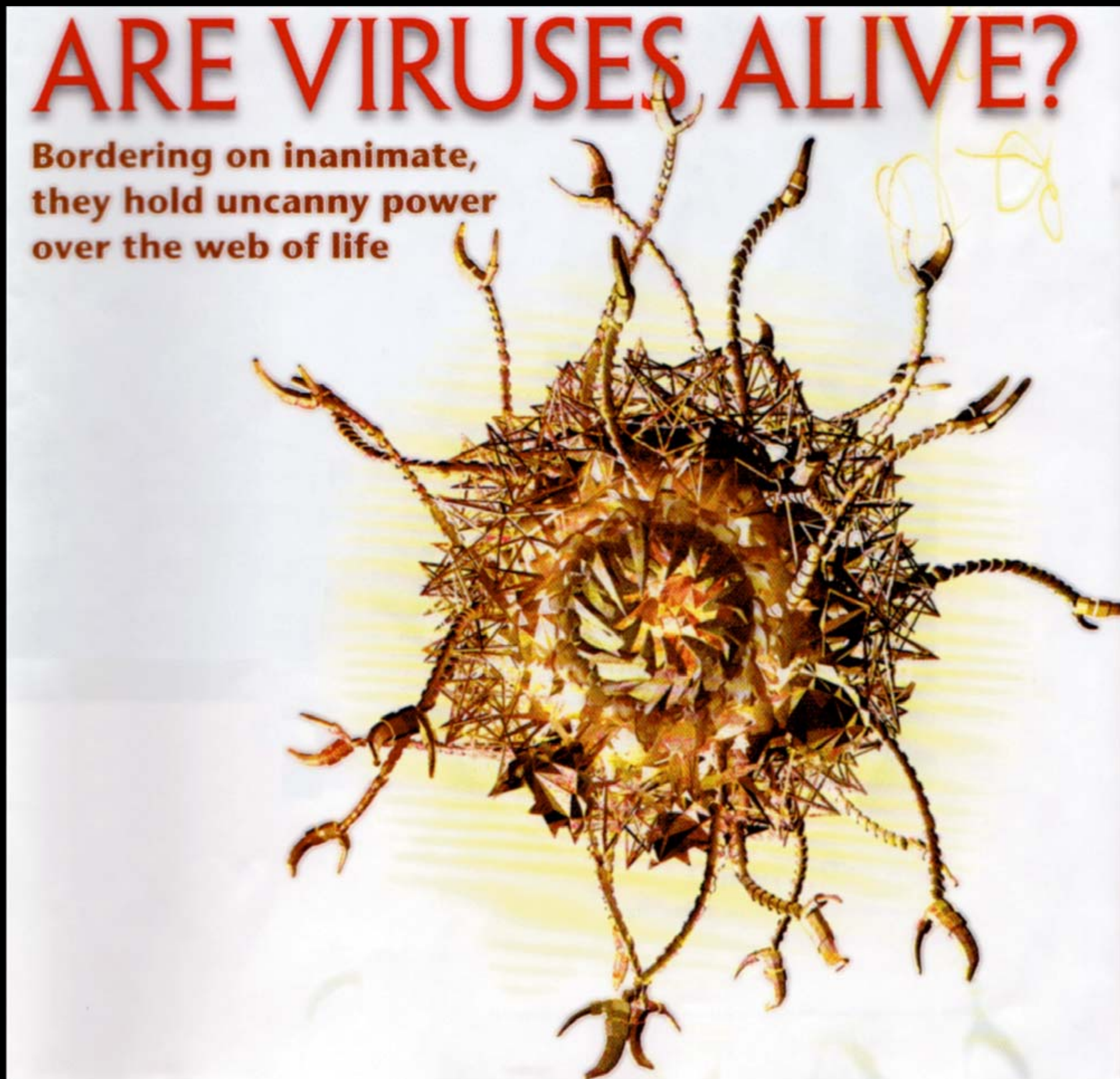
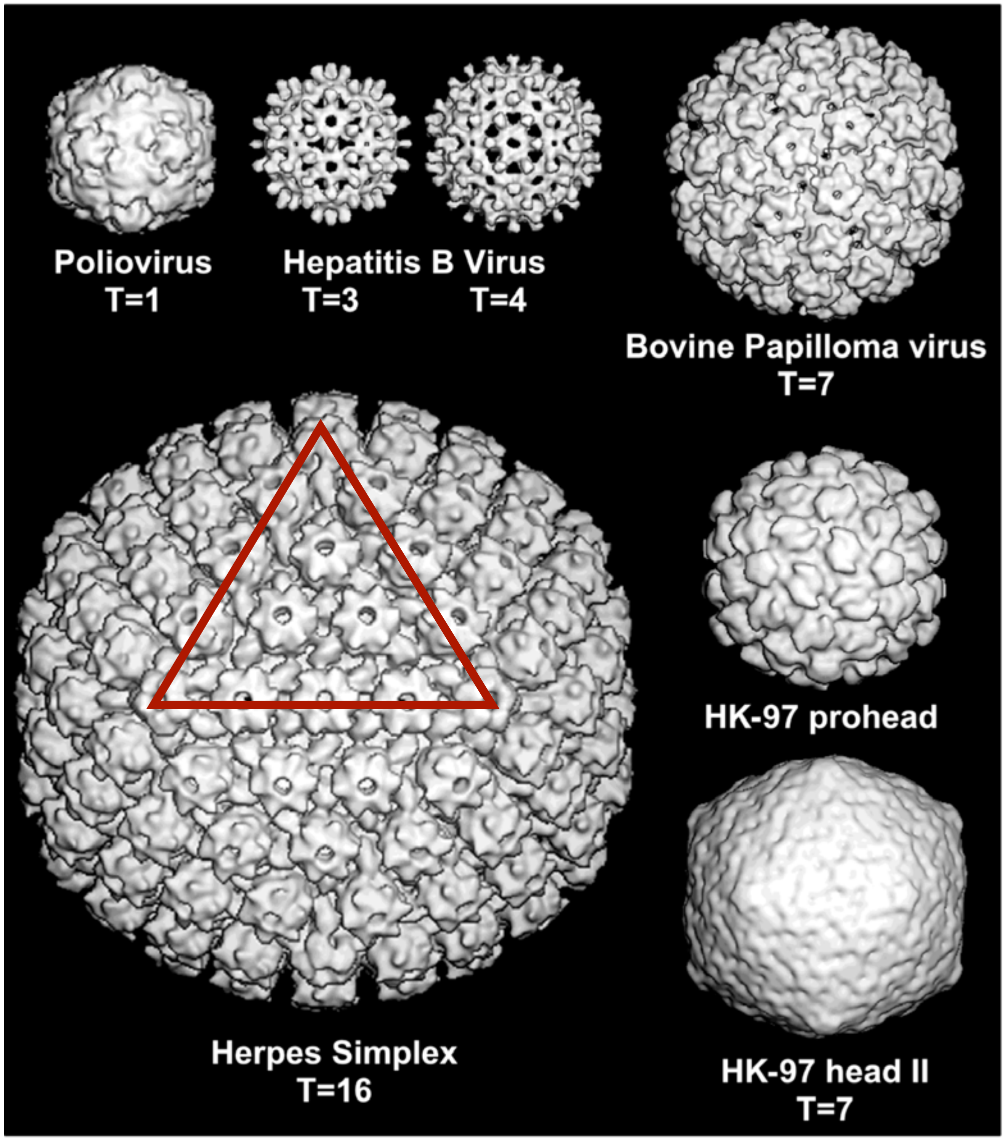


Scientific American December, 2004

Icosahedral Symmetry



Virions are assembled from many copies of a (few) small protein(s)

Economical use of the viral genome

Prodigal use of host resources

Paradoxical Requirements for Virus Capsids

1. Assembled from MANY small components
2. High Fidelity (size, shape, contents)
3. Assemble on biological time scale
4. Assemble at the right time
5. Stability
6. Instability

Correlating assembly and structure

Why is it valuable to rigorously describe assembly?

1. Gaps in a description suggest where unknown host and/or viral factors are involved in the reaction
2. It allows us to identify new mechanistic targets for (future) antivirals
3. We can control self-assembly to generate altogether new nanostructures from viral proteins

Why are biologists obsessed with regulation?

Statistical effects generate relatively broad, non-biological distributions

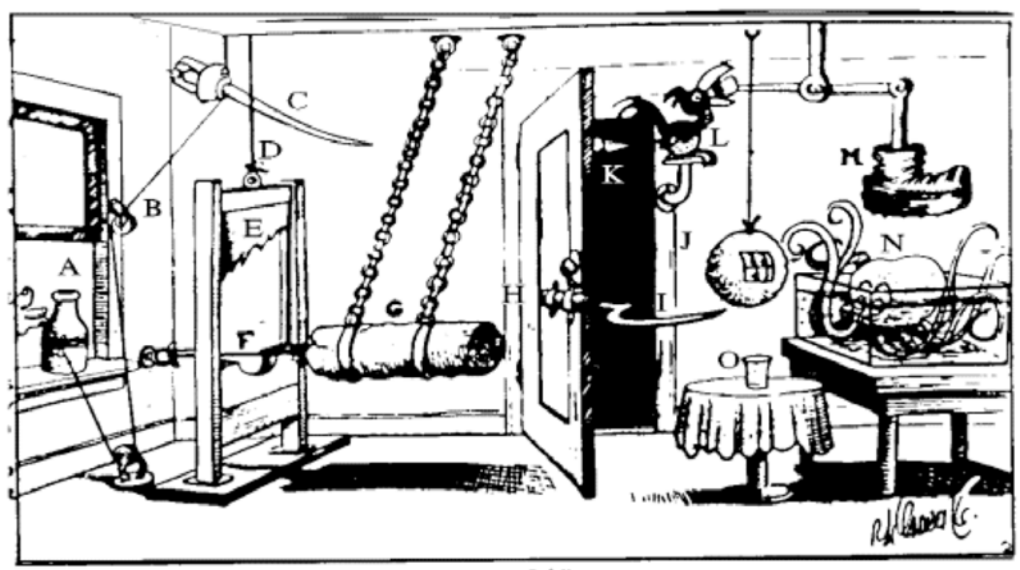
Regulation allows diverse reactions to become temporally linked

Pluses and minuses of reductionism

We characterize salient factors without confounding details

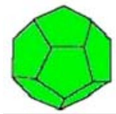
We risk oversimplification

Every should be made as simple as possible, but no simpler **A. Einstein**

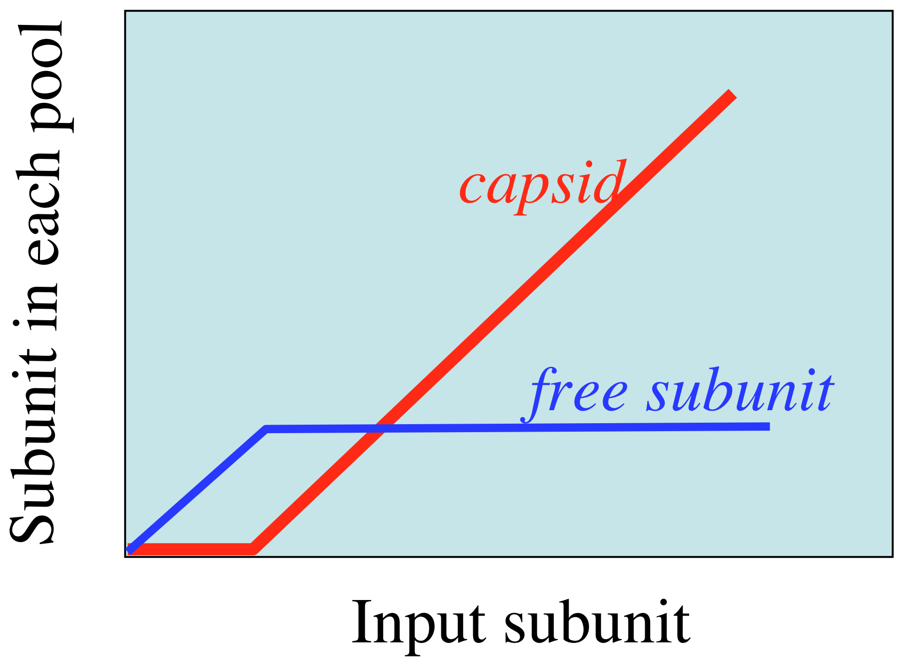


The law of mass action (assumes equilbrium)

$$K_{capsid} = \frac{[Capsid]}{[subunits]^n}$$



where n is the number of subunits



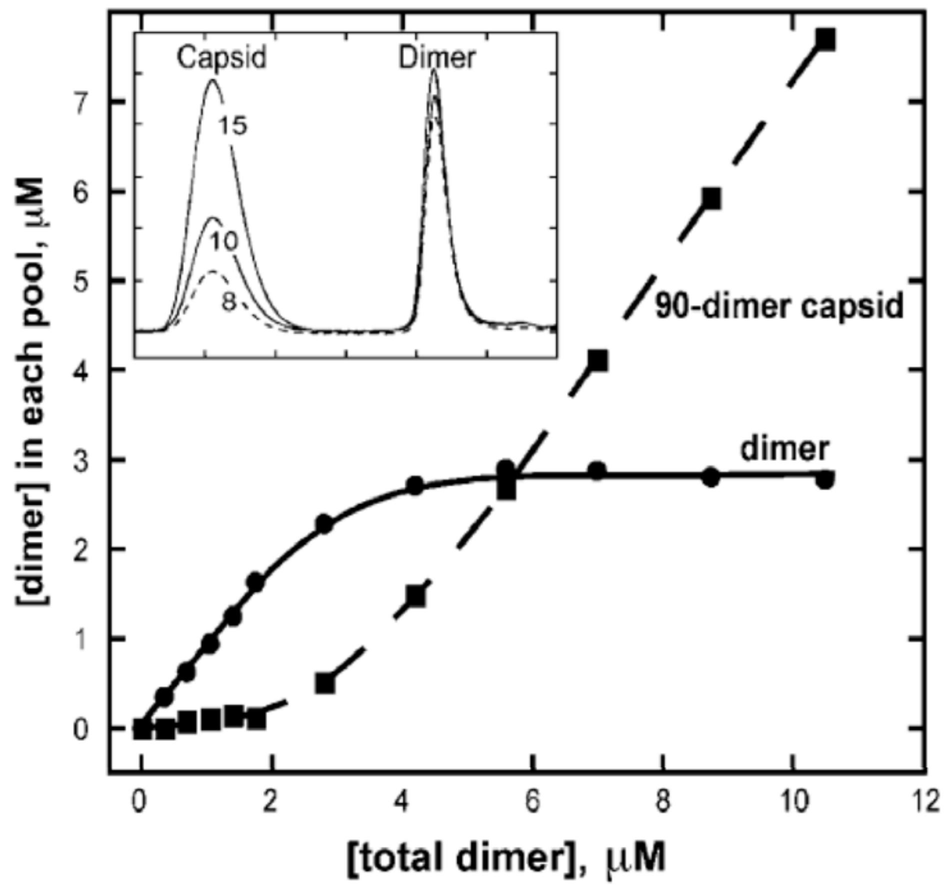
Because ***n*** is large

- [subunit] has a pseudo-critical concentration
- [capsid] is non-linear

The law of mass action at work

Cowpea Chlorotic Mottle Virus

$$K_{capsid} = \frac{[T=3\ capsid]}{[CP\ dimers]^{90}}$$

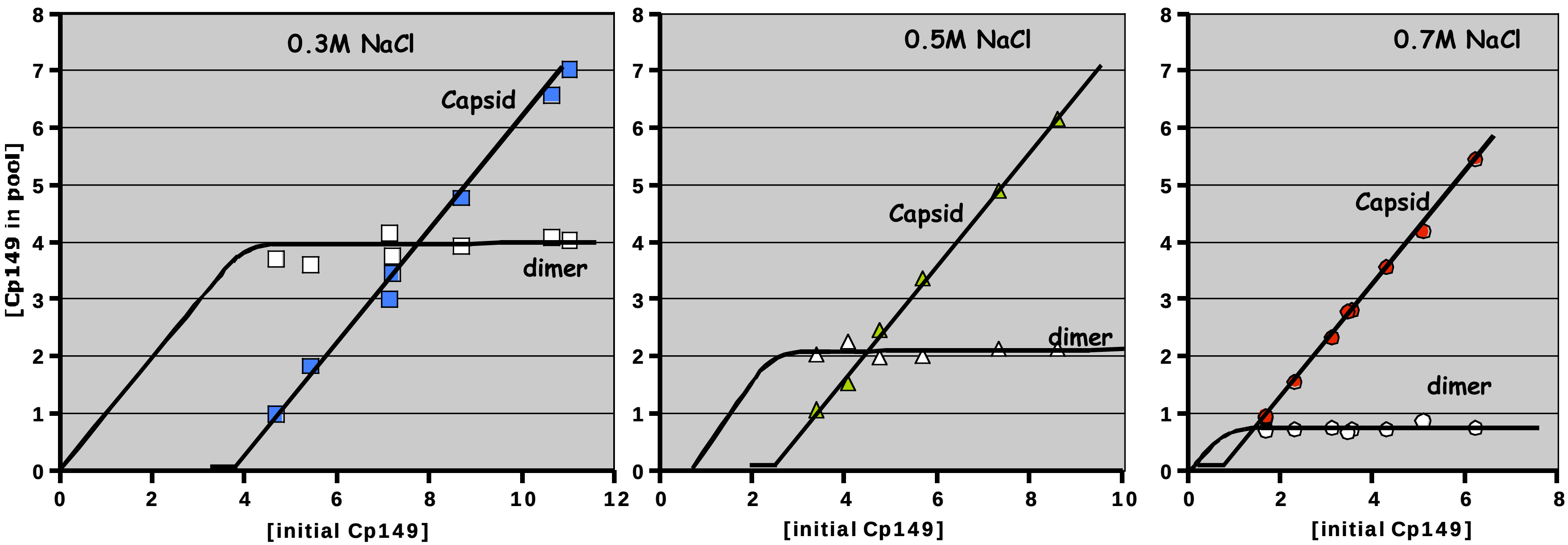


Johnson, Tang, Nyame, Willits, Young, and Zlotnick (2005) Nano Letters **5**, 765-70

The law of mass action at work

Hepatitis B virus

$$K_{capsid} = \frac{[T=4\ Capsid]}{[Cp\ dimers]^{120}}$$

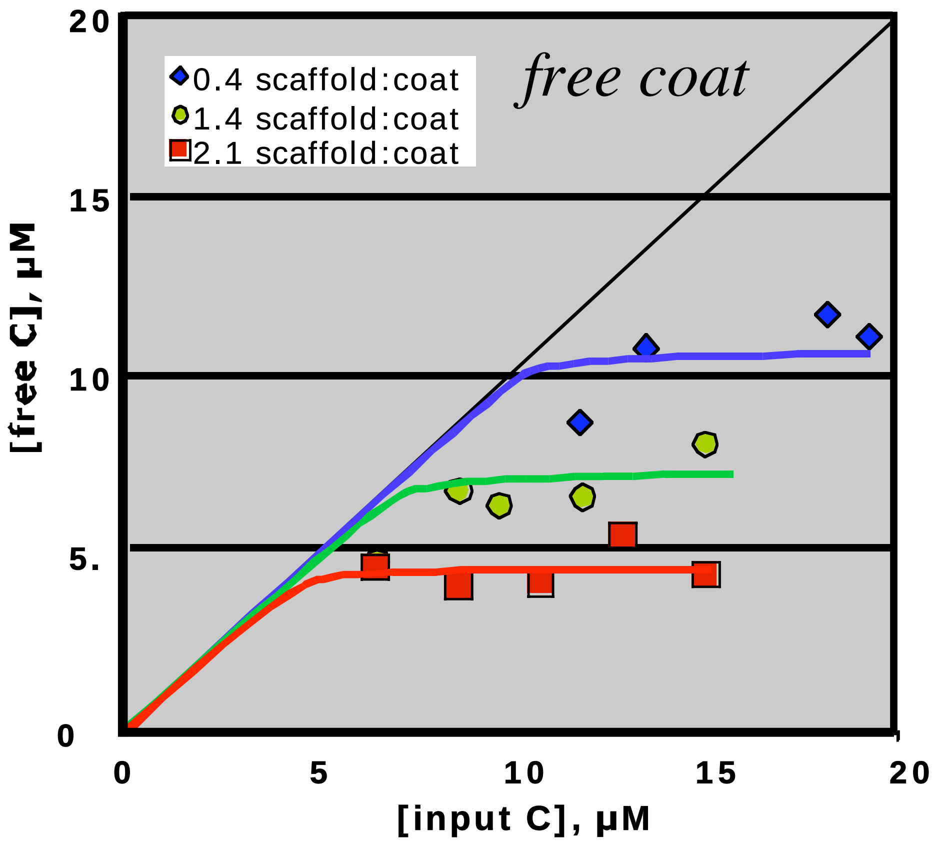


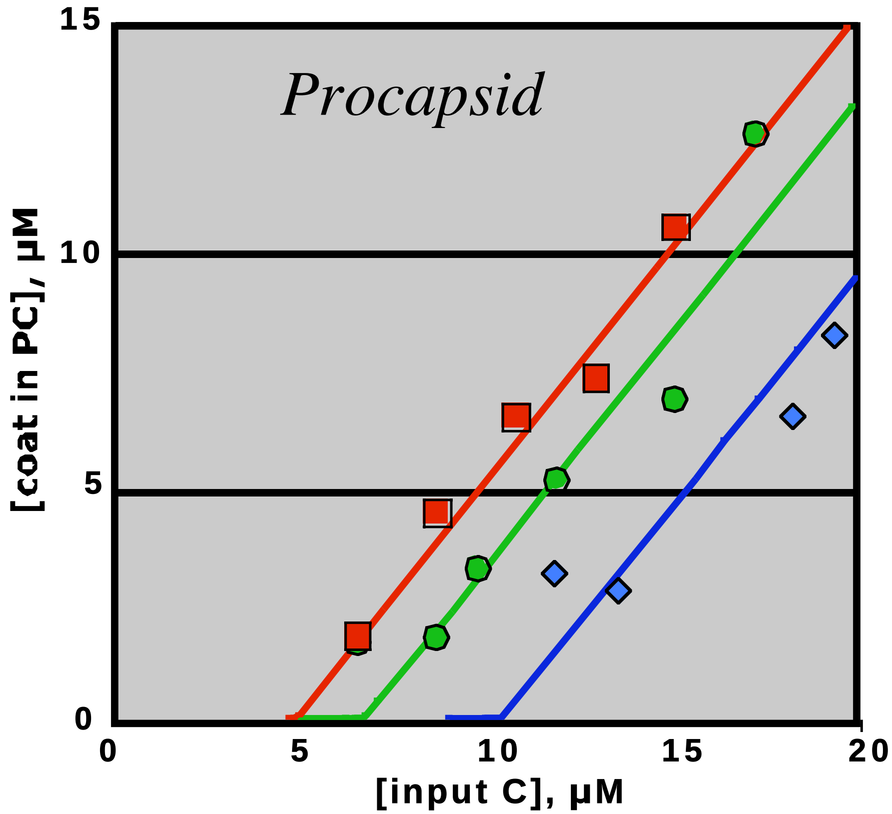
Ceres and Zlotnick (2002) Biochemistry **41**, 11525-31

The law of mass action at work

Bacteriophage P22

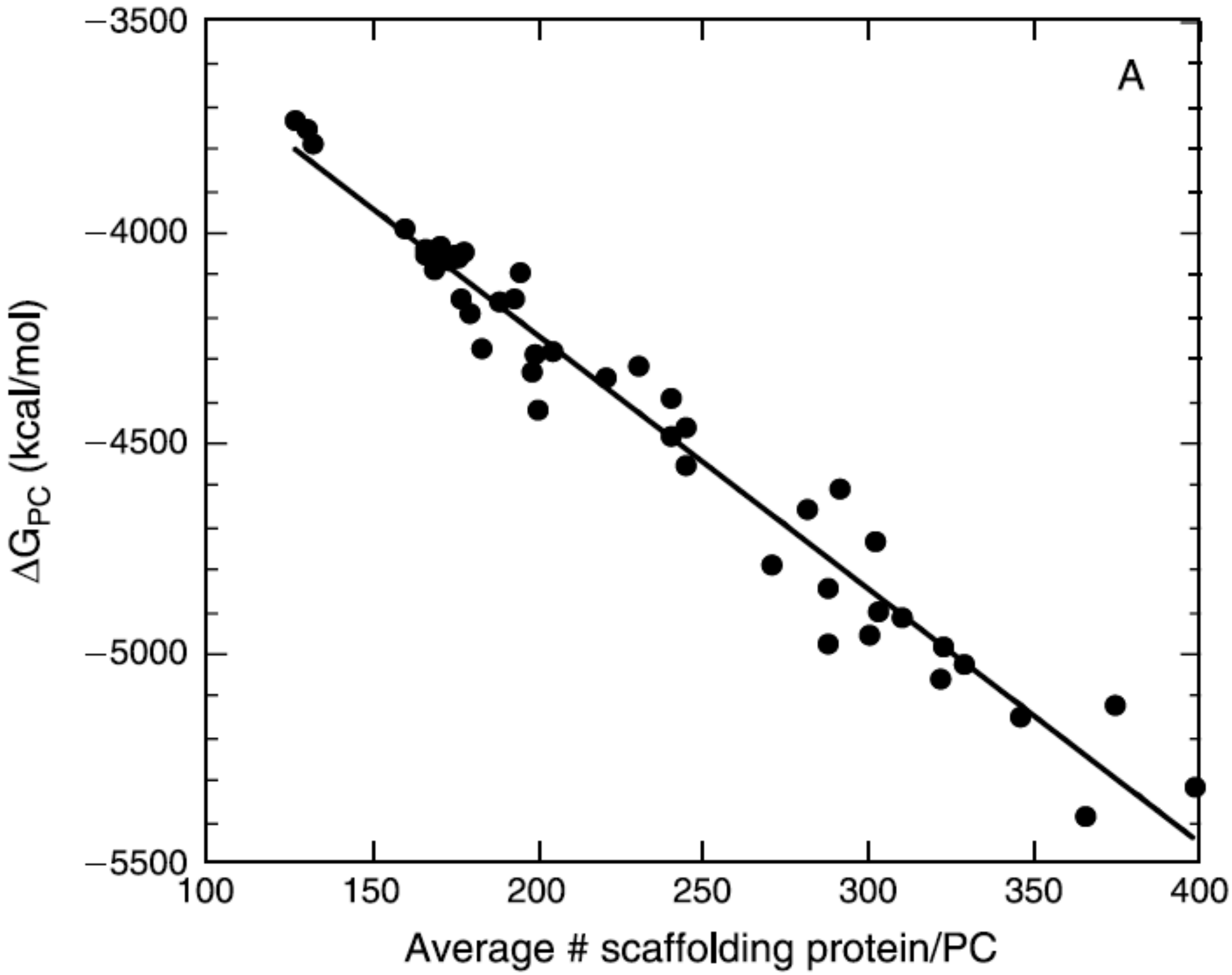
$$K_{capsid} = \frac{[T=7\ Procapsid]}{[CP]^{420}[scaffold]^{n}}$$





Parent, Zlotnick, and Teschke (2006) J Mol Biol 359, 1097-106

Scaffold stabilizes P22 procapsid



The law of mass action at work

Physical implications:
- reactions reach equilibrium
- pseudo-critical concentration
- K_{capsid} can be decomposed into component energies

    (i.g. for HBV, $\Delta G_{contact} \sim -RT \ln(K_{capsid})/240$

- weak association energy yields a globally stable capsid

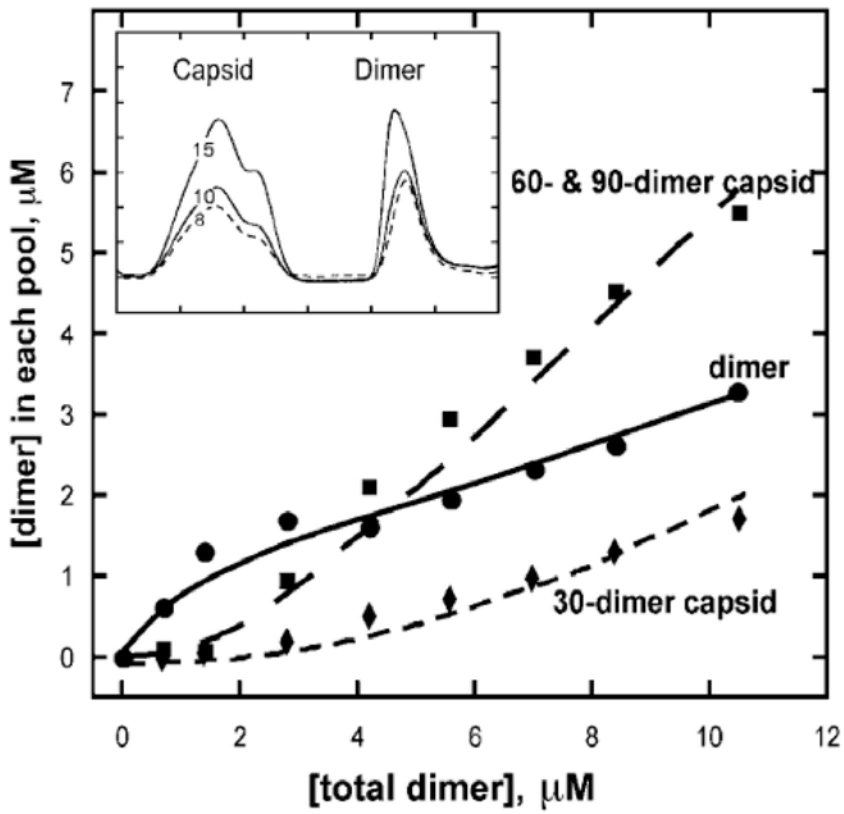
Biological implications:
- If excess subunit accumulates, empty capsids will form
- To prevent inopportune assembly concentration must be controlled or a regulatory factor must control reaction

ALLOSTERY!

Zlotnick, Johnson, Wingfield, Stahl, Endres (1999) Biochemistry 38, 14644-52
Ceres and Zlotnick (2002) Biochemistry 41, 11525-31

When the law of mass action can't be applied!

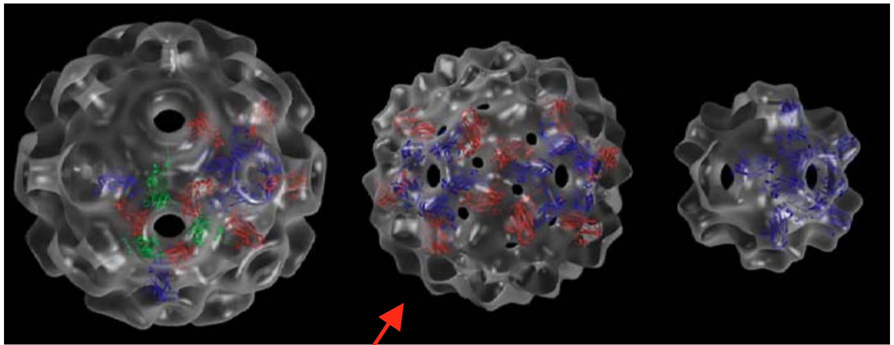
What if a reaction doesn't reach equilibrium? CCMV NΔ34



NO PSEUDO-CRITICAL CONCENTRATION

Concentrations are not stable

Products are heterogeneous



an aggregate of pentamers

Tang, Johnson, Dryden, Young, Zlotnick, Johnson (2006) J Struct Biol 154, 59-67

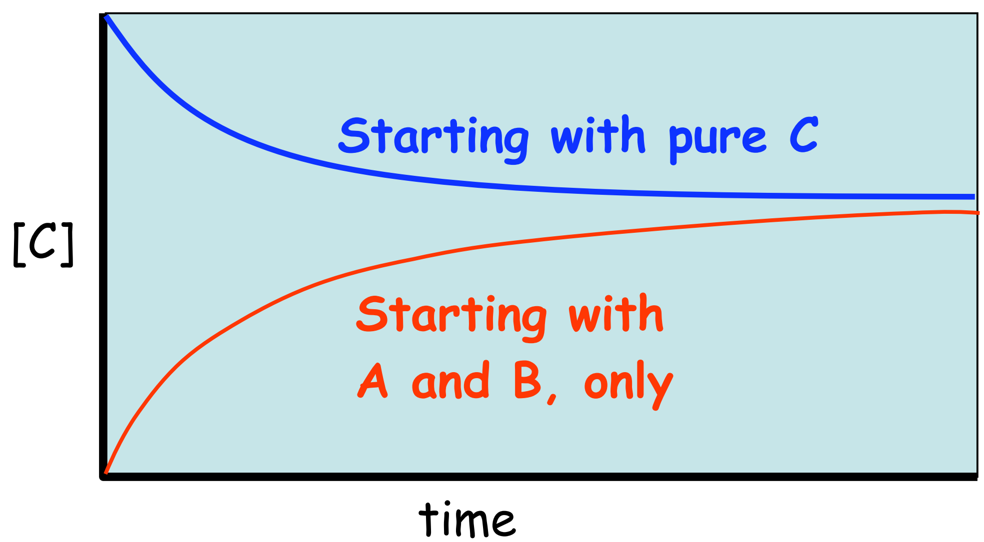
Johnson, Tang, Nyame, Willits, Young, and Zlotnick (2005) Nano Letters **5**, 765-70

Another way of looking at equilibrium

For simple cases,

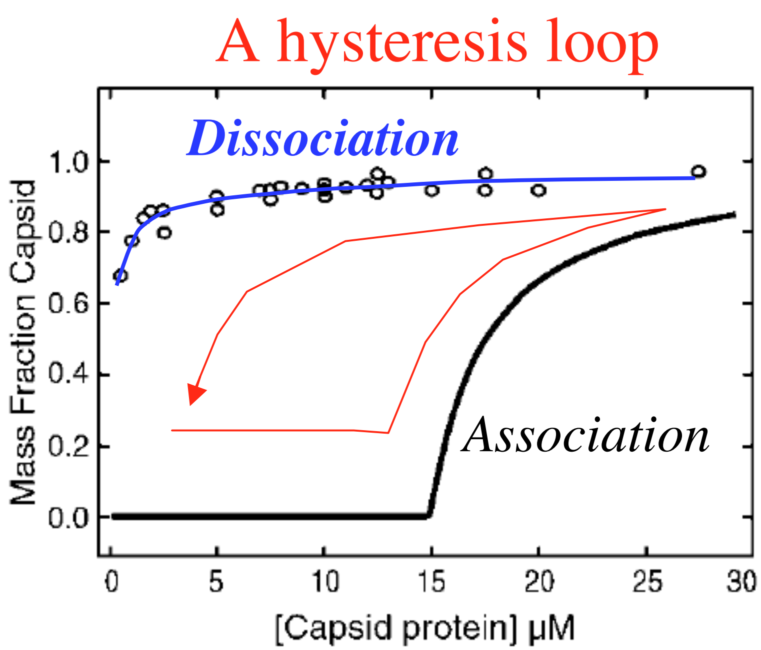
association and dissociation equilibrate to the same final point

$$A + B \rightleftharpoons C$$



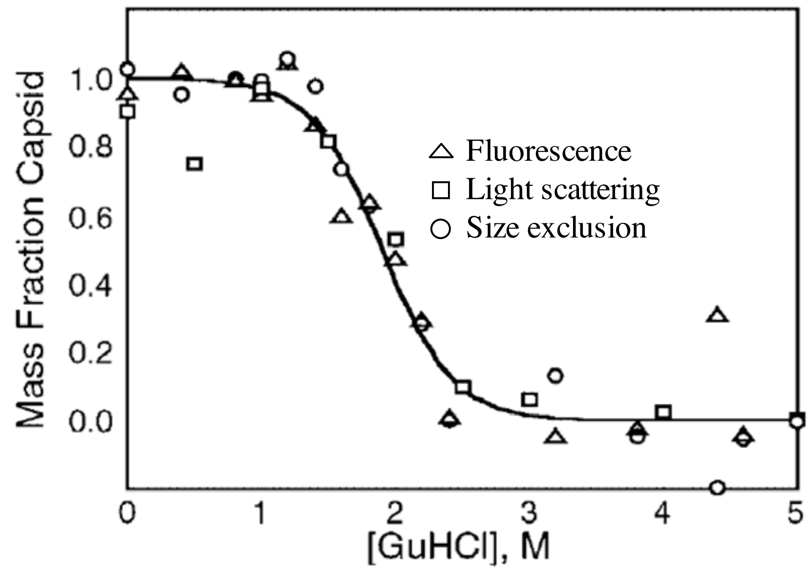
Viruses are NOT simple association reactions

For HBV



$\Delta G_{contact,\ assembly} = -3.1$ kcal/mol

$\Delta G_{contact,\ GuHCl} = -5.8$ kcal/mol



Singh and Zlotnick (2003) JBC 278, 18249-55

More anomalous stability

Weber, Da Poian, and Silva noted similar effects in phage R17, and other oligomers

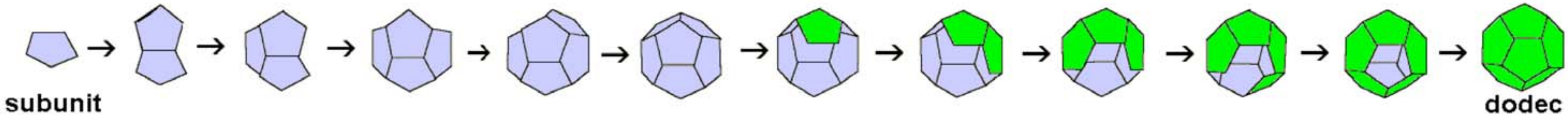
Biophys J 76, 167-73 (1996)

Phage P22 procapsids are stable for prolonged periods (±scaffold)

Prevelige, personal communication

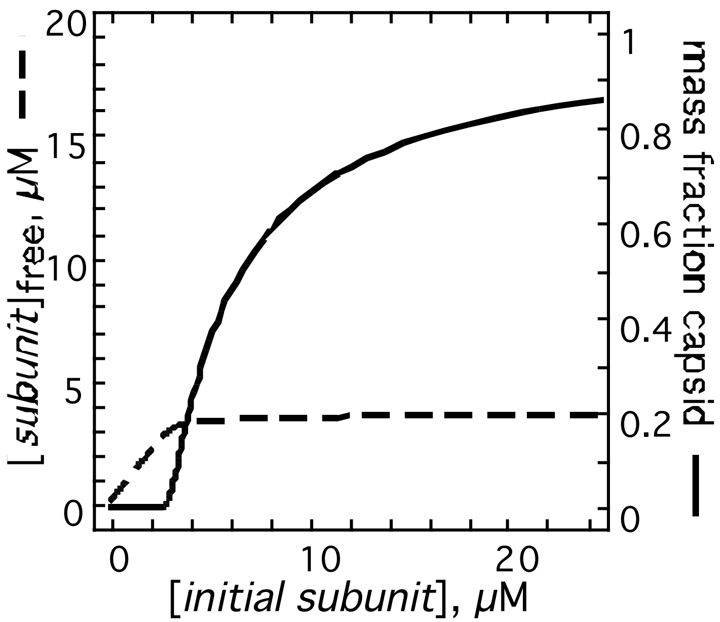
Teschke, personal communication

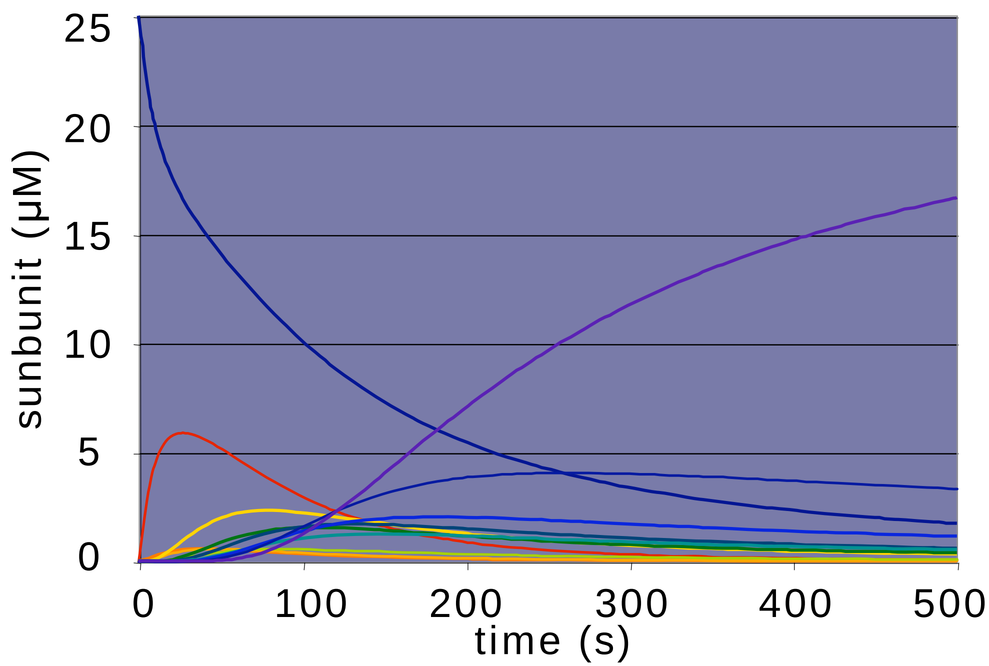
Dodecahedral model



At equilibrium: only monomer & capsid are observed
Isotherms are marked by a pseudo-critical concentration

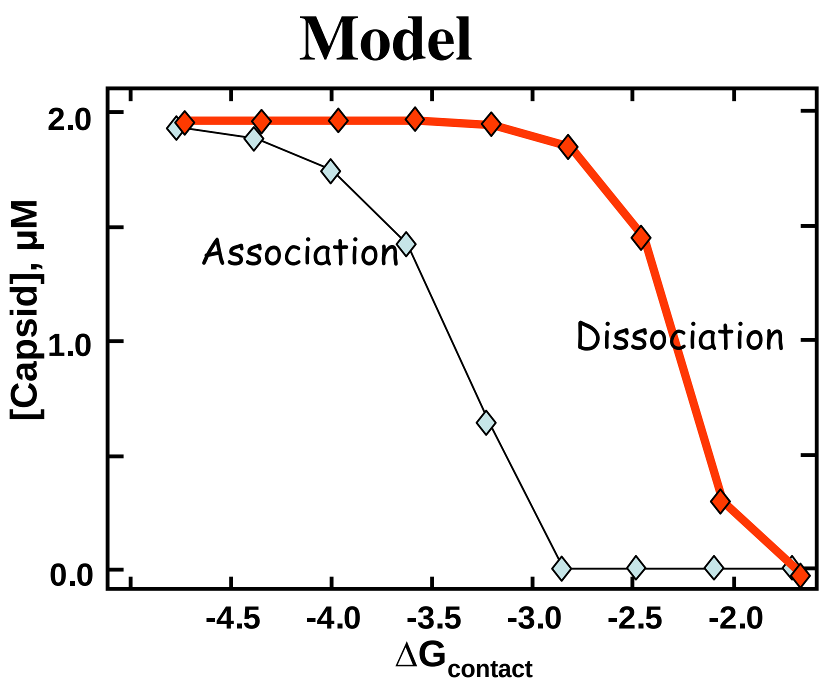
Transiently, intermediates accumulate and are consumed
Kinetics are sigmoidal, **equilibrium** is rapidly acheived





Zlotnick (1994) J Mol Biol 241, 59-67; Endres and Zlotnick (2002) Biophys J 83, 1217-30
Similar results with a different approach from Russell Schwartz

Hysteresis is built into capsids



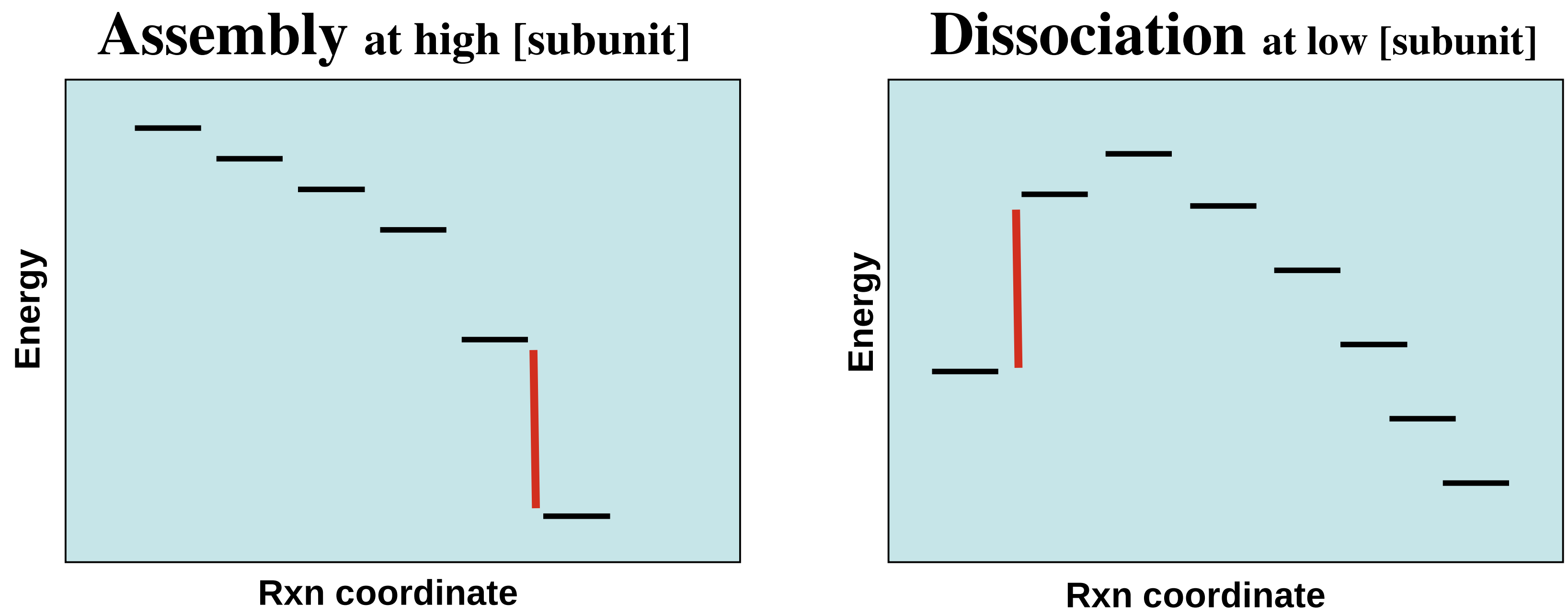
In simulations

• association reactions equilibrate
• interaction energy must be catastrophically weakened for dissociation

Hysteresis is also observed in coarse-grained dynamics simulations

Hagan & Chandler (2006) Biophys J, e-published

Why should hysteresis happen during dissociation but not association?



Hysteresis is a KINETIC EFFECT that derives from capsid closure

Implications for disassembly

Because of hysteresis

- Capsids are stable under conditions that don't support assembly
- Capsids persist in inhospitable environments

AND

- A catalyst will be required for dissociation

Some generalizations

A thermodynamic-kinetic description of CAPSID assembly is consistent with:

- Pseudo-critical concentration
- Weak local interactions
- Transient intermediates (rare at equilibrium)
- Hysteresis
- MD simulations of assembly

And implies:

- regulated activation of assembly (not just nucleation)
- regulated dissociation

Evaluating nucleation and assembly path

HBV

Nucleation -- trimer of dimers

Elongation -- one dimer at a time

Zlotnick et al (1999) Biochemistry 38, 14644-52

CCMV

Nucleation -- a pentamerof dimers +1

Elongation -- mixture of dimers and PODs

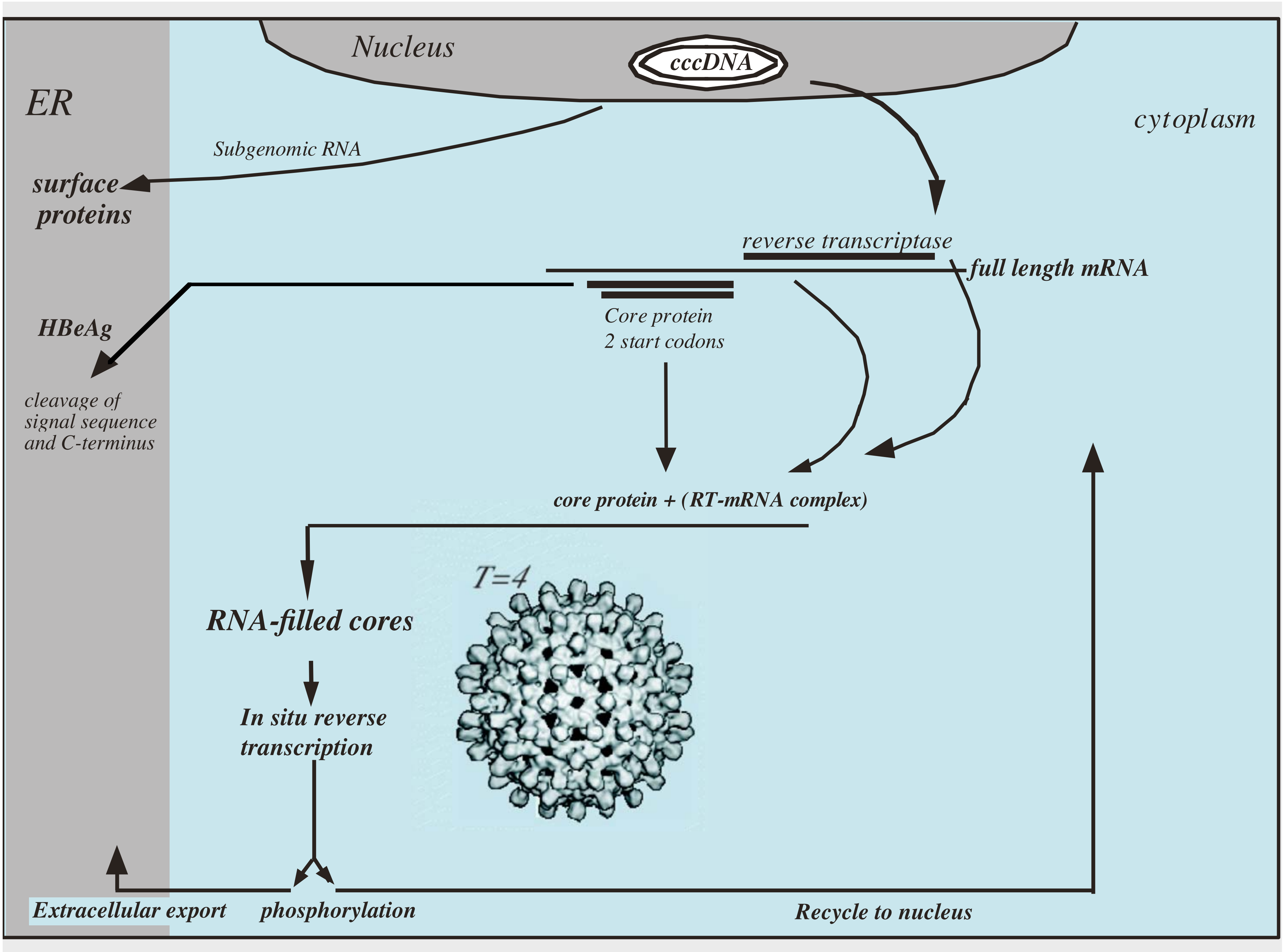
Johnson, Tang, Nyame, Willits, Young, Zlotnick (2005) Nano Letters 5, 765-70

HPV11

Nucleation -- a dimer of pentamers

Elongation -- one pentamer at a time

Casini, Graham, Heine, Garcea, Wu (2004) Virology 325, 320-7


Nucleus
cccDNA
ER
cytoplasm
Subgenomic RNA
surface proteins
reverse transcriptase
full length mRNA
HBeAg
Core protein
2 start codons
cleavage of signal sequence and C-terminus
core protein + (RT-mRNA complex)
T=4
RNA-filled cores
In situ reverse transcription
Extracellular export
phosphorylation
Recycle to nucleus

Taking advantage of regulated assembly: HBV and HAP1

HBV is a major health issue

- ***~350 million chronic infections***
- ***cirrhosis and liver cancer***

Capsid Assembly is required for

- ***RNA packaging***
- ***DNA synthesis***
- ***Intracellular trafficking***
- ***Export from the host***

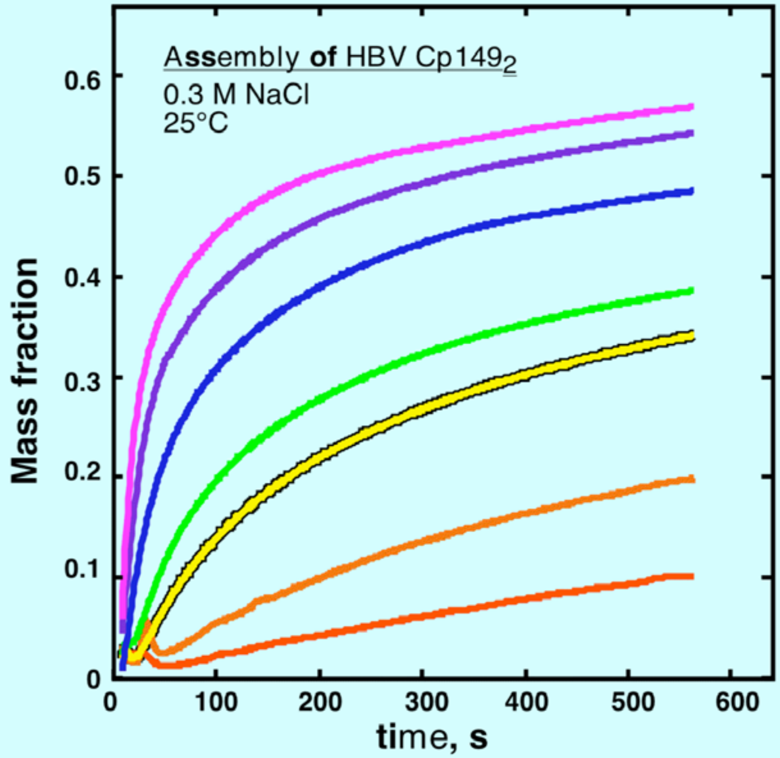
What does HAP1 do?

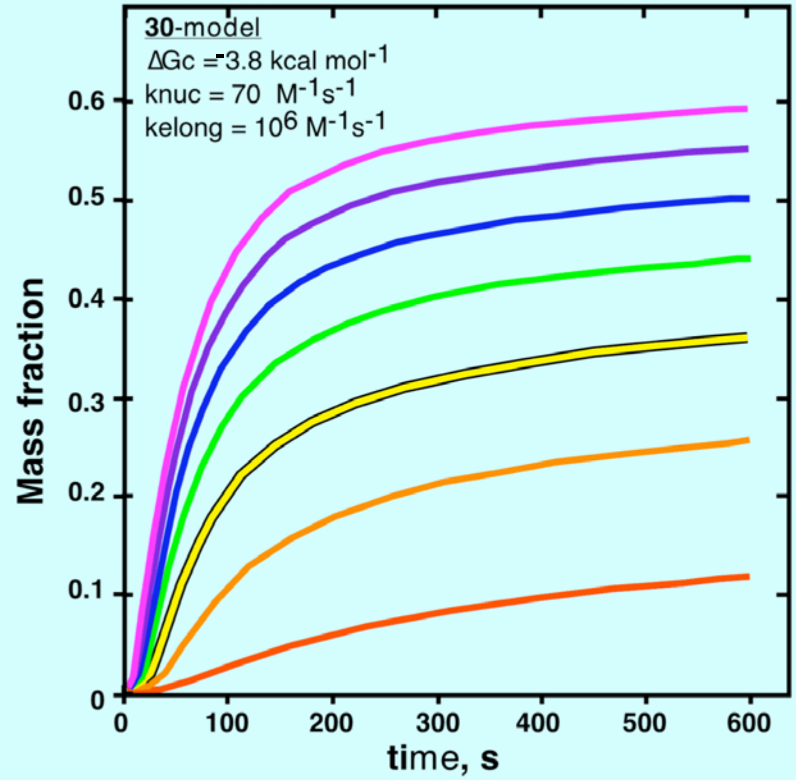
- speeds up assembly
  (i.e. it lowers the energy barrier to assembly)
- destabilizes 5-folds
- stabilizes 6-folds

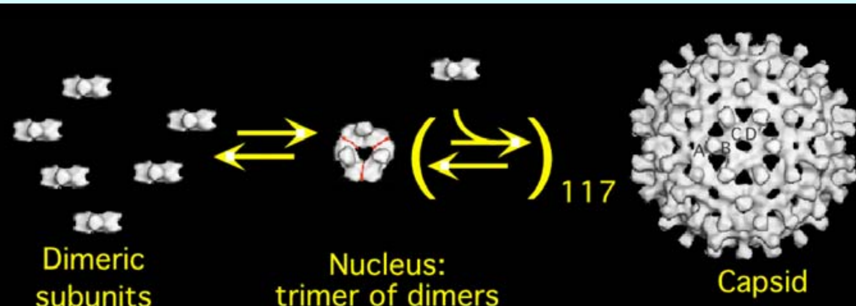
How does HAP1 do it?

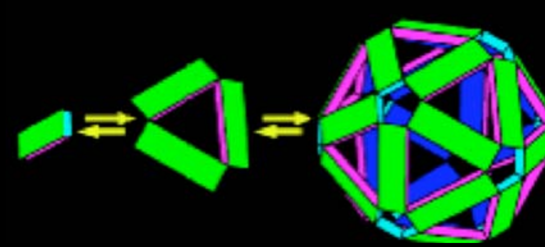
- Stabilizes the assembly active form
  (i.e. allosterically activates assembly)
- flattens 6-folds by putting a burr between C & D
- puckers 5-folds

Comparing Assembly:
A real 120-mer and a model 30-mer









Zlotnick, Johnson, Wingfield, Stahl, Endres (1999) Biochemistry 38, 14644-52

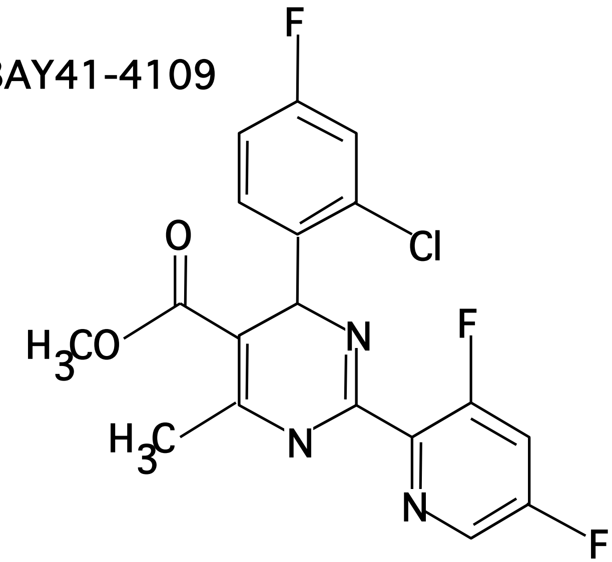
HAP1 activates and can misdirect HBV Cp assembly

Heteroaryldihydropyrimidines (HAP)

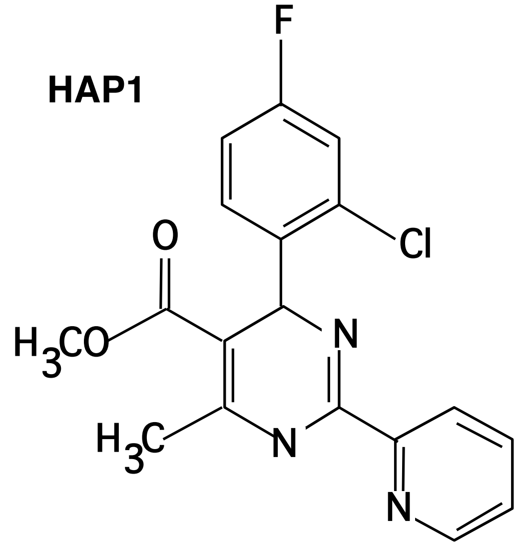
- decrease production of secreted virus
- decrease intracellular [HBV cores]
- intracellular depletion of Cp (proteosomal)

Deres et al (2003) Science 299, 893-6

BAY41-4109

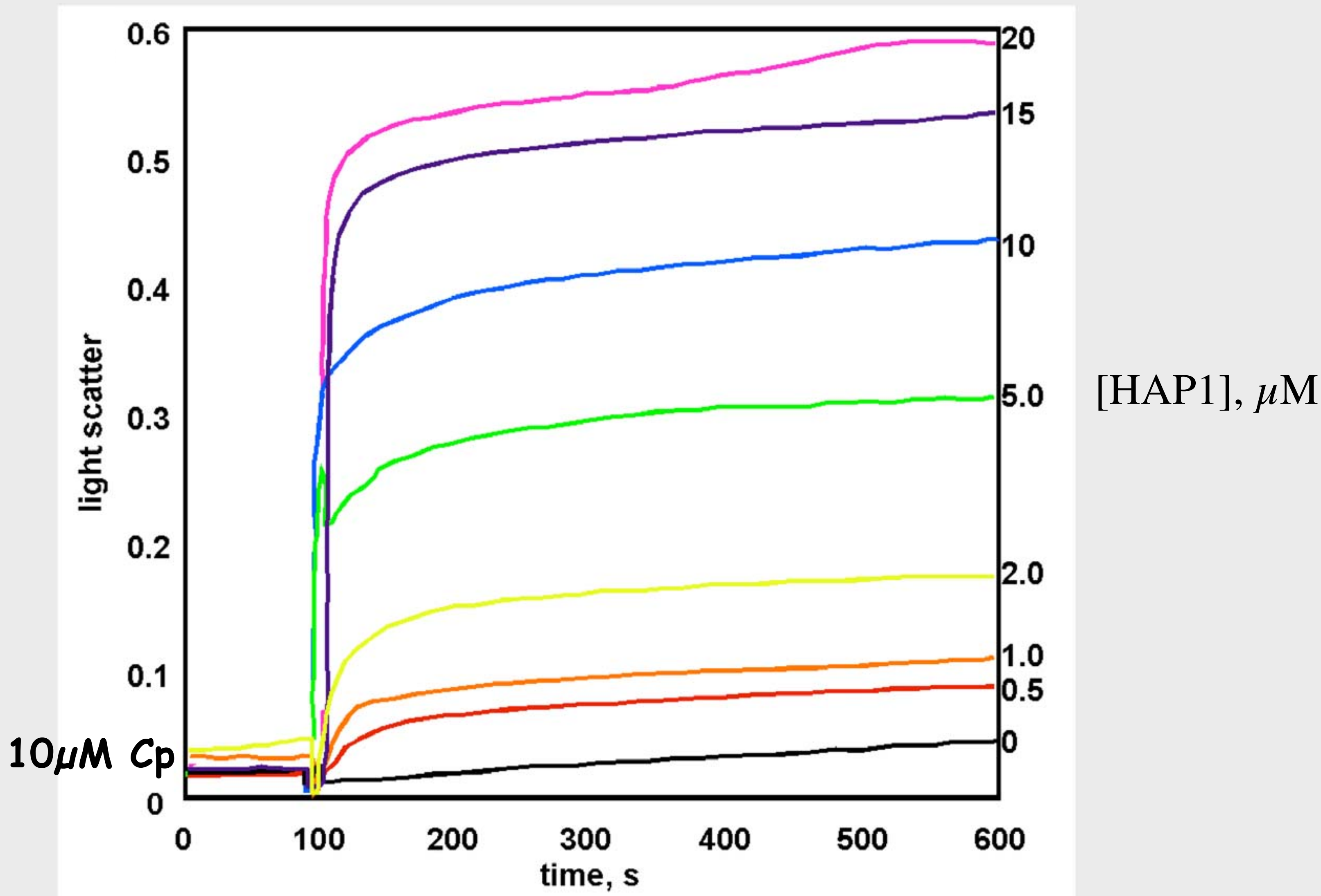


HAP1

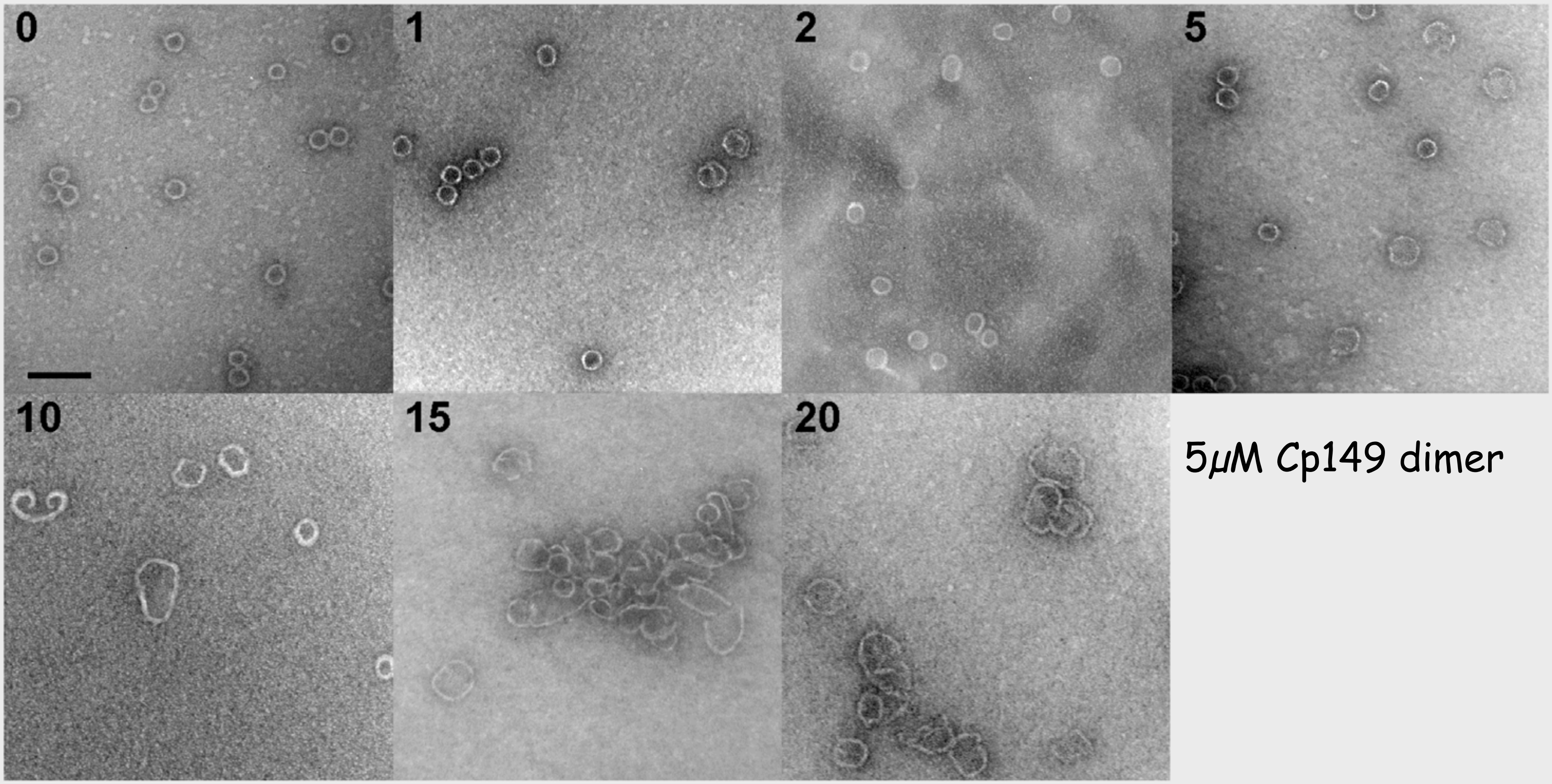


Stray, Bourne, Lewis, Punna, Finn, and Zlotnick (2005) PNAS102, 8138-43

HAP enhances the rate of Cp149 assembly

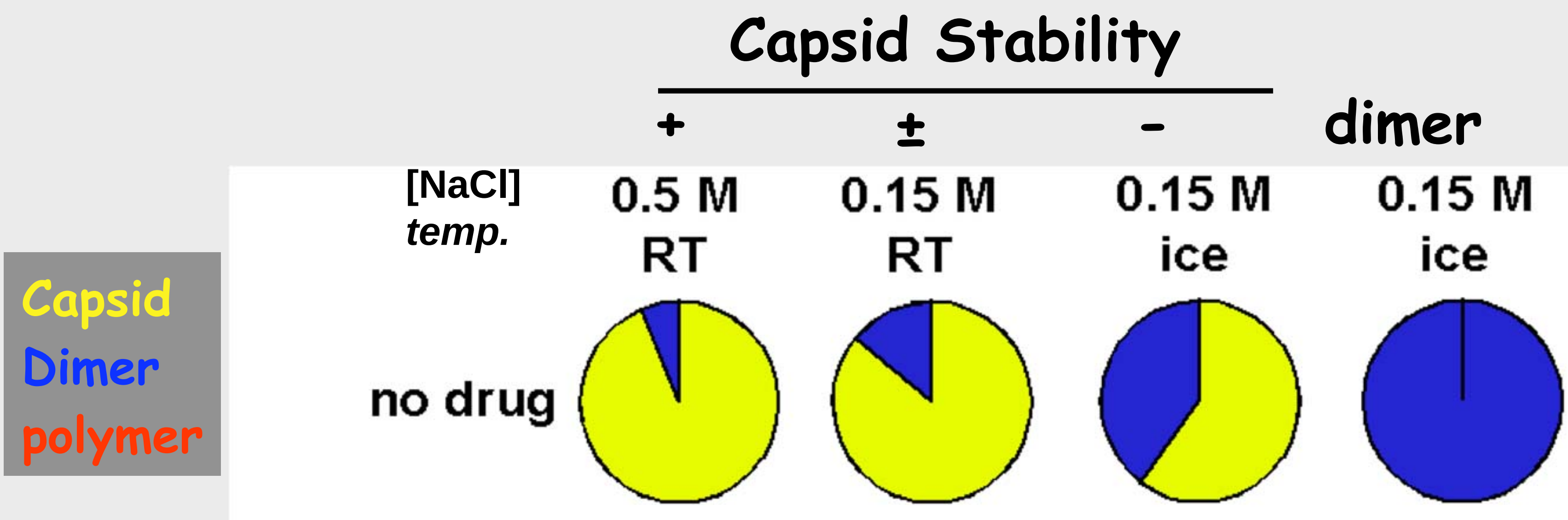


HAP1 leads to assembly of aberrant particles; near stoichiometric amounts of required.



5μM Cp149 dimer

HAP disrupts capsids by scavenging Cp to form non-capsid polymer



Summary

HAP binds capsid protein

- Enhances assembly (favors assembly-active state)
- excess HAP has radical effects on morphology

High [HAP] favors assembly of hexamers

$$\Delta G_{\text{hexamer},\mathbf{HAP}} < \Delta G_{\text{hexamer}}$$

Low [HAP] enhances rate but not extent of assembly

$$\Delta G_{\text{capsid},\mathbf{HAP}} \sim \Delta G_{\text{capsid}}$$

If $\Delta G_{\text{capsid}} = 12\Delta G_{\text{fivefold}} + 30\Delta G_{\text{hexamer}}$

THEN

$$\Delta G_{\text{fivefold},\mathbf{HAP}} > \Delta G_{\text{fivefold}}$$

Co-crystallization with HAP causes a change in unit cell dimensions

*C*2 with 1 particle per asymmetric unit

- HAP	150c	*a*= 558.4	***b*= 327.1**	*c*=562.2	β= 109.1
	3CA	*a*= 559.1	***b*= 328.8**	*c*=563.2	β= 109.1
+ HAP	150c	*a*= 528.5	***b*= 366.5**	*c*=540.1	β= 104.8
	3CA	*a*= 526.5	***b*= 363.5**	*c*=538.1	β= 105.0

T=4 capsid ~**340Å** diameter

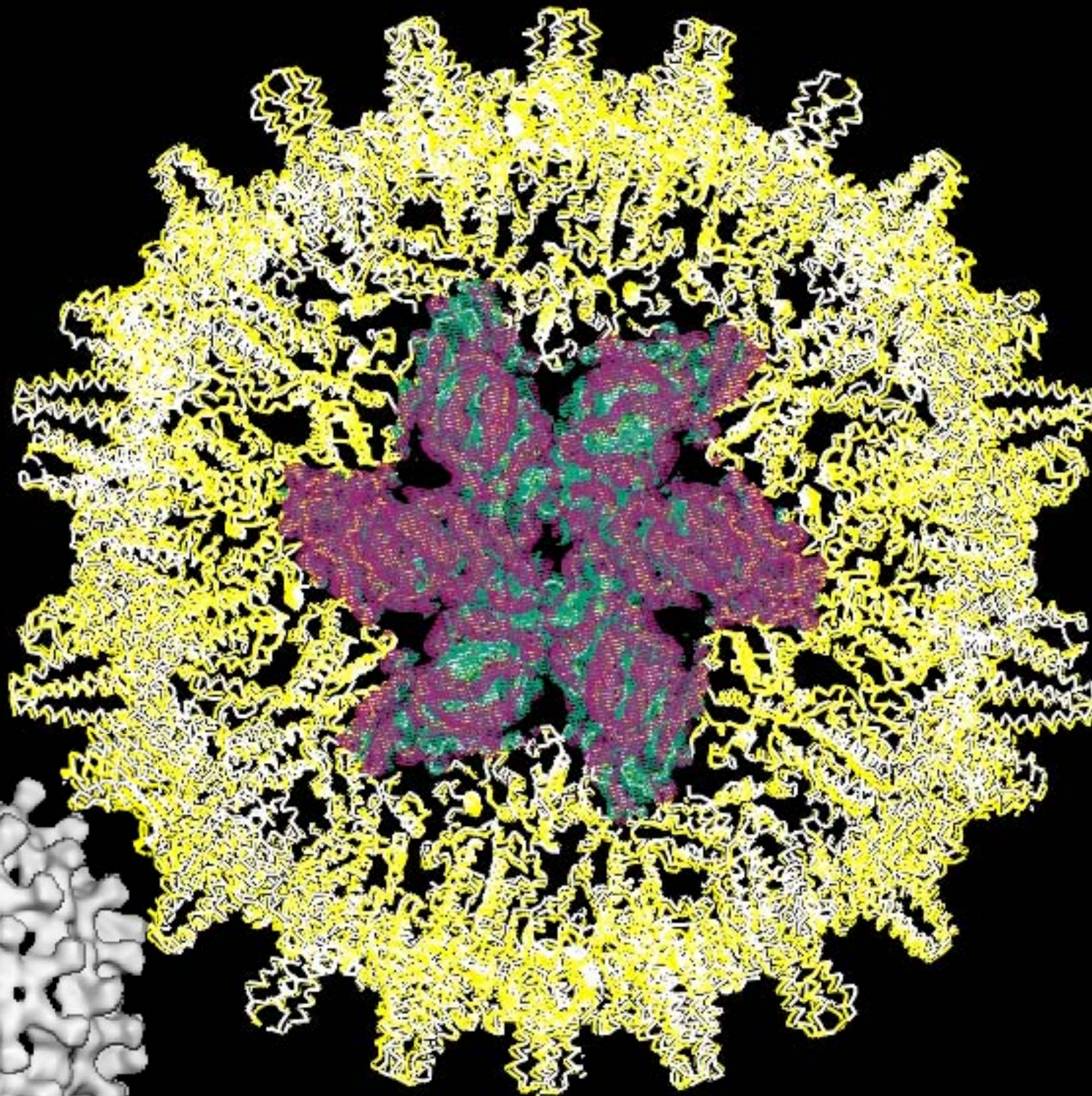
HAP1 alters quaternary structure

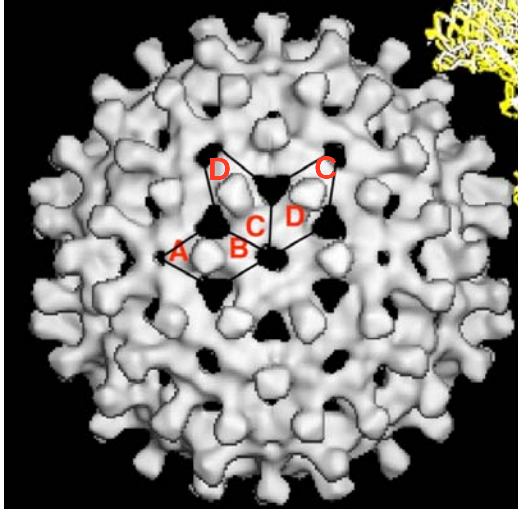
- HAP

+ HAP

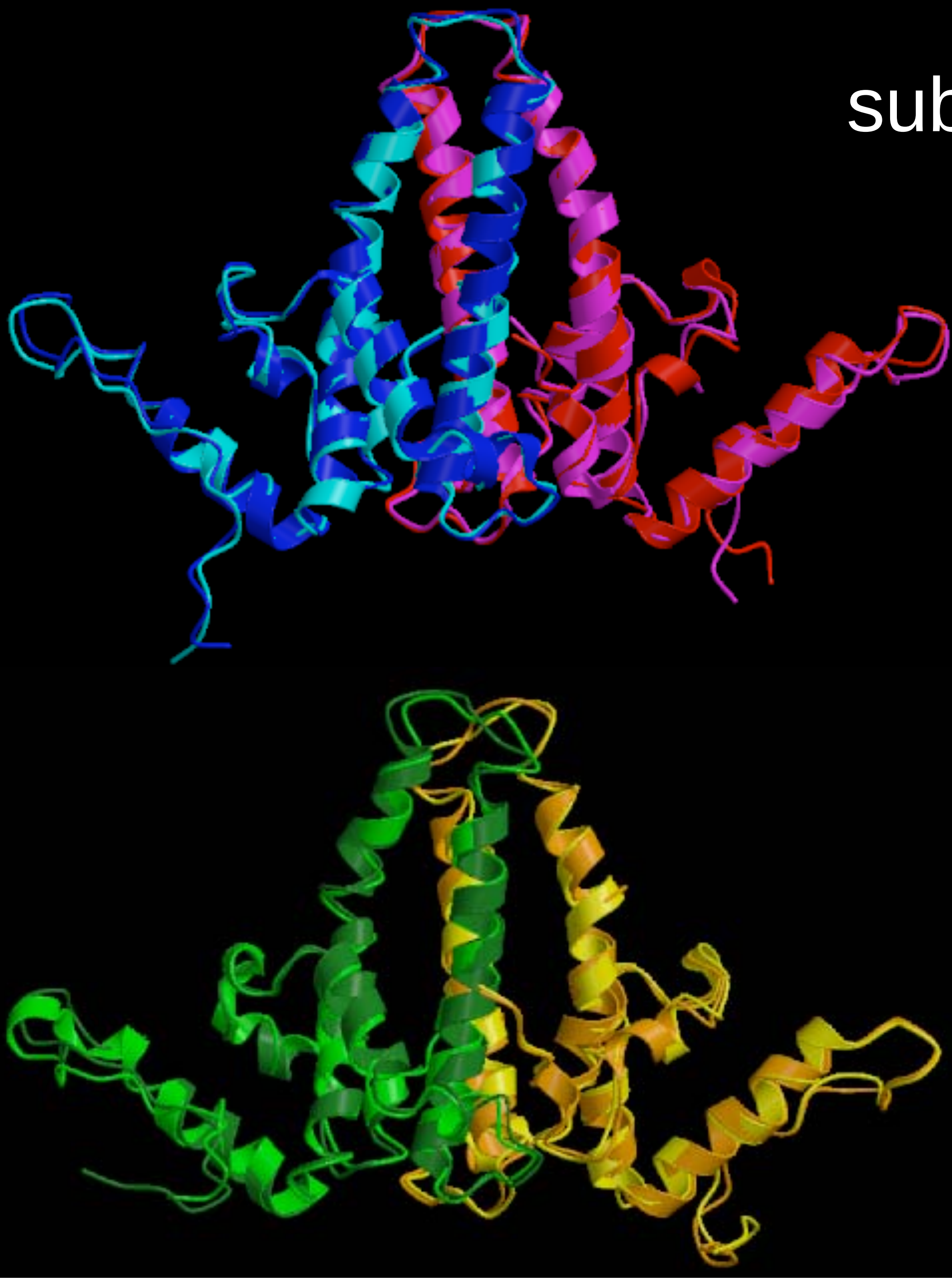
Density for -HAP

Density for +HAP



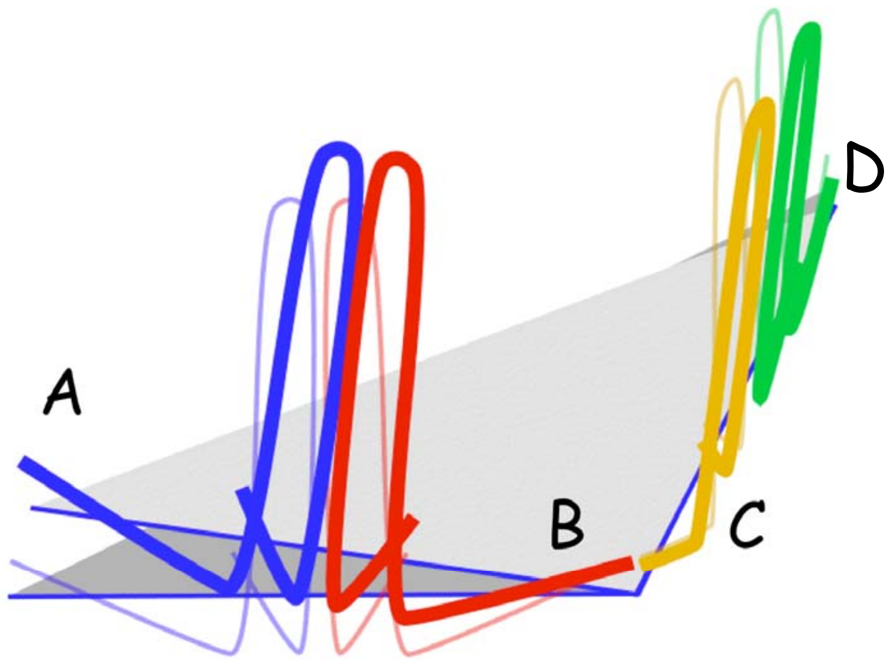


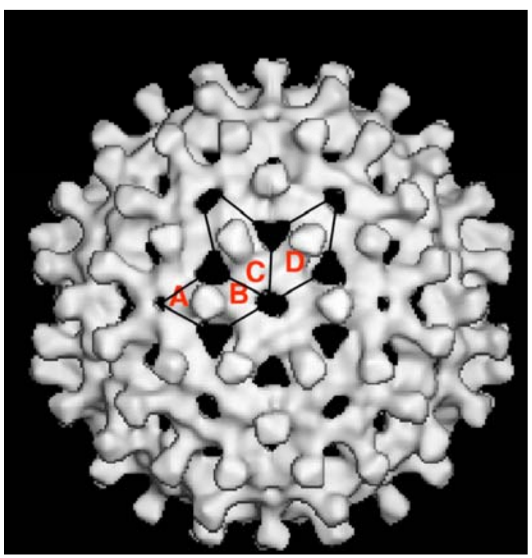
subunits/dimers display few differences



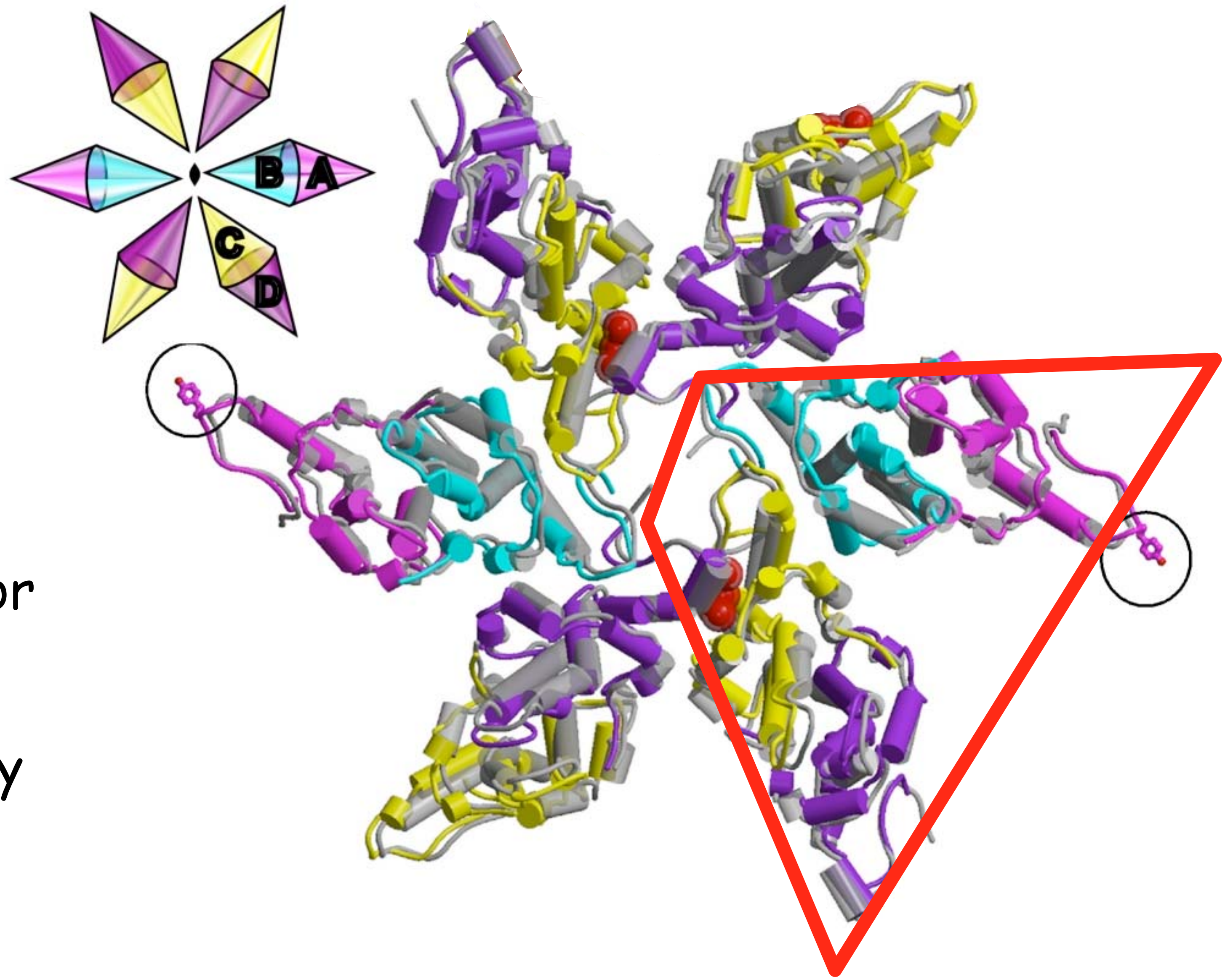
-HAP1	+HAP1
A	A
B	B
C	C
D	D

Subunits move as an AB+CD bloc





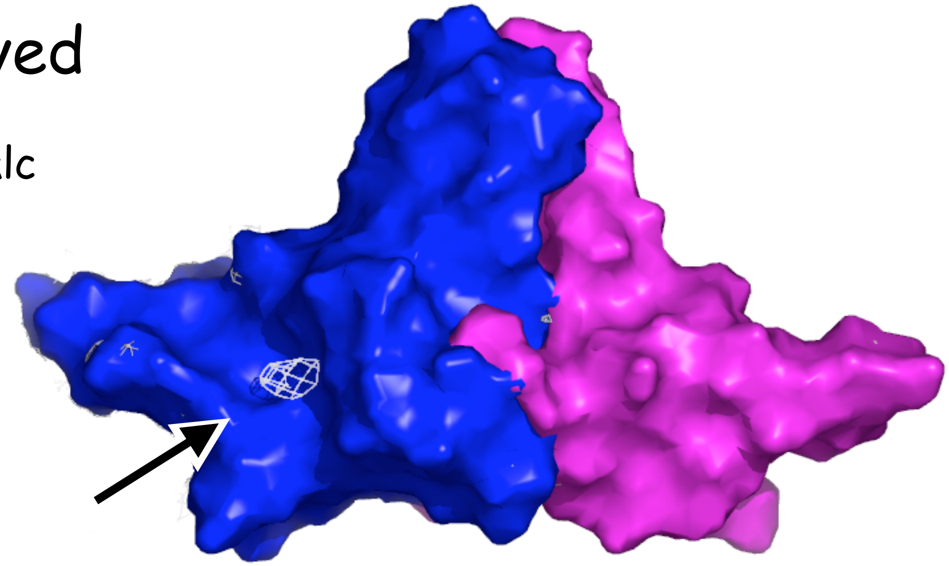
Subunits move as an AB-CD bloc

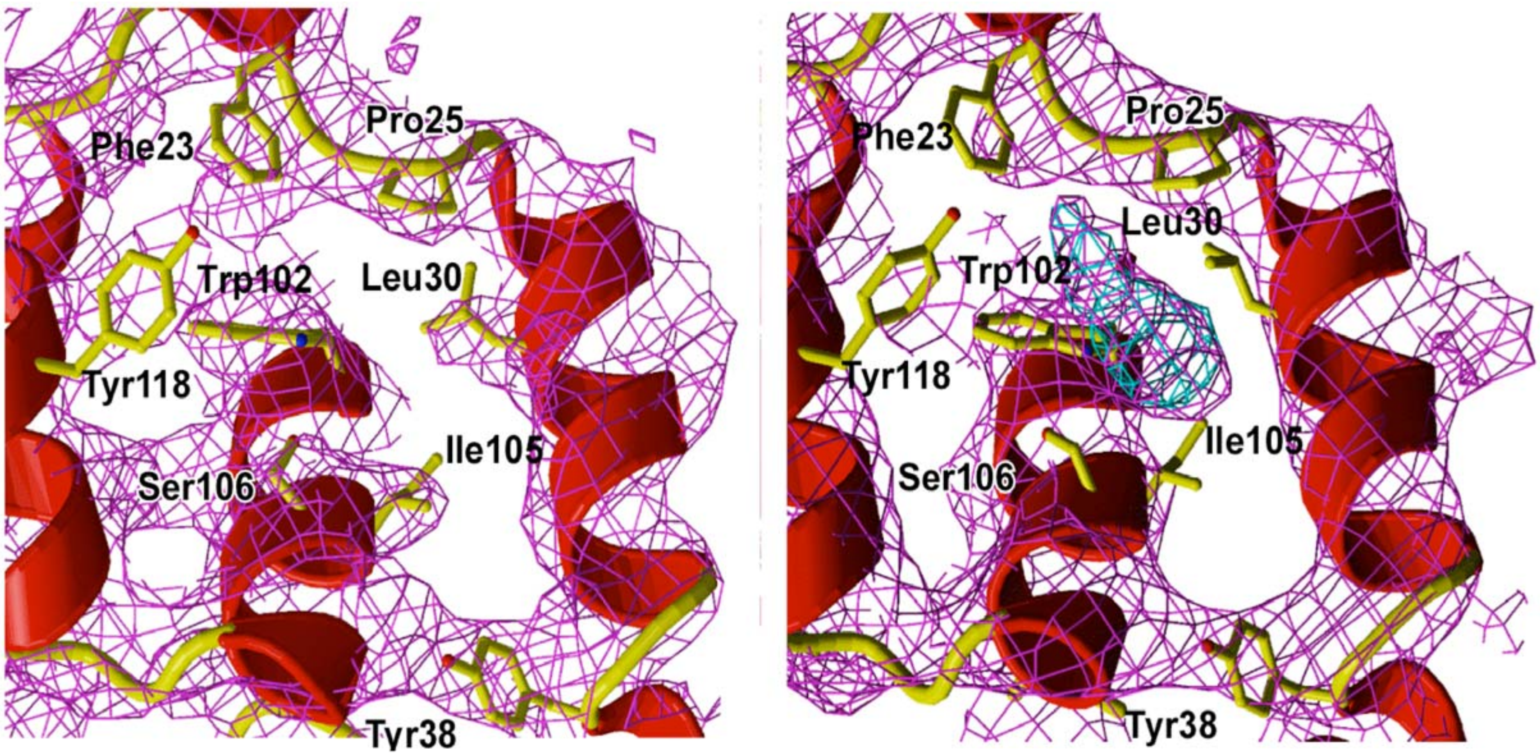


+HAP1- color

-HAP1 - gray

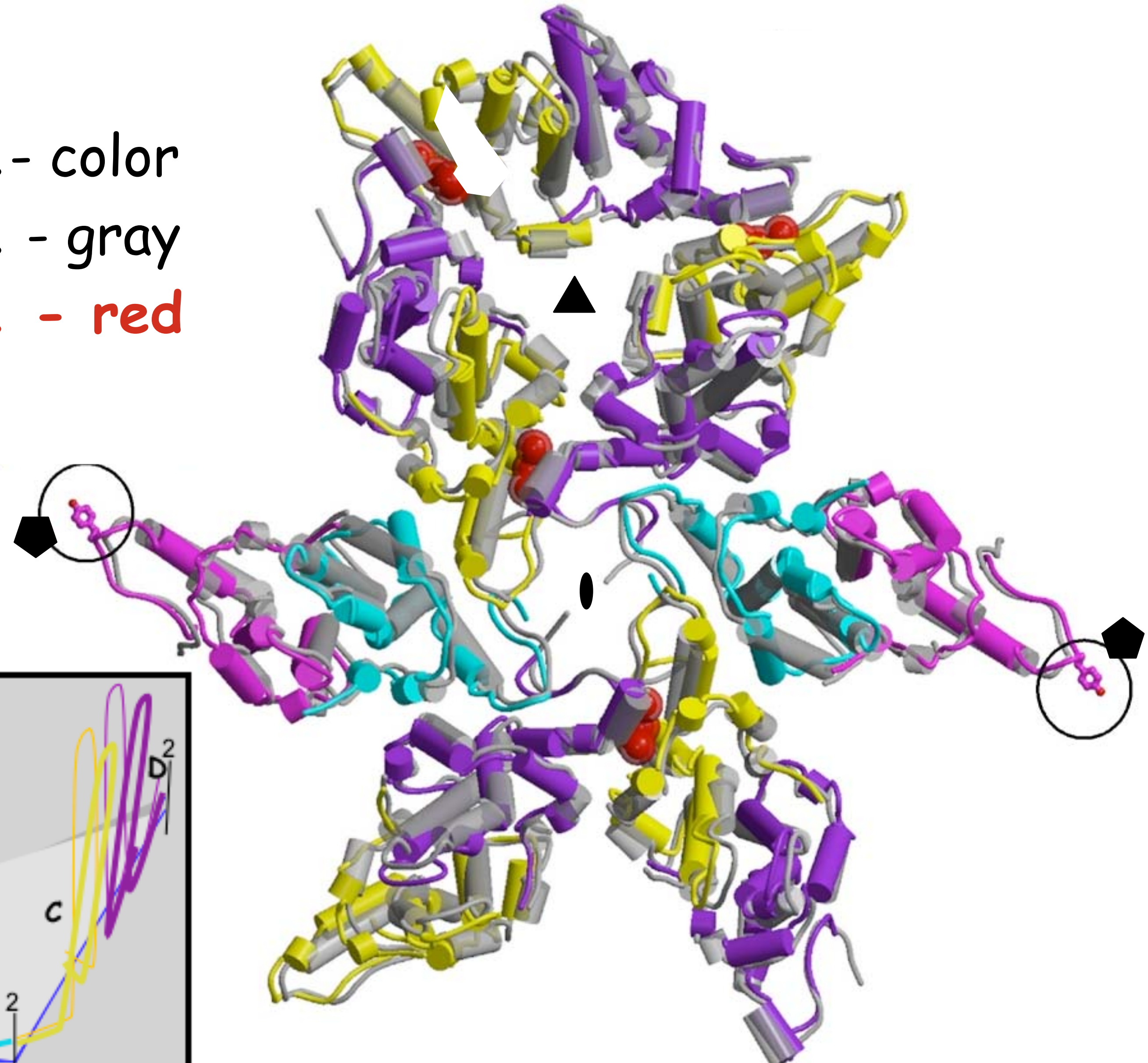
Putative HAP density observed as a 15σ density in a F_{obs}-F_{calc} map



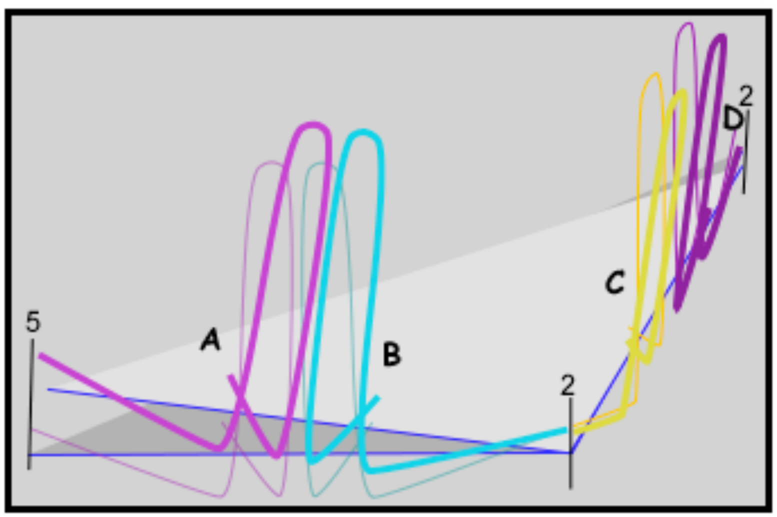


HAP1 in the *C* subunit surrounds the 3fold

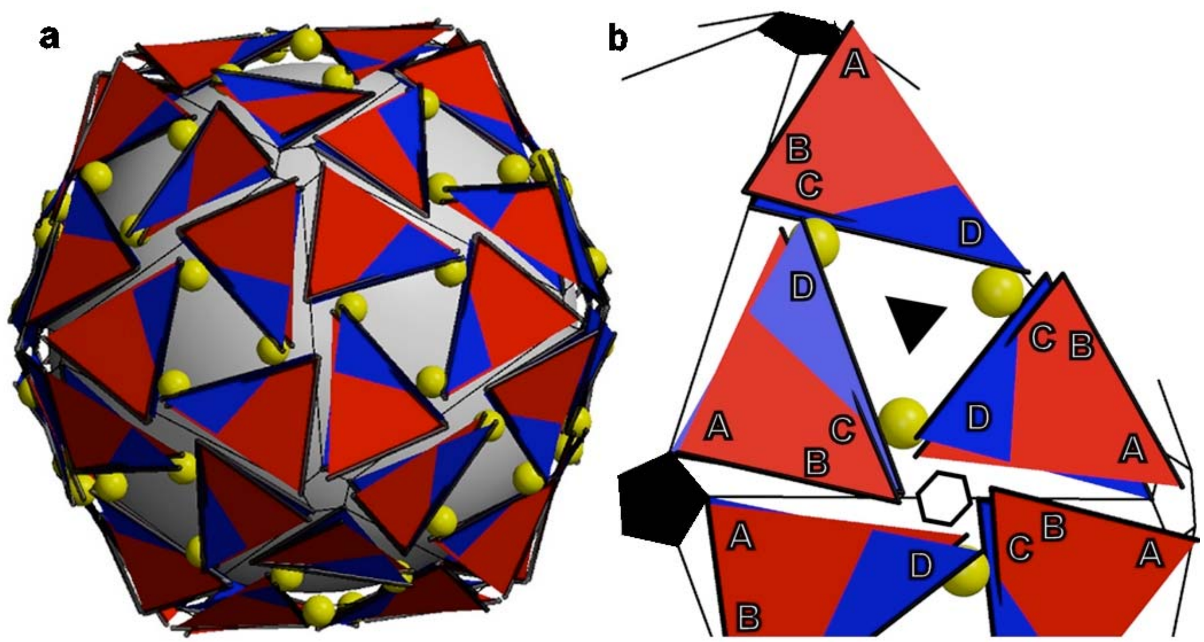
+HAP1- color
-HAP1 - gray
HAP1 - red



Subunits move as
an AB+CD bloc



Quaternary, not tertiary, structure changes
dimers acts as AB+CD blocs
HAP1 acts as a wedge to shift these blocs
The +HAP1 structure may be a snapshot in disassembly



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- stabilizes 6-folds

How does HAP1 do it?

- Stabilizes the assembly active form

  (i.e. allosterically activates assembly)
- flattens 6-folds by putting a burr between C & D

Some take home messages

- **Synergism between assembly studies and models**
  - Models must be adjusted to reflect chemical/biological details
  - Contact energy is weak **(a common theme)**
  - Dissociation displays hysteresis **(a common theme)**
- **HBV assembly is allosterically regulated (a common theme?)**
- **Assembly may be antagonized by drugs acting on different Cp conformations. (a common theme?)**

A clearer understanding of assembly is made possible by credible models and model-based predictions

Zlotnick lab (OUHSC)
- Christina Bourne
- Pablo Ceres
- Jennifer Johnson
- Sarah Katen
- Angela Lee
- Santanu Mukherjee
- Zach Porterfield
- Stephen Stray

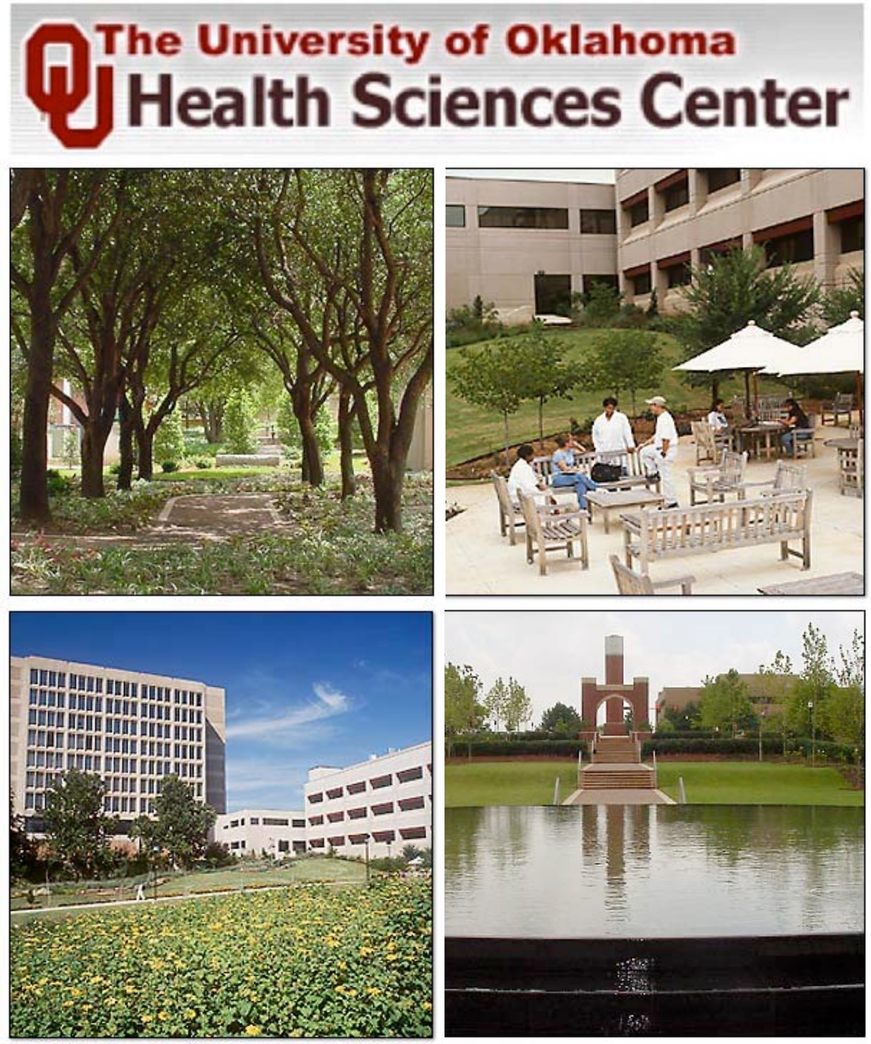
Brian Bothner (MSU)
- Jonathan Hilmer

Carol Teschke (UConn)
- Kristin Parent

Jack Johnson (Scripps)
- Jeff Speir
- Jinghua Tang

M.G. Finn (Scripps)

Mark Young (MSU)



Funding: NIH, NSF, American Cancer Society