Modeling and simulation of the mechanical response from nanoindentation test of DNA-filled viral capsids

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Abstract Viruses can be described as biological objects composed mainly of two parts: a stiff protein shell called a capsid, and a core inside the capsid containing the nucleic acid and liquid. In many double-stranded DNA bacterial viruses (aka phage), the volume ratio between the liquid and the encapsidated DNA is approximately 1:1. Due to the dominant DNA hydration force, water strongly mediates the interaction between the packaged DNA strands. Therefore, water that hydrates the DNA plays an important role in nanoindentation experiments of DNA-filled viral capsids. Nanoindentation measurements allow us to gain further insight into the nature of the hydration and electrostatic interactions between the DNA strands. With this motivation, a continuum-based numerical model for simulating the nanoindentation response of DNA-filled viral capsids is proposed here. The viral capsid is modeled as large-strain isotropic hyper-elastic material, whereas porous elasticity is adopted to capture the mechanical response of the filled viral capsid. The voids inside the viral capsid are assumed to be filled with liquid, which is modeled as a homogenous incompressible fluid. The motion of a fluid flowing through the porous medium upon capsid indentation is modeled using Darcy's law, describing the flow of fluid through a porous medium. The nanoindentation response is simulated using three-dimensional finite element analysis and the simulations are performed using the finite element code Abaqus. Force-indentation curves for empty, partially and completely DNA-filled capsids are directly compared to the experimental data for bacteriophage λ . Material parameters such as Young's modulus, shear modulus, and bulk modulus are determined by comparing computed force-indentation

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curves to the data from the atomic force microscopy (AFM) experiments. Predictions are made for pressure distribution inside the capsid, as well as the fluid volume ratio variation during the indentation test.

Keywords Capsid nanoindentation • AFM • Darcy's law • Finite element simulations • Spring constant • DNA-filled capsid

1 Introduction

The mechanical properties of complex structured viral capsids are not clearly understood. Our knowledge of the mechanical behavior of viruses is based on the experimental data derived from the nanoindentation probing performed by an atomic force microscope (AFM). Mechanical properties of only a few viral capsids have been investigated experimentally so far. To name a few, bacteriophages λ and $\phi 29$ [1, 2], plant virus CCMV [3], MVM virus [4], MLV, HIV [5, 6], HSV-1 [7] and HK97 [8]. Probing viral shells with an AFM tip generally results in either reversible deformation, often observed when the applied force is below a certain value, or an irreversible capsid rupture when the applied force is above this threshold value. This enables us to determine different parameters describing capsids' mechanical properties. The experimental data reveals distinct mechanical features for different classes of viruses, which can be directly correlated to the virion's replication cycle. These unusual properties also place viral capsids as great candidates for many nano-applications such as viral encapsidation and delivery of drugs and biomolecules. It is therefore of great importance to understand the relationship between the mechanical properties and biological functions of viral capsids.

Viruses are some of the smallest biological entities. A typical virus capsid has a diameter between 20 and 300 nm. The majority of capsids have an icosahedral symmetry and their protein subunits are arranged in a quasi-spherical structure [9]. A regular icosahedron is assembled of 20 equilateral triangular facets connected in a pattern with 12 pentagonal vertices. Typically, the capsid proteins are arranged in pentamers and hexamers (structures composed of five and six subunits) [10]. While hexamers assemble into flat surfaces, introduction of pentamers introduces an angle and the curvature needed to form an icosahedral-spherical capsid is obtained. This curvature varies with the number of hexamers determining the size of the capsid. Thus, small viruses are more rounded, i.e., with a geometry closer to a sphere, whereas large viruses are strongly faceted with a clear icosahedral shape. The bacterial virus, bacteriophage λ (or just phage λ), used in this work as a model system, is a medium-sized virus with a diameter of 63 nm. Its shape is between spherical and icosahedral symmetry [11].

The majority of phage capsids experience internal genome pressures of tens of atmospheres due to the tightly packaged double-stranded DNA. In case of phage λ , it has been shown that the capsid strength approximately matches the force exerted by the DNA on the capsid walls [2, 12]. This suggests that the capsid strength determines the maximum length of DNA that can be packaged, which in turn is also correlated with the strength of the packaging motor complex [2, 13]. Therefore, investigating the mechanical properties of DNA-filled versus empty capsids is of great interest.

The basic principle of AFM is that a microscopic cantilever with a probe tip sweeps over an area in liquid. AFM starts with scanning the sample, and when the probe tip is in contact with a virus on the surface, the cantilever deflection force is recorded as an electric

voltage along with the extent of deflection. Since both the viral capsid and the cantilever are bending, the measured deflection is not the true virus deformation. The deformation of the viral capsid can be obtained by calibration of the cantilever against the glass surface (aka glass curve) and subtracting this spring constant value from the total measured bending and spring constant. The critical force load at which the capsid collapses is referred to as the breaking force. For a range of the reversible deformations (not reaching the breaking force value), force-distance curves are collected providing an average value of the capsid's spring constant. Modeling of the empty and the filled viral capsids performed in this paper is focused on this latter case of spring constant measurements. Typical results from such AFM indentation experiments on phage are shown in Fig. 1. Modeling viral capsids and their mechanical response has become an active field of research. Different approaches have been developed and used to simulate the characteristic behavior of viral capsids. Both discrete and continuum approaches have been used. Two-dimensional models [4, 14–17], using thin shell approximation, and general three-dimensional models [3, 5, 6] have been used to model viral capsids. However, the majority of these simulations are modeling only an empty viral shell [3–6, 14–22].

In [2] it was experimentally demonstrated that upon AFM cantilever indentation of DNA-filled phage, water hydrating the packaged DNA is being displaced to the bulk through the pores in the capsid. It should be noted that all viral capsids are permeable to water and smaller ions. Thus, the measured force resisting the AFM indentation is dependent on the DNA hydration force (that is, the force required to move one molecule of water from the encapsidated DNA to the bulk) [23, 24]. The decrease in packaged DNA-DNA spacing upon indentation results in an exponential increase in the repulsive hydration and electrostatic interaction [23, 24]. The bending energy of DNA also contributes to the overall resistance to the AFM indentation of the capsid. The hydration force is in this case nearly equal to the osmotic pressure of DNA inside the capsid. The strength of the capsid is optimized to the maximum osmotic pressure resulting from packaging the wild-type λ -DNA length [2]. However, the experimental data in ref. [2] shows that only in the case of 100% wt λ -DNA length-filled capsids, the internal DNA hydration force significantly contributes to the overall capsid stiffness. In the case of 78% wt λ -DNA length-filled capsids, AFM determined capsid stiffness is similar to that of an empty phage capsid. To gain a better understanding of the fluid flow through the capsid wall pores and the mechanical response

Fig. 1 Typical results from an AFM experiment. Adapted from [2]

to the deformation of DNA-filled viral capsids, we perform in this work finite element (FE) method simulations on empty, partially DNA-filled (with 78% wt λ -DNA length) and 100% DNA-filled (wt λ -DNA length) bacteriophage λ capsids. The results of the simulations are directly compared to previously obtained experimental data [2].

The goal of this work is to develop a simulation model for DNA-filled viral capsids utilizing FE-analysis and determining the material constants in the models by direct comparison with the experimental AFM data [2]. When we refer to the fully filled capsids in the text, it implies wild-type λ -DNA length, with approximately 50% of the capsid volume is occupied by the DNA and the remaining volume is occupied by water molecules hydrating the DNA. Three models are used here. In the first simple model the filled capsid is assumed to behave as hyper-elastic material. In the second model, porous elastic material with voids filled with incompressible fluid is chosen to mimic the DNA. In the third, the most accurate model, in order to simulate the conditions of actual nano-indentation AFM probing, the fluid inside the capsid is allowed to flow through the viral capsid pores upon capsid deformation.

2 Model description

2.1 Large-strain isotropic hyperelastic model

The viral capsid is modeled as large-strain isotropic hyperelastic material. The Cauchy stress can be derived from the strain energy function $W = W(\mathbf{F})$ given by the deformation gradient $\mathbf{F}$, defined as,

$$\mathbf{F} = \frac{\partial \mathbf{x}}{\partial \mathbf{X}} \quad (1)$$

where $\mathbf{x}$ is the current position of the a material point and $\mathbf{X}$ is the reference position of the same point. For simplicity, we define the modified deformation gradient $\bar{\mathbf{F}}$ as,

$$\bar{\mathbf{F}} = J^{-\frac{1}{3}} \mathbf{F} \quad (2)$$

where $J = \det \mathbf{F}$. The principle of material frame indifference restricts the dependence of the strain energy function W on the deformation gradient $\mathbf{F}$ through the modified left Cauchy–Green deformation tensor $\bar{\mathbf{B}} = \bar{\mathbf{F}}\bar{\mathbf{F}}^T$. Then the strain invariants are defined as,

$$\bar{I}_1 = \text{tr} \bar{\mathbf{B}}, \quad \bar{I}_2 = \frac{1}{2} ((\text{tr} \bar{\mathbf{B}})^2 - \text{tr}(\bar{\mathbf{B}}^2)), \quad I_3 = \det \mathbf{B} \quad (3)$$

where $\mathbf{B} = \mathbf{F}\mathbf{F}^T$ and $\det \mathbf{B} = J^2$. For isotropic compressible material, the strain energy W is a function of $\bar{I}_1$, $\bar{I}_2$ and J . Biological materials are characterized by nearly incompressible behavior and that restricts $J = \det \mathbf{F} = 1$. Hence, strain energy function for an isotropic material is

$$W = \sum_{p,q} C_{pq} (\bar{I}_1 - 3)^p (\bar{I}_2 - 3)^q \quad (4)$$

where C_{pq} are material parameters and p and q integer numbers. Several particular forms of the strain energy potential could be used, e.g., the Ogden model, the Mooney–Rivlin, Yeoh, or neo-Hooke. As it was shown in [11, 25], the response obtained with these different models is similar to that using the non-linear neo-Hookean model and it is therefore significant to use the large deformation formulation, i.e., non-linear analysis. Since the

sensitivity to the constitutive law is very low, here the simplest constitutive model is chosen, namely the non-linear Hookean model, which provides sufficiently accurate results. The strain energy potential is a linear function of $\bar{I}_1$

$$W = C_{10}(\bar{I}_1 - 3) \quad (5)$$

where $2C_{10} = G$.

For the large-strain hyper-elastic material, which is considered here, the Cauchy stress can be derived from the strain energy function given by the deformation gradient. The logarithmic strain $\varepsilon = \ln \mathbf{V}$ is introduced as a strain measure, where $\mathbf{V} = (\mathbf{F}\mathbf{F}^T)^{1/2}$ is the left stretch tensor. The total strain can be resolved into volumetric and deviatoric parts according to

$$\varepsilon^{\text{vol}} = \ln J \quad , \quad \varepsilon^{\text{dev}} = \ln(J^{-\frac{1}{3}}\mathbf{V}). \quad (6)$$

2.2 Modeling the DNA-filled capsid-porous material

The viral capsid is filled with DNA chains surrounded by fluid. Porous elasticity, see [26, 27], is adopted here to capture the mechanical response from nanoindentation experiments on the DNA-filled viral capsid. It is assumed that the DNA distribution is uniform inside the capsid.

A porous material is a medium composed of grains from solid materials and voids, which consist of liquids and/or air and gases, see Fig. 2. The void ratio e of the porous material is defined as

$$e = \frac{V_v}{V_{gr}} \quad (7)$$

where V_{gr} is the volume of grains and V_v is the volume of the voids, see Fig. 2. For simplicity, the filled capsid is modeled as a porous elastic material composed of grains from solid materials and fluid-filled voids.

Fig. 2 Schematic representation. Porous medium consisting of solid grains and voids (to the left) Void ratio e (to the right)

In the present simple porous elastic model, it is assumed that the shear and the bulk behavior are uncoupled and the material is described by two elastic parameters, the shear modulus G and the bulk modulus K . For simplicity, it is assumed that the shear modulus G is constant and the bulk modulus linearly dependent on the mean stress or pressure p , see [27, 29],

$$K = \frac{p(1 + e)}{\kappa} \quad (8)$$

where κ is the elastic flexibility.

The model for the filled capsid consisting of an isotropic hyperelastic capsid and a porous elastic core contains four material parameters that need to be estimated. For the porous material assumed for the core of the capsid, the total volume change is the sum of the volume changes of the two constituents, i.e., the solid material and the liquid. This is due to the fact that the fluid is incompressible and the solid part (biological material) is nearly incompressible. Volume change is expressed in terms of the total volumetric strain ε^{vol} of the core being the sum of the volumetric strain of the solid material (viral genome) $\varepsilon_s^{\text{vol}}$ and the volumetric strain of the liquid $\varepsilon_l^{\text{vol}}$, i.e.,

$$d\varepsilon^{\text{vol}} = d\varepsilon_s^{\text{vol}} + d\varepsilon_l^{\text{vol}}. \quad (9)$$

Then the relationship for the bulk modulus becomes

$$\frac{1}{K} = \frac{1}{K_s} + \frac{1}{K_l} \quad (10)$$

where K is the total bulk modulus for the core, K_s is the bulk modulus for the solids and K_l is the bulk modulus of the liquid.

2.3 Liquid flow

The voids inside the viral capsid are assumed to be filled with liquid, which is modeled as a homogenous incompressible fluid. The motion for a fluid flowing through the porous medium is modeled by Darcy's law, which in the three-dimensional case is written as

$$\mathbf{q} = -k \text{grad } \varphi \quad (11)$$

where $\mathbf{q}$ is the specific flux vector with gradient components $(-\partial\varphi/\partial x, -\partial\varphi/\partial y, -\partial\varphi/\partial z)$ in the x , y , z directions, respectively, representing the volume of fluid passing through a unit area per unit time relative to solid. k is the hydraulic conductivity and φ is the hydraulic gradient defined as $\varphi = p/\rho g$, with ρ the density of the fluid, p the pressure and g the acceleration of gravity.

2.4 FE-model

The FE-model used is based on the FE code, Abaqus, and consists of three parts: the base, the indenter, and the filled viral capsid. Since the AFM cantilever is significantly stiffer than the biological material, the indenter can be considered as an analytical rigid part and it is modeled as a rigid semi-sphere with a radius of 20 nm. The indenter is restricted from moving in the horizontal xz -plane and from rotating around the x -, y - and z -axis. The base is modeled as a rigid surface restricted from moving in all translational and rotational directions. Even though the shape of the capsid is icosahedral, the geometry of the capsid is chosen as spherical, which is justified by the finding in our previous work, [11]. The capsid

is not allowed to rotate around the vertical y -axis and the bottom point of the capsid is fixed. The load is applied as a concentrated force in the negative vertical y -direction, see Fig. 3a.

The whole virus is modeled consisting of two parts. The first part is the empty capsid, modeled as a three-dimensional spherical shell with a diameter of 63.2 nm and a thickness of 2.8 nm [28]. The second part, called the core, which corresponds to the viral DNA and the liquid that hydrates it inside the capsid, is modeled as a sphere inside the spherical shell. These two parts have been merged together to form one solid sphere. The modeling consists of three steps, referred to as model 1, model 2, and model 3.

In model 1, both the capsid and the core are assumed to behave like a non-linear isotropic elastic material—a neo-Hookean material. First, the Young's modulus for the capsid E_{cap} is determined by simulating an empty viral capsid and comparing it to the experimental data for empty phage λ capsid. Then the calibrated value for E_{cap} is used in the next simulation where the Young's modulus for the core E_{core} is estimated by a direct comparison with the experimental data for partially filled and fully DNA-filled capsids.

Poisson's ratio ν varies between 0 and 0.5. Materials with a Poisson's ratio close to 0 are easily compressed, while materials with $\nu = 0.5$ are incompressible materials. In [11], simulations of the nanoindentation experiment showed that the mechanical response was not significantly affected by the value of Poisson's ratio ν and since the capsid is slightly compressible a reasonable numbers for the Poisson's ratio would be between 0.3 and 0.4. Therefore, ν is set to 0.4 in this work both for the capsid and for the core.

In the next model, model 2, the nanoindentation response of the capsid core is modeled as a porous elastic material with an incompressible void, where the voids are filled with linear viscous fluid. The solid material of the virus is described by four parameters, two for the capsid and two for the core. The osmotic pressure inside the capsid, caused by the strong bending of the DNA and the repulsive forces between the neighboring negatively charged chains is of order $2.0 \times 10^{-3} - 4.0 \times 10^{-3}$ GPa, see [12]. The osmotic pressure is added in to the model as an extra pressure at each node in the FE-model, both on the capsid and on the core. In the simulations, the osmotic pressure does not change when fluid flow occurs, but the effects are included in [10].

Fig. 3 FE-model, geometry, and mesh

In this model, the previously estimated values for the Young's modulus and Poisson's ratio for the empty viral capsid from model 1, are used. By tracing a cross-section image of a filled bacteriophage λ capsid, obtained with Cryo-EM single particle reconstruction (article in preparation), it was possible to estimate the void ratio e to be 0.96 for the 100%-DNA length filled capsid. In terms of volume percentage, it is 49% void and 51% solid material (that is, DNA). Therefore only two new parameters κ and G for the capsid core need to be estimated.

In the last model, model 3, the fluid is allowed to flow in the porous material of the core and out through the capsid wall. The fluid flow is modeled with Darcy's law, which describes the dynamics for a fluid flowing through a porous medium. The fluid is allowed to move freely in all directions. Initially, the velocity of the fluid is zero and there is no fluid flow in the core or through the boundaries before the indentation, since we assume that water hydrating the tightly packaged DNA is strongly bound to the DNA. Indeed, since the osmotic pressure inside the capsid is high, the DNA hydration force is high, which implies that significant energy is required to move the water from the DNA inside the capsid out to the bulk solution.

The absolute value of the osmotic pressure inside the capsid is significantly higher than it is in the bathing solution. However, there cannot be any outward net flow of water from the capsid prior to AFM indentation. Consider a situation in which DNA is confined at concentration $c(0)_{DNA}$ inside a rigid, immovable, semipermeable membrane (mimicking a phage capsid) permeable to water and salt but not to DNA. The "outside" (bathing) solution is open to the atmosphere and is hence at a hydrostatic pressure of 1 atm. Because of this high concentration of osmolyte (DNA) inside the capsid, water is drawn into the fixed volume and a large osmotic pressure is developed to equalize the chemical potential of the water throughout the system (in the "inside" and "outside" solutions). This osmotic pressure due to the compressed water inside the rigid volume (and withstood by the rigid walls of the container) is often described as a DNA repulsion pressure mediated through the hydration interaction. We assume that the DNA is hexagonally packed. It follows that a fixed concentration of DNA inside the rigid walls $c(0)_{DNA}$ —associated with a fixed amount of DNA and a fixed confining volume—corresponds to a particular fixed value of interaxial DNA-DNA spacing, $d(0)$. At this point (before the capsid is indented by an AFM cantilever), there is no net flow of water between "inside" and "outside" of the capsid. There is pressure build-up inside the capsid from the water that has moved inside in an attempt to dilute the confined DNA. At the same time, rigid and unstretchable capsid walls will exert an equal amount of counterbalancing pressure on the DNA and water until there is no net flow of water into the capsid. However, during the AFM cantilever indentation, water will be "pushed" out from the DNA volume (with the DNA-DNA d -spacing decreasing), reducing the water density and increasing the DNA-DNA repulsion pressure and the osmotic pressure inside. This scenario is implemented in our model. The boundary condition for the fluid in this model is a pressure boundary condition given by the reference pressure. Initially the pressure is set to be the osmotic pressure both on the capsid and on the core, see Fig. 3b. The outer boundary surface of the capsid is exposed to the atmospheric pressure. Since there is no pressure difference between the core and capsid at this point (before the capsid is indented by an AFM cantilever), there is no net flow of water between core and the capsid. However, upon the AFM cantilever indentation, the pressure difference between the core and capsid will occur and water will be pushed out from the DNA volume, reducing the water density (fluid volume fraction). The normal fluid flow through the boundaries is controlled by defining the flow velocity as a function of pore pressure. The fluid flows

proportionally to the difference between the current pore pressure on the surface obtained from the solution and the reference pore pressure value, which is set to the atmospheric pressure value. The proportionality coefficient in model 3 is specified as $1.0 \times 10^{-3} \text{ m}^3/\text{Ns}$, according to [30].

In this model, several new parameters, such as the permeability of the core and the capsid are introduced. These parameters have not yet been calibrated to the experimental data and therefore only qualitative behavior has been studied. By assuming the material behavior of the core described in section and using the estimated value of the elastic flexibility κ from model 2 together with the bulk modulus for water from [10], we can calculate a value for the DNA bulk modulus. The osmotic pressure is added to the FE-model as described in model 2, i.e., as an extra pressure of $2.0 \times 10^{-3} \text{ GPa}$ at each node in the FE-model for the filled (100% wild-type λ -DNA length) and $1.56 \times 10^{-3} \text{ GPa}$ for the partially DNA-filled capsid (78% of wild-type DNA length). It is reasonable to assume that the DNA and the capsid will always be surrounded by liquid and therefore the liquid saturation ratio is set to its maximum value of 1. The remaining parameter is the permeability. Values found in [31] for the permeability parameter vary between $0.2 \times 10^{-6} \text{ m/s}$ and $3.0 \times 10^{-6} \text{ m/s}$. Therefore, we chose the value of $k = 1.0 \times 10^{-6} \text{ m/s}$ for the core and the capsid. The values of hydraulic conductivity could be estimated if there were experimental data for the amount of water that has left the capsid at the end of the nanoindentation experiment, when the maximum indentation depth is reached.

3 Numerical results

In this section, results from the simulations are presented. The nanoindentation response of filled capsid has been modeled in two steps. The Young's modulus for the capsid, which is calibrated in the first step by simulating an empty viral capsid, is used in the next step. The material parameters of the capsid core are evaluated by direct comparison of the simulated data to the experimental data for DNA-filled viral capsids. Models 1 and 2 can be seen as intermediate calibrating steps required for model 3. In each step, some of the calibrated material constants are kept and used for the next model.

The experimental data available for the DNA-filled phage capsid indentation provides only the slope of a straight line, representing the force–displacement curve. A straight line has been fitted to the experimental data of force–distance curves in order to obtain the experimental spring constant [2]. This spring constant value is directly compared to the one obtained in these simulations. The glass curve, which is the deformation of the cantilever only, is also presented with a slope. However, for empty λ capsids, a complete set of experimental data is available, where the deformation of the capsid is subtracted from the overall deformation and individual data points of force–distance measurement are available for comparison with simulations [2].

To determine which simulation data provides the most accurate description of the experimental data, the least-square method with regard to the distance between the simulated curves and a straight line with slope given by the measured capsid spring constant, is used. In some simulations, this resulted in curves partially outside the two deviation lines corresponding to -10% and $+10\%$ deviation from the of the breaking force obtained from the experiment. Therefore, an additional constraint of keeping the curve within these two deviation lines was introduced. More details about the calibration procedure can be found in [32].

Table 1 Experimental data used for Phage λ

Genome length %	Capsid breaking force nN	Std err.	Max indentation before breaking nm	Capsid spring constant N/m
0	0.8	0.01	5.7	0.13
78	0.9	0.02	6.1	0.15
100	1.6	0.01	7.6	0.23

3.1 Results: Model 1

The force and its standard deviation, the indenter displacement, and the slope of the force-displacement curve are presented in Table 1. The experimental data is adopted from [2].

First the mechanical response of the empty capsid is studied. Five different simulations were performed for different values of the Young's modulus. Simulations 2 and 3 are conducted with a 20-node-brick quadratic element mesh with 22,241 nodes forming 7,984 tetrahedron elements, whereas in simulations 1, 4, and 5 a mesh with 11,547 nodes forming 5,096 tetrahedron elements is used. The results are seen in Fig. 4a. The black circles are points from the experimental data set, the solid black line represents the straight line with the slope given in Table 1, and the dotted black line represents the least-squares method

Fig. 4 Calibration of the bacteriophage λ . **a** Capsid stiffness. **b** Filled with 78% DNA, and **c** filled with 100% DNA. The dashed lines represent the 10% imposed deviation

linearization of the experimental data set. The two black dashed lines represent the 10% deviation of the breaking force. When the distances from the simulations to the experimental individual data points are considered, simulation 5 with $E_{\text{cap}} = 1.75$ GPa provides the best fit. Therefore, $E_{\text{cap}} = 1.75$ GPa is used in the following simulations. It was observed that the mesh sensitivity is insignificant, therefore in the following simulations, the coarser mesh is used.

Force–distance curves used to estimate the Young’s modulus for the core material in phage λ packaged with 78% of its wild-type DNA length are shown in Fig. 4b. The simulation with $E_{\text{core78}} = 0.95 \times 10^{-3}$ GPa is the best fit when considering the distance to the given slope. Force–displacement curves for the wild-type phage λ -DNA length (100%) packaged in phage λ are shown in Fig. 4c. The simulation with $E_{\text{core100}} = 8.0 \times 10^{-3}$ GPa provides the best fit, with the least-squares method fitted line being closest to the experimental line. It is also the best fit when both comparison methods are used.

3.2 Results: Model 2

In these simulations, the initial G value was set to a value calculated from the calibrated parameters in model 1 for 78% and 100%-DNA length packaged phage λ and the relationship between E , G , and κ in a homogeneous isotropic material.

$$G = \frac{E}{2(1 + \nu)}. \quad (12)$$

The following values for the shear modulus were obtained, $G_{78} = 3.39 \times 10^{-4}$ GPa and $G_{100} = 3.04 \times 10^{-3}$ GPa. As expected, these values are an overestimation of the shear modulus in a porous elastic material adopted in model 2, but these values provide a starting point in the calibration procedure. Two parameter studies, one for the elastic flexibility κ and one for the shear modulus G , were performed. The results for these studies showed that the force–displacement curve is almost not affected when the elastic flexibility κ is varied, whereas the experimental data is fitted when the shear modulus is within the range of $10^{-6} < G < 10^{-4}$ GPa.

The parametric study for 78%-DNA length packaged in phage λ was performed in [32]. The shear modulus G_{78} was varied between $10^{-5} < G < 10^1$ GPa in steps of 10 GPa. The curve obtained with $G_{78} = 1 \times 10^{-5}$ GPa was the only one that was within the two deviation lines in Fig. 5a corresponding to -10% and $+10\%$ deviation from the experimental data line. Therefore, this value was chosen. The force–displacement curves with this value of G are shown in Fig. 5a, for three different values of the elastic flexibility κ . The best fit when comparing the simulation to the experimental line is obtained with $\kappa = 10$. These values are used in the following simulations for the 100%-DNA length-filled phage λ . Force–displacement curves for different values of the shear modulus G are shown in Fig. 5b. The best fit to the experimental data line is the simulation with $G_{100} = 2.1 \times 10^{-3}$ GPa, which is also the best fit when both methods are considered. The osmotic pressure inside the phage capsid is 20 atmospheres for 100% wild-type DNA-filled phage, see [2], which is equivalent to 2.026×10^{-3} GPa. The osmotic pressure is thus added to the model as an extra pressure of 2.0×10^{-3} GPa at each node in the FE-model for the filled (100% DNA) and 1.56×10^{-3} GPa for the partially filled capsid (with 78% of wild-type DNA length). The value of 1.56×10^{-3} GPa is an estimation of the osmotic pressure when 22% of the DNA length is removed from the capsid. As seen from Fig. 6, the maximum pressure is found just below the indenter and for the case of partially filled capsid (78%-DNA length), the maximum

Fig. 5 Calibration of the bacteriophage λ filled with **a** 78% and **b** 100% DNA

value of 6.0×10^{-3} GPa is obtained, whereas in the case with (100 %)-DNA length-filled capsid, the maximum pressure value is 7.3×10^{-3} GPa. In the rest of the capsid, the average pressure is around 3.25×10^{-3} GPa for partially filled capsid and 4.25×10^{-3} GPa for completely filled capsid at the end of the indentation. This high pressure is caused by the DNA hydrating fluid when the capsid is compressed by the AFM tip, reinforcing the capsid against an external AFM deformation, as was also observed in [2]. However, the hydration fluid provides a significant resistance to the AFM indentation only for the 100% wt λ -DNA length-filled phage capsid.

3.3 Results: Model 3

Using the estimated value of the elastic flexibility κ from model 2 together with the bulk modulus for water $K_f = 2.15$ GPa (10) gives a value for the bulk modulus of the DNA, $K_s = 8.33 \times 10^{-4}$ GPa.

Fig. 6 Pressure distribution (GPa) at the end of the nanoindentation test for **a** 78% and **b** 100% DNA-filled bacteriophage λ (model 2)

The pressure distribution at the end of the nanoindentation test is shown in Fig. 7. As can be observed in the figure, the highest pressure occurs just beneath the indenter and for the case with partially DNA-filled capsid (78% of wild-type DNA length), the maximum value is 5.0×10^{-3} GPa, whereas in the case of filled capsid (100% of wild-type DNA length), the maximum pressure value under the indenter is 6.2×10^{-3} GPa. As expected, both maximum values are somewhat lower than the corresponding maximum values in model 2. This is due to the fluid flow through the pores in the capsid wall, which reduces the pore pressure and the total pressure inside the capsid.

The fluid void ratio distribution for the partially filled capsid (78% DNA) at the end of the nanoindentation experiment is shown in Fig. 8a, whereas the total fluid void ratio distributions for the 100%-DNA-filled capsid at a vertical displacement of 3.5 nm (at the end of the nanoindentation test) are presented in Fig. 8b, c. The void ratio at the beginning of the test was set to 0.96 for the 100% DNA-filled capsid and to 1.2 for the 78% DNA-filled capsid. It decreases as expected during the test, since the fluid is allowed to flow through the capsid wall. For the partially filled capsid, the fluid void ratio value at the end of the test is approximately 0.5 almost everywhere inside the capsid, except for the region beneath the indenter where the maximum value reaches 0.70. In terms of volume percentage, this means that at the end of the test we have approximately 33% fluid and 67% DNA (compared to 60% fluid and 40% DNA in the beginning of the test).

For the 100% DNA-filled capsid, the value at the end of the test is about 0.4 inside the capsid and the maximum value is 0.59. In terms of volume percentage, this means that at the end of the test there is approximately 28% fluid and 72% DNA (compared to 49% fluid and 51% DNA in the beginning of the test). It is interesting to note that the fluid void ratio is largest in the same regions where the largest principal logarithmic strain is maximized, see Fig. 8d. Rupture of the capsid occurs in the region with the maximum principal logarithmic strain. However, when the capsid is filled with DNA, these regions are inside the capsid (see Fig. 8d) and are no longer on the capsid wall. This makes the virus stiffer and higher forces per area are needed in order to rupture a DNA-filled capsid compared to an empty

Fig. 7 Pressure distribution (GPa) at the end of the nanoindentation test for **a** 78% and **b** 100% DNA-filled bacteriophage λ (model 3)

Fig. 8 Total fluid void ratio distribution **a** at the end of the nanoindentation test for 78% DNA-filled, **b** at vertical displacement 3.5 nm for 100% DNA-filled, **c** at the end of the nanoindentation test for 100% DNA-filled, and **d** the distribution of the largest principal logarithmic strain at the end of the nanoindentation test for 100% DNA-filled (model 3, bacteriophage λ)

capsid. This finding of DNA-induced capsid strength against external deformation has been observed in the AFM experiments [2].

4 Discussion

This study is a first attempt to model DNA-filled viral capsids within the framework of finite strain continuum mechanics including water that hydrates encapsidated DNA. This model provides predictions for the pressure distribution inside the capsid during the nanoindentation test mimicking an AFM experiment. In the model, the core of the capsid contains both DNA strands and liquid, which is allowed to flow both inside the capsid and out through the capsid walls during the indentation. The osmotic pressure inside the capsid,

due to the hydration of the DNA strands, is also taken into account in this model. The first two simple models are the intermediate steps toward the final model 3.

Model 1 in this work consists of two parts, modeling an empty and then DNA-filled viral capsid. The empty capsid has been modeled before with two-dimensional models by [4, 14–17] using thin-shell approximation and with three-dimensional continuum models, assuming linear elastic material model i.e., at small strain by [3, 5]. The theory of shells is an engineering approximation, which reduces the original three-dimensional problem to a simpler two-dimensional problem. The theory of shells does not satisfy all the field equations for equilibrium, kinematics, and constitutive relations. The numerical solution such as FE solution of shells involves by itself approximations to an already approximated model, the validity of which is thus limited. For some applications, where the thickness is very small compared to the shell diameter, it could provide a realistic solution. However, in order to obtain reasonable results using thin shell theory, this ratio should be at least a few orders of magnitude. This is not the case for the dimensions of the majority of viral capsids. The thickness of most capsids is in the range of 5–30 times smaller than the radius.

In addition, the deformation that viral capsids undergo during the nanoindentation test is finite (large) compared to the thickness and the radius for most of the AFM-tested viruses listed above. Furthermore, the lateral shear deformation experienced by the capsid is not negligible. Thin-shell theory precludes lateral shear deformation. The deformation that occurs under the tip is the local deformation. This deformation is then spread to the overall structure. Neglecting the shear force results in a less accurate description of the global deformation.

Continuum models provide a useful and reliable representation of the overall deformation scenario that capsids undergo when external forces are applied. Three-dimensional continuum models, assuming linear elastic material model, i.e., within small strain limit, have been used before for simulating nanoindentation tests on spherical capsids [3, 5]. It was shown and emphasized in [25] that the use of linearized small strains is inadequate for such analysis when they conducted three-dimensional finite element simulation on empty capsids. In all of these investigations, the simulations have been performed only on empty viral capsids.

This study models DNA-filled capsids versus empty capsids. Comparing the values for the Young's modulus for partially filled (78% of wild-type DNA length) and filled (100% wild-type DNA length) obtained from model 1, it is noteworthy that the Young's modulus value for the 100% -DNA length-filled phage λ is nearly 10 times larger than this value for the 78% -DNA-filled capsid (measured spring constant values are increased by 1.5–2 times). This indicates that packaging of the last 22% of DNA provides a remarkable increase in the stiffness of the filled viral capsid. This indicates the increase in the pressure inside the capsid and thereby a higher force is required in order to break the capsid. Such capsid strength reinforcement can be related to an evolutionary optimization of the capsid strength versus packaged DNA length. Thus, viral capsids filled with the wild-type DNA length can survive external mechanical stress in nature better than shorter DNA length mutants [2].

Models 2 and 3 are the first finite-strain continuum mechanical models that include the liquid effects and osmotic pressure inside the viral capsid. These models include several material parameters. The experimental data used in model 2 need to be supplemented with an additional filled capsid cross-section image in order to estimate the real void ratio e . Model 3 includes, in addition to model 2, the fluid flow as the capsid is being indented. The values of hydraulic conductivity could be estimated if there were existing experimental data of the amount of water that has left the capsid at the end of the nanoindentation test.

However, the proposed model shows that it is able to quantitatively describe the behavior of the DNA-filled viral capsid during the nanoindentation experiment including the process of fluid displacement through the capsid walls. This reveals interesting data that can be of relevance to survival of viruses between infection events [2, 33, 34].

5 Conclusions

A continuum-based numerical model for simulation of the nanoindentation response of DNA-filled viral capsids is presented in this work. Several important and realistic features of the AFM nanoindentation experiment are incorporated in this model. In the model, the interior of the capsid contains both DNA and liquid. Liquid hydrating the DNA is allowed to flow both inside the capsid and out through the capsid walls during the indentation. The osmotic pressure inside the capsid, due to the strong confinement of the DNA, is accounted for in the model by adding an initial stress at each integration point. The first two simple models are steps toward the complete model 3. Predictions are made for the pressure distribution inside the capsid, the pore pressure, and the fluid/DNA ratio at the end of the indentation. The model has shown good capability to realistically represent the conditions of the AFM nanoindentation experiment and predict the behavior of the DNA-filled capsids. The obtained results provide new insight into the complicated mechanical behavior of the genome-filled viral capsids.

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