Experimental report

03/11/2019

Proposal: **8-03-970** **Council:** 10/2018

Title: Packaging of RNAs and synthetic polyelectrolytes in viral capsids

Research area: Physics

This proposal is a new proposal

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Samples: deuterated polystyrene sulfonate
Purified RNA from in vitro transcription
Capsid protein of the plant virus CCMV

Instrument	Requested days	Allocated days	From	To
D22	3	2	11/07/2019	13/07/2019

Abstract:

RNA viruses are ubiquitous pathogens that possess a relatively simple structure consisting of genomic RNAs packaged into a generally spherical protein shell called capsid. Despite a great deal of work on the structural and molecular biology of viruses the physicochemical mechanisms underlying the in vivo viral life cycles are still elusive. In particular, how viral RNAs (and not non-viral ones or other molecules) get selectively encapsidated is still a mystery. We are currently investigating these issues by studying the in vitro capsid formation in the presence of either RNAs or synthetic polyelectrolytes mimicking RNAs. By using complementary approaches (cryoEM, TR-SAXS, SANS) our end goal is to understand the physical principles of viral assembly. Here, we plan to study the packaging efficiency of a panel of different RNAs and poly(styrene sulfonic acid) polyelectrolytes mixed with capsid proteins of a plant virus, by precisely measuring the mass ratio between proteins and packaged RNAs or polyelectrolytes. The expected results will allow us to test if viral RNAs are more efficiently packaged than non-homologous RNAs and to compare between differently charged polyelectrolytes.

Final report of the experiment n° 8-03-970

Packaging of RNAs and synthetic polyelectrolytes in viral capsids

Motivation

Viruses usually selectively encapsidate their genomic material (either RNA or DNA) during part of their life cycle. For the model plant virus CCMV (Cowpea chlorotic mottle virus) however, there is no known packaging signal indicating a selective encapsidation of their RNAs. Our goal was to use the SANS technique as an original method to test the existence of a specificity of encapsidation. We investigated the encapsidation efficiency of different RNAs and polyelectrolytes in order to find a potential selectivity of the capsid protein (CP) of the CCMV virus. The particularity here was that samples were placed in physicochemical conditions where the structures formed were not well-structured capsids but amorphous objects called nucleoprotein complexes (NPCs). This metastable state shall allow us to probe fine encapsidation differences.

Study of the RNA encapsidation under NPCs conditions

First, the structures formed by RNAs (either endogenous or exogenous) mixed with CP under NPC conditions were studied (Fig. 1). The samples were placed in 67% D_2O Tris-HCl buffer (pD 7.5) in order to contrast match the scattering signal of the RNAs. So, only the signal of the CPs is measured. From the characteristic oscillation pattern, we can see that all the samples already contain nearly spherically symmetric objects. This was a surprise as we were expecting amorphous NPCs. This means that the NPC conditions were not obtained. Possibly, the presence of D_2O, which is known to spur protein structuration, is the reason. The well-structured objects were present for every concentration measured. We were also expecting an effect of the concentration.

We were also interested in learning more about the impact of the presence of divalent cations on the virions structure. We compared RNAs mixed with CPs in presence (or not) of Ca^{2+} ions (Fig. 2). Even though spherical capsids were already formed, we can see that the presence of Ca^{2+} induces a shift of the form factor that correspond to a shrinkage of the capsid. So, Ca^{2+} ions do have an effect on the CCMV capsid formation by bridging subunits together.

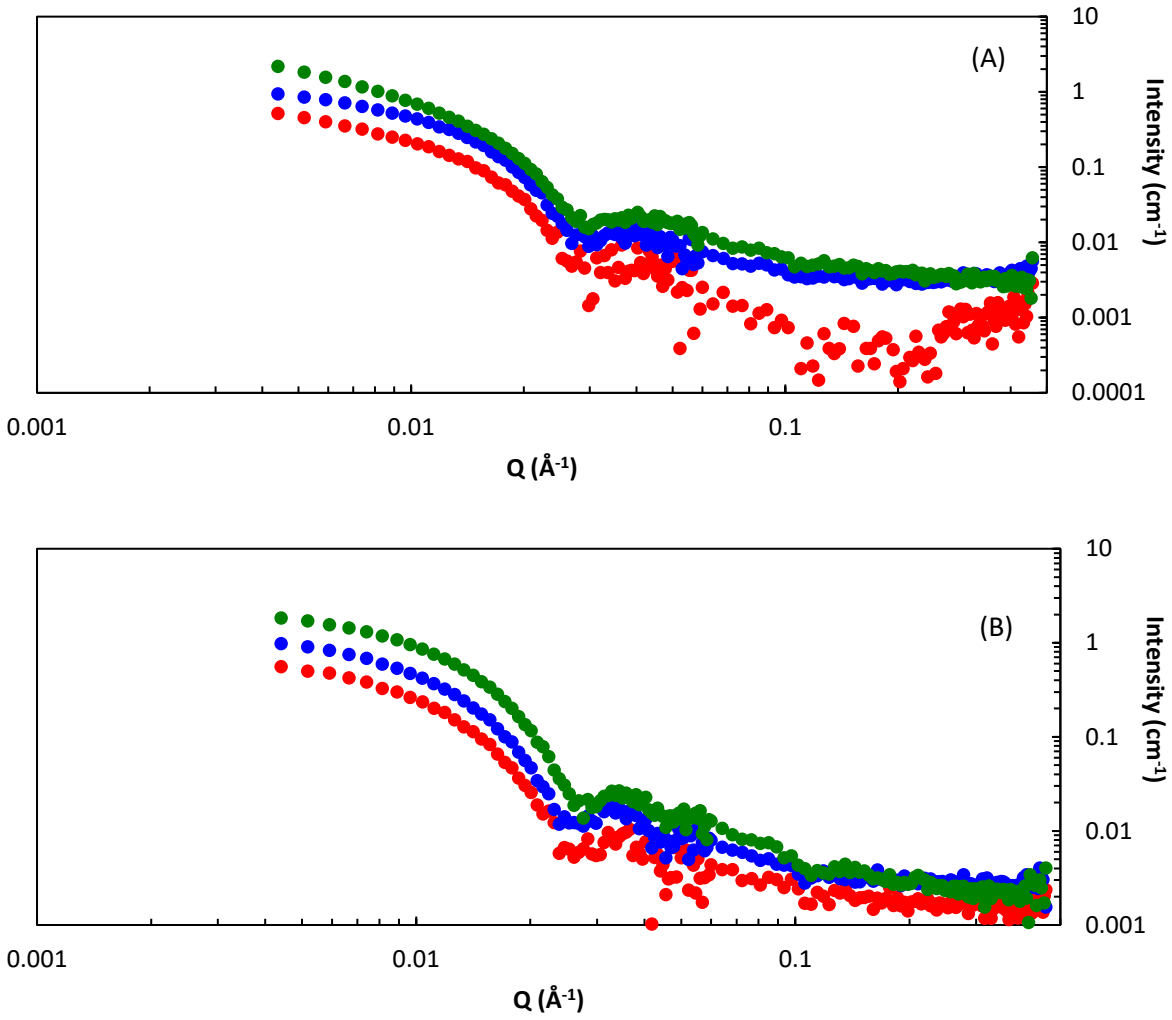

Figure 1: SANS curves of CP mixed with either endogenous (A) or exogenous (B) RNAs in 67% D_2O Tris-HCl buffer (pD 7.5). 3 proteins concentrations were chosen: 1 $g.L^{-1}$ (red), 2 $g.L^{-1}$ (blue), 4 $g.L^{-1}$ (green)

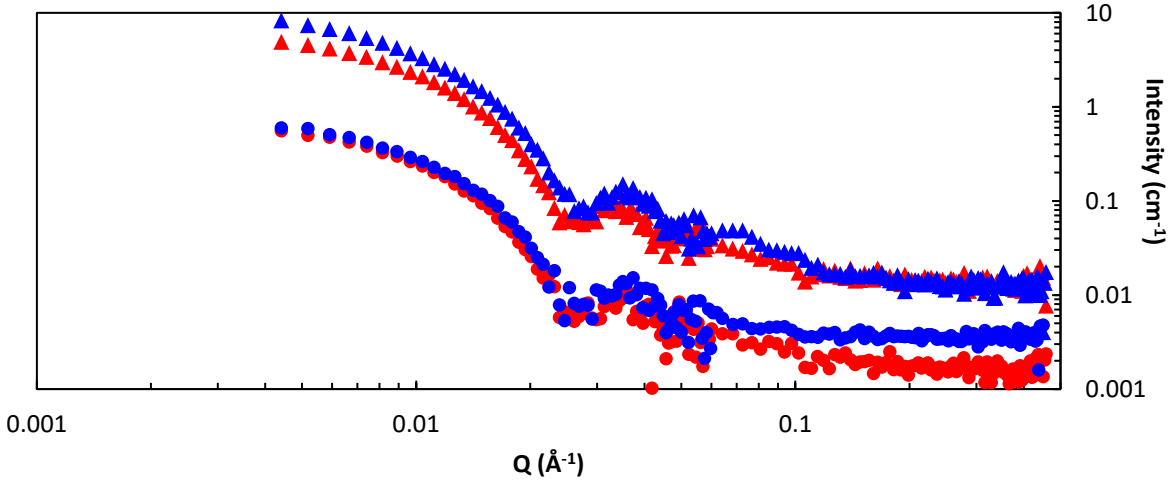

Figure 2: SANS curves of CPs mixed with endogenous RNAs in 67% D_2O Tris-HCl buffer (pD 7.5). 2 proteins concentrations were chosen: 1 $g.L^{-1}$ (circles), 2 $g.L^{-1}$ (triangles), with (blue) and without (red) Ca^{2+} ions.

Study of polymer encapsidation under NPCs conditions

Finally, we looked at the impact of the size and structure of encapsidated polymers by comparing the structure of capsids packaging different deuterated polyelectrolytes mimicking RNAs (either long, short, or branched polymers). By comparing the scattering curves under the same conditions (Fig. 3), we can see that capsids containing short and long polymers are very similar while, in the presence of branched polymers, they are a lot more polydisperse. This can be explained by the lack of flexibility of the branched polymers that compels the CPs to form partially closed capsids.

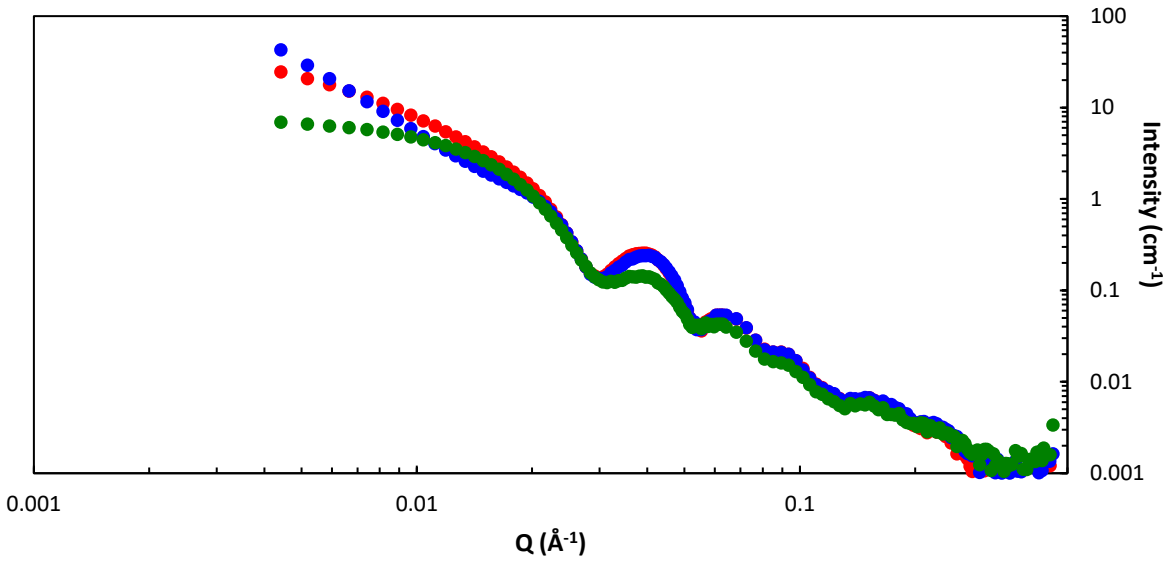

Figure 3: SANS curves of CPs mixed with different d-PSS in 100% D_2O Tris-HCl buffer (pD 7.5). 3 d-PSS were chosen: short polymer (162 kDa, red), long polymer (665 kDa, blue), branched polymer (160 kDa, green).