Superantigens are a family of bacterial and viral toxins that are able to induce a massive T-cell activation [1,2]. Compared to conventional antigens where only 0.0001-0.01% of T-cells are activated, superantigens are able to activate 10-30% of T-cells. Superantigen-induced T-cell activation results in an extensive production of inflammatory cytokines, such as tumor necrosis factor and interleukin-2. Consequently, superantigens are responsible for acute diseases, including toxic shock syndrome and food poisoning. In addition, there are reports that superantigens may also be implicated in autoimmune diseases.

Superantigens act by cross-linking MHC class II molecules with T-cell receptors. Binding to the MHC class II molecules can occur through either the α- or the β-chain of the MHC class II molecule. Alternatively, both chains may be simultaneously utilised. Binding to the β-chain requires the presence of a zinc ion at the C-terminal domain of the superantigen.

Streptococcus dysgalactiae-derived mitogen (SDM) was found to activate T-cells bearing Vβ1 and Vβ23 variable regions [3]. Sequence alignment showed approximately 30% homology with other superantigens, in particular SPEC. Moreover, phylogenetic analysis suggested that SDM belongs to a separate family, distinct from other known superantigens. To gain insights into the structure of SDM and to facilitate further functional studies, crystal structure determination was initiated.

Crystals of SDM were grown at 16°C using PEG 4K and lithium nitrate in the mother liquor [4]. For successful crystallisation, the N-terminus 6XHis-tag was removed. Data to 1.9 Å were collected at station X11 and processed with the HKL package to give a 96.3% complete data set with an Rsym of 4.8%. The structure was solved by molecular replacement using a poly-alanine model of SPEC. Refinement was carried out with CNS to 1.95 Å due to high Rsym between 1.95-1.90 Å. The current structure (Figure 1) has an Rcryst of 22.4% (Rfree=26.8% for 5% of the reflections excluded from the refinement). A strong and persistent density at the C-terminal domain of the molecule was assigned to the presence of a zinc ion. Anomalous data collected close to the zinc absorption edge and inductively coupled plasma mass spectrometry (ICP-MS) measurements confirmed the presence of zinc in the structure. Structural comparisons with other superantigens showed alterations in the T-cell receptor binding site. Mutagenesis experiments for key residues assumed to be involved in TcR binding according to structure-based superposition revealed no changes in the mitogenic activity. Thus, SDM may exhibit differences in its interactions and binding mode with TcR. Further mutagenesis studies based on the structure are underway to precisely locate the TcR binding site. The presence of the metal binding site strongly suggests that SDM is able to bind to the β-chain of the MHC class II molecules. Mutation of an His residue from the zinc-binding site to Ala resulted in a toxin with less mitogenic activity compared to the native one.
Figure 1: Crystal structure of SDM. Bound zinc is shown as brown sphere. Figure was drawn with Pymol.

References