ESRF	**Experiment title:** London Cancer Group BAG – AGT	**Experiment number**: LS-1950
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Report:

Primary hyperoxaluria type 1 (PH1) is the more common of the two well-characterized hereditary hyperoxalurias. It is characterised by excessive synthesis and excretion of oxalate and glycolate and the deposition of insoluble calcium oxalate in the kidney and urinary tract as nephrocalcinosis and/or urolithiasis. PH1 is caused by a deficiency of the liver-specific intermediary metabolic enzyme alanine:glyoxylate aminotransferase (AGT). AGT is a pyridoxal phosphate-dependent enzyme that catalyses the transamination/detoxification of glyoxylate to glycine. At the level of enzyme phenotype, about one third of patients have disease because AGT is mistargeted from peroxisomes to mitochondria. Such an organelle-to-organelle enzyme trafficking defect is without parallel in human genetic disease.

Crystals of AGT with a variety of the 30 known mutations were grown. All adopt a tetragonal habit. Initially though, it appeared that the different proteins grew in different, but related, tetragonal or orthorhombic space groups. Native data was collected on AGT5 (Pro11Leu), which crystallised in the smallest cell (1 molecule per asymmetric unit). Attempts to solve this by molecular replacement using 1c0n (a selenocysteine lyase chosen from its supposed

structural similarity) as the search model failed. This was not surprising though, due to the low sequence identity between the two proteins.

Seleno-methionine labelled protein was produced. There are 11 methionines in the 22kD protein and we achieved an incorporation of >95%, as judged by mass spectrometry. We were initially unable to grow any crystals using this protein, but finally succeeded by microseeding methods using unlabelled protein crystals as the seeds. The first attempt to collect data on these crystals failed when the diffraction limit of the crystals was found to be much lower than for the native protein. We suspect that this was due to oxidation of the Se-Met in the crystal and the subsequent damage to the lattice. The second attempt was much more successful from a diffraction point of view, with data extending to 2.5Å. We were unable to find a crystal with the small tetragonal unit cell, and therefore took several data sets that indexed as either the large tetragonal cell or the large orthorhombic form. Analysis of the anomolous signal from the data indicated that there was something amiss and further scrutiny of the intensity statistics showed that the crystals were probably all multiple/twinned in a manner that gave rise to the super cells. An exhaustive check of all crystals showed them to be similar.

Small tetragonal Se-Met labelled crystals have now been grown and the data should be able to be solved fairly quickly.