Integrative structural study of antibiotic-inactivating enzyme from *Stenotrophomonas maltophilia*

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An opportunistic bacterial pathogen *Stenotrophomonas maltophilia* causes a serious number of infections worldwide. This species possesses significant antibiotic resistance that has been further broadened owing to the ability to acquire antibiotic-resistance genes and mutations [1]. Based on a bioinformatic analysis of its sequenced genomes, we selected for our research a not yet experimentally characterised protein, sequentially related to antibiotic-inactivating enzymes.

The target protein was expressed in *Escherichia coli* strain Lemo21 (DE3) and subsequently purified using affinity and size-exclusion chromatography. Results from the spectrophotometric assay in the UV-VIS range indicated that the protein catalysed the enzymatic modification of antibiotics.

Moreover, the enzyme was crystallized and a dataset of diffraction images was collected at a synchrotron radiation source. The diffraction was strongly anisotropic: the proposed high-resolution cutoff, reported in *Aimless* [2] using the criterion of $CC_{1/2} > 0.30$, was in a range from 2.43 Å to 1.92 Å, depending on the direction in the reciprocal space. Hence, the anisotropic correction was carried out with *STARANISO* [3]. The phase problem was solved using molecular replacement in *MoRDa* [4], followed by refinement of the structure model in *REFMAC*5 [5]. Paired refinement on the anisotropic data was performed with *PAIREF* [6] to determine the diffraction limit of 1.95 Å.

The asymmetric unit contains two homodimers. Each dimer is associated through two disulfide bridges. Nonetheless, the further analysis of oligomerization, conducted with mass structural spectrometry and small-angle X-ray scattering, revealed that the enzyme is in a monomeric state in solution. The overall molecular structure is analogous to the family of tetracycline destructases [7] or the reductase from the biosynthesis pathway of abyssomicines [8]. However, the arrangement of the putative substrate-binding pocket differs significantly. The crystal structure provides the basis for a further *in silico*, *in vitro* or *in crystallo* investigation of the complexes with antibiotic substrates or potential inhibitors.

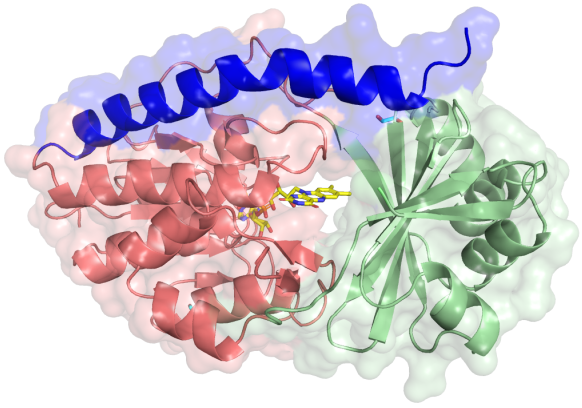

Figure 1. Crystal structure of a monomer of the antibiotic-inactivating enzyme from *Stenotrophomonas maltophilia*. The FAD-binding domain is coloured in red, the substrate-binding domain in green, and the C-terminal helix in blue. Flavin adenine dinucleotide (FAD) is displayed in stick representation (carbon atoms in yellow).

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