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Light-Driven Biocatalytic Oxidation and Reduction Reactions: Scope and Limitations

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Dedicated to Jan-Erling Bäckvall on the occasion of his 60th birthday

The quest for practical regeneration concepts for nicotinamide-dependent oxidoreductases continues. Recently we proposed the use of visible light to promote the direct reductive regeneration of a flavin-dependent monooxygenase. With this enzyme (PAMO-P3) light-driven enantioselective Baeyer–Villiger oxidations were performed. In spite of the significant reduction in the complexity achieved, catalytic performance of the novel approach did not meet the requirements for an efficient biocatalytic oxygenation system. Driven by this ultimate goal, we further investigated the limiting factors of our particular system. We discovered that oxidative uncoupling of the flavin-regeneration reaction from enzymatic O_2-activation accounts for the futile consumption of approximately 95% of the reducing equivalents provided by the sacrificial electron donor, EDTA. Furthermore, it was found that the apparent turnover frequency (TOF) for PAMO-P3 in the present setup is approximately two orders of magnitude lower than in conventional setups that use NADPH as reductant. This finding was traced to sluggish electron transfer kinetics that arose from an impeded interaction between PAMO-P3-bound FAD and the reducing catalyst. The limiting factors and potential approaches for their circumvention are discussed. Furthermore, we broadened the light-driven regeneration approach to the class of flavin-dependent reductases. By using the Old Yellow Enzyme homologue YqjM as a model system, a significantly higher catalytic turnover for the enzyme catalyst was achieved, which we assign to a higher accessibility of the prosthetic group as well as to the absence of oxidative uncoupling.

Introduction

The use of enzymes as catalysts in synthetic organic chemistry has increased steadily during the last few decades.[1–5] In particular those enzymes that do not require cofactor regeneration constitute simple and practical systems that can be handled even by chemists who have no background in enzymology, which is one reason why they are used most often. Examples include hydrolytic enzymes such as esterases, lipases, proteases and epoxide hydrolases as well as C–C bond-forming enzymes such as oxynitrilases or aldolases. They often show a remarkably broad substrate scope as well as high regio- and enantioselectivity. In the relevant applications, tedious protective group methodology is not necessary; this makes the use of enzymes even more attractive. Indeed, protective groups can be introduced selectively in multifunctional compounds by enzyme catalysis, and selective deprotection is also possible.[6] If these traditional methods fail, directed evolution can be applied,[7–13] as for example in the control of *R* or *S* selectivity, on an optional basis.[14–16]

Oxidoreductases (E.C. 1.x.x.x) have been used to catalyse selective oxidation or reduction, albeit to a much lesser extent than hydrolases.[1–5] Such a limitation appears odd, especially in view of the fact that oxidoreductases are often complementary to their chemical counterparts, and allow for chemo-, regio-, and stereoselective transformations that are not possible (or not easily possible) by synthetic catalysts. Examples are stereoselective Baeyer–Villiger (BV) reactions, enantioselective reduction of "difficult" prochiral ketones and chemoselective oxidation of polyolic substrates. The fact that oxidoreductases are not used by organic chemists as extensively as they deserve to be is, to a significant extent, due to their dependence on redox cofactors.[1–5] In particular, the relatively unstable nicotinamide cofactors NAD(P)H and NAD(P)$^+$, which serve as sources of redox equivalents, are prohibitively expensive, and therefore cannot be applied stoichiometrically. For this reason several regeneration methods that enable the substoichiometric use of these cofactors have been developed. One approach that is commonly used in industry is whole-cell catalysis,[17–20] which relies on the microbial cell to both express the enzyme and regenerate the cofactor. However, this requires equipment that is usually not available in organic chemistry laboratories. Another approach is based on enzyme-coupled systems,[21–26] and makes use of such regeneration enzymes as glucose-, formate-, or alcohol dehydrogenases, which of course make the overall system more complicated. Finally, chemical or electrochemical approaches or combinations thereof have been reported, but again each system has advantages and disadvantages.[27–31] Conceptually, the most elegant approach completely circumvents the use of a nicotinamide cofactor as a carrier for reduction equivalents. For example, electrochemical reduction coupled to P450 monooxygenases has been described.[32–38] Moreover, chemical and electrochemical methods for the direct re-

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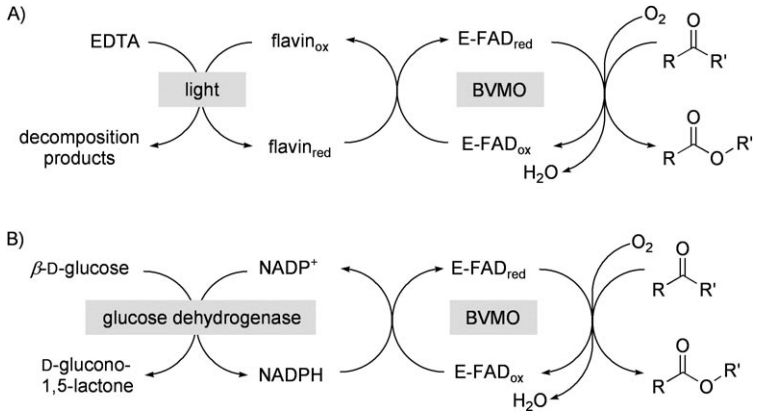
generation of flavin-dependent monooxygenases have been established.[30,39,40] The electrochemical methods, although successful, likewise add to the complexity of the system and generally require special equipment and training. Several review articles that cover the field of cofactor regeneration methods have appeared recently.[41]

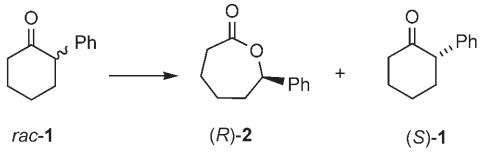
We recently proposed and demonstrated the use of visible light for the reductive regeneration of flavin-dependent enzymes, which eliminates the need for nicotinamide cofactors as carriers for reduction equivalents, and also greatly simplifies the overall system.[42] It had been known for decades from the work of Frisell[43] and Massey[44] that flavins can be photochemically reduced by using simple sacrificial electron donors such as amines (e.g., ethylenediaminetetraacetate, EDTA). We therefore exploited these observations in the development of a light-driven stereoselective biocatalytic oxidation,[42] specifically the enantioselective monooxygenase-catalyzed Baeyer–Villiger reaction of ketones. We chose a mutant of phenylacetone monooxygenase (PAMO-P3) from *Thermobifida fusca* as the flavin-dependent Baeyer–Villigerase (BVMO), which we had previously engineered to catalyze enantioselective BV reactions of selected ketones.[22,45] The light-driven system (Scheme 1 A)



Scheme 1. Comparison of a simplified, light-driven regeneration approach (A) for a Baeyer–Villiger monooxygenase (BVMO, e.g., PAMO) with the traditional enzyme-coupled regeneration approach (B). The latter system employs the common glucose dehydrogenase to catalyse the regeneration of the reduced nicotinamide cofactor (NADPH). By using visible light, the use of a coupled enzyme and the nicotinamide can be avoided, thereby reducing the complexity of the setup; E-FAD: enzyme-bound FAD.

constitutes a simplification of the traditional enzyme-coupled process based on the use of a regenerating enzyme, such as glucose dehydrogenase (Scheme 1 B).

Among the substrates that were tested in BV reactions, racemic 2-phenylcyclohexanone (*rac*-**1**) was subjected to kinetic resolution. To our initial disappointment, the attempt to initiate the reaction by shining white light (simple light bulb) on a mixture of *rac*-**1**, EDTA, flavin adenine dinucleotide (FAD), and catalytic amounts of the mutant monooxygenase PAMO-P3 failed.[42] However, several groups had postulated that in the natural system, the nicotinamide cofactor stays bound to a BV monooxygenase during the catalytic cycle, which suggests that this sustains a catalytically active conformation of the enzyme.[29,46,47] In our previous study, we, therefore, repeated the light-based system (Scheme 1 A) in the presence of catalytic amounts of NADP$^+$, which indeed resulted in notable catalytic turnover. The maximal theoretical conversion of 50% in the kinetic resolution of *rac*-**1** to lactone (*R*)-**2** was essentially reached, with excellent enantioselectivity (48% conversion, 97% *ee*; Scheme 2).[42] The observed enantioselectivity turned



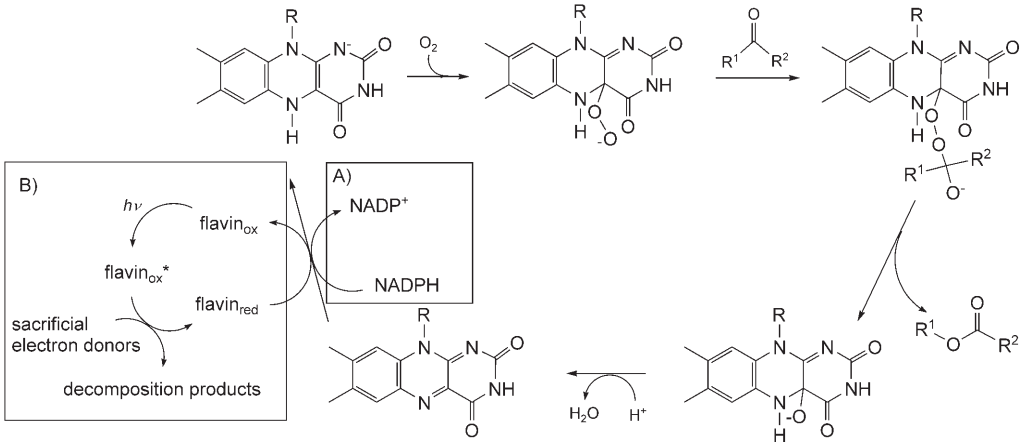
Scheme 2. Kinetic resolution of 2-phenylcyclohexanone (*rac*-**1**) by a Baeyer–Villiger reaction.

out to be identical to that observed in previous experiments that utilized whole cells,[22,45] or an in vitro system based on conventional cofactor regeneration. Other ketones were shown to behave similarly; again this strongly indicates an unaltered mechanism for the actual oxidation.

In our initial study we performed several control experiments, which corroborated the mechanism as outlined in Scheme 1 A.[42] For example, reactions in the absence of any one of the components used, for instance, light, EDTA, FAD, or PAMO-P3 resulted in no turnover. It was also shown that the buffer component, Tris is not the source of electrons. Moreover, a putative flavo-H_2O_2-shunt pathway was excluded. Other control experiments showed that FAD can be replaced by riboflavin or flavin mononucleotide (FMN), which proved that no significant exchange of reduced FAD with the protein-bound cofactor occurs. Finally, we demonstrated that an alternative mechanism based on the possible photoreduction of NADP$^+$ does not operate. The apparent necessity for nonenzyme-bound flavin is in accord with previous findings,[44] and it suggests a catalytic role of free flavin in the reduction of the enzyme-bound cofactor.

The mechanistic details of the light-driven BV reaction according to Scheme 1 A are shown in Scheme 3. From a practical viewpoint, the light-driven process constitutes a very simple system, but the catalytic performance, as measured by the reaction rate, fell short by one to two orders of magnitude relative to the normal maximum activity.[22,42,45]

In this study we pursued two goals. First, in order to determine the factors that lead to reduced catalyst activity, appropriate experiments were performed. We expected this information to be useful in defining prerequisites for a successful system and thus the scope of the concept. Second, on the basis of the gained knowledge, we wished to extend the applicability of the light-driven direct regeneration system by including another family of flavin-dependent enzymes, specifi-



Scheme 3. Proposed reactions to regenerate the enzyme-bound FAD during the catalytic cycle of a BVMO. Besides the natural reductive pathway that uses NADPH, the newly introduced pathway is shown. A) Native reduction by using NADPH; B) light-driven regeneration by using EDTA as a source of electrons.

cally the Old Yellow Enzyme[48–50] (OYE) homologue YqjM from *Bacillus subtilis* (E.C. 1.6.99.1).[51,52]

Results and Discussion

Light-driven Baeyer–Villiger reactions catalyzed by PAMO-P3

Even though the light-driven catalyst system appears to be superior to other direct cofactor-regeneration systems of similar complexity (Table 1, entry 1 vs. 2), its major disadvantage is the reduced activity relative to the use of an enzyme-coupled regeneration system (Table 1, entry 1 vs. 3). We showed earlier that H_2O_2 and other reactive oxygen species, which arise from undesired uncoupling of the regeneration reaction from the actual enzymatic oxidation process, are not the major cause of the problem. Rather, the uncoupling itself and/or slow electron-transfer kinetics were suspected to be rate limiting.[42]

Table 1. Performance of the light-driven BVMO regeneration in comparison with a native regeneration system, and an electrochemical system of comparable simplicity.

	Enzyme	Mediator	Source of reducing equivalents	TOF[a] [h^{-1}]	TON[b] Enzyme	TON[b] Mediator
1	PAMO[c]	FAD	EDTA/$h\nu$	10	96	9.6
2	StyA[30]	FAD	cathode	104	26	0.2
3	PAMO[22]	NADPH[d]	isopropanol	394	9471	400

[a] TOF: catalyst turnover frequency (initial rate); [b] TON: total turnover number; [c] this study; [d] NADPH regeneration by a coupled enzyme.[22]

In order to learn more about the light-driven BV reaction, and perhaps improve the catalytic performance of the system, further experiments were carried out. The effect of varying the concentration of enzyme in the reaction of 2-phenyl-cyclohexanone (**1**) was studied first. As expected, at low enzyme concentrations, a linear correlation was observed between the biocatalyst concentration and rate (data not shown).

As already mentioned, we discovered that in the absence of the oxidized nicotinamide cofactor NADP$^+$ no oxidation occurs under the conditions of the light-driven reaction. Thus, NADP$^+$ might function as an "allosteric regulator" by shifting the spatial geometry of PAMO-P3 into its catalytically active form. Indeed, we found a distinct increase of the overall rate upon increasing the in situ concentration of NADP$^+$ (Figure 1).

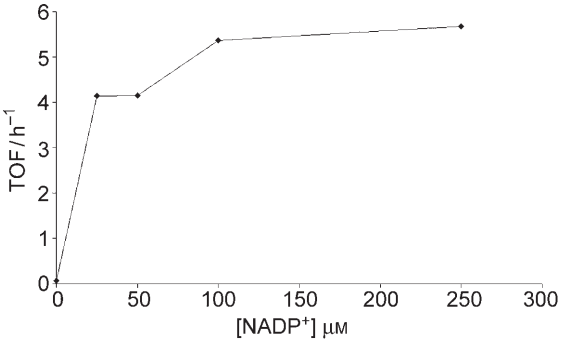


Figure 1. Influence of the variation of NADP$^+$ concentration on the rate of 2-phenylcyclohexanone oxidation. Conditions: 30 °C, 100 W light bulb, 10 μM PAMO-P3, 100 μM FAD, 25 mM EDTA, 1 mM substrate, 50 mM Tris-HCl (pH 7.4), 1% (*v*/*v*) acetonitrile. TOF was determined after 4 h. The substrate concentrations used here are different from those that were employed previously,[42] and therefore the values for TOF are different.

To ensure full saturation of PAMO with NADP$^+$, all further experiments were performed in the presence of 250 μM NADP$^+$. The observed saturation-type behavior supports the assumption of reversible binding of NADP$^+$ to the enzyme. A further parameter that might influence catalytic performance is the power of the light source used. We tested three tungsten bulbs that differed in their nominal power output under otherwise identical conditions (Table 2).

It was found that the 40 W light bulb was not powerful enough to efficiently induce turnover, but the 100 W and 200 W lamps led to TOFs of 5.67 and 7.12 h^{-1}, respectively. A change of the power of the light source from 100 W to 200 W

Table 2. Influence of power of the light source on the oxidation of *rac*-**1**. Conditions: 30 °C, 10 μM PAMO-P3, 100 μM FAD, 25 mM EDTA, 1 mM substrate, 250 μM $NADP^+$, 50 mM Tris-HCl (pH 7.4), 1% (*v*/*v*) acetonitrile.

Power of light source [W]	TOF $[h^{-1}]$[a]	Conversion [%][b]
40	< 0.1	< 1
100	5.67 ± 0.20	53.8 ± 1.6
200	7.12 ± 0.24	56.6 ± 1.7

[a] Determined after 4 h; [b] determined after 26 h; note that in PAMO-catalyzed kinetic resolution of *rac*-**1** the *S* enantiomer of the substrate is converted with a significantly lower rate.

only leads to a slight increase of the reaction rate and the final conversion. In fact, we found that the reaction mixtures were decolorized in the course of the reaction; this indicates that a significant fraction of FAD is in its reduced state. In any case, the decolorization was found to be at least partially reversible; this indicates insignificant decomposition of the flavins, and was further corroborated by the apparent independence of the yield on the intensity of light applied.

Of particular interest was the influence of concentration of the sacrificial electron donor used on the reaction rate and the final conversion. In a series of experiments, the reaction progress was monitored for various initial EDTA concentrations (Figure 2).

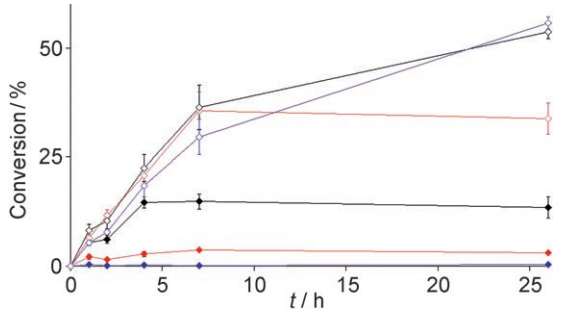
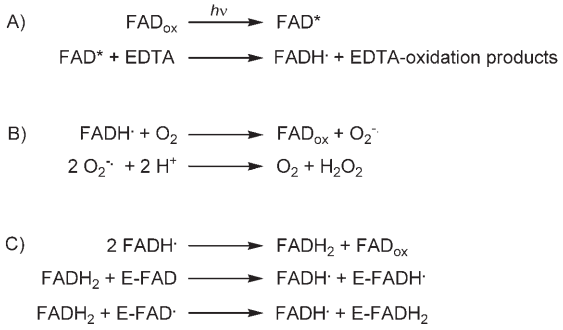


Figure 2. Influence of EDTA concentration on the light-driven PAMO-P3-catalyzed oxidation of 2-phenylcyclohexanone. Conditions: 100 W light bulb, 30 °C, 10 μM PAMO-P3, 100 μM FAD, 1 mM substrate, 250 μM $NADP^+$, 50 mM Tris-HCl (pH 7.4). [EDTA] = 1 mM (◆), 2 mM (◆), 5 mM (◆), 10 mM (◇), 25 mM (◇), 50 mM (◇).

Except for the lowest EDTA concentration (where the reaction probably ceased before the first sampling), the initial overall reaction rate was independent of the electron donor concentration. However, there is a distinct correlation between the initial EDTA concentration and the final yield of the reaction. Thus, the production of one equivalent of product necessitates the consumption of an approximate 30-fold molar excess of EDTA. In other words, over 95% of the reducing equivalents that are provided by EDTA are not productively coupled to the enzymatic reaction. This value even increases if multiple oxidation of one EDTA molecule is assumed.

Similar effects have been reported previously for chemical and electrochemical regeneration of a FAD-dependent monooxygenase.[30,40] Apparently, free-diffusing reduced species react quite fast with molecular oxygen; this results in uncoupling of the FAD-regeneration reaction from the enzymatic oxygenation reaction (Scheme 4).[44] Overall, not only are the reducing equivalents redirected into a futile reaction, but O_2 is also consumed, and reactive oxygen species are generated. We conclude that this undesired uncoupling represents a major limitation of the light-driven reaction.



Scheme 4. Schematic representation of the relevant reactions that occur in the light-driven regeneration of PAMO–$FADH_2$. A) The first elementary reaction is the light-driven excitation of flavins, which results in a high-redox-potential species that is capable of oxidizing EDTA. B) The resulting semi-quinone species reacts very fast with molecular oxygen; this results in the reformation of oxidized flavins (uncoupling reaction) with concomitant formation of the superoxide radical anion, which itself quickly disproportionates into H_2O_2 and O_2. C) Synproportionation of two semi-quinones results in the formation of the fully reduced flavin, which can proportionate with PAMO-bound FAD. After a second electron transfer from a free flavin to PAMO–FADH·, the reduced, catalytically active PAMO-bound-$FADH_2$ is formed.

Massey et al. reported that the photochemical reduction of flavoproteins (i.e., reduction of the enzyme-bound flavins, FAD, FMN, or riboflavin) is not strictly linked to the enzyme-specific type of flavin, but can be catalyzed by a variety of differnet flavins.[44,53] In our own experiments we could confirm this result; we found significant conversion using FAD, FMN, and riboflavin as freely diffusible regeneration catalysts (Figure 3).

Due to a strong interaction between FAD and the monooxygenase, a significant contribution of a putative exchange mechanism, wherein the reduced flavin binds to the apo-pro-

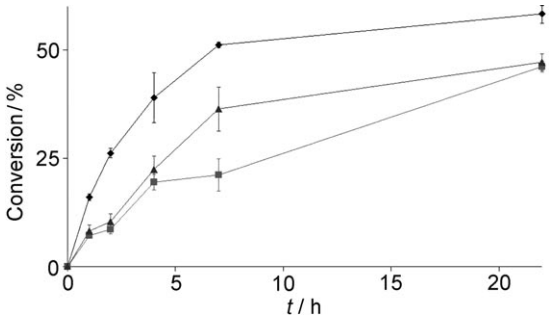


Figure 3. Light-driven oxidation of *rac*-**1** by using FMN (◆), riboflavin (■), or FAD (▲) as a cocatalyst. The concentration of the flavin was 100 μM; standard reaction conditions were applied (initial concentration of substrate: 1 mM).

tein to catalyze the BV-oxidation reaction, can be excluded. Overall, our results are in line with the mechanism previously proposed by Massey (Scheme 4).[44]

In further experiments the influence of the nature of flavins on the rate of the overall reaction was studied (Table 3). At least for FAD and FMN, saturation-like behavior can be assumed; this suggests a reversible binding mechanism prior to the actual electron-transfer step. Interestingly, the smaller FMN exhibits a significantly higher TOF value compared to FAD; this can be attributed to the decreased steric constraints (vide infra). Riboflavin, on the other hand reacts as fast as FAD—a finding that is currently difficult to rationalize.

Table 3. Influence of the external flavin and its concentration on the light-driven PAMO-P3 catalyzed oxidation of ketone **1**. Standard conditions were employed; conversion was determined after 22 h.

External flavin	[Flavin] [μM]	TOF [h^{-1}][a]	Conversion [%]
FAD	250	5.96 ± 0.18	48.5 ± 2.0
	100	5.67 ± 0.20	47.2 ± 2.0
	50	3.55 ± 0.31	25.7 ± 2.6
FMN	250	9.67 ± 0.34	54.1 ± 0.7
	100	10.67 ± 0.63	58.3 ± 2.1
	50	7.78 ± 0.39	45.1 ± 4.1
riboflavin[b]	150	5.99 ± 0.21	43.6 ± 0.5
	100	4.87 ± 0.21	46.1 ± 1.2
	50	4.10 ± 0.19	42.5 ± 3.2

[a] Determined after 4 h; [b] due to the low solubility of riboflavin in water, the highest concentration that was tested was 150 μM.

It needs to be pointed out that the catalytic performance of the present "optimized" setup still falls short of the native cycle by at least one order of magnitude. In addition to the above-discussed uncoupling, this can be explained by a sterically impeded interaction between the free flavin and the enzyme-bound FAD. This conclusion is guided by the crystal structure of PAMO, which shows the FAD deeply buried within the enzyme cavity with poor accessibility from the solvent phase (Figure 4). Due to an unfavorable geometry, both flavins cannot interact perfectly for electron transfer.[54]

The present observations show that the photochemically driven BVMO-catalysis is limited mainly by two factors: first, O_2-dependent uncoupling of the regeneration reaction from the desired oxygenation reaction occurs. Second, electron transfer between free and enzyme-bound flavins is relatively slow. In order to overcome the former limitation, one might consider covalent attachment of the free flavins to the enzyme via a flexible linker. Thus, the concentration of free flavins can be reduced, which could result in an optimized ratio of synthesis to uncoupling reaction. With regard to the second limitation, either smaller optimized electron shuttle molecules might be suitable, or enzyme engineering could lead to PAMO variants with increased accessibility of the enzyme-bound flavin to the solvent. Also, we suggest deazaflavins as redox mediators, which are known to be redox-stable towards molecular oxygen.[55] This would prevent the uncoupling reaction.

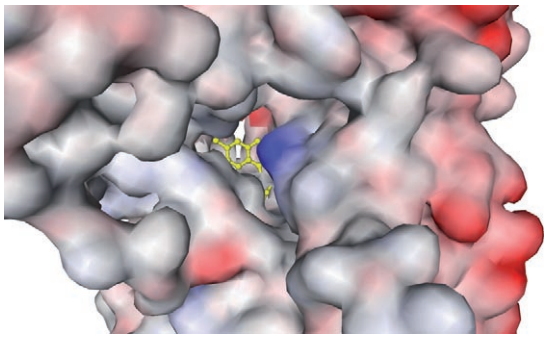


Figure 4. Close-up of the substrate access channel of PAMO (X-ray structure,[55] PDB ID: 1W4X), which shows the protein surface. The enzyme-bound FAD is displayed in yellow (ball-and-stick). Only the A ring of the isoalloxazine moiety of the flavin is visible, the rest of the cofactor is shielded by the protein.

Light-driven reduction of enones catalyzed by the Old Yellow Enzyme homologue YqjM

In order to further exploit the scope of the photochemical regeneration approach, we decided to evaluate an enzyme system in which both of the above limitations can be overcome. The Old Yellow Enzyme (OYE)[48] homologue YqjM from *Bacillus subtilis*[51,52] (E.C. 1.6.99.1) fulfills two requirements: as a reductase, YqjM is oxygen independent, and the YqjM-bound FMN is readily accessible from the surface (Figure 5). If successful, this would also corroborate some of the previous conclusions.

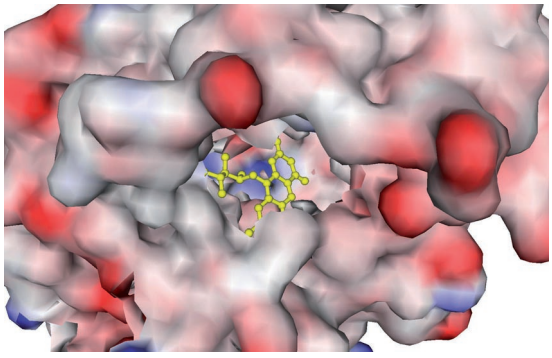
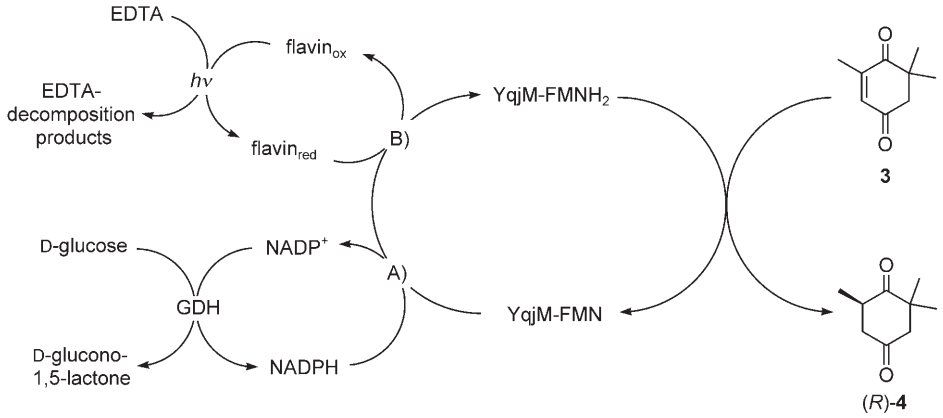


Figure 5. Close-up of the crystal structure of YqjM (PDB ID: 1Z41). The enzyme surface is shown with the well-exposed enzyme-bound FMN in a ball-and-stick depiction (yellow).

YqjM catalyzes the NADPH-dependent reduction of α,β-unsaturated carbonyl compounds such as *N*-ethylmaleimide and cyclohex-2-enone. We chose the prochiral compound ketoisophorone (**3**) as a substrate, which has not been described as a substrate for YqjM previously. The resulting product **4** is a key intermediate in carotinoid synthesis.[56] We envisioned the potentially enantioselective light-driven reduction to occur as outlined in Scheme 5.

We were pleased to find that irradiation with white light in the presence of free flavin (FMN) and EDTA gave the desired



Scheme 5. Enantioselective YqjM-catalyzed reduction of ketoisophorone **3**. A) Schematic comparison of the "traditional" regeneration, and B) the light-driven, simplified regeneration approach; GDH: glucose dehydrogenase.

product (*R*)-**4** in quantitative yield and with good enantioselectivity (88% *ee*). As expected, control experiments in the absence of free flavin showed no significant conversion.

Control experiments in the absence of light showed that a background reaction that is seemingly independent of reaction time occurs, albeit to a lesser extent (approximately 5%); this was an initially surprising result. The conspicuous conversion in the dark controls was then found to be due to the exposure of the reaction mixture to light during sample preparation and workup. Moreover, we discovered that in the absence of YqjM, reduced flavin is capable of reducing ketoisophorone to yield racemic product **4** (Figure 6). The reduction that is catalyzed directly by the free flavin occurs at approximately 10% of the rate of the YqjM-catalyzed reaction. This non-enzymatic background reaction also explains the somewhat lower optical purity of the product that was obtained from the light-driven pathway compared to the one from the "traditional" approach with a conventional cofactor regeneration system (glucose dehydrogenase, NADP; 88% *ee* vs. 92% *ee*, respectively). The slight decrease in enantioselectivity with increasing reaction time, even after full conversion, suggests a very slow racemization of the product under the reaction conditions.

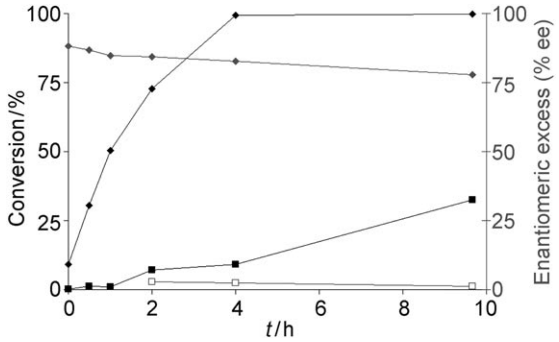


Figure 6. Light-driven enantioselective YqjM-catalyzed reduction of ketoisophorone. Conditions: 100 W light bulb, 30 °C, 303 μg mL^{-1} YqjM lysate (total protein content according to Bradford assay), 25 mM EDTA, 100 μM FMN, 1 mM ketoisopherone, 50 mM Tris (pH 7.4), 1% (*v*/*v*) acetonitrile. Conversion: YqjM catalyzed (◆), absence of YqjM (■); enantiomeric excess in favor of (*R*)-**4**: YqjM catalyzed (◈), absence of YqjM (□).

Compared to the PAMO system (see previous section), turnover numbers and especially turnover frequencies were dramatically higher, which underlines the potential of a flavin-dependent reductase in the light-driven direct cofactor regeneration (TON = 383; TOF = 194 h^{-1}).

Furthermore, all experiments performed with YqjM showed that NADP$^+$ is not required for an efficient reaction to proceed. This finding illuminates the difference between the YqjM and PAMO system. In the case of PAMO the nicotinamide cofactor stays bound to the enzyme during the actual oxidation reaction in the catalytic cycle. The addition of NADP$^+$ in the light-driven PAMO-system is required only in order to sustain a catalytically active conformation of the enzyme. In contrast, the YqjM system does not require such activation.

Conclusions

In this study we have demonstrated the general applicability of the proposed photochemical regeneration approach for flavin-dependent monooxygenases and reductases.[42] In the PAMO-based light-driven system, the major limitations were identified to be unproductive oxidative uncoupling of the light-driven flavin reduction reaction from the regeneration of the prosthetic group of the monooxygenase, and the somewhat sluggish electron-transfer kinetics, which can be related to poor access of the mediator to PAMO-bound FAD due to steric reasons. The use of O_2-stable mediator systems, such as deazaflavins,[53] might be suitable to overcome this limitation. Further investigations to address such issues will be necessary for a deeper mechanistic understanding and perhaps for improving catalytic performance.

In the case of the Old Yellow Enzyme homologue YqjM as a reductase, the efficiency of the light-driven regeneration turned out to be much higher, as expected from the results of the PAMO system. Thus, the higher performance can be explained by two factors: the reaction conditions without constant aeration allow for (partial) suppression of the undesired uncoupling reaction, and the more accessible prosthetic group of YqjM compared to PAMO results in more efficient electron transfer. We expect that further applications of our concept will follow in which other enzymes are targeted, and will hopefully lead to practical catalyst systems for application in synthetic organic chemistry.

Experimental Section

PAMO-catalyzed reactions: Preparation of PAMO and PAMO-catalyzed reactions were performed and analyzed by GC as described earlier.[42] Each series of experiments was performed from the same

PAMO preparation because of slightly varying activities in the light-driven reactions of different preparations. Therefore, some values reported here might be slightly different from those that were reported earlier.[42]

YqjM-catalyzed reactions: YqjM was expressed in *E. coli* Rosetta-(DE3) under otherwise unchanged conditions according to a known procedure.[51] After reconstitution of the crude lysate with FMN followed by removal of excess FMN by gel-filtration (PD-10 desalting columns, GE Healthcare) the protein was analyzed by SDS-PAGE by using a 12.5% gel. By using the densitograph function of BioDocAnalyze (Biometra, Göttingen, Germany) the YqjM content was determined to be 32% of the total protein content of the lysate. The total protein content of the lysate was determined by using a Bio-Rad Bradford assay reagent with bovine serum albumin as standard.[57] YqjM-catalyzed reactions were performed according to the procedure for the PAMO-catalyzed reactions. Samples were extracted with ethyl acetate and analyzed by GC (Achiral method: instrument: Agilent Technologies 6890N, carrier gas: 0.6 bar H_2, column: 15 m ZB1 (100% dimethylpolysiloxane, 0.25 mm inner diameter, 0.5 μm film), injector $T = 220\,^{\circ}C$, detector $T = 350\,^{\circ}C$, program: ramp 80 °C to 110 °C with $5\,^{\circ}C\,min^{-1}$, then $20\,^{\circ}C\,min^{-1}$ to 340 °C, retention times: 2.83 min (**3**), 3.05 min (**4**). Chiral method: Agilent Technologies 6890N, carrier gas: 0.6 bar H_2, column: 30 m BGB176 (20% 2,3-dimethyl-6-tert-butyldimethylsilyl-β-cyclodextrin dissolved in BGB-15), 0.25 mm inner diameter, 0.1 μm film, injector $T = 220\,^{\circ}C$, detector $T = 350\,^{\circ}C$, program: 100 °C (iso) 15 min, retention times: 12.11 min (3), 12.35 ((*R*)-**4**) min and 13.90 min ((*S*)-**4**).

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