

H₂ as a fuel for flavin- And H₂O₂-dependent biocatalytic reactions

Al-Shameri, Ammar; Willot, Sébastien J.P.; Paul, Caroline E.; Hollmann, Frank; Lauterbach, Lars

DOI

[10.1039/d0cc03229h](https://doi.org/10.1039/d0cc03229h)

Publication date

2020

Document Version

Final published version

Published in

Chemical Communications

Citation (APA)

Al-Shameri, A., Willot, S. J. P., Paul, C. E., Hollmann, F., & Lauterbach, L. (2020). H₂ as a fuel for flavin- And H₂O₂-dependent biocatalytic reactions. *Chemical Communications*, 56(67), 9667-9670.
<https://doi.org/10.1039/d0cc03229h>

Important note

To cite this publication, please use the final published version (if applicable).
Please check the document version above.

Copyright

Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

Takedown policy

Please contact us and provide details if you believe this document breaches copyrights.
We will remove access to the work immediately and investigate your claim.



H₂ as a fuel for flavin- and H₂O₂-dependent biocatalytic reactions†

Ammar Al-Shameri,^a Sébastien J.-P. Willot,^b Caroline E. Paul,^b Frank Hollmann^b and Lars Lauterbach^a

Cite this: *Chem. Commun.*, 2020, 56, 9667

Received 5th May 2020,
Accepted 14th July 2020

DOI: 10.1039/d0cc03229h

rsc.li/chemcomm

The soluble hydrogenase from *Ralstonia eutropha* provides an atom efficient regeneration system for reduced flavin cofactors using H₂ as an electron source. We demonstrated this system for highly selective ene-reductase-catalyzed C=C-double bond reductions and monooxygenase-catalyzed epoxidation. Reactions were expanded to aerobic conditions to supply H₂O₂ for peroxxygenase-catalyzed hydroxylations.

Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are key versatile cofactors for electron transfer and substrate conversion. Flavins are found as prosthetic groups in many biocatalysts including ene-reductases, monooxygenases, hydrogenases and oxidases. These flavoenzymes catalyze various industrially relevant reactions such as asymmetric hydrogenations and sulfoxidations, selective amine oxidations, and Baeyer-Villiger oxidations.¹

In their reduced form (FNMH₂ and FADH₂, respectively), flavins serve as electron donors for a broad range of biological redox reactions many of which are relevant in the organic synthesis.² In the case of preparative chemical applications (*i.e.*, biocatalysis), the stoichiometric reductant plays a crucial role in envisioning the economic feasibility and environmental impact of the reaction system. Mimicries of the natural, NAD(P)H-dependent regeneration pathways are currently the most widely used approach, but suffer from complex reaction schemes, the need for the nicotinamide cofactor and the formation of significant wastes originating from stoichiometric reductants such as glucose.³ In addition, some photo⁴ and electrochemical^{5,6} approaches to regenerate reduced flavins have been reported.

Hydrogen would be an ideal reductant, especially from an environmental point of view as it does not yield any waste-products and is sustainable when generated by electrolysis using renewable energy sources. The NAD⁺-reducing hydrogenase from *Ralstonia eutropha* (soluble hydrogenase, SH) uses molecular hydrogen (H₂) to reduce NAD⁺ to NADH and consists of a hydrogenase module (HoxHY) with a H₂ converting active site and a diaphorase module (HoxFUI₂) with a NAD⁺ binding site (Fig. 1).^{7–9} H₂ is oxidized at the NiFe active site with three biologically untypical CN[−] and CO ligands. The electrons are transferred *via* the FMN-a close to the active site within the hydrogenase module and the Fe-S cluster chain to the second FMN-b at the NAD⁺ binding site (Fig. 1). FMN-b serves here as a mediator between the one electron centers (Fe-S clusters) and the two-electron acceptor NAD⁺.¹⁰ The flavins of the SH are non-covalently bound. During SH purification, FMN-a is easily lost and can be reconstituted by adding free FMN to the purified enzyme, which significantly improves the NAD⁺ reduction activity.^{11,12} Experiments with separately produced hydrogenase and diaphorase modules showed that the FMN-a of the hydrogenase module can be reconstituted under reducing conditions and the FMN-b of the diaphorase module is released under reducing conditions.^{8,9} Purified SH shows a lag phase during activity, since most of the enzyme population is inactive and requires reverse electron transfer from NADH generated from an active enzyme population for reactivation.^{13,14}

In contrast to most other hydrogenases, the SH is O₂ tolerant; *i.e.* its catalytic activity is not impaired by ambient O₂.¹⁵ SH has been employed for the recycling of NAD(P)H in various enzymatic reactions *in vitro* and *vivo*.^{16–18} Compared with common NAD(P)H-regeneration systems such as formate dehydrogenase (generating CO₂ as a stoichiometric by-product) and glucose-6-phosphate dehydrogenase (yielding the corresponding gluconolactone), H₂-driven cofactor recycling has the advantage of being 100% atom efficient, by-product-free and cheap since it only relies on H₂ as reductant.^{19,20} Besides NAD⁺, SH is also known to reduce other electron acceptors such as ferricyanide, methylene blue and methyl viologen.¹³ This catalytic

^a Institute of Chemistry, Technische Universität Berlin, Strasse des 17. Juni 135, 10623 Berlin, Germany. E-mail: lars.lauterbach@tu-berlin.de

^b Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629HZ Delft, The Netherlands. E-mail: f.hollmann@tudelft.nl, c.e.paul@tudelft.nl

† Electronic supplementary information (ESI) available: Details of H₂-driven biotransformations; GC product analysis; kinetic parameters of SH for FMN and FAD; formation of H₂O₂. See DOI: 10.1039/d0cc03229h



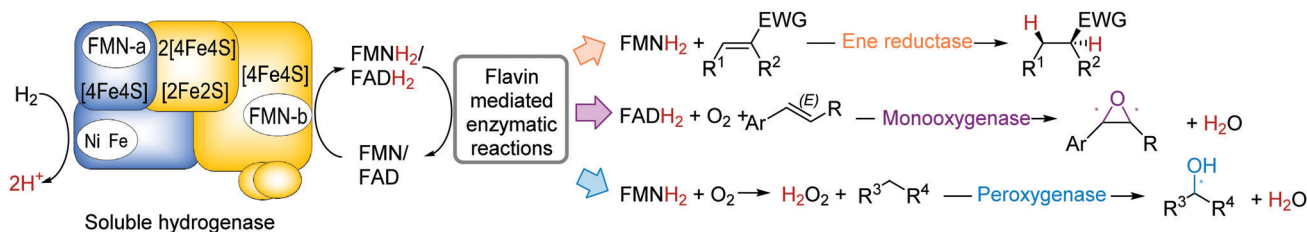


Fig. 1 H_2 -driven reduced flavin regeneration by soluble hydrogenase (SH) coupled with three biocatalysts to catalyze asymmetric hydrogenation, epoxidation and selective hydroxylation with high atomic efficiency. SH consists of a hydrogenase module (blue) with the NiFe active site responsible for H_2 conversion and a diaphorase module (yellow) with the NAD^+ binding site near FMN-b. Red H atoms show the fate of H atoms after H_2 oxidation and illustrating the atom efficiency of H_2 -driven flavin reduction. The resulted protons from H_2 splitting are released to the solution and electrons are transferred via FMN-a, Fe-S clusters and FMN-b to the NAD^+ binding site for FMN or FAD conversion to FMNH_2 and FADH_2 , respectively. Protons come from solution for flavin reduction. EWG = electron withdrawing group.

promiscuity in terms of electron acceptors may open up new avenues of H_2 -driven biocatalysis while avoiding costly nicotinamide cofactors. We therefore evaluated SH-driven reduction of flavins to promote a range of biocatalytic redox reactions of synthetic interest.

First, we investigated the kinetic parameters for the SH-catalyzed reduction of FMN and FAD (FADH_2/FAD and FMNH_2/FMN $E^\circ = -190$ mV) driven by H_2 oxidation ($\text{H}_2/2\text{H}^+$ $E^\circ = -412$ mV), which is thermodynamically favoured. The specific activity of SH for reducing FMN was 5.8 U mg^{-1} (20.3 s^{-1}), with a $K_{\text{m,FMN}}$ of $680 \text{ } \mu\text{M}$ (Fig. S1, ESI†). Adding catalytic amounts of NADH increased the FMN specific activity by 100% and led to the disappearance of the lag phase. The disappearance of the lag phase is explained by faster reactivation under reducing conditions and reverse electron transfer (see above). Surprisingly, SH exhibited a significantly lower activity on FAD (0.14 U mg^{-1} , 0.49 s^{-1}), which prohibited the precise determination of its K_{m} value. The higher activity towards the FMN compared with the longer FAD, as well as the increased FMN activity in the presence of NADH, support the reaction model, that the reduction occurs by temporarily substituting the prosthetic FMN-b by free flavin. After reduction of bound flavin, it is released again (Fig. 1). The FMN-b in the Rossmann fold is deeper submerged than the binding site of NAD^+ , as seen in the crystal structure of the related SH from *Hydrogenophilus thermoluteolus*,²¹ explaining the 17 times lower specific activities for FMN in comparison to NAD^+ as the substrate.¹⁵ The Fe-S clusters of the SH are buried in the protein matrix,²¹ which may explain only a low contribution of Fe-S cluster in flavin reduction. The kinetic experiments demonstrated that SH-catalysed reduction of flavins is indeed feasible. Recently, the hydrogenase Hyd1 from *Escherichia coli* was described to catalyse the H_2 -driven reduction of free flavins.²² However, Hyd1 shows 180 times lower specific activity for FMN in comparison to SH and contains only additional Fe-S clusters and no flavins besides the NiFe active center. This supports the hypothesis that FMN-b of SH plays an important role for improved H_2 -driven FMN reduction.

Encouraged by the high H_2 -driven reduction activity of the SH, we explored the H_2 -driven regeneration of reduced flavins to drive biocatalytic redox reactions (Fig. 1). As a first model reaction, we chose the stereoselective reduction of activated

$\text{C}=\text{C}$ -double bonds catalyzed by the ene reductase Old Yellow Enzyme from *Thermus scotoductus* (TsOYE).²³ In accordance with the substrate spectrum of TsOYE, a range of cyclic enones was reduced (Table 1).²⁴ Control reactions in the absence of either SH or H_2 under otherwise identical conditions gave no detectable conversion. In particular, ketoisophorone was fully converted (99% conversion, Table 1, entry 1), albeit yielding a product with a significantly reduced optical purity (37%). Similar observations were made with this product and are generally associated with racemization under the reaction conditions (Table S3, ESI†). Interestingly, the control reaction without FMN

Table 1 H_2 -driven asymmetric reduction of alkenes

Reaction scheme showing the catalytic cycle:

$\text{H}_2 \rightarrow 2\text{H}^+$ (via SH)

$\text{FMN} \rightarrow \text{FMNH}_2$ (via SH)

$\text{FMNH}_2 \rightarrow \text{FMN}$ (via TsOYE)

Chemical structures of the substrate and product are shown, with substituents $\text{R}^1, \text{R}^2, \text{R}^3, \text{R}^4$ and stereochemistry indicated.

R^1	R^2	R^3	R^4	Product/mM	ee/%
Me	=O	H	$(\text{CH}_3)_2$	21.8 ± 0.4	37 (<i>R</i>)
H	H	H	H	9.7 ± 0.2	n.a.
Me	H	(<i>S</i>) isopropenyl	H	1.9 ± 0.2	> 99, 98.1 de (<i>2R,5S</i>)
Me	H	(<i>R</i>) isopropenyl	H	1.1 ± 0.1	> 99, 93.2 de (<i>2R,5R</i>)
Me	H	H	H	17.8 ± 0.5	> 94 (<i>R</i>)

Total turnover numbers^a

TsOYE	SH	FMN
2625	8400	105

Reaction conditions: 2 mL of KPi buffer pH 7.5 containing: 200 μM FMN, 22 mM substrate. The buffer was purged with H_2 prior to adding the substrate, SH (2.5 μM) and TsOYE (8 μM). The reaction was performed at 30 °C for 4–16 hours. Maximum products yields are indicated. Conversions and ee values were determined by GC-FID analysis. n.a. = not applicable. SH = NAD^+ -reducing hydrogenase, TsOYE = ene reductase. ^a Total turnover numbers of mol 2-methylcyclohexanone per mol enzyme or cofactor were determined at end of experiment.



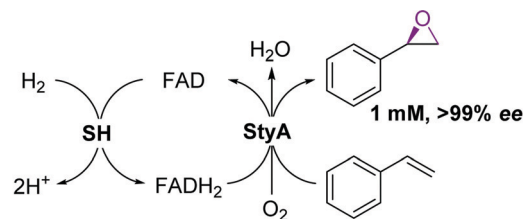
gave a low but detectable conversion, which may be indicative of a slow, direct electron transfer from SH to the flavin prosthetic group in TsOYE.²⁵ Pleasingly, already under non-optimized conditions, significant catalytic performances for both SH and FMN with TTN up to 8400 and 105, respectively, were observed (Table 1).

In order to demonstrate the O₂ tolerance of the SH for flavin regeneration and the extension to the FAD cofactor, we used our system to drive the epoxidation of styrenes catalyzed by styrene monooxygenase from *Pseudomonas* sp. VLB120.²⁶ Styrene monooxygenase is a two-component, flavin monooxygenase composed of StyA, the FADH₂-dependent monooxygenase subunit and StyB (a flavin reductase catalyzing the *in situ* generation of FADH₂ from FAD and NADH).²⁷ The FADH₂ regeneration system proposed here circumvents the nicotinamide cofactor together with the reductase subunit (StyB). Up to 1 mM of enantiopure (S)-styrene oxide was obtained upon incubation of StyA with SH and FAD under O₂/H₂ atmosphere for the reduction of styrene with the co-substrate O₂ (Scheme 1). The TTN of SH for FAD reduction was up to 333.

One major side reaction of the H₂-driven styrene epoxidation reaction was the oxidative uncoupling of the FADH₂ regeneration from the StyA-catalyzed epoxidation reaction. Due to the high reactivity of reduced flavin species with O₂, the StyA-related formation of 4a-hydroperoxoflavin yielding the desired epoxidation product competes with the spontaneous aerobic reoxidation of FADH₂ yielding H₂O₂.²⁸ Although this 'Oxygen Dilemma' severely impedes the StyA-catalyzed epoxidation reaction,²⁹ it is a promising approach to promote selective oxyfunctionalization reactions catalyzed by peroxygenases (UPOs). While UPOs rely on H₂O₂ as a stoichiometric oxidant, they are also rapidly inactivated in the presence of excess amounts of H₂O₂, thereby necessitating precise control over the *in situ* H₂O₂ concentration.³⁰ We therefore coupled the proposed SH-based flavin reduction system to the recombinant peroxygenase from *Agroclybe aegerita* (rAaeUPO) for selective hydroxylation reactions (Scheme 2 and Fig. S2, ESI†).^{31,32}

To minimize evaporative loss of the volatile reagents under the non-optimized reaction conditions for the proof-of-concept, we utilized the two liquid phase approach (*i.e.*, applying the substrate as a neat organic layer to the aqueous reaction mixture). Under these conditions, 1.5 mM of enantiopure (R)-1-phenylethanol was obtained from ethylbenzene. The overall robustness of the reaction process needed to be improved, as the reaction typically ceased after 1 h. We attributed this short reaction time to the poor stability of SH in the presence of hydrophobic solvents,³³ which could be alleviated by immobilization of the biocatalyst as demonstrated previously.³⁴ Hence, we were pleased to observe that using 95 nM of the Amberlite FPA54 immobilized SH under identical conditions resulted in an accumulation of 4.4 mM of the enantiomerically pure product. Under these conditions (substituting ethylbenzene with cyclohexane), up to 3.7 mM of cyclohexanol product were obtained (Scheme 2 and Table S4, ESI†). Notably, the TTN for SH increased from 750 to approximately 46 000.

In air, H₂ has a flammability range of 4–74% and can be explosive at concentrations of 19–57%.³⁵ An undesired combustion



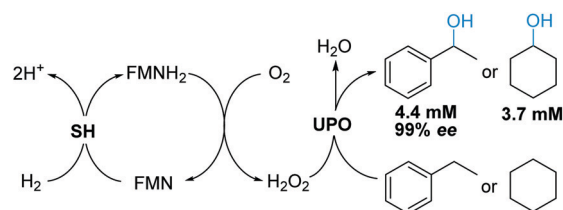
Total turnover numbers^a

StyA	SH	FAD
200	333	3.3

Scheme 1 H₂-driven asymmetric epoxidation of styrene. Reaction conditions: 1 mL of KPi buffer pH 7.5 containing 300 μM FAD, 5 mM substrate. The gas mixture was 10% O₂ 30% N₂ and 60% H₂. SH (3 μM) and styrene monooxygenase (5 μM) was added to start the reaction. The reaction was performed at 30 °C for 16 hours. Conversions and ee values were determined by GC-FID analysis. SH = NAD⁺-reducing hydrogenase, StyA = styrene monooxygenase. ^aTotal turnover numbers of mol styrene epoxide per mol enzyme or cofactor.

during H₂ driven catalysis can be prevented with mixtures that are very dilute in either H₂ or O₂. As the SH has a K_M for H₂ in the lower μM range,³⁶ this usually does not present a problem. Recently we developed a scalable platform that employs electrolysis to power biotransformations.³⁷ For H₂ driven flavin reactions, a similar setup could be used in future studies. The supply of the right amount of H₂ by electrolysis, which is continuously oxidized by the SH, would ensure a safe H₂ handling by avoiding the formation of explosive gas mixtures.³⁷

In conclusion, we have demonstrated as a proof of concept the H₂-driven reduction of flavins to promote selective biocatalytic reduction and oxidation reactions. The presented flavin recycling by H₂ oxidation enabled the efficient reduction of activated alkenes, selective hydroxylation of alkanes and asymmetric epoxidation of styrene. The cofactor regeneration by a



Total turnover numbers^a

UPO	SH	FMN
11250	750	225
	46000 (immobilized)	

Scheme 2 H₂-driven hydroxylation of ethylbenzene and cyclohexane. Reaction conditions: 750 μL of KPi buffer pH 7.5 containing 200 μM FMN was added to 750 μL of pure substrate in a two-phase system. The reaction was performed in pressure secured closed vials with 7 mL excess of headspace filled with gas composed of 40% O₂ and 60% H₂. The reaction was performed at 30 °C for 4 hours. Sampling was taken from the organic phase. 95 nM of immobilized SH on 210 mg Amberlite™ FPA54 and 4 μM UPO were added to start the reaction. Conversions and ee values were determined by GC-FID analysis. SH = NAD⁺-reducing hydrogenase, UPO = unspecific peroxygenase. ^aTotal turnover numbers of mol ethylbenzene per mol enzyme or cofactor.



hydrogenase resulted in 100% atom efficiency without by-products, with a high turnover rate of up to 20.3 s^{-1} and a total turnover number of up to 46 000. The electron delivery strategy circumvents NAD(P)H as the reductant and shortens previous multi-protein systems with reductases to a two-enzyme system. The O_2 tolerance of the hydrogenase allowed aerobic reactions with a monooxygenase, whereas the spontaneous aerobic re-oxidation of FMNH₂ to H₂O₂ fueled peroxxygenase-catalyzed hydroxylation reactions. The general applicability of the NAD(P)H-free, H₂-driven flavin regeneration system was demonstrated using different oxidoreductases (*TsOYE*, *StyA* and *UPO*) and substrates (e.g., 2-methylcyclohexenone, styrene, ethylbenzene). The ability to use H₂ as a fuel for flavin recycling opens up opportunities for highly atom-efficient biocatalysis, providing a versatile platform for future biotransformations with flavin- and H₂O₂-dependent enzymes.

A. A.-S. and L. L. received funding from the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG, project number 284111627) and the Einstein foundation. Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC 2008 – 390540038 – UniSysCat and by the Fonds der Chemischen Industrie. We thank Oliver Lenz for generous support by using his lab equipment, Changzhu Wu (University of Southern Denmark) for his support in providing Amberlite FPA54, and Ewald Jongkind (TU Delft) for his GC sampling assistance. We thank the Netherlands Organization for Scientific Research for financial support through a VICI grant (no. 724.014.003).

Conflicts of interest

There are no conflicts to declare.

Notes and references

- G. de Gonzalo and M. W. Fraaije, *ChemCatChem*, 2013, **5**, 403–415.
- E. Romero, J. R. Gómez Castellanos, G. Gadda, M. W. Fraaije and A. Mattevi, *Chem. Rev.*, 2018, **118**, 1742–1769.
- W. Zhang and F. Hollmann, *Chem. Commun.*, 2018, **54**, 7281–7289.
- L. Schmermund, V. Jurkaš, F. F. Özgen, G. D. Barone, H. C. Büchenschütz, C. K. Winkler, S. Schmidt, R. Kourist and W. Kroutil, *ACS Catal.*, 2019, **9**, 4115–4144.
- F. Hollmann, K. Hofstetter, T. Habicher, B. Hauer and A. Schmid, *J. Am. Chem. Soc.*, 2005, **127**, 6540–6541.
- R. Ruinatscha, C. Dusny, K. Buehler and A. Schmid, *Adv. Synth. Catal.*, 2009, **351**, 2505–2515.
- L. Lauterbach, O. Lenz and A. Vincent Kylie, *FEBS J.*, 2013, **280**, 3058–3068.
- L. Lauterbach, Z. Idris, K. A. Vincent and O. Lenz, *PLoS One*, 2011, **6**, e25939.
- L. Lauterbach, J. Liu, M. Horch, P. Hummel, A. Schwarze, M. Haumann, A. Vincent Kylie, O. Lenz and I. Zebger, *Eur. J. Inorg. Chem.*, 2011, 1067–1079.
- M. Horch, L. Lauterbach, O. Lenz, P. Hildebrandt and I. Zebger, *FEBS Lett.*, 2012, **586**, 545–556.
- K. Schneider and H. G. Schlegel, *Biochem. Biophys. Res. Commun.*, 1978, **84**, 564–571.
- E. van der Linden, B. W. Faber, B. Bleijlevens, T. Burgdorf, M. Bernhard, B. Friedrich and S. P. Albracht, *Eur. J. Biochem.*, 2004, **271**, 801–808.
- K. Schneider and H. G. Schlegel, *Biochim. Biophys. Acta*, 1976, **452**, 66.
- T. Burgdorf, O. Lenz, T. Buhrke, E. van der Linden, A. K. Jones, S. P. J. Albracht and B. Friedrich, *J. Mol. Microbiol. Biotechnol.*, 2005, **10**, 181–196.
- L. Lauterbach and O. Lenz, *J. Am. Chem. Soc.*, 2013, **135**, 17897–17905.
- A. Al-Shameri, N. Borlinghaus, L. Weinmann, P. Scheller, B. M. Nestl and L. Lauterbach, *Green Chem.*, 2019, **21**, 1396–1400.
- T. H. Lonsdale, L. Lauterbach, S. Honda Malca, B. M. Nestl, B. Hauer and O. Lenz, *Chem. Commun.*, 2015, **51**, 16173–16175.
- A. K. Holzer, K. Hiebler, F. G. Mutti, R. C. Simon, L. Lauterbach, O. Lenz and W. Kroutil, *Org. Lett.*, 2015, **17**, 2431–2433.
- J. Ratzka, L. Lauterbach, O. Lenz and M. B. Ansorge-Schumacher, *Biocatal. Biotransform.*, 2011, **29**, 246–252.
- L. Lauterbach and O. Lenz, *Curr. Opin. Chem. Biol.*, 2019, **49**, 91–96.
- Y. Shomura, M. Taketa, H. Nakashima, H. Tai, H. Nakagawa, Y. Ikeda, M. Ishii, Y. Igarashi, H. Nishihara, K.-S. Yoon, S. Ogo, S. Hirota and Y. Higuchi, *Science*, 2017, **357**, 928–932.
- S. J. Srinivasan, S. E. Cleary, C. E. Paul, M. A. Ramirez and K. A. Vincent, *ChemRxiv*, 2020, DOI: 10.26434/chemrxiv.12213398.
- D. J. Opperman, L. A. Piater and E. van Heerden, *J. Bacteriol.*, 2008, **190**, 3076–3082.
- J. Bernard, E. van Heerden, I. W. C. E. Arends, D. J. Opperman and F. Hollmann, *ChemCatChem*, 2012, **4**, 196–199.
- D. J. Opperman, B. T. Sewell, D. Lithauer, M. N. Isupov, J. A. Littlechild and E. van Heerden, *Biochem. Biophys. Res. Commun.*, 2010, **393**, 426–431.
- S. Panke, M. G. Wubbolts, A. Schmid and B. Witholt, *Biotechnol. Bioeng.*, 2000, **69**, 91–100.
- K. Otto, K. Hofstetter, M. Röthlisberger, B. Witholt and A. Schmid, *J. Bacteriol.*, 2004, **186**, 5292–5302.
- V. Massey, *J. Biol. Chem.*, 1994, **269**, 22459–22462.
- D. Holtmann and F. Hollmann, *ChemBioChem*, 2016, **17**, 1391–1398.
- B. O. Burek, S. Bormann, F. Hollmann, J. Z. Bloh and D. Holtmann, *Green Chem.*, 2019, **21**, 3232–3249.
- R. Ullrich, J. Nüske, K. Scheibner, J. Spantzel and M. Hofrichter, *Appl. Environ. Microbiol.*, 2004, **70**, 4575–4581.
- P. Molina-Espeja, S. Ma, D. M. Mate, R. Ludwig and M. Alcalde, *Enzyme Microb. Technol.*, 2015, **73–74**, 29–33.
- J. Ratzka, L. Lauterbach, O. Lenz and M. B. Ansorge-Schumacher, *J. Mol. Catal. B: Enzym.*, 2012, **74**, 219–223.
- N. Herr, J. Ratzka, L. Lauterbach, O. Lenz and M. B. Ansorge-Schumacher, *J. Mol. Catal. B: Enzym.*, 2013, **97**, 169–174.
- R. G. Keefe, M. J. Axley and A. L. Harabin, *Arch. Biochem. Biophys.*, 1995, **317**, 449–456.
- Z. M. Shapiro and T. R. Moffette, *Hydrogen flammability data and application to PWR loss of coolant accident*, US Department of Energy, 1957, OSTI ID: 4327402.
- A. Al-Shameri, M.-C. Petrich, K. Junge Puring, U.-P. Apfel, B. M. Nestl and L. Lauterbach, *Angew. Chem., Int. Ed.*, 2020, **59**, 10929–10933.

