Supporting information for:

Highly Efficient Production of Chiral Amines in Batch and Continuous Flow by Immobilized ω-Transaminases on Controlled Porosity Glass Metal-Ion Affinity Carrier

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1. Abbreviations

AsR-ωTA	(*R*)-selective ω-transaminase from *Arthrobacter* species
Cv-ωTA	(*S*)-selective ω-transaminase from *Chromobacterium violaceum*
CPG	controlled porosity glass
DMAP	dimethylaminopyridine
DMSO	dimethylsulfoxide
EtOAc	ethyl acetate
EziG^3-AsR	AsR-ωTA immobilized on EziG^3 Fe Amber
EziG^3-Cv	Cv-ωTA immobilized on EziG^3 Fe Amber
GC	gas chromatography
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
KOH	potassium hydroxide
$MgSO_4$	magnesium sulfate
MOPS	3-(*N*-morpholino)propanesulfonic acid
n.a.	not applicable
n.d.	not determined
PLP	pyridoxal 5'-phosphate
rac-α-MBA	*rac*-α-methylbenzylamine
RT	room temperature
SEM	scanning electron microscopy
TOF	turnover frequency
TON	turnover number

2. General information

2.1 Equipment

For the immobilization of enzymes on carrier material, a C-star orbital shaker no. 12846016 (Thermo Fisher Scientific, UK) was used. Biorad protein assay dye reagent concentrate was purchased from Carl Roth (Karlsruhe, Germany). Biotransformations were performed in an Eppendorf Thermomixer compact 5350 (Germany). Continuous flow experiments were performed with a Dionex P680 HPLC pump unit (Thermo Fischer Scientific, UK). Scanning electron microscopy (SEM) was performed using a FEI Verios 460 scanning electron microscope (Amolf, University of Amsterdam, The Netherlands).

2.2 Analytics

Conversions were determined by GC using a 7890A GC system (Agilent Technologies), equipped with a FID detector using H_2 as carrier gas with a DB1701 column from Agilent (30 m, 250 µm, 0.25 µm). The enantiomeric excess of derivatized amines was measured using a ChiraSil DEX-CB column from Agilent (25 m, 320 µm, 0.25 µm).

DB1701 30m method: constant pressure 6.9 psi, T injector 250 °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.

ChiraSil DEX-CB method: constant flow 1.4 mL/min, T injector 250 °C, split ratio 20:1, T initial 100 °C, hold 2 min; gradient 1 °C/min up to 130 °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 S 1. GC retention time of reference compounds.

Entry	Compound	Retention time [min]	GC column
1	*rac*-α-MBA	10.9	DB1701 30m
2	Acetophenone	11.7	DB1701 30m
3	(*S*)-α-MBA	31.6	ChiraSil DEX-CB
4	(*R*)-α-MBA	32.3	ChiraSil DEX-CB

3. Calculations and terminology

The data from the immobilization studies were interpreted using the following calculations to define parameters such as immobilization yield, immobilized enzyme activity, turnover frequency (TOF), and turnover number (TON):

3.1 Immobilization yield

In order to determine how much of the enzyme is immobilized during the process, a Bradford assay (UV absorption at 595 nm, section 6.2) was performed before ($A_{595\ initial}$) and after the immobilization process ($A_{595\ final}$) for calculating the amount of enzyme bound to the beads, i.e. the immobilization yield (Equation 1).

Equation 1

$$immobilization\ yield\ [\%] = \frac{\left(A_{595\ final} - A_{595\ initial}\right)}{A_{595\ initial}} \times 100\%$$

3.2 Turnover number (TON)

After immobilization, the immobilized enzyme was tested for its activity. The conversion of substrate per amount of immobilized enzyme in a given time gives the turnover number (TON) as is defined below in Equation 2.

Equation 2

$$Turnover\ of\ immobil.enz.(TON) = \frac{\left(\frac{Conv.of\ substrate\ [\%]}{100}\right) \times Added\ substrate\ [\mu mol]}{Amount\ of\ immobil.enzyme\ [\mu mol]}$$

In relation to Equation 2, the amount of immobilized enzyme can be determined by taking into account the amount of enzyme that was added to the carrier beads (before immobilization). Using the immobilization yield, as reported in Equation 1, it is possible to calculate the quantity of enzyme that remained bound to the beads at the end of the immobilization process. Then, it is possible to calculate the exact quantity of enzyme used in each reaction from the amount of beads that was employed (with the enzyme immobilized). Details are given in Equation 3.

Equation 3

$$Amount\ of\ immobil.enzyme\ [\mu mol] = \frac{\left(\frac{immobil.yield\ [\%]}{100} \times mass\ initial\ added\ enzyme\ \ [\mu g]\right)}{Enzyme\ molecular\ mass\ [\frac{\mu g}{\mu mol}]}$$

In which the mass initial added enzyme [µg] is the amount of enzyme added to the beads at the beginning of the immobilization process.

3.3 Immobilized enzyme activity or turnover frequency (TOF)

When the turnover number obtained from the activity tests is plotted against time, the slope of this graph (considering the linear range) indicates the immobilized enzyme activity or turnover frequency (TOF). This is shown in Equation 4.

Equation 4

$$Immobilized\ enzyme\ activity\ (or\ TOF) = \frac{TON}{time\ [min]}$$

3.4 Reaction rate in flow reactors

In flow reactors, several parameters relate to the reaction rate. An important parameter is space velocity (SV, in units of reciprocal time), which is defined by the volumetric flow rate of the reactant stream (V_o, specified at the inlet conditions of temperature and pressure with zero conversion), and the catalyst volume (V_c).[1] Often catalyst volume (V_c) is equally related to the reactor volume (V_r), which depends on the packing density of the catalyst particles.

Equation 5

$$SV\ (space\ velocity) = \frac{V_o}{V_r}$$

Space time (τ, in units of time) is the inverse of space velocity and it gives the time required to process one reactor volume:

Equation 6

$$\tau\ (space\ time) = \frac{1}{SV} = \frac{V_r}{V_o}$$

The space time yield (STY) refers to the quantity of product produced per quantity of catalyst per unit time. If the catalyst is well-packed in the full reactor, then the catalyst volume (V_c) can be equated to the reactor volume (V_r).

Equation 7

$$STY\ (space\ time\ yield) = \frac{product\ produced\ [g]}{(V_r\ \times time)}$$

Calculation of space-time yield for the flow process described in the manuscript:

$$STY = \frac{5.05\ [g]}{(157\ [\mu L] \times 96\ [h] \times 10^{-6}\ [L\ \mu L^{-1}])} = 335\ g\ L^{-1}h^{-1}$$

4. Expression and purification of ω-transaminases

Expression and purification of the ω-transaminases used in this study is described in the main manuscript. The SDS-Page analysis can be seen below:

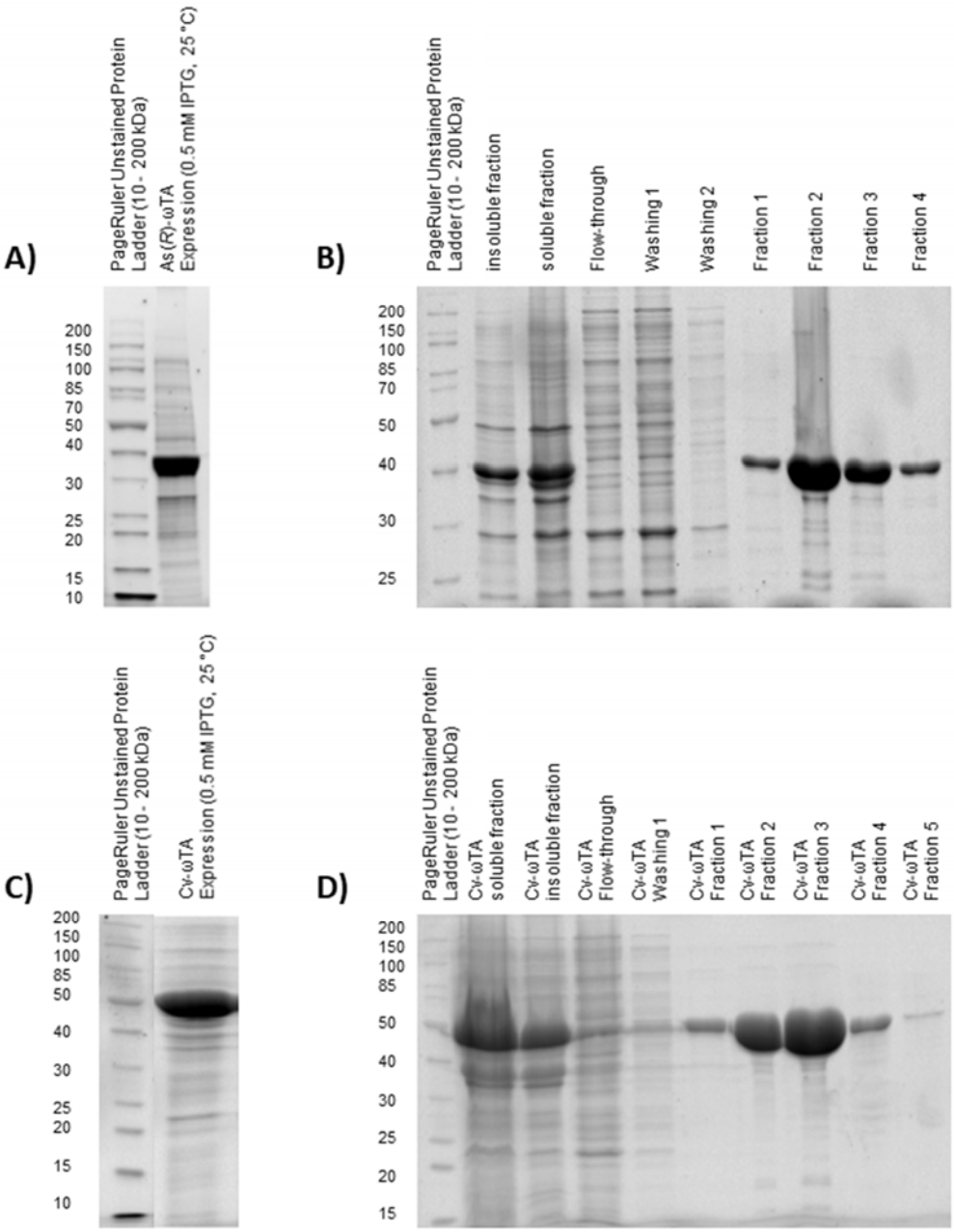

Figure S 1. SDS PAGE analysis of samples taken from A) expression of C-term His_6-tagged AsR-ωTA, B) purification of AsR-ωTA, C) expression of N-term His_6-tagged Cv-ωTA, and D) purification of Cv-ωTA.

5. EziG product specifications

Table S 2. EziG product specifications: particle size 75-125 µm (100-300 mesh), chelated Fe^{3+} >10 µmol/g.

Entry	Product	Surface	Pore diameter [nm]	Pore volume [mL/g]	Bulk density [g/mL]	pH range	pH optimum
1	EziG[1] Fe Opal	Directly derivatized hydrophilic glass	50±5	ca. 1.8	0.25-0.32	5-10	7-9
2	EziG[2] Fe Coral	Hydrophobic polymer	30±5	ca. 1.8	0.21-0.25	5-10	7-9
3	EziG[3] Fe Amber	Semi-hydrophobic copolymer	30±5	ca. 1.8	0.21-0.25	5-10	7-9

6. Immobilization of ω-transaminases

6.1 Immobilization of ω-transaminases on EziG materials

Immobilization of ω-transaminases was performed as described in Material & Methods (main manuscript).

6.2 Bradford assay

Biorad protein assay dye reagent concentrate was diluted 5 times with MilliQ water and filtered over a paper filter. The stock solution was freshly prepared before use and kept in the dark at 4 °C. Albumine calibration was performed in the standard range of 200-1000 µg mL^{-1} protein. For lower protein concentration (<25 µg mL^{-1}) the low-concentration assay of 1-20 µg mL^{-1} was used. Samples were prepared by mixing 980 µL stock solution and 20 µL protein sample (low-concentration assay: 800 µL stock and 200 µL protein sample) followed by incubation for 5-10 minutes at RT. Absorption at 595 nm was measured and plotted against the protein concentration. Diluted enzyme samples were then measured in the same fashion in order to determine their concentration.

6.3 Optimization of immobilization conditions

Immobilization of ω-transaminases on EziG carrier material was optimized for application in aqueous environment in terms of type of immobilization buffer, buffer concentration, pH and cofactor concentration. AsR-ωTA was chosen as a model enzyme. In all experiments stated in this section, only the immobilization conditions were changed. Unless stated otherwise, the reaction conditions for the kinetic resolution assay were as depicted in Scheme 1 (main manuscript).

6.3.1 Influence of the incubation time on the immobilization

AsR-ωTA was immobilized (preparative scale) on three types of EziG carrier materials. The immobilization was performed in KPi buffer (100 mM, pH 8) at 4 °C. The enzyme was diluted in 10 mL buffer supplied with PLP (1 mM) and EziG carrier material was added. The suspension was shaken (120 rpm) for 2 hours, aliquots (20 µL) of the buffer solution were taken over time and protein concentration was determined by the method of Bradford (Figure 2A, main manuscript, Table S 3). The immobilization yield was determined as described in section S3.

Table S 3. Immobilization in time for AsR-ωTA on EziG carriers. Immobilization conditions: AsR-ωTA (12 mg, 322 nmol, purified), EziG material (120 mg, 10% enzyme loading, w w^{-1}), KPi (10 mL, 100 mM, pH 8), PLP (1 mM), 4 °C, orbital shaker (90 rpm), 2 h incubation time.

Entry	Carrier material	Time [min]	A_{595}[a]	Immobilization yield [%]
1	EziG[1] Fe Opal	0	0.7	0
2		15	0.31	56
3		30	0.18	86
4		60	0.1	92
5		120	0.01	100
6	EziG[2] Fe Coral	0	0.7	0
7		15	0.34	50
8		30	0.19	72
9		60	0.09	88
10		120	0.00	100
11	EziG[3] Fe Amber	0	0.75	0
12		15	0.25	66
13		30	0.10	87
14		60	0.06	93
15		120	0.00	100

[a] Absorption at 595 nm with Bradford assay.

6.3.2 Influence of buffer concentration (ionic strength) on the immobilization

Immobilization of AsR-ωTA on analytical scale on EziG carrier materials in KPi buffer was performed with increased buffer concentration. Bradford assay was made before and after 2 hour incubation of the carrier materials with the enzyme (Figure 2B, main manuscript, Table S 4). The immobilization yield was determined as described in Calculations and terminology.

Table S 4. Immobilization of AsR-ωTA on EziG carrier materials in KPi buffer with increasing KPi buffer concentration. The immobilization yield was determined using the Bradford assay (A_{595}). Immobilization conditions: AsR-ωTA (1 mg, 27 nmol), EziG carrier material (10 mg, 10% enzyme loading, w w^{-1}), KPi buffer (1 mL, **buffer concentration varied**, pH 8), PLP (0.1 mM), 4 °C, orbital shaker (120 rpm), 2 h incubation time.

Entry	Carrier material	Buffer strength [mM]	A_{595} after 2 hours[a]	Immobilization yield [%]
1	EziG[1] Fe Opal	100	0.08	89
2		200	0.26	64
3		300	0.49	30
4		400	0.60	14
5		500	0.67	4
6		750	0.73	0
7		1000	0.75	0
8	EziG[2] Fe Coral	100	0.07	90
9		200	0.11	85
10		300	0.12	83
11		400	0.19	72
12		500	0.24	65
13		750	0.31	56
14		1000	0.30	58
15	EziG[3] Fe Amber	100	0.03	98
16		200	0.12	83
17		300	0.17	76
18		400	0.21	70
19		500	0.24	66
20		750	0.32	55
21		1000	0.32	54

[a] Absorption at 595 nm with Bradford assay.

6.3.3 Influence of external PLP in the immobilization process

Immobilization of AsR-ωTA (analytical scale) was performed on EziG carrier materials while varying the PLP concentration in the immobilization buffer. Bradford assay was made before and after 2 hour incubation of the carrier materials with the enzyme (Figure 2C, main manuscript, Table S 5). The immobilization yield was determined as is described in Calculations and terminology.

Table S 5. Immobilization of AsR-ωTA on EziG carrier materials in KPi buffer with external PLP. Immobilization conditions: AsR-ωTA (1 mg, 27 nmol), EziG material (10 mg, 10% enzyme loading, w w^{-1}), KPi (1 mL, 100 mM, pH 8), PLP (**external concentration varied**), 4 °C, orbital shaker (120 rpm), 2 h incubation time.

Entry	Carrier material	PLP concentration [mM]	Immobilization yield [%][a]
1	EziG[1] Fe Opal	0	96±1.1
2		0.1	95±1.1
3		0.3	90±0
4		0.5	86±1.1
5		0.7	83±1.1
6		1	69±0.5
7		2	42±4.3
8		3	35±6.4
9		5	35±2.1
10	EziG[2] Fe Coral	0.1	91
11		0.3	87±0.7
12		0.5	81±3.1
13		0.7	59±2
14		1	20±1.9
15		2	6±3.2
16		3	2±2.7
17		5	5±2.6
18	EziG[3] Fe Amber	0	98±1.1
19		0.1	98±1.1
20		0.3	94±0.5
21		0.5	73±3.7
22		0.7	47±12.3
23		1	36±6.4
24		2	14±2
25		3	2±2.1
26		5	2±2.1

[a] Values are displayed with absolute difference between two experiments.

6.3.4 Influence of type of buffer on the immobilization

AsR-ωTA was immobilized on EziG^3 Fe Amber (10% enzyme loading, w w^{-1}) in four different buffer types:

1) MOPS buffer (100 mM, pH 8)

2) KPi buffer (100 mM, pH 8)

3) HEPES buffer (100 mM, pH 8)

4) Tris buffer (100 mM, pH 8)

The immobilization conditions applied were as previously described. The activity of EziG^3-AsR was determined for the kinetic resolution of *rac*-α-MBA as described in *Optimized conditions for kinetic resolution with EziG-immobilized ω-transaminases* (Figure 2D, main manuscript, and Table S 6).

Table S 6. Activity of EziG^3-AsR for the kinetic resolution of *rac*-α-MBA using a variety of buffer salts for the immobilization process. Immobilization conditions: AsR-ωTA (1 mg, 27 nmol), EziG^3 Fe Amber (10 mg, 10% enzyme loading, w w^{-1}), immobilization buffer (1 mL), PLP (0.1 mM), 4 °C, orbital shaker (120 rpm), 1 h incubation time. Reaction conditions: EziG^3-AsR (10 mg, 10% enzyme loading, w w^{-1}), *rac*-α-MBA (100 mM), sodium pyruvate (50 mM), DMSO (5%, v v^{-1}), PLP (25 µM), HEPES buffer (0.5 mL reaction volume, 250 mM, pH 7), 30 °C, thermomixer (750 rpm).

Entry	Immobilization buffer	Reaction time [min]	Conversion [%][a,b]	TON[b,c]	TOF[d]
1	100 mM MOPS pH 8	0.5	13.5±0.2	250±7	364
2		1	23.1±0.7	427±23	
3		2	36.7±0.7	680±23	
4	100 mM KPi pH 8	0.5	9.7±0.5	179±10	317
5		1	17.8±0.9	330±17	
6		1.5	26.3±0.3	488±6	
7		2	33.1±1.2	613±23	
8	100 mM HEPES pH 8	0.5	7.7±0.2	142±3	272
9		1	15.5±0.7	288±14	
10		1.5	22.0±0.9	407±16	
11		2	28.9±0.6	535±11	
12	100 mM Tris pH 8	0.5	2.6±0.7	48±12	83
13		1	4.9±1.6	91±30	
14		1.5	6.9±2.2	128±40	
15		2	8.6±2.5	158±46	

[a] Conversion to acetophenone. [b] Values are depicted with standard deviation over three experiments. [c] Turnover is depicted as µmol of converted substrate per µmol of immobilized enzyme. [d] Turnover frequency or activity in units per µmol of immobilized enzyme.

6.3.5 Influence of the pH value on the immobilization

The pH of a buffer defines the ionogenic environment around the enzyme during the process of immobilization. We studied the influence of changing the pH value of the immobilization buffer on the final activity of EziG^3-AsR for the kinetic resolution of *rac*-α-MBA (Table S 7). No significant change in the activity of EziG^3-AsR was observed, when immobilizing in a pH range from 6.5 to 7.5.

Table S 7. Activity of EziG^3-AsR for the kinetic resolution of *rac*-α-MBA when immobilizing at different pH values. Immobilization conditions: AsR-ωTA (1 mg, 27 nmol), EziG^3 Fe Amber (10 mg, 10% enzyme loading, w w^{-1}), MOPS buffer (1 mL, 100 mM, **pH varied**), PLP (0.1 mM), 4 °C, orbital shaker (120 rpm), 1 h incubation time. Reaction conditions: EziG^3-AsR (10 mg, 10%, w w^{-1}), *rac*-α-MBA (100 mM), sodium pyruvate (50 mM), DMSO (5%, v v^{-1}), PLP (25 µM), HEPES buffer (0.5 mL reaction volume, 250 mM, pH 7), 30 °C, thermomixer (750 rpm).

Entry	Immobilization pH	Reaction time [min]	Conversion [%][a,b]	TON[b,c]	TOF[d]	*ee* %[e]
1	6.0	0.5	7.5±0.1	140±2	192	n.d.
2		1	12.4±0.3	230±11		n.d.
3		2	19.1±1.3	354±45		n.d.
1	6.5	0.5	10.8±0.3	201±12	379	n.d.
2		1	21.0±1.5	389±49		n.d.
3		2	40.5±0.6	750±21		n.d.
4	7	0.5	10.9±0.5	201±18	323	n.d.
5		1	17.3±1.5	321±51		n.d.
6		2	34.4±0.5	638±18		n.d.
7	7.5	0.5	10.7±0.6	198±12	365	14
8		1	21.0±1.3	388±24		29
9		2	38.6±1.7	715±31		65
10		3	46.4±0.8	860±15		92
11		5	49.1±0	909±0		98
12		10	49.2±0.1	912±2		>99

[a] Conversion to acetophenone. [b] Values are depicted with standard deviation over three experiments. [c] Turnover is depicted as µmol of converted substrate per µmol of immobilized enzyme. [d] Turnover frequency or activity in units per µmol of immobilized enzyme. [e] Enantiomeric excess of remaining (*S*)-α-MBA.

7. Kinetic resolution with EziG-immobilized ω-transaminases

7.1 Time study for the kinetic resolution of of *rac*-α-MBE using purified AsR-ωTA

Kinetic resolution with AsR-ωTA (purified enzyme) was followed in time (Table S 8). Both conversion to acetophenone and the *ee*% of remaining (*S*)-α-MBA were determined.

Table S 8. Activity test of purified AsR-ωTA. Reaction conditions: AsR-ωTA (1 mg, 27 nmol), *rac*-α-MBA (100 mM), sodium pyruvate (50 mM), DMSO (5% v v^{-1}), PLP (25 µM), HEPES buffer (0.5 mL reaction volume, 250 mM, pH 7), 30 °C, thermomixer (750 rpm).

Entry	Reaction time [min]	Enzyme in reaction [nmol]	Conversion [%][a,b]	TON[b,c]	TOF[d]	*ee* %[e]
1	0.5	27	25.8±0.2	478±4	n.d.	38
2	1	27	42.0±0.1	778±2		76
3	1.5	27	48.6±0	900±1		95
4	2	27	49.1±0.1	908±3		97

[a] Conversion to acetophenone. [b] Values are depicted with standard deviation over three experiments. [c] Turnover is depicted as µmol of converted substrate per µmol of immobilized enzyme. [d] Turnover frequency or activity in units per µmol of immobilized enzyme. [e] Enantiomeric excess of remaining (*S*)-α-MBA.

7.2 Time study for the kinetic resolution of *rac*-α-MBE using EziG^3-Cv

Kinetic resolution with EziG^3-Cv was followed in time (Figure 3B, main manuscript, Table S 9). Both conversion to acetophenone and the *ee*% of remaining (*R*)-α-MBA were determined.

Table S 9. Time study of EziG^3-Cv in kinetic resolution of *rac*-α-MBA. Reaction conditions: EziG-Cv (10 mg, 10% enzyme loading, w w^{-1}), *rac*-α-MBA (100 mM), sodium pyruvate (50 mM), DMSO (5% v v^{-1}), PLP (25 µM), HEPES buffer (0.5 mL reaction volume, 250 mM, pH 7), 30 °C, thermomixer (750 rpm).

Entry	Reaction time [min]	Conversion [%][a,b]	TON[b,c]	TOF[d]	*ee* %[e]
1	0.5	3.8±0.2	99±6	155	3
2	1	6.4±0.9	168±24		7
3	2	11.3±0.5	297±14		12
4	3	13.7±0.7	361±18		16
5	5	21.3±0.3	561±8		27
6	10	29.7±0.3	781±7		44
7	30	33.3±0.1	877±2		47
8	60	42.2±0.5	1111±13		72
9	180	49.4±0.1	1301±2		99

[a] Conversion to acetophenone. [b] Values are depicted with standard deviation over three experiments. [c] Turnover is depicted as µmol of converted substrate per µmol of immobilized enzyme. [d] Turnover frequency or activity in units per µmol of immobilized enzyme. [e] Enantiomeric excess of remaining (*R*)-α-MBA.

7.3 Time study for the kinetic resolution of *rac*-α-MBE using purified Cv-ωTA.

Kinetic resolution with Cv-ωTA (purified enzyme) was followed in time (Table S 10). Both conversion to acetophenone and the *ee*% of remaining (*R*)-α-MBA were determined.

Table S 10. Time study of Cv-ωTA (purified) in kinetic resolution of *rac*-α-MBA. Reaction conditions: Cv-ωTA (1 mg, 19 nmol), *rac*-α-MBA (100 mM), sodium pyruvate (50 mM), DMSO (5%, v v^{-1}), PLP (25 µM), HEPES buffer (0.5 mL reaction volume, 250 mM, pH 7), 30 °C, thermomixer (750 rpm).

entry	Reaction time [min]	Conversion [%][a,b]	TON[b,c]	TOF[d]	*ee* %[e]
1	0.5	14.3±0.1	376±1	304	20
2	10	24.8±0.3	652±8		37
3	30	42.5±0	1120±0		77
4	60	49.1±0	1293±1		95
5	120	49.9±0.1	1333±1		99

[a] Conversion to acetophenone. [b] Values are depicted with absolute difference between two experiments. [c] Turnover is depicted as µmol of converted substrate per µmol of immobilized enzyme. [d] Turnover frequency or activity in units per µmol of immobilized enzyme. [e] Enantiomeric excess of remaining (*R*)-α-MBA.

7.4 Temperature stability of purified AsR-TA

AsR-ωTA (purified enzyme) was employed in kinetic resolution as previously described. The activity was measured under same reaction conditions varying only the temperature of the reaction from 40 °C to 50 °C. The conversion to acetophenone was measured in time as well as the *ee*% of the remaining (*S*)-α-MBA (Figure S 2 and Table S 11).

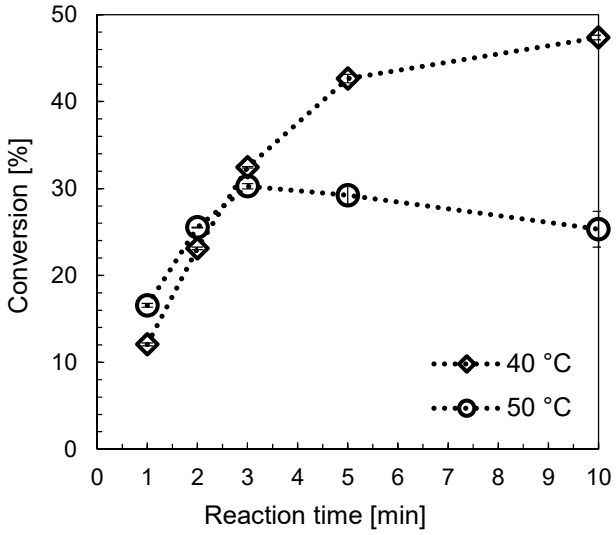

Figure S 2. Stability of purified AsR-ωTA in kinetic resolution of *rac*-α-MBA at 40 °C (diamonds) and 50 °C (circles). Conversion to acetophenone. Error bars display standard deviation over three experiments. Reaction conditions: AsR-ωTA (0.2 mg, 5.4 nmol, purified enzyme), *rac*-α-MBA (100 mM), sodium pyruvate (50 mM), DMSO (10%, v v^{-1}), PLP (25 µM), HEPES buffer (0.5 mL reaction volume, 250 mM, pH 7), **temperature varied**, thermomixer (750 rpm).

Table S 11. Stability of purified AsR-ωTA in kinetic resolution of *rac*-α-MBA at 40 °C, and 50 °C. Reaction conditions: AsR-ωTA (0.2 mg, 5.4 nmol, purified enzyme), *rac*-α-MBA (100 mM), sodium pyruvate (50 mM), DMSO (10%, v v^{-1}), PLP (25 µM), HEPES buffer (0.5 mL reaction volume, 250 mM, pH 7), **temperature varied**, thermomixer (750 rpm).

Entry	Reaction time [min]	Reaction T [°C]	Conversion [%][a,b]	*ee* %[c]
1	1	40	12.1±0.2	13
2	2		23.1±0.2	30
3	3		32.4±0.1	50
4	5		42.7±0.5	80
5	10		47.4±0.3	89
6	1	50	16.5±0.2	19
7	2		25.5±0	35
8	3		30.3±0.3	46
9	5		29.2±1.1	45
10	10		25.3±2.0	41

[a] Conversion to acetophenone. [b] Values are depicted with standard deviation over three experiments. [c] Enantiomeric excess of remaining (*S*)-α-MBA.

7.5 Procedure for performing the kinetic resolution of *rac*-α-MBE at high substrate concentrations

EziG^3-AsR was suspended in reaction buffer (0.5 mL final volume). Reaction buffer was prepared from three stock solutions:

Stock A: HEPES buffer (750 mM, pH 7) with 10% DMSO (v v^{-1})

Stock B: *rac*-α-MBA (1 M) with 10% DMSO (v v^{-1}) and sodium pyruvate (0.5 M) in HEPES buffer (750 mM, pH 7)

Stock C: 1 mM PLP in HEPES buffer (750 mM, pH 7)

To each reaction vial was added stock A, stock B, and stock C according to Table S 12. The reactions were shaken 30 °C in a thermoshaker (750 rpm) for 15 minutes. The immobilized enzyme was let to settle down and, then, the reaction buffer was transferred (pipetting) to another tube. The reaction buffer was basified with KOH (100 µL, 5 M) and extracted with EtOAc (2 x 500 µL). Analytics were performed as previously described (see; Analytics). Results are depicted in Figure 4A (main manuscript) and Table S 13.

Table S 12. Stock solutions prepared for kinetic resolution at higher substrate concentrations. From each stock solution was taken a specified amount and combined in one reaction. EziG^3-AsR was added to the vial and the volume was added up to 0.5 mL.

Entry	Final concentration of *rac*-α-MBA [mM]	Stock A [µL]	Stock B [µL]	Stock C [µL]
1	100	439	48.5	12.5
2	200	390	97.5	12.5
3	400	293	194.5	12.5
4	600	195	292.5	12.5
5	800	97.5	390	12.5
6	1000	0	487.5	12.5

Table S 13. EziG^3-AsR in kinetic resolution with high substrate loadings. Immobilization conditions: AsR-ωTA (1.7 mg, 46 nmol), EziG^3 Fe Amber (10 mg, 17% enzyme loading, w w^{-1}), PLP (0.1 mM), MOPS buffer (100 mM, pH 7.5, 1 mL), 4 °C, orbital shaker (120 rpm), 1 h incubation time. Reaction conditions: EziG^3-AsR (10 mg enzyme, 17% enzyme loading, w w^{-1}), *rac*-α-MBA (**concentration varied**), DMSO (10%, v v^{-1}), sodium pyruvate (0.5 equiv.), PLP (25 µM), HEPES buffer (0.5 mL reaction volume, 750 mM, pH 7), 30 °C, thermomixer (750 rpm), 15 minutes.

Entry	Final concentration of *rac*-α-MBA [mM]	Conversion [%][a,b]	TON[b,c]	*ee*%[d]
1	100	49.0±0.1	541±2	95
2	200	45.7±0.1	993±1	86
3	400	45.8±0	1991±2	85
4	600	37.6±1	2449±65	61
5	800	19.6±0.5	1702±47	18
6	1000	12.4±0.6	1349±65	5

[a] Conversion to acetophenone. [b] Values are depicted with standard deviation over three experiments. [c] Turnover is depicted as µmol of converted substrate per µmol of immobilized enzyme. [d] Enantiomeric excess of remaining (*S*)-α-MBA.

7.6 Biocatalyst recycling of EziG^3-AsR

EziG^3-AsR was repeatedly employed in the kinetic resolution of *rac*-α-MBA. After 15 minutes reaction time, the immobilized enzyme was let to settle down and, then, the reaction buffer was transferred (pipetting) to another tube. The buffer was basified (100 µL 5M KOH) and extracted with EtOAc (2 x 500 µL). Analytics were performed as previously described (see; Analytics). EziG^3-AsR was then re-suspended in fresh buffer and the reaction was run for another 15 minutes. This procedure was repeated for 16 reaction cycles in total (Figure 4B, main manuscript, Table S 14). The reactions were performed with addition of 25 µM PLP in each new reaction cycle. When no additional PLP was supplied conversions for the reaction dropped slightly (approx. 3% per cycle) indicating that a surplus of PLP is beneficial (data not shown).

Table S 14. Recycling experiments for EziG^3-AsR in kinetic resolution of *rac*-α-MBA. Reaction conditions: EziG^3-AsR (10 mg, 10% enzyme loading, w w^{-1}), *rac*-α-MBA (100 mM), sodium pyruvate (50 mM), DMSO (10%, v v^{-1}), PLP (25 µM), HEPES buffer (0.5 mL reaction volume, 250 mM, pH 7), 30 °C, thermomixer (750 rpm), 15 minutes per reaction cycle.

entry	cycle #	Total reaction time [min]	Conversion [%][a,b]	Product per cycle [µmol]	*ee*%[c]
1	1	15	47.8±0.1	23.9	>99
2	2	30	48.5±0	24.3	>99
3	3	45	48.9±0	24.5	>99
4	4	60	49.1±0.2	24.6	>99
5	5	75	49.5±0.1	24.8	>99
6	6	90	49.4±0	24.7	>99
7	7	105	49.7±0.3	24.9	>99
8	8	120	49.9±0	25.0	>99
9	9	135	47.5±0.7	23.8	>99
10	10	150	47.1±0.2	23.6	>99
11	11	165	48.3±0.3	24.2	>99
12	12	180	48.3±0.2	24.2	>99
13	13	195	48.4±0.2	24.2	98
14	14	210	48.4±0.4	24.2	97
15	15	225	47.2±0.5	23.6	>99
16	16	240	47.4±0.6	23.7	98
	Total			388.2	>99

[a] Conversion to acetophenone. [b] Values are depicted with standard deviation over three experiments. [c] Enantiomeric excess of remaining (*S*)-α-MBA.

8. References

[1] a) H. Scott Fogler, “Elements of Chemical Reaction Engineering”, 4th Ed., Prentice Hall PTR, 2005; b) C. G. Hill, ‘‘An Introduction to Chemical Engineering Kinetics & Reactor Design’’, J. Wiley, NY, 1977.