Experiment title: BAG - CNRS gif sur Yvette		Experiment number: LS 1659a
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Shifts: 2.5	**Local contact(s):** Hassan Belrhali	*Received at ESRF:*

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Report:

A- β-glucosyltransferase (BGT) is a DNA-modifying enzyme encoded by bacteriophage T4 which catalyses the transfer of glucose from uridine to 5-hydroxymethylcytosine in double-stranded DNA in presence of metal ions. From two BGT structures, one solved with its UDP product and the other with its UDPG substrate, a catalytic mechanism has been proposed and residues involved in both DNA binding and in stabilising a 'flipped-out' hydroxymethyl-cytosine nucleotide have been identified. The aim of this study is to confirm or/and extend the proposed catalytic mechanism. As BGT catalysis requires metal ions, we would like to identify and locate the metal site.

Two data sets have been collected on BGT crystals. A data set at 2.5 Å resolution of free form giving the first complete structure of BGT. This is very interesting as the previous structure was missing two large surface loop regions (residues 68-76 and 109-122). We can now compare the free and complexed forms in detail. A data set at 2.07 Å resolution of BGT-UDP complexed with Mg2+ ion. A metal site in the active site can now be described.

B- Cofactor A is a molecular chaperone involved in tubulin folding. We have shown that cofactor A binds to the b-tubulin polypeptide chain. The aim of this study is to solve the structure of this highly specific chaperone that may reveal extremely useful for the determination of b-tubulin structure in a cofactor A / b-tubulin complex. We obtained small crystals of cofactor A that we collected. A data set at 2.8 Å resolution. Now, we need to collect derivatives to solve the structure.

C- The HPr-kinase is a bacterial Ser/Thr kinase without sequence similarities with any other known protein. After entry of sugars in the cell via the PTS system, the glycolysis cycle produces FBP which activates phosphorylation of the small protein HPr on residue Ser-46 by the HPr-kinase. Further interaction of Ser-P-HPr with the transcription regulator CcpA activates the carbon catabolite repression signalisation pathway. The aim of this study is to ellucidate the structure of this new family of protein kinase and to understand its mechanism. A 2.7Å resolution data set has been obtained with a truncated form of HPr-kinase. We also have crystals with this protein containing 6 selenomethionine residues. We now need to collect a MAD data set to solve the structure of the protein.

We have crystals of full length HPr-kinase in complex with FBP and/or ATP analogues. Co-crystallization experiments with the HPr substrate are also underway in order to solve the structure of the complex.