Standard Project

Experimental Report

Proposal title: Kinetics of RNA packaging into virus-like particle		**Proposal number:** SC-3852
Beamline: ID02	**Date(s) of experiment:** from: 25/09/2014 to: 28/09/2014	**Date of report:** 24/02/2015
Shifts: 9	**Local contact(s):** Sylvain Prévost	*Date of submission:* *10/2013*

Objective & expected results:
The proposed experiments aimed at elucidating the packaging of RNA into a viral capsid. The mechanisms by which the viral proteins selectively capture the 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 the spatiotemporal processes with subsecond and nanometer resolutions for soft matter systems. We expected to unveil the kinetic differences between the packaging of RNA and that of synthetic linear polyelectrolytes previously investigated. Another objective was to study the disassembly of empty capsids for comparison with the assembly kinetics that we performed before.

Results and the conclusions of the study:
We carried out our experiments with the capsid proteins of a plant virus, the cowpea chlorotic mottle virus (CCMV), that spontaneously form a T=3 shell of ~28 nm in diameter upon mixing with purified viral RNA. Dimers initially stable in a 0.5 M buffer at pH 7.5 were rapidly mixed to viral RNA in the same buffer with a stopped-flow apparatus, then injected in a flow-through capillary. Exposure time was 5 ms and dead time between frames was geometrically increased from 33 ms to a few minutes. The kinetics turned out to be multiscale with a rapid event occurring over the first milliseconds and a relaxation lasting up to 15 minutes (see Figure **a**). The analysis is still ongoing to devise a comprehensive model encompassing the full process. However, we can conjecture that dimers bind rapidly to RNA upon a diffusion-limited process. This step takes place in a few tens of seconds at most. Afterwards, the amorphous aggregates relax into globular objects while still capturing a few dimers to complete their shell. This second step may last typically 15 minutes to be completed. The final pattern was not that of a regular icosahedral virus with marked oscillations in the 10^{-1}-nm^{-1} range of wavenumbers. We believe that a number of RNA chains were not packaged in the process and thereby contributed to the final scattering intensity. Figure **a** shows a kinetics with a protein concentration of 2.08 g/L. When the protein concentration was lowered down to 0.5 g/L, we clearly observed less globular objects at the end and the forward scattering intensity increased only by 2. The binding of dimers to RNA was also much slower and we could not easily identify two steps like before.

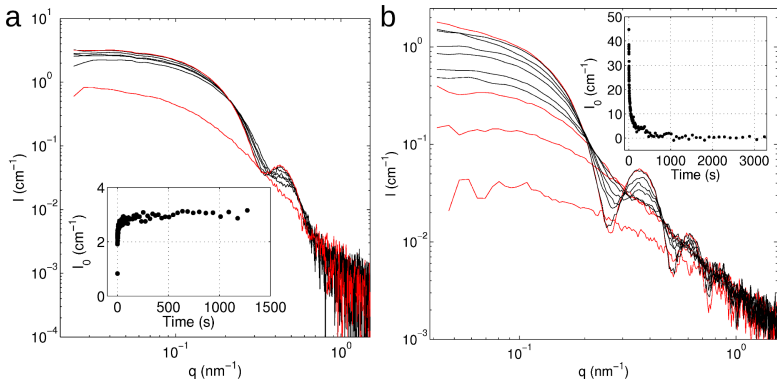

Figure. *TR-SAXS patterns for the assembly and disassembly of virus-like particles.* ***(a)*** *Packaging of viral RNA into CCMV capsids. Dissociated dimers were rapidly mixed with viral RNA in the same buffer solution at pH 7.5. About 100 patterns were collected with an exposure time of 5 ms.* ***(b)*** *Disassembly of empty CCMV capsids. Capsids initially at pH 4.8 were rapidly mixed with a buffer solution raising the pH to 7.5. As a result, the capsids disassembled through distinct steps into fully dissociated dimers. Similarly, more than 100 patterns were collected with an exposure time of 5 ms. The two insets show the time evolution of the estimated forward scattering intensity.*

The second series of experiments dealt with the disassembly of empty CCMV capsids. Upon a jump of pH from 4.8 to 7.5, icosahedral capsids disassembled into dimers. We carried out the experiments at 20 °C with a high protein concentration (2.9 g/L). The conditions of data collection were similar to those used for RNA packaging. Remarkably, we distinguished three steps in the disassembly process (Figure **b**): (i) capsids broke into 2 or 3 large pieces in less than a minute; (ii) these large pieces themselves were cut into two smaller intermediates that resembled a major intermediate identified previously by us with another viral system. This intermediate consisted of two pentamers of A-B dimers connected by a C-C dimer. It is amazing that such a structure is recovered in the disassembly of a yet different viral system. We hypothesize that it must be conserved in all T=3 capsids and our future experiments will aim at verifying this assumption. Over the last 20 minutes, the small intermediates were fully dissociated and we checked that the final pattern of the kinetics corresponded well to the form factor of a dimer.

Justification and comments about the use of beam time:
The experiments were successful and the vast majority of the runs yielded good results. Since the kinetics were multiscale, starting from a few milliseconds up to one or two hours, we both needed a stopped-flow apparatus and enough shifts to perform all the necessary calibrations, to explore long durations to reach the steady state, and to test enough sample conditions to draw a clear picture of the self-assembly processes. We shall here acknowledge our local contact for allowing us to work in very smooth conditions.