s8a.m1.p37 **The Crystal Structure of a Strepmyces sp.Cel12A.** A.Shaw, L. Gross, C. Mitchinson, R Bott. *Genencor International Inc., 925 Page Mill Rd. Palo Alto CA 94304 USA.*
Keywords: hydrolase, catalysis, X-ray structure.

Presented is the 3-dimensional structure of the catalytic core of a Streptomyces sp. Cel12A glucohydrolase, determined using X-ray crystallography, refined to 1.75Å resolution. Cel12 contains a glycoside hydrolase family 12 (GH12) catalytic core, Cel12A, and a C-terminal cellulose binding domain (CBD). When heterologously expressed in Streptomyces lividans, the CBD is proteolytically cleaved from the catalytic core, which readily crystallizes. The protein has the expected fold for a GH12 enzyme. Essentially it contains two b-sheets, of 6 and 9 strands, packed on top of one another. There are two disulfide bonds.

The concave surface of the 9-strand b-sheet forms a large crevice into which the substrate binds, and in which the active-site residues are located. In the active-site, we find a carboxylic acid trio, similar to that of family 7 glycoside hydrolases, in an apparent case of convergent evolution. The binding crevice is lined with aromatic amino-acid side-chains, which may play a role in substrate binding via stacking interactions with glucose residues.

s8a.m1.p38 **Neutron-Laue & Atomic Resolution X-ray Analysis of the Aspartic Proteinase Endothiapepsin**. L. Coates, P. T. Erskine and J. B. Cooper, *School of Biological Sciences, University of Southampton, Southampton, SO16 7PX, UK*, and D. Myles, *EMBL Grenoble Outstation*.
Keywords: aspartic proteinase, catalytic mechanism.

The aspartic proteinases are a family of enzymes involved in a number of important physiological and pathological processes. Current proposals for the catalytic mechanism are largely based on X-ray structures of inhibitor complexes but these proposals differ in the assignment of protonation states to the catalytic groups during the reaction. The recent development of the neutron quasi-Laue technique at ILL (Grenoble), has allowed us to collect neutron data to 1.95 Å on a complex of endothiapepsin with a transition state analogue[1]. In parallel, we have collected atomic resolution cryogenic X-ray data on the same complex to 1.1 Å resolution and on the native enzyme to 0.9 Å resolution. These X-ray datasets have been used to refine the structure and have revealed much of interest about the enzyme but so far have not defined the positions of hydrogen atoms at the catalytic centre clearly. In contrast, refinement with the neutron dataset has shown distinct sites of deuteration at the active site allowing us to discriminate between different models for the transition state complex.

[1] Cooper, J. B. and Myles, D. A. A., "A preliminary neutron Laue diffraction study of the aspartic proteinase endothiapepsin.", Acta Crystallogr. (2000) D56: 246-248.