ESRF	**Experiment title: Structural studies on cell-cycle proteins in complex with small molecule inhibitors**	**Experiment number**: **LS-1526**
Beamline: ID14-EH3 ID14-EH4	**Date of experiment**: from: 24-09-99 to: 26-09-99 from: 13-11-99 to: 15-11-99	**Date of report**: 23 August, 2000
Shifts: 1	**Local contact(s)**: Dr. E. Mitchell	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Martin Noble*, Dr. Jane Endicott* and Professor Louise Johnson

Laboratory of Molecular Biophysics

Rex Richards Building

South Parks Road

Oxford OX1 3QU, U.K.

Report:

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(In collaboration with AstraZeneca and members of the ADDI group, Newcastle Universty, U.K.)

Identification of Novel Purine and Pyrimidine Cyclin-Dependent Kinase Inhibitors with Distinct Molecular Interactions and Tumour Cell Growth Inhibition Profiles

Substituted guanines and pyrimidines were tested as inhibitors of cyclin B1/CDK1 and cyclin A3/CDK2 and soaked into crystals of monomeric CDK2. O6-Cyclohexylmethylguanine (NU2058) was a competitive inhibitor of CDK1 and CDK2 with respect to ATP, (Ki values: CDK1, 5 ± 1 μM; CDK2, 12 ± 3 μM) and formed a triplet of hydrogen bonds (ie NH-9 to Glu 81, N-3 to Leu 83, and 2-NH2 to Leu 83). Hydrogen bonding interactions between NU2058 and CDK2 are shown in Figure 1. The triplet of hydrogen bonding and CDK inhibition was reproduced by 2,6-diamino-4-cyclohexylmethoxy-5-nitrosopyrimidine (NU6027, Ki values: CDK1, 2.5 + 0.4 μM; CDK2, 1.3 + 0.2 μM). X-ray data collection and refinement statistics

pattern of selectivity disctinct from flavopiridol and olomoucine. These CDK inhibition and chemosensitivity data indicate that the distinct mode of binding of NU2058 and NU6027 has direct consequences for enzyme and cell growth inhibition.

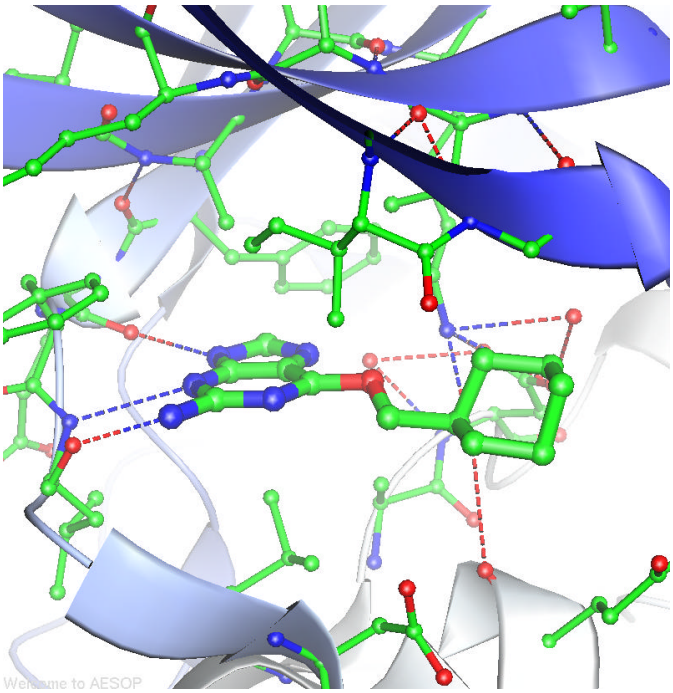

Figure 1 Interaction of NU2058 with monomeric CDK2

	CDK2/NU2058	CDK2/NU6027
cell dimensions (Å)	52.62, 71.05, 71.50	52.65, 69.90, 71.61
maximal resolution (Å)	1.95	1.85
observations	42 243	62 274
unique reflections, completeness (%)	17 940 (88.8)	22 399 (96.4)
R_{merge}[a]	0.065	0.057
mean $I/(I)$	12.3	16.3
highest resolution bin (Å)	2.04–1.95	1.93–1.85
completeness (%)	84.3	97.2
mean I/mean (I)	2.6	2.27
R_{merge}	0.313	0.372
protein atoms	2338	2338
residues	290	290
other atoms	174 water 18 NU2058	144 water 18 NU6027
resolution range (Å)	20.00–1.8	20.00–1.85
R_{conv}[b]	19.5	21.3
R_{free}[c]	26.9	28.1
mean main chain protein temperature factors (Å)^2	25.2	35.9
mean ligand temperature factors (Å)^2	16.9	42.6

[a] $R_{merge} = \Sigma_h\Sigma_j|I_{h,j} - \bar{I}_h|/\Sigma_h\Sigma_j|I_{h,j}|$ where $I_{h,j}$ is the intensity of the jth observation of unique reflection h. [b] $R_{conv} = \Sigma_h||F_{o_h}| - |F_{c_h}||/\Sigma_h|F_{o_h}|$ where F_{o_h} and F_{c_h} are the observed and calculated structure factor amplitudes for reflection h. [c] R_{free} is equivalent to R_{conv} but