Supporting information

Continuous flow for enantioselective cyanohydrin synthesis

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A. Procedure for synthesis of monolith

Polyethylene glycol 35 000 (8.67 g) was dissolved in 1 M HNO_3 (100 mL) and then tetraethoxysilane (82.8 mL) was added dropwise under stirring (500 rpm) in an ice bath. Cetyltrimethylammonium bromide (3.8 g) was added next and after mixing the solution was left to gel in teflon cylindrical forms at 40 ºC and aged for 10 days. The received alcogels were impregnated with 1 M ammonia water solution for 9 h at 90 ºC, rinsed with water, dried and calcinated at 550 ºC for 10 h (ramp of 1 ºC min^{-1}) to obtain silica rods (60 mm in length, 6 mm in diameter). The monoliths were then functionalized and clad with polymer resin (L285MGS-H285MGS type) to obtain single-rod microreactors.

B. Procedure for post-functionalization of silica monolith

The silica monoliths were modified for non-specific enzyme attachment by amino glutaraldehyde linkage. Before functionalization, monoliths were contacted with water vapor for 5 h and dried at 200 ºC for 2 h. Then amino groups were grafted onto the monolith surface by reacting with 3-aminopropyltrimethoxysilane (5 mmol per g of silica) in dry toluene (40 mL per g of silica) for 72 h at 85 ºC. After that monoliths were washed with ethanol at 50 ºC for 5 h and dried.

C. Procedure for production of *Arabidopsis thaliana* HNL (*At*HNL)

pET28a-*At*HNL expression plasmid containing the *At*HNL gene (GenBank accession number AAN13041, EC: 4.1.2.10) codon optimized for E.coli and with a polyhistidine tag (His6-tag) was obtained from the group of Martina Pohl (Institute of Bio- and Geosciences, Jülich, Germany). *E.coli* BL21(DE3) was transformed with the expression plasmid for the production of the His-tagged *At*HNL. A preculture was prepared by inoculating one single colony of *E. coli* BL21(DE3)-pET28a-*At*HNL in 10 mL of Lysogeny Broth (LB) medium with kanamycin (40 μg mL^{-1}) and incubated overnight at 37 ºC, 180 rpm. Subsequently, this preculture was used for the inoculation of 1 L of Terrific Broth (TB) medium containing kanamycin (40 μg mL^{-1}) and incubated overnight (Eppendorf/New Brunswick Scientific Incubator Shaker Excella E24 Series) at 37 ºC, 120 rpm. When the OD 600 reached 0.7-0.9 the protein expression was induced by adding 1 mL of 0.1 M isopropyl β-D-thiogalactoside (IPTG) per liter of culture (0.1 mM IPTG final concentration) and cultivation was continued at 25 ºC, 180 rpm for 20 h. Cells were harvested by centrifugation at 4 ºC, 3600 rpm during 20 min (Sorvall RC6, Thermo Fisher Scientific). The supernatant was discarded and the pellets were washed with 30 mL of 10 mM sodium phosphate buffer pH 7.0, frozen in liquid nitrogen and stored at -80 ºC.

D. Procedure for enzyme purification and chromatogram

The pellets containing *At*HNL were resuspended in lysis buffer (10 mM potassium phosphate buffer pH 7.4 + DNase) and lysed in a cell disruptor (Constant Systems Ltd.) at 1.5 kBar and 4 ºC to avoid protein denaturation. The cell free extracts were collected by centrifugation at 48,000×g, 1 h, 4 ºC (Sorvall RC 6, Thermo Fisher Scientific). The enzyme was purified by using a NGC Chromatography system (Bio-Rad) by immobilized metal ion chromatography (IMAC) with chelating Ni^{2+} Sepharose (HiTrap Chelating HP 5 mL, GE Healthcare) according to the manufacturer. 20 mM sodium phosphate + 0.5 M NaCl + 20 mM imidazole pH 7.4 was used for the enzyme binding and 20 mM sodium phosphate + 0.5 M NaCl + 0.5 M imidazole pH 7.4 was used for the enzyme elution. The purified *At*HNL was concentrated with a 10 kDa MWCO Amicon filter (Millipore) and desalted with a PD-10 desalting column (Cytiva) according to the supplier instructions.

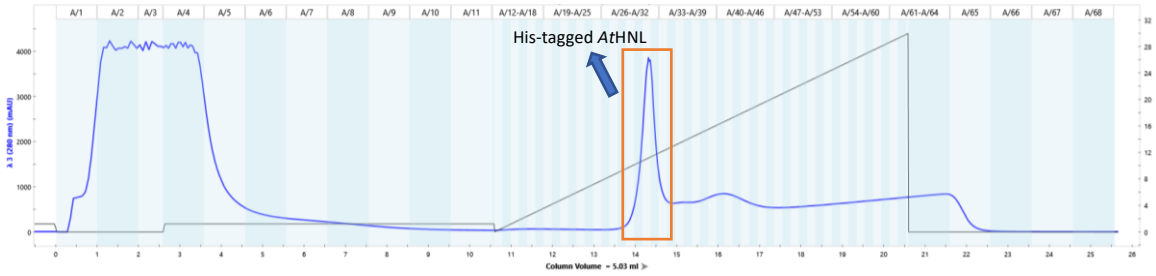


Fig. S1: NGC chromatogram for the detection of his-tagged *At*HNL

E. Procedure for enzymatic activity assay

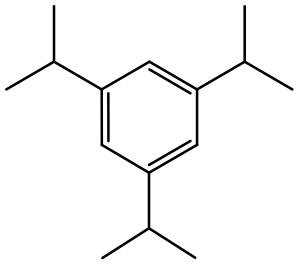
*At*HNL activity was determined spectrophotometrically (Agilent Technologies Cary 60 UV-VIS). The cleavage of *rac*-mandelonitrile into benzaldehyde and hydrogen cyanide was followed at 280 nm and 25 ºC in 1 cm quartz glass cuvettes. Briefly, 1400 µL of 50 mM citrate/phosphate buffer pH 5.0 and 200 µL of enzyme solution (in 5 mM phosphate buffer pH 6.5) were mixed and incubated for 30 s at 25 ºC. The reaction was started by adding 400 µL of 60 mM *rac*-mandelonitrile solution (80 µL of *rac*-mandelonitrile in 10 mL 3 mM citrate/phosphate buffer, pH 3.5). The enzymatic activity was calculated with the molar extinction coefficient of benzaldehyde (ε_{280} = 1.376 mM^{-1} cm^{-1}) and the background reaction (performed without enzyme) was subtracted in the final calculation.

One unit of *At*HNL activity is the amount of micromoles of *rac*-mandelonitrile converted per minute in citrate/phosphate buffer pH 5.0 at 25 ºC.

F. HPLC method and retention times

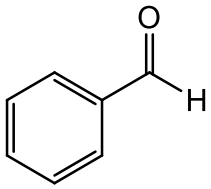
HPLC analysis were performed on Agilent Technologies 1200 series equipped with UV/Vis, column thermostat, autosampler, HPLC pumps and a Daicel Chiralpak AD-H column (250 × 4.6 mm, 5 μm). Samples (8 μL for **1c** or 25 μL for **1a, 1b, 1d**) were diluted in 1 mL heptane/isopropanol (95:5), dried over anhydrous $MgSO_4$ and centrifuged, then 10 μL of the sample was injected into the HPLC system. The applied HPLC method settings were as follows: mobile phase: heptane:isopropanol 95:5, UV detection wavelength: λ = 216 nm, oven temperature: 40 ºC, flow rate: 1 mL min^{-1}.

1,3,5-Triisopropylbenzene (ISTD)



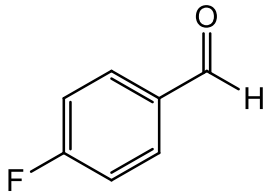
Retention time: ~2.9 min

Benzaldehyde (1a)



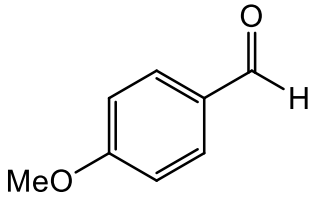
Retention time: ~5.0 min

4-Fluorobenzaldehyde (1b)



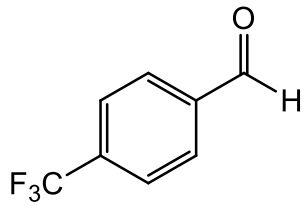
Retention time: ~5.5 min

4-Methoxybenzaldehyde (1c)



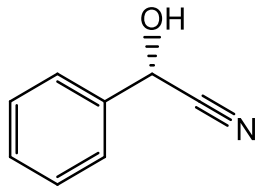
Retention time: ~7.2 min

4-(Trifluoromethyl)benzaldehyde (1d)



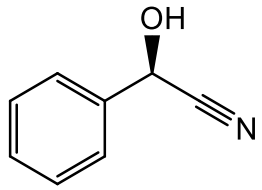
Retention time: ~5.1 min

(*S*)-Mandelonitrile ((*S*)-2a)



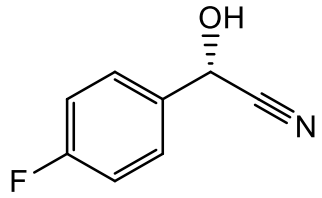
Retention time: ~11.5 min

(*R*)-Mandelonitrile ((*R*)-2a)



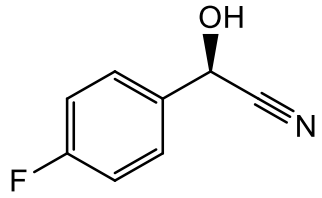
Retention time: ~12.9 min

(*S*)-4-Fluoromandelonitrile ((*S*)-2b)



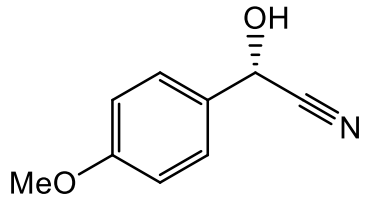
Retention time: ~11.4 min

(*R*)-4-Fluoromandelonitrile ((*R*)-2b)



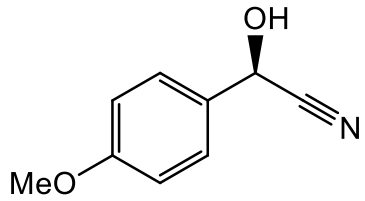
Retention time: ~12.9 min

(*S*)-4-Methoxymandelonitrile ((*S*)-2c)



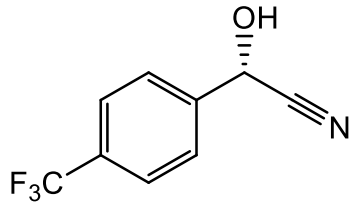
Retention time: ~18.8 min

(*R*)-4-Methoxymandelonitrile ((*R*)-2c)



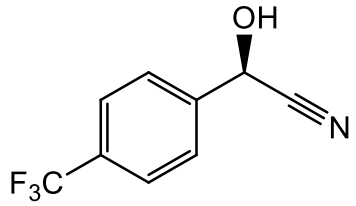
Retention time: ~21.1 min

(*S*)-4-(Trifluoromethyl)mandelonitrile ((*S*)-2d)



Retention time: ~9.4 min

(*R*)-4-(Trifluoromethyl)mandelonitrile ((*R*)-2d)



Retention time: ~11.1 min