Supporting Information

A multi-enzyme cascade for the production of high value aromatic compounds

Claudia Engelmann¹, Jens Johannsen², Thomas Waluga², Georg Fieg², Andreas Liese¹ and Paul Bubenheim*¹

¹ Institute of Technical Biocatalysis, Hamburg University of Technology, Denickestr. 15, 21073 Hamburg
² Institute of Process and Plant Engineering, Hamburg University of Technology, Am Schwarzenberg-Campus 4, 21073 Hamburg
* Correspondence: paul.bubenheim@tuhh.de
**Enzyme choice**

The activity of the different ADHs and the pH and temperature screening were determined via initial reaction rate measurements.

| No. | Enzyme   | Activity_{Aldehyde} | Activity_{Alcohol} |
|-----|----------|---------------------|--------------------|
| 1   | ADH-39L  | 0.616 U/mg          | 0.021 U/mg         |
| 2   | ADH-80   | /                   | /                  |
| 3   | ADH-99L  | /                   | /                  |
| 4   | ADH-125  | /                   | /                  |
| 5   | ADH-168  | 0.624 U/mg          | 0.082 U/mg         |
| 6   | ADH-97L  | 0.953 U/mg          | 0.030 U/mg         |

**Cond.:** Left: $c = 20$ mM (5% DMSO), Tris/HCl buffer (pH 8, 0.1 M), $c_{NAD^+} = 0.05$, $\lambda = 340$ nm, $T = 30$ °C. Right: Tris/HCl buffer (pH 8, 0.1 M), $c = 5$ mM, $c_{NADH} = 0.05$ mM, $\lambda = 340$ nm, $T = 30$ °C.

The different lipases from were screened batchwise in 1 mL in a tempered shaker and the reaction solution was analyzed via gas chromatography.

**Cond.:** $V_{CPME} = 1$ mL, $C_{Acid/Alcohol} = 100$ mM, $m_{Lipase} = 5$ mg, $T = 50$ °C.
Kinetics

The kinetics were investigated as described in the manuscript.
**Differential scanning fluorimetry**

Measurement of protein stability by differential scanning fluorimetry as described in the manuscript. The plots show the reaction progress of the in situ co-factor regeneration depending on the ADH concentration.

**Cond.:** Potassium phosphate buffer pH 8, $0.1 \text{ M}$, $V = 25 \text{ mL}$, $[\text{Aldehyde}] = 7.5 \text{ mM}$, $c_{\text{NADH}} = 0.15 \text{ mM}$, $m_{\text{ADH}} = 0.08/0.02 \text{ mg/mL}$, $m_{\text{FDH}} = 5 \text{ mg}$, Sodium formate $= 100 \text{ mM}$, $30 \degree \text{C}$.