Single particle cryoelectron microscopy and 3D reconstruction of viruses

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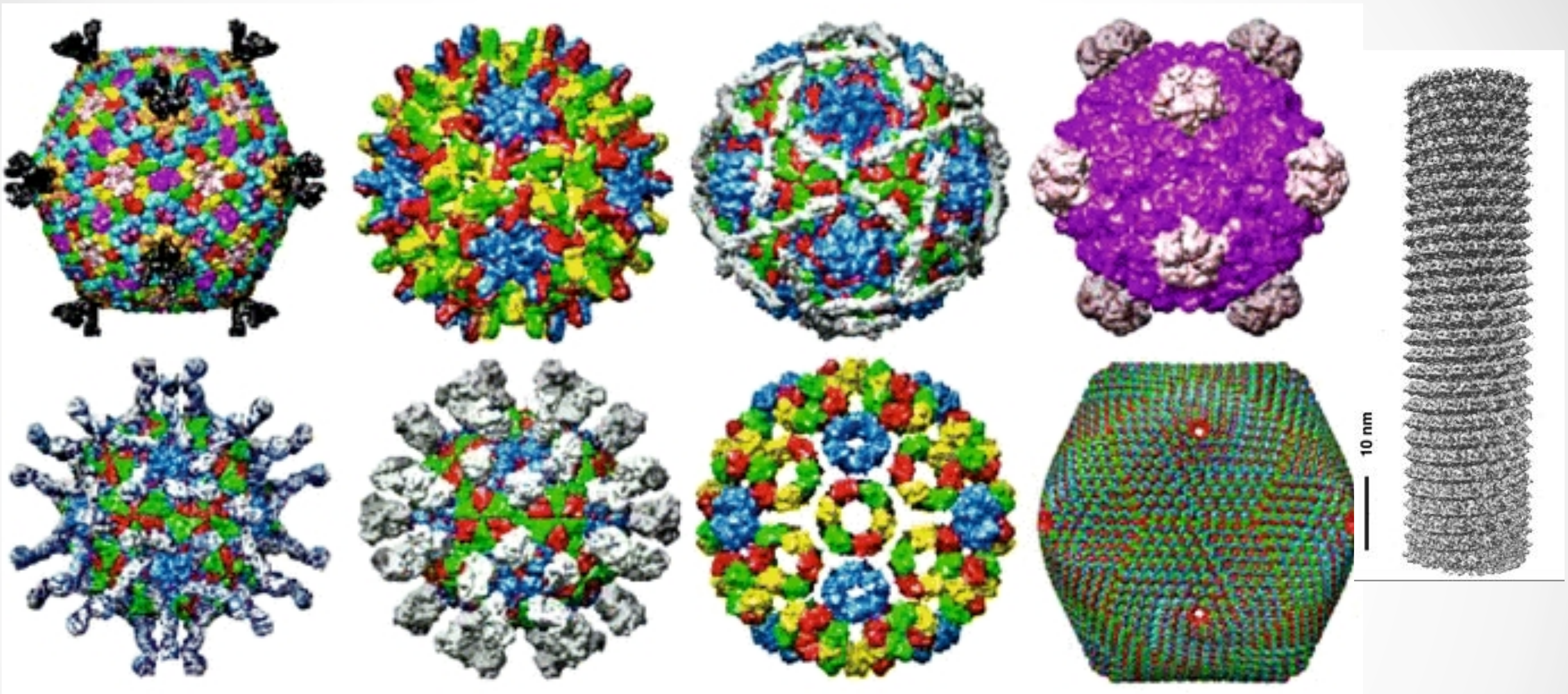
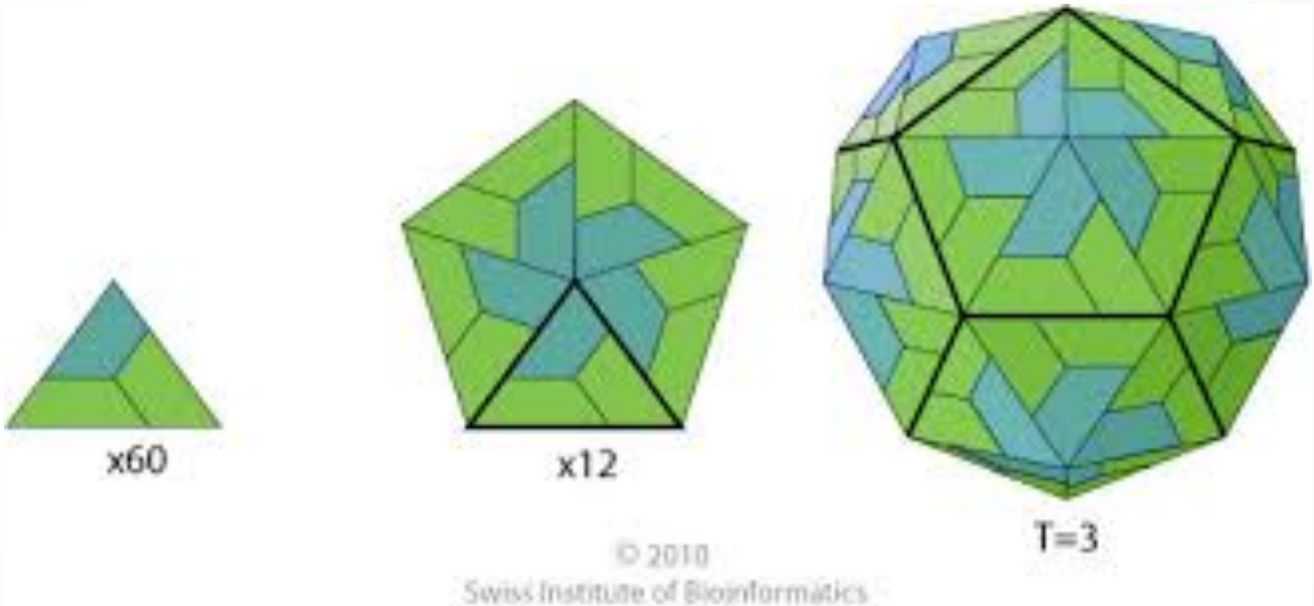
Virus Types



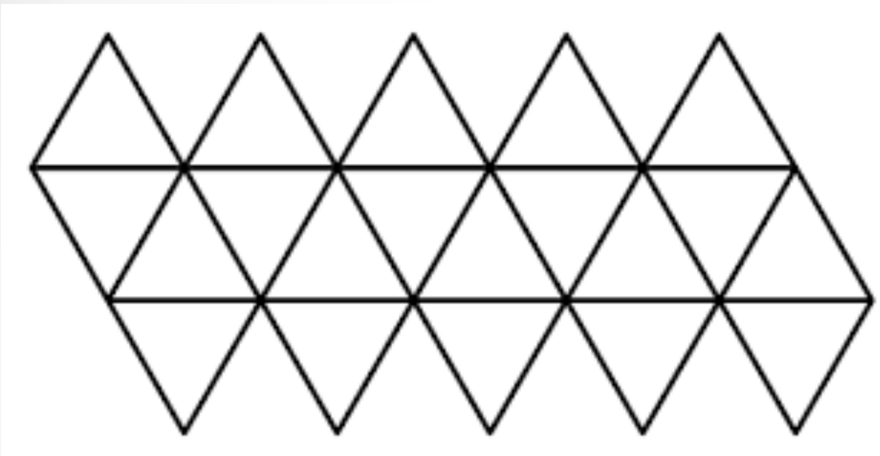
Composed of protein, nucleic acid (ss/ds DNA/RNA) and lipid components
Icosahedral, helical, oblate, head-tail structures
Genome codes for non-structural proteins, scaffolding proteins
Viral genome contributes less mass than viral proteins
Multiple copies make up capsid

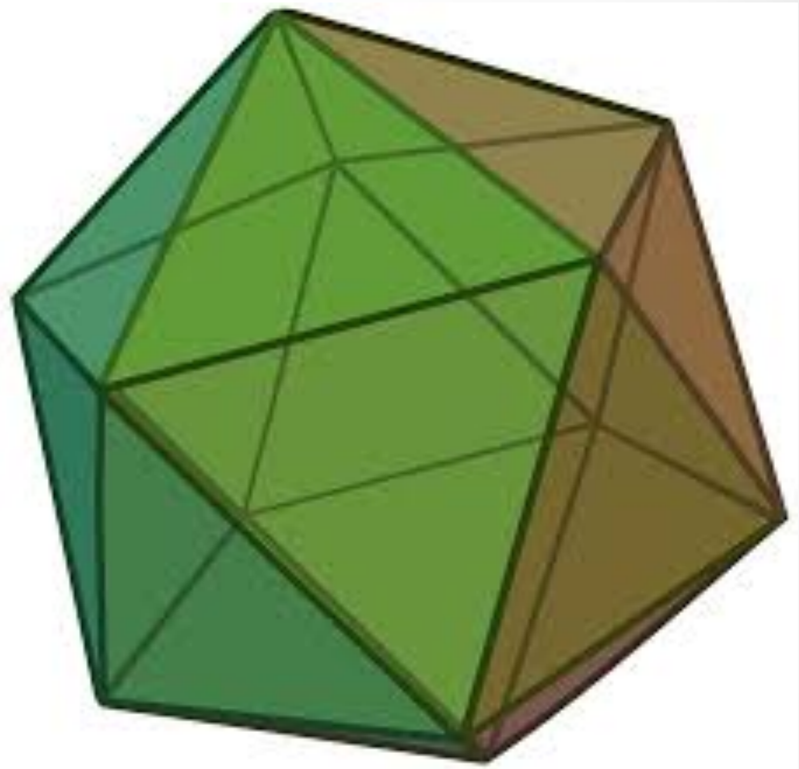
Images from VIPERdb

Icosahedral organization of virus capsids



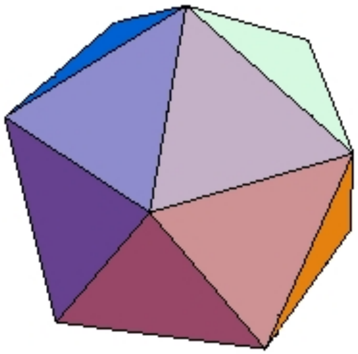
Icosahedral organization of virus capsids





Protein tertiary structure is non-symmetric
Need to form a stable, closed structure to protect the genome
Minimum free energy = maximum number of intersubunit bonds

Icosahedral organization of virus capsids



Constructed from 20 equilateral triangles

60 subunits form an icosahedron

Requires a greater number of subunits to form a same volume
(size of subunits smaller, less genetic information required)

Physical constraints prevent tight packing of subunits required for tetrahedral and octahedral geometry

Icosahedral organization of virus capsids

Early information from X-ray crystallography

Tomato Bushy Stunt Virus at 2.8 Å: Harrison *et al* (1978)

Southern bean Mosaic Virus at 2.8 Å: Abad-Zapetero *et al* (1980)

Satellite Tobacco Necrosis Virus at 3.0 Å: Liljas *et al* (1982)

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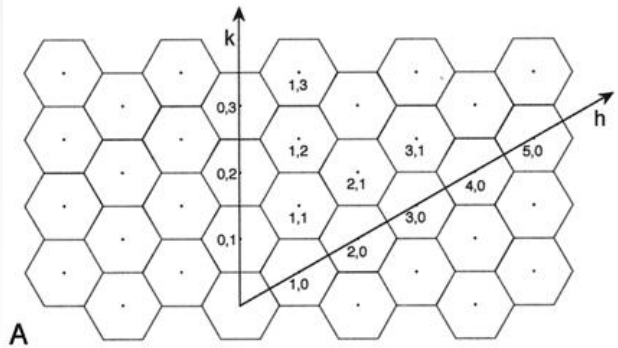
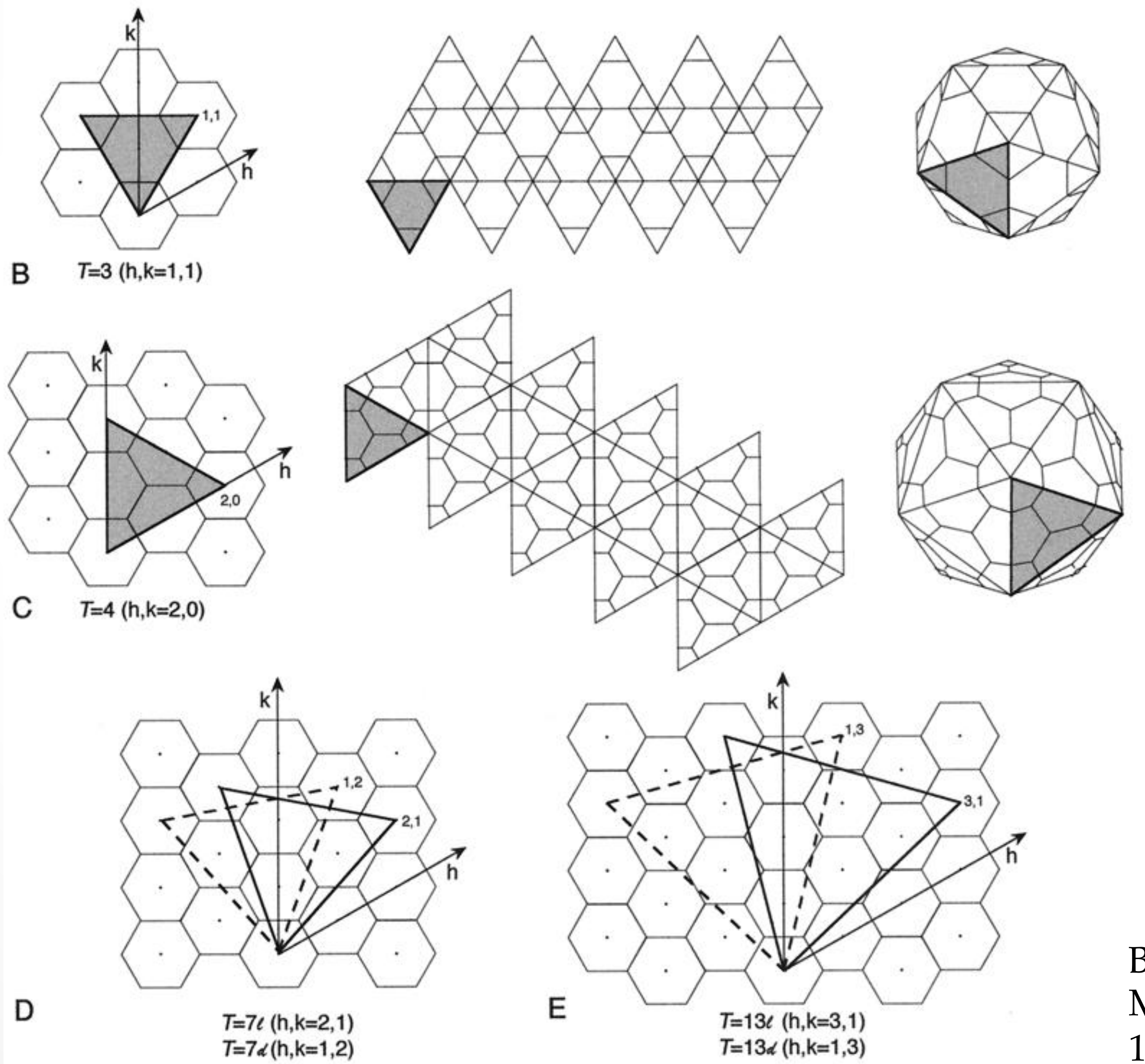
Adenovirus at 3.5 Å: Reddy *et al* (2010)



h k	T	Example
1 0	1	bacteriophage ϕX174
1 1	3	tomato bushy stunt virus
2 0	4	Sindbis virus
1 2	7*d*	polyoma virus
3 1	13*l*	reovirus
1 3	13*d*	infectious bursal disease virus
4 0	16	herpesvirus
5 0	25	adenovirus

Notations: *d* = *dextro* (right handed)
l = *laevo* (left handed)

F



Baker *et al.*,
Microbiol Mol Biol Rev,
1999

Icosahedral organization of virus capsids

Minimum of 60 subunits required

>60 subunits accommodated by expansion of triangular facets, and subdivision

Triangulation number

$T = H^2 + HK + K^2$

Three classes:

$H \geq 1, K = 0$ (T=1, 4, 9……)

$H = K \geq 1$ (T= 3, 12, 27……..)

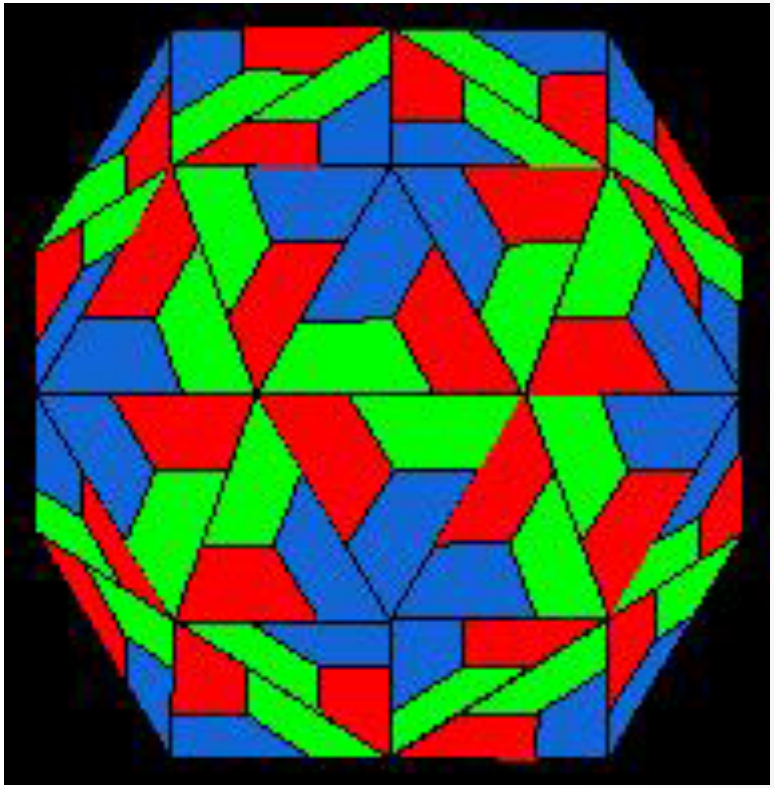
$H \neq K \geq 1$ (T = 7, 13, 19, 21….) skewed class, leavo or dextro enantiomorphic configurations
Polyomavirus (T=7d), Rotavirus (T = 13l)

Icosahedral organization of virus capsids

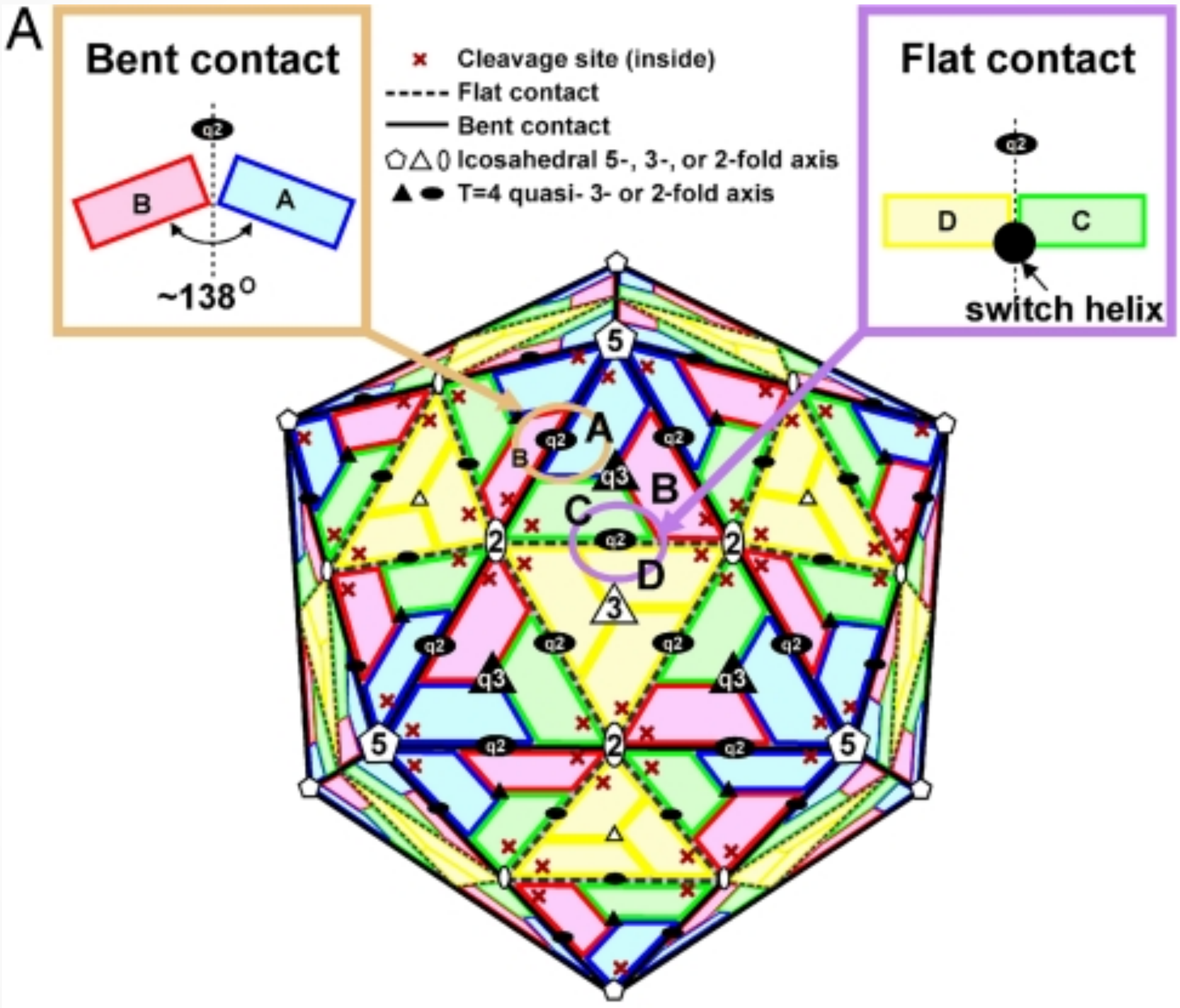
Icosahedral asymmetric unit (iASU)

Quasi-equivalence of subunits

12 pentamers, 10 (T-1) hexamers

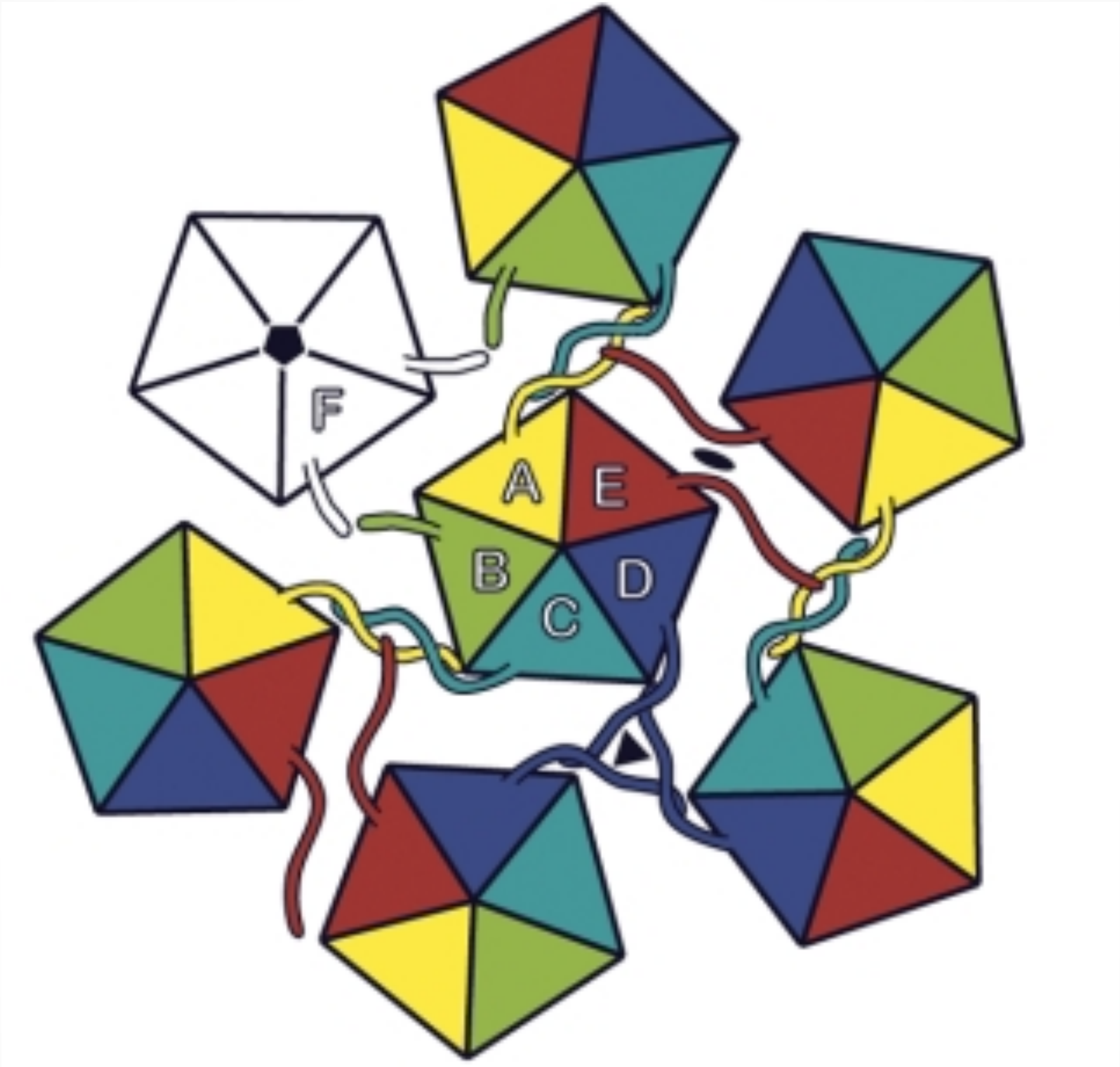


Icosahedral organization of virus capsids



Matsui *et al.*, PNAS, 2012

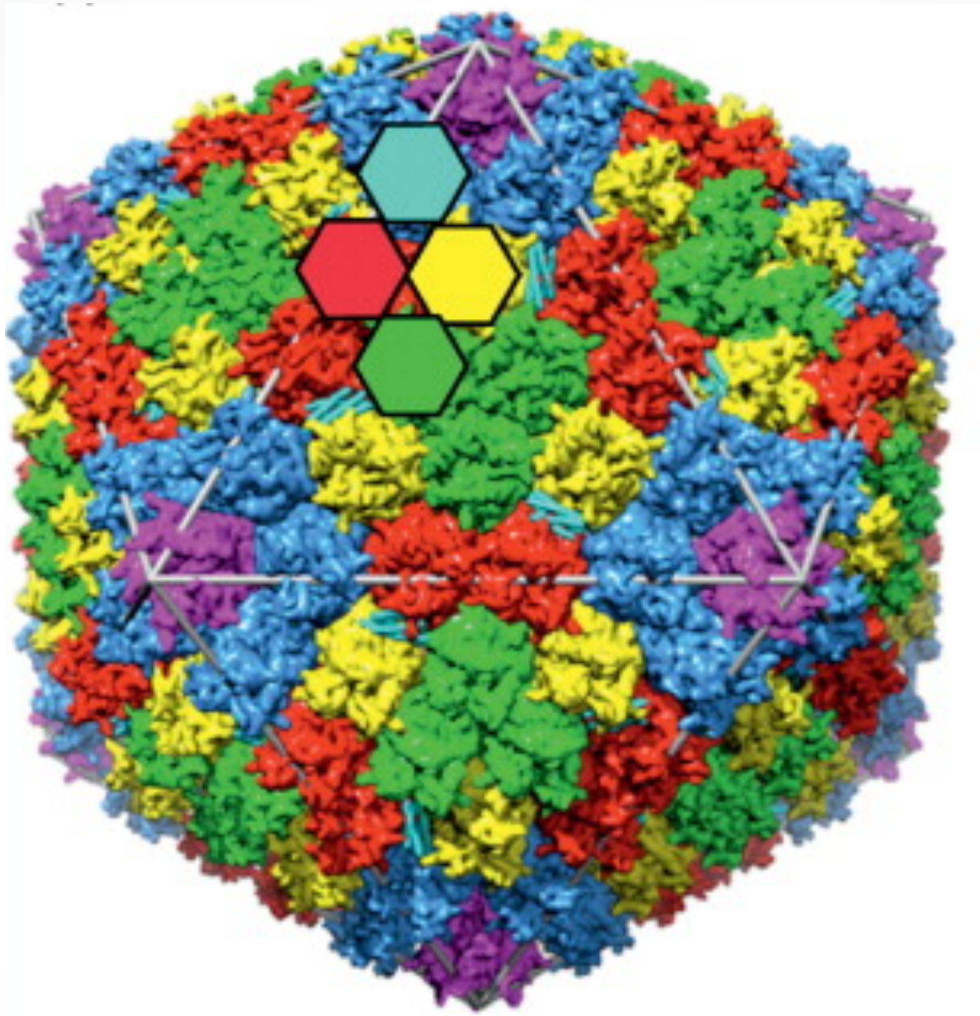
Departure from icosahedral organization



Bovine papillomavirus: 72 pentamers

Wolf *et al*., PNAS, 2010

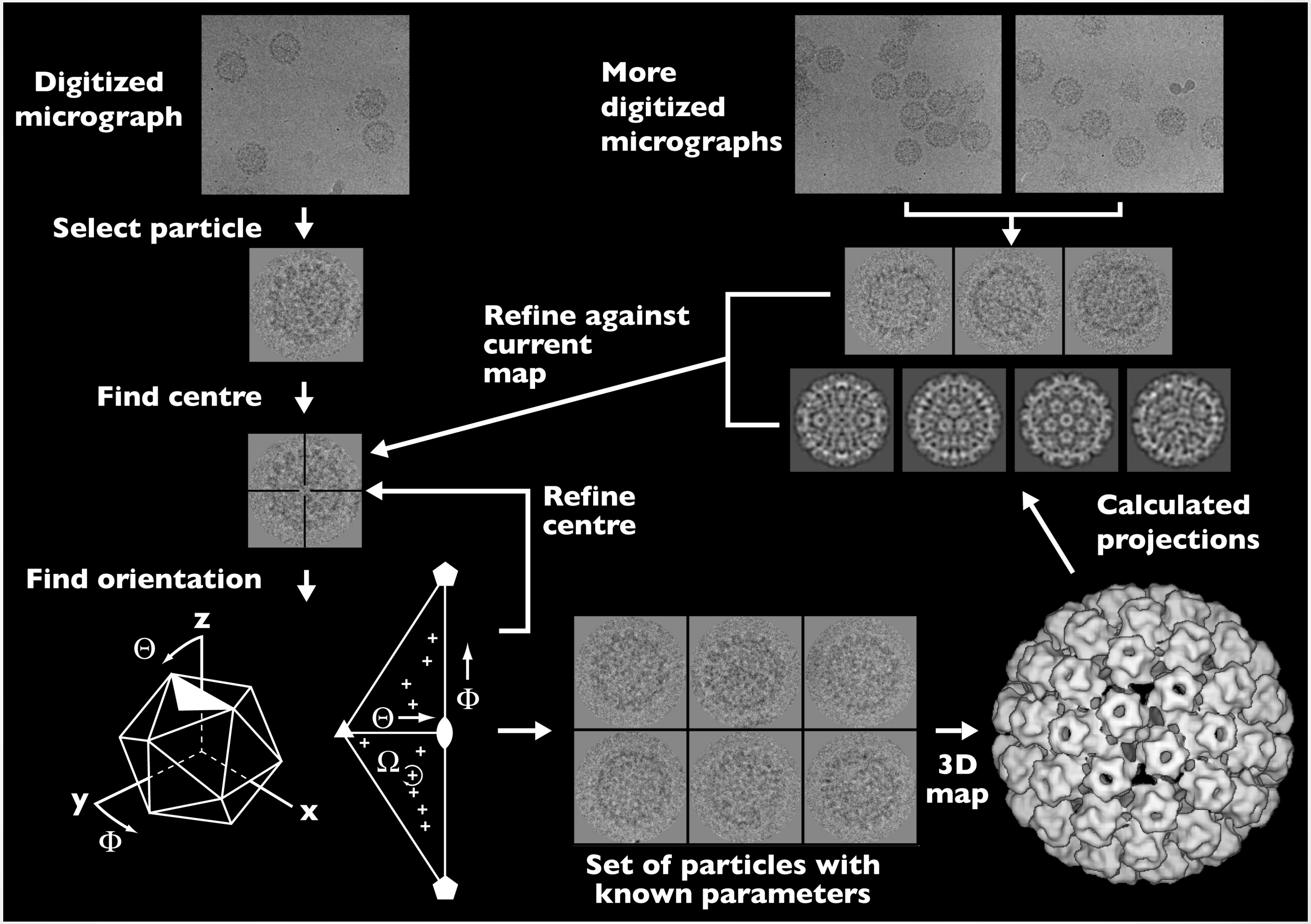
Departure from icosahedral organization



Adenovirus
240 trimers occupy hexavalent positions

Nemerow *et al.*, Curr Opin Virol, 2012

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses



Crowther, 1971

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

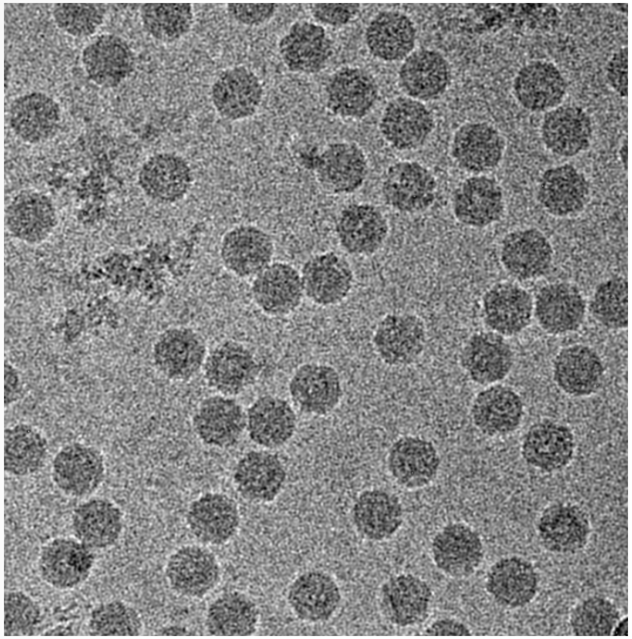
Negatively stained Tomato Bushy Stunt Virus

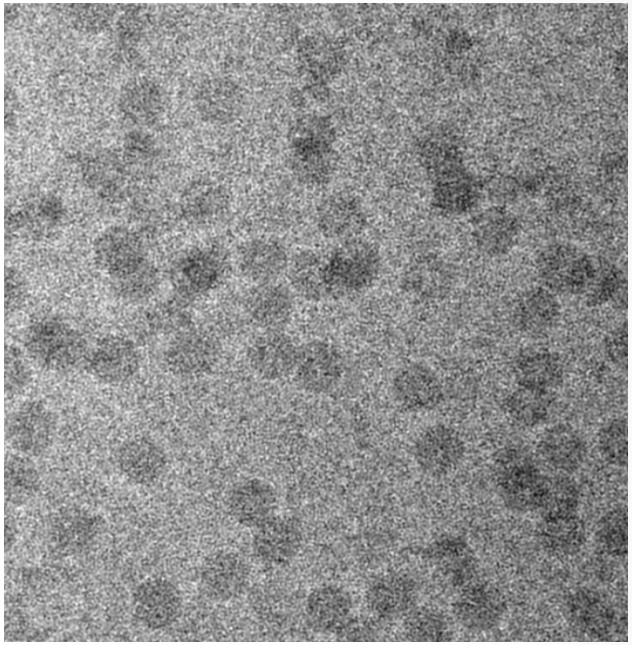
Common lines to find the orientations of 2D particles

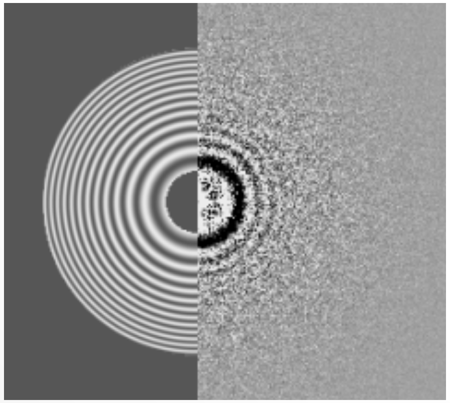
Fourier Bessel method to compute 3D map

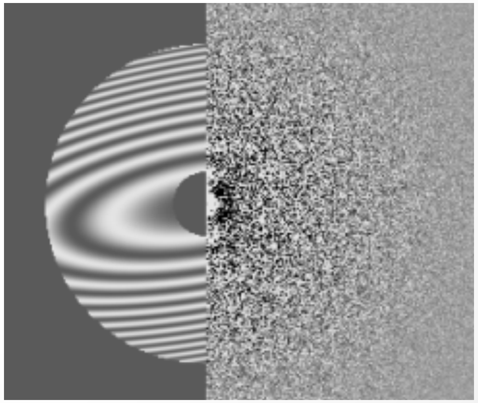
Crowther, 1971

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

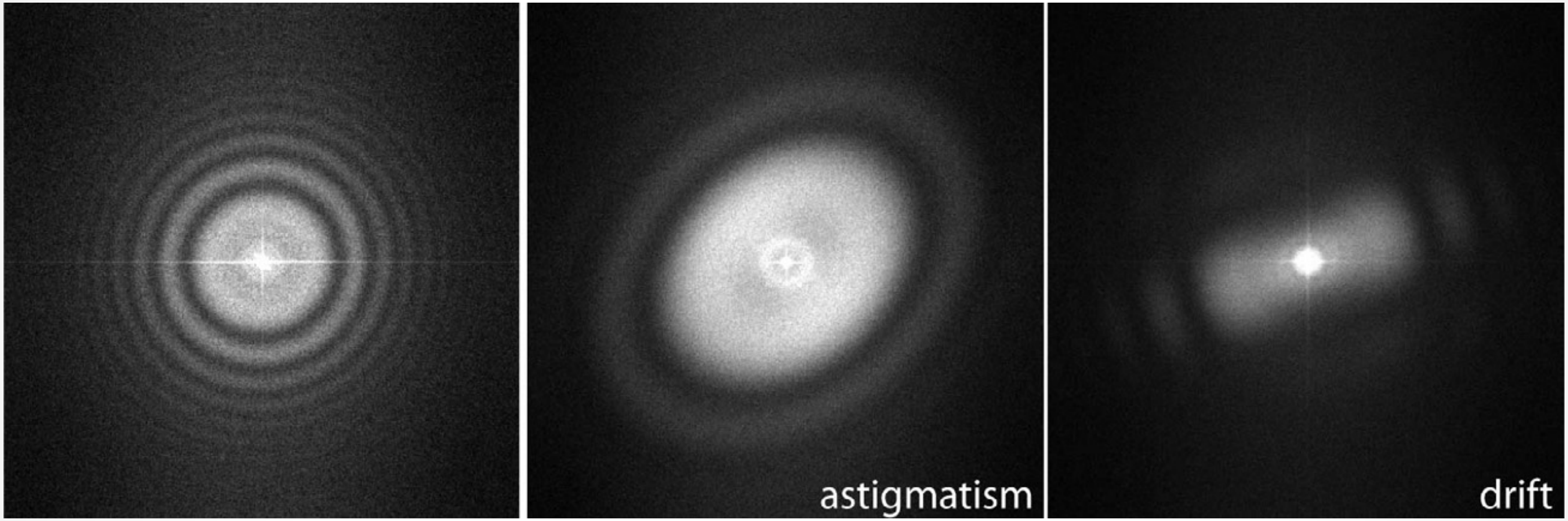






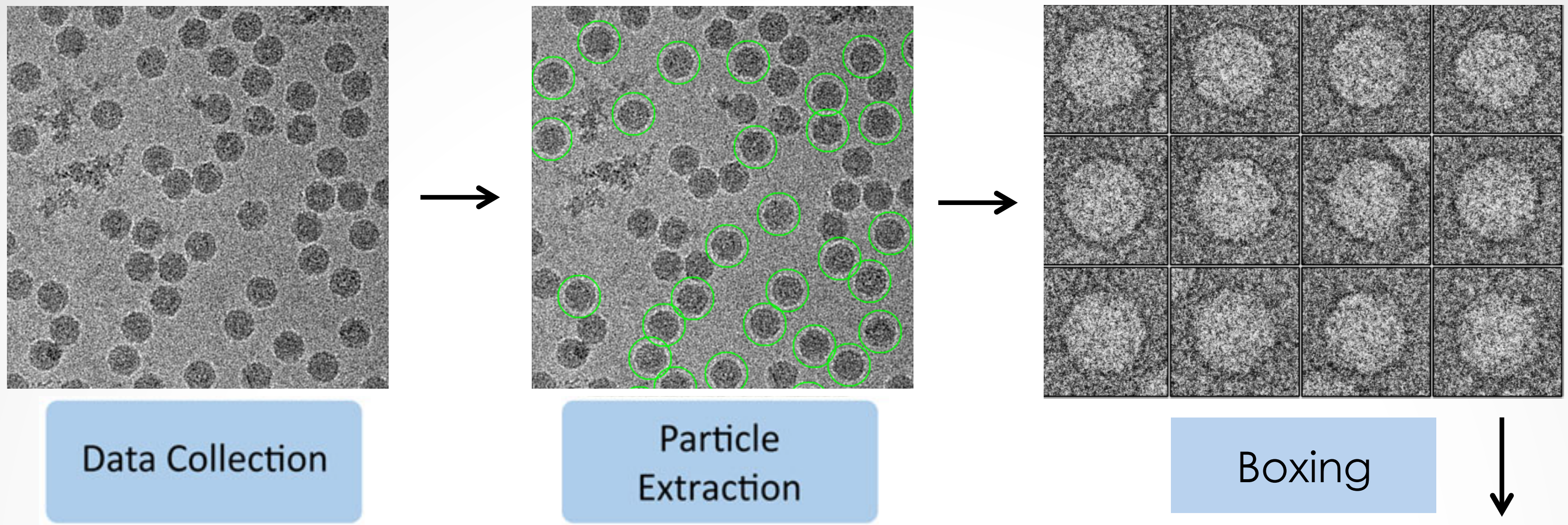


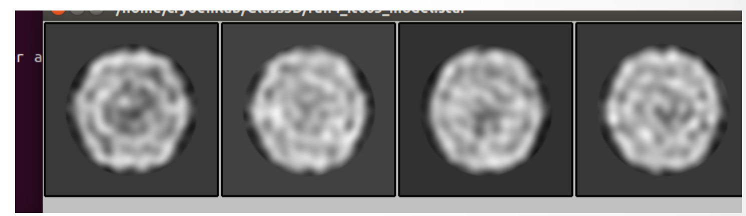
Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

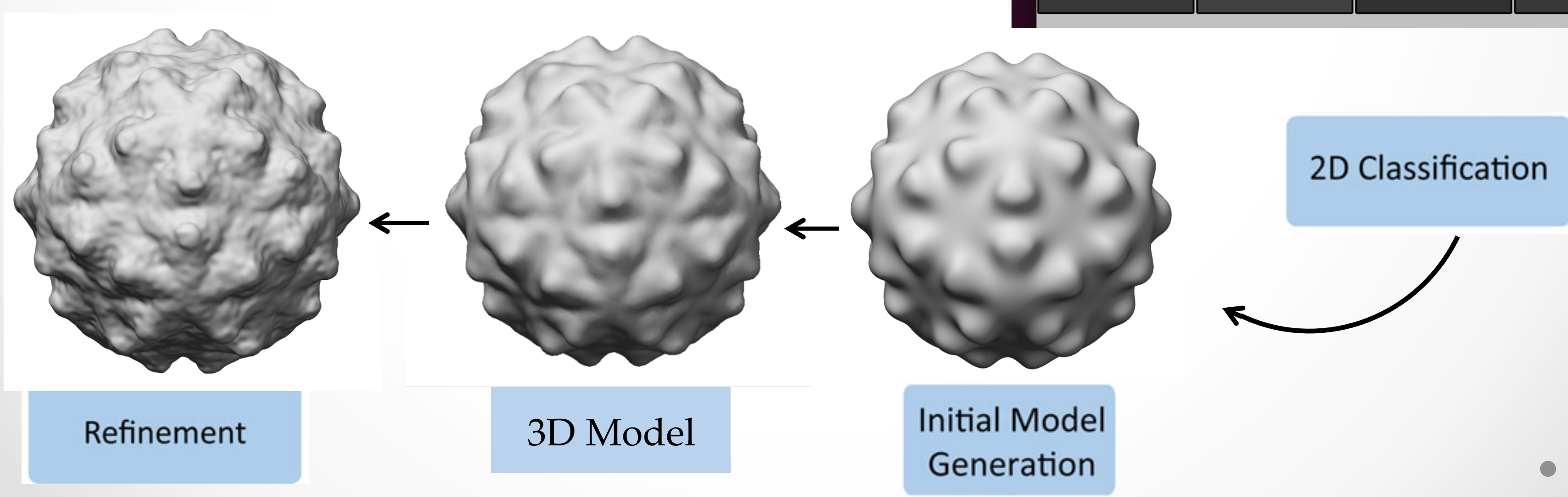


J. M. Carazo *et. al,* Archives of Biochemistry and Biophysics, 2015

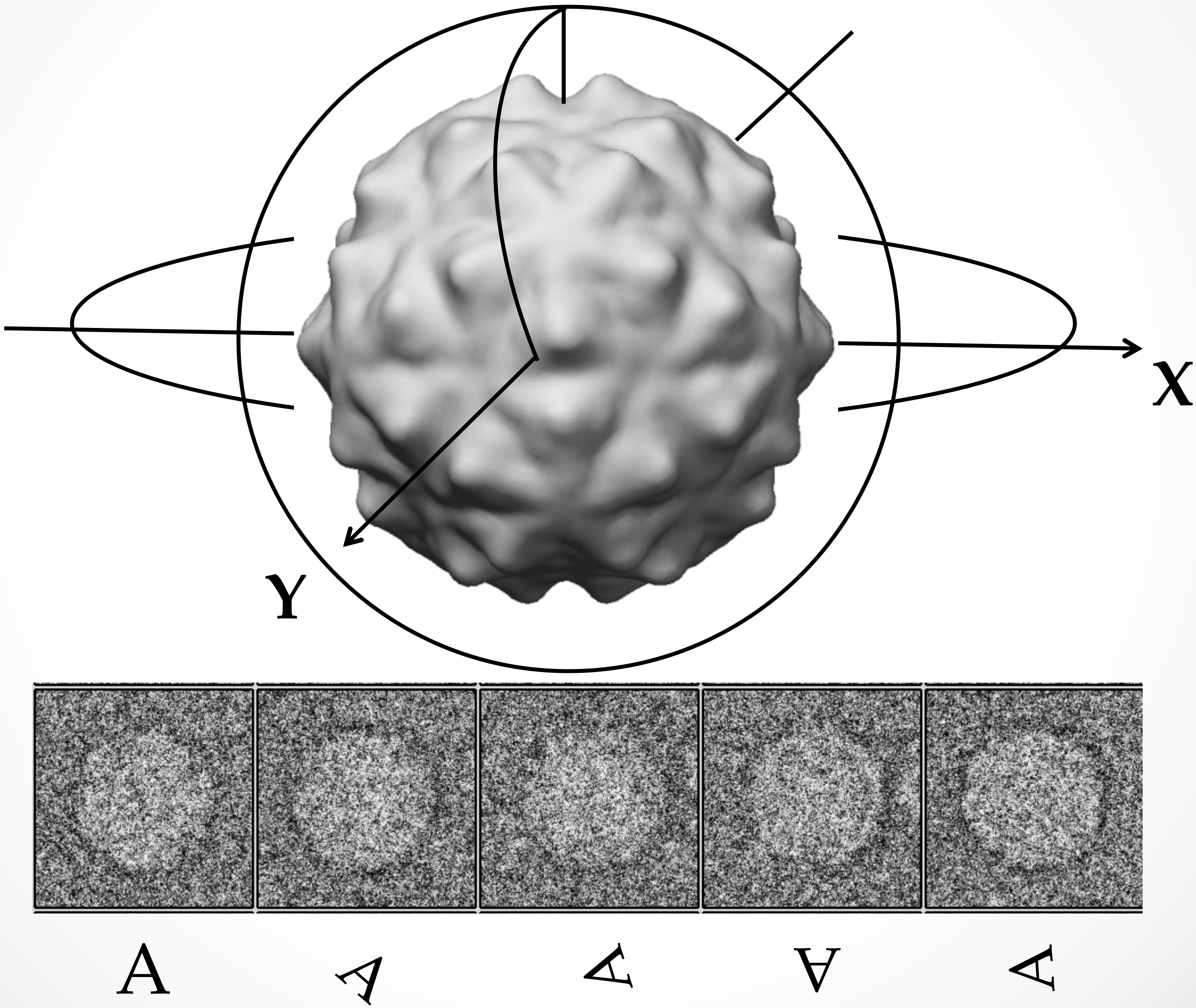
Steps in 3D reconstruction







Cryoelectron microscopy and 3D reconstruction of icosahedral viruses



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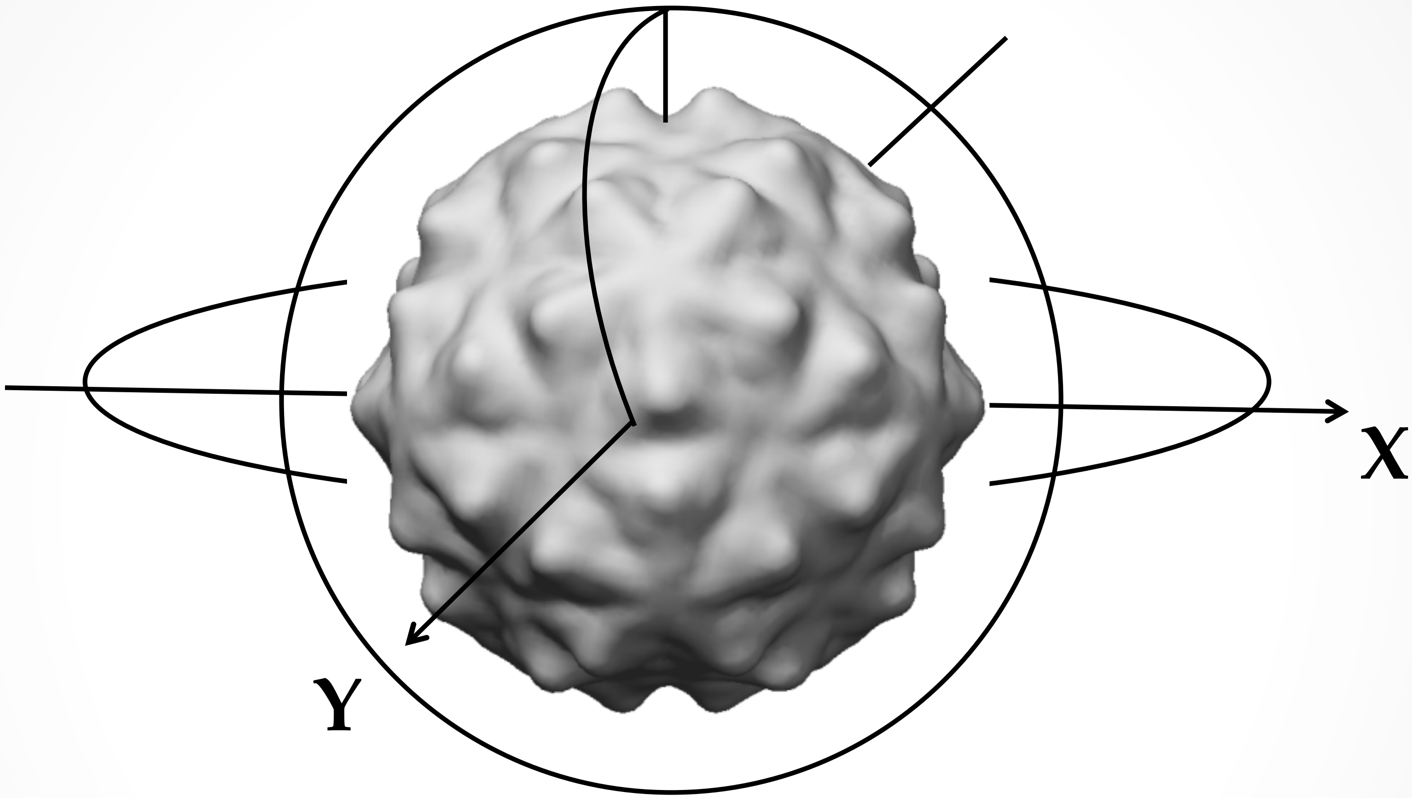


Assignment of 5 spatial parameters to each image:
x, y, θ, Φ, ω

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

How to generate an initial model?

1) Download a density map from EMDB, scale dimensions, filter to keep only icosahedral features

2) Randomly assign icosahedral orientations to a few hundred particles, generate model from raw data

3) Compute self-common lines for a few images, no reference model required

Good data minimizes possibilities of model bias

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

Particle orientation and center determination

Projection matching and class averages

Determine particle's projection angles (θ, Φ), in-plane rotation angle (ω), center (x, y)

Projections matched with particle images

2D class averaging

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

Particle orientation and center determination

Common lines method

60 equivalent icosahedral orientations: each define a plane in Fourier space

The line at which any two planes intersect is a self-common line

37 self common lines for any icosahedral particle

Particle's center should be identified correctly

No model required

Cross-common lines: between two particles

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

Reconstructing a 3D volume

1) Fourier space reconstructions

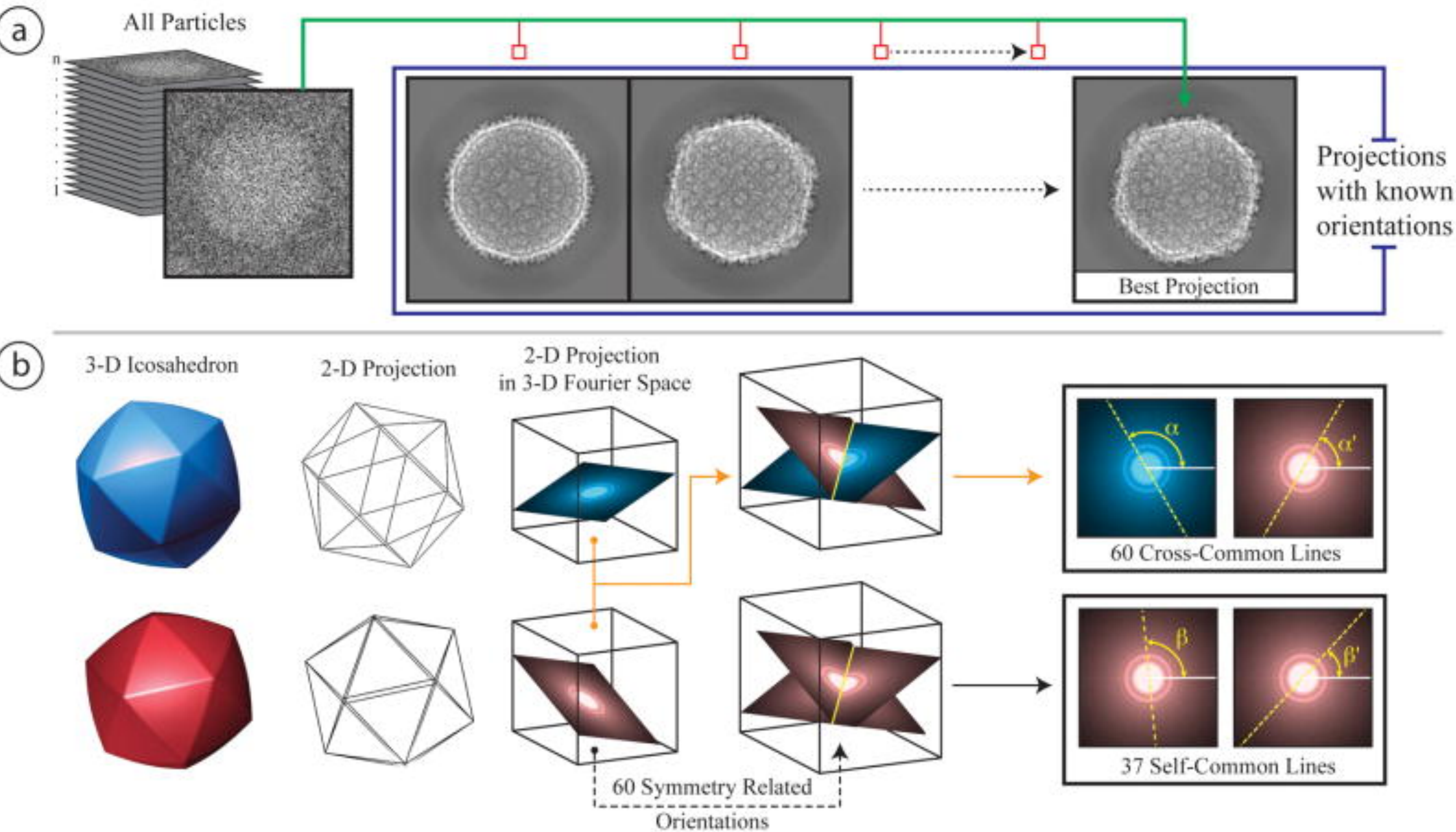
Central section theorem

2D FTs inserted into 3D matrix, based on particle spatial parameters

2) Real space reconstructions

Back projection

Non-crystallographic symmetry averaging: Extracting and averaging similar subunits



Chang *et al.*, Adv Exp Mol Biol, 2012

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

Symmetry-free reconstructions

Density for asymmetric/less symmetric regions blurred out

60-fold more images required

Optimization of SNR of asymmetric regions

Defocus/phase plate

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

How to generate a model for symmetry-free reconstructions?

Low-pass filtering an equivalent structure

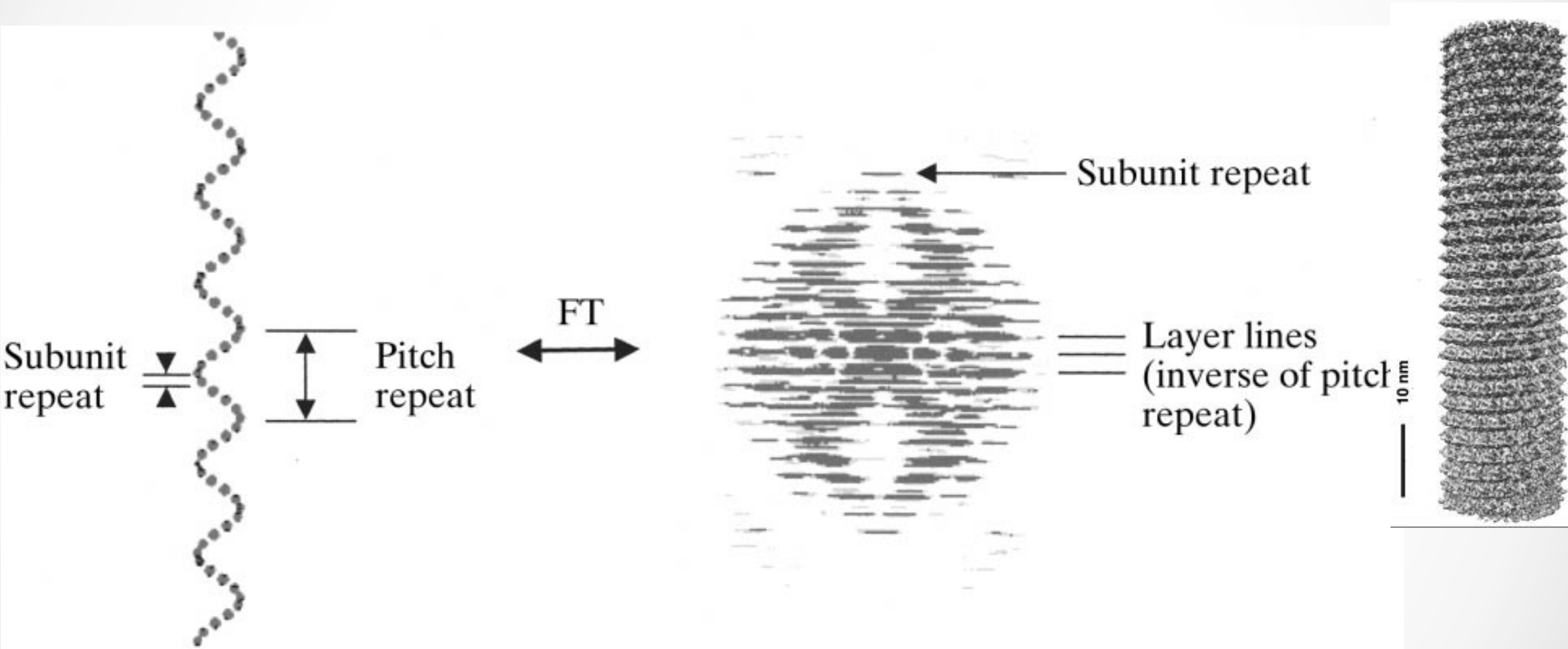
Geometric shape mimicking the component

Extracting shape information by lowering icosahedral threshold of entire map

Orientation assignment, 3D reconstruction

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

Helical reconstruction



Saibil H., Acta Cryst, 2000

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

Helical reconstruction

No of equivalent views a function of length of helix and number of units per helical repeat

In ideal situation, a single image is sufficient for a reconstruction

In reality, all helices distorted

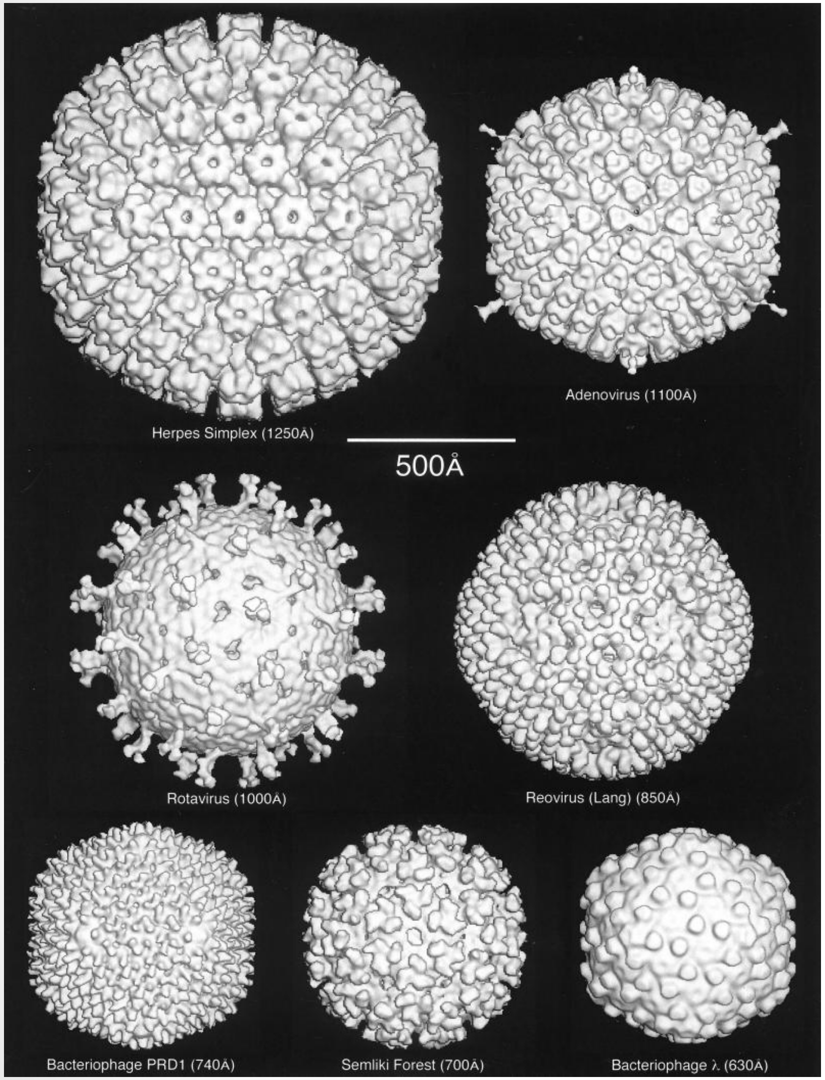
Images of helical fragments divided into short, overlapping segments

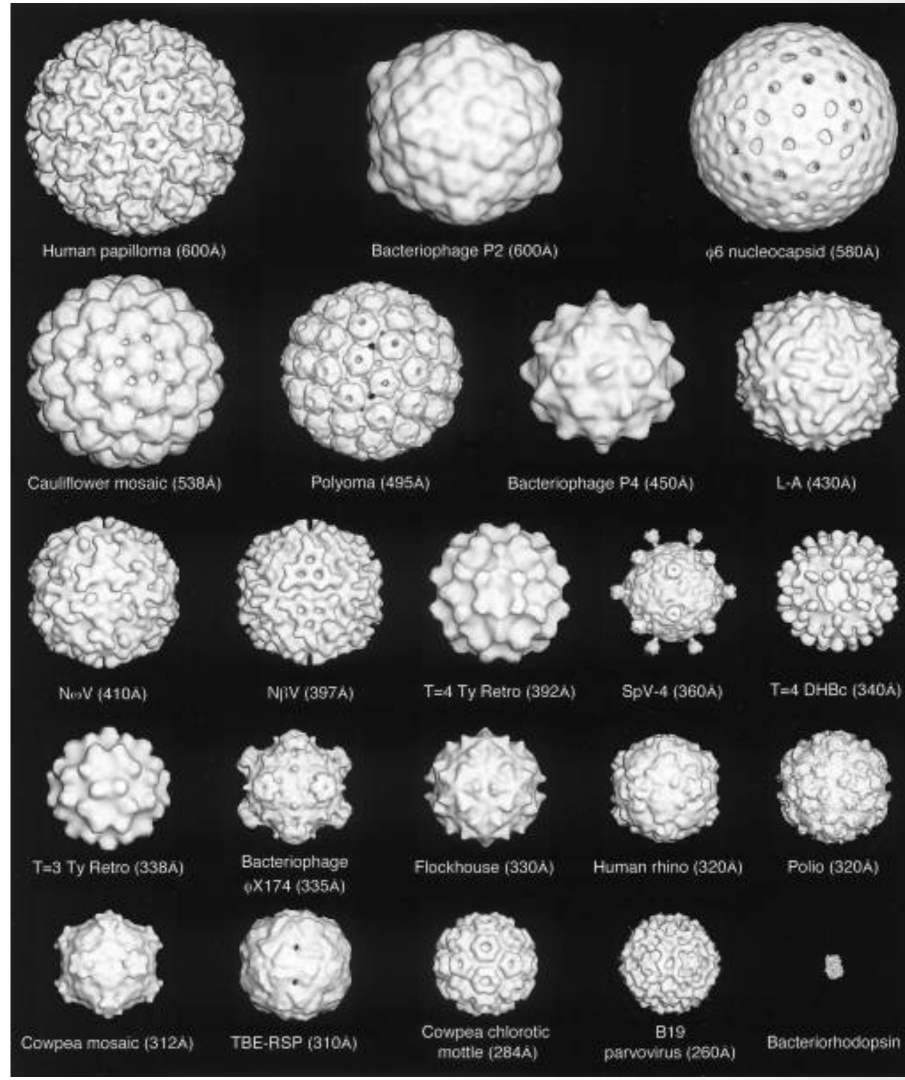
Segments aligned as separate images based on projection matching

Minimization of differences between symmetry-related elements

Egelman, 2012

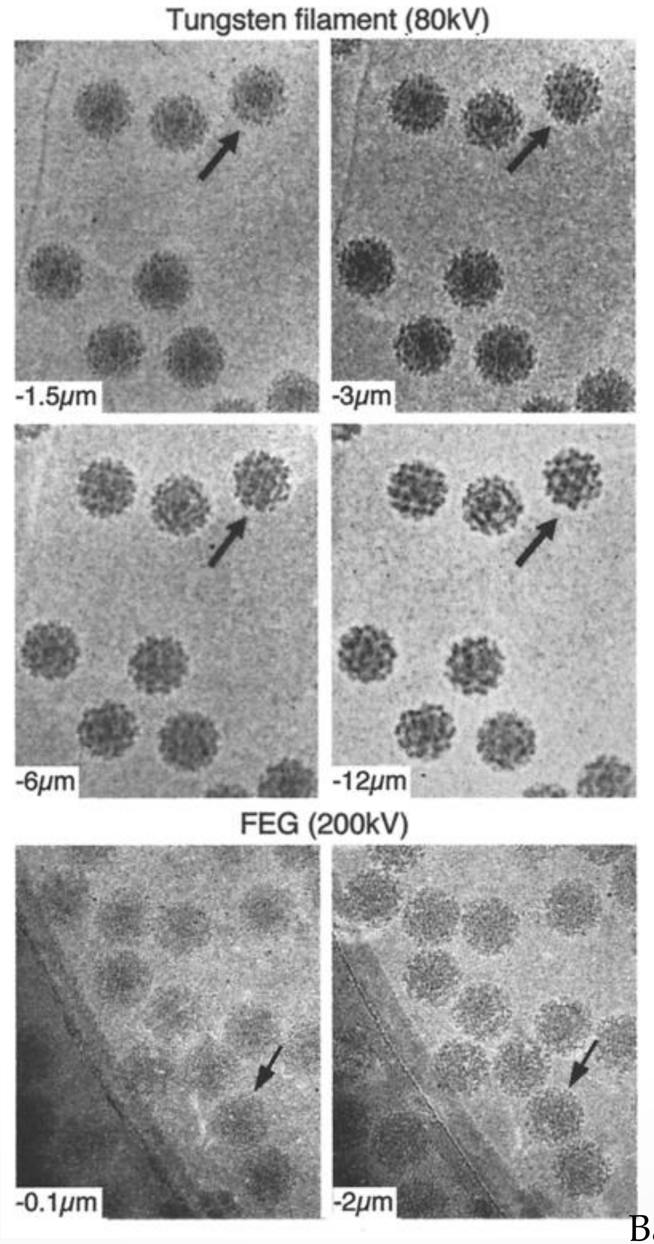
Cryoelectron microscopy and 3D reconstruction of icosahedral viruses





Baker *et al.*, Microbiol Mol Biol Rev, 1999

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses



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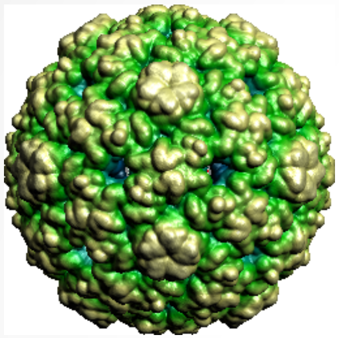
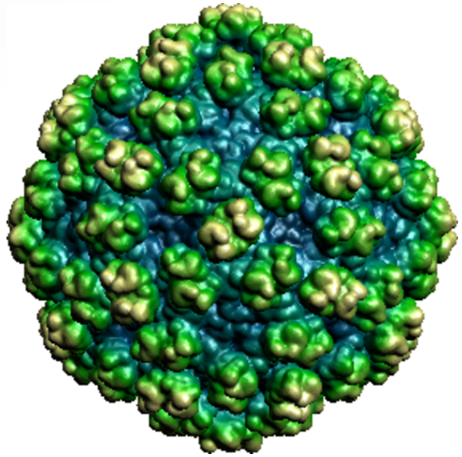
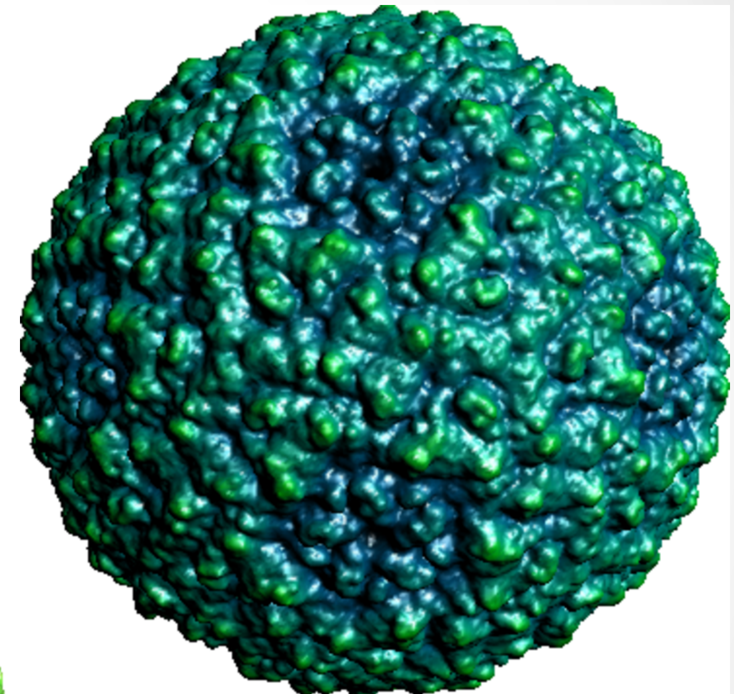
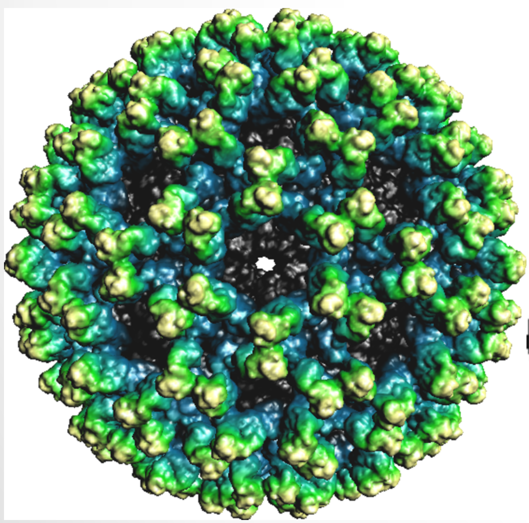
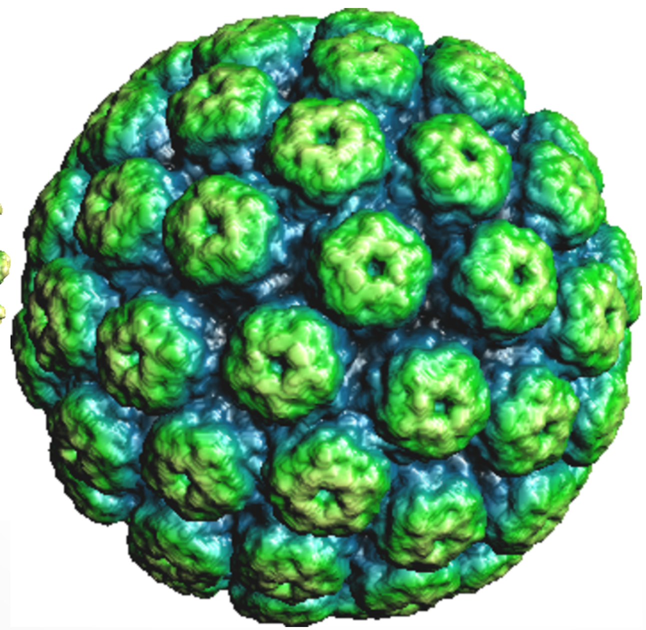




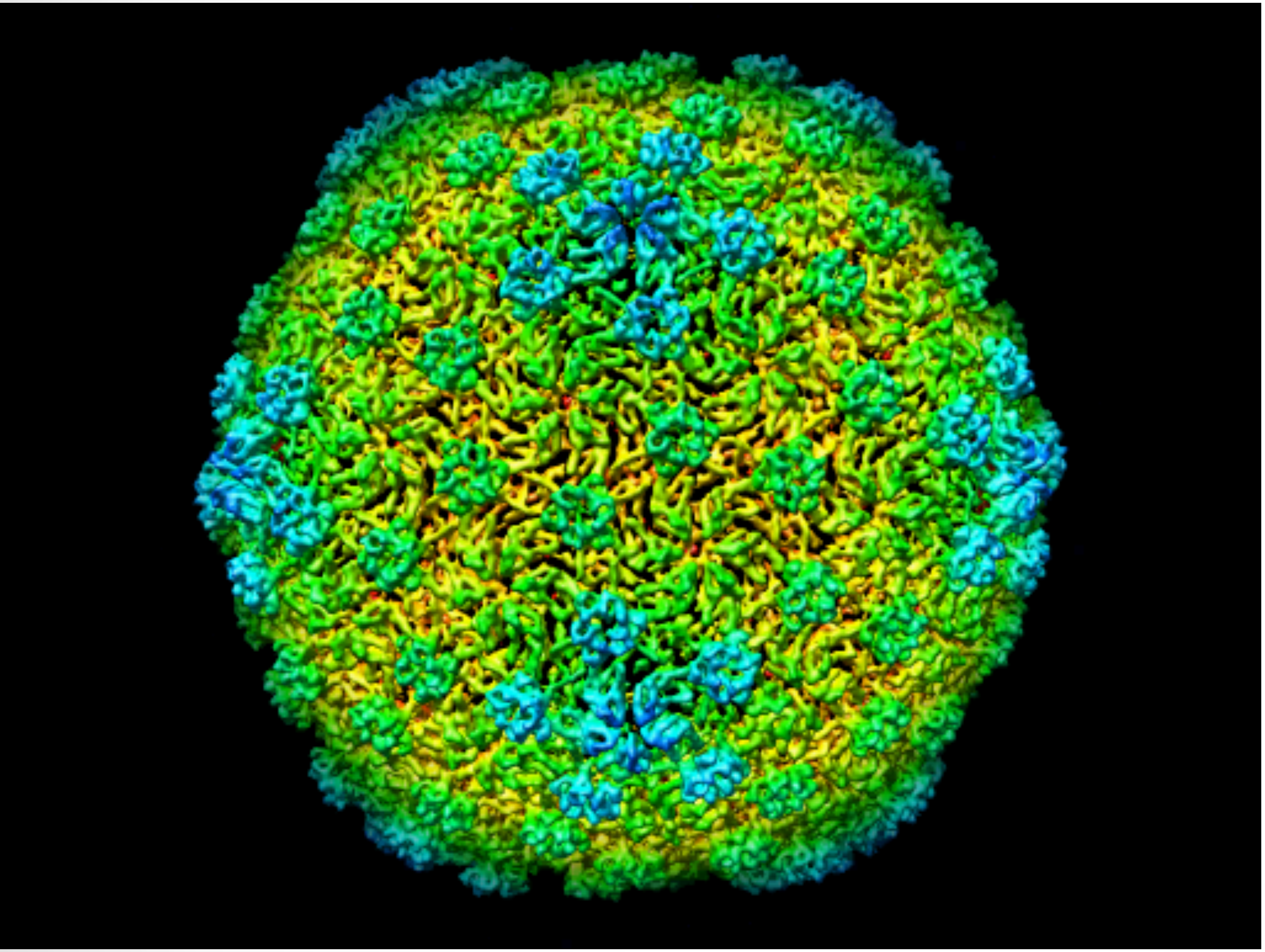






VIPERdb

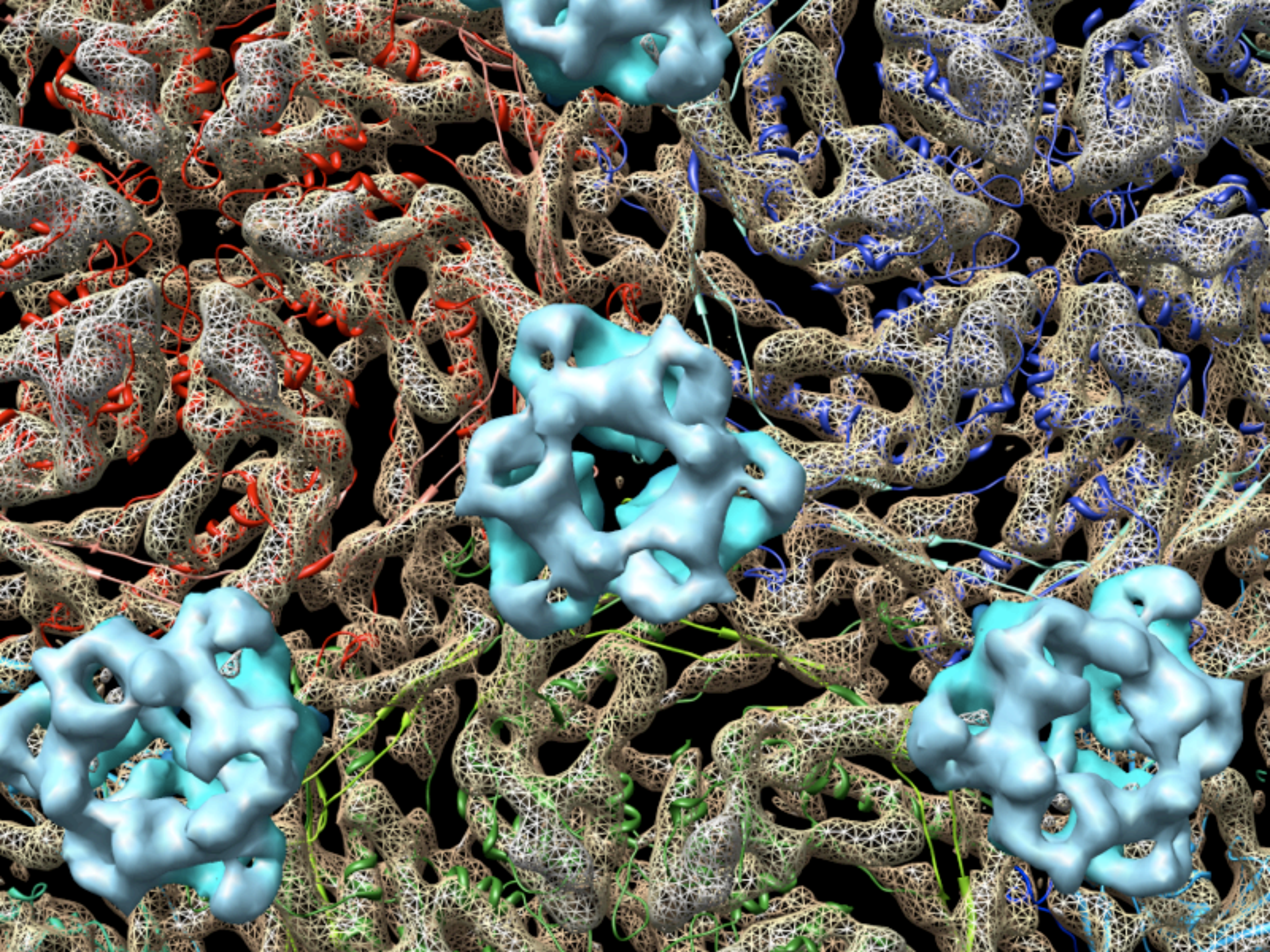
Cryoelectron microscopy and 3D reconstruction of icosahedral viruses



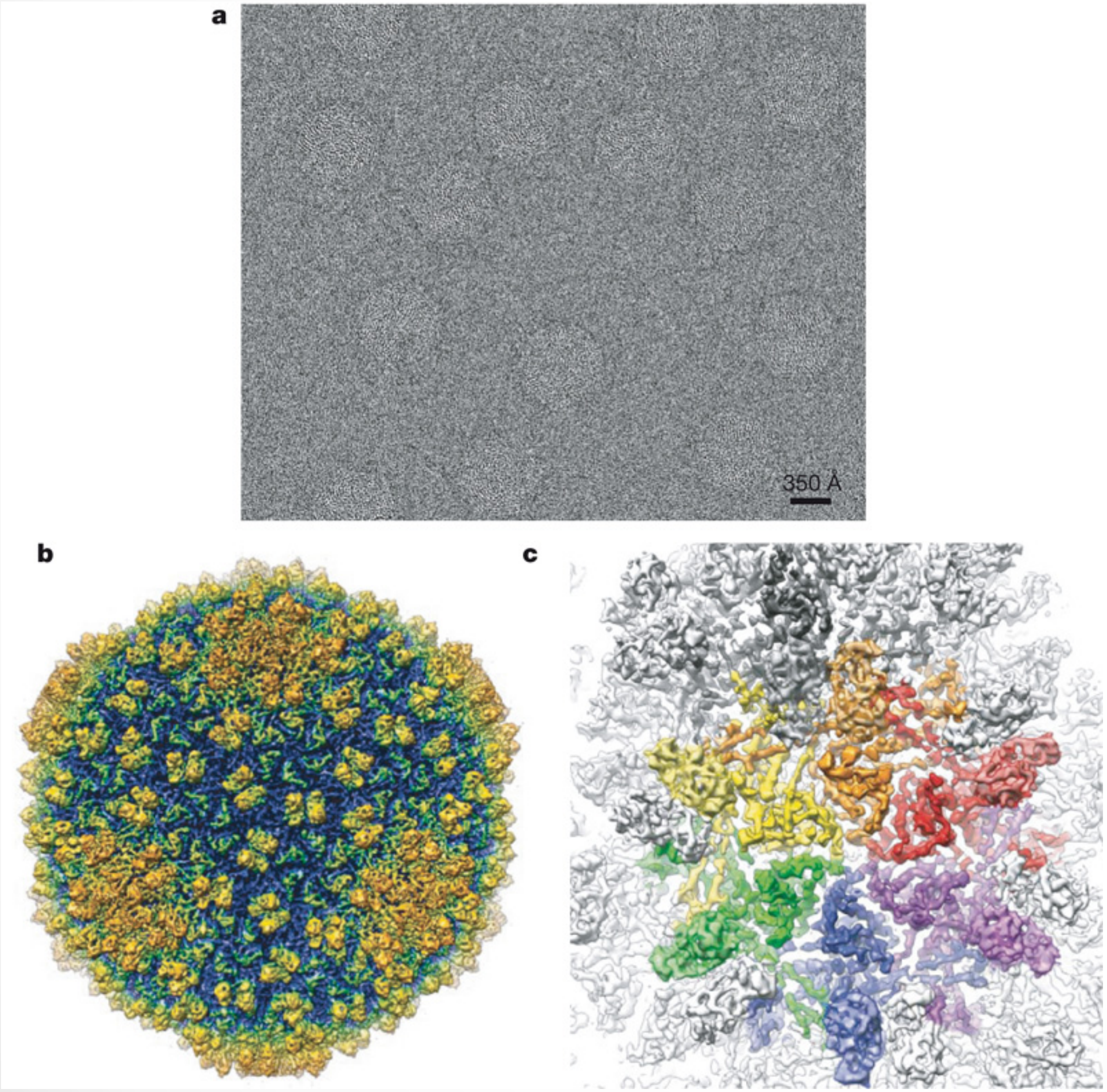
Subnanometer structures

Lambda phage stabilization by gpD

Lander *et al.*, Structure, 2008



Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

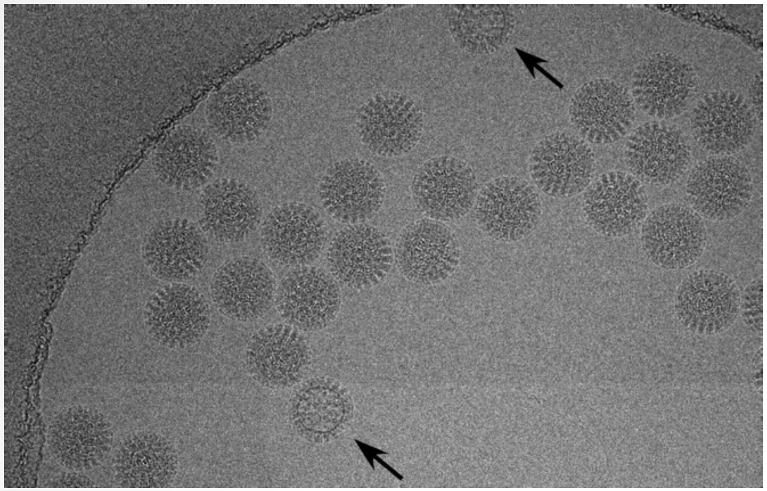


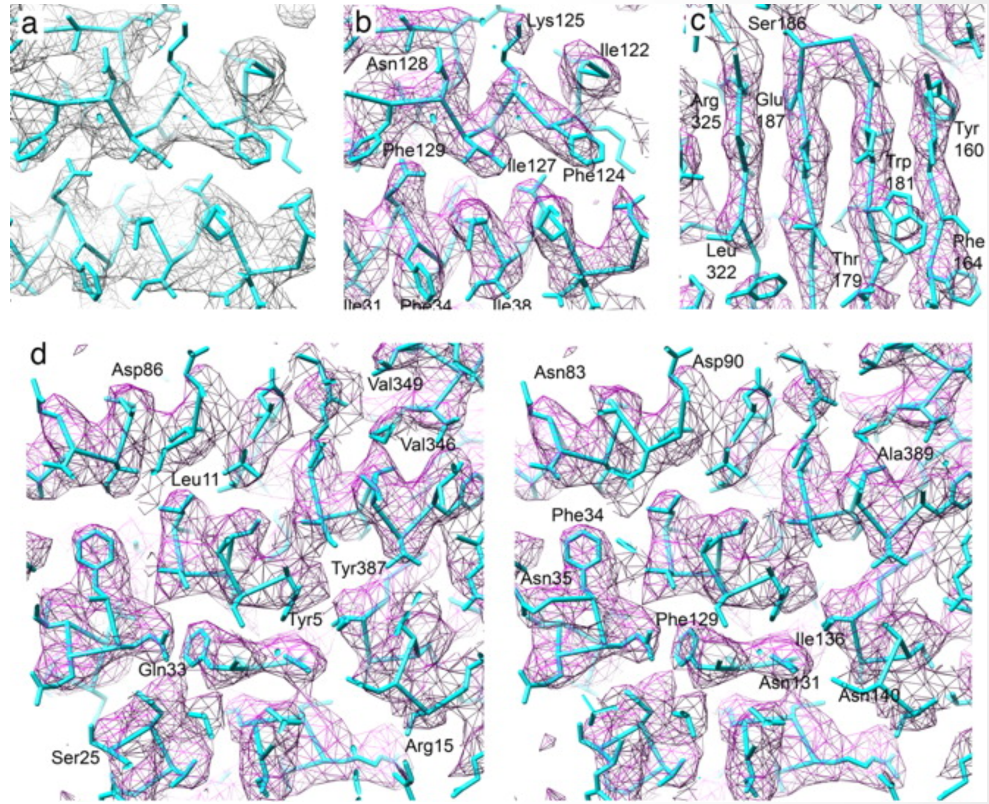
Epsilon15 virus at 4.5 Å

Jiang *et al.*, Nature, 2008

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

Rotavirus at 3.5 Å

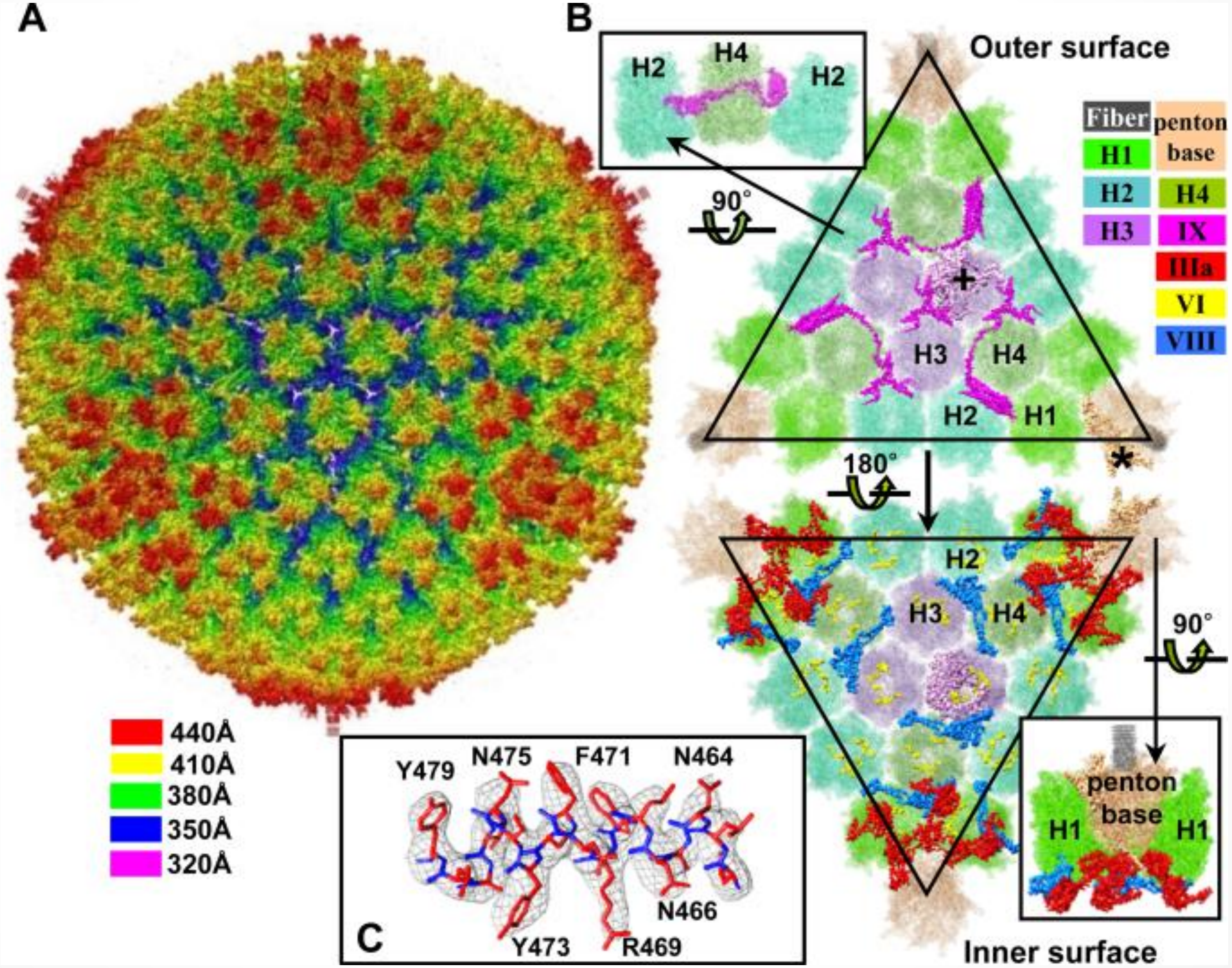




Zhang *et al.*, PNAS, 2008

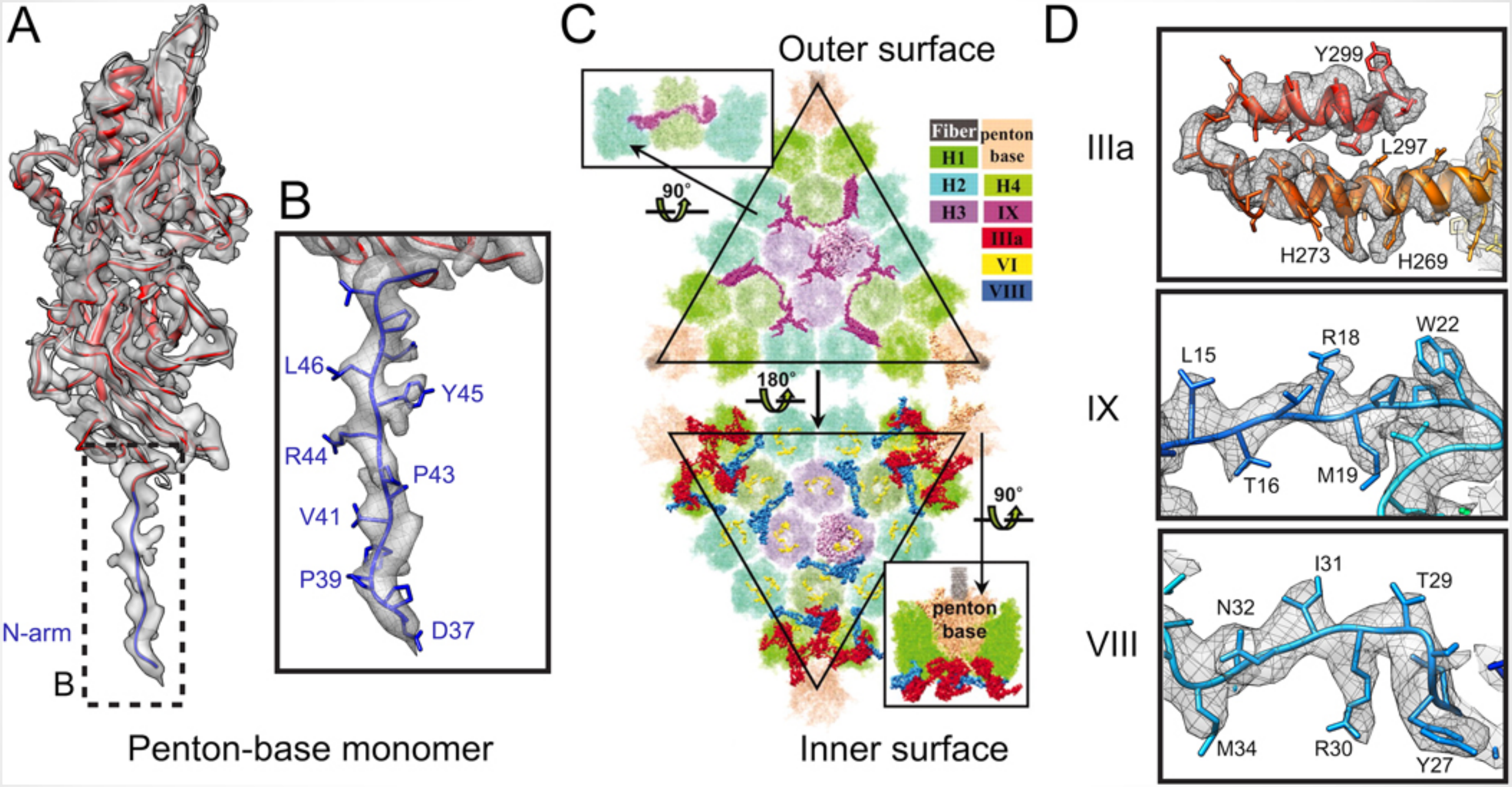
Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

Adenovirus at 3.6 Å



Liu *et al.*, Science, 2010

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses



Liu *et al.*, Science, 2010

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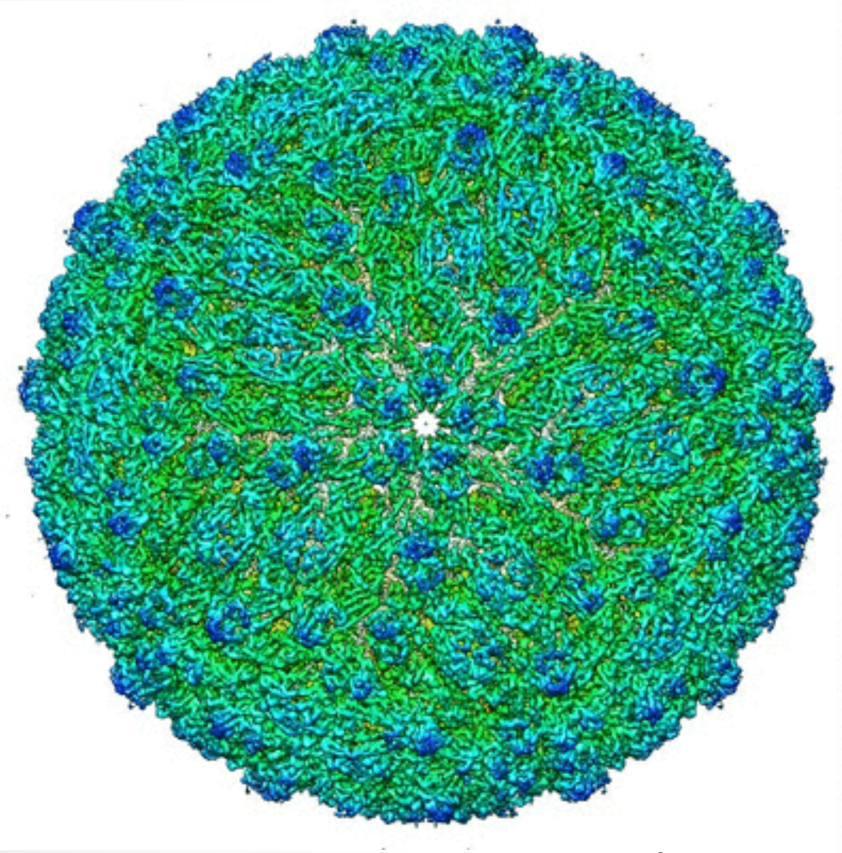
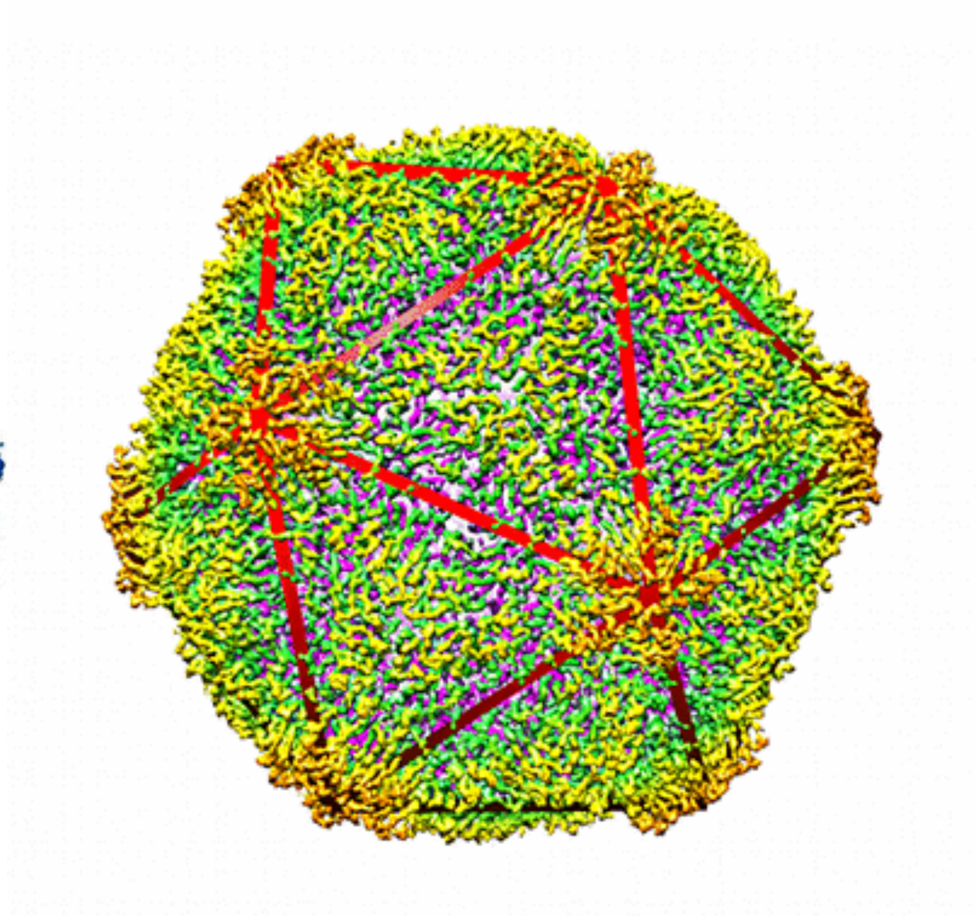


Zika virus at 3.7 Å
EMD8139
Kostyuchenko *et al*., Nature, 2016



Grapevine fanleaf virus at 2.8 Å
EMD3246

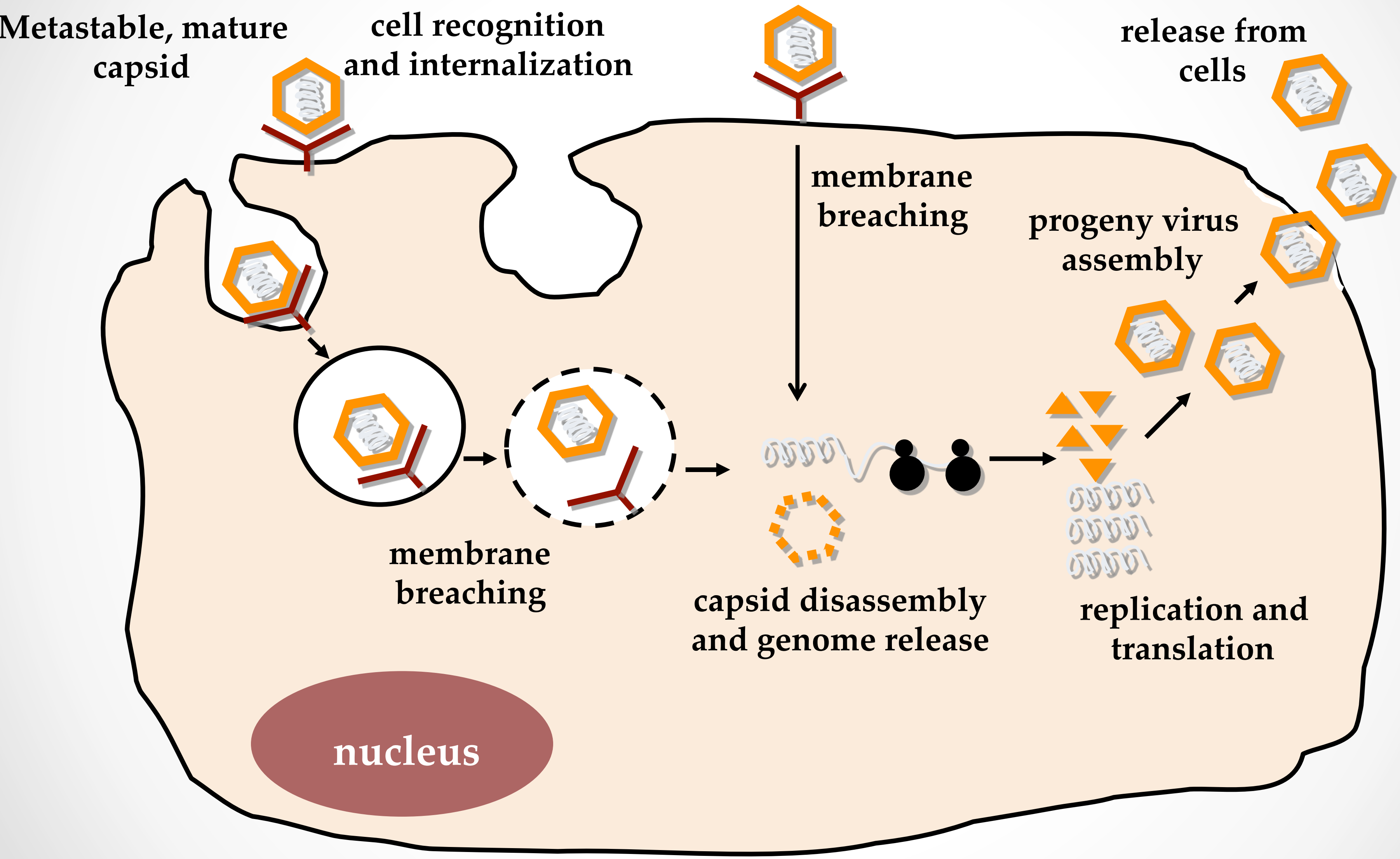
Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

Advantages of electron microscopy

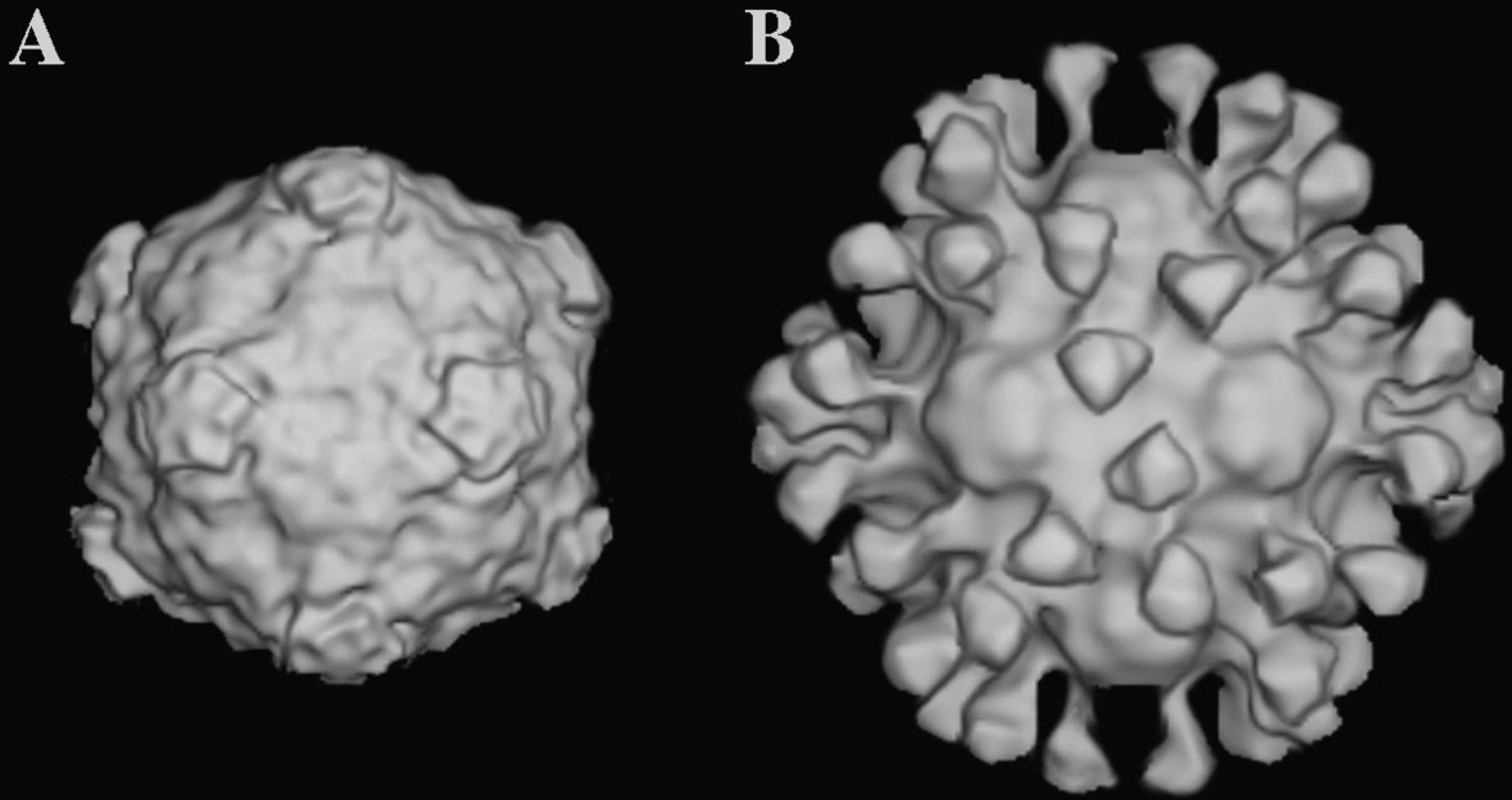
To capture unstable intermediates
- Difficult to crystallize
- Difficult to freeze crystals
- Data incompleteness

To capture dynamic processes by time-resolved methods

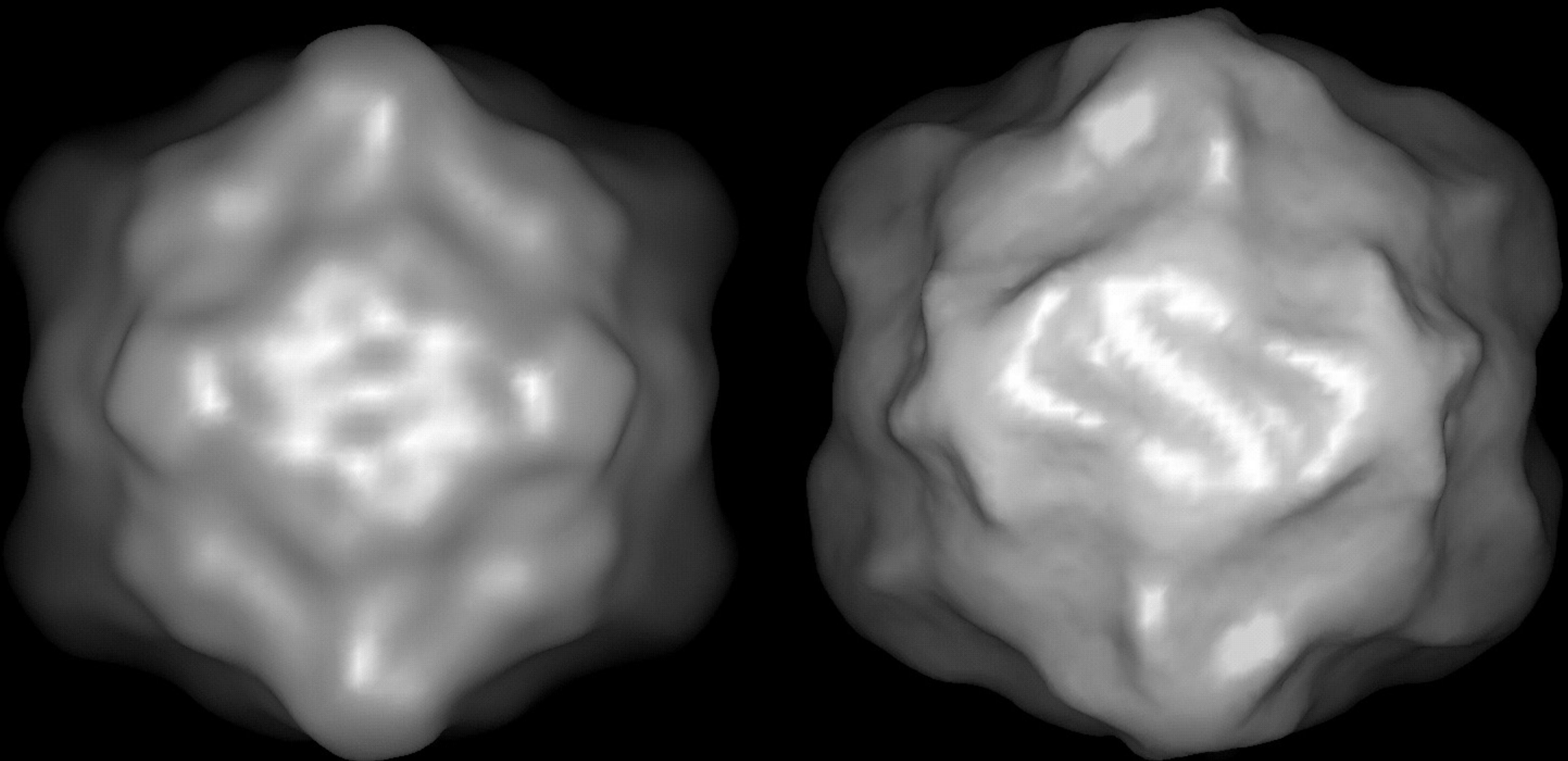
Virus life cycle



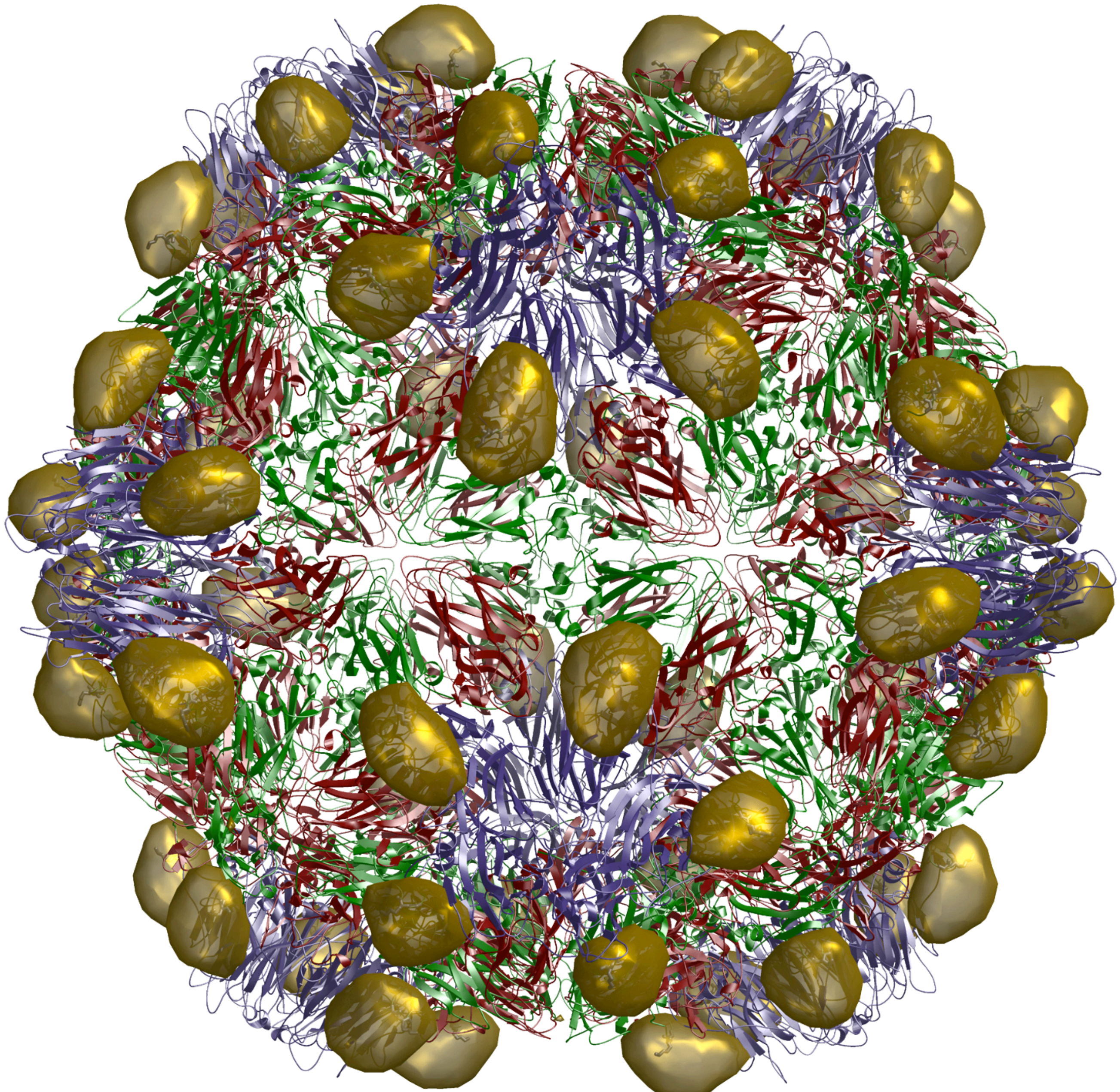
Binding of receptors/antibodies/other molecules to virus particles



Fab fragments from a monoclonal antibody bound to Cowpea Mosaic Virus (Wang *et al.*, Nature, 1992)



Nanogold bound to Cowpea Mosaic Virus
(Wang *et al*., Agnew Chem, 2002)



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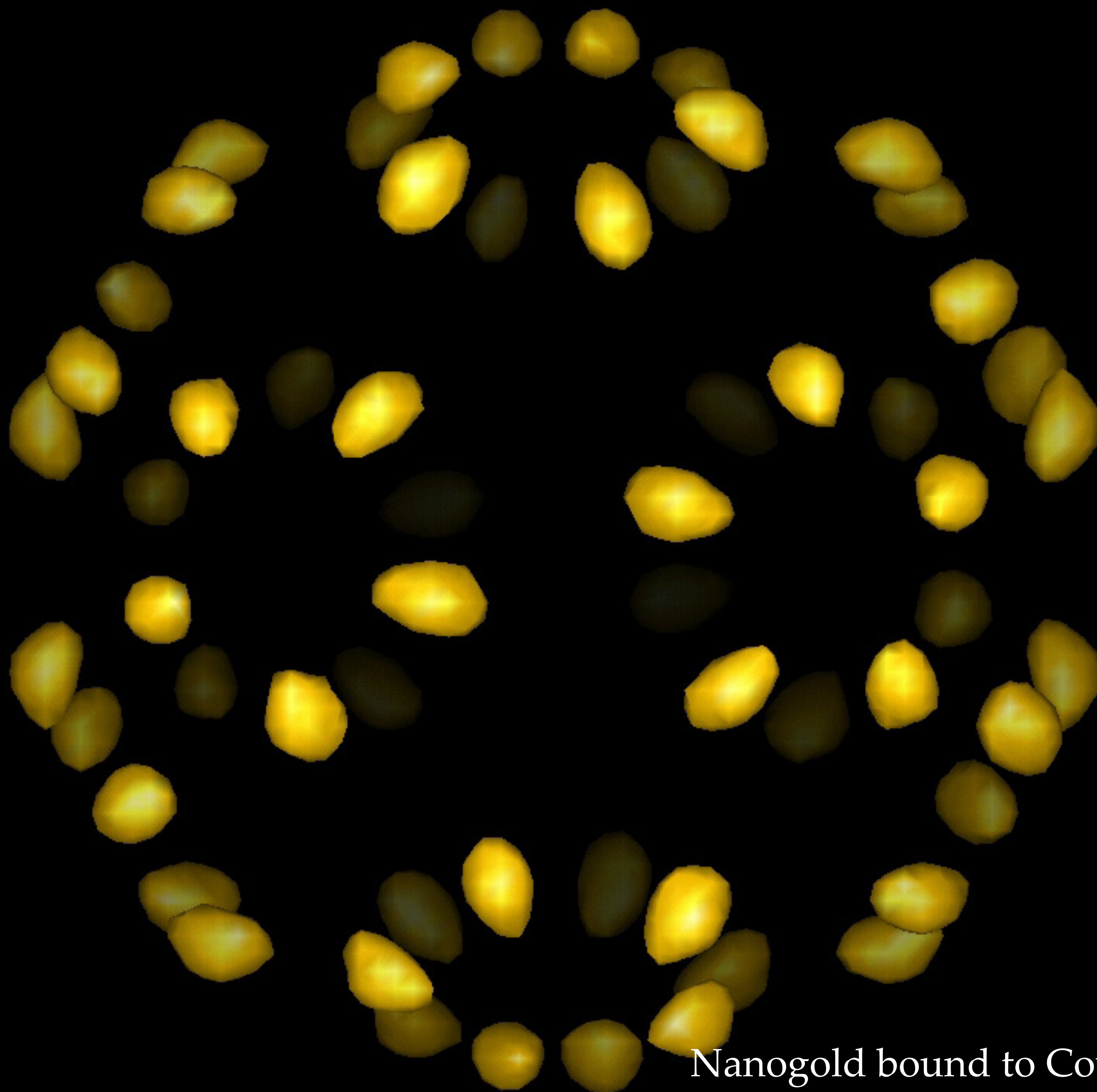

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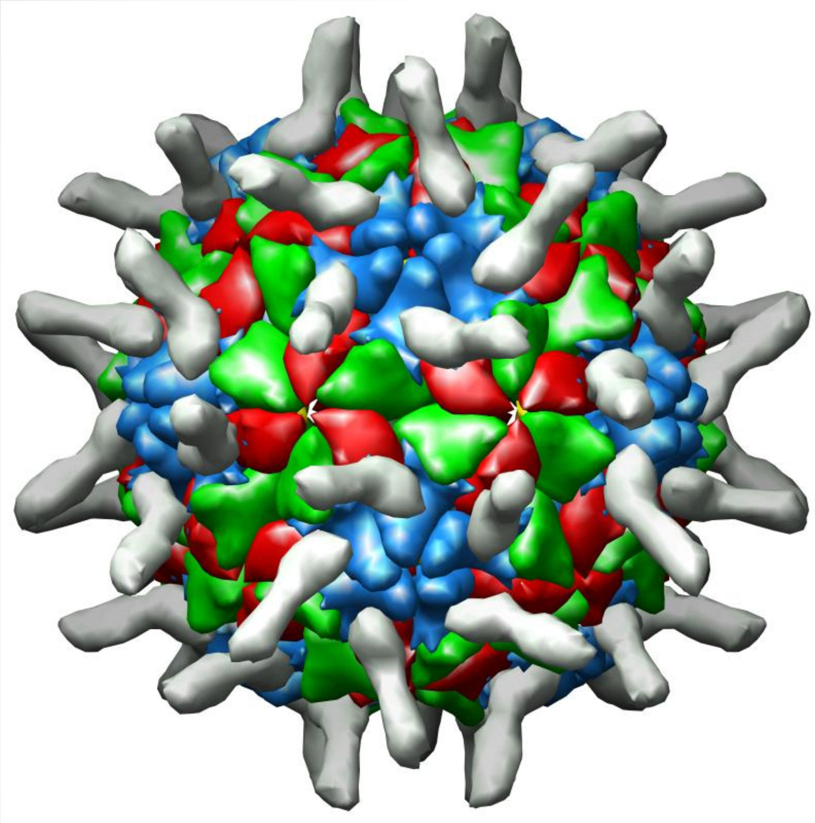
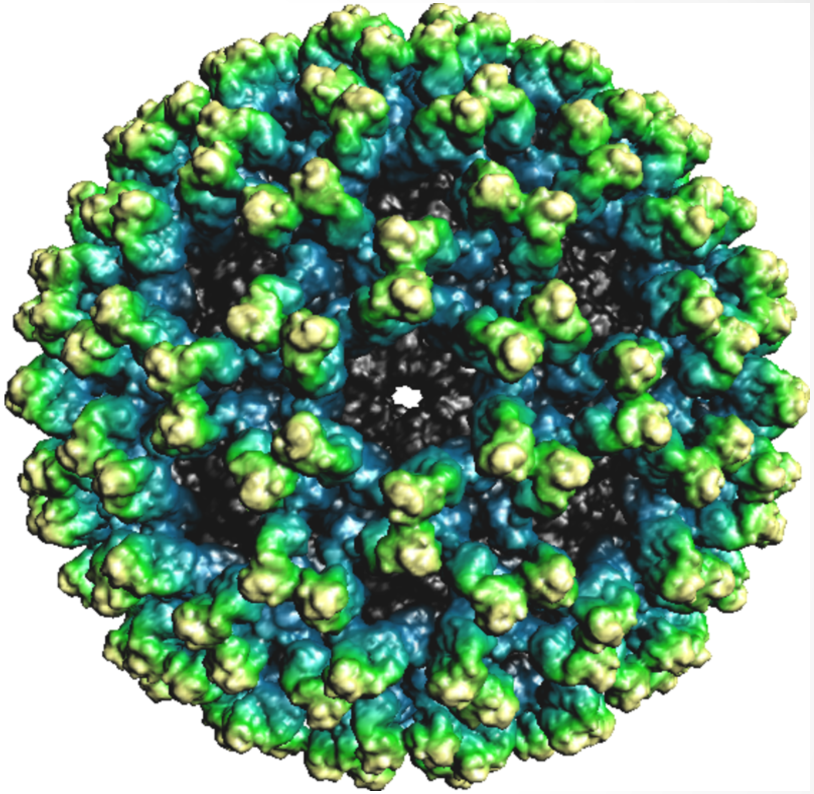
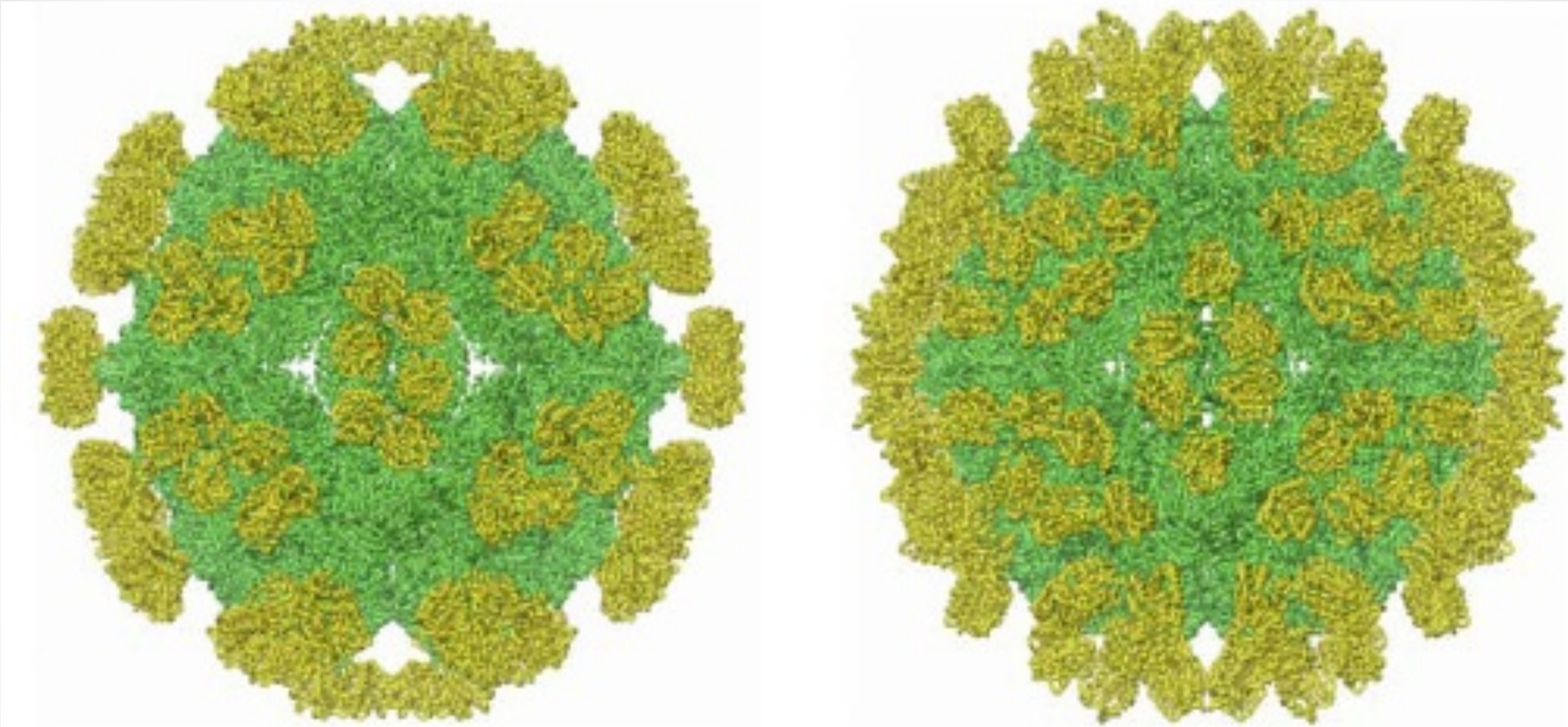


Poliovirus bound to receptor



CHIKV bound to antibody

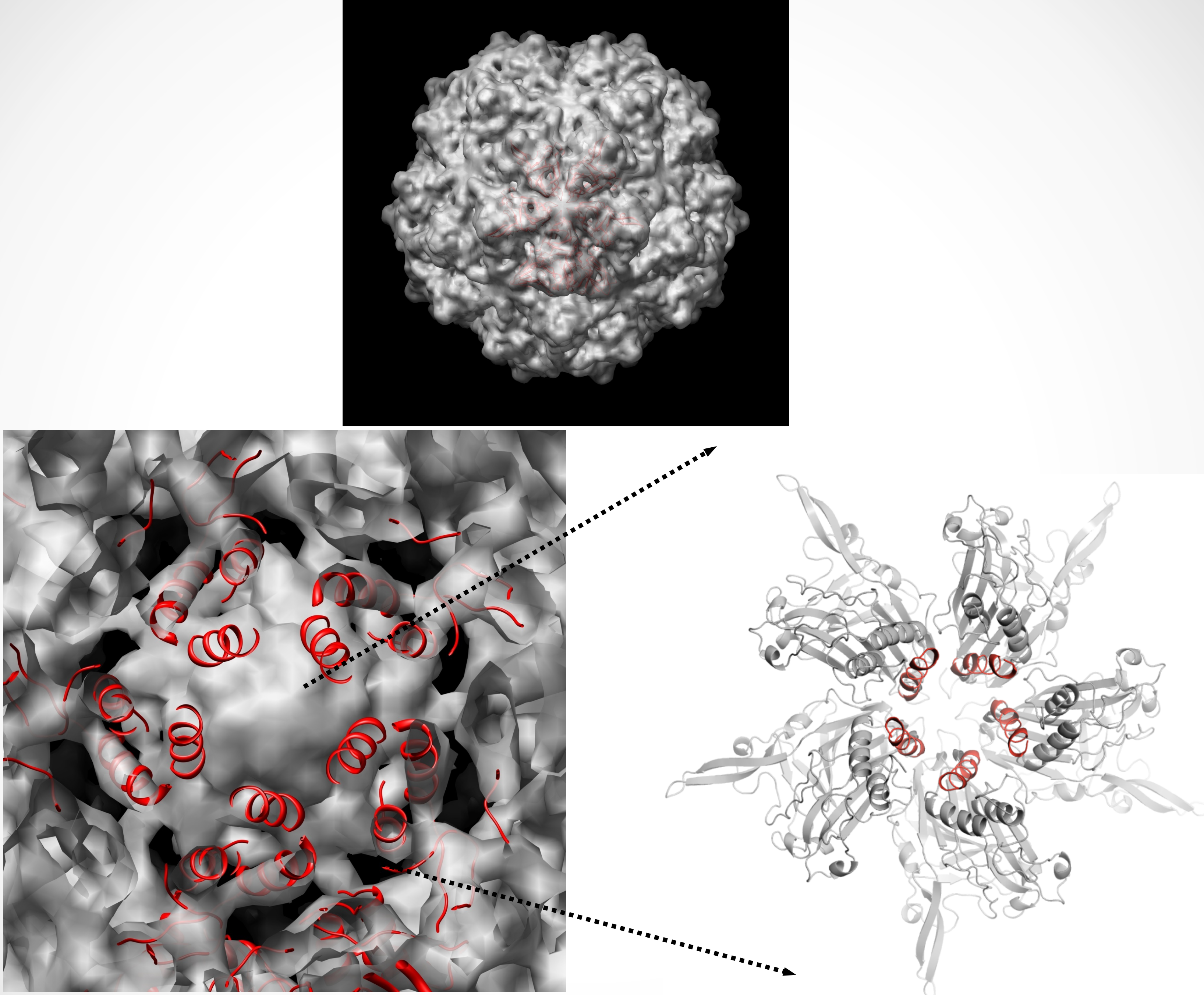
Zhang *et al.*, PNAS, 2008
VIPERdb

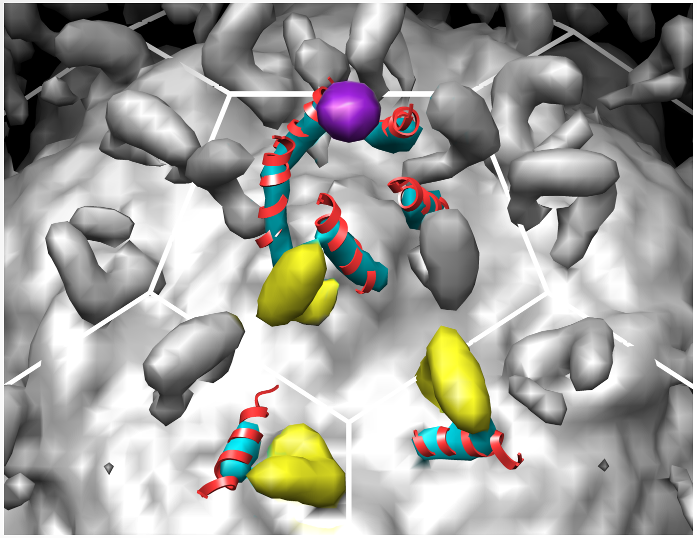


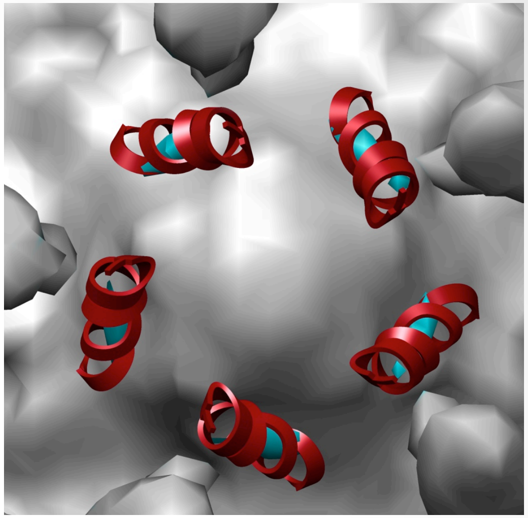
FHV conjugated to anthrax toxin receptor

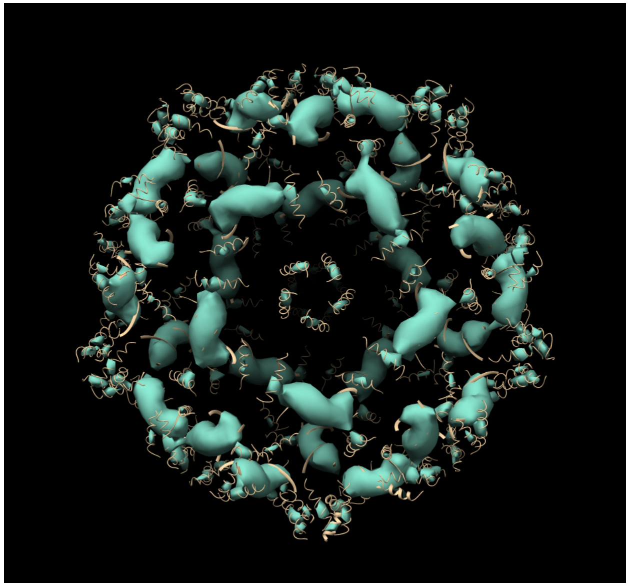
Manayani *et al.*, PLoS Pathogens, 2007

Membrane penetration – effect of quasi-equivalent peptides



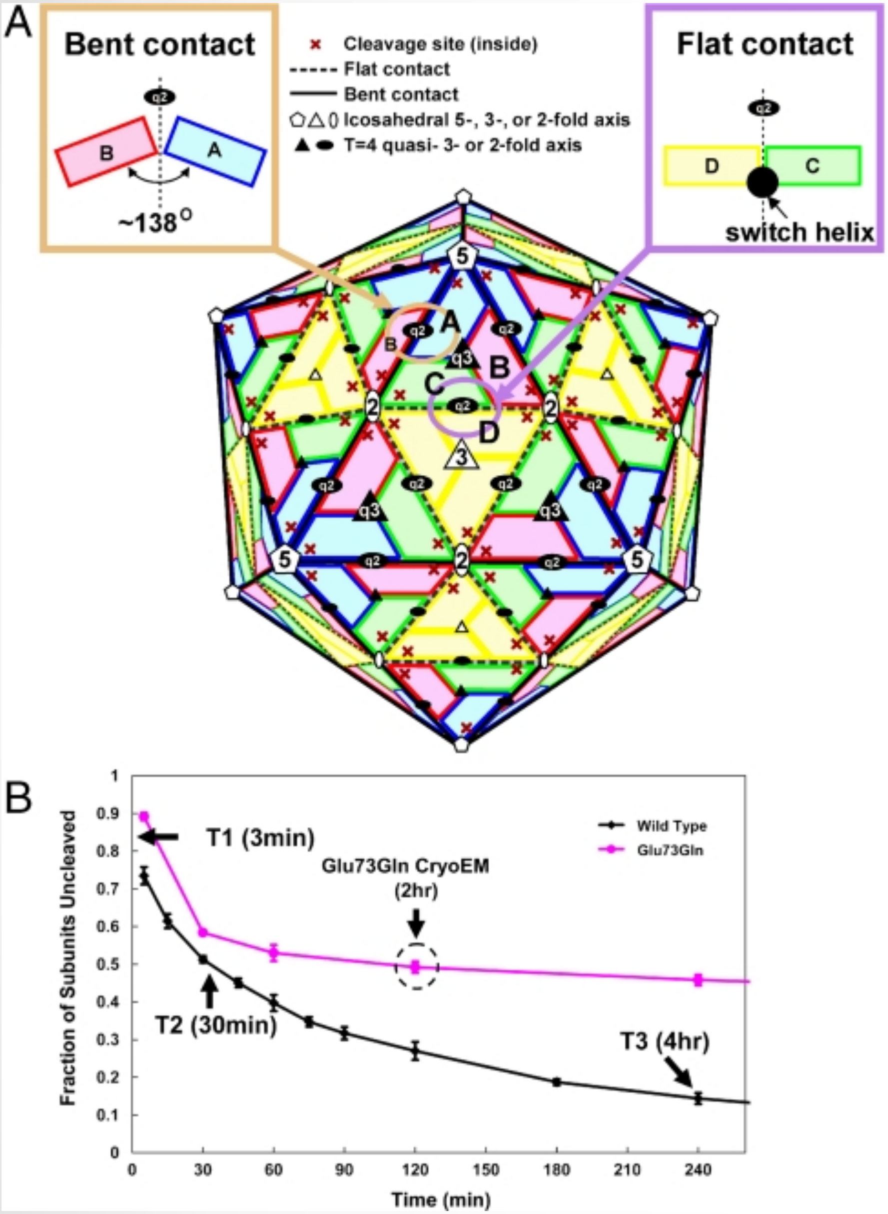


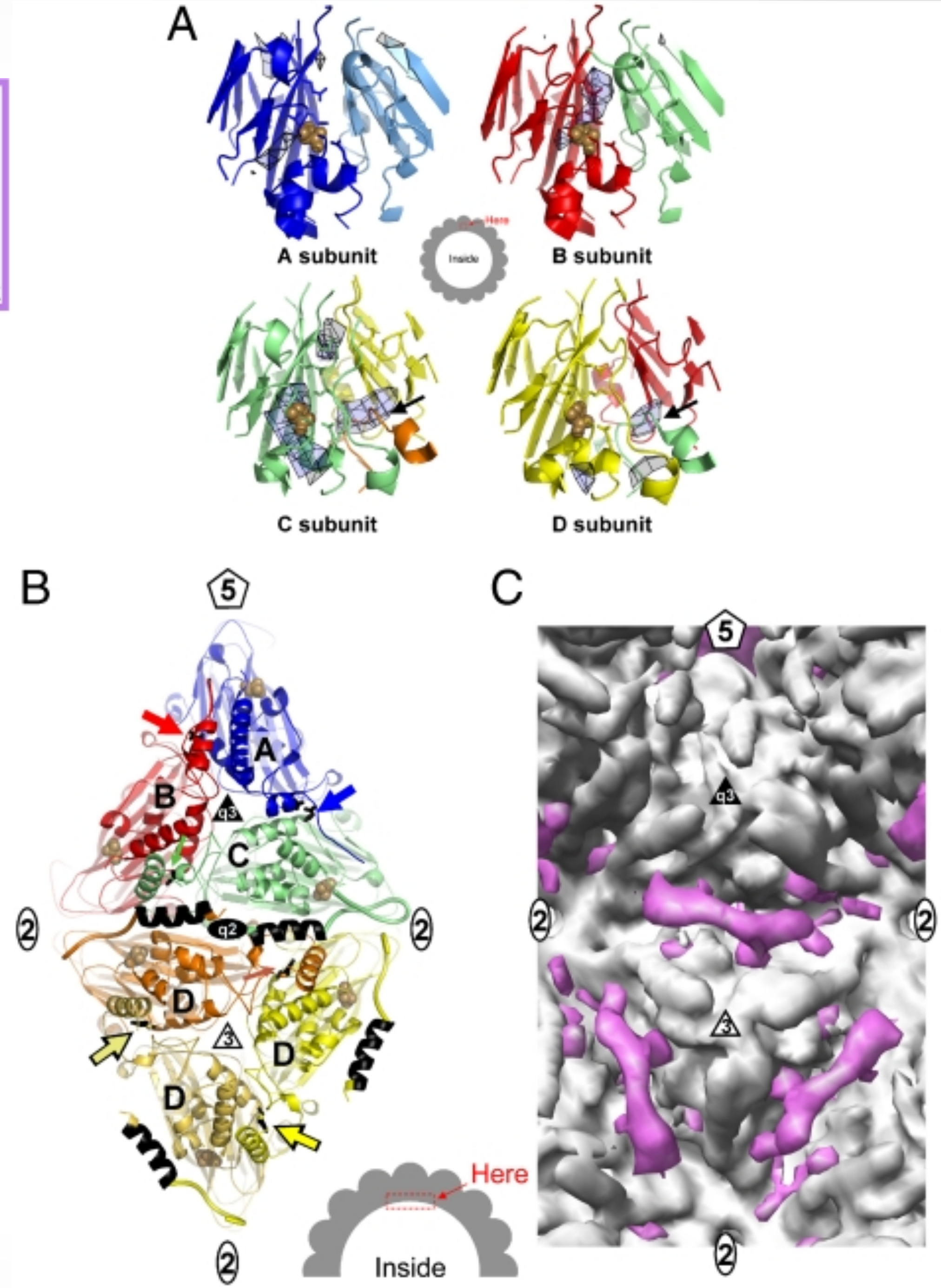




Banerjee *et al*, J Virol, 2009
Bajaj *et al*, JMB, 2016

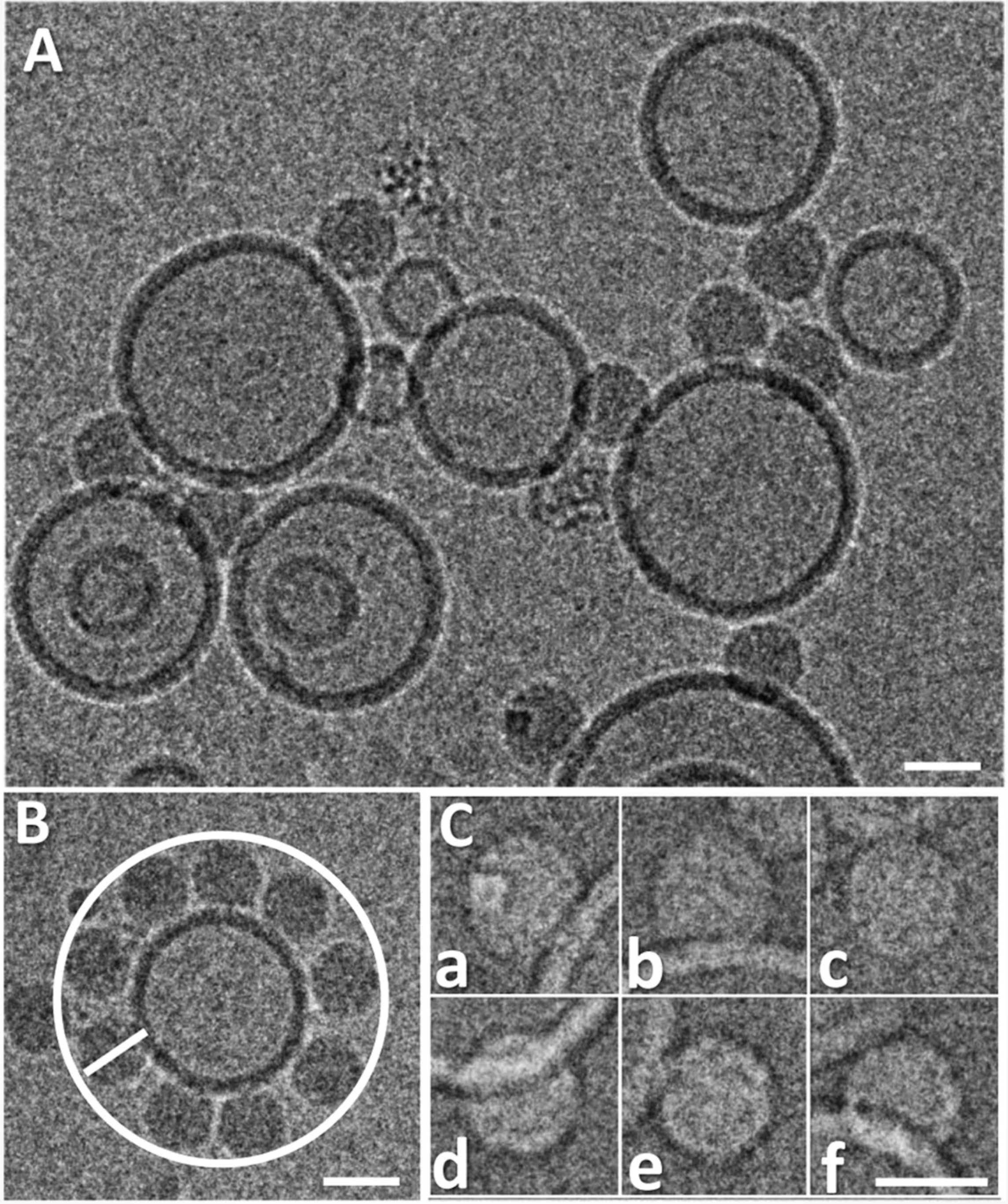
Time-resolved membrane penetration



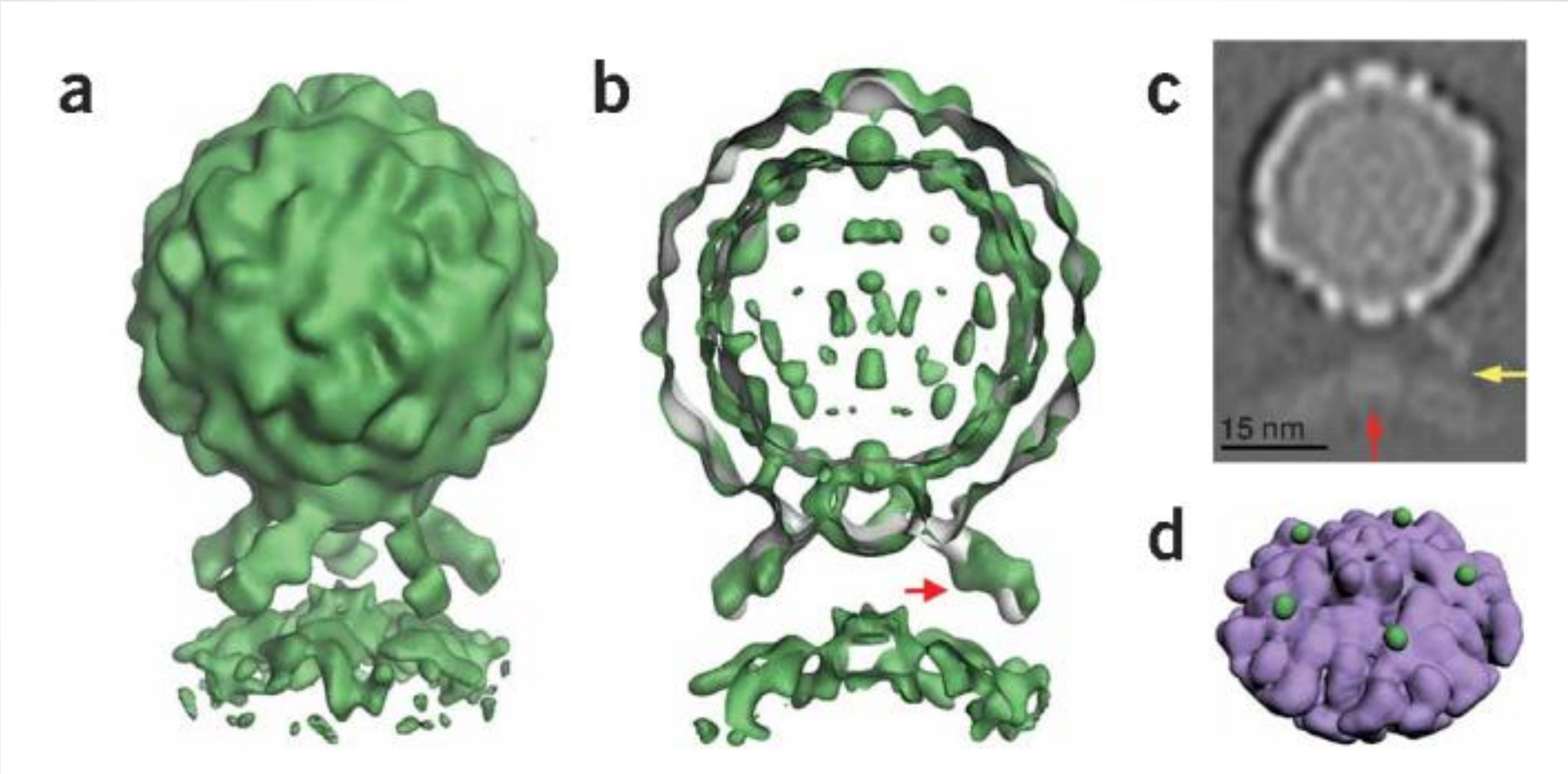


Matsui *et al.*, PNAS, 2012

Membrane interaction



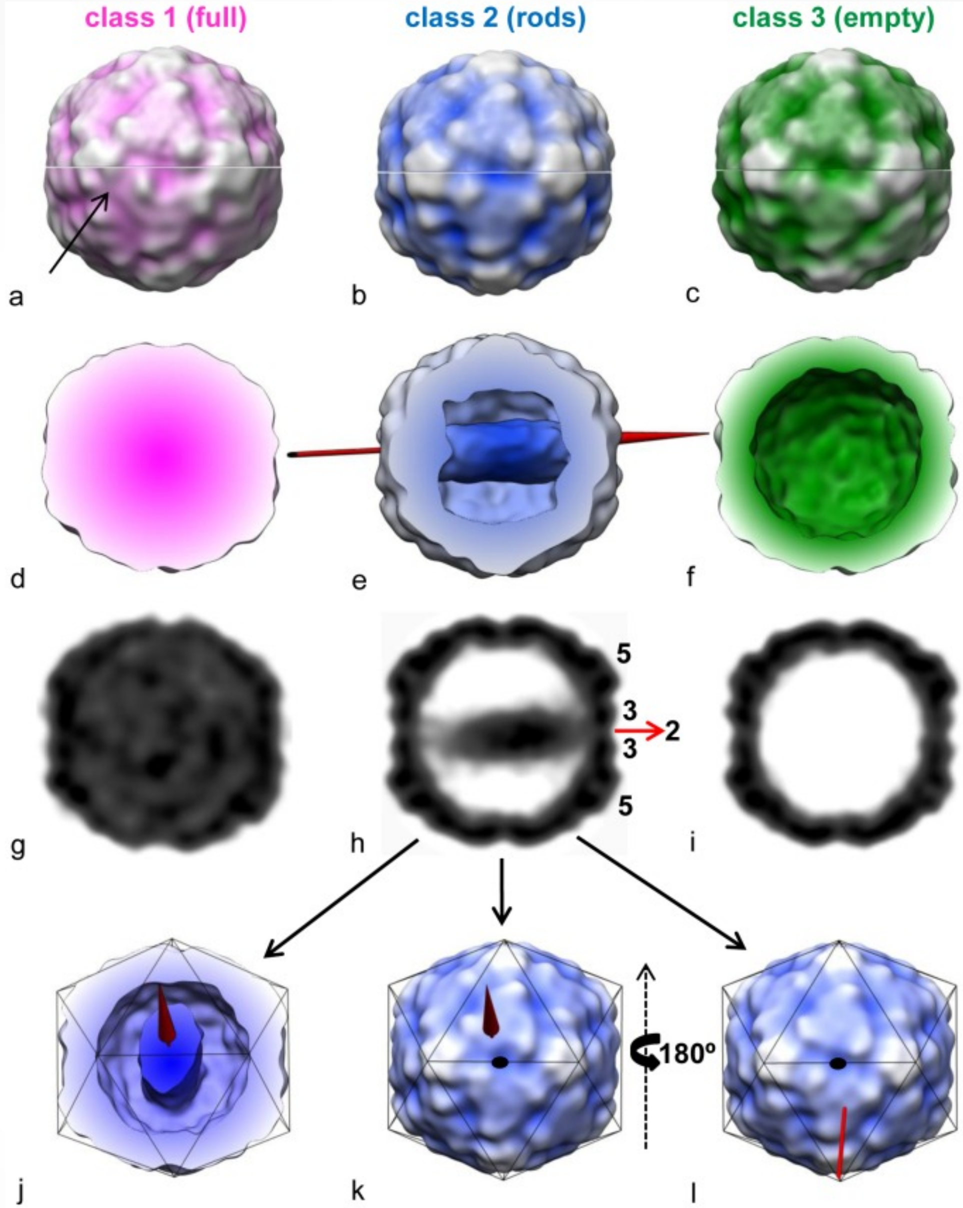
Membrane interaction by HRV2 Kumar *et al.*, JVI, 2014



Membrane interaction by poliovirus

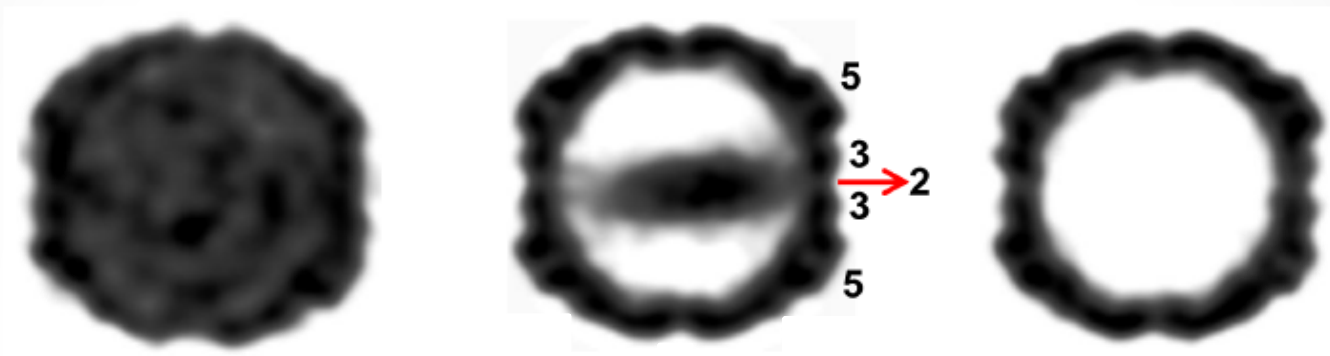
Bubeck *et al.*, JVI, 2005

Release of genome (uncoating)



Genome release intermediates of HRV2

Harutyunyan, Kumar *et al.*, PLoS Pathogens, 2013



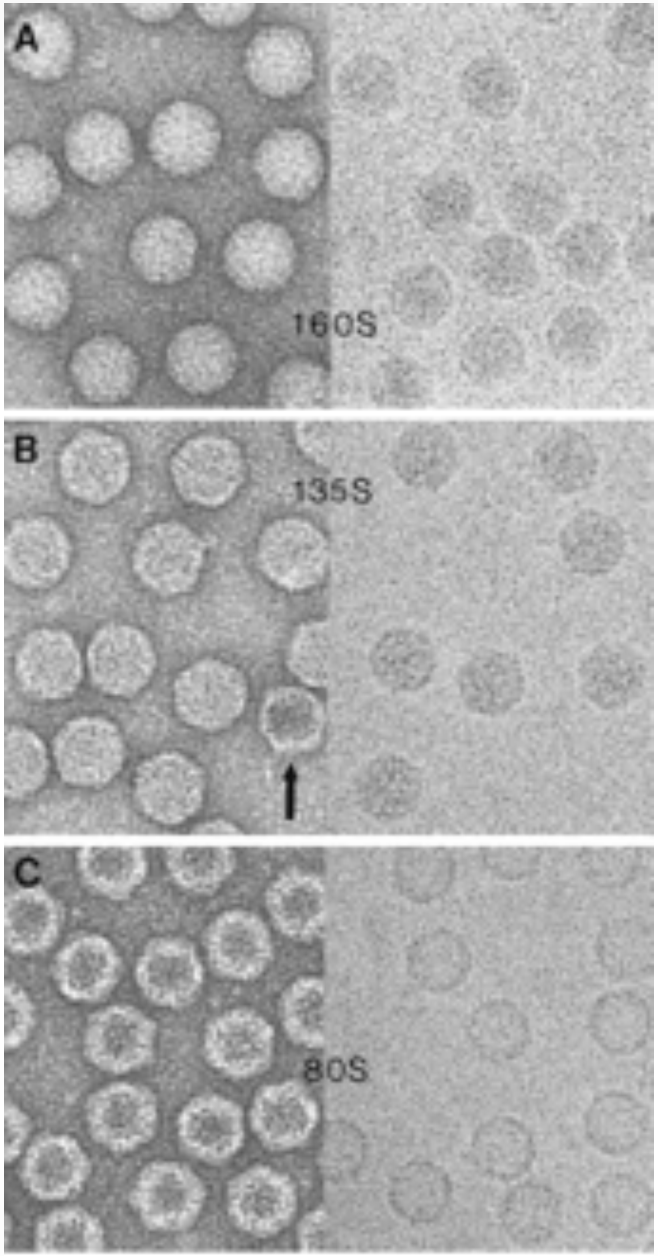
Native Virus

RNA releasing intermediate (including rod particle)

Empty Particle

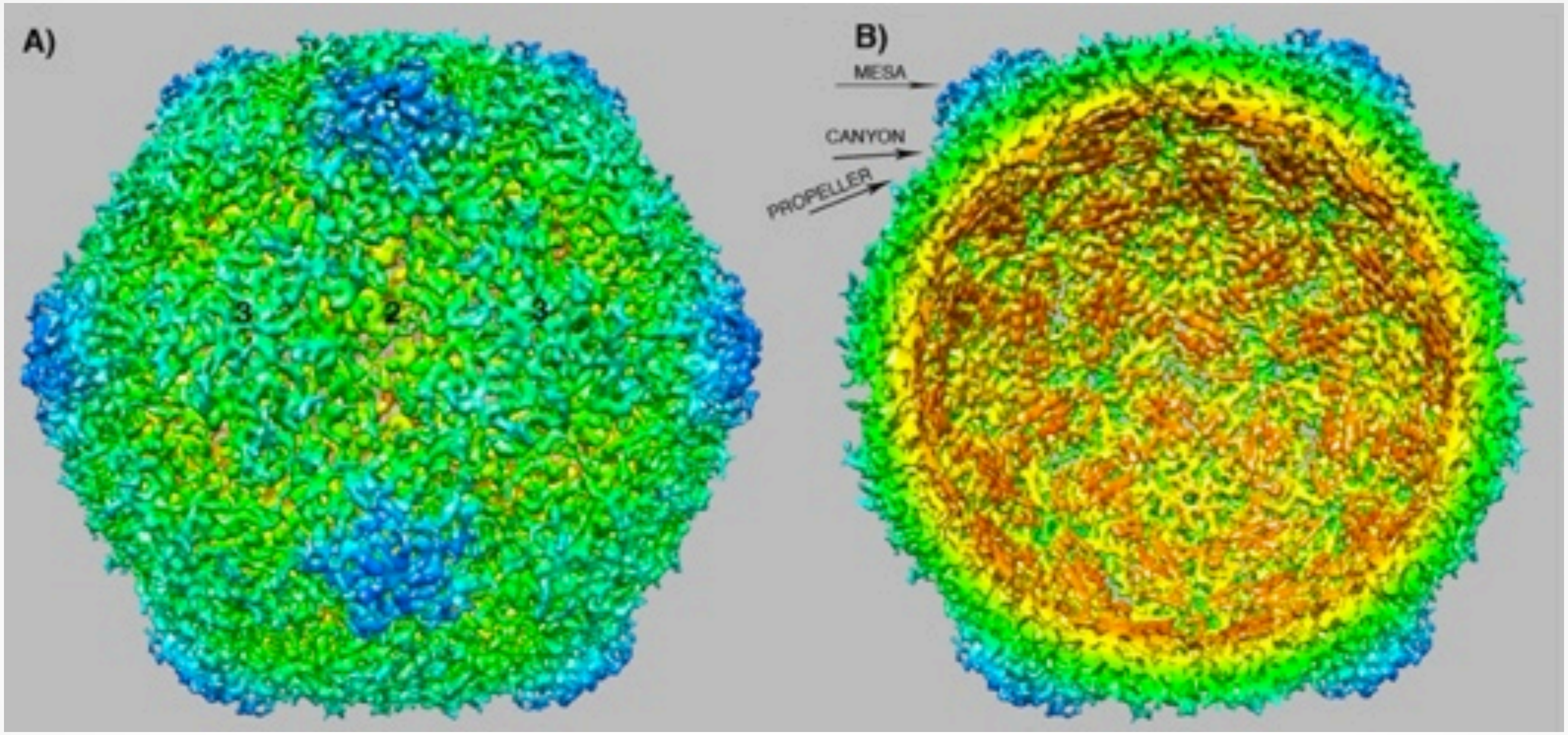
Genome release intermediates of HRV2

Harutyunyan, Kumar *et al.*, PLoS Pathogens, 2013



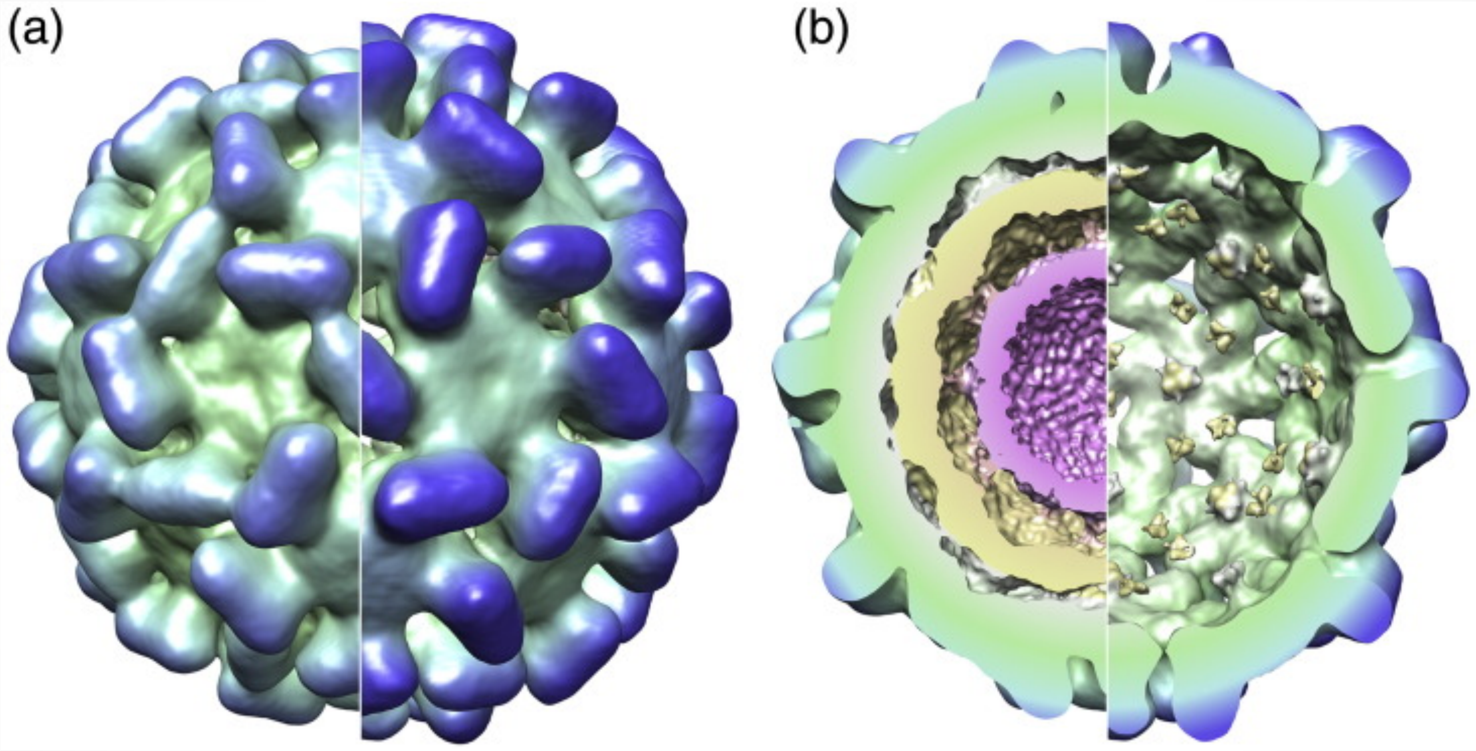
Genome release intermediates of poliovirus

Bubeck *et al.*, JVI, 2005



Genome release intermediates of poliovirus

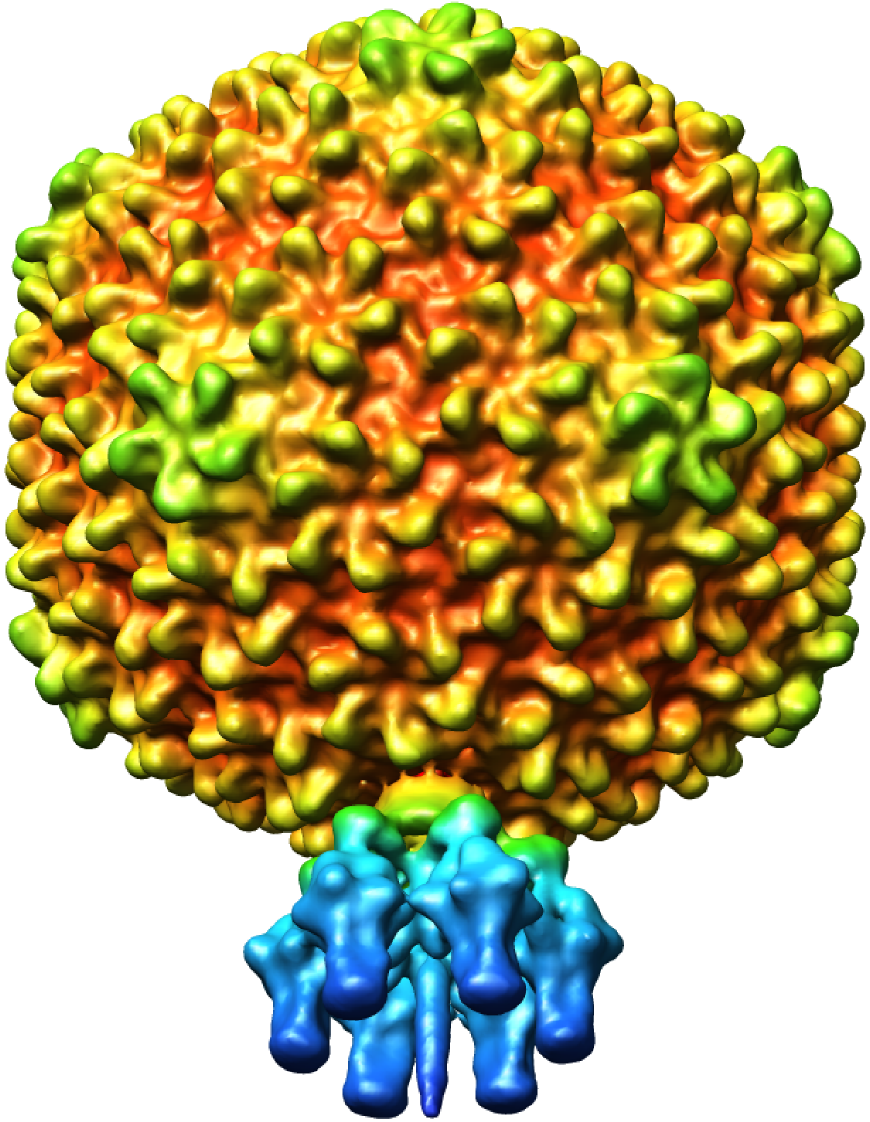
Butan *et al.*, JVI, 2014



Uncoating intermediate of a plant virus

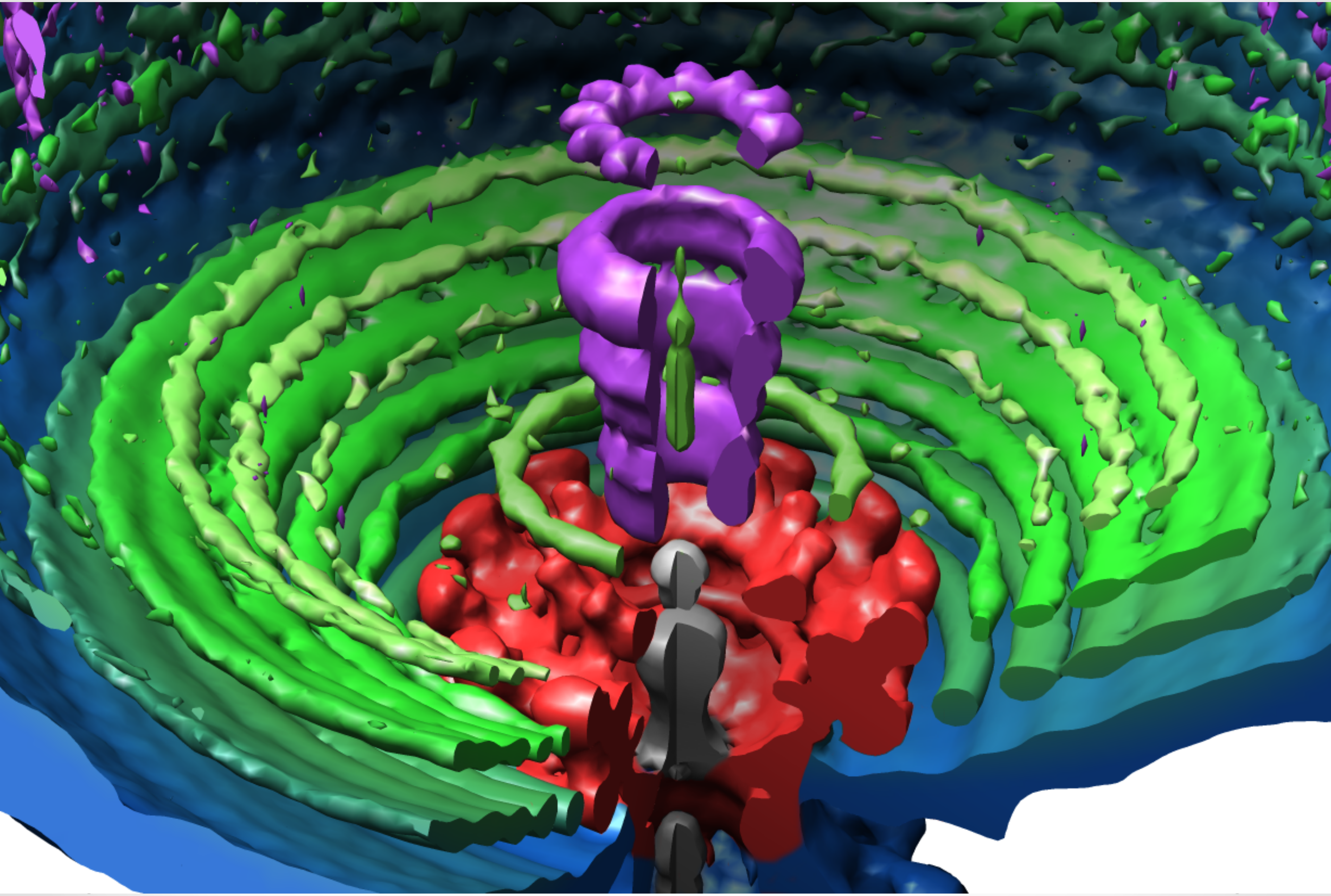
Bakker *et al,* JMB, 2012

Mechanism of DNA packaging



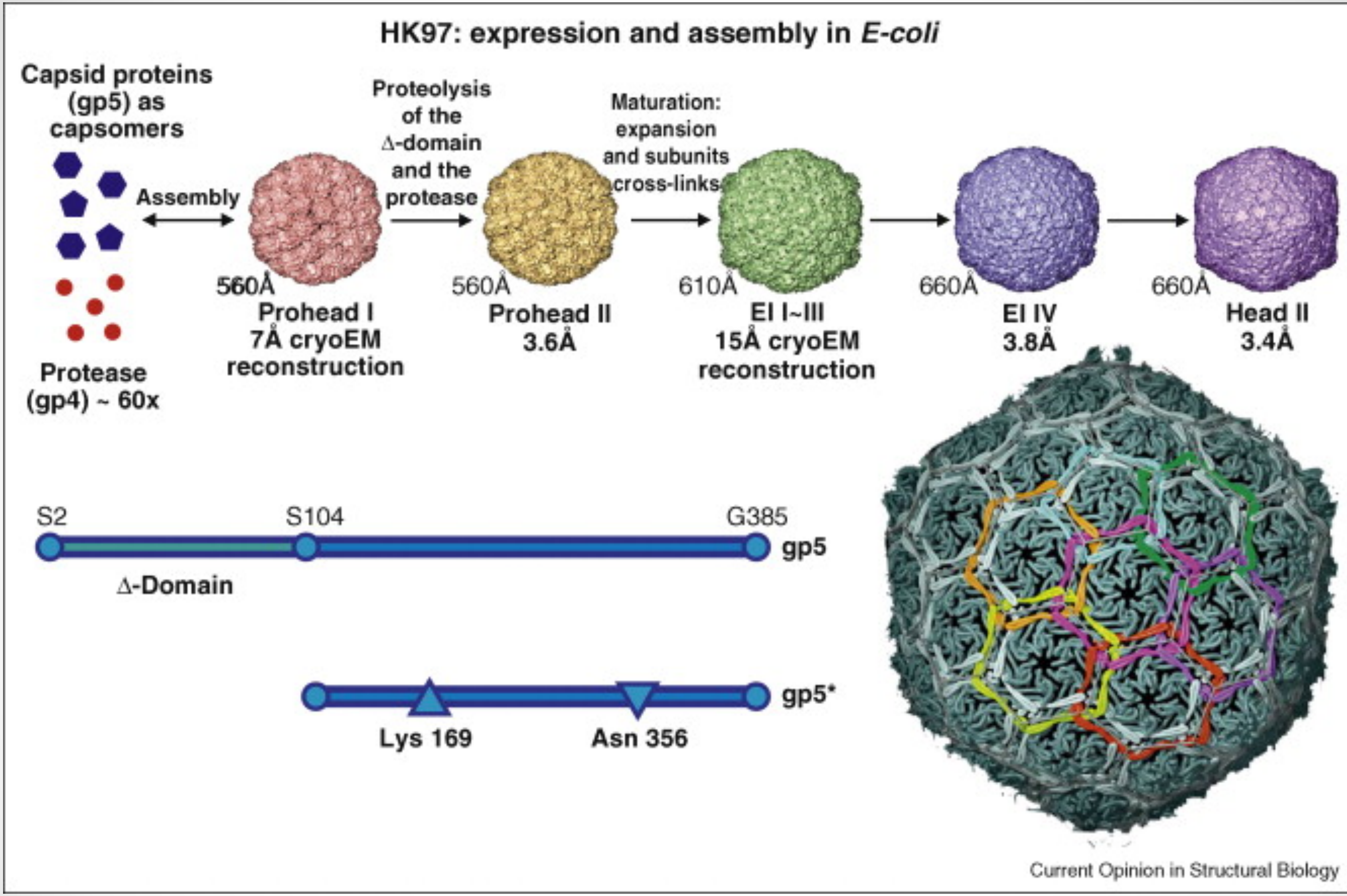
Asymmetric reconstruction of P22 bacteriophage

Lander *et al.*, Science, 2006



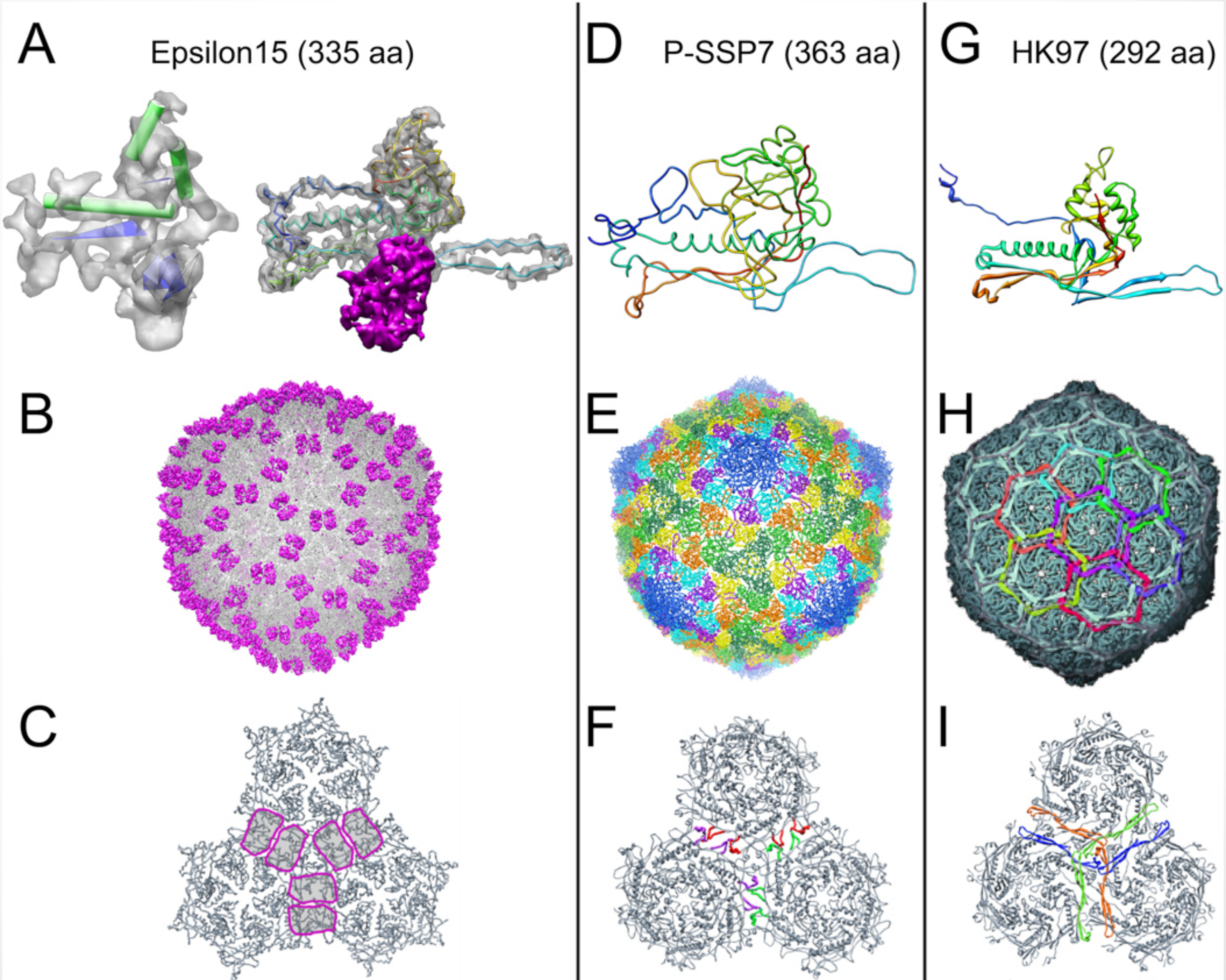
Lander *et al.*, Science, 2006

Assembly and maturation

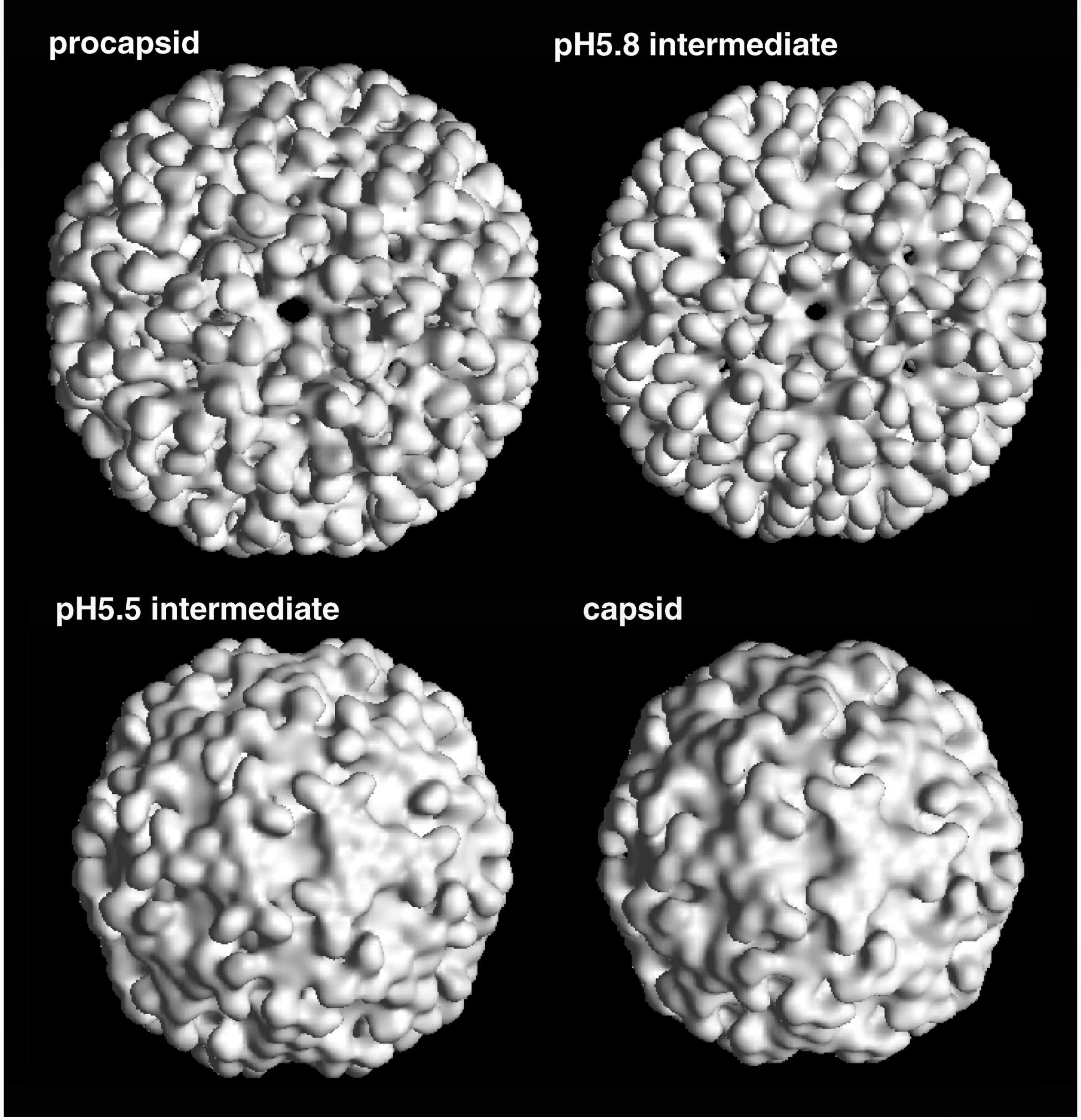


Johnson JE, Curr Opin Struct Biol, 2010

Cryoelectron microscopy and 3D reconstruction of icosahedral viruses

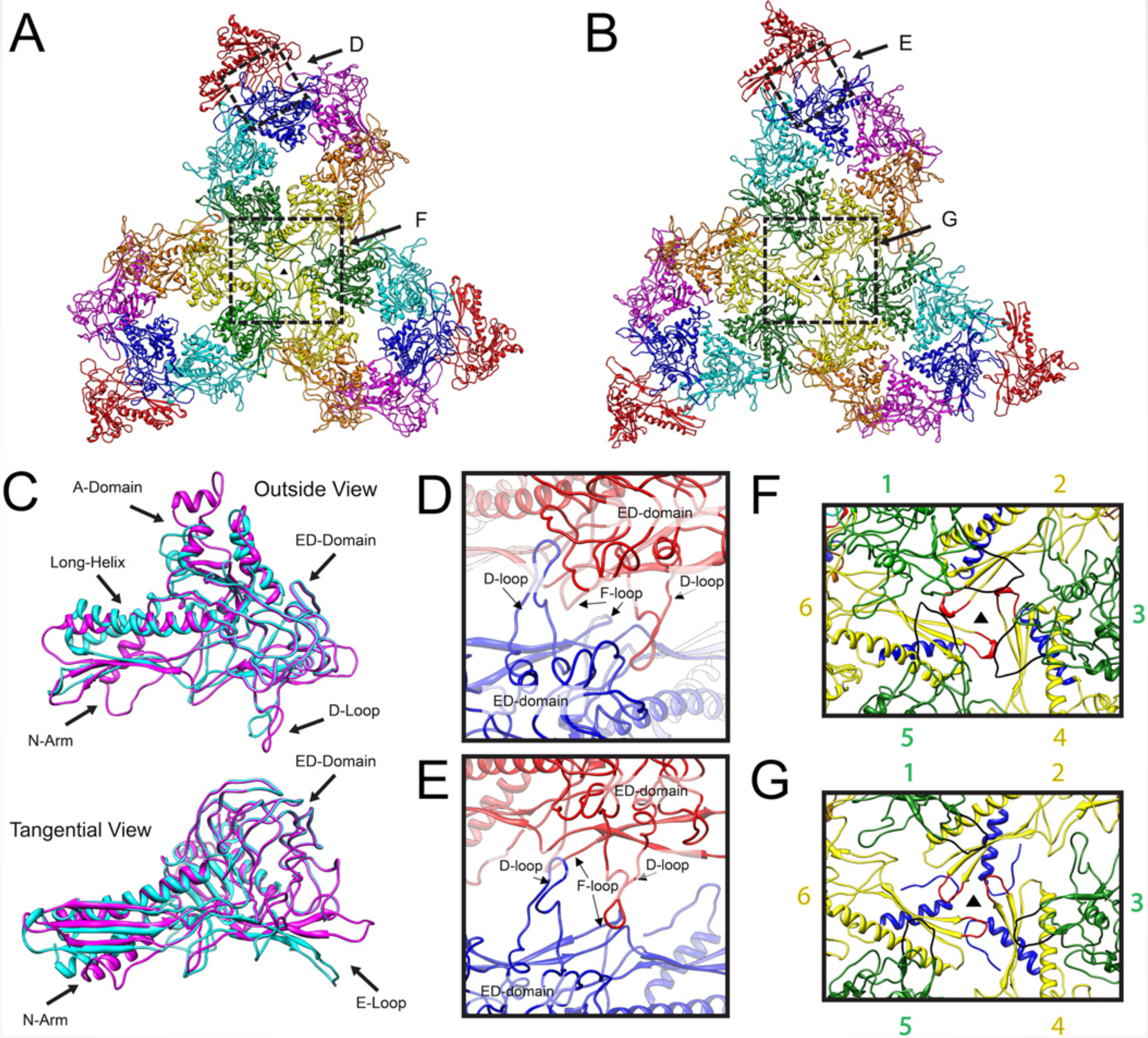


Hryc *et al.*, Curr Opin Virol, 2011



Maturation of NωV procapsid

Canady *et al.*, JMB, 2000



Maturation of P22 procapsid

Hryc *et al.*, Curr Opin Virol, 2011

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