Studying viruses using solution X-ray scattering

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Abstract

Viruses have been of interest to mankind since their discovery as small infectious agents in the nineteenth century. Because many viruses cause diseases to humans and agriculture, they were rigorously studied for biological and medical purposes. Viruses have remarkable properties such as the symmetry and self-assembly of their protein envelope, maturation into infectious virions, structural stability, and disassembly. Solution X-ray scattering can probe structures and reactions in solutions, down to subnanometer spatial resolution and millisecond temporal resolution. It probes the bulk solution and reveals the average shape and average mass of particles in solution and can be used to study kinetics and thermodynamics of viruses at different stages of their life cycle. Here we review recent work that demonstrates the capabilities of solution X-ray scattering to study in vitro the viral life cycle.

Keywords SAXS · Self-assembly · Virology · SV40 · HBV · Time-resolved SAXS

Introduction

Viruses are amongst the simplest biological forms in nature and some may be regarded in a simple sense as quite literally being an infective nucleic acid (Moreira and López-García 2009; Lwoff 1957; Villarreal 2004). A more common form of viruses is of nucleic acid encapsulated in a protein protective shell called a capsid. The capsids are usually made up of one or few types of subunits arranged in large structures. By doing so, the genetic material must be shorter and thereby easier to enclose (Caspar and Klug 1962). About half of virus capsids are icosahedral. Caspar and Klug showed in their work (Caspar and Klug 1962) that an icosahedral shell is the most stable way of enclosing space when the same subunit is used to form the shell. To do so, a quasi-equivalence must be taken into account in which not all the subunits are in an equal environment; hence, slight

deviations are allowed. Icosahedral capsids are classified into different structures based on the amount of subunits and capsid size. It is common to classify the icosahedral capsids by their triangulation (T) number. These numbers represent a ratio between capsid volumes by comparing the area of the triangular face of the icosahedron to that of the regular, and smallest, icosahedron ($T = 1$). The icosahedral capsid will have $60 \times T$ capsid proteins arranged so 12 will be in a 5-fold symmetry (pentamers) and $10(T - 1)$ in a 6-fold symmetry (hexamers).

Viruses were initially of interest due to their pathogenic properties. The first virus presented in the literature was the tobacco mosaic virus (TMV) (Lustig and Levine 1992). Back in 1892, Dmitry Iwanovsky was able to filter the sap from leaves infected by the tobacco mosaic disease, and used it to infect other healthy plants. This finding led him to conclude that there are pathogenic particles that are smaller than microbes and fungi. As such, Dmitry Iwanovsky is regarded as one of the founding fathers of virology (Lustig and Levine 1992). Since those initial discoveries, many other pathogens have been investigated. Pathogenic viruses are still of much interest owing to their impact on public health. Viruses such as the human immunodeficiency virus (HIV) and hepatitis B virus (HBV) pose major health concerns especially in third world countries. Besides diseases, viruses have an important role in biology. A big resurgence in viral studies, especially in bacteriophages, started in the 1940s after Max Dellbrück moved to Caltech. Bacteriophage studies lead to great discoveries such as the

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random nature of mutations, and the three-nucleotide reading frame (Keen 2015). Viruses can also be used for therapy (Lin et al. 2017), as gene vectors (Lundstrom 2018), or as building blocks for devices (Fischlechner and Donath 2007). Therefore, a better understanding of viral assembly mechanisms can have impact on many fields.

The first structural studies were on TMV, which was also the first virus to be crystallized (Kay 1986). Since then, additional approaches and methods were used to study viral structure and physical properties. Jacobson et al. wrote a review about methods that can be used to study structure, properties, and assembly of viral capsids (Kondylis et al. 2019). There are also many theoretical studies on capsid stability, structure, and assembly (van der Schoot and Zandi 2007; Perlmutter and Hagan 2015; Božič et al. 2012). Here we shall focus on the use of small-angle X-ray scattering (SAXS) to study viral assembly properties. Owing to the homogeneity of viral and virus-like particle (VLP) samples, SAXS can be used as a tool with both high spatial and high temporal resolution, sensitive to structural differences in the nanometer scale, and can follow reactions in the millisecond scale. The review starts with a brief description of the theory of SAXS. It is then followed by an analysis of published literature describing the in vitro viral life cycle, as studied by SAXS: VLP assembly (empty capsids), assembly on scaffolds, DNA structure in assembled capsids, maturation of virions, and virus disassembly.

Basic SAXS theory

SAXS results from elastic interactions between X-rays and matter. The measurable quantity in these experiments is the scattered intensity, which is the scattered amplitude squared. The elementary scattering unit is the electron, which is regarded as structureless. The scattering amplitude from N particles is

$$A(\mathbf{q}) = \sum_{j=1}^N f_j(q) e^{i\mathbf{q}\mathbf{r}_j} \quad (1)$$

where $\mathbf{q}$ is defined as the momentum transfer vector, whose magnitude is $q = \frac{4\pi}{\lambda} \sin(\theta)$, θ being half the scattering angle with respect to the incident beam and $\mathbf{r}_j$ is the position of each scattering element. f_j is the form factor of the particle; for an electron it can simply be taken as the Thomson scattering length, $r_0 = 2.82 \times 10^{-5}$ Å. For a molecule, the form factor becomes

$$f_j(q) = -r_0 \int \rho(\mathbf{r}) e^{i\mathbf{q}\mathbf{r}} d\mathbf{r} = \begin{cases} -r_0 Z & \text{for } q \rightarrow 0 \\ 0 & \text{for } q \rightarrow \infty \end{cases} \quad (2)$$

where $\rho(\mathbf{r})$ is the molecular electron density and Z is the total number of electrons. From Eq. 2, it can be seen that the form factor is the Fourier transform of the electron density.

If the molecules are arranged in a lattice then each of the lattice vectors $\mathbf{R}_n$ must be taken into consideration in the form factor, resulting in two separate contributions to the form factor: that of the molecule, i.e., the lattice subunit, and that of the lattice itself. The form factor is then multiplied by the lattice sum:

$$A^{\text{crystal}}(\mathbf{q}) = \sum_{j=1}^N f_j e^{i\mathbf{q}\mathbf{r}_j} \sum_{r=1}^k e^{i\mathbf{q}\mathbf{R}_r} \quad (3)$$

For large assemblies (like viruses), with repeating subunits (identical or not) that are shifted, rotated, and docked onto one another, the scattering amplitude is:

$$A(\mathbf{q}) = \sum_{j=1}^J \sum_{m=1}^{M_j''} \left[f_j(\mathbf{A}_{j,m}^{-1} \mathbf{q}) \sum_{k=1}^{K_{j,m}} e^{i\mathbf{q}\mathbf{R}_{j,m,k}} \right], \quad (4)$$

J is the number of different types of objects, which can either be geometry-based or atomic-based models, taken, for example, from PDB files. M_j'' is the number of unique orientations of object type j , determined by the rotation matrices $\mathbf{A}_{j,m}$. $K_{j,m}$ is the number of real-space translations, $\mathbf{R}_{j,m,k}$, of object j with orientation $\mathbf{A}_{j,m}$. D+ software (<https://scholars.huji.ac.il/uriraviv/software/d-software>), developed in our lab, can compute this type of amplitudes (Ginsburg et al. 2016; Ginsburg et al. 2019).

In solution, particles are distributed in random orientations; hence, the intensity is orientation averaged in reciprocal space. The scattering is sensitive to difference in electron densities between the particles and the solvent, $\Delta\rho(\mathbf{r})$. In the continuum limit, we get:

$$A(\mathbf{q}) = -r_0 \int \Delta\rho(\mathbf{r}) \exp(i\mathbf{q} \cdot \mathbf{r}) d\mathbf{r}, \quad (5)$$

The solution scattering intensity, I , as a function of q , is computed by squaring the amplitude and orientation average in reciprocal space:

$$I(q) = \frac{1}{4\pi} \int_0^{2\pi} \int_0^\pi |A(\mathbf{q})|^2 \sin\theta_q d\theta_q d\phi_q \quad (6)$$

where θ_q and ϕ_q are the polar and azimuth angles in $\mathbf{q}$ -space, respectively. For a more in-depth review of SAXS, see Li et al. (2016) and Boldon et al. (2015).

SAXS studies of virus' life cycle

Viral particles can be regarded as biological nanoparticles, and therefore can be used as building blocks for larger structures, such as cubic crystals (Asor et al. 2017). These types of viral structures can be used for electronics or biotechnology (Wen and Steinmetz 2016; Li et al. 2009; Niu et al. 2006) and will not be covered in this review. We will

try to illustrate how different steps in the viral life cycle in-vitro can be studied by SAXS. We shall not follow the full process of assembly, stability, maturation, and disassembly of the same virus but rather a few different viral capsids for the various steps.

Assembly of empty virus-like particles

Capsid assembly results in empty capsids (Michel et al. 2006; Fox et al. 1998) and provides important insight into virus assembly. The process of empty capsid assembly was previously studied by various methods such as light scattering and mass spectrometry (MS) techniques (Endres et al. 2005; Casini et al. 2004; Prevelige et al. 1993; Lutowski et al. 2018). The non-templated assembly pathways of HBV capsids was studied recently by SAXS (Asor et al. 2019b). For the $T = 3$ and $T = 4$ capsids formed by the capsid protein (cp) of HBV, it was shown that there are 1.8×10^{30} unique intermediates, making the problem of capsid assembly similar to Levinthal's paradox: viruses cannot sample the whole space of possible intermediates in a biologically relevant time scale, meaning some selection has to be applied (Zwanzig et al. 1992). A pool of intermediate models was generated using Monte Carlo simulations, and their computed solution scattering profiles were calculated using D+ software <https://scholars.huji.ac.il/uriraviv/book/d-0>; (Ginsburg et al. 2019). The models were computed by docking the atomic model of the cp into the lattice of each intermediate. When an energetic term was applied to select the stable species that dominated the reaction mixture, only a few hundred intermediates were chosen, mapping out a narrow reaction path through the immense reaction landscape. It was found that there is a narrow window of dimer-dimer contact free energy (between -4.2 and -5.4 kcal/mol) at which regular assembly occurs. The window limits can be achieved by controlling the ionic strength. Higher ionic strength increases the dimer-dimer association free energy. Below this energy, the energetic pathway leads to the dimer form. Above it, the assembly pathway can lead to kinetic traps in which the barriers for going back to regular assembly are high and irregular assembly continues, or trapped intermediates accumulate.

Slow dialysis to higher ionic strengths can lead to an increase in dimer-dimer contact energy without getting the assembly process into kinetic traps. Assembly yield increases with initial capsid protein concentration, ionic strength, and temperature. Under physiological assembly conditions, dimers and complete capsids comprised 99% of the assembly products. The state-of-the-art fitting procedures presented in this work (Asor et al. 2019b) show that the sensitivity of SAXS for studying assembly reactions in solution is such that the presence of up to 2% of small intermediates (60-mers or less), cannot be excluded.

Assembly of cargo-filled virus-like particles

The next step in studying the assembly of virus-like particles (VLPs) involves including a nucleic acid in the assembly process. Caspar and Klug suggested that if the complexity of viruses is to be divided into classes, the lowest class will be the infectious nucleic acid itself, and the second class will be a simple virus: nucleic acid encapsulated in a protecting protein capsid (Caspar and Klug 1962). Many groups studied the assembly of capsid proteins around nucleic acids, DNA, RNA, and charged polymers (Porterfield et al. 2010; van Rosmalen et al. 2018; Rayaprolu et al. 2017). Vega-Acosta et al. (2014) studied the electrostatic interactions between the negatively charged nucleic acid (or different polyanion) and the positively charged binding regions of the capsid. Two main mechanisms for assembly around nucleic acid are common: (1) When the protein-protein interactions are of the same order of magnitude as the protein-nucleic acid interactions, a nucleation and growth assembly mechanism takes place. During this mechanism, a slow step of nucleation is followed by a series of faster elongation steps. (2) When the protein-nucleic acid interactions are strong, the en masse mechanism governs the assembly. The proteins quickly adhere to the nucleic acid in an undefined aggregated-like structure, and then anneal into the full capsid (Hagan 2014).

The assembly of simian virus 40 (SV40) capsid proteins, VP1-pentamers (VP1₅), around a 500-mer RNA was studied by time-resolved SAXS (TR-SAXS) (Kler et al. 2012). Even though wild-type SV40 has a $T = 7$ symmetry and naturally binds 5200 base-pair circular dsDNA, short RNA leads to the simplest case of assembly, that is, a $T = 1$ capsid in which all of the pentamers have five-fold symmetry. The reaction was nearly complete within 2–3 s. No intermediates accumulated to a measurable amount. Even though 12 pentamers and 1 ssRNA are needed to form the capsid, the process is very fast and occurs with high fidelity. A two-state mechanism is supported by a clear isosbestic point in the SAXS curves. The nucleation step was 100 times slower than the elongation rate. A mechanism in which the capsid protein binds to the RNA, through long-range electrostatic interactions, and then diffuses along it to the nucleation site was purposed. Using SAXS and TR-SAXS, allowed for a complete kinetic analysis of the assembly process and showed that assembly followed the nucleation and growth mechanism.

The en masse mechanism was observed in the analysis of cowpea chlorotic mottle virus (CCMV) assembly around native nucleic acids and other polyanions (Chevreuil et al. 2018). Using analysis of the average mass indicated by the intensity at $q \rightarrow 0$, the average mass growth rate was followed in solution. The assembly process had two steps: a quick binding of capsid proteins onto a ssRNA,

through an en masse mechanism, to form nucleo-protein complexes (NPCs), followed by a slow maturation process into viral capsids, induced by lowering the pH (increasing the protein-protein interactions). The binding time scale was milliseconds, whereas the relaxation times to form the NPCs were seconds, indicating an en masse route. Lowering the pH made the interaction regime similar to the nucleation and elongation mechanism (NA-cp and cp-cp interactions in the same order of magnitude) and therefore the mechanism was slower, with binding and annealing times on the 1000-s time scale. The formation of mature capsids was followed by the gradual formation of a form factor; when oscillation minima become deeper and well defined the formation of more regular and homogeneous structures can be deduced. Furthermore, measuring with various temperatures revealed the en masse reaction energy barrier of $20 k_B T$.

When inspecting the assembly around polystyrene sulfonic acid (PSS) it was found that capsid-like structures were assembled at pH 7.5, though with a lower yield. The reason for that is the hydrophobic character of PSS. The result shows that the properties of the assembled cargo affect the final product and assembly conditions.

Capsid-confined DNA behavior

It is possible to also inspect the nucleic acid organization inside the capsid of DNA viruses and test its physical properties under the capsid induced confinement conditions. The persistence length of dsDNA is about 50 nm (Guilbaud et al. 2019; Manning 2006), which is similar to the diameter of many capsids. Bacteriophages use biological motors to pack the DNA in their capsids, thereby creating high pressure inside the capsid (Evilevitch et al. 2003; Kindt et al. 2001). The tight packaging of the DNA inside the λ bacteriophage, which takes up to 59% of the capsid's volume (Nurmemmedov et al. 2007), makes it well organized and a good candidate for SAXS analysis. When comparing the scattering patterns of empty and full λ bacteriophage capsids the DNA peak is clear at the higher q values (2.5 nm^{-1}). The position of the peak represents the d -spacing between the DNA strands in the capsid, so following its location under various conditions allows for the DNA structural and physical analysis. Qiu et al. (2011) were able to show the interplay between the bending energy and the electrostatic self-repulsion energy of packaged DNA inside λ -phage capsid as a function of DNA length and ionic strength. While self-repulsion prefers to separate the strands and increase the DNA-DNA spacing, the energetic cost of bending makes it preferable to have the DNA strands closely packed to decrease their curvature as much as possible. Both decreasing the repulsion and increasing the bending energy decrease the DNA-DNA spacing.

When 48.5 kbp DNA (full length) was used, decreasing the electrostatic repulsion by increasing ionic strength did not change the inter-DNA spacing in the capsid. This is due to the initial short spacing of $27.5 \text{ \AA}$, in which the repulsion regime is dominant and is not enough to overcome the DNA bending energy even at high ionic strength. When using 37.8-kbp dsDNA (78% of the full length), the inter-DNA spacing decreased from $30.3 \text{ \AA}$ by up to $1 \text{ \AA}$ with increasing ionic strength, owing to the larger free volume inside the capsid with the shorter DNA. By comparing the inter-DNA spacing to osmotic stress-distance equation of state, a repulsive energy of $0.8 k_B T/\text{bp}$ was calculated for a buffer containing 10 mM Mg^{2+} . This energy is an order of magnitude larger than the bending energy (about $0.06 k_B T/\text{bp}$ for an average radius of 10–15 nm). It is also possible to combine SAXS with other methods to study the organization of nucleic acid in capsids to gain insight into the biophysics of viral DNA. Liu et al. (2014) combined SAXS with isothermal titration calorimetry (ITC), atomic force microscopy (AFM), and electron microscopy performed at cryogenic temperatures (CryoTEM) to study the phase transition of wt- λ phage DNA with temperature. ITC showed a DNA phase transition close to physiological temperatures (33°C), indicated by a decrease in the absolute value of DNA ejection enthalpy. SAXS was used to corroborate their assumption that the phase transition was from ordered solid-like DNA to a more fluid disordered phase by the following analysis. The area underneath a scattering peak caused by an ordered phase represents the amount of DNA in that phase. Decrease of the peak area represents transition to a less ordered phase. SAXS showed a steep decrease in the amount of ordered DNA at the same critical temperature found in ITC, showing that the phase transition is indeed a solid to fluid-like transition. AFM results showed the same phenomenon, though in a less direct way, by measuring the stiffness of the capsid and how the stiffness decreases at the critical temperature. A combination of SAXS with CryoTEM data led to the conclusion that the phase transition occurred mostly in the center of the capsid rather than near the capsid walls. Owing to the high sensitivity of SAXS to ordered phases it becomes an effective tool for studying the highly ordered DNA inside viral capsids under biological relevant conditions. These studies revealed the energy required to pack the DNA and how favorable its ejection can be under physiological conditions.

Ionic effects on capsid stability

Ions can affect capsid stability. Calcium ions are known to stabilize SV40 (Stehle et al. 1996; Asor et al. 2019a) and CCMV (Speir et al. 1995). Assor et al. (2019b) used SAXS

to show that Ca^{2+} and disulfide bonds stabilize wtSV40 and SV40 VLP. EDTA was used as a chelating agent for calcium ions and DTT was used to reduce the disulfide bonds. wtSV40 swells when both DTT and EDTA are used; using only one of them does not lead to detectable structural changes. The amount of swelling was determined by fitting the SAXS data to a geometrical model, calibrated against an atomic model of the capsid of SV40 taken from the protein data bank (PDB), entry 1SVA (Stehle et al. 1996). This was done by first generating the scattering profile of the solvated PDB using the D+ program (<https://scholars.huji.ac.il/uriraviv/book/d-0>; Ginsburg et al. 2019) and then fitting its first 4 oscillations with a geometrical model of concentric spheres with varying electron densities, representing the electron density of the capsid and the DNA (Fig. 1). After the reference geometric model was obtained, it was possible to change its radius to fit the swellings of the virus. When using both DTT and EDTA were added, the radius of the capsid increased by 5% from 24.2 ± 0.2 to 25.5 ± 1.2 nm. This resulted in about a 17% volume increase. Applying osmotic pressure to the swollen capsids shrank them back. 20 kPa was enough to shrink the capsids, possibly by removing water from the capsid. Addition of CaCl_2 to SV40 VLPs reduced their radius to resemble wtSV40, owing to increasing the protein-protein association free energy rather than decreasing of the internal pressure. (Aramayo et al. 2005) used time-resolved SAXS to follow the effect of divalent ion removal on the swelling of Tomato Bushy Stunt Virus (TBSV). Combining the SAXS data with high-resolution crystallographic data and 3D reconstruction of CryoTEM images allowed for analysis of the structure. This work showed the importance of combining SAXS with other structural methods to obtain an in-depth mechanistic understanding.

Viral maturation

In their life cycle viruses go through maturation processes (Veesler and Johnson 2012; Garoff et al. 1998). Because these processes usually involve delicate morphological changes, and are dependent on environment (solution) conditions, SAXS can be a useful tool (Steven et al. 2005) to study them. Canady, Tsuruta, and Johnes (2001) showed how the *Nudaurelia capensis* ω virus (N ω V) decreases in size upon pH-triggered maturation. In steady-state measurements it was found that between pH 6.4 and 7.2, no structural changes occurred, and the virus-like particles had a radius of 23.7 nm, expected for the pro-capsid structure. Lowering the pH to 5.45 and 5.1 decreased the radius to 19.8 nm (the wild-type capsid radius), suggesting that maturation has occurred. At pH 6, the radius was 20.5 nm as a result of a mixture of the two species in solution: 18% pro-capsid like and 82%

Fig. 1 Calibration of geometric models with computed scattering curves of atomic models. Fitting the radii of the shells to that of the atomic model allowed for quicker analysis. **a** Atomic model (blue symbols, wine color cartoon), based on protein data bank (PDB) entry 1SVA (Stehle et al. 1996). The geometric centers of pentamers in the PDB was at radius of 21.3 nm for the vertices and 21.1 nm for the edges. We assumed a hydration layer with electron density of 364 e/nm^3 and thickness of 0.2 nm. The red curve and red cartoon show the best fitted concentric shell model **b** The electron density profile of the concentric shell models, shown in **a**. **c** Atomic model of a swollen capsid in which the geometric centers were multiplied by a factor of 1.1 (blue symbol, wine color cartoon) and the best fitted concentric shell model (red curve and red cartoon). **d** The electron density profile of the concentric shell models, shown in **c**. The wall thicknesses and outer radii used in the geometric models (red curves) are indicated in the figure. Figure and caption were adapted with permission from Asor et al. (2019a). Publication can be found in the following link: <https://pubs.acs.org/doi/10.1021/acsomega.8b02753>. All further permissions related to the material excerpted should be directed to the ACS

capsid like. This was tested by linearly combining the signals representing each structure and fitting them to the pH 6 signal. Time-resolved analysis clearly showed that the maturation process rate is pH-dependent. At pH 5, the reaction was essentially complete within 100 ms while at higher pH values, up to 6, the reaction took between seconds and minutes to complete. At pH 5.5, two intermediates were

observed; the radius of samples in solution was not a linear combination of both pro-capsid and capsid, and no isosbestic points were observed, indicating it was not a two-state reaction. It was possible to model the intermediate based on the structures of the pro-capsid and capsid as references to the starting and end point of the reaction, respectively. The first intermediate formed and consumed between 0 and 1.25 s and this took the second intermediate between 1.25 and 2.5 s. These fast-fleeting intermediates were spotted thanks to the high temporal resolution of SAXS. Testing the reversibility of the maturation process revealed that maturation is fully reversible in the first 30 s of the reaction when incubated at pH 5 and brought back to 7.2, indicating that the energy difference between the two structures is not significantly large. At longer incubation periods, reversibility was not possible due to autoproteolysis.

Thanks to advances in experimental resolution and data analysis Matsui et al. (2010), obtained insight into the electrostatic origin of the conformational change which occurs during the N ω V pH-triggered maturation. Fitting the data to model signals of uniform spheres showed a decrease of 2.8 Å from the 236 Å radius of the regular size at pH 6.5. The temporal resolution improved to 10 ms, allowing fast transitions to be probed. This resulted in characterization of three transitions in the maturation process: rapid collapse (< 10 ms), continuous size drop, and small transitions (2–3 min). Lee et al. (2004) achieved spatial resolution of viral capsids of 5–7 Å by using spherical shell models for the inner and outer radii for species, present during the maturation process of HK97 bacteriophage. These results further demonstrate the high-spatial resolution of SAXS.

Capsid disassembly

The final stage in the viral life cycle is the ejection of the nucleic acid for infection. To do so, the capsid must be metastable: stable enough to protect the nucleic acid but not too stable such that it cannot disassemble upon infection (Mateu 2013; Perlmutter and Hagan 2015). There are many factors that can trigger disassembly, such as binding to specific proteins or pH change (Evilevitch et al. 2003; Inoue et al. 2011). The disassembly process can be regarded as having two steps: nucleic acid ejection and capsid disassembly. As shown earlier, SAXS is highly sensitive to changes in solution between various form-factors, such as the difference between the subunits and the full capsid or the spacing between inter-DNA chains in the λ phage. Hence, the approach used to study viral assembly and DNA packaging can be applied to study viral disassembly. Bauer et al. (2015) used SAXS to follow the DNA ejection from λ and P22 phages, and human herpes-simplex 1 (HSV-1), which preceded capsid disassembly.

Following differential scanning microcalorimetry (DSC) results, SAXS was used to study the structure of the different capsids as a function of incubation time in various temperatures. The results clearly showed how the DNA peak disappeared when reaching a critical temperature, while the capsid oscillations remained intact. At higher temperatures the capsid disassembled. Law-Hine et al. (2015) studied the pH-induced disassembly of empty CCMV with time-resolved SAXS. Following how $I(q \rightarrow 0)$ changed with time they identified 3 phases of disassembly between 5 ms and 2000 s. The phase changes were identified by changes in the decay slopes of the $I(q \rightarrow 0)$ as a function of time curves. It was also noted that there is probably a swelling phase at time scales shorter than 5 ms. To further analyze the disassembly process, singular value decomposition (SVD) analysis was applied to time-resolved SAXS data. SVD reveals the minimal number of contributing species to the time-resolved data set. The first phase was clearly a two-state transition owing to the presence of isosbestic points, and SVD corroborated that. The second phase had 4 species in solution: Capsid, dimer and 2 intermediates. A kinetic model was developed, and using both global-analysis and SVD, the structure of the intermediates and rate constants were calculated. The followed disassembly mechanism was purposed: Swelling, disassembly into two big pieces with 4- or 5 5-fold symmetry and dimers, disassembly into smaller intermediates, and finally disintegration into dimers. This mechanism is not the reverse assembly mechanism, strengthening the fact that the process of disassembly is not the mirror image of assembly, as was shown previously (Singh and Zlotnick 2003). At high pH, wtSV40 swells, its minichromosome pokes a hole in the capsid and escapes, following which the capsid disintegrates (Asor et al. 2020).

Conclusion

We showed how small-angle X-ray scattering can be applied to study physical virology. The homogeneity of the capsid structure and assembly/disassembly reactions make this system highly suitable for SAXS analysis. SAXS is able to probe the viral life cycle, starting from assembly of empty viruses through packing of biological charged polymers, stability, maturation, and disassembly. Different viruses were used to study different steps in the viral life cycle. A plethora of data analysis approaches were applied ranging from simple analysis of peak location to cutting-edge direct fitting of structures based on thermodynamic analysis of macromolecular assembly.

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