ACS Catalysis

Research Article

pubs.acs.org/acscatalysis

Nonenzymatic Regeneration of Styrene Monooxygenase for Catalysis

Caroline E. Paul,[†] Dirk Tischler,[‡] Anika Riedel,[‡] Thomas Heine,[‡] Nobuya Itoh,[§] and Frank Hollmann*[,†]

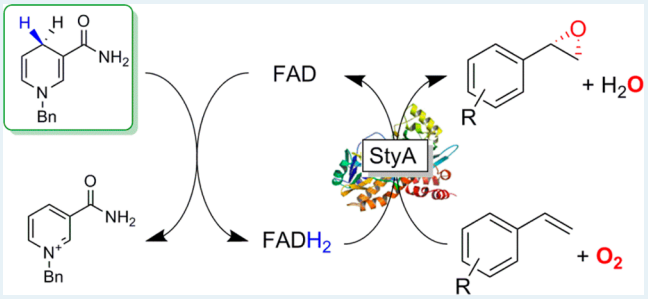
[†]Department of Biotechnology, Delft University of Technology, Julianalaan 136, 2628BL Delft, The Netherlands

[‡]Interdisciplinary Ecological Center, TU Bergakademie Freiberg Leipziger Straße 29, 09599 Freiberg, Germany

[§]Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan

Supporting Information

ABSTRACT: Styrene monooxygenase (SMO) is a two-component flavoprotein catalyzing the selective epoxidation of various C=C double bonds. For cell-free catalysis, traditionally a cascade of NAD(P)H:flavin oxidoreductase, nicotinamide cofactor (NADH), and NADH regeneration enzyme is required to supply StyA with the reduced flavin adenine dinucleotide cofactor ($FADH_2$) required for catalysis. Herein, we present a more direct and efficient $FADH_2$ regeneration system using the nicotinamide cofactor mimic 1-benzyl-1,4-dihydronicotinamide (BNAH) as the sole reductant. Thus, BNAH replaces two enzymes and the nicotinamide cofactor, resulting in a significantly simplified reaction system.



KEYWORDS: *asymmetric epoxidation, electron transfer, mNADHs, styrene monooxygenases, two-component flavin monooxygenases*

Styrene monooxygenases (SMOs) are highly interesting biocatalysts enabling the organic chemist to perform challenging enantiospecific epoxidation and sulfoxidation reactions (Scheme 1).[1−13] One of the major challenges for the practical application of these enzymes lies in their complicated molecular architecture. Essentially, SMOs utilize a reduced flavin cofactor ($FADH_2$) provided by a NAD(P)H:flavin oxidoreductase using reduced nicotinamide cofactors as reductant.[1−13] The latter are prohibitively expensive, thus requiring enzymatic in situ regeneration systems to allow for their use in catalytic amounts. As a result, rather complicated electron transport chains comprising two additional enzymes and the nicotinamide cofactor result (Scheme 1), which (unnecessarily) complicate the practical application of these enzymes.[10,13−15]

An additional major limitation for the preparative application of SMO lies in the so-called oxygen dilemma: on the one hand, molecular oxygen is necessary to complete the monooxygenase's catalytic cycle, but at the same time, O_2 also leads to uncoupling of the electron supply from the monooxygenation reaction. This oxygen dilemma is particularly severe because a diffusible reduced flavin is involved in the overall mechanism and is well-known to react very quickly with molecular oxygen, producing H_2O_2 (vide infra).[9,11,16]

Overall, to fully exploit the catalytic potential of SMO, a simpler, more robust electron transport chain needs to be established, and the sometimes very poor electron transfer yield (ETY, calculated as product formed per electron donor) from the sacrificial electron donor to the oxygenase must be improved significantly.

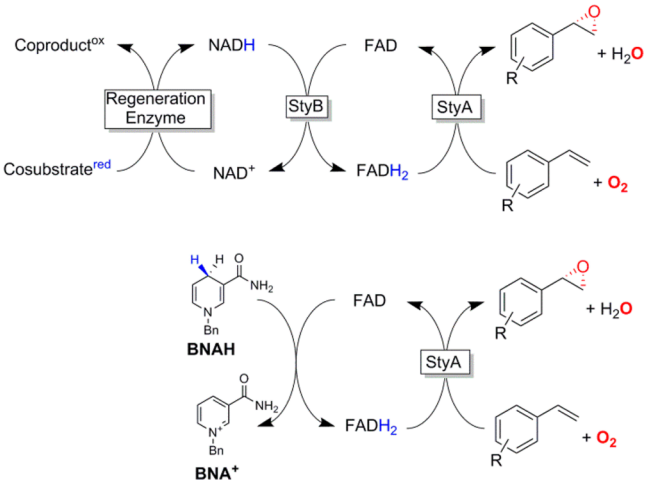
Previously, we have proposed that simpler reaction schemes (shorter electron transport chains) could significantly improve the electron transfer yield for SMOs by using e.g. $[Cp^*Rh(bpy)(H_2O)]^{2+}$ as a direct flavin reduction catalyst.[9,11,17] However, the mutual inactivation of the heavy-metal catalyst and enzymes[18] together with issues related to the cost of the catalyst and its toxicological properties, to our mind, exclude it from being a practical solution for the future. Nevertheless, recently we,[19−22] along with others before us,[23] have demonstrated that simple synthetic nicotinamides (mNADHs) are capable of reducing flavins at a high rate, thereby making them interesting stoichiometric reductants for flavoprotein-catalyzed C=C bond reductions and hydroxylations.

Encouraged by these promising preliminary results, we decided to evaluate the direct nonenzymatic regeneration of the reduced flavins using 1-benzyl-1,4-dihydronicotinamide (BNAH) as a stoichiometric reducing agent. Currently, in situ regeneration systems of BNAH are limited but are under investigation. We envisioned that the simpler reaction scheme (BNAH replacing the NAD(P)H:flavin oxidoreductase, the natural nicotinamide cofactor, and the putative NAD(P)H

Received: January 10, 2015
Revised: March 31, 2015
Published: April 2, 2015

DOI: 10.1021/acscatal.5b00041
ACS Catal. 2015, 5, 2961−2965

Scheme 1. Styrene Monooxygenase (SMO) Catalyzed Epoxidation of Styrenes: (Top) Molecular Architecture of the Electron Transport Chain Comprising a NAD(P)H:flavin Oxidoreductase (StyB) Catalyzing the Hydride Transfer from NAD(P)H to the Flavin;[a] (Bottom) Proposed Direct Regeneration of $FADH_2$ using Synthetic Nicotinamides[b]



[a]For in situ regeneration of the reduced nicotinamide cofactor typically an enzymatic regeneration system is used. [b]1-Benzyl-1,4-dihydronicotinamide (BNAH) as a stoichiometric reducing agent. In the proposed setup BNAH substitutes for two enzymes (StyB and the regeneration enzyme) and the nicotinamide cofactor (NADH).

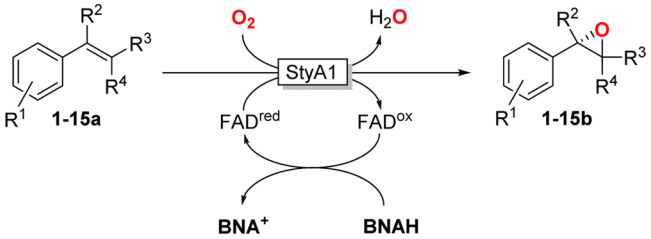
regeneration system) would lead to more robust and efficient reaction schemes (Scheme 1).

As a model enzyme we chose the styrene monooxygenase (SMO, EC 1.14.14.11) from *Rhodococcus opacus* 1CP (StyA1), which was recently discovered and described as a novel subtype among SMOs.[4,24−26] StyA1, together with its natural reductase StyA2B or together with a related reductase StyB, performs highly selective oxo functionalizations. However, it was also demonstrated that StyB does not deliver sufficient reducing equivalents for StyA1 to employ its maximum potential in biocatalysis. We suspected the oxygen dilemma to eventually account for this shortcoming.

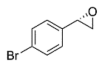
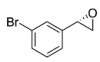
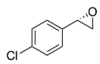
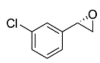
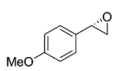
In a first set of experiments, we established for the first time that the BNAH could in fact replace the natural NADH, StyB reductase system and NADH regeneration system required to perform the biocatalytic epoxidation reaction. Control reactions in the absence of BNAH, FAD, or StyA1, respectively, yielded no detectable product formation. Encouraged by these results, we further explored the substrate scope of the novel, simplified reaction scheme. A selection of *α*-, *β*-, para-, and meta-substituted styrene derivatives (Table 1) as well as substituted aromatic sulfides (Table 2) were tested for conversion and enantioselectivity.

Admittedly, several reaction conversions for asymmetric epoxidation were low due to enzyme stability issues, seen over the time course in Figure 1 (additionally see Section 3, Figures S1−S5, in the Supporting Information), but with excellent ee values (up to >99%). Conversions could be improved through the addition of more StyA1. The best results were obtained with substrates **1a**, **3a**, **9a**, and **12a** in terms of conversion and ee. *cis*-*β*-Methylstyrene (**3a**), *α*-methylstyrene (**4a**), and meta-substituted styrene substrates (entries 6, 8, 10, and 12) generally afforded excellent ee values of >99%.

Table 1. Enantioselective Epoxidation of Styrene Derivatives with the StyA1/BNAH System[a]



1 R^1 = H, R^2 = H, R^3 = H, R^4 = H
2 R^1 = H, R^2 = CH_3, R^3 = H, R^4 = H
3 R^1 = H, R^2 = H, R^3 = CH_3, R^4 = H
4 R^1 = H, R^2 = H, R^3 = H, R^4 = CH_3
5 R^1 = *p*-Br, R^2 = H, R^3 = H, R^4 = H
6 R^1 = *m*-Br, R^2 = H, R^3 = H, R^4 = H
7 R^1 = *p*-Cl, R^2 = H, R^3 = H, R^4 = H
8 R^1 = *m*-Cl, R^2 = H, R^3 = H, R^4 = H
9 R^1 = *p*-F, R^2 = H, R^3 = H, R^4 = H
10 R^1 = *m*-F, R^2 = H, R^3 = H, R^4 = H
11 R^1 = *p*-CH_3, R^2 = H, R^3 = H, R^4 = H
12 R^1 = *m*-CH_3, R^2 = H, R^3 = H, R^4 = H
13 R^1 = *p*-OCH_3, R^2 = H, R^3 = H, R^4 = H
14 Allyl benzene
15 1,2-Dihydronaphthalene

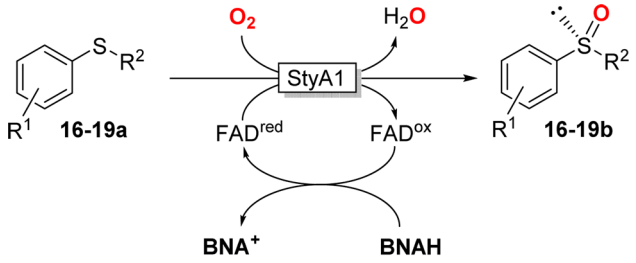
Entry	Product	TN (h^{-1})[b]	[Product yield] (mM)[b]	*ee* (%)[c]
1		433 175	1.30 0.02[d]	93 (*S*)
2		107	0.32	>99 (*S*)
3		390	1.17	94 (1*S*,2*S*)
4		83	0.25	99 (1*S*,2*R*)
5		130	0.39	66 (*S*)
6		123	0.37	>99 (*S*)
7		97	0.29	83 (*S*)
8		67	0.20	>99 (*S*)
9		333	1.00	>99 (*S*)
10		73	0.22	>99 (*S*)
11		107	0.32	n.d.
12		300	0.90	>99 (*S*)
13		163	0.49	98 (*S*)
14		133	0.40	>99 (*S*)
15		73	0.22	n.d.

DOI: 10.1021/acscatal.5b00041
ACS Catal. 2015, 5, 2961−2965

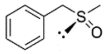
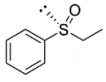
Table 1. continued

[a]Reaction conditions: Tris-HCl buffer (50 mM, pH 7.0), BNAH (10 mM), catalase (651 U), FAD (50 μM), styrene (4 mM), StyA1 (3.0 μM), final volume 1 mL, shaken at 900 rpm, 30 °C, 60 min. [b]The turnover number (TN) was calculated as [product yield]/[StyA1]. The product yield was measured by GC with calibration curves and dodecane (2 mM) as an internal standard; n.d. = not determined. [c]The enantiomeric excess was measured by chiral GC. [d]Reaction performed with StyA1 (0.114 μM) and StyB as previously described over 2 h,[25] without BNAH.

Table 2. Enantioselective Biocatalytic Sulfoxidation Reaction of Thioanisole Derivatives with the StyA1/BNAH System[a]



16 R^1 = H, R^2 = CH_3
17 R^1 = *o*-Cl, R^2 = CH_3
18 Benzyl methyl sulfide
19 R^1 = H, R^2 = CH_2CH_3

Entry	Product	TN (h^{-1})[b]	[Product yield] (mM)[b]	*ee* (absolute configuration) (%)[c]
16		887	2.66	>99 (*S*)
17		957	2.87	>99 (*S*)
18		583	1.75	99 (*S*)
19		870	2.61	64 (*S*)

[a]Reaction conditions: Tris-HCl buffer (50 mM, pH 7.0), BNAH (10 mM), catalase (651 U), FAD (50 μM), styrene (4 mM), StyA1 (3.0 μM), final volume 1 mL, shaken at 900 rpm, 30 °C, 60 min. [b]The turnover number (TN) was calculated as [product]/[StyA1]. The product yield was measured by GC with calibration curves and dodecane (2 mM) as an internal standard. [c]The enantiomeric excess was measured by chiral GC.

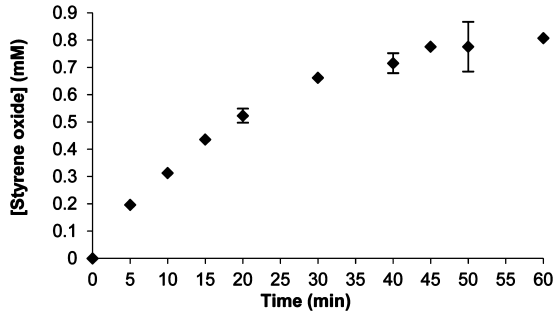
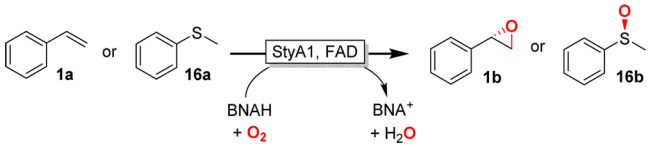


Figure 1. Time course of the StyA1-catalyzed epoxidation of styrene (**1a**). Standard reaction conditions: Tris-HCl buffer (50 mM, pH 7.0), BNAH (10 mM), catalase (651 U), FAD (50 μM), styrene (4 mM), StyA1 (2.2 μM), final volume 1 mL, shaken at 900 rpm, 30 °C, 90 min.

Table 3, electron transfer yields (ETYs) of up to 80% could be achieved, which is in stark contrast with respect to the ETYs

Table 3. Electron Transfer Yields (ETYs) for Different Substrates and Enzyme Concentrations[a]



[StyA1] (μM)	[BNAH] (mM)	[(*S*)-styrene epoxide] (mM)[b]	ETY (%)[c]
2.2	0.5	0.29	58
	0.75	0.38	51
	1	0.46	46
4.4	0.5	0.33	66
	0.75	0.43	58
	1	0.54	54
[StyA1] (μM)	[BNAH] (mM)	[(*S*)-methylphenyl sulfoxide] (mM)[b]	ETY (%)[c]
2.2	0.5	0.30	60
	0.75	0.42	56
	1	0.54	54
4.4	0.5	0.39	77
	0.75	0.64	85
	1	0.72	72

[a]General conditions: Tris-HCl buffer (50 mM, pH 7.0), BNAH (0.5, 0.75, or 1 mM), catalase (651 U), FAD (50 μM), styrene (4 mM), StyA1 (2.2 μM or 4.4 μM), final volume 1 mL, shaken at 900 rpm, 30 °C, 60 min. [b]The product yield was measured by GC with calibration curves and dodecane (2 mM) as an internal standard. [c]The electron transfer yield (ETY) was measured as ([product]/[BNAH]) × 100.

A series of sulfide substrates was also screened with the StyA1/BNAH system (Table 2). The oxidation of methyl phenyl sulfides by SMOs generally gives poor to good enantioselectivity, achieving between 45 and 92% ee. Surprisingly, we obtained excellent enantiomeric excess (>99% ee, compounds **16**–**18**) with high conversions, where no overoxidation was observed.

Interestingly, the performance (in terms of turnover number (TN) attainable for StyA1) of the simplified reaction scheme surpassed the performance of the "natural" system significantly (Table 1). We suspected the more efficient electron transfer to account for this observation and therefore performed a set of experiments under BNAH-limiting conditions. As shown in obtained in the traditional regeneration scheme (generally less than 5%).

Two observations from this set of experiments are particularly interesting to note: first, the ETY tended to be higher in the presence of elevated enzyme concentrations, and second, the ETY with thioanisole (being the "better substrate") was generally higher than that with styrene (being the "worse substrate"). Both observations support the assumption that the ETY is mostly determined by the efficiency with which StyA1 can utilize $FADH_2$ in competition with the spontaneous aerobic reoxidation of $FADH_2$. Thus, styrene monooxygenases were previously reported to stabilize the reactive flavin species in the presence of substrate,[24] and the data obtained here allow us to

DOI: 10.1021/acscatal.5b00041
ACS Catal. 2015, 5, 2961−2965

conclude that methyl phenyl sulfide does it even better than the natural substrate styrene.

Next, we investigated the influence of the FAD concentration on the overall reaction (Figure 2). Doubling the concentration

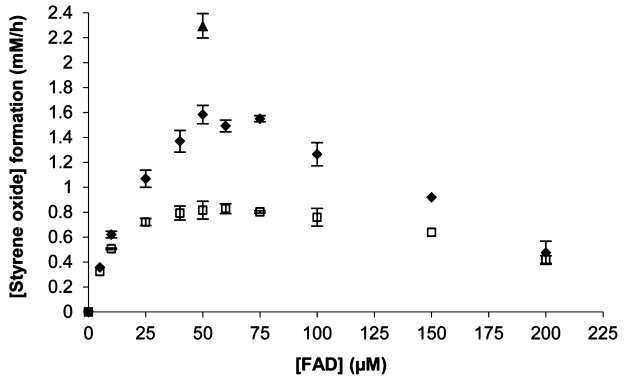


Figure 2. Initial styrene oxide formation at different FAD concentrations at StyA1 concentrations of 4.4 μM (▲), 2.2 μM (◆), and 1.1 μM (□). Error bars represent standard deviations. Reaction conditions: Tris-HCl buffer (50 mM, pH 7.0), BNAH (10 mM), catalase (651 U), FAD, styrene (4 mM), StyA1, final volume 1 mL, shaken at 900 rpm, 30 °C, 20 min.

of the enzyme from 1.1 to 2.2 μM at optimal FAD concentration (50 μM) doubled the formation of styrene oxide. Maximum styrene oxide formation rates were observed between 50 and 75 μM of FAD. Doubling again the enzyme concentration to 4.4 μM led to 2.3 mM of styrene oxide, a lower amount than expected, perhaps due to oxygen limitation. Under these conditions, an ETY of 90% was achieved (see the Supporting Information).

Interestingly, an optimal FAD concentration was observed. The increasing overall reaction rate up to approximately 50 μM FAD may easily be explained by the bimolecular character of the $FADH_2$ reduction reaction (BNAH + FAD + H^+ → BNA^+ + $FADH_2$) and the resulting rate dependence on both concentrations.[19] However, the decrease of the product formation rate at higher FAD concentrations is somewhat more difficult to explain. Nevertheless, we believe that the preferred aerobic reoxidation mechanism of reduced flavins proceeding via a synproportionation step sufficiently explains this observation.[27] The first step of the aerobic reoxidation is the fast synproportionation of FAD and $FADH_2$ to the intermediate semiquinone (eq 1). The following (equally fast) next steps are single electron transfer from the semiquinone to O_2 yielding fully reoxidized FAD and superoxide (eq 2), which undergoes disproportionation into H_2O_2 and O_2 (eq 3).

$$FADH_2 + FAD \underset{5 \times 10^8\ M^{-1}\ s^{-1}}{\overset{10^6\ M^{-1}\ s^{-1}}{\rightleftharpoons}} 2FAD^{\bullet} \quad (1)$$

$$FAD^{\bullet} + O_2 \xrightarrow{10^8\ M^{-1}\ s^{-1}} FAD + O_2^{\bullet -} \quad (2)$$

$$2O_2^{\bullet -} \xrightarrow{8 \times 10^7\ M^{-1}\ s^{-1}} H_2O_2 + O_2 \quad (3)$$

Apparently, the rate of the first step (semiquinone formation, eq 1) also depends on the FAD concentration. Furthermore, the equilibrium is shifted to the semiquinone side in the presence of higher FAD concentrations. Overall, this sufficiently explains the observed reduction in epoxidation rate (and concomitantly in the ETY; Figure 1).

Finally, a 100 mg scale reaction was performed with thioanisole as substrate. Using StyA1, BNAH, and FAD, a 53% yield was obtained with >99% ee of the (*S*)-methyl phenyl sulfoxide. This demonstrates that preparative application of the proposed simplified reaction scheme is principally feasible. At the present stage the poor thermal stability of the SMO used represents the major limitation en route to preparative scale.

In conclusion, we have developed a simpler, more robust, and efficient electron transport system for preparative-scale biocatalytic asymmetric (ep)oxidation to afford enantiopure epoxides and sulfoxides, using BNAH as a cheap and efficient reductant. As a comparison, BNAH allows an ETY of 66% in comparison to 2−5% with the same SMO and its natural reductase.[25]

We believe our system is not limited to styrene monooxygenases but can provide a more efficient catalytic process for two-component flavin monooxygenases (2CFMOs) in general, making them more attractive for practical use.[17,25,26,28−36] Indeed, preliminary experiments with the 2CFMO tryptophan 7-halogenase (PrnA) with BNAH gave promising results; further experiments are ongoing. We envision application of the proposed simplified regeneration system to further 2CFMOs, which is currently being developed in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

The following file is available free of charge on the ACS Publications website at DOI: 10.1021/acscatal.5b00041.

Details of the synthesis of the racemic products, reaction conditions, enzyme stability, ETY calculations, and GC methods and chromatograms (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail for F.H.: f.hollmann@tudelft.nl.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work is supported by the Marie Curie IEF "Biomimic" financed by the European Union through the seventh Framework People Programme (grant agreement number 327647).

■ REFERENCES

(1) Wu, S.; Chen, Y.; Xu, Y.; Li, A.; Xu, Q.; Glieder, A.; Li, Z. *ACS Catal.* **2013**, *4*, 409−420.
(2) Toda, H.; Imae, R.; Komio, T.; Itoh, N. *Appl. Microbiol. Biotechnol.* **2012**, *96*, 407−418.
(3) Toda, H.; Imae, R.; Itoh, N. *Tetrahedron: Asymmetry* **2012**, *23*, 1542−1549.
(4) Tischler, D.; Groning, J. A. D.; Kaschabek, S. R.; Schlomann, M. *Appl. Biochem. Biotechnol.* **2012**, *167*, 931−944.
(5) Julsing, M. K.; Kuhn, D.; Schmid, A.; Bühler, B. *Biotechnol. Bioeng.* **2012**, *109*, 1109−1119.
(6) Ruinatscha, R.; Karande, R.; Buehler, K.; Schmid, A. *Molecules* **2011**, *16*, 5975−5988.
(7) Lin, H.; Liu, Y.; Wu, Z.-L. *Tetrahedron: Asymmetry* **2011**, *22*, 134−137.

DOI: 10.1021/acscatal.5b00041
ACS Catal. 2015, 5, 2961−2965

(8) Ruinatscha, R.; Dusny, C.; Buehler, K.; Schmid, A. *Adv. Synth. Catal.* **2009**, *351*, 2505−2515.
(9) Hollmann, F.; Hofstetter, K.; Habicher, T.; Hauer, B.; Schmid, A. *J. Am. Chem. Soc.* **2005**, *127*, 6540−6541.
(10) Otto, K.; Hofstetter, K.; Rothlisberger, M.; Witholt, B.; Schmid, A. *J. Bacteriol.* **2004**, *186*, 5292−5302.
(11) Hollmann, F.; Lin, P. C.; Witholt, B.; Schmid, A. *J. Am. Chem. Soc.* **2003**, *125*, 8209−8217.
(12) Panke, S.; Held, M.; Wubbolts, M. G.; Witholt, B.; Schmid, A. *Biotechnol. Bioeng.* **2002**, *80*, 33−41.
(13) Schmid, A.; Hofstetter, K.; Feiten, H.-J.; Hollmann, F.; Witholt, B. *Adv. Synth. Catal.* **2001**, *343*, 732−737.
(14) Hollmann, F.; Hofstetter, K.; Schmid, A. *Trends Biotechnol.* **2006**, *24*, 163−171.
(15) Hofstetter, K.; Lutz, J.; Lang, I.; Witholt, B.; Schmid, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 2163−2166.
(16) Holtmann, D.; Fraaije, M. W.; Opperman, D. J.; Arends, I. W. C. E.; Hollmann, F. *Chem. Commun.* **2014**, *50*, 13180−13200.
(17) Unversucht, S.; Hollmann, F.; Schmid, A.; van Pée, K.-H. *Adv. Synth. Catal.* **2005**, *347*, 1163−1167.
(18) Poizat, M.; Arends, I. W. C. E.; Hollmann, F. *J. Mol. Catal. B: Enzym.* **2010**, *63*, 149−156.
(19) Paul, C. E.; Churakova, E.; Maurits, E.; Girhard, M.; Urlacher, V. B.; Hollmann, F. *Bioorg. Med. Chem.* **2014**, *22*, 5692−5696.
(20) Paul, C. E.; Arends, I. W. C. E.; Hollmann, F. *ACS Catal.* **2014**, *4*, 788−797.
(21) Paul, C. E.; Gargiulo, S.; Opperman, D. J.; Lavandera, I.; Gotor-Fernandez, V.; Gotor, V.; Taglieber, A.; Arends, I. W. C. E.; Hollmann, F. *Org. Lett.* **2013**, *15*, 180−183.
(22) Lutz, J.; Hollmann, F.; Ho, T. V.; Schnyder, A.; Fish, R. H.; Schmid, A. *J. Organomet. Chem.* **2004**, *689*, 4783−4790.
(23) Ryan, J. D.; Fish, R. H.; Clark, D. S. *ChemBioChem* **2008**, *9*, 2579−2582.
(24) Montersino, S.; Tischler, D.; Gassner, G. T.; van Berkel, W. J. H. *Adv. Synth. Catal.* **2011**, *353*, 2301−2319.
(25) Tischler, D.; Kermer, R.; Groning, J. A. D.; Kaschabek, S. R.; van Berkel, W. J. H.; Schlomann, M. *J. Bacteriol.* **2010**, *192*, 5220−5227.
(26) Tischler, D.; Eulberg, D.; Lakner, S.; Kaschabek, S. R.; van Berkel, W. J. H.; Schlomann, M. *J. Bacteriol.* **2009**, *191*, 4996−5009.
(27) Massey, V. *J. Biol. Chem.* **1994**, *269*, 22459−22462.
(28) Duffner, F. M.; Müller, R. *FEMS Microbiol. Lett.* **1998**, *161*, 37−45.
(29) Keller, S.; Wage, T.; Hohaus, K.; Hölzer, M.; Eichhorn, E.; van Pée, K.-H. *Angew. Chem., Int. Ed.* **2000**, *39*, 2300−2302.
(30) Kirchner, U.; Westphal, A. H.; Muller, R.; van Berkel, W. J. H. *J. Biol. Chem.* **2003**, *278*, 47545−47553.
(31) Dong, C. J.; Flecks, S.; Unversucht, S.; Haupt, C.; van Pee, K. H.; Naismith, J. H. *Science* **2005**, *309*, 2216−2219.
(32) van Berkel, W. J. H.; Kamerbeek, N. M.; Fraaije, M. W. *J. Biotechnol.* **2006**, *124*, 670−689.
(33) Joosten, V.; van Berkel, W. J. H. *Curr. Opin. Chem. Biol.* **2007**, *11*, 195−202.
(34) Flecks, S.; Patallo, E. R.; Zhu, X. F.; Ernyei, A. J.; Seifert, G.; Schneider, A.; Dong, C. J.; Naismith, J. H.; van Pee, K. H. *Angew. Chem., Int. Ed.* **2008**, *47*, 9533−9336.
(35) Chen, X. P.; van Pee, K. H. *Acta Biochim. Biophys. Sin.* **2008**, *40*, 183−193.
(36) van Pee, K.-H. *Curr. Org. Chem.* **2012**, *16*, 2583−2597.

DOI: 10.1021/acscatal.5b00041
ACS Catal. 2015, 5, 2961−2965