SC- 3654

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

Viruses are evolved examples of self-assembled structures. This ability to self assemble have been used to assemble structures for guided synthesis of inorganic and organic nanostructures, as cages for packaging cargos, and as vectors for gene therapy. Nevertheless, the mechanisms by which spherical viruses assemble from hundreds of capsid proteins around nucleic acid, as well as the dynamics of virus structure in solutions are yet unresolved.

Our model system for studying virus assembly and dynamics is the Simian Virus 40 (SV40), a small nonenveloped virus belonging to the polyomavirus family. SV40 viral capsid proteins encapsidate a circular double-stranded (ds) DNA genome of 5,200 base pairs compacted by histone octamers, forming a minichromosome structure. In vitro, the recombinant capsid protein, VP1, of SV40 may also assemble cooperatively around different nucleic acids, to form virus-like particles (VLPs) in which different scaffolds direct the assembly into different capsid geometries.

To resolve the high resolution structure of wt SV40, as well as its changes in response to different solution conditions, we are combining solution Small Angle X-ray Scattering (SAXS), unique SAXS data analysis tools that we are developing in our lab, and Monte Carlo simulations. By combining these methods we hope to better fit a high resolution structural model to the solution SAXS data from wt SV40 and gain insights into the organization of the minichromosome that until now could not be resolved.

We were able to follow the structural changes of wt SV40 under different solution conditions, mimicking the conditions in which disassembly naturally occurs. Our SAXS results showed a gradual change in the conformation of the virus from a compact structure at pH 3 to a completely disassembled structure at pH 10.7. In addition, our SAXS data indicated that wt SV40 swelled when chelating and reducing agents were added. The size of the swollen structure can be altered by applying external osmotic pressure, suggesting a method to estimate the strength of interactions between capsid proteins.

Using Time-Resolved SAXS (TR-SAXS) we were able to directly follow SV40 viral proteins encapsidating short ssRNA (524 nucleotides) and 5.2 kbs circular dsDNA molecules. The assembly process around ssRNA yields T = 1 icosahedral particles comprised of 12 protein subunits and one RNA molecule. The encapsidation reaction around dsDNA yields T = 7 icosahedral particles similar to the structure of wt SV40. The reaction time scale is in the order of minutes.

Assembly around RNA

The assembly reaction between VP1 pentamers and 524 nt ssRNA results in the formation of T = 1 structure. Our TR-SAXS results from ID02, using stopped flow setup, revealed that the assembly process around ssRNA is extremely fast, where the reaction is near equilibrium within 10sec, and already 1/3 complete after 35msec. Both TR-SAXS and Electrophoretic Mobility Shift Assay (EMSA) measurements indicated that the assembly process is very cooperative and effectively can be considered as a two state reaction. This was also suggested by the existence of the isosbestic point in the SAXS data and the distribution of ssRNA on the gel. Such a cooperative process can be assumed to propagate through slow nucleation phase at early stage of the reaction, following a series of fast elongation steps leading to the final product. Curve fitting analysis was used to extract the concentration of VLPs as a function of time. These data were then fitted to a two phases kinetic model using a master equation fitting. The Rate constant for the elongation phase, extracted from the model, is of the order 109 M^{-1} sec^{-1}. A detailed description of the measurement procedure, analysis technique, and results regarding the assembly process around ssRNA can be found in our paper: Kler et al., JACS, 134, 8823.

Assembly around dsDNA

TR-SAXS was used for the study of VLPs formation around 5.2 kbp circular dsDNA. Measurements were performed at ID02 ESRF using a flow through setup. TR-SAXS results (Figure 1) indicated that the assembly process for the encapsidation of dsDNA by VP1 pentamers followed a slower time scale than the assembly around ssRNA (minutes for DNA-VLP compare with milliseconds for the RNA-VLP). We have tried to analyze the time resolved data with the same procedure used for the RNA-VLP assembly (Kler et al., JACS, 134, 8823). Fitting to a linear combination of reactants and product signals, however, gave poor results especially for the early times in the reaction. We attribute this poor fit and the lack of a sharp isosbestic points, to the accumulation of intermediates throughout the reaction. If intermediates, including partial assemblies or amorphous VP1-DNA complexes, accumulated throughout the reaction, their contribution to the scattering intensities could not be ignored and an assembly model that includes their contribution has to be used. A possible way to identify the partial assemblies during the reaction may depend on finding the dominant structures at equilibrium when the dsDNA:VP1 ratio is lower than the correct stoichiometric ratio in the complete VLP (as shown in Figure 1 a. and b.)

We should note that ID02 beamline is a wonderful beamline and, as far as we know, is the most suitable beamline for our experiments.

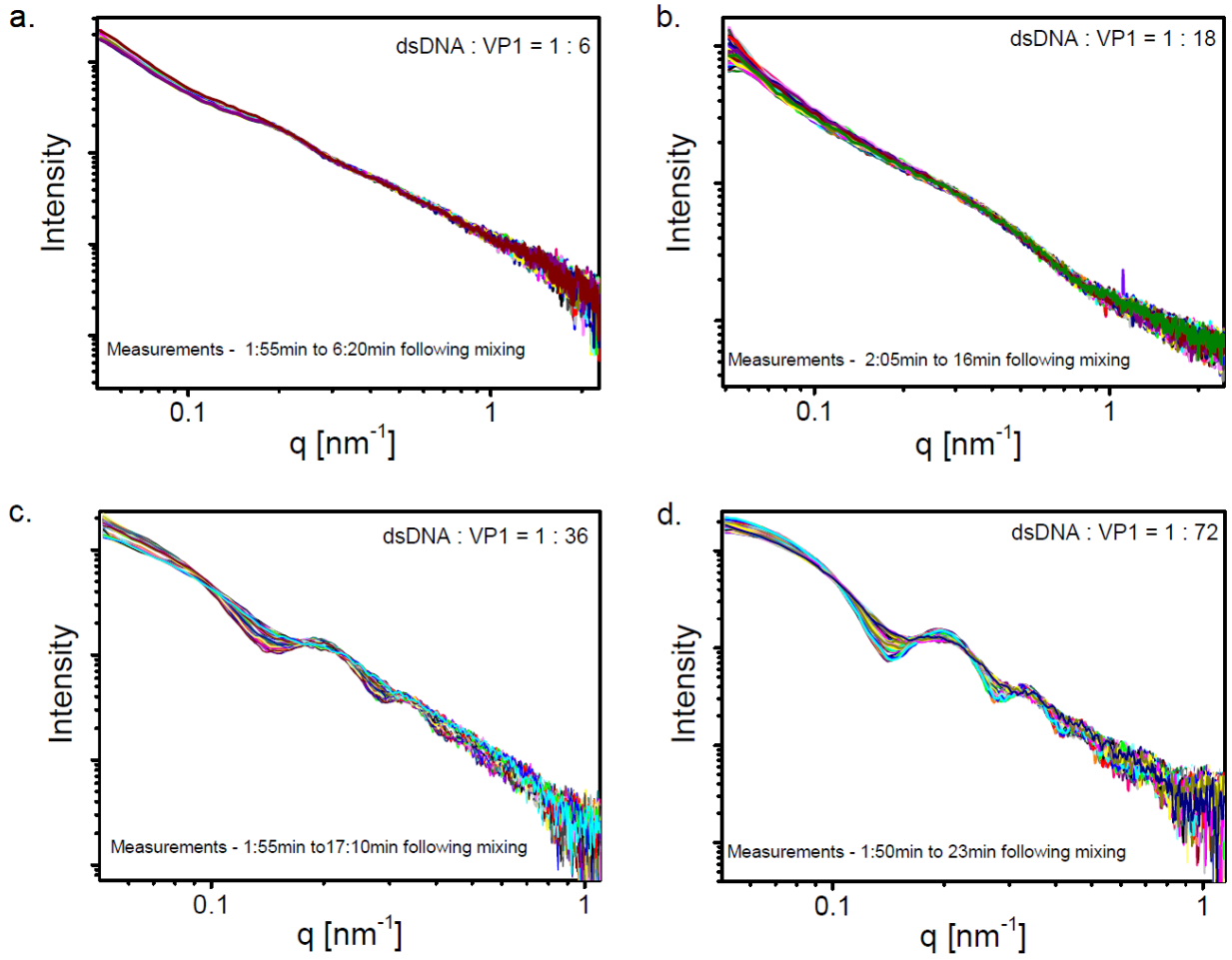

Figure 1. Kinetics of VP1 assembly around circular dsDNA. Radially integrated TR-SAXS curves of 0.21 mg/ml VP1 (2.7 μM) mixed with different molar ratios of 5.2kbp circular dsDNA in 150 mM NaCl, 50mM MOPS, pH 7.2 at room temperature. The first time point is approximately 2min following mixing and the last time point ranged between 6:20min and 23min (at different VP1:DNA ratios).