ESRF	**Experiment title:** How do viruses package their genome? A time-resolved study.	**Experiment number**: SC-4395
Beamline: ID02	**Date of experiment**: from: 21/10/2016 to: 24/10/2016	**Date of report**: 09/02/2017
Shifts: 9	**Local contact(s)**: Johannes MOELLER	*Received at ESRF:*

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Report:

Objective & expected results. The objective of the proposed experiment was to elucidate the dynamic pathways of genome packaging into viral capsids. The mechanisms by which viral proteins capture the right segments of nucleic acids remain unknown to date. Time-resolved small-angle X-ray scattering (TR-SAXS) is a technique of choice that can probe spatio-temporal processes with subsecond and nanometer resolutions for soft matter and biological systems. Previous experiments with the same viral system were carried out (SC-3852) but we needed to supplement the data with measurements in different ionic conditions. We thus expected to draw a clear picture as to how viral proteins bind to RNA and make up a closed shell.

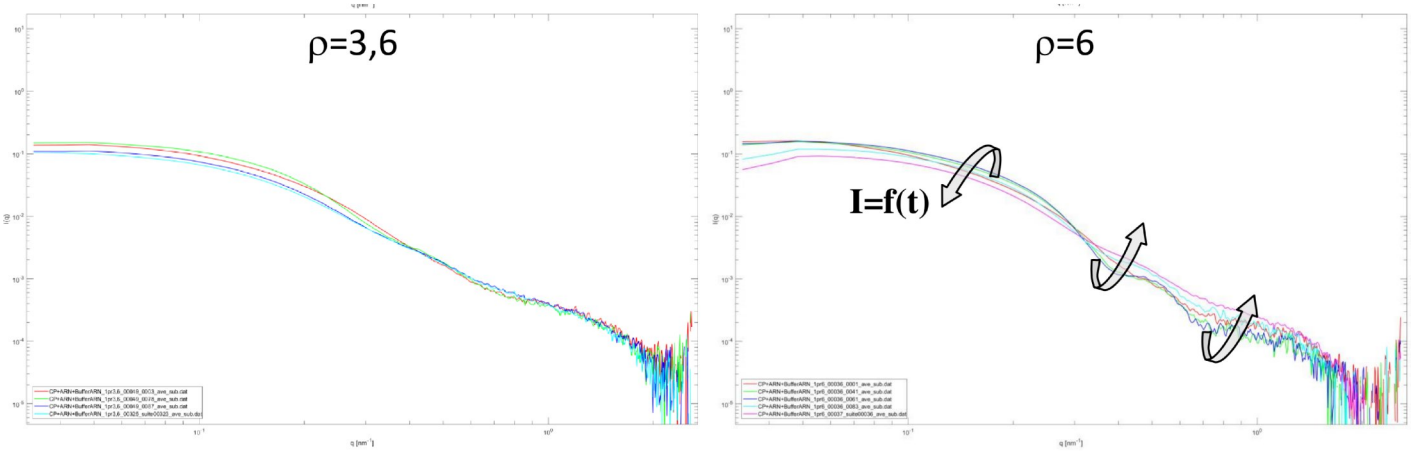

Figure 1 Association kinetics of CCMV dimers with RNA at two different protein-to-RNA mass ratios ρ. The buffer solution was maintained at pH 7.5 with an ionic strength of 0.1 M. Data were collected over an hour and only selected intensity curves are shown for the sake of clarity.

Results and conclusions. The experiments were carried out with proteins and RNA purified from a plant virus, the cowpea chlorotic mottle virus (CCMV). Proteins are stable in the form of dimers in buffer solution maintained at pH 7.5 and with an ionic strength I of 0.5 M. When dimers are mixed with RNA (pH 7.5 and I = 0.5 M), both components remain free in solution because the ionic strength is high enough to screen the electrostatic attraction between the cationic arms of dimers and the negatively charged backbone of RNA. By contrast, when I is lowered to 0.1 M, dimers bind to RNA and form amorphous aggregates. The first series of

measurements was dedicated to probing this association process by mixing a concentrated solution of dimers at I= 0.5 M with a RNA solution in such a way that the final ionic strength was 0.1 M. Figure 1 shows the scattering intensities collected for two different protein-to-RNA mass ratios ρ. Notice that in the native virion, $\rho = 4$. While we expected the dimers to bind rapidly to RNA and the process to end up within the first seconds, we observed that indeed the dimers bind as early as the first milliseconds, but also that the objects slowly anneal and release the excess of dimers. This pathway was unexpected and demonstrates that the binding energy between dimers and RNA is moderate, a few times the thermal energy at most. The transient objects exhibit a certain degree of order as suggested by the presence of oscillations on the early scattering patterns for $\rho = 6$. Afterwards, it seems that the objects lose their order while releasing gradually the excess of dimers.

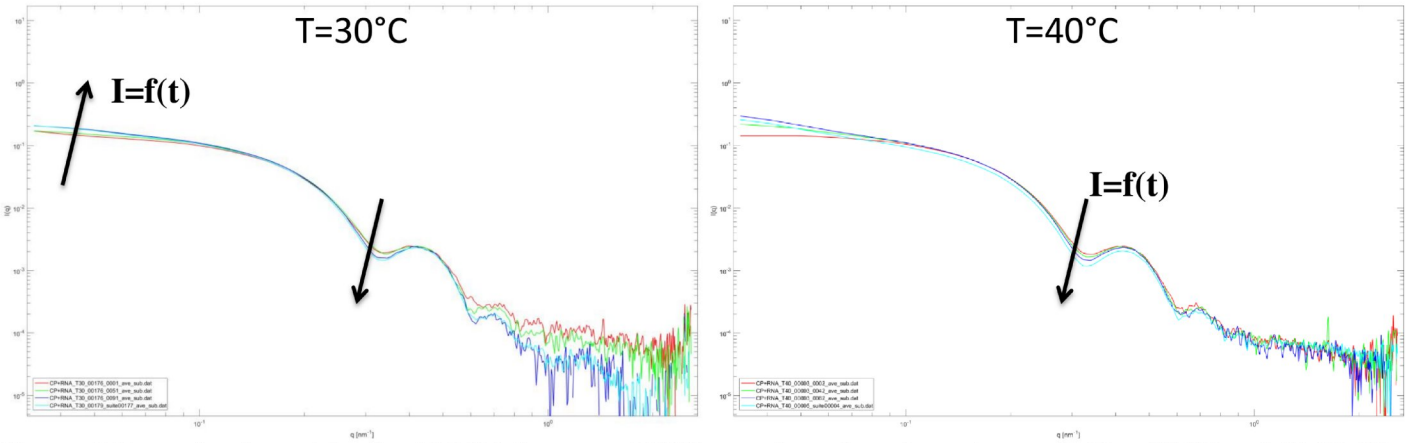

Figure 2 Scattering intensities for CCMV dimers and RNA premix undergoing a jump of pH at 30 °C and 40 °C. The final pH was around 5.2 and the ionic strength was maintained at 0.1 M.

In the second set of measurements, dimers and RNA were premixed at $\rho = 6$ in a buffer solution at pH 7.5 and I= 0.1M. The premix was rapidly mixed with a buffer solution at pH 4.8 and with the same ionic strength. Upon a decrease of pH, viral proteins capture free protons and their surface charge vanishes. As a result, the hydrophobic protein-protein attraction overcomes the electrostatic repulsion and proteins self-assemble to form a closed shell while remaining bound to RNA. Figure 2 depicts the corresponding scattering intensities at 30 °C and 40 °C. We can see that the oscillations are gradually more pronounced, which is characteristic of objects acquiring a spherical symmetry. In other words, the initial amorphous aggregates of protein-bound RNA anneal into icosahedral virions. The process is slow, it takes several hours, and we have not been able to collect the whole kinetics. By increasing the temperature, the process is slightly faster, which suggests that it can be accounted for by the classical theory of energy barrier crossing.

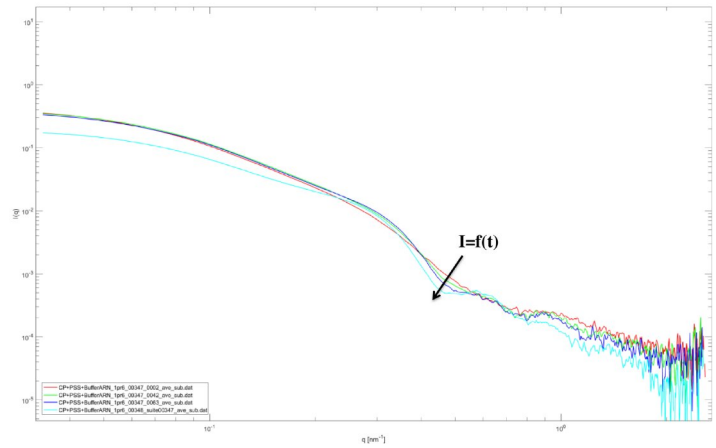

Figure 3 Scattering intensities for CCMV dimers mixed with 650-kDa PSS at pH 7.5 and I= 0.1 M.

In the last experiments, we mixed CCMV dimers with 650-kDa poly(styrene sulfonic acid) (PSS), a negatively charged and flexible homopolymer (Figure 3). The final objects are smaller and more globular than the aggregates resulting from the association of dimers and RNA in the same conditions. This is due to the hydrophobic nature of PSS backbone that promotes the collapse of the chains and allows the shell to close up more easily than in the case of RNA.

Comments on the use of beam time. The experiments were successful in overall and most of the data promise original information on the genome packaging process. Because the kinetics were multiscale, starting from a few milliseconds up to a few hours, we needed a stopped-flow device and enough shifts to perform the necessary calibrations as well as to probe the long time scales to reach the steady state. We shall acknowledge our local contact for allowing us to work in smooth conditions.