EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Elucidating the specificities of the PolD active site at the molecular level: determining the cryo-EM structures of PolD trapped in both its elongation and proofreading modes	**Experiment number**: MX2019
Beamline: CM01	**Date of experiment**: from: 11/12/17 to: 13/12/17	**Date of report**:
Shifts: 6	**Local contact(s)**: Gregory Effantin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Ludovic Sauguet
Pierre Raia *

Unité de Dynamique Structurale des Macromolécules Institut Pasteur – 25 rue du docteur Roux – 75015 Paris

Experimental Report

Proposal Summary :

PolD is an atypical archaeal DNA polymerase, which catalytic motor is strikingly different from all other known DNA polymerases (DNAPs). Recently, we determined the first crystal structures of both individual DP1 and DP2 catalytic subunits of PolD from *Pyrococcus abyssi*. Unexpectedly, we found that PolD shares a structural homology with the "two-barrels" family of RNA polymerases (RNAPs), which includes multisubunit transcriptases from all domains of life. This finding has important evolutionary implications as it bridges together DNA transcription and DNA replication within the same protein superfamily.

However, the DP1 and DP2 crystal structures were obtained separately and do not provide a comprehensive description of the molecular mechanisms of DNA polymerization and proofreading by PolD. Indeed, the DNA polymerase active site is located at the interface between the DP1 and DP2 subunits, and the interaction between both subunits is essential for the full activity of PolD. Unlike other DNAPs whose crystal structures have been solved in complex with various DNA substrates, the molecular basis of DNA and nucleotide-recognition by PolD remains poorly characterized. Elucidating the specificities of the PolD active site at the molecular level is required in order to fully exploit the biotechnological potential of this unusual thermostable DNAP.

We plan to resolve the structure of the full-length PolD complex bound to nucleotides, and various DNA substrates (including damaged-DNA) that have been designed in order to trap the polymerase either in its elongation or proofreading modes. These new structures will help us to i) decipher the molecular mechanisms of DNA-binding and catalysis by archaeal PolD, ii) unravel the

cooperativity mechanisms between the DNA polymerase elongation active site and the proofreading exonuclease active site, which are carried by two distinct subunits.

Scientific background :

DNA polymerases are molecular motors directing the synthesis of DNA from nucleotides. DNA polymerases play fundamental biological roles in genome replication, maintenance and repair. In addition to their fundamental biological functions, DNAPs play central roles in modern molecular biology and biotechnology. PolD is a recently discovered new family of DNAPs that is structurally and genetically unrelated to any other DNAP family. PolD exists in all Archaea, except Crenarchaea, and is a replicative polymerase responsible for initiating DNA synthesis. It is composed of a large catalytic subunit (DP2) and a smaller subunit with proofreading exonuclease activity (DP1).

We determined the first crystal structures of both individual DP1 and DP2 catalytic subunits of PolD from *Pyrococcus abyssi*. The structure of PolD is strikingly different from all other DNAPs characterized so far. In particular, the catalytic core of PolD is structurally distinct from the Klenow-like catalytic core that is shared by all other thermostable DNAPs marketed for PCR. Unexpectedly, we found that PolD shares a structural homology with the "two-barrels" family of RNAPs, which includes multisubunit transcriptases from all domains of life. This finding has important evolutionary implications as it bridges together DNA transcription and DNA replication within the same protein superfamily. **This work was recently published in Nature communications, cited by the Faculty F1000Prime, in a press release issued by CNRS, and highlighted by IFREMER as well as both ESRF and SOLEIL synchrotrons.**

However, the DP1 and DP2 crystal structures were obtained separately and do not provide a comprehensive description of the molecular mechanisms of DNA polymerization and proofreading by PolD. Indeed, the DNA polymerase active site is located at the interface between the DP1 and DP2 subunits, and the interaction between both subunits is essential for the full activity of PolD. Recently, we have solved a cryo-EM structure of the PolD full-length DP1-DP2 complex. **Elucidating the specificities of the PolD active site at the molecular level is required in order to fully exploit the biotechnological potential of this unusual thermostable DNAP**.

Experimental Report for our experiment on CM01 (11th to 14th December 2018) :

1) Grids:

Four grids were loaded on the Titan (with different protein concentration and salt concentration). These grids were tested on our in house F20 microscope. All four grids showed a homogeneous distribution of our particles, a good signal to noise ratio and no ice contamination. We decided to collect on the grid prepared with the lowest concentration of salt as a high concentration of salt may dissociate the PolD:DNA complex.

2) Data collection:

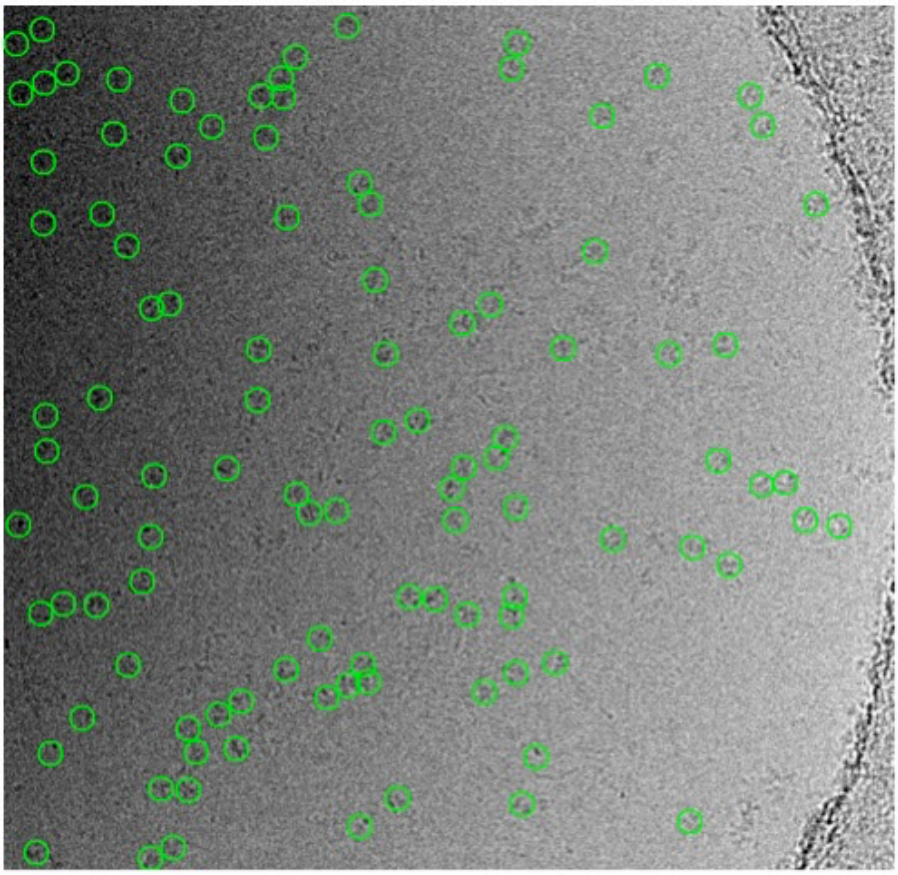
The following data collection parameters were selected: 105000 x, Spotsize 7, 15e-/pixel/second, 54e- total dose/40frames per movie/ defocus range (-1 to -4 um), energy filter 20 eV, beam width 1.2 um, 1.35 e-/A2/frame, 1.36 pixel size.

2100 frames were collected (2074 after removing bad frames).

3) Data processing: particle picking:

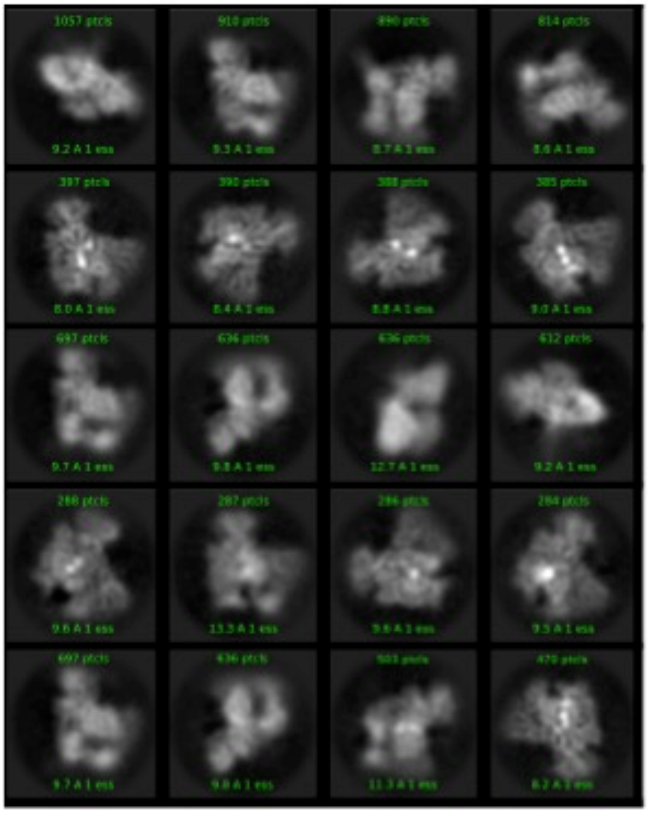
Particles were picked using Relion 2 and reviewed manually. About 100000 particles were selected for 2D classification. Several softwares were used for 2D classification but best results were obtained using Cryosparc.

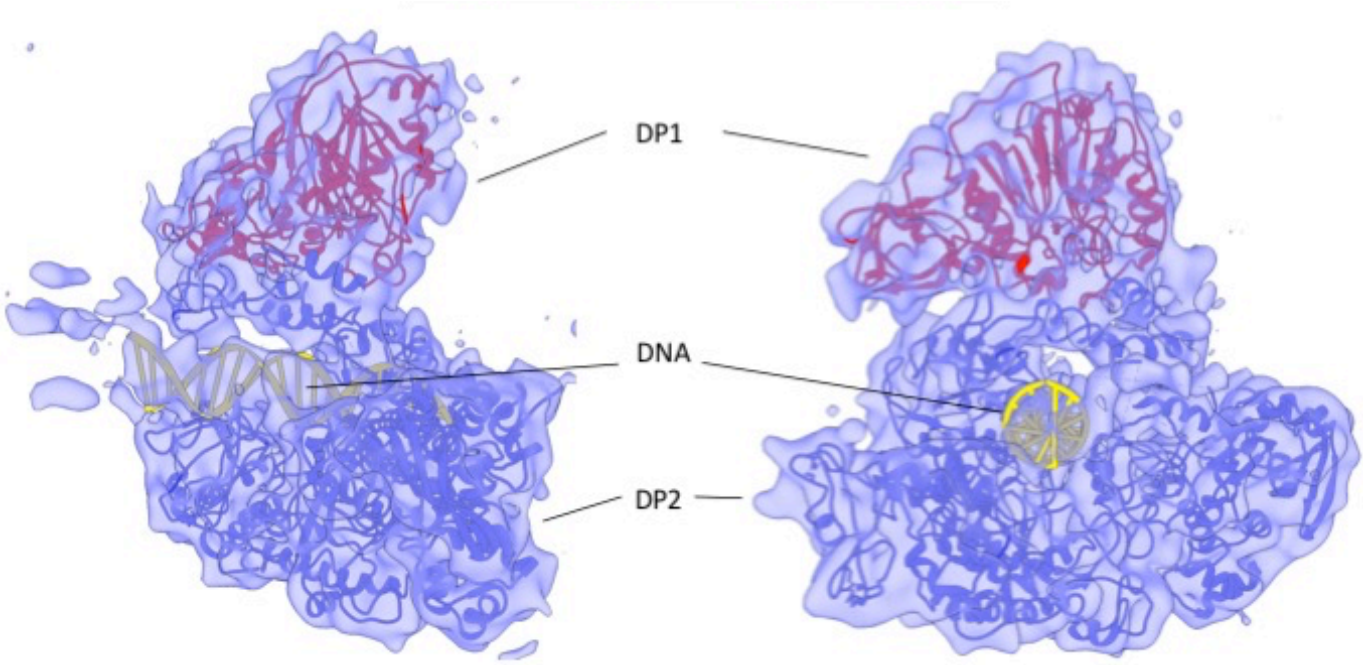
A representative frame of the dataset collected at ESRF is shown below (defocus -3,1um):



4) Data processing: model reconstruction:

2D and 3D classifications, and model refinement were performed using Cryosparc. This is still an ongoing work but a preliminary model has been reconstructed at 7 A resolution and shows density accounting for the presence of DNA in the active site. (See 2D classes & a preliminary 3D reconstruction below)





5) Ongoing work (using the current dataset):

We are working on improving the resolution of our model (currently 7A). In particular, we have observed that 2D classes calculated from particles extracted from movies obtained using a total dose of 54 electrons show less details than 2D classes calculated from particles obtained with a total dose of 25 electrons. Different programs are tested for model reconstruction.

6) Ongoing work (for future data collection):

We plan to prepare new grids using PolD bound to damaged DNA substrate in order to trap the polymerase in its proof-reading mode. In addition, we have reconstituted the ternary complex between PolD, DNA and the Proliferating Cellular Nuclear Antigen, PCNA, the main partner of PolD in the replication fork. We aim to prepare grids of the PolD:DNA:PCNA ternary complex.

Conclusion:

The beamtime on ESRF allowed us to obtain the first structure of the entire DP1:DP2 PolD complex bound with DNA. This result provides a comprehensive description of the substrate-bound PolD active site, allowing a detailed comparison with other DNA and RNA polymerases. This study will clarify whether PolD that constitutes a third class of DNA polymerases (two-barrel catalytic core), has evolved specific mechanisms of polymerization, DNA-binding, nucleotide selection, or did follow a converging evolution with the other two classes of DNA polymerases (Klenow-like and Polβ-like catal