Supporting Information

**Light-Driven Enzymatic Decarboxylation of Fatty Acids**

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Supporting Information
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Table of Contents

Experimental Procedures ............................................................................................................................................................ 3
  Chemicals ............................................................................................................................................................................. 3
  Cloning of the CvFAP ........................................................................................................................................................... 3
  Preparation of the cell free extract containing CvFAP (full-length/short-length) ......................................................... 3
  Purification of CvFAP (full-length/short-length) ............................................................................................................... 3
  Photocatalytic setup ............................................................................................................................................................. 4
  Enzymatic cascade reactions transforming triglycerides into alkanes ............................................................................. 4
  Preparative-scale synthesis of pentadecane ................................................................................................................... 4

Supplementary Figures ............................................................................................................................................................... 6

Supplementary Tables .............................................................................................................................................................. 17

Gas Chromatography ................................................................................................................................................................ 19

Author Contributions ................................................................................................................................................................. 19

References ................................................................................................................................................................................ 19
Experimental Procedures

Chemicals

Unless indicated otherwise all chemicals were purchased from Sigma-Aldrich, Fluka, Acros or Alfa-Aesar in the highest purity available and used without further treatment. Lipase from *Candida rugose* (890 units/mg) was bought from Aldrich (in 1989) and used directly.

Cloning of CvFAP

For production of CvFAP in *E. coli*, two constructs were designed based on a previously reported construct\(^1\). The constructs both consist of sequentially a 6x His-tag, thioredoxin (TrxA) tag, tobacco etch virus (TEV) protease cleavage site and the gene coding for CvFAP (GenBank: KY511411.1). The first construct comprises the full-length sequence (residues 1-654) of CvFAP, while the second construct lacks the residues encoding for a predicted chloroplast targeting sequence and thereby comprises residues 62-654 of CvFAP (Fig. S1). The sequence coding for CvFAP was codon optimized for expression in *E. coli*. The construct was synthesized by Baseclear (Leiden, the Netherlands) and cloned into a pET28a vector using *Nde*\(_I\) and *Hind*\(_III\) restriction sites. Competent *E. coli* BL21 (DE3) cells (NEB) were transformed with the plasmid for recombinant enzyme production.

Preparation of the cell free extract containing CvFAP (full-length/short-length)

10 mL pre-cultures were inoculated with *E. coli* BL21 (DE3) cells harboring the designed pET28a-His-TrxA-CvFAP plasmid. These cultures were grown overnight in terrific broth (TB) medium, containing 50 μg/mL kanamycin. The pre-cultures were used to inoculated large cultures (500 mL TB + 50 μg/mL kanamycin in 2 L shake flasks). Cells were grown at 37 °C, 180 rpm, until an OD\(_{600}\) between 0.7-0.8 was reached. Protein production was induced by the addition of 0.5 mM IPTG and the cells were left at 17 °C, 180 rpm, for about 20 hours. Cells were harvested by centrifugation (11000 g at 4 °C for 10 min), washed with Tris-HCl buffer (50 mM, pH 8, containing 100 mM NaCl) and centrifuged again. The cell pellet was resuspended in the same buffer, and 1 mM PMSF was added. Cells were lysed by passing them passed twice through a Multi Shot Cell Disruption System (Constant Systems Ltd, Daventry, UK) at 1.5 kbar, followed by centrifugation of the cell lysate (38000 g at 4 °C for 1 h). After centrifugation, 5% glycerol (w/v) was added to the soluble fraction, the cell extract was aliquoted, frozen in liquid nitrogen and stored at -80 °C.

The total protein content of the cell extract was determined by a BCA Assay (Interchim), using BSA as a standard. CvFAP production was analyzed by SDS-PAGE (Figure S2) using a Criterion™ Cell electrophoresis system (Bio-Rad). As a molecular weight marker, Precision Plus Protein Standard (Bio-Rad) was used. The gel was analyzed using a gel imaging system (GBox, Syngene, Cambridge, UK) and the amount of CvFAP in the cell extract was estimated from the relative intensity of the bands on gel.

As a control, a cell free extract of *E. coli* BL21 (DE3) cells harboring an empty pET28a vector was prepared according to the same protocol.

Purification of CvFAP (full-length/short-length)

Part of the cell extract of full-length and short-length CvFAP was used for purification. To the extracts, which were in a Tris-HCl buffer (pH 8, 50 mM, containing 100 mM NaCl), imidazole and NaCl were added to final concentrations of respectively 10 mM and 200 mM. In addition, a pinch of DNase and 1 mM MgCl\(_2\) were supplemented. The extracts were centrifuged (5000 g, 4 °C, 15 min) to remove any insoluble parts. Next, the cell extract was loaded on a 5 mL Ni-NTA column, equilibrated in a Tris-HCl buffer (pH 8, 50 mM) containing 300 mM NaCl, 10 mM imidazole and 5% (w/v) glycerol. After extensive washing with the same buffer, bound protein was eluted with a Tris-HCl buffer (pH 8, 50 mM), containing 300 mM NaCl, 500 mM imidazole and 5% (w/v) glycerol in a one-step elution. The eluate was incubated on ice with 1 mM FAD After 60 min of incubation, the enzyme solution was desalted twice using PD-10
SUPPORTING INFORMATION

Desalting columns (GE Healthcare) in order to remove excess FAD. Finally, the samples were concentrated using an Amicon® Ultra-15 centrifugal filter device (30 kDa cutoff, Millipore). The final enzyme solution was aliquoted, frozen in liquid nitrogen and stored at -80 °C.

As for the cell extract, the total protein content of the purified enzyme solution was determined by a BCA Assay, and the purification was analyzed by SDS-PAGE (Figure S2), as described above for the cell extract.

Photocatalytic setup

The photoenzymatic decarboxylation reactions catalysed by CvFAP were performed at 37 °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. Unless mentioned otherwise, 200 μL of DMSO containing 65.5 mM of palmitic acid, 100 μL of pure DMSO, 500 μL of Tris-HCl buffer (pH 8.5, 100 mM) and 200 μL of CvFAP stock solution (30 μM cell extract in Tris-HCl buffer) were added to a transparent glass vial (total volume 5.0 mL). The vial was sealed and exposed to blue LED light under gentle magnetic stirring. The homemade setup is shown in Figure S3. The final conditions of this reaction were: [palmitic acid] = 13.1 mM and [CvFAP] = 6 μM in Tris-HCl (pH 8.5, 100 mM), 30 % DMSO. At intervals, aliquots were withdrawn and the reagents were extracted with ethyl acetate (containing 5 mM of 1-octanol as internal reference) in a 2:1 ratio (v/v). The remaining organic phase was analysed using Gas Chromatography.

Enzymatic cascade reactions transforming triglycerides into alkanes

One-pot one-step approach: a certain amount of lipase from Candida rugosa in 300 μL of Tris-HCl buffer (pH 8.5, 100 mM), 200 μL of CvFAP stock solution (30 μM cell extract in Tris-HCl buffer), and 500 μL of triolein as an organic phase were added to a transparent glass vial (total volume 5.0 mL). The vial was sealed and exposed to blue LED light under gentle magnetic stirring at 37 °C. At intervals, 10 μL of organic phase was withdrawn and treated with 30 μL of NaOH (12M) at 70 °C for 1.5 h. Subsequently, the mixture was extracted with ethyl acetate (containing 5 mM of 1-octanol as internal reference) and analysed using Gas Chromatography.

One-pot two-step approach: a certain amount of lipase from Candida rugosa in 500 μL of Tris-HCl buffer (pH 8.5, 100 mM) and 500 μL of triolein as an organic phase were added to a transparent glass vial (total volume 5.0 mL). The hydrolysis of triolein was then performed at 37 °C for 12 hours in a thermal shaker (700 rpm). After 12 hours, 200 μL of CvFAP stock solution (30 μM cell extract in Tris-HCl buffer) was added and the mixture was exposed to blue LED light under gentle magnetic stirring at 37 °C. At the end of the reaction, 10 μL of organic phase was withdrawn and treated with 30 μL of NaOH (12M) at 70 °C for 1.5 h. Subsequently, the mixture was extracted with ethyl acetate (containing 5 mM of 1-octanol as internal reference) and analysed using Gas Chromatography.

Preparative-scale synthesis of pentadecane

The preparative-scale synthesis of pentadecane was performed on a 10 mL scale. For this reaction, 8 mL of Tris-HCl buffer (pH 8.5, 100 mM) containing CvFAP and 1.5 mL of palmitic acid stock solution in DMSO 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 37 °C. After 4 hours and after 8 hours, 1.5 mL of palmitic acid stock solution in DMSO was added again to the reaction mixture. The reaction continued overnight (12.5 h). The final conditions of this reaction were: [palmitic acid] = 60 mM and [CvFAP] = 6 μM in Tris-HCl (pH 8.5,100 mM), 36 % DMSO, 37 °C. The reaction was performed in 2 batches. The remaining substrates and products were extracted with diethyl ether (20 mL, 2×) and the organic phases were combined. After the removal of diethyl ether under vacuum in a rotovap, the product was suspended in water and treated with concentrated NaOH. The remaining mixture was filtered and the aqueous phase was extracted again with diethyl ether (10 mL, 2×), dried over MgSO₄ and removed afterwards under vacuum. The final product was weighed (155mg from 2 batches). GC analysis showed that 47.5 mM of pentadecane was obtained at the end of the reaction. Conversion: 79%. Isolated Yield: 61%.
Docking of fatty-acids

Molecular docking analyses were performed employing AutoDock Vina algorithm. The crystal structure of CvFAP (PDB: 5NCC) was used as rigid receptor. Ligand structures were prepared using AutoDock Tools setting a free torsions for all the C-C bonds.
**Supplementary Figures**

**A 6xHis-TrxA-TEV-CvFAP (full-length)**

ATGAAACTTCTCTGGTTCTCTCATGAGCCGTAATAATATTATCTCTACGTACAGCAGACGCTTGGTTCTATGAGCGATAAAATTATTCACCTGACTGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCCTCGTCGATTTCTGGGCAGAGTGGTGCGGTCCGTGCAAAATGATCGCCCCGATTCTGGATGAAATCGCTGACGAATATCAGGGCAAACTGACCGTTGCAAAACTGAACATCGATCAAAACCCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACTCTGCTGCTGTTCAAAAACGGTGAAGTGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTGAAAGAGTTCCTCGACGCTAACCTGGCCGGGATCGAGGAAAACCTGTACTTCCAATCCGCGTCTGCCGTTGAAGACATCCGTAAAGTCCTGTCCGATTCTTCGTCTCCTGGTGGCGGGTCAGAAATATGACTACATCCTGGTTGGCGGTGGCACCGCGGCGTGCTGGCTGGCAAACCGTCTGAGCGCTGACGGTTCCAAACGTGTACTGGTTCTGGAAGCAGGCCCGGATAACACCTCCCGCGACGTTAAGATTCCGGCGGCGATCACCCGGCTGTTCCGCTAGCCCCGCTGGATTGGAACCTGTTTTCCGAACTGCAGGAACAGCTCGCCGAACGTCAAATCTACATGGCACGCGGCCGTCTGCTGGGCGGCAGCTCTGCGACCACGCAACCCTGTACCATCGTGGCGCTGCAGGTACTACGACGCGTGGGGTGTGGAAGGCTGGTCTTCTGAAGATGTGCTGTCCTGGTTCGTCCAGGCAGAAACCAACGCGGATTTCGGTCCGGGCGCTTACCACGGTTCTGGTGGTCCGATGCGCGTTGAAAACCCGCGTTACACCAACAAACAGCTGCACACTGCTTTCTTCAAGGCTGCTGAAGAAGTTGGTCTTACCCCGAACTCCGATTTCAACGATTGGAGCCATGACCACGCCGGTTACGGCCTTTCAGGTGATGCAGGATAAAGGCACCCGCGCGGATATGTACCGTCAGTATCTGAAACCTGTGCTGGGTCGTCGCAACCTGCAGGTACTGACCGGCGCTGCAGTGACCAAAGTCAACATCGACCAGGCTGCGGGCAAAGCGCAGGCTCTGGGTGTTGAATTCTCCACCGACGGCCCAACCGGCGAACGCCTGTCTGCGGAACTGGCTCCGGGTGGTGAGGTCATCATGTGCGCAGGTGCTGTTCACACCCGTTCCTGCTGAAACATTCCGGCGTTGGCCCGTCTGCTGAGCTGAAAGAATTCGGCATCCCGGTTGTTAGCAACCTGGCTGGTGTTGGCCAGAACCTGCAGGATCAGCCGGCTGCCTGACCGCGGCTCCGGTTAAAGAAAAATACGACGGGTATTGCCATTTCTGATCACATCTACAACGAAAAAGGCCAGATCCGTAAACGTGCAATCGCATCCTACCTGCTGGGTGGTCGTGGCGGTCTGACTTCCACCGGTTGCGATCGCGGTGCCTTCGTTCGTACCGCGGGTCAGGCGCTGCCGGACCTGCAGGTTCGCTTCGTTCCAGGTATGGCGCTGGACCCGGACGGTGTTAGCACCTACGTTCGTTTTGCTAAATTCCAGAGCCAGGGTCTGAAATGGCCGAGCGGCATCACCATGCAGCTGATCGCTTGCCGTCCGCAGTCTACCGGCTCCGTCGGTCTTAAATCCGCTGACCCGTTTGCGCCGCCGAAACTGTCACCAGGTTACCTGACCGACAAAGACGGTGCTGATCTGGCTACCCTGCGTAAAAGGACACGACCGACCGGGAAAGCAACCATTGGTGCATCTGGCTGCTGCACCGGCGACCGTAGCTGCATAA

**B 6xHis-TrxA-TEV-CvFAP (short-length)**

ATGAAACTTCTCTGGTTCTCTCATGAGCCGTAATAATATTATCTCTACGTACAGCAGACGCTTGGTTCTATGAGCGATAAAATTATTCACCTGACTGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCCTCGTCGATTTCTGGGCAGAGTGGTGCGGTCCGTGCAAAATGATCGCCCCGATTCTGGATGAAATCGCTGACGAATATCAGGGCAAACTGACCGTTGCAAAACTGAACATCGATCAAAACCCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACTCTGCTGCTGTTCAAAAACGGTGAAGTGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTGAAAGAGTTCCTCGACGCTAACCTGGCCGGGATCGAGGAAAACCTGTACTTCCAATCCGCGTCTGCCGTTGAAGACATCCGTAAAGTCCTGTCCGATTCTTCGTCTCCTGGTGGCGGGTCAGAAATATGACTACATCCTGGTTGGCGGTGGCACCGCGGCGTGCTGGCTGGCAAACCGTCTGAGCGCTGACGGTTCCAAACGTGTACTGGTTCTGGAAGCAGGCCCGGATAACACCTCCCGCGACGTTAAGATTCCGGCGGCGATCACCCGGCTGTTCCGCTCCCCGCTGGACTGGAACCTGTTCTCTGAACTGCAGGAACAGCTTGCGGAACGTCAGATCTACATGGCGCGTGGCCGTCTGCAGTGACCAAAGTCAACATCGACCAGGCTGCGGGCAAAGCGCAGGCTCTGGGTGTTGAATTCTCCACCGACGGCCCAACCGGCGAACGCCTGTCTGCGGAACTGGCTCCGGGTGGTGAGGTCATCATGTGCGCAGGTGCTGTTCACACCCGTTCCTGCTGAAACATTCCGGCGTTGGCCCGTCTGCTGAGCTGAAAGAATTCGGCATCCCGGTTGTTAGCAACCTGGCTGGTGTTGGCCAGAACCTGCAGGATCAGCCGGCTGCCTGACCGCGGCTCCGGTTAAAGAAAAATACGACGGGTATTGCCATTTCTGATCACATCTACAACGAAAAAGGCCAGATCCGTAAACGTGCAATCGCATCCTACCTGCTGGGTGGTCGTGGCGGTCTGACTTCCACCGGTTGCGATCGCGGTGCCTTCGTTCGTACCGCGGGTCAGGCGCTGCCGGACCTGCAGGTTCGCTTCGTTCCAGGTATGGCGCTGGACCCGGACGGTGTTAGCACCTACGTTCGTTTTGCTAAATTCCAGAGCCAGGGTCTGAAATGGCCGAGCGGCATCACCATGCAGCTGATCGCTTGCCGTCCGCAGTCTACCGGCTCCGTCGGTCTTAAATCCGCTGACCCGTTTGCGCCGCCGAAACTGTCACCAGGTTACCTGACCGACAAAGACGGTGCTGATCTGGCTACCCTGCGTAAAAGGACACGACCGACCGGGAAAGCAACCATTGGTGCATCTGGCTGCTGCACCGGCGACCGTAGCTGCATAA

**Figure S1.** The designed construct for the expression of CvFAP. The construct consists of sequentially a 6xHis-tag (black background), thioredoxin (TrxA) tag (grey background), tobacco etch virus (TEV) protease cleavage site (black background) and the gene coding for CvFAP (grey background). A full-length construct comprising residues 1-654 of CvFAP; B short-length construct comprising residues 62-654 of CvFAP.
Figure S2. SDS-PAGE analysis of the cell free extracts and purified samples of CvFAP. From left to right: (1) CFE control cells not producing CvFAP; (2) CFE full-length CvFAP; (3) purified full-length CvFAP; (4) CFE short-length CvFAP; (5) purified short-length CvFAP; (6) Molecular weight marker.

Molecular weight of the full-length CvFAP construct (6xHis-TrxA-TEV-CvFAP(full-length)): 83.1 kDa; molecular weight of the short-length CvFAP construct (6xHis-TrxA-TEV-CvFAP(short-length)): 77.0 kDa.
**Figure S3.** Image of the homemade-LED light setup.

**Figure S4.** A representative blue light spectrum (a) and intensity (b) of the 15 levels of the homemade-LED light setup.
Figure S5. Decarboxylation of palmitic acid by short length-CvFAP. Reaction conditions: [palmitic acid] = 13 mM, [CvFAP] = 6 μM (▲), 3 μM (■) or 1.5 μM (●) in Tris-HCl buffer (pH 8.5, 100 mM), 30 % DMSO, 37°C, blue light illumination.

Figure S6. Decarboxylation of palmitic acid by full-length CvFAP. Reaction conditions: [palmitic acid] = 13 mM, [F-CvFAP] = 11 μM (△), 3 μM (□) or 1.5 μM (○) in Tris-HCl buffer (pH 8.5, 100 mM), 30 % DMSO, 37°C, blue light illumination.
Figure S7. Photoenzymatic decarboxylation of palmitic acid with varied DMSO content. Reaction conditions: [palmitic acid] = 13 mM, [S-CvFAP] = 6 μM in Tris-HCl buffer (pH 8.5, 100 mM) at 37°C, blue light illumination.

Figure S8. Photoenzymatic decarboxylation of palmitic acid at varied light intensities. Reaction conditions: [palmitic acid] = 13 mM, [S-CvFAP] = 6 μM in Tris-HCl buffer (pH 8.5, 100 mM) at 37°C, blue light illumination.
Figure S9. Photoenzymatic decarboxylation of palmitic acid at varied temperatures. Reaction conditions: [palmitic acid] = 13 mM, [S-CvFAP] = 6 μM in Tris-HCl buffer (pH 8.5, 100 mM) at 20°C (○), 30°C (□) or 37°C (△), blue light illumination.

Figure S10. A representative GC chromatogram of the decarboxylation of palmitic acid. 1-Octanol was used as internal standard.
Figure S11. Image of isolated pentadecane from the photoenzymatic decarboxylation of palmitic acid.

Figure S12. GC chromatogram showing the purity of pentadecane isolated from the photoenzymatic decarboxylation of palmitic acid. Rt = 7.091 min was pentadecane.
Docking study of fatty-acids

In accordance with previous observations CvFAP showed highest activity with long-chain fatty acids (C>14). These data were also confirmed by molecular docking analyses: lauric acid (C=12), for example, interacts predominantly with tyrosine-446 through hydrophobic interactions. As a consequence of this interaction the distance between the carboxylate and the FAD prosthetic group is significantly prolonged (Figure S13) compared to other fatty acids (Figures S14-S17).

Similarly, binding of oleic acid (cis-9-Octadecenoic acid) is not optimal for efficient transformation: the cis-double bond is positioned at the warp of the hydrophobic tunnel that leads to the active site, provoking the torsion of the rest of molecule and preventing the productive positioning of the carboxyl group in the active site (Figure S14). Elaidic acid (trans-9-Octadecenoic acid) is geometrically less impeded and binds in the active site in a correct productive conformation (Figure S15). Other unsaturated fatty acids (cis-6-Octadecenoic acid and trans-11-Octadecenoic acid) were also tested and, as predicted (Figures S16 and S17, respectively), they are converted with a good efficiency (Table S3).

Figure S13: Docking analysis of lauric acid (in light blue) in the active site of CvFAP. The distance shown with a yellow dashed line corresponds to 7.7 Å. Tyrosine 446 and FAD are shown.
Figure S14: Docking analysis of cis oleic acid (Δ9) (in dark blue) in the active site of CvFAP. The distance shown with a yellow dashed line corresponds to 6.3 Å. The double bond of the ligand is highlighted in dark blue. Tyrosine 446 and FAD are shown.

Figure S15: Docking analysis of elaidic acid (Δ9) (in dark blue) in the active site of CvFAP. The distance shown with a yellow dashed line corresponds to 5.0 Å. The double bond of the ligand is highlighted in dark blue. Tyrosine 446 and FAD are shown.
Figure S16: Docking analysis of trans-11-octadecenoic acid (in dark blue) in the active site of CvFAP. The distance shown with a yellow dashed line corresponds to 4.6 Å. The double bond of the ligand is highlighted in dark blue. Tyrosine 446 and FAD are shown.
Figure S17: Docking analysis of cis-6-octadecenoic acid (in dark blue) in the active site of CvFAP. The distance shown with a yellow dashed line corresponds to 3.4 Å. The double bond of the ligand is highlighted in dark blue. Tyrosine 446 and FAD are shown.
Further experiments on the stability of the CvFAP

To get more insight into the higher stability of CvFAP in crude cell extracts we have incubated the purified enzyme with free FAD, with cell extract (CFE) and with filtered cell extract. For the latter, we filtered the cell extract using a 10 kDa cutoff spin filter, in order to remove all proteins and only leave small molecules in the extract. The incubations were performed for 3 h in blue LED light or for 3 h in the dark, after which the activity with pentadecane was tested. In addition, the activity of the mixtures was tested without incubation, so directly after mixing the components. The purified enzyme incubated with filtered cell extract gave a slight increase of the reaction rate compared to the purified enzyme itself. The addition of cell extract to the purified enzyme gave the slightly higher activity compared to the cell extract itself. In addition, incubation of the cell extract or the purified enzyme with free FAD did not enhance the activity. Furthermore, illumination of the purified enzyme or cell extract in buffer (excluding the substrates) showed that the enzyme was slightly deactivated by light. All results can be found in the table below.

Table S1. investigation of the enzyme stability.

| Reaction | Condition | Product after 3h |
|----------|-----------|-----------------|
|          |           | 0h illumination | 3h illumination, prior to addition the substrate | 3h darkness prior, to addition the substrate |
|          | No addition | FAD | CFE | CFE filtered | [Pentadecane] [mM] | [Pentadecane] [mM] | [Pentadecane] [mM] |
| Reaction 1 | Purified enzyme | x | | | 1.57 | 0.64 | 0.94 |
| Reaction 2 | Purified enzyme | x | | | 1.73 | 0.66 | 1.02 |
| Reaction 3 | Purified enzyme | x | | | 11.77 | 7.8 | 10.07 |
| Reaction 4 | Purified enzyme | x | | | 2.93 | 0.95 | 1.69 |
| Reaction 5 | CFE | x | | | 9.45 | 7.54 | 8.43 |
| Reaction 6 | CFE | x | | | 10.21 | 7.16 | 8.37 |

Reaction conditions: [substrate] = 13 mM, [CvFAP] or [purified enzyme] = 3.0 μM, [FAD] = 6.0 μM, Tris-HCl buffer (pH 8.5, 100 mM), 30 % DMSO, blue light illumination (intensity = 13.7 µE L⁻¹ s⁻¹) for 3 hours. ‘CFE filtered’ means that we filtered the cell extract using a 10 kDa cutoff spin filter, in order to remove all proteins and only leave small molecules in the extract.

Table S2. Decarboxylation of palmitic acid under different atmosphere [a]

| Atmosphere | Conversion, % |
|------------|---------------|
| air        | 100           |
| N₂         | 99.1          |
| H₂         | 100           |
| Ar         | 98.4          |

[a] Reaction conditions: [palmitic acid] = 13 mM, [S-CvFAP] = 6 μM in Tris-HCl buffer pH 8.5 (100 mM) at 37°C, 3h, blue light illumination (intensity = 13.7 µE L⁻¹ s⁻¹). Conversion: \( \text{Conversion} = \frac{[\text{product}]_{\text{final}} + ([\text{product}]_{\text{final}} + [\text{substrate}]_{\text{final}})^{-1}}{2}; \) A gas balloon was used to provide the corresponding reaction atmosphere.
### Table S3. Photoenzymatic decarboxylation of isomers of oleic acid.

| Entry | Substrate                  | [Product] [mM] | Distance between carboxylate and FAD [Å] | TON (CvFAP) [mol mol⁻¹] |
|-------|----------------------------|----------------|------------------------------------------|--------------------------|
| 1     | cis-9-Octadecenoic acid, ∆9 | 6.93           | 6.3                                      | 1155                     |
| 2     | trans-9-Octadecenoic acid, ∆9 | 13.47          | 5.0                                      | 2245                     |
| 3     | cis-6-Octadecenoic acid, ∆6 | 14.71          | 3.4                                      | 2450                     |
| 4     | trans-11-Octadecenoic acid, ∆11 | 12.15         | 4.6                                      | 2025                     |

Reaction conditions: [substrate] = 15 mM, [CvFAP] = 6.0 μM, Tris-HCl buffer (pH 8.5, 100 mM), 30 % DMSO, blue light illumination (intensity = 13.7 µE L⁻¹ s⁻¹) for 4.5 hours. [a] Due to the lack of standard reference, the product concentration from other isomers (Entries 2-4). [b] TON: [product]_{final} × [CvFAP]⁻¹. Duplicated experiments were performed.
All compounds in the reactions were analyzed by Gas Chromatography (model: SHIMADZU GC-2014) equipped with column Cpsil 5 CB: (50m × 0.53mm × 1.0 µm), FID, N2 as the carrier gas.

Table S2. Details of GC analysis.

| Substrate                        | Retention time, min | Temperature profile                                      |
|----------------------------------|---------------------|----------------------------------------------------------|
| C_{12}H_{24}O_{2} (Lauric acid)  | Lauric acid: 7.06   | 70 °C hold 3.4 min, 25 °C /min to 190 °C hold 2.1 min, 25 °C /min to 230 °C hold 3.1 min, 30 °C /min to 320 °C hold 1.5 min. |
|                                  | Undecane: 6.28      |                                                          |
| C_{14}H_{28}O_{2} (Myristic acid)| Myristic acid: 11.4 | 70 °C hold 3.4 min, 25 °C /min to 190 °C hold 2.1 min, 25 °C /min to 230 °C hold 3.1 min, 30 °C /min to 320 °C hold 1.5 min. |
|                                  | Tridecane: 7.66     |                                                          |
| C_{16}H_{32}O_{2} (Palmitic acid)| Palmitic acid: 10.23| 110 °C hold 3.4 min, 25 °C /min to 190 °C hold 2.1 min, 25 °C /min to 230 °C hold 2.1 min, 30 °C /min to 320 °C hold 0.8 min. |
|                                  | Pentadecane: 7.08   |                                                          |
| C_{16}H_{34}O_{2} (Margaric acid)| Margaric acid: 11.74| 110 °C hold 3.4 min, 25 °C /min to 190 °C hold 2.1 min, 25 °C /min to 230 °C hold 4.1 min, 30 °C /min to 320 °C hold 2.0 min. |
|                                  | Hexadecane: 7.83    |                                                          |
| C_{18}H_{36}O_{2} (Stearic acid)| Stearic acid: 12.75 | 110 °C hold 3.4 min, 25 °C /min to 190 °C hold 2.1 min, 25 °C /min to 230 °C hold 4.1 min, 30 °C /min to 320 °C hold 2.0 min. |
|                                  | Heptadecane: 8.82   |                                                          |
| C_{20}H_{40}O_{2} (Arachidic acid)| Arachidic acid: 15.05| 110 °C hold 3.4 min, 25 °C /min to 190 °C hold 2.1 min, 25 °C /min to 230 °C hold 4.1 min, 30 °C /min to 320 °C hold 2.0 min. |
|                                  | Nonadecane: 10.48   |                                                          |
| C_{18}H_{34}O_{2} (Oleic acid)  | Oleic acid: 12.51   | 110 °C hold 3.4 min, 25 °C /min to 190 °C hold 2.1 min, 25 °C /min to 230 °C hold 4.1 min, 30 °C /min to 320 °C hold 3.0 min. |
|                                  | heptadec-6-ene: 8.74|                                                          |
| C_{19}H_{32}O_{2} (Δ9, 12)      | Linoleic acid: 12.62| 110 °C hold 3.4 min, 25 °C /min to 190 °C hold 2.1 min, 25 °C /min to 230 °C hold 4.1 min, 30 °C /min to 320 °C hold 2.0 min. |
| (Linoleic acid)                  | Heptadeca-6,9-diene: 8.55 |                                                          |

[a] Due to the lack of standard compound of Heptadeca-6,9-diene, the conversion of the reaction was calculated based on the consumption of linoleic acid.

Author Contributions

M. M. E. Huijbers and W. Zhang prepared the biocatalysts, performed the experiments and analysed the results. F. Tonin performed docking and simulation and analysis of the results. F. Hollmann conceived the study, analysed the results and prepared the manuscript.

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