

**EPN BAG report**

**Full-length Hantaan virus polymerase bound to 5' viral RNA**

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Hantaan virus is a dangerous human pathogen whose segmented negative-stranded RNA genome is replicated and transcribed by a virally-encoded multi-functional polymerase. We recently observed that, in the absence of viral RNA, Hantaan polymerase oligomerize in different multimers. It was thus interesting to analyze if these multimers were existing also in presence of viral RNA. The data collection performed on the ESRF Krios CM01 was revealed that binding to viral RNA disruption of the symmetric multimers. The 26.745 movies collected on the Gatan K3 at 105.000 x magnification, followed by advanced cryo-EM image processing using the movies realigned in scipion at ESRF, allowed to derive two high-resolution 2.6 Å cryo-EM maps. These maps and the atomic models built inside the maps reveal with great precision the conformational changes induced by viral RNA-binding. The 2 maps differ in the positioning of the canonical motifs that compose the active site, notably the motif E that switches from an alpha-helix to a beta-strand. These movements are likely to be related to the polymerase activity, notably the replication initiation. These results are greatly advancing our understanding on Hantaan virus polymerase structure and activity will be key to define antiviral compounds to counteract these life-threatening viruses.

We would like to really thank all the people who are maintaining and operating the ESRF CM01 Krios, in particular Romain Linares who was our local contact for this shift.

**We are in the process of writing an article relating these results and thus request this summary to remain confidential.**