catalysts

MDPI

Supplement

Ppo-Er1 a novel ene reductase from *Paenibacillus polymyxa*

Supporting Information

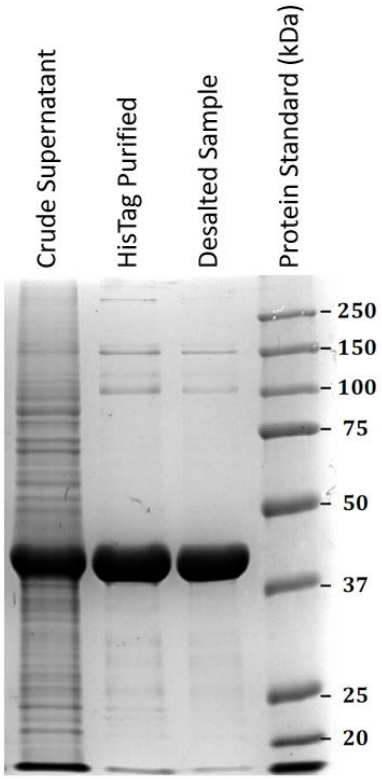


Figure S1: SDS-PAGE showing the purity of ene reductase Ppo-ER1 after each purification step.

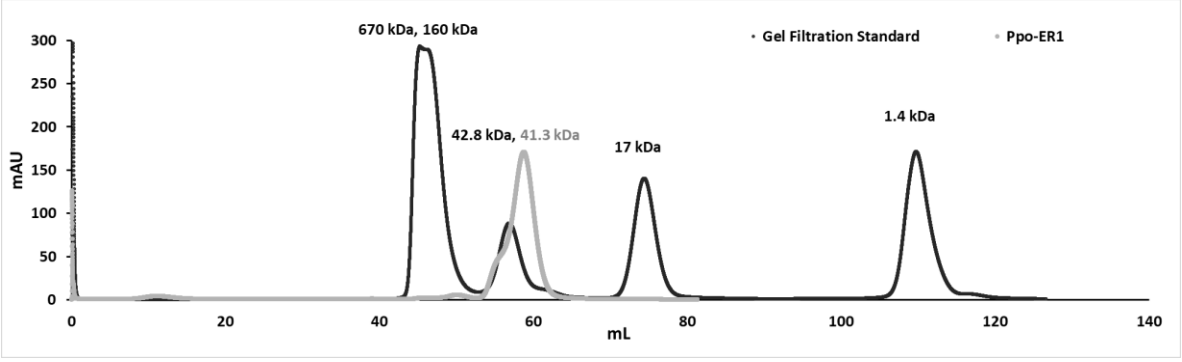


Figure S2: Gel filtration of Ppo-ER1 in comparison with the Gel Filtration Standard (1.4-670 kDa) from Bio Rad.

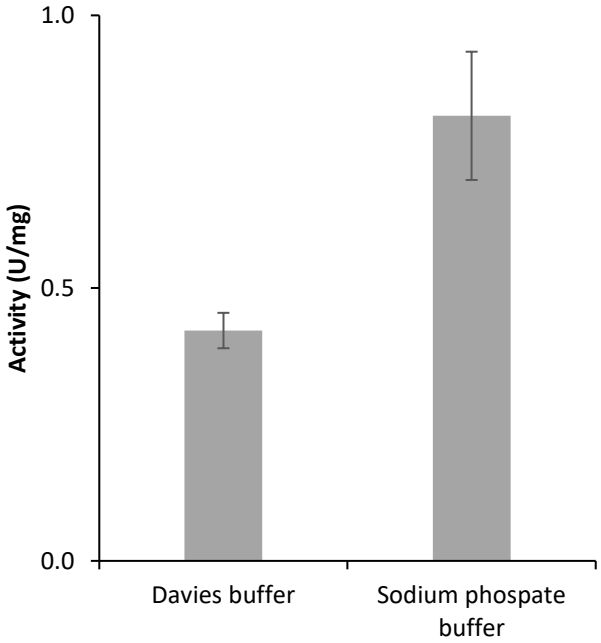


Figure S3: Comparison of the activity of Ppo-ER1 in Davies buffer and sodium phosphate buffer.

Table S1: Overview of the used GC-methods. For all measurements a 7890B GC System Agilent with FID detector was used.

Method	Column	Temperature profile	Constant Flow/Pressure	Split	Substrate Retention Time (min)	Product Retention Time (min)
A	DB5	40 °C 5 min -> 3 °C/min -> 60°C -> 40 °C/min -> 300 °C	25 psi	10:01	Cyclohexenone (8.8) 2-Methyl-2-cyclohexenone (12.0) 3-Methyl-2-cyclohexenone (13.5) Cyclopentenone (4.7) 3-Methyl-2-cyclopentenone (10.6) Hexenal (5.5) 2-Methyl-2-pentenal (4.7)	Cyclohexanone (7.0) 2-Methyl-2-cyclohexanone (9.6) 3-Methyl-2-cyclohexanone (9.7) Cyclopentanone (3.5) 3-Methyl-2-cyclopentanone (5.2) Hexanal (3.8) 2-Methyl-2-pentanal (2.9)
B	DB5	110 °C 10 min -> 60 °C/min -> 300 °C	25 psi	10:01	4-Phenyl-3-buten-2-one (9.8)	4-Phenyl-3-butanone (5.6)
C	DB5	40 °C 10 min -> 3 °C/min -> 60 °C -> 40 °C/min -> 300 °C	15.8 psi	10:01	Butyl acrylate (11.9) Ethyl crotonate (8.1)	Butyl propionate (12.9) Ethyl butyrate (5.9)
D	DB5	50 °C -> 10 °C/min -> 90 °C 10 -> 15 °C/min -> 120 °C -> 60 °C/min -> 300 °C	25 psi	10:01	Carvone (13.2)	Dihydrocarvone (10.5/10.9)
E	DB5	100 °C 8 min -> 10 °C/min -> 190 °C -> 60 °C/min -> 300 °C	25 psi	10:01	*trans*-β-Methyl-β-nitrosyrene (13.2)	2-Methyl-2-nitroethylbenzen (10.6)
F	DB5	110 °C 10 min -> 10 °C/min -> 180 °C -> 60 °C/min -> 300 °C	25 psi	10:01	Cinnamic acid (13.1)	3-Phenylpropionic acid (9.6)
G	DB5	40 °C 10 min -> 11 °C/min -> 110 °C -> 60 °C/min -> 300 °C	25 psi	10:01	Citraconic acid (11.1) Crotonic acid (6.6)	
H	DB5	100 °C 10 min -> 5 °C/min -> 140 °C -> 20 °C/min -> 220 °C -> 40 °C/min -> 300 °C	26.3 psi	10:01	Citral (7.2/8.5)	Citronellal (4.6)
I	DB5	40 °C 8 min -> 15 °C/min -> 140 °C -> 60 °C/min -> 300 °C	26.3 psi	10:01	Maleimide (11.6)	Succinimide (13.7)

Method	Column	Temperature profile	Constant Flow/Pressure	Split	Substrate Retention Time (min)	Product Retention Time (min)
J	DB5	100 °C 7 min -> 10 °C/min -> 200 °C -> 40 °C/min -> 300 °C	26.3 psi	10:01	Diethylbenzyldienmalonate (16.5)	Diethylbenzylmalonate (12.3)
K	DB5	95 °C 10 min -> 15 °C/min -> 180 °C -> 60 °C/min -> 300 °C	25 psi	10:01	Cinnamic aldehyde (10.6)	3-Phenylpropanal (5.9)
L	BGB-175	120 °C 10 min -> 5 °C/min -> 160 °C -> 20 °C/min -> 220 °C	27.9 psi	10:01	Carvone (8.2)	Dihydrocarvone ((R) 6.2/ (S) 6.9)
M	BGB-175	100 °C 10 min -> 5 °C/min -> 140 °C -> 20 °C/min -> 220 °C	26.3 psi	10:01	Citral (15.8/16.1)	Citronellal ((S) 8.3/ (R) 9.0))
N	BGB-175	90 °C 10 min -> 5 °C/min -> 140 °C -> 20 °C/min -> 220 °C	25.4 psi	10:01	2.Methyl-2-cyclohexenone (10.6)	2-Methyl-2-cyclohexenone ((S) 8.2/ (R) 9.7)
O	BGB-175	45 °C 10 min -> 5 °C/min -> 140 °C -> 20 °C/min -> 220 °C	16.9 psi	10:01	2-Methyl-2-pentenal 15.3)	2-Methyl-2-pentanal ((R) 13.1/ (S) 13.2)

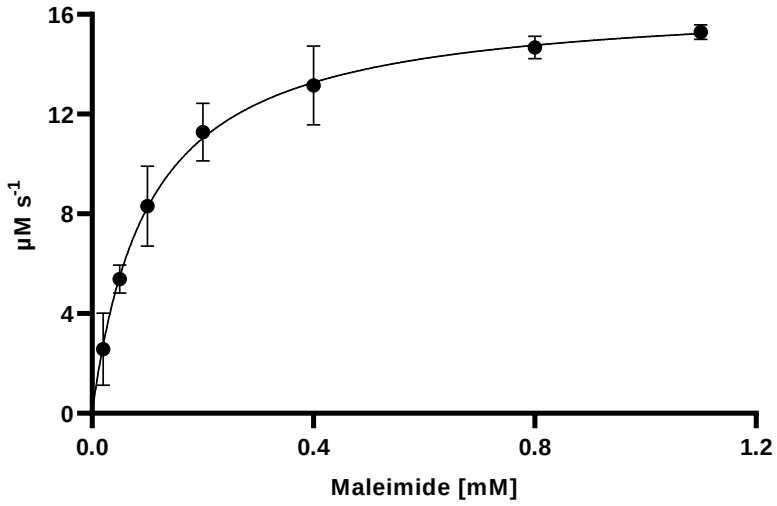


Figure S4: Michaelis-Menten kinetic for maleimide

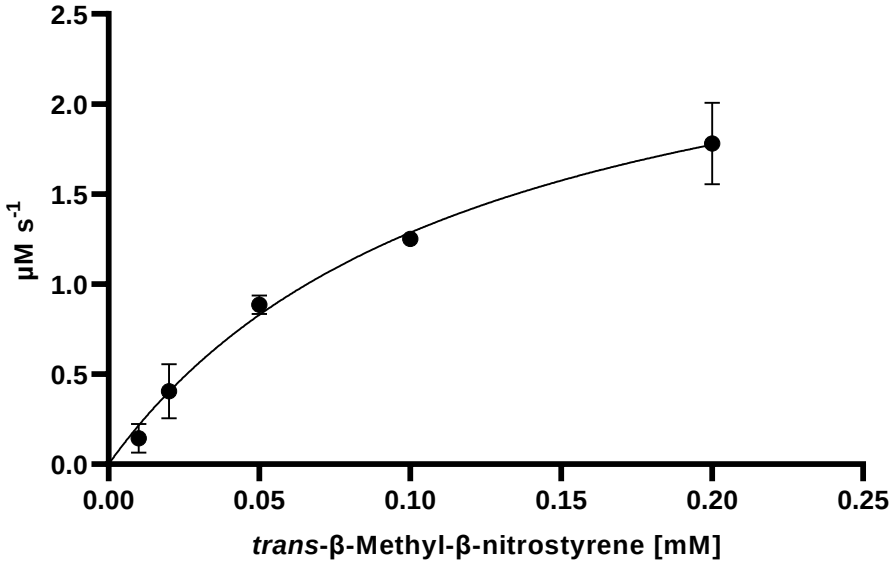


Figure S5: Michaelis-Menten kinetic for *trans*-β-Methyl-β-nitrostyrene

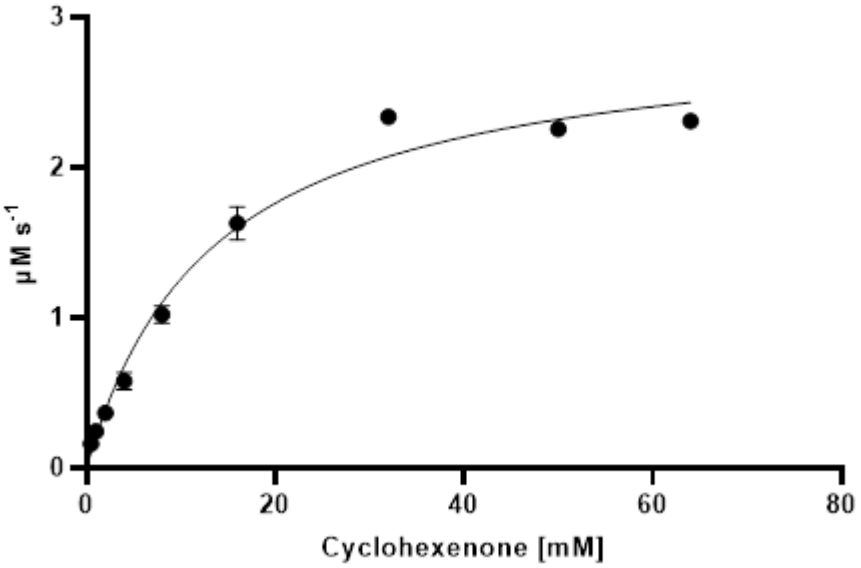


Figure S6: Michaelis-Menten kinetic for cyclohexenone

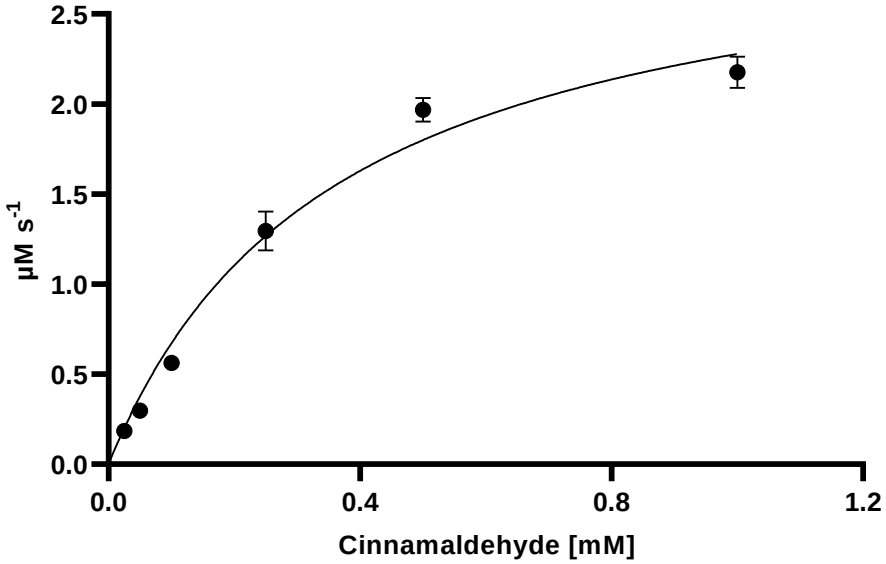


Figure S7: Michaelis-Menten kinetic for cinnamaldehyde

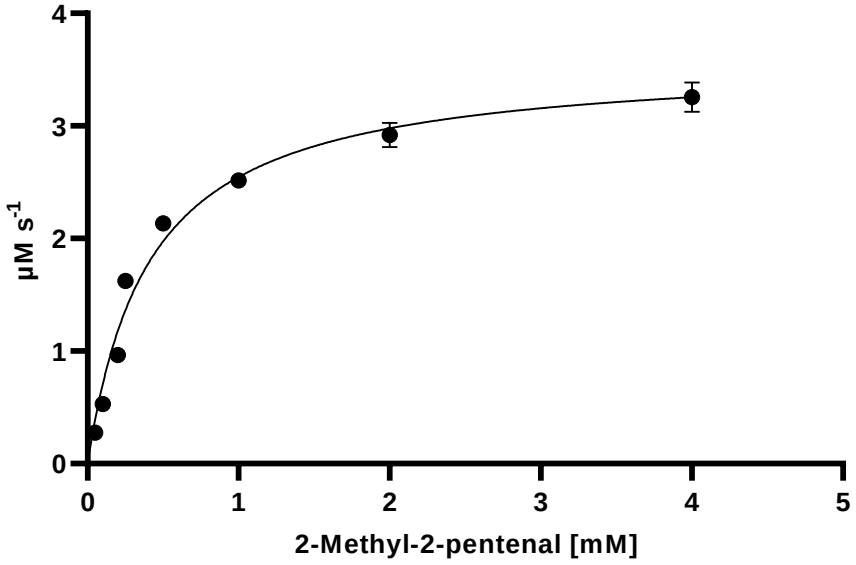


Figure S8: Michaelis-Menten kinetic for 2-methyl-2-pentenal.

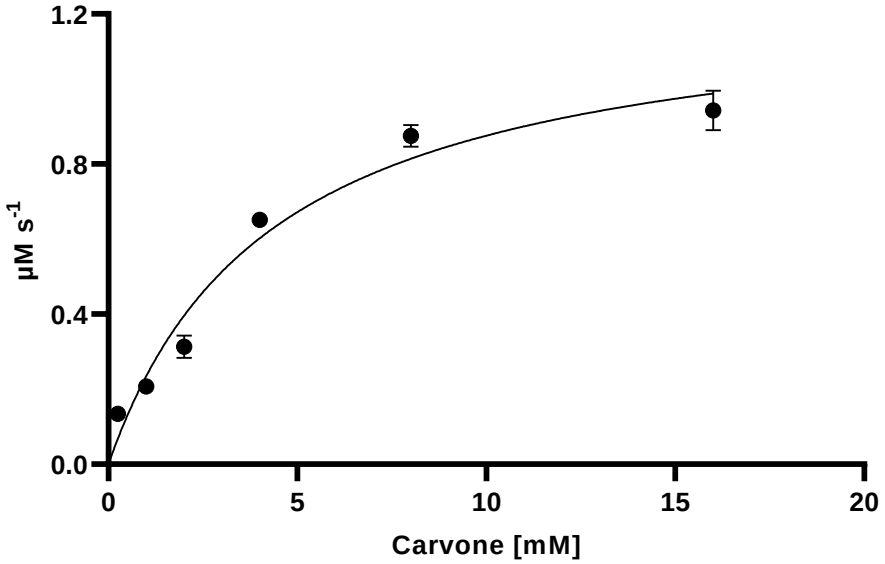


Figure S9: Michaelis-Menten kinetic for carvone.

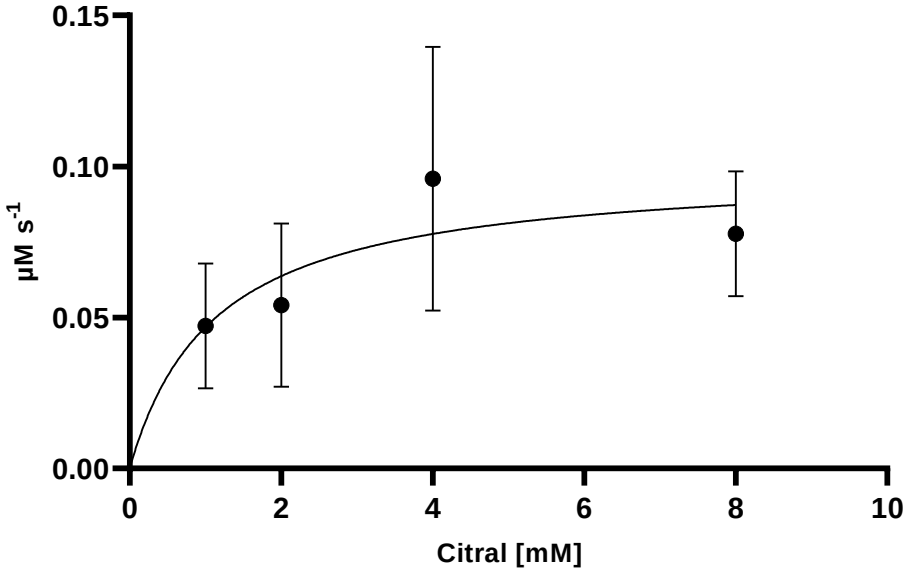


Figure S10: Michaelis-Menten kinetic for citral.

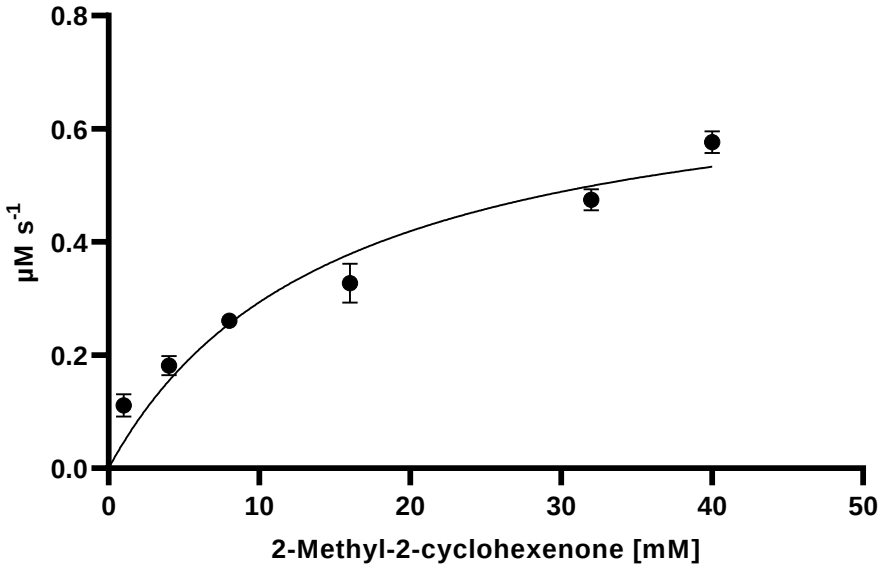


Figure S11: Michaelis-Menten kinetic for 2-methyl-2-cyclohexenone.

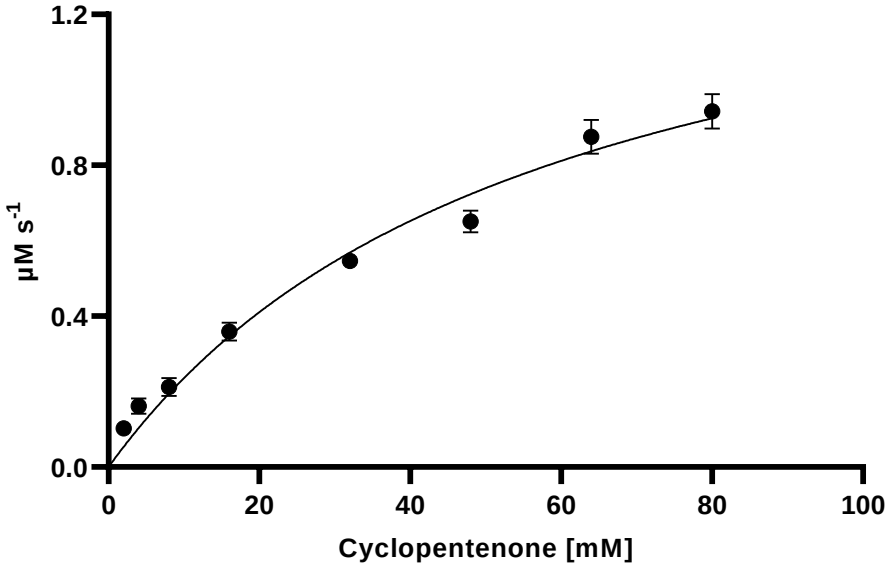


Figure S12: Michaelis-Menten kinetic for cyclopentenone.

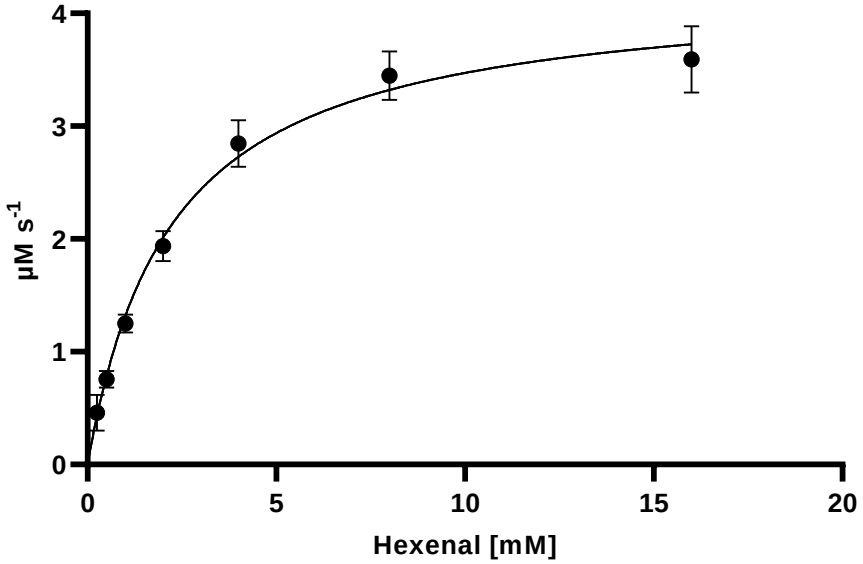


Figure S13: Michaelis-Menten kinetic for hexenal.

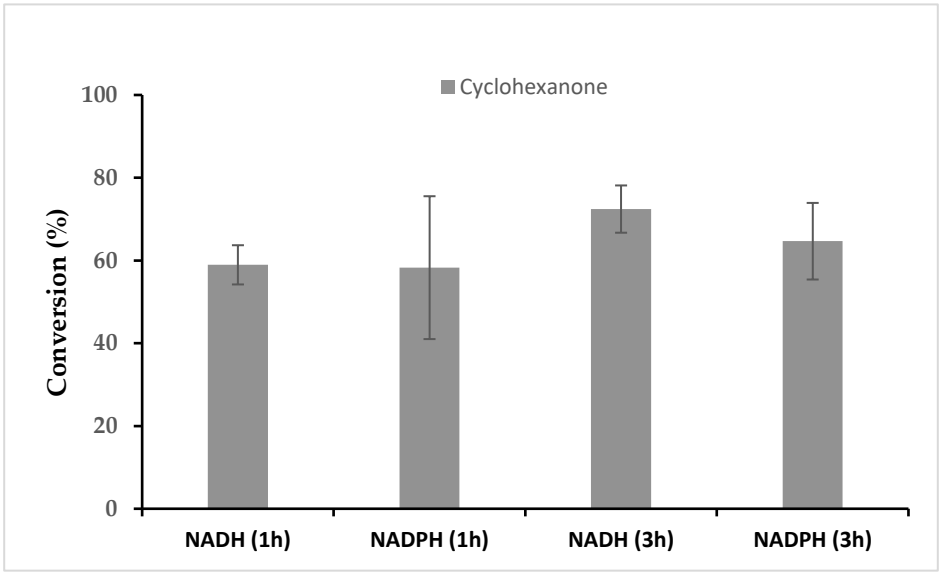
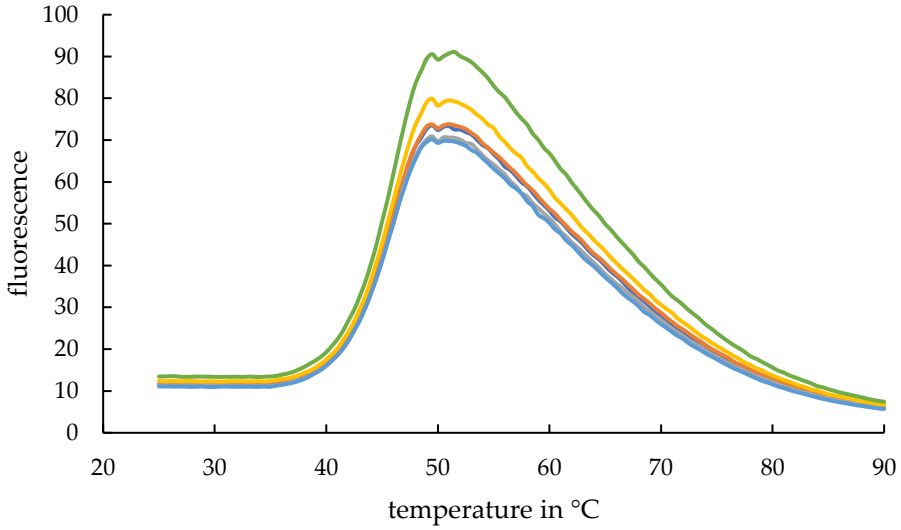


Figure S14: Comparison of cyclohexenone conversion using either NADH or NADPH as cofactor. Biocatalysis reactions were performed in triplicates with 5 mM cyclohexenone and equimolar NADH/NADPH.



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91 **Figure S15**: Melting curve measured with the *Thermo*FAD assay.

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