NewProt Deliverables

D6.1 (Experimental) validation year 1

Experimental validation of the PLP dependent transferase 3DM database

3DM was used to compare the amino acid distribution in an active site loop present in known ATAs in order to design a library of loop mutants. The mutants were prepared and then the activity against amines of varying steric bulk was accessed *via* a spectroscopic assay.

Comparison of the loop region of the ATA subfamilies within the 3DM database led to the discovery that one subfamily contained loop sequences of varying length. This led to the development of a loop exchange strategy whereby the loop of interest of one subfamily is exchanged for that of another subfamily in order to increase the substrate space within the active site. The results from this route so far indicate that introducing mutations which are novel to one subfamily into a different subfamily (where there is no president for such a mutation) leads to inactive mutants. This is in agreement with the ‘small, smart ’concept for library design with 3DM has been used successfully in the past in protein engineering.

Therefore 3DM should be used to modify the loop exchange library to focus only on loop mutants, which follow this concept, which could mean using the database to find a new template for the loop exchange. If successful this would validate the use of 3DM in the design of ‘small, smart libraries’ for protein engineering, but also show the power of 3DM as a tool for protein engineering template discovery.

Deliverables:

* Small library design, preparation and screening of 30 variants against 3 different substrates.
* Poster presentation ‘The use of a 3DM Database for the Design of a "*Small, but Smart*" Library of Loop Exchange Mutants of Amine Transaminases’at the 2nd Symposium on Protein Engineering for Biocatalysis, Greifswald, Germany.