MS5-P47 Characterisation of a phosphinothricin N-acetyltransferase from *Pseudomonas syringae*

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The herbicide glufosinate contains the glutamate analog phosphinothricin, which inactivates glutamine synthetase. Protection of crops against the herbicide is conferred by the *bar* gene, first isolated from *Streptomyces*, the product of which N-acetylates phosphinothricin [1,2] A putative phosphinothricin N-acetyl transferase from *Pseudomonas aeruginosa* PAC1, termed pita, displayed activity with methionine-sulfoximine but not phosphinothricin, although both compounds are glutamate analogs [3]. We cloned and expressed a putative phosphinothricin N-acetyltransferase with 41% sequence identity to pita, from *Pseudomonas syringae* pv. tomato DC3000, a strain responsible for bacterial speck in tomato and *Arabidopsis thaliana* [4]. Kinetic characterisation of this enzyme, termed syr_bar, demonstrated specificity for phosphinothricin, and not methionine sulfoximine. The structure of syr_bar was solved to 1.6Å resolution, revealing a dimeric arrangement, with a similar overall fold and active site structure to pita. We also solved the structure for syr_bar in complex with phosphinothricin to 2.6Å resolution, in a second crystal form. Comparison with pita from *P. aeruginosa,* and kinetic and crystallographic characterisation of syr_bar active site mutants, has provided insight into the determinants governing specificity for methionine-sulfoximine and phosphinothricin.

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MS5-P48 Dragonfly® screen optimizer helps researchers tackle tuberculosis

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The ability to crystallise proteins, nucleic acids or macromolecular complexes pose significant challenges to the protein crystallography community, from large scale screening assays for the determination of initial crystallization conditions, screen optimization and final screen set-up. Protein crystal optimization is vital to ensure high quality X-ray diffraction data for the solving of high resolution structure. This process involves the set-up of a series of complex screening combinations where the ratios of the individual components identified from primary crystallization studies are varied. In order to reduce the effort and tedium of this process, TTP Labtech have designed dragonfly® for crystallization screening as an addition to their successful mosquito liquid handling portfolio. This poster will describe an example of how the dragonfly has benefited drug discovery. Dr. Michal Blaszczyk at Cambridge University, UK has optimised the conditions for the crystallization of a target enzyme involved in the growth of the mycobacteria that cases tuberculosis (*Mycobacterium tuberculosis).* Extensive screening using TTP Labtech's mosquito Crystal produced the initial hits which went on to be optimised. Using dragonfly optimization of 20 conditions took 1 week rather than a more usual time of several weeks. This high throughput optimization screening method also reduced the volume of condition media required by nearly two thirds, mainly due to the ability to perform the screen in 96-well rather than 24-well, plates. The highly reproducible results have led to optimised crystals that diffract well. Dr Blaszczyk plans to use dragonfly to develop high throughput methods that have not been possible previously, for example, *in situ* screening for crystallization. This poster demonstrates that "dragonfly" is a valuable, compact, low cost addition to the crystallographer's bench. It eliminates lengthy and complicated plate set-up at the optimization stage of crystallization.