ESRF	**Experiment title: Structural studies on signal transduction pathways and cell cycle control.**	**Experiment number**: LS-1382 (LS-912)
Beamline: ID14-3 BM 14	**Date of experiment**: from: 5th March to: 6th March, 1999 from: 30th April to: 1st May, 1999	**Date of report**: 16th February, 2000.
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Report: (In collaboration with the groups of Dr. Laurent Meijer, CNRS Station Biologique, Roscoff, France and Dr. Gerhard Eisenbrand, University of Kaiserlautern, Germany). (Nature Cell Biology, (1999), 1: 60-67.)

Indirubin (靛玉红), the active constituent of the Chinese anti-leukaemia traditional herbal medicine recipe Dang Gui Lu Hui Wan (当归芦荟丸), selectively inhibits cyclin-dependent kinases –Crystal structure of a cdk2/indirubin-3'-monoxime complex.

We have identified indirubin and its analogues as potent and selective cyclin-dependent kinase (CDK) inhibitors in a screen of a collection of compounds derived from traditional Chinese medicinal plants. Indirubin had previously been identified as the active ingredient of Dang Gui Lu Hui Wan, a complex mixture of plants used to treat chronic myelocytic leukaemia. Indirubin-3'-monoxime inhibited the proliferation of Jurkat cells, arresting cells in G1 (low concentrations) or in G2/M (high concentrations) and triggering apoptosis. The crystal structures of CDK2 in complex with two indirubin derivatives show that these inhibitors interact with the kinase ATP binding site through van der Waals interactions and 3 hydrogen bonds. The CDK inhibitory properties of indigoïds may substantially contribute to their anti-leukaemic properties *in vivo*. This molecular mechanism of action provides a simple way for optimisation of indigoïds as anti-mitotic agents of therapeutic potential.

The structures of the CDK2/indirubin derivative complex was refined to yield crystallographic statistics given in Table 1. Indirubin-3'-monoxime (data collected at Elettra)

and indirubin-5-sulfonate act by competing with ATP for binding to CDK2. They share a similar binding mode: the lactam amide nitrogen donates a hydrogen bond to the peptide oxygen of Glu81, the NH group of Leu 83 donates a hydrogen bond to the lactam amide oxygen, and the cyclic nitrogen acts as a hydrogen bond donor to the backbone oxygen of Leu 83. As compared to previously reported CDK2-inhibitor structures, the indirubin derivatives make an additional hydrogen bond to the CDK2 backbone. The shape of the indirubin molecule complements the bent shape of the ATP binding site in the hinge region. The indirubin derivatives make a number of apolar contacts with conserved residues that line the ATP binding site. The sulfonate group of indirubin-5-sulfonate through its ability to make both charge and hyrdrogen bond interactions is complementary to the CDK2 structure at the back of the ATP binding cleft. In particular, the sulfonate group of indirubin-5-sulfonate interacts with the sidechain amino group of Lys33. In addition to this interaction the sulfonate oxygens contact the backbone nitrogen of Asp 145 and the amide group of Asn 132. Indirubin-5-sulfonate binding to CDK2 causes changes to the main chain conformation for residues Asp 145-Phe 146-Gly 147-Leu 148 (the conserved 'DFG' motif found in most protein kinases) so that unfavourable steric and charge effects are avoided. Gly 147 can be located in the CDK2/indirubin-5-sulfonate complex electron density maps, but the following residues that lead into the activation loop cannot be defined and the model resumes at Val 163. In contrast, the CDK2/indirubin-3'-monoxime structure is similar to the CDK2/ATP structure and shows a loop region that is ordered through to residue Ala 151. The indirubin-3'-monoxime monoxime group occupies the ATP ribose binding site and makes no direct interactions with CDK2. The details of the atomic interactions between this inhibitor class and CDK2 will be helpful in the synthesis of new, potent inhibitor analogues.

TABLE 1. Statistics of the datasets used and of the refined structures.

	CDK2/indirubin-5-sulfonic acid
Cell dimensions (Å), space group $P2_1\,2_12_1$	53.2, 69.5, 71.6
Maximal Resolution (Å)	1.90
Observations	38,756
Unique reflections, completeness (%)	19,244 (85.9%)
R_{merge}	0.045
mean I / σ(I)	17.2
Highest resolution bin (Å):	1.99-1.90
Completeness (%)	90.4%
mean I / mean σ(I)	1.66
R_{merge}	0.403
Protein atoms	2217
Residues	1-35, 44-147, 163-298
Other atoms	108 water 24 indirubin-5-sulfonic acid
Resolution range (Å)	20.00-1.90
R_{cony}[2]	0.22
R_{free}[3]	0.285
Mean protein temperature factors $(Å)^2$	47.55
Mean ligand temperature factors $(Å)^2$	44.1

FIGURE 1. CDK2/indirubin-5'-sulphonate complex.