



EMBL

Antenne de Grenoble

Laboratoire Européen de Biologie Moléculaire
European Molecular Biology Laboratory
Europäisches Laboratorium für Molekularbiologie

PROJECT SUMMARY REPORT

Access to Research Infrastructure Action

"Structuring the European Research Area Specific Programme"

The contract number is: RII3-CT-2004-506008.

Project title	Solution of the structure of a plakin domain fragment by MAD
Name of the Group Leader	Jose M DE PEREDA
Home Laboratory	Centro de Investigacion del Cancer. Univ. of Salamanca
E-mail address	Pereda@usal.es
Telephone	34 923 294819
Experiment number	14-U-856

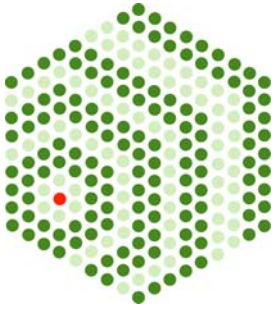
1. Project objectives (no more than 10 lines)

The goal of this experiment was the solution of the structure of a fragment of the N-terminal region of the plakin domain of plectin, by using multiple wavelength anomalous diffraction (MAD) methods and crystals of seleno-methionine substituted protein.

2. Main achievements and difficulties encountered (no more than 20 lines)

During this experiment we have collected a **two wavelength MAD dataset**, at peak (0.9785 Å) and remote (0.9185 Å) Se energies, from a single crystal of **Se-methionine substituted protein**. Data from the peak and remote wavelengths extend to 2.7 Å and 2.5 Å respectively. Using these MAD data the positions of seven selenium atoms corresponding to the six Met residues (one in two conformations) of the construct were found, and phases were calculated and extended to 2.5 Å resolution with ShelxC/D/E. After phase improvement with DM the mean figure of merit was 0.72 and a readily interpretable map was obtained. Using the experimental map and the program ARP/WARP a model consisting of five helical segments and a total of 149 residues was automatically built. The position of the methionine residues, derived from the localization of the Se atoms, was used for the unequivocal assignment of the protein sequence.

In addition a native dataset to 2.05 Å from non-derivatized protein was collected. The native and Se-Met crystals were isomorphous. This native data have been used to refine the structure to a Rwork of 21.0% and an Rfree of 26.1%. The refined model has 216 residues and 57 water



EMBL

Antenne de Grenoble

Laboratoire Européen de Biologie Moléculaire
European Molecular Biology Laboratory
Europäisches Laboratorium für Molekularbiologie

molecules. All main chain torsion angles of the nonglycine residues lie in the most favored and additional allowed regions of the Ramachandran plot.

In summary all the objectives of this experiment were successfully achieved and no significant problems were encountered.

Publication derived from this experiment:

Sonnenberg A, Rojas AM, de Pereda JM. (2007) "The structure of a tandem pair of spectrin repeats of plectin reveals a modular organization of the plakin domain." *J. Mol. Biol.*, 368(5):1379-1391