Biophotoelectrochemistry: From bioelectrochemistry to photosynthesis

FUNDAMENTALS OF BIOELECTROCHEMISTRY

Chapter 5: Artificial photosynthesis: hybrid systems

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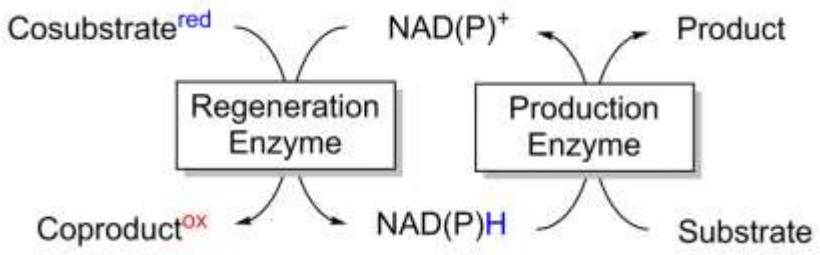
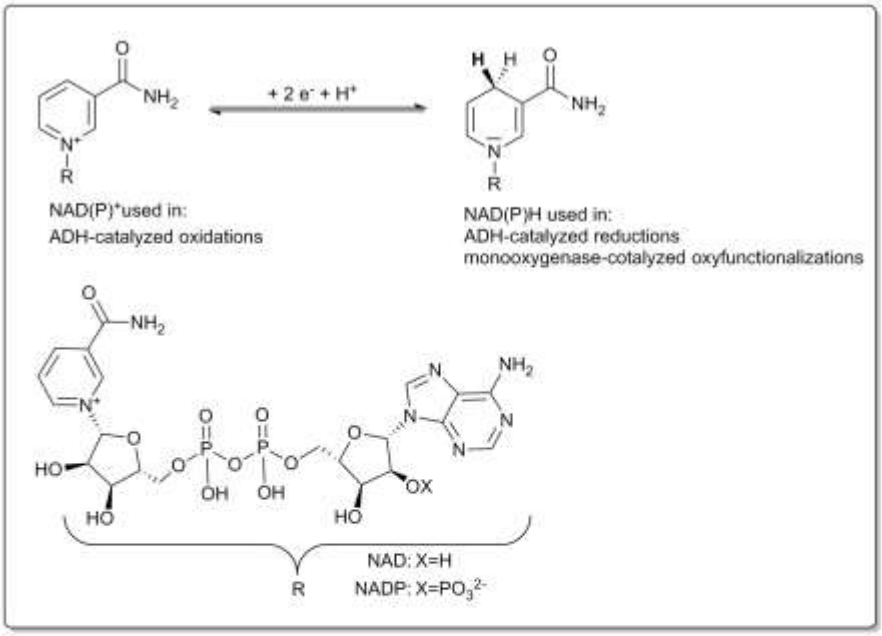
1. Introduction

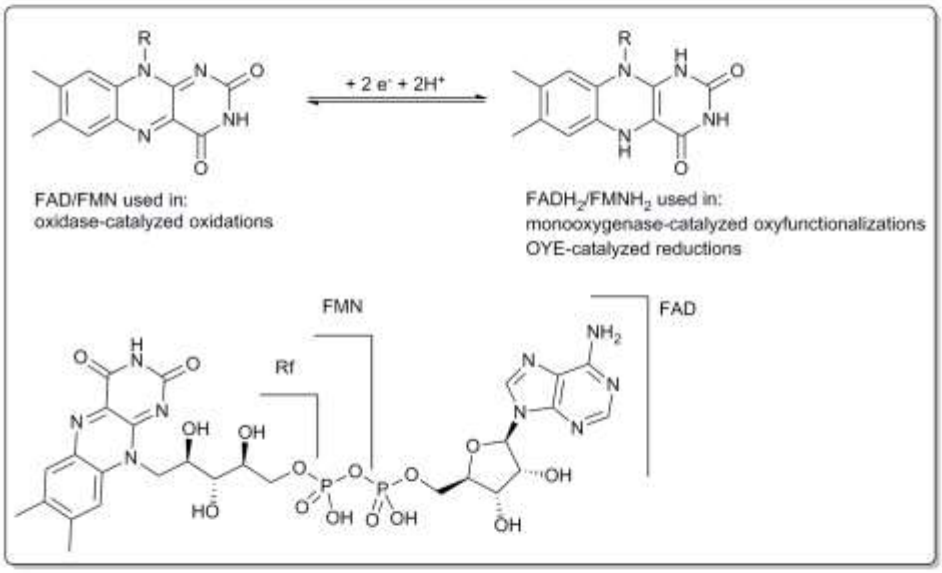
Oxidoreductases are very useful catalysts for organic synthesis as they often enable specific redox transformations at selectivities not accessible with established chemical catalysts.[1] To make full use of nature's arsenal of oxidoreductases, efficient methods that sustain the oxidoreductases' catalytic cycles are vital. In other words, electrons need to be delivered to or taken away from the enzymes' active sites. Within their natural environment (generally whole living cells) this task is accomplished by cofactors that 'wire' the oxidoreductase to cellular metabolism. The latter, however, has been optimized to sustain the cells survival and function and not to provide an efficient network for preparative application of the cell. From a chemist's perspective it is generally most desirable if the oxidoreductase of interest operates at full speed which obviously is not always in line with the cell's requirements. Therefore, it is not astonishing that from an early stage on, one branch of biocatalysis research has focussed on efficient regeneration systems.[2] Today, a broad range of different regeneration approaches are established, some of them on industrial scale. The majority of these approaches is biomimetic as they rely on a second enzymatic process to provide the redox equivalents needed for the oxidoreductase of interest. The redox mediator used in these systems typically is NAD(P)H (Scheme 1).

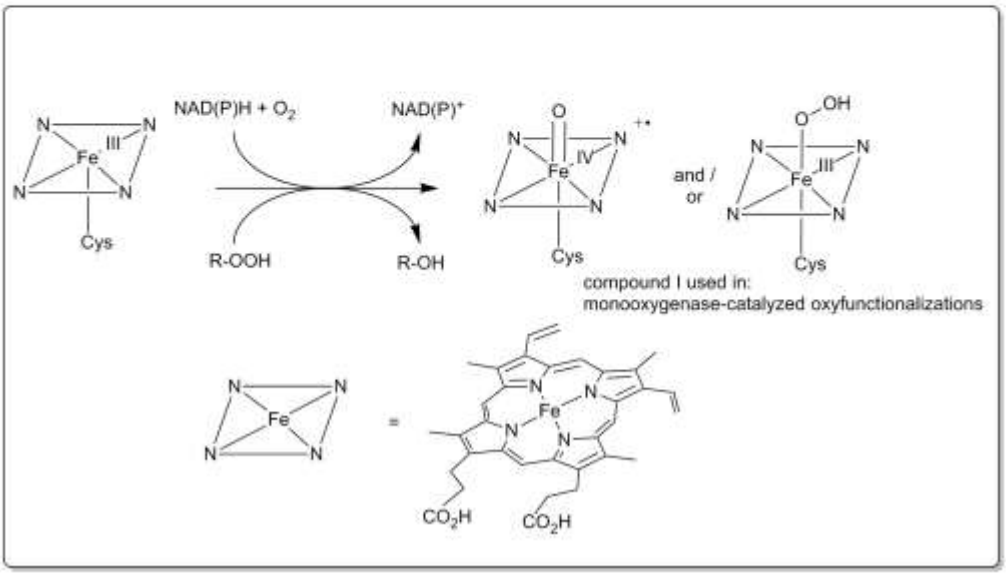


Scheme 1. General representation of established enzymatic regeneration systems for NAD(P)H-dependent production enzymes.

Most oxidoreductases known today rely on redox-active metals or organic molecules bound to the oxidoreductases' active sites to perform the actual reaction. Scheme 1 summarizes the most important cofactors and prosthetic groups discussed in this contribution.


+ 2 e⁻ + H⁺
NAD(P)⁺used in:
ADH-catalyzed oxidations
NAD(P)H used in:
ADH-catalyzed reductions
monooxygenase-cotalyzed oxyfunctionalizations
NAD: X=H
NADP: X=PO3 2-
R


+ 2 e⁻ + 2H⁺
FAD/FMN used in:
oxidase-catalyzed oxidations
FADH2/FMNH2 used in:
monooxygenase-catalyzed oxyfunctionalizations
OYE-catalyzed reductions
FMN
FAD
Rf


NAD(P)H + O2
NAD(P)+
R-OOH
R-OH
Cys
and / or
compound I used in:
monooxygenase-catalyzed oxyfunctionalizations

Scheme 2. Structures and basic electrochemistry of the most relevant oxidoreductase prosthetic groups and cofactors.

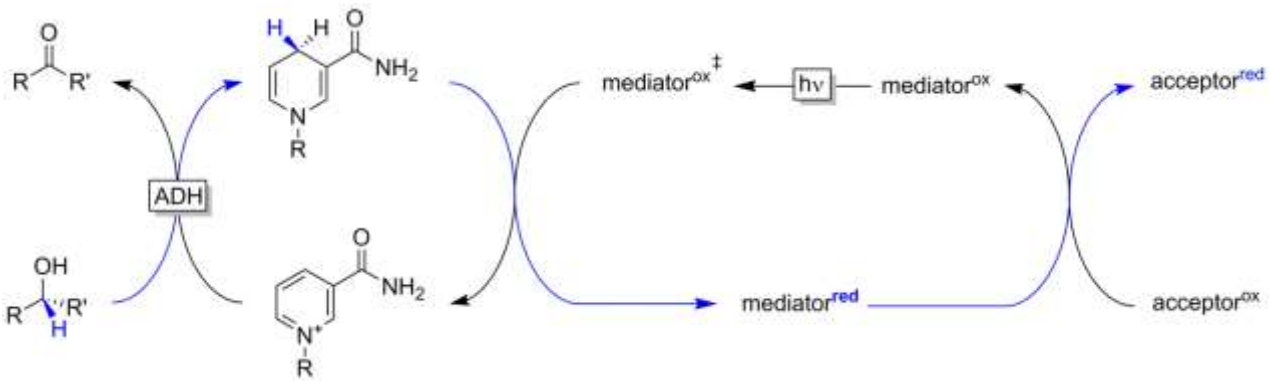
In addition to these established methodologies, electrochemical and photochemical *in situ* regeneration approaches are also under investigation.[3] Electrochemical regeneration has to take place indirectly using redox mediators as two electron transfer agents, which is further described in Chapter 1 and 2 of this book. Today, an increased interest in photochemical methods can be attested, which may be due to various motivations. Photochemical approaches in principle enable simplified reaction schemes, avoiding additional (enzymatic) regeneration catalysts. Photochemical processes frequently utilize homogeneously dissolved catalysts and thereby in principle overcome diffusion limitations often encountered with electrochemical approaches. Finally, light energy can serve as a 'catalyst' to accelerate chemical reactions but also as source of energy to make thermodynamically unfavourable transformations feasible.

The aim of this contribution is to provide a tutorial overview in photochemical regeneration of cofactors and oxidoreductases and to provide a critical review of the current trends in photobiocatalysis with a focus on processes utilizing isolated oxidoreductases.

2. Photocatalytic oxidative regeneration

2.1 $NAD(P)^+$ regeneration

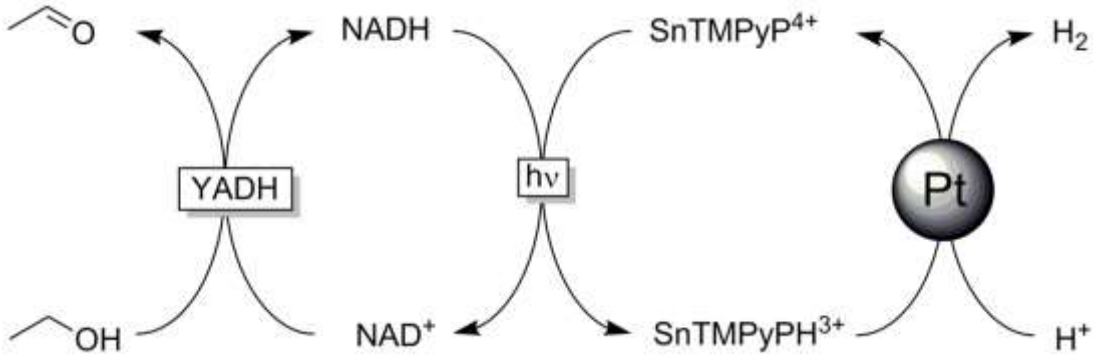
Dating back to the 1980s, photocatalytic oxidation of reduced nicotinamide cofactors to promote alcohol dehydrogenase (ADH)-catalyzed oxidations of alcohols, is one of the oldest man-designed photobiocatalytic reaction.[2a] Due to the rather negative redox potential of the NAD(P)H/$NAD(P)^+$ redox couple of -320 mV vs. SCE, most $NAD(P)^+$ regeneration reactions are thermodynamically feasible (especially if O_2 serves as terminal electron acceptor). Hence the following illustrations are true examples for photocatalysis. In other words, light is used to accelerate an exergonic reaction and not as an external energy source. The most frequent photocatalytic mechanism comprise photoexcitation of an oxidized mediator molecule, which in its photoexcited state reacts faster with NAD(P)H than the corresponding ground state (Scheme 3).



Scheme 3. General scheme for photocatalytic regeneration of oxidized nicotinamide cofactors.

Overall, a photoexcited mediator serves as hydride abstractor from NAD(P)H yielding the reduced mediator and the (desired) oxidized nicotinamide cofactor. Whether this hydride transfer occurs concerted as a true hydride mechanism or sequentially as sequence electron transfer – deprotonation – electron transfer (ECE mechanism, *vide infra*) is only poorly understood and probably also depends on the mediator used.

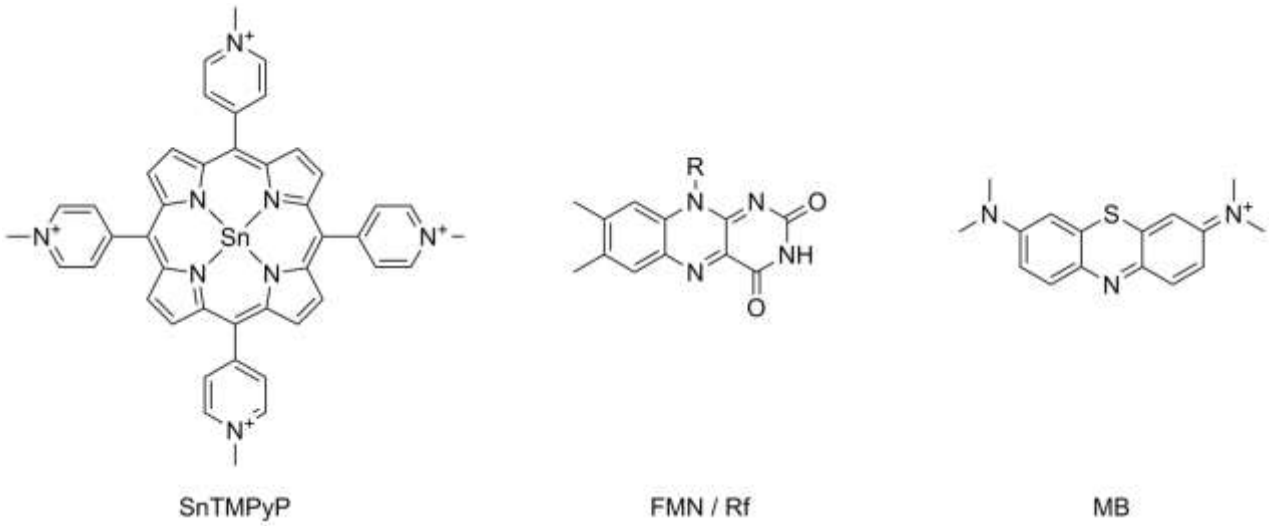
To use catalytic amounts of the mediator, generally a sacrificial electron donor is applied to re-oxidize the mediator and enable the next catalytic cycle. Generally, molecular oxygen serves as terminal electron acceptor yielding either hydrogen peroxide of water as by-product. Alternatively, Handman et al reported protons as terminal electron acceptors using Pt particles as H_2-evolution catalysts (Scheme 4).[4] A photoelectrochemical variant of this approach has also been reported.[5]



Scheme 4. Photo-chemo-enzymatic dehydrogenation of ethanol. Using the ADH from yeast (YADH), Sn-*meso*-tetrakis (N-methyl-4-pyridyl)porphine (SnTMPyP) as photocatalyst/mediator and Pt as H2-evolution catalysts.

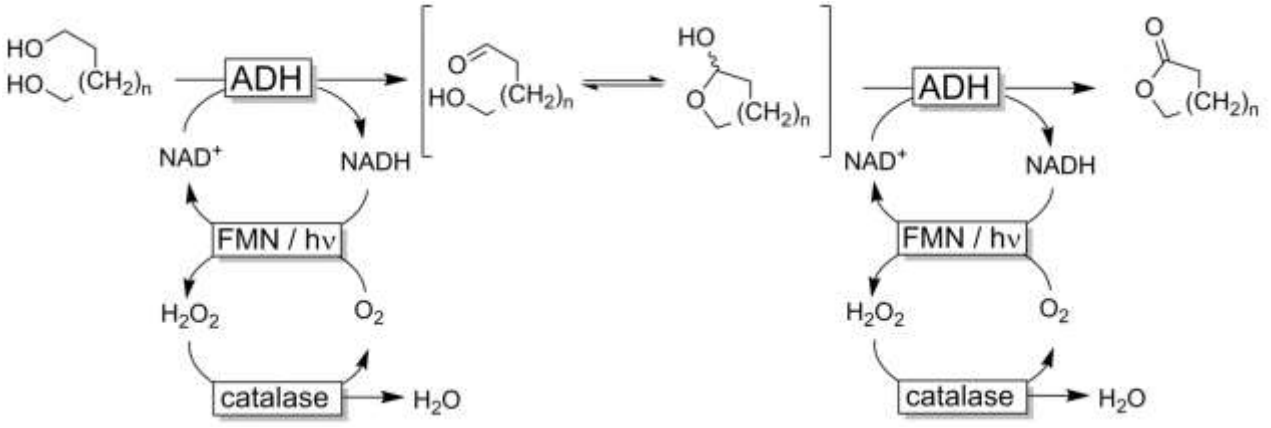
A selection of frequently used redox mediators following the reaction sequence in Scheme 3 is shown in Scheme 5.

For example, methylene blue or phenazonium methyl sulfate had been reported as very efficient NAD^+ regeneration catalyst under visible light illumination.[6] Turnover numbers (TNs) for the nicotinamide cofactor of up to 1125 had been reported, pointing towards a very efficient regeneration system. Interestingly, H_2O_2 was not detected, which the authors attribute to H_2O_2 being an even more efficient reoxidant of MBH_2 than O_2. Unfortunately, this very promising system was not followed up in later studies.



Scheme 5. Commonly used organic dyes for photoaccelerated oxidation of NAD(P)H. SnTMPyP: Sn-*meso*-tetrakis (N-methyl-4-pyridyl)porphine; FMN/Rf: Flavin mononucleotide / riboflavin; MB: methylene blue.

More recently we reported that visible light significantly accelerated the well-known aerobic reoxidation of both NADH and NADPH by simple flavins such as FMN and riboflavin.[7] In the absence of an external (visible) light source the reaction kinetics are painfully slow necessitating stoichiometric amounts of the flavin 'catalyst' to enable reasonable overall rates. Simple illumination with a commercial white light bulb dramatically accelerated this process overall, enabling catalytic turnover of both the flavin catalysts and the nicotinamide cofactors.[8] Using this setup (chiral) lactones became accessible through oxidation of diols (Scheme 6).[9]

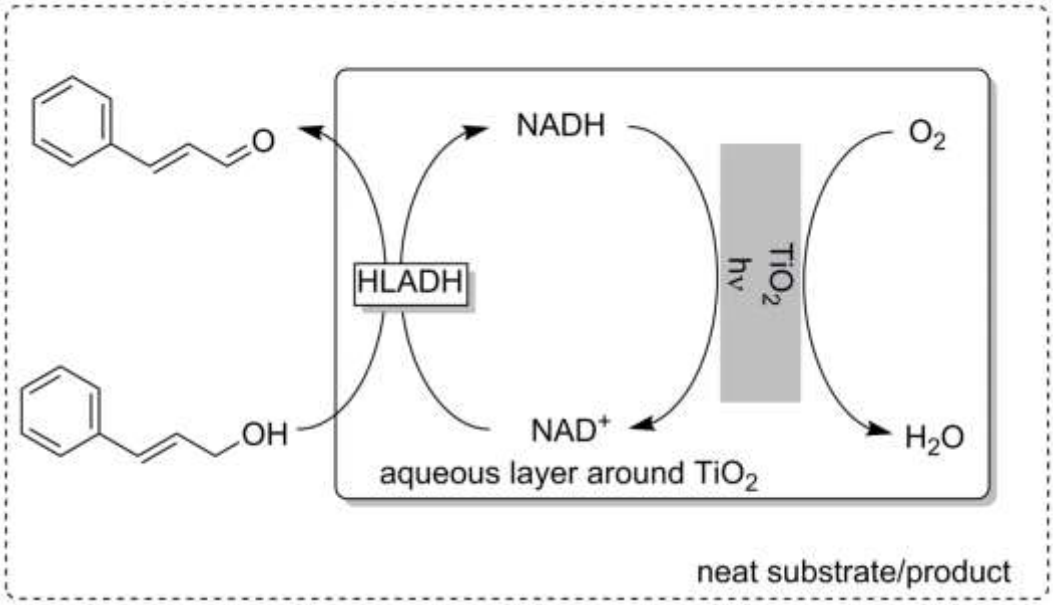


Scheme 6. Light-accelerated aerobic regeneration of NAD+ to promote ADH-catalyzed oxidative lactonization reactions.

A drawback of this system however is the formation of hydrogen peroxide, which for the sake of enzyme stability necessitated application of catalase.

Laccase mediator systems can be used for the *in situ* regeneration of $NAD(P)^+$.[9a, 10] Also here, under certain circumstances, visible light can accelerate this process.[11]

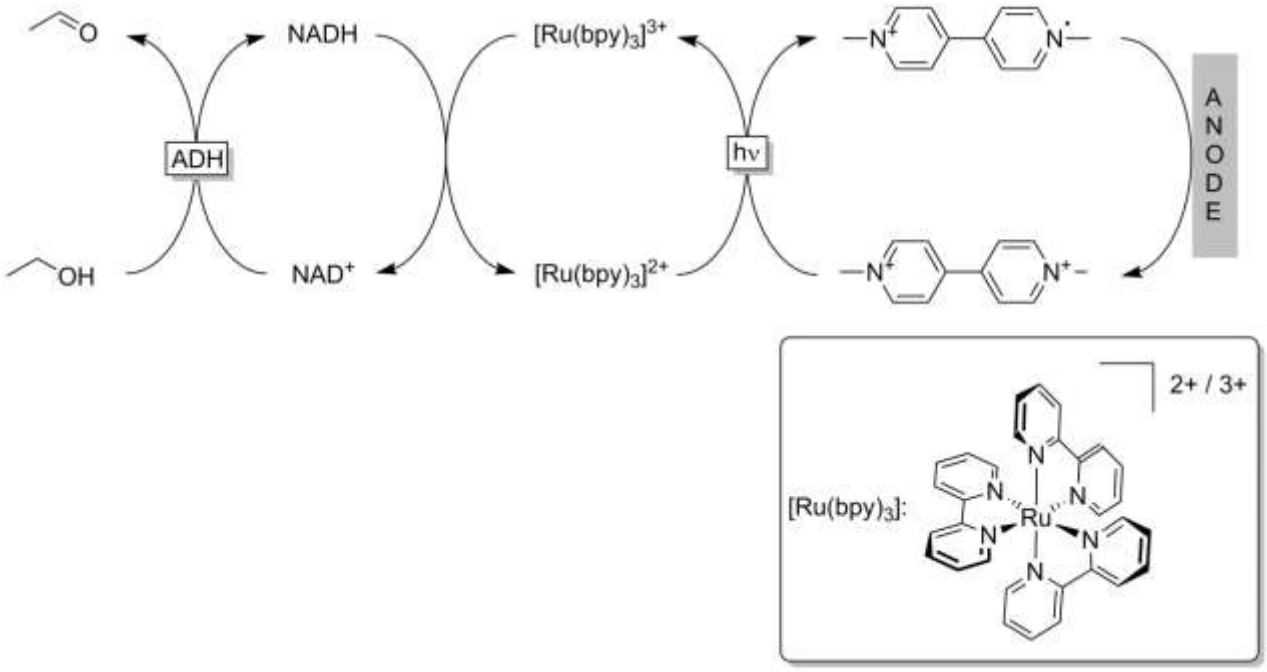
In addition to the aforementioned organic dyes, also inorganic semi-conductors have been used as photocatalysts to accelerate the (aerobic) reoxidation of NAD(P)H. For example, TiO_2 has been reported by Tanaka and coworkers for this purpose.[12] Next to its function as photocatalyst, TiO_2 also served as carrier material to immobilize the ADH (from horse liver, HLADH) and enabled using a near-neat reaction system (Scheme 7).



Scheme 7. Photocatalytic NAD^+ regeneration system using TiO_2 as regeneration catalyst.

This represents a very interesting approach, which however, has not been followed-up much in the literature. The use of TiO_2 as light-harvesting photosensitizer is limited due to its wide optical bandgap (3.2 eV) and thus restricted application under ultraviolet light less than 387 nm.

The photocatalytic NAD(P)H oxidation systems presented so far all rely on a reductive quenching mechanism. In other words, the photoexcited catalyst is able to oxidize NAD(P)H. An alternative mechanism relies on oxidative quenching; here the oxidized mediator reacts quickly with NAD(P)H and photoexcitation acts on the reduced mediator thereby facilitating its own reoxidation. Steckhan and coworkers pioneered this approach using a tribipyridylruthenium $[Ru(bpy)_3]^{2+}$ as photocatalyst / mediator. The Ru^{III} complex swiftly oxidises NAD(P)H (in an ECE mechanism) and the resulting Ru^{II} complex is photoexcited to allow for reoxidation with methyl viologen. The latter is reoxidised anodically (Scheme 8).[13]



Scheme 8. Photoelectrochemical regeneration of NAD^+ to promote ADH-catalysed oxidation reactions.

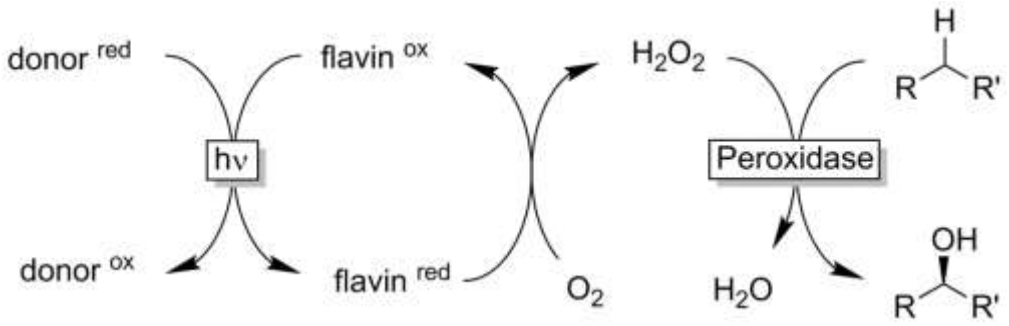
This system represents an interesting but not very practical approach (need for electrochemical and photochemical equipment, use of toxic viologenes). Furthermore, the turnover numbers (TNs) and frequencies (TFs) are too low to suggest economic feasibility.

In conclusion, a broad range of promising photochemical $NAD(P)^+$ regeneration systems have been reported. However, compared to alternative enzymatic regeneration systems they clearly fall back in terms of popularity.

2.2 $NAD(P)^+$ independent oxidative regeneration of oxidoreductases

Examples for the direct oxidative regeneration are few. Gray and coworkers reported a very interesting approach to oxidize heme-Fe(III) using a covalently attached Ru-photocatalyst.[14] This approach may be, if further developed, actually lead to O_2-independent P450-catalysis.

Another method for oxidative regeneration of P450 monooxygenases is to utilize the so-called hydrogen peroxide shunt pathway.[15] Here, the catalytically active oxyferryl species is formed directly from the resting state of the enzyme and H_2O_2, which circumvents the need of an expensive nicotinamide cofactor together with a regeneration system. The principal feasibility of this approach using photochemically generated H_2O_2 has been demonstrated.[16] So-called peroxygenases are (even more than P450 monooxygenases) of interest here as they utilize H_2O_2 as the natural oxidant.[17] The challenge to be met here is the poor stability of heme enzymes in the presence of excess H_2O_2 leading to oxidative inactivation of the prosthetic heme group.[17] We have addressed this issue via photocatalytic *in situ* generation of H_2O_2 using a flavin photocatalyst (Scheme 9).[18]



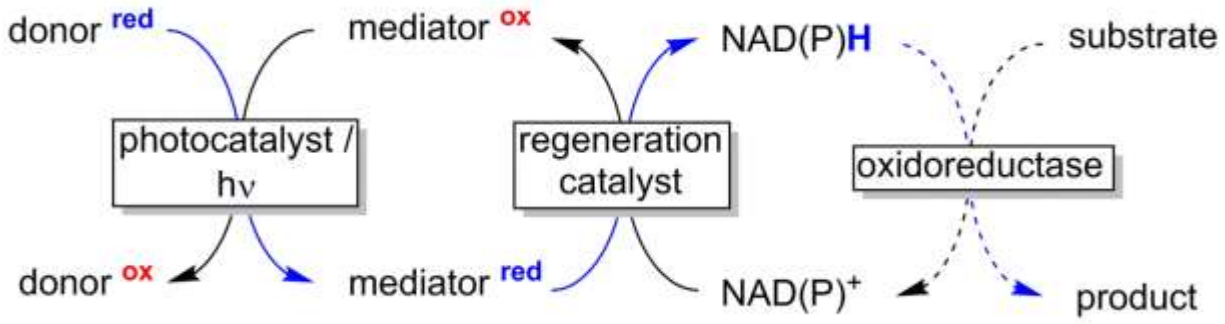
Scheme 9. Photocatalytic reduction of molecular oxygen to provide peroxidases with H2O2 for (stereoselective) oxyfunctionalization reactions.

Very promising catalytic performances (in terms of TFs and TNs of the catalysts applied) have been observed so far. These system are currently under further investigation in our laboratory and we are confident that they are going to become compatible alternatives to the established enzymatic, chemical and electrochemical systems.

3. Photocatlytic reductive regeneration

3.1 NAD(P)H regeneration

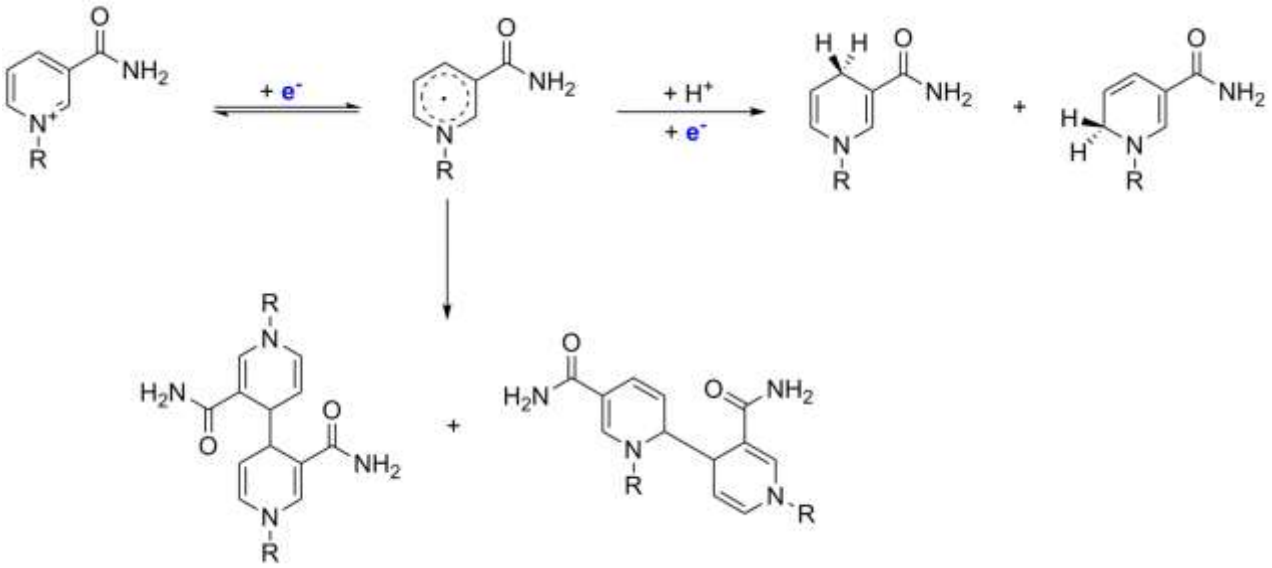
Reduced nicotinamide cofactors (NAD(P)H) play a central role in biocatalytic redox reactions. In nature NAD(P)H are the central reductants used for a vast range of different reduction and oxyfunctionalization reactions. Therefore it is also not very astonishing that *in situ* regeneration of NAD(P)H has also been in focus of research for many years now. Basically, the majority of photocatalytic NAD(P)H regeneration systems can be summarized by Scheme 10.



Scheme 10. General scheme of photocatalytic regeneration of reduced nicotinamide cofactors to promote NAD(P)H-dependent redox reactions.

A photosensitizer/photocatalyst is applied to liberate reducing equivalents from a sacrificial electron donor. The majority of sacrificial electron donors are low potential (high energy content) compounds, thus the electron transfer is thermodynamically feasible and the photocatalyst merely accelerates this step. Photosynthetic reactions, i.e. reaction schemes utilizing light energy to add thermodynamic driving force into an uphill electron transfer (e.g. from water mimicking natural photosynthesis) are very scare (*vide*

infra). Once liberated from the sacrificial electron donor, the reducing equivalents are transferred indirectly (i.e. via a mediator and a regeneration catalyst) to $NAD(P)^+$. The need for the regeneration catalyst ($NAD(P)^+$ reduction catalyst) is due to the redox chemistry of $NAD(P)^+$, which has been investigated in detail in the past for electrochemical NAD(P)H regeneration systems.[19] In essence, a sequence of single electron transfer, protonation and single electron transfer (overall corresponding to a hydride addition to $NAD(P)^+$) usually results in various undesired side reactions such as dimerization and formation of enzymatically inactive 1,2- and 1,6-isomers of NAD(P)H (Scheme 11).



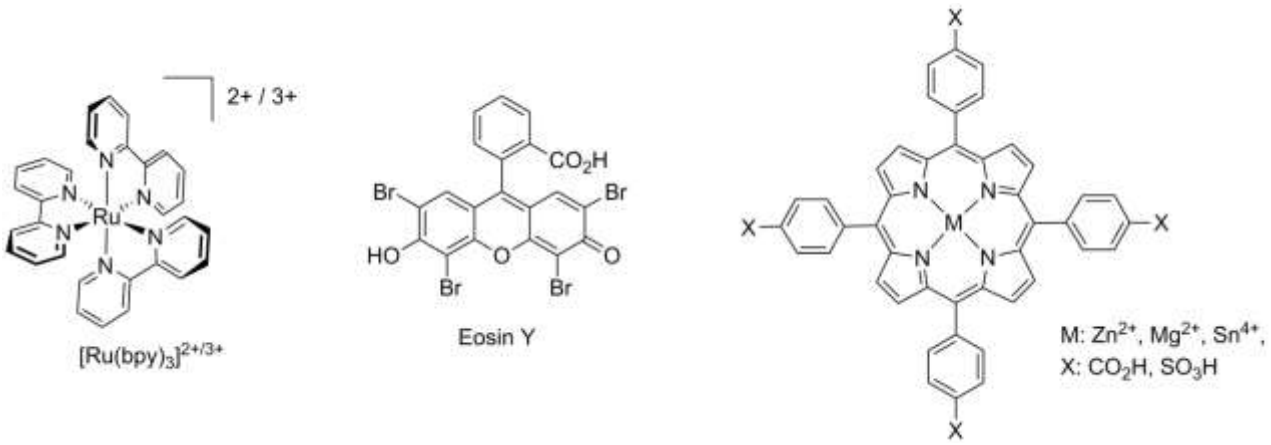
Scheme 11. Steps and products involved in the single electron reduction (either electrochemical of photochemical) of $NAD(P)^+$. The primary single electron transfer (SET) is followed by fast protonation and second SET of the intermediate radical species eventually yielding a mx of (enzymatically active) 1,4-NADH and (enzymatically inactive) 1,2- and 1-6-NADH. Also radical recombination yielding NAD dimers is observed.**[19]**

In the last three decades an enormous variety of photocatalytic NAD(P)H regeneration systems have been reported. A systematization can be made based on either the photocatalyst, the mediator, the NAD(P)H regeneration catalyst and of course based on the sacrificial electron donors used. In the following we will briefly comment on all of these aspects.

3.1.1 Photocatalysts used for NAD(P)H regeneration

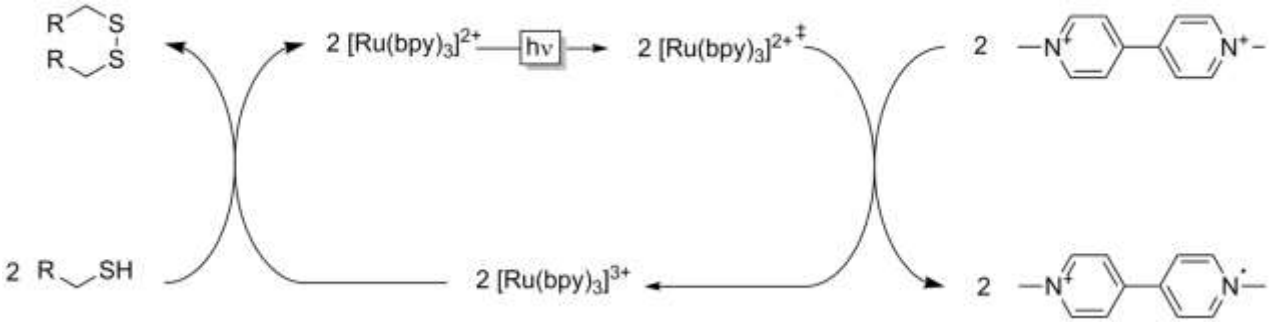
The most popular photocatalysts are (in)organic semiconductor materials, organometallic complexes and organic dyes. Scheme 12 shows the most prominent small molecules used as photosensitizers for photochemical NAD(P)H regeneration. Especially Tris bipyridine Ruthenium ($[Ru(bpy)_3]^{2+}$) has been used early on e.g. by Willner and coworkers[20] but finds renewed interest nowadays.[21] More recently, light-

absorbing organic dyes such as Eosin[22] and its derivatives,[23] proflavine,[24] oligothiophenes[25] are in focus. Similarly, arylene-vinylene polymers,[26] graphene derivates[27] and 'synthetic wood'[28] are under investigation. The same is true for 'bioinspired' porphyrin-based photocatalysts.[29]



Scheme 12. Popular photocatalysts for photochemical NAD(P)H regeneration.

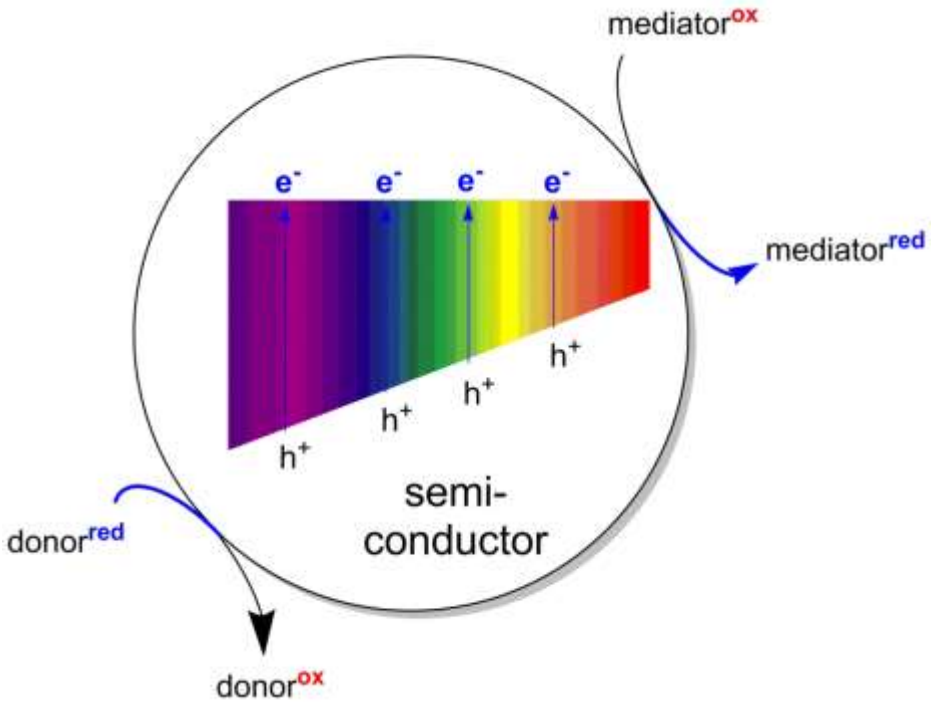
The predominant mechanism of action of the aforementioned molecular photocatalysts is oxidative quenching, i.e. upon photoactivation the reduced photcatalyst is able to reduce the intermediate electron acceptor. Scheme 13 exemplifies this at the example of the $[Ru(bpy)_3]^{2+}$-mediated electron transfer between thiols (sacrificial electron donor) and methyl viologen (mediator).



Scheme 13. General mechanism of light- and $[Ru(bpy)_3]$-promoted electron transfer from a sacrificial electron donor (e.g. thiol) to a mediator (e.g. methyl viologen). Oxidation of the sacrificial electron donor (e.g. a thiol) by the oxidized phocatalysts proceeds spontaneously. Upon absorption of a photon the resulting reduced photocatalyst is brought to an electronically excited state wherein it is able to reduce the mediator (e.g. viologen).

In parallel to the aforementioned molecular photocatalysts also semi-conductor-based photocatalysts have been developed. By far the most widely investigated photocatalyst is TiO_2 together with C-, P- or B-doped versions of it.[30] In addition, other inorganic semiconductors such as CdS[31], ZnS[32] or $W_2Fe_4Ta_2O_{17}$[33] as well as organic semiconductors such as graphitic carbon nitride [34] have been reported. The general scheme of semiconductor-based photocatalysts is depicted in Scheme 14. Interaction of the semiconductor with light leads to the promotion of an electron from the valence band into the conducting band. The

'electron hole' in the conducting band is filled by oxidation of a sacrificial electron donor (predominantly TEOA) whereas the electron promoted into the valence band eventually is transferred to the mediator.

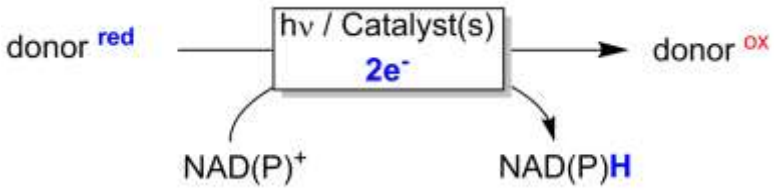


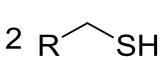
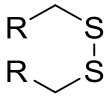
Scheme 14. Semiconductors as photocatalysts to enable the electron transfer from a sacrificial electron donor to a mediator.

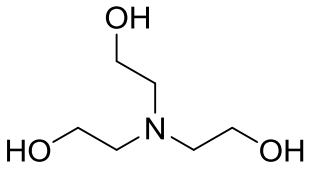
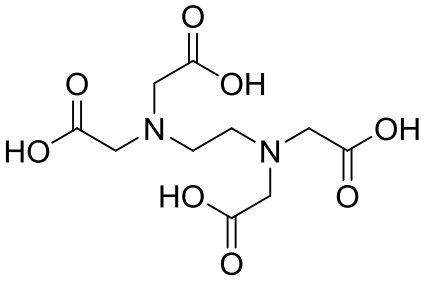
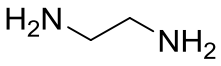
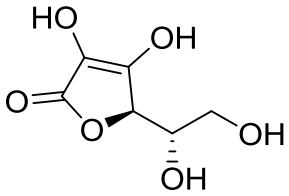
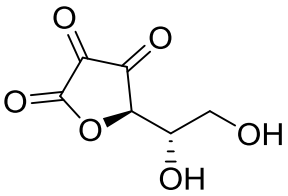
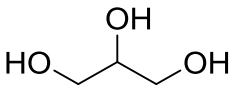
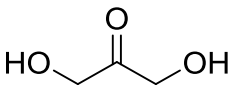
3.1.2 Sacrificial electron donors

For the catalytic reduction of $NAD(P)^+$ a stoichiometric source of electrons is necessary. For this task, two major substance classes have been established: Thiols (such as mercaptoethanol) and β-aminoalcohols and –acids (such as triethanolamine or EDTA). Next to these a few other sacrificial electron donors have been summarized in Table 1.

Table 1. Selection of most common sacrificial electron donors for the photocatalytic reduction of $NAD(P)^+$.



Donor ^{red}	**Donor ^{ox}**	**'E-factor'** $[g_{Waste} \times mol_{NAD(P)H}^{-1}]$ **Ref**
		154 (mercaptoethanol) [20]

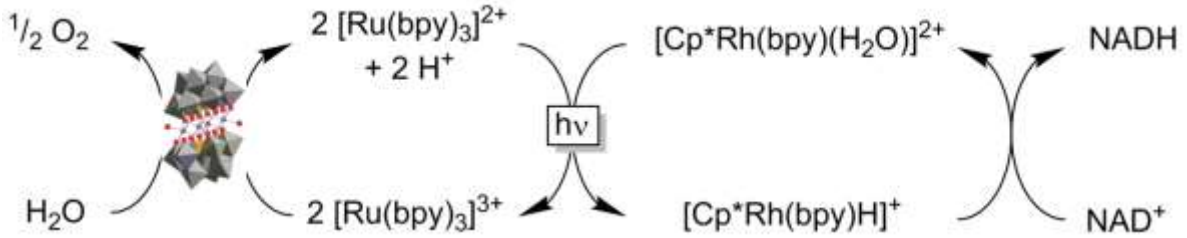
	NH_3 + 6 HCHO	65.7 [22-24, 26-27, 27c-e, 28, 29b-d, 30b, 30f, 34-35]
	 + 4 CO_2 + 4 HCHO	89 [25, 29c, 33]
		174 [29a]
		90 [32, 36]
HCO_2H	CO_2	44 [30a]
H_2O	½ O_2	32 [21]
Cathode	-	-[a] [37]

[a] No coproduct is generated. The value depends on the origin of the electrical power used.

While thiols are mostly relevant from a ‚historical' point of view, β-aminoalcohols and –acids are very popular today, possibly due to the usually high electron transfer rates observed with them. However, their use for future preparative applications has to be questioned due to several reasons. First of all these compounds are 'high energy compounds' already and therefore the overall reaction is already thermodynamically feasible. The role of the photocatalyst and of the light energy introduced into the

system therefore resides with catalysis, i.e. acceleration of a feasible reaction. Furthermore, the use of thiols or β-aminoalcohols and –acids is questionable from an atom economy point of view (i.e. generation of significant amounts of problematic by-products).

From an environmental point of view, water would be most desirable as sacrificial electron donor. Unfortunately, today, there are very few examples for this approach. Park and coworkers reported an interesting method using Co-polyoxometalates (such as $[Co_4(H_2O)_2(PW_9O_{34})_2]^{10-}$) as water oxidation catalysts together with $[Cp^*Rh(bpy)(H_2O)]^{2+}$ derivatives as NAD(P)H regeneration catalyst (*vide infra*).[21] To make the WOC-mediated oxidation of water feasible a $Ru(bpy)^{3+}$ catalyst was applied (Scheme 15) its re-oxidation was enabled after visible light-absorption. Though the turnover numbers in this system are still rather low and further shortcoming of transition metal catalysts (discussed below), this system represents an impressive proof of concept pointing towards water-driven redox biocatalysis!



Scheme 15. Coupling of water oxidation to NADH regeneration.

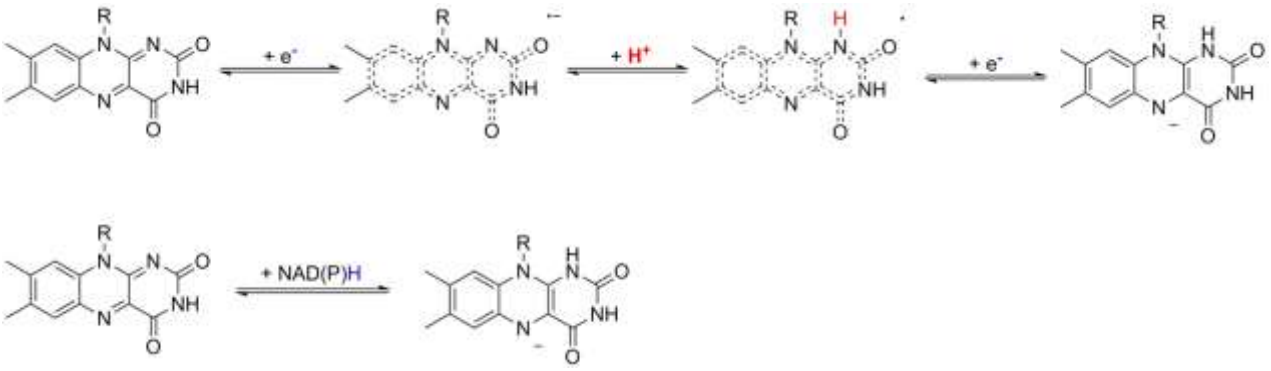
3.1.3 NAD(P)H regeneration catalysts

Once liberated from the sacrificial electron donor, the reducing equivalents have to be transferred to $NAD(P)^+$ to attain the desired *in situ* regeneration of the reduced cofactors (NAD(P)H). As mentioned above, this mostly cannot be achieved by direct electron transfer from the reduced photocatalyst to $NAD(P)^+$ as this electron transfer mostly comprises single electron transfer (SET) steps with the mechanistic challenges associated to it (Scheme 11). Therefore, a relay system transforming two SET steps and a protonation step into a single hydride step is necessary for efficient NAD(P)H regeneration. According to Steckhan such a NAD(P)H regeneration catalyst has to fulfil a range of requirements.[38] First of all, it has to act as hydride donor instead of mediating single electron transfer steps; this is to avoid NAD(P) radical formation and the undesired side reactions resulting thereof (Scheme 11). Secondly, the catalytically active form has to be formed at redox potentials less negative than the first $NAD(P)^+$ reduction potential (to avoid direct reduction of $NAD(P)^+$ by the source of reducing equivalents). Third, a successful NAD(P)H regeneration catalyst must selectively form the enzymatically active 1,4-NADH isomer only. Lastly, the NAD(P)H regeneration catalysts should not interfere with the other reactants in the system.

Today, principally two different NAD(P)H regeneration systems are available: (1) Ferredoxin-$NADP^+$ reductase (FDR) and (2) pentamethylcyclopentadienyl Rh complexes ($[Cp^*Rh(bpy)(H_2O)]^{2+}$).

3.1.3.1 Ferredoxin-$NADP^+$ reductase (FDR)

FDR (EC 1.18.1.2) is a flavoprotein whose natural role appears to be to mediate the electron transfer between ferredoxin (FD) and NADP. A catalytic flavin prosthetic group is responsible for the conversion of the two SET steps to a hydride step, which is enabled by the flexible redox chemistry of flavins (Scheme 16).



Scheme 16. Simplified flavin redox chemistry. Upper: Flavin reduction through two SET steps (via an intermediate, stabilized semiquinone radical); lower: flavin reduction through (e.g. NAD(P)H-mediated) one-step hydride transfer.

This reaction is reversible, which is why FDR mediates both the reduction of $NADP^+$ by two reduced FDs and the oxidation of NADPH by two oxidized FDs. FDR is highly specific with respect to the nicotinamide cofactor used (accepting only the phosphorylated form) but exhibits a very relaxed substrate scope with respect to the one electron donor/acceptor. Next to ferredoxin also simple metals complexes and –salts as well as a range of quinones are converted. An exception to this is the previously mentioned photocatalyst $[Ru(bpy)_3]^{2+}$. Therefore, all photocatalytic systems using FDR as NADPH regeneration system utilize additional mediators (facilitating the electron transfer from the reduced photocatalysts to FDR).[20, 25, 29a, 30a] Amongst them methyl viologen, as pioneered by Willner and coworkers, is by far the most popular and efficient one.[20, 25, 29a, 30a] Very promising catalytic turnovers of the single catalysts have been reported as early as the 1980s reaching several thousand for $[Ru(bpy)_3]^{2+}$ and hundred thousand for FDR. The turnover numbers for NADP as well as the viologen mediator are less promising ranging from a few dozen to hundreds. Possibly these lower numbers together with the high toxicity of viologenes and the limitation to FDR to NADPH-regeneration explains why at present this approach is not the dominating one.

3.1.3.2 $[Cp^*Rh(bpy)(H_2O)]^{2+}$

The organometallic compound $[Cp^*Rh(bpy)(H_2O)]^{2+}$ has been reported as early as the 1980s by Steckhan and coworkers to be an efficient catalysts for indirect electrochemical regeneration of NAD(P)H.[39] Several features make $[Cp^*Rh(bpy)(H_2O)]^{2+}$ and its derivates interesting catalysts for the photochemical regeneration of NAD(P)H: (1) first of all $[Cp^*Rh(bpy)(H_2O)]^{2+}$ is capable of both single electron and of hydride transfer reactions. Hence, it can accept two successive electrons (and one proton) from an electrochemical or an photochemical cathode forming a hydrido species ($[Cp^*Rh(bpy)H]^+$) serving as hydride reductant for $NAD(P)^+$; thereby avoiding NAD(P) radicals and the undesired side reactions related to it (Scheme 11). (2) Furthermore, the active hydrido species coordinates to the carbonyl group of $NAD(P)^+$ and thereby is positioned close to the C4-atom resulting in highly regioselective hydride transfer and minimizing the undesired formation of enzymatically inactive 1,2- and 1,6-NAD(P)H isomers.[40]

Therefore, it is not very astonishing that $[Cp^*Rh(bpy)(H_2O)]^{2+}$ is frequently used as NAD(P)H regeneration catalyst.[21, 22b, 22c, 23-24, 26-27, 29b, 29c, 30b-d, 30f, 31-35, 37, 41]

Despite its great success in non-enzymatic NAD(P)H regeneration, it should be mentioned here that $[Cp^*Rh(bpy)(H_2O)]^{2+}$ exhibits some significant drawbacks that on the long term may severely limit its practical usefulness. First of all, like many transition metals, the Rh central atom is rather expensive (and future projections of the Rh prices point towards even higher prices). At the same time, turnover numbers reported for this catalyst (so far) range between 10 and 1000. As a consequence, the cost contribution of this catalyst alone to the final product is very significant. Certainly, cheaper catalysts (e.g. based on abundant metals) with improved catalytic performance would give the field a fresh impetus! Another issue related to $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (and other transition metals) is the frequently observed mutual inactivation of the metal catalyst and the biocatalyst needing further attention.[42] In this respect, new catalysts such as Cobaloximes may become interesting in the future.[22a]

Finally, Table 2 gives a representative overview over the performance of some photobiocatalytic systems involving NAD(P)H regeneration. Especially comparing the TN reported for the nicotinamide (but also for the photo- and NAD(P)H-regeneration catalysts) are still orders of magnitude away from economic feasibility.

Table 2. Selection of photochemical NAD(P)H regeneration systems.

Photosensitizer	**Mediator**	**Electron donor**	**NAD(P)H regeneration rate [mM h^{-1}]**	**TN (NAD(P))**	**Ref**
Eosin Y	$[Cp^*Rh(bpy)(H_2O)]^{2+}$	TEOA	1.42	23	[22b]
ZnTPPS	$[Cp^*Rh(bpy)(H_2O)]^{2+}$	TEOA	0.23	n.d.	[29b]
$Ru(bpy)_3^{2+}$	FDR	Mercaptoethanol	n.d.	15	[20c]

CCGCMAQSP	$[Cp^*Rh(bpy)(H_2O)]^{2+}$	TEOA	0.091	89	[27a]
Graphene-BODIPY	$[Cp^*Rh(bpy)(H_2O)]^{2+}$	TEOA	0.11	116	[27d]
Proflavin	$[Cp^*Rh(bpy)(H_2O)]^{2+}$	TEOA	1.28	33	[24]
Synthetic wood	$[Cp^*Rh(bpy)(H_2O)]^{2+}$	TEOA	0.28	4.5	[28]
Carbon nitride	$[Cp^*Rh(bpy)(H_2O)]^{2+}$	TEOA	2	5	[34b]
H-SiNWs	$[Cp^*Rh(bpy)(H_2O)]^{2+}$	TEOA	0.4	1.4	[35]
CdS / TiO_2	$[Cp^*Rh(bpy)(H_2O)]^{2+}$	TEOA	0.25	3.6	[30f]
SnC	$[Cp^*Rh(bpy)(H_2O)]^{2+}$	EDTA	0.056	1.0	[29c]

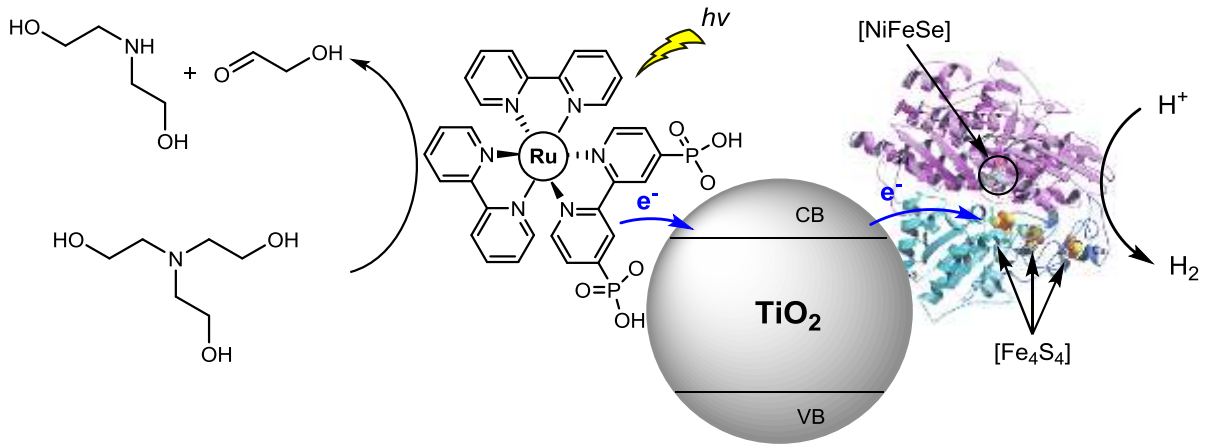
TEOA: triethanolamine; CCGCMAQSP: chemically converted graphene coupled with multianthraquinone-substituted porhyrin; FDR: Ferredoxin-$NADP^+$ reductase; BODIPY = (1-picolylamine-2-aminophenyl-3-oxy-phenyl-4,40-difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-s-indacene-triazine); H-SiNWs= hydrogen-terminated silicon nanowires; SnC = tin(IV)-meso-tetrakis(N-methylpyridinium)-chlorin

4. Direct regeneration of oxidoreductases

NAD(P)H is the most important redox mediator in all living systems. However, many oxidoreductases do not directly rely on NAD(P)H as reductant. For example, monooxygenases utilize NAD(P)H to generate reduced species (e.g. reduced heme- or flavin-species) that activate molecular oxygen for the actual oxygenation reaction. Hence, NAD(P)H 'only' serves as reductant and is mostly not directly involved in the catalytic mechanism and substitution of NAD(P)H by artificial reduction catalysts appears a straightforward method to substantially simplify the catalytic mechanism.

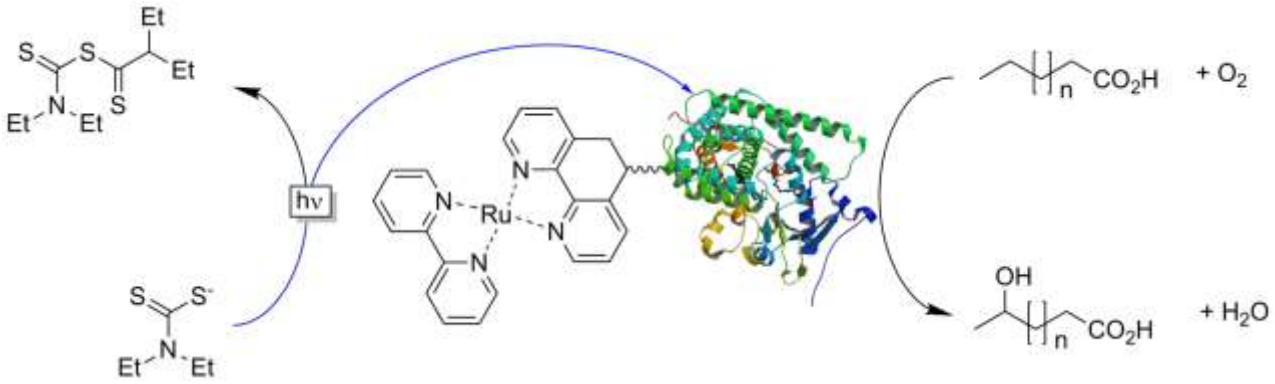
For this purpose two catalyst system have been evaluated: (1) flavins and (2) Ru complexes, which will be discussed in somewhat more detail below. Very recentlty, Park and coworkers reported on eosin Y to directly regenerate P450 BM3.[43]

Reisner and coworkers developed a photocatalytic hydrogen evolution system in which a [NiFeSe]-hydrogenase was attached directly on Ru dye-sensitised TiO_2 nanoparticles (Scheme 17). [44] Other photosensitizers such as polymeric carbon nitride, eosin Y and cyanobacterial photosystem II were also utilized to directly regenerate the hydrogenase. [45]



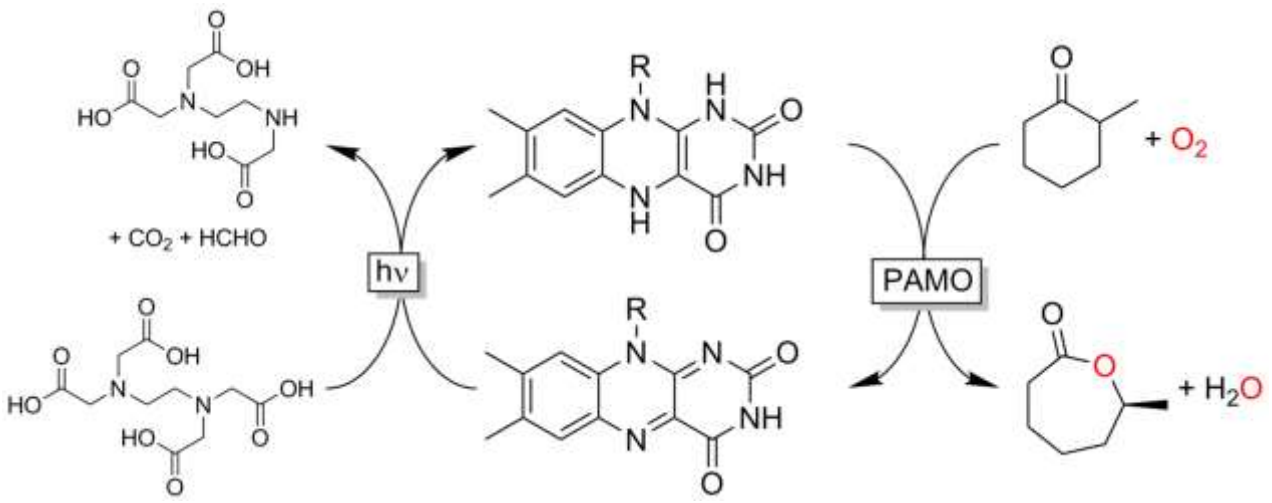
Scheme 17. Schematic representation of photocatalytic hydrogen generation with a [NiFeSe]-hydrogenase and Ru dye-sensitized TiO_2.

Cheruzel and coworkers have extensively studied various Ru(II) photosensitizers to directly reduce heme groups in P450 monooxygenases for catalysis (Scheme 17).[46] Variations of the Ru(II)-photosensitizers described above were covalently linked to the heme-dependent mononooxygenase from *Bacillus megaterium* (P450 BM3), which enabled efficient direct electron transfer from (*in situ* photogenerated Ru(I)) to the heme-iron.



Scheme 17. Direct photochemical reduction of the heme group of P450 BM3 to achieve reductive activation of molecular oxygen for catalysis.

Alternatively, flavins (such as riboflavin, flavin mononucleotde, FMN or flavin adenine dinucleotide, FAD) can also be used as photosensitizer/reductant for enzymes. Flavins are a class of biological cofactors which act as a redox center for many oxidoreductases. They themselves are photoexcitable under visible light and thus can act as photosensitizers for the reductive regeneration of flavin-containing enzymes for catalysis. This was first demonstrated by us, using a flavin-dependent monooxygenase (phenylacetone monooxygenase, PAMO).[47] Upon photoexcitation, flavins are capable of utilizing simple reductants e.g. mentioned in Table 1 and transfer the electrons to the enzymes' active sites (Scheme 18).[48]



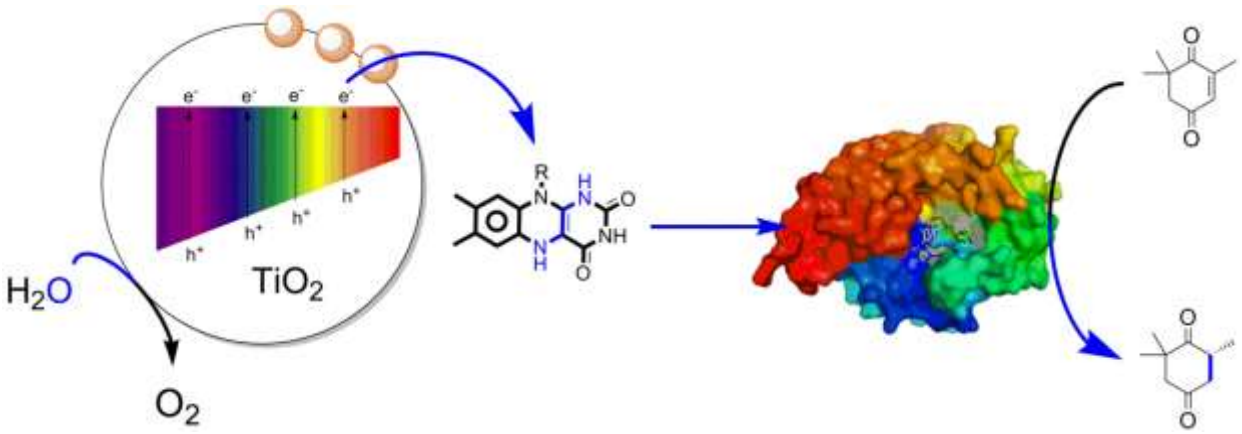
Scheme 18. Using FAD as photosensitizer to couple EDTA oxidation to a monooxygenase reaction.

Even though the principal feasibility of this setup could be demonstrated with promising conversions and enantioselectivities, the performance of the artificial system fell back significantly behind the performance of the natural cycle using NADPH. Probably oxidative decoupling of the regeneration reaction from the enzymatic reaction due to the rapid reaction of reduced flavin species with molecular oxygen account for the poor performance. [16a, 49] In fact, less than 10% of the electrons provided by the sacrificial electron donor (EDTA) were used productively i.e. for the enzymatic Baeyer-Villiger oxidation. The majority of reducing equivalents was channelled into direct oxygen reduction yielding H_2O_2. A possible solution to this *Oxygen Dilemma* may be to use deazaflavins. [50] Alternatively, the *Oxygen Dilemma* can also be used productively by using the H_2O_2 generated to promote peroxygenase-catalyzed oxyfunctionalization reactions (see above).

A third alternative is to simply circumvent the *Oxygen Dilemma* by utilizing O_2-independent enzymes such as Old Yellow Enzymes (OYEs) for enantioselective reduction of conjugated C=C-bonds.[51] Members of this enzyme class contain a catalytic flavin (FMN) in their active site, which in its reduced form performs a Michael-type hydride addition to conjugated C=C-double bonds. Various studies have demonstrated that the native nicotinamide cofactor may be substituted by other reductants such as viologenes,[52] 'smart cosubstrates', [53] chemically reduced flavins[54] or synthetic nicotinamide analogues.[55]

The reduction of OYEs using flavins as photocatalysts and electron mediators was established using EDTA as sacrificial electron donor.[47b, 56] Indeed, it could be demonstrated that the electron transfer yield (i.e. the percentage of electrons used productively) in the absence of molecular oxygen was close to the theoretical value. Furthermore, the enzyme properties (such as enantioselectivity or enzyme activity) was apparently not impaired by the 'unnatural' reaction conditions. One drawback from an environmental point of view however was the nature of the byproducts formed (see Table 1). However, very recently, we could

demonstrate that productive coupling of the OYE-catalyzed reduction reaction to a photochemically operated water oxidation catalyst is feasible (Scheme 19).



Scheme 19. Photochemical water oxidation used to promote biocatalytic reduction reactions.

5. Conclusions

Photobiocatalysis is a dynamic and rapidly evolving area of research. As outlined in this contribution, many different approaches are currently explored by various research groups.

The scope of photobiocatalysis lies in more robust and simplified regeneration schemes; especially if shortened electron transport chains are enabled by direct regeneration of the oxidoreductases' active sites. Today, however, the traditional enzymatic regeneration systems still outperform their photochemical counterparts in terms of turnover numbers of the catalytic components used. We need to intensify our research efforts in order to improve the efficiency of the reaction systems. A particular focus here should lie on (1) improving the turnover numbers of the catalysts used (only if the catalysts are used efficiently, i.e. if their cost contribution to the final product is sufficiently low, 'real life' application of the systems developed will occur) and (2) increasing the reagent payload (today, most reports deal with low substrate concentrations around 10 mM, which clearly is orders of magnitude away from practical feasibility). The time for proof-of-concept studies is slowly running out; now it is time to make the systems truly practical!

6. Acknowledgements

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