ESRF	**Experiment title:** **Crystal Structure of MHC class I molecule HLA-B*2705 in complex with a nonameric peptide**	**Experiment number**: TC - 88
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Report:

Human leukocyte antigen (HLA) molecules are located on the surface of most nucleated cells. As major histocompatibility complex (MHC) class I molecules, they play a key role in the recognition of pathogens. Their biological function is to present peptides derived from intracellular digested proteins to T-cell receptors on CD8+ T cells. Cells invaded by a pathogen present pathogen-derived peptides which targets these cells for destruction. More than 20 years ago, the association of HLA-B27 to spondyloarthropathies, a group of chronic inflammatory disorders that include ankylosing spondylitis (AS) and reactive arthritis, was recognised. However, the molecular basis for this association is still not known. The evidence so far implicates HLA-B27 heavy chains, possibly in a misfolded form, and/or hypothetical arthritogenic peptide(s) binding to this molecule, as causative agents [1]. Recent studies revealed that only certain subtypes show disease association, while other subtypes are not or only rarely present in patients [2]. Particularly interesting are the subtypes B*2705, which shows strong disease association, and B*2709 with no or only very weak disease association, as both differ in only one amino acid. In order to understand the role of HLA-B27 in arthritic diseases, we initiated the crystal structure determination of HLA-B*2705 and B*2709.

HLA-B*2705 and B*2709 molecules consist of a heavy chain, a light chain (β_2-microglobulin) and a peptide. They were produced by separate expression of both protein chains as inclusion bodies in *E. coli* followed by refolding of the complex in presence of synthetically produced peptide. We identified a nonameric and a decameric peptide that support refolding with both subtypes, and succeeded in crystallising HLA-B*2705 with the nonameric peptide and B*2709 with the decameric peptide. Due to their small size, both crystal forms diffract to only 4 to 3 Å on a rotating anode with poor quality. Data sufficient for the crystal structure determination of HLA-B*2705 could be collected at the microfocus beamline ID13. The micro-focused beam turned out to especially valuable as we were able to collect the complete data set from the well-grown tip of a needle-shaped crystal. The rest of the needle, which showed severe twinning and presence of satellite needles, would have caused deterioration of the diffraction quality if radiated with a less focused X-ray beam.

A complete data set to 2.1 Å resolution could be collected from one crystal at 100 K. The structure was solved by molecular replacement. In the meantime we collected data of the not disease associated subtype B*2709. The structure determination is in progress. A comparison of the structures of the disease associated subtype B*2705 and the not disease associated subtype B*2709 will reveal whether structural differences are responsible for the different patterns of disease association.

[1] P. Bowness, N. Zaccai, L. Bird, E.Y. Jones (1999). HLA-B27 and disease pathogenesis: new structural and functional insights. *Expert reviews in molecular medcine*, http://www-ermm.cbcu.cam.ac.uk, short code: txt001pbo

[2] M. D'Amato et al. (1995). *Eur. J. Immunol.* **25**, 3199-3201.