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Supporting Information

Hydrogen-Borrowing Alcohol Bioamination with Coimmobilized Dehydrogenases

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1. Abbreviations

ADH	alcohol dehydrogenase
AA-ADH	alcohol dehydrogenase originated from *Aromatoleum aromaticum*
AmDH	amine dehydrogenase
Ch1-AmDH	chimeric amine dehydrogenase generated through domain shuffling of Bb-PhAmDH variant and L-AmDH variant.[1] The former was originated from the L-phenylalanine dehydrogenase from *Bacillus badius*; the latter was originated from the L-leucine dehydrogenase from *Bacillus stearothermophilus*.
NOx	Recombinant nicotinamide oxidase
ee	enantiomeric excess
EtOAc	ethyl acetate
n.d.	not determined

2. General Information

(*S*)-alcohol substrates **1a**, **4a**, and **5a** were purchased from Sigma-Aldrich (Steinheim, Germany). (S)-alcohol substrate **2a** and **3a** were synthesised as previously reported.[2]

Nicotinamide cofactor (NAD^+) was purchased from Melford Biolaboratories (Chelsworth, Ipswich, UK).

Lysozyme from chicken egg white (3.2 mg, Sigma L6876, lyophilized powder, protein 95%, >40000 U/mg protein) was purchased from Sigma-Aldrich (Steinheim, Germany). The Ni^{2+} affinity columns (HisTrap FF, 5 mL) were purchased from GE Healthcare Bio-Sciences (Munich, Germany).

Controlled porosity glass EziG Fe-Amber affinity beads were provided by EnginZyme AB (Stockholm, Sweden).

3. Expression and purification of recombinant AA-ADH in *E. coli* host cells.

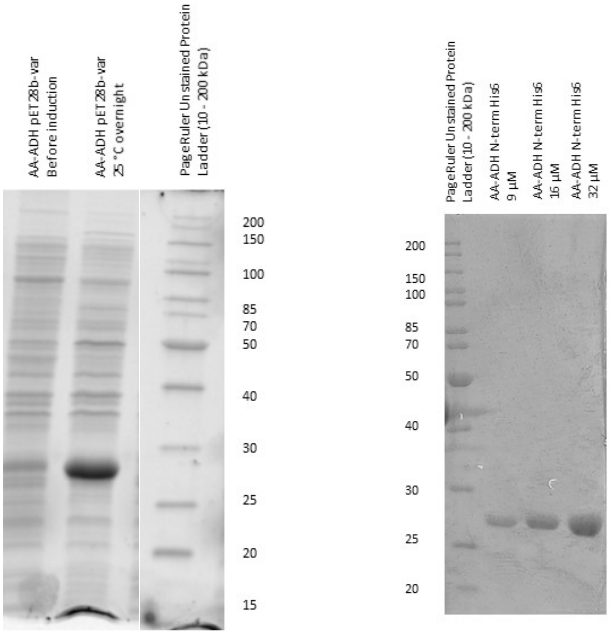


Figure S1. SDS PAGEs of the expression of AA-ADH in *E. coli* (left) and of the purification of AA-ADH via Ni^{2+} affinity chromatography (right). The SDS-Pages were visualized using a gel imaging system from Biorad.

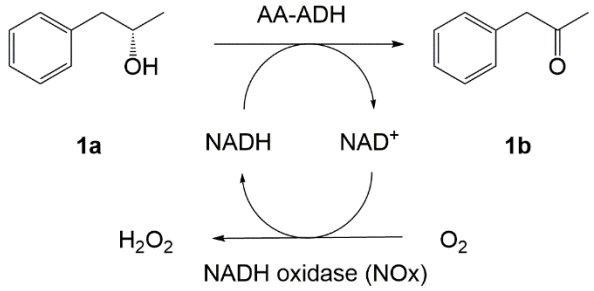
For recombinant expression of the N-term His_6-tagged protein AA-ADH,[2] 800 mL of LB medium supplemented with the appropriate antibiotic (50 µg mL^{-1} kanamycin) was inoculated with 15 mL of an overnight culture of *E. coli* BL21 DE3 cells harboring the pET28b plasmid with the genes for the expression of the protein. Cells were grown at 37 °C until an OD_{600} of 0.6 – 0.9 was reached and expression of protein was induced by the addition of IPTG (0.5 mM final concentration). Protein expression was carried out overnight at 25 °C. After harvesting of the cells (4 °C, 4500 rpm, 20 min), the remaining cell pellet was frozen for long storage or was used directly in the next step. Pellet was re-suspended (5 mL buffer g_{pellet}^{-1}) in Lysis buffer (50 mM KH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0) containing lysozyme 1 mg mL^{-1}) and incubated at 4 °C for 45 minutes prior to cell disruption by ultrasonication (pulse ON 10 sec., pulse off 20 sec., total time 12 min., amplitude 45 %). After centrifugation (4 °C, 15000 rpm, 1 h) the supernatant was filtered through a 0.45 µm filter and protein purification was performed by Ni-NTA affinity chromatography using pre-packed Ni-NTA HisTrap FF columns (5 mL column, GE Healthcare) according to the manufacturer´s instructions. After loading of the filtered lysate, the column was washed with sufficient amounts of washing buffer (60 mL, 50 mM KH_2PO_4, 300 mM NaCl, 25 mM imidazole, pH 8.0), and bound protein was recovered with elution buffer (50 mM KH_2PO_4, 300 mM NaCl, 200 mM imidazole, pH 8.0). The process of purification was analyzed by SDS-PAGE and fractions containing sufficient purified protein were pooled and dialyzed overnight against potassium phosphate buffer (50 mM, pH 8,). Protein solutions were concentrated and the concentration was determined spectrophotometrically using an extinction coefficient at 280 nm of 22523 M^{-1} cm^{-1} for AA-ADH. A protein yield of 45 mg AA-ADH per liter of cell culture was obtained.

Note: the amine dehydrogenase Ch1-AmDH was expressed and purified as described previously.[1b, 2]

4. Activity control experiments for AA-ADH

To 450 µL disrupted cell extract (obtained from 0.5 g cells in 3 mL KPi) was added 50 µL of NAD^+ (final concentration 1 mM) as stock solution in KPi (100 mM, pH 8). To this mixture was added NADH oxidase (NOx, 7 µL from a 398 µM stock solution) and (*S*)-1-phenylpropan-2-ol **1a** (1.45 µL, 20 mM). The reactions were shaken 30 °C in an incubator (170 rpm) and quenched after 21 hours with KOH (100 µL, 10 M). The water layer was extracted with EtOAc (2 x 400 µL) and the organic layer was dried over $MgSO_4$ and injected on GC (see Analytics, method: DB1701-60m-A).

Table S1. Activity tests of the expressed AA-ADH from *Aromatoleum aromaticum*. Number of individual experiments (n) is given for each reaction. Reaction conditions: disrupted cell extract (450 µL from 0.5 g cell pellet in 3 mL buffer) in KPi (100 mM, pH 8), NAD^+ (1 mM), NOx (purified, 7 µL from 398 µM stock), and (*S*)-1-phenylpropan-2-ol (**1a**, 20 mM), 21 h, 30 °C.



Entry	Expression T [°C]	n	Conversion [% 1b]
1	25	2	97
2	30	2	83
3	37	2	74

5. Initial experiments of co-immobilization of AmDH and ADH on EziG Fe-Amber ion affinity beads

Purified recombinant proteins Ch1-AmDH (29 µL from 35 mg mL^{-1} stock solution, 23 nmol) and AA-ADH (67 µL from 15 mg mL^{-1} stock solution, 35 nmol) were combined in Tris/HCl (1 mL, 50 mM, pH 8) at 4 °C. 40 mg of EziG Fe-Amber metal ion affinity beads (50 mg $g_{enzyme\ loading}^{-1}$) were suspended in the enzyme solution and the suspension was incubated for 2 hours at 4 °C (120 rpm). Bradford assay (980 µL of ready to use Bradford solution plus 20 µL sample, measuring absorbance at λ of 595 nm) was used to monitor the immobilization process over the time as well as to determine the final yield of immobilization (see equation below). The co-immobilized enzymes were collected by centrifugation (12000 rpm, 4 °C, 2 min.) and the remaining buffer solution was discarded. The immobilized enzymes were either stored at 4 °C or used directly in biotransformations.

The biotransformations were performed in NH_4Cl/NH_4OH buffer (0.5 mL, 2 M, pH 8.7) with 1 mM NAD^+ and (*S*)-**1a** (20 mM, 1.37 µL) as a substrate. The reactions were incubated for 48 hours at 30 °C, quenched with KOH (100 µL, 10 M), and extracted with EtOAc (2 x 500 µL). The organic layer was dried over $MgSO_4$ and injected on GC-FID (see Analytics, method: DB1701-60m-A).

Equation used for the determination of the percentage of yield of immobilization (based on Bradford assay):

$$Immobilization\ [\%] = \frac{A_{595\ before\ immobil.} - A_{595\ after\ immobil.}}{A_{595\ before\ immobil.}} * 100\ \%$$

where;

$A_{595\ before\ immobil.}$ is the absorbance related to the enzyme solution before immobilization measured at λ of 595 nm

$A_{595\ after\ immobil.}$ is the absorbance at λ of 595 nm measured on the supernatant after removal of the beads possessing the co-immobilized enzymes.

6. Optimization of enzyme molar ratio on carrier beads

Immobilization and biotransformations with purified Ch1-AmDH and AA-ADH on EziG Fe-Amber metal ion affinity beads were performed as described in section 5. The molar ratio of AmDH and ADH were varied according to Table S2 and Table S3.

In the first experiment the amount of ADH (35 nmol) was kept constant and the amount of AmDH (2.3-46 nmol) was varied. The enzyme loading (50 $mg_{enzymes}$ g_{beads}^{-1}) in each reaction was maintained by adding different amounts of bead material to each reaction vial. Full immobilization was achieved in all cases and the beads were used directly in biotransformations. Lower amounts of AmDH resulted in lower conversions of **1a** with an optimal enzyme molar ratio of 11 nmol AmDH and 35 nmol of ADH (Table S2).

In the second experiment the amount of AmDH (23 nmol) was kept constant and the amount of ADH (3.5-70 nmol) was varied. Conversions up to 94 % were achieved in 48 hours (Table S3).

Table S2. Enzyme ratio optimization of co-immobilized AmDH and ADH on EziG Fe-Amber metal ion affinity beads. Number of individual experiments (n) is given for each reaction. Immobilization conditions: Tris/HCl (50 mM, pH 8, 1 mL), EziG Fe-Amber (50 $mg_{enzymes}$ g_{beads}^{-1}), Ch1-AmDH (2.3-46 nmol), AA-ADH (35 nmol), 4 °C, 120 rpm, 2-3 h. Reaction conditions: ammonium chloride (2 M, pH 8.7, 0.5 mL), NAD^+ (1 mM), (*S*)-**1a** (20 mM), 30 °C, 170 rpm, 48 hours.

Entry	AmDH [nmol]	ADH [nmol]	n	Immobilization [%][a]	Conversion [% (*R*)-1c][b]	1b [%][b]	(*S*)-1a [%][b]	Average TTN_{AmDH}[b,c]	Average TTN_{ADH}[b,d]
1	2.3	35	3	> 99	5.6±2	8.8±0.9	85.5±2.9	246±88	16±5.8
2	3.4	35	3	> 99	14.4±5.4	8.8±0.8	76.9±6.2	417±158	41±16
3	5.7	35	3	> 99	32.1±3.4	8.4±0.1	59.5±3.5	560±60	92±9.8
4	11	35	3	> 99	90.4±0.1	4.5±0.1	5.1±0.1	788±1.2	259±0.4
5	23	35	3	>99	92.1±0.2	4.3±0.3	3.6±0.1	401±1	264±0.7
6	34	35	3	> 99	92.1±0.4	4.4±0.3	3.5±0.2	268±1.1	264±1
7	46	35	3	> 99	91.3±0.3	5.0±0.2	3.7±0.2	199±0.8	262±1

[a] Immobilization determined by Bradford assay (UV-VIS absorption at λ 595 nm). [b] Average values reported with standard deviation. [c] µmol of converted substrate per µmol of immobilized AmDH. [d] µmol of converted substrate per µmol of immobilized ADH.

Table S3.Enzyme ratio optimization of co-immobilized AmDH and ADH on EziG Fe-Amber metal ion affinity beads. Number of individual experiments (n) is given for each reaction. Immobilization conditions: Tris/HCl (50 mM, pH 8, 1 mL), EziG Fe Amber (50 $mg_{enzymes}$ g_{beads}^{-1}), Ch1-AmDH (23 nmol), AA-ADH (3.5-70 nmol), 4 °C, 120 rpm, 2-3 h. Reaction conditions: ammonium chloride (2 M, pH 8.7, 0.5 mL), NAD^+ (1 mM), (*S*)-**1a** (20 mM), 30 °C, 170 rpm, 48 hours.

Entry	AmDH [nmol]	ADH [nmol]	n	Immobilization [%][a]	Conversion [% (*R*)-1c][b]	1b [%][b]	(*S*)-1a [%][b]	Average TTN_{AmDH}[b,c]	Average TTN_{ADH}[b,d]
1	23	3.5	3	> 99	93.7±0.4	3.8±0.0	2.5±0.4	408±1.8	2688±12
2	23	5.2	3	> 99	93.7±0.2	3.9±0.3	2.5±0.2	408±0.7	1791±3
3	23	8.7	3	> 99	93.5±0.0	3.8±0.3	2.8±0.3	407±0.2	1073±0.5
4	23	17	3	> 99	92.9±0.2	4.1±0.3	3.1±0.2	405±1	533±1.3
5	23	35	3	> 99	92.1±0.2	4.3±0.3	3.6±0.1	401±1	264±0.7
6	23	52	3	> 99	91.7±0.4	4.3±0.1	4.0±0.4	399±1.5	175±0.7
7	23	70	3	> 99	91.2±0.2	4.4±0.1	4.4±0.2	397±0.7	131±0.2

[a] Immobilization determined by Bradford assay (UV-VIS absorption at λ 595 nm). [b] Average values reported with standard deviation. [c] µmol of converted substrate per µmol of immobilized AmDH. [d] µmol of converted substrate per µmol of immobilized ADH.

$$TTN_{AmDH} = \frac{\mu mol\ converted\ substrate}{\mu mol\ of\ AmDH\ in\ reaction}$$

$$TTN_{ADH} = \frac{\mu mol\ converted\ substrate}{\mu mol\ of\ ADH\ in\ reaction}$$

Example from Table S3, entry 1:

$$converted\ substrate\ [\mu mol] = \frac{93.7\ \%\ conv.}{100} * 20\ mM * 0.5\ mL = 9.37\ \mu mol$$

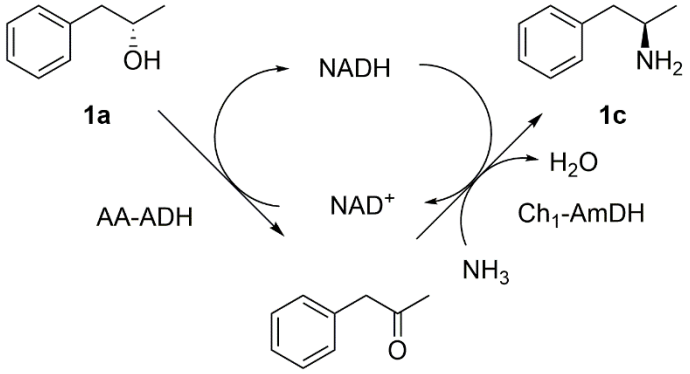
$$TTN_{AmDH} = \frac{9.37\ \mu mol}{23 * 10^{-3}\ \mu mol} = 408$$

$$TTN_{ADH} = \frac{9.37\ \mu mol}{3.485 * 10^{-3}\ \mu mol} = 2688$$

7. Control experiments with non-immobilized AmDH and ADH in solution

The biotransformations were performed with purified Ch1-AmDH and purified AA-ADH in NH_4Cl/NH_4OH buffer (0.5 mL, 2 M, pH 8.7), NAD^+ (1 mM), and (*S*)-**1a** (20 mM, 1.37 μL) as a substrate. The reactions were incubated for 48 hours at 30 °C, quenched with KOH (100 μL, 10 M), and extracted with EtOAc (2 x 500 μL). The organic layer was dried over $MgSO_4$ and injected on GC-FID (see Analytics, method DB1701-60m-A).

Table S4. Enzymes ratio control experiments of non-immobilized AmDH and ADH. Number of individual experiments (n) is given for each reaction. Reaction conditions: ammonium chloride (2 M, pH 8.7, 0.5 mL), NAD^+ (1 mM), Ch1-AmDH (2.3-46 nmol), AA-ADH (3.5-70 nmol), 20 mM (*S*)-**1a**, 30 °C, 170 rpm, 48 hours.



1b

entry	AmDH [nmol]	n	ADH [nmol]	Conversion [% (*R*)-1c]	1b [%]	(*S*)-1a [%]	TTN_{AmDH}	TTN_{ADH}
1	23	1	3.5	92.2	5.7	2.0	402	2646
2	23	1	5.2	92.6	5.2	2.1	404	1772
3	23	1	8.7	93.0	4.8	2.2	405	1067
4	23	1	17	93.0	4.4	2.6	405	533
5	23	1	35	92.4	4.7	2.9	402	265
6	23	1	52	92.8	4.5	2.7	404	178
7	23	1	70	92.7	4.9	2.5	404	133
8	2.3	1	35	2.8	8.7	88.5	123	8
9	3.4	1	35	6.6	9.0	84.4	192	19
10	5.7	1	35	22.7	8.5	68.9	395	65
11	11	1	35	92.1	3.5	4.5	802	264
12	34	1	35	93.4	3.3	3.3	271	268
13	46	1	35	93.1	3.5	3.4	203	267

8. Experiments on activity vs. substrate concentration with co-immobilized AmDH and ADH on EziG Fe-Amber metal ion affinity beads

Immobilization and biotransformations with purified Ch1-AmDH and AA-ADH on EziG Fe Amber affinity beads were performed as described in sections 5 and 6.

Reactions were performed with two sets of immobilized preparations. In the first set of experiments, 11 nmol of AmDH and 3.5 nmol of ADH were co-immobilized on the beads and used for each biocatalytic reaction. The reactions were run under our standard conditions at different substrate concentrations. The conversions were not higher than 40 % with this system and the productivity of the system decreased at increased substrate concentrations (Figure S2 and Table S5).

In the second set of experiments, 23 nmol of AmDH and 3.5 nmol of ADH were co-immobilized on the beads and used for each biocatalytic reaction. The reactions were run under our standard conditions at different substrate concentrations (Figure 1C in the main manuscript and Table S6).

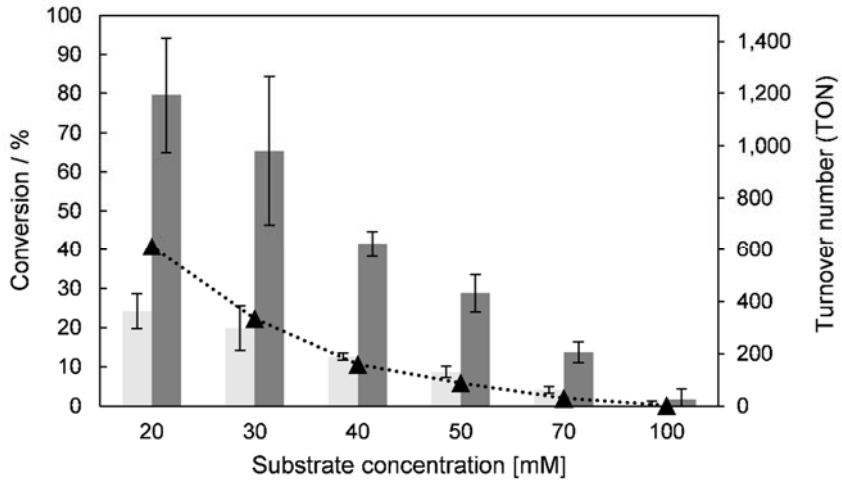


Figure S2. First set of experiments on activity of co-immobilized AmDH and ADH on EziG Fe-Amber metal ion affinity beads at varied substrate concentration. Conversion of (S)-**1a** to (R)-**1c** (black triangles), TTN_{ADH} (dark grey bars), and TTN_{AmDH} (light grey bars) are shown. Immobilization conditions: Tris/HCl (50 mM, pH 8, 1 mL), EziG Fe-Amber (50 $mg_{enzymes}$ g_{beads}^{-1}), Ch1-AmDH (11 nmol), AA-ADH (3.5 nmol), 4 °C, 120 rpm, 2-3 h. The co-immobilized enzymes were used directly in the biotransformation. Reaction conditions: ammonium chloride (2 M, pH 8.7, 0.5 mL), NAD^+ (1 mM), (*S*)-**1a** (20-100 mM), 30 °C, 170 rpm, 48 hours.

Table S5. Activity of co-immobilized AmDH and ADH on EziG Fe-Amber metal ion affinity beads at varied substrate concentration. Number of individual experiments (n) is given for each reaction. Immobilization conditions: Tris/HCl (50 mM, pH 8, 1 mL), EziG Fe-Amber (50 $mg_{enzymes}$ g_{beads}^{-1}), Ch1-AmDH (11 nmol), AA-ADH (3.5 nmol), 4 °C, 120 rpm, 2-3 h. Reaction conditions: ammonium chloride (2 M, pH 8.7, 0.5 mL), NAD^+ (1 mM), (*S*)-**1a** (20-100 mM), 30 °C, 170 rpm, 48 hours.

entry	AmDH [nmol]	ADH [nmol]	n	Substrate concentration [mM]	Conversion [% (*R*)-1c][a]	1b [%][a]	(*S*)-1a [%][a]	Average TTN_{AmDH}[a,b]	Average TTN_{ADH}[a,c]
1	11	3.5	3	20	41±8	7±0.1	52±8	363±67	1,194±219
2	11	3.5	3	30	22±7	6±0.1	72±7	298±86	980±285
3	11	3.5	3	40	11±1	5±0.1	84±1	189±15	623±48
4	11	3.5	3	50	6±1	5±0.3	89±1	131±22	431±71
5	11	3.5	3	70	2±0.4	4±0.0	94±0.4	62±12	205±40
6	11	3.5	3	100	0.2±0.3	4±0.0	96±0.2	7±13	24±42

[a] Average values reported with standard deviation. [b] µmol of converted substrate per µmol of immobilized AmDH. [c] µmol of converted substrate per µmol of immobilized ADH.

Table S6. Activity of co-immobilized AmDH and ADH on metal ion affinity beads at increased substrate concentration. Number of individual experiments (n) is given for each reaction. Immobilization conditions: Tris/HCl (50 mM, pH 8, 1 mL), EziG Fe Amber (50 $mg_{enzymes}$ g_{beads}^{-1}), Ch1-AmDH (23 nmol), AA-ADH (3.5 nmol), 4 °C, 120 rpm, 2-3 h. Reaction conditions: ammonium chloride (2 M, pH 8.7, 0.5 mL), 1 mM NAD^+, (*S*)-**1a** (20-100 mM), 30 °C, 170 rpm, 48 hours.

entry	AmDH [nmol]	ADH [nmol]	n	Substrate concentration [mM]	Conversion [% (*R*)-1c][a]	1b [%][a]	(*S*)-1a [%][a]	Average TTN_{AmDH}[a,b]	Average TTN_{ADH}[a,c]
1	23	3.5	3	20	91±3	5±0.3	4±2	406±11	2676±72
2	23	3.5	3	30	81±7	5±0.1	15±6	538±43	3541±285
3	23	3.5	3	40	59±3	4±0.2	36±3	527±30	3471±196
4	23	3.5	3	50	46±4	4±0.1	50±4	508±41	3346±271
5	23	3.5	3	70	16±3	4±0.0	80±3	255±53	1681±351
6	23	3.5	3	100	5±1	3±0.2	91±1	119±21	783±137

[a] Average values reported with standard deviation. [b] µmol of converted substrate per µmol of immobilized AmDH. [c] µmol of converted substrate per µmol of immobilized ADH.

9. Activity at increased enzyme loading with co-immobilized AmDH and ADH on metal ion affinity beads

Immobilization and biotransformations with purified Ch1-AmDH and AA-ADH on EziG Fe-Amber metal ion affinity beads were performed as described in sections 5 and 6.

Each reaction was performed with the same amount of total enzyme by varying the amount of beads added to each reaction vial. An enzyme molar ratio of 23 nmol AmDH and 8.7 nmol ADH was used and the substrate concentration was fixed at 20 mM.

Table S7. Activity of co-immobilized AmDH and ADH on EziG Fe-Amber metal ion affinity beads at varied enzyme loading. Number of individual experiments (n) is given for each reaction. Immobilization conditions: Tris/HCl (50 mM, pH 8, 1 mL), EziG Fe Amber (10-100 $mg_{enzymes}$ g $_{beads}^{-1}$), Ch1-AmDH (23 nmol), AA-ADH (8.7 nmol), 4 °C, 120 rpm, 2-3 h. Reaction conditions: ammonium chloride (2 M, pH 8.7, 0.5 mL), NAD^+ (1 mM), (*S*)-**1a** (20 mM), 30 °C, 170 rpm, 48 hours.

entry	Enzyme loading [mg/g beads]	n	Conversion [% (*R*)-1c][a]	1b [%][a]	(*S*)-1a [%][a]	Average TTN_{AmDH}[a,b]	Average TTN_{ADH}[a,c]
1	10	3	89±0.4	7±0.3	4±0.1	387±1.6	1546±6.5
2	20	3	92±0.1	5±0.1	3±0.0	402±0.6	1607±2.3
3	30	3	93±0.1	4±0.1	3±0.0	404±0.6	1618±2.5
4	50	3	93±0.2	4±0.2	2±0.0	406±0.8	1626±3.3
5	70	3	93±0.1	4±0.1	2±0.0	407±0.4	1627±1.7
6	100	3	94±0.1	4±0.1	2±0.0	407±0.4	1629±1.4

[a] Average values reported with standard deviation. [b] µmol of converted substrate per µmol of immobilized AmDH. [c] µmol of converted substrate per µmol of immobilized ADH.

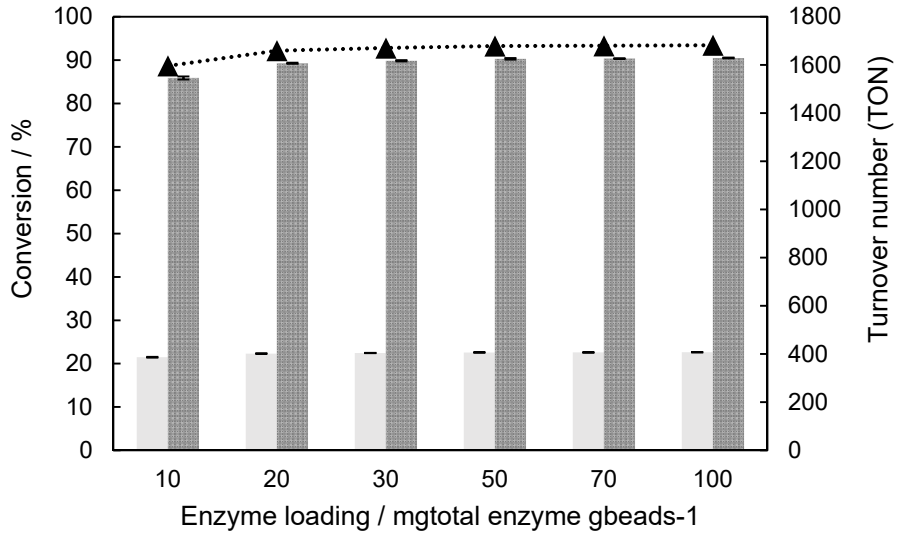


Figure S3. Influence of enzyme loading on EziG Fe-Amber beads (10-100 mg $_{total\ enzyme}$ g $_{beads}^{-1}$; AA-ADH/Ch1-AmDH 8.7, 23 molar ratio). Conversion of (*S*)-**1a** to (*R*)-**1c** (black triangles), TON_{ADH} (dark grey bars), and TON_{AmDH} (light grey bars) are shown. Immobilization conditions: Tris/HCl (50 mM, pH 8, 1 mL), 4 °C, 120 rpm, 2-3 h. General reaction conditions: NH_4Cl buffer (2 M, pH 8.7, 0.5 mL), NAD+ (1 mM), at 30 °C, 170 rpm, 48 hours. The data represents the average of three experiments and error bars the standard deviation (n=3).

10. Preparative scale reaction with co-immobilized AmDH and ADH.

Purified recombinant proteins Ch1-AmDH (800 µL from 50 mg mL^{-1} stock solution, 920 nmol) and AA-ADH (101 µL from 90 mg mL^{-1} stock solution, 350 nmol) were combined in Tris/HCl (20 mL, 50 mM, pH 8) at 4 °C. 500 mg of EziG Fe-Amber metal ion affinity beads (100 mg $g_{\text{enzyme loading}}^{-1}$) was suspended in the enzyme solution and the suspension was incubated for 3 hours at 4 °C with mild shaking (90 rpm). Bradford assay (980 µL of ready to use Bradford solution plus 20 µL sample, measuring absorbance at λ of 595 nm) was used to monitor the immobilization process over the time as well as to determine the final yield of immobilization. The co-immobilized enzymes were collected by sedimentation on ice (30 min.) and the remaining buffer solution was discarded. The immobilized enzymes were used directly in the preparative scale reaction.

The preparative scale reaction was performed in a 50 mL round-bottom flask in NH_4Cl/NH_4OH buffer (20 mL, 2 M, pH 8.7) with 1 mM NAD^+ and (*S*)-**1a** (20 mM, 52.4 µL, 0.39 mmol) as a substrate. The reaction was incubated for 24 hours at 30 °C. Work-up of the reaction was performed by separating the beads from the reaction mixture by pipetting. Acidification (pH 2, universal pH indication paper) of the water layer and extraction of the remaining alcohol and ketone intermediate was performed (EtOAc, 1 x 15 mL). The water layer was basified (pH 12, universal pH indication paper) and extracted three times with EtOAc (3 x 15 mL). The organic layer was dried over $MgSO_4$ and the solvent evaporated to yield a slightly yellow oil (42 mg, 0.31 mmol, 80% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) is reported in Figure 4.

A small sample of the reaction after 24 hours (0.5 mL) was quenched with KOH (100 µL, 10 M), and extracted with EtOAc (2 x 500 µL). The organic layer was dried over $MgSO_4$ and injected on GC-FID (see Analytics, method: DB1701-60m-A, 93% conversion). The enantiomeric excess of the amine product (*R*)-**1c** was determined after derivatization to acetamido. Derivatization of the samples was performed by adding 4-dimethylaminopyridine into acetic anhydride (40 µL of 50 mg mL−1 stock solution). The samples were shaken in an incubator at RT for 30 minutes. Afterwards, water (300 µL) was added and the samples were shaken for an additional 30 minutes. After centrifugation, the organic layer was dried with $MgSO_4$. Enantiomeric excess was determined by GC with a Variant Chiracel DEX-CB column.

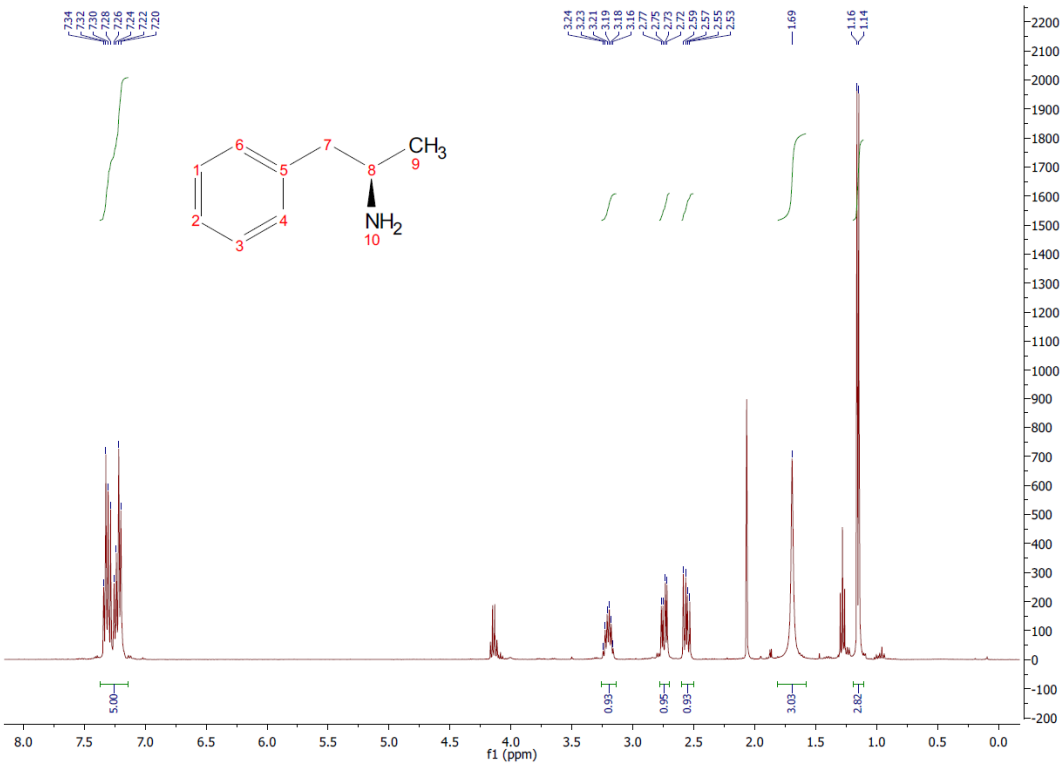


Figure 4. ^{1}H NMR of (*R*)-1-phenyl-2-aminopropane (400 MHz, Chloroform-d).

11. Recycling experiments with co-immobilized AmDH and ADH on metal ion affinity beads

Immobilization and biotransformations with purified Ch1-AmDH and AA-ADH on EziG Fe Amber affinity beads were performed as described in sections 5 and 6.

Recycling of the co-immobilized enzyme preparation was performed as follows. Each reaction cycle was performed for 24 h with 20 mM of (*S*)-**1a**. The reaction mixture was separated from beads by centrifugation (2 min, 10 000 rpm) and extracted as previously described. The co-immobilized enzymes on the beads were then reused directly by adding fresh ammonium chloride buffer (2M, pH 8.7, 1 mM NAD^+) and 20 mM of (*S*)-**1a**. This procedure was repeated for 5 consecutive cycles (in triplicate).

Table S8. Recycling experiments of co-immobilized AmDH and ADH on EziG Fe-Amber metal ion affinity beads. Number of individual experiments (n) is given for each reaction. Immobilization conditions: Tris/HCl (50 mM, pH 8, 1 mL), EziG Fe-Amber (50 $mg_{enzymes}$ g_{beads}^{-1}), Ch1-AmDH (23 nmol), AA-ADH (8.7 nmol), 4 °C, 120 rpm, 2-3 h. Reaction conditions: ammonium chloride (2 M, pH 8.7, 0.5 mL), NAD^+ (1 mM), (*S*)-**1a** (20 mM), 30 °C, 170 rpm, 48 hours.

entry	# cycles [24 hours]	n	Conversion [% (*R*)-1c][a]	Conversion [mM (*R*)-1c] [a]	Average TON_{AmDH}[a,b]	Average TON_{ADH}[a,c]
1	1	3	92±0.2	18±0.0	401±0.6	1604±3
2	2	3	87±0.2	18±0.0	381±0.9	1523±4
3	3	3	44±9	9±2	190±39	762±156
4	4	3	13±2	3±0.5	57±10	228±41
5	5	3	5±1v	1±0.2	20±4	78±16

[a] Average values reported with standard deviation. [b] µmol of converted substrate per µmol of immobilized AmDH. [c] µmol of converted substrate per µmol of immobilized ADH.

12. Additional detailed discussion

a) Comparison of different immobilized enzyme-systems for the synthesis of α-chiral amines

The herein described dual-enzyme hydrogen-borrowing amination of alcohols (with co-immobilized dehydrogenases) offers several advantages compared to other established methods:

1) It is true that transaminases have been successfully immobilized on different matrices, including metal-ion affinity beads as EziG.[3] However, the bio-amination of alcohols using transaminase (even with co-immobilized enzymes) would require a total of three enzymes and, at least, stoichiometric alanine as sacrificial amine donor.[4] Then, 2 cofactors are required (PLP and NAD) instead of the sole NAD. Hence, in general, more waste is generated. Furthermore, conversions (and TONs) with the transaminase-based system are significantly lower than the ones for the ADH-AmDH system.

2) In the case of immobilization of lipases for α-chiral amine synthesis, kinetic resolution or dynamic kinetic resolution of racemic amines is applied. First, these enzymes do not enable a direct conversion of alcohols into optically active amines. Secondly, resolution techniques of racemates generate in general more waste than direct asymmetric synthesis from precursors.

b) Comparing the co-immobilization of ADH-AmDH on EziGs and other carrier materials for carrying out the hydrogen-borrowing amination (pros and cons)

In relation to the comparison between different immobilization techniques, existing methods are effective but not general. It is very often required to test and optimize several parameters. Moreover, a pre-purification step is mandatory if a pure (o partially purified) enzyme is required for the particular application. This point is of particular importance for redox-neutral biocatalytic processes (like a hydrogen-borrowing amination), wherein cross-redox activities due to enzyme impurities must be avoided; otherwise, the system may become unbalanced. In this context, metal-ion affinity immobilization allows for a one-step purification and immobilization. Hence, it becomes more practical and convenient than other (covalent and non-covalent) non-selective immobilization methods.

EziGs are glass particles with size-controlled macro- or meso-pore (CPG) and can be coated with organic, inorganic as well as inorganic-organic hybrid materials. The advantages of using metal-ion derivatized CPGs (like EziGs) are: [3, 5]

- the wider applicability (*i.e.* organic solvents and aqueous buffer at pH < 10) as the carrier material is chemically and dimensionally stable under these conditions;

- the low solution flow resistance of the interconnecting pore structure, which facilitates mass transfers of substrate and product (very important in heterogeneous catalysis);

- the shorter time required for immobilization (under optimized conditions 20 – 30 minutes compared to many hours);

- the possibility to (co)-immobilized with excellent selectivity the desired enzyme(s) from a crude mixtures (e.g. cell lysate);

- the properties of retaining quantitative enzyme activity upon immobilization (as observed with different classes of enzymes;

- the general applicability of the immobilization method to diverse families of enzymes because the terminal His-tag of the enzyme is the only part virtually interacting with the matrix.

- when enzyme activity is lost, the EziGs can be regenerated by washing with EDTA and loading of fresh metal ions (e.g. Fe(III), Co(II))

EziG also performs better if compared to other porous carriers (e.g. Accurel ®) in terms of time for immobilization and volumetric productivity of the immobilized catalyst after swelling. [3] A cheaper alternative to EziG might be the derivatization of CPG with amine-containing derivatives (*e.g.* aminopropyl-CPG). Then, the enzyme is immobilized to the matrix using glutaraldehyde as linking reagent between the amine group present on the enzyme surface (e.g. lysine residues) and the amine moiety on the CPG particles. The drawback is the non-specific (i.e. random) binding, which leads to reduce or even loss of the activity. Furthermore, as the binding is non-selective, pre-purification of the enzyme(s) might be required for some applications.

Finally, other cheaper carrier materials for enzyme immobilization are organic polymer (plastic) material, based on monomers such as styrene, ethylene, propylene, acrylate and derivatives thereof. These polymers are functionalized with functional groups such as amines, aldehydes, epoxy, etc. The functionalized plastic can be prepared as porous material. The drawback of these materials as carrier is their swelling, which occur during the reaction. In contrast, CPG coated with organic polymer (hybrid-CPG) are incompressible and non-swelling (justifying the higher manufacturing costs for some applications). Another difference between hybrid-CPG and organic polymers as carrier material is the more elevated resistance of the former to higher temperatures, which may be required to accelerate the kinetics of a particular biocatalytic reactions.

13. Analytics

GC analysis was performed according to previously described procedures.[2]

The conversion for the hydrogen-borrowing amination of alcohols was determined by GC using a 7890A GC system (Agilent Technologies), equipped with a FID detector using H_2 as carrier gas with a DB-1701 column from Agilent (30 m or 60 m, 250 µm, 0.25 µm).

DB1701-30m-A: constant pressure 13.5 psi, T injector 300 °C, split ratio 40:1, T initial 80 °C, hold 6.5 min;gradient 10 °C/min up to 160 °C, hold 5 min; gradient 20 °C/min up to 200 °C, hold 2 min; gradient 20°C/min up to 280 °C, hold 1 min.

DB1701-30m-B: constant pressure 13.5 psi, T injector 300 °C, split ratio 40:1, T initial 60 °C, hold 6.5 min;gradient 20 °C/min up to 100 °C, hold 1 min, gradient 20 °C/min up to 280 °C, hold 1 min.

DB1701-60m-A: constant pressure 13.5 psi, T injector 300 °C, split ratio 40:1, T initial 80 °C, hold 6.5 min, gradient 5 °C/min up to 160 °C, hold 2 min; gradient 20 °C/min up to 280 °C, hold 1 min.

DEX-CB: constant flow 1.4 mL/min, T injector 250 °C, split ratio 40:1, T initial 100 °C, hold 2 min; gradient 1 °C/min up to130 °C, hold 5 min; gradient 10 °C/min up to 170 °C, hold 10 min.; gradient 10 °C/min up to 180 °C, hold 1 min.

Table S9. GC-FID retention time of substrates and products in this study.

entry	Compound	GC method	Retention time [min]
1	(***S***)-**1a**	DB1701-60m-A	20.4
2	**1b**	DB1701-60m-A	20.5
3	(***R***)-**1c**	DB1701-60m-A	18.7
4	(***R***)-**1c**	DEX-CB	33.7
5	(***S***)-**2a**	DB1701-60m-A	22.1

6	**2b**	DB1701-60m-A	22.0
7	**(*R*)-2c**	DB1701-60m-A	20.1
8	**(*S*)-3a**	DB1701-60m-A	25.3
9	**3b**	DB1701-60m-A	24.2
10	**(*R*)-3c**	DB1701-60m-A	24.5
11	**(*S*)-4a**	DB1701-30m-B	5.6
12	**4b**	DB1701-30m-B	5.1
13	**(*R*)-4c**	DB1701-30m-B	4.0
14	**(*S*)-5a**	DB1701-30m-A	5.5
15	**5b**	DB1701-30m-A	5.2
16	**(*R*)-5c**	DB1701-30m-A	4.2

14. References

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