Experimental report

27/09/2018

Proposal: 8-03-931 **Council:** 4/2018

Title: Revealing radial distribution of DNA density packaged in the Herpes Simplex type 1 virus

Research area: Biology

This proposal is a continuation of 8-03-889

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Samples: HSV-1 virus-like inactive particles

Instrument	Requested days	Allocated days	From	To
D11	6	2	13/06/2018	16/06/2018

Abstract:

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) [1] virus-like particles. This experiment consisted of two days of beam time at D11 (June 2018).

Three kinds of samples were measured under different conditions: A-capsids, C-capsids and buffers matching the capsid samples. C-capsids are protein capsids with the viral genome packed inside, A-capsids are empty capsids (no DNA), the structure of the capsid is however mostly identical to the one of C-capsids. All the samples were prepared with 50 mM Tris-Cl and 10 mM magnesium sulfate at pH 7.5. The wavelength used was 6 ångströms and the Q range goes from 0.0015 to 0.45 reciprocal ångströms. Standard banjo cuvettes of 1 mm pathlength (300 uL) were used. The data were corrected using common procedures and then radially averaged to show the 1-D scattering in absolute units (cm^-1).

The experiment was a success regarding the implementation of the contrast matching technique. Figure 1 shows the scattering of C-capsids, A-capsids and a corresponding buffer at room temperature at the protein contrast matching point. The scattering of the A-capsids (just protein) is week in comparison with the scattering of the C-capsids. $I(Q_{min})\approx 3\ cm^{-1}$ for A-capsids whiles $I(Q_{min})\approx 20\ cm^{-1}$ for C-capsids for samples with similar concentration of capsids. This implies that the structure factor of the encapsidated DNA can be isolated from the contribution from the protein capsid. This enables relative detailed structural studies of the packing structure.

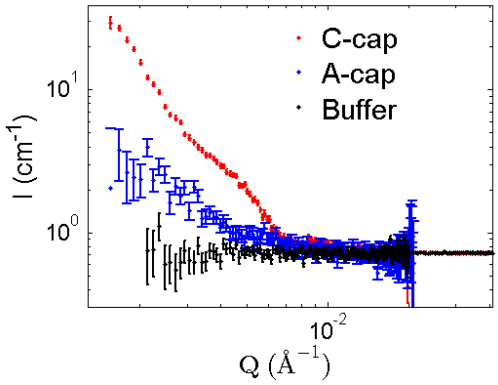
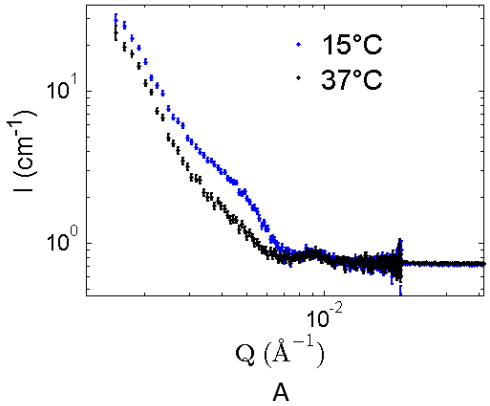


Figure 1: SANS curve for C-capsids, A-capsids and buffer at room temperature and at the protein matching point (approx. 43% D_2O).

Previous works in the group have demonstrated that temperature can dramatically modify the packing structure of the DNA. The experiment also proved that SANS is sensible to such structural variations. Figure 2A shows the scattering of C capsids at the protein contrast match point at 15 and 37 °C. Figure 2B shows the scattering at the same temperature of A capsids at 100% D_2O where the contrast is the highest and the incoherent background is the lowest. The figure suggests that the scattering from the protein capsid (and therefore its structure) remains practically constant with temperature whiles the scattering attributed only to the encapsidated DNA undergoes a dramatic change with temperature. This change must be related with structural changes in the packing structure.



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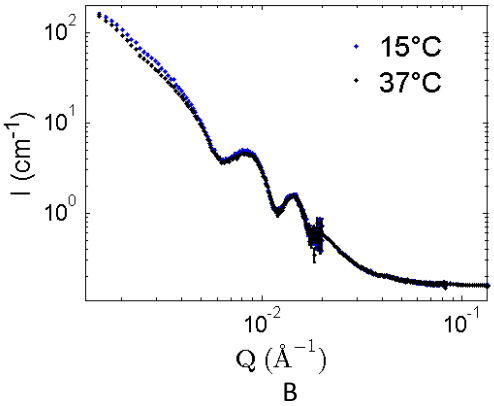

B

Figure 2: Temperature-dependent scattering of A-capsids (A) at full contrast and C-capsids at the protein matching point.

We tried to model the data in different ways to extract information about the packing structure of DNA and its evolution with temperature. Despite the promising results presented above, at the early stages of the data analysis we realized that the scattering we recorded seemed to suggest the presence of particles in our samples larger than the capsids (which are known to have an approximated radius of 60 nm [2]).

We used a core-multishell model to fit the data. These models represent the capsids as spheres with a core and a number of shells. Each part has a different scattering length density (SLD), see figure 3A. The available fitting parameters of the model are the radius of core and shells, the SLDs, an overall scale constant and an incoherent background. Figure 3B shows the data from C-capsids at the protein matching point alongside to two fits for the data using a core-shell model. The red line is a fit in which the maximum allowed radius for the particle was 60 nm. The magenta line is a fit in which no constrains were imposed on the size of the particle. The figure shows that a particle of the expected capsid radius cannot reproduce the measured scattering data. However, a scattering profile from a particle of almost 3 times the size of the capsids fits the data rather well.

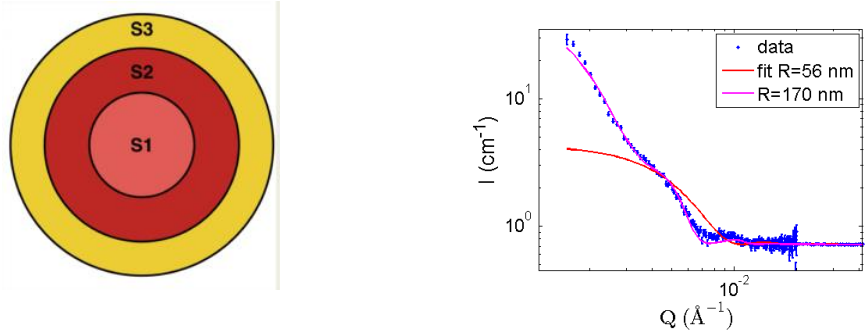

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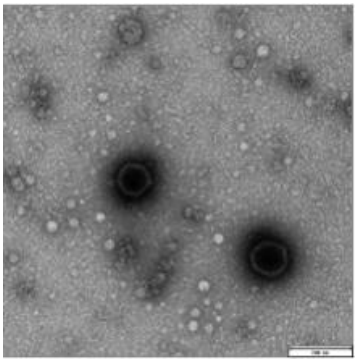
Figure 3: (A) Representation of a core-2-shell model with 3 SLDs (S1, S2, S3). (B) SANS curve of C-capsids at the protein matching point along with two fits using a core-shell model. The red line is a fit with a particle radius of 56 nm, close to the expected capsid radius, and the magenta line is a fit with a particle radius of 170 nm.

Other data analysis methods consistently suggested particles of larger radius than the protein capsids. Specifically, a fit of the low Q data using the Guinier approximation to extract the radius of gyration and a calculation of the expected scattering profile using EM 3D density maps of HSV-1 particles under identical conditions.

We are confident that our sample preparation gives solutions of capsids without significant contamination from other particles since we tested the purity of our first batches with negative stain TEM and running SDS gels stained with Coomassie. Figure 4A shows how no particles bigger than the capsids are detectable with TEM. Figure 4B shows the result of a SDS gel electrophoresis. After thermally breaking the capsids, the SDS gel process separates the different components by size. The different bands found are consistent with an original sample composed only by the capsids.



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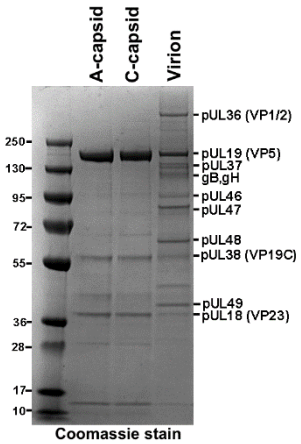

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Figure 4: (A) Representative negative stain TEM image of C-capsids. C-capsids are the black spots (DNA) surrounded by a corona (protein capsid). (B) Result of a SDS gel electrophoresis with Coomassie stain ran in A-capsids, C-capsids and Virions (natural HSV-1 particles: C-capsids + tegument proteins +virus membrane).

Since contamination of our neutron scattering samples is unlikely, the easiest explanation for the apparent size of the particles in the samples is an aggregation of the capsids. Capsid aggregation has not been detected before in similar samples prepared by the group for X-ray scattering and other techniques. However, our samples were at the highest concentration ever tried and they were prepared in D_2O buffer. It is known that both increasing concentration and D_2O content favors aggregation of proteins [3], [4].

To test this hypothesis, we performed dynamic light scattering measurements using a Brookhaven instrument on some of the same samples measured on D11. A fit of the autocorrelation function using cumulant methods (performed by the control software of the instrument) gave estimations for the diameter of the particles consistently higher than the expected 120 nm for every sample. Figure 5 shows an example of the results of this fit for the D11 sample of C capsids at the protein match point (black line). Light scattering data was collected overnight and so the diameter of the suspended particles was estimated as a function of time after a strong pipetting of the sample and loading in the glass cuvette. The apparent diameter of this particles remains virtually constant with a mean value of 291 $\pm$ 13 nm (the error was taken as the standard deviation of all the data points). Since proteins are less prompt to aggregate as concentration decreases, we diluted the sample and remeasured it overnight. The original concentration was approximatively 1.75e10^13 capsids/ml and the new one 1.2e10^13 (roughly 30% dilution). Figure 4 shows that at the reduced concentration the size of the particles is dramatically reduced to an average of 127 $\pm$ 11 nm (blue line) which is very close to the expected size of the capsids. Another two D11 samples were measured with similar results. We conclude that concentrations of 1.2e10^13 the time for aggregation is considerably lower and single capsids stay suspended in the solution for at least 15 hours.

The samples for this beam time were directly dialyzed against the final D_2O/H_2O buffer from the original H_2O. The impact of D_2O on aggregation can be reduced in future experiments by performing a stepwise dialysis from a H_2O water buffer to the final D_2O/H_2O in which each step increases the D_2O by 5% or 10% [5].

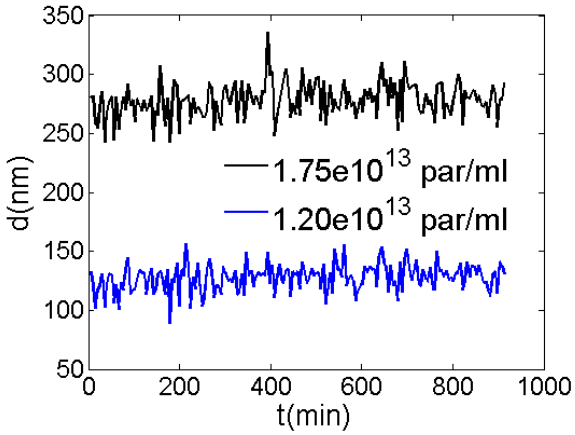

Figure 5: Estimation of the diameter of the particles suspended in a C-capsids sample at the protein matching point using dynamic light scattering at two different concentrations.

In summary the experiment was a technical success. However, to achieve the full potential of the proposed experiment we will need further measurements in samples in which the aggregation is not present and the particle interaction can be safely ignored.

References

[1]-U. Sae-Ueng et al.; Nature chemical biology;10(10); 861-7 (2014). **[2]-** M. McElwee et al.; PLoS Biol 16(6); e2006191 (2018). **[3]-** P. Cioni et al.; Biophys. J. ; 82 ; 3246-3253 (2002). **[4]-**A.L. Fink ; Folding and design; 3:R9-R23 (1998). **[5]-**M. Lebendiker et al.; FEBS Letters; 588; 236-246 (2014).