-Supporting Information-

Immobilisation of *Arabidopsis thaliana* hydroxynitrile lyase (*At*HNL) on EziG Opal

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Table of contents

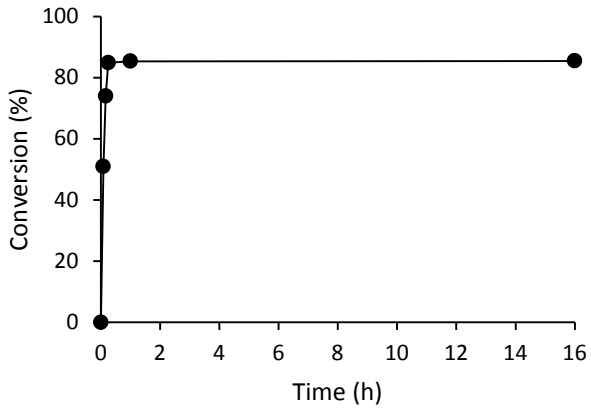

Figure S1. Leaching assay for AtHNL on EziG Opal (2 U mg-1). Immobilization was performed by adsorption and drying. *At*HNL on EziG1 was removed after 15 minutes of reaction. Conditions: Ratio benzaldehyde : HCN in citrate/phosphate buffered MTBE, pH 5, 1:4, benzaldehyde (100 μL, 1 mmol), 2 ml HCN solution in citrate/phosphate buffered MTBE (1.5 - 2 M) pH 5, 27.5 μL (0.1 mmol) 1,3,5-triisopropylbenzene as internal standard (I.S.) and a teabag filled with *At*HNL immobilized on 50 mg EziG1. The reaction was stirred at 900 rpm at room temperature.

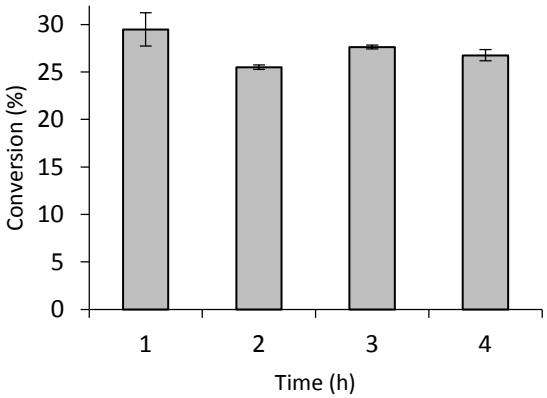

Figure S2. Blank reaction in batch. Conditions: Ratio benzaldehyde : HCN in citrate/phosphate buffered MTBE, pH 5, 1:4, benzaldehyde (100 μL, 1 mmol), 2 ml HCN solution in citrate/phosphate buffered MTBE (1.5 - 2 M) pH 5, 27.5 μL (0.1 mmol) 1,3,5-triisopropylbenzene as I.S. and 50 mg of dried EziG Opal. The reaction was stirred at 900 rpm at room temperature. Error bars correspond to the standard deviation of duplicates (n=2).

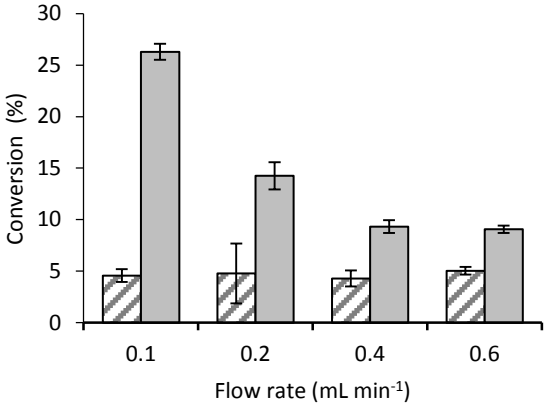

Figure S3. Blank reaction in flow. Grey bars are the reactions performed with wet EziG Opal (carrier + 0.306 mL of citrate/phosphate buffer pH 5). Dashed bars are the reactions performed with dried EziG1. Conditions: Ratio benzaldehyde : HCN in citrate/phosphate buffered MTBE, pH 5, 1:4, benzaldehyde (100 μL, 1 mmol), 2 ml HCN solution in citrate/phosphate buffered MTBE (1.5 - 2 M) pH 5, 27.5 μL (0.1 mmol) 1,3,5-triisopropylbenzene as I.S. and 150 mg EziG1. The reactions were stirred at 900 rpm at room temperature. Error bars correspond to the standard deviation of duplicates (n=2).

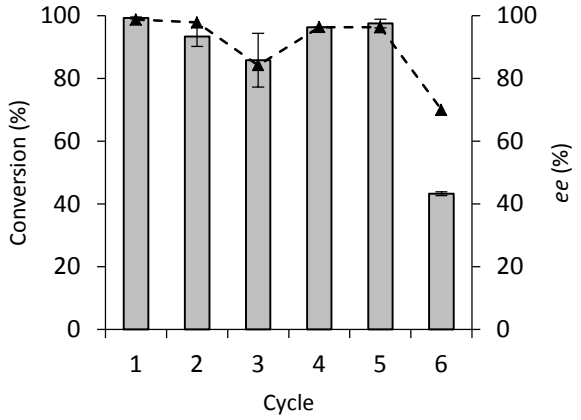

Figure S4. Recycling of AtHNL immobilised on EziG Opal (20 U mg-1) in eight successive cycles. Immobilization was performed by incubation and drying. Conversion (bars), enantiomeric excess (dotted line and triangles). Conditions: Ratio benzaldehyde : HCN in citrate/phosphate buffered MTBE, pH 5, 1:4, benzaldehyde (100 µL, 1 mmol), 2 ml HCN solution in citrate/phosphate buffered MTBE (1.5 - 2 M) pH 5, 27.5 µL (0.1 mmol) 1,3,5-triisopropylbenzene as I.S. and a teabag filled with *At*HNL immobilized on 50 mg EziG1. The reaction was stirred at 900 rpm at room temperature. The enzyme was washed for 1 minute with 100 mM citrate/phosphate buffer saturated MTBE pH 5 after each cycle. Error bars correspond to the standard deviation of duplicates (n=2).

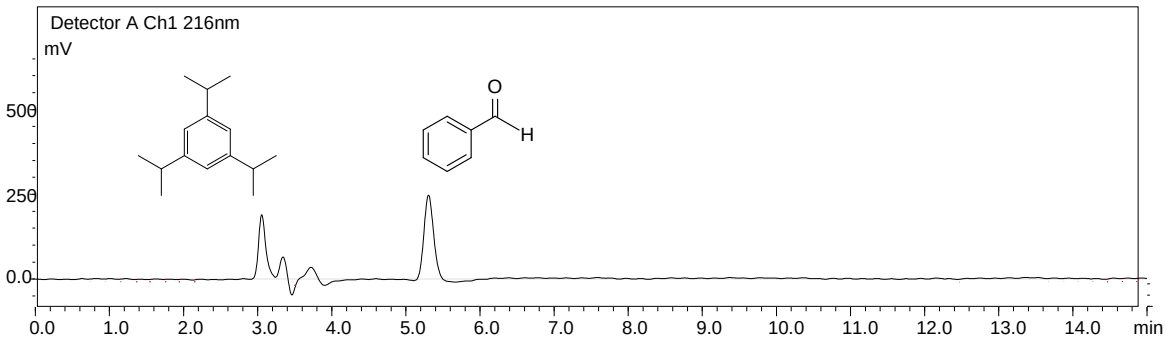

Figure S5. HPLC detection of benzaldehyde and 1,3,5-triisopropylbenzene during 8 hours of incubation. Conditions: Ratio benzaldehyde : HCN in buffered MTBE pH 4 ~1:4, 100 µL benzaldehyde (1 mmol), 2 mL HCN in acetate buffered MTBE pH 4**.** The reaction was stirred at 1000 rpm at 5 °C.

*At*HNL - Gene sequence	*At*HNL - Aminoacid sequence
5′GGRAAATTTACCTCTAGAATAATTTTGTTTACTTTAAGA AGGAGATATACCATGGGCAGCAGCCATCATCATCATCAT CACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGAG AGGAAACATCACTTCGTGTTAGTTCACAACGCTTATCATG GAGCCTGGATCTGGTACAAGCTCAAGCCCCTCCTTGAATC AGCCGGCCACCGCGTTACTGCTGTCGAACTCGCCGCCTCC GGGATCGACCCACGACCAATCCAGGCCGTTGAAACCGTC GACGAATACTCCAAACCGTTGATCGAAACCCTCAAATCT CTTCCAGAGAACGAAGAGGTAATTCTGGTTGGATTCAGCT TCGGAGGCATCAACATCGCTCTCGCCGCCGACATATTTCC GGCGAAGATTAAGGTTCTTGTGTTCCTCAACGCCTTCTTG CCCGACACAACCCACGTGCCTTCTCACGTTCTGGACAAGT ATATGGAGATGCCTGGAGGTTTGGGAGATTGTGAGTTTTC ATCTCATGAAACAAGAAATGGGACGATGAGTTTATTGAA GATGGGACCAAAATTCATGAAGGCACGTCTTTACCAAAA TTGTCCCATAGAGGATTACGAGCTGGCAAAAATGTTGCAT AGGCAAGGGTCATTTTTCACAGAGGATCTATCAAAGAAA GAAAAGTTTAGCGAGGAAGGATATGGTTCGGTGCAACGA GTTTACGTAATGAGTAGTGAAGACAAAGCCATCCCCTGC GATTTCATTCGTTGGATGATTGATAATTTCAACGTCTCGA AAGTCTACGAGATCGATGGCGGAGATCACATGGTGATGC TCTCCAAACCCCAAAAACTCTTTGACTCTCTCTCTGCTATT GCCACCGATTATATGTAAGCGGCCGCACTCGAGCACCAC CACCACCACCACTGAGATCCGGCTGCTAACAAGCCCGAA AGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAAC TAGCAWACCCCTTGGGGCCTCTAAACGGTCTTGAGGGTTT TTTGCTGAAGGAGGAACTATATCCGGATTGGCGAATGGG ACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGG -3′	MGSSHHHHHHSSGLVPRGSHMERKHH FVLVHNAYHGAWIWYKLKPLLESAGH RVTAVELAASGIDPRPIQAVETVDEYSK PLIETLKSLPENEEVILVGFSFGGINIALA ADIFPAKIKVLVFLNAFLPDTTHVPSHV LDKYMEMPGGLGDCEFSSHETRNGTMS LLKMGPKFMKARLYQNCPIEDYELAKM LHRQGSFFTEDLSKKEKFSEEGYGSVQR VYVMSSEDKAIPCDFIRWMIDNFNVSKV YEIDGGDHMVMLSKPQKLFDSLSAIAT DYM

Table S1. *At*HNL gene and amino acid sequences

EnginZyme

EziG™ Instruction Manual

General enzyme immobilization for biocatalysis applications

EziG is used for immobilization of His-tagged enzymes, performed in a single step from cell lysate (intracellular expression) or cell-free culture supernatant (extracellular expression). Binding by the His-tag enriches the enzyme in the immobilization process, and offers a non-destructive binding which results in high retention of catalytic activity. The carrier material is inert and suitable in organic solvents as well as aqueous media. It also has excellent fluid properties which minimizes diffusion limitations, to give an effective heterogeneous biocatalyst which is suitable for use in batch reactions and flow chemistry applications.

Carrier Materials

EziG is has a core made of controlled porosity glass (CPG), which is: inert, has low flow resistance due to an interconnecting accessible pore structure, and does not swell. The material is therefore excellent for use as catalyst carrier since it creates large areas for attachment, minimizes diffusion problems, and is applicable in both aqueous and organic environments. By coating the porous surface with an organic polymer a hybrid material (hybCPG) is constructed with tailoring possibilities of the surface, in terms of hydrophobicity and functionalization. CPG (EziG 1) and hybCPG (EziG 2 & 3) are therefore well suited enzyme carriers. The surface properties of the carrier are important for retained activity of the immobilized enzyme; although the enzyme loading may be comparable the activity may differ between EziG 1, 2 and 3. The available surface options are therefore essential for an effective immobilized preparation of the target enzyme.

Affinity Tag Binding

Affinity tags, such as the His_6-tag, are widely used for protein attachment purposes; IMAC is a general method for protein purification. EziG utilizes affinity tags for enzyme immobilization in biocatalytic applications.

EziG Enzyme Immobilization

1. **Prepare cell lysate or culture supernatant with overexpressed His_6-enzyme**
   - Remove all cell debris and use a suitable buffer. **20 mM phosphate or HEPES, 500 mM NaCl, pH 7-8, is recommended.**
   - **Tip:** Increase the binding selectivity by addition of imidazole (25-75 mM).
2. **Add EziG and incubate for 30 min**
   - Orbital shaker, end-over-end or propeller stirring is preferred. **Do not use magnetic stirring** since it grinds the material, destroying the EziG.
   - **Tip:** The first time an enzyme is immobilized, use an excess of enzyme to evaluate the maximum loading for the enzyme in question. Thereafter, binding can be done with the optimum amount of EziG, 15-60% w/w is expected.
3. **Rinse to remove host cell protein**
   - Use the optimal buffer and pH (between 5-10), for the enzyme in question.
   - For drying and storage, vacuum is needed to remove all water from the porous material.
   - **Tip:** In some cases, lyophilization or vacuum drying may deactivate the enzyme. Omit drying if this is observed.

Biocatalysis with your EziG-enzyme

- **Use the EziG immobilized enzyme preparation in your tank or column reactor**
  - **Do not use magnetic stirring** since it grinds the material, destroying the EziG; propeller and end-over-end stirring, or shaking, is recommended.
  - Use pH 5-10 or organic solvents.
  - Use in batch or continuous flow system.
  - After a batch reaction, filter or let the beads sink to the bottom of the vessel, and reuse.
  - **Tip:** Use in situ product removal by applying organic solvent simultaneously when using flow systems.
  - **Tip:** Local pH-effects may cause enzyme leaching. Use a higher ionic strength, pH and/or buffer concentration if this is observed.