BIOPHYSICAL CHEMISTRY

Unravelling capsid transformations

The interactions between a virus capsid and its cargo are essential for viral infection as well as in the design of synthetic virus-like particles. Now a combination of analytical techniques has unravelled key steps in the transformation of a model virus and the release of its RNA cargo.

Masaki Uchida and Trevor Douglas

Viruses are the most abundant biological entities on the planet and are a major repository of genetic diversity and biological carbon[1]. Although most viruses make use of a limited range of hosts, the vast number of different viruses, and subtle variations among nearly identical viruses, makes them a significant threat to human health. The study of virus structure, infection and replication has therefore been a cornerstone in the development of modern biochemistry.

The past 40 years has witnessed a tour de force of structural virology during which crystallography and cryo-TEM reconstructions have provided a wealth of information on a vast range of viruses[2–4]. However, these techniques provide information on population-averaged structures. Subtle individual variations within the population ensemble — such as a sub-population with a different structure — are extremely difficult to resolve. These sub-populations might be crucial for understanding the pathway along which viruses recognize their host, infect and replicate. Writing in *Nature Chemistry*, A. J. R. Heck, W. H. Roos and co-workers use a combination of analytical techniques to characterize interactions in individual virus particles as the outer protein coat reversibly disassembles to release the RNA cargo[5].

The development of soft ionization techniques such as electrospray ionization have ushered in a new era of mass spectrometry (MS). These methods allow the direct interrogation of the mass of non-covalently associated macromolecular complexes in their native states, which is known as native mass spectrometry[6–8]. Further advances have now made it possible to measure very large protein complexes including virus capsids, which have native molecular masses in the mega-dalton range. Native MS can resolve individual species within a population; a simultaneous 'single' molecule view of the whole ensemble. This makes it a powerful tool for revealing the steps in viral assembly and disassembly processes as they usually involve heterogeneous species and transient intermediates.

Atomic force microscopy (AFM) is another valuable tool for investigating these macromolecular complexes. A unique feature of AFM, over other microscopy techniques, is that it can elucidate not only structural information but also probe the mechanical properties by nanoindentation. For viruses, poised as they are between stability to ensure structural integrity and instability to enable release of their nucleic acids, the mechanical properties are key to their

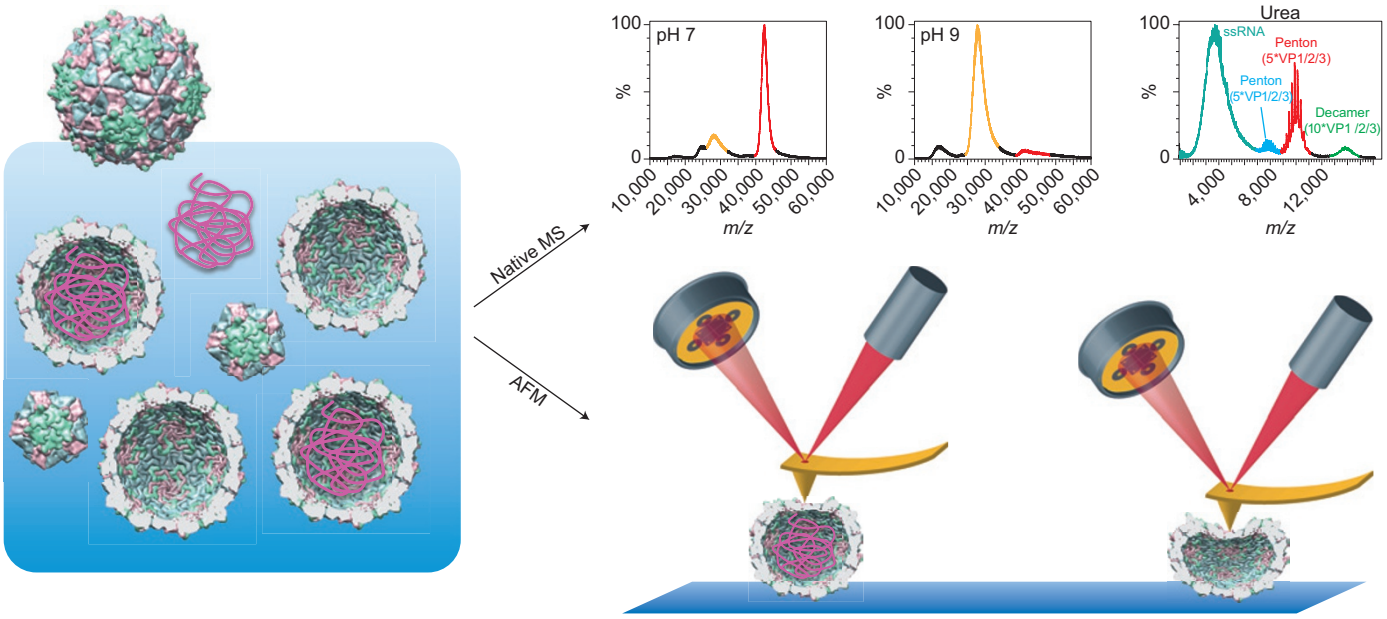

Figure 1 | Untangling heterogeneous populations of a virus with mass spectrometry (MS) and atomic force microscopy (AFM). Top: Heterogeneous populations of viral capsids in solution can be interrogated by native MS to measure the mass of individual particles. The relative amounts of virion (red) and empty capsid (yellow) are shown at pH 7 and pH 9. Chemical denaturation by urea demonstrates that pentons and penton dimers are the main disassembly products of the capsid. Bottom: Complementary information on the mechanical properties of individual particles can be assessed by nanoindentation using AFM. Particles with a high spring constant and high breaking force (left) can be distinguished from those with a low spring constant and low breaking force (right).

function[9]. The viral genome, in addition to coding for required gene products, also plays a role in assembly and in the overall mechanical stability of the capsid, which can be interrogated using AFM techniques.

Heck, Roos and colleagues elegantly demonstrate that native MS and AFM are complementary approaches that can be combined to reveal an intermediate state formed during the transformations of an intact virus particle known as a virion. This process involves disassembly of the virion to release viral RNA, and reassembly of the outer coat protein as an empty capsid. To study virion capsid transformations the team used a model virus system called the Triatoma virus (TrV), which is a picorna-like virus and similar to the virus that causes Polio. TrV infects *Triatoma infestans* insects, which can carry Chagas disease, via an oral–faecal route. The midgut of the insect is acidic whereas the hindgut is alkaline. The large pH changes experienced by the virus as it passes through the insect gut are postulated to play an important role in infectivity.

Investigating the stability of TrV virions by native MS over weakly acidic to alkaline pH revealed that the virion remains intact under acidic and neutral conditions; however, raising the pH to between 8 and 9 leads to the release of viral RNA and the detection of empty capsids (Fig. 1). Furthermore, pentons (a sub-structure made from structural proteins VP1, VP2 and VP3) are detected at alkaline pH and at a high concentration of urea that promotes denaturation. This suggests that the penton is an assembly intermediate of the TrV capsid. This was further confirmed by AFM nanoindentation in which the capsid was broken into the twelve pentons. Additional collision induced dissociation, and ion mobility mass spectrometry experiments showed that the penton has a sheet-like structure, rather than a globular shape, and that the VP2 protein plays a crucial role in the assembly of pentons into a capsid. Extraction of this level of structural information is an astonishing demonstration of how far the use of MS can be extended in the study of macromolecular dynamics and assembly.

Atomic force microscopy was used in a complementary role to native MS by revealing structural information and mechanical properties of the viral capsid that were not accessible using native MS; AFM is inherently a single-molecule technique whereas MS measures the ensemble of molecules. Measuring a large number of virions by AFM allows the distribution of mechanical properties (such as spring constant and breaking force) to be investigated. The AFM results showed a clear shift of distribution that correlated with changes in pH.

The combination of two techniques showed the presence of three distinct forms of the TrV capsid; intact virion, reversible intermediate and empty capsid. The intact virion shows a high spring constant (that is, stiffer) and high breaking force, whereas the empty capsid has low spring constant and low breaking force. The intermediate has the same mass as the intact virion indicating that it still contains the viral genome (and is therefore indistinguishable by native MS), but it has a lower spring constant than the intact virion and its mechanical properties are similar to the empty capsid. This suggests that the genome–capsid interaction is diminished in this intermediate form. Based on the data obtained through this study, Heck, Roos and colleagues propose a dual role for the genomic RNA in the viral assembly–disassembly process. The genome stabilizes the virion by electrostatic interactions with the capsid at neutral pH. On an increase in pH the interaction weakens and eventually the densely packed genome triggers the uncoating of the capsid.

Recently there has been a shift from thinking about viruses solely as disease-causing agents and their potential as nanoscale materials has been recognized[10]. Viral systems have been developed for gene and drug delivery, diagnostic imaging and vaccine development, as well as energy generation and storage. Addressing the differences between the properties of individual particles and the properties that arise from averaging across a diverse population of structures is key to understanding the behaviour of natural and synthetic virus particles. Heck, Roos and co-workers have shown that this is indeed possible. Furthermore, these results indicate the importance of the interactions between capsid and cargo are a key factor for establishing controlled packing and release. This combination of techniques could be a powerful tool not only for a fundamental study of virus infectivity but also for the development of virus-based nanomaterials. ❐

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References

1. Suttle, C. A. *Nature* **437,** 356–361 (2005).
2. Bloomer, A. C., Champness, J. N., Bricogne, G., Staden, R. & Klug, A. *Nature* **276,** 362–368 (1978).
3. Harrison, S. C., Olson, A. J., Schutt, C. E., Winkler, F. K. & Bricogne, G. *Nature* **276,** 368–373 (1978).
4. Johnson, J. E. & Chiu, W. *Curr. Opin. Struct. Biol.* **10,** 229–235 (2000).
5. Snijder, J. *et al. Nature Chem.* **5,** 502–509 (2013).
6. Siuzdak, G. *et al. Chem. Biol.* **3,** 45–48 (1996).
7. Benesch, J. L. P., Ruotolo, B. T., Simmons, D. A. & Robinson, C. V. *Chem. Rev.* **107,** 3544–3567 (2007).
8. Heuvel, R. H. v. d. & Heck, A. J. *Curr. Opin. Chem. Biol.* **8,** 519–526 (2004).
9. Michel, J. P. *et al. Proc. Natl Acad. Sci. USA* **103,** 6184–6189 (2006).
10. Douglas, T. & Young, M. *Science* **312,** 873–975 (2006).

Li-O_2 BATTERIES

An agent for change

The rechargeable Li-O_2 battery has low energy efficiency, which is mainly due to kinetic difficulties in the electrochemical oxidation of the insulating discharge product, Li_2O_2. Now a redox mediator, acting as an electron–hole transfer agent, has been used to promote this oxidation reaction.

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One of the greatest challenges of the twenty-first century is unquestionably the conversion of energy and its storage. If the needs of modern society are to be met, while also considering environmental concerns and energy security, then breakthroughs in battery science and technology are required.

All batteries are composed of two electrodes connected by an ionically conductive material (electrolyte). The amount of electrical energy per mass (specific energy) or volume (energy density) that a battery can deliver is determined by both the voltage and capacity of the cell,