	Experiment title: FRANKFURT BAG: DFPase Cleavage Mechanism	**Experiment number**: LS-1930
Beamline: ID 14 – EH1	**Date of experiment**: from: 7 October 02 to: 8 October 02	**Date of report**: 22 February 02
Shifts: 3	**Local contact(s)**: Dr. Stéphanie Monaco	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Fritzsch, Günter, Max-Planck-Institut für Biophysik, Frankfurt a.M., Germany

Dr. Koepke, Jürgen, Max-Planck-Institut für Biophysik, Frankfurt a.M., Germany

*** Dr. Roy Lancaster, Max-Planck-Institut für Biophysik, Frankfurt a.M., Germany**

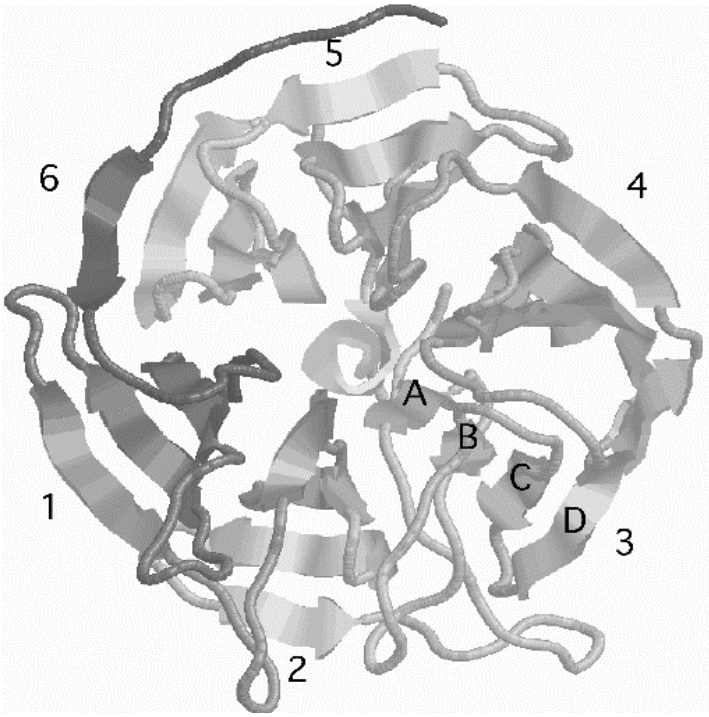
*** Diem, Ralf, Max-Planck-Institut für Biophysik, Frankfurt a.M., Germany**

*** Vicky Katsemi, Institut für Biophysikalische Chemie, J. W. Goethe-Universität Frankfurt am Main, Germany**

Report:

Diisopropylfluorophosphatases (DFPases) are enzymes capable of detoxifying organophosphate-based chemical warfare agents. The DFPase reported here was originally isolated from squid head ganglion of *Loligo vulgaris.* It is the first example of a structural characterization of a squid-type DFPase and the second crystal structure of a phosphotriesterase determined to date. The enzyme hydrolyzes the organophosphates

diisopropylfluorophosphate, soman, tabun, sarin, and cyclosarin. In our group, the X-ray crystal structure of DFPase has been refined to 1.8 Å resolution.



The overall folding of DFPase resembles a ß-propeller. Shown is a view of the molecule down the pseudo six-fold axis.

To get more insight into the functional mechanism of hydrolysis, the mutant DFPase F314A with the inhibitor diisopropylmethyl-phosphonate was co-crystallized from PEGs 8,000 and 20,000. X-ray data have been collected at cryo conditions. Both data sets suffer from low quality. The crystals belong to the same space group as the crystals of the native protein: $P2_12_12_1$. The unit cell parameters of the PEG 8,000 and PEG 20,000 variants differ considerably: 40.6Å x 82.7Å x 87.3Å and 40.1Å x 83.1Å x 86.4Å, respectively. After employing the molecular replacement method and simulated annealing in CNS, the refinement of the PEG 8,000 data set diffracted up to 2.3 Å and yielded an R_{free} = 54% (R_{cryst}=44%). The crystal of the PEG 20,000 data set showed a high mosaic spread of 1.8°. For this reason, the data have been evaluated in XDS. The internal agreement of the data was very low with an R_{symm} of 15.9%. The model of the native structure could be refined directly against the new data to 2.9 Å resolution using Refmac5, however, the resulting agreement factor was only R_{free} = 36% (R_{cryst}=29.9). Subsequently applied simulated annealing in CNS ended up with the same R_{free} but a slighly worse R_{cryst} of 33.6%. Visual inspection of the two models (Refmac5 and CNS) showed in some areas significant shifts of the backbone atoms, while other stretches visible in the native structure were completely missing. Large difference densities along these poorly defined regions have been observed even after extensive rebuild. The presence of the phosphonate molecule in the binding pocket has not been demonstrated.