



|   |   |                                      |
|---|---|--------------------------------------|
|   | <b>Experiment title:</b><br>The mechanism of isopenicillin N synthase | <b>Experiment number:</b><br>LS 1387 |
| <b>Beamline:</b><br>ID14 EH4  | <b>Date of experiment:</b> 17-6-1999<br>from: to:                     | <b>Date of report:</b><br>20-3-2000  |
| <b>Shifts:</b><br>1 (12 hr)   | <b>Local contact(s):</b><br>Sean McSweeney                            | <i>Received at ESRF:</i>             |
| <b>Names and affiliations of applicants (* indicates experimentalists):</b><br>Dr P. L. Roach*<br>Dr I. J. Clifton*<br>J. M. Elkins*<br>J. M. Ogle*<br>P. J. Rutledge<br>Professor Sir Jack Baldwin |   |                                      |

## Report:

With this time on EH4 we have carried out further time resolved studies on the enzyme isopenicillin N synthase. Data was collected from crystals of Fe-IPNS with the substrate analogues  $\delta$ -(L- $\alpha$ -aminoadipoyl)-L-cysteinyl-D-vinylglycine (ACvG) and  $\delta$ -(L- $\alpha$ -aminoadipoyl)-L-cysteine D- $\alpha$ -hydroxy isovaleryl ester (ACoV).

These crystals had been subjected to high-pressure oxygen gas prior to flash-freezing and data collection in the attempt to bring about turnover in the active site.

Specifically, seven datasets were collected from IPNS:Fe<sup>2+</sup>:ACvG crystals that had been pressurised for a wide range of time periods, and from three crystals of IPNS:Fe<sup>2+</sup>:ACoV exposed for 20 s, 30 s and 30 s respectively. Ten datasets were collected in all.