SQUEEZING SELF-ASSEMBLED NANOCONTAINERS: AFM NANOINDENTATION STUDIES OF VIRAL CAPSIDS

KITP
June 2006

Collaborators

UCLA

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- Melissa Gibbons, Bill Klug (Mech Eng)
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Vrije Universiteit Amsterdam

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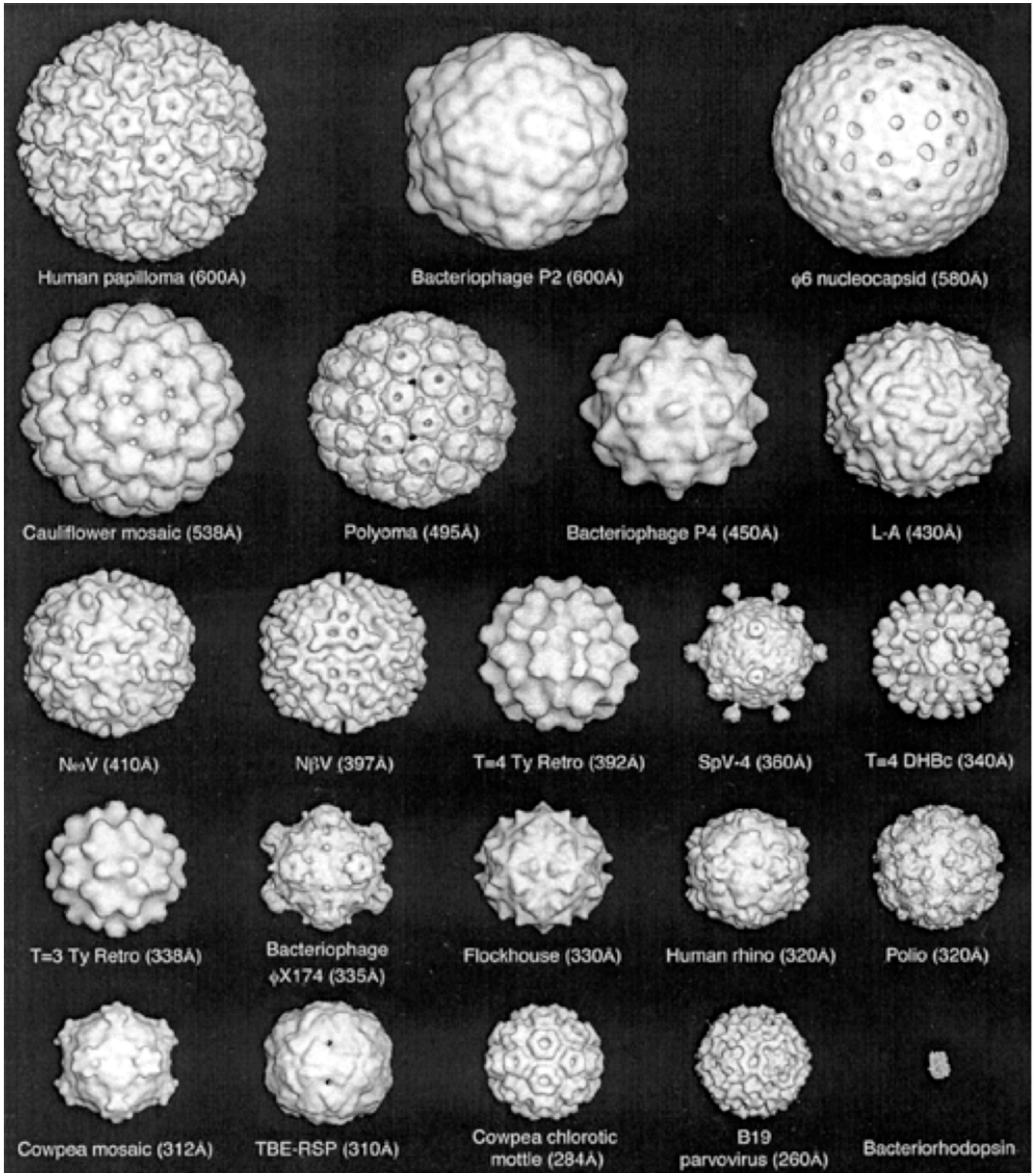
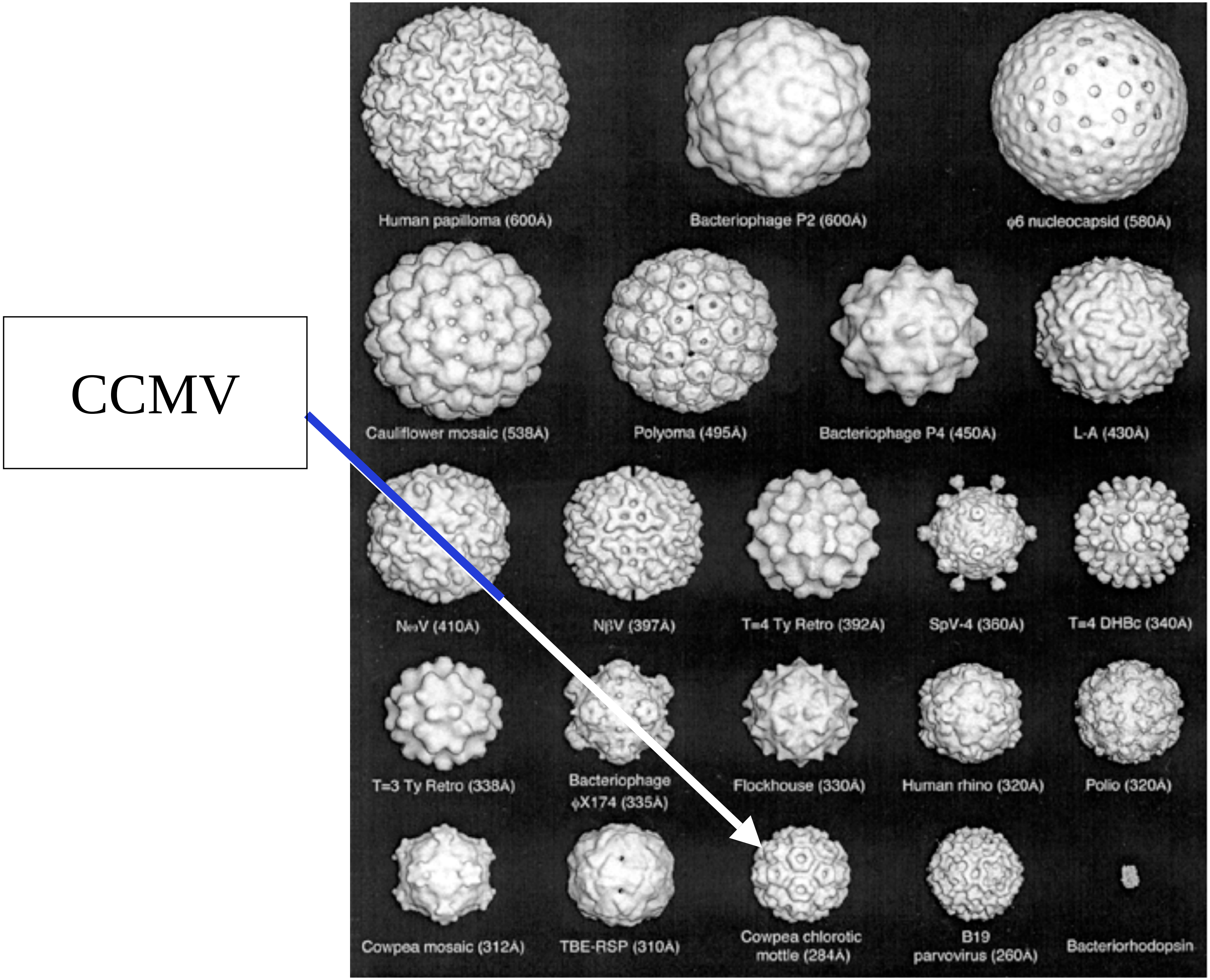
- Can we model the mechanical properties of capsids? Finite element analysis; Phenomenological models


Human papilloma (600Å)
Bacteriophage P2 (600Å)
φ6 nucleocapsid (580Å)
Cauliflower mosaic (538Å)
Polyoma (495Å)
Bacteriophage P4 (450Å)
L-A (430Å)
NωV (410Å)
NβV (397Å)
T=4 Ty Retro (392Å)
SpV-4 (360Å)
T=4 DHBc (340Å)
T=3 Ty Retro (338Å)
Bacteriophage φX174 (335Å)
Flockhouse (330Å)
Human rhino (320Å)
Polio (320Å)
Cowpea mosaic (312Å)
TBE-RSP (310Å)
Cowpea chlorotic mottle (284Å)
B19 parvovirus (260Å)
Bacteriorhodopsin


CCMV
Human papilloma (600Å)
Bacteriophage P2 (600Å)
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Cauliflower mosaic (538Å)
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T=3 Ty Retro (338Å)
Bacteriophage φX174 (335Å)
Flockhouse (330Å)
Human rhino (320Å)
Polio (320Å)
Cowpea mosaic (312Å)
TBE-RSP (310Å)
Cowpea chlorotic mottle (284Å)
B19 parvovirus (260Å)
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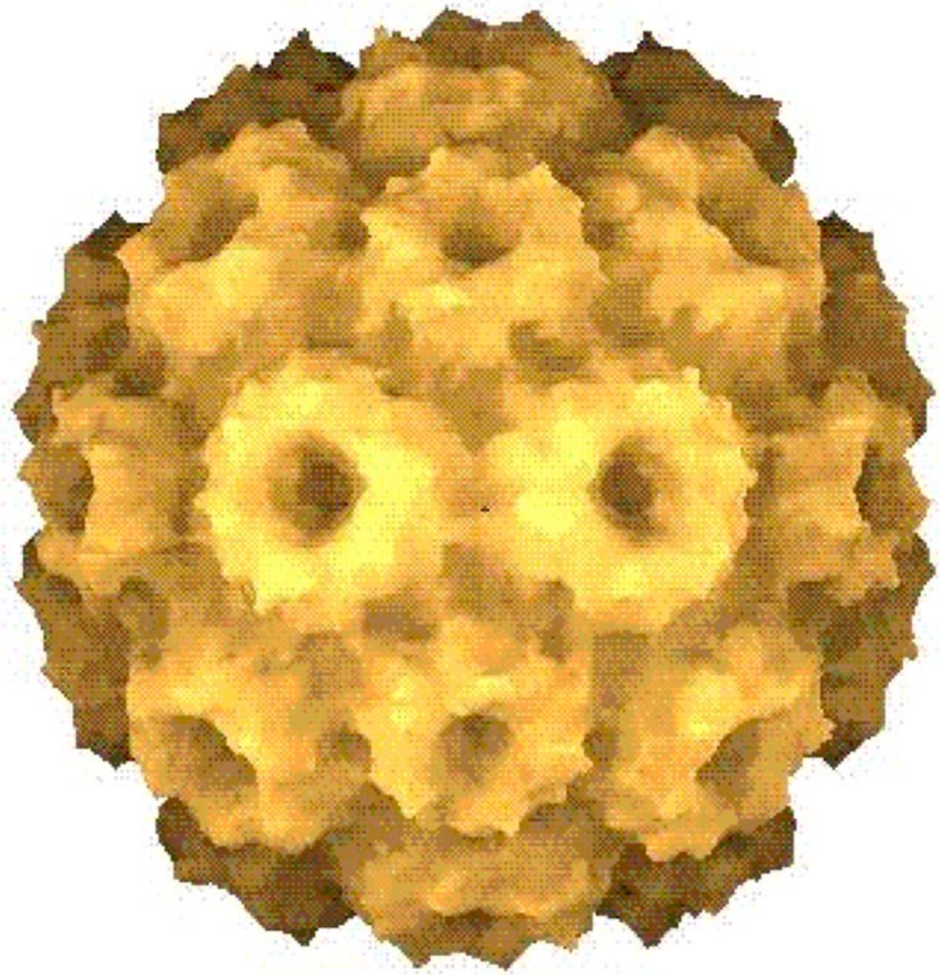
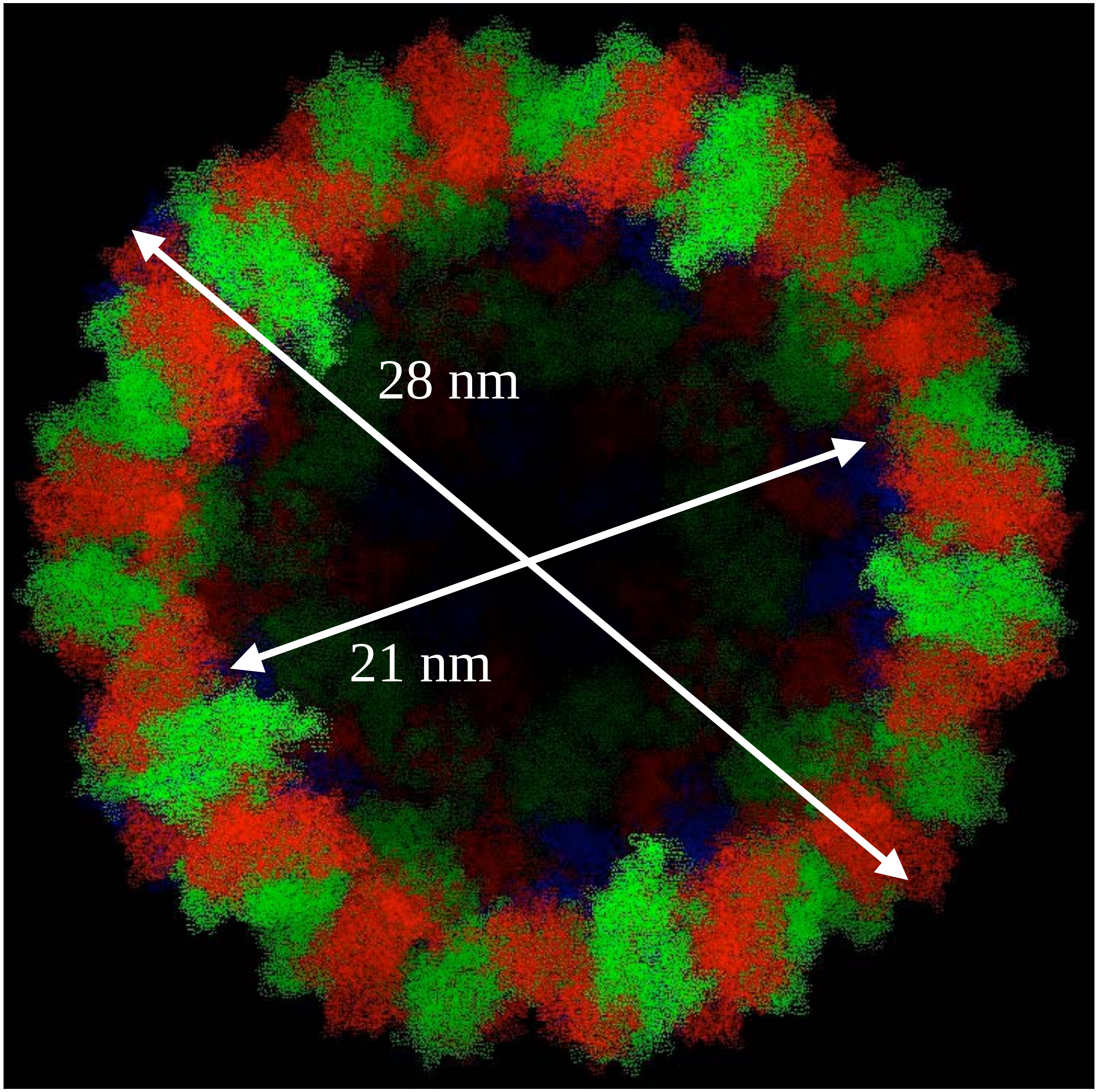


CCMV
Cowpea chlorotic mottle virus

PROPERTIES OF CCMV

- 28-nm diameter capsid made up of 180 copies of a single protein


28 nm
21 nm

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- Genome is ss RNA

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PROPERTIES OF CCMV

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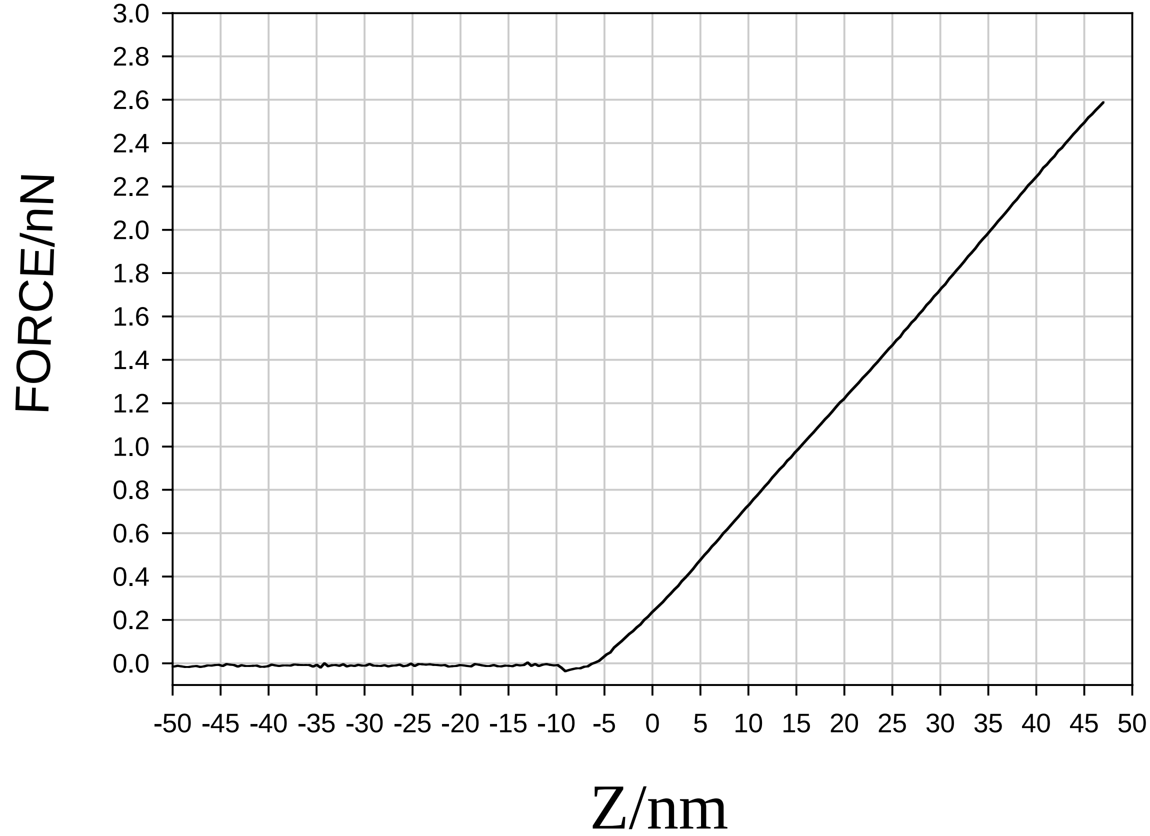
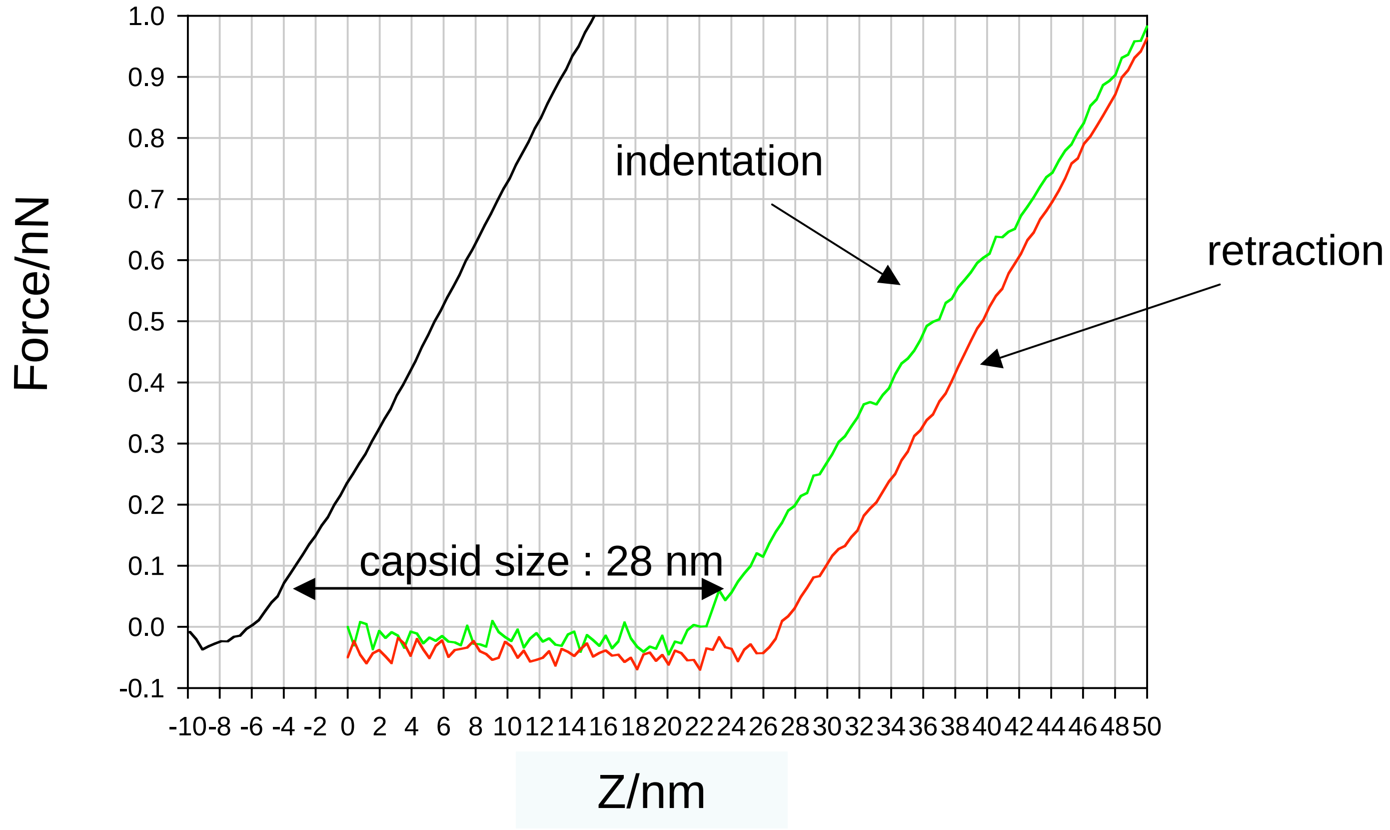
- Empty capsids can also be self-assembled
- **Capsid undergoes reversible radial expansion on change of pH**

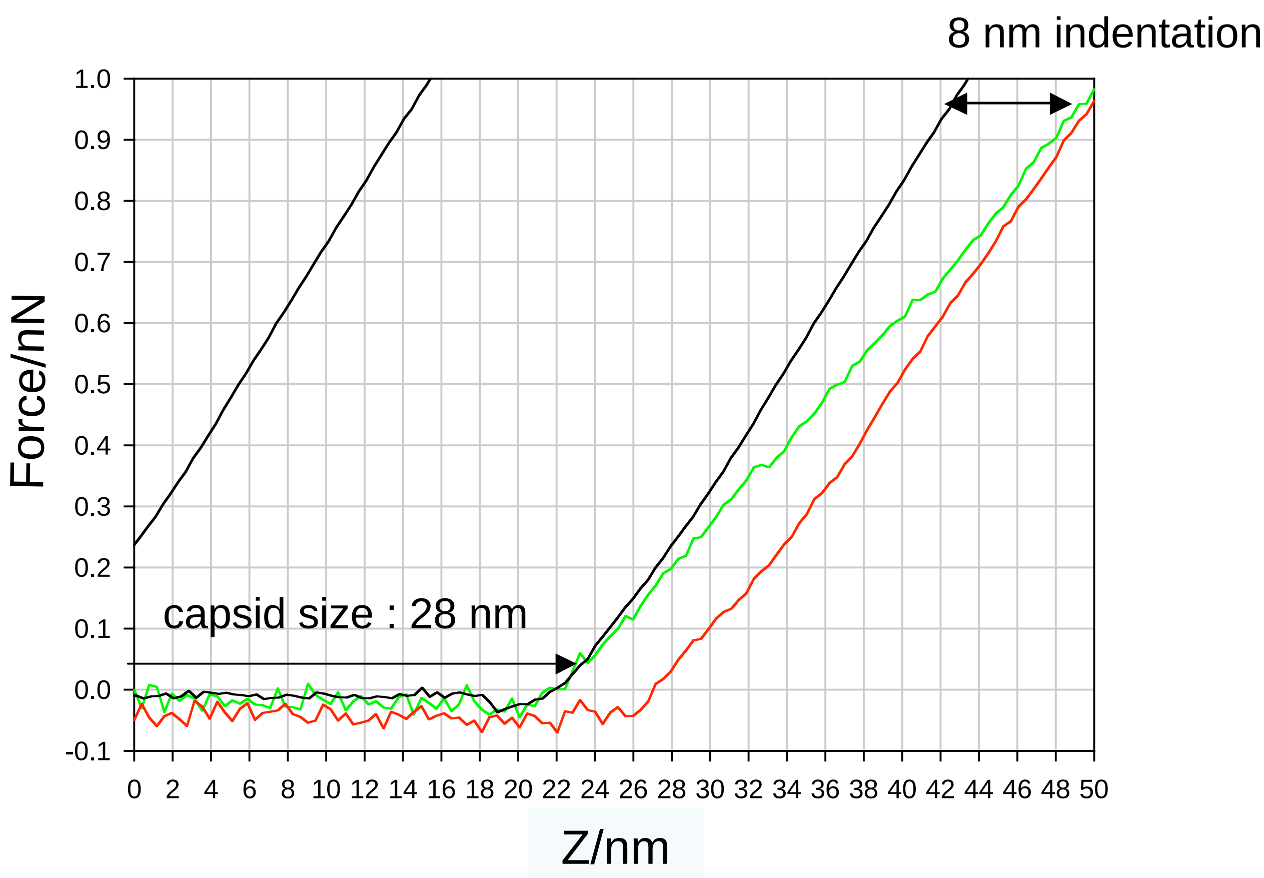
NANO-INDENTATION EXPERIMENT

Force Curve on Glass Surface

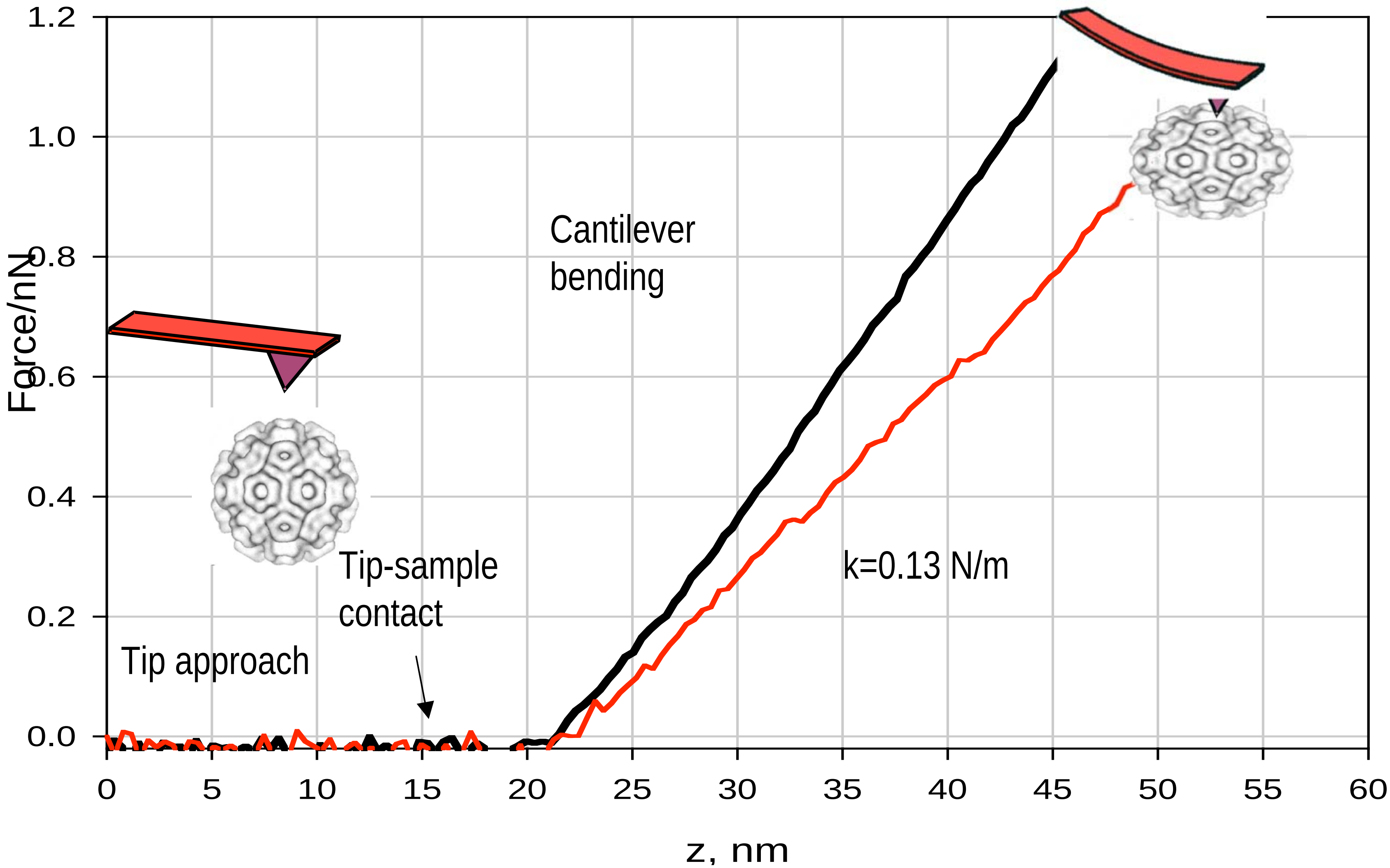




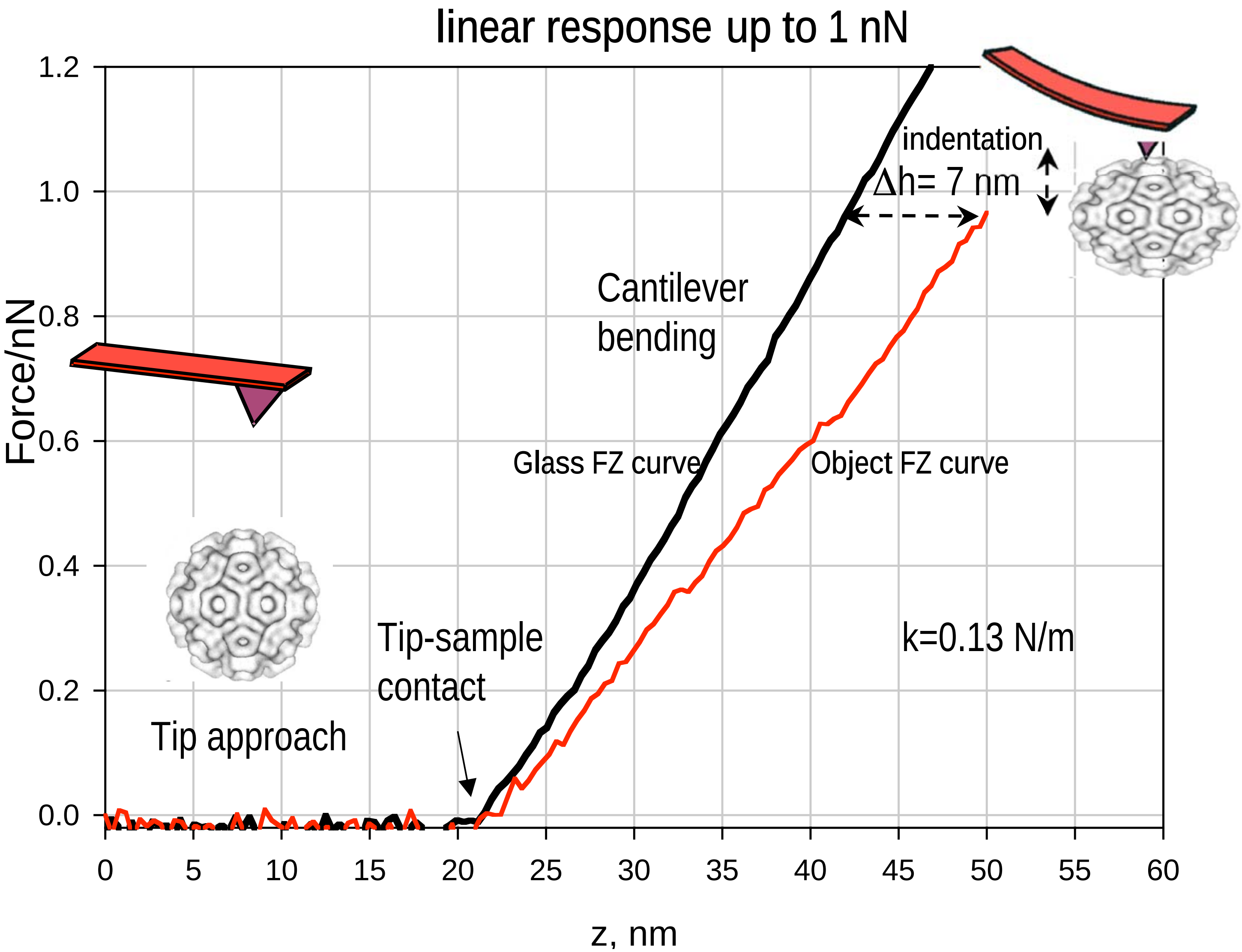
Force/nN
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-10 -8 -6 -4 -2 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
Z/nm
indentation
retraction
capsid size : 28 nm


8 nm indentation
capsid size : 28 nm
Force/nN
Z/nm
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50

CCMV – empty capsid



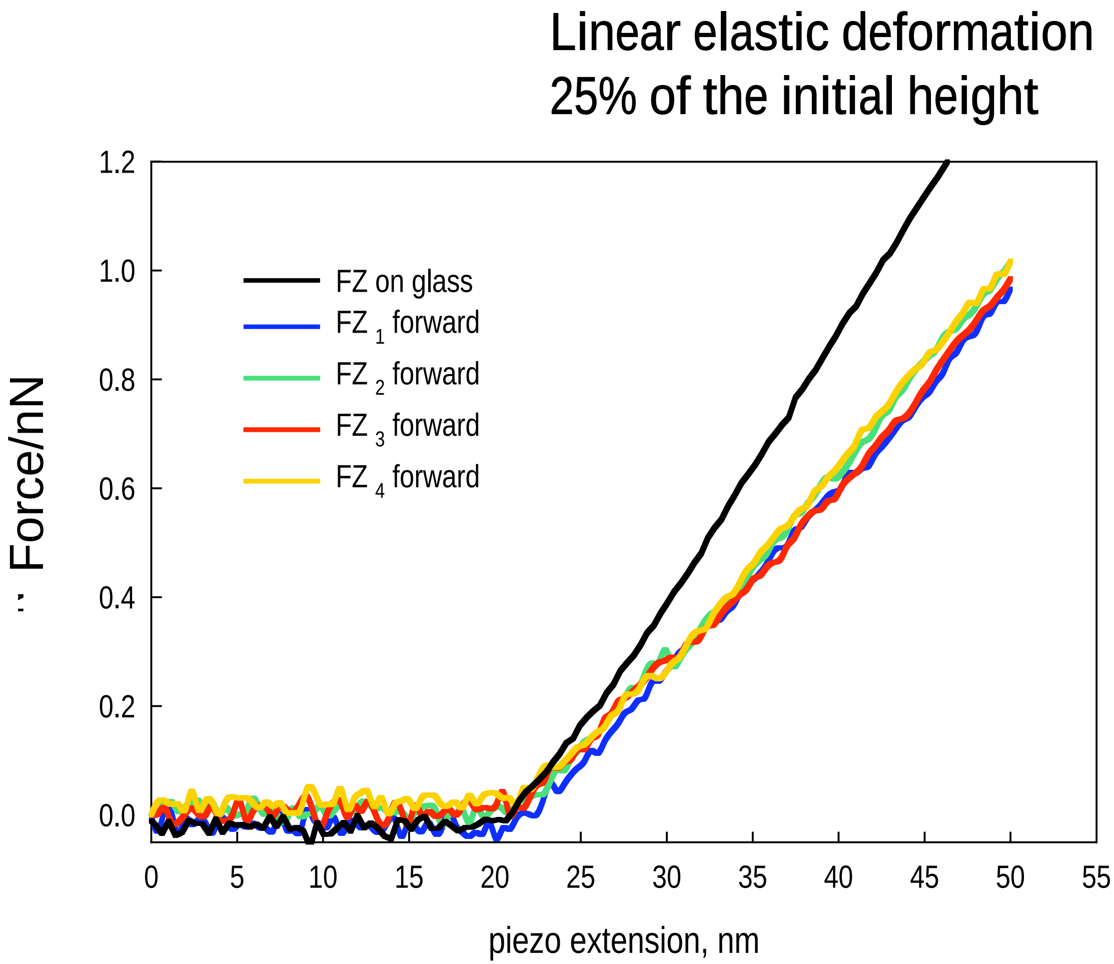
CCMV – empty capsid



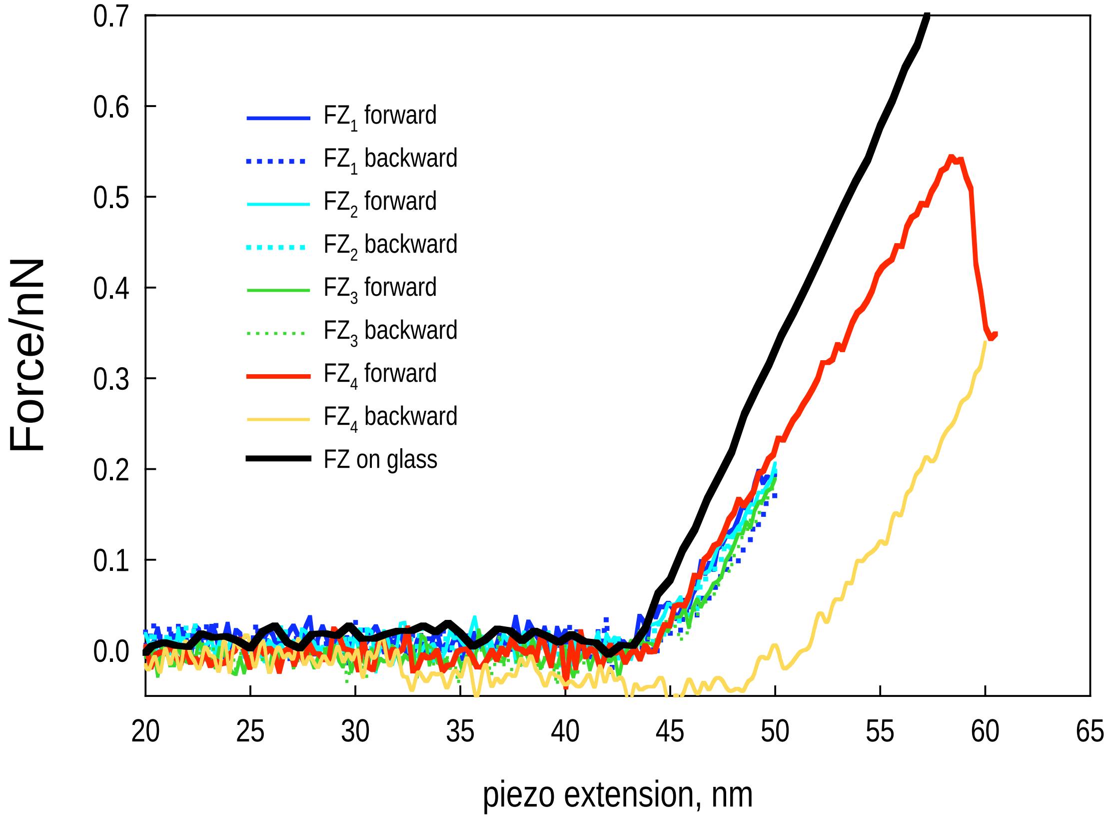
- Spring constant of the capsid is calculated from

$$k_{capsid} = \frac{k_{cant} k_{eff}}{k_{cant} - k_{eff}}$$

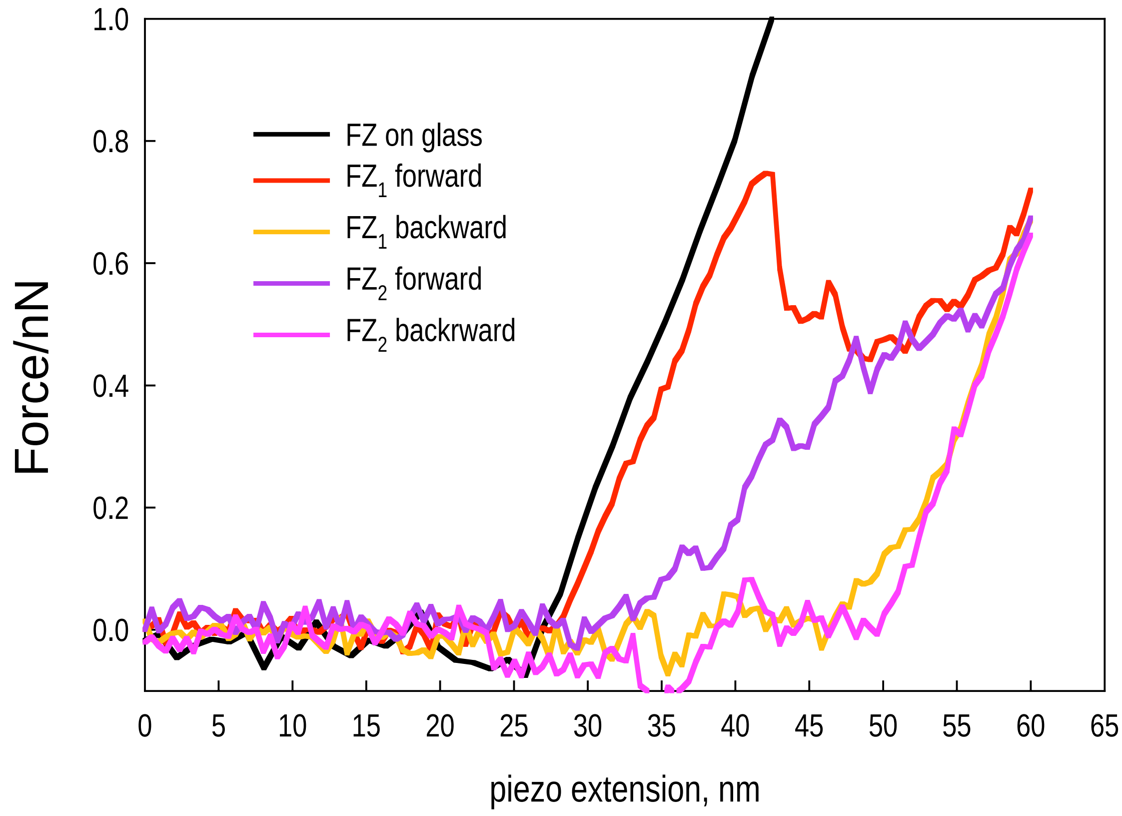
where $k_{cant}=0.05$ N/m



Linear elastic deformation
25% of the initial height
Force/nN
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
35
40
45
50
55
piezo extension, nm
FZ on glass
FZ 1 forward
FZ 2 forward
FZ 3 forward
FZ 4 forward

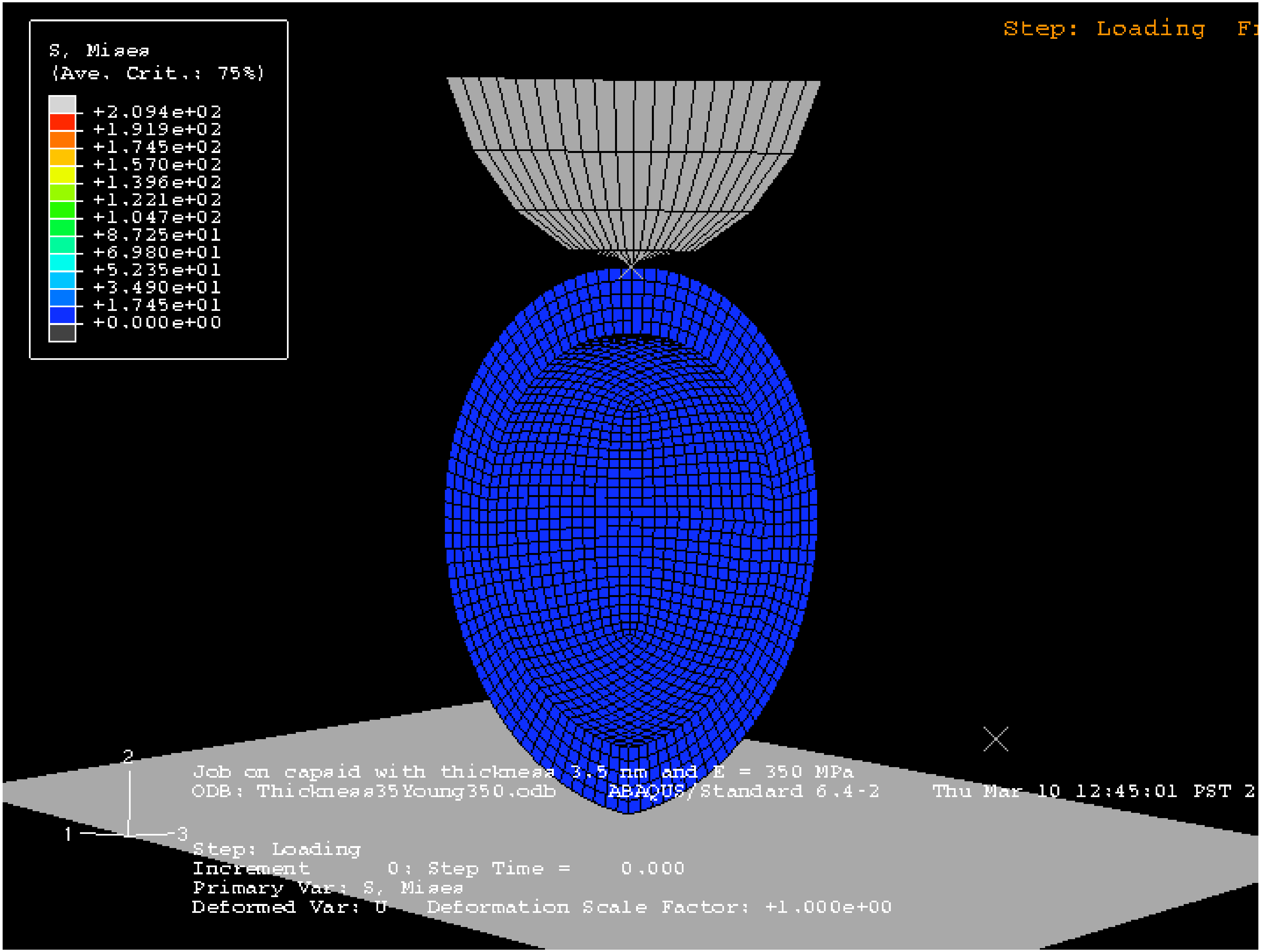


Force/nN
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
20
25
30
35
40
45
50
55
60
65
piezo extension, nm
FZ1 forward
FZ1 backward
FZ2 forward
FZ2 backward
FZ3 forward
FZ3 backward
FZ4 forward
FZ4 backward
FZ on glass



Force/nN
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
35
40
45
50
55
60
65
piezo extension, nm
FZ on glass
FZ1 forward
FZ1 backward
FZ2 forward
FZ2 backrward

The indentation of a thick homogeneous shell by an AFM tip can be modeled with finite-element analysis


S, Mises
(Ave. Crit.: 75%)
+2.094e+02
+1.919e+02
+1.745e+02
+1.570e+02
+1.396e+02
+1.221e+02
+1.047e+02
+8.725e+01
+6.980e+01
+5.235e+01
+3.490e+01
+1.745e+01
+0.000e+00
Step: Loading
Job on capsid with thickness 3.5 nm and E = 350 MPa
ODB: Thickness35Young350.odb ABAQUS/Standard 6.4-2 Thu Mar 10 12:45:01 PST 2
2
1
3
Step: Loading
Increment 0: Step Time = 0.000
Primary Var: S, Mises
Deformed Var: U Deformation Scale Factor: +1.000e+00

COMPRESSION OF A HOMOGENEOUS THIN SPHERICAL SHELL

RADIUS ***R*** THICKNESS ***h***

YOUNG'S MODULUS ***Y***

COMPRESSION DISTANCE ***d***

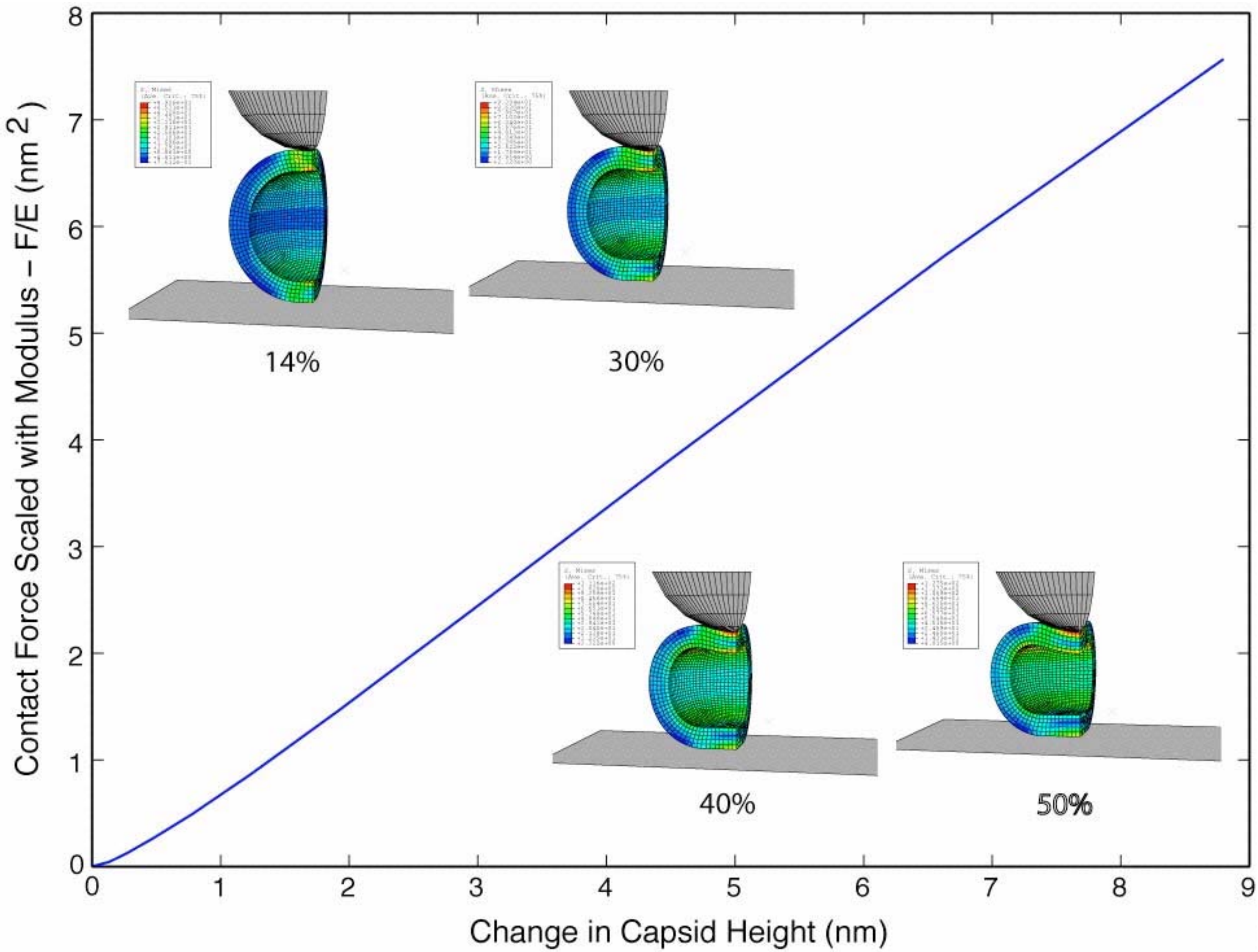
COMPRESSION BOTH STRETCHES

AND BENDS SHELL

$$F \sim (Yh^2/R)d = k_{capsid}d$$

Finite-element analysis of thick shells shows:

- The thin-shell approximation is reasonable to indentations of 15-20%
- Nonlinearity appears when the the capsid buckles



Contact Force Scaled with Modulus – F/E (nm 2)
Change in Capsid Height (nm)
0
1
2
3
4
5
6
7
8
9
14%
30%
40%
50%

Finite-element analysis also allows us to examine the Conditions of the experiment, such as:

- The effect of the tip size
- The effect of the tip position

and to estimate local stresses.

Values of Young’s Modulus of the order of 150 MPa are found, typical soft plastics such as Teflon

SPRING CONSTANTS (N/m)

EMPTY

WT	0.15 ± 0.01
subE	0.19 ± 0.02

FULL

WT	0.20 ± 0.02
salt stable	0.31 ± 0.02

PHAGE PROCAPSID

ϕ**29**	0.30

In both mutants studied, one lysine residue in the 180-protein capsid has been replaced by an arginine

X-ray crystal analysis shows that this one mutation produces 660 new inter-subunit interactions (hydrogen bonds, salt bridges) per particle at the center of the 20 hexameric capsomers

J. A. Speir, et al., *J. Virol*. **80,** 3582 - 91 (2006)

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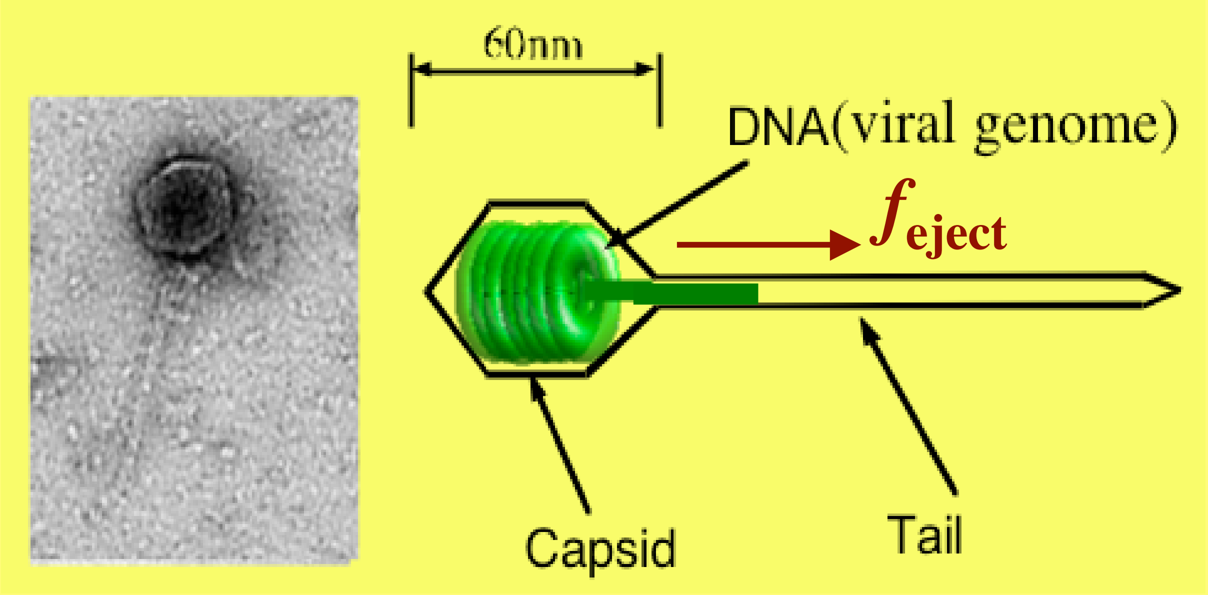
PHAGE PROCAPSID

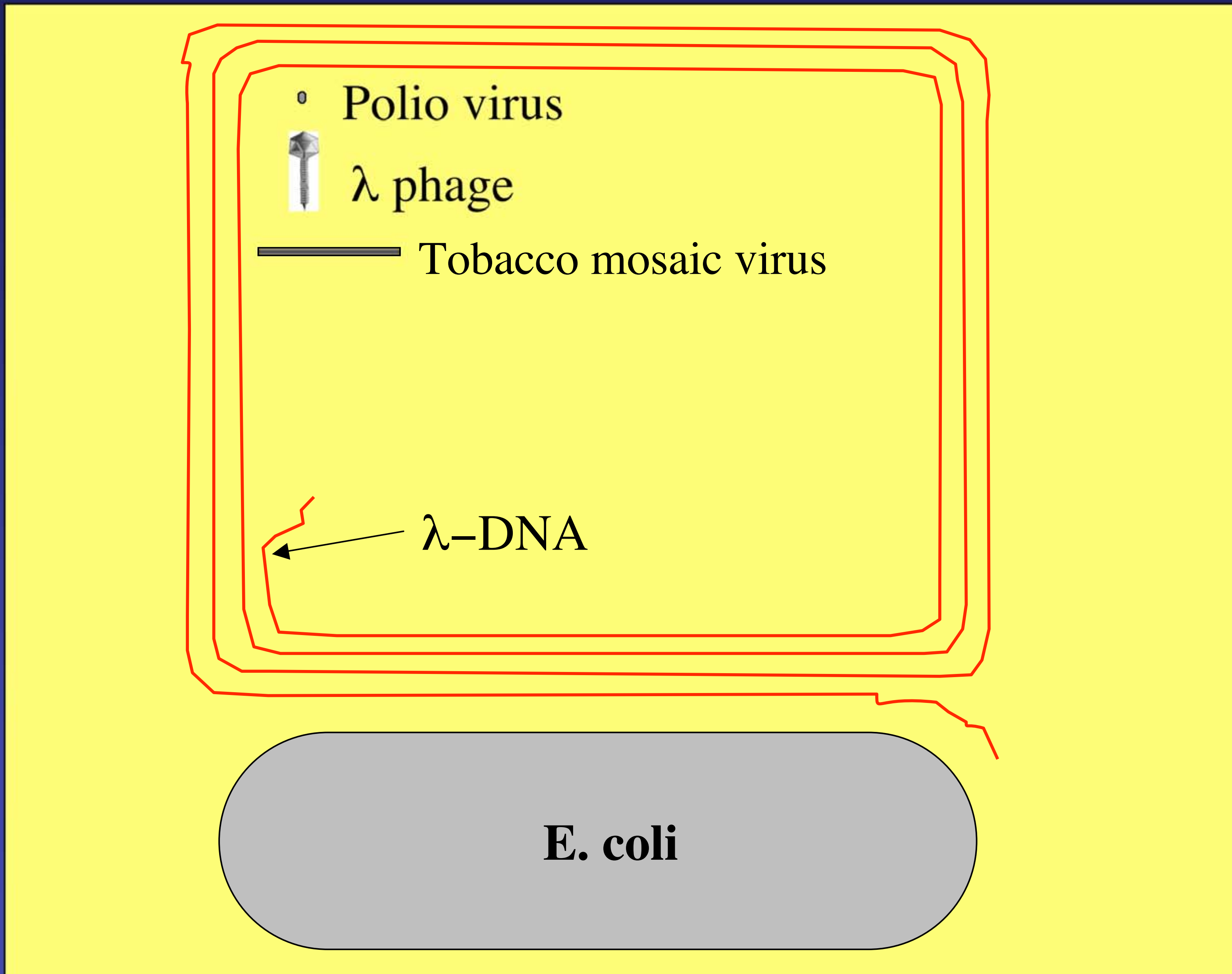
ϕ29	0.30

E = 2 GPa

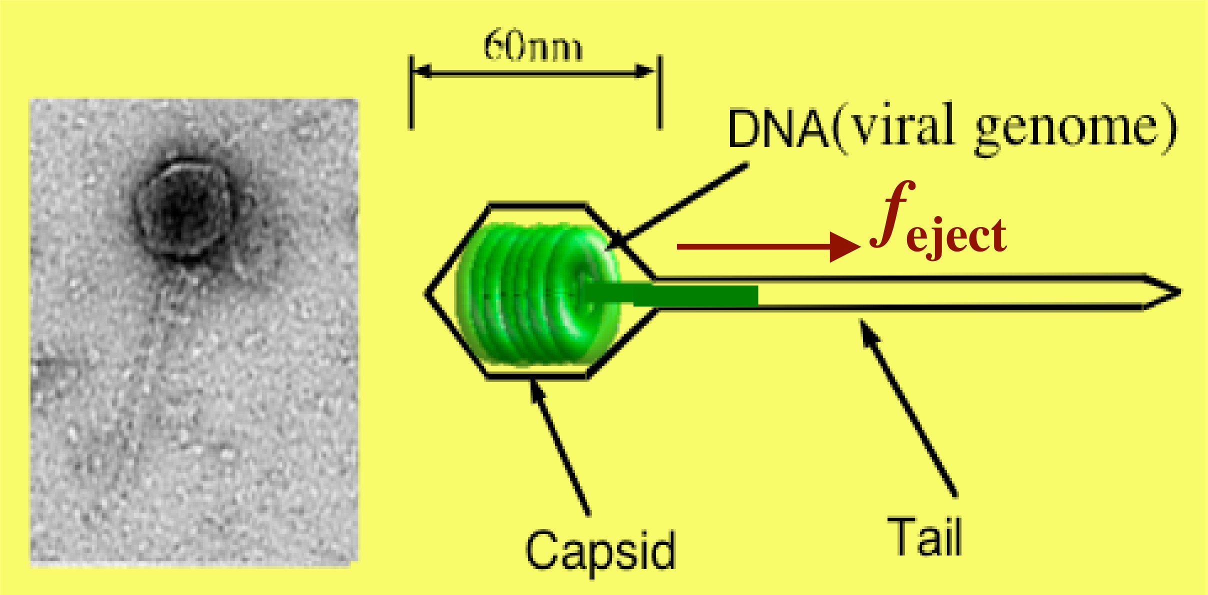
Bacteriophage lambda



Viral genome vs capsid size



Bacteriophage lambda

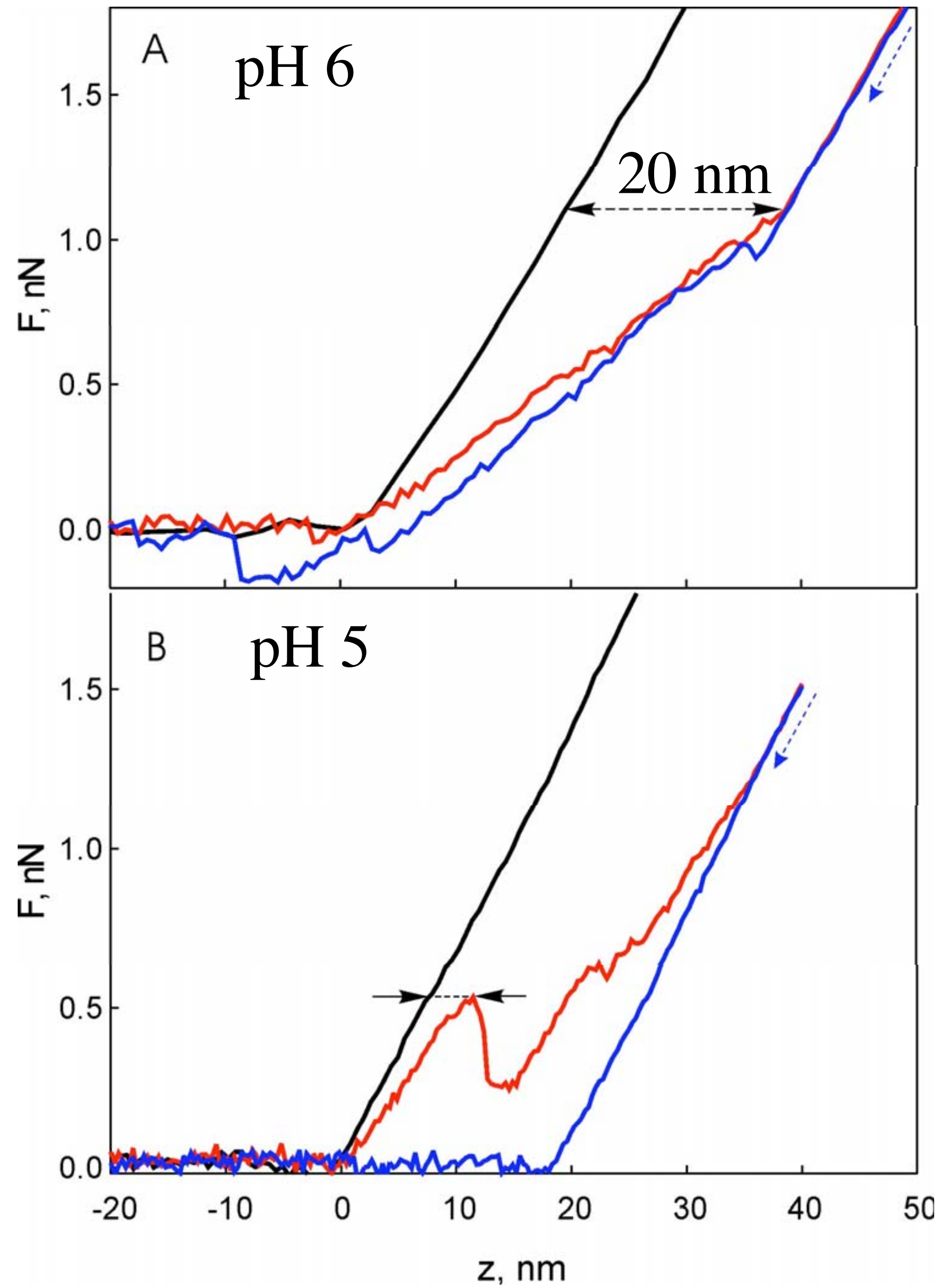


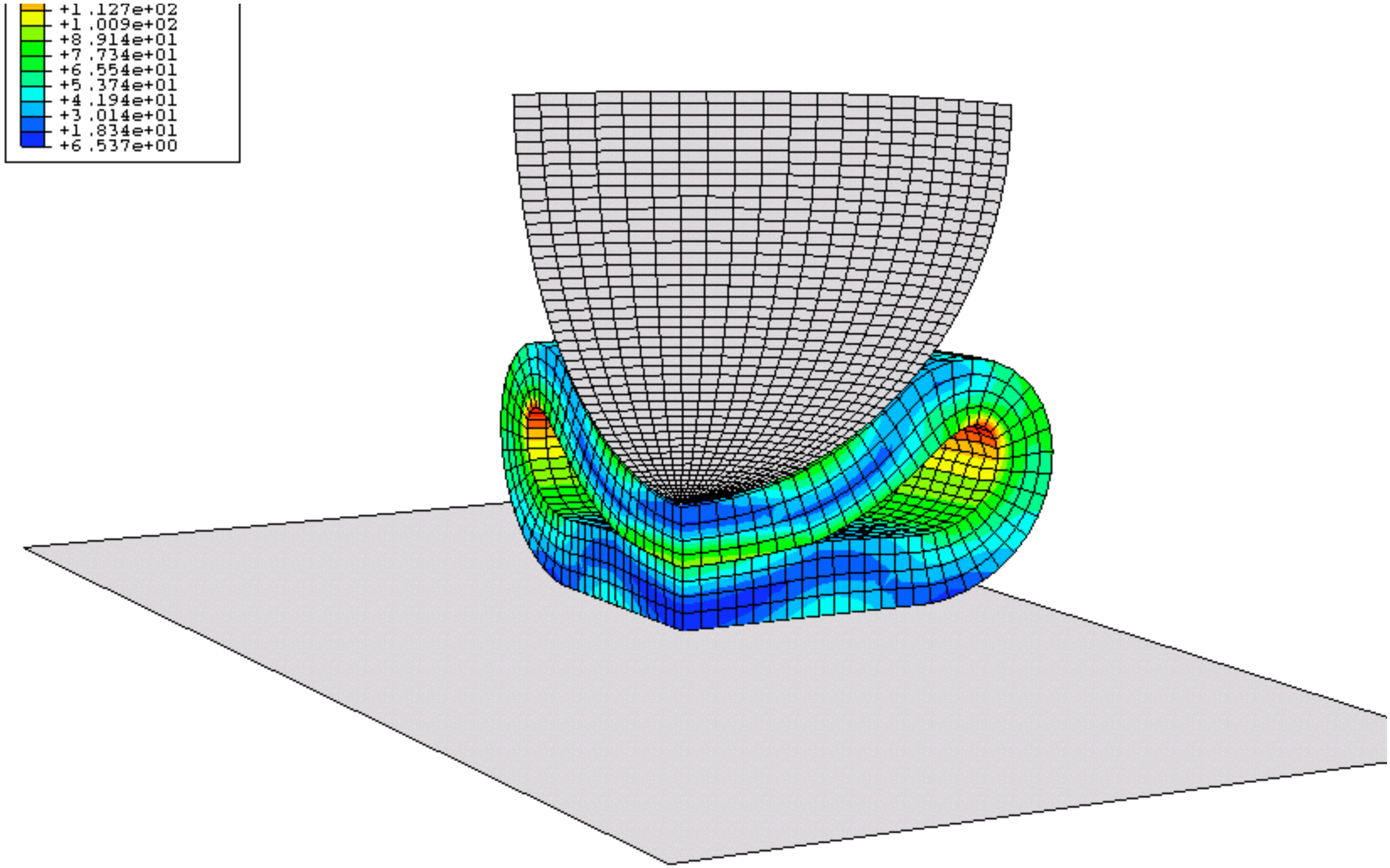
- DNA in capsid is highly stressed
- Electrostatic Repulsion
- Bending Energy - Persistence Length 50 nm

J. T. Kindt, Tzlil, S., A. Ben Shaul, W. M. Gelbart, PNAS 98:13671, 2001

A. Evilevitch, L. Lavelle, C. M. Knobler, E. Raspaud , W.M. Gelbart, PNAS 103: 9292, 2003

Empty capsids




+1.127e+02
+1.009e+02
+8.914e+01
+7.734e+01
+6.554e+01
+5.374e+01
+4.194e+01
+3.014e+01
+1.834e+01
+6.537e+00

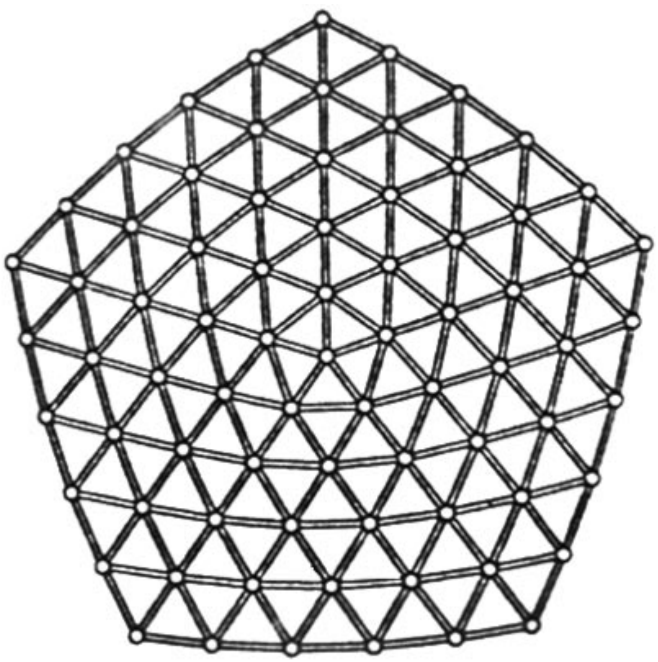
Lidmar, Mirny, Nelson *PRE 2004*

Föppl-von Kármán Number $\gamma = YR^2/\kappa$

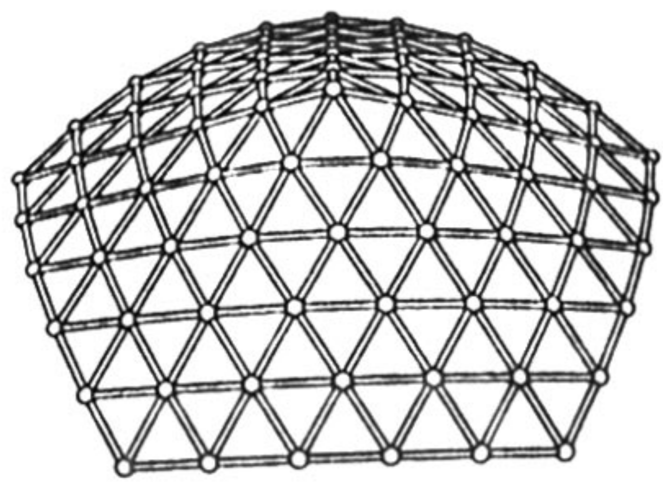
Y = 2-*D Young's modulus* κ =*Bending modulus*

$\gamma < 250$

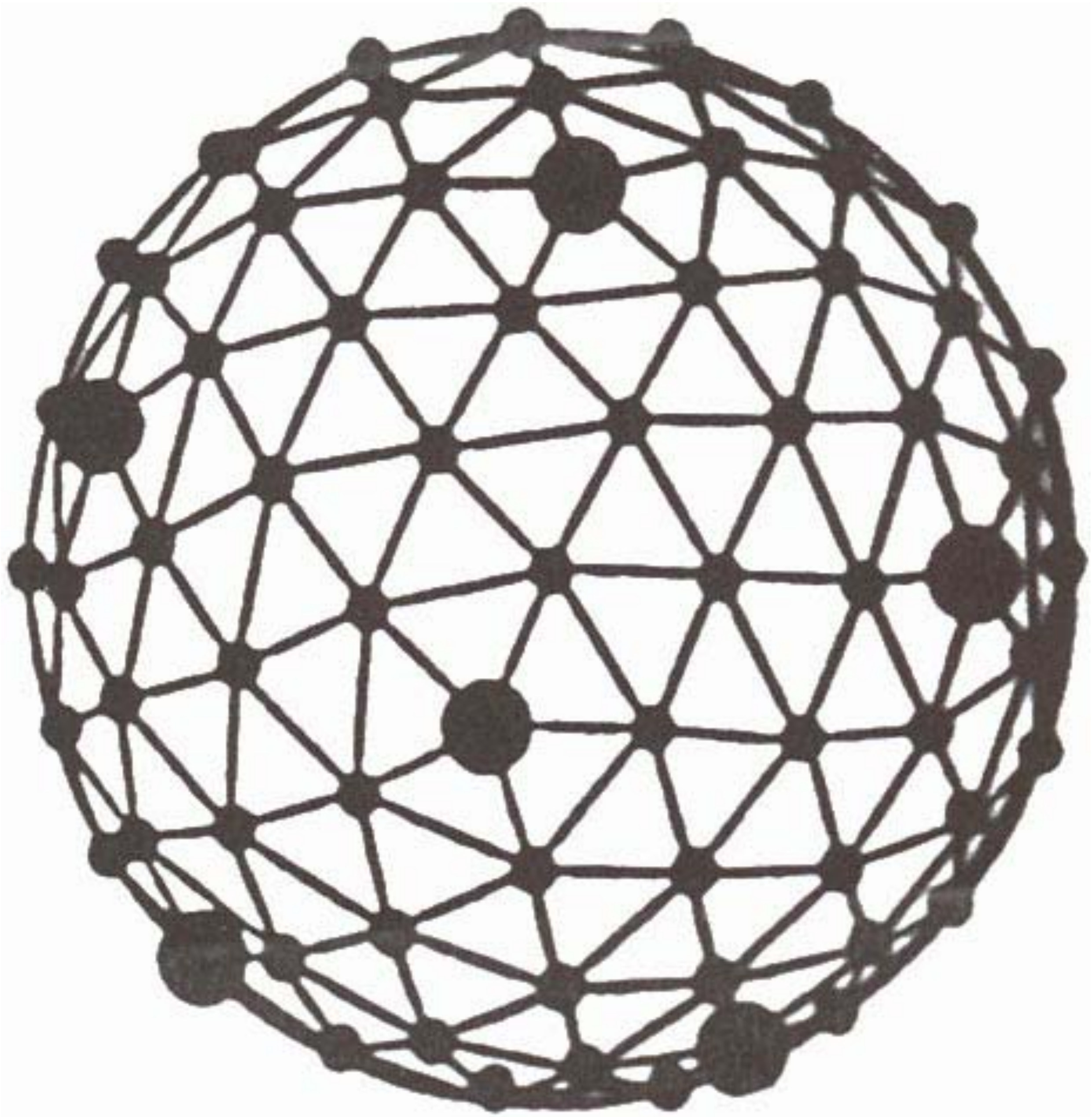
$\gamma > 250$



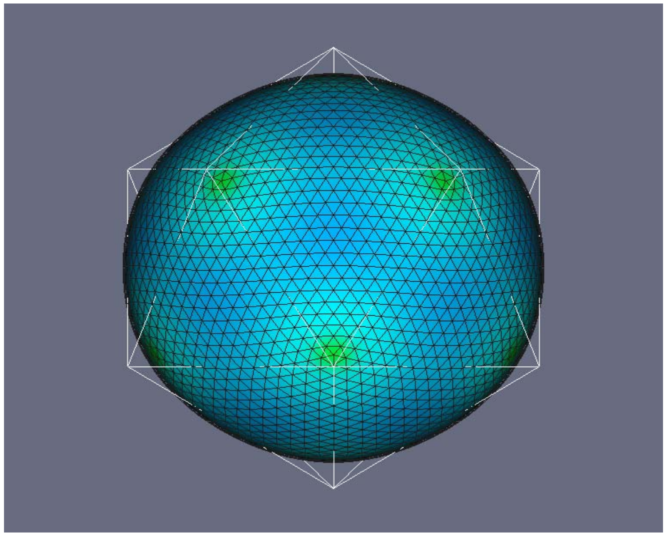
(a)



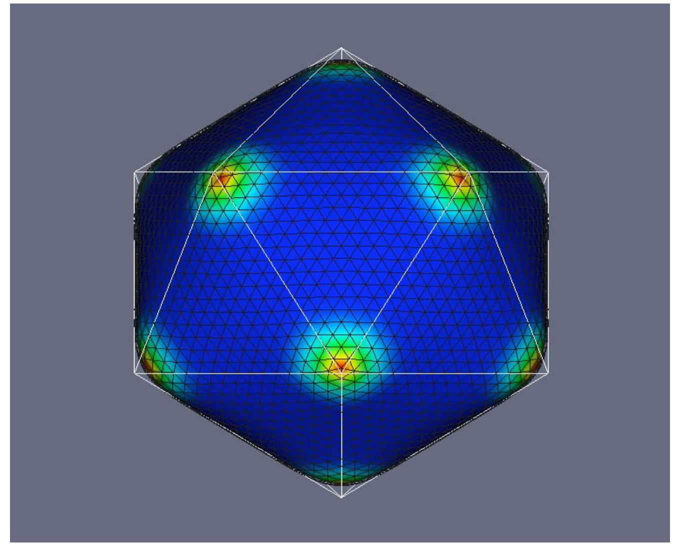
(b)



$\gamma = 100$



$\gamma = 1200$



W. S. Klug, et al., “Failure of Viral Shells,”
submitted to PRL

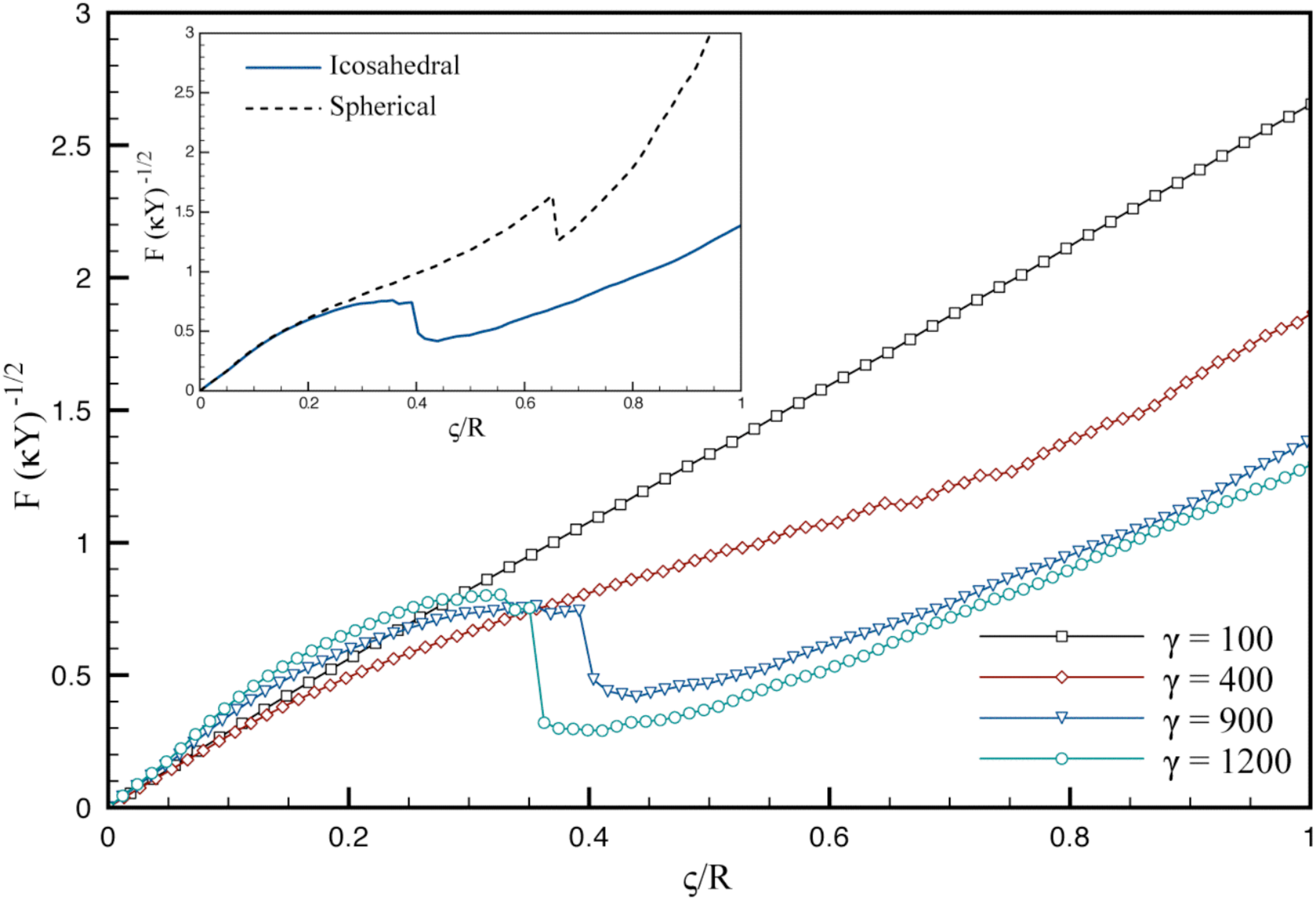
Deformation of thin spherical shell

$$F \propto \sqrt{\kappa Y}\,\frac{\zeta}{R}$$

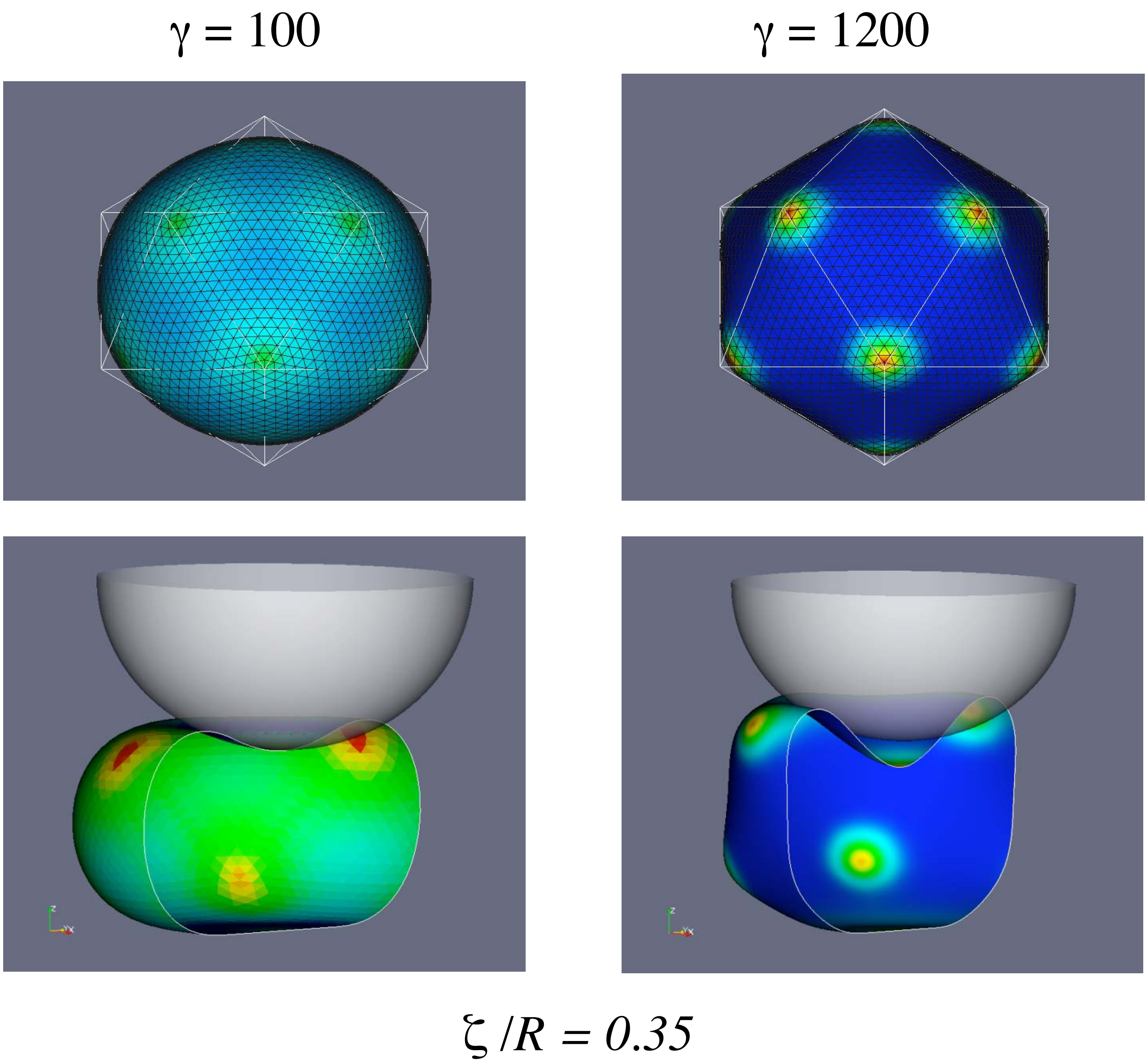
Y - 2D Young's Modulus (in-plane elasticity)

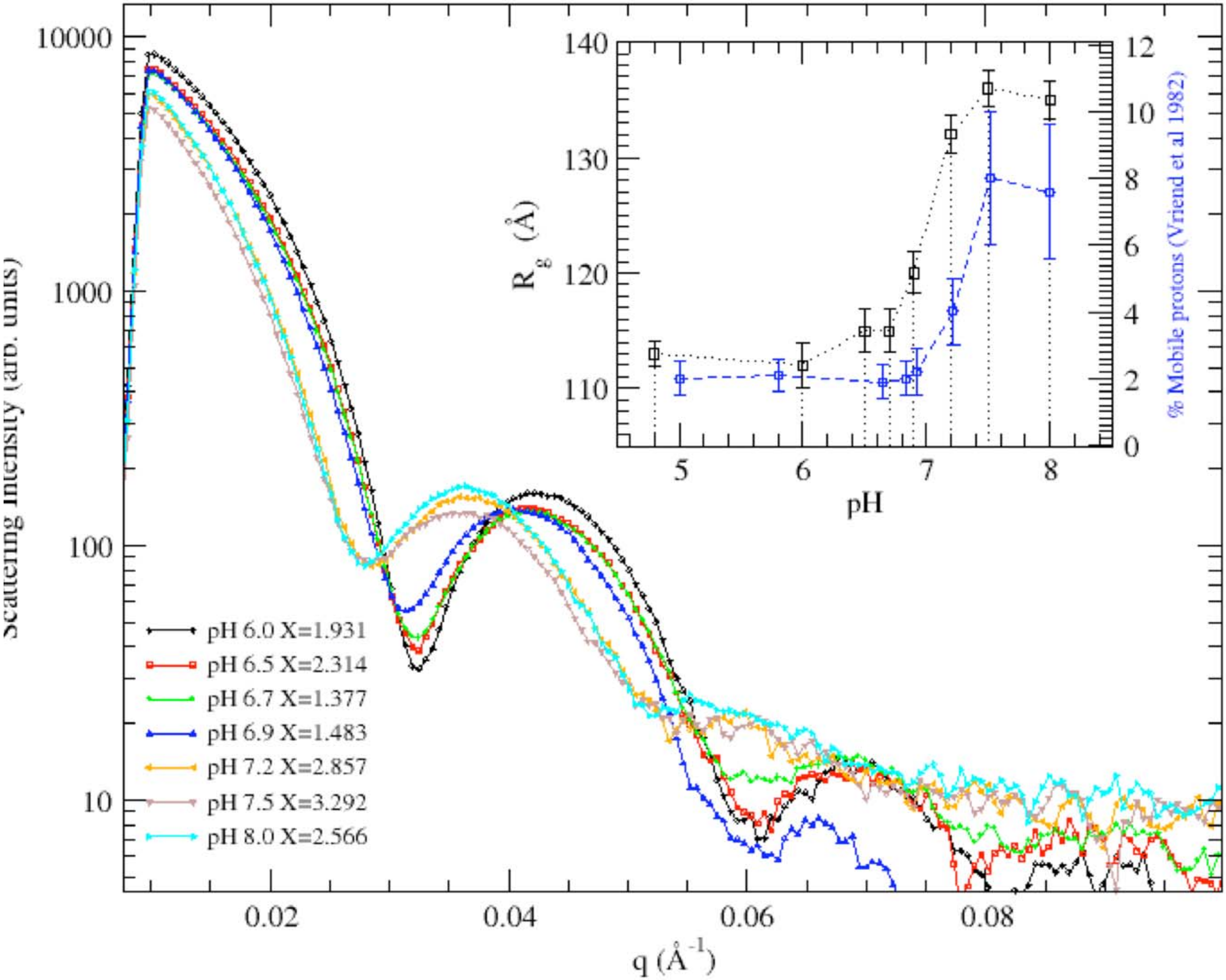
κ - bending energy (out of plane)

z - indentation   R - radius



F (κY)^-1/2
ς/R
Icosahedral
Spherical
γ = 100
γ = 400
γ = 900
γ = 1200


γ = 100
γ = 1200
ζ /R = 0.35



Scattering intensity (arb. units)
10000
1000
100
10
0.02
0.04
0.06
0.08
q (Å^{-1})
pH 6.0 X=1.931
pH 6.5 X=2.314
pH 6.7 X=1.377
pH 6.9 X=1.483
pH 7.2 X=2.857
pH 7.5 X=3.292
pH 8.0 X=2.566
R_g (Å)
140
130
120
110
5
6
7
8
pH
% Mobile protons (Vriend et al 1982)
12
10
8
6
4
2
0

CCMV

Pores 2 nm in size

pH = 5
native CCMV
d = 28 nm

pH = 7.5
swollen CCMV
expands by 10%

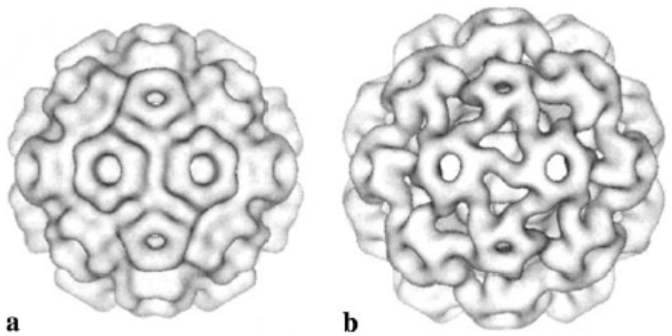


Fig. 2. Cryo-EM image reconstructions of (a) native CCMV and (b) swollen CCMV particles. The capsids are placed with icosahedral twofold axis perpendicular to the page. The swollen structure expands by ~10% from the native form, with the largest changes occurring at the icosahedral and quasi-twofold axes.

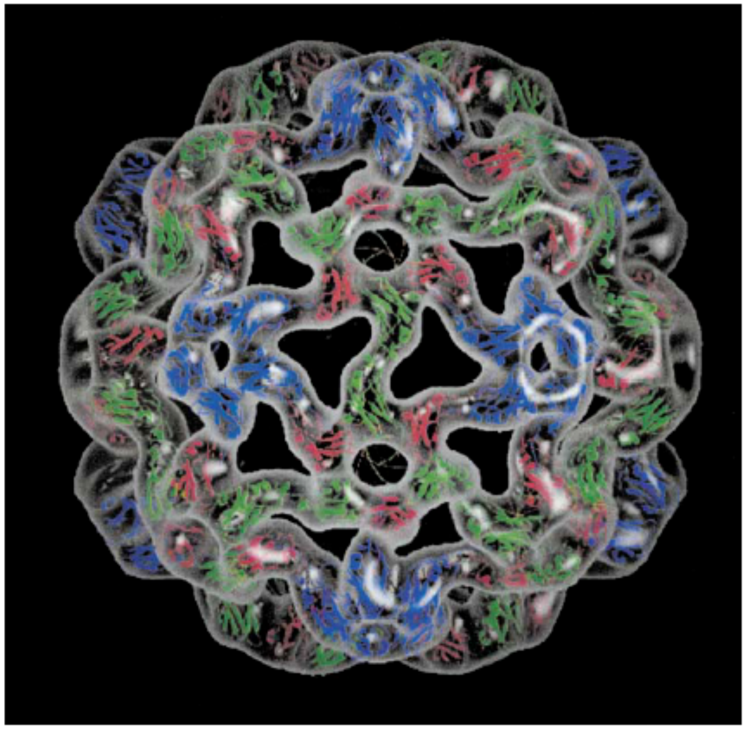
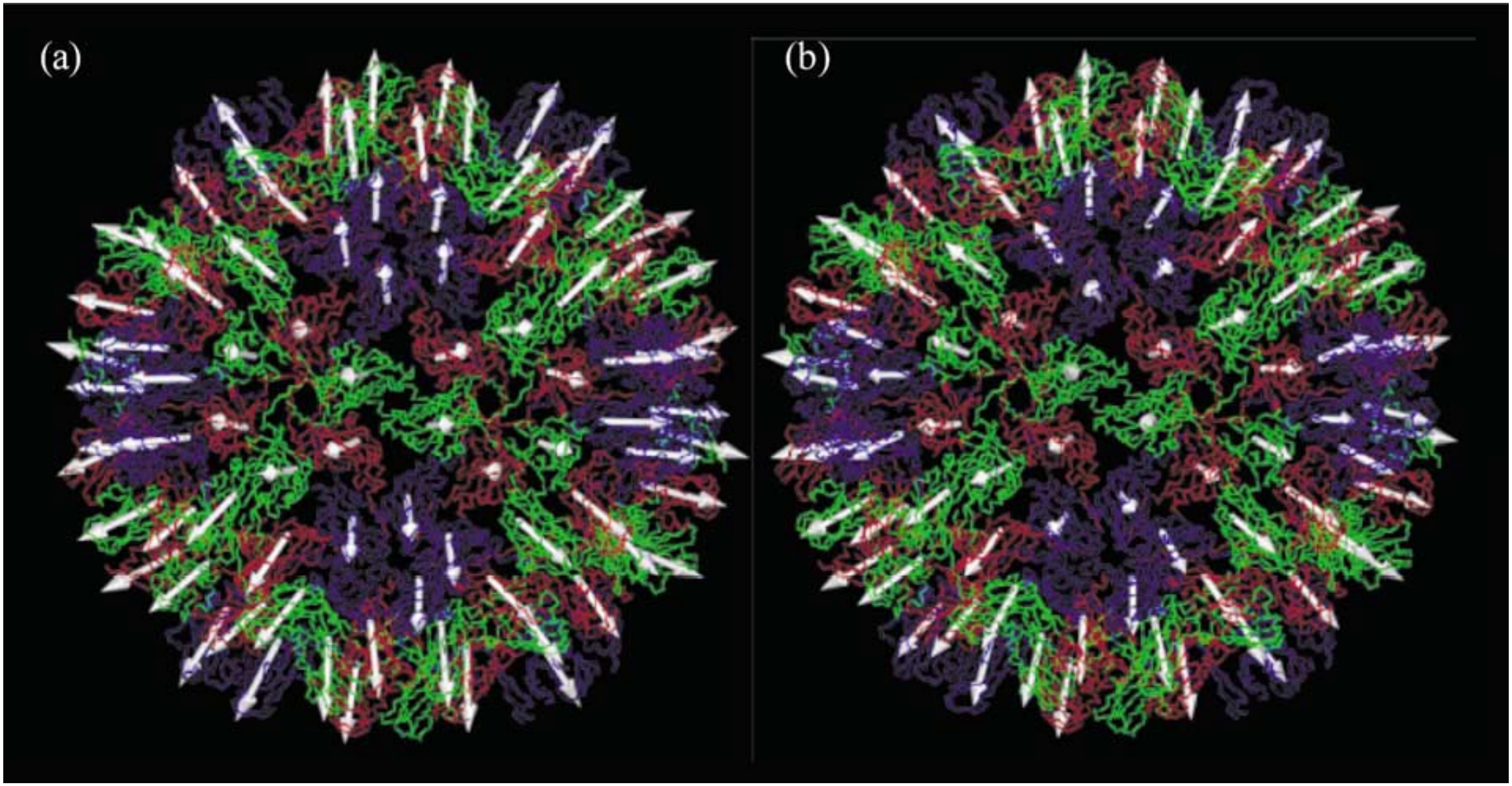


Fig. 4. Electron density fitted with the final model for the swollen form of CCMV.



Amplitude and direction of motion from

(a) structural data (b) normal mode 24

F. Tama and C.L. Brooks III, *J. Mol. Bio.* **318**, 733-47 (2002)

CONCLUSIONS

- At pH 5, CCMV capsids are remarkably elastic for indentations of up to 25%
- Buckling, followed by breakage, is observed at higher indentations. The nature of the failure is not yet understood
- The linear behavior and buckling are consistent with finite element analysis studies of **thick** homogeneous shells

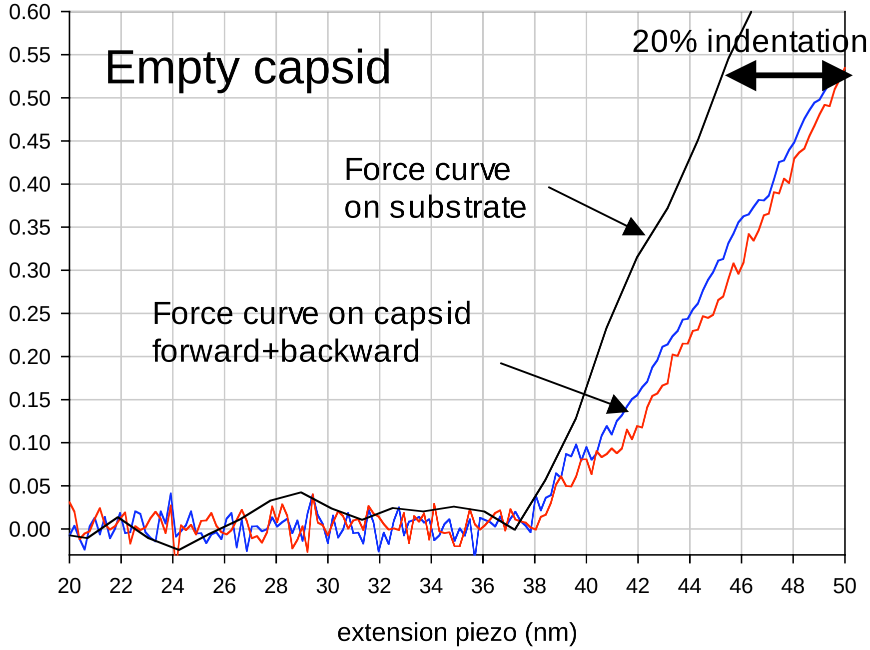
- The remarkable elasticity at pH 6 can be related to a decrease in the Föppl-von Kármán Number as the result of a soft mode
- Not surprisingly, the coupling of the RNA to the capsid increases the stiffness
- Differences in elasticity can be correlated with capsid protein mutations

A Landau-Ginsburg treatment of a weakly deformed spherical elastic shell subject to a soft mode instability (Guérin & Bruinsma) shows that the force deformation relation is:

$$F \propto \sqrt{\kappa Y^{*}}\,\frac{\zeta}{R}$$

with Y^{*} a reduced effective Young's Modulus

Small indentation at pH 5

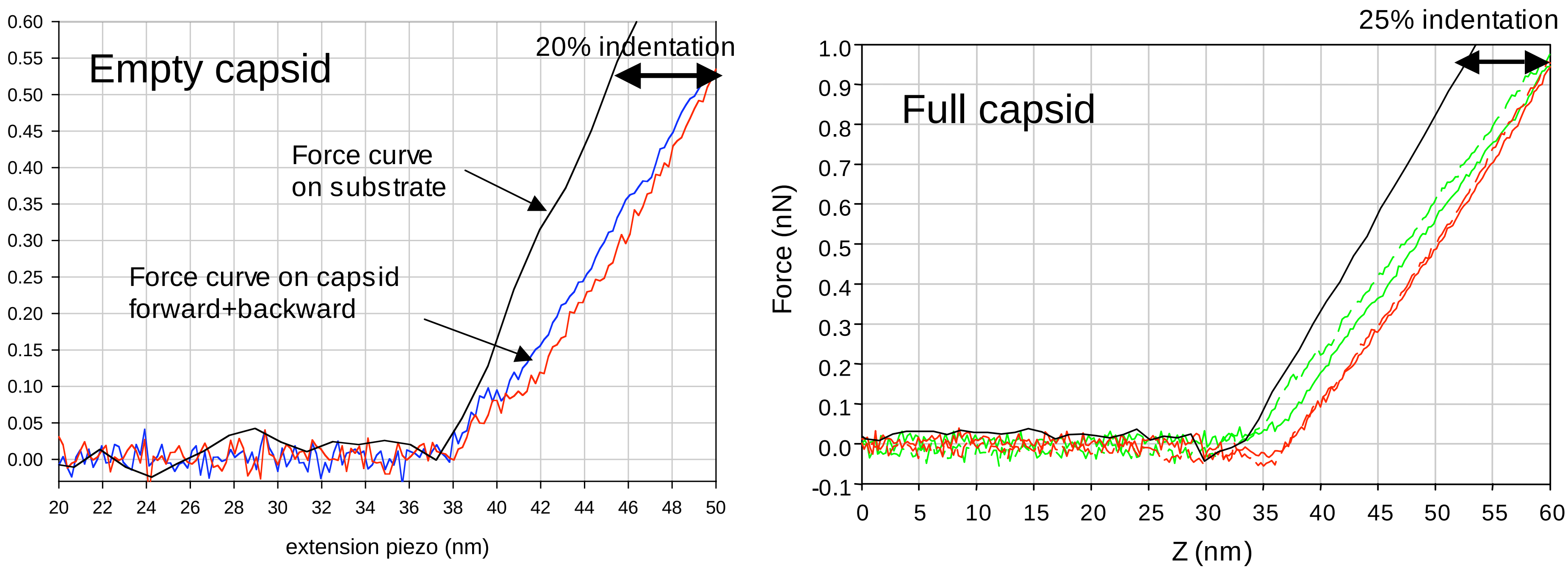


linear and reversible regime

capsid unchanged

***k*(empty) ~ 0.15-0.18 N/m**

Small indentation at pH 5



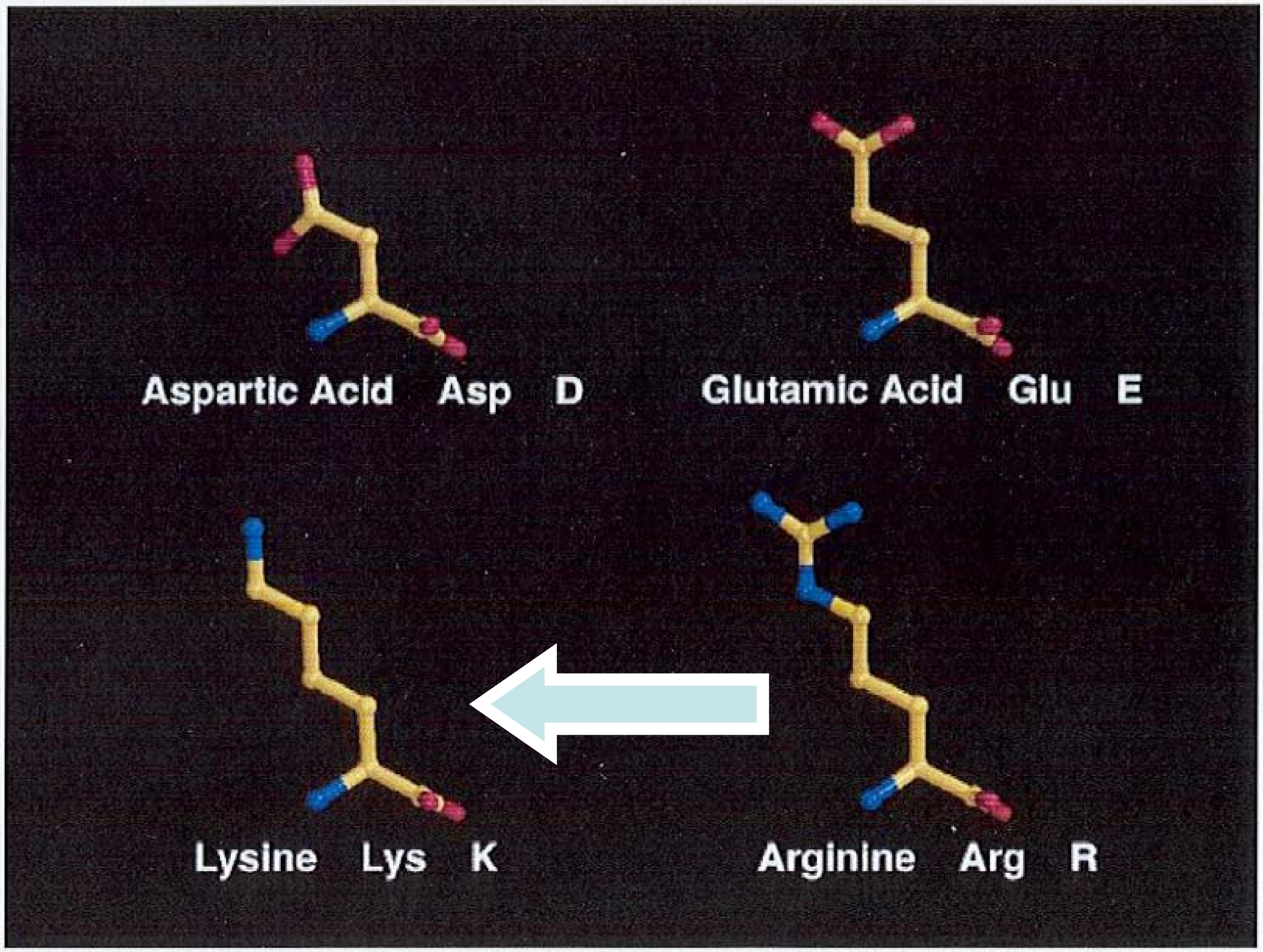
⇒ **linear and reversible regime**

capsid unchanged

***k*(empty) ~ 0.15 N/m**

***k*(full) ~ 0.20 N/m**

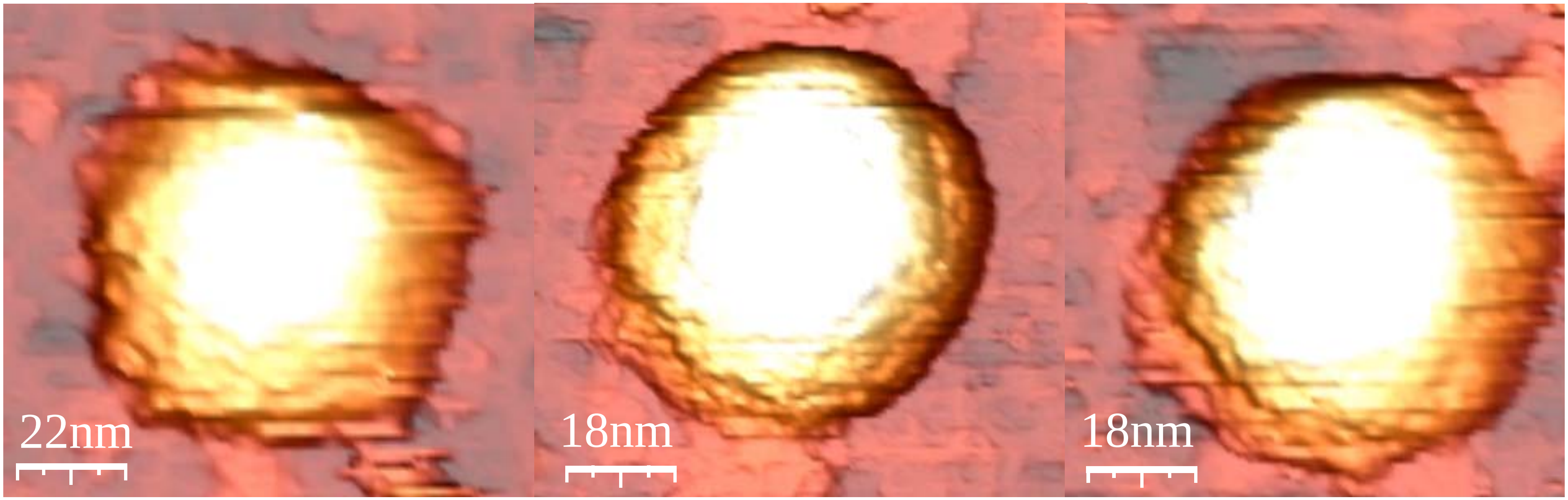
Charged Amino Acids



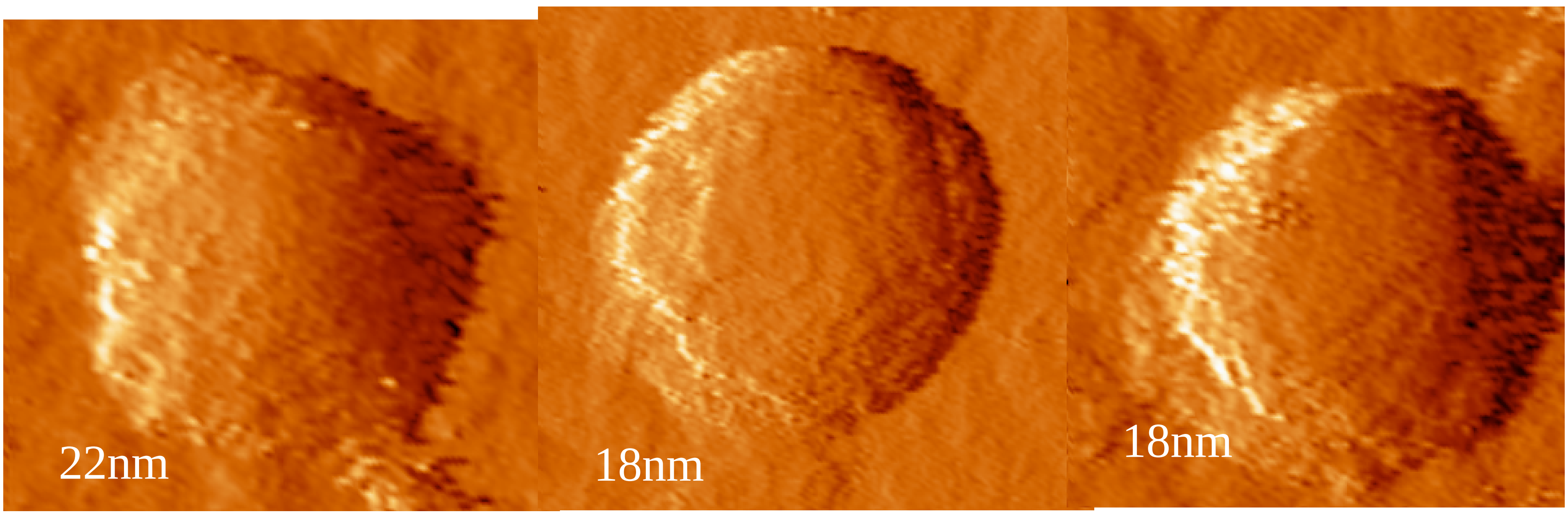
THERE ARE THREE TYPES OF ICOSHEDRAL SITES ON THE CAPSID CORRESPONDING TO THE 2-, 3- AND 5-FOLD AXES

THEY HAVE ROUND, HEXAGONAL AND PENTAGONAL PROJECTIONS ON THE SURFACE

IF THE ADSORPTION OF THE CAPSID IS RANDOM, THEN THESE DISTINCT SHAPES SHOULD BE SEEN IN THE AFM IMAGES AND THEY SHOULD APPEAR IN THE RATIO 30:20:12



TOPOGRAPHIC IMAGES



DERIVATIVE IMAGES

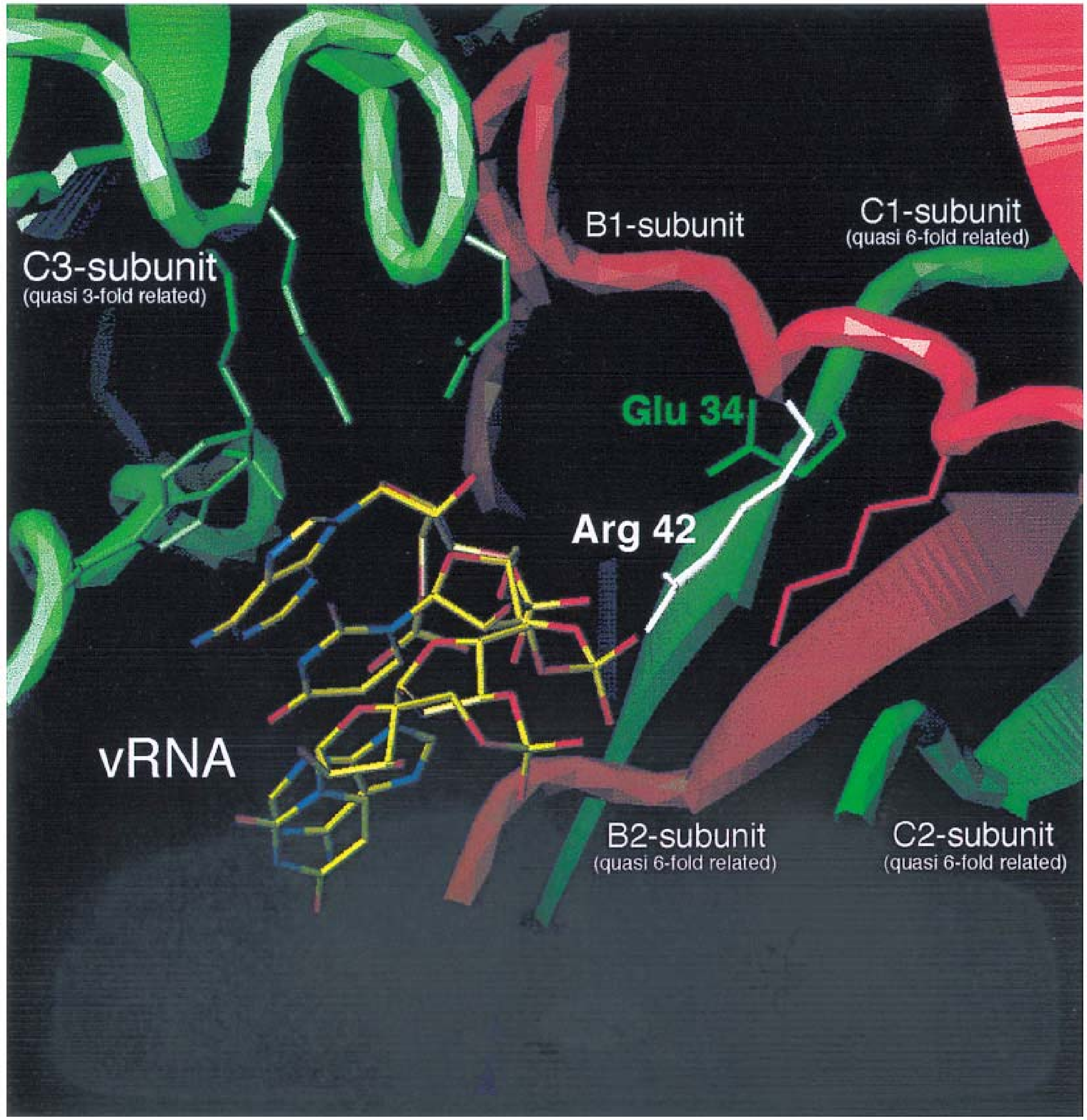
	Capsid Heights (nm)		
	Pentagonal	Hexagonal	Circular
Full	25.4 +/- 0.3	27.7 +/- 0.2	27.5 +/- 0.3
Empty	24.6 +/- 0.3	28.6 +/- 0.3	28.7 +/- 0.2

JUMPING MODE AFM MEASUREMENTS

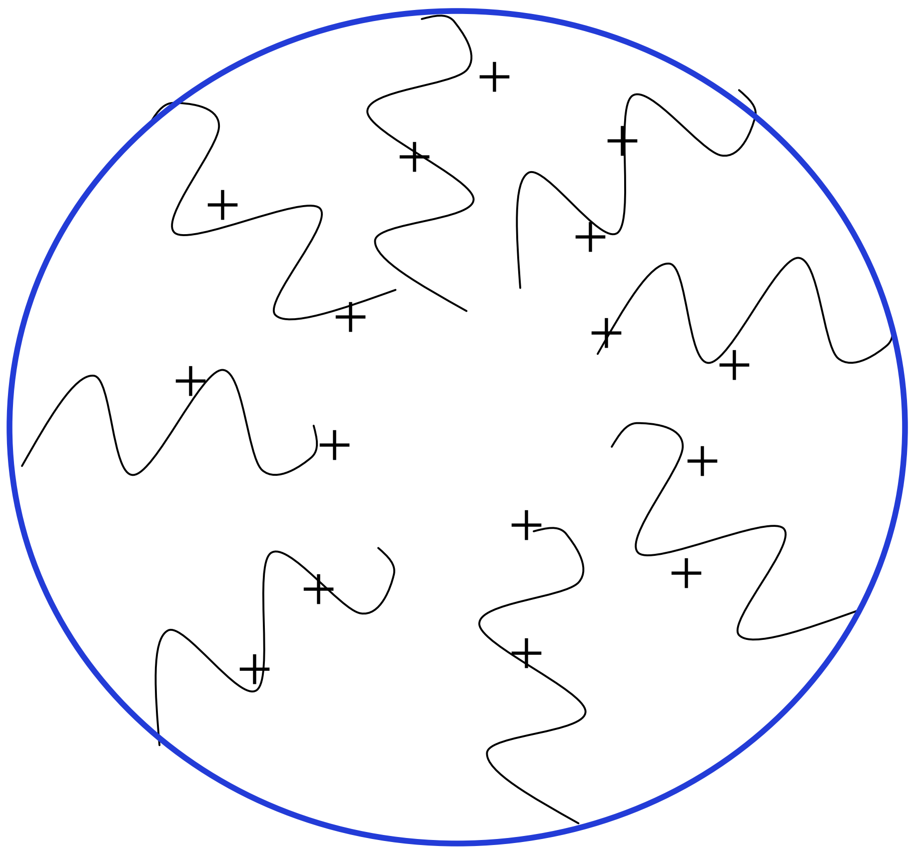
IMAGING BY RASTER SCAN OF
FORCE VS DISTANCE FROM SURFACE
ONLY NORMAL FORCES APPLIED--NO SHEAR

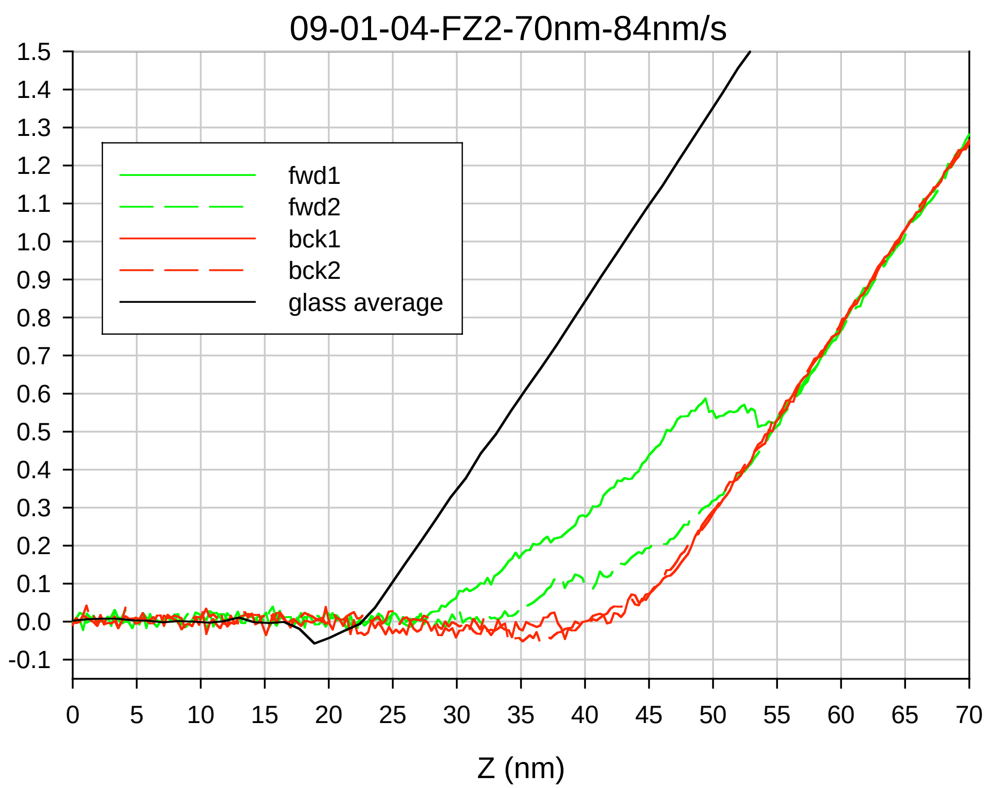
NANO-INDENTATION MEASUREMENTS
REPEATED FORCE VS DISTANCE
MEASUREMENTS AT A SINGLE POINT

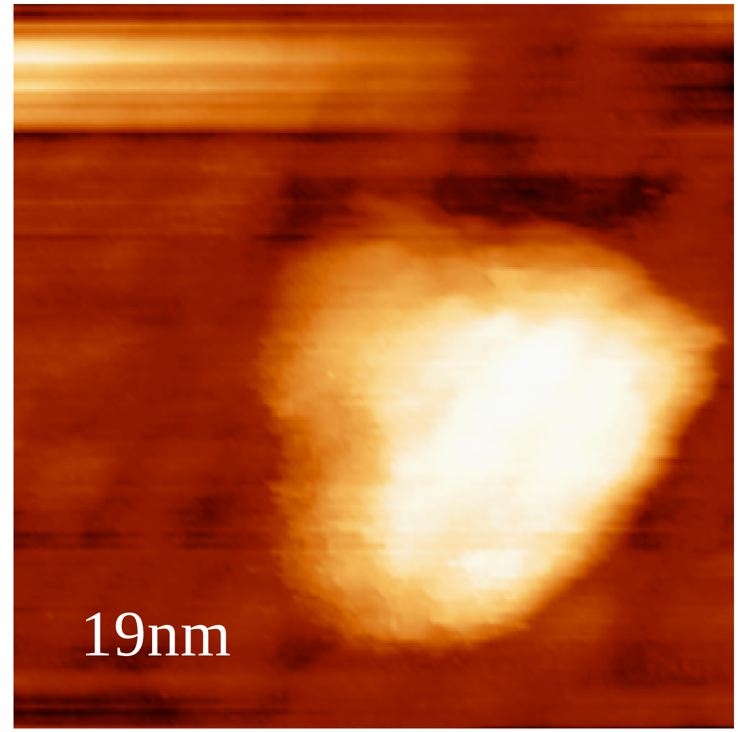
In the mutant,
a lysine residue
is replaced by
an arginine.
The residue
interacts more
strongly with the
glutamic acid of
a neighboring protein

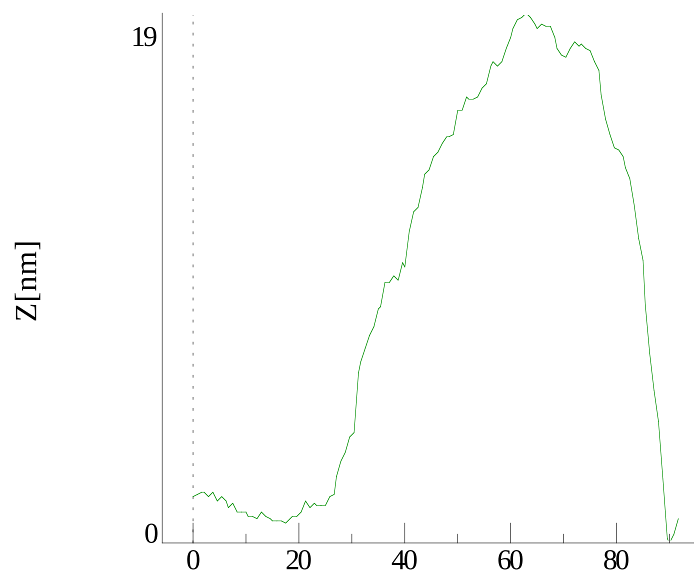


Capsid proteins have short acidic tails that project into capsid

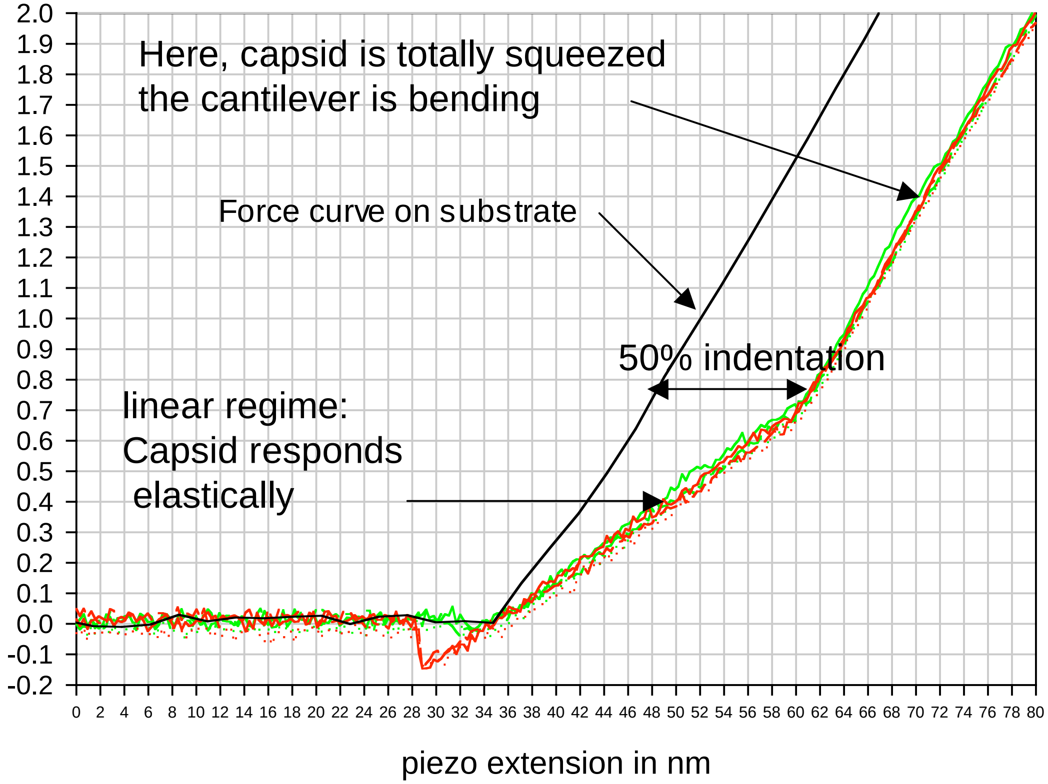


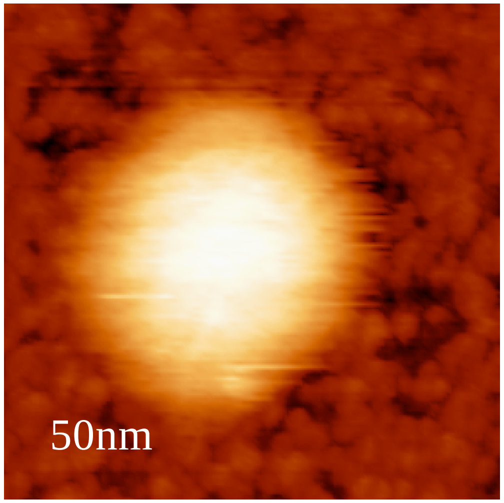

09-01-04-FZ2-70nm-84nm/s
fwd1
fwd2
bck1
bck2
glass average
Z (nm)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70


19nm

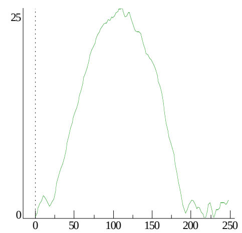

Z[nm]
19
0
0
20
40
60
80

Empty wt capsid at pH 6

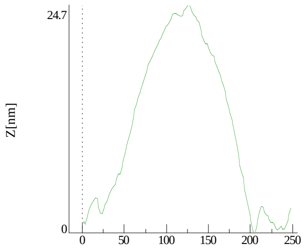


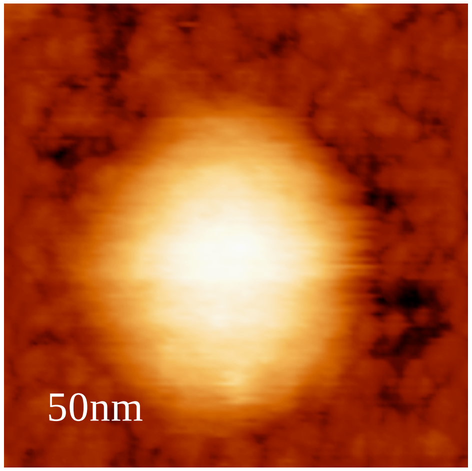


before



after

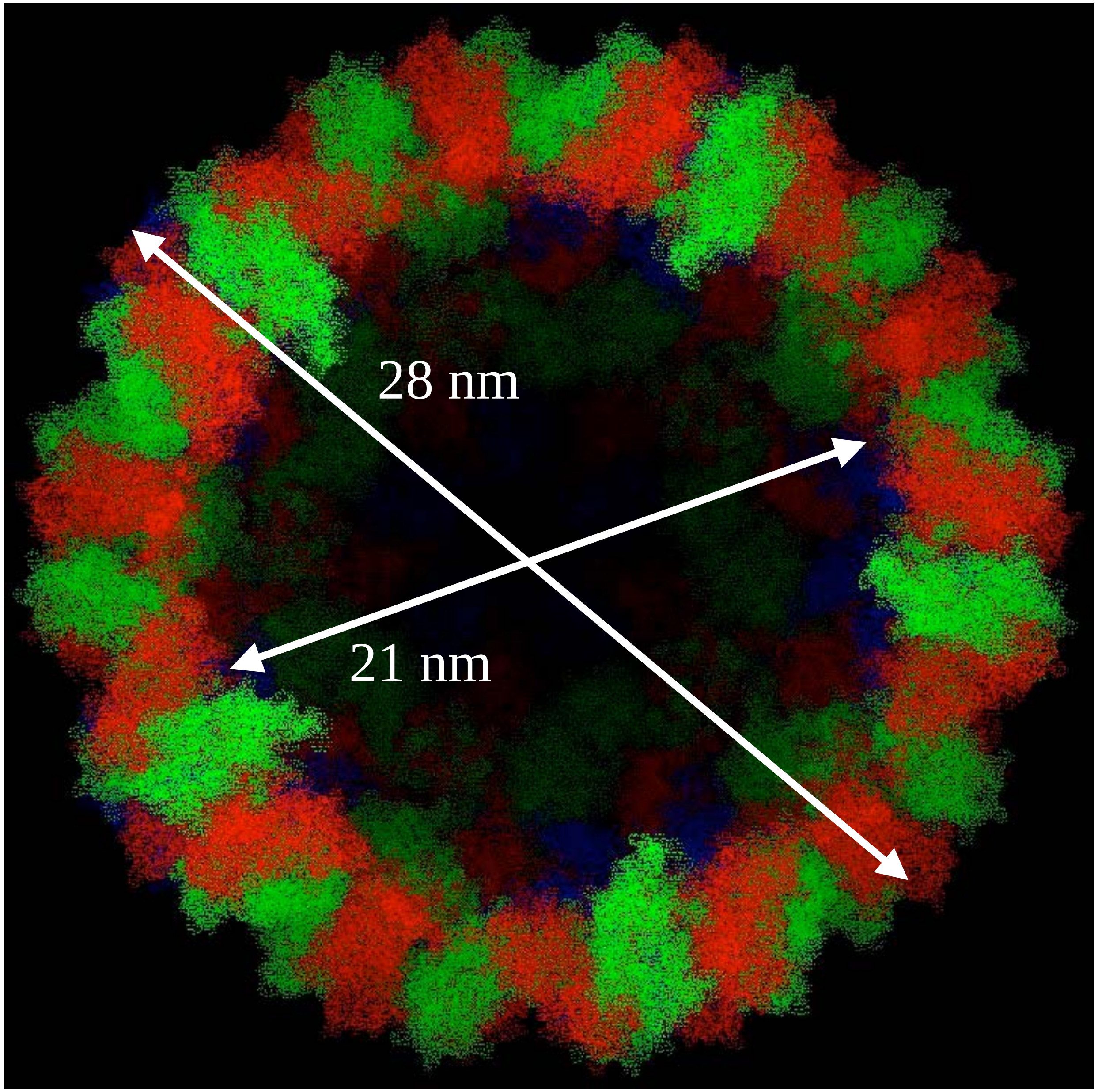




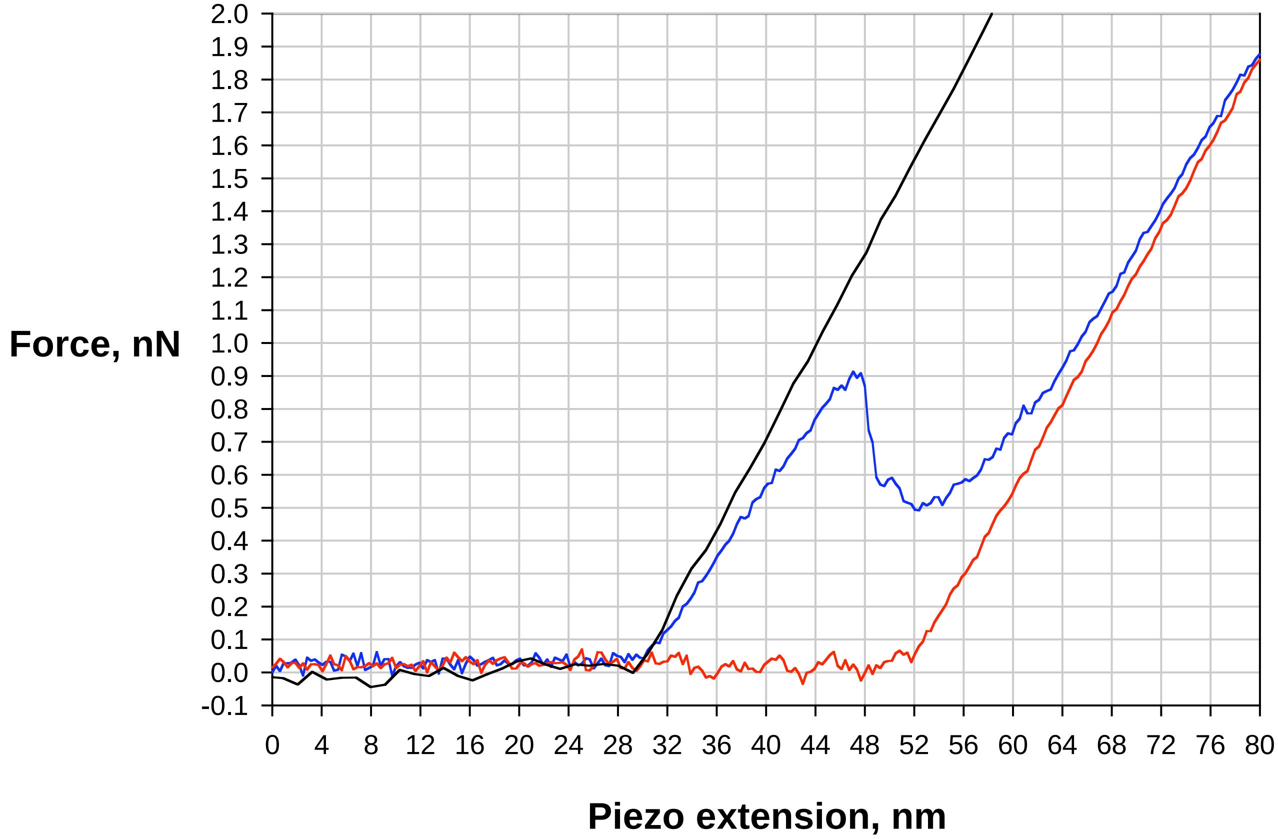
k ~ 0.05 N/m

No jump at 1 nN + preserved height

⇒ **highly elastic capsid**


28 nm
21 nm

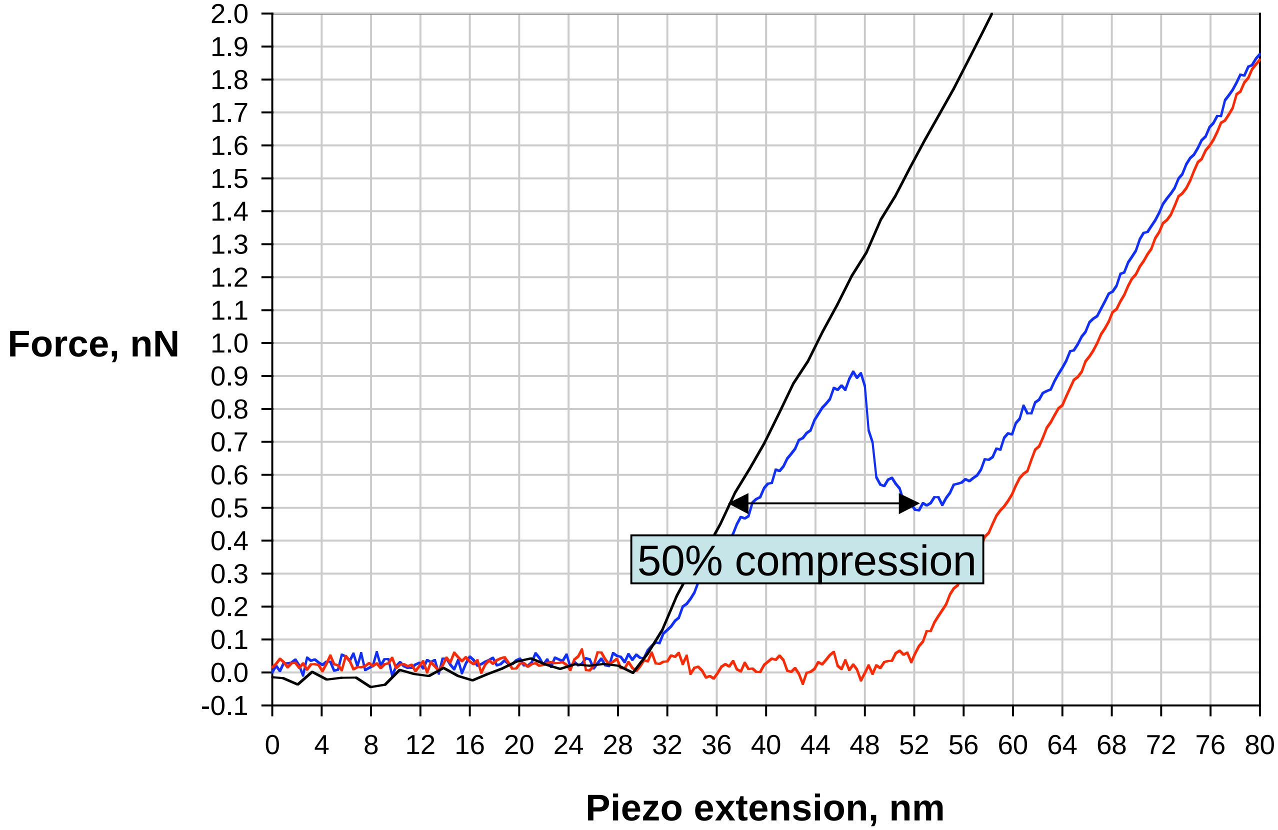
Large compression at pH 5



Break at 0.9-1 nN, then nonlinear behavior

Re-imaging shows capsid damaged

Large indentation at pH 5



Break at 0.9-1 nN, then nonlinear behavior
Re-imaging shows capsid damaged

What is the state of the RNA in the capsid?

We know that the *ds* DNA in λ Phage is hexagonally ordered at crystalline density

Is the *ss* RNA ordered?
Is it nonuniformly distributed and bound to the capsid protein?

As a 1st approximation, assume that the RNA behaves like an isotropic fluid

Then, $k = k_{empty} + k_{RNA}$ where k_{RNA} is a tension that arises from the Laplace pressure of the confined fluid, with

$$k_{RNA} = PR/2$$

We find that $\Delta k = k_{full} - k_{empty}$

Corresponds to a Laplace pressure of

100 atm

Is this reasonable?

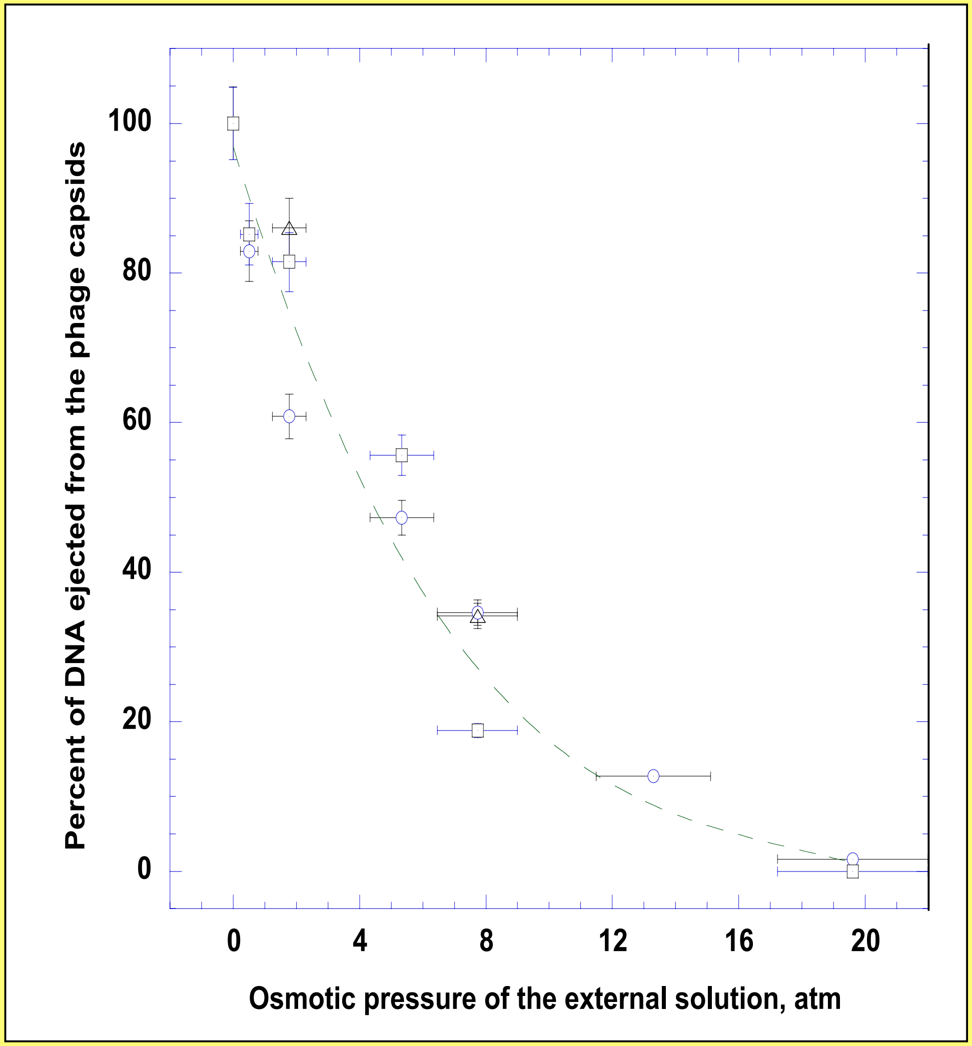
We find that $\Delta k = k_{full} - k_{empty}$

Corresponds to a Laplace pressure of 100 atm -- **very unlikely**

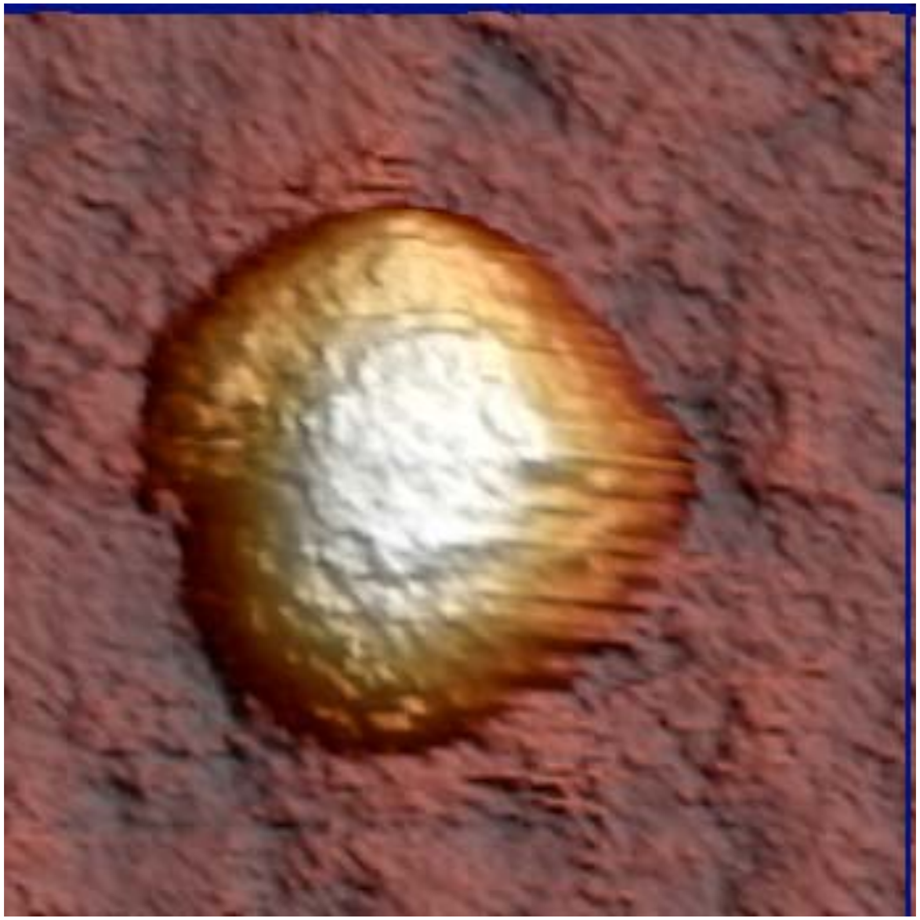
At a similar density (base/nm^3) the pressure in λ is about 2 atm

Must consider the interaction between the capsid protein tail and RNA

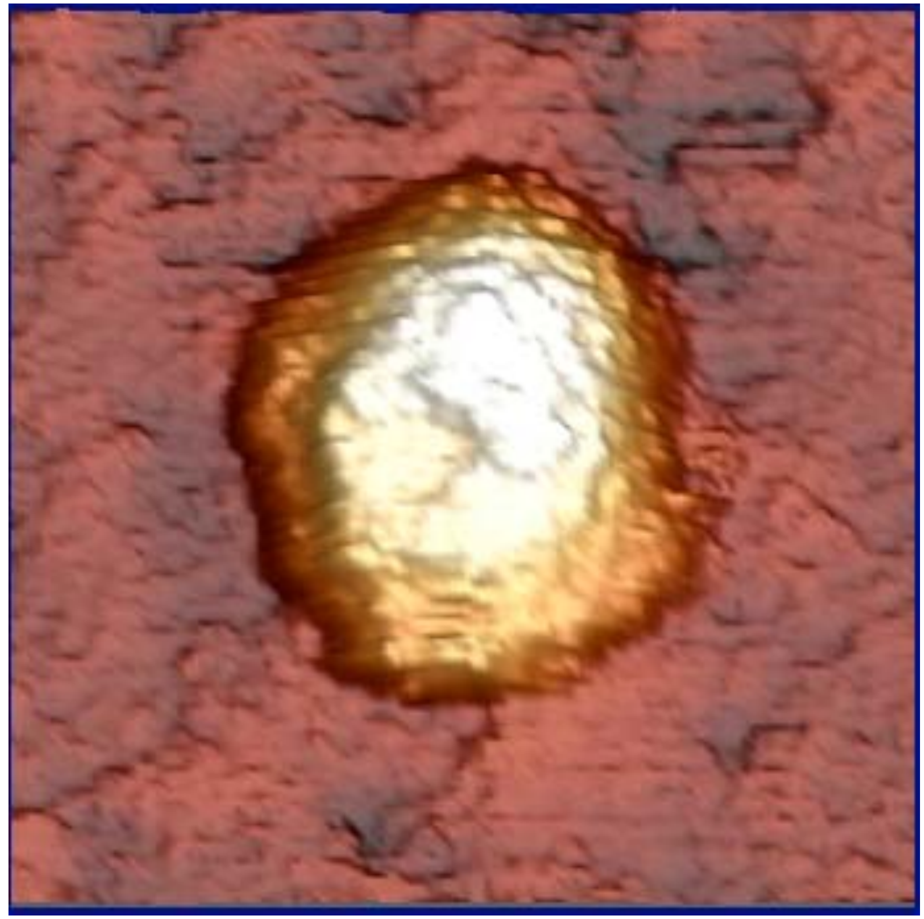
xtent of Ejected DNA *vs* Osmotic Pressure in Solutio



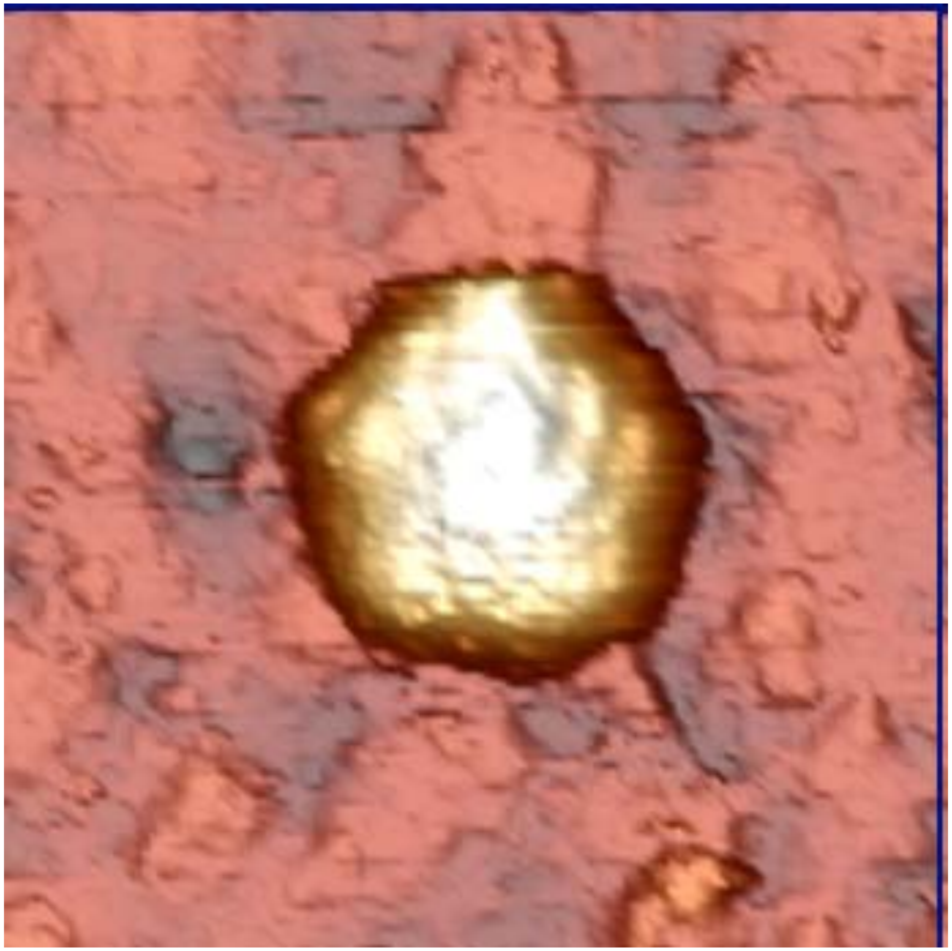
Large compression



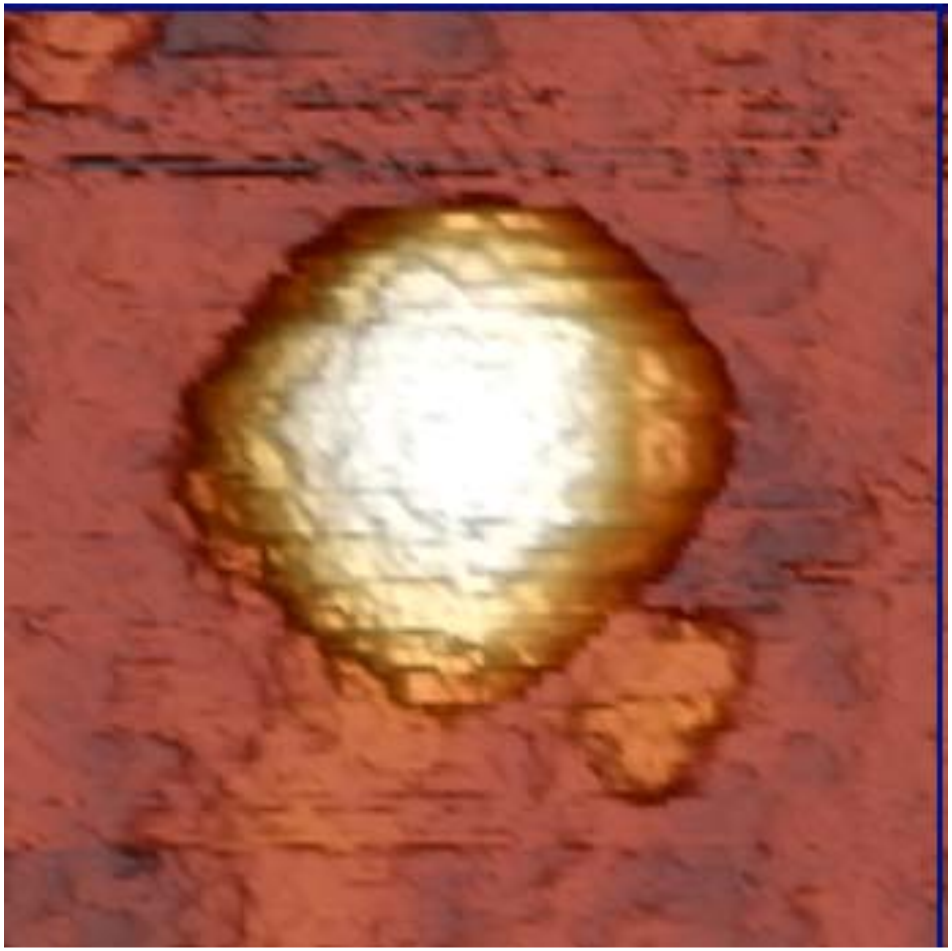
BEFORE



AFTER



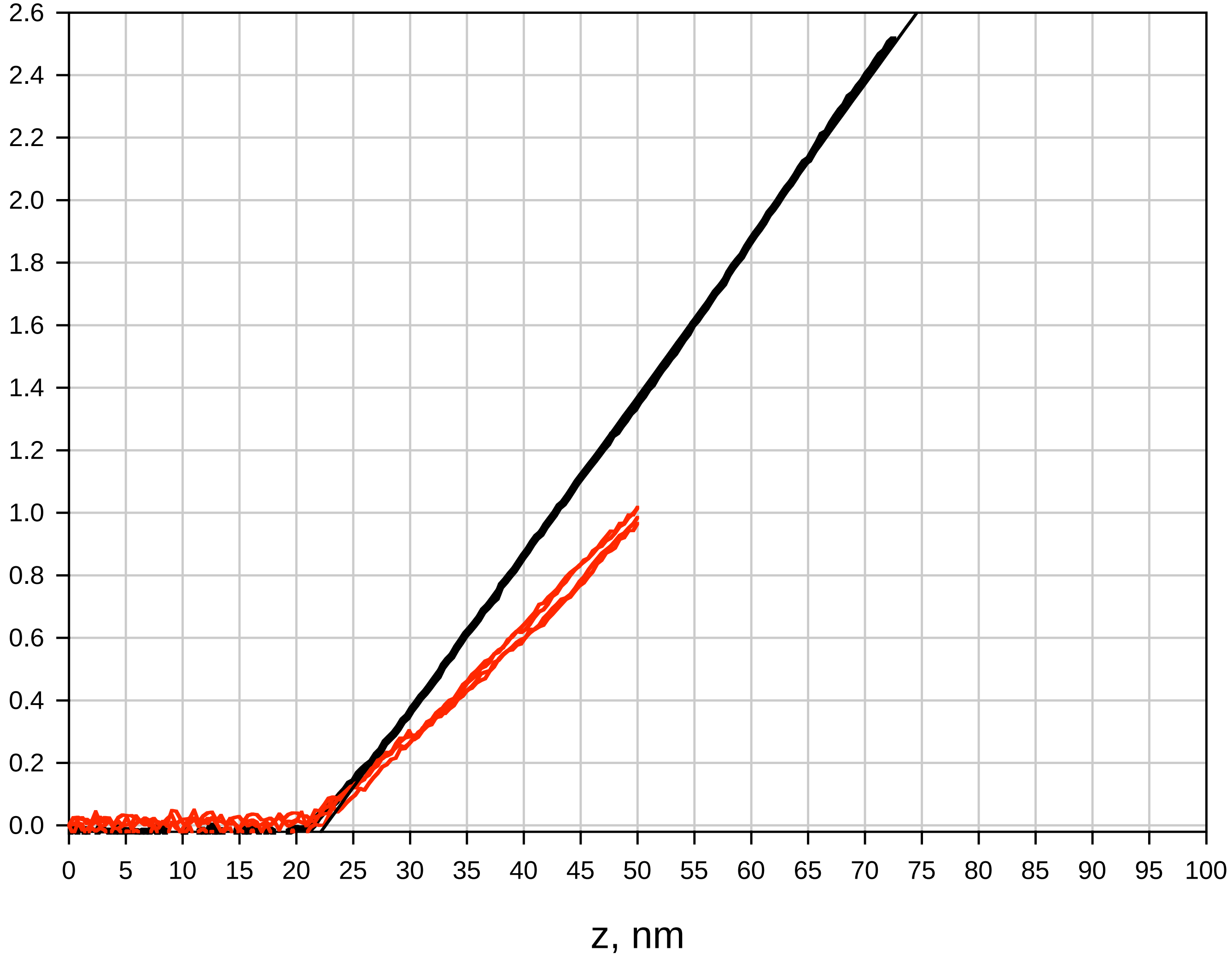
Empty capsid of CCMV
oriented on a hexamer



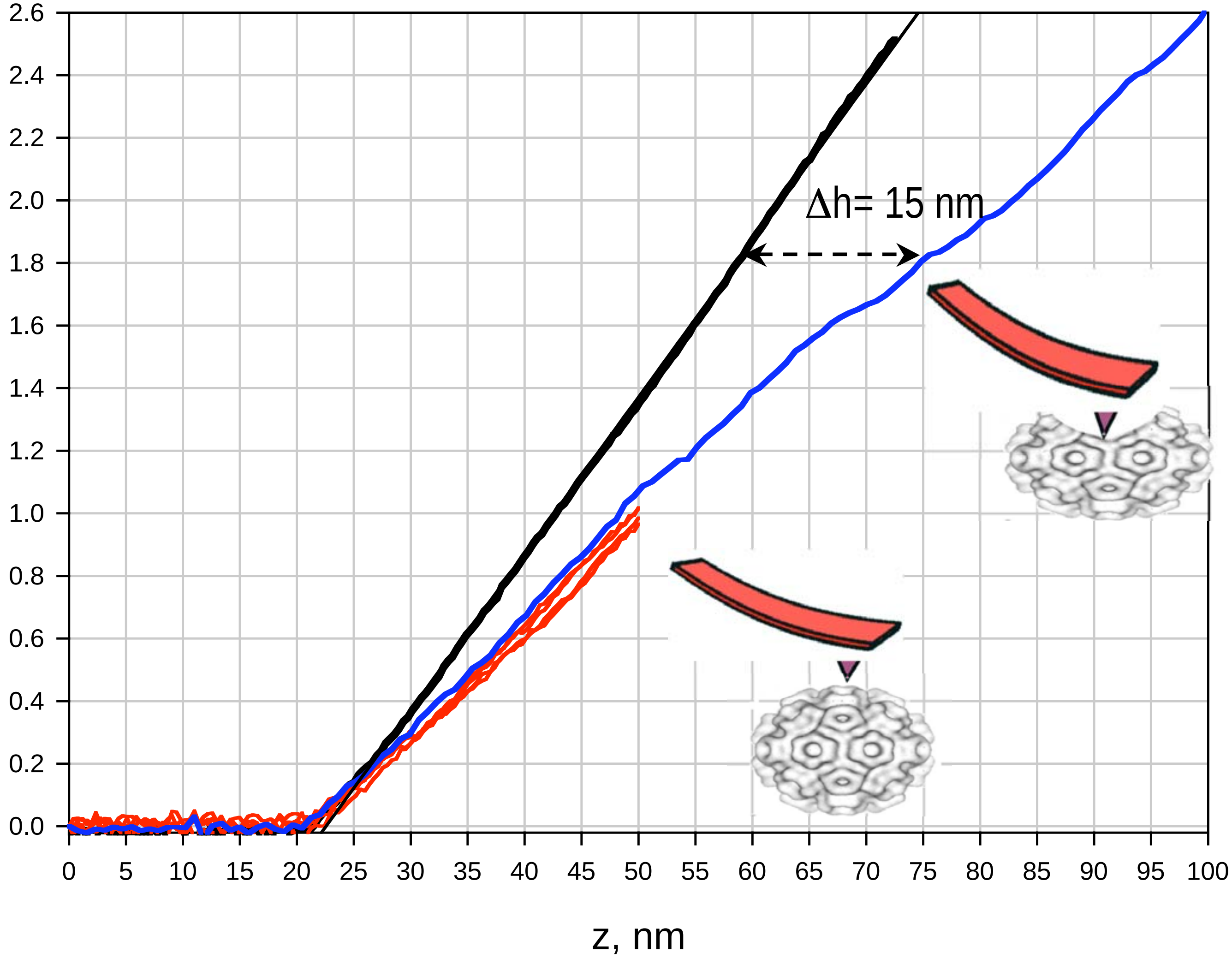
Empty capsid of CCMV
oriented on a pentamer

Force and tip used for imaging:
K(cantilever) ~ 0.05-0.07 N/m⇒ F~ **50-100 pN**
Conical tip : **15-20 nm** of curvature radius (Olympus Research)

CCMV – empty capsid



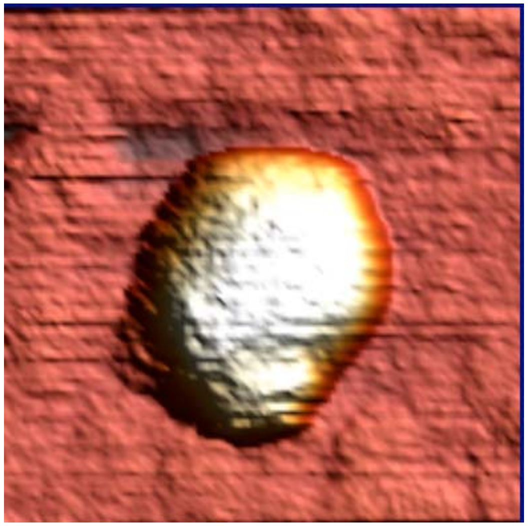
CCMV – empty capsid

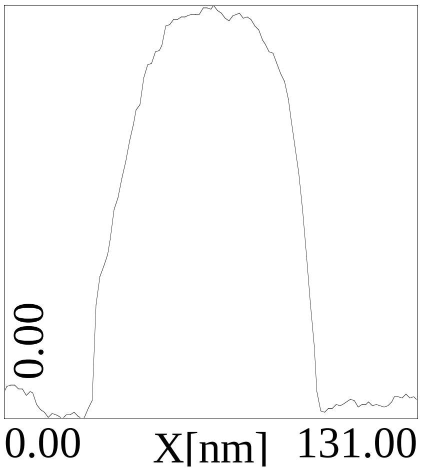


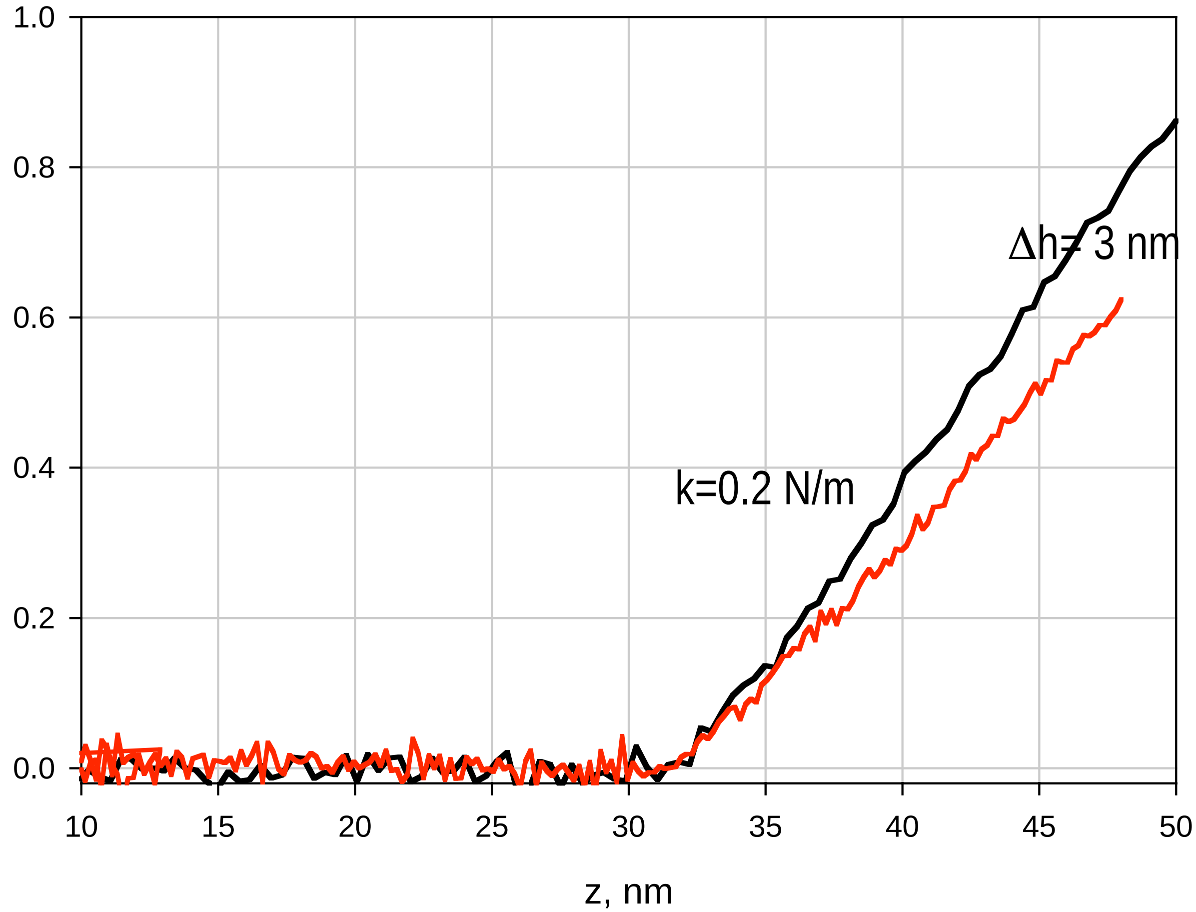
Nonlinear response

Buckling of the capsid

CCMV – full capsid

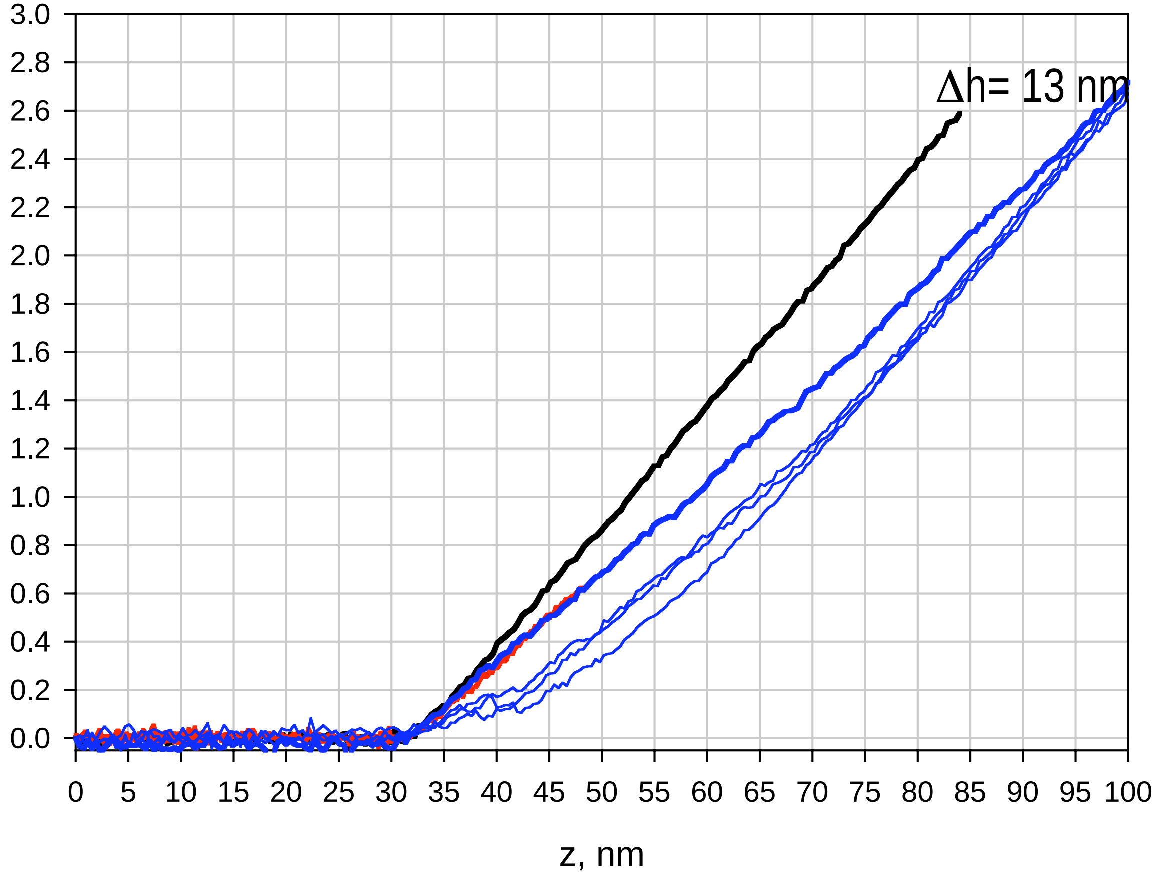






For 6 different viruses
k = 0.2 N/m ± 0.04

CCMV – full capsid



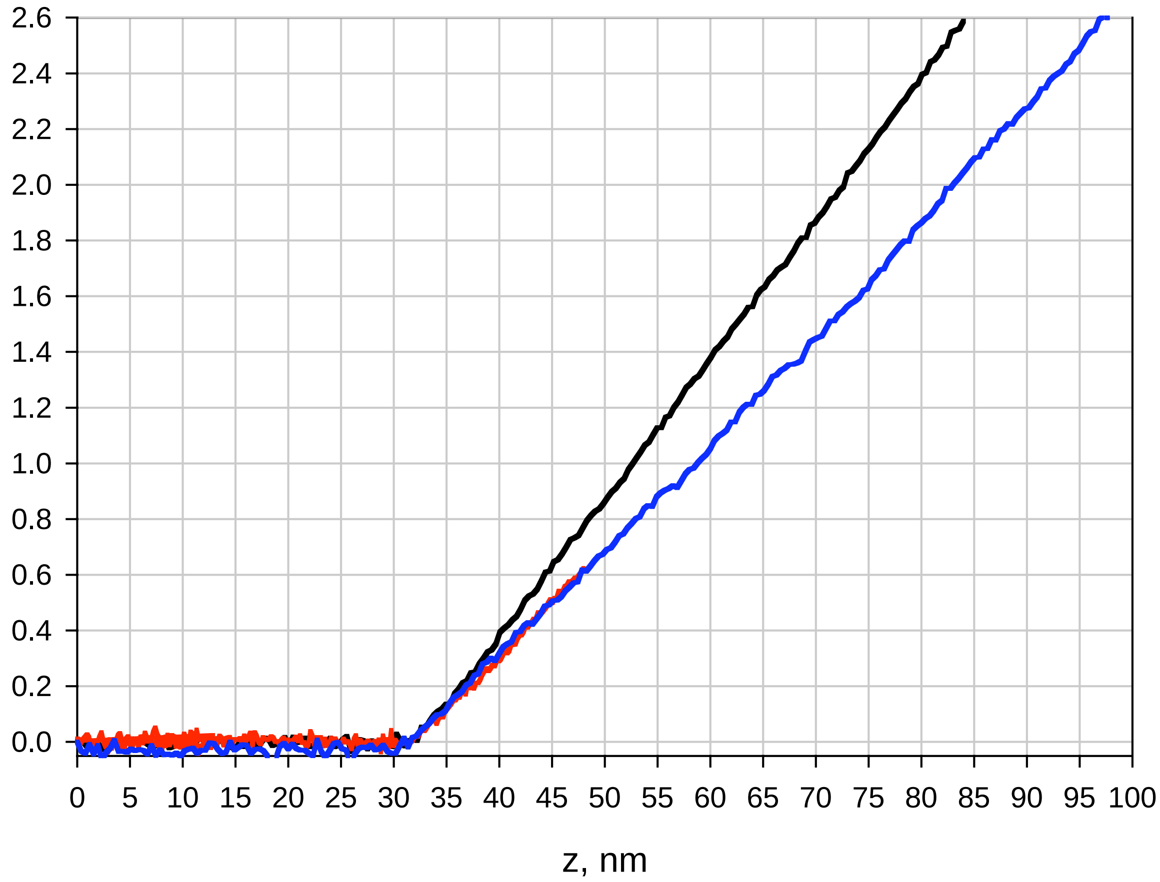
First conclusions and questions

1. CCMV shell demonstrate amazing elastic behavio
   - linear elastic deformation on large scale
   - buckling?

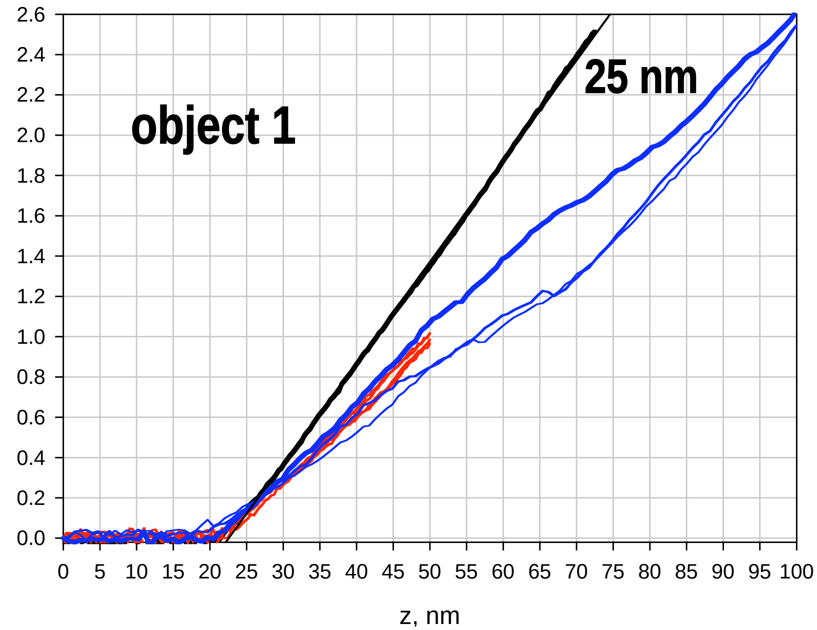
2. CCMV virus filled with RNA respond in different way under external applied pressure
   - higher spring constant
   - is this due to shell RNA binding?
   - could be a measure for the pressure inside the capsid?

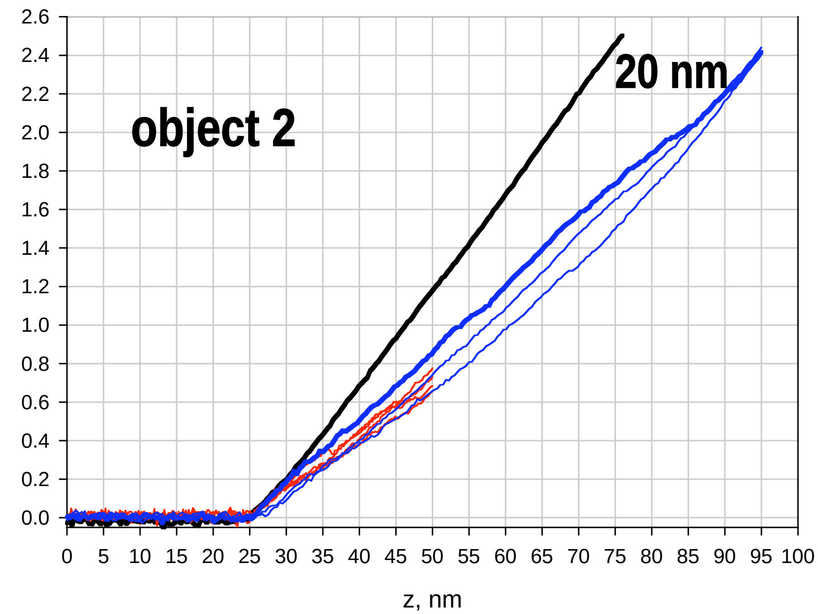
CCMV – full capsid

Respond linearly like a thin shell…



CCMV – empty shell





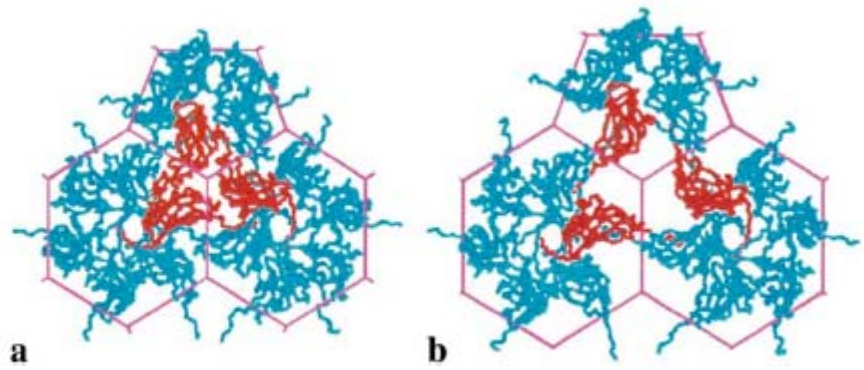
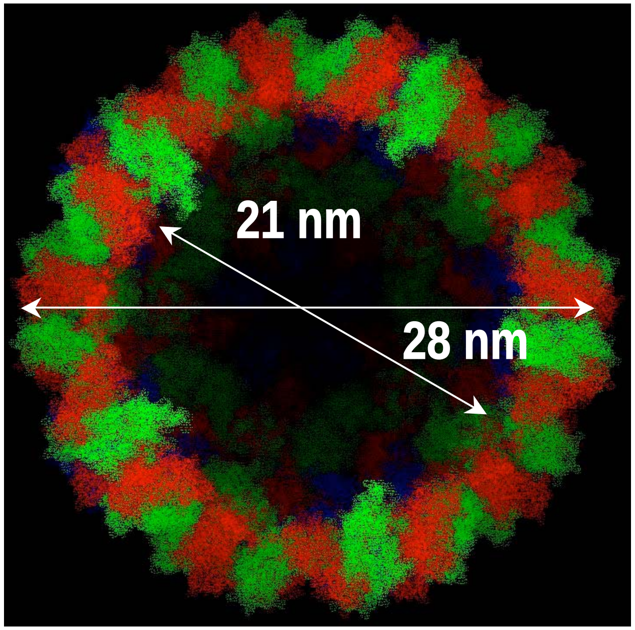
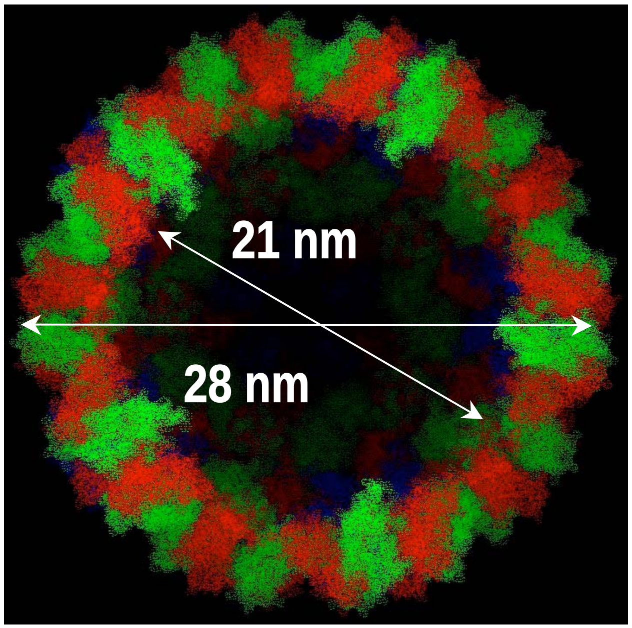


Fig. 5. Pentameric, hexameric, and dimeric contacts in the (a) crystal and (b) swollen structures.



CCMV – empty shell



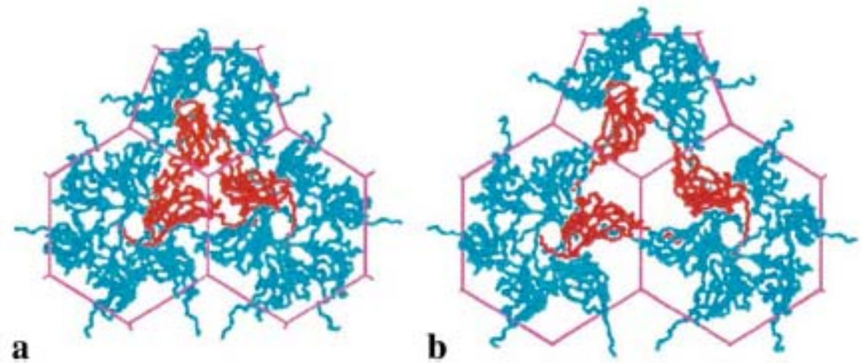


Fig. 5. Pentameric, hexameric, and dimeric contacts in the (a) crystal and (b) swollen structures.

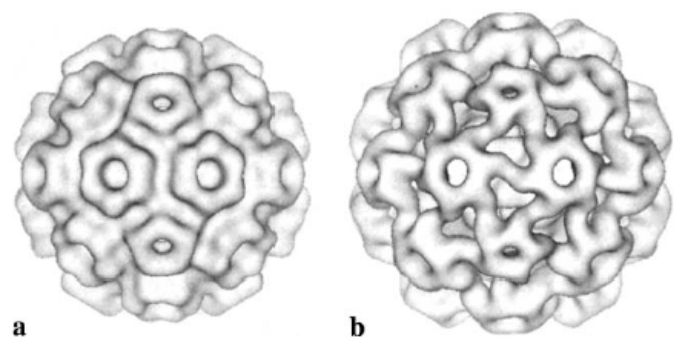
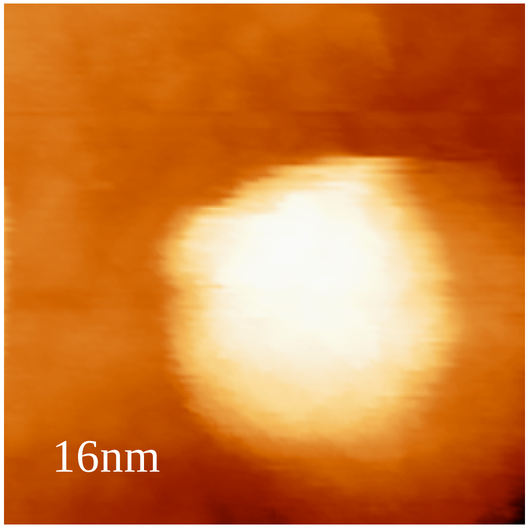
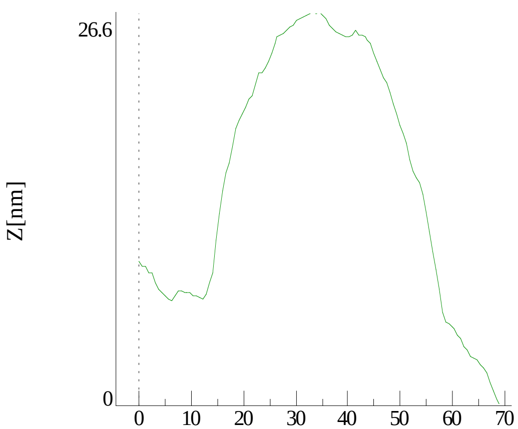


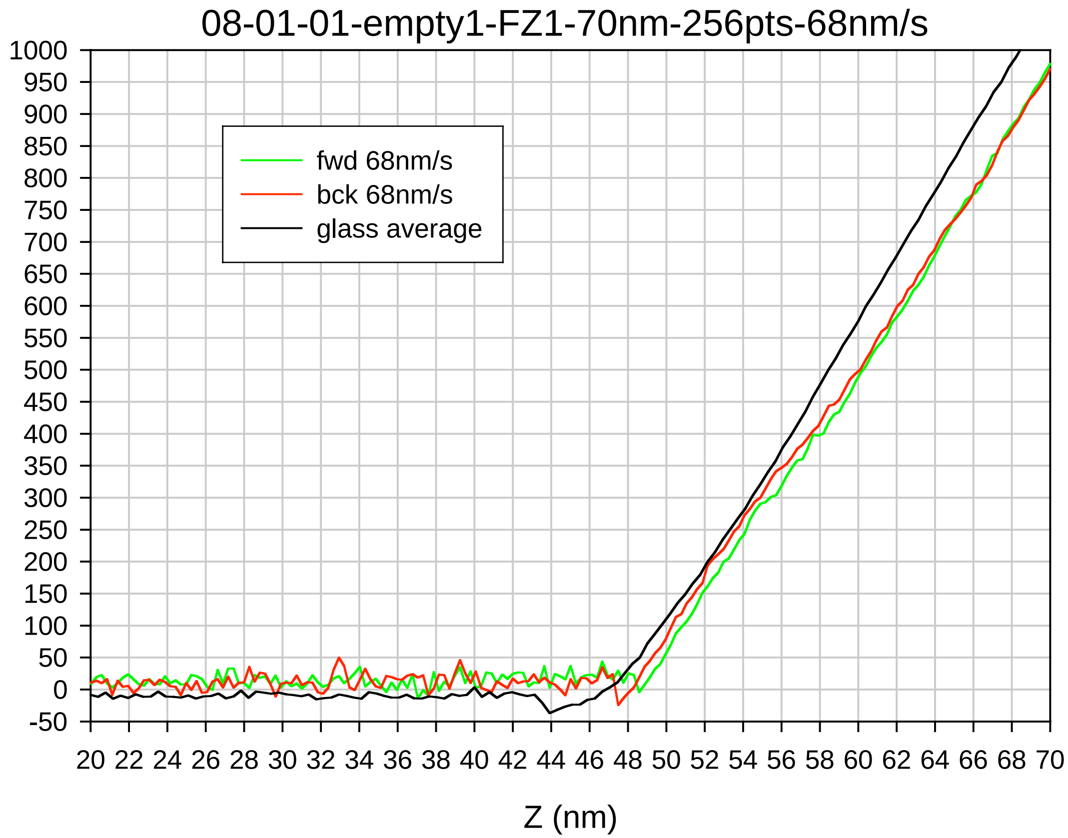
Fig. 2. Cryo-EM image reconstructions of (a) native CCMV and (b) swollen CCMV particles. The capsids are placed with icosahedral twofold axis perpendicular to the page. The swollen structure expands by ~10% from the native form, with the largest changes occurring at the icosahedral and quasi-twofold axes.

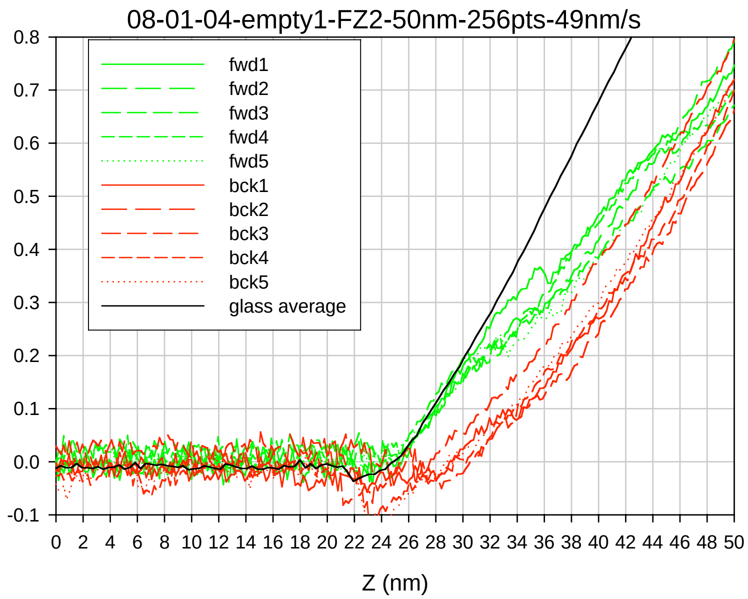
08-01-04-empty1

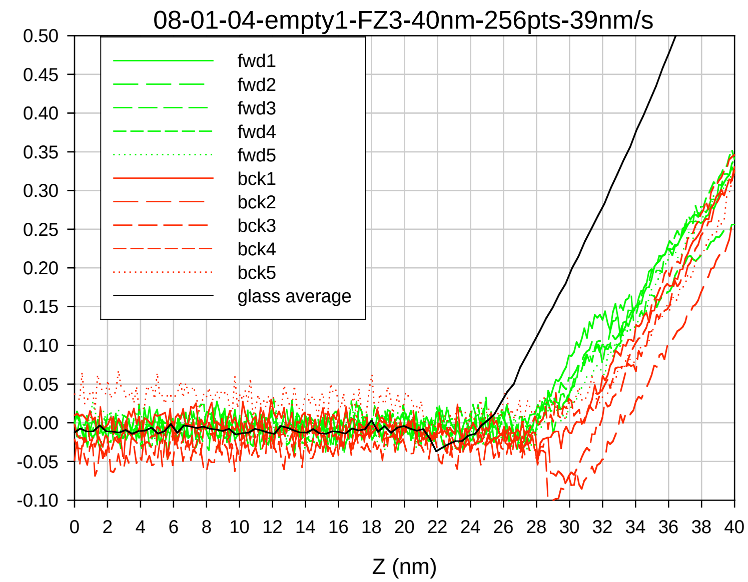
initially





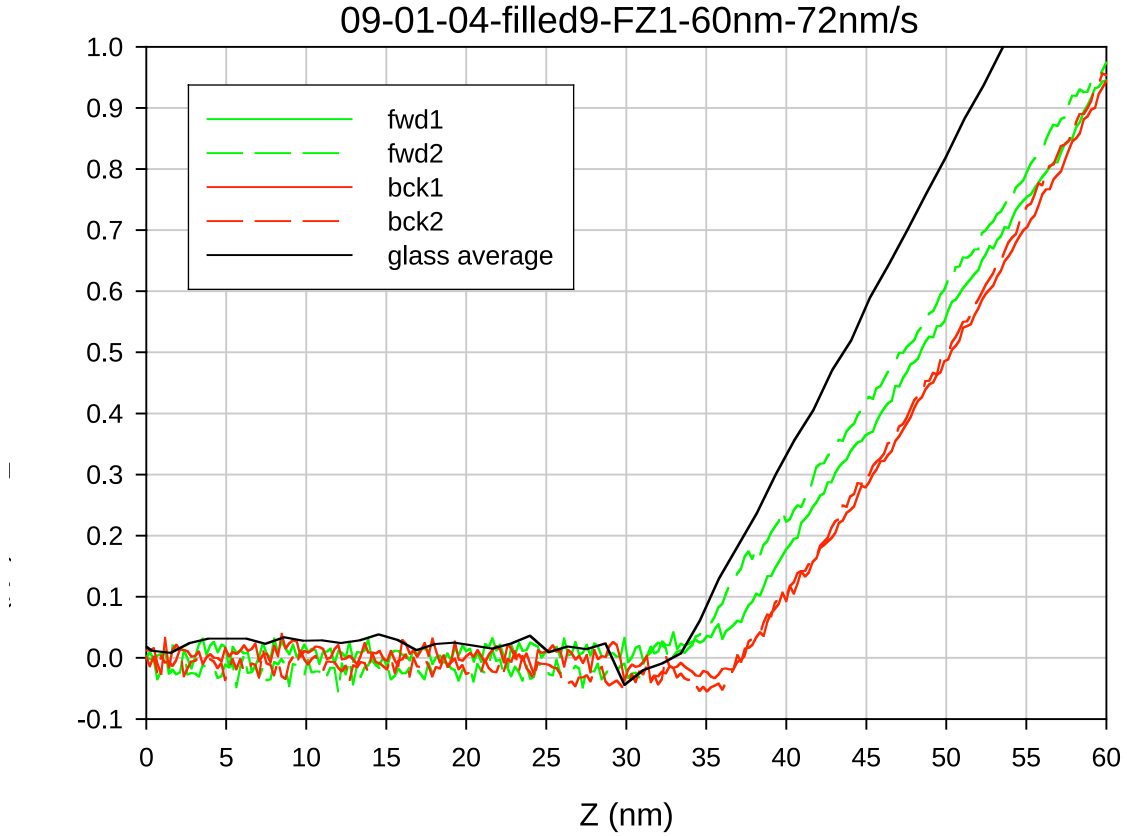



08-01-04-empty1-FZ2-50nm-256pts-49nm/s
fwd1
fwd2
fwd3
fwd4
fwd5
bck1
bck2
bck3
bck4
bck5
glass average
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
Z (nm)


08-01-04-empty1-FZ3-40nm-256pts-39nm/s
fwd1
fwd2
fwd3
fwd4
fwd5
bck1
bck2
bck3
bck4
bck5
glass average
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Z (nm)

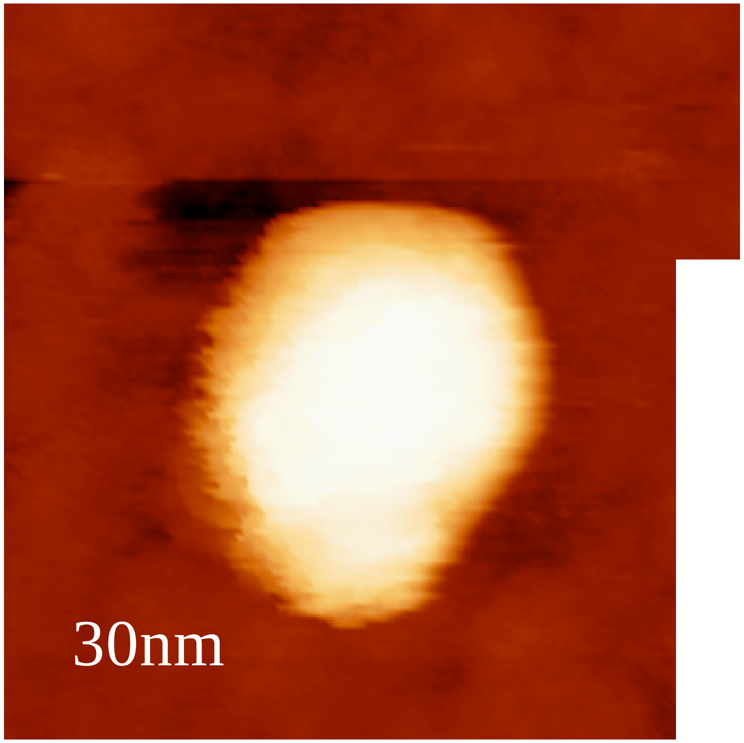
FILLED CAPSID

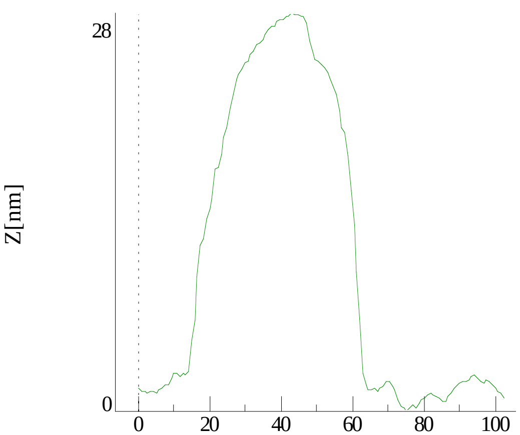
WT -NOT MUTANT

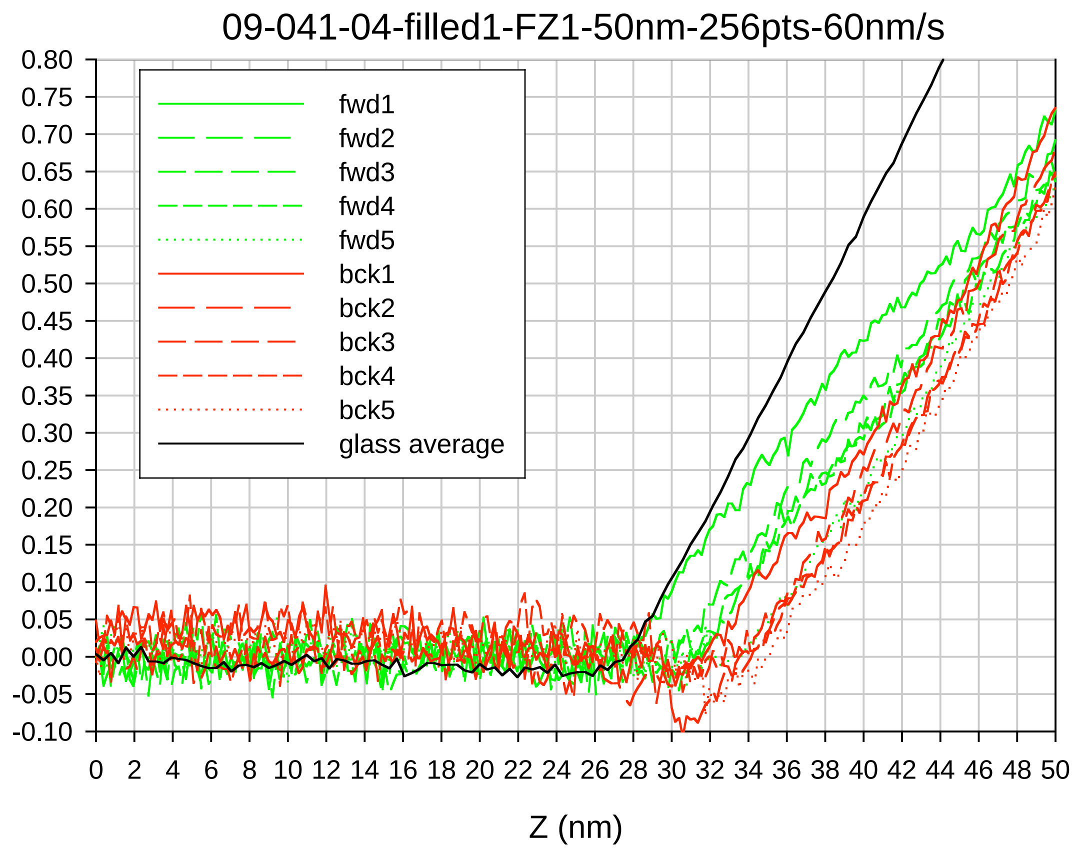


FILLED CAPSID

WT -NOT MUTANT







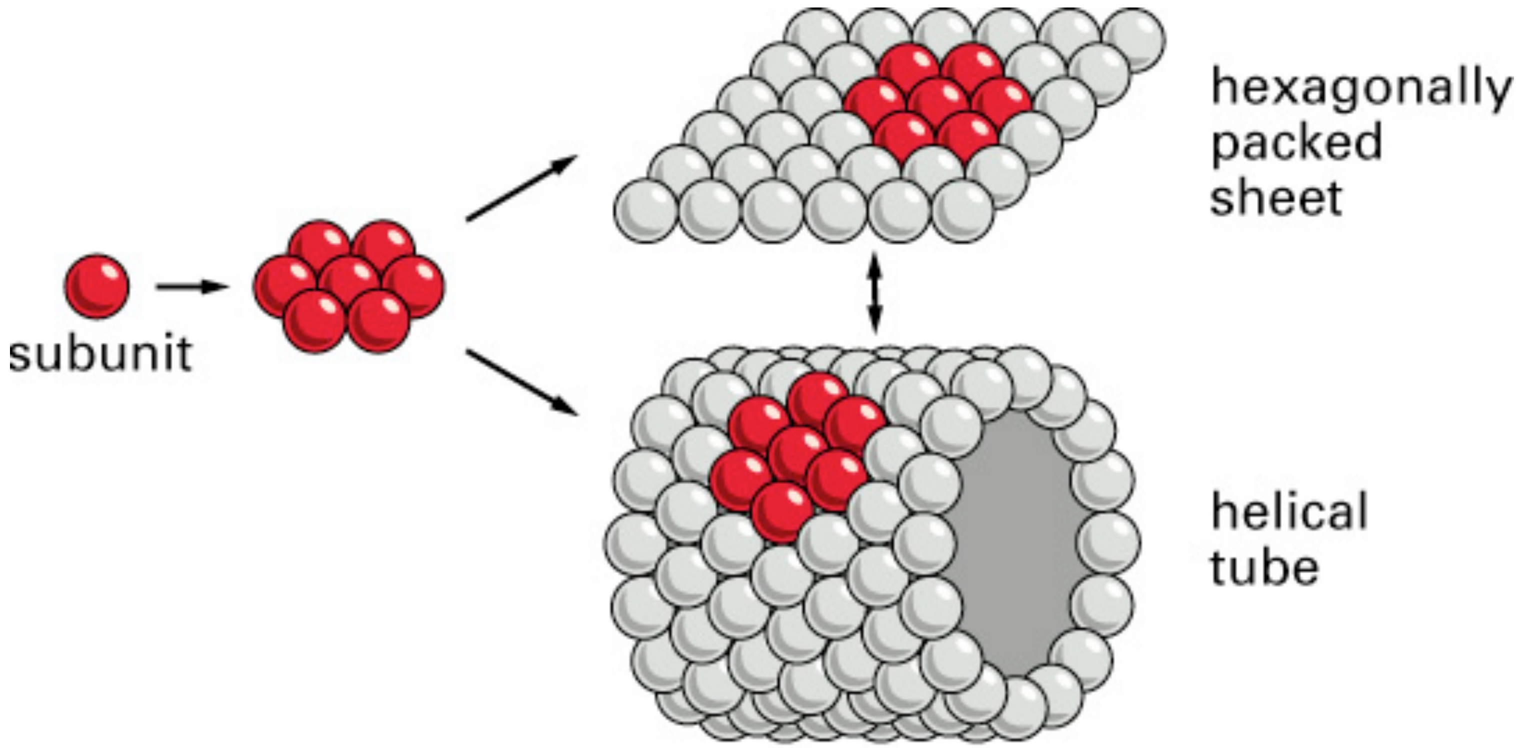


Figure 3–30. Molecular Biology of the Cell, 4th Edition.

Capsids consist of just a few proteins. In the case of CCMV (cowpea chlorotic mosaic virus) capsids consist of 180 copies of a single protein.

Many capsids have icosahedral symmetry and have diameters of the order of 100 nm

Some capsids can self-assemble in solution… around their genomes or empty

⇨ Capsid proteins can also self-assemble into other forms such as sheets or "onions", depending upon pH, ionic strength, temperature and concentration

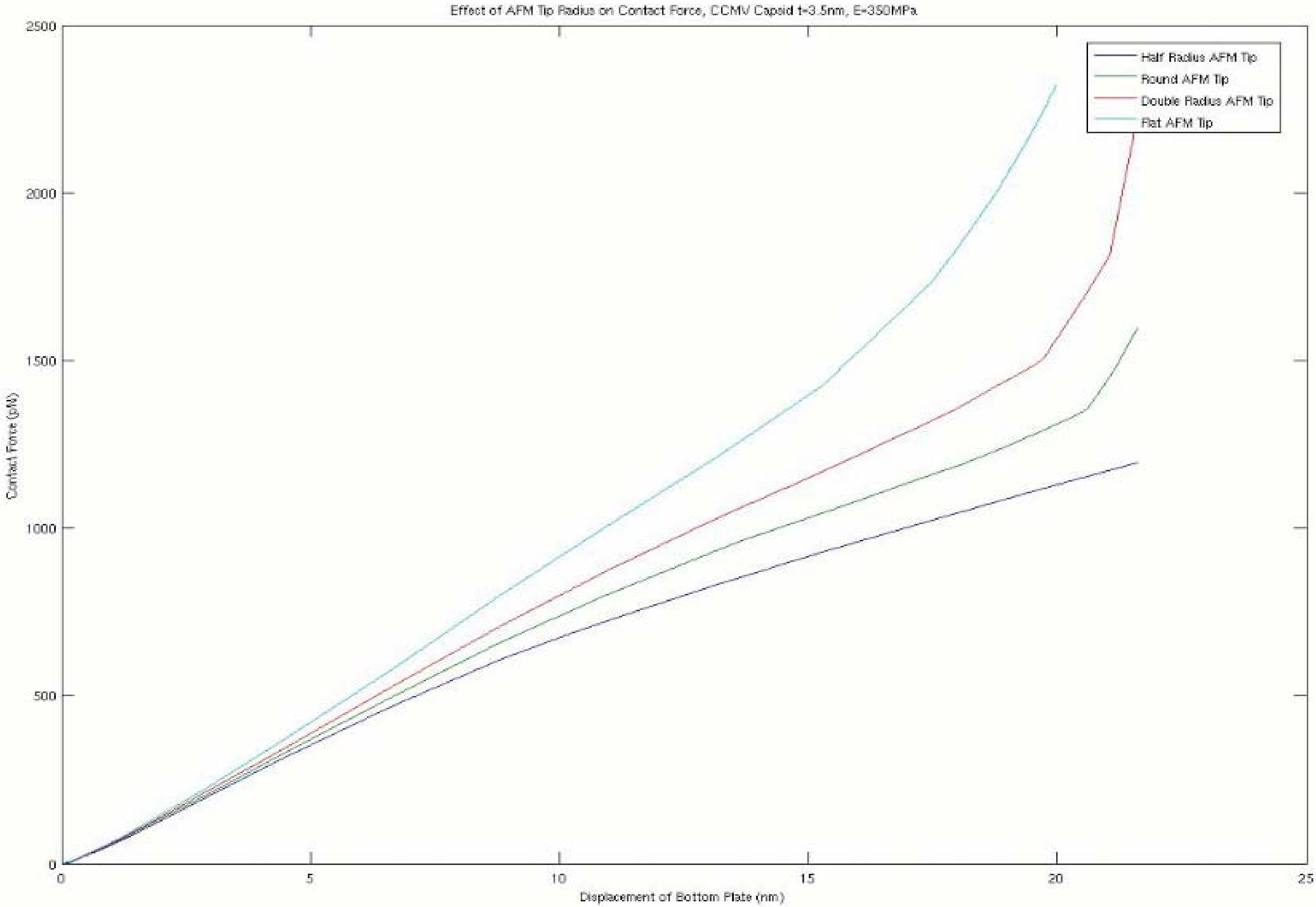
Some capsids can self-assemble in solution…
around their genomes or empty

- ⇨ Some capsids can undergo a *reversible* isotropic expansion with change of pH

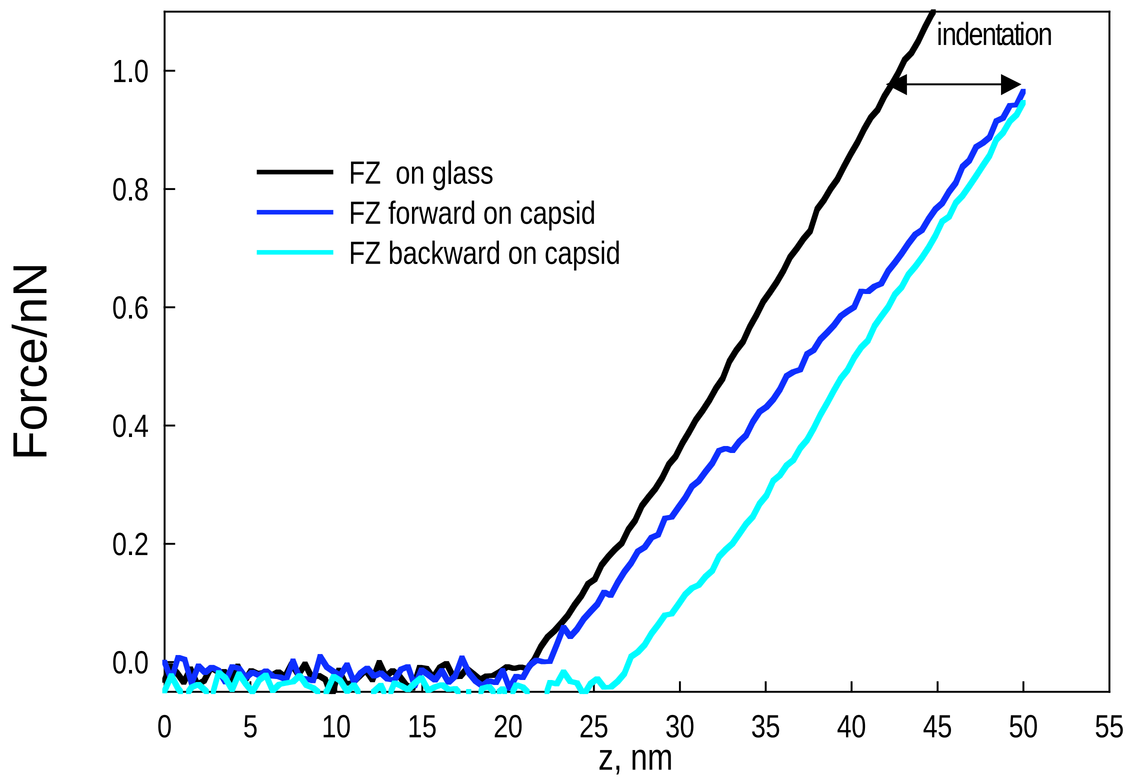
Some capsids can self-assemble in solution… around their genomes or empty

Capsid proteins can also self-assemble into other forms such as sheets or “onions”, depending upon pH, ionic strength, temperature and concentration

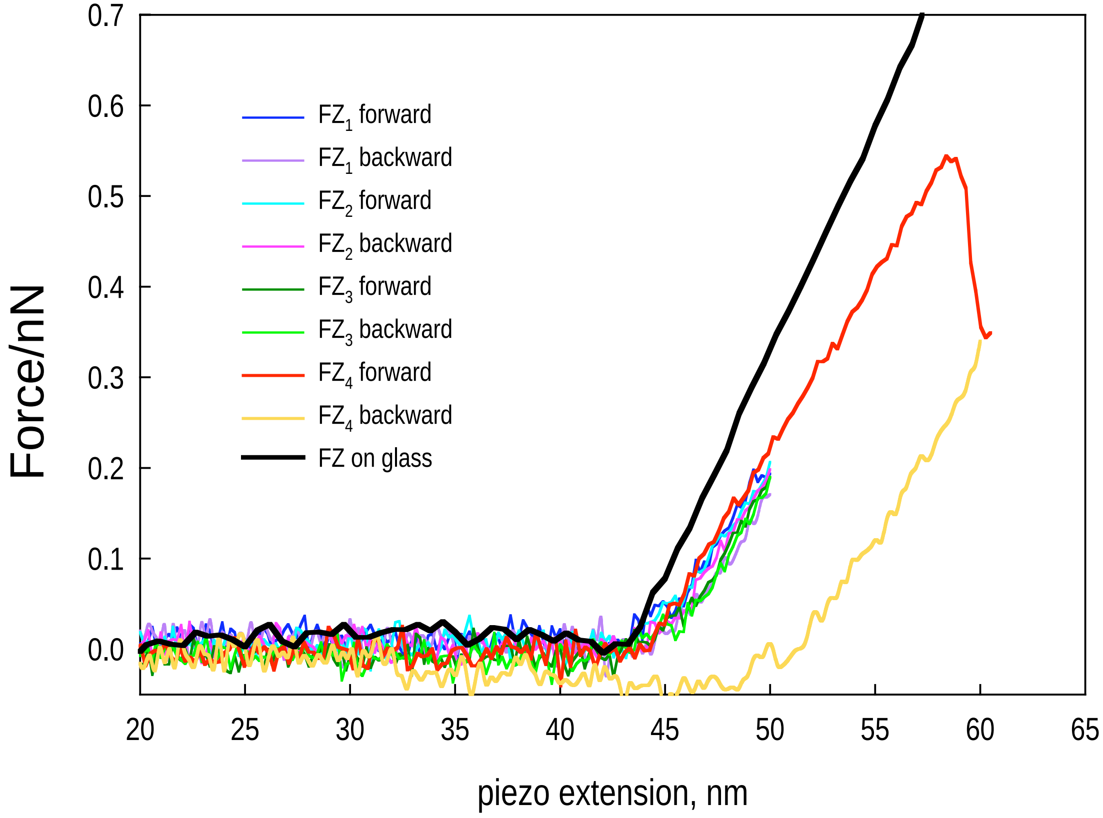
⇨ Some capsids can undergo a *reversible* isotropic expansion with change of pH

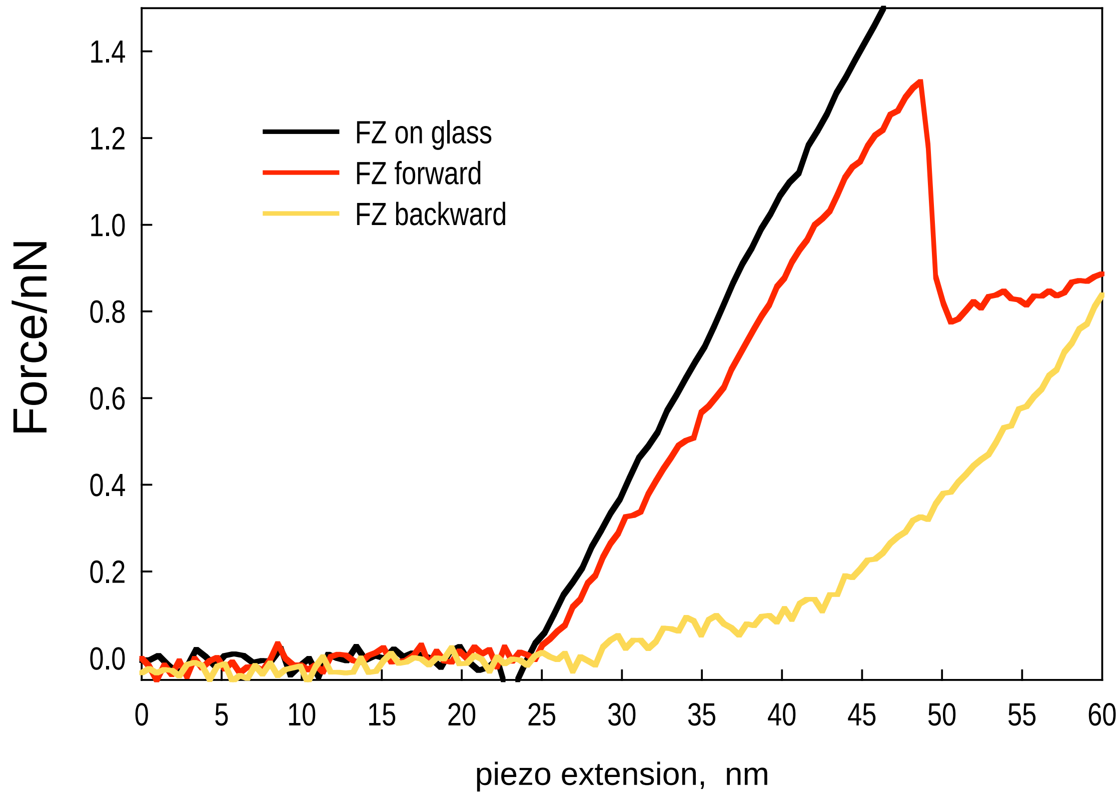


Effect of AFM Tip Radius on Contact Force, CCMV Capsid t=3.5nm, E=350MPa
Half Radius AFM Tip
Round AFM Tip
Double Radius AFM Tip
Flat AFM Tip
Contact Force (pN)
Displacement of Bottom Plate (nm)
0
500
1000
1500
2000
2500
0
5
10
15
20
25



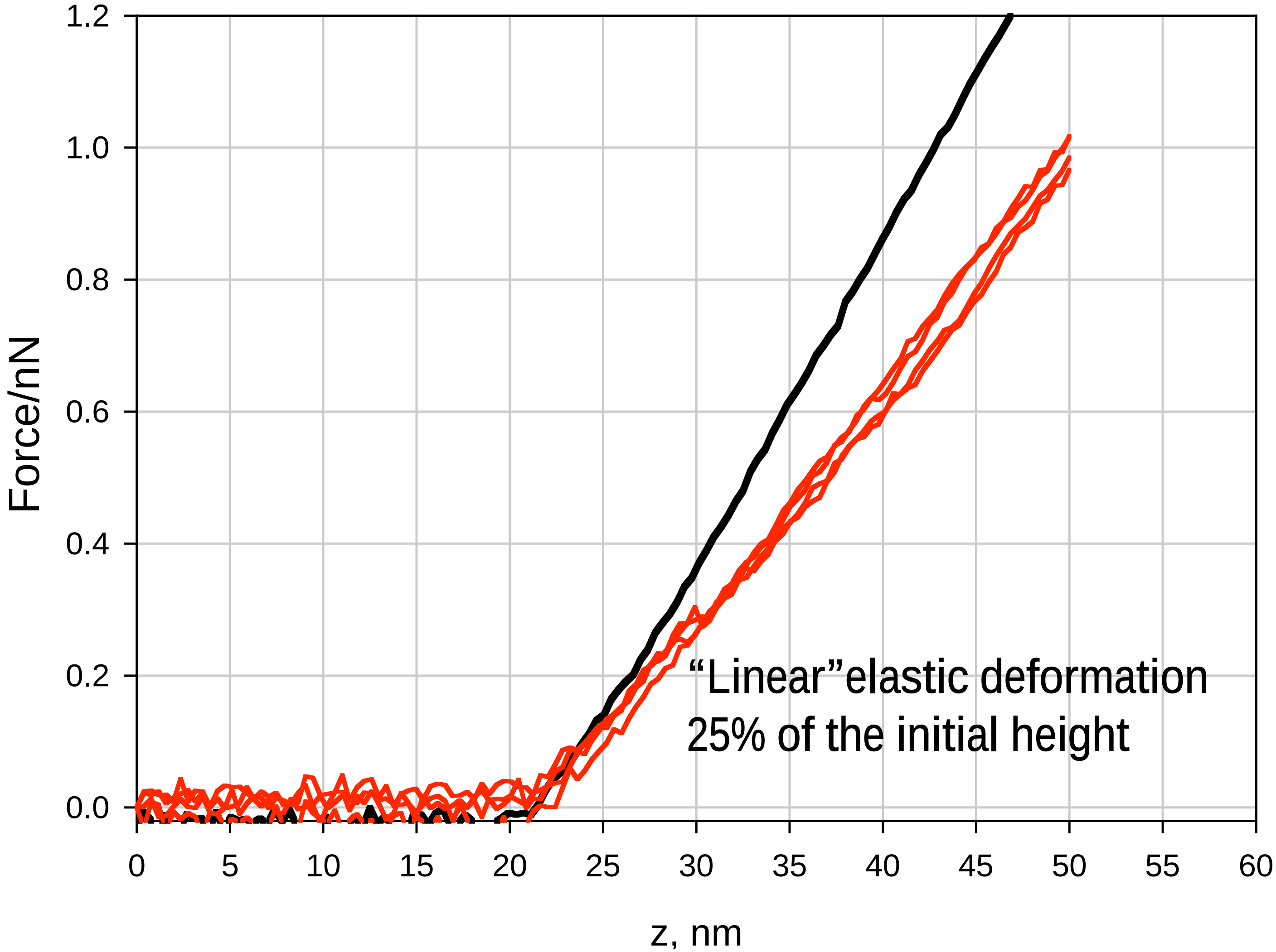
Force/nN
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
35
40
45
50
55
z, nm
indentation
FZ on glass
FZ forward on capsid
FZ backward on capsid

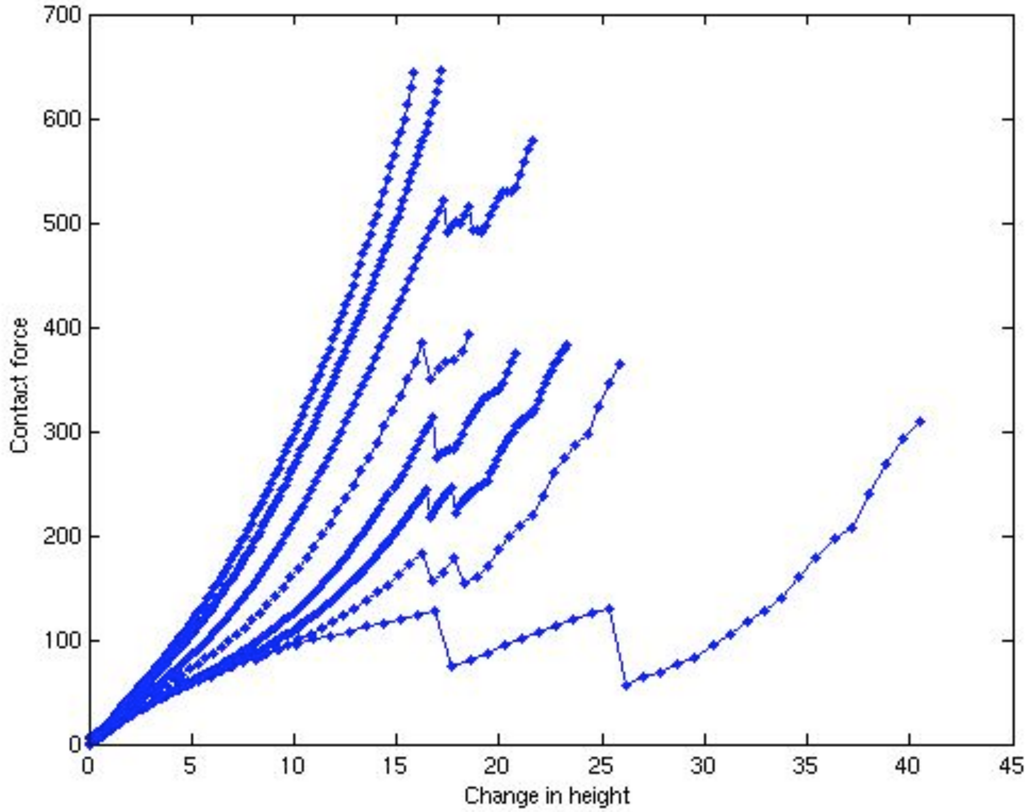

Force/nN
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
20
25
30
35
40
45
50
55
60
65
piezo extension, nm
FZ1 forward
FZ1 backward
FZ2 forward
FZ2 backward
FZ3 forward
FZ3 backward
FZ4 forward
FZ4 backward
FZ on glass



Force/nN
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
FZ on glass
FZ forward
FZ backward
0
5
10
15
20
25
30
35
40
45
50
55
60
piezo extension, nm

CCMV – empty capsid



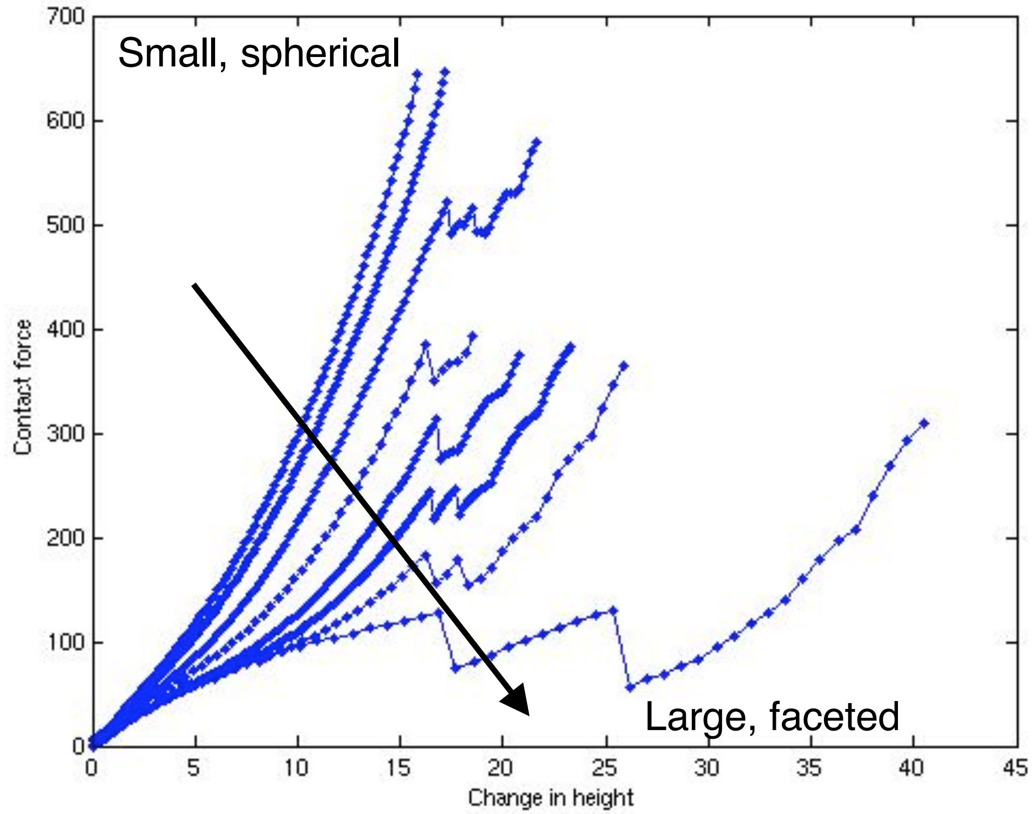


Contact force
Change in height
0
100
200
300
400
500
600
700
0
5
10
15
20
25
30
35
40
45

J.-P. Michel and C. M. Knobler
Dept. of Chemistry & Biochemistry, UCLA
http://virus.chem.ucla.edu

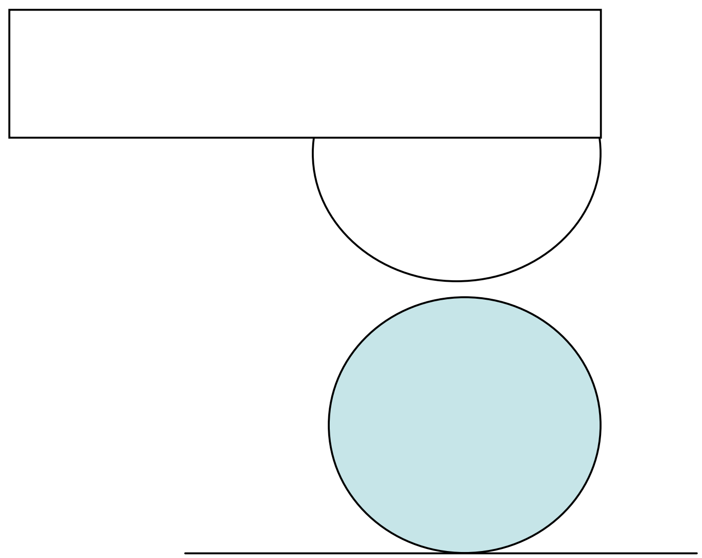
I.L. Ivanovska, G. J. L. Wuite, C. F. Schmidt
Dept. of Physics, Vrije Universiteit, Amsterdam

M. M. Gibbons and W. S. Klug
Department of Mechanical Engineering
UCLA



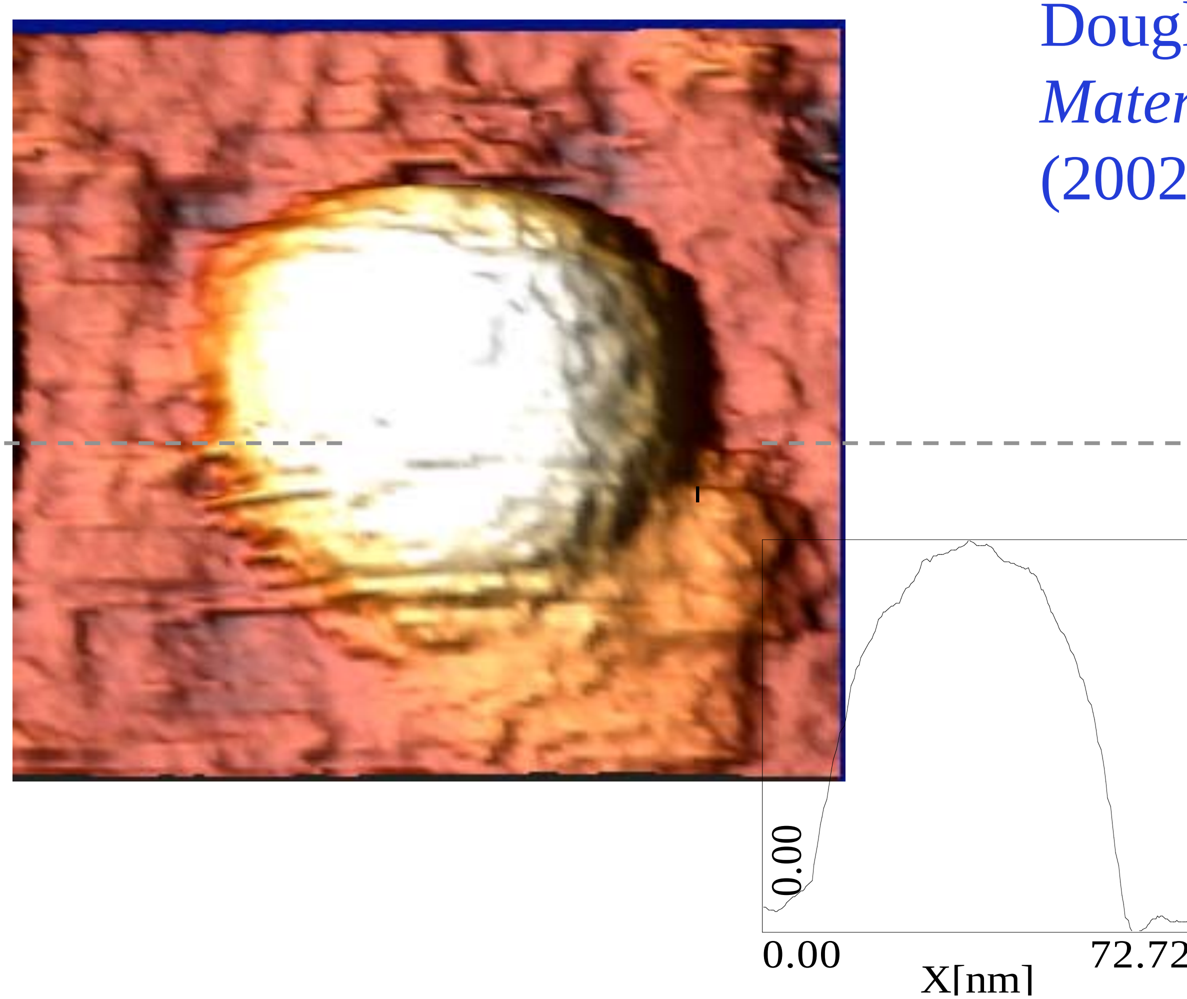
Small, spherical
Large, faceted
Contact force
Change in height
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
45

In the case of CCMV, the radius of curvature of the tip is comparable to that of the virus



subE Mutant

Douglas, et al. *Adv. Mater*. **14**, 415 (2002)



JUMPING MODE TOPOGRAPHIC IMAGE OF EMPTY CCMV CAPSID

APPLIED FORCE ~ 100 pN