



	Experiment title: Single crystal enzymology of isopenicillin N synthase: The fourth dimension	Experiment number: LS-507
Beamline: ID02	Date of experiment: from: 11/12/96 to: 14/12/96	Date of report: 22/2/97
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Report

Two crystal forms of IPNS with the ferrous iron cofactor and the substrate, ACV, bound at the active site have been obtained. Exposure of either of these crystal forms to dioxygen results in catalysis which, due to crystal packing constraints, leaves the products trapped in the active site.

Previous EPR studies using nitric oxide as an analogue of dioxygen indicated binding of NO to the Fe(II):ACV:IPNS complex to yield an octahedral complex, which showed line broadening in the presence of H₂¹⁷O suggesting water was also bound to the iron centre. When exposed to NO, the crystals also react to form the ternary Fe:NO:ACV:IPNS complex with a similar visible spectrum to that observed in solution. Data collected on ID02 allowed us to solve the crystal structure of the Fe:NO:ACV:IPNS complex to a resolution of 1.45 Å, which reveals that NO binds to the iron centre *trans* to

Asp-216, indicating this is the likely oxygen binding site. Other subtle movements in the protein structure in response to NO binding were also identified. The NO is bound to the iron in a non-linear orientation (with an Fe-N-O angle of 120°) and the NO oxygen atom is equidistant (3.3 Å) from both the ACV valine nitrogen and cysteinyl β carbon atom from each of which a hydrogen atom must be removed to close the β -lactam ring.

Work is in progress with data collected on oxygenated samples and the electron density maps show clear changes in structure.

TABLE 1. Data collected from LS-507

Crystal type	Resolution limit (Å), R_{merge}	Notes
Fe:ACV:NO:IPNS	1.45, 8.2	$R_{\text{cryst}}=20.66$ $R_{\text{free}}=21.20$
Fe:AC-Ala:NO:IPNS	1.65, 11.5	$R_{\text{cryst}}=20.07$ $R_{\text{free}}=23.94$
Fe:ACV:IPNS, hex form, 4 hours exposure to O ₂	1.7	
Fe:ACV:IPNS, hex form, 3 hours exposure to O ₂	1.7	
Fe:ACV:IPNS, hex form, 2 hours exposure to high pressure O ₂	1.6	
Fe:ACA:IPNS, hex form, 6 hours exposure to O ₂	1.9	
Fe:ACA:IPNS, hex form, 4 hours exposure to O ₂	1.8	