Report on the use of ID14:2, 1-3 december, 3 shifts

5-fluorouracil is one of the five-most used drugs in the chemical combat against cancer, in spite of its severe side-effects. These side effects are due to the rapid breakdown of the drug in the pyrimidine catabolic pathway. The rate limiting enzyme in this pathway is dihydropyrimine dehydrogenase (DPD) and the enzyme is a target for improvement of cancer therapy. This enzyme consists of more than 1000 amino acids per subunit. We have determined the structure to 1.9 Å resolution based on MAD phasing using the anomolous signal of Fe due to the presence of four Fe-S clusters, with data collected at the ESRF (Dobritzsch et al., 2001). The experiment performed during this visit extended the study to the analysis of an enzyme-inhibitor complex in order to map the architecture of the active site.

ternary complex of DPD(WT) / 6-aminouracil / NADPH
wavelength: 0.931 Å, distance: 200 mm, Δφ: 0.8°, images: 1-24, 150-320, detector: MAR-CCD

resolution	30-2.48 Å	(2.58-2.48 Å)	
completeness	95.5 %	(89.8 %)	
R_{sym}	0.100	(0.358)	
I/σ	4.3	(1.4)	SCALA
multiplicity	3.1	(2.6)	

results:
6-aminouracil is only partially bound in active site, no full occupancy; active site loop is open, NADPH is bound close to FAD, but not in conformation suitable for catalysis

ternary complex of DPD(C671A mutant) / uracil acetic acid / NADPH
wavelength: 0.931 Å, distance: 215 mm, Δφ: 0.8°, images: 1-222, detector: MAR-CCD

resolution	30-2.6 Å (used only to 3.3 Å)	(3.36-3.30 Å)	
completeness	98.3 %	(92.9 %)	
R_{sym}	0.109	(0.463)	
I/σ	6.1	(1.4)	SCALA
multiplicity	3.5	(3.2)	

results:
data are useful only to a resolution of 3.3 Å, high mosaicity, inhibitor is bound in active site; the active-site loop is open, most likely due to sterical constraints (carboxymethyl-moiety of inhibitor), NADPH is bound close to FAD in for catalysis required conformation;