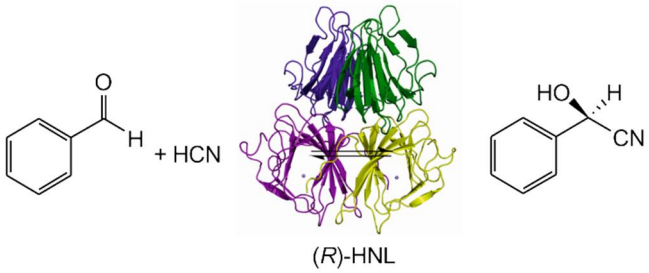
Hydroxynitrile lyases (HNLs) are powerful carbon-carbon bond forming enzymes. The reverse of their natural reaction - the stereoselective addition of hydrogen cyanide (HCN) to carbonyls ï yields chiral cyanohydrins, versatile building blocks for the pharmaceutical and chemical industry**.** To meet the requirements for industrial application, hydroxynitrile lyases need to fulfil several criteria: (i) availability of sufficient quantities of proteins with constant quality and batch-to-batch reproducibility at low cost, (ii) broad substrate scope, (iii) high stability under acidic pH and high solvent stability and (iv) activity at low temperatures. One task of ACIB within the Kyrobio project is the provision of improved hydroxynitrile lyases obtained either from new sources or by enzyme engineering of known enzymes.

Recently, bacterial HNLs have been discovered by our group, which represent a completely new type: HNLs with a cupin fold. Due to various benefits of cupins (like e.g. high yield recombinant expression in *Escherichia coli*) the class of cupin HNLs provides a new source for interesting, powerful hydroxynitrile lyases. Unfortunately, the activity and substrate scope of the cupin HNLs known at the start of Kyrobio was quite limited (*Gt*HNL from *Granulicella tundricola* (*Gt*HNL) catalysed the synthesis of (*R*)-mandelonitrile with a good conversion of 80% and an enantiomeric excess of 90%, Hajnal et al., 2013).



In one WP of the project novel cupin-HNLs from other bacterial sources should be explored by data base search based on the protein sequence of *Gt*HNL. The most promising target after screening for HNL activity, *Ac*HNL from *Acidobacterium capsulatum* ATCC 51196, catalysed the synthesis of (*R*)-mandelonitrile with 97.3 % conversion and 94.7 % *ee* after 24 h, which is a significant improve compared to *Gt*HNL. The results were presented at the Biotrans 2013 and published in June 2014 in CSBJ.

Wiedner R, Gruber-Khadjawi M, Schwab H, Steiner K. 2014. Discovery of a novel (*R*)-selective bacterial hydroxynitrile lyase from *Acidobacterium capsulatum*. *Comp Struct Biotechnol J.*, in press, DOI: 10.1016/j.csbj.2014.07.002.

In another WP, the activity and enantioselectivity of *Gt*HNL was significantly improved by site-saturation mutagenesis of active site amino acids (designed evolution) and random mutagenesis. The combination of beneficial mutations resulted in a variant with 490-fold increased specific activity in comparison to the wild type at the same reaction conditions. More importantly, this variant is a highly competitive alternative for the synthesis of chiral cyanohydrins, such as 2-chlorobenzaldehyde cyanohydrin, (*R*)-2-hydroxy-4-phenylbutyronitrile and (*R*)-2-hydroxy-4-phenyl-3-butene nitrile, which serve as intermediates for the synthesis of pharmaceuticals.

These results have been presented at the ECB16 in Edinburgh in July 2014, and will be submitted within the next few weeks for publication and a patent application has been filed.