NRAMM Workshop, CIMBio, La Jolla CA

November 8 - 13, 2009

NIAMS

Machines with Moving Parts – See How They Run.

Alasdair C. Steven

Laboratory of Structural Biology, NIAMS-NIH

Sources of Variability in

Electron Micrographs of Macromolecular Complexes

1) Viewing Geometry

2) Intrinsic Variability of Individual Complexes

3) Noise

a) Heterogeneity of composition

b) Multiple discreet conformers

c) Continuous variability :

global breathing; local fluctuations

• Multiple Particle Analysis – Multiple Conformations -
Time-resolved Cryo-EM

• Thermo-cryo-electron microscopy

* Resolution is Inhomogeneous.

• The smallest feature we have been able to see (0.9 kDa)
and the largest feature we have been unable to see (90 kDa)

• A Machine with Many Moving Parts

HSV assembly

LSBR-NIAMS

Naiqian Cheng

Bernard Heymann

Benes Trus

Giovanni Cardone

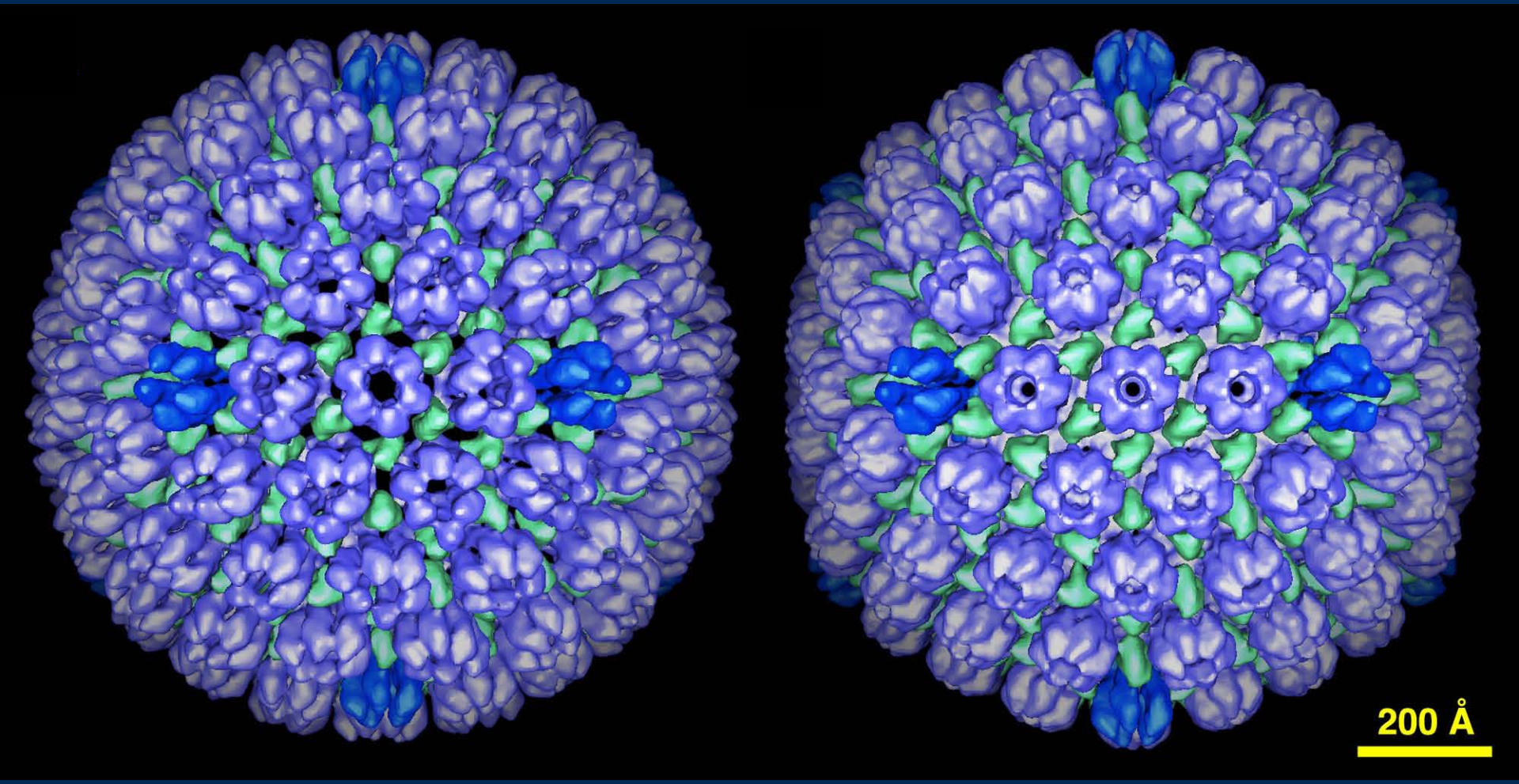
Univ. Virginia Med. School

Jay Brown

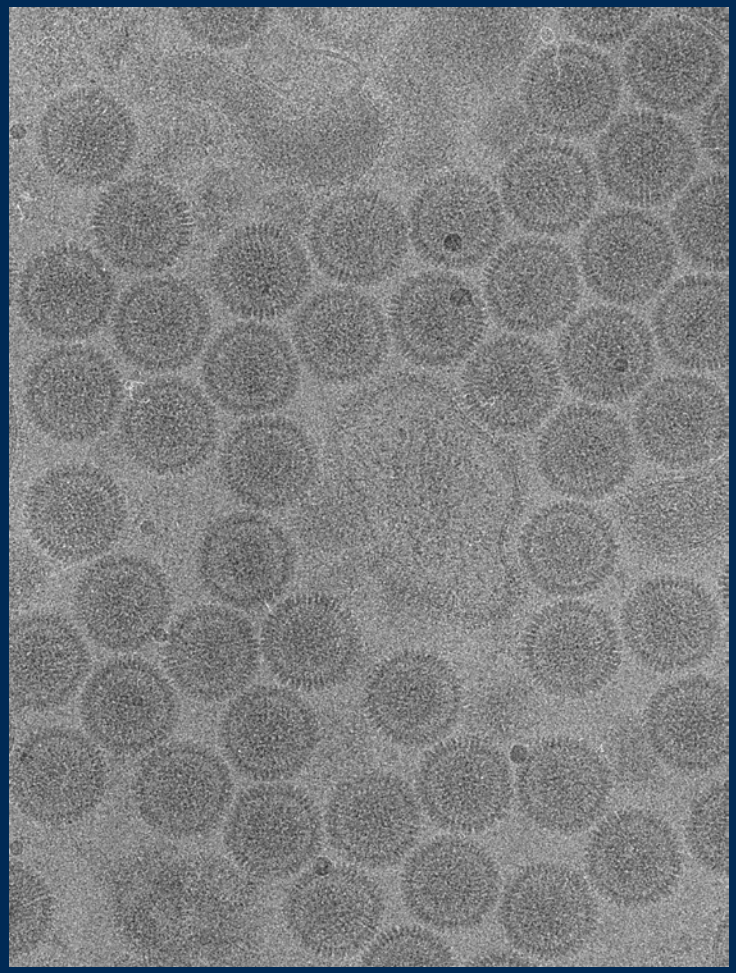
William Newcomb

procapsid

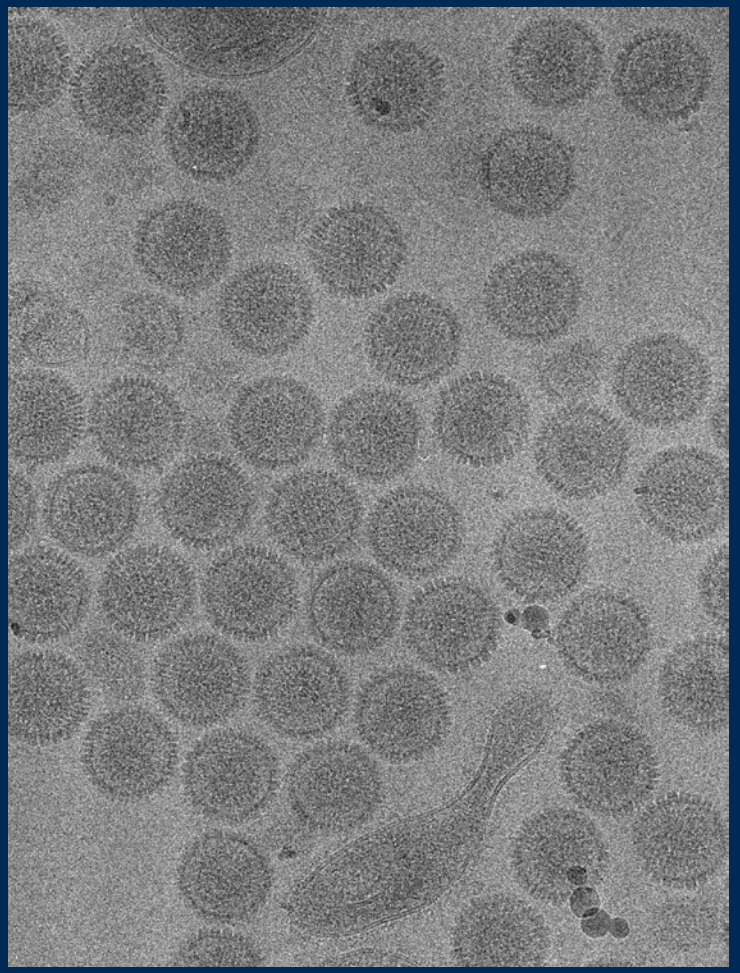
mature capsid



Heymann et al. (2003) *Nature Struct. Biol. 10:334-344*

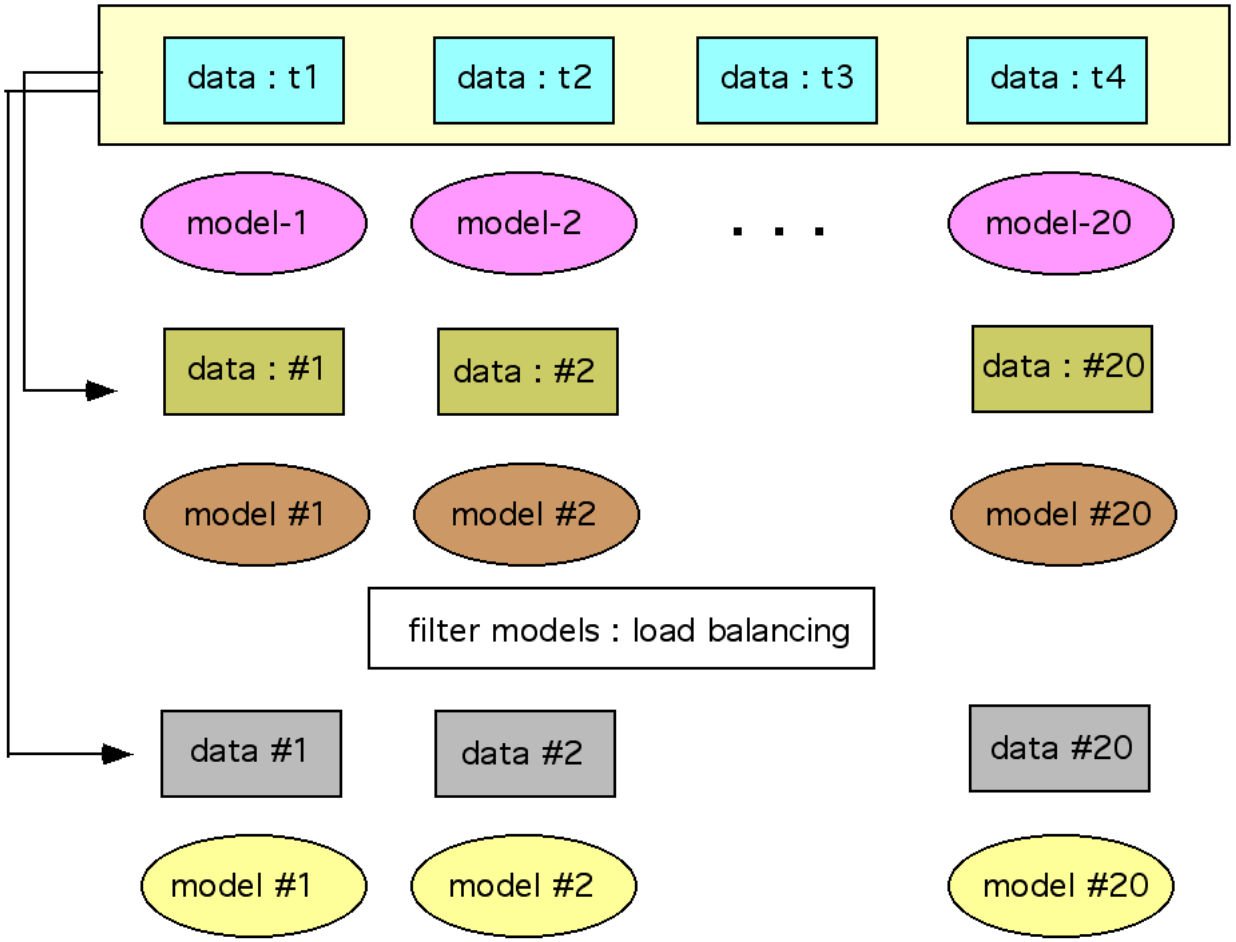


0 hr



48 hr

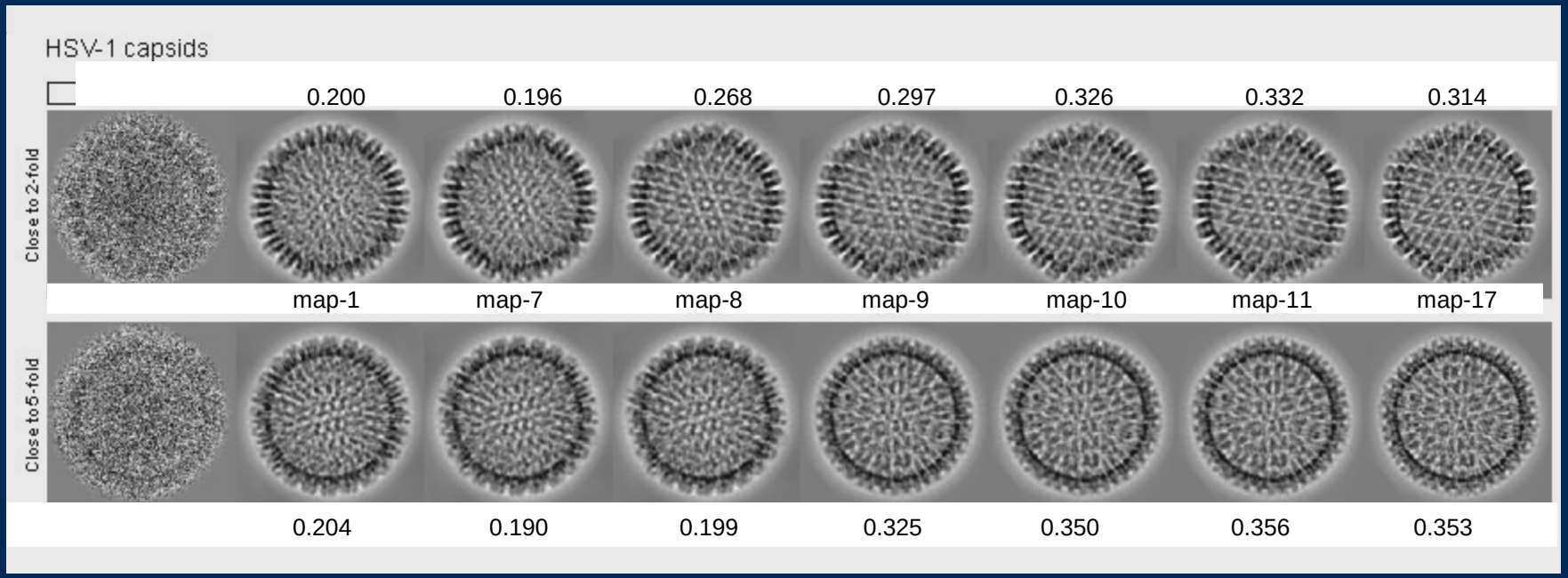
Classification Strategy (HSV)



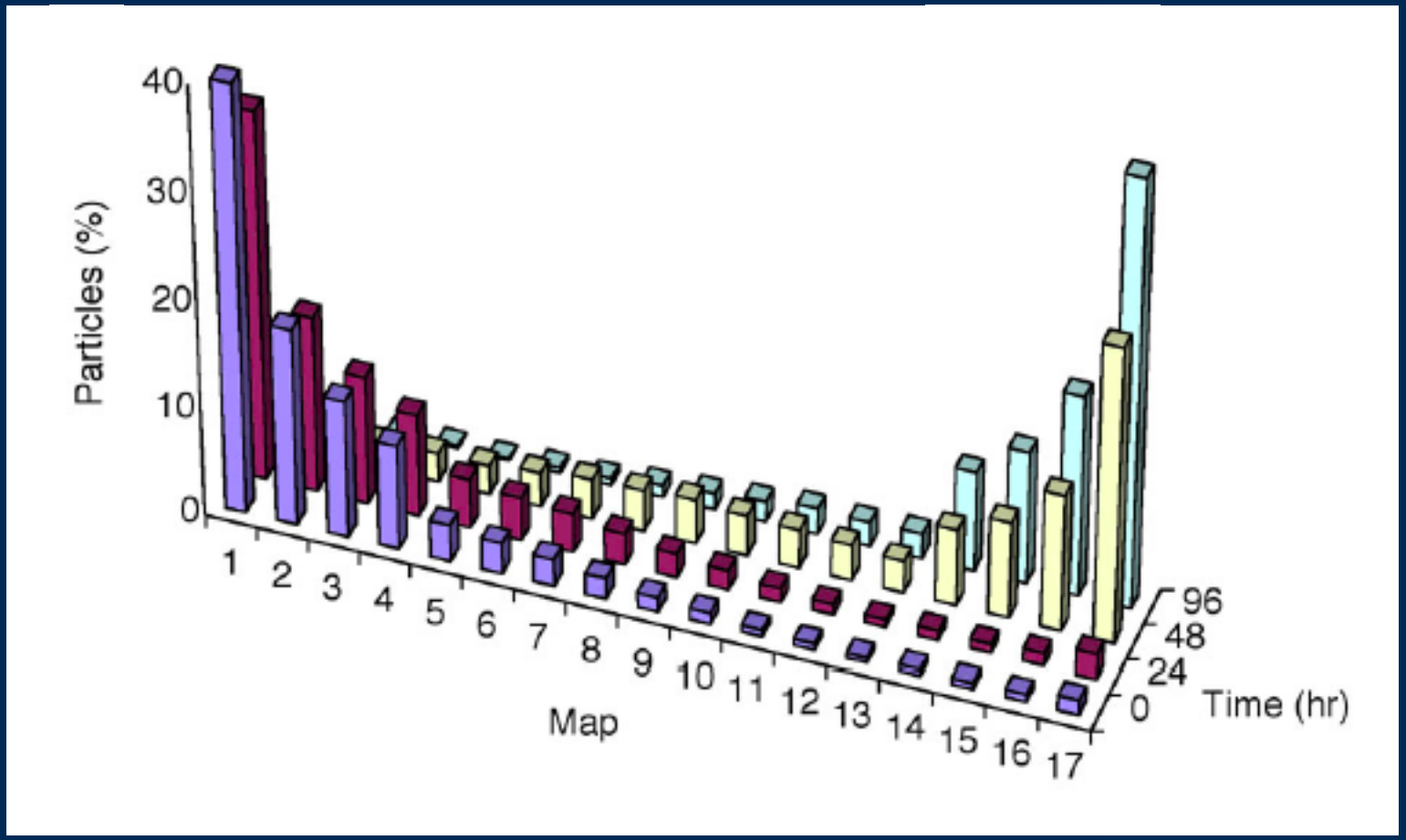
5 iterations

finally - standardize radial scaling of models (1-2% shifts)and reduce to 17 "well defined" models (not all distinct)

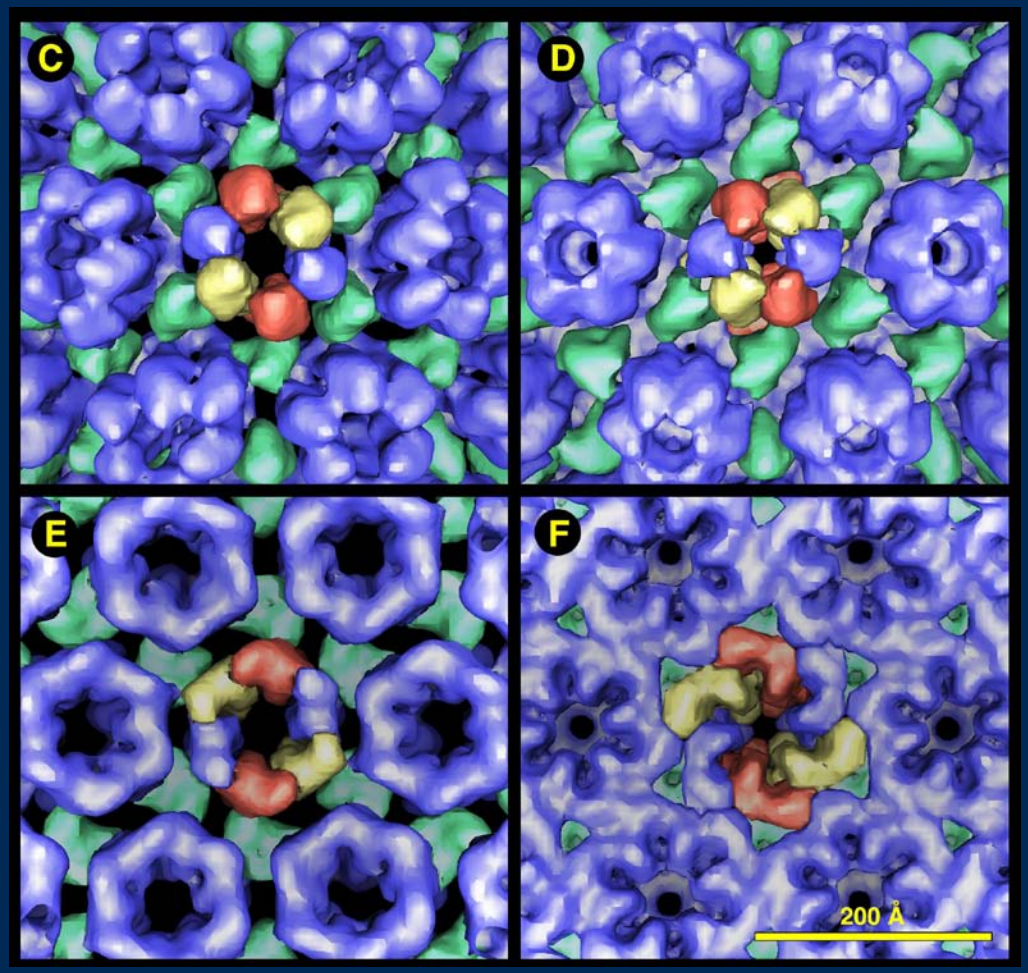
Multi-model discrimination by projection matching (MPA = Multi-particle analysis)

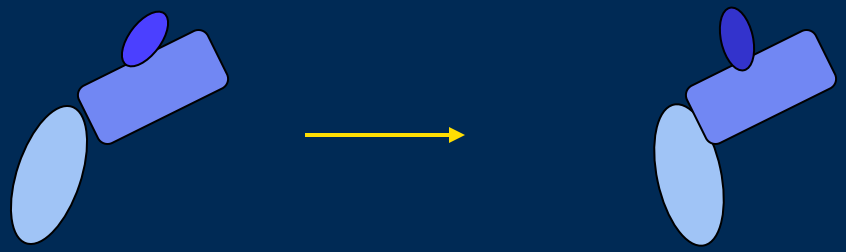


Kinetics of HSV capsid maturation



Rotating Domains





- Multiple Particle Analysis – Multiple Conformations - Time-resolved Cryo-EM

- *Issues*

- Number of states (models)?
- Where to get starting models?
- Need good SNR for reliable classification (iterative supervised classification)
- Need **a LOT** of data
- Can do kinetic modelling

- Thermo-cryo-electron microscopy

Naiqian Cheng, Lyuben Marekov - LSBR, NIAMS

Philip Ross - LMB, NIDDK

James Conway - Dept. Structural Biology, U. Pittsburgh

Robert Duda, Brian Firek, Roger Hendrix
- Dept. Biological Sciences, U. Pittsburgh

Jack Johnson, Bill Wikoff, Lu Gan, Kelly Lee *et al.*
- The Scripps Research Institute

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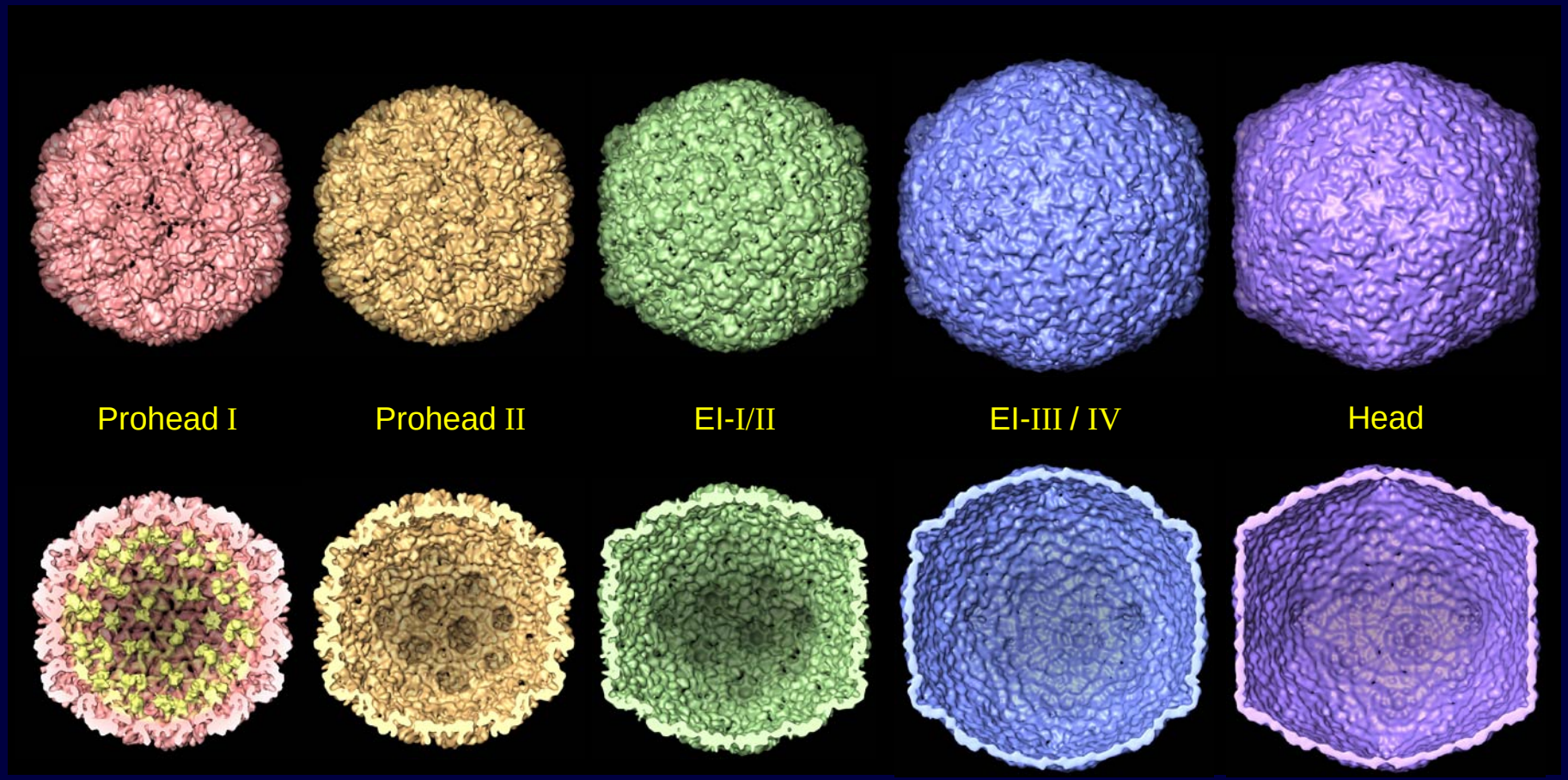
Maturation pathway : Five structural states

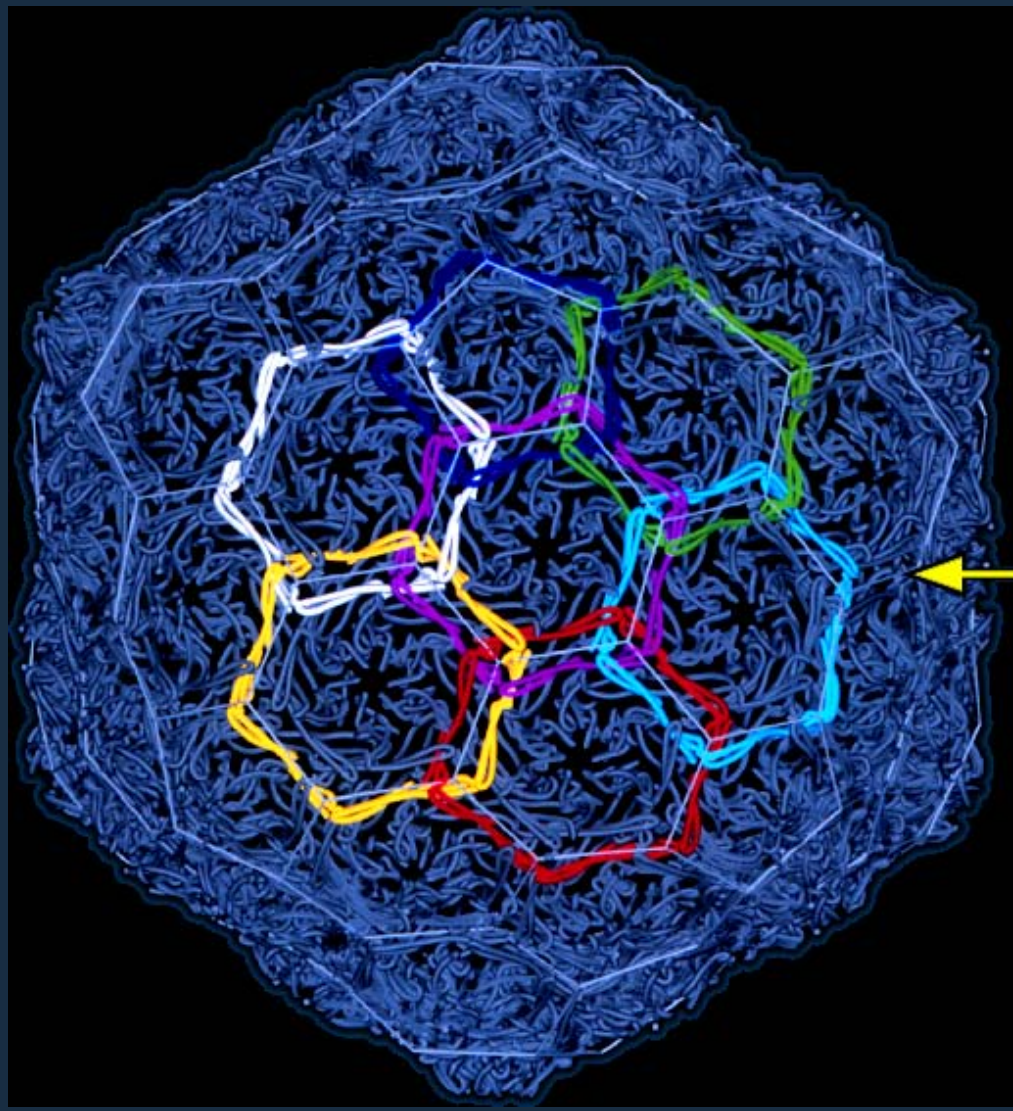


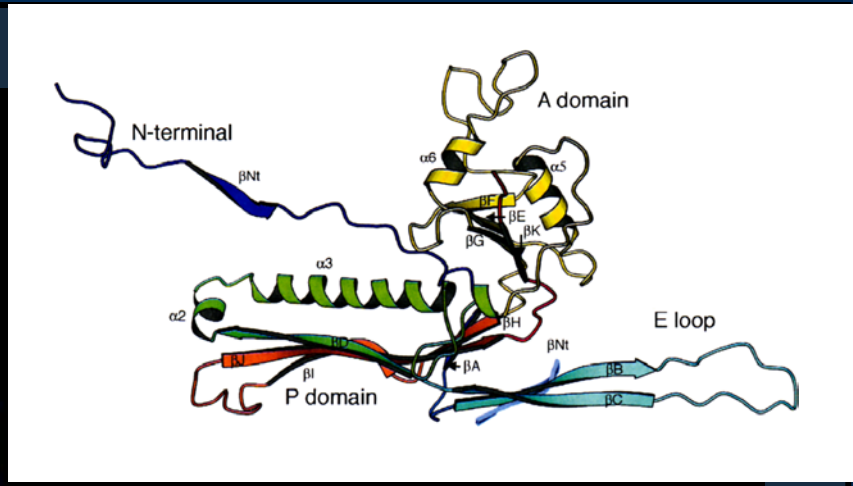
cleavage

expansion

cross-linking

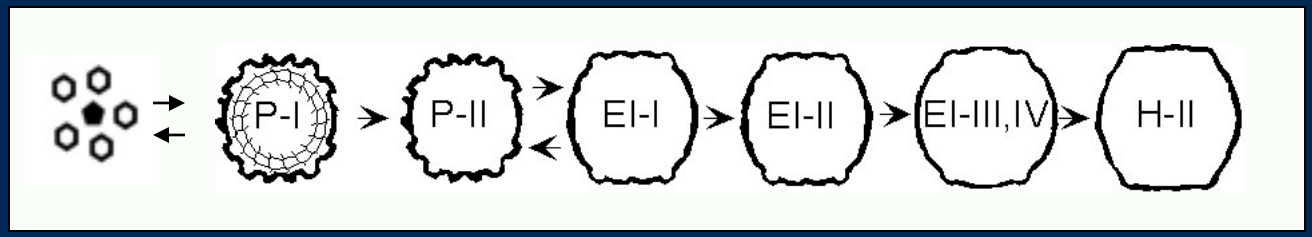
Gp5* (res. 103 - 385)

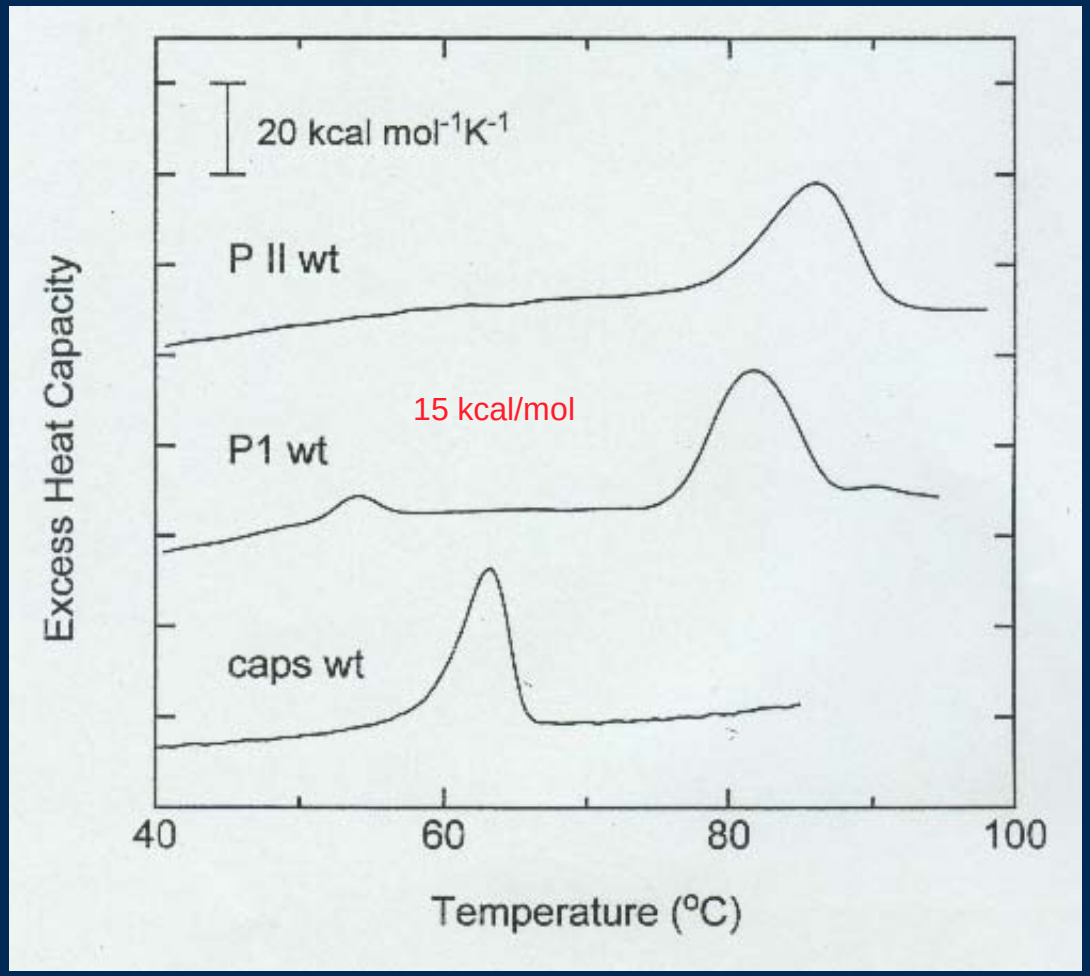




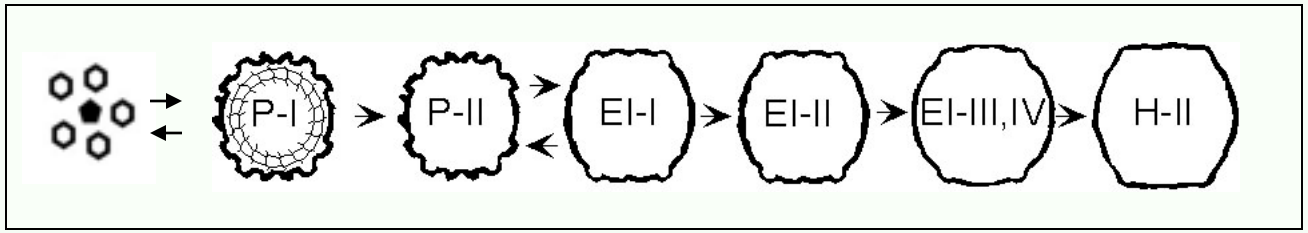
Covalent cross-linking results in **protein catenanes** (inter-linked rings)

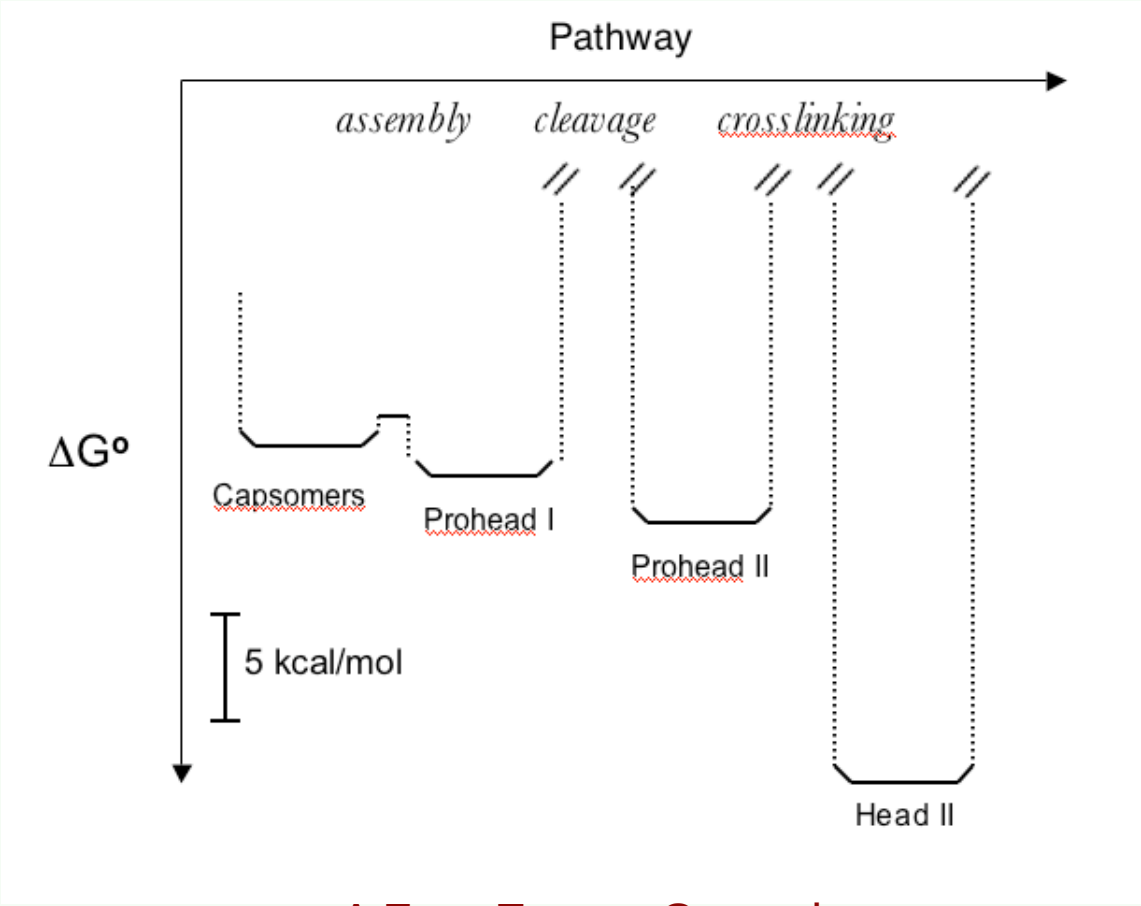
From: Helgstrand *et al* (2003) J Mol Biol 334, 885-899





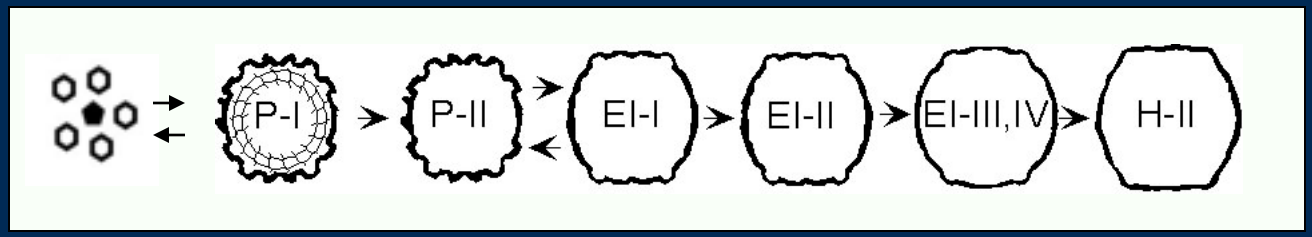
Capsomer Assembly and Proteolysis Enhance Thermal Stability

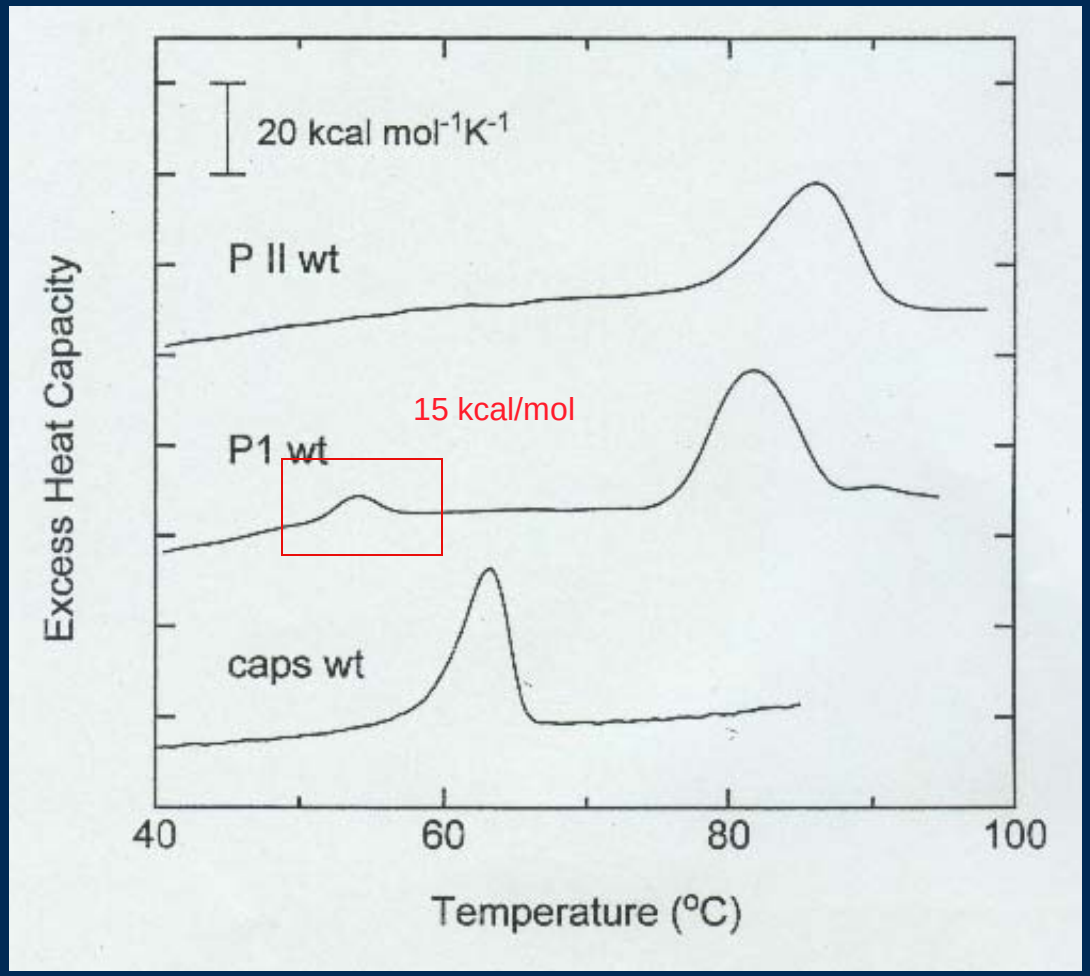




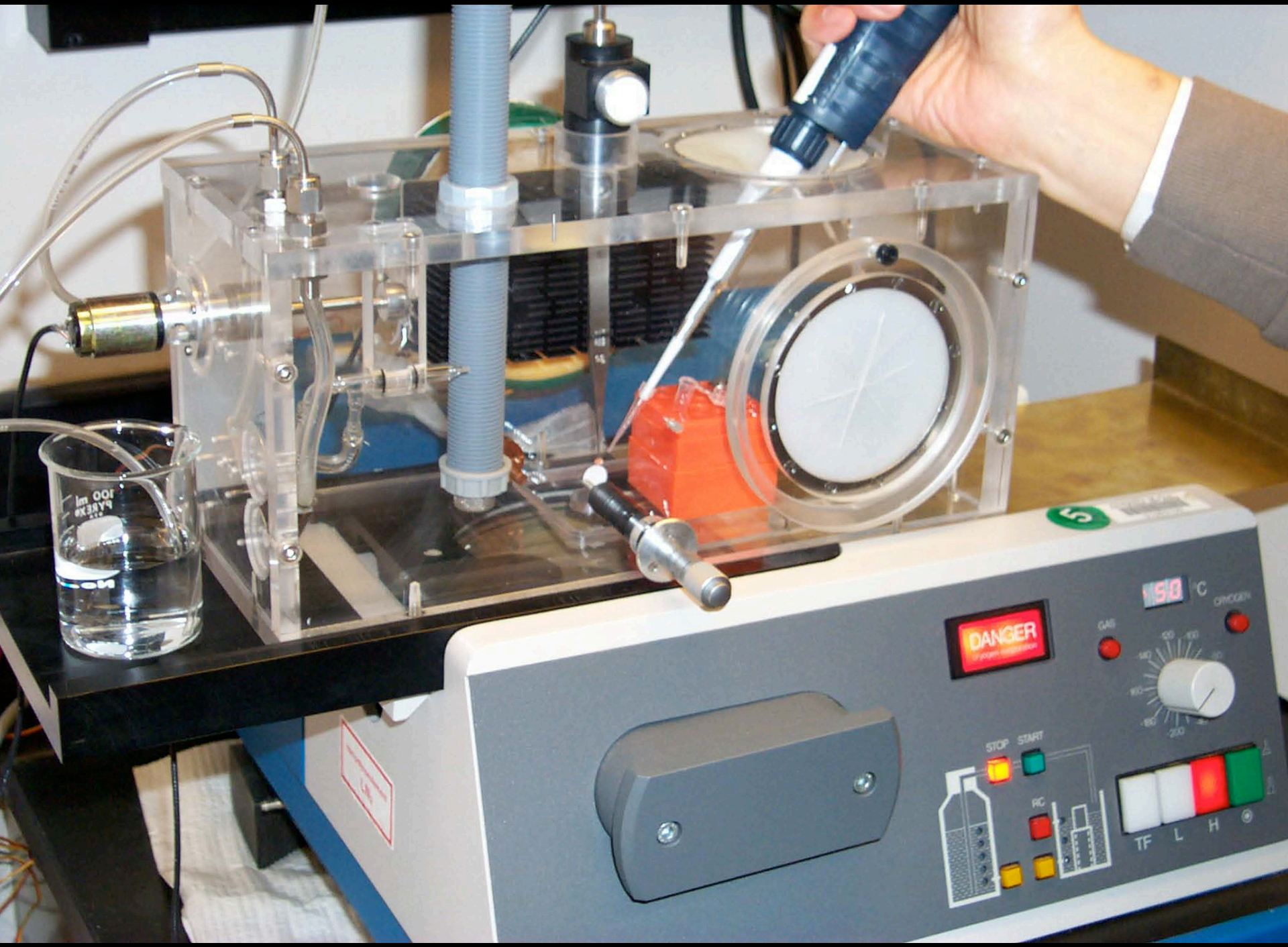
A Free Energy Cascade

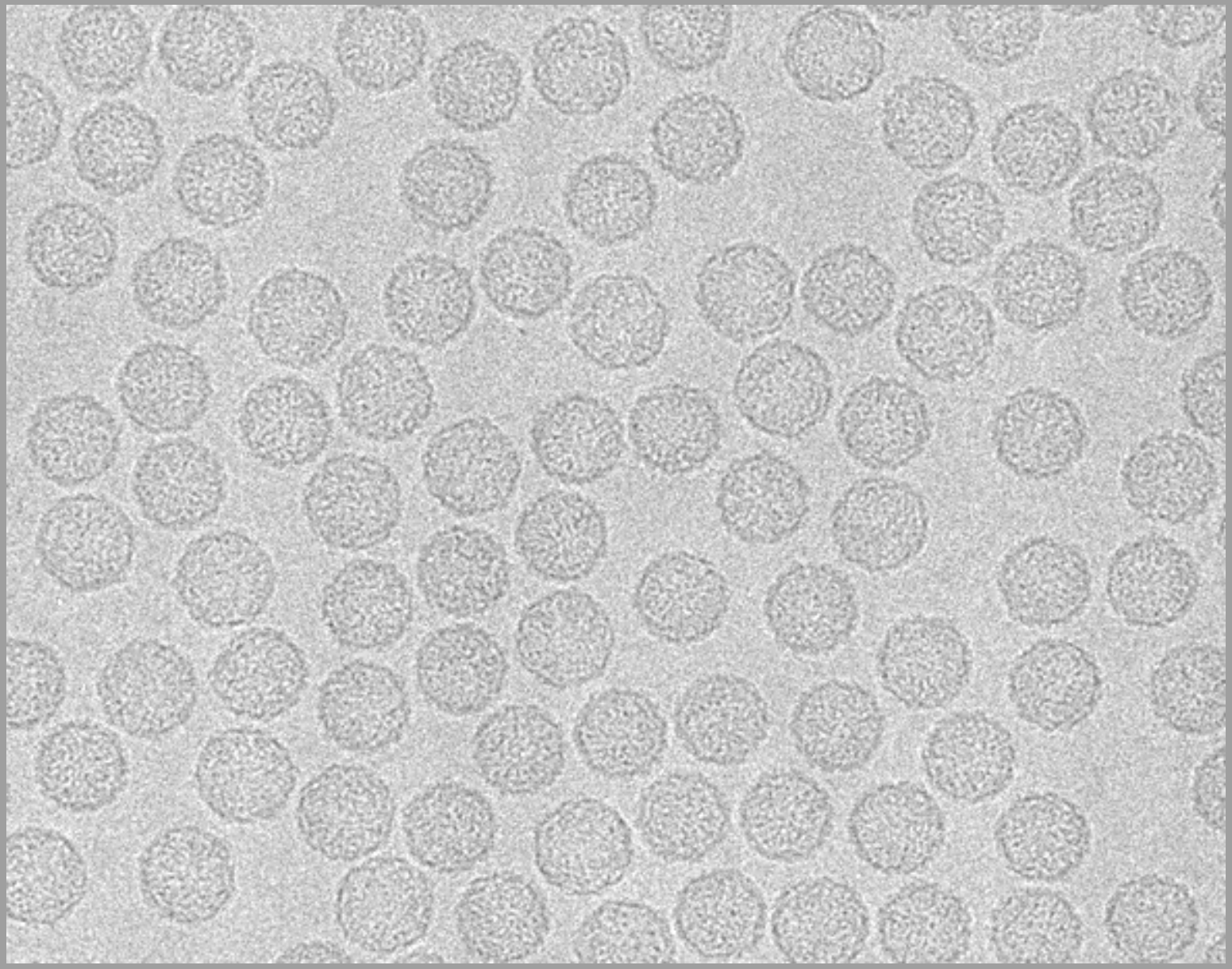
Ross et al. JMB 364, 512 (2006)



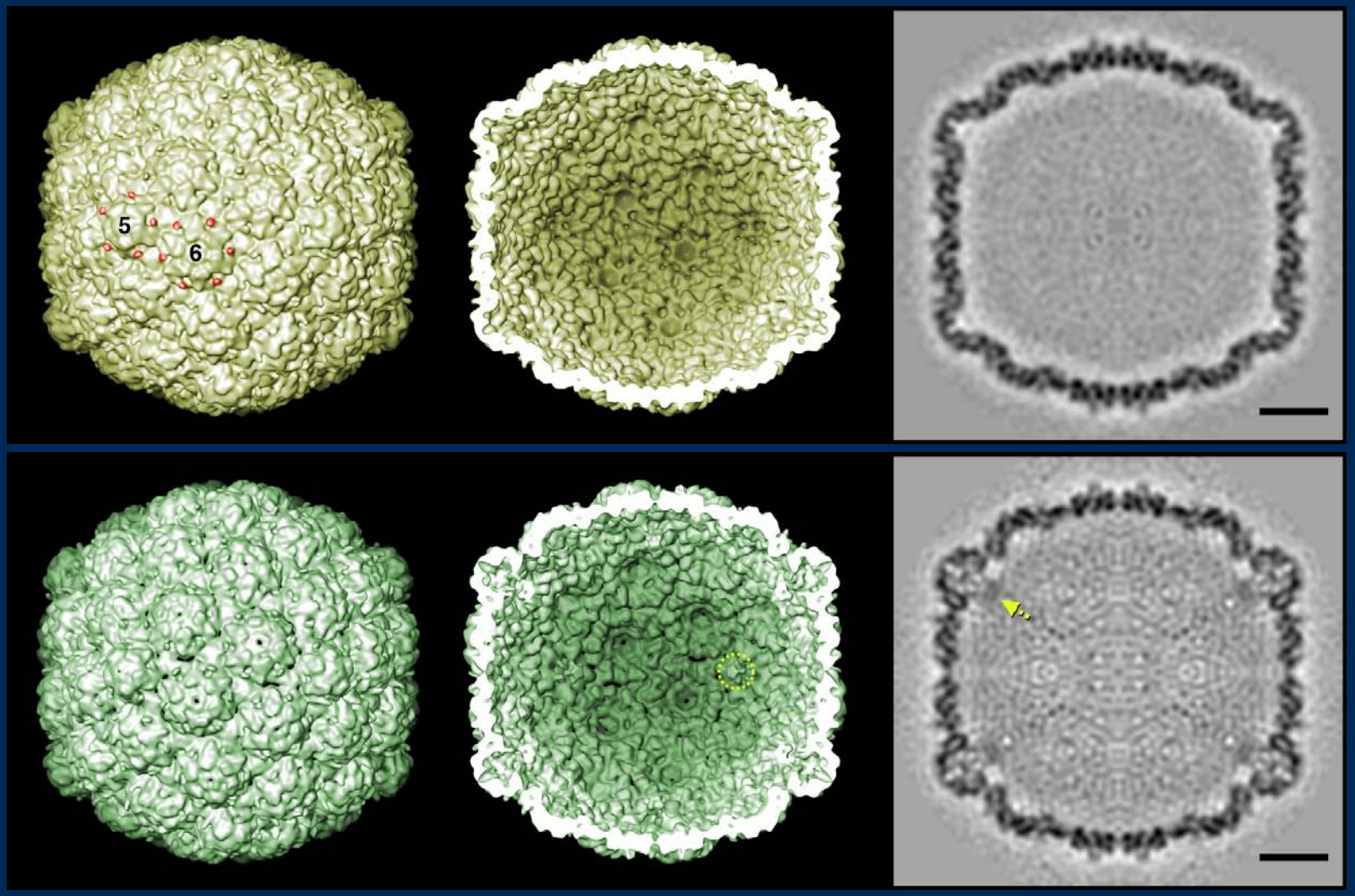


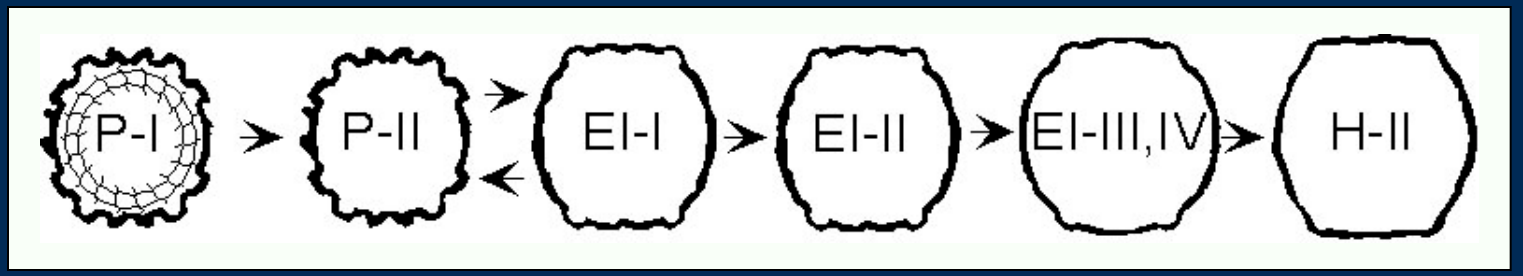
Capsomer Assembly and Proteolysis Enhance Thermal Stability


DANGER
STOP
START
TF
L
H



Visualization of the 53-degree phase transition





The 53^{o} event of Prohead I represents a reversible phase transition

After this transition, the capsid has the pentamers of Prohead I and the hexamers of Expansion Intermediate I

The Δ-domains of the hexamers but not the pentamers are disordered

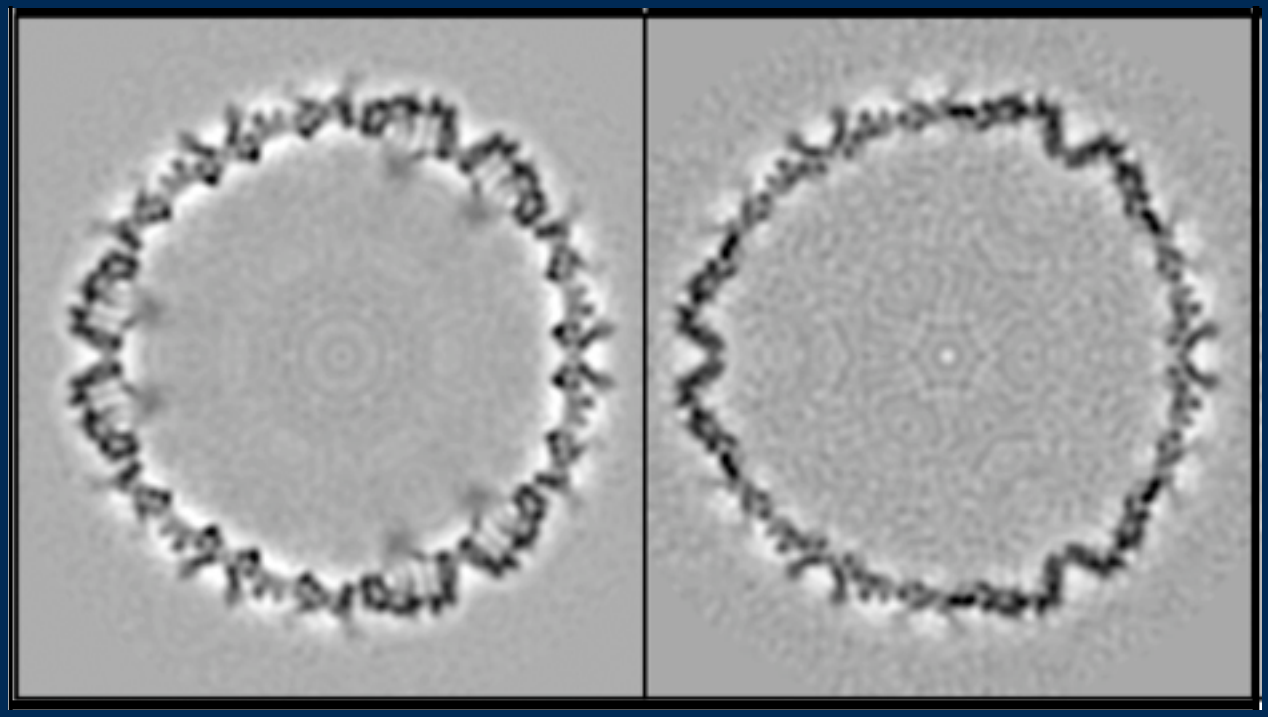
The Δ-domains restrain Prohead I from embarking on maturation

•Thermo-Cryo-Electron Microscopy

- *Issues*
- Thermally excited states short-lived
- At high temperatures, rapid drying of thin film
  - made environmental chamber

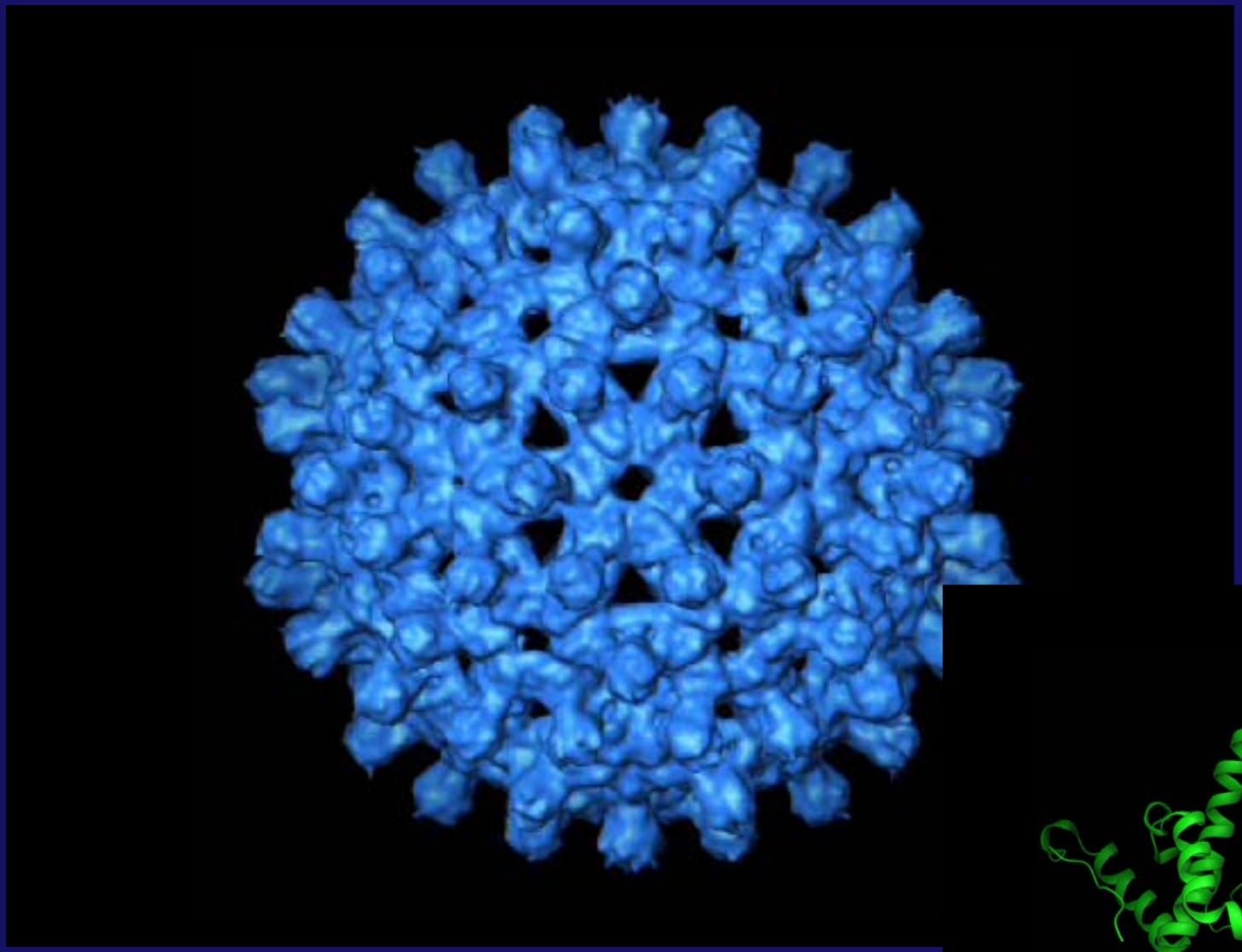
* Apply Multiple Particle Analysis

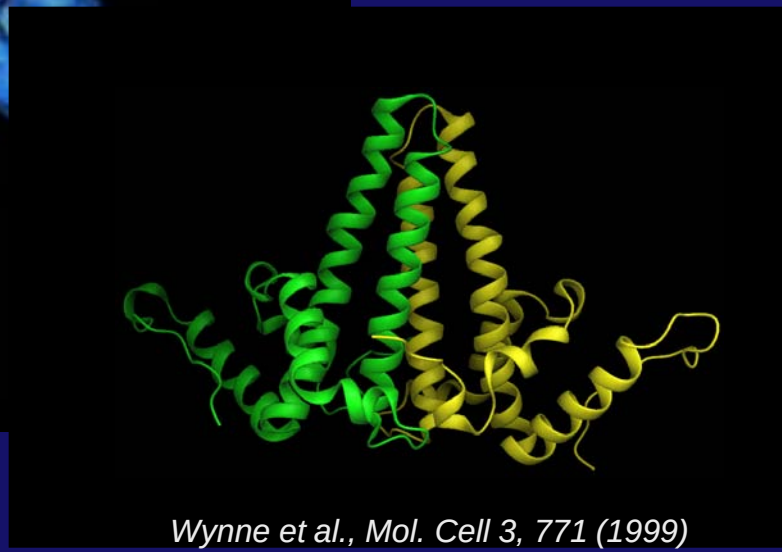
- Resolution is inhomogeneous in density maps



- The smallest molecular feature that we have been able to see – a nonapeptide of < 1 kDa.

HBV Cp149 T=4 capsid structure



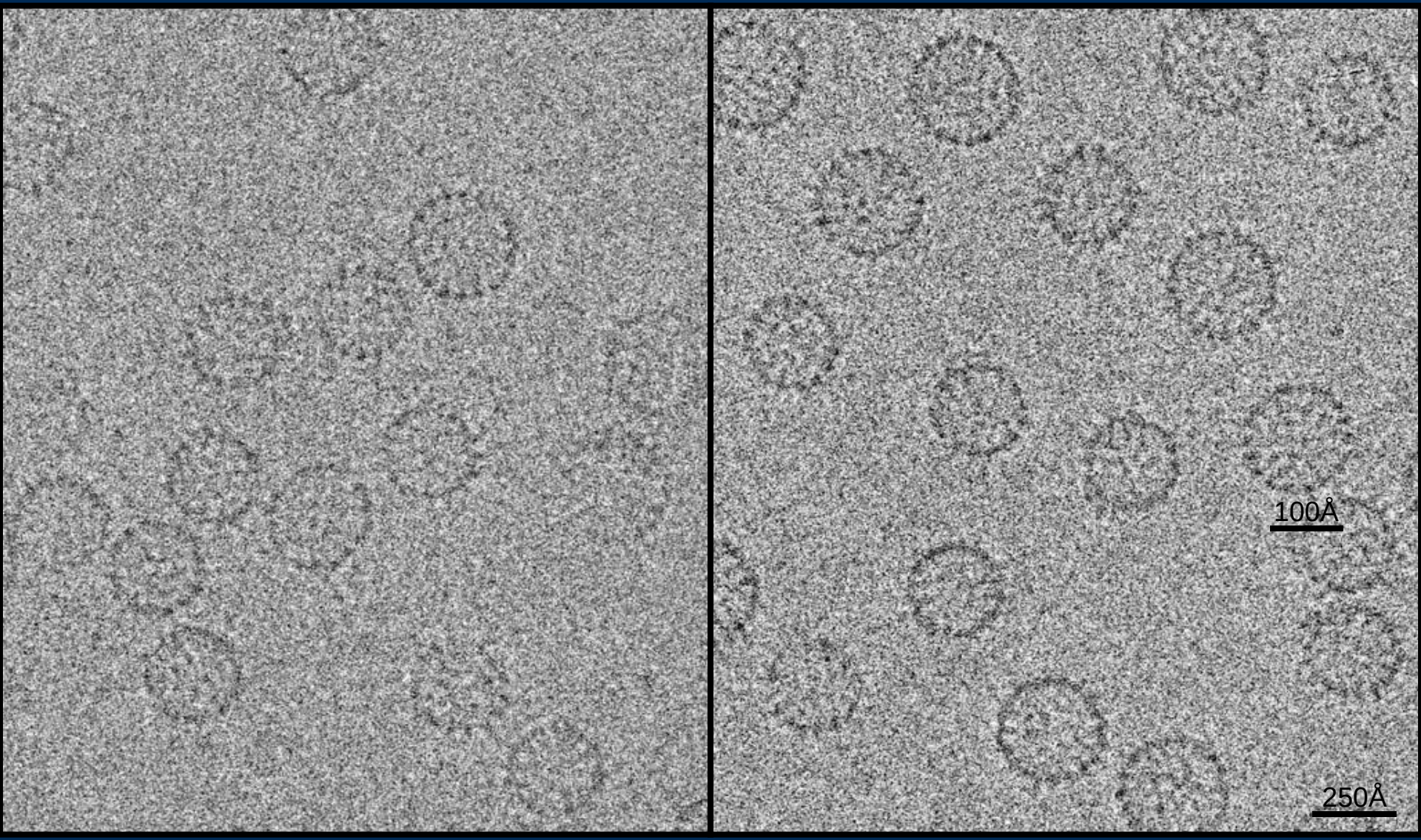


Wynne et al., Mol. Cell 3, 771 (1999)

Visualizing the HBV Linker Peptide

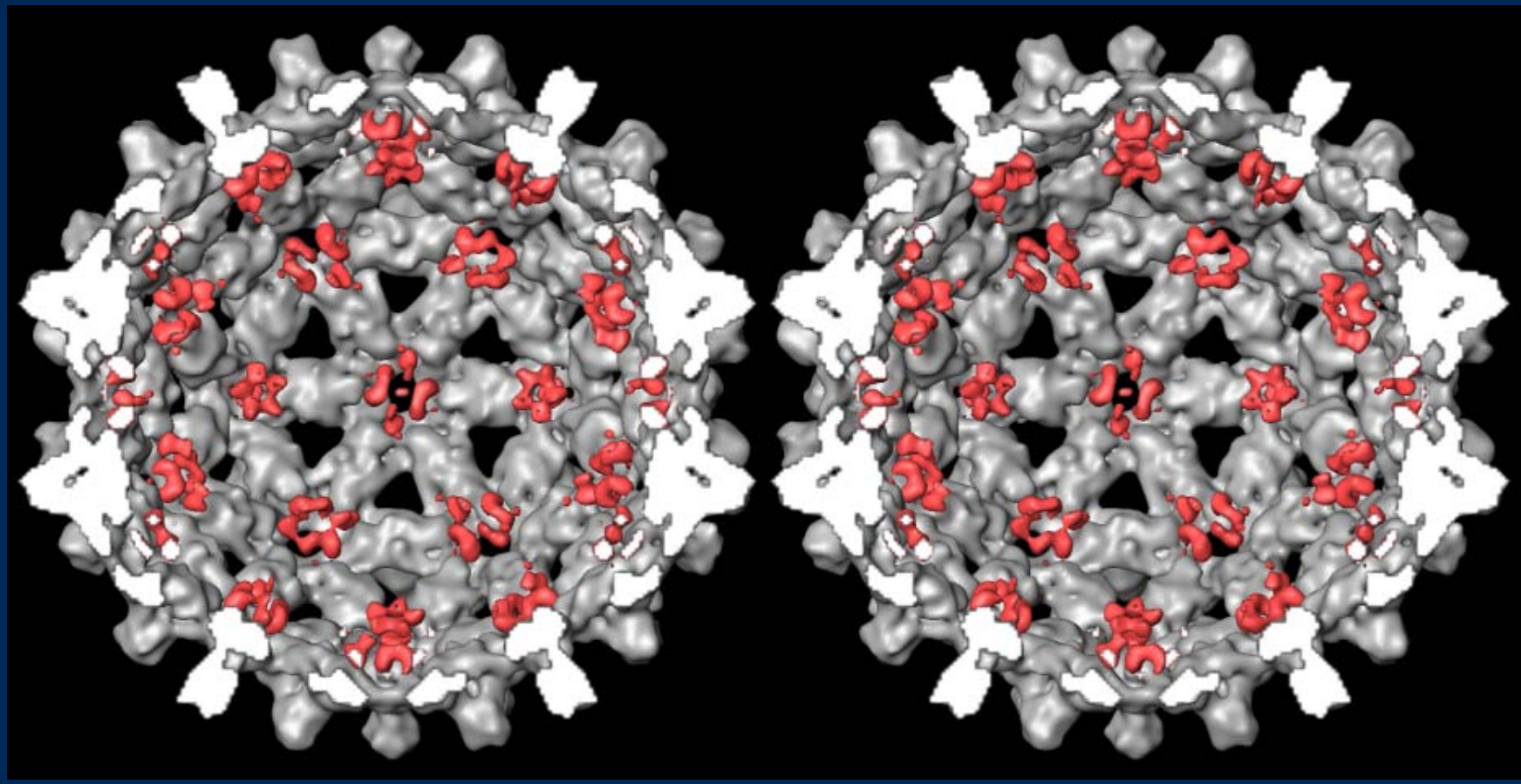
Cp140

Cp149



HBV Linker : T=4 capsids

Cp140 + Difference



X-eye stereo

HBV Linker - homology

HBV 141-149

141 149

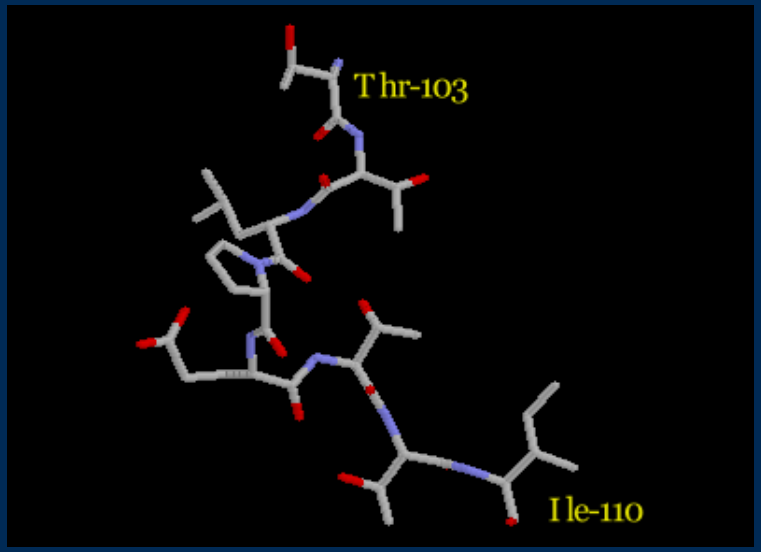
S T L P E T T V V

103 110

T T L P E T T I

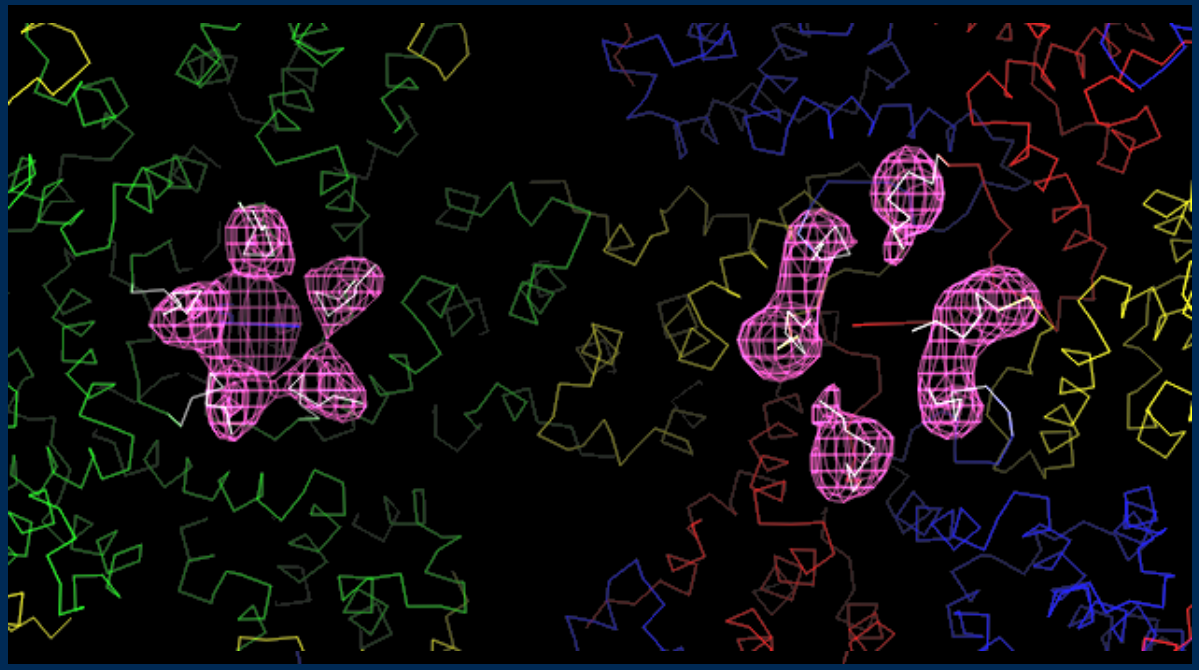
Cellobiose dehydrogenase

P. chrysoporium: extracellular flavocytochrome
(Hallberg et al, 2000, Struct. Fold. Des 8, 79-88)



HBV Linker - Fitting

	141								149
HBV 141-149	S	T	L	P	E	T	T	V	V
	103								110
Cellobiose dehydrogenase	T	T	L	P	E	T	T	I	



HBV T=4 xtal structure: Wynne et al 1999, Mol. Cell 3, 771-780

- We were able to see a nonapeptide of < 1 kDa by difference imaging
- 7/9 of the nonapeptide were not seen in the crystal structure
- limited resolution (and good SNR), an advantage in this case

- shorten Cp beyond residue 140 or remove linker – no capsids assembled

- A Machine with Many Moving Parts

- The largest feature we have been unable to see (90 kDa)

Laboratory of Structural Biology Research,

NIAMS - NIH

Gregory Effantin

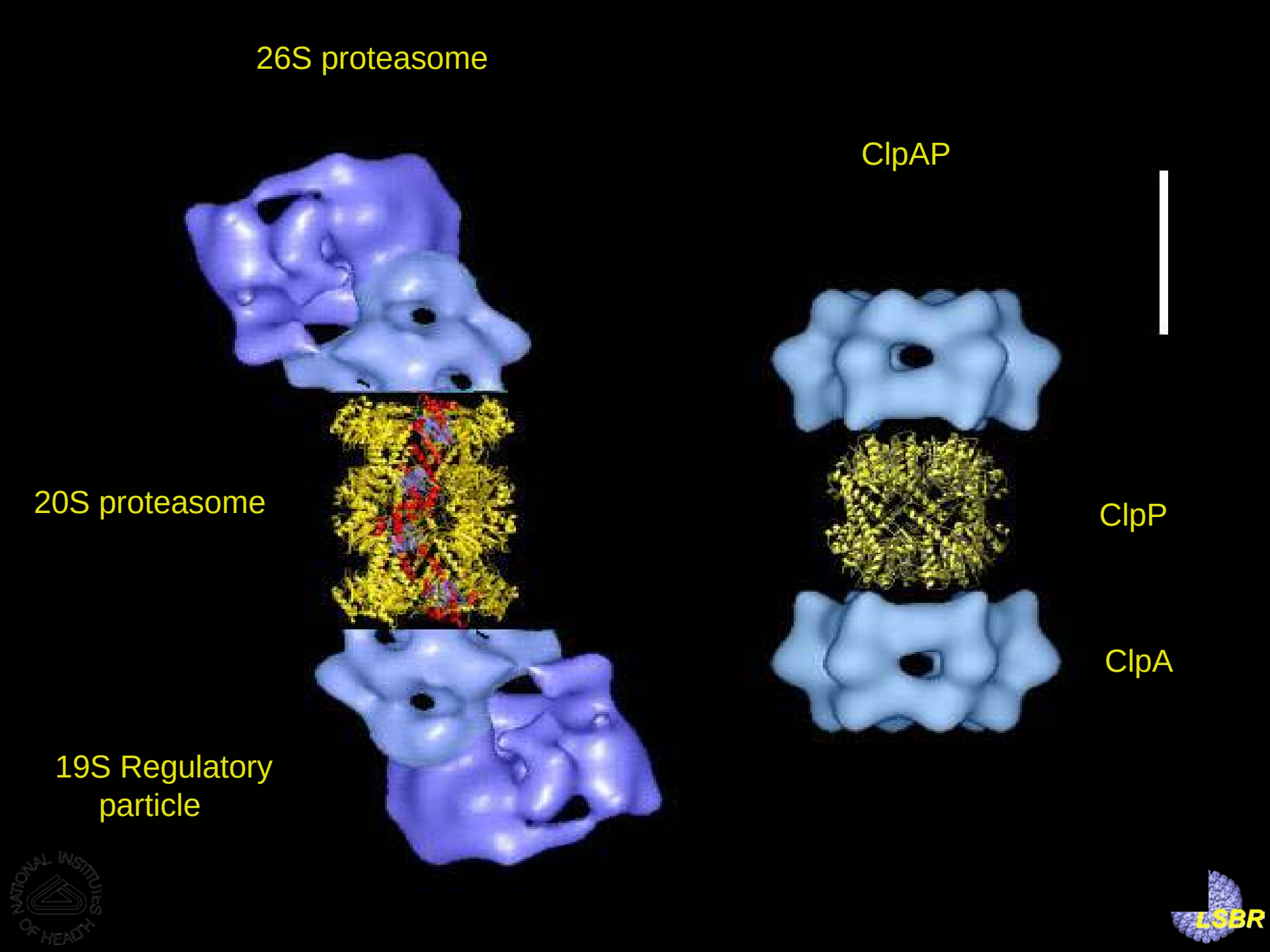
Takashi Ishikawa (now ETH Zurich)

Laboratory of Cell Biology, NCI-NIH

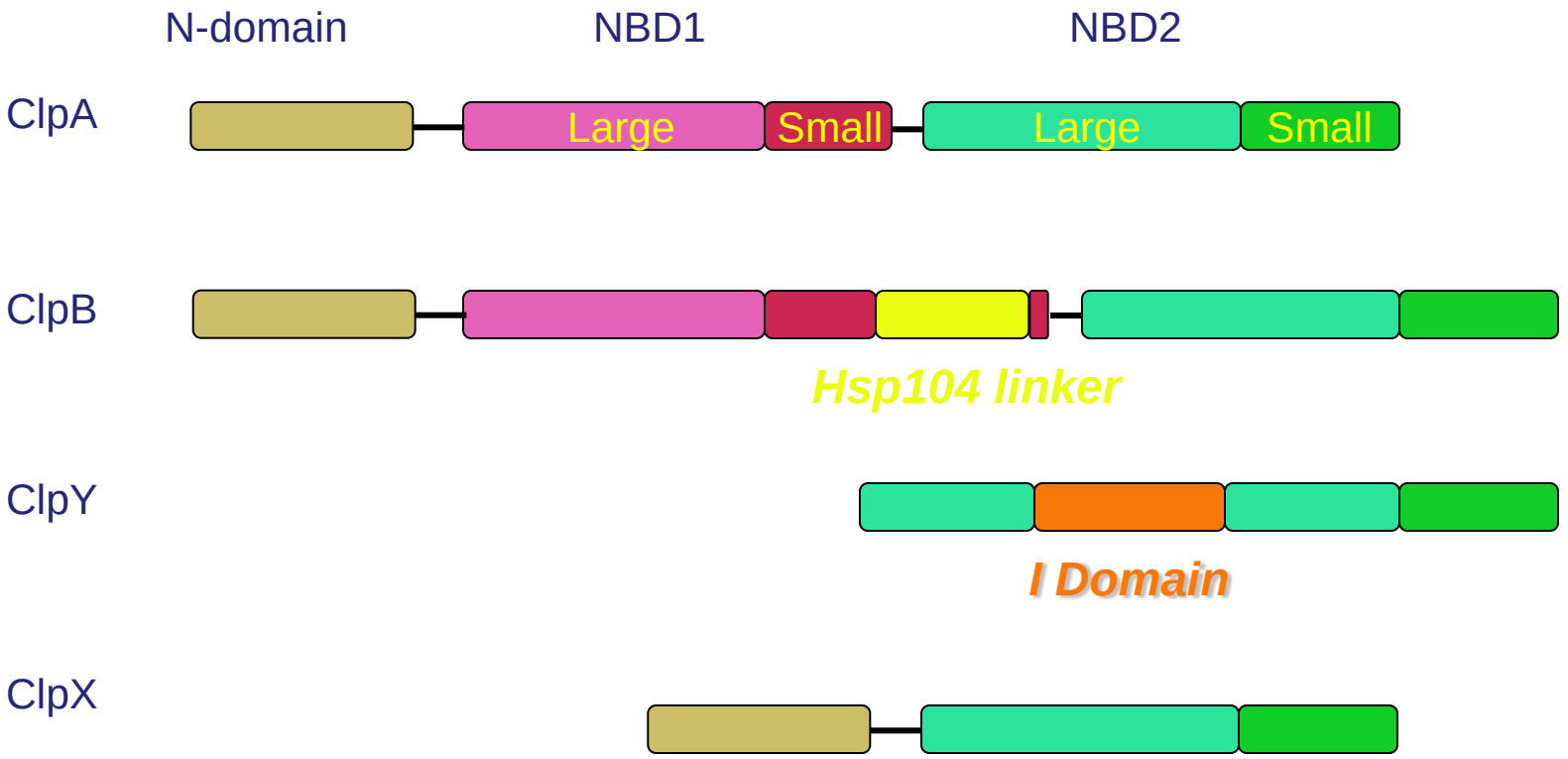
Gian Marco De Donatis

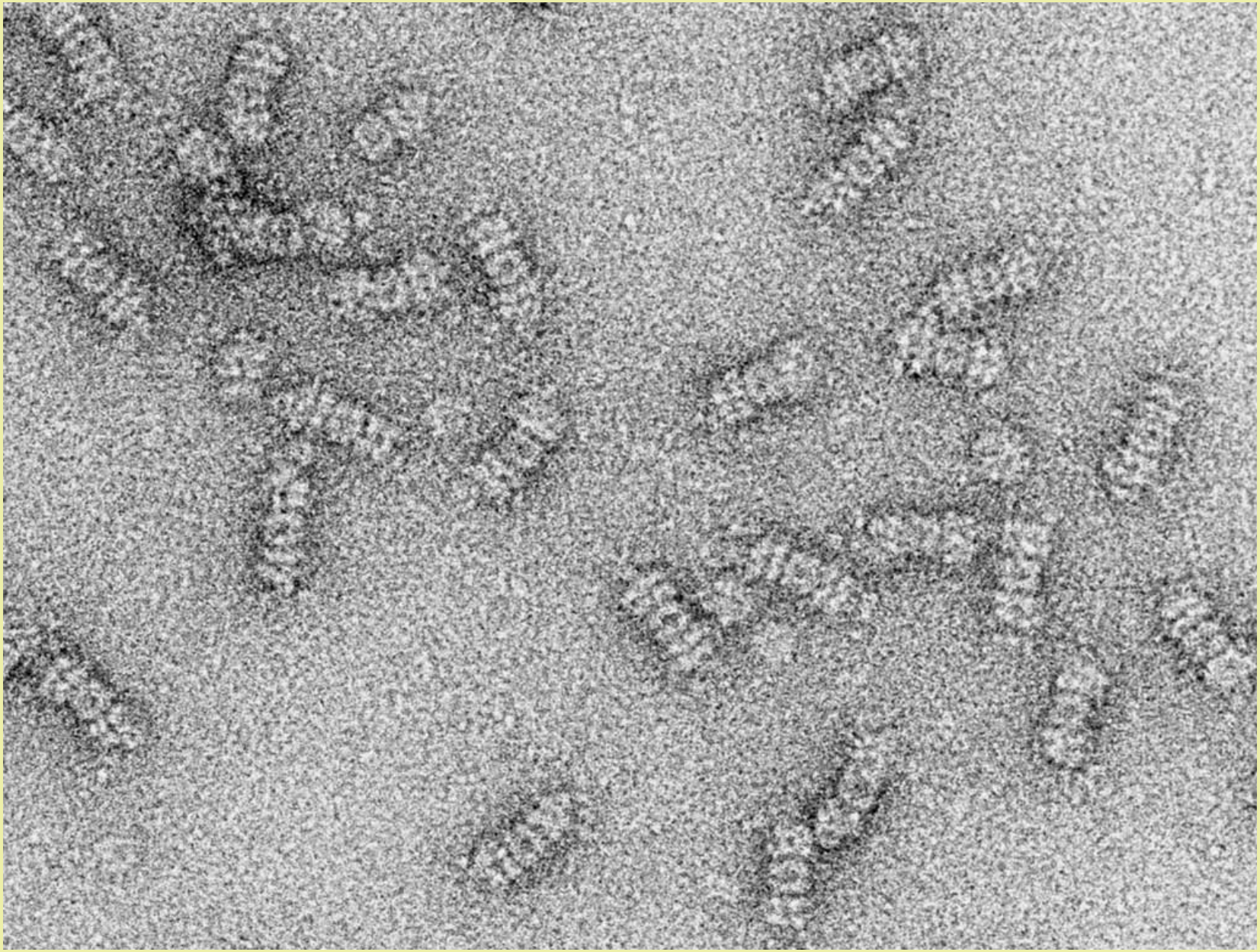
Michael Maurizi

David Belnap, Fabienne Beuron, Martin Kessel, Joaquin Ortega


26S proteasome
ClpAP
20S proteasome
ClpP
ClpA
19S Regulatory particle

Domain architecture of Clp ATPase proteins



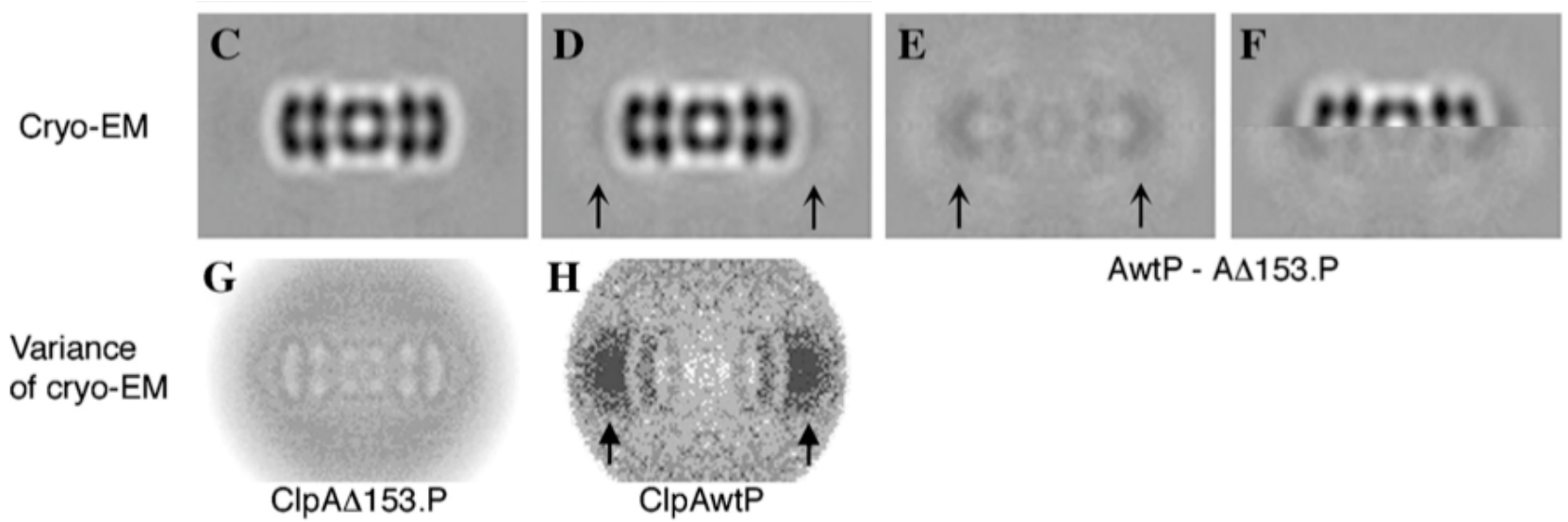


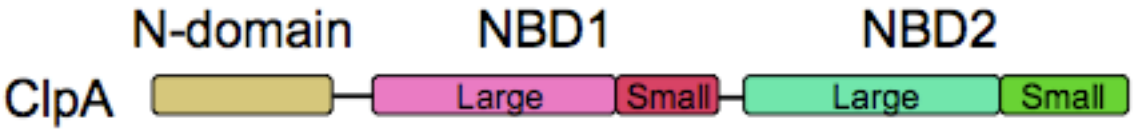
Issues

- Getting side-views in vitrified specimens
- The 6 : 7 symmetry mismatch, pseudo-symmetry
- With ClpA alone, mistaking side-views for top views
- Highly mobile N-domains

The N-domains are highly mobile in solution

From Ishikawa et al., JSB 2004





How were the various populations sorted?

- Visual screening in manual particle picking
- Multiple particle analysis *aka* multi-reference alignment based on correlation coefficients

  - Crunch questions:

    how many particles (references) to use? *Be conservative*

    How to get starting models? *Easier in time-course experiments*

How was the averaging done?

- In the usual way, but typically omit bottom third (lowest cccs)

What were the thought processes and decisions along the way?

(optimism, depression, pragmatism)$_n$

Get good biochemical collaborators.

How were the various problems that were encountered solved?

How were bad images identified?

I recommend more extensive use of focal pairs and even focal triplets, pending the advent of the ideal phase-plate.

Stronger signal => more reliable identification of views and more reliable discrimination between competing models. You don’t have to include the 2nd & 3rd exposure in final map.

What is in the pipeline in terms of new approaches?

More extensive use of variance mapping

Time-resolved studies – 4D cryoEM

Closer integration of SPA and tomography

For more confidence-inspiring averaging of subtomograms, we need better resolution in the primary tomograms

What resolution is useful?

High resolution is good: high information content is better

Keep a close eye on current and emerging biological/biochemical/genetic data on your molecule of interest.

What is the question you are trying to answer?

What does not work?

Fitting crystal structures of globular subunits into low resolution EM density maps