

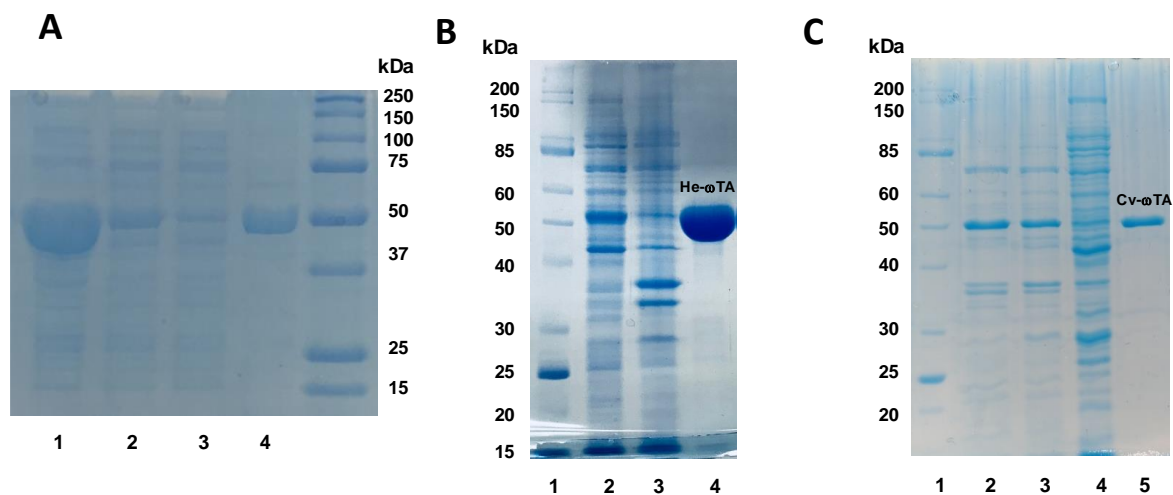
## Self-sufficient flow-biocatalysis by co-immobilization of pyridoxal 5'-phosphate and $\omega$ -transaminases onto porous carriers

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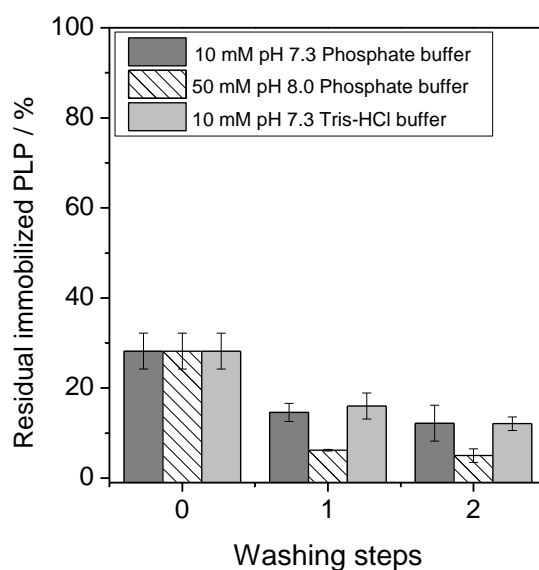
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## 1. Protein purification



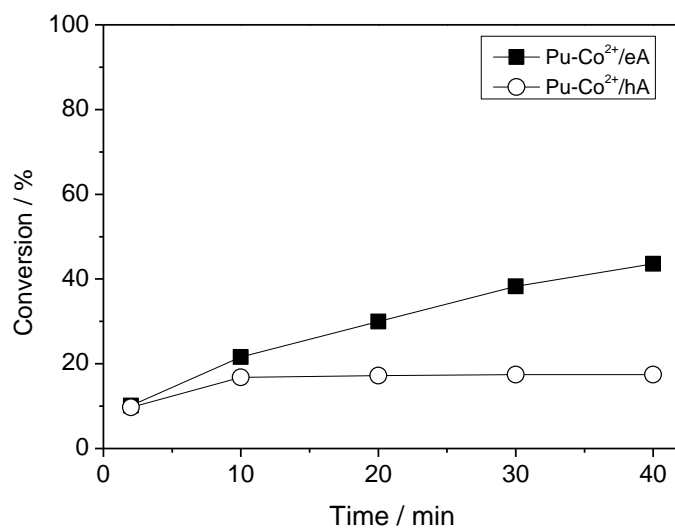
**Figure S1. SDS-PAGE of the purification of the enzymes.** A) Pf- $\omega$ TA. Line 1: crude extract, line 2: soluble fraction after cell disruption by sonication, line 3: flow through after IMAC purification, line 4: purified enzyme and line 5: molecular weight markers (BioRad Precision Plus Protein All Blue Standard) B) He- $\omega$ TA. Line 1: molecular weight markers (NEB Broad Range 10-200 kDa), line 2: crude extract, line 3: pellet after sonication and line 4: purified He- $\omega$ TA. C) Cv- $\omega$ TA. Line 1: molecular weight markers (NEB Broad Range 10-200 kDa), line 2: crude extract, line 3: soluble fraction after sonication, line 4: insoluble fraction after sonication and line 5: purified Cv- $\omega$ TA.

## 2. Cofactor immobilization

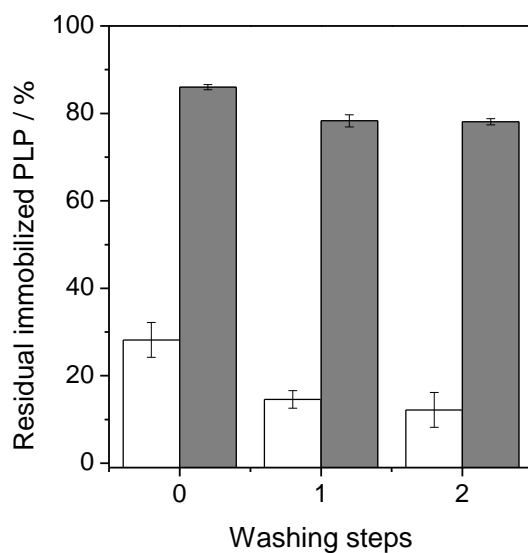


**Figure S2.** Optimization of ionic strength and pH of the buffer for the PLP immobilization. Pu-Co<sup>2+</sup>/eA beads were incubated with 1 mM PLP prepared in different buffered solutions for 1 hour at room temperature.

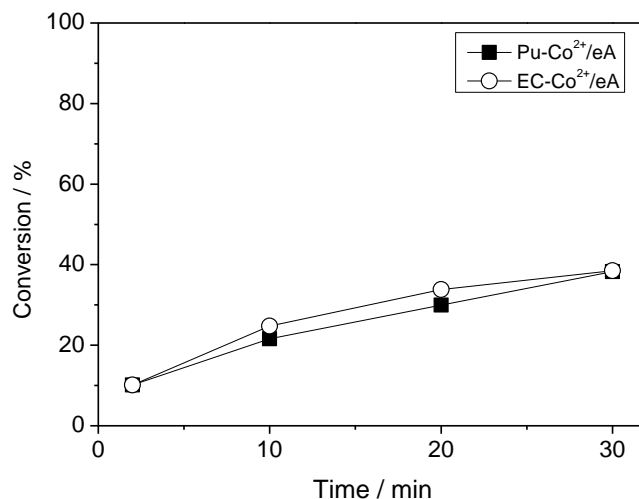
### 3. Enzyme kinetics



**Figure S3.** Effect of amine group on the enzyme kinetics. He- $\omega$ TA was immobilized onto Pu-Co<sup>2+</sup>/eA and Pu-Co<sup>2+</sup>/hA beads at 1 mg x g<sup>-1</sup>. PLP was co-immobilized at  $1.7 \pm 0.09 \mu\text{mol} \times \text{g}^{-1}$  onto Pu-Co<sup>2+</sup>/eA and  $6.5 \pm 0.01 \mu\text{mol} \times \text{g}^{-1}$  onto Pu-Co<sup>2+</sup>/hA.



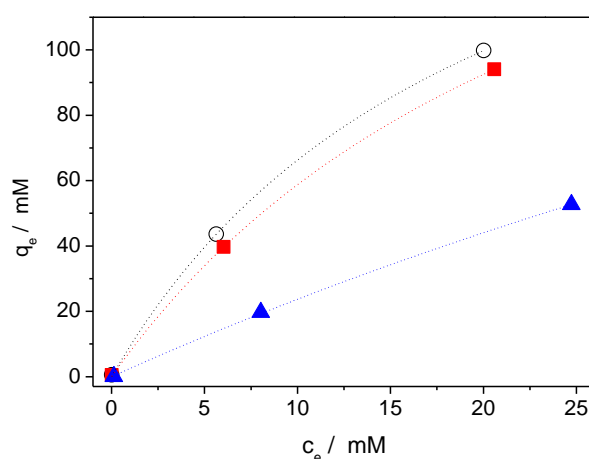
**Figure S4.** Immobilization of PLP on different methacrylate-based carriers. Pu-Co<sup>2+</sup>/eA (white bars) and EC-Co<sup>2+</sup>/eA (grey bars) were incubated with 1 mM PLP (1:10 v/w) in 10 mM phosphate buffer at pH 7.3. Washing steps were carried out with the same buffer.



**Figure S5.** Effect of methacrylate-based carrier on the enzyme kinetics. He- $\omega$ TA was immobilized onto Pu-Co<sup>2+</sup>/eA<sup>+</sup> and EC-Co<sup>2+</sup>/eA beads at 1 mg x g<sup>-1</sup>. PLP was co-immobilized at 1.7 ± 0.09  $\mu$ mol x g<sup>-1</sup> onto Pu-Co<sup>2+</sup>/eA and 7.8 ± 0.03  $\mu$ mol x g<sup>-1</sup> onto EC-Co<sup>2+</sup>/eA.

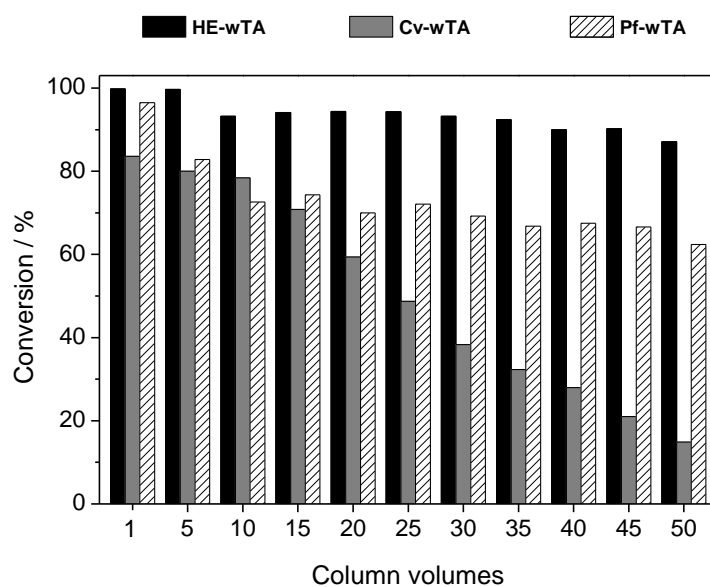
#### 4. Langmuir Isotherms

0.1 g of the three different aminated carriers was incubated with 1mL of (0-50 mM) PLP solution in sodium phosphate buffer pH 7.3 for 1 hour. The PLP concentration in the supernatant was determined to calculate the non-bound and the bound PLP concentration. These concentration values were fitted into the Langmuir isotherm equation:  $q_e = (q_{max} \times C_e) / (K_d + C_e)$ , where  $q_e$  is the bound PLP at equilibrium,  $C_e$  is the non-bound PLP at equilibrium,  $q_{max}$  is the maximum binding capacity of the carrier and  $K_d$  the PLP dissociation constant.



**Figure S6.** Adsorption isotherms of PLP. Three methacrylate-based carriers activated with PEI (white circles), ethanolamine (red squares) and hydroxylamine (blue triangles) were used to immobilize PLP. The Y-axis shows the PLP load at the equilibrium ( $q_e$ ) and the X-axis the free PLP at the equilibrium ( $c_e$ ) in the supernatant. Experimental data were fitted to the Langmuir equation ( $q_e = q_{max} \times C_e / (K_d + C_e)$ ), where  $q_{max}$  means the maximum load of the carrier and  $K_d$  the equilibrium constant.

## 5. Flow deamination reactions



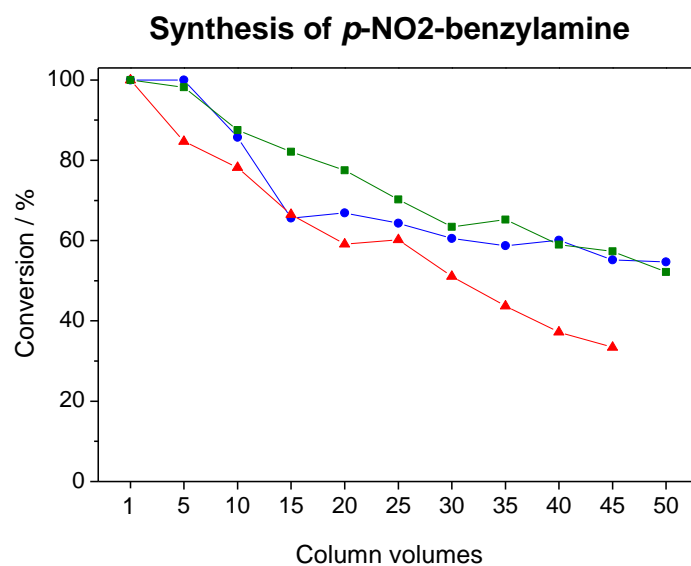
**Figure S7.** Stability test of the biocatalysts with immobilized  $\omega$ -transaminases ( $5 \text{ mg} \times \text{g}^{-1}$ ) onto EC-Co<sup>2+</sup>/PEI. Reactions using nor immobilized PLP neither exogenously added. The continuous flow reaction was performed at  $1.45 \text{ mL} \times \text{min}^{-1}$ . Each column volume corresponds to 1 minute.

## 6. Preparation of biocatalyst for stability assay

**Table S1.** Immobilization and activity parameters of co-immobilized Pf- $\omega$ TA and PLP on EC-Co<sup>2+</sup>/PEI. The activity was monitored at high shaking orbital speed (800 rpms).

Enzyme	Immobilization yield (%)	Cofactor load ( $\mu\text{mol}_{\text{PLP}} \times \text{g}_{\text{carrier}}^{-1}$ )	Relative recovered activity (%)
	$92 \pm 0.26$	0	$78 \pm 1.2$
PfwTA	$91 \pm 0.04$	1	$120 \pm 10$
	$94 \pm 0.22$	10	$111 \pm 15$

## 7. Flow synthesis of amines



**Figure S8.** Continuous synthesis of *p*-nitrobenzylamine adding free PLP (blue circles), no adding PLP (red triangles) and co-immobilizing PLP (green squares). He- $\omega$ TA was first immobilized on EC-Co<sup>2+</sup>/PEI at 5 mg x g<sup>-1</sup>. The flow reactions were performed at 0.725 mL x min<sup>-1</sup>. Each column volume corresponds to 2 minutes.

## 8. Raw data of sustainable metrics for the continuous synthesis of cyanamylamine

**Table S2.** Raw data of sustainable metrics extracted from Figure 7. Mass of all reactant was calculated for each reaction cycle to determine the total mass of each one after the 50 reaction cycles under the flow-conditions described for the synthesis of amines.

			Cynamaldehyde	Alanine	Cinamylamine	Pyruvate	PLP	DMSO	Water	Biocatalyst
			Mw	132,16	89,09	133,19	88,06	274,142	78,13	18
			mM	10	500			0	128	1
			volumen	1,4	1,4	1,4	1,4	1,4	1,4	1,4
<b>IMMOBILIZED PLP</b>									(g)	(g)
Cycles	Conversion	Reaction Media	Reactants (µmol)							
1	1	1	0,00	686,00	14,00	14,00	0,00	179,20	1,40	1,00
5	1	1	0,00	2744,00	56,00	56,00	0,00	716,80	5,60	0,00
10	0,82	1	12,60	3442,60	57,40	57,40	0,00	896,00	7,00	0,00
15	0,8	1	14,00	3444,00	56,00	56,00	0,00	896,00	0,00	0,00
20	0,77	1	16,10	3446,10	53,90	53,90	0,00	896,00	7,00	0,00
25	0,7	1	21,00	3451,00	49,00	49,00	0,00	896,00	28,00	0,00
30	0,63	1	25,90	3455,90	44,10	44,10	0,00	896,00	35,00	0,00
35	0,62	1	26,60	3456,60	43,40	43,40	0,00	896,00	0,00	0,00
40	0,62	1	26,60	3456,60	43,40	43,40	0,00	896,00	35,00	0,00
45	0,63	1	25,90	3455,90	44,10	44,10	0,00	896,00	140,00	0,00
50	0,64	1	25,20	3455,20	44,80	44,80	0,00	896,00	175,00	0,00
<b>Total µmoles</b>			193,90	34493,90	506,10	506,10	0,00	8960,00		
<b>total grams</b>			0,03	3,07	0,07	0,04	0,00	0,70	434,00	1,00
<b>Total E factor</b>	<b>6510</b>	Specific Contribution	0,38	45,59		0,66	0,00	10,39	6438,46	14,84
		Relative Contribution (%)	0,01	0,70		0,01	0,00	0,16	98,90	0,23
<b>E factor no solvent</b>	<b>61</b>	Specific Contribution	0,38	45,59		0,66	0,00			14,84
		Relative Contribution (%)	0,62	74,17		1,08	0,00			24,14
<b>Atom economy</b>	<b>60</b>									
			cynamaldehyde	Alanine	Cinamylamine	pyruvate	PLP	DMSO	water	
			Mw	132,16	89,09	133,19	88,06	274,142	78,13	18
			mM	10	500			0,1	128	1
			volumen	1,4	1,4	1,4	1,4	1,4	1,4	1,4
<b>FREE PLP</b>									(g)	(g)
Cycles	Conversion	Reaction Media	Reactants (µmol)							
1	1	1	0,00	686,00	14,00	14,00	0,14	179,20	1,40	1,00
5	1	1	0,00	2744,00	56,00	56,00	0,56	716,80	5,60	0,00
10	0,82	1	12,60	3442,60	57,40	57,40	0,70	896,00	7,00	0,00
15	0,5	1	35,00	3465,00	35,00	35,00	0,70	896,00	0,00	0,00
20	0,55	1	31,50	3461,50	38,50	38,50	0,70	896,00	7,00	0,00
25	0,5	1	35,00	3465,00	35,00	35,00	0,70	896,00	28,00	0,00
30	0,52	1	33,60	3463,60	36,40	36,40	0,70	896,00	35,00	0,00
35	0,5	1	35,00	3465,00	35,00	35,00	0,70	896,00	0,00	0,00
40	0,51	1	34,30	3464,30	35,70	35,70	0,70	896,00	35,00	0,00
45	0,53	1	32,90	3462,90	37,10	37,10	0,70	896,00	140,00	0,00
50	0,55	1	31,50	3461,50	38,50	38,50	0,70	896,00	175,00	0,00
<b>Total µmoles</b>			281,40	34581,40	418,60	418,60	7,00	8960,00		
<b>total grams</b>			0,04	3,08	0,06	0,04	0,002	0,700	434,00	1,00
<b>Total E factor</b>	<b>7871</b>	Specific Contribution	0,67	55,26		0,66	0,03	12,556	7784,288	17,94
		Relative Contribution (%)	0,01	0,70		0,01	0,00	0,160	98,893	0,23
<b>E factor no solvent</b>	<b>75</b>	Specific Contribution	0,67	55,26		0,66	0,03			17,94
		Relative Contribution (%)	0,89	74,12		0,89	0,05			24,06
<b>Atom economy</b>	<b>27</b>									