

EPN BAG report

10-12 May 2023

This session was divided into 2 sessions (3 shifts each)

Allison Ballandras infimp (10-12/05/23) 1 day

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.

Jan Kadlec PRC2-ULT1 (10-12/05/23) 1 day

This project aims to determine the cryo-EM structure of an important epigenetic regulatory complex in plants. We found that this regulator can further interact with a transcription factor that antagonizes its catalytic activity on chromatin. The sample thus consists of three subunits of the complex and the extra transcription factor. The sample is cross-linked with BS3 to prevent its disassembly. We previously collected datasets at the IBS Glacios that revealed very clear 2D classes. The preliminary 3D reconstruction did not reach resolution sufficient for model building and it was not clear whether the transcription factor is present. After data collection on CM01, we obtained very nice maps covering the epigenetic complex, that allow easy model building (based on the Alphafold model). This is the first time this complex is solved in plants. However, for the moment we were not able to identify density corresponding to the transcription factor that might be linked to the epigenetics complex in a flexible way.