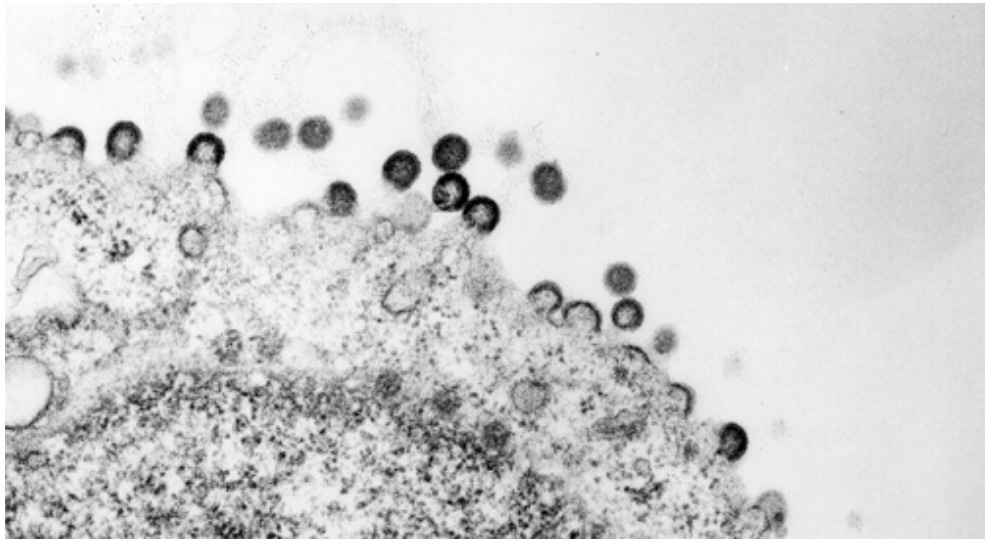
Lecture 3: Sizing Up Viruses

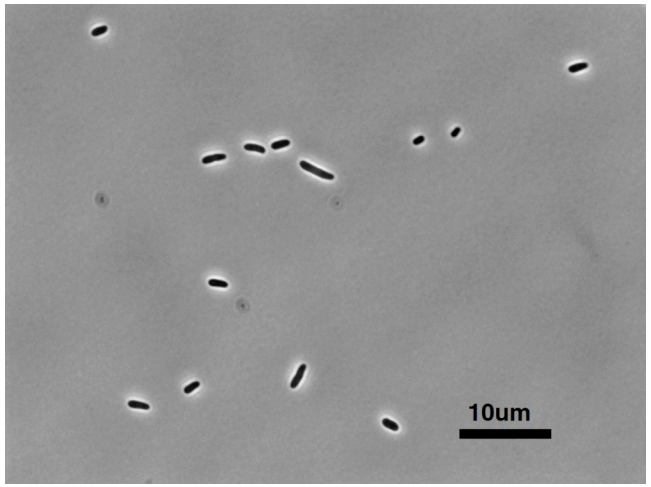
The Parts List of a Virus



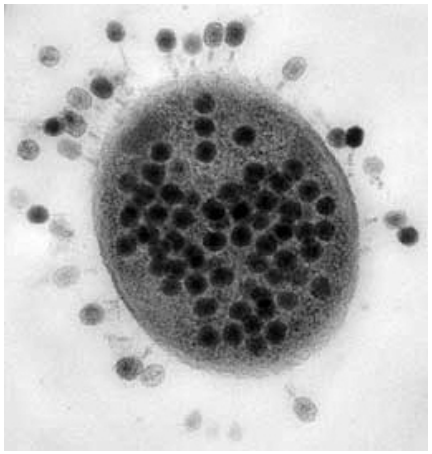
http://faculty.washington.edu/jais/microscopy.html

Cells and the Viruses That Infect Them

Bacteria

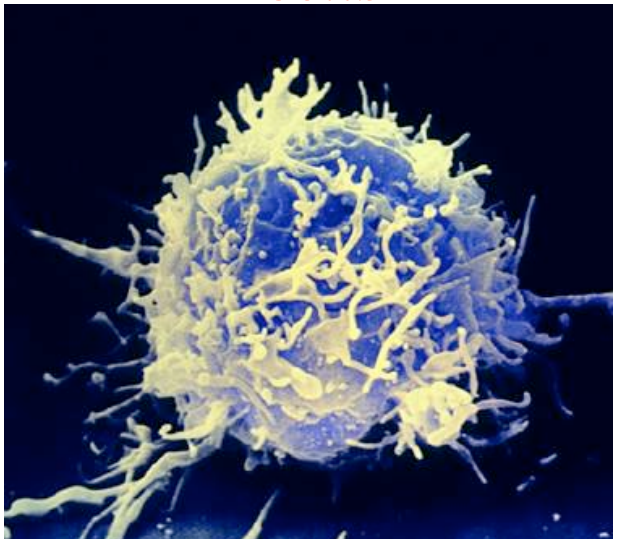


+phage

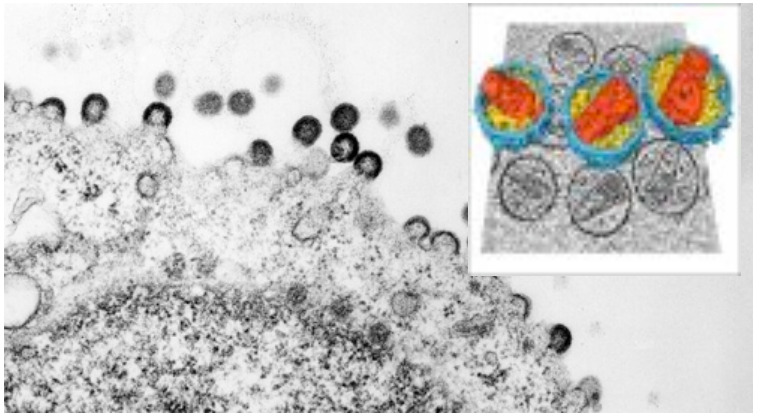


Note: the viral parts list is very small (10-100 pieces) with ger roughly of 10kb. They accomplish so much with so little.

T cells



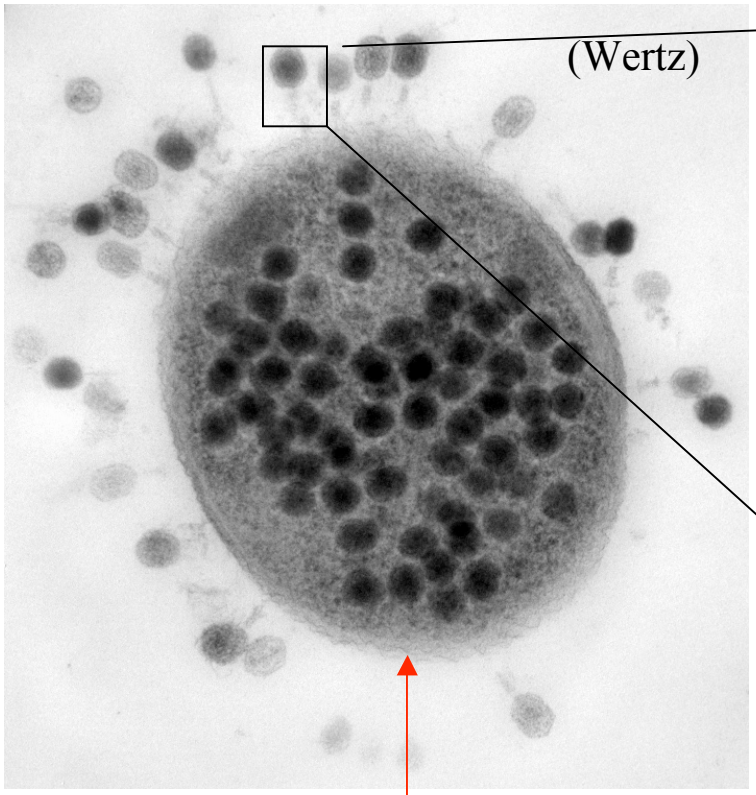
+HIV

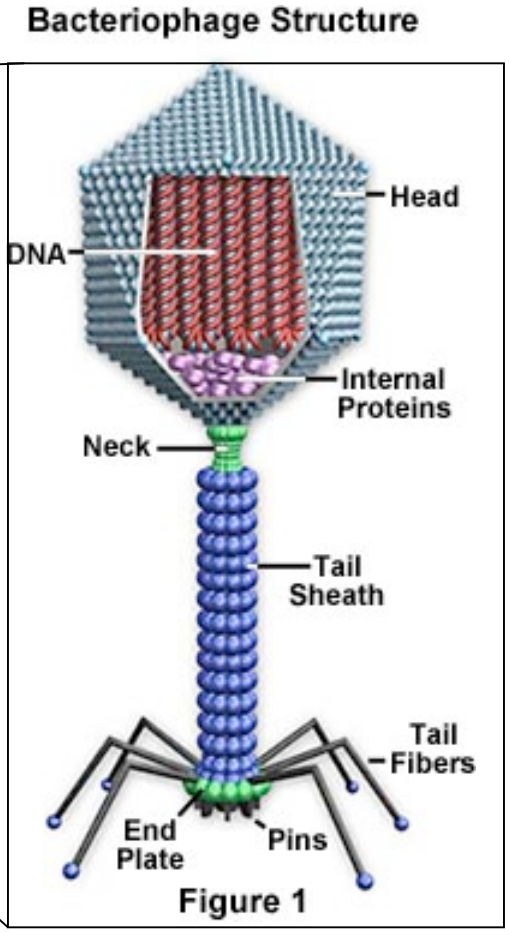


http://faculty.washington.edu/jais/microscopy.html

"Phage and the Origins of Molecular Biology"

Adapted from *Molecular Expressions*

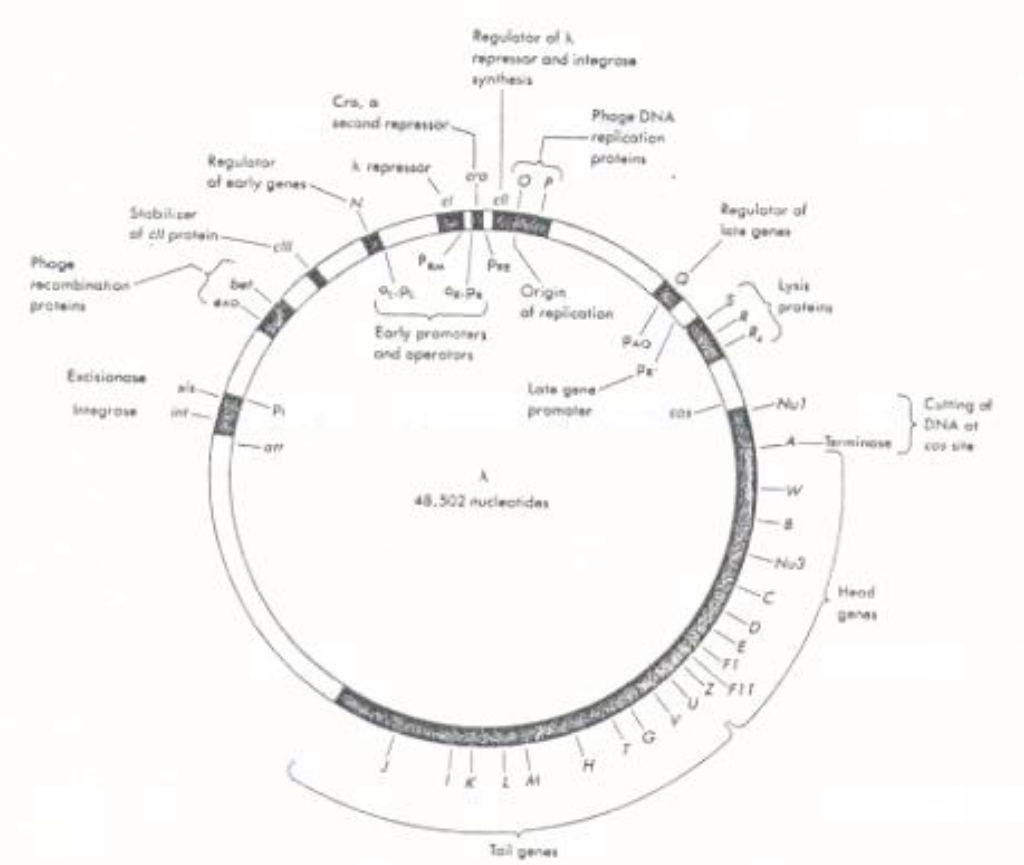


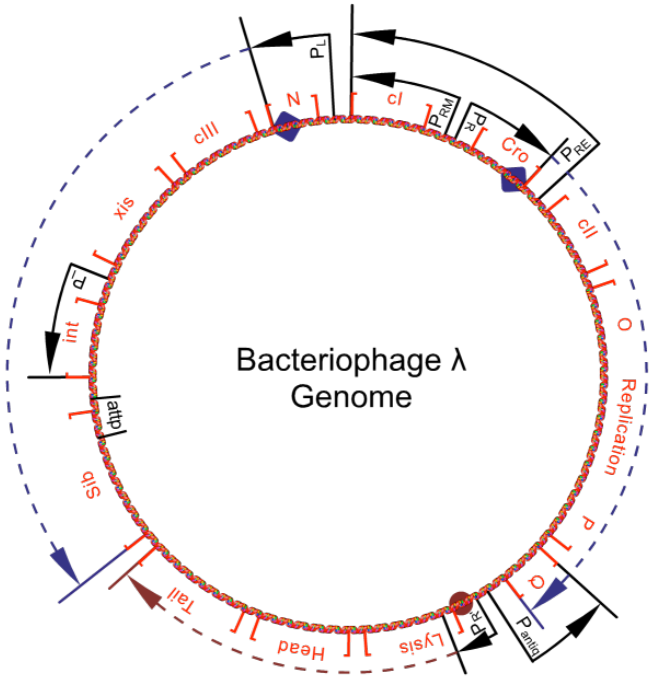


An infected bacterium: note that virus injects DNA and leaves body behind

Analogy: Put 500m of Golden Gate B suspension cable in the back of a Fe

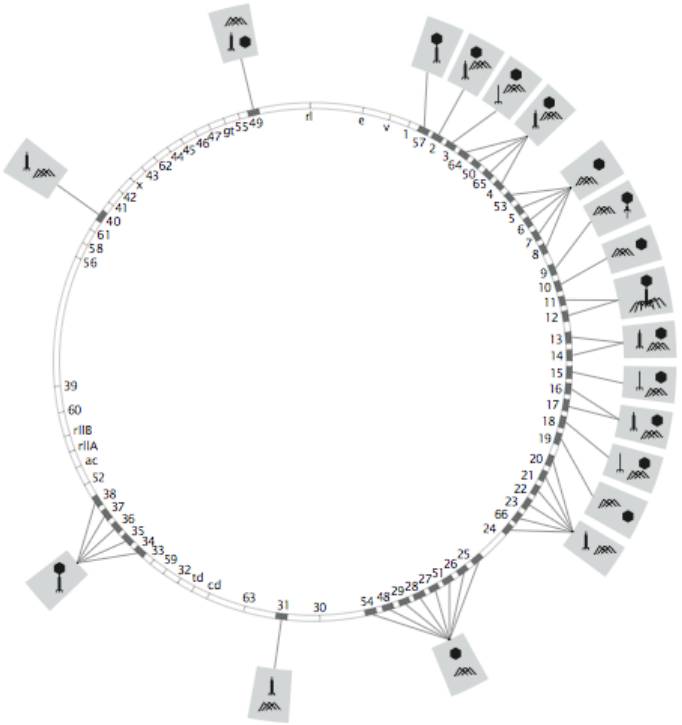
Views of the Lambda Phage Genome

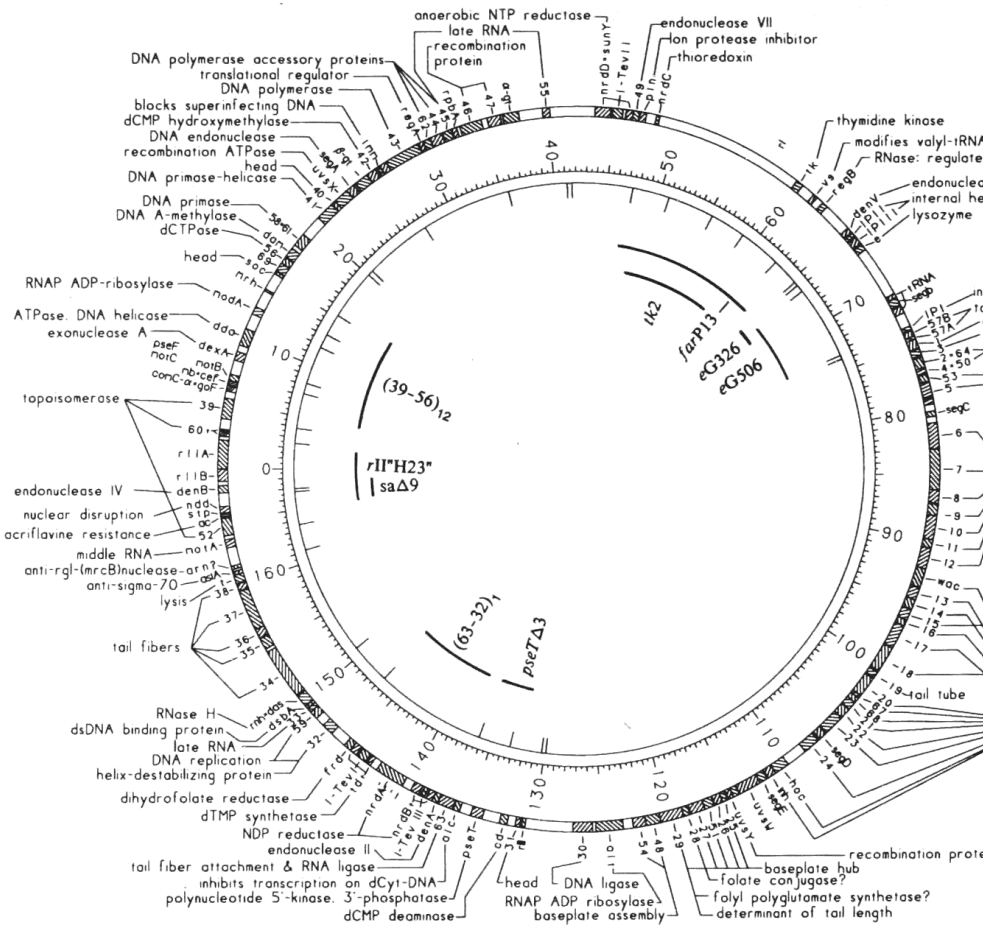




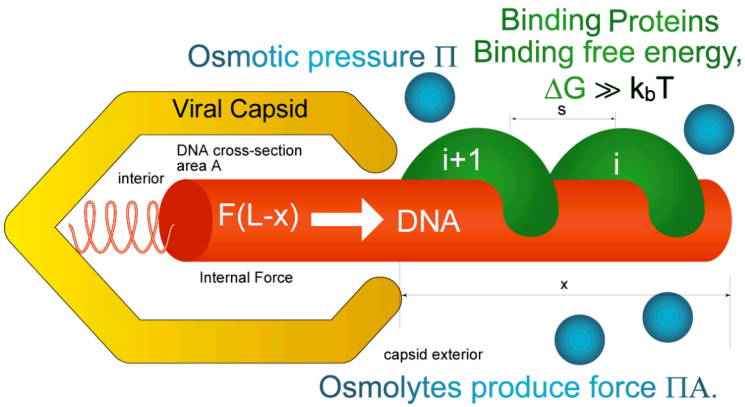
Phage T4 Genome

- *Despite their supposedly simplicity and the existence of sequenced genomes, there is still much that we don't know about what goes on in these genomes.*
- *168,903 base pairs in the genome*

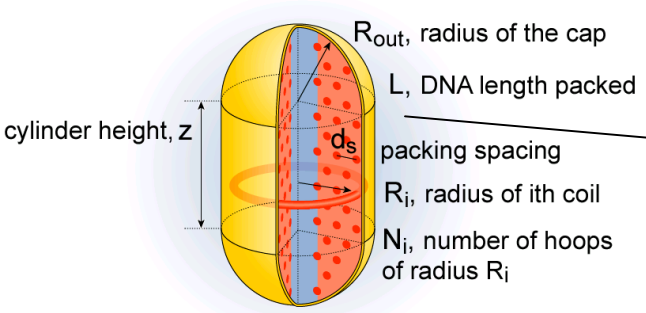




Life Cycle of a Bacteriophage

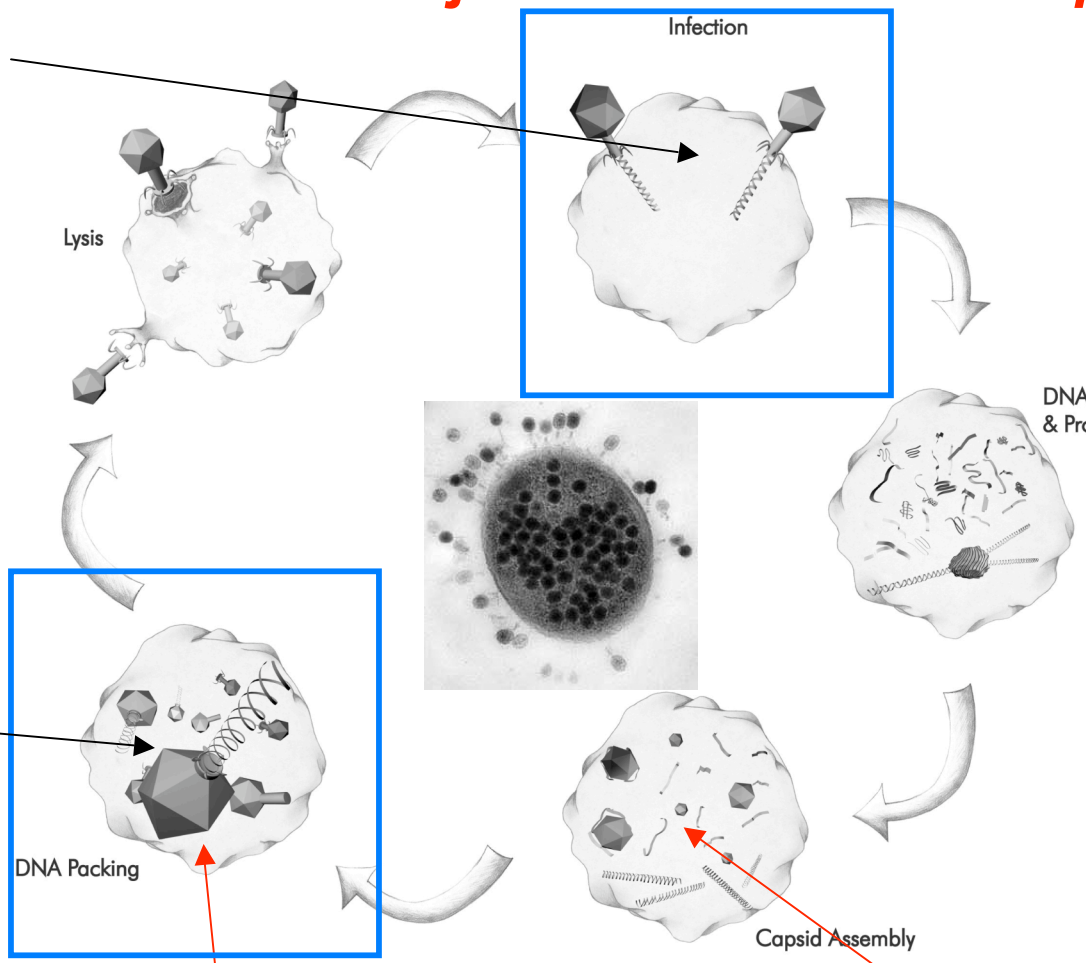


Forceful ejection



Construct a physical model of these processes.

Rate of ejection: ≈ 100 - 10000b



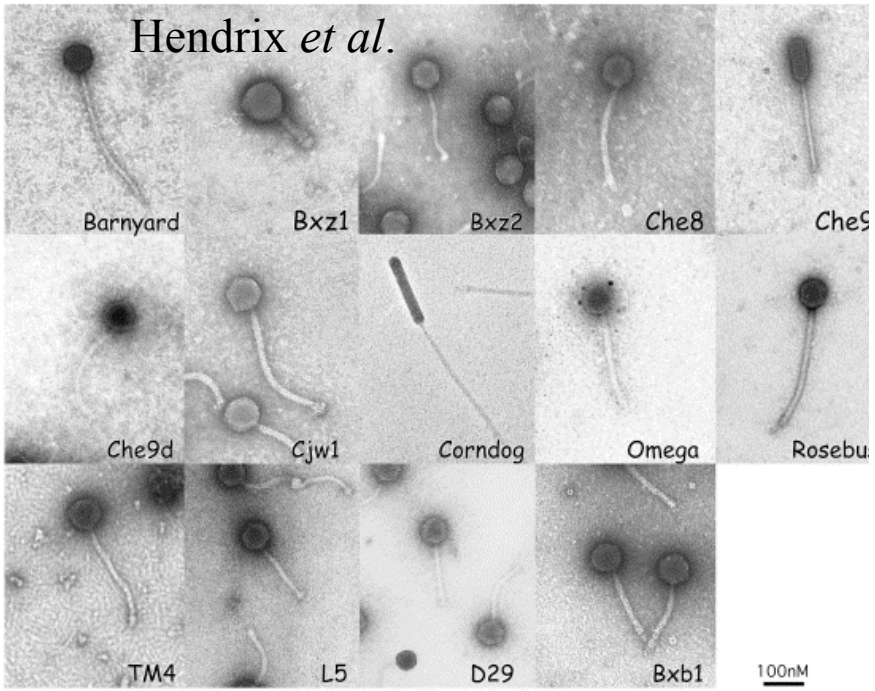
Rate of packing: 100bp/sec

"Some assembly required"

Self-assem

Who Are the Real Predators Out There?

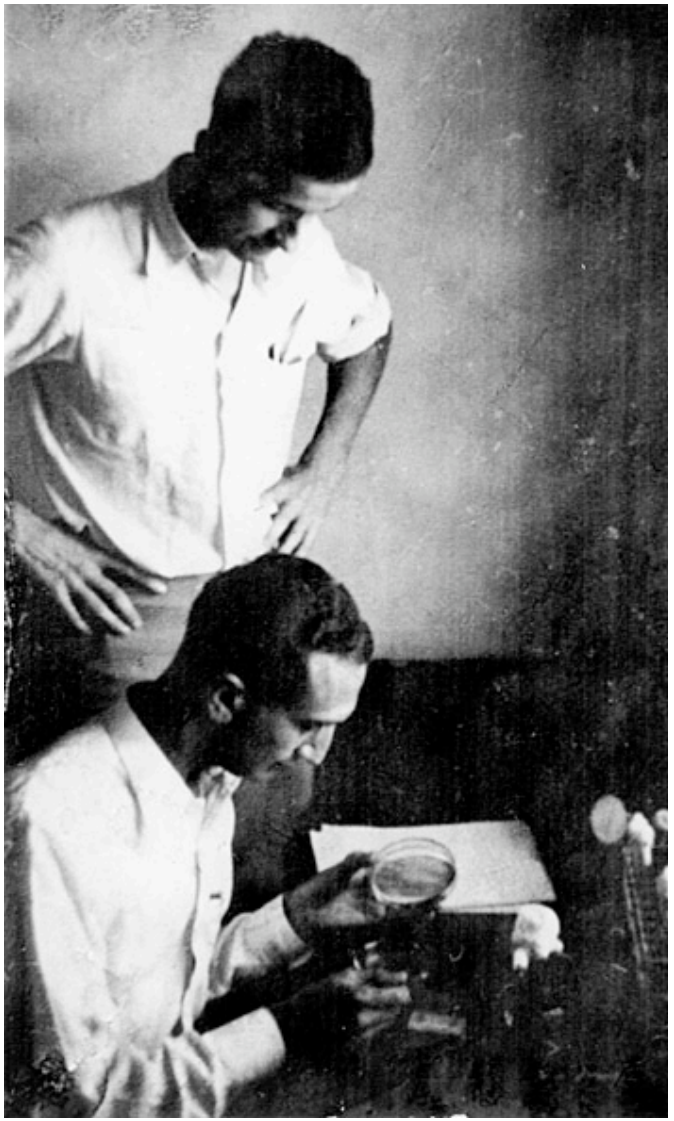
The density of bacteriophage in the ocean is 50 x 10^6/ml while the density of sharks is much less than 10^-6/ml!



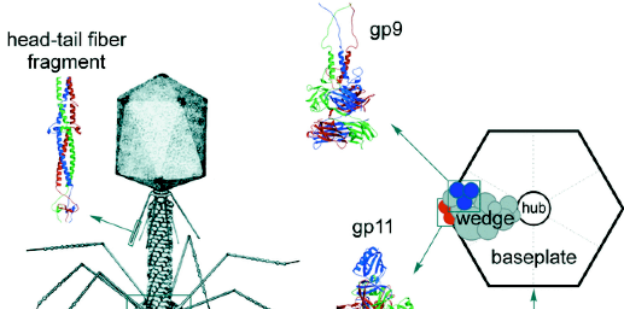
It is estimated that 40% of the bacterial background is infected everyday by bacteriophage - that is a predator!

Phage as Model Systems for Physical Biology

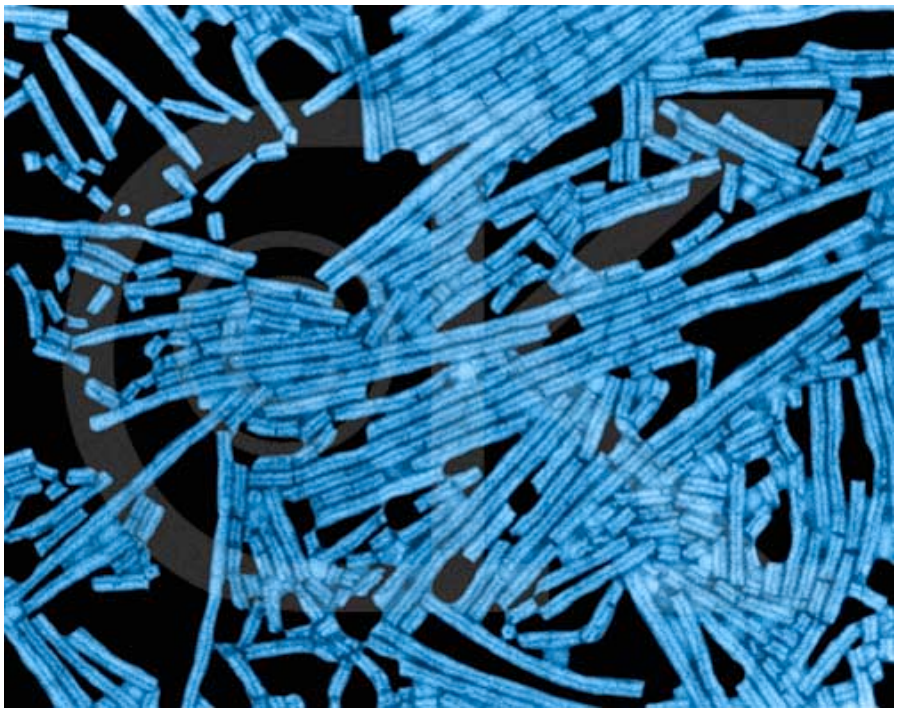
- *Phage provide a setting within which we can ask sharp, quantitative questions and test precise models of biological phenomena.*
- *Some universal biological themes: macromolecular assembly in a crowded environment, orchestration in space and time, polymer translocation, gene expression, evolution, nature's nanotechnology etc.*
- *Close to having a full census (molecular inventory) and full structure of phage.*
- *An opinion: phage have not outlived their ability to teach!*

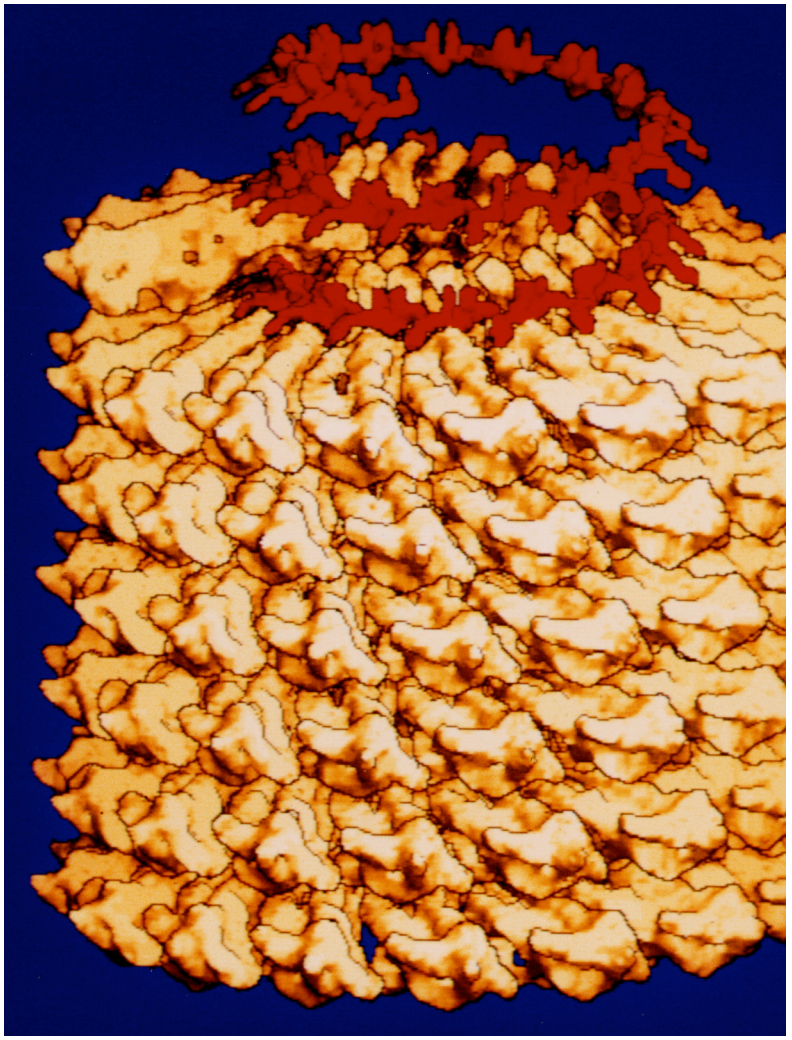


Rossmann *et al.*



Plant Viruses and the "Reconstitution of L

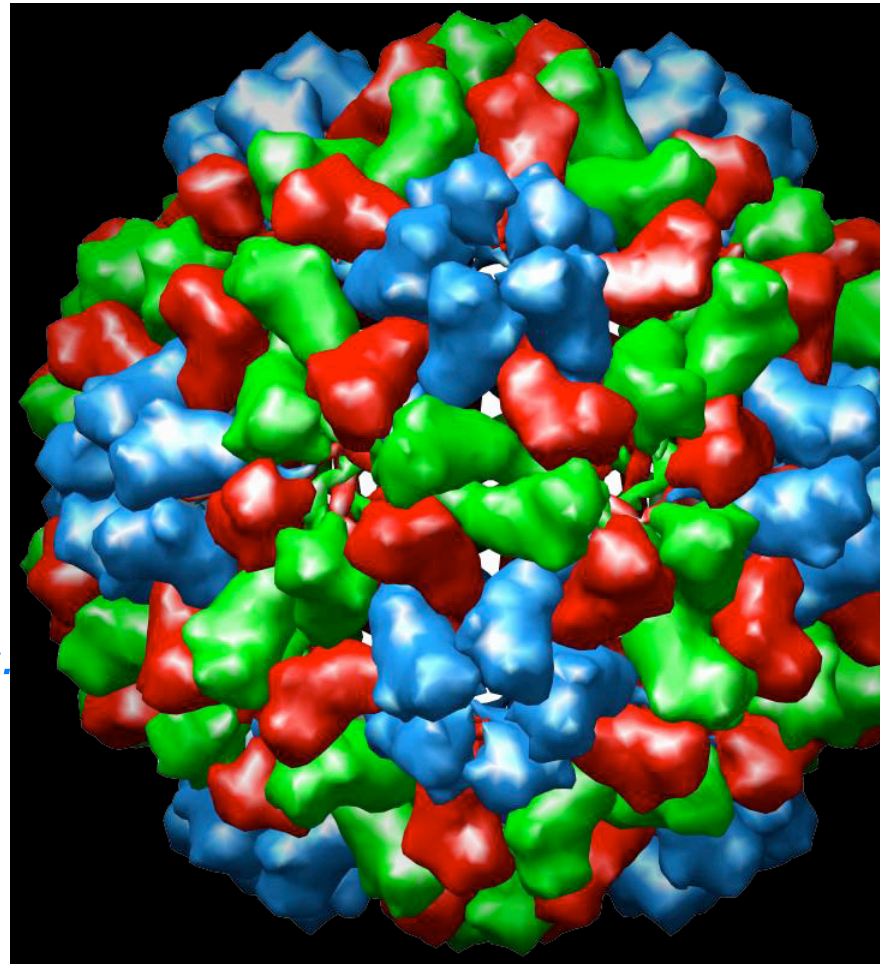




- *1955 Fraenkel-Conrat and Williams - first example of complex biological entity reconstituted outside off cell.*
- *> 2000 protein units co-assemble with RNA molecule.*
- *Headlines: "Life created in test tube!"*

Plant Viruses and the "Reconstitution of L

Fig. 7. Leaf symptoms of cowpea chlorotic mottle virus.



- *Cowpea chlorotic mottle virus - plant virus.*
- *4 separate RNA molecules constitute its genome.*
- *Structure known with atomic resolution.*

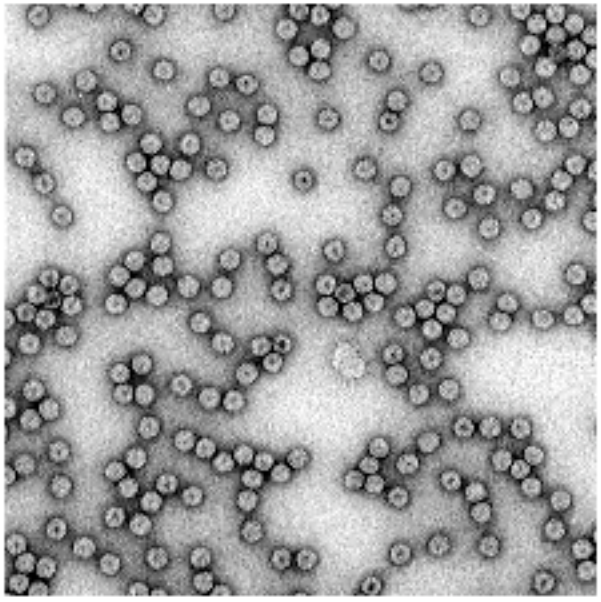
Reconstitution of CCMV

Formation of an Infectious Nucleoprotein from Protein and Nucleic Acid Isolated from a Small Spherical Virus[1]

J. B. BANCROFT
ERNEST HIEBERT

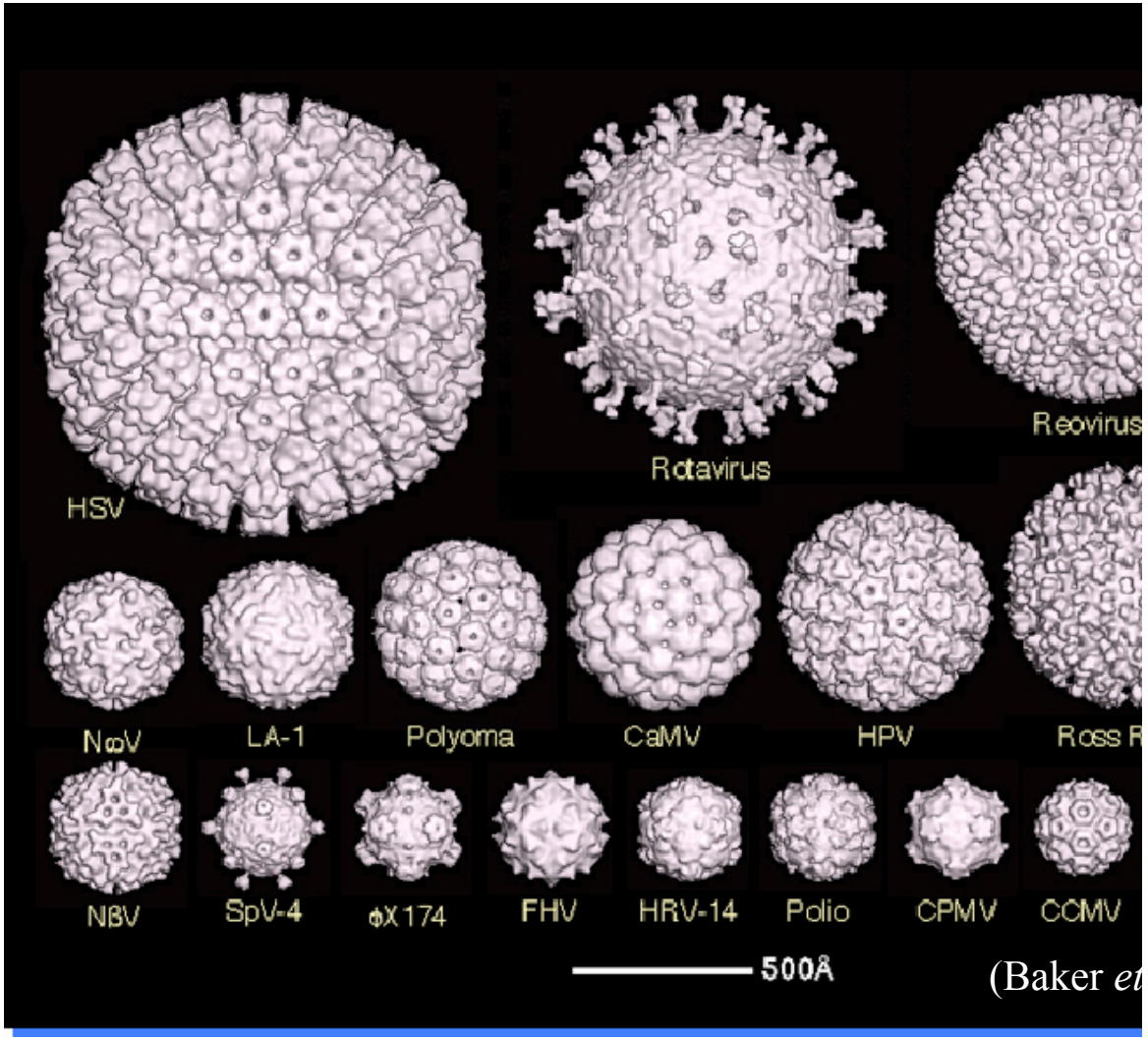
Department of Botany and Plant Pathology
Lilly Hall of Life Sciences
Purdue University
Lafayette, Indiana 47907

Accepted April 4, 1967



Structure of Viruses

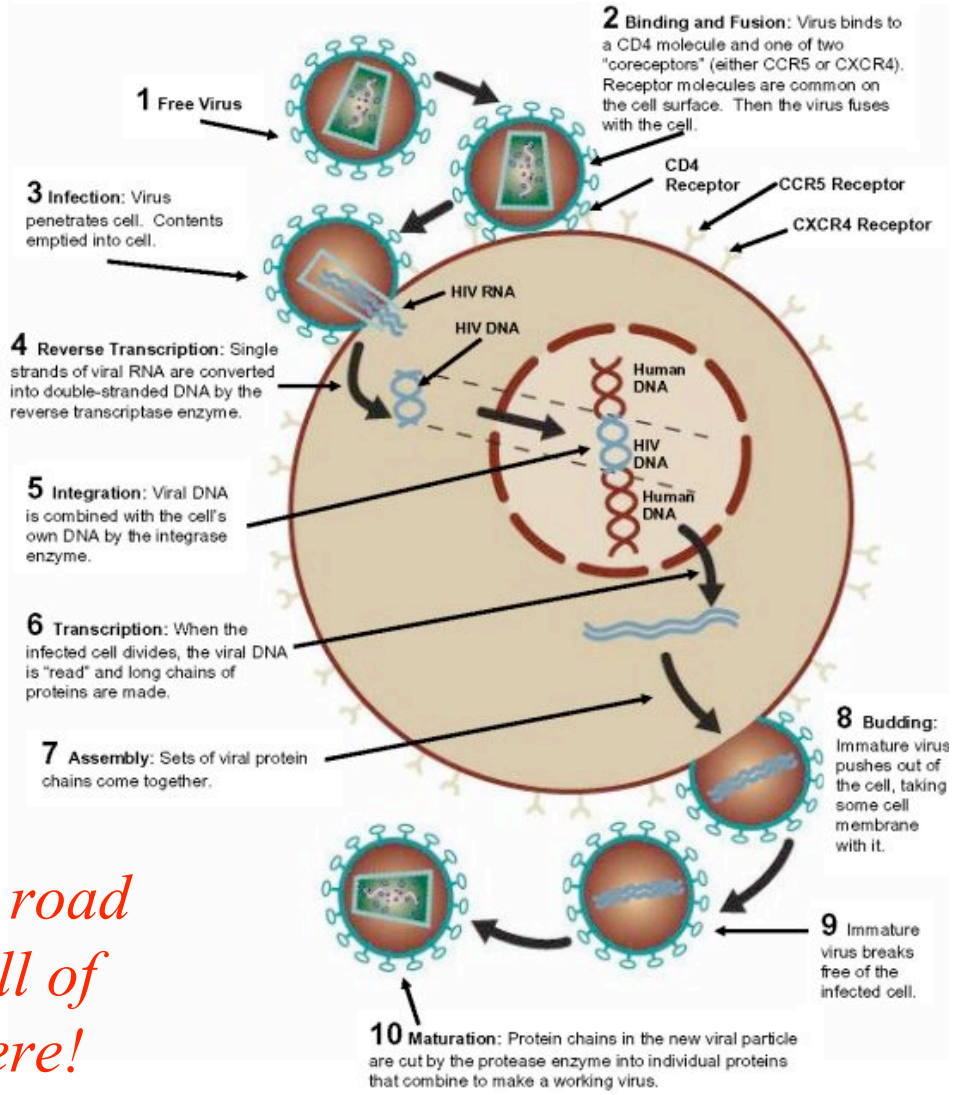
- *Characteristic size scale is 30-100 nm.*
- *Structures are known at "atomic resolution" - see Viper website.*
- *Highly symmetric - think hard about what this implies about assembly!*



(Baker *et*

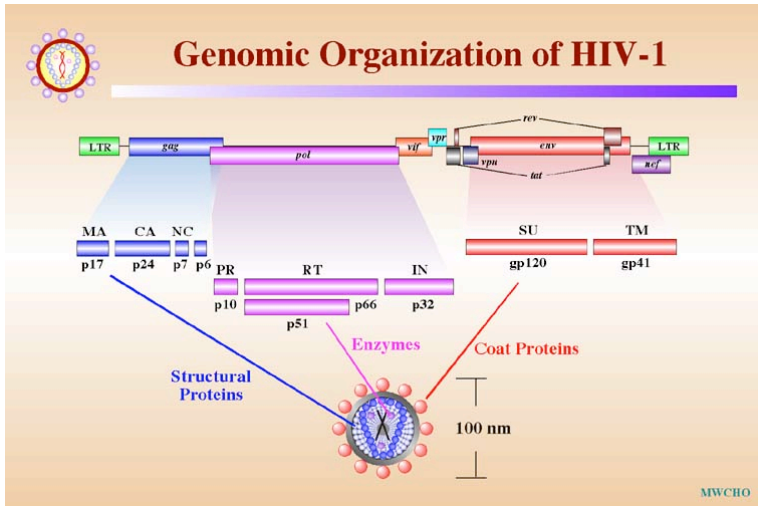
The HIV Life Cycle

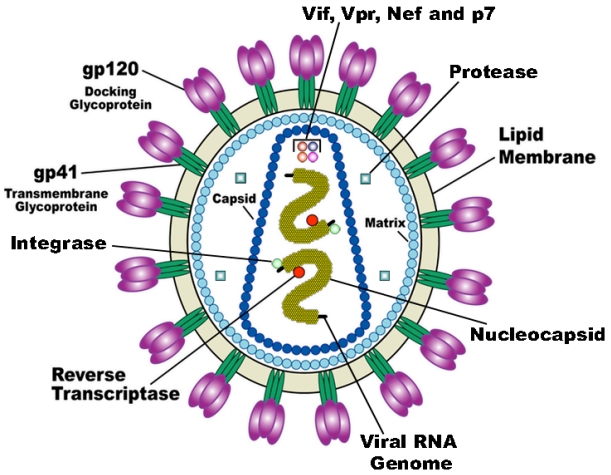
- *Key point: viruses hijack the host cell to make new viruses.*
- *Once again, many of the great themes of biology are played out in this microcosm.*
- *HIV a convenient and intriguing prism through which to view all of biology.*
- *There are many interesting physical processes that take place in this life cycle that will catch our fancy: binding, membrane fusion, transcription and its control, integration, assembly, budding!*

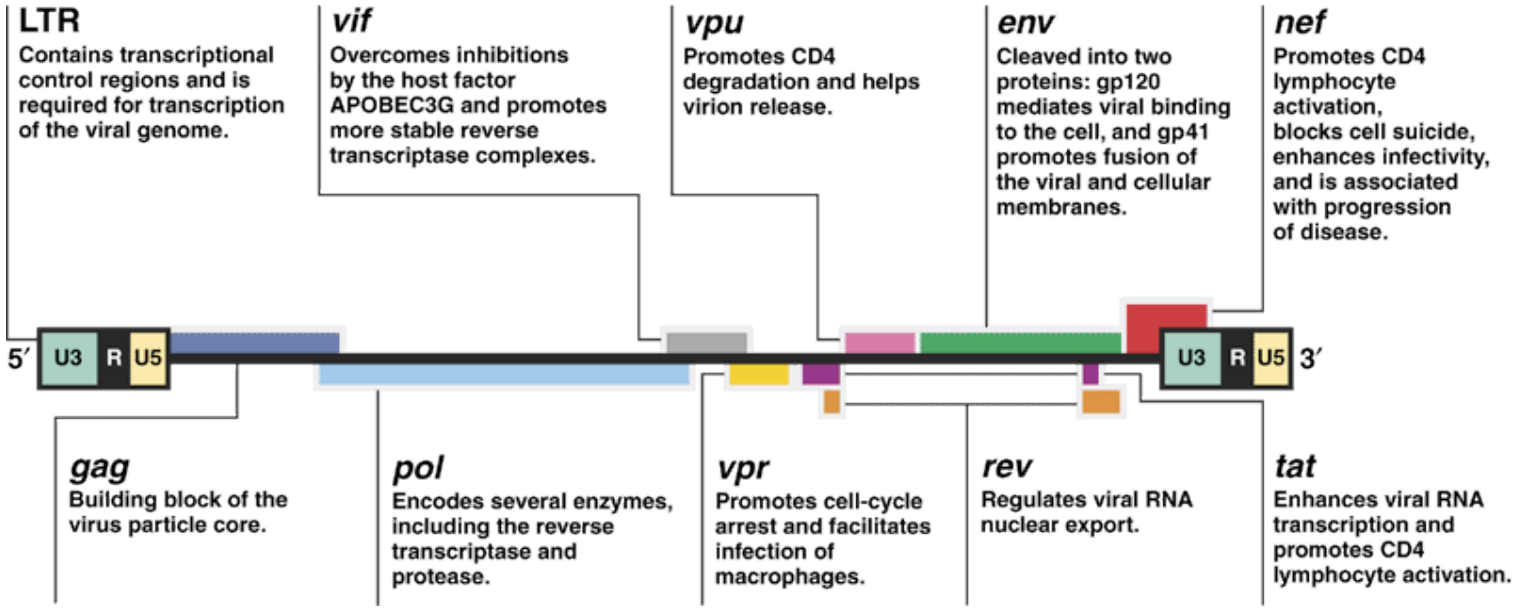


This one picture literally provides a road map for the entire course. Almost all of our big themes are revealed right here!

Views of the HIV Genome



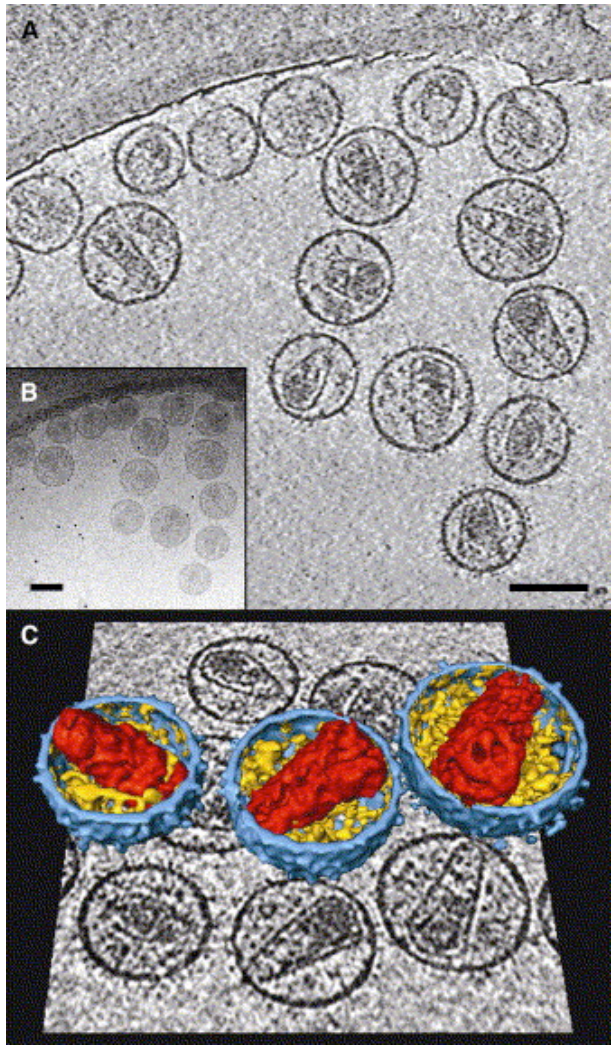




http://www.gladstone.ucsf.edu/gladstone/html/connections/fig1.html

Making a Virus: Mature Virions

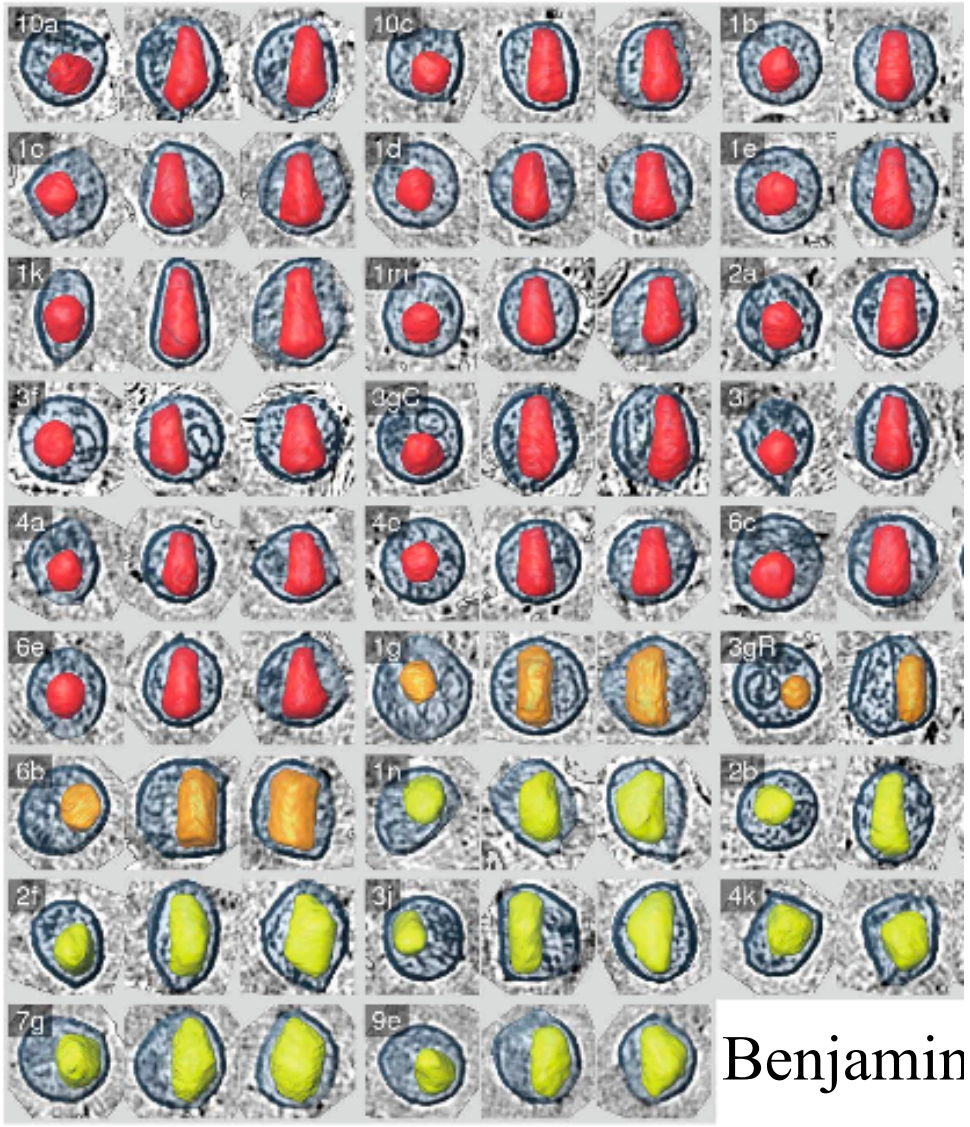
- *Cryo electron microscopy provides a window on virion structure.*



Fuller *et al.*

Structural Heterogeneity of HIV Particles

- *Mean diameter approximately 120nm.*
- *Average volume approximately 45,000 nm^3*
- *Approximate mass per virus particle is 1fg (650 Mda)*

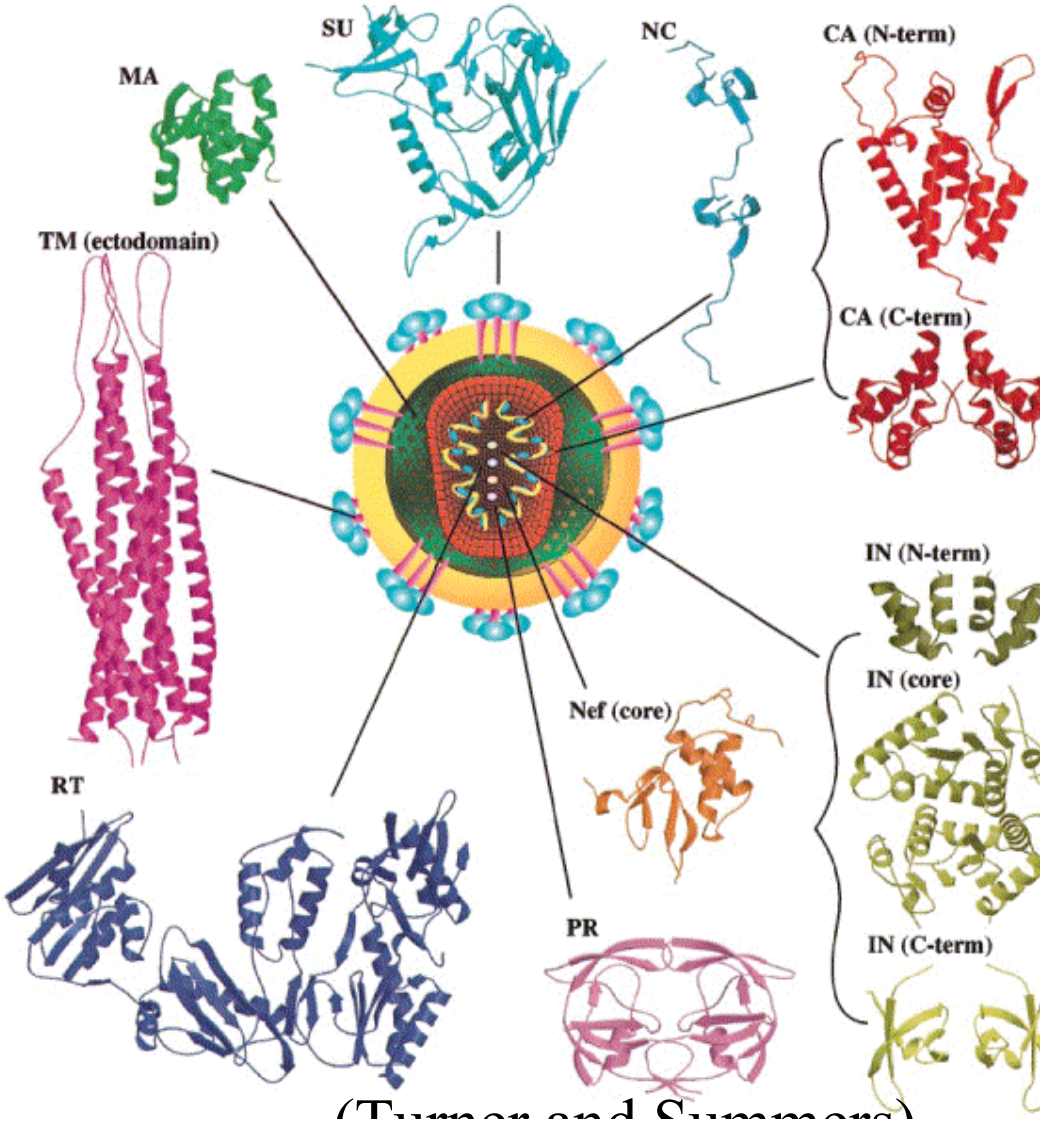


Each picture is 160nm wide.

Benjamin

The HIV Protein Parts List

- *This is a nice and thought provoking picture, but…*
- *Be careful, which features of this cartoon do we REALLY know?*
- *When shown cartoons like this, you have to ask yourself what is known and how. Compare the cartoon to the actual data - what is the data? Try to make a cartoon sometime and it will reveal lots about what we don't know.*

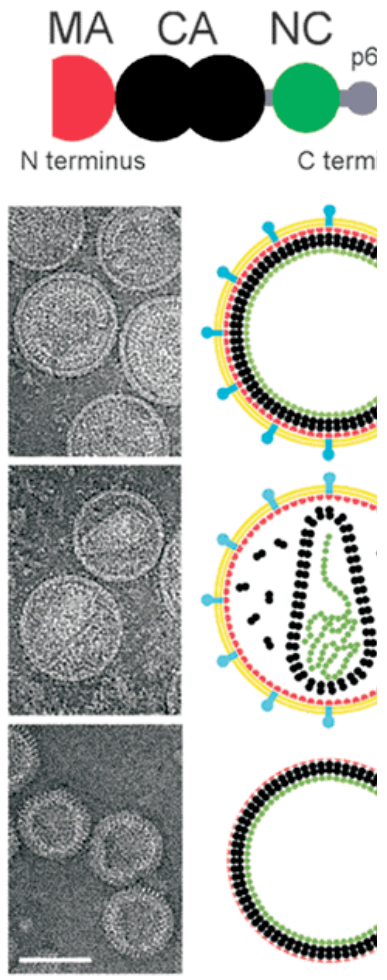


(Turner and Summers)

Making a Virus

- *Yogi Berra said "You can observe a lot just by watching." We will pursue a corrolary: you can learn a lot just by estimating.*
- *Estimation question: how many Gag proteins does it take (roughly) to make an HIV virion? We begin by considering the immature virus.*
- *Does the cartoon make sense?*
- *To figure out the number of Gag proteins, we need an estimate of the area per protein.*

$$N_{Gag} \approx \frac{A_{virus}}{A_{lipid}} \approx \frac{4\pi r_{virus}^2}{\pi r_{protein}^2}$$



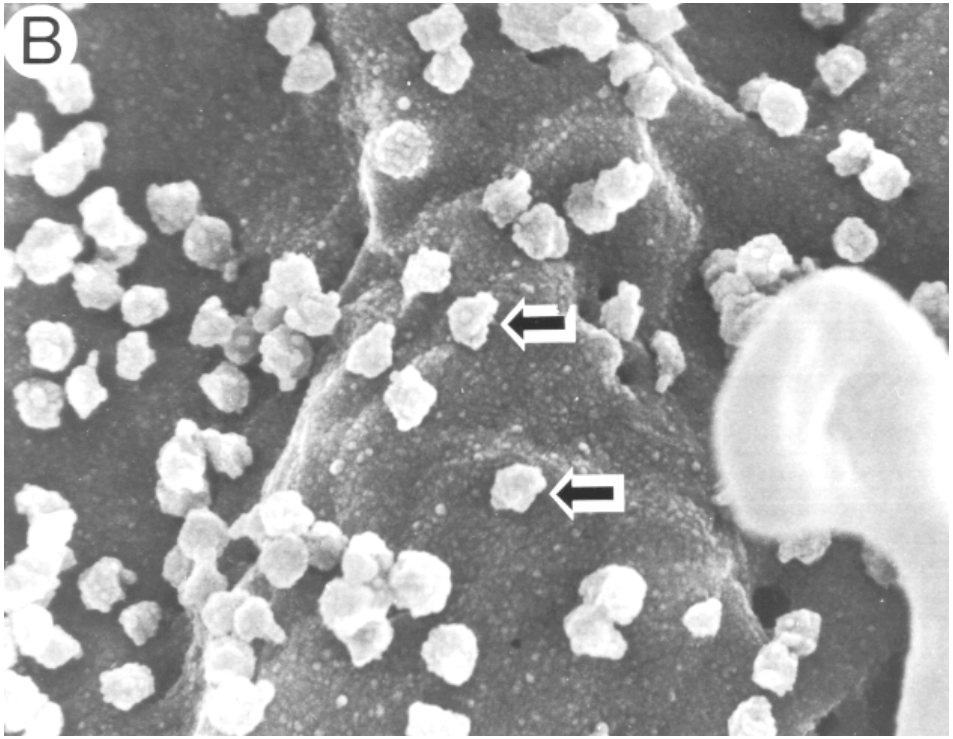
Fuller *et al.*

Important Yogi Berra remarks

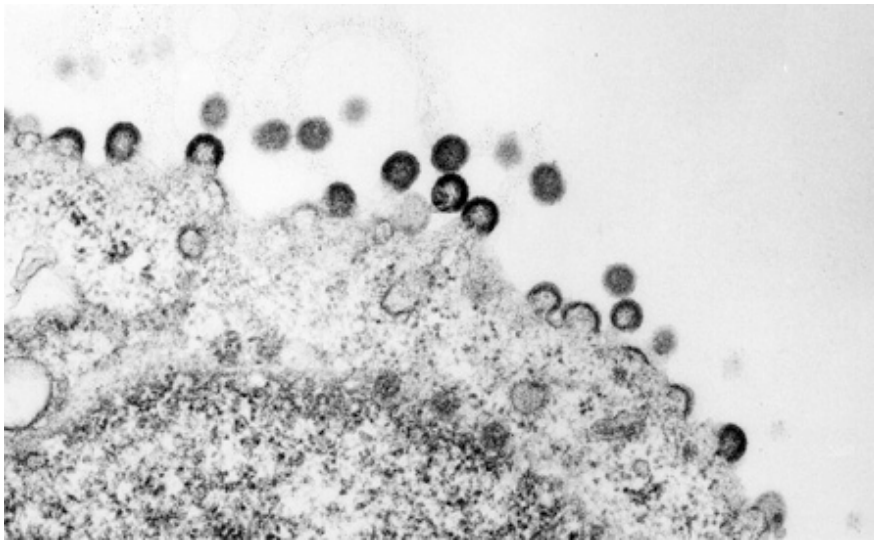
- "Nobody goes there anymore; it's too crowded."
- "If you come to a fork in the road, take it."
- "You should always go to other people's funerals; otherwise, they won't come to yours."
- "It was impossible to get a conversation going; everybody was talking too much."
- "You've got to be very careful if you don't know where you're going, because you might not get there."

Acquiring a Membrane: How HIV Gets Its Lipids

http://www.homepage.montana.edu/~spincus/sp_hiv.html

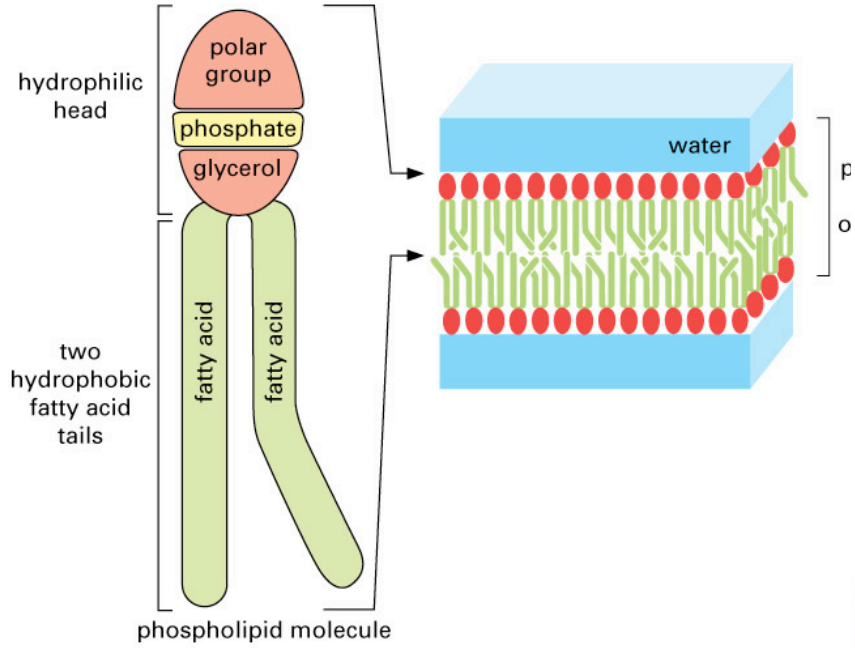


http://faculty.washington.edu/jais/microscopy.l



This scanning electron microscope picture demonstrates HIV budding (arrows) from the surface of an infected T-lymphocyte magnified 80,000X.

Lipid Composition of the HIV Membrane



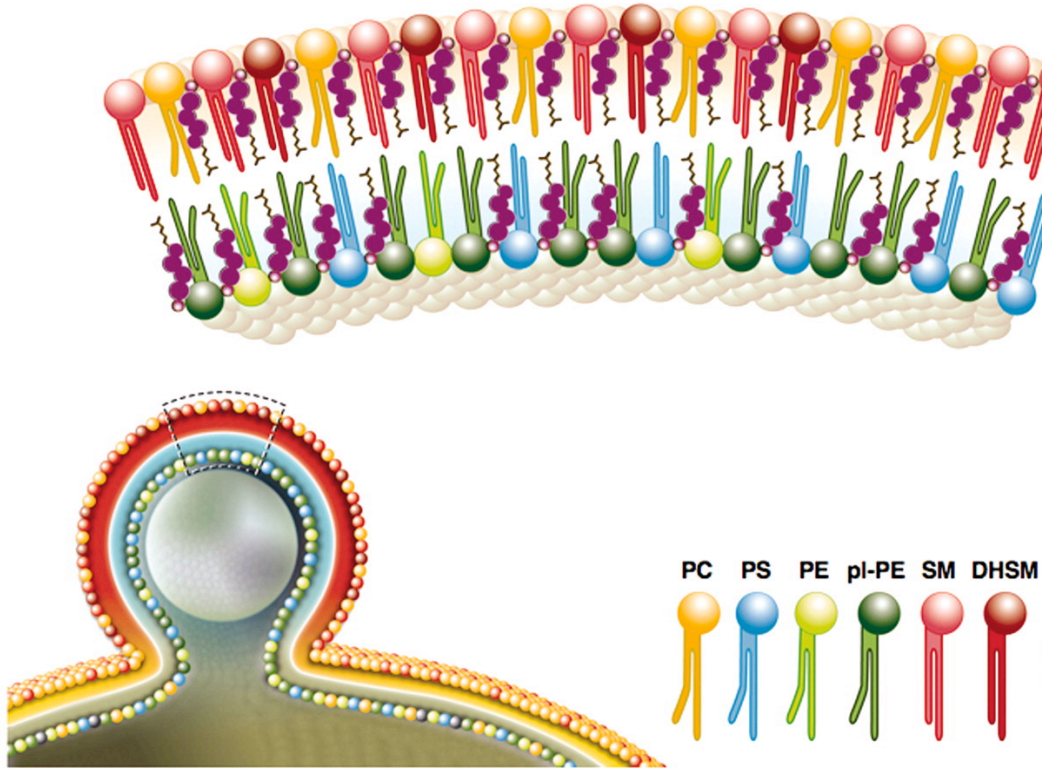


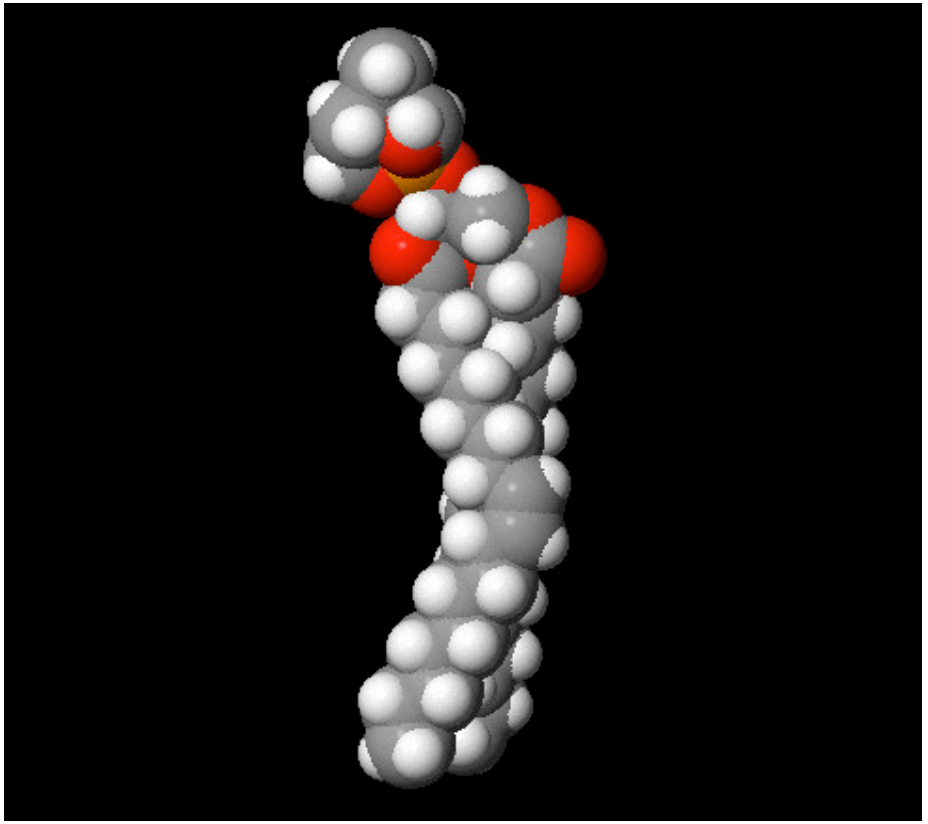
Figure 2-20 Essential Cell Biology, 2/e. (© 2004 Garland Science)

- *Fact of life: lipids come in many different shapes and chemical forms.*
- *HIV lipid census provides a partial picture of the virion.*

Brugger *et al.*

What Are Lipids Like?

- *Typical sizes: 2.5nm*
- *Typical masses: 750 Da*
- *Area per molecule: 1/4 nm^2*
- *A useful place to find stuff out is Avanti Polar Lipids.*



Lipid Content of HIV Particles

- *The actual molecular census of the HIV virus.*

Table 1. Phospholipid composition of MT-4 cells and HIV-1

	MT-4 cells (mol % ± SD)	HIV-1 (mol % ± SD)
PC	43.0 ± 2.9	16.0 ± 1.0
SM + DHSM	10.4 ± 1.6	33.1 ± 1.2
PE	17.0 ± 1.5	8.2 ± 1.3
pl-PE	15.9 ± 0.5	27.0 ± 3.3
PS	7.4 ± 0.8	15.5 ± 2.2

Lipids were extracted and analyzed for phospholipid content as described in *Materials and Methods*. Values are expressed either as mol percentage of a given phospholipid to total phosphate (MT-4 cells) or as mol percentage of a given phospholipid to the total of all phospholipids quantified (HIV-1).

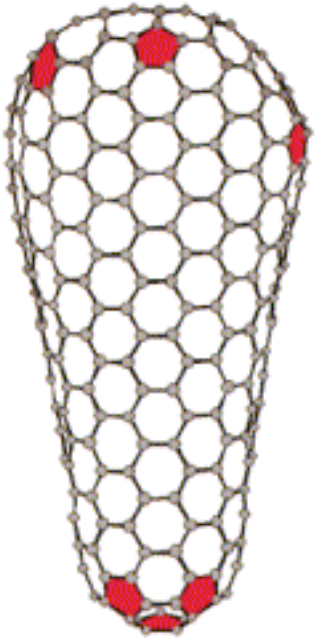
Table 2. The lipid composition of HIV-1

	Lipid molecules per average HI virion
PC	26,000
SM	37,000
DHSM	17,000
PE	13,000
pl-PE	44,000
PS	25,000
Chol	134,000
Cer	160
HC	600

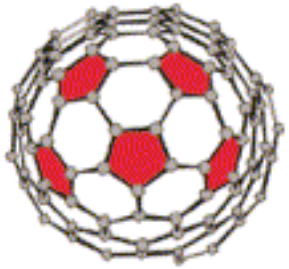
For details, see *Supporting Text*.

Capsid Structure

- *Fascinating structure of the internal capsid of HIV.*
- *Shape conferred by geometric rules about 5-fold defects.*



Side View

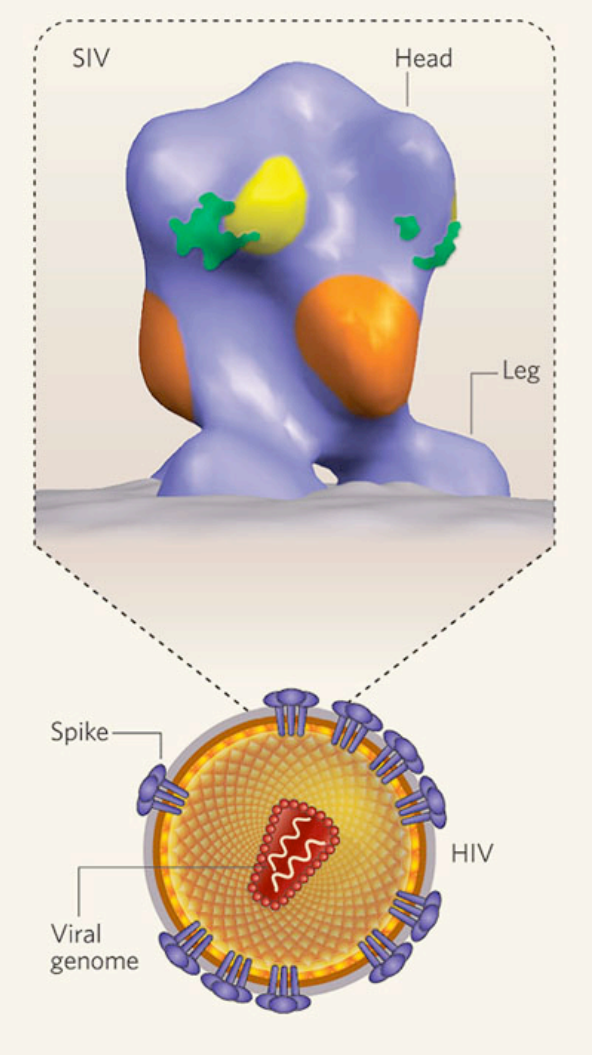


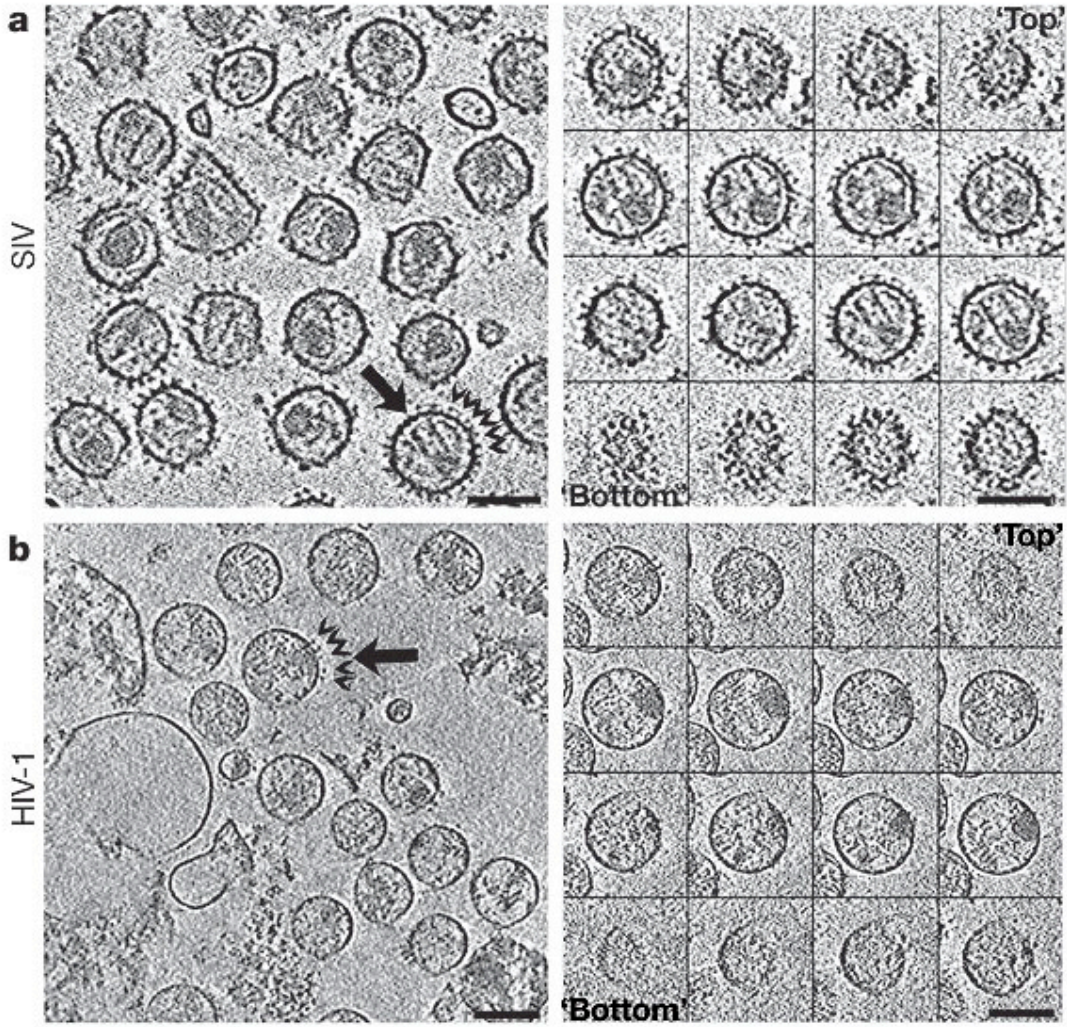
Bottom View

Sundquist *et al*.

Distribution of Spike Complexes

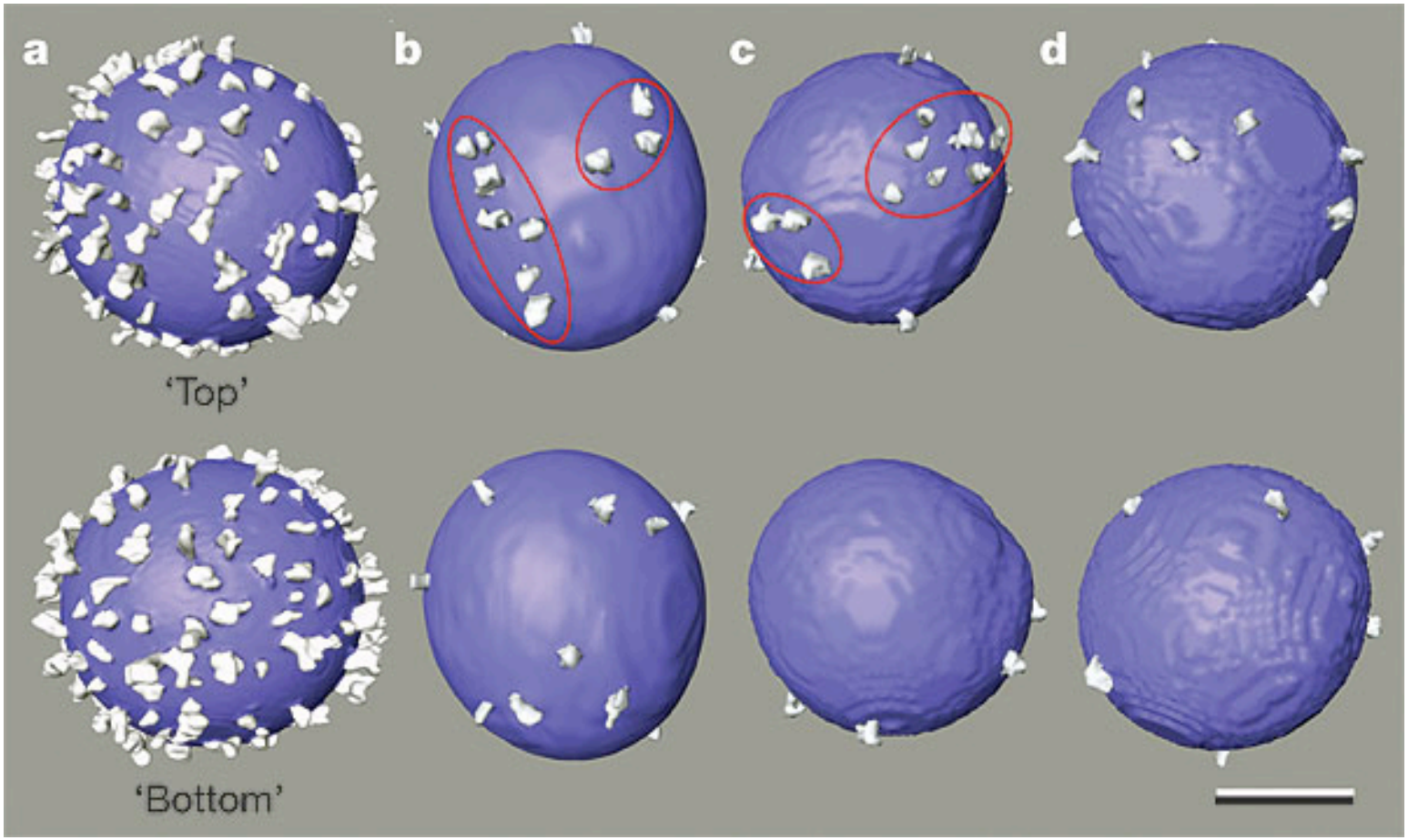
Zhu *et al.*, Nature 2006





Distribution of Spike Proteins?

Zhu *et al*., Nature 2006



Viruses and the Great Themes of Biolo

- *How do viruses make new viruses?*
- *How do viruses evolve? (including to evade the host defenses)*
- *How do cells defend against viruses?*
- *How do cells make decisions and how is that decision making altered by the presence of viruses?*

Korber *et al.*