

## Electronic Supplementary Information

### **FTIR microscopy for direct observation of conformational changes on immobilized $\omega$ -transaminase: effect of water activity and organic solvent on biocatalyst performance**

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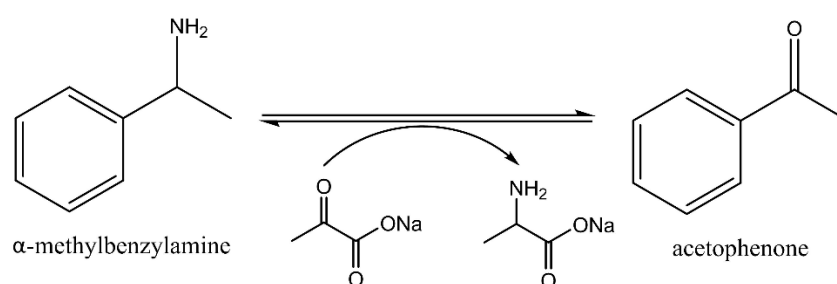
## 1. EziG specifications

Table S1. EziG carriers' specifications: all EziG products are based on controlled porosity glass.

Carrier	Surface	Particle size [ $\mu\text{m}$ ]	Pore diameter [nm]	Coating	pH range	Chelated $\text{Fe}^{3+}$ [ $\mu\text{mol/g}$ ]
EziG <sup>1</sup> -Opal	hydrophilic	75 - 125	50 $\pm$ 5	long amino alkyl chain	5 - 10	>10
EziG <sup>2</sup> -Coral	hydrophobic	75 - 125	30 $\pm$ 5	polyvinyl benzyl chloride	5 - 10	>10
EziG <sup>3</sup> -Amber	semi-hydrophilic	75 - 125	30 $\pm$ 5	copolymer of styrene and acrylonitrile	5 - 10	>10

All information is from EziG™ Product Data Sheet.

## 2. Activity assay in aqueous media of all immobilized ATA-117-His-tag formulations



Scheme S1. Model reaction used in the spectrophotometric assay of the activity in aqueous media of all immobilized His<sub>6</sub>-ATA-117 formulations pretreated according to the protocols of Table 1 (manuscript). The reaction was monitored by following the formation of acetophenone in a microplate reader by measuring the increase in absorbance at 245 nm.

### 3. Spectroscopic characterization of EziG carriers by means of FTIR microscopy

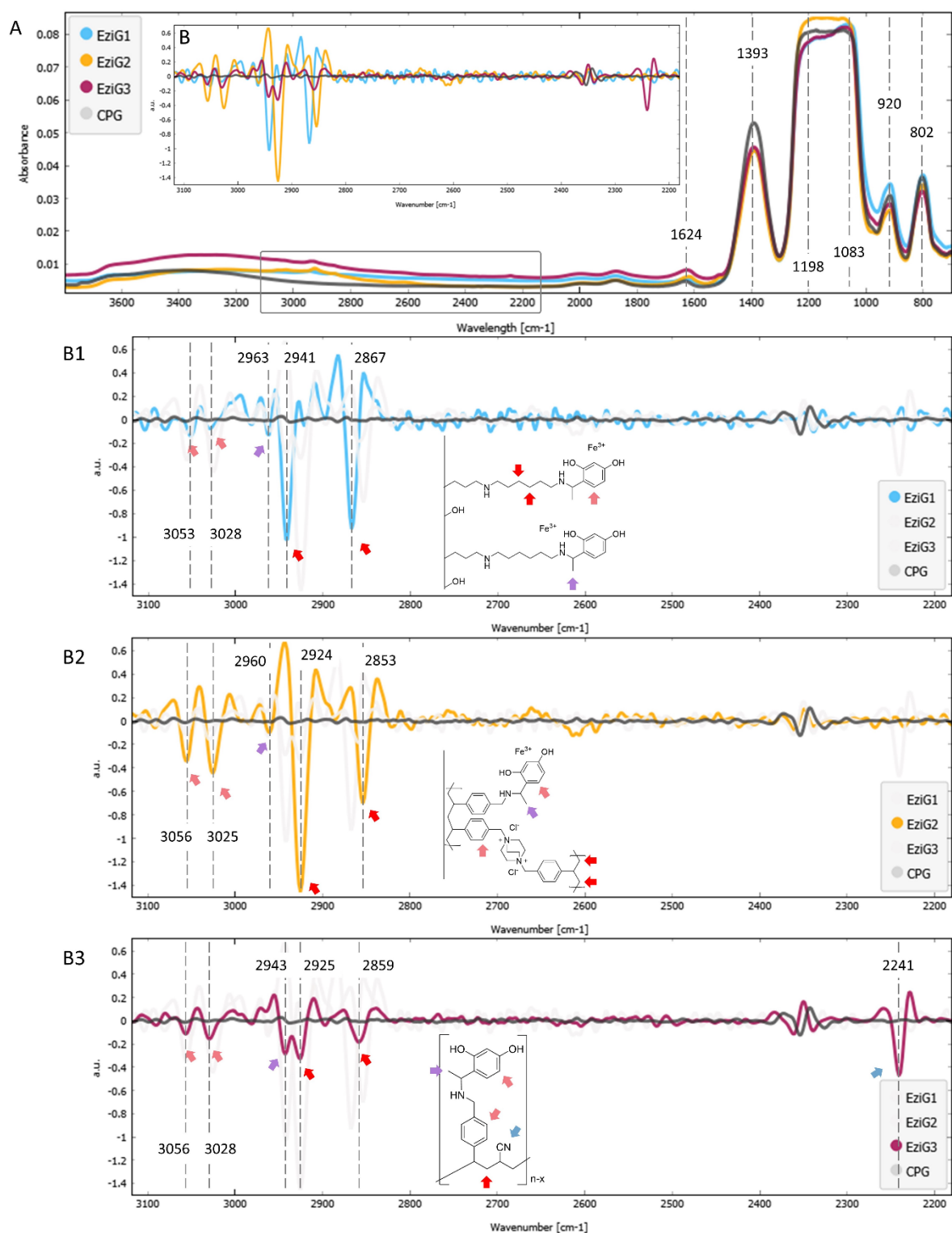
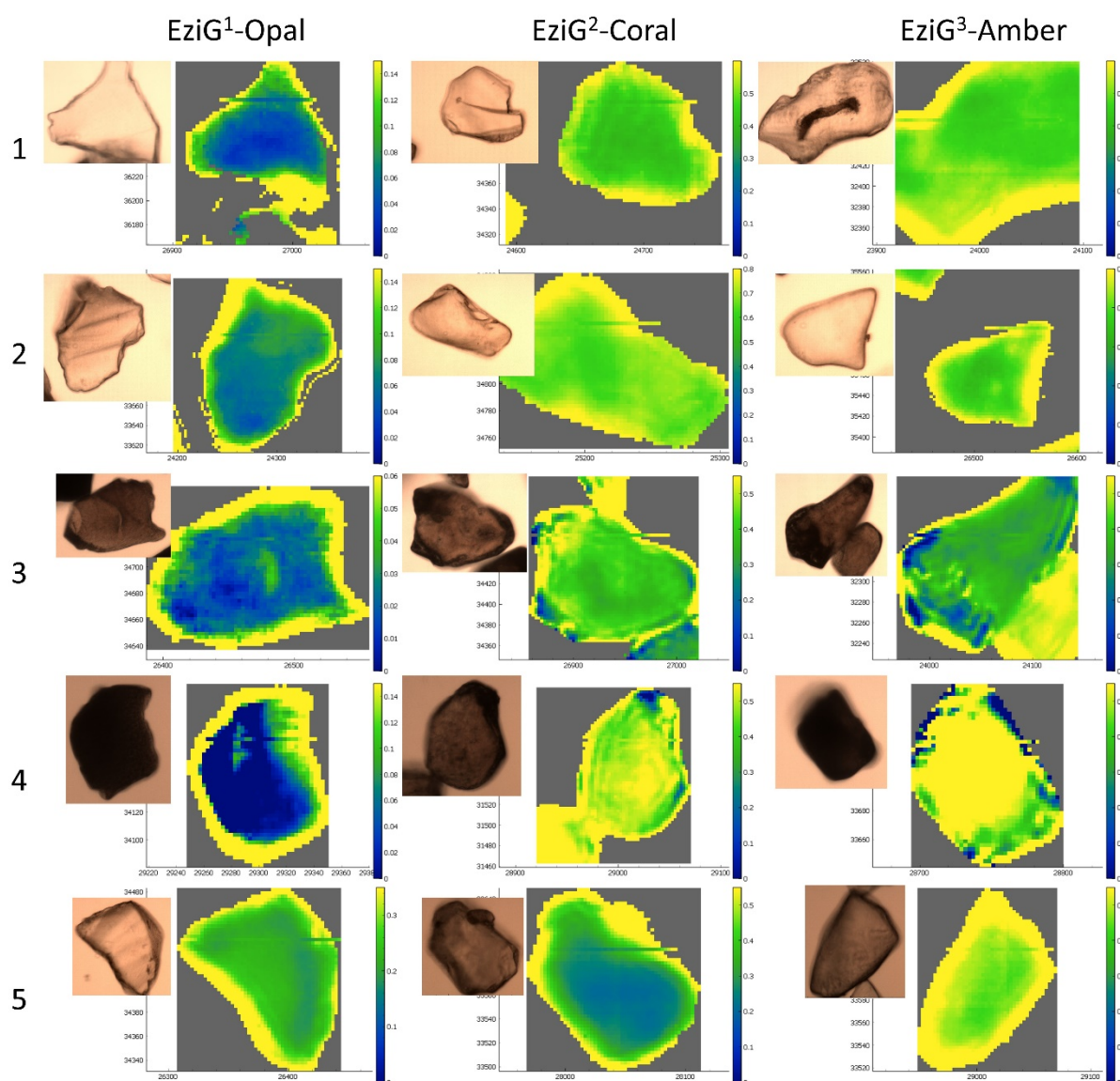


Figure S1. A: FTIR spectra (3800–700  $\text{cm}^{-1}$  region) of EziG<sup>1</sup>-Opal (blue), EziG<sup>2</sup>-Coral (orange), EziG<sup>3</sup>-Amber (dark red), and uncoated CPG (gray); black dashed lines indicate the FTIR spectral vibrations of  $\text{H}_2\text{O}$  bending at 1624  $\text{cm}^{-1}$ , B-O-B at 1393  $\text{cm}^{-1}$ , asymmetric Si-O stretching at 1198 and 1083  $\text{cm}^{-1}$ , Si-O-B stretching at 920  $\text{cm}^{-1}$  and Si-O-Si  $\text{cm}^{-1}$  stretching at 802  $\text{cm}^{-1}$ . B: Second derivative FTIR spectra (vector normalized in the spectra region 3120–2180  $\text{cm}^{-1}$ )

of the region where the most diagnostic differences are seen. In B1 (EziG<sup>1</sup>-Opal), B2 (EziG<sup>2</sup>-Coral) and B3 (EziG<sup>3</sup>-Amber) panels the main functional groups in the coating polymer composition are highlighted. Each of the spectra contains an insertion of the possible formula of the polymer coating <sup>4,5</sup>: the peaks at 3053, 3056 cm<sup>-1</sup> and 3028, 3025 cm<sup>-1</sup> were attributed to the aromatic C-H stretches; peaks at 2963, 2960 and 2943 cm<sup>-1</sup> were associated with C-H stretches in CH<sub>3</sub>; peaks at 2941, 2925, 2924 cm<sup>-1</sup> and 2087, 2859, 2853 cm<sup>-1</sup> from CH<sub>2</sub> asymmetric and symmetric stretches; the peak at 2241 cm<sup>-1</sup> that can be observed only for EziG<sup>3</sup>-Amber is attributed to C-N stretching.



Scheme S2. The optical images of the carriers with the overlap of the chemical infrared images generated by the ratio of the integration in the specific region of Amide I and Amide II bands (~1710-1480 cm<sup>-1</sup>) and the band identifying the glass carrier (~1500-1300 cm<sup>-1</sup>). The colour scale bar ranges from higher biocatalyst concentration (yellow areas) to not-detectable concentrations (blue areas).

#### 4. Fluorescence microscopy

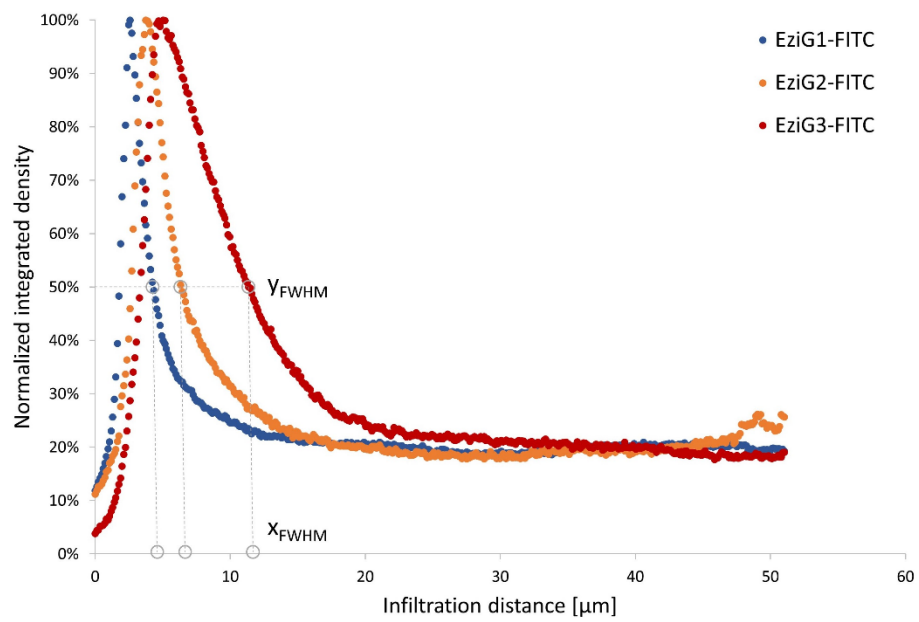


Figure S3. Radial cross-section fluorescence profile of EziG carriers shown in Figure 4.

$$FWHM = R - X_{FWHM}$$

## 5. FTIR investigation of the secondary structure of immobilized and pure ATA-117

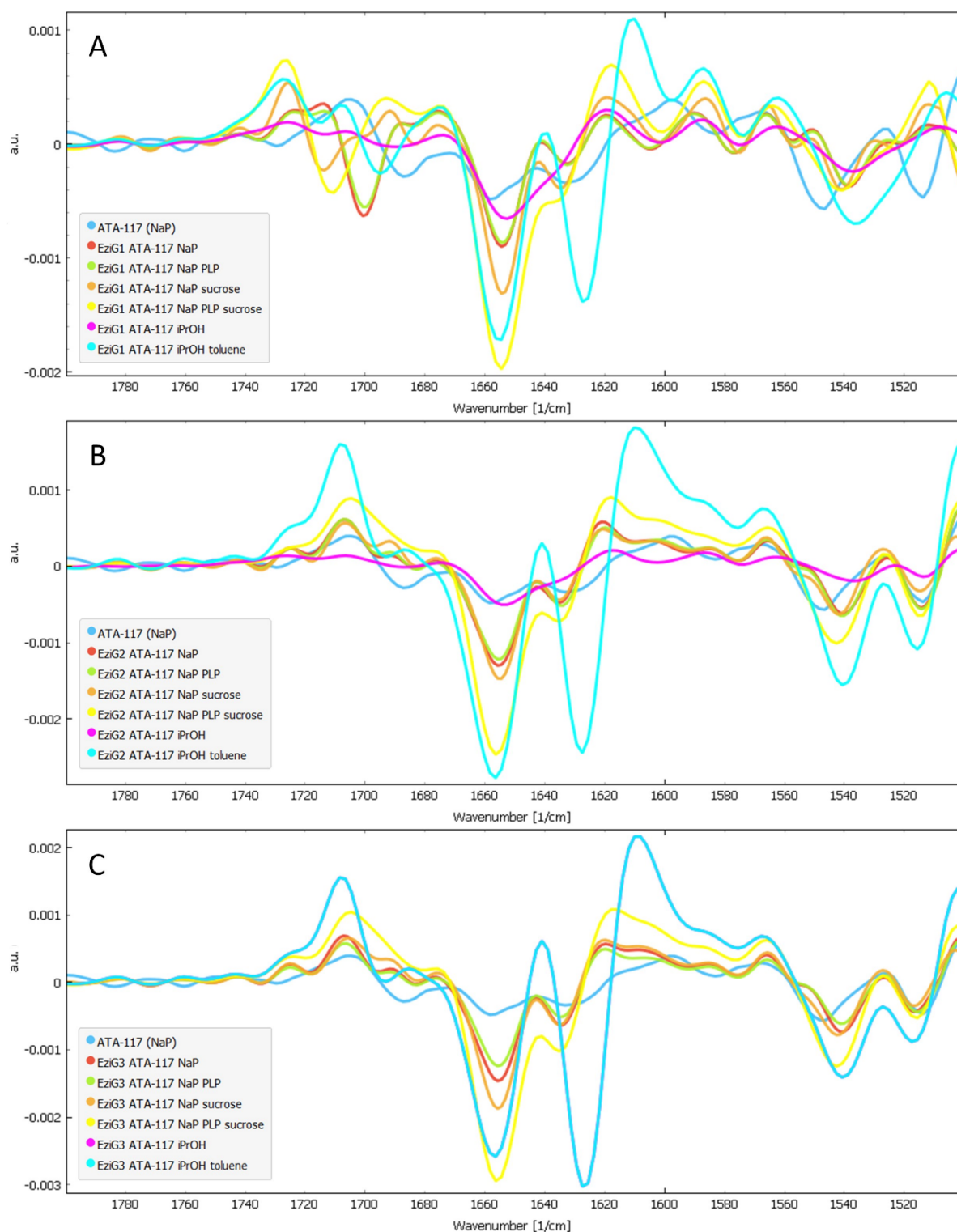


Figure S4. Savitzky-Golay's second derivative spectra for Amide I and II – comparison between different washes. The comparison was presented between native ATA-117 (resuspended in NaP buffer), immobilized ATA-117, but also the buffer used for the purification and resuspension of the enzyme. The spectra were grouped as follow: A. EziG<sup>1</sup>-Opal, B. EziG<sup>2</sup>-Coral, C. EziG<sup>3</sup>-Amber (samples were dried under vacuum at 100 mbar, 25°C for 24h).

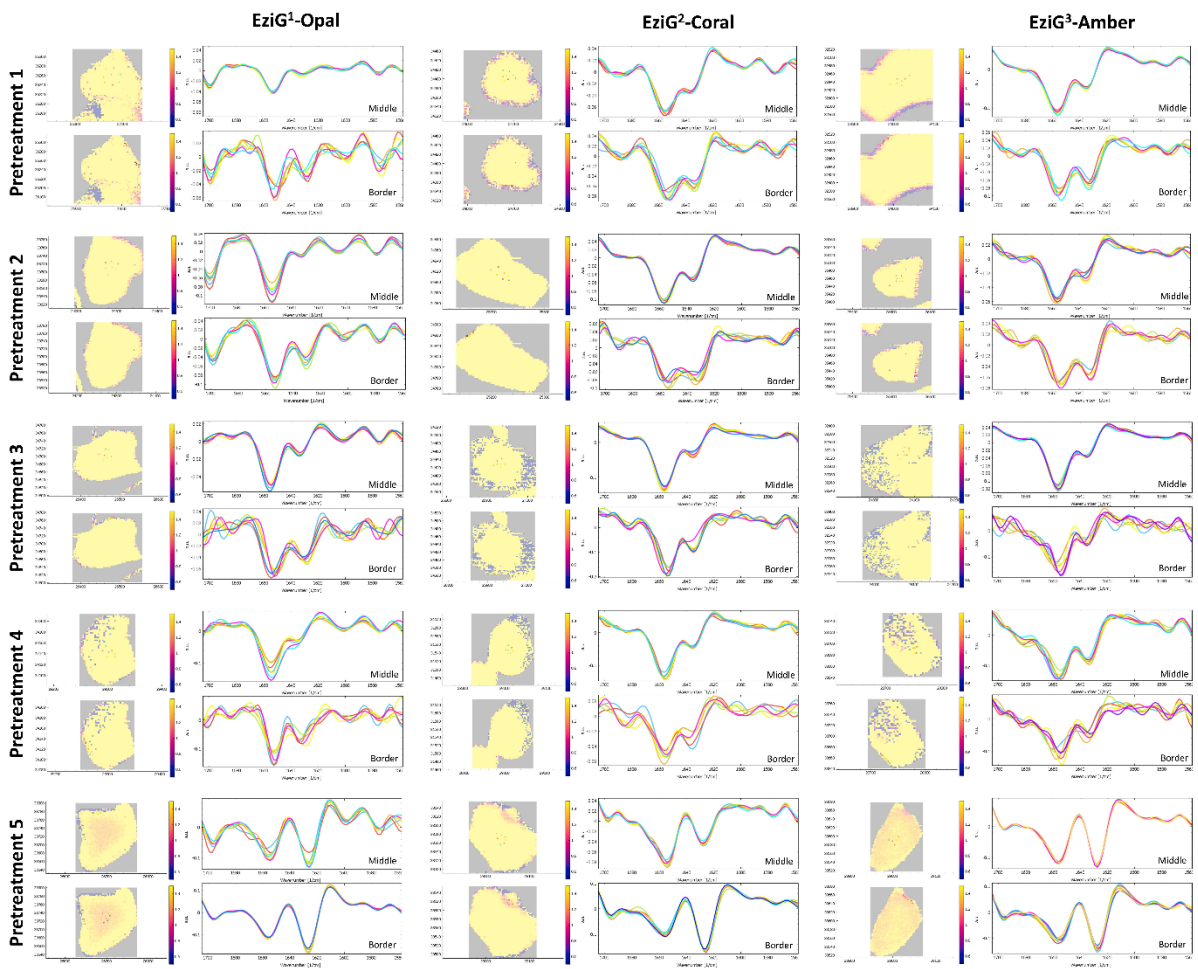
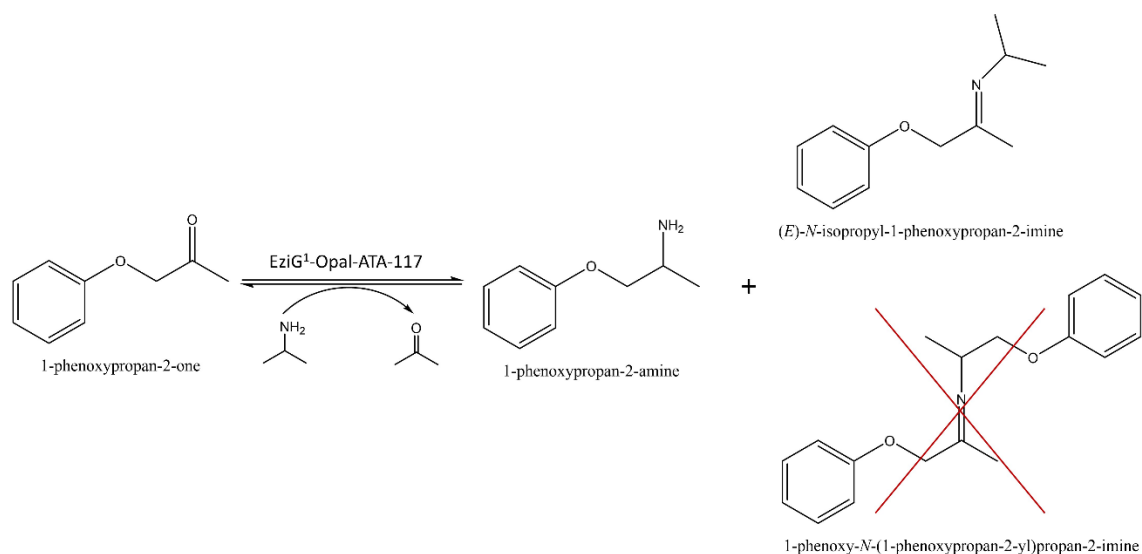


Figure S5. Savitzky-Golay's second derivative spectra for Amide I and II – comparison between different washes looking to representative spots of FRIR images at the border and at the center of the carrier. The integral from baseline of Amide I and Amide II and for the glass carrier were calculated and ratioed.



## 6. EziG-immobilized ATA 117: activity in toluene



Scheme S3. Model reaction used to evaluate the effect of the water on the performance of the immobilized ATA-117 on EziG<sup>1</sup>-Opal; reaction was performed in toluene. The formation of the imine between the ketone substrate and the formed 1-phenoxypropan-2-amine was not observed.

Table S2. Values of water activity measured after 24h of equilibration of some reference mixtures. All samples were incubated in 4 mL vial at 25°C for 24h. The achievement of the equilibrium was confirmed by the constant values of  $a_w$  observed across the time (see materials and methods).

Solvent	Pretreatment of EziG <sup>1</sup> -Opal-ATA-117		$a_w$
	Rinse with	Drying	
<b>Control mixtures</b>			
dry toluene	no	no	0.22±0.01
dry toluene	iPrOH followed by dry toluene	under vacuum 100mbar, 25°C, 24h	0.22±0.01
dry toluene	iPrOH followed by dry toluene	no	0.81±0.01
water-saturated toluene	no	no	0.60±0.01

## Bibliography

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