ESRF	**Experiment title:** Structural studies on kinase substrate interactions	**Experiment number:** LS-647
Beamline: D2AM	**Date of experiment:** from: 12-3-97 to: 13-3-97	**Date of report:** 27-8-97
Shifts: 3	**Local contact(s):** Dr. Jean-Luc Ferrer	*Received at ESRF:* 2 9 AOUT 1997

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Report:

To date, the only information on the mode of recognition of peptide substrates by protein kinases has derived from the binding of an inhibitor peptide to cyclic AMP dependent protein kinase. It has not been possible to grow crystals of CDK2 bound to nucleotide and peptide substrates, and so we have used the y-subunit of phosphorylase kinase (PHK) as a model system to gain insights into this aspect of kinase function. This approach builds upon the previously reported structure of a truncated form of the y-subunit of PHK (PHKγt)[1].

Here, we report crystals of a ternary complex containing PHKγt, a nucleotide analogue (AMPPNP), and a peptide substrate. These crystals grow in space group $P3_221$, with cell dimensions a=b=65.3Å, c=145.8Å, and have been used to collect a 2.6 Å dataset at beam line D2AM of the ESRF. A model has been built and refined against this data to a final free R-factor of 29.9%.

The peptide substrate used contains the phosphorylatable serine residue with three residues at the N- and C-terminal ends, and shares sequence identity with the optimal substrate peptide identified by Songyang et *al.*[2], and with the natural substrate peptide (residues 1 l-17 of glycogen phosphorylase) .

The binding of a substrate peptide to PHKγt resembles the binding of the inhibitor peptide to cyclic AMP dependent protein kinase (cAPK) [3] for residues up to and including the site of phosphorylation. Residues C-terminal to this bind in a different orientation. This allows the formation of a a short anti-parallel P-sheet between the substrate and the "activation segment" (figure la). The involvement of the activation segment in peptide binding identifies one of the mechanisms by which its conformation may influence kinase activity.

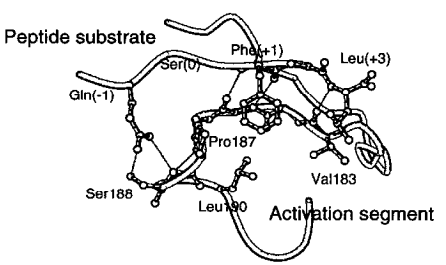

Figure la)

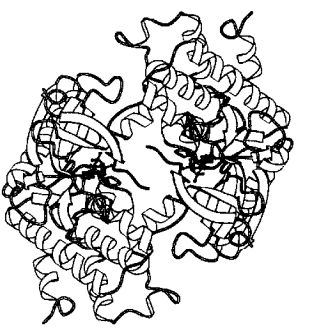

Figure lb)

This novel crystal form of PHKγt contains an intimate dimeric association, shown in figure lb, between two molecules related by a crystallographic two-fold axis. Although this association may be artefactual, it is possible that it reflects an association which occurs in the hexadecameric PHK holoenzyme.

References:

1. Owen, D.J., Noble, M.E., Garman, E.F., Papageorgiou, A.C. & Johnson, L.N. Structure 3,467-482 (1995).

2. Songyang, Z., *et al. Mol. and Cell. Biol.* 16, 6486-6493 (1996).

3. Knighton, D.R., *et al. Science 253, 407-413* (1991).