ESRF	**Experiment title: Structure determination of the serine/threonine protein phosphatase KAP using MAD data.**	**Experiment number:** LS-486
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Report:

KAP (kinase associated protein phosphatase) is a dual specificity protein phosphatase that functions to regulate progression through the eukaryotic cell cycle. This is achieved by dephosphorylating phosphothreonine 160 within the activation segment of cyclin dependent kinase-2 (CDK-2), hence inactivating the kinase. The activity of KAP is specific for monomeric CDK-2; the interaction of cyclin A with CDK-2 prevents the dephosphorylation reaction *in vitro*. The suggests that *in vivo*, the levels of cyclin A regulate indirectly, the degree of phosphorylation of CDK-2 through the activities of the opposing kinases and phosphatases.

KAP belongs to the family of dual specificity phosphatases, a sub-family of the protein tyrosine phosphatase (PTP) family, characterised by a characteristic sequence motif: $C(X)_5RS$. Apart from this region of sequence similarity, no other sequence similarity is observed between KAP and other members of this family. We initiated a project to determine the structure of KAP in order to understand its relationship to the PTPs and its ability to recognise CDK-2 specifically. The protein was produced from an over-expression vector in *E.coli* using a modified pET28a vector (Hanlon and Barford, 1998) and crystallised.

The enzyme (mol. wt 25 kDa) crystallised in space group $P6_522$ with a = b = 74.7 A, c = 139.2 A., with one molecule per asymmetric unit. The structure was solved using a combination of MAD and MIRAS phasing methods. MAD data were collected at BM14 using a CCD detector to 3.2 A using a mercury derivatised (EMTS) crystal at 3 wavelengths. There are three mercury sites in the protein and the coordinates of these sites were determined using direct methods with the SHELX program. Patterson peaks corresponding to these sites were observed in the anomalous Patterson maps and these superimposed upon identical sites in a mercury difference Patterson map. The phases, determined using MLPHARE, from the MAD experiment alone were of sufficient quality to outline the overall trace of the molecule. A portion of this map is shown below. However, additional experimental data were included to improve the overall phasing power and map quality. These included data collected at BM14 and also at station PX9.6 in Daresbury. The BM14 data included isomorphous data sets using the mercury derivative and two other derivatives, namely gold and selenate. Selenate binds to the catalytic site and replaces a sulphate ion present within the crystallisation solution.

The structure has now been solved to 2.5 A resolution and refined with an R_{work} of 0.23 using 8 379 reflections (R_{free} is 0.33). Further refinement is in progress. The overall structure of KAP is similar to the core elements of the PTPs, despite sharing no sequence similarity. KAP contains a central four stranded P-sheet composed of parallel P-strands surrounded on both sides by one and four α-helices. However, KAP appears to be a truncated form of the PTPs and lacks the phosphotyrosine recognition domain that in the PTPs determines the specificity for phosphotyrosine containing proteins. However, like the PTPs, the base of the catalytic site of KAP is formed by the PTP signature motif and essentially all catalytic site residues of PTP 1B have counterparts within the KAP catalytic site.

An unexpected finding in the structure of KAP was that the catalytic Cys residue (Cys 140) forms an intramolecular disulphide bond with Cys 79. This observation probably explains why very low catalytic activity is observed with bacterially expressed KAP. Blocking the Cys 140 side chain with a disulphide would completely inhibit enzyme activity. The physiological significance of this disulphide is not obvious. Based upon this observation we have now prepared two mutants of KAP, namely a Cys 140 -> Ser mutant and a Cys 79 -> Ser mutant. The latter enzyme displays markedly higher catalytic activity than the wild type enzyme as the catalytic Cys residue does not participate within a disulphide bond. Crystals of the Cys 140 -> Ser mutant have been obtained and we aim to compare the structure of this mutant with that of the wild type protein.