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Following in real-time the structural changes during SV40 and Hepatitis B virus assembly

SV40

Virus particles are a remarkable example of self-assembled nanobiomaterial-based machines. Viruses encapsulate and release genetic materials at different points of their life cycle, at which they are exposed to a wide range of pH values. To assemble and disassemble, viruses should be both stable and dynamic. The interactions between the capsid and its cargo are therefore crucial for understanding the function of viruses and for designing viral-based nanotechnology platforms and functional nanobiomaterials. As we show in our recent paper on pH Stability and Disassembly Mechanism of Wild-Type Simian Virus 40 in *Soft Matter* result from the project, pH can dramatically affect the stability and infectivity of wild-type Simian Virus 40 (wt SV40), a member of the polyomavirus family. wtSV40 is a fascinating virus because it has a minichromosome, containing about 20 nucleosomes and 5.2 kb circular DNA, which is highly dynamic and hence difficult to study.

Using time-resolved cryo-TEM and state-of-the-art synchrotron time-resolved solution X-ray scattering at ID02 beamline, together with novel X-ray scattering data analysis tools, developed in our lab in the past decade, we unraveled the disassembly reaction mechanism of wtSV40, triggered by a pH jump, at high temporal and structural resolutions. The pathway discovered in our paper is likely to be similar in other members of the polyomavirus family.

We have also showed that the virus has a wide pH stability and infectivity range, making it very attractive nanobiomaterial for various bionanotechnological applications and delivery systems.

Our study also provides insight into possible approaches for designing antiviral drugs and directing, tuning, and controlling the structure and properties of viruses or virus-like nanoparticle assemblies, which can serve as functional nanobiomaterials for bionanotechnological applications.

The novel approach developed in this study could be used for investigating other complex self-assembled nanobiomaterials.

Another paper on the Effect of Calcium Ions and Disulfide Bonds on Swelling of SV40 was published in ACS Omega.

HBV

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, 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'.

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.

Part of this project was published in ACS Nano and another paper, ***Rapidly Forming Early Intermediate Structures Dictate the Pathway of Capsid Assembly***, was deposited in the chemrxiv and is now under review.