ESRF	**Experiment title:** London Cancer Group BAG – Inositol Synthase.	**Experiment number**: LS-1950
Beamline: ID14-4	**Date of experiment**: from: 06/06/01 to: 08/06/01	**Date of report**: 31/08/01
Shifts: 3	**Local contact(s)**: Raimond Ravelli	*Received at ESRF:*

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Report:

This year we have determined the first structure of an inositol synthase at 1.95Å resolution bound to the cofactor NAD and to a zinc ion. The three-dimensional structure of inositol synthase was first determined at 2.6Å resolution from multiwavelength anomalous diffraction data measured on station ID14-4, using a crystal of a selenomethionine-substituted form of the enzyme. The selenium sites were identified during the data collection and an interpretable electron density map was produced shortly afterwards. Whilst still on the ID14-4 beamline we then collected a high resolution native dataset to 1.95Å. The selenomethionine structure was refined to an R-factor of 23.7 and an R-free of 25.0 and then used as a starting point for building into the 1.95Å resolution native dataset. The native structure was refined to an R-factor of 21.9 and R-free of 24.7.
The enzyme is tetrameric with each subunit comprising an NAD-binding domain and a dimerisation domain. Pairs of dimers associate through an extended ß-sheet to generate a tetramer with D_2 symmetry. Inositol synthase converts D-glucose-6-phosphate to L-inositol-1-phosphate via a three step mechanism. The active site is located within a deep cleft at the junction between the NAD-binding domain and a dimerisation domain. Using site-directed mutagenesis we have identified several active site residues required for catalysis and given some insight into the complex mechanism of both eukaryotic and prokaryotic inositol synthases.

Figure Legend – MOLSCRIPT rendering of the inositol synthase tetramer, each protomer is coloured differently. Four NAD cofactors are drawn as sticks and a zinc atom shown as a green sphere.