Report on the use of BM-14, 6-8 october, 6 shifts

1. Thioredoxin, glutaredoxin and thioredoxin reductase are part of the system maintaning and regulating the proper redox state in the cell. Deficiencies in this system often are associated with human disease. In this project, we wish to determine the structure of a novel member of the glutaredoxin family, NrdH from E. coli. This protein belongs, based on sequence to the glutaredoxin family, however has a thioredoxin-like activity profile. Attempt to solve the structure of this protein by MR have failed and we therefore performed a MAD experiment using selenomethionine substituted protein.

Space group $P2_1$

Table 1. Data collection and refinement statistics

	SeMet MAD		
	λ1 peak	λ2 inflection	λ3 remote
Wavelength (Å)	0.9786	0.9787	0.9184
X-ray source	BM14 ESRF	BM14 ESRF	BM14 ESRF
Resolution (Å)	25.0 – 2.9	25.0 – 2.9	25.0 – 2.9
R_{sym} (%)	6.2	7.4	6.2
I/σ(I)	12.8	12.9	14.5
Completeness (%)	95.0 (70.9)	99.8 (98.5)	99.5 (99.0)
Unique reflections	5629	3134	3117
Phasing power (iso/ano)	2.5 / 3.9	1.5 / 3.4	2.9
Rcullis (iso/ano)	0.59 / 0.49	0.85 / 0.60	0.64

Structure is solved and refined to 1.7 Å resolution. Manuscript in preparation.

2. Anthracyclines are important chemotherapeutic agents of the polyketide class of antibiotics produced by actinomycetes. In Streptomyces species, these antibiotics are synthesized by a number of enzymes, encoded by open reading frames denoted *rdmA* to *rdmF*. *RdmE* encodes a FAD-dependent monooxygenase which functions as an aromatic polyketide hydroxylase. As one step towards the elucidation of the pathway for the synthesis of this class of antibiotics, we have crystallised Rdme with bound substrate and intend to determine the structure of this hydroxylase. Due to lack of sequence homology to other hydroxylases of known structure, we have to resort to MAD/MIR techniques for phase determination. Production of Se-methionine substituted protein in Streptomyces has not yet been successful.

During this visit we performed a three-wavelength MAD experiment using a crystal of the enzyme soaked in mother liquid containing 0.5 M NaBr. The data quality was not very good, R-sym values were above 10%. The analysis of the data did not reveal any Br sites in the crystal.