



HAL
open science

Exciting Enzymes: Current State and Future Perspective of Photobiocatalysis

Véronique Alphand, Willem J H van Berkel, Valentina Jurkaš, Selin Kara,
Robert Kourist, Wolfgang Kroutil, Francesco Mascia, Marc M Nowaczyk,
Caroline E Paul, Sandy Schmidt, et al.

► **To cite this version:**

Véronique Alphand, Willem J H van Berkel, Valentina Jurkaš, Selin Kara, Robert Kourist, et al..
Exciting Enzymes: Current State and Future Perspective of Photobiocatalysis. *ChemPhotoChem*,
2023, 7 (7), pp.e202200325. 10.1002/cptc.202200325 . hal-04495561

HAL Id: hal-04495561

<https://hal.science/hal-04495561>

Submitted on 8 Mar 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Exciting Enzymes: Current State and Future Perspective of Photobiocatalysis

Véronique Alphand,^[a] Willem J. H. van Berkel,^[b] Valentina Jurkaš,^[c] Selin Kara,^[d] Robert Kourist,^[e] Wolfgang Kroutil,^[c, f, g] Francesco Mascia,^[h, i] Marc M. Nowaczyk,^[j] Caroline E. Paul,^[k] Sandy Schmidt,^[l] Jelena Spasic,^[h, i] Paula Tamagnini,^[h, i] and Christoph K. Winkler^{*[c]}

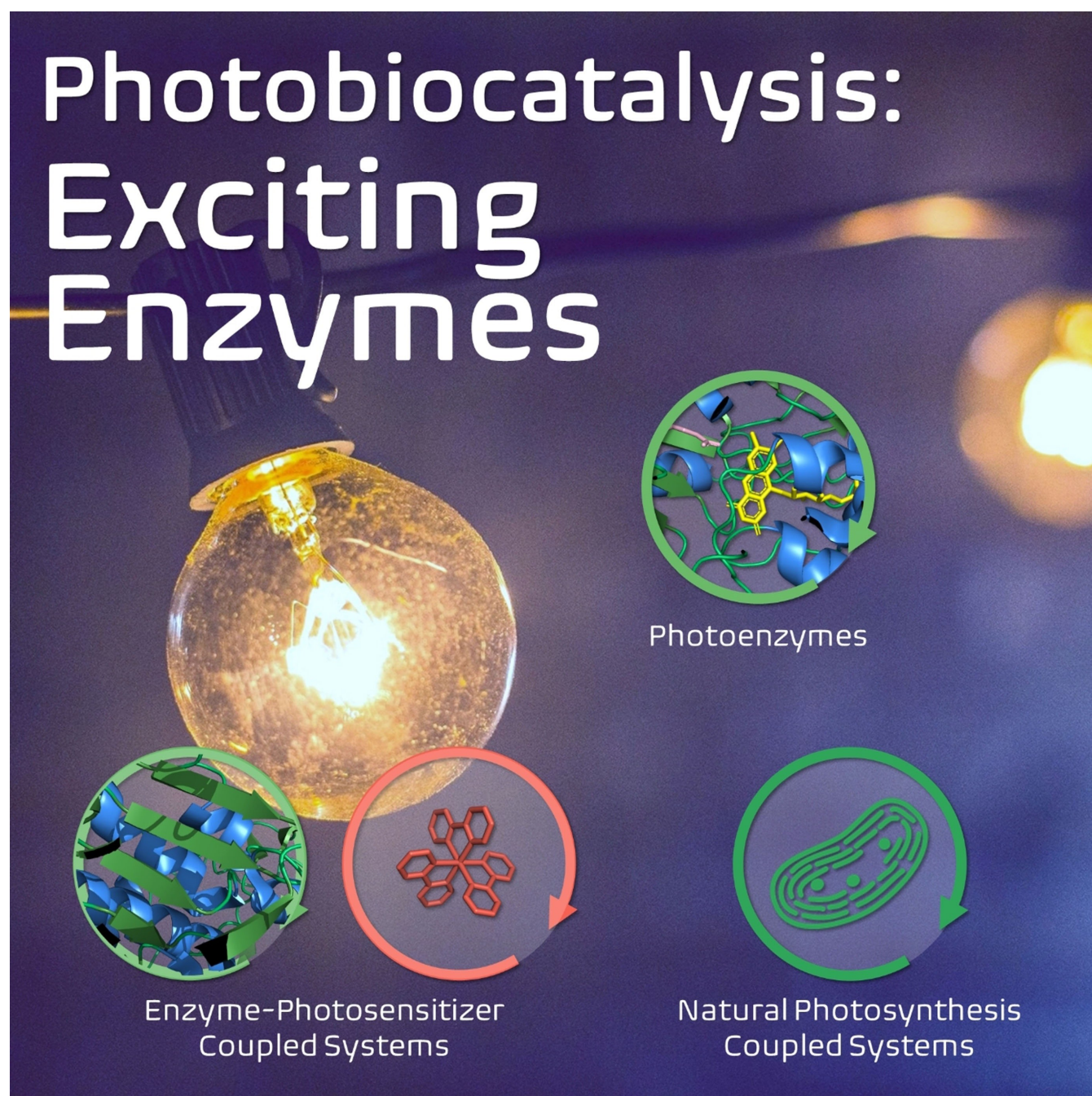


Image background by Pexels via Pixabay.

The recent increase of interest in photocatalysis spread to biocatalysis and triggered a rush for the development of light-dependent enzyme-mediated or enzyme-coupled processes. After several years of intense research on photobiocatalysis, it is time to evaluate the state of the field in a structured manner. In this Perspective, we suggest to group photobiocatalysis into distinct disciplines and provide principal guidelines and standards for the reporting of photobiocatalytic research results as well as advice on performing photobiocatalytic reactions. Over-

all, we assess that the field contributes to the diversity of biocatalytic reactions while offering the selectivity of enzymes to photocatalysis. We foresee that the ongoing excitement for light-dependent enzymatic processes will lead to the discovery of novel photobiocatalytic mechanisms to complement biocatalysis with new bond-forming reactions and will provide additional innovative strategies to utilize light as a possible benign energy source.

1. Introduction

Applying light-dependent processes in the context of biocatalysis holds the promise of updating the vast reaction scope of photocatalysis with the exquisite selectivities of enzymes, while utilizing light as benign energy source. Due to this motivation, photobiocatalysis has recently attracted considerable attention, as summarized in a number of review articles.^[1–3] Within the past years the authors of this contribution have collaborated

within the framework of the Marie Skłodowska-Curie Photo-BioCat training network to investigate key aspects of photobiocatalysis: the development of novel synthetically applicable light-dependent biocatalytic reactions, the investigation of biocatalytic applications of photosynthetic microorganisms and the establishment of strategies to run such processes in lab-scale and at larger scale.^[4] Within this Perspective, we want to summarize the experience that we gathered during the project, by evaluating the positioning of photobiocatalysis within the field of preparative biocatalysis, as well as by sharing the lessons learned and to provide an outlook towards future developments of photobiocatalysis.

There are three fundamentally different ways to utilize visible light for enzyme-catalyzed processes. Photoenzymes, which constitute the first discipline (Figure 1, PE), employ light as part of their mechanism. Apart from light-driven enzymatic processes involved in photosynthesis, natural light-dependent enzymes converting an organic substrate into a product are rare (Figure 1a). (i) Photolyases are involved in DNA repair where they resolve cyclobutene pyrimidine dimers formed during exposure to ultraviolet light.^[5–6] (ii) Protochlorophyllide oxidoreductases (LPORs) catalyze a light-dependent reduction during chlorophyll biosynthesis.^[7–8] (iii) Fatty acid photo-decarboxylases (FAPs) catalyze the cleavage of fatty acids and other carboxylic acids to produce the corresponding *n*-1 alkanes (Figure 1a).^[9] All these enzymes rely either on a chlorophyll-like tetrapyrrole or on a flavin cofactor as a chromophore.^[10] While the high specificity of photolyases and LPORs prevents their broad application in biocatalysis, FAPs have received considerable attention for the synthesis of biodiesel and high-value chemicals such as optically pure building blocks.^[11–15] The three truly photocatalytic enzymes (besides the enzymes involved in photosynthesis) are complemented by enzymes that show light-dependent, promiscuous reactivities (Figure 1b) and artificial photoenzymes (Figure 1c). The latter groups of enzymes remain largely unexplored but hold many opportunities for biocatalysis.^[16]

The second discipline consists of enzymes that are combined with photosensitizers in so-called enzyme-photocatalyst coupled systems (Figure 1, EPC). On the one hand, this approach exploits the diverse reactivity of chemical photocatalysis with the often-outstanding selectivity of enzymes, giving rise to highly interesting chemoenzymatic cascade reactions (Figure 1d).^[23] On the other hand, photocatalysis is used to provide reduction- or oxidation equivalents for

- [a] Dr. V. Alphand
Aix Marseille Univ., CNRS, Centrale Marseille
iSm2 UMR7313, 13397 Marseille (France)
- [b] Prof. Dr. W. J. H. van Berkel
Laboratory of Food Chemistry, Wageningen University
Bornse Weilanden 9, 6708 WG Wageningen (The Netherlands)
- [c] Dr. V. Jurkaš, Prof. Dr. W. Kroutil, Dr. C. K. Winkler
Institute of Chemistry, University of Graz
Heinrichstraße 28, 8010 Graz (Austria)
E-mail: christoph.winkler@uni-graz.at
Homepage: <http://biocatalysis.uni-graz.at/>
- [d] Prof. Dr. S. Kara
Department of Biological and Chemical Engineering
Biocatalysis and Bioprocessing Group, Aarhus University
Gustav Wiedes Vej 10, 8000 Aarhus (Denmark)
- [e] Prof. Dr. R. Kourist
Institute of Molecular Biotechnology, Graz University of Technology
Petersgasse 14, 8010 Graz (Austria)
- [f] Prof. Dr. W. Kroutil
Field of Excellence BioHealth, University of Graz
8010 Graz (Austria)
- [g] Prof. Dr. W. Kroutil
BioTechMed Graz
8010 Graz (Austria)
- [h] Dr. F. Mascia, Dr. J. Spasic, Prof. Dr. P. Tamagnini
i3S – Instituto de Investigação e Inovação em Saúde
Universidade do Porto & IBMC
Instituto de Biologia Molecular e Celular
R. Alfredo Allen 208, 4200-135 Porto (Portugal)
- [i] Dr. F. Mascia, Dr. J. Spasic, Prof. Dr. P. Tamagnini
Departamento de Biologia Faculdade de Ciências, Universidade do Porto
Rua do Campo Alegre, Edifício FC4, 4169-007 Porto (Portugal)
- [j] Prof. Dr. M. M. Nowaczyk
Department of Biochemistry, University of Rostock
Albert-Einstein-Str. 3, 18059 Rostock (Germany)
- [k] Prof. Dr. C. E. Paul
Department of Biotechnology, Delft University of Technology
Van der Maasweg 9, 2629 HZ Delft (The Netherlands)
- [l] Asst. Prof. Dr. S. Schmidt
Department of Chemical and Pharmaceutical Biology
Groningen Research Institute of Pharmacy, University of Groningen
Antonius Deusinglaan 1, 9713 AV Groningen (The Netherlands)

© 2023 The Authors. ChemPhotoChem published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

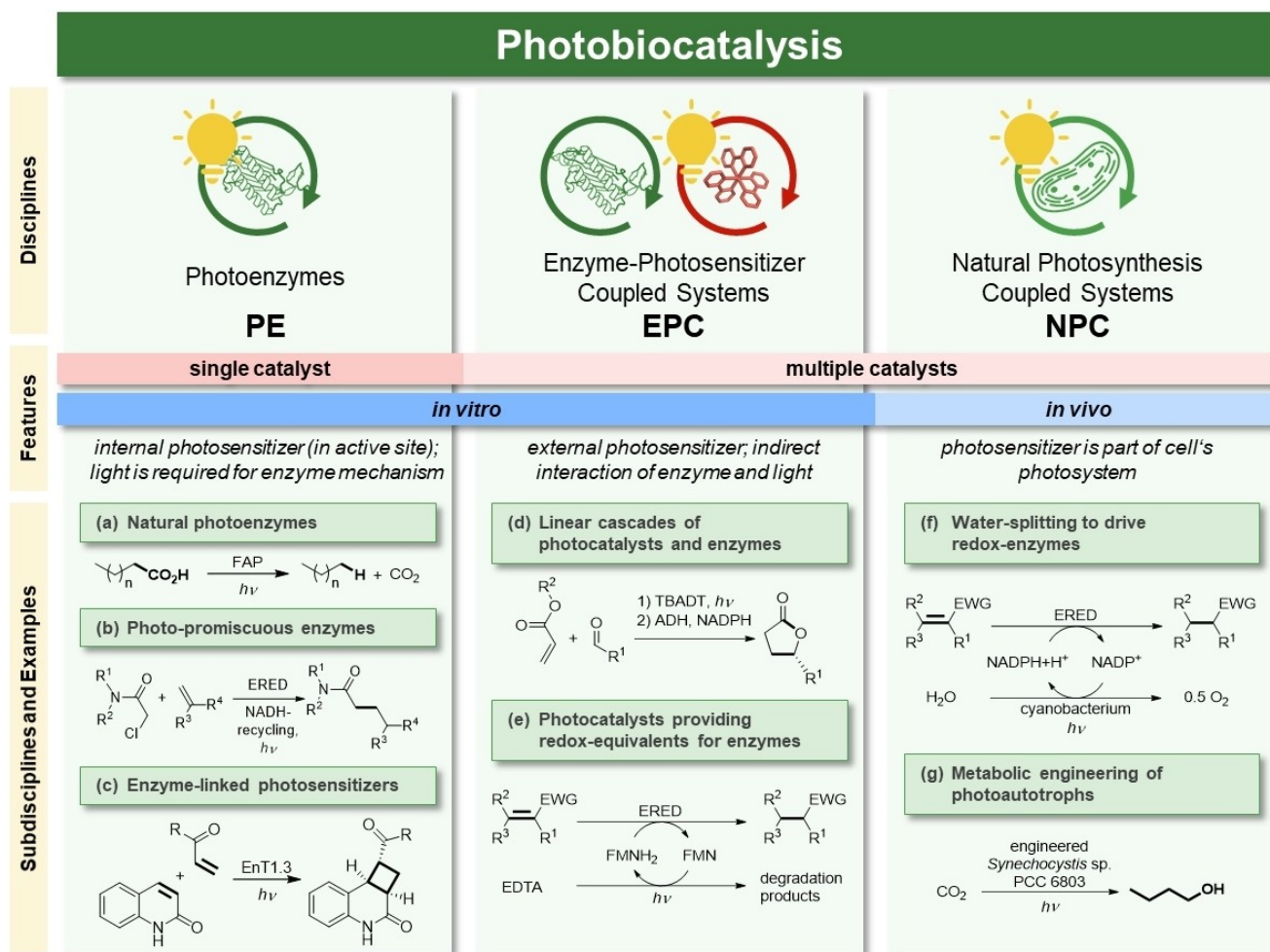


Figure 1. The three disciplines of photobiocatalysis: PE: photoenzymes, including (a) natural photoenzymes (e.g., FAP)^[9] as well as (b) photo-promiscuous enzymes (e.g., ERED catalyzed C–C bond formation)^[17] and (c) enzymes that are linked to a photosensitizer (e.g., [2 + 2] cycloaddition by an enzyme with a non-canonical amino acid),^[18] all requiring light in their mechanism. EPC: enzyme-photosensitizer coupled systems, that either (d) combine photocatalytic reactions with an enzyme in a synthetic cascade (e.g., photocatalytic C–C bond formation and biocatalytic keto-reduction),^[19] or (e) use external photosensitizers to provide redox equivalents for a biocatalyst (e.g., regeneration of FMNH₂ for EREDs).^[20] NPC: natural photosynthesis coupled systems, that utilize living photoautotrophic cells, either (f) to supply redox enzymes with cofactors, electrons or oxygen, based on photosynthetic water splitting (e.g., reduction of C=C bonds using EREDs),^[21] or (g) for their ability to produce chemicals from CO₂ and light (e.g., production of 1-butanol from CO₂).^[22] FAP: fatty acid photodecarboxylase; ERED: ene-reductase; EWG: electron-withdrawing group; TBADT: tetra-*n*-butylammonium decatungstate; ADH: alcohol dehydrogenase.

biocatalytic redox reactions and thus substitutes the organic auxiliary co-substrates that are currently employed for enzymatic cofactor recycling (Figure 1e).^[24–27] A noteworthy situation are processes that involve flavin. In case the photoactive flavin is bound in the active site, such processes belong to the discipline PE (e.g., Figure 1b). In this case, flavin acts as photocatalyst and the subsequent biocatalytic reaction is not light-dependent, the process constitutes a linear cascade and is part of the discipline EPC (e.g., isomerization of C=C-bonds followed by enzymatic reduction).^[28] Despite their elegance, most photocatalytic systems to provide enzymes with redox-equivalents rely on the oxidation of amines or buffer components, whereas the coupling of oxidoreductases to photocatalytic water oxidation *in vitro* is still challenging.^[29–30]

Finally, the third discipline utilizes natural photosynthesis (Figure 1, NPC).^[31–33] Water-splitting by photosystem II provides

electrons in the form of reduced nicotinamides (or other electron mediators), which can be used by oxidoreductases, as demonstrated on heterologous EREDs (ene-reductases) in 2016 (Figure 1f),^[21] and many more systems since.^[31–32] Engineering of the metabolism of photoautotrophic organisms allows to use CO₂, their primary carbon-source, as chemical building block, e.g., in the production of 1-butanol (Figure 1g). The recent expansion of the molecular toolbox for cyanobacteria has greatly facilitated the recombinant expression of the genes of oxidoreductases in different cyanobacterial species.^[32–33]

2. Challenges and Opportunities

All disciplines of photobiocatalysis are faced with a series of challenges that need to be addressed when a new process or

catalyst is developed. In addition, issues such as low catalytic turnover numbers, the need for high catalyst loadings, substrate and/or product inhibition or the mutual deactivation of catalysts and enzymes are not specific to photobiocatalysis but must be considered as they may occur in any (chemo)enzymatic reaction.

One key issue in photobiocatalysis is the well-documented photo-lability of photoenzymes and their cofactors (e.g., flavins) or even chemo-photocatalysts.^[34–36] For example, when working with FAPs (Figure 1a), the substrate must always be present, as it has a protective effect against self-attack of the excited cofactor onto residues in the active site.^[36–37] This is not required for LPOR, since in this case the substrate gets excited. A major hurdle for EPC-systems (Figure 1d) is the frequent incompatibility of the reaction conditions (e.g., solvents, wavelengths, temperatures, pH, concentrations) that are required by the applied chemo-photocatalysts and enzymes. Such issues may be overcome by finding an optimal trade-off or via an extended search for compatible catalysts.^[38] Furthermore, the combination of oxygen-dependent enzymes with photoredox-catalysts consistently raises the 'oxygen dilemma', as the photocatalyst is quenched by the oxygen that is required for the enzymatic reaction, i.e., the application of light leads to uncoupling.^[39] In addition, while great progress has been made in the engineering of cyanobacteria and other photoautotrophic organisms for NPC-systems, cloning and expression of enzymes (in particular heterologous) are still laborious and genetic stability remains an issue.^[32,40] Indeed, the complexity of the regulation of gene expression^[41] and the photosynthetic electron transport chain (PETC) needs to be fully understood and optimized for larger scales.^[42–44]

However, it must be emphasized that the above-mentioned challenges are mirrored by the manifold opportunities that the implementation of light into biocatalytic processes offers. (Visible) light is non-toxic, generates no waste, and is abundantly available as green energy-source.^[45] Besides this, one of the most intriguing opportunities that photobiocatalysis offers is its promise to enable new synthetic transformations, sometimes even new to nature.^[16] Hurdles of PEs have been overcome via engineering, e.g., the often restricted substrate and reaction scope of naturally occurring photoenzymes (Figure 1a),^[7,9,12] was successfully expanded by rational protein design^[13,46] or reaction engineering.^[47] The poor photostability of many photobiocatalytic systems however remains a problem, and solutions besides medium engineering must be found.^[37,48] Meanwhile, the light-dependent promiscuity of natural enzymes, especially EREDs was broadly explored, giving access to novel stereoselective C–C bond formation processes (Figure 1b).^[17,49–53] Directed evolution of the ERED biocatalyst allowed to improve the enzymes quantum yield.^[54]

Another way to realize new non-natural chemistry is the combination of photo- and biocatalysts, each contributing their strengths.^[16] This can be done either via the direct incorporation of photocatalysts in a protein scaffold (Figure 1c),^[16,18,55–58] or in so-called photochemo-enzymatic cascade reactions (EPC-systems; Figure 1d).^[1,19,23,59–60] As an example of the former case, recently, a triplet state energy transfer PE that performs [2 + 2]

cycloadditions was constructed by incorporation of a photoactive non-canonical amino acid into a protein scaffold,^[18] a development that raises the opportunity to transfer the vast reaction scope of triplet sensitization chemistry to biocatalysis. In this context, also the conjugation of photocatalysts to protein scaffolds via covalent bonds was successfully applied.^[16]

EPC-systems often combine photocatalytic bond-forming steps to provide a scaffold for a follow-up biocatalytic reaction (Figure 1d).^[19] Hyster and co-workers developed strategies via photoexcitation for forming carbon-centered radicals from organohalides and acetates, using flavin-dependent EREDs (Figure 2a),^[61] demonstrating the ability of this enzyme family to control the stereochemical outcome of radical reactions.^[62] This approach has been further expanded to an ERED-based photoenzymatic platform capable of harnessing nitrogen-centered radicals for C–N bond formation (Figure 2b).^[63]

Although the concept of using light and photocatalysts to harness new-to-nature chemistry from an existing and often engineered enzyme scaffold is a rather recent development in photobiocatalysis, we believe that it will open up a new era in sustainable organic synthesis.^[1,16,61,64]

Next to using light for catalysis, various strategies have been developed to efficiently light-regulate enzyme activity using natural photoreceptors or optochemical tools, that are promising for the application in biocatalysis. While external stimuli, such as light, have long been known to be suitable to change the activity of 'switchable' enzymes,^[65–66] applying light regulation to biocatalytic problems, such as controlling cross-reactivities in cascade reactions^[67] or selectivity^[68] offers a new approach for realizing complex (multistep) chemical reactions. Applying light to enzyme regulation might even allow for controlling the activity of each enzyme in the reaction independently from each other, therewith further highlighting the future potential of photobiocatalysis. Note that this approach mimics natural light-activated enzymes, such as the blue-light activated histidine kinases.^[69]

In a different approach, the energy of light is made available for biocatalysis by whole-cell autotrophic cells,^[32]

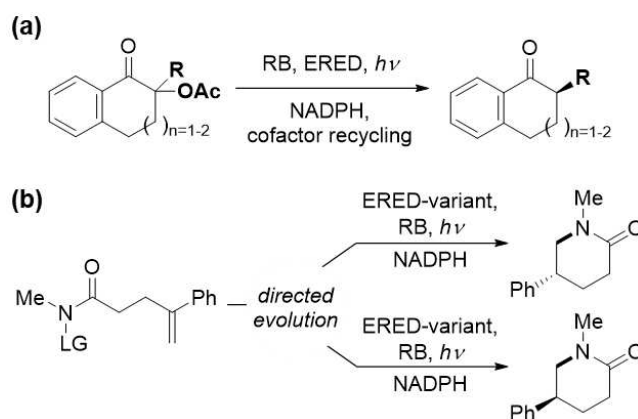


Figure 2. EPC-systems using enzymes for asymmetric radical reactions. (a) EREDs catalyzing the deacetoxylation of α -acetoxyketones by using photoexcited RB^[53] and (b) photoenzymatic platform enabling radical C–N bond formations.^[63] RB: rose bengal; LG: leaving group.

providing reducing equivalents in the form of NAD(P)H^[21] or reduced ferredoxins,^[41] without the need to oxidize organic auxiliary substrates (NPC-systems). Note that this contributes to an enhanced atom economy, as water is the only consumed reagent. Besides EREDs that served as proof-of-concept (Figure 1f),^[21,43] the versatility of light-driven whole-cell biotransformations was demonstrated on alcohol dehydrogenases,^[70–71] imine reductases,^[72] heme-independent monooxygenases^[73] and cytochrome P450 monooxygenases (MOs).^[41] Notably, in case the target enzyme is a monooxygenase, both products of photosynthesis, NADPH and oxygen are utilized. Using *Synechocystis* sp. PCC6803 as host for a Baeyer–Villiger monooxygenase (BVMO), high activities of up to 25 U_{g_{CDW}⁻¹ were reached (Figure 3a).^[42,74] The biocatalytic application of cytochrome P450 MOs usually requires the provision of additional enzymes as redox partners. Within cyanobacteria, endogenous ferredoxins that are linked to the photosynthetic electron transport chain can be utilized for this task (Figure 3b).^[41]}

To circumvent the challenging genetic manipulation of cyanobacteria, efforts to shuttle the electrons for cofactor recycling to the extracellular space headed the development of the coupling of formate dehydrogenase to formate-exporting cells of the microalgae *Chlamydomonas reinhardtii*,^[75] and, quite recently, employing acetone/isopropanol as mediators for the export of reducing equivalents from the cyanobacterium *Synechococcus elongatus* PCC 7942 to extracellularly allocated oxidoreductases.^[76]

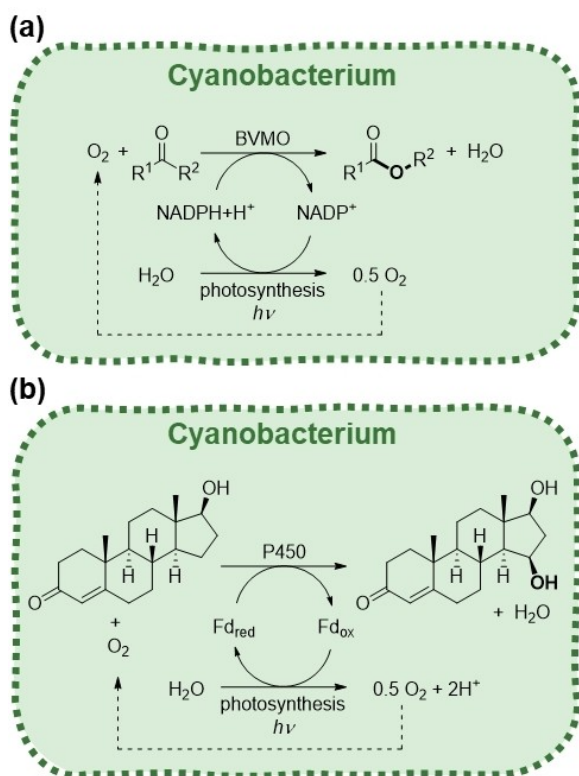


Figure 3. The application of cyanobacterial cells (*Synechocystis* sp. PCC 6803) for the synthesis of (a) lactones and (b) 15β-hydroxytestosterone.^[41–42,74]

To investigate all these opportunities of photobiocatalysis, one crucial challenge remains. Being a comparably young discipline, well-characterized illumination equipment or photobioreactors are not broadly available^[77] and the community still needs to define a good practice regarding the reporting of all experimental data that is required for the reproduction of photo(bio)catalytic reactions (see next sections).^[78–79]

3. Performing Photobiocatalytic Reactions

Photobiocatalysis needs to move beyond proof-of-concept experiments and towards more robust scalable systems. The particularity of photochemical reactions is that they need to be supplied with light, preferably under well-characterized and reproducible conditions.^[27] Therefore, the first consideration in the planning of a light-dependent reaction must be the way of delivering uniform, controlled illumination to all parts of the reaction vessel. As light is most often provided externally, reactors with a greater surface-to-volume ratio enable more homogenous light-distribution and deeper light penetration.^[77] This can be enhanced using internal light sources (e.g., wireless light emitters).^[80–82] The light source can be either sunlight or artificial light (tungsten lamps, fluorescent lamps, lasers or light emitting diodes – LEDs).^[77] Artificial light sources enable tighter control of light intensity to tune the reactivity while avoiding effects such as light-induced degeneration of cofactors, photosensitizers or the enzyme.^[36,43]

An increasing number of commercial photoreactors are available, but they often come with high price tags or low throughputs.^[83] For this reason, many research groups turn to custom solutions, however, the illumination conditions of any reactor must be well characterized, in order to guarantee replicability and reproducibility on other equipment. As the number of photons at a specific location in a reactor decreases exponentially with the distance from the light source, photo(bio)catalytic reactions are notably difficult to scale in batch. Application of flow technology enables uniform illumination, regardless of the reactor scale.^[84] While the transfer of batch protocols to flow brings its own challenges,^[85] flow methods are likely to find applications in photobiocatalysis.^[86–88]

As for any biocatalytic transformation, thoroughly characterized and tailored reaction parameters are required, including controlled temperature, agitation, light wavelength, and intensity, and both time of reaction and illumination. Classically, reaction engineering is approached via parallel screening of the different reaction conditions, which is enabled by photoreactors that can handle several reactions in parallel, e.g., at analytical scale,^[83] or in a 96 well-plate format.^[54]

Notably, the light intensity in photobiocatalysis must be treated differently than in traditional photochemistry where the catalyst amount is often increased to concentrations that allow full absorption of the supplied irradiation, i.e., not wasting any photon. This leads to high apparent quantum yields and an efficient utilization of the supplied light (note the difference to quantum yield, which is independent of the supplied irradiation

power). In contrast, biocatalysis operates at comparably low catalyst concentrations (usually well below 0.1 mol%). This leads to absorption of a smaller fraction of the supplied light and to lower apparent quantum yields ($\leq 1\%$). Therefore, instead of a high catalyst concentration, often a large excess (100-fold) of photons is applied to keep the majority of the chromophore at the excited state, i.e., not wasting any molecule of catalyst.

Finally, reaction temperature is also an important factor that needs to be considered, especially as it may increase due to the heat produced by the light source.

4. Standardized Reporting of Photobiocatalytic Reactions

Novel fields of research, such as photobiocatalysis, are often investigated by only a hand-full of laboratories, using their own in-house solutions to run and investigate reactions, which often raises replicability and reproducibility issues for other researchers. As the field matures to broader application, it is therefore necessary to establish standards for reporting the technical and chemical details of photobiocatalytic reactions. As such guidelines proved to be important for photocatalysis^[78–79] and biocatalysis,^[89] herein we highlight the parameters that are of special interest for photobiocatalysis (Figure 4).

4.1. Light source emission spectrum and intensity

Photons are the energy input that drive photochemical processes, and the rate of such reactions strongly correlates with the number of absorbed photons.^[78–79] For this reason, it is essential to report the provided amount of light (photon flux), e.g., by determining it via chemical actinometry.^[90] Unspecific, not-quantifiable terms such as “blue light” should be avoided and either the emission spectrum or at least the peak wavelength (or mean emission wavelength) and the spectrum half width (width of the emission spectrum at half intensity) must be reported. Specifications from commercial providers should be mentioned.

4.2. Absorption spectrum of the photo(bio)catalyst

Not every photon hitting the reaction solution is absorbed, and not every absorbed photon leads to catalysis. Therefore, the extinction coefficient, and the absorption spectrum of the photochemically active species (e.g., enzyme-cofactor complex, charge-transfer complex or photocatalyst) at the illumination wavelength/spectrum must be reported.^[78–79]

4.3. Reactor geometry

As the light intensity in the reaction vessel decreases with an increasing distance to the light source, the minimal information that must be reported is the exact distance of the two.^[78–79] The

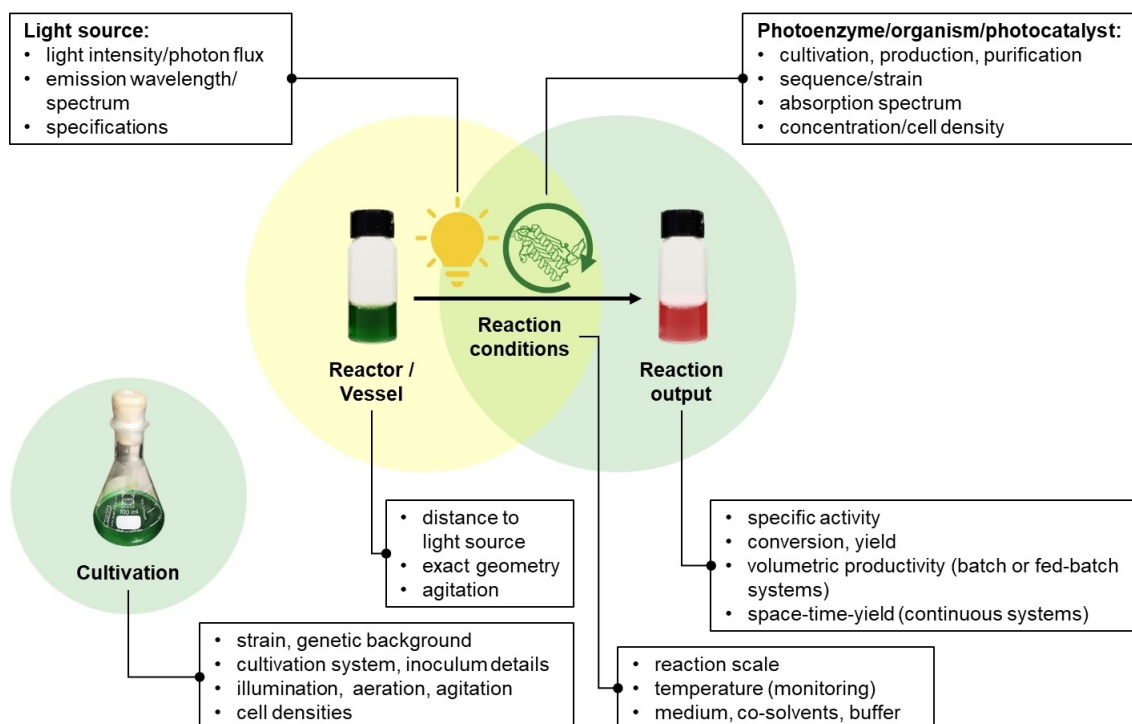


Figure 4. Key information required for reporting photobiocatalytic reactions.

influence of the reaction vessels shape on the outcome of biocatalytic reactions is well established,^[89] and therefore, ideally the exact reactor geometry, as well as details regarding stirring or shaking should be reported. Applied filters, the type of illumination (internal or external) and specifications from commercial providers should be mentioned.^[77]

4.4. Agitation

In line with the requirement for details on the reaction vessel, also information regarding the form of agitation (i.e., stirring or shaking) must be reported. This includes the stirring/shaking form, speed and/or shaking thrust.^[77,89]

4.5. Reaction temperature

Temperature is a crucial reaction parameter, especially for enzymatic reactions, that is often overlooked.^[89] Due to the fact that the light source might also significantly heat the reaction vessel, a careful monitoring and control of the reaction temperature is important for photobiocatalytic reactions. Photobioreactors that are well-agitated will lead to uniform temperatures in the reactor vessel.

4.6. Details on photoenzymes (PE)

As it should be a standard in biocatalysis, details regarding the production (cloning, expression, purification, and characterization) of the enzyme must be reported.^[80] This includes the supplied catalysts DNA sequence, genetic construct and database accession number (e.g., UniProtKB).

4.7. Details on living cells as photocatalysts (NPC-systems)

Like for any whole-cell biotransformation, the correct identification of the strains/sub-strains and their genetic background is of uttermost importance, as well as the description of the modules utilized for their genetic engineering.^[80] This should include information about the ORFs (open reading frames; e.g., sequence, database accession number, source organism, IUBMB EC number, protein tag, codon optimization) and details of the regulatory elements used (promoter, RBS, terminator, and any additional elements). Since genetic instability might also be an important issue, the doubling time should be noted. The transformation method to obtain engineered strains should be described and stated, as well as if the modules are on a self-replicative plasmid or integrated into the genome.^[42–43,71] As for the *in vitro* reactions, the cultivation parameters should be accurately described, especially including the light source,^[82] spectrum/wavelength, intensity and regimen,^[78] detailed media composition and incubation temperatures. The bioreactor geometry and characteristics (e.g., open or closed), stirring, and aeration (mode of supply, flow and identity of the supplied

gas) should be stated as well.^[73] For inducible systems, inducer, inducer concentration, and induction and collection time points should be mentioned. In addition, the pre-inoculum/inoculum details (e.g., cell densities) are important to be reported.

4.8. Further reaction conditions

Important information on the reaction conditions of photobiotransformations include cell density (OD₇₃₀, chlorophyll and/or cell dry weight), physiological state (exponential- or stationary phase, proliferating cells, resting cells) and form (free or immobilized) in case whole photoautotrophic cells are used, or the catalyst concentration and form (whole cells, cell-free extracts, purified or immobilized enzyme) in case a photoenzyme is applied.^[80] Furthermore, the reaction scale (volume, substrate and product concentration)^[91] and the reaction temperature, pressure, medium and applied co-solvents^[92] should be reported. Especially when working with whole-cells, toxicity issues caused by substrates or products should be reported together with potential side reactions, including those arising from the native metabolism of the whole-cell biocatalyst.^[72]

4.9. Performance indicators

Finally, standards for performance indicators of the reaction output are required. As a minimum, parameters such as the specific activity of whole-cell preparations (normalized per [mg of Chl *a*] or [g of CDW]), conversion and product yield must be communicated. The volumetric productivity should be reported for batch or fed-batch systems and space-time-yield for continuous systems. Concentrations of starting material and products should be measured, and the used calibration curves should be provided.

4.10. Negative results

As we believe that photobiocatalysis will contribute to the future of sustainable organic synthesis, it is unavoidably important to facilitate this progress by also sharing negative results with the photobiocatalysis community so that energy, time, and resources are saved.

Note, that reporting data following the FAIR principles (findable, accessible, interoperable, reusable) will allow its easy access and efficient fully automated reuse.^[79]

5. Outlook of Using Light in Enzymatic Systems

Each discipline of photobiocatalysis offers opportunities with a varied degree of associated challenges. Although the number of known natural PEs (Figure 1a) is low at the moment, the recent discovery of a new PE-class, namely FAPs^[9,93–94] indicates that there might be more to come, especially as natural light-

dependent enzymes were not in the focus of research until recently. Such new enzymes would again trigger an avalanche of opportunities. In this context, the exploitation of natural and non-natural photoactive cofactors within enzymes may provide even more options to explore.

From a synthetic point of view, using biocatalysts is most beneficial when outstanding stereo- and regioselectivity are required.^[95–96] Asymmetric organocatalysis and metal-organocatalysis involved in photoreactions,^[97–98] either linked to an enzyme (Figure 1c, PE) or in a chemoenzymatic cascade (Figure 1d, EPC), may here stimulate the creation of further hybrid catalysts, as exemplified by the incorporation of a photosensitizer as a non-natural amino acid in a protein.^[18] Following such examples, chiral as well as achiral organo- and metal-organocatalysts can be linked to protein backbones generating novel promising catalysts for asymmetric syntheses. Current limitations of the quantum yield and narrow absorption spectra of photoenzymes will be improved using antennae complexes for light harvesting.^[99–101]

The possibilities that originate from the combination of photocatalytic reactions (natural and chemical) with further biocatalytic steps (EPC-systems, Figure 1d) can be easily extended far beyond what is described today. Challenges remain when the reactivities triggered by light, e.g., the generation of excited oxygen, interferes with other reactions in the cascade, or light induces destruction of the applied enzymes. Better knowledge of the mechanisms of light dependent damaging of the enzyme will help to tackle this issue.^[102]

The coupling of natural photosynthesis to redox enzymes (NPC-systems, Figure 1f) for cofactor recycling bears the potential to be further improved in efficiency by engineering of the whole-cell catalysts, e.g., by the deletion of genes encoding for competing electron sinks.^[43–44] These findings will also stimulate the coupling of natural photosynthesis with intracellular cascades for accessing molecules of interest directly from CO₂ and light.^[103–104]

As in photochemistry in general, reactions on larger scale need special attention. In this regard, performing photobiocatalysis is a promising option and the current first examples will be complemented by further applications.^[84–88]

6. Conclusion

Photobiocatalysis started with the excitement that is inherent to new scientific topics. Now, after a few years of studies, the conclusion is clear: Photobiocatalysis has come to stay. While biocatalysis and biotechnology benefit from the expanded scope of chemical transformations that photocatalysis contributes, performing photochemistry in the active sites of enzymes benefits from the high selectivity that is enabled by the defined environment (shape and electronics) within the biocatalyst.^[1–3] Today, new engineering methods and the continuously growing experience with organisms that perform photosynthesis allow their genetic manipulation and the development of strains that couple biocatalysis with natural photo-

synthesis.^[31–33] Modern biocatalytic and biochemical methods such as bioconjugation,^[16,97] the use of non-canonical amino acids,^[18,58] and directed evolution^[54] contribute strongly to the development of novel and versatile photobiocatalytic processes and only a small fraction of the available reaction space has been investigated yet.^[16] The open space left is continuously populated with an increasing number of biochemically and synthetically useful processes. However, clear standards for the reporting of photobiocatalytic experiments^[78–79,89] and access to well-characterized affordable illumination equipment^[77] are required for a broad application of photobiocatalysis. All these developments underline the expectation that light-dependent enzymatic processes will become a standard tool in biocatalytic laboratories.

Disclaimer

The opinions expressed in this publication are the view of the authors and do not necessarily reflect the opinions or views of *ChemPhotoChem*, the Publisher, Chemistry Europe, or the affiliated editors.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 764920. The University of Graz and the Field of Excellence BioHealth are acknowledged for financial support.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: photocatalysis · biocatalysis · photoenzymes · photoautotrophic organisms · reaction engineering · cascade reactions

- [1] Y. Z. Peng, Z. C. Chen, J. Xu, Q. Wu, *Org. Process Res. Dev.* **2022**, *26*, 1900–1913.
- [2] C. J. Seel, T. Gulder, *ChemBioChem* **2019**, *20*, 1871–1897.
- [3] L. Schmermund, V. Jurkas, F. F. Ozgen, G. D. Barone, H. C. Büchschütz, C. K. Winkler, S. Schmidt, R. Kourist, W. Kroutil, *ACS Catal.* **2019**, *9*, 4115–4144.
- [4] Marie Skłodowska-Curie grant agreement No. 764920, DOI: 10.3030/764920.
- [5] Z. Liu, L. Wang, D. Zhong, *Phys. Chem. Chem. Phys.* **2015**, *17*, 11933–11949.
- [6] J. I. Lucas-Lledo, M. Lynch, *Mol. Biol. Evol.* **2009**, *26*, 1143–1153.

- [7] L. Schmermund, S. Bierbaumer, V. K. Schein, C. K. Winkler, S. Kara, W. Kroutil, *ChemCatChem* **2020**, *12*, 4044–4051.
- [8] C. Reinbothe, M. E. Bakkouri, F. Buhr, N. Muraki, J. Nomata, G. Kurisu, Y. Fujita, S. Reinbothe, *Trends Plant Sci.* **2010**, *15*, 614–624.
- [9] D. Sorigue, B. Legeret, S. Cuine, S. Blangy, S. Moulin, E. Billon, P. Richaud, S. Brugiere, Y. Coute, D. Nurizzo, P. Muller, K. Brettel, D. Pignol, P. Arnoux, Y. Li-Beisson, G. Peltier, F. Beisson, *Science* **2017**, *357*, 903–907.
- [10] A. Taylor, D. J. Heyes, N. S. Scrutton, *Curr. Opin. Struct. Biol.* **2022**, *77*, 102491.
- [11] W. Zhang, J. H. Lee, S. H. H. Younes, F. Tonin, P. L. Hagedoorn, H. Pichler, Y. Baeg, J. B. Park, R. Kourist, F. Hollmann, *Nat. Commun.* **2020**, *11*, 2258.
- [12] M. M. E. Huijbers, W. Zhang, F. Tonin, F. Hollmann, *Angew. Chem. Int. Ed.* **2018**, *57*, 13648–13651; *Angew. Chem.* **2018**, *130*, 13836–13839.
- [13] J. Xu, Y. Hu, J. Fan, M. Arkin, D. Li, Y. Peng, W. Xu, X. Lin, Q. Wu, *Angew. Chem. Int. Ed.* **2019**, *58*, 8474–8478; *Angew. Chem.* **2019**, *131*, 8562–8566.
- [14] T. M. Hedison, D. J. Heyes, N. S. Scrutton, *Curr. Res. Chem. Biol.* **2022**, *2*, 100017.
- [15] R. Wu, X. Li, L. Wang, D. Zhong, *Angew. Chem. Int. Ed.* **2022**, *61*, e202209180.
- [16] W. Harrison, X. Huang, H. Zhao, *Acc. Chem. Res.* **2022**, *55*, 355–359.
- [17] C. G. Page, S. J. Cooper, J. S. DeHovitz, D. G. Oblinsky, K. F. Biegasiewicz, A. H. Antropow, K. W. Armbrust, J. M. Ellis, L. G. Hamann, E. J. Horn, K. M. Oberg, G. D. Scholes, T. K. Hyster, *J. Am. Chem. Soc.* **2021**, *143*, 97–102.
- [18] J. S. Trimble, R. Crawshaw, F. J. Hardy, C. W. Levy, M. J. B. Brown, D. E. Fuerst, D. J. Heyes, R. Obexer, A. P. Green, *Nature* **2022**, *611*, 709–714.
- [19] F. F. Özgen, A. Jorea, L. Capaldo, R. Kourist, D. Ravelli, S. Schmidt, *ChemCatChem* **2022**, *14*, e202200855.
- [20] M. M. Grau, J. C. van der Toorn, L. G. Otten, P. Macheroux, A. Taglieber, F. E. Zilly, I. W. C. E. Arends, F. Hollmann, *Adv. Synth. Catal.* **2009**, *351*, 3279–3286.
- [21] K. Koninger, A. Gomez Baraibar, C. Mugge, C. E. Paul, F. Hollmann, M. M. Nowaczyk, R. Kourist, *Angew. Chem. Int. Ed.* **2016**, *55*, 5582–5585; *Angew. Chem.* **2016**, *128*, 5672–5675.
- [22] X. F. Liu, R. Miao, P. Lindberg, P. Lindblad, *Energy Environ. Sci.* **2019**, *12*, 2765–2777.
- [23] F. F. Ozgen, M. E. Runda, S. Schmidt, *ChemBioChem* **2021**, *22*, 790–806.
- [24] N. Yang, Y. Tian, M. Zhang, X. Peng, F. Li, J. Li, Y. Li, B. Fan, F. Wang, H. Song, *Biotechnol. Adv.* **2022**, *54*, 107808.
- [25] E. H. Edwards, K. L. Bren, *Biotechnol. Appl. Biochem.* **2020**, *67*, 463–483.
- [26] J. Kim, C. B. Park, *Curr. Opin. Chem. Biol.* **2019**, *49*, 122–129.
- [27] S. H. Lee, D. S. Choi, S. K. Kuk, C. B. Park, *Angew. Chem. Int. Ed.* **2018**, *57*, 7958–7985; *Angew. Chem.* **2018**, *130*, 8086–8116.
- [28] Z. C. Litman, Y. Wang, H. Zhao, J. F. Hartwig, *Nature* **2018**, *560*, 355–359.
- [29] A. Bachmeier, B. J. Murphy, F. A. Armstrong, *J. Am. Chem. Soc.* **2014**, *136*, 12876–12879.
- [30] M. Mifsud, S. Gargiulo, S. Iborra, I. W. C. E. Arends, F. Hollmann, A. Corma, *Nat. Commun.* **2014**, *5*, 1–6.
- [31] L. Malihan-Yap, H. C. Grimm, R. Kourist, *Chem. Ing. Tech.* **2022**, *94*, 1628–1644.
- [32] J. Jodlbauer, T. Rohr, O. Spadiut, M. D. Mihovilovic, F. Rudroff, *Trends Biotechnol.* **2021**, *39*, 875–889.
- [33] D. Jaiswal, D. Sahasrabudhe, P. P. Wangikar, *Curr. Opin. Biotechnol.* **2021**, *73*, 314–322.
- [34] B. König, S. Kümmel, E. Svobodová, R. Cibulka, *Phys. Sci. Rev.* **2018**, *3*, 15.
- [35] J. A. Macia-Agullo, A. Corma, H. Garcia, *Chem. Eur. J.* **2015**, *21*, 10940–10959.
- [36] B. Lakavath, T. M. Hedison, D. J. Heyes, M. Shanmugam, M. Sakuma, R. Hoeven, V. Tilakaratna, N. S. Scrutton, *Anal. Biochem.* **2020**, *600*, 113749.
- [37] Y. Wu, C. E. Paul, F. Hollmann, *ChemBioChem* **2021**, *22*, 2420–2423.
- [38] S. Bierbaumer, L. Schmermund, A. List, C. K. Winkler, S. M. Glueck, W. Kroutil, *Angew. Chem. Int. Ed.* **2022**, *61*, e202117103.
- [39] D. Holtmann, F. Hollmann, *ChemBioChem* **2016**, *17*, 1391–1398.
- [40] A. Sengupta, H. B. Pakrasi, P. P. Wangikar, *Appl. Microbiol. Biotechnol.* **2018**, *102*, 5457–5471.
- [41] F. Mascia, S. B. Pereira, C. C. Pacheco, P. Oliveira, J. Solarczek, A. Schallmeyer, R. Kourist, V. Alphand, P. Tamagnini, *Green Chem.* **2022**, *24*, 6156–6167.
- [42] E. Erdem, L. Malihan-Yap, L. Assil-Companioni, H. Grimm, G. D. Barone, C. Serveau-Avesque, A. Amouric, K. Duquesne, V. de Berardinis, Y. Allahverdiyeva, V. Alphand, R. Kourist, *ACS Catal.* **2022**, *12*, 66–72.
- [43] L. Assil-Companioni, H. C. Büchenschütz, D. Solymosi, N. G. Dyczmons-Nowaczyk, K. K. F. Bauer, S. Wallner, P. Macheroux, Y. Allahverdiyeva, M. M. Nowaczyk, R. Kourist, *ACS Catal.* **2020**, *10*, 11864–11877.
- [44] J. Spasic, P. Oliveira, C. Pacheco, R. Kourist, P. Tamagnini, *J. Biotechnol.* **2022**, *360*, 152–159.
- [45] T. P. Yoon, M. A. Ischay, J. Du, *Nat. Chem.* **2010**, *2*, 527–532.
- [46] J. Xu, J. Fan, Y. Lou, W. Xu, Z. Wang, D. Li, H. Zhou, X. Lin, Q. Wu, *Nat. Commun.* **2021**, *12*, 3983.
- [47] W. Zhang, M. Ma, M. M. Huijbers, G. A. Filonenko, E. A. Pidko, M. van Schie, S. de Boer, B. O. Burek, J. Z. Bloh, W. J. van Berkel, *J. Am. Chem. Soc.* **2019**, *141*, 3116–3120.
- [48] L. C. P. Goncalves, H. R. Mansouri, E. L. Bastos, M. Abdellah, B. S. Fadiga, J. Sa, F. Rudroff, M. D. Mihovilovic, *Catal. Sci. Technol.* **2019**, *9*, 1365–1371.
- [49] K. F. Biegasiewicz, S. J. Cooper, X. Gao, D. G. Oblinsky, J. H. Kim, S. E. Garfinkle, L. A. Joyce, B. A. Sandoval, G. D. Scholes, T. K. Hyster, *Science* **2019**, *364*, 1166–1169.
- [50] X. Huang, B. Wang, Y. Wang, G. Jiang, J. Feng, H. Zhao, *Nature* **2020**, *584*, 69–74.
- [51] H. Fu, J. Cao, T. Qiao, Y. Qi, S. J. Charnock, S. Garfinkle, T. K. Hyster, *Nature* **2022**, *610*, 302–307.
- [52] M. A. Emmanuel, N. R. Greenberg, D. G. Oblinsky, T. K. Hyster, *Nature* **2016**, *540*, 414–417.
- [53] K. F. Biegasiewicz, S. J. Cooper, M. A. Emmanuel, D. C. Miller, T. K. Hyster, *Nat. Chem.* **2018**, *10*, 770–775.
- [54] B. T. Nicholls, D. G. Oblinsky, S. I. Kurtoic, D. Grosheva, Y. Ye, G. D. Scholes, T. K. Hyster, *Angew. Chem. Int. Ed.* **2022**, *61*, e202113842.
- [55] X. Liu, F. Kang, C. Hu, L. Wang, Z. Xu, D. Zheng, W. Gong, Y. Lu, Y. Ma, J. Wang, *Nat. Chem.* **2018**, *10*, 1201–1206.
- [56] F. Kang, L. Yu, Y. Xia, M. Yu, L. Xia, Y. Wang, L. Yang, T. Wang, W. Gong, C. Tian, X. Liu, J. Wang, *ACS Catal.* **2021**, *11*, 5628–5635.
- [57] T. D. Schwochert, C. L. Cruz, J. W. Watters, E. W. Reynolds, D. A. Nicewicz, E. M. Brustad, *ChemBioChem* **2020**, *21*, 3146–3150.
- [58] Y. Fu, J. Huang, Y. Wu, X. Liu, F. Zhong, J. Wang, *J. Am. Chem. Soc.* **2021**, *143*, 617–622.
- [59] W. Zhang, E. F. Fueyo, F. Hollmann, L. L. Martin, M. Pesic, R. Wardenga, M. Hohne, S. Schmidt, *Eur. J. Org. Chem.* **2019**, *2019*, 80–84.
- [60] X. Guo, Y. Okamoto, M. R. Schreier, T. R. Ward, O. S. Wenger, *Chem. Sci.* **2018**, *9*, 5052–5056.
- [61] T. K. Hyster, *Synlett* **2019**, *31*, 248–254.
- [62] D. Grosheva, T. K. Hyster, in *Flavin-Based Catalysis: Principles and Applications* (Eds.: R. Cibulka, M. Fraaije), John Wiley & Sons, Ltd, Weinheim, Germany, **2021**, pp. 291–313.
- [63] T. Hyster, Y. Ye, J. Cao, D. Oblinsky, D. Verma, C. Prier, G. Scholes, *ChemRxiv* **2021**, preprint DOI: 10.26434/chemrxiv-2021-t85fh.
- [64] L. E. Meyer, B. E. Eser, S. Kara, *Curr. Opin. Green Sustain. Chem.* **2021**, *31*, 100496.
- [65] M. Aizawa, K. Namba, S. Suzuki, *Arch. Biochem. Biophys.* **1977**, *180*, 41–48.
- [66] G. Montagnoli, S. Monti, L. Nannicini, M. P. Giovannitti, M. G. Ristori, *Photochem. Photobiol.* **1978**, *27*, 43–49.
- [67] C. Claassen, T. Gerlach, D. Rother, *Adv. Synth. Catal.* **2019**, *361*, 2387–2401.
- [68] L. Schmermund, S. Reischauer, S. Bierbaumer, C. K. Winkler, A. Diaz-Rodriguez, L. J. Edwards, S. Kara, T. Mielke, J. Cartwright, G. Grogan, B. Pieber, W. Kroutil, *Angew. Chem. Int. Ed.* **2021**, *60*, 6965–6969; *Angew. Chem.* **2021**, *133*, 7041–7045.
- [69] T. E. Swartz, T. S. Tseng, M. A. Frederickson, G. Paris, D. J. Comerci, G. Rajashekar, J. G. Kim, M. B. Mudgett, G. A. Splitter, R. A. Ugalde, F. A. Goldbaum, W. R. Briggs, R. A. Bogomolni, *Science* **2007**, *317*, 1090–1093.
- [70] A. Sengupta, A. V. Sunder, S. V. Sohoni, P. P. Wangikar, *J. Biotechnol.* **2019**, *289*, 1–6.
- [71] V. Jurkaš, C. K. Winkler, S. Poschenrieder, P. Oliveira, C. C. Pacheco, E. A. Ferreira, F. Weissensteiner, P. De Santis, S. Kara, R. Kourist, P. Tamagnini, W. Kroutil, *Eng. Microbiol.* **2022**, *2*, 100008.
- [72] H. C. Büchenschütz, V. Vidimce-Risteski, B. Eggbauer, S. Schmidt, C. K. Winkler, J. H. Schrittwieser, W. Kroutil, R. Kourist, *ChemCatChem* **2019**, *12*, 726–730.
- [73] A. Hoschek, B. Buhler, A. Schmid, *Angew. Chem. Int. Ed.* **2017**, *56*, 15146–15149; *Angew. Chem.* **2017**, *129*, 15343–15346.

- [74] S. Böhmer, K. Köninger, Á. Gómez-Baraibar, S. Bojarra, C. Mügge, S. Schmidt, M. Nowaczyk, R. Kourist, *Catalysts* **2017**, *7*, 240.
- [75] J. Löwe, A. Siewert, A. C. Scholpp, L. Wobbe, H. Gröger, *Sci. Rep.* **2018**, *8*, 10436.
- [76] V. Jurkas, F. Weissensteiner, P. De Santis, S. Vrabl, F. A. Sorgenfrei, S. Bierbaumer, S. Kara, R. Kourist, P. P. Wangikar, C. K. Winkler, W. Kroutil, *Angew. Chem. Int. Ed.* **2022**, *61*, e202207971.
- [77] S. N. Chanquia, G. Vernet, S. Kara, *Eng. Life Sci.* **2021**, *22*, 712–724.
- [78] H. E. Bonfield, T. Knauber, F. Levesque, E. G. Moschetta, F. Susanne, L. J. Edwards, *Nat. Commun.* **2020**, *11*, 804.
- [79] D. Ziegenbalg, A. Pannwitz, S. Rau, B. Dietzek-Ivansic, C. Streb, *Angew. Chem. Int. Ed.* **2022**, *61*, e202114106.
- [80] M. Heining, R. Buchholz, *Biotechnol. J.* **2015**, *10*, 1131–1137.
- [81] M. Heining, A. Sutor, S. C. Stute, C. P. Lindenberger, R. Buchholz, *J. Appl. Phycol.* **2015**, *27*, 59–66.
- [82] M. Hobisch, J. Spasic, L. Malihan-Yap, G. D. Barone, K. Castiglione, P. Tamagnini, S. Kara, R. Kourist, *ChemSusChem* **2021**, *14*, 3219–3225.
- [83] C. K. Winkler, S. Simić, V. Jurkaš, S. Bierbaumer, L. Schermund, S. Poschenrieder, S. A. Berger, E. Kulterer, R. Kourist, W. Kroutil, *ChemPhotoChem* **2021**, *5*, 957–965.
- [84] J. D. Williams, C. O. Kappe, *Curr. Opin. Green Sustain. Chem.* **2020**, *25*, 100351.
- [85] S. Simic, M. Jakstaite, W. T. S. Huck, C. K. Winkler, W. Kroutil, *ACS Catal.* **2022**, *12*, 14040–14049.
- [86] S. N. Chanquia, A. Valotta, H. Gruber-Woelfler, S. Kara, *Front. Catal.* **2022**, *1*, 816538.
- [87] A. Valotta, L. Malihan-Yap, K. Hinteregger, R. Kourist, H. Gruber-Woelfler, *ChemSusChem* **2022**, *15*, e202201468.
- [88] L. A. D. Benincá, A. S. França, G. C. Brêda, R. A. C. Leão, R. V. Almeida, F. Hollmann, R. O. M. A. de Souza, *J. Mol. Catal.* **2022**, *528*, 112469.
- [89] L. Gardossi, P. B. Poulsen, A. Ballesteros, K. Hult, V. K. Svedas, D. Vasic-Racki, G. Carrea, A. Magnusson, A. Schmid, R. Wohlgemuth, P. J. Halling, *Trends Biotechnol.* **2010**, *28*, 171–180.
- [90] H. J. Kuhn, S. E. Braslavsky, R. Schmidt, *Pure Appl. Chem.* **2004**, *76*, 2105–2146.
- [91] A. Hoschek, I. Heuschkel, A. Schmid, B. Bühler, R. Karande, K. Bühler, *Bioresour. Technol.* **2019**, *282*, 171–178.
- [92] A. Hoschek, J. Toepel, A. Hochkeppel, R. Karande, B. Bühler, A. Schmid, *Biotechnol. J.* **2019**, *14*, e1800724.
- [93] Y. Zeng, X. Yin, L. Liu, W. Zhang, B. Chen, *J. Mol. Catal.* **2022**, *532*, 112717.
- [94] R. Ge, P. Zhang, X. Dong, Y. Li, Z. Sun, Y. Zeng, B. Chen, W. Zhang, *ChemSusChem* **2022**, *15*, e202201275.
- [95] C. K. Winkler, J. H. Schrittwieser, W. Kroutil, *ACS Cent. Sci.* **2021**, *7*, 55–71.
- [96] S. Mondal, F. Dumur, D. Gigmes, M. P. Sibi, M. P. Bertrand, M. Nechab, *Chem. Rev.* **2022**, *122*, 5842–5976.
- [97] Y. S. Zubi, B. Liu, Y. Gu, D. Sahoo, J. C. Lewis, *Chem. Sci.* **2022**, *13*, 1459–1468.
- [98] R. Verma, P. Jindal, J. Prasad, S. L. Kothari, N. P. Lamba, A. Dandia, R. K. Khangarot, M. S. Chauhan, *Top. Curr. Chem.* **2022**, *380*, 48.
- [99] F. Feyza Ozgen, M. E. Runda, B. O. Burek, P. Wied, J. Z. Bloh, R. Kourist, S. Schmidt, *Angew. Chem. Int. Ed.* **2020**, *59*, 3982–3987; *Angew. Chem.* **2020**, *132*, 4010–4016.
- [100] S. Y. Hwang, D. Song, E. J. Seo, F. Hollmann, Y. You, J. B. Park, *Sci. Rep.* **2022**, *12*, 9397.
- [101] P. T. Cesana, C. G. Page, D. Harris, M. A. Emmanuel, T. K. Hyster, G. S. Schlau-Cohen, *J. Am. Chem. Soc.* **2022**, *144*, 17516–17521.
- [102] H. Poddar, D. J. Heyes, G. Schiro, M. Weik, D. Leys, N. S. Scrutton, *FEBS J.* **2022**, *289*, 576–595.
- [103] J. Zhou, T. Zhu, Z. Cai, Y. Li, *Microb. Cell Fact.* **2016**, *15*, 2.
- [104] J. Behler, D. Vijay, W. R. Hess, M. K. Akhtar, *Trends Biotechnol.* **2018**, *36*, 996–1010.

Manuscript received: December 12, 2022
Revised manuscript received: March 3, 2023
Version of record online: May 2, 2023