ESRF	**Experiment title: Single crystal enzymology of Isopenicillin N synthase.**	**Experiment number:** LS507
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Report: Structure of Isopenicillin N synthase complexed with substrate and the mechanism of penicillin formation. Peter L. Roach, Ian J. Clifton, Charles M. H. Hensgens, Norio Shibata, Christopher J. Schofield, Janos Hajdu and Jack E. Baldwin, Nature, 387, 827830 (1997).

The biosynthesis of penicillin and cephalosporin antibiotics in microorganisms requires the formation of the bicyclic nucleus of penicillin. Isopenicillin N synthase (IPNS), a non-haem iron-dependent oxidase, catalyses the reaction of a tripeptide *L*-δ-(α-aminoadipoyl)-*L*-cysteinyl-D-valine (ACV) and dioxygen to form isopenicillin N and two water molecules. Mechanistic studies suggest the reaction is initiated by ligation of the substrate thiolate to the iron centre, and proceeds through an enzyme-bound monocyclic intermediate. Here we report the crystal structure of IPNS complexed to ferrous iron and ACV, determined to 1.3 Å resolution. Based on this structure we propose a mechanism for penicillin formation which involves initial ligation of ACV to the iron centre creating a vacant iron coordination site into which dioxygen may bind. Subsequently, iron-dioxygen and iron-oxo species remove the requisite hydrogens from ACV without the direct assistance of protein residues. The crystal structure with the dioxygen analogue, NO, and ACV bound to the active site iron supports this hypothesis.