Natural Flavins

Drenth, Jeroen; Fraaije, Marco W.

Published in:
Flavin-Based Catalysis

DOI:
10.1002/9783527830138.ch2

2

Natural Flavins: Occurrence, Role, and Noncanonical Chemistry

Jeroen Drenth and Marco W. Fraaije

University of Groningen, Molecular Enzymology Group, Nijenborgh 4, Groningen 9747AG, The Netherlands

2.1 Introduction

Flavin-dependent enzymes are versatile biocatalysts involved in numerous cellular oxidation and reduction reactions over a wide range of substrates, many of which are of key importance to primary and secondary metabolism. Since their discovery about a century ago [1], these flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) utilizing oxidoreductases have been well studied, and currently much is known about flavin chemistry. Some examples of the wide range of reactions catalyzed by flavins include: reduction of activated C=C double bonds [2], oxidation of alcohols [3, 4], oxidations and reductions of aldehydes [5, 6] and lactols [7, 8], as well as (cyclic) alkane hydroxylation [9], aromatic hydroxylation [10, 11], Baeyer–Villiger oxidation [12, 13], epoxidation, sulfoxidation, phosphite ester, selenide, organoboron, and amine oxidations [14], dehalogenation, halogenation [15], decarboxylation [16, 17], and even light production [18]. Part of the chemical versatility is derived from the ability to undergo both one- and two-electron reduction/oxidation reactions, to form several thermodynamically and kinetically accessible stable redox states [19]. The mode of flavin reactivity and substrate specificity is dictated by the protein part of the enzyme, as it finetunes the flavin redox potential, directs specific substrates to the cofactor, and stabilizes certain redox states and covalent adducts [19–21]. Most enzymes do this through noncovalent interactions, but there are cases where the flavin cofactor is actually covalently bound to the protein scaffold, enabling more drastic changes in flavin reactivity and substrate acceptance. Sometimes, enzymes may even stabilize superoxidized flavin states, as in the case of the recently discovered flavin-N5-oxide ($Fl_{N5[O]}$) [22]. Modified flavins can greatly enhance the "canonical" activity of flavoenzymes. Canonical flavin chemistry is hydride transfer to and from the N5 locus and molecular oxygen activation at C4a, and is responsible for almost all the reported activities. But modified flavins can also harbor new activities. A recently discovered means of steering the flavin reactivity is prenylation of the isoalloxazine catalytic core structure [23]. This highly modified cofactor is of key importance in reversible decarboxylations. In addition

Flavin-Based Catalysis: Principles and Applications, First Edition.
Edited by Radek Cibulka and Marco W. Fraaije.

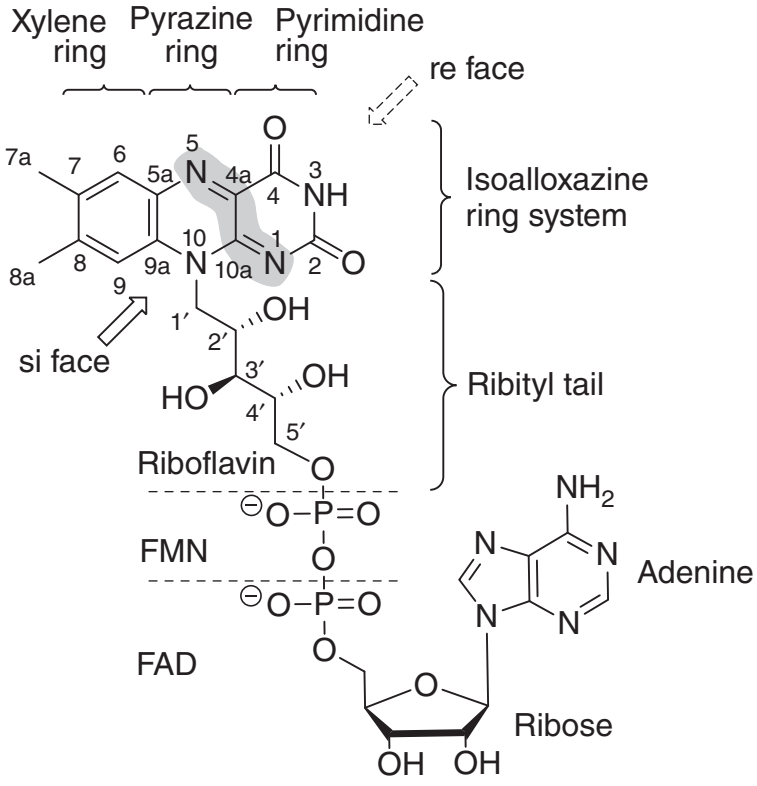


Figure 2.1 Flavin structure. The catalytic core structure of all flavins is the 7,8-dimethylisoalloxazine ring system, which comprises an electron-rich xylene ring, an electron-deficient pyrimidine ring, and a bridging electrophilic pyrazine ring. The catalytically important locus N5–C4a–C10a–N1 is highlighted in gray shading. Riboflavin, FMN, and FAD also have a ribityl unit fused to the 7,8-dimethylisoalloxazine moiety at N10. The ribityl tail of flavin mononucleotide (FMN) is decorated with a 5′-phosphoryl group and flavin adenine dinucleotide (FAD) is further lengthened with adenosine monophosphate.

to the riboflavin-derived cofactors also the 5-deazaflavin F_{420} exists in nature. This flavin analogue is synthesized mainly by archaea and Actinobacteria from an intermediate in riboflavin biosynthesis. This underexplored cofactor, with its extraordinarily low redox potential, could be a very valuable tool in biocatalysis and medicinal chemistry [24, 25]. This chapter will deal with F_{420} and the "exotic" cases of flavin chemistry found in nature, where the cofactor is covalently modified or performs noncanonical flavin chemistry, further expanding the toolbox of flavin biocatalysis (Figure 2.1).

2.2 Flavin Biosynthesis

Riboflavin, the precursor of FMN and FAD, also known as vitamin B_2, is essential to all known organisms, but not all organisms – including humans – can

actually synthesize this important cofactor (Figure 2.1). Plants and many fungi and bacteria, however, can produce this cofactor from one equivalent of guanosine triphosphate (GTP) and two equivalents of ribulose-5-phosphate (R5P), both common metabolites in primary metabolism [26, 27]. The first steps in biosynthesis are the hydrolytic removal of C8 from the guanine ring from GTP as formate and the hydrolytic release of pyrophosphate (PP_i), both catalyzed by GTP cyclohydrolase II [26]. In bacteria, the product of these reactions, 2,5-diamino-6-(5-phospho-D-ribosylamino)pyrimidin-4(3*H*)-one ((**2**) in Figure 2.2), then undergoes deamination at C2 of the ring, and subsequent NAD(P)H-dependent reduction of the phosphoribosyl sidechain (reactions B and D in Figure 2.2, respectively) to form 5-amino-6-(1-D-ribitylamino)uracil. In bacteria, both reactions are catalyzed by a bifunctional enzyme [28]. Fungi, however, first reduce the phosphoribosyl side chain of 2,5-diamino-6-(5-phospho-D-ribosylamino)pyrimidin-4(3*H*)-one and then deaminate the reduced product. Both reactions, depicted by arrows C and E in Figure 2.2, are catalyzed by separate enzymes [29]. The 5′-phosphoryl group is then removed by a phosphatase [30] and the product, 5-amino-6-(1-D-ribitylamino) uracil, is then condensed with L-3,4-dihydroxybutan-2-one 4-phosphate to form 6,7-dimethyl-8-(D-ribityl)lumazine by 6,7-dimethyl-8-ribityllumazine synthase [31–33] (arrow G in Figure 2.2). L-3,4-dihydroxybutan-2-one 4-phosphate is formed from R5P in a peculiar reaction, involving several tautomerizations and a sigmatropic rearrangement, catalyzed by L-3,4-dihydroxybutan-2-one 4-phosphate synthase [33–35]. The last step in the biosynthesis pathway is catalyzed by riboflavin synthase, which catalyzes the unusual dismutation of two 6,7-dimethyl-8-(D-ribityl)lumazine equivalents to form one equivalent of riboflavin and one equivalent of its precursor, 5-amino-6-(1-D-ribitylamino)uracil [36]. In the case of FMN, the 5′-carbon is decorated with a phosphoryl group, and in the case of FAD this is further elongated by an adenosine-5′-monophosphate (AMP) moiety. Both steps are either done by bifunctional FAD synthases in bacteria or by riboflavin kinase and FAD synthase in fungi and plants, respectively [37].

2.3 Covalently Bound Flavin Cofactors

Cofactors can be transiently or permanently bound to enzymes. Coenzymes or cosubstrates are only temporarily bound to the enzyme during a catalytic cycle, as is normally the case for coenzyme A, coenzyme Q, and nicotinamides. On the other hand, prosthetic groups are tightly and permanently bound to the enzyme, and may only dissociate upon enzyme denaturation. Examples are metal ions, heme groups, iron–sulfur clusters, thiamine pyrophosphate (TPP), pyridoxal phosphate (PLP), and biotin. In some cases, the nicotinamide cofactors can also serve as a prosthetic group [38]. Most of these cofactors are tightly but not covalently bound to the enzyme. Some prosthetic groups, however, are actually covalently linked to the polypeptide, as is the case for lysine bound biotin and lipoic acid, which enables these cofactors to swing in between different active sites of an enzyme complex [39]. Other covalently bound cofactors are formed *in situ* out of amino acid side chains,

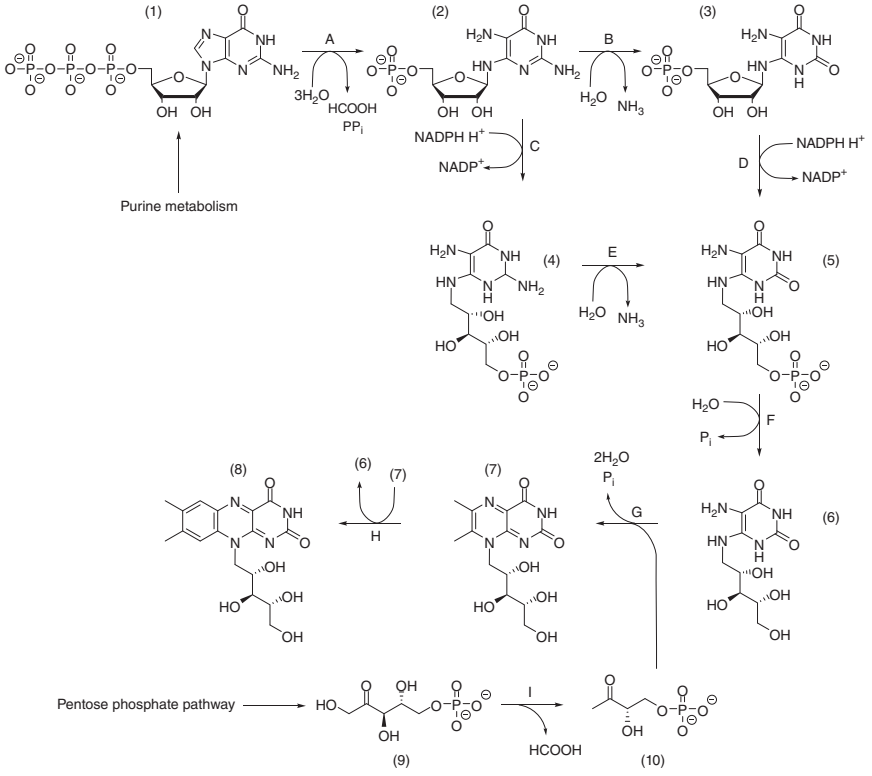


Figure 2.2 Riboflavin biosynthesis pathway. Riboflavin (**8**) is synthesized out of one equivalent of GTP (**1**) and two equivalents of ribulose-5-phosphate (R5P (**9**)). **A**: The C8 and two phosphate groups of GTP are hydrolyzed by GTP cyclohydrolase II, forming pyrophosphate, formate, and 2,5-diamino-6-(5-phospho-D-ribosylamino)pyrimidin-4(3*H*)-one (**2**). **B**: In bacteria (**2**) is diaminated at the C2 position by diaminohydroxy-phosphoribosylaminopyrimidine deaminase, forming 5-amino-6-(5′-phosphoribosylamino)uracil (**3**) and ammonia. **C**: In fungi (**2**) is first reduced to form 2,5-diamino-6-(5-phospho-D-ribitylamino)pyrimidin-4(3*H*)-one (**4**), which is catalyzed by 2,5-diamino-6-(ribosylamino)-4(3*H*)-pyrimidinone 5′-phosphate reductase. **D**: 5-amino-6-(5-phosphoribosylamino)uracil reductase then catalyzes the NADPH-dependent reduction of the ribose moiety of (**3**), forming 5-amino-6-(5′-phospho-D-ribitylamino)uracil (**5**). **E**: 2,5-diamino-6-(5-phospho-D-ribitylamino)-pyrimidin-4(3*H*)-one deaminase then catalyzes the deamination of (**4**), creating (**5**). **F**: (**5**) Is dephosphorylated to form amino-6-(1-D-ribitylamino)uracil (**6**), catalyzed by 5-amino-6-(5-phospho-D-ribitylamino)uracil phosphatase. **G**: Condensation of (**6**) and L-3,4-dihydroxybutan-2-one 4-phosphate (**10**) by 6,7-dimethyl-8-ribityllumazine synthase, forms 6,7-dimethyl-8-(D-ribityl)lumazine (**7**). **H**: two equivalents of (**7**) are condensed to form one equivalent of riboflavin (**8**) and one equivalent of (**6**). **I**: 3,4-dihydroxy 2-butanone 4-phosphate synthase catalyzes the formation of (**10**) out of R5P (**9**).

like topaquinone and analogous cofactors [40–42]. FMN and FAD are special in the sense that these cofactors can serve both as coenzyme and prosthetic group, and as a prosthetic group they can be either covalently or noncovalently attached to the enzyme. In fact, hemes and flavins are the only cofactors, which can be found as both covalently and noncovalently bound prosthetic groups. It is estimated that about 10% of the flavoproteins contain a covalently bound flavin [43].

2.3.1 Types and Occurrence of Covalent Protein–Flavin Bonds

The first covalent flavoprotein, succinate dehydrogenase, was identified in 1955 and later experimental work revealed that it contained 8α-N^3-histidyl-FAD [44, 45]. Thus far, seven additional covalent flavin–enzyme linkages have been identified after this discovery. Covalent bonds can be formed between histidine, cysteine, tyrosine, aspartate, and the C8a and C6 positions of the isoalloxazine ring system or between the 5′-phosphoryl group of FMN and a threonine residue, and the resulting linkages found in nature are 8α-N^1-histidyl-FAD/FMN, 8α-N^3-histidyl-FAD/FMN, 8α-*S*-cysteinyl-FAD/FMN, 8α-*O*-tyrosyl-FAD, 8α-*O*-aspartyl-FAD, 6-*S*-cysteinyl-FMN, and phosphoester-threonyl-FMN, respectively (see Figure 2.3) [43, 46, 47]. In some cases, FAD is linked to the protein via two covalent bonds. The bicovalent 8α-N^1-histidyl-6-*S*-cysteinyl-FAD is found in several enzymes, among which are gluco-oligosaccharide oxidase (GOOX), aclacinomycin oxidoreductase, berberine bridge enzyme (BBE), cannabidiolic acid synthase, hexose oxidase, chito-oligosaccharide oxidase (ChitO) [48–53]. The 8α-*N*-histidyl-FAD linkage is the most abundant form, found in a large array of proteins. This type of linkage is especially prevalent in enzymes that belong to the vanillyl alcohol oxidase (VAO) family. In fact, studies on the VOA family in the genome database predict that one in four proteins contains a covalent FAD, due to the presence of a conserved active-site histidine [54, 55]. Apart from the 8α-N^1-histidyl-FAD and 8α-N^3-histidyl-FAD linkages, some VOA members also

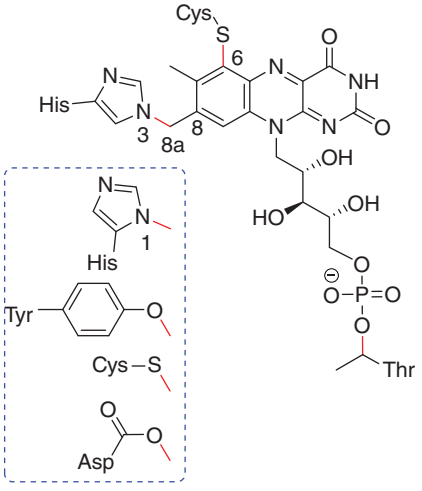


Figure 2.3 Identified covalent flavin–protein linkages. 8α-N^1-histidyl-FAD/FMN, 8α-N^3-histidyl-FAD/FMN, 8α-*S*-cysteinyl-FAD/FMN, 8α-*O*-tyrosyl-FAD, 8α-*O*-aspartyl-FAD, 6-*S*-cysteinyl-FMN, 8α-N^1-histidyl-6-*S*-cysteinyl-FAD, and phosphoester-threonyl-FMN.

contain 8α-N^1-histidyl-6-*S*-cysteinyl-FAD and 8α-*O*-tyrosyl-FAD. Another large protein family, the glucose oxidase/methanol oxidase/cholesterol oxidase (GMC) family, contains far less members with a covalently bound flavin cofactor. This suggests that the fold type of the enzyme might be important for promoting covalent flavin–protein bonds. Strikingly, the residues in the VAO family that form the covalent bond between the polypeptide and C8a of FAD are not fully conserved. The histidine residue, which is responsible for 8α-N^1-histidyl-FAD bond, is found near the N-terminus, which is part of the FAD-binding domain, whereas the histidine responsible for the 8α-N^3-histidyl-FAD is found in different positions on the cap domain; the same holds for the tyrosine that forms 8α-*O*-tyrosyl-FAD. No consensus sequence for covalent flavin incorporation has been identified. In fact, most binding motifs in covalent flavoproteins are similar to their noncovalent counterparts [43, 46].

The 8α- and 6-*S*-cysteinyl bound flavins are less widespread than the 8α-*N*-histidyl bound flavins; moreover, the 8α-*O*-tyrosyl-FAD, 8α-*O*-aspartyl-FAD and phosphoester threonyl FMN are thus far only identified in isolated cases. Histidyl-FAD/FMN and 8α-*S*-cysteinyl-FAD are found in all domains of life, whereas all the other bond types have only been identified in bacterial proteins. Extensively studied proteins containing 8α-*S*-cysteinyl-FAD are monoamine oxidase (MAO) and bacterial monomeric sarcosine oxidase (MSOX) [56, 57], and a well-studied example of a 6-*S*-cysteinyl-FMN-containing enzyme is trimethylamine dehydrogenase (TMADH) [58, 59]. 8α-*O*-tyrosyl-FAD, 8α-*O*-aspartyl-FAD, and phosphoester threonyl FMN are found in *p*-cresol methyl hydroxylase (PCMH) [60, 61], chloramphenicol halogenase [62], and the NprB/C subunits of a Na^+-translocating NADH:quinone reductase [63, 64], respectively.

2.3.2 The Mechanisms of Protein–Flavin Bond Formation

A wealth of structural and biochemical data on covalently bound flavins has led to a consensus on the general mechanisms of covalent flavinylation at the isoalloxazine ring system. The formation of a covalent bond between the protein and the C8a and C6 positions was long believed to be a fully autocatalytic post-translational process, but recent studies have shown that covalent tethering at the 8-methyl group might be assisted by a small helper protein in some cases.

2.3.2.1 Formation of the Protein–Flavin Bond at the C8a Position

The proposed general mechanism for the autocatalytic formation of a covalent bond between the C8a of the isoalloxazine ring system and the nucleophilic side chain of tyrosine, cysteine, aspartate, and histidine is shown in Figure 2.4, as was first proposed by Walsh [65]. The first step in this mechanism is the deprotonation of the 8a-methyl group by an active-site base, forming an iminoquinone methide intermediate. This intermediate was first discovered by Bullock and Jardetzkey in high-temperature deuterium exchange nuclear magnetic resonance spectroscopy (NMR) experiments [66], and was later also found in biomimetic 8α-functionalization studies of 2′,3′,4′,5′-tetraisobutyrylriboflavin with morpholine and imidazole, using base catalysis [67]. The nucleophilic amino acid residue then

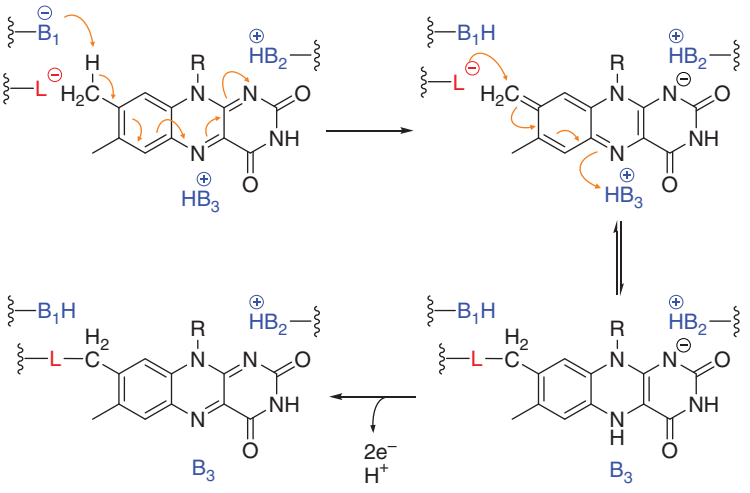


Figure 2.4 Proposed mechanism for the formation of a covalent flavin–enzyme bond at the C8a position of the isoalloxazine ring system. B_1, B_2, and B_3 are active-site bases and L^- is the nucleophilic side chain of tyrosine, histidine, cysteine, or aspartate. B3 might be an amino acyl side chain or an acidic water molecule. The formed product can be 8α-N^1-histidinyl-flavin, 8α-N^3-histidinyl-flavin, 8α-*S*-cysteinyl-flavin, 8α-*O*-tyrosyl-flavin, or 8α-*O*-aspartyl-flavin. Source: Adapted from Heuts et al. [43].

performs a nucleophilic attack on the C8a-position, accompanied by a concerted protonation of N5, creating a covalent adduct between the protein and reduced flavin hydroquinone. Oxidation of the hydroquinone results in the oxidized 8α-peptidyl–flavin resting state. The formation of the reduced flavin intermediate was observed when the apo-form of bacterial monomeric sarcosine oxidase (MSOX), expressed and purified from a riboflavin auxotrophic *Escherichia coli* strain, was reconstituted with FAD *in vitro*. This aerobic reconstitution process, forming 8α-*S*-cysteinyl-FAD, produced a stoichiometric amount of hydrogen peroxide with apparent second-order kinetics, which suggests an autocatalytic process [57]. The same experiments were conducted for VAO, showing the same results [68]. The formation of reduced and quinone methide intermediates and the production of stoichiometric amounts of hydrogen peroxide *in vitro* show that this is an autocatalytic process. Autocatalytic 8-*S*-cysteinyl–flavin formation was also seen for the A394C mutant of putrescine oxidase from *Rhodococcus erythropolis* NCIMB 11540. This enzyme normally binds FAD noncovalently as a prosthetic group, but the introduced cysteine, which faces the C8a position of FAD, can actually form a covalent bond [69].

Replacement of FAD by 1-deaza and 5-deaza analogues does not lead to covalent bond formation in MSOX, as well as in *p*-cresol methyl hydroxylase (PCMH) and 6-hydroxy-D-nicotine oxidase (6-HDNO) [70], as can be explained by the lower redox potentials and lower electrophilicity, making the 8a-protons less acidic. Some flavin analogues with redox potentials higher than 5-deaza FAD were shown to covalently bind PCMH [71].

In some cases, covalent flavinylation might need some stabilizing factors, like helper proteins. PCMH is a $\alpha_2\beta_2$ tetramer, containing two flavoprotein α-subunits (PchF) and two c-type cytochrome β-subunits (PchC), and can only form a covalent 8α-*O*-tyrosyl-FAD bond when the full complex is formed. Apo-PchF can be noncovalently reconstituted with FAD, but the cofactor and the enzyme are only covalently linked in the presence of PchC. Binding of PchC to holo PchF causes a conformational change that leads to the covalent bond formation. The reduced flavin that is formed in this process, in turn, reduces the cytochrome, which also happens in its normal catalytic scheme [72, 73]. The autocatalytic formation of the histidyl-N^1-FAD bond in purified 6-HDNO needs the presence of additives like glycerol-3-phosphate *in vitro* [70, 74]. For PCMH, one could still argue that covalent bond formation is fully autocatalytic, as the whole tetramer that is needed for the 8α-*O*-tyrosyl-FAD bond formation is also necessary to form a normal catalytically active unit. And for 6-HDNO one could say the same, as these small molecules naturally occur in living cells and do not directly contribute to the catalytic process. Studies on human succinate dehydrogenase, however, have suggested that a small ~10 kDa protein, SdhAF2, is needed as an assembly factor for the formation of the covalent flavin–protein bond [75]. This factor has homologues in all kingdoms of life, like Sdh5 in yeast and SdhE in bacteria [76]. Structural, biochemical, and mutagenesis experiments on SdhE and Sdh5 have shown that this protein has a conserved motive for binding succinate dehydrogenase and quinol:fumarate reductase (RGXXE) and does not bind FAD itself [77–79]. Moreover, circular dichroism studies show that these flavoproteins are already completely folded and contain FAD before treatment with SdhE. Therefore, it is believed to stabilize the protein in a locked active-site conformation, promoting autocatalysis [80, 81]. The lack of SdhE homologues in some extreme thermophiles, containing succinate dehydrogenases that share high homology with their mesophilic counterparts, agrees with this hypothesis [82].

2.3.2.2 Formation of the 6-*S*-Cysteinyl–Flavin Bond

The formation of the 6-*S*-cysteinyl–FMN bond in trimethyl amine dehydrogenase (TMADH) is believed to happen autocatalytically by nucleophilic attack of the Cys30-thiolate anion on the C6 position. Tautomeric rearomatization creates a reduced 6-*S*-cysteinyl–FMN intermediate. This hydroquinone then donates electrons to a nearby [4Fe–4S] cluster by two consecutive 1-electron-transfer reactions, forming the oxidized 6-*S*-cysteinyl–flavin ground state [58] (Figure 2.5).

2.3.2.3 Formation of the Phosphoester Threonyl–FMN Bond

Unlike covalent bond formation at the isoalloxazine ring system, phosphoester threonyl–FMN bond formation is nonautocatalytic and needs assistance from an FMN transferase that incorporates FMN into the polypeptide. When the Na^+-translocating NADH:quinone oxidoreductase (Na^+-NQR) from *Vibrio cholera* was heterologously expressed in *E. coli*, no flavin incorporation was seen [83]. This led to the discovery of a "chaperone" protein, ApbE, through bioinformatic analysis of the genomes of all nqr operon-containing bacteria. AphE was later

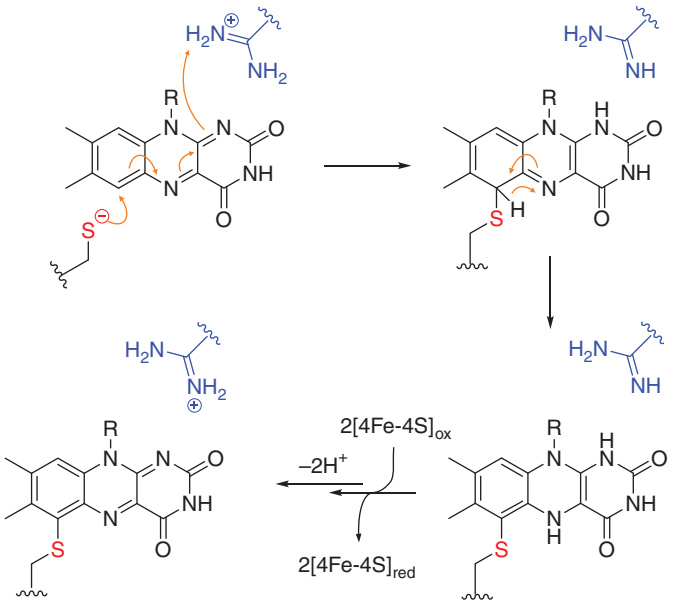


Figure 2.5 Proposed mechanism for the formation of 6-*S*-cysteinyl-FMN. The cysteinyl thiolate anion attacks C6 of the isoalloxazine ring system. The positively charged arginine (blue) assists by stabilizing the negative charge on N1. Source: Adapted from Scrutton et al. [58].

renamed as Ftp (flavin-trafficking protein). Coexpression of the *Vibrio harveyi* NqrC (a subunit of Na^+-NQR) and ApbE (Ftp) genes in *E. coli* resulted in the production of a covalently flavinylated NqrC, and also *in vitro* covalent flavinylation was seen when incubating FAD, ApbE, and NqrC in one pot [84]. A homologue of ApbE (Ftp) from *Treponema pallidum* (Ftp_Tp) was shown to have metal-dependent FAD pyrophosphatase activity, hydrolyzing FAD into AMP and FMN, as well as flavin-transferase activity. Mutating the active-site residue asparagine-55 to tyrosine changed its activity to a FAD-binding protein. And mutating the metal-binding aspartate-284 to an alanine abolished catalytic activity [85]. The same loss of activity was seen for ApbE when it was incubated with ethylenediaminetetraacetate (EDTA) [84]. Crystal structures of *Salmonella enterica* and *T. pallidum* ApbE indeed showed that these enzymes can bind FAD [85, 86]. This led to the conclusion that Ftps are not just chaperones, but are enzymes that catalyze the formation of the phosphoester threonyl–FMN bond. A consensus sequence for the covalent targeting was identified as: DgxtsAT/S, in which T/S is the residue that is covalently modified [87]. A conserved histidine, which is in close proximity to the FAD pyrophosphate in Ftp proteins, is believed to activate threonine or serine as a nucleophile for flavin transfer, or acts as a general acid to activate AMP as a leaving group. Mutagenesis studies indeed showed that this histidine is essential for catalysis [85]. Furthermore, it is also believed that a lysine (Lys-211) in Na^+-NQR is involved in activating the threonine or serine as nucleophile by polarization. A possible mechanism is shown in Figure 2.6.

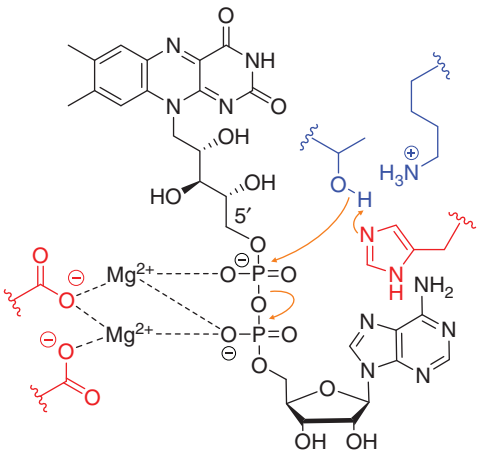


Figure 2.6 Phosphoester threonyl–FMN bond formation. Residues in red are from AphE/Ftp and residues in blue are from Na^+-NQR.

2.3.3 The Function of Covalent Flavinylation

Covalent flavinylation can serve different purposes in enzymes. Studies on enzymes with covalently bound flavins suggest a number of roles, namely increasing the redox potential, structural integrity, holoenzyme lifetime, flavin and substrate orientation, and flavin reactivity.

2.3.3.1 Redox Potential

The protein environment can alter the redox potential (midpoint potential, E_h or E_m) of flavins by noncovalent interactions [88, 89], but not as drastically as compared to covalent modifications. Substitutions at positions 7 and 8 of the isoalloxazine system (*meta* and *para* to N5) with electron-withdrawing and -donating substituents have a dramatic effect on the redox potential, and the correlation follows a linear Hammet relationship [90]. Mutagenesis studies on several enzymes with flavins tethered to the polypeptide chain through C8a showed that these enzymes have a higher redox potential than their noncovalent counterparts. The same is observed at the C6 position. The high redox potential of covalent flavoenzymes, which is in many cases above 0, results in a very limited array of electron acceptors. Therefore, most of these enzymes are indeed oxidases, using dioxygen as electron acceptor. A drastic drop in redox potential from +55 mV to −65 mV and an accompanying 10-fold decrease in catalytic activity were seen for VAO when the His-422, responsible for the covalent FAD linkage, was replaced by an alanine. The structure and protein stability, however, seem not to be affected by this mutation, suggesting that covalent flavinylation is purely influencing the redox potential in these enzymes [91, 92]. A similar trend was seen for PCMH when the covalent-anchoring point Tyr-384 was mutated to a phenylalanine. This mutation prevented covalent binding and resulted in a drop in redox potential (from +84–93 mV to +34–48 mV) and an astonishing drop in k_{cat} (from 121 to 3.8 s^{-1}), whereas the K_m remained virtually unchanged [71, 93]. The same effects were also seen in studies on cholesterol oxidase type II (CholO), where drastic drops in turnover numbers and redox potential were seen and there were no deviations in crystal structures [94, 95]. Changes in turnover

number can be correlated to a decrease in the flavin reduction rate when the redox potential is lowered.

Bicovalent attachment at both C8a and C6 results in an even higher redox potential, as is seen in *E. coli* reticuline oxidase, GOOX, and ChitO [96, 97]. Breaking either covalent attachment results in a decrease in reduction rate and decrease in redox potential. So, both covalent-anchoring events contribute to the elevation in redox potential.

2.3.3.2 Structural Integrity and Holoenzyme Lifetime

Covalent flavinylation increases the redox potential and therefore also influences the reduction rate and the electron-acceptor scope, but it can also effect the stability and substrate scope in some cases. A clear effect on the substrate scope and holoenzyme lifetime is seen in enzymes with a bicovalently anchored flavin, which have a very open active site that allows the conversion of bulky substrates and polymers, without loss of the cofactor [50, 97]. Removal of the C6-cysteinyl bond in ChitO influences the redox potential, as was discussed in Section 2.3.3.1, but also influences the K_m of the substrate, indicating that this bond is also important for the correct positioning of FAD in the active site for Michaelis complex formation with the substrate. A similar effect is seen for MSOX [98]. ChitO mutants with only one covalent attachment tend to be less stable and aggregate over time; moreover, mutants with none of the covalent linkages hardly express at all, which shows the stabilizing effects these attachments have on the protein [97]. Lower stability is also seen for CholO and 6-HDNO [70, 99].

2.4 Naturally Occurring Riboflavin Analogues and Modified Flavins – Roles and Occurrence

Several flavin analogues have been identified over the last couple of decades. These analogues are made by variations in biosynthesis or by enzyme-mediated modifications of FMN or FAD. Some are modified in such a way that they are better catalysts for the job than the standard riboflavin derivatives FMN and FAD, but others are believed to be artifacts of faulty catalysis.

2.4.1 6-Hydroxy and 7-Methyl-8-Hydroxyflavins

6-Hydroxyflavin mononucleotide (6-hydroxyFMN; Figure 2.7a) is one of these cases where a flaw in the catalytic mechanism results in an inactive flavin derivative. It was discovered in two mutants of trimethylamine dehydrogenase from *Methylophilus methylotrophus* (sp. W_3A_1). Active-site mutants W355L and C30A – which is normally responsible for a C6-cysteinyl flavin–protein bond – showed substrate-induced and substrate-independent formation of this compound, respectively, which severely reduced the oxidative demethylation activity [46]. Wild-type enzyme seemed to also produce 6-hydroxyFMN, but in much lower quantities. This is yet another advantage of covalent flavinylation: controlling the reactivity of the bound flavin.

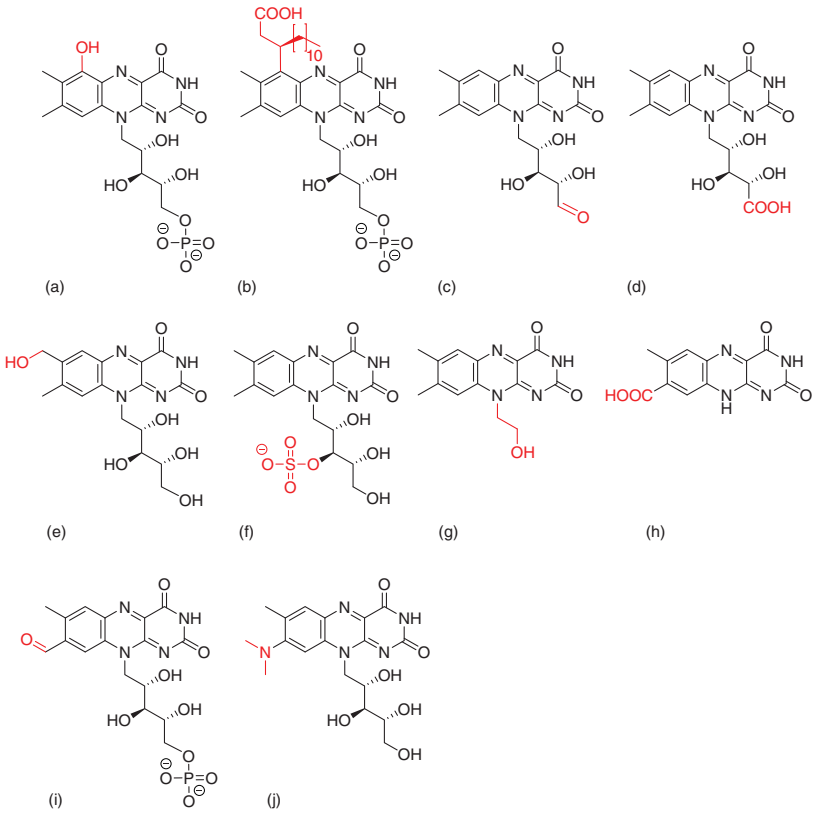


Figure 2.7 Structures of natural modified flavins. (a) 6-hydroxyFMN, (b) 6-(3′-(R)-myristyl)FMN, (c) 7,8-dimethyl-l0-(2,3,4-trihydroxy-4-formylbutyl)isoalloxazine (a chizoflavin), (d) 7,8-dimethyl-l0-(2,3,4-trihydroxy-4-carboxybutyl)isoalloxazine (a chizoflavin), (e) Nekoflavin; 7α-hydroxymethyl riboflavin, (f) riboflavin-3′-sulfate, (g) 10-hydroxymethyl-7,8-dimethylisoalloxazine (hydroxymethyl flavin), (h) 8-carboxy lumichrome, (i) 8-formylFMN, (j) roseoflavin.

At first, it was thought to be conceived by hydration of the electrophilic imino-quinone methide, which is the intermediate responsible for C8a and C6 covalent flavin–protein bonds, but a later isotope labeling study found that the 6-hydroxyl group was derived from molecular oxygen [100].

Also, 6-hydroxy modified FAD and FMN analogues were detected in an electron-transferring flavoprotein (ETF) from *Peptostreptococcus elsdenii* and glycolate oxidase from pig liver, respectively. This was a serendipitous discovery, due to the peculiar green color of these compounds at pH 9 [101]. The reason for the existence of these compounds and a possible function in these enzyme systems was never found.

Not long after the discovery of 6-hydroxyFAD in *P. elsdenii* FTR, the orange 7-methyl-8-hydroxyFAD was isolated from the same organisms as a prosthetic group of an NADH dehydrogenase [102]. The enzymes with this cofactor were

actually not active, and therefore it was thought that both newly identified cofactors in *P. elsdenii* were artifacts that were somehow created in the purification process.

2.4.2 6-(3′-(*R*)-Myristyl)Flavin Mononucleotide

6-(3′-(*R*)-myristyl)flavin mononucleotide (MyrFMN or Myristylated FMN; Figure 2.7b) is another covalently modified FMN, which is also believed to be an artifact of a faulty enzyme-mediated chemical reaction. It was found in bioluminescent bacteria in several genera, like *Photobacteria*, *Aliivibrio*, and *Vibrio* that use the FMN-dependent bacterial luciferase [103, 104], and a direct correlation between light production and MyrFMN production was seen. Bacterial luciferase catalyzes the oxidation of long-chain aldehydes to long-chain fatty acids, like myristic acid (tetradecanoic acid), through a chemically induced electron exchange luminescence (CIEEL) mechanism, which produces a short-lived excited state of FMN [105]. It is thought that rearrangement of the alkoxy radical to a carbon radical results in the covalent attachment of myristic acid to FMN [104]. Luciferase is strongly inhibited by MyrFMN [106, 107], and a gene product from *LuxF*, a homologue to the β-subunit of luciferase, is believed to be a scavenger for MyrFMN [104], and coexpression greatly enhances light production.

2.4.3 Chizoflavins

Two riboflavin derivatives, termed chizoflavins, were isolated in the culture broth of *Schizophyllum commune*, which had the 5′-hydroxyl group oxidized to an aldehyde or carboxylic acid functionality, yielding 7,8-dimethyl-l0-(2,3,4-trihydroxy-4-formylbutyl)isoalloxazine and 7,8-dimethyl-l0-(2,3,4-trihydroxy-4-carboxybutyl) isoalloxazine, respectively [108, 109], see Figure 2.7c,d. An nicotinamide adenine dinucleotide 2′-phosphate (NADPH)-dependent enzyme was later identified as the 5′-aldehyde-forming enzyme [110]. Thus far, no physiological role has been assigned to these chizoflavins.

2.4.4 7-Hydroxymethyl and 8-Hydroxymethyl Riboflavin

Nekoflavin (Figure 2.7e) was discovered in cat choroids as a pigment molecule [111]. This compound was later identified as 7α-hydroxymethyl riboflavin [112]. Both nekoflavin and 8α-hydroxymethyl riboflavin are found in human and rat urine, and may be pigments necessary for vision. Both 7-carboxy and 8-carboxylumichrome, also found in milk, are probably degradation products of these compounds (Figure 2.7h). Hydroxymethyl flavin, shown in Figure 2.7g, is also found in urine as a degradation product of riboflavin, which is probably produced by symbiotic bacteria in the urinal tract [113].

2.4.5 Plant Root Iron Uptake Cofactors

Some plants excrete reduced riboflavin and riboflavin analogues that contain either a 3′ or 5′-sulfate group from their roots upon iron starvation, as was seen for sugar

beet [114]. It is hypothesized that reduced flavin is used to reduce iron(III) to iron(II) and the riboflavin sulfates are then used for iron uptake. See Figure 2.7f.

2.4.6 Roseoflavin – An Antimicrobial Flavin Analogue

Roseoflavin, 8-dimethylamino-8-demethyl-D-riboflavin (RoF), was originally isolated from *Streptomyces davawensis* in 1974 and was found to have antimicrobial properties [115]. RoF is especially active against Gram-positive bacteria, and has a minimal inhibitory concentration (MIC) of 1.56 μg ml^{-1} for *Bacillus subtilis* and 0.25–6.25 μg ml^{-1} for *Staphylococcus aureus* [115, 116].

Although the exact nature of the antimicrobial effect is not well understood, it is postulated that roseoflavin inhibits FMN- and FAD-dependent enzymes as a competitive inhibitor, after being converted to roseoflavin-5′-phosphate and roseoflavin adenine dinucleotide [117–120]. Another possible mode of action is the binding of roseoflavin in FMN-dependent riboswitches that control the expression of the riboflavin biosynthesis machinery [121].

RoF is synthesized from FMN in several steps by three enzymes. The first enzyme, RosB, oxygenates FMN on the 8α-position to form 8-formyl-FMN, which is then further oxidized to 8-demethyl-8-carboxylFMN and then a thiamine-dependent decarboxylation/glutamate-dependent amination results in 8-demethyl-8-aminoriboflavin-5′-phosphate (AFP). RosB is a flavodoxin-like enzyme, composed of four subunits, which has evolved from an electron- or hydride-transferring flavoprotein to an "FMN 8-aminase" [122, 123]. AFP is subsequently dephosphorylated to form 8-amino-8-demethyl-D-riboflavin (AF) [124]. And AF is then subjected to two *S*-adenosyl methionine-dependent N-methylations, catalyzed by 8-amino-8-demethyl-D-riboflavin dimethyltransferase (RosA), to form roseoflavin [125].

2.4.7 8-Formyl Flavins

Formyl flavin analogues were reported three times in the last twenty years. A mutagenesis study on two strictly conserved active-site arginines in an FMN-dependent lactate oxidase from *Aerococcus viridans* showed the slow formation of so-called *8-formylFMN* (8-demethyl-8-formylFMN) [126]. In another study, 8-formylFAD (8-fFAD) was found in wild-type formate oxidase from *Aspergillus oryzae*. This enzyme seems to slowly and autocatalytically produce 8-fFAD after reconstitution of the apoenzyme with FAD, which makes the enzyme 10-fold more active [127]. A rationale for the increasing activity would be the increase in redox potential due to the electron-withdrawing group on the 8-position, as is also the case for flavins covalently attached to the polypeptide. It is actually thought that the 8-formyl group is formed through the same mechanism as covalent protein–flavin formation, involving the quinone–methide tautomer, which is hydrated to form the 8-fFAD product. Substitution of lysine-87, which is believed to act as a base in this mechanism, with an alanine prohibited the formation of 8-fFAD, thus strengthens this suggested mechanism [127].

8-fFAD was also discovered in the heterodimeric human electron-transferring flavoprotein (hETF) [128]. This enzyme is responsible for the transfer of electrons from at least 13 different flavin-dependent dehydrogenases to the mitochondrial respiratory chain through a noncovalently bound FAD cofactor. This noncovalently bound FAD cofactor undergoes the same time-dependent 8-formyl formation in the active site as was shown for formate oxidase, and seems to be pH dependent. Higher pH values promote 8-fFAD formation. The 8-fFAD cofactor in hETF seemed to reside in the semiquinonic state for longer period than is seen for FAD, which slightly lowered the observed rates. But, surprisingly, the 8-fFAD-containing hETF had a fivefold enhanced affinity to human dimethylglycine dehydrogenase, which shows that 8-fFAD must modulate the binding affinity somehow. Furthermore, it was shown that two mutations that prohibit the formation of 8-fFAD are a cause of glutaric aciduria type II, a rare genetic metabolic disease [128]. Thus, formation of this 8-formylFAD compound results in better enzyme activities and its absence can even result in a life-threatening chronic disease.

2.4.8 Prenyl-FMN

Another recent discovery of a highly modified flavin was done while investigating a decarboxylase in the ubiquinone biosynthesis pathway of fungi and bacteria. Biochemical and crystallographic studies on *Aspergillus niger* FdcI and the *E. coli* homologue UbiD have detected a prenylated FMN in their active sites [129, 130]. The prenylated FMN, prenyl-FMN (prFMN), is provided by an FMN-prenyl transferase, which is UbiX in *E. coli* and Pad1 in *A. niger*. The substrate for the transferase is dimethylallyl-monophosphate and is initially linked to N5 by nucleophilic attack of the nitrogen atom on C1′, releasing phosphate through nucleophilic displacement. The active site then assists in a conformational change of the olefin adduct through the formation of carbocation species, which then promotes the formation of the C6–C3′ dimethylallyl bond [131], see Figure 2.8a. After the formation of holo-UbiD/Fdc1 the reduced prenyl-FMN undergoes oxidative maturation, most probably through proton-coupled electron transfer [129]. Oxidized prenyl-FMN has an azomethine ylide character, and two isomeric forms, a secondary N5-ketamine and an N5-iminium adduct, have been observed in the crystal structure of Fdc1, with a very different ring structure. The hexameric holoenzyme catalyzes the reversible decarboxylation of cinnamic acid *in vitro*, which is placed directly on top of the azomethine ylide. The substrate and cofactor undergo a 1,3-dipolar cycloaddition – the first to be described in nature – and the formed substrate–prFMN$^{\text{iminium}}$ pyrrolidine adduct is then decarboxylated through a Grob-type fragmentation reaction. A second prFMN$^{\text{iminium}}$ pyrrolidine adduct is then formed, upon protonation of C2′, which results in product (styrene) release through a *retro*1,3-cycloaddition [130], see Figure 2.8b. The stabilized iminium adduct of prFMN is the first discovered modification that facilitates noncanonical flavin chemistry, as it enables covalent 1,3-cycloaddition catalysis – something not seen before in nature.

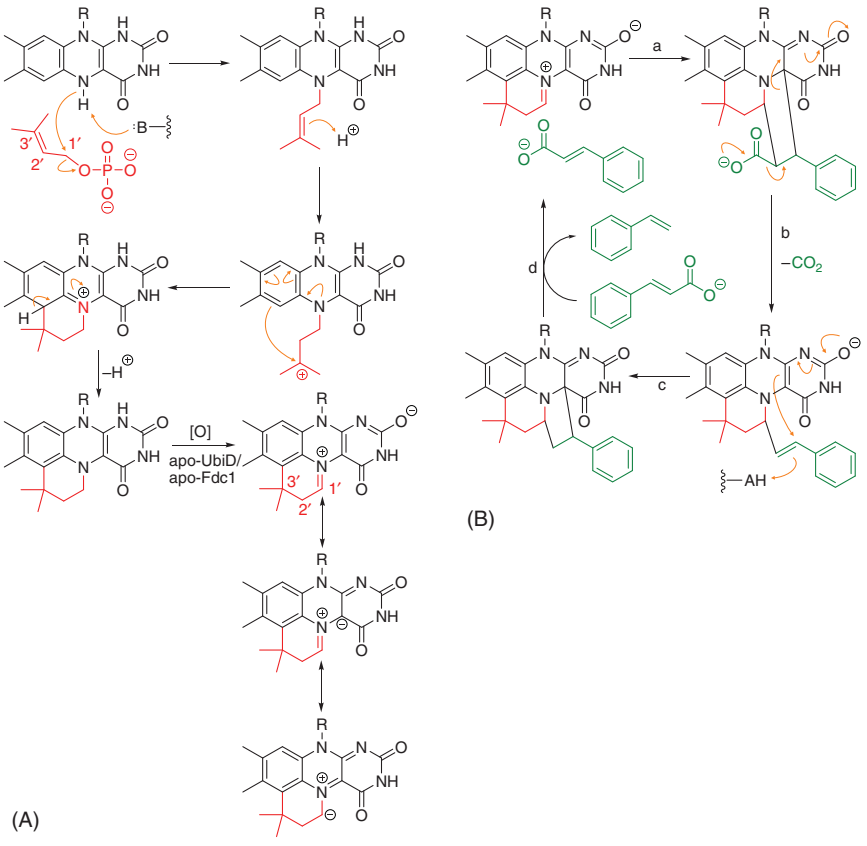


Figure 2.8 Prenyl-FMN formation and UbiD/Fdc1 decarboxylase activity. (a) the UbiX/Pad1 catalyzed formation of prenyl-FMN and oxidative maturation in UbiD/Fdc1. (b) Catalytic cycle of UbiD/Fdc1 with cinnamic acid. a: formation of substrate–prFMN^{iminium} pyrrolidine adduct through a 1,3-dipolar cycloaddition, b: Grob-type fragmentative decarboxylation, c: intermediate–prFMN^{iminium} pyrrolidine adduct formation and protonation, d: product release by retro 1,3-dipolar cycloaddition. Source: Adapted from Marshall et al. [132] and Payne et al. [130].

2.4.9 Artificial Cofactors and Novel Catalytic Activity

Artificial flavin analogues have been used extensively as active-site probes for the elucidation of flavoenzyme mechanisms and flavin chemistry [90, 133–136]. Artificial flavins have also been successfully introduced as a means to create or change enzyme activity. The dehalogenase activity of iodotyrosine deiodinase could be changed to nitroreductase activity by exchanging FMN with 5-deazaFMN, using $NaBH_4$ as the reductant [137]. Unlike most native nitroreductases, which convert the substrate only to the hydroxylamine, this artificial enzyme could fully reduce its substrate to the amine product.

Similarly, monooxygenase activity could be introduced to a riboflavin-binding protein by exchanging riboflavin with several *N*-alkylated flavins. These artificial flavoenzymes could perform H_2O_2-driven sulfoxidations, for which

the enantioselectivity could be switched by using different *N*-alkylated flavin analogues [138].

Three F_{420}-dependent reductases, MSMEG_2027, MSMEG_6848, and MSMEG_3356, are capable of using both F_{420} and FMN as a cofactor to either reduce or oxidize the aflatoxins AFG1 and AFG2 [139]. In other words, also natural systems can adopt different activities by switching naturally occurring cofactors. Cofactor exchange may continue to be an interesting method of creating novel activities in existing flavoproteins.

2.5 N5-substrate and N5-oxygen Adducts

The canonical reactivity of flavin cofactors is redox chemistry through N5-hydride transfer or oxygen activation at C4a. Most dehydrogenases, oxidases, and monooxygenases utilize these catalytic strategies. The previous section discussed prenyl-FMN and its ability to do 1,3-dipolar cycloadditions. The stabilized N5-iminium ion in a cyclic azomethine ylide functionality of this highly modified flavin is the cause of this novel reactivity. Although the majority of enzymes containing unmodified FMN or FAD cofactors perform the standard "canonical" redox chemistry, a few flavoenzymes have recently been shown to catalyze redox-neutral processes with covalent flavin-N5-substrate intermediates. The best-studied enzyme in flavin-dependent covalent catalysis is uridine 5′-diphosphate (UDP)-galactopyranose mutase (UGM), which catalyzes the isomerization to UDP-galactopyranose to UDP-galactofuranose.

2.5.1 Redox-neutral Covalent Catalysis

A wealth of studies have resulted in the elucidation of the mechanism of UGM. Crystal structures indicate that the substrate, UDP-galactopyranose, binds close to reduced FAD, and that the O4 of FAD hydrogen bonds with the sugar moiety [140]. Positional isotope exchange then showed that the O-glycosidic bond is cleaved before isomerization, which implies that a highly electrophilic oxocarbenium cation is formed [141]. This can then react with the nucleophilic N5-position of the isoalloxazine ring, creating a covalent intermediate between N5 and the anomeric carbon. A covalent FAD-galactose intermediate was indeed seen in a crystal structure [142]. The N5–anomeric carbon bond promotes sugar-ring opening by iminium ion formation, which later acts as an electron sink for ring closure. Nucleophilic attack by a phosphate oxygen of UDP forms UDP-galactofuranose and the reduced flavin ground state, ending the catalytic cycle. See Figure 2.9.

A similar iminium-adduct intermediate is seen in alkyl-dihydroxyacetone phosphate synthase, which is involved in the biosynthesis of important ether lipids. In this enzyme, a covalent N5–substrate adduct is formed by a nucleophilic attack from C1 of the enolate form of the substrate, acyldihydroxyacetone phosphate, which is stabilized by active-site histidine residues. Strikingly, the flavin needs to be in the oxidized state for catalysis, contrary to UGM. The iminium adduct acts as an electron sink for further catalysis and promotes ether formation with fatty alcohols [144].

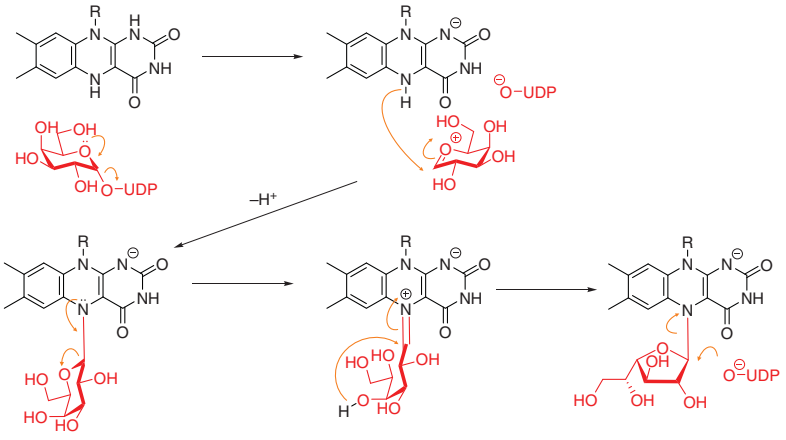


Figure 2.9 Proposed UGM mechanism. Source: Adapted from Tanner et al. [143].

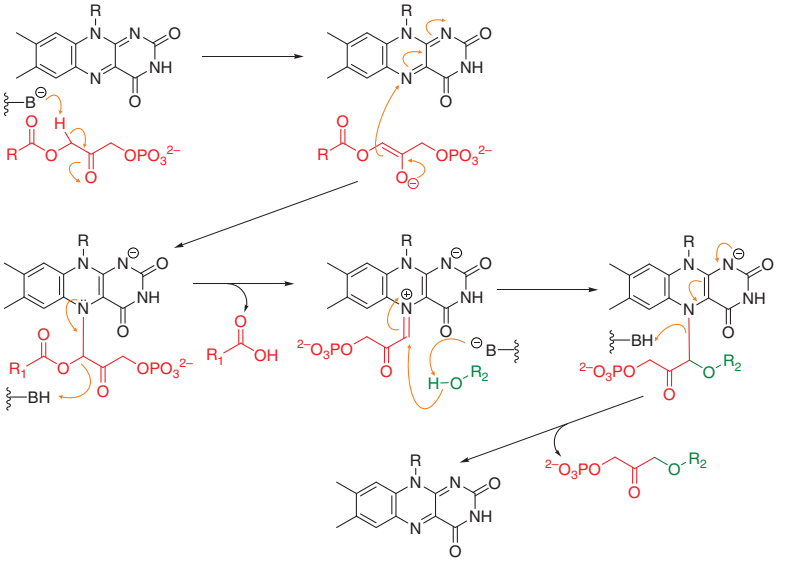


Figure 2.10 Proposed alkyl-dihydroxyacetone phosphate synthase (ADPS) mechanism. Source: Adapted from Sobrado [145].

Another fascinating observation is that the active-site architecture and the whole structure are very similar to those of VAO enzymes. This shows that minor evolutionary changes can greatly alter protein function. See Figure 2.10.

Two other bacterial enzymes, namely tRNA methyl-transferase (TrmFO) [146] and flavin-dependent thymidylate synthase (FDTS) [147], catalyze the methylenetetrahydrofolate-dependent methylation of uracil in tRNA or dUMP, respectively. Both enzymes utilize the flavin cofactor as a covalent methyl-transfer catalyst, again exploiting the flavin N5-imminium intermediate, but the two

structurally unrelated enzymes catalyze the reaction differently [148–150]. This shows that covalent catalysis might be more abundant in nature and that highly unrelated enzymes can exploit the same N5-iminium adduct in different ways.

2.5.2 N5-oxygen Adducts

The majority of flavin-dependent monooxygenases utilizes the C4a-(hydro)peroxyflavin intermediate as a form of activated oxygen [20]. A few flavoenzymes, however, were found to utilize an N5-peroxy flavin intermediate. The first discovered enzyme that was shown to harbor an N5-oxide ($Fl_{N5[O]}$) is EncM from *Streptomyces maritimus*, which is involved in the enterocin biosynthesis pathway [151]. UV/VIS spectroscopic and mass spectrum analysis showed that the covalently bound FAD prosthetic group was present as an N5-oxide adduct [22, 152]. This $FAD_{N5[O]}$ forms a covalent adduct with an enolic carbon of the substrate, which then results in the oxygenated substrate and oxidized FAD. The formed alcohol then reduces FAD and forms a ketone product, which subsequently undergoes a Favorskii-type rearrangement, inside the enzyme. The reduced flavin can then again form the N5-oxide by reacting with molecular oxygen. See Figure 2.11.

The C4a-peroxyflavin is thought to be formed by a single-electron transfer between the C4a carbon and oxygen upon "face-on" approach. Apolar or amphipathic oxygen-binding pockets around this flavin locus facilitate this reaction. O_2-pressurized X-ray crystallography showed that EncM has such a binding pocket on the *re*-face of FAD, facing the N5 in a reactive position [153]. The same was seen for the crystal structure of another N5-oxide containing protein, RutA, where O_2 has a distance of 2.1 Å from N5 and a dihedral angle of 99° with respect to the FMN plane, ideal for covalent bond formation [154]. The current opinion is that N5 reacts with oxygen in a similar fashion as C4a, in which the reduced hydroquinone form and oxygen have a single-electron transfer, resulting in a negatively charged semiquinone radical, in which the radical resides on the N5. Radical pairing yields flavin N5-hydroperoxide, which can form N5-oxide by elimination of water [22, 154]. See Figure 2.12.

Escherichia coli RutA is a group C monooxygenase, which catalyzes the first step in the Rut pathway, which is the conversion of uracil to 3-ureindoacrylic acid [155, 156]. Reactions with isotopically labeled oxygen, oxygen-pressurized crystallography,

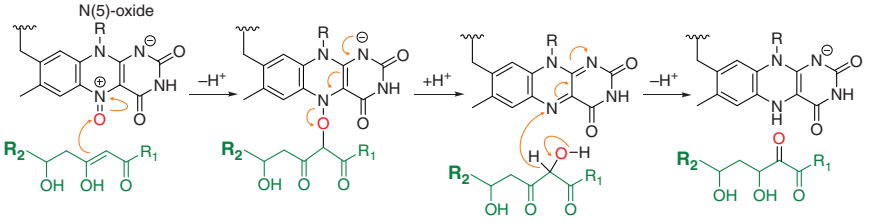


Figure 2.11 EncM mechanism involving a crucial $FAD_{N5[O]}$. Source: Adapted from Teufel et al. [22].

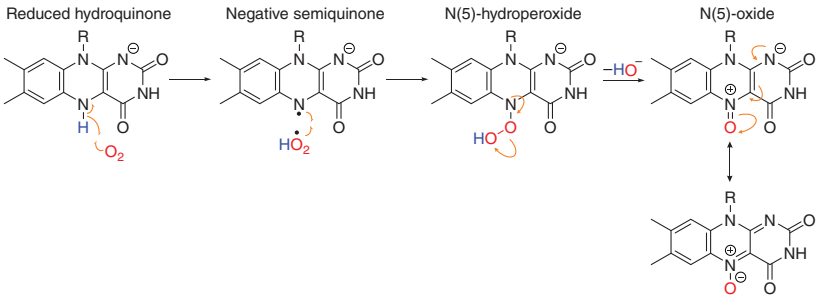


Figure 2.12 Proposed mechanism for the formation of flavin N(5)-oxide. Source: Adapted from Matthews [154].

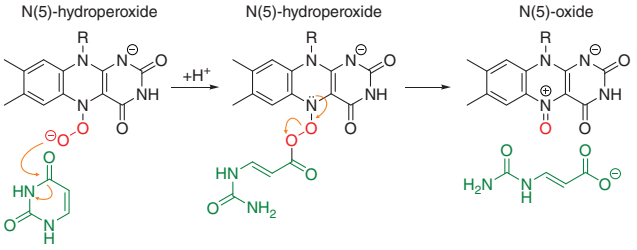


Figure 2.13 Proposed mechanism for RutA. Source: Adapted from Adak and Begley [156].

and quantum mechanical modeling suggest that the N5-peroxyflavin is responsible for substrate oxygenation through a peroxyacid intermediate, forming the product and flavin N5-oxide, as is shown in Figure 2.13 [154, 156]. It was shown that two other members from the same group C monooxygenase also exploit the N5-peroxide/N5-oxide for catalysis. These enzymes are DszA from *R. erythropolis* [157] and HcbA1 from *Nocardioides* sp. Strain PD653 [158]. Both enzymes use the N5-peroxyflavin as a weak nucleophile, oxygenating dibenzothiophene sulfone and hexachlorobenzene through nucleophilic aromatic substitutions.

The identification of three monooxygenases from the same group as N5-peroxyflavin utilizing enzymes in different bacterial species suggests that more enzymes in this superfamily might exploit this type of catalysis. It could be that the N5-oxide intermediate was overlooked in previously characterized monooxygenases from this and other superfamilies. The UV/VIS-spectrum of the N5-oxide intermediate is in fact very similar to that of oxidized flavin. EncM is structurally not related to the group C monooxygenases, but has a mechanism that also proceeds through the N5-oxide intermediate. This indicates that this flavin chemistry is not restricted to a specific class of flavoproteins.

2.6 F_{420} – A Natural Deazaflavin

F_{420} is sometimes seen as a rare cofactor, but this naturally occurring deazaflavin is actually widespread in nature, as it is found in methanogenic, halophilic, and sulfate-reducing archaea as well as many Actinobacteria [159–161]. It was first discovered in lysates of *Methanobacterium bryantii* in which it is responsible for the characteristic blue-green fluorescence at 420 nm, hence the name [162]. The extremely low redox potential and electrochemical and photochemical properties make F_{420} an attractive cofactor for various other purposes [163].

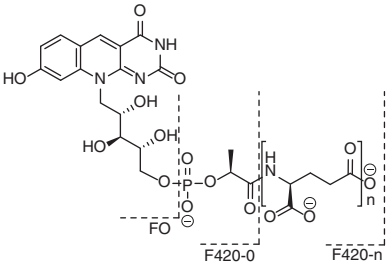
2.6.1 Structure and Properties of F_{420}

The structure of F_{420} was solved in the same decade as its discovery [164]. It has a tricyclic 8-hydroxy-5-deazaisoalloxazine catalytic core structure, which is attached to a ribityl moiety at N10. This 8-hydroxy-5-deazariboflavin compound, called FO (or Fo), is further decorated with a polar 5′-phospho-L-lactyl-γ-L-glutamyl tail, see Figure 2.14. The number of glutamyl residues varies depending on the organism and ranges from 2 in methanogens to sometimes 9 in *Mycobacteria* [165]. The 5-deaza and 8-hydroxy modifications have a large effect on the chemical properties of this flavin analogues, as compared to FMN and FAD. The redox potential of free F_{420}, being −340 mV, is dramatically lower than that of FMN, FAD, and NAD(P)H, and can be even −385 mV under some physiological conditions [166, 167]. Furthermore, F_{420} is an obligate hydride transfer agent, as semiquinone radical states cannot be stabilized in the absence of N5 [168]. This makes reduced F_{420} ($F_{420}H_2$) a relatively stable compound under aerobic conditions. This deazaflavin cofactor has a distinctive absorbance maximum at 420 nm, which shifts to lower wavelengths at lower pH values, with an isobestic point at 400 nm ($\varepsilon_{400\,nm} = 25.7\,mM^{-1}\,cm^{-1}$). It has a fluorescence emission maximum at 470 nm [164].

2.6.2 Physiological Functions of F_{420}

F_{420} has several important roles in methanogens. Reduced F_{420} is of key importance in the CO_2-reducing pathway, and therefore these organisms exploit several means of reducing F_{420}, like molecular hydrogen, formate, and secondary alcohols, catalyzed by Frh, Ftd, and Adf, respectively [169–173]. $F_{420}H_2$ is then used as a cofactor

Figure 2.14 Structure of F_{420}.



for Mer and Mtd that reduce methenyl-tetrahydromethanopterin [174, 175]. It can also be used in the methanogenic electron transport chain, oxygen detoxification, sulfur mobilization, and $NADP^+$-reduction for biosynthesis [176–180]. Although not much is known about most of these organisms, it is believed that F_{420} also performs a crucial role in the central metabolism of sulfate-reducing archaea and halobacteria [181].

F_{420} is also present in various Actinobacteria, in which it does not seem to have a role in central metabolism. Knockouts in the F_{420}-biosynthesis machinery did not affect *Mycobacteria* under optimal growth conditions, but prohibited survival under oxidative and nitrosative stress [182, 183]. Therefore, it is believed that this cofactor plays an important role in stress relief. An F_{420}-dependent glucose-6-phosphate dehydrogenase seems to be the source of reduced cofactor for the protection against stress [183–185]. Some enzymes in a subgroup of the flavin/deazaflavin oxidoreductases (FDORs; with a β-roll topology), the FDOR-As, can reduce menaquinone with reduced F_{420}, assuring that the respiratory chain stays in a reduced state [182]. A set of prodrugs were found to be activated by these F_{420}-dependent reductases, releasing toxic nitrogenoxides into the cytosol [186]. Another *Mycobacterial* F_{420}-dependent enzyme, hydroxymycolic acid dehydrogenase, is directly involved in the biosynthesis of virulence factors that make these cells less susceptible to antibiotics [187]. Several F_{420}-dependent reductases from the FDOR superfamily are involved in secondary metabolism of *Mycobacteria*, and perform fatty-acid modifications and reduce degradation products of heme, some of which may form potent antioxidants [188]. Members of the same superfamily are involved in the biosynthesis of tetracycline and its homologues, as well as lincosamide and pyrrolobenzodiazapine antibiotics in *Streptomyces* species [189–191].

2.6.3 F_{420} Biosynthesis

F_{420} in synthesis utilizes 5-amino-6-ribitylamino-2,4[1*H*,3*H*]-pyrimidinedione from the riboflavin biosynthesis pathway. But this compound is instead condensed with tyrosine to form the 7,8-didemethyl-8-hydroxyriboflavin compound FO. FO synthesis is catalyzed by either two separate enzymes in archaea, CofG, and CofH, or by the bifunctional FbiC in Actinobacteria, which proceeds through a radical mechanism that involves *S*-adenosyl methionine [192, 193]. FO is decorated with a 5′-phosphoryl-L-lactyl tail or phosphoryl-enolpyruvyl tail from enolpyruvyl-diphospho-5′-guanosine (EPPG) or L-lactyl-2-diphospho-5′-guanosine (LPPG) by the action of CofD in archaea or FbiA in Actinobacteria. LPPG and EPPG can be synthesized from phosphoenol pyruvate or lactate and GTP by CofC or FbiD. Dehydro-F_{420-0}, the product of FbiA, is reduced by the FMN-dependent bifunctional enzyme FbiB to form F_{420-0}. The same enzyme then finalizes the synthesis by attaching several (2–9) glutamyl residues to F_{420}, forming F_{420-n} [194]. See Figure 2.15 for an overview of the biosynthetic pathway. A peculiar F_{420} variant was found in the Gram-negative, endofungal bacterium *Paraburkholderia rhizoxinica*. This bacterium produces 3PG-F_{420}, which has a phosphoglyceryl tail, instead of a phospholactyl tail [195].

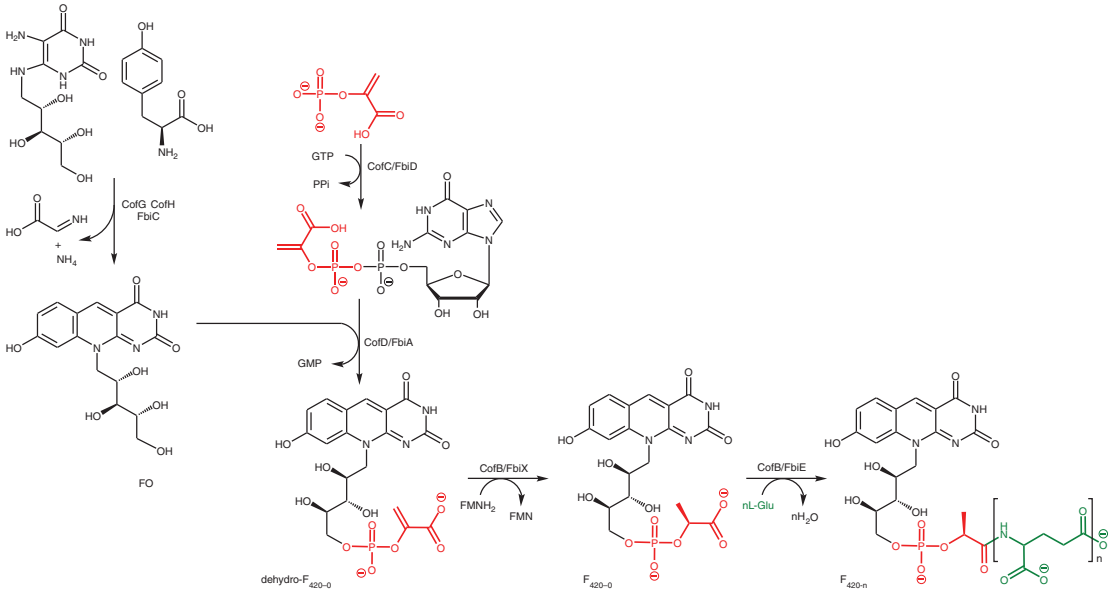


Figure 2.15 F_{420}-biosynthesis pathway in Actinobacteria. Fbi enzymes are found in Actinobacteria and the corresponding Cof enzymes are found in archaea.

2.6.4 F_{420}-dependent Enzymes in Biocatalysis

The very low redox potential and its stability under aerobic conditions make F_{420} a very attractive cofactor for biocatalytic purposes. The members of the FDOR and luciferase-like hydride transferases (LLHT; having TIM barrel topology) superfamily show a diverse range of compounds that can be converted, some of which are recalcitrant to other enzymatic systems. F_{420}-dependent ene reductases, imine reductases, and secondary alcohol dehydrogenases in these superfamilies could be valuable redox catalysts in the future, as well as many more enzymes that have yet to be discovered. Glucose-6-phosphate dehydrogenases and F_{420}:NADPH oxidoreductases, as well as other enzymes, could be used as cofactor recycling systems [25]. The special photochemical properties of deazaflavins could also be exploited for light-driven reactions, omitting the necessity for cofactor recycling systems or yielding completely new catalytic mechanisms.

2.7 Conclusion

The flavin cofactors FMN and FAD can convert a wealth of compounds through their canonical N5-hydride transfer and C4a-(hydro)peroxide exploiting catalytic strategies. Oxidases, monooxygenases, and reductases that exploit this chemistry are capable of aromatic, aliphatic, and heteroatom oxygenations and oxidations, dehalogenation, halogenation, and even light production. Covalent flavin–protein adducts and other modified flavins, like 8-formyl and prenylated flavins, can greatly enhance the catalytic properties of FMN and FAD. The modifications that nature made to flavins can serve as inspiration for man-made artificial cofactor systems that can boost the field of biocatalysis. Cofactor replacement studies with artificial cofactors have already shown novel activities in existing enzymes.

Noncanonical flavin chemistry exploits the catalytic power of the N5 locus on the isoalloxazine ring, and expands the known flavin chemistry even further. Redox-neutral covalent catalysis, through N5-imminum intermediates, opens up a whole new field of flavin chemistry. This is even more expanded by covalent modifications, like the highly modified prenyl-FMN cofactor, making it possible to do reversible decarboxylations of aromatic compounds. N5-peroxy and N5-oxide catalysis also shows that flavins utilize several pathways for oxygenation. The notion that different types of flavin chemistry can exist in one family of enzymes, and that at the same time one family of enzymes can have a mass of different catalytic strategies, shows that flavins are far more versatile and – maybe – less predictable than we thought. This makes one wonder what is still to be discovered in the field of flavin catalysis.

References

1 Warburg, O. and Christian, W. (1932). Ein zweites sauerstoffübertragendes Ferment und sein Absorptionsspektrum. *Naturwissenschaften* 20: 688.

2 Scholtissek, A., Tischler, D., Westphal, A.H. et al. (2017). Old yellow enzyme-catalysed asymmetric hydrogenation: linking family roots with improved catalysis. *Catalysts* 7: 130.

3 Romero, E. and Gadda, G. (2014). Alcohol oxidation by flavoenzymes. *Biomol. Concepts* 5: 299–318.

4 Pickl, M., Fuchs, M., Glueck, S.M. et al. (2015). The substrate tolerance of alcohol oxidases. *Appl. Microbiol. Biotechnol.* 99: 6617–6642.

5 Koshiba, T., Saito, E., Ono, N. et al. (1996). Purification and properties of flavin- and molybdenum-containing aldehyde oxidase from coleoptiles of maize. *Plant Physiol.* 110: 781–789.

6 Dijkman, W.P. and Fraaije, M.W. (2014). Discovery and characterization of a 5-hydroxymethylfurfural oxidase from *Methylovorus* sp. strain MP688. *Appl. Environ. Microbiol.* 80: 1082–1090.

7 Wilson, R. and Turner, A.P.F. (1992). Glucose oxidase: an ideal enzyme. *Biosens. Bioelectron.* 7: 165–185.

8 Martin, C., Trajkovic, M., and Fraaije, M.W. (2020). Production of hydroxy acids: selective double oxidation of diols by flavoprotein alcohol oxidase. *Angew. Chem. Int. Ed.* 59: 4869–4872.

9 Li, L., Liu, X., Yang, W. et al. (2007). Crystal structure of long-chain alkane monooxygenase (LadA) in complex with coenzyme FMN: unveiling the long-chain alkane hydroxylase. *J. Mol. Biol.* 376: 453–465.

10 Entsch, B. and Van Berkel, W.J.H. (1995). Structure and mechanism of para-hydroxybenzoate hydroxylase. *FASEB J.* 9: 476–483.

11 Chakraborty, S., Ortiz-Maldonado, M., Entsch, B. et al. (2010). Studies on the mechanism of *p*-hydroxyphenylacetate 3-hydroxylase from *Pseudomonas aeruginosa*: a system composed of a small flavin reductase and a large flavin-dependent oxygenase. *Biochemistry* 49: 372–385.

12 Donoghue, N.A., Norris, D.B., and Trudgill, P.W. (1976). The purification and properties of cyclohexanone oxygenase from *Nocardia globerula* CL1 and *Acinetobacter* NCIB 9871. *Eur. J. Biochem.* 63: 175–192.

13 Fürst, M.J.L.J., Gran-Scheuch, A., Aalbers, F.S. et al. (2019). Baeyer-Villiger monooxygenases: tunable oxidative biocatalysts. *ACS Catal.* 9: 11207–11241.

14 Walsh, C.T. and Chen, Y.-C.J. (1988). Enzymic Baeyer-Villiger oxidations by flavin-dependent monooxygenases. *Angew. Chem. Int. Ed. Eng.* 27: 333–343.

15 Van Pée, K.H. and Unversucht, S. (2003). Biological dehalogenation and halogenation reactions. *Chemosphere* 52: 299–312.

16 Sutton, W.B. (1957). Mechanism of action and crystallization of alctic oxidative decarboxylase from *Mycobacterium phlei*. *J. Biol. Chem.* 226: 395–405.

17 Zhao, Y. (2012). Auxin biosynthesis: a simple two-step pathway converts tryptophan to indole-3-acetic acid in plants. *Mol. Plant* 5: 334–338.

18 Brodl, E., Winkler, A., and Macheroux, P. (2018). Molecular mechanisms of bacterial bioluminescence. *Comput. Struct. Biotechnol. J.* 16: 551–564.

19 Massey, V. (2000). The chemical and biological versatility of riboflavin. *Biochem. Soc. Trans.* 28: 283–296.

20 Huijbers, M.M.E., Montersino, S., Westphal, A.H. et al. (2014). Flavin dependent monooxygenases. *Arch. Biochem. Biophys.* 544: 2–17.

21 Romero, E., Rubén, J., Gómez, R. et al. (2018). Same substrate, many reactions: oxygen activation in flavoenzymes. *Chem. Rev.* 118: 1742–1769.

22 Teufel, R., Stull, F., Meehan, M.J. et al. (2015). Biochemical establishment and characterization of EncM's flavin-N5-oxide cofactor. *J. Am. Chem. Soc.* 137: 8078–8085.

23 Wang, P.H., Khusnutdinova, A.N., Luo, F. et al. (2018). Biosynthesis and activity of prenylated FMN cofactors. *Cell Chem. Biol.* 25: 560.e6–570.e6.

24 Greening, C., Ahmed, F.H., Mohamed, A.E. et al. (2016). Physiology, biochemistry, and applications of F_{420}-and Fo-dependent redox reactions. *Microbiol. Mol. Biol. Rev.* 80: 451–493.

25 Shah, M.V., Antoney, J., Kang, S.W. et al. (2019). Cofactor F_{420}-dependent enzymes: an under-explored resource for asymmetric redox biocatalysis. *Catalysts* 9: 868.

26 Foor, F. and Brown, G.M. (1980). GTP Cyclohydrolase II from *Escherichia coli*. *Methods Enzymol.* 66: 303–307.

27 Mailänder, B. and Bacher, A. (1976). Biosynthesis of rirboflavin; structure of the purine precursor and origin of the ribityl side chain. *J. Biol. Chem.* 251: 3623–3628.

28 Richter, G., Fischer, M., Krieger, C. et al. (1997). Biosynthesis of riboflavin: characterization of the bifunctional deaminase-reductase of *Escherichia coli* and *Bacillus subtilis*. *J. Bacteriol.* 179: 2022–2028.

29 Hollander, I. and Brown, G.M. (1979). Biosynthesis of riboflavin: reductase and deaminase of *Ashbya gossypii*. *Biochem. Biophys. Res. Commun.* 89: 759–763.

30 Haase, I., Sarge, S., Illarionov, B. et al. (2013). Enzymes from the haloacid dehalogenase (HAD) superfamily catalyse the elusive dephosphorylation step of riboflavin biosynthesis. *ChemBioChem* 14: 2272–2275.

31 Kis, K., Volk, R., and Bacher, A. (1995). Biosynthesis of biboflavin. Studies on the reaction mechanism of 6,7-dimethyl-8-ribityllumazine synthase. *Biochemistry* 34: 2883–2892.

32 Neuberger, G. and Bacher, A. (1986). Biosynthesis of riboflavin. enzymatic formation of 6,7-dimethyl-8-ribityllumazine by heavy riboflavin synthase from *Bacillus subtilis*. *Biochem. Biophys. Res. Commun.* 139: 1111–1116.

33 Volk, R. and Bacher, A. (1988). Biosynthesis of riboflavin. The structure of the four-carbon precursor. *J. Am. Chem. Soc.* 110: 3651–3653.

34 Neuberger, G. and Bacher, A. (1985). Biosynthesis of riboflavin. An aliphatic intermediate in the formation of 6,7-dimethyl-8-ribityllumazine from pentose phosphate. *Biochem. Biophys. Res. Commun.* 127: 175–181.

35 Volk, R. and Bather, A. (1990). Studies on the 4-carbon precursor in the biosynthesis of riboflavin; purification and properties of L-3,4-dihydroxy-2-butanone-4-phosphate synthase. *J. Biol. Chem.* 265: 19479–19485.

36 Winestock, C.H., Aogaichi, T., and Plaut, G.W.E. The substrate specificity of riboflavin synthetase. *J. Biol. Chem.* 233: 2866–2874.

37 Abbas, C.A. and Sibirny, A.A. (2011). Genetic control of biosynthesis and transport of riboflavin and flavin nucleotides and construction of robust biotechnological producers. *Microbiol. Mol. Biol. Rev.* 75: 321–360.

38 Oppenheimer, N.J. (2010). NAD^+ and $NADP^+$ as prosthetic groups for enzymes. In: *eLS*. Wiley: Chichester, UK.

39 Reche, P. and Perham, R.N. (1999). Structure and selectivity in post-translational modification: attaching the biotinyl-lysine and lipoyl-lysine swinging arms in multifunctional enzymes. *EMBO J.* 18: 2673–2682.

40 Xie, L. and Van Der Donk, W.A. (2001). Homemade cofactors: self-processing in galactose oxidase. *PNAS* 98: 12863–12865.

41 Dooley, D.M. (1999). Structure and biogenesis of topaquinone and related cofactors. *J. Biol. Inorg. Chem.* 4: 1–11.

42 Mure, M. (2004). Tyrosine-derived quinone cofactors. *Acc. Chem. Res.* 37: 131–139.

43 Heuts, D.P.H.M., Scrutton, N.S., McIntire, W.S. et al. (2009). What's in a covalent bond? *FEBS J.* 276: 3405–3427.

44 Kearney, E.B. and Singer, T.P. (1955). On the prosthetic group of succinic dehydrogenase. *Biochim. Biophys. Acta* 17: 596–597.

45 Walker, W.H., Singer, T.P., Ghisla, S. et al. (1972). Studies on succinate dehydrogenase. 8alpha-histidyl-FAD as the active center of succinate dehydrogenase. *Eur. J. Biochem.* 26: 279–289.

46 Mewies, M., McIntire, W.S., and Scrutton, N.S. (1998). Covalent attachment of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) to enzymes: the current state of affairs. *Protein Sci.* 7: 7–21.

47 Starbird, C.A., Maklashina, E., Cecchini, G. et al. (2015). Flavoenzymes: covalent versus noncovalent. In: *eLS*. Wiley: Chichester, UK.

48 Huang, C.H., Lai, W.L., Lee, M.H. et al. (2005). Crystal structure of glucooligosaccharide oxidase from *Acremonium strictum*: a novel flavinylation of 6-S-cysteinyl, 8α-N1-histidyl FAD. *J. Biol. Chem.* 280: 38831–38838.

49 Alexeev, I., Sultana, A., Mäntsälä, P. et al. (2007). Aclacinomycin oxidoreductase (AknOx) from the biosynthetic pathway of the antibiotic aclacinomycin is an unusual flavoenzyme with a dual active site. *Proc. Natl. Acad. Sci. U.S.A.* 104: 6170–6175.

50 Winkler, A., Hartner, F., Kutchan, T.M. et al. (2006). Biochemical evidence that berberine bridge enzyme belongs to a novel family of flavoproteins containing a bi-covalently attached FAD cofactor. *J. Biol. Chem.* 281: 21276–21285.

51 Rand, T., Qvist, K.B., Walter, C.P. et al. (2006). Characterization of the flavin association in hexose oxidase from *Chondrus crispus*. *FEBS J.* 273: 2693–2703.

52 Heuts, D.P.H.M., Janssen, D.B., and Fraaije, M.W. (2007). Changing the substrate specificity of a chitooligosaccharide oxidase from *Fusarium graminearum* by model-inspired site-directed mutagenesis. *FEBS Lett.* 581: 4905–4909.

53 Taura, F., Sirikantaramas, S., Shoyama, Y. et al. (2007). Phytocannabinoids in *Cannabis sativa*: recent studies on biosynthetic enzymes. *Chem. Biodivers.* 4: 1649–1663.

54 Leferink, N.G.H., Heuts, D.P.H.M., Fraaije, M.W. et al. (2008). The growing VAO flavoprotein family. *Arch. Biochem. Biophys.* 474: 292–301.

55 Fraaije, M.W., Van Berkel, W.J.H., Benen, J.A.E. et al. (1998). A novel oxidoreductase family sharing a conserved FAD-binding domain. *Trends Biochem. Sci.* 23: 206–207.

56 Edmondson DE, Binda C, Mattevi A. The FAD Binding sites of human monoamine oxidases A and B. *Neurotoxicology* 2004; 25: 63–72.

57 Hassan-Abdallah, A., Bruckner, R.C., Zhao, G. et al. (2005). Biosynthesis of covalently bound flavin: isolation and in vitro flavinylation of the monomeric sarcosine oxidase apoprotein. *Biochemistry* 44: 6452–6462.

58 Scruttons, N.S., Packman, L.C., Mathews, F.S. et al. (1994). Assembly of redox centers in the trimethylamine dehydrogenase of bacterium Wfi, properties of wild-type enzyme and a C30A mutant expressed from a cloned gene in *Escherichia coli*. *J. Biol. Chem.* 269: 13942–13950.

59 Packman, L.C., Mewies, M., and Scrutton, N.S. (1995). The flavinylation reaction of trimethylamine dehydrogenase: analysis by directed mutagenesis and electrospray mass spectrometry. *J. Biol. Chem.* 270: 13186–13191.

60 Scott Mathews, F., Chen, Z.W., Bellamy, H.D. et al. (1991). Three-dimensional structure of p-cresol methylhydroxylase (flavocytochrome c) from *Pseudomonas putida* at 3.0-Å resolution. *Biochemistry* 30: 238–247.

61 Mclntire, W., Edmondson, D.E., Hopper, D.J. et al. (1981). 8a-(O-tyrosyl)flavin adenine dinucleotide, the prosthetic group of bacterial p-cresol methylhydroxylase. *Biochemistry* 20: 3068–3075.

62 Podzelinska, K., Latimer, R., Bhattacharya, A. et al. (2010). Chloramphenicol biosynthesis: the structure of CmlS, a flavin-dependent halogenase showing a covalent flavin–aspartate bond. *J. Mol. Biol.* 397: 316–331.

63 Hayashi, M., Nakayama, Y., Yasui, M. et al. (2001). FMN is covalently attached to a threonine residue in the NqrB and NqrC subunits of Na^+-translocating NADH-quinone reductase from *Vibrio alginolyticus*. *FEBS Lett.* 488: 5–8.

64 Steuber, J., Vohl, G., Casutt, M.S. et al. (2014). Structure of the *V. cholerae* Na^+-pumping NADH:quinone oxidoreductase. *Nature* 516: 62–67.

65 Walsh, C.T. (1980). Flavin coenzymes: at the crossroads of biological redox chemistry. *Acc. Chem. Res.* 13: 148–155.

66 Bullock, F.J. and Jardetzky, O. (1965). An experimental demonstration of the nuclear magnetic resonance assignments in the 6,7-dimethylisoalloxazine nucleus. *J Org Chem* 30: 2056–2057.

67 Bramson, N.H., Thomas, N., DeGrado, W.F. et al. (1980). Biomimetic 8a functionalization of riboflavin. *J. Am. Chem. Soc.* 102: 7157–7159.

68 Jin, J., Mazon, H., van den Heuvel, R.H.H. et al. (2008). Covalent flavinylation of vanillyl-alcohol oxidase is an autocatalytic process. *FEBS J.* 275: 5191–5200.

69 Van Hellemond, E.W., Mazon, H., Heck, A.J. et al. (2008). ADP competes with FAD binding in putrescine oxidase. *J. Biol. Chem.* 283: 28259–28264.

70 Brandschs, R. and Bichler, V. (1991). Autoflavinylation of Apo6-hydroxy-nicotine oxidase. *J. Biol. Chem.* 266: 19056–19062.

71 Efimov, I. and Mcintire, W.S. (2004). A study of the spectral and redox properties and covalent flavinylation of the flavoprotein component of p-cresol methylhydroxylase reconstituted with FAD analogues. *Biochemistry* 43: 10532–10546.

72 Cunane, L.M., Chen, Z.-W., Mcintire, W.S. et al. (2005). p-Cresol methylhydroxylase: alteration of the structure of the flavoprotein subunit upon its binding to the cytochrome subunit. *Biochemistry* 44: 2963–2973.

73 Kim, J., Fuller, J.H., Kuusk, V. et al. (1995). The cytochrome subunit is necessary for covalent FAD attachment to the flavoprotein subunit of p-cresol methylhydroxylase. *J. Biol. Chem.* 270: 31202–31209.

74 Koetter, J.W.A. and Schulz, G.E. (2005). Crystal structure of 6-hydroxy-D-nicotine oxidase from *Arthrobacter nicotinovorans*. *J. Mol. Biol.* 352: 418–428.

75 Hao, H.-X., Khalimonchuk, O., Schraders, M. et al. (2009). SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science* 325 (80): 1139–1142.

76 McNeil, M.B., Clulow, J.S., Wilf, N.M. et al. (2012). SdhE is a conserved protein required for flavinylation of succinate dehydrogenase in bacteria. *J. Biol. Chem.* 287: 18418–18428.

77 Lim, K., Doseeva, V., Demirkan, E.S. et al. (2004). Crystal structure of the YgfY from *Escherichia coli*, a protein that may be involved in transcriptional regulation. *Proteins Struct. Funct. Bioinform.* 58: 759–763.

78 Eletsky, A., Jeong, M.Y., Kim, H. et al. (2012). Solution NMR structure of yeast succinate dehydrogenase flavinylation factor Sdh5 reveals a putative Sdh1 binding site. *Biochemistry* 51: 8475–8477.

79 McNeil, M.B. and Fineran, P.C. (2013). The conserved RGxxE motif of the bacterial FAD assembly factor SdhE is required for succinate dehydrogenase flavinylation and activity. *Biochemistry* 52: 7628–7640.

80 Starbird, C.A., Maklashina, E., Sharma, P. et al. (2017). Structural and biochemical analyses reveal insights into covalent flavinylation of the *Escherichia coli* complex II homolog quinol:fumarate reductase. *J. Biol. Chem.* 292: 12921–12933.

81 Maklashina, E., Rajagukguk, S., Starbird, C.A. et al. (2015). Binding of the covalent flavin assembly factor to the flavoprotein subunit of complex II. *J. Biol. Chem.* 291: 2904–2916.

82 Kounosu, A. (2014). Analysis of covalent flavinylation using thermostable succinate dehydrogenase from *Thermus thermophilus* and *Sulfolobus tokodaii* lacking SdhE homologs. *FEBS Lett.* 588: 1058–1063.

83 Barquera, B., Häse, C.C., and Gennis, R.B. (2001). Expression and mutagenesis of the NqrC subunit of the NQR respiratory Na+ pump from *Vibrio cholerae* with covalently attached FMN. *FEBS Lett.* 492: 45–49.

84 Bertsova, Y.V., Fadeeva, M.S., Kostyrko, V.A. et al. (2013). Alternative pyrimidine biosynthesis protein ApbE is a flavin transferase catalyzing covalent attachment of FMN to a threonine residue in bacterial flavoproteins. *J. Biol. Chem.* 288: 14276–14286.

85 Deka, R.K., Brautigam, C.A., Liu, W.Z. et al. (2015). Evidence for posttranslational protein flavinylation in the syphilis spirochete *Treponema pallidum*: structural and biochemical insights from the catalytic core of a periplasmic flavin-trafficking protein. *MBio* 6: 1–9.

86 Boyd, J.M., Endrizzi, J.A., Hamilton, T.L. et al. (2011). FAD binding by ApbE protein from *Salmonella enterica*: a new class of FAD-binding proteins. *J. Bacteriol.* 193: 887–895.

87 Bogachev, A.V., Baykov, A.A., and Bertsova, Y.V. (2018). Flavin transferase: the maturation factor of flavincontaining oxidoreductases. *Biochem. Soc. Trans.* 46: 1161–1169.

88 Zhou, Z. and Swenson, R.P. (1996). The cumulative electrostatic effect of aromatic stacking interactions and the negative electrostatic environment of the flavin mononucleotide binding site is a major determinant of the reduction potential for the flavodoxin from *Desulfovibrio vulgaris*. *Biochemistry* 35: 15980–15988.

89 Zhou, Z. and Swenson, R.P. (1995). Electrostatic effects of surface acidic amino acid residues on the oxidation-reduction potentials of the flavodoxin from *Desulfovibrio vulgaris* (Hildenborough). *Biochemistry* 34: 3183–3192.

90 Edmondson, D.E. and Ghisla, S. (1999). Electronic effects of 7 and 8 ring substituents as predictors of flavin oxidation-reduction potentials. In: *Flavins and Flavoproteins: Proceedings of the Thirteenth International Symposium, Konstanz, Germany* (29 August–4 September 1999), 71–76.

91 Fraaije, M.W., Van Den Heuvel, R.H.H., Van Berkel, W.J.H. et al. (1999). Covalent flavinylation is essential for efficient redox catalysis in vanillyl-alcohol oxidase. *J. Biol. Chem.* 274: 35514–35520.

92 Fraaije, M.W., Van Den Heuvel, R.H.H., Van Berkel, W.J.H. et al. (2000). Structural analysis of flavinylation in vanillyl-alcohol oxidase. *J. Biol. Chem.* 275: 38654–38658.

93 Efimov, I., Cronin, C.N., and McIntire, W.S. (2001). Effects of noncovalent and covalent FAD binding on the redox and catalytic properties of p-cresol methylhydroxylase. *Biochemistry* 40: 2155–2166.

94 Lim, L., Molla, G., Guinn, N. et al. (2006). Structural and kinetic analyses of the H121A mutant of cholesterol oxidase. *Biochem. J.* 400: 13–22.

95 Motteran, L., Pilone, M.S., Molla, G. et al. (2001). Cholesterol oxidase from *Brevibacterium sterolicum*; the relationship between covalent flavinylation and redox properties. *J. Biol. Chem.* 276: 18024–18030.

96 Winkler, A., Kutchan, T.M., and Macheroux, P. (2007). 6-S-cysteinylation of bi-covalently attached FAD in berberine bridge enzyme tunes the redox potential for optimal activity. *J. Biol. Chem.* 282: 24437–24443.

97 Heuts, D.P.H.M., Winter, R.T., Damsma, G.E. et al. (2008). The role of double covalent flavin binding in chito-oligosaccharide oxidase from *Fusarium graminearum*. *Biochem. J.* 413: 175–183.

98 Hassan-Abdallah, A., Zhao, G., and Jorns, M.S. (2006). Role of the covalent flavin linkage in monomeric sarcosine oxidase. *Biochemistry* 45: 9454–9462.

99 Caldinelli, L., Iametti, S., Barbiroli, A. et al. (2005). Dissecting the structural determinants of the stability of cholesterol oxidase containing covalently bound flavin. *J. Biol. Chem.* 280: 22572–22581.

100 Lu, X., Nikolic, D., Mitchell, D.J. et al. (2003). A mechanism for substrate-induced formation of 6-hydroxyflavin mononucleotide catalyzed by C30A trimethylamine dehydrogenase. *Bioorg. Med. Chem. Lett.* 13: 4129–4132.

101 Mayhew, S.G., Whitfield, C.D., Ghisla, S. et al. (1974). Identification and properties of new flavins in electron-transferring flavoprotein from *Peptostreptococcus elsdenii* and pig-liver glycolate oxidase. *Eur. J. Biochem.* 44: 579–591.

102 Ghisla, S. and Mayhew, S.G. (1976). Identification and properties of 8-hydroxyflavin: adenine dinucleotide in electron-transferring flavoprotein from *Peptostreptococcus elsdenii*. *Eur. J. Biochem.* 63: 373–390.

103 Bergner, T., Tabib, C.R., Winkler, A. et al. (1854). Structural and biochemical properties of LuxF from *Photobacterium leiognathi*. *Biochim. Biophys. Acta, Proteins Proteomics* 2015: 1466–1475.

104 Tabib, C.R., Brodl, E., and Macheroux, P. (2017). Evidence for the generation of myristylated FMN by bacterial luciferase. *Mol. Microbiol.* 104: 1027–1036.

105 Eckstein, J.W., Hastings, J.W., and Ghisla, S. (1993). Mechanism of bacterial bioluminescence: 4a,5-dihydroflavin analogs as models for luciferase hydroperoxide intermediates and the effect of substituents at the 8-position of flavin on luciferase kinetics. *Biochemistry* 32: 404–411.

106 Moore, S.A. and James, M.N.G. (1995). Structural refinement of the non-fluorescent flavoprotein from *Photobacterium leiognathi* at 1.60 Å resolution. *J. Mol. Biol.* 249: 195–214.

107 Wei, C.J., Lei, B., and Tu, S.C. (2001). Characterization of the binding of *Photobacterium phosphoreum* P-flavin by *Vibrio harveyi* luciferase. *Arch. Biochem. Biophys.* 396: 199–206.

108 Tachibana, S. and Murakami, T. (1975). The isolation and some properties of new flavins ('schizoflavin') formed by *Schizophyllum commune*. *J. Nutr. Sci. Vitaminol. (Tokyo)* 21: 61–63.

109 Tachibana, S., Murakami, T., and Ninomiya, T. (1975). Identification of the chemical structures of schizoflavins as 7,8-dimethyl-10-(2,3,4-trihydroxy-4-formylbutyl)isoalloxazine and 7,8-dimethyl-10-(2,3,4-trihydroxy-4-carboxybutyl)isoalloxazine. *J. Nutr. Sci. Vitaminol. (Tokyo)* 21: 4.

110 Tachibana, S. and Oka, M. (1982). NADPH-dependence of vitamin B2-aldehyde-forming enzyme. *J. Nutr. Sci. Vitaminol.* 28: 335–342.

111 Matsui, K. (1965). Nekoflavin, a new flavin compound, in the choroid of cat's eye. *J. Biochem.* 57: 201–206.

112 Matsui, K. and Kasai, S. (1996). Identification of nekoflavin as 7a-hydroxyriboflavin. *J. Biochem.* 119: 441–447.

113 Owen, E.C. and West, D.W. (1970). Metabolites of riboflavine in milk, urine and tissues of animals in relation to alimentary symbiotic bacteria. *Br. J. Nutr.* 24: 45.

114 Susin, S., Abián, J., Sanchez-Baeza, F. et al. (1993). Riboflavin 3′-and 5'-sulfate, two novel flavins accumulating in the roots of iron-deficient sugar beet (*Beta vulgaris*). *J. Biol. Chem.* 268: 2095–20965.

115 Otani, S., Takatsu, M., Nakano, M. et al. (1974). Roseoflavin, a new antimicrobial pigment from *Streptomyces*. *J. Antibiot. (Tokyo)* 27: 86–87.

116 Mansjö, M. and Johansson, J. (2011). The riboflavin analog roseoflavin targets an FMN-riboswitch and blocks *Listeria monocytogene* s growth, but also stimulates virulence gene-expression and infection. *RNA Biol.* 8: 674–680.

117 Langer, S., Nakanishi, S., Mathes, T. et al. (2013). The flavoenzyme azobenzene reductase AzoR from *Escherichia coli* binds roseoflavin mononucleotide (RoFMN) with high affinity and is less active in its RoFMN form. *Biochemistry* 52: 4288–4295.

118 Grill, S., Busenbender, S., Pfeiffer, M. et al. (2008). The bifunctional flavokinase/flavin adenine dinucleotide synthetase from *Streptomyces davawensis* produces inactive flavin cofactors and is not involved in resistance to the antibiotic roseoflavin. *J. Bacteriol.* 190: 1546–1553.

119 Pedrolli, D.B., Nakanishi, S., Barile, M. et al. (2011). The antibiotics roseoflavin and 8-demethyl-8-amino-riboflavin from *Streptomyces davawensis* are metabolized by human flavokinase and human FAD synthetase. *Biochem. Pharmacol.* 82: 1853–1859.

120 Shinkai, S., Kameoka, K., Honda, N. et al. (1986). Coenzyme models. 40. Spectral and reactivity studies of roseoflavin analogs: correlation between reactivity and spectral parameters. *Bioorg. Chem.* 14: 119–133.

121 Lee, E.R., Blount, K.F., and Breaker, R.R. (2009). Roseoflavin is a natural antibacterial compound that binds to FMN riboswitches and regulates gene expression. *RNA Biol.* 6: 187–194.

122 Schwarz, J., Konjik, V., Jankowitsch, F. et al. (2016). Identification of the key enzyme of roseoflavin biosynthesis. *Angew. Chem. Int. Ed.* 55: 6103–6106.

123 Konjik, V., Brünle, S., Demmer, U. et al. (2017). The crystal structure of RosB: insights into the reaction mechanism of the first member of a family of flavodoxin-like enzymes. *Angew. Chem. Int. Ed.* 56: 1146–1151.

124 Mora-Lugo, R., Stegmüller, J., and Mack, M. (2019). Metabolic engineering of roseoflavin-overproducing microorganisms. *Microb. Cell Factories* 18: 1–13.

125 Jankowitsch, F., Kühm, C., Kellner, R. et al. (2011). A novel N,N-8-amino-8-demethyl-D-riboflavin dimethyltransferase (RosA) catalyzing the two terminal steps of roseoflavin biosynthesis in *Streptomyces davawensis*. *J. Biol. Chem.* 286: 38275–38285.

126 Yorita, K., Matsuoka, T., Misaki, H. et al. (2000). Interaction of two arginine residues in lactate oxidase with the enzyme flavin: conversion of FMN to 8-formyl-FMN. *Proc. Natl. Acad. Sci. U.S.A.* 97: 13039–13044.

127 Robbins, J.M., Souffrant, M.G., Hamelberg, D. et al. (2017). Enzyme-mediated conversion of flavin adenine dinucleotide (FAD) to 8-formyl FAD in formate oxidase results in a modified cofactor with enhanced catalytic properties. *Biochemistry* 56: 3800–3807.

128 Augustin, P., Toplak, M., Fuchs, K. et al. (2018). Oxidation of the FAD cofactor to the 8-formyl derivative in human electron transferring flavoprotein. *J. Biol. Chem.* 293: 2829–2840.

129 Marshall, S.A., Fisher, K., Cheallaigh, A.N. et al. (2017). Oxidative maturation and structural characterization of prenylated FMN binding by UbiD, a decarboxylase involved in bacterial ubiquinone biosynthesis. *J. Biol. Chem.* 292: 4623–4637.

130 Payne, K.A.P., White, M.D., Fisher, K. et al. (2015). New cofactor supports a,b-unsaturated acid decarboxylation via 1,3-dipolar cycloaddition. *Nature* 522: 497–501.

131 White, M.D., Payne, K.A.P., Fisher, K. et al. (2015). UbiX is a flavin prenyltransferase required for bacterial ubiquinone biosynthesis. *Nature* 522: 502–506.

132 Marshall, S.A., Payne, K.A.P., Fisher, K. et al. (2019). The UbiX flavin prenyltransferase reaction mechanism resembles class I terpene cyclase chemistry. *Nat. Commun.* 10: 1–10.

133 Massey, V., Ghisla, S., and Moore, E.G. (1979). 8-Mercaptoflavins as active site probes of flavoenzymes. *J. Biol. Chem.* 254: 9640–9650.

134 Biemann, M., Claiborne, A., Ghisla, S. et al. (1984). 4-Thioflavins as active site probes of flavoproteins. Reactions with sulfite. *J. Biol. Chem.* 259: 13355–13362.

135 Ghisla, S. and Massey, V. (1986). New flavins for old: artificial flavins as active site probes of flavoproteins. *Biochem. J.* 239: 1–12.

136 Massey, V., Ghisla, S., and Yagi, K. (1986). 6-Thiocyanatoflavins and 6-mercaptoflavins as active-site probes of flavoproteins. *Biochemistry* 25: 8103–8112.

137 Su, Q., Boucher, P.A., and Rokita, S.E. (2017). Conversion of a dehalogenase into a nitroreductase by swapping its flavin cofactor with a 5-deazaflavin analogue. *Angew. Chem. Int. Ed.* 56: 10862–10866.

138 De Gonzalo, G., Smit, C., Jin, J. et al. (2011). Turning a riboflavin-binding protein into a self-sufficient monooxygenase by cofactor redesign. *Chem. Commun.* 47: 11050–11052.

139 Lapalikar, G.V., Taylor, M.C., Warden, A.C. et al. (2012). Cofactor promiscuity among F420-dependent reductases enables them to catalyse both oxidation and reduction of the same substrate. *Catal. Sci. Technol.* 2: 1560–1567.

140 Karunan Partha, S., van Straaten, K.E., and Sanders, D.A.R. (2009). Structural basis of substrate binding to UDP-galactopyranose mutase: crystal structures in

the reduced and oxidized state complexed with UDP-galactopyranose and UDP. *J. Mol. Biol.* 394: 864–877.

141 Zhang, Q. and Liu, H.-W. (2001). Mechanistic investigation of UDP-galactopyranose mutase from *Escherichia coli* using 2-and 3-fluorinated UDP-galactofuranose as probes. *J. Am. Chem. Soc.* 123: 6756–6766.

142 Mehra-Chaudhary, R., Dai, Y., Sobrado, P. et al. (2016). In crystallo capture of a covalent intermediate in the UDP-galactopyranose mutase reaction. *Biochemistry* 55: 833–836.

143 Tanner, J.J., Boechi, L., Andrew McCammon, J. et al. (2014). Structure, mechanism, and dynamics of UDP-galactopyranose mutase. *Arch. Biochem. Biophys.* 544: 128–141.

144 Razeto, A., Mattiroli, F., Carpanelli, E. et al. (2007). The crucial step in ether phospholipid biosynthesis: structural basis of a noncanonical reaction associated with a peroxisomal disorder. *Structure* 15: 683–692.

145 Sobrado, P. (2012). Noncanonical reactions of flavoenzymes. *Int. J. Mol. Sci.* 13: 14219–14242.

146 Urbonavič, J., Phane Skouloubris, S., Myllykallio, H. et al. (2005). Identification of a novel gene encoding a flavin-dependent tRNA:m 5 U methyltransferase in bacteria-evolutionary implications. *Nucleic Acids Res.* 33: 3955–3964.

147 Myllykallio, H., Lipowski, G., Leduc, D. et al. (2002). An alternative flavin-dependent mechanism for thymidylate synthesis. *Science (80-)* 297: 105–107.

148 Hamdane, D., Argentini, M., Cornu, D. et al. (2011). Insights into folate/FAD-dependent tRNA methyltransferase mechanism: role of two highly conserved cysteines in catalysis. *J. Biol. Chem.* 286: 36268–36280.

149 Myllykallio, H., Skouloubris, S., Grosjean, H. et al. (2009). Folate-dependent thymidylate-forming enzymes: parallels between DNA and RNA metabolic enzymes and evolutionary implications. In: *DNA and RNA Modification Enzymes: Comparative Structure, Mechanism, Functions, Cellular Interactions and Evolution* (ed. H. Grosjean). Landes Bioscience.

150 Mishanina, T.V., Yu, L., Karunaratne, K. et al. (2016). An unprecedented mechanism of nucleotide methylation in organisms containing thyX. *Science (80-)* 351: 507–510.

151 Xiang, L., Kalaitzis, J.A., and Moore, B.S. (2004). EncM, a versatile enterocin biosynthetic enzyme involved in Favorskii oxidative rearrangement, aldol condensation, and heterocycle-forming reactions. *Proc. Natl. Acad. Sci. U.S.A.* 101: 15609–15614.

152 Teufel, R., Miyanaga, A., Michaudel, Q. et al. (2013). Flavin-mediated dual oxidation controls an enzymatic Favorskii-type rearrangement. *Nature* 503: 552–556.

153 Saleem-Batcha, R., Stull, F., Sanders, J.N. et al. (2018). Enzymatic control of dioxygen binding and functionalization of the flavin cofactor. *Proc. Natl. Acad. Sci. U.S.A.* 115: 4909–4919.

154 Matthews, A., Saleem-Batcha, R., Sanders, J.N. et al. (2020). Aminoperoxide adducts expand the catalytic repertoire of flavin monooxygenases. *Nat. Chem. Biol.* 16: 556–563.

155 Mukherjee, T., Zhang, Y., Abdelwahed, S. et al. (2010). Catalysis of a flavoenzyme-mediated amide hydrolysis. *J. Am. Chem. Soc.* 132: 5550–5551.

156 Adak, S. and Begley, T.P. (2017). RutA-catalyzed oxidative cleavage of the uracil amide involves formation of a flavin-N5-oxide. *Biochemistry* 56: 3708–3709.

157 Adak, S. and Begley, T.P. (2016). Dibenzothiophene catabolism proceeds via a flavin-N5-oxide intermediate. *J. Am. Chem. Soc.* 138: 6424–6426.

158 Adak, S. and Begley, T.P. (2019). Hexachlorobenzene catabolism involves a nucleophilic aromatic substitution and flavin-N5-oxide formation. *Biochemistry* 58: 1181–1183.

159 Eirich, L.D., Vogels, G.D., and Wolfe, R.S. (1979). Distribution of coenzyme F_{420} and properties of its hydrolytic fragments. *J. Bacteriol.* 140: 20–27.

160 Ney, B., Ahmed, F.H., Carere, C.R. et al. (2017). The methanogenic redox cofactor F_{420} is widely synthesized by aerobic soil bacteria. *ISME J.* 11: 125–137.

161 Selengut, J.D. and Haft, D.H. (2010). Unexpected abundance of coenzyme F_{420}-dependent enzymes in *Mycobacterium tuberculosis* and other actinobacteria. *J. Bacteriol.* 192: 5788–5798.

162 Cheeseman, P., Toms-Wood, A., and Wolfe, R.S. (1972). Isolation and properties of a fluorescent compound, Factor420, from *Methanobacterium* strain M.o.H. *J. Bacteriol.* 112: 527–531.

163 Edmondson, D.E., Barman, B., and Toliin, G. (1972). On the importance of the N-5 position in flavin coenzymes. properties of free and protein-bound 5-deaza analogs. *Biochemistry* 11: 1133–1138.

164 Wolfe, R.S., Vogels, G.D., and Eirich, L.D. (1978). Proposed structure for coenzyme F_{420} from *Methanobacterium*. *Biochemistry* 17: 4583–4593.

165 Ney, B., Carere, C.R., Sparling, R. et al. (2017). Cofactor tail length modulates catalysis of bacterial F_{420}-dependent oxidoreductases. *Front. Microbiol.* 8: 1902.

166 Jacobson, F. and Walsh, C. (1984). Properties of 7,8-didemethyl-8-hydroxy-5-deazaflavins relevant to redox coenzyme function in methanogen metabolism. *Biochemistry* 23: 979–988.

167 de Poorter, L.M.I., Geerts, W.J., and Keltjens, J.T. (2005). Hydrogen concentrations in methane-forming cells probed by the ratios of reduced and oxidized coenzyme F_{420}. *Microbiology* 151: 1697–1705.

168 Walsh, C. (1986). Naturally occurring 5-deazaflavin coenzymes: biological redox roles. *Acc. Chem. Res.* 19: 216–221.

169 Schauer, N.L. and Ferry, J.G. (1986). Composition of the coenzyme F_{420}-dependent formate dehydrogenase from *Methanobacterium formicicum*. *J. Bacteriol.* 165: 405–411.

170 Tzeng, S.F., Wolfe, R.S., and Bryant, M.P. (1975). Factor 420-dependent tyridine nucleotide-linked hydrogenase system of *Methanobacterium ruminantium*. *J. Bacteriol.* 121: 184–191.

171 Aufhammer, S.W., Warkentin, E., Berk, H. et al. (2004). Coenzyme binding in F_{420}-dependent secondary alcohol dehydrogenase, a member of the bacterial luciferase family. *Structure* 12: 361–370.

172 Widdel, F., Rouvière, P.E., and Wolfe, R.S. (1988). Classification of secondary alcohol-utilizing methanogens including a new thermophilic isolate. *Arch. Microbiol.* 150: 477–481.

173 Vitt, S., Ma, K., Warkentin, E. et al. (2014). The F_{420}-reducing [NiFe]-hydrogenase complex from *Methanothermobacter marburgensis*, the first X-ray structure of a group 3 family member. *J. Mol. Biol.* 426: 2813–2826.

174 Hagemeier, C.H., Shima, S., Thauer, R.K. et al. (2003). Coenzyme F_{420}-dependent methylenetetrahydromethanopterin dehydrogenase (Mtd) from *Methanopyrus kandleri*: a methanogenic enzyme with an unusual quarternary structure. *J. Mol. Biol.* 332: 1047–1057.

175 Shima, S., Warkentin, E., Grabarse, W. et al. (2000). Structure of coenzyme F_{420} dependent methylenetetrahydromethanopterin reductase from two methanogenic archaea. *J. Mol. Biol.* 300: 935–950.

176 Johnson, E.F. and Mukhopadhyay, B. (2005). A new type of sulfite reductase, a novel coenzyme F_{420}-dependent enzyme, from the methanarchaeon *Methanocaldococcus jannaschii*. *J. Biol. Chem.* 280: 38776–38786.

177 Bäumer, S., Ide, T., Jacobi, C. et al. (2000). The $F_{420}H_2$ dehydrogenase from *Methanosarcina mazei* is a redox-driven proton pump closely related to NADH dehydrogenases. *J. Biol. Chem.* 275: 17968–17973.

178 Welte, C. and Deppenmeier, U. (2011). Re-evaluation of the function of the F_{420} dehydrogenase in electron transport of *Methanosarcina mazei*. *FEBS J.* 278: 1277–1287.

179 Berk, H. and Thauer, R.K. (1997). Function of coenzyme F_{420}-dependent NADP reductase in methanogenic archaea containing an NADP-dependent alcohol dehydrogenase. *Arch. Microbiol.* 168: 396–402.

180 Seedorf, H., Dreisbach, A., Hedderich, R. et al. (2004). F420H2 oxidase (FprA) from *Methanobrevibacter arboriphilus*, a coenzyme F_{420}-dependent enzyme involved in O_2 detoxification. *Arch. Microbiol.* 182: 126–137.

181 Möller-Zinkhan, D., Börner, G., and Thauer, R.K. (1989). Function of methanofuran, tetrahydromethanopterin, and coenzyme F_{420} in *Archaeoglobus fulgidus*. *Arch. Microbiol.* 152: 362–368.

182 Gurumurthy, M., Rao, M., Mukherjee, T. et al. (2013). A novel F_{420}-dependent anti-oxidant mechanism protects *Mycobacterium tuberculosis* against oxidative stress and bactericidal agents. *Mol. Microbiol.* 87: 744–755.

183 Purwantini, E. and Mukhopadhyay, B. (2009). Conversion of NO_2 to NO by reduced coenzyme F_{420} protects mycobacteria from nitrosative damage. *Proc. Natl. Acad. Sci. U.S.A.* 106: 6333–6338.

184 Bashiri, G., Squire, C.J., Moreland, N.J. et al. (2008). Crystal structures of F_{420}-dependent glucose-6-phosphate dehydrogenase FGD1 involved in the activation of the anti-tuberculosis drug candidate PA-824 reveal the basis of coenzyme and substrate binding. *J. Biol. Chem.* 283: 17531–17541.

185 Nguyen, Q.-T., Trinco, G., Binda, C. et al. Discovery and characterization of an F_{420}-dependent glucose-6-phosphate dehydrogenase (Rh-FGD1) from *Rhodococcus jostii* RHA1. *Appl. Microbiol. Biotechnol.* 101: 2831–2842.

186 Manjunatha, U., Boshoff, H.I.M., and Barry, C.E. (2009). The mechanism of action of PA-824. *Commun. Integr. Biol.* 2: 215–218.

187 Purwantini, E. and Mukhopadhyay, B. (2013). Rv0132c of *Mycobacterium tuberculosis* encodes a coenzyme F_{420}-dependent hydroxymycolic acid dehydrogenase. *PLoS One* 8: e81985.

188 Ahmed, F.H., Carr, P.D., Lee, B.M. et al. (2015). Sequence–structure–function classification of a catalytically diverse oxidoreductase superfamily in *Mycobacteria*. *J. Mol. Biol.* 427: 3554–3571.

189 M-ST, K., DA, Y., Coats, J.H. et al. (1989). Isolation and identification of 7,8-didemethyl-8-hydroxy-5-deazariboflavin, an unusual cosynthetic factor in streptomycetes, from *Streptomyces lincolnensis*. *J. Antibiot. (Tokyo)* 42: 475–478.

190 Li, W., Chou, S., Khullar, A. et al. (2009). Cloning and characterization of the biosynthetic gene cluster for tomaymycin, an sjg-136 monomeric analog. *Appl. Environ. Microbiol.* 75: 2958–2963.

191 Coats, J.H., Li, G.P., Kuo, M.-S.T. et al. (1989). Discovery, production and biological assay of an unusual flavenoid cofactor involved in lincomycin biosynthesis. *J. Antibiot. (Tokyo)* 42: 472–474.

192 Choi, K.P., Kendrick, N., and Daniels, L. (2002). Demonstration that fbiC is required by *Mycobacterium bovis* BCG for coenzyme F_{420} and FO biosynthesis. *J. Bacteriol.* 184: 2420–2428.

193 Philmus, B., Decamps, L., Berteau, O. et al. (2015). Biosynthetic versatility and coordinated action of 5′-deoxyadenosyl radicals in deazaflavin biosynthesis. *J. Am. Chem. Soc.* 137: 5406–5413.

194 Bashiri, G., Antoney, J., Jirgis, E.N.M. et al. (2019). A revised biosynthetic pathway for the cofactor F_{420} in prokaryotes. *Nat. Commun.* 10: 1558.

195 Braga, D., Last, D., Hasan, M. et al. (2019). Metabolic pathway rerouting in *Paraburkholderia rhizoxinica* evolved long-overlooked derivatives of coenzyme F_{420}. *ACS Chem. Biol.* 14: 2088–2094.