Standard Project

Experimental Report

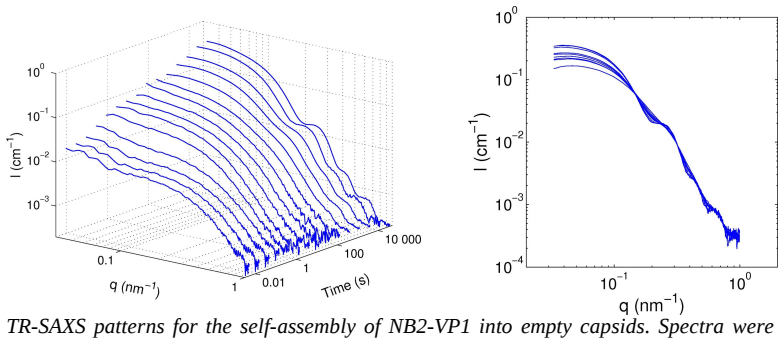
Proposal title: Kinetic pathways of self-assembling viral capsids encapsulating synthetic polyelectrolytes		**Proposal number:** SC-3267
Beamline: ID02	**Date(s) of experiment:** from: 04/11/2011 to: 07/11/2011	**Date of report:** 22/03/2012
Shifts: 9	**Local contact(s):** Jérémie Gummel	*Date of submission:* *04/2011*

Objective & expected results:

The proposed experiments aimed at elucidating the self-assembly of pseudo-viral particles made up of a spherical viral capsid enclosing or not a polyelectrolyte. Time-resolved small-angle X-ray scattering (TR-SAXS) is a technique of choice that can probe the spatio-temporal processes with subsecond and nanometer resolutions for soft matter systems. We expected to compare the mechanisms of self-assembly for empty capsids and capsids enclosing polyelectrolytes, and for the latter case, to determine whether the proteins bind first to the polyelectrolyte and relax towards a closed shell, or if the shell grows with the polyelectrolytes inside.

Results and the conclusions of the study:

We started our study with capsid proteins of a calicivirus (NB2-VP1) that spontaneously form a T = 3 shell of ~30 nm in diameter upon lowering the pH from 9.0 to 6.0. The proteins at pH 9.0 were rapidly mixed to a buffer with a stopped-flow apparatus and then injected in a flow-through capillary. Exposure time was 20 ms for the first minutes, and was subsequently increased to 50 ms. The kinetics turned out to be multiscale with events occurring within the first seconds and relaxation times lasting up to a few hours (see Figure). Spectra collected over long time scales exhibited isobestic points, which suggests a slow mass transfert between two populations of objects in solution. A singular value decomposition (SVD) analysis confirmed the existence of two major species and a global fitting procedure was successfully applied to extract the reaction rates and the basis spectra associated with the species in solution. The analysis is still ongoing to devise a comprehensive model encompassing both the short and long time scales. The initial protein concentration for the kinetics shown on the Figure was 1.59 mg/mL and static measurements at pH 9.0 showed that the proteins were associated in dimers (~114 kDa). When we used a smaller concentration (0.40 mg/mL), the self-assembly process stalled and hardly any capsids were formed after more than an hour, while a higher concentration (2.58 mg/mL) led to a fast process that almost terminated after two minutes. It is worth mentioning here that over short and long time scales the patterns were perfectly repeatable.



TR-SAXS patterns for the self-assembly of NB2-VP1 into empty capsids. Spectra were measured with 20 ms and 50 ms exposures after rapid mixing of the proteins with a buffer lowering the pH from 9.0 to 6.0. (left) Evolution from 4 ms to 8 hours. (right) Spectra collected between 522 s and 3 hours revealed isobestic points suggesting a two-state mass transfert mechanism.

The second series of experiments dealt with proteins purified from cowpea chlorotic mottle virus (CCMV). The proteins also self-assemble upon a jump of pH from 7.2 to 4.8 into T = 3 icosahedral shells. In the presence of a negatively-charged polyelectrolyte though, spherical shells spontaneously form at pH 7.2 and enclose the polyelectrolyte. We examined both processes by using poly(styrene sulfonic acid) (PSS) as the polyelectrolyte with various masses: 8 kDa, 130 kDa and 1.6 MDa. Without PSS, the self-assembly kinetics resembled that of NB2-VP1, namely, a multiscale process in which dimers gather into a reduced number of intermediate species before forming closed shells. Global fitting procedures on these data is still ongoing. In the presence of PSS, the kinetics is radically different. We mixed PSS and the proteins in a mass ratio of 1:9 for all the masses available, at a final pH of 7.2 and 4.8 for comparison with the case of empty capsids. Clearly, the proteins bound to PSS in a very short time scale, below the millisecond, because of the strong electrostatic interactions. At this stage, the assemblies were amorphous and very extended, probably like pearl chains. Their size shrank slowly over several tens of minutes while releasing bound proteins - I(q=0) was decreasing -, and the proteins eventually formed globular compact shells containing PSS. The shell polydispersity depended on the PSS mass and pH, but in many cases, oscillations were visible on the SAXS patterns.

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 several 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 the local contact for allowing us to work in very smooth conditions.