EPN cryo-EM BAG report 2020 EMBL - Kowalinski group

Adenosine deaminase acting on transfer RNA (ADAT) is an essential eukaryotic enzyme that catalyzes the deamination of adenosine to inosine at the first position of tRNA anticodons. The enzyme consists of a heterodimer with two deaminase domains, one of them lacking the catalytic residues. While the crystal structure of the catalytic subunit ADAT2 has been solved, this homodimer is inactive. The role of the pseudo-deaminase subunit ADAT3 and its additional N-terminal domain is not clear. To address this question, we would like to solve the structure of the Trypanosoma brucei ADAT2-ADAT3 dimer bound to a tRNA substrate.

During our allocated time at ESRF we were able to collect 9399 exposures. Preprocess, particle picking and extraction were performed by Warp. Extracted particles were 2D classified (Figure 1 A) and filtered, and 3D models were built and refined in Cryosparc, to a resolution of 4.35 A (Figure 1 B). Although our 2D classes look crisp and show secondary structure features, during the refinement we notice a strong preferred orientation of our particles, represented by the peak in the distribution map (Figure 1 C). CryoEF estimated the distribution efficiency of our data to 0.63, that, combined with a poor Fourier space coverage also reported by the program, indicates that our model quality would be limited. The current map fits well with the expected size of the complex, although the resolution does not yet allow building a model based on the homology models available. Currently we are investigating procedures to eliminated orientation bias and envisage an additional Krios data collection with the improved sample.

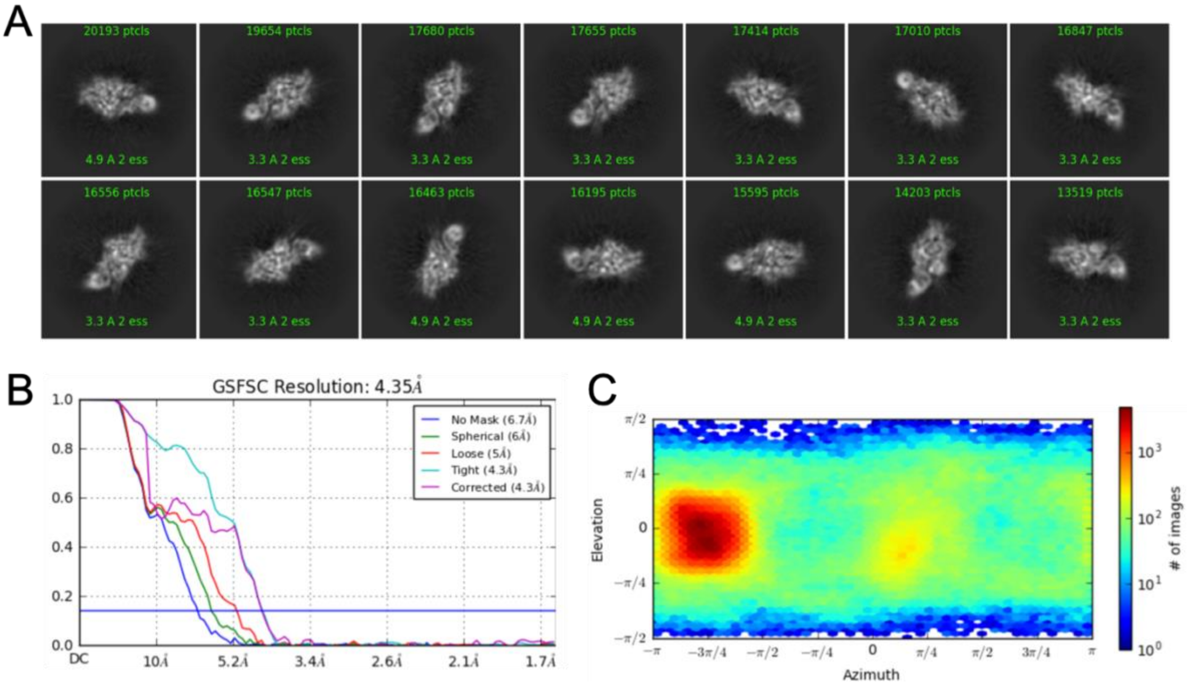

***Figure 1:* Adat23 tRNA complex Krios data process. (A)** Representative 2D classes calculated by Cryosparc, from the 1.072.282 particles picked by Warp, where we can see well defined classes with features. **(B)** The Fourier shell correlation graphic calculated by Cryosparc during the 3D homogeneous refinement, indicating a resolution of 4.35A **(C)** Angular distribution of the particles orientation during the refinement, where we can notice a preferred orientation of our particles in the grid, as well as an absence of top views (particles oriented with 90° or -90° of elevation).