E. coli Nickel-Iron Hydrogenase 1 Catalyses Non-native Reduction of Flavins: Demonstration for Alkene Hydrogenation by Old Yellow Enzyme Ene-reductases**

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Abstract: *A new activity for the [NiFe] uptake hydrogenase 1 of Escherichia coli (Hyd1) is presented. Direct reduction of biological flavin cofactors FMN and FAD is achieved using H_2 as a simple, completely atom-economical reductant. The robust nature of Hyd1 is exploited for flavin reduction across a broad range of temperatures (25–70°C) and extended reaction times. The utility of this system as a simple, easy to implement $FMNH_2$ or $FADH_2$ regenerating system is then demonstrated by supplying reduced flavin to Old Yellow Enzyme "ene-reductases" to support asymmetric alkene reductions with up to 100% conversion. Hyd1 turnover frequencies up to 20.4 min^{-1} and total turnover numbers up to 20 200 were recorded during flavin recycling.*

Academic and industrial fields are increasingly looking to biotechnology to make chemical manufacturing more sustainable.[1] Enzymes provide many advantages: they are renewable, biodegradable, nonhazardous, and provide high selectivity. Furthermore, the once-limited scope of known enzyme reactions is rapidly expanding, aided by enzyme engineering and ongoing discovery and characterization of new enzymatic functions.[2,3]

Old Yellow Enzyme (OYE) ene-reductases are gaining prominence in industrial biotechnology for catalysis of asymmetric alkene reductions. OYEs contain a tightly bound FMN prosthetic group which transfers electrons from an external reductant to an activated alkene (Supporting Information, Figure S2). Most commonly, OYEs are supplied

with reducing equivalents via the expensive cofactors NADPH or NADH, and hence they are typically operated with a cofactor recycling system for the reduced nicotinamide cofactors such as glucose/glucose dehydrogenase (GDH). OYE ene-reductases can also accept reducing equivalents from synthetic analogues of NADH,[4] although work is still needed on effective recycling systems for these artificial cofactors. There are also reports[5,6] of electron uptake from reduced flavins, $FMNH_2$ or $FADH_2$ (oxidized and reduced forms are shown in Scheme 1). Presumably the tightly bound
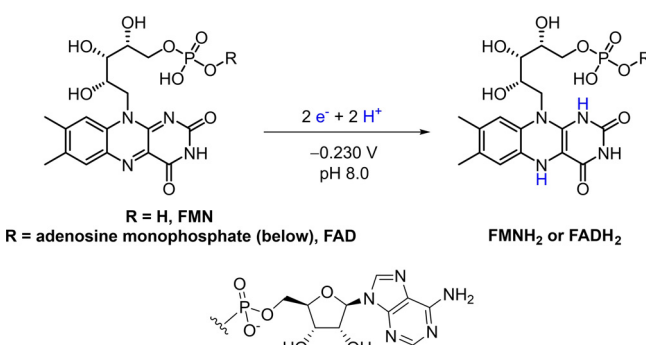
Scheme 1. Oxidized (left) and reduced (right) FMN or FAD cofactors.

prosthetic flavin in OYEs is sufficiently exposed to allow this promiscuity in terms of reductant. Supply of a catalytic quantity of oxidized FMN or FAD, together with a recycling system for reduced flavin is preferable to stoichiometric addition of $FMNH_2$ or $FADH_2$, both in terms of lowering cost and minimizing waste. Reduced flavins have been recycled in situ by means of photochemistry, electrochemistry or metal catalysis,[6] which can suffer from biocompatibility challenges (such as mutual inactivation, mismatched ideal solvent, pH, or temperature).[7,8] Milder biocatalytic approaches to flavin recycling are cumbersome (Supporting Information, Figure S3),[7,9,10] requiring both an NAD(P)H-dependent reductase to produce $FMNH_2$ or $FADH_2$ at the expense of NAD(P)H[11] and GDH/glucose for recycling the NAD(P)H.

Use of H_2 for cleaner enzymatic NAD(P)H cofactor recycling has been demonstrated.[12–14] The soluble hydrogenase from *Cupriavidus necator* (formerly *Ralstonia eutropha*) natively uses H_2 to provide electrons for NAD^+ reduction at a prosthetic flavin cofactor.[13] Reduction of external flavin substrates by this enzyme under H_2 has long been known,[15] and presumably occurs at the NAD^+ binding

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site. The multi-subunit soluble hydrogenase has recently been demonstrated as a possible recycling system for reduced flavin,[16] but the enzyme is complex to express and lacks stability.[17,18]

This inspired us to test whether a simple hydrogenase (Figure 1) could be suitable for H_2-driven flavin reduction. The thermodynamic potential for the H^+/H_2 couple (-0.472 V, pH 8) relative to the flavin potential (-0.230 V, pH 8),[19] makes reduction of flavin by H_2 thermodynamically favorable. We selected *E. coli* [NiFe]-hydrogenase (Hyd1), which is a good H_2 oxidizer[20,21] and well-characterized in terms of X-ray crystal structures[22,23] and spectroscopy.[21,24]
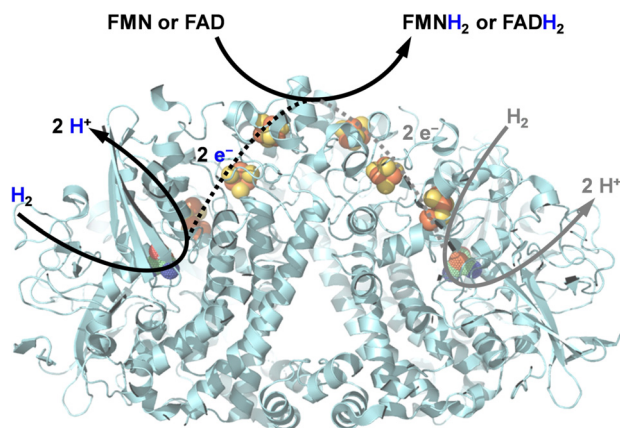

Figure 1. Flavin reduction by Hyd1. H_2 oxidation at the [NiFe] active site (green, red, blue) provides 2 electrons that are transferred to the protein surface via FeS clusters (yellow, orange). The figure, showing the homodimer of HyaAB units, was prepared using PyMOL 2.3.4 (PDB: 6FPW).

Hyd1 is natively expressed in *E. coli* and, unlike many hydrogenases,[25] it is O_2-tolerant[21] and active over a wide pH range.[26] Like other uptake hydrogenases, the basic unit of Hyd1 is a heterodimer of the large subunit (HyaB) housing the [NiFe] active site, and the small subunit (HyaA) housing the iron-sulfur cluster electron transfer relay. Natively, Hyd1 exists as a homodimer, $(HyaAB)_2$ and is coupled to a cytochrome electron acceptor. Our isolated enzyme comprises predominantly the dimeric HyaAB[27] and our preparation lacks the cytochrome (Supporting Information, Figure S1).

The H_2 oxidation activity of Hyd1 is typically measured using the artificial electron acceptor benzyl viologen in colourimetric assays.[26] Electrons from H_2 oxidation at the [NiFe] active site (Figure 1) are relayed through FeS clusters where, evidence suggests, benzyl viologen reduction occurs, rather than directly at the [NiFe] active site.[28] The fact that electron transfer from hydrogenases to electrodes is also well-established[21,25] encouraged us to explore scope for other non-natural electron transfer reactions of robust Hyd1 from *E. coli*. We demonstrate that both FMN and FAD can accept electrons from H_2 oxidation by Hyd1 to generate $FMNH_2$ and $FADH_2$ respectively, and show that Hyd1 can be used as an effective $FMNH_2$ regeneration system to support asymmetric alkene reduction by three OYE-type ene-reductases.
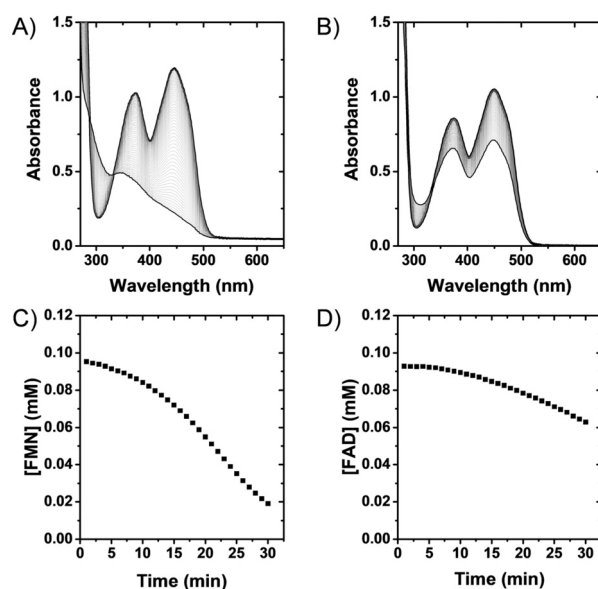

Figure 2. Activity assay for H_2-driven Hyd1 reduction of flavin measured by in situ UV/Vis spectroscopy. A) Hyd1 reducing FMN. B) Hyd1 reducing FAD. C) Calculated [FMN] based on $\lambda_{max} = 445$ nm ($\varepsilon = 12.50$ mM^{-1} cm^{-1}). D) Calculated [FAD] based on $\lambda_{max} = 450$ nm ($\varepsilon = 11.30$ mM^{-1} cm^{-1}). Reaction conditions: General Procedure A in Tris-HCl buffer (50 mM, pH 8.0, 25°C).

Figure 2 shows the results of in situ UV/Vis spectrophotometric assays to explore H_2-driven FMN and FAD reduction by Hyd1 (produced and isolated in accord with the Supporting Information, Methods Section S1.2; reaction follows General Procedure A). The flavin moiety of FMN gives λ_{max} at 445 nm and FAD at 450 nm, both of which bleach upon two-electron reduction[29,30] (Figure 2 A,B; see the Supporting Information, Figure S6 for spectra of fully reduced FMN). The decrease in oxidized flavin concentration over time was used to calculate initial enzyme activity (Figure 2 C,D). Control experiments indicated that omission of Hyd1 or H_2 led to negligible flavin reduction (Supporting Information, Figures S7–S9).

Upon addition of Hyd1, a lag phase was observed during FMN and FAD reduction, which is attributed to the well-characterized H_2-dependent activation phase for aerobically purified Hyd1.[21] Later experiments (when indicated) used Hyd1 that was first activated under a H_2 atmosphere.[31] The lag phase was followed by a decrease in absorbance consistent with $FMNH_2/FADH_2$ formation, and clear isosbestic points at 330 nm corroborate a lack of side products. Specific initial activities for FMN and FAD reduction (76 and 32 nmol min^{-1} mg^{-1} Hyd1, respectively) were determined during the linear reaction phase. The higher activity for reduction of FMN compared with FAD cannot be attributed to thermodynamic driving force since both cofactors have similar reduction potentials,[19] but could relate to the cofactors' ability to interact at the protein surface.

Hyd1 is known to be robust which inspired us to test H_2-driven flavin reduction activity at different temperatures (25–70°C, General Procedure A). Figure 3 shows the conversions from reactions performed at different temperatures after
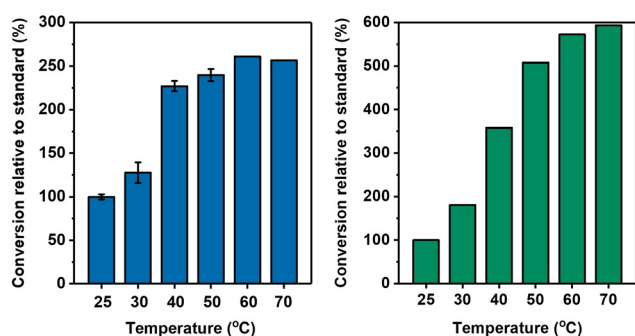
Figure 3. Hyd1-catalysed flavin reduction at different temperatures (left: FMN; right: FAD). Conversion relative to standard $= \frac{\text{Conversion at temp}}{\text{Conversion at 25 °C}} \times 100\%$. The FMN 25–50 °C bars represent the average of relative conversions calculated from duplicate experiments, with the range represented as error bars. Reaction conditions: General Procedure A (Supporting Information) in phosphate buffer (50 mM, pH 8.0). Conversion was calculated after 30 min using UV-visible spectroscopy.

30 minutes relative to a standard reaction performed at 25 °C. This standard temperature and stop time were selected to leave room for improvement in conversions of FMN and FAD at the higher temperatures. Reactions at 25–50 °C using FMN were performed twice, and the corresponding bars indicate the average relative conversion with the range of results represented with error bars (± 3–12%). This level of reproducibility is likely to extend to FAD owing to an identical reaction set up. Results for FMN and FAD may not be directly comparable due to different purity levels of the cofactors which were obtained from different suppliers. Conversion of FMN and FAD to the reduced forms after 30 min reaction time increased with temperature (Figure 3), suggesting that Hyd1 is likely to open new doors to cofactor recycling for flavoenzymes with optimal activity at higher temperatures.

To demonstrate the utility of Hyd1 in biotechnologically-relevant flavin recycling, we first coupled Hyd1-catalysed flavin reduction with the OYE-type ene-reductase from *Thermus scotoductus*, *Ts*OYE,[32,33] to catalyze enantioselective reduction of ketoisophorone (**1**) to (*R*)-levodione (**2**, Table 1). Reactions were conducted according to General Procedure B (Supporting Information) and monitored using chiral-phase GC-FID after extraction of the reaction mixture into ethyl acetate (Supporting Information, Figure S13). Enantiomeric excess (*ee*) was always >99% at the first time point but decreased to 86–92% from slow racemization under alkaline conditions as previously reported.[34] Control experiments confirmed good reproducibility (4.4% standard deviation) and that each component is required for conversion (Supporting Information, Tables S1,S2).

The highest Hyd1 turnover frequency (TOF, 20.4 min^{-1}) and quantitative conversion after 15 h were achieved with 0.5 mM FMN and 2 mM **1** at room temperature (entry 1, Table 1).

When 0.1 mM FMN was used with varying [**1**] (entries 2–5), a Hyd1 total turnover number (TTN) of up to 10 200 and 97 FMN turnovers (TN) were achieved after 24 h. This is comparable to the FMN TN reported for formate-driven Rh-catalyzed FMNH$_2$ recycling, however background, non-enan-

Table 1: H$_2$-driven enzymatic reduction of **1** under various conditions.[a]

Entry	[1] (mM)	[FMN] (mM)	Conv. to [%][b]	Hyd1 TOF [min^{-1}][c]	Hyd1 TTN[d]	FMN TN[d]
1	2	0.5	100	20.4	2100	4
2	2	0.1	100	7.8	2100	20
3	5	0.1	95 {100}	4.8	5200	50
4	10	0.1	62 {97}	5.4	10 200	97
5	20	0.1	24 {37}	5.4	7800	74
6[e]	20	0.1	{44}	8.4	9300	88
7[f]	10	0.1	{94}	9.6	9900	94
8[g]	20 then 24.2[h]	0.1	{29} then >99[i]	3.0	20 200[i]	240[i]

[a] Reaction conditions: In accord with General procedure B using 57 µg Hyd1, 72 µg *Ts*OYE in Tris-HCl (50 mM, pH 8), 1 vol% DMSO at room temperature (20 °C–22 °C). [b] GC conversion to **2** at 15 h {and 24 h}. [c] Hyd 1 turnover frequency (mol **2** per mol Hyd1 per min) was calculated after 60 minutes. [d] Hyd1 total turnover number (mol **2** per mol Hyd1) and FMN turnover number (mol **2** per mol FMN) were determined at the end of the reaction. [e] 4 bar H$_2$. [f] 35 °C; some evaporation of **1** and **2** was observed from GC-FID. [g] 71 µg Hyd1 was used. [h] Reaction was fed with additional 72 µg *Ts*OYE and 4.2 mM **1** at 66 h and 71 h, respectively. [i] Conversion, Hyd 1 TTN and FMN TN were determined at 134 hours, additional time point data in the Supporting Information, Figure S12.

tioselective reduction of **1** by [Cp*Rh(bpy)H]$^+$ meant a careful balance of catalysts was required in that case.[32] This was not an appreciable issue with our biocatalytic system (Supporting Information, Table S2). Increasing H$_2$ pressure to 4 bar boosted conversion and Hyd1 TOF from 5.4 min^{-1} to 8.4 min^{-1}, likely due to improved H$_2$ availability (entries 5,6).
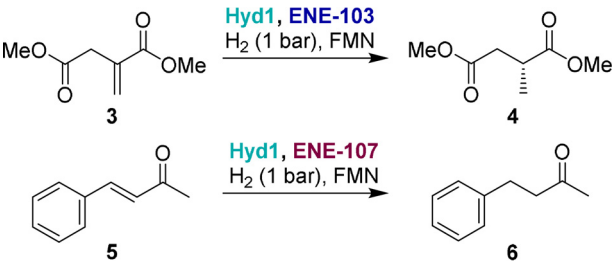
Like Hyd1, *Ts*OYE has enhanced activity at elevated temperatures,[33] therefore entry 4 was replicated at 35 °C (see entry 7). Hyd1 TOF nearly doubled to 9.6 min^{-1} and 94% conversion was achieved after 24 h, however GC-FID showed that some of **1** and **2** likely evaporated.

To test stability over time, entry 5 was replicated using 71 µg Hyd1, and as the reaction neared full conversion an additional 72 µg *Ts*OYE then 4.2 mM **1** was added (66 h and 71 h, respectively, see entry 8). Though the reaction likely still had active enzymes (Supporting Information, Figure S12), the reaction was stopped for analysis at 134 h (5.5 days) after which Hyd1 TTN reached 20 200 and FMN TN 240. This represents an improvement in stability over *R. eutropha* SH (TTN 8400) for flavin recycling with *Ts*OYE.[16] The 20 200 TTN is of an appropriate order of magnitude for use as a catalyst in the pharmaceutical and fine chemicals industries,[35] approaches values measured from commercial grade enzymatic processes,[36] and there remains room for further optimization to that end. The demonstrated continuous Hyd1 stability over time (Supporting Information, Figure S12) is an important performance benchmark for potential commercial applications, particularly in flow.[37] Furthermore, this appli-

cation is likely to extend to *Ts*OYE variants, which have demonstrated broad substrate acceptance, are robust in harsh conditions, and can switch enantioselectivity.[38]

We extended this system to two commercially available ene-reductases, ENE-103 and ENE-107 (Johnson Matthey), which are typically sold as a kit with GDH and formate dehydrogenase for NAD(P)H recycling. The alkene reductions demonstrated were dimethyl itaconate (**3**) reduction to dimethyl (*R*)-methyl succinate (**4**) by ENE-103 and 4-phenyl-3-buten-2-one (**5**) reduction to 4-phenyl-2-butanone (**6**) by ENE-107 (Table 2), using the same protocols established for *Ts*OYE. Control experiments to show that each component is required for substrate conversion are summarized in the Supporting Information, Tables S3,S4.

Table 2: H$_2$-driven enzymatic alkene reductions using commercial ene-reductases.[a]

Entry	Substrate	[FMN] (mM)	Ene-reductase	*t* [h]	Conv. [%][b]	*ee* [%]
1	**3**	0.1	ENE-103	42	81	> 99
2	**3**	0.5	ENE-103	42	98	> 99
3[c]	**5**	0.1	ENE-107	24	20 ± 1	n.a.[d]
4[c]	**5**	0.5	ENE-107	24	33 ± 3	n.a.[d]
5	**5**	0.1	ENE-107	40	35	n.a.[d]
6	**5**	0.5	ENE-107	40	100	n.a.[d]

[a] Reaction conditions: In accord with General procedure B using 142 µg Hyd1, 3 mg ene-reductase and 5 mM substrate in Tris-HCl (50 mM, pH 8), 1 vol% DMSO at room temperature (20 °C–30 °C). [b] GC conversions to **4** or **6**. [c] Entries 3 and 4 were performed in triplicate and are shown ±1 standard deviation, and were separate experiments from entries 5 and 6. [d] Not applicable.

With ENE-103, enantioselective (> 99 % *ee*) reduction to (*R*)-**4** improved from 81 % to 98 % conversion as FMN concentration was increased from 0.1 mM to 0.5 mM (entries 1,2). Conversion of **5** to **6** using ENE-107 was drastically improved when FMN concentration increased from 0.1 mM to 0.5 mM (compare entries 3 and 4, and entries 5 and 6), increasing from 35 % to 100 % conversion in the 40 hour experiment. These results highlight the straightforward application of different ene-reductases with Hyd1-catalysed flavin recycling, suggesting that this simplified H$_2$-driven system could be valuable in applications that require low waste, high catalyst stability and temperature tolerance.

Our work has shown a clean, atom-efficient way of driving commercial ene-reductase enzymes with flavin recycling in place of nicotinamide cofactor recycling. Further modifications to Hyd1, which is tolerant of mutagenesis,[23,31] might enhance its non-native flavin reduction activity. Other

promising synthetically interesting flavin-dependent enzymes, including halogenases (chlorination, bromination, iodination)[7] and flavoprotein monooxygenases (epoxidation, hydroxylation, Baeyer–Villiger oxidation)[39,40] are currently under-utilized in industrial biotechnology, perhaps due to the lack of available simplified flavin recycling systems. This proof-of-concept work shows that the robust Hyd1, tolerant to a range of conditions, is a promising catalyst to develop for clean flavin recycling in biotechnology.

Acknowledgements

Conflict of interest

The authors declare no conflict of interest.

[1] R. A. Sheldon, J. M. Woodley, *Chem. Rev.* **2018**, *118*, 801–838.
[2] H. A. Bunzel, X. Garrabou, M. Pott, D. Hilvert, *Curr. Opin. Struct. Biol.* **2018**, *48*, 149–156.
[3] K. Chen, F. H. Arnold, *Nat. Catal.* **2020**, *3*, 203–213.
[4] T. Knaus, C. E. Paul, C. W. Levy, S. De Vries, F. G. Mutti, F. Hollmann, N. S. Scrutton, *J. Am. Chem. Soc.* **2016**, *138*, 1033–1039.
[5] M. M. Grau, J. C. Van Der Toorn, L. G. Otten, P. Macheroux, A. Taglieber, F. E. Zilly, I. W. C. E. Arends, F. Hollmann, *Adv. Synth. Catal.* **2009**, *351*, 3279–3286.
[6] W. Zhang, F. Hollmann, *Chem. Commun.* **2018**, *54*, 7281–7289.
[7] J. Latham, E. Brandenburger, S. A. Shepherd, B. R. K. Menon, J. Micklefield, *Chem. Rev.* **2018**, *118*, 232–269.
[8] F. Rudroff, M. D. Mihovilovic, H. Gröger, R. Snajdrova, H. Iding, U. T. Bornscheuer, *Nat. Catal.* **2018**, *1*, 12–22.
[9] C. K. Winkler, K. Faber, M. Hall, *Curr. Opin. Biotechnol.* **2018**, *43*, 97–105.
[10] H. S. Toogood, N. S. Scrutton, *ACS Catal.* **2018**, *8*, 3532–3549.
[11] L. Sellés Vidal, C. L. Kelly, P. M. Mordaka, J. T. Heap, *Biochim. Biophys. Acta Proteins Proteomics* **2018**, *1866*, 327–347.
[12] H. A. Reeve, L. Lauterbach, O. Lenz, K. A. Vincent, *ChemCatChem* **2015**, *7*, 3480–3487.
[13] L. Lauterbach, O. Lenz, K. A. Vincent, *FEBS J.* **2013**, *280*, 3058–3068.

[14] J. Preissler, H. A. Reeve, T. Zhu, J. Nicholson, K. Urata, L. Lauterbach, L. L. Wong, K. A. Vincent, O. Lenz, *ChemCatChem* **2020**, *12*, 4853–4861.

[15] K. Schneider, H. G. Schlegel, *Cell* **1976**, *452*, 66–80.

[16] A. Al-Shameri, S. J.-P. Willot, C. E. Paul, F. Hollmann, L. Lauterbach, *Chem. Commun.* **2020**, *56*, 9667–9670.

[17] O. Lenz, L. Lauterbach, S. Frielingsdorf in *Methods in Enzymology* (Ed.: F. A. Armstrong), Academic Press, New York, **2018**, pp. 117–151.

[18] N. Herr, J. Ratzka, L. Lauterbach, O. Lenz, M. B. Ansorge-Schumacher, *J. Mol. Catal. B* **2013**, *97*, 169–174.

[19] S. Vogt, M. Schneider, H. Schäfer-Eberwein, G. Nöll, *Anal. Chem.* **2014**, *86*, 7530–7535.

[20] P. Wulff, C. C. Day, F. Sargent, F. A. Armstrong, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 6606–6611.

[21] M. J. Lukey, A. Parkin, M. M. Roessler, B. J. Murphy, J. Harmer, T. Palmer, F. Sargent, F. A. Armstrong, *J. Biol. Chem.* **2010**, *285*, 3928–3938.

[22] A. Volbeda, P. Amara, C. Darnault, J.-M. Mouesca, A. Parkin, M. M. Roessler, F. A. Armstrong, J. C. Fontecilla-Camps, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 5305–5310.

[23] R. M. Evans, E. J. Brooke, S. A. M. Wehlin, E. Nomerotskaia, F. Sargent, S. B. Carr, S. E. V. Phillips, F. A. Armstrong, *Nat. Chem. Biol.* **2016**, *12*, 46–50.

[24] R. Hidalgo, P. A. Ash, A. J. Healy, K. A. Vincent, *Angew. Chem. Int. Ed.* **2015**, *54*, 7110–7113; *Angew. Chem.* **2015**, *127*, 7216–7219.

[25] K. A. Vincent, A. Parkin, F. A. Armstrong, *Chem. Rev.* **2007**, *107*, 4366–4413.

[26] B. J. Murphy, F. Sargent, F. A. Armstrong, *Energy Environ. Sci.* **2014**, *7*, 1426–1433.

[27] P. Wulff, C. Thomas, F. Sargent, F. A. Armstrong, *J. Biol. Inorg. Chem.* **2016**, *21*, 121–134.

[28] C. Pinske, S. Krüger, B. Soboh, C. Ihling, M. Kuhns, M. Braussemann, M. Jaroschinsky, C. Sauer, F. Sargent, A. Sinz, et al., *Arch. Microbiol.* **2011**, *193*, 893–903.

[29] P. Macheroux in *Methods in Molecular Biology* (Eds.: S. K. Chapman, G. A. Reid), Humana Press, Totowa, **1999**, pp. 1–7.

[30] A. M. Edwards in *Methods in Molecular Biology* (Eds.: S. Weber, E. Schleiker), Humana Press, New York, **2014**, pp. 3–13.

[31] R. M. Evans, P. A. Ash, S. E. Beaton, E. J. Brooke, K. A. Vincent, S. B. Carr, F. A. Armstrong, *J. Am. Chem. Soc.* **2018**, *140*, 10208–10220.

[32] J. Bernard, E. van Heerden, I. W. C. E. Arends, D. J. Opperman, F. Hollmann, *ChemCatChem* **2012**, *4*, 196–199.

[33] D. J. Opperman, B. T. Sewell, D. Litthauer, M. N. Isupov, J. A. Littlechild, E. van Heerden, *Biochem. Biophys. Res. Commun.* **2010**, *393*, 426–431.

[34] A. Fryszkowska, H. Toogood, M. Sakuma, J. M. Gardiner, G. M. Stephens, N. S. Scrutton, *Adv. Synth. Catal.* **2009**, *351*, 2976–2990.

[35] J. Dong, E. Fernández-Fueyo, F. Hollmann, C. E. Paul, M. Pesic, S. Schmidt, Y. Wang, S. Younes, W. Zhang, *Angew. Chem. Int. Ed.* **2018**, *57*, 9238–9261; *Angew. Chem.* **2018**, *130*, 9380–9404.

[36] T. A. Rogers, A. S. Bommarius, *Chem. Eng. Sci.* **2010**, *65*, 2118–2124.

[37] C. Hammond, *Green Chem.* **2017**, *19*, 2711–2728.

[38] N. Nett, S. Duewel, L. Schmermund, G. E. Benary, K. Ranaghan, A. Mullholland, D. J. Opperman, S. Hoebenreich, *Mol. Catal.* **2021**, *502*, 111404.

[39] R. D. Ceccoli, D. A. Bianchi, D. V. Rial, *Front. Microbiol.* **2014**, *5*, 25.

[40] T. Heine, W. van Berkel, G. Gassner, K.-H. van Pée, D. Tischler, *Biology* **2018**, *7*, 42.