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Report for beam time SC-2791 at ESRF

Following in real time the structural changes during the assembly process of SV40 virus

Background

Viruses are sub-microscopic infectious agents that are unable to grow or reproduce outside a host cell. Viruses consist of two or three parts: all viruses have genetic information coded in either DNA or RNA; all have a protein coat (capsid) that protects these genes, and some have a lipid envelope that surrounds them when they are outside a cell. In contrast to bacterial viruses (Bacteriophages) that have an organized condensed DNA structure within the capsid, in animal, human and plant viruses the DNA or RNA organization, and in most cases the packaging structure order, as well as the forces directing assembly are still unknown. We focus in this proposal on the simian vacuolating virus 40 (SV40) that is a member of the polyomavirus family. The SV40 virus particle is small (~45nm), non-enveloped, with a capsid and viral shell structures that have been determined by cryo-transmission tomography and particle averaging, as well as crystallographic methods. The lattice symmetry of the SV40 capsid was found to be that of an irregular T=7d icosahedron of 72 capsomers with an average internal capsid diameter of 29.5nm (32nm upper limit)[1]. SV40 is a DNA virus consisting of a closed circular dsDNA of 5.2kb, stacked with 20-25 nucleosomes originating with the host cell. The nucleosomes can be approximated by cylinderers of 8.36nm in diameter[2] and 6nm in height[3]. This complex of DNA and nucleosomes is called a minichromosome. Experiments show that the minichromosome forms various self-assembled structures in different environments[4]. At high salt concentrations, the nucleusomes tend to fuse into 10 nm granules, while at lower salt concentration the granules open to 10 nm filament and then to nucleusome strings. Recent cryo-electron tomography experiments[5] show what seems like residual specific nucleusome packaging related to the icosahedral symmetry of the virus.

The SV40 virus can be self-assembled in vitro. Therefore it is an excellent model system to study DNA and protein assembly. SV40 and other viruses may also be used as delivery systems for genetic material, DNA and RNA, for research as well as for gene therapy applications. Recent studies[6] showed that in SV40 the DNA must be highly compacted for packaging. To date, the assembly process of condensed DNA and protein capsid into a virus is poorly understood.

In this project we assembled in-vitro SV40 virus capsid proteins around DNA condensed by nucleosomes (mini-cromosomal DNA particles). Our main goal was to study the **process of SV40 self-assembly**.

Using high resolution 3rd generation synchrotron small x-ray scattering (SAXS) source, we investigated the structure of SV40 and the mechanism by which condensed DNA and capsid protein self assemble into the mature SV40 virus.

Experimental method

This research was conducted in collaboration with the laboratory of Prof. Ariella Openheim, The Hematology department. Prof. A. Oppenheim has developed a unique SV40-based gene delivery system that is constructed in vitro from recombinant SV40 capsid proteins, produced in insect cells, and plasmid DNA. The lab of Prof. Openheim provided us with DNA molecules and recombinant SV40 major capsid protein VP1 as well as other reagents and protocols that they have developed.

In-situ high resolution solution SAXS in transmission mode was used to characterize the SV40 virus assembly. The structural information was studied on length scales ranging from a nanometer to some tens of nanometers, under controlled buffer conditions.

Steady state samples will be in flame sealed quartz capillaries, 1.5 mm in diameter, as was done in our earlier synchrotron experiments [7, 8].

The most important aspect of this study was the ability to follow the assembly process as a function of time, using the flow through setup at ID02.

Results

Our results show that solution x-ray scattering is a very sensitive method to detect small changes in the structure of SV40. The SAXS signal of the full particle is different from the empty capsid. This signal is mainly due to the form factor of the particles, related to the scattering particle's shape and size. We find that the form factor of the full particle is shifted towards lower scattering angles (or q values), indicating that the full particle is *larger* than the empty particles and have a slightly different shape (Figure 1).

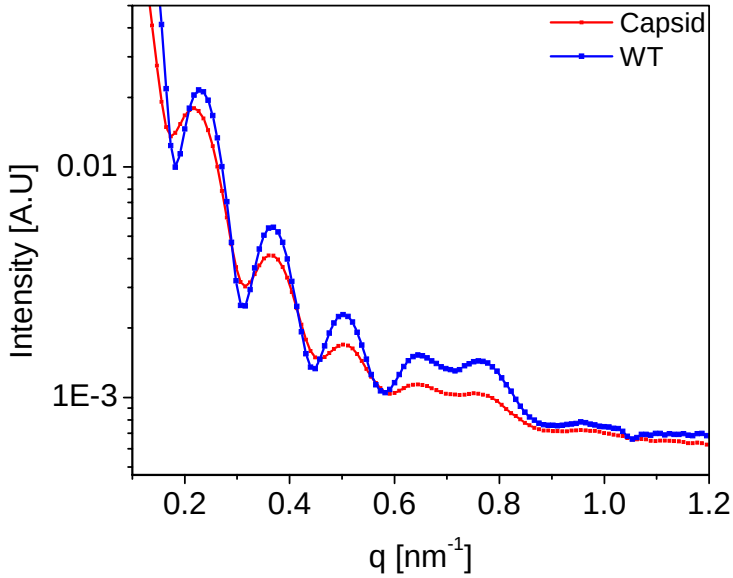

Figure 1. Radially integrated scattering intensity of empty capsid and wild type (WT) SV40.

The scattering amplitude is the Furrier transform of the sample's electron density and the measured intensity is the square of the amplitude. In solution the sample is isotropic and therefore all possible orientations contribute to the scattering amplitude and this has to be taken into account. We have been able to simulate the x-ray scattering experiment to model the structure of the viral particles. By fitting the model to the data we were able to determine the size of the full and empty particles at sub-nanometer resolution. We find that the empty particle is smaller by ca. 1.6 nm, indicating that the signal is extremely sensitive to the particle size; we note that the resolution of transmission electron microscopy is an order of magnitude lower. Our data shows that we can use SAXS to determine the viron structure. Part of this data is now published in:

Szekely P., Ginsburg, A., Ben Nun, T. and Raviv, U. "*Solution X-Ray Scattering Form Factors of Supramolecular Self-Assembled Structures*", Lamgmuir, **26**, 13110-13129, 2010.

The next step was to follow the virus assembly process by monitoring the evolution of the SAXS signal with time following introduction of DNA to capsid protein solution (Figure 2).

Our time-dependent low resolution experiments showed that in the early stages of the assembly the particles have a diameter of about 52 nm where is in the end of the assembly process we found that the diameter is about 47 nm.

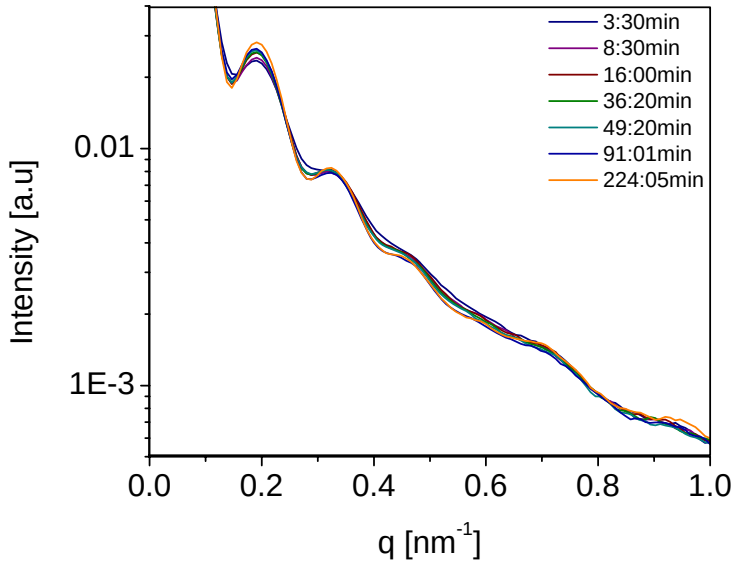

Figure 2. Time dependent radially integrated intensities of assembled DNA + VP1 in assembly buffer. The time indicated is from the mixing moment.

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