	Experiment title: New insights into the mode of inhibition of human mitochondrial monoamine oxidase B from high resolution crystal structures	**Experiment number**: LS2183 MX129
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Report:

The results obtained thanks to the experiments carried out at the ESRF are being published:

1. Binda, C., Li, M., Hubálek, F., Restelli, N., Edmondson, D.E., Mattevi, A. (2003) New insights into the mode of inhibition of human mitochondrial monoamine oxidase B from high resolution crystal structures, *Proc. Natl. Acad. Sci.*, in press.

2. Hubálek, F., Binda, C., Li, M., Mattevi, A., Edmondson, D.E., (2003) Polystyrene micro-bridges used in sitting drop crystallisation release 1,4-diphenyl-“-butene, a novel inhibitor of human MAO B. *Acta Cryst D*, in press

As a summary of the work carried out at the ESRF, we include here the abstract of the two articles

New insights into the mode of inhibition of human mitochondrial monoamine oxidase B from high resolution crystal structures

Monoamine oxidase B (MAO B) is an outer mitochondrial membrane-bound enzyme that catalyses the oxidative deamination of arylalkylamine neurotransmitters and has been a target for a number of clinically-used drug inhibitors. The 1.7 Å structure of the reversible isatin-MAO B complex has been determined which forms a basis for the interpretation of the enzyme's structure bound to either reversible or irreversible inhibitors. 1,4-Diphenyl-2-butene is found to be a novel reversible MAO B inhibitor which occupies both the entrance and substrate cavity space in the enzyme. Comparison of these two structures identifies Ile199 as a "gate" between the two cavities. Rotation of the side chain allows for either separation or fusion of the two cavities. Inhibition of the enzyme with N-(2-aminoethyl)-*p*-chlorobenzamide results in the formation of a covalent N(5) flavin adduct with the phenyl ring of the inhibitor occupying a position in the catalytic site overlapping that of isatin. Inhibition of MAO B with the clinically-used *trans*-2-phenylcyclopropylamine results in the formation of a covalent C(4a) flavin adduct with an opened cyclopropyl ring and the phenyl ring in a parallel orientation to the flavin. The peptide bond between the flavin substituted Cys397 and Tyr398 is in a *cis* conformation, which allows the proper orientation of the phenolic ring of Tyr398 in the active site. The flavin ring exists in a twisted, non-planar conformation which is observed in the oxidized form as well as in both the N(5) and in the C(4a) adducts. An immobile water is H-bonded to Lys296 and to the N(5) of the flavin as observed in other flavin-dependent amine oxidases. The active site cavities are highly apolar, however, hydrophilic areas exist near the flavin which direct the amine moiety of the substrate for binding and catalysis. Small conformational changes are observed on comparison of the different inhibitor–enzyme complexes. Future MAO B drug design will need to consider "induced fit" contributions as an element in ligand-enzyme interactions.

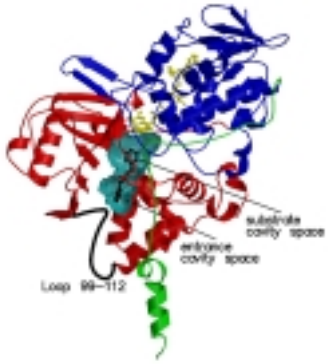

Figure 1. Overall three-dimensional structure of human MAO B monomeric unit in complex with 1,4-diphenyl-2-butene. The FAD-binding is in blue, the substrate-binding domain in red, and the C-terminal membrane binding region in green. The inhibitor binds in a cavity (shown as a cyan surface) which results from the fusion of the entrance and substrate cavities.

Polystyrene micro-bridges used in sitting drop crystallisation release 1,4-diphenyl-“-butene, a novel inhibitor of human MAO B.

In the course of protein structure determinations of the membrane-bound enzyme monoamine oxidase B (MAO B) by X-ray crystallography, a compound was found in the active site of the enzyme that consists of two phenyl rings separated by four carbons. This compound was identified by chromatography and by mass spectrometry to be 1,4-diphenyl-2-butene and found to be a component of the polystyrene micro-bridges that are used in protein crystallization. This compound is present at a level of ~0.3 mg (~1.5 μmoles)/micro-bridge and functions as a competitive inhibitor of MAO B with a Ki of 35 μM. The presence of detergents in the crystallization solutions facilitates the extraction of this compound from the polymer medium.

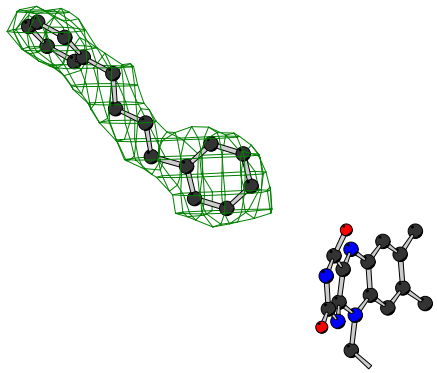

Figure 2. The unbiased 2Fo-Fc electron density map at 2.3 Å resolution (shown in stereo at 1 σ contour level) for the 1,4-diphenyl-2-butene inhibitor bound to MAO B