ESRF	**Experiment title:** Structural Studies on cyclin-dependent kinase 2 (CDK2) in complex with specific CDK inhibitors	**Experiment number:** LS-762
Beamline: BM14	**Date of experiment:** from: 06- 12-97 to: 07- 12-97	**Date of report:** 25-2-98
Shifts: 3	**Local contact(s):** Dr. Gordon Leonard.	*Received at ESRF:* 02 MAR

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Report: Sequential activation of the CDKs directs progress through the eukaryotic cell cycle (1). 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. Two different classes of CDK inhibitors were tested. The first class is a series of purine-based CDK inhibitors with IC_{50} values in the low μM range that have been identified by our collaborators at Newcastle University (unpublished results). We have previously determined structures for ten of these compounds bound to CDK2. 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 (2) has informed another round of compound synthesis. The second class of inhibitors have been chemically synthesised following their isolation from herbal medicine sources. This project is in collaboration with Dr. L. Meijer (Roscoff, France) and Dr. G. Eisenbrand (Kaiserslautern, Germany). Five compounds of the first class, (the "NU" series) and two of the second (the

"E" series) were tested in the present beamtime allocation. CDK2 crystals were soaked in mother liquor solution containing added compounds at concentrations ranging from 0.1mM to 5mM for between 6 and 20 hours.

Multiple crystals were tested following soaking in NU6036. The presence of this compound in the soak solution severely compromised the integrity of the crystals. A partial dataset was collected for NU2048 but has not been processed. CDK2/NU6021 and CDK2/NU6027 datasets were recollections to improve the quality of the datasets on compounds of particular interest. The structures are currently being refined. The CDK2/NU6021 model is being refined at 1.4Å resolution. The model consists of residues 1-35 and 44-298, NU6021 and 206 waters. Rf= 25.5%, Rc= 23.1%. The CDK2/NU6027 model is being refined to 1.9Å resolution. . This model consists of residues l-35 and 44-298, and NU6027, Rf=30.7%, Rc= 25.7%. Finally, a dataset was collected on CDK2 crystals soaked in compound NU2017. The CDK2/NU2017 model has been refined against this data at 1.4Å resolution. It contains residues l-35 and 44-298, NU2017 and 273 waters. Rf= 22.2%, Rc= 18.6%. The two datasets collected on compounds in our second class E221 and E226 proved to be native.

Further compounds are now being designed for crystallographic studies and to determine using biochemical methods the effects of compound binding on CDK2-cyclin association and activation of kinase activity.

References

1. Morgan, D.O., (1995), Nature 374: 131-134.
2. Lawrie, A.M., et al., (1997), Nature Struct. Biol. 4: 796-801.