ESRF	**Experiment title:** Mechanism of Isopenicillin N Synthase	**Experiment number:** LS-825
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Report:

The successful determination of the structure of IPNS with both Fe(II) and ACV bound at the active site [ref. 1] opened up the possibility of carrying out time-resolved experiments to observe the oxidation of it's tripeptide substrate, *L*-δ-(α-aminoadipoyl)-*L*-cyteinyl-*D*-valine (ACV) to form isopenicillin N in the crystals. Conditions for achieving turnover of the enzyme required optimisation, as the reaction in the crystalline state is slow and the product, isopenicillin N, is relatively unstable. We therefore co-crystallised IPNS with the substrate and with two analogues, *L*-δ-(α-aminoadipoyl)-*L*-cyteinyl-*D*-α-aminobutyrate (ACAb) and *L*-δ-(α-aminoadipoyl)-*L*-cyteinyl-*D*-S-methylcysteine (ACSMC). The rationale behind these experiments is that turnover of appropriately deuterated ACAb results in the more stable cepham product. ACSMS was designed to trap the proposed Fe(IV) intermediate in the active site of the enzmye.

Data was collected on crystals as follows:

Substrate	Oxidation Period	Resolution Limit	Rmerge
ACV	500 mins	1.30 Å	0.10
ACAb	0 mins	1.40 Å	*0.06*
ACSMC	320 mins	1.35 Å	*0.09*

Refinement of these structures is proceeding.

References

1. Structure of the Isopenicillin N Synthase Complexed with Substrate and the Mechanism of Penicillin Formation. P. L. Roach, I. J. Clifton, N. Shibata, C. M. Hensgens, J. Hajdu, C. J. Schofield and J. E. Baldwin, *Nature, 387, 8274330 (1997).*