ESRF	**Experiment title:** **SOUTHAMPTON / PORTSMOUTH BAG**	**Experiment number**: LS-1812
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Shifts: 6	**Local contact(s)**: THOMPSON Andrew	*Received at ESRF:*

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Report:

5-Aminolevulinate dehydratase (ALAD), sometimes referred to as porphobilinogen synthase, catalyses the second step in the biosynthesis of tetrapyrroles involving the condensation of two 5-aminolaevulinic acid (ALA) molecules to form the pyrrole porphobilinogen (PBG). We have determined the X-ray structure of yeast ALAD showing for the first time that the enzyme forms a large homo-octameric structure in which each subunit adopts the TIM barrel fold with an extended N-terminal arm. The monomers associate to form compact dimers and four of these interact, principally via their arm regions, to form the octamer. In the active site of each subunit are two lysine residues, one of which forms a Schiff base link to the substrate. The enzyme has a well defined zinc binding site at the catalytic centre formed by 3 cysteines (α-site). The enzyme is strongly inhibited by lead ions and we have shown that these replace the zinc ion bound at the active site. The structure of *E. coli* ALAD was then determined at high resolution revealing for the first time the nature of a regulatory metal binding site (β-site) possessed by many ALADs. Our work revealed how known clinical mutations of human ALAD, some of which are harmful, would affect the structure of the enzyme.

Work on analysis of active-site directed mutants and complexes with inhibitors that form a Schiff base complex with the enzyme has yielded important information on the substrate binding sites and catalytic mechanism. During the experiment at ESRF, data were collected on a complex of yeast ALAD with a di-keto inhibitor (4,7-dioxo-sebacic acid) to 1.8 Å resolution. The data have an R-merge of 13.6 % and are 99.5 % complete. The inhibitor appears to be unique in that it forms two Schiff bases at the active site with the two invariant lysines. One half of the inhibitor occupies the P-site of the enzyme and is in a conformation very similar to that found in the other complexes we have analysed. The second half of the molecule defines interactions which substrate most probably makes at the more elusive A-site of the enzyme with unprecedented clarity. These include salt-bridges with conserved arginines.

Data to 3.0 Å were collected on human erythroid porphobilinogen deaminase (PBGD) allowing this structure to be solved by molecular replacement for ongoing refinement. The data from this crystal form (which has 4 molecules per asymmetric unit) have an R-merge of 9.6 % and are 95.0 % complete.

Data were collected on crystals of the vsr mismatch restriction endonuclease which extend to 2.2 Å resolution and are 80 % complete with an R-merge of 12.1 %. This structure has been solved by molecular replacement and is currently being refined. Data to 1.9 Å resolution were collected on a crystal form of serum amyloid P-component grown at high calcium ion concentration with the objective of studying the structural basis for the known effects of calcium on the protein's solubility. These data are 96 % complete with an R-merge of 17.2 % - the structure is currently being refined. Data to 2.4 Å were collected on crystals of plant chloroplast chaperonin Cpn60 α-subunit. The structure is currently being analysed by molecular replacement. The data have an R-merge of 14.0 % and are 99.4 % complete.

During the same experimental run datasets were also collected on three putative inhibitor complexes of the C-C bond hydrolase MhpC to around 2.5 Å resolution. These datesets have R-merge values in the range of 8 – 9 % and are in excess of 94 % complete but inspection of the final electron density maps revealed no signs that the ligands had bound to the enzyme.

A near-atomic resolution dataset was also collected on a G76S mutant of bovine inositol monophosphatase which extended to 1.3 Å resolution and is currently being analysed. This loop mutant, which was engineered to study the folding pathway of the enzyme, has the advantage of diffracting to higher resolution than the native enzyme.

Preliminary low resolution datasets were obtained from test crystals of Southampton virus proteinase and from an oligonucleotide complex of gene 5 protein.