

EPN BAG report
CarH sample
2-4 June 2023

The grids were previously screened on a Glacios microscope equipped with a 200 kV FEG, an autoloader and a K2-summit direct electron detector. A small dataset was then collected on one grid (~1,000 images) and processed with RELION, leading to a 3.9 Å structure of the CarH complex. A similar grid from the same freezing session with the same protein batch was selected for the high-end data collection on CM01 Titan Krios with Quantum-K3 camera (plus the grid already collected on the Glacios as a back-up). The automated data collection was set up on the first grid with EPU: suitable squares were selected, an acquisition pattern with 3 shots per hole was set up with AFIS activated to minimise stage movement and speed up the data collection. The EPU run went very smoothly without any issues and 39,932 movies could be collected over the 48 hours allocated slot. After transfer of the data on a HPC cluster, processing of the data with RELION was performed: ~7.5 millions particles were initially extracted (~190 particles per micrographs) and submitted to several rounds of 2D and 3D classification, allowing the selection of a very small subset of 98,311 particles of high homogeneity with high-resolution features. After further CTF refinement and particle polishing rounds, a 2.4 Å 3D map was obtained, allowing to build unambiguously an atomic model of the complex. These results are part of a wider study and will be incorporated in a publication in the coming months.