EPN BAG report
RESC sample
10-12 October 2022

In trypanosomatida, the majority of mitochondrial messenger RNAs is edited for maturation. In this process, the RNA editing substrate binding complex (RESC) forms the plaform for the RNA-guided RNA editing. RNA editing is the insertion and removal of uridines in the pre-mRNA based on guide RNA templates. The heterodimer formed by proteins RESC1 and RESC2 forms the core of RESC. We solved an apo-cryo-EM structure of RESC1-RESC2 heterodimer at 3.4 Å with data collected at the Titan Krios at EMBL Heidelberg. We collected data at the ESRF CM01 Titan Kros to the RESC1-RESC2 heterodimer bound to gRNA. During our allocated time at ESRF we were able to collect 4420 exposures with a 30-degree stage tilt. The data was process combining RELION, WARP and CryoSPARC software suites. The resolution of the reconstruction extended to 4.7 Å resolution (Figure 1A). Despite lower resolution than the apo reconstruction, the map showed density inside the RNA binding site of RESC2 (Figure 1B) and a subtraction map allowed to place the 5'-GTPof the gRNA bound to RESC2 (figure 1C).

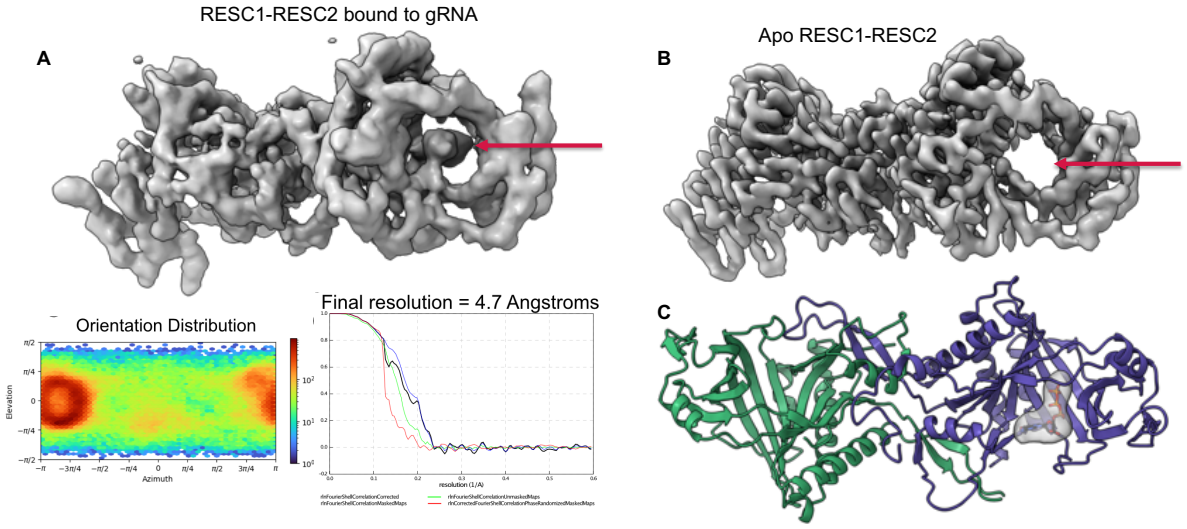

Figure 1: **Structure of RESC1-RESC2 heterodimer bound to gRNA collected at CM01. (A)** Final Cryo-EM reconstruction of RESC1-RESC2 heterodimer bound to gRNA**,** with the orientation distribution and the final FSC. The red arrow indicates the extra density, which is absent in the apo map. **(B)** Previous Cryo-EM reconstruction of RESC1-RESC2 heterodimer without gRNA, with the red arrow indicating the empty barrel. **(C)** The final model of RESC1-RESC2 heterodimer bound to gRNA, with the 5'-triphosphate of the gRNA bound in the interior of RESC2's barrel. The density used to build the gRNA is the result of a subtraction between bound and apo maps.

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Model deposition: EM maps are available at EMDB: EMD-16592 (apo) and EMD-16593 (gRNA bound) and the apo model is deposited at PDB: 8CDP.