

Blending Crystallography and CryoEM to Study STIV: A Virus That Thrives in Boiling Acid

NRAMM Workshop on
Advanced Topics in EM
La Jolla, November 14, 2007

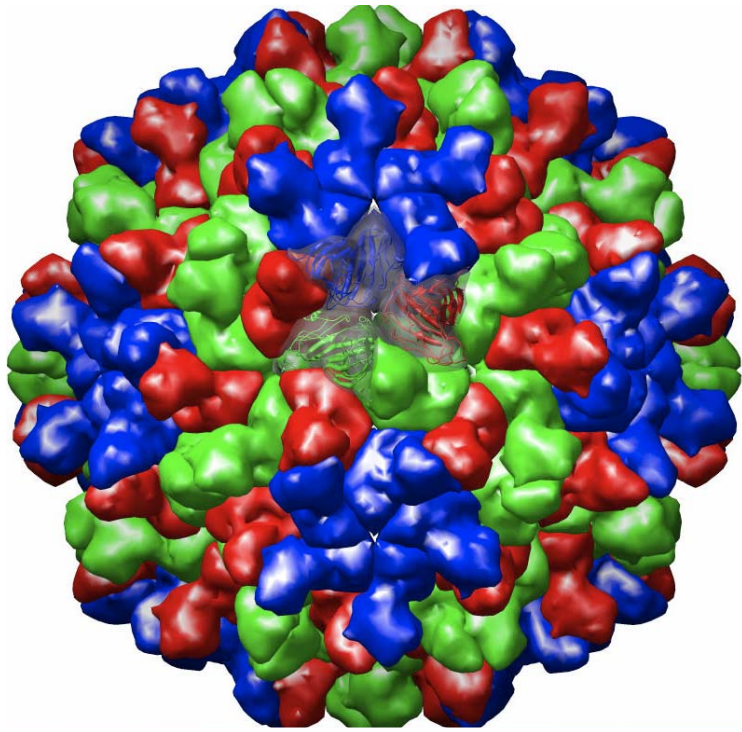
Reza Khayat and Jack Johnson
The Scripps Research Institute
La Jolla, CA

The real goal of automation

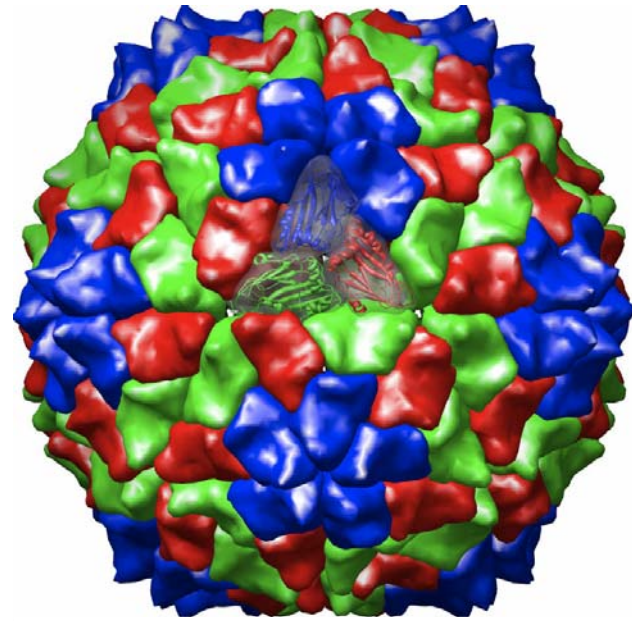


Yet another virus!

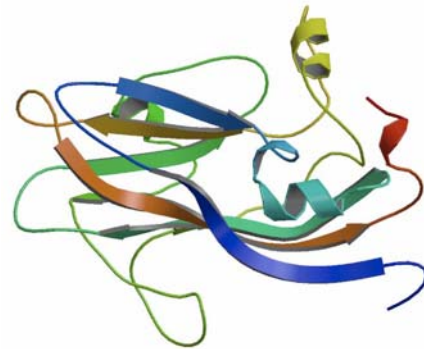
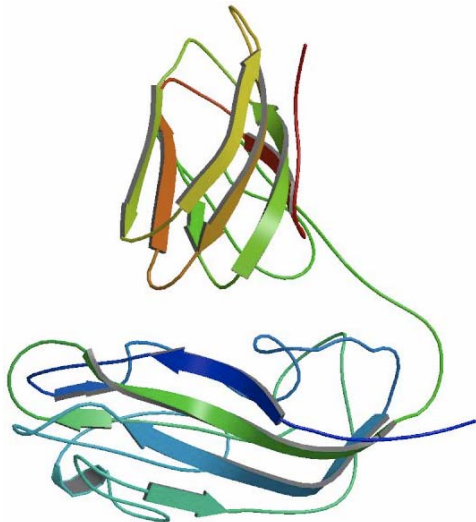
EM, ca. 2050

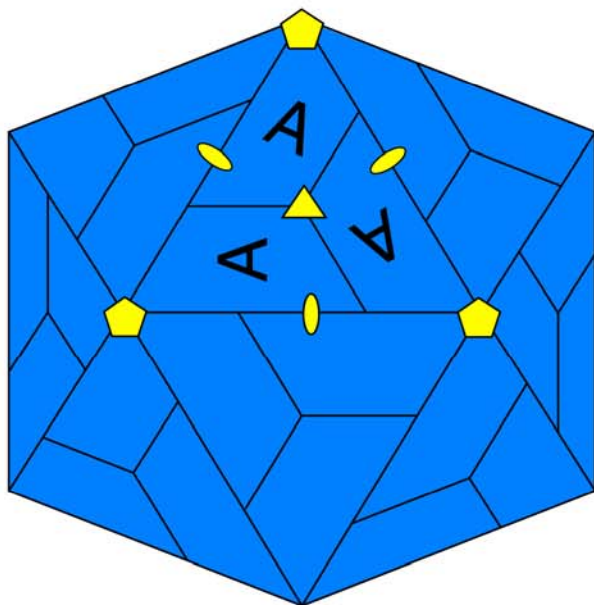
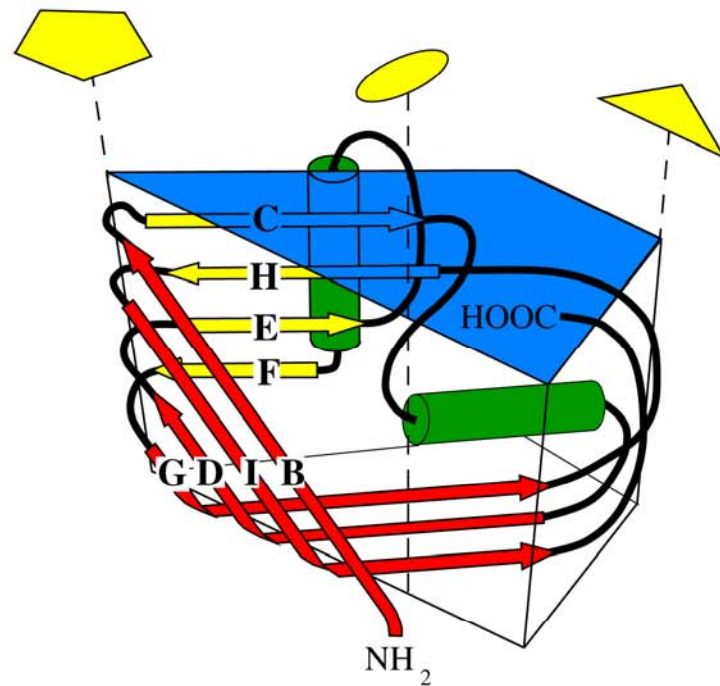
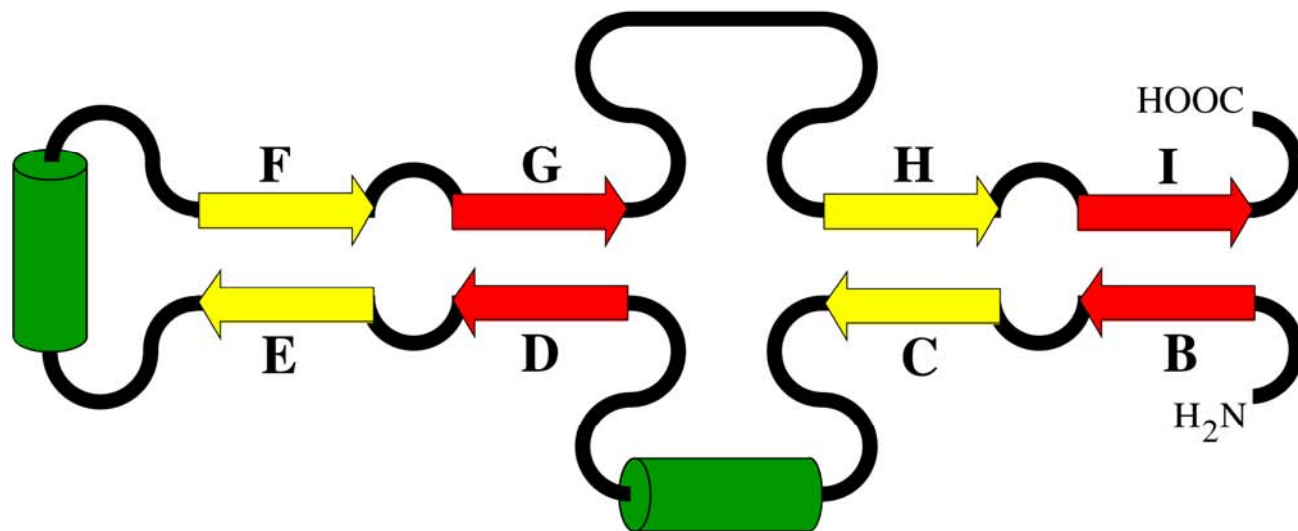


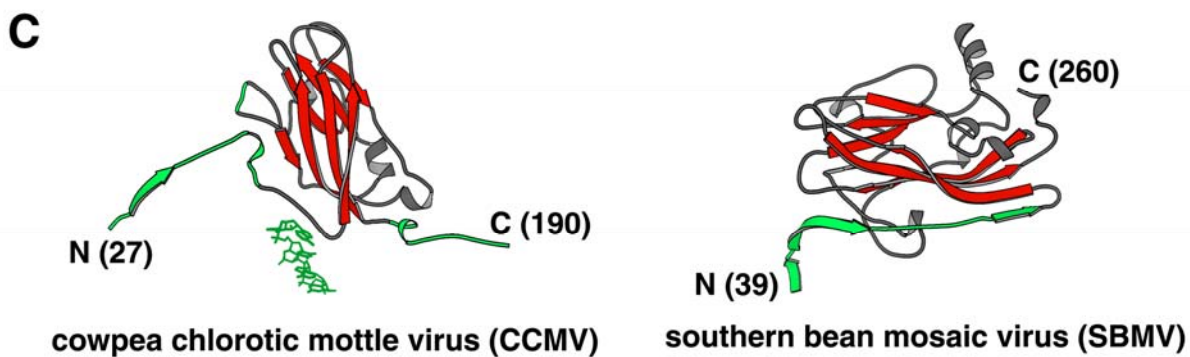
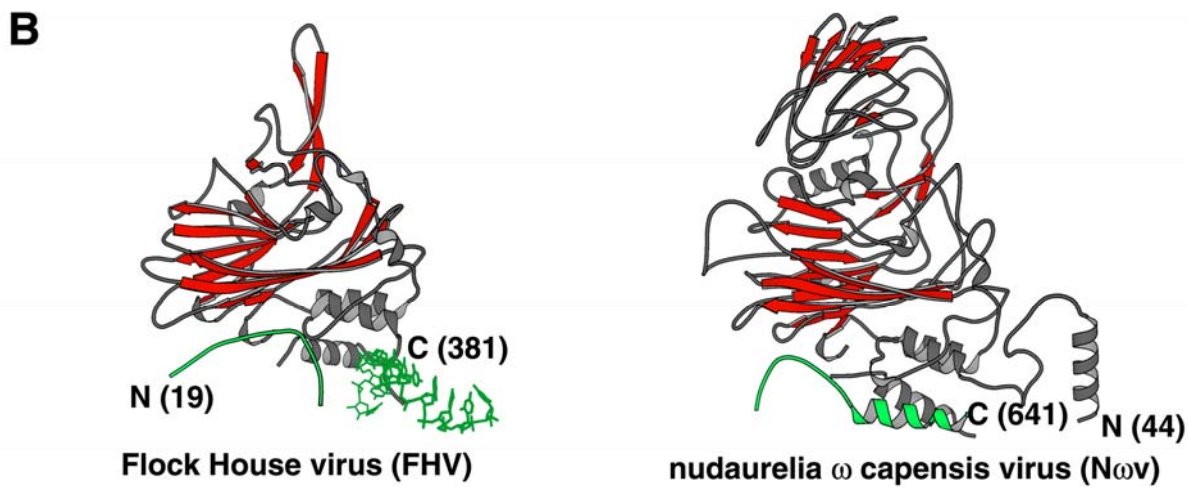
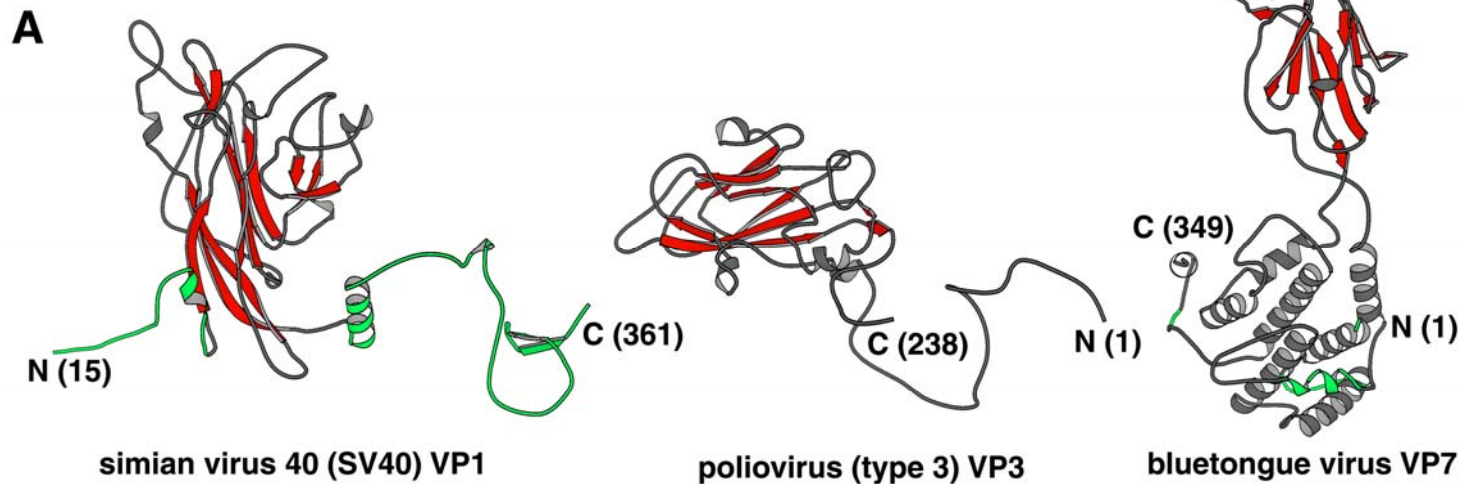
TBSV Harrison et al 1978

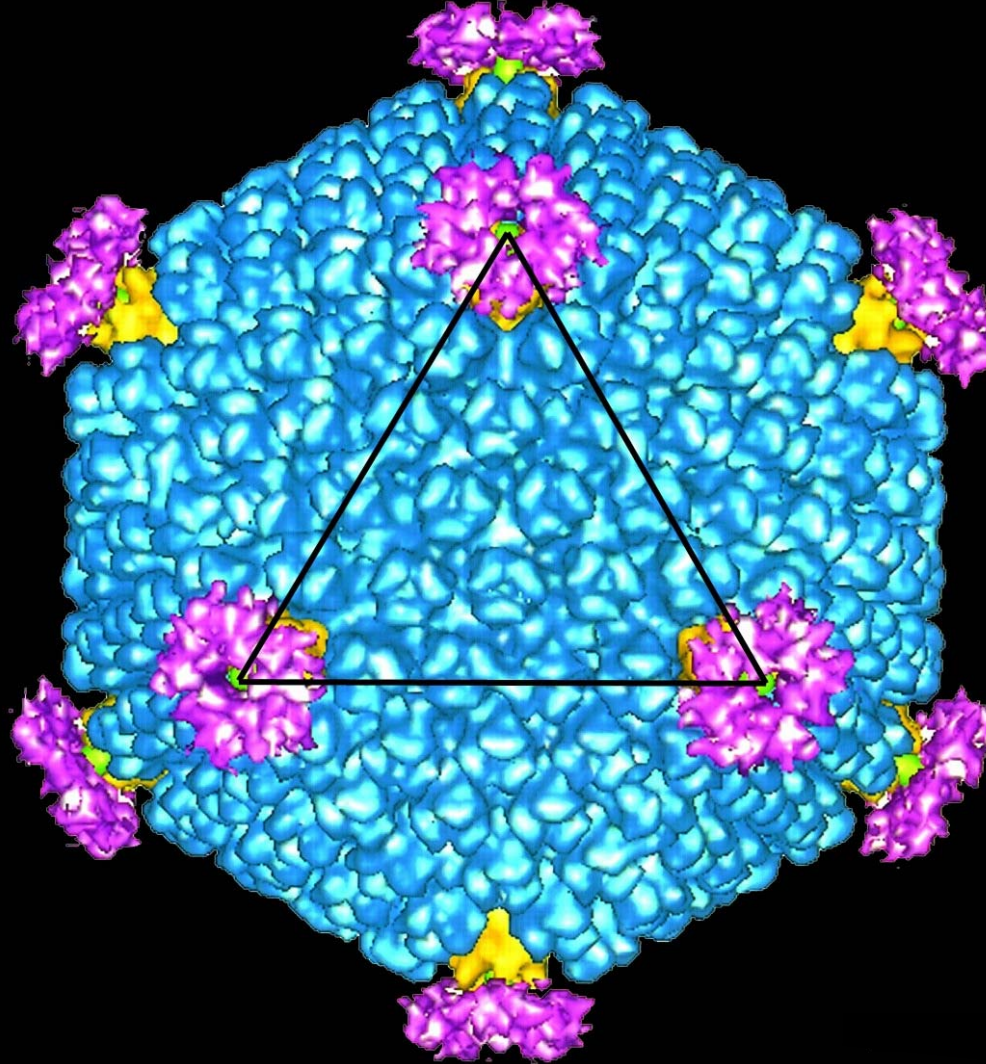


SBMV Rossmann et al
1980



A**B****C**





CryoEM reconstruction Adenovirus (Fuller and Burnet)

Articles

Science, Vol 232, Issue 4754, 1148-1151

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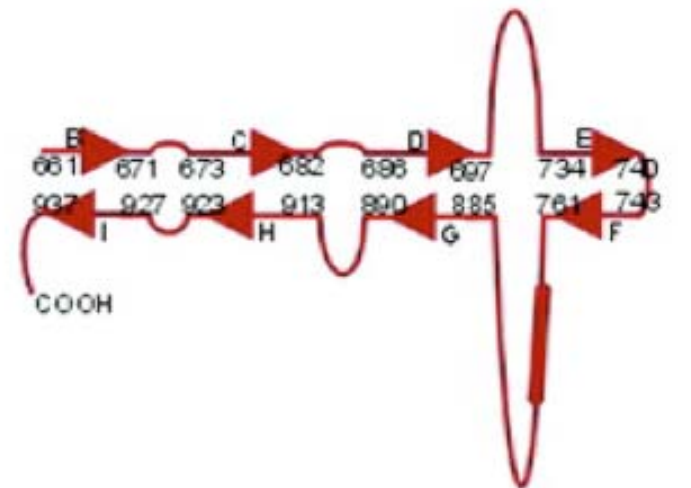
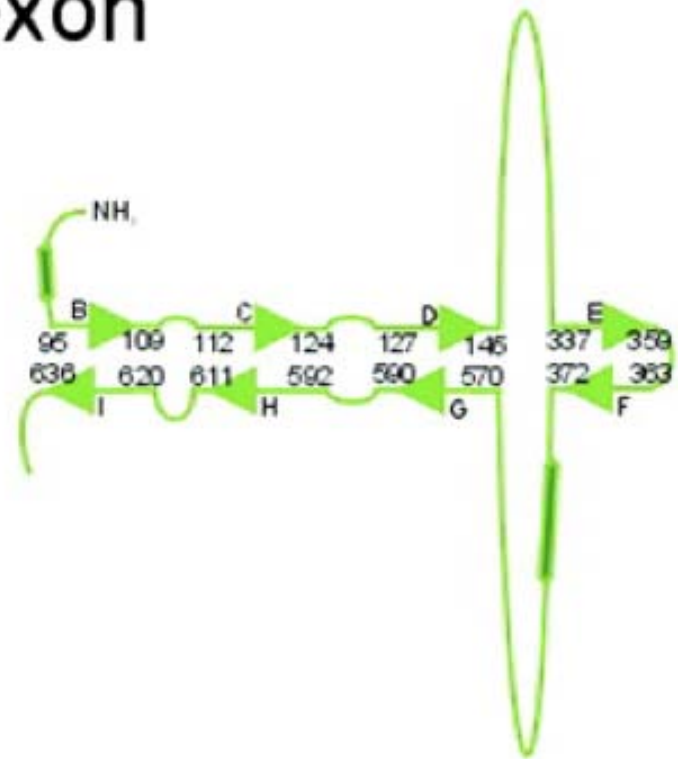
articles

Three-dimensional structure of the adenovirus major coat protein hexon

MM Roberts, JL White, MG Grutter, and RM Burnett

The three-dimensional crystal structure of the adenovirus major coat protein is presented. Adenovirus type 2 hexon, at 967 residues, is now the longest polypeptide whose structure has been determined crystallographically. Taken with our model for hexon packing, which positions the 240 trimeric hexons in the capsid, the structure defines 60% of the protein within the 150×10^6 dalton virion. The assembly provides the first details of a DNA-containing animal virus that is 20 times larger than the spherical RNA viruses previously described. **Unexpectedly, the hexon subunit contains two similar beta-barrels whose topology is identical to those of the spherical RNA viruses, but whose architectural role in adenovirus is very different.** The hexon structure reveals several distinctive features related to its function as a stable protective coat, and shows that the type-specific immunological determinants are restricted to the virion surface.

Adenovirus hexon



Viral Evolution Revealed by Bacteriophage PRD1 and Human Adenovirus Coat Protein Structures

Stacy D. Benson,* Jaana K. H. Bamford,†
Dennis H. Bamford,† and Roger M. Burnett*‡

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3601 Spruce Street
Philadelphia, Pennsylvania 19104

†Department of Biosciences and
Institute of Biotechnology
University of Helsinki
FIN-00014 Helsinki
Finland

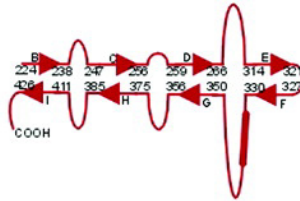
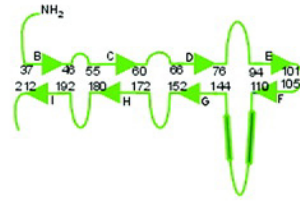
The structure and evolution of the major capsid protein of a large, lipid-containing DNA virus

Narayanasamy Nandhagopal*, Alan A. Simpson*, James R. Gurnon[†], Xiadong Yan*, Timothy S. Baker*, Michael V. Graves^{‡§}, James L. Van Etten^{†¶}, and Michael G. Rossmann*^{||}

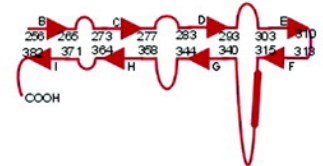
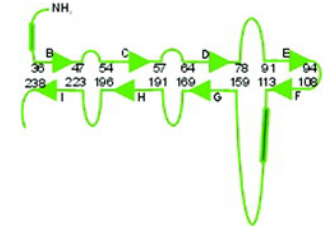
*Department of Biological Sciences, Purdue University, West Lafayette, IN 47907; [†]Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583; and [‡]Nebraska Research Initiative Center for Biotechnology, and [¶]Nebraska Center of Virology, University of Nebraska, Lincoln, NE 68588

Structural Conservation

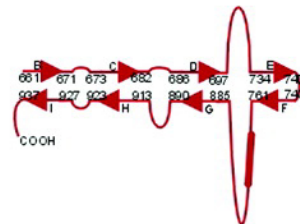
PBCV-1 Vp54



PRD1 P3



Adenovirus hexon

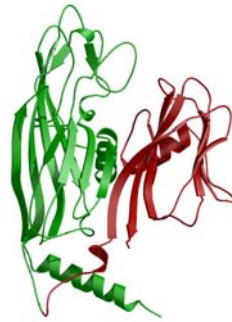


- Eight stranded jelly-roll.
- No sequence conservation.
- Jelly-roll contains an α -helix between strands five and six.
- Similar loops between β -strands.

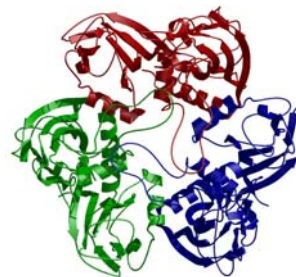
Nandhagopal et al., 2002

Double Barrel Viral Subunits circa 2002

monomer



pseudo
hexamer

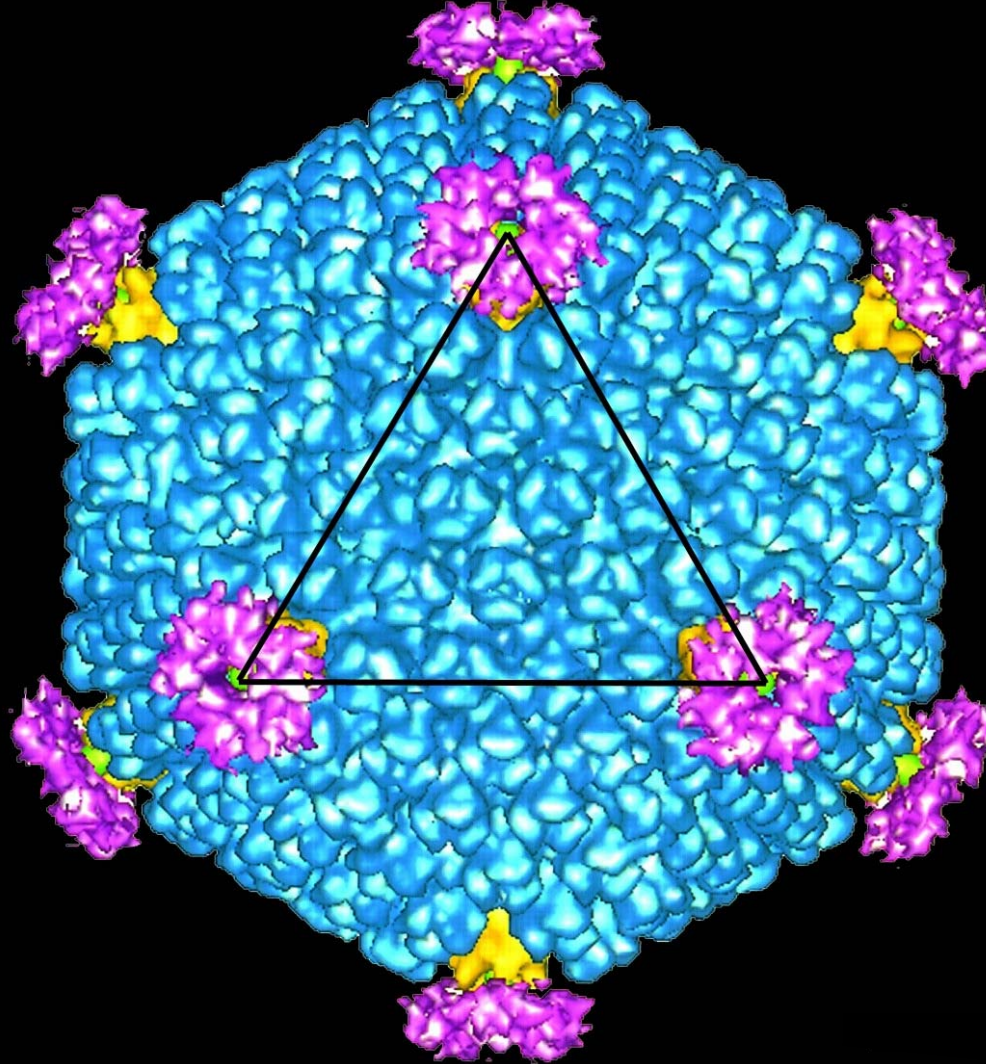


adenovirus
hexon

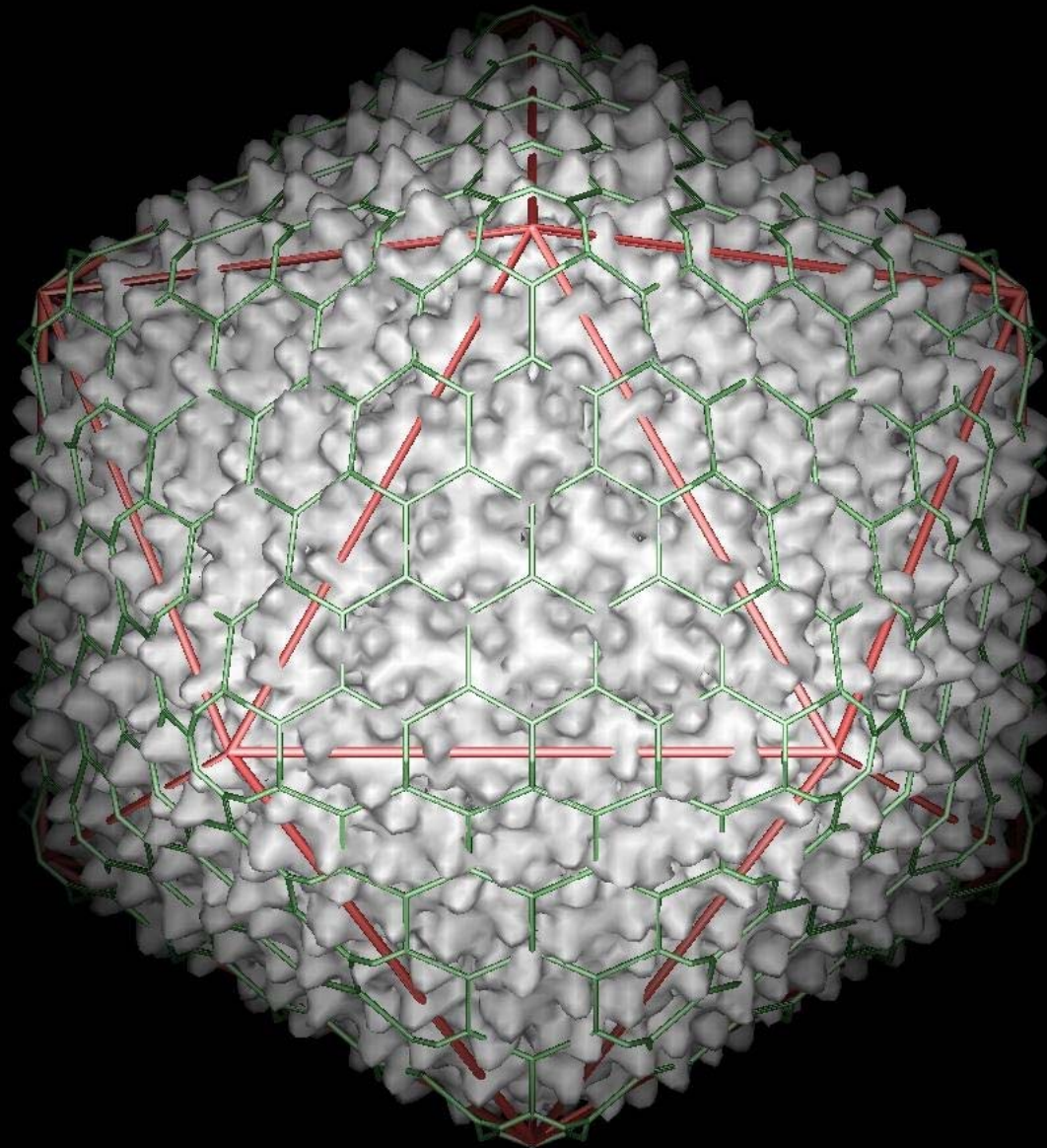
PRD1
P3

PBCV-1
Vp54

Paramecium bursaria
Chlorella virus type 1

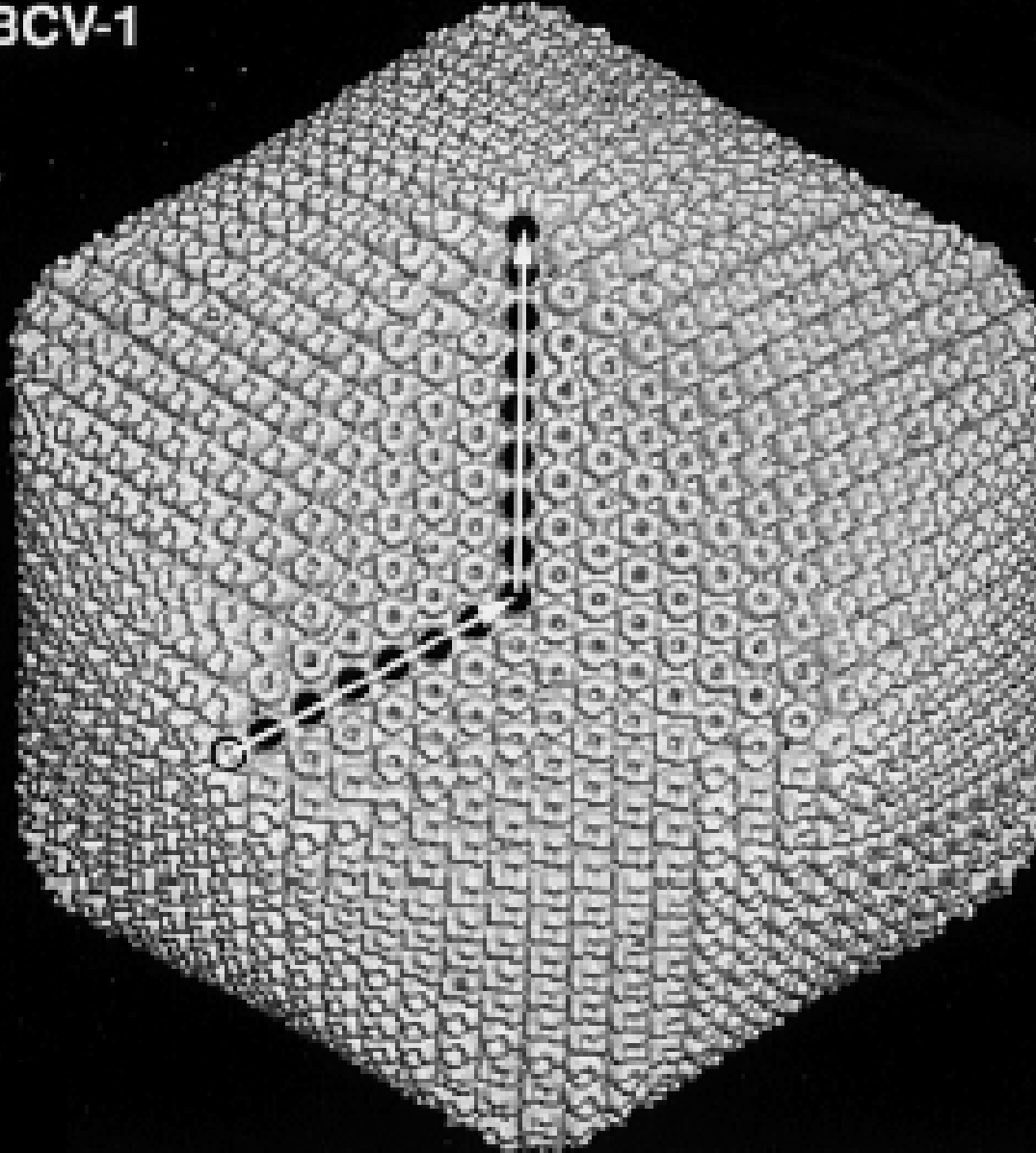


CryoEM reconstruction Adenovirus (Fuller and Burnet)



PRD1 (Bamford et al)

PBCV-1



Paramecium bursaria chlorella virus, Diameter=1900Å, $T=169$

Sulfolobus sulfotaricus

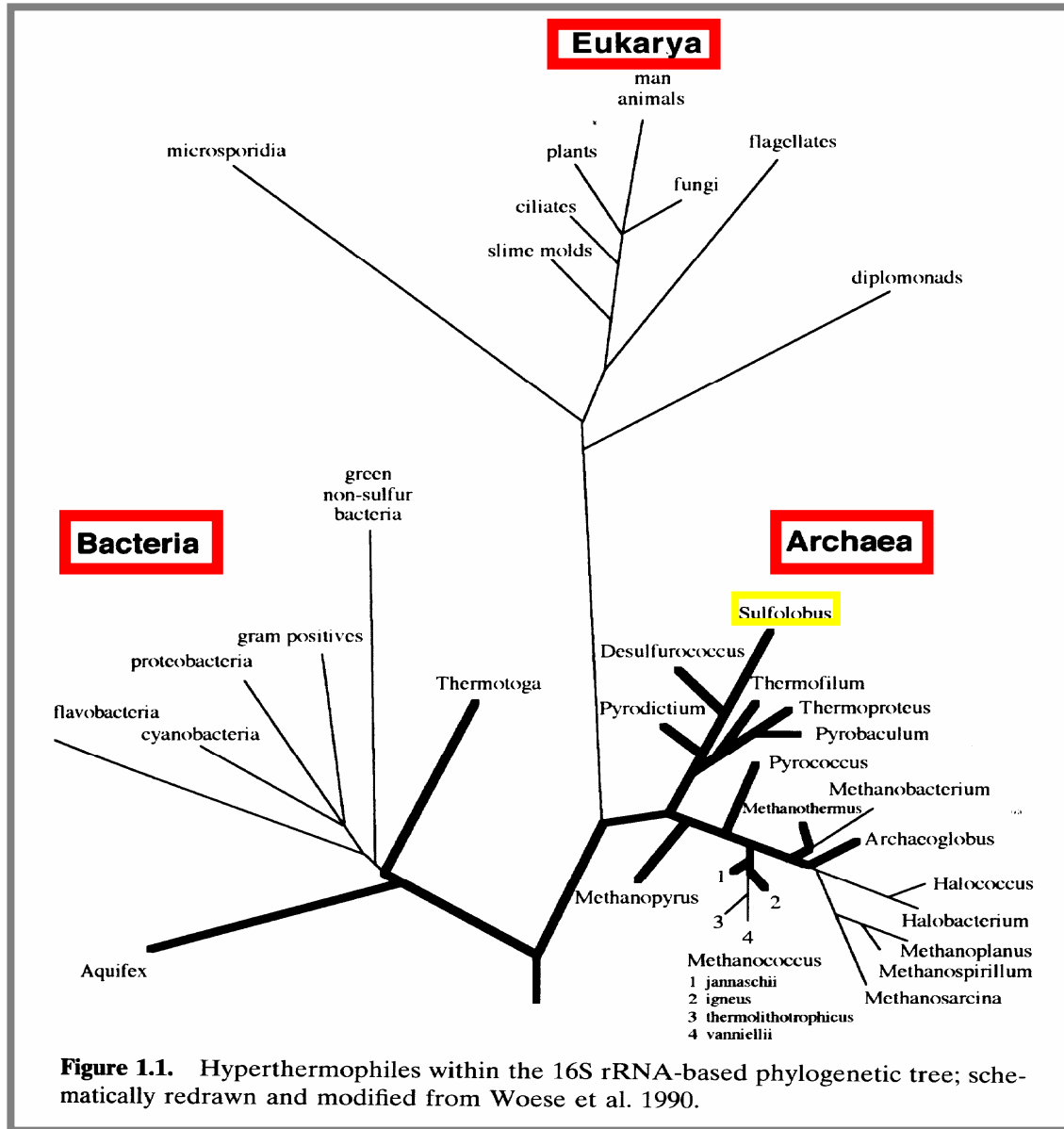
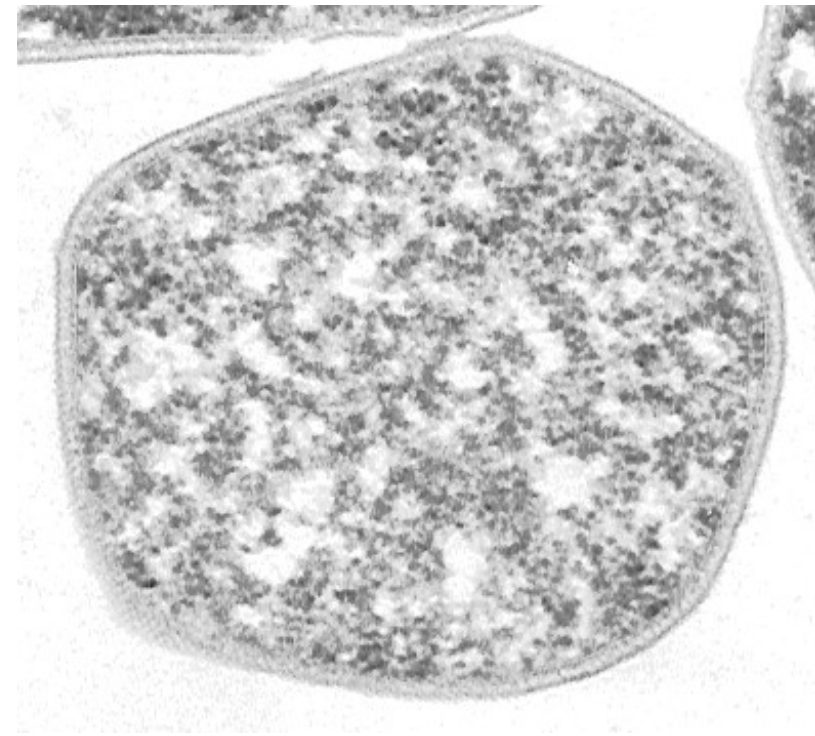


Figure 1.1. Hyperthermophiles within the 16S rRNA-based phylogenetic tree; schematically redrawn and modified from Woese et al. 1990.

Sulfolobus

- Extreme **THERMOPHILE** archaea
 - ~ 80C, pH+ 3.0 optimum
- Easy to grow
- Completely sequenced
 - 3Mbp Genome





NO BULL
ENTER

EXCURSION





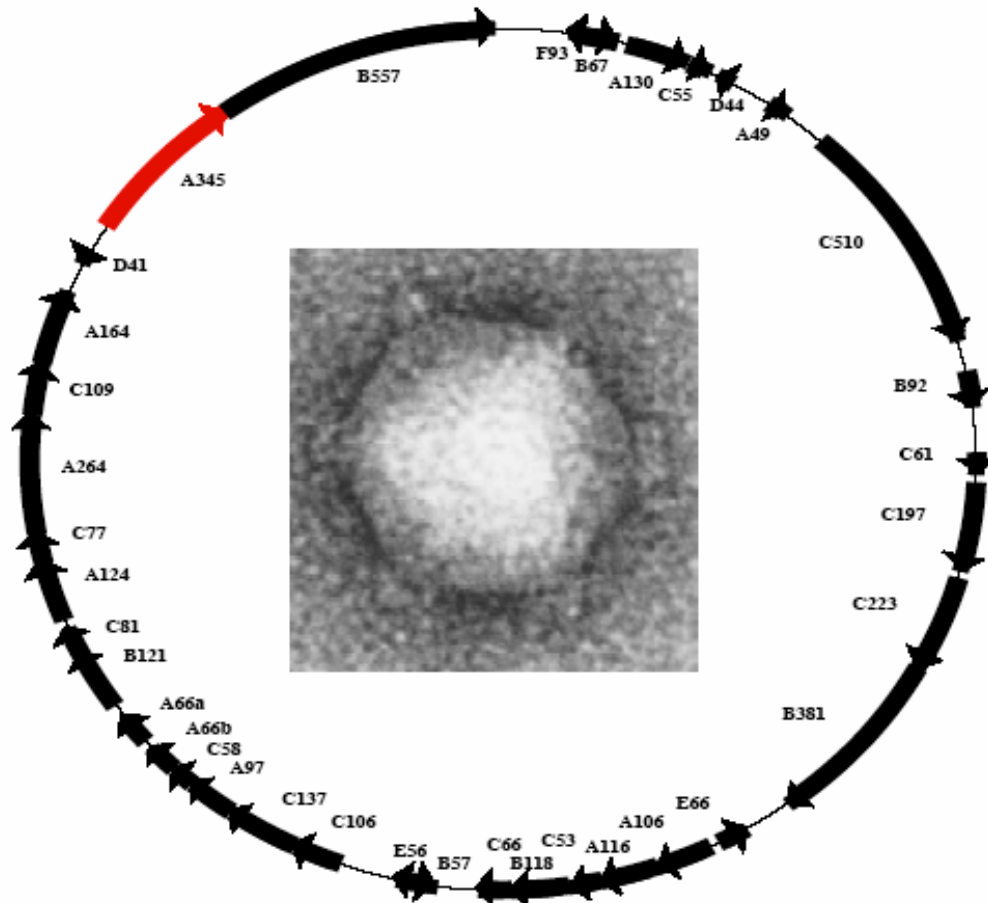




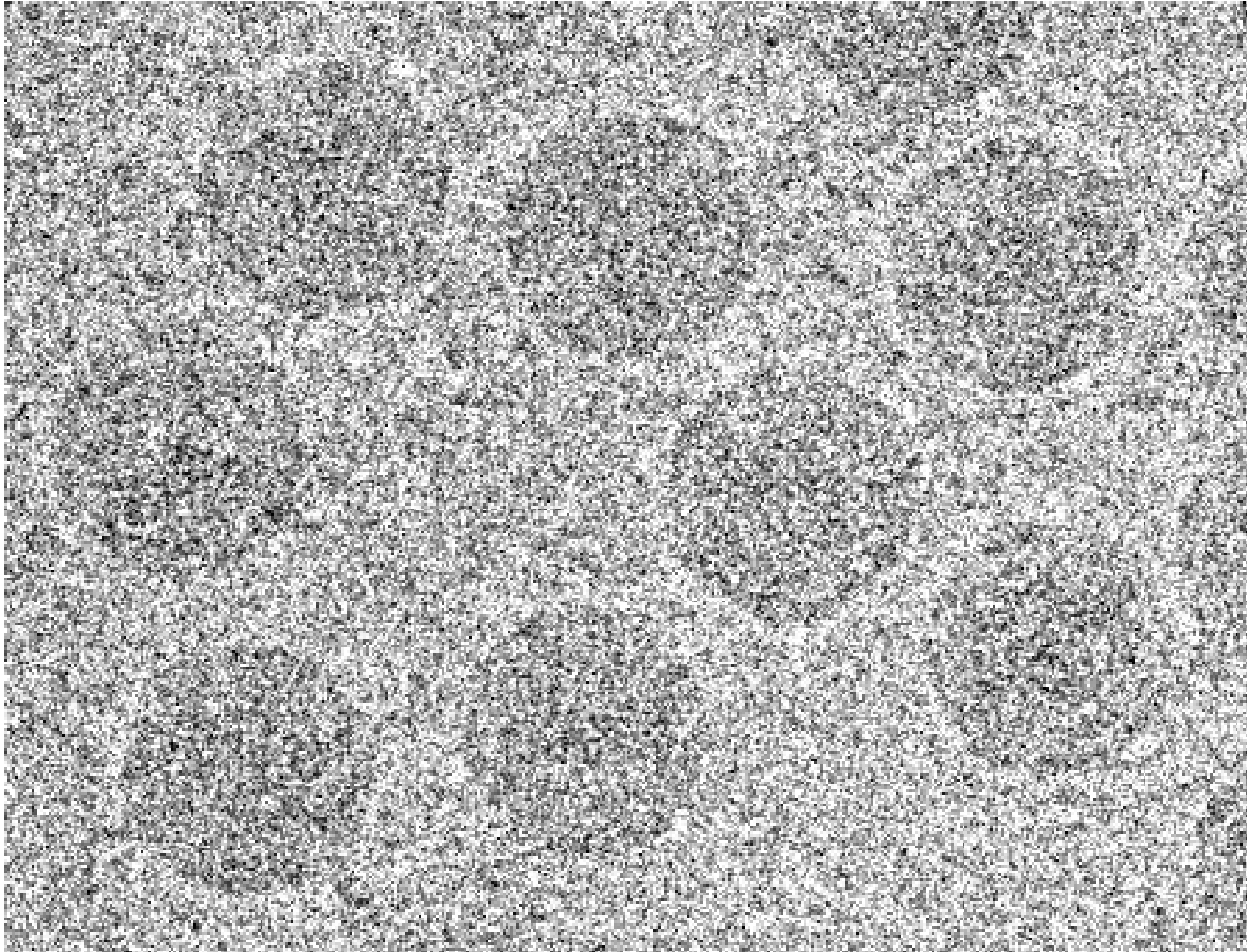
An electron micrograph showing two individual STIV particles. Each particle is roughly spherical and has a distinct, darker outer shell (turret) and a lighter, more granular inner core. The background is a dark, noisy field of small particles.

EM image of STIV negatively stained with 2% Uranyl Acetate. Maximum dimension including turrets 1000Å

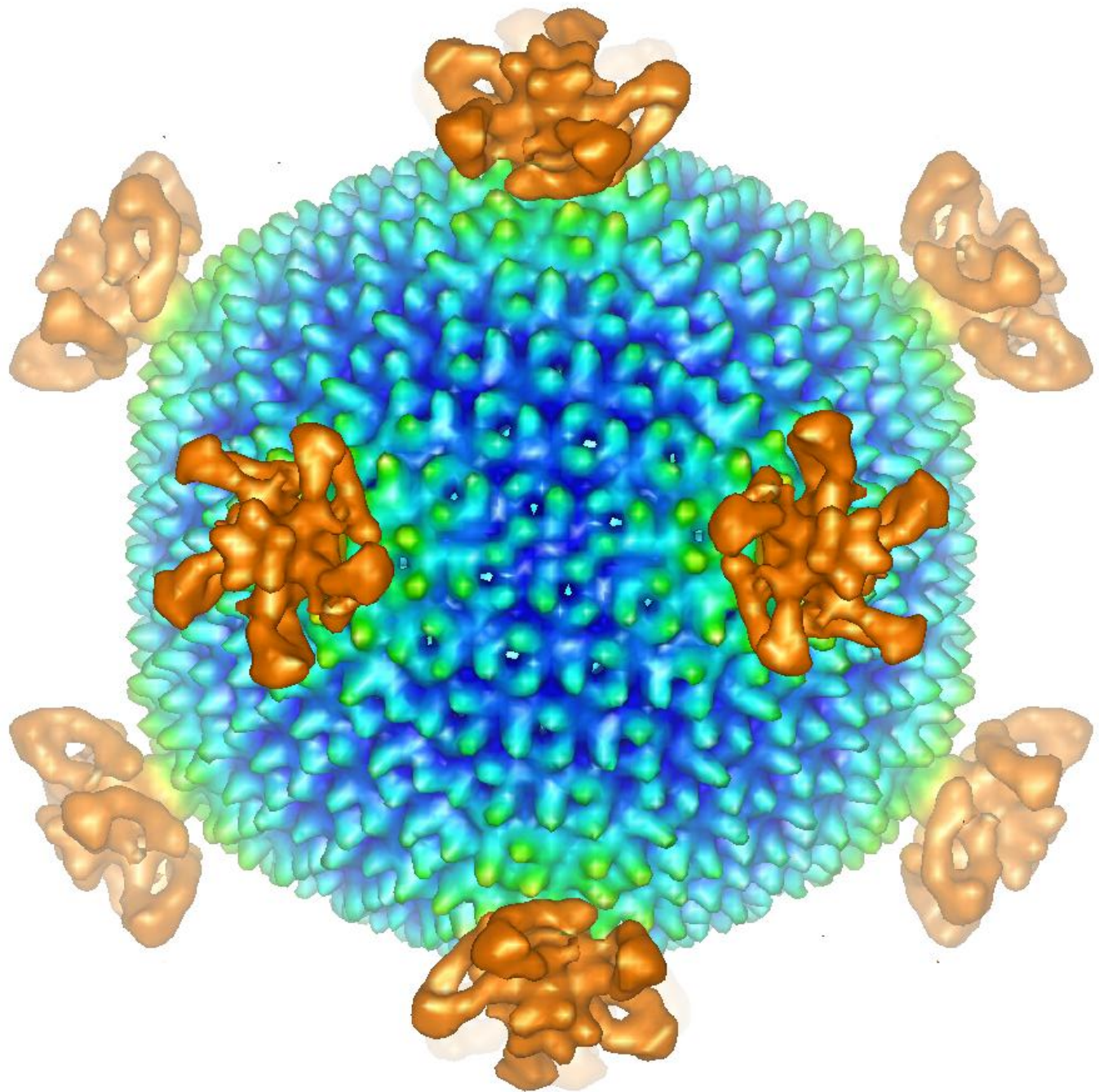
Genome sequence



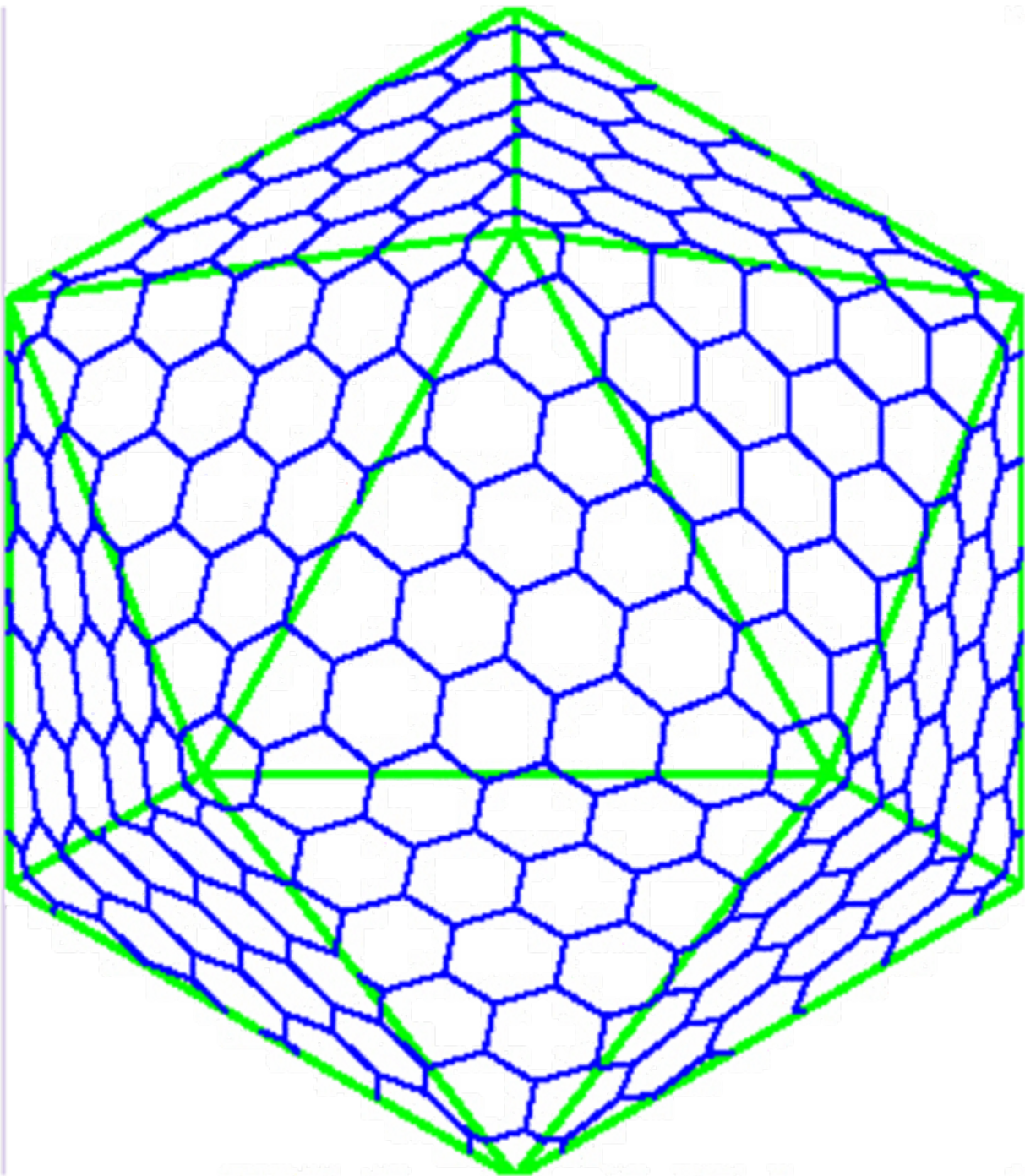
- 17663 bp
- 36% G+C
- 36 ORF's larger than 40 aa
- Largest ORF 138 kD
- Smallest ORF 5.1 kD
- **Coat Protein 37.8 kD**
- No similarity to known proteins

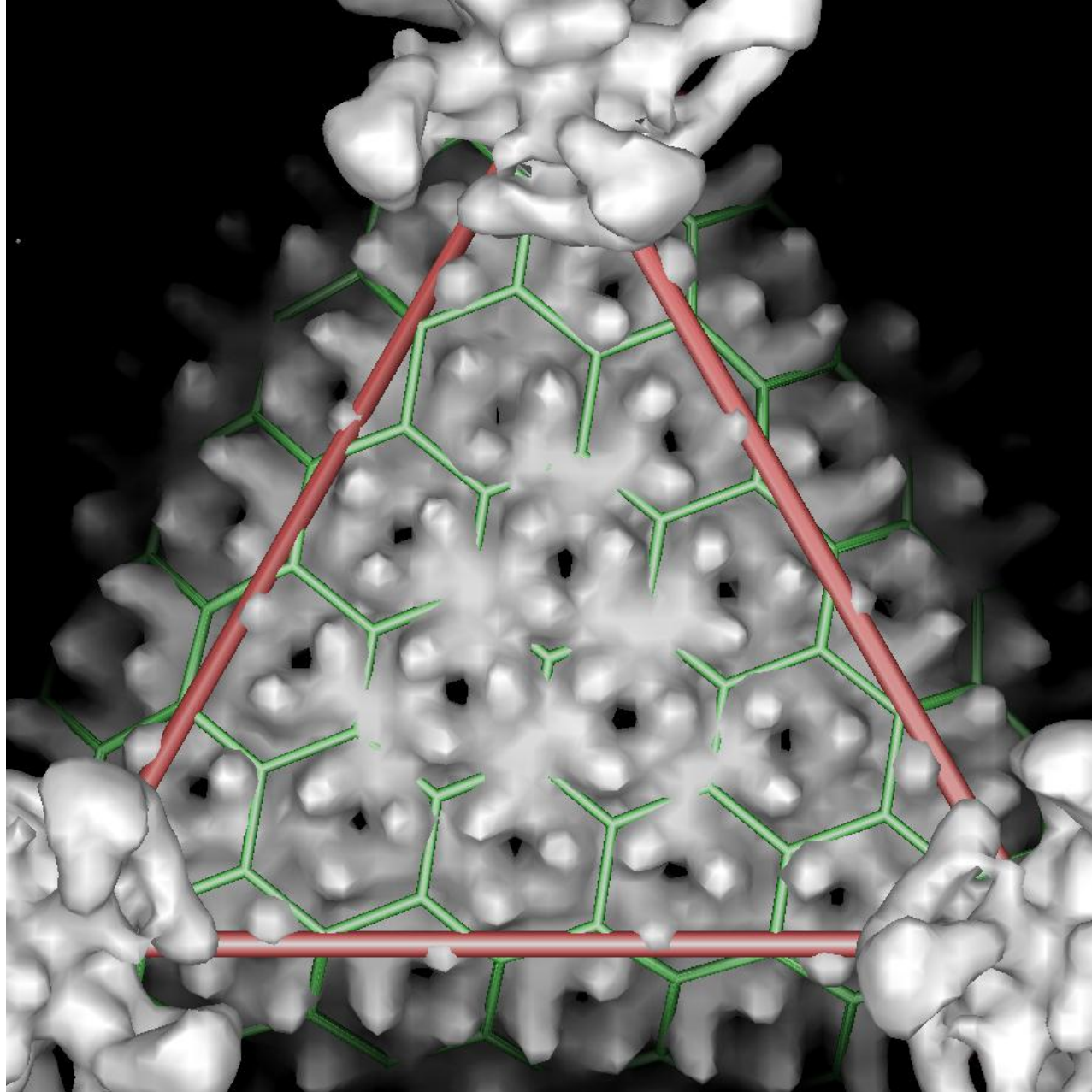


EM, Cryo, Low-dose image of STIV Particles



- Reconstruction performed with SPIDER
- Reconstruction based on ~250 particles
- Resolution of Reconstruction 27Å
- Particle diameter with turrets 1000Å
- Particle diameter without turrets 740Å
- Capsid thickness 64Å



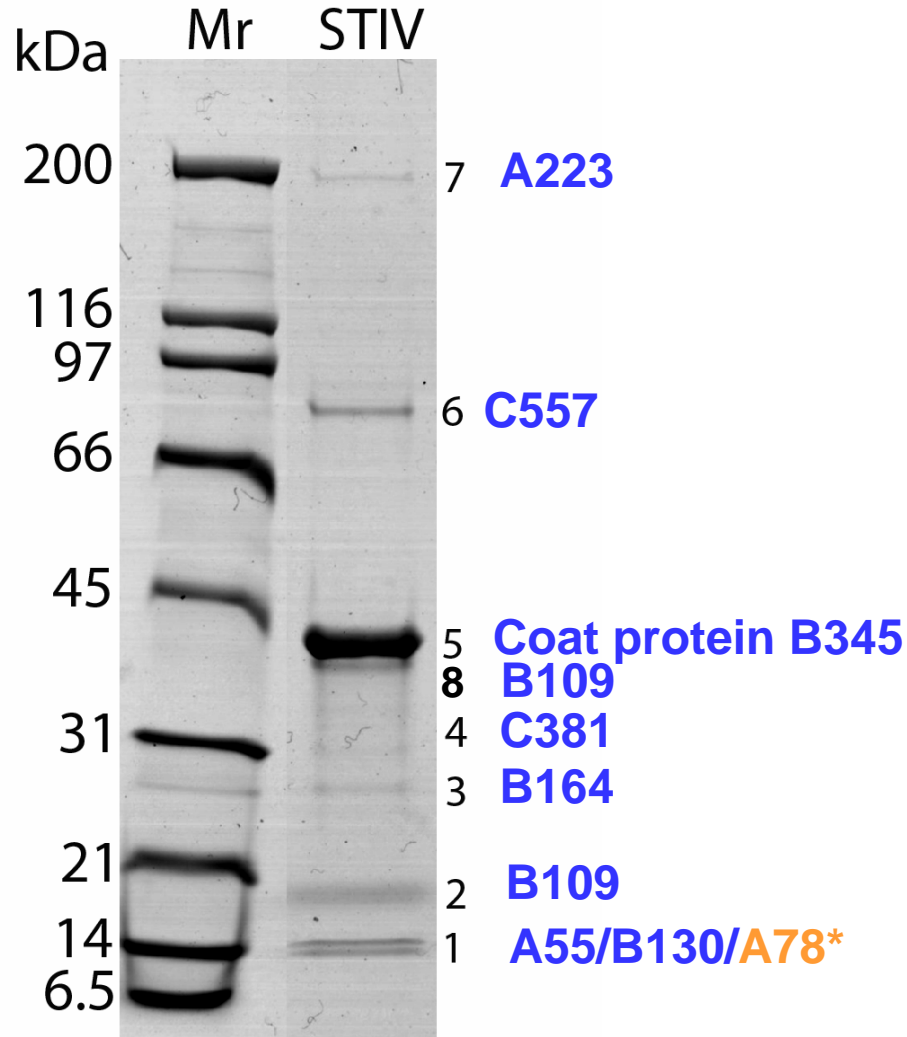


Yellowstone Virus $T=31$

A comparison among PRD1, adenovirus, PBCV-1 and STIV

	PRD1	adenovirus	PBCV-1	STIV
family	<i>Tectiviridae</i>	<i>Adenoviridae</i>	<i>Phycodnaviridae</i>	?
dsDNA linear genome	15k bp	36k bp in type2	330k bp	circular 19.5k bp
Diameter/Å	700	920	1,900	1,000
pseudo T	25	25	169	31
major capsid protein	P3	hexon	Vp54	A345
MW per monomer	43 kD	109 kD	54 kD	38 kD
5-fold vertex complex	(P31)5(P5)3(P2)1	penton base, fiber	minor proteins	?
membrane	inside	no	inside	inside
cell entry	no	endocytosis	no	?
DNA translocation	membrane tube	nuclear pore	injection	?
host	many Gram-negative bacteria	human	unicellular green algae	<i>Sulfolobus islandicus</i>

STIV, *sulfolobus turisticosahedral virus* ; PBCV-1, *Paramecium bursaria Chlorella virus type 1*



Coomassie-stained SDS-PAGE of the structural proteins prepared from purified STIV virions. A 4 to 20% gradient gel was used for electrophoresis. Numbers indicate the excised bands. Mr, protein molecular mass marker (kilodaltons).

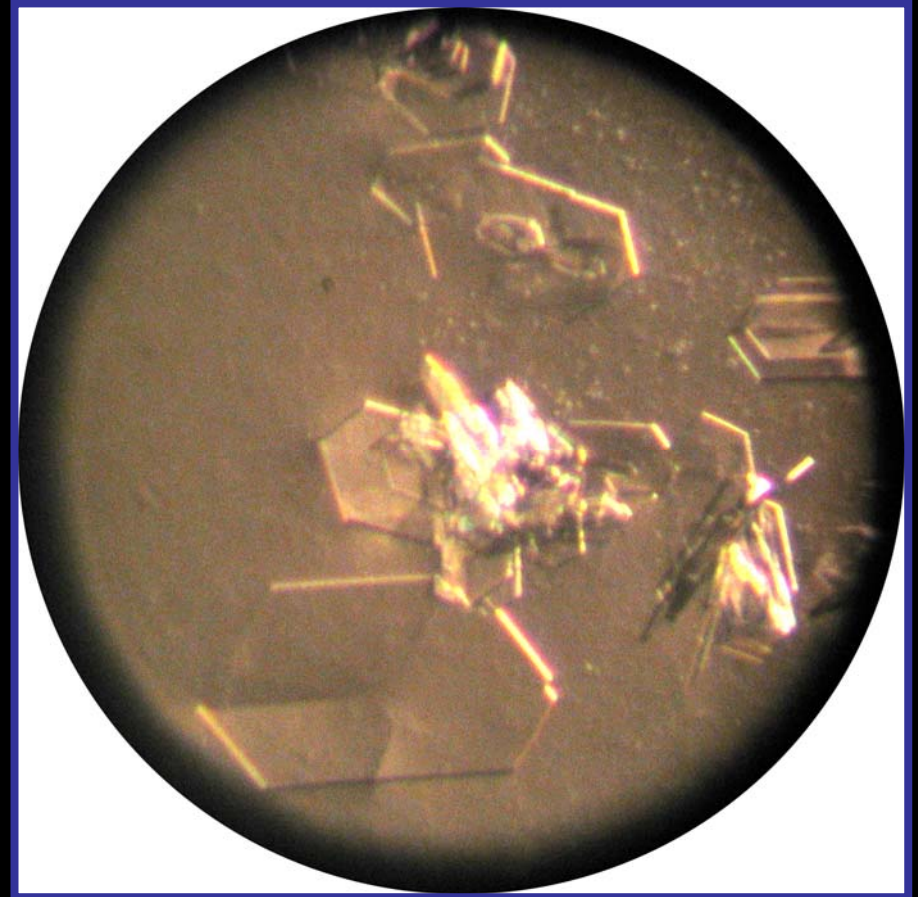
Crystal Information

Crystallization condition

- 16% PEG 3350
- 10% MPD
- 0.1M Bicine 9.0
- 5mM β -Me
- 0.2mM Am. Citrate 4.5
- 50mM Na_2SO_4
- 5% Glycerol
- 0.1M Gd-HCl
- 20.4mM DDAO

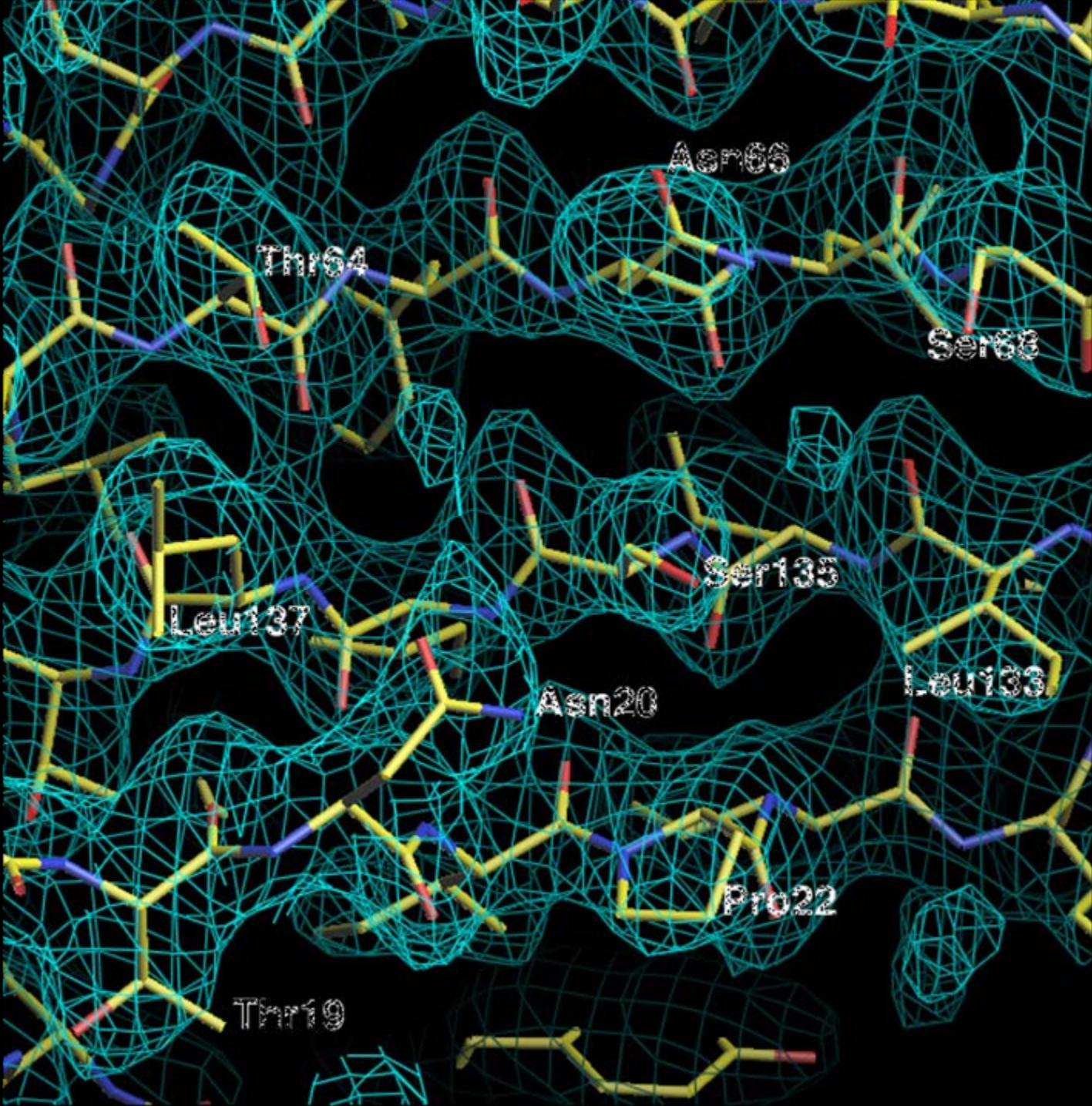
Crystal Growth

- Grown in 3 to 4 days
- Flash frozen in N_2 (*l*)

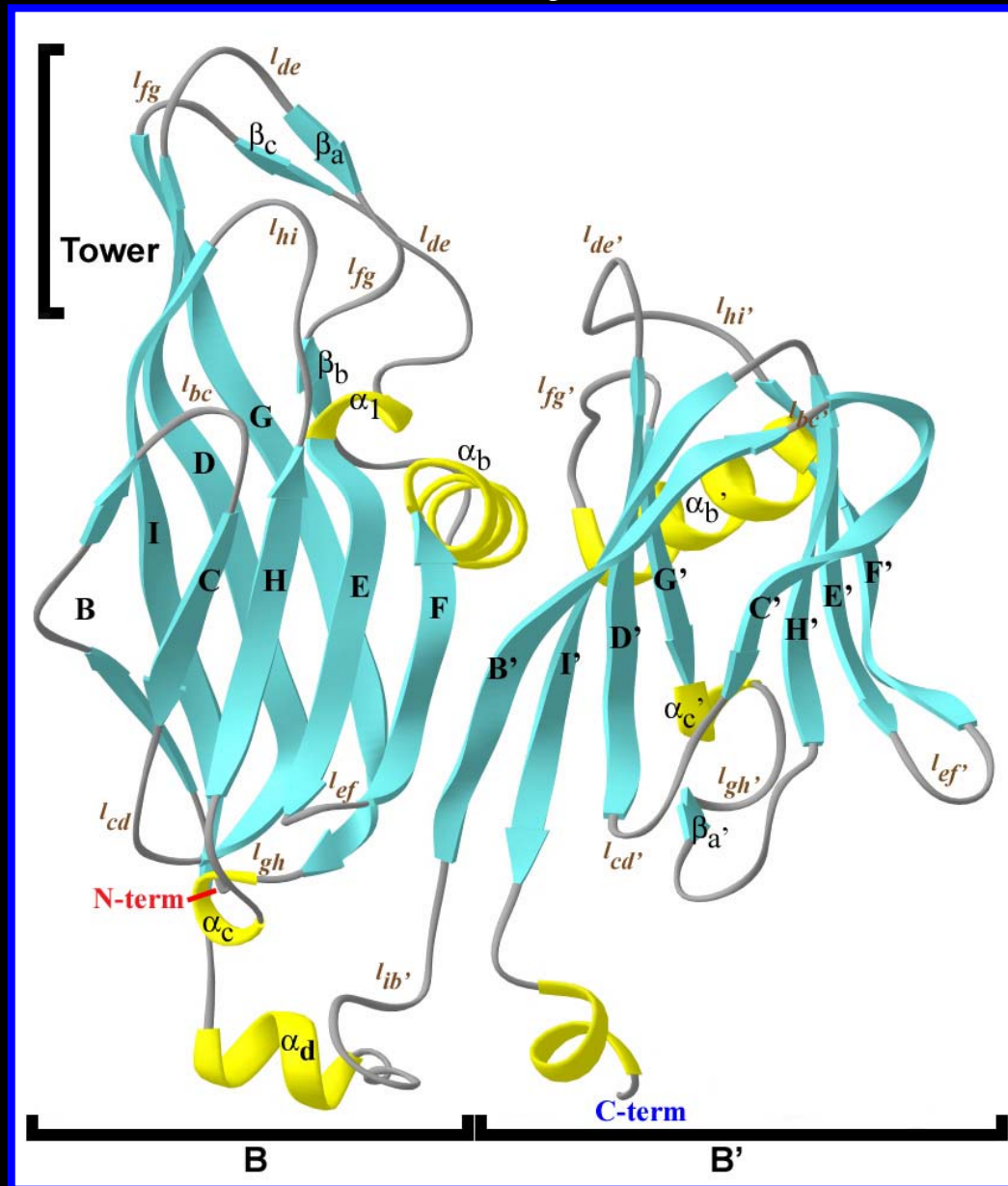


Summary of Xray Data		
	λ_{1a}	λ_{1b}
Wavelength (Å)	0.97957	0.97957
Max resolution (Å)	2.6	2.0
Measured Reflections	941,689	821,050
Space Group	C2	C2
Cell parameters	$a=241.2, b=82.9, c=115.8$ Å $\alpha=\gamma= 90^\circ, \beta=116$	$a=241.5, b=82.9, c=115.8$ Å $\alpha=\gamma= 90^\circ, \beta=116^\circ$
Resolution range (Å)	30-2.6	30-2.0
Unique Reflections	62,522	92,518
Completeness (%) ^b	97.7 (97.6)	83.1 (54.2)
R_{merge} (%) ^{a,b}	8.0 (27.9)	7.1 (29.1)
I/σ	14.1 (2.9)	16.6 (3.15)
Multiplicity	3.6	4.7
Solvent Fraction	0.58	0.58
Substructures (Se)	14 Se	---
^a $R_{\text{merge}} = \sum_h \sum_i I_{hi} - \bar{I}_h / \sum_h \sum_i I_{hi}$, ^b Values in parenthesis refer to the highest resolution bin.		

Summary of Refinement Statistics	
Resolution range (Å)	30 - 2.0
Number of Reflections	81,614
Completeness (%)	72.8
R factor (%) ^b	20.7
Free R factor (%)	24.6
RMSD bond length (Å)	0.006
RMSD bond angle (°)	1.38
${}^b R = \frac{\sum_h F_h^o - F_h^c }{\sum_h F_h^o}$	

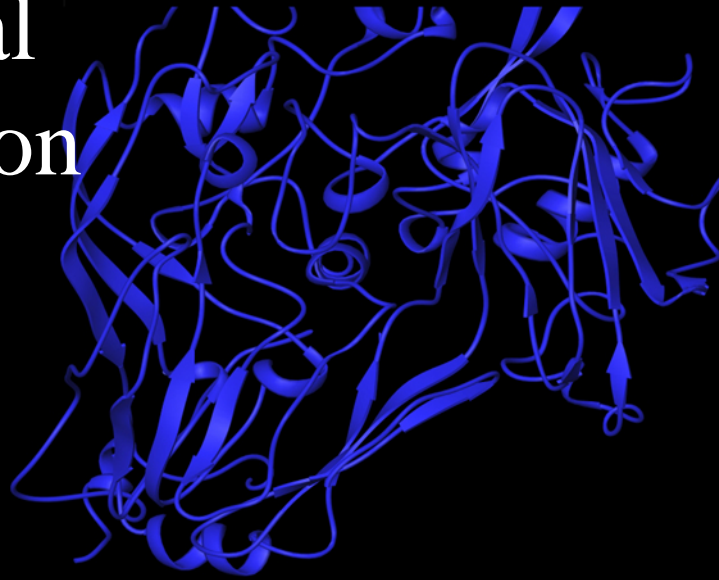


STIV MCP Crystal Structure

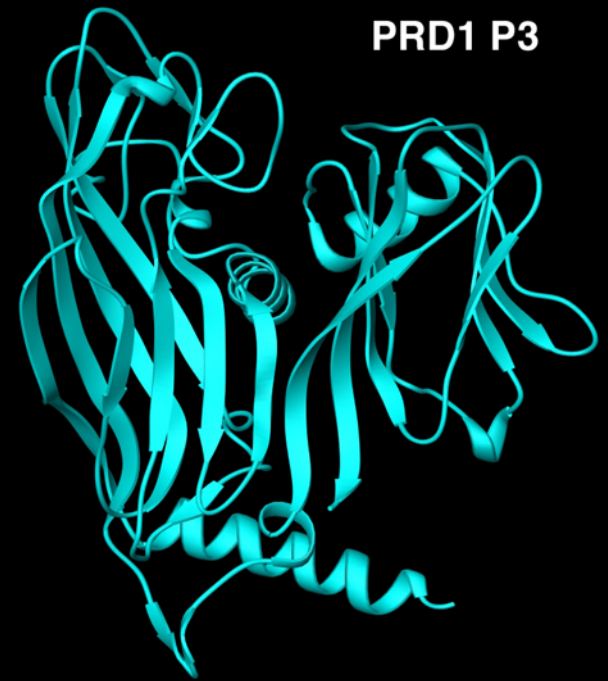


Structural Comparison

Adenovirus Hexon



PRD1 P3



PBCV1 vp54



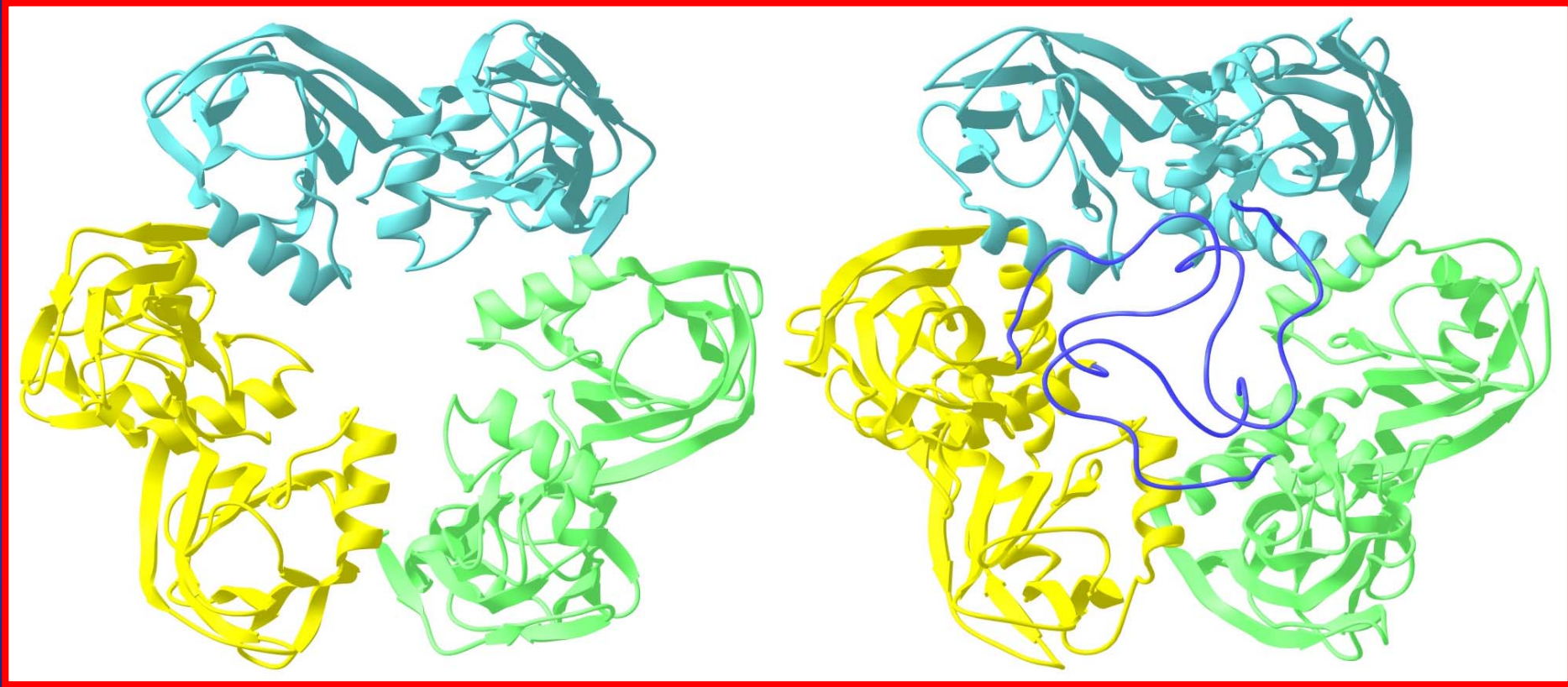
STIV MCP





STIV Trimer model

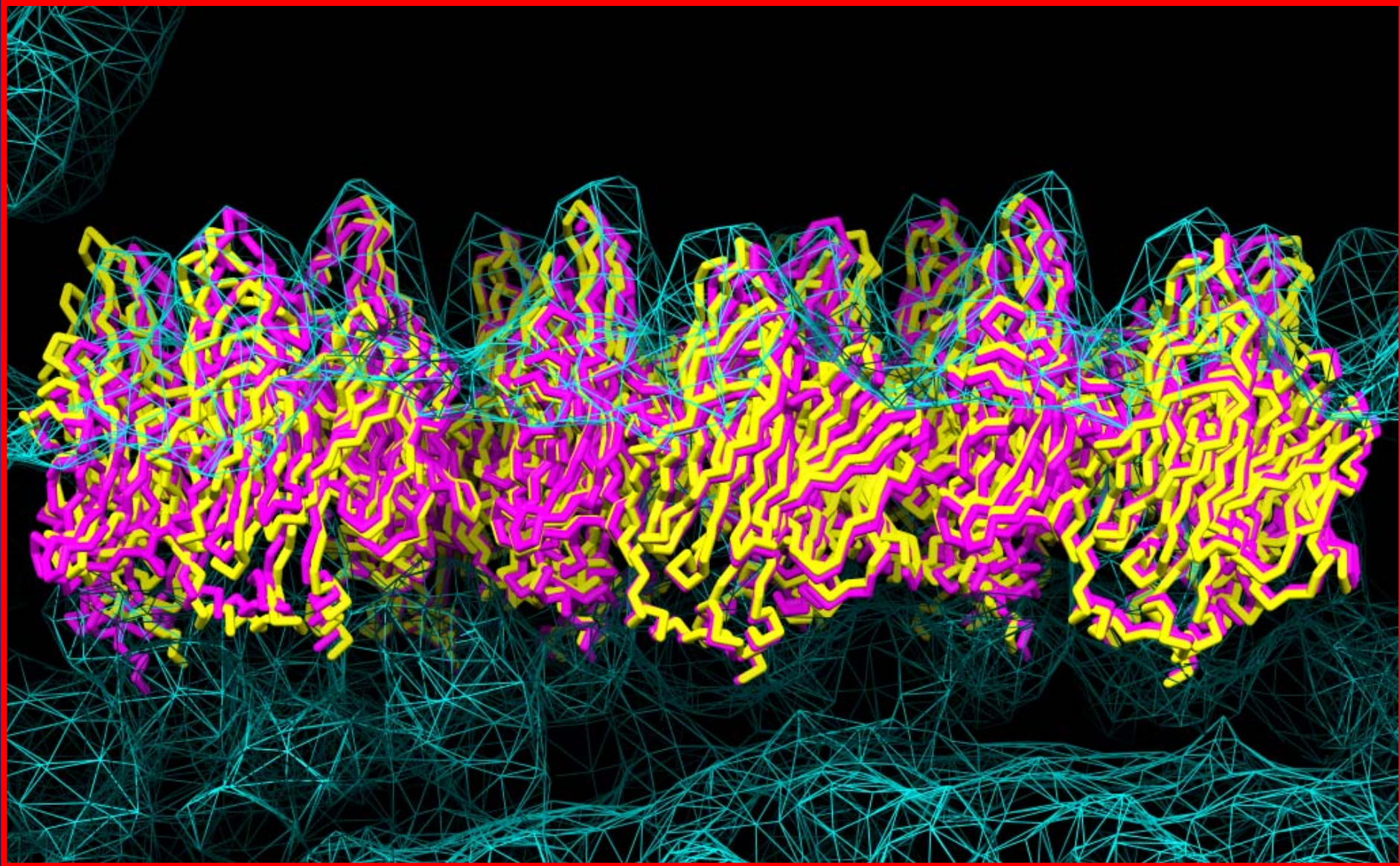
PRD1 x-ray structure

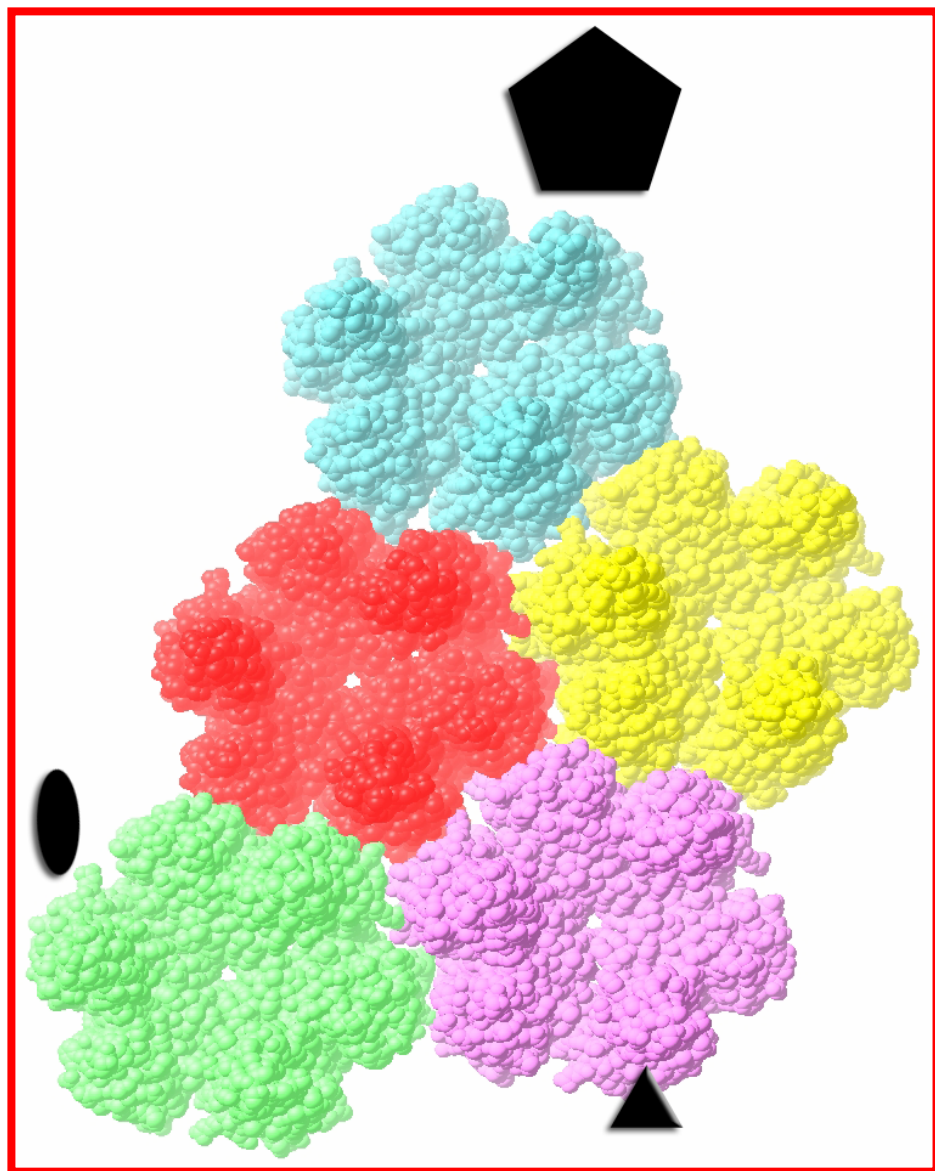


Refined Pseudo-atomic models

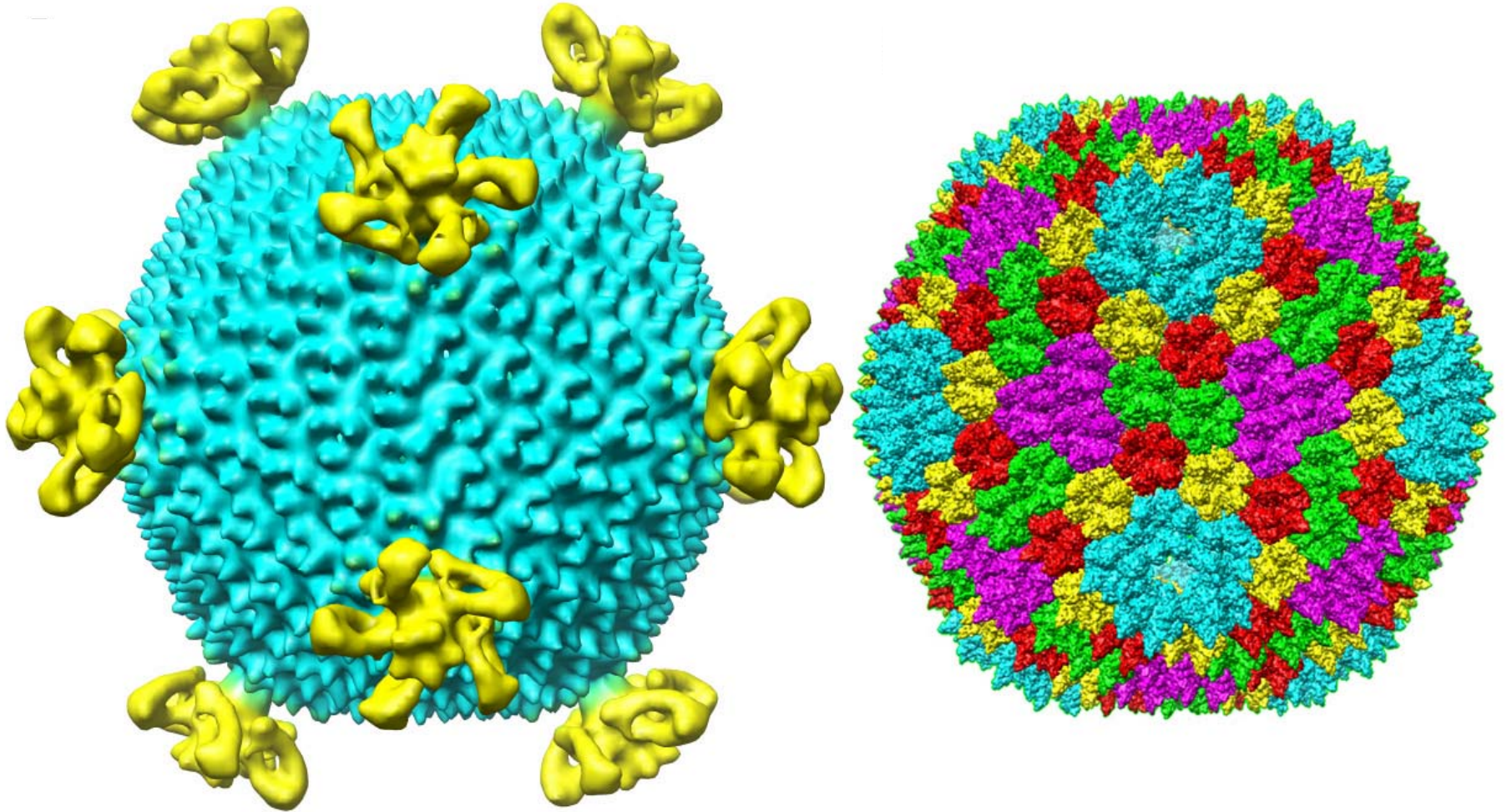
Method 1	
R factor (%) ^a	32.8
Temp. factor (²)	276
Method 2	
R factor (%) ^a	29.9
Temp. factor (²)	258
^a $R = \frac{\sum_h F_h^o - F_h^c }{\sum_h F_h^o}$	

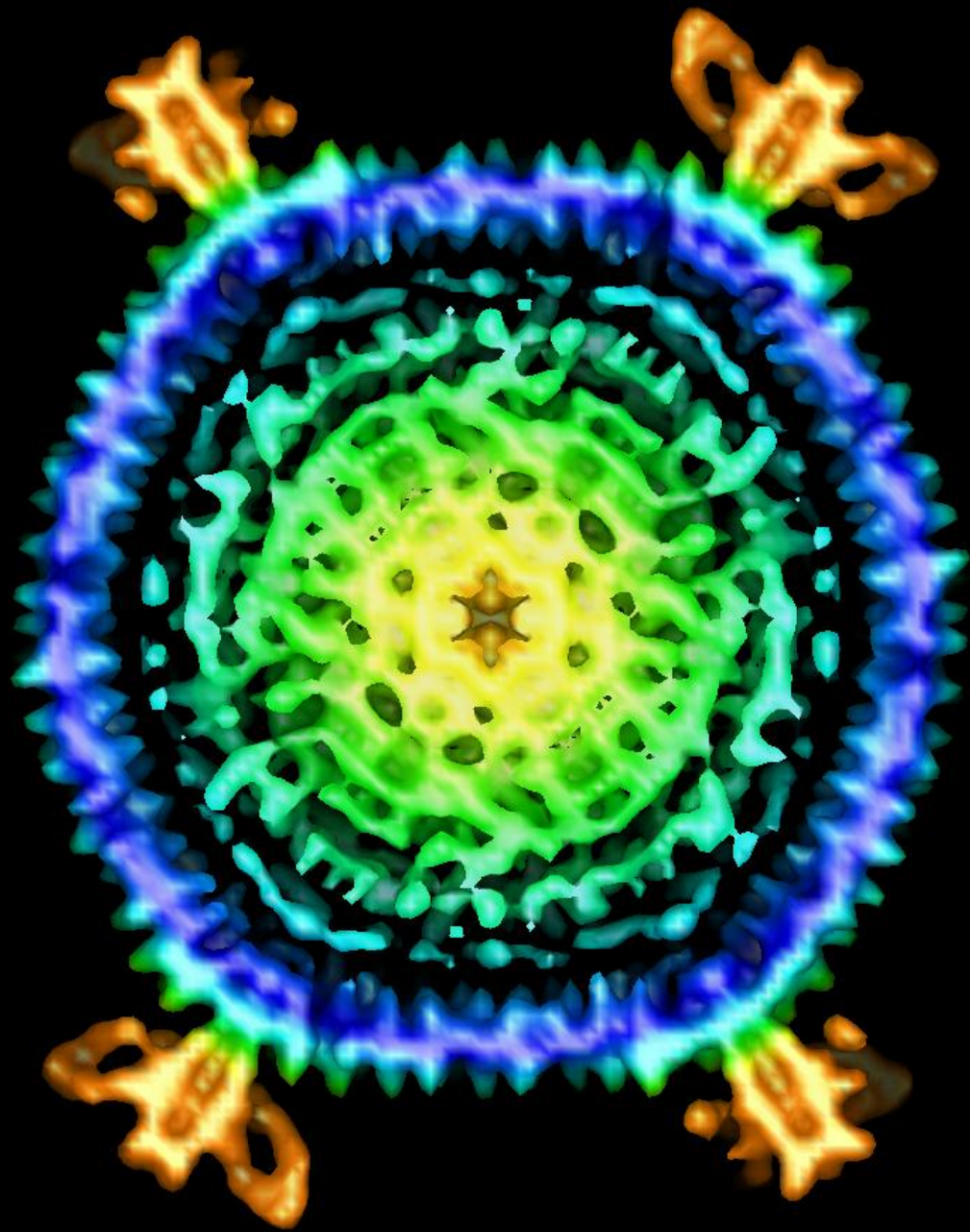
Refined Icosahedral ASU



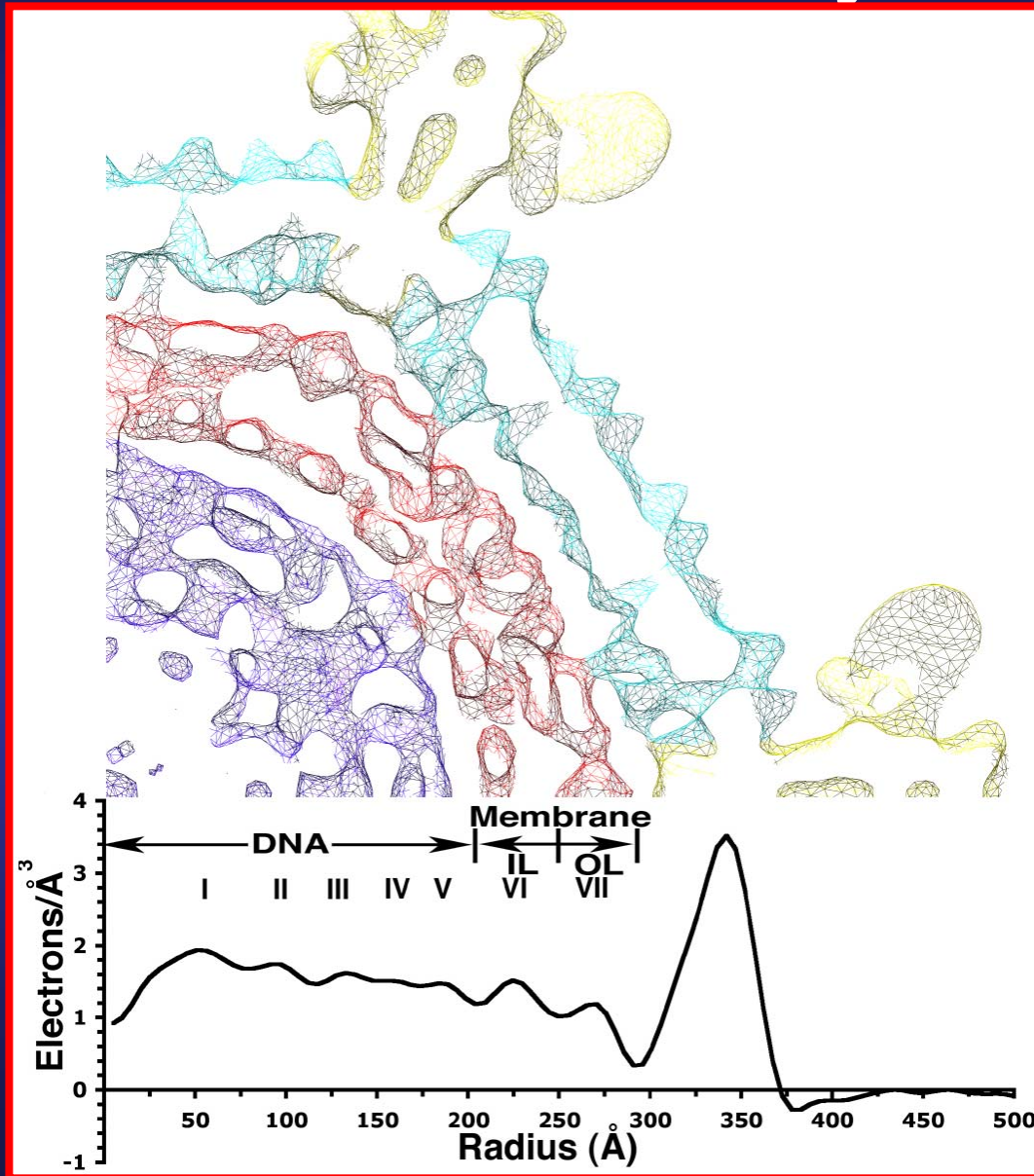


Pseudo-atomic model of shell

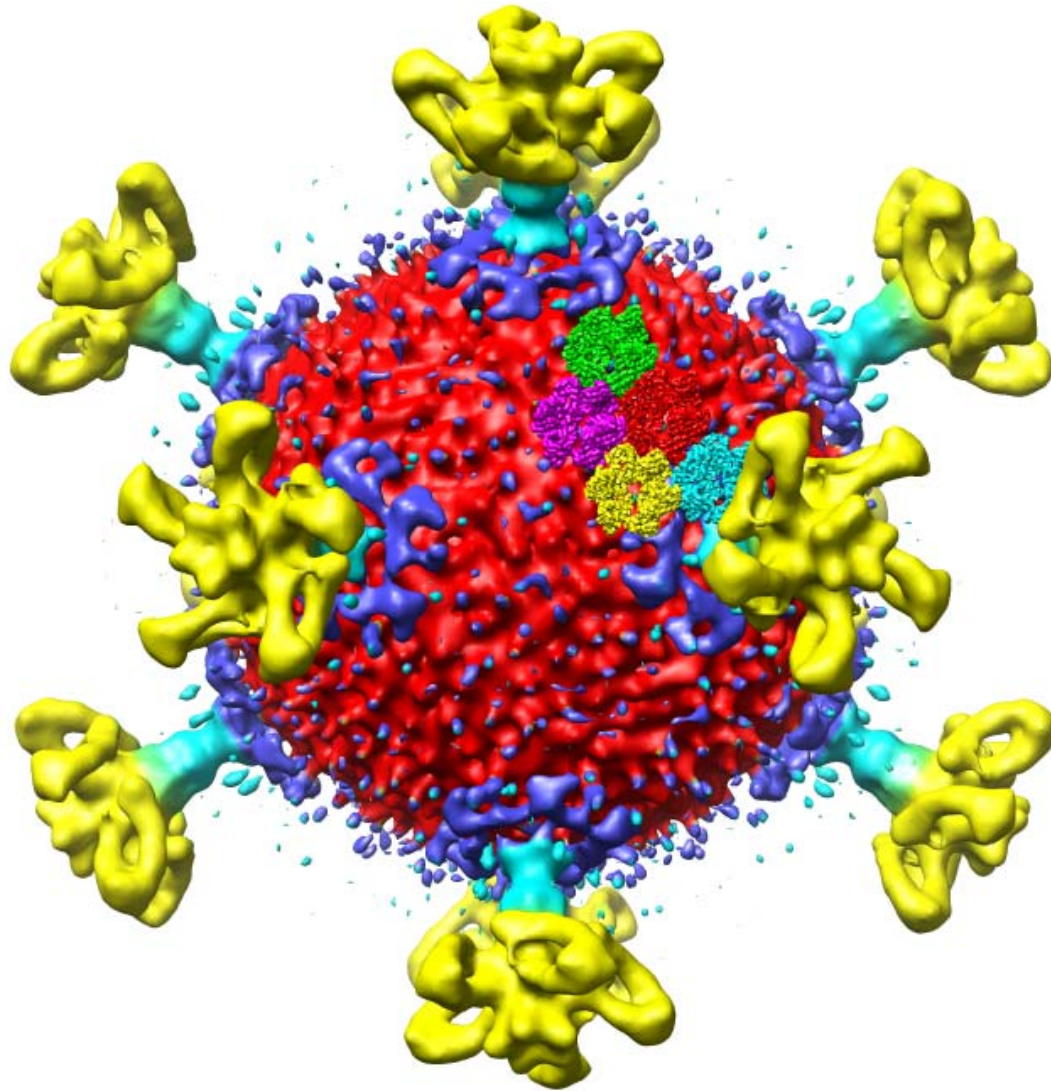




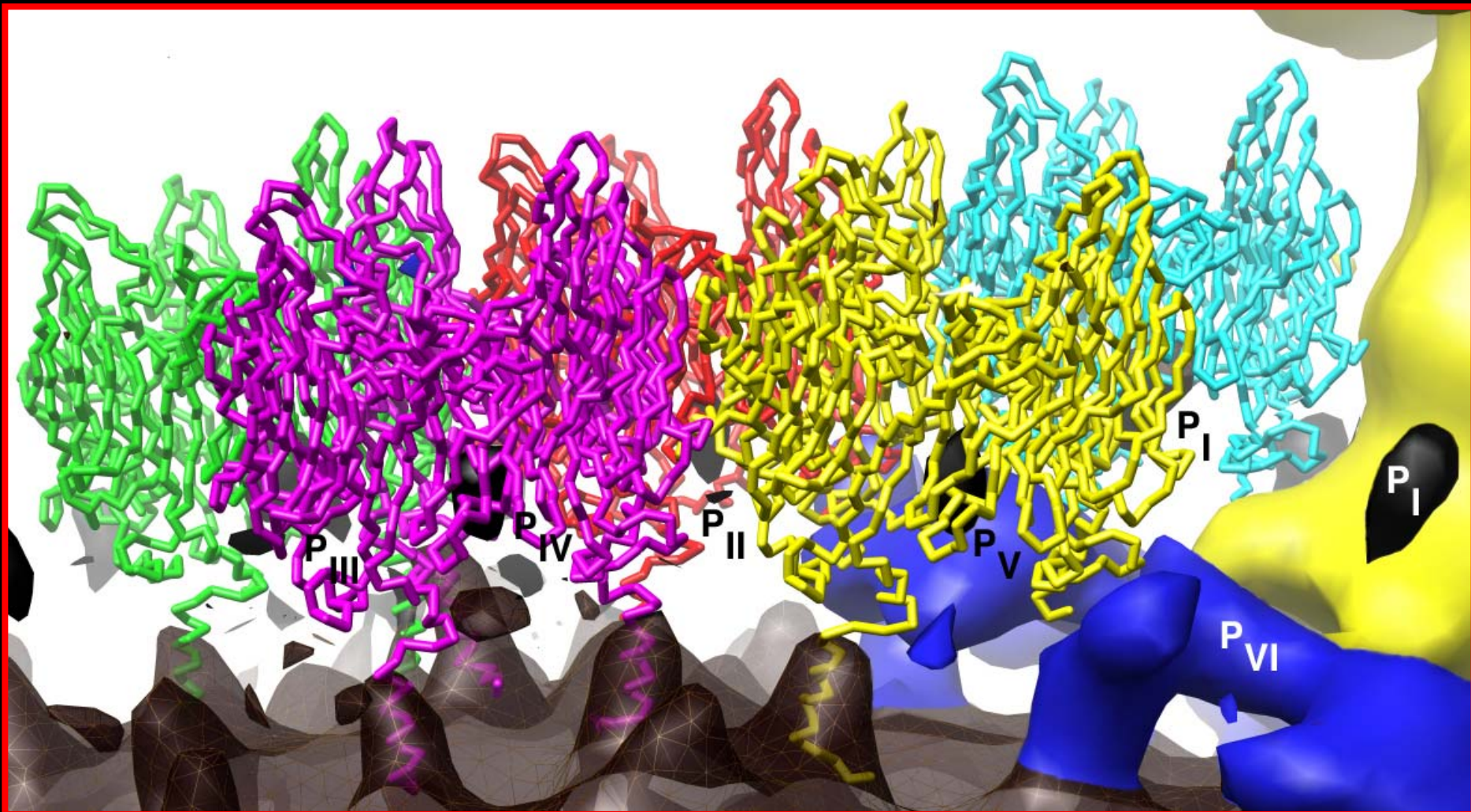
Radial Electron Density Profile



Difference Map



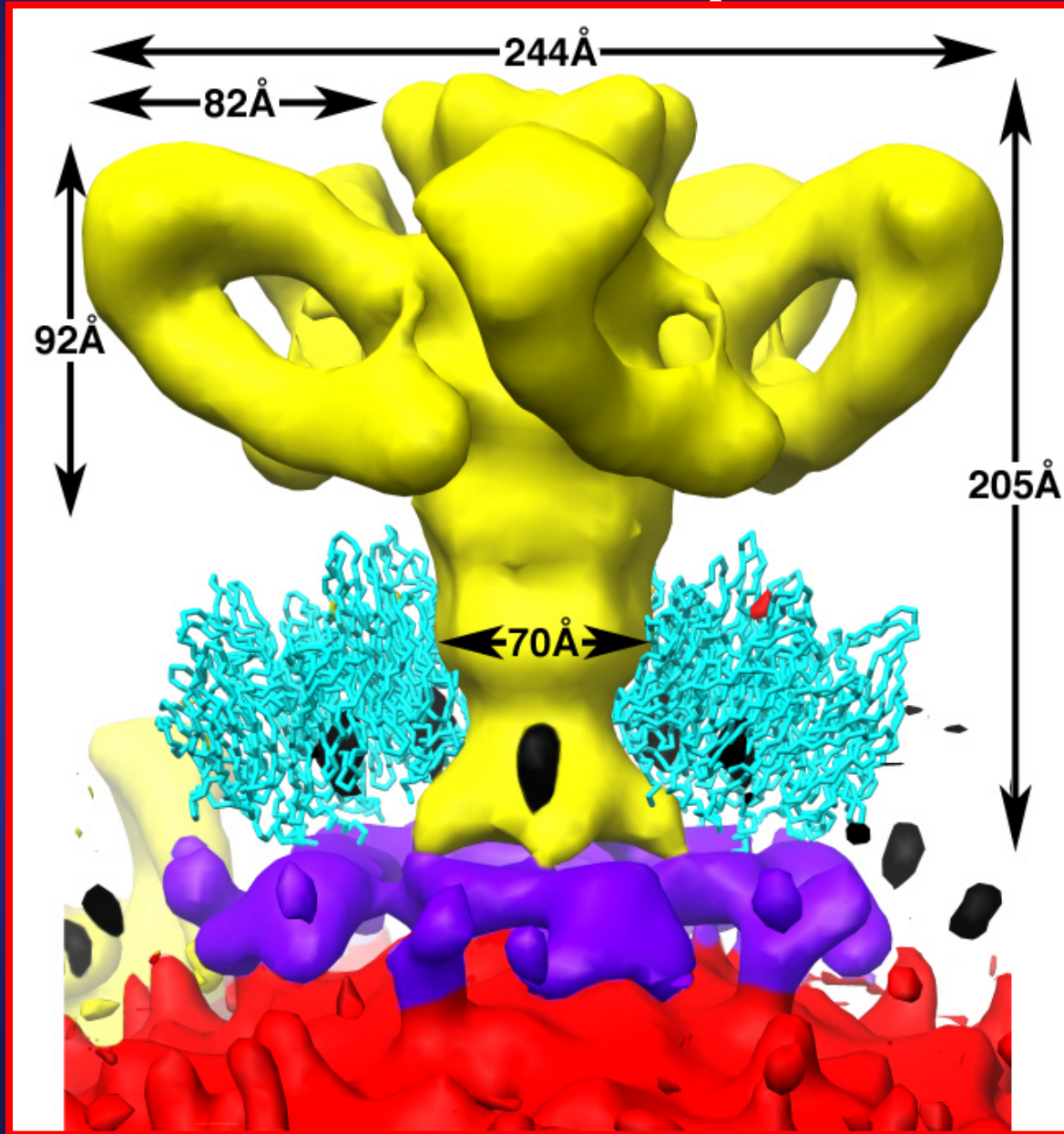
Difference Map



$P_{VI} = 19.8$ KDa

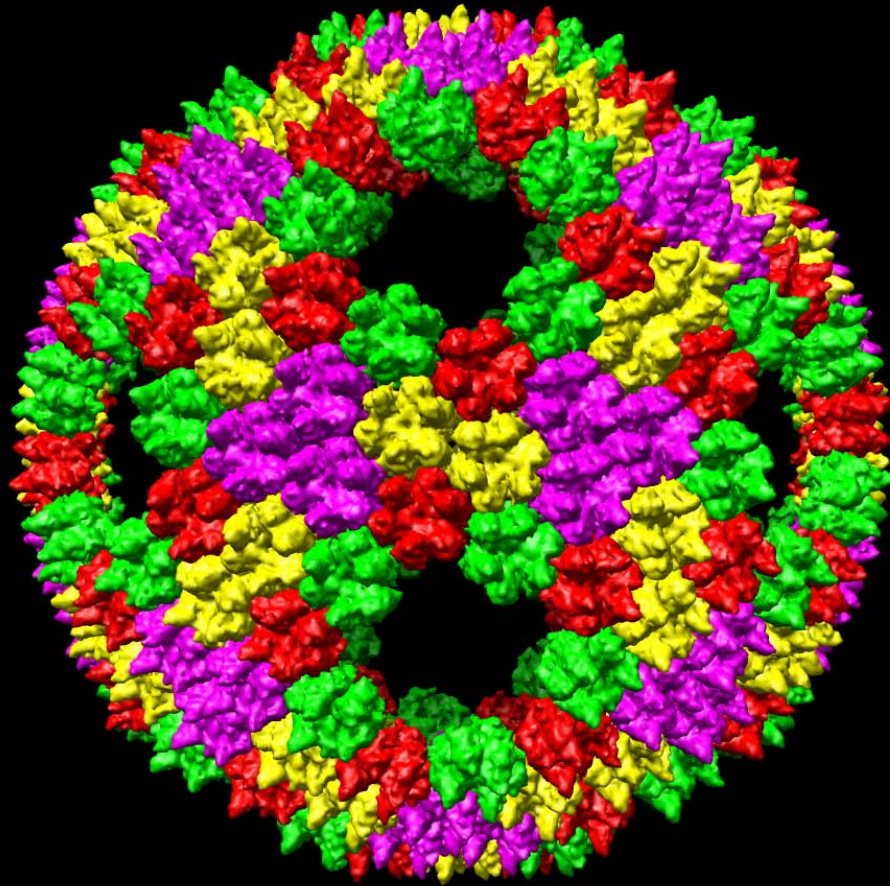
C-term = 2.7 KDa

Vertex Complex

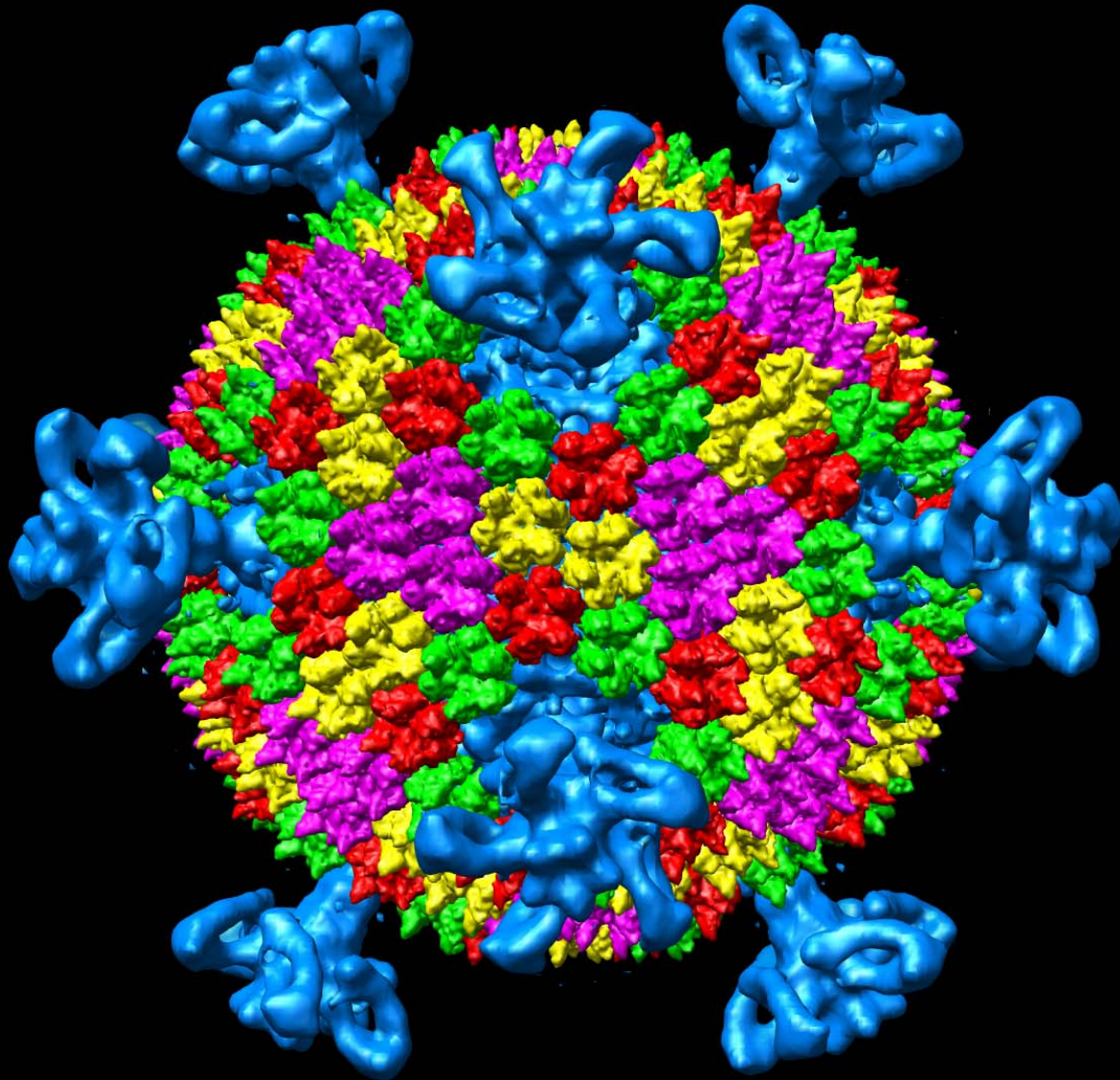


~127.4 KDa

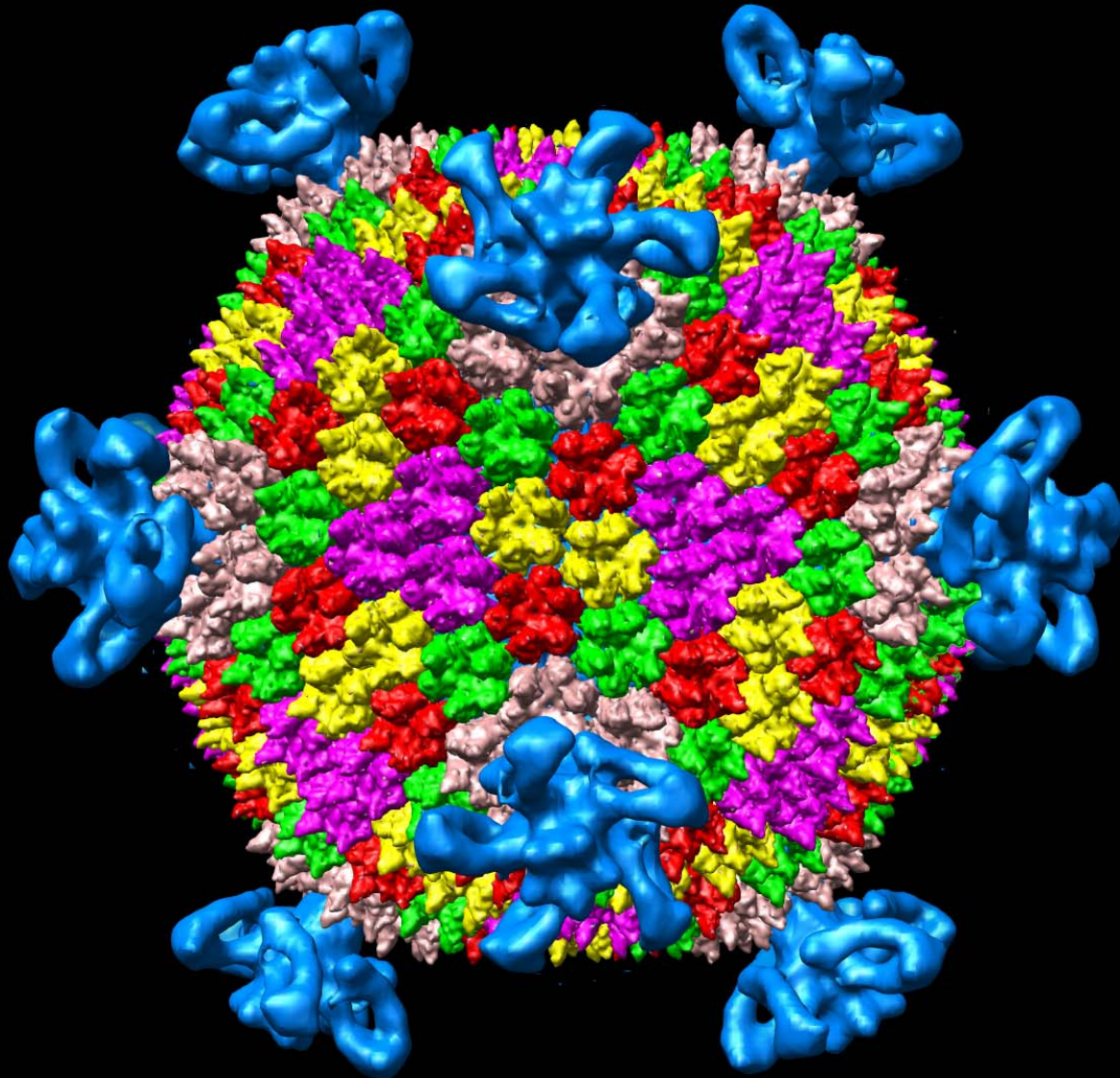
Assembly



Assembly



Assembly

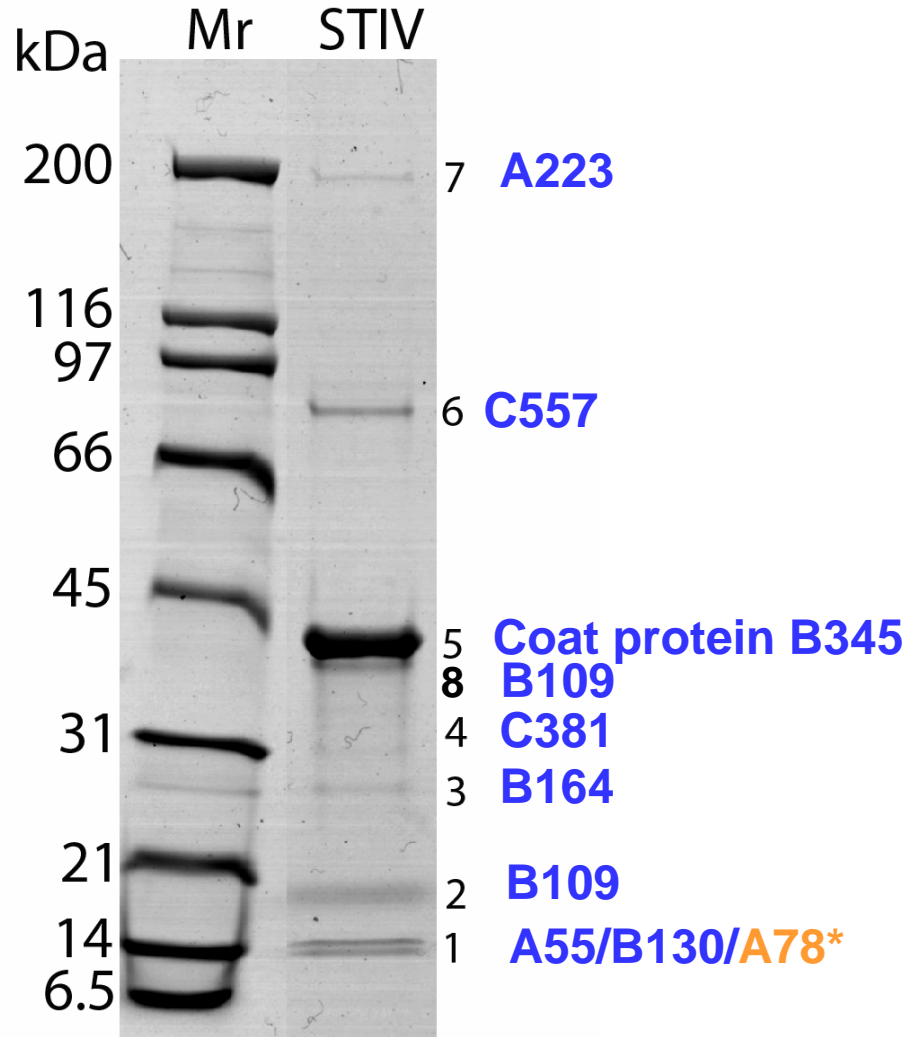


Proteomics of STIV

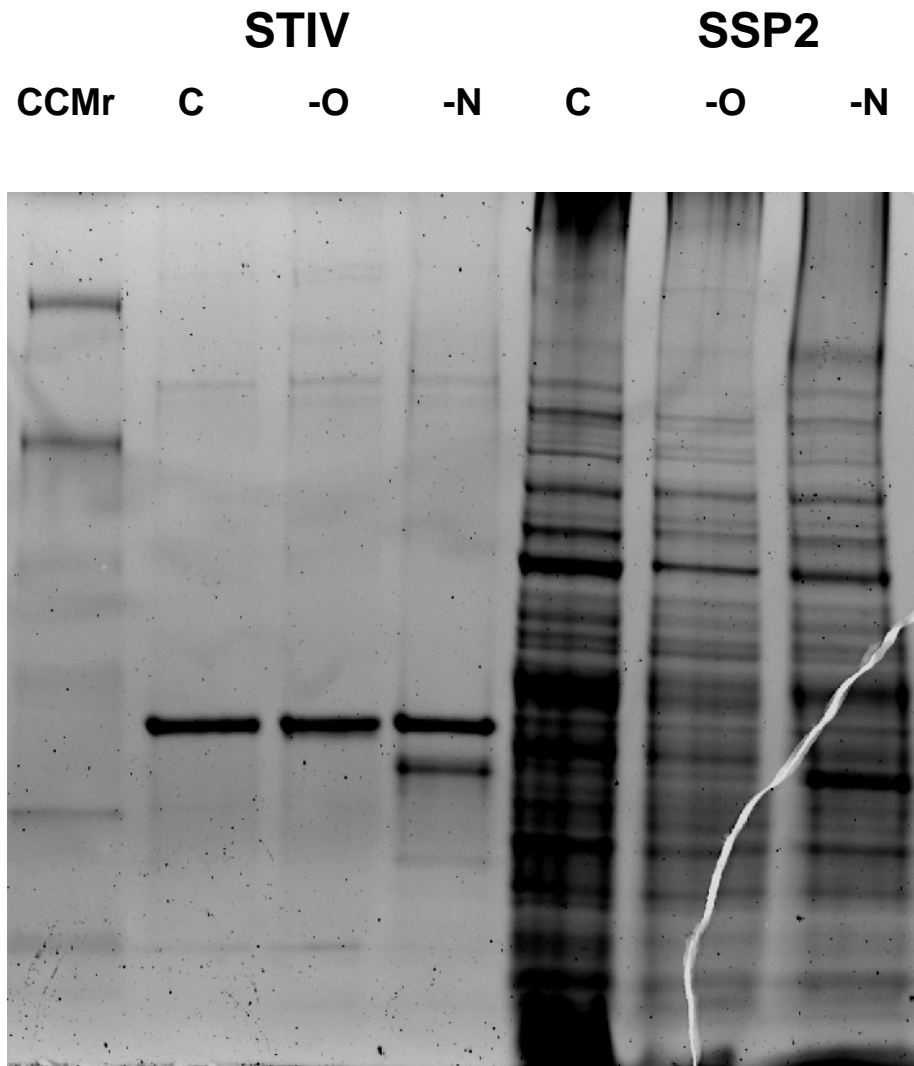
JOURNAL OF VIROLOGY, Aug. 2006, p. 7625–7635 Vol. 80, No. 15

Characterization of the Archaeal Thermophile *Sulfolobus* Turreted Icosahedral Virus Validates an Evolutionary Link among Double-Stranded DNA Viruses from All Domains of Life

Walid S. A. Maaty, Alice C. Ortmann, Mensur Dlakic', Katie Schulstad, Jonathan K. Hilmer, Lars Liepold, Blake Weidenheft, Reza Khayat, Trevor Douglas, Mark J. Young, and **Brian Bothner**

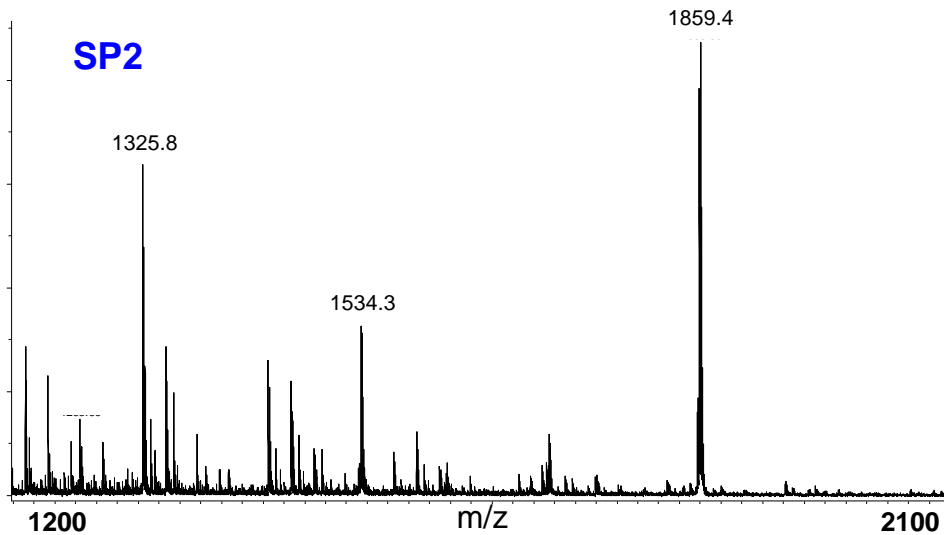


Coomassie-stained SDS-PAGE of the structural proteins prepared from purified STIV virions. A 4 to 20% gradient gel was used for electrophoresis. Numbers indicate the excised bands. Mr, protein molecular mass marker (kilodaltons).

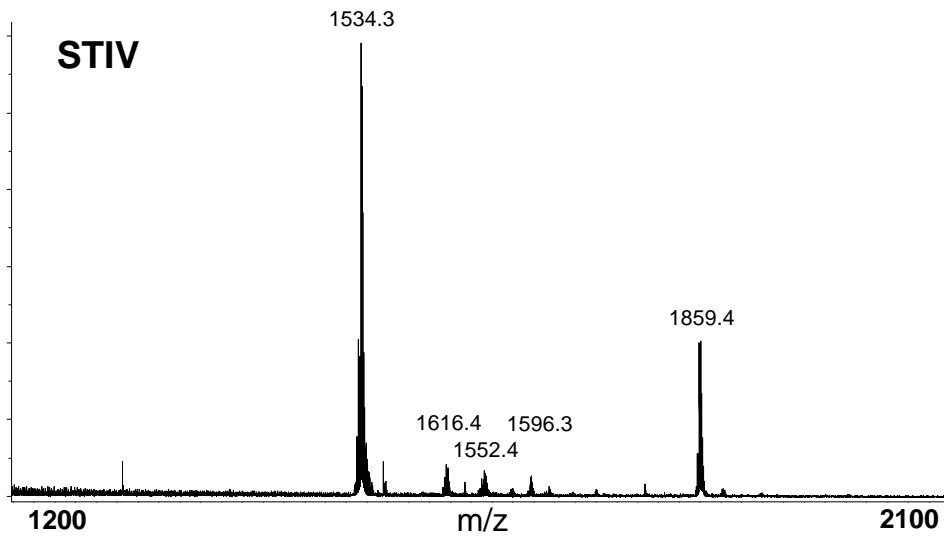


Pro-Q Glycoprotein stained SDS-PAGE of the structural proteins prepared from intact STIV virions and whole cell lysate of SSP2 bacteria after treatment with O or N deglycosidase enzymes. CCMr, CandyCane glycoprotein marker.

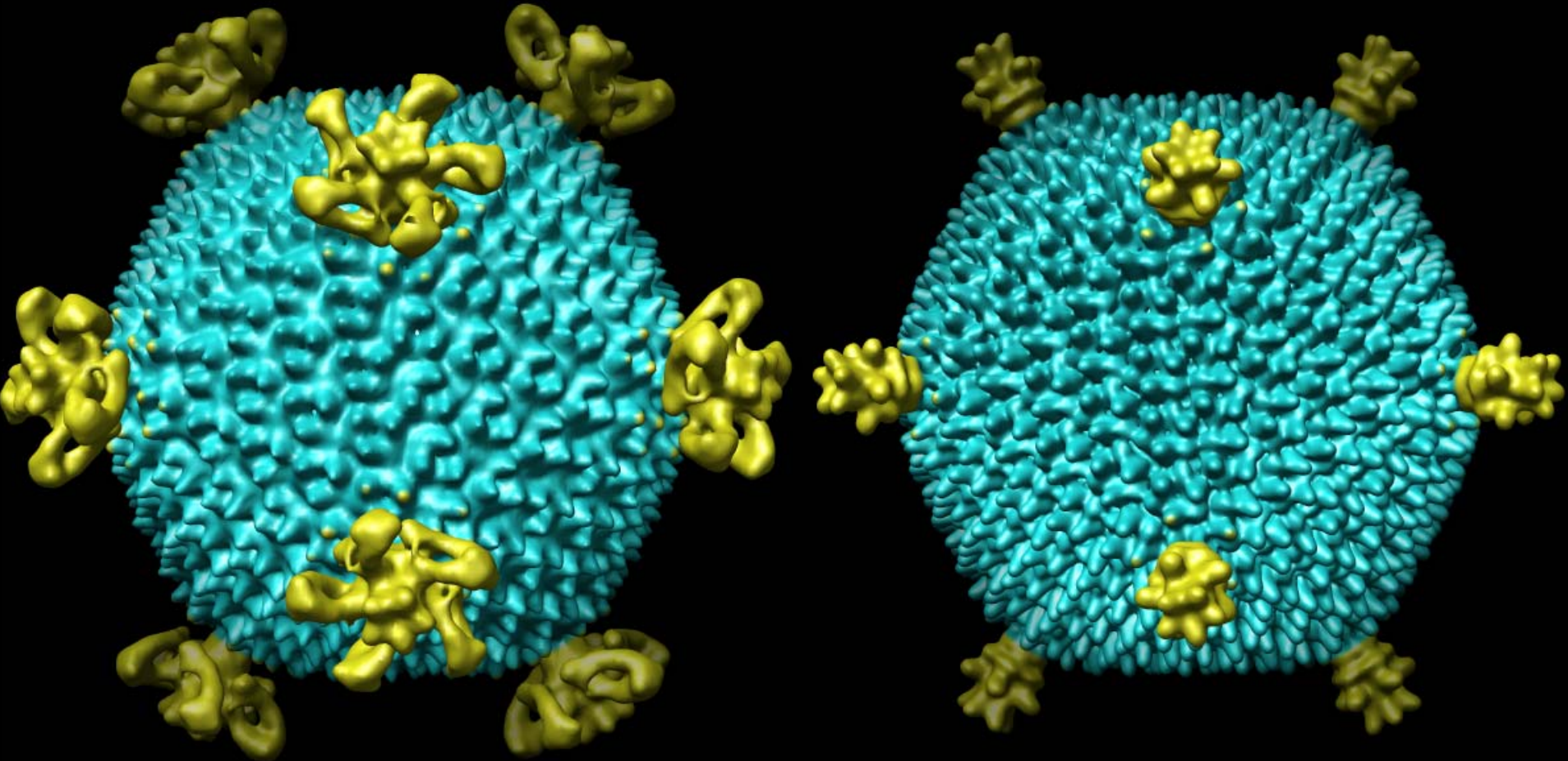
STIV Preferentially Selects Lipids from the Host Cell



Total lipid extracts from SP2 cells and STIV analyzed using negative ion electrospray. The solvent for analysis was Methanol:Chloroform 3:1. This solvent system can be used to differentiate neutral and acidic lipids.

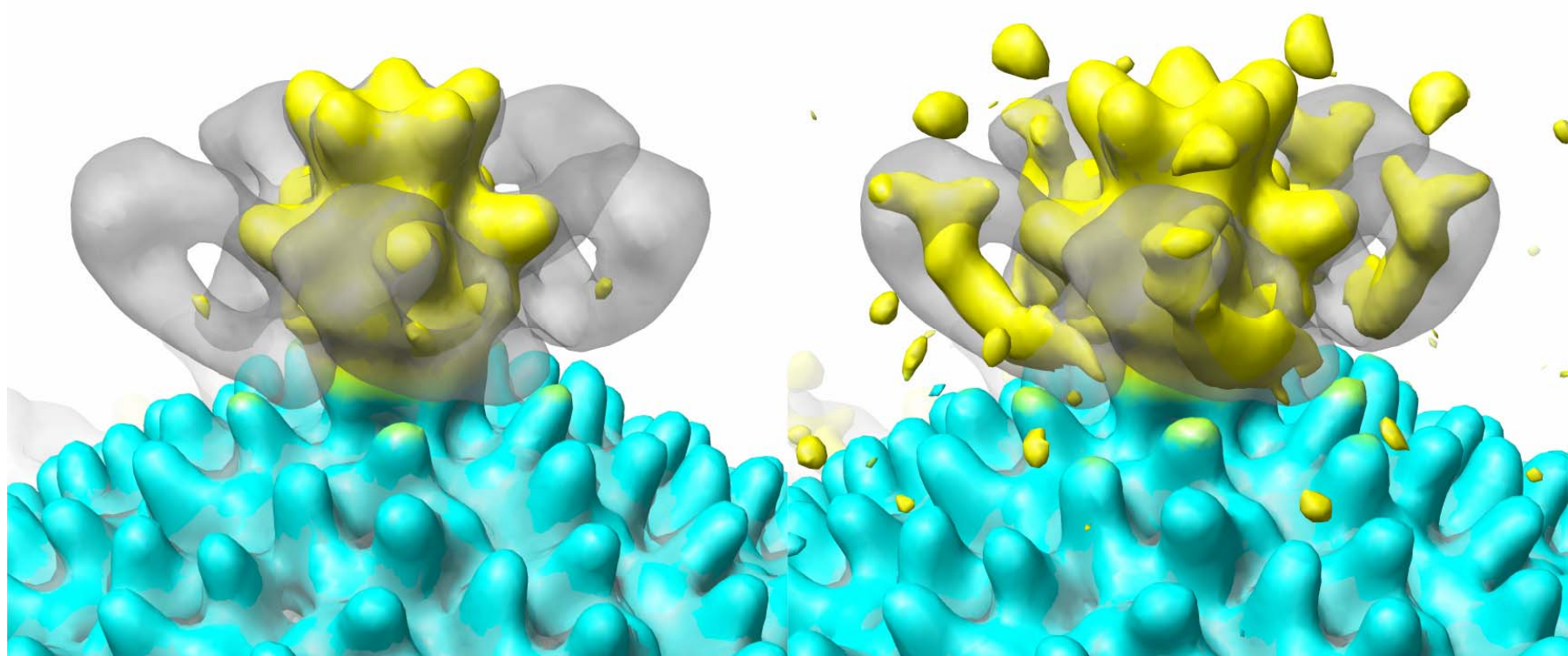


Morphological Differences

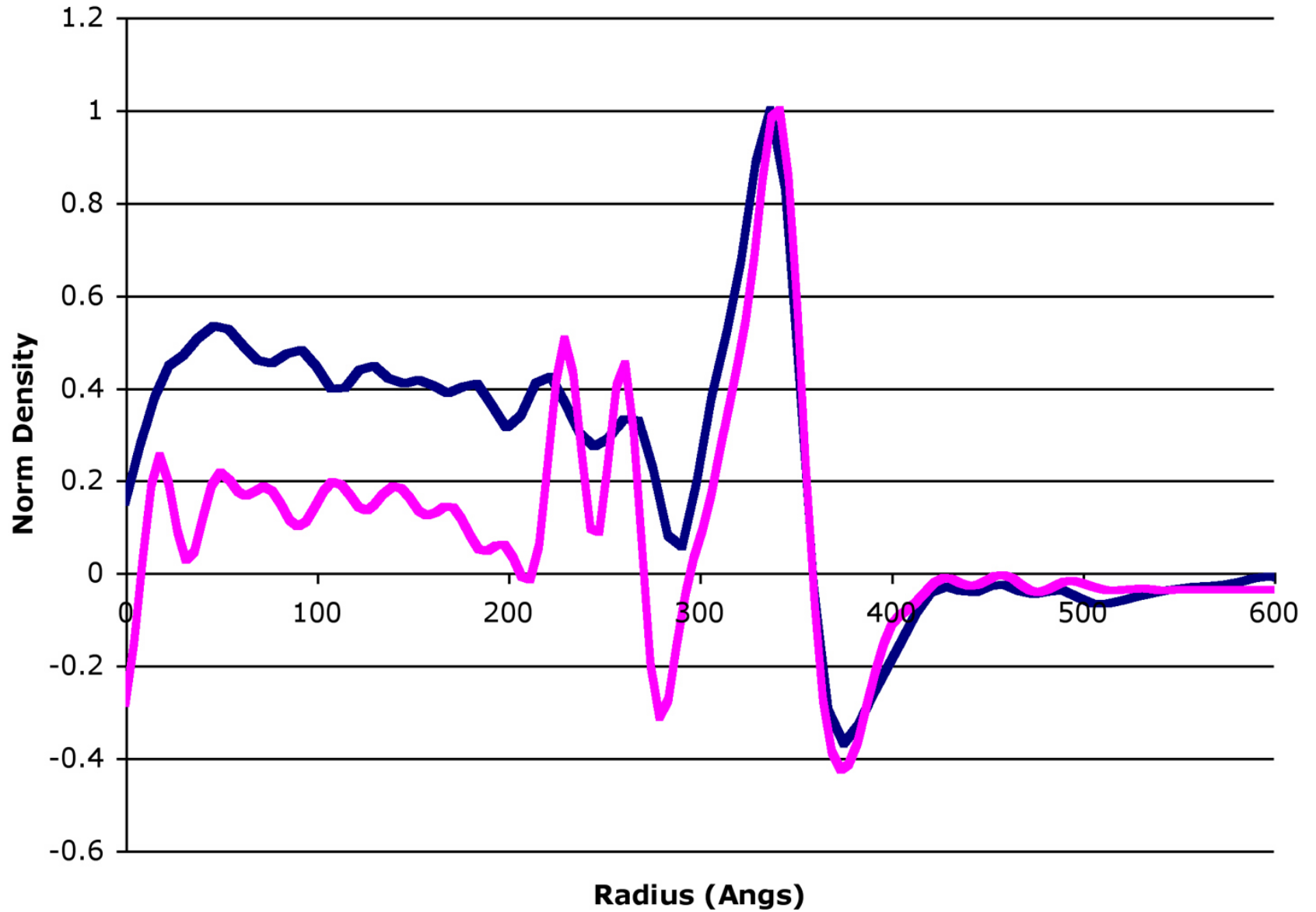


27Å

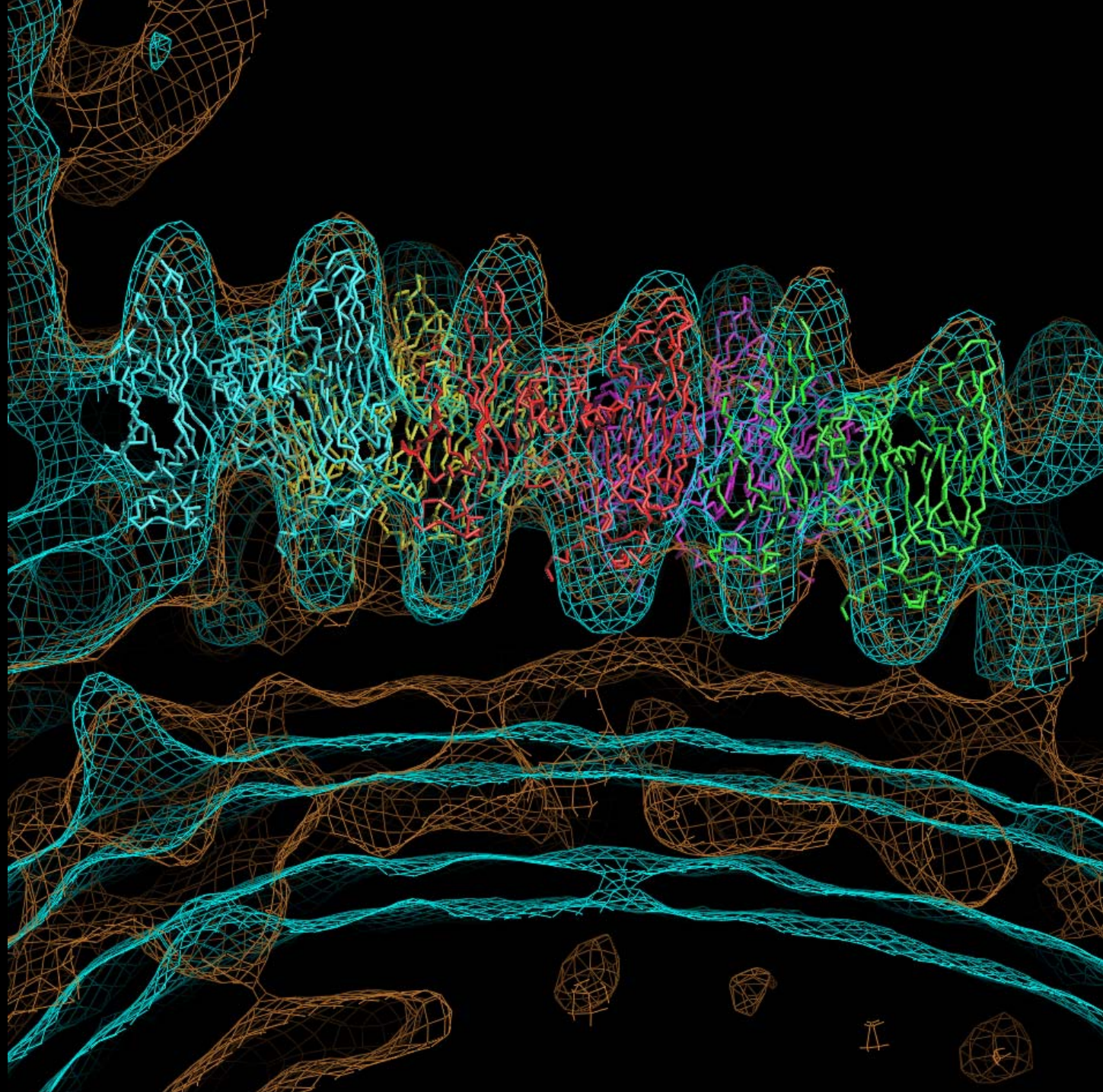
12.5Å



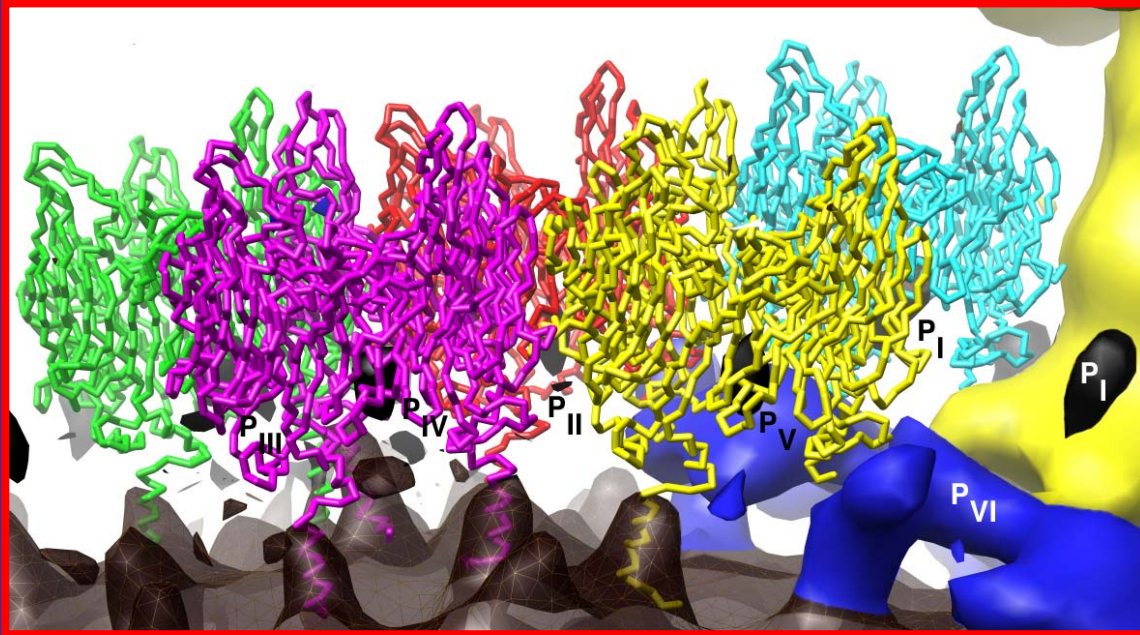
Radial Density



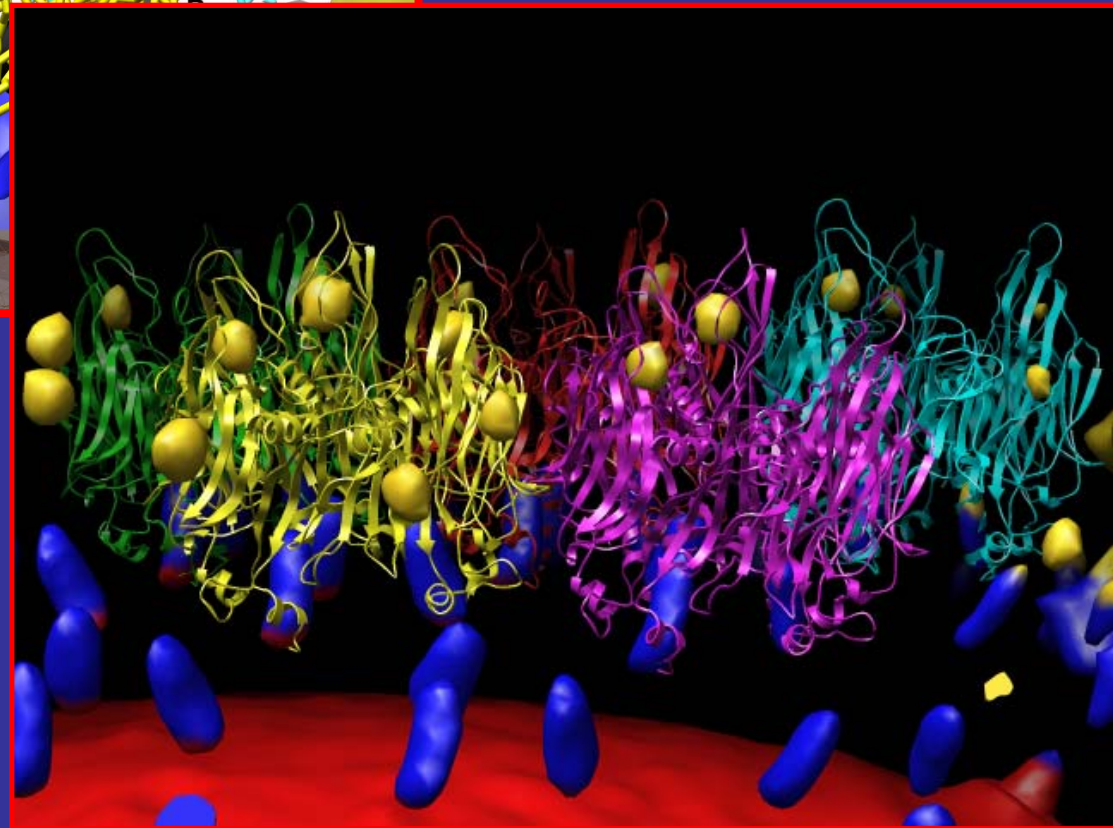
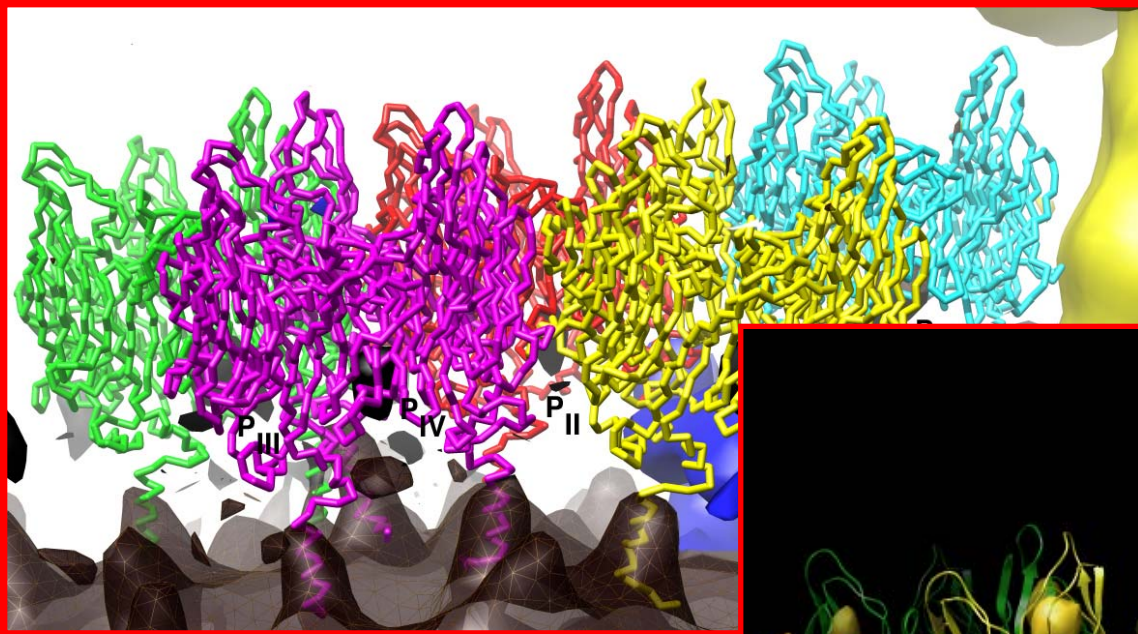
Original New Data



Compare Difference Maps

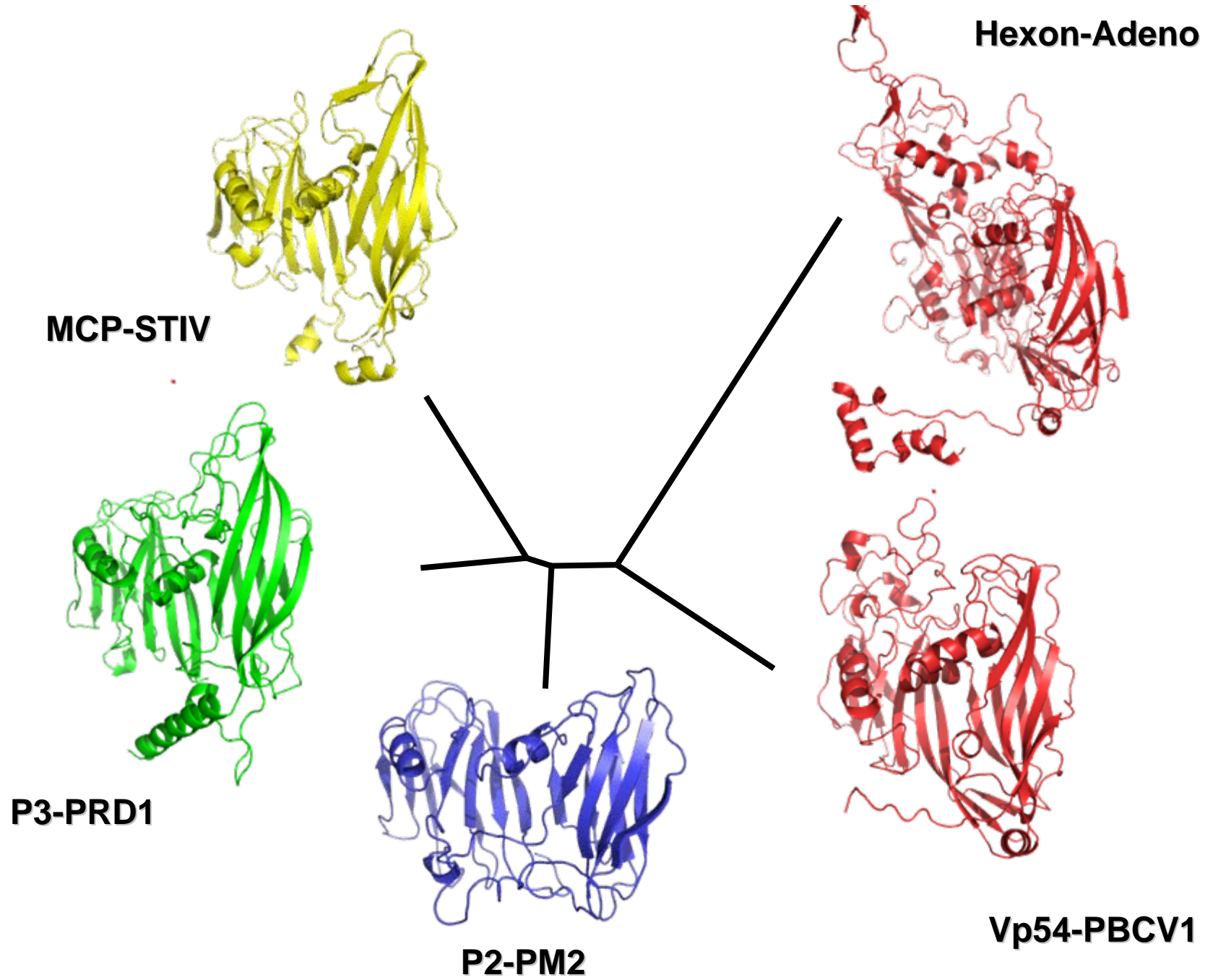


Compare Difference Maps



Adenovirus-like capsids

- All are dsDNA viruses
- Subunits contain the “double barrel” fold.
- Subunits form trimers that are pseudo hexamers.
- Particles have large T numbers.
- Particles have protruding pentons.
- All but adenovirus have an internal membrane.
- Are there tape measure proteins in all the particles as observed in PRD1?



Provisional members of the PRD1 -adeno lineage

Bacterial viruses

PRD1- group (*Tectiviridae*) Gram negative hosts

Bam35- group (*Tectiviridae*) Gram positive hosts

PM2 (*Corticoviridae*) Gram negative hosts

Thermus virus 77 (no classification yet) *

Archaeal viruses

Sulfolobus turreted icosahedral virus (STIV)

Halovirus SH1*

Lower eukaryotic viruses

Paramecium bursaria Chlorella virus 1 (*Phycodnavirideae*)

Mimivirus (? *Mimiviridae*)

Invertebrate and vertebrate viruses

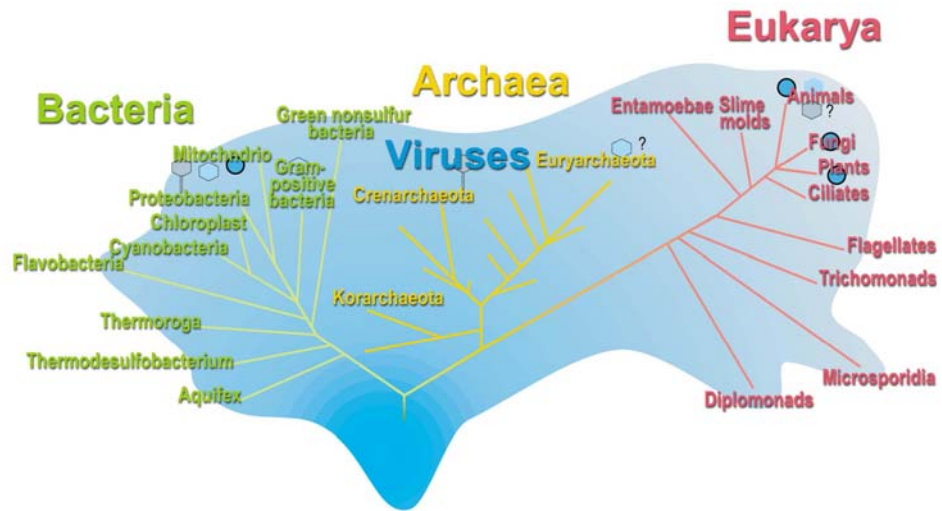
Chilo iridescent virus (*Iridoviridae*)

African swine fever virus (*Asfarviridae*)

Vaccinia virus (*Poxviridae*) ?

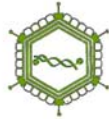
Nucleopolyhedrosisvirus (*Baculoviridae*) ?

[Human adenovirus (*Adenoviridae*)]



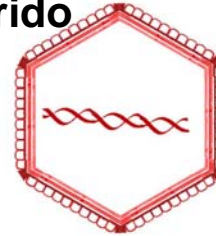
Lineage 1

PRD1

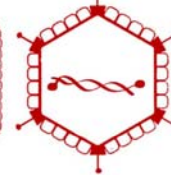


PRD1 / Adeno

irido



adeno



Lineage 2

phi6

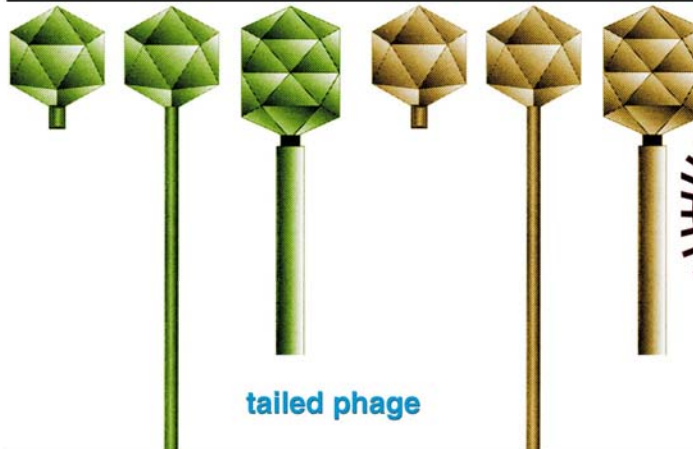


dsRNA

BTV



Lineage 3

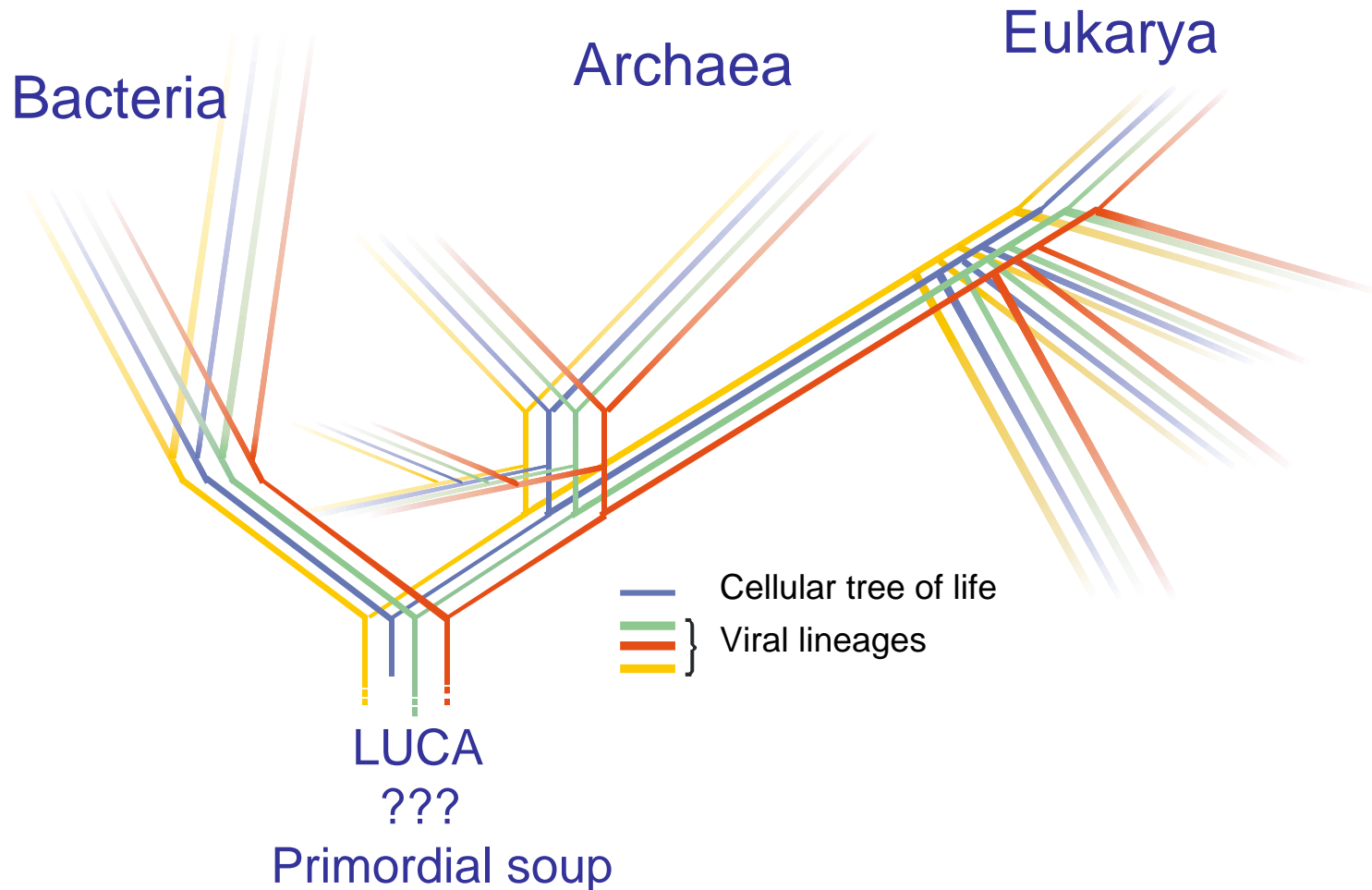


tailed phage



herpes

POLYPHYLETIC ORIGIN OF VIRAL LINEAGES



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