Experimental report

18/06/2021

Proposal: 8-03-960 **Council:** 10/2018

Title: Solid-to-fluid like DNA transitionin phage capsid controls viral infectivity. Revealing structure of intra-capsid DNA in phage with SANS

Research area: Biology

This proposal is a new proposal

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Local contacts: Ralf SCHWEINS

Samples: Phage Lambda particles (capsid+DNA)

Instrument	Requested days	Allocated days	From	To
D11	5	3	09/10/2019	12/10/2019

Abstract:

Bacteriophage λ (phage λ) is a virus which infects Escherichia coli (E. coli) by injecting viral double-stranded (ds) DNA into the bacterial cytoplasm without capsid disassembly. The ejection dynamics was recently found define the course of the infection. Rapid synchronized DNA injections from tens of pages into a single host cell prompts a latent infection in which the viral DNA is integrated into the host without affecting its vital functions. At the same time, slow desynchronized injections lead to lytic infection, where new viruses are reproduced within the cell leading to cell lysis and release of new infectious virions.
The encapsidated DNA undergoes a structural transition which seems evolutionary adapted to increase the mobility of DNA and facilitate ejection but the pre- and post-transition packed structure is unknown. We want to use neutrons to study the specifics of the genome structure inside the Phage λ and how this structure and structural transitions affect the dynamics of virus infection and replication or latency.

ILL Experimental Report

Experiment number: 8-03-960
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 the ILL experiment

Herpes virus capsids were purified according to established 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 TNE buffer (1mM EDTA, 500mM NaCl, 50mM Tris) and stored at 4°C.

At ILL chemistry lab

Preparation of D2O-TNE buffers (100% and 43% D2O, 1mM EDTA, 500mM NaCl, 50mM Tris). Dialysis of protein capsid samples in 100% and 43% D2O. The samples are non-infectious capsids 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.

Results

Initially, the herpesvirus empty (A) capsids were measured at full contrast (dialyzed in 100% D_2O). The A capsids were dialyzed for 4 hours (2 times) and overnight. For this sample, 3 detectors were used at 1m, 8m and 39m (Fig.1).

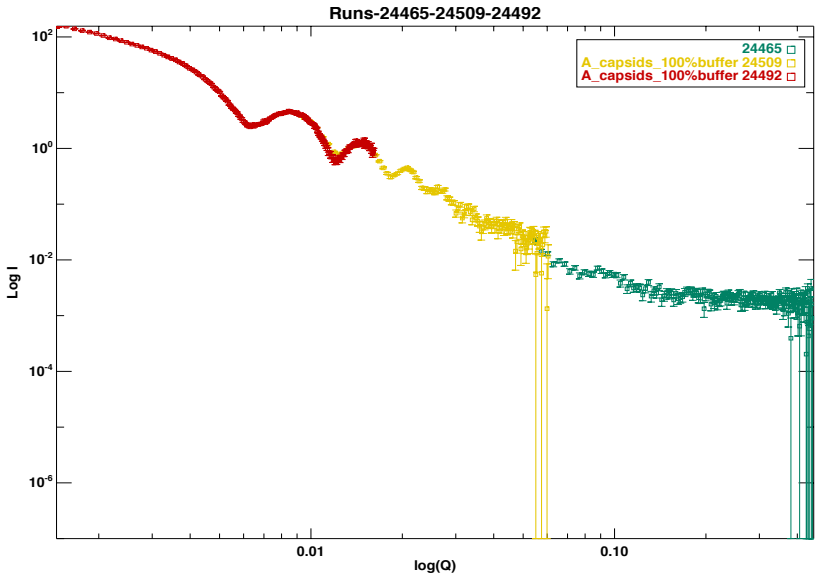

Figure 1: A capsids in full contrast (100% D2O) in 3 detector distances. Red curve is 39m detector, yellow curve is 8m detector and green curve is 1m detector.

To determine the contrast match point, the A capsids were dialyzed at 43% D2O-TNE buffer (500mM NaCl), for 4 hours (2 times) and overnight. We observed an increase in intensity at low q values. This was concerning result at first so an additional 4-hour dialysis step of the sample was performed. The fourth dialysis did not cause any change in the scattering pattern, indicating that the 3-dialysis protocol is sufficient for sample preparation.

The DNA-filled (C) capsids were measured in 3 detector configurations, 1m, 8m and 39m to cover the entire q range. The C capsids were dialyzed in a similar manner as the A capsids and were treated with DNase I (100μg/ml and 20mM of MgCl2) to remove released DNA from the capsids. In addition, the C capsids were measured at a temperature range of 20, 25, 30, 37 and 40^{0}C in order to determine changes in the structure of the intracapsid DNA.

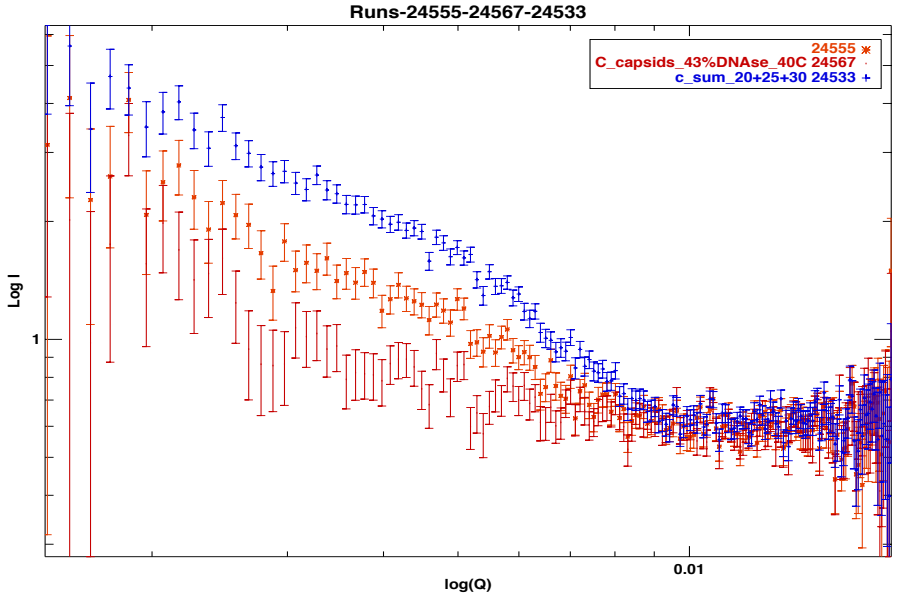

Figure 2: C capsids in 43% D2O in 39m detector configuration. Blue curve is the sum of the curves from temperatures 20, 25 and 30C. Orange curve shows the intensity decrease at 37C and red curve is the level of intensity at 40C.

A curve in the low q range was observed at 20, 25 and 30^{0}C while the intensity begun to drop at 37^{0}C and above (Fig.2). This decrease in the signal can be an effect of particle aggregation and sedimentation out of beam, as well as the sensitivity of C capsids to large temperatures. No difference was observed from 20°C to 30°C. Furthermore, in the current experiment, no DNA ''peak'' was observable at 10^{-2} q value in any of the temperatures tested (Fig.3), probably the signal from aggregates hides the signal from "peak" of DNA. However, we believe that there is a tendency for a feature in this q range but the data are very noisy with the current configuration. To determine whether there is a DNA ''peak'' we suggest an additional measurement of the C capsids in 43% D2O using the 20m detector and expose the sample to the beam for 2-2.5h. This configuration should provide the required coverage for the intracapsid DNA with improved resolution and better statistics. Additionally, we could try to reduce D2O concentration to try to avoid particle aggregation in order to look for the aforementioned DNA "peak", even more, in the modelling of data we might to use the core-shell model to consider the presence of the capsid, it will help us to follow step by step, by changing the temperature, the signature of DNA structural reorganization into capsid. These modifications will be performed during the upcoming beamtime on July 9 to July 12, 2021.

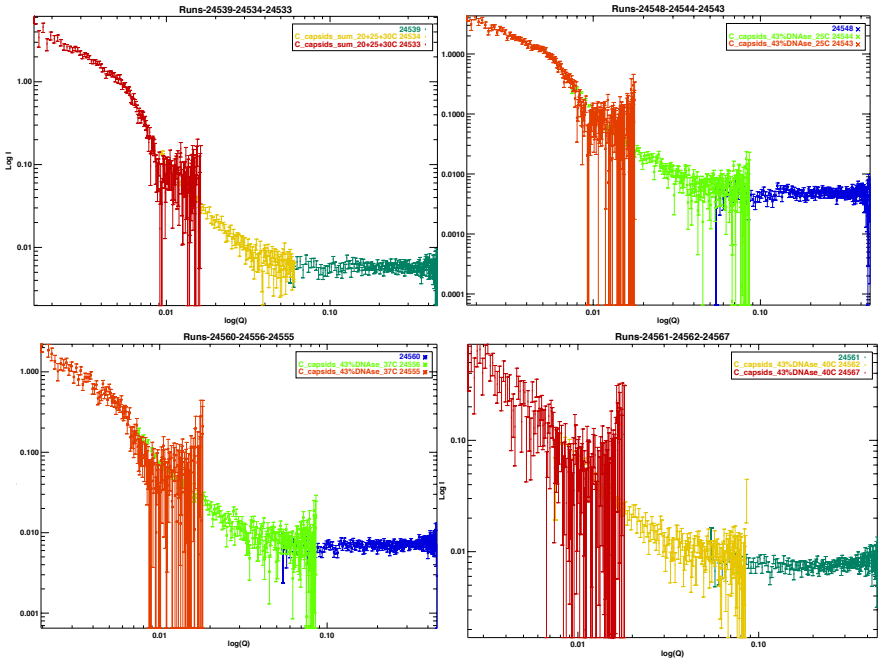

Figure 3: Scattering patterns of C capsids in 43% D2O in 1m, 8m and 39m detector configurations as well as different temperatures. Top left, 20^{0}C, top right, 25^{0}C, bottom left, 37^{0}C and bottom right, 40^{0}C.

The last sample measured, were the C capsids of a recombinant herpesvirus that contains only 91% of the wild type virus genome. In principle, the A capsids of the virus are structurally identical to the wild type measured above. The 91% C capsids were dialyzed and treated with DNase I similarly to the above samples. It was observed that the 91% C capsids had higher scattering intensity which may be related to sample quality (Fig.4) and higher particle concentration. In addition, similar curves with the wild type C capsids were observable with the 91% C capsid sample. At 20 and 30^{0}C the scattering intensity was similar and decreased with increasing temperature to 37^{0}C and even further at 40^{0}C (Fig.4). All data have background subtracted.

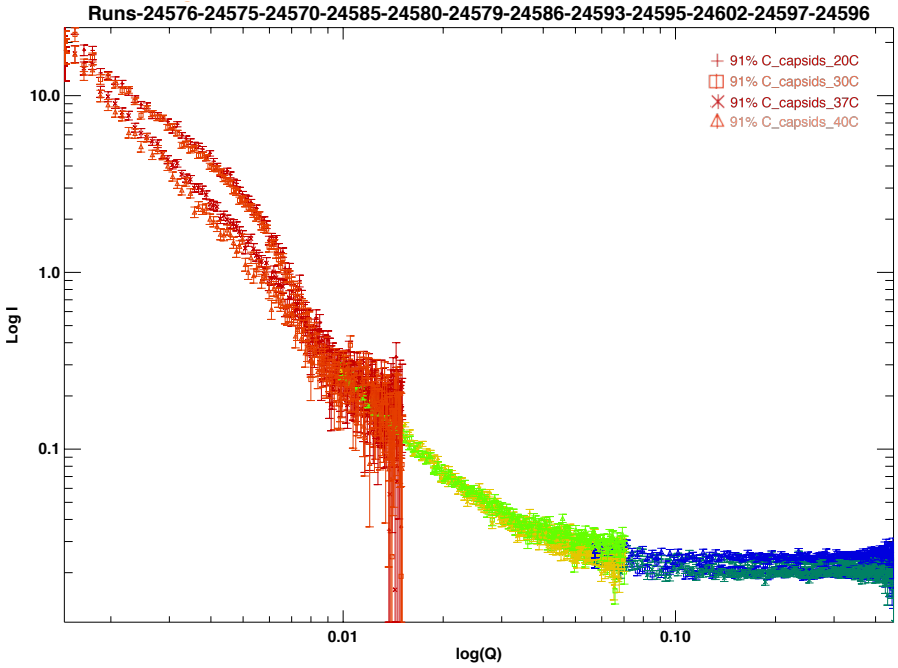

Figure 4: Scattering patterns of mutant C capsids (91% DNA) in 43% D2O in 1m, 8m and 39m detector configurations as well as different temperatures.