SC-4389

Following in real-time the structural changes during the assembly process of 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.

About half of known virus families have icosahedral capsids. Icosahedral viruses are diverse in size, protein fold, genome, and host. Their self-assembly is fundamental to virology. Yet this reaction is poorly understood because its complexity places it at the borders of physics, chemistry, and biology. Nonetheless, capsid assembly has recently emerged as an antiviral target.

Our aim is to resolve the mechanisms by which viruses assemble from hundreds of capsid proteins around nucleic acid, as well as the dynamics of virus structure in solutions.

Our model systems for studying virus assembly and dynamics are Hepatitis B virus (HBV) and the Simian Virus 40 (SV40). Chronic HBV infects 240M people leading to 780,000 deaths each year.

Chronic HBV infection is based on the circular viral DNA resident in the nucleus. This DNA is the template for pre-genomic RNA (pgRNA) for virion production and mRNAs for viral protein synthesis. *In vivo*, 120 copies of the homodimeric HBV core protein assemble around a complex of pgRNA and the viral reverse transcriptase (RT). Together, the capsid and its contents comprise the HBV core.

Inside the capsid, RT synthesizes the relaxed circular dsDNA genome from the linear pgRNA template. The DNA-filled core either acquires an envelope or recycles to the nucleus to add to the cccDNA pool. The 183-residue core protein (Cp183) has an assembly domain (residues 1-149, Cp149) and a C-terminal nucleic acid-binding peptide. In response to solution conditions, purified Cp183 can be induced to form empty or RNA-filled capsids; purified Cp149 only forms empty capsids.

SV40 is 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.

Results.

We measured HBV Cp149 assembly kinetics and steady state structures formed in 50 mM HEPES at 37°C with different [NaCl] (Figs.).

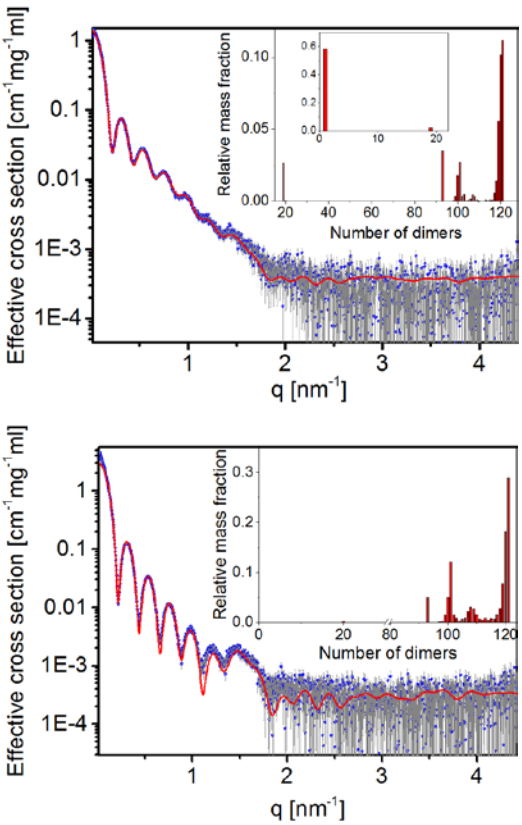

Figure 1. Radially integrated steady state *SAXS data (symbols) of ca. 2 mg/ml Cp149, in 50 mM HEPES and 100 (top) or 300 (bottom) mM NaCl, at 37°C.* The red curves are the calculated model based on analysis with ***D+***. The relative molar fraction as a function of number of dimers are shown at the insets and were obtained by maximum entropy optimization.

In an initial experiment we measured the solution SAXS of HBV core protein assembly domain dimer, Cp149, and compared it with the curve calculated, using our home-developed state of the art analysis software, ***D+***, from the pdb file 2G33-CD and other small intermediates (Fig. 2). This sample was intended to be pure dimer. It had been freshly treated with urea to dissociate residual capsid and then dialyzed into 50mM HEPES. Nonetheless, we found a very small fraction of capsids, indicating the importance of SEC purification of Cp149 dimer immediately before SAXS.

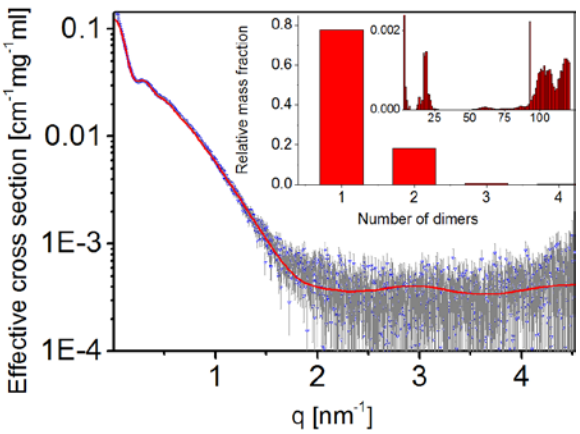

Figure 2. Radially integrated steady state *SAXS data (symbols) from 2 mg/ml Cp149, in 50 mM HEPES at 10°C.* The red curve is the D+ calculated model with the relative molar fractions as a function of the number of dimers, shown at the insets, obtained by maximum entropy optimization.

Unassembled dimer control led to two important results: a small fraction of dimers-of-dimer and dimer conformation. The concentration of dimers-of-dimer indicates a pairwise dissociation constant of 4mM, in excellent agreement with our expectation for Cp149 under these conditions. Furthermore, we found that the dimer component is similar to the structure of dimer from capsid (e.g. 2G33) and free dimer structure (3KXS), but is distinctly different from predictions for HBeAg.

Steady state SAXS spectra of equilibrated assembly reactions demonstrate our ability to dissect simple mixtures (Fig. 2). Using our analysis program ***D+*** we initially calculated the solution x-ray scattering curves from the pdb files of dimer and T=4 capsid, and all intermediates that could be generated by sequentially removing one dimer at a time from a T=4 particle. The steady state spectra formed in the presence of 100 and 300 mM NaCl were modeled using ***D+*** combined with maximum entropy algorithms of our time-resolved analysis program, ***T+,*** to determine the relative molar fraction of dimers, intermediates, and fully formed capsids (Fig. 2). Interestingly, the ***D+*** fits for these data were based on the assumption of dimer and T=4 particles as the only geometries, leading ***D+*** to identify a major intermediate consisting of a T=4 particle missing about 30 subunits, i.e. an excellent approximation of a T=3 particle. In 100 mM NaCl and 300 mM NaCl, as predicted the amount of dimer decreased and the amount of capsid increased. In both cases, about 20% of the capsids were T=3 particles.

Time-resolved SAXS measurements are more difficult to generate and analyze (Fig. 3). The goal of this experiment was to demonstrate our ability to manipulate assembly rate by adjusting Cp149 concentration and buffer conditions. For observation of assembly kinetics we mixed reactants at 4°C and then rapidly increased temperature to 37°. As HBV assembly is entropy driven, we reasoned this approach would provide well-defined kinetics. As assembly progressed, the spectra showed progressively stronger oscillations as the capsid-sized components increased. We did not reach steady state in this experiment. Equilibrium typically requires longer times (ca. 24 hr).

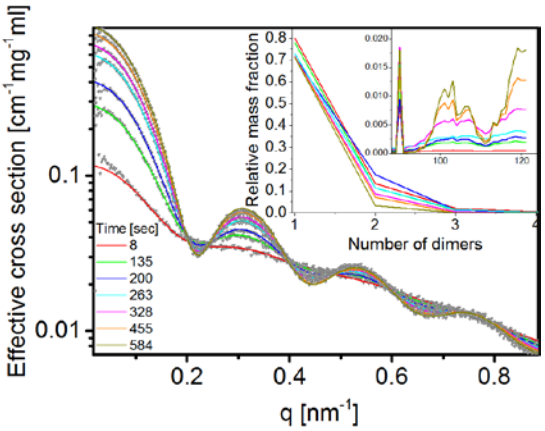

Figure 3. Radially integrated TR-SAXS data (symbols) of 2 mg/ml Cp149. Kinetic experiments in 50 mM HEPES and 100 mM NaCl, at 37°C. The curves are the calculated model based on analysis with ***D+***. The insets show the relative mass fraction of each of the structural intermediates at different time points, as indicated by the color of the curves at the main figure.

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. Returning the pH to 7 reassembled to the same structure as the original virus. However, reassembly did not occur when we waiting for more than 30 min at pH 10.7. Using TR-SAXS setup, we were able to follow the disassembly of wt SV40 as a function of time.

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 was in the order of 2-3 minutes.