ESRF	**Experiment title:** Structural Studies on cyclin-dependent kinase 2 (CDK2) in complex with specific CDK inhibitors	**Experiment number:** LS-912
Beamline: ID14 3	**Date of experiment:** from:24-04-98 to: 26-04-98	**Date of report:** 25-8-98
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Report: Sequential activation of the CDKs directs progress through the eukaryotic cell cycle. Loss of CDK regulation has been genetically linked to the development of human cancers. There is a strong interest in the design of potent and specific CDK inhibitors for use in cancer treatment. Specific CDK inhibitors will be important tools to probe the roles of this kinase family in cell cycle control and apoptosis. A novel series of purine-based CDK inhibitors with IC_{50} values in the low μM range, have been identified through the Anticancer Drug Discovery Initiative, (ADDI), at Newcastle University (unpublished results). Knowledge of the binding mode of these compounds and of the microbial alkaloid staurosporine (a potent but non-specific protein kinase inhibitor) within the CDK2 ATP-binding site has informed another round of compound synthesis. In collaboration with Dr. Laurent Meijer (CNRS, Roscoff) and Dr. Gerhardt Eisenbrand (Kaiserlautern) we have studied a second inhibitor series (E series) derived from plant alkaloids. These compounds inhibit CDC2/cyclin B with measured IC_{50}s in the low nanomolar range. A third inhibitor series (in collaboration with Dr. Laurent Meijer and Dr. Konrad Konig (Hamburg) was also tested.

In total, ten compounds were tested in the present beamtime allocation.

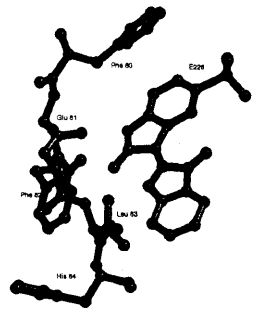
Space group: $P2_12_12_1$

	CDK2/6023	CDK2/6027	CDK2/2048	CDK2/2056
Cell dimensions (Å)	53.09,71.08	52.65,69.89	53.17,71.26	52.86, 71.07
	72.11	71.63	72.26	71.84
Max. Res. (Å)	1.6	1.85	1.45	1.3
Obs	83,887	62,274	116,798	152,792
Unique reflts,	35,444	22,3 99	47,628	63,571
completeness (%)	96.2	96.4	95.9	96.3
R_{merge}	0.056	0.057	0.038	0.044
mean I / σ(I)	13.6	16.3	22.0	18.8
Highest res. bin (Å):	1.67-1.60	1.93-1.85	1.52-1.45	1.36-1.30
Completeness (%)	94.0	97.2	99.2	97.2
mean I / mean σ(I)	1.6	2.3	2.1	1.6
R_{merge}	0.306	0.372	0.339	0.439

Three further CDK2 datasets were collected from soaks on this compound series. NU6036 was native, NU2005 has yet to be processed; it is closely related to a compound for which the structure has been determined An initial processing of the CDK2/NU2077 data shows that the compound has bound, the data has to be reprocessed. One example of the E series, E226 was tested.

	CDK2/e226
Cell dimensions (Å)	53.19,69.52
	71.56
Max. Res. (Å)	1.90
Obs	45,914
Unique reflts,	20,308
completeness (%)	90.7
R_{merge}	0.046
mean I / σ(I)	18.52
Highest res. bin (Å):	1.99-1.90
Completeness (%)	95.0
mean I / mean (I)	1.8
R_{merge}	0.399

e226 bound to CDK2



A third series of inhibitors with a different backbone were tested. Two soaks were carried out. One dataset was native, data for the second CDK2/inhibitor soak is yet to be processed. A dataset to 1.3 Å was also collected on native CDK2.

We collected datasets on ten different CDK2/inhibitor complexes and a high resolution native dataset. Rapid data collection was possible because of the very bright beam and the CCD detector. We also tested crystals of AP2-μ2, a subunit of the endocytosis complex, and crystals of mutants of the cell cycle control protein $p13^{suc1}$. A dataset was collected for the mutant S 13G. The second mutant $p13^{suc1}$(delta 87-89) is of interest because it is constitutively dimeric. Unfortunately we were not able to extend the resolution of our previous dataset. The crystals diffract poorly beyond 3.5 Å.