IN SILICO-ASSISTED EVOLUTION OF TRANSAMINASES AND CHIRAL AMINE SCALE-UP

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Abstract

Chiral amines are important building blocks for a large range of industrially valuable compounds. An increasing number of chiral amines are prepared using enzymatic methods to avoid complex chemical synthesis [1]. Among several enzymatic approaches employed for the synthesis of optically active amines, transaminase (TAm) enzymes offer an attractive route to *R* and *S* chiral amines starting from readily available *pro*-chiral ketones. Rational protein engineering is a promising approach for achieving significant increase in the efficiency of biocatalysts tailored for specific industrial applications [2]. The present poster highlights *in silico* rational design as a rapid method to increase TAm activity towards hindered ketones. A significant improvement over wild type activity was achieved by screening small mutant libraries using high throughput UHPLC and colorimetric assays with ~30-fold increase in activity.

Introduction

The preparation of optically pure sterically hindered amines is a current thrust area in the pharmaceutical industry. Biocatalytic synthesis of hindered amines through stereo selective transamination of their corresponding hindered ketones (small bulky ketones and bulky-bulky ketones) offers a green and much more efficient alternative to metal-catalysed asymmetric reduction. A transamination project was investigated at Almac as part of an API synthesis programme as shown in **Figure 1**.

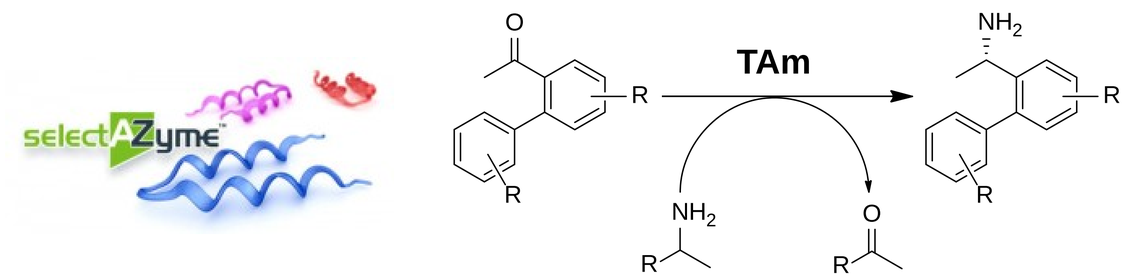

Figure 1: Investigation of Almac's transaminase panel to generate novel amines as part of an API synthesis project

Candidate Identification

Almac's diverse in-house TAm enzyme panels were screened for TAms exhibiting activity against the hindered ketone shown above. Hits were identified using sensitive colorimetric assays and UHPLC analyses allowing fast and reliable hit characterisation.

Enzyme Engineering

The classic approach towards enzyme optimization using random mutation often results in screeening of large mutant libraries. Molecular modelling and bioinformatic analysis provide a powerful tool to build focused libraries and overcome the need to screen large number of clones. Our *in silico* rational protein engineering approach uses molecular docking and molecular dynamics (MD) simulations in combination with information about correlated mutation networks within the protein sequences as shown in **Figure 2**.

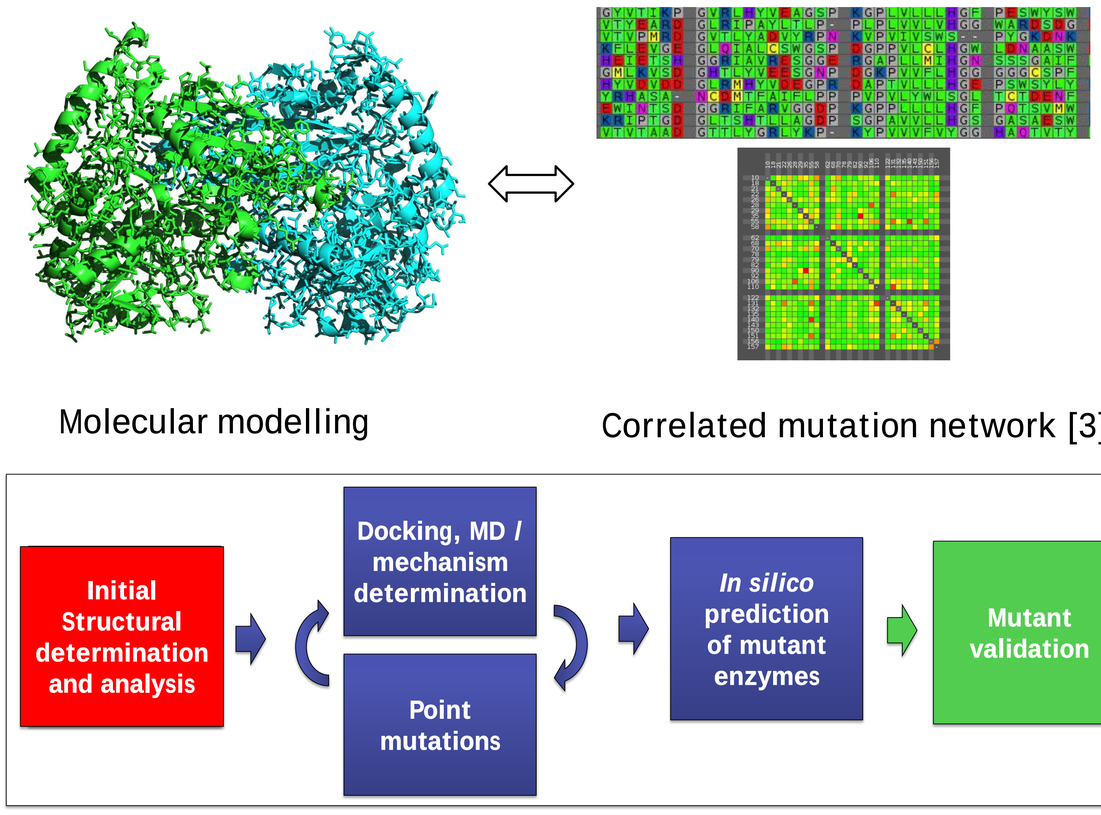

Figure 2: Combining molecular modelling and bioinformatic analysis to generate focused mutant libraries

Results

In order to increase transaminase activity towards hindered ketones a focused library of 70 transaminase mutants containing single and combinatorial mutations was synthesized. The results from the mutant library screen are shown in **Figure 4** (Red = no activity, Yellow = similar activity to WT and Green = enhanced activity).

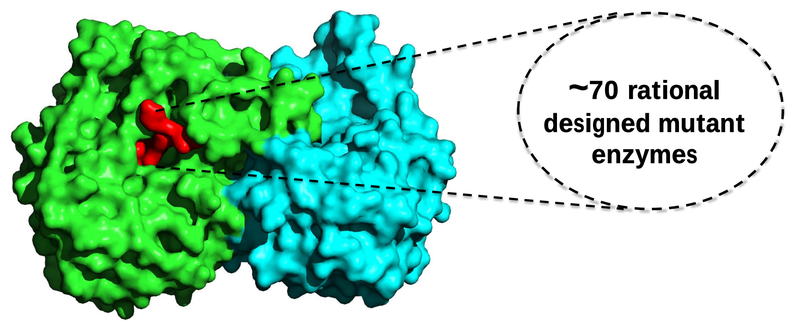

Figure 3: A focused mutant library was synthesised containing single and combinatorial mutations in the active site

The expression analysis revealed that soluble protein expression was mainly retained in the mutant library and is shown in **Figure 4**. Only changes in one of the mutated amino acid residue were leading to insoluble protein formation.

	1	2	3	4	5	6	7	8	9	10	11	12
A	WT	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11
B	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22	M23
C	M24	M25	M26	M27	M28	M29	M30	M31	M32	M33	M34	M35
D	M36	M37	M38	M39	M40	M41	M42	M43	M44	M45	M46	M47
E	M48	M49	M50	M51	M52	M53	M54	M55	M56	M57	M58	M59
F	M60	M61	M62	M63	M64	M65	M66	M67	M68	M69	M70	M71

Antibody-dot blot expression analysis

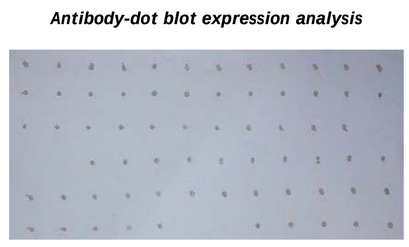

Figure 4: Activity and antibody-dot blot expression analysis of the mutant library

Over half of the mutants in the library showed activity against the tested ketone with best mutants reaching up to 30-fold increase in activity as shown in **Figure 5**. In all of the cases the enantiomeric purity remained at >99%.

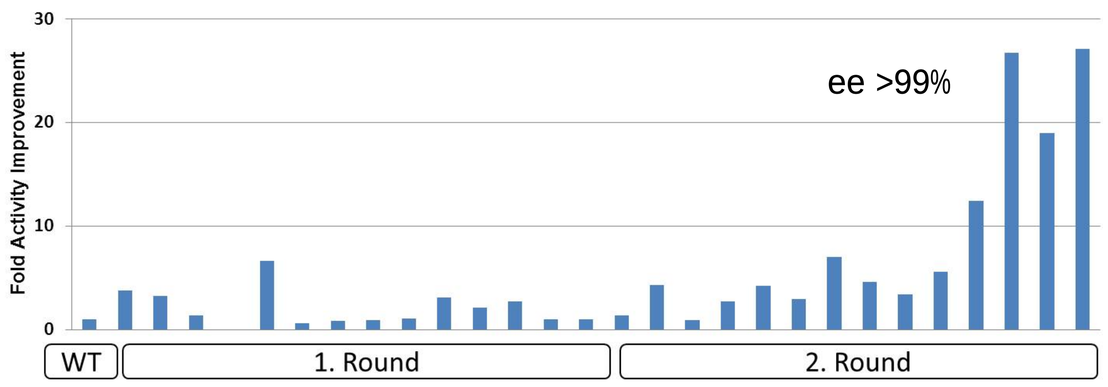

Figure 5: Focused library appraoch resulted in 30-fold activity increase towards the selected hindered ketone

Summary

Transaminases can show low activity towards industrial important ketones making it necessary to optimise the reaction by enzyme engineering. Focused libraries based on molecular modelling and bioinformatic analysis are a powerful alternative to the screening of large numbers of random mutant libraries, thereby lowering the cost and timelines to access superior biocatalysts.

[1] R. C. Simon, *et* al, *ACS Catal.* 2014, 4(1)
[2] K. Steiner, *JComput. Struc. Biotechnol. J.* 2012, 2(3).
[3] R. Kourist, *Chembiochem*. 2010 Aug 16;11(12)

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