s8a.m1.p23 **Status Report of the Strucure Determination of Baker's Yeast 12S Phosphofructokinase1 R-State.** I. Mechin[a], G. Kopperschläger[b], W.R. Rypniewski[a], [a]*European Molecular Biology Laboratory (EMBL) c/o DESY, Germany,* [b]*Institue of Biochemistry, Medical Faculty, University of Leipzig, Germany.*
Keywords: phosphofructokinase, allostery, glycolysis.

Extensive crystallographic studies have been carried out on bacterial phosphofructokinase1 but until now no eukaryotic PFK1 has been crystallised in a form suitable for X-ray analysis. PFK1 catalyses the conversion of fructose 6-phosphate into fructose 1,6-bisphosphate in the presence of MgATP. It is an allosteric key enzyme in glycolysis, with various effectors, both activators and inhibitors. In baker's yeast PFK1 is a heterohectamer ($\alpha_4\beta_4$) of molecular weight approximately 800 kDa (21S). As a result of a early gene duplication each eukaryotic subunit consists of two homologous parts, each repeat similar to one prokaryotic subunit. One of them is thought to have lost catalytic function and acquired a regulatory role. In yeast a second gene duplication event is supposed forming two different types of subunits called α and β. Yeast PFK1 octamer consists of 16 prokaryotic like units with heterogeneous terminal regions responsible for subunit association.

The 12S form of yeast PFK1 is obtained by limited proteolytic cleavage of the native enzyme by chymotrypsin in the presence of ATP, leading to the truncation of C- and N- terminal parts[1]. As a consequence the 21S octamer dissociates to form two 12S tetramers of identical size composed of partially degraded α' and β' subunits. This truncated form is catalytically active, possesses similar kinetic properties to the native enzyme and shows no micro-heterogeneity.

The size of yeast PFK1 makes the crystallography a challenge but structure solution is feasible. The 12S enzyme was crystallised under a variety of conditions[2]. The crystals have been characterised. Native diffraction data have been collected to 2.9 Å under cryogenic conditions, on the EMBL beamline at the DORIS storage ring in Hamburg. Because molecular replacement using the bacterial subunit has failed until now, work is in progress to record derivative datasets. Cross-linked crystals obtained by the glutaraldehyde vapour diffusion technique were already soaked in presence of heavy atom coumpounds and tested. First results will be presented.

[1] Kopperschläger G., Bär J. and Stellwagen E. "Limited proteolysis of yeast phosphofructokinase, sequence locations of cleavage sites created by the action of different proteinases", Eur. J. Biochem, (1993), 217:527-533.
[2] Obmolva G., Kopperschläger G., Heinisch J. and Rypniewski W. R. "Crystalisation and preleminary X-ray analysis of the 12S form of phosphofructokinase from *Saccharomyces cerevisiae*", Acta. Cryst., (1998), D54:96-98.
[3] Lusty C. J. "A gentle vapor-diffusion technique for cross-linking of protein crystals for cryocrystallography.", J. Apll. Cryst., (1999), 32:106-112

s8a.m1.p24 **Structural studies of the NDP kinase/antiviral nucleotide analogues interaction.** P. Meyer[a], B. Schneider[b], S. Sarfati[c] D. Deville-Bonne[b], M. Veron[b], B. Canard[d] & J. Janin[a]. [a]*Laboratoire d'Enzymologie et de Biochimie Structurales CNRS bât. 34, F-91198 Gif/Yvette cedex,* [b]*Unité de Régulation Enzymatique des Activités cellulaires et* [c]*Unité de Chimie Organique, Institut Pasteur, F-75724 Paris cedex 15,* [d]*Laboratoire d'Architecture et Fonction des Macromolécules Biologiques, Campus de Luminy, 13288 Marseilles cedex 09.*
Keywords: nucleotide phosphorylation, HIV, CH--O bond.

Nucleoside analogues such as azido thymidine (AZT) are used against AIDS as inhibitors of the reverse transcriptase (RT) of HIV virus. To be active they must be phosphorylated to their tri-phosphate derivative forms by kinases from the host cell. The last step in this phosphorylation cascade is catalysed by NDP kinase. Studies of the phosphorylation of the diphosphates of natural nucleosides and their antiviral analogs demonstrate the importance of the 3'-hydroxyl in the catalytic efficiency of NDP kinase. The comparison of the X-ray structure of NDP kinase bound to TDP and AZT-diphosphate[1] has provided further understanding of the role of the 3'-hydroxyl. It forms extensive interaction with the protein and gives a hydrogen bond to the oxygen bridging the beta and gamma phosphates. Whereas most antiviral nucleoside analogues are modified at the 3' position, some are better substrate of NDP kinase than others. Thus, we have determined the crystallographic structure of NDP kinase bound to nucleotide analogues. The structure of d4T (2',3'-didehydro-dideoxy-thymidine) triphosphate bound to the H122G mutant (lacking the catalytic imidazole group) of *Dictyostelium* NDP kinase is the first structure showing a bound tri-phosphate. It suggests that a CH---O hydrogen bond replaces the –OH…O bond present with natural substrates and contributes to catalysis. The structure of alpha-boranophosphate derivatives bound to wild-type *Dictyostelium* NDP kinase provides structural information on a potentially new anti-HIV drug. Boranophosphate nucleoside derivatives are better substrates of NDP kinase than AZT-diphosphate and they are efficiently incoprporated into viral DNA by wild type and by drug-resistrant HIV reverse transcriptase.

[1] Xu, Y., Sellam, O., Morera, S., Sarfati, S., Biondi, R., Veron, M. and Janin, J. (1997). *PNAS USA* 94: 7162-5.