Supporting Information

Chemoenzymatic cascade for stilbene production from cinnamic acid catalyzed by ferulic acid decarboxylase and an artificial metathease

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Catalyst preparation

Coupling of the Grubbs-Hoveyda type catalyst to FhuA ΔCVF^{tev} was performed under nitrogen in a *MBraun* glovebox using the previously published protocols.[1] Grubbs-Hoveyda type catalyst with maleimide linking unit was prepared as published previously.[1] AquaMet catalyst is commercially available and was used as received.

Product analysis

GC-FID analyses were performed using a *Shimadzu* GC-2010 device equipped with an Optima 17ms column (CS-Chromatographie). Hydrogen was used as carrier gas. GC-MS analyses were recorded on a *Shimadzu* GCMS-GP2010 Plus machine using the same column as above. Helium was used as the carrier gas.

FDC1 cloning, expression and analytics

Media

All media components were obtained from AppliChem. LB (tryptone 10 g·L^{-1}, NaCl 10 g·L^{-1}, yeast extract 5 g·L^{-1}) and TB (peptone 12 g·L^{-1}, yeast extract 24 g·L^{-1}, glycerol 4 g·L^{-1}, KH_2PO_4 2.31 g·L^{-1}, K_2HPO_4 12.5 g·L^{-1}) media were used for cultivation and enzyme production. If required, kanamycin was supplemented at a concentration of 50 mg·L^{-1} (LB_{Kan}, TB_{Kan}).

Cloning

Ferulic acid decarboxylase from *Saccharomyces cerevisiae* (FDC1; UniProt Q03034) and a truncated version of phenylacrylic decarboxylase (tPAD1)[2] were ordered as a bicistronic synthetic construct (Thermo Fisher Scientific) with both genes being codon optimized for expression in *E. coli*. Cloning into the pET28a(+) vector was performed by restriction enzyme digestion via *NcoI* and *BamHI*, followed by ligation using T4 DNA ligase (all enzymes from New England Biolabs).

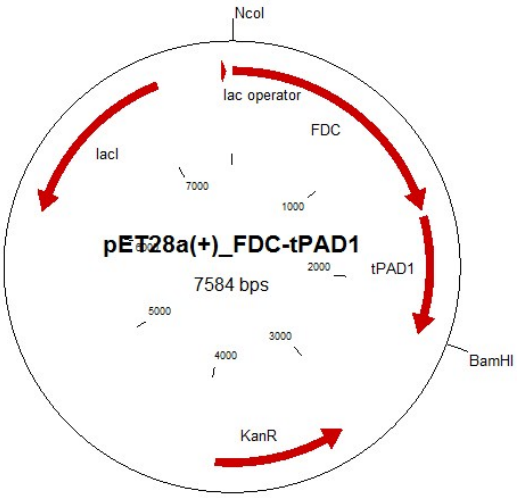

Figure S1: Vector map of FDC1-tPAD1 in pET28a(+). The genes FDC1 and tPAD1 consist of 1536 and 729 bps, respectively.

Expression

FDC1 and tPAD1 from *Saccharomyces cerevisiae* were produced in *E. coli* BL21 Gold (DE3). 5 mL LB_{Kan} medium were inoculated from a glycerol stock and incubated at 37 °C, 250 rpm overnight. The preculture was used to inoculate 500 mL TB_{Kan} medium. Cells were grown at 30 °C until they reached an OD_{600} of 0.8 – 1.0. Then, gene expression was induced by addition of 0.5 mM isopropyl-β-D-thiogalactopyranosid (IPTG). The induction was carried out at 30 °C, 250 rpm for 20 h. Cells were harvested by centrifugation at 3220 g, 4 °C for 20 min. The cell pellet was stored at -20 °C until used.

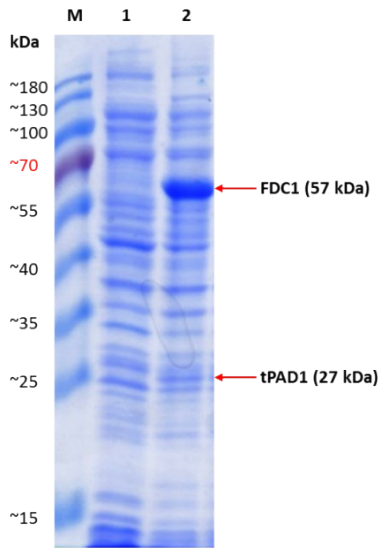

Figure S2: SDS-PAGE of FDC1-tPAD1. The molecular weight of FDC1 and tPAD1 is 57 and 27 kDa, respectively. **M** = marker (PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, ThermoFisher); **1** = sample before induction; **2** = sample after induction with 0.5 mM IPTG (20 h).

Purification of FDC1

FDC1 was purified using a His-Tag column as described by Lin *et al*. with an ÄKTAprime Plus chromatography system (GE Healthcare).[2] Protein concentration of the elution fractions was determined by BCA assay and fractions containing high protein amount were pooled and desalted through a HiTrap desalting column (GE Healthcare) into sodium phosphate buffer (100 mM, pH 8.0).

Determination of FDC1 concentration in cell-free extracts

FDC1 concentration in cell-free extracts was determined using SDS-PAGE. A standard curve was prepared using purified FDC1 in a range from 1 – 40 µM. ImageJ 1.52d (Rasband, W.S., U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018) was used to calculate protein concentration.

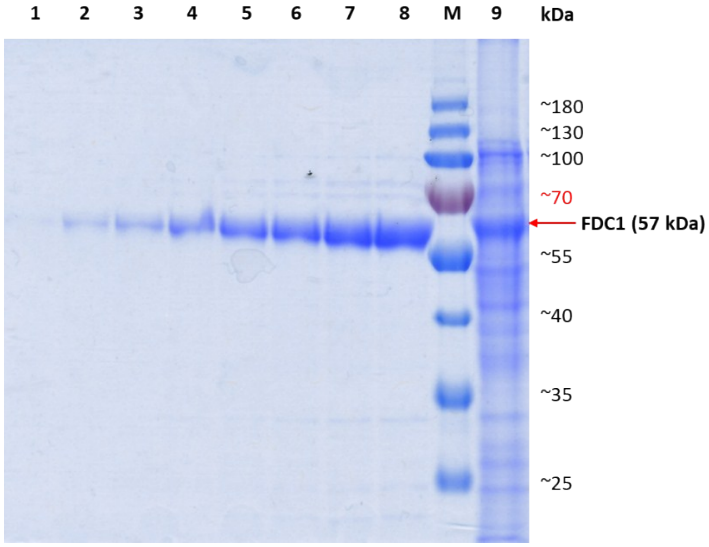

Figure S3: SDS-PAGE of FDC1-tPAD1 cell lysate for protein concentration determination. The molecular weight of FDC1 is 57 kDa. **1 – 8** = standard curve (1, 2.5, 5, 10, 15, 20, 30, 40 µM). **9** = FDC1-tPAD1 cell lysate. **M** = marker (PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, ThermoFisher).

FhuA ΔCVF^{tev} production, extraction, coupling and analytics

Expression and extraction

Production of FhuA ΔCVF^{tev} and extraction using SDS were performed as previously described.[1, 3]

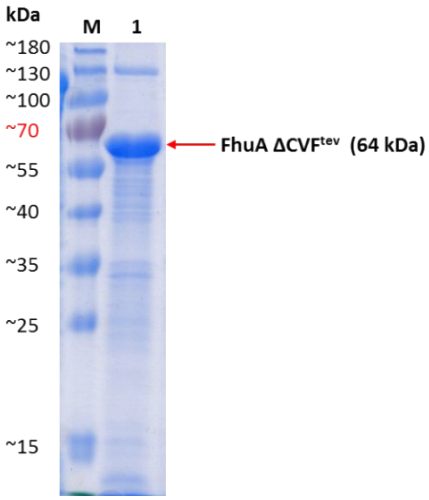

Figure S4: SDS-PAGE of FhuA ΔCVF^{tev} extraction. The molecular weight of FhuA ΔCVF^{tev} is 64 kDa. **M** = marker (PageRuler^{TM} Prestained Protein Ladder, 10 to 180 kDa, ThermoFisher); **1** = FhuA ΔCVF^{tev} in 1.25 (w/w)% SDS.

Determination of protein concentration

The Pierce^{TM} BCA Protein Assay Kit (ThermoFisher Scientific) was used to determine protein concentrations.

Coupling of Grubbs-Hoveyda type catalyst to FhuA ΔCVF^{tev}

Covalent coupling of the Grubbs-Hoveyda type catalyst to FhuA ΔCVF^{tev} was performed as described previously using thiol-ene click reaction.[1]

Determination of coupling efficiency

Coupling efficiency was determined using the fluorescence dye ThioGlo1® (Berry & Associates) as previously described.[3]

Procedures for catalysis

Procedure for decarboxylation

Cell-free extracts were prepared by sonication and centrifugation (10 min, 15.000 g, 4 °C) of cells harboring FDC1 and tPAD1. For decarboxylation of cinnamic acid derivatives, the substrate was added to a final concentration of 12 mM in sodium phosphate buffer (100 mM, pH 6.0, 137 mM NaCl, 5 (v/v)% THF). Typically, cell-free extracts contained 15 µM FDC1 (as determined by quantitative SDS-PAGE analysis; see: Determination of FDC1 concentration in cell-free extracts). The reaction mixture was stirred for 1 h at 35 °C, followed by extraction with dichloromethane (DCM) and analysis by GC-FID.

Procedure for cross-metathesis (CM)

CM reaction mixtures contained 3 mol % of catalyst and 12 mM substrate in sodium phosphate buffer (100 mM, pH 6.0, 50 mM NaCl, 0.1 (w/w)% SDS). The reaction was run for 4 h at 35 °C, followed by extraction with DCM and analysis by GC-MS-FID.

Procedure for concurrent and sequential cascade reaction

In the concurrent setup, 15 µM FDC1 in cell-free extracts (as determined by quantitative SDS-PAGE analysis; see: Determination of FDC1 concentration in cell-free extracts) and 1 mol % biohybrid catalyst or AquaMet catalyst were used for conversion of 12 mM substrate in 500 µL sodium phosphate buffer (100 mM, pH 6.0, 50 mM NaCl, 0.04 (w/w)% SDS, 2 (v/v)% THF). The reaction was run for 4 h at 35 °C.

In sequential cascade reactions, the decarboxylation step was performed as described above. Subsequently, 3 mol % of freshly coupled biohybrid catalyst or AquaMet catalyst were added and the reaction was continued for 4 h at 35 °C.

AquaMet Catalyst

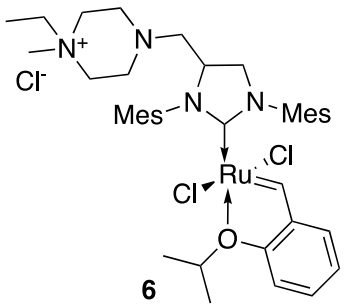

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Figure S4. Molecular structure of AquaMet catalyst **6**.

ICP-AES measurements

For ICP-AES analysis, the sample was treated with 10 mL aqua regia (3:1 HCl:HNO_3 mixture) under heating (80-100 °C) for 48 h. The resulting mixture was filtered and analyzed. The background was subtracted from the obtained ppm values and the dilution was taken into account.

Table S1. Results of the ICP-AES analysis.

Entry	Sample	ppm Ru (measured)	ppm Ru (in the sample)
1	Aqua regia	0.139	-
2	Buffer solution containing AquaMet (Table 2, entry 3)	3.804	36.65
3	Catalysis with AquaMet (Table 2, entry 3)	2.565	24.26
4	Catalysis with BHC (Table 2, entry 8 or 9)	0.242	1.03
Explanation: Entry 1 (Background): Sample of aqua regia as used for dissolving the other samples. Entry 2: Aqueous phase of a reaction mixture containing AquaMet. Entry 3: After catalysis with AquaMet, the aqueous phase was extracted with DCM. The DCM extract was then analyzed by ICP-AES. Entry 4: After catalysis with biohybrid catalyst (BHC). The aqueous phase was extracted with DCM. The DCM extract was then analyzed by ICP-AES.			

Appendix

Amino acid sequence of FDC1–tPAD1 (*S. cerevisiae*, UniProt Q03034 and P33751)

ATGGGC**CATCACCATCATCATCAC**CGTAAACTGAATCCGGCACTGGAATTTCGTGATTTTATTCAGGTT
CTGAAAGATGAGGATGACCTGATTGAAATCACCGAAGAAATTGATCCGAATCTGGAAGTTGGTGCCA
TTATGCGTAAAGCATATGAAAGCCATCTGCCTGCACCGCTGTTTAAAAACCTGAAAGGTGCAAGCAAA
GACCTGTTTAGCATTCTGGGTTGTCCGGCAGGTCTGCGTAGCAAAGAAAAAGGTGATCATGGTCGTAT
TGCCCATCATCTGGGTTTAGATCCGAAAACCACCATCAAAGAGATCATCGATTATCTGCTGGAATGCA
AAGAGAAAGAACCGCTGCCTCCGATTACCGTTCCGGTTAGCAGCGCACCGTGTAAAACCCATATTCTG
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<u>GGAATAA</u>**<u>AGGAGG</u>**<u>TATACC</u>ATGCTGCTGTTTCCGCGTCGTACCAATATTGCATTTTTCAAAACCACCG
GCATCTTTGCAAATTTTCCGCTGCTGGGTCGTACCATTACCACCAGTCCGAGCTTTCTGACCCATAAAC
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GTATTCTGGATTGTTTTGGTATTCATGCCGATACCTTTCCGCGTTGGGAAGGTATTAAAAGCAAATAA

FDC1 (1st gene) and tPAD1 (2nd gene), His-Tag marked in bold

Linkerregion underlined, rbs marked in bold

References

1. F. Philippart, M. Arlt, S. Gotzen, S.-J. Tenne, M. Bocola, H.-H. Chen, L. Zhu, U. Schwaneberg and J. Okuda, *Chem. Eur. J.*, 2013, **19**, 13865 – 13871.
2. F. Lin, K. L. Ferguson, D. R. Boyer, X. N. Lin and E. N. G. Marsh, *ACS Chem. Biol.*, 2015, **10**, 1137-1144.
3. D. F. Sauer, Y. Qu, M. A. S. Mertens, J. Schiffels, T. Polen, U. Schwaneberg and J. Okuda, *Catal. Sci. Technol.*, 2019, **9**, 942-946.