Letter

An Engineered Self-sufficient Biocatalyst Enables Scalable Production of Linear Alpha Olefins from Carboxylic Acids

Chen Lu, Fenglin Shen, Shuaibo Wang, Yuyang Wang, Juan Liu, Wen-Ju Bai, and Xiqing Wang

ACS Catal., **Just Accepted Manuscript** • DOI: 10.1021/acscatal.8b01313 • Publication Date (Web): 23 May 2018

An Engineered Self-sufficient Biocatalyst Enables Scalable Production of Linear Alpha Olefins from Carboxylic Acids

Chen Lu,[†§] Fenglin Shen,[†§] Shuaibo Wang,[§] Yuyang Wang,[‡] Juan Liu,[‡] Wen-Ju Bai,*[ξ] Xiqing Wang*[§]

[§] College of Bioscience and Biotechnology, Yangzhou University, Yangzhou, Jiangsu 225009, China

[‡] Testing Center, Yangzhou University, Yangzhou, Jiangsu 225009, China

[ξ] Department of Chemistry, Stanford University, Stanford, California 94305-5080, USA

ABSTRACT: Fusing the decarboxylase $OleT_{JE}$ and the reductase domain of P450BM3 creates a self-sufficient protein, OleT-BM3R, which is able to efficiently catalyze oxidative decarboxylation of carboxylic acids into linear α-olefins (LAOs) under mild aqueous conditions using O_2 as the oxidant and NADPH as the electron donor. The compatible electron transfer system installed in the fusion protein not only eliminates the need for auxiliary redox partners, but also results in boosted decarboxylation reactivity and broad substrate scope. Coupled with the phosphite dehydrogenase based NADPH regeneration system, this enzymatic reaction proceeds with improved product titers of up to 2.51 g L^{-1} and volumetric productivities of up to 209.2 mg L^{-1} h^{-1} at low catalyst loadings (~0.02 mol%). With its stability and scalability, this self-sufficient biocatalyst offers a nature-friendly approach to deliver LAOs.

KEYWORDS: Green chemistry, Biocatalysis, Protein engineering, Oxidative decarboxylation, α-Olefins

Linear α-olefins (LAOs) are next-generation fuels and key feedstock chemicals for the production of surfactants, lubricants, detergents, and polymers.[1-5] Currently, these olefins are predominantly produced from petroleum via ethylene oligomerization that only yields even-numbered terminal olefins.[6-9] Considering dwindling fossil fuel reserves, pursuing a renewable route to access LAOs is of urgency.[10,11] Pleasingly, abundant and bioavailable fatty acids (FAs) may provide an alternative source for the sustainable production of LAOs through straightforward decarbonylative dehydration or oxidative decarboxylation,[12] which can lead to both even- and odd-numbered LAOs. To achieve this goal, transition-metal catalysts including palladium, rhodium, iridium, and iron have been developed.[13-20] While impressive, these approaches usually suffer from harsh reaction conditions (T $\geqslant$110 °C) and require in situ distillation of the product to maintain acceptable α-selectivity. Furthermore, activation of the FAs with stoichiometric anhydrides is often needed, resulting in extra waste. Nowadays, intensifying environmental concerns urge scientists to investigate chemical transformations using green solvents, such as water, or eco-friendly methods, including electrochemistry[21-24] and biocatalysis.[25-28] Accordingly, we aimed for a green strategy to supply LAOs from FAs.

Recently, a cytochrome P450 enzyme, $OleT_{JE}$, was found to catalyze the oxidative decarboxylation of saturated FAs into terminal olefins using H_2O_2 as the oxidant.[29] H_2O_2 deactivated this biocatalyst at millimolar concentration and these H_2O_2-dependent $OleT_{JE}$ systems had relatively low catalytic efficiency and narrow substrate scope (FAs ≥ C8).[30-35] When coupled with redox partners, $OleT_{JE}$ could catalyze the same reaction with O_2 as the oxidant and NAD(P)H as the electron donor.[36-39] Addition of putidaredoxin reductase and putidaredoxin (CamAB) to the reaction could increase the reactivity and turnover number of $OleT_{JE}$.[37-39] However, preparation of the CamAB-containing lysate and calibration of the lysate versus $OleT_{JE}$ complicated the reaction system and would limit its application. Previous effort to create a self-sufficient enzyme by fusing $OleT_{JE}$ to P450RhF reductase domain achieved H_2O_2-independent catalysis, but this engineered enzyme unfortunately displayed low activity, only being able to decarboxylate FAs C12-C18.[36] In this study, a more compatible electron transfer system is installed in an $OleT_{JE}$-containing fusion protein, resulting in a self-sufficient biocatalyst with increased decarboxylation reactivity. Our approach, employing O_2 as the

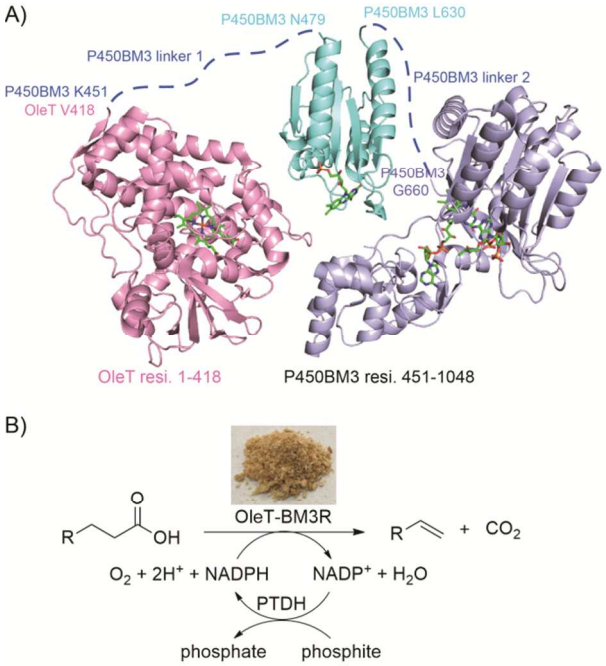


Figure 1. (A) Structural schematic of OleT-BM3R shown as the cartoon representation. OleT-BM3R is composed of $OleT_{JE}$ (residues 1-418, PDB code 4L54, pink), FMN binding domain (residues 479-630 of P450BM3, PDB code 1BVY, cyan) and FAD/NADPH-binding domain (residues 660-1048 of P450BM3, PDB code 4DQL, blue) of P450BM3. The cofactors, heme, FMN, FAD and $NADP^+$ are displayed as sticks. (B) Oxidative decarboxylation of carboxylic acids to olefins by OleT-BM3R (pictured in lyophilized form) that uses O_2 with NADPH recycling. PTDH = phosphite dehydrogenase.

oxidant and NADPH as the electron donor, converts structurally diversified carboxylic acids to their corresponding olefins under mild aqueous conditions. This stable fusion protein reduces the effort required for protein production and purification and simplifies the reaction system, making it more suitable for synthetic and industrial application.

To start, we chose CYP102A1 (P450BM3) from *Bacillus megaterium* as the template for the construction of the self-sufficient $OleT_{JE}$ fusion protein. The heme domain of P450BM3 is fused with the reductase domain (BM3R) as a single polypeptide, establishing an efficient electron transfer to the heme iron that is not contingent on encountering a discrete redox partner.[40,41] Powered by this efficient electron transfer chain, P450BM3's hydroxylase activity is >1000 fold higher than those of other P450 fatty acid hydroxylases.[42] Superimposition of their crystal structures revealed a similar structural fold between $OleT_{JE}$[30] and the heme domain of P450BM3[43] (RMSD=3.6 Å) (Figure S1). The replacement of the heme domain of P450BM3 with $OleT_{JE}$ (residue 1-418) resulted in a soluble protein, named OleT-BM3R (Figure 1A). Preliminary experiments showed that the fusion protein OleT-BM3R exhibited high decarboxylation activity towards stearic

Table 1. The oxidative decarboxylation of various saturated FAs by OleT-BM3R with NADPH recycling[a]

Entry	Substrate	Product	Yield [%][b]
1	$CH_3(CH_2)_{18}CO_2H$ (**1a**)	$CH_3(CH_2)_{16}CH{=}CH_2$ (**2a**)	70
2	$CH_3(CH_2)_{16}CO_2H$ (**1b**)	$CH_3(CH_2)_{14}CH{=}CH_2$ (**2b**)	73
3	$CH_3(CH_2)_{14}CO_2H$ (**1c**)	$CH_3(CH_2)_{12}CH{=}CH_2$ (**2c**)	60
4	$CH_3(CH_2)_{12}CO_2H$ (**1d**)	$CH_3(CH_2)_{10}CH{=}CH_2$ (**2d**)	52
5	$CH_3(CH_2)_{10}CO_2H$ (**1e**)	$CH_3(CH_2)_8CH{=}CH_2$ (**2e**)	46
6	$CH_3(CH_2)_9CO_2H$ (**1f**)	$CH_3(CH_2)_7CH{=}CH_2$ (**2f**)	58
7	$CH_3(CH_2)_8CO_2H$ (**1g**)	$CH_3(CH_2)_6CH{=}CH_2$ (**2g**)	70
8	$CH_3(CH_2)_7CO_2H$ (**1h**)	$CH_3(CH_2)_5CH{=}CH_2$ (**2h**)	47
9	$CH_3(CH_2)_6CO_2H$ (**1i**)	$CH_3(CH_2)_4CH{=}CH_2$ (**2i**)	40
10	$CH_3(CH_2)_5CO_2H$ (**1j**)	$CH_3(CH_2)_3CH{=}CH_2$ (**2j**)	70
11	$CH_3(CH_2)_4CO_2H$ (**1k**)	$CH_3(CH_2)_3CH{=}CH_2$ (**2k**)	68
12	$CH_3(CH_2)_3CO_2H$ (**1l**)	$CH_3CH_2CH{=}CH_2$ (**2l**)	11
13	$CH_3(CH_2)_2CO_2H$ (**1m**)	$CH_3CH{=}CH_2$ (**2m**)	14

[a]Reaction conditions: purified OleT-BM3R (3 μM), substrate (1 mM), catalase (100 U mL^{-1}), NADPH (200 μM), PTDH (2 μM), sodium phosphite (0.01 M), 1 mL scale, rt, 12 h. [b]determined by GC or headspace GC (see the supporting information).

acid (FA C18:0) in the presence of oxygen and NADPH at ambient temperature [turnover number (TON) = 2520, turnover frequency (TOF) = 472 h^{-1}, NADPH coupling efficiency = 61%]. In contrast, when the same reaction was performed with $OleT_{JE}$ and free BM3R, the TOF and NADPH coupling efficiency were only 65 h^{-1} and 29%, respectively. These results prove an enhanced electron transfer from BM3R to OleT in our fusion protein. Compared with other redox systems for the decarboxylation of FAs with $OleT_{JE}$, this self-sufficient enzyme showed advantages in turnover number, rate of reaction, and NADPH coupling efficiency (Table S1). When this reaction was coupled to a phosphite dehydrogenase (PTDH) based NADPH regeneration system, [44-46] the overall decarboxylation activity (TON = 2167) was maintained. Isolated BM3R was susceptible to temperature-induced activity loss (inactivation rate = 0.22 min^{-1} at 30 °C),[47] but the OleT-BM3R fusion protein was stable at 37 °C for 24 h (Figure S2). Moreover, purified OleT-BM3R can be lyophilized (Figure 1B) and conveniently stored at -20 °C for months without significant activity loss. These results demonstrate that $OleT_{JE}$ is able to complement BM3R in

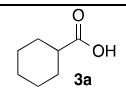
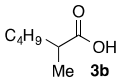
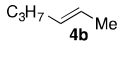
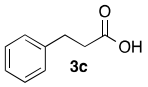
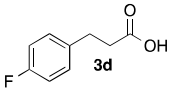
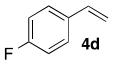
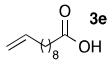
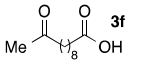
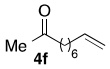
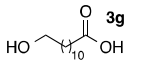
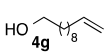
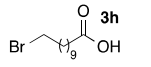
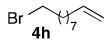
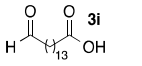
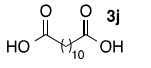
P450BM3, together forming a structurally stable and functionally active entity.

With the engineered OleT-BM3R in hand, we proceeded to explore the reactivity and scope of the oxidative decarboxylation of FAs (Table 1). Gratifyingly, both long- and medium-chain saturated FAs **1a-k** successfully participated in this transformation, delivering the desired LAOs **2a-k** up to 73% yield (entries 1-11). Short-chain fatty acids **1l-m** also worked albeit with meager yields (entries 12-13). These results indicate good compatibility between OleT and BM3R in the fusion protein. Notably, utilizing naturally occurring even-numbered saturated FAs afforded prohibitively expensive odd-numbered terminal olefins, such as **2a** and **2b**, which are inaccessible through the oligomerization of ethylene. Meanwhile, α- and β-hydroxylations occurred alongside the desired decarboxylation process. Formation of the nonvolatile α/β-hydroxylated products was also dependent on chain length (Table S2). The catalytic cycle of $OleT_{JE}$ involves abstraction of a Cα/Cβ hydrogen atom of the carboxylic acid by the iron (IV)-oxo heme π-cation radical intermediate (Compound I), followed by a competition between the ·OH rebound and carbon-carbon scission that delivers the corresponding hydroxylation and decarboxylation products.[30,48-50] This mechanism guarantees the exclusive formation of α-alkenes from the decarboxylation pathway. As a result, this mild enzymatic approach displays advantages over synthetic methods, which employ transition-metal catalysts under harsh conditions and thus partially form the thermodynamically stable internal alkenes.

To extend the synthetic application of this enzymatic transformation, we further explored its substrate scope and functional group tolerance (Table 2). To our delight, both cyclic and acyclic α-branched carboxylic acids **3a-b** underwent the expected reaction, providing the corresponding olefins in reasonable yields (entries 1-2). Similarly, phenylpropanoic acids **3c-d** smoothly delivered the respective vinylbenzenes **4c-d** with improved yields (entries 3-4) and only traces of hydroxylation products were formed in these reactions (Table S2). Functional groups like olefins and ketones were tolerated and a free-hydroxyl moiety was also untouched during the reaction (entries 5-7). Remarkably, sensitive groups such as bromo and aldehyde, which are vulnerable under conditions using transition-metal catalysts, survived due to our mild enzymatic conditions (entries 8-9). When diacid **3j** was examined, the anticipated oxidative decarboxylation occurred on both acids (entry 10). Interestingly, only terminal diene was formed. Although no ω-alkenoic acid intermediate was found, its α/β-hydroxy derivatives were detected (Table S2), indicating that the dicarboxylic acid underwent tandem decarboxylation via ω-alkenoic acid intermediate.

To testify its potential for preparative application, this enzymatic transformation was performed in a concentrated manner with stearic acid as a substrate (Table 3). Simply increasing the substrate concentration from 1 mM to 10 mM led to a slightly diminished yield but dramatically improved product titer and volumetric productivity

Table 2. Further substrate scope study.[a]

Entry	Substrate	Product	Yield [%][b]
1	 **3a**	 **4a**	30
2	 **3b**	 **4b**	30
3	 **3c**	 **4c**	78
4	 **3d**	 **4d**	44
5	 **3e**	 **4e**	71
6	 **3f**	 **4f**	48
7	 **3g**	 **4g**	42
8	 **3h**	 **4h**	40
9[c]	 **3i**	 **4i**	11[d]
10	 **3j**	 **4e**	42

[a]Reaction conditions: see conditions in Table 1. [b]determined by headspace GC. [c]substrate (10 mM), sodium phosphite (0.05 M), 30 mL scale, 20 h. [d]isolated yield.

(entry 1 vs 2). Compared to the previous OleT/CamAB/FDH cascade system,[37] our newly developed OleT-BM3R/PTDH coupled system offers higher product titer (1.40 g L^{-1} vs. 0.93 g L^{-1}) and volumetric productivity (116.7 mg L^{-1} h^{-1} vs. 42.5 mg L^{-1} h^{-1}), presumably because of the greater TOF (472 h^{-1} vs. 53 h^{-1}) and NADPH coupling efficiency (61% vs. 25%). Meanwhile, simultaneously elevating the concentrations of both catalyst and substrate further benefited the product titer and volumetic productivity at the cost of the conversion (entries 3-4). The product titer of our OleT-BM3R/PTDH system could reach as high as 2.51 g L^{-1} while the volumetric productivity climbed up to 209.2 mg L^{-1} h^{-1} at a low catalyst loading (~0.02 mol%) (entry 4). Notably, cell-free lysate allowed the production of 1.26 g L^{-1} (5.28 mM, 53% yield) 1-heptadecene from 10 mM stearic acid without the

Table 3. Oxidative decarboxylation of stearic acid at various substrate concentrations by OleT-BM3R with NADPH recycling.[a]

Entry	OleT-BM3R [μM]	Substrate [mM]	Yield [%]	Titer [g L^{-1}]	Volumetic productivity [mg L^{-1} h^{-1}]
1	3	1	73	0.17	14.5
2	3	10	59	1.40	116.7
3	9	20	40	1.91	159.2
4	9	40	26	2.51	209.2
5	5b	10	53	1.26	105.0
6	3c	10	54	1.29	107.5

[a]Reaction conditions: OleT-BM3R (indicated), catalase (100 U mL^{-1}), NADPH (200 μM), PTDH (2 μM), sodium phosphite (0.05 M), 1 mL scale, rt, 12 h; [b]Cell-free lysate without NADPH supplement. [c]The activity of lyophilized OleT-BM3R was tested after 2-month storage at -20 °C.

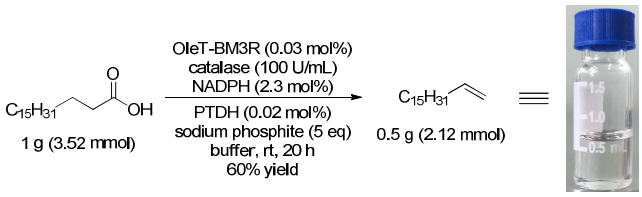


Figure 2. Gram scale enzymatic oxidative decarboxylation of stearic acid.

need for protein purification (entry 5). This cell-free lysate system is economically desirable as no additional NADPH is required. Additionally, lyophilized OleT-BM3R exhibited comparable reactivity after two-month storage at -20 °C (entry 6). Moreover, our enzymatic system proved scalable, and 1g of stearic acid was successfully converted to 1-heptadecene in 60% yield (Figure 2). As the obtained product is particularly expensive, our enzymatic reaction provides this LAO not only through a sustainable approach, but also in a cost-effective fashion. Overall, the outstanding stability and scalability of this self-sufficient biocatalyst demonstrates encouraging potential for preparation of LAOs.

In summary, we engineered a catalytically self-sufficient fusion protein, OleT-BM3R, for oxidative decarboxylation of FAs without the need for auxiliary redox partners. The simplified reaction could be set up with less effort in protein production and purification and performed under mild conditions in aqueous solution using O_2 as the oxidant and NADPH as the electron donor. It could also use inexpensive sodium phosphite as the final electron source when coupled with a PTDH based NADPH regeneration system. A similar structure between $OleT_{JE}$ and the heme domain of P450BM3, a good compatibility of $OleT_{JE}$ and BM3R, and the structural elements in P450BM3 that facilitate domain-domain interactions and electron transfer processes altogether contribute to the boosted decarboxylation reactivity and broad substrate scope of the fusion protein. The stability and scalability of this enzyme make it a useful biocatalytic platform for the sustainable synthesis of LAOs. In addition, high ferricyanide and cytochrome c reduction rates (9850 min^{-1} and 3330 min^{-1}, respectively) were determined for this fusion protein, indicating that it was powered by the rapid electron transfer in BM3R.[51] But a comparable reaction rate of P450BM3-catalyzed fatty acid hydroxylation (usually TOF>400 min^{-1}) was not observed.[42] Thus, further engineering this fusion protein for enhanced interaction and electron transfer between the FMN binding domain and OleT, which might additionally benefit its catalytic performance, is worth future exploration. Meanwhile, production of LAOs in microbial platforms with this biocompatible catalyst is also underway in our laboratories.

AUTHOR INFORMATION

Corresponding Author

*E-mail: xiqingwang@yzu.edu.cn, bai1@stanford.edu

Author Contributions

†These authors contributed equally.

ASSOCIATED CONTENT

Supporting Information includes Experimental Procedures GC/Headspace GC results and ^{1}H Spectra data. This material is available free of charge via the Internet at http://pubs.acs.org.

ABBREVIATIONS

LAOs, linear α-olefins; FAs, fatty acids ; TON, turnover number; TOF, turnover frequency; PTDH, phosphite dehydrogenase; NADP, nicotinamide adenine dinucleotide phosphate.

ACKNOWLEDGMENT

We thank Christopher A. Kalnmals (Stanford University) for critical reading of the manuscript. X. W. acknowledges the financial support from National Natural Science Foundation of China (Grants 31400649 and 31670792), Natural Science Foundation of Jiangsu Province (Grant BK20140477), Natural Science Research Grant from Department of Education of Jiangsu Province (Grant 14KJB180026), and Talent Support Program of Yangzhou University.

REFERENCES

(1) Rude, M. A.; Schirmer, A. New Microbial Fuels: a Biotech Perspective. *Curr. Opin. Microbiol.* **2009**, *12*, 274-281.

(2) Franke, R.; Selent, D.; Börner, A. Applied Hydroformylation. *Chem. Rev.* **2012**, *112*, 5675-5732.

(3) Ray, S.; Rao, P. V. C.; Choudary, N. V. Poly-α-Olefin-Based Synthetic Lubricants: a Short Review on Various Synthetic Routes. *Lubr. Sci.* **2012**, *24*, 23-44.

(4) Peralta-Yahya, P. P.; Zhang, F.; del Cardayre, S. B.; Keasling, J. D. Microbial Engineering for the Production of Advanced Biofuels. *Nature* **2012**, *488,* 320-328.

(5) Herbert, M. B.; Marx, V. M.; Pederson, R. L.; Grubbs, R. H. Concise Syntheses of Insect Pheromones Using Z-Selective Cross Metathesis. *Angew. Chem. Int. Ed.* **2013**, *52,* 310-314.

(6) Skupinska, J. Oligomerization of α-Olefins to Higher Oligomers. *Chem. Rev.* **1991**, *91,* 613-648.

(7) Small, B. L.; Brookhart, M. Iron-Based Catalysts with Exceptionally High Activities and Selectivities for Oligomerization of Ethylene to Linear α-Olefins. *J. Am. Chem. Soc.* **1998**, *120,* 7143-7144.

(8) Ittel, S. D.; Johnson, L. K.; Brookhart, M. Late-Metal Catalysts for Ethylene Homo- and Copolymerization. *Chem. Rev.* **2000**, *100,* 1169-1204.

(9) McGuinness, D. S. Olefin Oligomerization via Metallacycles: Dimerization, Trimerization, Tetramerization, and Beyond. *Chem. Rev.* **2011**, *111,* 2321-2341.

(10) Murray, J.; King, D. Climate Policy: Oil's Tipping Point Has Passed. *Nature* **2012**, *481,* 433-435.

(11) Straathof, A. J. Transformation of Biomass into Commodity Chemicals Using Enzymes or Cells. *Chem. Rev.* **2014**, *114,* 1871-1908.

(12) Dawes, G. J. S.; Scott, E. L.; Le Nôtre, J.; Sanders, J. P. M.; Bitter, J. H. Deoxygenation of Biobased Molecules by Decarboxylation and Decarbonylation – a Review on the Role of Heterogeneous, Homogeneous and Bio-catalysis. *Green Chem.* **2015**, *17,* 3231-3250.

(13) Miller, J. A.; Nelson, J. A.; Byrne, M. P. A Highly Catalytic and Selective Conversion of Carboxylic Acids to 1-Alkenes of One Less Carbon Atom. *J. Org. Chem.* **1993**, *58,* 18-20.

(14) Gooßen, L. J.; Rodriguez, N. A Mild and Efficient Protocol for the Conversion of Carboxylic Acids to Olefins by a Catalytic Decarbonylative Elimination Reaction. *Chem. Commun.* **2004**, *6,* 724-725.

(15) Le Nôtre, J.; Scott, E. L.; Franssen, M. C. R.; Sanders, J. P. M. Selective Preparation of Terminal Alkenes from Aliphatic Carboxylic Acids by a Palladium-Catalysed Decarbonylation–Elimination Reaction. *Tetrahedron Lett.* **2010**, *51,* 3712-3715.

(16) Kraus, G.; Riley, S. A Large-Scale Synthesis of α-Olefins and α,ω-Dienes. *Synthesis* **2012**, *44,* 3003-3005.

(17) Maetani, S.; Fukuyama, T.; Suzuki, N.; Ishihara, D.; Ryu, I. Iron-Catalyzed Decarbonylation Reaction of Aliphatic Carboxylic Acids Leading to α-Olefins. *Chem. Commun.* **2012**, *48,* 2552-2554.

(18) Liu, Y.; Kim, K. E.; Herbert, M. B.; Fedorov, A.; Grubbs, R. H.; Stoltz, B. M. Palladium-Catalyzed Decarbonylative Dehydration of Fatty Acids for the Production of Linear Alpha Olefins. *Adv. Synth. Catal.* **2014**, *356,* 130-136.

(19) Chatterjee, A.; Hopen Eliasson, S. H.; Törnroos, K. W.; Jensen, V. R. Palladium Precatalysts for Decarbonylative Dehydration of Fatty Acids to Linear Alpha Olefins. *ACS Catal.* **2016**, *6,* 7784-7789.

(20) Chatterjee, A.; Jensen, V. R. A Heterogeneous Catalyst for the Transformation of Fatty Acids to α-Olefins. *ACS Catal.* **2017**, *7,* 2543-2547.

(21) Little, R. D.; Schwaebe, M. K. Reductive Cyclizations at the Cathode. *Top. Curr. Chem.* **1997**, *185,* 1-48.

(22) Little, R. D.; Moeller, K. D. Organic Electrochemistry as a Tool for Synthesis. *Electrochem. Soc. Interface.* **2002**, *11,* 36-42.

(23) Frontana-Uribe, B. A.; Little, R. D.; Ibanez, J. G.; Palma; A.; Vasquez-Medrano, R. Organic Electrosynthesis: a Promising Green Methodology in Organic Chemistry *Green Chem.* **2010**, *12,* 2099-2119.

(24) Francke, R.; Little, R. D. Redox Catalysis in Organic Electrosynthesis: Basic Principles and Recent Developments. *Chem. Soc. Rev.* **2014**, *43,* 2492-2521.

(25) Hammer, S. C.; Knight, A. M.; Arnold, F. H. Design and Evolution of Enzymes for Non-natural Chemistry. *Curr. Opin. Green Sustain. Chem.* **2017**, 7, 23-30.

(26) Arnold, F. H. Directed Evolution: Bringing New Chemistry to Life. *Angew. Chem. Int. Ed.* **2018**, 57, 4143 – 4148.

(27) Romney, D. K.; Murciano-Calles, J.; Wehrmüller, J. E.; Arnold, F. H. Unlocking Reactivity of TrpB: A General Biocatalytic Platform for Synthesis of Tryptophan Analogues. *J. Am. Chem. Soc.* **2017**, 139, 10769-10776.

(28) Prier, C. K.; Zhang, R. K.; Buller, A. R.; Brinkmann-Chen, S.; Arnold, F. H. Enantioselective, Intermolecular Benzylic C-H Amination Catalysed by an Engineered Iron-Haem Enzyme. *Nat. Chem.* **2017**, 9, 629-634.

(29) Rude, M. A.; Baron, T. S.; Brubaker, S.; Alibhai, M.; Del Cardayre, S. B.; Schirmer, A. Terminal Olefin (1-Alkene) Biosynthesis by a Novel P450 Fatty Acid Decarboxylase from *Jeotgalicoccus* Species. *Appl. Environ. Microbiol.* **2011**, *77,* 1718-1727.

(30) Belcher, J.; McLean, K. J.; Matthews, S.; Woodward, L. S.; Fisher, K.; Rigby, S.E.; Nelson, D. R.; Potts, D.; Baynham, M. T.; Parker, D. A.; Leys, D.; Munro, A. W. Structure and Biochemical Properties of the Alkene Producing Cytochrome P450 $OleT_{JE}$ (CYP152L1) from the *Jeotgalicoccus* sp. 8456 Bacterium. *J. Biol. Chem.* **2014,** *289,* 6535-6550.

(31) Zachos, I.; Gassmeyer, S. K.; Bauer, D.; Sieber, V.; Hollmann, F.; Kourist, R. Photobiocatalytic Decarboxylation for Olefin Synthesis. *Chem. Commun.* **2015**, *51,* 1918-1921.

(32) Hsieh, C. H.; Makris, T. M. Expanding the Substrate Scope and Reactivity of Cytochrome P450 OleT. *Biochem. Biophys. Res. Commun.* **2016**, *476,* 462-466.

(33) Matthews, S.; Tee, K. L.; Rattray, N. J.; McLean, K. J.; Leys, D.; Parker, D. A.; Blankley, R. T.; Munro, A. W. Production of Alkenes and Novel Secondary Products by P450 $OleT_{JE}$ Using Novel H_2O_2-Generating Fusion Protein Systems. *FEBS lett.* **2017**, *591,* 737-750.

(34) Hsieh, C. H.; Huang, X.; Amaya, J. A.; Rutland, C. D.; Keys, C. L.; Groves, J. T.; Austin, R.N.; Makris, T.M. The Enigmatic P450 Decarboxylase OleT Is Capable of, but Evolved To Frustrate, Oxygen Rebound Chemistry. *Biochemistry* **2017**, *56,* 3347-3357.

(35) Matthews, S.; Belcher, J. D.; Tee, K. L.; Girvan, H. M.; McLean, K. J.; Rigby, S. E.; Levy, C. W.; Leys, D.; Parker, D. A.; Blankley, R. T.; Munro, A. W. Catalytic Determinants of Alkene Production by the Cytochrome P450 Peroxygenase $OleT_{JE}$. *J. Biol. Chem.* **2017**, *292,* 5128-5143.

(36) Liu, Y.; Wang, C.; Yan, J.; Zhang, W.; Guan, W.; Lu, X.; Li, S. Hydrogen Peroxide-Independent Production of α-Alkenes by $OleT_{JE}$ P450 Fatty Acid Decarboxylase. *Biotechnol. Biofuels* **2014, 7,** 28.

(37) Dennig, A.; Kuhn, M.; Tassoti, S.; Thiessenhusen, A.; Gilch, S.; Bülter, T.; Haas, T.; Hall, M.; Faber, K. Oxidative Decarboxylation of Short-Chain Fatty Acids to 1-Alkenes. *Angew. Chem. Int. Ed.* **2015**, *54,* 8819-8822.

(38) Wang, J. B.; Lonsdale, R.; Reetz, M. T. Exploring Substrate Scope and Stereoselectivity of P450 Peroxygenase $OleT_{JE}$ in Olefin-Forming Oxidative Decarboxylation. *Chem. Commun.* **2016**, *52,* 8131-8133.

(39) Dennig, A.; Kurakin, S.; Kuhn, M.; Dordic, A.; Hall, M.; Faber, K. Enzymatic Oxidative Tandem Decarboxylation of Dioic

Acids to Terminal Dienes. *Eur. J. Org. Chem.* **2016**, *2016,* 3473-3477.

(40) Narhi, L. O.; Fulco, A. J. Identification and Characterization of Two Functional Domains in Cytochrome P450BM-3, a Catalytically Self-Sufficient Monooxygenase Induced by Barbiturates in *Bacillus megaterium*. *J. Biol. Chem.* **1987**, *262,* 6683-6690.

(41) Govindaraj, S.; Poulos, T. L. The Domain Architecture of Cytochrome P450BM-3. *J. Biol. Chem.* **1997**, *272,* 7915-7921.

(42) Boddupalli, S. S.; Estabrook, R. W.; Peterson, J. A. Fatty Acid Monooxygenation by Cytochrome P450BM-3. *J. Biol. Chem.* **1990**, *265,* 4233-4239.

(43) Girvan, H. M.; Seward, H. E.; Toogood, H. S.; Cheesman, M. R.; Leys, D.; Munro, A. W. Structural and Spectroscopic Characterization of P450 BM3 Mutants with Unprecedented P450 Heme Iron Ligand Sets: New Heme Ligation States Influence Conformational Equilibria in P450 BM3. *J. Biol. Chem.* **2007**, *282,* 564-572.

(44) Johannes, T. W.; Woodyer, R. D.; Zhao, H. Directed Evolution of a Thermostable Phosphite Dehydrogenase for NAD(P)H Regeneration. *Appl. Environ. Microbiol.* **2005**, 71, 5728-5734.

(45) Woodyer, R. D.; van der Donk, W. A.; Zhao, H. Optimizing a Biocatalyst for Improved NAD(P)H Regeneration: Directed Evolution of Phosphite Dehydrogenase. *Comb. Chem. High Throughput Screening* **2006**, 9, 237-245.

(46) Johannes, T. W.; Woodyer, R. D.; Zhao, H. Efficient Regeneration of NADPH Using an Engineered Phosphite Dehydrogenase. *Biotechnol. Bioeng.* **2007**, 96, 18-26.

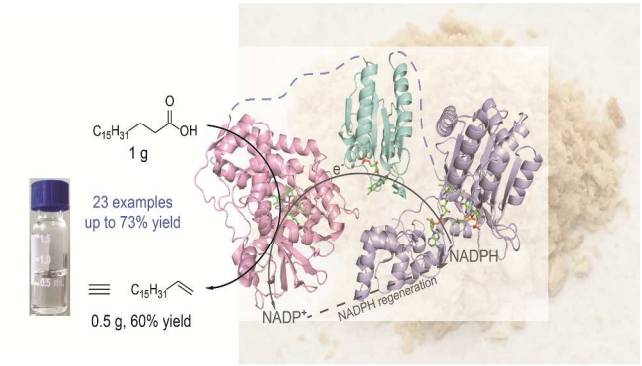
(47) Jamakhandi, A. P.; Jeffus, B. C.; Dass, V. R.; Miller, G. P. Thermal Inactivation of the Reductase Domain of Cytochrome P450 BM3. *Arch. Biochem.Biophys.* **2005**, 439, 165-174.

(48) Grant, J. L.; Hsieh, C. H.; Makris, T. M. Decarboxylation of Fatty Acids to Terminal Alkenes by Cytochrome P450 Compound I. *J. Am. Chem. Soc.* **2015**, 137, 4940–4943.

(49) Faponle, A. S.; Quesne, M. G.; de Visser; S. P. Origin of the Regioselective Fatty-Acid Hydroxylation versus Decarboxylation by a Cytochrome P450 Peroxygenase: What Drives the Reaction to Biofuel Production? *Chem. Eur. J.* **2016**, 22, 5478-5483.

(50) Grant, J. L.; Mitchell, M. E.; Makris, T. M. Catalytic Strategy for Carbon–Carbon Bond Scission by the Cytochrome P450 OleT. *Proc. Natl. Acad. Sci. USA.* **2016**, 113, 10049-10054.

(51) Klein, M. L.; Fulco, A. J. Critical Residues Involved in FMN Binding and Catalytic Activity in Cytochrome $P450_{BM-3}$. *J. Biol. Chem.* **1993**, 268, 7553-7561.


$C_{15}H_{31}$
O
OH
1 g
23 examples
up to 73% yield
$C_{15}H_{31}$
0.5 g, 60% yield
e⁻
NADPH
NADP+
NADPH regeneration