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ELECTRONIC SUPPORTING INFORMATION

Development of an amine transaminase-lipase cascade for chiral amide synthesis under flow conditions

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Content:

1. Immobilisation of pure protein – Immobilisation Yield [%] and Protein Loading [mg/g]

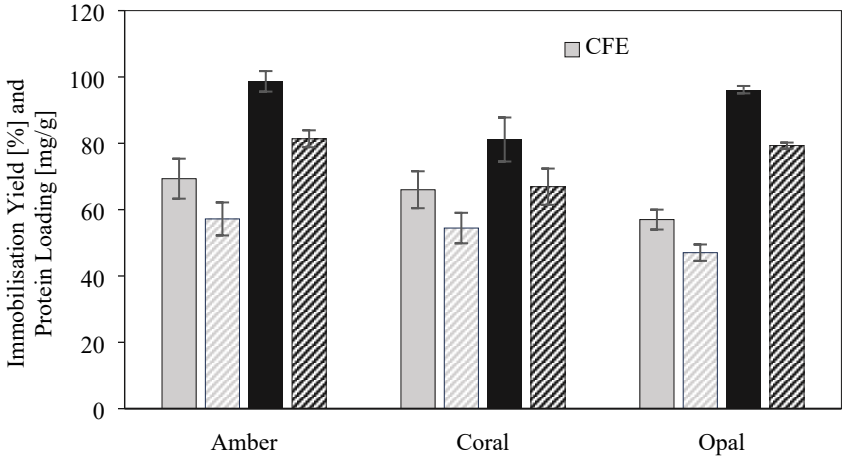

Figure S1. Immobilisation yield (filled) and protein loading (dashed) of the ArRmut11ATA on EziG Amber, Coral and Opal. Immobilisation from CFE: 0.5 mL of 15 mg/mL of CFE resuspended in 20 mM sodium phosphate buffer with 0.3 mM PLP, pH 8.0, was added to 10 mg of carrier material. Immobilisation from pure protein: 434 µL of the pure protein solution in 50 mM MOPS buffer with 0.3 mM PLP, pH 7.6, was added to 10 mg of carrier material. In both cases: Tubes were covered with foil and immobilisation was performed on an end-over-end rotator, 20 rpm, at room temperature. 50 µL of the enzyme supernatant were withdrawn after 2 h for kinetic analysis and diluted for the spectrophotometric assay containing 10 mM 1-phenylethylamine and 10 mM pyruvate in 20 mM sodium phosphate buffer pH 8.0. Total volume for the spectrophotometric assay was 200 µL. All the measurements were performed in duplicates and the results are presented as the mean of the individual samples.

2. Initial rates of immobilised and free enzyme

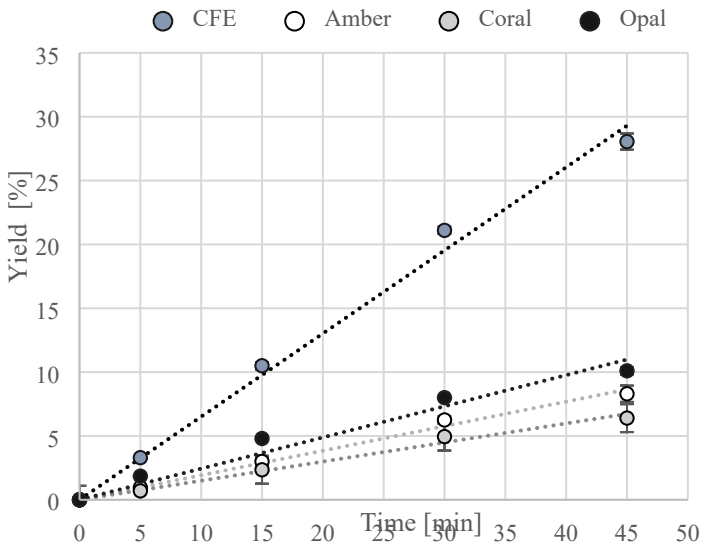

Figure S2. The recovered activity gives the percentage of activity that is retained after immobilisation. The recovered activity was obtained from the specific activity of immobilised enzyme compared to the specific activity of CFE in aqueous based assay. The specific activity was determined using initial reaction rates, considering the amount of enzyme used in the reaction. For the immobilised enzyme, the immobilisation yield was included in the calculations to consider only the amount of immobilised enzyme. Biotransformation conditions: 50 mM 1-phenoxypropan-2-one, 250 mM IPA, 5 v/v% DMSO in 20 mM sodium phosphate buffer, pH 8. CFE (7.5 mg) and ArRMut11ATA-EziG (10 mg of EziG with 0.5 mL of 15 mg/mL CFE) in 1 mL total reaction volume, 37 °C, 1200 rpm. The reactions were run for 0, 5, 15, 30 and 45 min. Each reaction was performed in duplicate, and a single reaction was conducted for each time point. Yields were determined using GC after quenching with 5 M NaOH and extraction (yield considering total amine formation, including both (R)- and (S)-enantiomers).

3. Water profile of ArRmut11ATA immobilised on Amber

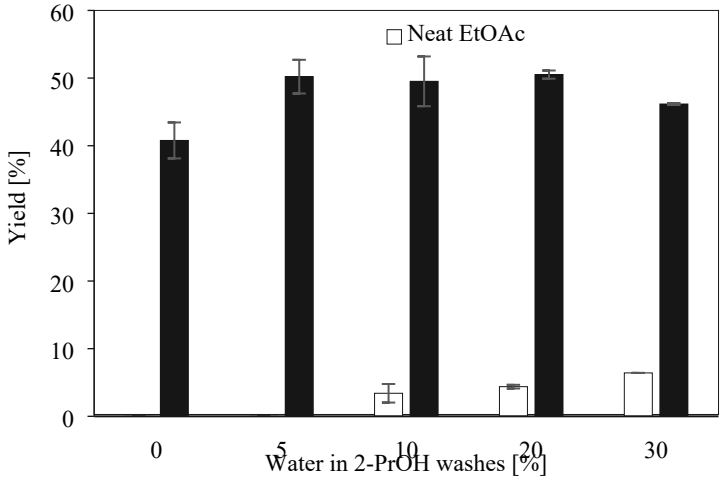

Figure S3. Yield data from the transformation of 1-phenoxypropan-2-one using ArRMut11ATA immobilised on Amber containing varying levels of water (0-30 v/v%). Biotransformation conditions: 100 mM 1-phenoxypropan-2-one, 250 mM IPA, neat or water saturated ethyl acetate, ArRMut11ATA-EziG (10 mg of EziG with 0.5 mL of 15 mg/mL CFE) in 1 mL total reaction volume, 18 h reaction time, 37 °C, 1200 rpm. Reactions were performed in duplicate, and yields were determined using GC, after derivatisation of the samples with acetic anhydride.

4. Activity in organic solvent

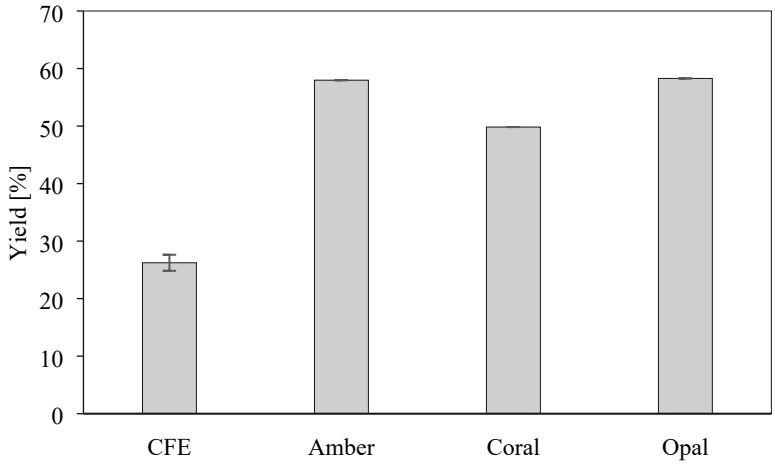

Figure S4. Activity of the CFE and immobilised catalysts in organic solvent based on the conversion of 1-phenoxypropan-2-one to 1-phenoxypropan-2-amine. Biotransformation conditions: 100 mM 1-phenoxypropan-2-one, 250 mM IPA, 3% water in ethyl acetate, 1 mL total reaction volume, 18 h, 37 °C, 1200 rpm. The amount of CFE used in the reactions was 7.5 mg, corresponding to 0.825 mg of target protein. The amount of immobilised catalysts was 10 mg, which corresponds to an amount of immobilised target protein of 0.621, 0.588 and 0.495 mg for Amber, Coral and Opal, respectively. Reactions were performed in duplicate, and yields were determined using GC, after derivatisation of the samples with acetic anhydride.

5. Labelled image of the flow setup

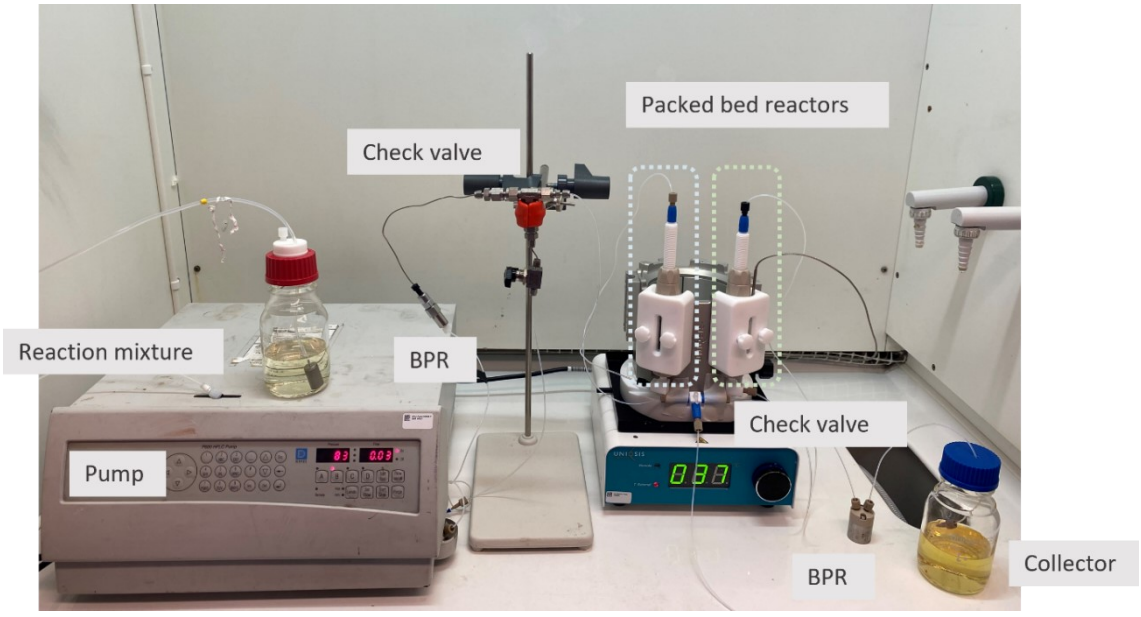

Figure S5. Labelled image of the flow setup.

6. GC chromatogram of the 1-phenoxypropan-2-one incubated with isopropylamine

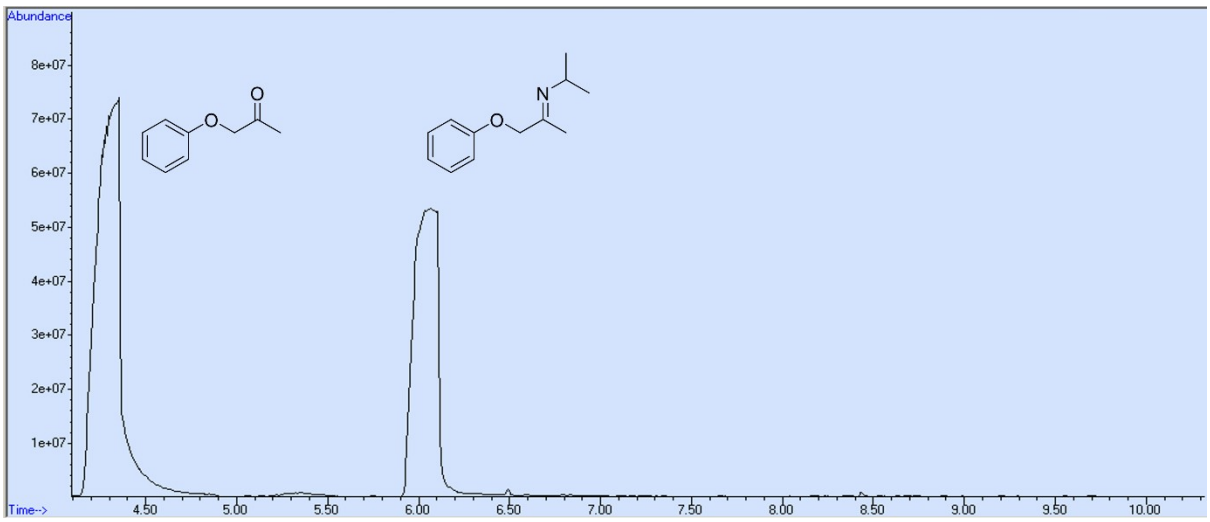

Figure S6. GC-FID chromatogram of 1-phenoxypropan-2-one (peak at 4.3 min) and *N*-isopropyl-1-phenoxypropan-2-imine (peak at 6.0 min).

7. MS analysis of the 1-phenoxypropan-2-one and *N*-isopropyl-1-phenoxypropan-2-imine

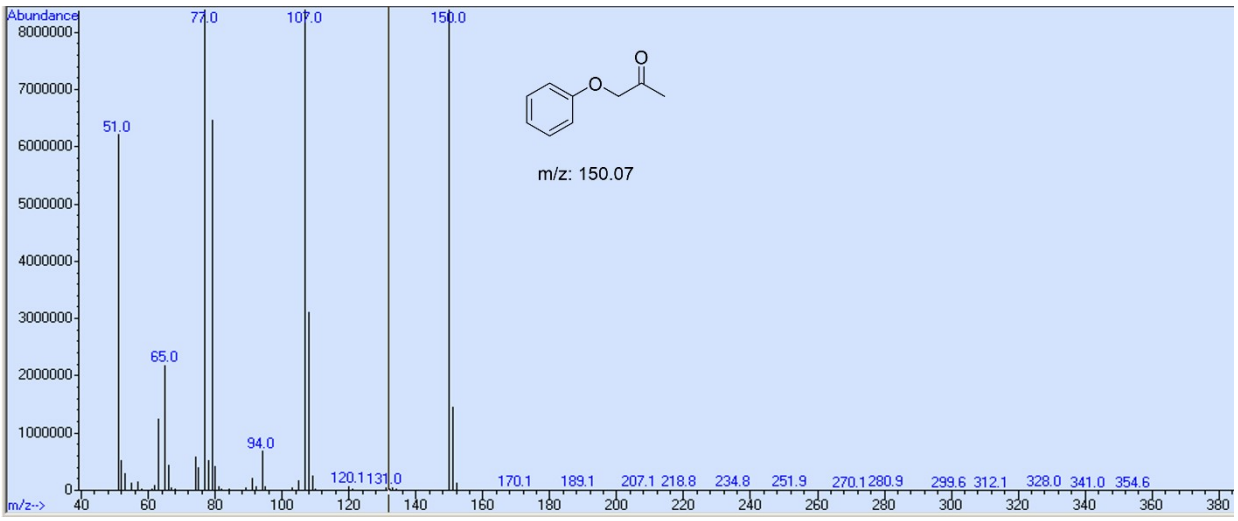

Figure S7. MS analysis of 1-phenoxypropan-2-one (peak at 4.3 min). Calculated m/z: 150.1, obtained: 150.0.

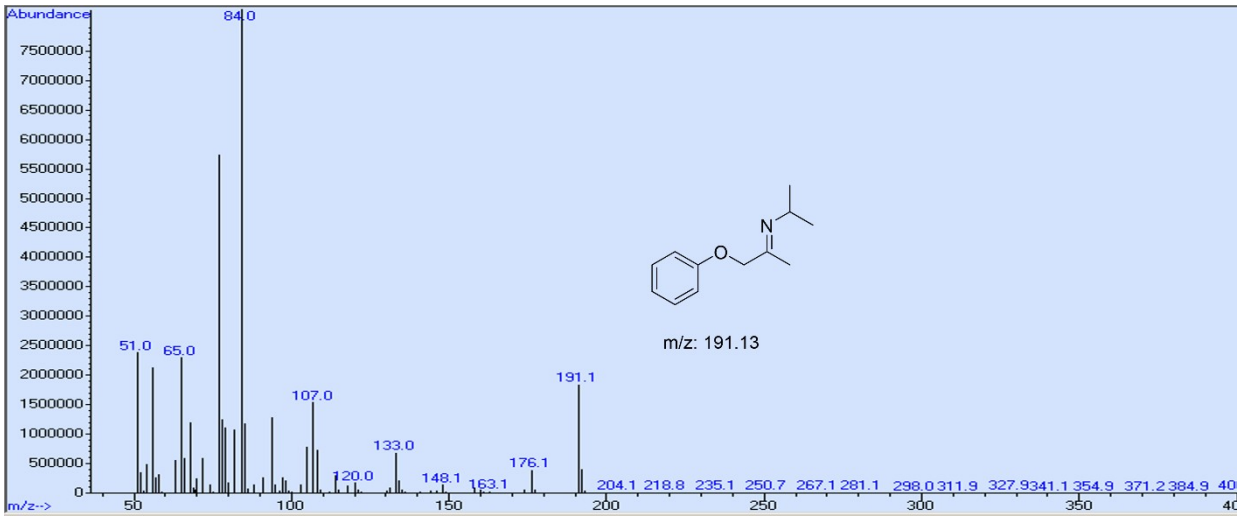

Figure S8. MS analysis of *N*-isopropyl-1-phenoxypropan-2-imine (peak at 6.0 min). Calculated m/z: 191.1, obtained: 191.1.

8. ^{1}H-NMR spectrum of the incubated 1-phenoxypropan-2-one with isopropylamine

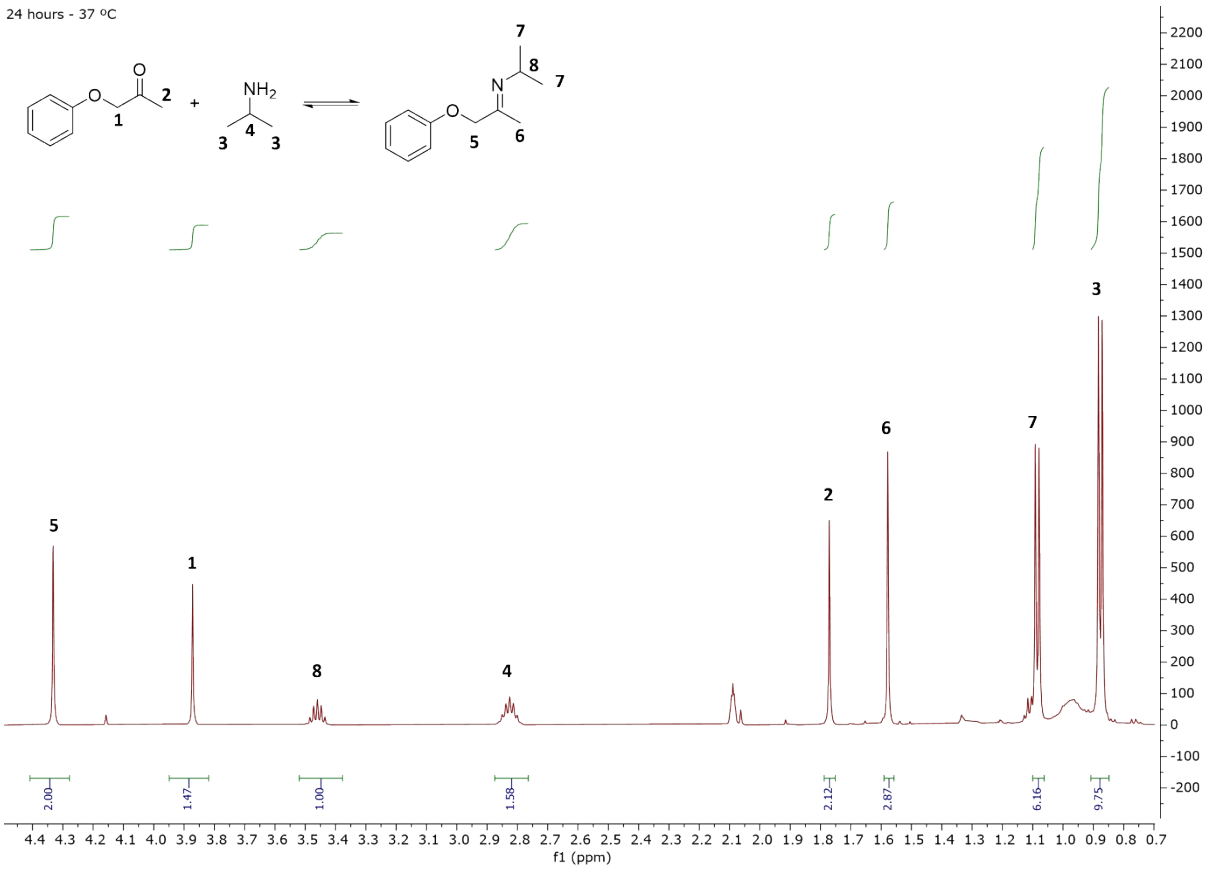

Figure S9. ^{1}H-NMR of the reaction mixture in deuterated toluene.

9. Transaminase stability – Temperature study in flow

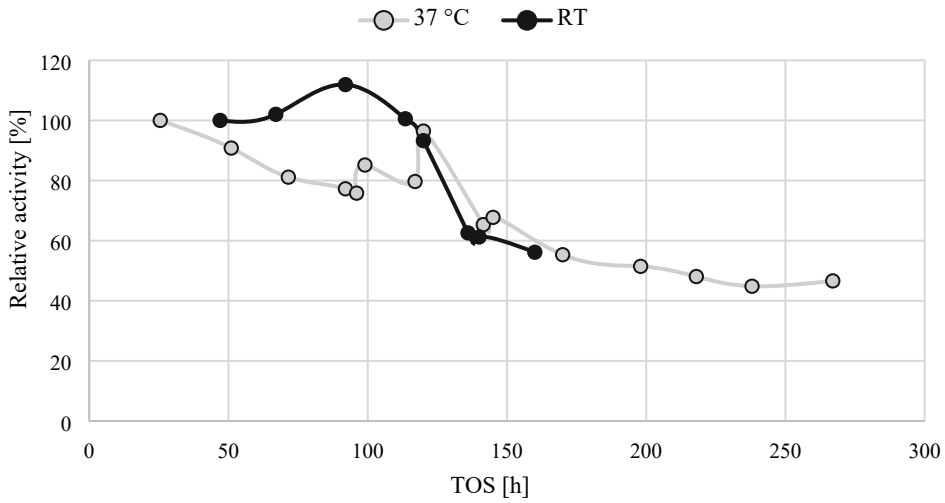

Figure S10. Stability comparison of the cascade (in flow) at two different temperatures, 37 °C and room temperature.