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CHEMPHOTOCHEM Communications

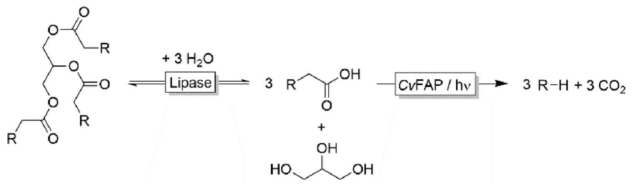
Photoenzymatic Production of Next Generation Biofuels from Natural Triglycerides Combining a Hydrolase and a Photodecarboxylase

Yunjian Ma,[a] Xizhen Zhang,[b] Wuyuan Zhang,[c] Peilin Li,[a] Yongru Li,[a] Frank Hollmann,*[c] and Yonghua Wang*[a]

A photobiocatalytic cascade transforming natural triglycerides into alkanes/alkenes is proposed. Starting from natural triglycerides, free fatty acids have been obtained using lipases. The free fatty acids were then, in a photoenzymatic step, decarboxylated into the C1-shortened alkanes using a recently described photodecarboxylase from *Chlorella variabilis* NC64A. This cascade produced alkanes from various natural (waste) oils in significant amounts (up to 24 g L^{-1}) and may provide a basis for valorisation of waste oils into a next generation of biodiesel.

The term Biodiesel usually refers to a class of vegetable oil-derived fatty acid esters (fatty acid methyl or ethyl esters, FAMEs and FAEEs, respectively) to substitute fossil-based alkanes for energetic use such as in transportation or heating. FAMEs and FAEEs are derived from the corresponding triglycerides via catalytic transesterification with methanol or ethanol.[1] Though increasingly being used worldwide, the synthesis of FAMEs and FAEEs is hampered by the reversibility of this reaction necessitating significant molar surpluses of the alcohol to shift the thermodynamic equilibrium.[1]

An alternative approach to valorise (waste) vegetable oils into biofuel is to convert them into the corresponding (C1-shortened) alkanes via decarboxylation (Scheme 1). First, the irreversible nature of the reaction facilitates process design. Second, the alkanes exhibit, compared to the corresponding FAMES and FAEEs, higher specific caloric values.



Scheme 1. Envisioned bienzymatic cascade to transform natural triglycerides into alkanes.

Very recently, Beisson and co-workers reported on an enzyme from the microalga *Chlorella variabilis* NC64A (*Cv*FAP) catalysing the photocatalytic decarboxylation of fatty acids,[2] which ever since has caused some attention.[3] *Cv*FAP contains a (photo)catalytic flavin prosthetic group. Spectroscopic and modelling evidence suggests that the photoexcited flavin ($\lambda =$ 450 nm) abstracts one electron from the acid starting material, which then rapidly eliminates CO_2 leaving an alkyl radical behind. The latter forms the alkane after back-transfer of one electron from the flavin semiquinone and protonation. Typical radical by-products have not been observed, due to the confinement of the alkyl radical in the enzyme's active site.[3b]

To utilise natural oils as starting material, we have proposed a bienzymatic cascade comprising a hydrolase (lipase) to cleave the ester functions of the triglyceride and generating the starting material for *Cv*FAP (Scheme 1).

The aim of this study was to expand the scope of the photoenzymatic reaction to 'real' substrates and to outline the current limitations of the reaction setup.

*Cv*FAP was produced via recombinant expression in *Escherichia coli* following a previously established protocol (see SI for a full description of the expression procedure).[3c] Having the photodecarboxylase at hand, we compared its catalytic performance using it either as cell free extract (*Cv*FAP CFE) or as whole cells (*Cv*FAP@*E. coli*) (Figure 1). Interestingly, product concentrations using *Cv*FAP@*E. coli* were significantly higher than those obtained from experiments using *Cv*FAP CFE indicating that confinement of the photodecarboxylase within the *E. coli* cells resulted neither in significant diffusion limitation of the reagents over the cell membrane nor in reduced activity due to light scattering or absorption. For further experiments we therefore used *Cv*FAP@*E. coli*.

Next, we investigated the *in situ* hydrolysis of triglycerides combined to *Cv*FAP-catalysed decarboxylation of the resulting fatty acids. Envisioning a one-pot-one-step cascade, we focused on lipase-catalysed hydrolysis due to the suspected compati-

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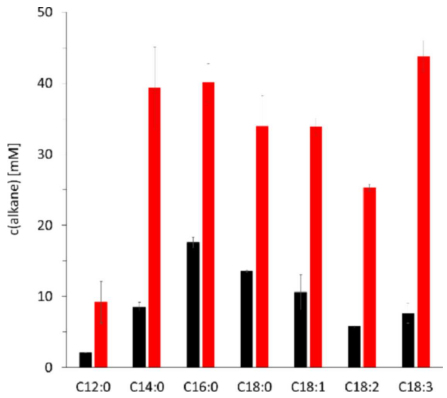

Figure 1. Comparison of the photodecarboxylation activity of *Cv*FAP as cell free extract (CFE *Cv*FAP; black) and as whole cells (*Cv*FAP@*E. coli*; red). (C12:0): Dodecanoic acid; (C14:0): Tetradecanoic acid; (C16:0): Palmitic acid; (C18:0): Stearic acid; (C18:1): Oleic acid; (C18:2): Linoleic acid; (C18:3): Linolenic acid. Reaction conditions: 700 µL of Tris-HCl buffer (pH 8.5, 100 mM) containing CFE *Cv*FAP or wet *Cv*FAP@*E. coli* and 300 µL of free fatty acid stock solution in DMSO were added to a transparent glass vial (total reaction volume was 1 mL). The final conditions of this reaction were: DMSO 30% (v:v), [CFE *Cv*FAP] = 8 µM or [Wet *Cv*FAP@*E. coli*] = 0.25 g mL^{-1}, [Free fatty acid] = 50 mM, 30 °C, blue LED, 24 h.

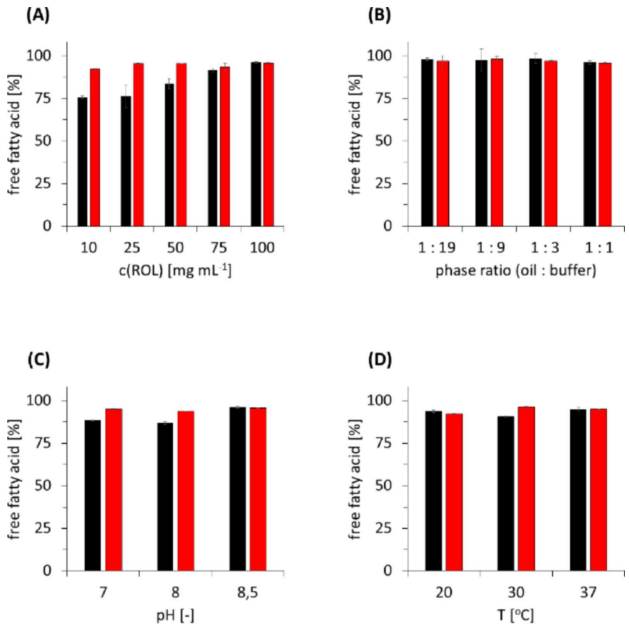

Figure 2. Basic characterisation of ROL under the reaction conditions required for the one-pot–one-step hydrolysis/decarboxylation reaction. Red: waste cooking oil; Black: soybean oil. Reaction conditions: (A): reaction volume: 1 mL in total, 500 µL oil, 500 µL Tris-HCl buffer (pH 7.0, 100 mM), 37 °C, 500 rpm, 24 h; (B): reaction volume: 1 mL in total, 950–500 µL Tris-HCl buffer (pH 7.0, 100 mM), 100 mg ROL, 37 °C, 500 rpm, 24 h; (C): reaction volume: 1 mL in total, 500 µL oil, 500 µL Tris-HCl buffer (100 mM), 100 mg ROL, 37 °C, 500 rpm, 24 h; (D): reaction volume: 1 mL in total, 500 µL oil, 500 µL Tris-HCl buffer (pH 8.5, 100 mM), 100 mg ROL, 500 rpm, 24 h. At the end of the reaction the phases were separated via centrifugation, a sample was taken from the hydrophobic phase and analysed by HPLC.

bility of both enzyme systems. As biocatalyst we chose the immobilised lipase from *Rhizopus oryzae* (ROL).[4] As starting material we evaluated native soybean oil and waste cooking oil (Figure S4). While the native soybean oil was essentially pure triglyceride, the waste cooking oil was already significantly hydrolysed (approx. 60% of the fatty acids were present in free form). Pleasingly, ROL proved to be very active and robust under the reaction conditions envisioned for the bienzymatic cascade. Already at comparably low lipase loadings of 10 mg mL^{-1} (immobilised enzyme, the immobilised enzyme of ROL contains 20 mg pure enzyme per gram, the hydrolytic activity of immobilised ROL enzyme is 200000 U g^{-1} immobilised enzyme) more than 75% hydrolysis of soybean oil was achieved within 24 h. Using 100 mg mL^{-1} (immobilised enzyme), hydrolysis was almost quantitative (Figure 2a). In case of waste cooking oil, the extent of hydrolysis was always somewhat higher, due to the higher initial content of free fatty acids. The volume ratio of fatty acid/oil phase to the aqueous reaction buffer had no significant influence on the hydrolysis (Figure 2b). The same is true for the pH- and T-range of the hydrolysis reaction (Figure 2c and d). Under optimised conditions, full hydrolysis of both, waste cooking oil and soybean oil was achieved within 24 h (Figure S5).

We also tested the stability of ROL under illumination with blue light and found no significant influence of blue light on the activity of ROL over time (Figure S6). Overall, ROL and *Cv*FAP appeared to be highly compatible.

Therefore, we next used both catalysts in a one-pot-one-step reaction cascade. As shown in Figure 3, this approach indeed lead to the near-complete hydrolysis/decarboxylation of both soybean oil and waste cooking oil.

The alkane composition of the products corresponded well with the fatty acid composition of the starting materials (Table 1) indicating that the majority of the carboxylic acids (esters) present in the starting material were transformed into the corresponding (C1-shortened) alkanes.

Table 1. Comparison of the fatty acid composition of the starting material and the alkane/alkene composition of the corresponding product.[a]

	Fatty acid	Abundance [%]	Alkane/alkene (concentration [mM])	Abundance [%]
Soybean oil	C16:0	11.8	C15 (8.3)	11.3
	C18:0	3.7	C17 (0.7)	1.0
	C18:1	25.8	C17:1 (23.8)	32.2
	C18:2	51.4	C17:2 (36.9)	49.8
	C18:3	5.2	C17:3 (3.3)	4.5
	others	2.2	0.9	1.2
	Sum	100	74.1	100
Waste cooking oil	C16:0	20.0	C15 (8.9)	16.3
	C18:0	5.4	C17 (4.9)	9.0
	C18:1	40.0	C17:1 (23.1)	42.2
	C18:2	26.9	C17:2 (15.6)	28.5
	C18:3	2.2	C17:3 (1.2)	2.2
	others	5.5	1.0	1.8
	Sum	100	54.7	100

[a] Reaction conditions: 950 µL of Tris-HCl buffer (pH 8.5, 100 mM) containing *Cv*FAP@*E. coli*, 100 mg ROL and 50 µL of oil were added to a transparent glass vial (total reaction volume is 1 mL). The final conditions of this reaction were: [*Cv*FAP@*E. coli*] = 0.35 g mL^{-1}, [ROL] = 0.1 g mL^{-1}, [Oil] = 50 µL mL^{-1}, 30 °C, blue LED, 24 h. (C16:0): Palmitic acid; (C18:0): Stearic acid; (C18:1): Oleic acid; (C18:2): Linoleic acid ; (C18:3): Linolenic acid, (C15): Pentadecane; (C17): Heptadecane; (C17:1): (Z)-heptadec-8-ene; (C17:2): (6Z,9Z)-heptadeca-6,9-diene; (C17:3): (3Z,6Z,9Z)-heptadeca-3,6,9-triene. Abundance = relative amount (of total fatty acids or alkanes).

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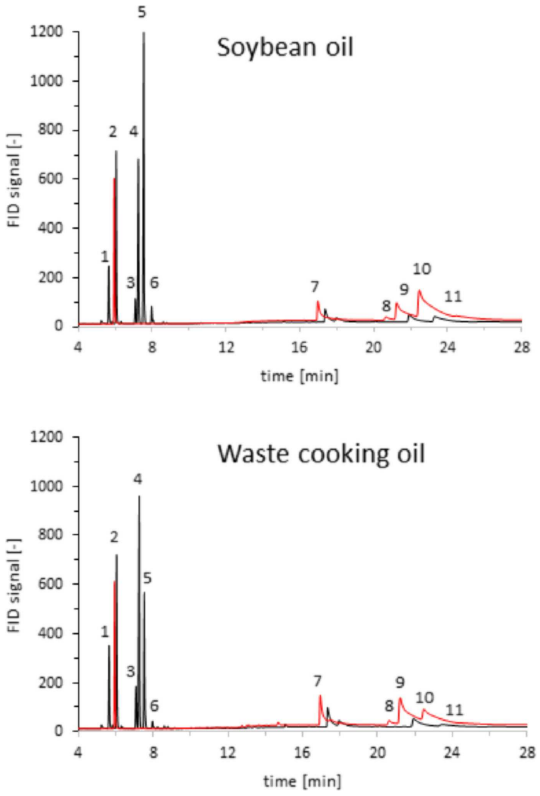

Figure 3. Component analysis of soybean oil and waste cooking oil before (black) and after decarboxylation (red). Samples were analysed via gas chromatography, annotation of the FID signals was based on authentic standards: **1**: Pentadecane (C15), **2**: n-Octanol (internal standard), **3**: Heptadecane (C17), **4**: (Z)-heptadec-8-ene (C17:1), **5**: (6Z,9Z)-heptadeca-6,9-diene (C17:2), **6**: (3Z,6Z,9Z)-heptadeca-3,6,9-triene (C17:3), **7**: Palmitic acid (C16:0), **8**: Stearic acid (C18:0), **9**: Oleic acid (C18:1), **10**: Linoleic acid (C18:2), **11**: Linolenic acid (C18:3)). Reaction conditions: 950 µL of Tris-HCl buffer (pH 8.5, 100 mM) containing *Cv*FAP@*E. coli*, 100 mg ROL and 50 µL of oil were added to a transparent glass vial (total reaction volume is 1 mL). The final conditions of this reaction were: [*Cv*FAP@*E. coli*] = 0.35 g mL^{-1}, [ROL] = 0.1 g mL^{-1}, [Oil] = 50 µL mL^{-1}, 30 °C, blue LED, 24 h.

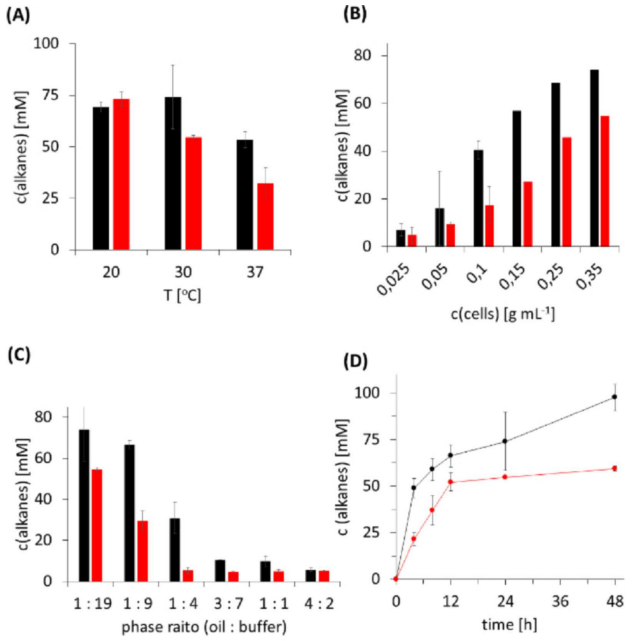

Figure 4. Optimisation of the one-pot-one-step hydrolysis/decarboxylation cascade of soybean oil (black) and waste cooking oil (red). The influence of temperature (A), concentration of wet *Cv*FAP@*E. coli* (B), substrate addition (C) and time course of the cascade reaction (D). The final conditions of this reaction were: [*Cv*FAP@*E. coli*] = 0.025–0.35 g mL^{-1}, [ROL] = 0.1 g mL^{-1}, [Oil] = 50–800 µL mL^{-1}, Tris-HCl buffer (pH 8.5, 100 mM), 20–37 °C, blue LED, 24 h.

Encouraged by these findings, we further characterised the cascade reaction. As shown in Figure 4A, product formation decreased with increasing reaction temperature, which we attribute to the rather poor thermal stability of the wild-type *Cv*FAP.[2a] Quite expectedly, the photocatalyst concentration also positively influenced the product formation (Figure 4B). At first sight unexpected was that increasing substrate amounts negatively influenced the overall product formation (Figure 4C). This can, however, be rationalised by taking the pH of the reaction mixture into account. In case of waste cooking oil, higher acid concentrations lead to a significant drop of pH whereas in case of soybean oil, this effect was achieved indirectly after ROL-catalysed hydrolysis. The more acidic pH values of the aqueous phases resulted in significantly decreased *Cv*FAP activity. These findings also underline the importance of an optimised activity ratio of hydrolase and photodecarboxylase to maintain overall slightly basic reaction conditions as required by *Cv*FAP. To avoid accumulation of the fatty acid (and concomitant acidification of the reaction buffer) the photodecarboxylase activity should be higher than the hydrolase activity. Taking these boundaries into account, the cascade reaction starting from soybean oil proceeded continuously for 48 h yielding 100 mM of the corresponding alkane products (Figure 4D) corresponding to a product concentration in the range of 24 g L^{-1}.

To confirm the broad applicability of the proposed one-pot-one-step hydrolysis/decarboxylation cascade, we broadened the substrate range to various other natural oils. In total 17 commercially available oils were evaluated (Table 2). Pleasingly, most of the oils evaluated were converted into the corresponding alkane mixtures at significant conversions. Using triolein as basis, the overall concentration of fatty acids was estimated to be around 150 mM. Obviously, this number is only a rough estimate as the densities and fatty acid compositions of the oils used here may considerably vary from the numbers for triolein; nevertheless, we believe that this comparison gives a realistic order of magnitude to assess the efficiency of the cascade reaction. The estimated product concentrations varied between 3.9 mM and 74.1 mM corresponding to approximately 2.6% and 49.4% yield (average yield: 31.3 ± 12.1%). Coconut oil gave poor conversion, which most likely can be attributed to its carboxylic acid composition consisting of more than 75% of C14 and shorter carboxylic acids. These shorter carboxylic acids are rather poor substrates for CvFAP thereby rationalising the poorer alkane yield in this case. Reactions performed at 30 °C gave higher product yields compared to those conducted at 37 °C, consistent with the observation in Figure 4A.

Finally, a semi-preparative scale transformation of soybean oil was conducted. From 15 mL reaction scale (in 6 batches),

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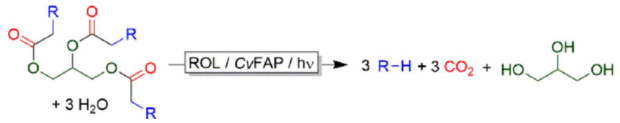
Table 2. Photoenzymatic hydrolysis/decarboxylation of various oils in a one-pot-one-step cascade.[a]



Entry	Substrate	Reaction temperature [°C]	Product [mM]
1	Waste cooking oil	30	54.7 ± 0.8
2		37	32.3 ± 7.8
3	Soybean oil	30	74.1 ± 15.5
4		37	53.4 ± 3.9
5	Tea seed oil	30	56.9 ± 7.3
6		37	44.8 ± 2.7
7	Rice bran oil-1[b]	30	71.4 ± 1.1
8		37	49.6 ± 5.9
9	Rice bran oil-2[b]	30	61.8 ± 2.8
10		37	41.9 ± 3.1
11	Rapeseed oil	30	62.2 ± 4.5
12		37	38.3 ± 0.5
13	Rapeseed oil (low erucic	30	50.8 ± 11.0
14	acid)	37	26.1 ± 8.7
15	Cottonseed oil	30	67.9 ± 9.0
16		37	47.5 ± 3.9
17	Olive oil	30	46.0 ± 7.5
18		37	38.2 ± 2.1
19	Peanut oil	30	54.4 ± 1.2
20		37	34.5 ± 0.5
21	Canola oil	30	49.2 ± 3.2
22		37	39.0 ± 0.9
23	Sunflower seed oil	30	73.0 ± 14.0
24		37	46.9 ± 1.1
25	Sea buckthorn oil	30	31.6 ± 3.6
26		37	13.6 ± 2.5
27	Linseed oil	30	73.0 ± 4.8
28		37	37.5 ± 2.5
29	Coconut oil	30	8.2 ± 0.1
30		37	3.9 ± 0.004
31	Corn oil	30	67.3 ± 8.3
32		37	44.7 ± 5.5
33	Palm oil (melting point	30	70.0 ± 10.3
34	24 °C)	37	33.6 ± 2.6

[a] Reaction conditions: 950 µL of Tris-HCl buffer (pH 8.5, 100 mM) containing *Cv*FAP@*E. coli*, 100 mg ROL and 50 µL of oil were added to a transparent glass vial (total reaction volume was 1 mL). The final conditions of this reaction were: [*Cv*FAP@*E. coli*] = 0.35 g mL^{-1}, [ROL] = 0.1 g mL^{-1}, [Oil] = 50 µL mL^{-1}, blue LED, 24 h. [b] Two different batches from different suppliers were used.

0.91 g final product (56.1 mM alkanes, 28.1 % conversion and 21.2 % isolated yield) was isolated (Figure S11).

Overall, we have demonstrated the potential of use the *Cv*FAP to produce biofuels from (waste) oils. Despite the rather early stage of development, very significant product titres have been achieved already demonstrating the synthetic potential of this procedure. We are convinced that further optimisation of the reaction setup and of the biocatalyst(s) will yield a practical approach to valorise non-edible triglycerides and acids into biofuels. Compared to the state-of-the-art, this methodology excels by the lower energy demand (especially if sunlight is used) and the irreversible nature of the reaction (making large molar excesses of alcohols unnecessary). Therefore, we are convinced that this may lead to a practical system for the preparation of a next generation of bio-based fuels.

Experimental Section

Chemical Reagents and Materials

All chemicals were purchased from Sigma-Aldrich, TCI or Aladdin in the highest purity available and used without further purification. Lipase ROL (*Rhizopus oryzae* lipase, 20 mg ROL per gram of immobilisate, A_{spec} = 200000 Ug^{-1}) was a gift by Guangdong VTR Bio-Tech Co., Ltd (Zhuhai, China). Waste cooking oil, soybean oil and other vegetable oils were friendly provided by Guangzhou Zhizhiyuan Oil Industry Co., Ltd. (Guangzhou, China). Water was purified with a Millipore (Bedford, MA) Milli-Q water system.

Experimental Set-Up and Operating Conditions

For the detection of alkanes and free fatty acids: An Agilent 7890B GC system (Agilent Technologies, Palo Alto, CA, USA) was used together with an KB-FFAP GC column (Kromat Corporation, 4 Providence Court, Delran, NJ08075, USA. 30 m length × 0.25 mm I. D. × 0.25 µm film thickness) Method: injector temperature: 250 °C; split mode: 30 : 1; detector temperature: 280 °C; GC oven temperature program: initial 110 °C, hold for 3.4 min, then from 110 °C to 190 °C at a ramp rate of 25 °C min^{-1}, hold for 2.1 min, from 190 to 230 °C at a ramp rate of 25 °C min^{-1}, then hold for 2 min, from 230 to 250 °C at a ramp rate of 30 °C min^{-1}, then hold for 12 min. Retention time are shown in Table S1.

For the detection of products after enzymatic hydrolysis of oils: The products were analysed using HPLC (Waters, 1525) equipped with a phenomenex luna silica column (250 mm × 4.6 mm i.d., 5 µm particle size, Phenomenex Corporation, Torrance, CA, America) and a refractive index detector (Waters, 2414). Injection quantity: 10 µL. The mobile phase was a mixture of *n*-hexane, 2-propanol and formic acid (18 : 1 : 0.003, v : v : v) and performed with a flow rate of 1 mL min^{-1} at 30 °C. The retention time for triglyceride; free fatty acid; 1,3-glycerol diester; 1,2-glycerol diester were 3.66 min, 4.04 min, 4.75 min and 6.05 min, respectively. Waters 2695 integration software was employed to calculate the peak-areas percentages.

Preparation of the Cell-Free Extract Containing *Cv*FAP (CFE *Cv*FAP) and Whole-Cell *Cv*FAP (*Cv*FAP@*E. coli*)

Production and isolation of recombinant *Cv*FAP enzyme conducted in Escherichia coli according to the method previously reported with minor modification.[3b,c]

Cultivation Protocol

Pre-culture: Pre-culture was inoculated with *E. coli* BL21 (DE3) cells harboring the designed pET28a-His-Trx-FAP plasmid, and overnight culture (ONC) in terrific broth (TB) medium (TB medium: (yeast extract 2.4 % (w/v), peptone 1.2 % (w/v), glycerol 0.4 % (v/v), 72 mM K_2HPO_4, 17 mM KH_2PO_4) containing 50 µg mL^{-1} kanamycin, incubation at 37 °C at 200 rpm.

Main culture: The main culture consisted of 500 mL TB and 50 µg mL^{-1} kanamycin in 2 L Erlenmeyer flask, inoculated with the corresponding volume of the ONC constituting a start OD600 of 0.1. The main culture was incubated at 37 °C, 200 rpm for approx. 3 until 4 h to reach an OD600 value of 0.7–0.8.

Induction: After reaching the desired OD600, protein production was induced by the addition of 0.5 mM IPTG and the cells were left at 17 °C and 180 rpm for about 20 h.

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Harvest: For the cell free extract containing *Cv*FAP (CFE *Cv*FAP), the cell pellet was collected by centrifugation (4000×g, 30 min, 4 °C) followed by supersonic treatment. Washed with Tris-HCl buffer (50 mM, pH 8, containing 100 mM NaCl), and centrifuged again (10000×g, 20 min, 4 °C). The cell pellet was resuspended in the same buffer, and 1 mM PMSF and 5% glycerol (w/v) was added to the soluble fraction, the cell extract was separated into aliquots, frozen in liquid nitrogen, and stored at −20 °C. For the whole cell *Cv*FAP (*Cv*FAP@*E. coli*), the cell pellet was collected by centrifugation (4000×g, 30 min, 4 °C). Washed with Tris-HCl buffer (50 mM, pH 8, containing 100 mM NaCl), and centrifuged again (10000×g, 20 min, 4 °C). The cell pellet was resuspended in the same buffer, and 1 mM PMSF and 5% glycerol (w/v) was added to the soluble fraction, the *Cv*FAP@*E. coli* frozen in liquid nitrogen, and stored at −20 °C. 1 g of wet *Cv*FAP@*E. coli* corresponds to 250 mg dry *Cv*FAP@*E. coli*. As a control, a whole cell of *E. coli* BL21 (DE3) cells harboring an empty pET28a vector was prepared according to the same protocol.

Free Fatty Acids Standard Photoenzymatic Decarboxylation Reactions Catalysed by CFE *Cv*FAP and *Cv*FAP@*E. coli*

The free fatty acids standard photoenzymatic decarboxylation reactions catalysed by CFE *Cv*FAP and *Cv*FAP@*E. coli* were performed at 30 °C (unless indicated otherwise) in a total volume of 1.0 mL Tris-HCl buffer (pH 8.5, 100 mM) containing 30% DMSO as cosolvent.

For CFE *Cv*FAP photoenzymatic decarboxylation reaction, unless mentioned otherwise, 700 μL of Tris-HCl buffer (pH 8.5, 100 mM) containing CFE *Cv*FAP and 300 μL of free fatty acid stock solution in DMSO were added to a transparent glass vial (total reaction volume is 1 mL).The final conditions of this reaction were: DMSO 30% (v:v), [CFE *Cv*FAP] = 8 μM, [Free fatty acid] = 50 mM. Reaction mixtures were thermostatted at the reaction temperatures indicated, stirred at 500 rpm and illuminated with blue LEDs (10 W, 220 V) for 24 h, the reaction temperature is 30 °C. Afterwards, the entire reaction mixtures were extracted with ethyl acetate (containing 25 mM of 1-octanol as internal reference) in a 2:1 ratio (v:v) and analysed via GC chromatography.

For *Cv*FAP@*E. coli* photoenzymatic decarboxylation reaction, unless mentioned otherwise, 700 μL of Tris-HCl buffer (pH 8.5, 100 mM) containing 0.25 g wet *Cv*FAP@*E. coli* and 300 μL of free fatty acid stock solution in DMSO were added to a transparent glass vial (total reaction volume is 1 mL).The final conditions of this reaction were: DMSO 30% (v:v), [Wet *Cv*FAP@*E. coli*] = 0.25 g mL^{-1}, [Free fatty acid] = 50 mM. Reaction mixtures were thermostatted at the reaction temperatures indicated, stirred at 500 rpm and illuminated with blue LEDs (10 W, 220 V) for 24 h, the reaction temperature is 30 °C. Afterwards, the entire reaction mixtures were extracted with ethyl acetate (containing 25 mM of 1-octanol as internal reference) in a 2:1 ratio (v:v) and analyzed via GC chromatography. The homemade experimental setup is shown in Figure S3.

Enzymatic Hydrolysis Reactions of Oils

The enzymatic hydrolysis reactions of oils were performed in a 5 mL glass bottle containing 500 μL of oil, 500 μL Tris-HCl buffer (pH 8.5, 100 mM) together with 100 mg of Lipase ROL (*Rhizopus oryzae* lipase on resin). The reaction flasks were placed into oil bath with 500 rpm of the rotate speed at 30 °C for 24 h. After the reaction is over, the mixture was centrifuged at 10000×g for 3 minutes. the upper layer (reservoir) will be used for high performance liquid chromatography (HPLC) analysis. Absorbing 30 μL upper reservoir into the 1.5 mL chromatographic bottle, then dissolve and mix well with 970 μL mobile phase (mixture of *n*-hexane, 2-propanol and formic acid (18:1:0.003, v:v:v)) for HPLC analysis.

One-Pot-One-Step Hydrolysis/Decarboxylation Reaction Cascade Transforming Oils into Alkanes/Alkenes

A one-pot-one-step hydrolysis/decarboxylation reaction cascade transforming oils into alkanes / alkenes: 950 μL of Tris-HCl buffer (pH 8.5, 100 mM) containing 0.35 g wet *Cv*FAP@*E. coli*, 100 mg ROL and 50 μL of oil were added to a transparent glass vial (total reaction volume is 1 mL). The final conditions of this reaction were: [Wet *Cv*FAP@*E. coli*] = 0.35 g mL^{-1}, [ROL] = 0.1 g mL^{-1}, [Oil] = 50 μL mL^{-1}, Reaction mixtures were thermostatted at the reaction temperatures indicated, stirred at 500 rpm and illuminated with blue LEDs (10 W, 220 V) for 24 h. Afterwards, the entire reaction mixtures were extracted with ethyl acetate (containing 25 mM of 1-octanol as internal reference) in a 2:1 ratio (v:v) and analysed via GC chromatography. The homemade experimental setup is shown in Figure S3.

Preparative-Scale Synthesis of Alkanes/Alkenes

The preparative-scale synthesis of alkanes / alkenes was performed on a 15 mL scale, For this reaction, 14 mL of Tris-HCl buffer (pH 8.5,100 mM) containing 4.0 g wet *Cv*FAP@E. coli, 200 mg of Lipase ROL (Rhizopus oryzae lipase on resin) and 1.0 mL of soybean oil were added to a transparent glass vial (total volume 15 mL). The vial was sealed and exposed to blue LED light under gentle magnetic stirring at 30 °C. The reaction continued overnight (24 h). The final conditions of this reaction were: [soybean oil] = 67 μL mL^{-1}, [Lipase ROL] = 13 mg mL^{-1} and [Wet *Cv*FAP@E. coli] = 0.26 g mL^{-1}, 30 °C. The reaction was performed in 6 batches. GC analysis showed that 56.1 mM of alkanes / alkenes were obtained at the end of the reaction. The remaining substrates and products were extracted with ethyl acetate (30 mL, 2×) and the organic phases were combined. After the removal of diethyl ether under vacuum in a rotary evaporator, the product was suspended in water and treated with concentrated NaOH. The remaining mixture was filtered and the aqueous phase was extracted again with ethyl acetate (10 mL, 2×), dried over Na_2SO_4 and removed afterwards under vacuum. The crude products were purified by using a silicon column (heptane with 1% ethyl acetate as the eluent). The final product was weighed (0.91 g from 6 batches). Conversion: 28.1%. Isolated Yield: 21.2%.

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Conflict of Interest

The authors declare no conflict of interest.

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