Electronic Supplementary Information

Liquid immobilisation concept for enzymes by thermomorphic solvent systems

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1 Reagents and materials

The lipase Amano Lipase PS was purchased from Amano Pharmaceuticals Ltd. (Nagoya, Japan) and p-nitrophenyl palmitate (98%+) and p-nitrophenyl palmitate for calibrations (Pestanal®, analytical standard) were from Sigma-Aldrich (St. Louis, MO, USA). All non-aqueous solvents used in this work were obtained from Acros Organics (Geel, Belgium). All of them were 99% pure or better with the exception of hexanol which had a purity of 98%+. All other chemicals employed herein were purchased from commercial suppliers and were of the highest purity available. Photometric measurements were recorded on a Specord S600 from Analytik Jena AG (Jena, Germany).

2 Quantification of *p*-nitrophenyl palmitate and *p*-nitrophenol

Yields of *p*-nitrophenyl palmitate and *p*-nitrophenol were determined by high performance liquid chromatography (HPLC). Analyses were performed on a computer-controlled Merck-Hitachi HPLC system (VWR International, Darmstadt, Germany) containing the following modules: an interface L-7000, a pump L-7100, a diode array detector L-7450, an auto sampler L-7200 with a 100 µl sample loop, a solvent degasser L-7612 and a high-pressure gradient mixer. The administration of the device, data recording and analysis was performed with the LaChrom Software version 3.2.1. The column from Merck (Darmstadt, Germany) has an inner diameter of 4.6 mm, a length of 250 mm and is packed with LiChrospher® 100 RP-18 (5 µm). Acetonitrile and water with 1% acetic acid were used as the eluent for separation.

Flow rates and eluent concentration were as shown in Table S1. Ultraviolet detection occurred at λ(pNPP)=276 nm and λ(pNP)=313 nm.

Table S1: Flow rates and eluent concentration.

Time (min)	Flow rate (ml/min)	Water (%)	Acetonitrile (%)
0	0.0	100	0
2	1.0	90	10
3	2.5	0	100
7	2.5	0	100
8	1.0	100	0

3 Hydrolysis of *p*-nitrophenyl palmitate (pNPP) in the TMS system

In a typical experiment, 23.0 g water, 15.5 g methanol and 5.5 g hexanol were weighed into the reaction vessel. After adding 330 µl of triallylamine, the TMS was heated up to 45°C to generate one phase. The pH value was adjusted to pH 7 with hydrochloric acid. A solution of 15 mg *p*-nitrophenyl palmitate in 0.5 g hexanol was added and the reaction was started by adding a solution of 1 mg Amano Lipase PS in 1000 µl water. In order to monitor the reaction course, 250 µl samples were taken from the reaction mixture for HPLC analysis. A solution of 250 µl methanol and 5% trifluoroacetic acid (TFA) was added to stop the reaction. Methanol was important to have a monophasic solution during HPLC analysis. The TFA effected the denaturation of the enzyme.

4 Recycling experiment

In a typical experiment, 46.0 g water, 31.0 g methanol and 11.0 g hexanol were weighed into the reaction vessel. After adding 660 µl of triallylamine, the TMS was heated up to 45°C to generate one phase. The pH value was adjusted to pH 7 with hydrochloric acid. A solution of 30 mg *p*-nitrophenyl palmitate (pNPP) in 1.0 g hexanol was added and the reaction was started by adding a solution of 6 mg Amano Lipase PS in 2000 µl water. After 30 min a sample for HPLC analysis was taken as described above and the reaction mixture was cooled down to 5°C. After phase

separation the organic phase was replaced by a fresh one of a new, parallel pre-prepared TMS system. The TMS system was heated up to 45°C and the reaction was started again by the addition of 30 mg pNPP in 1.0 g hexanol.

5 Recycling experiment with higher substrate concentration

In a typical experiment, a solution of 12.0 g hexanol and 300 mg *p*-nitrophenyl palmitate (pNPP) was prepared at 45°C. Into the reaction vessel 8.5 g methanol, 9.0 g water and 590 µl of triallylamine were added and heated up to 45°C. Both solutions were combined. The pH value of the monophasic TMS system was adjusted to pH 7 with hydrochloric acid. The reaction was started by adding 10 mg Amano Lipase PS. During reaction samples for HPLC analysis were taken as described above to monitor the reaction course. Finally, the reaction mixture was cooled down to 5°C for 2 h. After phase separation the aqueous phase was again heated up to 45°C. The reaction was started again by adding a fresh organic phase of a new, parallel pre-prepared TMS system containing 300 mg pNPP.

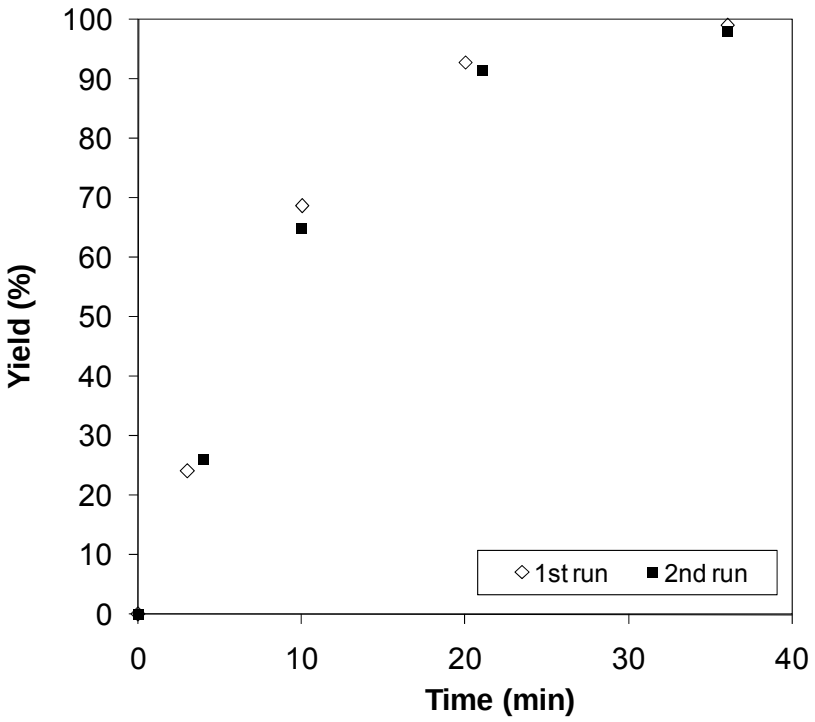

Fig S1. **Results of catalyst recycling runs with higher substrate concentration.**