	Experiment title:	Experiment number:
	Tricorn Protease. A new Class of Proteolytic Enzymes	LS-1326
Beamline: ID14-3	**Date of experiment:** from: 22.4.99 to: 24.4.99	**Date of report:** 30.8.99
Shifts: 6	**Local contact(s):** Wim Burmeister and Dumon Laurance	*Received at ESRF:* - 1 SEP. 1999

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Report:

We collected data sets on three potential derivatives as well as a native data set that is complete to 2.8Å resolution.

So far we did not get enough phase information to solve the structure by MIR methods and it is planned to do a Se-MAD experiment (see below).

Native crystals diffract to a resolution of better than 1.8Å resolution. This is surprising given the large unit cell (310Å, 170Å, 220Å) and the high solvent content of 62% of the crystals. This resolution limit is a significant improvement over the data collected at other synchrotron light sources. We could however collect only data to 2.8Å for two reasons. Firstly the crystals are only diffracting to their maximum resolution until about six hours of exposure. This time is just enough to collect a complete data set to 2.8Å resolution from a single crystal. Secondly the large unit cell results in a very close spacing of reflections and using the small MAR CCD detector at ID14-3 will only allow reflections up to about 2.6Å to be resolved. In order to get the best possible native data set (better than 2Å resolution) we therefore need to collect data on multiple crystals using a larger CCD detector. Our Experience at ID14-3 suggests that we need to collect data from 15-20 crystals with 0.1 degree oscillations in order to get a data set that is complete to 2Å resolution. The high

resolution data will be very important for solving the structure. Based on sequence analysis we expect the protein to consist almost exclusively of beta strands. The chain tracing of beta strand structures will be significantly simplified by high resolution and high quality data. It will also allow us to use automated chain tracing protocols that will speed up the model building process (more than 7000 amino acids in the asymmetric unit).
Phasing of the data was as yet not possible. We collected a series of data sets on crystals that were soaked in heavy metal solutions and attempted to solve the structure by MIR. We could identify a tungsten complex compound that is usable in the resolution range from 100-12Å. We plan now to do a MAD experiment on Se-Met crystals and use the tungsten low resolution phase information to identify the up to 288 Se sites in the asymmetric unit.
We expect to get enough phase information to phase the structure to at least 3.5Å and then extend phases by averaging and solvent flattening. The positions of the Se sites will help dramatically in tracing the 1071 amino acids of the monomer.