Self-Assembly of Viral Capsids around ssRNA

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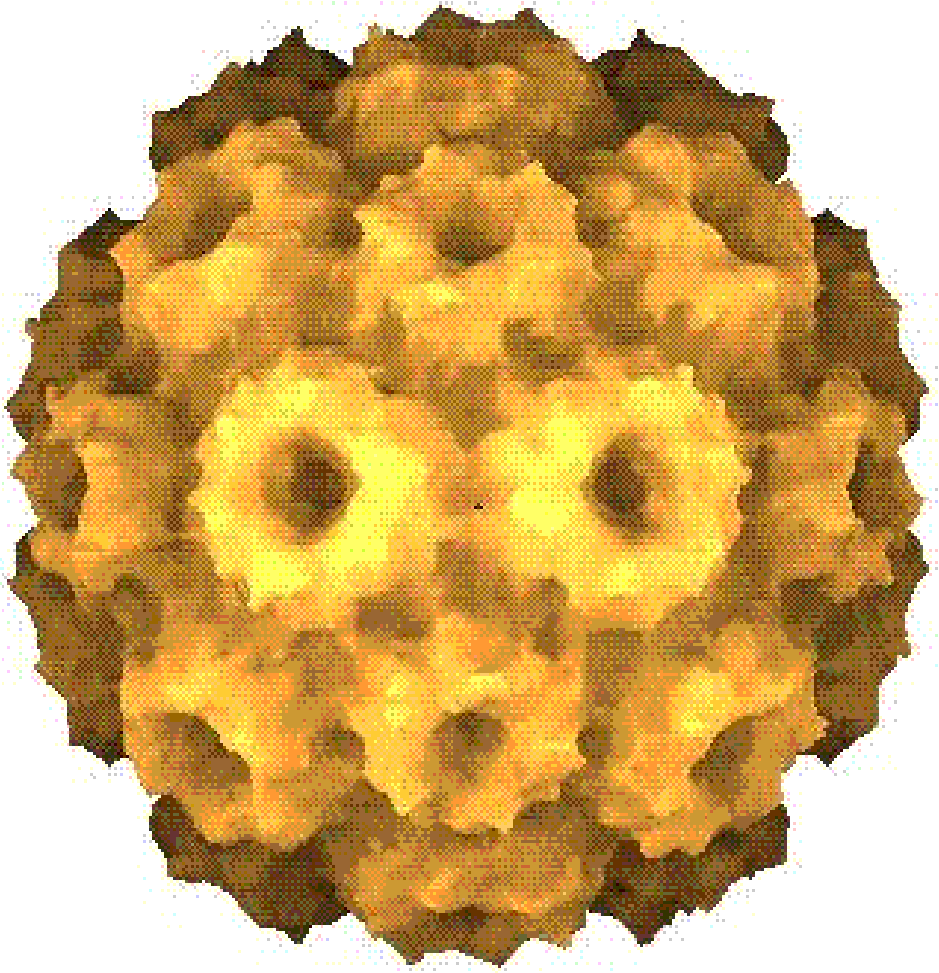
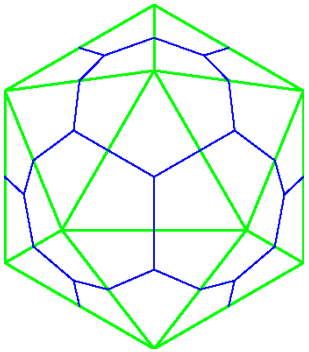


CCMV
Cowpea chlorotic mottle virus

CCMV CAPSID PROTEIN CAN FORM ICOSAHDERAL CAPSIDS CONTAINING 120, 180 OR 240 PROTEINS

T= 2

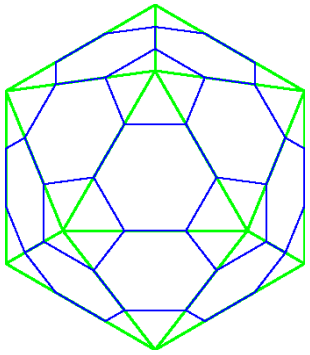
120 proteins



Pseudo K-K structure

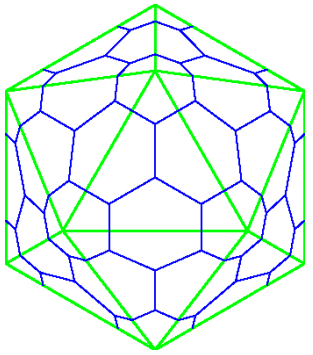
T= 3

180 proteins



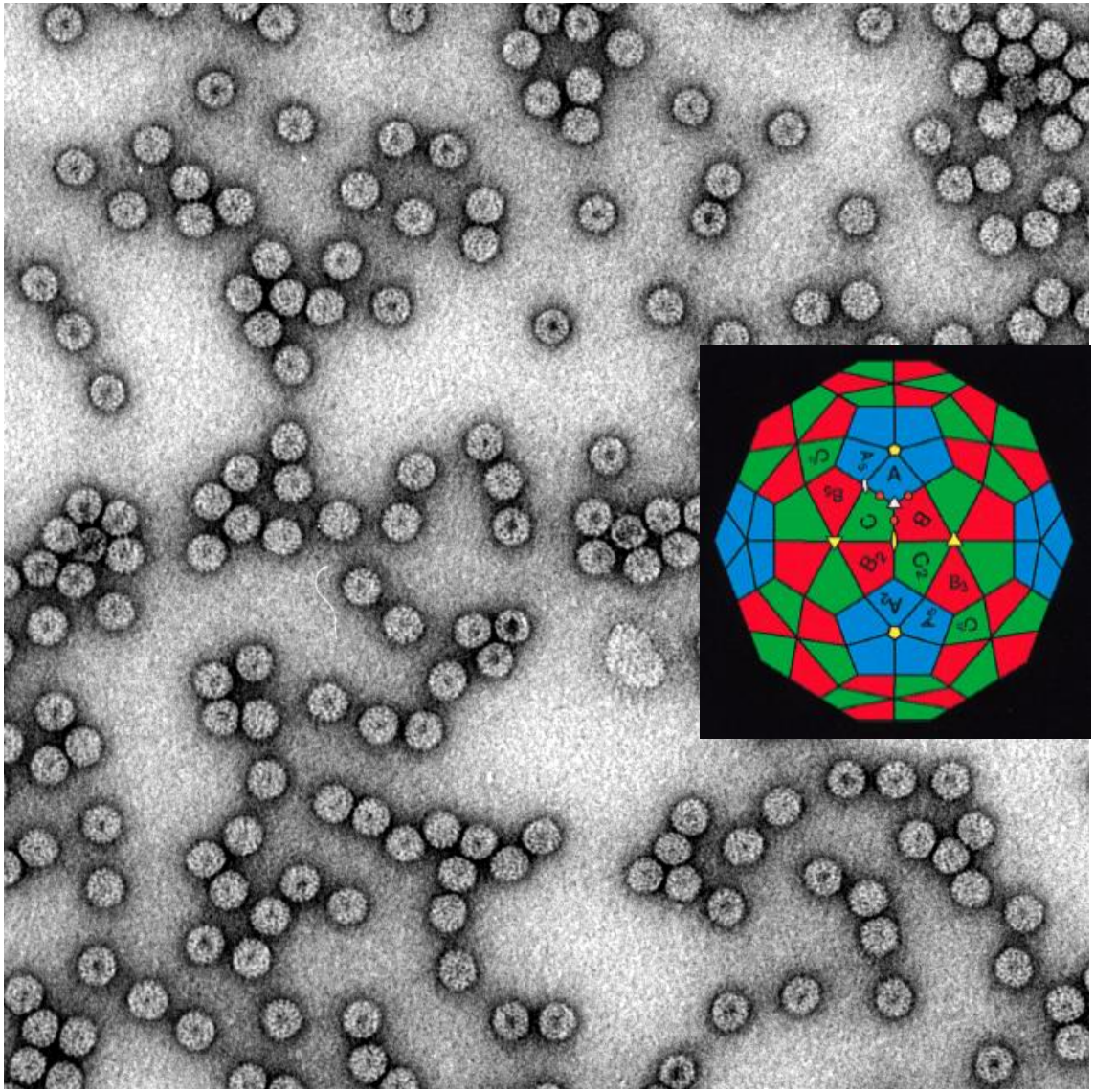
T= 4

240 proteins



Number of proteins = 60*T*

CCMV



WT Capsid

T=3 composed of 60x3 = 180 copies of a single protein arranged into

12 pentamers

20 hexamers

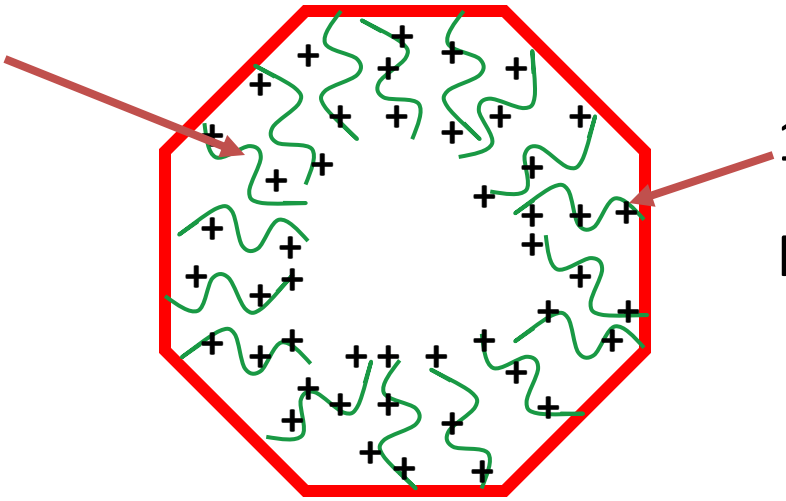
Genome

Multipartite, 4 RNAs
Capsids contain either RNA1, RNA2 or RNA3+RNA4 – 3000 nt/capsid

BASIC PROTEIN TAILS PROTRUDE INTO CAPSID --A TOTAL OF 1800 + CHARGES

INTERACT WITH THE RNA PHOSPHATE BACKBONE

180 PROTEINS



10 + CHARGES

PER PROTEIN

CCMV ASSEMBLY

MAKE-A-VIRUS KIT

PARTS LIST:

1 CCMV RNA (3000 NT LONG)

1 CAPSID PROTEIN (190 RESIDUES LONG)

MAKE-A-VIRUS KIT

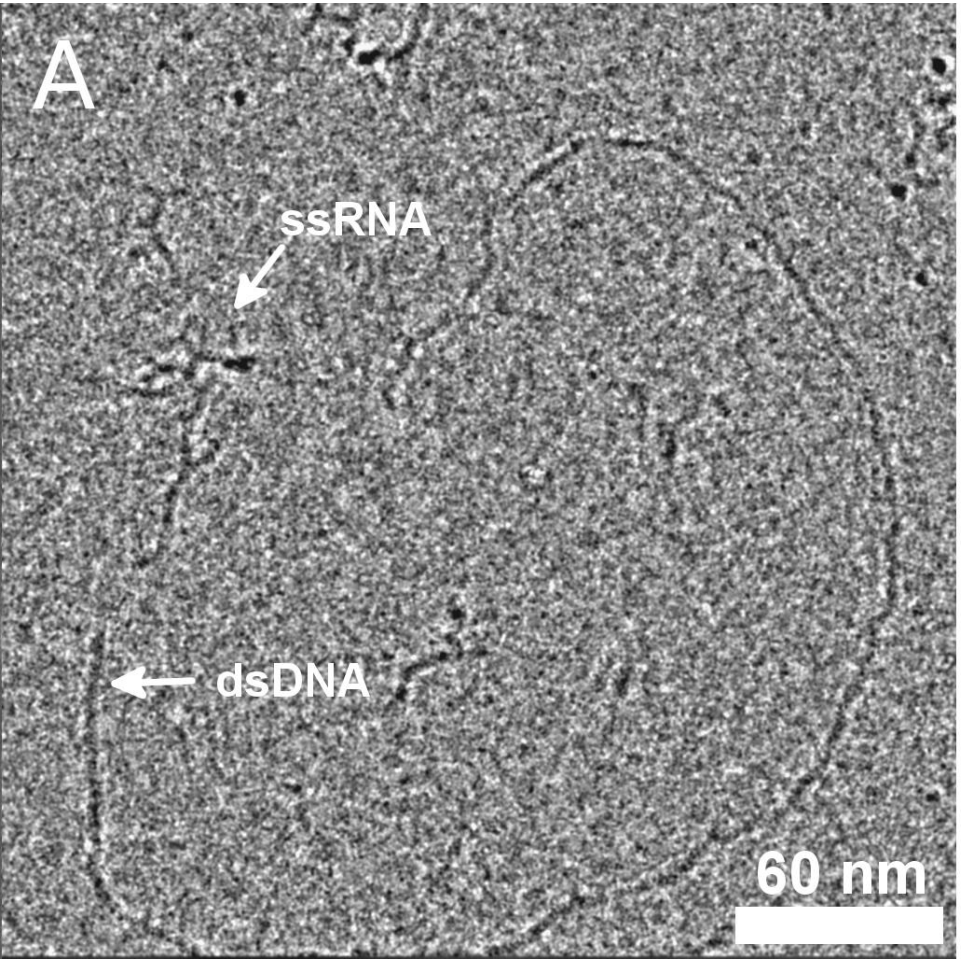
ASSEMBLY INSTRUCTIONS

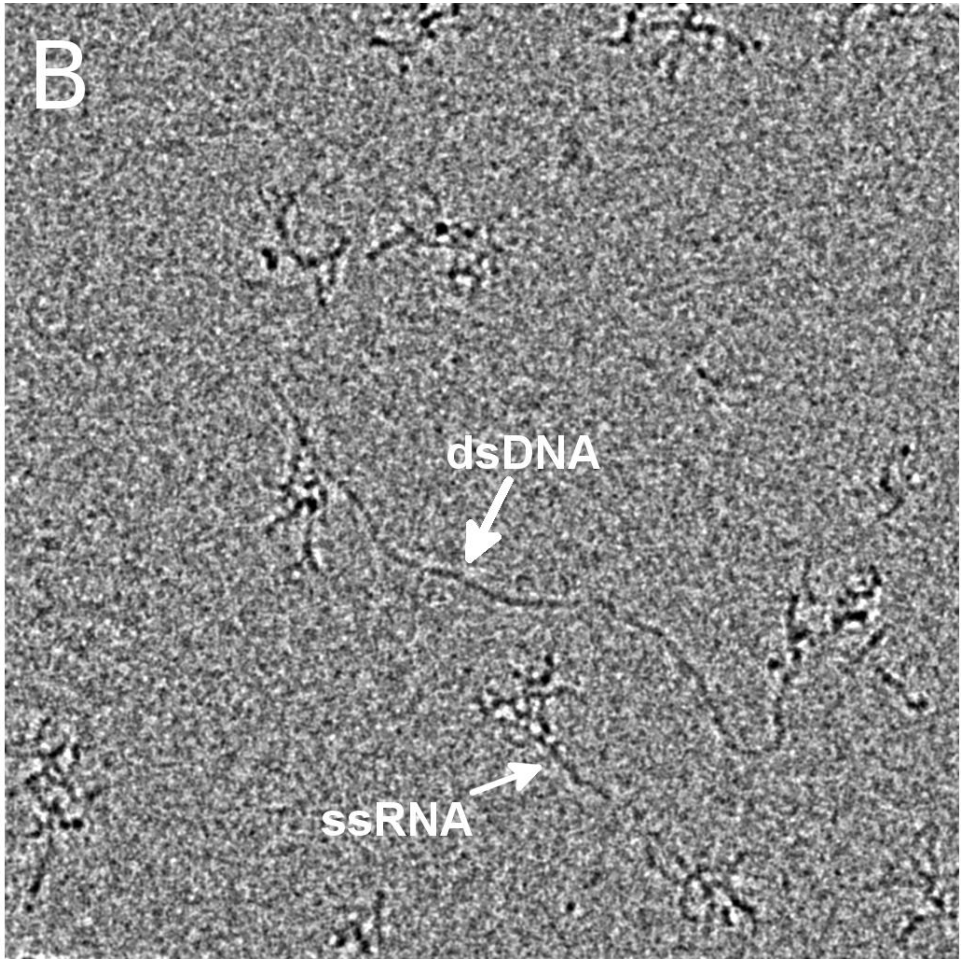
1. MIX RNA AND PROTEIN IN BUFFER SOLUTION at pH 7

2. HAVE A CUP OF COFFEE

3. COLLECT VIRUS

COMPARISON BETWEEN 2117 nt *ss*RNA AND 2141 bp *ds*DNA CRYO-EM (A. Gopal)





TE buffer pH 7.4

with Mg^{2+}

CCMV and Self-Assembly

- CCMV is one of the viruses that can self-assemble *in vitro*…
- packaging its genome, 3000 nt *ss*RNA...
  - or RNAs from other viruses…
    - other anionic polymers…
    - functionalized quantum dots…
    - nanoemulsion droplets…

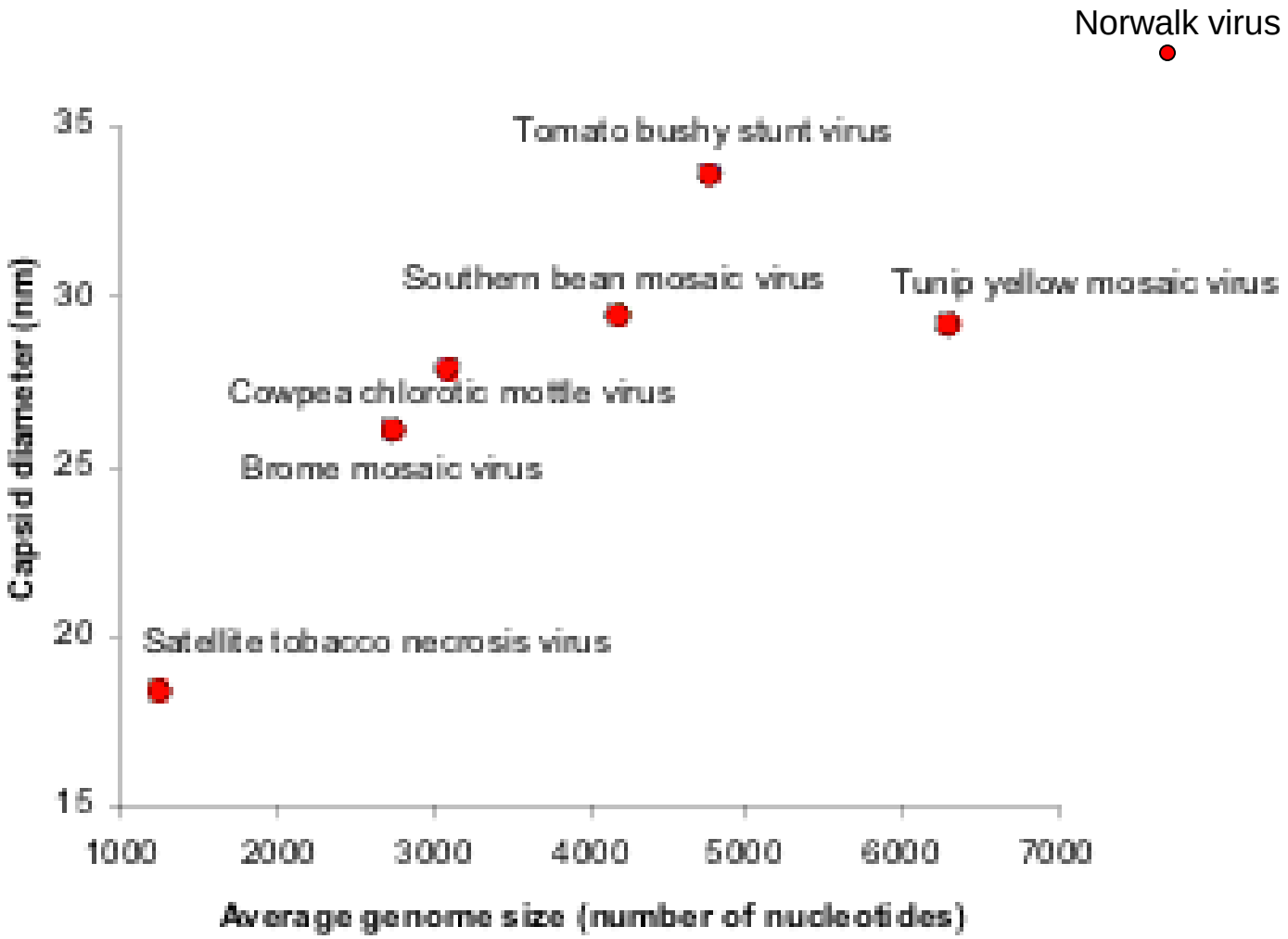
CCMV and Self-Assembly

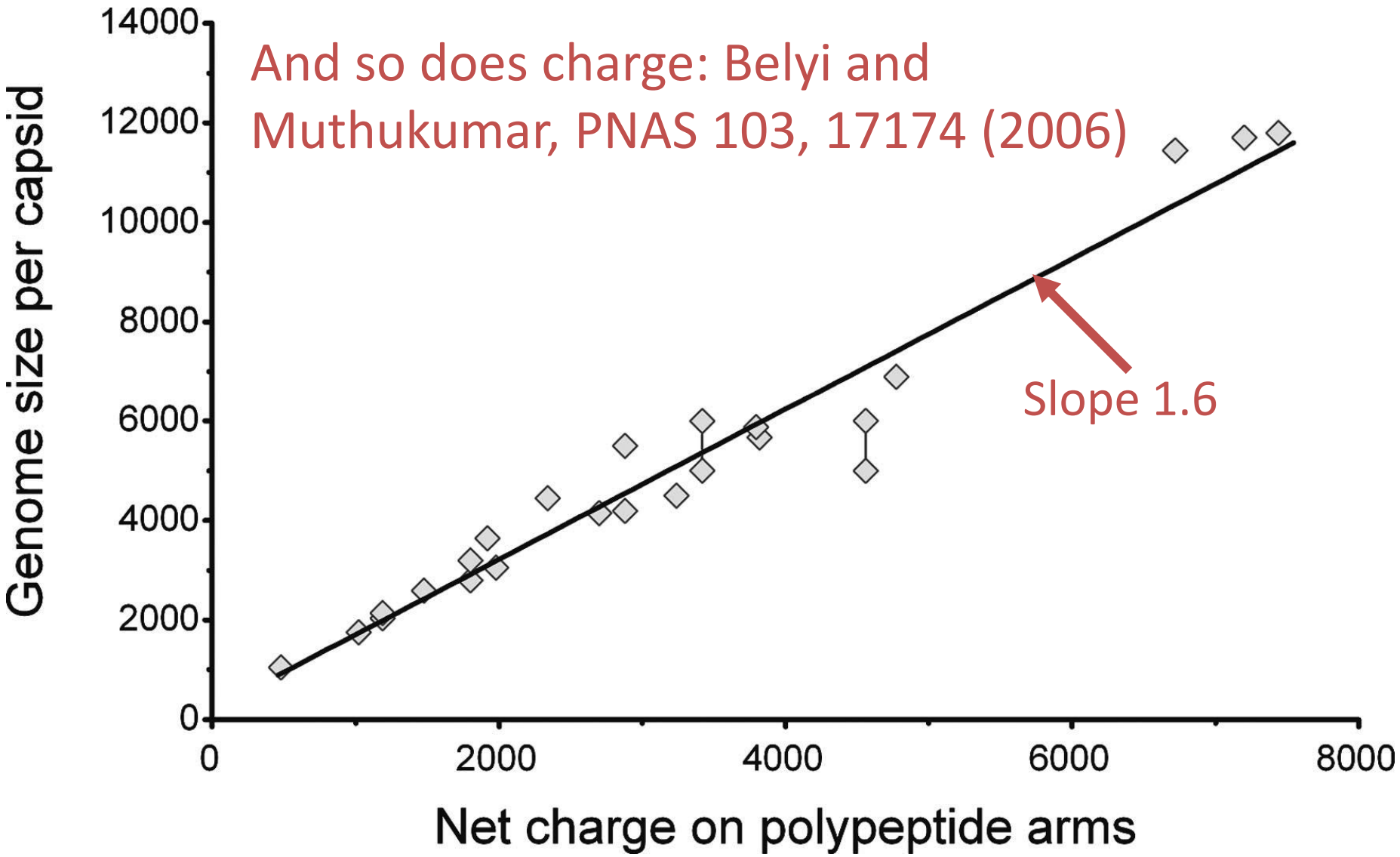
- CCMV is one of the viruses that can self-assemble *in vitro*…
- packaging its genome, 3000 nt *ss*RNA...
- or RNAs from other viruses…
- other anionic polymers…

- HOW DOES ASSEMBLY TAKE PLACE?

WHAT DETERMINES THE SIZE OF A VIRUS?

GENOME SIZE PLAYS A ROLE…




And so does charge: Belyi and Muthukumar, PNAS 103, 17174 (2006)
Slope 1.6
Genome size per capsid
14000
12000
10000
8000
6000
4000
2000
0
0
2000
4000
6000
8000
Net charge on polypeptide arms

Study Packaging of RNAs by CCMV Capsid Protein

How does capsid size vary with RNA length?

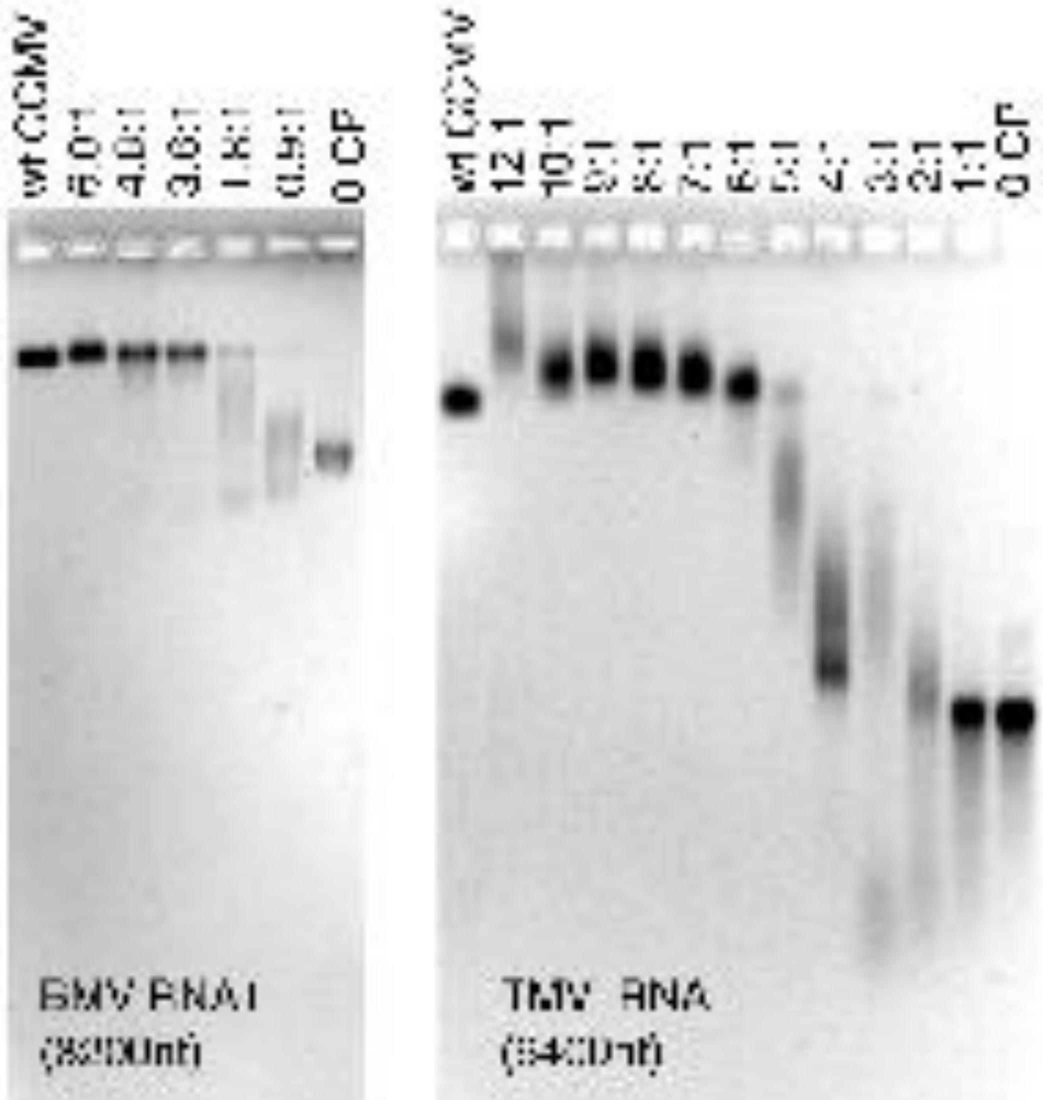
How does efficiency of packaging depend on RNA length?

Study Packaging of RNAs by CCMV Capsid Protein

- BMV (Brome mosaic virus) RNA1 (3200 nt)
- Truncated BMV RNA1s (at 3' end) (140 to 2000 nt)
- Ligated BMV RNAs 1+ 3 (4500, 5300 nt)
- TMV RNA (6400 nt)
- Sindbis Replicon (9000 nt)
- Sindbis (12,000 nt)

ASSEMBLY PROTOCOL

- BRING CAPSID PROTEIN AND RNA TOGETHER IN BUFFER SOLUTION (*p*H 7.2) AT DIFFERENT MOLAR RATIOS
- AFTER A WAIT OVERNIGHT FOR ASSEMBLY, ANALYZE PRODUCTS BY GEL ELECTROPHORESIS AND ELECTRON MICROSCOPY


wt CCMV
0 CP
wt CCMV
12:1
10:1
1:1
0 CP
BMV RNA1
TMV RNA

"Magic Ratio"

The number ratio of proteins required for complete encapsidation of the RNAs ranges from 15 to 1200 but in every case complete encapsidation occurs at the same *weight* ratio $w_P/w_{RNA} = 6$, *independent of RNA length*

RATIO CORRESPONDS TO CHARGE NEUTRALITY

moles positive charge $N^+ = \xi N_P$

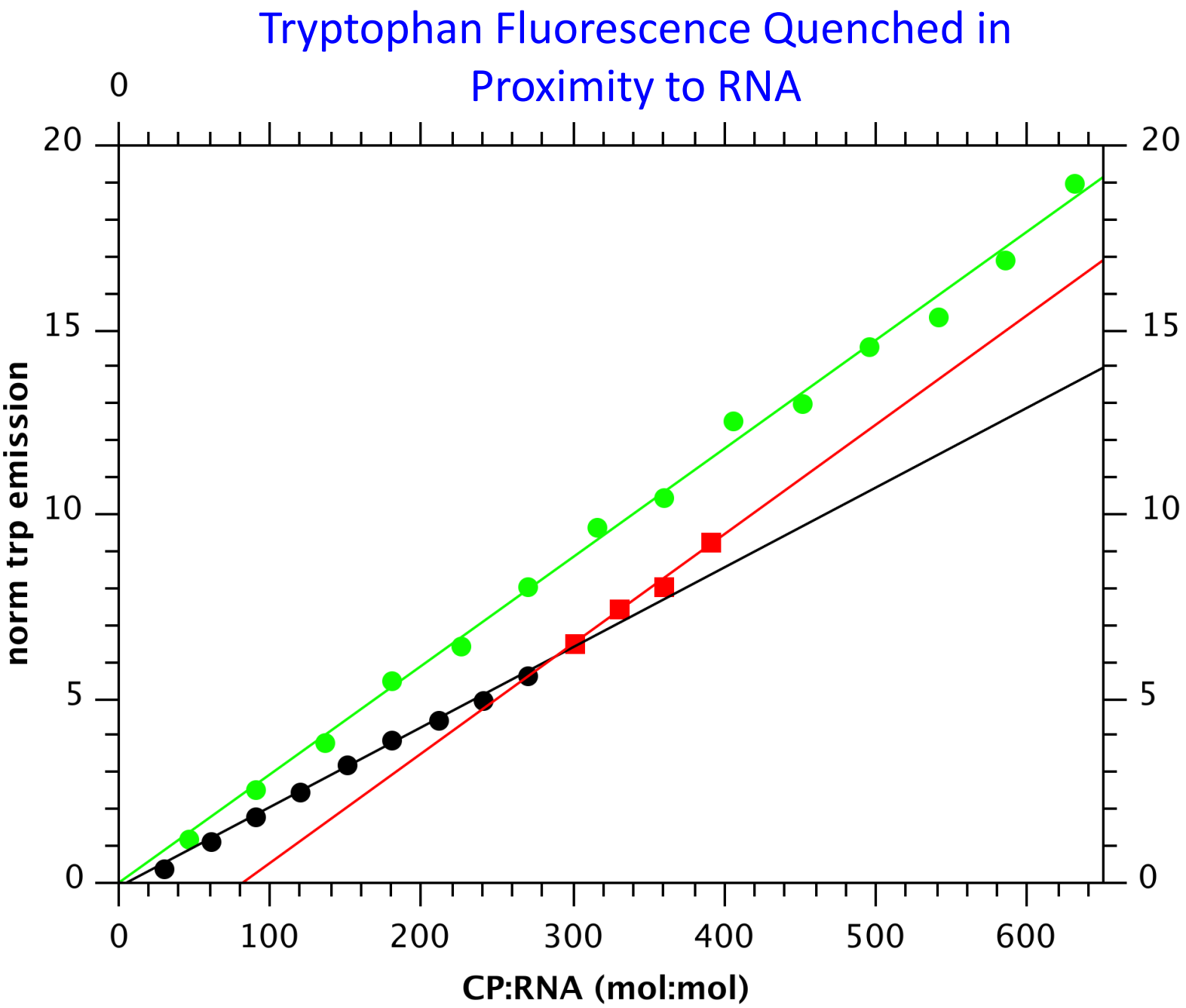
moles negative charge $N^- = L_{RNA}\, N_{RNA}$

Setting $N^+ = N^-$

gives $\dfrac{N_P}{N_{RNA}} = \dfrac{1}{\xi} N_{RNA}$ $\qquad \dfrac{w_P}{w_{RNA}} = \dfrac{1}{\xi} \dfrac{M_P}{\langle M_{nucleotide} \rangle}$

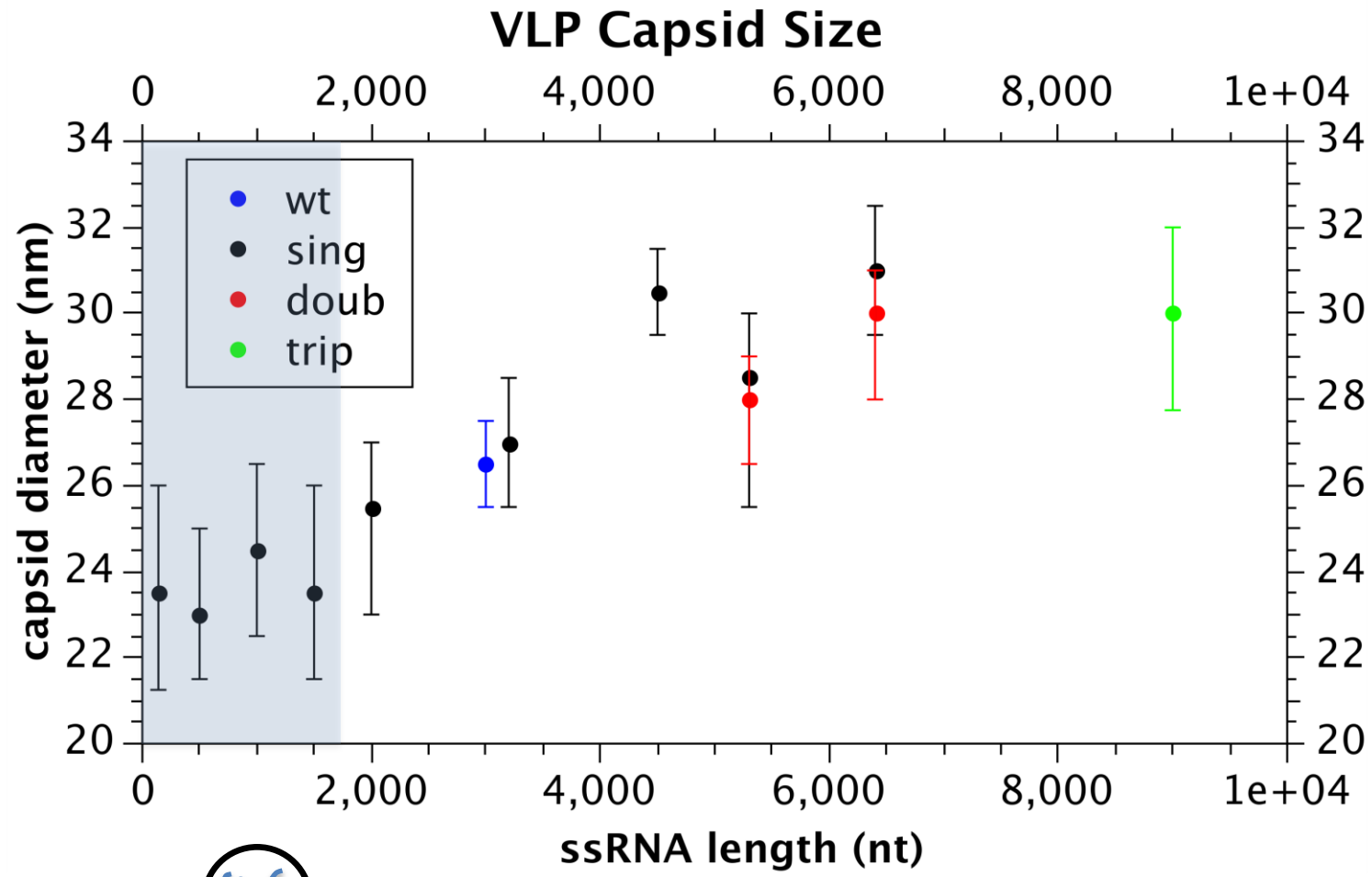
Which for $\xi = 10$

gives $\dfrac{w_P}{w_{RNA}} = 6$

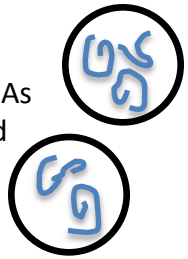

Tryptophan Fluorescence Quenched in Proximity to RNA
norm trp emission
CP:RNA (mol:mol)
0
5
10
15
20
0
100
200
300
400
500
600

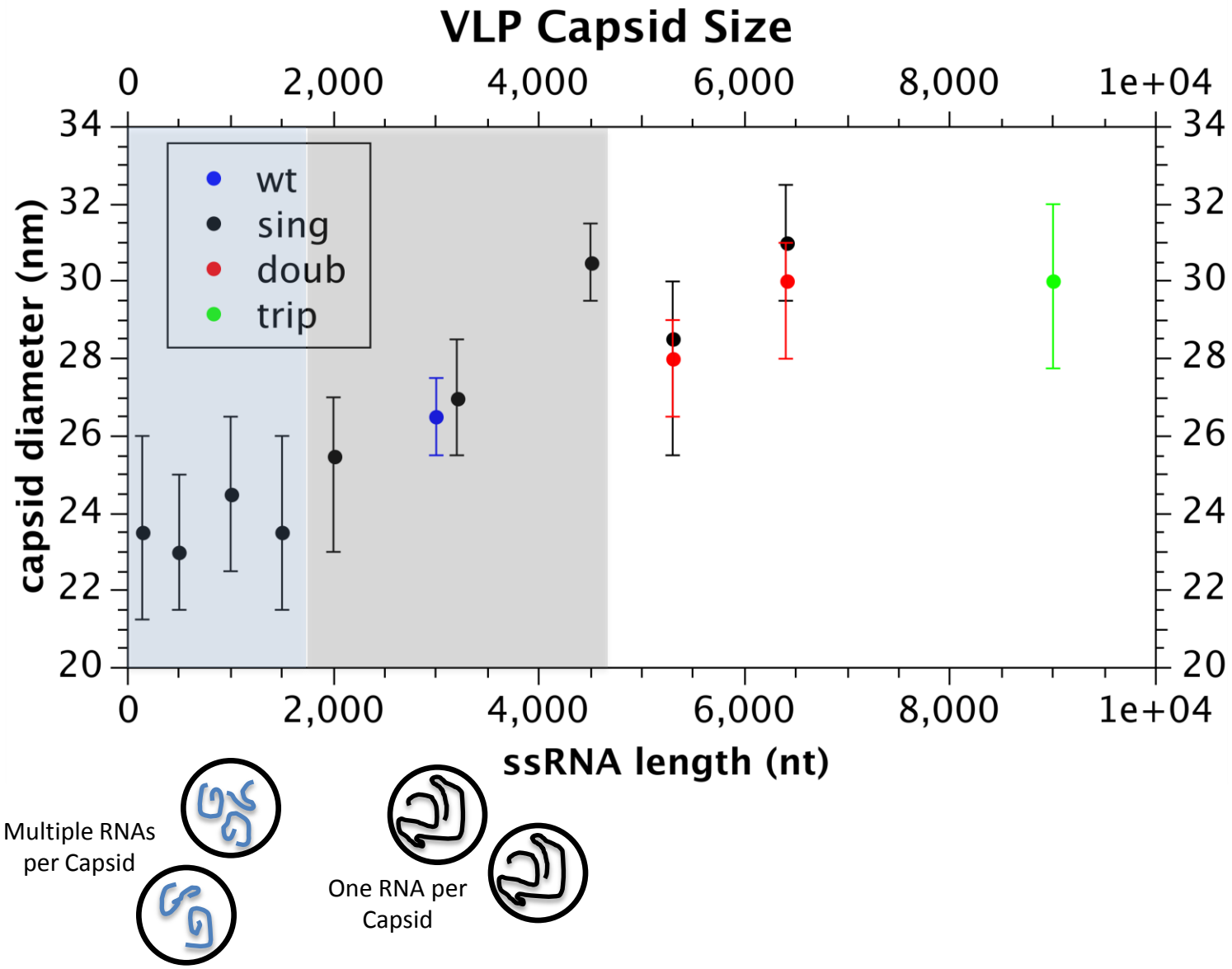
The “magic ratio” is *not* the stoichiometric ratio

- For a T =3 capsid containing a single RNA the stoichiometric ratio is 180 proteins per RNA
- But the magic ratio is 300:1 – an excess of protein is required

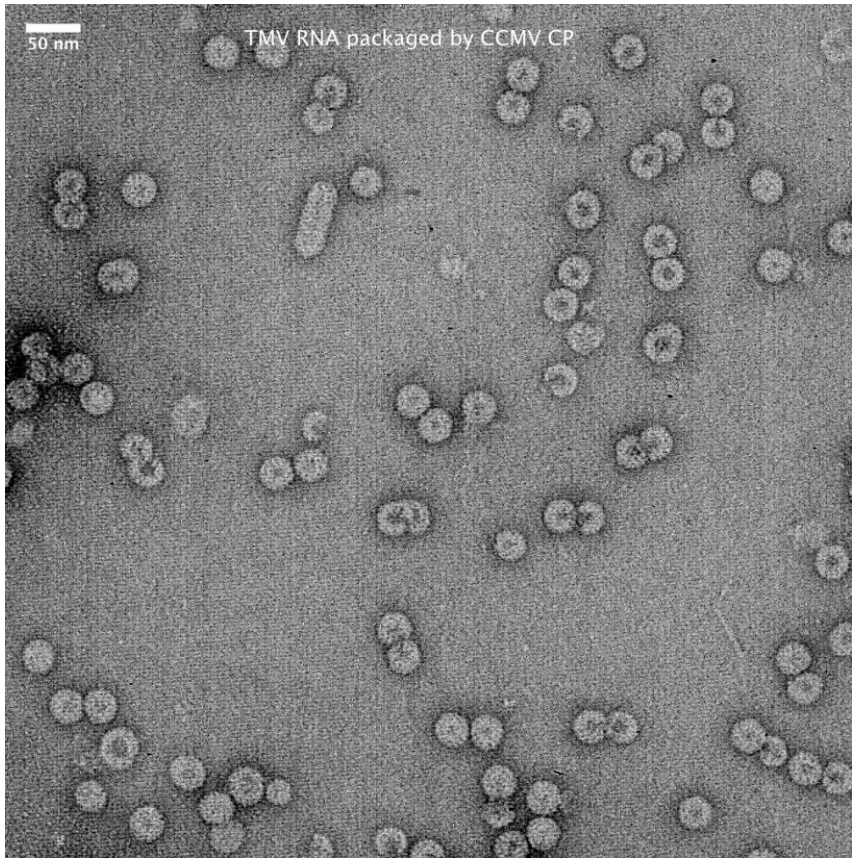


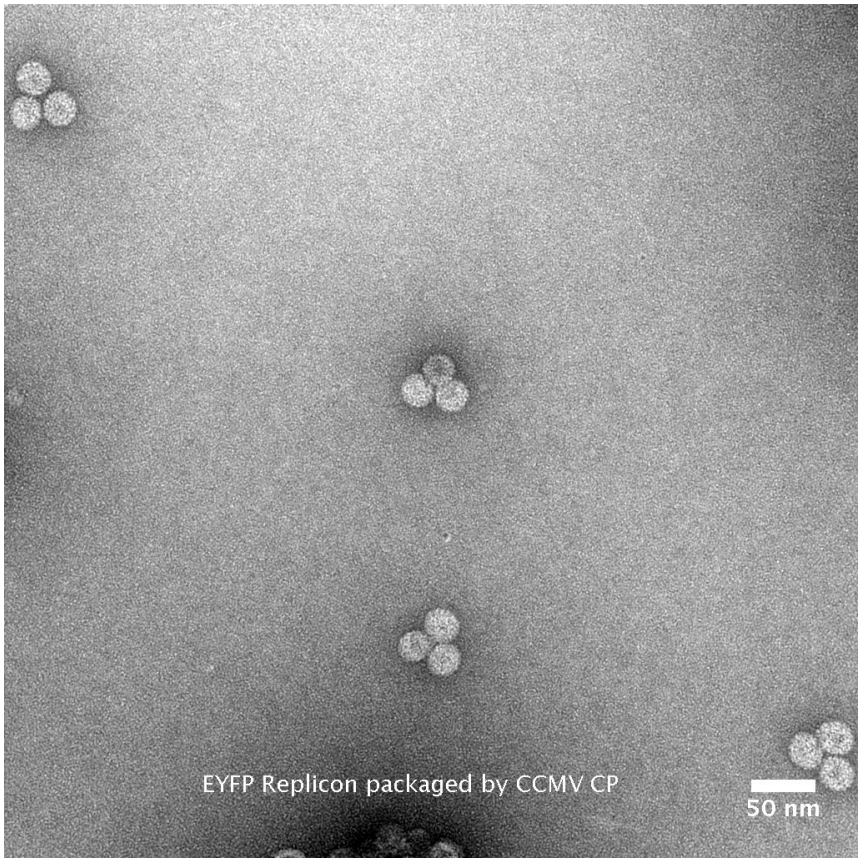
Multiple RNAs per Capsid



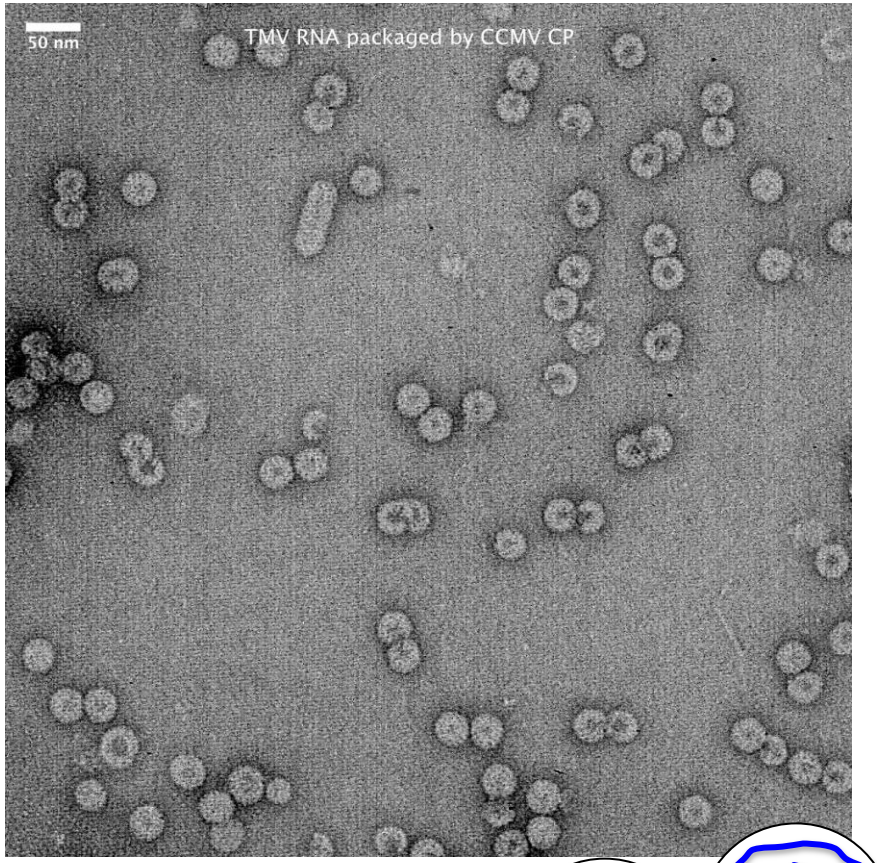

VLP Capsid Size
0
2,000
4,000
6,000
8,000
1e+04
34
32
30
28
26
24
22
20
capsid diameter (nm)
wt
sing
doub
trip
ssRNA length (nt)
Multiple RNAs per Capsid
One RNA per Capsid

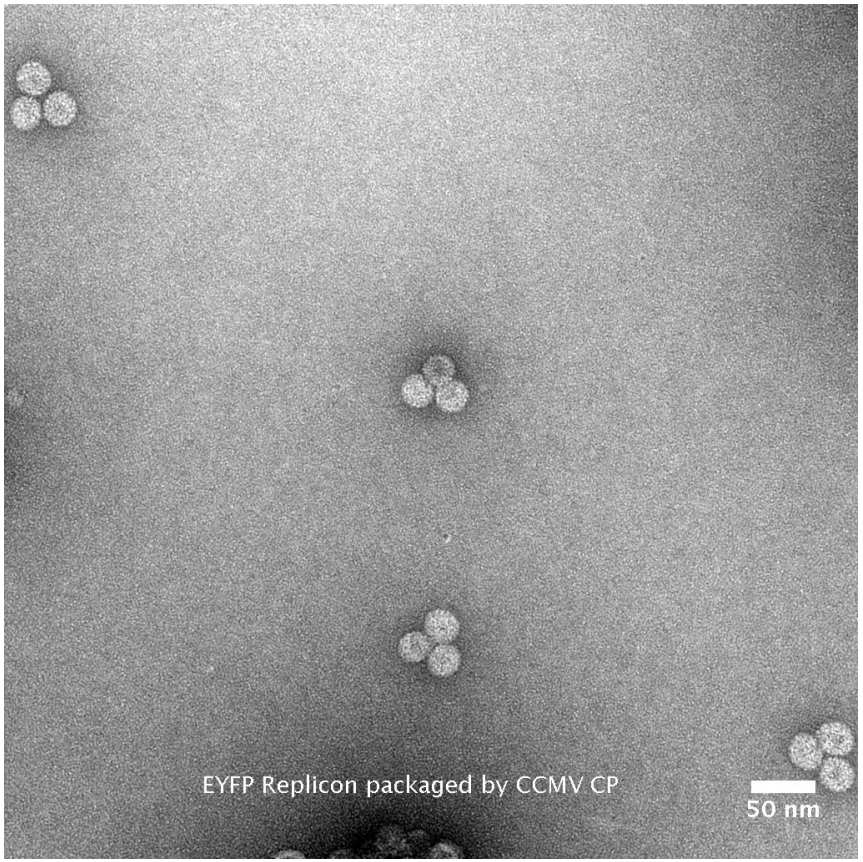
RNAs with length ≥ 4500 nt assemble into multiplets

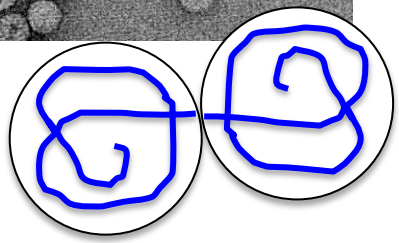


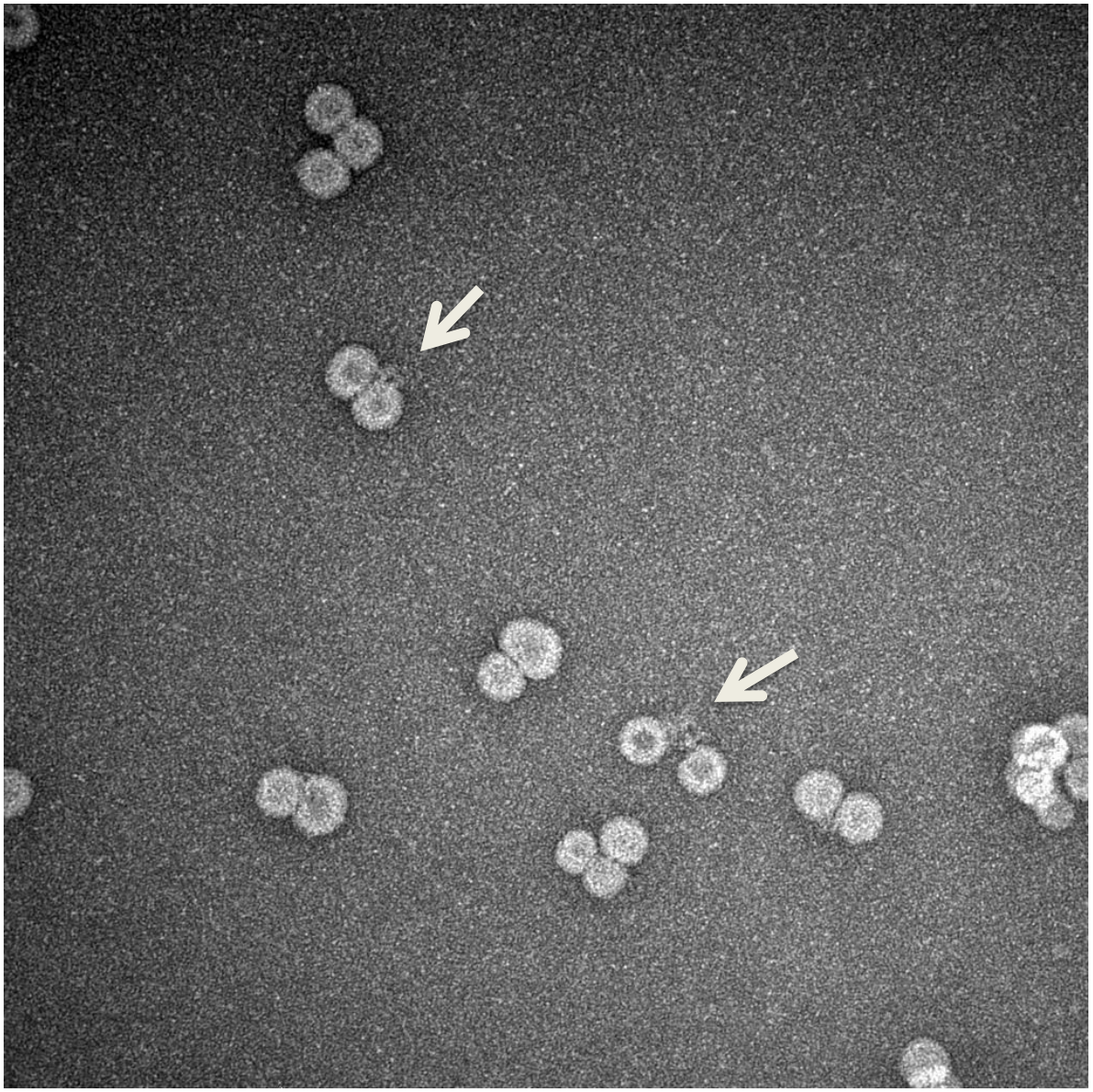


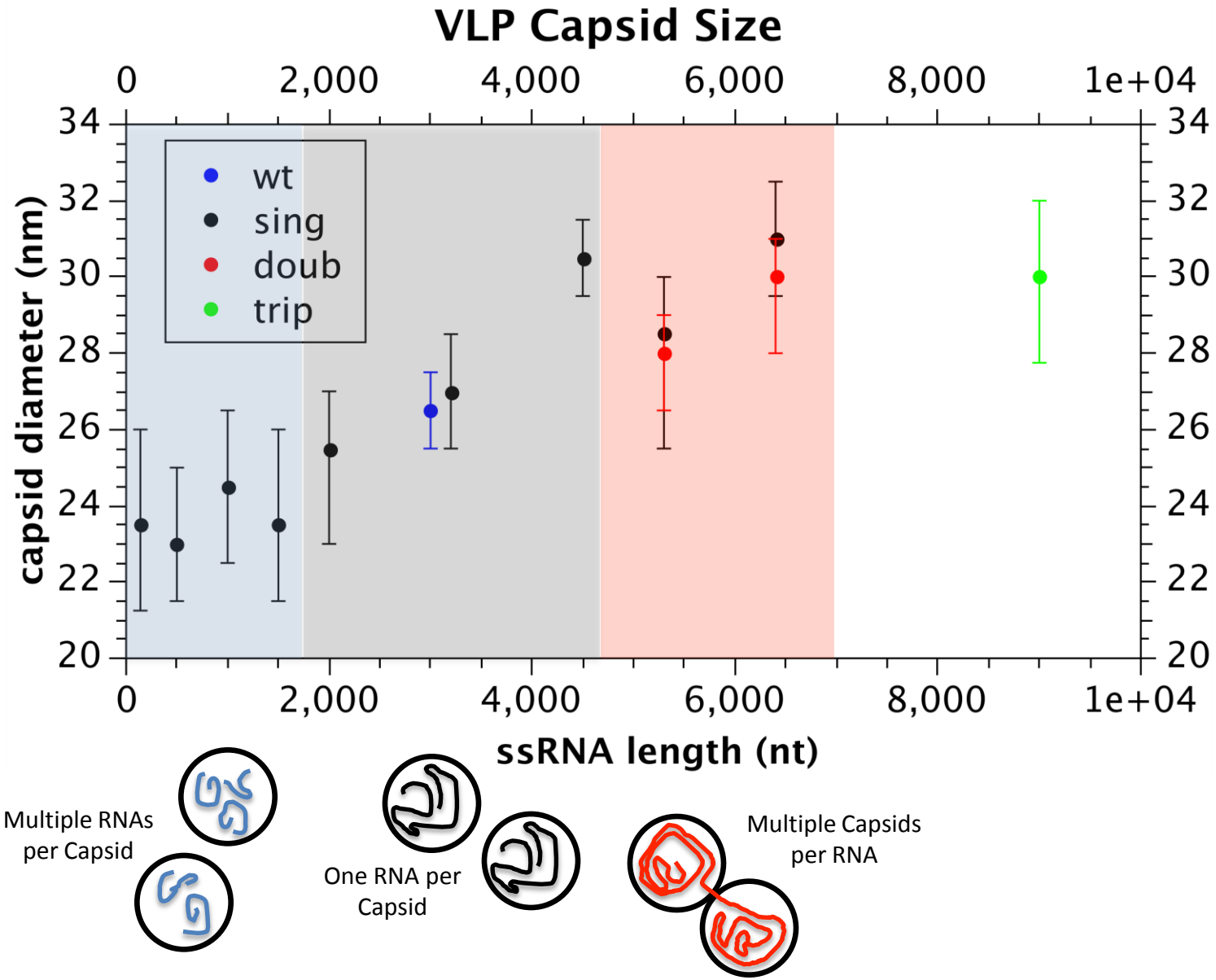
RNAs with length ≥ 4500 nt assemble into multiplets

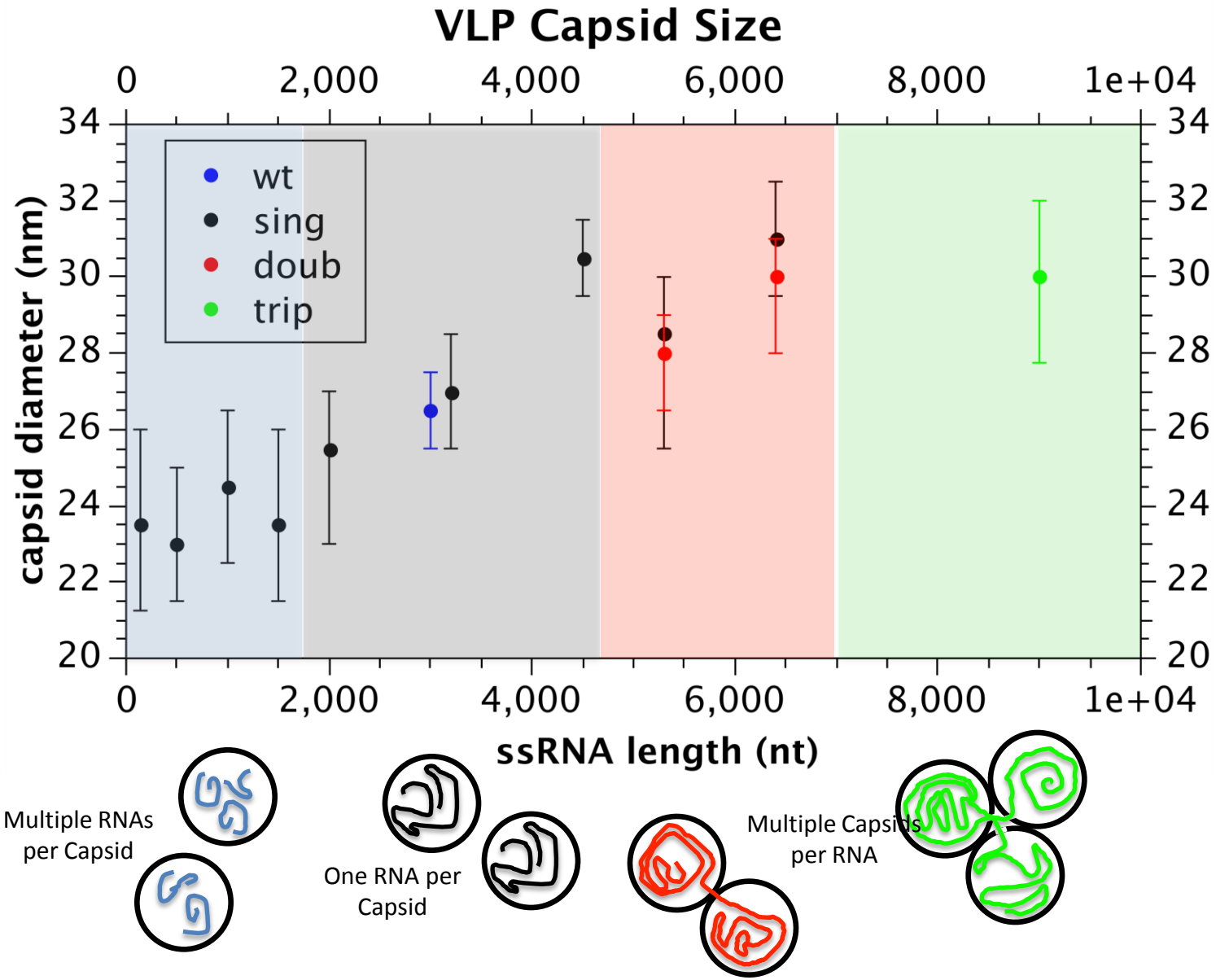


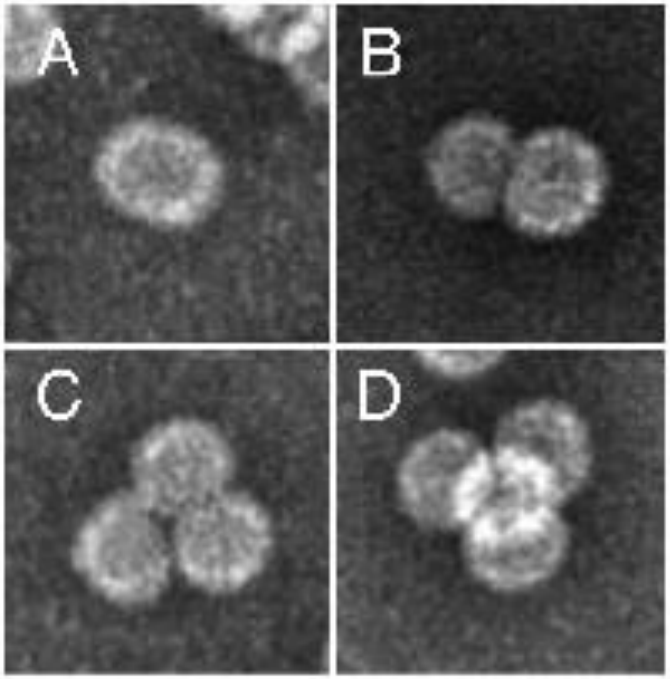


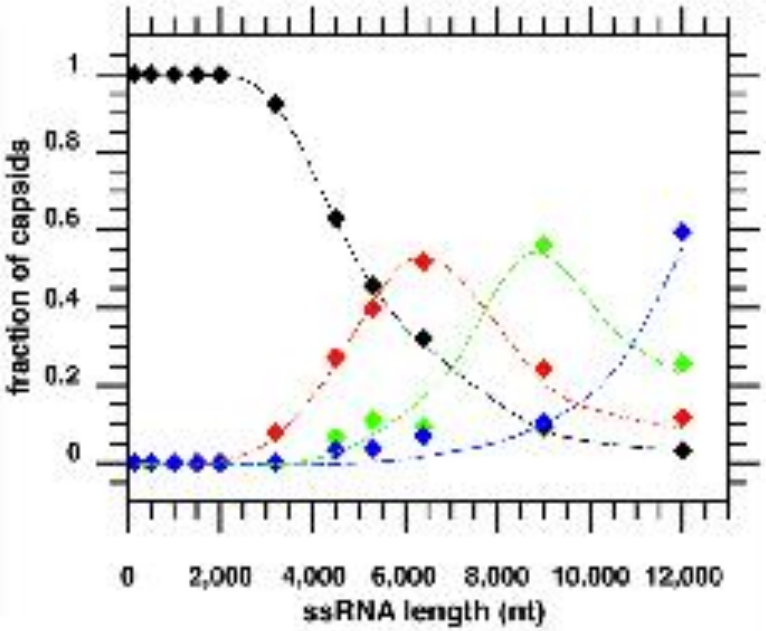





VLP Capsid Size
0
2,000
4,000
6,000
8,000
1e+04
34
32
30
28
26
24
22
20
capsid diameter (nm)
wt
sing
doub
trip
ssRNA length (nt)
Multiple RNAs per Capsid
One RNA per Capsid
Multiple Capsids per RNA


VLP Capsid Size
0
2,000
4,000
6,000
8,000
1e+04
34
32
30
28
26
24
22
20
capsid diameter (nm)
wt
sing
doub
trip
ssRNA length (nt)
Multiple RNAs per Capsid
One RNA per Capsid
Multiple Capsids per RNA


A
B
C
D


fraction of capsids
1
0.8
0.6
0.4
0.2
0
0
2,000
4,000
6,000
8,000
10,000
12,000
ssRNA length (nt)

"Magic Ratio" Allows "Head-to-Head" Packaging Competition Experiments

- Mix equal weights of two RNAs of different lengths with sufficient protein to completely package *only one* of them
- Treat assemblies with RNase to remove unpackaged RNA
- Identify packaged RNA by disassembly of VLPs and electrophoresis or by packaging fluorescently labeled RNA

Competition Between Full-Length (3.2 knt) BMV RNA1 and Truncated RNAs

Competition Between Full-Length (3.2 knt) BMV RNA1 and Truncated RNAs

- 0.5 knt copackages with RNA1

Competition Between Full-Length (3.2 knt) BMV RNA1 and Truncated RNAs

- 0.5 knt copackages with RNA1
- 1.0 knt *none* packaged

Competition Between Full-Length (3.2 knt) BMV RNA1 and Truncated RNAs

- 0.5 knt *co*packages with RNA1
- 1.0 knt *none* packaged
- 1.5 knt *none* packaged

Competition Between Full-Length (3.2 knt) BMV RNA1 and Truncated RNAs

- 0.5 knt copackages with RNA1
- 1.0 knt *none* packaged
- 1.5 knt *none* packaged
- 2.0 knt *out*competes – efficiency 1.18

Competition Between Full-Length (3.2 knt) BMV RNA1 and Truncated RNAs

- 0.5 knt copackages with RNA1
- 1.0 knt *none* packaged
- 1.5 knt *none* packaged
- 2.0 knt *out*competes – efficiency 1.18
- 2.5 knt efficiency 0.40

Competition Between Full-Length (3.2 knt) BMV RNA1 and Truncated RNAs

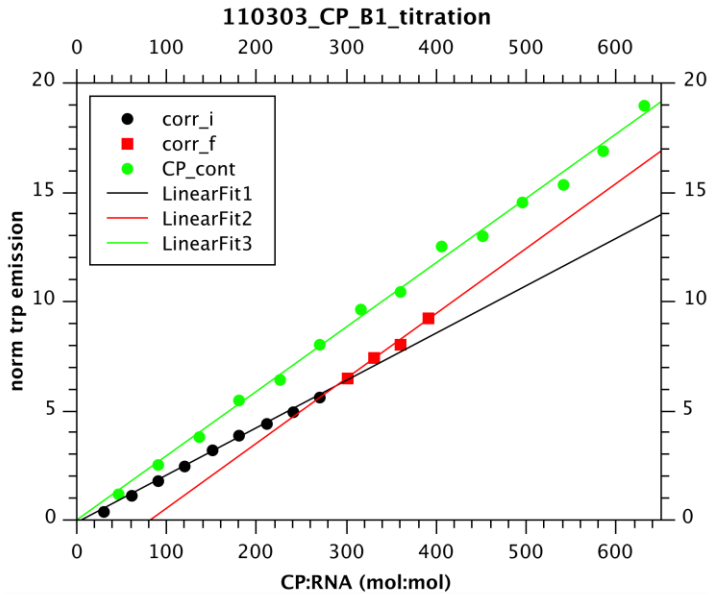
- 0.5 knt copackages with RNA1
- 1.0 knt *none* packaged
- 1.5 knt *none* packaged
- 2.0 knt *out*competes – efficiency 1.18
- 2.5 knt efficiency 0.40
- CCMV RNA1 efficiency 0.33!

Among the experiments in the works…

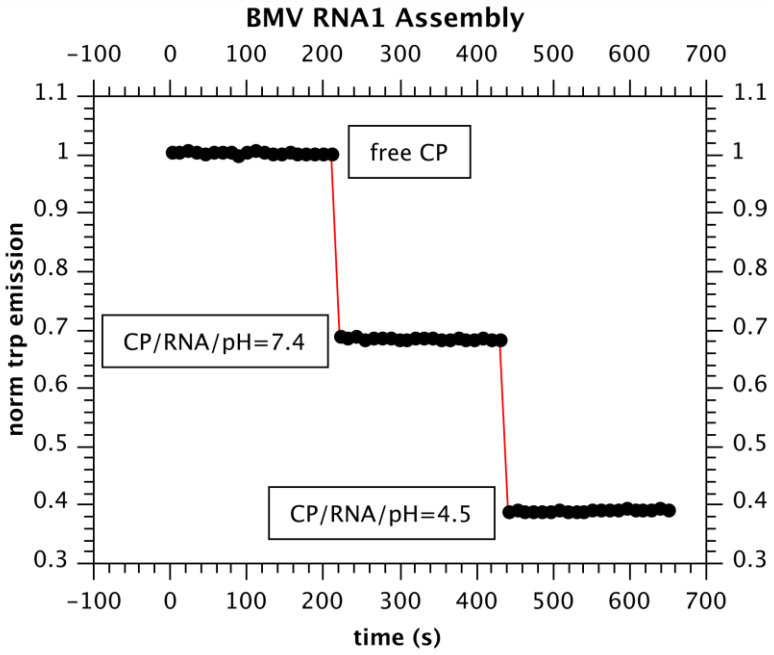
Demonstrate that the TLS packaging signal on RNA1 has no effect

Compete viral with non-viral RNAs of the same length – we predict that the viral RNA will outcompete the nonviral RNA

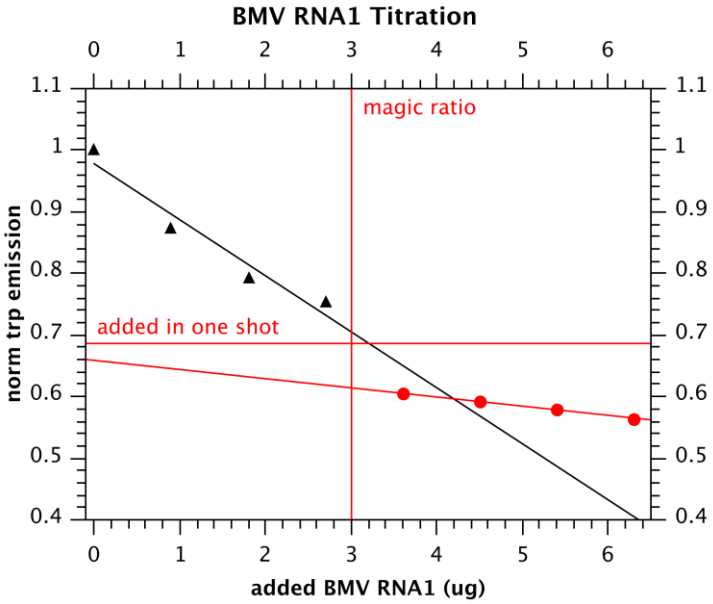
Compete RNA3 with RNA4

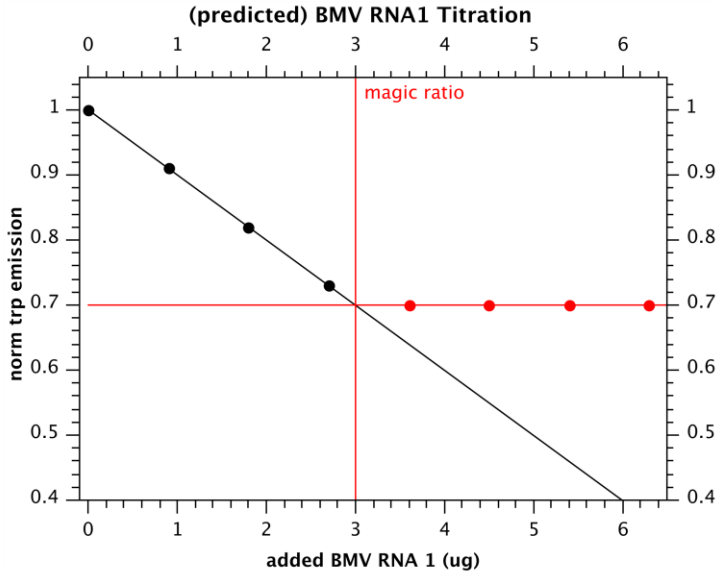


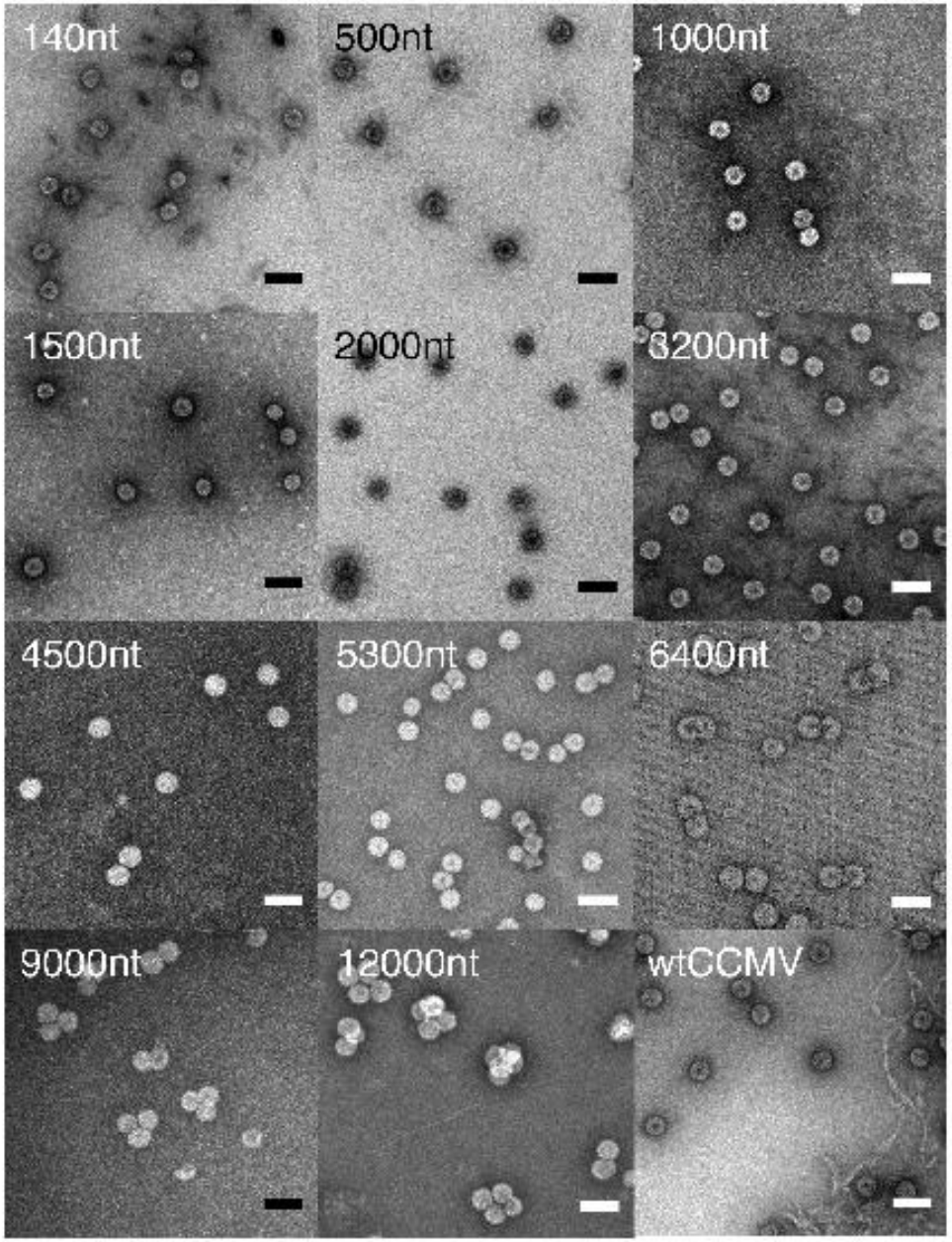
$$\frac{Slope_{FREE}}{Slope_{QUENCH}} = \frac{0.0295}{0.0217} = 1.36$$

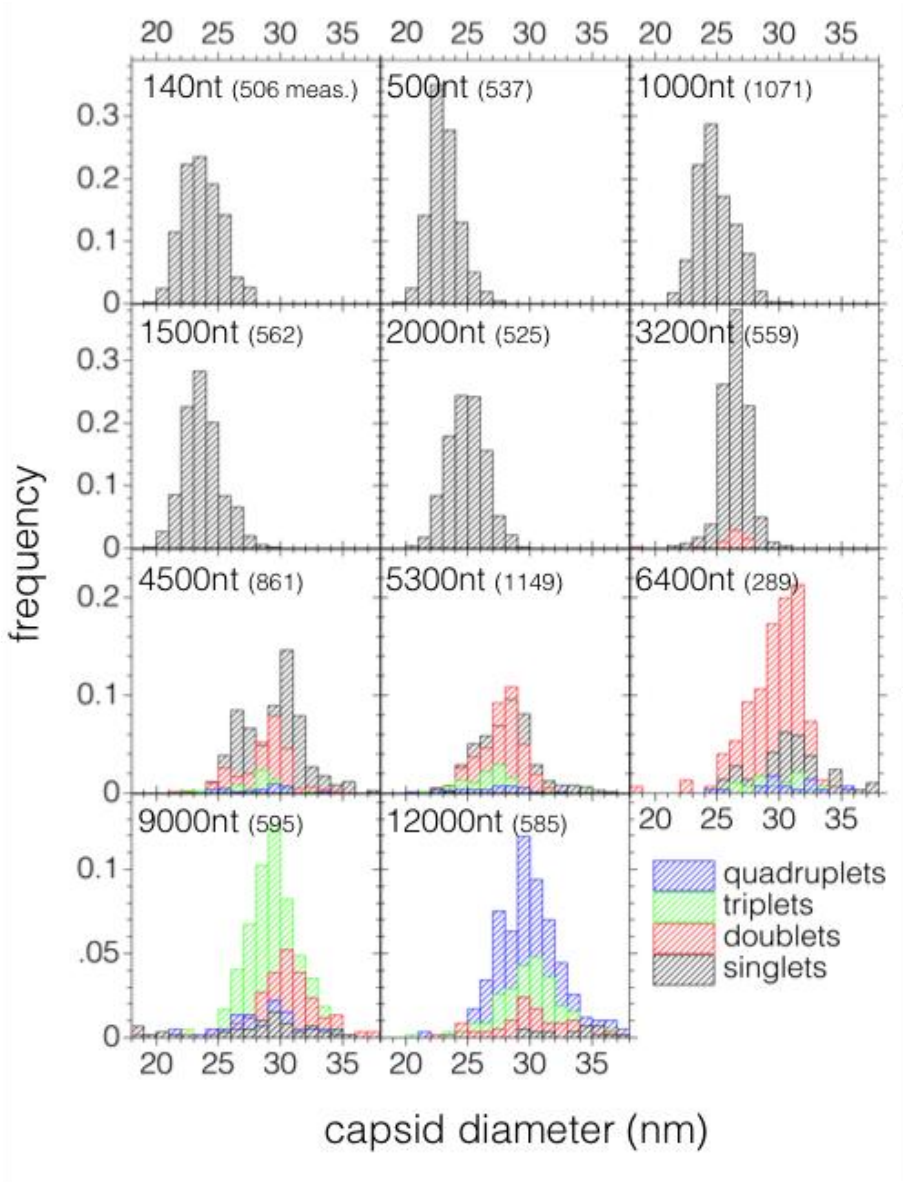


$$\frac{I_{FREE}}{I_{QUENCH}} = \frac{1}{0.7} = 1.43$$

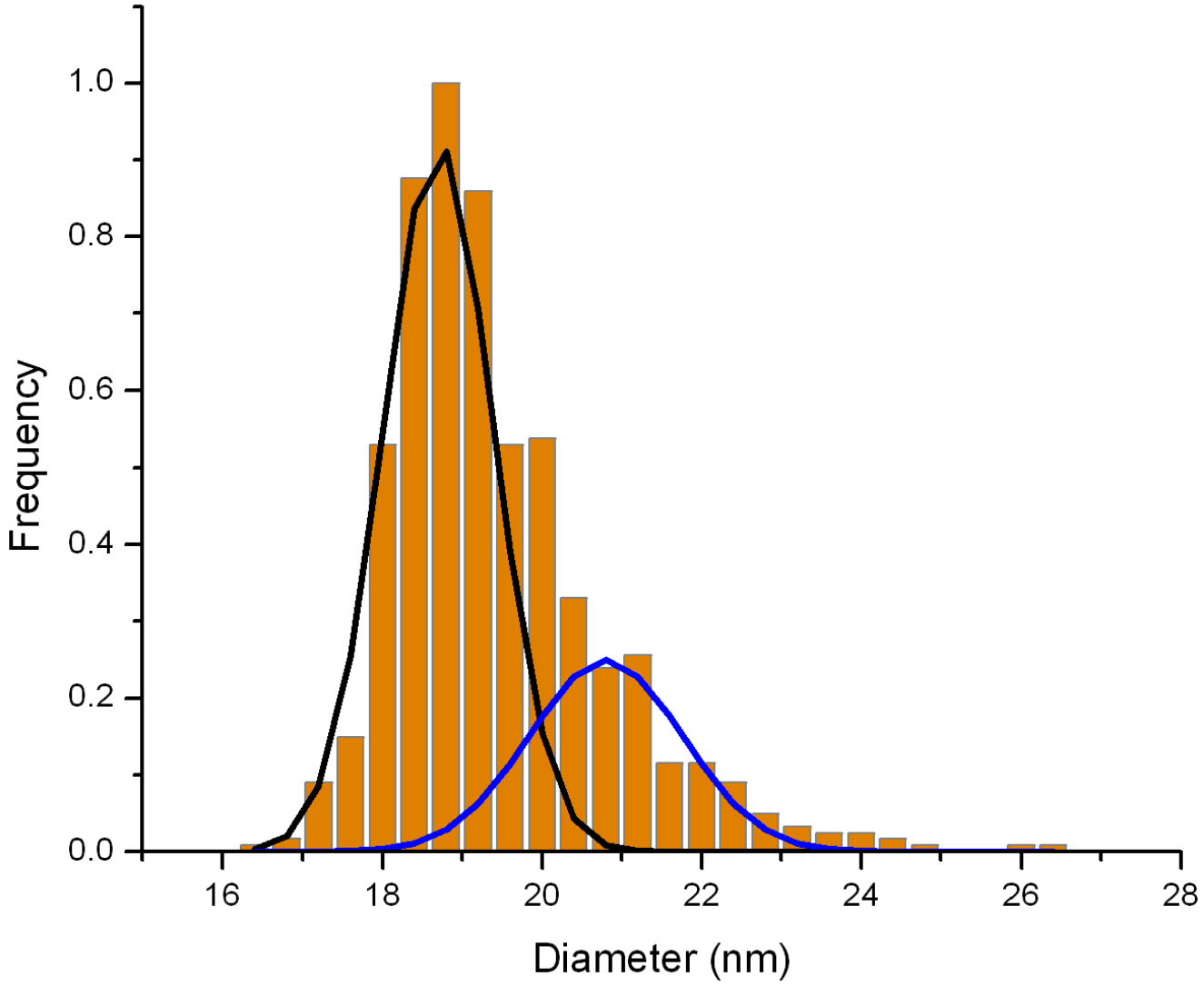

BMV RNA1 Titration
magic ratio
added in one shot
norm trp emission
added BMV RNA1 (ug)

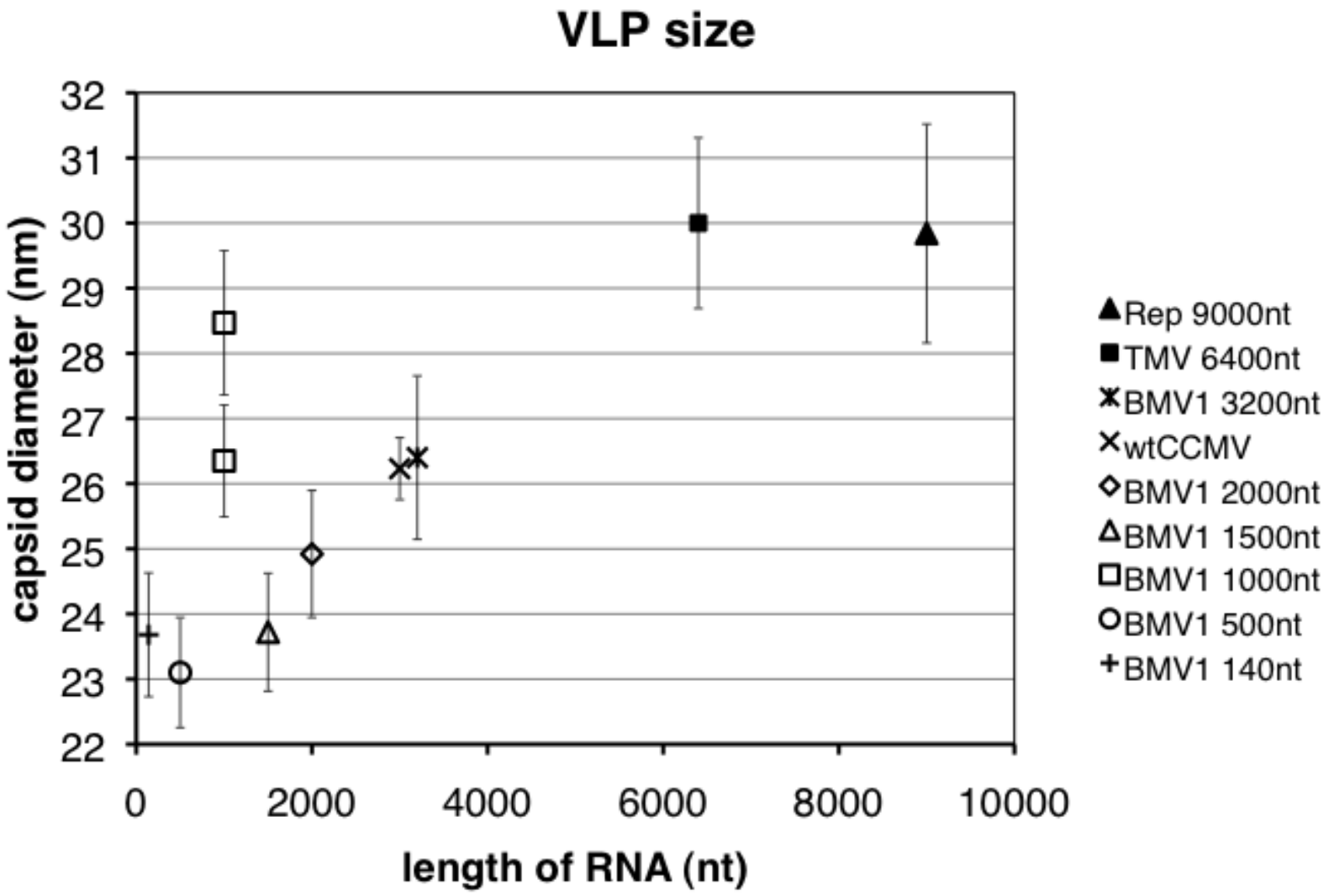

(predicted) BMV RNA1 Titration
magic ratio
norm trp emission
added BMV RNA 1 (ug)


140nt
500nt
1000nt
1500nt
2000nt
3200nt
4500nt
5300nt
6400nt
9000nt
12000nt
wtCCMV

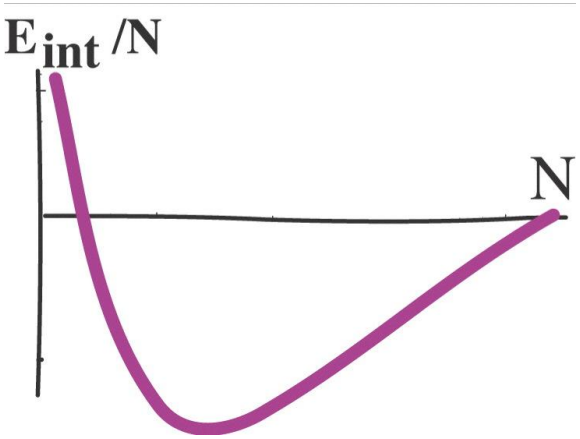

140nt (506 meas.)
500nt (537)
1000nt (1071)
1500nt (562)
2000nt (525)
3200nt (559)
4500nt (861)
5300nt (1149)
6400nt (289)
9000nt (595)
12000nt (585)
quadruplets
triplets
doublets
singlets
frequency
capsid diameter (nm)

45 kD PSS Forms T = 1 Capsids

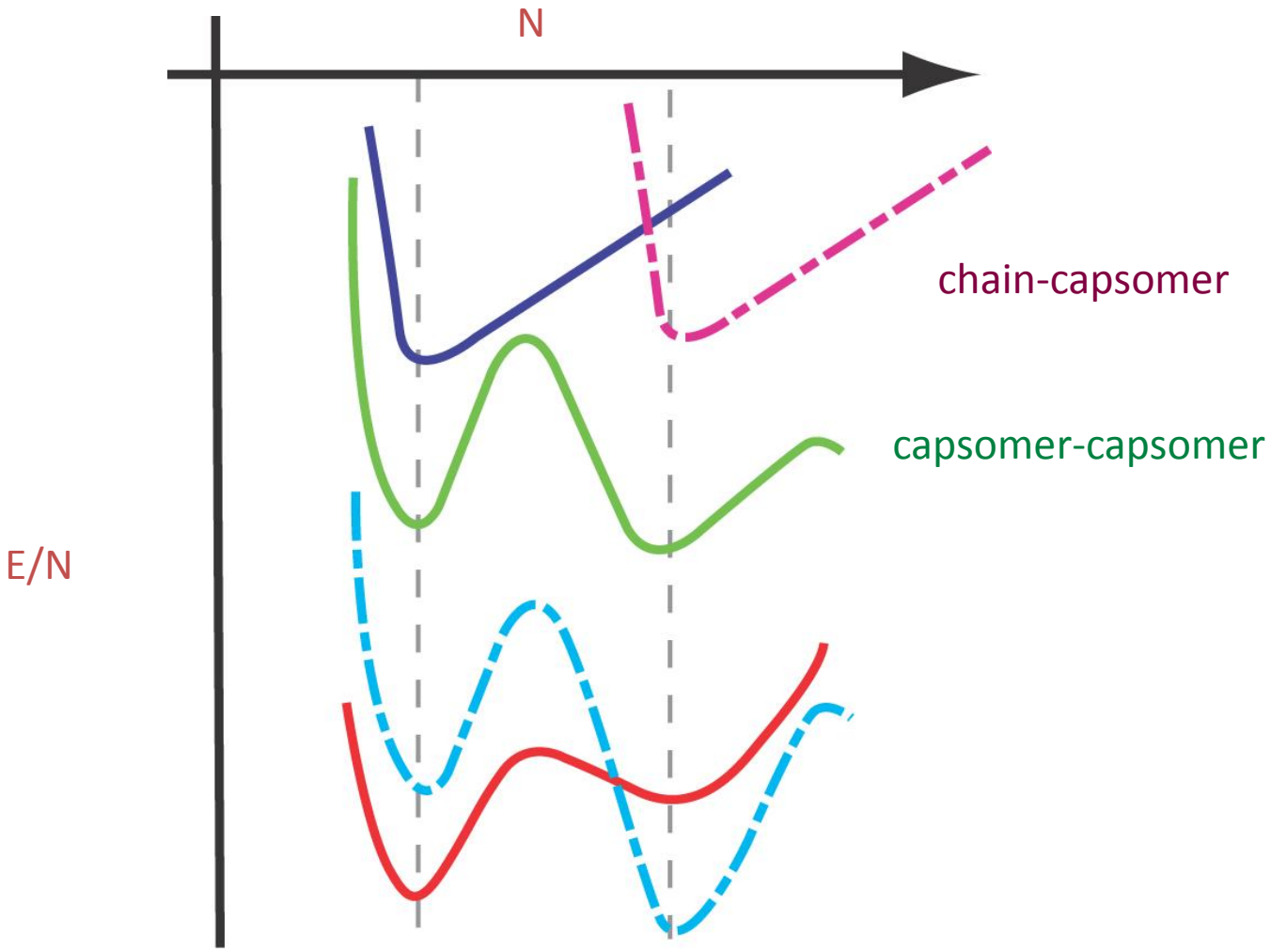


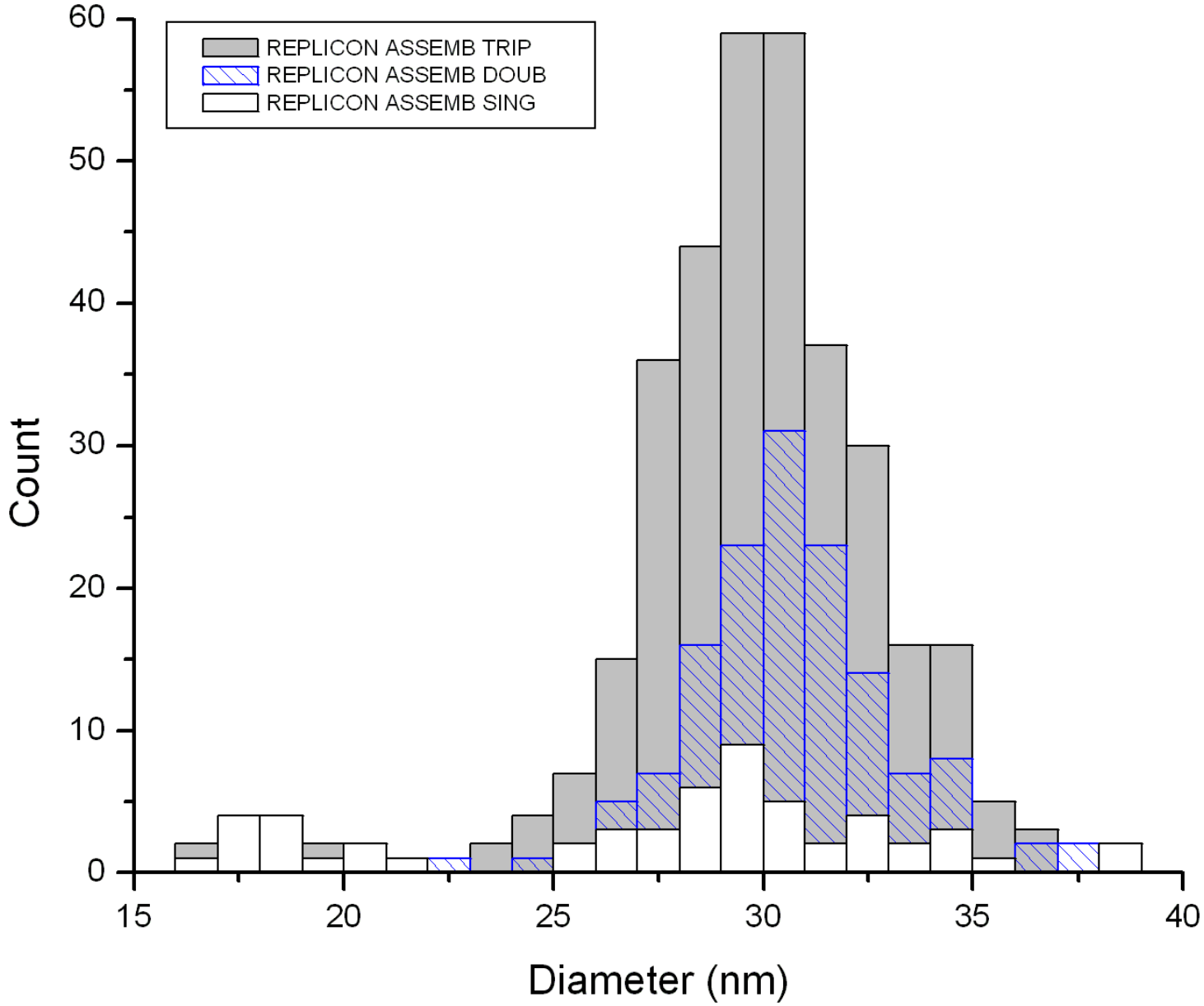

VLP size
capsid diameter (nm)
32
31
30
29
28
27
26
25
24
23
22
0
2000
4000
6000
8000
10000
length of RNA (nt)
Rep 9000nt
TMV 6400nt
BMV1 3200nt
wtCCMV
BMV1 2000nt
BMV1 1500nt
BMV1 1000nt
BMV1 500nt
BMV1 140nt

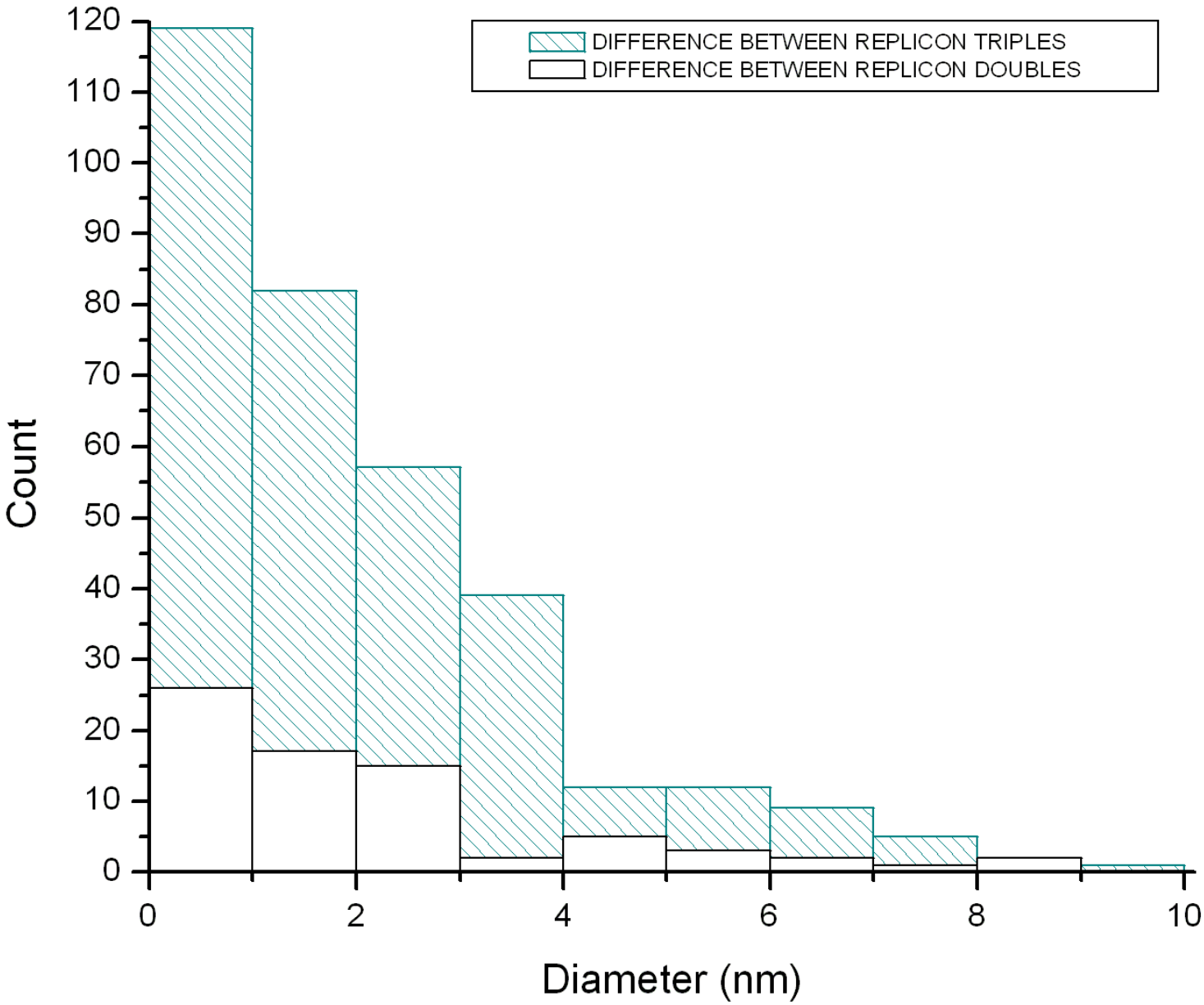
The Interaction Energy Between Polymer and a Capsid Has a Minimum at a Particular Chain Length



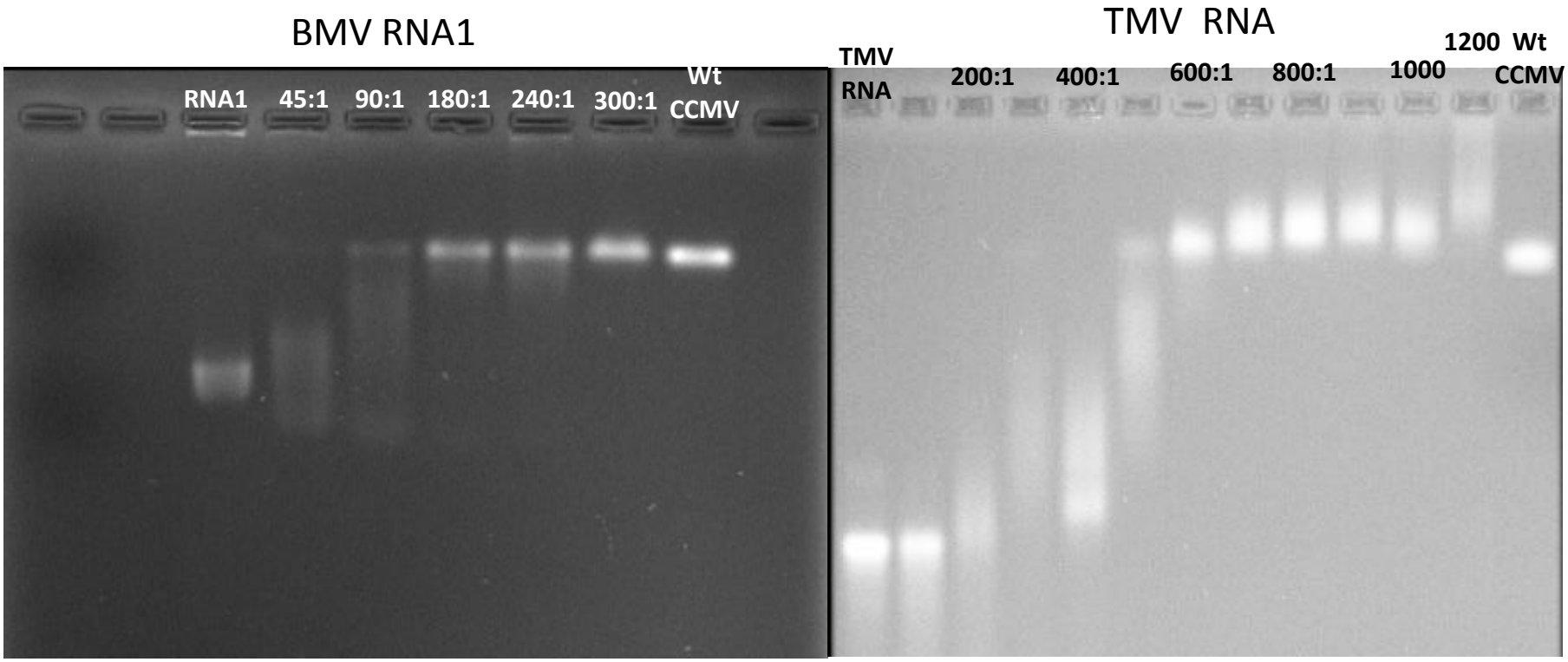
Superposition of the chain-capsid energy curve onto the capsomer-capsomer interaction energy curve determines preferred capsid size.

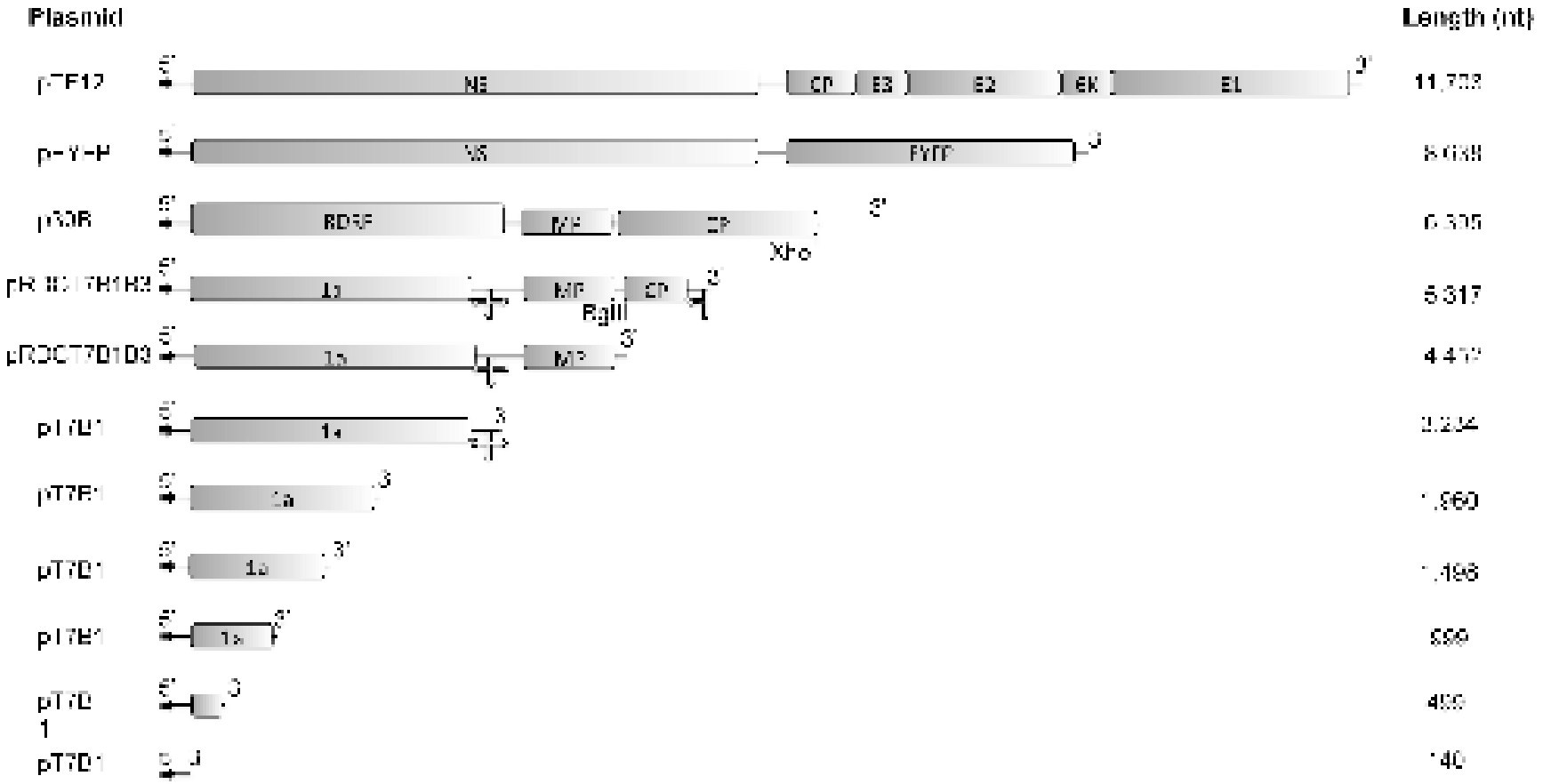


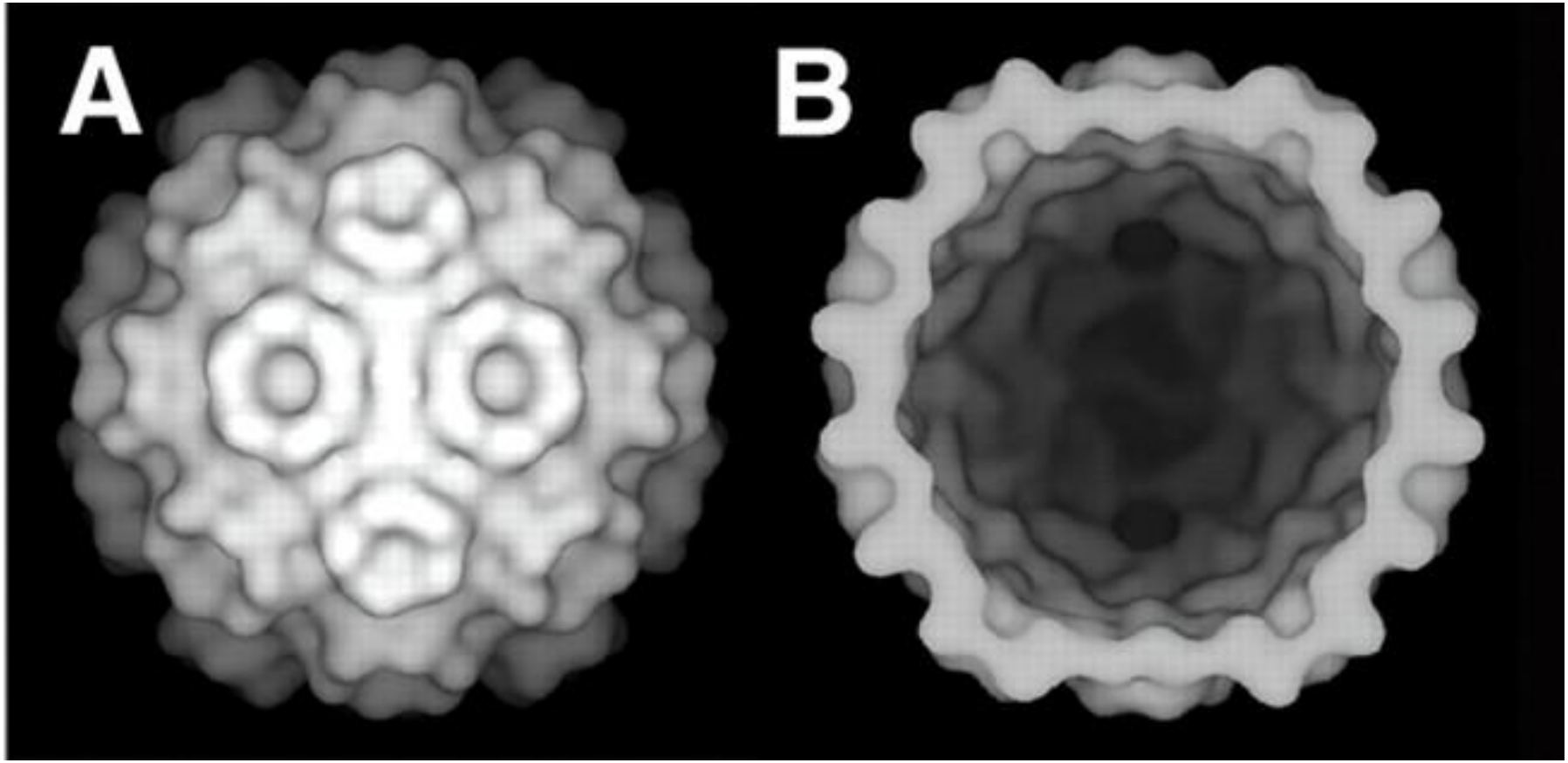

REPLICON ASSEMB TRIP
REPLICON ASSEMB DOUB
REPLICON ASSEMB SING
Count
0
10
20
30
40
50
60
15
20
25
30
35
40
Diameter (nm)


DIFFERENCE BETWEEN REPLICON TRIPLES
DIFFERENCE BETWEEN REPLICON DOUBLES
Count
120
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
Diameter (nm)

RNA-CP Titrations

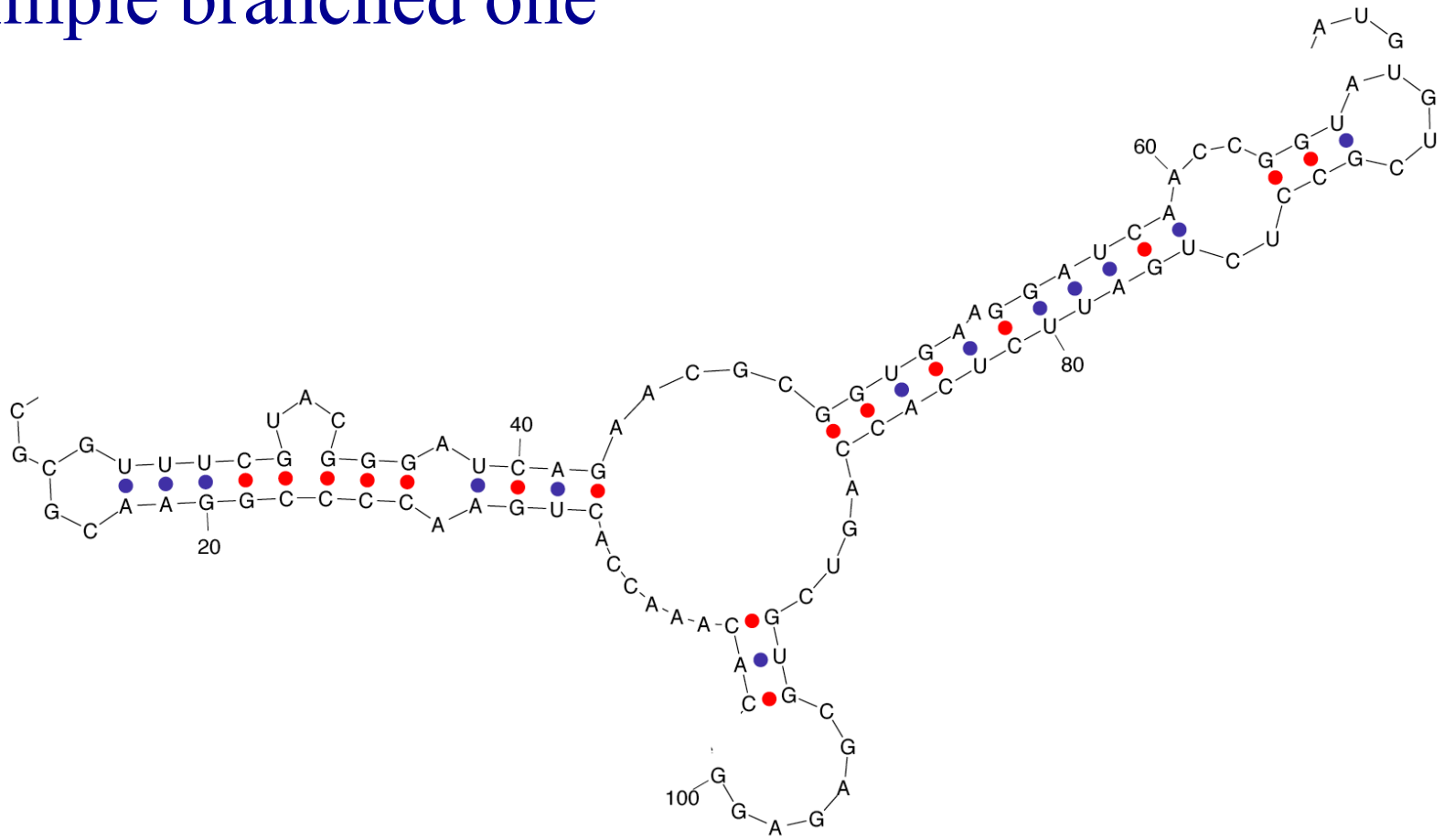



Plasmid
Length (nt)
NS
CP
E3
E2
6K
E1
NS
RDRP
MP
CP
XhoI
1a
MP
CP
BglII
1a
MP
1a
1a
1a
1a

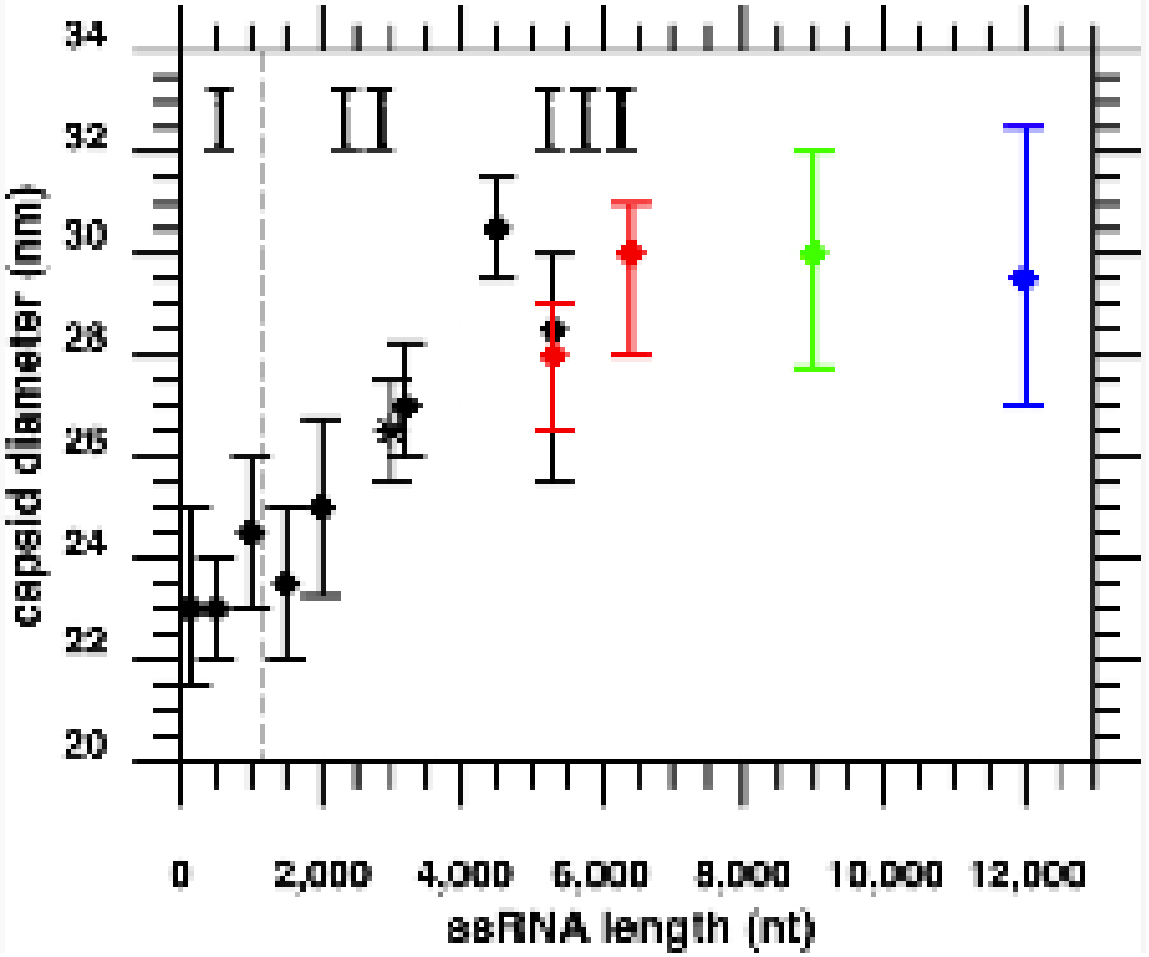


T Douglas, M Young Science 2006;312:873-875

RNA is not a linear polymer, nor is it a simple branched one



(5’)CACAAACCACUGACCCCGGAACGCGUUUCGUACGGGAUCAGAA
CGCGGUGAAGGUCAACCGGUAUGUCGCCUCUGAUUCUCACCAGU
CGUGCGAGAGG(3’)


I
II
III
34
32
30
28
26
24
22
20
capsid diameter (nm)
0
2,000
4,000
6,000
8,000
10,000
12,000
ssRNA length (nt)