

P031 Expanding the Substrate Range of Monoamine Oxidase
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Monoamine oxidase (MAO-N) from *Aspergillus niger* was previously subjected to directed evolution and saturation mutagenesis, resulting in a variant with enhanced activity towards amines such as (S)- α -methylbenzylamine and (S)-1-methyltetrahydroisoquinoline ((S)-MTQ). This enantioselective variant was used as a starting point to identify further variants for the deracemisation of various chiral amines.

The X-ray crystal structure of human MAO-B has been reported. We used the 3-D structure as a reference to generate a MAO-N model in which (S)-MTQ was modelled into the active site to reveal all the residues located at a distance up to 7 Å from the substrate. Eleven such residues were identified. Saturation mutagenesis was then performed either at single or double sites. The variant libraries were screened using a colorimetric high-throughput plate-based assay. Several amines were screened and full kinetic data was obtained from the promising hits using purified protein in a microtitre plate-based format.

To date, 3 libraries have been screened and 3 variants from the same library identified, resulting in up to a 37-fold increase in activity towards 1,2,3,4-tetrahydro-1-naphthylamine. These variants will be subjected to whole cell deracemisation experiments and structural characterisation to understand the nature of the mutation. We also intend carrying out recombination of mutants once the remaining libraries have been screened in order to maximize the enzyme activity.