Report for MX-2123 (9 shifts on CM01)

ESRF CM01 Krios 22nd Oct 2018
Grid number SLC12A6B-u353
apix 1.067
dose 37.8
frames 40
defocus -1.4 to -2.8 (0.2 steps)
2 days 30' tilt, 1 day flat.
should be i010
images: 3155

Data processing was carried out in Relion 2 and cisTEM.

Prior to MX-2123, datasets had been obtained at eBIC (Diamond Light Source, Didcot, UK) without stage tilt, where the maximum resolution obtained was 6.4 Å. At the time it was believed that resolution was limited due to extreme orientation bias (**Figure 1**).

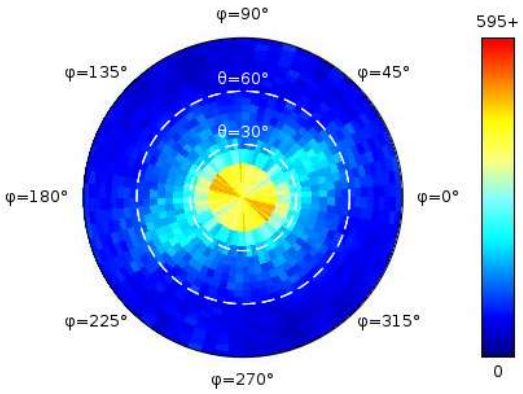

Figure 1. Angular distribution of previously obtained dataset, at eBIC, limited to 6.4 Å, with C2 symmetry applied. Data processing for 3D refinement was performed in cisTEM.

Recent publications have suggested that by tilting the grid on the stage, new orientations can be acquired and therefore alleviating the orientation bias[1]. As such, a tilted dataset was attempted during the MX-2123 visit.

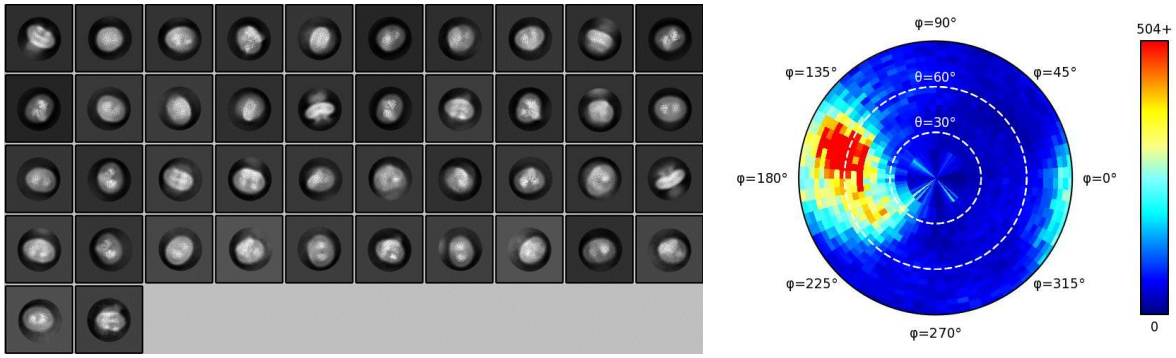

Figure 2. Left - 2D classification performed in Relion 2 with 473 355 selected particles, acquired from the MX-2123 dataset. **Right** – Angular distribution of the MX-2123 dataset, obtained from 3D refinement with cisTEM.

Whilst the tilted dataset at MX-2123 generated a wider spread of orientations (**Figure 2. Right**), the resulting resolution was still 6.4 Å. One of the potential factors limiting higher resolution is the inherent flexibility of the sample, especially with the transmembrane domain isolated in detergent.