The MX2260 cryo-EM data was collected on CM01 on a sample of Thogoto virus polymerase in complex with the vRNA promoter in vitrified ice on Quantifoil 2/1 300 Au grids. We used a nominal magnification of 165 000 x, an equivalent to 0.827 Å/pix, and this allowed us to use a small beam size and to obtain five exposures per one hole position. The fluence of an exposure was ~50e/Å^2 in 50 frames. Altogether, we obtained ~5300 movies with a defocus range of -1 to -2.6 µm. The overall low number of movies was due to technical issues on the CM01 beamline.

The cryo-EM movies were aligned and dose weighted by MotionCor2 package (Figure 1A). A total number of ~1,655,000 individual single particle images were picked by the WARP package and sorted by 2D classification in RELION (Figure 1B). Two main 3D classes of the target RNA-Thogoto viral polymerase complex (Figure 2A) were identified and refined to ~3 Å resolution at the 0.143 gold standard Fourier Shell Correlation (FSC) cut off (Figure 2B). The maps allow the first atomic model of Thogoto polymerase to be constructed, although certain domains are missing.

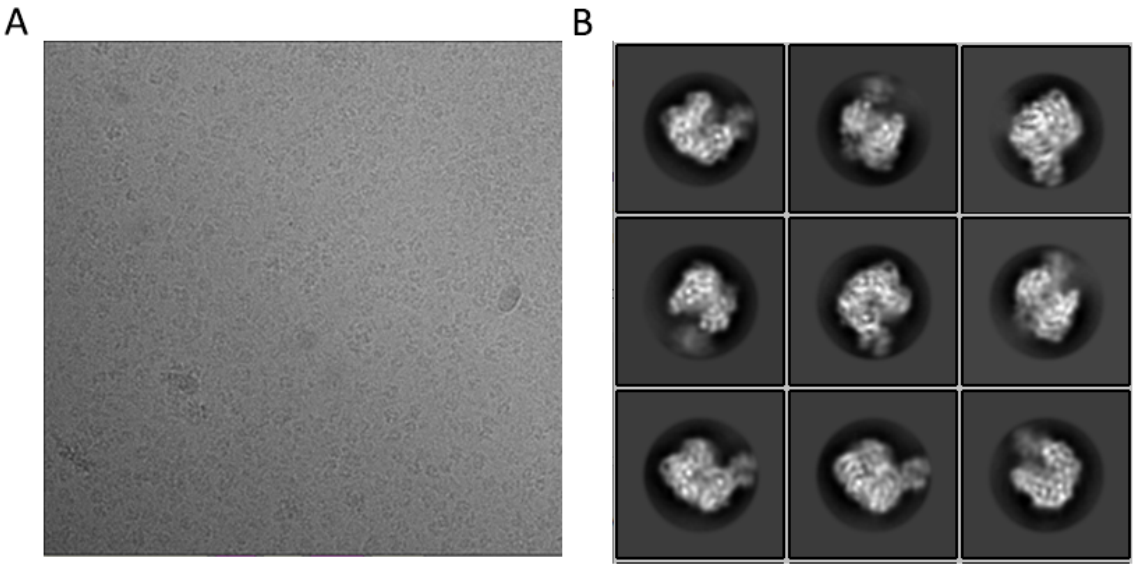

Figure 1: A) Example of aligned micrograph at ~-2.6 µm defocus. B**)** 2D class averages of different views of the Thogoto-vRNA polymerase complex showing secondary structures.

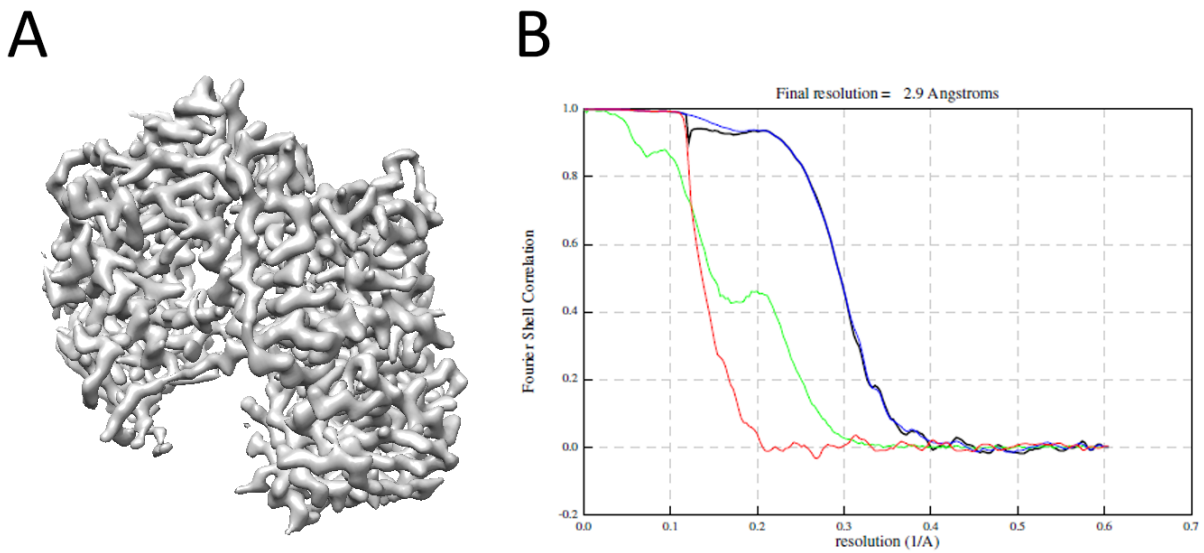

Figure 2: A) Visualization of one of the identified class 3D refinement. **B)** FSC curve for the observed 3D reconstructions.