Boulder lecture #3: William M. Gelbart

MAKING VIRUSES FROM SCRATCH

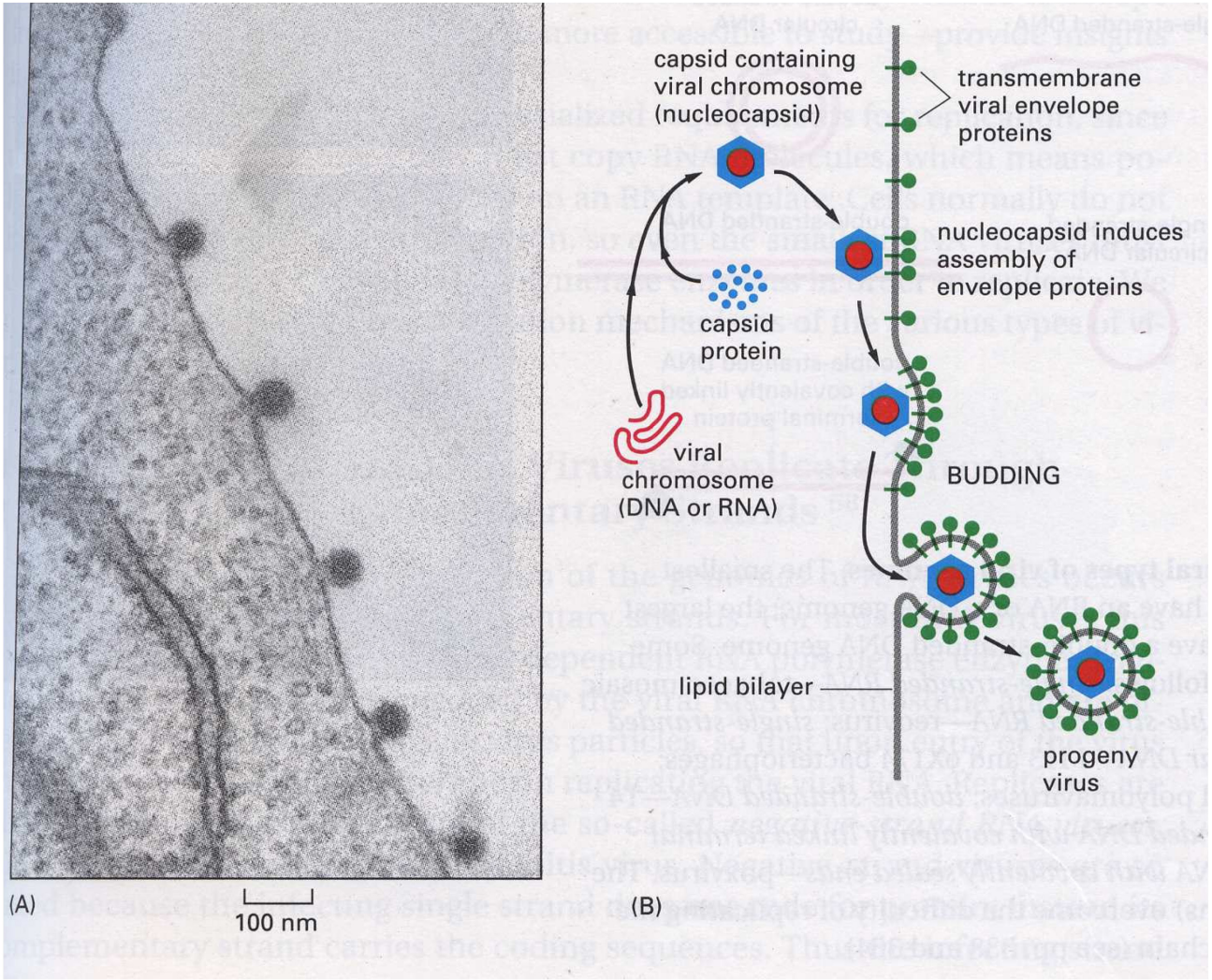
Making dsDNA, motor-packaged, viruses

(e.g., lambda)

Making ssRNA viruses:

“naked” viruses (CCMV)

“enveloped” viruses (Sindbis)

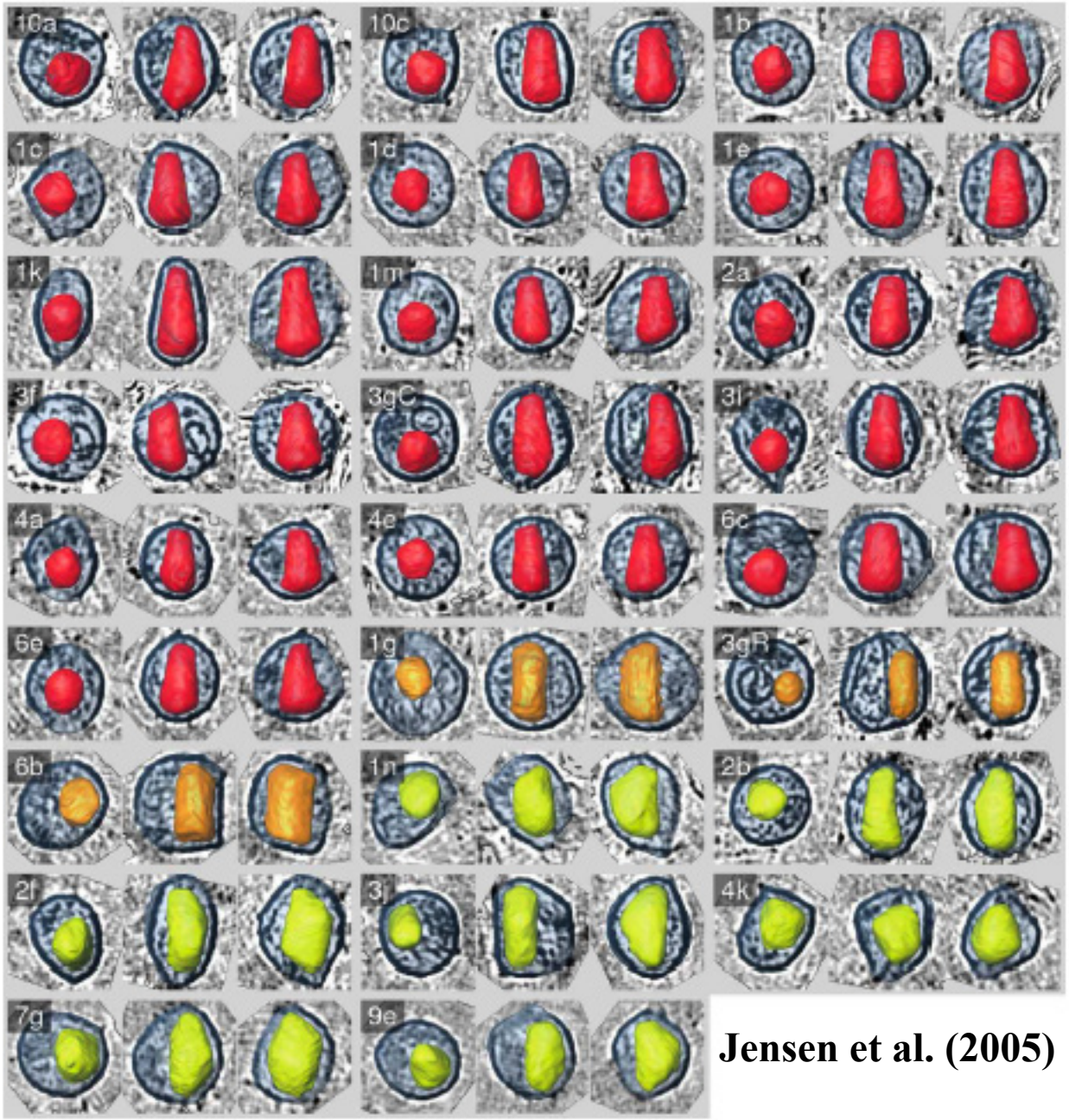


Many viral infections, like those of the alphaviruses, involve the capsid being fully formed in the host cell cytoplasm and then being wrapped by the plasma membrane and "budding".

Others are self-assembled only in interaction with the cell membrane…

Schematic showing the self-assembly of a ***retrovirus***, for which capsid formation requires simultaneous, direct, interactions between viral RNA, capsid proteins, and the host cell membrane (with associated "spike" proteins); capsids are polymorphic and weakly coupled to envelope

HIV polymorphism….



Jensen et al. (2005)

50 years ago, a virus -- Tobacco Mosaic Virus (TMV) -- was reconstituted for the first time *in vitro*,

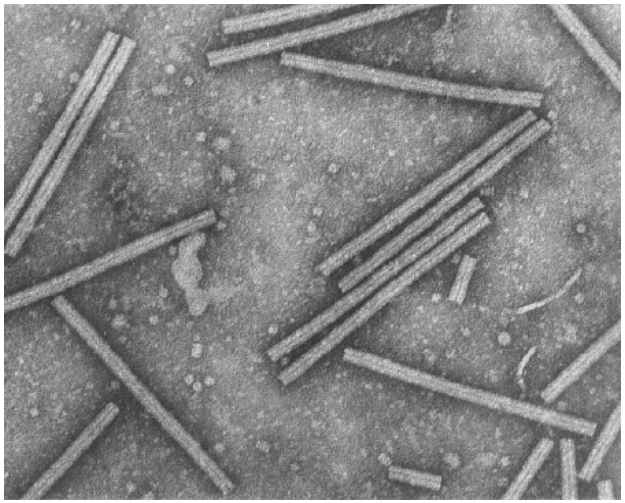
i.e., the virus was "synthesized" -- via a spontaneous self assembly process -- from its purified protein and nucleic acid components

*RECONSTITUTION OF ACTIVE TOBACCO MOSAIC VIRUS FROM ITS INACTIVE PROTEIN AND NUCLEIC ACID COMPONENTS**

By H. Fraenkel-Conrat and Robley C. Williams

VIRUS LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY

Communicated June 17, 1955



12 years later, in 1967, the first"spherical" virus was reconstituted *in vitro*: Cowpea Chlorotic Mottle Virus (**CCMV**)

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

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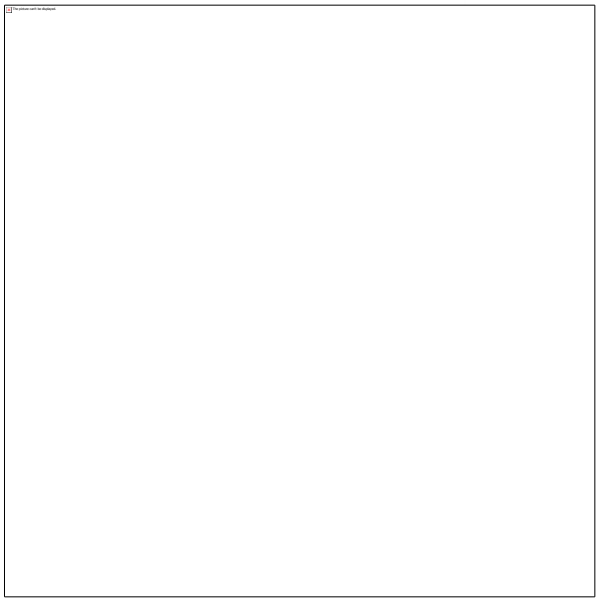
Accepted April 4, 1967



Other ssRNA plant viruses have been reconstituted, but not as "cleanly" as in the case of CCMV….

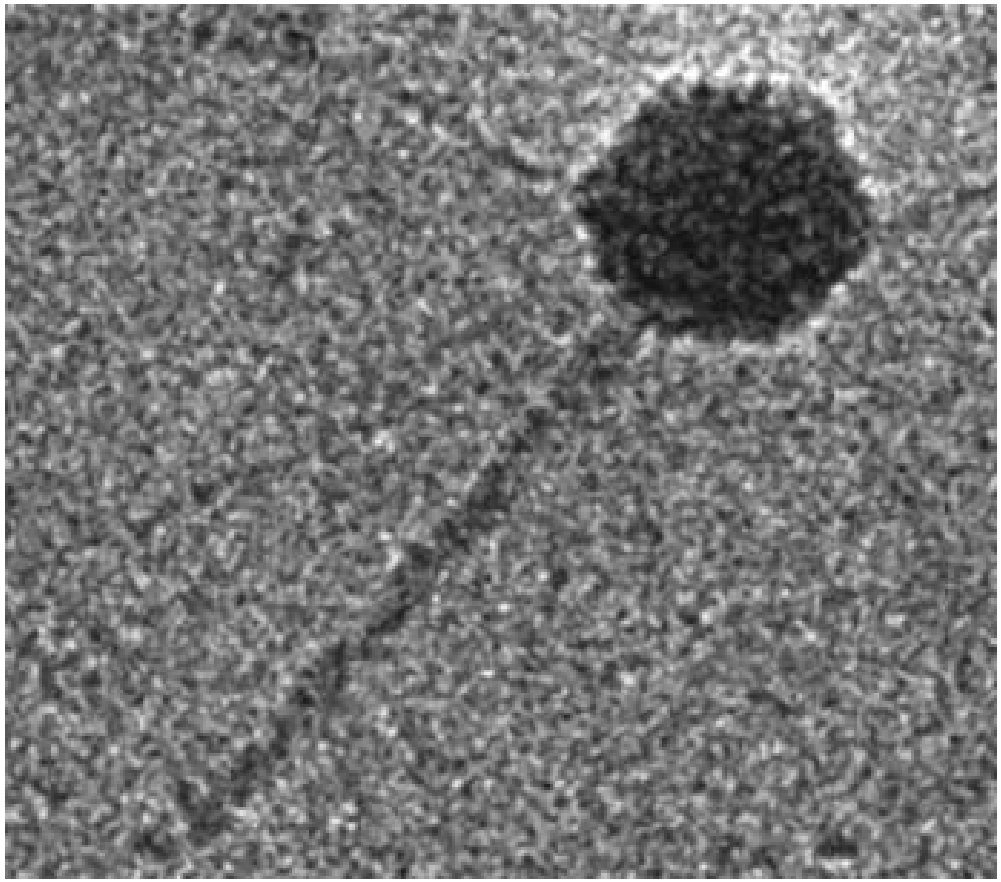
(Bancroft et al.)

Virus[b]	Particle				Protein		RNA						
	Diameter mμ	S	Particle weight ×10⁻⁶	Isoel. pt. pH	Mol. wt. ×10⁻⁵	A_{280} (0.1%)	% of particle weight	S	Mol. wt. ×10⁻⁶	A:G:U:C			
CCMV	25	88.3	4.6	3.65	19.6	1.2	24		1.1	25	26	28	20
									0.7				
									0.3[c]				
BMV	26	86	4.7	7.9	20.3	0.76	21.4	27	1.0				
								22	0.7	27	28	24	21
								14	0.3				
BBMV	26	84	4.6	5.5	20.7	0.22	22		1.1[c]	27	25	29	19
CMV (Y or S)	28	95.6	~5.5		32		18		1.0[c]				
AMV Bottom	18×58	99	7.3		32.6		~18	24	1.3				
								20	0.86				
Top a	18×36	68	3.9		32.6		18	17	0.63[c]				
TRV l[d]	26×180	295			24		5		2.3				
s[d]	26×70	198			24		5		0.9				
TMV	18×300	190	39.4	3.5	17.5	1.3	5		2.05	28	24	28	20

What about dsDNA bacterial viruses?

(They are much more complicated [!], and require that lots of work be done)

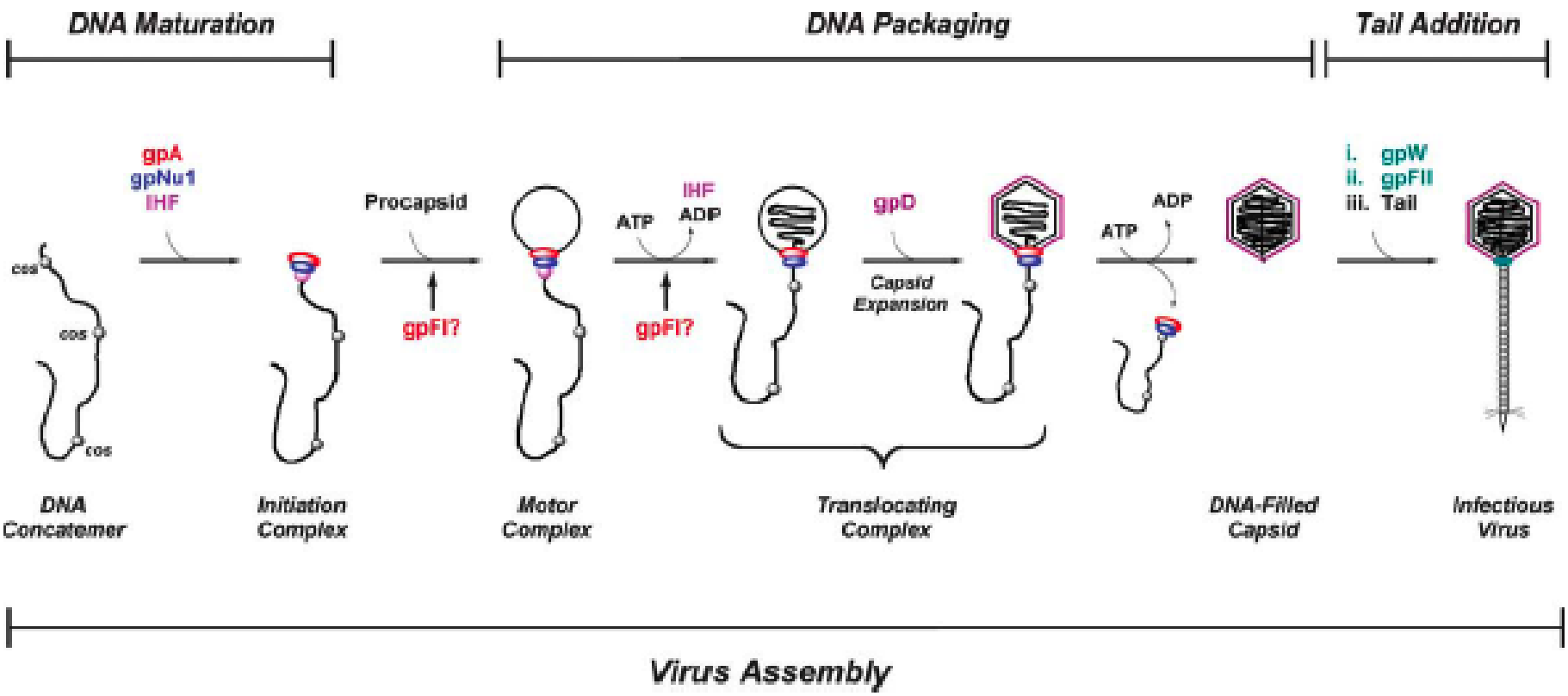
“tailed” ds DNA bacteriophage (λ, here)



single molecule of dsDNA inside

But a *large* “parts list”…

“Synthesis” of lambda (λ) [Catalano et al.]



Lambda “parts list”…

Component	Optimized concentration *in vitro*	Estimated concentration *in vivo*[a]
Terminase (2)	25 nM[b]	40 nM[b]
IHF (2)	50 nM	15 μM–30 μM
DNA (mature genomes)	3 nM	170 nM −330 nM
gpFI	0	70 μM
Procapsids (4)	30 nM	170 nM–330 nM
gpD	2 μM	150 μM
gpW	10 μM	Not reported[c]
gpFII	10 μM	8–16 μM
Tails (11)	10 nM	170 nM–330 nM

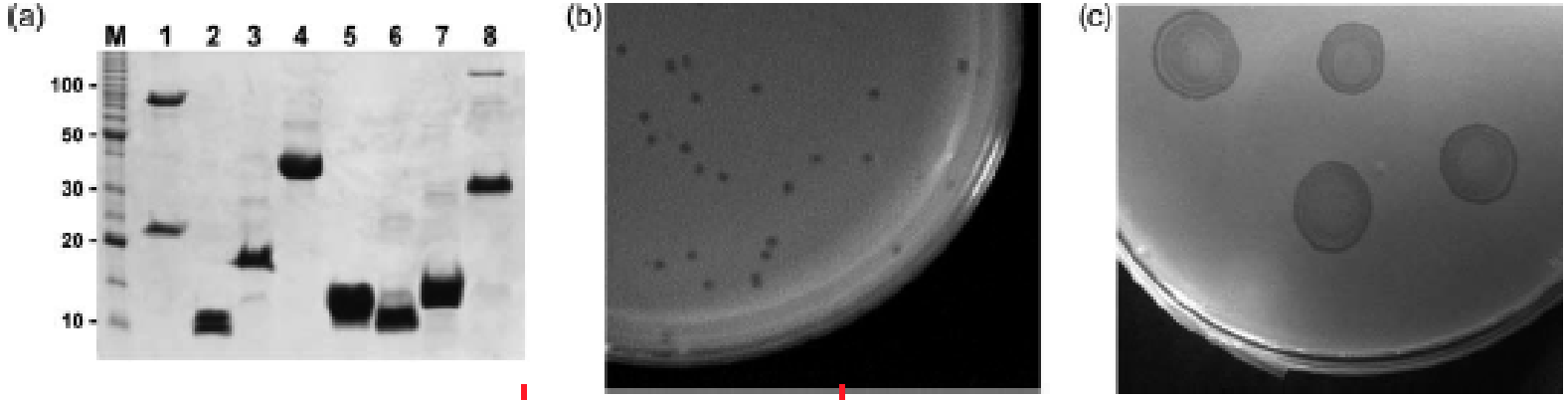
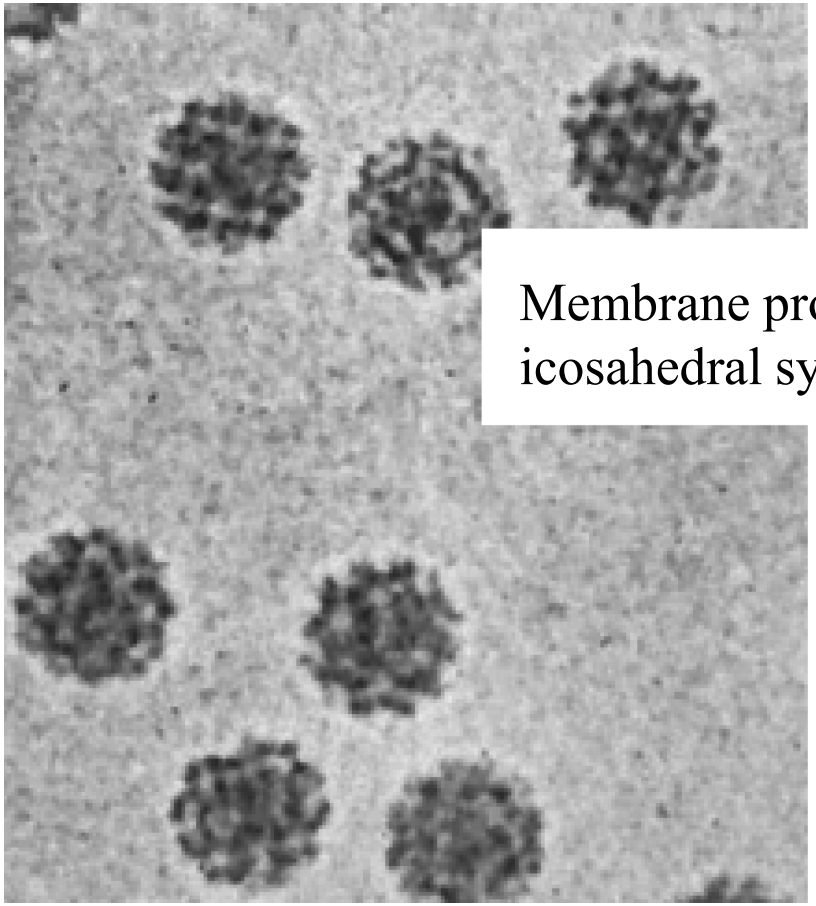
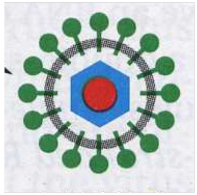


Figure 2. Virus assembly using purified components. (a) Purified proteins used in this study. M, protein markers. (1) Terminase holoenzyme, composed of gpA (72.2 kDa) and gpNu1 (20.4 kDa) subunits, (2) *E. coli* integration host factor (IHF), composed of α (9.5 kDa) and β (8.5 kDa) subunits, (3) gpFI, (4) procapsids, composed of gpE, gpB*, pX1 and pX2 proteins; the gpE major capsid protein (37 kDa) is clearly visible, (5) gpD, (6) gpW, (7) gpFII and (8) viral tails, composed of gpZ, gpU, gpV, gpG, gpT, gpH, gpM, gpL, gpK, gpI, and gpJ proteins; the gpV major tail protein (31 kDa) and the gpJ and gpH minor tail proteins (135 kDa and 80 kDa, respectively) are clearly visible. (b) Plaque assay shows viral plaques in a bacterial lawn. Virus was assembled *in vitro* as described in Materials and Methods and plated on *E. coli* LE392 cells. (c) *In vitro* assembled virus lysogenizes *E. coli*. Virus was assembled *in vitro* and used to infect *E. coli* as described in Materials and Methods. Lysogenic bacteria were isolated from phage-infected cells and induced to yield a zone of lysis in the bacterial lawn.[36]

What about an *enveloped* animal virus?
Can we make one?

E.g., Semliki Forest Virus (SFV)





Membrane proteins have same icosahedral symmetry as capsid!

single molecule of ssRNA inside

Baker et al. (1999)

ADVANTAGES OF THE ALPHAVIRUSES (SFV, Sindbis…)

1. Simplest ("the H-atom") of enveloped animal viruses:
   * Nothing is inside nucleocapsid but a *single* RNA molecule
   * Only a single capsid protein makes up the capsid shell
   * Membrane proteins (two) have full symmetry of capsid

2. Genetically related to bromoviruses (e.g., "our" CCMV), the simplest of the plant viruses (the simplest of *all* viruses?)

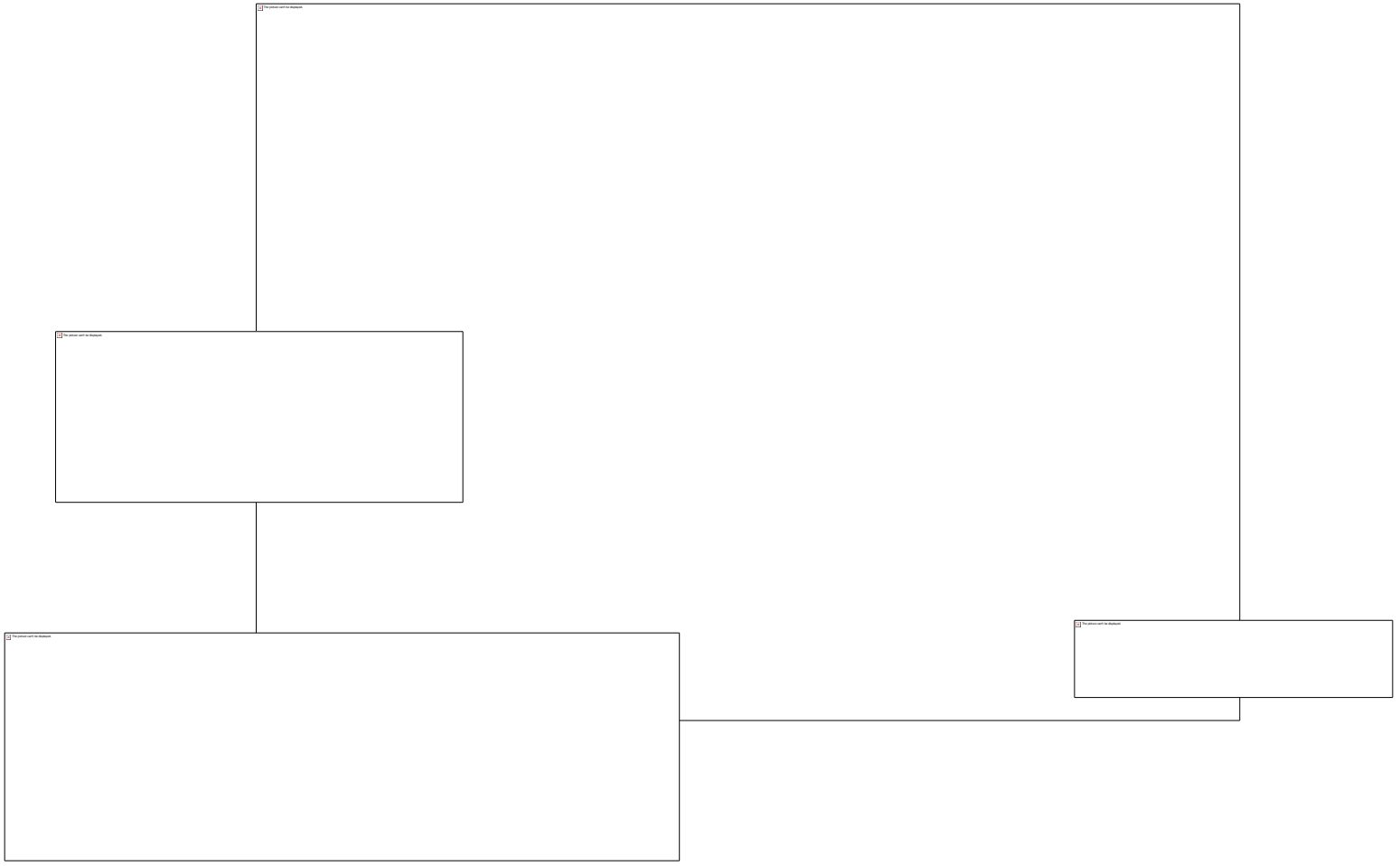
3. cDNA clones are available for RNA genomes

4. Natural expression vectors, since structural genes are under control of separate and *strong*, "subgenomic", promoter

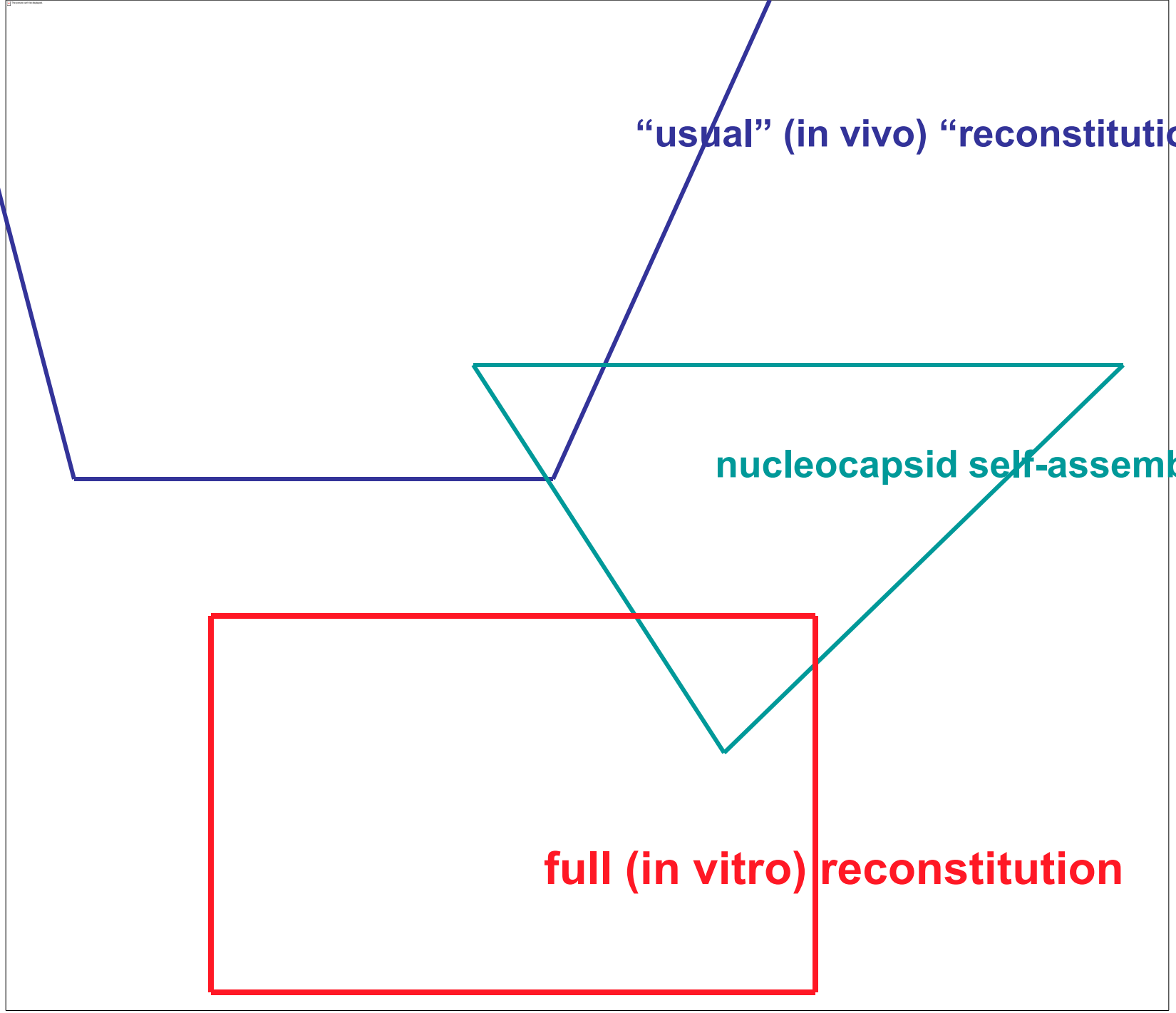
5. Purified viruses can be disassembled by detergent into intact nucleocapsids and envelopes

…NATURAL CANDIDATE FOR FIRST ANIMAL VIRUS TO BE RECONSTITUTED *IN VITRO*

The “parts list” of an enveloped virus…



Putting the parts together…


“usual” (in vivo) “reconstitution”
nucleocapsid self-assembly
full (in vitro) reconstitution

Alphavirus RNA is 11,700 nt, and -- like the bromovirus RNAs -- can be self-assembled into nucleocapsids by *in vitro* mixing with capsid protein in buffer

Sindbis: Virus of Choice

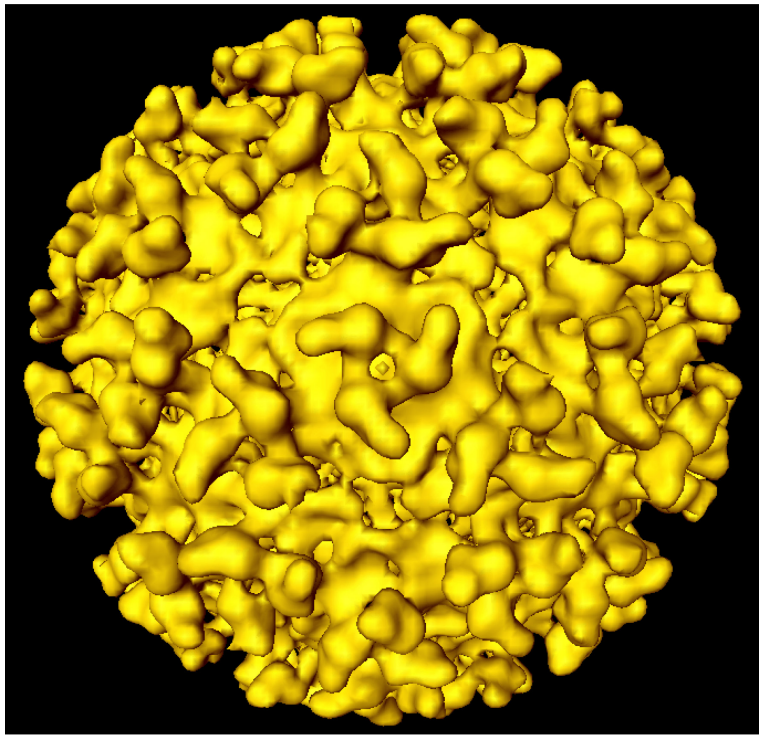
- Arguably the simplest animal virus: ***two-stage assembly*** life cycle

- Its nucleocapsid has been self-assembled *in vitro*

- One-to-one correspondence between each capsid protein and spike protein

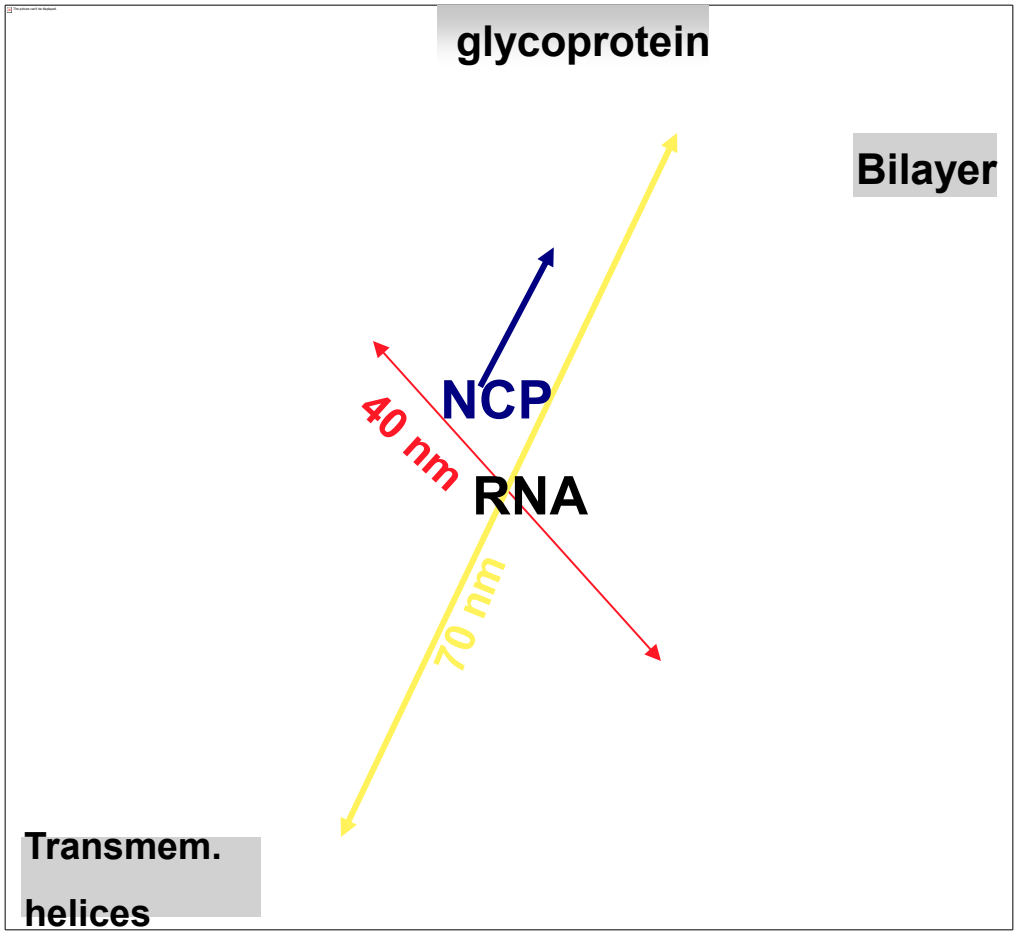
- Strong interaction between capsid and spike proteins established *in vitro*

- Relatively benign



Sindbis reconstruction from cryo EM images (R. Kuhn)

Sindbis cross-section



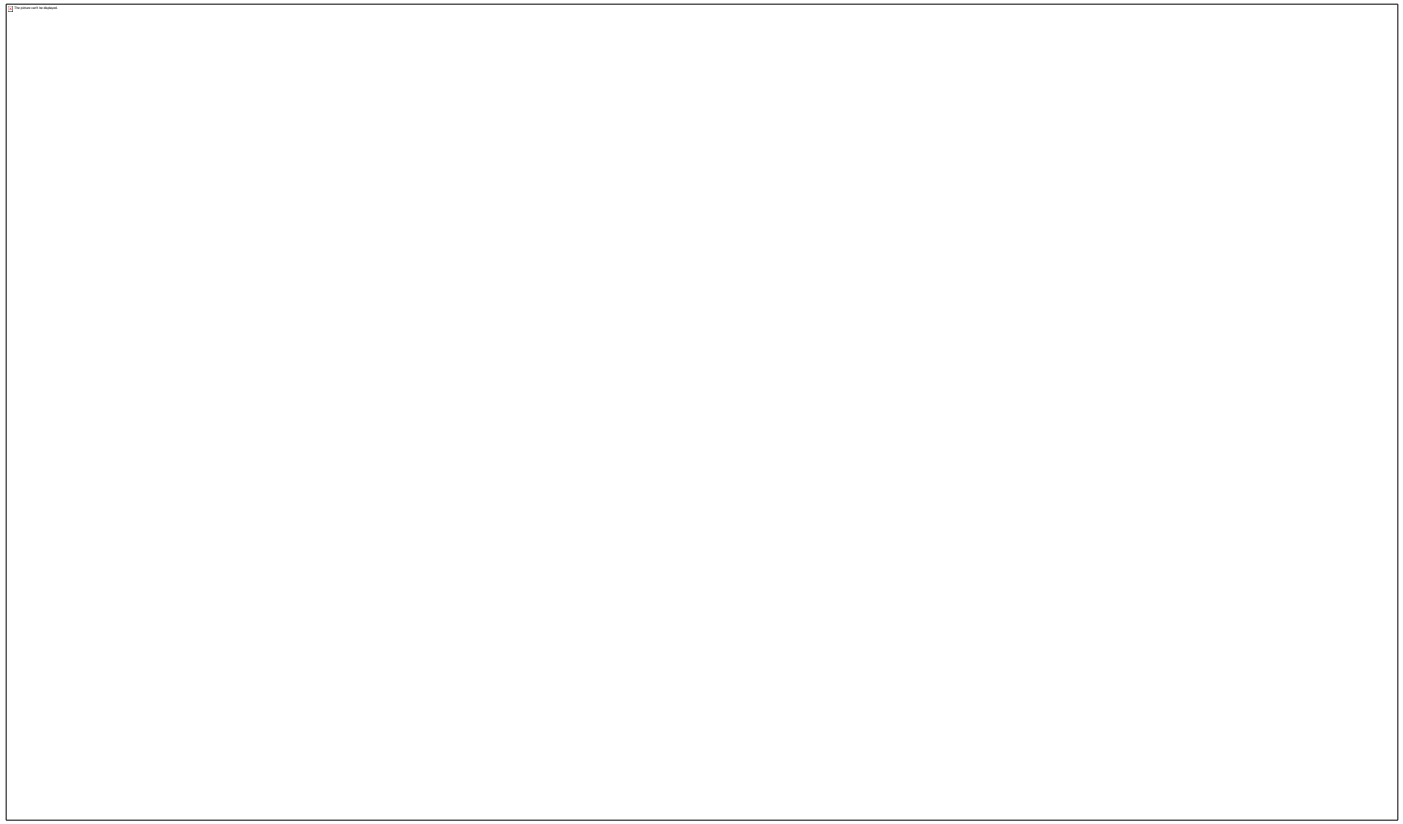
Zhang et al. (2002)

We “peel off” envelope from nucleocapsid using detergent; then re-assemble it around the NC by removing detergent

Peeling off the membrane is possible!

Dissociation of the viral envelope from the nucleocapsid is stepwise (for SFV and Triton X-100 system)

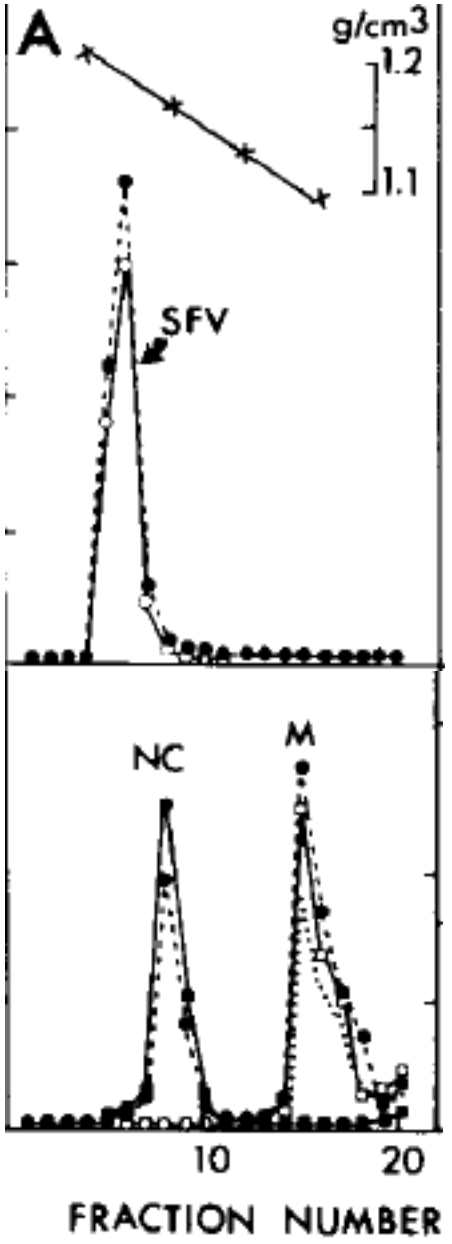
Helenius and Söderland, *Biochimica et Biophysica Acta*, 307 (1973), 287-300.

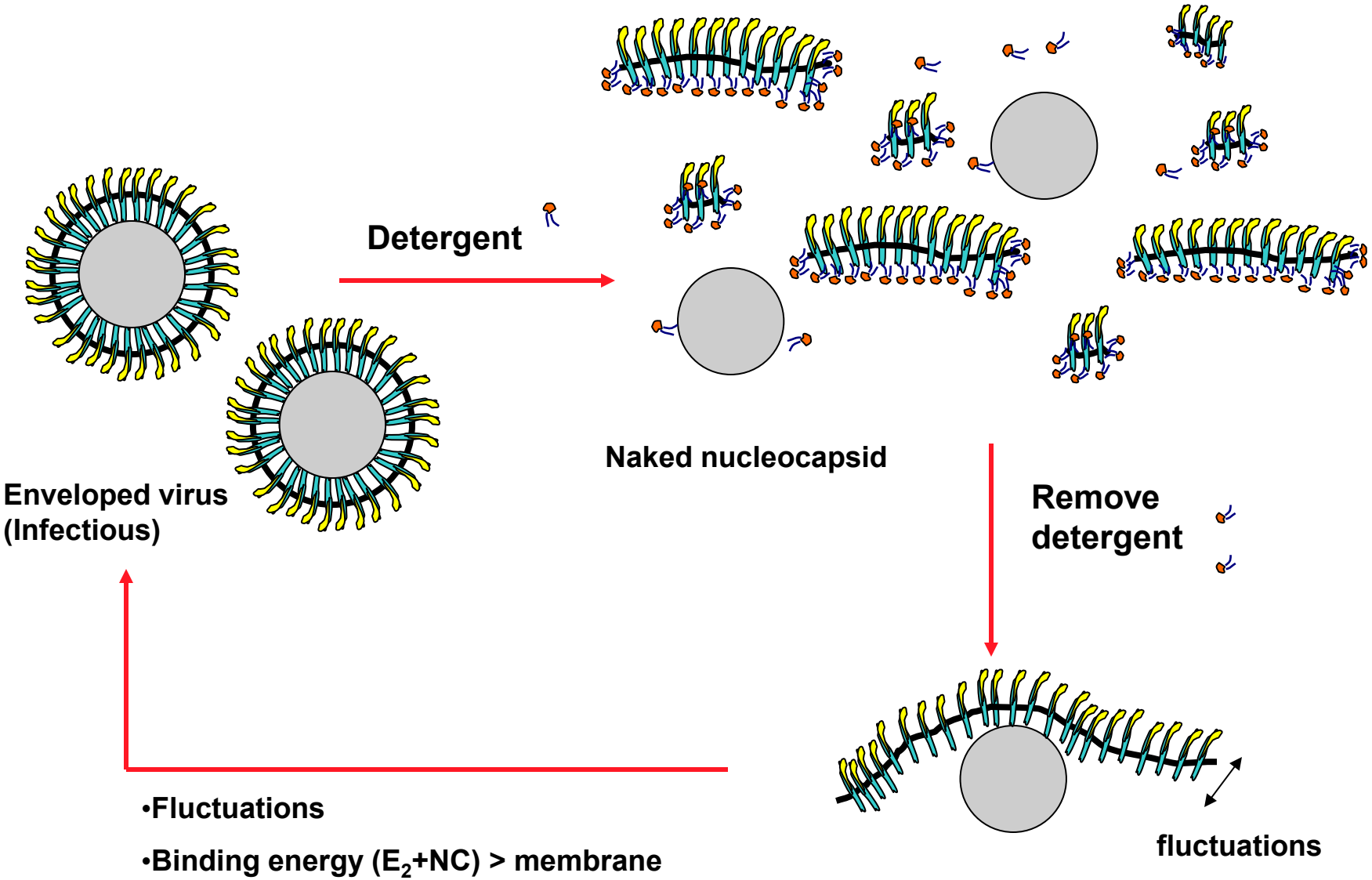


BREAKING DOWN THE VIRUS INTO ITS NUCLEOCAPSID (NC) AND MEMBRANE (M) COMPONENTS

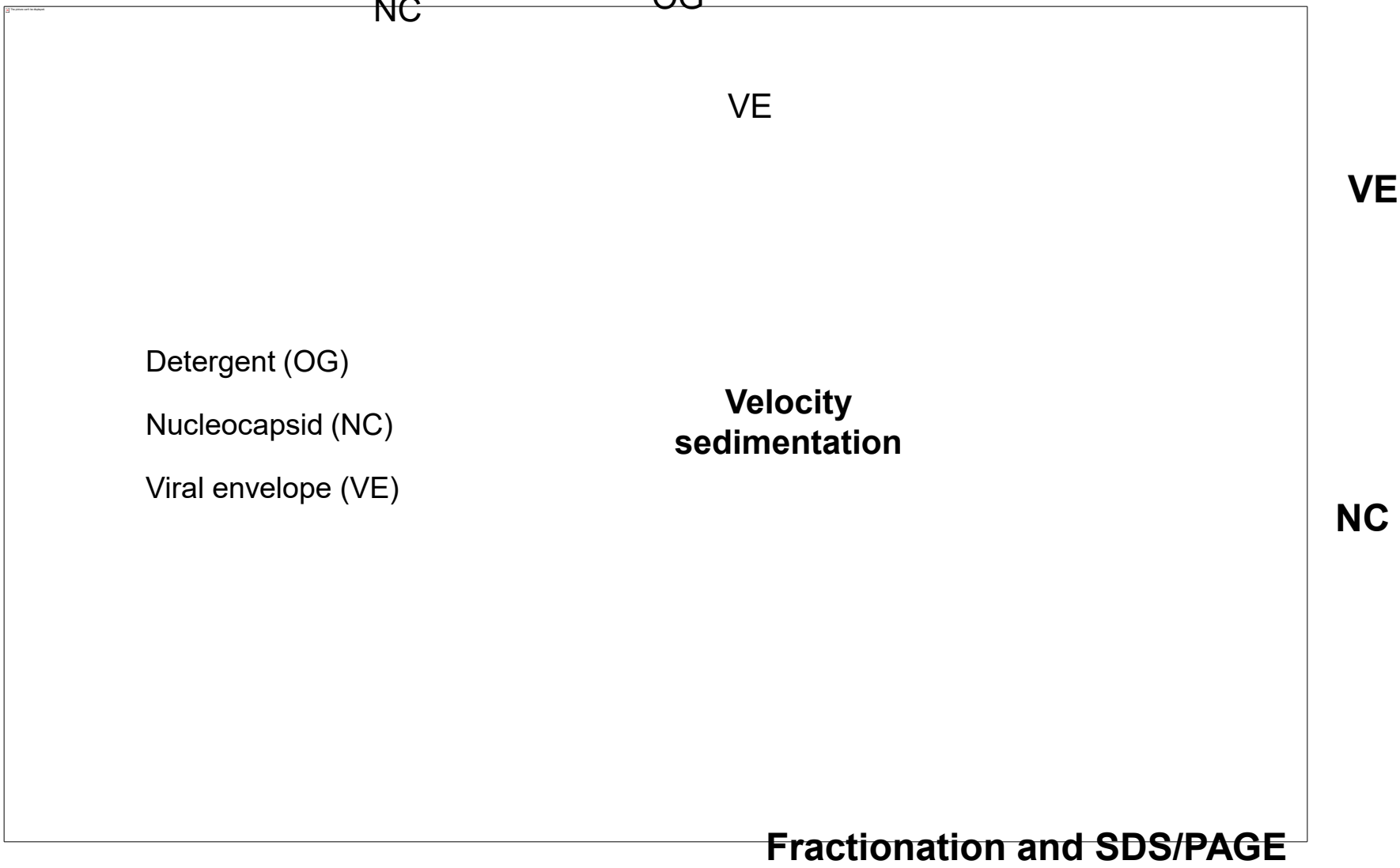
DETERGENT TREATMENT OF SFV OR SINV VIRIONS:

2 distinct bands (NC and M) arise in sucrose gradient centrifugation when just the right amount of detergent is added




Detergent
Naked nucleocapsid
Enveloped virus
(Infectious)
Remove
detergent
fluctuations
•Fluctuations
•Binding energy (E_2+NC) > membrane bending energy

Physical separation of nucleocapsids and viral membranes after detergent treatment



- Can also separate the viral envelopes from nucleocapsids by equilibrium sedimentation
- Mix the purified components on centricons, to rid of detergent and allow intact virions to reform
- Show that infectious particles have been reconstituted from ***non***-infectious components

Determine number of infectious virions before and after detergent treatment, and then after detergent removal

Plaque assay: allows us to count ***individual*** infectious virions

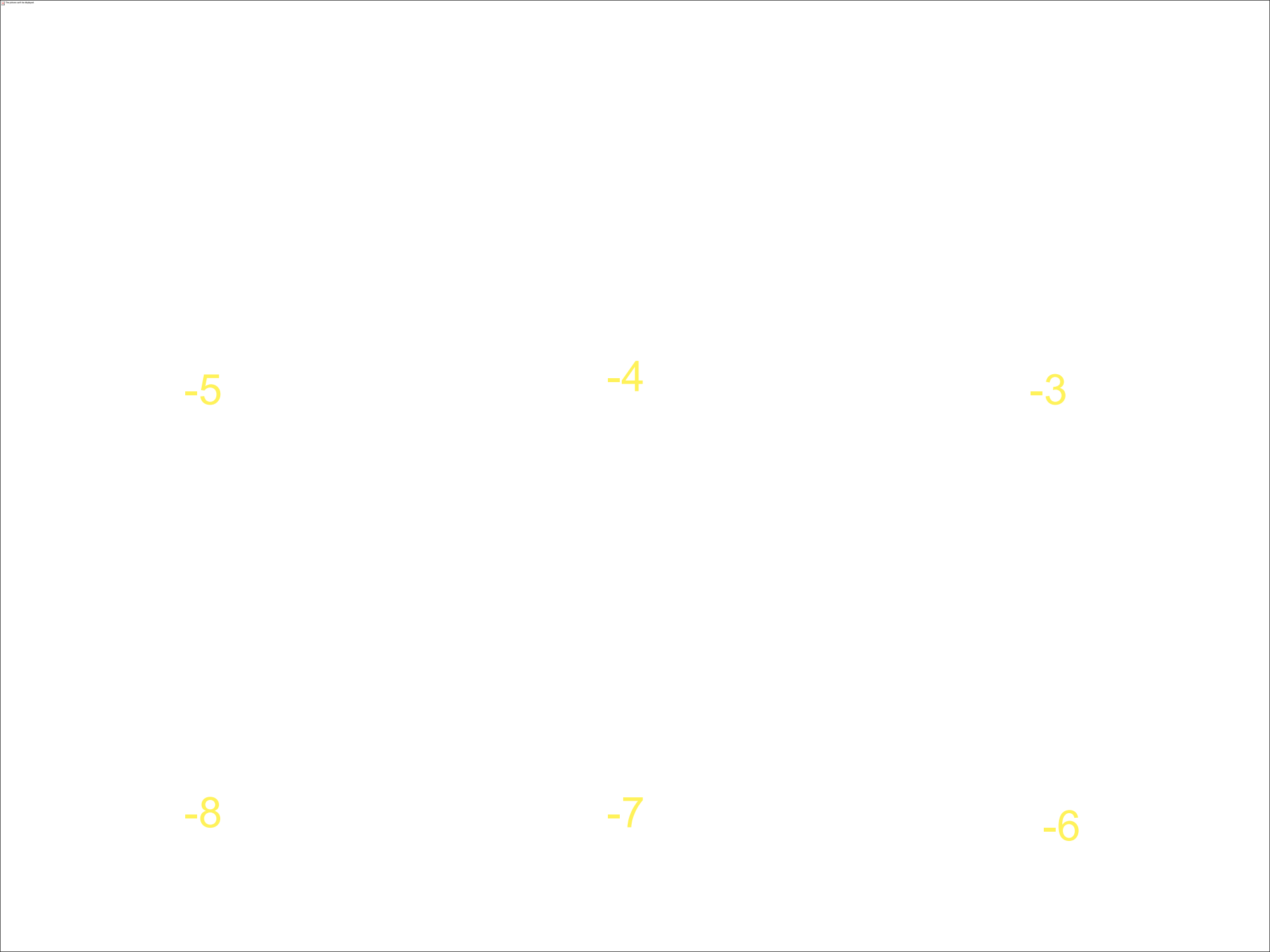
Plaque: a region of infected cells, detectable by naked eye

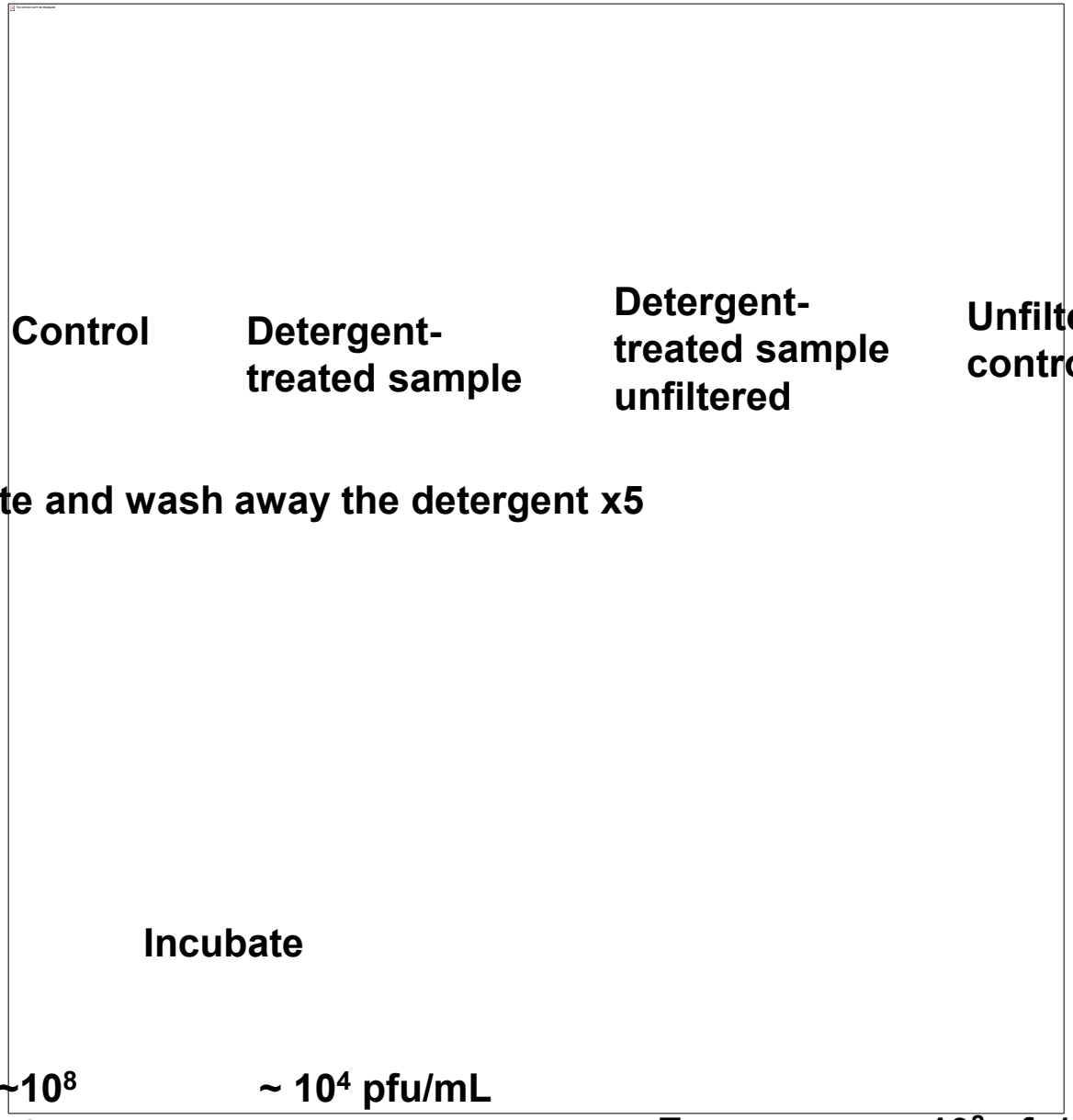
Substrate: a monolayer of host (2-day-old BHK) cells

Need to be able to count the cells that are infected: serial (powers of ten) dilutions lead to small (distinct, and hence countable) numbers (1's, 10's, 100's) of infectious virions (hence infected cells: plaques) per plate

Only interested in the number of infected cells that were *initially* infected by the virus: agarose is added to decrease the mobility of the virions in the medium

Plaques are visualized by a stain


-5
-4
-3
-8
-7
-6



Control

Detergent-treated sample

Detergent-treated sample unfiltered

Unfiltered control

Concentrate and wash away the detergent x5

Incubate

Plaque assay:

~10^8 pfu/mL

~ 10^4 pfu/mL

Zero

~ 10^8 pfu/mL

Partial recovery !!!

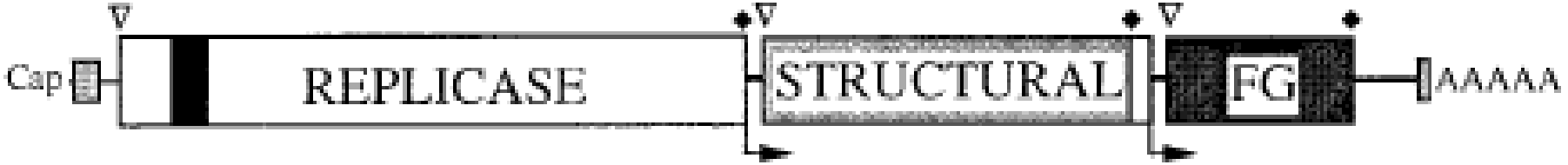
Future experiments

Increase the number of dissociated components -- nucleocapsids (NCs) and viral envelopes (VEs) -- and the yield of reconstituted virions

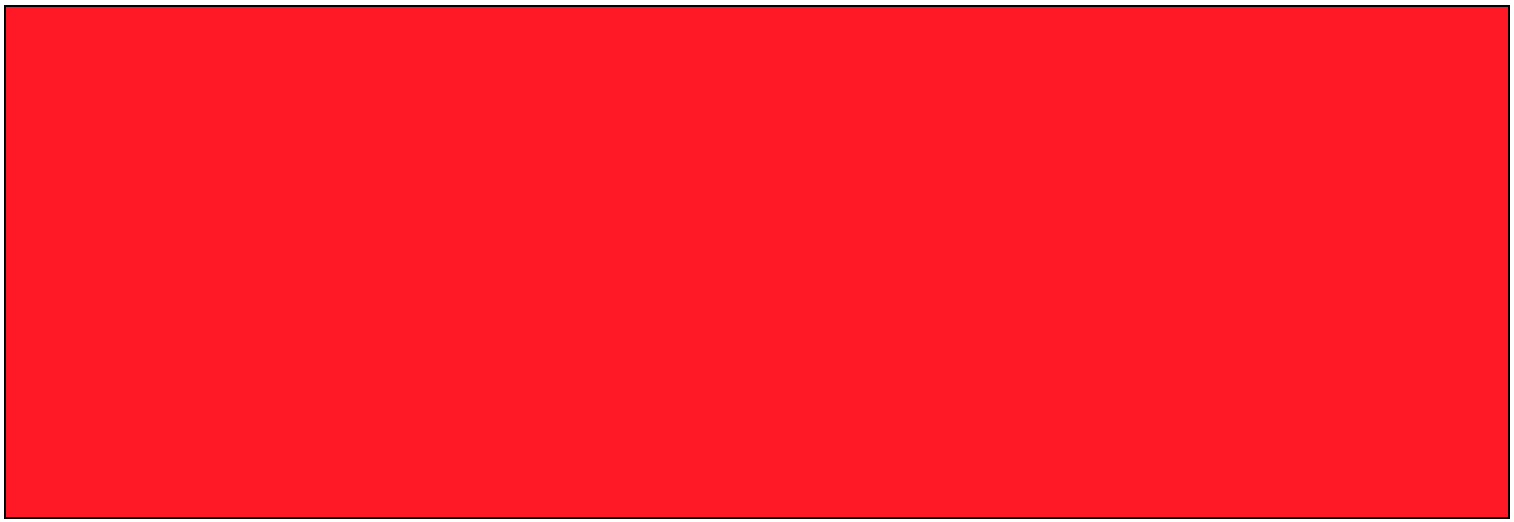
Use VEs to make giant unilamellar vesicles (GUVs) -- in a solution of NCs -- and watch infectious particles form as virions "bud" out of the vesicles

Make recombinant NCs *in vitro* (taking advantage of the special Sindbis genome…) and add them to VEs

The key: a second, subgenomic, promoter….



Of special interest is the fact that foreign genes (FG) can be added under the conrrol of a second subgenomic promoter, leaving fully intact the original genes. Here the solid diamonds denote stop codons for translation, and the open triangles denote promoter sequences. [From Frolov et al., PNAS, 1996]




reconstitution

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