s1.m6.p8 **Crystallographic Studies on the Reaction Cycle of Isopenicillin N Synthase.** Wei Ge, Rob Adlington, Peter Rutledge, Jack Baldwin, *Oxford University, Chemistry Department, University College, OX1 4BH, UK. E-mail: wei.ge@univ.ox.ac.uk*

Keywords: IPNS; Crystallographic; X-Ray

There has been intense interest in all aspects of penicillin since Alexander Fleming discovered it in 1929. The biosynthetic route to the penicillin and cephalosporin antibiotics starts with three amino acids - L-valine, L-cysteine and L-a-aminoadipic acid. These three amino acids are assembled into the linear tripeptide L-a-aminoadipoyl-L-cysteinyl-D-valine (ACV), which is converted to bicyclic isopenicillin N (IPN), the biosynthetic precursor to all penicillins and cephalosporins, by a single enzyme, Isopenicillin N synthase (IPNS).

IPNS is a non-haem iron oxygenase. The IPNS catalysed reaction is an oxidative cyclisation. The reaction stoichiometry involves the loss of four hydrogen atoms from ACV, concomitant with the reduction of one equivalent of molecular oxygen to two molecules of water. IPNS requires one equivalent of ferrous iron for full activity. IPNS - like the ring expansion enzymes - has been the subject of extensive research interest over many years, because this oxidative bicyclisation reaction is unique in nature and is of key commercial importance.

My project focuses on the synthesis and enzymatic investigations of δ-(L-α-aminoadipoyl)-(3S-methyl)-L-cysteine D-a-hydroxyisovaleryl ester (AmSCOV, 14) and δ-(L-α-aminoadipoyl)-(3R-methyl)-L-cysteine D-S-methyl-cysteine ester (AmCOmC,35) that may allow the trapping and observation of an Fe(II) oxene-containing intermediate in the IPNS system.

s1.m6.p9 **How to pick the best low-hanging fruit in medically important target genomes.** Helliwell J.R., Cianci M., Raftery, J and Rosmarin, I., *Dept of Chemistry, University of Manchester, M13 9PL, UK. E-mail: john.helliwell@man.ac.uk*

Keywords: Structural genomics; Bioinformatics; Boolean algebra gene selection

The original promise of structural genomics, every gene a protein structure, remains unrealised. We have developed a Boolean method for targetting 'high return' genes, which is general, and applied it to the medically important M.tuberculosis target genome along with that of M. leprae so as to keep essential genes, but also deleting eukaryotic homologues [1]. From that analysis, out of thousands of genes, we have a set of 65 Clusters of Orthologous Groups comprising 95 proteins. From within that select group we have now also considered those genes that have a low predicted protein 'foldability' [2] and that are also probable transmembrane proteins, which have a lower crystallisation probability. Thus, overall, we have selected new 'rational-drug-design' gene candidates whilst recognising the problem cases for protein crystallographic study; these problem cases are evocatively referred to as being the 'high hanging fruit' ie the difficult pickings.

This is a contribution from the North West Structural Genomics Consortium (www.nwsgc.ac.uk) *University of Manchester Chemistry Node.*

[1] James Raftery and John R. Helliwell Spoilt for choice: protein target selection in a time of plenty *Acta Cryst* (2002). D**58**, 875-877

[2] Uversky VN, Gillespie JR, and Fink, AL Why are "natively unfolded" proteins unstructured under physiologic conditions? *Proteins* 2000; **41**:415-427.