ESRF	**Experiment title:** **The structures of family 2 Inorganic Pyrophosphatases**	**Experiment number**: LS1684 LS1504
Beamline: ID14-4 BM30 ID14-1	Date of experiment: from: 15 Feb 2000 to: 17 Feb 2000 (LS1504) from: 9 Apr 2000 to: 10 Apr 2000 (LS1684) from: 3 Jun 2000 to: 5 Jun 2000 (LS1684)	**Date of report**: Sep 2000
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Report:

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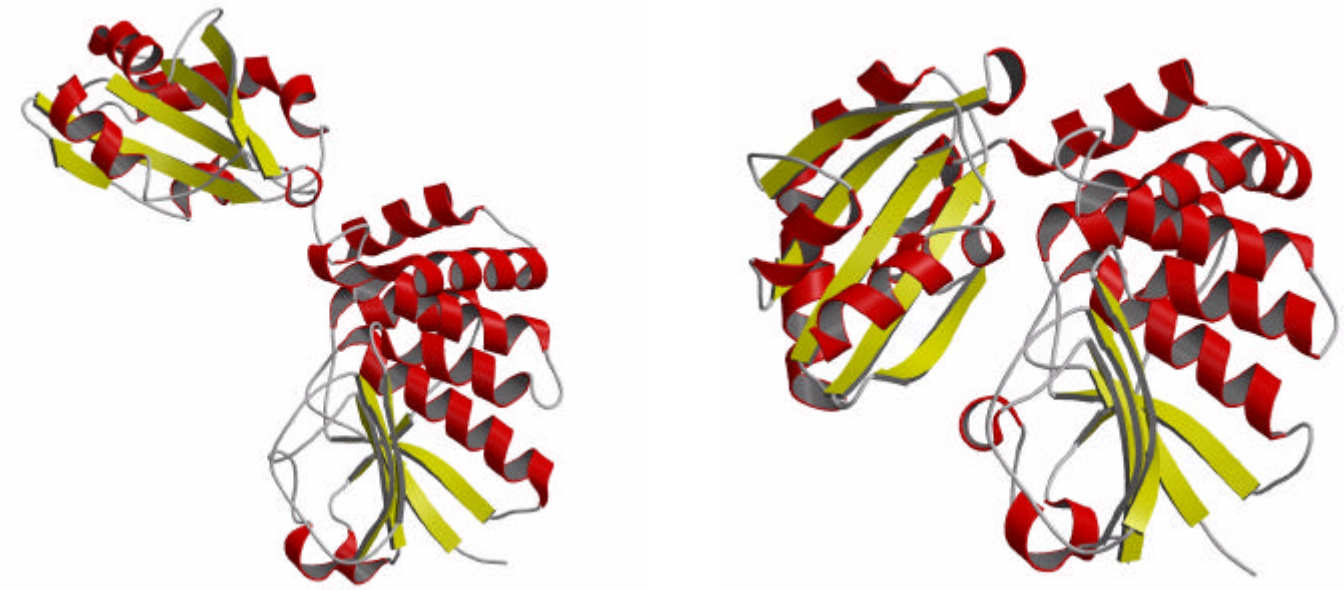
Inorganic pyrophosphatases (PPases) are ubiquitous enzymes, catalysing the hydrolysis of the toxic by-product pyrophosphate, produced during the biosynthesis of many important biomolecules, e.g. DNA, RNA and protein polypeptide. Pyrophosphate hydrolysis is thermodynamically favourable and thus provides a thermodynamic driving force to cellular processes such as DNA synthesis. PPases have been shown to be essential for the growth of the cell. There are two classes of inorganic pyrophosphatase, the second class, so called "family 2", was only discovered 3 years ago. In the first experiment (ID14-4, LS1504) we collected a MAD data set on the SelenoMet derivative of *Bacillus subtilis* PPase, a member of the family 2 class. The structure was solved using the program Shake'n'Bake from the diffraction data collected at the peak wavelength. Selenium positions were refined against all three wavelengths of the MAD data set using the program SHARP. The structure reveals four

copies of the protein in the asymmetric unit as two homodimers. Each monomer consists of a single polypeptide folded into two domains. A putative active site has been located in the N-terminal domain. Together, the two domains form a very open structure.

In the second experiment (BM30, LS1684), a MAD data set to 3.0 Å resolution was collected on a SelenoMet derivative of a second family 2 member, the *Streptococcus gordoni* PPase. This structure was solved using all three wavelengths in the program SOLVE, taking a total of 36 minutes of CPU to find all possible Selenium sites (Solve score of 96). The data were later extended to 1.5 Å resolution using data collected on ID14-1 (LS1684). The structure reveals a homodimer in the asymmetric unit, again with each polypeptide folding into two domains. However, in the *S.g.* structure the N-terminal and C-terminal domains are tightly packed together to form a complex, representing a rotation of approximately 90 - 100° of the C-terminal domain about a 2 - 3 amino-acid residue “hinge” region.

These are the first structures of family 2 PPases. It is clear that the family 2 PPase is different from the family 1 PPase in every respect. To date, only one species has been found to have both a family 1 and family 2 PPase: the Collera pathogen.

(a) **(b)**



The 3.0 Å structure of *Bacillus subtilis* inorganic pyrophosphatase (a) in the open form and (b) the 1.5 Å structure of *Streptococus gordoni* inorganic pyrophosphatase in the ‘closed’ conformation. The N-terminal domain (right in both figures) is in the same relative orientation.