Review

Cofactor F_{420}-Dependent Enzymes: An Under-Explored Resource for Asymmetric Redox Biocatalysis

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Abstract: The asymmetric reduction of enoates, imines and ketones are among the most important reactions in biocatalysis. These reactions are routinely conducted using enzymes that use nicotinamide cofactors as reductants. The deazaflavin cofactor F_{420} also has electrochemical properties that make it suitable as an alternative to nicotinamide cofactors for use in asymmetric reduction reactions. However, cofactor F_{420}-dependent enzymes remain under-explored as a resource for biocatalysis. This review considers the cofactor F_{420}-dependent enzyme families with the greatest potential for the discovery of new biocatalysts: the flavin/deazaflavin-dependent oxidoreductases (FDORs) and the luciferase-like hydride transferases (LLHTs). The characterized F_{420}-dependent reductions that have the potential for adaptation for biocatalysis are discussed, and the enzymes best suited for use in the reduction of oxidized cofactor F_{420} to allow cofactor recycling in situ are considered. Further discussed are the recent advances in the production of cofactor F_{420} and its functional analog F_{O-5'}, which remains an impediment to the adoption of this family of enzymes for industrial biocatalytic processes. Finally, the prospects for the use of this cofactor and dependent enzymes as a resource for industrial biocatalysis are discussed.

Keywords: cofactor F_{420}; deazaflavin; oxidoreductase; hydride transfer; hydrogenation; asymmetric synthesis; cofactor biosynthesis

1. Introduction

Enzymes that catalyze the asymmetric reduction of activated double bonds are among the most important in biocatalysis, allowing access to chiral amines from imines (C=N), sec-alcohols from ketones (C=O), and enantiopure products derived from enoates (C=C). To date, the reduction of imines, ketones and enoates has been achieved largely using enzymes that draw their reducing potential from the nicotinamide cofactors NADH and NADPH; e.g., imine reductases, ketoreductases and Old Yellow Enzymes [1–4]. However, there has been recent interest in an alternative reductive cofactor, cofactor F_{420} (8-hydroxy-5-deazaflavin) [5,6].

Cofactor F_{420} is a deazaflavin that is structurally similar to flavins (Figure 1), with a notable difference at position 5 of the isoalloxazine ring, which is a nitrogen in flavins and a carbon in deazaflavins. Additionally, while C-7 and C-8 are methylated in riboflavin, they are not in cofactor F_{420}: C-7 is hydroxylated and C-8 is unsubstituted. These structural differences cause significant differences in the electrochemical properties of cofactor F_{420} and flavins: a −360–340 mV the redox mid-point.

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potential of cofactor $F_{420}$ is not only lower than that of the flavins (−205 mV to −220 mV), but it is also lower than that of the nicotinamides (−320 mV) [7]. Additionally, as a consequence of the substitution of N-5 for a carbon, cofactor $F_{420}$ cannot form a semiquinone (Figure 1), which means that unlike other flavins, cofactor $F_{420}$ can only perform two-electron reductions.

Cofactor $F_{420}$ was originally described in methanogenic archaea, where it plays a pivotal role in methanogenesis [8,9]. Cofactor $F_{420}$ has since been described in a range of soil bacteria supporting a range of metabolic activities, including catabolism of recalcitrant molecules (such as picric acid) and...
a range of metabolic activities, including catabolism of recalcitrant molecules (such as picric acid) and the production of secondary metabolites, such as antibiotics [7]. A comprehensive review of the biochemistry and physiological roles of cofactor F_{420} was recently published by Greening and coworkers [7]. This review considers the potential of F_{420}-dependent enzymes in industrial biocatalysis, focusing on the enzyme families relevant to biocatalytic applications and the reactions that they catalyze. Cofactor recycling strategies and cofactor production are also discussed, with a focus on the prospects for achieving low-cost production at scale in the latter case.

2. Families of F_{420}-Dependent Enzymes Relevant to Biocatalysis

With respect to their prospective biocatalytic applications, the two most important families of F_{420}-dependent enzymes are the Flavin/Deazaflavin Oxidoreductase (FDOR) and Luciferase-Like Hydride Transferase (LLHT) families, albeit F_{420}-dependent enzyme from other families have also been shown to have catalytic activities of interest (e.g., TomJ, the imine reducing flavin-dependent monooxygenase or OxyR, the tetracycline oxidoreductase) [10,11]. The FDOR and LLHT families are large and contain highly diverse flavin/deazaflavin-dependent enzymes. In both families, there are enzymes with preferences for flavins, such as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), as well as those that use cofactor F_{420} [12,13]. Moreover, there are F_{420}-dependent FDORs that have been shown to be able to promiscuously bind FMN and use it in oxidation reactions [14]. In this section, the FDOR and LLHT families and the classes of reaction that they catalyze are discussed.

2.1. The FDOR Superfamily

The FDOR superfamily (PFAM Clan CL0336) can be broadly divided into two groups: the FDOR-As (which includes a sub-group called the FDOR-AAs) and the FDOR-Bs. The FDOR-As are restricted to Actinobacteria and Chloroflexi and to date no FDOR-As have been described that use cofactors other than F_{420} [7,12]. The FDOR-Bs are found in a broader range of bacterial genera than the FDOR-A enzymes, and in addition to F_{420}-dependent enzymes, this group also includes heme oxygenases, flavin-sequestering proteins, pyridoxine 5’ oxidases and a number of proteins of unknown function [12,15–17]. Both groups of FDOR are highly diverse, with many homologs often found within a single bacterial genome (e.g., Mycobacterium smegmatis has 28 FDORs) [18]. In addition, the majority of the enzymes of this family are yet to be characterized with respect to either their biochemical or physiological function, and therefore the FDORs represent a currently under-explored source of enzymes for biocatalysis.

The FDOR enzymes share a characteristic split β-barrel fold that forms part of the cofactor-binding pocket. The majority of the protein sequences of enzymes currently identified as belonging to this family are small single-domain proteins. The topologies of the two FDOR subgroups are broadly similar (Figure 2), with the split-barrel core composed of 7–8 strands and with 4–5 helices interspersed. All FDOR-Bs studied so far have been demonstrated to be dimeric, with stands β2, β3, β5 and β6 making up the core of the dimer interface (Figure 2). In structures of full-length FDOR-As solved to date, the N-terminal helix (if present) lies on the opposite face of the beta sheet to that in FDOR-Bs. Thus, the N-terminus occupies part of the dimer interface region and prevents interaction between the sheets of adjacent monomers. In contrast to the FDOR-Bs, the oligomerization state of the FDOR-As is more varied. While a number of FDOR-As have been determined to be monomeric [18], the deazaflavin-dependent nitroreductase (DDN) from M. tuberculosis forms soluble aggregates through the amphipathic N-terminal helix [19]. DDN and the FDOR-AA subgroup have been shown to be membrane-associated [20–22], and FDOR-AAs have been associated with fatty acid metabolism [12]. No structures of FDOR-AAs have been solved to date.
Figure 2. Representative structures of F$_{420}$-dependent FDOR-A (PDB: 3R5Z, panels A and C) and FDOR-B (PDB: 5JAB, panels B and D). Both are predominantly composed of a single $\beta$-sheet forming a split barrel. The N-terminal helices are spatially displaced between the two families, falling on opposite faces of the $\beta$-sheet.

2.2. The LLHT Family:

The LLHT family form part of the Luciferase-Like Monooxygenase family (PFAM PF00296). They adopt an ($\alpha/\beta$)$_8$ TIM-barrel fold with three insertion regions, IS1–4 (Figure 3). IS1 contains a short loop and forms part of the substrate cleft. IS2 contains two antiparallel $\beta$-strands, and IS3 contains a helical bundle at the C-terminus of the $\beta$-barrel and contains the remainder of the substrate-binding pocket (Figure 3). All structures solved to date from the LLHT family contain a non-prolyl $\text{cis}$ peptide in $\beta$3 [23–26]. Recent phylogenetic reconstructions have shown that the F$_{420}$-dependent LLHTs form two clades: the F$_{420}$-dependent reductases and the F$_{420}$-depended dehydrogenases [27]. The F$_{420}$-reductases contain methylenetetrahydromethanopterin reductases (MERs), which catalyze the reversable, ring-opening cleavage of a carbon-nitrogen bond during the biosynthesis of folate in some archaea [28–30]. The F$_{420}$-dependent dehydrogenases can be further divided into three subgroups. The first contains F$_{420}$-dependent secondary alcohol dehydrogenases (ADFs) and the hydroxymycolic acid reductase from $M$. tuberculosis [31]. The second contains the F$_{420}$-dependent glucose-6-phosphate dehydrogenases (FGDs) from Mycobacteria and Rhodococcus, while the third appear to be more general sugar-phosphate dehydrogenases [27]. In contrast to the heterodimeric structure of bacterial luciferase, the F$_{420}$-dependent dehydrogenases form homodimers with the dimer interface burying a relatively large portion of the surface area of the monomers ($\approx$2000 Å$^2$, roughly 15%
of the total surface area) [24–26]. A number of enzymes involved in the F\textsubscript{420}-dependent degradation of nitroaromatic explosives, such as picrate and 2,4-dinitroanisole, appear to belong to the LLHT family as well [32,33].

**Figure 3.** Structure of representative luciferase-like hydride transferase (LLHT) (PDB: 1RHC). (A) A 3D representation of the biologically relevant dimer (panel A). Monomer of an LLHT with insertion sequences IS1–4 highlighted, along with the helical bundle composed of \( \alpha_{7–9} \) (panel B). Topology diagram showing \((\alpha/\beta)_8\) fold with insertion sequences highlighted: IS1, red; IS2, orange; IS3, light green, IS4, pink. The helical bundle of \( \alpha_{7–9} \) is highlighted in purple (panel C).

### 2.3. Cofactor F\textsubscript{420}-Dependent Reactions with Relevance to Biocatalysis

From the perspective of biocatalysis, cofactor F\textsubscript{420}-dependent enzymes catalyze a number of key reductions including the reduction of enoates, imines, ketones and nitro-groups (Table 1; Figure 4).
Figure 4. Representative cofactor F420-dependent oxidoreductions with the potential for adaptation to biocatalytic applications. Included are: nitroreduction, enoate reduction, ketoreduction and imine reduction (from top to bottom). For clarity, only the dehydropiperidine ring of the thiopeptide is shown and partial structures for biliverdin-IXα and phthiodiolone dimycocerosates are shown.

For enoate reductions, a small number of FDORs have been studied. However, the substrate range for most of these enzymes is yet to be fully elucidated. The ability of the mycobacterial FDORs to reduce activated C=C double bonds was first identified when DDN was shown to be responsible for activating the bicyclic nitroimidazole PA-824 in *M. tuberculosis*. These enzymes were then shown to also reduce enoates in aflatoxins, coumarins, furanocoumarins and quinones [6,12,14,16,34–38]. Recent studies have shown that these enzymes are promiscuous and can use cyclohexen-1-one, malachite green and a wide range of other activated ene compounds as substrates [35]. However, there have been a few FDOR studies to date that have examined their kinetic properties and stereospecificity. In one of these studies, FDORs from *Mycobacterium hassiacum* (FDR-Mha) and *Rhodococcus jostii* RHA1 (FDR-Rh1 and FDR-Rh2) were shown to reduce a range of structurally diverse enoates with conversions ranging from 12 to >99% and e.e. values of up to >99% [6]. Interestingly, it has been proposed that both the hydride and proton transfer from F420H2 in these reactions was directed to the same face of the activated double bond (Figure 5), which results in the opposite enantioselectivity compared to that of the FMN-dependent Old Yellow Enzyme family of enoate reductases [6]. This suggests that the F420-dependent FDORs may provide a stereocomplementary enoate reductase toolbox. However, other studies suggest that protonation of the substrate is mediated by solvent or an enzyme side-chain (as it...
is in Old Yellow Enzyme) [37]. Further structure/function studies are needed to fully understand the mechanistic diversity of this family of enzymes.

The LLHT family contains several enzymes with alcohol oxidase or ketoreductase activity (Table 1; Figure 4). The F420-dependent glucose-6-phosphate dehydrogenases of several species have been investigated [25,26,39]. Although an extensive survey of their substrate ranges has yet to be conducted, it has been demonstrated that glucose is a substrate for the Rhodococcus jostii RHA1 enzymes [26]. An F420-dependent alcohol dehydrogenase (ADH) from Methanogenium liminatans has been shown to catalyze the oxidation of the short chain aliphatic alcohols 2-propanol, 2-butanol and 2-pentanol (85, 49 and 23.1 s\(^{-1}\) \(k_{\text{cat}}\), 2.2, 1.2 and 7.2 mM \(K_M\) respectively) [40], but it was unable to oxidize primary alcohols, polyols or secondary alcohols with more than five carbons. It is unclear whether these alcohol oxidations are reversible, but in the oxidative direction, these reactions provide enzymes that can be used to recycle reduced cofactor F420 (see Section 4). Alcohol oxidation can also be used to produce ketones as intermediates in biocatalytic cascades that can then be used in subsequent reactions, such as those catalyzed by transaminases or amine dehydrogenases in chiral amine synthesis [1,41–43] or by ketoreductases or alcohol dehydrogenases in chiral sec-alcohol synthesis (i.e., deracemization or stereoinversion of sec-alcohols). This approach can be achieved in a one pot cascade if different cofactors are used for the oxidation and reduction (Figure 6) [44].

At least one F420-dependent ketoreductase has been described. The mycobacterial F420-dependent phthiodiolone ketoreductase catalyzes a key reduction in the production of phthiocerol dimycocerosate, a diacylated polyketide found in the mycobacterial cell wall [45]. Although the physiological role of
this enzyme has been elucidated, biochemical studies of the catalytic properties and substrate range are required to assess this enzymes’ potential for use as a biocatalyst.

**Figure 6.** Proposed scheme for one-pot, enzyme cascades for deracemization/stereoinversion of sec-alcohols (top) and chiral amine synthesis (bottom) using cofactor F$_{420}$-dependent alcohol oxidation.

F$_{420}$-dependent enzymes have also been shown to reduce imines (Table 1; Figure 4). An FDOR fromr *Streptomyces tateyamensis* (TpnL) is responsible for the reduction of dehydropiperidine in the piperidine-containing series a group of thioppeptide antibiotics produced in this bacterium (Figure 4). TpnL was identified as the F$_{420}$-dependent dehydropiperidine reductase responsible for the reduction of dehydropiperidine ring in thiostrepton A to produce the piperidine ring in the core macrocycle of thiostrepton A [45]. TpnL activity was affected by substrate inhibition at concentrations higher than 2 μM of thiostrepton A, preventing the measurement of the $K_M$. $k_{cat}/K_M$ was measured at 2.80 × 10$^4$ M$^{-1}$ S$^{-1}$ [45]. The substrates for phthiodiolone ketoreductase and TpnL are large secondary metabolites and, as yet, it is unclear if it will accept smaller substrates or substrates with larger/smaller heterocycles (e.g., dehydropyrroles).

Another F$_{420}$-dependent imine reductase (TomJ) has been described from *Streptomyces achromogenes* that reduces the imine in 4-ethylidene-3,4-dehydropyrrole-2-carboxylic acid during the production of the secondary metabolite tomaymycin, which has been shown to have potentially interesting pharmaceutical properties [11]. Additionally, the reduction of a prochiral dihydropyrrole to a pyrrole is a reaction with a number of biocatalytic applications [5].

Nitroreductases have the potential application in the reduction of a prochiral nitro group to form a chiral amine [46]. The LLHT family F$_{420}$-dependent nitroreductase Npd from *Rhodococcus* catalyzes the two-electron reduction of two nitro groups in picric acid during catabolism of the explosive TNT (Table 1; Figure 4) [47]. While this stops short of reducing the nitro group to an amine, this catalytic activity may contribute to a reductive cascade that achieves this conversion.

The final class of reaction for consideration in this review is the unusual, reversible ring-opening/ closing reaction catalyzed by the MERs (Figure 4; Table 1). This reaction is required for folate biosynthesis in some archaea [23,28–30]. However, ring-closing reactions of this type could be used for producing N-containing heterocycles, which are intermediates in the synthesis of numerous pharmaceuticals [48,49]. The promiscuity of the MERs has not yet been investigated, and so the potential to re-engineer these enzymes is not fully understood.
Table 1. Characterized F<sub>420</sub>-dependent enzymes with activities that could be adapted for biocatalytic applications.

| Reaction                               | Family | Reference          |
|----------------------------------------|--------|--------------------|
| Enolate reduction                      |        |                    |
| Aflatoxins                             | FDOR   | [14,18,34]         |
| Coumarins                              | FDOR   | [14,34,35]         |
| Quinones                               | FDOR   | [36]               |
| Biliverdin reduction                   | FDOR   | [12,16]            |
| Nitroimidazoles                        | FDOR   | [36]               |
| Cyclohexenones                         | FDOR   | [6,34,38]          |
| Citral/Neral/Geranial                  | FDOR   | [6]                |
| Carvone                                | FDOR   | [6]                |
| Ketoisophorone                         | FDOR   | [6]                |
| Alcohol oxidation/ketoreduction        |        |                    |
| Glucose-6-phosphate                    | LLHT   | [26,50]            |
| Phthiodiolone dimycocerosate           | LLHT   | [51]               |
| Isopropanol                            | LLHT   | [40]               |
| Imine reductions                       |        |                    |
| Dehydropiperidine (in thiopeptins)     | FDOR   | [45]               |
| 4-ethylidene-3,4-dihydropyrrole-2-carboxylic acid | Flavin-dependent monooxygenase | [11] |
| Nitroreductions                        |        |                    |
| Picrate                                | LLHT   | [47,50]            |
| 2,4-DNP                                 | LLHT   | [48,50]            |
| Ring opening/closing                   |        |                    |
| C-N bond cleavage/formation in         |        |                    |
| methylenetetrahydromethanopterin      | LLHT   | [23,28–30]         |

3. Cofactor Recycling for Cofactor F<sub>420</sub>

Cofactor recycling is essential for the practical application of the F<sub>420</sub>-dependent enzymatic processes in biocatalysis. There are various strategies for cofactor regeneration for NADH and NADPH, including enzymatic, chemical, electrochemical and photochemical methods [52]. In this section, the potential enzymes for the regeneration of cofactor F<sub>420</sub> are discussed. As most of the industrially relevant F<sub>420</sub>-dependent reactions are asymmetric reductions, F<sub>420</sub>-dependent oxidases are required for cofactor regeneration. Figure 7 shows the characterized enzymes that catalyze F<sub>420</sub>-dependent oxidations that could be applied in cofactor F<sub>420</sub> reduction.

Emulating methods developed for nicotinamide cofactors, both formate dehydrogenase (FDH) and glucose 6-phosphate dehydrogenase (G6PD) enzymes are attractive enzymatic routes for cofactor reduction both in vitro [53–56] and in vivo [57,58]. Fortunately, F<sub>420</sub>-dependent G6PDs and FDHs have been identified and characterized. The F<sub>420</sub>-dependent G6PD from *Mycobacteria* (FGD) is one potential cofactor F<sub>420</sub>-recycling enzyme. FGD is the only enzyme in these bacteria known to reduce oxidized cofactor F<sub>420</sub>. The intracellular concentration of G6P in *Mycobacteria* is up to 100-fold higher than it is in *E. coli*, which provides a ready source of reducing power for F<sub>420</sub>-dependent reduction reactions [59]. FGD from *Rhodococcus jostii* and *Mycobacterium smegmatis* have been studied and expressed in *E. coli*, both the enzymes were stable in vitro assays [26,39,60]. Both FGDs have been expressed in engineered *E. coli* producing cofactor F<sub>420</sub> together with FDORs [38,59]. FGDs have been shown to efficiently regenerate reduced cofactor F<sub>420</sub> both in vivo and in vitro. However, the cost of the glucose-6-phosphate and the need to separate reaction products from the accumulated FGD byproduct (6-phosphoglucono-δ-lactone) may prove to be impediments for the adoption of FGD as a recycling system for cofactor F<sub>420</sub> in the in vitro biotransformations.
Figure 7. Cofactor F\textsubscript{420}-dependent oxidation reactions that could be exploited to produce reduced cofactor F\textsubscript{420}.

Formate is an excellent reductant for cofactor recycling, with FDH-dependent cofactor reduction yielding carbon dioxide, a volatile byproduct that can be easily removed from the reaction mixture, thereby simplifying the downstream processing of the product of interest. Additionally, formate is a low-cost reagent, leading to favorable process economics. Most methanogens have the capability to use formate as sole electron donor using F\textsubscript{420}-dependent formate dehydrogenase [61]. The soluble F\textsubscript{420}-dependent FDH from \textit{Methanobacterium formicium} has been expressed in \textit{E. coli} [62], purified and studied in vitro with the reduction of 41.2 µmol of F\textsubscript{420} min\textsuperscript{-1} mg\textsuperscript{-1} of FDH, with non-covalently bound FAD required for optimal activity [8]. \textit{Methanobacterium ruminantium} FDH reduces cofactor F\textsubscript{420} at a much slower rate than \textit{M. formicium}: 0.11 µmol of F\textsubscript{420} min\textsuperscript{-1} mg\textsuperscript{-1} of FDH [8]. As yet, the use of F\textsubscript{420}-dependent FDHs for in vitro cofactor recycling has been sparsely studied. However, as these enzymes are soluble and can be heterologously expressed, they represent a promising system for use in cofactor F\textsubscript{420}-dependent biocatalytic processes.

Another potential recycling system for cofactor F\textsubscript{420} is the F\textsubscript{420}:NADPH oxidoreductase (Fno), which couples the reduction of cofactor F\textsubscript{420} with oxidation of NADPH. Methanogenic archaea use this enzyme to transfer reducing equivalents from hydrogenases to produce NADPH via F\textsubscript{420}, while in bacteria it functions in the opposite direction, that is, to provide the cell with reduced F\textsubscript{420} via NADPH [63]. Fno is also required for the production of reduced F\textsubscript{420} for tetracycline production in \textit{Streptomyces} [63]. The Fno enzymes from the thermophilic bacteria \textit{Thermobifida fusca} and the thermophilic archaeon \textit{Archeoglobus fulgidus} have been expressed in \textit{E. coli} [64,65]. These enzymes are thermostable, with their highest activity observed at 65 °C. As the redox midpoint potentials of NADP and cofactor F\textsubscript{420} are very similar, it is perhaps unsurprising that pH has a significant influence on the equilibrium of the reaction, with the reduction of NADPH\textsuperscript{2+} favored at high pH (8–10) and the reduction of F\textsubscript{420} favored at low pH (4–6) [64,65]. The Fno \textit{Streptomyces griseus} has also been purified and characterized, and also displayed pH-dependent reaction directionality [66]. Fno may be an excellent enzyme for the in vivo reduction of cofactor F\textsubscript{420}, where NADPH would be provided from
central metabolism. However, for its use as a cofactor $F_{420}$ recycling enzyme in vitro, Fno would need to be coupled with an NADPH regenerating enzyme, such as an NADPH-dependent formate dehydrogenase [67]. This added complexity and cost may limit the use of Fno-dependent cofactor $F_{420}$ recycling in vivo.

Hydrogenotrophic archaea, including methanogens and sulfate-reducing archaea, possess an essential, cofactor $F_{420}$-dependent hydrogenase (FhrAGB) [68–71]. These nickel/iron enzymes could potentially be used in vivo to allow the direct $H_2$-dependent reduction of cofactor $F_{420}$. However, as these heterododecameric enzymes have complex cofactor requirements (four $[4Fe\,4S]$ clusters, and NiFe center and FAD), are oxygen-sensitive and tend to aggregate [71], it is unclear if they can be made suitable for in vitro use.

4. Cofactor Production

The lack of a scalable production system for cofactor $F_{420}$ has been noted as a major impediment to the adoption of $F_{420}$-dependent enzymes by industry [5]. Cofactor $F_{420}$ is available as a research reagent (http://www.gecco-biotech.com), but its production at scale is not yet economic. In fact, most research laboratories with an interest in cofactor $F_{420}$-dependent enzymes synthesize and purify the cofactor themselves using slow-growing $F_{420}$ producing microorganisms, most commonly methanogens and actinobacteria (Table 2). The economic production of cofactor $F_{420}$ at large scale is not feasible using natural producers as they are ill-suited to industrial fermentation and generally lack the genetic tools required to improve cofactor $F_{420}$ yield.

| Source | $F_{420}$ Yield (µmol/g Cell Weight) | Growth Conditions | Ref |
|--------|--------------------------------------|-------------------|-----|
| Methanobacterium thermoautotrophicum | 0.42 $^{a,c}$ | Grown at 60°C using complex media in fermenter, under pressurized hydrogen | [9] |
| Methanobacterium formicium | 0.27 $^{a,c}$ | Grown at 37°C using complex media in fermenters | [9] |
| Methanospirillum hungatii | 0.41 $^{a,c}$ | Grown at 37°C using complex media in fermenters | [9] |
| Methanobacterium strain M.o.H | 0.53 $^{a,c}$ | Grown at 40°C using complex media in fermenters | [9] |
| Methanobacterium thermoautotrophicum | 1.7 $^e$ | Grown using complex media in fermenters, under pressurized hydrogen gas | [73] |
| Streptomyces fissculus | 0.62 $^a$ | Grown using complex media in fermenters | [73] |
| Streptomyces coelicolor | 0.04 $^a$ | Grown using complex media in fermenters | [73] |
| Streptomyces griseus | 0.008 $^{a,c}$ | Growth conditions not mentioned in the publication | [74] |
| Rhodococcus rhodochrous | 0.11 $^a$ | Grown using complex media in fermenters | [73] |
| Mycobacterium smegmatis | 0.30 $^a$ | Grown using complex media in fermenters | [73] |
| Mycobacterium smegmatis | 3.0 $^d$ | Overexpression of $F_{420}$ pathway genes, cultivation in complex media at 37°C in shake flasks | [72] |
| Escherichia coli | 0.38 $^b$ | Overexpressing $F_{420}$ pathway genes, grown in minimal media at 30°C in shake flasks. | [59] |

$^a$ Mol weight of $F_{420}$ with 1 glutamate tail is 773.6 Da, which was used to convert values published as µg of $F_{420}$, noting that micro-organisms produce mixture of $F_{420}$ with different number of glutamates (1–9) attached.

$^b$ Concentration estimated through absorbance at 400 nm and using extinction coefficient of 41.4 mM$^{-1}$ cm$^{-1}$ [73].

$^c$ $F_{420}$ concentration per g of wet cell weight. $^d$ Concentration of $F_{420}$ not mentioned in the publication, but $F_{420}$ yield was stated to be 10 times higher than wild-type M. smegmatis. $^e$ Concentration estimated through absorbance at 400 nm and using extinction coefficient of 25.7 mM$^{-1}$ cm$^{-1}$ [74].

Recently, there have been significant advances towards the scalable production of the cofactor for $F_{420}$-dependent enzymes. M. smegmatis has been engineered to overexpress the biosynthetic genes for cofactor $F_{420}$ production, leading to a substantial improvement in yields (Table 2) [72]. However, M. smegmatis is not ideally suited as a fermentation organism as it is slow growing, forms clumps...
during cultivation and is not recognized as GRAS (generally regarded as safe). More recently, the biosynthetic pathway for cofactor F$_{420}$ has been successfully transplanted to E. coli [59], allowing the heterologous production of the cofactor at levels similar to those of the natural F$_{420}$ producers (Table 2) [59], accumulated to 0.38 µmol of F$_{420}$ per gram of dry cells.

There is scope to further improve the production of F$_{420}$ in E. coli. Cofactor F$_{420}$ does not appear to be toxic to E. coli [59], which suggests that there is little interaction between F$_{420}$ and the enzymes E. coli (although this is yet to be confirmed experimentally). The thermodynamics of cofactor F$_{420}$ production are favorable (Appendix A), suggesting that there are no major thermodynamic impediments to improving yield. Interestingly, the first dedicated step of cofactor F$_{420}$ production (catalyzed by CofC/FbiD) is not energetically favorable and may consequently be sensitive to intracellular metabolite concentrations. In addition to the engineering considerations that this may impose, it may also be responsible for the biochemical diversity of this step in different microorganisms. In different microbes, the CofC/FbiD-dependent step uses 2-phospholactate [75], 3-phosphoglycerate [76] or phosphoenolpyruvate [59] as a substrate, which may reflect the relative abundance of those metabolites in various bacteria and archaea and the thermodynamic constraints on this step.

Another recent advance is the production of a synthetic analog of cofactor F$_{420}$, called F$_{O}$-5'-phosphate (F$_{OP}$). F$_{OP}$ was derived from F$_{O}$, the metabolic precursor of cofactor F$_{240}$, which is phosphorylated using an engineered riboflavin kinase [38]. F$_{OP}$ has also been shown to function as an active cofactor for cofactor F$_{240}$-dependent enzymes activities, albeit there is a penalty in the rates of these reactions [38]. Drenth and coworkers prepared F$_{O}$ by chemical synthesis, using a method developed by Hossain et al. [77]. However, it is likely that the engineered kinase for the phosphorylation of F$_{O}$ could be introduced into an organism that over-produces F$_{O}$ allowing for the production of F$_{OP}$ by fermentation. This semisynthetic pathway would have the advantage that it needs only two biosynthetic steps, instead of the four steps needed for cofactor F$_{420}$ production, and demands less metabolic input from the native host metabolism (e.g., no glutamate is required) [38]. The production of F$_{OP}$ also opens the possibility of making deazaflavin analogs of FMN and FAD, which would be electrochemically more like F$_{420}$ than flavins, but may still bind FMN and FAD- dependent enzymes and potentially allow access to new chemistry with already well-characterized enzymes.

5. Prospects

Reduced cofactor F$_{420}$ is electrochemically well suited for biocatalytic applications, and the small number of F$_{420}$-dependent enzymes characterized to date show promise as potential biocatalysts (as discussed above). However, before these enzymes can be widely and effectively used as biocatalysts, further research is needed to better characterize them as the biochemistry of cofactor F$_{420}$-dependent enzymes remains under-explored. The LLHT and FDOR families are a rich source of highly diverse enzymes with considerable potential for biocatalysis, albeit much of the research to date has focused on the physiological roles of these enzymes, rather than their in vitro enzymology. Although some of these enzymes have been shown to have small molecule substrates, those involved with secondary metabolite biosynthesis tend to act on high molecular weight substrates and it is not yet clear whether they will accept lower molecular weight molecules.

To be cost competitive, cofactor F$_{420}$ needs to have effective recycling systems. The enzymes for cofactor recycling have already been identified, although there have been a few studies investigating their performance in this role. Moreover, alternative cofactor recycling strategies, such electrochemical or photochemical recycling, have not yet been investigated for cofactor F$_{420}$. The production of cofactor F$_{420}$ at scale and at low cost remains a roadblock for the use of these enzymes by industry. However, considerable progress has been made on this front in the last few years and it is likely that low cost cofactor F$_{420}$, or F$_{420}$ surrogates, will soon be available. Additionally, the availability of F$_{420}$-producing bacteria with tools for facile genetic manipulation, along with a growing number of empirically determined protein structures, opens up the prospect of improving this class of enzymes using in vitro evolution and rational design. It is notable that there is still some uncertainty concerning
the mechanistic detail of F₄₂₀-dependent reactions, which need to be addressed through a detailed structure/function analysis to enable a rational design of these enzymes.

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**Abbreviations**

5AD: 5′-Deoxyadenosine; 5ARPD: 5-amino-6-(o-ribitylamino)uracil; 5ARPD4HB: 5-amino-5-(4-hydroxybenzyl)-6-(o-ribitylimino)-5,6-dihydrouracil; dF₄₂₀: Dehydro coenzyme F₄₂₀-0 (oxidized); EPPG: Enolpyruvyl-diphospho-5′-guanosine; F₇₈: 7,8-Didemethyl-8-hydroxy-5-deazariboflavin; F₄₂₀: Coenzyme F₄₂₀-0 (oxidized); F₄₂₀-1: Coenzyme F₄₂₀-1 (oxidized); F₄₂₀-2: Coenzyme F₄₂₀-2 (oxidized); F₄₂₀-3: Coenzyme F₄₂₀-3 (oxidized); FMN: Flavin mononucleotide (oxidized); FMNH₂: Flavin mononucleotide (reduced); GDP: Guanosine diphosphate; GMP: Guanosine monophosphate; Glu: l-Glutamate; GTP: Guanosine triphosphate; Glu: l-Glutamate; H⁺: Proton; ImiAce: 2-iminocacetate or Dehydroglycine; Met: l-Methionine; NH₂: Ammonium; PEP: Phosphoenolpyruvate; Pi: Phosphate; PPi: Diphosphate; SAMe: S-Adenosyl-l-methionine; Tyr: l-Tyrosine.

**Appendix A Thermodynamics of F₄₂₀ Biosynthesis**

The thermodynamic properties of each of the steps in cofactor F₄₂₀ biosynthesis were estimated to evaluate the feasibility of increasing the production of the cofactor in an engineered microorganism. The pathway assembled by Bashiri et al. [59] in *E. coli* was used (i.e., PEP was used as substrate for CofC). The standard transformed Gibbs free energy (ΔᵣG°) of each step were calculated under the physiological conditions (25 °C, pH 7, and ionic concentration of 0.25 M) as described elsewhere [78,79]. The overall Gibbs free energy (ΔᵣG) was then calculated by summing up all individual ΔᵣG° (Table A1). The Gibbs free energy of metabolite formation (ΔᵣG) for each metabolite in the pathway was obtained (Supplementary Information) from comprehensive lists of metabolites whose ΔᵣG were estimated using a group contribution method [80,81]. The ΔᵣG for each metabolite was then converted into its transformed type (ΔᵣG°) method of Alberty [78]. The data were collected from relevant biochemical databases and the literature for any metabolite with missing ΔᵣG [82–84]. Owing to possessing different protonation states, the inconsistencies in ΔᵣG of certain metabolites such as the glutamates in F₄₂₀-ₙ among databases and the literature are inevitable. Thus, ΔᵣG° for reactions containing metabolites with varying ΔᵣG were calculated considering the differences in their ΔᵣG leading to the generation of a total of four sets of ΔᵣG°. Finally, the mean and standard deviations were calculated for these sets to yield the variation in each reaction as well as in the overall pathway (Table A1).

The data shown in Table A1 confirms that the overall cofactor F₄₂₀ biosynthesis pathway is thermodynamically feasible under the given conditions. However, certain steps in this pathway impose a thermodynamic barrier with respect to the physiological conditions examined. For example, CofC/H seems to be one of the major thermodynamically unfavorable steps in the whole pathway possibly due to the energy-dependent synthesis of EPPG, one of the precursors for making F₄₂₀. CofC/H combined appears to be the most thermodynamically favorable step in the whole pathway driving the biosynthesis of F₀₉, the other key precursor for F₄₂₀ biosynthesis. Interestingly, the formation of F₄₂₀-2 molecule seems to be the most favorable step among other F₄₂₀ molecules downstream of the pathway. It should be noted that the thermodynamic calculations were only performed up to three steps of F₄₂₀ molecule production (i.e., F₄₂₀-3) largely because of the high levels of inconsistencies of the data available for ΔᵣG of higher F₄₂₀ molecules.
Table A1. Standard transformed Gibbs free energy of reaction ($\Delta G^\circ$), for the F$_{420}$ biosynthesis pathway, calculated based on Gibbs free energy of metabolite formation ($\Delta G^\circ$) calculated at 25 °C, pH of 7, and ionic concentration of 0.25 M.

| Enzyme       | Reaction a                                                                 | $\Delta G^\circ$ (kJ) b       |
|--------------|----------------------------------------------------------------------------|-------------------------------|
| CofC/FbiD    | PEP + GTP $\rightarrow$ EPPG + PP$_i$                                      | +71.27(±67)                   |
| CofC/FbiC    | 5ARPD + Tyr + SAMe $\rightarrow$ 5ARPD4HB + ImAcet + Met + 5AD            | −1192.39(±0)                  |
| CofH/FbiC    | 5ARPD4HB + SAMe $\rightarrow$ F$_O$ + NH$_4^+$ + Met + 5AD               | +71.90(±36)                   |
| CofD/FbiA    | F$_O$ + EPPG $\rightarrow$ dF$_{420}$-0 + GMP                             | −31.3(±128)                   |
| CofX/FbiB    | dF$_{420}$-0 + FMNH$_2$ $\rightarrow$ F$_{420}$-0 + FMN                   | −74.59(±87)                   |
| CofE/FbiB    | F$_{420}$-0 + GTP + Glu $\rightarrow$ F$_{420}$-1 + GDP + P$_i$          | −7.50(±24)                    |
| CofE/FbiB    | F$_{420}$-1 + GTP + Glu $\rightarrow$ F$_{420}$-2 + GDP + P$_i$          | −39.44(±35)                   |
| CofE/FbiB    | F$_{420}$-2 + GTP + Glu $\rightarrow$ F$_{420}$-3 + GDP + P$_i$          | −21.99(±38)                   |
| Overall      | PEP + 5ARPD + Tyr + (2) SAMe + FMNH$_2$ + (3) Glu + (4) GTP $\rightarrow$ F$_{420}$-3 + (2) Met + (2) 5AD + ImAcet + NH$_4^+$ + FMN + (3) GDP + (3) P$_i$ + GMP + PP$_i$ | −1224.05(±82) |

a For simplicity, protons were omitted in these equations and subsequent calculations as the $\Delta G^\circ$ of a proton under the set conditions is ~0.08 kJ. However, all $\Delta G^\circ$ calculations are based on a balanced equation. b The mean values of four sets and their standard deviations in parenthesis shown for each reaction. c $\Delta G^\circ$ of 5ARPD4HB has only been reported in MetaCyc inferred by computational analysis. Including it in the calculations of $\Delta G^\circ$ for CofC and CofH results in −225.88(±0) and −894.62(±36), respectively. d Hydrolysis of PP, (H$_3$P$_2$O$_7$)$^{3-}$ + H$_2$O $\rightarrow$ 2 HPO$_4^{2-}$ + H$^+$ yields a $\Delta G^\circ$ of −17 kJ/mole, resulting in less than 2% change in the overall $\Delta G^\circ$.

References
1. Patil, M.D.; Grogan, G.; Bommarius, A.; Yun, H. Oxidoreductase-catalyzed synthesis of chiral amines. ACS Catal. 2018, 8, 10985–11015. [CrossRef]
2. Toogood, H.S.; Scrutton, N.S. New developments in ‘ene’-reductase catalysed biological hydrogenations. Curr. Opin. Chem. Biol. 2014, 19, 107–115. [CrossRef] [PubMed]
3. Cosgrove, S.C.; Brzezniak, A.; France, S.P.; Ramsden, J.I.; Mangas-Sanchez, J.; Montgomery, S.L.; Heath, R.S.; Turner, N.J. Imine reductases, reductive aminases, and amine oxidases for the synthesis of chiral amines: Discovery, characterization, and synthetic applications. In Enzymes in Synthetic Biology; Scrutton, N., Ed.; Elsevier Academic Press Inc.: San Diego, CA, USA, 2018; Volume 608, pp. 131–149.
4. Bai, D.Y.; He, J.Y.; Ouyang, B.; Huang, J.; Wang, P. Biocatalytic asymmetric synthesis of chiral aryl alcohols. Prog. Chem. 2017, 29, 491–501.
5. Taylor, M.; Scott, C.; Grogan, G. F-420-dependent enzymes-potential for applications in biotechnology. Trends Biotechnol. 2013, 31, 63–64.
6. Mathew, S.; Trajkovic, M.; Kumar, H.; Nguyen, Q.-T.; Fraaije, M.W. Enantio- and regioselective ene-reductions using F$_{420}$H$_2$-dependent enzymes. Chem. Commun. 2018, 54, 11208–11211. [CrossRef]
7. Greening, C.; Ahmed, F.H.; Mohamed, A.E.; Lee, B.M.; Pandey, G.; Warden, A.C.; Scott, C.; Oakeshott, J.G.; Taylor, M.C.; Jackson, C.J. Physiology, biochemistry, and applications of F$_{420}$- and Fo-dependent redox reactions. Microbiol. Mol. Biol. Rev. 2016, 80, 451–493. [CrossRef]
8. Tzing, S.F.; Bryant, M.P.; Wolfe, R.S. Factor 420-dependent pyridine nucleotide-linked formate metabolism of Methanobacterium ruminantium. J. Bacteriol. 1975, 121, 192–196.
9. Eirich, L.D.; Vogels, G.D.; Wolfe, R.S. Distribution of coenzyme F$_{420}$ and properties of its hydrolytic fragments. J. Bacteriol. 1979, 140, 20–27.
10. Wang, P.; Bashiri, G.; Gao, X.; Sawaya, M.R.; Tang, Y. Uncovering the enzymes that catalyze the final steps in oxytetracycline biosynthesis. J. Am. Chem. Soc. 2013, 135, 7138–7141. [CrossRef]
11. Li, W.; Chou, S.C.; Khullar, A.; Gerratana, B. Cloning and characterization of the biosynthetic gene cluster for tomatymin, an SJG-136 monomeric analog. Appl. Environ. Microbiol. 2009, 75, 2958–2963. [CrossRef]
12. Ahmed, F.H.; Carr, P.D.; Lee, B.M.; Afriat-Jurnou, L.; Mohamed, A.E.; Hong, N.-S.; Flanagan, J.; Taylor, M.C.; Greening, C.; Jackson, C.J. Sequence–structure–function classification of a catalytically diverse oxidoreductase superfamily in mycobacteria. J. Mol. Biol. 2015, 427, 3554–3571. [CrossRef] [PubMed]
13. Selengut, J.D.; Haft, D.H. Unexpected abundance of coenzyme F$_{420}$-dependent enzymes in Mycobacterium tuberculosis and other actinobacteria. J. Bacteriol. 2010, 192, 5788–5798. [CrossRef] [PubMed]
14. Lapalikar, G.V.; Taylor, M.C.; Warden, A.C.; Onagi, H.; Hennessy, J.E.; Mulder, R.J.; Scott, C.; Brown, S.E.; Russell, R.J.; Easton, C.J.; et al. Cofactor promiscuity among F420-dependent reductases enables them to catalyse both oxidation and reduction of the same substrate. Catal. Sci. Technol. 2012, 2, 1560–1567. [CrossRef]

15. Harold, L.K.; Antoney, J.; Ahmed, F.H.; Hards, K.; Carr, P.D.; Rapson, T.; Greening, C.; Jackson, C.J.; Cook, G.M. FAD-sequestering proteins protect Mycobacteria against hypoxic and oxidative stress. J. Biol. Chem. 2019, 294, 2903–2912. [CrossRef] [PubMed]

16. Ahmed, F.H.; Mohamed, A.E.; Carr, P.D.; Lee, B.M.; Condic-Jurkic, K.; O'Mara, M.L.; Jackson, C.J. Rv2074 is a novel F420/H2-dependent biliverdin reductase in Mycobacterium tuberculosis. Protein Sci. 2016, 25, 1692–1709. [CrossRef] [PubMed]

17. Mashalidis, E.H.; Mukherjee, T.; Śledź, P.; Matak-Vinković, D.; Boshoff, H.I.; Abell, C.; Barry, C.E. Rv2607 from Mycobacterium tuberculosis is a pyridoxine 5′-phosphate oxidase with unusual substrate specificity. PLoS ONE 2011, 6, e27643. [CrossRef] [PubMed]

18. Taylor, M.C.; Jackson, C.J.; Tattersall, D.B.; French, N.; Peat, T.S.; Newman, J.; Briggs, L.J.; Lapalikar, G.V.; Campbell, P.M.; Scott, C.; et al. Identification and characterization of two families of F420/H2-dependent reductases from Mycobacteria that catalyse aflatoxin degradation. Mol. Microbiol. 2010, 78, 561–575. [CrossRef]

19. Cellitti, S.E.; Shaffer, J.; Jones, D.H.; Mukherjee, T.; Gurumurthy, M.; Bursulaya, B.; Boshoff, H.I.; Choi, I.; Nayyar, A.; Lee, Y.S.; et al. Structure of DDN, the deazaflavin-dependent nitroreductase from Mycobacterium tuberculosis involved in bioreductive activation of PA-824. Structure 2012, 20, 101–112. [CrossRef]

20. De Souza, G.A.; Leversen, N.A.; Målen, H.; Wiker, H.G. Bacterial proteins with cleaved or uncleaved signal peptides of the general secretory pathway. J. Proteom. 2011, 75, 502–510. [CrossRef]

21. He, Z.; De Buck, J. Cell wall proteome analysis of Mycobacterium smegmatis strain mc2 155. BMC Microbiol. 2010, 10, 121. [CrossRef]

22. Sinha, S.; Kosalai, K.; Arora, S.; Namane, A.; Sharma, P.; Gaikwad, S.; Shima, S.; Ermler, U. Structural basis of the hydride basis of coenzyme and substrate binding. J. Biol. Chem. 2011, 286, 29411–29419. [CrossRef] [PubMed]

23. Aufhammer, S.W.; Warkentin, E.; Ermler, U.; Hagemeier, C.H.; Thauer, R.K.; Shima, S. Crystal structure of methylenetetrahydromethanopterin reductase (MER) in complex with coenzyme F420: Architecture of the F420/FMN binding site of enzymes within the nonprolyl cis-peptide containing bacterial luciferase family. Protein Sci. 2005, 14, 1840–1849. [CrossRef] [PubMed]

24. Aufhammer, S.W.; Warkentin, E.; Berk, H.; Shima, S.; Thauer, R.K.; Ermler, U. Coenzyme binding in F420-dependent secondary alcohol dehydrogenase, a member of the bacterial luciferase family. Structure 2004, 12, 361–370. [CrossRef] [PubMed]

25. Bashiri, G.; Squire, C.J.; Moreland, N.J.; Baker, E.N. Crystal structures of F420-dependent glucose-6-phosphate dehydrogenase FGDI involved in the activation of the anti-tuberculosis drug candidate PA-824 reveal the basis of coenzyme and substrate binding. J. Biol. Chem. 2008, 283, 17531–17541. [CrossRef] [PubMed]

26. Nguyen, Q.T.; Trinco, G.; Binda, C.; Mattevi, A.; Fraaije, M.W. Discovery and characterization of an F420-dependent glucose-6-phosphate dehydrogenase (Rh-FGD1) from rhodococcus jostii rha1. Appl. Microbiol. Biotechnol. 2017, 101, 2831–2842. [CrossRef] [PubMed]

27. Mascotti, M.L.; Kumar, H.; Nguyen, Q.T.; Ayub, M.J.; Fraaije, M.W. Reconstructing the evolutionary history of F420-dependent dehydrogenases. Sci. Rep. 2018, 8, 17571. [CrossRef] [PubMed]

28. Ceh, K.; Demmer, U.; Warkentin, E.; Moll, J.; Thauer, R.K.; Shima, S.; Ermler, U. Structural basis of the hydride transfer mechanism in F420-dependent methylenetetrahydromethanopterin dehydrogenase. Biochemistry 2009, 48, 10098–10105. [CrossRef]

29. Shima, S.; Warkentin, E.; Grabarse, W.; Sordel, M.; Wicke, M.; Thauer, R.K.; Ermler, U. Structure of coenzyme F420-dependent methylenetetrahydromethanopterin reductase from two methanogenic archaea. J. Mol. Biol. 2000, 300, 935–950. [CrossRef]

30. Vaupel, M.; Thauer, R.K. Coenzyme F420-dependent N-5, N-10-methylenetetrahydromethanopterin reductase (MER) from Methanobacterium thermototrophicum strain marburg: Cloning, sequencing, transcriptional analysis and functional expression in Escherichia coli of the mer gene. Eur. J. Biochem. 1995, 231, 773–778.

31. Purwanti, E.; Mukhopadyhay, B. Rv0132c of Mycobacterium tuberculosis encodes a coenzyme F420-dependent hydroxyymycolic acid dehydrogenase. PLoS ONE 2013, 8, e81985. [CrossRef]
32. Fida, T.T.; Palamuru, S.; Pandey, G.; Spain, J.C. Aerobic biodegradation of 2,4-dinitroanisole by Nocardioides sp. Strain js1661. Appl. Environ. Microbiol. 2014, 80, 7725–7731. [CrossRef] [PubMed]

33. Ebert, S.; Rieger, P.G.; Knackmuss, H.J. Function of coenzyme $F_{420}$ in aerobic catabolism of 2,4,6-trinitrophenol and 2,4-dinitrophenol by Nocardioides simplex FJ2-1A. J. Bacteriol. 1999, 181, 2669–2674. [PubMed]

34. Lapalikar, G.V.; Taylor, M.C.; Warden, A.C.; Scott, C.; Russell, R.J.; Oakeshott, J.G. $F_{420H_2}$-dependent degradation of aflatoxin and other furanocoumarins is widespread throughout the Actinomycetales. PLoS ONE 2012, 7, e30114. [CrossRef] [PubMed]

35. Greening, C.; Jirapanjawat, T.; Afroze, S.; Ney, B.; Pandey, G.; Lee, B.M.; Russell, R.J.; Jackson, C.J.; Oakeshott, J.G.; et al. Mycobacterial $F_{420H_2}$-dependent reductases promiscuously reduce diverse compounds through a common mechanism. Front. Microbiol. 2017, 8. [CrossRef]

36. Guo, F.; Berglund, P. Transaminase biocatalysis: Optimization and application. Green Chem. 2017, 19, 333–360. [CrossRef]

37. Mohamed, A.E.; Ahmed, F.H.; Arulmozhiraja, S.; Lin, C.Y.; Taylor, M.C.; Krausz, E.R.; Jackson, C.J.; Coote, M.L. Protonation state of $F_{420H_2}$-dependent anti-oxidant mechanism protects Mycobacterium tuberculosis against oxidative stress and bactericidal agents. Mol. Microbiol. 2013, 87, 744–755. [CrossRef]

38. Miller, A.F.; Park, J.T.; Ferguson, K.L.; Pitsawong, W.; Bommarius, A.S. Informing enantiomerically pure alcohols and amines via biocatalytic deracemisation methods. Cat. Sci. Technol. 2019, 9, 10–1039. [CrossRef]

39. Heiss, G.; Hofmann, K.W.; Trachtmann, N.; Walters, D.M.; Rouviere, P.; Knackmuss, H.J. $Npd$ gene functions of Rhodococcus (opacus) erythropolis. J. Bacteriol. 2016, 208, 5770–5780. [CrossRef]

40. Wichmann, R.; Vasic-Racki, D. Cofactor regeneration at the lab scale. In Technology Transfer in Biotechnology; Springer: Berlin, Germany, 2005; pp. 225–260. [CrossRef] [PubMed]

41. Tishkov, V.I.; Popov, V.O. Catalytic mechanism and application of formate dehydrogenase. Biochemistry 2004, 69, 1252. [CrossRef] [PubMed]

42. Eguchi, T.; Kuge, Y.; Inoue, K.; Yoshikawa, N.; Mochida, K.; Uwajima, T. NADPH regeneration by glucose dehydrogenase from Gluconobacter scleroides for L-leucovorin synthesis. Biosci. Biotechnol. Biochem. 1992, 56, 701–703. [CrossRef] [PubMed]
Catalysts 2019, 9, 868

55. Demir, A.S.; Talpur, F.N.; Sopaci, B.; Kohring, G.-W.; Celik, A. Selective oxidation and reduction reactions with cofactor regeneration mediated by galactitol-, lactate-, and formate dehydrogenases immobilized on magnetic nanoparticles. *J. Biotechnol.* 2011, 152, 176–183. [CrossRef] [PubMed]

56. Wong, C.-H.; Whitesides, G.M. Enzyme-catalyzed organic synthesis: NAD(P)H cofactor regeneration by using glucose-6-phosphate and the glucose-5-phosphate dehydrogenase from *Leuconostoc mesenteroides*. *J. Am. Chem. Soc.* 1981, 103, 4890–4899. [CrossRef]

57. Lee, W.-H.; Park, J.-B.; Park, K.; Kim, M.-D.; Seo, J.-H. Enhanced production of e-caprolactone by overexpression of NADPH-regenerating glucose 6-phosphate dehydrogenase in recombinant *Escherichia coli* harboring cyclohexanone monoxygenase gene. *Appl. Microbiol. Biotechnol.* 2007, 76, 329–338. [CrossRef] [PubMed]

58. Berrios-Rivera, S.J.; Bennett, G.N.; San, K.Y. Metabolic engineering of *Escherichia coli*: Increase of NADH availability by overexpressing an NAD+ dependent formate dehydrogenase. *Metab. Eng.* 2002, 4, 217–229. [CrossRef]

59. Bashiri, G.; Antoney, J.; Jirgis, E.N.M.; Shah, M.V.; Ney, B.; Copp, J.; Sreebhavan, S.; Palmer, B.; Middledlitch, M.; et al. A revised biosynthetic pathway for the cofactor F420 in prokaryotes. *Nat. Commun.* 2019, 10, 1558. [CrossRef]

60. Purwantini, E.; Daniels, L. Purification of a novel coenzyme F420-dependent glucose-6-phosphate dehydrogenase from *Mycobacterium smegmatis*. *J. Bacteriol.* 1996, 178, 2861–2866. [CrossRef]

61. Costa, K.C.; Wong, P.M.; Wang, T.; Lie, T.J.; Dodsworth, J.A.; Swanson, I.; Burn, J.A.; Hackett, M.; Leigh, J.A. Protein complexing in a methanogen suggests electron bifurcation and electron delivery from formate to heterodisulfide reductase. *Proc. Natl. Acad. Sci. USA* 2010, 107, 11050–11055. [CrossRef]

62. Novotná, J.; Neužil, J.; Hošek, Z. Spectrophotometric identification of 8-hydroxy-5-deazaflavin: NADPH oxidoreductase activity in *Streptomyces* producing tetracyclines. *FEMS Microbiol. Lett.* 1989, 59, 241–245. [CrossRef]

63. Kunow, J.; Schwörrer, B.; Stetter, K.O.; Thauer, R.K. A F420-dependent NADP oxidoreductase in the extremely thermophilic sulfate-reducing *Archaeoglobus fulgidus*. *Arch. Microbiol.* 1993, 160, 199–205.

64. Eker, A.P.M.; Hesses, J.K.C.; Meerwaldt, R. Characterization of an 8-hydroxy-5-deazaflavin: NADPH oxidoreductase from *Streptomyces griseus*. *Biochim. Biophys. Acta* 1989, 990, 80–86. [CrossRef]

65. Seelbach, K.; Riebel, B.; Hummel, W.; Kula, M.R.; Tishkov, V.I.; Égorov, A.M.; Wandrey, C.; Kraegl, U. A novel, efficient regenerating method of NADPH using a new formate dehydrogenase. *Tetrahedron Lett.* 1996, 37, 1377–1380. [CrossRef]

66. Kumar, H.; Nguyen, Q.T.; Binda, C.; Mattevi, A.; Fraaije, M.W. Isolation and characterization of a thermostable F420:NADPH oxidoreductase from *Thermobifida fusca*. *J. Biol. Chem.* 2017, 292, 10123–10130. [CrossRef] [PubMed]

67. Shuber, A.P.; Orr, E.C.; Recny, M.A.; Schendel, P.F.; May, H.D.; Schauer, N.L.; Ferry, J.G. Cloning, expression, and nucleotide sequence of the formate dehydrogenase genes from *Methanobacterium thermoautotrophicum* delta-H. *Proc. Natl. Acad. Sci. USA* 1999, 96, 12942–12947. [CrossRef]

68. Alex, L.A.; Reeve, J.N.; Ormejohnson, W.H.; Walsh, C.T. Cloning, sequence determination, and expression of the genes encoding the subunits of the nickel-containing 8-hydroxy-5-deazaflavin reducing hydrogenase from *Methanobacterium thermotogae*. *Biochemistry* 1990, 29, 7237–7244. [CrossRef]

69. Tersteegen, A.; Hedderich, R. *Methanobacterium thermotogae* encodes two multisubunit membrane-bound NiFe hydrogenases - transcription of the operons and sequence analysis of the deduced proteins. *Eur. J. Biochem.* 1999, 264, 930–943. [CrossRef]

70. Hocking, W.P.; Stokke, R.; Roalvkn, I.; Steen, I.H. Identification of key components in the energy metabolism of the hyperthermophilic sulfate-reducing archaeon *archaeoglobus fulgidus* by transcriptome analyses. *Front. Microbiol.* 2014, 5, 20. [CrossRef]

71. Vitt, S.; Ma, K.; Warkentin, E.; Moll, J.; Pierik, A.J.; Shima, S.; Ermrler, U. The F-420-reducing NiFe-hydrogenase complex from *methanothermobacter marburgensis*, the first x-ray structure of a group 3 family member. *J. Mol. Biol.* 2014, 426, 2813–2826. [CrossRef]

72. Bashiri, G.; Rehan, A.M.; Greenwood, D.R.; Dickson, J.M.; Baker, E.N. Metabolic engineering of cofactor F420 production in *Mycobacterium smegmatis*. *PLoS ONE* 2010, 5, e15803. [CrossRef]

73. Isabelle, D.; Simpson, D.R.; Daniels, L. Large-scale production of coenzyme F420-5,6 by using *Mycobacterium smegmatis*. *Appl. Environ. Microbiol.* 2002, 68, 5750–5755. [CrossRef] [PubMed]
74. Eker, A.P.M.; Pol, A.; van der Meyden, P.; Vogels, G.D. Purification and properties of 8-hydroxy-5-deazaflavin derivatives from *Streptomyces griseus*. *FEMS Microbiol. Lett.* 1980, 8, 161–165. [CrossRef]

75. Grochowski, L.L.; Xu, H.M.; White, R.H. Identification and characterization of the 2-phospho-L-lactate guanylyltransferase involved in coenzyme F-420 biosynthesis. *Biochemistry* 2008, 47, 3033–3037. [CrossRef] [PubMed]

76. Braga, D.; Lasta, D.; Hasan, M.; Guo, H.; Leichnitz, D.; Uzum, Z.; Richter, I.; Schalk, F.; Beemelmanns, C.; Hertweck, C.; et al. Metabolic pathway rerouting in *Paraburkholderia rhizoxinica* evolved long-overlooked derivatives of coenzyme F_{420}. *ACS Chem. Biol.* 2019, 2088–2094. [CrossRef]

77. Hossain, M.S.; Le, C.Q.; Joseph, E.; Nguyen, T.Q.; Johnson-Winters, K.; Foss, F.W. Convenient synthesis of deazaflavin cofactor F_0 and its activity in F_{420}-dependent NADP reductase. *Organ. Biomol. Chem.* 2015, 13, 5082–5085. [CrossRef]

78. Alberty, R.A. Calculation of standard transformed formation properties of biochemical reactants and standard apparent reduction potentials of half reactions. *Arch. Biochem. Biophys.* 1998, 358, 25–39. [CrossRef]

79. Alberty, R.A. Calculation of standard transformed gibbs energies and standard transformed enthalpies of biochemical reactants. *Arch. Biochem. Biophys.* 1998, 353, 116–130. [CrossRef]

80. Benedict, M.N.; Gonnerman, M.C.; Metcalf, W.W.; Price, N.D. Genome-scale metabolic reconstruction and hypothesis testing in the methanogenic archaeon *Methanosarcina acetivorans* C2A. *J. Bacteriol.* 2012, 194, 855–865. [CrossRef]

81. Jankowski, M.D.; Henry, C.S.; Broadbelt, L.J.; Hatzimanikatis, V. Group contribution method for thermodynamic analysis of complex metabolic networks. *Biophys. J.* 2008, 95, 1487–1499. [CrossRef]

82. Henry, C.S.; Dejongh, M.; Best, A.A.; Frybarger, P.M.; Linsay, B.; Stevens, R.L. High-throughput generation, optimization and analysis of genome-scale metabolic models. *Nat. Biotechnol.* 2010, 28, 977–982. [CrossRef]

83. Nazem-Bokaee, H.; Gopalakrishnan, S.; Ferry, J.G.; Wood, T.K.; Maranas, C.D. Assessing methanotrophy and carbon fixation for biofuel production by *Methanosarcina acetivorans*. *Microb. Cell Fact.* 2016, 15, 10. [CrossRef] [PubMed]

84. Caspi, R.; Billington, R.; Fulcher, C.A.; Keseler, I.M.; Kothari, A.; Krummenacker, M.; Latendresse, M.; Midford, P.E.; Ong, Q.; Ong, W.K.; et al. The metacyc database of metabolic pathways and enzymes. *Nucleic Acids Res.* 2018, 46, D633–D639. [CrossRef] [PubMed]