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Following in real-time the structural changes during Hepatitis B virus assembly

The assembly of a virus capsid (the protein shell that protects the genome of viruses, made of many copies of one or a few proteins) is a critical step in the lifecycle of viruses and a remarkable example of macromolecular self-assembly reaction. Hepatitis B Virus (HBV), is a well-characterized endemic pathogen and a promising target for antiviral drug development. In-vivo, 90% of the HBV particles are empty. Antiviral agents (including molecules that are now in clinical trials) act by manipulating the mechanism of capsid assembly reaction. The assembly reaction of HBV capsid can be recapitulated in vitro.

The mechanism of capsid assembly has remained poorly understood because it involves a large number of capsid protein subunits (120 in HBV), a huge number of possible intermediates (about 10^{30} in HBV), and many more potential assembly pathways, which are impossible to explore. Assembly reaction, however, can be very rapid (msec) and therefore very difficult to follow. To resolve the underlying mechanism of virus assembly it is critical to resolve the early steps of assembly.

To explore the pathways that virus capsid subunits follow to form stable 120-subunit HBV empty capsids, we have used state-of-the-art Time-Resolved Small Angle X-ray Scattering at ID02 beamline and data analysis methods, developed in our lab in the past decade. Our computational approach includes umbrella sampling of Monte Carlo simulations of assembly to generate a realistic library of intermediates, calculating scattering curves of atomic intermediates models, maximum entropy optimization analysis to fit observed SAXS with calculated curves, and thermodynamic analysis of macromolecular assemblies. These are all incorporated into our home-developed program, D+. From rigorous analyses of our data, and examination of the free energy landscape, we find that an increase of 1 k_BT in the interaction strength between subunits can dramatically affect the reaction rates, accumulation of intermediates, and assembly mechanism. Remarkably, under the conditions that we tested, the path of assembly was determined in less than a second.

Under mild assembly conditions (Figure 1), after a 10 sec lag phase, the reaction appeared two-state from dimer to 120-dimer capsid. The energy landscape directs the reaction to follow a narrow minimum free energy path through the most compact and stable intermediates. There is a relatively high and broad energy barrier, facilitating multiple reversible steps, following which the energy decreases towards the full capsid with no local minima, consistent with a heterogeneous nucleation mechanism. At aggressive assembly conditions (in which the interaction energy between subunits is stronger by only 1 k_BT), a diverse array of small to mid-size intermediates accumulated within the first 250 msec. Capsids then assembled by either slow elongation of the mid-size intermediates or by establishing new 'capsid assembly lines'.

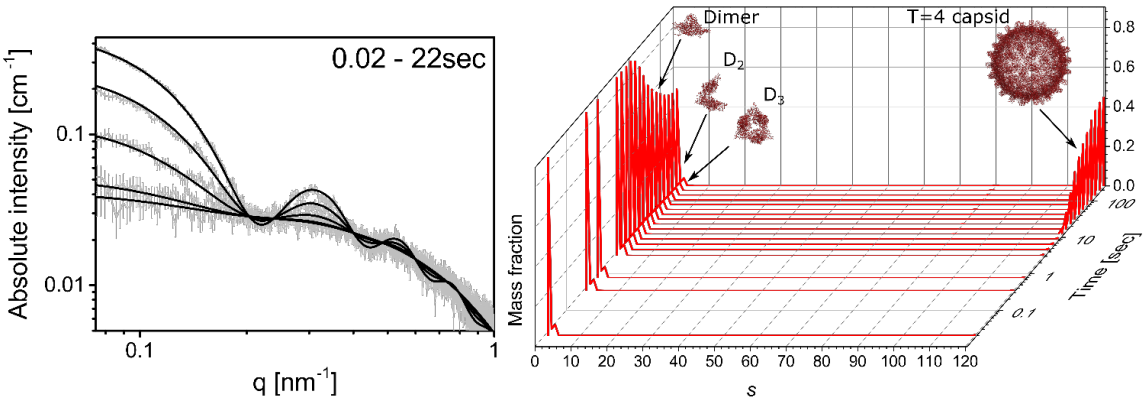

Figure 1. TR-SAXS data (left) under mild assembly conditions. Later times correspond to more oscillatory signals. The temporal evolution of the mass fraction of intermediates as a function of the size, s, of capsid intermediates (s = number of subunits).

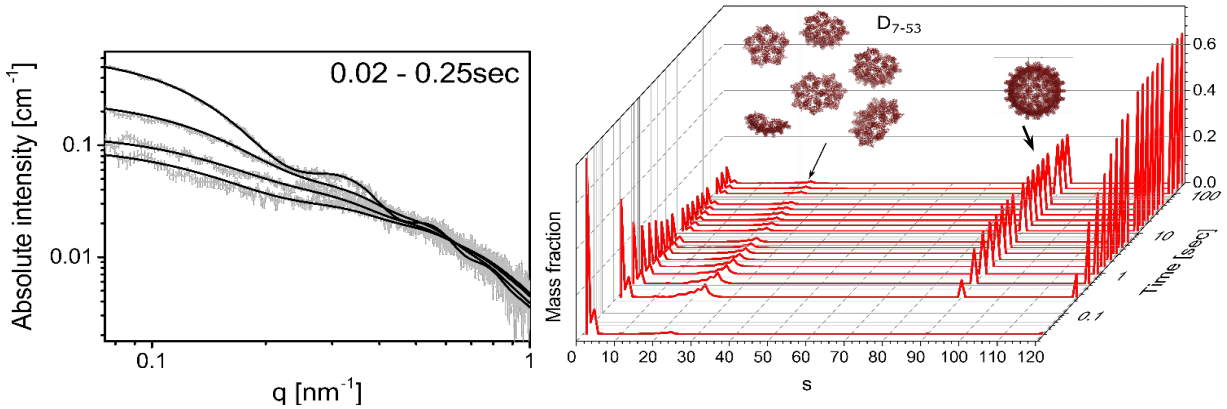

Figure 2. TR-SAXS data (left) under aggressive assembly conditions. The temporal evolution of the mass fraction of intermediates as a function of the size, s, of capsid intermediates (s = number of subunits).

The experimental and analysis approach developed and established in this work can be used to characterize other complex multicomponent macromolecular assembly reactions. The mechanistic insight gained by our analyses provides a means to direct, tune, and control complex self-assembly reactions.

The effect of zinc ions on the assembly and the mechanism of capsid disassembly were investigated by adding GuHCl. We observed the zinc ions created barrier for assembly and produced small oligomers. GuHCl disassembled the capsid but our analysis suggests that the disassembly pathway is different than the assembly pathway.

Part of this project was published in ACS Nano and JACS.