Supporting information

In situ reactive extraction with oleic acid for process intensification in amine transaminase catalyzed reactions

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1.) Catalyst preparation

1.1) Transformation und cultivation procedure of *Escherichia coli* BL21 (DE3) with pET29a_Cv2025-His6

Chemically competent *Escherichia coli* BL21 (DE3) cells were transformed using a standard heat-shock protocol. Initially, 1 µL of plasmid DNA was transferred to 50 µL thawed competent cells. The mixture was incubated on ice for 30 minutes. To initiate the transformation, the mixture was heated for 90 seconds at 42°C. After the heat-shock, the cells were cooled on ice for 5 minutes and 500 µL lysogeny broth (LB) medium was added. The transformed cells were incubated at 37°C and 300 rpm. After 1 hour incubation, 50 µL of the transformation mixture was plated onto LB agar plates containing 50 µg/mL kanamycin and incubated at 37°C overnight. For strain propagation, 50 mL LB medium was inoculated with a single colony and incubated at 37°C and 120 rpm in 250 mL shake flasks with baffles. The next day, 1 L auto-induction medium (12 g/L peptone, 24 g/L yeast extract, 91 mM potassium phosphate buffer, 2 g/L lactose, 6.3 g/L glycerol, 0.5 g/L glucose, 10 mg/L kanamycin) was inoculated in 5 L shake flasks with baffles from the pre-culture (1:200) and incubated at 37°C and 80 rpm for 3 h. Subsequently, the cultivation was continued at 20°C and 80 rpm for 48 h. The production of Cv2025 was checked via SDS-PAGE (expected molecular weight 52 kDa).

1.2) Enzyme purification via immobilized metal-affinity chromatography (IMAC)

The crude extract was prepared in equilibration buffer (50 mM potassium phosphate buffer pH 7.5, 0.2 mM pyridoxal-5'-phosphate (PLP)) from a 10% (w/v) cell suspension after cell disruption by ultrasonification in a flow cell at 4°C (70 % amplitude, 0.5 cycle time, 30 min). Afterwards, the cell suspension was centrifuged (21500 rpm, 30 min, 4°C). Protein purification was achieved via affinity chromatography (ÄKTApurifier, GE Healthcare, Chicago, USA) using a Ni-NTA superflow column material (Qiagen, Hilden, Germany). After a washing step (50 mM potassium phosphate buffer, 0.2 mM PLP, 25 mM imidazole, pH 7.5), the enzyme was eluted from the column with elution buffer (50 mM potassium phosphate, 0.2 mM PLP, 300 mM imidazole, pH 7.5). The elution fraction was desalted on a Sephadex G-25 column (GE Healthcare) with 10 mM potassium phosphate buffer pH 7.5 + 0.2 mM PLP and frozen in a crystallization dish at -20°C for lyophilization. The purification of the amine transaminase Cv2025 in the elution fraction was verified via SDS-PAGE with > 90% purity. The protein content of the lyophilizate was determined between 25-50 % (w/w).

2.) Plasmid information

Plasmid information Cv2025-His6

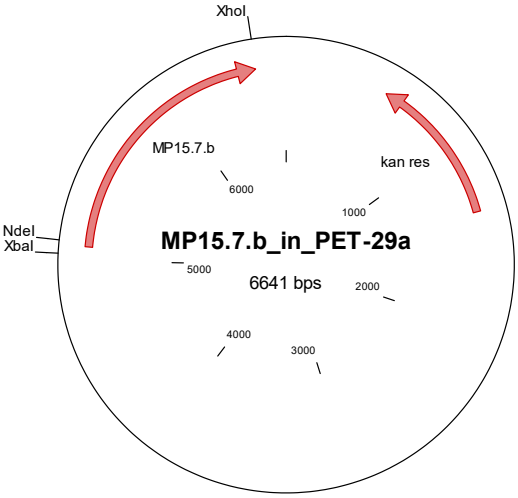

Figure S2.1: Plasmid of Cv2025-His6 in PET-29a.

The gene sequence of Cv2025-His6 in PET-29a is given below (coding sequence for enzyme highlighted in red, coding sequence for the His_6-tag underlined):

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Protein sequence of *Cv*2025-His6:

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EEFEQTLKARGLA

3.) Reaction analytics

3.1) HPLC analytics with online derivatization for determination of metaraminol in pure substance systems

The metaraminol in the pure substance system was quantified via HPLC measurement using the online derivatization method with orthophtalic aldehyde and mercaptoethanol as described in the main manuscript, chapter 2.2. The used inline derivatization procedure is given in table S3.1, where vial 99 contained 0.1 ml OPA reagent and 0.9 ml of 0.4M borate buffer at pH 10.4 and vial 100 contained distilled water. The derivatized samples were injected onto the HPLC and the method was rum for 6 minutes. The eluents were (A) 2.5 mM acetic acid in water with 10 % methanol at pH 6 and (B) methanol. The gradient elution is given in table S3.2.

Table S3.1.1: Derivatization procedure for quantification of metaraminol in pure substance systems

	Derivatization procedure
1	DRAW 4.5 µL from vial 100
2	DRAW 1.5 µL from sample
3	DRAW 0.5 µL from air
4	NEEDLE wash in vial 99
5	DRAW 4.5 µL from vial 100
6	MIX 11.0 µL in seat
7	WAIT 1.00 min
8	INJECT
9	DRAW and EJECT 100 µL from vial 99

Table S3.1.2: Gradient elution used in the quantification of metaraminol in pure substance systems

Time	Eluent A	Flow
[min]	[%]	[ml/min]
0.00	90	0.4
1.00	10	0.4
2.00	10	0.4
5.00	90	0.4

The calibration curve for metaraminol and an exemplary chromatogram are shown below (Agilent 1200, DAD 334nm, Machery Nagel Nucleodur C18ec pre-column, 2 x 4 mm, 40°C)

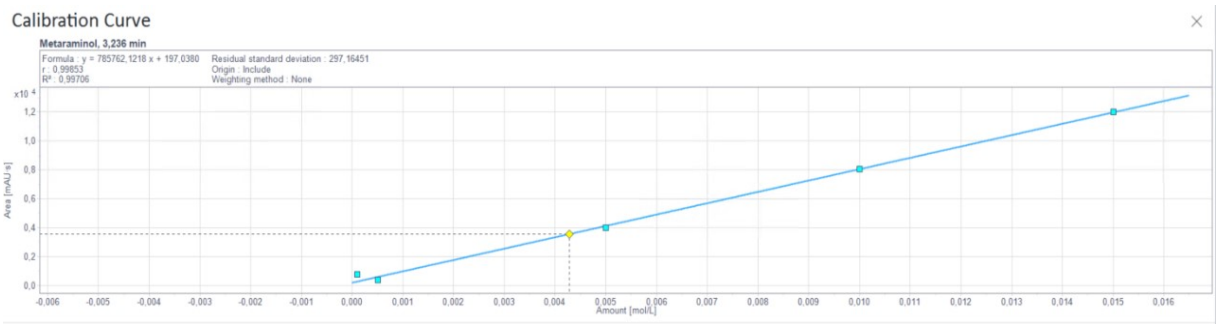

Figure S3.1.1: Calibrations for HPLC quantification of metaraminol.

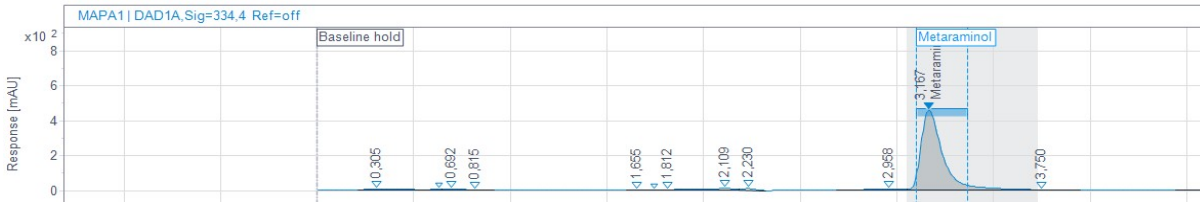

Figure S3.1.2: Typical HPLC chromatogram for the quantification of metaraminol using online derivatization with orthophtalic aldehyde and mercaptoethanol. The retention time of metaraminol was 3.16 min.

3.2) UHPLC analytics for the determination of (*R*)-3-OH-PAC and metaraminol in the enzymatic reaction system

The reaction components were quantified using two different UHPLC methods as the retention and separation for the reaction product metaraminol was ideal in an isocratic elution, while the substrate (*R*)-3-OH-PAC was analyzed using a gradient method. Calibrations for both reaction components are shown below (Agilent 1290 Infinity II, DAD 220 nm, Agilent Zorbax Eclipse plus C18 column, 2 x 100 mm, 1.8 µm, 20 °C, 5 µL injection volume).

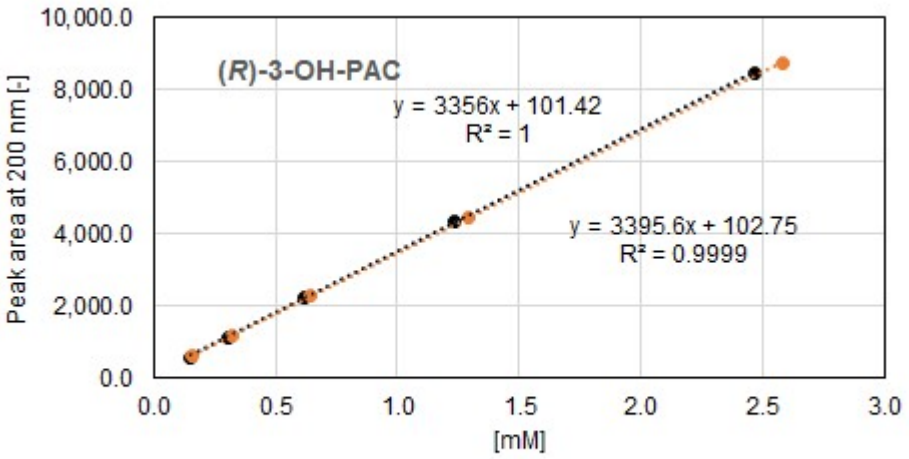

Figure S3.2.1: Calibrations for HPLC quantification of (*R*)-3-OH-PAC. Double determination between 0.125 mM and 2.5 mM in 50 % acetonitrile (v/v) in ultra-pure water. Gradient method (0.5 mL/min, 10-90 % acetonitrile in a linear gradient over 5 min, 90-10% H_2O + 0.1% diethylamine + 0.075% trifluoroacetic acid).

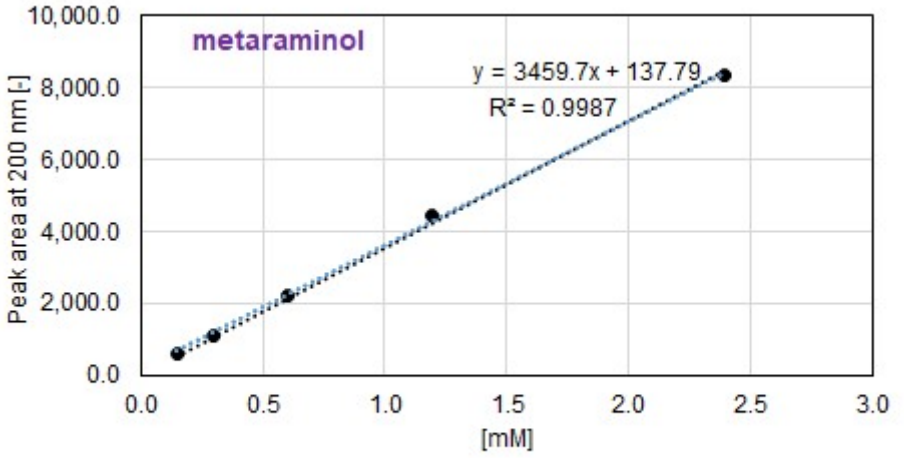

Figure S3.2.2: Calibrations for HPLC quantification of metaraminol. Double determination between 0.125 mM and 2.5 mM in 50% acetonitrile (v/v) in ultra-pure water. Isocratic method (0.3 mL/min, 15% acetonitrile + 85% H_2O + 0.1% diethylamine + 0.075% trifluoroacetic acid).

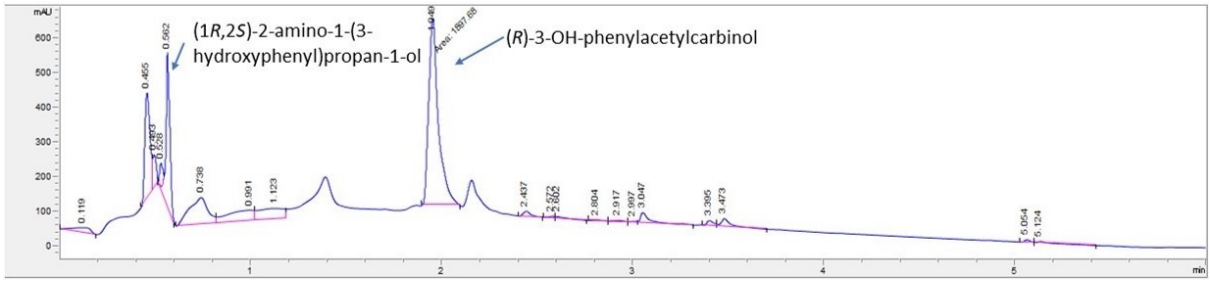

Figure S3.2.3: HPLC chromatogram of transamination of (*R*)-3-OH-phenylacetylcarbinol to (1*R*,2*S*)-2-amino-1-(3-hydroxyphenyl)propan-1-ol (metaraminol) using a gradient elution (0.5 mL/min, 10-19% acetonitrile in a linear gradient over 5 min, 90-10% H_2O + 0.1% diethylamine + 0.075% trifluoroacetic acid). The retention time of (*R*)-3-OH-PAC was 1.95 min. This method was used for (*R*)-3-OH-PAC detection in biotransformations.

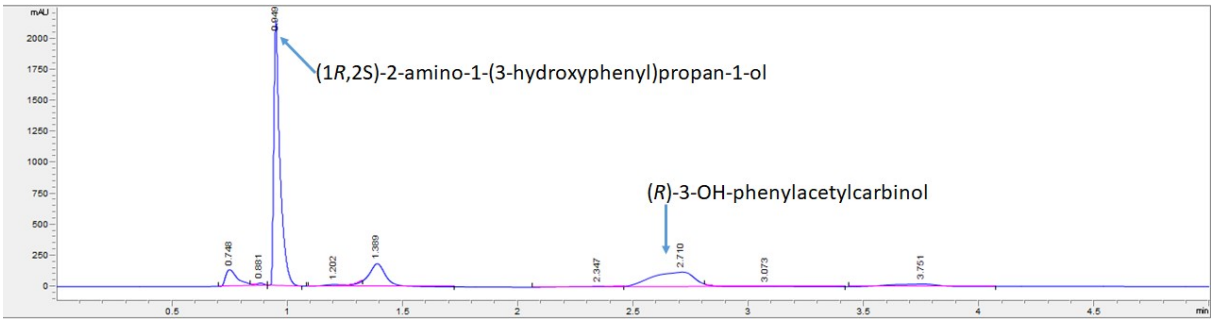

Figure S3.2.4: Typical HPLC chromatogram of transamination of (*R*)-3-OH-phenylacetylcarbinol to (1*R*,2*S*)-2-amino-1-(3-hydroxyphenyl)propan-1-ol (metaraminol) using an isocratic elution (0.3 mL/min, 15% acetonitrile + 85% H_2O + 0.1% diethylamine + 0.075% trifluoroacetic acid). The retention time of metaraminol was 0.94 min. This method was used for metaraminol detection.

4.) Raw data for key experimental results

4.1) Reactive extraction experiments (Figure 2)

Table S4.1: Measured concentration of metaraminol in the aqueous phase before and after reactive extraction with 0.1, 0.25 M and 0.5 M oleic acid in saturated 1-octanol. Where c0,aq is the initial metaraminol concentration in the aqueous phase, pH is the resulting pH after extraction and c1,aq is the measured concentration after extraction. All experiments contain different amounts of added NaOH according to the experimental description. For extraction 1 ml of aqueous and 1 ml of organic phase were contacted.

Initial aqueous concentration	Extraction with 0.1 M oleic acid		Extraction with 0.25 M oleic acid		Extraction with 0.5 M oleic acid	
c0,aq	pH	c1, aq	pH	c1, aq	pH	c1, aq
[mol/L]		[mol/L]		[mol/L]		[mol/L]
0.01005	5.03	0.00970	4.99	0.00956	4.98	0.00895
0.01005	5.22	0.00953	5.21	0.00917	5.14	0.00880
0.00998	5.78	0.00833	5.43	0.00862	5.28	0.00844
0.01010	6.13	0.00885	5.62	0.00808	5.51	0.00780
0.01012	6.32	0.00736	5.8	0.00734	5.66	0.00723
0.01013	6.56	0.00652	6.02	0.00664	5.81	0.00644
0.01005	6.75	0.00556	6.09	0.00597	5.9	0.00599
0.01001	6.93	0.00464	6.31	0.00508	5.91	0.00536
0.01000	7.14	0.00385	6.53	0.00396	6	0.00484
0.00995	7.21	0.00297	6.8	0.00362	6.08	0.00428
0.01000	7.41	0.00225	6.87	0.00293	6.22	0.00377
0.01001	7.71	0.00158	6.94	0.00225	6.33	0.00313
0.01008	7.82	0.00116	7.1	0.00169	6.43	0.00269
0.01007	7.99	0.00090	7.13	0.00130	6.5	0.00221
0.01006	8.16	0.00065	7.22	0.00097	6.66	0.00178
0.01015	8.29	0.00058	7.34	0.00076	6.9	0.00144
0.01009	8.35	0.00048	7.53	0.00059	6.99	0.00108
0.01026	8.39	0.00042	7.56	0.00048	7.07	0.00076
0.01025	8.42	0.00039	7.63	0.00041	7.18	0.00012
0.01006	8.43	0.00041	7.74	0.00037	7.28	0.00010
0.01014	8.46	0.00034	7.8	0.00033	7.47	0.00047

4.2) Single step *in situ* reactive extraction coupled with the enzymatic reaction (figure 8)

Table S4.2.1: **Exemplary measured concentrations of the reaction components within single-step transamination reaction to metaraminol using L-alanine as amine donor. The concentrations were derived from HPLC analytics using external calibration curves. Different charges of (*R*)-3-OH-PAC were used (> 98% pure, HPLC). Metaraminol = (1*R*,2*S*)-3-[-2-Amino-1-hydroxy-propyl]phenol (167.21 g/mol), (*R*)-3-OH-PAC ((*R*)-3-OH-phenylacetylcarbinol) = 1-hydroxy-1-(3-hydroxyphenyl)propan-2-one (166.18 g/mol), n.q. = not quantified, reex = back extraction phase.**

Control 40 mL aqueous reaction phase, no organic phase, 30°C, pH 7.6			
Reaction component	Weighed portion	c0, aq	c1, aq
	[mg]	[mmol/L]	[mmol/L]
(*R*)-3-OH-PAC	261.0	45.9	35.8
L-alanine	885.9	n.q.	n.q.
metaraminol	-	n.q.	8.4

Table S4.2.2: **Measured concentrations of the reaction components within single-step reactive extractions integrated with the transamination reaction to metaraminol. The concentrations were derived from HPLC analytics using external calibration curves. Different charges of (*R*)-3-OH-PAC were used (> 98% pure, HPLC). Metaraminol = (1*R*,2*S*)-3-[-2-Amino-1-hydroxy-propyl]phenol (167.21 g/mol), (*R*)-3-OH-PAC = 1-hydroxy-1-(3-hydroxyphenyl)propan-2-one (166.18 g/mol), n.q. = not quantified, reex = back extraction phase.**

Single-step *in situ* reactive extraction 40 mL aqueous reaction phase, 10 mL organic phase containing 0.1 M oleic acid, 30°C, pH 7.6, 40 mL back extraction phase (50 mM tartaric acid)				
Reaction component	Weighed portion	c0, aq	c1, aq	c1, reex
	[mg]	[mmol/L]	[mmol/L]	[mmol/L]
(*R*)-3-OH-PAC	269.0	43.8	15.5	5.5
L-alanine	892.4	n.q.	n.q.	n.q.
metaraminol	-	n.q.	7.3	4.5
Single-step *in situ* reactive extraction 40 mL aqeuous reaction phase, 10 mL organic phase containing 0.25 M oleic acid, 30°C, pH 7.6, 40 mL back extraction phase (50 mM tartaric acid)				
Reaction component	Weighed portion	c0, aq	c1, aq	c1, reex
	[mg]	[mmol/L]	[mmol/L]	[mmol/L]
(*R*)-3-OH-PAC	264.2	40.9	10.1	4.1
L-alanine	891.0	n.q.	n.q.	n.q.
metaraminol	-	n.q.	7.4	10.1

4.3) Repeated *in situ* reactive extractions coupled with the enzymatic reaction (figure 9)

Table S4.3: **Measured concentrations of the reaction components within single-step transamination reactions to metaraminol using L-alanine as amine donor. The concentrations were derived from HPLC analytics using external calibration curves. Different charges of (*R*)-3-OH-PAC were used (> 98% pure, HPLC). Metaraminol = (1*R*,2*S*)-3-[-2-Amino-1-hydroxy-propyl]phenol (167.21 g/mol), (*R*)-3-OH-PAC = 1-hydroxy-1-(3-hydroxyphenyl)propan-2-one (166.18 g/mol), n.q. = not quantified, reex = back extraction phase.**

Repeated *in situ* reactive extraction 40 mL aqueous reaction volume, 10 mL organic phase containing 0.25 M oleic acid, 30°C, pH 7.6 10 mL back extraction phase (50 mM tartaric acid)								
			1. extraction step		2. extraction step		3. extraction step	
Reaction component	Weighed portion	c0, aq	c1, aq	c1, reex	c2, aq	c2, reex	c3, aq	c3, reex
	[mg]	[mM]	[mM]	[mM]	[mM]	[mM]	[mM]	[mM]
(*R*)-3-OH-PAC	265.3	37.9	14.2	7.3	5.9	2.4	3.2	1.5
L-alanine	893.8	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
metaraminol	-	n.q.	5.4	34.7	4.6	31.3	3.4	22.2