

EPN BAG report
Infimp and TET protease samples
18-20 November 2022

Sample infimp – session 18-20/11/22 and 10-12/05/23 (24h each session)

Aim of the project: characterization of the molecular interactions responsible for the influenza RNA dependent RNA polymerase (RdRp) subcomplex PA-PB1 nuclear import. RanBP5 is the karyopherin involved in PA-PB1 nuclear import, while the last RdRp subunit is imported via a different pathway. We have purified the stable RanBP5-PA-PB1 complex and collected cryo-EM data on the complex (24h, [session 18-20/11/22](#)), allowing the 3D reconstruction of RanBP5 to 3.8 Å. Unfortunately, we could not get a high-resolution structure of the full RanBP5-PA-PB1 complex, even though we saw a density for PA-PB1, it is rather undefined. To improve the data quality, we are exploring strategies to rigidify the complex and allow better positioning of PA-PB1 in regards to RanBP5. One strategy consists of adding a small ligand interacting with PA-PB1 to stabilize them. Initial cryoEM data collected on a Glacios microscope confirmed the presence of each partner and gave promising 2D classes, we therefore collected data on CM01 with this new sample (24h, [session 10-12/05/23](#)). Image analysis validated that the complex is formed with all the partners involved, however we could not achieve a resolution that allows the positioning of PA and PB1 without doubts in the EM map. Altogether, we need to further optimize our sample to solve that intriguing biological question.

Sample TET protease– session 18-20/11/22

The grids were previously screened on a Glacios microscope equipped with a 200 kV FEG, an autoloader and a K2-summit direct electron detector. A small dataset was then collected on one grid (~2,500 images) and processed with RELION, leading to a 3.1 Å structure of the TET protease complex. A similar grid from the same freezing session with the same protein batch was selected for the high-end data collection on CM01 Titan Krios with Quantum-K3 camera (plus the grid already collected on the Glacios as a back-up). The automated data collection was set up on the first grid with EPU: 11 suitable squares were selected, an acquisition pattern with 3 shots per hole was set up with AFIS activated to minimise stage movement and speed up the data collection. The EPU run went through the night without any issues and 13,492 movies could be collected over the 24 hours allocated slot. After transfer of the data on a HPC cluster, processing of the data with RELION was performed: ~2.5 millions particles were initially extracted (~185 particles per micrographs) and submitted to several rounds of 2D and 3D classification, allowing the selection of a subset of 243,691 particles of high homogeneity and high-resolution. After further CTF refinement and particle polishing rounds, a 2.1 Å 3D map was obtained, allowing to build unambiguously an atomic model of the complex. These results are part of a bigger study and will be incorporated in a publication in the coming months.