ENZYME ENGINEERING AT ALMAC: case studies of enzyme discovery and engineering

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Key Words: Biocatalysis solutions, INSIGHT enzyme discovery, enzyme engineering, AI

Enzyme engineering has been increasingly transforming the chemical and GMP API manufacture landscape. In silico methodologies are responsible for the development of a new generation of improved biocatalysts by intelligently exploring the enzymes multidimensional fitness landscapes, obtaining variants at a fraction of the time and cost compared to traditional protocols. Hereby, we present a few examples of enzymes obtained with our INSIGHT™ enzyme discovery and enzyme engineering platforms.

A successful enzyme development campaign avoids unnecessary costs by starting with an active candidate enzyme. With INSIGHT™ (Figure 1) we sourced new carbonyl reductases for the reduction of bulky-bulky ketone substrates into alcohols. Physics-based, Bioinformatics and AI methods were used to identify important reaction motifs and substrate-binding motifs to screen vast Almac proprietary metagenomic libraries. The identified hits show high activities for conversion of these ketones to industrially relevant alcohols.

Enzyme engineering is utilized to improve enzyme activity, selectivity and/or stability according to the needs of the reaction process. Three different examples of enzyme engineering will be presented. A methionine sulfoxide reductase was evolved to accept bulky sulfoxide substrates bearing non-methyl substituents on the sulfur atom. The best variant was found to catalyze these sulfoxides kinetic resolution of with an ee up to 99% [1]. A cyclase enzyme was first discovered and later engineered to catalyse the cyclization of an alcohol. For a reaction scale of 50g/L of substrate and 2.5% of enzyme loading, with some variants up to 142-fold improved in activity over the WT enzyme.
Finally using Almac's smart site saturation mutagenesis, a nitrilase was developed for one-pot, enantioselective, dynamic kinetic resolution for (R)-2-methoxymandelic acid. After process development an ee of 97% and 70% isolated yield were obtained on multigram scale [2].

References:
[1] *ACS Catal.* 2023, 13, 7, 4742–4751
[2] *Org. Process Res. Dev.* 2022, 26, 3, 849–858.

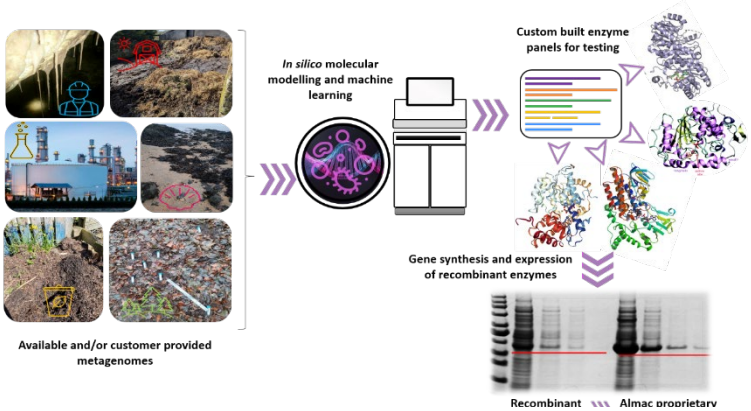

Figure 1 – General overview of the INSIGHT™ platform.