-Supporting Information 1-

Batch and flow nitroaldol synthesis catalysed by *Granulicella tundricola* hydroxynitrile lyase immobilised on Celite R-633

José Coloma [1,2], Lidwien Teeuwisse [1], Muhammad Afendi [1], Peter-Leon Hagedoorn [1] and Ulf Hanefeld [1]*

1 Biokatalyse, Afdeling Biotechnologie, Technische Universiteit Delft, Van der Maasweg 9, 2629 HZ Delft, The Netherlands; J.L.ColomaHurel@tudelft.nl (J.C); L.C.Teeuwisse@student.tudelft.nl (L.T.); MuhammadFarhanbinMuhammadAfendi@student.tudelft.nl (M.A); P.L.Hagedoorn@tudelft.nl (P.-L.H); u.hanefeld@tudelft.nl (U.H)

2 Universidad Laica Eloy Alfaro de Manabí, Avenida Circunvalación s/n, Manta 13-05-2732, Ecuador

***Corresponding Author:** Ulf Hanefeld
Tel: +31(0)15-2789304; Fax: +31(0)15-2781415; Email: U.Hanefeld@tudelft.nl.
Biokatalyse, Afdeling Biotechnologie, Technische Universiteit Delft, Van der Maasweg 9, 2629 HZ Delft, The Netherlands

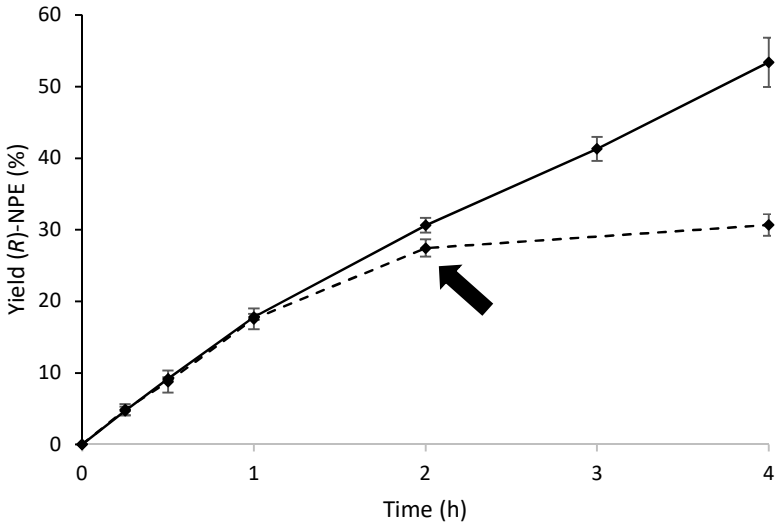


Figure S1. Leaching assay for *Gt*HNL on Celite R-633 (10 U/50 mg Celite R-633). Conditions: Ratio benzaldehyde : nitromethane, 1:50, 20 mM benzaldehyde in MTBE saturated with 100 mM KPi pH 7, 1 M nitromethane, 20 mM 1,3,5-tri-isopropylbenzene as internal standard (I.S) in MTBE saturated with 100 mM KPi pH 7 and *Gt*HNL-3V immobilised on 50 mg Celite R-633. The immobilised *Gt*HNL was freely placed into the glass reaction vial. Reaction volume 1 mL. The reaction was shaken at 1200 rpm at 30 °C. Continuous line and diamonds is the enzyme catalysed reaction (10 U), dashed line and diamonds is the reaction where the immobilised enzyme (10 U) was removed after 2 hours. Error bars correspond to the standard deviation of duplicates (n=2). The yield of (*R*)-NPE was calculated by the ratio of (*R*)-NPE concentration to the initial benzaldehyde concentration.

Table S1: *Gt*HNL- A40H/V42T/Q110H gene sequence

***Gt*HNL-A40H/V42T/Q110H**	***Gt*HNL-A40H/V42T/Q110H – Aminoacid sequence**
5′CCATGGAGATTAAACGTGTTGGTTCTCAGGC TTCTGGTAAAGGTCCGGCTGATTGGTTCACTGG TACTGTTCGTATCGATCCGCTGTTTCAGGCTCC GGATCCGGCATTAGTAGCTGGTCACTCTACTAC CTTTGAACCGGGTGCTCGTACTGCTTGGCATAC TCATCCGTTAGGTCAGACTCTGATTGTAACTGC TGGTTGTGGTTGGGCTCAGCGTGAAGGTGGTGC TGTTGAAGAAATTCATCCGGGTGATGTTGTATG GTTCTCTCCAGGTGAAAAACACTGGCATGGTGC TGCACCAACTACCGCTATGACCCACCTGGCTAT CCACGAACGTCTGGATGGTAAAGCTGTTGATTG GATGGAACACGTTACTGATGAACAGTACCGTCG TTAAGCTT -3′	M E I K R V G S Q A S G K G P A D W F T G T V R I D P L F Q A P D P A L V A G H S T T F E P G A R T A W H T H P L G Q T L I V T A G C G W A Q R E G G A V E E I H P G D V V W F S P G E K H W H G A A P T T A M T H L A I H E R L D G K A V D W M E H V T D E Q Y R R A

Aminoacids in red show the mutations at positions 40, 42 and 110.