ESRF	**Experiment title:** Structure determination of arylamine N-acetyltransferases, and inhibited complexes thereof	**Experiment number:** LS-953
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Report:

Arylamine N-acetyltransferase (NAT) is an enzyme found in eukaryotes and a range of bacteria including *Salmonella typhimurium, E. coli,* and *Mycobacterium tuberculosis.* **NAT** enzymes are dependent upon coenzyme-A, are of approximately 30kDa in size, and are anticipated to have a novel fold. Although the identity of the endogenous substrate is not established, the broad selectivity of NAT enzymes gives them activity against a wide range of xenobiotics, including antibiotics. Since it is known that the anti-tubercular agent isoniazid is a NAT substrate, and that acetylation of isoniazid by human NAT inactivates the drug, it may be that effective inhibition of bacterial NAT provides a route to addressing some instances of antibiotic resistance. The aim of the experiment is to determine the structure of NAT from *S. typhimurium,* and inhibited forms thereof.

In order to solve the structure of NAT, we collected a total of 7 datasets representing three different potential heavy atom derivatives of NAT at various different wavelengths. The first candidate was a substrate like arylamine substituted with a bromine. This compound

forms an irreversibly inhibited complex with NAT, retaining the bromine atom of the inhibitor. An EXAFS scan was used to determine the optimal wavelength to collect peak and remote wavelength datasets. It was apparent from the EXAFS scan that the bromine anomalous signal was very weak, and data reduction showed that the merging R-factor for Friedel reflections was not higher than for symmetry related reflections, indicating poor occupancy by the bromine compound. It was decided therefore not to collect an inflection point dataset for this derivative.

Two mercury containing compounds were then used: one of which resembled the product of a NAT reaction, and the other being ethylmercury thiosalicylate (EMTS). For both compound, EXAFS scans were performed, and optimal peak wavelengths collected. For the EMTS, two further wavelengths were collected (remote and inflection point), and for the other compound only an inflection point dataset was collected. Both of the mercury compounds show higher values for Ranom than Rsym up to a resolution of 4.5 angstroms. Initial attempts to solve heavy atom structures for these datasets have been unsuccessful. We intend to pursue this problem both computationally using recent advances (the SOLVE package and the program REVISE), and by further data collection.