interaction of mTNF with TNF-R75 contributes in a specific way to the systemic toxicity. In order to investigate in more detail these receptor-specific interactions, we have established the 3D-structure of mTNF. The latter was expressed in soluble form in E. coli, and purified by established procedures. Crystals suitable for X-ray studies were produced by the hanging drop vapor diffusion technique at 5 °C using methoxypolyethylene glycol 2000 and isopropanol as precipitants. The crystals belong to the space group P1 with unit cell dimensions a = 49.45 Å, b = 49.92 Å, c = 52.32 Å, $\alpha$= 114.63°, $\beta$ = 104.15°, $\gamma$= 90.44° and one trimer in the asymmetric unit. Crystals were stable to X-rays and diffracted beyond 2.8 Å. Cooling the crystals during data collection was necessary, since the crystals dissolved at room temperature.

The 3D-structure of mTNF was solved by molecular replacement using hTNF as a model. The structure provides insights into the difference in murine receptor specificity between hTNF and mTNF. Also, a firm basis is now avaliable to explain the structure/function relationship of receptor-specific mTNF muteins.

**PS04.10.11 CRYSTAL STRUCTURE OF CheR, BACTERIAL CHEMOTAXIS RECEPTOR METHYLTRANSFERASE.** Snezana Djordjevic and Ann Stock. Center for Advanced Biotechnology and Medicine, Department of Biochemistry, UMDNJ, Piscataway, New Jersey 08854, and Howard Hughes Medical Institute.

CheR is a methyltransferase which catalyzes the *S*-adenosyl-L-methionine-dependent transfer of methyl groups to specific glutamate side chains on a set of membrane chemoreceptor proteins that mediate bacterial chemotaxis. Methylation/demethylation of the receptors is involved in cellular adaptation to the concentrations of the chemoeffectors. An increase in attractant concentration in the medium results in an increase in the level of receptor methylation whereas a negative signal, such as removal of an attractant or an increase in repellent concentration is followed by a net decrease in receptor methylation.

The methyltransferase from *Salmonella typhimurium*, has been over-expressed in *E. coli* and purified to homogeneity. The enzyme was crystallized in the presence of the product of the methylation reaction, *S*-adenosyl-L-homocysteine. Multiple rounds of microseeding were required to obtain crystals suitable for X-ray diffraction studies. The crystal lattice belongs to a monoclinic space group $P2_1$ with unit cell dimensions of a = 55.0 Å, b = 48.0 Å, c = 63.2 Å and $\beta$ = 112.3°. There is one monomer of 283 residues per asymmetric unit and Matthew's value is 2.4 $Å^3$/Da. An X-ray diffraction native data set to 2 Å, with an $R_{sym}$ of 4.9 %, has been collected on an R-axis II image plate detector. Initial phases were obtained to 3 Å using several mercurial and one platinum derivative crystals which gave an overall figure of merit of 0.71. An MIR electron density map was subjected to density modification procedure. This map contained interpretable elements of secondary structure and enabled us to build a partial model. Subsequent electron density maps were calculated by combining MIR and partial model phases. CheR is a mixed $\alpha/\beta$ protein with two distinct domains. We have identified electron density corresponding to *S*-adenosyl-L-homocysteine associated with the larger domain.

**PS04.10.12 CRYSTAL STRUCTURE OF THE SH2 DOMAIN OF THE PROTEIN SHC: pH-DEPENDENT SELF-ASSOCIATION.** Klaus Fütterer, Stéphane Réty, Richard A. Grucza, Gabriel Waksman, Washington University School of Medicine, Department of Biochemistry and Molecular Biophysics, Saint Louis, MO 63110, USA.

The Src Homologous and Collagen-like (SHC) protein plays an essential role in signal transduction pathways leading to the activation of the protein Ras. SHC contains a Src Homology 2 (SH2) domain, the function of which is to specifically recognize tyrosine phosphorylated peptide ligands. The structure of an SH2 domain can be described as a central $\beta$-sheet flanked by two $\alpha$-helices (G. Waksman et al., J. Kuriyan, 1992, Nature 358: 646-653). The peptide binding surface of SH2 domains in general consists of two binding pockets on either side of the central $\beta$-sheet. While the binding pocket for the phosphotyrosine is conserved, its counterpart accommodating the third amino acid C-terminal to the phosphotyrosine varies in shape and depth between different SH2 domains, reflecting specificity for different peptide ligands.

This contribution presents the crystal structure of the SH2 domain of SHC determined by multiple isomorphous replacement at a resolution of 2.5 Å (S. Réty et al., G. Waksman, 1996, Protein Science, in press). The SH2 domain of SHC reveals a fold similar to other SH2 domains. The peptide binding surface of SHC-SH2 resembles that of the SH2 domain of Src (G. Waksman et al., J.Kuriyan, 1993, Cell 72: 779-790) in that it presents a clearly delineated pocket for the third residue C-terminal to the phosphotyrosine, but it is deeper and more distinct in SHC than in Src.

A novel feature of the SH2 domain of SHC is the observation of an intermolecular disulfide bond accompanied by an extensive dimer interface between two symmetry-related molecules. The potential dimerization of SHC-SH2 in solution was studied under reducing conditions using analytical ultracentrifugation and polyacrylamide gel electrophoresis. The results suggest that the SH2 domain of SHC dimerizes in a pH-dependent manner, where low pH conditions (~pH 4.5) are conducive to dimer formation. Dimerization of SHC may have important biological implications, possibly promoting the assembly of large heteromultimeric signaling complexes.

**PS04.10.13 STRUCTURE AND IMPORTANCE OF THE DIMERIZATION DOMAIN IN ELONGATION FACTOR TS FROM *THERMOS THERMOPHILUS*.** Youxing Jiang*, Steffen Nock†, Martina Nesper†, Mathias Sprinzl†, Paul B. Sigler#‡, *The Department of Chemistry, #The Department of Molecular Biophysics and Biochemistry and the Howard Hughes Medical Institute, Yale Univ., CT 06511, USA, †Laboratorium fur Biochemie, Universitat Bayreuth, ‡To whom correspondence should be addressed

Elongation factor Ts (EF-Ts) functions as a nucleotide exchange factor which catalyze the GDP/GTP exchange in elongation factor Tu. *Thermus thermophilus* elongation factor Ts forms a homodimer in solution. The crystal structure of the EFTs dimerization domain has been solved and refined to a resolution of 1.7 Å. This high resolution structure gives a clear picture of the dimerization interactions of EFTs. The three-stranded anti-parallel $\beta$-sheet of each subunit comes together to form a $\beta$-sandwich held together by a disulfide bond between the Cysl90 of each subunit and by the hydrophobic core constructed by Ile60, His62, Ile64, Gly71, Leu73 and Phel92. Based on the structure, two mutants, C190A and L73D, were designed to disrupt the dimer interface. The activity analyses show that monomeric EF-Ts loses the ability to catalyze exchange of GDP/GTP in EF-Tu which indicates that *Thermus thermophilus* EF-Ts functions as a homodimer.