Summary of the results obtained on Hantaan virus polymerase structure based on the data collected the 26-28/09/20 on MX-2260

The *Bunyavirales* is a very large and diverse order of segmented negative stranded RNA viruses comprising more than 500 species classified in ten families[1]. Spread worldwide, arthropods and rodents are their natural reservoirs and man is occasionally infected resulting in severe diseases including meningitis, encephalitis and hemorrhagic fevers. Hantaan virus, the subject of this study, is causing haemorrhagic fever with renal syndrome and has a mortality rate of 15% in human. As for other bunyaviruses, no licensed drug is available to counteract its infection and it is thus crucial to analyze essential steps of its viral cycle to understand its mechanism of function and identify anti-viral targets. In this context, we are focusing our interest on an essential viral protein: the RNA dependent RNA polymerase. This enzyme performs RNA replication, that gives rise to full-length copies of the genome, and transcription, that produces translation competent viral mRNAs. Transcription initiation is performed by a "cap-snatching" mechanism, whereby short 5' capped RNA segments are bound and cleaved by the viral polymerase and then used to prime synthesis of mRNA[2]. If the biological function of hantaan virus polymerase are known, a detailed understanding of the mechanism underlying Hantaan virus polymerase activities were impeded by the lack of structure. It was therefore crucial to determine this structure by cryo-EM to shed light on ignored aspects of bunyavirus viral cycle.

Before the data collection on the Krios, we optimized expression, purification and cryo-EM grid preparation with great care. If data collection and image processing of 1160 micrographs collected on the IBS Glacios equipped with a Falcon II had enabled to obtain 2D class averages displaying clear secondary structures on the core region, it had also revealed the presence of more flexible peripherical regions that were impeding high-resolution 3D structure determination. Data collection of 7262 micrographs on the high-end ESRF CM01 Krios the 26-28/09/20, on the session MX-2260, was thus essential for the project. It enabled to determine the structure of the Hantaan virus polymerase core at 3.5 Å resolution. In addition, it revealed the position and the binding mode of the 5' extremity of the viral RNA. This information is essential for the next steps of the project. Indeed, understanding the binding mode of the 5' and 3' promoters are pre-requisite to stabilize the Hantaan virus polymerase in stalled functional states, as recently observed for Influenza and La Crosse virus polymerases[3–5].

In a nutshell, the Krios session MX-2260 was a key step in the project, both in an academic point of view to understand the behavior of this enzyme that is essential to the viral cycle, but also to permit in the future drug-design targeting the viral polymerase of this life-threatening virus.

FIGURES

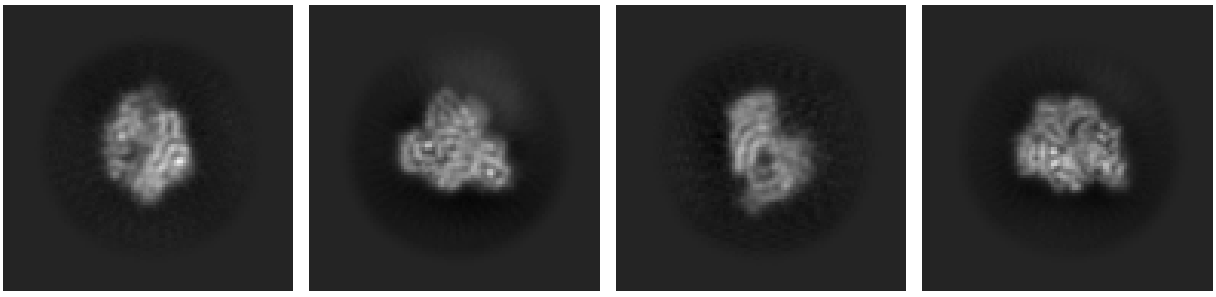

Figure 1: 2D class averages from the data collected on the CM01 ESRF Titan Krios

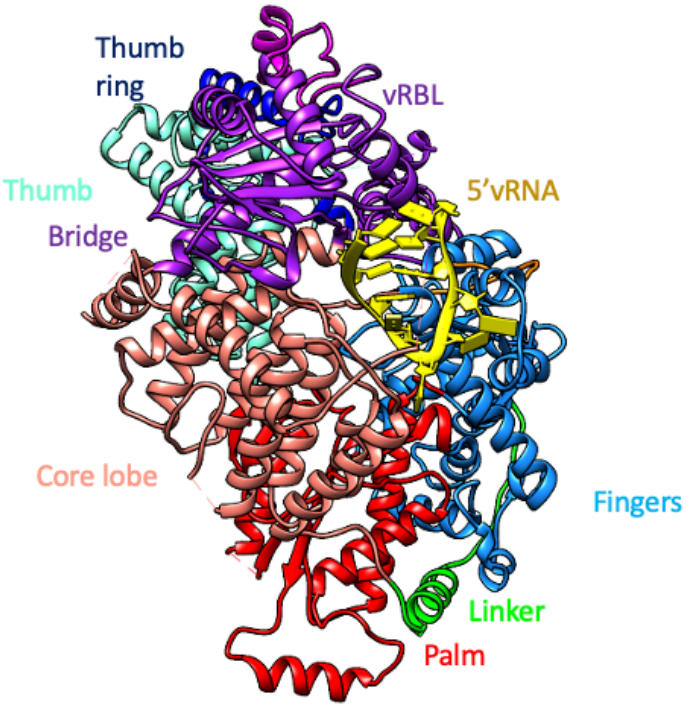

Figure 2: Hantaan virus polymerase core structure in complex with the 5'vRNA

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