ESRF	**Experiment title:** MAD with Selenium on the crystals of the catalytic domaine of bovine α1,3galactosyltransferase	**Experiment number:**
Beamline: ID14-EH4	**Date of experiment:** from: 16.09.1999 to: 17.09.1999	**Date of report:**
Shifts:	**Local contact(s):** R. Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Louis Gastinel

AFMB

CNRS UPR 9039

Report:

The catalytic domain of bovine α1,3galactosyltransferase (280 amino acids) produced *in E. coli*, has been crystallized using the native protein and the seleno-methionine derivatized protein in the P4(1)2(1)2 space group with one molecule per asymetric unit. Three different data sets of a single seleno-methionine crystal were recorded to solve the structure by using MAD technique using the three wavelenghts, e1= 0.9795 (peak), e2= 0.98000 (inflection) and e3=0.9324 (remote). The seleno-methionine crystals diffracted up to 2.8 Å. A clear solution was found with SOLVE , with 8 sites (of the 10 theoretical seleno-methionine sites) and with <m>=0.50 and a score =31.86. An electron density map clearly identified zones of protein density. Solvant flattening using DM with 60% solvant increased considerably the quality of the electron density map. The protein structure was solved to 2.8Å using only the experimental MAD phases and refined to Rfactor=23%, Rfree=28% with relatively good geometry.

Data sets of native α1,3galactosyltransferase was recorded on the same beam line with good statistics up to 2.0 Å resolution. We are currently using the structural model obtained to 2.8 Å to solve the native structure at 2.0 Å resolution. Data sets of crystals soaked with substrates and substrate analogs were recorded to 2.5 Å resolution in order to reveal the amino acids involved in the catalytic pocket interacting with the substrate.