Supplementary Materials

Comparison of Four Immobilization Methods for Different Transaminases

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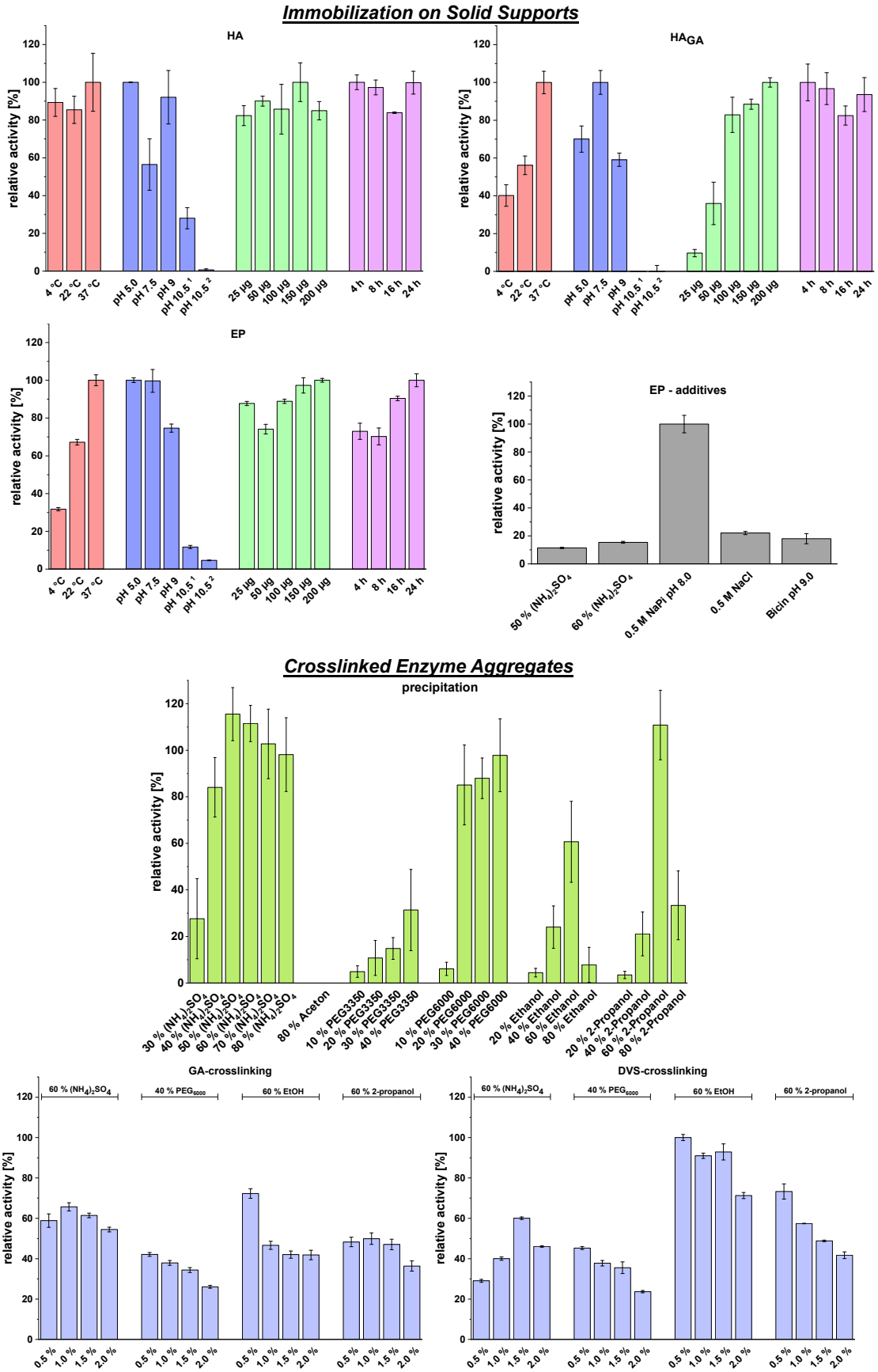
All experimental data, which are visualized in the graphs or omitted for space and presentation reasons were published in PUB (Publikationen an der Universität Bielefeld) at doi 10.4119/unibi/2968396.

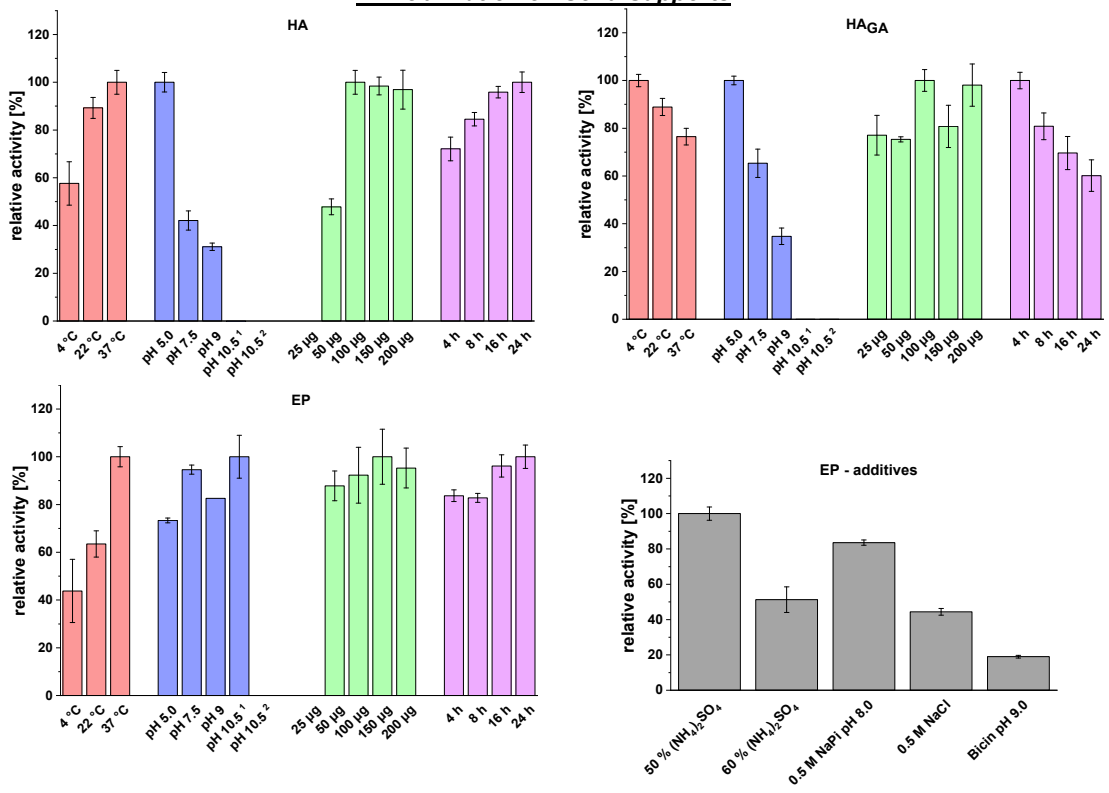
1. Establishment of optimal immobilization conditions for each transaminase

ATA-Bmu

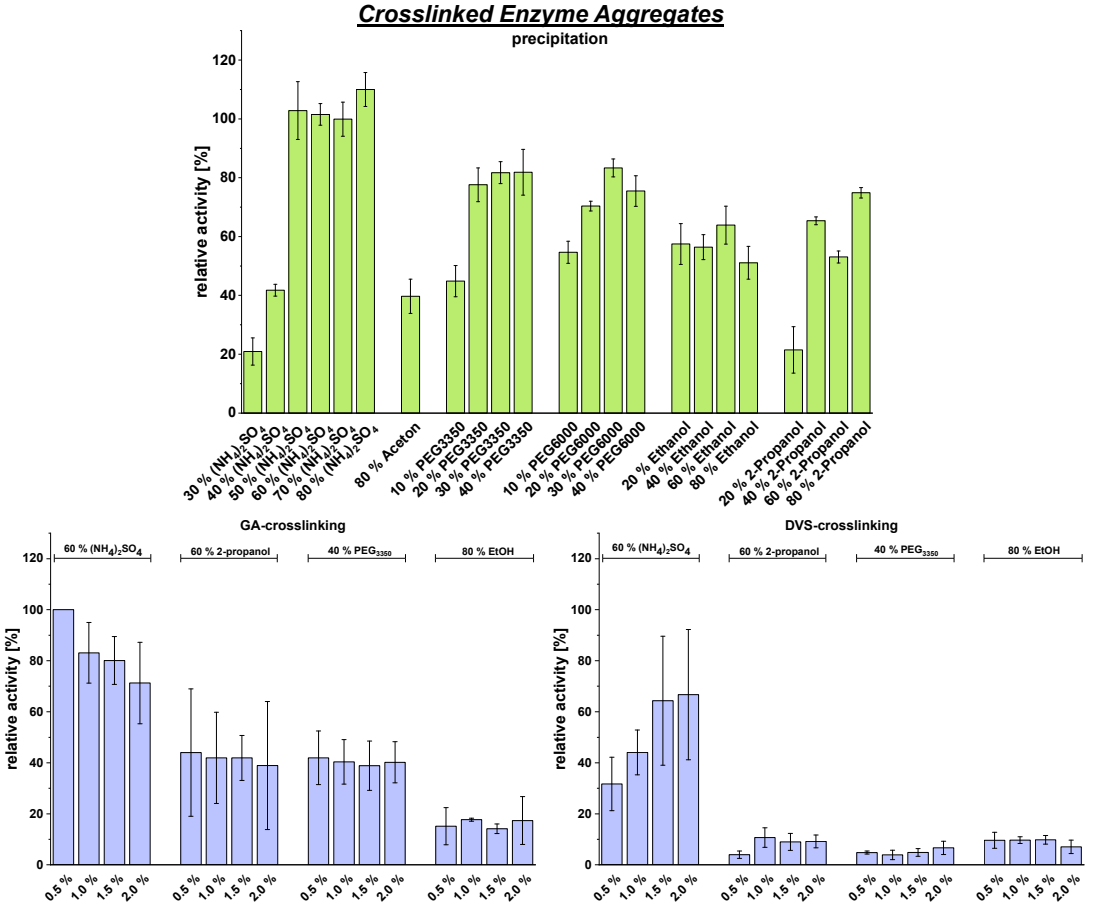


ATA-Vfl

Immobilization on Solid Supports

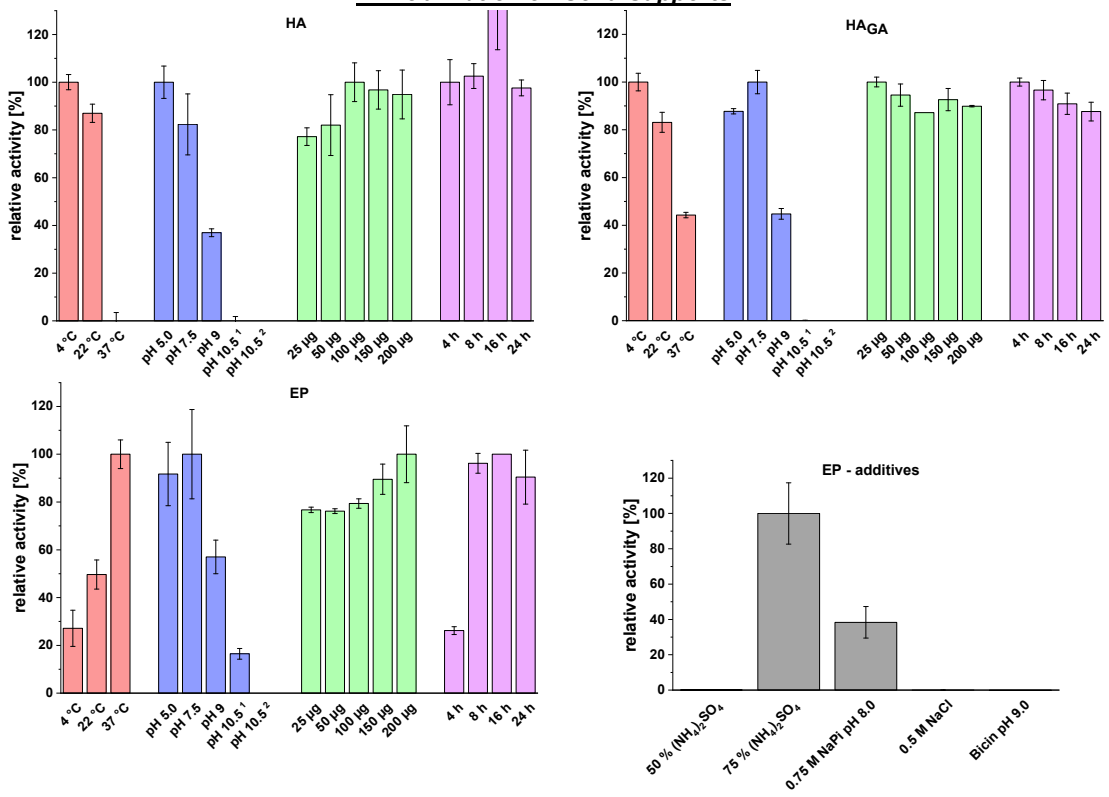


Crosslinked Enzyme Aggregates



ATA-3FCR-5M

Immobilization on Solid Supports



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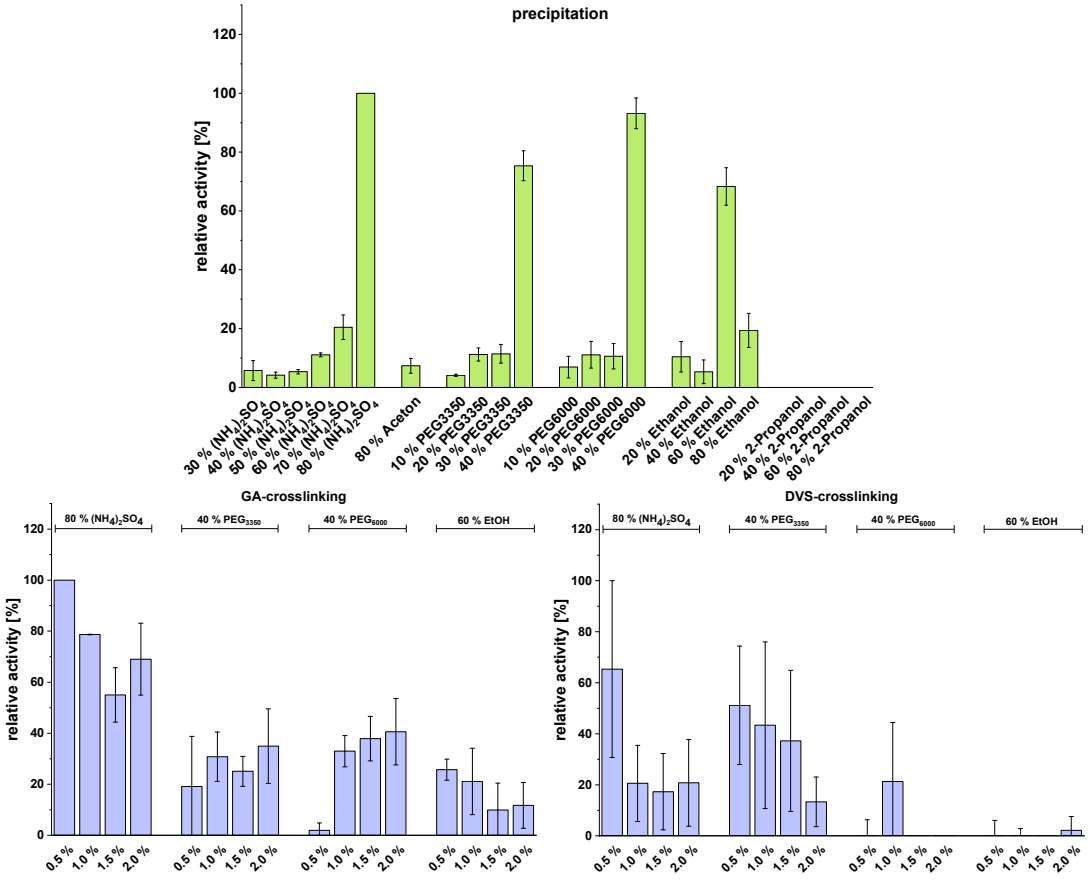

ATA-Lsy

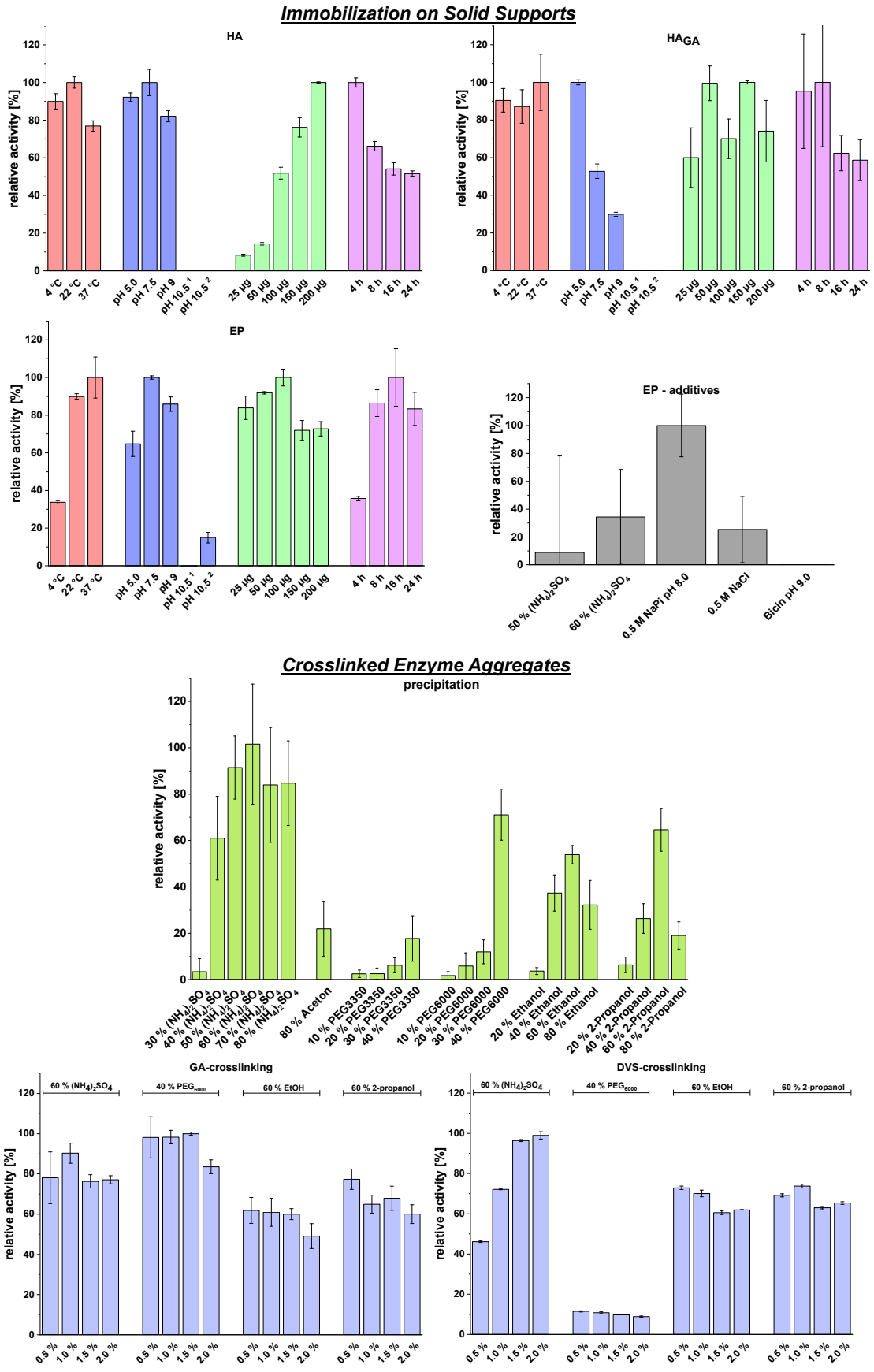

Figure S1: Establishment of optimal immobilization conditions. The optimal immobilization conditions for all transaminases (1) ATA-Bmu, 2) ATA-Vfl, 3) ATA-3FCR-5M, 4) ATA-Lsy) were determined by extensive screening

of various conditions. For the solid supports (amine(HA)-, glutaraldehyde-functionalized amine(HA_{GA}) and epoxy(EP)-beads) immobilization was analyzed at different temperatures (red), pH (blue), durations (pink) and enzyme amounts per mg bead (green). Different additives were also studied for efficient immobilization on epoxy beads (grey). The activity resulting from the best condition in each series of experiments (e.g., pH-screen) was set as 100 % and the others within this series were referred to it. Except for the varied conditions in each series, fixed standard conditions (50 mM bicine buffer, pH 9.0, 20 μM enzyme solution, 100 μg enzyme per mg bead, 37 °C, 24 h) were used. For each experiment, 5 mg beads were mixed with the appropriate volume of immobilization solution and incubated as mentioned. Beads were then washed and cross-linked (see methods) and activity was subsequently determined under standard conditions (see methods). For the preparation of the cross-linked enzyme aggregates (CLEAs), different additives were first analyzed for their ability to precipitate proteins that can be resolubilized in an active form (green). The activities were referred to the highest activity in the whole set, which was set as 100 %. Therefore, 100 μg protein (10 μM) was incubated with the respective additive in phosphate buffer (final concentration: 100 mM) in a total volume of 500 μl at 4 °C for 1 h. Subsequently, the precipitated enzymes were centrifuged and resolubilized in buffer (50 mM bicine pH 9.0, 0.1 mM PLP), and the activity was determined under standard conditions (see methods). Chosen precipitants that resulted in the highest activities of the dissolved enzymes were used to screen two cross-linkers (glutaraldehyde (GA) and divinylsulfone (DVS)) at different concentrations. The activities were referred to the highest activity obtained within both cross-linker-experiments, which was set as 100 % (blue). For this purpose, GA and DVS was added to the respective enzyme solution after precipitation with the chosen precipitants (see before and methods) and the solution was incubated for further 4 h at 4 °C. Subsequently, the generated CLEAs were centrifuged, washed (3x 50 mM Tris pH 8.0, 0.1 mM PLP), and the activity was determined under standard conditions (see methods). All experiments were performed in at least triplicates, and standard deviations are shown as error bars. Additional information is provided in the methods.

The optimal immobilization conditions for each transaminase resulting from the extensive screening (Figure S1) are listed in Table S1.

Table S1: Optimal immobilization conditions for each transaminase. After evaluating the extensive screening of various immobilization conditions (Figure S1), optimal conditions were identified for each transaminase. The optimal conditions were selected based on the highest activity of the immobilized protein (U/g beads) achieved rather than other parameters such as activity recovery or binding efficiency (Table S2). However, in some cases, binding efficiencies were low, resulting in protein loss, whereby the amount of protein applied per mg of bead could be reduced in these cases.

Trans-aminase	Type	Optimal immobilization condition						
		Temperature [°C]	pH	µg protein per mg bead	Duration [h]	Additive	Precipitant	Cross-linker
ATA-Vfl	HA	22-37	5.0	100	24	-	-	-
	HA_{GA}	4	5.0	100	4	-	-	-
	EP	37	7.5/10.5	100	24	50 % $(NH_4)_2SO_4$	-	-
	CLEA	4	7.5		1+4	-	60 % $(NH_4)_2SO_4$	0.5 % GA
ATA-Bmu	HA	4-37	5.0	25	4	-	-	-
	HA_{GA}	37	7.5	100	4	-	-	-
	EP	37	5.0/7.5	25	24	0.5 M NaPi	-	-
	CLEA	4	7.5		1+4	-	60 % $(NH_4)_2SO_4$ or 60 % EtOH	0.5 % DVS
ATA-3FCR-5M	HA	4	5.0	100	4	-	-	-
	HA_{GA}	4	5.0/7.5	25	4	-	-	-
	EP	37	5.0/7.5	200	8	75 % NH_4SO_4	-	-
	CLEA	4	7.5		1+4*	-	80 % $(NH_4)_2SO_4$	0.5 % GA
ATA-Lsy	HA	4-22	5.0/7.5	200	4	-	-	-
	HA_{GA}	4-37	5.0	50	4	-	-	-
	EP	37	7.5	100	16	0.5 M NaPi	-	-
	CLEA	4	7.5		1+4	-	60 % $(NH_4)_2SO_4$ or 40 % PEG-6000	0.5 % GA

*In contrast to other proteins, prolonged CLEA-formation (>> 6 h) leads to complete inactivation of ATA-3FCR-5M.

Table S2: Immobilization parameters. Each protein was immobilized in a larger scale (40 mg beads) using the optimized conditions (Table S1). The protein concentration and the activity in all solutions were determined. Based on this, various parameters (defined in the experimental section) could be determined, allowing an assessment of the immobilization quality.

Transaminase	Specific activity [a] [U/mg]	Type	Starting activity [b] [U]	Observed activity [c] [U]	Specific activity of biocatalyst [d] [U/g bead]	Binding efficiency [e] [%]	Activity recovery [f] [%]
ATA-Vfl	4.24	HA	36.2	2.1	52.8	97.6	5.8
		HA_{GA}	34.9	2.5	62.4	52.7	7.2
		EP	39.6	2.0	49.2	64.0	5.0
		CLEA	0.9	0.1	-	16.0	12.7
ATA-Bmu	1.97	HA	5.8	1.0	25.5	97.1	17.4
		HA_{GA}	11.7	2.2	54.6	69.3	18.7
		EP	5.1	1.0	25.9	97.7	20.2
		CLEA	0.12	0.02	-	82.8	15.6
ATA-3FCR-5M	1.90	HA	11.4	1.7	43.6	95.4	15.3
		HA_{GA}	3.6	1.8	44.1	98.2	49.7
		EP	25.6	0.6	14.6	77.3	2.3
		CLEA	0.33	0.06	-	22.5	17.3
ATA-Lsy	0.99	HA	13.2	1.9	47.8	46.9	14.5
		HA_{GA}	8.3	1.4	34.1	94.6	16.4
		EP	12.3	1.4	35.6	62.6	11.6
		CLEA	0.16	0.01	-	28.6	8.1

[a] The specific activity of the tagged and non-tagged proteins did not differ significantly. The reaction behavior of the two enzymes was similar. [b] Starting activity is the total activity initially applied with the stock solution for the immobilization procedure (in Units). [c] Observed activity is the actual measured activity of the immobilized enzyme (immobilizate, in Units). [d] The specific activity of biocatalyst is the observed activity of the immobilized enzyme per g of bead support. [e] Binding efficiency is the percentage ratio between the total amount of immobilized enzyme (protein amount in the starting solution minus protein amount in the supernatant) and the total protein amount initially applied with the starting solution. [f] Activity recovery is the percentage ratio between the observed activity and the starting activity.

2. Methods and additional data on transaminase stability and activity

General setup of sample incubation

In this study, enzyme activities and stabilities were analyzed as they have a great impact on the application. For this purpose, the transaminases were immobilized on beads in larger batch-preparations (approximately 0.8-1 g beads per replicate) as described in the methods and then suspended in water and distributed by pipetting into several vessels to obtain 5 mg beads per vessel (always in duplicates). By that one immobilization batch was used for most approaches. In the case of CLEAs, all preparations were performed separately. After removal of the supernatant, different buffers were added depending on the preparation. Before each activity assay, the immobilizates were washed sufficiently (3 times 50 mM Tris, 0.1 mM PLP). In the case of soluble transaminases, highly concentrated solutions (7.5 mg/mL) were used to enable a sufficient dilution factor afterwards (finally in the activity assay between 240 and 480 depending on the activity of the respective transaminase; diluted with water) to exclude effects in the activity assays of the additives and solvents applied. These solutions were mixed with the respective buffers (see below) and diluted prior to activity assays. 25 µL of the diluted proteins (final protein amount of 1-10 µg) were mixed with 50 µL of 3x enzyme buffer (150 mM Tris pH 8.0, 0.3 mM PLP), after which the reaction was started with 75 µL of 2x reaction buffer (50 mM Tris pH 8.0, 1.0 % DMSO, 5 mM 1-PEA, 5 mM pyruvate).

Long-term storage of enzymes is of great interest and therefore needs to be characterized under different conditions. Soluble and immobilized enzymes were stored in different buffers (Bicine, HEPES, sodium phosphate and Tris; each at pH 8.0), at different pH values (5.5 (acetate buffer), 6.0 (sodium phosphate), 8.0 (bicine), 10.0 (CAPS) and with different additives at 4 and 20 °C under exclusion of light with 0.1 mM PLP and exposed to air. The residual activity of the ATA stored under the above specified conditions was analyzed every two weeks for a total of 56 days and was always related to the standard storage condition (50 mM bicine, 0.1 mM PLP, pH 8.0, 4 °C). Long-term storage under operating conditions was performed by adding either 20 mM *rac*-1-phenylethylamine (1-PEA) (to obtain a final concentration of 10 mM (*S*)- or (*R*)-1-PEA for (*S*)- or (*R*)-selective transaminases, respectively) or 10 mM alanine (L- or D-alanine for (*S*)- or (*R*)-selective transaminases, respectively). All samples were stored in the dark to exclude effects of light to transaminase and/or cofactor stabilities [63,91].

Thermostability was analyzed by incubating the enzymes at different temperatures in standard buffer (50 mM bicine, 0.1 mM PLP, pH 8.0) or under operating conditions (standard buffer supplemented with 20 mM *rac*-1-PEA or 10 mM L- or D-alanine). The activity was determined after 6 hours of incubation.

The reason for the choice of the two amine donors was that they behave differently: **i)** Alanine is deaminated slower compared to 1-PEA (70 %) [92], **ii)** The product of alanine, pyruvate, is rapidly used by ATAs and the product of 1-PEA, acetophenone, is slowly used as an amine acceptor in the second half [92]. However, alanine can also be highly destabilizing compared to 1-PEA, as shown for example for the melting temperature of the wildtype transaminase from *Chromobacterium violaceum* [7,8].

Another critical factor is stability upon presence of various organic solvents. Those can be used, for example, to dissolve substrates or products, to permanently extract products, to allow reactions to take place, or to minimize side reactions. To analyze the operational solvent stability within reactions, different solvents and additives ($(NH_4)_2SO_4$, DMSO, ethanol, isopropanol, acetonitrile (ACN)) were added to the standard reaction (50 mM Tris, 0.1 mM PLP, 0.5 % DMSO, 2.5 mM *rac*-1-PEA, 2.5 mM pyruvate, pH 8.0) at different concentrations (0, 10, 25, 50 %). In addition, the effect of these solvents was analyzed under short-term storage and resting conditions (resting solvent stability). Therefore, the proteins were stored with different solvents at different concentrations (see above) at 20 °C for 6 h. To

minimize the final concentrations of the solvents in the activity assay and to exclude effects on the activity, the solutions were highly diluted (soluble ATAs, see first paragraph) or, in case of immobilized ATAs, the immobilizates were thoroughly washed (3 times 50 mM Tris, 0.1 mM PLP).

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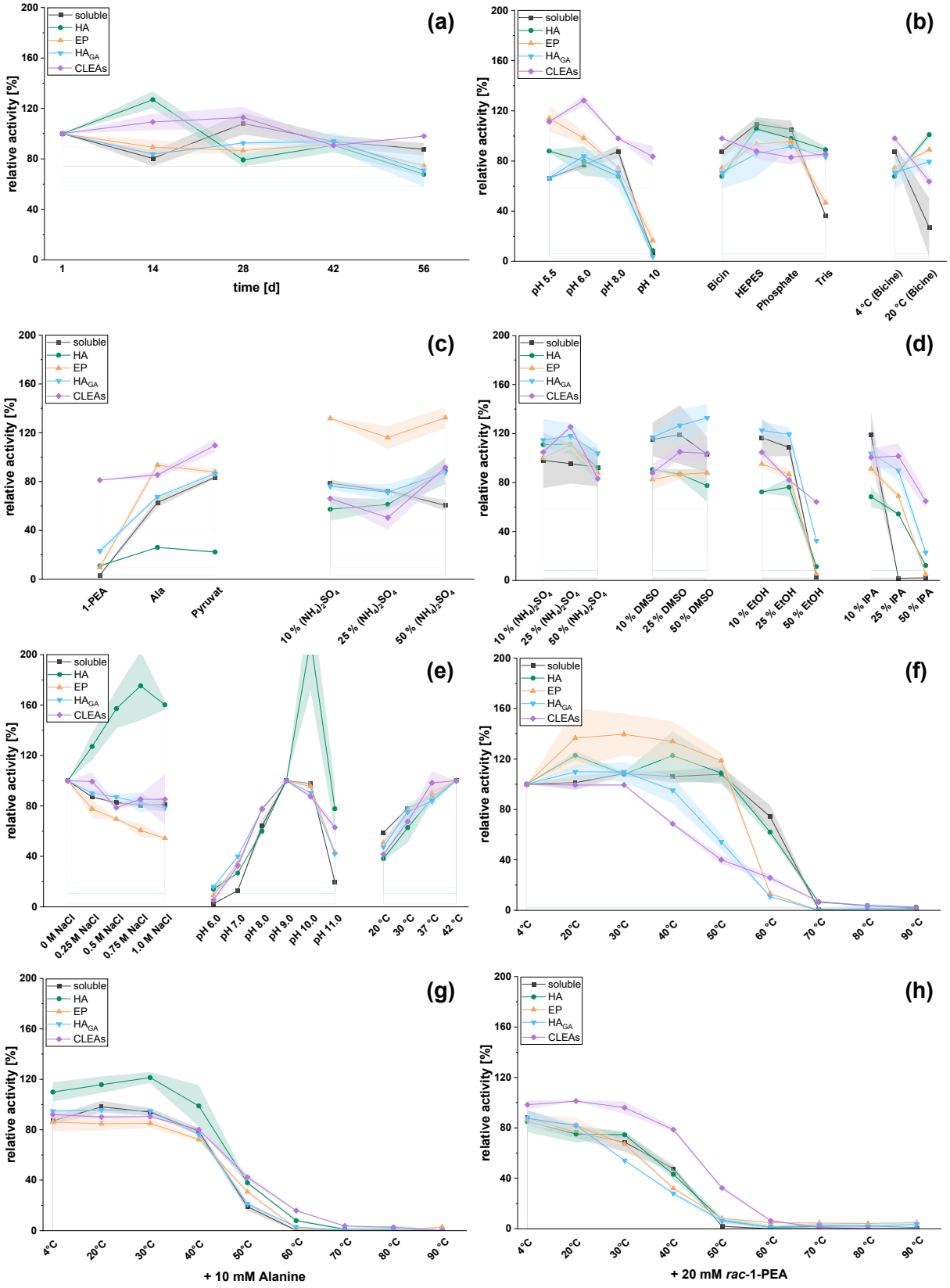
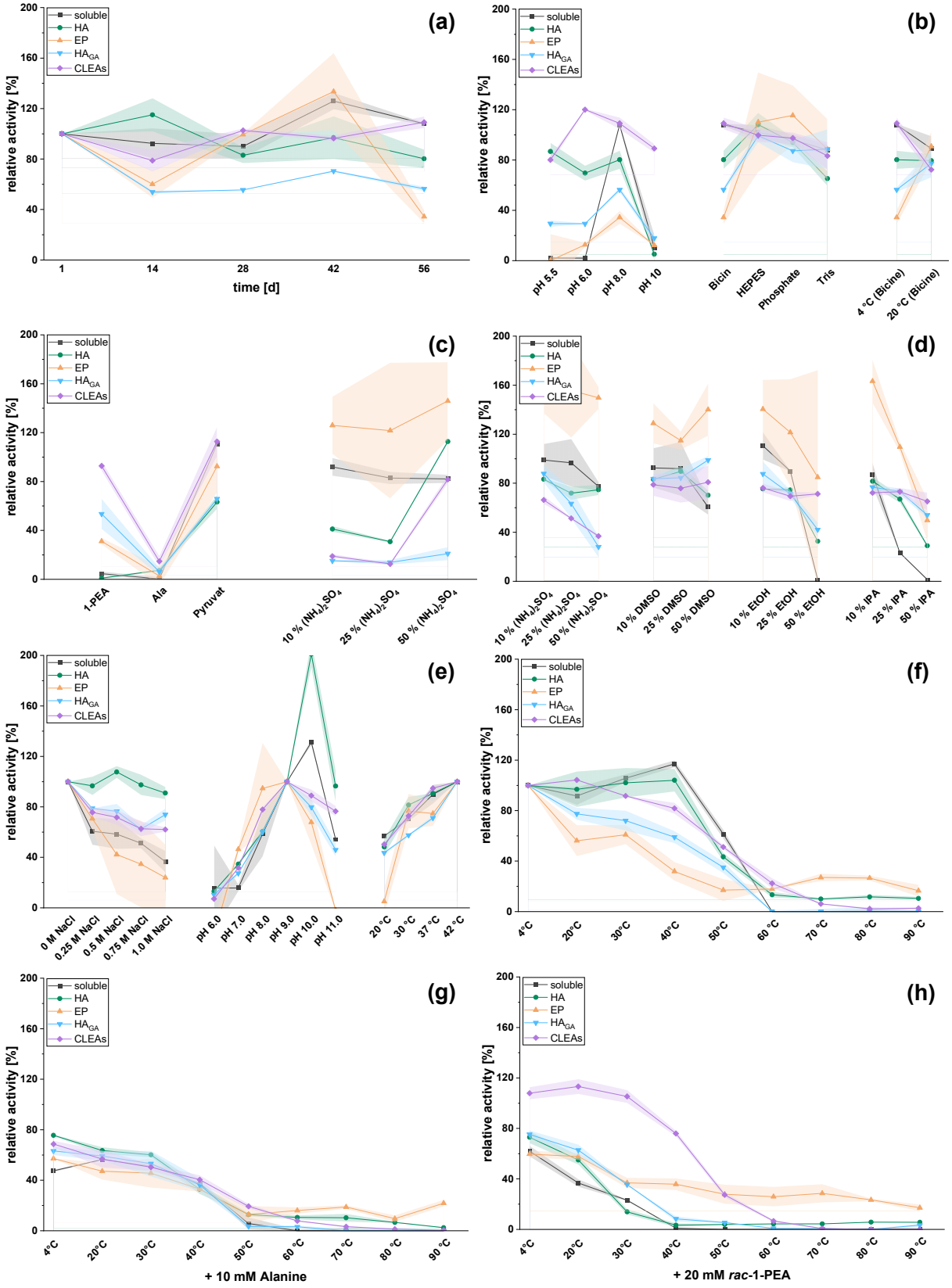
ATA-Vfl


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
soluble
HA
EP
HA$_{GA}$
CLEAs
relative activity [%]
time [d]
pH 5.5
pH 6.0
pH 8.0
pH 10
Bicin
HEPES
Phosphate
Tris
4 °C (Bicine)
20 °C (Bicine)
1-PEA
Ala
Pyruvat
10 % (NH$_4$)$_2$SO$_4$
25 % (NH$_4$)$_2$SO$_4$
50 % (NH$_4$)$_2$SO$_4$
10 % DMSO
25 % DMSO
50 % DMSO
10 % EtOH
25 % EtOH
50 % EtOH
10 % IPA
25 % IPA
50 % IPA
0 M NaCl
0.25 M NaCl
0.5 M NaCl
0.75 M NaCl
1.0 M NaCl
pH 6.0
pH 7.0
pH 8.0
pH 9.0
pH 10.0
pH 11.0
20 °C
30 °C
37 °C
42 °C
4 °C
20 °C
30 °C
40 °C
50 °C
60 °C
70 °C
80 °C
90 °C
+ 10 mM Alanine
+ 20 mM rac-1-PEA

ATA-3FCR-5M


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
soluble
HA
EP
HA$_{GA}$
CLEAs
relative activity [%]
time [d]
1
14
28
42
56
pH 5.5
pH 6.0
pH 8.0
pH 10
Bicin
HEPES
Phosphate
Tris
4 °C (Bicine)
20 °C (Bicine)
1-PEA
Ala
Pyruvat
10 % (NH$_4$)$_2$SO$_4$
25 % (NH$_4$)$_2$SO$_4$
50 % (NH$_4$)$_2$SO$_4$
10 % DMSO
25 % DMSO
50 % DMSO
10 % EtOH
25 % EtOH
50 % EtOH
10 % IPA
25 % IPA
50 % IPA
0 M NaCl
0.25 M NaCl
0.5 M NaCl
0.75 M NaCl
1.0 M NaCl
pH 6.0
pH 7.0
pH 8.0
pH 9.0
pH 10.0
pH 11.0
20 °C
30 °C
37 °C
42 °C
4°C
20°C
30°C
40°C
50°C
60 °C
70 °C
80 °C
90 °C
+ 10 mM Alanine
+ 20 mM *rac*-1-PEA

ATA-Lsy

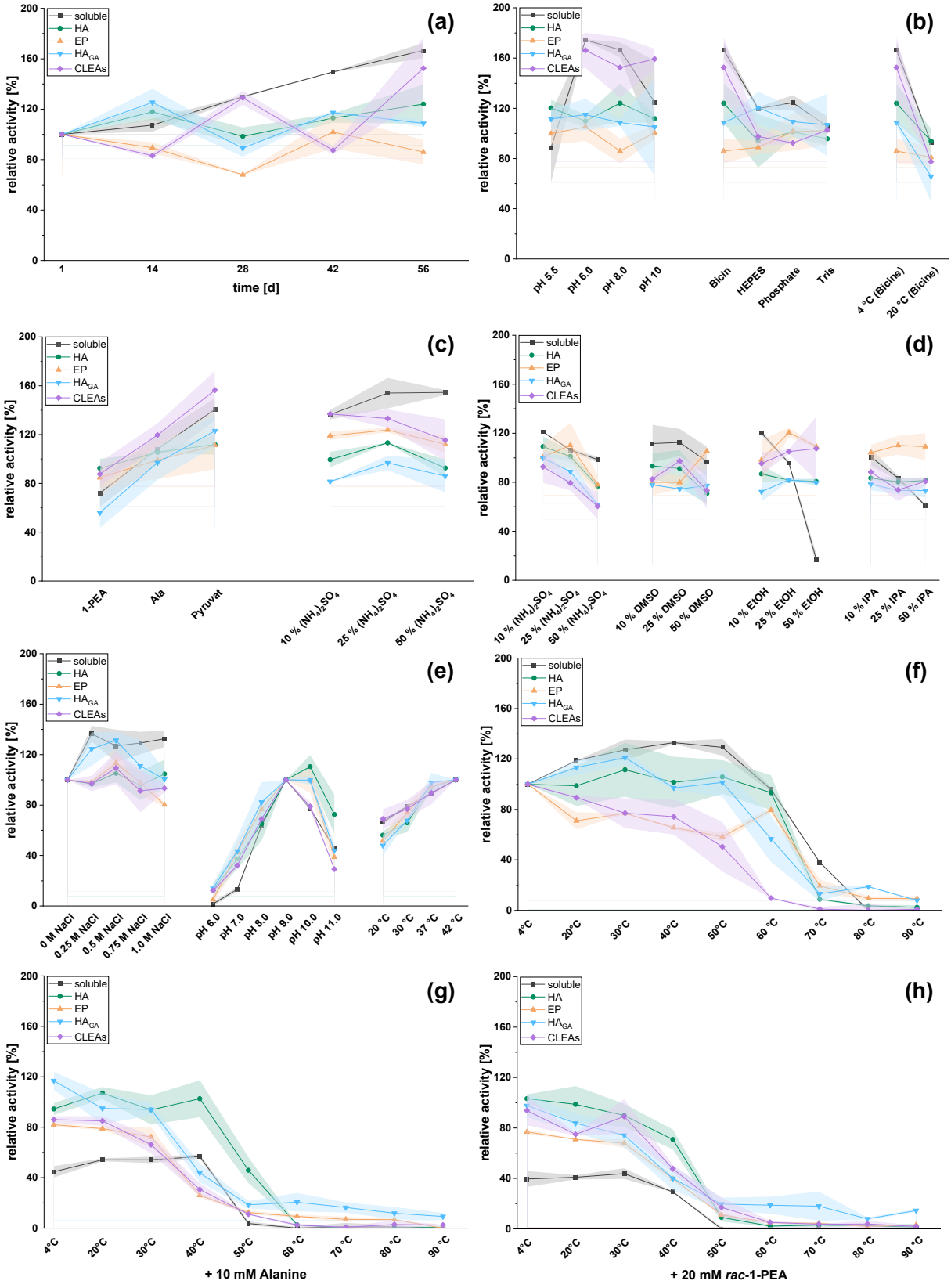

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
soluble
HA
EP
HA$_{GA}$
CLEAs
relative activity [%]
time [d]
1
14
28
42
56
pH 5.5
pH 6.0
pH 8.0
pH 10
Bicin
HEPES
Phosphate
Tris
4 °C (Bicine)
20 °C (Bicine)
1-PEA
Ala
Pyruvat
10 % (NH$_4$)$_2$SO$_4$
25 % (NH$_4$)$_2$SO$_4$
50 % (NH$_4$)$_2$SO$_4$
10 % DMSO
25 % DMSO
50 % DMSO
10 % EtOH
25 % EtOH
50 % EtOH
10 % IPA
25 % IPA
50 % IPA
0 M NaCl
0.25 M NaCl
0.5 M NaCl
0.75 M NaCl
1.0 M NaCl
pH 6.0
pH 7.0
pH 8.0
pH 9.0
pH 10.0
pH 11.0
20 °C
30 °C
37 °C
42 °C
4°C
20°C
30°C
40°C
50°C
60 °C
70 °C
80 °C
90 °C
+ 10 mM Alanine
+ 20 mM *rac*-1-PEA

Figure S2: Stability and reactivity of ATA-Vfl, ATA-3FCR-5M and ATA-Lsy under different conditions. The stabilities and reactivities of 1) ATA-Vfl, 2) ATA-3FCR-5M and 3) ATA-Lsy as soluble (black) and immobilized enzyme (on HA-beads (green), EP-beads (yellow), HAGA-beads (blue) or as CLEAs (purple)) under different conditions are presented analogously to Figure 1 in the main manuscript: **a)** storage stability under standard storage conditions (pH 8.0, bicine buffer, 0.1 mM PLP, 4°C, dark, exposed to air), **b-c)** storage stability over 56 days under various conditions (different pH, buffer and additives) while the standard storage conditions (see before) were fixed except for the varied condition, **d)** resting solvent stability after storage at room temperature for 6 h with different additives, **e)** reactivity at different salt concentrations, pH and temperatures while the standard reaction conditions (see below) were fixed except for the varied condition, **f-h)** thermostability: Incubation for 6 h without additives and with alanine or racemic 1-phenylethylamine (*rac*-1-PEA) at different temperatures. In all cases except e), the immobilizates were washed thoroughly (3x 50 mM Tris, 0.1 mM PLP) and the activity was subsequently detected under standard reaction conditions (50 mM Tris pH 8.0, 0.5 % DMSO, 0.1 mM PLP, 2.5 mM *rac*-1-PEA, 2.5 mM pyruvate). All reactions were done in duplicates and the standard deviations are shown as filled areas. Additional information can be obtained from the aforementioned description as well as from the methods.

3. Post-cross-linking and storage of dried beads

Post-cross-linking

Transaminases can be destabilized under operating conditions, as the presence of high amine concentrations allows catalysis of the first half reaction, which can lead to loss of the unbound PMP intermediate and thus dissociation of the two monomers and finally to inactivation. Furthermore, when only one monomer is covalently bound to a solid support, the other can be subsequently removed. Therefore, we aimed to analyze the effects of intermolecular cross-linking of the monomers after bead-immobilization to maintain the multimeric structure. After immobilization on the different beads, the enzymes were post-cross-linked with 0.5 % glutaraldehyde (1 h, 20 °C) and, after several washing steps (3x 50 mM Tris, 0.1 mM PLP), used for all series of experiments (i.e., temperature, storage, solvent stability experiments) analogous to the non-post-cross-linked beads. As a general result, post-cross-linking effects were dependent on the immobilization strategy and the enzyme used, with the least effect on ATA-3FCR-5M immobilized on HA_{GA}-beads. However, in all sets of experiments, the activity of the post-cross-linked immobilizates was lower than the activity of the non-post-cross-linked immobilizates (Figure S3), whereas the stability did not change significantly (data not shown): On average for all proteins and reactions, post-cross-linking decreased the activity to about 45 % (HA-), 55 % (EP-), and 75 % (HA_{GA}-beads). Therefore, this technique was disadvantageous.

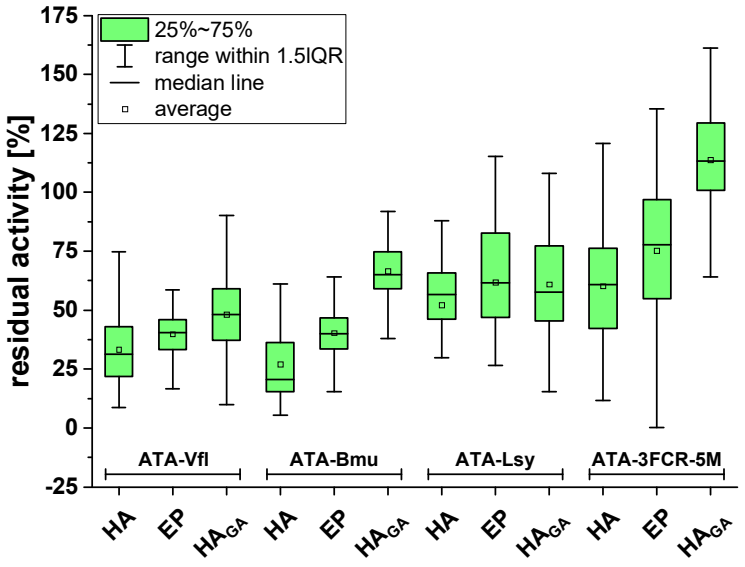


Figure S3: Effect of post-cross-linking on specific activity of the immobilizates. The transaminases were immobilized on solid supports (HA-, EP, HA_{GA}-beads) and subsequently cross-linked using 0.5 % glutaraldehyde (1 h, 20 °C). The beads were used for all sets of experiments after several washing steps (3x 50 mM Tris, 0.1 mM PLP), analogous to non-post-cross-linked beads and the observed activity was set in relation to that of the non-post-cross-linked beaded. The Boxplots visualize the distribution of all observed relative activities.

Storage of dried beads

Compared to dissolved materials, dried materials are potentially easier to transport and to store, saving costs and energy. They are also less subject to the effects of surrounding solvents and might be more stable due to the absence of interactions. To analyze the effect of drying the immobilized enzymes, all immobilized enzymes with and without post-cross-linking were dried after immobilization (supernatant was discarded and beads were dried at 40 °C under vacuum for 30 min) and the storability and thermostability were analyzed. Prior to the determination of the immobilized activity, the dried beads were suspended in the appropriate buffer. Generally, the activities of all dried immobilizates were found to consistently exhibit a mean activity decrease of approximately 75-85 % compared to the beads stored in buffer (Figure S4), excluding the drying of the immobilized beads. The loss of activity might be explained by conformational changes or denaturation of the enzymes upon drying and thus buffer removal. Immobilization and increased rigidification do not allow these enzymes to return to their native conformation, resulting in a loss of their enzyme activity.

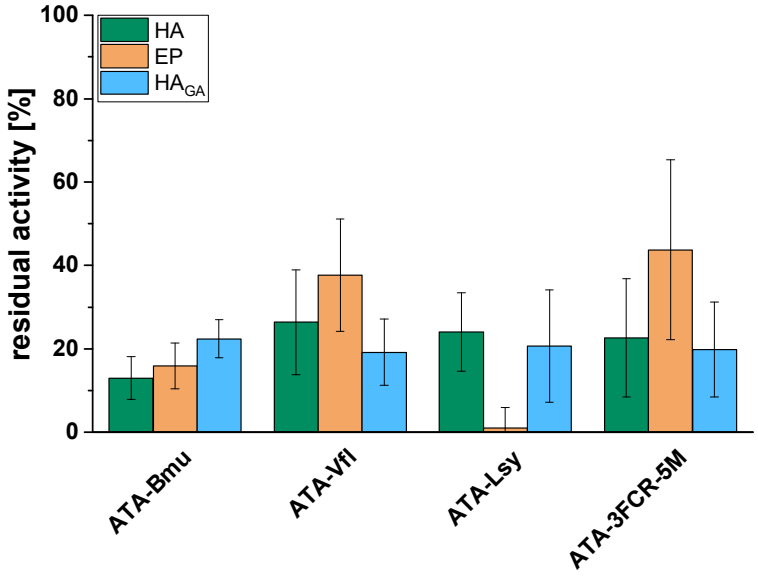


Figure S4: Effect of drying on specific activity of the immobilizates. After immobilization of the transaminases on solid supports (HA-, EP, HA$_{GA}$-beads), the supernatant was removed, and the beads were dried (40 °C, 30 min under vacuum). The activity was determined after suspending the beads in appropriate buffer, and the specific activity of the immobilizates was related to the activity of non-dried beads. Visualized are the mean values over all relative specific activities of the immobilizates and their standard deviations in error bars.

4. Kinetic resolution catalyzed by the final immobilizates

Chiral HPLC runs

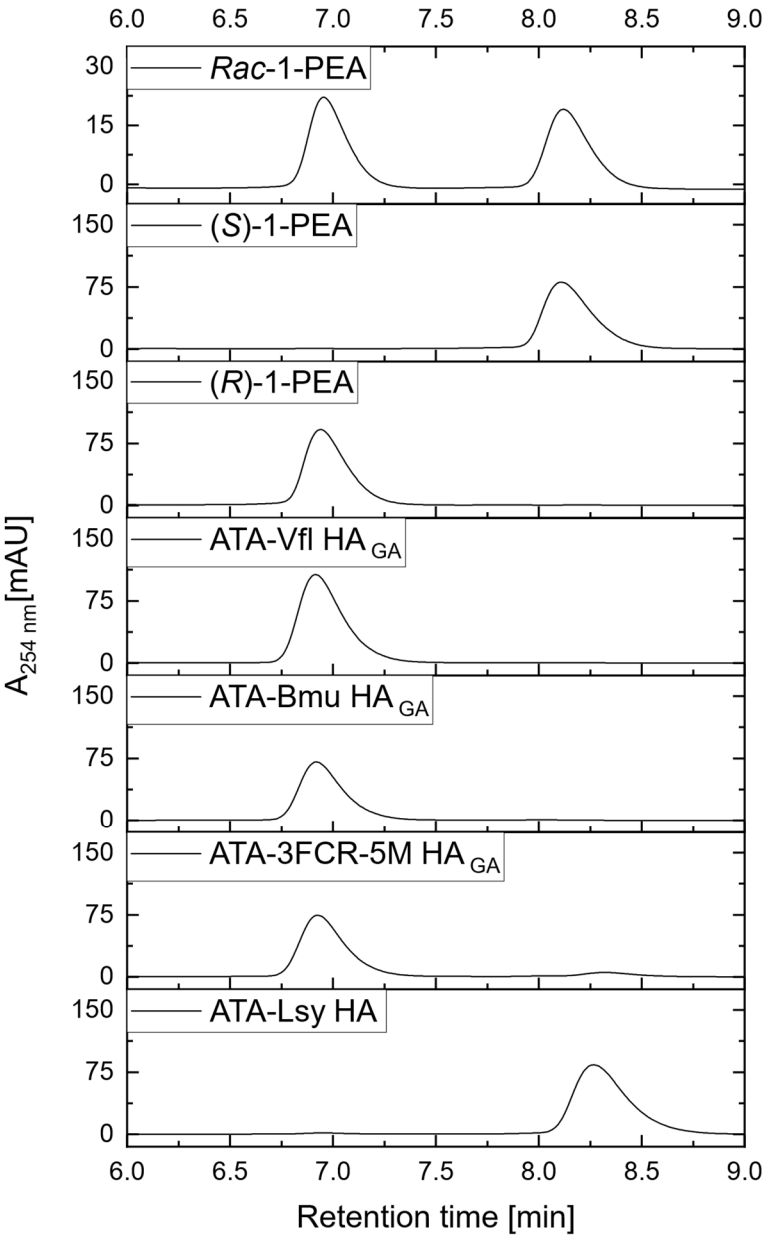
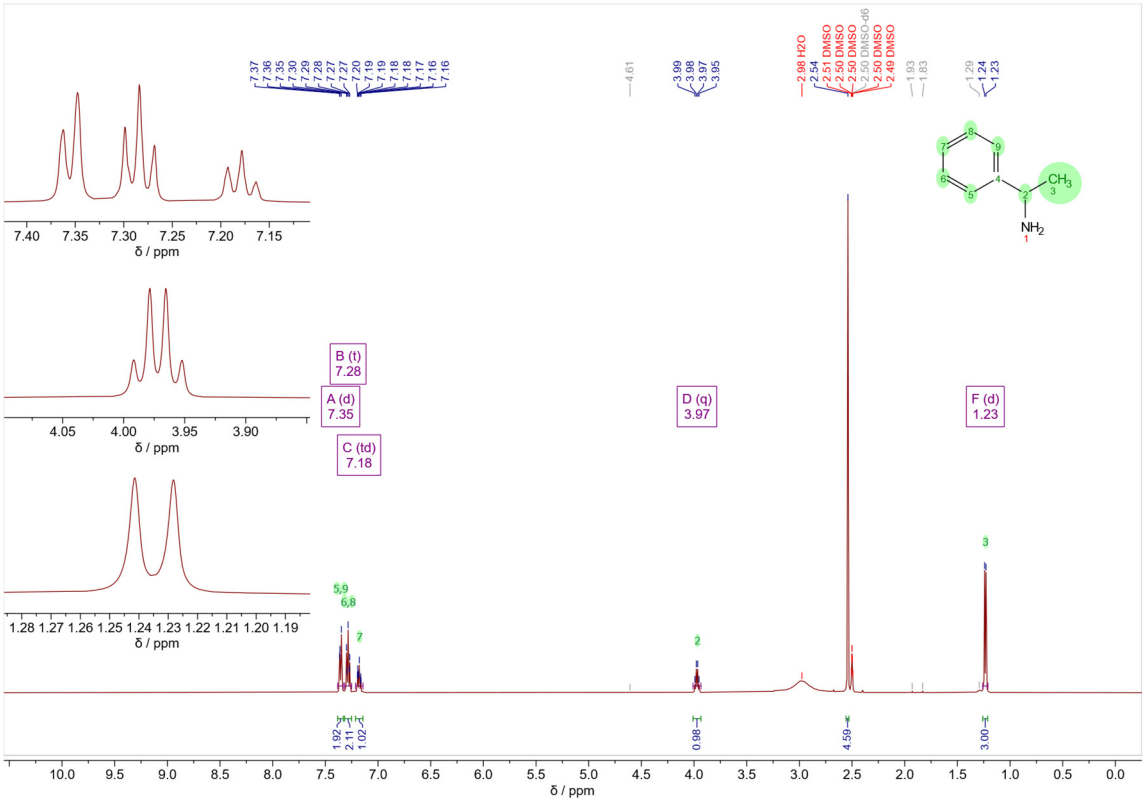


Figure S5: Chiral HPLC runs. The products of the kinetic resolutions catalyzed by the immobilized transaminases ATA-Vfl, ATA-Bmu, ATA-3FCR-5M and ATA-Lsy on HAGA- or HA-beads were extracted from the reaction solutions after complete conversion and analyzed by chiral HPLC (additional information in the main manuscript and methods). The enantioselectivity was maintained by immobilization as the (*S*)-enantiomer of *rac*-1-Phenylethylamine (*rac*-1-PEA) was converted by ATA-Vfl, ATA-Bmu and ATA-3FCR-5M and the (*R*)-enantiomer by ATA-Lsy. The enantiomeric excess was high in all reactions (ATA-Vfl HAGA: 99.8 %*ee*; ATA-Bmu HAGA: 98.8 %*ee*; ATA-3FCR-5M HAGA 87.8 %*ee*; ATA-Lsy HA: 98.2 %*ee*). Racemic as well as enantiomerically pure (*S*) and (*R*)-1-PEA served as control.

^{1}H-NMR



^{13}C-NMR

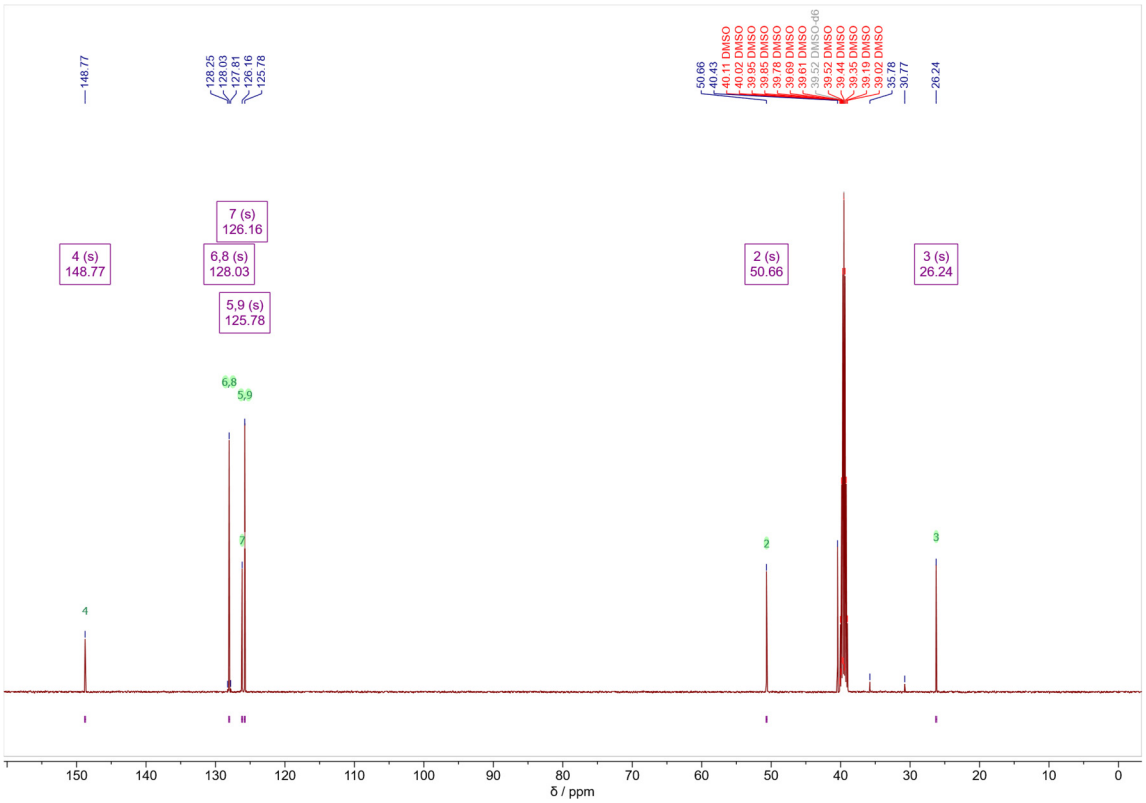


Figure S6: Exemplary NMR-analysis of (*S*)-1-PEA. The final extracted product of the kinetic resolution catalyzed by ATA-Lsy ((*S*)-1-phenylethylamine) was analyzed by NMR (upper panel: ^{1}H-NMR; lower panel: ^{13}C-NMR) in deuterated DMSO. ^{1}H NMR (500 MHz, DMSO) δ = 7.35 (d, *J*=7.0, 2H, Phenyl-C^{o}**H**), 7.28 (t, *J*=7.7, 2H, Phenyl-C^{m}**H**), 7.18 (td, *J*=7.0, 1.4, 1H, Phenyl-C^{p}**H**), 3.97 (q, *J*=6.6, 1H, benzyl-C**H**), 1.23 (d, *J*=6.6, 3H, -C**H**$_3$). ^{13}C NMR (126 MHz,

DMSO) δ = 148.77 (Phenyl-**C**^{i}), 128.03 (Phenyl-**C**^{m}), 126.16 (Phenyl-**C**^{p}), 125.78 (Phenyl-**C**^{o}), 50.66 (Benzyl-**C**), 26.24 (-**C**H_3).

Behavior of acetophenone and 1-phenylethylamine in bead-containing solutions

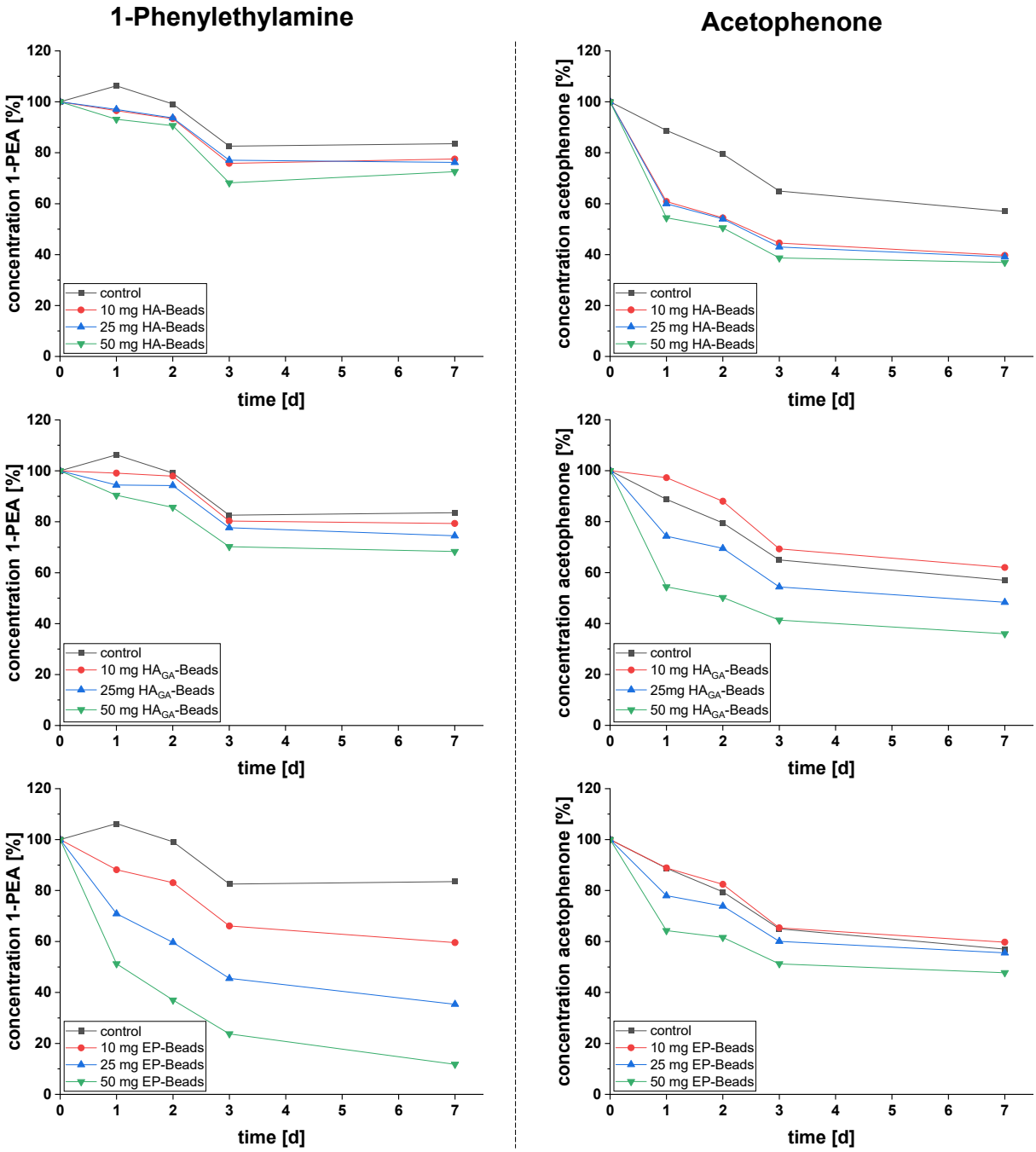


Figure S7: Behavior of acetophenone and 1-phenylethylamine in bead-containing solutions. Reaction solution (1 mL in 2 mL reaction vessels, 50 mM Tris; 0.1 mM PLP, 0.5 % DMSO, 10 mM *rac*-1-phenylethylamine, 10 mM acetophenone, pH 8.0)) was added to different amounts (10 mg (red), 25 mg (blue), 50 mg (green)) of beads (HA, HA$_{GA}$, EP) and incubated with shaking at 37 °C for seven days. On several days, the concentration of *rac*-1-phenylethylamine (1-PEA) and acetophenone was quantified by analytical HPLC and related to the starting concentrations. A control (reaction solution without beads) was treated analogously. All beads were treated as usual (sufficient solubilization, washing and blocking).

5. Cloning of transaminases

Table S3: Components and concentrations of the add-on- and OE-PCR. For the Add-On- and Overlap-Extension(OE)-PCR the PCR kit 2xKAPA HiFi HotStart ReadyMix (Roche) was used. First, the primers (synthesized from Thermo Scientific) were used in the Add-On-PCR to add sequences to the transaminase genes which are complementary to the sequences in pET-24b-X-AH. Second, the generated transaminase-amplicons were applied as megaprimers in the OE-PCR without further purification.

Component	Add-On PCR (25 µL reaction)	OE-PCR (50 µL reaction)
2x HiFi HotStart Ready Mix	1x	1x
10 µM forward primer[a]	0.3 µM	-
10 µM reverse primer[b]	0.3 µM	-
Transaminase plasmid[c]	1 ng	-
pET24b-X-AH[d]		15 ng
Amplicons of Add-ON-PCR		950 ng

[a] Forward primer used*: 1) generation of ATA-Bmu-AH: aaggagatatacatatg cta *tcttacaacgaagcgaaattc*, generation of ATA-3FCR-5M-AH: aaggagatatacatatg *ctgaaaaacgaccaactgg*, 3) generation of ATA-Lsy-AH: aaggagatatacatatg t *tgtccgatgaaccg*,

[b] Reverse primer used*: 1) generation of ATA-Bmu-AH: gagtacataaactagcact *atccttcgcaaccggaac*, generation of ATA-3FCR-5M-AH: gagtacataaactagcact *acccagaacggctttgac*, 3) generation of ATA-Lsy-AH: gagtacataaactagcact *cagcggtgttgcataacg*

[c] Transaminase plasmids used: 1) pET22b-ATA-Bmu, 2) pET22b-ATA-3FCR-5M, 3) pET24b-ATA-Lsy

[d] pET24b-X-AH was prepared and previously designated as pET24b-X-CTPSR-H6 in [46].

* Underlined sequences: complementary sequences added for OE-PCR); italic sequences: sequences for the amplification of the transaminase.

Table S4: PCR program for the add-on- and OE-PCRs.

Step	Cycles	Add-On-PCR		OE-PCR	
		Temperature [°C]	Duration [sec]	Temperature [°C]	Duration [sec]
Initial denaturation		95	180	95	180
Denaturation	4	98	20	-	-
Annealing		61/59*	20	-	-
Extension		72	120	-	-
Denaturation	24	98	20	98	20
Annealing		72	20	45	30
Extension		72	180	72	480
Final extension		72	360	72	600
Storage		4	-	4	-

* The annealing temperature used 61 °C for ATA-Bmu-AH and ATA-3FCR-5M-AH and 59 °C for ATA-Lsy-AH generation.

6. Nucleic acid sequences

The sequences of ATA-Vfl, ATA-Vfl-AH and Δ72-hFGE were previously published [46].

Green: Transaminase; Blue: Aldehyde-Tag; Purple: His-Tag; Black: Sequences of respective vectors.

Open reading frames of ATA-Bmu, ATA-3FCR-5M and ATA-Lsy in respective plasmids without aldehyde-tag

1) ATA-Bmu in pET22b

atgatttcttacaacgaagcgaaattctggcacccgatgctgcacccgaacgaaatgaaacgtcgtaaaccgatccgtatcgttcgtggtgacggttgctacgttttcga
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ctga

2) ATA-3FCR-5M in pET22b

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3) ATA-Lsy in pET24b

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Open reading frames of ATA-Bmu, ATA-3FCR-5M and ATA-Lsy in pET24b-X-AH

1) ATA-Bmu

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2) ATA-3FCR-5M

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ccaccaccactga

3) ATA-Lsy

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gcaggtcgtaccattcgtggcaccacaccgggtccgattacccaggcaattgataatgcatattgggcaatgcgtgaaaccgatcgttatgcaacaccgctgagtgcta
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7. Purification of Δ72-hFGE

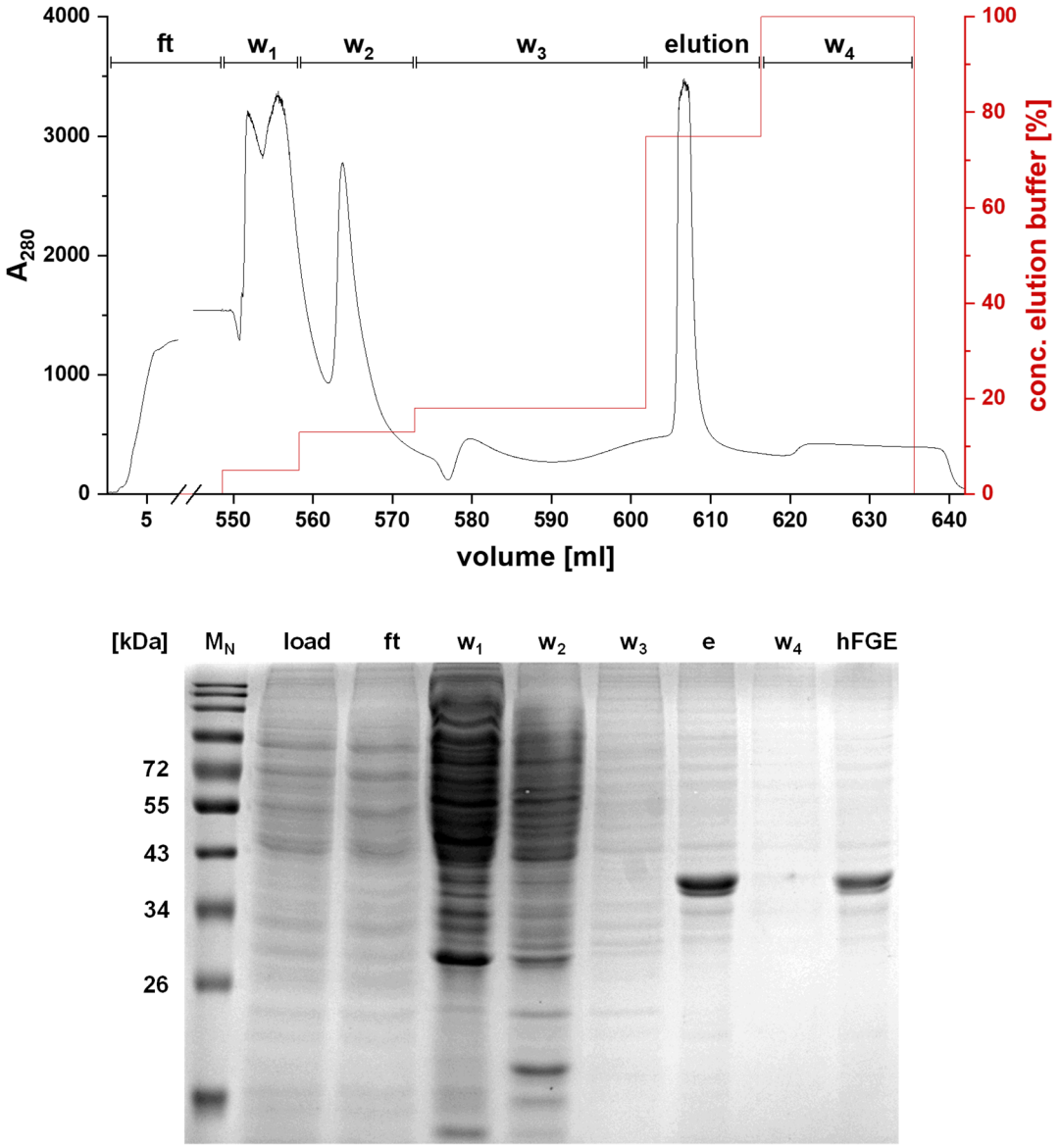

Figure S8: Purification of Δ72-hFGE. Purification of Δ72-hFGE was performed as described in the methods. Left: Chromatogram showing the absorbance at 280 nm (black) and the applied step gradient (red). The respective fractions (flowthrough: ft; wash steps 1-4: w_1-w_4; elution of Δ72-hFGE: elution) are indicated. Right: SDS-gel showing each fraction of the purification, in addition with the load (filtered supernatant of the insect expression culture) and the final dialyzed and concentrated Δ72-hFGE (hFGE). M_N: Color Prestained Protein Standard (#P7719; NEB)

8. Fluorescent-labeling of FGly

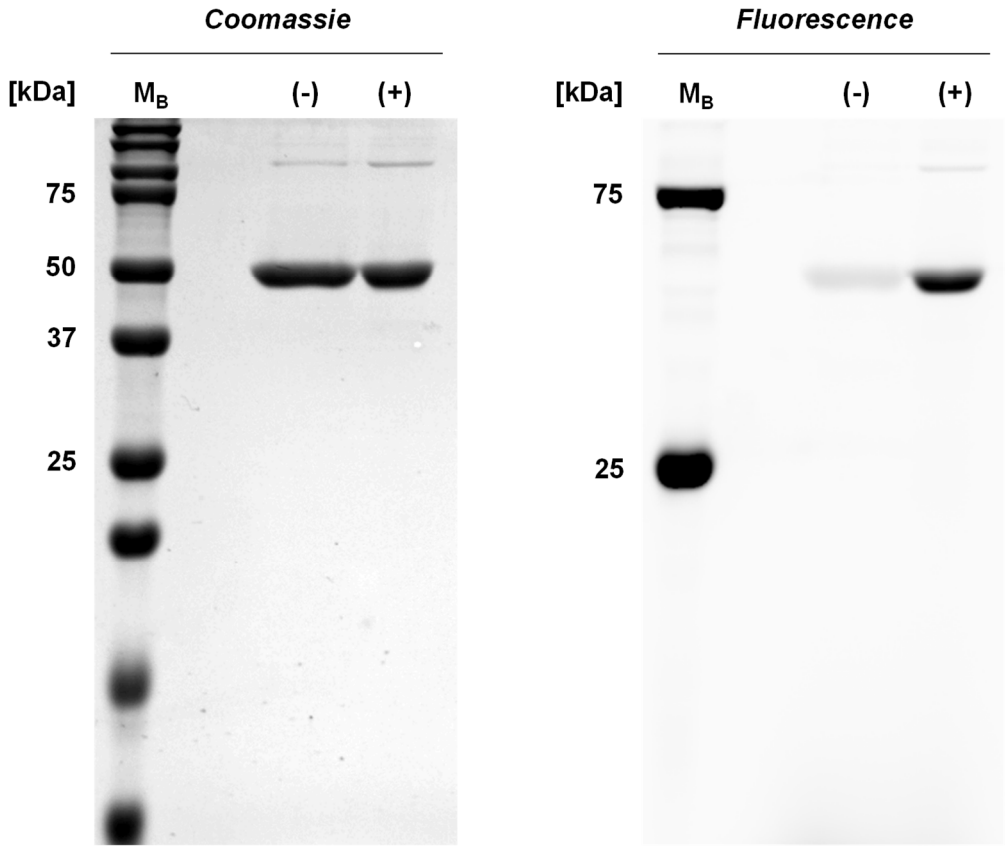

Figure S9: Fluorescent-labeling of Formylglycine (exemplary for ATA-Vfl-AH). To prove the conversion of the aldehyde-tag by the Δ72-hFGE, equimolar amounts of the converted (+) and unconverted (-) ATA-Vfl-AH (approx. 50 kDa) were analyzed by fluorescent-labeling and SDS-PAGE as described in the methods. ATA-Vfl-AH was selected to show exemplary the qualitative prove of conversion for all tagged transaminases. Left: Coomassie-staining of the SDS-gel. Right: In-gel fluorescence of the SDS-gel. MB: prestained molecular weight protein standard with two fluorescent bands at 25 and 75 kDA (#1610374; Bio-Rad).

9. Supporting references

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