalyzing the carbonyl reduction of both, the starting material and the products are not regenerated and therefore remain inactive.

Non-flavin-dependent reductases

In addition to the above-mentioned flavoenzymes also metal-dependent oxidoreductases can be regenerated *via* direct (NAD(P)H-independent) electron transfer. Especially, the Armstrong group contributed a range of photocatalytic systems such as the direct reductive regeneration of Ni-dependent CO-dehydrogenases to reduce CO_2 into CO, which could be used, for example, in Fischer-Tropsch-like syntheses of alkanes [49–52].

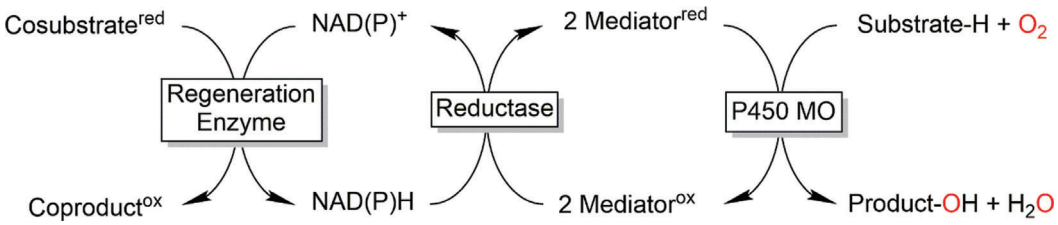
Formate dehydrogenase (FDH) is also widely studied especially by the Reisner lab for the reduction of CO_2 into formate. For example, dye-sensitized semiconductors combined with a formate dehydrogenase enable accumulation of millimolar concentrations of formate at the expense of triethanolamine (TEOA) as sacrificial electron donor [53]. More elegantly, water would serve as electron donor, which was demonstrated by the same group by combining the FDH-catalyzed reduction reaction in a divided cell to the photosystem-catalyzed oxidation of water [54].

Photobiocatalytic H_2 production utilizing hydrogenases have also been investigated intensively by these groups [55–60]. The turnover numbers observed with hydrogenases tend to be excellent ranging in the millions range. Finally, selective dehalogenations are worth mentioning here [61].

Direct reductive regeneration of monooxygenases

Monooxygenases catalyze a broad range of synthetically useful oxidation/oxyfunctionalization reactions for which classical chemical synthesis has not yet developed efficient catalysts. (Stereo)selective hydroxylation of non-activated sp^3-C-H-bonds for example is a reaction where especially the so-called P450 monooxygenases excel [62–65].

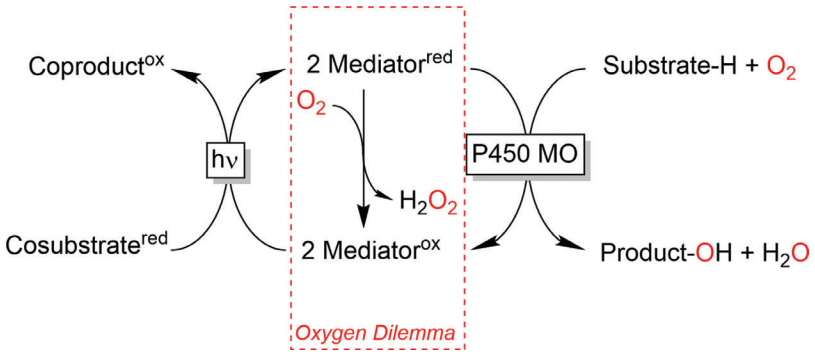
The catalytic mechanism of monooxygenases comprises reduction of the prosthetic group in the first step followed by reductive activation of molecular oxygen yielding a highly reactive oxyferryl species (in case of P450 monooxygenases) or an organic hydroperoxide (in case of flavin-dependent monooxygenases), which mediates the desired oxyfunctionalization reaction. The reducing equivalents needed in this mechanism are usually derived from reduced nicotinamide cofactors *via* more or less complex electron transport chains. Especially P450 monooxygenases, due to their O_2-activation mechanism comprising a sequence of single electron transfer, O_2-binding and -reduction followed by a second single electron transfer step and water elimination, require a relay system to transform the hydride donation step from NAD(P)H into the required single electron transfer steps. As a result, especially in case of P450 monooxygenases, the electron transfer chain tends to be rather complex (Scheme 9.7).



Scheme 9.7: Generalized molecular architecture of the electron transport chains of P450 monooxygenases.

Therefore, it is not very astonishing that especially P450 monooxygenases have also been investigated envisaging direct electron transfer from various electron donors. The aforementioned radical character of most reduced photosensitizers now appears beneficial in view of direct regeneration of P450 monooxygenases yielding simplified regeneration schemes (Scheme 9.8) [66].

Various photosensitizers/mediators have been evaluated in the past decade. Among them porphyrins [67–69] and further organic dyes such as eosin Y [70, 71] or (deaza)flavins [72, 73]; most popular, however, are photoactive Ru-complexes [74–83] Wiring P450 monooxygenases to the natural photosystem for light-driven, water-utilizing reactions have also been reported [84, 85]. Direct, photocatalytic regeneration of some flavin-dependent [86–88] and Cu-dependent monooxygenases [89] has also been studied.



Scheme 9.8: Direct, photochemical regeneration of P450 monooxygenases. The photoreduced mediators can either reduce the P450 monooxygenase (desired reaction) or they can react with dissolved O_2 (undesired uncoupling reaction, *Oxygen Dilemma*).

Despite the promise of simplified and therefore more efficient regeneration of monooxygenases, it, has so far not been kept. Although a broad range of interesting oxyfunctionalization reactions have been reported, the product titers tend to be in the lower millimolar, sometimes micromolar range. Obviously, this severely limits the preparative usefulness of direct (photochemical) regeneration approaches of P450 monooxygenases.

The major limitation of these approaches lies with their radical character. In contrast to hydride-reducing agents (such as NAD(P)H), single electron donors (radicals) readily react with (triplet) O_2. Hence, under aerobic conditions, reduced photocatalysts can either deliver their reducing equivalents to the monooxygenases (desired electron transfer pathway) or can directly react with dissolved O_2 (thereby uncoupling the electron supply from the monooxygenase reaction) (Scheme 9.8) [39].

So far, no satisfactory solution to the *Oxygen Dilemma* has been proposed, leaving this an open question in photochemically driven monooxygenase reactions.

9.2.2 Oxidative regeneration

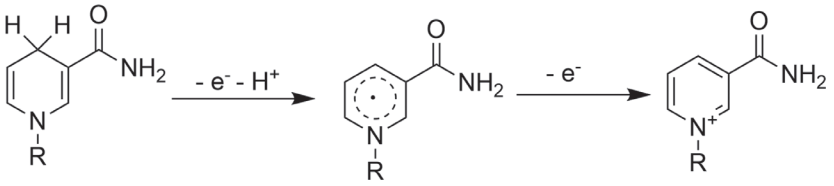
In oxidative regeneration of redox enzymes, again, principally NAD(P)-dependent and NAD(P)-independent approaches can be distinguished.

9.2.2.1 Photochemical NAD(P)$^+$ regeneration to drive ADH-catalyzed oxidation reactions

Compared to reductive use of ADHs, their application in the oxidative direction is far less common. One reason is that the oxidation of secondary alcohols usually destroys chiral information, whereas the reverse reaction, that is, the reduction of

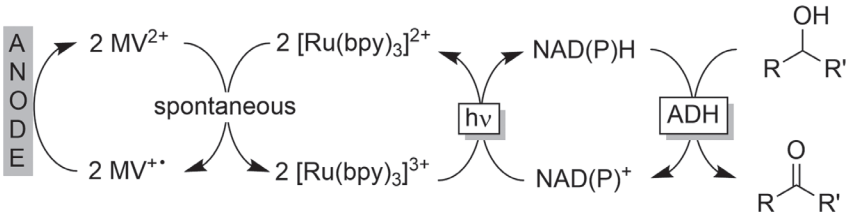
ketones, leads to the formation of chiral (ideally enantiomerically pure) secondary alcohols. This also explains why the number of reported enzymatic $NAD(P)^+$ regeneration systems falls back significantly behind the NAD(P)H regeneration systems. In essence, NADH-oxidases[[90–93]] and ADH-catalyzed NAD(P)H oxidation[[94–97]] prevail.

Nevertheless, a range of photochemical $NAD(P)^+$ regeneration systems have been reported. In contrast to the reverse reaction (photochemical reduction of $NAD(P)^+$), selectivity issues play no role in the reaction mixtures are the desired product ($NAD(P)^+$) is aromatic and thereby thermodynamically stable without product isomers (Scheme 9.9).



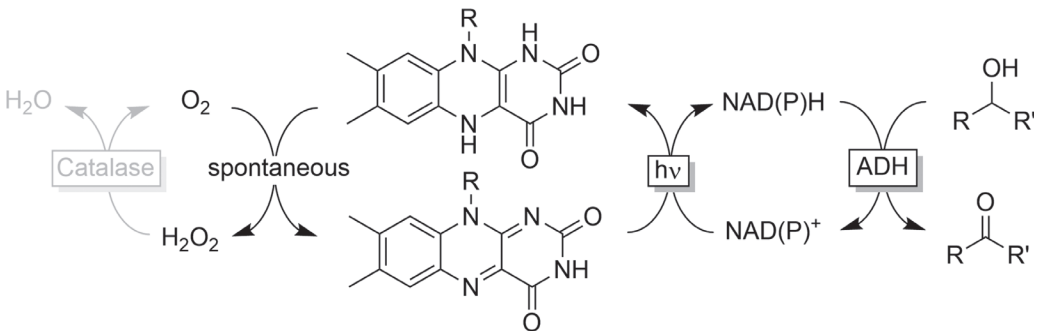
Scheme 9.9: ECE mechanism of NAD(P)H oxidation.

In an early contribution, Steckhan and coworkers reported a photoelectrochemical NAD(P)H oxidation system based on photoexcited $[Ru(bpy)_3]^{3+}$ complexes (Scheme 9.10) [98]. The reducing equivalents transferred to the photoexcited Ru complexes were then, in a spontaneous cascade, transferred to an anode. Unfortunately, this system proved to be rather complex and not efficient enough to be of preparative use.



Scheme 9.10: The photoelectrochemical $NAD(P)^+$ regeneration system proposed by Steckhan and coworkers to promote ADH-catalyzed oxidation reactions.

Later, we reported that photoexcited flavins are very efficient catalysts to oxidize NAD(P)H to $NAD(P)^+$ [99, 100]. The spontaneous hydride transfer from NAD(P)H to oxidized flavins is actually known since decades.[[101–104]] The sluggish reaction rate, however, demanded large molar surpluses of the flavin "catalyst" to achieve acceptable overall reaction rates. Simple illumination of the reaction system with blue light ($\lambda = 450$ nm, that is, the absorption maximum of oxidized flavins) increased the reaction rate by orders of magnitude, thereby enabling truly catalytic use of the flavin photocatalyst (Scheme 9.11) [105].



Scheme 9.11: Photochemical NAD(P)$^+$ regeneration system using photoexcited flavins. Please note, the mechanism shown here is highly simplified. Most likely, flavin-semiquinone radicals formed by SET from NAD(P)H to the photoexcited flavin are formed reacting with O_2 in a sequence of SETs.

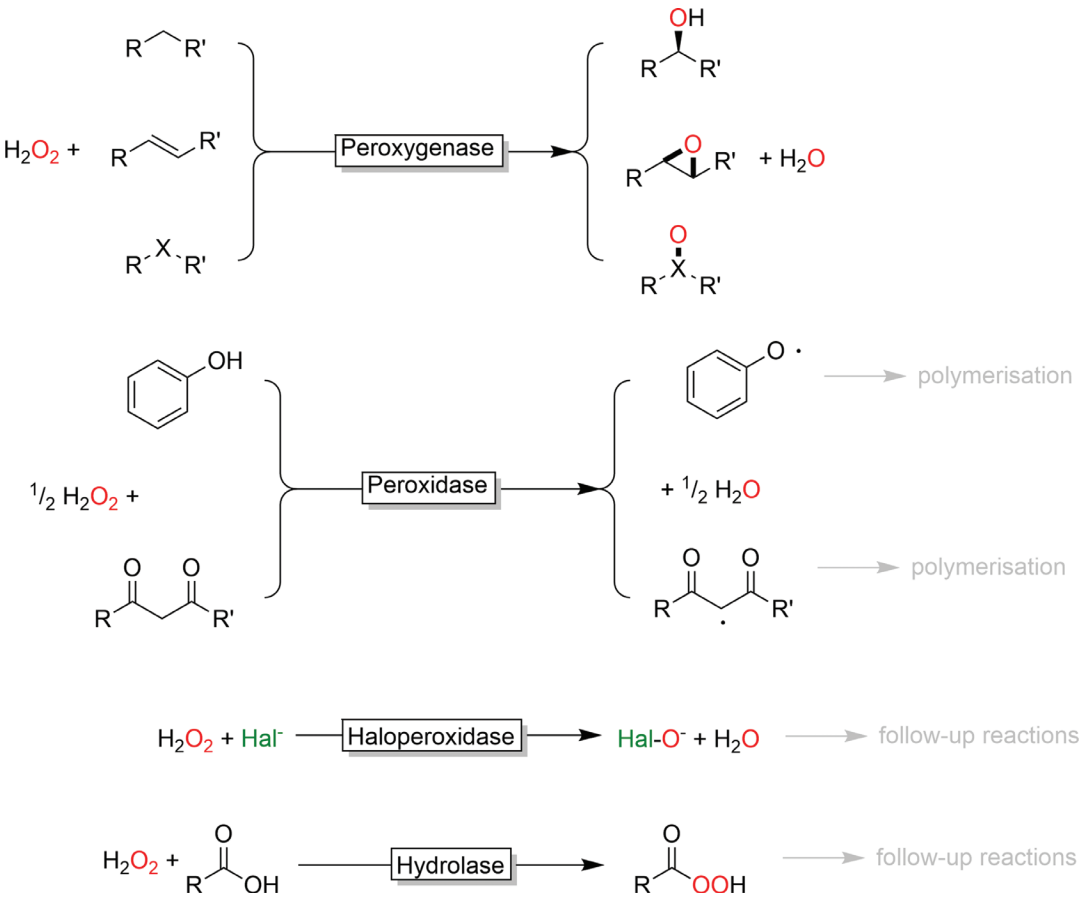
This approach is also applicable to various other photoactive redox dyes such as methylene blue, rose Bengal or Meldola's blue [106].

9.2.2.2 Photochemical regeneration of H_2O_2-dependent enzymes

As mentioned above, photochemical systems in the presence of molecular oxygen tend to uncouple. In other words, the reduced photocatalysts/mediators (mostly being radicals) react swiftly with molecular oxygen directly. In the case of photochemical NAD(P)$^+$ regeneration systems this is the desired reaction. In cases where the reducing equivalents should be delivered to a biocatalyst (i.e,. to a monooxygenase), this represents an undesired side reaction. In some cases, this side reaction dominates over the desired electron flow, leading to a waste of up to 95% of the reducing equivalents (*Oxygen Dilemma*). The final product of this uncoupling reaction is H_2O_2.

A range of enzymes (so-called peroxizymes), however, can use H_2O_2 productively in their catalytic mechanisms [107]. Hence, the *Oxygen Dilemma* can be used productively to promote peroxizyme-catalyzed oxidation reactions!

Peroxygenases (UPO for unspecific peroxygenases) are the most prominent peroxizymes [108, 109]. UPOs catalyze a very broad range of synthetically useful oxyfunctionalization reactions such as regio- and stereospecific hydroxylations and epoxidations as well as stereospecific heteroatom oxygenations (Scheme 9.12). As heme-dependent enzymes, however, they are also prone to rapid oxidative inactivation in the presence of H_2O_2 [106]. Therefore, a range of *in situ* H_2O_2 generation approaches have been developed in the past to balance the H_2O_2 concentration to the UPO activity and thereby minimize oxidative inactivation [107]. Most prominent at present are enzymatic systems based on oxidases (i.e., enzymes that couple the oxidation of their substrate to the reduction of O_2 to H_2O_2). In the past decade, we and others have developed a range of photocatalytic systems to drive peroxygenase- and peroxidase-reactions. A summary is given in Table 9.4. Obviously, using water as cosubstrate would be the most attractive



Scheme 9.12: Peroxizymes utilize H_2O_2 to catalyze or initiate catalytic oxidation reactions.

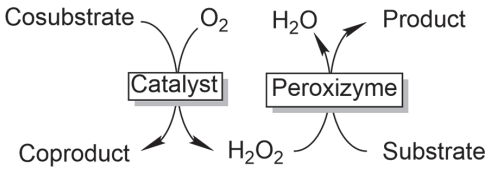
application of photocatalysis with peroxizymes. However, the current state-of-the-art is hampered by the rather sluggish water oxidation rates, making the resulting reaction systems rather slow. Next-generation water oxidation catalysts are highly desired!

9.3 Photobiocatalytic cascades combining chemical and biocatalytic transformations

Next to the various examples using photocatalysis to provide redox enzymes with redox equivalents for catalysis, there is also a growing interest in combining photochemical with biocatalytic transformations.

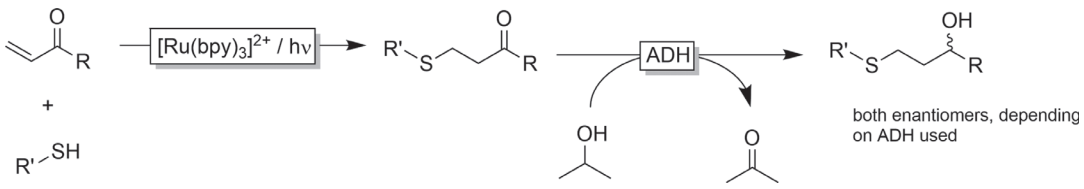
Castagnolo and coworkers, for example, reported that a photo-catalyzed thio-Michael addition yielding saturated ketones can be completed by an ADH-catalyzed, stereoselective reduction of the carbonyl group yielding enantiomerically pure 1,3-mercaptoalkanols in a one-pot setup (Scheme 9.13) [121].

Table 9.4: Selection of peroxizyme reactions driven by photocatalytic H_2O_2 generation.



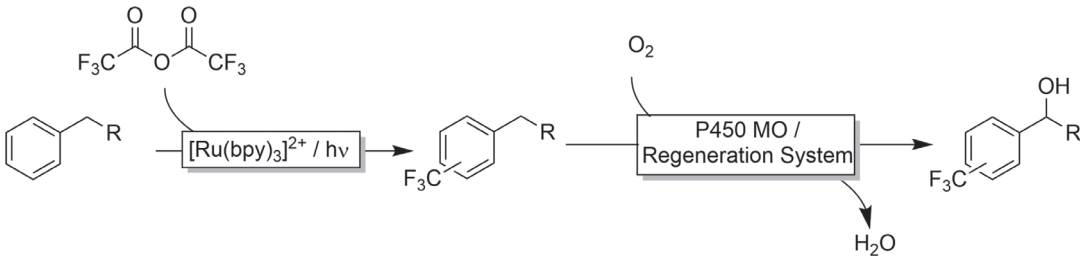
Catalyst	Cosubstrate	Coproduct	Peroxizyme	Product	TTN (Enzyme)	Remarks	ref
Flavin	EDTA	EDTriA / H_2CO / CO_2	*Cf*CPO	thioanisole sulfoxide	22,000	Using a 2LPS approach significantly improved product formation	[110, 111]
Flavin	EDTA	EDTriA / H_2CO / CO_2	*Aae*UPO	various	<40,000		[112]
Flavin	EDTA	EDTriA / H_2CO / CO_2	OleT	1-alkenes			[113, 114]
Flavin-modified cathode	H_2O	O_2	*Aae*UPO	1-phenyl ethanol	123,000	Photoelectrochemical approach	[115]
Au-TiO_2	MeOH	CO_2	*Aae*UPO	various	>60,000		[116]
Au-TiO_2	H_2O	O_2	*Aae*UPO	various	>30,000		[117]
Various dyes / FDH	HCO_2H	CO_2	*Aae*UPO	1-phenyl ethanol	>40,000	Combining color-complementary redox dyes allows for better usage of the visible light range	[118]
Flavin	EDTA	EDTriA / H_2CO / CO_2	cytochrome P450 peroxygenases	Hydroxy myristic acid	200	Low, due to poor solubility of the reagents	[119]
Flavin	MES	n.d.	*Am*VHCPO	Various halogenated phenols and anilines	2,000		[120]

EDTA: ethylenediamine tetraacetate; EDTriA: ethylenediamine triacetate; MES: 2-(*N*-morpholino)ethanesulfonic acid;
*Am*VHCPO: V-dependent haloperoxidase from *Acaryochloris marina*;



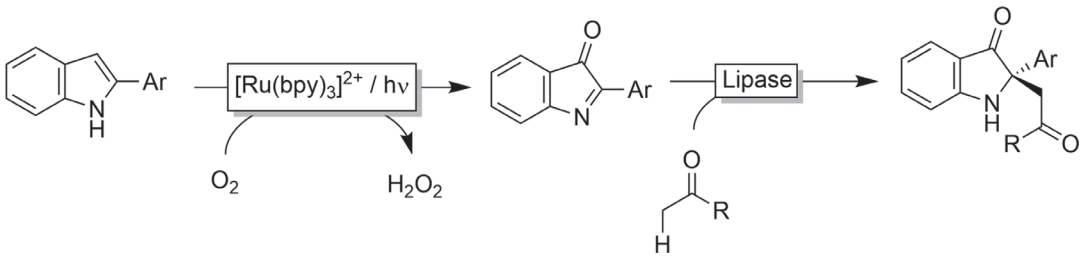
Scheme 9.13: Photoenzymatic cascade combining photoaccelerated thio-Michael addition and stereoselective, ADH-catalyzed ketoreduction.

The group around Cheruzel investigated the photocatalytic trifluoromethylation of alkylarenes followed by P450 monooxygenase-catalyzed hydroxylation of the intermediate product (Scheme 9.14) [75].



Scheme 9.14: Cascade of photocatalytic trifluoromethylation of arenes followed by P450 monooxygenase-catalyzed hydroxylation.

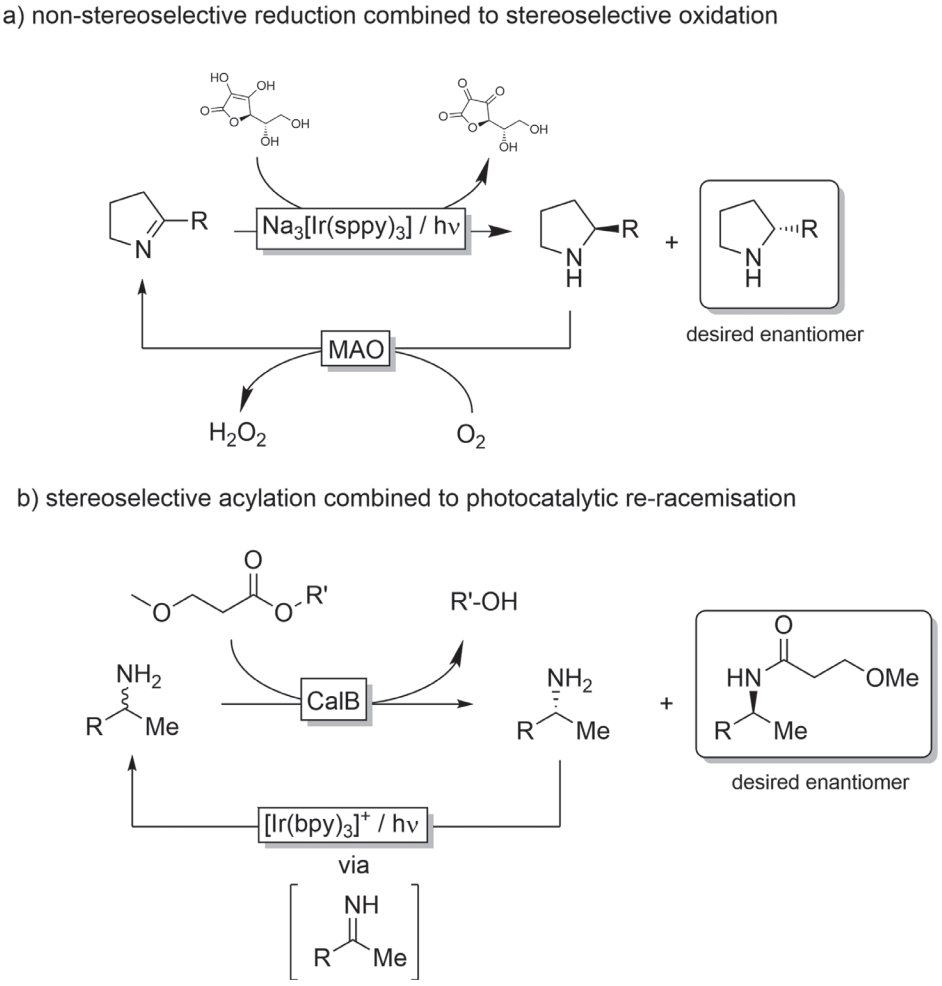
Another example of photocatalytic C-C-bond formation coupled to a selective biocatalytic reaction step was reported recently by He and coworkers (Scheme 9.15) [122]. Here the authors combined the photocatalytic oxidation of 2-arylindoles to 2-arylindol-3-ones combined with an enantioselective, lipase-catalyzed addition of enolisable ketones yielding enantioenriched 2,2-disubstituted indol-3-ones.



Scheme 9.15: Photocatalytic oxidation of 2-arylindoles to 2-arylindol-3-ones coupled to a lipase-catalyzed C-C-bond formation.

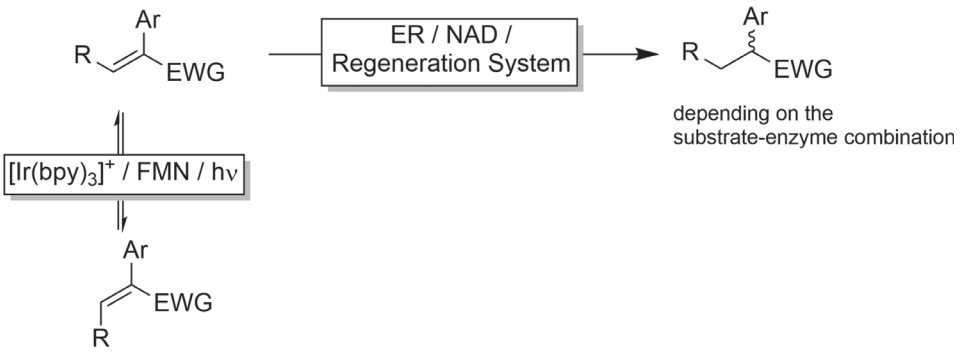
Photochemical reactions are generally not stereoselective, a fact that can be exploited in the deracemization of chiral alcohols and amines if combined with a stereoselective enzymatic step. Interestingly, photoactivatable Ir-complexes have been

reported for this purpose. In the first example, a photoexcited Ir-complex mediated the (ascorbate-driven), non-stereoselective reduction of (cyclic) imines. In combination with the well-known monoamine oxidase (MAO) catalyzing the stereoselective oxidation of the resulting amine, a deracemization was achieved (Scheme 9.16a) [123, 124]. In another example, an Ir-complex was used to racemize amines *via* a photoaccelerated H-borrowing reaction (i.e. catalyzing the H-atom abstraction and non-selective re-donation from an amine). Through combination with an enantioselective, lipase-catalyzed acylation step, complete transformation of racemic amines into enantiomerically pure amides was achieved (Scheme 9.16b) [125].



Scheme 9.16: Examples for photobiocatalytic deracemization reactions combining non-selective photocatalytic redox reactions with enantioselective, biocatalytic kinetic resolutions.

Another elegant combination of photocatalysts with biocatalysis was reported by Hartwig and coworkers combining photocatalytic *E/Z*-isomerization of conjugated carbonyl groups with stereoselective reduction of the *E*-configured C = C-double bond by ene reductases (Scheme 9.17) [126].



Scheme 9.17: Photocatalytic E/Z isomerization for the complete conversion of trisubstituted alkenes by ene-reductases.

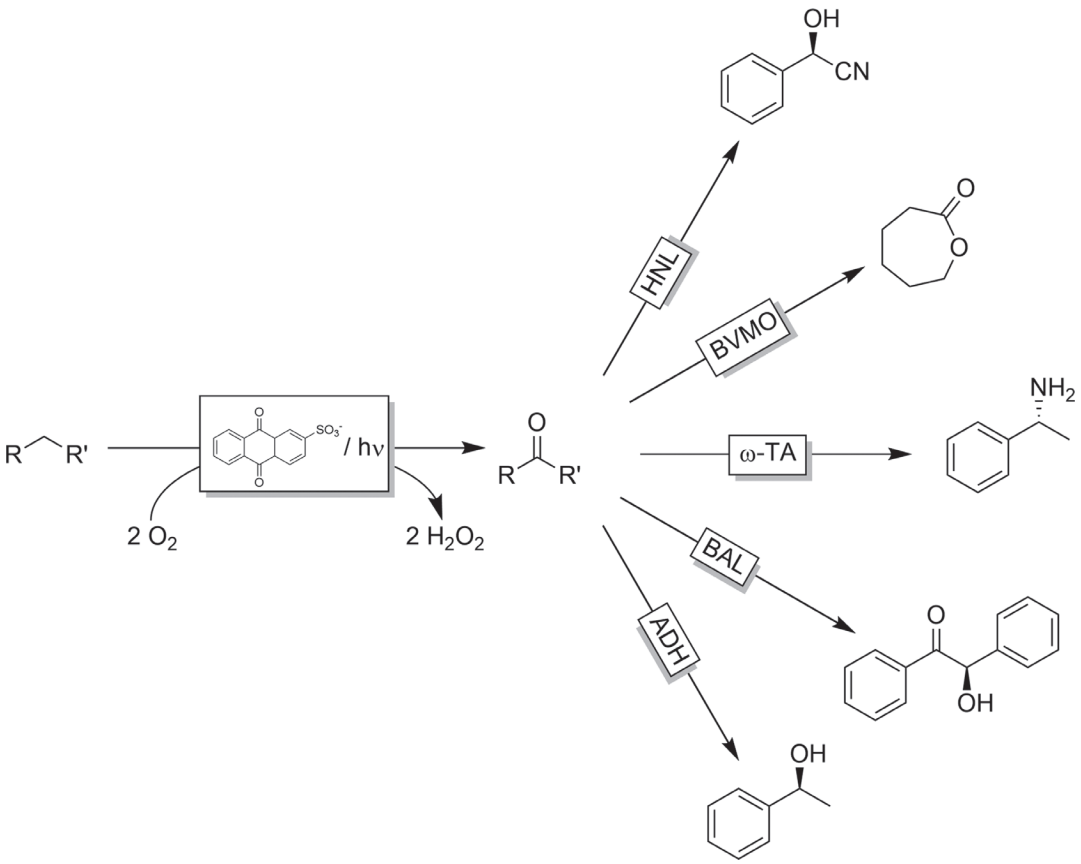
Finally, we have developed a range of photocatalytic oxidation reactions yielding prochiral ketones and aldehydes, which then in a subsequent biocatalytic step were converted into optically pure cyanohydrins, alcohols, amines, lactones, benzoins and others. (Scheme 9.18).[[127–129]] The reactions generally gave better results (conversion) if the cascades were performed sequentially, that is, performing the photocatalytic oxidation reaction followed by the biocatalytic reaction step. Reasons for this were manifold ranging from compatibility issued of the photo- and biocatalysts to cross-reactivities.

Similar compatibility issues were also observed by Kourist and coworkers combining OleT-catalyzed decarboxylation of ω-fatty acids with Ru-catalyzed metathesis of the resulting terminal alkene [113].

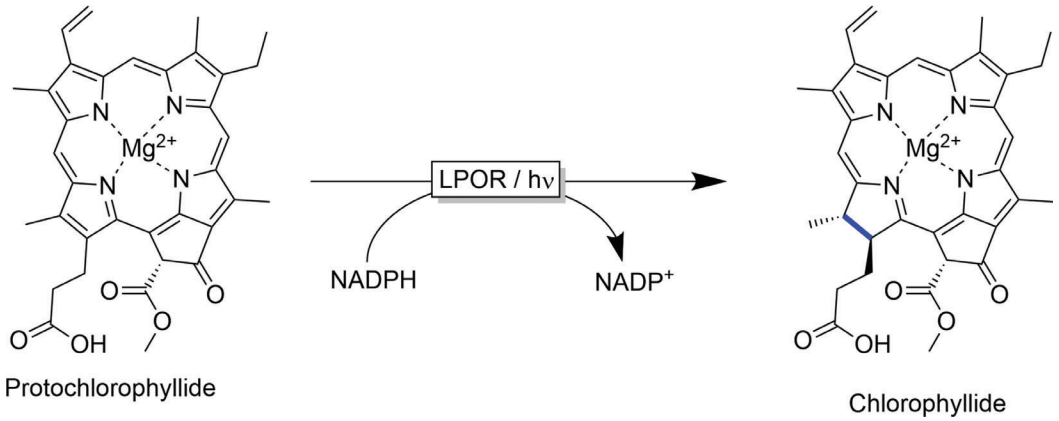
9.4 Photoactivated enzymes

Today, only a handful of enzymes necessitating light activation are known. The most important enzymes obviously are photosystem I and photosystem II, playing a fundamental role in life as we know it [130, 131]. In addition, photolyases involved in DNA repair[132, 133] and protochlorophyllide-reductases (light-dependent protochlorophyllide oxidoreductase (LPOR), Scheme 9.19)[134] involved in chlorophyll synthesis are known. In case of LPORs, the substrate itself functions as photosensitizer [10, 135, 136].

In the past years, photodecarboxylases are enjoying increasing interest. The first example of these photoactivated fatty acid decarboxylases (*Cv*FAP) was discovered from *Chlorella variabilis* NC64A by Beisson and coworkers [137, 138]. *Cv*FAP contains a flavin cofactor, which upon photoexcitation mediates the first single electron transfer from the enzyme-bound carboxylic acid initiating a sequence of CO_2 extrusion and back-transfer of the initially abstracted electron to the newly formed C-centered radical [137, 139]. Soon, *Cv*FAP and its mutants found broad interest as catalyst for the synthesis of (fuel) alkanes from carboxylic acids[[140–146]] or for the synthesis of

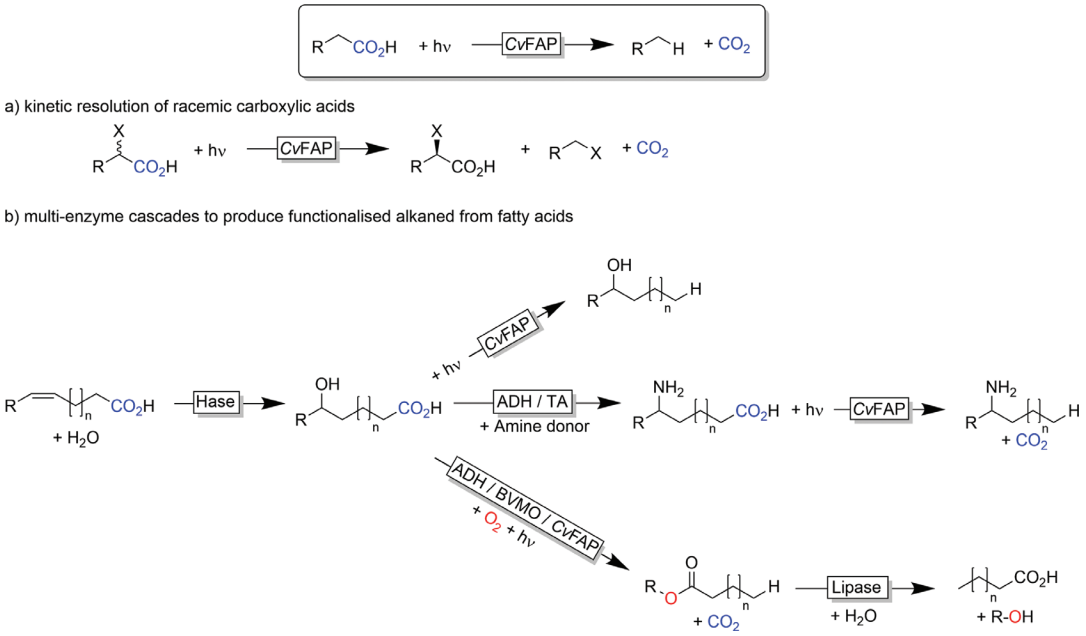


Scheme 9.18: Sequential photobiocatalytic cascades to transform non-functionalized alkanes into various (optically pure), functionalized products.



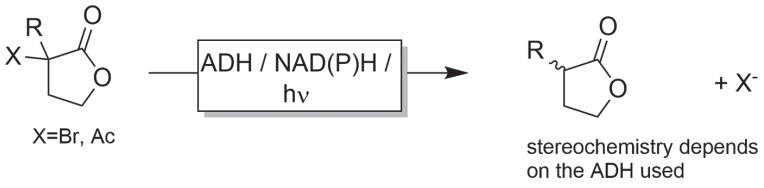
Scheme 9.19: LPOR (light-dependent protochlorophyllide oxidoreductase)-catalyzed reduction of protochlorophyllide to chlorophyllide.

chiral compounds via kinetic resolution of racemic hydroxyl carboxylic acids [147]. In combination with other enzymes, *Cv*FAP can also be applied to synthesize fine chemicals such as secondary alcohols[148] or polymer building blocks[149] from unsaturated fatty acids (Scheme 9.20).



Scheme 9.20: Selection of non-fuel applications of *Cv*FAP.

Very recently Hyster and coworkers realized the potential of photoexcited cofactors to introduce "non-natural" reactivities to cofactor dependent enzymes. In a first study, they utilized photoexcited reduced nicotinamide cofactors to catalyze the enantioselective dehalogenation or deacetylation of α-substituted lactones (Scheme 9.21) [150, 151]. Later, the same group expanded this concept to flavin-dependent enzymes[150, 152], also demonstrating that the application of photocatalysis can turn an ene-reductase into a ketoreductase [153].



Scheme 9.21: Turning an ADH into a dehalogenase using photochemistry.

9.5 Conclusions

Photobiocatalysis is a dynamically evolving field of research opening up new synthetic possibilities for the organic chemist.

To fully unfold this potential, compatibility will be the most pressing issue to be addressed. The high reactivity of photoexcited species frequently leads to inactivation of the biocatalyst and photocatalysts themselves. As a consequence, turnover numbers of the catalysts still tend to be rather low thereby limiting the preparative value of the systems.

Envisioning sustainable production system it will be of utmost importance to focus on the source of electrons used for photobiocatalytic reactions. Today, mainly energy-rich sacrificial electron donors are still very common. In the long term water as sacrificial electron donor (enabled by photo energy) will be inevitable to attain sustainable reaction schemes.

Next to protein engineering approaches also reaction engineering approaches, that is, through physical separation of photo- and biocatalysts, right now appear to be the most promising solution to the compatibility issue.

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