	Experiment title:	Experiment number:
	Crystallographic studies on the interactions between collagen type II peptides and molecules from the immune system	MX-691
Beamline: ID 23-2	**Date of experiment**: from: 10/12/2007 16:00 to: 11/12/2007 8:00	**Date of report**: 28-4-2008
Shifts: 2	**Local contact(s)**: Christoph MUELLER-DIECKMANN	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Huseyin Usyal*, Raymond Yengo*, Marjolein Thunnissen* Dep. Molecular Biophysics, kemicentrum, Lund University. Getingevägen 60 S22100 Lund Sweden

Report:

Our goal was to collect data on a MHC class II Aq molecule with a bound peptide derived from collagen type II. The structure of this complex will give us information on the interactions the peptide makes with the MHC and give clues on the recognition of the glycosylated peptide an it will give insight in how post-translational modifications are recognized in autoimmune diseases. The main problem in this project has been the production of stable MHC protein without an additional leucine zipper domain but we are able now to reliable produce the MHC molecule with good yields for crystallization purposes. We have obtained needle type crystals of the MHC-peptide complex but unfortunately during the experiment on ID23-2 it became clear that these crystals diffract very anisotropically between 6 and 3Å. We tested and collected data on about 15 different crystals but they all showed the pronounced anisotropic diffraction and the datasets collected gave only reliable statistics to about 5 to 6Å. The crystals are also very sensitive to radiaton damage and had to be translated during data collection. We were able to deduce that the spacegroup of the crystals is tetragonal (P4 or higher) with unit cell dimensions a=b=115.5 Å, and c=179 Å. Assuming the spacegroup is P4, the Matthews's coefficient calculation suggests then four molecules per asymmetric unit with 59% solvent content. It is clear that for obtaining relable data these crystals need to be optimized further and this is under progress.

In the remaining time several other crystals from different proteins were tested. A number of these crystals had severe problems like limited resolution or internal disorder We were able to collect two more datasets. The first dataset was one of a complex of the cancer-repressor WT1 in complex with DNA. The crystals were spacegoup trigonal with celldimensions a=b=46.64Å and c = 72.40Å. The data extended to 2.4 Å with an Rmerge=5.1%. Unfortunately these cell dimensions indicate that there is no space for the full complex and these crystals contain only DNA and not the protein WT1.

The second dataset is of a complex between the β-glucosidase BglB from *Thermotoga maritima* and an inhibitor. The structure of the 710-residue enzyme had previously been solved using data collected at ID29 (D.T. Logan et al., manuscript in preparation). The present crystals, of an inactive mutant D242A, had been grown in the presence of a product of the synthesis reaction that this enzyme can carry out, namely β-D-hexylglucoside. Crystals belong to space group $C222_1$ with unit cell dimensions a = 74.9, b = 128.8, c = 177.5 Å. The crystals were radiation sensitive but nevertheless a data set to 2.3 Å could be collected in 9 segments of 30 0.5° oscillations each, translating the elongated crystals to expose a fresh volume in each segment. The data were processed using XDS and scaled using XSCALE. The data are 92.3% complete to 2.3Å with R_{merge} = 12.3 % (67.8 % for the highest resolution shell) and a multiplicity of 3.8. The average I / σ(I) for the dataset is 8.9 (1.5 for the highest resolution shell). The structure was solved by refining the apo structure against this data set. Electron density could be seen for β-D-hexylglucoside in the active site, binding in an unexpected orientation. These data will be incorporated in the manuscript describing the BglB structure (D.T. Logan et al., in preparation).