Atomic Force Microscopy of virus capsids uncovers the interplay between mechanics, structure and function

Pedro José de Pablo

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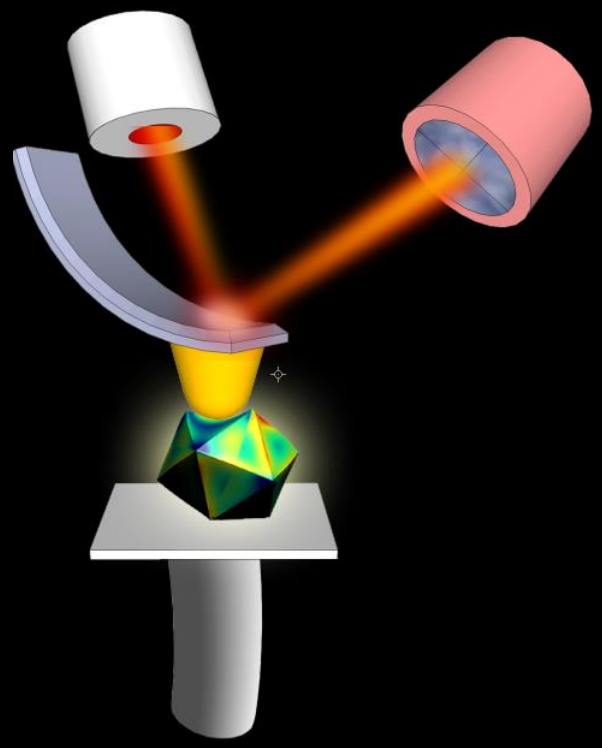
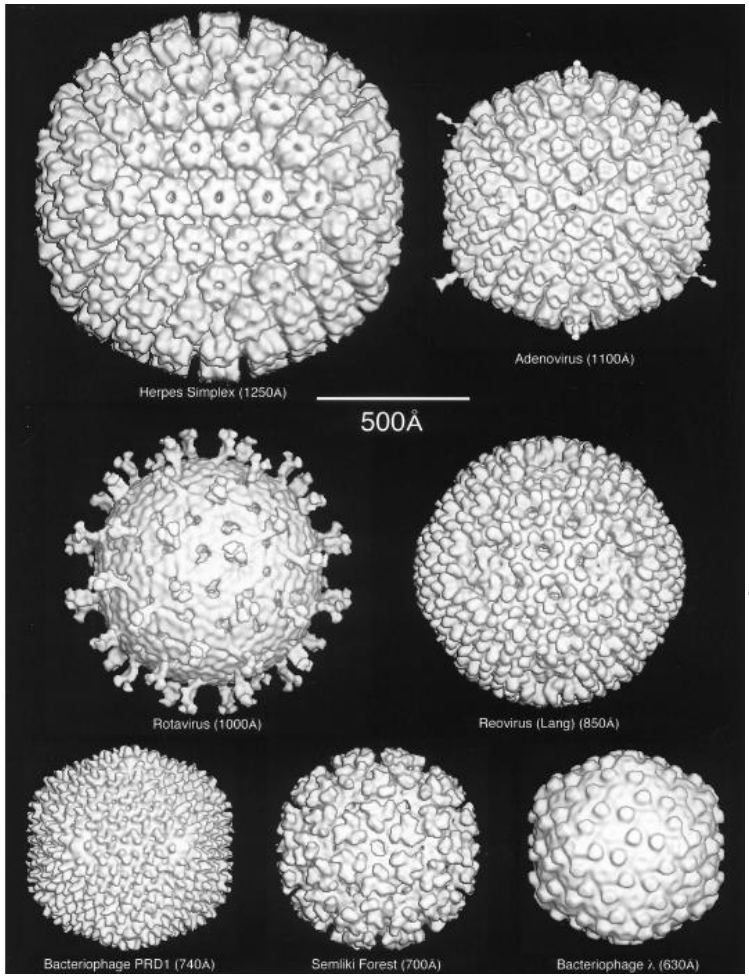
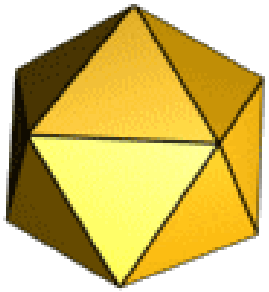


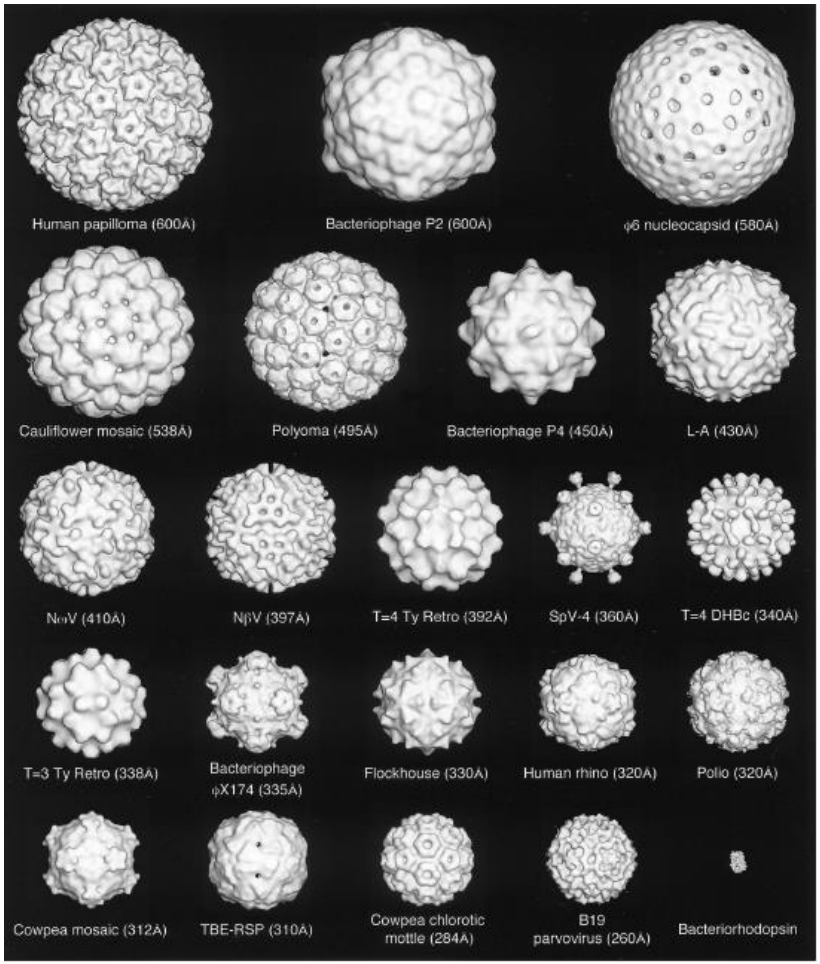
Topics today

1. Introduction
2. Mechanics of human adenovirus: capsid and core
3. Genome release: watching a virus undress
4. Mechanical role of cementing proteins: tuning particles stability with symmetrical morphogenesis
5. Summing up

Viruses





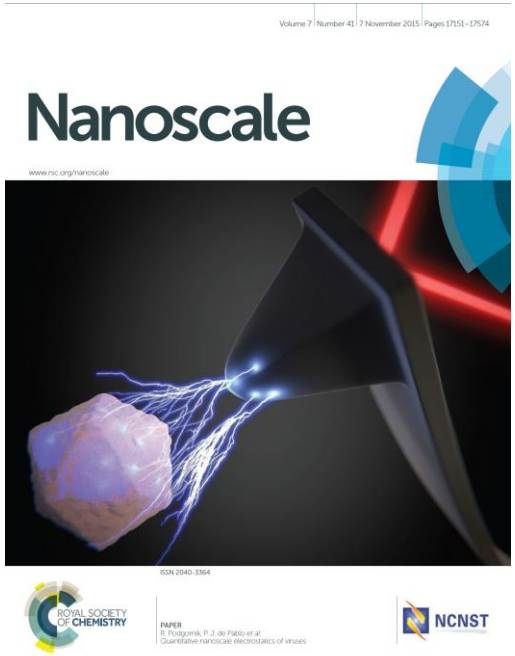


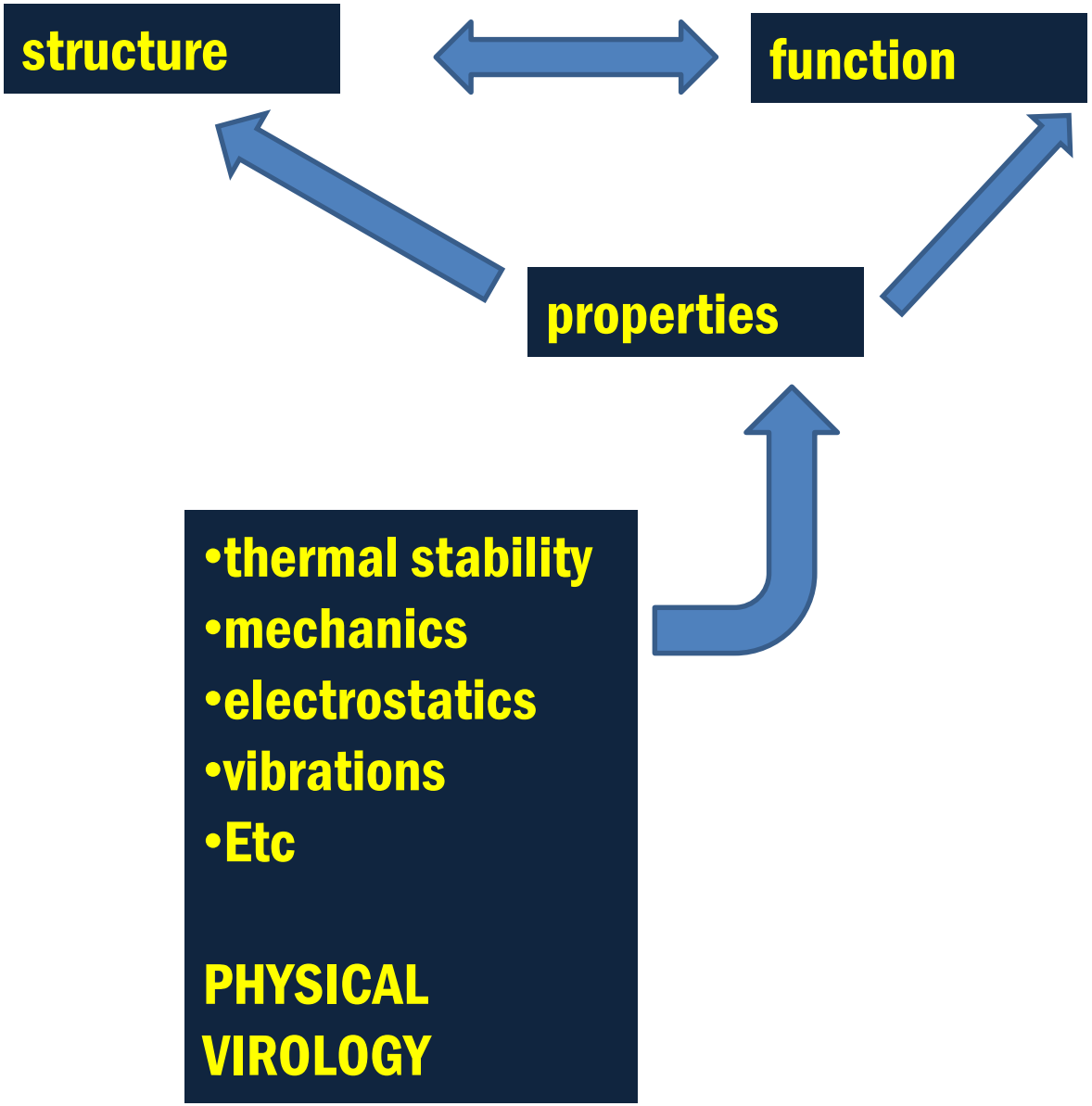
T. S. BAKER,[1*] N. H. OLSON,[1] AND S. D. FULLER[2,3] CROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Dec. 1999, p. 862–922

X-ray, EM

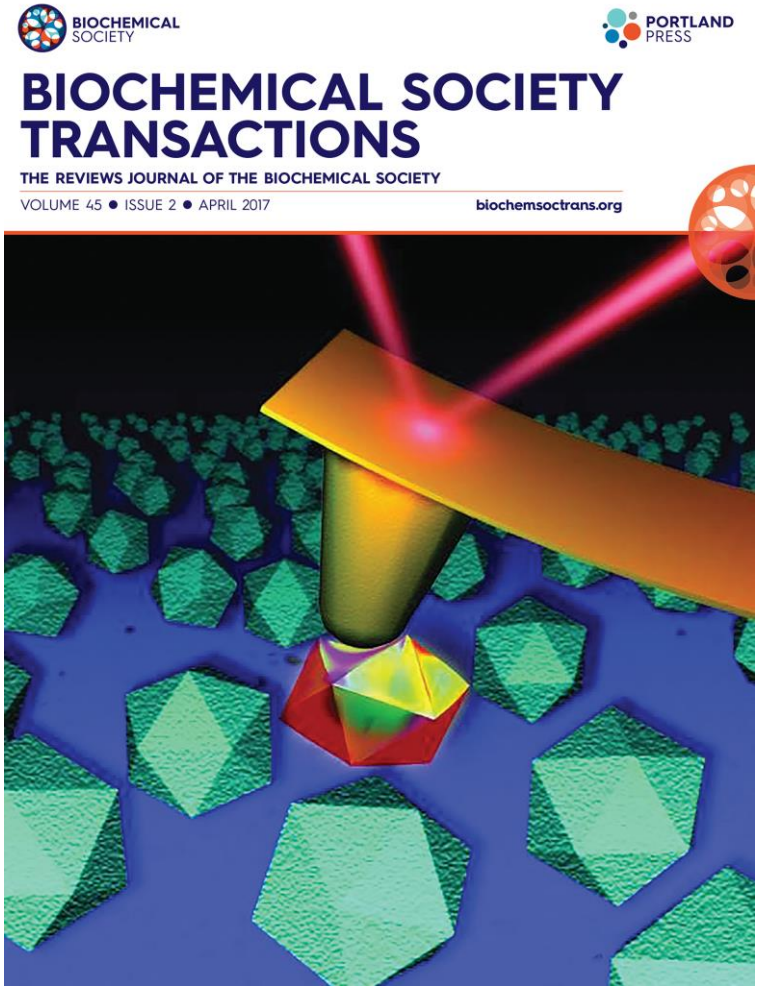
structure ⟷ **function**


NANO MICRO
small
Physical Virology
A 3D AFM topography rendition of a phage phi29 (red) adsorbed on mica (blue) is shown by P. J. de Pablo and coworkers, where part of the viral DNA (yellow) has been ejected through the tail. The phi29 bacteriophage translocates part of its DNA into the host by releasing the elastic energy arising from the internal pressure created during the DNA-packing process. Pushing with the AFM tip on the phage enables the direct measurement of the stiffness to estimate its internal pressure, just as a tyre may be pressed with fingers.
15/2012
WILEY-VCH
Direct Measurement of Phage phi29 Stiffness Provides Evidence of Internal Pressure
P. J. de Pablo et al.


Volume 7 | Number 41 | 7 November 2015 | Pages 17151–17574
Nanoscale
www.rsc.org/nanoscale
ISSN 2040-3364
ROYAL SOCIETY OF CHEMISTRY
PAPER
R. Podgornik, P. J. de Pablo et al.
Quantitative nanoscale electrostatics of viruses
NCNST


structure
function
properties
•thermal stability
•mechanics
•electrostatics
•vibrations
•Etc
PHYSICAL VIROLOGY

Single molecule techniques provide complementary information to structural biology



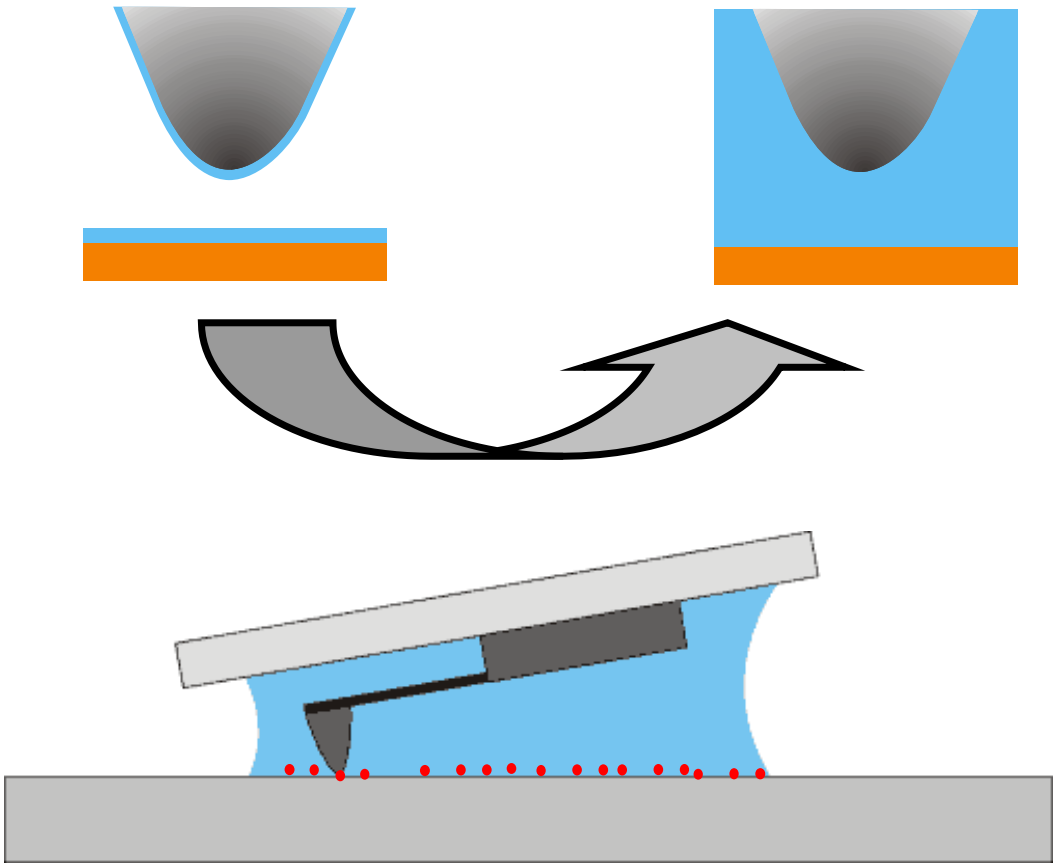
Atomic Force Microscopy

Binnig, Quate, Gerber, PRL 1986

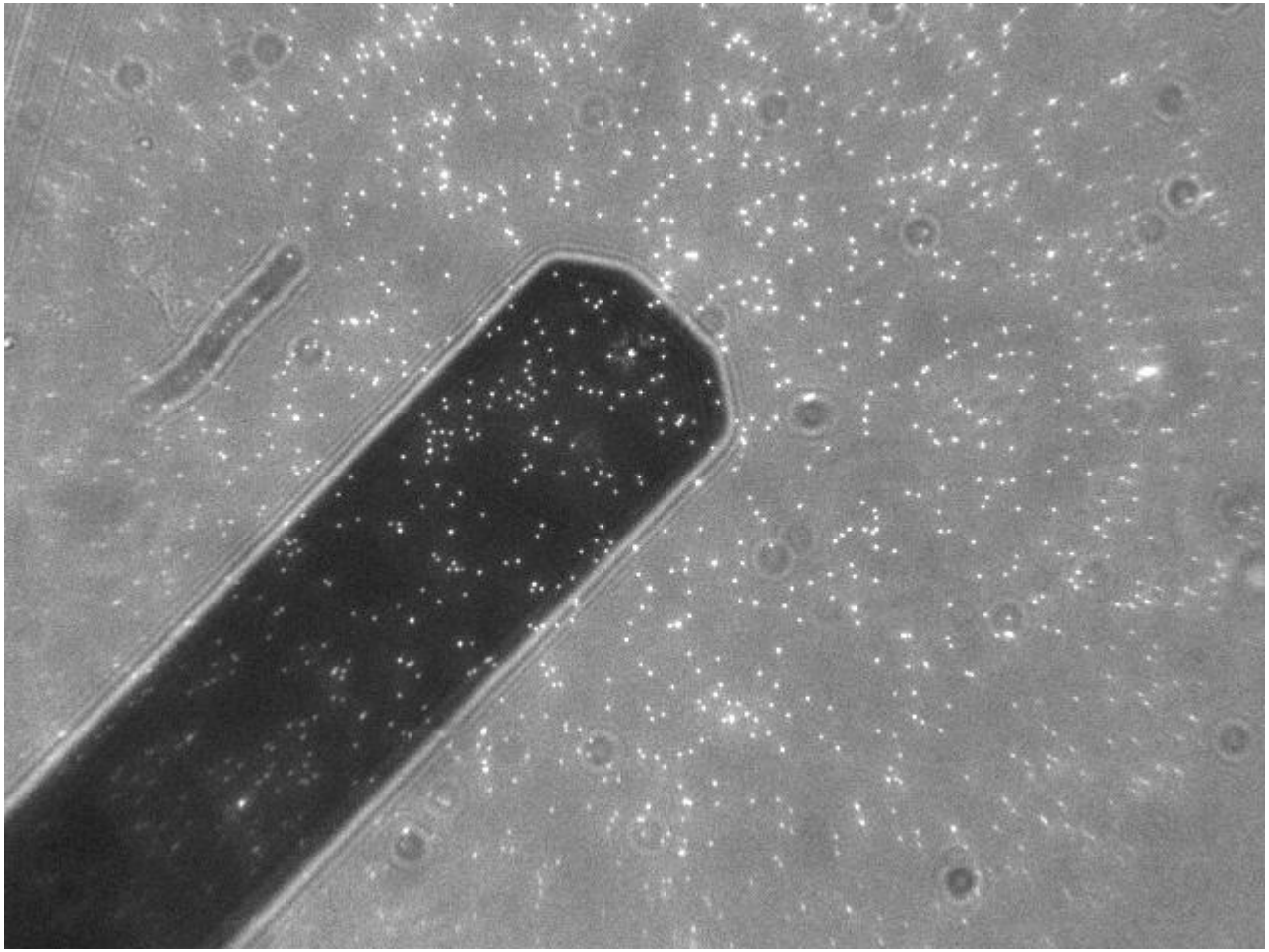
Physiological conditions
Functional protein shells

Mechanics
Manipulation
Real time experiments

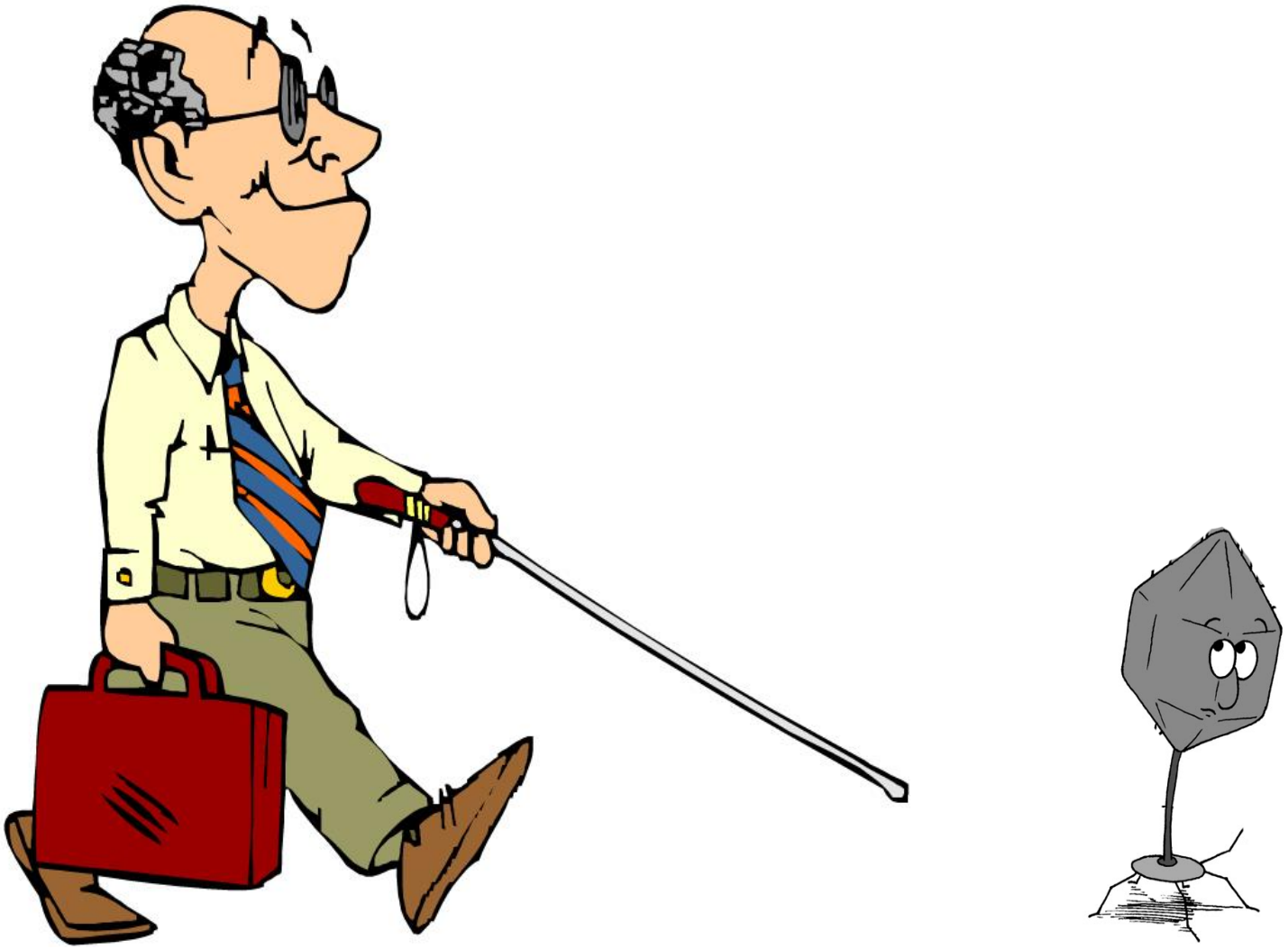
Atomic Force microscopy *in liquids*



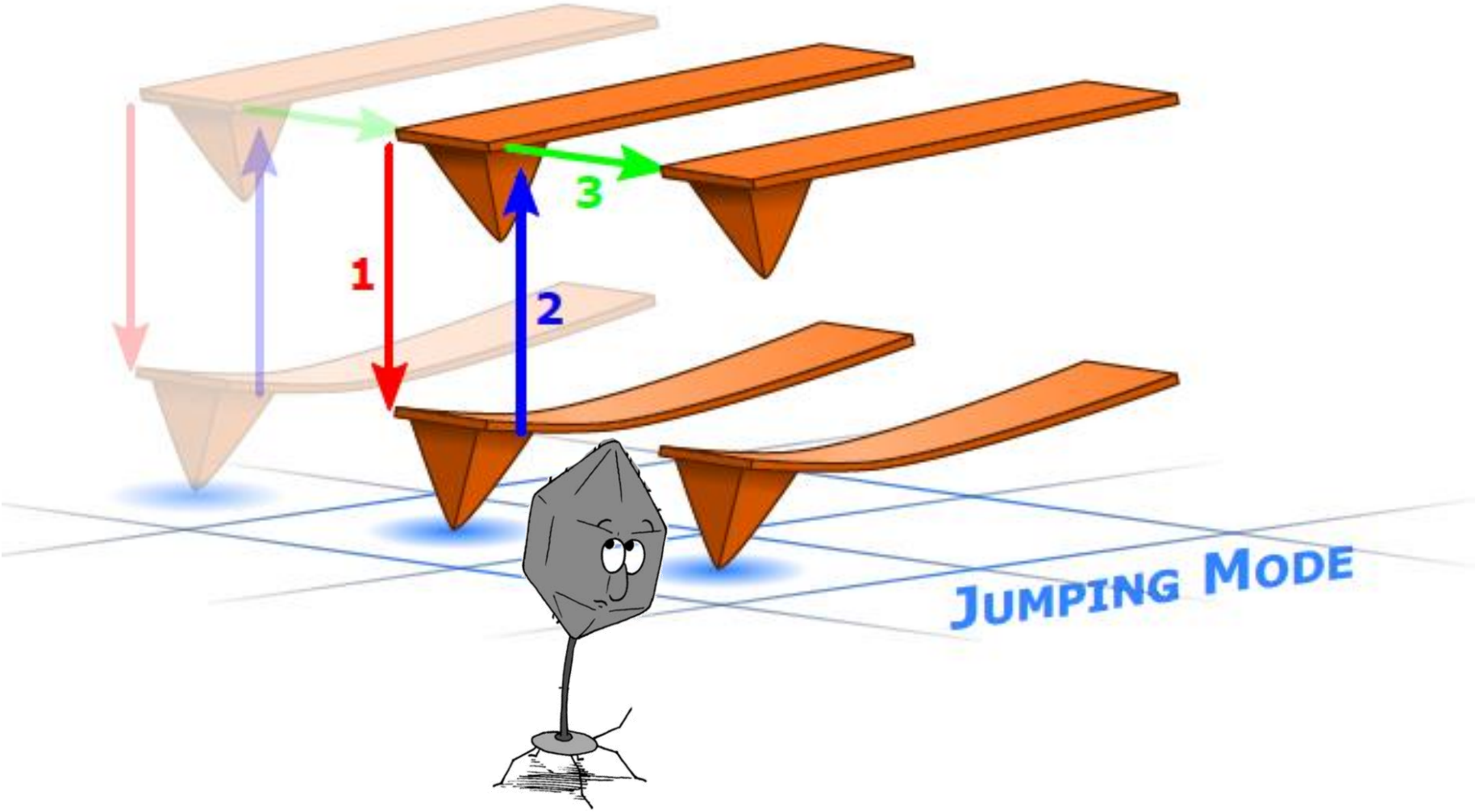
Cantilever/virus size



Atomic Force Microscopy
scanning probe



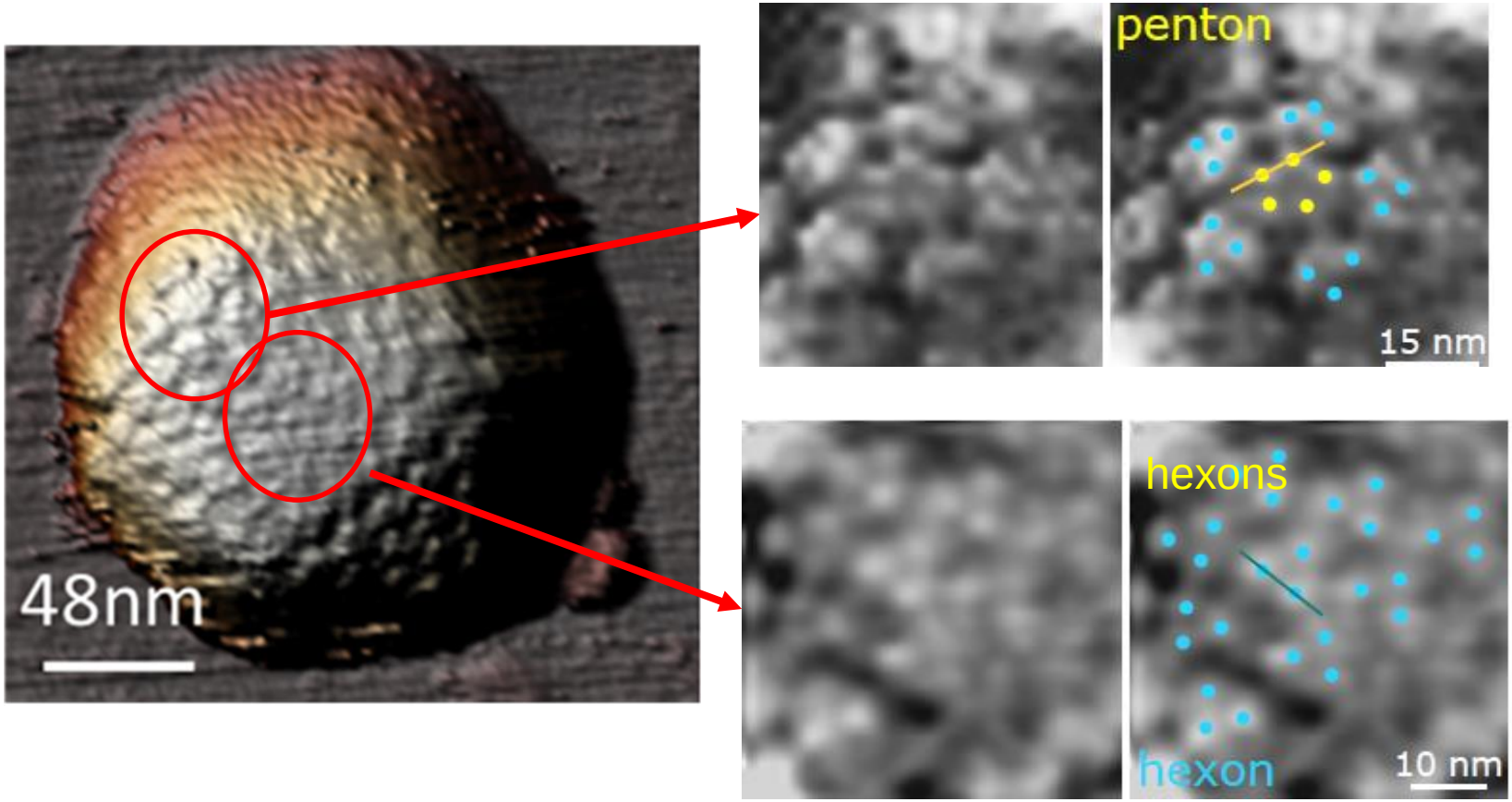
AFM imaging of viruses



De Pablo et al, APL 1998

Ortega-Esteban *et al.* Ultramicroscopy 2012

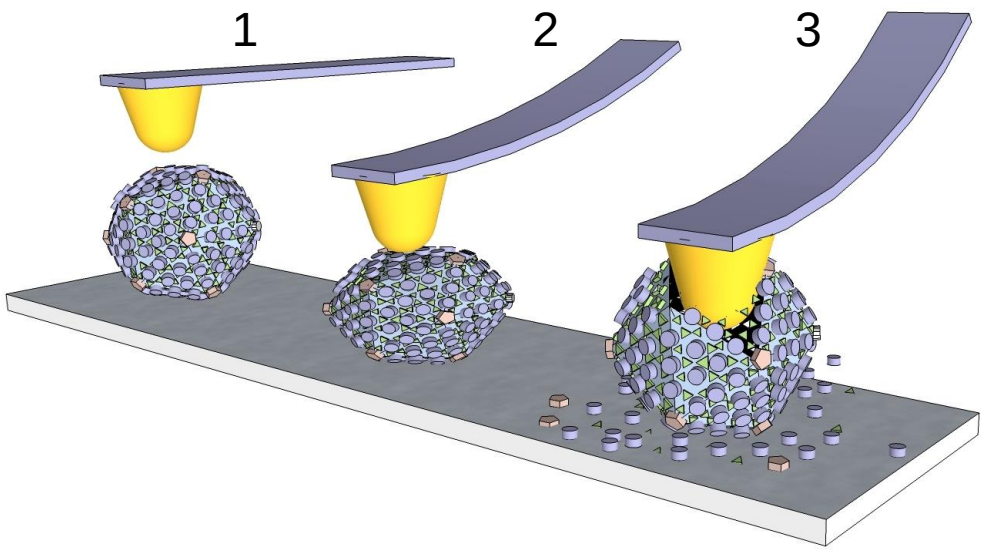
High resolution AFM of adenovirus

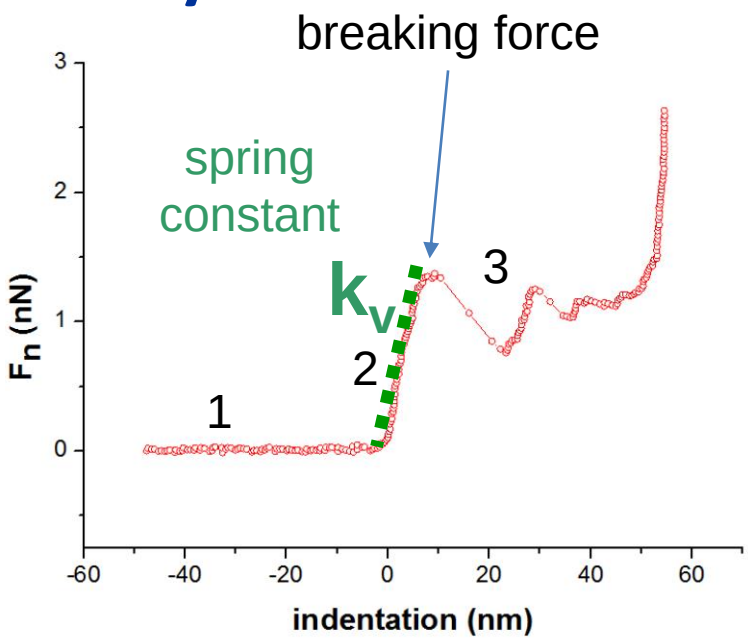


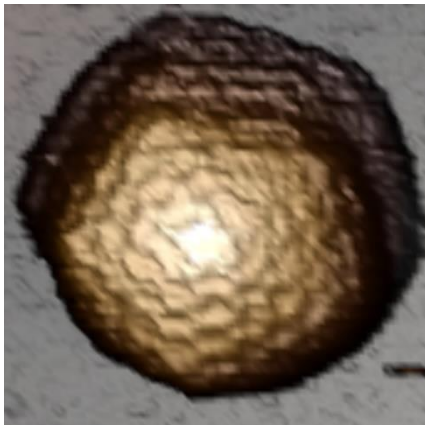
SCIENTIFIC REPORTS | 3 : 1434 | DOI: 10.1038/srep01434

Single indentation assay

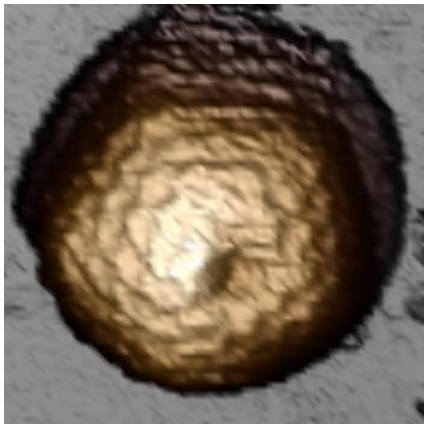
Single indentation assay





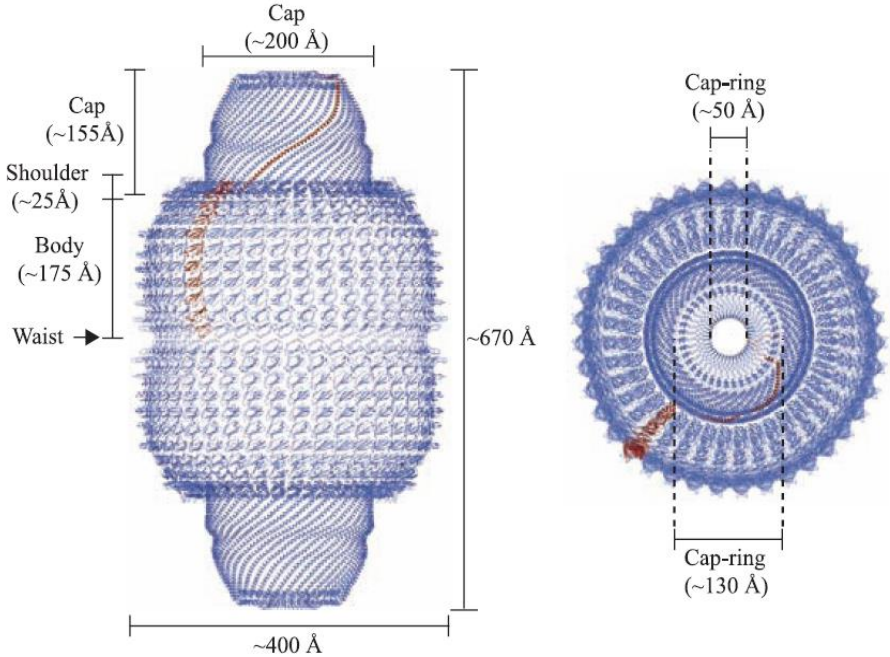


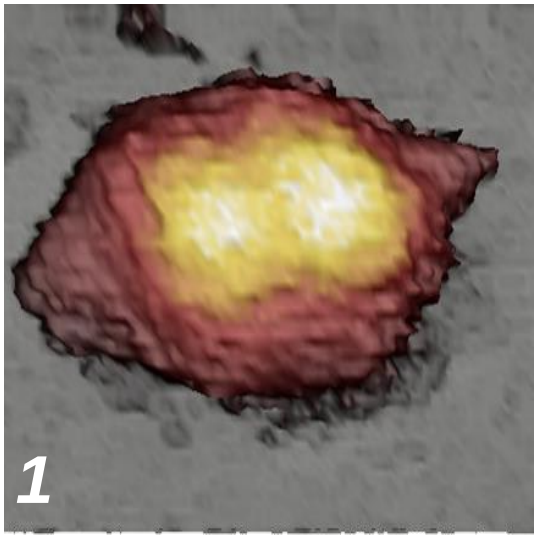
before

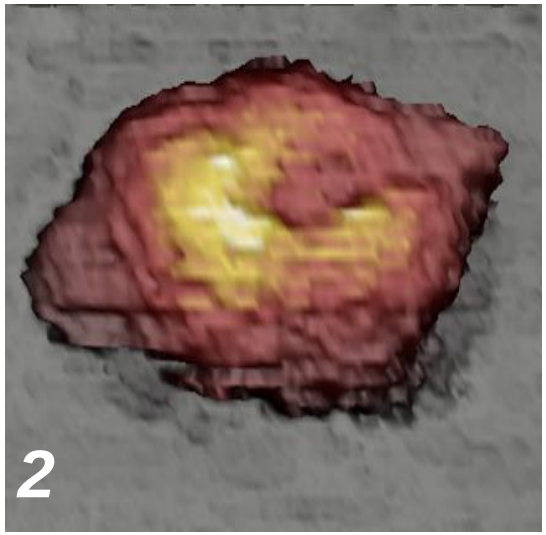


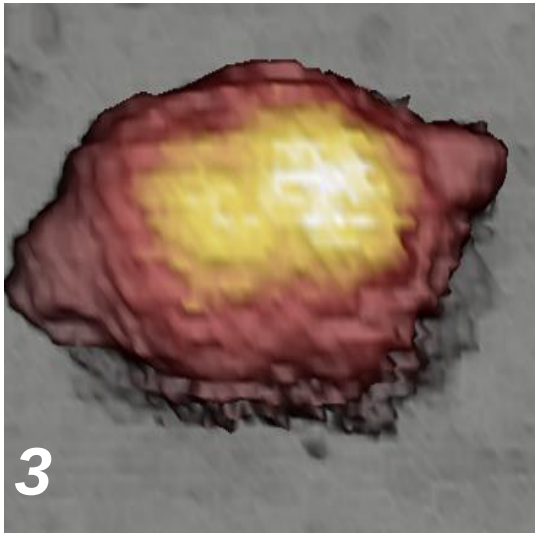
after

Self-recovery of vault particles









Llauró et al Biophysical Journal 2014

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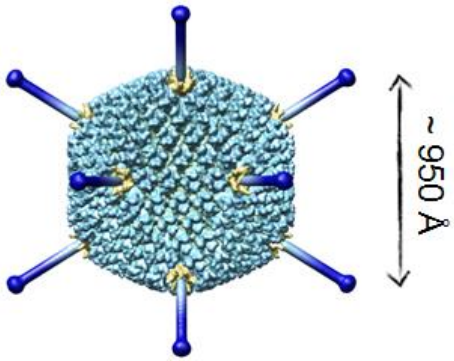
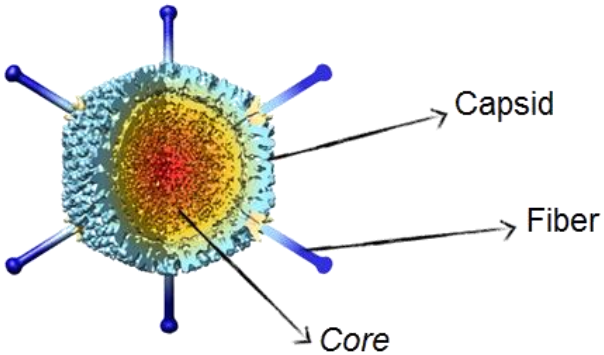
Human adenovirus



Capsid 240 hexons, 12 pentons, proteins IIIa, VI, VIII, IX

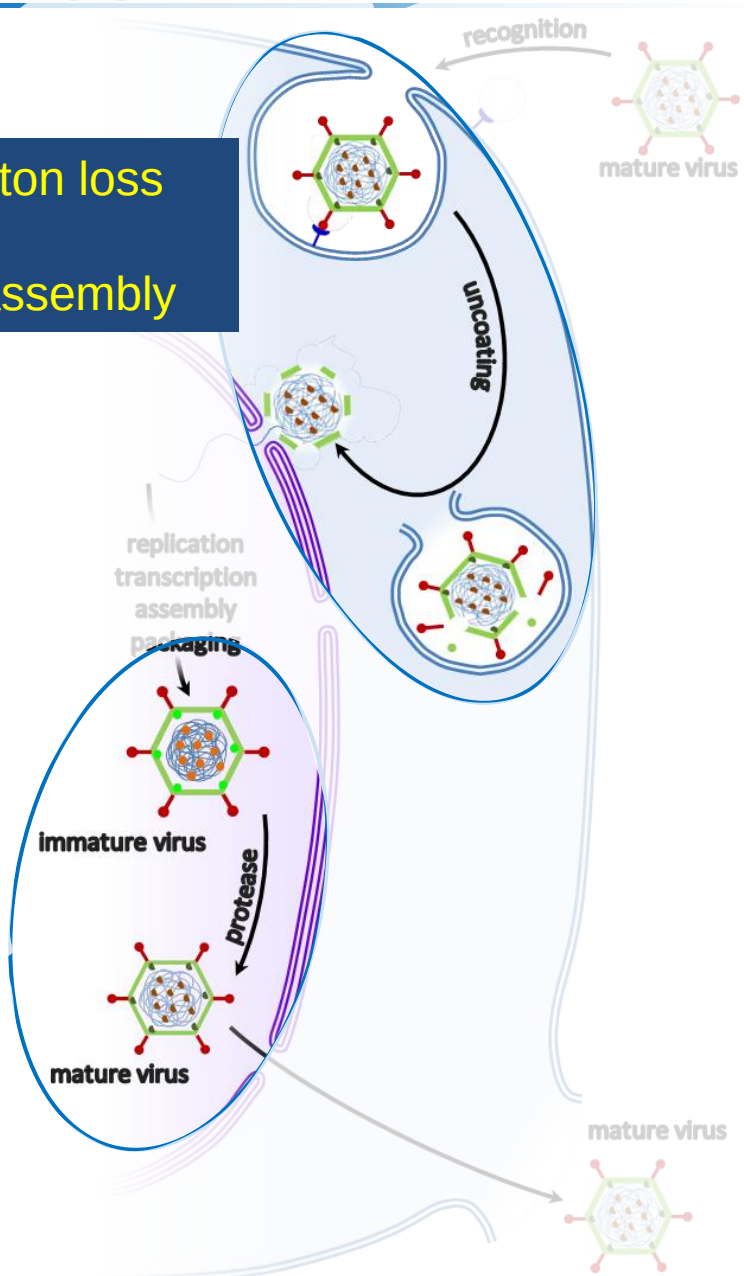
Fiber flexible, specific host recognition

Core 35kbp dsDNA, proteins TP, VII, μ



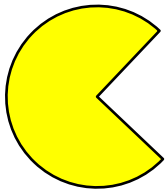
50% DNA + 50% histone-like proteins

Penton loss and disassembly

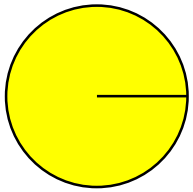


Greber *et al.* Cell 1993

Maturation changes the core

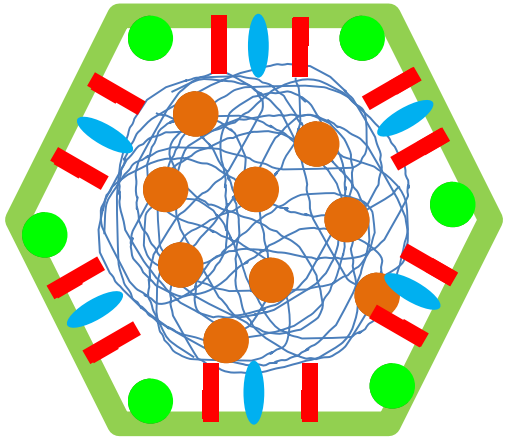


protease



cleaved protein

protease



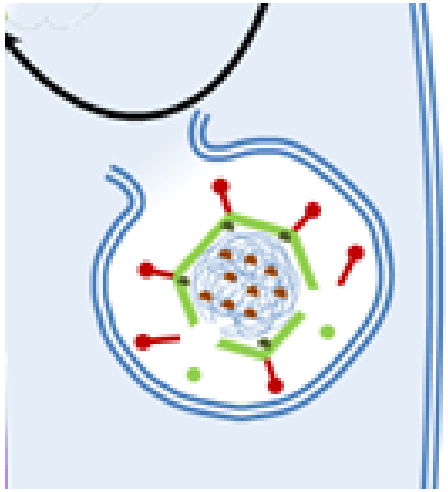
Immature non infectious

Mature infectious

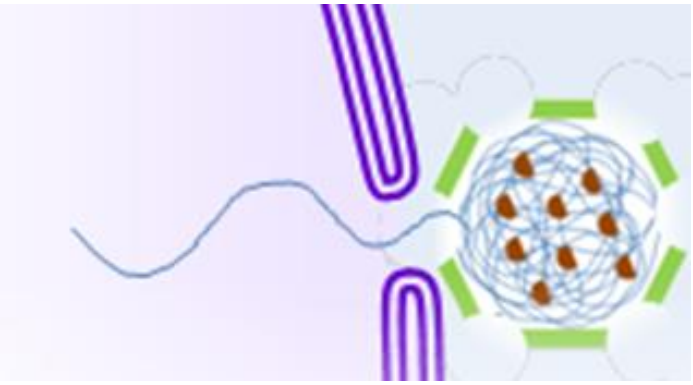
Pérez-Berná *et al.* JMB 2009

DNA

- **Does DNA modulate the mechanical properties of adenovirus particles?**
- **Interplay between physical properties and virus function?**

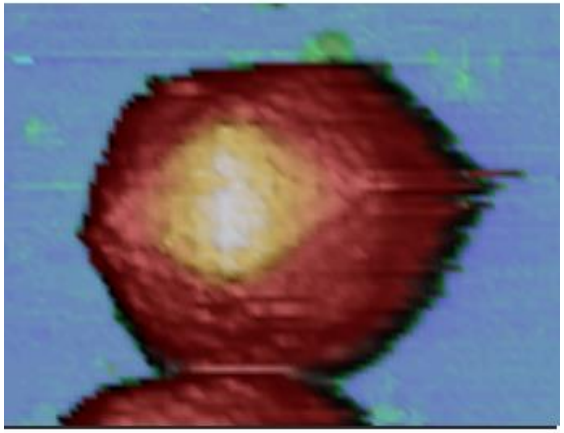


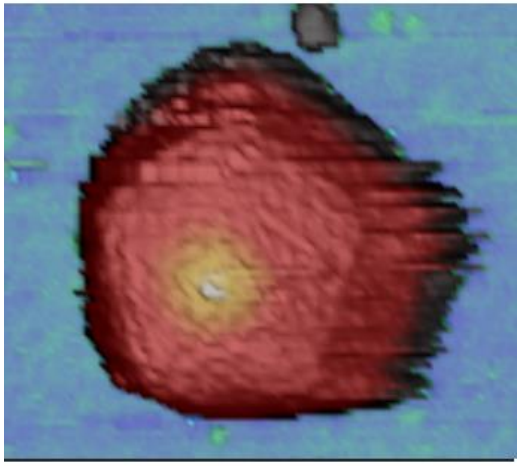
disassembly

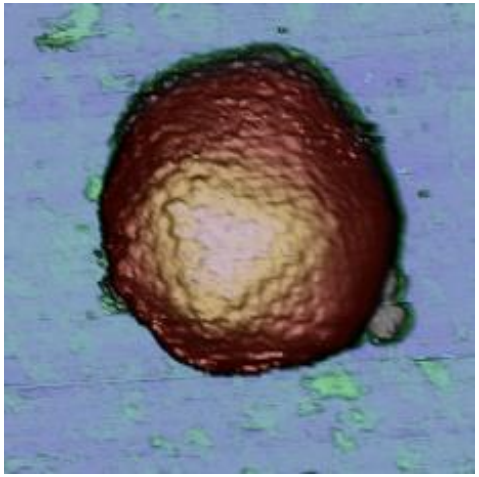


DNA diffusion

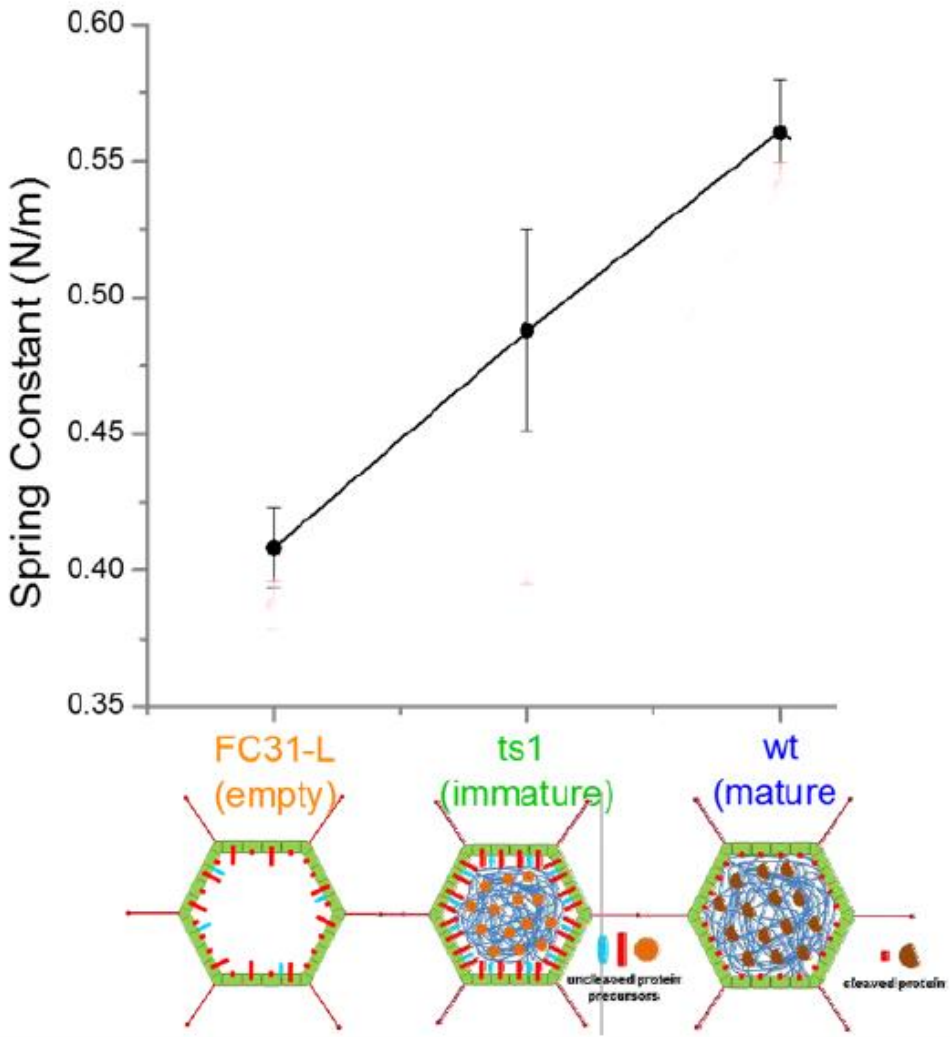
Adsorption geometries



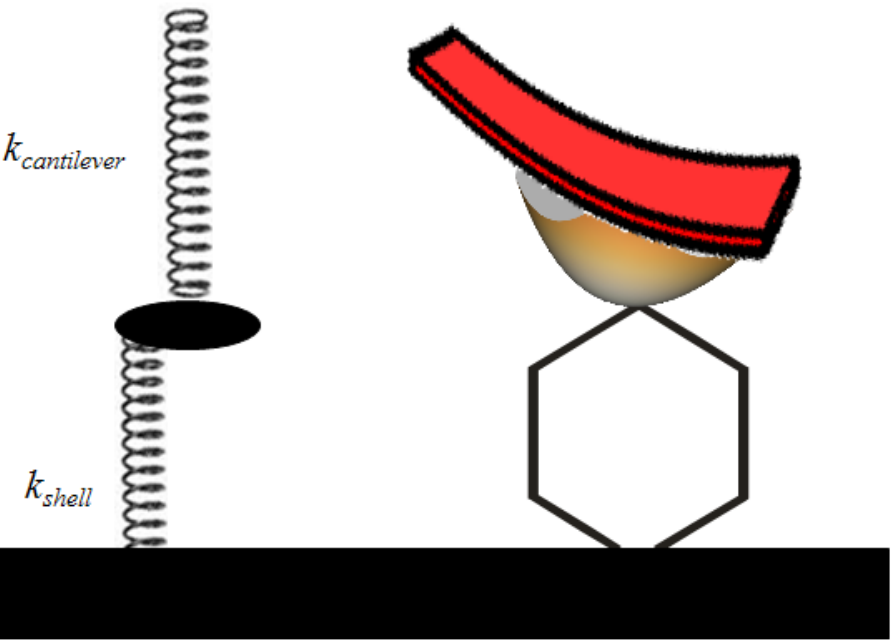


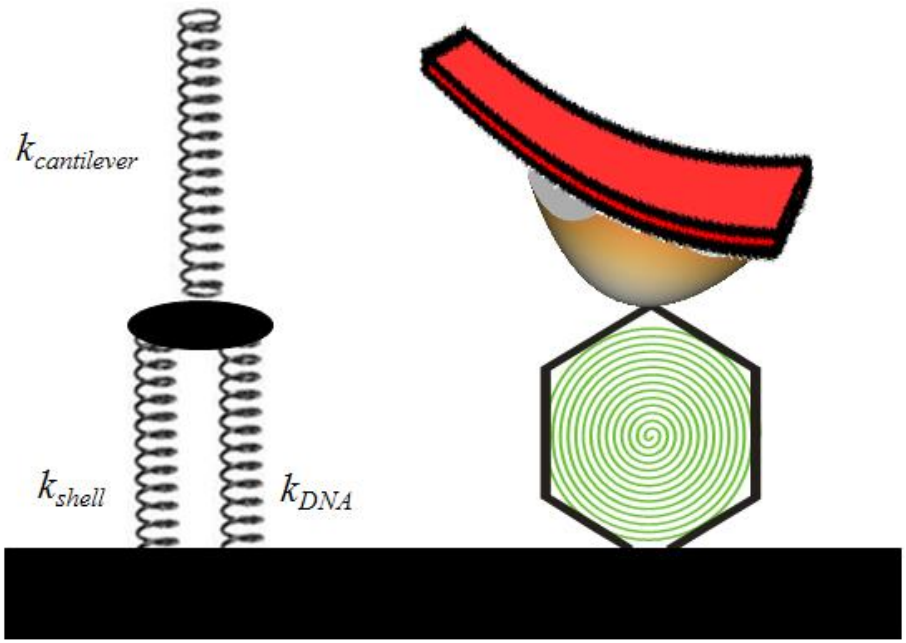


Mechanical evolution

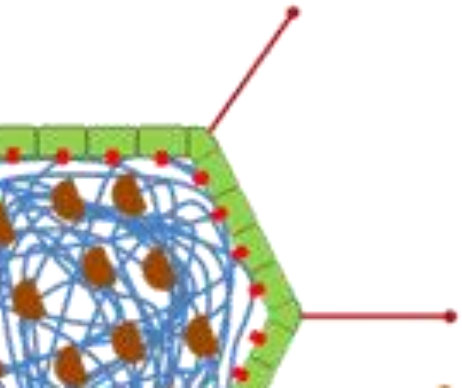


Interpretation

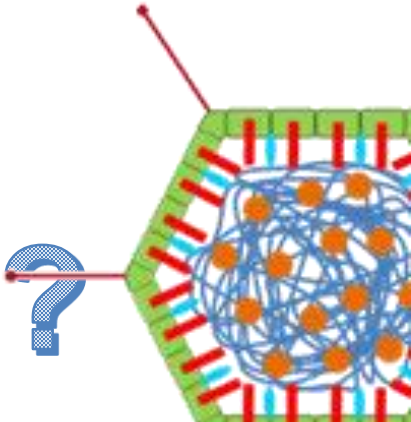




$$k_{virus}=k_{shell}+k_{DNA}$$



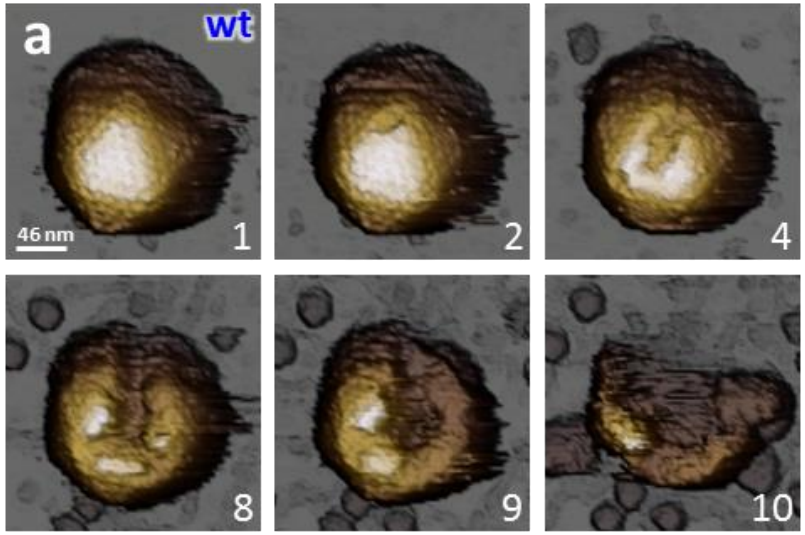
$$k_{DNA}\ (mature) > k_{DNA}(immature)$$

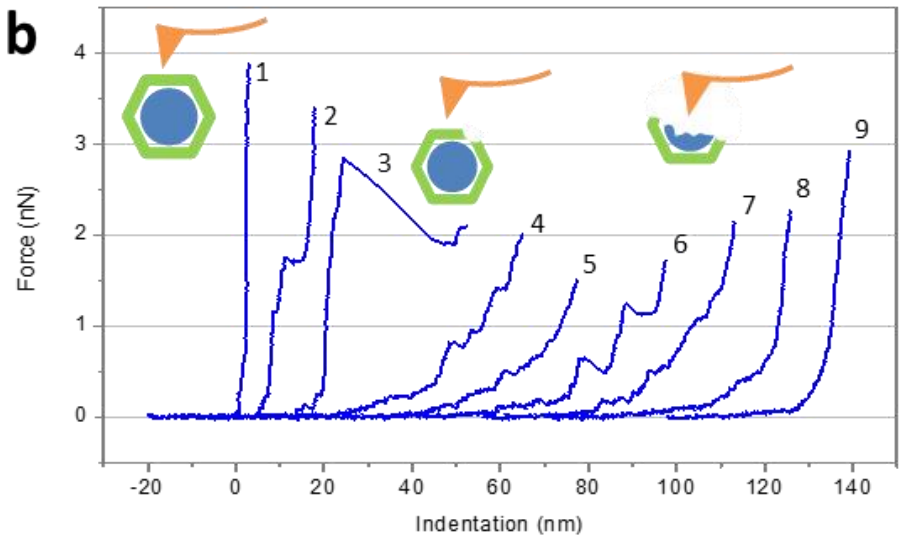


Crack-open the shell

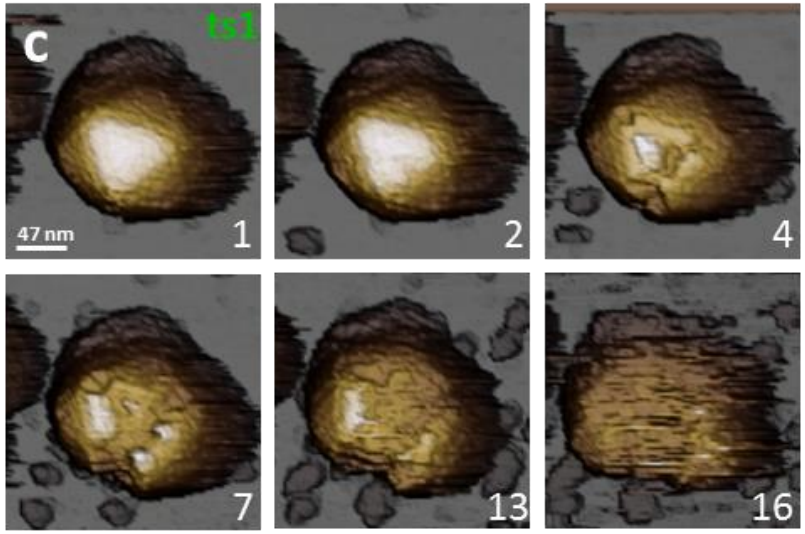
Crack-opening the shell

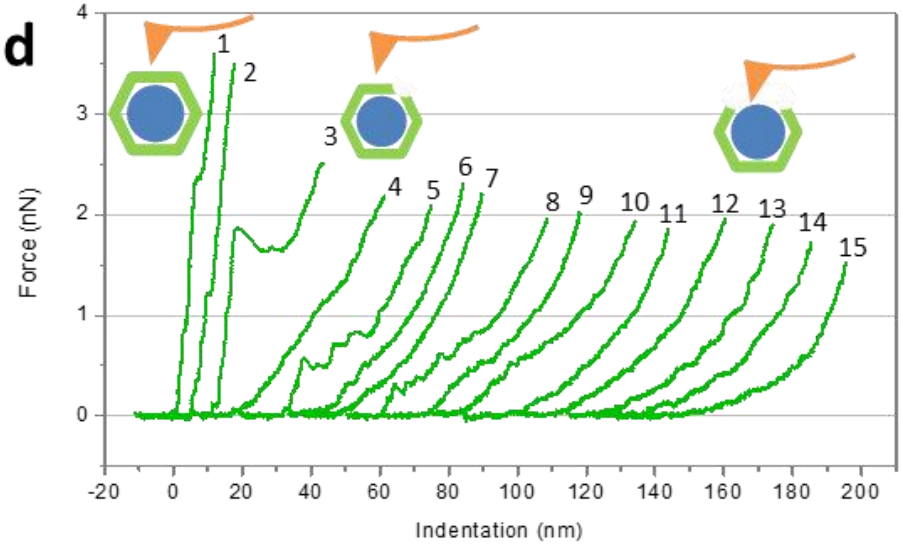
mature



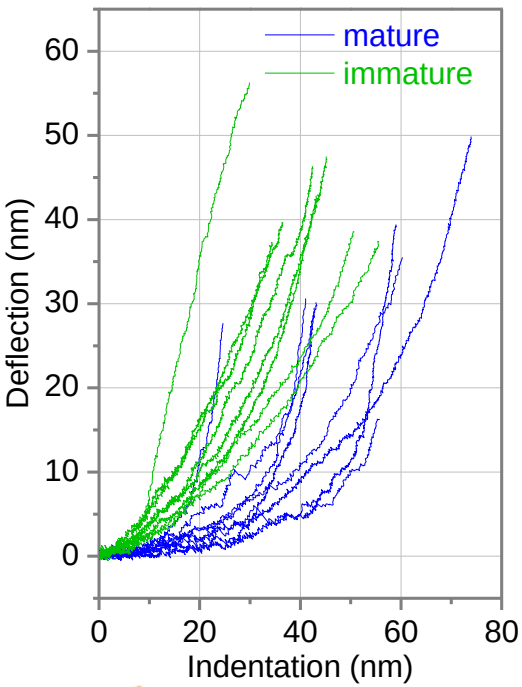


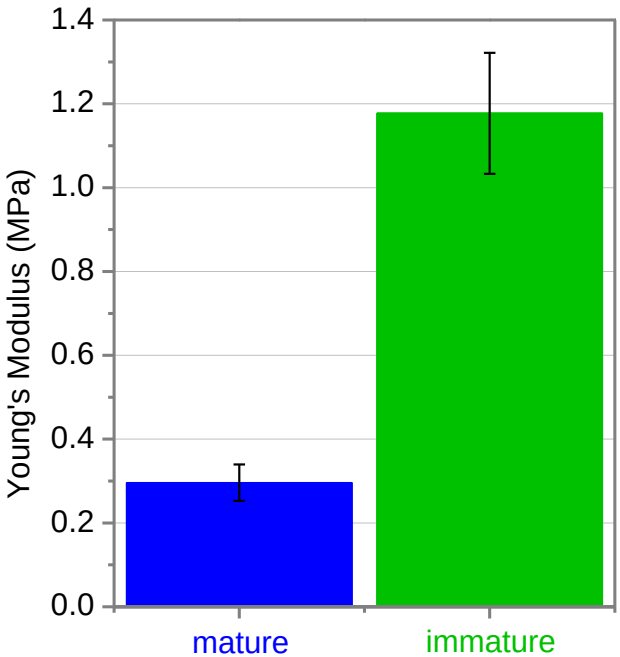
inmature





Mechanics of cores





Dimitriadis

Biophys J. 2002

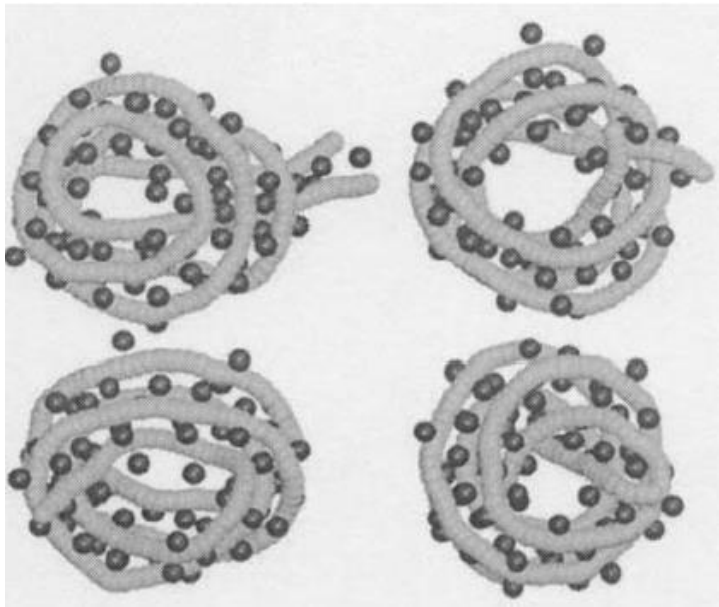
$E_{mature} < E_{immature}$

Pressurization?

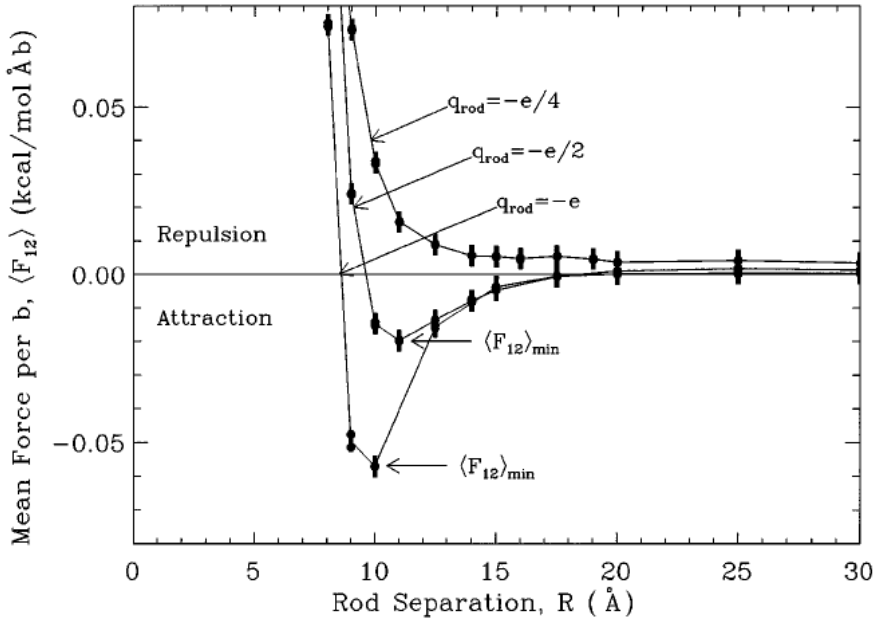
$k_{mature} > k_{immature}$

DNA condensate

Adding counterions to DNA induce toroidal condensates (3+)

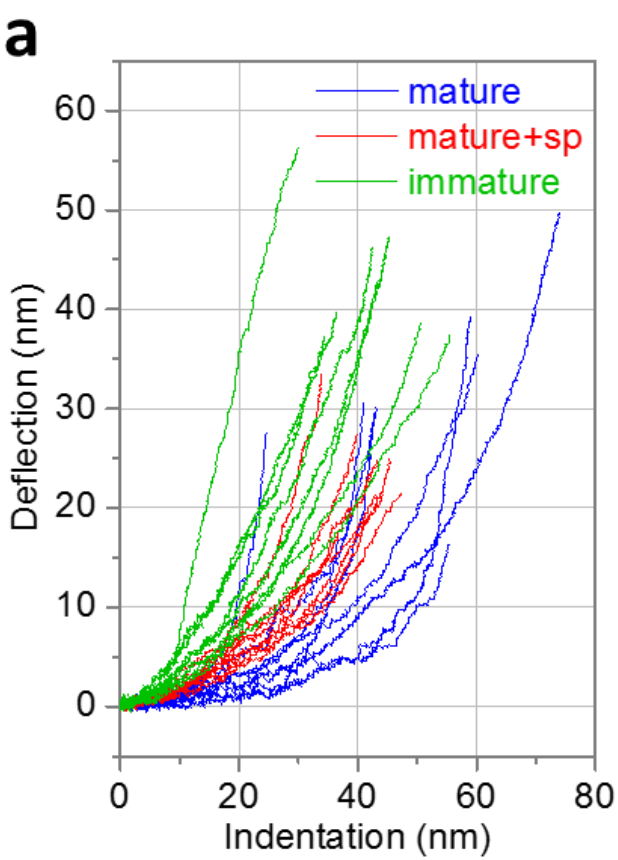


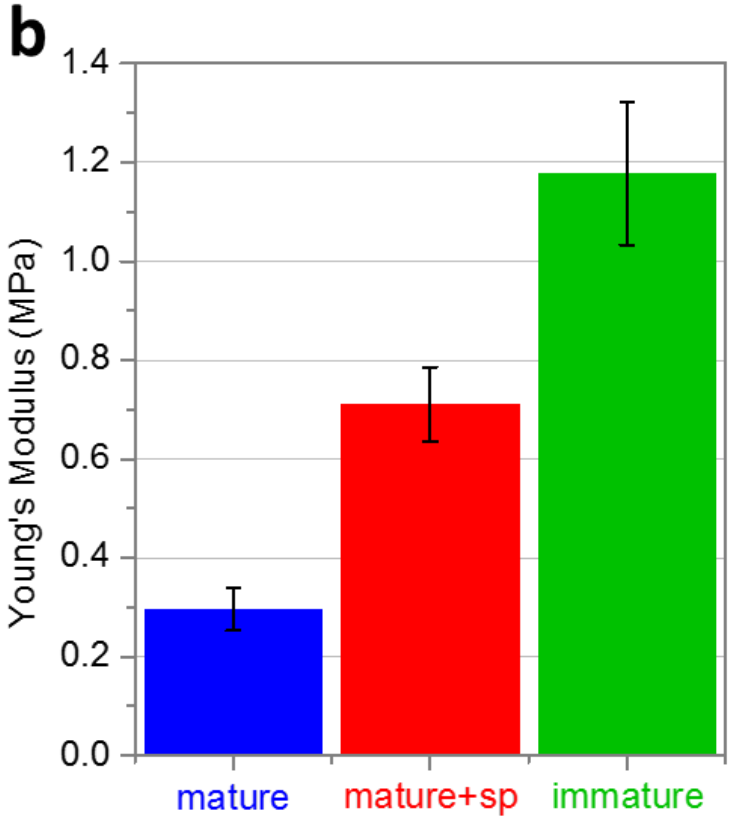
M. J. Stevens (2001)



Gronbech-Jensen et al. PRL 1997

Core mechanics





$E_{mature} < E_{mature\ spermidine} < E_{immature}$

Pressure estimation

Irrespective of the physical origin

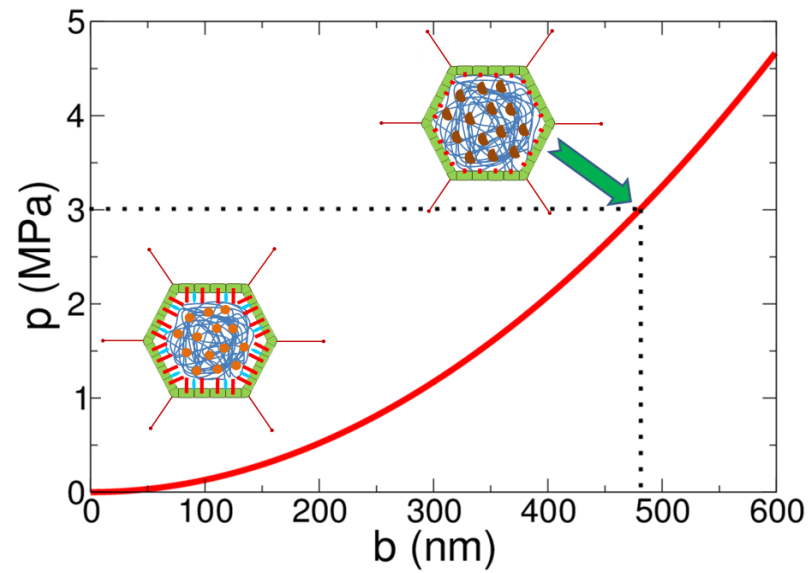
Vella et al. The Indentation of Pressurized Elastic Shells: From Polymeric Capsules to Yeast Cells. **2011,** *Journal of The Royal Society Interface*.

$$k_1 = \frac{\pi}{2} k_0 \frac{(\tau^2 - 1)^{\frac{1}{2}}}{\operatorname{arctanh}\left[(1 - \tau^{-2})^{\frac{1}{2}}\right]}.$$

$$\tau = \frac{pR_1}{k_0}$$

p=3±1 MPa

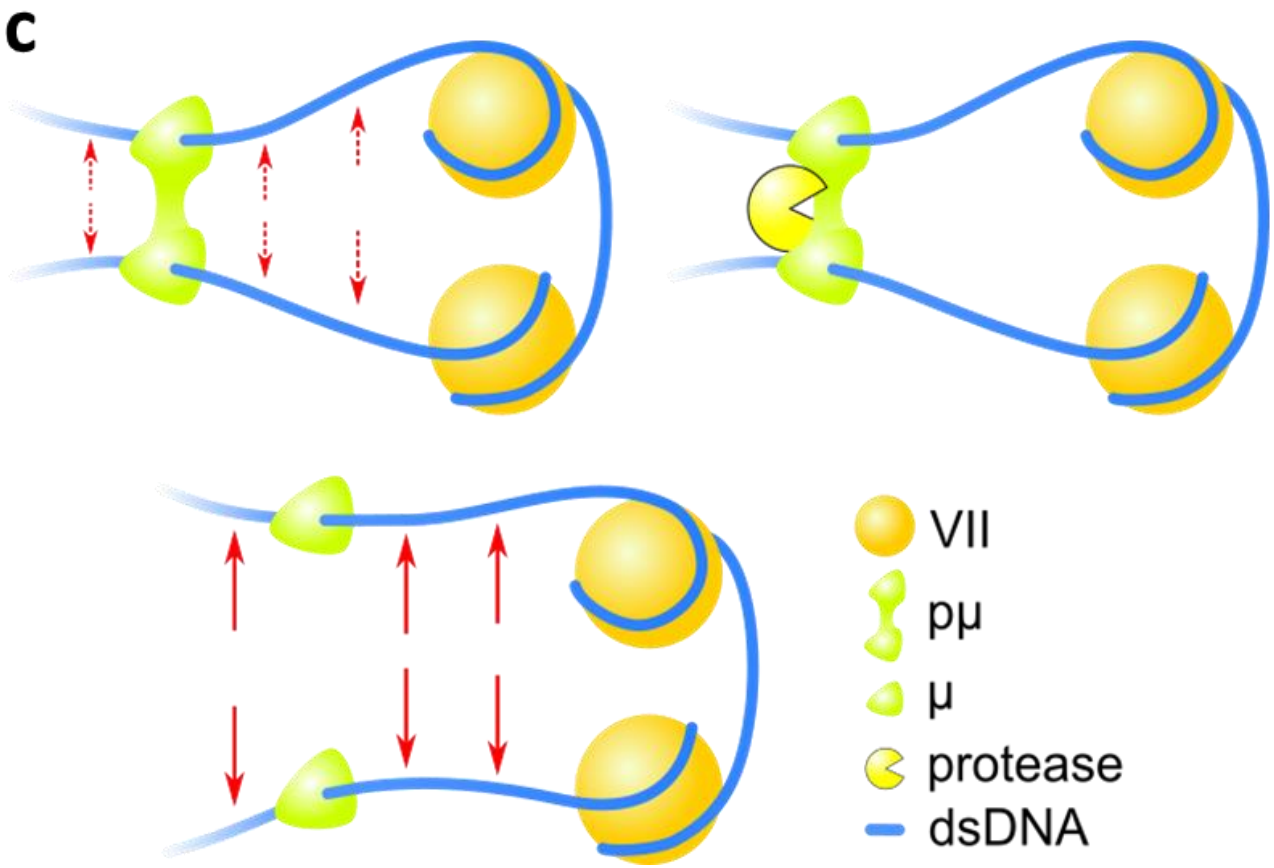
Unbranched polymer



persistence length *b*/2

$$F \approx k_B T (R_g/R)^{1/\nu}$$

$$P = -\left.\frac{\partial F}{\partial V}\right|_T$$

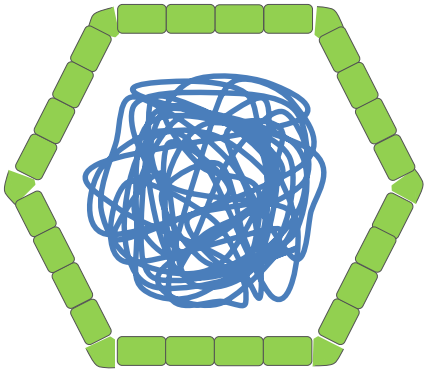


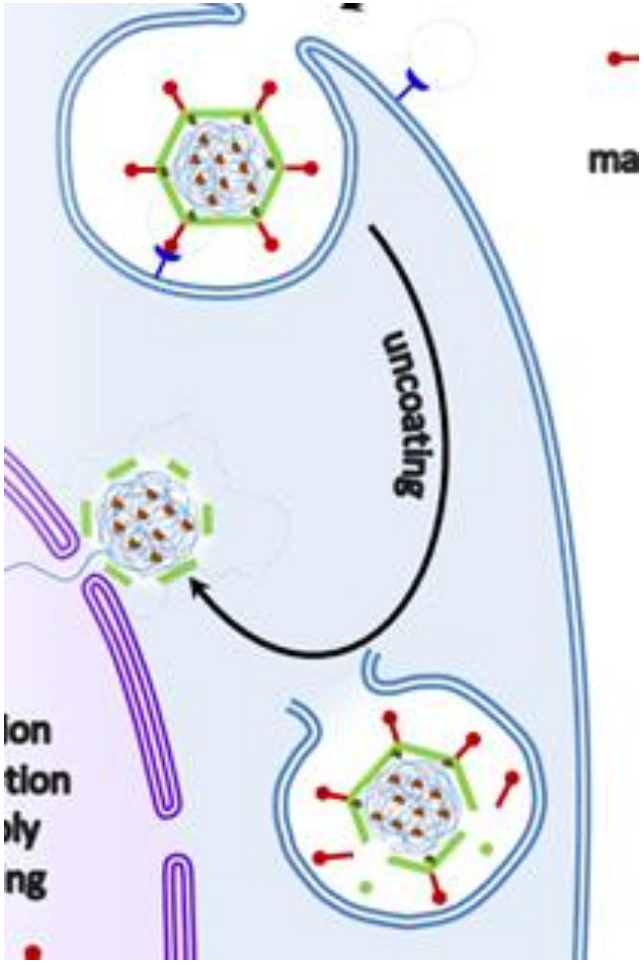
DNA-DNA repulsion pressurizes the shell after maturation

Biological implications

Pentons are the weakest capsomers

W. Klug et al, PRL 10/2012; 109(16):168104.
Ortega-Esteban Sci. Rep. 2013, 3, 14434

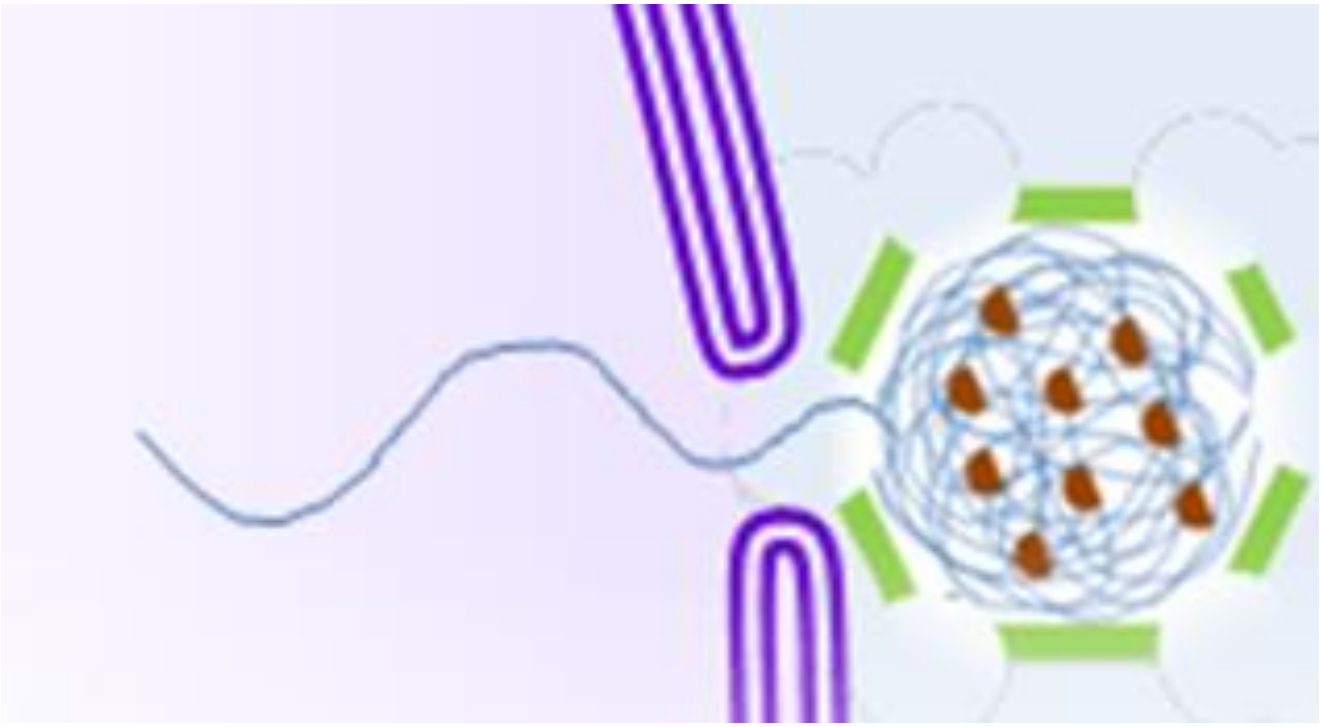




Ortega-Esteban et al ACS Nano 2015

We propose that pressure helps to pop-off pentons at the early endosoome

Biological implications

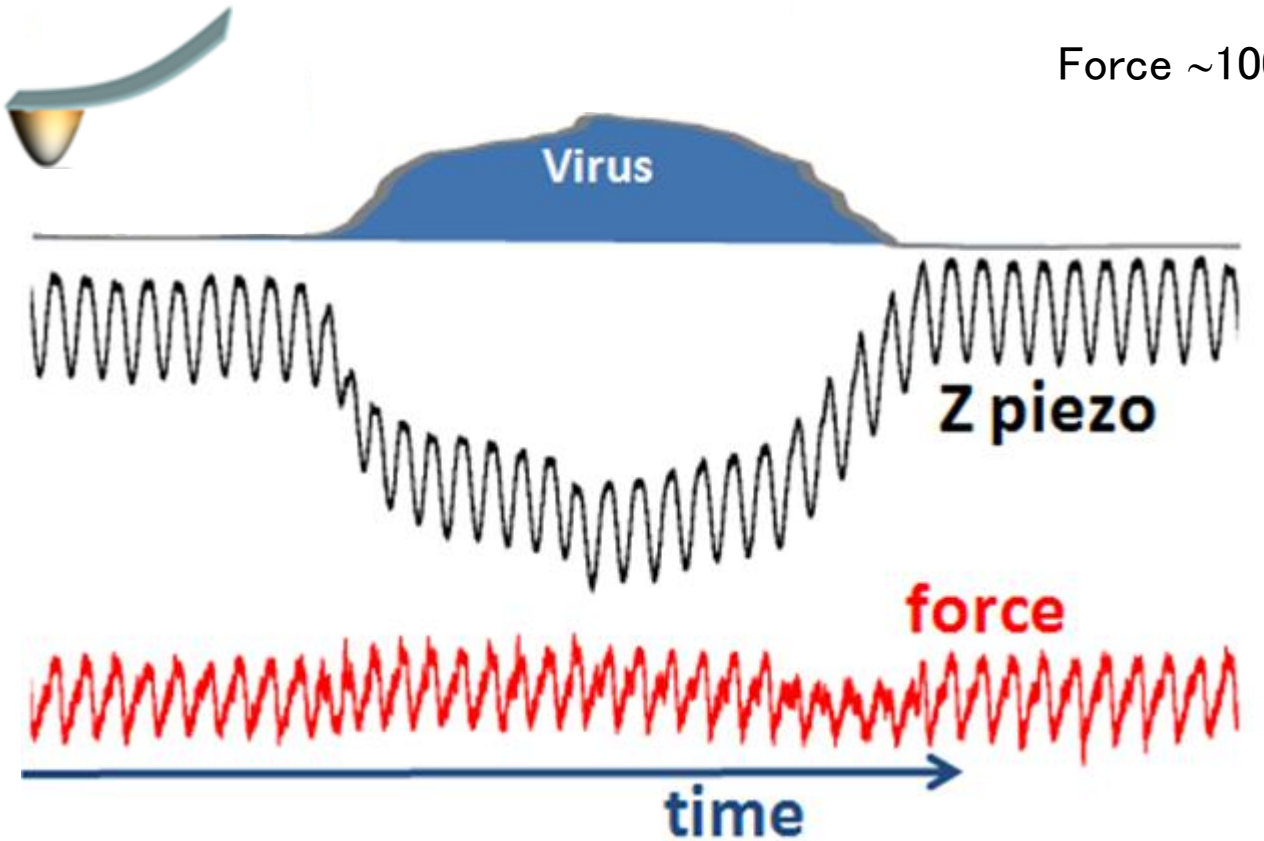


diffusion of DNA?

Fatigue

[redacted]

Multiple indentation assay below the breaking force (fatigue)



30 times less than breaking force!

Ortega-Esteban *et al.* Ultramicroscopy 2012

SCIENTIFIC REPORTS | 3 : 1434 | DOI: 10.1038/srep01434

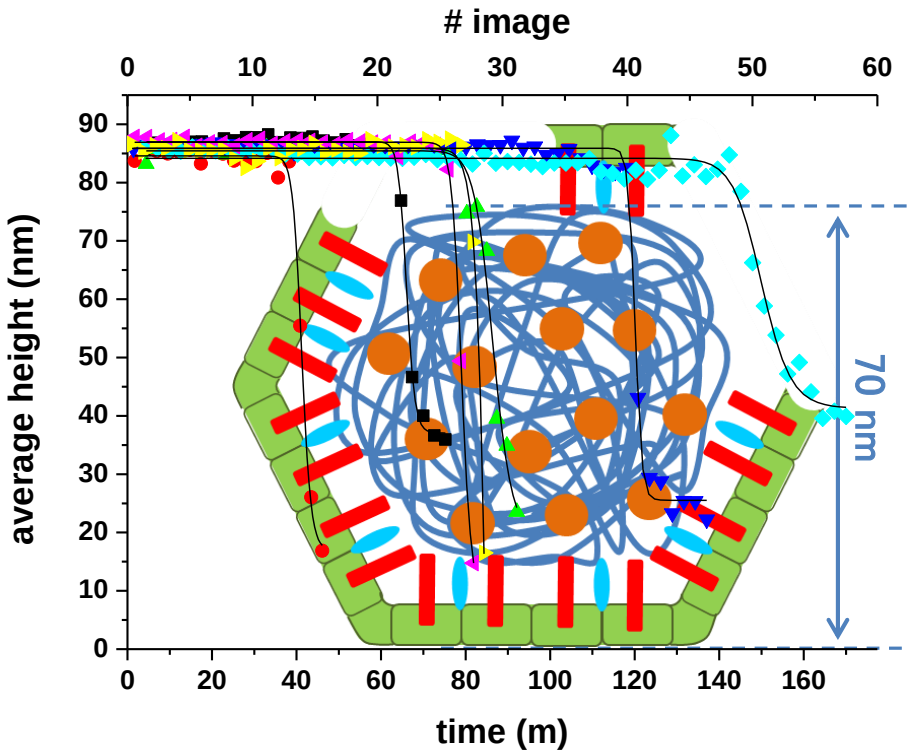
Uncoating dynamics

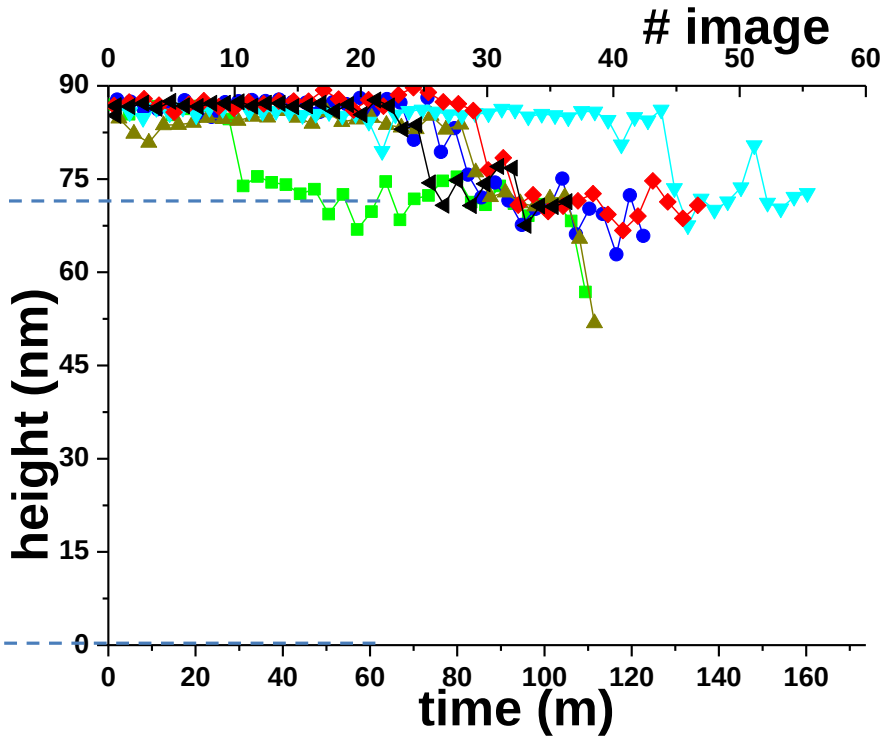
Mature

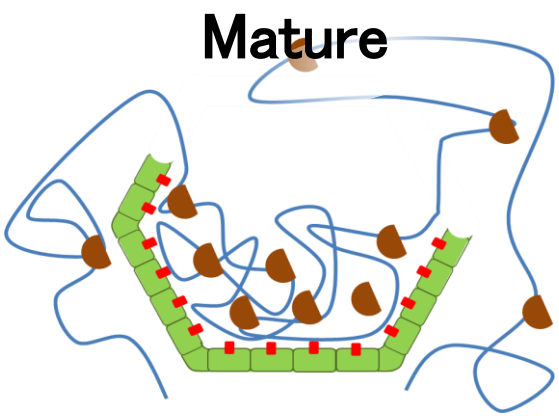
Immature

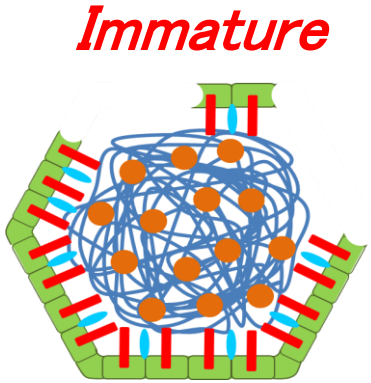
SCIENTIFIC REPORTS | 3 : 1434 | DOI: 10.1038/srep01434

Core exposure





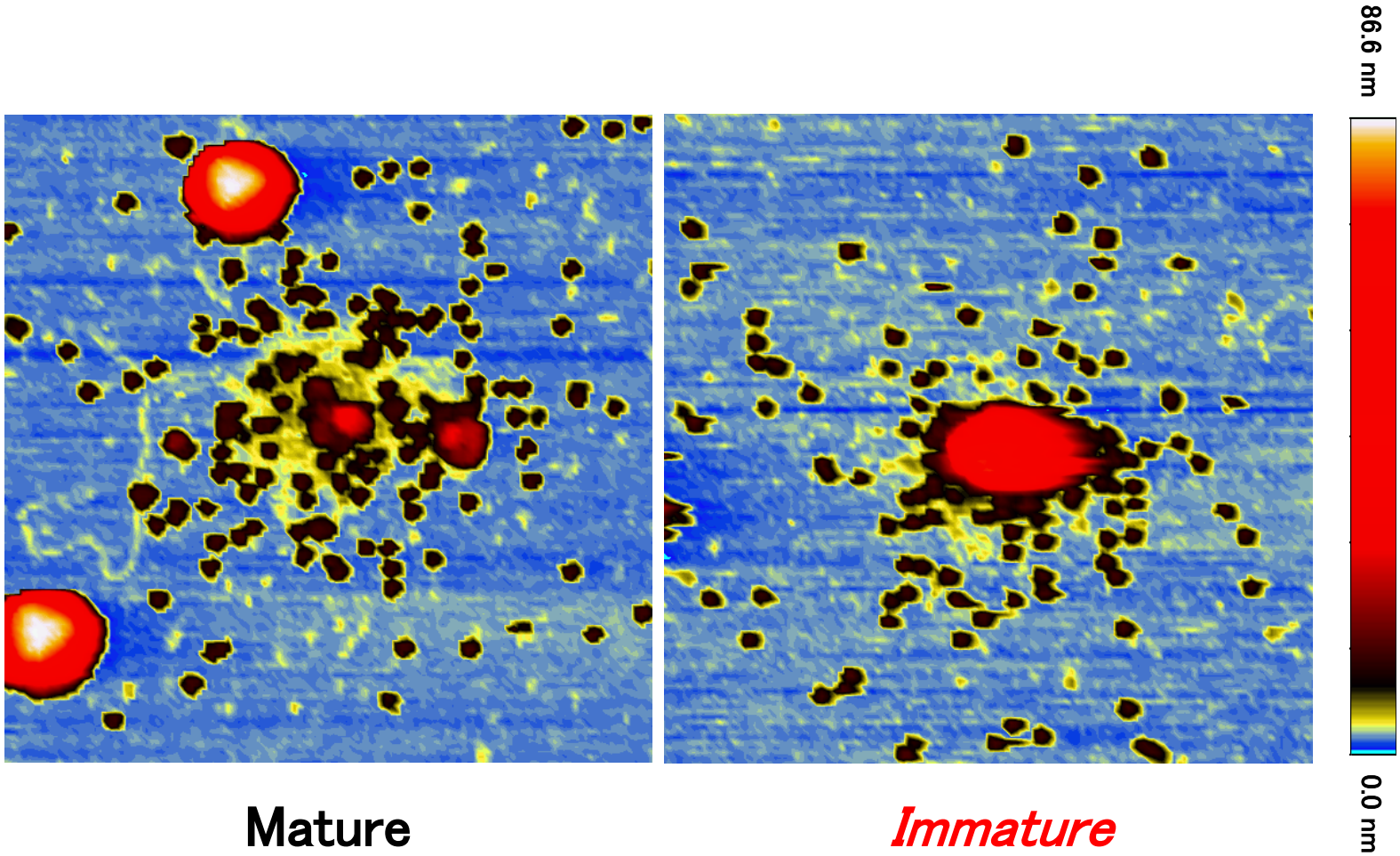




Can we visualize genome uncoating?

SCIENTIFIC REPORTS | 3 : 1434 | DOI: 10.1038/srep01434

Core exposure



Can we visualize the genome uncoating?

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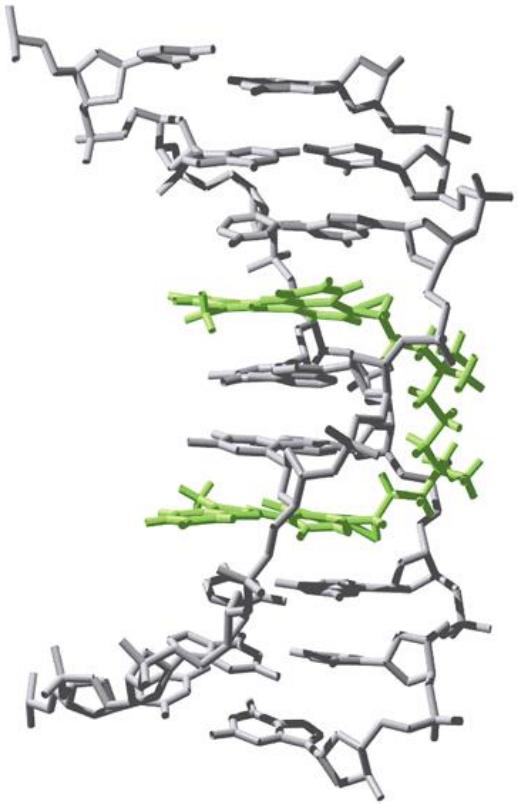
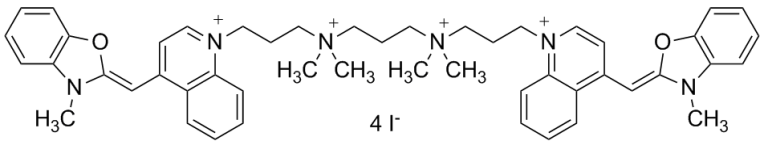
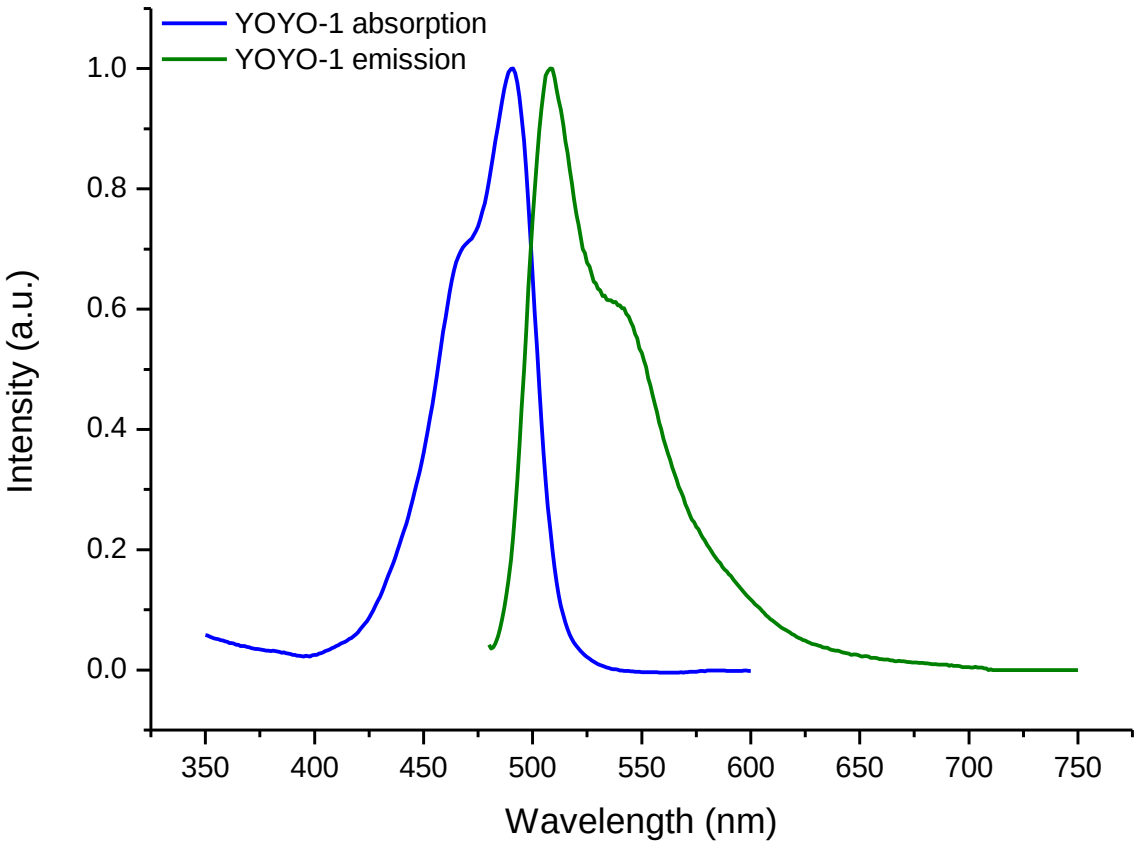
Topics today

1. Introduction
2. Mechanics of human adenovirus: capsid and core
3. Genome release: watching a virus undress
4. Mechanical role of cementing proteins: tuning particles stability with symmetrical morphogenesis
5. Summing up

Fluorescence

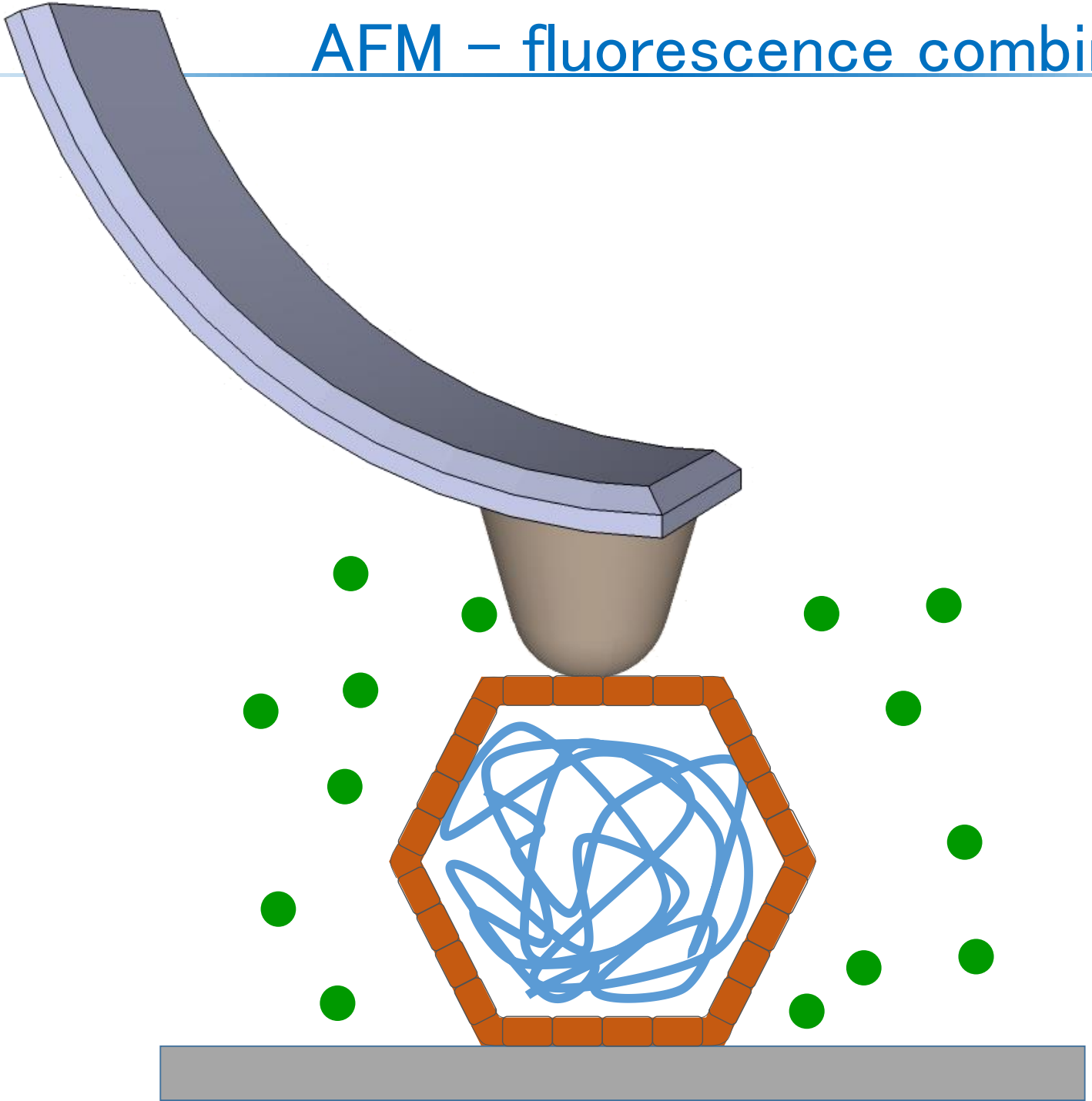




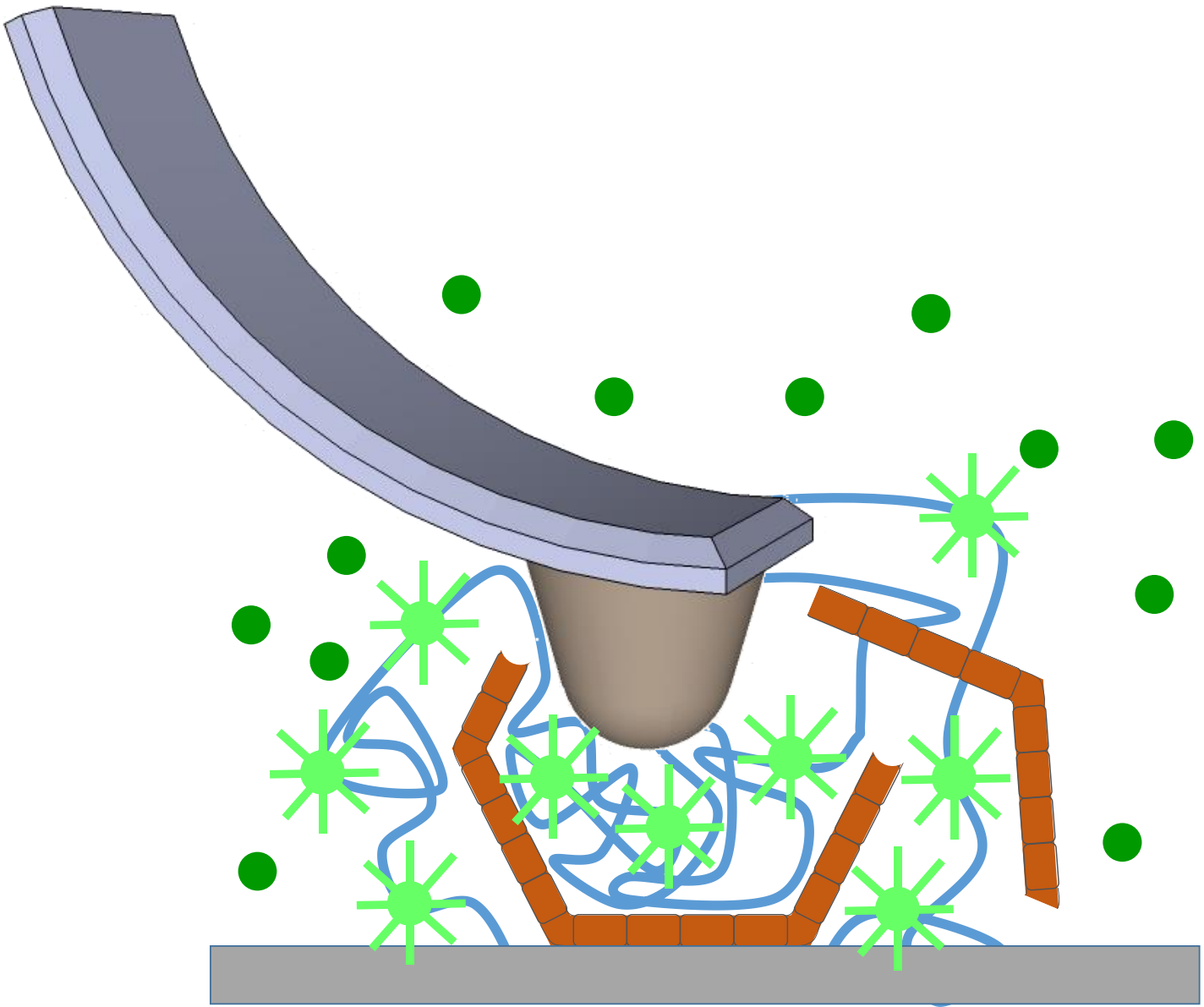


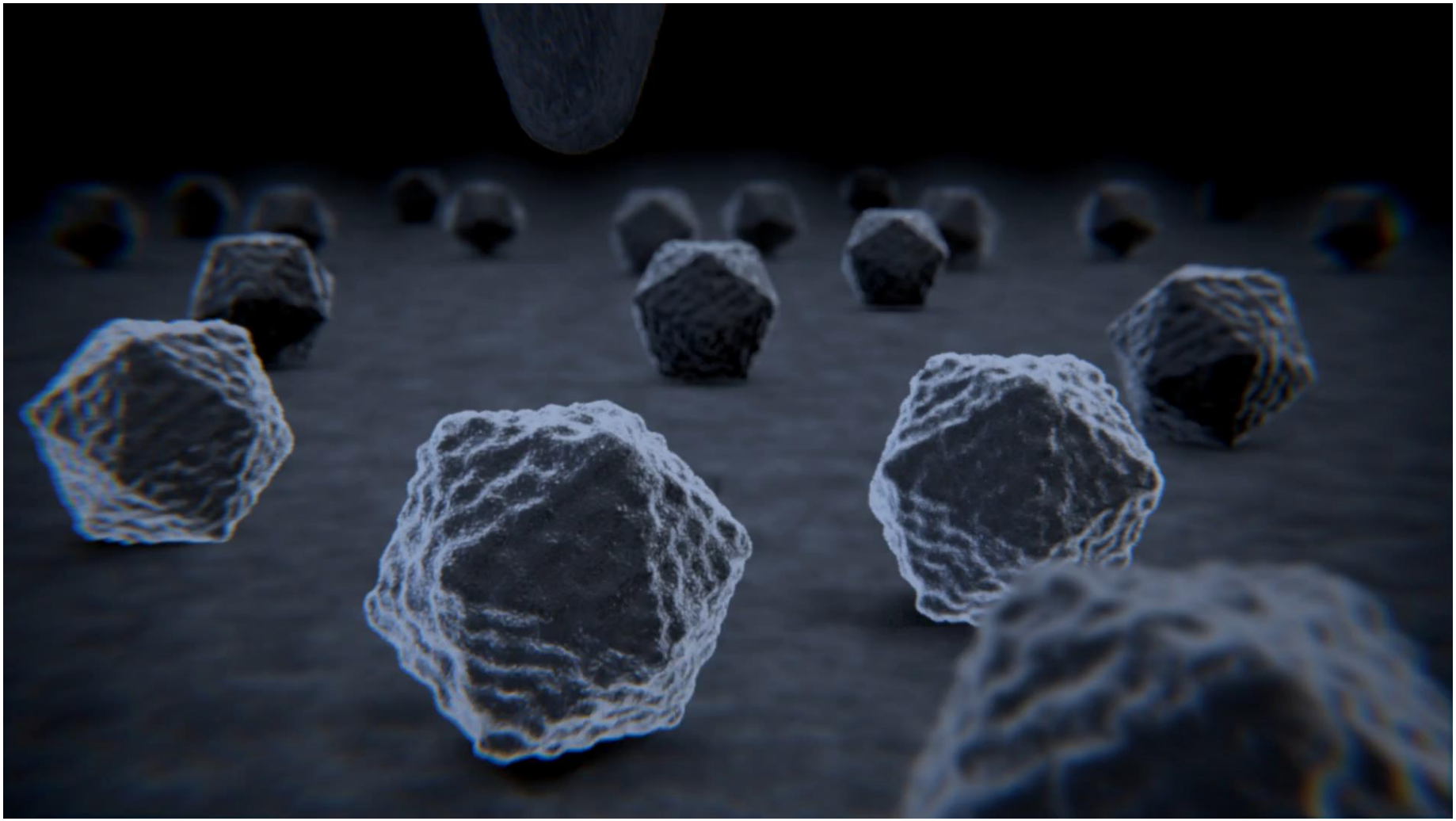
YOYO-1

AFM – fluorescence combination

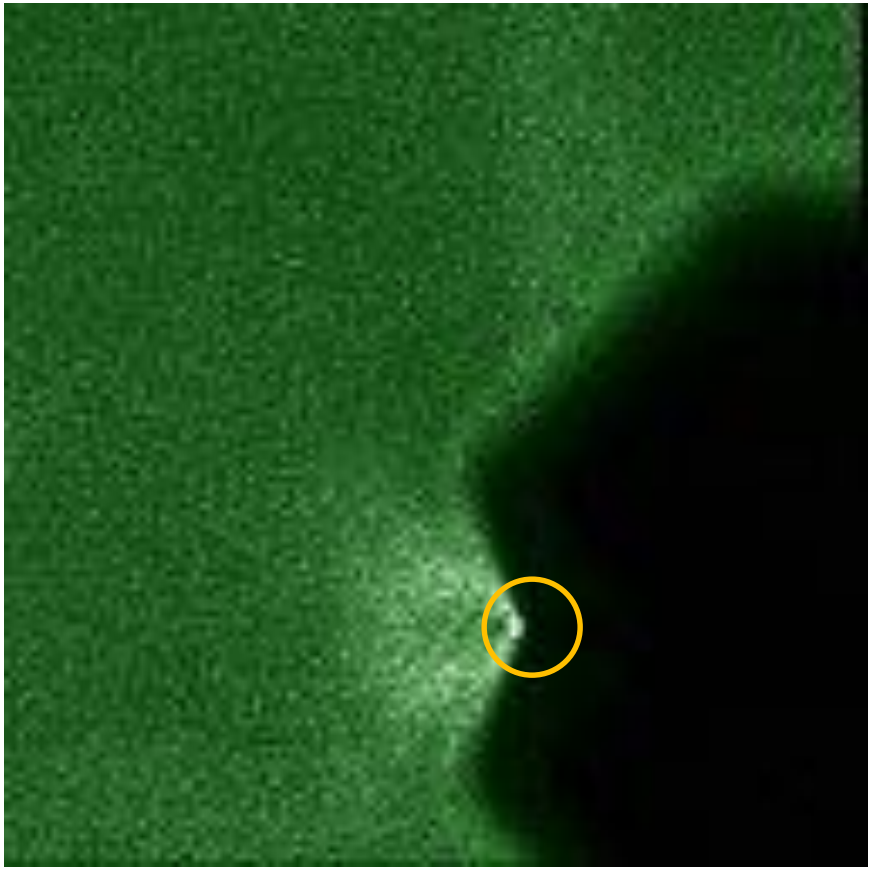


AFM – fluorescence combination

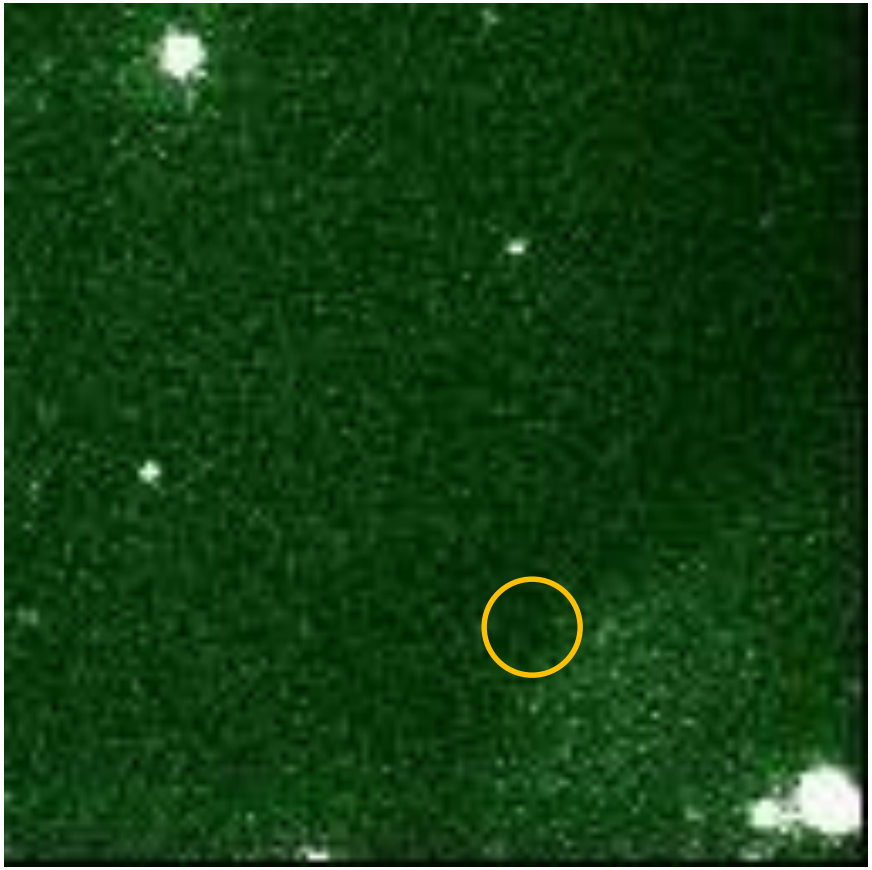




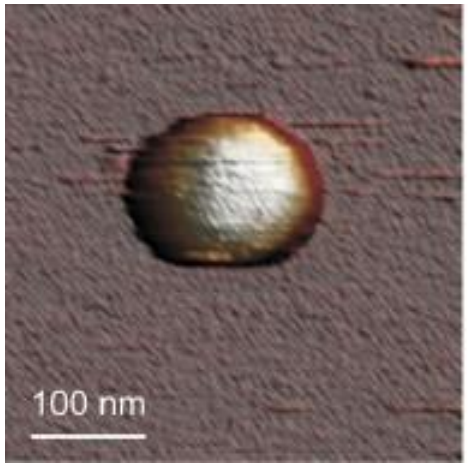
AFM induced unpacking of adenovirus

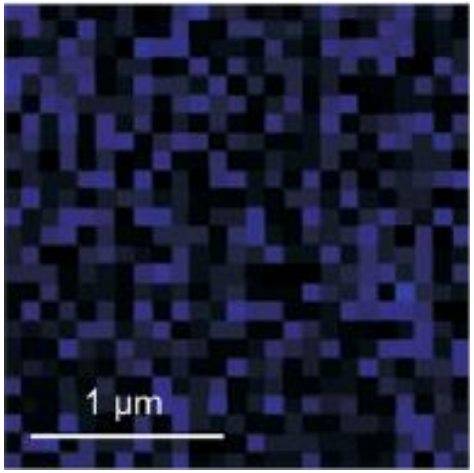


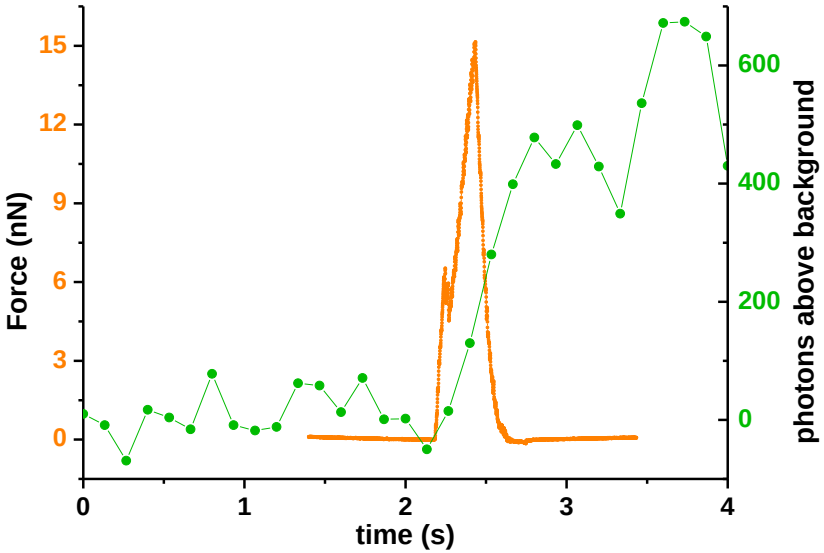
AFM induced unpacking of adenovirus

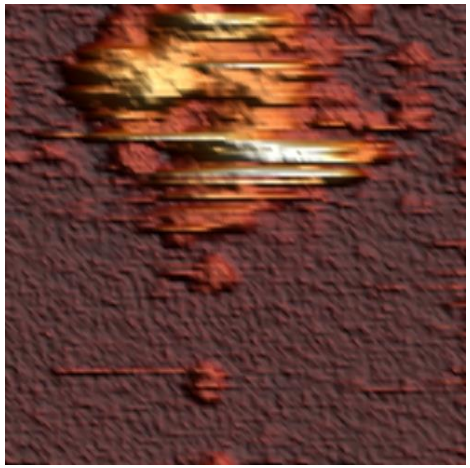


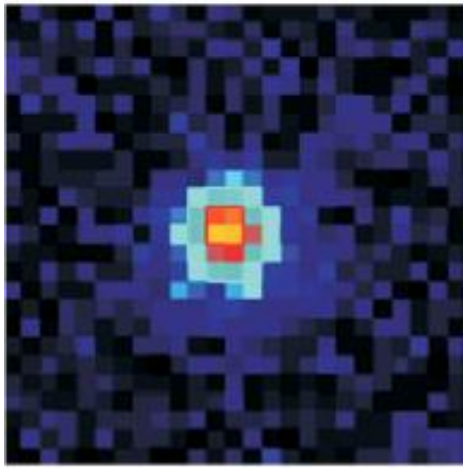
AFM forced unpacking of Adenovirus







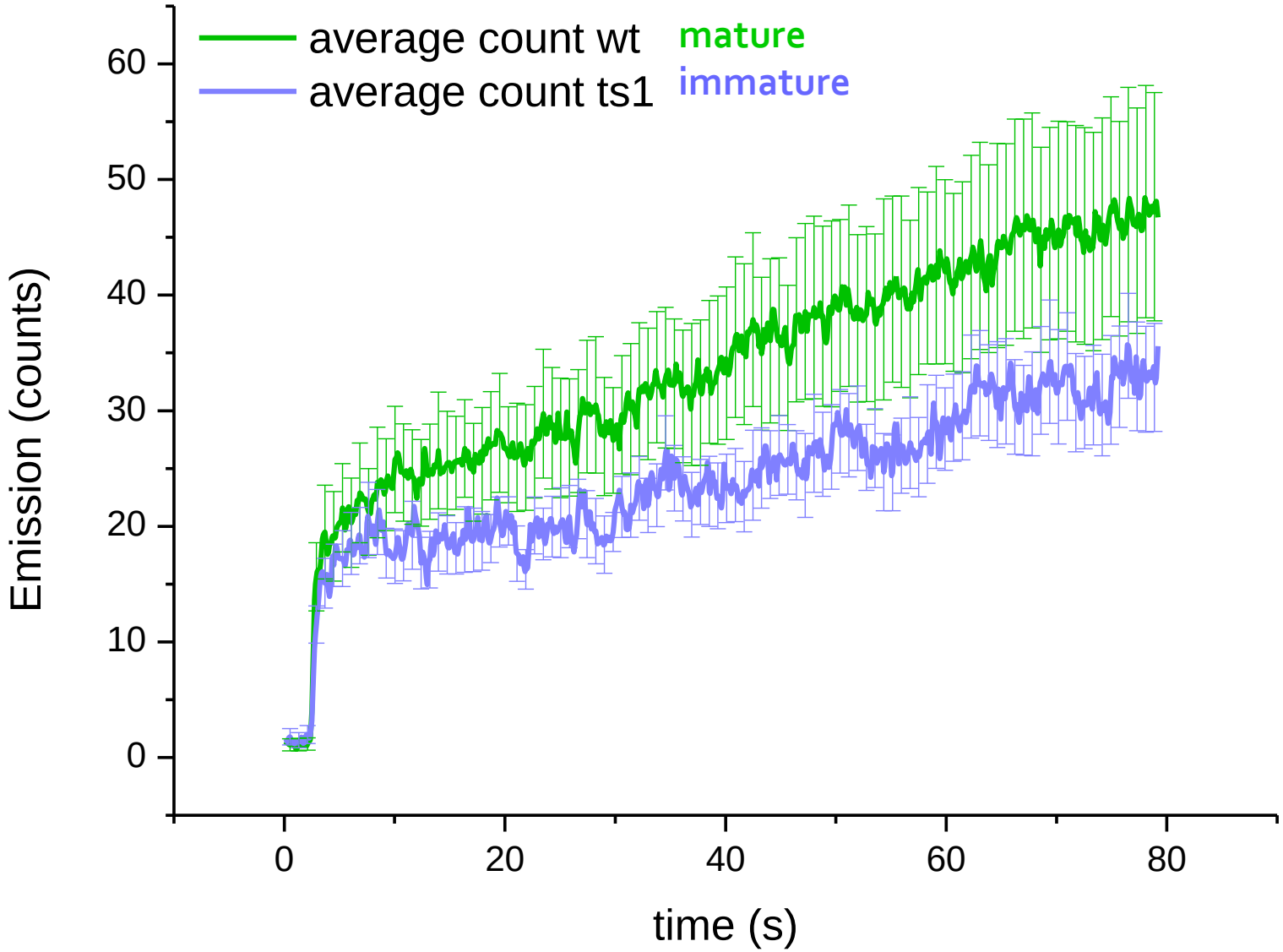




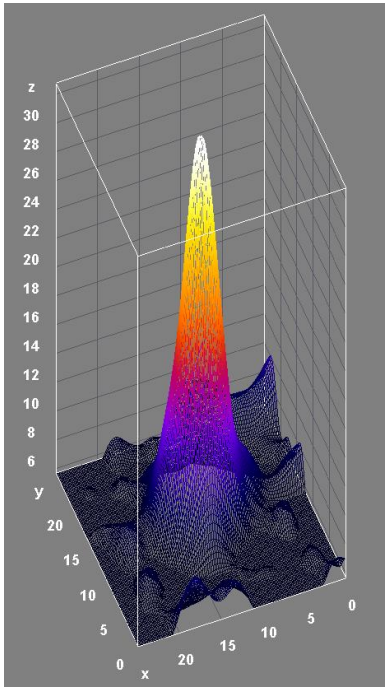
simultaneous single particle fluorescence with AFM

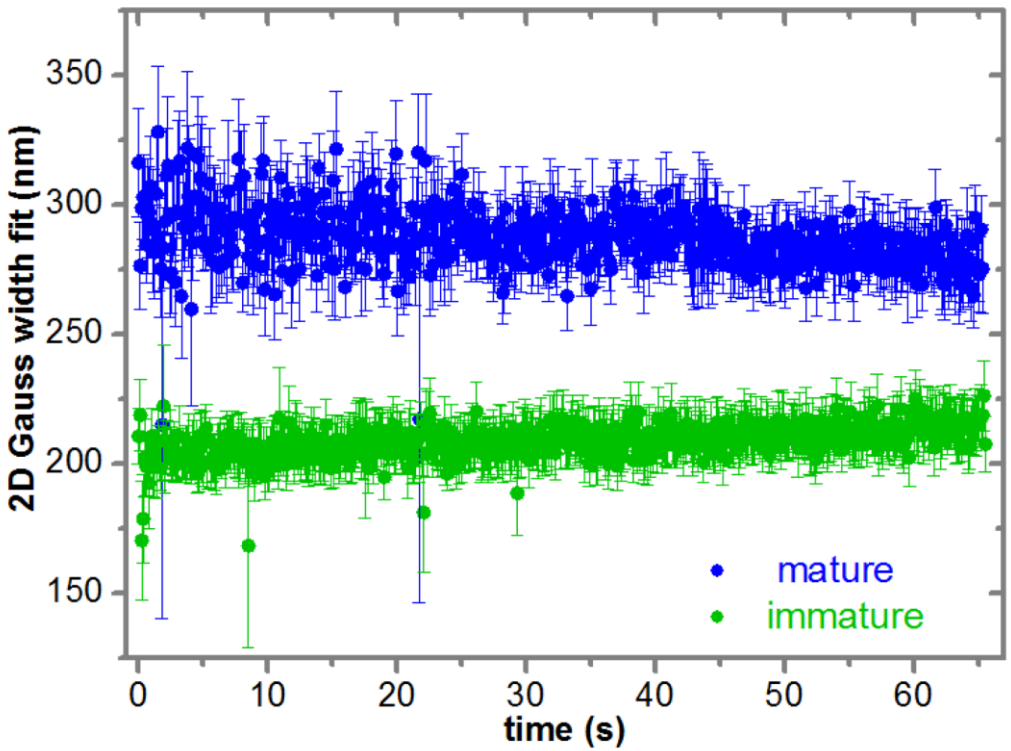
observe DNA release with YOYO-1

Quantifying DNA release



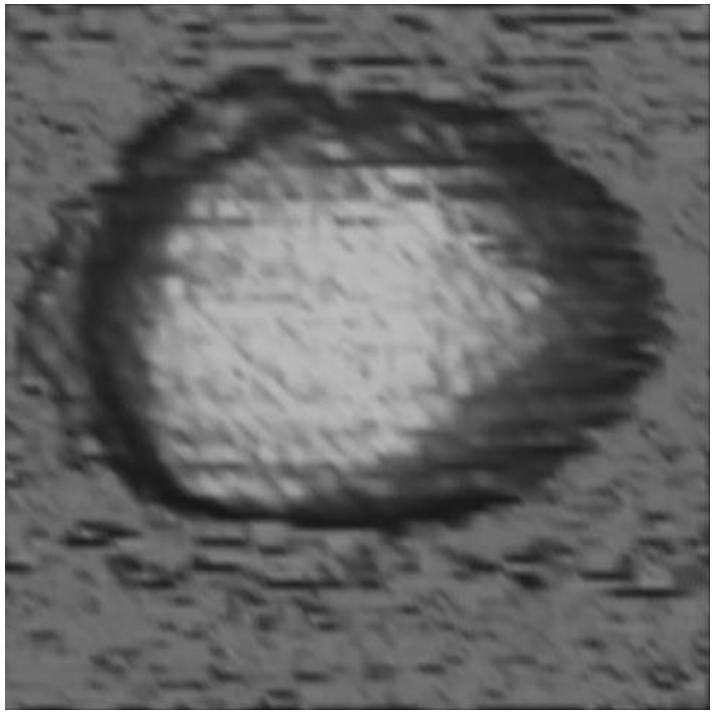
Quantifying DNA release

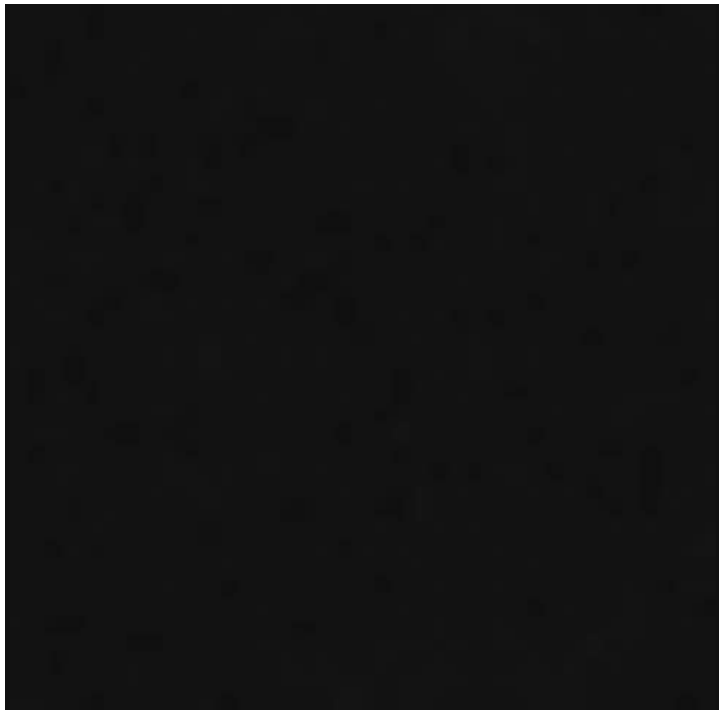




Ortega-Esteban, de Pablo, Schaap et al. ACS Nano 2015

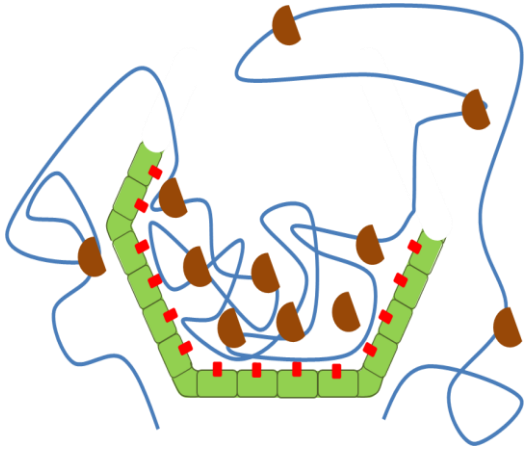
Controlled capsid disassembly



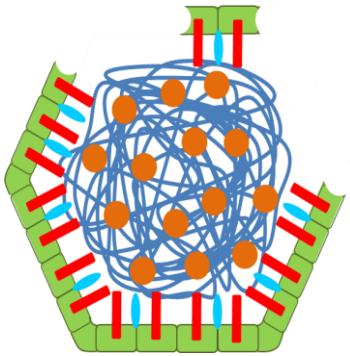


Quantifying DNA release

Mature



Immature



- Mature core spreads more the genome

- Immature emits less photons

Ortega-Esteban, de Pablo, Schaap et al. ACS Nano 2015

Topics today

1. **Introduction**
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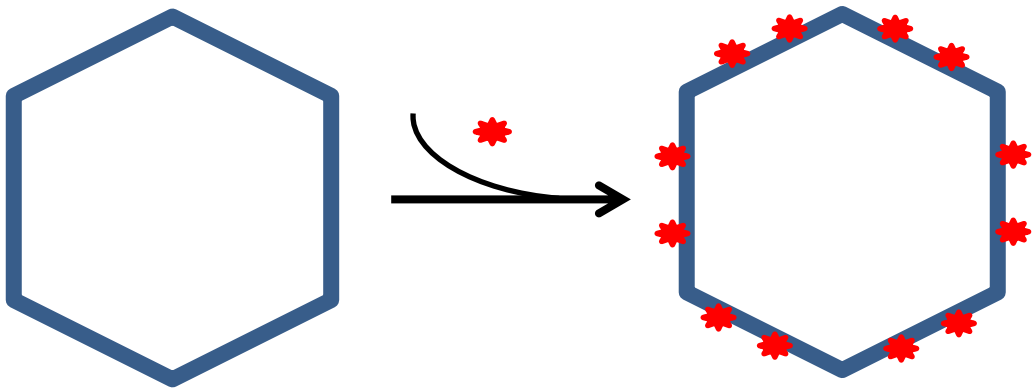
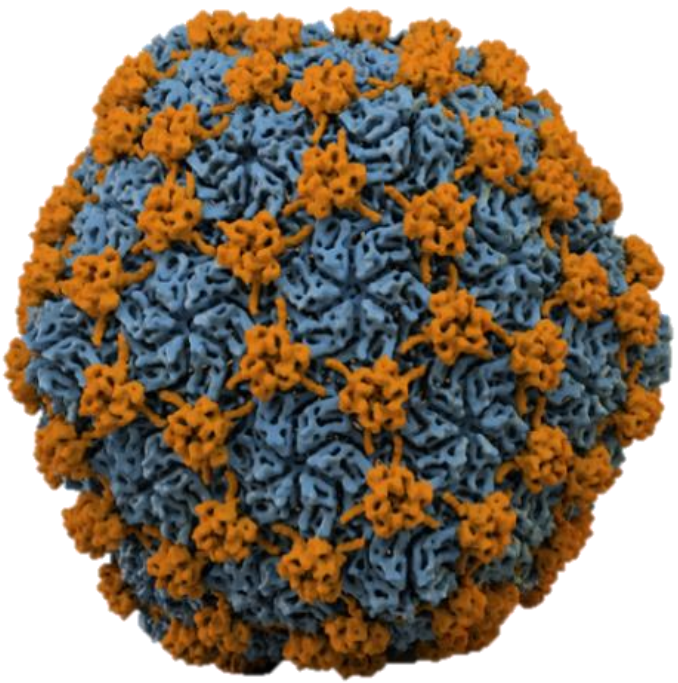
Cementing/decorative proteins

An alternative strategy to strengthen virus capsids during maturation



Lambda phage



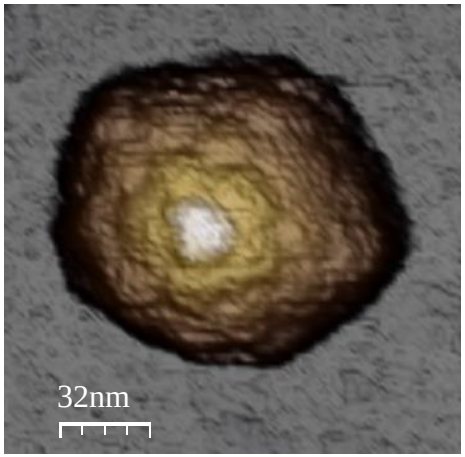
C.G Lander

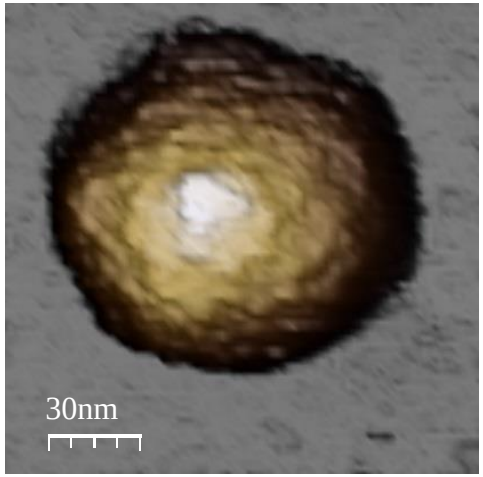
- 60 nm in diamter
- 420 gpE + 415 gpD.

  72 capsomers
- DNA ~ 48.5 kbp ~ 14.5 µm .

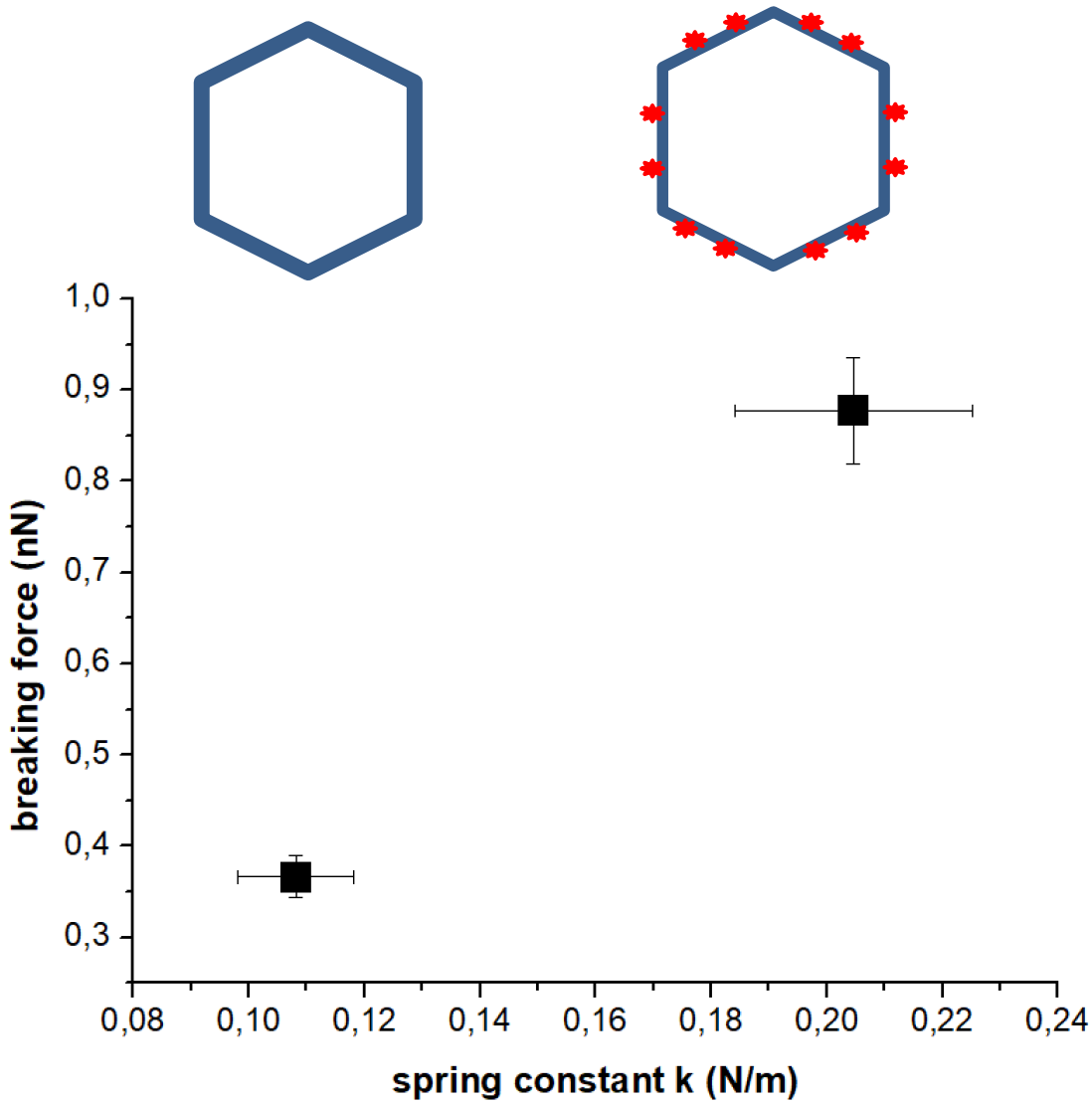
Single indentation assay

undecorated





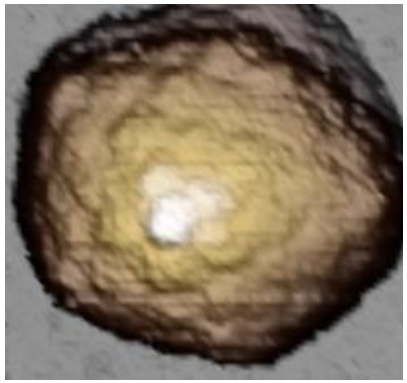
decorated



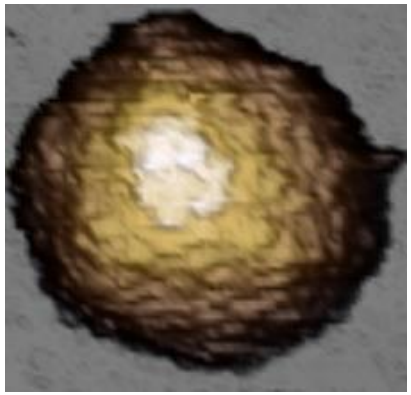
Nature Communications 5, 4520 (2014)

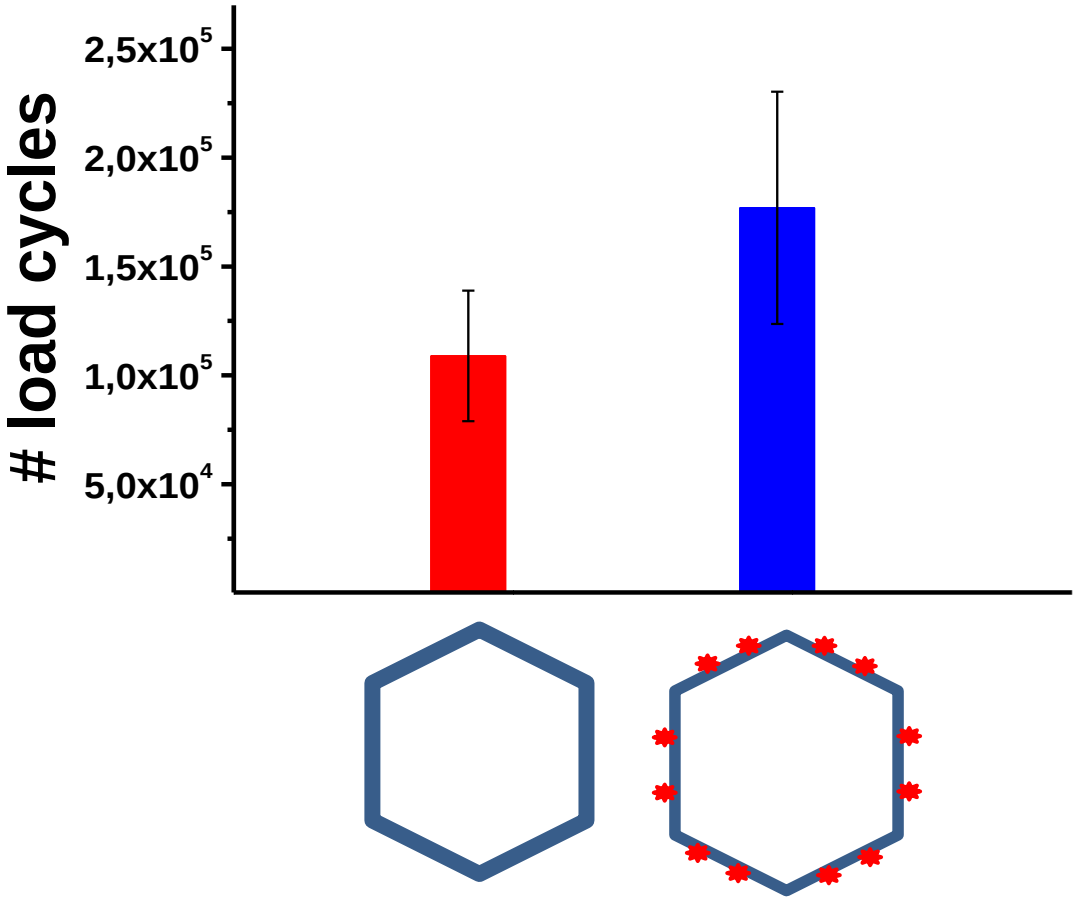
Mechanical fatigue

undecorated



decorated

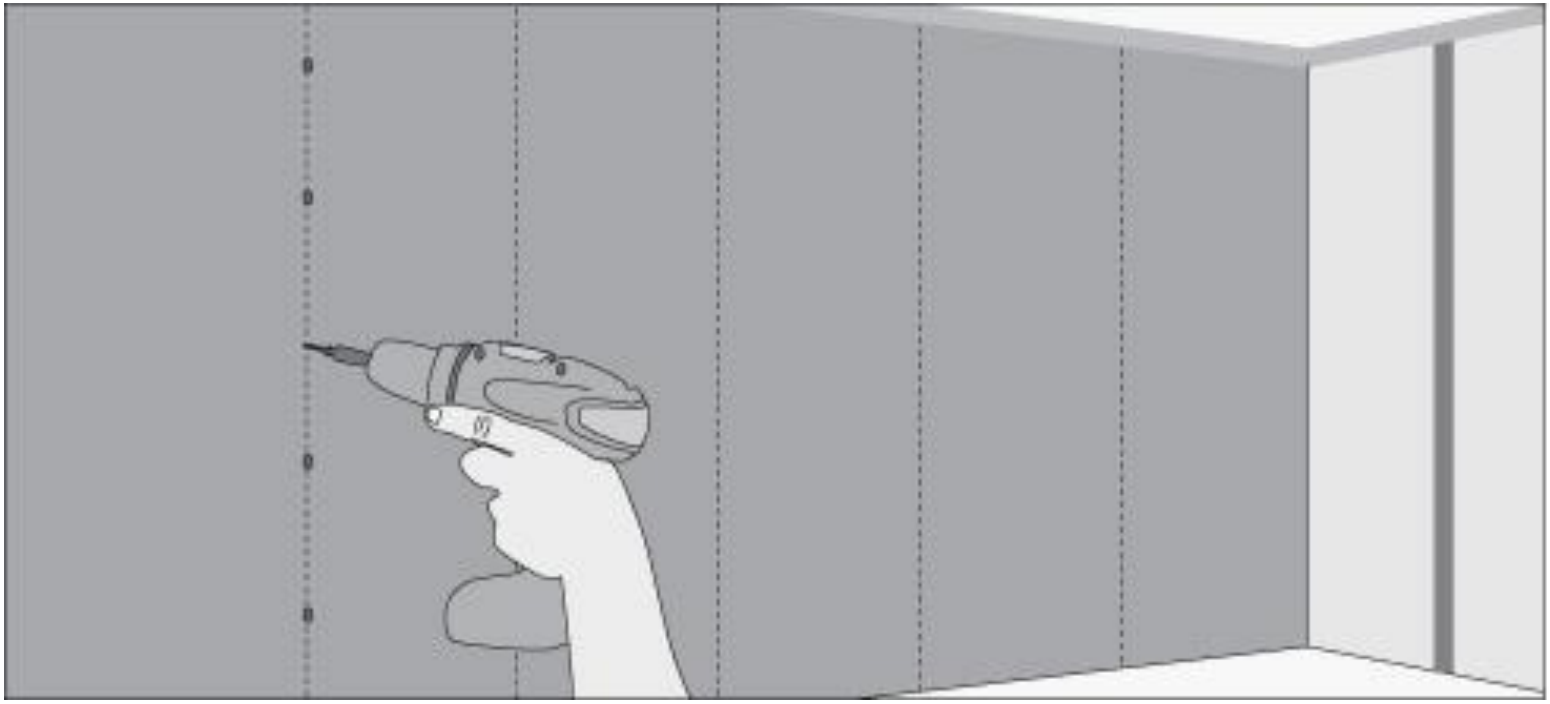




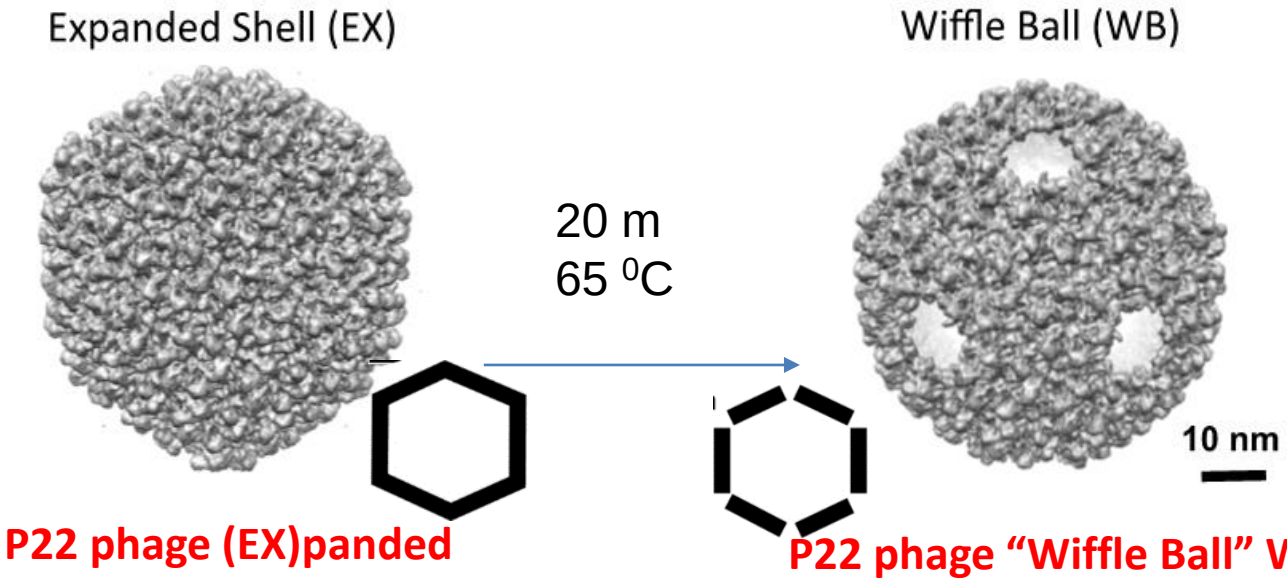
Nature Communications 5, 4520 (2014)

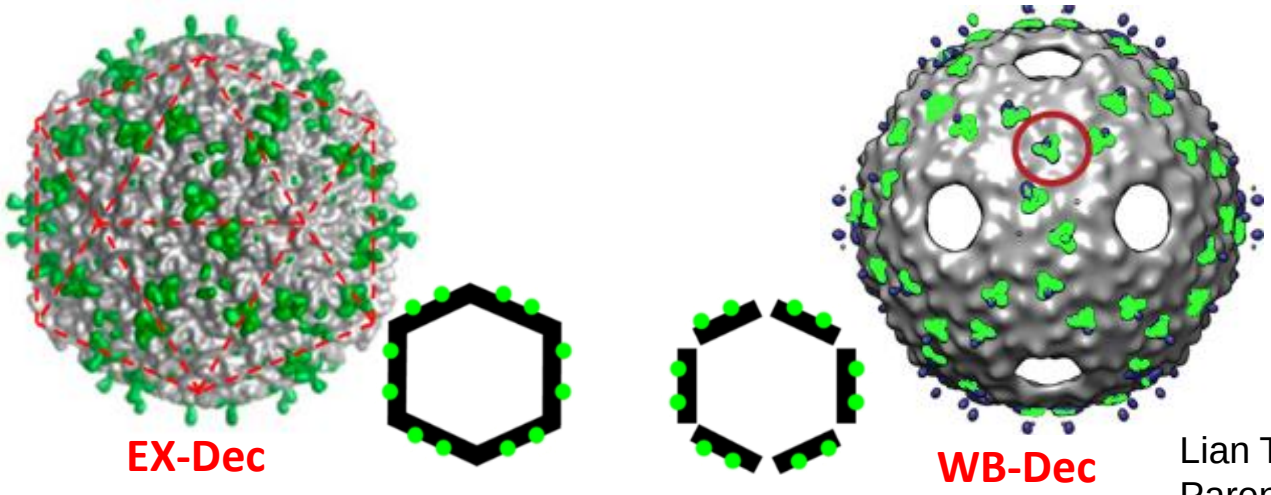
Decorated particles are mechanically more robust that undecorated

Can we use cementing proteins to recover weaken protein shells?



Tuning viral capsid nanoparticle stability with simmetrycal morphogenesis

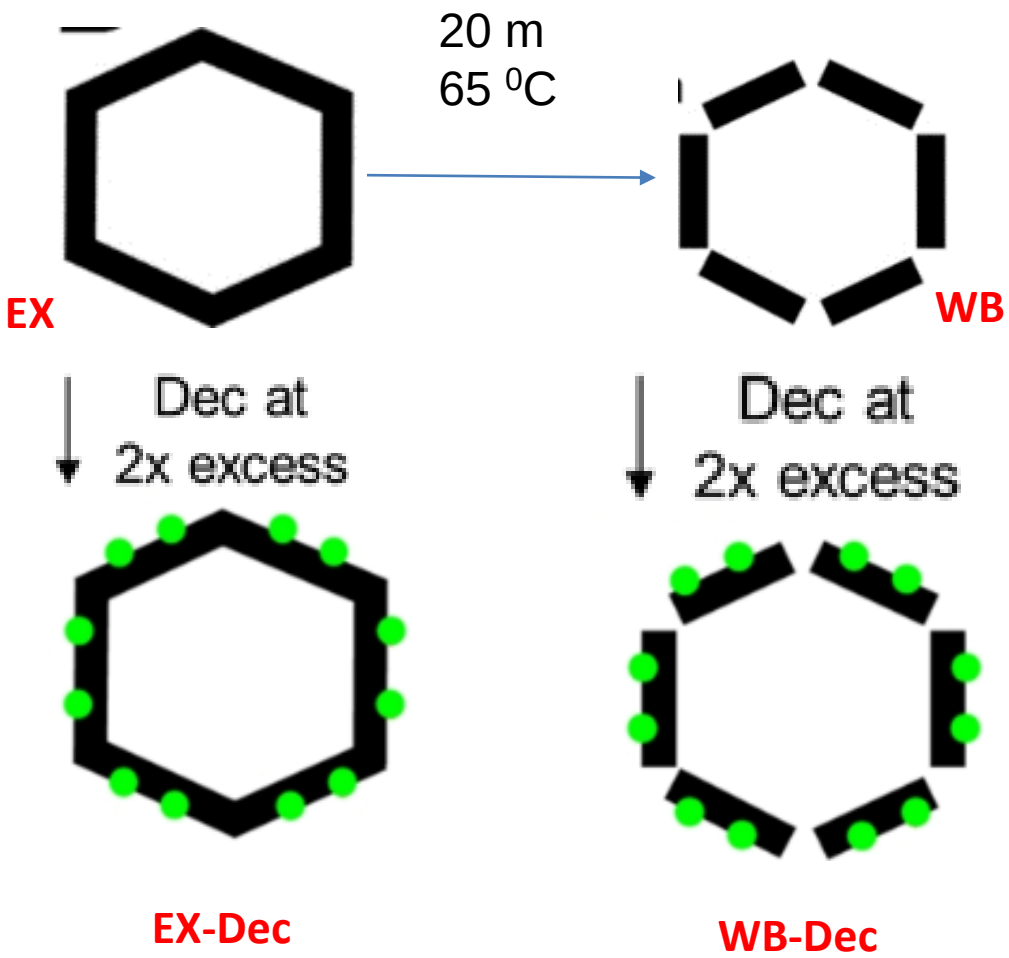




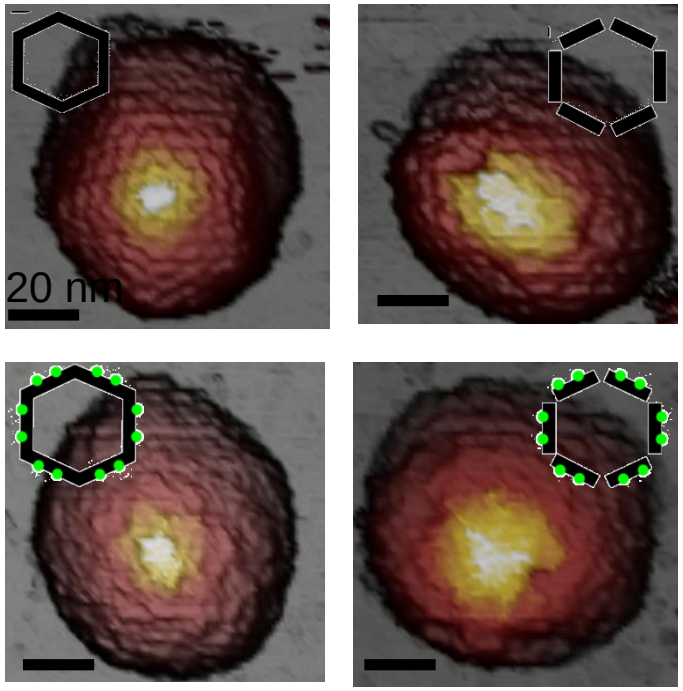
Lian Tang et al Structure 2006
Parent et. Al
Structure 2010, Biomaterials 2012

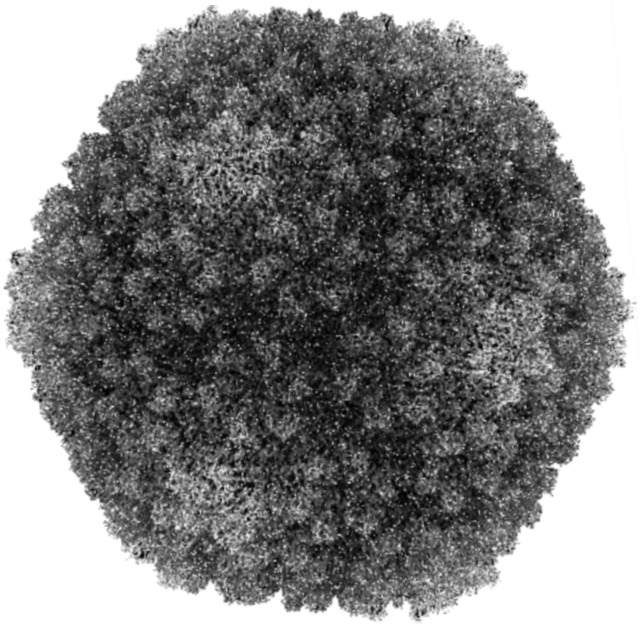
P22 binds phage L Dec proteins at quasi-three fold locations

P22 particles



Collapse of p22 particles after adsorption on the surface





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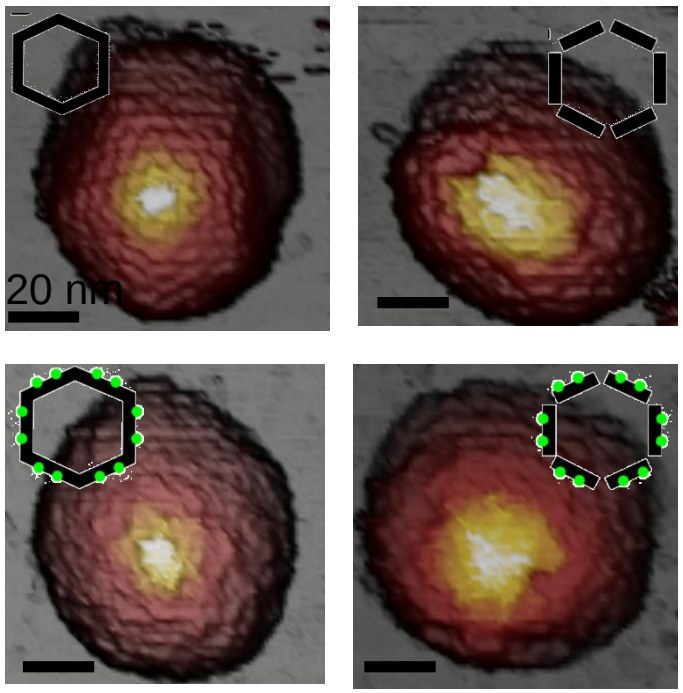

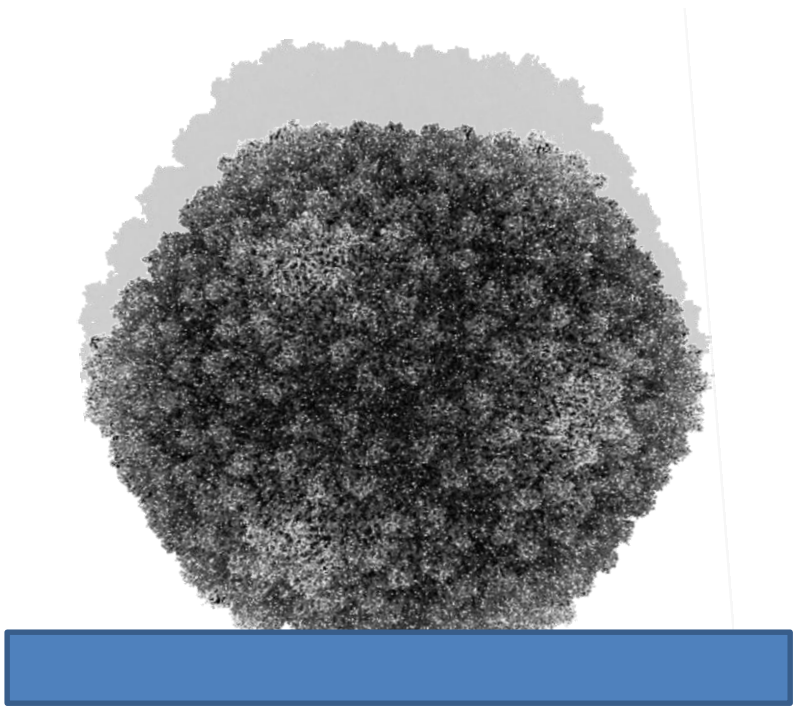

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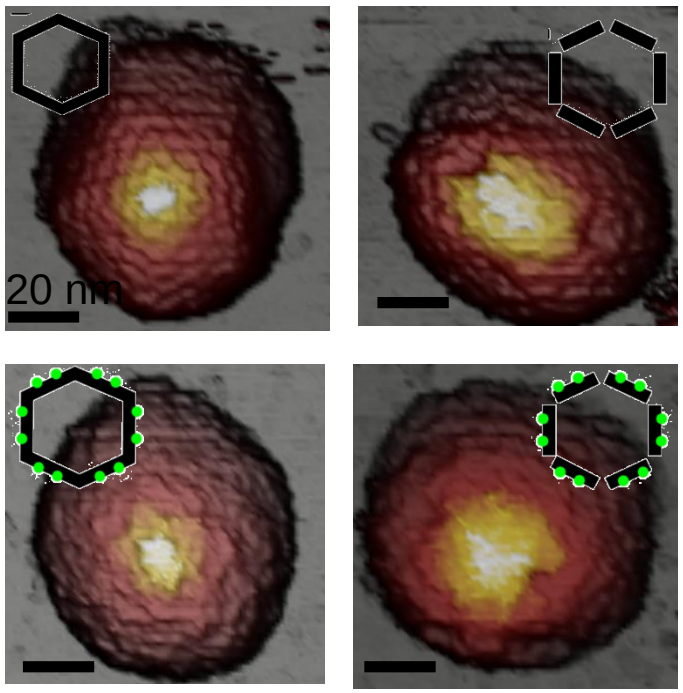
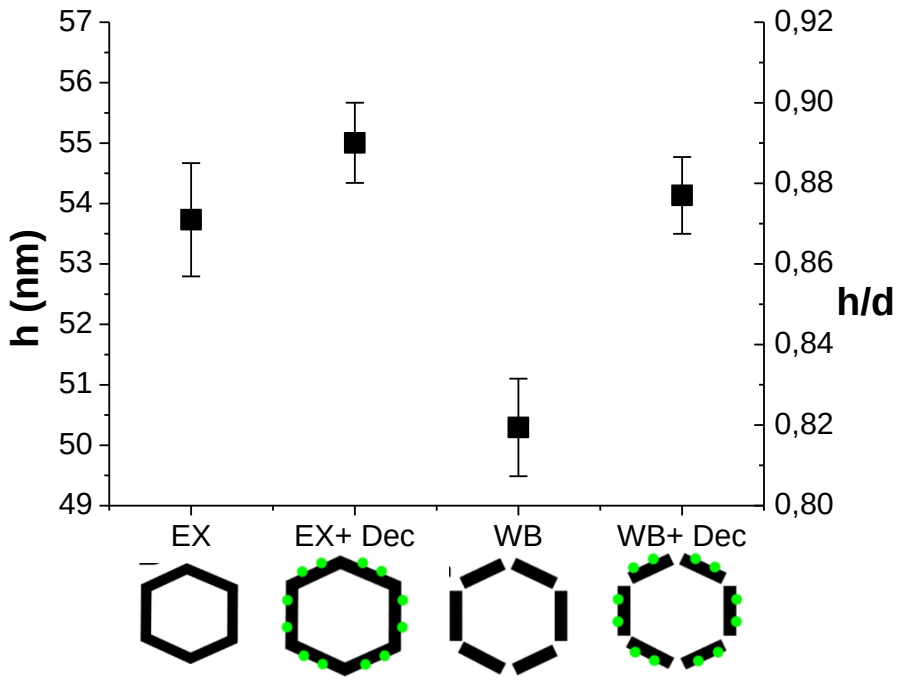
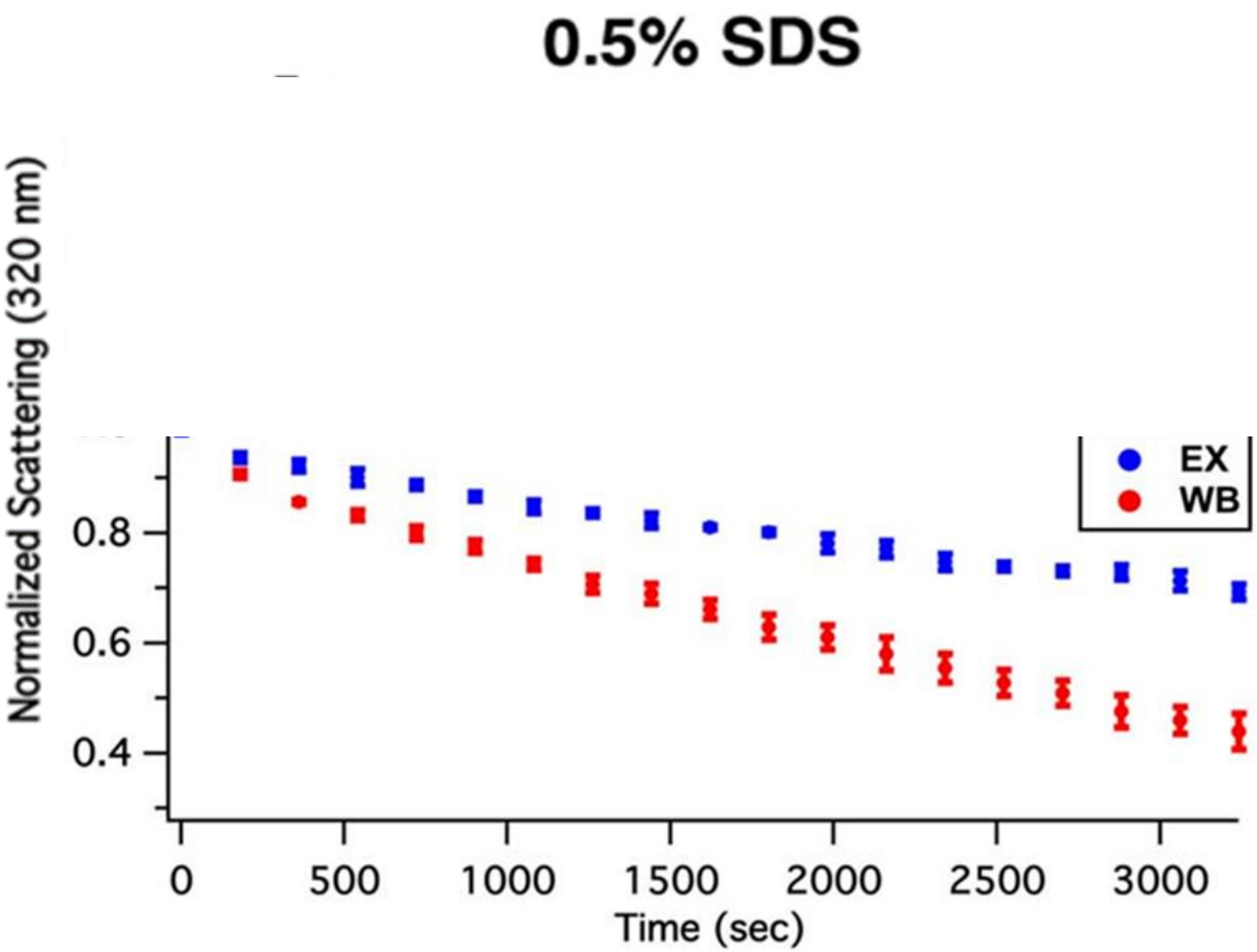




Which structure is more stable?



Llauró et al. ACS Nano 2016

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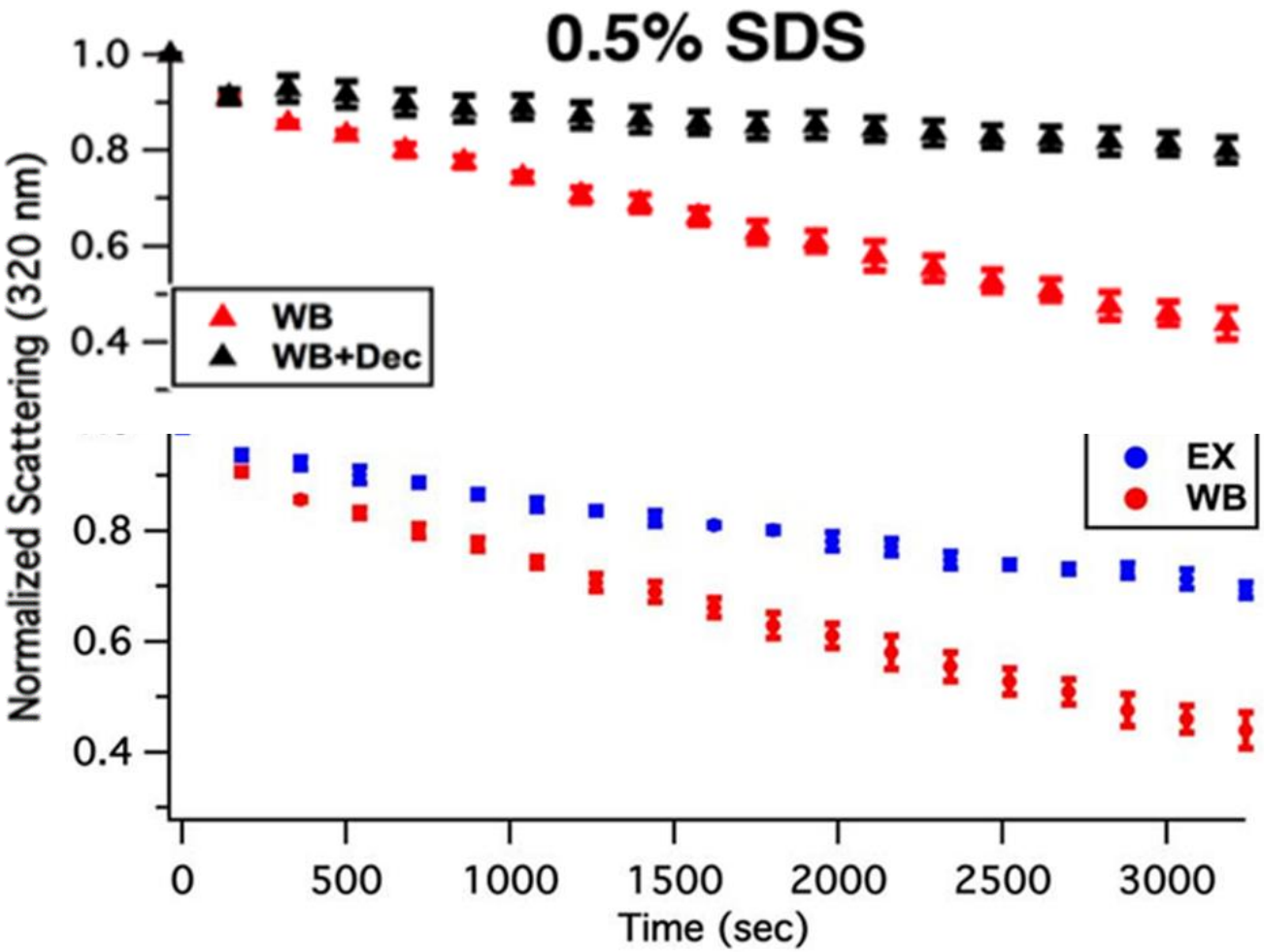
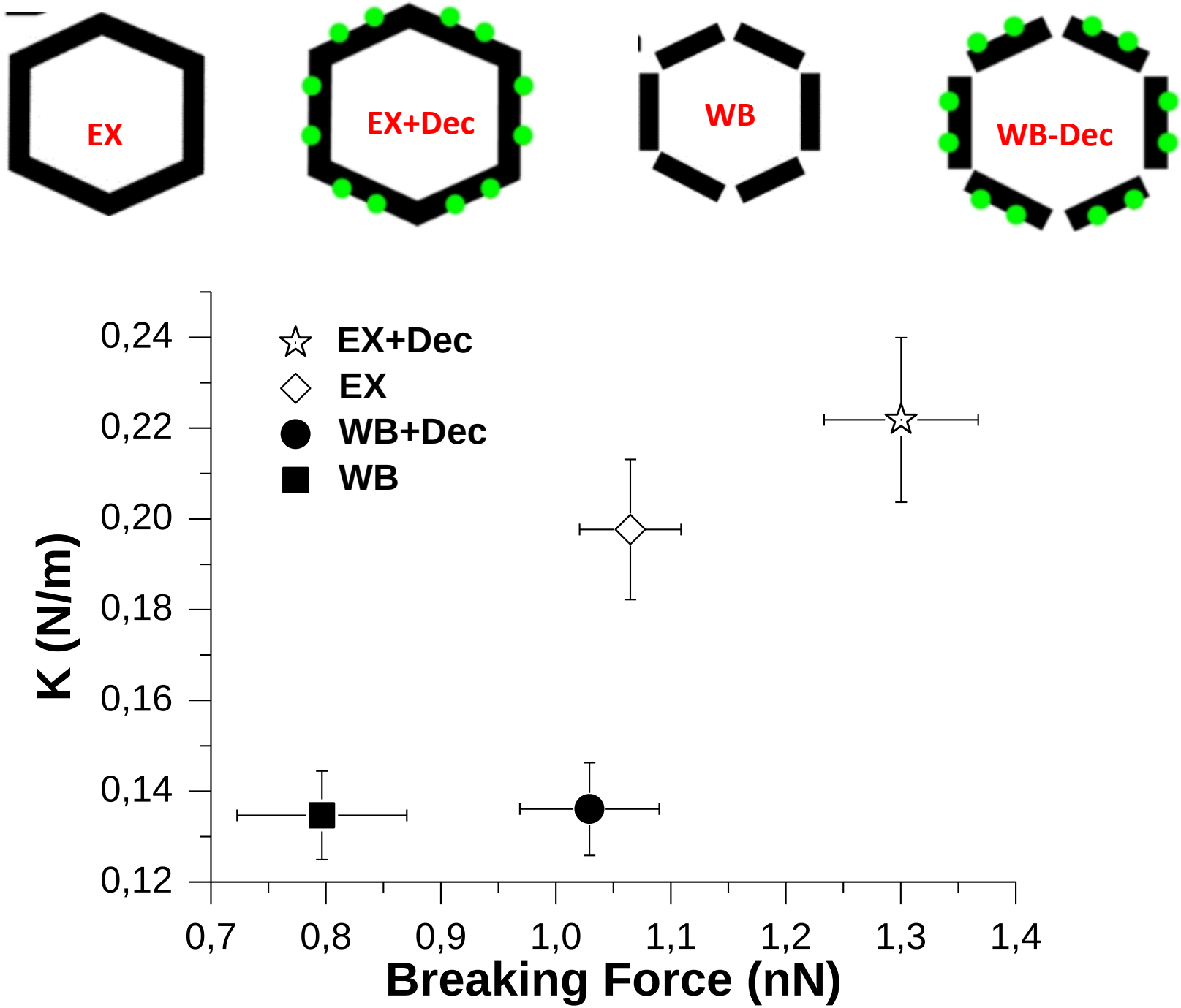
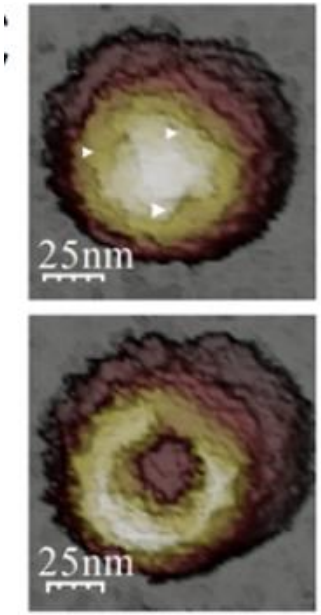
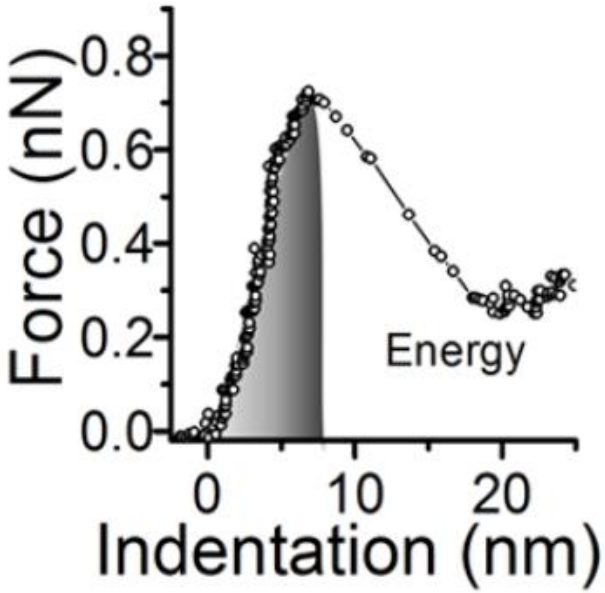


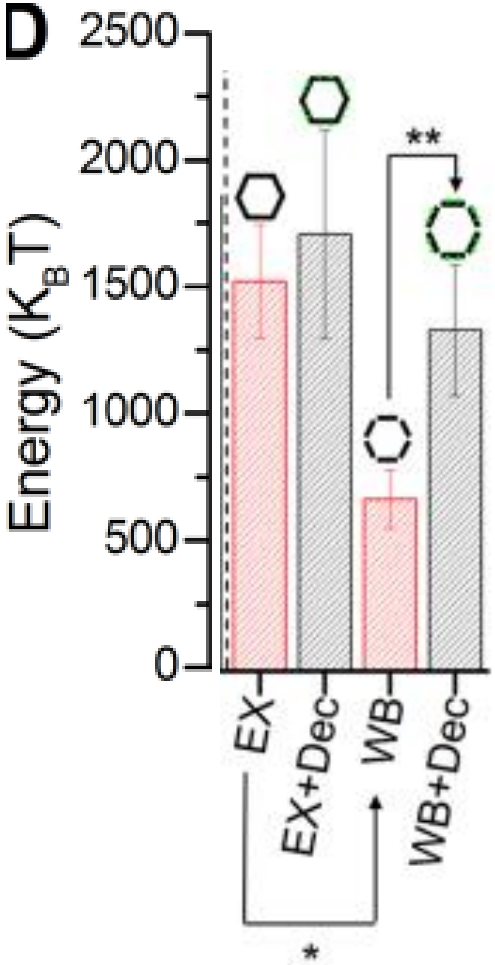
Llauró et al. ACS Nano 2016


EX
EX+Dec
WB
WB-Dec
EX+Dec
EX
WB+Dec
WB
K (N/m)
0,24
0,22
0,20
0,18
0,16
0,14
0,12
0,7
0,8
0,9
1,0
1,1
1,2
1,3
1,4
Breaking Force (nN)

How much work is used to crack the particles?

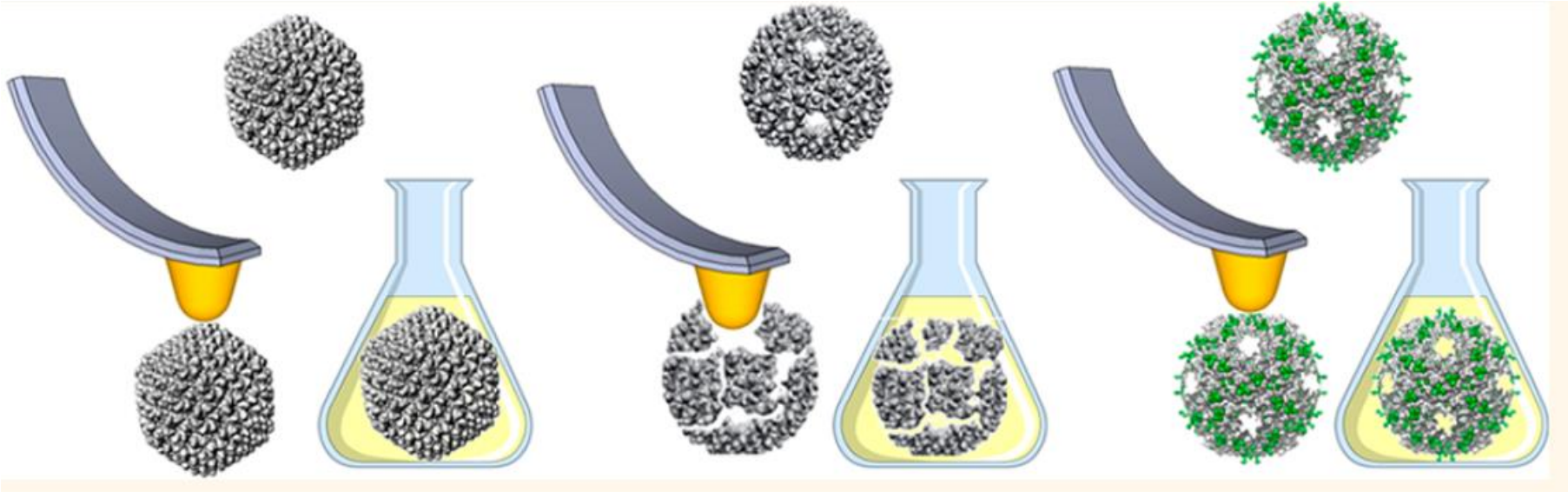






Llauró et al. ACS Nano 2016

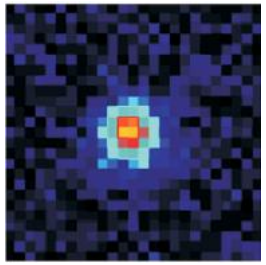
Cementing proteins improve capsid performance

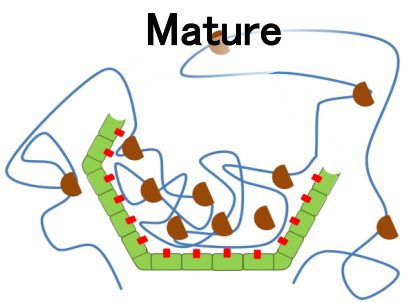


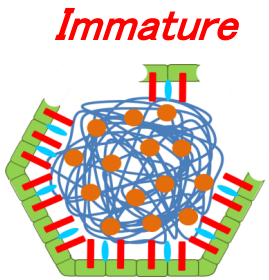
Summary

Core mechanics indicates adenovirus pressurization that helps for disassembly and genome delivery

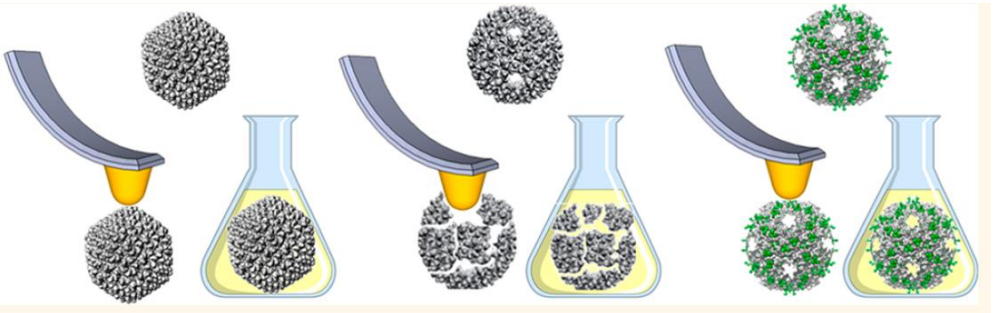
Genome condensation influences on diffusion







Cementing proteins recovers weak particles



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Carolina Carrasco
Alvaro Ortega
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Arvind Raman
Carlos E. Catalano
Carmen San Martín
Daniel Luque
Dave Evans
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José Ruiz Castón
Mark van Raaij
Mauricio García Mateu
Nuria Verdaguer
Rudi Podgornik
Salvatore Cannistraro
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Thank you!