ESRF	**Experiment title:** The structure determination of *E. coli* nitroreductase	**Experiment number**: LS1504 LS1684
Beamline: ID14-4 ID14-1	**Date of experiment**: from: 15.2.00 to: 17.2.00 from: 3.6.00 to: 5.6.00	**Date of report**: Sep 2000
Shifts: 6 6	**Local contact(s)**: Dr Raimond Ravelli Dr Hassan Belrhali	*Received at ESRF:*

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E. coli nitroreductase (NTR) is a dimeric flavoprotein that catalyses the oxidation of NAD(P)H and the reduction of aromatic nitro groups via a ping-pong mechanism. It has been proposed to use NTR in an anticancer therapy, in which the patient has been injected with CB1954, a harmless prodrug containing NO_2 groups. NTR can be expressed exclusively in tumour cells using an adenovirus expression system, where it reduces CB1954 to produce a cytotoxic agent, killing the cell very rapidly. For a more effective treatment, there is a need for a more efficient enzyme. We wish to engineer NTR to be catalytically faster and to produce only one reduced product (currently, two are produced, only one of which is useful). We have obtained three crystal forms of NTR - tetragonal, orthorhombic and monoclinic, all grown with the same crystallisation conditions (different crystal forms often appearing in the same drop). We have solved the structures of all three forms (see table below).

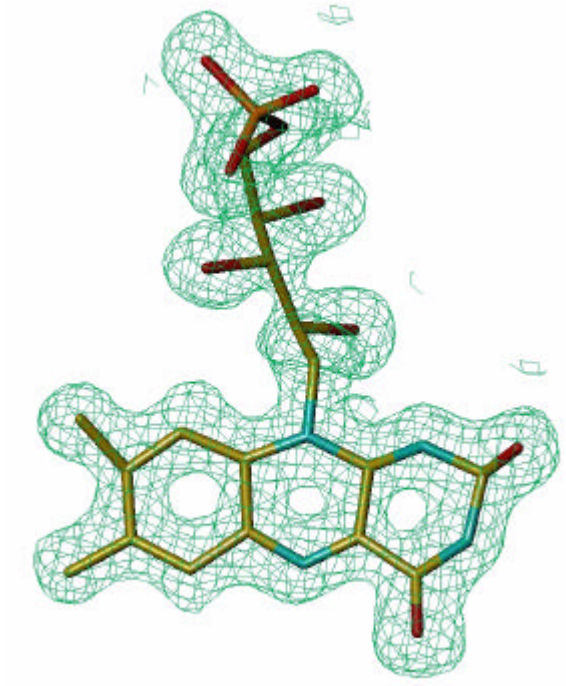
Crystal Properties	**Orthorhombic**			**Tetragonal**		**Monoclinic**
Spacegroup	$P2_12_12_1$			$P4_12_12$		$P2_1$
Cell Dimensions	58, 120, 144			57, 57, 262		72, 58, 117, β=104
Solvent Content	50.5%			43.1%		48.0%
Copies in ASU	4			2		4

Data Collection[1]	**MAD**			**SAD**	**Nat**	**Nat**
	IP	PK	RM	PK		
Wavelength (Å)	0.9793	0.9795	0.9393	0.979	0.934	0.934
No. Observations	119,730	120,033	124,872	106,233	328,334	1,165,744
No. Unique Reflections	71,817	71,764	72,966	54,146	47,571	85,832
Completeness (%)	95.6 (80.0)	95.7 (80.2)	97.2 (91.4)	96.6 (92.7)	94.7 (88.2)	98.2 (92.3)
Redundancy	2	2	2	2	2	7
I/sigI	14.4	14.6	15.2	15.6	13.3	5.1
R(sym)	5.8%	5.9%	5.2%	4.3%	5.5%	5.9%
Resolution	2.4	2.4	2.4	2.0	1.7	1.8

Structure Determination		
Figure of Merit	0.75	0.52
No. of Se Atoms Found	16 / 20	8 / 10

[1] MAD and SAD data sets were collected on Selenomethionyl derivative. Abbreviations Nat = Native IP = Inflection Point PK = Peak RM = Remote

All three forms have been refined with low R-factors.



The figure shows the refined 2Fo-Fc density for the flavin mononucleotide in a partially refined tetragonal nitroreductase model. The three ring isoalloxazine ring is clearly defined. The map is calculated to 1.7 Å resolution.