ESRF	**Experiment title:** Structural bases of activation of cyclin-dependent kinase 5 (Cdk5).	**Experiment number:** **LS1933**
Beamline: ID14-3 ID29	**Date of experiment:** from 28-04-2001 to 30-04-2001 from 23-06-2001 to 25-06-2001	**Date of report:** 25-07-01
Shifts to BAG: **12** **6**	**Local contact(s):** Edward Mitchell Andy Thompson	*Received at ESRF:*

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Cyclin-dependent kinases (CDKs) constitute a family of serine/threonine kinases whose activity is regulated by the interaction with proteins known as cyclins (Morgan, 1995). Although most CDKs have been implicated in the regulation of the cell division cycle, emerging evidence indicates that certain members of this family are involved in other processes. An important example is represented by CDK5, whose function is critical during neuronal development (Smith et al., 2001). CDK5 substrates include the neuron-specific cytoskeletal proteins Neurofilament and Tau, the LIS1 and Dynein associated protein Nudel, the synaptic protein Synapsin 1 and the Munc18/Syntaxin1A complex. CDK5 has also been shown to phosphorylate proteins involved in functions of the mature brain. CDK5 phosphorylates DARPP32, a neuronal striatum specific protein regulating dopamine signaling, rendering it an inhibitor of PKA. Phosphorylation of DARPP32 by CDK5 was further shown to dampen the effect of chronic cocaine treatment indicating a possible regulatory role of CDK5 in drug addiction.

Deregulation of CDK5 activity upon conversion of p35 to p25 has been implicated in neurodegenerative diseases. p25 is a 208 residue C-terminal proteolytic product of p35. The accumulation of p25 in human brain tissues correlates with AD affection. Conversion of p35 to p25 by calpain can be induced by a variety of neurotoxic conditions including ischemia, excitotoxicty, and beta amyloid peptide. Despite the fact that both p35 and p25 efficiently activate CDK5, p25 displays distinct properties from p35. First, p35 is a very unstable protein with a half-life of 20 to 30 minutes while p25 displays a substantially longer half-life. In addition, p35 localizes to cellular membranes by virtue of an N-terminal myristoylation motif. In neurons, p35 is present throughout the entire cell including the tips of processes. p25 lacks the myristoylation site and is concentrated in the cell body and nucleus in neurons. These properties of p25 lead to deregulation of CDK5 kinase activity *in vivo*, which is associated with tau hyper-phosphorylation, cytoskeletal disruptions, and apoptotic death of neurons. Thus, conversion of p35 to p25 may play a role in the pathogenesis of AD.

Recently, we have determined the structure of the CDK5-p25 complex (Tarricone et al., In the press). We are now concentrating on the possibility of collecting co-crystals of CDK5 with kinase inhibitors. This effort may improve our understanding of the structural bases of selective CDK inhibition, and might contribute to the discovery of drugs to fight AD. Crystals of CDK5/p25 are relatively poor diffractors, are brittle, and tend to grow as twinned plates. During our experiments in Grenoble we were able to collect a single high-quality data set from crystals soaked in different CDK inhibitors. In particular, we were able to collect a dataset from a crystal soaked in Indirubin. Compared with other previously collected datasets, this is of particularly high quality, and of considerably higher resolution that those previously available. The structure is being refined, and indirubin is clearly visible in the electron density (Tarricone et al. In preparation).

References

Morgan, D. O. (1995). Principles of CDK regulation. Nature *374*, 131-4.

Table: Summary of CDK5/p25 data collection (ID14-3, 28-04-01 to 30-04-01)

Crystal	complex with indirubin
Space Group	P21
Unit cell	a=87.3 b= 142.7 Å, c=82.5 Å, beta=94.2°
Resolution	2.3 Å
N° measurements	160456
N° reflections	51760
Completeness	98.8 %
Rsym	7.5 %