Synergistic Catalysis of Tandem Michael Addition/Enantioselective Protonation Reactions by an Artificial Enzyme

Zhou, Zhi; Roelfes, Gerard

Published in:
ACS Catalysis

DOI:
10.1021/acscatal.1c02298

ACS Catalysis

pubs.acs.org/acscatalysis

Letter

Synergistic Catalysis of Tandem Michael Addition/Enantioselective Protonation Reactions by an Artificial Enzyme

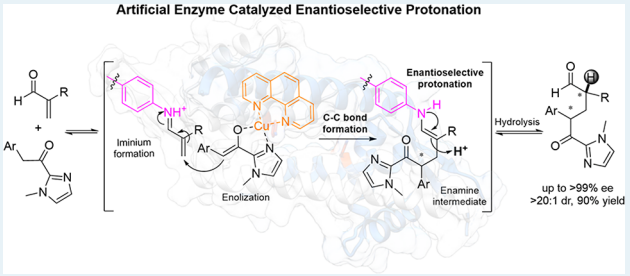
Zhi Zhou* and Gerard Roelfes*

Cite This: *ACS Catal.* 2021, 11, 9366–9369

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Enantioselective protonation is conceptually one of the most attractive methods to generate an α-chiral center. However, enantioselective protonation presents major challenges, especially in water. Herein, we report a tandem Michael addition/enantioselective protonation reaction catalyzed by an artificial enzyme employing two abiological catalytic sites in a synergistic fashion: a genetically encoded noncanonical *p*-aminophenylalanine residue and a Lewis acid Cu(II) complex. The exquisite stereocontrol achieved in the protonation of the transient enamine intermediate is illustrated by up to >20:1 dr and >99% ee of the product. These results illustrate the potential of exploiting synergistic catalysis in artificial enzymes for challenging reactions.



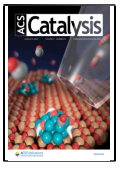
KEYWORDS: *artificial enzymes, enantioselective protonation, synergistic catalysis, noncanonical amino acids, Lewis acid catalysis, stop-codon suppression*

Tertiary carbon stereocenters are ubiquitous in biologically active natural products and pharmaceuticals. Enantioselective protonation is conceptually one of the most efficient methods for generating a tertiary carbon stereocenter.[1−4] However, enantioselective transfer of a proton presents tremendous challenges, especially in aqueous solvents: protons are difficult to control due to their small size, protons in water are highly mobile, proton transfer is generally very fast, and the products can be prone to racemization.[5−7] Nature has evolved several efficient enzymes such as decarboxylases and esterases that catalyze enantioselective protonation reactions.[8−11] Approaches for nonenzymatic enantioselective protonation mainly rely on the stereoselective protonation of prochiral enolates. These are either preprepared or formed *in situ* in transition-metal-catalyzed or organocatalytic conjugate addition reactions, which are mostly performed in organic solvents.[12−17] Complementary to enolate chemistry, enantioselective protonation of enamine intermediates is an attractive alternative.[18−21] Here, we report tandem Michael addition/enantioselective protonation reactions in water catalyzed by an artificial enzyme with excellent enantioselectivity in proton transfer to an enamine intermediate. Key to the activity and stereoselectivity of the artificial enzyme is the simultaneous activation of both the Michael donor and acceptor by two abiological catalytic groups.

Artificial enzymes are proteins that contain abiological catalytic groups. They have emerged as a promising approach toward the biocatalysis of reactions that have no equivalent in nature.[22−26] The well-defined secondary coordination sphere around the catalytic sites and substrates provided by the protein offers fascinating opportunities to optimize both the catalytic activity and selectivity of the artificial enzymes. Some examples of tandem Friedel−Crafts alkylations/enantioselective protonation reactions catalyzed by artificial metalloenzymes have been reported, albeit with moderate ee values.[27−29]

Recently, we have introduced a novel artificial enzyme design that involved combining two abiological catalytic sites capable of synergistic catalysis of Michael addition reactions in a single protein.[30] Synergistic catalysis is a powerful concept that involves simultaneous activation of two reacting substrates by separate catalytic groups.[31−33] The synergistic artificial enzyme was based on the Lactococcal multidrug resistance Regulator (LmrR).[34−36] It contained a noncanonical *p*-aminophenylalanine (pAF) residue, introduced using expanded genetic code methods, that can function as a nucleophilic catalyst[37] and a Lewis acidic Cu(ll) complex of the ligand 1,10-phenanthroline (Cu(II)-phen), bound between the indole rings of two tryptophan residues in the hydrophobic pore of LmrR.[29,38] The simultaneous action of these two catalytic sites, which are in close proximity and in a well-defined orientation with respect to each other, resulted in a high catalytic activity and excellent enantioselectivity in Michael addition reactions. Having established the power of the synergistic catalysis concept in artificial enzymes, we sought to apply this concept to the

Received: May 21, 2021
Revised: June 28, 2021
Published: July 13, 2021



catalysis of the challenging tandem Michael addition/enantioselective protonation reaction in water.

The artificial enzyme was assembled as described previously.[30] In this study, we used the variant LmrR_V15pAF, which contains a pAF residue at position 15. pAF was introduced indirectly: first *p*-azidophenylalanine was introduced via amber stop-codon suppression using the pEVOL-pAzF plasmid, followed by a Staudinger reduction of the azide to the amine with TCEP during purification.[37,39] We have found before that this indirect method gives rise to higher yields of LmrR_V15pAF in comparison to using direct pAF incorporation.[37] The Lewis acidic Cu(II)-phen complex was then introduced via self-assembly with LmrR_V15pAF in a buffered solution.[38] In this design, iminium ion formation with the aniline side chain of pAF results in activation of the α-substituted acrolein, while the nucleophilic enolate is generated by activation of the Michael donor by Cu(II)-phen. This results in a Michael addition to the activated acrolein derivative, followed by an enantioselective proton-transfer reaction to the transient enamine intermediate to give the chiral aldehyde product (Figure 1).

Initial reactivity tests were performed with 2-acylimidazole (**1a**) as the Michael donor and methacrolein (**2a**) as the Michael acceptor. LmrR_V15pAF/Cu(II)-phen was prepared by combining Cu(II)-phen (6 mol %) with LmrR_V15pAF (5 mol %), in MES buffer at pH 6.5 for 1 h. No catalytic activity was observed when one of the components was omitted from the artificial enzyme or when the combination of wild type LmrR, without a pAF residue, and Cu(II)-phen was used (Table 1, entries 1−3). When the artificial enzyme was used, the Michael addition/enantioselective protonation product **3a** was obtained in 26% yield, 3:1 dr, and 89% and 32% ee for the major and minor diastereomer, respectively (Table 1, entry 4).[40] At shorter reaction times higher diastereoselectivity and enantioselectivity were obtained, suggesting that a slow epimerization of the chiral product occurs over time (Table 1, entry 5). This is well-known for ketones and aldehydes containing a stereocenter at the α position.

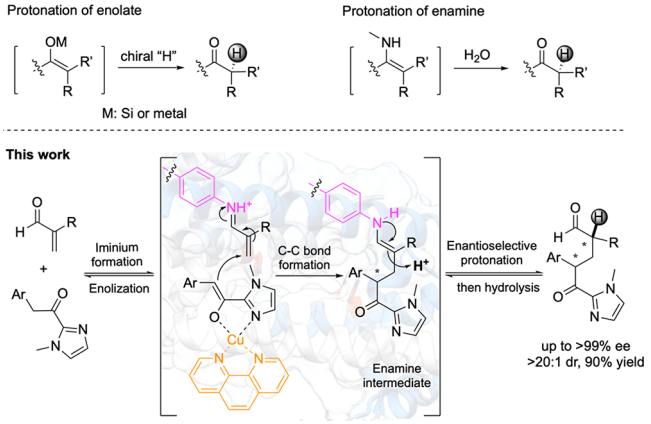
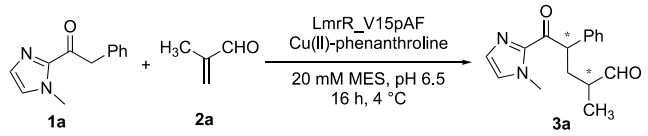


Figure 1. (top) Enantioselective protonation of enolate or enamine intermediates. (bottom) Tandem Michael addition and enantioselective protonation catalyzed by an LmrR-based artificial enzyme that combines a noncanonical pAF residue, which activates the α-substituted enal via iminium ion formation, and a Lewis acidic Cu(II) complex catalyzing the enolization of the Michael donor. After the Michael addition reaction, the resulting enamine intermediate undergoes enantioselective protonation.

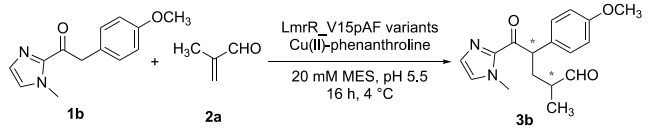
Table 1. Results of the LmrR_V15pAF/Cu(II)-phen Catalyzed Tandem Michael Addition/Enantioselective Protonation Reactions[a]



entry	catalyst		yield (%)[b]	dr[c]	ee (%)[c,d]
1	LmrR_pAF		<1	ND	ND
2		Cu(II)-phen	<1	ND	ND
3	LmrR	Cu(II)-phen	<1	ND	ND
4	LmrR_pAF	Cu(II)-phen	26 ± 3	3:1	89 ± 0/32 ± 1
5[e]	LmrR_pAF	Cu(II)-phen	14 ± 2	6:1	93 ± 0/24 ± 2

[a]Conditions used: LmrR_V15pAF or LmrR (5 mol %; 50 μM), 1.2 equiv of Cu(II)-phen (6 mol %; 60 μM), **1a** (1 mM), **2a** (10 mM), in MES buffer (20 mM, pH 6.5), NaCl (150 mM), at 4 °C for 48 h, unless noted otherwise. Yields and ee values are the averages of independent experiments, performed at least in triplicate. [b]Determined by HPLC analysis. [c]dr and ee values were determined by chiral HPLC. [d]ee values of major diastereomer. [e]Reactions carried out for 16 h. Errors represent standard deviations based on at least three independent experiments. ND = not determined.

Table 2. Study of Reaction Conditions and Effect of LmrR Mutations[a]



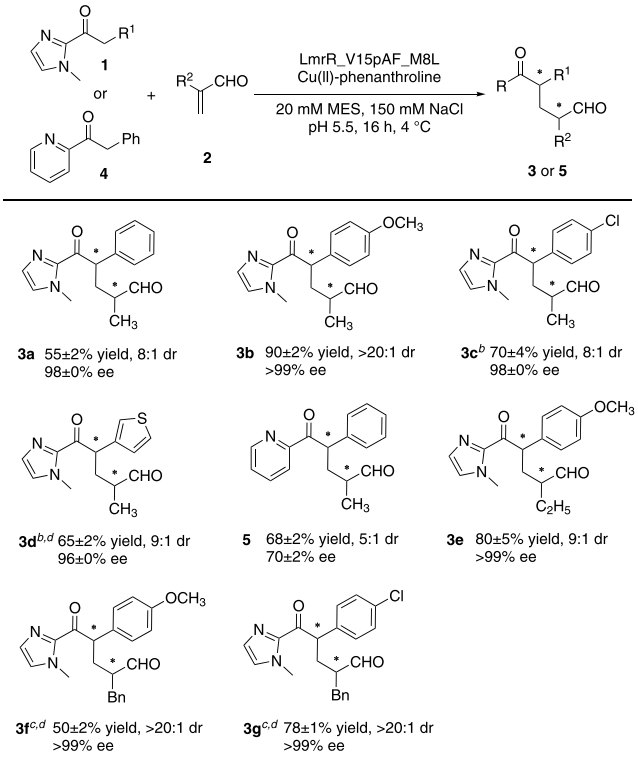
entry	enzyme + Cu(II)-phen	yield (%)[b]	dr[c]	ee (%)[c,d]
1[e]	LmrR_V15pAF	44 ± 2	10:1	99 ± 0
2	LmrR_V15pAF	28 ± 3	10:1	99 ± 0
3[f]	LmrR_V15pAF	16 ± 1	9:1	99 ± 0
4	LmrR_V15pAF_W96A	5 ± 1	3:1	18 ± 1
5	LmrR_V15pAF_M8W	40 ± 1	15:1	99 ± 0
6	LmrR_V15pAF_M8I	58 ± 3	18:1	>99
7	LmrR_V15pAF_M8L	65 ± 2	20:1	>99
8[g]	LmrR_V15pAF_M8L	33 ± 2	5:1	99 ± 0
9[h]	LmrR_V15pAF_M8L	88 ± 1	8:1	99 ± 0
10[e]	LmrR_V15pAF_M8L	90 ± 2	>20:1	>99

[a]Conditions used: LmrR_V15pAF or mutants (2.5 mol %; 25 μM), 1.2 equiv pf Cu(II)-phen (3 mol %; 30 μM), **1b** (1 mM), **2a** (10 mM), in MES buffer (20 mM, pH 5.5), NaCl (150 mM), at 4 °C for 16 h, unless noted otherwise. Yields and ee values are the average of independent experiments, performed at least in triplicate. [b]Determined by HPLC. [c]dr and ee values were determined by chiral HPLC. [d]ee values for major/minor diastereomer. In cases where the dr was high, the ee of the minor diastereomer was not determined. [e]5 mol % of LmrR_V15pAF variant and 6 mol % of Cu(II)-phen. [f]1 mol % of LmrR_V15pAF and 1.2 mol % of Cu(II)-phen. [g]Reaction performed at 18 °C. [h]Reaction performed for 48 h. Errors represent standard deviation based on independent experiments performed at least in triplicate. The ee of the minor diastereomers are not shown in this table but in Table S2 of the Supporting Information.

Next, the *p*-methoxy-substituted ketone **1b** was used as a Michael donor, as in our previous study this substrate was found to be more reactive (Table 2).[30] After optimization of the reaction conditions, using a combination of 2.5 mol % of LmrR_V15pAF with 3 mol % of Cu(II)-phen, a higher dr value

https://doi.org/10.1021/acscatal.1c02298
ACS Catal. 2021, 11, 9366−9369

Table 3. Substrate Scope[a]



[a]Conditions used: LmrR_V15pAF_M8L (5 mol %; 50 μM), 1.2 equiv of Cu(II)-phen (6 mol %; 60 μM), **1** or **4** (1 mM), **2** (10 mM), in MES buffer (20 mM, pH 5.5), NaCl (150 mM), at 4 °C for 16 h, unless noted otherwise. Yield and ee values are the average of independent experiments, performed at least in triplicate. Error values represent the standard deviations. [b]Reactions for 7 h. [c]Reaction with **1** (1 mM), **2** (2 mM), in MES buffer (20 mM, pH 5.5), NaCl (150 mM), at 4 °C for 24 h. [d]ee and dr values were determined after conversion by a Wittig reaction. The ee of the minor diastereomers are not shown in this figure but in Scheme S1 in the Supporting Information.

and excellent ee values of 99% and 72% for the major and minor diastereomers, respectively, were obtained (Table 2, entry 2). Using 1 mol % of LmrR_V15pAF did not affect the stereoselectivity of the reaction (Table 2, entries 1 and 3). When LmrR_V15pAF_W96A, which is lacking the Cu(II)-phen binding site, was used, a strong decrease in the yield and stereoselectivity was observed. This shows that the precise positioning of the Cu(II)-bound enolate in relation to the activated enal is of key importance to catalysis (Table 2, entry 4).

Mutations of the methionine at position 8 were known to potentially improve the catalytic activity of the artificial enzyme.[29,30] Hence, some M8 mutants of LmrR_V15pAF were evaluated in catalysis (Table 2, entries 5−7). The variant LmrR_V15pAF_M8L exhibited both improved stereoselectivity and reactivity in comparison to LmrR_V15pAF with a 65% yield, >20:1 dr, and >99% ee for the major diastereomer. Higher reaction temperatures and longer reaction times were found to have a negative effect on the diastereoselectivity and reactivity (Table 2, entries 8 and 9). Using a slightly higher concentration of artificial enzyme improved the reaction, resulting in >99% ee, >20:1 dr, and 90% yield (Table 2, entry 10).

The substrate scope of the tandem Michael addition/enantioselective protonation was investigated using various α-substituted enals and 2-acylimidazoles or 2-acylpyridine with LmrR_V15pAF_M8L/Cu(II)-phen, using the optimized conditions (Table 3). Using the *p*-chlorophenyl (**1c**)- and 3-thiophene-containing (**1d**) 2-acylimidazoles gave the products **3c** and **3d**, respectively, with excellent enantioselectivities, albeit with a slightly lower dr in comparison to **3a** and **3b**. Replacing the 2-acylimidazole moiety with 2-acylpyridine (**4**) gave the product **5** with moderate results. In addition to methacrolein, α-benzyl- and α-ethylacrolein were also well tolerated in this reaction, giving products **3f**, **3g**, and **3h**, respectively, with excellent ee, dr, and yield.

Generally, it was found that both the diastereoselectivity and enantioselectivity decreased somewhat with a longer reaction time, indicative of epimerization of the product (Table S3). This was not unexpected in view of the known lability of α stereocenters. Control experiments involving incubating enantioenriched **3g** with protein LmrR_V15pAF_M8L, LmrR, and pH 5.5 MES buffer separately suggested that both the artificial enzyme and the medium contribute to the epimerization process.

In conclusion, here we have shown that the tandem Michael addition/enantioselective protonation reaction in water is catalyzed efficiently by an LmrR-based artificial enzyme that exploits synergistic catalysis by two abiological catalytic groups. The combination of using a genetically encoded noncanonical amino acid to activate the electrophile and a supramolecularly bound metal complex to activate the nucleophile and deliver it with high precision to one prochiral face of the activated electrophile makes it possible to perform challenging reactions, such as the reaction presented here, with excellent diastereo- and enantioselectivities. These results illustrate the power of synergistic artificial enzymes, and we envision this concept to be broadly applicable for the biocatalysis of new-to-nature reactions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscatal.1c02298.

Complete experimental procedures and methods, tables and a scheme as described in the text, protein sequences, HPLC chromatograms, and NMR spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Zhi Zhou − *Stratingh Institute for Chemistry, University of Groningen, 9747 AG Groningen, The Netherlands;* orcid.org/0000-0001-7926-118X; Email: z.zhou@rug.nl

Gerard Roelfes − *Stratingh Institute for Chemistry, University of Groningen, 9747 AG Groningen, The Netherlands;* orcid.org/0000-0002-0364-9564; Email: j.g.roelfes@rug.nl

Complete contact information is available at: https://pubs.acs.org/10.1021/acscatal.1c02298

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by The Netherlands Organisation for Scientific Research (NWO, project 724.013.003), the European Research Council (ERC advanced grant 885396), and The

Netherlands Ministry of Education, Culture and Science (Gravitation program no. 024.001.035).

REFERENCES

(1) Fehr, C. Enantioselective Protonation of Enolates and Enols. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2566−2587.
(2) Mohr, J. T.; Hong, A. Y.; Stoltz, B. M. Enantioselective Protonation. *Nat. Chem.* **2009**, *1*, 359−369.
(3) Oudeyer, S.; Brière, J. F.; Levacher, V. Progress in Catalytic Asymmetric Protonation. *Eur. J. Org. Chem.* **2014**, *2014*, 6103−6119.
(4) Phelan, J. P.; Ellman, J. A. Conjugate Addition-Enantioselective Protonation Reactions. *Beilstein J. Org. Chem.* **2016**, *12*, 1203−1228.
(5) Weerasinghe, A. J.; Amin, S. A.; Barker, R. A.; Othman, T.; Romano, A. N.; Parker Siburt, C. J.; Tisnado, J.; Lambert, L. A.; Huxford, T.; Carrano, C. J.; Crumbliss, A. L. Borate as a Synergistic Anion for Marinobacter Algicola Ferric Binding Protein, FbpA: A Role for Boron in Iron Transport in Marine Life. *J. Am. Chem. Soc.* **2013**, *135*, 14504−14507.
(6) Kieffer, M. E.; Repka, L. M.; Reisman, S. E. Enantioselective Synthesis of Tryptophan Derivatives by a Tandem Friedel-Crafts Conjugate Addition/Asymmetric Protonation Reaction. *J. Am. Chem. Soc.* **2012**, *134*, 5131−5137.
(7) Li, Y. P.; Li, Z. Q.; Zhou, B.; Li, M. L.; Xue, X. S.; Zhu, S. F.; Zhou, Q. L. Chiral Spiro Phosphoric Acid-Catalyzed Friedel-Crafts Conjugate Addition/Enantioselective Protonation Reactions. *ACS Catal.* **2019**, *9*, 6522−6529.
(8) Matsumoto, K.; Tsutsumi, S.; Ihori, T.; Ohta, H. Enzyme-Mediated Enantioface-Differentiating Hydrolysis of α-Substituted Cycloalkanone Enol Esters. *J. Am. Chem. Soc.* **1990**, *112*, 9614−9619.
(9) Hirata, T.; Shimoda, K.; Kawano, T. Asymmetric Hydrolysis of Enol Esters with Two Esterases from Marchantia Polymorpha. *Tetrahedron: Asymmetry* **2000**, *11*, 1063−1066.
(10) Miyamoto, K.; Ohta, H. Purification and Properties of a Novel Arylmalonate Decarboxylase from Alcaligenes Bronchisepticus KU 1201. *Eur. J. Biochem.* **1992**, *210*, 475−481.
(11) Matoishi, K.; Ueda, M.; Miyamoto, K.; Ohta, H. Mechanism of Asymmetric Decarboxylation of α-Aryl-α- Methylmalonate Catalyzed by Arylmalonate Decarboxylase Originated from Alcaligenes Bronchisepticus. *J. Mol. Catal. B: Enzym.* **2004**, *27*, 161−168.
(12) Sibi, M. P.; Patil, K. Enantioselective H-Atom Transfer Reactions: A New Methodology for the Synthesis of B2-Amino Acids. *Angew. Chem., Int. Ed.* **2004**, *43*, 1235−1238.
(13) Poisson, T.; Yamashita, Y.; Kobayashi, S. Catalytic Asymmetric Protonation of Chiral Calcium Enolates via 1,4-Addition of Malonates. *J. Am. Chem. Soc.* **2010**, *132*, 7890−7892.
(14) Mohr, J. T.; Nishimata, T.; Behenna, D. C.; Stoltz, B. M. Catalytic Enantioselective Decarboxylative Protonation. *J. Am. Chem. Soc.* **2006**, *128*, 11348−11349.
(15) Leow, D.; Lin, S.; Chittimalla, S. K.; Fu, X.; Tan, C. H. Enantioselective Protonation Catalyzed by a Chiral Bicyclic Guanidine Derivative. *Angew. Chem., Int. Ed.* **2008**, *47*, 5641−5645.
(16) Vora, H. U.; Rovis, T. N-Heterocyclic Carbene Catalyzed Asymmetric Hydration: Direct Synthesis of α-Protio and α-Deuterio α-Chloro and α-Fluoro Carboxylic Acids. *J. Am. Chem. Soc.* **2010**, *132*, 2860−2861.
(17) Kitanosono, T.; Sakai, M.; Zhu, L.; Kobayashi, S. Enantioselective Aza-Michael/Protonation Reactions in Water. *Chem. Lett.* **2019**, *48*, 783−786.
(18) Fu, N.; Zhang, L.; Luo, S. Catalytic Asymmetric Enamine Protonation Reaction. *Org. Biomol. Chem.* **2018**, *16*, 510−520.
(19) Fu, N.; Zhang, L.; Luo, S.; Cheng, J. P. Chiral Primary-Amine-Catalyzed Conjugate Addition to α-Substituted Vinyl Ketones/Aldehydes: Divergent Stereocontrol Modes on Enamine Protonation. *Chem. - Eur. J.* **2013**, *19*, 15669−15681.
(20) Fu, N.; Zhang, L.; Li, J.; Luo, S.; Cheng, J. P. Chiral Primary Amine Catalyzed Enantioselective Protonation via an Enamine Intermediate. *Angew. Chem., Int. Ed.* **2011**, *50*, 11451−11455.
(21) Zhang, L.; Fu, N.; Luo, S. Pushing the Limits of Aminocatalysis: Enantioselective Transformations of α-Branched β-Ketocarbonyls and Vinyl Ketones by Chiral Primary Amines. *Acc. Chem. Res.* **2015**, *48*, 986−997.
(22) Hilvert, D. Design of Protein Catalysts. *Annu. Rev. Biochem.* **2013**, *82*, 447−470.
(23) Schwizer, F.; Okamoto, Y.; Heinisch, T.; Gu, Y.; Pellizzoni, M. M.; Lebrun, V.; Reuter, R.; Köhler, V.; Lewis, J. C.; Ward, T. R. Artificial Metalloenzymes: Reaction Scope and Optimization Strategies. *Chem. Rev.* **2018**, *118*, 142−231.
(24) Nanda, V.; Koder, R. L. Designing Artificial Enzymes by Intuition and Computation. *Nat. Chem.* **2010**, *2*, 15−24.
(25) Drienovská, I.; Roelfes, G. Expanding the Enzyme Universe with Genetically Encoded Unnatural Amino Acids. *Nat. Catal.* **2020**, *3*, 193−202.
(26) Leveson-Gower, R. B.; Mayer, C.; Roelfes, G. The Importance of Catalytic Promiscuity for Enzyme Design and Evolution. *Nat. Rev. Chem.* **2019**, *3*, 687−705.
(27) García-Fernández, A.; Megens, R. P.; Villarino, L.; Roelfes, G. DNA-Accelerated Copper Catalysis of Friedel-Crafts Conjugate Addition/Enantioselective Protonation Reactions in Water. *J. Am. Chem. Soc.* **2016**, *138*, 16308−16314.
(28) Mansot, J.; Aubert, S.; Duchemin, N.; Vasseur, J. J.; Arseniyadis, S.; Smietana, M. A Rational Quest for Selectivity through Precise Ligand-Positioning in Tandem DNA-Catalysed Friedel-Crafts Alkylation/Asymmetric Protonation. *Chem. Sci.* **2019**, *10*, 2875−2881.
(29) Villarino, L.; Chordia, S.; Alonso-Cotchico, L.; Reddem, E.; Zhou, Z.; Thunnissen, A. M. W. H.; Marechal, J.-D.; Roelfes, G. Cofactor Binding Dynamics Influence the Catalytic Activity and Selectivity of an Artificial Metalloenzyme. *ACS Catal.* **2020**, *10*, 11783−11790.
(30) Zhou, Z.; Roelfes, G. Synergistic Catalysis in an Artificial Enzyme by Simultaneous Action of Two Abiological Catalytic Sites. *Nat. Catal.* **2020**, *3*, 289−294.
(31) Allen, A. E.; MacMillan, D. W. C. Synergistic Catalysis: A Powerful Synthetic Strategy for New Reaction Development. *Chem. Sci.* **2012**, *3*, 633−658.
(32) Patil, N. T.; Shinde, V. S.; Gajula, B. A One-Pot Catalysis: The Strategic Classification with Some Recent Examples. *Org. Biomol. Chem.* **2012**, *10*, 211−224.
(33) Afewerki, S.; Córdova, A. Combinations of Aminocatalysts and Metal Catalysts: A Powerful Cooperative Approach in Selective Organic Synthesis. *Chem. Rev.* **2016**, *116*, 13512−13570.
(34) Agustiandari, H.; Lubelski, J.; Van Den Berg Van Saparoea, H. B.; Kuipers, O. P.; Driessen, A. J. M. LmrR Is a Transcriptional Repressor of Expression of the Multidrug ABC Transporter LmrCD in Lactococcus Lactis. *J. Bacteriol.* **2008**, *190*, 759−763.
(35) Madoori, P. K.; Agustiandari, H.; Driessen, A. J. M.; Thunnissen, A. M. W. H. Structure of the Transcriptional Regulator LmrR and Its Mechanism of Multidrug Recognition. *EMBO J.* **2009**, *28*, 156−166.
(36) Roelfes, G. LmrR: A Privileged Scaffold for Artificial Metalloenzymes. *Acc. Chem. Res.* **2019**, *52*, 545−556.
(37) Drienovská, I.; Mayer, C.; Dulson, C.; Roelfes, G. A Designer Enzyme for Hydrazone and Oxime Formation Featuring an Unnatural Catalytic Aniline Residue. *Nat. Chem.* **2018**, *10*, 946−952.
(38) Bos, J.; Browne, W. R.; Driessen, A. J. M.; Roelfes, G. Supramolecular Assembly of Artificial Metalloenzymes Based on the Dimeric Protein LmrR as Promiscuous Scaffold. *J. Am. Chem. Soc.* **2015**, *137*, 9796−9799.
(39) Mayer, C.; Dulson, C.; Reddem, E.; Thunnissen, A. M. W. H.; Roelfes, G. Directed Evolution of a Designer Enzyme Featuring an Unnatural Catalytic Amino Acid. *Angew. Chem., Int. Ed.* **2019**, *58*, 2083−2087.
(40) Zhang, D.-H.; Knelles, J.; Plietker, B. Iron-Catalyzed Michael Addition of Ketones to Polar Olefins. *Adv. Synth. Catal.* **2016**, *358*, 2469−2479.

https://doi.org/10.1021/acscatal.1c02298
ACS Catal. 2021, 11, 9366−9369