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Mechanical modeling of viral capsids

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Abstract As revealed by techniques of structural biology and single-molecule experimentation, the protein shells of viruses (capsids) are some of nature's best examples of highly symmetric multiscale self-assembled structures, with impressive mechanical properties of strength and elasticity. Mechanical models of viral capsids built "from the bottom up," i.e., from all-atom models in the context of molecular dynamics and normal mode analysis, have chiefly focused on unforced vibrational capsid dynamics. Due to the size of viral capsids, which can contain several hundred thousand atoms, the computer power needed for these types of methods has only recently reached the level required for all-atom simulations of entire viral capsids. Coarse-grained normal mode analysis has provided a simplified means of studying the unforced vibrational dynamics of viral capsids. Recent focus on "top-down" mechanical models of viral capsids based on two- and three-dimensional continuum elasticity have provided a theoretical complement to single molecule experiments such as atomic force microscopy, and have advanced the fundamental understanding of the forced mechanics. This review serves to assess the current state of modeling techniques for the study of the mechanics of viral capsids, and to highlight some of the key insights gained from such modeling. In particular, a theme is established of a link between shape—or geometry—and the global mechanical properties of these hierarchical multiscale biological structures.

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Introduction

The use of biological materials in modern materials science is a rapidly growing field. The nanometer-sized protein shells, or *capsids*, of viruses are attractive candidates for the design of new materials, primarily because they assemble from a small set of components to form simple structures with precisely determined geometries and sizes. In recent years, several novel uses for viruses in nanoscale materials have been proposed. One idea is to use soft materials, as found in the biological world, to organize inorganic materials, creating a class of new hybrid materials. Accordingly, Lee et al. [1] engineered a highly oriented, self-supporting composite material assembled from a bacteriophage—a bacterial virus—(M13) and ZnS solution. In another study, Blum et al. [2] engineered cysteine residue placement in the subunit structure of cowpea mosaic virus, such that the capsid is utilized as a scaffold for the binding of gold nanoparticles in specific, alterable, and reproducible configurations. Similarly, viruses themselves have been organized by binding them to nucleotide chains [3]. Viral capsids have also been used as nanocages for constrained nanomaterials synthesis [4, 5]. By slightly altering the charges on the interior of the capsid proteins, the chemical reactivity can be tuned to match the reactivity of the material in question. These examples serve as a first look at ways in which viruses may be employed outside of the biological world.

To fully embrace viral capsid structures as components of new materials, the mechanical properties and behavior must be fully elucidated. The major theme of this review is that mechanical models of viral capsids will serve as a centerpiece to that end. Yet, apart from theoretical modeling, much is known about the mechanical properties of viral capsids simply from observing them experimentally.

To begin with, viral capsids are a particularly impressive result of two-dimensional protein assembly, which serve to contain and protect viral genome molecules (either DNA or RNA). These protein shells assemble from multiple copies of only a few capsid proteins (in some cases only one) held together by non-covalent forces into very regular structures that are, in most cases, either cylindrical or spherical in shape. In rare cases, capsid self-assembly is achievable in vitro from purified protein components [6]. Because of their regularity, the structures of many capsids have been determined to very high resolution (better than 3 Å) through combined X-ray crystallography and cryo-electron microscopy (cryo-EM) studies. Figure 1 shows representations of two viruses that have been extensively studied both structurally and mechanically: the plant virus, cowpea chlorotic mottle virus (CCMV), and the bacteriophage ϕ29. The structure of CCMV is known at a resolution of about 3 Å [7], and so the atomic coordinates are known with a high degree of certainty, as shown in Fig. 1a. The low-resolution representation of the CCMV capsid is shown in Fig. 1b, enabling the icosahedral nature of the capsid to be clearly seen. The structure of the ϕ29 capsid is not yet known at atomic resolution, thus the only data available is a density map created from low-resolution cryo-EM data. The density map as determined by Morais et al. [8] is shown in Fig. 1c.

The structure of spherical capsids is ordered according to the symmetry of an icosahedron, with capsid proteins grouped locally into fivefold and sixfold *capsomers*. Fivefold capsomers (pentamers) adopt the role of the 12 icosahedral vertex positions, whereas the sixfold capsomers (hexamers) fill in the 20 icosahedral face regions. The pentamer–hexamer count can vary according to the "*T*-number" classification proposed by Caspar and Klug [12] in the 1960's, which groups the total number of 60*T* capsid proteins into 12 pentamers and 10(*T*–1) hexamers. The *T*-number index can only adopt certain integer values, 1, 3, 4, 7, 9, 13, …. Among the different spherical viruses, capsid diameters vary roughly from about 20 nm to a few hundred nm. Constituent proteins are roughly similar in size for most viruses, such that capsid size tends to increase directly with *T* number. For most viruses, capsid size is uniformly and precisely determined and is consistently reproduced under physiological conditions. One notable exception is the human immunodeficiency virus, HIV-1, the capsid of which is polymorphic, marked by variation in size and shape [13]. Interestingly, similarities in the multiscale structural ordering extend to sub-protein, molecular spatial scales as well. For example, as revealed in structures determined by X-ray crystallography and cryo-EM, the coat proteins of many plant, insect, and animal viruses having icosahedral structure are formed around the same core structural motif, that of the so called "jelly roll" β barrel [14].

Despite their precise ordering, significant configurational changes have been observed at various stages throughout the viral life cycle for some capsids. For instance, many viruses first assemble as "procapsids" which then mature into infectious capsids. Maturation can involve structural transitions where proteins are rearranged (e.g., HIV-1 [15]), existing covalent bonds are broken (e.g., flock house virus (FHV) [16]), or new covalent bonds are formed (e.g., HK97 [17]). Structural transitions in capsids can also be triggered by environmental changes, as is the case for the pH controlled swelling of CCMV [7, 18] and the pH triggered release of a pentamer by tymovirus [19]. Similarly, calcium-mediated deformation of simian virus 40 (SV40) is thought to play a role in its infection process [20]. The mechanics of these structural transitions is not well understood nor is it generally known how these conformational changes can be controlled by mechanical force stimulus. A notable exception is the maturation of FHV which is influenced by pressure on the capsid [16].

Recently, technological advances in single-molecule experimentation have enabled direct measurement of the strength and elasticity of viral capsids. Measurement of the

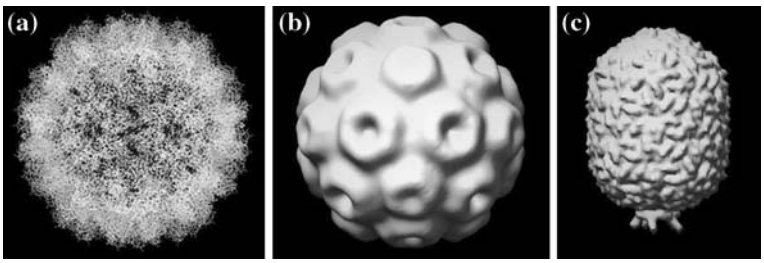

Fig. 1 Representations of the CCMV (**a**) and (**b**) and ϕ29 (**c**) viral capsids. The structure of CCMV has been determined to atomic resolution, and therefore the atomic positions are known with a high degree of precision, as shown in the atomic representation (**a**). The low-resolution representation of CCMV in (**b**) shows the icosahedral structure. The capsid structure of ϕ29 has not beed determined to atomic resolution, thus only low-resolution representations such as density maps are available, and shown in (**c**). Renderings of CCMV and ϕ29 were generated with the molecular imaging software, Chimera [9]. The CCMV atomic coordinates were downloaded from the RCSB Protein Data Bank [10], and the ϕ29 density map was downloaded from EMBL-EBI [11]

forces required to package DNA into bacteriophage ϕ29 using optical tweezers has shown that its capsid is strong enough to sustain effective pressures estimated on the order of 60 atm [21–24]. Traditional experimental methods for determining elastic properties of materials involve stretching and compressing them. Atomic force microscopy (AFM) allows for this to be done with single molecules and their assemblies. AFM has been used to study the mechanical properties of the capsids of several viruses: ϕ29 [25], CCMV [26, 27], parvovirus minute virus [28], and murine leukemia virus [29]. In fact the technique is useful for a general class of protein assemblies and has also been used recently to study buckling of microtubules [30]. In these experiments, capsids are indented with the AFM cantilever tip, producing a force-deflection curve. Figure 2 shows force-indentation results from experiments performed on CCMV at two different pH levels [26, 27]. At pH 5 a jump in force is observed at a compression of about 30% and accompanied by a permanent decrease in stiffness (Fig. 2b) indicating an irreversible mechanical failure of the capsid. In contrast, the pH 6 force response remains linear without significant hysteresis throughout the complete indentation range, even to the point where the capsid is compressed such that the top and bottom inner surfaces of the capsid shell are pressed into contact. Thus, at pH 6 CCMV is almost perfectly elastic, and effectively *indestructible* under nanoindentation.

The collection of experiments on viral capsids reveal a number of mechanical properties that contribute to their allure for materials applications. Namely, their surprising strength and flexibility as found by AFM experiments, even under large deformations, sets them apart from most traditional materials. Additionally, the ability to control and alter the material properties of some viral capsids as a function of adjustable environmental conditions, such as pH, makes them versatile. And yet, viral capsids are not weak; as noted above, some viral capsids are able to sustain extremely high pressures [21–24]. Finally, viral capsids can exhibit some degree of structural flexibility, as they may undergo conformational changes in which their size can expand or contract, and may self-assemble from pure components. Effective utilization of these properties requires a full understanding of the underlying mechanisms. Complementary theories are needed to bring about the understanding of these mechanisms, and mechanical models provide an excellent foundation for determining the properties that control these behaviors.

To date, the use of bottom-up mechanical models such as molecular dynamics (MD) and normal mode analysis (NMA) have been the most widely used methods to study the unforced vibrational dynamics of viral capsids. These methods tend to involve an all-atom description of capsid structure, although coarse-graining has gained in use. In fact, viral capsids, which are large macromolecules with many thousands of atoms, are nearly impossible to study with these methods without some form of coarse-graining. Because these methods are meant to study the unforced vibrational dynamics of a system, they give an indirect look at the mechanics of viral capsids. One of the important results to come out of the coarse-grained studies of viral capsids is that the overall structure, and not the atomic detail, is the main factor in controlling the unforced mechanics. With the development of new direct experimental methods of probing viral capsids, such as the AFM experiments described above, mechanical models are needed to describe the forced mechanical behavior. In the last few years, top-down approaches such as continuum modeling have been developed and used as a theoretical complement to describe the response of viral capsids under forced conditions, with the results suggesting that the overall shape of the capsid also plays a dominant role in governing the forced mechanical behavior. Ultimately, it is likely that novel multiscale methods will be required to capture both the global structural response of the capsid in addition to the detailed atomic response that will govern complex behaviors such as failure.

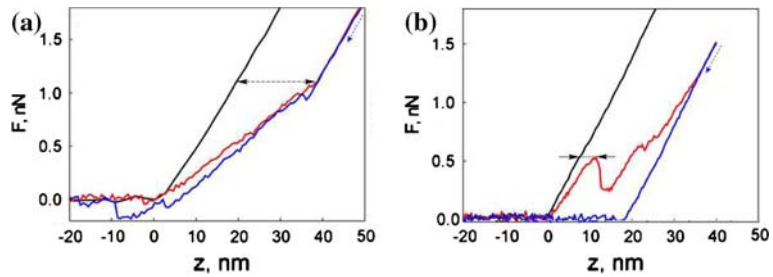

Fig. 2 Indentation force plotted versus substrate displacement for CCMV capsids at pH 6 (left) and pH 5 (right) [26, 27]. For a corresponding force, the indentation is given by the distance between the black curve (cantilever alone) and the colored curves (cantilever and capsid in series). Loading is described by the red curves, unloading by the blue curves. The large drop in force and presence of significant hysteresis in the pH 5 case is indicative of capsid failure. (Figure adapted from [27].)

Modeling viral capsids from the bottom up

Experimental methods such as X-ray crystallography and cryo-EM used to determine the atomic structures of viral capsids show that they are not entirely static. At the atomic scale, thermal motions are detectable and the equilibrium structure describes the average position of all atoms over time. These structural methods are also able to detail changes in the atomic positions in response to the binding of external molecules, such as antiviral complexes, and the structural rearrangements seen during conformational changes. In order to understand both the equilibrium fluctuations and structural changes, mechanical models of viral capsids were first developed to study their unforced vibrational dynamic behavior almost twenty years ago. Methods such as molecular dynamics (MD) and normal mode analysis (NMA) represent a bottom-up approach to modeling viral capsids; the models were initially used with atomic level detail, and more recently, coarse-grained models have been introduced.

Molecular dynamics

Beyond studying the equilibrium fluctuations of viral capsids, MD has been used to model several specific types of problems: assembly [31, 32], stability [33, 34], and antiviral activity [35–38], to name a few. MD is useful for the modeling of very short time scale dynamics (on the order of pico and nanoseconds), and captures both anharmonic and harmonic motions. The main challenge in the application of MD to viral capsids is the number of atoms in the system. There is no general rule for estimating the number of atoms per protein subunit, and thus the number of atoms for different viruses with the same T-number may widely vary. However, among several representative $T = 3$ viruses, the total number of atoms varies from about 150,000 to 250,000. Consequently, most MD simulations performed on viral capsids are limited to single subunits, with symmetry conditions enforced so as to capture the icosahedral environment.

Viral capsids have been studied using MD for almost 20 years, with the first simulation, performed in 1988, consisting of a single human $T = 1$ rhinovirus subunit in vacuo, with an antiviral compound bound to the subunit in order to determine the dynamic effects of the binding [39]. This simulation was performed on ~8000 atoms, and the results showed large edge effects as no boundary conditions were applied, and thus the icosahedral environment of the entire capsid was not modeled. In 1991, a method was developed to model the complete capsid environement by taking advantage of the rotational symmetry boundary conditions present in icosahedral viruses, and MD was again applied to the human rhinovirus subunit in vacuo [35], and fully solvated [36]. In this way, the physical environment of the entire capsid is modeled, while the smaller number of atoms in a single subunit is utilized. The use of the rotational symmetry boundary conditions showed a significant reduction in the edge effects as atoms near the boundaries remained close to the crystal coordinates. However, the total simulation time only varied from 15 ps [35] to 60 ps [36].

Until very recently, MD simulations performed on viral capsids were limited to the asymmetric unit (the collection of subunits that comprise one icosahedral unit, and in the case of $T = 1$ capsids, a single subunit) alone, as detailed above. The first molecular dynamics simulation on an entire virus was completed in 2006 [40]. The simulation was performed on the $T = 1$ satellite tobacco mosaic virus (STMV). The $T = 1$ capsid structure is the smallest among spherical viruses. The simulation was performed with both the capsid proteins and a highly simplified model of the nucleic acid, which was built from identical RNA segments to match the icoshedrally averaged electron density of RNA, in a completely solvated environment, with ~1,060,000 total atoms, ~900,000 of which were water. The time scale of the simulation was 10 ns. The aim of the simulation was to investigate the stability of the viral capsid with and without the RNA present, and it was found that the capsid without the RNA was unstable after 10 ns. The capsid was, however, extremely stable with the RNA present in the simulation. Unlike some viruses that have been observed to self-assemble in vitro into empty capsids (no nucleic acid packaged), empty STMV has not been observed [40].

In order to perform MD on viral capsids, an all-atom structure needs to be available for the system of interest. These are not available for all viral capsids. All-atom descriptions necessarily limit the size of the system that can be studied, and at the time scales sampled by MD, large deformations of a capsid, such as conformational changes, cannot be modeled. Even the studies that use the rotational symmetry boundary condition method to model only a small fraction of the capsid are limited in the types of motions that are output; any type of asymmetric or global motion will not be captured. Bottom-up descriptions such as MD are advancing very slowly in terms of their capability. The use of MD to model externally applied forces, such as would be present in experimental methods such as AFM, is probably not feasible. Experimental methods such as AFM incur large deformations and occur on a time scale of milliseconds to seconds. These deformation and time scales are simply inaccessible by all-atom methods, unless some form of coarse-graining is applied. Aware of these limitations, a new coarse-grained MD method has been recently developed to study the dynamic stability of larger viral capsids, extending the timescale to the microsecond range [65].

Normal mode analysis

Normal mode analysis (NMA) was developed as a more computationally efficient alternative to MD. Initially, NMA simulations were all-atom and involved the same interatomic potential field as MD, but by using a quadratic approximation for the potential energy, the problem is reduced to a normal mode analysis on a structure and only outputs harmonic motions. For an excellent reference on both the theory and applications of NMA, see Cui and Bahar [41]. The potential energy, or dynamical, matrix is formulated from the second derivatives of the potential function. In this way, the time propagation of MD is eliminated, and the computationally expensive step is diagonalizing the dynamical matrix, after which the output eigenvalues (frequencies) and eigenvectors (normal modes) are easily extracted. However, for larger systems, the diagonalization becomes a major bottleneck as the size of the dynamical matrix grows proportionally to the size of the system being studied. Thus, the use of all-atom NMA was limited for many years to small proteins due to computational limitations.

It has been shown that the lowest modes tend to represent coordinated global motions of the structure being studied, and are therefore most relevant for large motions such as conformational changes [42]. Generally, NMA is used to find normal modes that capture conformational changes of proteins [43], as an aid in the refinement of low-resolution cryo-EM density data [44–46], and to describe the general unforced dynamics of macromolecules [47, 48]. As applied to viral capsid structures, NMA is used to study maturation dynamics and conformational changes such as capsid swelling [49–51]. The time scales associated with the NMA frequencies are longer than that of MD, but are still limited to the microsecond range.

An early all-atom NMA study was performed on the rod shaped tobacco mosaic virus (TMV), in which one full unit that is necessary for symmetry—seventeen subunits that make up on turn—was modeled for the analysis [52]. The standard CHARMM force field was used [53]. In 2005, van Vlijmen and Karplus [54] reported the first atomic-level NMA calculations on a full icosahedral virus capsid, also using a full interatomic potential. However, all-atom NMA calculations using standard interatomic potentials remain quite rare due to the computational demands, the development of simplified potentials, and the use of coarse-grained models.

In 1996, Tirion [55] introduced a simplified pairwise Hookean potential to replace the complex interatomic potential, and the method became known as the elastic network (EN) model. The potential involves a single parameter that is equal for all interactions, thus the need for all atoms is avoided. This allows for NMA to be applied to coarse-grained models, and consequently for larger systems such as viral capsids to be studied. In addition, the intital energy minimization required when using the complex interatomic potential is also avoided. The EN model is useful for the lowest modes of vibration as the high frequency modes cannot be modeled well with simplified potentials.

In two studies, Tama and Brooks [50, 51] used coarse-grained NMA with the EN model to study the conformational changes observed in icosahedral virus capsids. In 2002, they reported that a coarse-grained NMA of the CCMV capsid, which undergoes a pH induced swelling with ~10% size increase in the radial direction, showed that just a few of the lowest symmetric normal modes contributed to the swelling displacement [50]. The goal was to model several pathway intermediate structures, to get a better idea of the structural changes during swelling. The CCMV capsid was coarse-grained using the C^{α} atoms, the simplified Hookean potential was used, and the rotations-translations of blocks (RTB) method [56, 57] was used to simplify the problem by assuming each individual subunit was rigid. The normal modes are computed as a linear combination of the rotations and translations of the rigid blocks. In 2005, the same methods were applied to study conformational changes of other viruses [51]. It was shown that for viruses with known conformational changes (CCMV, HK97, and NωV) one symmetric mode captures the bulk of the motion. The next few lowest symmetric modes are needed to more accurately describe the known conformational pathway. They argue that this shows the method is robust, and can be predictively applied to other viruses for which both conformational states are not known, or for viruses for which it is not known if conformational changes exist. Also in 2005, Rader et al. [49] applied coarse-grained NMA to study the conformational changes that the HK97 virus undergoes during maturation. It was shown that the first eleven modes capture over 98% of the observed maturation pathway. The low-frequency modes also showed regions of relative flexibility, at substrate recognition sites, and relative stiffness, at anchors or hinge sites. This study proposed a maturation pathway ultimately leading to a cross-linked "chain-mail" complex.

If EN models are used in a normal mode analysis, at any level of coarse-graining, the need for the initial minimization of the potential energy is eliminated, but the results are less physical. As the leading constant in the Hookean potential is the only tunable parameter in the model, the value is found by fitting the normal mode results to experimental vibrational data. There is then a loss of some predictive power. Similarly, using NMA to predict the pathways of large conformational changes is only possible if the initial and final states are known, as it is not obvious which of the lowest modes capture the relevant pathway. The only exception to this might be viral capsid swelling, which appears to involve only symmetric motion, and thus

comprised of only the symmetric modes. However, van Vlijmen and Karplus [54] argue that symmetric modes, which Tama and Brooks [50, 51] use in their coarse-grained NMA analyses of viral capsids, are not necessarily the true pathway of a conformational change, and non-symmetric modes may need to be studied to fully understand the pathway.

It is fair to say that NMA is currently the best method available to study unforced vibrational macromolecular dynamics. Currently, all-atom NMA simulations are very complex, so coarse-grained models are the preferred method for large systems such as viral capsids. Not only has NMA established coarse-graining as a useful step in studying large systems, but as a necessary one as well. Coarse-graining allows for systems without full atomic descriptions to be studied, in addition to highlighting the fact that the atomic details are not a main factor in determining the global motions. One of the main conclusions that has been drawn from the numerous coarse-grained NMA studies is that the overall shape of a structure governs its unforced vibrational dynamics [42]. This may have far-reaching implications for modeling viral capsid systems under forced conditions. In fact, it is reasonable to assume that shape governs forced mechanics, and based on recent computational studies of viral capsids where only the shape is preserved, this appears to be precisely the case.

Modeling viral capsids from the top down

Recent single molecule experiments such as atomic force microscopy have given rise to a number of new top-down two- and three-dimensional continuum elasticity models involving the mechanical response of viral capsids. As discussed above, a major result of previous coarse-grained NMA studies was that the most important factor determining a structure's unforced vibrational response was its shape, not atomic detail. Similarly, here it is argued that the same is true for forced mechanical response, as the results of several recent continuum studies of viral capsid mechanics seem to support.

Two-dimensional continuum modeling

From the number of viral capsid structures determined experimentally by X-ray crystallography and cryo-EM, it has been observed that icosahedral capsids, which come in a variety of sizes, have what appears to be a size dependent shape character. Smaller viruses tend to appear more spherical and smooth, whereas larger viruses tend to show faceting and visible ridges. Recent computational models of icosahedral capsids have been created on the basis of the theory of thin elastic shells to study the shapes and shape transitions of the icosahedral viruses. These models treat the total energy of the system as a sum of both bending and stretching contributions. Building off of previous work on the stability of planar elastic sheets [58], Lidmar, Mirny and Nelson (LMN) [59] hypothesized that the pentamers of an icosahedral capsid act as fivefold disclinations, which are necessary to form a closed shell from an otherwise hexameric lattice. They showed that the degree of faceting is dependent on the relative stiffness of bending and stretching and the size of the shell. In particular, the energy minimizing shape of the capsid shell transitions from roughly spherical to icosahedrally faceted with an increase in value of a dimensionless parameter, the Föppl-vonKármán (FVK) number $\gamma = YR^2/\kappa$, where Y is the two-dimensional Young's modulus, κ is the bending rigidity, and R is the average radius of the capsid. There is a critical value of the FVK number at which a transition occurs that can be described as buckling. For sub-critical FVK numbers bending stiffness dominates over stretching and the capsid shape is roughly spherical. Above this transition, the structure has a lower energy if the surface is bent near the disclination, thereby relaxing the otherwise dominant stretching energy. This effect is argued to be a cause for the visible faceting. As the subunits of different viruses have similar molecular structure, they should be expected to have similar mechanical properties. Thus, the spherical or faceted shape of a virus is predicted to scale with its size. Following the LMN study, Nguyen et al. [60, 61] built on the thin elastic shell theory to include spontaneous curvature in an attempt to discover non-spherical equilibrium capsid shapes, such as the conical capsid shape often seen in HIV. The two-dimensional icosahedral models of Zandi and Reguera [62] showed that in addition to the size dependent shape character of viral capsids, there is a size dependent stress character; the degree of stress concentration at the pentamer sites grows with increasing T-number.

Icosahedral models of viruses have very recently been extended to model the mechanical response of capsids under applied loads. Simulations of AFM experiments have been performed to study the effect of the shape transitions implied by the FVK number. Vliegenthart and Gompper [63] simulated indentation of T-number lattice triangulations, which for sufficiently large T-numbers are consistent with the Föppl-vonKármán continuum elastic description. Using a molecular dynamics approach to solve for equilibrium shapes, they showed that the buckling instabilities of fivefold disclinations can be excited during indentation, leading to discontinuous drops in indentation force. Furthermore Vliegenthart and Gommper argue based on their results that "size effects" may be important for smaller capsids, causing their force-indentation character to differ from that predicted by continuum mechanics. Using a

thin-shell continuum finite element approach, Klug et al. [27] recently modeled AFM experiments on the empty CCMV viral capsid modeled as an icosahedron with varying values of the FVK number. The shells with FVK numbers below the buckling threshold (more round) show linear behavior consistent with pH 6 experiments, while the shells with FVK numbers above the buckling threshold (more faceted) show sharp discontinuities in the force due to inversion of buckled fivefold disclinations, reminiscent of the failure of capsids seen experimentally at pH 5. In particular it is argued that the pH indued swelling transition of CCMV detailed earlier has the effect of softening the mechanical response of the capsid by lowering the effective Young's modulus of the capsid, and thus lowering the FVK number. This is proposed as a mechanism responsible for the presense and absense of failure in AFM indentation experiments at pH 5 and 6 respectively.

The results of the LMN study support the notion established by NMA studies [42] of a strong link between shape and mechanical properties for macromolecular assemblies. Indeed, whereas NMA studies have suggested that shape governs mechanics, the LMN model represents an example of the reverse, i.e., how mechanics can govern shape. The results of Klug et al., and Vliegenthart and Gompper go further by examining the link between shape and mechanical response of icosahedral capsids under the influence of externally applied mechanical forces. The one common feature of these two-dimensional elastic shell models is the assumption that because of their geometry the coat proteins most naturally fit together in a planar hexagonal lattice, which represents a stress-free reference configuration. Thus the presence of fivefold disclinations necessarily introduces pre-stresses in the closed capsid shell. This assumption resides at a midpoint within the multiscale hierarchy of capsid structure, at a length scale above that of primary and secondary molecular structure, but below that of the global capsid assembly. It may perhaps be surprising then, that other recent continuum models working purely at the global structural scale also seem to do well in explaining features of capsid mechanics observed in experiments.

Three-dimensional continuum modeling

As a key theoretical complement to the recent experimental advances in single molecule experiments such as atomic force microscopy (AFM), three-dimensional continuum modeling has emerged as a simple but powerful tool in understanding viral capsid mechanics. Because the problem has complex boundary conditions and geometry, analytical solutions are not feasible without generalizations that would result in a loss of precision, and so finite element methods have been used to simulate the AFM experiments.

In 2004, the first AFM study on a viral capsid along with continuum model elasticity studies was reported by Ivanovska et al. [25]. The virus studied was the bacteriophage $\phi 29$, which has icosahedral endcaps and a cylindrical center section made from a ring of subunits arranged into hexameric groups. It was shown that the empty capsids had a linear response up to displacements of ~30% of the capsid height, and was completely reversible unless the capsid was displaced to the point of failure, at which drops in the contact force were seen. In order to extract material parameters for the capsid, a three-dimensional continuum elastic finite element model was created, on which the AFM experiment was simulated. The Young's modulus was varied until the slopes of the experimental and simulated contact force curves matched. Similar studies were performed on the plant virus CCMV and subsequently modeled using three-dimensional continuum elastic finite elements by Michel et al. [26], and Gibbons and Klug [64]. It was shown by Gibbons and Klug in a series of parametric studies that the observed linear behavior may be understood as a combination of several geometric effects; mainly that the thickness of the capsid and size of the AFM tip (which is on the order of the capsid size itself) largely determine the mechanical response to applied loads [64]. Interestingly, the response was not sensitive to the constitutive law chosen, as long as large strain measures were used. (The use of linearized small strains was shown to be inadequate for such analyses.) From these studies, it appears that the geometry of the capsid and AFM tip, rather than local material response, have the most impact on the resulting force curves.

Three-dimensional continuum models were also used to model immature and mature murine leukemia virus (MLV) capsids [29]. AFM studies on the immature and mature capsid showed quantitative and qualitative differences in the contact force response curves; the immature capsid is 20 nm thick, and showed a response similar to Hertzian contact behavior, while the mature virus, which is only 4 nm thick, showed a linear contact force response. The nominal external radius in both cases is the same, at ~50 nm. These results agree very well with the contact force curves of the AFM experiments.

Similar AFM studies were performed on the minute virus of mice (MVM) with and without the DNA genome enclosed [28]. The studies showed that the capsid without the genome showed force responses that were equal for all three symmetry faces, but showed an anisotropic increase in stiffness when the DNA was enclosed. A corresponding continuum model was created in which the capsid was modeled as an icosahedral shell with the average thickness of the capsid, with the DNA modeled as circular disks that add thickness to the capsid at different points on a given face of the icosahedron. They were able to show the anisotropic increase in stiffness with the capsid and DNA model.

Continuum finite element models for both empty and full capsids have served as a sufficient means of capturing some of the most important mechanical behaviors observed experimentally. Although icosahedral viruses can have very complex topographies, these features do not appear to play a main role in the observed elastic response of the capsids; rather, the details will more likely play an important role in failure mechanisms, which may depend more heavily on the specific regions that are weak, and are likely to be directly related to the strength of individual capsomer–capsomer interactions. The studies cited above have shown that continuum models that take into account the overall shape of viral capsids do quite well at reproducing experimentally observed contact force curves. This serves as an inspiration for further continuum and coarse-grained approaches, specifically to investigate mechanisms of failure.

Conclusions

Molecular dynamics and normal mode analysis have revealed important mechanical properties of viral capsids by studying the unforced vibrational dynamics. One of the main conclusions of molecular modeling is that the overall shape of viral capsids, and not the atomic detail, governs the unforced mechanical response. In particular, coarse-grained NMA models are able to capture the dynamic behavior of viral capsids, such as conformational changes, without the fine scale detail used in MD simulations. Despite the success of these bottom-up methods, there are limitations to both. Computational expense is a large problem that has only recently been addressed with coarse-grained methods, the time scales on which both MD and NMA are valid are still quite small, and perhaps most importantly, the methods are limited to unforced dynamic problems. The latter limitation excludes the study of a range of problems for which experimental data is now available, such as the newly developed AFM experiments that were detailed above. Additionally, the trend towards coarse-grained models has moved the models closer to continuum descriptions, and while the ability to describe movements at atomistic detail is lost, the bulk dynamic behavior is retained, and this suggests that continuum models may provide insights into the forced mechanical behavior of viral capsids without a loss of physicality.

It has been demonstrated that continuum models provide a wealth of information about the response of viral capsids to applied loads. By modeling the global shape of viral capsids, without atomic level structural information, both thin and thick shell mechanics seem to consistently predict what is observed experimentally. Results of several continuum finite element studies have demonstrated that the shell-like geometry of the capsid is the most important factor in determining the mechanical response to external forces, while the models are not highly dependent on the constitutive modeling details. These results are complementary to the results of the molecular mechanical models, which have shown that the *details* of the atomic structure and interatomic potentials are not essential to capture the unforced vibrational behavior. In particular it is important to recognize the consistency between the insensitivity to constitutive law for continuum models and the insensitivity to interatomic potentials for the molecular models. This provides some justification for the modeling assumption of constitutive homogeneity. It may seem incredible that continuum elasticity theory would be meaningful for a nanometer-scale molecular structure such as a viral capsid. Yet, the results of recent modeling studies establish that this is precisely the case.

Yet, caution should be exercised in celebrating the sucesses of continuum theory. The established continuum models of capsid mechanics are not without limitations, the largest of which is the exclusion of dynamic behavior. In particular, thermal forces and fluctuations are not considered, and although these details may not be important to the quasi-static global mechanical response of the viral capsids, time sensitive behaviors are not captured. Dynamic behaviors are extremely important when studying assembly and disassembly, and viscoelastic/rheological behavior. Furthermore, in the quest for deeper understanding of the material properties of capsid shells, some questions that simple continuum elasticity models have difficulty answering will need to be addressed. For instance, elasticity is—by definition—incapable of explaining the irreversible damage observed in the experiments on CCMV [26] and $\phi 29$ [29]; what are the mechanisms that lead to irreversible failure at one pH, and nigh-invulnerability at another? More generally, how are the "microscopic" conformational changes of individual proteins manifested in the "macroscopic" structural mechanics of a large protein aggregate? Perhaps continuum methods can be stretched and extended via multiscale modeling to provide answers to these questions.

As informative as bottom-up methods such as MD and NMA are on short time scales and at atomic levels, top-down continuum models hold the most promise for understanding viral capsid behavior under the variety of applications they now serve in. Yet to overcome the limitations of the current continuum methods, incorporating atomic level detail when needed may be necessary. Thus, the development of multiscale methods will most likely be needed to understand complex dynamic behavior of viral capsids, such as conformational changes. NMA has been used to describe the pathway of such deformations, but perhaps continuum and multiscale methods will deepen the understanding about the molecular mechanisms behind these behaviors. For example, continuum models have

been used to study the conformational changes of the CCMV capsid, and have shown that relevant modes may be exictable when forces are applied, and this exictation will manifest as a change in material properties [27]. The exact molecular mechanisms responsible are as of yet unknown. Additionally, multiscale models will be needed to model the forced mechanical response of viral capsids beyond the elastic regime, to study the failure of viral capsids, as failure will be initiated at the atomic level due to the breaking of bonds.

Note: Just before this article went to print, an article appeared detailing AFM experiments on the bacteriophage λ, studying the effects of packed genome on the strength of the capsid [66]. We would also like to report that this same group is preparing an article on the subject of viral capsid failure.

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