Photo-Biocatalysis: Biotransformations in the Presence of Light

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ABSTRACT: Light has received increased attention for various chemical reactions but also in combination with biocatalytic reactions. Because currently only a few enzymatic reactions are known, which per se require light, most transformations involving light and a biocatalyst exploit light either for providing the cosubstrate or cofactor in an appropriate redox state for the biotransformation. In selected cases, a promiscuous activity of known enzymes in the presence of light could be induced. In other approaches, light-induced chemical reactions have been combined with a biocatalytic step, or light-induced biocatalytic reactions were combined with chemical reactions in a linear cascade. Finally, enzymes with a light switchable moiety have been investigated to turn off/on or tune the actual reaction. This Review gives an overview of the various approaches for using light in biocatalysis.

KEYWORDS: photocatalysis, biocatalysis, biotransformation, cascades, cofactor recycling

1. INTRODUCTION

The Review discusses the progress in photo-biocatalysis in recent years, emerging concepts, and the possibilities for using biocatalysis in combination with light for sustainable synthesis of organic compounds. In order to understand the rise of the young research field of photo-biocatalysis, one has to consider first the developments in the individual fields, biocatalysis and photocatalysis.

In the last 20 years, biocatalysis has become a recognized and green tool in organic synthetic chemistry because of the generally mild and environmentally friendly conditions required for the reactions.1–6

Within the third wave of biocatalysis, important developments have turned biocatalysis into an alternative to metal or organocatalysis.7 Especially, the progress in the field of directed evolution8 enabled the straightforward modification of enzymes and their adaption to different reaction conditions. Enzymes can be engineered to accept non-natural substrates, to produce new products, and to withstand extreme temperatures or pH values.

The possibility of carrying out specific, stereoselective, and complex reactions with enzymes at ambient temperature and pH values as well as in the presence of water or organic solvents led to an increasing influence of biocatalysis in the pharmaceutical and chemical industry.9–13

In the same period of time, photocatalysis has also developed into a widely respected field of research. Photocatalysis, using (transition) metal or organic catalysts, has become a highly investigated topic.14–21 For many reactions such as cycloadditions,22–26 C–H activation,27,28 C–C bond formations via cross-coupling,27,29–31 and halogenations,32,33 photocatalytic reactions were found that show an extended substrate scope and proceed under milder conditions compared to the light-independent alternatives. Another important advancement in photocatalysis was the discovery of numerous photocatalysts that use visible light as an energy source.15,34 In addition to various photocatalytic materials, TiO2-based materials in particular occupy an outstanding position in photocatalysis.35–37 From the ever-expanding range of photocatalysts and the scope of application in photochemical synthesis, one can speak currently about the heyday of photocatalysis. Furthermore, today, light can be obtained from electricity, generated from renewable energy sources, which makes it an optimal reagent for environmentally friendly synthesis processes.38

It is therefore not surprising that attempts are being made to combine the advantages of photocatalysis with biocatalysis. This brings together two of the most research-intensive fields in catalysis of the past decade. Accordingly, there was much progress in combining photochemical principles and catalysis with biocatalysis. But is photo-biocatalysis currently an efficient extension or alternative to conventional biocatalysis and can it broaden the field of photocatalysis in a meaningful way?
This requires a closer look at the individual areas that have developed in photo-biocatalysis in recent years, ranging from the field of light-driven enzymes to light-activated cofactor recycling and the use of light-dependent organisms.

In recent years, numerous enzymes have been found that show a completely different or extended reaction spectrum under irradiation with light. Furthermore, methods have been published that focus on the light-driven activation of redox-enzymes. Especially, the required electron donors, photosensitizers, and mediators, which are suitable for a direct or indirect transfer of photoinduced electrons to an enzyme, were investigated. In this context, the photochemical regeneration of cofactors also plays a crucial role, as it is a very effective and simple method to combine photocatalytic with biocatalytic transformations. Consequently, numerous biocatalytic redox reactions were coupled with a photocatalytic step, which is catalyzed by a photosensitizer. For example, porphyrins, iridium and ruthenium transition-metal complexes, eosin Y, or xanthenes are used as photosensitizers, which enable a direct transfer of electrons to a redox prosthetic group of an enzyme, or indirectly pass the electrons to the enzyme through mediators, or enable the indirect photochemical regeneration of the natural cofactor. Compared to the broad applications in this field of photo-biocatalysis, the applications of naturally light-driven enzymes in photo-biocatalysis are currently of minor importance.

Scheme 1. Light-Driven Biocatalytic Transformations of the Four Known Photoenzymes

(A) Schematic representation of the photosystem with photosystem I (PSI) and II (PSII). Under illumination, water is oxidized by PSII, and the resulting electrons are transferred to PSI. There, a light-dependent transmembrane electron transfer takes place, at the end of which NADPH is synthesized. The resulting proton drives the production of ATP by the ATP-synthase; Cyt b6f = cytochrome b6f complex, Fdx = ferrodoxin. (B) Blue-light-catalyzed repair of the cyclobutane pyrimidine dimer (CPD) via retro-cycloaddition by a photolyase. (C) Light-driven stereoselective reduction of the C=C bond of protochlorophyllide (pchlide) by a protochlorophyllide-reductase from Dinoroseobacter shibae under formation of chlorophyllide (chlide), a precursor of chlorophyll. Only the C17=C18 double bond of pchlide, indicated with corresponding numbers, is reduced by the enzyme. (D) Blue-light-catalyzed decarboxylation of saturated and unsaturated fatty acids by a fatty acid photodecarboxylase from Chlorella variabilis NC64A.
However, to date, neither the photochemical activation of enzymes nor the photochemical regeneration of cofactors has become a standard approach, yet. One of the limiting factors in this area has been the relatively poor transfer kinetics of photoexcited electrons to the enzyme, in connection with low TTN (total turnover number) and TOF (turnover frequency) of the photocatalyst or enzyme. Another problem arises from the generation of strong oxidants and reactive free radicals. However, these problems are addressed (i) by an increasing number of modified structures and properties of the above-mentioned photosensitizers, which may allow a more efficient transport of electrons, or (ii) by enzyme engineering to simplify the electron acceptance and to improve the recognition and turnover of the substrates.

As an alternative to these artificial systems to utilize light for reaction energy, a new branch of photo-biocatalysis has emerged that uses the photosystem in photo-autotrophic organisms to supply enzymes with energy, whereby in vivo photo-biocatalysis is enabled. The photosystem converts light energy into redox equivalents, whereby the cell provides highly specialized electron transport chains, mechanisms for controlling reactive species, and regenerates in vivo parts of the system in the case of irreversible damage.

A challenge for applying photo-biocatalytic reactions is the upscaling to larger volumes and higher concentrations: parameters such as the intensity of light and those connected to that the light’s penetration depth cannot easily be scaled. In addition, the lack of standardized photoreactors and light sources complicates comparison of experiments between laboratories.

Previous reviews in the field of photo-biocatalysis are specific for the electron transfer, activation of redox-enzymes, photoregeneration of cofactors, or structural and mechanistic aspects. In this Review, a broad overview of the use of photo-biocatalysis in synthetic chemistry and the current possibilities and potential is given, for which synthetic options exist so far, and substrates and functional groups can be addressed by means of photo-biocatalysis. For a broad overview of the field of photo-biocatalysis, the first part of the Review will focus on photoenzymes requiring light for their natural catalytic activity. This very small group of enzymes catalyzes versatile reactions and was recently enlarged with the discovery of a new photoenzyme in 2016. Then, enzymes displaying new reaction mechanisms and promiscuous activity under visible-light irradiation will be discussed. Especially, nicotinamide-dependent and FMN/FAD-dependent enzymes were reported to increase their biocatalytic reaction repertoire and to improve the chemical reaction). The next section focuses on the coupling of photocatalysts with biocatalytic transformations. This section is separated into two parts. The first part deals with systems, in which redox equivalents are provided via photocatalysis for the subsequent biocatalytic reaction (i.e., energy is provided with light). The second part of the section deals with cascades, in which a photochemical reaction is coupled with a biocatalytic transformation (i.e., light enables the chemical reaction). The next section deals with the exploitation of the photosystem coupled with biocatalytic transformations. The section first focuses on phototrophic organisms that provide redox equivalents for biocatalytic reactions via photosynthesis. Subsequently, biocatalytic cascades involving a photo-biocatalytic transformation will be discussed. Finally, the last section will introduce photo-switchable enzymes that exhibit catalytic activity.

The respective examples and methods in the individual sections will comprehensively reflect the respective research area; however, a special focus was placed on the most recent reaction examples.

2. NATURAL LIGHT-DRIVEN CATALYTIC ENZYMES

Photoenzymes are enzymes that require a steady flux of light to catalyze a chemical reaction; thus, light is required directly in the reaction catalyzed by the enzyme. In the absence of light, photoenzymes remain completely catalytically inactive. To this day, four types of photoenzymes were discovered, which are the photosystem, photolyases, photoredox enzymes, and photodecarboxylases (Scheme 1). It is presumed that most of possible previously existing photoenzymes were sorted out by evolution and that today’s photoenzymes are only the last survivors of this process. Nevertheless, there are many light-controlled regulatory processes in nature such as light-induced promoters; thus, the utilization of light-induced protein rearrangements can be widely found in nature. Consequently, it is even more striking that so few light-driven enzymes have been identified. Although the protein bacteriorhodopsin is important for archaea, most notably by halobacteria, to generate energy from light, it actually acts as a proton pump; thus, it captures light energy to move protons across the membrane out of the cell via E/Z-isomerization within the protein. The resulting proton gradient is subsequently converted into chemical energy. Consequently, bacteriorhodopsin does not catalyze a transformation of a substrate to a product and is consequently not detailed here further.

The photosystem represents the first group of photoenzymes and consists of photosystem I (PSI) and photosystem II (PSII), which are light-driven enzymes (Scheme 1A). Together, PSI and PSII enable the process of photosynthesis, one of the most important biological processes on earth. PSI and PSII are located in the thylakoid membranes of plants, cyanobacteria, or algae and allow the conversion of light energy into chemical energy (NADPH and ATP) and the production of molecular oxygen. The first step involves the photo-oxidation of water to molecular oxygen (1/2 O2 per water molecule), the generation of two electrons, and the release of two protons by the PSII. PSI then channels the released electrons to a plastoquinone (PQ) while also binding two further protons from the stroma. This process provides the proton gradient across the thylakoid membrane that is required for ATP synthesis. The electrons (and protons) are then channeled via an electron-transfer chain of different redox mediators to a cytochrome b6f complex that releases the bound protons to the lumen (aiding in the generation of the proton gradient) and transfers the electrons via an electron-transfer chain to PSI. In PSI, a light-driven transmembrane electron transfer takes place, whereby the electrons are transferred via a ferredoxin to a different metabolic electron sink, including production of the reduced nicotinamide cofactor NADPH by ferredoxin-NAD” oxidoreductase.

Because of the very complex structure of the photosystem and its highly specific reaction, the use of the photosystem as an in vitro biocatalyst seems to be unlikely. PSI on its own consists of up to 36 proteins, to which up to 381 cofactors are noncovalently bound. The most widespread use of the photosystems is currently the application of natural photosystem-functionalized photoelectrodes as semiartificial photoelectrochemical devices. The most common application of
PSII, which has been immobilized on an electrode (photoanode), is the photoelectrochemical water oxidation. In this case, it is also possible to couple the water oxidation of the PSII with the hydrogen production of a hydrogenase in a photo-bio-electrochemical cell, which may be used in the future in synthetic applications. The simultaneous use of the PSII as the photoanode and the PSI as the photocathode also enables a number of bio-photovoltaic applications.

In addition, alcohol-dehydrogenases or ene-reductases are heterologously expressed in engineered cyanobacteria and coupled with the natural photosystem of these organisms. The photosystem is thus indirectly involved in biocatalysis by acting coupled with the natural photosystem of these organisms. The late (MTHF) or 8-hydroxy-7,8-didemethyl-5-deazariboflavin (GMC) oxidoreductase family, were found in the algae species. The photosystem enables a number of bio-photovoltaic applications.

The third group of photoenzymes consists of protochlorophyllide-reductases (PORs), which play a key role in the biosynthesis of chlorophyll. PORs catalyze the reduction of protochlorophyllide (pchlide) to chlorophyllide (chlide, Scheme 1C), whereby the reaction can be catalyzed by two different types of pchlide-reductases, which differ structurally and evolutionarily from each other. On the one hand there are dark-operative, oxygen-sensitive PORs (DPORs), which reduce pchlides independently of light in an ATP-dependent process, and on the other hand, there are the light-dependent, oxygen-insensitive PORs (LPORs) that reduce pchlide in a strictly light-dependent process by using NADPH as a cofactor. LPORs are found in both higher plants, cyanobacteria, algae, and also in one anoxic phototrophic bacterium.

LPORs catalyze the sequential reduction of the C17–C18 double bond of the D-ring of pchlide by trans-addition of a hydride and a proton along the double bond. Already in the dark, a ternary complex of substrate, cofactor, and enzyme, which acts as a photoreceptor during the reaction. Under illumination, an endergonic light-driven hydride transfer takes place from the pro-S face of the NADPH to the C17 position of the pchlide, whereby a charge-transfer complex is formed. This is followed by a light-independent proton transfer to C18, which is presumably provided by a tyrosine residue in the active site.

In recent years, LPORs have been studied for their substrate specificity, but so far, all remained limited to chlorophyll and protochlorophyllide derivatives. The previous studies suggest that the chelated metal is the pro-S face of the NADPH, and the stereochemistry of the side groups on the isocyclic ring are important for substrate binding and positioning. No studies on substrates apart from porphyrin derivatives were reported yet.

The above-mentioned photoenzymes showcase that enzymes can catalyze sophisticated light-dependent reactions; however, none of these catalysts have been applied in biocatalysis yet, maybe because of their limited or unexplored substrate spectrum. This is in contrast to the fourth group of light-dependent enzymes that was recently discovered in microalgae. These photoenzymes, belonging to an algae-specific clade of the glucose-methanol-choline (GMC) oxidoreductase family, were found in the microalgae Chlorella variabilis NC64A and Clamydomonas reinhardtii and play a role in the lipid metabolism. The enzymes are identified as light-driven fatty acid photo-decarboxylases (FAPs) catalyzing the decarboxylation of free fatty acids to n-alkanes or alkenes in the presence of blue light (Scheme 1D). The FAPs mainly produce 7-heptadecenoic acid in the lipid metabolism of the microalgae starting from cis-vaccenic acid. It has recently been shown that a preparative scale synthesis of pentadecane (61% yield, TON of CrFAP 7916) is possible starting with dodecanoic acid employing the photodecarboxylase of Cllorella variabilis NC64A and that the enzyme has a solvent tolerance of up to 50 vol % DMSO. Thus, the FAPs may provide an alternative pathway for the production of jet fuels. The enzymes are also able to synthesize C11 to C19 alkanes or alkenes from the corresponding fatty acids, whereby a higher efficiency was found for C16–C17 chains. In the reaction, saturated fatty acids are converted to the corresponding alkanes; thus, no terminal double bond is introduced by the decarboxylation as in an elimination reaction. In the dark as well as with red light, no decarboxylation is observed; thus, a light-control of the
A biocatalytic process is feasible. The duration of the reaction can be precisely controlled. The chromophore of the FAPs is a FAD, whereas the absorption maximum of the flavin (467 nm) is slightly higher than in most flavoproteins or free flavins (445–450 nm).\textsuperscript{107} The photoactivation of the FAPs by blue light suggests that exclusively the light-excited FAD in the active site of the enzyme is responsible for the catalytic decarboxylation.\textsuperscript{52,66,51}

The photoenzymes discussed here catalyze very different reactions and follow different mechanisms, which demonstrates the diversity of the photoenzymes. Thus, there may be unexplored potential in photoenzymes to further optimize both chemical and biocatalytic processes by making them even more ecological by using light as an energy source or by finding completely new biosynthetic approaches. For this purpose, it is necessary to advance the search for other light-driven enzymes and to further investigate the already existing photoenzymes, their functionality, and their potential for biocatalysis.

3. ENZYMES WITH PROMISCUOUS ACTIVITY IN THE PRESENCE OF LIGHT

Often, protein engineering is needed to produce synthetically useful biocatalysts for non-natural reactions.\textsuperscript{108} However, it was shown that photo-chemocatalytic redox reactions can be integrated into enzyme-controlled reactions for the in situ generation of radical intermediates. This allows the generation of promiscuous, non-native catalytic transformations, without the need for genetic manipulations or the preparation of artificial biohybrid catalysts. By this approach, existing enzymes

\footnotesize{Scheme 2. Radical Dehalogenation of Halolactones Catalyzed by Nicotinamide-Dependent Alcohol-Dehydrogenases in the Presence of Light}\textsuperscript{a}

\textsuperscript{a}(A) Proposed mechanism of radical dehalogenation. (B) Substrate scope of dehalogenation by RasADH or LKADH.\textsuperscript{40} LKADH led to the (R)-enantiomer and RasADH to the (S)-enantiomer.\textsuperscript{40} (C) Stereoselective radical dehalogenation catalyzed by the ketoreductase LKADH expanded to α-bromoamides using eosin Y as exogenous photocatalyst.\textsuperscript{112}
can serve as chiral scaffolds for directing the stereoselectivity of synthetically valuable photoredox reactions.

NAD(P)H-dependent enzymes are of special interest, as it is known that NAD(P)H and its mimics are redox-active due to their 1,4-dihydropyridine moiety and that they can be excited by visible light.\textsuperscript{109−111} Thereby, NAD(P)H turns from a ground-state, weak single-electron reductant to a strong single-electron reductant that can reduce a range of functional groups upon photoexcitation with blue light. Consequently, NAD(P)-H-dependent enzymes may bind and transform a substrate that was not susceptible to reduction via natural enzyme activity.

Such photoinduced enzyme promiscuity was first reported for stereoselective radical dehalogenation of halolactones with nicotinamide-dependent ketoreductases.\textsuperscript{40} Upon photoexcitation with blue light (460 nm), alcohol-dehydrogenases from\textit{Ralstonia} sp. (RasADH) and\textit{Lactobacillus kefiri} (LKADH) converted racemic α-halo lactones to optically enriched dehalogenated lactones reaching up to 95% conversion, a yield of 81%, and an e.e. of 96% (Scheme 2A; for substrate scope, see Scheme 2B). Experimental evidence confirmed the role of NADPH as both a single-electron reductant and a hydrogen atom source. The racemic background reaction with free NADPH in solution was negligible, because the formation of an electron-donor−acceptor complex, responsible for the initial electron transfer to occur, was preferred in the enzyme active site because of stabilization of the photoexcited NADPH. However, the requirement for formation of an electron-donor−acceptor complex limited the scope only to lactone substrates.

\begin{scheme}
\centering
\includegraphics[width=\textwidth]{Scheme3.png}
\caption{Radical Deacetylation Catalyzed by an NADPH-Dependent Ene-Reductase and Rose Bengal as Photocatalyst in the Presence of Light\textsuperscript{a}}
\end{scheme}

\textsuperscript{a}(A) Proposed mechanism of radical deacetylation. (B) Substrate scope of radical deacetylation catalyzed by NtDBR and Rose bengal as photocatalyst.\textsuperscript{112}
This concept was expanded by employing an exogenous xanthene-based photocatalyst which enabled electron transfer without the formation of an electron-donor–acceptor complex (Scheme 3A).\(^{112}\) Rose bengal as photocatalyst was excited by light irradiation followed by electron transfer from NADPH onto the photocatalyst to form an anion radical of the photocatalyst. The electron transfer from the photocatalyst to 3 is energetically nonfavored in solution but enabled by hydrogen bonding of the substrate binding in the active site of the enzyme. Calculations suggested that the hydrogen bond formed between a tyrosine residue and the substrate 3 within the active site of the enzyme reduces the redox potential of 3. This makes electron transfer from the photocatalyst to the substrate feasible ($\Delta G^\circ = +3.45$ kcal mol$^{-1}$). After the electron transfer, a spin-center shift is presumed, which leads to the elimination of acetate under the decomposition of the ketyl radical. The resulting acyl radical is further bound to in the chiral environment of the enzyme. Therefore, the following hydrogen atom transfer (HAT) from the enzyme-bound NADPH occurs highly enantioselective. The binding of the substrate within the active site ensured that radicals form only in the chiral environment of the active site. According to the mechanism published and presented here, the reaction needs formerly just to be initiated by theoretically a single photon. The transformation was demonstrated with Rose bengal as photocatalyst and the ene-reductase from \textit{Nicotiana tabacum} (NiDBBR) enabling radical deacetylation of $\alpha$-acetoxytetralone (Scheme 3B). Under green light irradiation (530 nm), the desired product was generated in up to 87% yield with 86% e.e. A sacrificial catalytic equivalent of NADPH was needed in addition to light to generate the Rose bengal radical anion, which can interact with the enzyme-bound substrate in situ, as well as for hydrogen transfer to terminate the intermediate free radical. The generality of this approach was demonstrated by enantioselective dehalogenation of previously unreactive $\alpha$-bromoamides with a ketoreductase variant from \textit{L. kefiri} in the presence of an exogenous photoredox catalyst, with eosin Y providing the highest yield of up to 71% with 90% e.e. (Scheme 2C). Therefore, it can be envisaged to use this approach to expand the catalytic scope of a wide variety of NAD(P)H-dependent enzymes.\(^{112}\)

Flavin is on the one hand a cofactor of enzymes, including enzymes with native photocatalytical properties (Section 2), and on the other hand, it is also a well-characterized photocatalyst on its own.\(^{113}\) Consequently, it was expected that non-native photocatalytic activity would be found in flavoproteins. In a recent study, selected flavoproteins were investigated to catalyze the photoactivation of metal-based prodrugs by converting the Pt$^{IV}$ and Ru$^{III}$ complexes into Pt$^{II}$- or Ru$^{II}$-OH$_2$ species, commonly catalyzed by free FNM and FAD.\(^{114}\) Four flavoproteins were selected, harboring diverse chemical environments surrounding the flavin binding pockets, which control solvent and substrate accessibility to the active site as well as the photooxidation properties of flavin: miniSOG (mini singlet oxygen generator),\(^{115}\) NOX (NADH oxidase from \textit{Thermus thermophilus} HB27),\(^{116}\) GOX (glucose oxidase from \textit{Aspergillus niger}),\(^{117}\) and GR (glutathione-reductase from \textit{Saccharomyces cerevisiae}).\(^{118}\) It was found that the protein scaffold had a pronounced effect on the catalysis: the negatively charged electrostatic surface and the deep flavin binding pocket of GOX prevented interaction with the negatively charged substrate, whereas the miniSOG and NOX, with positive and neutral shallow flavin binding pockets, respectively, promoted the light-triggered reaction. The activity of GR, which has a neutral but deep pocket and is the only one that does not generate reactive oxygen metabolites, was highly dependent on the electron donor. In the presence of MES, the conversion was inefficient, whereas NADPH led to significantly better conversion. It is also worthy to mention that NOX showed the ability to activate the prodrug even without light if NADH was the electron donor, whereas with MES as the electron donor, light was still required.

Similarly to NAD(P)H-dependent proteins, scaffolds of different flavoproteins may be able to borrow new properties to reactions catalyzed by flavins, such as higher selectivity, reducing undesired side-reactions, lowering the activation energy of the substrate, and producing optically pure compounds, thereby expanding the scope and applicability of flavin-catalyzed photo-reactions.

### 4. PHOTO-CHEMOCATALYSIS COUPLED WITH BIOCATALYTIC TRANSFORMATIONS

#### 4.1. Photo-Chemocatalysis Providing Redox Equivalents for Biocatalysis

Probably, the highest number of reported photo-biocatalytic systems consist of a photo-chemocatalytic reaction providing redox equivalents for a biocatalytic transformation catalyzed by an oxidoreductase (Scheme 4).\(^{39,50,119}\)

<table>
<thead>
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<th><img src="image" alt="Scheme 4. Photocatalytic Generation of Redox Equivalents for a Redox-Enzyme in a Parallel Cascade" /></th>
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</thead>
</table>

Thus, the reaction requiring light does not occur in the environment of a protein. In general, a photosensitizer (or photomediator)\(^{150}\) is coupled to a sacrificial electron donor (e.g., EDTA or water) for providing the reduced cofactor [e.g., NAD(P)H or FMNH$_2$] or oxidant (e.g., H$_2$O$_2$) driving the photo-biocatalytic redox reaction. In detail, when the photosensitizer is excited by light, its electrons change into the excited state enables the photosensitizer to grab electron(s) or a hydride from an electron donor. This is followed by a direct electron (hydride) transfer to the cofactor or indirectly via a mediator, which then regenerates the cofactor.\(^{15,39}\)

A wide range of photosensitizers, including semiconductor quantum dots, chlorophylls, metal nanoparticles, and organic dyes were used for the activation of redox-enzymes by direct or indirect transfer of photoinduced electrons.\(^{120–122}\) In these systems, sacrificial electron donors such as ethylenediaminetetraacetate (EDTA), triethanolamine (TEA), ascorbic acid (AA), or even water\(^{123,124}\) were used to recycle the mediator or cofactor and thus to enable the next catalytic cycle.
Additionally, photoelectrochemical water oxidation coupled to enzymatic reduction reactions, H₂ evolution, or CO₂ reduction have been investigated in recent years. Direct or indirect photocatalytic activation of redox-enzymes was recently summarized in an extensive review. Thus, herein, only an overview over fundamental concepts will be given, and recent

Table 1. Biocatalytic Reactions Combined with Photocatalytic Cofactor Regeneration

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction system</th>
<th>Photosensitizer(s) / mediator</th>
<th>Electron Source</th>
<th>Enzyme</th>
<th>In vitro or in vivo</th>
<th>Product</th>
<th>Optical purity</th>
<th>TTN and/or TOF[h]</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CdSe QDs</td>
<td>AA[a]</td>
<td>ferredoxin NADP⁺-reductase (FNR)</td>
<td>In vitro</td>
<td>H₂</td>
<td>-</td>
<td>TOF 1440 h⁻¹</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>TCPP[c]</td>
<td>TEOA[g]</td>
<td>ADH</td>
<td>In vivo</td>
<td>MeOH</td>
<td>-</td>
<td>-</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>FMN</td>
<td>EDTA[c]</td>
<td>OYE</td>
<td>In vivo</td>
<td>&gt;69% e.e.</td>
<td>TTN 10.9</td>
<td>138</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>V-TiO₂ / FMN</td>
<td>H₂O</td>
<td>OYE</td>
<td>In vivo</td>
<td>&gt;85% e.e.</td>
<td>TTN 658, TOF 197 h⁻¹</td>
<td>124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Ru(II) or Ir(III) complexes / [Ru(bpy)₃]₂⁺(CH₃)₂CHO (MV)[f]</td>
<td>TEOA [g]</td>
<td>OYE</td>
<td>In vivo</td>
<td>&gt;50% yield</td>
<td>TOF 125 h⁻¹</td>
<td>123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>RB[h]</td>
<td>TEOA[g]</td>
<td>OYE</td>
<td>In vivo</td>
<td>&gt;99% e.e.</td>
<td>TTN 235, TOF 52 h⁻¹</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>FAD (catalytic amounts of NADP⁺)</td>
<td>EDTA</td>
<td>PAMO-P3</td>
<td>In vitro</td>
<td>97% e.e.</td>
<td>enzyme (96), mediator (9.6)</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Rutile Au-TiO₂</td>
<td>H₂O</td>
<td>UPO</td>
<td>In vitro</td>
<td>&gt;99% e.e.</td>
<td>TON 18.8 x10⁴</td>
<td>142</td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>Au-TiO₂</td>
<td>MeOH</td>
<td>UPO</td>
<td>In vitro</td>
<td>&gt;98% e.e.</td>
<td>TTN &gt;71 x10⁴</td>
<td>143</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>FMN</td>
<td>EDTA</td>
<td>OLE₇[b]</td>
<td>In vitro</td>
<td>&gt;99% conv.</td>
<td>-</td>
<td>-</td>
<td>145</td>
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<tr>
<td>11</td>
<td>FMN</td>
<td>EDTA</td>
<td>OLE₇[b]</td>
<td>In vitro and in vivo</td>
<td>&gt;99% conv.</td>
<td>-</td>
<td>-</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Ru(II)-dilime</td>
<td>DTC[i]</td>
<td>P450 B3M</td>
<td>In vitro</td>
<td>&gt;95% yield</td>
<td>-</td>
<td>TTN 935</td>
<td>147</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>TiO₂ and MV[ia]</td>
<td>AA</td>
<td>[FeFe]-hydrogenase and matrularse</td>
<td>In vitro and in vivo</td>
<td>H₂</td>
<td>-</td>
<td>TOF 22 000 h⁻¹</td>
<td>149</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>EY[i]</td>
<td>TEOA</td>
<td>P450</td>
<td>In vivo</td>
<td>-</td>
<td>TTN −180</td>
<td>150</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1 summarizes different enzymatic biotransformations coupled to various types of photosensitizers and sacrificial electron donors, which have been performed either in vitro or in vivo. The most straightforward approach for such systems is the use of photochemical reactions to reduce (recycle) the cofactors NAD(P)H or FMNH₂, which are subsequently used in a biocatalytic reaction.¹²⁵−¹²⁹ Several photochemical systems have been developed for this purpose. In most cases, the NADPH is recycled using a photosensitizer to harvest light energy and a mediator to transfer the electrons to the cofactor. Such a system, consisting of a graphene-based light-harvesting photocatalyst and the metal-based mediator [Cp*Rh(bpy)H]⁺, was used for the light-driven activation of NAD(P)H-dependent alcohol-dehydrogenases (ADHs), enabling an ecofriendly synthesis of chiral alcohols (Scheme 5A).¹³⁰ Further studies reported biohybrid complexes consisting of CdSe quantum dots and ferredoxin NADP⁺-reductase for photochemical regeneration of NADPH. In one example, the regeneration system was coupled with an alcohol-dehydrogenase to convert different aldehydes to the corresponding alcohols. In the presence of 100 mM ascorbic acid (AA), an average TOF of 1440 h⁻¹ and a quantum yield of 5.8% were obtained (Table 1, Entry 1, cf. Section 4.2).¹³¹ Another example is the photocatalytic reduction of C=C bonds using nitrogen doped carbon nanodots as photocatalysts, for the reduction of artificial nicotinamide analogues in combination with a rhodium-based complex as mediator.¹²⁸ Thus, this is a first approach replacing NAD(P)H in a light-driven recycling process with synthetic NADH mimics (mNADHs). The possibility to tune mNADHs by synthetic modifications to the respective conditions and systems makes them an attractive new research field in photo-biocatalysis. It is possible to synthesize more stable, less expensive, and more reactive NADH analogues in comparison to the natural cofactor.¹³²,¹³³ Although the efficiency of regeneration of mNADHs is not yet comparable to that of natural cofactors, their modified structures show promising properties for light-driven recycling systems.¹²⁸

In another case, the photosensitizer TCPP [5,10,15,20-tetrakis(4-carboxyphenyl) porphyrin] was immobilized on thiol-containing silica microspheres coated with polydopamine/polyethylenimine and was applied for visible-light NADH regeneration.¹²⁰ After optimization of the reaction conditions, an NADH yield of up to 82% was achieved after illumination for 60 min. The approach represents one of the most efficient light-driven NADH regeneration systems (Table 1, Entry 2). Similar systems were developed for the regeneration of FMNH₂ or FADH₂.¹³⁴ Furthermore, the [Cp*Rh(bpy)H₂O]²⁺ mediator was also applied for the regeneration of NADPH for monoxygenases, which overall catalyze oxidations. One example is the oxidative C–O bond cleavage, catalyzed by the cytochrome P450 monoxygenase BM3 from Bacillus subtilis (Scheme 5B and Table 1, Entry 14).¹³⁵

It has already been described in 1978 that flavins (FAD or FMN) can be reduced in a light-dependent reaction, and therefore, it is not surprising that flavin-dependent systems have been widely applied for both oxidations and reduc-
The illumination of flavins by light leads to an excited molecule capable of extracting electrons from sacrificial electron donors such as EDTA. Once the electrons are liberated from the sacrificial electron donor, the reducing equivalents are transferred indirectly via the flavin to NAD(P)⁺.

However, photoreduced flavins are quite reactive and easily react with molecular oxygen, leading to an increase of the concentration of H₂O₂ which may deactivate the enzyme, leading to secondary reactions or H₂O₂ being utilized as oxidant. However, S-dezaflavin, a flavin derivative, in which NS has been exchanged with a carbon atom, has been investigated as an alternative because of its potential to avoid hydrogen peroxide formation. Nevertheless, the majority of photocatalytic regeneration approaches has been performed with flavins. The performance of flavins together with EDTA as sacrificial electron donor was intensely investigated in recent years. A typical reaction was performed with 100 μM photosensitizer (FMN) and 25 mM EDTA, leading to a total turnover number (TTN) of 1.09 × 10⁴ and a turnover frequency (TF) of 210 min⁻¹ regarding the performance of the ene-reductase YqjM from Bacillus subtilis in the photosynthetic system (Table 1, Entry 3).

The system was expanded with the addition of titanium-dioxide-based (Au−TiO₂ or V−TiO₂) photocatalysts to the flavin-based regeneration of ene-reductases. This allowed the use of water as the ultimate electron donor and was coupled to the Old Yellow Enzyme (OYE, ene-reductase) from Thermus scotodiscus SA-01 (TsOYE) (Scheme 6A). It has been found that the activity of V−TiO₂ coupled to the TsOYE under irradiation with polychromatic light of wavelengths longer than 385 nm gave very promising results, thus representing a system that can be in theory directly driven by sunlight (Table 1, Entry 4). Additionally, in recent years, numerous photosensitive transition-metal complexes have been investigated as electron donors for the regeneration of FMN. In particular, the use of various Ir- and Ru-based complexes as photosensitizers in combination with various mediators and artificial electron donors enabled the efficient light-driven biocatalytic reduction of α,β-unsaturated compounds by ene-reductases (Table 1, Entry 5). Carbon nanodots were investigated as alternative photocatalysts. Recently, also the NAD(P)⁺-free activation of the flavin-based OYE has been investigated by using Rose bengal as a photosensitizer (Scheme 6B). Most of the investigated organic dyes are inexpensive and easy to use in these systems. Consequently, enantiopure (R)-2-methycyclohexanone was obtained with a yield of 90%, an e.e. of >99%, and a TOF of 256 h⁻¹, thereby overcoming high production costs and the complex structure of the previously investigated systems (Table 1, Entry 6).

An alternative to recycling the cofactor outside of the enzyme is light-enabled recycling of the cofactor directly within the enzyme. This has been realized in vitro for the flavin cofactor FADH₂ (Scheme 7). The flavin-dependent halogenase PyrH from Streptomyces rugosporus was chosen as a model system. This enzyme halogenates regioselectively the S-position of tryptophan using chloride or bromide. In the natural system, the FADH₂ needs to be recycled (e.g., by a flavin-reductase). Here it was shown that FADH₂ can be recycled being tightly bound to the enzyme at the expense of light and a sacrificial electron donor (EDTA) following a concept as mentioned before. Because the flavin is tightly bound in the protein, futile cycles leading to the spontaneous generation of H₂O₂ were reduced. Consequently, the reaction proceeded without the addition of catalase, resulting in simpler

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Scheme 6. Photochemical Regeneration of Enzyme-Bound FMN (Prosthetic Group) as Mediator Coupled with Biocatalytic C=C Bond Reduction Catalyzed by Ene-Reductases

(A) Catalytic water oxidation by using a water-oxidation catalyst (Au−TiO₂) coupled to the ene-reductase-catalyzed reduction of 4-ketoisophorone to (R)-levodione. (B) Light-driven activation of ene-reductases by photochemical regeneration of FMN as mediator, using Rose bengal (RB) as photocatalyst.

Scheme 7. Light-Dependent Cofactor Recycling within the Halogenase PyrH

The flavin-dependent halogenase PyrH from Streptomyces rugosporus was chosen as a model system. This enzyme halogenates regioselectively the S-position of tryptophan using chloride or bromide. In the natural system, the FADH₂ needs to be recycled (e.g., by a flavin-reductase). Here it was shown that FADH₂ can be recycled being tightly bound to the enzyme at the expense of light and a sacrificial electron donor (EDTA) following a concept as mentioned before. Because the flavin is tightly bound in the protein, futile cycles leading to the spontaneous generation of H₂O₂ were reduced. Consequently, the reaction proceeded without the addition of catalase, resulting in simpler
reaction conditions. In total, 70% of 0.5 mM tryptophan was regionselectively converted to 5-chlorotryptophan (5-Cl-Trp) within 25 h. Conversion was not detectable in the dark, providing evidence for light-driven regeneration and catalysis.

A flavin-dependent system for the photo-chemocatalytic regeneration of the FAD-dependent system was also used to deliver the required electrons for oxygen-dependent reactions catalyzed by monooxygenases.140 This was demonstrated using a Baeyer–Villiger monoxygenase in combination with light, FAD, and EDTA as electron donor (Scheme 8 and Table 1, Entry 7).141

Scheme 8. Photo-Chemocatalytic Regeneration of Reduced Flavin Cofactor for a Baeyer–Villiger Monoxygenase (BMVO)141

The photocatalytic reactions discussed in the following part deal with the generation of oxidizing reagents or with the supply of the electrons required by the enzyme. In the first example, the inorganic Au–TiO2 photocatalyst was used to produce H2O2 for an unspecfic peroxygenase (UPO, Table 1, Entry 8).142 Because the application of peroxygenases in general suffers from the poor robustness of the peroxygenases in the presence of hydrogen peroxide, the approach allows the in situ production of H2O2 under illumination through reductive activation of ambient oxygen, whereby the amounts of peroxide can be tuned to ensure that the enzyme remains highly active and stable. With this approach, the stereoselective hydroxylation of ethylbenzene to (R)-1-phenyl-ethanol was achieved leading to 11 mM of product with an e.e. of >98% and TTN of more than 71 000 (Scheme 9A and Table 1, Entry 9).143

In an alternative method, H2O2 was produced using enzymatic formate oxidation. A formate-dehydrogenase from Candida boidinii oxidized formate to CO2 under formation of NADH from NAD+ (Scheme 9B). In a second catalytic step, molecular oxygen was transformed to H2O2 at the expense of the produced NADH, in the presence of a photocatalyst (such as FNM, phenosafranine, or methylene blue) and visible light. The produced H2O2 is then available for activation of the UPO. However, the ChFDH/NAD/HCO2H system coupled with the photocatalyst is not yet suitable for preparative application, as photobleaching of the organic photocatalysts was found, and the formate-dehydrogenase is not stable under the reaction conditions. Especially, flavin-derived photo-
catalysts led to a rapid inactivation of formate-dehydrogenase. For this reason, the Au–TiO2-based photochemical production of H2O2 seems to be the better alternative.144

Another enzyme that can utilize photocatalytically produced H2O2 is the fatty acid decarboxylase OleT from Jeotgalicoccus sp. (Table 1, Entries 10 and 11). In this case, H2O2 was generated by visible-light illumination of an EDTA/FMN system. Subsequently, OleT consumed the H2O2 for the oxidative decarboxylation of fatty acids or α-hydroxy fatty acids under formation of the corresponding alkenes or alkenols. In addition to decarboxylation, hydroxylation of the fatty acids also occurs to a minor extent.145,146

The already mentioned group of P450 enzymes perform a functionalization of inactivated C–H bonds. The enzymes utilize molecular oxygen, which is formally activated by two electrons that are provided by a reductase and ultimately are derived from NADPH. Besides the already mentioned direct photocatalytic recycling of NAD(P)H (Scheme 5) or the oxygen-independent methods using H2O2 (Table 1, Entries 10 and 11), cofactor-independent photocatalytic methods for the direct supply of electrons were developed. In this approach, the P450 BM3 from Bacillus megaterium, which is a heme thiolate containing monoxygenase fused to a NADPH-dependent reductase, was used as model enzyme for the covalent attachment of a Ru(II) photosensitizer. The photosensitizer provides electrons under illumination; thus, the reaction is no longer dependent on the reductase, and it becomes possible to perform P450 reactions upon visible-light irradiation.147 The obtained hybrid enzyme showed higher stability and was successfully activated under visible-light irradiation, leading to the hydroxylation of lauric acid with a TTN of 935 and an initial reaction rate of 125 mol of product/mol of enzyme−1 min−1 (Table 1, Entry 12).

In contrast to the utilization of metal complex photosensitizers, studies with photoactive nanoparticles coupled to biocatalysts have been performed.46,148 One example highlights the efficient wiring of the FAD-dependent glucose-dehydrogenase (FAD-GDH) to PbS quantum dot (QD)-sensitized opal-TiO2 (IO-TiO2) electrodes by means of an Ocomplex-containing redox polymer for the visible-light-driven glucose oxidation.36 Interestingly, the biohybrid signal chain, which was switched on with light, triggered a multistep electron-transfer cascade from the enzyme toward the redox polymer and finally to the IO-TiO2 electrode. The system enables a precise control of the biocatalytic reaction at the electrode interface.

Although many in vitro photo-chemocatalytic approaches coupled to biocatalytic transformations have been published, only a handful of examples are reported in vivo. Recently, the first example of a whole-cell reaction was reported using recombinant E. coli coupled to the photocatalytic H2 production as illustrated in Scheme 10A (Table 1, Entry 13).149

The extracellular photosensitizer TiO2 was coupled to E. coli BL21 (DE3) expressing the [FeFe]-hydrogenase HydA from Clostridium acetobutylicum NBRC 13948 (ATCC824) using methyl viologen (MV) as electron mediator. The addition of MV using whole cells and TiO2 enhanced H2 production from 28.6 to 117 μmol of H2 in 5 h.149

The second example shows a cofactor-free light-driven approach for P450 catalysis.150 The light-driven catalysis is based on in vivo photoreduction of the P450 by different light-harvesting complexes such as fluorescent dyes. The cofactor-
free in vivo photoreduction uses eosin Y (EY) as photosensitizer to mediate the electron transfer directly to the heme domain of P450s (mainly the variant BM3m2: Y51F/F87A of the P450 BM3). Triethanolamine (TEOA) was used as electron donor. By measuring the Fe−CO absorption band at 450 nm, characteristic for the reduced P450 heme domain, the authors demonstrated that the reduction of the heme domain only occurs in the presence of light and TEOA. Whole-cell biotransformation with BM3m2, EY, and TEOA under illumination resulted in a turnover number for BM3m2 of 16 in 18 h with 7-ethoxycoumarin as substrate, whereas no conversion was observed in darkness. Other BM3 variants and substrates showed conversion in the same range, and the results indicated the possibility of cofactor-free, light-driven P450 catalysis (Scheme 10B). Although several substrates and different P450s have been investigated, the overall product formation remained in a range of 50–80 μM within 20–24 h, thus showing that such in vivo systems still have to be improved.

4.2. Cascades Combining Photo-Chemocatalytic and Biocatalytic Transformation. This section summarizes combinations of a photo-chemocatalytic and a biocatalytic transformation in linear cascades, thus, cascades in which both steps contribute to the formation of the final product. In contrast to the previous Section 4.1, here the substrate is first transformed by the photocatalyst/light prior to the enzymatic reaction or vice versa (Scheme 11).
Numerous chemo-enzymatic cascades have been published, in which biocatalytic and chemocatalytic transformations proceed in a one-pot simultaneous or sequential fashion. Nevertheless, performing reactions catalyzed by enzymes and chemical catalysts in a one-pot fashion simultaneously is still challenging due to diverging reaction conditions. Particularly, the combination of different solvent requirements of enzymes and transition-metal catalysts represents a hurdle. The combination of a photochemical synthetic step with a biocatalytic transformation additionally leads to the problem that the biocatalysts may be inhibited due to the formation of highly reactive oxygen species or radical species in the presence of light. This may be the reason that the number of examples of biocatalytic transformations merged with photochemical catalysis is currently very low. Nevertheless, four examples were published in the last two years. The first one-pot sequential cascade combines a photocatalytic thiol-Michael addition followed by a biocatalytic keto reduction (Scheme 12A). In this way, 1,3-mercaptoalkanols were synthesized with high (S)- and (R)-stereoselectivity starting from $\alpha,\beta$-unsaturated ketones and thiols. 1,3-Mercaptoalkanols belong to the volatile sulfur compounds (VSCs), which are responsible for the aroma of many beverages and foods. The first step of the cascade constitutes the addition of substituted thiols to substituted vinyl ketones with terminal double bonds by visible-light catalysis using [Ru(bpy)$_3$Cl$_2$] as photocatalyst. This transformation was followed by an enantioselective reduction of the ketone group by alcohol-dehydrogenase variants under the formation of 1,3-mercaptoparalkanols with moderate to good yields and high e.e. values up to 99%. The enantioselective reduction of the carbonyl group was performed using commercial enzymes, which possess complementary stereoselectivity, thus allowing access to both stereoisomers. The photocatalytic step delivers high conversions within 5 min, wherein for the subsequent reduction of the keto group, another 24 h is required.

The second photoredox–biocatalytic cascade was developed for the enantioselective synthesis of amines (Scheme 12B).

Scheme 11. Concepts for Linear Cascades Combining a Photochemically Catalyzed Transformation and a Biocatalytic Transformation

![Diagram]

An intermediate is formed in the photocatalytic step, which serves as a substrate for the biocatalytic transformation (or vice versa).

Scheme 12. Cascades Combining Photo-Chemocatalytic and Biocatalytic Transformations—Part 1

(A) Synthesis of 1,3-mercaptoparalkanols in a photocatalyzed thio-Michael addition and a subsequent biocatalytic reduction of the carbonyl group. (B) Synthesis of enantiomerically enriched amines by linking a simultaneous photocatalyzed reduction of imines with an enantioselective oxidation by a monoamine oxidase.
Employing \( \text{Na}_3\text{Ir(ppy)}_3 \) as photocatalyst, cyclic imines were converted into the racemic amines in the first step of the cascade by visible-light-driven reduction. In the second step, one of the enantiomers of the amine was enantioselectively oxidized to the imine by the monoamine oxidase (MAO-N-9, used as a whole-cell catalyst overexpressed in \( \text{E. coli} \)) at the expense of molecular oxygen. The constant cycling of nonselective reduction and enantioselective oxidation leads overall to deracemization. The cascade is only feasible in the presence of ascorbic acid, which transfers a hydrogen atom to the unstable \( \alpha \)-aminoalkyl radical, formed by a photoinduced electron transfer. Unfortunately, the cascade is limited to a few 1-pyrrolines with phenyl, alkyl, or benzyl substituents in position 2. The yields are very high for all tested imines with up to 95%. In addition, e.e. values of up to 99% were obtained for cyclic imines with alkyl substituents.

By the addition of thiol donors [4-mercaptophenylacetic acid (MPAA-PEG) and 3-mercaptopropionic acid (MPA-PEG) derivatives], the substrate spectrum was extended to 1-methyl-3,4-dihydroisoquinoline. Because aromatic imines are able to stabilize the radical intermediate formed during the reaction, no HAT took place for this substrate without thiols. However, in the presence of MPAA-PEG and MPA-PEG, a polarity-matched HAT became possible. The nucleophilic \( \alpha \)-aminoalkyl radical reacts with the S–H bond of the added sulfur compounds to form an electrophilic thiyl radical. The subsequent reaction between this thiyl radical and nucleophilic ascorbic acid is much more favored and leads to the formation of the desired amines.

The third cascade combines a visible-light photocatalyzed isomerization of alkenes and the subsequent double-bond reduction by an ene-reductase. C–H functionalization of alkane and alkene derivatives by combining a light-driven oxofunctionalization with sodium anthraquinone sulfate (SAS) and an enzymatic transformation.

\[ \text{Scheme 13. Cascades Combining Photo-Chemocatalytic and Biocatalytic Transformations—Part 2}^{157} \]

(A) Visible-light photocatalyzed isomerization of alkenes and the subsequent double-bond reduction by an ene-reductase.\(^{157}\) (B) C–H functionalization of alkane and alkene derivatives by combining a light-driven oxofunctionalization with sodium anthraquinone sulfate (SAS) and a enzymatic transformation.\(^{158}\)
reaction using FMN or a cationic Ir\([\text{dmppy})_2(\text{dtbb})]\)PF\(_6\) complex. The development of a suitable isomerization system was one of the critical steps, as organic photocatalysts such as riboflavin enable efficient isomerization but at the same time inhibit the ene-reductases or the glucose-dehydrogenase recycling system. For optimization, 2-phenylbut-2-enedioic acid dimethyl ester was used as a model substrate. The substrate scope in this study was limited to related diesters and cyanacrylates, which can be converted by the investigated ene-reductases with moderate to high yields and high enantioselectivity with up to 99% e.e. However, as ene-reductases are well-characterized enzymes,\(^{162}\) this method may be extended to numerous other substituted alkenes.

In the fourth cascade, a C–H functionalization of alkanes was achieved by combining a light-driven oxofunctionalization of alkanes leading to aldehydes and ketones with a subsequent asymmetric biocatalytic transformation of these carbonyl functionalities (Scheme 13B).\(^ {158}\) The photocatalyst converting alkanes to the corresponding aldehydes or ketones was sodium anthraquinone sulfate (SAS), whereby in particular toluene, aryl alkane, or cycloalkene derivatives were used. For the subsequent enzymatic reaction, a number of enzyme classes were evaluated: 4-hydroxyacetophenone monoxygenases (HAPMO) forming formic esters, cyclohexanone monooxygenases (CHMO) leading to lactones, hydroxynitrile-lyase (HNL) forming chiral cyanohydrines, benzaldehyde-lyases (BAL) for the production of chiral acyloins, aryl alcohol oxidase (AAO) producing carboxylic acids, ene-reductases (ERED or OYE) forming chiral cyclohexanones, alcohol-dehydrogenases (ADHs) producing enantiopure alcohols, and transaminases forming amines with high e.e. The cascade demonstrates the potential that arises through the combination of photo and biocatalysis, because a large number of chiral products with a wide variety of functional groups can be obtained in two steps from simple, inexpensive, achiral starting materials. Preparative syntheses of \((R)-\)mandelonitriles and \((R)-\)benzoin on a gram scale were performed with excellent e.e. and yields. The problem of inhibition of the biocatalysts by reactive species that are formed during the photocatalytic step was solved by a two-phase system or by a temporal and spatial separation of the catalysts.

These four recently published cascades demonstrate the combination of photo-chemoredox reactions with biocatalytic transformations. In the following two linear cascades, two enzymes catalyze the two steps in the linear sequence, whereby
for one step, light is required for cofactor recycling or providing the oxidant for the enzyme.

In the first example, isobutanol was prepared in two steps from 2-ketosiovalerate using a keto acid decarboxylase (KDC) and an alcohol-dehydrogenase (ADH). For the ADH reaction, NADPH was recycled by a photoredox system based on biohybrid complexes of the ferredoxin NADP⁺-reductase (FNR) from *Chlamydomonas reinhardtii* and CdSe quantum dots (QD; Scheme 1A, cf. Section 4.1). The CdSe QD absorbs visible light undergoing charge separation to generate an electron–hole pair. The electron can be transferred to the FAD cofactor in the bound FNR, which leads to a photocatalytic regeneration of NADP⁺. The NADPH concentration increased linearly with illumination time up to 2 h, until all the available NADP⁺ was reduced. The QD ground state is regenerated by the sacrificial electron-donor ascorbic acid. In this study, the QD-FNR complexes were coupled to recycle NADPH, which was consumed by the alcohol-dehydrogenase TaADH from *Thermoanaerobium brockii* for the reduction of isobutyraldehyde. The reaction showed a quantum yield of 4.8–5.8%. This reaction was incorporated in a cascade employing the keto acid decarboxylase (KDC) from *Lactococcus lactis* for the decarboxylation of 2-ketosiovalerate to give isobutyraldehyde. Illumination of the KDC/TaADH/QD-FNR solutions for 1 h produced an isobutanol concentration 3.7-fold higher than the initial NADP⁺ concentration, indicating that a proof-of-concept was achieved, but that applications are far away. So far, only product concentrations below 1 μM have been achieved. The biggest limitation of this system is the low stability of the QD-FNR complex.

Another approach refers to the photochemical water oxidation used for in situ generation of H₂O₂ (Section 4.1). The oxyfunctionalization step was extended in a cascade leading to chiral alcohols and amines (Scheme 1B). The photoenzymatic oxidation of toluene to benzaldehyde was coupled to an enzymatic benzoin condensation using the benzaldehyde-lyase from *Pseudomonas fluorescens* coupled to an enzymatic benzoin condensation using the benzaldehyde-lyase from *Pseudomonas fluorescens*. Furthermore, acetophenone formed by the photoenzymatic oxyfunctionalization of ethylbenzene was subjected to reductive amination using the transaminases from *Aspergillus terreus* (At-ω-TA) and *(S)-selective Bacillus megaterium* (Bm-ω-TA). Both cascades were performed in a one-pot sequential fashion; thus, the photoenzymatic oxidation to the corresponding aldehyde or ketone was performed first, followed by addition of the biocatalysts needed for the second transformation.

Finally, the light-driven generation of H₂O₂ using EDTA and FMN was utilized for the H₂O₂-dependent decarboxylation of a ω-hydroxy fatty acid performed by OleT (Section 4.1, Table 1, Entries 10 and 11), and the synthesized alkenes were subsequently converted to the corresponding long-chain terminal diols in a [Ru]-catalyzed metathesis reaction (Scheme 1C). The cascade was performed in a biphasic buffer/isoctane one-pot reaction. First, the light-driven enzymatic step was performed in the aqueous medium, and then, the alkenes were extracted into the upper organic phase, where the metathesis reaction took place. However, no greater conversion than 20% was achieved. This can be explained with the incompatibility of the cell-free extracts with the metathesis catalyst. In addition, the organic phase must be shielded during the illumination to protect the catalyst from light-induced destruction.

In a nonsynthetic application, a cascade was developed for signal amplification for a high-throughput colorimetric bioassay to detect L-DOPA. The discussed cascades, combining photo-chemocatalytic and biocatalytic transformations, show that this field of photo-biocatalysis has great potential to provide green, environmentally friendly, and alternative synthetic routes to a variety of chemical substrates.

### 5. PHOTO-BIOCATALYSIS COUPLED WITH BIOCATALYTIC TRANSFORMATIONS

#### 5.1. Whole-Cell Photo-Biocatalysis Coupled with Biocatalytic Transformations

Whole-cell biotransformations offer several advantages, particularly regarding the availability of reduced nicotinamide cofactors [NAD(P)H] from the metabolism as well as the stability of enzymes, their regeneration due to the constant expression and the avoidance of enzyme purification steps. Although NAD(P)H can in general be recycled via the metabolism of glucose or other auxiliaries, oxygenic photosynthetic–photoautotrophic organisms such as cyanobacteria (prokaryotes), purple bacteria (prokaryotes), algae (eukaryocytes and prokaryocytes), and plants (eukaryocytes) offer a very appealing option to regenerate NAD(P)H via light splitting at the expense of light-giving protons and molecular oxygen as the only side-products (Scheme 15).

Scheme 15. Regeneration of NADPH with Photo-Autotrophic Organisms and Coupling with NADPH-Dependent Biotransformations

![Scheme 15](image)

NADPH is generated by the light-dependent water splitting.

The solar energy captured by photosynthetic pigments such as chlorophyll a (Chl a) is converted into electrochemical energy to regenerate NADPH from NADP⁺ via photosynthetic electron-transfer reactions. The linkage between photosynthetic electron flow and the oxidoreductase would involve either ferredoxin or NADPH via the ferredoxin–NADPH-reductase (FNR, cf. Section 2 and Scheme 1A). In this section, the focus will be on wild-type and recombinant prokaryotes. Cyanobacterial whole-cell biotransformations are particularly suitable for NADPH-dependent reactions, namely reductive reactions and oxyfunctionalization. Simultaneously to NADPH generation, molecular oxygen is formed, which could be directly consumed in oxygen-dependent biotransformations.

#### 5.1.1. Whole-Cell Biotransformation Driven by Photosynthetic Water-Splitting in Wild-Type Cyanobacteria

In 2000, cells from *Synechococcus elongatus* PCC 7942 were shown to reduce several aryl methyl ketones to the corresponding (S)-
alcohols with up to >99% e.e. and 90% yield within 3–9 days (Scheme 16A and Table 2, Entry 1). As the reaction rate of the reduction of α,α-difluorooctophenone with the same cyanobacterium increased under illumination with light from fluorescent lamps, this was claimed to be the first study on light-mediated regulation of an asymmetric reduction (Table 2, Entry 2). The herbicide DCMU, a N-phenyl urea derivative and inhibitor of photosystem II, was shown to reduce the reaction rate and influence the stereoselectivity. Thus, illumination improved not only the chemical yield but also the enantiomeric purity, whereby an explanation for the effect on the optical purity may be that more than one ADH is involved. In a comparative study, Anabaena variabilis, Nostoc muscorum, and again Synechococcus elongatus PCC 7942 were shown to reduce prochiral ketones such as ethyl 4-chloroacetate, 4-chlorooctophenone, 2'-3'4'-5'-6'-pentfluoroacetophenone, and ethylbenzoylacetate in an asymmetric fashion (Table 2, Entry 3). Although all of these cyanobacteria were able to reduce the prochiral ketones also in the absence of light, the optical purity of the outcome varied depending on the cultivation conditions (with or without light), which may be linked to different expression levels of the involved alcohol-dehydrogenases. Recombinant expression of an alcohol-dehydrogenase from the cyanobacterium Synechococcus elongatus PCC 7942 showed good to excellent enantioselectivities (>99.8% e.e.) toward several prochiral ketones and confirmed that NADPH is the preferred cofactor. In another example, the whole-cell wild-type strain Nostoc muscorum PTCC 1636, isolated from North Iran paddy fields, transformed hydrocortisone into androstane and pregnane derivatives (yields not reported, Table 2, Entry 4). Furthermore, morphologically different strains of cyanobacteria Arthospira maxima, Nostoc cf-muscorum, and Nodularia sphaerocarpa were exploited for enantioselective bioreduction of different diethyl oxophosphonates (Scheme 16B, Entry 5). Best results were obtained with Nodularia sphaerocarpa giving diethyl (S)-2-hydroxy-2-phenylethylphosphonate with 99% conversion and an optical purity of 92%.

In another comparative study, seven wild-type cyanobacterial species were analyzed for chemoselective reduction of cinnamaldehyde to cinnamyl alcohol (Scheme 16C and Table 2, Entry 6). The reduction of cinnamaldehyde by Synechocystis sp. PCC 6803, Synechocystis sp. PCC 6714, and Fischerella muscicola UTEX 1301 produced the desired product. Among these, Synechocystis sp. PCC 6803 proved to be the most efficient strain, giving a high conversion (>98%) after ca. 4 days of incubation under illumination. The amount of 3.8 mg of cells (corresponding to 0.076 mg of Chl a) converted to 1 mg of substrate. Byproducts were obtained in the reduction of cinnamaldehyde using Anabaena sp. PCC 7120, Plectonema boryanum IAM M101, and Synechococcus elongatus PCC 7942, presumably because of the presence of native ene-reductases. For comparison, the reductions of cinnamaldehyde by cyanobacteria were also performed in the dark, and among

**Scheme 16. Selected Examples of Photo-Biocatalysis by Whole-Cell Wild-Type Cyanobacteria**

(A) Various ketones were reduced to their corresponding alcohols by native alcohol-dehydrogenases in Synechococcus elongatus PCC 7942. (B) Phosphonate synthesis. (C) Selective reduction of cinnamaldehyde by different cyanobacterial strains.
Table 2. Photo-Biocatalytic Reduction of Ketones or Aldehydes Using Whole-Cell Wild-Type Cyanobacteria

<table>
<thead>
<tr>
<th>entry</th>
<th>organism</th>
<th>substrate</th>
<th>product</th>
<th>comment</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Synechococcus elongatus</em> PCC 7942</td>
<td>2′,3′,4′,5′,6′-pentfluoroacetophenones</td>
<td>corresponding (S)-alcohols</td>
<td>&gt;90% conversion after 9 days, 37 mg(_\text{CDW}), 0.57 mmol sub., e.e. &gt; 90%</td>
<td>165</td>
</tr>
<tr>
<td>2</td>
<td><em>Synechococcus elongatus</em> PCC 7942</td>
<td>αα′-difluoroacetophenone</td>
<td>(R)-1-phenyl-2,2-difluor ethanol</td>
<td>77% conversion, 1 g L(^{-1}) cells, 10 μmol sub., e.e. 66%</td>
<td>81</td>
</tr>
<tr>
<td>3</td>
<td><em>Anabaena variabilis</em></td>
<td>ethyl 4-chloroacetate</td>
<td>corresponding chiral alcohols</td>
<td><em>Anabaena variabilis</em>: &gt;99% conversion</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td><em>Nostoc muscorum</em></td>
<td>4-chloroacetophenone</td>
<td>with 5 mM 2′-3′-4′-5′-6′-pentfluoroacetophenone</td>
<td><em>Synechococcus elongatus</em> PCC 7942:</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Synechococcus elongatus</em> PCC 7942</td>
<td>2′-3′-4′-5′-6′-pentfluoroacetophenone</td>
<td>ethylbenzoylacetate</td>
<td>e.e. from 93 to &gt;99.8% (depending on sub.), max. product: 5 mM ethyl (S)-4-chloro-3-hydroxybutanoate</td>
<td></td>
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<tr>
<td>4</td>
<td><em>Nostoc muscorum</em> PTCC 1636</td>
<td>hydrocortisone</td>
<td>androstane</td>
<td>three metabolites purified: 11β-hydroxylandrostan-4-en-3β,17-one; 11β,17β-dihydroxyandrost-4-en-3-one; 11β,17α,20β,21-tetrahydroxypregn-4-en-3-one</td>
<td>170</td>
</tr>
<tr>
<td>5</td>
<td><em>Arthrospira maxima</em></td>
<td>(S)-2-oxopropylphosphonate</td>
<td>diethyl (S)-2-hydroxy-2-phenylethyl phosphate</td>
<td><em>Nodularia Sphaerocarpa</em>: major interesting results, diethyl (S)-2-hydroxy-2-phenylethylphosphonate, e.e. 92, 99% conversion</td>
<td>171</td>
</tr>
<tr>
<td></td>
<td><em>Nostoc muscorum</em></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td><em>Nodularia sphaerocarpa</em></td>
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<tr>
<td>6</td>
<td><em>Synechocystis</em> sp. PCC 6803</td>
<td>cinnamaldehyde</td>
<td>cinnamyl alcohol, dihydrocinnamaldehyde</td>
<td>cinnamyl alcohol was preferentially synthesized by six of these strains under illumination with red LEDs. <em>Synechocystis</em> sp. PCC 6803: most efficient, &gt;98% conversion after ca. 4 days, 3.8 mg(_\text{CDW}), 1 mg sub.</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td><em>Synechocystis</em> sp. PCC 6714</td>
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<td></td>
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<tr>
<td></td>
<td><em>Fischerella muscicola</em> UTEX 1301</td>
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<td></td>
<td><em>Anabaena</em> sp. PCC 7120</td>
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<td></td>
<td><em>Anabaena</em> cylindrica IAM M1</td>
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<tr>
<td></td>
<td><em>Plectonema boryanum</em> IAM M101</td>
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<td></td>
</tr>
<tr>
<td></td>
<td><em>Synechococcus elongatus</em> PCC 7942</td>
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<td></td>
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</tbody>
</table>

\(^\text{a}\)CDW = cell dry weight; CWW = cell wet weight; sub. = substrate.
the tested strains, only the reduction employing *Synechocystis* sp. PCC 6803 was mainly light-dependent. The examples demonstrate the synthetic potential of wild-type cells; however, for applications, the reactions need to be tuned to obtain higher titer. Moreover, these reactions are restricted to enzymes encoded in the genome of the wild-type. The strong influence of the cultivation conditions on the expression of different alcohol-dehydrogenases makes the biotransformations rather complex and limits the flexibility.

5.1.2. Light-Driven Whole-Cell Biotransformations in Recombinant Phototrophic Bacteria. The development of the first molecular tools for the engineering of cyanobacteria paved the way for the recombinant expression of oxidoreductases in cyanobacteria. This allowed production of a non-native enzyme in the cyanobacteria, enabling the optimization of possible production strains. Recombinant genes can now be introduced either using episomal replicating plasmids or genomic integration. A number of available vectors, promoters, and other genetic elements enable controlled enzyme expression in cyanobacteria. It should be mentioned that essential tools such as tight, inducible promoters are still not widely available in cyanobacteria.

Among the several cyanobacterial strains, non-filamentous unicellular strains are generally the most utilized for engineering. Among those, *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 are the most studied, and more information toward feasible recombinant strains is available.

The proof-of-concept of a photosynthetic biotransformation with recombinant oxidoreductases in cyanobacteria was realized by expression of the ene-reductase YqjM from *Bacillus* sp. in *Synechocystis* sp. PCC 6803 under the control of the light-induced promoter PpsbA2 (Scheme 17A). The produced cells converted a series of seven substrates with reaction rates up to 91 U g<sub>cdw</sub>⁻¹. YqjM accepts both NADH and NADPH as cofactors. In a semipreparative scale reaction, recombinant cells reduced 2-methyl-N-methylmaleimide (20 mM) to (R)-2-
methyl-N-Methylsuccinimide within 4 h of reaction time leading to 80 mg of isolated product with 99% e.e. and 80% yield after a simple extraction. Recombinant cells of *Synechocystis* sp. PCC 6803, expressing the cyclohexanone monooxygenase from *Acinetobacter* sp., showed that the approach can be also used for monooxygenases (Scheme 17B). With a substrate concentration of 5 mM, three different methyl-substituted ketones were converted with a specific activity between 2 to 5 U g<sub>CDW</sub>⁻¹. Interestingly, conversions were similar to those without aeration, showing that photosynthesis can supply the oxygen for monooxygenase reactions. Endogenous alcohol-dehydrogenases led to an undesired keto reduction as side-reaction. It remains open to which extent this can be suppressed by choosing appropriate cultivation conditions or inactivation of the responsible genes.

Additionally, the functional expression of the alkane monooxygenase AlkBGT from *Pseudomonas putida* Gpo1 in *Synechocystis* sp. PCC 6803 was reported (Scheme 17C). With light and without external supplementation of O<sub>2</sub>, the chemo and regioselective hydroxylation of nonanoic acid methyl ester to ω-hydroxynonanoic acid methyl ester was demonstrated. The reaction was driven by O<sub>2</sub> and reduction equivalents from photosynthetic water oxidation. The specific production rate under anaerobic light condition was 3.7 ± 0.5 mmol min⁻¹ g<sub>CDW</sub>⁻¹.

A recent study reported the asymmetric reduction of acetophenone to 1-phenylethanol by *Synechococcus elongatus* PCC 7942 whole cells, containing the overexpressed NADPH-dependent *Lactobacillus kefir* alcohol-dehydrogenase. Completion of the reduction to (R)-1-phenylethanol with 99% e.e. was reached within 6 h with a cell density of 0.66 g L⁻¹ coupling the optimum light and CO<sub>2</sub> amount. A higher CO<sub>2</sub> concentration enhanced the growth and biotransformation efficiency.

In addition to cyanobacteria, other phototrophic strains have been investigated. A recombinant strain of the bacterium *Rhodobacter sphaeroides* bearing an alcohol-dehydrogenase from *Leifsonia sp.* converted 3-chloroacetophenone to (R)-1-(3-chlorophenyl) ethanol with >99% e.e. (Scheme 17D). Although the reaction rate of 7.85 mM⁻¹ h⁻¹ g<sub>cells</sub>⁻¹ was quite high, it should be mentioned that instead of being light-driven in the strict sense (autotrophic), the approach is rather light-assisted (mixotrophic), as the microorganism lacks a photosynthetic apparatus against reactive oxygen species that are necessarily associated with catalytic water splitting. The addition of electron donors such as sodium thiosulfate was required.

### 5.1.3. Further Approaches for Light-Driven Whole-Cell Biotransformations

Artificial photosynthesis in heterotrophic bacteria such as *E. coli* is a tempting idea. However, it should be noticed, that cyanobacteria not only produce the photosynthetic machinery but also provide elaborate protection mechanisms against reactive oxygen species that are necessarily associated with catalytic water splitting. It remains to be investigate, to which extent these systems can be established successfully in other organisms. Nevertheless, several approaches using organic photosensitizers or membrane-permeable transport molecules demonstrated the feasibility of driving photocatalytic redox reactions in *E. coli*. A comparative view of the main concepts mentioned in Sections 5.1.1, 5.1.2, and 4.1 is displayed in Scheme 18. The use of phototrophic organisms as discussed in Section 5.1.1 and 5.1.2 is shown in Scheme 18A. In another approach, photosynthesis using the cyanobacteria or algae was coupled to redox reactions outside of the cell to produce reduced organic molecules for cofactor regeneration (Scheme 18B). This was shown recently for the green algae *Chlamydomonas reinhardtii*, which produces formic acid in the dark. Therefore, the cells were first “charged” by illumination and then further incubated in the dark, which led to the excretion of formic acid (ca. 3 mM). The formate was used on the outside of the cell for the recycling of NADH from NAD⁺ by formate-dehydrogenase, which was in turn consumed by the amine-dehydrogenase EsLeuDH-DM from *Exiguobacterium sibiricum*.

<table>
<thead>
<tr>
<th>Scheme 18. Approaches for Utilization of Light Energy toward Enzyme-Catalyzed Reactions</th>
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<tr>
<td><strong>A)</strong> NADPH-dependent enzymes as oxidoreductases are coupled to the photosynthetic conversion of light energy into chemical redox equivalents. (B) Exploitation of formate production from <em>Chlamydomonas reinhardtii</em> to regenerate NADH using formate-dehydrogenase from <em>Candida boidinii</em>. NADH is further used to convert ketones into the corresponding amines catalyzed by amine-dehydrogenase EsLeuDH-DM from <em>Exiguobacterium sibiricum</em>.</td>
</tr>
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</table>

“(A) NADPH-dependent enzymes as oxidoreductases are coupled to the photosynthetic conversion of light energy into chemical redox equivalents.”

“(B) Exploitation of formate production from *Chlamydomonas reinhardtii* to regenerate NADH using formate-dehydrogenase from *Candida boidinii*. NADH is further used to convert ketones into the corresponding amines catalyzed by amine-dehydrogenase EsLeuDH-DM from *Exiguobacterium sibiricum*.”
containing the ATP-synthase. The ATP was required for the phosphorylation of 3-phosphoglycerate by a kinase giving 1,3-bisphosphoglycerate, which was further converted by known enzymes to glucose. As starting material, ribulose-1,5-bisphosphate was used. This light-induced ATP formation system resulted in the production of 5.2 μg mL⁻¹ glucose in 0.78 mL of reaction buffer within 7 h. Glucose was not detectable without green LED illumination, proving the direct link between the enzymatic cascade generated and the light source.

Another cascade made use of the photodecarboxylase mentioned already in Section 2. In the cascade, the preparation of alkanes from triglycerides was achieved by a lipase-catalyzed hydrolysis of the triolein to the free oleic acid (and glycerol) combined with a decarboxylation, catalyzed by the fatty acid photodecarboxylase from Chlorella variabilis NC64A (CvFAP) upon blue-light illumination (Scheme 19B). The two-step cascade using 20 mM triolein gave a conversion of 83% and a respectable TON for the photodecarboxylase of 8280. Both steps were performed simultaneously.

6. PHOTOSWITCHABLE PROTEINS WITH CATALYTIC ACTIVITY

This part of the Review focuses on strategies to design light-controllable biocatalysts; thus, the enzymatic activity does not rely on light but can be switched on/off or regulated by light. Using light as an external modulator of enzyme activity may have several advantages over traditional modulators. In comparison to parameters such as temperature and pH, optical methods enable better tuning of reaction rates by users, because light can be precisely controlled in space, time, and intensity. Additionally, the use of light allows that the reaction vessel can stay physically closed from the beginning to the end of the reaction, so there is no chance that something else might affect the reaction. Such spatiotemporal control mechanisms of catalytic activities are central to the further development of one-pot reaction processes involved in biocatalysis. The spectrum of strategies described here can be generally applied to any enzyme, regardless of the morphology of their active site or catalytic mechanism, providing that the enzyme is photosensitive.

Strategies that enable photosensitization of enzymes can generally be divided in to two categories: genetically encoded tools and chemical functionalization of a protein or its surroundings with photosensitive molecules, termed “photoswitches”. The first category relies on photosensory domains derived from photosensitive proteins, often photoreceptors or fluorescent proteins. Photosensitive proteins are mostly modular, thus consisting of at least two domains: absorption of light induces conformational changes in the photosensory domain that are directly transduced to the effector domain, thereby changing its activity. This method is called optogenetics, as optically active domains are directly genetically fused to the protein sequence, producing a chimeric photosensitive protein. This method is straightforward, as no chemical modifications are needed, and the conformational changes are fully reversible. However, it is still difficult to predict the effect of domain fusion on the catalytic activity of the effector and to choose the appropriate type and site for domain fusion. A recent review described the advances in strategies for designing optogenetic tools with a focus on protein catalytic activity, including protein activation via dimerization or oligomerization, reconstitution of split proteins, regulation of protein accessibility, and other allosteric regulation.

In the past decade, a lot of biophysical, structural, and biochemical research has focused on the photosensitive light–oxygen–voltage (LOV) domain that detects blue light with a flavin cofactor. Even though the LOV domain and the bilin domain have been successfully employed with a number of different effector proteins, mainly to control cellular pathways, optogenetic design of a biocatalytically useful enzyme has not been reported, yet.

The second approach to introduce photosensitiveness in enzymes is through functionalization with molecular photoswitches or introduction of caged compounds in the system. Molecular photoswitches are a class of chemical structures that can readily isomerize between distinct geometries upon irradiation with light, a process known as “photoswitching”. A detailed review on the control of biological systems by molecular photoswitches has been published.

For efficient application, the photoswitching compounds need a fast switching process and large extinction coefficient with a high quantum yield. For this purpose, aromatic azobenzene molecules are the most studied photoswitches in biological applications. They are present as the cis (Z)- and trans (E)-isomers, which can interconvert both photochemi-
cally and thermally. Their conformational change upon light irradiation is fast and quite dramatic, as the isomerization from the extended trans to the bent cis form upon irradiation with UV light (320−350 nm) shortens the end-to-end length of the molecule by around 0.7 nm (Scheme 20A). The trans-isomer is easily recovered by irradiation with visible light of 400−450 nm or by thermal relaxation. These properties of azobenzene make it a crucial player in light-modulation research, and only in the last two years, several reviews were published focused on azobenzene modulators.190,191 Other known photoisomerizing molecules include stilbenes, spyropyrans, diarylethenes, thiophenfulgides, and hemithioindigos.189,192,193

As the UV light that is required for the photoswitching of azobenzene molecules is known to damage biological molecules, azobenzene compounds with bulky electron-rich substituents (Scheme 20B) were designed and effectively photoisomerized with red light (617 nm) and blue light (450 nm). The generality of these red-light photoswitches was confirmed with effects on the structure of a variety of internally cross-linked peptides.194 Because surface-exposed cysteines are often a target for covalent modifications of proteins, Cysteineome was created, a curated database of proteins with targetable cysteine residues along with covalent modulators, which facilitates identification of the most promising biocatalysts modified this way.195 In 2010, the first study was published where azobenzene derivatives were used to cross-link selected residues to modulate enzymatic activity.196 More than 30 variants of endonuclease PvuII were generated guided by the sGAL program, which is designed to find pairs of surface-exposed sites, suitable to introduce cysteine-mediated chemical cross-links into proteins.197 Variants with double cross-links showed a bigger difference in catalytic activity between the cis
and trans configurations of the photoswitch as opposed to variants with single cross-links. The effect was more pronounced on variants that carried the cross-links close to the catalytic center, and with additional amino acid substitutions, up to 16-fold relative change in activity could be accomplished. Mechanistic studies showed that the effect observed was mainly due to the differences in $V_{\text{max}}$ and not $K_{\text{m}}$.

A 3-fold fully reversible change of lipase activity on the hydrolysis of para-nitrophenyl palmitate was reported by cross-linking residues crucial for controlling the $\alpha$-helix structure of the lid domain with an azobenzene photoswitch (Scheme 20C).\textsuperscript{198}

In some cases, the protein-attached azobenzene does not need to act as a cross-link to affect an enzyme’s catalytic activity. A series of monofunctional azobenzene alkylmaleimides with varying spacer lengths were synthesized and attached through a single substitution close to the active site in bacterial histone deacetylase-like amidohydrolase. It was shown that the spacer length had a significant effect on photocatalysis: in one case, the cis conjugate was more active, in another, the trans, whereas the variants with the shortest spacer lengths led to a complete inhibition of the enzyme in both configurations.\textsuperscript{199} The cis state of the azobenzene group had an unusually long half-life of 30 h, and in a follow-up study, it was revealed that the state was stabilized by interactions with a histidine and a hydrophobic cluster on the protein surface.\textsuperscript{200} The improved stability allowed the photoswitch to be controlled by single light pulses rather than continuous illumination with UV light.

A structure-based computation-guided predictive model for the reversible control of enzyme activity using covalently attached cross-linked azobenzene groups was demonstrated by a 3-fold change in activity of a cytosine deaminase converting the prodrug 5-fluorocytosine to 5-fluourouracil (Scheme 20D).\textsuperscript{201} However, even though this method is quite general, introduction of a cysteine might not always be the best strategy, so new methodologies that target non-cysteine residues are being developed.\textsuperscript{202}

In some cases, photoswitchable azobenzenes even allow the control of the enzyme’s enantioselectivity. Covalent anchoring of azobenzene derivatives to residue 295 of the Lipase 2 from Bacillus thermodenaturans triggered a (slight) preference for the $S$-isomer under UV light and a (slight) preference for the $R$-isomer under visible light (Scheme 20E). This showcases a strategy for reversible and noninvasive modulation of biocatalytic properties in situ, which could be useful to control remotely a branching point in enzymatic cascades.\textsuperscript{203}

A tunable enzymatic–microbial hybrid fuel-cell was reported based on a surface-displayed, photoswitchable alcohol-dehydrogenase II (ADHII) from Zymomonas mobilis.\textsuperscript{204} Several mutants of ADHII were constructed by site-specific incorporation of the unnatural amino acid para-azido-l-phenylalanine (pAzF), which had around 80% lower enzyme activity compared to the wild-type. The BEPPY photoswitch [1-((E)-1,3-bis(4-((E)-phenyl-diazenyl)phenyl)triaz-2-en-1-yl)pent-4-yn-1-one; Scheme 20F] was incorporated in the protein by ligation to the azide of pAzF via a copper(I)-mediated alkyne–azide “click” cycloaddition reaction and allowed to control the access of the substrate to the active site. A UV-deactivated/visible-light-activated system based on a V66BEPPY mutant and a UV-activated/visible-light-deactivated system based on an L258BEPPY mutant was demonstrated, reaching an almost 5-fold observed change in the power output of the assembled microbial fuel-cell.

Several studies address the application of photoswitchable inhibitors that are designed by attaching a photosensitive unit such as azobenzene to a known reversible enzyme inhibitor. This allows for control of the steric of the inhibitor and thereby the access of the inhibitor to the binding site. In the past, photoswitchable inhibitors were most often studied with proteases as target enzymes.\textsuperscript{189,205} Recently, anchored photosensitive inhibitors that tether the inhibitor close to its active site were developed. A water-soluble adhesive photoswitch that selectively binds to a carbonyl anhydrase and photochemically modulates its activity has been described by attaching a sulphonamide inhibitory motif and a guanidium-based molecular glue unit that can adhere to the protein to the azobenzene. UV irradiation of this complex photoisomizes the azobenzene unit, resulting in undocking of the inhibitory motif from the active site (aided by the substrate), and visible light allows the inhibitor to access the active site again, restoring the enzyme’s intrinsic activity almost completely. It was found that the inhibition worked selectively even under physiological conditions in cell lysates, and it was suggested that, because of the cell-membrane permeability of the molecular glue, photochemical modulation of intracellular proteins might be possible.\textsuperscript{206} The same inhibitory motif attached to an azobenzene together with maleimide was used in design of three probes for classical conjugation with a cysteine residue (Scheme 20G). The probes differed in linker size between the azobenzene and maleimide groups and were screened in silico to identify the optimal sites from potential 250 residues for mutation into cysteine on human carbonic anhydrase II. Six combinations were evaluated experimentally; two of these resulted in light-responsive enzymes that showed 5.6- and 1.5-fold increases in activity in the presence of UV irradiation.\textsuperscript{207}

An alternative to the mentioned systems, an anthraquinone–enzyme–peptide hybrid was reported that allowed phototriggered self-destruction. The activity of this hybrid was comparable to the activity of the wild-type enzyme, but after 10 min of irradiation, it was irreversibly switched off due to anthraquinone-induced self-degradation.\textsuperscript{208}

A further alternative method is represented by photoresponsive polymer–enzyme switches, where photoinduced changes in the size and hydration of an azobenzene-derivatized dimethylacrylamide polymer can be used to regulate substrate access and enzyme activity of endoglucanase 12A.\textsuperscript{209} A similar polymer was used with heparinase III from Pedobacter heparinus, which was covalently linked to the azobenzene moiety of the polymer via a mutated cysteine residue (K130C). The enzyme was employed for degradation of heparin at a 10 mL scale, and it was shown to produce low-molecular-weight heparin with a lower and more uniform molecular weight compared to the wild-type enzyme. Activity was UV-induced by depolymerization and switched off by exposure to visible light and heating, which caused precipitation of the catalyst, facilitating product recovery.\textsuperscript{210}

Engineering photoresponsive environments that can photoswitch enzyme activity has the advantage of being generally applicable to enzymes no matter how much is known about their structure. A light-responsive cascade of two enzymes was reported by embedding glucose oxidase and hemin within an azobenzene-modified peptide-based matrix. Because aromatic stacking is the main driving force in the self-assembly of the
matrix, UV-induced photosomerization caused disintegration of the hydrogel within 12 h, causing a drop of 40% in activity. Visible irradiation for 1 day (24 h) restored the matrix structure and some catalytic activity, but the reversibility declined during repeated UV−vis switches, most likely because of the length of irradiation that was required.193

Controlled catalysis in light-responsive nested vesicle reactors was developed by incorporation of diacetylene functional groups in the lipids of the membrane of the inner vesicles. This was illustrated by separation of β-galactosidase and its nonfluorescent fluorogenic substrate in different compartments. Nanopore formation in the membrane of the inner compartment was induced by UV irradiation causing diacetylene polymerization, allowing the diffusion of the substrate, which exposed it to hydrolysis by the enzyme, generating the fluorescent fluorescein. Irradiation time modulated the extent of polymerization, controlling the substrate release rate and therefore the rate of the reaction. Because no enzyme modifications are involved in this method, it may be generally applied to many biocatalysts, especially for spatiotemporal control of cascade reactions.211

Optical switches have also been used to introduce a rapid shift in pH to control enzyme activity. Irradiation of 2-nitrobenzaldehyde causes a pH jump in the solution by release of caged protons. This is a good general strategy for control of enzymes with a significant pH profile, shown in an example of acid phosphatase whose relative activity was increased from 3 to 78% by a light-induced pH shift from pH 8 to 6.212 The same system has been applied for remote activation of oxidase-like activity of nanoyzmes.213 A dual photoreponsive system was designed for precise control of chemical reactions by optically controlling pH and temperature. Graphene oxide, which absorbs near-infrared and coverts it to thermal energy, was chosen as a thermoregulator, and malachite green carbinol base, which releases OH− under UV light, was chosen as a pH regulator. With dual control in near-infrared and UV, a broad range of temperatures and pHs could easily be adjusted to regulate the activity of a range of enzymes (acid phosphatase, alkaline phosphatase, cellulase, amylase). When light was removed, the system recovered to the original state and could be recycled many times.214

7. CONCLUSION AND OUTLOOK

Probably because of the rather small number of currently known catalytically active enzymes requiring light for the actual reaction (photosystem, photolyase, decarboxylation, pchlide-reductase), the number of concepts exploiting photo-chemo-catalytic reactions in combination with biocatalysis is greater than the number of example with photo-biocatalytic steps. Mostly the photo-chemocatalytic processes provide either the cofactor in the appropriate redox state or a redox cosubstrate or are coupled to the biocatalytic step in a linear sequence. When looking at enzymatic photoreactions, the photosystem is exploited to provide mainly reduced nicotinamide cofactor in phototrophic strains, for which the molecular engineering is still challenging. Using enzymes as a chiral environment for a photocatalytic reaction is starting to be investigated, exploiting the unique possibility to initiate single-electron-transfer reaction steps. Finally, photoswitchable catalysts have been investigated, which may be switched on or off, depending on the need.

From the studies described until now, it becomes clear that there are new opportunities but also various challenges such as mostly low TTNs and TOFs. Another challenge arises from the possible generation of strong oxidants and reactive free radicals (e.g., from oxygen) in the presence of light, which might lead either to an undesired side-reaction or damage of the enzyme. The latter may be solved by using living cells (e.g., cyanobacteria, algae) which allow repair of a defunctionalized photosystem. For this case, sufficient supply with CO2 has to be ensured for the cells. Introducing artificial photosynthesis in heterotrophic bacteria such as E. coli is a tempting thought. However, it should be noticed that bacteria such as cyanobacteria not only produce the photosynthetic machinery but also provide elaborate protection mechanisms against reactive oxygen species that are associated with catalytic water splitting. It remains to be investigated, to which extent these photosystem systems may be introduced successfully in other organisms.

A possible option to broaden the field of photo-biocatalysis is to incorporate non-native amino acids as new catalytic residues, enabling the use of non-native cofactors and thus showing new properties.215−218 This will allow insertion of light-activated or caged groups as well as non-native, photocaged amino acids into the enzymes leading to the development of new light-dependent reaction centers in enzymes and thus to new light-dependent biocatalysts, as the restriction to the natural amino acids is eliminated.219−221

An important point is the demonstration of the reaction on larger scale. Because most of the discussed reactions were shown on analytical scale, the next logical step is to prove their synthetic applicability in larger volumes and at higher concentrations. However, in contrast to classical biotransformations, which usually allow a straightforward volumetric upscaling, parameters such as the intensity of the light and, connected to that, the light’s penetration depth are crucial for photocatalysis.48 This may be addressed with new LED photoreactors,222 with flat panel photoreactors or flow systems.48 Alternatively, internal light sources such as WLE (wireless light emitters)223,224 or light guides (e.g., optical fibers) may be applied.225−227 Furthermore, neither of the mentioned systems allow the fast and reliable evaluation of different illumination and reaction conditions, as only a single sample can be tested at a time.49 Consequently, in order to push photo-biocatalysis to the next level, it is crucial to develop efficient photoreactors for large scale and to establish standardized small scale reactors for the reproducible parallel screening of different reaction conditions. Anyway, light can be considered as a unique environmentally compatible option to initiate reactions that are otherwise not feasible. Basic concepts have been developed but need to be extended, and novel reactions and concepts can be expected to come up.

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Notes
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