Electronic Supplementary Information (ESI)

Table S1: Examples of biocatalysis with recombinant whole cells in pure organic media

entry	enzyme	reaction	organic solvent	max. substrate conc. [mol/L]	max. conversion [%]	source
1	RrADH-a	ketone reduction	several	2	< 5	[1]
2	CPCR	ketone reduction	isopropanol	4	94	[2]
3	ADH_{Tt1} ADH_{Tt2}	ketone reduction	cyclohexanol	3.7	97	[3]
4	AtHNL	hydroxynitrile lyase	MTBE	0.5	100	[4]

RrADH-a: *Rhodococcus ruber* alcohol dehydrogenase a, CPCR: *Candida parapsilosis* carbonyl reductase, ADH_{Tt}: alcohol dehydrogenase from *Thermus thermophilus* HB27, AtHNL: *Arabidopsis thaliana* hydroxyl nitrile lyase

Table S2: Stereoselectivity of BAL and RADH catalyst in MTBE after 24h

entry	enzyme	reaction	product conc. [mM]	*ee/de* [%]
1	BAL catalyst	carboligation	66.0	99.6/-
2	RADH catalyst	ketone reduction	98.2	> 99/> 99

Optimal benzaldehyde and acetaldehyde concentrations for carboligation with BAL

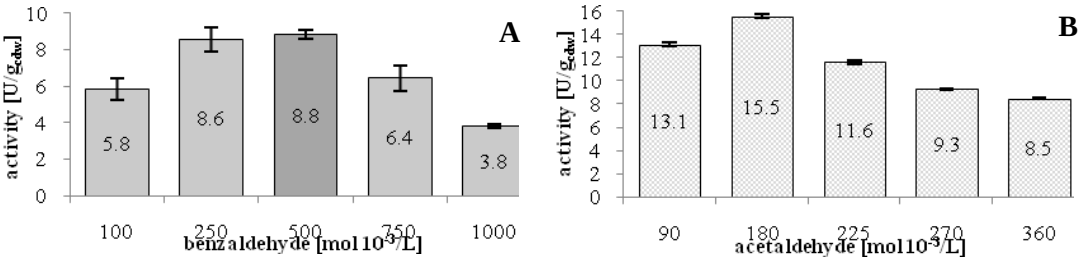

Fig. S1 Cell-specific initial rate activities of the BAL whole-cell catalyst in MTBE at several concentrations of (A) benzaldehyde and (B) acetaldehyde. 500 mM benzaldehyde is optimal (8.8±0.5 U g_{cdw}^{-1},Fig. S1A). Concentrations higher than 500 mM caused decreased activities possibly due to catalyst inactivation or inhibition. A regio isomer of (2*R*)-HPP, namely phenylacetyl carbinol, was detected at 100 mM benzaldehyde but vanished at higher benzaldehyde concentrations. The latter finding is in accordance with an earlier study, where the performance of BAL cells was tested in an MTBE/buffer biphasic system at elevated benzaldehyde concentrations.[5] For acetaldehyde, 180 mM is optimal with 15.6±0.2 U g_{cdw}^{-1}maximal BAL activity as depicted in Fig. S1B. Concentrations higher than 180 mM led to catalyst inactivation or inhibition. Stillger *et al.* did not observe inactivation of isolated BAL in aqueous media with acetaldehyde concentrations <150 mM either, nor did they test higher acetaldehyde concentrations.[6] The mixtures were light brown possibly due to media components rinsed from the lyophilised cell pellet. However, especially at higher acetaldehyde concentrations, the mixture turned darkish brown after prolonged incubation. This phenomenon had previously been observed for lipases and was directly attributed to enzyme deactivation by reaction of the acetaldehyde with free amino groups of proteins forming Schiff bases and Michael adducts.[7]

Table S3: Final values of preparative production of (2*R*)-HPP at 1 L scale in a micro-aqueous system

(2*R*)-HPP			**(*R*)-benzoin**			**benzaldehyde**		**sum**
conc. [mM]	*ee* [%]	conv. [%]	conc. [mM]	*ee* [%]	conv. [%]	conc. [mM]	conv. [%]	conc. [mM]
443.5	99.6	90.9	7.7	>99	1.6	36.5	7.5	487.7

Reaction conditions: 20 g L^{-1} BAL cells (lyophilised), 0.5 mol benzaldehyde (53 g), 0.18 mol acetaldehyde (10 mL) in MTBE (total volume 1 L). Reaction was started by the addition of 20 mL 1 M TEA, pH 10.0. After 90 mM (2*R*)-HPP was produced, 5 mL (90 mM) acetaldehyde was added to the reaction (pulses at 30, 60, 90, 150 and 270 min.). The course of the reaction was similar to that previously observed in5 mL scale. Purification: (2*R*)-HPP was purified from the crude by solubilising in ethyl acetate and precipitating it three times with petroleum ether. The crystals were washed and dried at room temperature. Final isolated yield was 68 % (51 g) with an *ee* of 99.6 %.

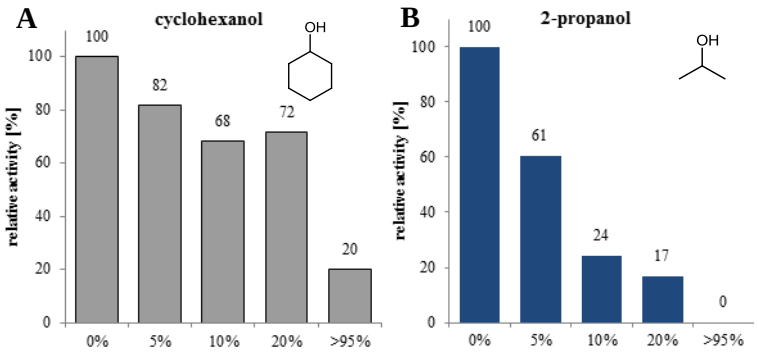

Fig. S2 Relative initial rate activity of BAL catalyst in the presence of varying amounts of cyclohexanol (A) and 2-propanol (B).

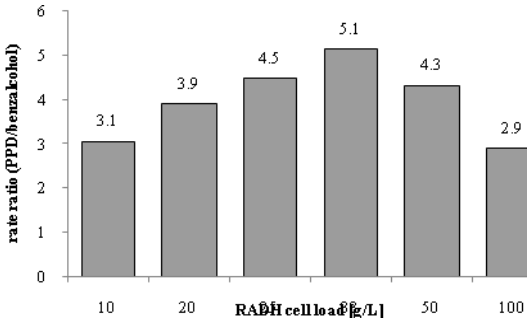

Fig. S3 Ratio of the cell-specific formation rates of (1*R*,2*R*)-PPD and benzyl alcohol at different RADH catalyst loads (BAL cell load constant at 25 g L^{-1}). Here, the initial reaction rates of RADH towards (1*R*,2*R*)-PPD and benzyl alcohol formation were recorded. Then, the ratio of these two cell-specific reaction rates were plotted versus the RADH catalyst load.

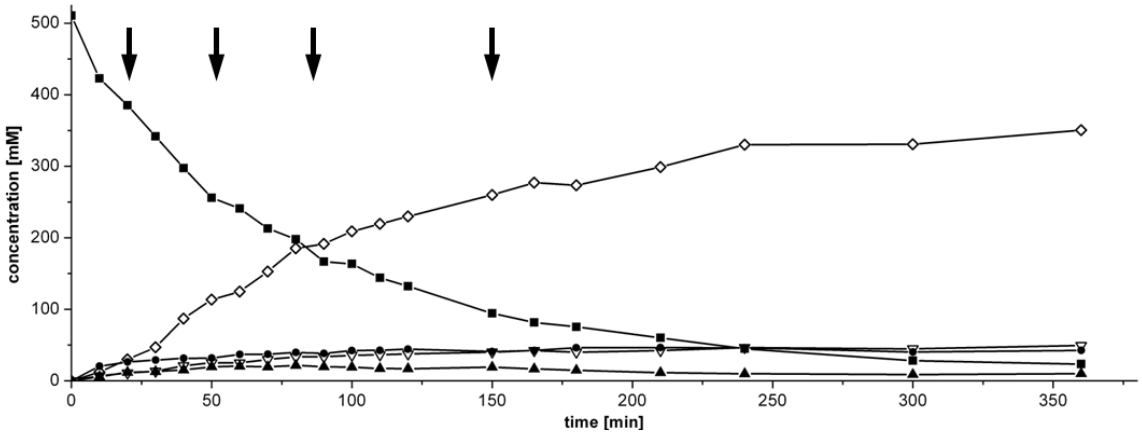

Fig. S4 Simultaneous cascade with BAL + RADH catalyst on preparative scale (20 mL). Production of (1*R*,2*R*)-PPD (◊) from benzaldehyde (■) and acetaldehyde with (2*R*)-HPP (●) as intermediate; *(R)*-benzoin (▲) and benzyl alcohol (Δ) are formed as side products. Acetaldehyde doses of 90 mM were pulsed after 20, 50, 85 and 150 min as indicated by arrows. For reaction conditions see experimental chapter of main paper

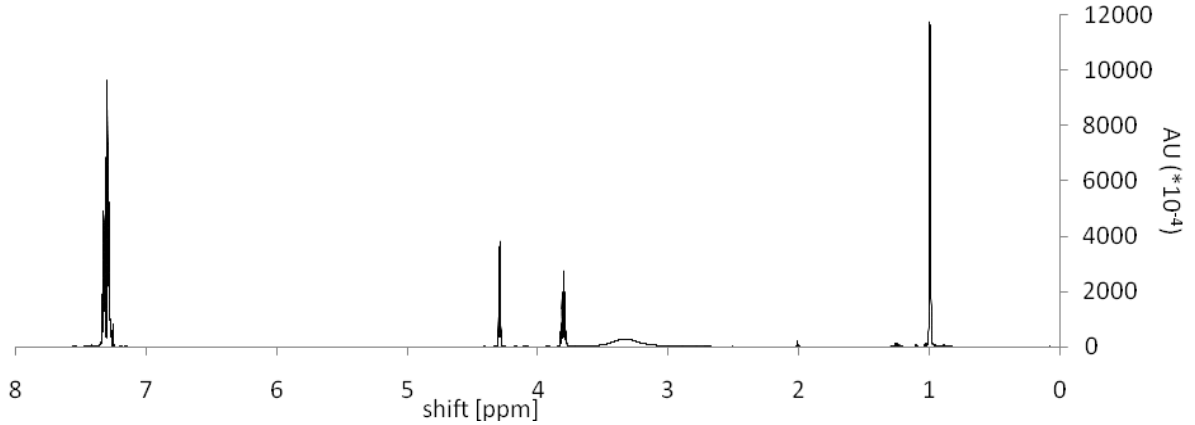

Fig. S5 ^{1}H-NMR of purified (1*R*,2*R*)-PPD (600 MHz, $CDCl_3$).0.996 (d, 3J1,2 = 6.0 Hz, 3 H, CH3); 3.33 (brs 2 H, OH); 3.80 (dq, 3J2,3 = 7.2 Hz, 3J2,1 = 6.0 Hz, 1 H, C2-H); 4.29 (brd, 3J3,2 = 7.8 Hz, 1 H, C3-H); 7.25-7.35 (m, 5 H, arom.-H)

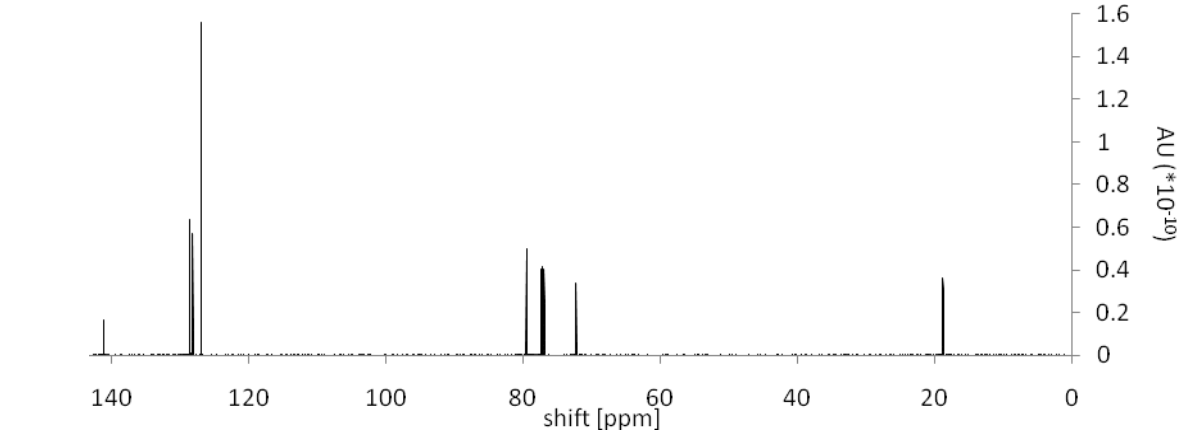

Fig. S6 ^{13}C-NMR spectrum of purified (1*R*,2*R*)-PPD (151 MHz, $CDCl_3$).18.8 (C-3), 72.2 (C-2), 79.5 (C-1), 126.9 (arom.-CH), 128.1 (arom.-C), 128.5 (arom.-C), 141.1 (arom.-C);three solvent signals at 76.9, 77.1, 77.3

Table S4: Calculation of space-time yields (STY)

	reaction volume [L]	final product concentration [mM]	molecular weight [g/mol]	product mass [g]	reaction time [h]	reaction time [d]	STY g L^{-1} d^{-1}	source
sequential cascade	0.005	440.0	152	0.339	9	0.375	178.3	this work
sequential cascade*	0.005	426.7	152	0.329	7	0.292	222.4	this work
simultaneous cascade	0.005	362.9	152	0.279	8	0.333	161.5	this work
simultaneous cascade*	0.005	358.5	152	0.276	4	0.166	326.9	this work
preparative sim. cascade	0.02	350.6	152	0.8744**	6	0.25	174.9	this work
published cascade	0.39	0.004	152	0.242**	120	5 (4 + 1)	0.124	[8]

For STY calculation of the sequential cascade the corrected values for reaction volume and product concentration were applied (dilution factor 1.37). In the process published by Kihumbu *et al.*,(2*R*)-HPP was produced according to Demir *et al.* where 285 mg was produced within 4 days in 100 mL volume.[9] For reduction with ADH-T, only 150 mg (2*R*)-HPP was applied and reduced within 1 day in 100 mL volume. However, for easier comparability, the reduction process was scaled up by a factor of 1.9 (285 mg (2*R*)-HPP in 190 mL volume). The corresponding isolated yield of (1*R*,2*R*)-PPD would then be 85 % (242 mg) in 390 mL volume as depicted in Tab.S3.
*same experiment but calculated after different reaction times **isolated yield

Table S5: Calculation of E-factor with and without work-up

	product mass	mass of reaction solution	E-factor w/o work-up	mass of work-up solutions	mass of solutions for chromatography	mass of silica	total mass of waste	E-factor with work-up
preparative simultaneous cascade	0.8744 g	18.65 g	21.3	89.7 g	1476.2 g	100 g	1684.5 g	1927

References

1. G. de Gonzalo, I. Lavandera, K. Faber and W. Kroutil, *Org. Lett.*, 2007, **9**, 2163-2166.
2. A. Jakoblinnert, R. Mladenov, A. Paul, F. Sibilla, U. Schwaneberg, M. B. Ansorge-Schumacher and P. Dominguez de Maria, *Chem. Commun.*, 2011, **47**, 12230-12232.
3. A. Hibino and H. Ohtake, *Proc. Biochem.*, 2013, **48,** 838-843.
4. K. E. Scholz, D. Okrob, B. Kopka, A. Grünberger, M. Pohl, K. E. Jaeger and U. Krauss, *Appl. Environ. Microbiol.*, 2012.
5. P. Dominguez de Maria, T. Stillger, M. Pohl, P. Kiesel, A. Liese, H. Gröger and H. Trauthwein, *Adv. Synth.. Catal.*, 2007, **350**, 165-173.
6. T. Stillger, M. Pohl, C. Wandrey and A. Liese, *Org. Proc. Res. Dev.*, 2006, **10**, 1172-1177.
7. B. Franken, T. Eggert, K. E. Jaeger and M. Pohl, *BMC Biochem.*, 2011, **12**, 10.
8. D. Kihumbu, T. Stillger, W. Hummel and A. Liese, *Tetrahedron: Asymmetry*, 2002, **13**, 1069-1072.
9. A. S. Demir, M. Pohl, E. Janzen and M. Müller, *J. Chem. Soc., Perkin Trans. 1*, 2001, **1**.