ESRF	Experiment title: Very Short Patch Repair Enzymes – EcVSR.	Experiment number: LS-1682 a
Beamline: ID14-2	Date of experiment: from: 26/02/00 to: 27/02/00	Date of report: 21/08/00
Shifts: 1	Local contact(s): Wilhelm Burmeister	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Karen Bunting – Institute of Cancer Research.
Mark Roe – Institute of Cancer Research.

Report:

Previously we had collected a 3.0Å data set on ID14-3. The structure had been solved by molecular replacement. However the refinement was not straightforward, several parts of the model were apparently seriously wrong and others were not visible at all. The crystals, although of a large size, had a very high solvent content (~70%), did not freeze well and decayed in the beam. Therefore, we decided to try and improve the data. A second set was collected on ID14-2. Initially the data diffracted to 2.4Å, but rapidly decayed to 2.7Å. The data was processed to 2.75Å with reasonable statistics. The side chains and loops were all removed and the protein rebuilt from the core outwards. The DNA came back especially clearly and shows the presence of a Hoogstein base pair near the lesion. This was not seen in the original Morikawa structure, possibly due to the non-natural substrate DNA used. This refinement proceeded more smoothly, but is now stuck at an R_{free} of around 37%. The waters are now being included. We intend to publish this year.

ESRF	Experiment title: EthenoCytosine DNA – Uracil Glycosylase Complex	Experiment number: LS-1682
Beamline: ID14-2	Date of experiment: from: 26/02/00 to: 27/02/00	Date of report: 21/08/00
Shifts: 0.3	Local contact(s): Wilhelm Burmeister	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Mark Roe – Institute of Cancer Research.

Report:

Data was collected on a small crystal of uracil DNA glycosylase (MUG) containing DNA with an ethenocytosine lesion. The protein had been mutated to prevent processing of the lesion (as verified during in vivo experiments). However, the data showed that the ethenocytosine had indeed been cleaved in the crystal. We suspect this to be due to the effective high concentration of protein in the crystal. A second version of the protein with a double mutation has been crystallised and the structure (hopefully) will be solved soon.