

Irreversible growth model for assembling irregular capsids

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Support: DOE

Overview of talk

1. Introduction
2. Model
3. Results
4. Problem of HIV capsid
5. Future directions?

1. Introduction: capsids

Capsid: virus's external shell, made from many copies of the same protein(s).

Quasiequivalence

Polymorphism

Equilibrium models?

Non-equilibrium assembly

Quasiequivalence

Only 60 units can be symmetry equiv.; most viruses have more

An ***approximate symmetry***: same bonding interaction in similar but not identical environments.

[Caspar and Klug, 1962]

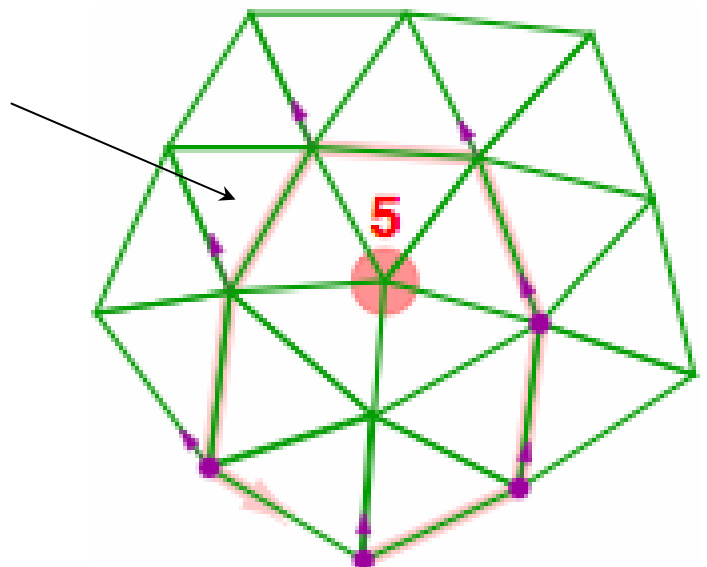
Disclinations (remark)

Physics viewpoint: actual network is ideal reference (triangular) lattice + topological defects.

Each 5 vertex a ***disclination***:

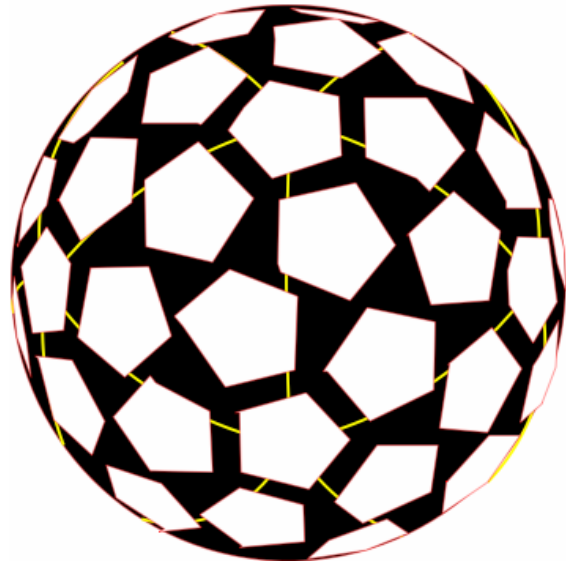
walk a loop around it, local axes rotate by $1/6$.

transporting orientation
along a loop around a 5

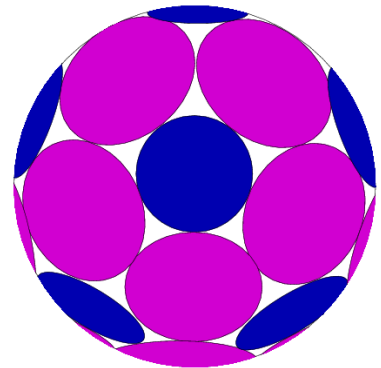


more on quasiequivalence

blatant violation: SV40
(papillomavirus, etc)
 $T=7$ but *pentamers*
in 6-fold coordination



maybe nonspecific bonding
("sticky disks") + orientation
interaction as perturbation?
[Bruinsma-Gelbart-Zandi]



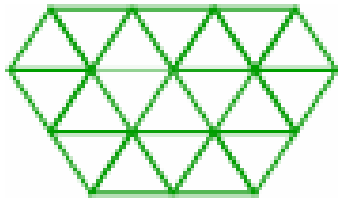
(show $T=3$ here:)

maybe a more complex / fragmentary approximate
symmetry? [Twarock]

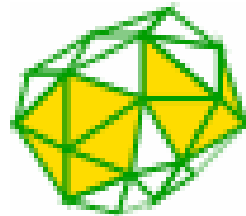
Polymorphism

Corollary of quasiequivalence:
same local bonding permits, in principle,
a large variety of shapes:

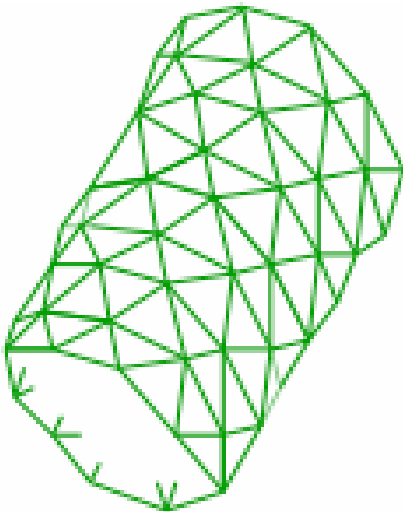
plane



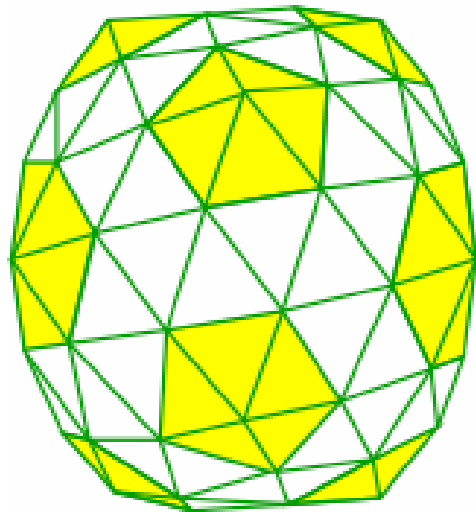
irreg. closed shell



tube



large (T7) icosahedron



Polymorphism (cont'd)

Too few nice polymorphic systems known (e.g. CCMV).

Biologists focus on “typical”; and (till recently), methods entailed averaging assuming symmetry (diffraction = spatial, Cryo-EM = orientational)

Our question: What determines the actual size/shape out of all these possibilities? Our model, in fact, selects no single result, hence we focused on retroviruses which experimentally are an ensemble.

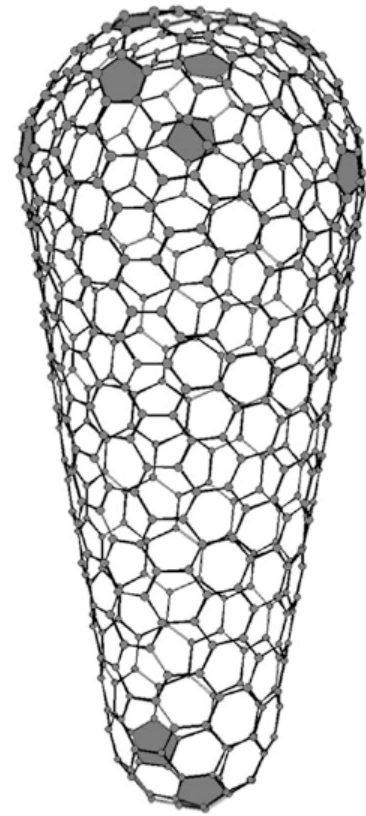
HIV capsid and quasiequivalence

Fullerene-like model: triangle
net w/o global symmetry

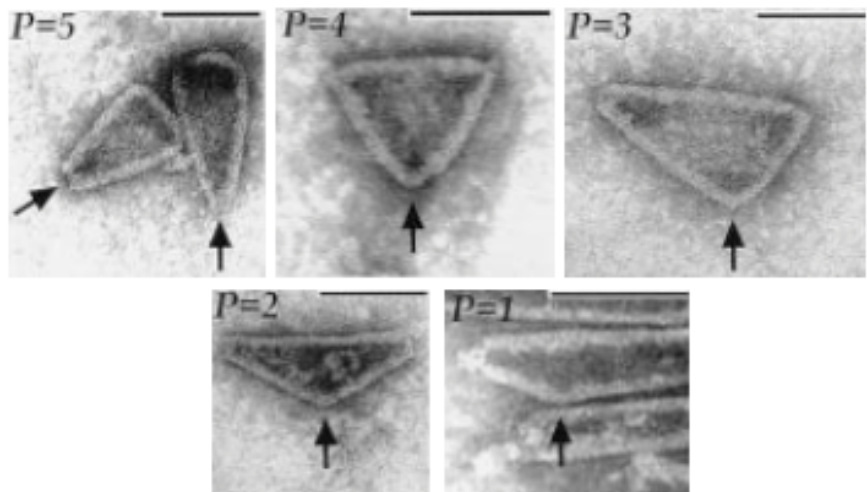
Cone angle quantized

(depends if 1,2,...5, or 6

5-fold vertices at small end)



Observed angles agree!



Ganser et al, Science 283, 80 (1999).

Equilibrium models?

- Local rules [Berger, ~Twarock]

Antithesis of quasiequivalence. protein has several conformations, each is a different flavor of “tile” with completely different matching rules for joining to its neighbor.

Too baroque for us.

- Bruinsma-Gelbart-Zandi: similar

Hamiltonian to ours. Energy minima are large icosahedra. But can system anneal to optimum? We explored opposite limit.

2. Model

Complementary deg's of freedom:

- discrete (bond network)
- continuous (coordinates in 3D)

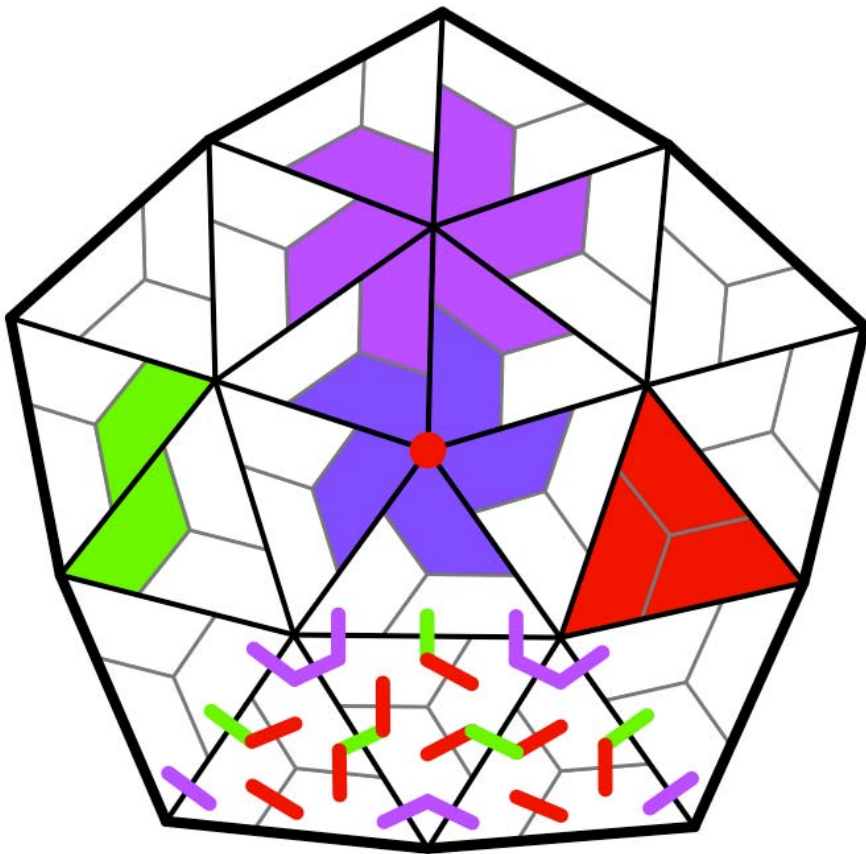
Corresponding parameters:

- transition rates (form new bonds)
- Elastic Hamiltonian

Feedback on each other

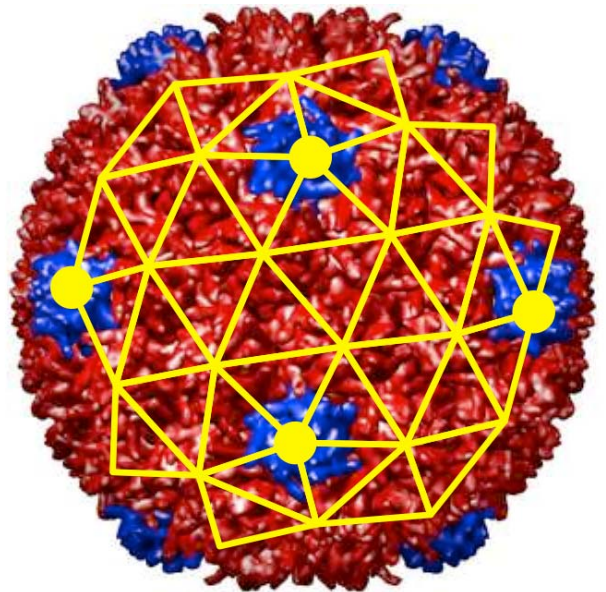
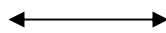
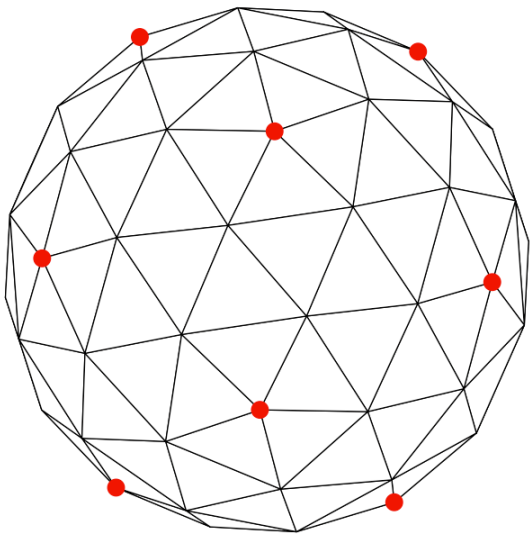
Representation?

There are 3 kinds of bonds
(Quasi 6, quasi 3, or quasi 2 axis)
2 kinds required to hold together.



Representation(cont'd)

We chose triangles (thus, trimer bond) as a representation; presuming it won't matter at a Coarser-grained level (?)



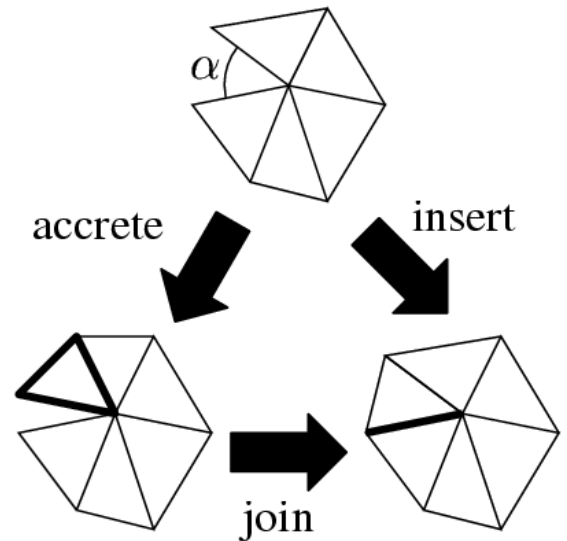
Ideal T=7

HK97 capsid

Model: growth rules

Three possible steps
(forward only)

- Accretion rate k_A is constant
- Other rates depend on angle α



Insertion rate k_I max. at $\alpha = 60^\circ$

Joining rate k_J max. at $\alpha = 0^\circ$

Form is

$$k_{I,J} / k_A = \Gamma_{I,J} e^{-(\alpha - \alpha_{I,J})^2 / 2\sigma^2}$$

Warning: need

$$\Gamma_{I,J} \approx 100, \quad \sigma \approx 12^\circ,$$

for best results.

Kinetic trap? (side note)

- Zlotnick [J Mol Biol 241, 59 (1994) and subsequent]

All the units may aggregate into valid partial capsids, then no way to finish them.

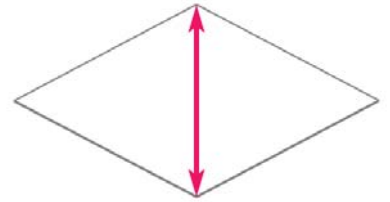
- Our regime: assume needs e.g. RNA to nucleate, hence only one partial capsid, no kinetic trap.

Instead, worry is partial capsids that are valid but incompletable.

Model: elastic Hamiltonian

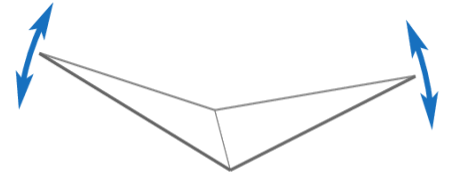
Stretching stiffness Y

$$H_{stretch} = \sum_{\langle i,j \rangle} \frac{\sqrt{3}Y}{4} (r_{i,j} - r_0)^2$$



Bending stiffness K

$$H_{bend} = \sum_{\langle a,b \rangle} \frac{2K}{\sqrt{3}} (1 - \cos(\theta_{a,b} - \theta_0))$$



discretization of continuum elasticity?
Yes, but triangles are the protein
units (not arbitrary grid).

Ratio of elastic constants

A key parameter:

Foppl-Karman length

$$\ell_f = \sqrt{\frac{\kappa}{Y}}.$$

Nelson and collaborators emphasized

Foppl-Karman **ratio**,

(here R is capsid radius)

$$\gamma \equiv \left(R / \ell_f\right)^2,$$

It determines **facetedness** of Icosahedral capsids.

But the concept is more general:
defines a healing length of the
distortions due to 5-fold vertices.

ℓ_f

And controls rate of crevice failure
mode (see later) in our simulations.

Observed HIV faceting matches

$$\gamma \approx 550$$

Estimate elastic constants

Ivanovska et al [PNAS 101, 7600 (2004)] AFM indentation:

$Y=3.5$ N/m?

Maeda & Fujime [Macromolecules 18, 2340 (1985)] persistence length of tubular phage fd 3.9 microns:

we infer $Y < 0.17$ N/m.

$$A_i \equiv \frac{\partial^2 H_{elastic}}{\partial \alpha_i^2} \approx 0.1 \quad Y r_o^2$$

Implies rms fluctuations

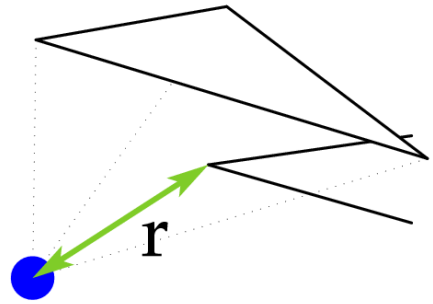
$$\sigma = 2.2^\circ \quad (\text{need } 10^\circ)$$

Model: steric repulsion

Just a technical necessity:

$$H_{steric} = \sum_{I,j} V_{steric} \left(\left| r_I^\Delta - r_j \right| \right)$$

Here I = all triangles,
 j = each vertex of I ;
 Δ = tip of “mast”
erected under tri. I



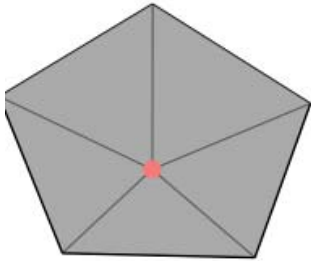
We used the form

$$V_{steric}(r) = k_{steric} \left(\ell_{steric}^2 - r^2 \right)^2, \quad r < \ell_{steric},$$

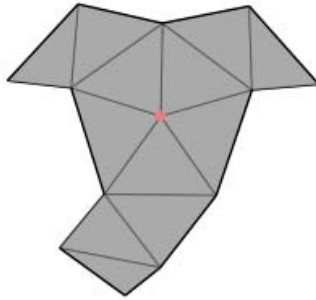
$$V_{steric}(r) = 0, \quad r > \ell_{steric}.$$

with $\ell_{steric} \approx 0.65 r_0$.

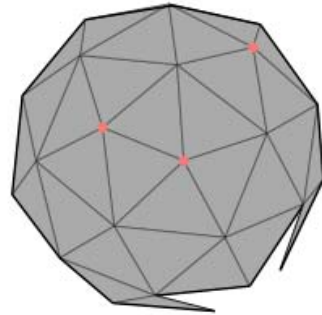
Examples of growth (A)



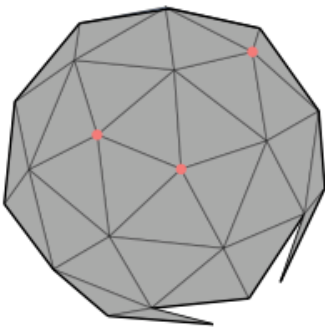
step 1



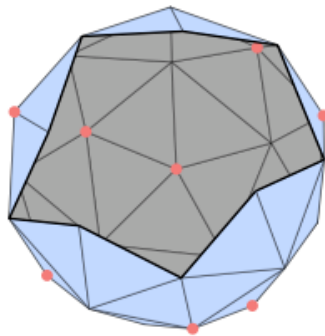
step 8



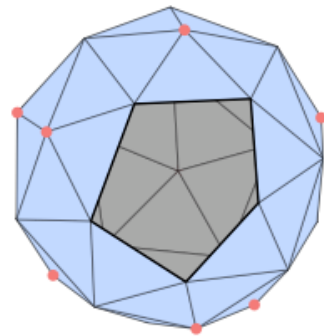
step 28



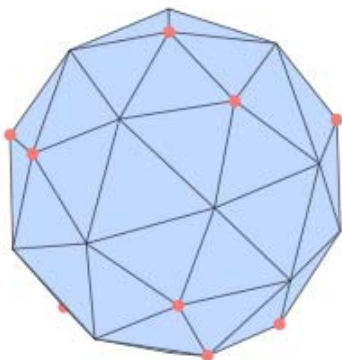
step 28



step 41

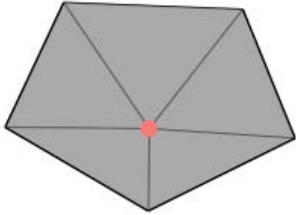


step 49

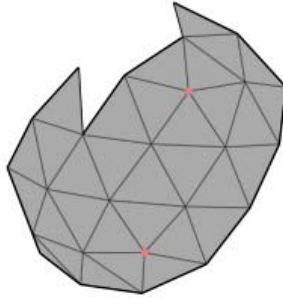


step 52

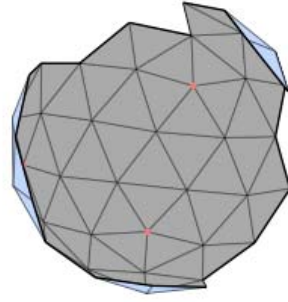
Growth: example B



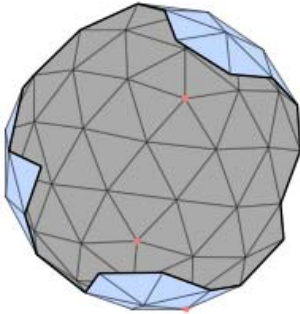
step 1



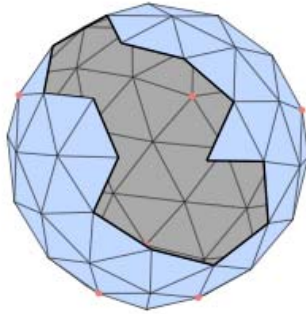
step 28



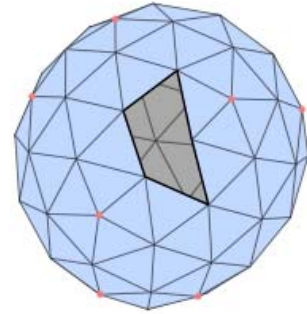
step 59



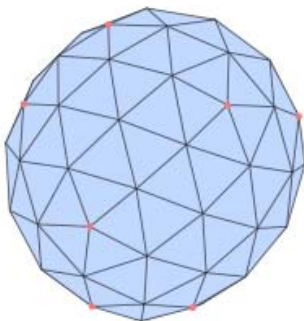
step 85



step 114



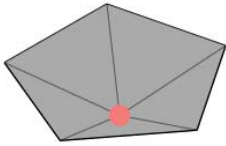
step 125



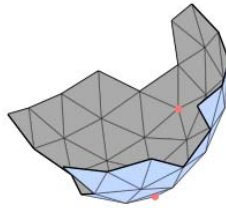
step 128

Usual result is
roughly spherical

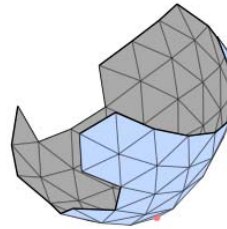
Growth: example C



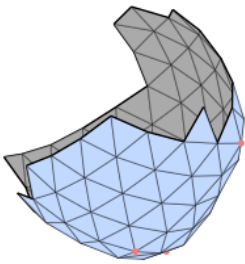
step 1



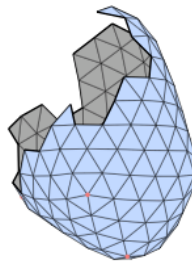
step 53



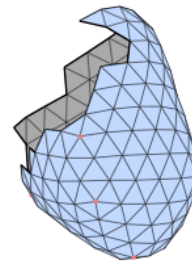
step 92



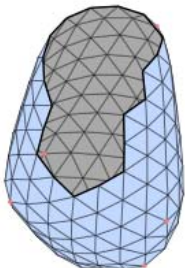
step 145



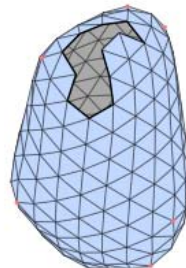
step 259



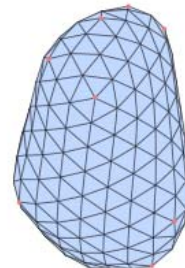
step 283



step 338

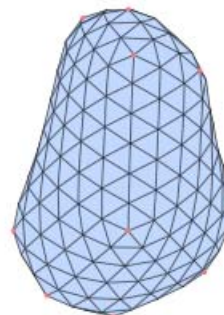


step 400



step 412

By chance, this came out roughly conical like HIV. Another view:



step 412

3. Results overview

- Success:
fraction completed,
failure modes, optimal parameter
values for completion
- Size:
relation to spontaneous
curvature parameter
- Shape:
how faceted (rel. to bending /
stretching stiffness ratio);
evaluate Gaussian curvature
within a loop on the surface

Results: failure modes

Growth rules can lead to partial capsid which is not part of any valid complete capsid.

We assumed irreversible growth

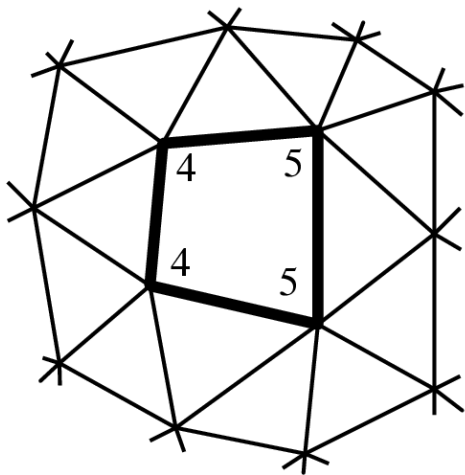
⇒ this is a failure.

Two kinds:

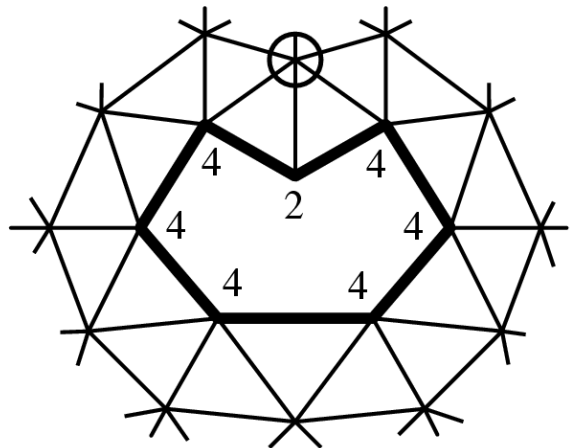
- Failure to close last hole
- Fingering, double sheet (gross failure)

Failure mode :unfillable hole

Any way to fill must have 7-vertex.
In (b), the circled vertex should
have been made a 5-vertex:
too late now! Will end up like (a).



(a)



(b)

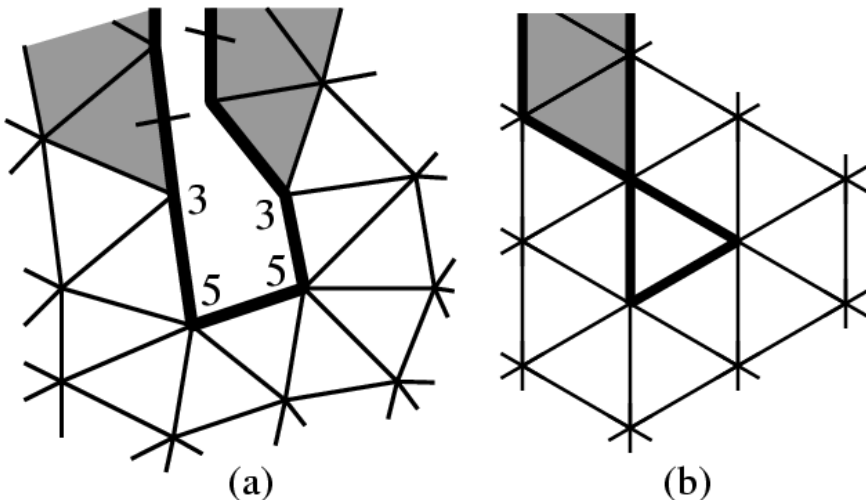
No one seems to know if such a
small hole is innocuous or lethal.

Failure mode: crevice

If joinings/insertions don't dominate, we get fingering: fatal.

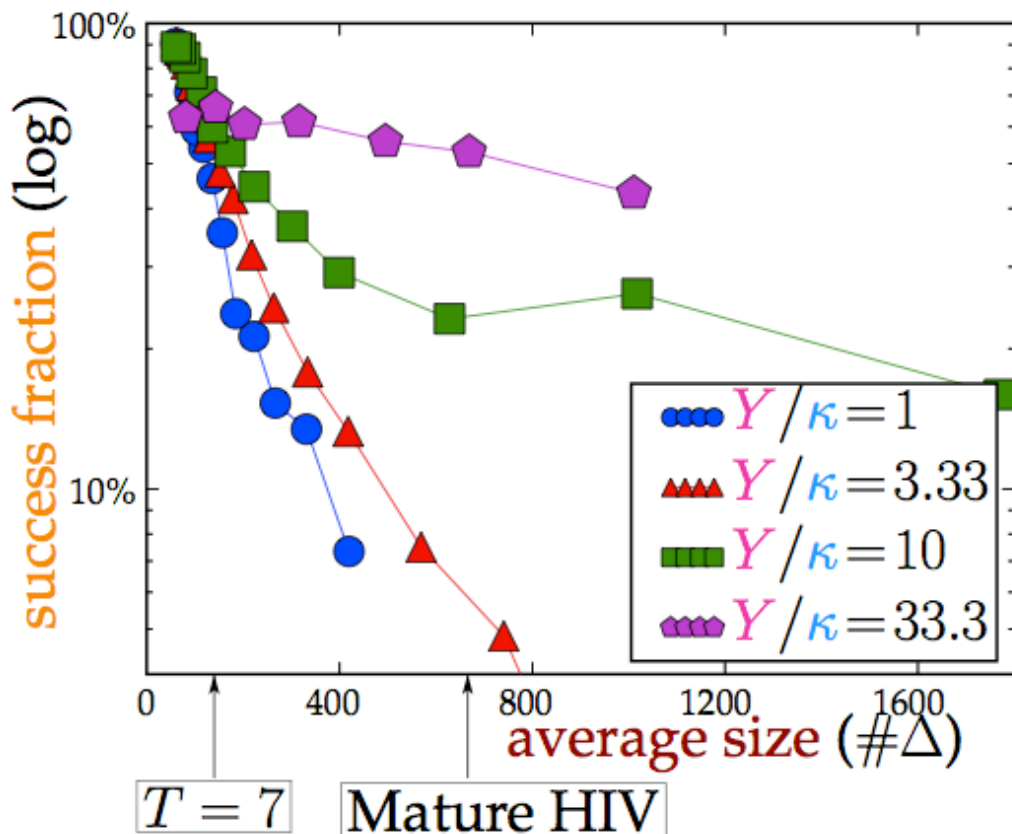
(Maybe one side grows over the other, forming a double surface).

This (and the other failure mode) can be recognized by an overlap when the border is flattened onto a triangular reference lattice.



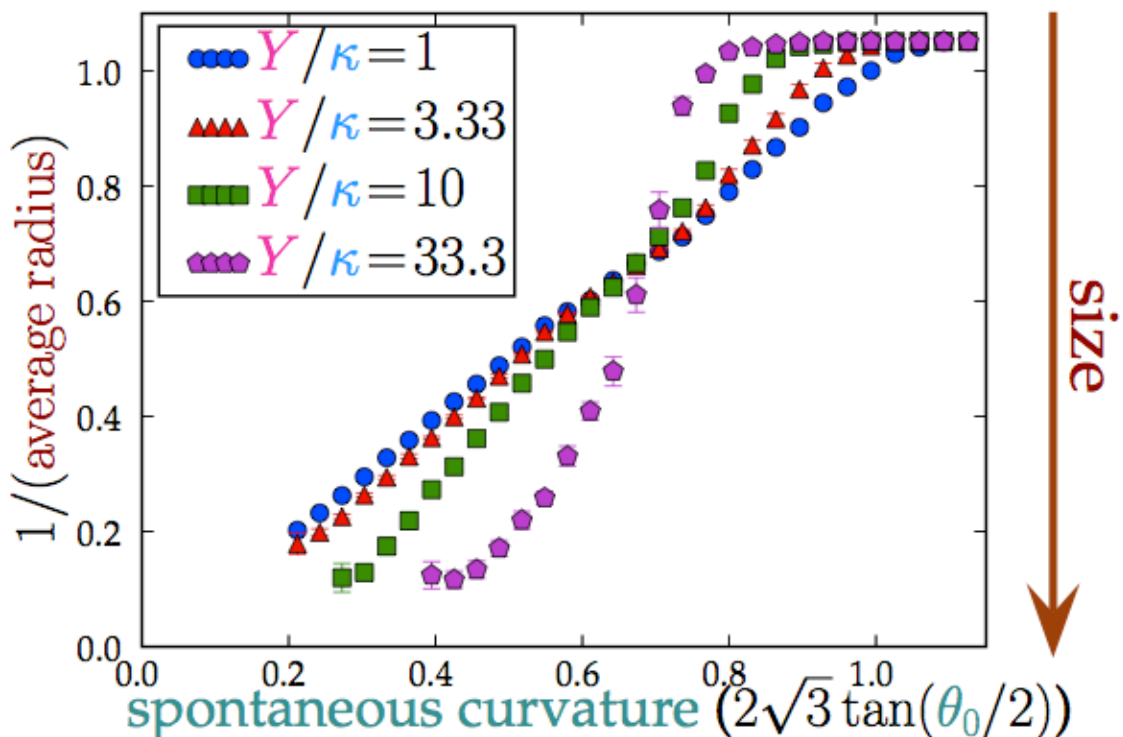
Results: success

Roughly, there is <1% chance per step to make a mistake



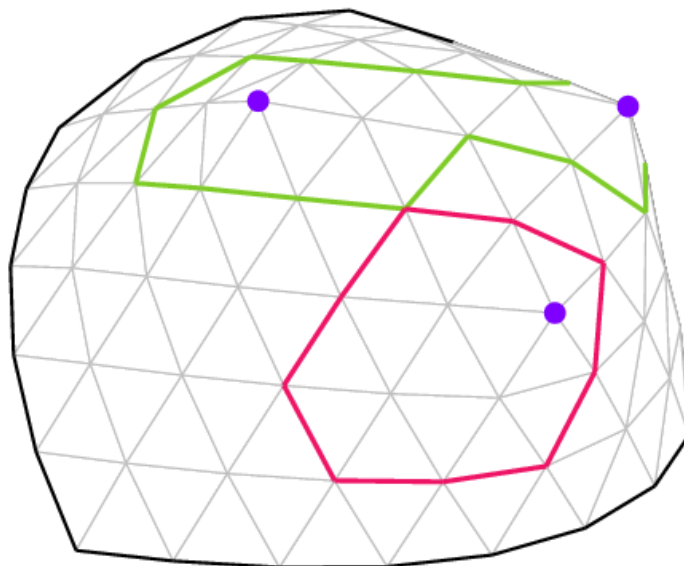
Results: size

The spontaneous dihedral angle sets a radius in the obvious way. Results are close: non-equilibrium makes little difference to this. (exception: strongly faceted regime where bending stiffness is tiny)



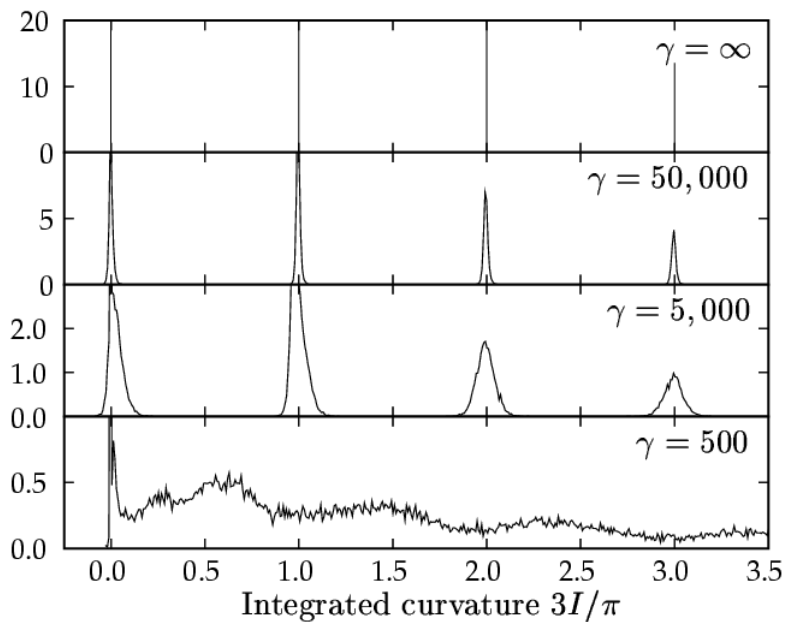
Results: shape

- Given any loop on the capsid surface, calculate enclosed Gaussian curvature using data only on that loop. Here, **path 1** encloses one pentamer, total enclosed curvature is $\approx (\pi/3)$. **Path 2** encloses 2 pentamers, etc.



Shape (continued)

In faceted capsids curvature is concentrated near pentamers



Given triangulated mesh (e.g from cryo-EM tomography), one can tighten these loops to “lassoo” locations of pentamers (even w/o single unit resolution)

Shape (IPR)

Inverse participation ratio (borrowed from localization)

of local curvature, another metric characterizing facetedness.

(D. Nelson metrics don't distinguish Overall non-sphericity vs faceting).

Conclusions (growth model)

- Our trimer-based model with irreversible growth steps can grow closed capsids, with the right parameter choices
- With trimer units (e.g. poliovirus) "insertion" step (= cooperative binding) seems necessary
- Capsid size depends mainly on Spontaneous curvature
- Growth of large ($T > 3$) capsids fails less if larger bending stiffness. (note maturation steps in which virus gets more faceted, e.g. phage HK97)
- Success probability decays exponentially with capsid size.

4. HIV capsids: overview

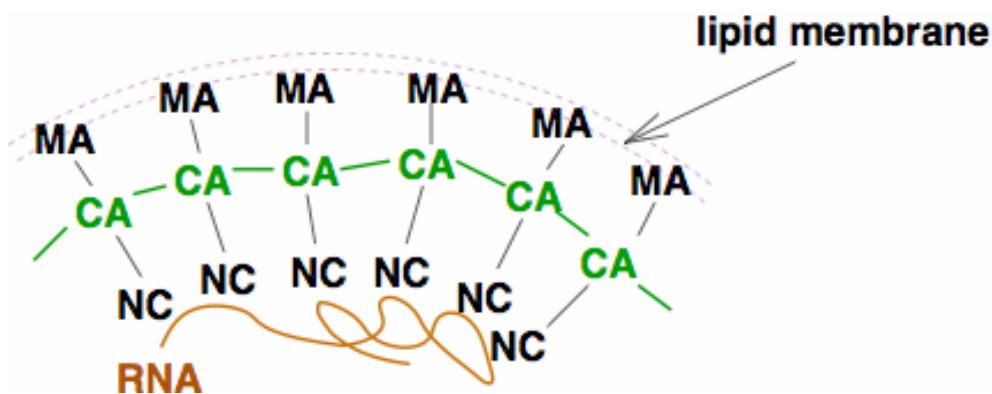
e.g. RSV (Rouse Sarcoma) or HIV.
Single-strand RNA, encapsulated
(thanks to Vogt group, Cornell):

- Cartoon of retrovirus assembly
- Why weird cone shape?

Immature capsid

Gene product Gag=MA+CA+NC

- MA (“matrix”) likes membrane
- CA (“capsid”) forms triang. Lattice
- NC (“nucleocapsid”) likes RNA



Spherical shells of Gag:

- Don't always need lipid
- Any RNA will do (not too short)
- RNA spread in thin layer under Gag layer and membrane

Cleavage step

- Protease cuts MA/CA/NC apart
- CA dissolves(?) into interior
- NC stays bound to RNA,
condenses in interior
- MA stays on membrane (forget it)

Mature capsid

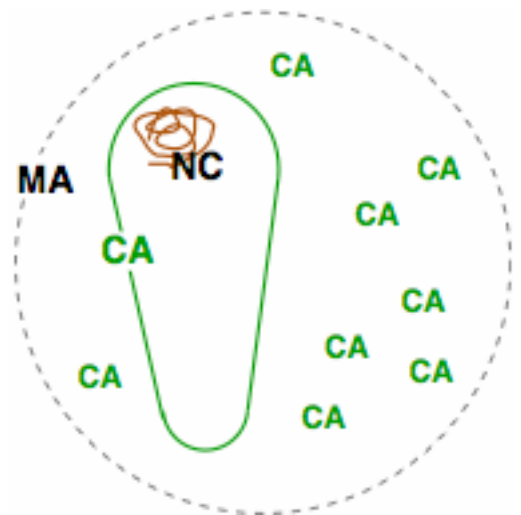
- CA forms non-spherical capsid (“cone”) inside membrane
- HIV case: large and small round caps, cone in between

Only ~30% (1500/4500) of CA used in cone

Sometimes a 2nd cone (rarely, a 3rd)

(Briggs et al, 2004)

RNA just 1% capsid volume:
interior not pressurized
(contrast phages: ~50%)

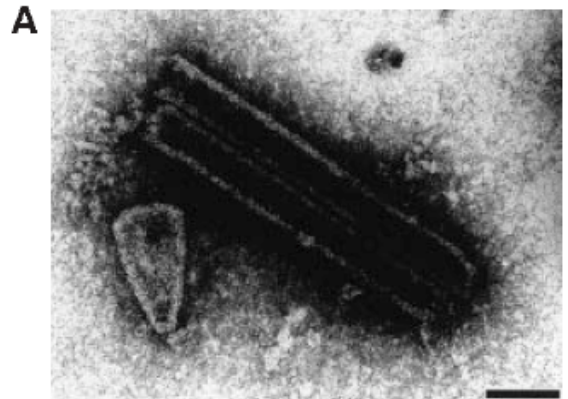


HIV capsids

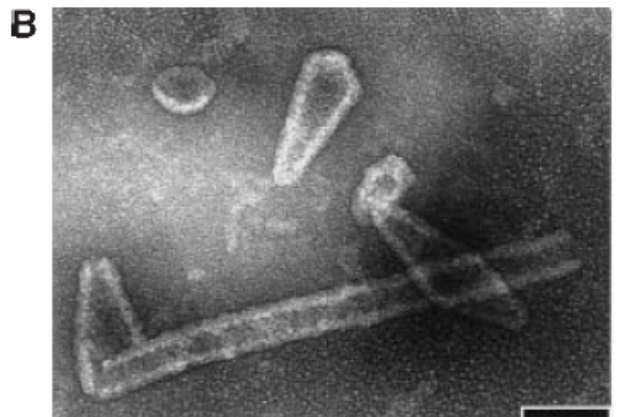
Ganser et al, Science 283, 80 (1999).

Assembly in vitro w/o lipid membrane

A: CA-NC with
random (TMV) RNA

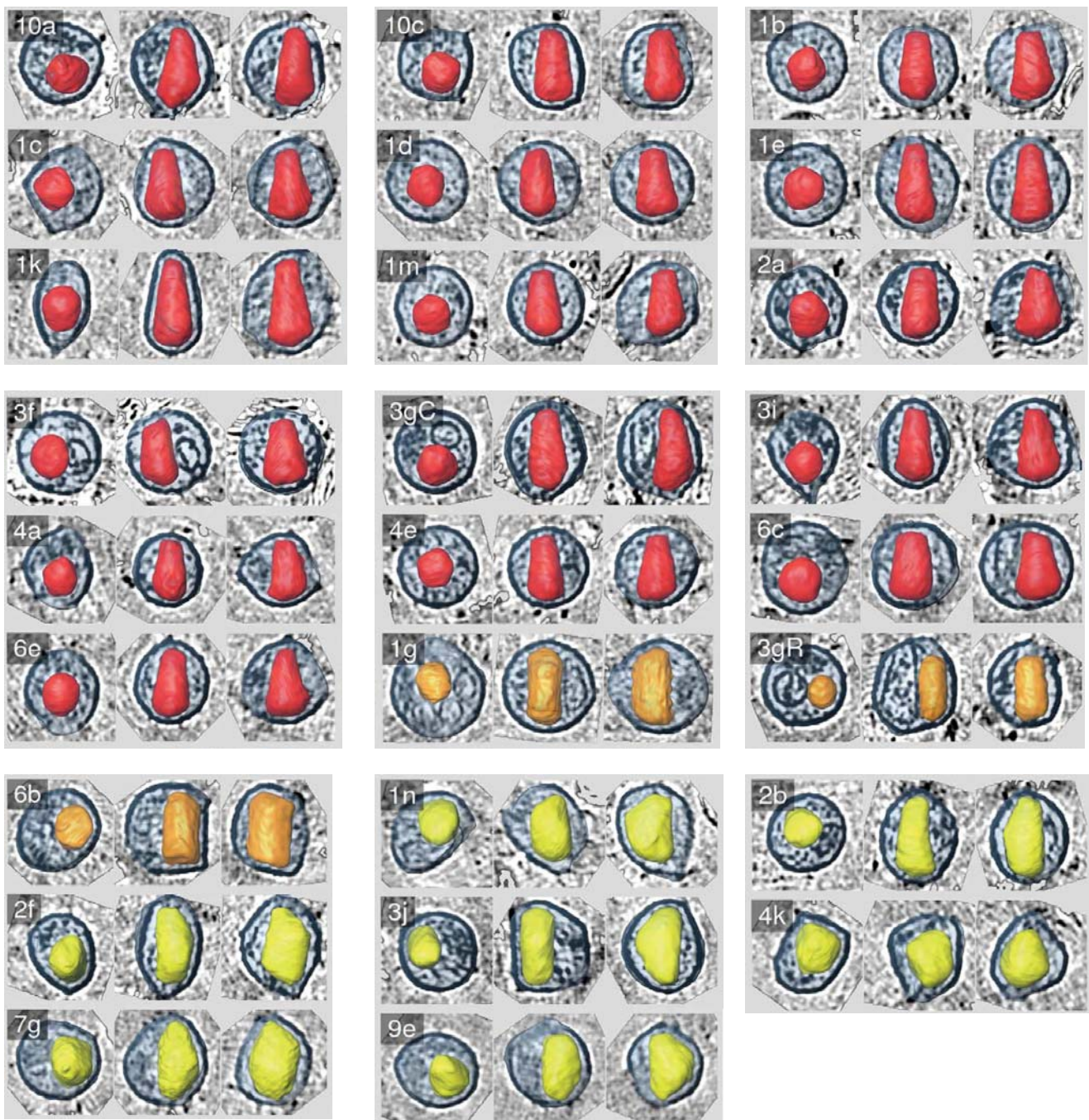


B: CA-NC w/o RNA
but huge (1 M) [NaCl]



Cryo-EM tomography of HIV capsids

Benjamin et al, J. Mol. Bio. 346, 577 (2005)
[Jensen lab]



Why HIV cones?

T. Nguyen: equilibrium model

Coexistence of 2 preferred radii

But: need fine-tuning of CA number

Our fantasy:

- nucleate on RNA at pointy end (catalyzing 5's while in contact);
- deplete [CA] => "joining" likelier (i.e. 5's) => fat end closes.

However: a. RNA is at the fat end,
b. [CA] is depleted only 30%.

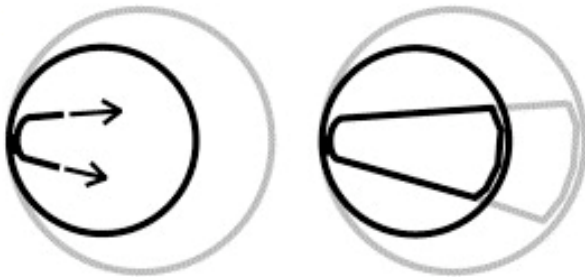
Jordan et al 2005 (Jensen lab):

- Starts along the membrane (fat end) (MA-CA interact?)
- Grow till sides converge.

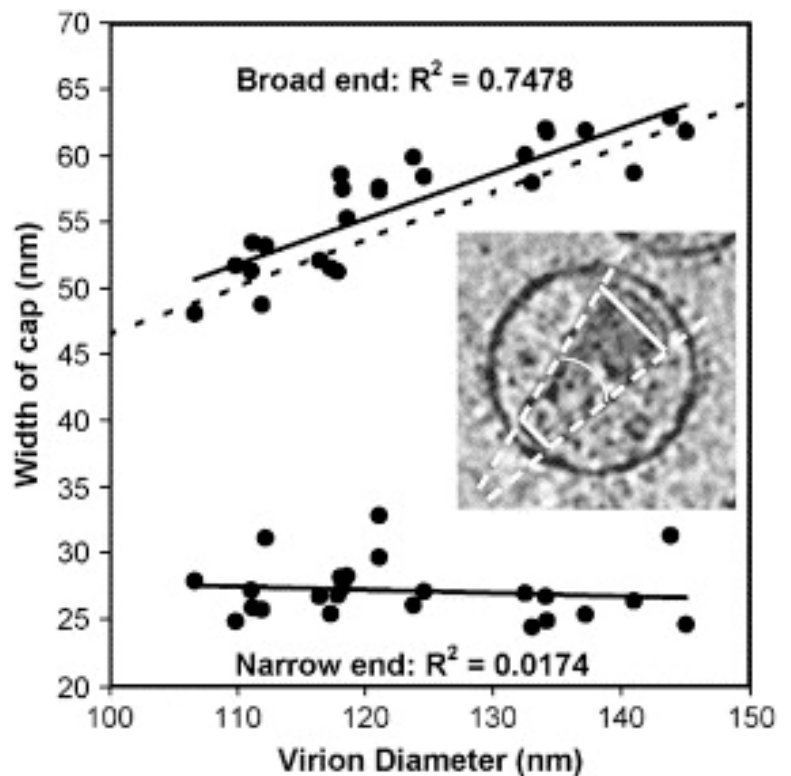
Briggs et al [Structure 14,15(2006)]:

Small end 1st (fixed radius)

-- then limited by membrane.



Observations are consistent:

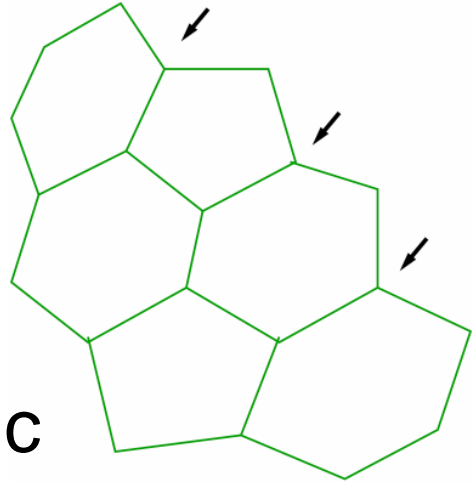


5. New directions (our simulations)

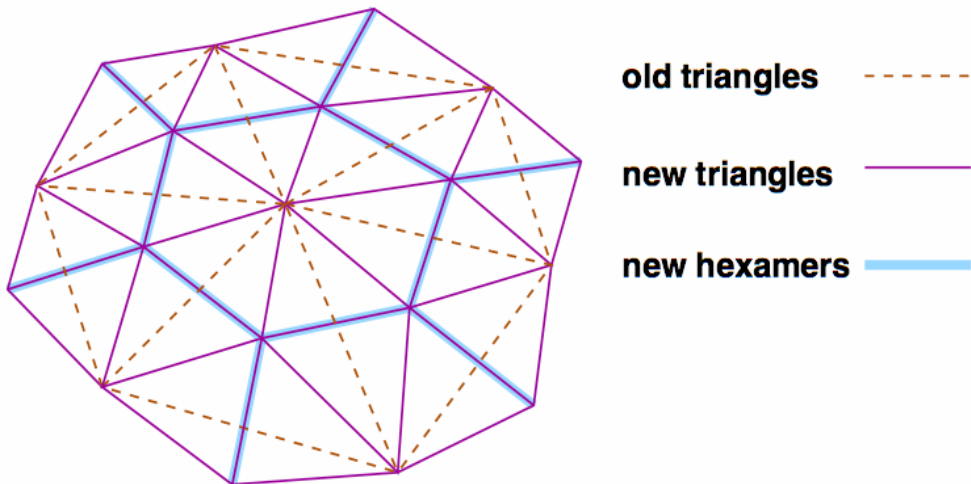
- Improvements in growth model
- Study state space (equil. models)
 - cost of small deviations from icosahedral placement of 5's?
 - annealing of network after initial formation?
- Connect parameters to (more) microscopic protein interactions
- Symmetric ($T > 3$) assembly tricks (scaffolding, non harmonic bending potential)

Improved model: growth?

Not universal: dual net behaves differently



No longer need magic bias for insert/join.
Just accrete at any 2/3 filled spot . (Still need pentamer/hexamer ratio)

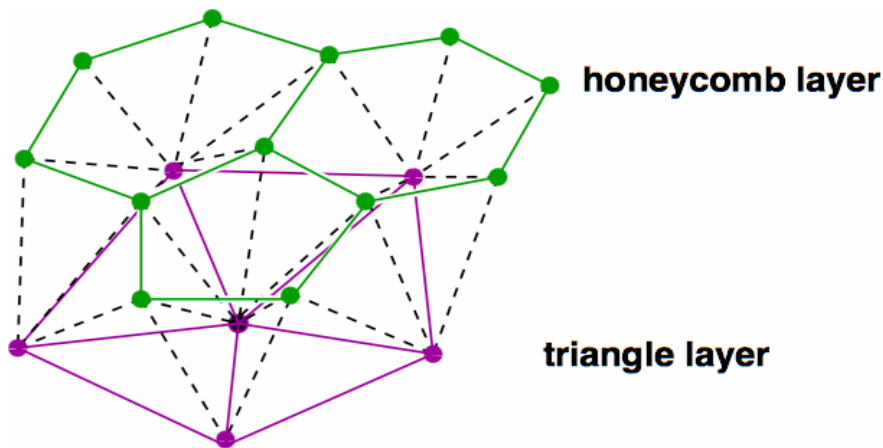


Improved model: elastic?

How generalize bending elasticity from triangle model?

Connect to more microscopic interactions a la Rapoport?

Our version: “masts” erected over dual lattice; i.e. 3D triangulation.



Simplification: make each protein a triangle (generically is trapezoid).

Corollary: now $\ell_f < \text{mast height}$ (old model allowed unphysically large bending stiffness)

State space of capsids

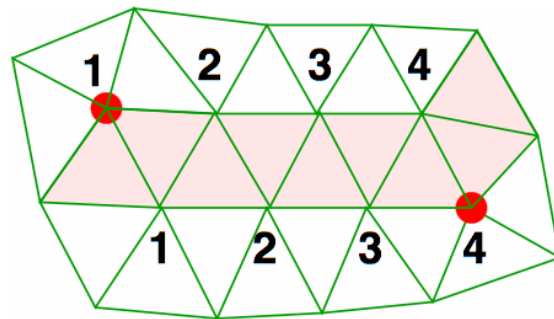
Analytical/mathematical aspect:
given “moves of the game”, can
you get there from here? How to
enumerate/classify valid shells?

Recall: (closed) capsid network is
fully specified by locations of the
5's (disclinations) between domains
of triangular lattice.

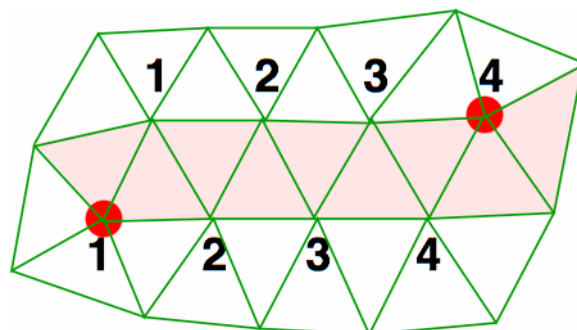
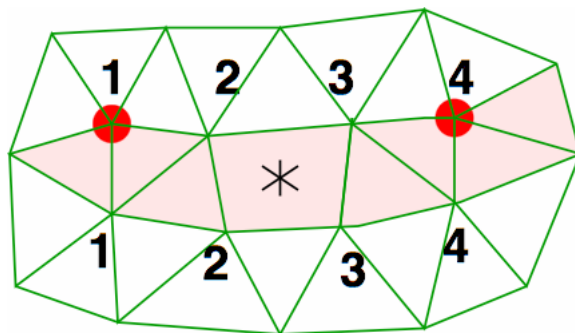
Moving 5's around?

Can't move just one while keeping triangulation with 5 and 6 vertices.

This way (sliding a strip) only move that conserves no. of triangles.



Intermediate state has dislocation.



Wish list for experiments

- General: measurements of atypical/defective capsids shed the most light on assembly mechanism!
- HIV: does immature CA-CA have different binding sites than mature?
- Immature net fully dissolved before mature cone forms?
- Which end of cone is first?
- Are there rearrangements after net is formed? (does CA in cone exchange w/CA in fluid?)
- Are there small holes in net? (what can get through?)