Supporting information

Strategies for Transferring Photobiocatalysis to Continuous Flow Exemplified by the Photodecarboxylation of Fatty Acids

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1 Experimental Methods

1.1 Chemicals and Reagents
Unless stated otherwise, solvents, reagents and all media components (yeast extract, tryptone, glycerol) were obtained from commercial sources and used without further purification.

1.2 Instrumentation
All photodecarboxylation reactions were run in a custom-built photoreactor, fitted with commercial LEDs. The reactions were analyzed by gas chromatography, using an Agilent Technologies 7890A GC-FID system, equipped with an HP-5 (30 m x 0.25 mm x 0.25 µm) column using the following method: total runtime 20.5 min; Temperature program: 100 °C for 0.5 min, then 10 °C/min to 300 °C; Injection volume 1 or 5 µL, unless stated otherwise. GC-MS measurements were carried out on a 7890A GC System (Agilent Technologies, Santa Clara, CA, USA), equipped with a5975C mass selective detector and an HP-5MS column (5% phenylmethylsiloxane, 30 m x 0.20 mm x 0.25 µm, J&W Scientific, Agilent Technologies) using He as the carrier gas. Injector temperature: 250°C; split ratio: 90:1; Injection volume: 1 µL; Flow rate: 0.7 mL/min; Temperature program: 100 °C for 0.5 min, then 10 °C/min to 300 °C (mixtures of decanoic acid and nonane, as well as undecanoic acid and decane were analyzed with an altered temperature program: 40 °C for 2 min, then 10 °C/min to 300 °C). Cell lysis was performed with the Branson 450 Digital Sonifier using 2 min 30 s total pulsation time, 2 s pulses, and 4 s of pause, at 30% amplitude. OD values were measured on an Eppendorf BioPhotometer Plus spectrophotometer. UV-Vis spectra of reaction mixture components (Figure S13) were taken on a Cary 60 UV-Vis Spectrophotometer (Agilent Technologies). The Bradford assay measurements were taken on a Molecular Devices SpectraMax M2 multi-mode microplate reader. Protein purification was performed using an Äkta Pure chromatography system, equipped with the round fraction collector F9-R. Lyophilization was performed on a Martin Christ Freeze Dryer ALPHA 1-4. The photon flux of the used light sources was determined by reineckate actinometry experiments. The syringe pumps used in flow experiments were KD Scientific Legato 100 or 200 Series. The KD Scientific 200 Series was equipped with a 6/10 multi-rack.
1.3 Enzyme Expression and Purification

The gene containing the coding sequence for the fatty-acid photodecarboxylase (GenBank: KY511411), along with a His6-tag, a thioredoxin (TrxA) tag, and a tobacco etch virus (TEV) protease cleavage site was ordered subcloned in pET28a(+) (General Biosystems), flanked by the NdeI and HindIII restriction sites. The protein lacks the residues encoding for a predicted chloroplast targeting sequence and thereby comprises residues 62-654 of FAP. The plasmid amplification, transformation, and the preparation of the cryostock of the recombinant strain was performed as described in a previous report. Terrific broth (TB) medium for cell cultivation was prepared by dissolving yeast extract (72 g), tryptone (36 g), and glycerol (12 mL) in deionized water (2.7 L) in one flask (TB base), and KH₂PO₄ (6.95 g) and K₂HPO₄ (37.6 g) in deionized water (0.3 L) in another (10 × KPi solution). The two medium components were autoclaved separately and combined prior to inoculation with the overnight culture. An overnight culture was prepared by charging Sarstedt tubes (50 mL) with TB medium (15 mL), adding kanamycin (15 µL of a 50 mg/mL stock), and inoculating with the glycerol stock containing E. coli carrying the pET28a-His-Trx-FAP plasmid (5 µL). The resulting cultures were shaken overnight at 30 °C. The empty cultivation flasks were heated to 200 °C for 2 h and cooled down to room temperature before being charged with the TB base (540 mL of base for 2 L flasks) and the 10 × KPi solution. The TB base and the 10 × KPi solution were autoclaved separately overnight. Upon cooling, 10 × KPi solution was added to each flask containing the TB base, in the clean bench. Upon that, kanamycin (600 µL, 50 mg/mL stock) and overnight culture (1% inoculate) were also added to each of the cultivation flasks. The inoculated media were shaken at 37 °C, 120 rpm, until the OD₆₀₀ value reached 1-1.2. The flasks were allowed at 4 °C for approximately 1 h. After cooling, expression was induced in each flask with IPTG (600 µL, 1 M stock) inside the clean bench, and the cultures were shaken overnight (approximately 20 h) at 17 °C, and 120 rpm, in the dark. The resulting mixtures were centrifuged for 10 min at 8000 rpm (12000 × g) and 4 °C, and the sedimented whole cells were lysed. The lysis was performed by two cycles of sonication, described previously in detail (see instrumentation). Upon lysis, the cell pellets were sedimented by centrifugation for 20 min at 17000 rpm (34700 × g) at 4 °C. Subsequently, the supernatant was either frozen in liquid nitrogen and lyophilized (protected from light) or purified. The CvFAP purification procedure was performed as follows. The cells were suspended in lysis buffer (50 mM Tris·HCl, 100 mM NaCl, pH 8) and sonicated under previously described conditions (see instrumentation).
resulting mixture was centrifuged for 20 min at 17000 rpm (34700 \times g) and 4 °C. The supernatant was separated and incubated with 50 \mu M FAD on ice for 1 h, protected from light. Imidazole, NaCl, and hexanoic acid were added to it subsequently (final concentrations were 10 mM, 300 mM, and 10 mM respectively). The final lysate solution was filtered through a 0.45 \mu m filter before purification. The filtration and all following steps until the freezing of protein solutions were carried out at 4 °C in the dark, or under dim white light. The lysate was loaded onto two connected 5 mL His-Trap™ FF columns (1 mL·min\(^{-1}\) flow rate) and eluted with five column volumes of the wash buffer (50 mM Tris·HCl, 300 mM NaCl, 10 mM imidazole, 50 \mu M FAD, 10 mM hexanoic acid, pH 8), and afterwards with five column volumes of the elution buffer (50 mM Tris·HCl, 300 mM NaCl, 500 mM imidazole, 50 \mu M FAD, 10 mM hexanoic acid, pH 8). Both the washing and elution steps were performed at 5 mL·min\(^{-1}\) flow rates. Tubes containing both the flow-through and the purified protein were collected. Prior to desalting, the solution was concentrated using an Amicon ultracentrifugation device (30 kDa cutoff). Afterwards, the protein solution (2.5 mL), was loaded onto a PD-10 desalting column, and the elution was done with the Tris·HCl buffer (3.5 mL, 100 mM, pH 8.5). Glycerol was added to the final eluent (5% v/v) and the final eluate was aliquoted into microcentrifuge tubes containing 100 \mu L of the final protein solution. The resulting aliquots were frozen under liquid nitrogen and further stored at – 80 °C.

### 1.4 Protein Analysis

SDS-PAGE analyses were performed as follows: Samples were dissolved in urea (6 M) and the protein concentration was first determined by Bradford assay. The protein samples (10 \mu L) were mixed with the Bradford reagent (490 \mu L of a 1:5 v/v dilution of commercial reagent and water). The samples were diluted if necessary to \approx 0.5 mg·mL\(^{-1}\). The final protein solutions were mixed with the equal volume of a Laemmli buffer (2x concentrate). The obtained mixtures were heated at 95 °C for 5 min and centrifuged briefly. The samples were loaded onto a 10% Bis-Tris gel, along with the PageRuler™ marker (5 \mu L). The electrophoresis was run at 140 V, using a Tris/MOPS buffer. The gels were stained using a Coomassie Blue solution. The Bradford assay for the determination of the concentration of purified CvFAP was determined as follows. A standard series of bovine serum albumin (\geq 96\%, Sigma Aldrich) was prepared in the Tris·HCl buffer (100 mM, pH 8.5) in six different concentrations (0.5, 0.35, 0.2, 0.1, 0.075, 0.05 mg·mL\(^{-1}\)) in triplicates. The CvFAP samples were diluted with the buffer so
that their concentrations fit into the specified concentration range. All protein samples (10 µL) were mixed with the working reagent (200 µL) in microtiter plates and the color was left to develop for 10 min at room temperature. The reagent was prepared by mixing one volume of the protein dye reagent concentrate (Bio-Rad) with four volumes of double distilled water and passing through a 0.22 µm filter. The absorbance measurements were taken at 595 nm.

1.5 General Procedure for the Decarboxylation of Palmitic Acid in Batch
A 1.5 mL screw cap vial was charged with Tris-HCl buffer (100 mM, pH 8.5), the lyophilized lysate or purified CvFAP dissolved in the same buffer (700 µL total volume), 65 mM palmitic acid in DMSO (200 µL) and DMSO (100 µL), unless stated otherwise. The reactions were illuminated for the designated amount of time, using 455 nm LEDs at 42 µmol·L⁻¹·s⁻¹ photon flux density (unless stated otherwise), and with constant shaking of 500 rpm. Reactions were run in triplicates, unless stated otherwise. Aliquots (500 µL) were taken from each reaction mixture, diluted with brine (500 µL), and acidified with hydrochloric acid (6 M, 50 µL). The mixtures were extracted with ethyl acetate containing n-decanol (5 mM) (2 × 500 µL, 300 µL were withdrawn after the first extraction, and 500 µL after the second extraction). All mixtures were centrifuged after each extraction step. The combined organic phases (total volume 800 µL) were dried over anhydrous Na₂SO₄. Each sample (100 µL) was then derivatized with a mixture of BSTFA/pyridine (1:1 v/v, 200 µL) and heated at 60 °C, 600 rpm shaking, for 1 h and analyzed by GC-FID.

1.6 General Flow Photodecarboxylation Procedure Under Homogeneous Conditions
An FEP tube (1/16” outer diameter, 0.03” inner diameter) at a length of 70 cm (320 µL volume) was placed in a photoreactor chamber. The overall illuminated path length was 58 cm (264 µL volume). The reaction mixtures were pumped through the tube with the aid of a syringe pump from 1 mL syringes. The reaction mixtures were prepared by mixing the solution of palmitic acid in Tris/Ammoeng (600 µL, 26 mM palmitic acid, 2% Ammoeng 102, Tris-HCl buffer, 100 mM, pH 8.5) with Tris-HCl buffer (600 µL, 100 mM, pH 8.5) and CvFAP (13.2 µL of 41.7 mg·mL⁻¹ solution, 60 µM final concentration, unless stated otherwise), so that the final volume was 1.2 mL. The mixtures were transferred to 1 mL syringes, and the air bubbles were removed. As the total tube volume was calculated to be 320 µL, 820 µL of the reaction mixture
was pumped through the tube, and 500 µL were collected in 2 mL microcentrifuge tubes. The illumination was performed at 455 nm or 528 nm at 10.7 µE·s⁻¹ photon flux, unless stated otherwise. The workup and analysis were performed as described in the general procedure for decarboxylation of palmitic acid in batch.

1.7 General Flow Photodecarboxylation Procedure Under Heterogeneous Conditions

An FEP tube (1/8" outer diameter, 0.062" inner diameter) was cut to a length of 19.6 cm. The part of the tube within the fittings was marked and the parts of the tube inaccessible to light were filled with sand, the illuminated part in-between was filled with Eupergit C250 L beads and the mass of the packed beads was measured (70-80 mg). The contents of the packed tube were hydrated until no bubbles were observed. To determine the volume of the packed reactor, an aqueous solution of methylene blue was pumped through the reactor and the time needed for the dye to flow through the illuminated part of the tube at a constant flow rate was measured. The packed reactor volume was found to be 225 µL. The system was then washed with deionized water until no traces of the dye was detectable anymore. Subsequently, the tube was washed with Tris·HCl buffer (100 mM, pH 8.5; 1 mL) at a rate of 50 µL·min⁻¹. The enzyme solution for immobilization was prepared by diluting the purified CvFAP stock solution (227 µL, 41.7 mg·mL⁻¹) with buffer to 1.2 mL (when 1 mL was loaded onto the column) or to 0.7 mL (209 µL of 24.2 mg·mL⁻¹ stock solution) when 0.5 mL was loaded onto the column with Tris·HCl buffer (100 mM, pH 8.5). From the prepared dilutions, 1 mL or 0.5 mL were introduced to the beads at the appropriate flow rate depending on desired residence time (e.g., 3.75 µL·min⁻¹ for a 60 min residence time). Washing with Tris·HCl buffer (100 mM, pH 8.5; 2.5 mL) followed to remove non-bound enzyme. The enzyme flow-through and the wash were collected and kept for the Bradford assay. To ensure that the enzyme is in contact with the substrate prior to the start of illumination, the reactor was washed with palmitic acid (13 mM) in buffer/Ammoeng 102 (1% v/v; 1 mL) at a flow rate of 50 µL·min⁻¹. The illumination was then started (455 nm, 2.7 µE·s⁻¹ photon flux, unless stated otherwise) and the palmitic acid solution (13 mM) in buffer/Ammoeng 102 was continued to be pumped through the column (in 500 µL fractions), at a flow rate dependent on desired residence time. The reaction mixtures were collected directly in 2 mL microcentrifuge tubes. To the collected fractions, HCl (aq., 6 M, 50 µL) and brine (500 µL) were added, and the extraction was
performed with EtOAc (containing 5 mM n-decanol; 2 × 500 µL, 300 µL withdrawn the first time, 500 µL the second time). Samples were centrifuged between extractions (18845 × g, 1 min) and combined organic phases (800 µL) were dried over anhydrous Na₂SO₄ and centrifuged again (18845 × g, 1 min). The derivatization was performed by mixing a BSTFA (1% TMSCl)/pyridine mixture (200 µL, 1:1 v/v) and the organic phases (100 µL) in 1.5 mL glass crimp vials and heating at 60 °C, 600 rpm, for 60 min. GC-FID analysis was performed using an HP-5 column (30 m × 0.25 mm × 0.25 µm) and the ACHIRAL method with 5 µL injection volume (see instrumentation).

1.8 General Procedure for the Immobilization of CvFAP onto Different Commercial Solid Supports in Batch
A stock solution of CvFAP (32.5 ± 0.8 mg·mL⁻¹) in Tris·HCl (100 mM, pH 8.5) was thawed at 4 °C in the dark and was subsequently diluted with the same buffer (32.6 µL to 530 µL) to a final concentration of 2 mg·mL⁻¹. The enzyme solution (500 µL) was added to a 1.5 mL screw cap vial charged with beads (10 mg of Eupergit C250 L, Eupergit C, Sepabeads EC-OD, Sepabeads EC-BU, Dowex 66). The remaining volume from each starting solution replicate was taken for Bradford analysis. The vials were shaken in an Eppendorf Thermomixer at 4 °C, and 600 rpm in the dark over 15 h for all supports apart from Dowex 66, which was shaken for 30 min. The immobilization of CvFAP on each type of support was performed in triplicates. After the elapsed time, the immobilization supernatant was pipetted out of the solution, and analyzed by Bradford assay. The resulting immobilized catalyst was washed with Tris·HCl (100 mM, pH 8.5) buffer (2 × 500 µL) also at 4 °C, under dim light. For the biotransformation, Tris·HCl buffer (100 mM, pH 8.5, 700 µL), DMSO (100 µL), palmitic acid in DMSO (65 mM, 200 µL), were added to the vials with immobilized enzyme at room temperature. The reaction mixtures were illuminated at 455 nm (42 µmol·L⁻¹·s⁻¹ photon flux density) for 1 h, at 25 °C, 500 rpm. Upon the elapsed time, the workup and analysis were done as outlined previously in the general decarboxylation procedure.

1.9 General Procedure for the Immobilization of CvFAP onto EziG beads in Batch
The immobilization of CvFAP from cell lysate was performed as follows. Solutions of cell lysate (10 mg·mL⁻¹, 0.36 U) in corresponding buffers were prepared at 4 °C under dim light. The lysate solutions (500 µL) were then added to 1.5 mL screw cap vials charged with EziG beads (10 mg). The remaining volume from each starting solution replicate was taken for Bradford
analysis. The vials were shaken in an Eppendorf Thermomixer at the stated temperature and time, at 900 rpm, in the dark. The immobilization of CvFAP on each type of EziG beads was performed in triplicates. After the elapsed time, the immobilization supernatant was analyzed by Bradford assay. The resulting immobilized catalyst was washed with Tris-HCl buffer (100 mM, pH 8.5; 2 × 500 µL) at 4 °C under dim light. For the biotransformation, Tris-HCl buffer (100 mM, pH 8.5, 700 µL), DMSO (100 µL), palmitic acid in DMSO (65 mM, 200 µL), were added to the vials with immobilized enzyme at room temperature. The reaction mixtures were illuminated at 455 nm (42 µmol·L⁻¹s⁻¹ photon flux density) for 1 h at 25 °C and 500 rpm. The workup and analysis were done as outlined in the general decarboxylation procedure. The immobilization of purified CvFAP was performed as follows. Solutions of purified CvFAP were prepared in corresponding buffers (4 mg·mL⁻¹). The resulting CvFAP solutions (500 µL) were added to 1.5 mL screw cap vials charged with 10 mg of EziG beads. Further treatment and analysis were done as described for the immobilization from cell lysate.

1.10 Procedure for the Immobilization of CvFAP onto Polyacrylamide Beads
Polyacrylamide beads were prepared as outlined in a previous report. The beads (10 mg) were placed into six 2 mL microcentrifuge tubes each along with HPLC-grade H₂O (300 µL). Afterwards, EDC·HCl (28.8 mg in 600 µL of H₂O) and N-hydroxysuccinimide (17.3 mg in 600 µL of H₂O) were added and the mixture were shaken in a benchtop shaker at 25 °C, 900 rpm, for 30 min. The beads were centrifuged (5000 × g, 3 min) and the supernatant was carefully removed using a 200 µL pipette. The washing was performed by adding HPLC-grade H₂O (3 × 1.5 mL) and pipetting the supernatant out (with centrifugation between the washing steps). To each tube with activated beads, CvFAP (500 µL containing 2 mg, 82.3 µL of the 24.3 mg/mL stock solution) was added and the coupling step was performed at 25 °C, 900 rpm, over 2 h. Before the coupling reagents were added, aliquots of the mixtures were taken (10 µL) for the Bradford assay. After the elapsed time, the supernatants were pipetted out of the tubes (to be analyzed by Bradford assay) and the beads were washed with HPLC-grade H₂O (2 × 1.5 mL). Three samples were lyophilized overnight, protected from light, and the activity of the other three samples (suspensions in H₂O) was tested immediately. The activity test was carried out as described previously.
1.11 Activity Assay of CvFAP from Cell Lysate

A 1.5 mL screw cap vial was charged with Tris-HCl buffer (500 µL, 100 mM, pH 8.5), the lyophilized lysate dissolved in the same buffer (200 µL solution, 5 mg of lyophilized lysate dry weight), 65 mM palmitic acid in DMSO (200 µL), and DMSO (100 µL). The reactions were illuminated for the designated amount of time, using a 455 nm light source (photon flux density 424 µmol ·L⁻¹ ·s⁻¹), at 23 °C and 500 rpm shaking speed. All reactions were performed in triplicates. The workup and analysis were further performed as outlined in the general decarboxylation procedure.
2 Supplementary Figures

**Figure S1.** The loss of activity of CvFAP in the photodecarboxylation of palmitic acid over time, under blue light irradiation. The enzyme was irradiated for the designated time before addition of substrate. Irradiation conditions before substrate addition: 455 nm light source wavelength, 42 µmol·L⁻¹·s⁻¹, Tris·HCl buffer (100 mM, pH 8.5, 686 µL), CvFAP (14.2 µL, 32.5 ± 0.8 mg·mL⁻¹), 25 °C, 500 rpm. Reaction conditions after addition of substrate: palmitic acid (13 mM), Tris·HCl buffer (100 mM, pH 8.5, 700 µL total volume), DMSO (30% v/v), total volume 1 mL, 455 nm irradiation (42 µmol·L⁻¹·s⁻¹), 25 °C, 1 h, 500 rpm. Displayed data points represent values calculated from triplicate experiments.
Figure S2. The effect of additives on the photodegradation of CvFAP after one minute of illumination in the absence of substrate, shown as reaction conversion after the addition of substrate. Irradiation conditions: CvFAP (14.2 µL, 32.5 ± 0.8 mg·mL⁻¹), 455 nm light (42 µmol·L⁻¹·s⁻¹), Tris·HCl (100 mM, pH 8.5, up to 700 µL), 1 min, 25 °C, 500 rpm. The concentrations of additives were as follows: FAD (50 µM), hexanoic acid (10 mM), BSA (6 mg·mL⁻¹). Reaction conditions: palmitic acid (13 mM), Tris·HCl buffer (100 mM, pH 8.5, 700 µL total volume), DMSO (30% v/v), total volume 1 mL, 455 nm irradiation (42 µmol·L⁻¹·s⁻¹), 25 °C, 1 h, 500 rpm. Displayed data points represent values calculated from triplicate experiments.
Figure S3. Photodecarboxylation of palmitic acid catalyzed by CvFAP in cell lysate – wavelength screening at 24 h and 20 min of reaction time. Reaction conditions: palmitic acid (13 mM), lyophilized lysate (15 mg, 0.39 U), Tris·HCl buffer (100 mM, pH 8.5), 30% v/v DMSO, 1 mL volume. The reaction was run at 25°C, 500 rpm, for designated time. The applied photon flux density was in the range 160.8–424.7 µmol·L⁻¹·s⁻¹ depending on the wavelength of the light source. Displayed conversions correspond to median values of duplicate experiments.
Figure S4. The dependence of conversion on time in the photodecarboxylation of palmitic acid catalyzed by purified CvFAP. Reaction conditions: palmitic acid (13 mM), CvFAP (final concentration 6 µM, concentration of stock solution 16.6 ± 0.8 mg·mL⁻¹), Tris·HCl buffer (100 mM, pH 8.5), 30% v/v DMSO, 1 mL volume. The reactions were run at 25 °C, 500 rpm. The wavelength of the light source was 455 nm, photon flux density 42 µmol·L⁻¹·s⁻¹. Displayed concentrations are mean values calculated from duplicate experiments.
Figure S5. The structural formulas of additives used for the dissolution of palmitic acid in Tris-HCl buffer (100 mM, pH 8.5).
Figure S6. Comparison of activity of CvFAP-containing cell lysate before and after passing through an ultrafiltration device (50 kDa cutoff). Activity test conditions: palmitic acid (13 mM), lyophilized cell lysate (5 mg of dry weight), Tris-HCl buffer (100 mM, pH 8.5, 700 µL total volume), DMSO (30% v/v), total volume 1 mL, 455 nm irradiation (420 µmol·L⁻¹·s⁻¹), 23 °C, time, 500 rpm. The reactions were illuminated for the designated amount of time, using a 455 nm light source ( photon flux density 424 µmol L⁻¹·s⁻¹, 10% duty cycle), at 23 °C, 500 rpm shaking speed. All reactions were performed in triplicates.
Figure S7. Immobilization of CvFAP from concentrated cell lysate onto EziG beads - buffer screening: MOPS (100 mM, pH 8), HEPES (100 mM, pH 8), KPi (100 mM, pH 8), Tris·HCl (100 mM, pH 8.5), Loading buffer (Tris·HCl, 50 mM, pH 8, 300 mM NaCl, 10 mM imidazole, 10 mM hexanoic acid, 50 µM FAD). Solid fill bars (■) correspond to protein loading and pattern bars (□) to conversion. Immobilization conditions: EziG (10 mg), lyophilized cell lysate (10 mg·mL⁻¹, 0.36 U) in the indicated buffer, total volume 0.5 mL, 4 °C, 900 rpm, 1 h. Activity test conditions: carrier/enzyme (≈ 10 mg), palmitic acid (13 mM), Tris·HCl buffer (100 mM, pH 8.5, 700 µL total volume), DMSO (30% v/v), total volume 1 mL, 455 nm irradiation (42 µmol L⁻¹·s⁻¹), 25 °C, 1 h, 500 rpm. Displayed errors correspond to standard deviations of triplicate determinations.
Figure S8. Immobilization of CvFAP from concentrated cell lysate onto EziG beads - Screening of different EziG beads (Opal, Coral, Amber) and immobilization time in HEPES (100 mM, pH 8) buffer. Solid fill bars (■) correspond to protein loading and pattern bars (□) to conversion. Immobilization conditions: EziG (10 mg), lyophilized cell lysate (10 mg·mL\(^{-1}\), 0.36 U) in HEPES (100 mM, pH 8) buffer, total volume 0.5 mL, 4 °C, 900 rpm, time. Activity test conditions: carrier/enzyme (≈ 10 mg), palmitic acid (13 mM), Tris·HCl buffer (100 mM, pH 8.5, 700 µL total volume), DMSO (30% v/v), total volume 1 mL, 455 nm irradiation (42 µmol L\(^{-1}\)s\(^{-1}\)), 25 °C, 1 h, 500 rpm. Displayed errors correspond to standard deviations of triplicate determinations.
**Figure S9.** SDS-PAGE analysis of cell lysate during immobilization of CvFAP on EziG Opal. M – Protein Ladder, im – eluted immobilized protein, L1 – cell lysate before immobilization, L2 – cell lysate after immobilization. The immobilized proteins were stripped from the beads (10 mg) by mixing with Tris·HCl buffer containing imidazole (50 mM, pH 8, 500 mM imidazole, 300 mM NaCl, 200 µL) for 30 min at 25 °C, 900 rpm. The molecular weight of the used CvFAP preparation is 77 kDa (the corresponding blots are highlighted).
Figure S10. Abundance of palmitic acid in fractions collected after passing through a tube packed with EziG Opal. Palmitic acid (13 mM) was dissolved in Tris·HCl (100 mM, pH 8.5) containing Ammonoeng 102 (1% v/v). The mixture was pumped through the packed tube at a rate of 24 µL·min⁻¹, and 500 µL fractions were collected. The values on the y-axis refer to the peak ratios of substrate and internal standard (GC-FID).
Figure S11. FAD assay of supernatants after incubation of CvFAP on EziG Opal with: T - Tris·HCl (100 mM, pH 8.5), TA - Ammoeng 102 (1% v/v) in Tris·HCl (100 mM, pH 8.5), Li - LiCl (300 mM), Na - NaCl (300 mM), K - KCl (300 mM), Mg - MgCl₂ (300 mM). All salts were dissolved in Ammoeng 102 (1% v/v) in Tris·HCl (100 mM, pH 8.5). CvFAP on EziG Opal (≈10 mg, ≈12% w/w) was incubated with corresponding solutions at 25 °C, 900 rpm, for 30 min. Errors correspond to standard deviation of triplicate determinations.
Figure S12. The ratio of absorbances of CvFAP at 455 nm and 528 nm. The series of CvFAP dilutions was prepared in Tris·HCl (100 mM, pH 8.5) at five concentrations (10, 25, 50, 75, 100 µM; 100 µL volume). Absorbance measurements were made in a 96-well plate using a SpectraMax M2 plate reader.
Figure S13. The UV/Vis spectra of components of the photodecarboxylation reaction mixture. Reaction mixture: palmitic acid (13 mM), CvFAP (6 µM), Ammoeng 102 (1% v/v), Tris·HCl (100 mM, pH 8.5); CvFAP: CvFAP (6 µM) in Tris·HCl (100 mM, pH 8.5); Palmitic acid: palmitic acid (13 mM) in Tris·HCl (100 mM, pH 8.5); Ammoeng 102, 1% v/v); Ammoeng 102: Ammoeng 102 (1% v/v) in in Tris·HCl (100 mM, pH 8.5); Pentadecane: pentadecane (13 mM) in ethyl acetate. For all samples apart from pentadecane, the Tris·HCl (100 mM, pH 8.5) buffer was taken as a background. For pentadecane, ethyl acetate was the background.
### Supplementary Tables

**Table S1.** Screening of organic solvents as co-solvents for the dissolution of palmitic acid.

| Solvent         | Palmitic acid concentration (mM) | Co-solvent fraction (vol%) | Solution formed |
|-----------------|----------------------------------|----------------------------|-----------------|
| Tetrahydrofuran | 13                               | 5                          | −               |
|                 |                                  | 10                         | +               |
|                 |                                  | 20                         | +               |
|                 |                                  | 30                         | +               |
|                 |                                  | 20                         | +               |
|                 |                                  | 30                         | +               |
|                 |                                  | 20                         | +               |
|                 |                                  | 30                         | +               |
|                 | 50                               | 30                         | +               |
| n-Propanol      | 20                               | 5                          | −               |
|                 |                                  | 10                         | +               |
|                 |                                  | 20                         | +               |
|                 |                                  | 30                         | +               |
|                 |                                  | 20                         | +               |
|                 |                                  | 30                         | +               |
|                 |                                  | 20                         | +               |
|                 |                                  | 30                         | +               |
|                 | 50                               | 30                         | +               |
| i-Propanol      | 20                               | 5                          | −               |
|                 |                                  | 10                         | −               |
|                 |                                  | 20                         | +               |
|                 | 30                               | 30                         | +               |
|                 |                                  | 30                         | +               |
|                 | 50                               | 30                         | +               |
| Ethanol         | 13                               | 5                          | −               |
|                 |                                  | 10                         | −               |
|                 |                                  | 20                         | +               |
|                 | 30                               | 30                         | +               |
|                 |                                  | 30                         | −               |
|                 | 50                               | 30                         | −               |
| 1,4-Dioxane     | 13                               | 30                         | −               |
| Acetonitrile    | 13                               | 30                         | −               |
Table S2. Activity test of CvFAP in presence of different Co-solvents.a

| Solvent              | Co-solvent concentration (% v/v) | Area (n-decanol) | Area (pentadecane) | Area (palmitic acid) | Conversion (%)b |
|----------------------|----------------------------------|------------------|--------------------|----------------------|-----------------|
| THF                  | 10                               | 306.3 ± 1.9      | 11.9 ± 2.3         | 684.3 ± 26.5         | 1.7             |
|                      | 20                               | 285.4 ± 11.7     | < 1                | 665.3 ± 57.9         | 0               |
|                      | 30                               | 261.9 ± 1.5      | < 1                | 571.2 ± 7.7          | 0               |
| n-PrOH               | 10                               | 304.4 ± 3.6      | 22.5 ± 1.8         | 636.1 ± 5.0          | 3.4             |
|                      | 20                               | 272.7 ± 6.9      | < 1                | 659.2 ± 15.7         | 0               |
| Triton X-100         | 1                                | 304.2 ± 13.4     | 66.9 ± 4.0         | 568.0 ± 16.9         | 10.5            |
|                      | 5                                | 279.6 ± 1.3      | 53.1 ± 3.4         | 530.5 ± 6.9          | 9.1             |
|                      | 10                               | 243.3 ± 11.4     | 25.6 ± 1.4         | 468.1 ± 14.5         | 5.2             |
| Tween 20             | 5                                | 401.9 ± 4.2      | 64.3 ± 1.8         | 418.7 ± 3.8          | 15.4            |
|                      | 10                               | 402.4 ± 16.0     | 62.5 ± 12.2        | 349.5 ± 60.3         | 17.9            |
| Ammonoeng 100        | 1                                | 318.5 ± 3.9      | 49.3 ± 3.1         | 636.8 ± 11.1         | 7.2             |
|                      | 5                                | 306.2 ± 0.9      | < 1                | 707 ± 5.5            | 0               |
|                      | 10                               | 301.5 ± 3.7      | < 1                | 686.1 ± 3.3          | 0               |
| Ammonoeng 102        | 1                                | 308.0 ± 2.0      | 102.1 ± 3.7        | 568.6 ± 7.2          | 15.2            |
|                      | 5                                | 310.1 ± 3.8      | 18.1 ± 0.8         | 700.4 ± 6.4          | 2.5             |
|                      | 10                               | 307.2 ± 0.9      | 10.7 ± 0.4         | 669.5 ± 4.0          | 1.6             |
| DMSOc                | 30                               | 323.2 ± 1.1      | 130.2 ± 4.4        | 602.8 ± 9.6          | 17.8            |

aReaction conditions: palmitic acid (13 mM), Tris-HCl (100 mM, pH 8.5), co-solvent (stated concentration), CvFAP (6 µM, from 1.78 ± 0.04 mg/mL or 24.3 ± 0.5 mg/mL stock solution) total volume 1 mL, 455 nm irradiation (42 µmol L⁻¹s⁻¹), 25 °C, 1 h, 500 rpm. Displayed errors correspond to standard deviations of triplicate determinations.

bThe conversion was calculated from peak areas, no standard deviation is shown.

cThe reference co-solvent for activity of CvFAP in the photodecarboxylation of palmitic acid
Table S3. Screening of ionic liquids and surfactants as co-solvents for the dissolution of palmitic acid.

| Solvent                        | Palmitic acid concentration (mM) | Co-solvent fraction (vol%) | Solution formed |
|--------------------------------|----------------------------------|----------------------------|-----------------|
| Ammoeng 100                    | 13                               | 1                          | +               |
|                                |                                  | 5                          | +               |
|                                |                                  | 10                         | +               |
|                                |                                  | 1                          | –               |
|                                | 20                               | 5                          | +               |
|                                |                                  | 10                         | +               |
|                                |                                  | 1                          | –               |
|                                | 30                               | 5                          | +               |
|                                |                                  | 10                         | +               |
|                                |                                  | 1                          | –               |
|                                | 50                               | 5                          | –               |
|                                |                                  | 10                         | +               |
| Ammoeng 102                    | 13                               | 1                          | +               |
|                                |                                  | 5                          | +               |
|                                |                                  | 10                         | +               |
|                                |                                  | 1                          | –               |
|                                | 20                               | 5                          | +               |
|                                |                                  | 10                         | +               |
|                                |                                  | 1                          | –               |
|                                | 30                               | 5                          | +               |
|                                |                                  | 10                         | +               |
|                                |                                  | 1                          | –               |
|                                | 50                               | 5                          | –               |
|                                |                                  | 10                         | +               |
| Tris-(2-hydroxyethyl)-methylammonium methylsulfate | 13 | 10 | – |
| 1-Ethyl-3-methylimidazolium acetate | 13 | 10 | – |
| 1-Butyl-3-methylimidazolium acetate | 13 | 10 | – |
| Tween 20                       | 13                               | 2.5                        | –               |
|                                |                                  | 5                          | +               |
|                                | 20                               | 5                          | +               |
|                                |                                  | 5                          | –               |
| Tween 40                       | 13                               | 2.5                        | –               |
|                                |                                  | 5                          | –               |
| Tween 80                       | 13                               | 2.5                        | –               |
|                                |                                  | 5                          | –               |
| TPGS                           | 13                               | 1% (w/v)                   | –               |
| Triton X-100                   | 13                               | 1% (w/v)                   | +               |

*a % (w/v)
Table S4. CvFAP-catalyzed decarboxylation of palmitic acid in presence and absence of co-solvent.

| Entry | Substrate     | Reaction medium                              | Conversion (%) |
|-------|---------------|----------------------------------------------|----------------|
| 1a    | Palmitic acid | 30% v/v DMSO in Tris·HCl (100 mM, pH 8.5)    | 20.0 ± 1.1     |
| 2b    | Palmitic acid | Tris·HCl (100 mM, pH 8.5)                    | 18.2 ± 0.8     |
| 3b    | Sodium palmitate | Tris·HCl (100 mM, pH 8.5)         | 19.7 ± 0.5     |

a palmitic acid (13 mM), Tris·HCl buffer (100 mM, pH 8.5, 700 µL total volume), CvFAP (6 µM), DMSO (30% v/v), total volume 1 mL, 455 nm irradiation (42 µmol L⁻¹s⁻¹), 25 °C, 1 h, 500 rpm.
b palmitic acid or sodium palmitate (13 mM), Tris·HCl buffer (100 mM, pH 8.5, 1 mL total volume), CvFAP (6 µM), total volume 1 mL, 455 nm irradiation (42 µmol L⁻¹s⁻¹), 25 °C, 1 h, 500 rpm.
c Conversions were calculated from peak areas in the chromatogram. Errors correspond to standard deviations of triplicate experiments.

Table S5. Effect of immobilization time and type of EziG beads on the protein loading and reaction conversion in the photodecarboxylation of palmitic acid.a

| Entry | EziG beads | Time (min) | Enzyme loading (% w/w) | Conversion (%)b |
|-------|------------|------------|-------------------------|-----------------|
| 1     | Opal       | 60         | 4.3 ± 1.0               | 0               |
| 2     | Opal       | 120        | 5.5 ± 1.1               | 0               |
| 3     | Opal       | 180        | 6.6 ± 1.6               | 0               |
| 4     | Coral      | 60         | 2.8 ± 2.9               | 0               |
| 5     | Coral      | 120        | 9.8 ± 1.7               | 0               |
| 6     | Coral      | 180        | 8.4 ± 1.7               | 0               |
| 7     | Coral      | 60         | 3.6 ± 1.7               | 0               |
| 8     | Amber      | 120        | 0.0 ± 5.9               | 0               |
| 9     | Amber      | 180        | 8.6 ± 1.6               | 0               |

a Immobilization conditions: EziG (10 mg), lyophilized cell lysate (40 mg·mL⁻¹, 0.35 U) in Tris·HCl buffer (100 mM, pH 8.5), total volume 0.5 mL, 4 °C, 900 rpm, time. Displayed errors correspond to standard deviations of triplicate determinations.
b Activity test conditions: carrier/enzyme (∼10 mg), palmitic acid (13 mM), Tris·HCl buffer (100 mM, pH 8.5, 700 µL total volume), DMSO (30% v/v), total volume 1 mL, 455 nm irradiation (42 µmol L⁻¹s⁻¹), 25 °C, 1 h, 500 rpm.
4 Chromatograms (GC-FID)

Figure S14. Chromatogram of the mixture of nonane, capric acid and n-decanol (internal standard) after derivatization of the ethyl acetate extract with BSTFA/Pyridine (1:1 v/v).

Figure S15. Chromatogram of the mixture of undecane, lauric acid and n-decanol (internal standard) after derivatization of the ethyl acetate extract with BSTFA/Pyridine (1:1 v/v).
Figure S16. Chromatogram of the mixture of tridecane, myristic acid and n-decanol (internal standard) after derivatization of the ethyl acetate extract with BSTFA/Pyridine (1:1 v/v).

Figure S17. Chromatogram of the mixture of pentadecane, palmitic acid and n-decanol (internal standard) after derivatization of the ethyl acetate extract with BSTFA/Pyridine (1:1 v/v).
Figure S18. Chromatogram of the mixture of decane, undecanoic acid and \textit{n}-decanol (internal standard) after derivatization of the ethyl acetate extract with BSTFA/Pyridine (1:1 v/v).

Figure S19. Chromatogram of the mixture of tetradecane, pentadecanoic acid and \textit{n}-decanol (internal standard) after derivatization of the ethyl acetate extract with BSTFA/Pyridine (1:1 v/v).
Figure S20. Chromatogram of the mixture of heptadecane, octadecanoic acid and \( n \)-decanol (internal standard) after derivatization of the ethyl acetate extract with BSTFA/Pyridine (1:1 v/v).
5  EI-MS spectra

Figure S21. EI-MS spectrum of the trimethylsilyl ester of decanoic acid.

Figure S22. EI-MS spectrum of the trimethylsilyl ester of undecanoic acid.
Figure S23. EI-MS spectrum of the trimethylsilyl ester of dodecanoic acid.

Figure S24. EI-MS spectrum of the trimethylsilyl ester of tetradecanoic acid.
**Figure S25.** EI-MS spectrum of the trimethylsilyl ester of hexadecanoic acid.

**Figure S26.** EI-MS spectrum of the trimethylsilyl ester of octadecanoic acid.
**Figure S27.** EI-MS spectrum of nonane.

**Figure S28.** EI-MS spectrum of decane.
Figure S29. EI-MS spectrum of undecane.

Figure S30. EI-MS spectrum of tridecane.
Figure S31. EI-MS spectrum of tetradecane.

Figure S32. EI-MS spectrum of pentadecane.
Figure S33. EI-MS spectrum of heptadecane.
6 Calibration Curves

6.1 Procedure for Preparation of Standard Series of Palmitic Acid and Pentadecane in DMSO (30% v/v) in Tris·HCl (100 mM, pH 8.5)

A standard series of palmitic acid solutions was prepared as outlined in Table S6.

Table S6. Measurements for the preparation of a standard series of palmitic acid.

| Sample | 50 mM palmitic acid [µL] | DMSO [µL] | Tris·HCl [µL] | Final concentration [mM] |
|--------|-------------------------|-----------|---------------|-------------------------|
| 1      | 3                       | 297       | 700           | 0.15                    |
| 2      | 36                      | 264       | 700           | 0.80                    |
| 3      | 69                      | 231       | 700           | 3.45                    |
| 4      | 102                     | 198       | 700           | 5.10                    |
| 5      | 135                     | 165       | 700           | 6.75                    |
| 6      | 168                     | 132       | 700           | 8.40                    |
| 7      | 201                     | 99        | 700           | 10.05                   |
| 8      | 234                     | 66        | 700           | 11.70                   |
| 9      | 267                     | 33        | 700           | 13.35                   |
| 10     | 300                     | 0         | 700           | 15.00                   |

The workup and analysis of the standard series samples was carried out as outlined in the general procedure.

Since pentadecane was found to be insoluble in DMSO, the standard series of solutions of pentadecane in 5 mM n-decanol (EtOAc) was prepared. The final concentrations of pentadecane that would be present in the organic phase after extraction were approximated with the following formula:

\[
c_{\text{final}} = \frac{n_{e1} + n_{e2}}{V_{\text{org}}} = \frac{3}{5} n_x + \frac{2}{5} n_x \cdot \frac{1}{700} \cdot \frac{1}{500} = \frac{0.0008}{1107.12 n_x}
\]

\[
n_{e1} = \frac{3}{5} n_x \quad \text{Molar quantity of pentadecane transferred in the first extraction step}
\]

\[
n_{e2} = \frac{2}{5} n_x \cdot \frac{1}{700} \cdot 500 \quad \text{Molar quantity of pentadecane transferred in the second extraction step}
\]

\[
V_{\text{org}} \quad \text{Total volume of combined organic phases}
\]
A standard series of pentadecane solutions was prepared as outlined in Table S7. The obtained solutions were subsequently derivatized and analyzed as specified in the general procedure.

**Table S7.** Measurements for the preparation of a standard series of pentadecane.

| Sample | 8.3 mM pentadecane [µL] | 5 mM decanol in ethyl acetate [µL] | Final concentration [mM] |
|--------|--------------------------|-----------------------------------|--------------------------|
| 1      | 10                       | 990                               | 0.083                    |
| 2      | 120                      | 880                               | 0.996                    |
| 3      | 230                      | 770                               | 1.910                    |
| 4      | 340                      | 660                               | 2.820                    |
| 5      | 450                      | 550                               | 3.736                    |
| 6      | 560                      | 440                               | 4.650                    |
| 7      | 670                      | 330                               | 5.564                    |
| 8      | 780                      | 220                               | 6.476                    |
| 9      | 890                      | 110                               | 7.390                    |
| 10     | 1000                     | 0                                 | 8.300                    |

### 6.2 Procedure for Preparation of Standard Series of Fatty Acids and Alkanes in Ammonoeng 102 (1% v/v)

Stock solutions of fatty acid or alkane (15 mM, 10 mL) were prepared in Ammonoeng 102 (1% v/v) in Tris·HCl buffer (100 mM, pH 8.5). The alkane stock solutions were obtained as a turbid stable emulsion. Preparation of both stocks required ultrasonication for several minutes to disperse the corresponding analyte. The standard series dilutions were made without replicates as shown in Table S8.

**Table S8.** Standard series of dilutions for fatty acids and alkanes in Ammonoeng 102 (1% v/v).

| Sample | 15 mM analyte (µL) | 1% Ammonoeng 102 (µL) | Final concentration (mM) |
|--------|--------------------|-----------------------|--------------------------|
| 1      | 5                  | 495                   | 0.15                     |
| 2      | 25                 | 475                   | 0.75                     |
| 3      | 115                | 385                   | 3.45                     |
| 4      | 170                | 330                   | 5.1                      |
| 5      | 225                | 275                   | 6.75                     |
| 6      | 280                | 220                   | 8.4                      |
| 7      | 335                | 165                   | 10.05                    |
| 8      | 390                | 110                   | 11.7                     |
| 9      | 445                | 55                    | 13.35                    |
| 10     | 500                | 0                     | 15                       |
The samples were prepared in 2 mL microcentrifuge tubes and workup and analysis were performed as described previously. GC-FID was used for analysis (ACHIRAL method, 5 µL injection volume).

6.3 Palmitic Acid and Pentadecane in DMSO (30% v/v) in Tris·HCl (100 mM, pH 8.5)

![Graph showing calibration curves for palmitic acid and pentadecane](image)

**Figure S34.** Calibration curve for palmitic acid after extraction from DMSO (30% v/v) in Tris·HCl (100 mM, pH 8.5).

**Figure S35.** Calibration curve for pentadecane from a standard series in ethyl acetate.
### 6.4 Stearic acid and Heptadecane in Ammoeng 102 (1% v/v)

![Graph](image)

**Figure S36.** Calibration curve for stearic acid after extraction from Ammoeng 102 (1% v/v in Tris·HCl buffer (100 mM, pH 8.5)).

![Graph](image)

**Figure S37.** Calibration curve for heptadecane from a standard series in ethyl acetate.
6.5 Palmitic Acid and Pentadecane in Ammoeng 102 (1% v/v)

**Figure S38.** Calibration curve for palmitic acid after extraction from Ammoeng 102 (1% v/v in Tris·HCl buffer (100 mM, pH 8.5)).

\[
y = 0.8564x + 0.0002 \\
R^2 = 0.999
\]

**Figure S39.** Calibration curve for pentadecane after extraction from Ammoeng 102 (1% v/v in Tris·HCl buffer (100 mM, pH 8.5)).

\[
y = 0.6753x - 0.074 \\
R^2 = 0.9976
\]
6.6 Pentadecanoic acid and Tetradeane in Ammoeng 102 (1% v/v)

Figure S40. Calibration curve for pentadecanoic acid after extraction from Ammoeng 102 (1% v/v in Tris-HCl buffer (100 mM, pH 8.5)).

\[ y = 0.8063x - 0.0251 \]
\[ R^2 = 0.999 \]

Figure S41. Calibration curve for tetradeane after extraction from Ammoeng 102 (1% v/v in Tris-HCl buffer (100 mM, pH 8.5)).

\[ y = 0.6284x - 0.0237 \]
\[ R^2 = 0.9988 \]
6.7 Undecanoic acid and Decane in Amмоeng 102 (1% v/v)

Figure S42. Calibration curve for undecanoic acid after extraction from Ammonoeng 102 (1% v/v in Tris-HCl buffer (100 mM, pH 8.5)).

\[ y = 0.5525x - 0.0395 \]
\[ R^2 = 0.9984 \]

Figure S43. Calibration curve for decane after extraction from Ammonoeng 102 (1% v/v in Tris-HCl buffer (100 mM, pH 8.5)).

\[ y = 0.4029x - 0.0172 \]
\[ R^2 = 0.9981 \]
6.8 Capric Acid and Nonane in Ammoeng 102 (1% v/v)

Figure S44. Calibration curve for capric acid after extraction from Amмоeng 102 (1% v/v in Tris·HCl buffer (100 mM, pH 8.5)).

\[ y = 0.5369x - 0.0167 \]
\[ R^2 = 0.9993 \]

Figure S45. Calibration curve for nonane after extraction from Amмоeng 102 (1% v/v in Tris·HCl buffer (100 mM, pH 8.5)).

\[ y = 0.3019x - 0.0092 \]
\[ R^2 = 0.9983 \]
6.9 Lauric Acid and Undecane in Ammoeng 102 (1% v/v)

Figure S46. Calibration curve for lauric acid after extraction from Ammoeng 102 (1% v/v in Tris·HCl buffer (100 mM, pH 8.5)).

\[ y = 0.6433x - 0.034 \]
\[ R^2 = 0.9987 \]

Figure S47. Calibration curve for undecane after extraction from Ammoeng 102 (1% v/v in Tris·HCl buffer (100 mM, pH 8.5)).

\[ y = 0.4274x - 0.0142 \]
\[ R^2 = 0.9976 \]
6.10 Myristic Acid and Tridecane in Ammoeng 102 (1% v/v)

Figure S48. Calibration curve for myristic acid after extraction from Ammoeng 102 (1% v/v in Tris-HCl buffer (100 mM, pH 8.5)).

\[ y = 0.7824x - 0.0152 \]

\[ R^2 = 0.9992 \]

Figure S49. Calibration curve for tridecane after extraction from Ammoeng 102 (1% v/v in Tris-HCl buffer (100 mM, pH 8.5)).

\[ y = 0.5198x - 0.0362 \]

\[ R^2 = 0.9942 \]
6.11 Calibration of BSA for the Bradford Assay

![Calibration curve for bovine serum albumin (BSA) in Tris·HCl buffer (100 mM, pH 8.5).](image)

**Figure S50.** Calibration curve for bovine serum albumin (BSA) in Tris·HCl buffer (100 mM, pH 8.5).

6.12 Calibration of FAD

6.12.1 Procedure

For the preparation of the standard series, a solution of FAD (50 µM) in Ammoeng 102 (1% v/v in Tris·HCl buffer) was prepared in a volumetric flask (4.3 mg in 100 mL). A standard series of dilutions was prepared as shown in Table S9.

**Table S9.** Preparation of the standard series of dilutions of FAD in Ammoeng 102 (1%).

| Sample | Stock solution (µL) | 1% Ammoeng 102 (µL) | Final concentration (µM) |
|--------|---------------------|----------------------|--------------------------|
| A      | 1000                | 0                    | 50.0                     |
| B      | 600                 | 400                  | 30.0                     |
| C      | 400                 | 600                  | 20.0                     |
| D      | 200                 | 800                  | 10.0                     |
| E      | 100                 | 900                  | 5.0                      |
| F      | 80                  | 920                  | 4.0                      |
| G      | 50                  | 950                  | 2.5                      |
CvFAP samples were heated at 100 °C for 10 min in a benchtop shaker and centrifuged at 14000 rpm for 1 min. Aliquots of each supernatant (200 µL) were transferred to a 96-well plate along with standard series solutions (200 µL). Ammoeng 102 (1% v/v in Tris-HCl buffer) was used as the background measurement. The absorbances were taken in the plate reader SpectraMax M2 at 450 nm, with 20 s shaking before measurement. All samples were prepared in triplicates.

6.12.2 Calibration curve

![Calibration curve for flavin adenine dinucleotide (FAD) in Ammoeng 102 (1% v/v in Tris-HCl buffer (100 mM, pH 8.5)).](image)

**Figure S51.** Calibration curve for flavin adenine dinucleotide (FAD) in Ammoeng 102 (1% v/v in Tris-HCl buffer (100 mM, pH 8.5)).
7 CvFAP Gene Sequence

- **Start Codon**
- **His6-tag**
- **Thioredoxin TrxA (Uniprot: P0AA25)**
- **TEV Protease** is a highly specific cysteine protease that recognizes the amino-acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-(/Ser) and cleaves between the Gln and Ser residue.
- **Residue 62-654 of FAP**
- **Stop Codon**

CATATGAAATCTTTCACCAATACACCACCATCACGTTTCTTCTATGAGCGATAAATATTACTACCTGACTGACGACAGTCTTTGACACCGGATATCGTACTCTAACAAACCGGAGCAGGGAAACGTGATGCTGCTGTTACCCGACGCGGACGGGTAGTCGACCGCGACCGGTTGCTTCTTCTACTACCTGGCTGCTGAGCTGAAAGGATTCGACCGGCTTACCCCGGAAACTGTCACCAGTTACCTGACCGACAAAGACGGTGCTGATCTGGCTACCCTGCGTAAAGGCATCCATTGGGCACGTGATGTTGCGCTAGCTCTGCTCTGTCCGAATACCTGGATGGTGAGCTGTTCCCAAGTAGCGGCGTTGTTTCTGATGATCAGATCGATGAATATATCCGTCGTTCTATCCACTCGTCCAACGCTATCACTGGCACCTGTAAAATGGGTAACGCAGGTGACAGCAGCTCTGTGGTAGACAACCAGCTGCGTGTTCACGGTGTTGAAAGGGCCTGCGCGTTGTTGACGCTAGCGTTGTTCCGAAAATTCCGGGTGGTCAGACCGGTGCGCCGGTAGTTATGATCGCTGAACGCGCAGCAGCTCTGCTGACGGGGAAAGCAACCATTGGTGCATCTGCTGCTGCACCGGCGACCGTAGCTGCA

TAA AAGCTT
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