New and Notable

Capsid Deformations Reveal Complex Mechano-Chemistry

Konstantin I. Popov
and Garegin A. Papoian*
Department of Chemistry and Biochemistry, Institute for Physical Science and Technology, University of Maryland, College Park, Maryland

Viral genomes are commonly protected by capsids with beautiful geometries and symmetries. However, despite the relative simplicity of the capsid shapes, many fundamental questions remain unanswered about capsids mechanical properties and their self-assembly mechanisms, which, in turn, have important biological implications. Therefore, gaining deeper insights into the nature of viral shells in particular, and nanoscale shells in general, could provide foundations for further advances in a variety of applications in nanomaterials, bioscience, and medicine.

It has been well known since Crick and Watson's groundbreaking article in 1956 (1) that nearly-spherical shells of small viruses, called capsids, are likely to be composed of identical multiprotein units, called capsomers, with a total number that is a multiple of 12, packed into a regular pseudo-crystalline structure with icosahedral symmetry. Capsomers are often found to be ~10 nm in diameter. Therefore, conceptual understanding of capsid shapes and their mechanical properties should start from the theory of thin elastic shells with icosahedral symmetry and, ideally, also take into account the internal mechano-chemical properties of capsomers.

The classical, continuous theory of thin elastic shells (2) serves as a convenient starting point for gaining insights into the way viral capsids deform under various mechanical stresses. For example, a ping-pong or a tennis ball may be compressed in various ways (see Fig. 1 *a*) (2,3), under the load of either a rigid plane or a spatially localized object. When a rigid plane indents the shell, first the shell's surface area in contact with the plane flattens, then buckles inwards at higher loads, minimizing its elastic energy (3). This buckling is a first-order transition, showing hysteresis and irreversibility when the deformations are large. In the opposite limit a sharp object may be used to make an indentation, where a circular fold appears at low loads (Fig. 1 *a*), with the extent of the deformation being proportional to the applied force (3). Contrary to the case of contact with a plane, this transition is continuous and reversible. However, at higher loads, the fold further transforms into a polygonal structure, composed by a number of inflexible ridges (Fig. 1 *a*). Similar structures become also energetically favored for large deformations by a rigid plane. Interestingly, the shape of the polygon further evolves as the load continues to increase.

The next step in understanding the nature of viral shells and their deformations is to recall that the capsid surface is composed of capsomers as units. In the simplest approximation, the mechanical network of the shell may be locally characterized by elastic stretching and bending deformations. When the spherical topology is additionally imposed, minimization of the network's energy results in the capsid's icosahedral shape and determines many of its mechanical properties (4,5). This can be understood in the following way: When a regular planar triangular lattice is mapped onto a spherical surface, an excess of vertices is produced. Therefore, to form a smooth lattice, it is necessary to remove some of the original planar points or, in other words, to introduce so-called topological defects. Introducing such defects strongly affects the elastic energy of the shell in the ground state (in particular, increasing the stretching energy (4)). However, because the shell is flexible, some vertices buckle out of the plane to lower their energies by forming conical structures. Detailed calculations show that increasing the radius of the shell makes these bucklings more favorable and sharper, leading to more faceted structures (Fig. 1 *b*) (4,5).

When the local curvature becomes comparable to the capsomer size, for example during strong deformations, the key assumptions of the theory of elasticity may no longer hold. Therefore, the structural chemistry of proteins comprising the capsomers, and the corresponding interprotein interactions, need to be taken into account. Kononova and coworkers (6) combined single-molecule atomic force microscopy technique with long-timescale biomolecular simulations to describe the mechano-chemistry of Cowpea Chlorotic Mottle Virus capsid, investigating structural transitions and mechanisms when viral shells are mechanically deformed. During the past decade, single-molecule atomic-force microscopy experiments and associated molecular-dynamics simulations have become a powerful tool for investigating folding-unfolding processes of single proteins and mechanical responses of more complex biomolecular assemblies (7,8).

As noted above, a viral capsid is characterized by a complicated microscopic structure, where the capsid's mechanical properties depend on multiscale couplings across the shell, both in the neighborhoods of individual capsomers and involving larger-scale collective excitations. The relaxation processes following capsid deformations occur on millisecond-to-second timescales, requiring high-performance computational approaches for their simulation. By using

Submitted July 30, 2013, and accepted for publication September 26, 2013.

*Correspondence: gpapoian@umd.edu

Editor: Matthias Rief.

0006-3495/13/11/2233/2 $2.00

http://dx.doi.org/10.1016/j.bpj.2013.09.055

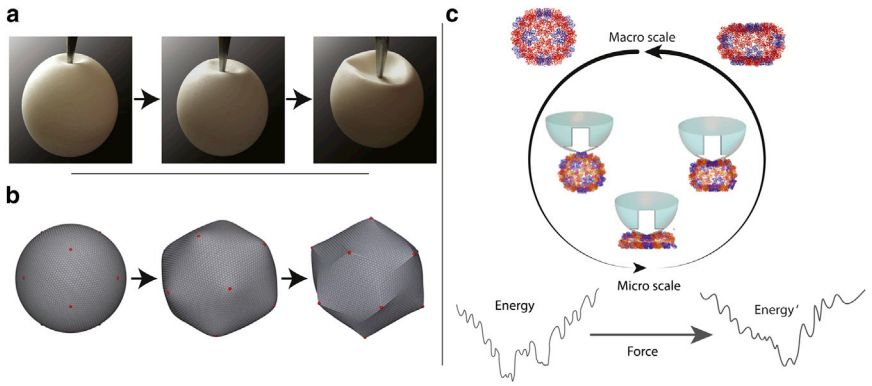

FIGURE 1 (*a*) A standard ping-pong ball was indented by a sharp metal object illustrating deformations of the elastic shell at different loads. (*b*) Snapshot of faceting of the shell is shown (5). (*c*) Schematic representation of capsize evolution under the deformation is shown (the drawing was composed using snapshots from Kononova (6)).

a coarse-grained model of a protein, highly accelerated by graphics processing units, Kononova et al. (6) (to our knowledge for the first time) made a direct comparison of the experimental results and computer simulations performed under similar conditions to study a force load response of a Cowpea Chlorotic Mottle Virus capsid. Using various data analysis techniques, the authors carefully studied the energy profiles of proteins and capsomers in response to the applied deformations, revealing detailed molecular motions during capsid force indentation, both in equilibrium (under a small load) and out-of-equilibrium (under a large load).

Such a detailed description of capsid deformation dynamics has revealed many interesting aspects, which were not apparent in the earlier works. On the macroscale level, deformational behavior is quite intuitive: at low values of indentation depth, deformations are reversible; after exceeding some critical value, mechanical responses become irreversible. However, because of the complexity of the capsomer structure, it turns out that the deformation process strongly depends on the local geometry of mechanical perturbation and the specific capsid orientation under the tip. Another important conclusion is that the deformation of the capsid shell is highly dynamic and displays multiple stages of mechanical response.

At the first stage of reversible deformations, where the capsid shows almost elastic behavior, the external force excites multiple modes of capsomer motions. During this period, a large number of proteins are located near the tip-shell contact area. Increasing the load brings the deformation to the second stage—an irreversible (i.e., permanent) deformation. At this stage, local rearrangements of the capsid proteins start to occur and capsomers' motions shift out-of-equilibrium. A further increase of the indentation depth leads to remodeling of the underlying proteins' energy landscapes (9,10), resulting in small conformational rearrangements of individual proteins, but significant alterations of some interprotein interfaces (Fig. 1 *c*). The subsequent large reorganization of the capsid structure results in irreversible deformations.

Interestingly, the deformation pathways significantly depend on the rate of capsid compression. For example, at low-force loading some assistance from the thermal kicks is needed to overcome energy barriers between successive structural transformations, leading to the collapsed states in multiple stages and producing a series of characteristic force peaks on force-deformation curves. On the contrary, a lot of energy is injected into the system at high loading rates, helping to overcome energy barriers and resulting in smoother force-deformation curves (6). Another interesting observation, which cannot be explained with the classical elasticity theory of thin shells, is the strong coupling of the in-plane and out-of-plane deformations (6). As the indentation increases, the out-of-plane deformations no longer dominate, becoming strongly coupled and even difficult to distinguish from the in-plane deformations.

Many related interesting questions may be further explored in future researches, including whether nonnative interactions and hydrodynamic couplings play a significant role in capsid's mechanical response, and also using molecular simulations to investigate the mechano-chemistry of capsids with different geometries and elastic constants.

REFERENCES

1. Crick, F. H., and J. D. Watson. 1956. Structure of small viruses. *Nature.* 177:473–475.
2. Landau, L. D., and E. M. Lifshits. 1975. Theory of Elasticity, Vol. 7, 3rd Ed. Butterworth-Heinemann, London, UK.
3. Pauchard, L., and S. Rica. 1998. Contact and compression of elastic spherical shells: the physics of a 'ping-pong' ball. *Philos. Mag. B.* 78:225–233.
4. Lidmar, J., L. Mirny, and D. R. Nelson. 2003. Virus shapes and buckling transitions in spherical shells. *Phys. Rev. E.* 68:051910.
5. Sknepnek, R., and M. Bowick. 2013. Pathways to faceting of vesicles. *Soft Matter.* 9:8088–8095.
6. Kononova, O., J. Snijder, …, V. Barsegov. 2013. Structural transitions and energy landscape for cowpea chlorotic mottle virus capsid mechanics from nanomanipulation in vitro and in silico. *Biophys. J.* 105:1893–1903.
7. Engel, A., and D. J. Müller. 2000. Observing single biomolecules at work with the atomic force microscope. *Nat. Struct. Biol.* 7:715–718.
8. Mickler, M., R. I. Dima, …, M. Rief. 2007. Revealing the bifurcation in the unfolding pathways of GFP by using single-molecule experiments and simulations. *Proc. Natl. Acad. Sci. USA.* 104:20268–20273.
9. Frauenfelder, H., S. G. Sligar, and P. G. Wolynes. 1991. The energy landscapes and motions of proteins. *Science.* 254:1598–1603.
10. Materese, C. K., C. C. Goldmon, and G. A. Papoian. 2008. Hierarchical organization of Eglin-c native state dynamics is shaped by competing direct and water-mediated interactions. *Proc. Natl. Acad. Sci. USA.* 105:10659–10664.