

Viral Structure and Mechanics

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Abstract

The prospect of understanding and controlling the structure, formation and properties of nanoscale virus capsids has challenged researchers from disciplines as diverse as biology, chemistry, mechanical engineering and physics for over half a century. In this review, I will describe a number of theoretical approaches to this. The most well-known approach is that of Caspar and Klug, developed in the 1950's and 60's, and I will outline both the original idea and recent extensions of it. I will then focus on an alternative approach developed by Nelson *et al.* that combines notions from crystallography and continuum mechanics, detailing the main ideas and further developments based on these. I will also touch on key AFM nanoindentation experiments of viral capsids, exploring how they can be understood within Nelson *et al.*'s theoretical framework.

1 Introduction

The study of biological systems in the context of materials science is an emerging paradigm at the intersection of a number of science and engineering fields [1, 2]. In particular, elucidating the mechanical properties of nano- or micro- scale biological structures is crucial not only for the understanding of their functions, but also for guiding the design of bio-inspired materials.

Viruses and the macromolecular protein shells (*capsids*) that make up polyhedral viruses are one such class of structures, having dimensions on the order of tens of nanometers. A good deal of work spanning over 40 years has focused on understanding viral capsids from a variety of scientific perspectives. From a fundamental point of view, various mechanisms have been proposed to explain the structure and formation of icosahedral viral capsids, starting from Caspar and Klug's seminal work in the 1950's and 60's [3] inspired by the notion of *tensegrity*. From a more engineering point of view, a number of researchers have recently begun to use novel experimental tools and theoretical approaches to understand the mechanical properties of empty and filled (e.g. with DNA) capsids. Because viruses do not have active metabolisms, both of these points of view can utilize various thermodynamic and geometric approaches without having to deal with many complex non-equilibrium phenomena, unlike most other biological systems (e.g. [4]).

In this article, I will review some recent work in the field of virology in the context of mechanics, focusing on the fundamental point of view. In particular, I will introduce the idea of tensegrity and the development of the prevalent Caspar-Klug model of virus structure (section 3), outline various extensions of Caspar-Klug theory for the understanding of the structure and formation of viral capsids (section 4.1), and explore an alternative approach that elegantly combines notions from crystallography and continuum mechanics (section 4.2). I will also touch on various experiments and related theoretical developments that attempt to understand the mechanical properties of unfilled and filled viral capsids (section 5), as well as recent developments that build on these insights in the context of materials engineering (section 6).

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2 Introduction to Viruses

Viruses, as we currently understand them, fall into four morphological classes: viruses with helical structure (such as the rod-shaped tobacco mosaic virus), viruses with polyhedral structure (such as the icosahedral viruses discussed here, including the bushy stunt virus studied by Caspar in 1956; the bacteriophage $\phi 29$ discussed later in this paper is an elongated icosahedron), composite viruses (combining, for example, an icosahedral ‘head’ and a helical ‘tail’), and viruses with more complex structures [5]. These are often optimized for a number of functions, including those key to self-replication: attachment to and penetration of a target cell, and delivery of the viral nucleic acid for host cell production of even more viruses. (The host cell subsequently ruptures – a process known as *lysis* – and the viruses are released in a ‘budding’ process.) The unique structural and mechanical properties of capsids are key to protecting the viral genetic information (e.g from external pressures or chemical groups), as well as withstanding the high internal pressures developed due to the tightly-packed DNA or RNA inside the capsid.

Understanding these structures, formation mechanisms, mechanical properties and functions is interesting not only from an academic viewpoint, but may also have important consequences in developing approaches to detect and combat diseases. For example, one might imagine engineering innocuous antiviral drugs with structures similar to viral capsids, thus potentially preventing virus attachment to target cells by saturating key binding sites. Another approach may be to use insights into the mechanism of viral formation to develop chemical approaches to inhibit the synthesis process. In addition, work in this area may lead to novel biomedical devices, such as highly sensitive real-time virus protein detectors [6]. From the point of view of materials science and engineering, viruses and virus-inspired structures could be utilized as specific and structurally robust self-replicating nanoscale ‘packages’ or ‘carriers’, for example.

3 Tensegrity and Caspar-Klug Theory

A number of experiments in the 1950’s sought to elucidate the structure of viruses using a variety of techniques. Based on X-ray diffraction (XRD), electron microscopy (EM) and chemical studies of the size, shape and composition of plant viruses, Crick and Watson proposed that small viruses are formed by the packing of many identical protein sub-units [7]. This has the evolutionary advantage that the viral genome need only be large enough to code for one or a few protein subunits. Further work by a number of researchers showed not only that these viruses possess icosahedral symmetry (which requires 60 structural subunits), but that they were made up of *more* than 60 protein units [8, 9]. This was, to say the least, very puzzling.

In 1962, Caspar and Klug proposed one resolution of this puzzle using a conceptual framework motivated by the principle of tensegrity¹. This is a mechanical design principle pioneered by Kenneth Snelson and Buckminster Fuller in the 1960s, in which structures are designed such that the competition between forces – tension versus compression – throughout has a self-stabilizing effect:

“The universal comprehensive tension system could be interspersed locally with islands of compression, in the form of struts, in such a manner that the islanded compression struts would not touch one another. Yet these struts would force the tension network into outward patterning from the center of the total structural system in such a manner that the individual compression struts would not touch one another, yet would hold the tension network outwardly in firm spherical patterning.” [12]

¹While tensegrity inspired the field of work described in this review, concepts of tensegrity are rarely considered in the study of viral structure and properties today. However, tensegrity as a design principle turns out to be ubiquitous: for example, carbon nanotubes can be thought of in terms of tensegrity (and these have a direct parallel in the viral world – rod-like viruses). Obviously, buckyballs (C_{60} fullerenes) are also carbon-based analogues of polyhedral viral capsids. Other biological structures have deep links to the idea of tensegrity, as well: for example, cell cytoskeletons and the “architecture of life” [10]. More recently, actuated nanocolumns have been thought of in terms of tensegrity [11].

A well-known example of a design employing this idea is the geodesic dome. A *geodesic* is the shortest path between two points – for example, on the surface of a sphere – and the surface of a geodesic dome can be thought of as the intersection of multiple such geodesics.² In particular, the dome surface is tiled with identical triangular elements that are situated in one of two different *quasi-equivalent* environments on the surface (*pentamers* or *hexamers*).

This idea motivated Caspar and Klug’s seminal work on understanding the structure of polyhedral viruses. In particular, Caspar and Klug proposed that the structural subunits of viral capsids (*capsomers*) are pentamers (at the 12 five-fold symmetric vertices of the icosahedral capsid) or hexamers (at the other six-fold symmetric vertices of the capsid) of identical proteins experiencing slightly different chemical bonding environments; that is, they are quasi-equivalent [3, 13]. These thermodynamically *self-assemble*³ via non-covalent bonding to form a structure with icosahedral symmetry – just as in a geodesic dome – consisting of 60 asymmetric subunits (the capsomers); crucially, this structure can contain more than 60 protein subunits, as observed experimentally. Geometrically, one obtains this capsid structure by triangulating the surface of an icosahedron, with protein subunits located at the corners of the triangular facets. This model predicts that the number of protein subunits (S) is given by the relation $S = 60T = 60(h^2 + hk + k^2)f^2$, where h and k are coprime integers and f is a positive integer, and T is the “triangulation number” – that is, the number of triangular facets per icosahedral face.

The beauty of this model is that it provides an elegant means of viewing viral capsids. In particular, it explains the icosahedral symmetry of viral capsids consisting of more than 60 protein subunits in a very general framework, starting from very simple assumptions, and makes a specific prediction for the number of protein subunits that can make up these capsids. This has been verified in many subsequent experiments using XRD and 3D reconstruction from cryo-EM [14, 15, 16]. Over the past few decades, further refinements have been made to Caspar and Klug’s model, and a number of exceptions have been found [17, 18] – but it largely holds and is used as a guiding principle by many researchers.

4 Structure and Formation of Viral Capsids

4.1 Building on Caspar and Klug

Recent work has explored the capsid self-assembly process suggested by Caspar and Klug even further. For example, Bruinsma *et al.* have come up with a phenomenological Hamiltonian describing capsomers as hard disks with ‘sticky’ edges free to interact on the surface of a sphere⁴. Including a relevant mixing entropy and minimizing the resulting free energy allows one to study the stable structures predicted by this model [19]. Perhaps unsurprisingly, some of these structures are in line with Caspar and Klug’s predictions, while some are not. A number of improvements can be made to this model – for example, it does not distinguish between pentagonal and hexagonal capsomers.

Incorporating disks of two different sizes – with no energy cost to ‘switch’ between the two – into the analytic model and optimizing the surface coverage of disks seems to lead to increasing agreement with

²It is no surprise, then, that these exhibit closely-packed surfaces with exceptional structural integrity – for example, any stresses applied to the surface are more likely to be spread out over more structural elements. Furthermore, because they rely on an extended ‘open’ network of small components, these structures have very small densities. Biological systems employing these design principles presumably have a significant evolutionary advantage over those that do not.

³To my knowledge, Caspar and Klug’s paper was the first use of this term in its modern form. Today, self-assembly is a prevalent theme in much of modern-day condensed matter physics and materials science.

⁴This is an extension of an older mathematical problem dealing with the optimal coverage of a sphere by N caps, the Tammes problem (also biologically inspired – interested readers are referred to the work of the Dutch botanist P. M. L. Tammes). The problem of viral capsid assembly also has deep connections to the ‘Thomson problem’, put forth by J. J. Thomson over a century ago: *how does one arrange N electrons on a sphere?* In this case, one replaces the Coulomb interactions between the particles with an interaction potential more relevant to capsid proteins, such as a van der Waals interaction.

Caspar-Klug structures. Zandi *et al.* have included this crucial detail in Monte-Carlo simulations of this model system, with additional refinements – for example, the disks in their model interact via a Lennard-Jones potential, as opposed to the hard-disk interaction of the previous model [20]. Encouragingly, the energy minima of this updated model correspond to Caspar-Klug structures, supporting the idea that they form spontaneously through free-energy minimization (although Zandi *et al.* also find that single-capsomer structures may also exhibit icosahedral symmetry, possibly explaining the experimental observation of all-pentagonal icosahedral capsids [21]).

It is interesting, however, that incorporating a significant energy cost ($\gg k_B T$) for disks to switch between the pentamer state and the hexamer state – perhaps the more realistic scenario, since energy is required for a pentamer to bind an additional protein subunit – reduces the agreement between Zandi *et al.*'s simulation and Caspar-Klug theory. Rather, the stable structures predicted with this additional refinement seem to agree with exceptions to Caspar-Klug theory that have also been observed experimentally [22], indicating that the energetics of the molecular interactions in capsid self-assembly play a key role in determining their structure.

4.2 Combining Continuum Mechanics and Crystallography

While virus sizes are typically $\sim 50\text{nm}$, a range of sizes exist. Smaller capsids are able to store less genetic information, but require less subunits to assemble and can be mechanically stronger because of their rounded, versus faceted, shape. Observing the shapes of viral capsids of varying sizes indicates that, as a general rule, smaller capsids appear more spherical while larger capsids appear more faceted [15]. Another theoretical perspective to viral structure does away with the details of the self-assembly process, utilizing ideas from *spherical crystallography* – the study of ordered structures on curved surfaces – and continuum mechanics. Specifically, Nelson *et al.* are able to explain not only the observed icosahedral ground-state structure and size-dependent faceting of capsids, but also put forward a theoretical means by which crucial materials parameters of virus capsids can be predicted [23, 24]. This approach is outlined in the remainder of this section.

Hard-core particles on a plane prefer to pack in a hexagonal lattice network. In a vein similar to Caspar and Klug's triangulation of the icosahedron, one can consider virus capsid structure as being the result of folding up this lattice net onto the surface of a sphere. By applying Euler's formula relating the number of faces F , vertices V , and edges E of this tessellation [25], one finds that at least 12 vertices of the tessellated spherical surface are five-fold disclinations at which the local axes are rotated – the pentamers. Thus, this approach suggests that the icosahedral symmetry associated with viruses results from the “geometrical frustration” associated with spherical crystallography of protein subunits, with disclinations situated at the 12 vertices.⁵ (Because the energy associated with a disclination is quite large, disclination-disclination interactions are repulsive, and it is not surprising that they will be arranged so as to maximize the spacing between them.)

Nelson *et al.* have incorporated this salient feature of geometrical frustration – the existence of icosahedrally arranged disclinations – into a continuum mechanics model of the capsid. In particular, the continuum mechanics of thin shells is a well-studied problem [30], and Nelson *et al.*'s model considers the unfilled virus capsid as a thin spherical shell (the filled case is discussed in the next section). Motivation for this comes from previous work by Seung and Nelson [31], in which they considered a tessellated flat thin disk – characterized by a 2D Young's modulus Y , radius R , and bending rigidity κ – possessing a single 5-fold disclination. The bending and in-plane stretching energies associated with this system depend on the system size and

⁵In general, trying to do “crystallography on a sphere” necessarily involves topological defects, such as disclinations, dislocations, vacancies and interstitials [26, 27, 28]. This notion has been verified experimentally under certain conditions [29].

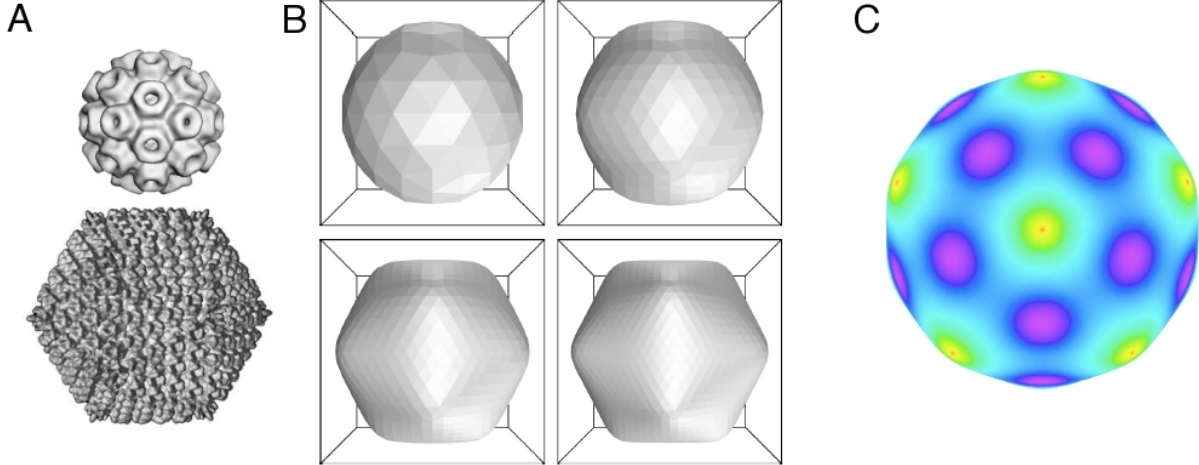


Figure 1: (A) Reconstructed cryo-EM images of CCMV (top, from [32]) and adenovirus (bottom, from [33]), showing size-dependent faceting. Images are not to scale – CCMV size is $\sim 284\text{\AA}$, while adenovirus size is $\sim 1100\text{\AA}$ [15]. (B) Numerically calculated virus shapes, for capsids of increasing γ , using the method of Nelson *et al.* as discussed in the text (from [23]). (C) Logarithmic color plot of total elastic energy (violet = low, red = high) for a viral capsid above the buckling transition, using the method of Nelson *et al.* as discussed in the text (from [24]).

materials properties, and the total elastic energy is given by:

$$E = \frac{1}{2} \int [\kappa(\nabla^2 f(\mathbf{r}))^2 + 2\mu u_{ij}^2 + \lambda u_{kk}^2] dS \quad (1)$$

where $f(\mathbf{r})$ describes the deflection – and thus $\nabla^2 f$ describes the mean curvature, μ and λ are the Lamé coefficients, and u_{ij} is the stress tensor. The 2D Young's modulus is given by $Y = 4\mu(\mu + \lambda)/(2\mu + \lambda)$. Thus the first term in eq. 1 represents the bending energy, while the second two terms represent the stretching energy.

Minimizing this nonlinear energy is equivalent to solving the Foppl-von Kármán differential equations for the large deflections of an elastic plate. It is useful to define a dimensionless parameter, the Foppl-von Kármán number, that describes the competition between in-plane stretching and bending: $\gamma = YR^2/\kappa$. In the case of the flat thin disk, Seung and Nelson found that for $\gamma > 154 = \gamma_b = YR_b^2/\kappa$, the disclination energy (which dominates the energy of the system) goes as $\kappa \ln(R/R_b)$, while for $\gamma < \gamma_b$ the energy goes as YR^2 . Driven by energy minimization, the disk undergoes a size-dependent buckling transition between the flat geometry and a conical geometry, with the cone vertex defined by the disclination.

For the case relevant to viral capsids, in which the starting geometry is a thin *spherical* shell with 12 interacting disclinations, Lidmar, Mirny and Nelson found a similar buckling transition [23]. They have quantified this numerically, using a well-developed discretization scheme relevant to this system; the results of this are shown in figure 1. (The existence of this transition in the spherical viral case is not so obvious: while it is intuitively clear that buckling of a disk from a flat geometry to a curved geometry may minimize the shell energy, it is not so clear that buckling of an *already curved* shell will necessarily lead to energy minimization. Indeed, Lidmar *et al.* find this buckling transition to be less sharp than that of a flat disk.)

A clear size-dependent buckling transition from a rounded capsid to a faceted capsid is observed when considering the variation of either the total energy or the mean squared asphericity ($\langle\langle\Delta R^2\rangle\rangle/\langle R\rangle^2$, representing

the deviation of the capsid from a perfect sphere) with γ ($\gamma_b \sim 140$) – just as observed. The agreement between this theoretical treatment and experimental observations is mainly qualitative, partly due to the lack of experimental data for Y and κ . However, it is possible to use this model to derive an estimate for Y/κ for various virus capsids, given experimental data for their real-space structure. This is done by measuring the RMS deviation of the numerically predicted structure from the observed structure, as a function of γ . Interestingly, sharp minima in the RMS deviation are observed for two ‘test’ viruses: these are at $\gamma = 1480$ for the HK97 bacteriophage and $\gamma = 547$ for the Yeast virus L-A, with the RMS deviation ≤ 2 in both cases. Experimental measurements should be useful in verifying the accuracy of these predictions. Indeed, there is a dearth of much-needed experimental data on the elastic properties of viral capsids. While Nelson *et al.*’s model elegantly incorporates the role of disclinations – crucial for nanoscale systems – and capsid thin-shell mechanics, I am not aware of any experimental studies that directly verify their key predictions of the shapes of viruses; that is, quantitative investigations correlating the structure of viral capsids to the Foppl-von Kármán number.

Various theoretical extensions of this model have been explored. One of the key assumptions adopted by Lidmar, Mirny and Nelson is the lack of a spontaneous curvature term in their treatment of the capsid energy. Nguyen, Bruinsma and Gelbart have incorporated this into the continuum elasticity model of Nelson *et al.*, mapping out a shape ‘phase’ diagram involving not only icosahedral capsids, but also spherocylindrical capsids – such as $\phi 29$, the “elongated icosahedron” described in the introduction [34, 35]. Many of these shapes are *not* the result of unconstrained energy minimization – the focal point of previous theoretical studies – but result only under specific constraints on capsid assembly, such as a constant volume constraint. These results have compelling connections to experiments: indeed, a number of viral structures, such as the HIV-1 retrovirus, have non-icosahedral shapes that are well described by this generalized continuum theory.

Addressing additional assumptions in this continuum elasticity approach could lead to many more exciting insights into viral structure; for example, incorporating interactions between the viral capsid proteins and the enclosed DNA or RNA molecules may lead to better understanding of the structure of viruses such as SV40 or CCMV, which have been observed to take on a variety of shapes under different conditions (e.g. see sections C and D of [34] and references therein). Furthermore, a fundamental assumption made in these models is that viral capsids are well represented by thin elastic shells. While the molecular-scale details of capsids can be treated in this framework, being incorporated into mechanical parameters such as Y and κ , their geometric aspects may not be. For example, the CCMV capsid shell is known to have an average thickness of 3.8nm, greater than 13% of its outer diameter [36]; it is unclear whether thin-shell elasticity models are appropriate to such systems. Theoretical studies of the influence of more realistic ‘thick’ shells on the results of Nelson *et al.* remain to be done, although these have been explored in the context of nanoindentation experiments, discussed in the next section.

Because they have a significant degree of crystallinity inherent in their structure, it is interesting to consider what the vibrational modes of viral capsids and other periodic structures on curved surfaces are. Other extensions of the continuum elasticity theory of Nelson *et al.* explore the normal modes of these structures e.g. via computer-based normal mode analysis [37]. For example, Widom, Lidmar and Nelson have studied the phonon spectrum of viral capsids undergoing this size-dependent buckling transition using a simplified mass-and-spring network model [24]. They confirmed that the buckling transition of viral capsids is less ‘sharp’ than that of a flat thin disk, interpreting this as a soft mode transition⁶. Further work has extended this analysis in the framework of the Landau theory of phase transitions [39]. More work remains to be done, however, and a number of discrepancies exist between some theoretical investigations and experiments. For example, recent inelastic Brillouin light-scattering experiments [40] did not pick up any vibrational modes of viral capsids in the frequency range theoretically predicted in [37]; further experiments using complementary probes and spectroscopies should help elucidate this and other details. For example, Raman spectroscopy is increasingly being used to study the high- and low-frequency vibrations of viruses, useful for understanding

⁶This has deep connections to structural phase transitions in various other solid-state systems – see, for example, ref. [38].

the makeup and environment of viral capsids [41, 42].

4.3 Other Approaches and Future Work

Other approaches to understanding capsid structure exist. For example, Vernizzi and de la Cruz have shown that icosahedral shapes can result from energy minimization of ionic ‘rafts’ interacting via electrostatics, as opposed to the Lennard-Jones interactions considered by Zandi *et al.* [43]. Interestingly, upon making the connection between the ionic strength of these rafts and the crystal Young’s modulus [44], the results of this approach seems to agree with the work of Nelson and others described above. It is currently not clear which picture more accurately represents the details of capsid subunit interactions, and the role of electrostatics continues to be explored [45].

In other work, Rapaport has used molecular dynamics simulations to study general organizational principles in the assembly of polyhedral virus protein shells [46], while Twarock and others have developed new mathematical techniques (e.g. based on group theory and tiling theory) to study and predict viral capsid structures [47]. Theoretical techniques such as the Landau theory of crystallization, in which the order parameter represents a critical system of density waves, have also been used to take into account details such as the asymmetry of viral protein subunits [48].

Many details remain to be filled in, especially in our understanding of the *dynamics* of capsid formation. The mechanism by which capsids form has begun to be studied from a biochemical point of view, with much success and some surprises [49]. For example, Zlotnick and others have experimentally and theoretically investigated the role of molecular interactions, fluctuations, and the chemical kinetics of virus assembly [50, 51, 52], looking at reaction pathways that reach equilibrium [53] or those that become kinetically trapped, forming intermediates [54, 55]. Complementary mean-field theories of virus nucleation and growth are yielding insights into this, exploring for example hydrophobic and electrostatic effects [56, 57]. Molecular dynamics simulations are beginning to study not only the structure, but also the growth mechanisms of capsids [58, 59]. In other work, Hicks and Henley have taken a similar approach as Nguyen, Bruinsma and Gelbart, also incorporating a spontaneous curvature term into the model of Nelson *et al.* described in the previous subsection. They explore capsid assembly in a non-equilibrium framework, proposing an irreversible growth mechanism by which these aggregates form, in which protein subunits accrete to a growing edge individually [60].

5 Mechanical Properties of Virus Capsids

5.1 Empty Capsids

The approach of Nelson *et al.* outlined in the previous section provided an elegant means of understanding the structure of unfilled viral capsids via the Foppl-von Kármán number (γ). While this approach requires significant further development, it and similar approaches have proved useful in understanding experiments.

Atomic Force Microscopy (AFM) nanoindentation experiments are ubiquitous in nanomechanics; in their simplest form, a well-characterized AFM tip is used to controllably indent a nanostructure with spatial resolutions on the order of nanometers. The cantilever deflection force is measured as a function of the vertical piezo position; the resulting curve is known as a force-distance (FZ) curve. Recent AFM nanoindentation studies of spherocylindrical $\phi 29$ [61] capsids have addressed basic questions about the mechanical properties of these structures: for example, how strong are they? How do they deform? What are their mechanical limits? These studies quantify crucial physical quantities: for example, empty $\phi 29$ capsids deform linearly and reversibly under uniaxial pressure (up to 30% of their height) for a loading force $\leq 0.6\text{nN}$, possess a

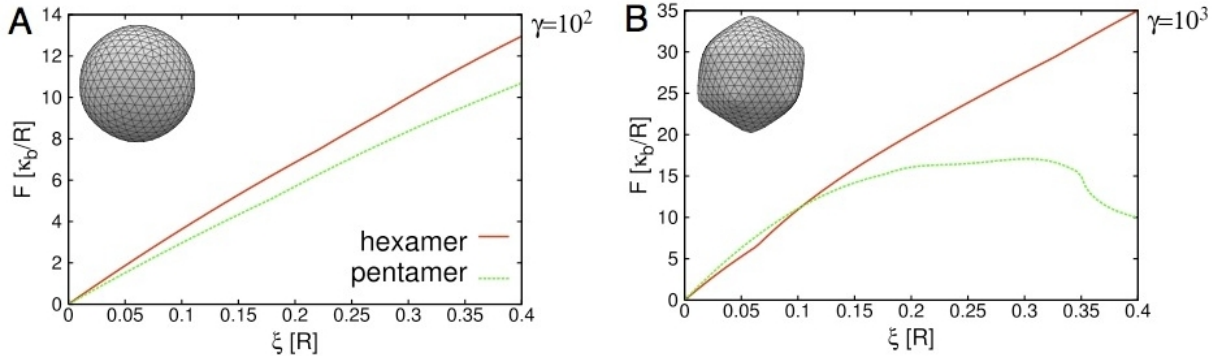


Figure 2: Simulated force-distance relationships of viral capsids (locally deformed at hexamers and pentamers), for increasing values of γ . (A) shows the linear behavior characteristic of a spherical capsid, while (B) shows the differing responses of hexamers and pentamers of capsids above the buckling threshold. This reflects the different elastic energies associated with these structural subunits after the capsid has buckled. From [64].

Young’s modulus $Y \sim 1.2 - 1.8\text{GPa}$ ⁷, and interestingly, exhibit a bimodal distribution of elastic constants [61]. The height statistics of these studies are in accord with the virus structure, as well: for example, the height distribution of spherocylindrical $\phi 29$ capsids is strongly bimodal (peaks separated by $\sim 10\text{nm}$) while that of icosahedral CCMV capsids unimodal or only slightly bimodal, corresponding to differences in height associated with pentagonal or hexagonal faces binding to the substrate. Ivanovska *et al.* have used a similar approach to study icosahedral CCMV virus capsids [62, 63], with results consistent with the data and analysis of $\phi 29$ capsids. In addition, they have studied the pH-dependence of CCMV capsid mechanical failure, relating this to a soft mode instability (see the brief discussion of soft modes in section 4) and elucidating details involving inter- and intra-molecular interactions of capsid proteins.

A number of different groups have explored this system theoretically. For example, Buenemann and Lenz [64] performed numerical simulations of triangulated capsid surfaces consisting of a network of vertices connected by harmonic springs, determining the starting structures via energy minimization – an approach similar to that used by Nelson *et al.* and extensions thereof. Using this, they were able to calculate FZ curves of specific viral capsids and compare these to experimental FZ curves, with excellent agreement (figure 2). (Note that such calculations are done by varying the capsid indentation and calculating the resulting force using energetics, *opposite* to the experimental procedure in which the deflection is measured as a function of force.) Crucially, these simulations explain the bimodality in the elastic constants of $\phi 29$ capsids, suggesting that it results from the difference in the mechanical properties of pentamers versus hexamers – that is, it represents the different elastic energies characterizing the different capsomers. Interestingly, this bimodality is predicted to be strongly dependent on γ , being significant only for large enough γ – this is in agreement with analysis done by Ivanovska *et al.*

Vliegthart and Gompper have used a similar approach to Buenemann and Lenz to investigate this

⁷The capsid spring constant was calculated as $k_{shell} = k_c k_{eff} / (k_c - k_{eff})$, where k_c is the cantilever spring constant and k_{eff} is the effective spring constant deduced from FZ measurements – physically, this models the capsid and the cantilever as being two springs in series. Strictly speaking, this is accurate only for $k_c \sim k_{shell}$; in the experiments, $k_c \sim 0.05\text{N/m}$ while $k_{shell} \geq 0.15\text{N/m}$. The Young’s modulus is then calculated using a thin-shell continuum mechanics [30] for a thin spherical elastic shell of Poisson ratio $\nu = 0.3$, using the formula $Y = Rk_{shell}/2.25h^2$. with R and h being the shell thickness and radius. It is important to note that a key assumption made in this analysis is that the underlying substrate does not influence the measurement in any significant way – simulation-based techniques may help elucidate the full impact of this. For example, the Young’s modulus may, in reality, be much smaller than the measured quantity.

problem, using a slightly simpler triangulation scheme restricted only to icosahedral viruses [65]. While they are not able to perform detailed quantitative comparisons to the experimental data, they too observe strong finite-size effects in the structure and properties of viral capsids. As with efforts to understand the structure and formation of capsids, a variety of other approaches have been used to understand their mechanical properties, as well. For example, Zandi *et al.* have used their extension of Caspar-Klug theory, described in section 4.1, to study stress distributions in and the mechanical response behavior of capsids – with relevance, for example, for experiments studying the mechanical failure and rupture of capsids (e.g. through “osmotic shock”) [66].

One particularly puzzling aspect of the $\phi 29$ and CCMV nanoindentation experiments is the observation of linear response, even for large deformations. Recent work has shown this to be partly due to geometric factors neglected in most models used to understand the structure and mechanical properties of viral capsids; these rely on the assumption that viral capsids are accurately modeled as *thin* shells, as noted earlier. While an analysis of the validity of this assumption in determining the structure of capsids is still lacking, recent finite-element simulations by Gibbons and Klug have focused on nanoindentation experiments using the general framework of nonlinear three-dimensional continuum elasticity theory, explicitly studying the influence of parameters such as the capsid shell thickness [67]. Interestingly, they find that while simple thin-shell models can be used to accurately determine the capsid Young’s modulus to within an order of magnitude, the linearity of capsid force-indentation response is heavily dependent on the tip size and shell thickness. It is clear that work along these lines will be crucial for understanding the limits of continuum elasticity theory when applied to these nanoscale systems.

5.2 Filled Capsids

Under “real” conditions, viral capsids typically store genetic information (RNA or DNA) to enable them to replicate, and a significant amount of research has focused on the mechanical properties of filled capsids. Packing the relevant biomolecules within the viral cavity is non-trivial; indeed, it is well known that DNA packs very, very tightly within capsids [68], this process having been characterized as being similar to packing “500 meters of the Golden Gate Bridge cable into the back of a FedEx truck” [69]! In many cases, including that of $\phi 29$, the viral DNA is “pulled” into the capsid by a portal “motor”, which consumes energy in the form of ATP. This process has been studied beautifully using optical tweezers: in a seminal experiment, Smith *et al.* measured the forces exerted on the viral DNA as it was packaged, noting that the internal force due to the confined DNA developed up to ~ 50 pN [70]. This translates to an internal pressure of ~ 6 MPa, which the capsid sustains without rupture or significant mechanical damage. Assuming a shell thickness of 1.6nm from structural studies, this means that the capsid shell has a tensile strength ≥ 100 MPa.

Fully understanding this process is challenging, and involves understanding the mechanical properties of DNA molecules themselves: a subject of much current work [71]. For example, the persistence length – the characteristic length scale over which DNA biopolymers are ‘stiff’ – is $\xi_p \sim 50$ nm, similar in size to the viral capsid that contains it. What this means is that to fully understand the internal pressures developed by storing DNA, one must consider the energetics of bending the DNA molecules at these tiny length scales, as well as the repulsive electrostatic interactions the tightly-coiled DNA is forced to undergo with itself. Entropic contributions associated with the configuration of these molecules under various conditions tend not to be too important. Purohit *et al.* have considered the energetics of this process [72], comparing the elastic bending energy, given by (d_s is the spacing between DNA loops, $N(R)$ is the number of hoops at radius R , and R and R_c are the innermost DNA loop and viral capsid radii, respectively)

$$E_{el} \sim \frac{\xi_p k_B T}{d_s} \int_R^{R_c} \frac{N(R')}{R'} dR' \quad (2)$$

with the DNA self-interaction energy, given by (L is the length of DNA in the capsid, and c is a solvent-

dependent parameters)

$$E_{self} \sim L(c + d_s)e^{-d_s/c} \tag{3}$$

and minimizing the total energy with respect to d_s . This simple analysis gives quite close agreement to the experimental data of Smith *et al.*, and predicts internal capsid pressures of tens of atmospheres similar to those estimated experimentally [73].

Biologically, the ability for virus capsids to withstand these high pressures is crucial – the high internal pressures enabled by strong capsids aid in ejecting the DNA into a target cell (although as it turns out, this is not sufficient for *full* ejection, and better understanding the mechanism and dynamics of DNA ejection is of enormous current interest [74, 75, 76].) In the previous subsection, I noted some of the remarkable mechanical properties of empty viral capsids, deduced using AFM experiments and various theoretical approaches; the mechanical properties of filled capsids have similarly been studied. Michel *et al.* and Carrasco *et al.* have found, perhaps unsurprisingly, that many of the qualitative elastic properties found for empty capsids are still exhibited in filled capsids – for example, linear elasticity for small deflections [62, 77]. A simple elasticity model of the capsid as a thin spherical shell at equilibrium ($\nabla \cdot \sigma = 0$, where σ is the stress tensor) [30] loaded from within by a pressure p_i and from the outside by a pressure p_o can be used to show that the rupture stress of viral capsids is much larger than that developed during the genome packaging process, using phenomenological parameters to take into account inter- and intra-molecular interactions [72]. Further refinements to this – for example, incorporating disclinations à la Nelson *et al.*; or considering thick shells, as discussed in previous sections; or perhaps analyzing the stress distributions developed by packaged biomolecules, and incorporating any non-isotropic stresses into models – should assist in better understanding these properties and their limits further.

Michel *et al.* and Carrasco *et al.* also find that capsids are mechanically reinforced when filled with DNA or RNA. Intriguingly, this reinforcement is not isotropic, but depends strongly on the crystallographic orientations of the capsid – presumably due to molecular interactions between the DNA/RNA and the capsid inner shell. As noted in the previous subsection, chemical interactions between the capsid proteins and other species are not well studied, and a good deal of work continues to build on these experiments. Further experiments by Carrasco *et al.* have used protein engineering to tailor the interactions between packaged DNA and capsid proteins; unsurprisingly, capsids made of proteins that have been designed to not interact with packaged DNA do not exhibit as marked a difference with empty capsids in their mechanical properties as ‘regular’ capsids do [78]. This elegantly highlights the importance of considering molecular-scale interactions. Theoretically, the techniques outlined in previous sections (e.g. finite-element simulations, or continuum elasticity theory) have been used to better understand these and other observations [79, 66].

6 Applications and Conclusion

It is now possible to use this understanding of the structure, formation mechanisms, mechanical properties, and molecular-scale interactions associated with viral capsids to make tailored nanostructures for specific applications. For example, insights into the assembly of spherical viruses are enabling novel chemical synthesis techniques by which ‘artificial’ nanoscale capsids can be controllably produced, mimicking viruses [80]. Virus proteins themselves have been used as carriers of non-viral materials, such as minerals and organic polymers [81], oil nanodroplets [82], gold and magnetic iron oxide nanoparticles [83, 84, 85], and semiconductor quantum dots [86]. These could be used in a wide variety of applications that take advantage of the specificity, rigidity and self-replicating characteristics of viruses, including drug delivery and as chemical microreactors. For this to be the case, a good deal of work remains to be done, particularly in perfecting the chemistry needed to synthesize viruses and virus-like particles.

Many questions dealing with the fundamental physical and mechanical aspects of viruses still remain open, however, and a number of these have been raised in this paper. What are the energetic details of the

capsid self-assembly process? Is there anything special about structures that deviate from Caspar and Klug's predictions? Will further experiments – for example, using AFM nanoindentation – enable the formation of a 'library' of viruses, detailing their sizes, shapes, and mechanical properties, thus enabling the direct testing of theoretical frameworks such as that developed by Nelson *et al.*? What are the limitations of thin-shell continuum elasticity models used to describe viral capsids, and how can they be improved? (For example, it would be interesting to extend Nelson *et al.*'s model to the case of thick shells. Would similar buckling behavior still be predicted?) How do molecular interactions – both between different capsid proteins, and between capsid proteins and encapsulated nucleic acids – influence the structure and properties of capsids? What are the collective vibrational modes of viral capsids, and how can this be used to both understand their structure, as well as potentially identify them *in vivo*? To what extent are electrostatic interactions, versus Lennard-Jones interactions, responsible for the structure and properties of viral capsids? Can the dynamics of capsid formation be thought of in terms of critical phenomena, or from a biochemical point of view, for example? What are the details of viral genome ejection? There is no doubt that these and related questions will continue to challenge and inspire researchers in this exciting field.

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