Report for experiment LS-2006

In this experimental run three proposals from the Dept. of Biochemical Sciences of the University of Roma La Sapienza have been joined together. A description of the experiements carried out for the three proposals is therefore provided.

1. LS-2006 Title:Structure determination of PGIP (Poligalacturonase Inhibiting Protein): a leucin rich repeat protein of plant origin involved in resistance.

In our last experiment we tried a MAD experiment on a potassium tetrachloroplatinate (II) soak which we had previously measured as a potential isomorphous derivative. We took a florescence spectrum of the soaked crystal and noticed an a encouraging peak at 1.0716 Å wavelenght. We collected many frames at the peak wavelenght and calculated a Patterson map. Unfortunatly the peaks observed in the Patterson map were not sufficient intense to permit us to carry out a MAD experiment.
Because of the presence of 12 sulfur atoms in the PGIP2 sequence, we decide to tried a S-SAD experiment, we collected 360 frames, but the crystal suffered considerable radiation damage and we have had to stop data collection. Peaks of the Patterson map were not very strong, probaly due to the multeplicity of the collected reflections. We tested two other possible heavy atom derivatives: an uranyl acetate and a lutetium chloride. We collected two complete data sets and after processing the data we could not observe interpretable peaks in the Patterson map.

2. LS-2023 (Ilari) Title: x-ray structure of flavohaemoglobin

In thist experiment we tested many crystals of flavoaemoglobin, unfurtunatly none of them diffracted so well to carry out a complete data collection; this is probably due to a crystal damage occourred during the tansport to the ESRF Synchrotron.

3. LS-2062 (Vallone) Title: Structure of a thermophilic archaeal recombinanat amidase from Sulfolobus solfataricus.
We had previously obtained crystals of S. solfataricus amidase (Nastopoulos et al. 2001) in the rombohedral R3 space group of which we have collected a native and derivative (pHMB) data set at ESRF. Analysis of the data resulted in the conclusion that all the crystals we had exposed were affected by merohedral twinning, showing a twinning fraction close to 50%. We have subsequently attempted to define new crystallization protocols also using protein batches obtained from the E. coli soluble fraction, and we have grown another crystal form in the orthorhombic system, which shows no signs of twinning and diffracted at 2.7 Å during a test performed at Elettra.
We have therefore not used the R3 crystal form during this run, which was initially planned as MAD on the mercury derivative, but we have exposed one native crystal in the new orthorombic space group. The crystal, in spite of its small size (50 μm in its largest dimension), diffracted to 2.4 Å and yielded a native data set complete to 2.5 Å resolution. Also the S. solofolobus amidase could be solved taking advantage of the intrisic sulfur anomalous signal since 5% of its aminoacic content (i.e. 24 residues over a total of 237) is made up by methionines and cysteines.
We are currently attempting to improve the size and diffraction quality of the orthoromic crystal form.
References:

Nastopoulos V, Vallone B, Politi L, Scotto D'Abusco A, Scandurra R, Tsernoglou D. (2001) Crystallization and X-ray diffraction measurements of a thermophilic archaeal recombinant amidase from Sulfolobus solfataricus MT4. Acta Crystallogr D Biol 57:1036-7.