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Supporting Information

Towards Preparative Chemoenzymatic Oxidative Decarboxylation of Glutamic Acid

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Supplementary Information

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Materials. L-glutamic acid (99.5% pure), sodium L-glutamate monohydrate (99% pure), NaBr (99.0% pure), H_2O_2 (50%, (wt-%)), citric acid, Na_3VO_4 (99.0% pure), MCD (monochlorodimedone), $HClO_4$ (70% pure) and H_2SO_4 (95-97% pure) were purchased from Sigma-Aldrich, 3-cyanopropanoic acid (95% pure) was provided by Interchim Uptima. The enzyme vanadium chloroperoxidase was produced in house (see below).

***Ci*VCPO production.** Vanadium chloroperoxidase from *Curvularia inaequalis* (*Ci*VCPO) was obtained from heterologous expression in recombinant *Escherichia coli* by following a modified procedure.[1]Two 50 mL pre-precultures of LB medium containing 100 µg/mL of ampicillin were inoculated with 5 µL *E. coli* TOP10 pBADgIIIB VCPO glycerol stock [1] and incubated overnight at 37°C and 180 rpm. Further, two precultures of 200 mL and 500 mL were inoculated with pre-preculture. The overexpression was carried out in 15 L stirred tank fermenter. 13 L of TB medium containing 100 µg/mL of ampicillin was inoculated with 200 mL of pre-culture (OD_{600} =4.08) to an OD of approx. 0.05 and incubated at 37°C and 180 rpm. At OD_{600} = 1.0 (approx. 3 h), 15 mL 20% L-arabinose was added to reach a final inducer concentration of 0.02%. After induction, the culture was incubated for additional 24 h at 25°C and 180 rpm. The wet bacterial pellets obtained after centrifugation were washed with 50 mM Tris/H_2SO_4 buffer pH 8.2 and stored at -20°C until processed as described below.

***Ci*VCPO purification.** About 30 g of wet cells were thaw on a hot water bath and re-suspended in 50 mM Tris/H_2SO_4 buffer pH 8.2 (0.5 g cells per mL). A spatula tip of DNase was added to the re-suspended cells which were disrupted by sonication. The obtained suspension was centrifuged (10000 rpm, 20 min) and the supernatant was incubated for 2 × 20 min at 70°C (20 min were counted after the solution reached 70°C). Each heat treatment was followed by centrifugation (10000 rpm, 20 min) to remove the denatured proteins. The supernatant was concentrated and desalted by exchanging the buffer 4 times with Tris/H_2SO_4 buffer (50 mM, pH 8.2 + 100 µM Na_3VO_4) using Amicon membrane filters (30 kDa cut-off). The concentrated enzyme solution was stored at -20°C in small aliquots (0.5 mL) until further use. Purity of the preparation was determined by SDS-Page comparing the band intensities by Image Lab™ software (version 6.0.1). The stock enzyme solution (approx. 10 mL) had a specific activity (based on monochlordimedone assay) of 177 U/mg protein, a protein concentration of 2.85 mg/mL of which 78% VCPO and a VCPO concentration of 33 µM.

HPLC. Glutamic acid samples were analysed after appropriate dilution (0.05-5 mM range) in MQ water by a HPLC (Shimadzu) comprising of LC-20AD pump, SIL-20AC HT autosampler, SPD-10A VP UV/Vis detector, and a CTO-20AC temperature-control unit. The column used was a Crownpak CR(+)/CR(-) column (150 × 4.0 mm, Chiral Technologies) with $HClO_4$ (pH 1.5, 10ºC, 0.5 mL/min, 210 nm). Retention time glutamic acid 13.1 min.

3-cyanopropanoic acid samples were analysed after appropriate dilution (0.1-100 mM range) in MQ water by a HPLC (Shimadzu) comprising of a LC-20AT pump, SIL-20A HT autosampler, RID 10A detector and a CTO-20A temperature-control unit. Detection was achieved by refractive index detector set at 40º C. The column used was an organic acid column CARBOSep CoreGel 87H3 (7.8 × 300 mm, Transgenomic) with H_2SO_4 (10 mM, 60ºC, 0.75 mL/min). Retention time 3-cyanopropanoic acid 14.1 min.

The yield in H_2O_2 (%) was calculated with the following formula: yield = $[CPA]_{t=x}$ / ($[H_2O_2]_{t=x}$ / 2) × 100 where $[CPA]_{t=x}$ is the concentration of CPA at time x and $[H_2O_2]_{t=x}$ is the concentration of H_2O_2 added up to time x.

Product extraction from the semi-preparative reaction. The reaction mixture was acidified with H_2SO_4 at pH 3, saturated with NaCl and extracted with ethyl acetate (2×100 mL). The collected organic layers were dried over $MgSO_4$ and the solvent was evaporated. The ^{1}H NMR showed 3-cyanopropanoic acid as the main product (612 mg, 31% isolated yield, 96% purity). The ^{1}H NMR (water suppression) of the aqueous phase revealed the presence of unextracted nitrile. To increase the yield another extraction was performed. For this, the aqueous phase was further acidified to pH 1 with H_2SO_4, extracted with diethyl ether (3×70 mL), dried over $MgSO_4$, the solvent was evaporated and analysed by NMR. An additional 215 mg of 3-cyanopropanoic acid was isolated (97% pure based on NMR). Overall, 827 mg off-white oily solid (96-97% 3-cyanopropanoic acid, 42% isolated yield) was obtained.

NMR. NMR spectra were recorded by using an Agilent 400 MHz (^{1}H, 9.4 Tesla) spectrometer operating at 399.67 MHz for ^{1}H at 298 K. Water suppression was performed by using a PRESAT pulse sequence. 3-cyanopropanoic acid: ^{1}H NMR (400 MHz, D_2O): δ = 2.81-2.78 (t, 2H), δ = 2.76-2.74 (t, 2H); ^{13}C NMR (400 MHz, D_2O): δ = 174.9, 120.7, 29.3, 12.4.

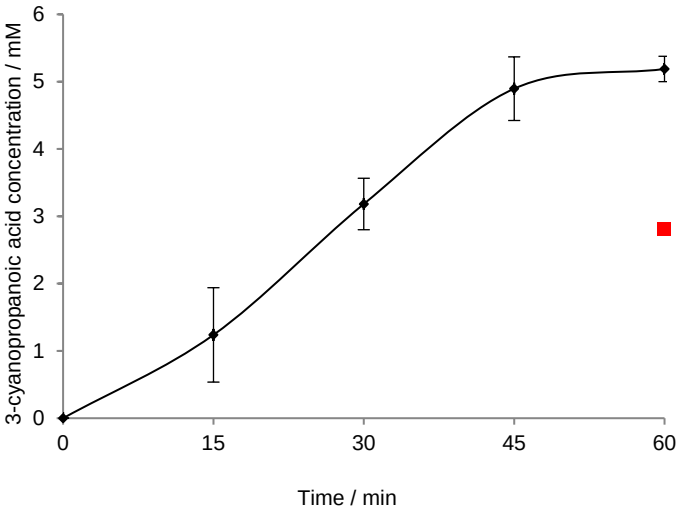

Figure S1. The conversion of 5 mM glutamic acid into CPA by continuous addition of H_2O_2 (◆) and by addition of H_2O_2 all (12 mM) at the beginning of the reaction (■). Reaction conditions: [Glutamic acid] = 5 mM, [NaBr] = 0.5 mM, [*Ci*VCPO] = 55 nM, H_2O_2-dosage: 12 mM × h^{-1} (from a 0.5 M stock solution), 20 mM sodium citrate buffer (pH 5.6), room temperature (22°C). The error bars represent the range of duplicate experiments.

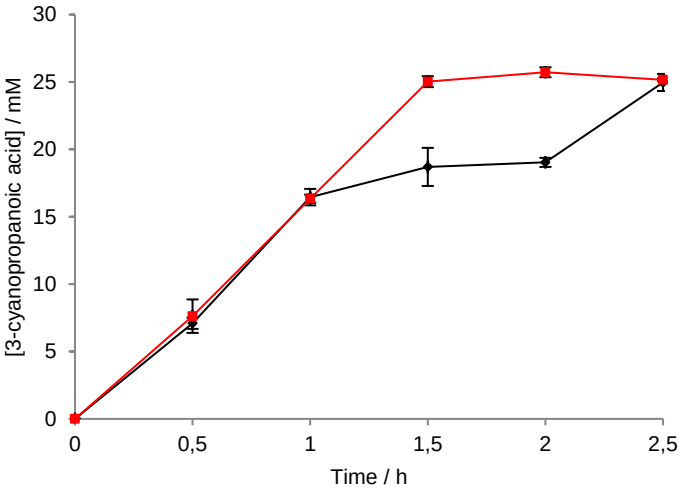

Figure S2. The conversion of 25 mM glutamic acid (◆) and sodium glutamate (■) into 3-cyanopropanoic acid. Reaction conditions: [Glutamic acid/Sodium glutamate] = 5 mM, [NaBr] = 0.5 mM, [*Ci*VCPO] = 55 nM, H_2O_2-dosage: 39 mM × h^{-1} (from a 1 M stock solution), 20 mM sodium citrate buffer (pH 5.6), room temperature (22°C). The error bars represent the standard deviation of triplicate experiments.

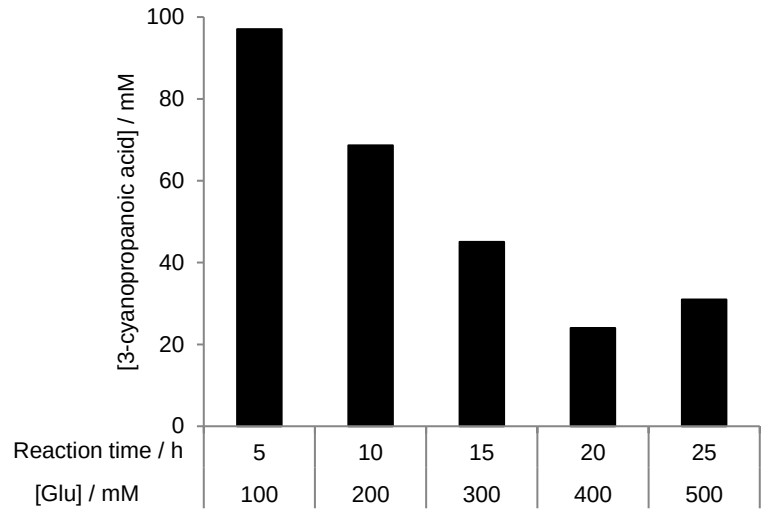

Figure S3. The conversion of sodium glutamate (different concentrations) into 3-cyanopropanoic acid. Reaction conditions: [NaBr] = 0.5 mM, [*Ci*VCPO] = 55 nM, H_2O_2-dosage: 39 mM × h^{-1} (from a 1 M stock solution), 20 mM sodium citrate buffer (pH 5.6), room temperature (22°C).

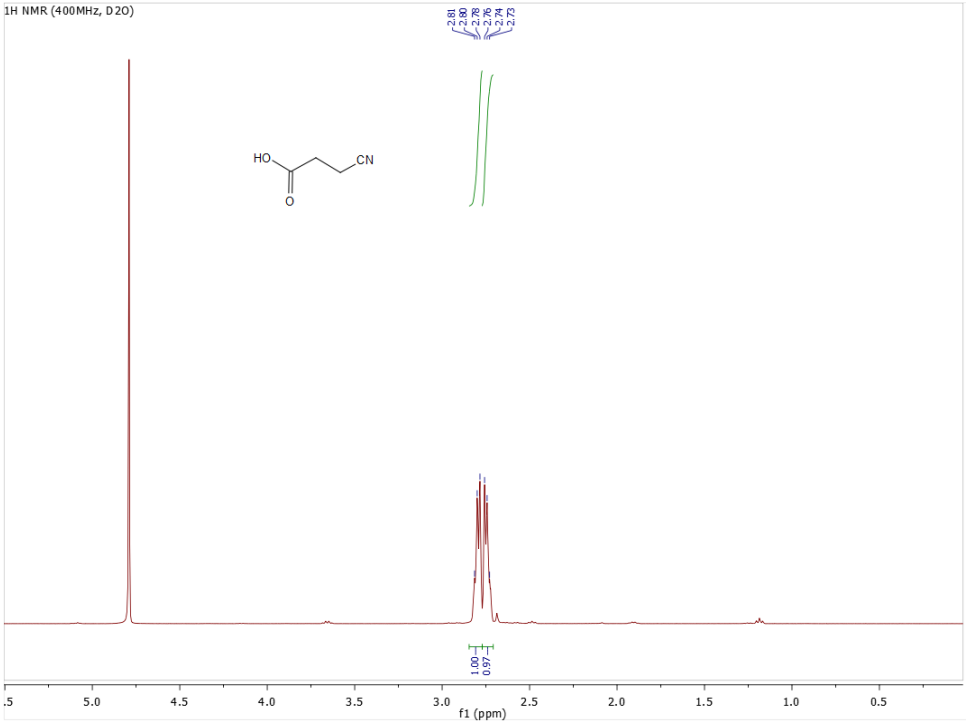

Figure S4. ^{1}H NMR of isolated 3-cyanopropanoic acid. The impurities (1.0-2.5 and 3.7 ppm, acetic acid and ethanol) derive from hydrolysis of the extraction solvent (ethyl acetate).

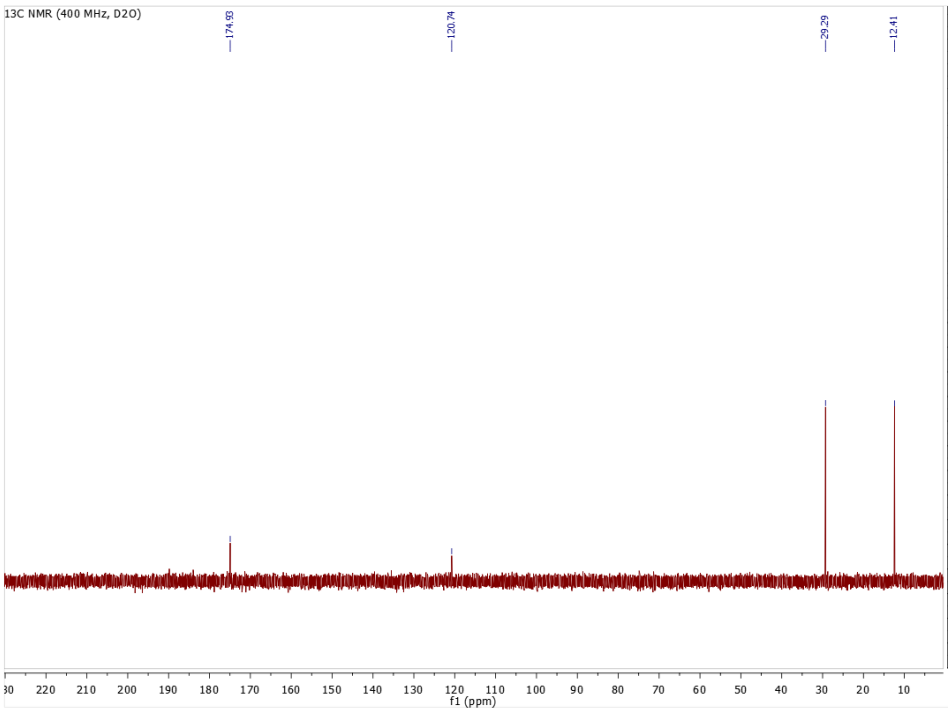

Figure S5. ^{13}C NMR of isolated 3-cyanopropanoic acid.

[1] Z. Hasan, R. Renirie, R. Kerkman, H. J. Ruijssenaars, A. F. Hartog, R. Wever, *J. Biol. Chem.* **2006**, *281*, 9738-9744.