ESRF	**Experiment title:** X-RAY DATA COLLECTION ON SPECTRIN REPEAT NO. 16	**Experiment number:** LS-490
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Names and affiliations of applicants (*indicates experimentalists):

K. DJINOVIĆ CARUGO*
J. PASCUAL
M. WILMANNS*

EMBL-HEIDELBERG
MEYERHOF STR. 1
69012 HEIDELBERG
GERMANY

Report:

STRUCTURE OF THE SPECTRIN REPEAT

The cell's shape is determined by an integrated system of molecules called cytoskeleton. Spectrin is the major component of the membrane-associated cortical cytoskeleton and interacts with actin (Hartwig, 1994).

Its primary structure is dominated by homologous 106-residue repeats. Secondary structure prediction based on sequence information has suggested that the repeat is made of three α-helices (A, B and C) separated by two loop regions (AB and BC). Tertiary structure models hate been proposed for the folding of the repeat into a left-handed antiparallel triple-helical coiled-coil. In marked contrast, the structure of a homodimer of the 13th repeat of *Drosophila* α-spectrin that was solved by X-ray crystallography showed an intermolecular triple helical bundle where helices A and B from a molecule packed against helix C' of the other molecule in the dimer and the proposed BC loop appeared in an a-helical conformation as part of a continuous helix B-C (Yan *et al.*, 1993).

The importance of the spectrin repeat to the elastic properties of the membrane skeleton and to the structural and functional integrity of the normal red cell is demonstrated by the fact that it is a target to mutations which cause hemolytic anemias, such as some cases of hereditary elliptocytosis, pyropoikilocytosis and spherocytosis characterized by abnormally shaped erythrocytes.

We have undertaken the determination of the crystal structure of the 16th repeat from chicken brain α-spectrin that folds into a stable and monomeric helical structure as shown by spectroscopic methods. Crystals were grown at room temperature in hanging drops containing 0.05 M sodium acetate pH=4.25, 1.9 M NaCl and 20% glycerol, with a size of 0.2x0.05x0.02 mm. Data collected with native crystals have been obtained at a resolution of 2.0 Å, 85% completeness and R-merge of 7.7 %. Protein crystallized in the space group p212121 with a unit cell of a=47.36, b=25.54, c=80.86 (α=β=γ= 90). Heavy metal derivative search in order to refine the model is currently under progress.

Hartwig, J.H. (1994). Actin binding proteins 1: Spectrin superfamily. *Protein Profile* 1, 71 l-762.

Yan, Y., Winograd, E., Viel, A., Cronin, T., Harrison, S.C. and Branton, D. (1993). Crystal structure of the repetitive segments of spectrin. *Science 262, 2027-30.*