

The Mechanics of Viral DNA Packaging

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

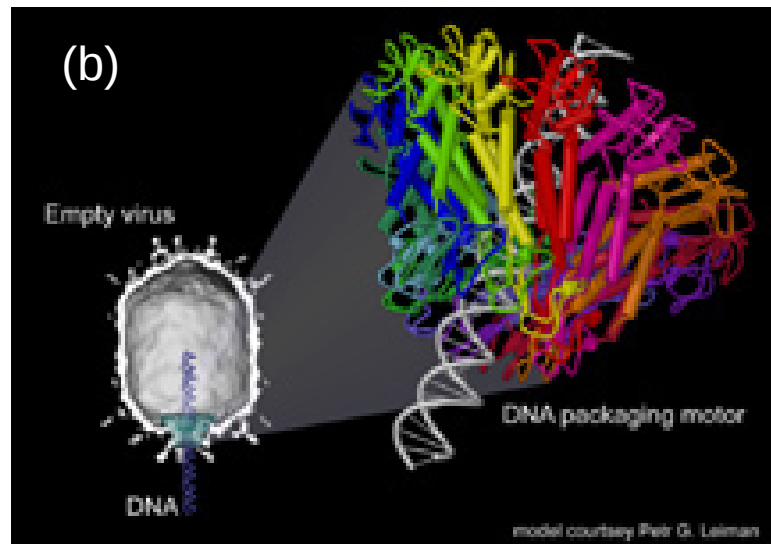
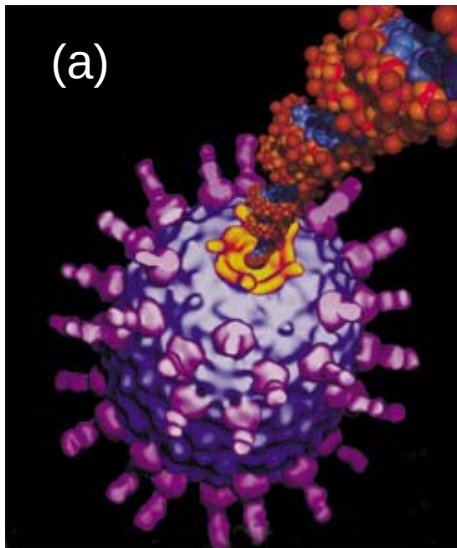


PHYSICAL CHEMISTRY SEMINAR
UCLA, May 19, 2003

Michael Ortiz
UCLA, 05/03

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.

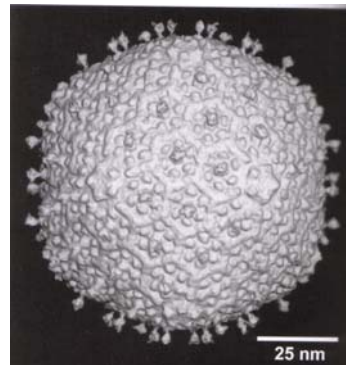


- a) Translocation double-stranded DNA into the capsid (prohead) of the *Bacillus subtilis* ø29 bacteriophage, cover of Nature, **408** (2000)
- b) Structure of the ATP-hydrolysis driven portal motor as determined by X-ray crystallography (3.2 Å resolution) Simpson *et al.*, *Nature*, **408**, 745-750 (2000)

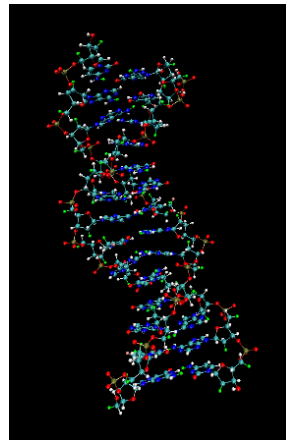


Viral DNA encapsidation

- Packing viral DNA in capsid is extremely tight: Length of T4 phage genome = $54\text{ }\mu\text{m}$; capsid diameter = 50 nm (1080-fold linear compression).



$D \approx 50\text{ nm}$



$L \approx 54\text{ }\mu\text{m}$

$\sim$



$D \approx 9''$

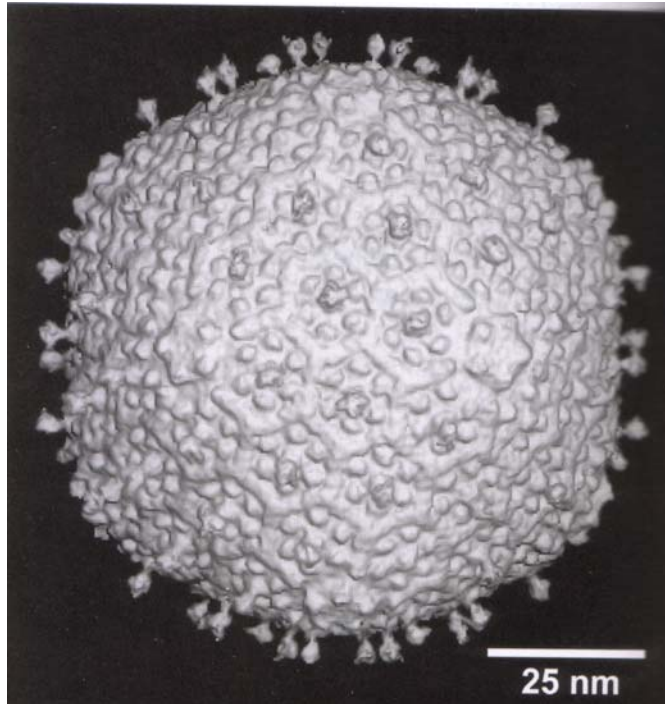


$L \approx 810'$

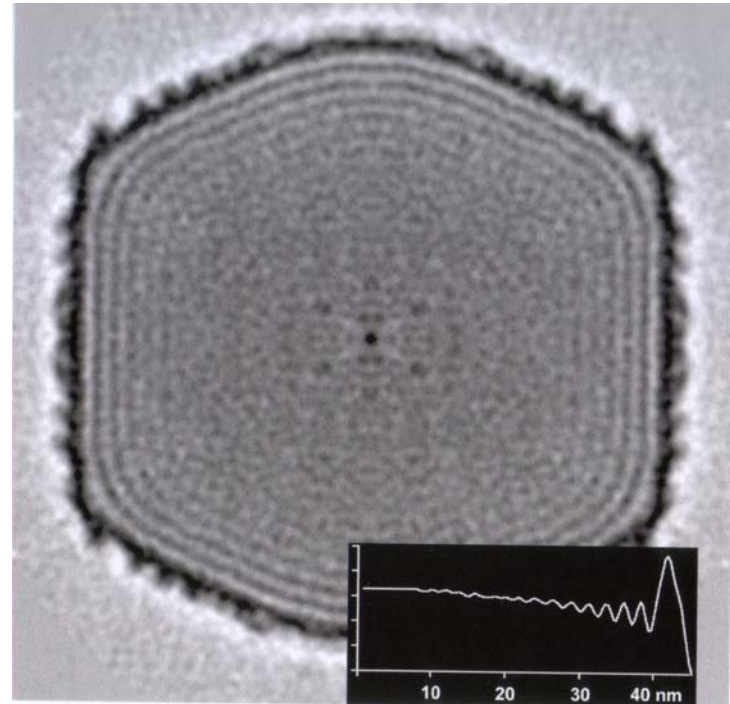
- **Related problems:** DNA condensation, DNA supercoiling (chromatin); crushing of cylindrical shells, paper crumpling. . .



Structure of encapsidated viral DNA



(a)



(b)

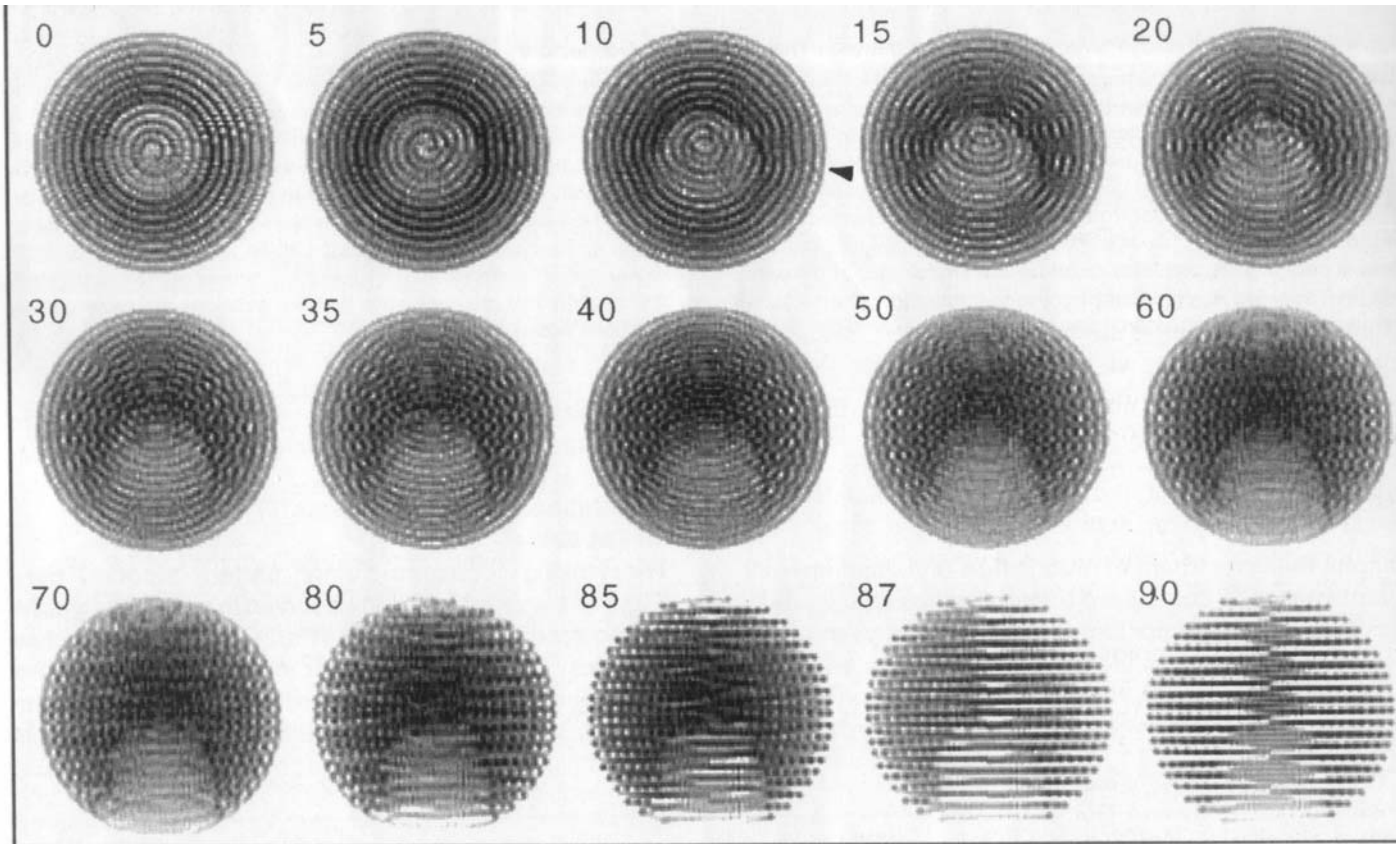
a) Cryo-EM image of DNA-filled bacteriophage T4 capsid.

b) Structure of encapsidated genome.

Olson et al., *Virology*, **279**, 385-391 (2001).



Structure of encapsidated viral DNA



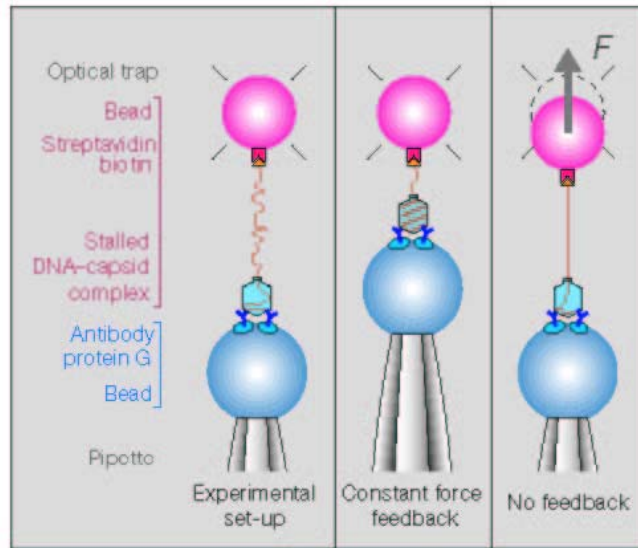
Cryo-EM images of encapsidated DNA structure in bacteriophage T7 capsid for viewing directions varying from 0° (axial view) to 90° (side view) angle to the capsid axis.

Cerritelli *et al.*, *Cell*, **91**, 271-290 (1997).

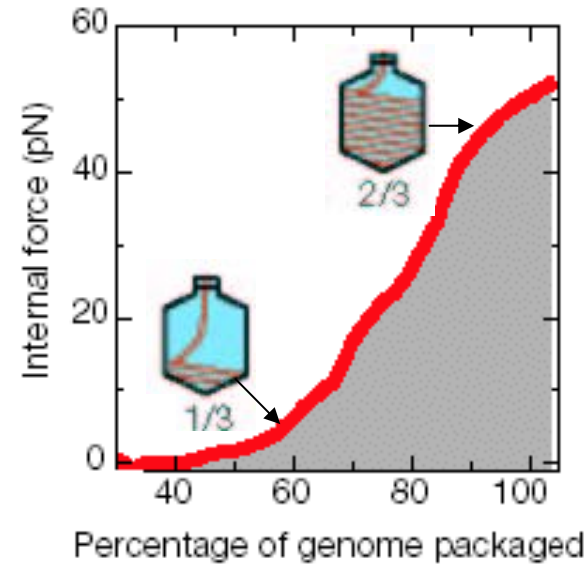
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DNA packaging forces in $\phi 29$ phage



(a)



(b)

- 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.

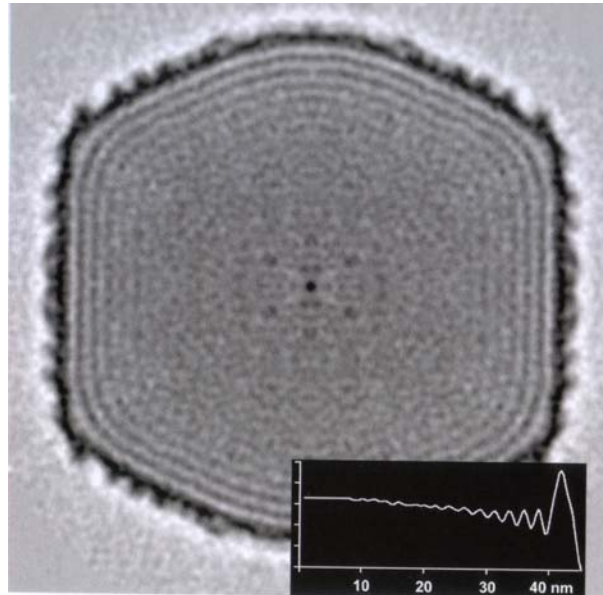
Smith *et al.*, *Nature*, **413** (6857) 748-752 (2001).

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Viral DNA encapsidation

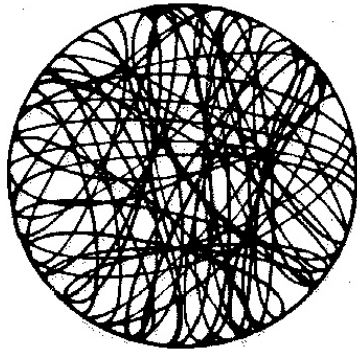
- **Problem:** Determine the likely conformations of encapsidated viral genome and attendant macroscopic properties (e. g., forces, pressure)



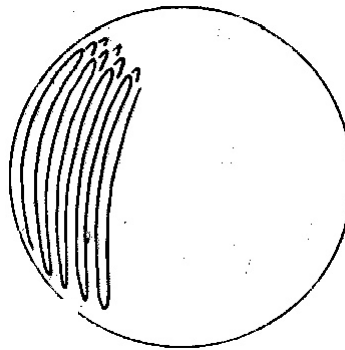
Structure of T4 encapsidated genome
Olson et al., *Virology*, **279**,
385-391 (2001).



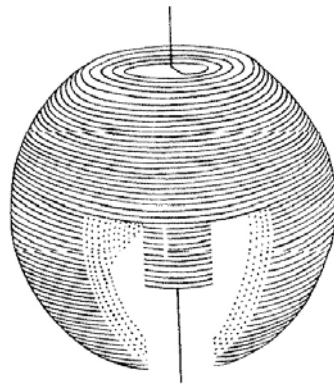
Viral DNA encapsidation



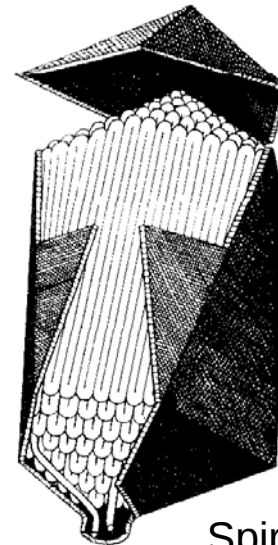
Ball on string



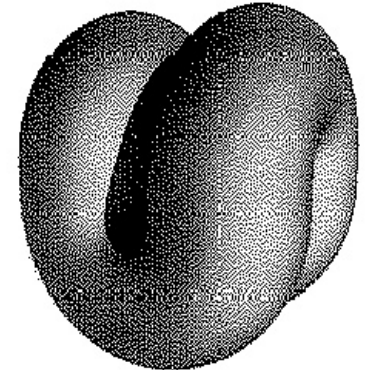
Folded chain



Inverse spool



Spiral fold



Bifolded toroid

Klimenko *et al.*, *J. Mol. Bio.*, **23**, 523-533 (1967)

Earnshaw and Harrison, *Nature*, **268**, 598-602 (1977)

Richards *et al.*, *J. Mol. Bio.*, **78**, 255-259 (1973)

Black *et al.*, *PNAS USA*, **82**, 7960-7964 (1985)

Hud, *Biophys. J.*, **69**, 1355-1362 (1995)



Viral DNA encapsidation

“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).

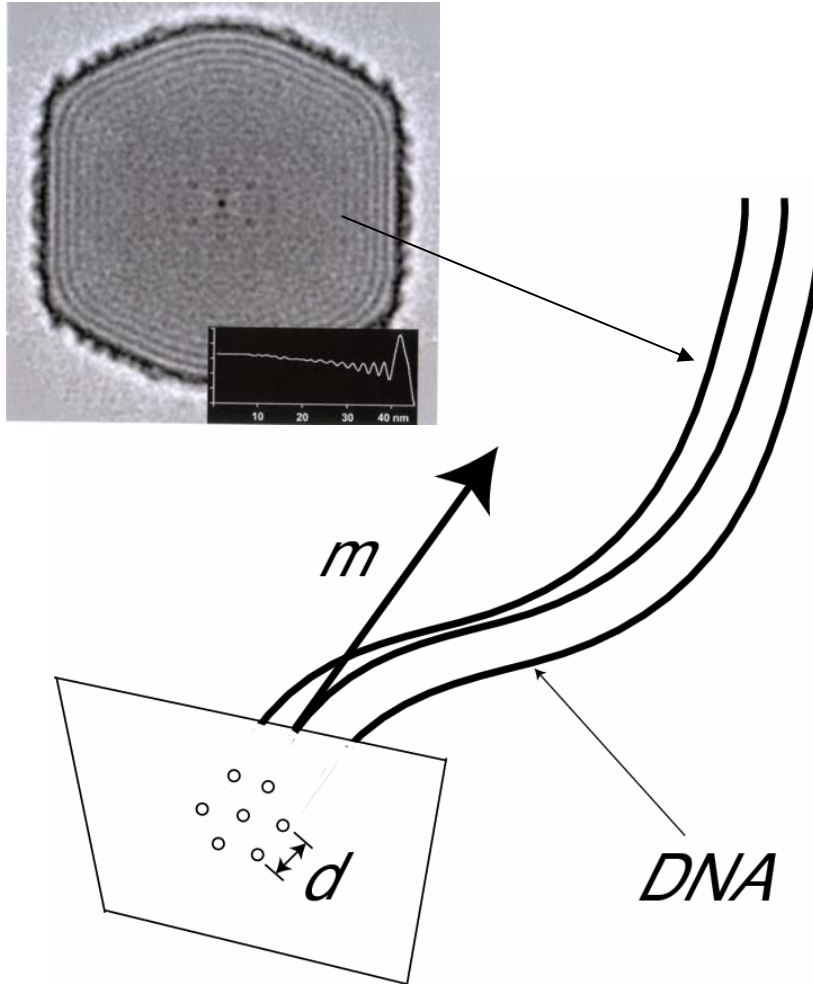


Viral DNA encapsidation

- **Approach:**
 - i) Continuum **field theory** of DNA packaging.
 - ii) **Energy minimization.**
 - iii) Boundary-value problem.
 - iv) Equivalent variational (**nonconvex**) problem.
 - v) Nonlinear analysis: Relaxation,
 Γ -convergence, optimal scaling.
 - vi) Numerical analysis: Lattice-field models.
- **Related theories:** Statistical field theories, lattice-field theories, micromagnetics, liquid crystals.



The DNA director field - Definition



- Local tangent direction:

$$t(x) = \frac{m(x)}{|m(x)|}$$

- Local DNA density (L/V):

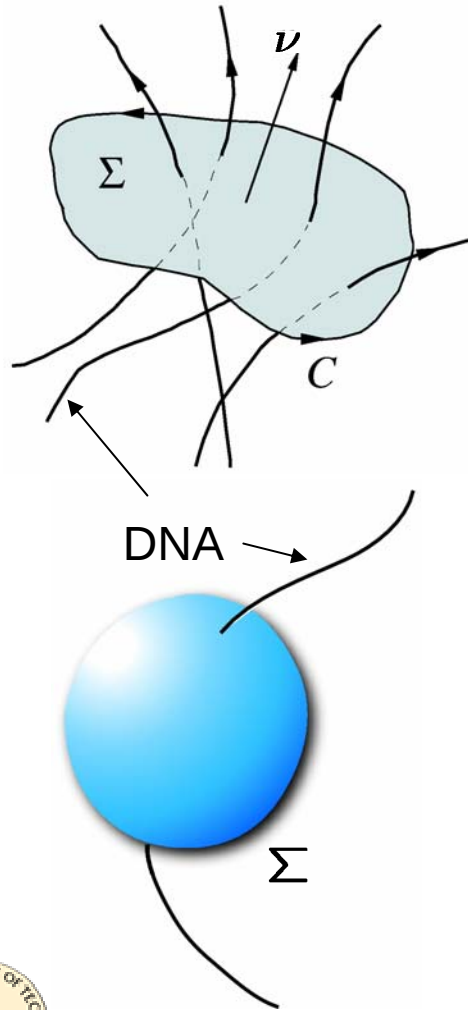
$$|m(x)| \sim \frac{2}{\sqrt{3}} \frac{1}{d^2(x)}$$

- DNA length in set U :

$$L(U) = \int_U |m| dx$$



Director field - Divergence constraint



- Number of signed crossings through Σ :

$$N(\Sigma) = \int_{\Sigma} \mathbf{m} \cdot \boldsymbol{\nu} dS$$

- For any closed surface:

$$\int_{\Sigma} \mathbf{m} \cdot \boldsymbol{\nu} dS = 0$$

- At points where $\mathbf{m}$ is differentiable:

$$\nabla \cdot \mathbf{m} = 0$$

- Across smooth surfaces of discontinuity:

$$[[\mathbf{m}]] \cdot \boldsymbol{\nu} = 0$$



Elastic energy

- Serret-Frenet triad: $\{\mathbf{t}, \mathbf{n}, \mathbf{b}\}$.

- Serret-Frenet formulae:

$$\mathbf{t}' = \kappa \mathbf{n}; \quad \mathbf{n}' = \tau \mathbf{b} - \kappa \mathbf{t}; \quad \mathbf{b}' = -\tau \mathbf{n}$$

where $f' = \mathbf{t} \cdot \nabla f \equiv$ arc-length derivative of f .

- Curvature: $\kappa^2 = |\mathbf{t}'|^2$.

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

- Strain-energy density:

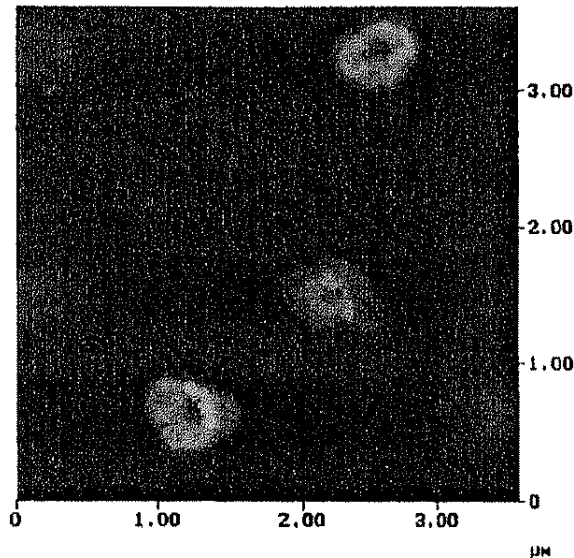
$$W(\mathbf{m}, \nabla \mathbf{m}, \nabla \nabla \mathbf{m}) = \left\{ \frac{A}{2} \kappa^2 + \frac{B}{2} \tau^2 \right\} |\mathbf{m}|$$

where $A = ak_B T$, $B = bk_B T$.

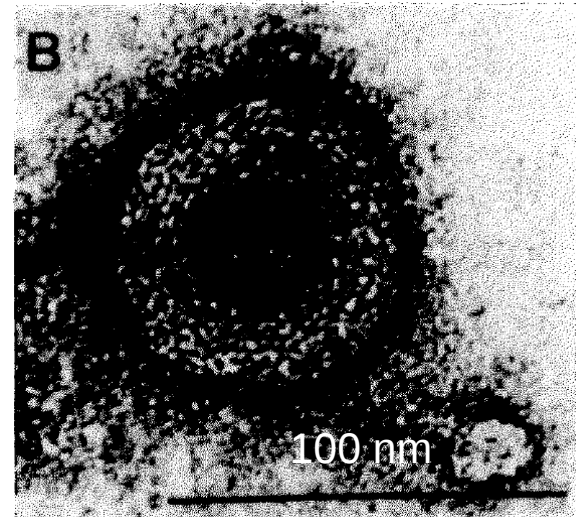


Cohesive energy

- Cohesive energy: Net effect of all electrostatic interactions (e. g., negatively charged phosphates, electrolytes, 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:

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

Rau, Lee, and Parsegian, *PNAS*, **81** (1984)

- Polyvalent cations $\rightarrow$ attractive potential:

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

ϕ_0 ($k_B T/\text{nm}^3$)	μ_0 ($k_B T/\text{nm}$)	μ_{∞} ($k_B T/\text{nm}$)	u_{∞} (nm^{-2})
0.10	1.56	4.98×10^5	51.3

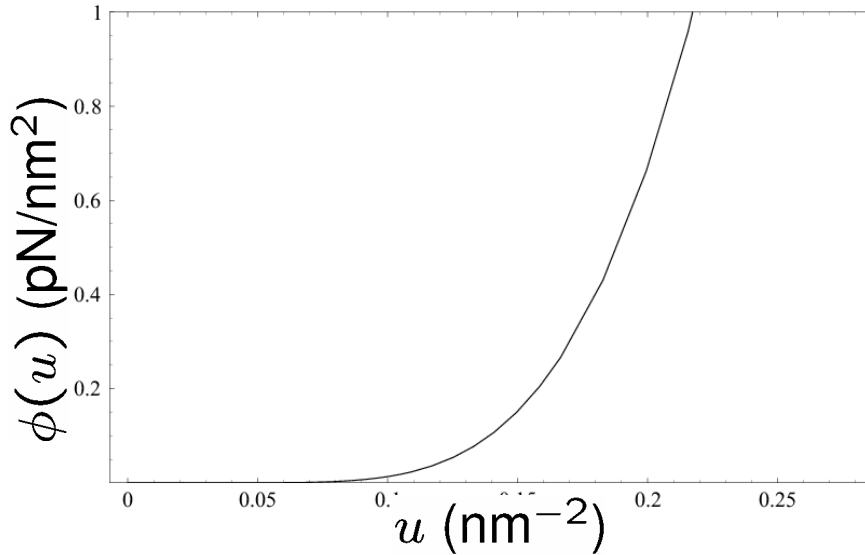
Kindt et al., *PNAS*, **98** (2001)

Rau and Parsegian, *Biophys. J.*, **61** (1992)

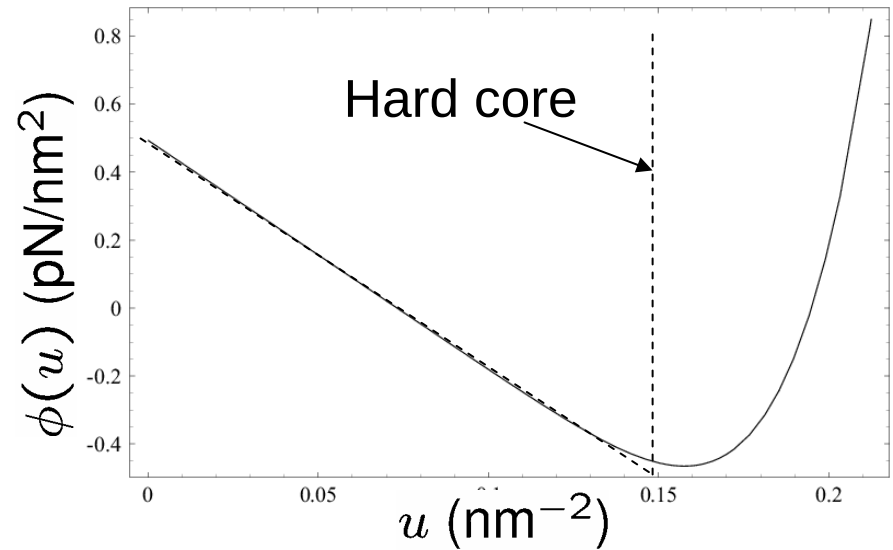
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Cohesive energy



Repulsive energy



Attractive 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:

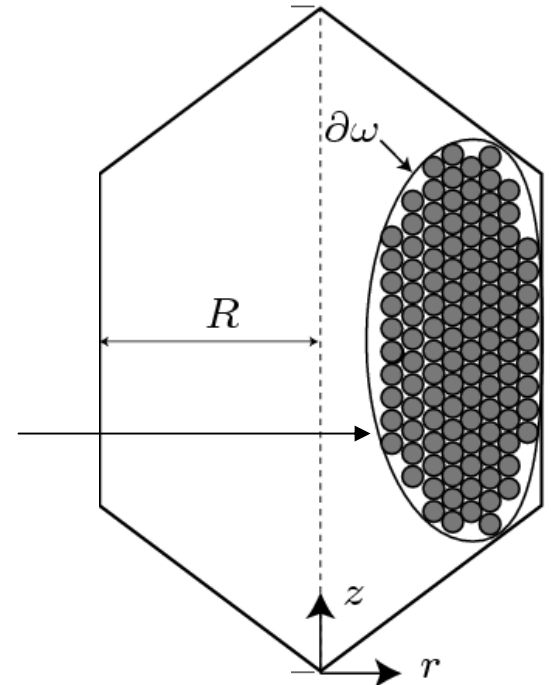
$$\phi(u) \rightarrow \phi(u) + \frac{|\nabla \phi(u)|}{\sqrt{2u/\sqrt{3}}}$$

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:

$$E(\mathbf{m}) = \int_{\Omega} [W(\mathbf{m}, \nabla \mathbf{m}, \nabla \nabla \mathbf{m}) + \phi(|\mathbf{m}|)] dx$$

- **Problem:** $\inf E(\mathbf{m})$

subject to: $\nabla \cdot \mathbf{m} = 0$ in Ω

$$\mathbf{m} \cdot \boldsymbol{\nu} = 0 \quad \text{on } \partial\Omega$$

$$\int_{\Omega} |\mathbf{m}| dx = L$$

- Recall: W degenerate, ϕ 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:** $\inf E(t)$

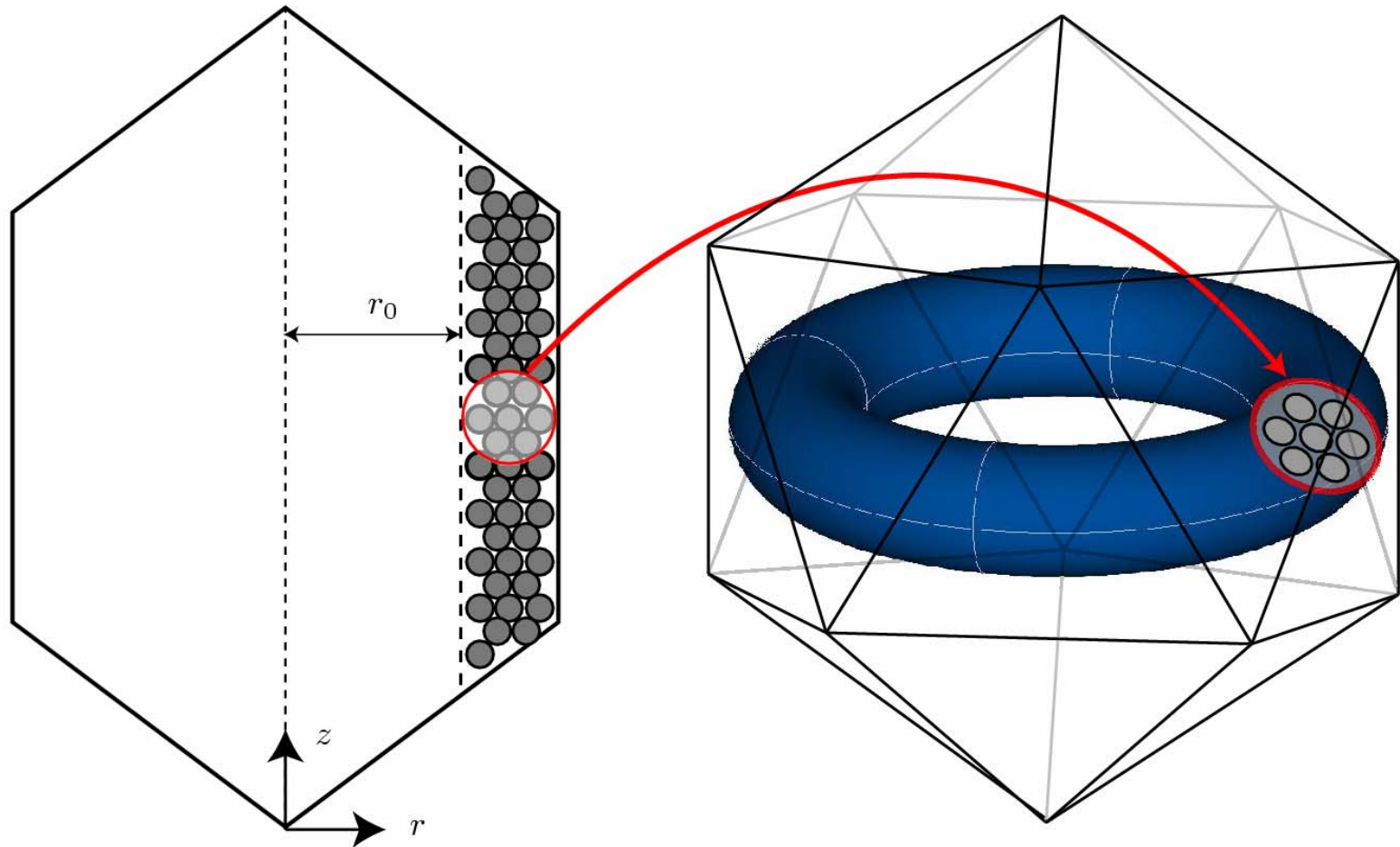
subject to: $\nabla \cdot t = 0 \quad \text{in } \Omega$

$$|t| = 1 \quad \text{in } \Omega$$

$$t \cdot \nu = 0 \quad \text{on } \partial\Omega$$



Inverse-spool construction



Inverse-spool construction

- Suppose: $\mathbf{m} = u(r, z)\mathbf{e}_\theta$

- Curvature: $\kappa^2 = r^{-2}$

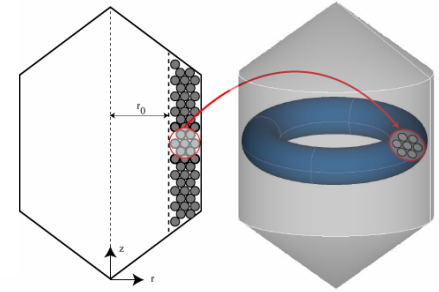
- Strain-energy density: $W(r, u) = \frac{Au}{2r^2}$

- 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 :

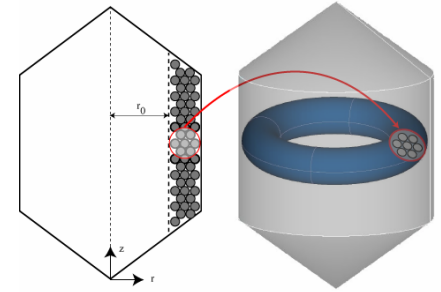
$$E^*(F) = - \inf_u I(u, F)$$

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

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

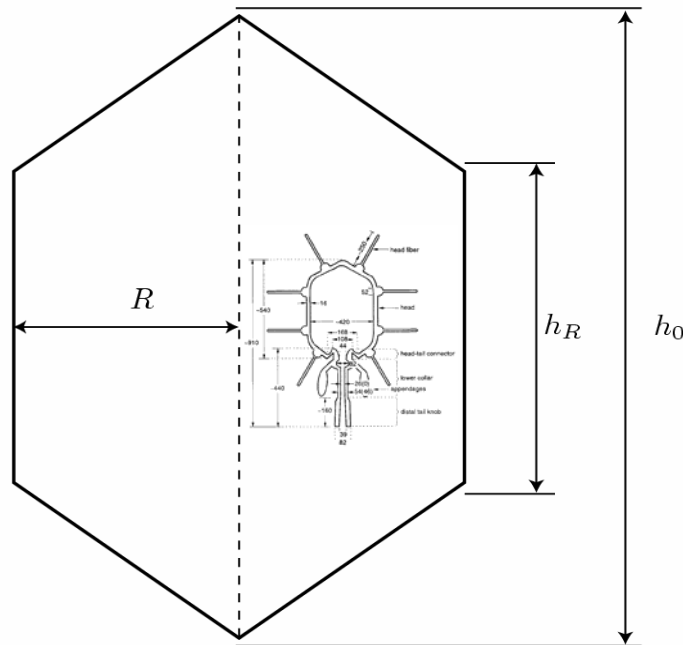
- Energy: $E(L) = \sup_F \{ FL - E^*(F) \}$

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

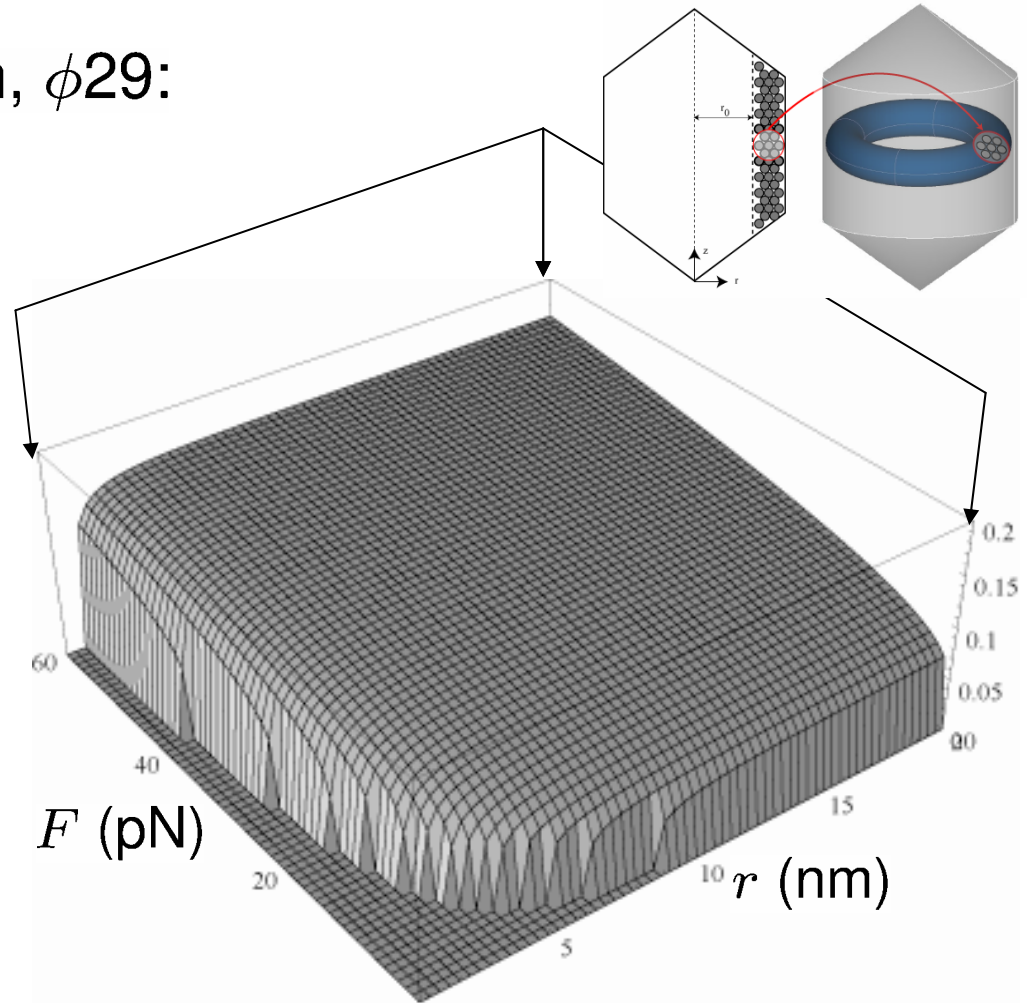


Inverse-spool construction

- Repulsive interaction, ϕ_{29} :



Capped Cylinder Model



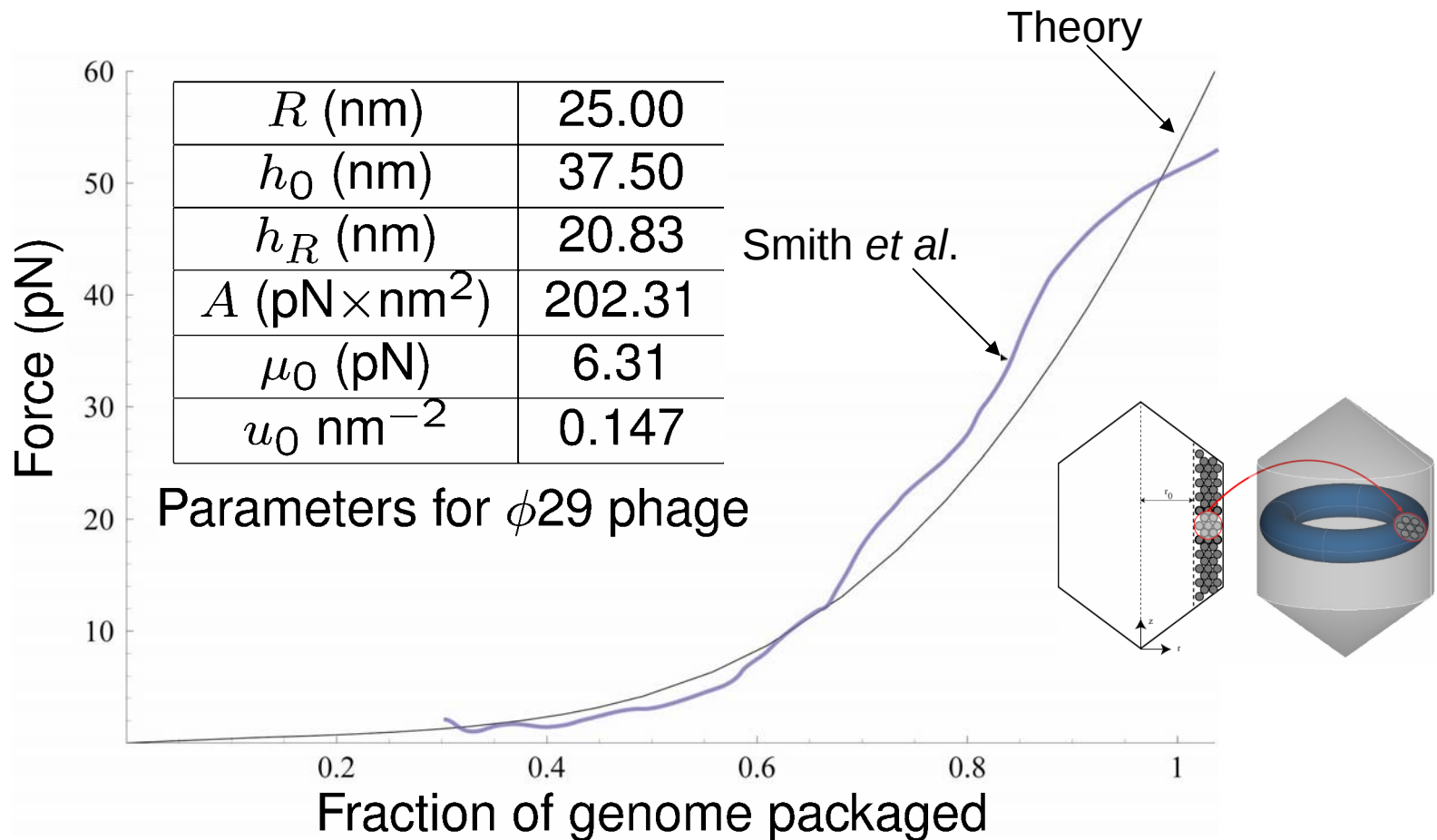
$$u(r, F)$$

Tao *et al.*, *Cell*, **95** (1998)

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Inverse-spool construction



Smith et al., *Nature*, **413** (6857) 748-752 (2001)



Spool construction – Surface energy

- Hard-core interaction, surface energy, ϕ_{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:

$$Au_0 \log r + (\phi_0 - Fu_0)r^2 +$$

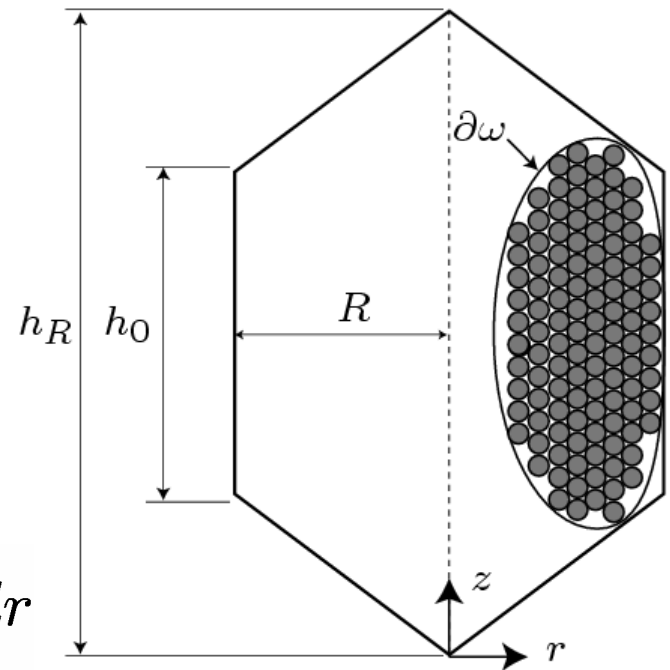
$$2\gamma r \cos \alpha = C \Rightarrow \alpha(r)$$

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

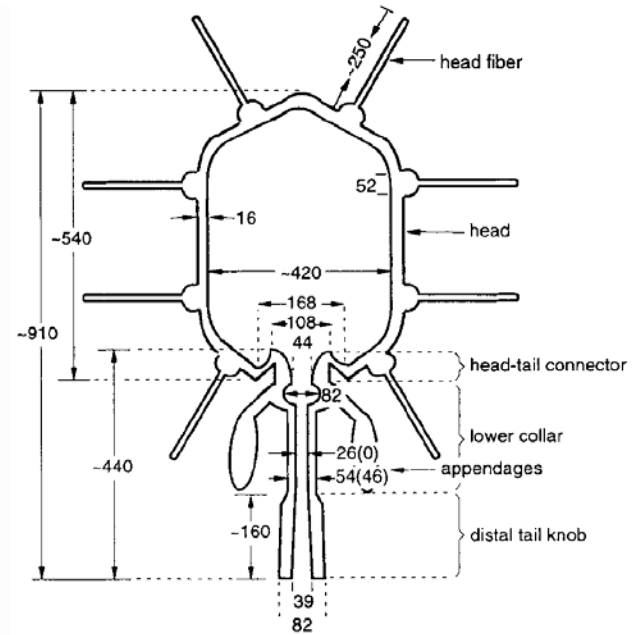
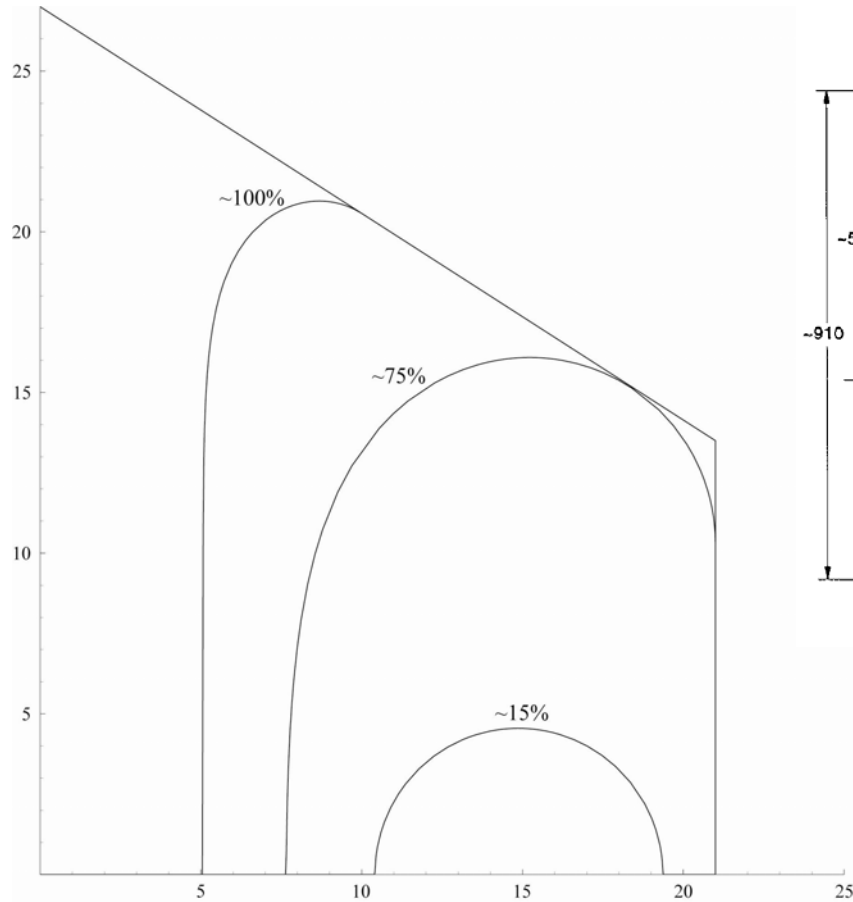
- Solution:

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

$$\text{where } \alpha(R_{\text{out}}) = 0$$



Spool construction – Surface energy



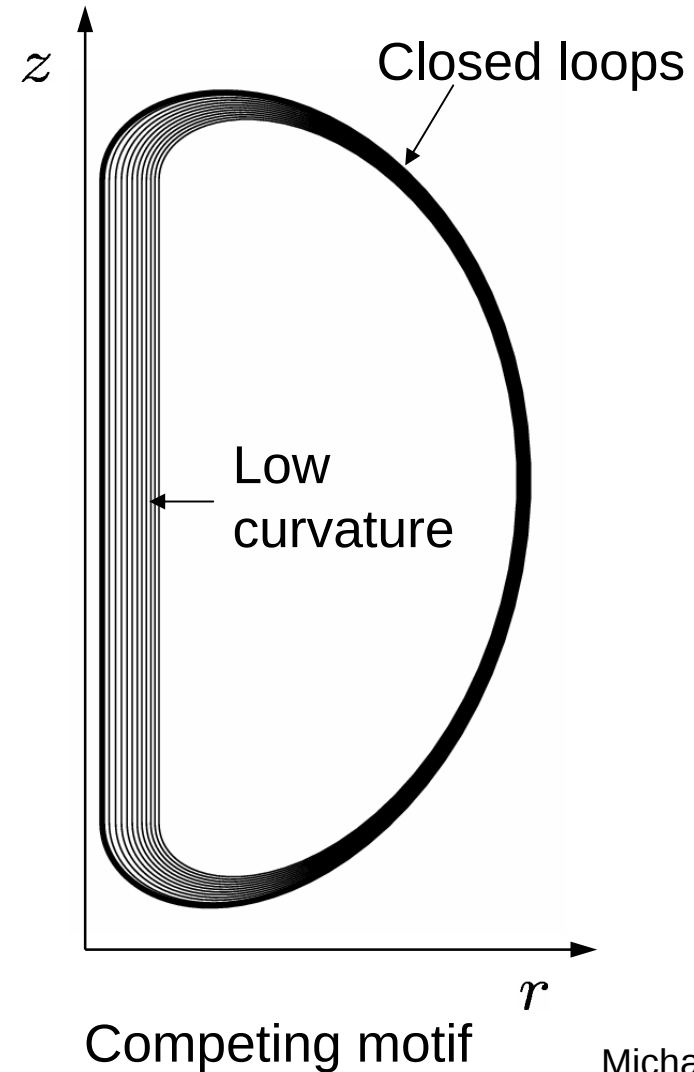
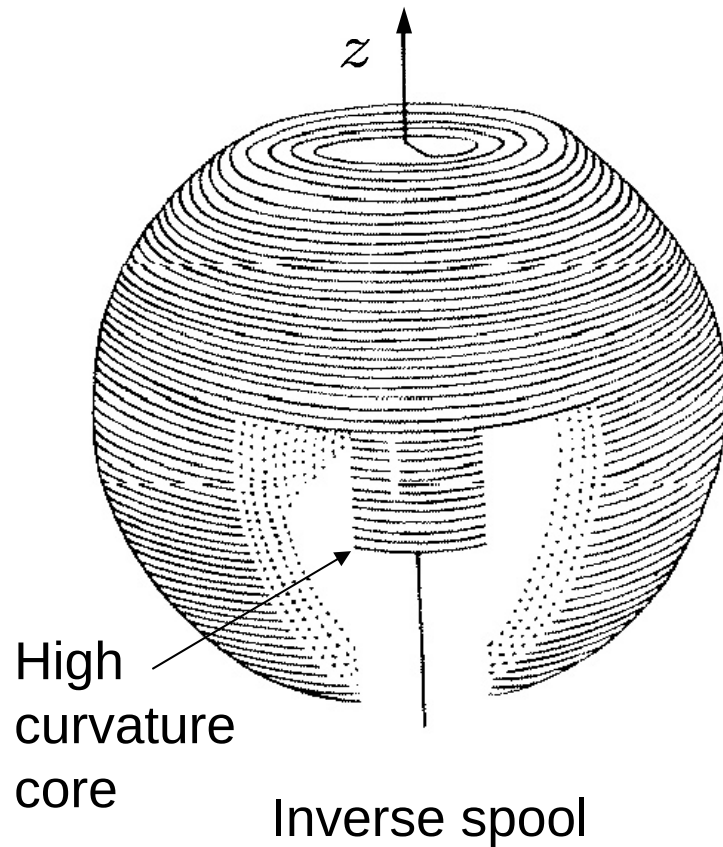
Tao *et al.*, *Cell*,
95 (1998)

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

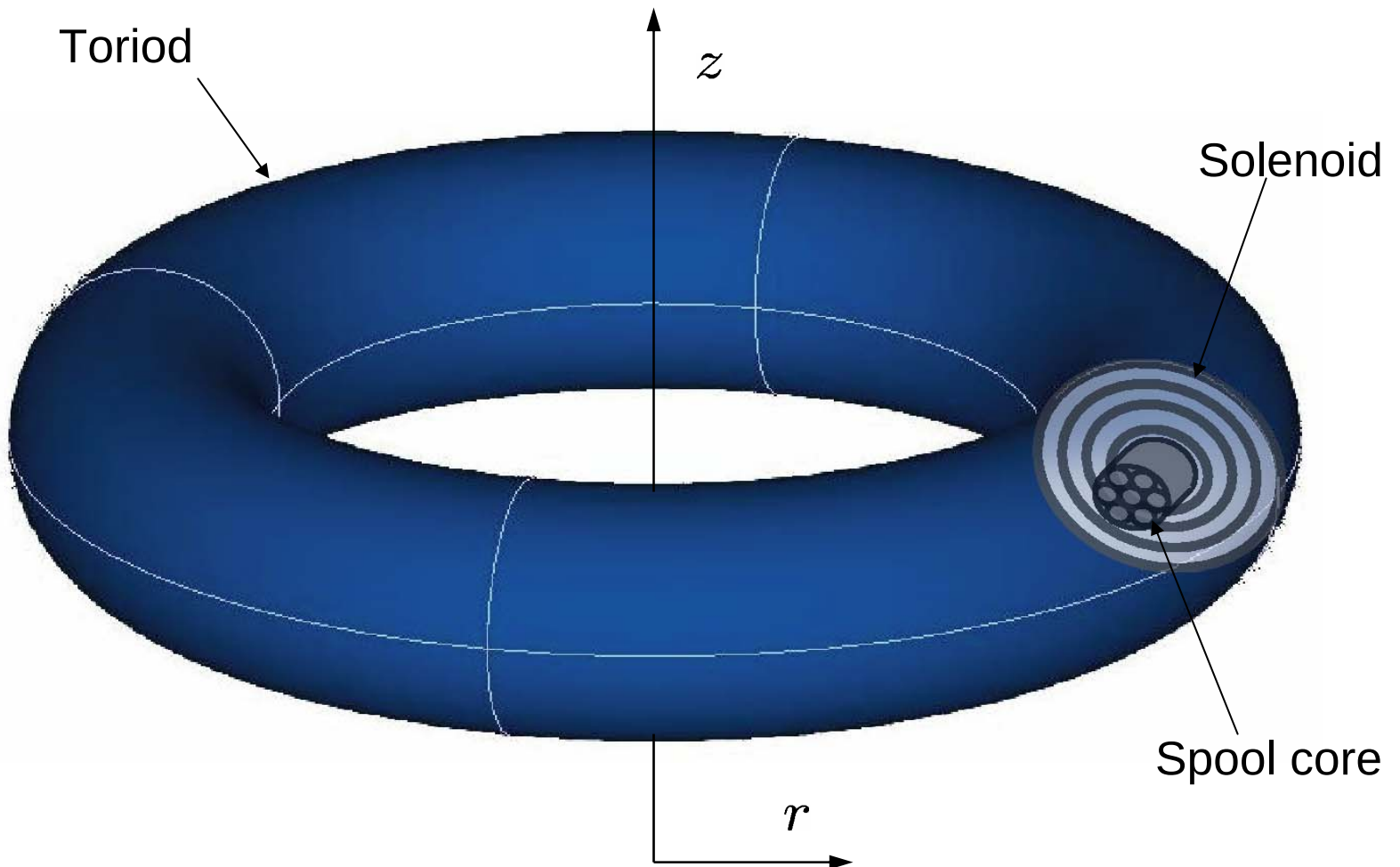
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Can the inverse spool be beaten?



Torsionless toroidal solenoid construction



Torsionless toroidal solenoid construction

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

- 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.

$$t_r = -\frac{\partial v_\theta}{\partial z}, \quad t_z = \frac{1}{r} \frac{\partial}{\partial r}(r v_\theta)$$

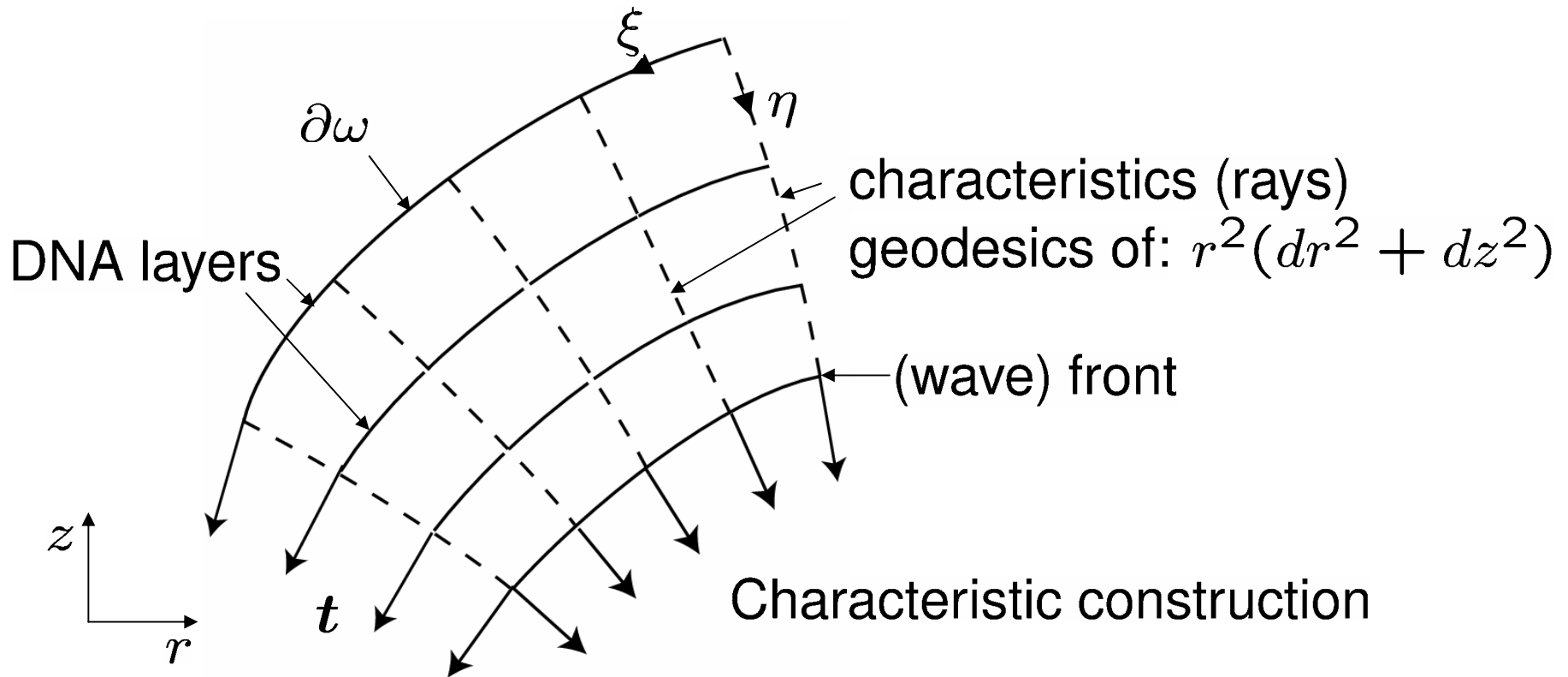
- Tangency BC: $v_\theta = 0$, on $\partial\omega$
- Modulus constraint: Let $\eta = r v_\theta$. Then,

$$\sqrt{\left(\frac{\partial \eta}{\partial r}\right)^2 + \left(\frac{\partial \eta}{\partial z}\right)^2} = r$$

Inhomogeneous **eikonal** equation.



Torsionless toroidal solenoid construction

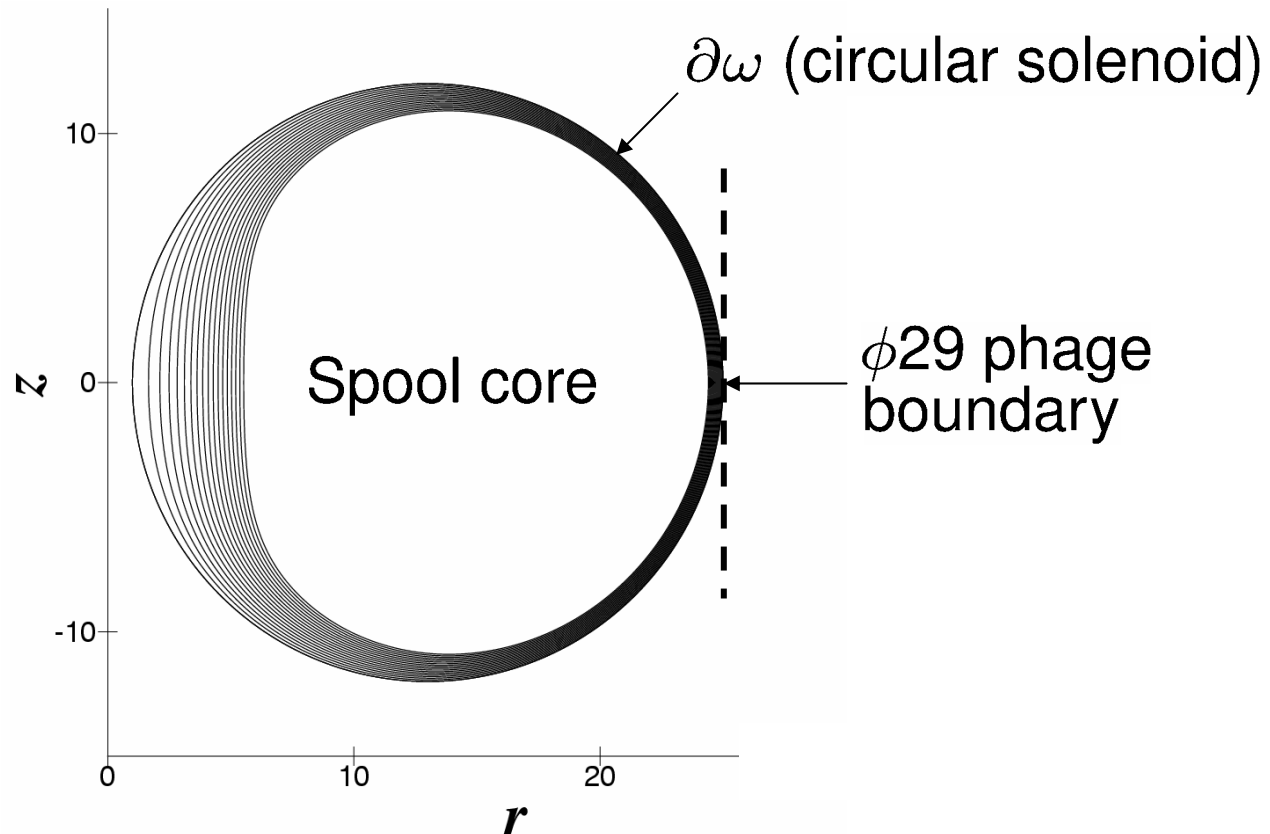


- Construction terminates when:

$$\int_{\eta=N} \frac{A}{2} \kappa^2 h_{\xi} h_{\eta} d\xi = \int_{\eta=N} \frac{A}{2} \frac{1}{r^2} h_{\xi} h_{\eta} d\xi$$



Torsionless toroidal solenoid construction



- $\Delta E = -1.45 \times 10^3 \text{ pN} \times \text{nm}$, **solenoid wins!**
- Work to pack $\phi 29$ genome $\sim 7.5 \times 10^4 \text{ pN} \times \text{nm}$ (Smith *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:

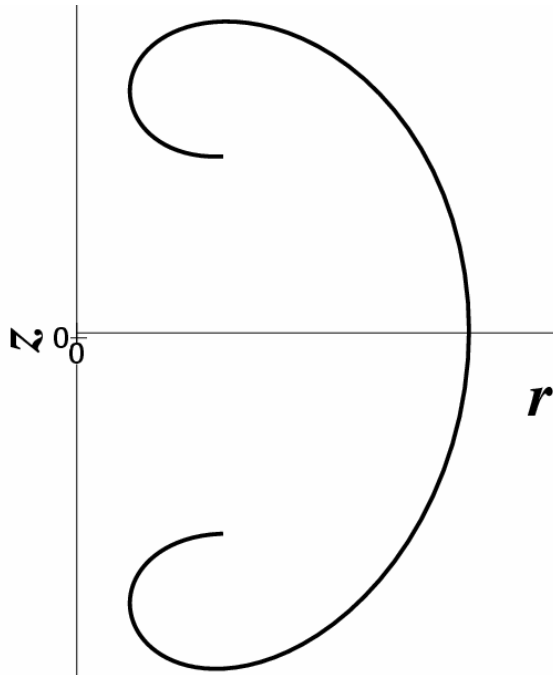
$$W(r, t) = \frac{Au_0}{2} \left\{ \kappa^2 \wedge \frac{1}{r^2} \right\}$$

Spool phase
Solenoid phase

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



Solenoid/spool mixtures - Interfaces



Segment $(-\pi \leq \varphi \leq 3\pi)$
of equilibrium interface
(prolate cycloid)

- Interface driving force:

$$f = \frac{Au_0}{2} \left(\frac{1}{r^2} - \kappa^2 \right)$$

- Equilibrium interfaces:

$$\kappa^2 = \frac{1}{r^2}$$

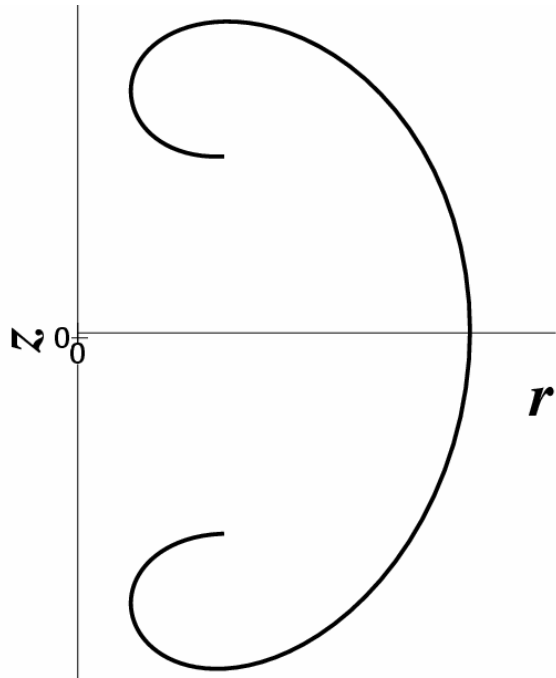
- Parametric equations:

$$r(\varphi) = r_0 \exp(\sin \varphi - \sin \varphi_0)$$

$$z(\varphi) = z_0 + \int_{\varphi_0}^{\varphi} r(\psi) \sin \psi \, d\psi$$



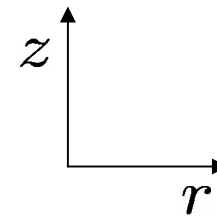
Solenoid/spool mixtures – Front relaxation



Segment ($-\pi \leq \varphi \leq 3\pi$)
of equilibrium interface
(prolate cycloid)

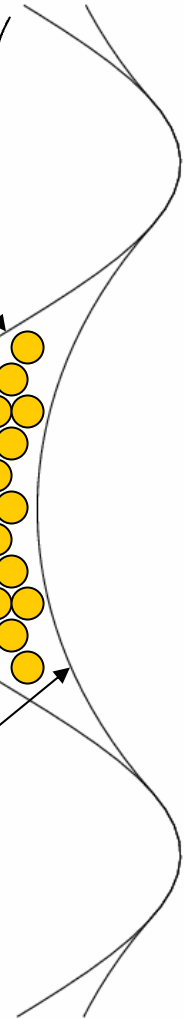


Unrelaxed front
($\kappa > r^{-1}$)

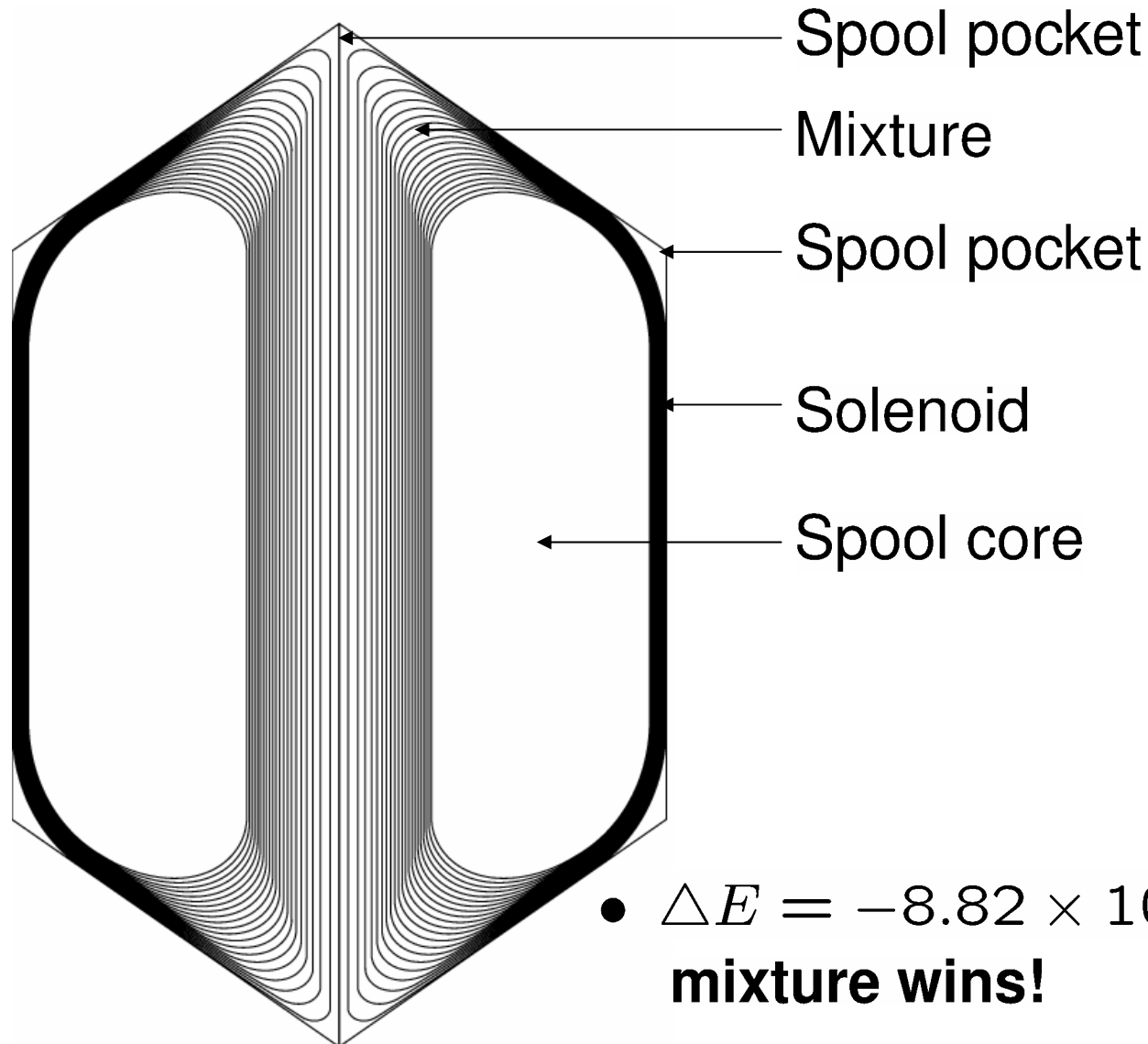


Spool pocket

Relaxed front
($\kappa = r^{-1}$)



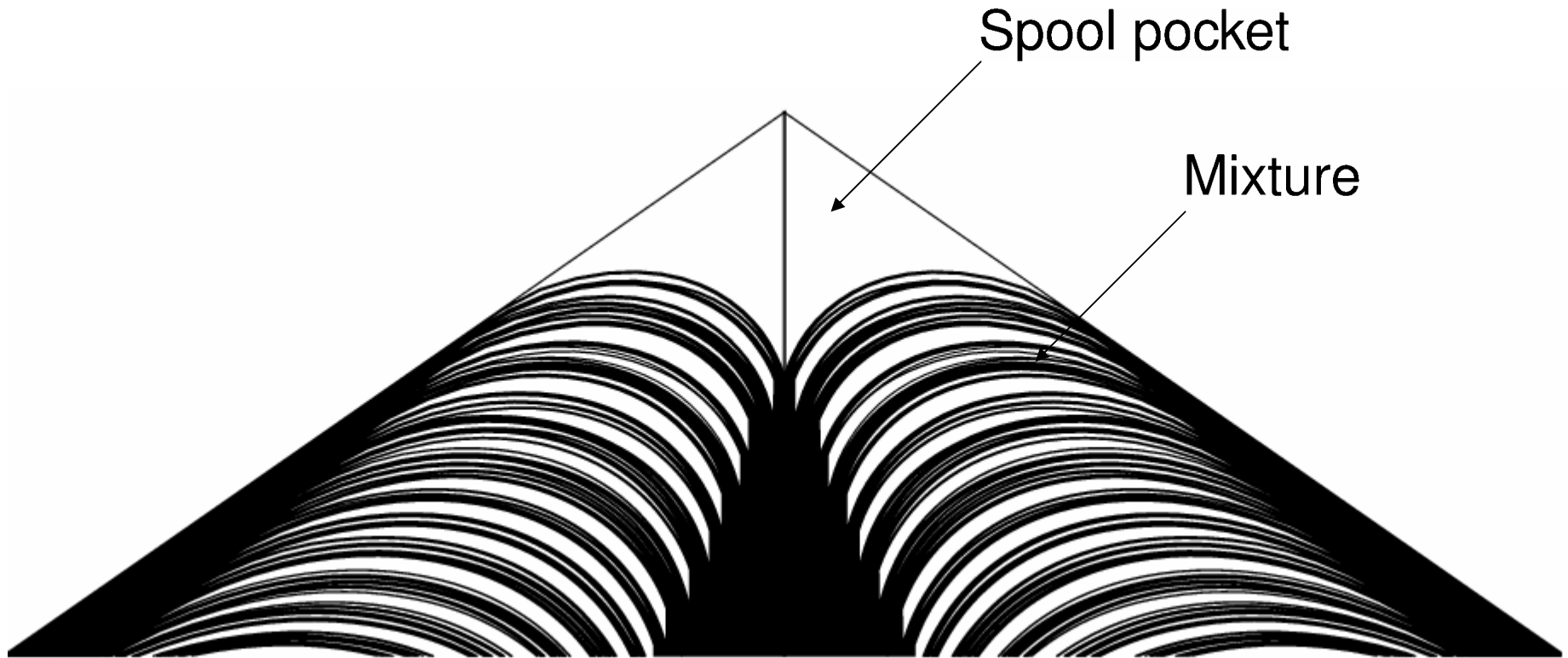
Solenoid/spool mixtures – ø29 phage



- $\Delta E = -8.82 \times 10^3 \text{ pN} \times \text{nm}$
mixture wins!



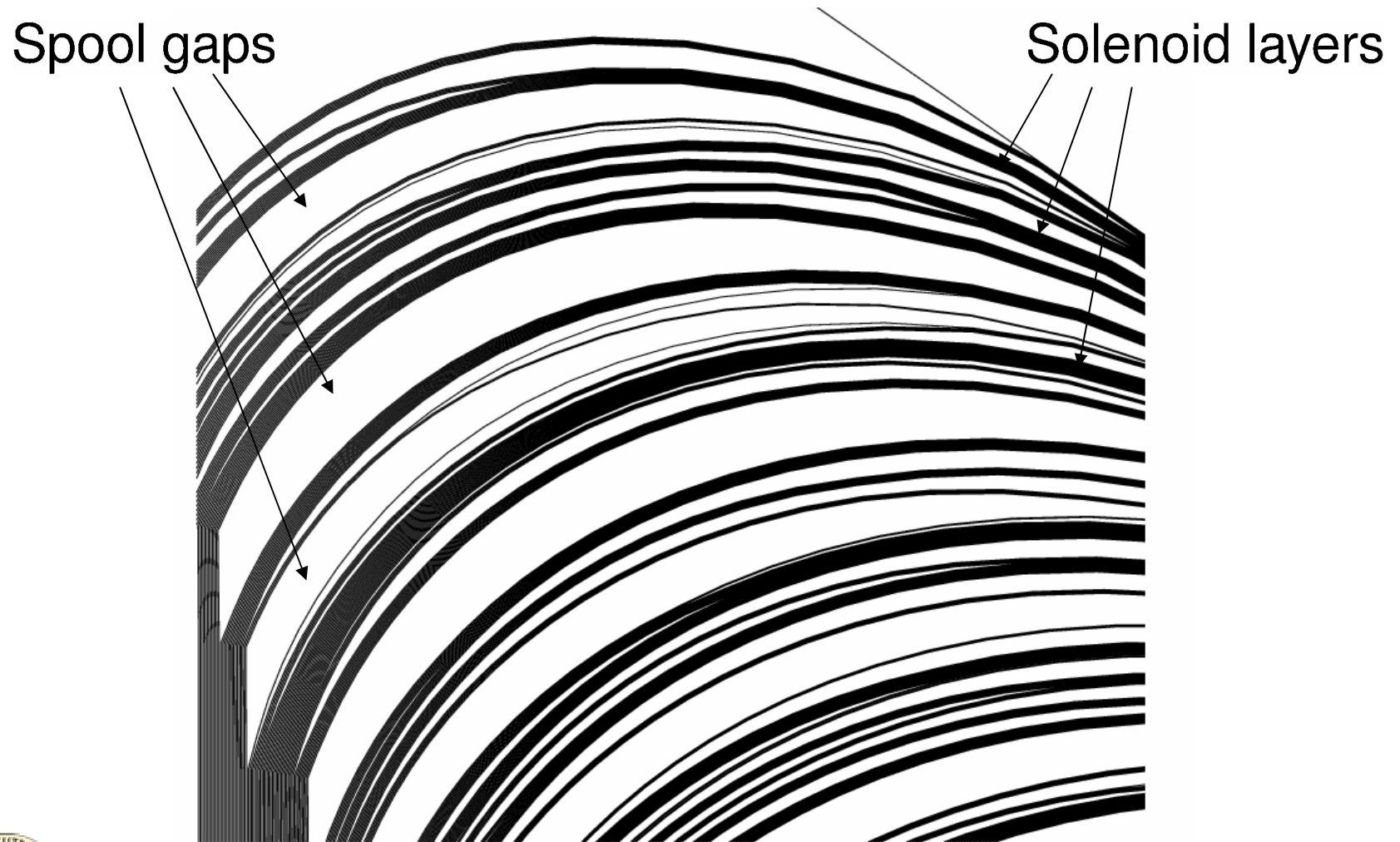
Solenoid/spool mixtures – ø29 phage



Close-up view of top apex region



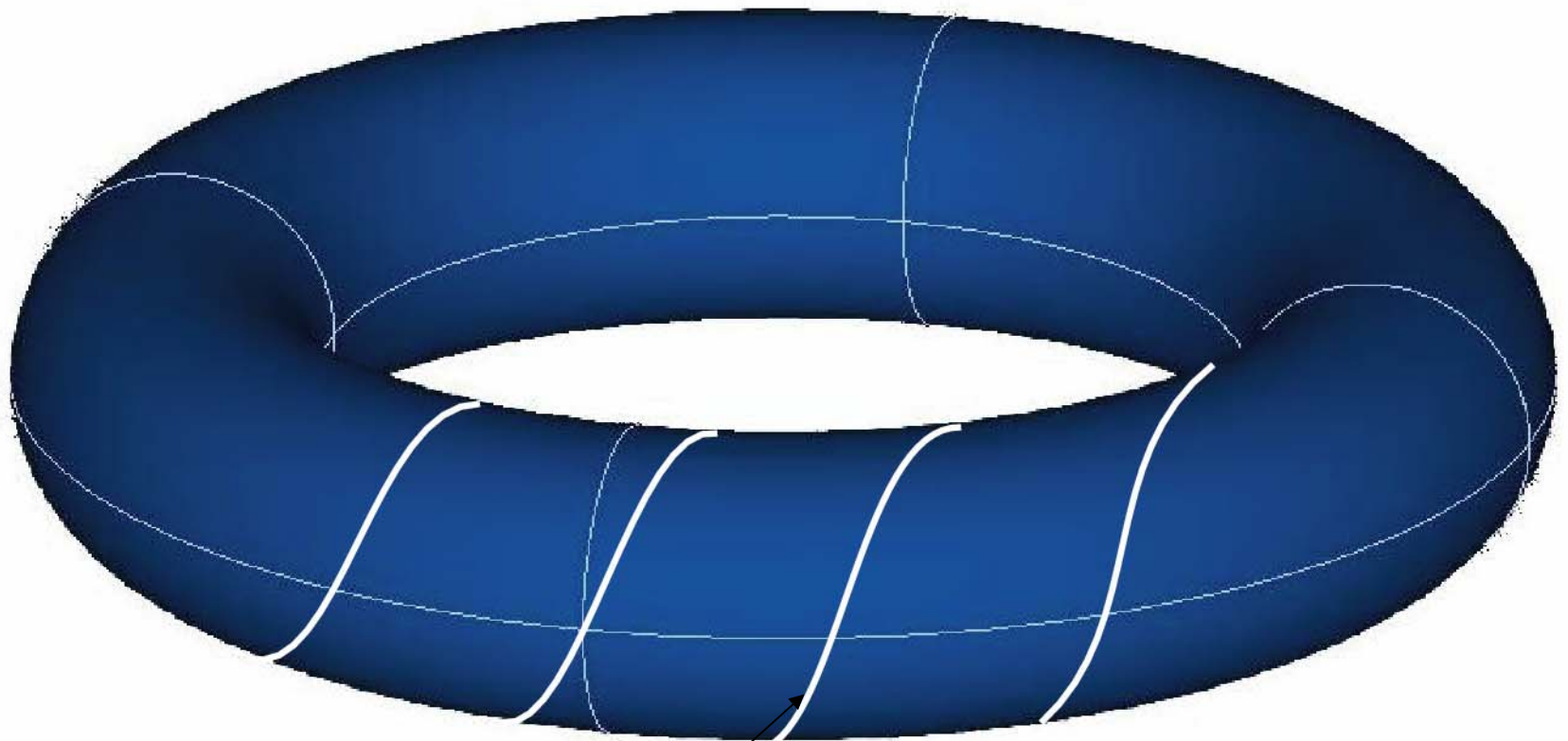
Solenoid/spool mixtures – ø29 phage



Close-up view of mixture region



Outlook - Toroidal solenoids

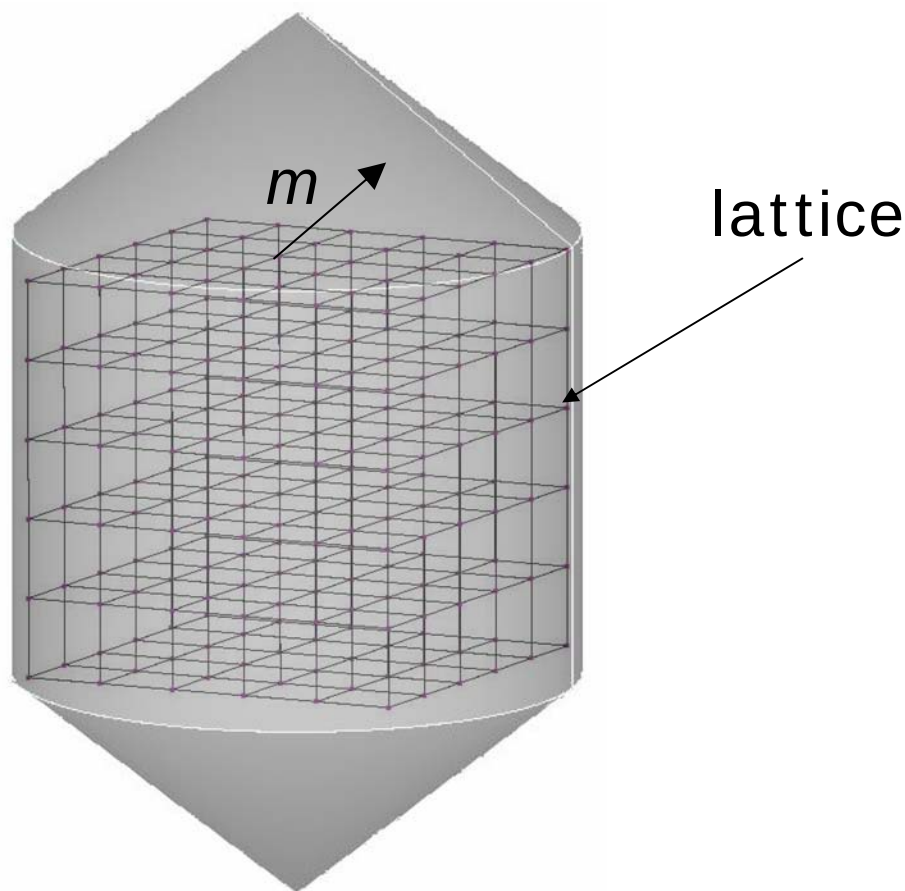


Toroidal helix

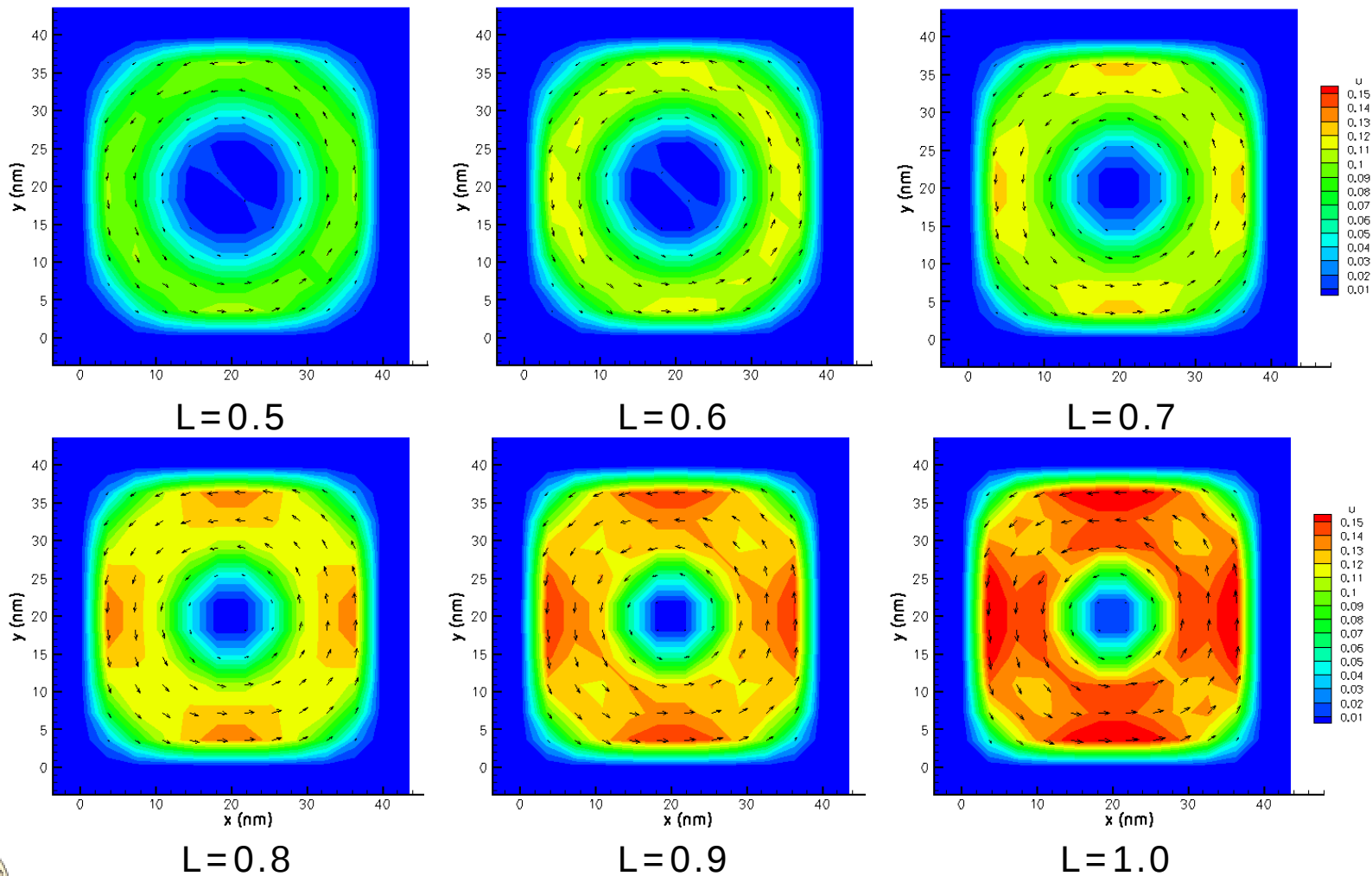


Outlook – Lattice models

- Discretize Ω , $m \Rightarrow$ *lattice model*. Relax energy by Monte Carlo, simulated annealing, genetic algorithms. . . .



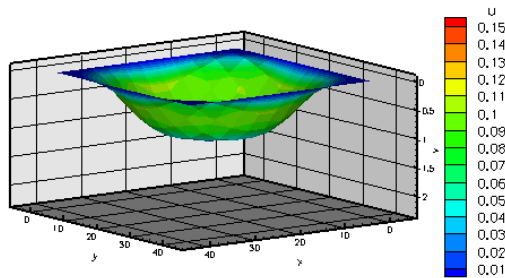
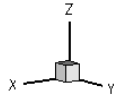
Outlook – Lattice models



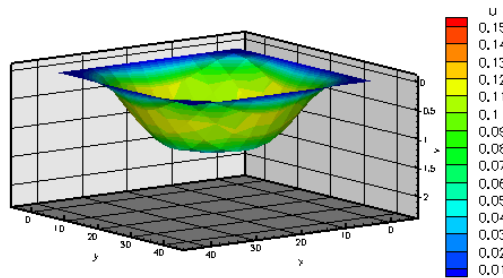
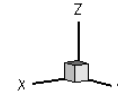
DNA density contour plots and director field

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UCLA 05/03

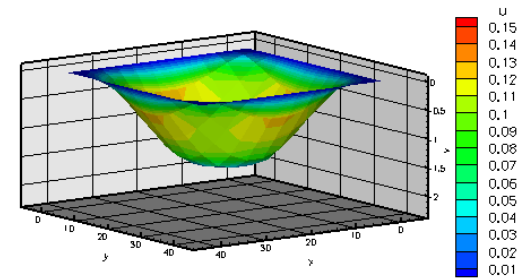
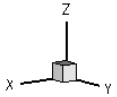
Outlook – Lattice models



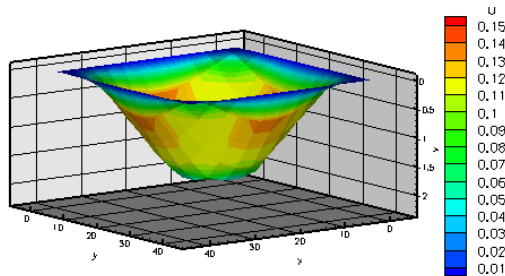
$L=0.5$



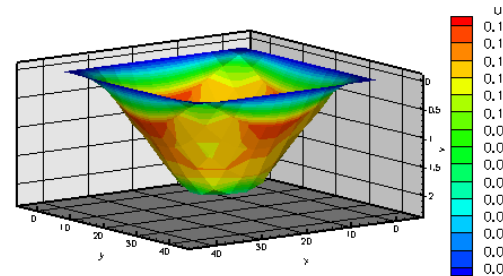
$L=0.6$



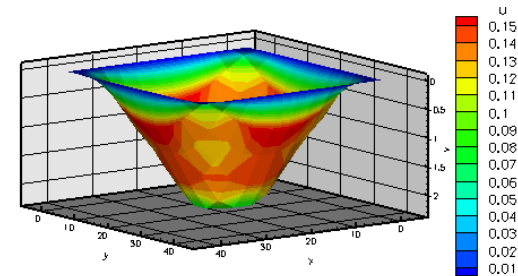
$L=0.7$



$L=0.8$



$L=0.9$



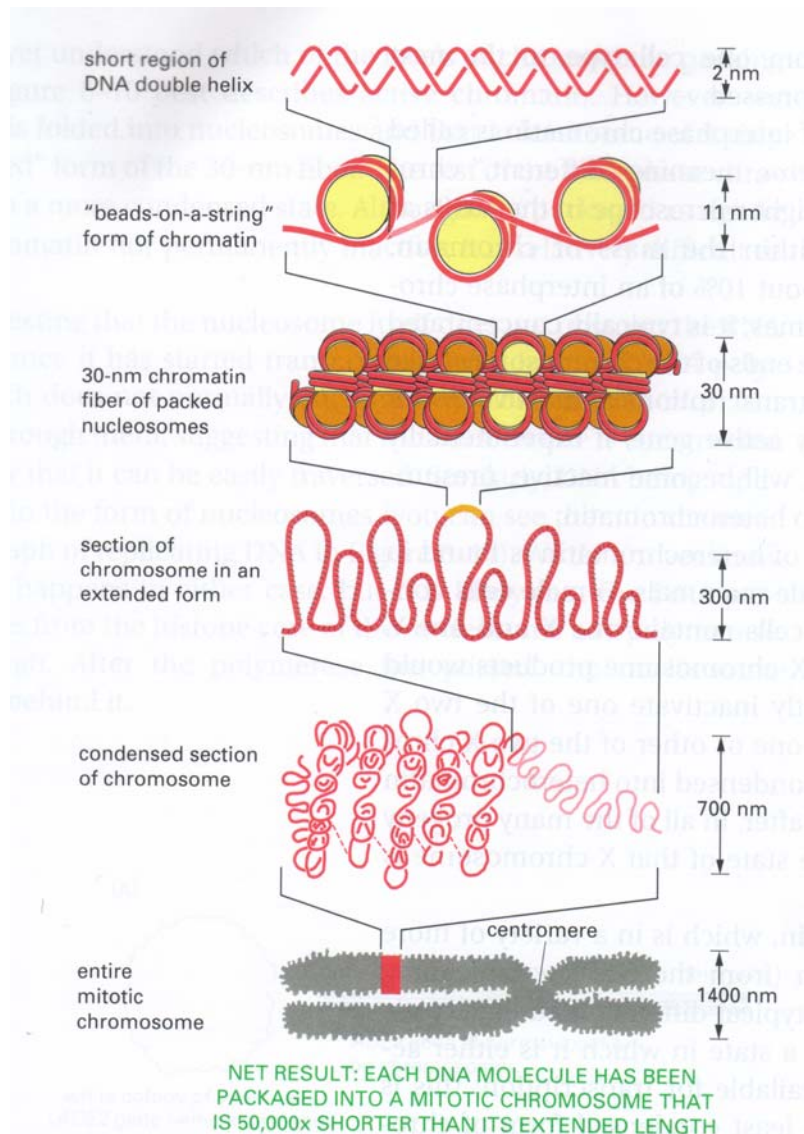
$L=1.0$

DNA density contour plots
and vector potential



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Outlook – Related problems

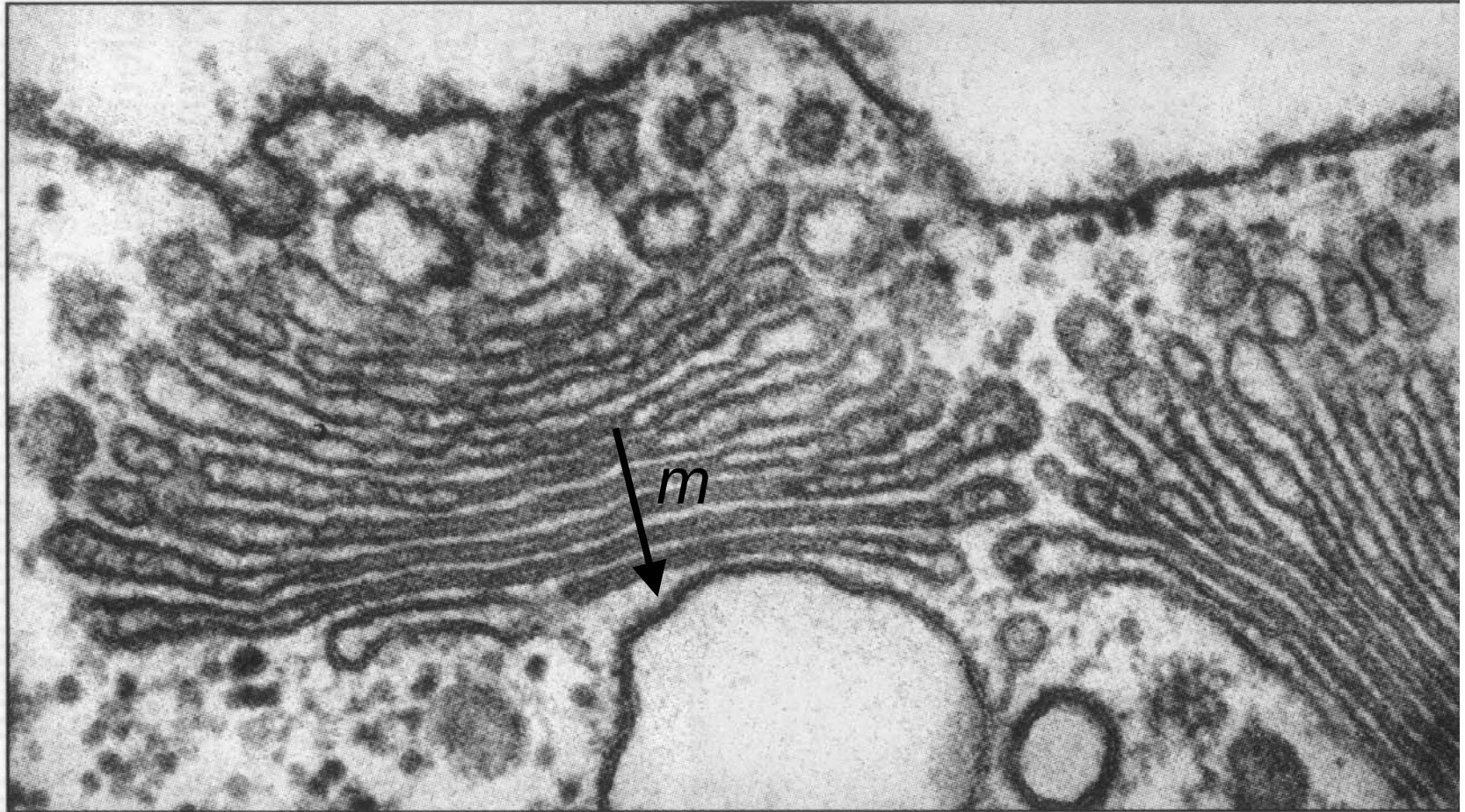


Levels of chromatic packing. Orders of chromatin packing thought to give rise to the highly condensed mitotic chromosome. The folding of naked DNA into nucleosomes is the best understood level of packing. The structures corresponding to the additional layers of chromosome packing are more speculative.

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



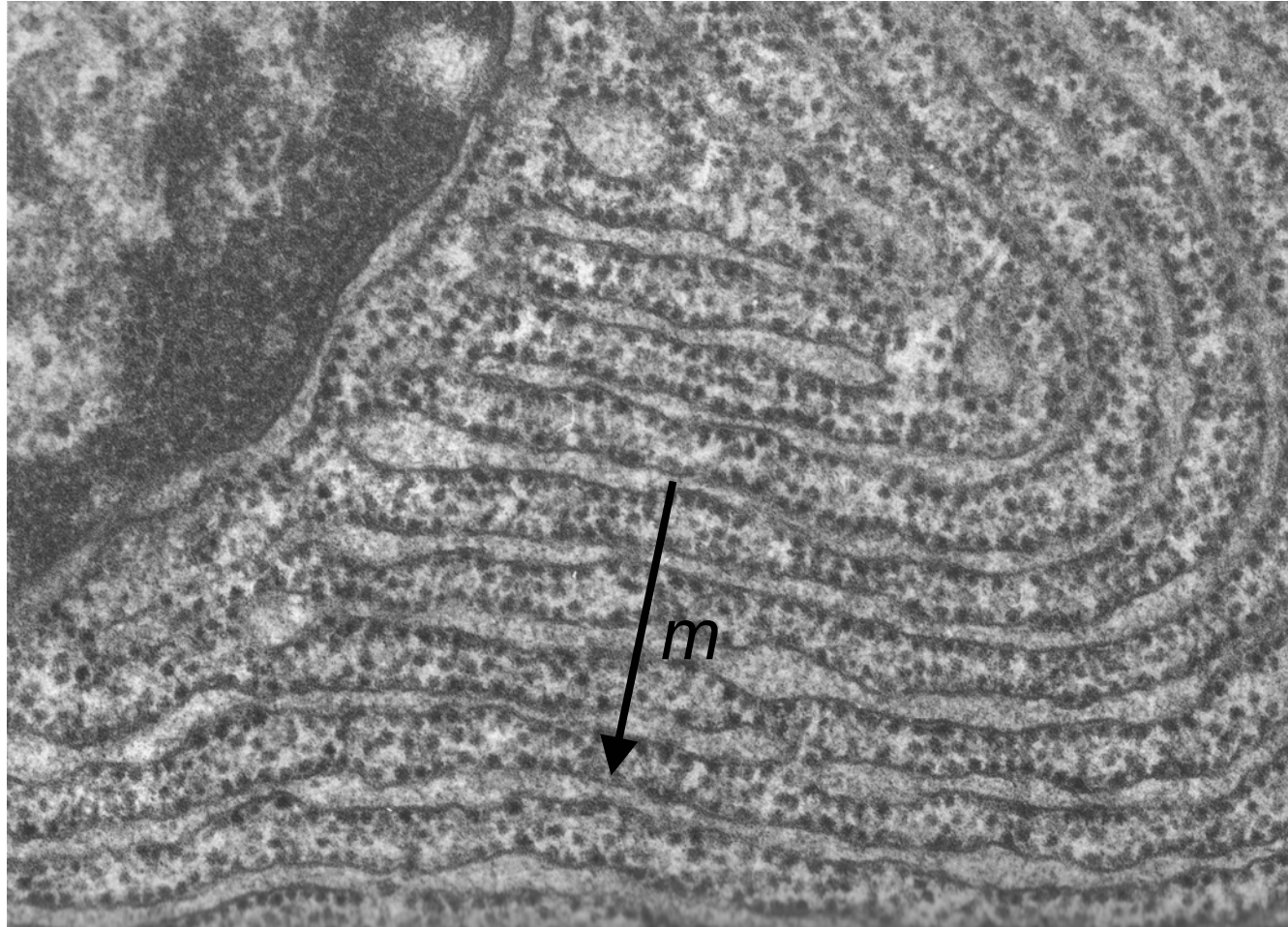
Outlook – Related problems



Golgi apparatus, secretory animal cell
(G. Palade)



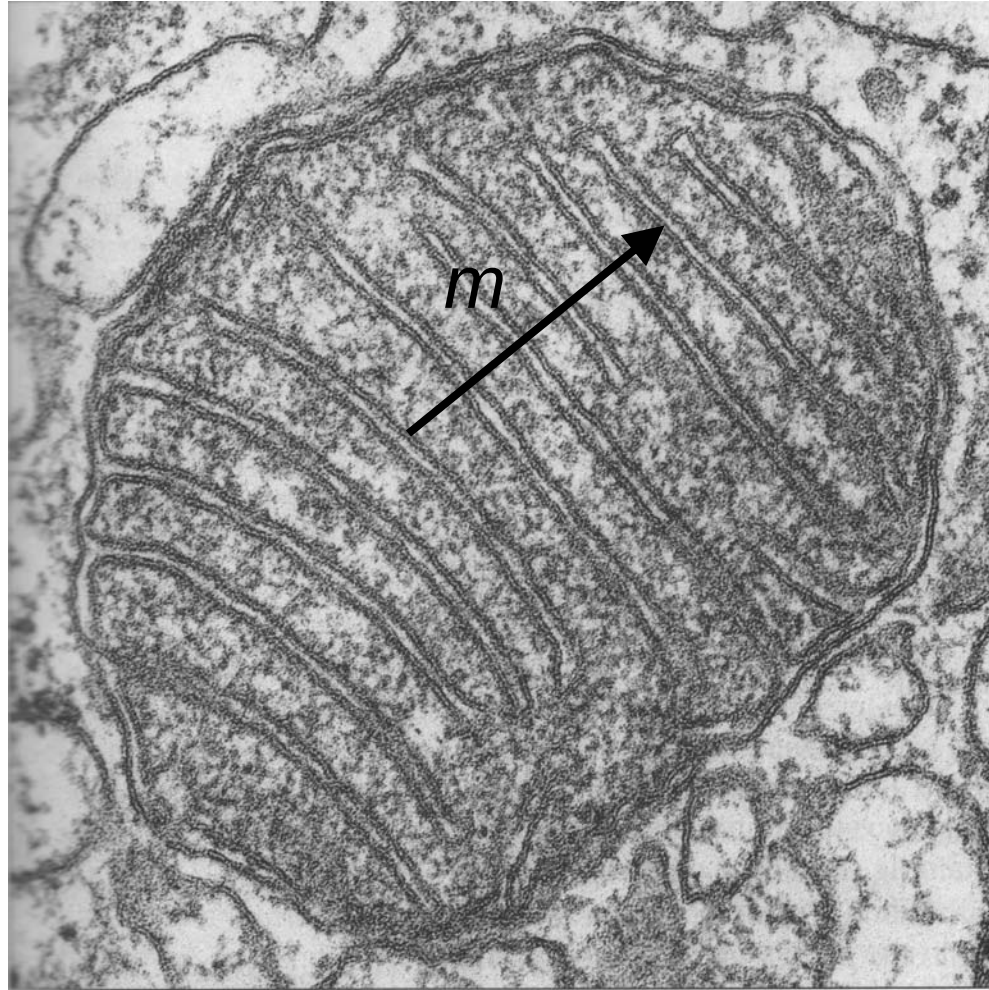
Outlook – Related problems



Endoplasmic reticulum, canine pancreas cell
(L. Orci)



Outlook – Related problems



Cross section of mitochondrion
(D.S. Friend)



Summary and concluding remarks

- Approach affords a clean variational characterization of viral DNA encapsidated conformations.
- Analysis:
 - What is the optimal DNA arrangement?
 - How does the energy scale with L , A , size of Ω ?
- Computation: lattice models.
- Director-field approach applies to other related problems involving the packing of rods and membranes.

