ESRF	**Experiment title: Crystal structure of TdT complexed with its different natural substrates.**	**Experiment number**: LS-1445
Beamline: ID14-4	**Date of experiment**: from: 23-Sept-1999 to: 24-Sept-1999	**Date of report**: 25-Fev-2000
Shifts: 3	**Local contact(s)**: J. Lescar	*Received at ESRF:*

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Report:

During these 3 shifts, data were collected for a binary complex between Terminal desoxyribonucleotidyl Transferase (TdT) and a brominated oligonucleotide $(5\text{-Br-dU})_9$

First a fluorescence spectrum was collected to determine the three different wavelengths to be used.
Then three different crystals were collected at these three wavelengths. The best crystal was the second one, with a full data set up to 3.0 Å resolution. The Rmerge was still around 20% in the 3.3-3.2 Å resolution shell.

The program SOLVE (Terwilliger) was used to determine the bromine atom positions, but did not succeed. Instead, the anomalous difference maps were calculated at 3.5 Å (using MIR phases from the native protein) and the top peaks were compared to the positions where the single stranded DNA is supposed to bind, in the preliminary model that we have. This is made possible through the deep structural homology between polymerase β and TdT, which was discovered during the course of the work on the native protein.
Three bromine atoms were unambiguously assigned in the anomalous Fourier difference map.
In addition, an isomorphous difference map was calculated using F_{cmplx} - F_{native}. Four different phosphates were identified in this map, at exactly the positione that were expected.
Therefore, we unambiguously conclude that the oligonucleotide is bound in the crystal.
However, the isomorphous signal $F(\lambda_1)$-$F(\lambda_2)$ or $F(\lambda_2)$-$F(\lambda_3)$ showed no peak at the expected positions whatsoever. We believe this is due to the intrinsic smallness of the signal to be measured for bromine atoms (about 1-1.5 electrons).
Even though these data have not been useful for phasing yet, they will be used in the next future to build the single stranded DNA in the active site, once the native structure is completely refined.

Moreover, we were able to collect data for the other binary complex TdT + dNTP, in the presence of Zn++ ions. Fourier difference maps with the native data set and MIR phases showed unambiguously electron density in the dNTP binding site.

In the next future, we plan to collect data for the ternary complex TdT + ddNTP + oligonucleotide.

Publication:

Crystallization of the catalytic domain of murine Terminal desoxynucleotidyl Transferase. Acta Cryst. D submitted.
N. Sukumar, J.B. Boulé, N. Expert-Bezançon, N. Jourdan, J. Lescar, F. Rougeon, C. Papanicolaou & M. Delarue**.**