Experimental report

13/09/2021

Proposal: 9-13-975 **Council:** 10/2020

Title: Quasistatic packaging of linear and star-shaped polyelectrolytes intoviral capsids with the contrast variation method

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: CCMV capsid protein
Deuterated poly(styrene sulfonic acid)

Instrument	Requested days	Allocated days	From	To
D22	3	2	30/06/2021	02/07/2021

Abstract:

Viruses are ubiquitous pathogens that possess a relatively simple structure comprising at least genomic nucleic acids packaged into a protein shell called capsid. Genome packaging is still an elusive process in all known viruses and it is for example remarkable that viruses manage to select specifically their genome during their assembly within the highly crowded intracellular environment. In this framework, synthetic homopolyelectrolytes are very helpful to biophysicists to understand and to model the packaging mechanisms by ruling out the specificity arising from the primary and secondary structures of viral RNA. In the present proposal, we aim at elucidating the packaging pathway in quasistatic conditions by probing separately the scattering intensities of a polyelectrolyte being packaged and those of the subunits building up the shell, using the contrast variation method. Such innovative measurements will allow us to relate the conformation of the polyelectrolyte with the advancement state of the capsid, which cannot be achieved otherwise than by small-angle neutron scattering with contrast variation.

Report on experiment 9-13-975
Quasistatic packaging of linear and star-shaped polyelectrolytes into viral capsids with the contrast variation method

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Context and motivation

The simplest viruses are made of a nanometer-scaled protein shell called the capsid protecting the genome in the form of single-stranded ribonucleic acid (ssRNA). Cowpea chlorotic mottle virus (CCMV) is a $T = 3$ icosahedral ssRNA plant virus, widely used for biophysical studies as a model system due to its structural simplicity, its ease of handling, and its low cost of production. CCMV subunits are capable of packaging nonviral ssRNA but also synthetic, negatively-charged polyelectrolyte via a self-assembly process. While at high ionic strength, free subunits and cargo (ssRNA or polyelectrolyte) weakly interact due to the screening of electrostatic interactions, at low ionic strength, subunits spontaneously form a spherical shell enclosing the cargo.

The experiment aimed at elucidating the packaging pathway **in quasistatic conditions**, that is, by slowly lowering the ionic strength through dialysis. The contrast variation method was used in order to **probe separately the scattering intensities of the ssRNA or polyelectrolyte being packaged and those of the subunits building up the shell**.

Materials and methods

CCMV capsid proteins, ssRNA and polyelectrolyte were initially in a high ionic strength buffer, i.e., 1 or 5 M NaCl, 10 mM Tris-DCl pD 7.5. Under such conditions, capsid proteins are known to be in dimeric form and will be hereafter referred to as subunits. Deuterated poly(styrene sulfonic acid) (dPSS) was used as polyelectrolyte with molecular weights of 10 and 660 kDa, which corresponds to 47 and 3,000 repeat units, respectively. The second genomic RNA segment called C2 was used in its perdeuterated form obtained by *in vitro* transcription. C2 comprised about 2,800 nucleotides.

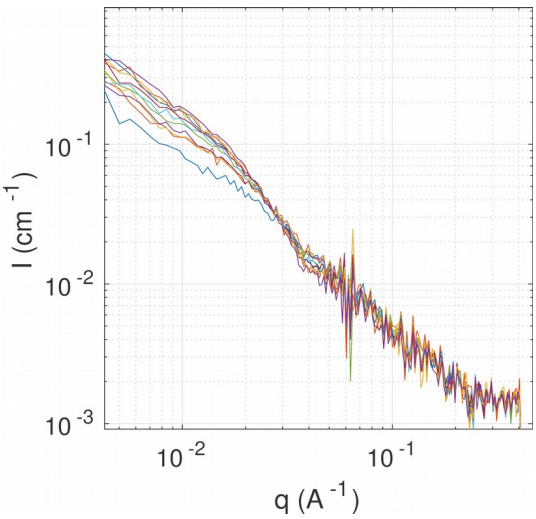

Figure 1 | SANS patterns for subunits alone in 100% D_2O. Subunit concentration is 1 g.L^{-1}. Curves evolve from bottom to top over 9 hours.

Small-angle neutron scattering (SANS) measurements were carried out by dialyzing the samples in high ionic strength buffer against low ionic strength buffer containing 0.1 M NaCl, 10 mM Tris-DCl pD 7.5, with a custom-made experimental setup designed by the staff of the D22 beamline. A 1-mm-thick quartz cuvette in contact with two side reservoirs through membranes contained the samples. Thanks to the presence of two detectors, the wavenumbers q covered the range from 4×10^{-3} to 0.4 Å^{-1} in a single acquisition. Exposure time was varied from 5 to 15 minutes and a single dialysis experiment could last up to 9 hours.

Assembly of subunits alone

In a previous study, we observed that free subunits could form long nanotubes upon a decrease of ionic

strength down to 0.1 M [1]. Therefore, we carried out the dialysis of free subunits at 1 g.L^{-1} in order to probe the formation of nanotubes. The buffers contained 100% D_2O in such a way that subunits had a finite contrast with solvent (recall that proteins are contrast-matched with 43% D_2O). Figure 1 depicts some of the collected scattering patterns: Even though the forward scattering intensity I_0 increased as expected, the variation remained moderate (factor two or three) whereas other measurements performed by small-angle X-ray scattering (SAXS) displayed a ten-fold increase of I_0 and showed oscillations at the medium q-values characteristic of the circular cross-section of nanotubes. The mild evolution might be due to the presence of heavy water that may alter the interactions between subunits and inhibit their assembly into nanotubes.

Polyelectrolyte packaging

The second series of measurements was devoted to the packaging of dPSS into capsids. We set the subunit-to-dPSS mass ratio to 4.0 in such a way that the charge balance was identical regardless of the length of dPSS. Figure 2 shows the scattering patterns of subunits in the presence of 660-kDa dPSS in 100% D_2O. Because dPSS is perdeuterated, its scattering intensity vanished while we could probe solely that of subunits. It turned out that at the beginning some particles were already assembled even though ionic strength was 1 M and should have completely screened the electrostatic interactions between subunits and dPSS. Even more surprisingly, the scattering patterns remained noisy after 6 hours, while our previous measurements under the same final conditions revealed smooth oscillations in the medium q-values, which is characteristic of well-ordered, spherically-symmetric objects. A second observation is that I_0 decreased instead of increasing as expected for the assembly of capsids. Once again, it looks as though heavy water was disturbing the interactions between subunits and/or those between subunits and dPSS.

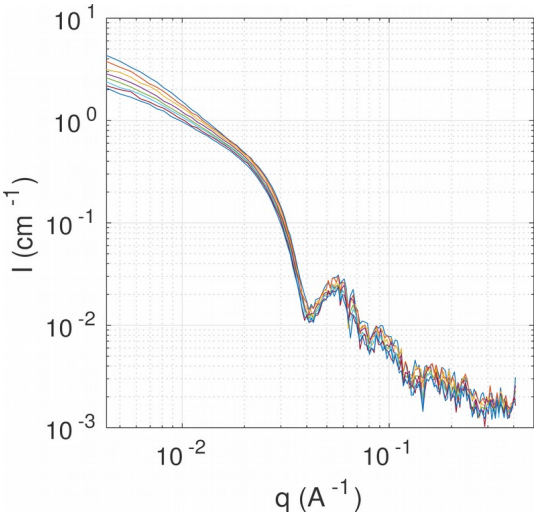

Figure 2 | SANS patterns for 660-kDa dPSS packaged into capsids in 100% D_2O. Subunit concentration is 2 g.L^{-1} and subunit-to-dPSS mass ratio is 4. Curves evolve from top to bottom over more than 6 hours.

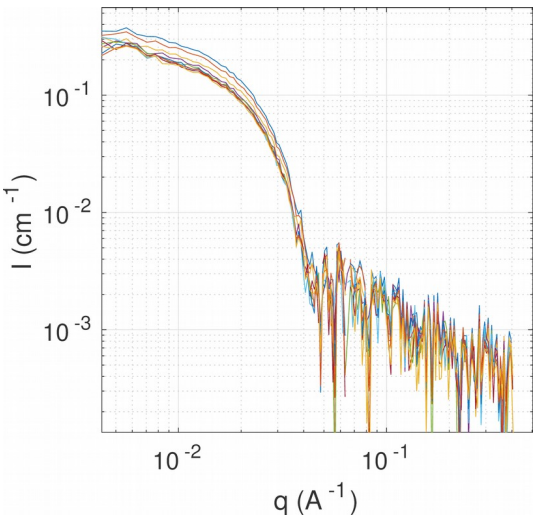

Figure 3 | SANS patterns for 10-kDa dPSS packaged into capsids in 100% D_2O with an initial ionic strength of 5 M. Subunit concentration is 0.5 g.L^{-1} and subunit-to-dPSS mass ratio is 4. Curves evolve from top to bottom over more than 8 hours.

Measurements performed with a much sorter polyelectrolyte, i.e., 10-kDa dPSS, in 100% D_2O, produced similar results (Fig. 3): A few particles were assembled at the beginning, the scattering patterns did not display any structural ordering over more than 8 hours, and the forward scattering intensity was slightly decreasing, which indicates that the objects released subunits instead of capturing them.

Genomic RNA packaging

At last, we investigated the packaging of viral RNA. The segment C2 of the viral genome was synthesized with perdeuterated nucleotides. Thus, in 43% D_2O, subunits were contrast-matched and the

scattering intensity solely arose from C2. The subunit-to-RNA mass ratio was set to 4.5 in such a way to have a slight excess of free subunits. Figure 4 gives the scattering patterns of C2 RNA in the presence of subunits. Unexpectedly, the forward scattering intensity I_0 drops by more than one order of magnitude after 5 hours. An explanation may be that RNA was being adsorbed on the dialysis membrane, which resulted in a decrease of its concentration. Indeed, we noticed during synthesis that RNA was readily trapped in centrifugal devices equipped with membrane. However, the radius of gyration of the objects in solution seemed to decrease with time, which would be consistent with the compaction associated with RNA packaging into capsids. Due to a high level of noise, no clear feature was visible in the medium q-values. The effect of heavy water was probably mild since the solvent contained only 43% D_2O and the backbone of RNA is less hydrophobic than that of dPSS.

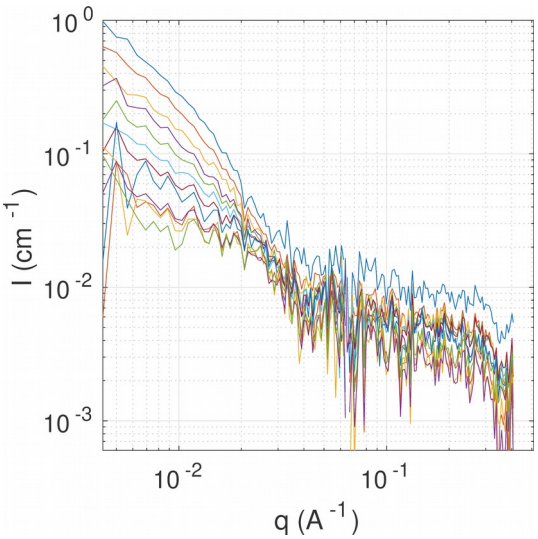

Figure 4 | SANS patterns for C2 RNA packaged into capsids in 43% D2O with an initial ionic strength of 1 M. Subunit concentration is 2.25 $g.L^{-1}$ and subunit-to-RNA mass ratio is 4.5. Curves evolve from top to bottom over more than 5 hours.

Conclusions

If dialysis can be performed while measuring scattering intensities, we faced up difficulties related to isotopic effects and nonspecific adsorption. As mentioned earlier, the backbone of dPSS is highly hydrophobic, and since heavy water tends to enhance hydrophobic interactions, **dPSS may collapse readily and favor the formation of a protein shell around it, even at very high ionic strength**. However, while our preliminary experiments in light water showed that low ionic strength further promotes the capsid assembly, it seems that it was not the case in heavy water. Notice that **in our previous measurements in heavy water [1,2], particles were assembled first in light water then dialyzed in heavy water prior to experiments**. The second issue was most likely due to the nonspecific adsorption of RNA on the dialysis membranes. Membranes with very low binding affinity with RNA must be used for this kind of experiments.

[1] L. Marichal... G. Tresset, *Biophys. J.* in press.
[2] G. Tresset *et al.*, *Phys. Rev. Lett.* **113** (2014) 128305.