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Supporting Information

Alternative Approach towards Poly-ε-Caprolactone through a Chemoenzymatic Synthesis: Combined Hydrogenation, Bio-Oxidations and Polymerization without Isolation of Intermediates

Severin Wedde,[a] Philipp Rommelmann,[a] Christian Scherkus,[b] Sandy Schmidt,[c] Uwe T. Bornscheuer,[c] Andreas Liese,*[b] and Harald Gröger*[a]

[a] Chair of Organic Chemistry I, Faculty of Chemistry, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld, Germany
[b] Institute of Technical Biocatalysis, Hamburg University of Technology, Denickestr. 15, 21073 Hamburg, Germany
[c] University of Greifswald, Institute of Biochemistry, Dept. of Biotechnology & Enzyme Catalysis, Felix-Hausdorff-Str. 4, 17487 Greifswald, Germany

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1. Material and methods

1.1 Chemicals

6-Hexanoid acid (Acros Organics, Geel, Belgium)
β-Nicotinamide adenine-dinucleotide phosphate disodiumsalt (GERBU Biotechnik GmbH, Heidelberg, Germany)
β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)
ε-Caprolactone (Alfa Aesar GmbH & Co KG, Karlsruhe, Germany)
Acetone (VWR International GmbH, Darmstadt, Germany)
Acetophenone (MERCK Schuchardt OHG, Hohenbrunn, Germany)
Ampicillin sulfate (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)
Casein peptone (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)
Cyclohexane (VWR International GmbH, Darmstadt, Germany)
Cyclohexanol (Merck KGaA, Darmstadt, Germany)
Cyclohexanone (VWR International GmbH, Darmstadt, Germany)
Chloroform (VWR International GmbH, Darmstadt, Germany)
deuterated Chloroform (Deutero GmbH, Kastellaun, Germany)
D-glucose monohydrate (VWR International GmbH, Darmstadt, Germany)
Dipotassium hydrogenphosphate (Applichem GmbH, Darmstadt, Germany)
Ethyl acetate (Fisher Scientific GmbH, Schwerte, Germany)
Isopropyl-β-D-thiogalactopyranoside (GERBU Biotechnik GmbH, Heidelberg, Germany)
Hydrogen (Linde Gas, Bielefeld, Germany)
Kanamycin sulfate (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)
Lipase B (CAL-B, Novozym 435) immobilized on acrylic resin (Sigma Aldrich, Steinheim, Germany)
Magnesium chloride (Applichem GmbH, Darmstadt, Germany)
Methylcyclohexane (VWR International GmbH, Darmstadt, Germany)
Methyl *tert*-butyl ether (MTBE) (VWR International GmbH, Darmstadt, Germany)
Phenol (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)
Potassium dihydrogenphosphate (Sigma Aldrich, Steinheim, Germany)
Polycaprolactone ØM_w ~ 14,000, ØM_n ~10,000 (Sigma Aldrich, Steinheim, Germany)
Polycaprolactone ØM_n ~45,000 (Sigma Aldrich, Steinheim, Germany)
Polystyrene (M_P 66,000; 28,000; 12,600; 9,130; 6,100; 4,920; 3,470; 2,280; 1,250, 162 g/mol) (PSS Polymer Standards Service GmbH, Mainz, Germany)
Polyvinylpyrrolidone, average mol wt 40,000 (Sigma Aldrich, Steinheim, Germany)
Rhodium(III) chloride hydrate (38-40% Rh) (Sigma Aldrich, Steinheim, Germany)
Silica, mesostructured (SBA-15) (Sigma Aldrich, Steinheim, Germany)
Sodium borohydride (MERCK Schuchardt OHG, Hohenbrunn, Germany)
Sodium chloride (VWR International GmbH, Darmstadt, Germany)
Sodium hydroxide (VWR International GmbH, Darmstadt, Germany)
Sodium sulfate anhydrous (Fisher Scientific GmbH, Schwerte, Germany)
SYLGARD® 184 (Sigma Aldrich, Steinheim, Germany)
Tetrahydrofurane (BASF, Ludwigshafen, Germany)
Toluene (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)
Yeast extract (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)
Trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane (Sigma Aldrich, Steinheim, Germany)

1.2 Bacterial strains and plasmids

E. coli BL21(DE3) [*fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS*]
λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5
(New England Biolabs GmbH, Frankfurt (Main), Germany)

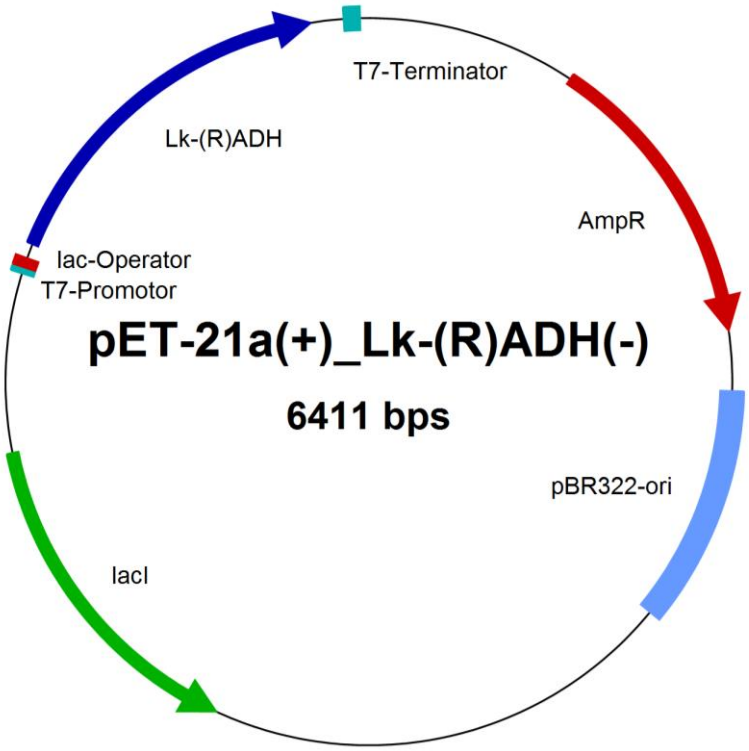


Figure S1: Vector map of pET-21a(+)_Lk-(R)ADH(-)

Coding sequence of the NADP-dependent *(R)*-specific alcohol dehydrogenase from *Lactobacillus kefir* DSM 202587 (GenBank: AY267012.1) on pET-21a(+)_Lk-(R)ADH(-) (cloning of this vector was described previously[1]):

ATGACTGACCGTTTGAAAGGTAAGGTAGCAATTGTAACTGGCGGTACCTTGGGAATTGGCTTGGCAATCGCTGAT
AAGTTTGTTGAAGAAGGCGCAAAGGTTGTTATTACCGGCCGTCACGCTGATGTAGGTGAAAAAGCTGCCAAATCA
ATCGGCGGCACAGACGTTATCCGTTTTGTCCAACACGATGCTTCTGATGAAGCCGGCTGGACTAAGTTGTTTGAT
ACGACTGAAGAAGCATTTGGCCCAGTTACCACGGTTGTCAACAATGCCGGAATTGCGGTCAGCAAGAGTGTTGAA
GATACCACAACTGAAGAATGGCGCAAGCTGCTCTCAGTTAACTTGGATGGTGTCTTCTTCGGTACCCGTCTTGGA
ATCCAACGTATGAAGAATAAAGGACTCGGAGCATCAATCATCAATATGTCATCTATCGAAGGTTTTGTTGGTGAT
CCAACTCTGGGTGCATACAACGCTTCAAAAGGTGCTGTCAGAATTATGTCTAAATCAGCTGCCTTGGATTGCGCT
TTGAAGGACTACGATGTTCGGGTTAACACTGTTCATCCAGGTTATATCAAGACACCATTGGTTGACGATCTTGAA
GGGGCAGAAGAAATGATGTCACAGCGGACCAAGACACCAATGGGTCATATCGGTGAACCTAACGATATCGCTTGG
ATCTGTGTTTACCTGGCATCTGACGAATCTAAATTTGCCACTGGTGCAGAATTCGTTGTCGACGGTGGCTACACT
GCTCAATAG

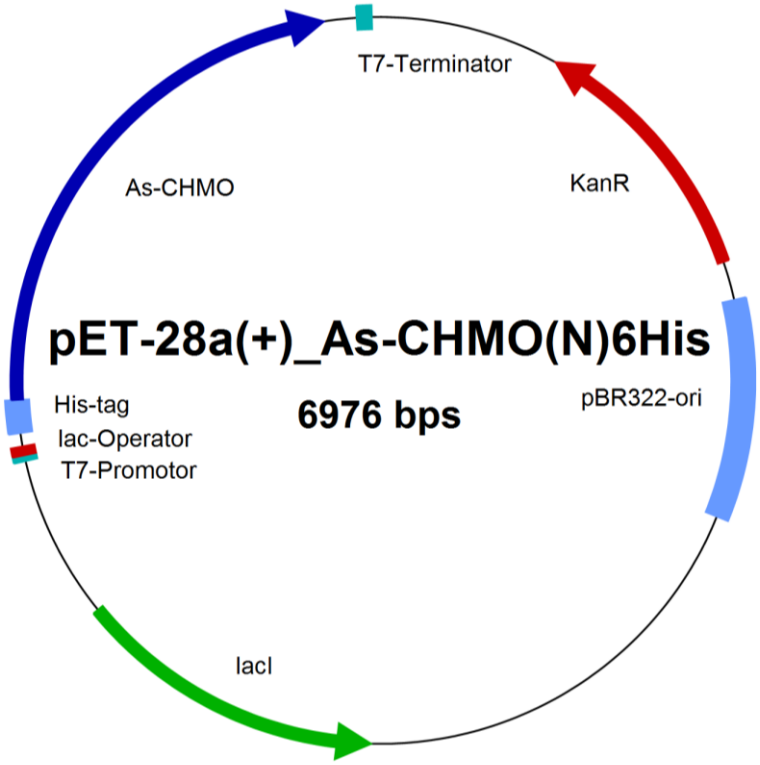


Figure S2: Plasmid map of pET-28a(+)_As-CHMO(N)6His

Coding sequence of the cyclohexanone monooxygenase from *Acinetobacter sp.* NCIMB9871 (GenBank: AB006902.2) on pET-28a(+)_As-CHMO(N)6His (cloning of this vector was described previously[2]):

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACT
GGTGGACAGCAAATGGGTCGCGGATCCATGTCACAAAAAATGGATTTTGATGCTATCGTGATTGGTGGTGGTTTT
GGCGGACTTTATGCAGTCAAAAAATTAAGAGACGAGCTCGAACTTAAGGTTCAGGCTTTTGATAAAGCCACGGAT
GTCGCAGGTACTTGGTACTGGAACCGTTACCCAGGTGCATTGACGGATACAGAAACCCACCTCTACTGCTATTCT
TGGGATAAAGAATTACTACAATCGCTAGAAATCAAGAAAAAATATGTGCAAGGCCCTGATGTACGCAAGTATTTA
CAGCAAGTGGCTGAAAAGCATGATTTAAAGAAGAGCTATCAATTCAATACCGCGGTTCAATCGGCTCATTACAAC
GAAGCAGATGCCTTGTGGGAAGTCACCACTGAATATGGTGATAAGTACACGGCGCGTTTCCTCATCACTGCTTTA
GGCTTATTGTCTGCGCCTAACTTGCCAAACATCAAAGGCATTAATCAGTTTAAAGGTGAGCTGCATCATACCAGC
CGCTGGCCAGATGACGTAAGTTTTGAAGGTAAACGTGTCGGCGTGATTGGTACGGGTTCCACCGGTGTTCAGGTT
ATTACGGCTGTGGCACCTCTGGCTAAACACCTCACTGTCTTCCAGCGTTCTGCACAATACAGCGTTCCAATTGGC
AATGATCCACTGTCTGAAGAAGATGTTAAAAAGATCAAAGACAATTATGACAAAATTTGGGATGGTGTATGGAAT
TCAGCCCTTGCCTTTGGCCTGAATGAAAGCACAGTGCCAGCAATGAGCGTATCAGCTGAAGAACGCAAGGCAGTT
TTTGAAAAGGCATGGCAAACAGGTGGCGGTTTCCGTTTCATGTTTGAAACTTTCGGTGATATTGCCACCAATATG
GAAGCCAATATCGAAGCGCAAAATTTCATTAAGGGTAAAATTGCTGAAATCGTCAAAGATCCAGCCATTGCACAG
AAGCTTATGCCACAGGATTTGTATGCAAAACGTCCGTTGTGTGACAGTGGTTACTACAACACCTTTAACCGTGAC
AATGTCCGTTTAGAAGATGTGAAAGCCAATCCGATTGTTGAAATTACCGAAAACGGTGTGAAACTCGAAAATGGC
GATTTCGTTGAATTAGACATGCTGATATGTGCCACAGGTTTTGATGCCGTCGATGGCAACTATGTGCGCATGGAC
ATTCAAGGTAAAAACGGCTTGGCCATGAAAGACTACTGGAAAGAAGGTCCGTCGAGCTATATGGGTGTCACCGTA
AATAACTATCCAAACATGTTCATGGTGCTTGGACCGAATGGCCCGTTTACCAACCTGCCGCCATCAATTGAATCA
CAGGTGGAATGGATCAGTGATACCATTCAATACACGGTTGAAAACAATGTTGAATCCATTGAAGCGACAAAAGAA
GCGGAAGAACAATGGACTCAAACTTGCGCCAATATTGCGGAAATGACCTTATTCCCTAAAGCGCAATCCTGGATT
TTTGGTGCGAATATCCCGGGCAAGAAAAACACGGTTTACTTCTATCTCGGTGGTTTAAAAGAATATCGCAGTGCG
CTAGCCAACTGCAAAAACCATGCCTATGAAGGTTTTGATATTCAATTACAACGTTCAGATATCAAGCAACCTGCC
AATGCCTAA

1.3 Preparation of hydrogenation catalyst

The synthesis of the catalyst Rh(0)-SBA-15 was carried out according to the reported literature protocol[3]. In a 500 ml round bottom flask, mesoporous silica (SBA-15, 1.0 g) was suspended in distilled water (50 mL). This suspension was stirred vigorously for 2 hours. In a 250 mL round bottom flask, polyvinylpyrrolidone (0.02 g) was dissolved in distilled water (100 mL). Sodium borohydride (0.10 g, 2.64 mmol) was added and this colorless solution was then added quickly in one portion to a dark red solution of rhodium(III) chloride hydrate (0.10 g) in distilled water (100 mL) under vigorous stirring. After precipitation of a black solid, this Rh(0) suspension was added to the SBA-15 suspension. The reaction mixture was then stirred vigorously for 5 hours at room temperature. After vacuum filtration through a frit (pore size 4), the black residue was washed with distilled water (3 x 60 mL) and dried at 100 °C in a drying oven for 16 h. The catalyst Rh(0)-SBA-15 (1.00 g, 96%) was obtained as a grey, voluminous powder, which was stored dark and dry.

1.4 Hydrogenation experiments (according to Tab. 1)

!!! CAUTION: Experiments with hydrogen gas represent a substantial safety risk and must be conducted strictly in accordance with the appropriate safety procedures and in conjunction with the use of proper equipment !!!

All hydrogenation experiments were carried out in a 25 mL Schlenk flask equipped with a hydrogen balloon. Varying amount of phenol (1 mmol to 4 mmol) was dissolved in distilled water to obtain different substrate concentrations between 0.5 M and 2.0 M. After addition of the hydrogenation catalyst Rh(0)-SBA-15 (0.5 mol% to 1.9 mol% of Rh) to the phenol solution, the Schlenk flask was evacuated until the solvent boiled slightly and refilled with argon. A second evacuation step proceeded followed by ventilation with gas from the hydrogen balloon. The reaction mixture was heated to 75 °C. After stirring for 17 hours to 24 hours, the reaction was quenched by cooling down the mixture to room temperature and by removing the hydrogen balloon. After removal of the hydrogen atmosphere from the reaction mixture, the resulting mixture was filtered through a frit (pore size 4) under vacuum. The residue was washed with distilled water, and this wash liquid was added to the filtrate. The catalyst was recycled by drying the residue at 100 °C in a drying oven for at least 5 hours. For quantification of the conversion, distilled water was added to the liquid phase, which resulted from the combination of the filtrate and the wash liquid, to obtain a defined volume. 500 µL of this solution was transferred to a microtube and extracted with ethyl acetate (3 x 500 µL) on a vortexer (VWR International GmbH, Darmstadt, Germany) for 1 min at maximum speed. The organic layers were combined in a glass vial and analyzed by GC (see section 1.14).
For ^{1}H-NMR analysis of the reaction mixture, the filtrate solution was extracted with ethyl acetate. The solvent from the organic layer was removed in vacuum.

Phenol: ^{1}H-NMR (500 MHz, CD_2Cl_2): δ [ppm] = 7.24 (dd, J = 8.5 Hz, J = 7.5 Hz, 2H, H^{meta}), 6.92 (t, J = 7.4 Hz, 1H, H^{para}), 6.83 (d, J = 7.7 Hz, 2H, H^{ortho}), 4.97 (s, 1H, OH).

Cyclohexanone: ^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 2.33 (t, J = 6.7 Hz, 4H, C(O)CH_2), 1.86 (q, J = 6.2 Hz, 4H, C(O)CH_2CH_2), 1.71 (m, 2H, C(O)CH_2CH_2CH_2).

Cyclohexanol: ^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 3.61 (m, 1 H, C(OH)H), 1.89 (m, 2H, C(OH)C$H^{äq}$), 1.73 (m, 2H, C(OH)CH_2C$H^{äq}$), 1.55 (m, 1H, C(OH)CH_2CH_2C$H^{äq}$), 1.26 (m, 4H, C(OH)CH^{ax}CH^{ax}), 1.17 (m, 1H, C(OH)CH_2CH_2CH^{ax}).

The data corresponds to the ones reported in literature.

1.5 Production of whole-cell catalysts and enzyme crude extracts containing recombinant Lk-ADH:

A glycerol stock of *E. coli* BL21(DE3) containing the plasmid pET-21a(+)_Lk-(R)ADH(-) encoding the gene of the NADP-dependent *(R)*-specific alcohol dehydrogenase from *Lactobacillus kefir* DSM 202587 (GenBank: AY267012.1 – cloning was described previously[1]) was used for inoculation of 30 mL LB medium (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl)[4] containing 50 µg/ml ampicillin. Cultivation was performed in a 300 mL shaking flask with baffles at 37 °C on an orbital shaker (Infors GmbH, Einsbach, Germany) at 140 rpm overnight. The overnight culture was used as an inoculum to adjust 200 mL terrific broth medium (12 g/L casein peptone, 24 g/L yeast extract. 4 g/L glycerol and separately autoclaved 2,31 g/L KH_2PO_4, 12,54 g/L K_2HPO_4 [5]) containing 50 µg/ml ampicillin to OD_{600} 0.05. Cultivation was performed in a 1 L shaking flask with baffles at 37 °C and 140 rpm. *Lk*-ADH expression was induced between OD_{600} 0.6 and 0.8 with 0.05 mM ITPG. Cultivation was continued at 25 °C for 6 hours for production of whole-cell catalyst and for 20 hours for enzyme production as crude extract. After cultivation cells were harvested by centrifugation at 4,000g for 10 min at 4 °C and washed twice with KP_i-buffer (pH 7, 50 mM, 200 mg/L $MgCl_2$). The cell pellet was adjusted with KP_i-buffer to yield a 250 g_{cww}/L stock solution.

In whole-cell catalytic biotransformations the stock solution was used directly as resting cells whereas in biotransformations using crude extract, cells were disintegrated by sonifcation on ice for 3x 5 min with an ultrasonic homogenizer (Bandelin Sonoplus UW2070; 5x10 % cycle, 15 % power; BANDELIN electronic GmbH & Co. KG, Berlin, Germany). Cell debris were removed by centrifugation at 10,000 g for 10 min at 4 °C. The achieved supernatant consists of the soluble crude extract containing the overexpressed *Lk*-ADH. For biotransformations the activity of crude extract was determined spectrophotometrically (in analogy to standard protocols reported, e.g., in reference [6]) at 340 nm by consumption of NADPH through oxidation toward $NADP^+$ with a spectrophotometer V630 (JASCO Germany GmbH, Gross-Umstadt, Germany). 460 µL KP_i-buffer (pH 7, 50 mM, 200 mg/L $MgCl_2$) were mixed with 500 µL acetophenone (22 mM in KP_i-buffer). After performing a blank measurement 30 µL of a freshly prepared $NADP^+$-solution (8 mM in KP_i-buffer) were added under rigorous mixing. The reaction was started by the addition of 10 µL of 1:100 dilution of Lk-ADH crude extract under rigorous mixing. The slope (ΔE) of the observed linear range of minimal 60 seconds was used for activity calculation using the following equation:

$$A = (\Delta E \cdot V \cdot f)/(\varepsilon \cdot v \cdot d)$$

with the enzyme activity A (U/mL), the time depending change in extinction ΔE (1/min), the total volume V (1 mL), the volume of enzyme solution v (0,01 mL), the dilution factor f (100), the extinction coefficient ε of NADPH at 340 nm (6.300 L/mol·cm) and the layer thickness d (1 cm). Determination of enzyme activity was done in quintuplicate.

1.6 Production of whole-cell catalysts and enzyme crude extracts containing recombinant As-CHMO:

A glycerol stock of *E. coli* BL21(DE3) containing the plasmid pET-28a(+)_As-CHMO(N)6His encoding the gene of the CHMO from *Acinetobacter sp.* NCIMB 9871 with an N-terminal 6x histidine-tag (GenBank: AB006902.2 – cloning was described previously[8]) was used for inoculation of 30 mL LB medium[15] containing 100 µg/ml kanamycin. Cultivation was performed in a 300 mL shaking flask with baffles at 37 °C on an orbital shaker (Infors GmbH, Einsbach, Germany) at 140 rpm overnight. The overnight culture was used as an inoculum to adjust 200 mL terrific broth medium[TB] containing 100 µg/ml kanamycin to OD_{600} 0.05. Cultivation was

performed in a 1 L shaking flask with baffles at 37 °C and 140 rpm. CHMO expression was induced between OD_{600} 0.6 and 0.8 with 0.05 mM ITPG. Cultivation was continued at 25 °C for 5 to 6 hours for production of whole-cell catalyst and for 20 hours for enzyme production as crude extract. After cultivation cells were harvested by centrifugation at 4,000g for 10 min at 4 °C and washed twice with KP_i-buffer (pH 7, 50 mM, 200 mg/L $MgCl_2$). The cell pellet was adjusted with KP_i-buffer to yield a 250 g_{cww}/L stock solution.
In whole-cell catalytic biotransformations the stock solution was used directly as resting cells whereas in biotransformations using crude extract, cells were disintegrated by sonifcation on ice for 3x 5 min with an ultrasonic homogenizer (Bandelin Sonoplus UW2070; 5x10 % cycle, 15 % power; BANDELIN electronic GmbH & Co. KG, Berlin, Germany)**.** Cell debris were removed by centrifugation at 10,000 g for 10 min at 4 °C. The achieved supernatant consists of the soluble crude extract containing the overexpressed *Lk*-ADH. For biotransformations the activity of crude extract was determined spectrophotometrically at 340 nm by consumption of NADPH through oxidation toward $NADP^+$ with a spectrophotometer V630 (JASCO Germany GmbH, Gross-Umstadt, Germany). 460 µL KP_i-buffer (pH 7, 50 mM, 200 mg/L $MgCl_2$) were mixed with 500 µL cyclohexanone (20 mM in KP_i-buffer). After performing a blank measurement 30 µL of a freshly prepared $NADP^+$-solution (8 mM in KP_i-buffer) were added under rigorous mixing. The reaction was started by the addition of 10 µL As-CHMO crude extract under rigorous mixing. The slope (ΔE) of the observed linear range of minimal 60 seconds was used for activity calculation using the following equation:

$$A = (\Delta E \cdot V \cdot f)/(\varepsilon \cdot v \cdot d)$$

with the enzyme activity A (U/mL), the time depending change in extinction ΔE (1/min), the total volume V (1 mL), the volume of enzyme solution v (0,01 mL), the dilution factor f (1), the extinction coefficient ε of NADPH at 340 nm (6.300 L/mol·cm) and the layer thickness d (1 cm). Determination of enzyme activity was done in quintuplicate.

1.7 Enzymatic biotransformations in pure aqueous system using hydrogenation crude product as substrate (according to Fig. 1)

!!! CAUTION: Experiments with hydrogen gas represent a substantial safety risk and must be conducted strictly in accordance with the appropriate safety procedures and in conjunction with the use of proper equipment !!!

According to the procedure described in section 1.4, phenol (188.2 mg, 2 mmol) was hydrogenated to cyclohexanol, using 1 mol% Rh from Rh(0)-SBA-15 and 2 mL distilled water within a reaction time of 24 hours. After removal of the hydrogen atmosphere from the reaction mixture and subsequent removal of the catalyst from the resulting mixture by filtration under vacuum, the residue was washed with distilled water. This wash liquid was added to the filtrate, and the volume of the resulting liquid phase (filtrate and wash liquid) was increased to a total volume of 8 mL by addition of distilled water. This solution was diluted 1:1 with KPi-buffer (pH 7, 100 mM, 8 mL) to obtain a 125 mM solution of crude cyclohexanol in KPi-buffer (pH 7, 50 mM, 16 mL). For the biotransformation, 2 mL of the diluted cyclohexanol solution from the hydrogenation was added to a 10 mL round bottom flask. Recombinant As-CHMO crude extract (3.82U) and Lk-ADH crude extract (40 U), both freshly produced by cultivation and cell disintegration as described in section 1.5 and 1.6, were added. The reaction was started by addition of $NADP^+$ (10 mM, 200 µL). Enzyme activities and amount of cofactor is in accordance to previous work[7]. KPi-buffer (pH 7, 50 mM, 200 mg/L $MgCl_2$) was added to the reaction mixture to obtain a final volume of 5 mL and a final substrate concentration of 50 mM. The reaction mixture was stirred at 25 °C in a temperature controlled water bath on a magnet stirrer

(RCT basic, IKA®-Werke GmbH & CO. KG, Staufen, Germany) using a 20 mm long stirring bar (VWR International GmbH, Darmstadt, Germany) by rotating with 500 rpm. To measure the rate of the reaction, aliquots of 500 µL (aqueous phase) were transferred to a microtube, extracted with ethyl acetate (3 x 500 µL) on a vortexer (VWR International GmbH, Darmstadt, Germany) for 1 min at maximum speed and centrifuged at 13,000 rpm for 2 min at room temperature in a microcentrifuge (Heraeus Holding GmbH, Hanau, Germany). The organic layers were combined in a glass vial and analyzed by GC (see section 1.14).

1.8 Biotransformations in a two-phase system using enzyme crude extract (according to Fig. S5):

Biotransformations were performed in a 10 mL round-bottom flask with 5 mL aqueous phase in which the biotransformation takes place and 5 mL of an organic solvent (methylcyclohexane, cyclohexane or MTBE) for *in situ* product removal. Reactions were performed at 25 °C in a temperature controlled water bath on a magnet stirrer (RCT basic, IKA®-Werke GmbH & CO. KG, Staufen, Germany). 50 mg cyclohexanol (100 mM) was weighed out in a 5 mL volumetric flask and dissolved by addition of 500 µL of a 4 mM $NADP^+$-KPi-buffer (pH 7, 50 mM, 200 mg/L $MgCl_2$) solution (final $NADP^+$ concentration 0.4 $mM)_i$. Recombinant As-CHMO crude extract (3.82U) and Lk-ADH crude extract (40 U), both freshly produced by cultivation and cell disintegration as described in section 1.5 and 1.6, were added and filled up to 5 mL with KPi-buffer (pH 7, 50 mM, 200 mg/L $MgCl_2$). Enzyme activities and amount of cofactor is in accordance to previous work[7]. After rigorous mixing the reaction solution was completely transferred to a fresh 10 mL round-bottom flask, in which the reaction mixture was stirred by a 20 mm long stirring bar (VWR International GmbH, Darmstadt, Germany) by rotating with 340 rpm. The choice of an organic phase for *in situ* product removal was investigated by adding 5 mL either methylcyclohexane or cyclohexane or MTBE as a second (upper) phase. Simultaneously a biotransformation without the addition of an organic phase was performed as reference. To prevent evaporation of the organic solvents during reaction, all reaction flasks were closed by a cap, as in a previous study we could not observe any oxygen limitation in a closed reaction flask. All different biotransformations were done in duplicate.

Reactions were stopped after 23 h. As the organic phases looked more like an emulsion (Fig. S6) for analytics of the organic and aqueous phase, the reaction medium was centrifuged for phase separation at 10,000g for 10 min at 15 °C. 1 mL of the organic phase was directly analyzed by gas chromatography (GC). After removal of the organic phase as well as the interphase containing parts of the crude extract, 0.5mL of the aqueous phase was taken and extracted three times with 0.5 mL ethylacetate (with 2 mM acetophenone as an internal standard) by mixing on a vortexer (Scientific Industries Inc., Bohemia, NY, USA) for 1 min at maximum speed. Phase separation was achieved by centrifugation for 1 min at 21,500g in a microcentrifuge (VWR International GmbH, Darmstadt, Germany). The organic phases were combined and filled up to 2 mL with ethylacetate (2 mM acetophenone) in a volumetric flask. The organic phases were collected and dried using appropriate amounts of sodium sulphate (anhydrous) till the solution was devoid of water. Afterwards conversion was determined by GC (see section 1.15).

1.9 Preparation of polydimethylsiloxane thimbles

Polydemiethylsiloxane thimbles were prepared based on the protocol previously described in literature [8]. Three glass vials (40 mm high, 20 mm outer diameter) were placed upside down in a desiccator with a small beaker glass in the middle containing three drops of tri-chloro(1H, 1H, 2H, 2H-perfluorooctyl)silane on the bottom of the desiccator. By applying a static vacuum of approx. 50 mbar over night the trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane covers the

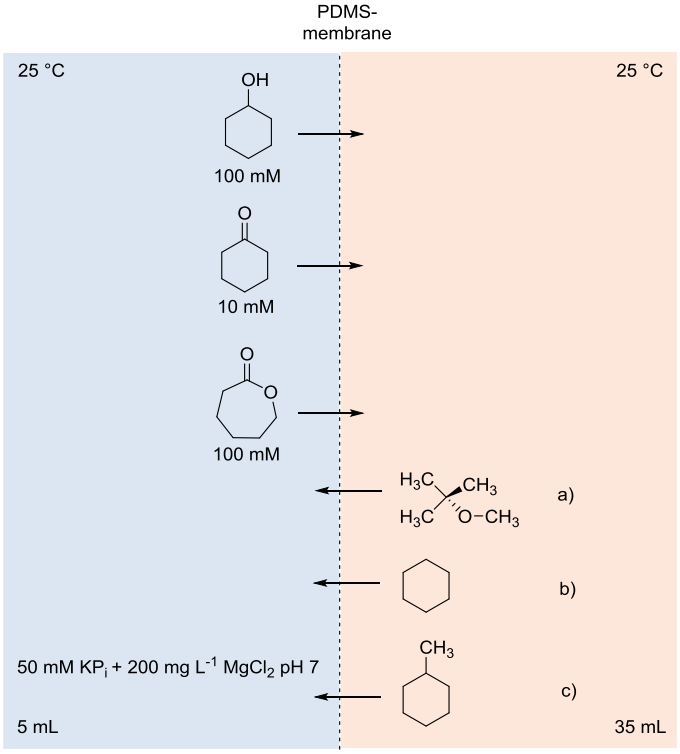
glass vials and enables an easy removal of the thimbles from the glass vials, which functions as a kind of casting mold. After pretreatment of the glass mold the elastomer and curing agent of Sylgard 184 were mixed intensively inside the 10 gram clip-pak after removal of the clip. The mixture was then transferred to a glass jar (66 mm high, 21,5 mm inner diameter) and rigorously mixed with a spatula and degassed in a fume hood. After complete degassing (approx. 1.5 h) a silane-covered glass vial is gripped with a tweezer at the top of the vial and dipped in the Sylgard 184 mixture to cover the complete outer surface of the glass vial. Due to safety concerns, it is highly recommended to wear appropriate gloves during this procedure. Furthermore it is recommended to prevent the silane-covered glass vial from any contact with the gloves and other stuff, as the Sylgard 184 mixture may drip off the glass at these contact points, which results in an unequal membrane thickness. After coating the surface of the glass vial, excessive elastomer solution drops down the vial, which is recollected in the glass jar. When droplet formation stops after a few minutes, the glass vial is placed upside down on an aluminium foil covering the outer bottom of a crystallizing dish. For curing of the PDMS-thimbles the glass vials were placed on the aluminium foil for 1 h at 65 °C in an oven. Afterwards the dipping-curing was repeated twice, with the exception that the final thermal curing step was performed for 40 min at 120 °C. When the glass vials cooled down to room temperature the tops of the PDMS-thimbles were cut around the notch of the glass vial with a scalpel. Subsequent the glass vials were placed in *n*-hexane, in which the PDMS-thimble swells up and thereby detaches from the glass surface. After a while the glass vial can be carefully removed with a tweezer from the swollen thimble. The PDMS-thimbles were dried under the fume hood, where they are shrink back to the size of the glass vial (33 mm high, 20 mm outer diameter with a membrane thickness of 200 – 250 µm).

For use in diffusion experiments and biotransformations a 10 mL single use syringe (B. Braun Melsungen AG, Melsungen, Germany) with an outer diameter of 17,5 mm was used for fixation of the PDMS-thimble in a 50 mL round bottom flask NS 29 in 35 mL organic solvent (methylcyclohexane, cyclohexane or MTBE). Therefore the plunger of the syringe was removed and the closed bottom with the needle adaptor (nozzle) was removed at the 2 mL mark with a scissors to obtain a pipe with a smooth rim, which was subsequently covered by the PDMS-thimble. The PDMS-thimble was fixed a few millimeters away from the rim with an adhesive tape (3M Temflex 1500 Vinyl Plastic Electrical Tape, 3M Deutschland GmbH, Neuss, Germany). For diffusion experiments and biotransformations the PDMS-thimble was filled with 5 mL of the aqueous phase and fixed by the small wings of the syringe in the 50 mL round-bottom flask NS 29 filled with 35 mL of the organic phase. The volume of 35 mL outside the thimble membrane was chosen, as the levels inside and outside the thimble were equal, thus enabling maximum surface area for exchange.

For each experiment a new PDMS-thimble was produced, to prevent deviations due to recycling and incorporation of reaction components in the PDMS-thimble.

Figure S3: Picture of the glass vial pretreated with the trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane and the glass jar for thimble preparation (left) as well as completed thimble (in the front),the modified syringe, the PDMS-thimble fixed at the syringe (middle) and the application of the PDMS-thimble in a 50 mL round-bottom flask with the two stirring bars (8 mm long inside thimble and 15 mm long outside the thimble) (right).

1.10 Diffusion experiments and investigation of permeability of the PDMS-membrane



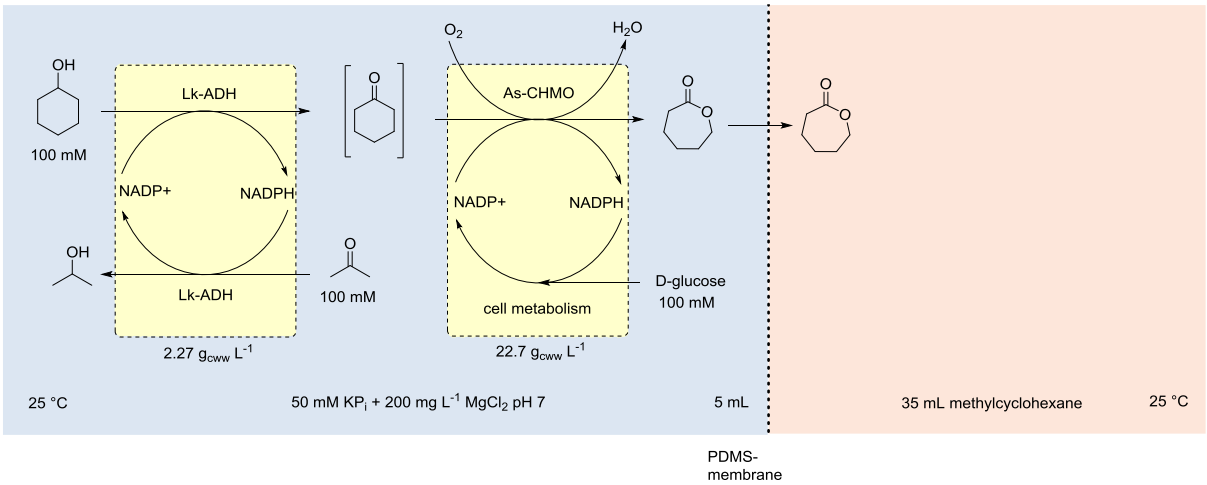
Scheme S1: Investigation of the selectivity of the PDMS-membrane for the reaction components cyclohexanol (100 mM), cyclohexanone (10 mM) and ε-caprolactone (100 mM) dissolved in 5 mL KP_i (50 mM, 200 mg/L $MgCl_2$, pH 7) inside the PDMS-thimble as well as for 35 mL of the extracting solvents outside the PDMS-thimble a) MTBE, b) cyclohexane or c) methylcyclohexane, respsectively.

For investigating the suitability of the PDMS-membrane for the selective *in situ* product removal diffusion experiments were performed. 5 mL of a KP_i-buffer solution (pH 7, 50 mM, 200 mg/L $MgCl_2$) containing 60 mM cyclohexanol, 10 mM cyclohexanone and 60 mM ε-caprolactone was filled together with an 8 mm long stirring bar in the PDMS-thimble fixed at the syringe as described in section 1.9. The thimble was dipped in 35 mL methylcyclohexane, cyclohexane or MTBE (with a 15 mm long stirring bar), respectively. Diffusion of the three components from the aqueous reaction compartment through the PDMS-membrane into the different organic solvents was monitored by taking samples of the organic phase after 3 h, 4.5 h and 23 h and analyzed by GC as described in section 1.15. Diffusion experiments were done in duplicate.
To investigate if the PDMS-membrane is impermeable for the extracting solvent to protect the enzymes from deactivation, the same experimental setup was used with the exception that pure buffer was used as aqueous phase. After 23 h 0.5 mL of the aqueous phase of each thimble-system was extracted three times with 0.5 mL deuterated chloroform by rigorous mixing for 1 min on a vortexer (Scientific Industries Inc., Bohemia, NY, USA). Subsequently phase separation was enforced by centrifugation for 1 min at 21,500g in a microcentrifuge (VWR International GmbH, Darmstadt, Germany). The organic phases were collected and dried using appropriate amounts of sodium sulphate (anhydrous) till the solution was devoid of water. Subsequently the organic phases were checked for the extracting solvent by ^{1}H-NMR (DRX 500, Bruker AXS GmbH, Karlsruhe, Germany).
Besides analysis of diffusion the used PDMS-thimbles were checked visually for leakage. Therefore the thimbles were drained and dried. Subsequently they were filled first with water and afterwards with *n*-hexane.
Furthermore the thimbles were investigated regarding loss of one of the reaction components cyclohexanol, cyclohexanone and ε-caprolactone by incorporation in the PDMS-membrane. Therefore the thimbles were incubated in 50 mL *n*-hexane in a closed beaker for 3.5 h. 1 mL of the *n*-hexane was then analyzed *via* GC.

Although it is not expected that the PDMS-membrane is permeable for polycaprolactone a diffusion experiment was performed for verification. Therefore 326 mg polycaprolactone was weighed out in a 50 mL round-bottom flask NS29 and subsequently dissolved with a 15 mm stirring bar in 35 mL of methylcyclohexane to obtain the outer compartment. Afterwards the PDMS-thimble was filled with 5 mL KP_i-buffer (pH 7, 50 mM, 200 mg/L $MgCl_2$) and an 8 mm long stirring bar was added. The thimble was fixed with the help of the syringe hanging in the organic phase. Both phases were mixed at 340 rpm. After 23 h the 0.5 mL of the aqueous phase were extracted three times with 0.5 mL deuterated chloroform, dried using appropriate amounts of sodium sulphate (anhydrous) till the solution was devoid of water and analyzed by ^{1}H-NMR.

To verify if the PDMS-membrane became asymmetric by the contact of the thimble-inside with the trichloro(1H,1H,2H,2H-perfluorooctyl)silane coated glass, the same experiments as described above were performed with a PDMS-thimble, whose sites were flipped, so that the original inner site was now facing outwards.

1.11 Biotransformations in PDMS-thimble system using recombinant whole-cell catalyst for synthesis of ε-caprolactone (according to Fig. 2):



Scheme S3: Two-cell based biotransformation of 100 mM cyclohexanol towards ε-caprolactone in the PDMS-thimble system with *in situ* product removal by methylcyclohexane outside the PDMS-thimble. The reference reaction was performed in a pure aqueous system (equal to the aqueous phase in the PDMS-thimble).

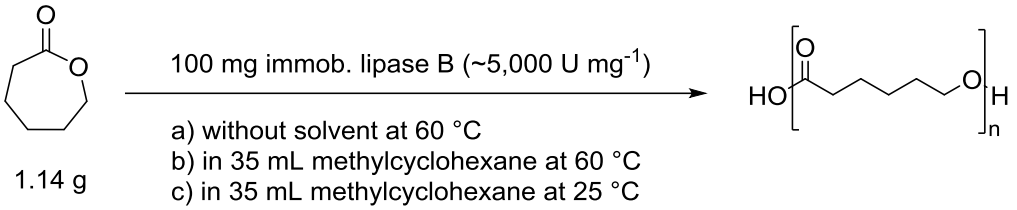
50 mg cyclohexanol (100 mM) was weighed out in a 5 mL volumetric flask. A previous study showed that in a two-cell system the addition of equimolar amounts of acetone and D-glucose is useful for a faster reaction and this acetone concentration is acceptable for the enzymes. Acetone enables in the Lk-ADH whole-cell catalyst the recycling of $NADP^+$, whereas the addition of glucose enables the intracellular recycling of NADPH in the As-CHMO whole cell catalysts [Schmidt]. Thus, 29 mg acetone (100 mM) was weighed and dissolved by adding 500 µL of a 1 M D-glucose-KPi-buffer (pH 7, 50 mM, 200 mg/L $MgCl_2$) solution (final glucose concentration 100 mM). In previous work it was also shown, that an optimal ratio of Lk-ADH whole-cells and As-CHMO whole-cells of 1:10 (m/m) is most efficient [Schmidt]. Thus 45,4 µL of the 250 g_{cww}/L Lk-ADH stock solution (final concentration 2,27 g_{cww}/L) and 454 µL of the 250 g_{cww}/L As-CHMO stock solution (final concentration 22,7 g_{cww}/L) – each freshly produced as described in section 1.5 and 1.6 – were added to yield an overall biocatalyst loading of 25 g_{cww}/L. After filling up to 5 mL with KPi-buffer (pH 7, 50 mM, 200 mg/L $MgCl_2$) the solution was transferred either in 10 mL round-bottom flask (reference experiment in a pure aqueous system) or in the PDMS-thimble.

Biotransformations in a pure aqueous system were performed in a 10 mL round-bottom flask closed by a cap with 5 mL aqueous phase at 25 °C in a temperature controlled water bath on a magnet stirrer (RCT basic, IKA®-Werke GmbH & CO. KG, Staufen, Germany) with a 1.5 cm stirring bar (VWR International GmbH, Darmstadt, Germany) at 240 rpm. Biotransformation in the PDMS-system were performed in a 50 mL round-bottom flask closed by a cap with 5 mL aqueous phase inside the PDMS-thimble stirred by an 8 mm long stirring bar (VWR International GmbH, Darmstadt, Germany). The PDMS-thimble is dipped in 35 mL methylcyclohexane, which are stirred by a 16 mm long stirring bar (VWR International GmbH, Darmstadt, Germany) at 25 °C in a tempered water bath on a magnet stirrer (RCT basic, IKA®-Werke GmbH & CO. KG, Staufen, Germany) at 240 rpm. Both biotransformations were performed in duplicate.

Reactions were stopped after 23 h. 1 mL of the organic phase was directly analyzed by GC as described in section 1.15. 0.5mL of the aqueous phase was taken and extracted three times with 0.5 mL ethylacetate (with 2 mM acetophenone as an internal standard) by mixing on a vortexer (Scientific Industries Inc., Bohemia, NY, USA) for 1 min at maximum speed and phase

separation for 1 min at 21,500g in a microcentrifuge (VWR International GmbH, Darmstadt, Germany). The organic phases were combined and filled up to 2 mL with ethylacetate (2 mM acetophenone) in a volumetric flask. After mixing the liquid was dried using appropriate amounts of sodium sulphate (anhydrous) till the solution was devoid of water and analyzed by GC according to section 1.15.
Furthermore the tightness of the PDMS-thimble was verified. Therefore the PDMS-thimble was emptied and dried under the fume hood. Afterwards it was filled with water and then with *n*-hexane whereby the outside of PDMS-thimble was checked for formation of liquid droplets.

1.12 Polymerization experiments (according to Fig. S9 and Fig. S10)

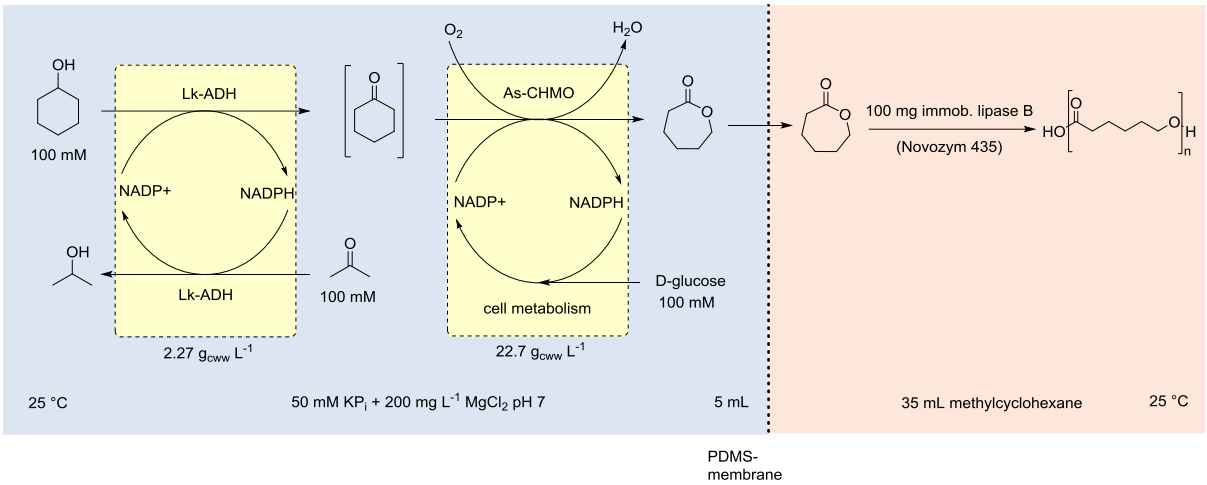


Scheme S2: Polymerization of ε-caprolactone towards poly-ε-caprolactone with immobilized lipase B from Candida antarctica (Novozym 435) without solvent at 60 °C (as described in literature [9]), with 35 mL methylcyclohexane as solvent at 60 °C and under the desired reactions conditions for the reaction sequence in methylcyclohexane at 25 °C to achieve temperature compatibility with the preceding whole-cell catalytic double oxidation.

The lipase B catalyzed polymerization of ε-caprolactone to polycaprolactone is a well-known process usually performed in the temperature range of 60 °C to 70 °C [10]. For our idea of the combination of whole-cell catalyzed double oxidation of cyclohexanol to ε-caprolactone with *in situ* product removal and polymerization towards polycarpolactone in a one-pot manner, the polymerization must take place for one thing in the used extracting solvent methylcyclohexane and for another thing at 25 °C. To combine polymerization efficiency regarding conversion and degree of polymerization was performed based on a published protocol with a mass ratio of $m_{monomer}$ to $m_{catalyst}$ ratio of 11.4 to 1 at 60 °C without any solvent [9]. Thus 1.14 g ε-caprolactone were weighed out in a 100 mL round-bottom flask and 100 mg immobilized lipase B (Novozym 435) were added together with a 15 mm long stirring bar. Reaction took place under a reflux condenser in an oil bath (Silicone oil M200, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) at 60 °C on a magnetic stirrer at 500 rpm for 23 h (reaction a). The same reaction was also performed with 1.14 g ε-caprolactone dissolved in 35 mL methylcyclohexane for one thing at 60 °C (reaction b) and for another thing at 25 °C (reaction c).
All reactions were cooled to room temperature and the polymeric crude products were completely dissolved by addition of 50 mL chloroform. Lipase B beads (300 – 900 µm) were removed from the crude product by filtration over a glass frit (POR 3/G3, 16- 40 µm, DURAN Group GmbH, Wertheim/Main, Germany) preheated to 40 °C with the help of vacuum. The retentate was washed with 20 mL chloroform. From the filtrate the chloroform (boiling point 61 °C) and in case of reaction b and c also the methylcyclohexane (boiling point 101 °C) was removed with a rotary evaporator (Rotavapor RII, BÜCHI Labortechnik GmbH, Germany; pumping unit PC 500 series, VACUUBRAND GMBH + CO KG, Wertheim, Germany) by successive reducing the pressure at 40 °C bath temperature to 50 mbar and subsequent increase of the water bath temperature to 60 °C. Afterwards an aliquot of approx. 10 mg of each crude product was transferred in a NMR-tube and dissolved in 1 mL deuterated chloroform for ^{1}H-NMR analysis. Conversion was determined by the ratio of the ε-caprolactone peak at 4,22 ppm, dimer (6-hydroxycaproic acid dimers) at 4,10 ppm and the polycaprolactone

peak at 4,05 ppm. Furthermore an aliquot of each crude product was analyzed by GPC as described in section 1.16.

1.13 Biotransformations in PDMS-thimble system using recombinant whole-cell catalyst for synthesis of poly-ε-caprolactone (according to Fig. 3):



Scheme S4: Two-cell based biotransformation of 100 mM cyclohexanol towards ε-caprolactone in the PDMS-thimble system with *in situ* product removal combined by polymerization to polycaprolactone *via* lipase B in methylcyclohexane outside the PDMS-thimble. The reference reaction was performed in a pure aqueous system towards ε-caprolactone (equal to the aqueous phase in the PDMS-thimble).

Biotransformations towards the production of poly-ε-caprolactone in the PDMS-thimble system were performed as described in section 1.11 with the exception of adding 100 mg immobilized lipase B from *Candida antarctica* (Novozym 435) to the 35 mL methylcyclohexane outside the PDMS-thimble. As reference the pure aqueous system towards the production of ε-caprolactone was used, as lipase B has no activity in a pure aqueous system. Both reactions were done in duplicate.

For determination of conversion of the caprolactone species 0.5 mL of the aqueous phase was extracted with ethylacetate as described in the previous chapter and analyzed by GC. In contrast to the previous experiments the organic phase couldn´t be analyzed *via* GC due to the presence of polycaprolactone, which do not evaporate (without decomposition). Thus the organic phase was analyzed by ^{1}H-NMR. Therefore the thimble with the aqueous phase was removed. To the organic phase 20 mL of chloroform were added to ensure that the polymeric products were completely solubilized before removal of lipase B by filtration through a glass frit (frit (POR 3/G3, 16- 40 µm, DURAN Group GmbH, Wertheim/Main, Germany) preheated to 40 °C with the help of vacuum in a clean round-bottom flask whose empty weight was noted. To remove residual product, the retentate was washed with 20 mL chloroform. Afterwards chloroform (boiling point 61 °C) and methylcyclohexane (boiling point 101 °C) was removed with a rotary evaporator by successive reducing the pressure at 40 °C water bath temperature to 50 mbar and subsequent increase of the water bath temperature to 60 °C. Under these conditions either cycylohexanol (boiling point 161 °C) nor cyclohexanone (boiling point 155 °C) nor ε-caprolactone (boiling point 237 °C) were removed. The weight of the flask with the crude product was determined for calculation of the weight of the crude product. Afterwards approx. 10 mg of the crude product were dissolved in 1 mL deuterated chloroform and analyzed by ^{1}H-NMR (DRX 500, Bruker AXS GmbH, Karlsruhe, Germany). The composition of the crude product was determined by the ratio of the peak integrals of ε-caprolactone (4,22 ppm, 2H), 6-hydroxycaproic acid dimer (4,10 ppm, 2H), polycaprolactone (4,05 ppm, 2H) and cyclohexanol

(3,61 ppm, 1H) taking each number of nuclei in account. Overall conversion was then calculated based on the amount of substance of the reaction components in the aqueous and organic phase. The degree of polymerization was analyzed *via* GPC as described in section 1.16.
Additionally the tightness of the PDMS-thimble was verified. Therefore the PDMS-thimble was emptied and dried under the fume hood. Afterwards it was filled with water and subsequently with *n*-hexane whereby the outside of PDMS-thimble was checked for formation of liquid droplets.

Figure S4: Application of the PDMS-thimble in a biotransformation: Inside the PDMS-thimble the double-oxidation of cyclohexanol towards ε-caprolactone takes place. The latter one diffuses over the PDMS-membrane into the outer compartment containing methylcyclohexane and lipase B for *in situ* product removal towards polycaprolactone.

Cyclohexanol: ^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 3.61 (m, 1H, C(OH)*H*), 1.89 (m, 2H, C(OH)C$H^{äq}$), 1.73 (m, 2H, C(OH)CH_2C$H^{äq}$), 1.55 (m, 1H, C(OH)CH_2CH_2C$H^{äq}$), 1.26 (m, 4H, C(OH)CH^{ax}CH^{ax}), 1.17 (m, 1H, C(OH)CH_2CH_2CH^{ax}).

6-Hexanoic acid: ^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 3.65 (t, 2H, C(OH)CH_2), 2,36 (t, 2H, C(OOH)CH_2), 1.63 (m, 4H, C(OH)CH_2CH_2, C(OH)$CH_2CH_2CH_2$CH_2), 1.41 (m, 2H, C(OH)CH_2CH_2CH_2)

ε-Caprolactone: ^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 4.22 (m, 2H, OCH_2), 2,63 (m, 2H, C(O)CH_2), 1.85 (m, 2H, CH_2CH_2CH_2), 1.76 (m, 4H, $COCH_2$CH_2, C(O)CH_2CH_2)

Polycaprolcatone: ^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 4.05 (t, 2H, OCH_2), 2.30 (t, 2H, C(O)CH_2), 1.64 (m, 4H, C(O)CH_2CH_2, C(O)$CH_2CH_2CH_2$CH_2), 1.38 (m, 2H, C(O)CH_2CH_2CH_2)

1.14 GC analytics of hydrogenation (according to Tab. 1) and biotransformation of hydrogenation crude product as substrate (according to Fig. 1)

GC analysis of samples from the phenol hydrogenation reactions was performed on a GC-2010 (Shimadzu Deutschland GmbH, Duisburg, Germany) with a chiral BGB-174 column (BGB Analytik AG, Boeckten, Switzerland) with the following temperature profile: starting with a plateau at 70 °C for 1 min followed by heating up to 180 °C with 40 °C/min, then heating up to 190 °C with 2 °C/min and finally heating up to 200 °C with 40 °C/min (SPLI1: 230 °C; flow control: linear velocity; linear velocity: 46.9 cm/sec; split ratio 10; injection volume: 1 µL; FID: 230 °C, carrier gas: nitrogen). Retention times of the analytes were as followed: cyclohexanol 3.2 min, cyclohexanone 3.6 min, phenol 4.2 min, ε-caprolactone 5.7 min. The conversion was determined by relative analytics, as it has been shown that phenol, cyclohexanol, cyclohexanone and ε-caprolactone were extracted by ethylacetate in identical ratio. Furthermore the different signal intensities of the different substances were taken into account by a calibration line as well as the dilution factor due to extraction.

1.15 GC analytics of diffusion experiments (according to Fig. S7) and biotransformations (according to Fig. 1, Fig. 2, Fig. 3 and Fig. S5):

GC analytics of samples was performed by a GC-2010 (Shimadzu Deutschland GmbH, Duisburg, Germany) with a BP5MS-column (SGE Analytical Science Europe Ltd., Milton Keynes, Great Britain) with the following temperature profile: starting with a plateau at 70 °C for 3.8 min followed by heating up to 200 °C with 40 °C/min (SPLI1: 230 °C; flow control: linear velocity; linear velocity: 46.9; split ratio 100; injection volume: 1 µL; FID: 230 °C, carrier gas: nitrogen). Retention times of the analytes were as followed: cyclohexanol 3.2 min, cyclohexanone 3.3 min, acetophenone 5.5 min, ε-caprolactone 6.0 min, 6-hydroxyhexanoic acid 6.8 min. The conversion was determined by relative analytics, as it has been shown that cyclohexanol and ε-caprolactone were extracted by ethylacetate in identical ratio. Furthermore the different signal intensities of the different substances were taken into account by a calibration line as well as the dilution factor due to extraction.

1.16 GPC analytics of polymers (according to Fig. 3):

5 to 7 mg dried crude product or commercial polycaprolactone (ØM_w ~ 14,000, ØM_n ~10,000 and ØM_n ~45,000) as reference were dissolved in 997 µL tetrahydrofurane (THF) and 3 µL toluene as an internal flow standard. GPC was performed with THF and 1 mL/min flow rate and polymers were separated on a GMHHR-L Mixed Bed column (Viscotek, Houston, TX, USA) in series to a PLgel 3 µm MIXED-E column (Agilent Technologies, Santa Clara, CA, USA). A refractive index (RI) detector (K2301, KNAUER Wissenschaftliche Geräte GmbH, Berlin, Germany) was used for monitoring the course of separation. The system was calibrated with molecular weight standards of polystyrene (M_P 66,000; 28,000; 12,600; 9,130; 6,100; 4,920; 3,470; 2,280; 1,250, 162 g/mol).

2. Results

2.1 Biotransformations in two-phase systems

In an initial study we did some solvent screening which revealed a positive effect of the addition of small amounts of co-solvents with high logP-values to a pure aqueous system, especially on the reaction rate [11]. By increasing the amount of co-solvent to obtain a two-phase system, the positive effect of the solvents is abolished, as conversion decreased in the two-phase system in comparison to a pure aqueous system at 100 mM substrate concentration. Figure S5 also show, that MTBE has a more deactivating effect than cyclohexane and methylcyclohexane.

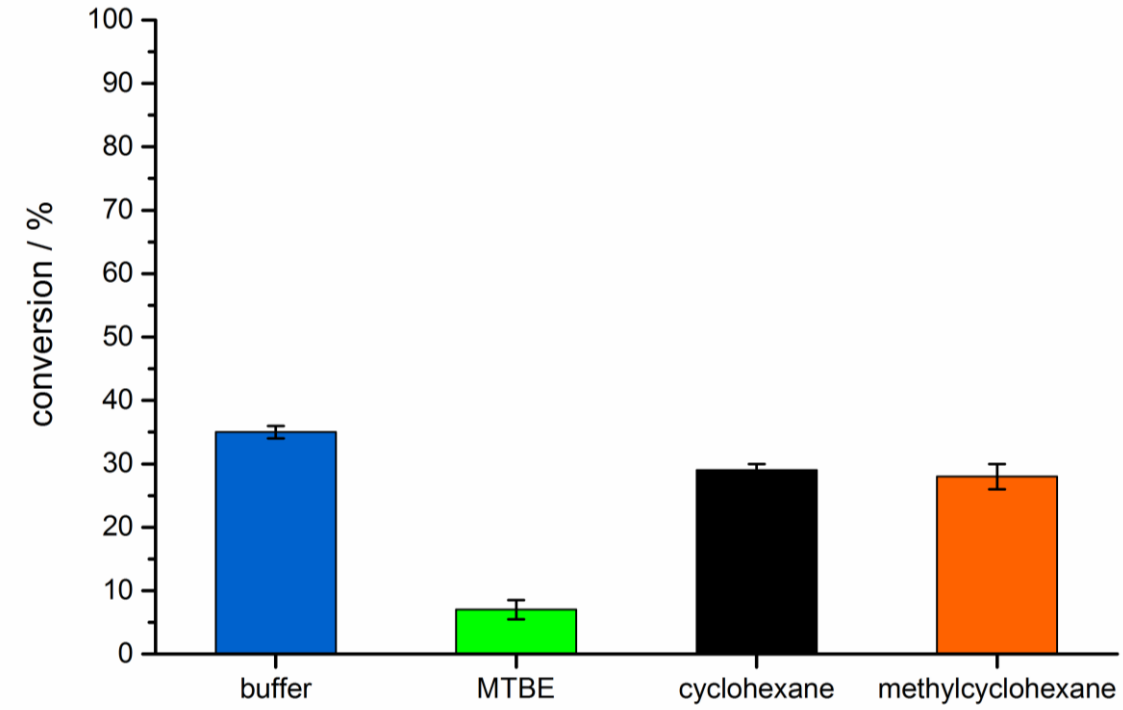


Figure S5: Comparison of the enzymatic conversion of 100 mM cyclohexanol towards ε-caprolactone in a pure aqueous system and different two-phase systems (1:1 volume ratio) consisting of organic solvents with different logP-values: MTBE (logP: 0,94), cyclohexane (logP: 3,44), methylcyclohexane (logP: 3,88) after 23 h reaction time.

Figure S6 shows on the macroscopic level that the decrease in conversion might be correlated to the phase boundary. After 23 h of biotransformation the organic phase looks more like an emulsion which indicates that the enzymes were deactivated by unfolding and partially dissolved or complexed by the organic solvent.

Figure S6: Picture of the reaction media after 23 h: left flask contains the pure aqueous system whereas the right flask contains a buffer-cyclohexane two-phase system.

2.2 Diffusion experiments and investigation of permeability of the PDMS-membrane

As the PDMS-thimbles were mechanically stable and showed no leakage after 23 h in the different extracting solvents (methylcyclohexane, cyclohexane, MTBE), they were in principle suitable for our desired application.

Diffusion experiments showed that the PDMS-membrane is permeable for ε-caprolactone as well as for the substrate cyclohexanol and the intermediate cyclohexanone, independent of the applied organic solvent used for *in situ* extraction and the orientation of the PDMS-membrane (Fig. S7). Although it would be more efficient to use a membrane, which is exclusively permeable for ε-caprolactone – which we could not find on the market and in extensive discussions with membrane manufacters – diffusion of the substrate cyclohexanol and the intermediate cyclohexanone is not critical, as the membrane is permeable in both directions and the substrate concentration can be kept low by applying a fed-batch mode. Furthermore, if expression of the Lk-ADH and the As-CHMO could be adjusted to an optimal ratio within one

whole-cell catalyst, the intermediate cyclohexanone will not accumulate, as it can be directly converted to ε-caprolactone within the same cell.
The distributional steady state of the three reaction components was obtained in each of the extracting solvents after at least 3 h. In all three cases the highest amount of cyclohexanol and cyclohexanone were found in the organic phase, whereas in the case of ε-caprolactone the distribution between both compartments dramatically depends on the extracting solvent used. Regarding the *in situ* product removal MTBE would be the solvent of choice as 58 % of ε-caprolactone was found in the organic phase whereas in the case of cyclohexane and methylcyclohexane percentage amount of substance is in the range of approx. 18 %, which suggest the assumption of an inversely proportional correlation between the amount of extracted ε-caprolactone and the logP-value of the extracting solvent.

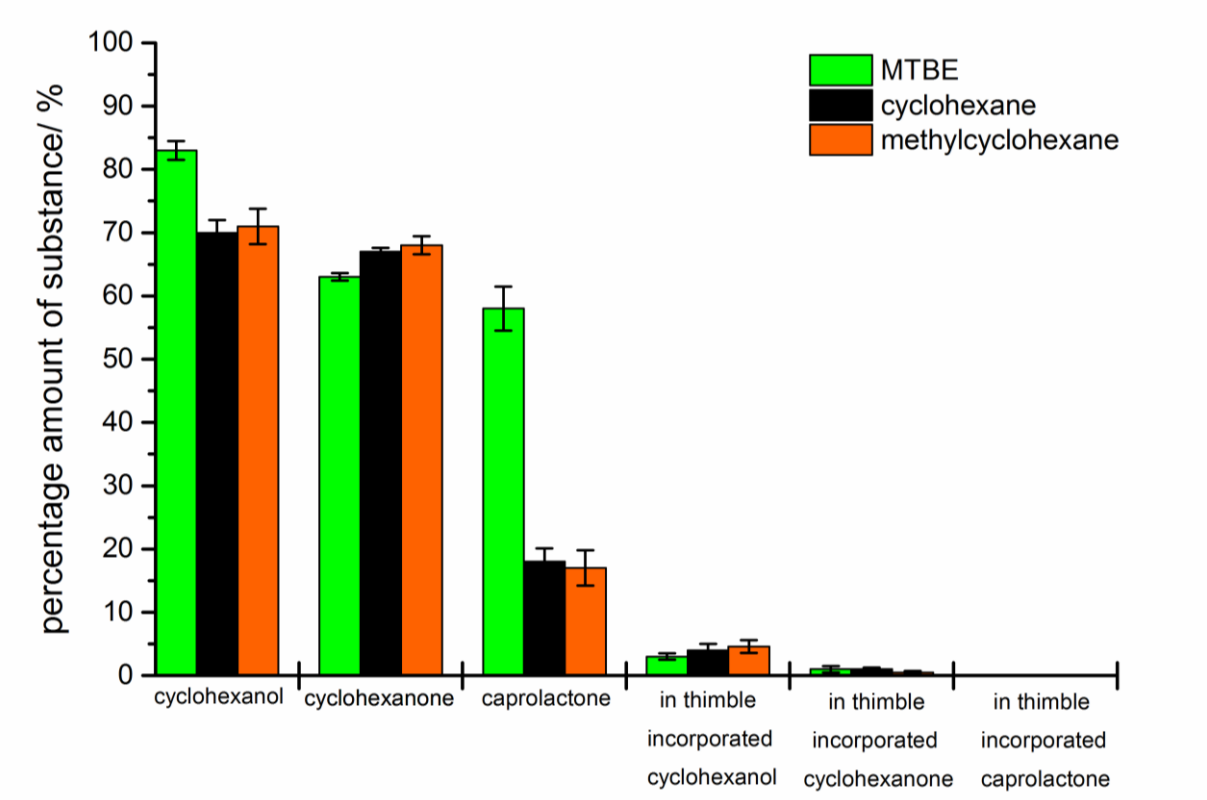


Figure S7: Percentage amount of the reaction components cyclohexanol, cyclohexanone and ε-caprolactone in the organic compartment after 3 h, as well as the percentage amount incorporated in the PDMS-membrane.

Analysis of the *n*-hexane "washing solution" revealed incorporation of small amounts of cyclohexanol and cyclohexanone, which was also observed in the biotranformation at 40 mM substrate loading shown in Figure 2. Nevertheless the loss due to incorporation is negligible at high substrate loadings or in a fed-batch process.
Regarding extracting ε-caprolactone from the aqueous phase, MTBE would be the solvent of choice. Nevertheless ^{1}H-NMR data from the aqueous phase shown in Figure S8 revealed that the PDMS-membrane is permeable for MTBE (Figure S8 A: peak at 3.21 ppm and 1.19 ppm) and cyclohexane (Figure S8 B: peak at 1.42 ppm).

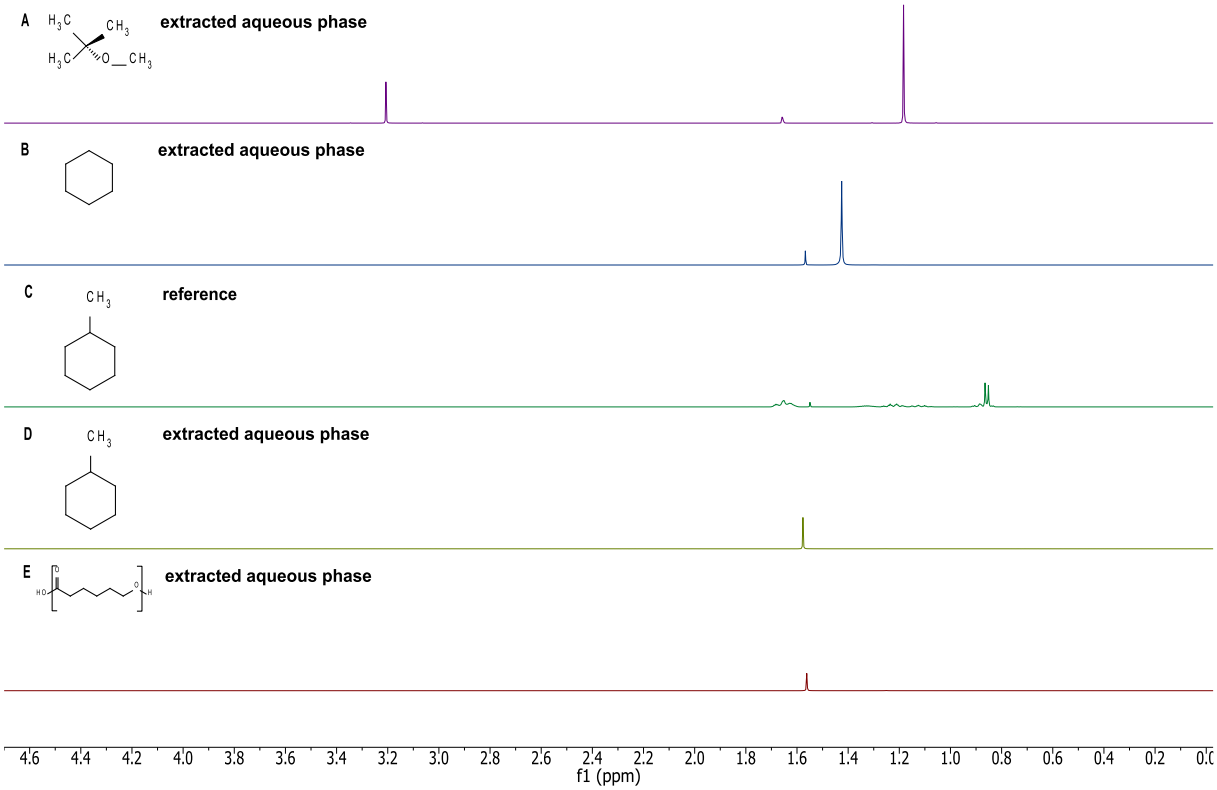


Figure S8: ^{1}H-NMRs of the aqueous phases for investigation of impermeability of the PDMS-membrane for the different extracting solvents and polycaprolactone: A) ^{1}H-NMR of the aqueous phase using MTBE (3.21 ppm, 1.19 ppm) as extracting solvent; B) ^{1}H-NMR of the aqueous phase using cyclohexane (1.42 ppm) as extracting solvent; C) ^{1}H-NMR of pure methylcyclohexane (1.84 – 1.49 ppm, 1.49 – 0,58 ppm, 0.87 ppm); D) ^{1}H-NMR of the aqueous phase using methylcyclohexane as extracting solvent; E) ^{1}H-NMR of the aqueous phase and polycaprolactone dissolved in methylcyclohexane located outside the thimble.

Comparison of ^{1}H-NMRs in Figure S8 C of pure mehtylcyclohexane as reference with peaks at 1.84 – 1.49 ppm, 1.49 – 0,58 ppm, 0.87 ppm and Figure S8 D of extracted aqueous phase, reveals that the PDMS-membrane is impermeable for methylcyclohexane.

As the PDMS-membrane is only completely impermeable for methylcyclohexane, methylcyclohexane is the solvent of choice for *in situ* extraction of ε-caprolactone from the aqueous compartment harboring the biocatalyst, although mehtylcyclohexane has the lowest extraction capability of the tested solvents.

Diffusion experiments furthermore confirmed that the PDMS-membrane is impermeable for polycaprolactone as no polycaprolactone peaks (4.05 ppm, 2.30 ppm, 1.64 ppm and 1.38 ppm) could be detected in the ^{1}H-NMR of the extracted aqueous phase.

2.3 Polymerization experiments

Polymerization of ε-caprolactone to polycaprolactone by lipase B is summarized by a very comprehensive review[9]. Nevertheless no information are available if polymerization takes place in methylcyclohexane and at room temperature. Thus, we investigated first the effect of solvent on the conversion and on the degree of polymerization.

Figure S9 show the conversion determined by ^{1}H-NMR without solvent at 60 °C as described by Uyama et al.[9]. Our data with 97 % oligomer and polymer and 3 % dimer are in accordance to the data from Uyama *et al.* with 99 % conversion. By using methylcyclohexane as solvent at 60 °C conversion is slighty decreased and the percentage amount of dimer increased,

showing a slight negative effect of methylcyclohexane on the polymerization. An additional reduction of the reaction temperature from 60 °C to 25 °C resulted in a higher amount of oligomer and polymer, as no dimer is detectable, but 5 % ε-caprolactone. Although efficiency is decreased due to the addition of methylcyclohexane as a solvent and reduction of the reaction temperature from 60 °C to 25 °C polymerization leads to approx. 95 % product, showing that polymerization is in principle compatible with the conditions in the one-pot thimble-system.

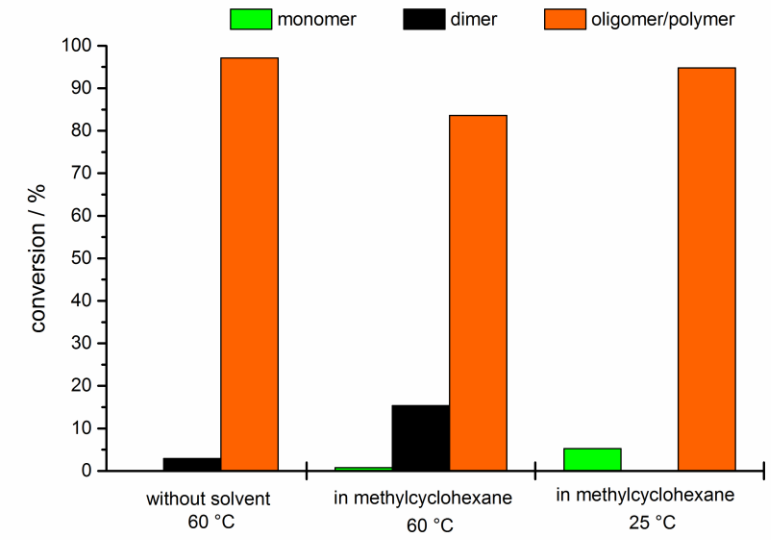


Figure S9: Influence of the reaction conditions of polymerization on conversion.

Besides conversion also the degree of polymerization is of industrial importance, thus we investigated also the influence of the different reaction conditions of polymerization on the degree of polymerization. Figure S10 shows that the degree of polymerization under the different reaction conditions do not dramatically differ, so that combination of *in situ* extraction with polymerization is in principle possible.

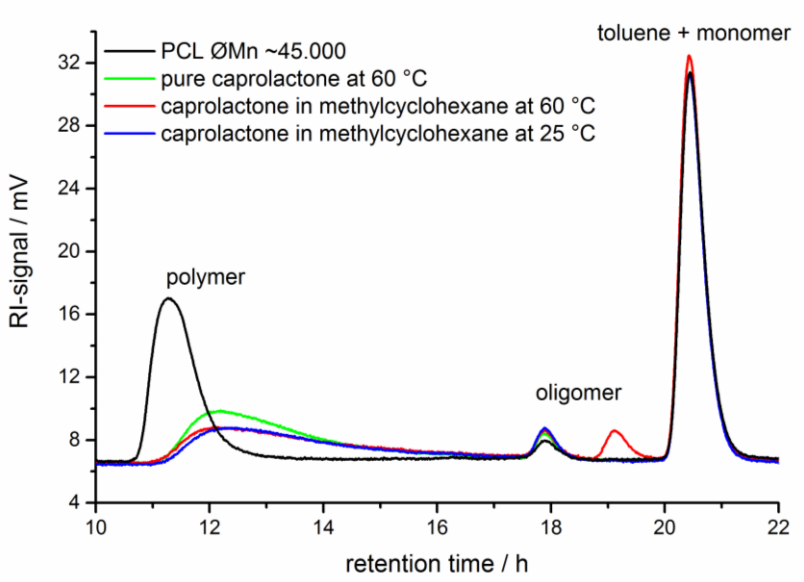


Figure S10: Influence of the solvent methylcyclohexane and the temperature on the degree of polymerization of PCL. Polymerization without solvent (A, described in [9]) and with the addition of methylcyclohexane (B), which should be used for *in situ* product extraction, both at 60 °C. For implementation of an enzymatic reaction sequence in a one-pot reaction, polymerization must take place at 25 °C in mehtylcyclohexane (C) as the enzymes from the double oxidation are not heat stable.

After successful *in situ* extraction by methylcyclohexane over the PDMS-membrane (Fig. S7, Fig. 2) and polymerization of ε-caprolactone in methylcyclohexane by lipase B (Fig. S9 and Fig. S10), the next step was to combine *in situ* extraction and polymerization in a biotransformation (Fig. 3).

3. References

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