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4.1 Introduction

Flavins **1** are naturally occurring compounds with an isoalloxazine skeleton, which act as cofactors in various enzymes, mainly in the form of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (Figure 4.1a) [1, 2]. Studies aimed at the genetic reconstruction of the last universal common ancestor and its basal metabolism suggest that flavins belong to the most ancient enzyme cofactors [3]. An ancestral origin may also be recognized from the adenosine-5′-monophosphate (AMP) moiety of FAD, which could be a vestige of the RNA world. Also, the isoalloxazine ring itself may have evolved from RNA-related building blocks (Figure 4.1b) [4]. The unique structure of the isoalloxazine ring and its variable reactivity result in the exceptional versatility of the flavins and flavoenzymes in different types of reactions, such as oxidations, oxygenations, and reductions, but also, for example, in halogenations or photocycloelimination reactions [1a, 5]. Moreover, the flavins family described in literature contains a vast number of derivatives, some of which occur in nature, some are synthetic.

In addition to their oxidized form (**1**), flavins (isoalloxazines) can also exist in the form of a stable radical (semiquinone) ($\mathbf{1_{H\bullet}}$) and their fully reduced form ($\mathbf{1_{H2}}$) (Scheme 4.1) [1a], and, thus, easily undergo one or two electron redox reactions. In addition, all redox forms can be subject to acid–base equilibria due to the existence of basic and acidic centers. In their electron-poor oxidized form, flavins are readily attacked by nucleophiles, while the electron-rich reduced form reacts with electrophiles. Flavins are also photoactive substances that typically absorb light at ~450 nm (see Chapter 3). In nature, flavins participate in the function of photoreceptors and are also involved in some photochemical reactions [6]. Excitation further enhances the ability of flavins to act as oxidizing or reducing agents. Therefore, flavins are frequently used in photocatalysis [7].

The properties of flavins can be modified upon changing their structure. For example, deazaflavins **2**, which are in nature represented by the deazaflavin

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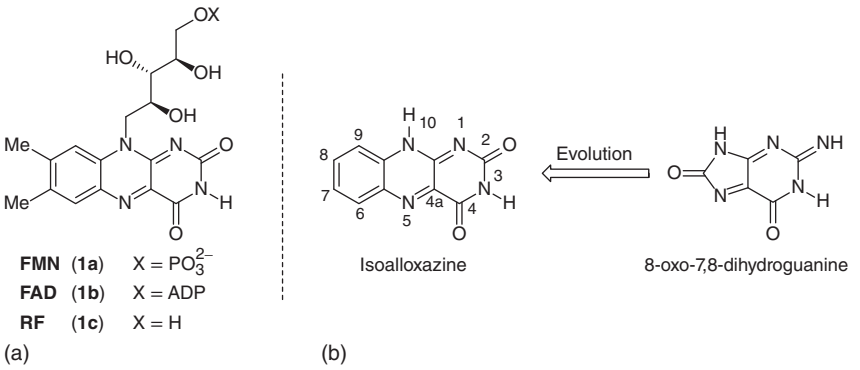
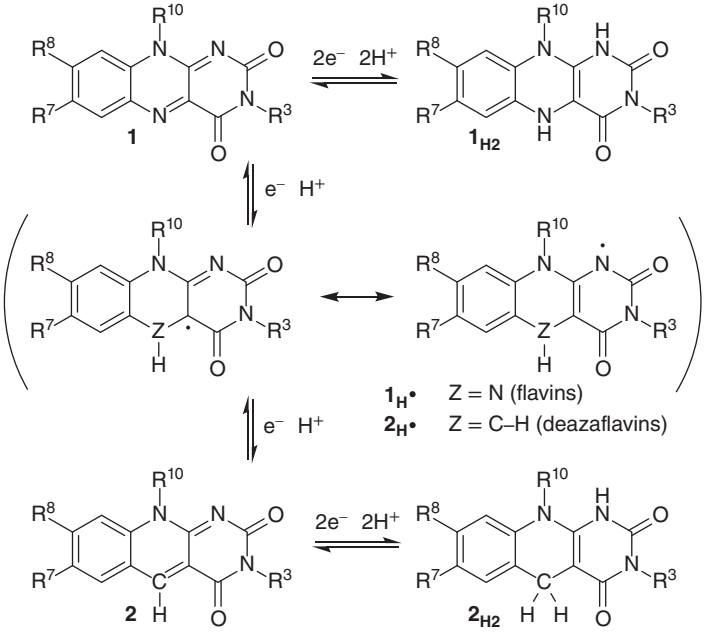


Figure 4.1 The structure of flavin (isoalloxazine) natural compounds (a), isoalloxazine ring with numbering and its prebiotic redox model (b) Source: Walsh and Wencewicz [1], Fraaije and Mattevi [1], and Fagan and Palfey [2]. (b) Modified from Nguyen and Burrows [4].



Scheme 4.1 The redox forms of flavins **1** and deazaflavins **2**. Source: Modified from Walsh and Wencewicz [1a].

cofactors F_0 or F_{420}, have a carbon atom instead of nitrogen atom in position 5 of the isoalloxazine nucleus [8]. Deazaflavins have a less stable semiquinone $\mathbf{2_{H\bullet}}$ and thus predominantly catalyze two-electron redox processes (see Chapter 2). The reduced forms of deazaflavins $\mathbf{2_{H2}}$ are excellent hydride donors and behave more like NAD(P)H analogues and not as flavins with a native isoalloxazine nucleus [8a, 9]. Another example of flavin derivatives are isomeric alloxazines **3** (Figure 4.2),

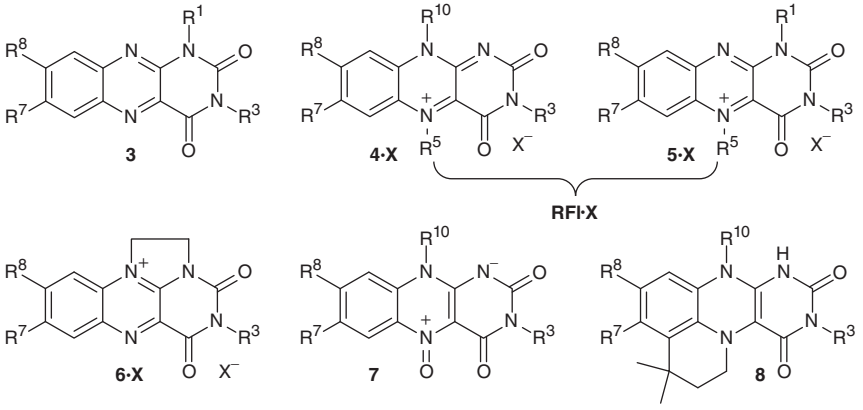


Figure 4.2 The structure of selected flavin derivatives important for catalysis in natural and artificial systems. **RFl•X** is often-used abbreviation for 5-alkylisoalloxazinium **4•X** and 5-alkylalloxazinium salts **5•X**. R means substituent in position 5.

which substantially differ from isoalloxazines **1** in terms of their photochemical properties (see Chapter 3) [10].

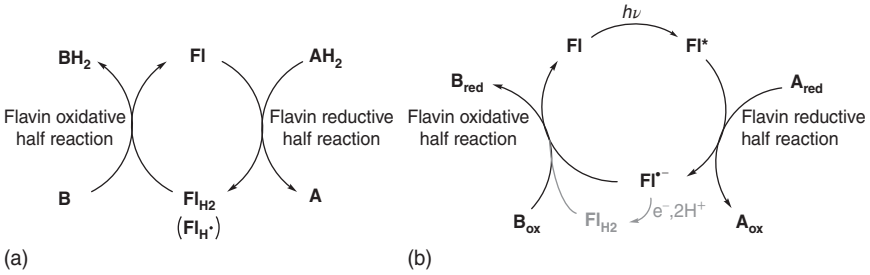
The reactivity of flavins is often modified by substitution at the C7 and/or C8 position or by changing the isoalloxazine skeleton [11]. For example, the introduction of a quaternary center (see flavinium salts **4•X**–**6•X**, Figure 4.1), which increases the reactivity toward nucleophiles, has been crucial for the development of flavin-based organocatalysis (see Chapter 5) [12]. Another approach is to design supramolecular artificial systems with flavin reactivity modified in an analogous manner to enzymes, i.e. hydrogen bonding or aromatic π–π stacking interactions (see Chapter 1) [13]. Alternatively, flavins have been attached to solid surfaces or incorporated in polymeric structures (see Chapter 6) [14].

Flavin cofactors are tightly but noncovalently bound in most flavoenzymes. Their properties such as electrochemical potential, acid–base equilibria, or stability are strongly influenced by their protein microenvironment [5a, 15]. On the other hand, a significant number of flavoenzymes harbor a covalently protein-bound flavin cofactor. Such covalent tethering has been found to tune the reactivity of the flavin so that it fulfills its catalytic role [16]. For example, it has been shown that a covalent attachment increases the redox potential, allowing enzymes to oxidize compounds that otherwise cannot be oxidized [17]. C8 modifications have also been recently shown to be essential in boosting enzyme activity [18] and (an additional) C6 covalent attachment is crucial for protecting the inactivation of a flavoenzyme and/or productive substrate positioning [19]. The recently reported N5-oxide **7** is another nice example by which nature has broadened the reaction scope of flavoenzymes [20]. An even more drastic modification is the prenylation of FMN (see structure **8** in Figure 4.2) for which a dedicated flavin-prenylation enzyme machinery has evolved [21]. The prenyl-FMN cofactor allows enzymes to act as (de)carboxylases. Clearly, nature has exploited chemical modifications to enlarge the catalytic space that a flavin cofactor can cover (see Chapter 2 for details).

The aim of this chapter is to draw attention to the ways in which members of the flavin family are involved in catalysis, regardless of whether the reactions take place in enzymes or reactions driven by artificial catalytic systems (see Section 4.2). However, due to the limited space, only the most important flavin functions have been selected. For a more detailed overview, we refer to the relevant chapters in this book focused on the individual types of bio-, organo-, or photocatalysis. For catalytic systems, in addition to the action of the catalyst itself, its regeneration closing the entire catalytic cycle is also essential. If the catalyst is not restored, the process stops after the first cycle. Therefore, Section 4.3 of this chapter deals with the regeneration of flavin catalysts. Finally, Section 4.4 shows examples of how the various types of flavin catalysis can inspire different fields of catalysis.

4.2 Modes of Catalysis

In redox reactions, flavins **Fl**[1] operate in each catalytic cycle via two half-reactions (Scheme 4.2a). In the reductive half-reaction, the substrate is oxidized, while the flavin is reduced. In the oxidative half-reaction, another substrate is reduced and the flavin goes back into its oxidized form [1a, 5]. In principle, only one half-reaction provides the key chemical transformation, while the other serves to regenerate the flavin catalyst. For example, after oxidation of the substrate (alcohol to form a carbonyl compound) by the flavin species, the reduced flavin is reoxidized by oxygen. In addition to the reactions in the ground state, there are catalytic processes involving flavins in their excited state, in which flavin excitation by a suitable light source precedes the key redox process (Scheme 4.2b) [7a]. While formal two-electron oxidation and reduction reactions prevail in the absence of light, single-electron processes are mainly involved in photocatalysis.



Scheme 4.2 Half-reactions in flavin (**Fl**) catalysis under dark conditions (a) and flavin photo(bio)catalysis (b). In light-dependent processes also excited fully reduced flavin or excited semiquinone can be involved, which is not shown here (see Section 4.2.1.2). Flavinium salts $\mathbf{Fl}^+$ can also participate both in light-dependent catalysis and catalysis under dark conditions. Source: (b) Modified from König et al. [7].

1 **Fl** refers to all neutral flavin derivatives in this Chapter, while $\mathbf{Fl}^+$ means positively charged flavinium species.

Redox reactions represent the vast majority of flavin-catalyzed transformations. However, there are some flavin-based nonredox processes such as the photocycloaddition of alkenes to cyclobutanes using a flavin photosensitizer [22] and iminium catalysis with FMN in UDP-galactopyranose mutase [23]. Also, there are procedures involving electron transfer, but they are not redox reactions overall,[2] such as the cycloreversion of cyclobutane to form alkenes in photolyases [24].

In most catalytic systems, the flavin catalyst is directly involved in the key transformation, whether it exchanges a hydride, oxygen atom, electron, or energy (when excited) with the substrate. Very often, flavins form covalent bond(s) with the molecule to be transformed (see Section 4.2.1). On the other hand, there are also processes where the flavin catalyst generates a reactive agent, which is responsible for the key reaction. The latter case is discussed separately in Section 4.2.2.

4.2.1 Reactions Based on the Direct Transformation of a Substrate by Flavin

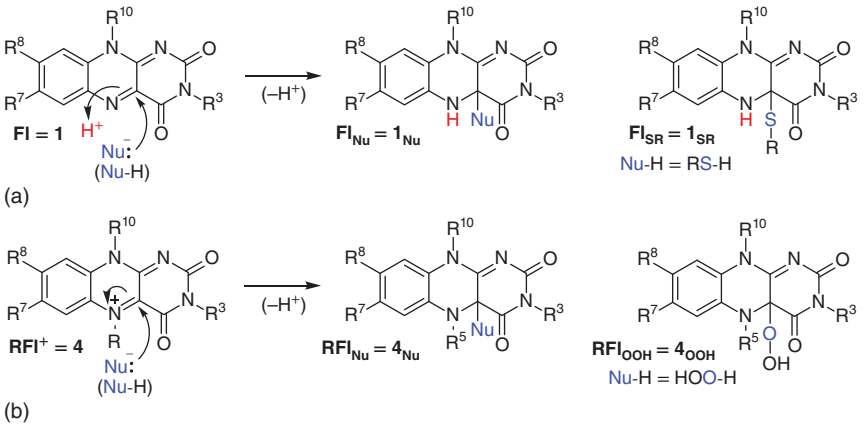
4.2.1.1 Organocatalysis and Biocatalysis in the Absence of Light

In flavin-based catalytic transformations proceeding under dark conditions, most substrates interact with the flavin moiety via a covalent bond, and thus, they are activated for the subsequent transformation. Eventually, the flavin is primarily activated, which is followed by an atom exchange with the substrate. For this purpose, the original flavins **1** with isoalloxazine core have two reactive positions at C4a and N5.

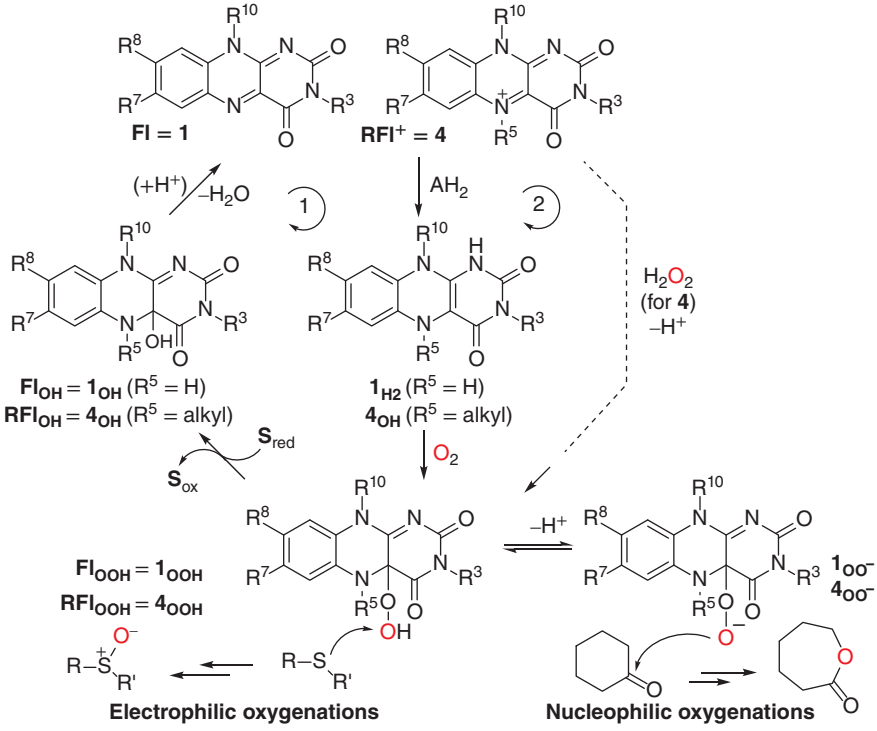
The C4a-position of oxidized flavin cofactors is subject to the reversible addition of strong nucleophiles such as thiolate to form an alkylsulfanyl adduct, an intermediate in dithiol oxidases/disulfide reductases (Scheme 4.3a) [25]. Analogously, the C4a-position of 5-alkylisoalloxazinium **4** and 5-alkylalloxazinium **5** species reacts with nucleophilic species such as amines, hydroxide, and hydrogen peroxide (Scheme 4.3b) [26]. The latter gives 5-alkylflavin-4a-hydroperoxides $\mathbf{RFl_{OOH}}$, which act as an oxidizing agent in artificial oxygenases (see Scheme 4.4) [12, 27]. The same species can also be formed upon the reaction of 5-alkyldihydroflavins $\mathbf{RFl_{red}}$ with oxygen, which is analogous to natural systems (Scheme 4.4, cycle 1). It should be noted that the reaction of reduced flavin cofactors with oxygen occurs via a two-step mechanism involving electron transfer, followed by radical recombination (see Chapter 1 for reactions with nucleophiles) [28].

The stability of 5-unsubstituted "natural" flavin-4a-hydroperoxide $\mathbf{Fl_{OOH}}$ differs significantly outside and inside an enzyme as it is stabilized by a protein environment [29]. This is the reason why 5-alkyl analogues $\mathbf{RFl_{OOH}}$ are used in artificial systems [30]. Nevertheless, the stabilization of nonalkylated flavin-4a-hydroperoxide has been recently achieved also in an organocatalytic process [31]. In addition to flavin-4a-hydroperoxides, the N5-peroxide involved in the enterocin biosynthetic enzyme [32] and flavin-10a-hydroperoxide [33] used in organocatalysis should be mentioned.

2 Such reactions belong to "redox neutral reactions," i.e. reactions that do not need external reducing or oxidizing agent.



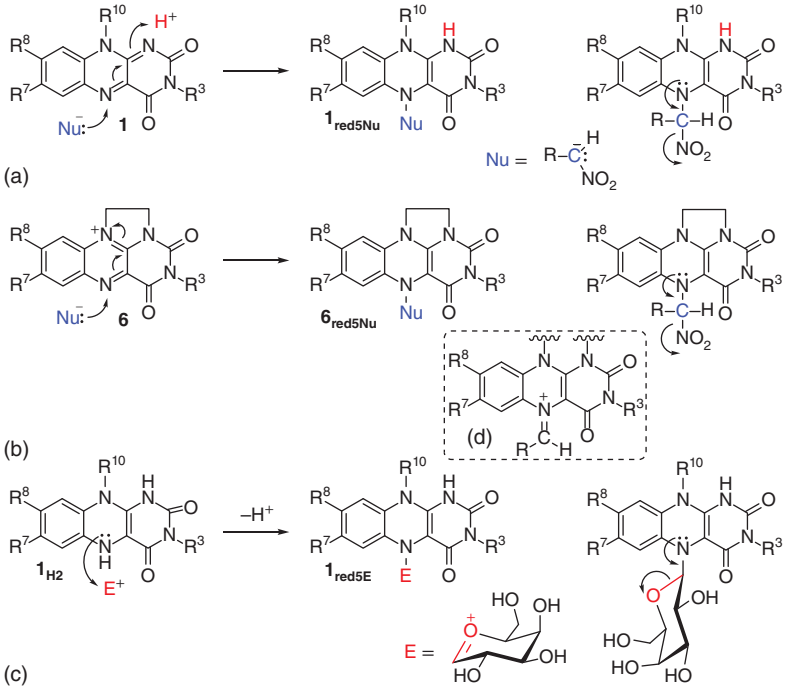
Scheme 4.3 The addition of nucleophiles to flavin (isoalloxazine) **1** (a) or flavinium (isoalloxazinium) **4** (b) with important adduct examples. Addition to alloxazinium **5** is not shown here. Source: (a) Modified from Schulz et al. [25]. (b) Chan and Bruice [26], Kemal and Bruice [26], and Hoegy and Mariano [26].



Scheme 4.4 Catalytic cycles with oxygen (cycle 1) and hydrogen peroxide (cycle 2), and an overview of monooxygenation reactions occurring with flavin **1** and isoalloxazinium **4** in natural and artificial systems. Analogous catalysis can be done also with alloxazinium **5** (not shown here). Source: Gelalcha [27], Iida et al. [12], Cibulka [12], and de Gonzalo and Fraaije [12].

Flavin-4a-hydroperoxides are involved in biocatalytic and organocatalytic monooxygenation reactions taking place via both electrophilic and nucleophilic mechanisms (see Chapters 5 and 7) [27]. In electrophilic oxygenations, the oxygen atom in flavin hydroperoxide (R)$\mathbf{Fl_{OOH}}$ is attacked by an electron-rich substrate, such as an organic sulfide, tertiary amine, alkene, or arene, which are transformed into sulfoxides, *N*-oxides, epoxides, or hydroxyarenes. During nucleophilic oxygenation, deprotonated flavin hydroperoxide (R)$\mathbf{Fl_{OO-}}$ attacks the electron-poor center in ketones resulting in a Baeyer–Villiger oxidation reaction (Scheme 4.4).

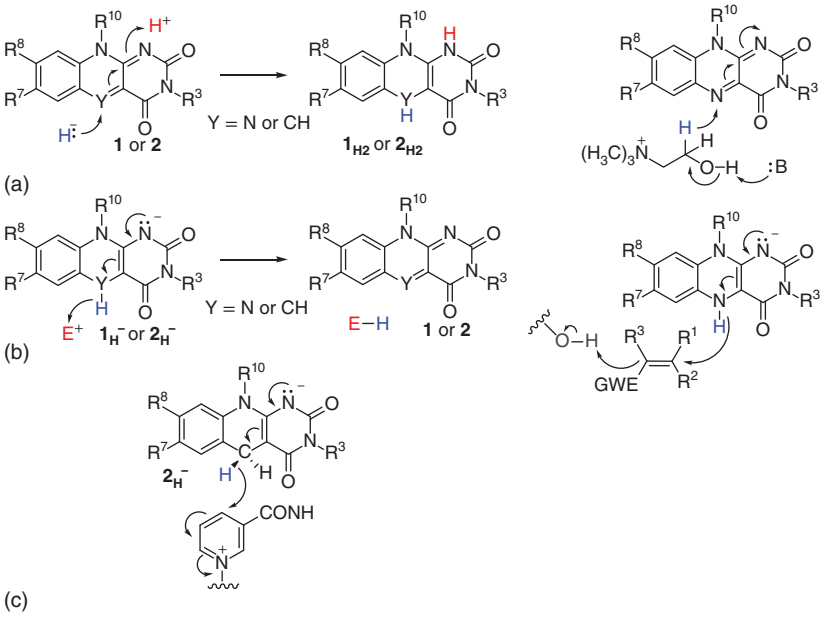
The N5-position in neutral flavins **1** is subject to carbanion addition reactions, for example in nitroalkane oxidase; a nitroalkane anion forms an adduct with the flavin (Scheme 4.5a) [34]. Another rare case in which a flavoenzyme catalyzes its reaction via formation of a N5 adducts through a nucleophilic attack was found for alkyldihydroxyacetonephosphate synthase [36]. An analogous addition reaction was observed in an artificial system using ethylene-bridged flavinium ion **6** (Scheme 4.5b) [35], which is also able to add other nucleophiles such as triphenylphosphine [37]. Upon the carbanion addition reaction, the attached carbon atom is formally oxidized, forming an aldehyde after the nitrite anion leaves and



Scheme 4.5 The reactivity of the flavin N5-position in biocatalytic (a) and organocatalytic (b) transformations toward nucleophiles. The reduced flavin cofactor has been found to react with electrophiles (c). Activation via N5 usually leads to iminium intermediate (d). Source: (a) Modified from Fitzpatrick [34]. (b) Modified from Thapa et al. [35]. (c) Modified from Zhang and Liu [23].

undergoes hydrolysis, thus completing the Neff reaction. N5 adducts can also be formed from the reduced flavin upon the reaction with an electrophile as proposed in the UDP-galactopyranose mutase mechanism (Scheme 4.5c) [23]. The adduct with galactopyranose is attacked by an oxygen of the phosphate group from UDP that starts the process continuing via an iminium intermediate and finally resulting in UDP-galactofuranose and reduced flavin cofactor.

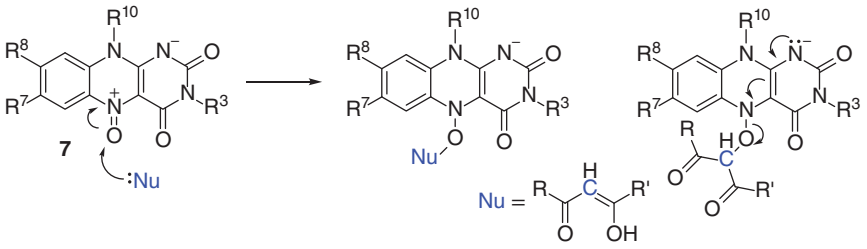
The N5-position in flavins **1** is able to accept and release a hydride (Scheme 4.6) [1a]. Flavoprotein dehydrogenases and oxidases typically employ a hydride transfer mechanism transferring two electrons and a proton from the substrate to the flavin moiety. Hydride transfer also starts the reduction of activated double bonds provided by flavoprotein reductases or the oxygenations catalyzed by flavoprotein monooxygenases. The C5-position in deazaflavins **2** behaves in an analogous manner [8a].



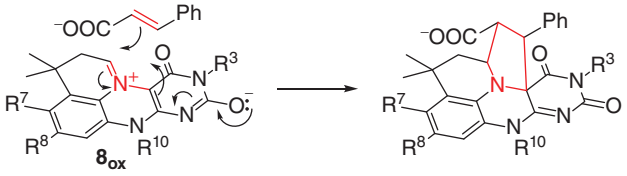
Scheme 4.6 Hydride transfer to/from the N5-position of flavin and deazaflavin, and an example of the oxidative process in choline oxidase (a) and double-bond saturation in reductases (b). Deazaflavin is also known to participate in hydride transfer reactions (c). Source: Modified from Walsh and Wencewicz [1].

A special example of the formation of an N5 adduct is the use of N5-oxide **7** for the addition of C-nucleophiles in the enterocin biosynthetic enzyme (Scheme 4.7) [38].

A more drastic N5 modification is present in the recent discovery of prenyl-FMN cofactor **8**. This decorated FMN cofactor is involved in a 1,3-dipolar cycloaddition reaction within the decarboxylation reaction mediated by decarboxylases (Scheme 4.8) [39].



Scheme 4.7 Participation of the N5-oxide during the formation of a flavin N5-adduct. Source: Modified from Teufel et al. [38].



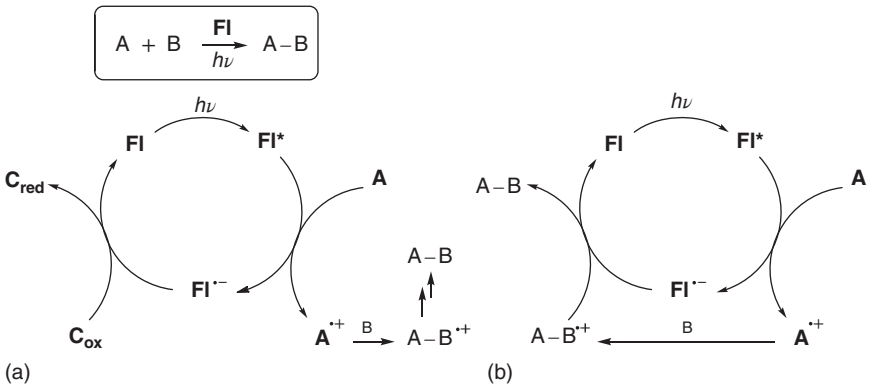
Scheme 4.8 Prenyl-FMN cofactor $\mathbf{8_{ox}}$ in its dipole form undergoing a 1,3-dipolar cycloaddition reaction in decarboxylases. Source: Modified from Payne et al. [39].

4.2.1.2 Flavin-Based Catalysis in an Excited State

Oxidative processes are the domain of flavin-based photocatalysis [7]. An excited flavin accepts an electron from an electron-rich substrate generating a reactive radical species, which undergoes further transformations (Scheme 4.9a). This is known as reductive quenching as the flavin is reduced during this process. The flavin species is then regenerated by sacrificial oxidant, mostly by molecular oxygen as the oxidative processes often occur under an air or oxygen atmosphere. Nevertheless, other sacrificial agents like potassium persulfate or nitrobenzene have been successfully used to reoxidize the flavin catalyst (see Section 4.3). The catalytic cycle can also be completed via electron transfer from an intermediate and therefore does not require an external oxidizing agent (Scheme 4.9b) [40]. It should be mentioned that the flavin radical anion is a relatively strong base and often it is protonated during the catalytic cycle. This is known as proton-coupled electron transfer (PCET) when both processes are concerted. PCET has been proposed in the flavin-based decarboxylation reaction [40, 41].

A typical aerobic photochemical transformation mediated by flavins is the oxidation of benzylalcohols to give aldehydes [7]. In addition to the product, the overall reaction produces a stoichiometric amount of hydrogen peroxide. Formally, it is reminiscent of reactions catalyzed by flavoprotein oxidases. Recently, other electron transfer oxidative procedures such as decarboxylation [40], cyclization [41], and sulfoxidation [42] have been reported (see Chapters 10–12 for details).

In the reductive quenching process, the flavin can participate both in its singlet excited and triplet excited states [43]. Nevertheless, in the singlet excited state with a lifetime usually in range of ns, flavins are susceptible to back-electron transfer resulting in the flavin and starting substrate [44]. Therefore, electron transfer from the



Scheme 4.9 General scheme of flavin **Fl** photocatalysis based on reductive quenching. The catalytic cycle using a sacrificial oxidant C_{ox} (a) and redox neutral process (b). In photocatalysis, also flavinium salts $\mathbf{Fl}^+$ can be involved analogously. Source: (b) Modified from Ramirez et al. [40].

singlet excited state has been shown as a nonproductive pathway in many cases, such as aerobic benzyl alcohol oxidation catalyzed by riboflavin tetraacetate (**1d**, **RFTA**). In this process, the productive pathway runs through flavin triplet state characterized by a significantly longer lifetime (μs) [44, 45]. On the other hand, the authors could not exclude the participation of flavin in its excited singlet state in some cases.

The power of oxidizing species in singlet and triplet excited states can be calculated from the ground-state reduction potential $E_{red}(Fl/Fl^{\bullet-})$ and energy of the first singlet excited state $E_{0,0}(S1)$ or first triplet excited state $E_{0,0}(T1)$, respectively [46]. From these values, the thermodynamics of reductive photoinduced electron transfer can be calculated using Eq. (4.1).

$$\Delta G = E_{red}{}^{*}(Fl^{*}/Fl^{\bullet-}) - E_{ox}(S^{\bullet+}/S) - \varpi \tag{4.1}$$

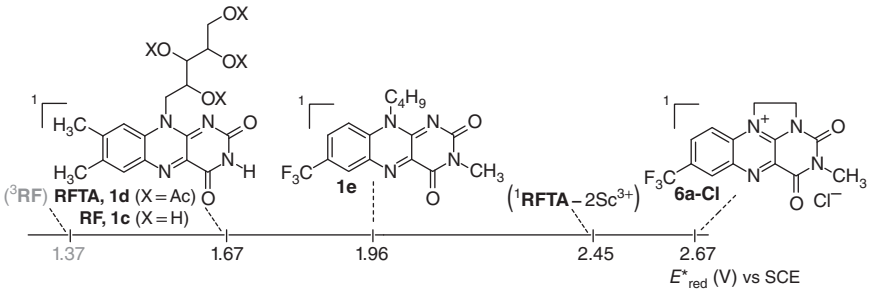
where ϖ is an electrostatic term, which is estimated to be 0.06 eV in acetonitrile, $E_{ox}(S^{\bullet+}/S)$ is oxidation potential of a substrate, and $E_{red}{}^{*}(Fl^{*}/Fl^{\bullet-})$ is the excited-state reduction potential of flavin calculated using Eq. (4.2).

$$E_{red}{}^{*}(Fl^{*}/Fl^{\bullet-}) = E_{red}(Fl/Fl^{\bullet-}) + E_{0,0} \tag{4.2}$$

where Fl^{*} represents either the S1 or T1 excited state with an $E_{0,0}$ value of $E_{0,0}(S1)$ or $E_{0,0}(T1)$, respectively.

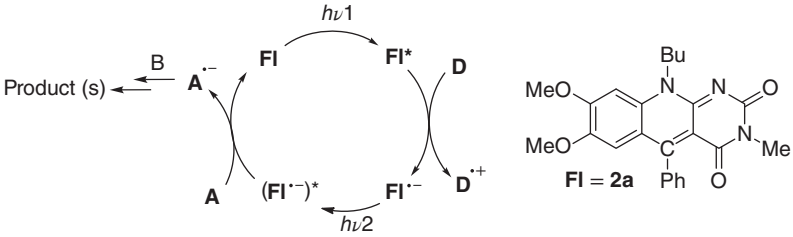
For riboflavin tetraacetate (**1d**, **RFTA**), which is mostly used as a photocatalyst, the singlet excited-state reduction potential is 1.67 V vs. SCE based on the ground-state reduction potential (–0.81 V vs. SCE) and $E_{0,0}(S1) = 2.48$ eV [47]. The value 1.37 V vs. SCE for the triplet excited state of riboflavin (**1c**, **RF**) can be calculated using $E_{red}(RF/RF^{\bullet-}) = -0.80$ V vs. SCE and $E_{0,0}(T1) = 2.17$ eV [43]. These values limit the application of neutral flavins **1** (isoalloxazines) to relatively electron-rich substrates. However, the oxidative power of flavins can be increased upon substitution with an electron-withdrawing group [48] or coordination to a metal ion (e.g. scandium, which achieves a value up to 2.45 V vs. SCE) (Scheme 4.10) [49]. Analogously, the introduction of a quaternary nitrogen in flavinium salt **6a•Cl** leads to a species with

an extremely high oxidation power as evident from the excited-state reduction potentials 2.67 and 2.43 V vs. SCE for singlet and triplet excited states, respectively [50]. The strongly oxidizing properties of excited flavins allow them to be used in the oxidation of heteroatoms as well as various benzylic substrates, including toluenes and ethylbenzenes bearing electron-withdrawing groups (see Chapter 10).



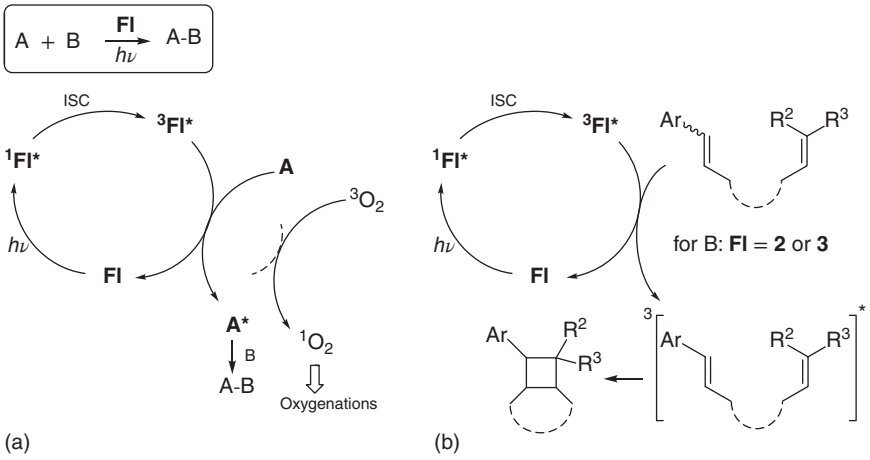
Scheme 4.10 Excited-state reduction potentials of some selected flavin derivatives. Source: Modified from Fukuzumi et al. [49] and Wolf et al. [47].

Flavin, in its excited fully reduced deprotonated form, is involved in the reductive splitting of pyrimidine base dimers to restore the function of damaged DNA by photolyases [24]. Analogously, several photolyase models have been designed and studied [51]. This process is based on reductive quenching, which is allowed by the very negative excited-state oxidation potential estimated to be –2.8 V vs. SCE for reduced deprotonated **FMN** [52]. The other reduced forms of flavin derivatives have been shown to be extremely potent in reductive processes. The excited-state oxidation potential of deazaflavin radical anion $(\mathbf{2a}^{\bullet-})^*$, which characterizes its reductive power, was estimated to be $E_{ox}{}^*(\mathbf{2a}^*/\mathbf{2a}^{\bullet-}) = -3.3$ V vs. SCE [53], and is among one of the strongest reducing agents reported to date [54]. The excited deazaflavin radical anion is produced via consecutive photoinduced electron transfer (*con*-PET[55]; Scheme 4.11) consisting of the generation of the deazaflavin radical anion via electron transfer from a tertiary amine (= donor) to the deazaflavin excited by the first photon, followed by excitation of this species by a second photon. The $(\mathbf{2a}^{\bullet-})^*$ species was found to reduce 4-halogenanisoles providing its dehalogenation [53].



Scheme 4.11 The *Con*-PET procedure using deazaflavin **2a** in the presence of a sacrificial electron donor (D = DIPEA). Source: Modified from Ghosh et al. [55] and Graml et al. [53].

Because of their long-lived triplet states, flavins are also involved in energy transfer processes, usually known as photosensitization reactions. Flavins, in their excited triplet state, transfer energy to the substrate, which becomes excited and undergoes a chemical reaction (Scheme 4.12a). A typical example of triplet energy transfer mediated by the flavins is the generation of singlet oxygen $^1O_2$, which is produced via energy transfer from flavin usually with relatively high efficiency; Φ_Δ = 0.3–0.9 depending on the substitution and whether it is isoalloxazine **1**, deazaflavin **2**, or alloxazine **3** [57]. This may be improved upon by the introduction of a heavy atom into the flavin skeleton due to the heavy atom effect [58]. Singlet oxygen has been found to be involved in various flavin-based oxygenation reactions, such as sulfoxidation [59] or benzylic oxygenation [60]. An example of the direct photosensitization of a substrate is the intramolecular [2 + 2] photocycloaddition of styrene dienes mediated by 1-butyl-7,8-dimethoxy-3-methylalloxazine (**3a**) or 10-butyl-3-methyldeazaflavin (**2b**), which starts by the generation of the excited substrate via energy transfer from an excited flavin species (Scheme 4.12b) [22, 56]. Photocycloaddition of styrene dimers is not efficiently provided by **RFTA** [$E_{0,0}$(T1) = 2.17 eV] because of its low triplet energy as compared to **2b** [$E_{0,0}$(T1) = 2.41 eV] and **3a** [$E_{0,0}$(T1) = 2.28 eV].



Scheme 4.12 General flavin-based catalytic cycle involving energy transfer (a) and intramolecular [2 + 2] photocycloaddition mediated by flavins (b) via an energy transfer mechanism. Source: (b) Mojr et al. [22, 56].

Most flavin derivatives in their oxidized form absorb light in the visible-light or near-UV region (see Chapter 3). Therefore, they are excited directly upon irradiation with light in biological processes. This is also a reason for the wide application of flavins in visible light photocatalysis, which is advantageous because of the light sources available. On the other hand, the fully reduced form does not absorb visible light. Therefore, reduced flavin cofactor (FAD) in photolyases enters an excited state via energy transfer from an antenna system containing among others 8-hydroxydeazaflavin [6, 61]. This sensitizer absorbs light in the region from 300

to 500 nm. Another approach to the excited species not absorbing in the region of the light source is the formation of an electron donor–acceptor (EDA) complex resulting in the red-shifted absorption maximum [46]. For example, the formation of a reduced FMN–haloketone complex has been used in photobiocatalytic dehalogenation/cyclization reaction cascade using reductases (see Chapter 12 for details) [62].

4.2.2 Flavin Catalyst–Activated Reactive Species Used to Transform the Substrate

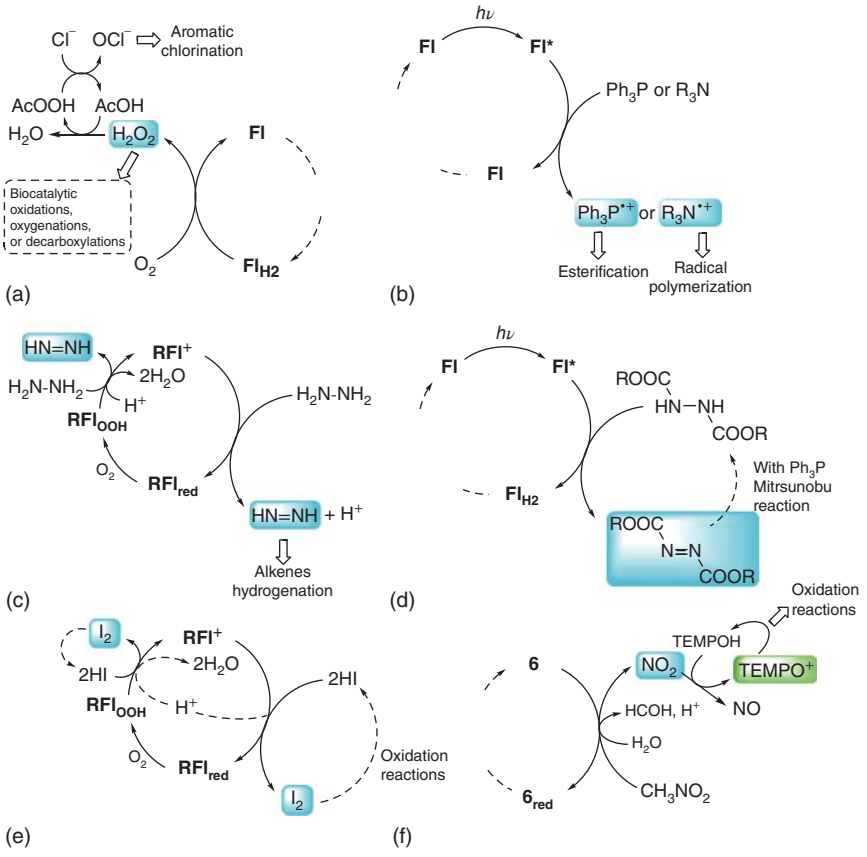
In biological systems, flavins usually directly interact with a substrate and transform it into a product or an intermediate. An exception to this is if the flavin cofactor transforms another into an active form, which carries out a chemical reaction. In artificial procedures, various useful reactive agents are generated either in the flavin reductive or oxidative half-reaction.

Hydrogen peroxide produced during flavin reoxidation by oxygen is a special example. Often, it is a nonuseful byproduct. Nevertheless, it can also be involved in an oxidative transformation. In biocatalytic systems, hydrogen peroxide is utilized by various enzymes, including peroxidases, peroxygenases, or cytochrome P450 for oxidative transformations [63]. A typical example of an artificial system is the halogenase-inspired oxidative chlorination of electron-rich arenes. In this procedure, hydrogen peroxide produced by a flavin-based photocatalytic cycle transforms acetic into peracetic acid, which generates electrophilic chlorine species from chloride (Scheme 4.13a) [64].

Typically, the reactive agents in artificial systems are formed in the flavin reductive half-reaction. One-electron oxidation of triphenylphosphine or a tertiary amine by a riboflavin derivative excited by blue light produces radical cations (Scheme 4.13b) [65, 66]. Triphenylphosphine radical cation ($Ph_3P^{\bullet+}$) has been shown to participate in an esterification reaction by activating either the alcohol via formation of an alkoxytriphenylphosphonium species or acid to form acyloxytriphenylphosphonium (after another SET) [65]. Regarding $R_3N^{\bullet+}$ generated by flavin, it is known to initiate the radical polymerization of vinyl substrates [66].

Diimide is a powerful reagent used for various double-bond hydrogenation reactions. This species is generated from hydrazine via oxidation using 5-alkylflavinium salts (**RFl•X**) (Scheme 4.13c) [67]. Flavinium $\mathbf{RFl^+}$ acts as a catalyst and it is regenerated by oxygen via flavin-4a-hydroperoxide $\mathbf{RFl_{OOH}}$, which has been shown to also contribute to the hydrazine → diimide oxidation reaction. Dialkyl azodicarboxylates, used in combination with triphenylphosphine during the Mitsunobu reaction, have been shown to be regenerated from their corresponding hydrazine derivative via a photocatalytic oxidation reaction using 3-methylriboflavin tetraacetate (**1f**) (Scheme 4.13d) [68].

5-Alkylalloxazinium salts **5•X** have been used for the regeneration of an iodine catalyst from I^- under aerobic conditions [69] via oxidation by the salt itself and oxygenation by flavin hydroperoxide $\mathbf{5_{OOH}}$, which is formed during the regeneration of the alloxazinium salt (Scheme 4.13e; **RFl = 5**).



Scheme 4.13 Examples of various reactive species generated by flavin bio- (a), organo- (c, e, f), and photocatalysis (a, b, d). Source: (a) Modified from Hering et al. [64]. (b) März et al. [65] and Encinas and Previtali [66]. (c) Smit et al. [67] and Imada et al. [67]. (d) Modified from Marz et al. [68]. (f) Modified from Thapa et al. [35].

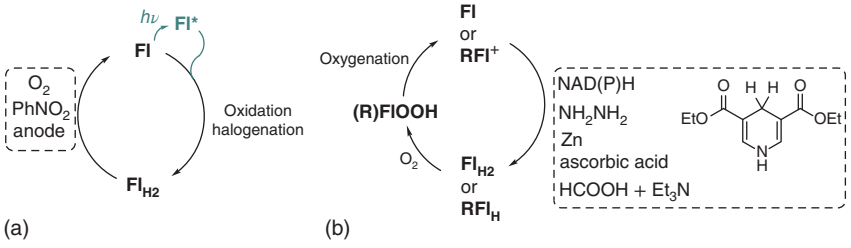
Ethylene-bridged flavinium salts **6-X** generates NO_x during the Neff reaction with nitromethane (see Section 4.2.1.1), providing a source of NO_2 used as a reoxidizing agent in the TEMPO-mediated oxidation of cyclic ethers, alcohols, and terminal diols to form lactones, aldehydes or ketones, and lactones, respectively (Scheme 4.13f) [35].

4.3 Flavin Catalysts Regeneration

Oxygen is by far the most commonly used regenerating agent for oxidized flavins in oxidative processes. When it is used, hydrogen peroxide is usually formed. With the exception of cases when it is the substrate/agent for another oxidative process (see Section 4.2.2), hydrogen peroxide is normally an unwanted byproduct, which

is toxic to biological systems and it is removed by catalases [70]. In photocatalysis, it can cause undesirable side reactions or catalyst bleaching. Therefore, there have been attempts to support hydrogen peroxide disproportionation to oxygen and water in flavin-based photocatalytic systems and thus eliminate any undesired processes, using heme-type iron(II) complexes [71], iron(II) perchlorate [60], or molecular sieves [68].

In biocatalysis, the reactive hydrogen peroxide often inhibits enzymes, including catalase, which has led to the development of oxygen-free procedures, for example the indirect regeneration of glucose oxidase using benzoquinone/hydroquinone and an anode as a terminal electron acceptor [72]. Attempts to replace molecular oxygen as a flavin reoxidizing agent in photocatalysis have led to a system using nitrobenzene, which is able to convert the fully reduced flavin into its oxidized form via oxidation to semiquinone and its disproportionation. Most products of nitrobenzene reduction, i.e. nitrosobenzene, azoxide, and azobenzene, can do the same job [65]. Systems using potassium persulfate [73] or an excited ruthenium complex [74] have also been described (Scheme 4.14a). In redox-neutral processes, the flavin is regenerated by an intermediate such as the *p*-toluenesulfonyl radical formed during flavin-based decarboxylative cyanation [40] with tosyl cyanide or a cysteine radical during the conversion of fatty acids into *n*-alkanes by fatty acid photodecarboxylase [76].



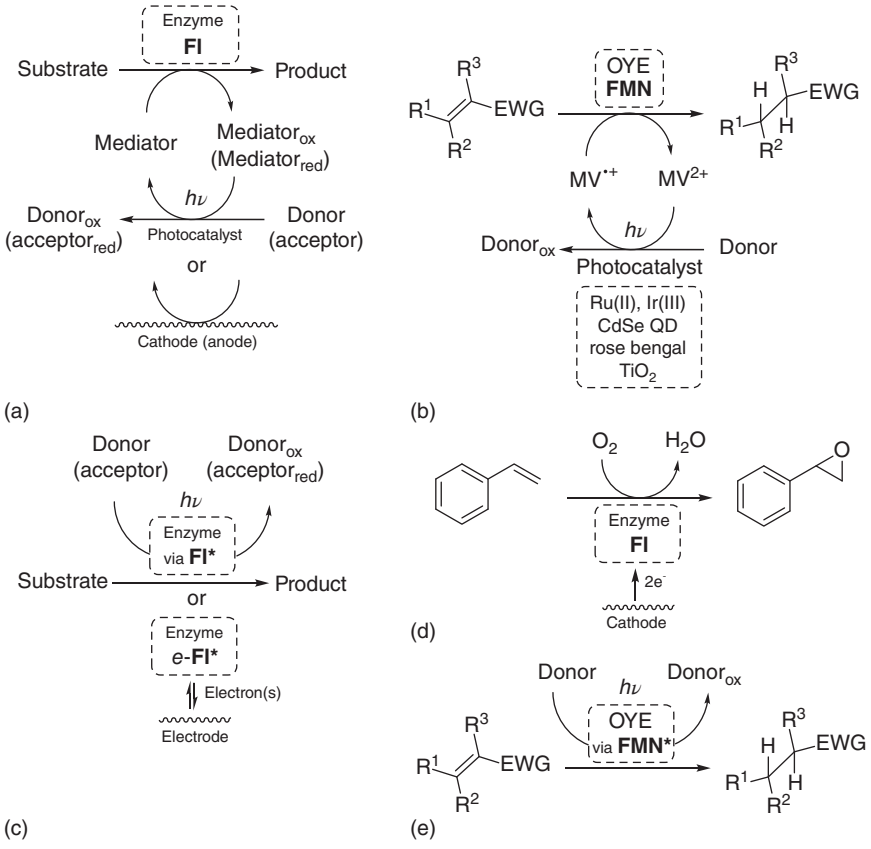
Scheme 4.14 Regeneration of flavin catalysts **Fl** or **RFl**$^+$ in oxidations (a) and monooxygenations (b). Source: (b) Modified from Imada et al. [75].

To form flavin hydroperoxides both in natural and artificial monooxygenases, the reduced flavin should be generated, which then reacts with oxygen to produce the active oxygenation species [27] (see Chapters 5 and 7). The reduction of oxidized cofactors, FAD or FMN, in flavoenzymes is typically carried out using NAD(P)H. In artificial systems, 5-alkylflavinium salts are reduced by hydrazine [77], zinc [78], Hantzsch ester [79], formic acid [80], or ascorbic acid (Scheme 4.14b) [75].

A key problem using monooxygenases as well as reductases on a large scale is the supply of the expensive NAD(P) cofactor in its desired oxidation state. This can be solved using whole cells or an enzymatic cofactor regeneration system such as glucose [81] and phosphite dehydrogenases [82] or NAD(P)H oxidase [83] with their corresponding sacrificial agents, glucose, phosphite, or oxygen, respectively. There are also chemical, photochemical, or electrochemical NAD(P) regeneration systems [84], some of them use flavin derivatives for NAD(P)$^+$ generation from NADPH such

as ethylene-bridged flavinium salts **6-X** in mannitol-1-dehydrogenase-catalyzed oxidation [85].

Another approach to avoid the need of large amounts of NAD(P) for flavin regeneration is to develop NAD(P)-independent systems [84]. In principle, either direct or indirect electron transfer from/to the flavin cofactor is used for recycling its active form. During the indirect regeneration process, electron transfer between the sacrificial donor/acceptor and flavin occurs via a redox mediator (Scheme 4.15a). The terminal donor/acceptor can be a chemical compound or an electrode. Electron transfer between the donor/acceptor and mediator can be a photocatalytic process. An example is the regeneration of FMN during the hydrogenation of activated double bonds by Old Yellow Enzymes (OYE) using a viologen (e.g. methyl viologen; MV) as an electron mediator and triethanol amine (TEOA) or ethylenediaminetetraacetate (EDTA) as a sacrificial donor with the first electron transfer provided by light and a photocatalyst such as an Ru(II) or Ir(III) complex [89], rose bengal [90],



Scheme 4.15 Indirect (a, b) and direct (c–e) systems used for flavin cofactor **Fl** regeneration with their corresponding examples. Source: (b) Mifsud et al. [86] and Burai et al. [86]. (d) Modified from Ruinatscha et al. [87]. (e) Modified from Grau et al. [88].

CdSe quantum dots or TiO_2 (Scheme 4.15b) [86]. Another example is amino acid oxidase immobilized together with a viologen mediator on the cathode surface, which was used for the amination of pyruvate [91]. As a mediator, an external photoactive flavin (riboflavin, FMN, or deazaflavin) can also be used, thus taking an electron by photoinduced electron transfer directly from a donor without the need of an additional photocatalyst [92]. A system using the regeneration of OYE by added FMN, light, and EDTA as sacrificial reductant works via this mechanism [88].

The direct regeneration of the flavin cofactor also uses photo- or electrochemistry (Scheme 14.15c–e). In electrochemical systems, the electrons are directly transferred from/to the cofactor in an immobilized enzyme such as the styrene monooxygenase-catalyzed epoxidation of vinylbenzenes, which takes electrons from the cathode (Scheme 14.15d) [87]. In direct photochemical regeneration, the photoexcitation of flavin cofactors is utilized enabling electron transfer from a donor or to an acceptor. Examples can be found among either Baeyer–Villiger oxidations provided by monooxygenases [93], hydrogenations provided by reductases (Scheme 14.15e) [88], or halogenations [94].

4.4 Development of New Flavin-Based Catalytic Methodologies

Flavin catalysis has been on the rise for several decades and further expansion is expected in the coming years. The reason is not only the development of the particular disciplines themselves: bio-, organo-, and photocatalysis, but also thanks to a uniform reagent, the possibility of inspiration and transfer of catalytic modes among them.

While more and more flavoenzymes are targeted for enzyme-engineering studies to tune them for biocatalytic applications, there are also opportunities to engineer flavoenzymes by altering the cofactor itself. By such cofactor-engineering approach, totally new catalytic properties can be introduced into flavin-dependent enzymes. In fact, before mature techniques were developed to engineer proteins through molecular biology tools, such cofactor-engineering studies were already performed. Pioneering studies by Massey and coworkers have shown that replacing natural flavin cofactors with chemically modified flavins can significantly alter enzyme activity [5]. Except for using such flavin derivatives for probing the active site of flavoenzymes, it has also resulted in drastic changes in catalytic properties. For example, by replacing FMN in old yellow enzyme with 8-cyano-FMN, the NADPH-dependent enone reductase was essentially turned into an oxidase [95]. A similar approach was recently taken when converting a dehalogenase into a reductase. By replacing FMN in a deiodinase with 5-deaza-FMN, the ability to dehalogenate was strongly reduced while installing nitroreductase activity [96]. In fact, the effect obtained by modifying the flavin cofactor was far greater when compared with protein-engineering attempts of the same enzyme for the same goals. Another example was the replacement of riboflavin in riboflavin-binding protein. While this protein is normally not catalytically active but merely meant

for vitamin storage, when complexed with N5-alkylated flavins it is able to catalyze H_2O_2-driven enantioselective oxidations [97]. These examples demonstrate that it can be powerful to include the option of altering the flavin cofactor when engineering flavoenzymes. Yet, there are challenges linked to such a cofactor-engineering approach. All example studies above involved the replacement of FMN or riboflavin, while there are relatively few examples of studies in which the FAD is modified. This relates to the fact that it is often not easy to replace a flavin cofactor and/or prepare a FAD derivative. Flavin cofactors are typically tightly bound, which makes it difficult to prepare the apo protein [98]. Alternatively, one can produce flavoproteins in hosts that are deficient in synthesis of riboflavin. This allows production of flavoproteins devoid of their cofactor that can be reconstituted with any desired flavin derivative [99]. Feeding riboflavin derivatives in the growth medium of such riboflavin-auxotrophic strains is even a more direct method to produce flavoenzymes with a modified flavin cofactor [100].

Various studies have also shown that nature has exploited the approach of modifying the flavin cofactor. A recently discovered example is the prenyl-FMN cofactor. Clearly, such a drastically altered flavin cofactor has an enormous effect on the reactions feasible, switching from redox reactions to (de)carboxylations. But there are also milder natural flavin modifications that are exploited in enzymes to tune activities. In 2017, Robbins et al. discovered that a fungal FAD-containing formate oxidase forms in a self-catalytic process 8-formyl FAD. It was found that this irreversible modification results in a 10-fold higher enzyme activity [18]. This autocatalytic modification at the 8-position and its effect on activity resembles the previously reported role of naturally occurring protein-FAD linkages [17]. While the first covalent flavin-protein linkages were discovered about 70 years ago, several new covalent attachment types were discovered in the last two decades [16]. Even more extreme is the observation that, while FMN was discovered almost a century ago, prenyl-FMN was only identified 5 years ago [101]. It will be interesting to see in the coming years what other flavin modifications are still hidden in nature.

Nature has also found ways to exploit flavin for their photochemical properties. A large number of flavoproteins are known to be involved in light sensing [102]. But there are also flavoenzymes that are even light driven. Recently, two new examples of FAD-containing enzymes that can be activated by blue light were described. A bacterial hydroxylase was found to be dependent on photoreduction for its activity, a feature that had never been observed before for a flavoprotein monooxygenase [103]. The other example comes from a green algae, which was found to harbor a FAD-containing fatty acid decarboxylase. This enzyme was found to rely on photoexcitation of the FAD cofactor to perform decarboxylations with a quantum yield of >80% [104]. The latter enzyme has attracted attention in the field of biocatalysis as it allows facile synthesis of alkanes from (fatty) acids [105]. While the mechanisms of these newly discovered photoenzymes are still poorly understood, they may allow development of truly novel biocatalytic applications. Except for natural light–fueled enzymes that are biocatalysts, recent work has revealed that "regular" flavoenzymes can also be exploited for photobiocatalysis (see Chapter 12). Future will tell whether

such light-triggered biocatalytic reactions will deliver robust catalytic procedures that can be used on industrial scale.

The field of flavin organocatalysis is typically inspired by enzyme catalysis. This is documented by oxygenations using 5-alkylflavin-4a-hydroperoxides, which mimic the function of monooxygenases [12]. The use of artificial monooxygenations in "noncanonical" chemistry or using reactive flavinium salts to generate reactive intermediates is an area that will certainly continue to develop. Pioneering work using stable N5-unmodified flavin-4a-hydroperoxide outside an enzyme [31] shows another possible approach for flavin organocatalysis based on neutral isoalloxazine **1** and alloxazine **2**, which are characterized by higher stability compared to flavinium salts. Catalytic procedures using flavin-N5 reactivity like artificial nitroalkane oxidase [35] are also expected to be designed based on new findings in enzymology [106]. Intriguingly, it is still a mystery why quite a few microbes produce the deazaflavin-based cofactor F_{420}. There may be deazaflavoenzymes that perform chemistries that we still do not know about, perhaps reductions that nicotinamides cannot do or light-dependent reactions. The use of deazaflavins in organo- or photocatalysis inspired by new findings from biological systems or use of an unusual deazaflavin reactivity in catalysis can be expected. One-electron reduction provided by excited deazaflavinsemiquinone is a recent example [53].

Early photocatalysis with flavins has been linked to pyrimidine dimers splitting in photolyases [51]. Although a number of photolyase models have been reported, it should be mentioned that the main reaction in flavin photocatalysis has long been benzylic oxidation, which mimics oxidases operating in the absence of light (see Chapter 10). Another artificial process might be designed using simple flavins with enhanced reactivity by photoexcitation instead of ground-state flavin cofactor activated by enzyme environment.

It can also be expected that flavin photocatalysis, like photocatalysis in general, will look for applications among known synthetic procedures to make them easier to use; or even to make possible new transformations, which are not allowed in dark. However, recent findings in flavin-based light-dependent processes, occurring in photoenzymes and photoreceptors, are expected to become another stimulus for new photocatalytic methods development. Decarboxylative procedures are the first examples [40, 41]. Combination of photocatalysis with electrochemistry is also expected to be a viable approach [107]. In short, there are still many new avenues that can be explored by flavin-based (bio)chemistry.

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