Experimental report

17/06/2022

Proposal: 8-03-965 **Council:** 10/2018

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

Research area: Biology

This proposal is a new proposal

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Local contacts: Ralf SCHWEINS

Samples: HSV-1 particles (protein capsid +DNA)

Instrument	Requested days	Allocated days	From	To
D11	6	3	09/07/2021	12/07/2021

Abstract:

The icosahedral protein capsid of Herpes Simplex virus type 1 contains the tightly packed viral DNA. During the Herpes infection the DNA is ejected inside the nucleus of the host cell where it starts the virus replication (lytic infection) or is integrated in the cell nucleus and remain there indefinitely but does not replicate (latent infection).
Our latest research suggest that there is a direct link between the structure of the encapsidated DNA, the dynamics of ejection and the fate of the infection, this is, whether the infection will be lytic and latent.
External factors, like temperature or ionic strength in the solution, can affect the structure of the DNA and therefore the latent/lytic switch.
We want to study the structure of the encapsidated DNA under different conditions as controlling the switch between lytic and latent infections is a way to stop the virus from spreading.

ILL Experiment Report

Experiment number: 8-03-965
Instrument: D11
Local Contact: Ralf Schweins

Experiment title: **Revealing radial distribution of DNA density packaged in the Herpes Simplex type 1 virus.**

Sample preparation

Prior to ILL experiment

Herpes virus capsids were purified according to stablished protocols. Briefly, infected Vero cell cultures were lysed using a hypotonic buffer. The viral capsids were released from the infected cell nuclei via sonication and the isolated capsids were diluted in TNA buffer (1mM EDTA, 500mM NaCl, 10mM Tris, pH=8.0) and stored at 4°C.
C-capsids are DNA filled particles and A-capsids are empty particles, i.e., just the protein coat.

At ILL chemistry lab

Preparation of D_2O-TNE buffer (43% D_2O, 1mM EDTA, 500 mM NaCl, 10 mM Tris). We performed the buffer exchange from 100% H_2O to 43% D_2O by proving three methods: dialysis (2 times for 4 hours and overnight), NAP-5 columns and direct dilution. The samples are non-infectious capsid of herpes simplex virus type 1. The viral membrane envelope and proteins have been removed during the purification process in Lund University and the resulting capsid particles are non-bio-hazardous. We pre-spin slightly the sample before load banjo cells in order to avoid the presence of protein debris in the sample.

Results

The main goal of this experiment is describing the possible coexistence of viral DNA in two phases into capsid. Initially, A- and C-capsids, after further dialysis, were loaded to banjo cells at 37°C, being the critical temperature where we expected to find any signature of viral DNA phase transition. As part of the preparation of this set of samples, we added DNase+$MgCl_2$ just before to load banjo cells in order to cut the partially expelled DNA from capsids. The used method of buffer exchange in D11 SANS (38m, 8m and 2m distance to detector) beamline caused particle aggregation, easily to visualize by eye (Fig. 1). The scattering intensity, I(q), for the buffer, A- and C-capsid is shown in Fig. 2. In all q-range, the buffer is flat as we expected but A-capsids seems to expect to scatter more than C-capsids, which is an unexpected result because there is evidence in the literature about the fact that 43% D_2O is the contrast match point for the protein coat. It seems that we corroborate the right contrast match point for herpes virus particles. We noticed for C-capsids there is not a plateau at low-q's which is commonly means a monodisperse solution of particles, however, there is an increasing intensity signal as q becomes smaller which means the formation of aggregates of particles as Fig. 1 is showing.

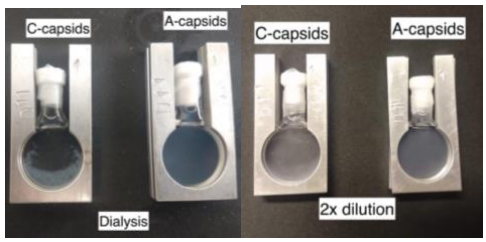

Figure 1. Photos showing how look like the banjo cell after several hours of exposure to neutron beam al D11 guideline at 37°C. Both, C- and A-capsids shown aggregation, which more noticeable for the former, T=37°.

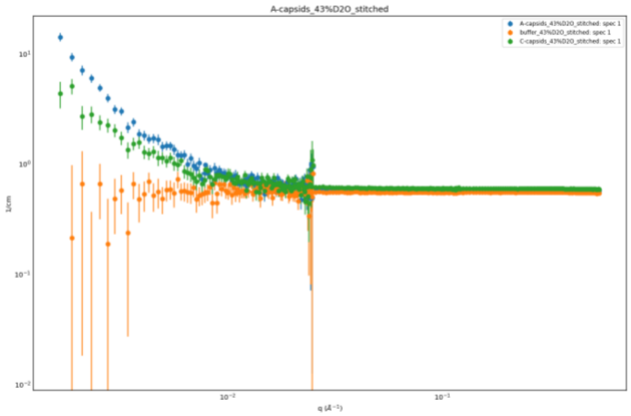

Figure 2. Scattering intensity of A- (blue symbols) and C-capsids (green symbols) in 43%D_2O (orange symbols). The buffer exchange is done though dialysis, T=37C. We had similar results with NAP-5 columns and direct dilution.

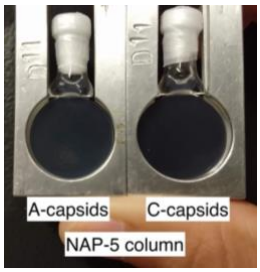

Figure 3. A- and C-capsids without addition of DNase+$MgCl_2$. There are not visible aggregates by eye, T=37°C.

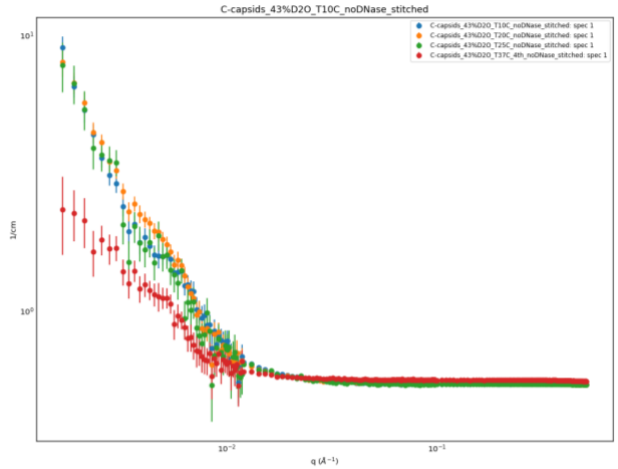

Figure 4. Scattering intensities at several temperatures for C-capsids. The sample preparation for T= 10°, 20° and 25°C comes from a 2x dilution and T=37°C comes from NAP-5 column, that explains the difference in the scattering intensity at low-q´s because the concentration is lower for NAP-5 column.

To verify if the presence of DNase+$MgCl_2$ was causing particle aggregation, we decided to avoid the addition of those components, the result of this proof is shown in Fig. 3, which does not have viewable aggregate for both A- and C-capsids. So, we decided to measure the scattering intensity at several temperatures without the addition of DNase+$MgCl_2$. The resulting I(q) is shown in Fig. 4. The scattering intensity at T=10°, 20° and 25°C (blue, orange and green symbols) comes from a 2x dilution and the scattering at T=37°C comes from a NAP-5 column which has a lower concentration of particles because the column causes some lost of sample, that is the reason of low intensity for this measurement. Unfortunately to our purposes, we still do not have a plateau at low-q´s, so that, we have the formation of aggregates in solution which limit the goal of this project: describe to possible coexistence of viral DNA in two phases into the capsid which should be described through a form factor whose first maximum should be located close to 0.01$Å^{-1}$, regrettably in that region the not clear evidence of the aforementioned form factor. As far as we know, the formation of aggregates is a consequence of particle interaction, which should be accounted by the structure factor whose main effect is in the low-q scale. The structure factor takes into consideration the ionic strength, temperature and concentration, hopefully, if we are able to estimate the structure factor for this set of measurements, we will be able to say something about particle interaction and the effect of D_2O in aggregates formation, as well as attempt to eliminate the contribution of structure factor from scattering intensity, then focus on the possible structural changes of viral DNA.