Viruses

7.88J The Protein Folding Problem

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Viruses

- Are parasites: require a host to survive
- All living organisms (animals, plants, bacteria) have viruses
- Occupy the “gray area” between living and non-living organisms
- Can “sleep” for years outside or inside their host cells

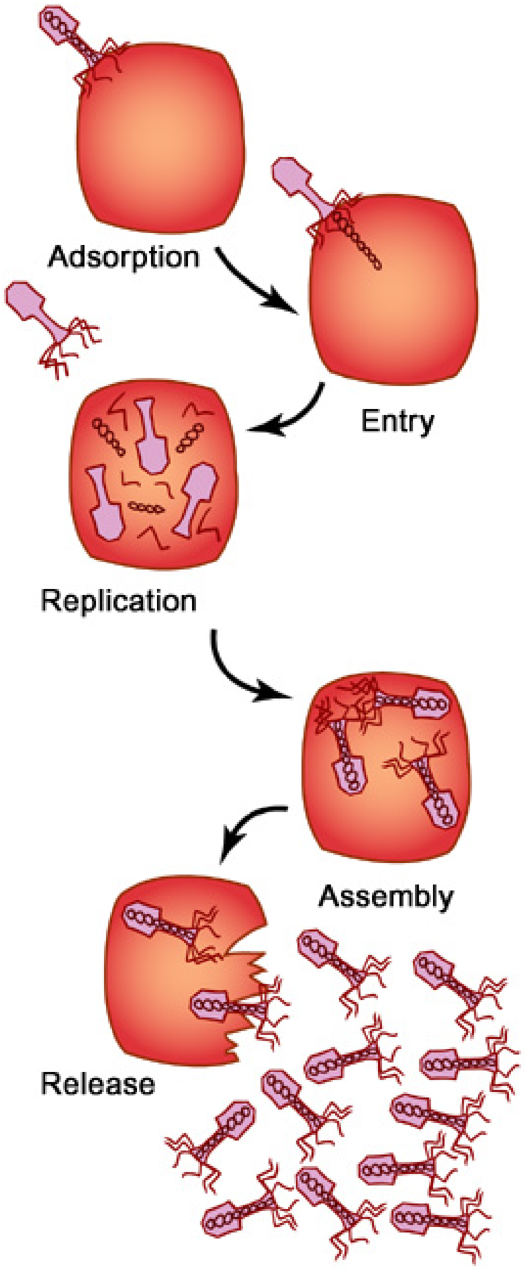
Human Viruses

- Influenza
- Chickenpox
- Smallpox
- Polio
- Herpes
- Hepatitis C
  - In 1999, **170m + 3m/yr**
    - > 80% chronic infection of the liver
      - Cirrhosis, fibrosis and cancer
  - Kills 500,000 /yr
- Human Immunodeficiency Virus (AIDS)
  - In 1999, **42m**
  - Kills 3m /yr

- Enter through nose, mouth, breaks in the skin, body fluids, etc.

http://www.economist.com/science/displayStory.cfm?story_id=2173183

Virus Life Cycle



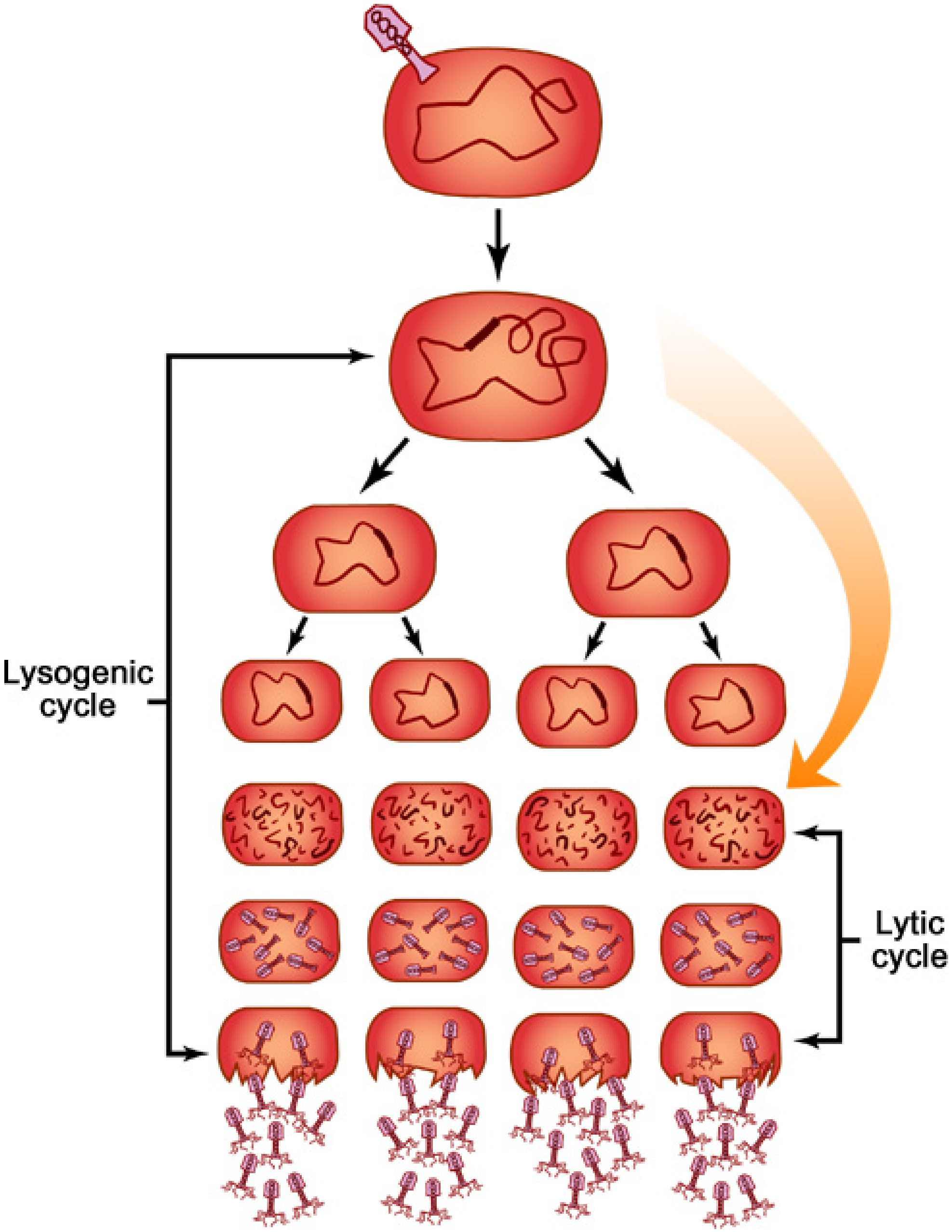
- All viruses require a host cell to replicate
- Follow same basic pattern
  - Deliver virus' genomic material into host cell
  - Subvert cell's biosynthetic machinery into producing new viral particles
  - New virus particles self-assemble in the infected cell
  - New virus particles leave infected cell to infect fresh host cells
    - Lysis – host cell destroyed
    - Budding – host cell not destroyed

Example: Common Cold Virus

- Enters through nose, attaches to cells lining sinuses
- Attacks cells, rapidly reproduces
- Host cells lyse, virus spreads to bloodstream and lungs
  - Fluid flows into nasal passages: runny nose
  - Viruses in fluid attacks cells lining throat: sore throat
  - Viruses in bloodstream attack muscle cells: aches
- Immune system releases pyrogens
  - Body temperature increases
  - Viral reproductive rate decreases

Lysogenic Cycle

- Herpes, HIV do not reproduce immediately
  - Mix their genetic material with that of host
  - “Sleep” through many rounds of reproduction
  - Await some environmental or predetermined genetic signal

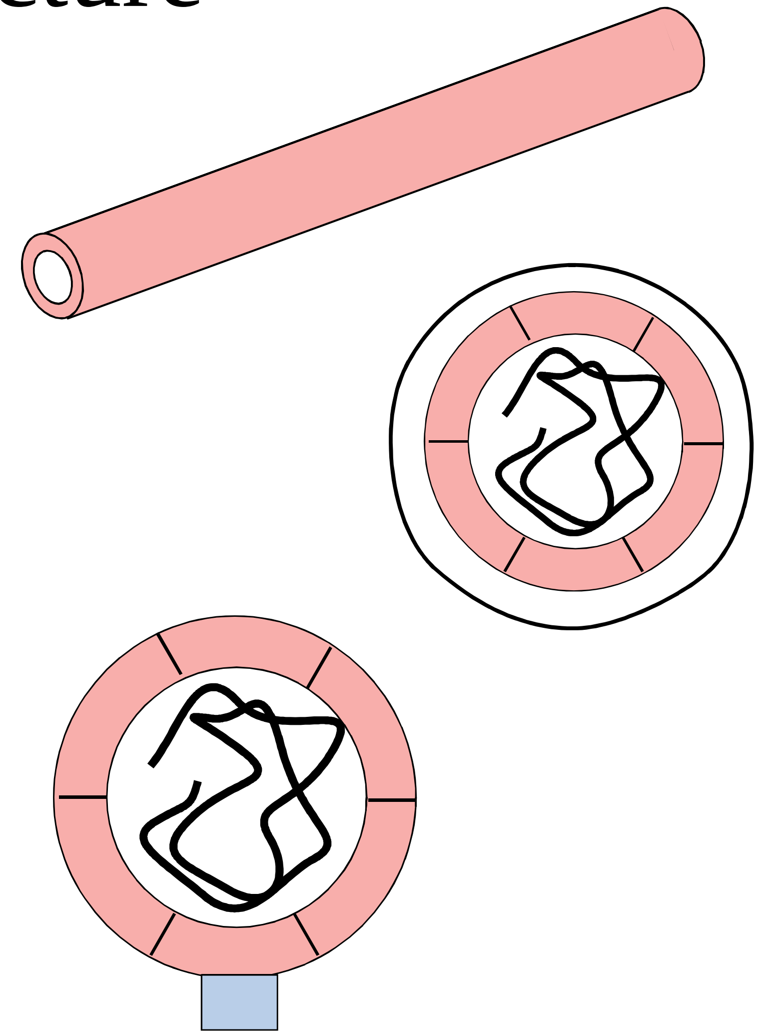


Good News & Bad News

- Viruses kept in check by
  - Limited “host range”
  - Host defense mechanisms

- Viruses evolve very rapidly

Virus Structure



- Size
  - 17 nm – 3000 nm diameter
- Basic shape
  - Rod-like
  - “Spherical”
- Protective Shell - Capsid
  - Made of many identical subunits
  - Symmetrically organized
  - 50% of weight
  - Enveloped or non-enveloped
- Genomic material
  - DNA or RNA
  - Single- or double-stranded
  - No unique structure

Virus Structure

- All viruses have some mechanism for
  - Host Recognition
    - Some kind of protein on coat or envelope that “feels” or “recognizes” proper host cells
  - Genomic Material Delivery
    - Enveloped: cell fusion event
    - Non-enveloped: more complex strategies & specialized structures

X-ray Crystallography of Viruses

- Symmetry of protein shells makes them uniquely well-suited to crystallographic methods

- Viruses are the largest aggregates of biological macromolecules whose structures have been determined at high resolution

History

- In 1953, Crick & Watson proposed … principles of virus structure
  - Key insight:
    - Limited volume of virion capsid => nucleic acid sufficient to code for only a few sorts of proteins of limited size
  - Conclusion:
    - Identical subunits in identical environments
    - Icosahedral, dodecahedral symmetry

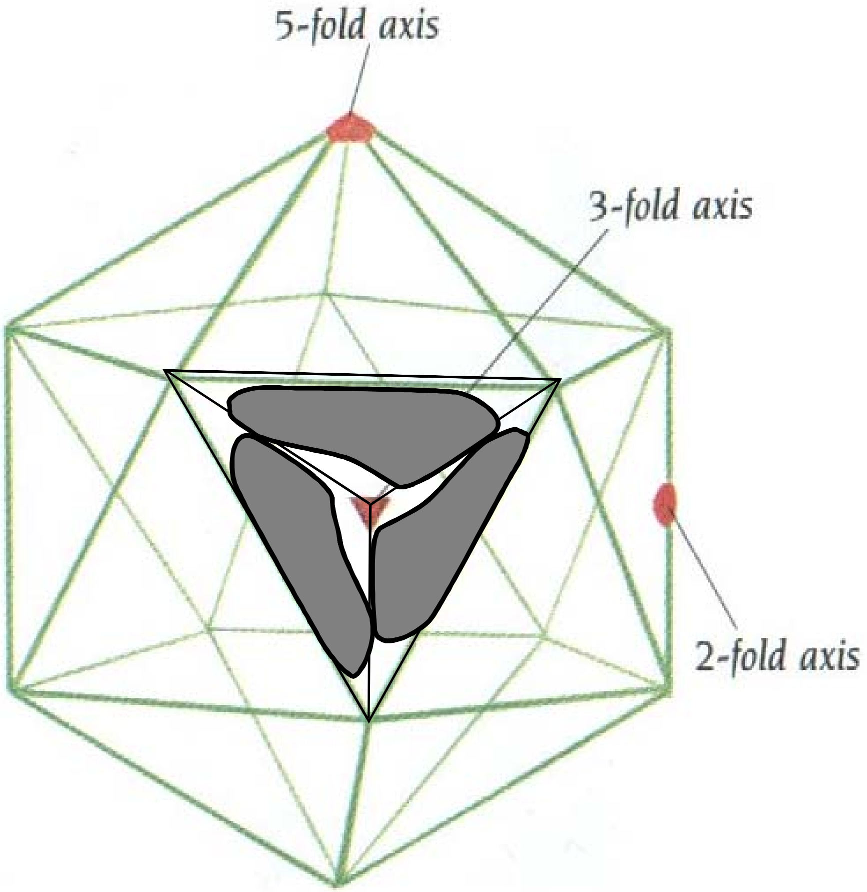
History con’t

- In 50’s & 60’s Klug and others confirmed that several (unrelated) “spherical” viruses had icosahedral symmetry
  - (Used negative staining & electron microscopy)

- Conclusion:
  - Icosahedral symmetry is preferred in virus structure

Icosahedral Symmetry

- 12 vertices
- 20 faces
  (equilateral triangles)
- 5-3-2 symmetry axes
- 60 identical* subunits
  in identical environments
  can form icosahedral shell
  * asymmetric



But …

- Clear evolutionary pressure to make larger capsid
  - Using larger subunits helps very little
  - Using more subunits helps a lot

- Not possible to form icosahedral shell (of identical units in identical environments) with more than 60 subunits

- Viruses with more than 60 subinits were observed

- Question:
  - How can >60 subunits form an icosahedral shell?
  - Will any number of subunits work?
  - If so, how would they be organized?

Quasi-equivalence

- In 1962, Caspar & Klug proposed the theory of "quasi-equivalence"
  - Not all protein subunits are equivalent
    - "Identical" subunits in slightly different environments
  - Only certain numbers of subunits will work

$n = 60\ T$ where

$T = P\ f^2$

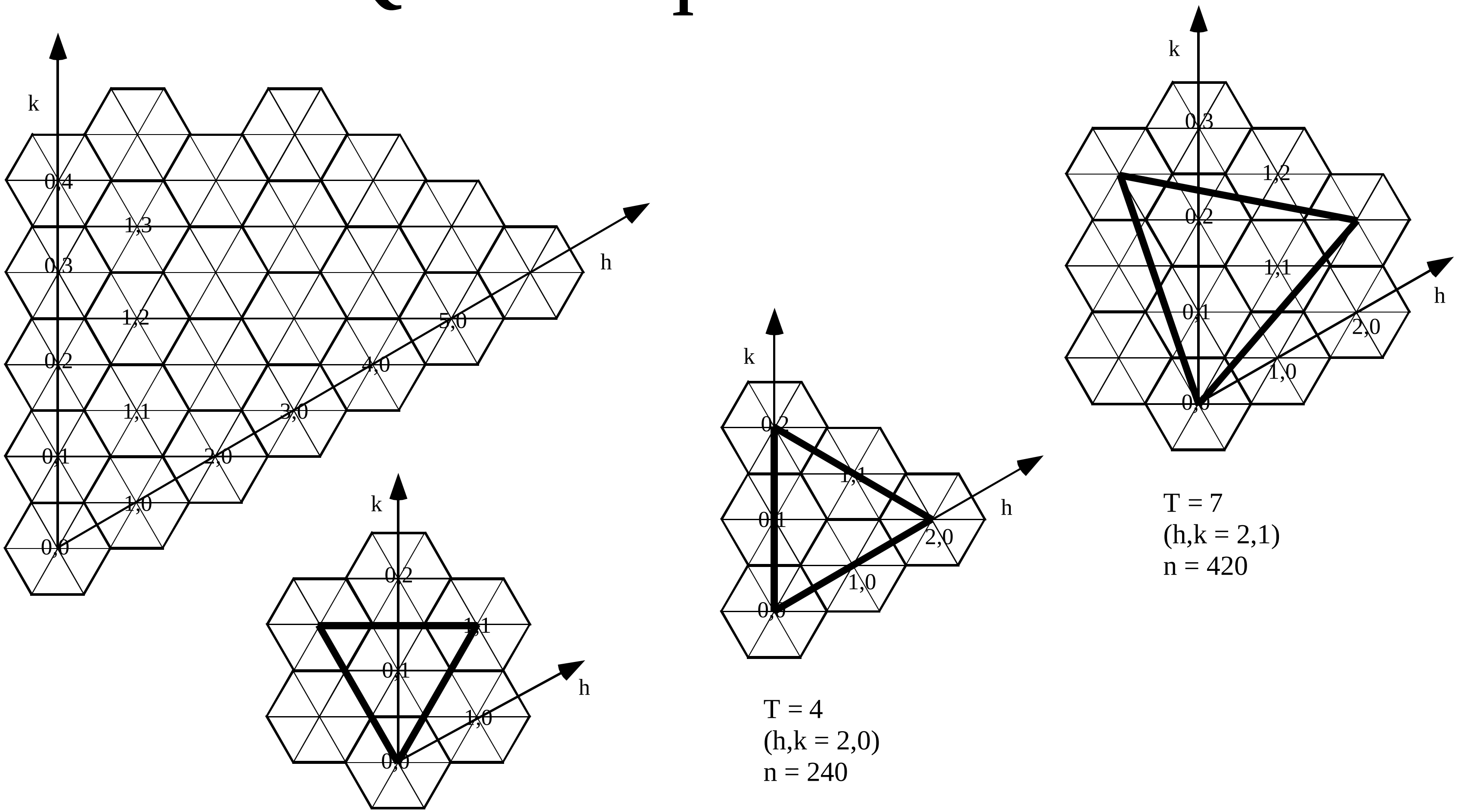
$P = (1, 3, 4, 7, 13, \ldots)$

$= h^2 + hk + k^2$ $\qquad h,k = 1, 2, 3, \ldots$

$f = 1, 2, 3, \ldots$

Caspar & Klug, Cold Spring Harbor, 1962

Quasi-equivalence



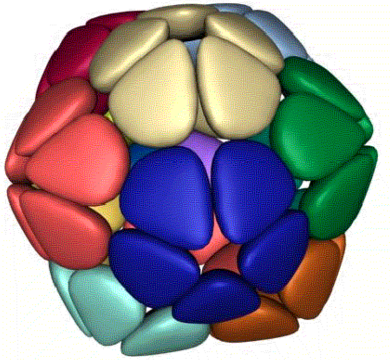
T = 3
(h,k = 1,1)
n = 180

T = 4
(h,k = 2,0)
n = 240

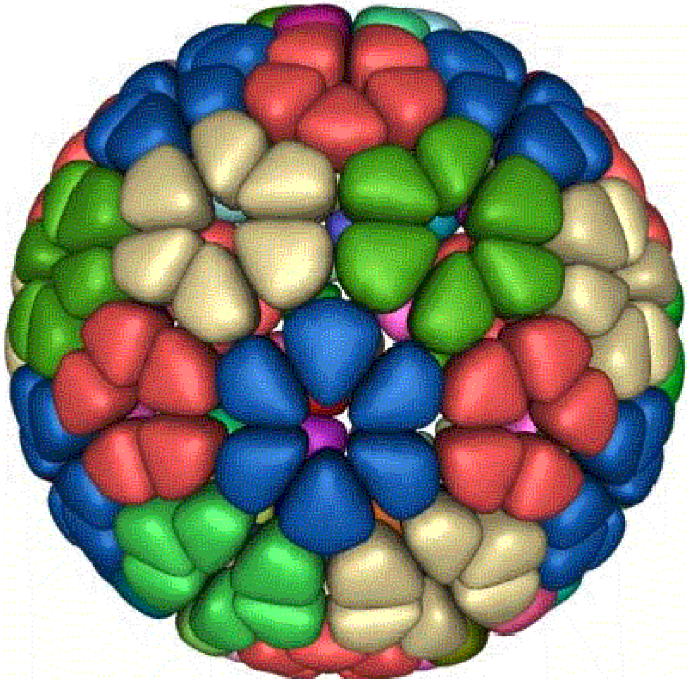
T = 7
(h,k = 2,1)
n = 420

Adapted from Caspar, and Klug. “Principles of icosahedral virus structure,” *Cold Spring Harbor Symp*. 27 (1962).

Quasi-equivalence



- Subunits are in “minimally” different environments
  - Pentamers at vertices
  - Hexamers elsewhere



- Predicts packing arrangements of larger capsids
  - Shift from T1 to T4 packing

  => 8-fold increase in volume

Experimental Confirmation

- The capsids of many (most?) “spherical” viruses exhibit spatial organization consistent with the quasi-equivalence principle

- However, some don’t

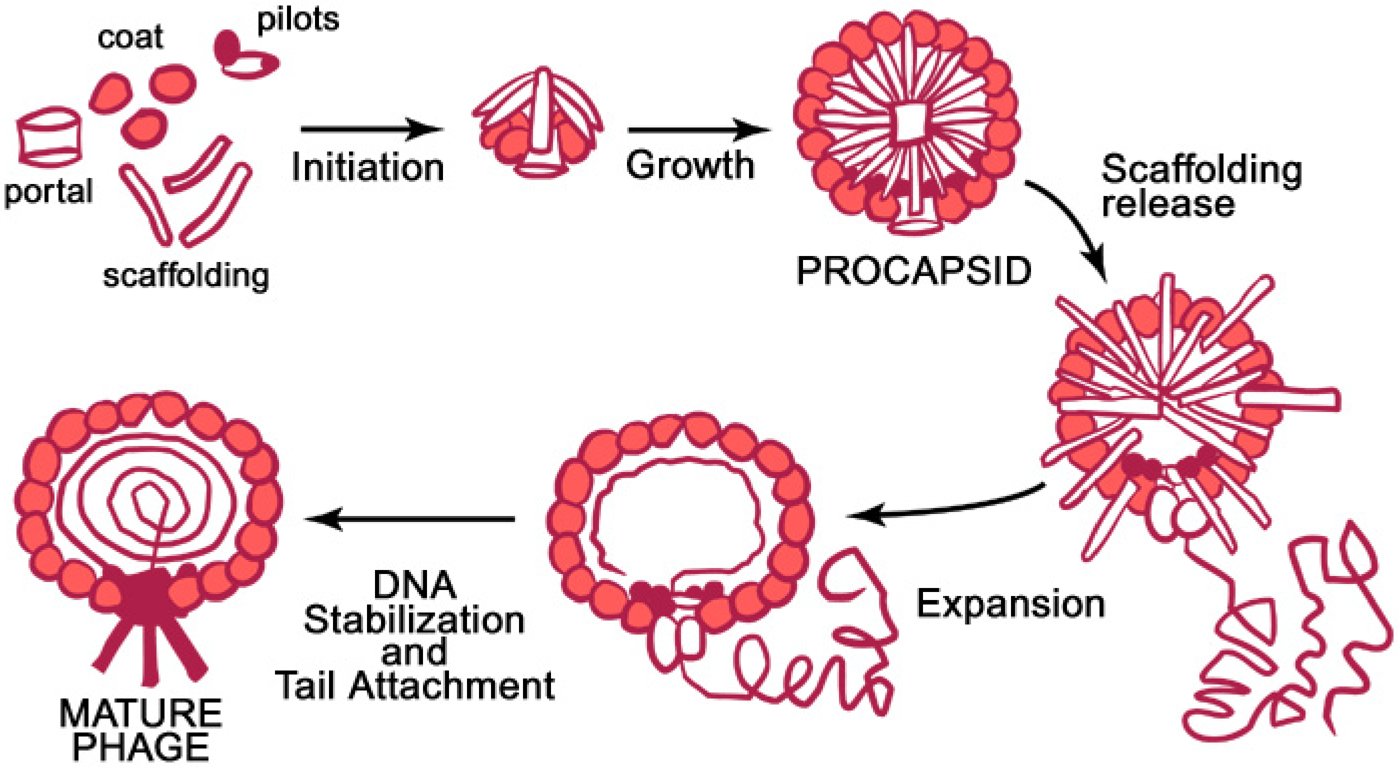
Similarity to Geodesic Domes

- Buckminster Fuller: architect
- An “optimal” structure ?
  - Area/weight
- Widely copied

Prof. King:

- Assembly
- Scaffolding proteins
- Procapsid state
- Maturation transition
- Remainder of pathway
  - Host recognition
  - Genomic material delivery

P22 Pathway


coat
pilots
portal
scaffolding
Initiation
Growth
PROCAPSID
Scaffolding release
Expansion
DNA Stabilization and Tail Attachment
MATURE PHAGE

Maturation Transition

- During / after DNA insertion

- Irreversible
  - Post-transition is a lower energy state

- A conformational change
  - Change in radius
    - Increase ~15% (P22 & HK97)
  - More angular
  - Holes in faces close

Corresponding images may be found in: Jiang, W., et.al. “Coat protein fold and maturation transition of bacteriophage P22 Seen at subnanometer resolutions.” *Nature Structural Biology* (21 January 2003).

Holes in Faces Close

- Skewed hexamers become more regular

Corresponding images may be found in:

Zhang, et.al., “Visualization of the Maturation Transition in Bacteriophage P22 by Electron Crymicroscopy.” *J. Mol.Biol.* 297 (2000): 615-626.

P22 Coat Protein

- Conformation change during maturation transition
- Contains 3 major helices
- Helices change relative orientation

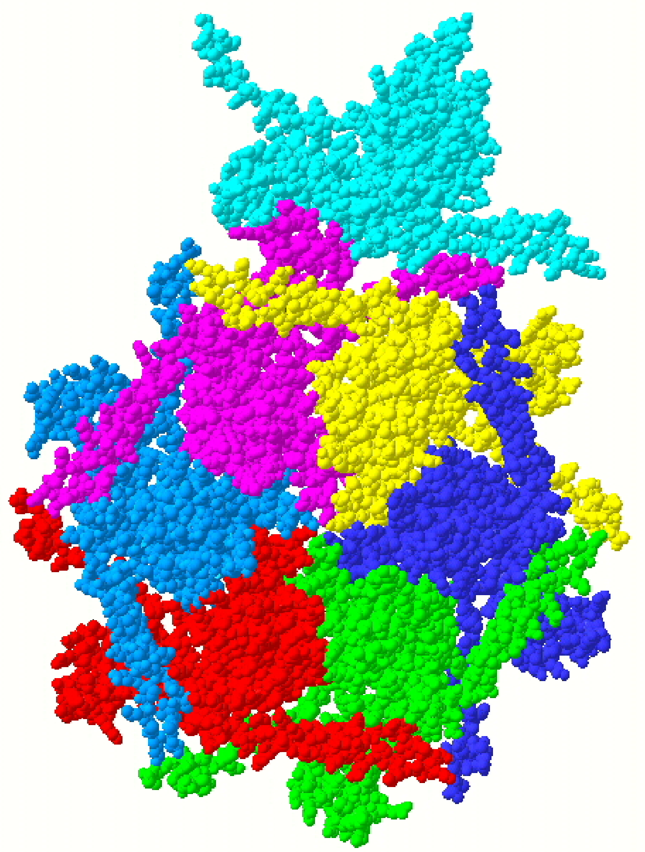
Corresponding images may be found in:
Jiang, W., et.al. “Coat protein fold and maturation transition of bacteriophage P22
seen at subnanometer resolutions.” *Nature Structural Biology* (21 January 2003).

HK97 Asymmetric Unit, Capsid & Size

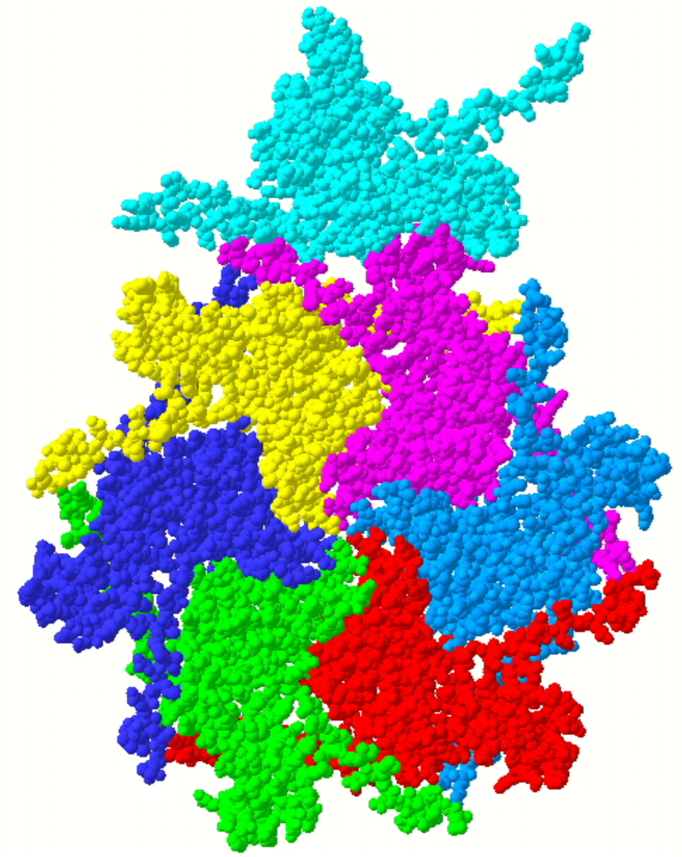
Corresponding images may be found in:

Wikoff, W. R., et.al. “Topologically Linked Protein Rings
in the Bacteriophage HK97 Capsid.” *Science* 289 (2000): 2129-2133.

HK97 Asymmetric Unit



Outside



Inside

HK97 Coat Protein

- 280 residues
  - (104-383)
- Structures
  - Domain A
  - Domain P
  - N-arm
  - E-loop
  - K169, N356

    form isopeptide bonds between subunits
- 3 major helices

Corresponding image may be found in:

Wikoff, W. R., et.al. “Topologically Linked Protein Rings in the Bacteriophage HK97 Capsid.” *Science* 289 (2000): 2129-2133.

HK97 & P22 Capsid Subunits

- Both have 3 major helices (despite < 20% sequence identity)
- Surprising degree of alignment

Corresponding images may be found in:

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But… during Maturation Transition

- In HK97, major helices do not move
  - rigid-body movements of capsid’s ASU’s match electron densities of procapsid

- In P22, major helices undergo significant movement
  - H1 & H2 remain relatively fixed (< 6 ^{o})
  - H3 undergoes a significant rotation (~ 47 ^{o})

Corresponding images may be found in:

Jiang, W., et.al. “Coat protein fold and maturation transition of bacteriophage P22 Seen at subnanometer resolutions.” *Nature Structural Biology* (21 January 2003).

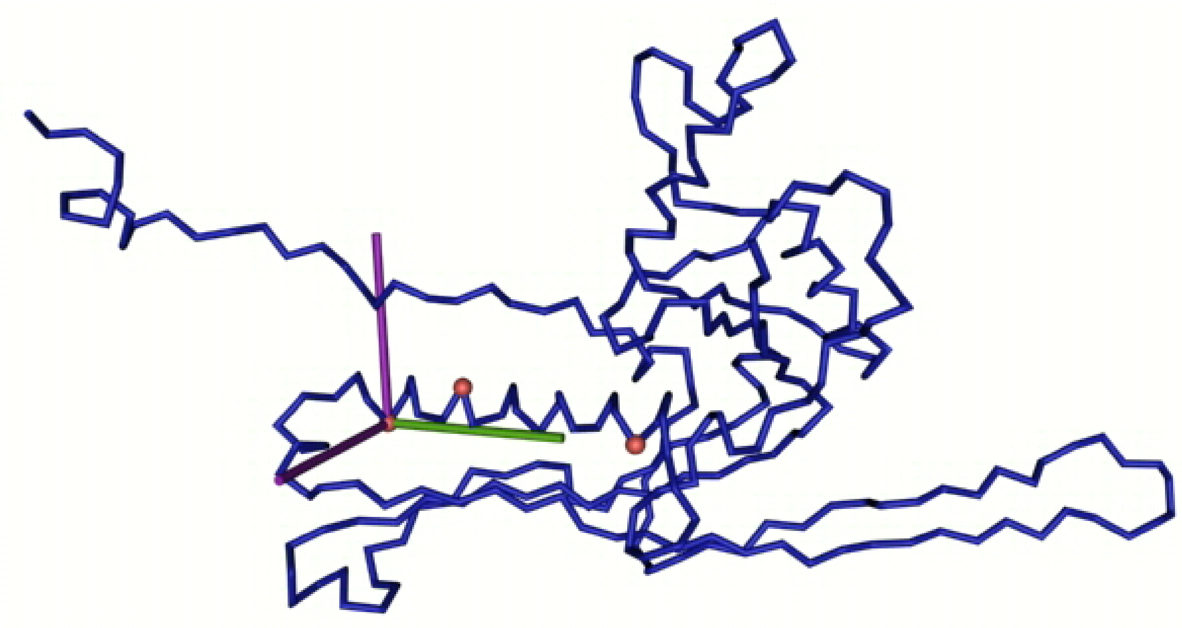
Questions

- How must the original coat protein change conformation in order to form:
  - the procapsid?
  - the capsid?

- During the maturation transition
  - How do the coat proteins change conformation?
  - Do the major helices in the coat proteins play a role?

Analysis of HK97 Capsid Asymmetric Unit (1HF6)

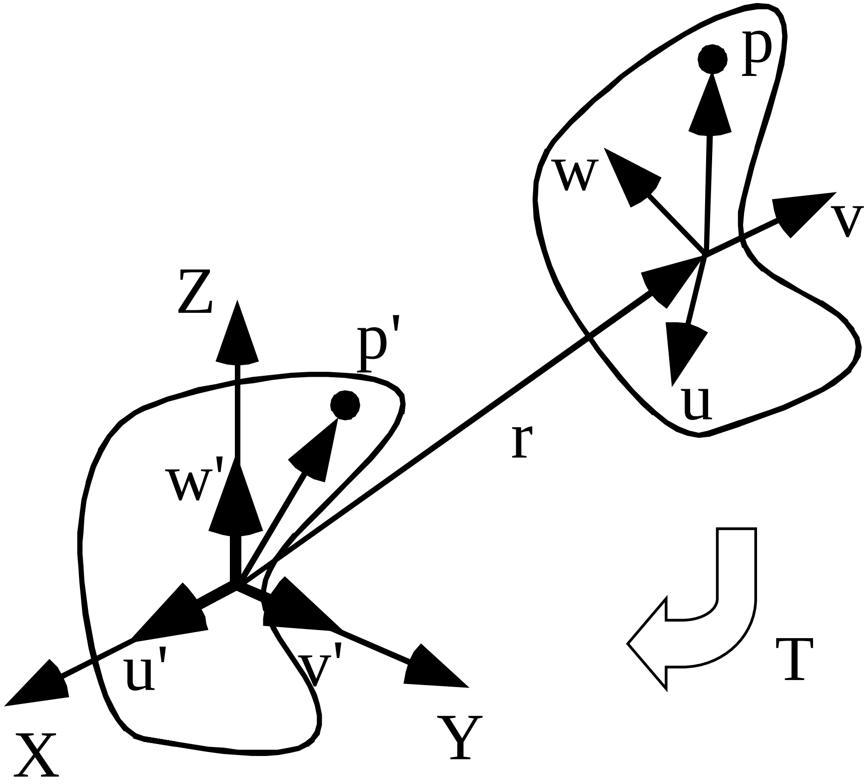
- Assumption: major helices relatively rigid
- Define coordinate system on H1 of each chain
  - key points: C_α 204, 222, 210



Transformation

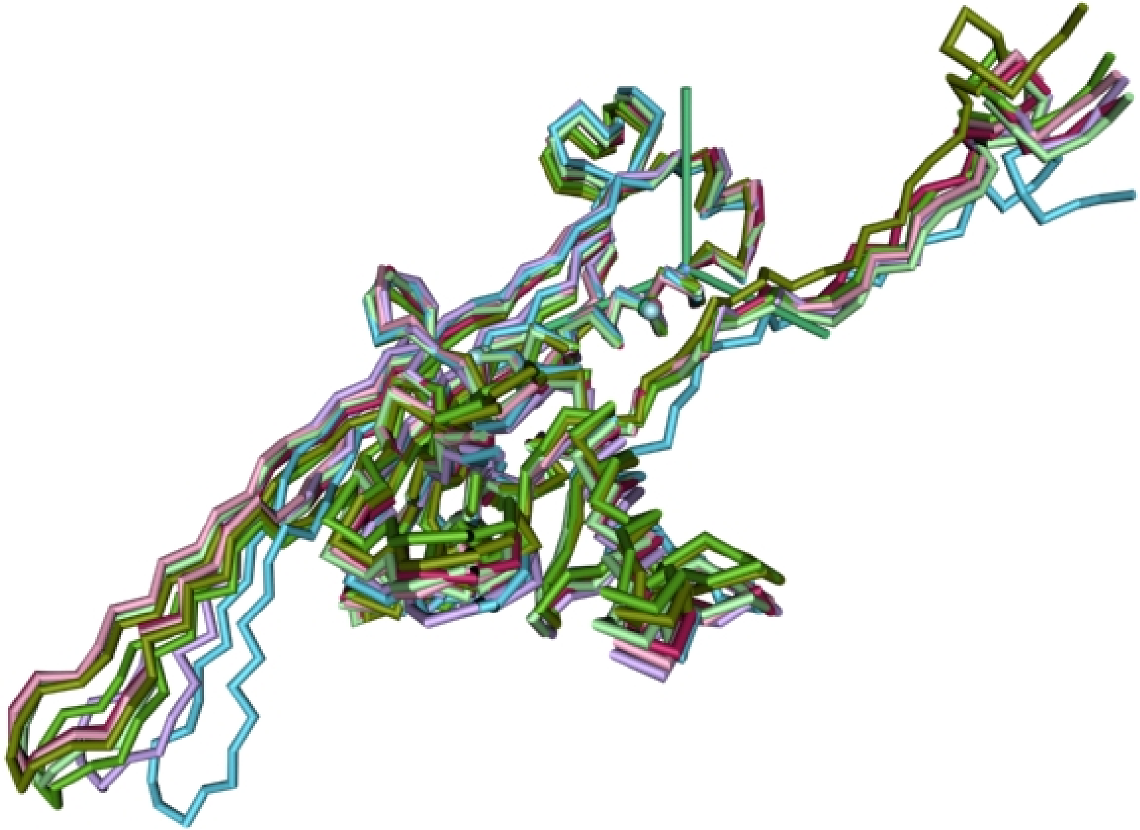
$$T = \begin{bmatrix} u_x & v_x & w_x & r_x \\ u_y & v_y & w_y & r_y \\ u_z & v_z & w_z & r_z \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

$$p = \begin{bmatrix} p_x \\ p_y \\ p_z \\ 1 \end{bmatrix} \qquad p' = Tp$$

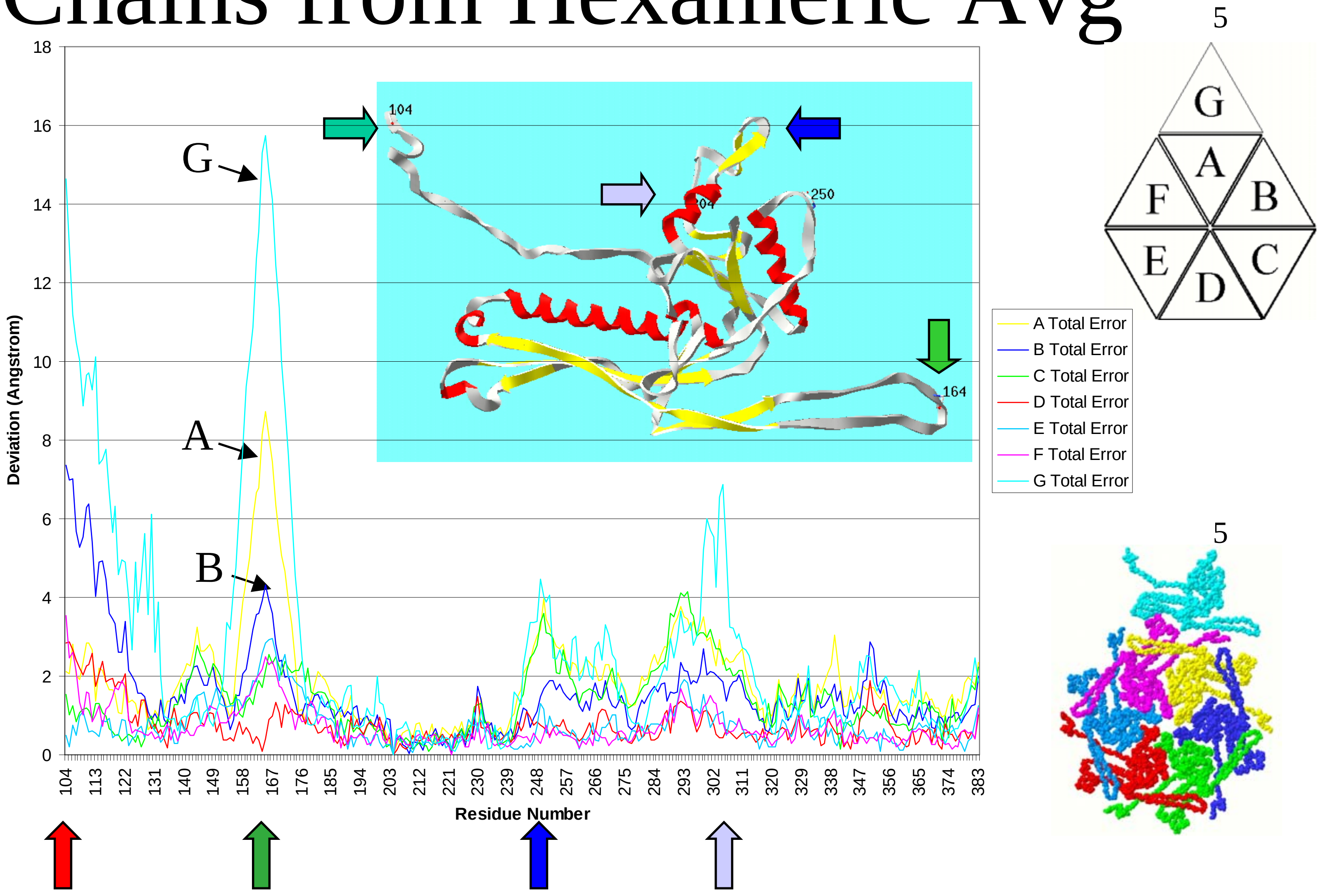


Superposition of Capsid Chains

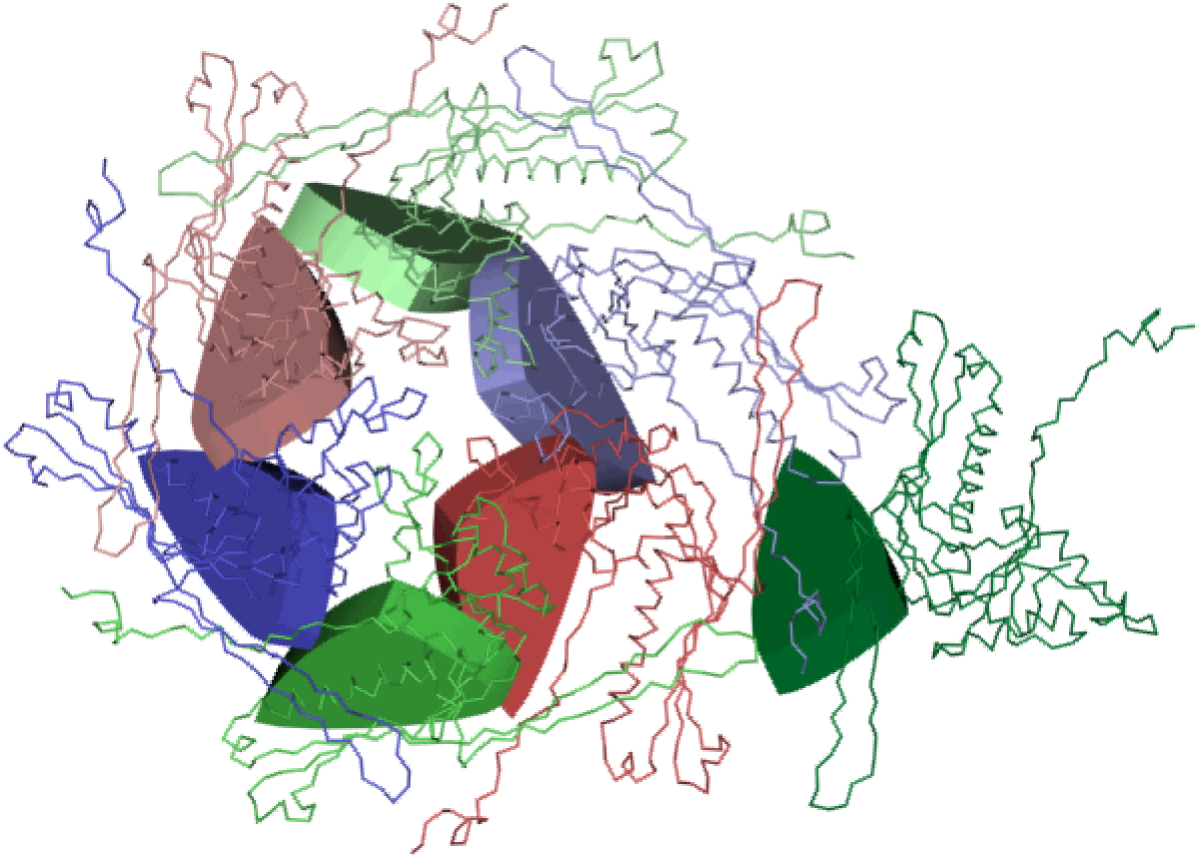
- Reveals how much chains differ
  - “deviation” from a (single) original shape in order to form a closed shell
  - a measure of “the limits of quasi-equivalence”
- Need a reference frame



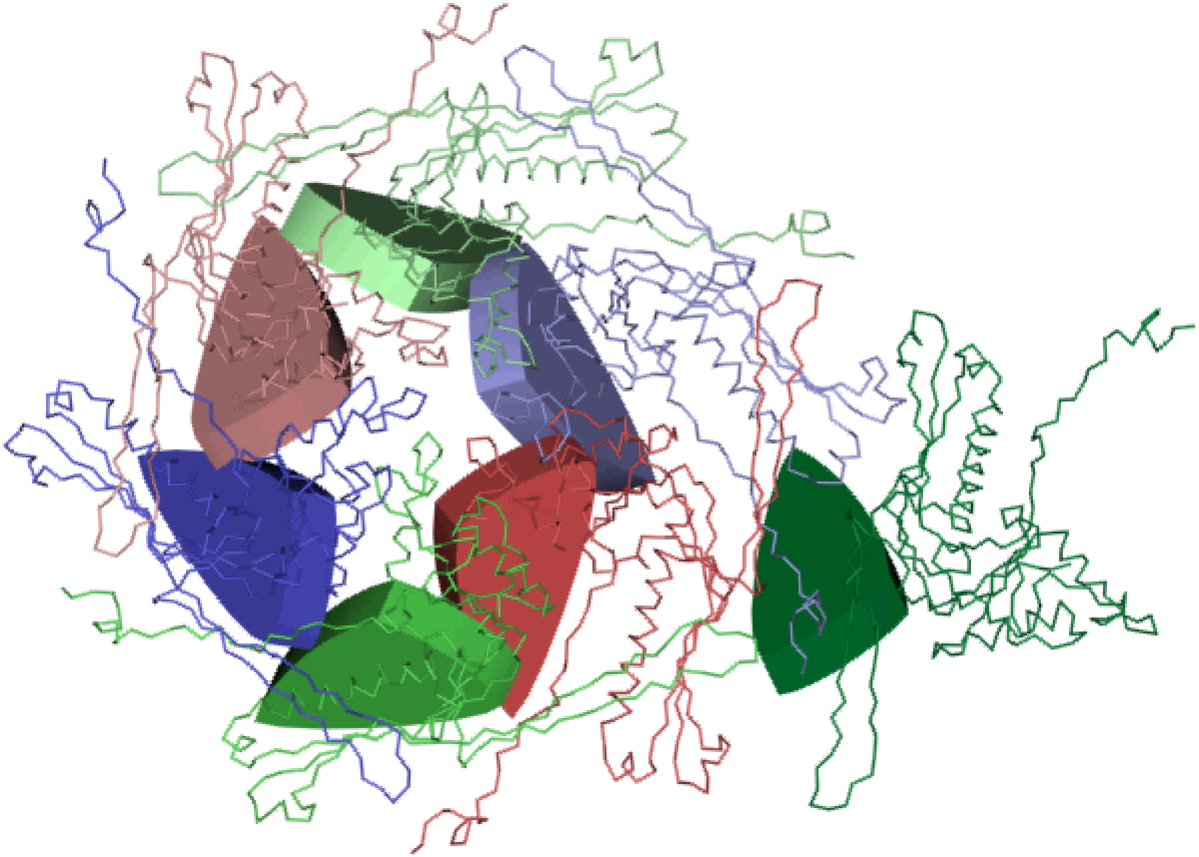
Deviation of HK97 Capsid Chains from Hexameric Avg



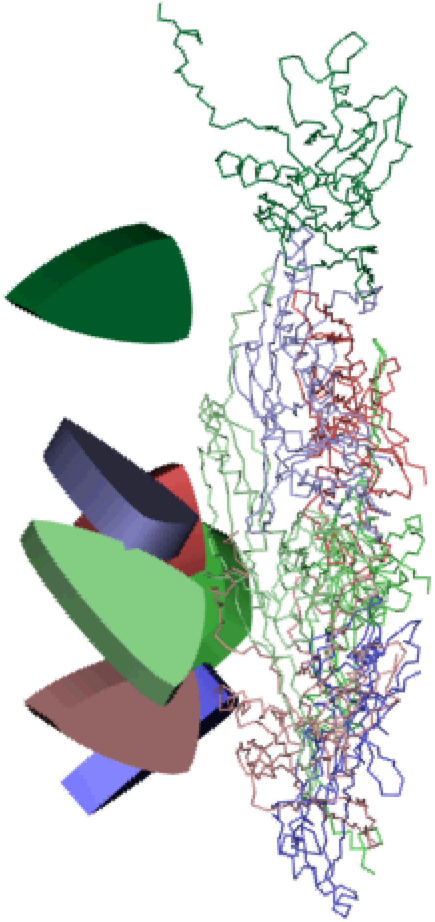
Movement of HK97 Helices during Maturation Transition



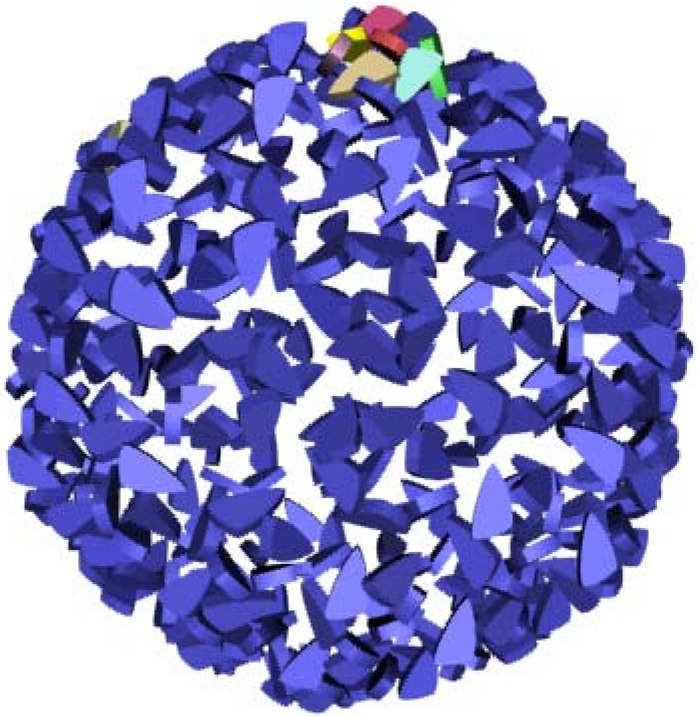
Movement of ASU Volumes during Maturation Transition



Movement of ASU Volumes during Maturation Transition



Simulation of Full Particle



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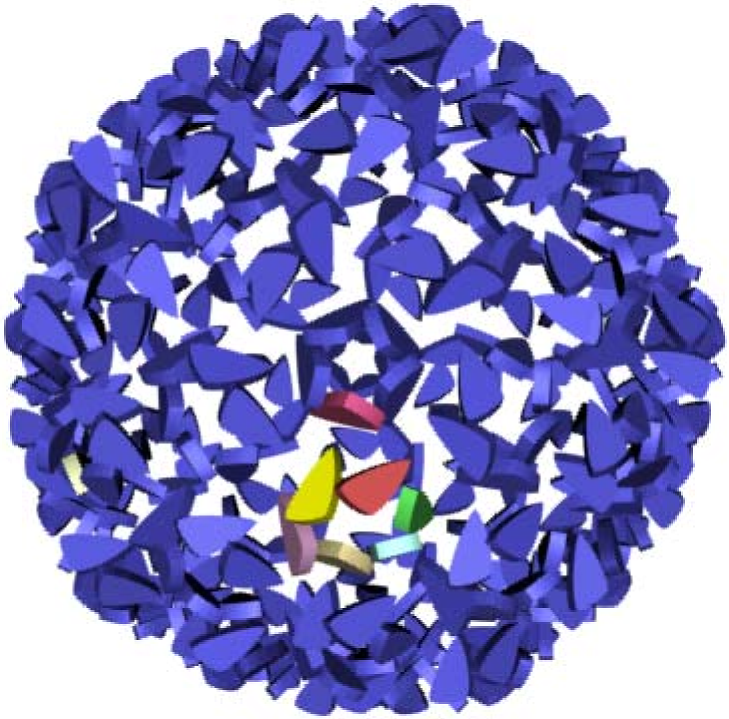

HK97 Contacts

- How do they change <u>during</u> maturation transition ?

[ChimeSCRIPT demo]