The Mechanics of Viral DNA Packaging

M. Ortiz and W.S. Klug
California Institute of Technology

Special thanks to: R. Phillips, P. Purohit (Caltech) and W.M. Gelbart (UCLA)

Solid Mechanics Seminar
Caltech, May 5, 2003
Viral DNA encapsidation

- As part of the viral infection cycle, viruses must package their newly replicated genomes within a capsid for delivery to other host cells.

  a) Translocation double-stranded DNA into the capsid (prohead) of the *Bacillus subtilis* ø29 bacteriophage, cover of Nature, **408** (2000)

Viral DNA encapsidation

- Packing viral DNA in capsid is extremely tight: Length of T4 phage genome = 54 μm; capsid diameter = 50 nm (1080-fold linear compression).

$D \approx 50 \text{ nm}$

$L \approx 54 \mu m$

$D \approx 9''$

$L \approx 810'$
Structure of encapsidated viral DNA

(a) Cryo-EM image of DNA-filled bacteriophage T4 capsid.
(b) Structure of encapsidated genome.
Cryo-EM images of encapsidated DNA structure in bacteriophage T7 capsid for viewing directions varying from 0° (axial view) to 90° (side view) angle to the capsid axis. Cerritelli et al., *Cell*, 91, 271-290 (1997).
DNA packaging forces in ø29 phage

(a) Experimental set up:
   a) One polystyrene microsphere captured by optical trap.
   b) Unpackaged end of DNA attached to microsphere.
   c) Second microsphere held by pipette, coated with antibodies against phage.

(b) Measurements:
   a) Force vs packaging rate.
   b) Packaging rate vs percentage of genome packaged.

Viral DNA encapsidation

- **Problem:** Determine the likely conformations of encapsidated viral genome.
- **Selection principle:** *energy minimization.*
- **Program:** i) Formulate energy function accounting for all energetic barriers (entropy, elasticity, electrostatic). ii) Characterize its minimizers.
- **Related problems:** DNA condensation, DNA supercoiling (chromatin); endoplasmic reticulum, Golgi apparatus, mitochondria; crushing of cylindrical shells, paper crumpling...
Encapsidated DNA models

Ball on string
Inverse spool
Folded chain
Spiral fold
Bifolded toroid

Black *et al.*, *PNAS USA*, **82**, 7960-7964 (1985)
Rod/bead-chain DNA models

“The direct elucidation of the statistical mechanics of a semi-flexible, highly charged chain confined to a domain of dimensions comparable to its persistence length and orders of magnitude smaller than its total length constitutes a daunting theoretical challenge” (Kindt et al., PNAS USA, 98, 13671-13674, 2001).

Need a more effective, analytically tractable, accounting device for describing the geometry of tightly packed DNA!
The DNA director field - Definition

- Local tangent direction:
  \[ t(x) = \frac{m(x)}{|m(x)|} \]

- Local DNA density \((L/V)\):
  \[ |m(x)| \sim \frac{2}{\sqrt{3}} \frac{1}{d^2(x)} \]

- DNA length in set \(U\):
  \[ L(U) = \int_U |m| \, dx \]
Director field - Divergence constraint

- Number of signed crossings through $\Sigma$:
  \[ N(\Sigma) = \int_{\Sigma} m \cdot \nu \, dS \]

- For any closed surface:
  \[ \int_{\Sigma} m \cdot \nu \, dS = 0 \]

- At points where $m$ is differentiable:
  \[ \nabla \cdot m = 0 \]

- Across smooth surfaces of discontinuity:
  \[ [m] \cdot \nu = 0 \]
Elastic energy

- Serret-Frenet triad: \( \{ t, n, b \} \).

- Serret-Frenet formulae:
  \[
  t' = \kappa n; \quad n' = \tau b - \kappa t; \quad b' = -\tau n
  \]
  where \( f' = t \cdot \nabla f \equiv \text{arc-length derivative of } f \).

- Curvature: \( \kappa^2 = |t'|^2 \).

- Torsion:
  \[
  \tau^2 = \frac{t''}{|t'|} \cdot \left( I - \frac{t' \otimes t'}{|t'|^2} \right) \cdot \frac{t''}{|t'|} - |t'|^2
  \]

- Strain-energy density:
  \[
  W(m, \nabla m, \nabla \nabla m) = \left\{ \frac{A}{2} \kappa^2 + \frac{B}{2} \tau^2 \right\} |m|
  \]
  where \( A = ak_B T, B = bk_B T \).
Cohesive energy

- Cohesive energy: Net effect of all electrostatic interactions (e.g., negatively charged phosphates, electrolites, hydration) in hexagonally packed DNA condensate.

Liu et al., Surface & Interface Analysis, 32, 15-19 (2001)

Arscott et al., Biopolymers, 30, 619-630 (1990)
Cohesive energy

- Hexagonal packing: \( u = |\mathbf{m}| = (2/\sqrt{3})d^{-2} \)
- DNA normally self-repulsive:

\[
\phi(u) = \mu_\infty (u + \sqrt{u_\infty u})e^{-\sqrt{u_\infty/u}}
\]


- Polyvalent cations \(\rightarrow\) attractive potential:

\[
\phi(u) = \phi_0 - \mu_0 u + (\mu_0 + \mu_\infty)(u + \sqrt{u_\infty u})e^{-\sqrt{u_\infty/u}}
\]

<table>
<thead>
<tr>
<th>( \phi_0 ) (( k_B T/\text{nm}^3 ))</th>
<th>( \mu_0 ) (( k_B T/\text{nm} ))</th>
<th>( \mu_\infty ) (( k_B T/\text{nm} ))</th>
<th>( u_\infty ) (( \text{nm}^{-2} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>1.56</td>
<td>4.98 ( \times 10^5 )</td>
<td>51.3</td>
</tr>
</tbody>
</table>

Kindt *et al.*, *PNAS*, 98 (2001)
Cohesive energy

- Approximate (hard core) model:
  \[
  \phi(u) = \begin{cases} 
  \phi_0 - \mu_0 u, & \text{if } u \leq u_0 \\
  +\infty, & \text{otherwise}
  \end{cases}
  \]
Surface energy

- Surface energy: Corrects for breaking of hexagonal packing regularity, e.g., at condensate boundaries:
  \[ \phi(u) \rightarrow \phi(u) + \frac{|\nabla \phi(u)|}{\sqrt{2u/\sqrt{3}}} \]

  Kindt et al., PNAS, 98 (2001)

- Hard-core model, sharp interface:
  \[ \gamma = \frac{\mu_0 \sqrt{u_0}}{2 \sqrt{2/\sqrt{3}}} = \text{constant} \]
  (energy per unit surface)
Variational problem (I)

- Total energy of encapsidated DNA:
  \[ E(m) = \int_{\Omega} [ W(m, \nabla m, \nabla \nabla m) + \phi(|m|) ] \, dx \]

- **Problem:** \( \inf E(m) \)
  subject to: \( \nabla \cdot m = 0 \) in \( \Omega \)
  \( m \cdot \nu = 0 \) on \( \partial \Omega \)
  \[ \int_{\Omega} |m| \, dx = L \]

- Recall: \( W \) degenerate, \( \phi \) non-convex \( \Rightarrow \) \( E \) not weak lower-semicontinuous \( \Rightarrow \) non-attainment \( \Rightarrow \) minimizing sequences \( \Rightarrow \) bounds, constructions.
Variational problem (II)

- Adopt approximate (hard core) cohesive model.
- Capsid filled with entire genome, \( m = u_0 \ t \).
- Total energy of encapsidated genome:
  \[
  E(t) = \int_{\Omega} W(t, \nabla t, \nabla \nabla t) \, dx
  \]
- Strain-energy density:
  \[
  W(t, \nabla t, \nabla \nabla t) = \left\{ \frac{A}{2} \kappa^2 + \frac{B}{2} \tau^2 \right\} u_0
  \]
- **Problem:**
  \[
  \inf_{t} E(t)
  \]
  subject to:
  \[
  \begin{align*}
  \nabla \cdot t &= 0 \quad \text{in } \Omega \\
  |t| &= 1 \quad \text{in } \Omega \\
  t \cdot \nu &= 0 \quad \text{on } \partial \Omega
  \end{align*}
  \]
Inverse-spool construction
Inverse-spool construction

- Suppose: \( m = u(r, z) e_\theta \)
- Curvature: \( \kappa^2 = r^{-2} \)
- Strain-energy density: \( W(r, u) = \frac{Au}{2r^2} \)
- Total energy (surface energy neglected):
  \[
  E(u) = \int_{a(r)}^{b(r)} \int_0^R \left\{ \frac{Au}{2r^2} + \phi(u) \right\} 2\pi r dr dz
  \]
- Enforce length constraint through multiplier \( F \):
  \[
  I(u, F) = \int_{a(r)}^{b(r)} \int_0^R \left\{ \frac{Au}{2r^2} + \phi(u) - Fu \right\} 2\pi r dr dz
  \]
Inverse-spool construction

- Minimize with respect to $u$:

$$E^*(F) = - \inf_u I(u, F)$$

$$= \int_0^R \phi^* \left( F - \frac{A}{2r^2} \right) 2\pi h(r) r dr$$

where: $u(r, z) = \partial \phi^* \left( F - \frac{A}{2r^2} \right)$

- Energy: $E(L) = \sup_F \{FL - E^*(F)\}$

- Length: $L(F) = \int_0^R \partial \phi^* \left( F - \frac{A}{2r^2} \right) 2\pi h(r) r dr$
Inverse-spool construction

- Repulsive interaction, $\phi_{29}$:

Capped Cylinder Model

Tao et al., *Cell*, 95 (1998)
Inverse-spool construction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R$ (nm)</td>
<td>25.00</td>
</tr>
<tr>
<td>$h_0$ (nm)</td>
<td>37.50</td>
</tr>
<tr>
<td>$h_R$ (nm)</td>
<td>20.83</td>
</tr>
<tr>
<td>$A$ (pN×nm²)</td>
<td>202.31</td>
</tr>
<tr>
<td>$\mu_0$ (pN)</td>
<td>6.31</td>
</tr>
<tr>
<td>$u_0$ nm⁻²</td>
<td>0.147</td>
</tr>
</tbody>
</table>

Parameters for φ29 phage

Smith et al., *Nature*, **413** (6857) 748-752 (2001)
Spool construction – Surface energy

- Hard-core interaction, surface energy, $\phi_{29}$:

$$ I(\omega, F) = \int_{\omega} \left( \frac{Au_0}{2r^2} + \phi_0 - Fu_0 \right) 2\pi r \, dr \, dz + \int_{\partial \omega} \gamma 2\pi r \, ds $$

- First integral:

$$ Au_0 \log r + (\phi_0 - Fu_0) r^2 + 2\gamma r \cos \alpha = C \Rightarrow \alpha(r) $$

where $z'(r) = -\cot \alpha(r)$

- Solution:

$$ z(r) = z(R_{out}) - \int_{R_{out}}^{r} \cot \alpha(r) \, dr $$

where $\alpha(R_{out}) = 0$
Spool construction – Surface energy

Capped cylinder model of $\phi 29$: Boundary contours of DNA condensate at various packing stages.

Tao et al., Cell, 95 (1998)
Can the inverse spool be beaten?

High curvature core

Inverse spool

Closed loops

Low curvature

Competing motif
Torsionless toroidal solenoid construction

Toroid

Solenoid

Spool core
Torsionless toroidal solenoid construction

- Toroid $\omega \times [0, 2\pi]$.

- Unit director field:
  
  \[ t_r = t_r(r, z), \quad t_\theta = 0, \quad t_z = t_z(r, z) \]

- Divergence constraint: Potential $v_\theta(r, z)$ s. t.
  
  \[ t_r = -\frac{\partial v_\theta}{\partial z}, \quad t_z = \frac{1}{r} \frac{\partial}{\partial r} (rv_\theta) \]

- Tangency BC: $v_\theta = 0$, on $\partial \omega$

- Modulus constraint: Let $\eta = rv_\theta$. Then,
  
  \[ \sqrt{\left(\frac{\partial \eta}{\partial r}\right)^2 + \left(\frac{\partial \eta}{\partial z}\right)^2} = r \]

Inhomogeneous eikonal equation.
Torsionless toroidal solenoid construction

DNA layers

characteristics (rays)
geodesics of: \( r^2(dr^2 + dz^2) \)

(wave) front

Characteristic construction

- Construction terminates when:

\[
\int_{\eta=N} A \frac{1}{2r^2} h_\xi h_\eta d\xi = \int_{\eta=N} \frac{A}{2} \kappa^2 h_\xi h_\eta d\xi
\]
Torsionless toroidal solenoid construction

- \( \Delta E = -1.45 \times 10^3 \text{ pN} \times \text{nm} \), \textbf{solenoid wins!}  
- Work to pack \( \phi 29 \) genome \( \sim 7.5 \times 10^4 \text{ pN} \times \text{nm} \) (Smith \textit{et al.}, 2001).
Can solenoids be beaten?

- Solenoid construction shows that energy can be reduced by replacing certain spool regions by solenoids.

- **Question**: What is the optimal arrangement of solenoid and spool regions?

- 'Two-well' strain-energy density:
  \[
  W(r, t) = \frac{Au_0}{2} \left\{ \kappa^2 \wedge \frac{1}{r^2} \right\}
  \]

  - Spool phase
  - Solenoid phase

- New construction: Allow for **fine solenoid/spool mixtures**.
Solenoid/spool mixtures - Interfaces

- Interface driving force:
  \[ f = \frac{A u_0}{2} \left( \frac{1}{r^2} - \kappa^2 \right) \]

- Equilibrium interfaces:
  \[ \kappa^2 = \frac{1}{r^2} \]

- Parametric equations:
  \[ r(\varphi) = r_0 \exp(\sin \varphi - \sin \varphi_0) \]
  \[ z(\varphi) = z_0 + \int_{\varphi_0}^{\varphi} r(\psi) \sin \psi \, d\psi \]
Solenoid/spool mixtures – Front relaxation

- Energy of mixture: \[ E(t, \chi) = \int_\omega \frac{A u_0}{2} \left\{ \chi \kappa^2 + (1 - \chi) \frac{1}{r^2} \right\} 2\pi r dr dz \]
- Divergence constraint: \[ \nabla \cdot (\chi t) = 0 \]
- Normal front advance: \[ \Delta n = \frac{\Delta \eta}{\chi r} \]
- Incremental energy: \[ \Delta E = \int_\gamma \frac{A u_0}{2} \left\{ \chi \kappa^2 + (1 - \chi) \frac{1}{r^2} \right\} 2\pi r \Delta n ds \]
- Optimal mixture:
  \[ \chi = \begin{cases} 
  1, & \text{if } \kappa < r^{-1} \\
  0, & \text{if } \kappa > r^{-1} 
\end{cases} \]
Solenoid/spool mixtures – Ø29 phage

- ΔE = −8.82 × 10³ pN×nm

mixture wins!
Solenoid/spool mixtures – ø29 phage

Close-up view of top apex region
Solenoid/spool mixtures – ø29 phage

Close-up view of mixture region
Concluding remarks

- Clean variational characterization of viral DNA encapsulated conformations

- Analysis:
  - What is the optimal DNA arrangement?
  - How does the energy scale with $L$, $A$, size of $\Omega$?

- Computation: Discretize $\Omega$, $m \Rightarrow$ lattice model. Relax energy by Monte Carlo, simulated annealing, genetic algorithms. . . .

- Apply director-field approach to related problems. . .
Concluding remarks

Levels of chromatic packing. Orders of chromatin packing thought to give rise to the highly condensed mitotic chromosome. The folding of naked DNA into nucleosomes is the best understood level of packing. The structures corresponding to the additional layers of chromosome packing are more speculative.

(Alberts et al., Essential Cell Biology, 1998)
Concluding remarks

Golgi apparatus, secretory animal cell
(G. Palade)
Concluding remarks

Endoplasmic reticulum, canine pancreas cell
(L. Orci)
Concluding remarks

Cross section of mitochondrion
(D.S. Friend)