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Sweating the assets of flavin cofactors: new insight of chemical versatility from knowledge of structure and mechanism

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Flavins are arguably one of the most versatile cofactors by virtue of the reactivity of the isoalloxazine ring system. A varied catalogue of reactions for the diverse family of flavoenzymes has been reported, leading to unifying concepts in (long-range) electron transfer, oxygen activation, photochemistry and substrate redox reactions. Recent examples of unprecedented flavin chemistry have been reported that uncover hidden depths of the flavoenzyme chemical repertoire. These include ring expansion of flavin through prenylation and formation of the superoxidized flavin-N5 oxide species. These and other new flavin based species are reviewed here and suggest further exciting discoveries await the flavoenzymology field.

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Introduction, flavin cofactors: old dogs and new tricks

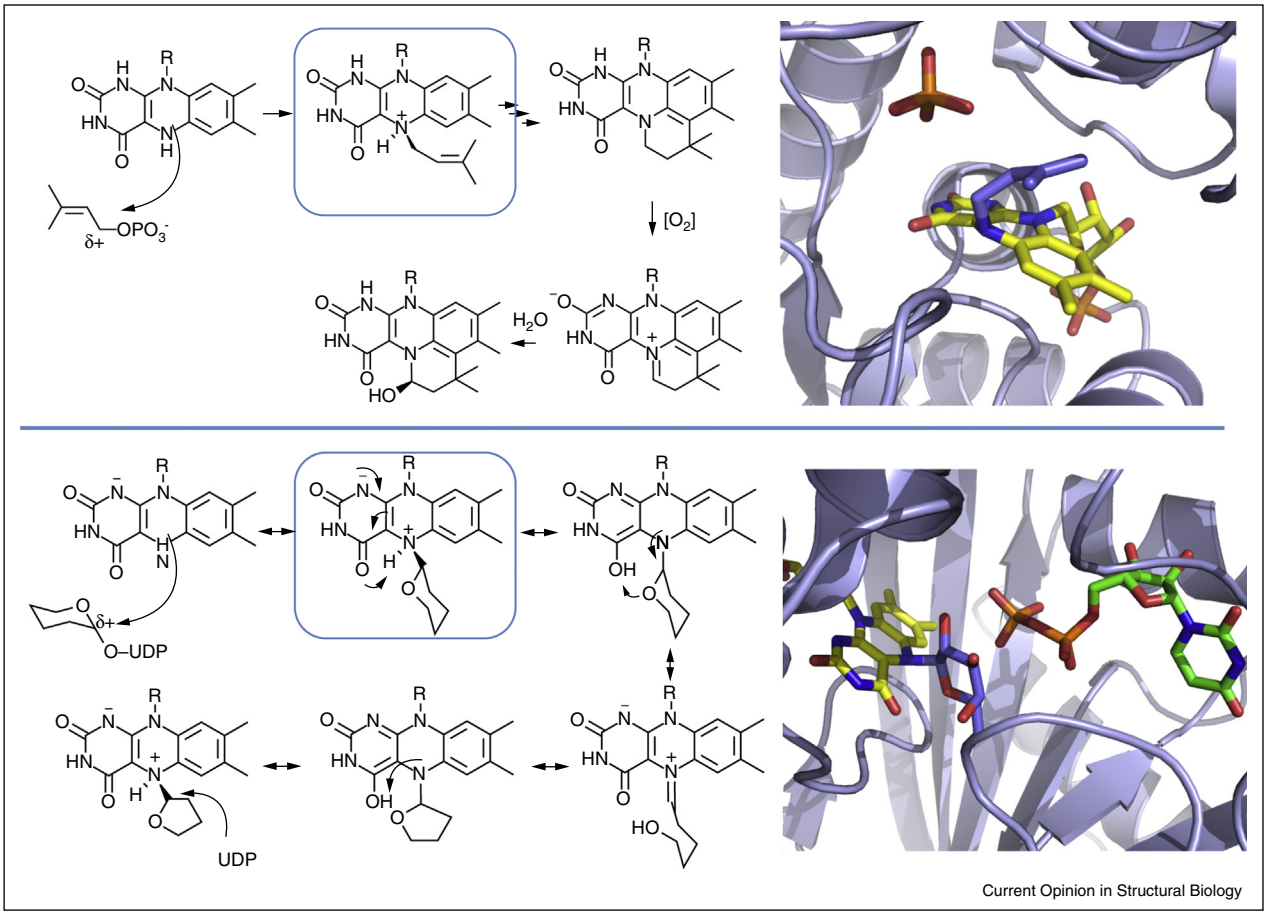
The chemical versatility of flavins has long been recognized, with these cofactors traditionally shown to be involved in a wide range of substrate reductions and oxidations [1,2]. This reactivity is attributed to the canonical flavin isoalloxazine ring, focused on the N5 and C4a atoms, the 'business end' of the flavin cofactor. By virtue of their various oxidation states (i.e. quinone, semiquinone and dihydroquinone), flavins sit at the crossroads of 1 and 2-electron chemistry. This versatility defines many established reaction classes, encompassing the oxidation of alcohols [3] and amines [4], long-range electron transfer chemistry, and multiple reactions involving the transfer of molecular oxygen [5] (e.g. Baeyer–Villiger oxidations, epoxidation, and oxidase reactions). This rich chemical repertoire might suggest the flavin potential is exhaustively documented leading one to ask how can the chemical versatility of flavin cofactors be expanded further to enrich the diversity of reaction types beyond that already established?

Over the years, mechanistic understanding of flavoprotein chemistry has emerged from integrated studies of structure, spectroscopy, kinetics, model chemistry and computation. Unifying concepts have emerged, but controversies remain where the balance of available data do not provide unequivocal evidence in support of a generally accepted reaction mechanism. Notable have been the current and historical debates concerning mechanisms of amine oxidation [6], or C–H bond breakage [7], based on reasoning from structural, computational and kinetic/spectroscopic investigations. These are rehearsed in some detail elsewhere, and are therefore not the focus of this article. Likewise, the versatility of flavins in 'non-chemical' transformations that is in photochemistry/light sensing [8] or long-range electron transfer (in selected cases involving complex bifurcation mechanisms [9,10]), has also been reviewed elsewhere. The purpose here is not to recapitulate this familiar territory. Instead, our aim is to establish to what extent fundamentally new aspects of flavin enzymology have arisen from integrated structural, computational and other experimental investigations reported in recent years (review period 2012–2016). Our focus in particular is on (i) new flavoprotein chemistry, (ii) deeper mechanistic insight of established flavin mechanisms, and (iii) exploitation of the above in the new world of synthetic and industrial biology.

New flavin cofactors empower new chemistry

The recent discovery that the reversible decarboxylase UbiD enzymes contain a highly modified FMN cofactor essential for activity confirms that an even wider scope for flavin chemistry is possible [11••]. These enzymes make use of a prenylated FMN cofactor, synthesized by the associated UbiX enzyme (Figure 1a). The latter covalently links the prenyl group of dimethylallyl-monophosphate to both the isoalloxazine N5 and C6 generating a fourth, non-aromatic ring [12••]. The UbiX mechanism proceeds via an unusual sp^3 N5-prenyl intermediate that could be visualized using kinetic crystallography. The UbiX prenylated FMN product is converted to the corresponding oxidized form in UbiD. This oxidative maturation leads to formation of

Figure 1



(a) Brief overview of the $FMNH_2$ prenylation reaction catalyzed by UbiX and the ensuing oxidative maturation in UbiD [11••,12••]. The prFMN cofactor in *holo*-UbiD is inactivated by hydrolysis. The reaction mechanism for UbiX could be elucidated using kinetic crystallography, with the first intermediate identified as an sp^3 N5-prenyl adduct (see inset; PDB 4ZAV [12••]). **(b)** Mechanism for UDP-galactopyranose mutase [13], which has some similarity to the UbiX-UbiD $FMNH_2$ prenylation as outlined in panel A. For clarity, only the ring structure of the sugar substrate is shown. Recently a structure of the covalent N5-sugar adduct could be obtained, revealing a similar sp^3 N5 adduct (see inset; PDB 5HHF [14•]).

a N5-prenyl C1′ iminium group, generating an azomethine ylide-like cofactor.

The prenylation of FMN by UbiX followed by the oxidative maturation in UbiD bears some chemical resemblance to the FMN dependent UDP-galactopyranose mutase [13], where recent efforts have also allowed visualization of a key sp^3 N5-galactose adduct (Figure 1b [14•]). In the latter enzyme, a N5-sugar iminium bond is created coupled to ring opening in the absence of any formal oxidation. The mutase reaction comes from the ability of distinct sugar alcohol groups to attack the N5-sugar iminium, a step that is somewhat similar to the inactivation of the UbiD cofactor by hydrolysis. In contrast to the proposed non-redox dependent formation of the N5-iminium species in UDP-galactopyranose, UbiD cofactor maturation via the N5-iminium is dependent on oxygen, leading to oxidation of the prenyl moiety via an unknown pathway.

The UbiD prFMN is proposed to support reversible decarboxylation via a 1,3 dipolar cyclo-addition reaction with the unsaturated substrate (a dipolarophile, Figure 2a). In fact, the prenylation and subsequent oxidation generating the prFMN azomethine ylide allows for formation of a covalent substrate–prFMN adduct. The latter is proposed to readily decarboxylate with development of a (transient) negative charge on the isoalloxazine ring system before protonation and product formation (via a retro 1,3 cyclo-addition step). Although present characterization of UbiD enzymes is limited to those family members specific for cinnamic acid-type substrates, other members work on benzoic acid derivatives. It has been proposed a similar mechanism could prevail in the latter [15].

As the oxidized prFMN cofactor is unstable when isolated from UbiD, it would appear UbiX has evolved to use reduced $FMNH_2$ in order to generate the more stable reduced prFMN form, allowing for cofactor transfer