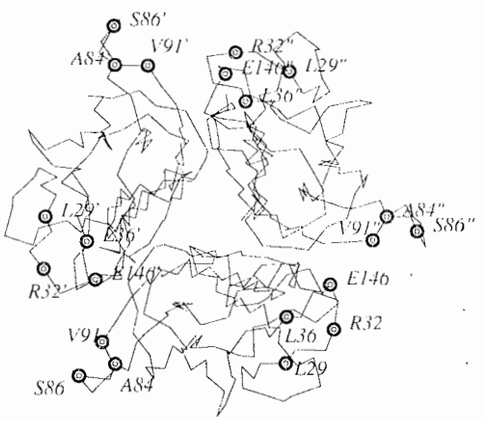
03-Crystallography of Biological Macromolecules

PS–03.09.02 X-RAY STRUCTURE OF THE [ALA-84→VAL] MUTANT OF HUMAN TUMOR NECROSIS FACTOR. By P. Saludjian[1], T. Prangé[1§], X. Van Ostade[2], J. Tavernier[2], W. Fiers[2] and J. Navaza[3], [1]Chimie Biomoléculaire (URA 1430 du CNRS) UFR-Biomédicale, 93012-BOBIGNY CEDEX, France and [§]LURE, Bât 209, Université Paris-Sud 91405-ORSAY. [2]Laboratory for Molecular Biology, State University of Gent, Ledeganckstraat 35, 9000 GHENT, Belgium. [3]Laboratoire de Physique, Faculté de Pharmacie, 92290- CHATENAY MALABRY, France.

In order to understand the reactivity of hTNF, the inactive mutant [Ala 84 Val] was prepared by mutagenesis and crystallized. This very conservative single mutation almost completely abolish the cytotoxic activity on L929 cells and further information was expected from its three dimensional structure analysis. The crystals belong to the trigonal class, space group $P3_12_12$ with two trimers (6 molecules) in the asymmetric unit. The parameters are roughly equivalent to that of the native protein and the structures are isomorphous. This case served as a test for a new molecular replacement method (AMoRe) in which the spheric harmonic coeficients are not just evaluated but acurately determined by solving their integral forms. The structure, proved to be resistant to classical methods, was easily solved by this way, using the tetragonal form coordinates. The diffraction data, collected at the DCI synchrotron in Orsay, were used in the structure refinement (XPLOR and PROLSQ techniques). The active site of hTNF, which is located at the monomer interfaces, show significant differences and the three cavities thus delimited by the trimerisation are not equivalent. The differences observed between the native and the mutant structures will be presented

View of the Cα tracing, down the local three-fold axis. The one residue-mutations which supress the hTNF activity are labeled by full circles. They all are located at the bottom of the trimer and on both sides of the crevasses formed upon trimerisation.



PS–03.09.03 X-RAY CRYSTALLOGRAPHIC STUDIES OF LEUKEMIA INHIBITORY FACTOR

A. J. McCoy*, V. Staton, A. Van Donkelaar, J. N. Varghese and P. M. Colman.

Biomolecular Research Institute, 343 Royal Parade, Parkville, 3052, Australia.

The aim of the work is to determine the tertiary structure of murine Leukemia Inhibitory Factor (LIF) expressed in *E. coli* using the techniques of X-ray crystallography.

LIF is a member of a group of polyfunctional regulatory molecules which includes interleukin-6 and interleukin-11. It is a molecule capable of such disparate effects as *inducing* the differentiation of the clonogenic cells of a mouse myeloid leukemic cell line (Tomida, Yamamoto-Yamaguchi & Hozumi, 1984) and *preventing* the the differentialtion commitment in normal embryonic stem cells (Williams, *et. al.* 1988).

The conditions under which recombinant murine LIF crystallises were determined by V. Staton in early 1991. The crystals are monoclinic and diffract to 2Å. Diffraction data have been collected using the synchrotron at the Photon Factory in Tsukuba (Japan), a Xentronics multi-wire area detector and a MAR detector. Twelve heavy atom derivatives have been prepared and phasing by multiple isomorphous replacement is proceeding.

Tomida, M., Yamamoto-Yamaguchi, Y. and Hozumi, M. (1984) *J. Biol. Chem.* **259**, 10978-10982.
Williams, R.L., Hilton, D.J., Pease, S., Wilson, T.A., Stewart, C.L., Gearing, D.P., Wagner, E.F., Metcalf, D., Nicola, N. and Gough, N. M. (1988) *Nature* **336**, 684-687

PS–03.09.04 LARGE MOTIONS IN SIGNAL TRANSDUCTIONS ASSOCIATED WITH CALMODULIN AND INITIAL RECEPTORS FOR ACTIVE TRANSPORT AND CHEMOTAXIS. By Florante A. Quiocho*, William E. Meador and Andrew Sharff, Howard Hughes Medical Institutes and Baylor College of Medicine, Houston, TX 77030.

Calmodulin (CaM) is the principal calcium-dependent transducer and regulator of a variety of eukaryotic cellular processes such as carbohydrate metabolism, cell division, cytoskeletal function, gene expression, ion channel regulation, and neurotransmitter synthesis and release. In many of these processes, CaM activates a plethora of intracellular enzymes, and the CaM-binding domains in several of them have been shown to reside in a region consisting of approximately 18-residue peptide segment. There is little identity in the amino acid sequences of these domains. Synthetic peptides based on these sequences have been shown to bind calmodulin in a calcium-dependent manner with the same 1:1 stoichiometry and tight affinity as the native proteins. To understand the atomic details of the mechanism of action of CaM, our laboratory has determined three X-ray structures of calmodulin — two of the calcium-activated, unbound forms and one in a form bound to a synthetic target peptide. In one of the largest conformational change ever observed for a protein, the binding of calmodulin to the target peptide causes a 5-residue turn of the central α helix of the native structure to unwind and expand with the consequence that the two domains come close together in a *cis* orientation and wrap around the α-helical target peptide. To emphasize this previously unrecognized and key feature of the linker function in molecular recognition and activation, we introduced the term "expansion joint". Thus calmodulin activates enzyme activity by gripping and pulling the peptide segment out of sterically hindering position.

Periplasmic binding proteins serve not only as initial ligand receptors for active transport and chemotaxis but also play a key role in initiating signal transduction in both processes. Ligand-induced conformational is essential in signal transduction. We have shown by x-ray crystallography that binding of maltodextrins to the receptor causes a combined large hinge-bending and small twist motions between the two domains, bringing the domains close together and enclosing the ligand bound in the cleft between the two domains. It is this "closed" liganded form that is presumably recognized by the membrane-bound components, triggering the signaling mechanism that triggers transport or chemotaxis.

