ESRF	**Experiment title:** Sso10b – an archaeal DNA-binding protein	**Experiment number**: LS-1821
Beamline: ID14-4	**Date of experiment**: from: 26/01/01 to: 27/01/01	**Date of report**:
Shifts: 3	**Local contact(s)**: Raimond Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***Professor Garry Taylor**

***Dr Rupert Russell**

Centre for Biomolecular Sciences

University of St Andrews

St Andrews

Fife KY16 9ST

Report:

Sso10b is a 10.5kDa DNA binding protein found in abundance in archaea. The protein forms a dimer, and is highly basic. There is evidence that the protein is involved in the compaction of DNA, and may represent some kind of early histone. The protein has homologues in bacteria and some eukarya.

Dr Malcolm White and Mr Ben Wardleworth at St Andrews have isolated, cloned and expressed sso10b. In collaboration with them, we have crystallised the protein which readily forms large hexagonal crystals belonging to space group $P6_122$ or its enantiomorph. In-house the crystals diffract to 2.6Å. Se-Met protein was produced for MAD phasing at ESRF.

We collected 3 wavelengths of data (peak λ=0.979Å, inflection λ=0.980Å, remote λ=0.939Å) from one frozen crystal with each set representing a 45º rotationof the crystal. Each dataset consisted of approximately 125,000 observations, representing 10,000 unique reflections to 2.7Å, with 100% completeness, and an Rmerge=0.061 overall and Rmerge=0.53 in the top shell. The structure was readily determined using 'solve' from one Se per monomer, and a dimer in the asymmetric unit. The spacegroup is $P6_522$. The structure has been refined currently to an R=0.23, Rfree=0.28.

Problems with ID14-4 meant that data collection did not start until late evening. The data were collected in <1 hour however.

ESRF	**Experiment title:** KDG aldolase from a hyperthermophile	**Experiment number**: LS-1821
Beamline: ID14-4	**Date of experiment**: from: 26/01/01 to: 27/01/01	**Date of report**:
Shifts: 3	**Local contact(s)**: Raimond Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***Professor Garry Taylor**

***Ms Helen Walden**

Centre for Biomolecular Sciences

University of St Andrews

St Andrews

Fife KY16 9ST

Report:

KDG aldolase catalyses the reversible aldol cleavage of 2-keto-3-deoxygluconate to pyruvate and glyceraldehyde in a non-phosphorylated pathway of glucose oxidation in the hyperthermophilic archaeon, *Sulfolobus solfataricus*. The enzyme is of biotechnological importance.

We had previously collected data from several crystal forms, but failed to solve the structure by molecular replacement using the homologue N-acetyl neuraminate lyase with which KDG aldolase shares 27% sequence identity. We therefore produced a Se-Met labelled protein, which is tetrameric with 4 Se atoms per monomer. The crystal form used at ESRF has one tetramer in the asymmetric unit.

We collected 3 wavelengths of data (peak λ=0.979Å, inflection λ=0.980Å, remote λ=0.939Å) from one frozen crystal with each set representing a 75º rotation of the crystal. Exposure time was one second per image. The cell dimensions were consistent with earlier data collections from crystals with 422 crystal class. Upon merging the data, it was clear that the crystals were now orthorhombic, $P2_12_12_1$, with a=83.5, b=131.1, c=132.4. Each dataset consisted of approximately 270,000 observations, representing 51,000 unique reflections to 2.5Å, with 98% completeness, and an Rmerge=0.068 overall and Rmerge=0.26 in the top shell. The structure was readily determined using 'solve' from 16 Se atoms, four per monomer. The structure has been traced and refined against the Se-Met data, and is now being refined against a 2.15Å dataset collected 2 years ago in Hamburg from a different crystal form with two tetramers per asymmetric unit.

Problems with ID14-4 meant that data collection did not start until late evening. The data were collected in <1 hour however.

ESRF	**Experiment title:** PK tag Fab-peptide complex	**Experiment number**: LS-1821
Beamline: ID14-4	**Date of experiment**: from: 26/01/01 to: 27/01/01	**Date of report**:
Shifts: 3	**Local contact(s)**: Raimond Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***Professor Garry Taylor**

***Ms Jane Potter**

Centre for Biomolecular Sciences

University of St Andrews

St Andrews

Fife KY16 9ST

Report:

The PK tag, or V5 tag as it is called in some quarters, is a continuous epitope on the P and V gene products of paramyxoviruses. In these viruses there is a stagger in the transcription of the P/V gene which creates two messages of different lengths coding two proteins that share an N-terminal domain, but which have quite different C-terminal domains. The very high affinity of a panel of monoclonal antibodies for a small peptide that is present in the N-terminal domain, has made the use of this peptide tag widespread. Our interest is ultimately to determine the structure of the P protein, however the struture of the Fab-peptide complex is also of geat interest. We plan to design other peptides with altered specificities that could be used in immunoaffinity purification. This is a collaboration with Prof Rick Randall and Mr Bernie Precious at St Andrews who first isolated the monoclonals and who have cloned most of the paramyxovirus proteins.

We had crystallised the Fab alone, and determined its structure using in-house data. Co-crystallisation with peptide produced a different crystal form, from which we collected 3.0Å data in-house that showed the peptide was present.

Problems with ID14-4 meant that data collection did not start on that line until late evening. These data were collected on ID14-2 where we were give a few hours in the morning of 26th Jan.

Data to 1.9Å were collected on ID14-2 from a frozen crystal rotated 180º. 271,919 observations yielded 35,529 unique reflections from a C2 cell a=176.9, b=40.3, c=64.4, β=98.7º. Rmerge=0.070, and 0.153 in the top shell. The refined structure, R=0.21 Rfree=0.25, shows beautiful density for most of the bound peptide, GKPIPNPLLGLDS.