Experimental report

06/02/2018

Proposal: 8-03-889 **Council:** 4/2016

Title: Density profile of DNA packaged inHerpes Simplex Virus type 1 revealed by contrast variation SANS

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: HSV-1 virus-like inactive particles

Instrument	Requested days	Allocated days	From	To
D11	6	2	05/12/2016	07/12/2016

Abstract:

In human Herpesviruses, the viral genome is a double-stranded DNA molecule which is several hundred times longer than the diameter of the capsid. This physical situation leads to genome bending stress and strong repulsive interactions resulting in internal capsid pressures reaching 20 atm. The energy and the structure of the confined viral genome are the two physical parameters that control genome ejection and packaging. Using contrast variation SANS we aim to provide a description of the viral genome packaging density in the capsid which in turn is correlated with its energy. Our unique approach uses measurements on viruses in liquid, which will allow us to apply the solution conditions mimicking the cellular environment.

In herpesviruses and many bacteriophages the viral DNA is tightly packaged into a protein capsid. This tight genome confinement leads to strong DNA-DNA repulsions and bending stress on the genome, generating pressure of tens of atmospheres on the capsid walls [1].

The objective of this project is to study the radial density distribution of encapsidated viral-DNA by measuring small angle neutron scattering from Herpes Simplex virus type 1 (HSV-1) and phage ʎ virus-like particles. This experiment consisted of two days of beam time at D11 (December 2016) with the aim of testing the quality of the system and the viability of the contrast matching technique applied to these samples.

Contrast match-points were determined using the NIST-NCNR contrast calculator software. The calculations included FASTA files for each capsid protein (detailing the amino acid sequence of the protein), the copy number of each protein and the DNA genome sequence [2]. The results of the calculations are very similar for HSV-1 and phage ʎ particles and they are summarized in figure 1 where SLD stands for scattering length density and complex for the DNA+capsid complex. The figure shows that the protein matching point, where the capsid protein and the solvent have the same SLD, is at approximately 43% D_2O, the DNA matching point is at 65% D_2O and the highest contrast with the different components and the complex is achieved in pure D_2O (full contrast).

Four samples of virus-like particles (none included the tegument proteins and the lipid envelopes found in naturally occurring viruses) suspended in D_2O/H_2O mixtures were measured. All of the mixtures containing 50 mM Tris-Cl and 10 mM Magnesium sulfate at pH 7.5 (which constitutes a TM buffer). The concentration of the virus-like particles was roughly the same for each sample, 10^{11} p f u/ml (plaque forming units). The samples were: HSV-1 like particles (DNA+capsid) in pure D_2O (full contrast), HSV-1 like particles at 43% D_2O, empty capsids of HSV-1 (no DNA) particles at 43% D_2O and phage ʎ like particles (DNA+capsid) at 43% D_2O.

Scattering intensities for each sample and the corresponding buffer were recorded in transmission by a two-dimensional position-sensitive ^{3}He detector. Three different instrument settings were used corresponding to a momentum transfer range $Q = \frac{4\pi}{\lambda \sin(\theta/2)}$ of $1.94\ 10^{-3} < Q < 0.44$ Å^{-1}. H_2O was used for instrumental calibrations and the intensities in absolute units (cm^{-1}) were obtained by applying the standard normalization and correction procedures [3].

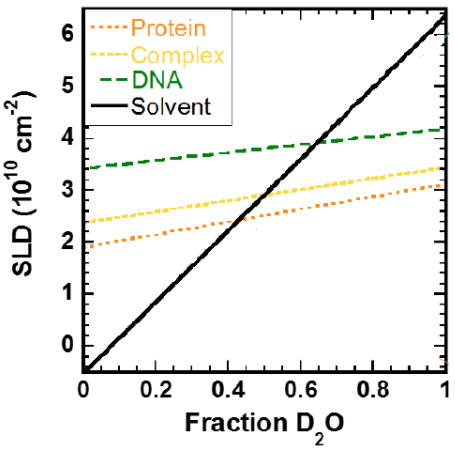
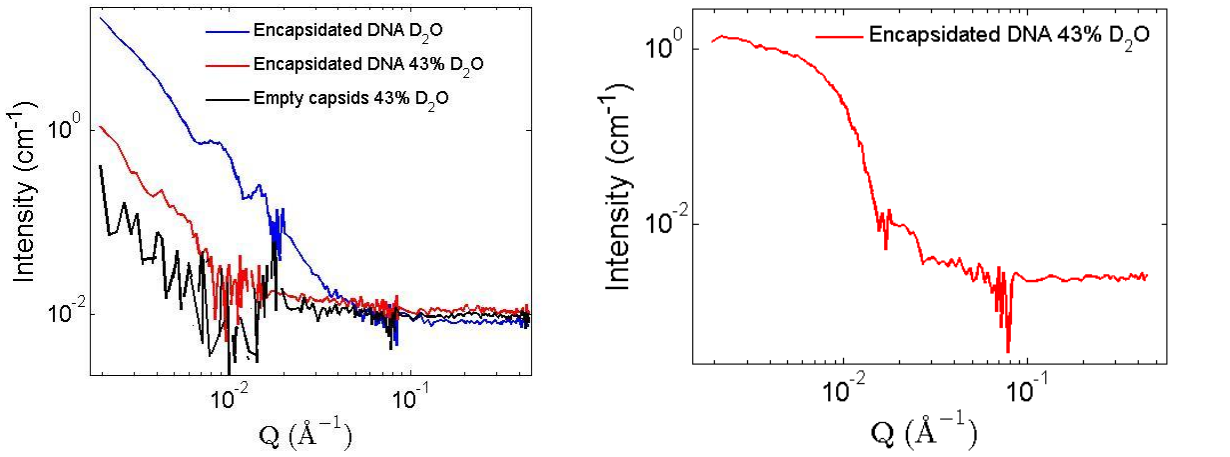


Figure 1: Calculated scattering length density of the buffer, the complex DNA+capsid, the DNA and the protein capsid as a function of the percentage of D_2O in the solvent.

The final SANS curves for each sample can be seen in figure 2. Figure 2a shows the data for the HSV-1 like particles. The intensity at Q=0 of the full contrast sample (in blue) is the highest as expected. The black curve in fig. 2a shows essentially no scattering from the contrast matched empty viral capsids. The red lines in fig. 2a show the scattering of the encapsidated DNA at 43% D_2O. Figure 2b shows the SANS curve of the encapsidated DNA for phage ʎ which is consistent with the red curve of figure 2a.

These results prove that it is possible to measure the structure-factor of virus like particles suspended in solution and that the contrast matched technique allows to discriminate between the contribution of the capsid and the DNA.

The data did not support a full quantitative analysis because the signal was too weak. We are improving the sample preparation method so increase the final concentration of virus-like samples which will allow to collect better quality data in future works.



(a) (b)

Figure 2: (a) SANS of HSV-1 like particles suspended in buffer: encapsidated DNA in 100% D_2O in blue; encapsidated DNA in a D_2O/H_2O mixture at 43% D_2O (estimated matching point of the protein capsid) in red; and empty capsids at 43% D2O in black. (b) SANS of phage λ like particles (encapsidated DNA) at 43% D_2O which is consistent with the red curve of figure 1a.

References

[1]- D.W. Bauer, J.B. Huffman, F.L. Homa, and A. Evilevitch. JACS, 135:1216–11221, 2013.

[2]- https://www.ncbi.nlm.nih.gov/nuccore/JQ673480

[3]-P. Lindner; Neutrons, X-Rays and Light: Scattering Methods Applied to Soft Condensed Matter, edited by P. Lindner and Th. Zemb (Elsevier, Amsterdam, 2002).