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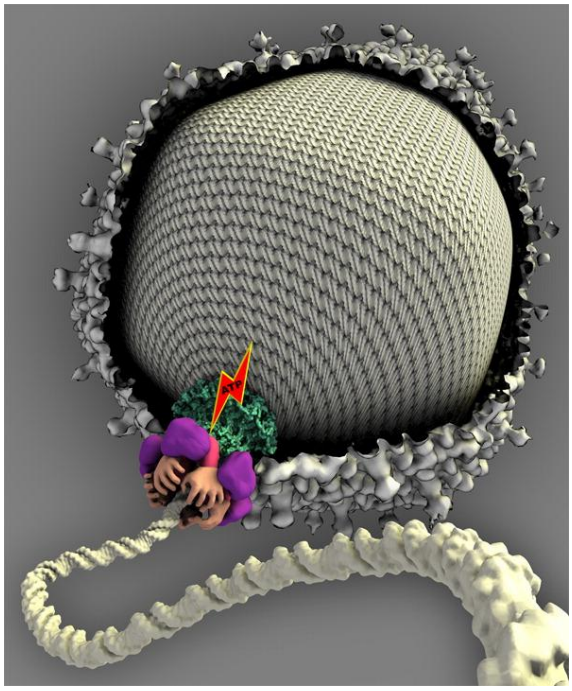
Seminar for the purposes of the subject Molecular Biophysics

DNA PACKING IN BACTERIOPHAGES



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1 Introduction

Viruses are small infectious agents that infect all types of organisms - bacteria, plants and animals alike. They define the boundary between living and non-living organisms, as they are able to grow and self-replicate, but only in the presence of another living organism. Viruses are essentially the simplest form of organisms, consisted only of a genome packed inside a protein container, which in the case of some viruses is surrounded by a layer of lipids. The genome of the virus can be consisted of ssRNA, dsRNA, ssDNA or dsDNA.

The virus attacks or infects a host cell by releasing its genome inside the cell. The machinery of the host cell then processes the genetic information of the virus and is tricked into producing all of the components for the new progeny viruses [1]. Once the protein container or so called *capsid* is assembled, the new genome is packed inside. The structure of the genome and the process of its packing inside the capsid have been a major point of interest for scientists for the past several decades. Once it was discovered that many of the viruses are able to package their genomes very tightly, it has resulted in a number of physical experiments regarding the forces and energies related to this process and many theories and computer simulations have been developed to complement them.

2 Bacteriophages

Bacteriophages (or shortly, phages) are intracellular parasites that infect bacteria. They multiply inside bacteria by making use of some or all of the host biosynthetic machinery. Bacteriophages can vary in size and structure, but they are all composed mainly of a nuceic acid and a protein shell. Depending upon the phage, the nucleic acid can be either DNA or RNA but not both and it can exist in a double stranded or single stranded form. The nucleic acid of the phage is packed tightly into the protein capsid, which is usually spherical and has a diameter with a typical length of tens of nanometers for most of the phages. Biophysicists argued for quite some time on the structure and composition of this capsid, mainly on the question wheter it is composed of only one very massive protein or a large number of molecular subunits bound together.

Francis Crick and James Watson were the first to provide an answer to this question, based solely on physical considerations. Considering a spherical phage with typical radius of 20 nm, it would imply a surface area of ≈ 5000 nm^2. Assuming that a protein with low molecular weight has a typical area of 10 nm^2, they concluded that a single massive protein or about 500 copies of the much small protein is required to make up the capsid. As size of the proteins that make up the capsis is believed to be proportional to the gene size, and we have seen that viruses have relatively short genes, Watson and Crick concluded that the protein capsid must be composed of hundreds of copies of a few protein structures, with a genome consisted of approximately tens of genes.

Watson and Crick were also among the first to predict the icosahedral symmetry of the protein capsid. The did so based on the assumption that the capsid was composed of many copies of a single gene product and considering the relevant symmetry operations that relate each of the protein subunits to the others in the capsid structure. After eliminating translations and mirror planes (due to the fact that proteins are finite and chiral structures), one is left with the cubic point groups, the largest of which is the icosahedral. It also displays the largest volume-to-surface ratio, which accounts for the large amount of nucleic acid able to be packed inside the small capsid. [2]

Bacteriophages come in many different sizes and shapes. The basic structural features of bacteriophages are illustrated in Figure 1, which depicts the phage called T4.

- Size - The typical size of phages ranges from 24-200 nm in length. T4 is among the largest phages - it is approximately 200 nm long and 80-100 nm wide. [3]

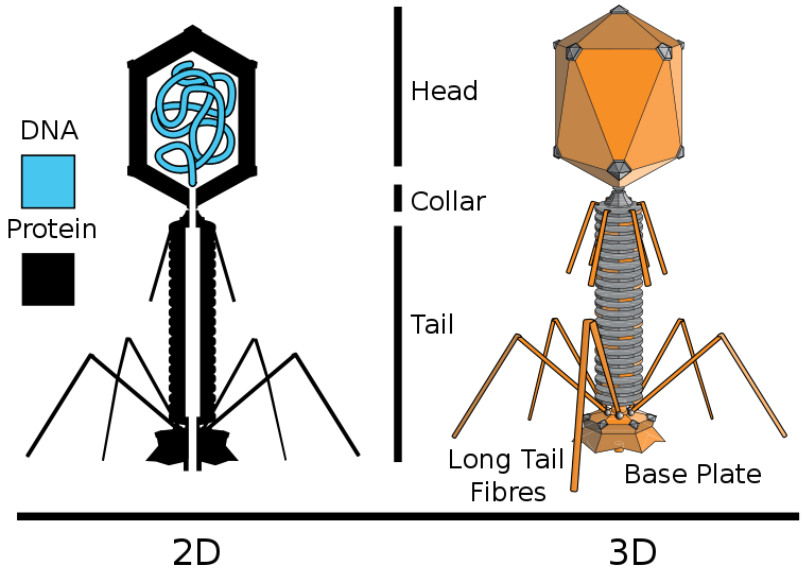

Figure 1: A typical structure of a bacteriophage [4]

- Head or Capsid - All phages contain a head structure which can vary in size and shape. The capsid of most bacteriophage shows pronounced icosahedral symmetry, while the shape goes from ideally spherical to more or less faceted for larger viruses. Inside the head the nucleic acid is packed to high densities. [3] [5] The capsids with icosahedral symmetry are characterized by the triangulation number T, which provides the number of protein unites it is consisted of. Bearing in mind that icosahedral symmetry yields twelve five-fold vertices, the triangulation number is related to number of units between two of the five-fold vertices:

$$T = h^2 + k^2 + hk \tag{1}$$

  where h and k are the number of steps we ought to take in the direction of the two unit vectors of the hexagonal lattice to travel from one to another five-fold vertex. The total number of protein units in the capsid is then $60T$.

- Tail - Many but not all phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. The size of the tail can vary and some phages do not even

have a tail structure. In the more complex phages like T4 the tail is surrounded by a contractile sheath which contracts during infection of the bacterium. At the end of the tail the more complex phages like T4 have a base plate and one or more tail fibers attached to it. The base plate and tail fibers are involved in the binding of the phage to the host cell. [3]

3 DNA packing in bacteriophages

The process of DNA packing in bacteriophages is probably one of their most intriguing properties, mainly because of the fact that they manage to pack it extremely tightly, and the resulting forces have important effects on the phage life cycle. Over the past several decades, a number of experiments have investigated the way DNA in viruses is packaged and ejected. The experiments have helped gain insights regarding the structure of both the portal motor as well as an example of the membrane puncturing device that leads to the delivery of the viral genome. Results of cryo-electron microscopy experiments have revealed the structure of certain viruses at various stages during self-assembly and the ordered arrangements of DNA in concentric circles within viral capsids. The force exerted by the portal motor during the process of viral packing has also been measured through single-molecule experiment.

The problem of DNA packing is intriguing not only on the grounds of sheer geometric crowding, but also because of the recognition that the regions within which DNA is packaged (such as in a viral capsid) have linear dimensions that are comparable to the persistence length of the DNA, resulting in a steep elastic energy cost to be paid to effect such packing. [6]

3.1 Experimental background

- Earnshaw and Harrison (1977) - characterize the tight packaging of DNA in viral capsids by the distance between the strands (2.8 nm in full capsids).

- Feiss et al. (1977) - identify limits on the amount of DNA that can be packaged into the capsid and suggest that adding more DNA than the upper limit makes the capsid unstable.

- Rau et al. (1984) - make measurements on large volumes of nonviral DNA that show that distance between strands values in the range of 2.53.0 nm correspond to a pressure of several tens of atmospheres.

- Shibata et al. (1987) - measure the rate of packaging for phage T3, under various temperatures and chemical conditions. The experiments showed that the packaging process is reversible - one-third of the genome was ejected back into solution upon early interruption of packaging.
- Cerritelli et al. (1997) - verify the tight packaging measured by Earnshaw and Harrison with cryoelectron microscopy and show that the DNA is apparently organized into circular rings within the capsids.
- Simpson et al. (2000) - reveal the structure of the portal motor in phage $\phi 29$.
- Smith et al. (2001) - reinforce the idea that a strong force builds up during packaging with real-time single-phage packaging experiments. They measured the rate of DNA packaging while subjecting the DNA to various resisting forces, quantifying the forces imposed by the packaging motor and the force resisting further packaging as a result of the confined DNA.
- Molineux (2001) - argues that the DNA of phage T7 is assisted into the cell by DNA binding proteins.
- Kanamaru et al. (2002) - reveal the structure of components involved during ejection.
- Evilevitch et al. (2003) - coerce the λ phage into ejecting its genome into a solution containing polyethilene glycol (PEG), that was used to create an external osmotic pressure. They found that various osmotic pressures of several tens of atmospheres could halt the ejection process resulting in fractional genome ejection. The fractional ejection reflects a balance of forces between the inside and outside of the capsid and shows that forces are still present during ejection at the same high levels as were observed during packaging. [7]

3.2 Orders of magnitude in phage physics

An interesting dimensionless quantity for roughly characterizing the packaging efficiency of bacteriophages is the packaging density ρ_{pack}:

$$\rho_{pack} = \frac{\Omega_{genome}}{\Omega_{capsid}} \tag{2}$$

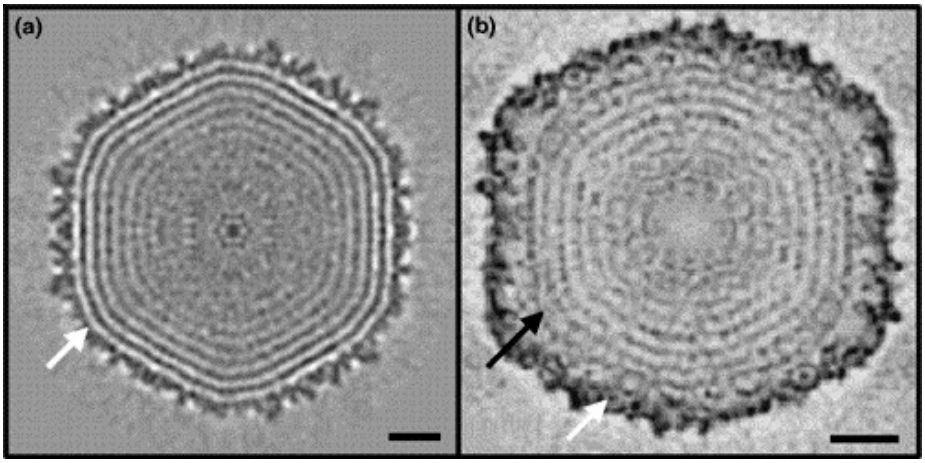

Figure 2: Electron microscopy of a bacteriophage sample shows concentric rings at spacing 2.5 nm.

where Ω_{genome} is the volume of the genetic material and Ω_{capsid} is the volume of the capsid. For double-stranded DNA bacteriophage, this result may be rewritten simply in terms of the number of basepairs in the phage DNA, N_{bp}, using the approximation that DNA is a cylinder of radius 1 nm and length 0.34 nm per basepair:

$$\rho_{pack} = \frac{0.34\pi N_{bp}}{\Omega_{capsid}[\mathsf{nm}^3]} \tag{3}$$

making the calculation of ρ_{pack} straightforward.

Virus type	Host type	Genome length (Kbp)	Diameter (nm)	ρ_{pack}
Bacteriophage T7	Bacteria	40	55	0.490
Bacteriophage ϕ29*	Bacteria	19.4	47	0.459
Bacteriophage T4	Bacteria	169	92	0.443
Bacteriophage $\lambda^{\dagger}$	Bacteria	48.5	63	0.419
Bacteriophage P22	Bacteria	41.7	63	0.319
Herpes Virus HSV1	Human	152	125	0.159
Human Adenovirus C	Human	36	80	0.143
Smallpox Virus 1‡	Human	186	220	0.036
Polyoma Virus SV40	Human	5.3	~50	0.083
Mimivirus§	Amoeba	~800	~400	0.026
Papillomavirus BPV1	Animal	7.9	60	0.070

Figure 3: Packaged volume fractions of some bacteriophage and eukaryotic viruses [7].

Figure 3 shows ρ_{pack} for a number of different viruses and it clearly shows that viruses that infect bacteria are more tightly packed than the viruses that infect eukaryotic cells. A likely reason for this difference in degree of genome compaction is the difference in infection strategies employed by the two types of viruses. Eukaryotic viruses infect a host cell through processes in which both the genetic material and the capsid are taken into the infected cell, while bacteriophage typically attach to the outside of the host and eject their DNA into the cytoplasm through a small channel. To transport their DNA quickly into the host, which itself is pressurized at ∼3 atm, bacteriophage may power the ejection with a large internal pressure.

A typical phage life cycle consists of: adsorption, ejection, genome replication, protein synthesis, self-assembly of capsid proteins, genome packaging inside the capsid and lysis of the bacterial cell (Figure 4).

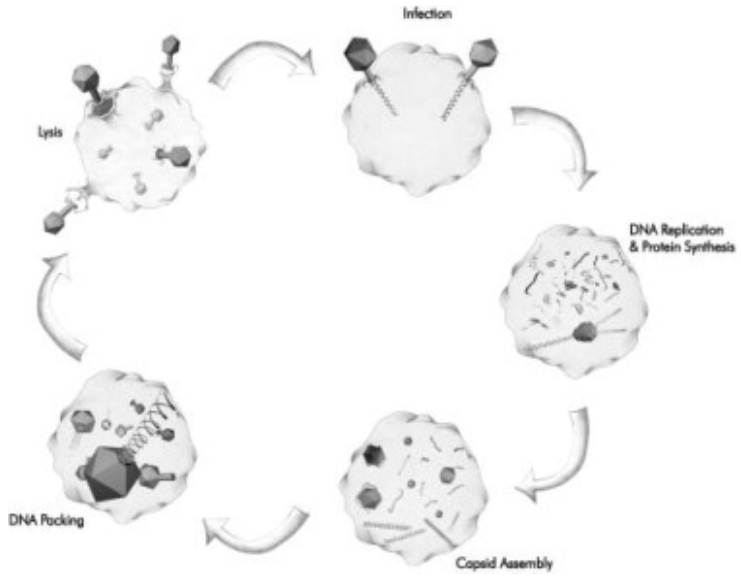

Figure 4: Life cycle of a bacteriophage. [7]

The bacteriophage life cycle is a dynamic process and it is of interest not only to consider its geometric parameters, but also to deal with the temporal scales that are involved as well.

The first step in the cycle is the adsorption of the phage onto the host cell. The frequency of this event depends on the abundance of available phage particles and their hosts. Since ∼50-300 new phage particles are released by a single infection, the destruction of the host cell proceeds exponentially once a cell is infected. After the phage has attached itself to the host its genome is generally released on timescales ranging from seconds to minutes. The time

between the adsorption of the phage and the appearance of the first progeny capsids is usually on the order of minutes. This period is known as the eclipse period and it is the time required to build up the concentration of the phage proteins to a level high enough to initiate self-assembly of the capsid, tail, and motor proteins that constitute a mature phage particle. Self-assembly is a highly concentration-dependent process, but once it starts it proceeds rapidly to completion in a few seconds.

The second step, the packaging process, is completed in$\sim$5-6 min. The packaging rate is on the order of 100 bp/s in the initial stages but it slows down as more of the genome is confined inside the capsid. That is, the rate of packaging depends on the force opposing the motor as it packages the genome. This internal force grows as the amount of genome packaged increases, and the magnitude of the force depends on the solvent conditions. After the progeny phage have been completely assembled, enzymes lyse the host cell and a new generation of phage are released. The process of adsorption to lysis is completed in $\sim$1 h [7].

4 The inverse spool model

As we have seen in the previous sections, the packed DNA is bound to take certain ordered conformations inside the viral capsid, the most probable being the inverse spool conformation. Following the experiments that have given rise to the idea of the inverse spool, a physical model has been developed in order to characterize the inverse spool conformation of the DNA and many computer simulations of viral packaging have supported the inverse spool model. It deals with the problem of DNA packing mainly through the energetics of the process and is based on the idea that the packing energy decomposes into two terms: the curvature energy of the DNA and the interaction among the DNA segments inside the viral capsid. Most of the theories that rely on the inverse spool model tend to evaluate these energy distributions through different sets of assumptions regarding the structure of the viral DNA.

For easier formulation, we may resort to the director field approach (instead of tracking the DNA segment-by-segment down the strand), which is able to give an efficient geometrical representation of complex DNA arrangments without requiring a detailed accounting of every infividual DNA segment. The director field yields the local direction and density of the DNA for every point of the system.

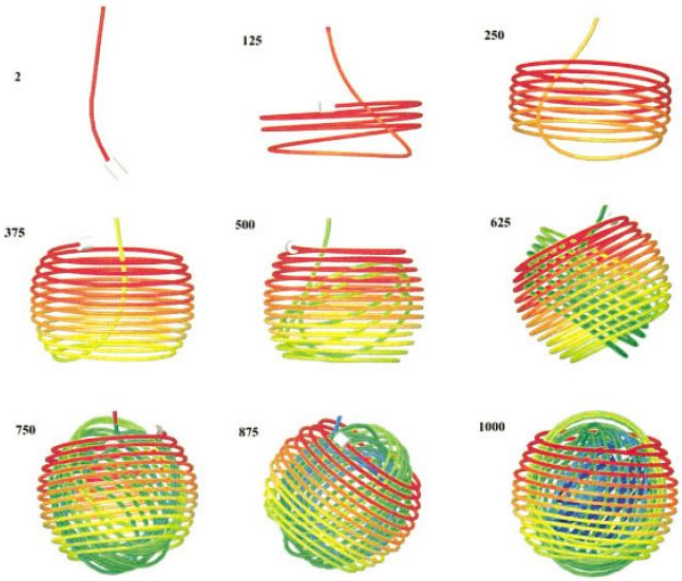

Figure 5: Computer simulations of packaging of viral DNA [8].

4.1 Energy in the inverse spool model

The main two contributions to the energy in the inverse spool model are the elastic curvature energy and the interaction energy. The elastic curvature energy is proportional to the square of the local DNA curvature and follows from the Euler-Kirchoff model of an elastic filament, whose parameters have been well estabilished and determined through series of experiments. The interaction energy displays a longer-ranged electrostatic contribution and a shorter-ranged hydration contribution and can be measured directly in osmotic stress experiments. The problem when trying to compare the theoretical basis and experimental results, is that in theory, the interaction energy is formulated thorugh the chemical potential, while the experimental variable is the osmotic pressure in the DNA arrays. There is, nevertheless a thermodynamical relation between the chemical potenital μ and osmotic pressure p at DNA density ρ:

$$\mu = \int \frac{dp}{\rho(p)} \tag{4}$$

but the relation between the osmotic pressure and the density of the DNA has been studied at only a limited interval of all the densities, which makes things relatively inaccurate when deconvoluting Eq. (4).

There is, however, a model that directly implements the experimental osmotic pressure as a parameter in the energy of the packaged DNA. The polymer nematic droplet model is a variant of the inverse spool model, which treats the DNA subphase within a continuum, mesoscopic framework. [9] The model has resulted from the notion that the packing of the DNA in phages is governed by the tendency of DNA to form local alignments whose orientational order is constrained by the phage capsid. The packed DNA can thus be treated as nematic liquid crystal, characterized by a director and density field.

Under these assumptions, the free energy of the DNA polymer is given by the Frank-Oseen ansatz as [9]:

$$f_D = \frac{1}{2}\left(K_1(\boldsymbol{\nabla}\cdot\mathbf{n})^2 + K_2(\mathbf{n}\cdot(\boldsymbol{\nabla}\times\mathbf{n}))^2 + K_3((\mathbf{n}\times(\boldsymbol{\nabla}\times\mathbf{n})\right)^2 \tag{5}$$

where the three terms correspond to splay, twist and bend, respectively. The splay coefficient K_1 and the twist coefficient K_2 are negligible when considering nematic polymers on the length scale of a viral capsid, so the bend term has the major contribution to the free energy of the system. $\mathbf{n}$ is the unit vector of the director field.

The bending elastic modulus K_3 has the form:

$$K_3 = K_c\rho^{(2)} + \frac{V(D)}{D} = k_BT\mathcal{L}_P\rho^{(2)} + K_0 \tag{6}$$

where K_c is the intrinsic elastic modulus of the polymer and $\mathcal{L}_P$ its persistence length. $\rho^{(2)}$ is the two-dimensional density of the polymers perpendicular to the director, D the average axial separation between the polymers and $V(r)$ the interaction potential. The two dimensional density is related to the three-dimensional density ρ through:

$$\rho^{(2)} = \rho\text{Ł}_{bp}\frac{L}{L_{bp}} \tag{7}$$

where L_{bp} is the phosphate-phosphate separation along the DNA and L is its total length.

The deformation free energy can thus be written as:

$$f_D = \frac{1}{2}k_BT\mathcal{L}_P\frac{\rho L_{bp}^2}{L}(\mathbf{n}\times(\boldsymbol{\nabla}\times\mathbf{n}))^2 \tag{8}$$

Assuming that the encapsidated DNA is in chemical equilibrium with its surroundings, so that it can be partitioned partly inside and partly outside the capsid, the total free energy can be written as:

$$f_{total} = f_0(\rho) + f_D - \rho\mu \tag{9}$$

where f_0 describes bulk DNA at density ρ and μ is the chemical potential, which depends on the osmotic pressure of the external compartment. An expression that contains the osmotic pressure can be derived from Eq. (9) and written as [9]:

$$p_0(\rho) + f_D = p_0(\rho) + \frac{1}{2} k_B T \mathcal{L}_P \frac{\rho L_{bp}^2}{L} (\mathbf{n} \times (\boldsymbol{\nabla} \times \mathbf{n}))^2 = p \tag{10}$$

where p_0 is the osmotic pressure inside the capsid and p is the external osmotic pressure. p_0 is the parameter that is measured through osmotic stress experiments, so Eq.(10) gives the direct relation of the observed parameter with the theoretical model.

We can further assume cylindrical symmetry in order to evaluate the expression $(\mathbf{n} \times (\boldsymbol{\nabla} \times \mathbf{n}))^2$. Thus:

$$\mathbf{n} = \hat{\phi} \tag{11}$$

$$\boldsymbol{\nabla} \times \mathbf{n} = \frac{\cos\theta}{r \sin\theta} \hat{\mathbf{r}} - \frac{1}{r} \hat{\theta} \tag{12}$$

$$\mathbf{n} \times (\boldsymbol{\nabla} \times \mathbf{n}) = \frac{\cos\theta}{r \sin\theta} \hat{\theta} + \frac{1}{r} \hat{\mathbf{r}} \tag{13}$$

and:

$$p_0(\rho) + \frac{1}{2} k_B T \mathcal{L}_P \frac{L_{bp}^2}{L} \frac{\rho}{r^2 \sin^2\theta} = p \tag{14}$$

The constraint on the length of the DNA inside the capsid is defined as: [9]

$$\frac{L(p)}{L_{bp}} = 2\pi \int_0^{\pi} \int_0^{R} \rho(r,\theta) r^2 \sin\theta \, d\theta \, dr \tag{15}$$

where R is the radius of the capsid.

Numerical solutions of Eq.(14) with the constraint Eq.(15) for different salt environments yield plots that are given in Figure 6.

The match of the numerical results curve from Eq.(14) and the curve obtained through evaluation of the length when omitting the bending energy, shows clearly that the bending energy does not contribute significantly to the energetics of the packaging process. The convergence of the curves at high pressures is a consequency of the behaviour of the DNA in the bulk, where the equation of state at high osmotic pressures becomes less dependent on the salt concentration.

The density distribution of the DNA inside the capsid is given in Figure 7.

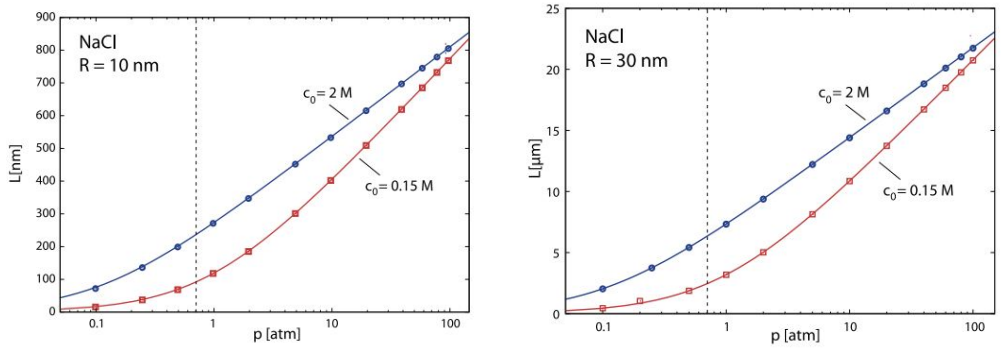

Figure 6: The length of the encapsidated DNA as a function of the osmotic pressure in salt solutions with 0.15 M and 2 M of NaCl, with persistence length set to 50 nm. The full lines are obtained by neglecting completely the DNA bending contribution (setting $\rho(r,\theta) = \rho_0$) and evaluating $L(p)$ as $4/3R^2\pi\rho_0(p)L_{bp}$ [9]

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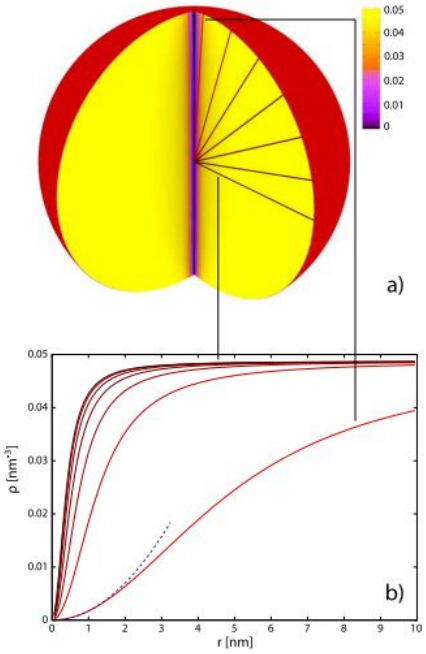

Figure 7: The DNA density distribution in the capsid. $R = 10nm$, $p = 0.5$ atm, $c_0 = 0.15$ M of NaCl, $L(p) = 68.5$ nm. The different curves in b) reperesent the density distribution along the directions indicated in the 3D cut-through view of the density given in a). [9]

5 Other models

Although the inverse spool model has been the most popular amongst the models of viral DNA packaging, other models can also be used to accurately describe the packaging process and the structure of the DNA inside certain phages:

a **Helical packaging** - the DNA in the capsid takes up the conformation of a helix on a sphere. A packaging model proposed in [10] defines the free energy as consited of three contributions: the entropy loss, the bending and twisting energies and the electrostatic energy contribution.

b **Toroidal packaging** - formation of toroids of DNA inside a viral capsid has been reported under certain conditions, such as upon partial ejection of the viral DNA that has previously taken the inverse spool conformation. The energy distribution is basically the same as in the inverse spool model, with the addition of a surface free energy of the toroid, which defines the shape and size of the DNA that has collapsed to form a toroidal globule.

c **Folded toroid** - this conformation is attractive from the standpoint of minimizing the total deformation energy, but it requires the DNA to spontaneously reorganize from a smaller unfolded toroid to the larger folded toroid near the end of the packaging, but it is not clear how easily such reorganization can be achieved within the confines of the capsid.

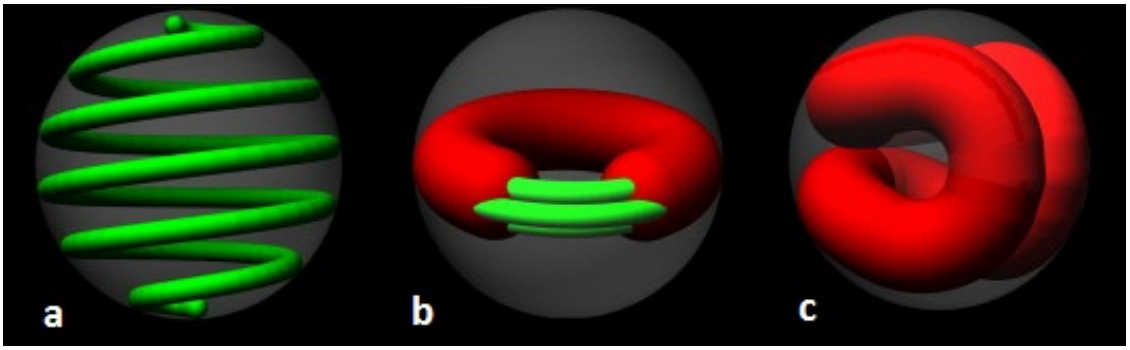

Figure 8: Graphical representation of the other models for the structure of DNA in viral capsids. [11]

6 The portal motor

A major part into the whole process of DNA packaging plays the portal motor, which is the driving force of the DNA into the capsid. One of the capsid's 12 fivefold vertices is a special portal vertex formed by the portal protein. During the translocation of the DNA, a viral enzyme, called terminase, is docked on the portal and the DNA is translocated through the portal channel. Terminase contains ATP[1], which powers the translocation machine [12]. The viral portal motor is one of the strongest mechanisms of nature, and its remarkable physical properties have been studied through a number of experiments.

6.1 Properties of the portal motor

- **Step size** - Step size is defined as a single step taken by the packaging motor, which is equivalent to the number of base pairs of DNA translocated per ATP hydrolyzed. The average step size is about 2 bp/ATP in phages 29 and T3. The prevailing hypothesis is that firing of each ATPase subunit of the packaging motor gives a push to translocate DNA, then a pause would follow during which the DNA is handed over to the next subunit in sequence, and then the firing of the second ATPase. It is, however, possible that ATPase firings may be continuous and pause occurs not between each firing but after all ATPase subunits are fired. Also, contrary to the thinking that step size is a whole and fixed number of base pairs, the motorsubunits may have to adjust to the imprecise DNA symmetry as well as the internal pressure built during translocation. Thus the step size may not be a whole number and may vary from step to step and depend on the extent of head filling [12].

- **Force** - Single-molecule studies have shown that the phage packaging motor generates enormous force in order to package DNA. Forces as high as ∼60 pN have been measured in phages ϕ29,λ, and T4, thus making the packaging motor one of the strongest force generating biological motors reported to date. Such high forces are apparently essential in order to pack the DNA against the enormous electrostatic repulsive forces (and bending and entropic energies) that rise when during the process of confining a highly negatively charged DNA polymer within the limited volume of the capsid [12].

[1]Adenosine-triphosphate (ATP) is a multifunctional nucleoside used in cells as coenzyme. It is often called the "molecular unit of currency" of intracellular energy transfer

- **Velocity** - The phage packaging motors show high rates of packaging. The T4 motor can achieve rates as high as ∼2000 bp/sec, the highest recorded to date. However, the translocation rate decreases as packaging proceeds and internal pressure builds. Measurements show that in phage λ, the motor velocity slows down by about three fold when ∼90% of the genome is packaged. In ϕ29, the packaging rate nearly falls to zero when 100% of the genome is packaged. The rate of translocation is proportional to the genome size of the respective phage. The ϕ29 motor packages its genome, which is 20000 bp long, at an initial rate of 100150 bp/sec, while the phage T4 motor packages a 170000 bp long genome at an average rate of ∼700 bp/sec. The λ motor packages the 48500 bp long chromosome at a rate of ∼600 bp/sec. This points to the conclusion that although the phages package different sized genomes, the motors may have evolved to adjust the rate and complete packaging in 2-5 min [12].

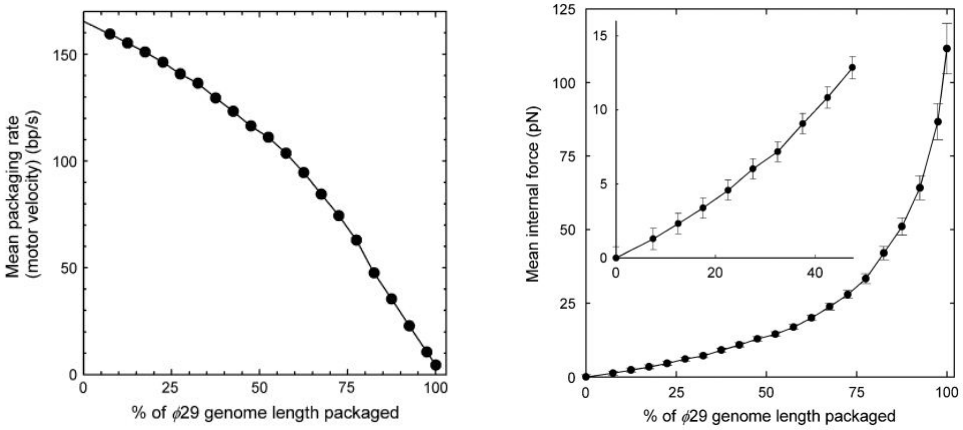

Figure 9: Plots of the mean packaging rate and internal force as function of the fractions of genome packed [13].

- **Power** - Phage packaging motors generate enormous power, with the T4 motor being the fastest and the most powerful. Even with a high external load force of 40 pN, the T4 motor can translocate DNA at a remarkable speed of ∼380 bp/sec. This is equivalent to a power of 15200 pN/bp/s, or 5.2×10^{-18} W. Scaling up the nanoscale T4 packaging motor to a macro-motor, the motor power density would be approximately twice that of a typical automobile engine [12].
- **Efficiency** - It has been determined that the phage packaging motor

converts about 30% of the ATP free energy into translocation of the viral DNA, while of the remaining 70%, a fraction likely supports conformational transitions, whereas the rest is probably released as heat, thus making an enormous entropic contribution to offset the ordered packing of DNA [12].

7 Conclusion

The packaging of DNA in viruses has proven to be one of their most remarkable physical properties. It has been noted that many bacteriophages are able to package their genomes extremely tightly and many experiments have succeeded in determining the physical properties of the packing process as well as the structure of the DNA inside the viral capsid and the structure of the portal motor. The force generated by the packaging motor has been directly measured using optical tweezers, which showed a continuous buildup of the internal pressure as packaging proceeds. Similarly, the pressurization of packaged phages has been confirmed by using osmotic pressure as a resistive force against DNA ejection, causing the ejection to cease and leaving partially filled capsid particles. The internal pressure within these packaged viruses is directly related to the free-energy cost of forcing the DNA into a small cavity and provides the energy for conversion to work in the injection of the DNA into the host cell. Cryo-electron microscopy and image processing have provided observational evidence supporting an axisymmetric packing motif known as the inverse spool conformation and electron microscopy with image reconstruction has show that the DNA inside certain viruses tends to adopt a spool- like conformation with several layers of order. The experimental results have lead to development of new theories, which alongside computer simulations are helping us understand this remarkable process and the consequences it has on the life cycle of the viruses.

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