

Report for the second microscope time of mx2001@CM01

During the winter of 2018, we took advantage of the second session of MX2001 to image the 5-HT3 receptor in the presence of serotonin (its natural ligand) and calcium.

Four grids were mounted and screened, and we collected on the best one. It was not as good-looking as the grid used for collection during the first session : there were fewer particles in each image. The reproducibility of grid preparation is still a bottleneck in our EM pipeline, as we don't have easy access to a screening microscope where we could unmount the grids and save them for later data collection on the ESRF Krios. One microscope like this will be installed on campus, and the closest one under operation is in Milano. For future data collection, we'll try to come with pre-screened grids to gain collection time! We used 9 second exposures and 45 frames, for a total dose of about dose of $\sim 50\text{e}^-/\text{\AA}^2$. 3 images per hole were taken, enabling a data collection of more than 3000 images during the 9 shifts. The defocus ranged from -0.8 to -2.5 μm . The microscope operation was very smooth.

We collected 3084 image stacks of good quality. A dataset of good quality (3.4-3.8 \AA range at the moment of writing this report) can be extracted from these images, and features two conformations of the 5-HT3 receptor. The two conformations can be separated in silico using focused classifications and tight masks. We have not yet published the structures derived from this dataset, which provide insight on the pre-active versus active equilibrium at a better resolution than previously ($>4 \text{\AA}$). Actually we are still treating the data, which will be an important part of a publication in preparation.

The data collected during the *first* microscope time (Nov 2017) has been included in a landmark publication for our team, and if I'm not wrong one of the first publications using data collected on CM01.

Polovinkin, Hassaine, Perot, Neumann, Jensen, Lefebvre, Corringer, Neyton, Chipot, Dehez, Schoehn & Nury. (2018) Conformational transitions of the serotonin 5-HT3 receptor. *Nature* 563:275-279