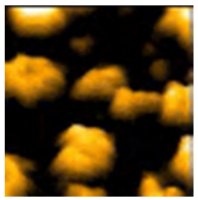
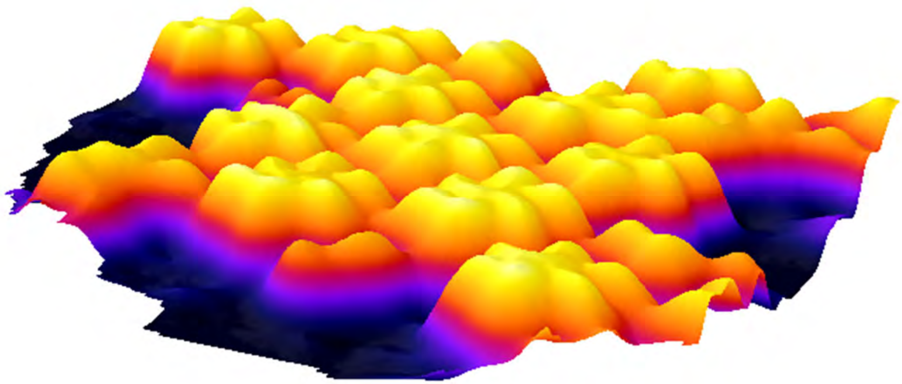
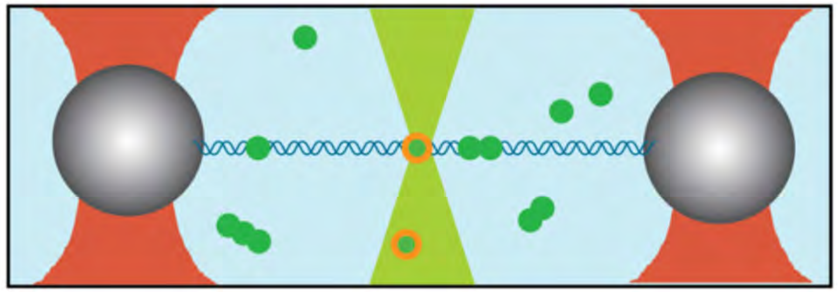
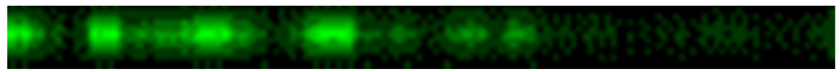
Advanced approaches to characterize stability and dynamics of single particles in real-time







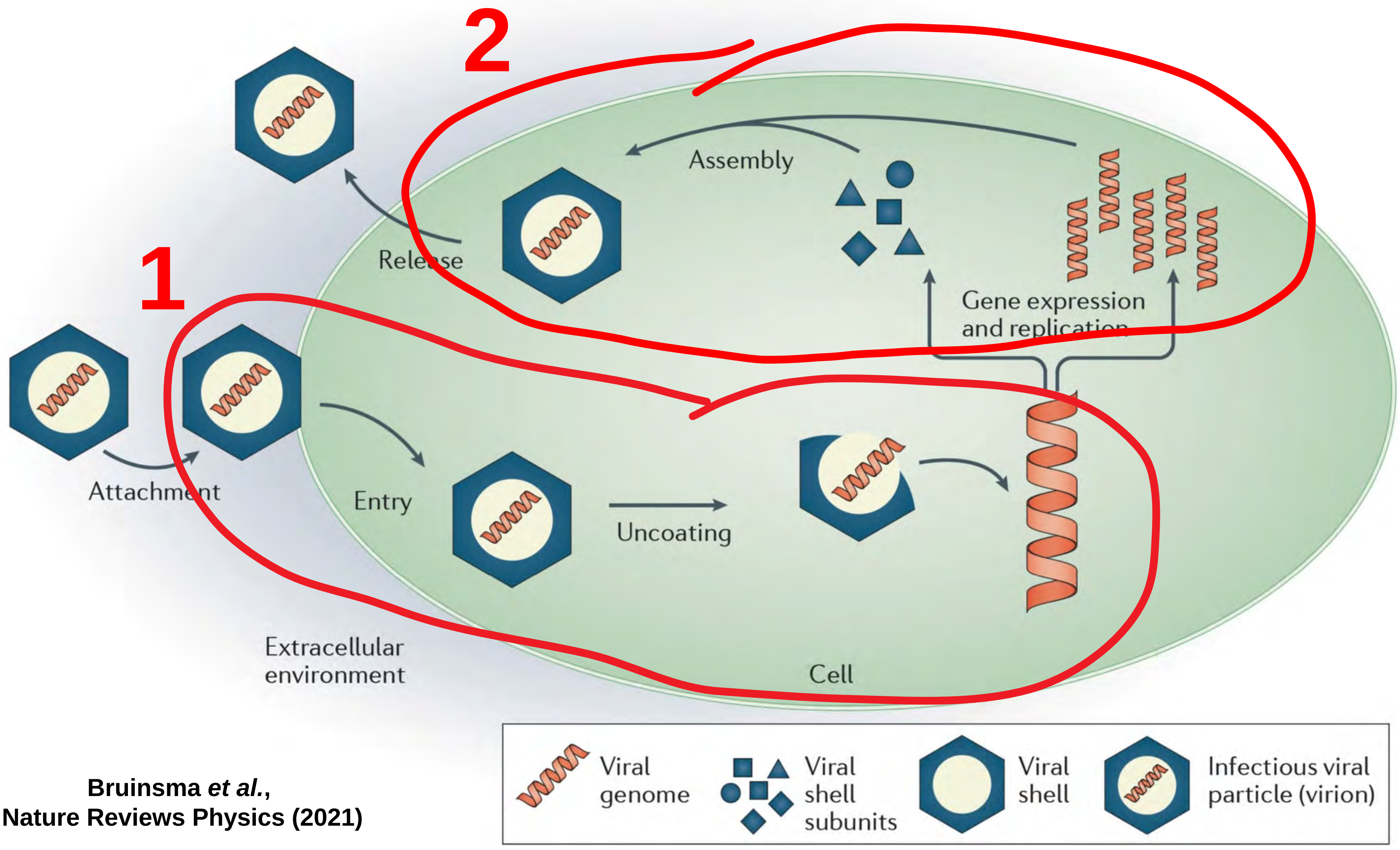


Wouter Roos

rijksuniversiteit groningen

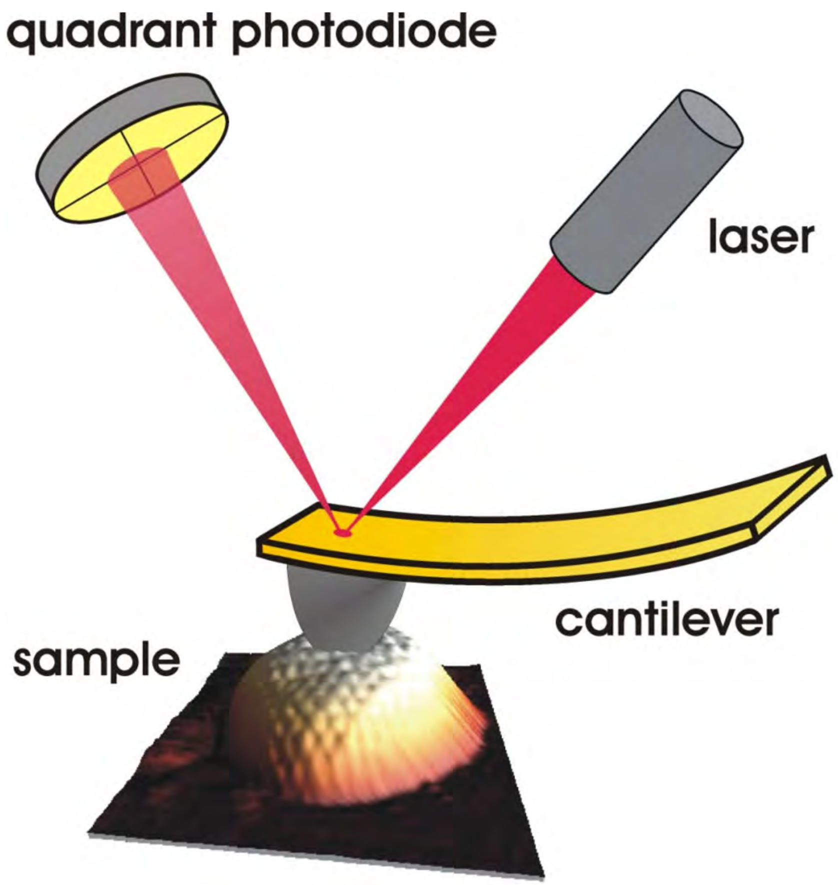
zernike institute for advanced materials

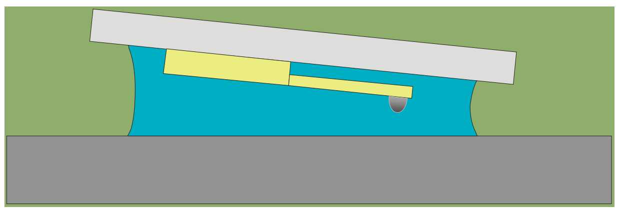
Viral life cycle



Bruinsma *et al.*,
Nature Reviews Physics (2021)

Atomic Force Microscopy





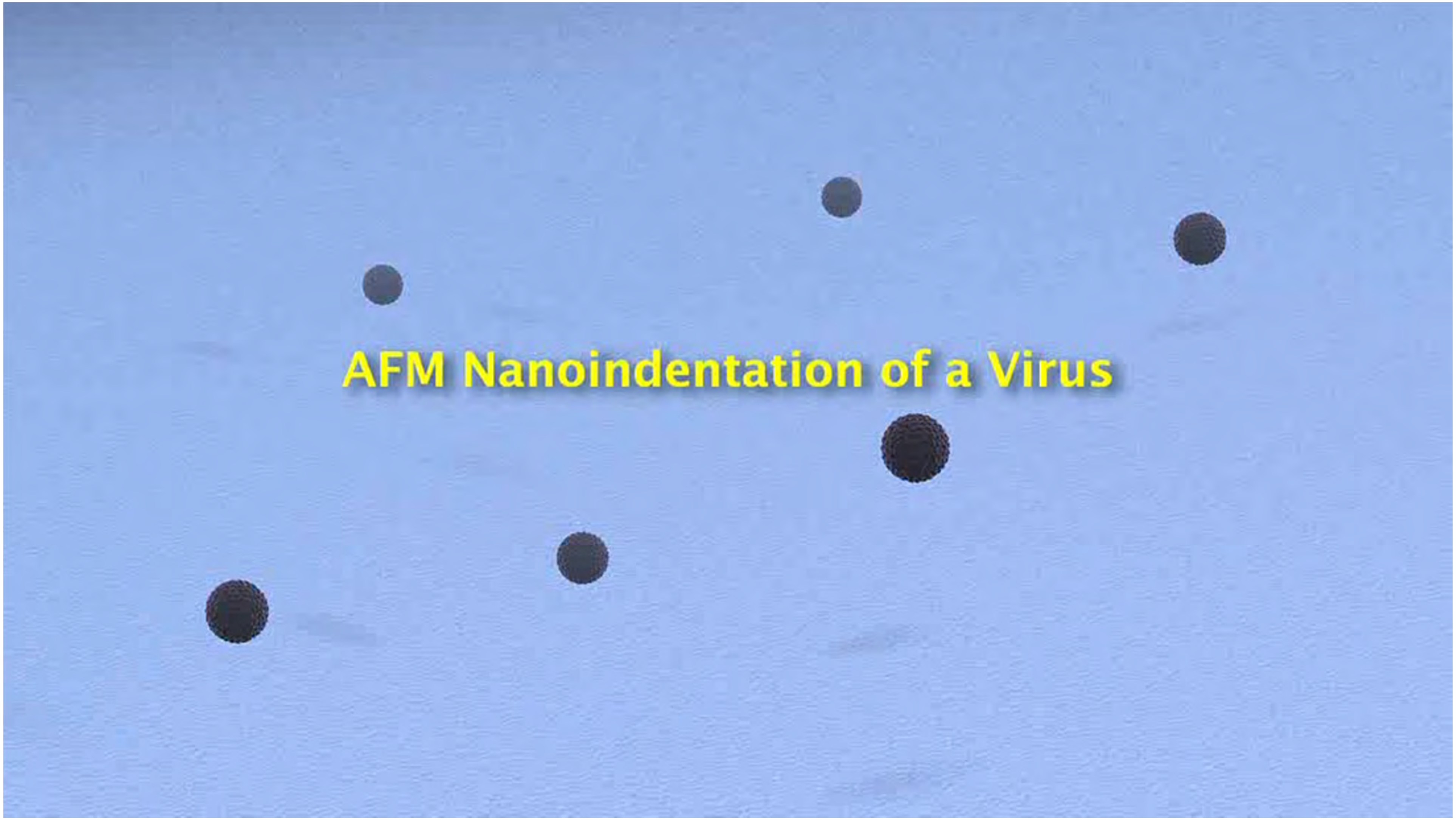
AFM:

- nm resolution

- force measurements

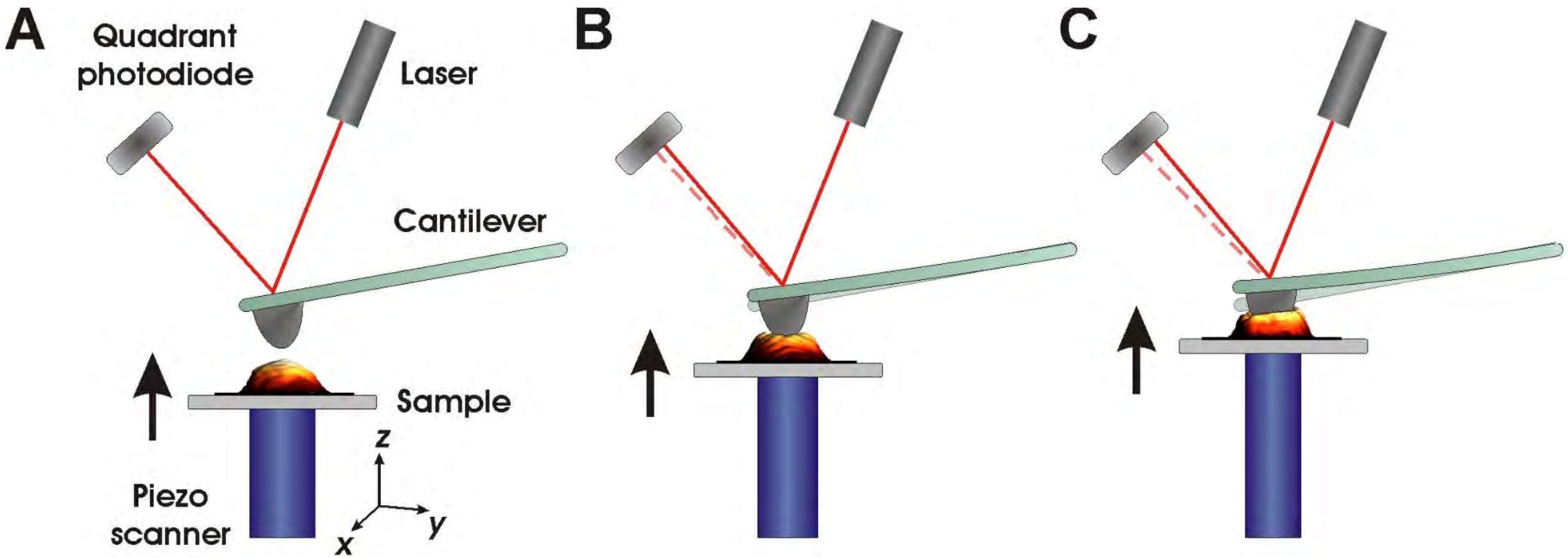
- dynamic measurements

- measurements in liquid

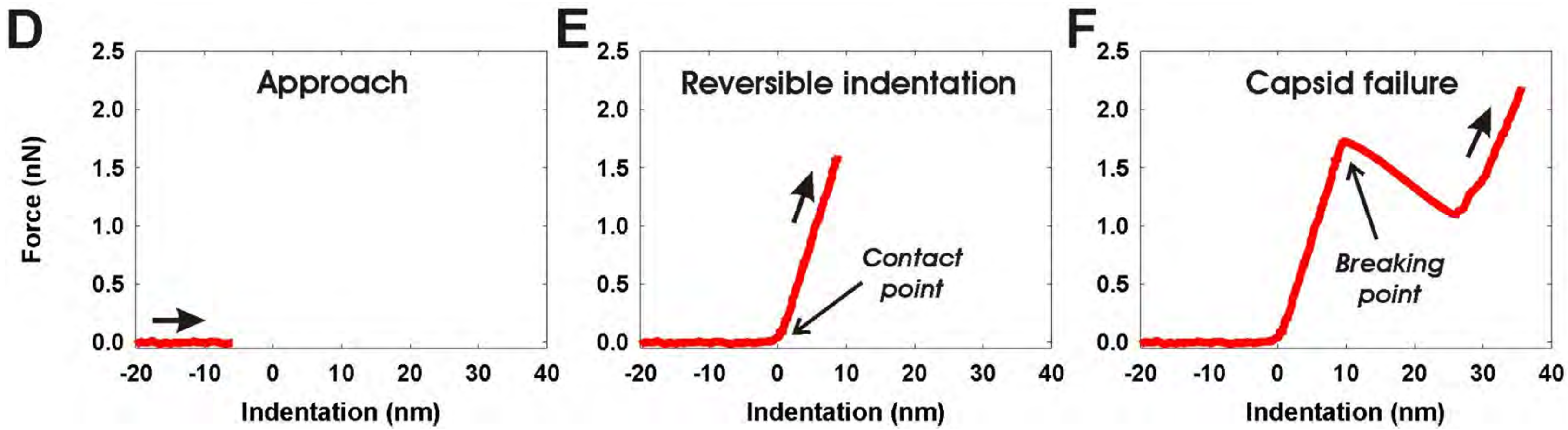


http://www.rug.nl/research/zernike/molecular-biophysics/

Atomic force microscopy-based mechanobiology



Roos *et al.*, Nature Physics 2010
Krieg *et al.*, Nature Reviews Physics 2019



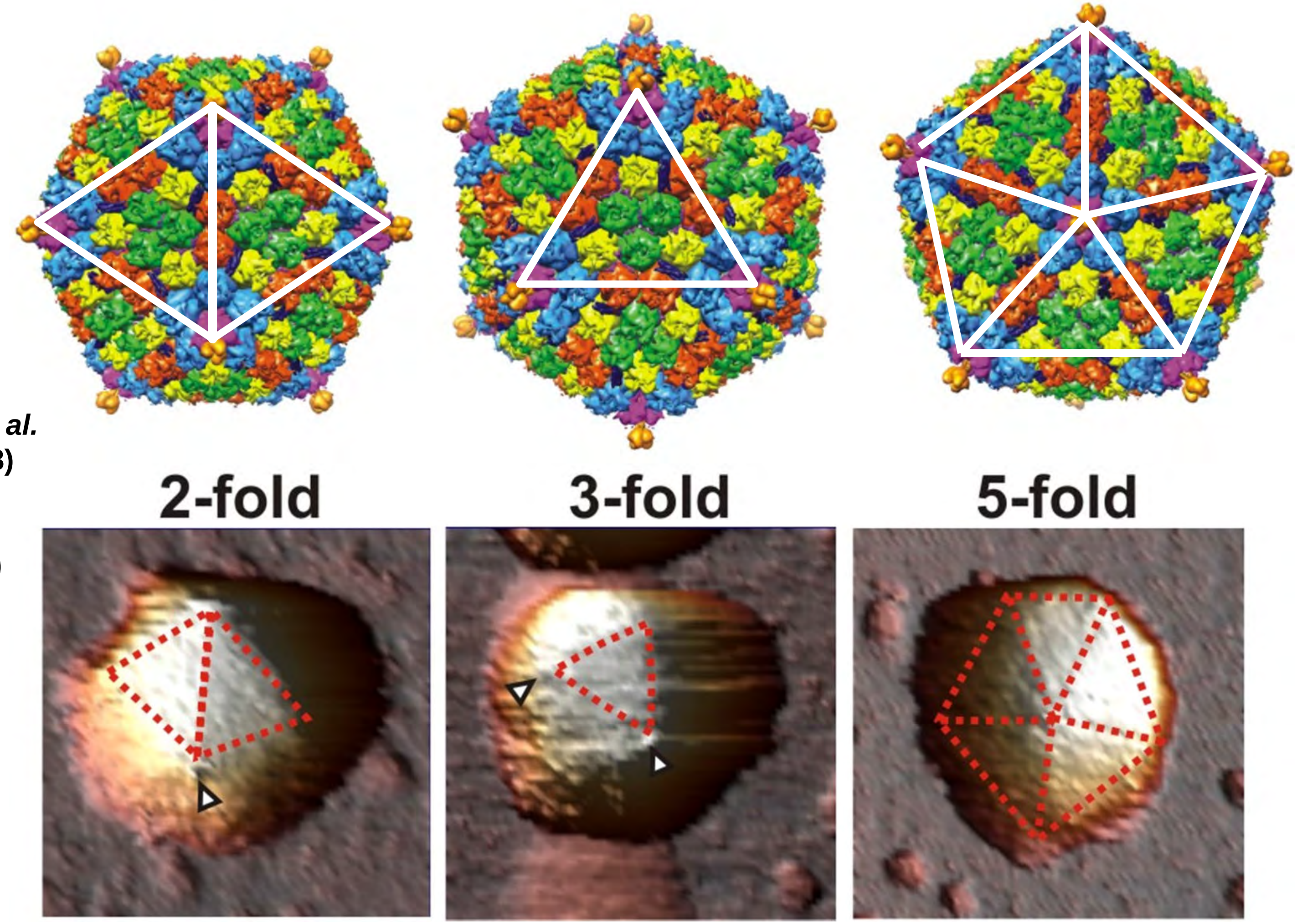
Linking virus mechanics to infectivity

Imaging Adenovirus along its principle symmetry axes

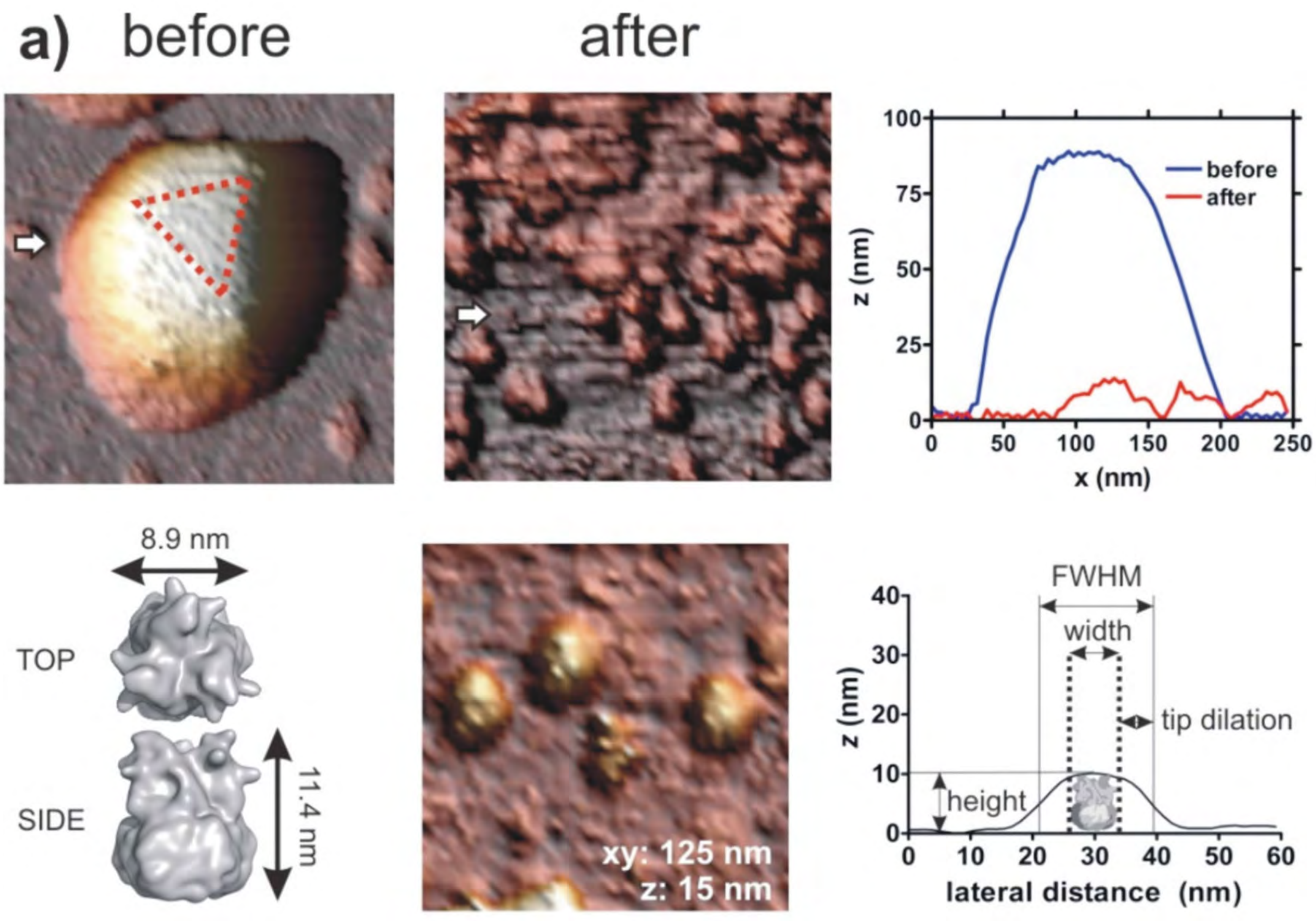
Snijder *et al.*
J Virol (2013)

Van Rosmalen *et al.*
J Biol Phys (2018)

Denning *et al.*
Nanoscale (2019)



Mechanical probing of Adenovirus



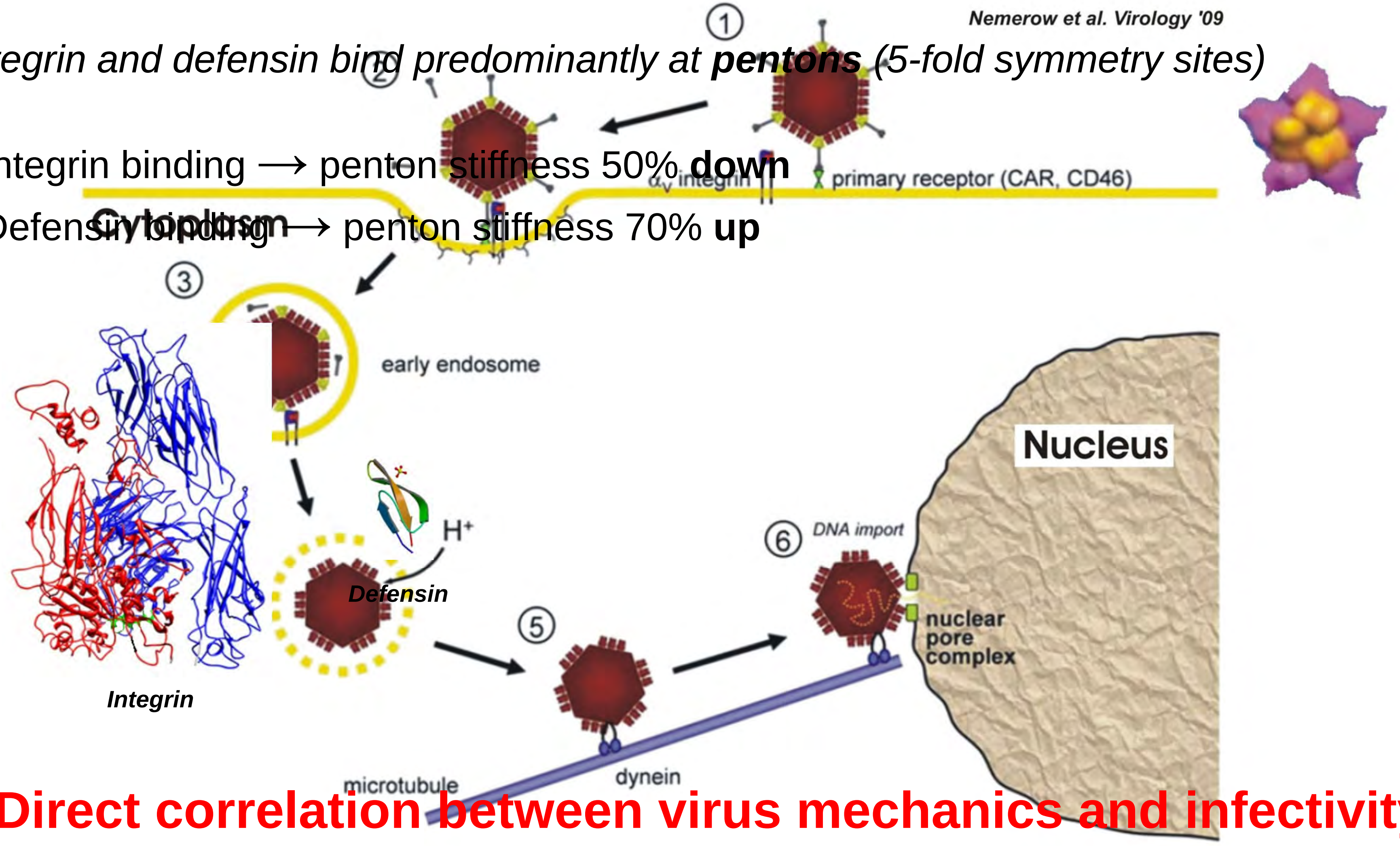
240
hexons
in
1 AdV

Host factors, mechanics & infectivity

- Integrin αvβ5 is cell surface receptor for adenovirus: promotes DNA uncoating
- Human alpha defensin (HD5, antimicrobial protein): blocks adenovirus infection

Integrin and defensin bind predominantly at ***pentons*** *(5-fold symmetry sites)*

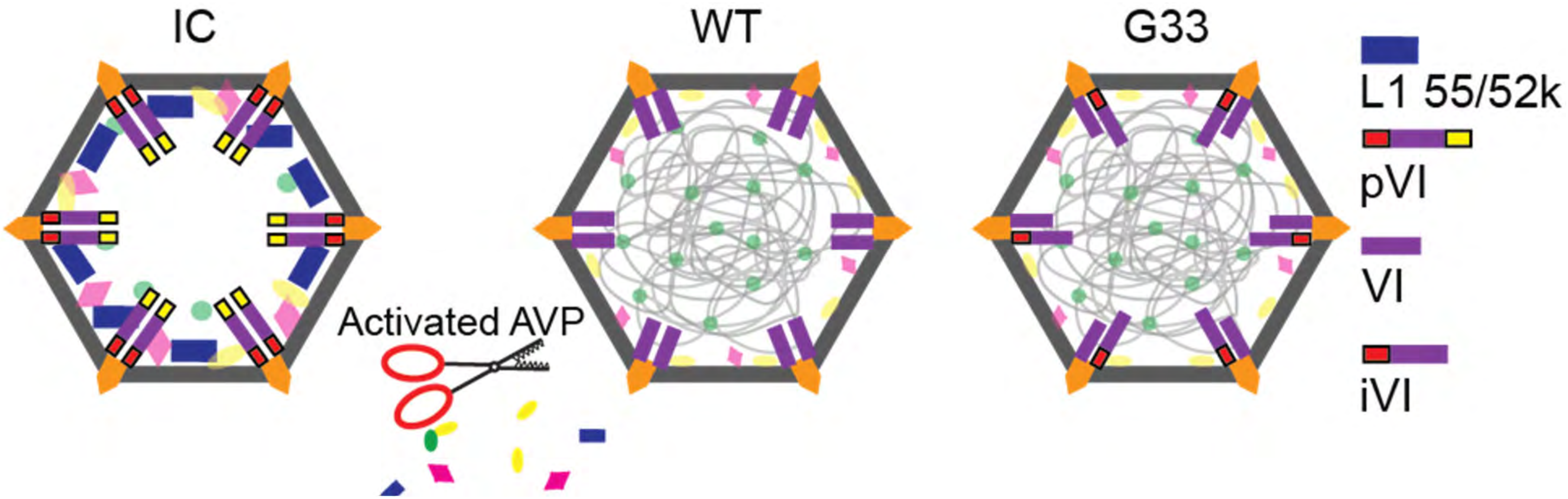
- Integrin binding ⟶ penton stiffness 50% **down**
- Defensin binding ⟶ penton stiffness 70% **up**



➡ **Direct correlation between virus mechanics and infectivity**

Maturation and infectivity

Adenovirus maturation



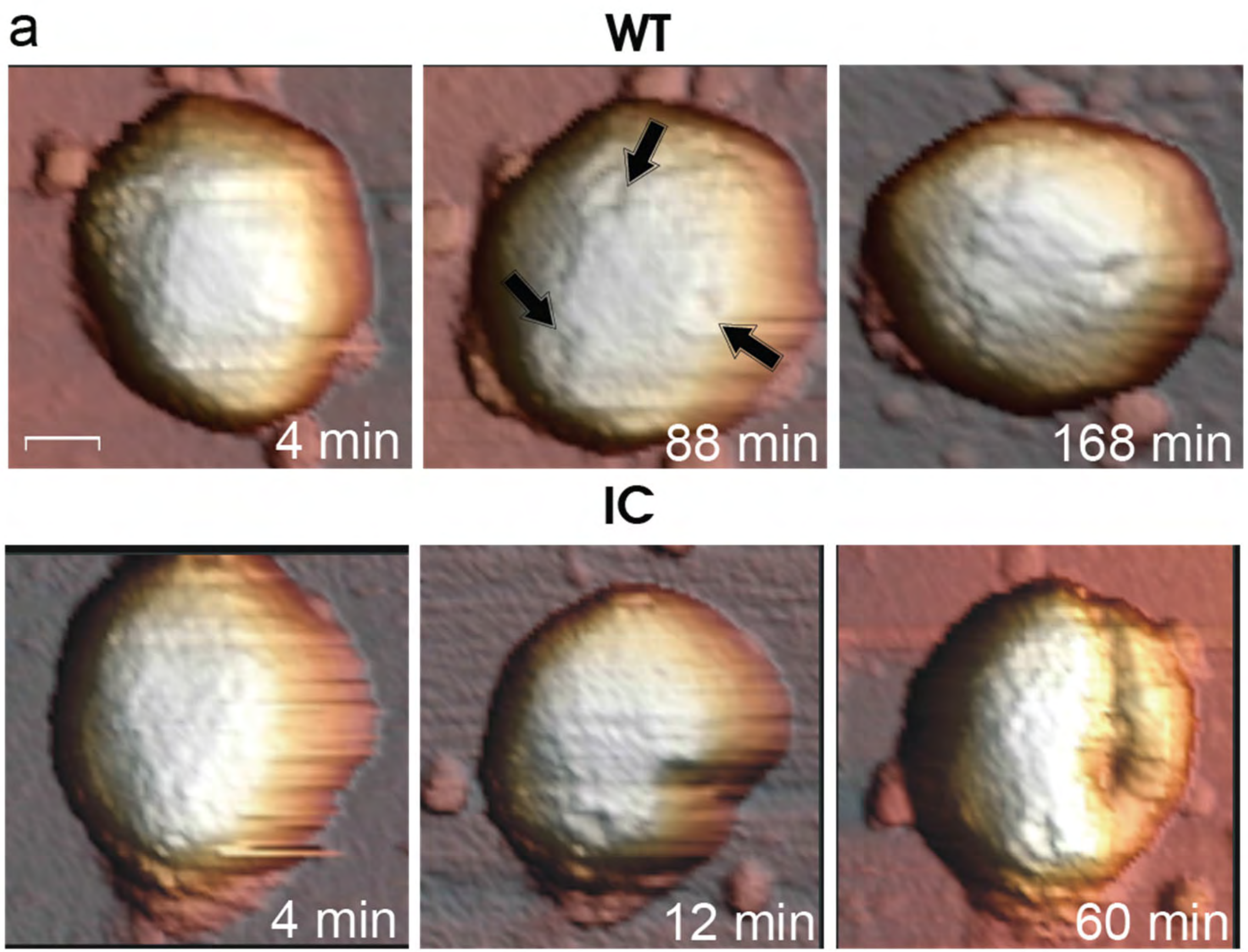
immature capsid

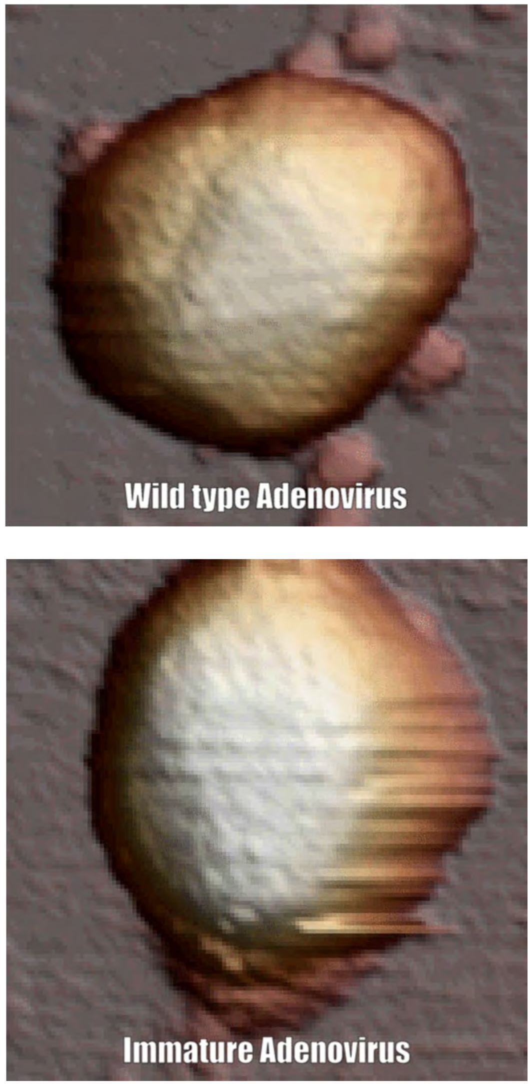
G33 mutant capsid

wild type mature capsid

Large difference in infectivity between WT and G33, but spring constant is the same

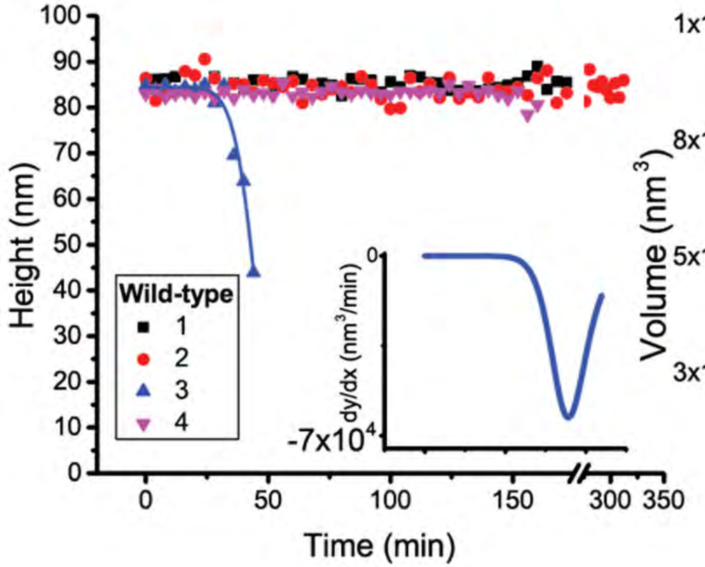
Maturation and infectivity

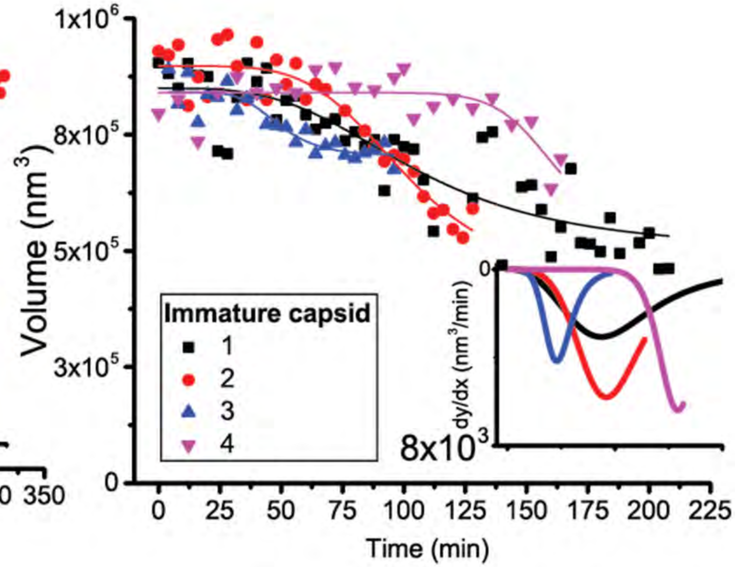


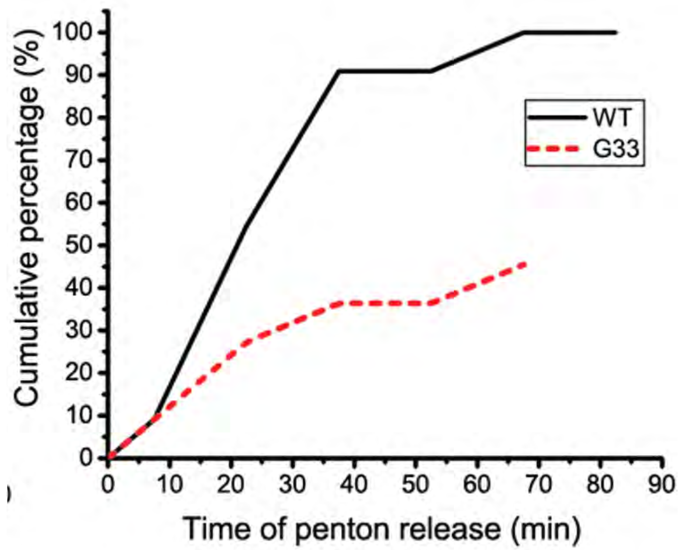


Denning *et al.* Nanoscale (2019)

Maturation and infectivity



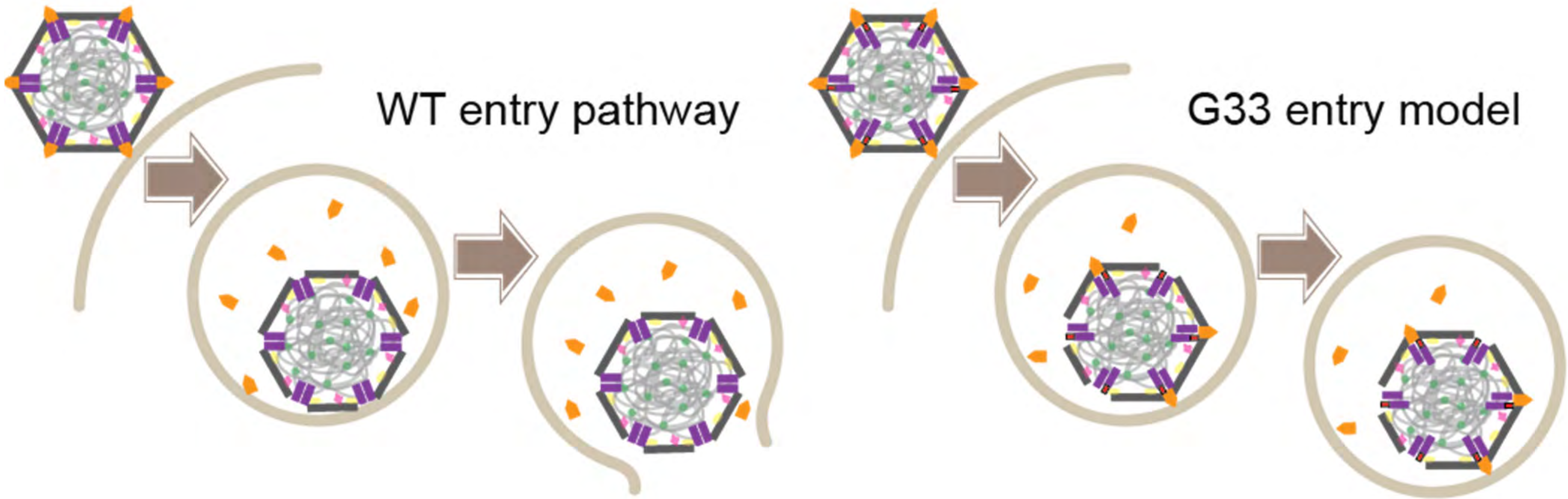




Clear difference
between penton stability

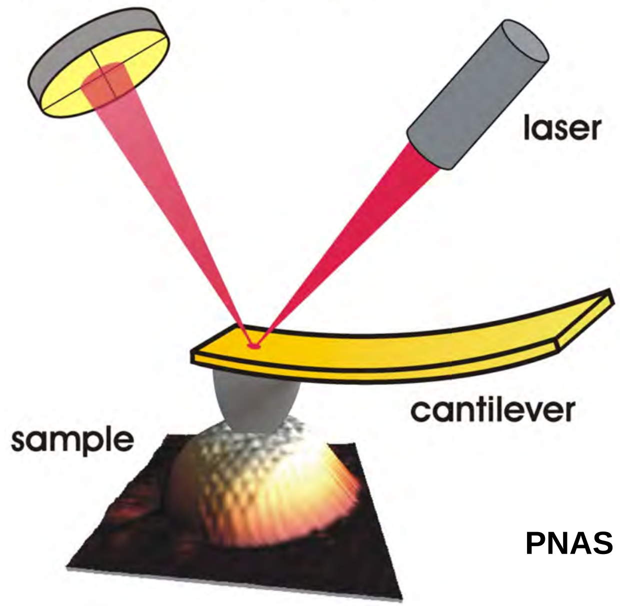
Maturation and infectivity

Full penton destabilization when the genome is present in addition to the successful maturation-linked proteolytic cleavage of preprotein VI.



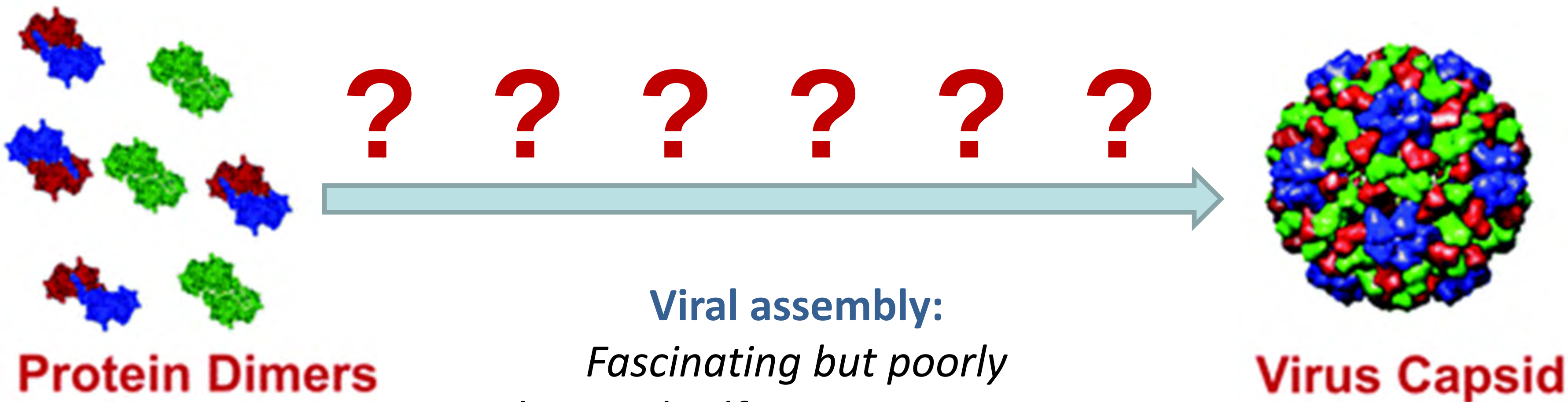
Maturation induces penton destabilization priming the capsid for endosomal release & disassembly

Viral structure and assembly



Viral structure:
Increasingly better view on mechanical & atomic structure

PNAS (2009), Nano Letters (2011), Nat Chem (2013), J Virol (2017), Nanoscale (2019)

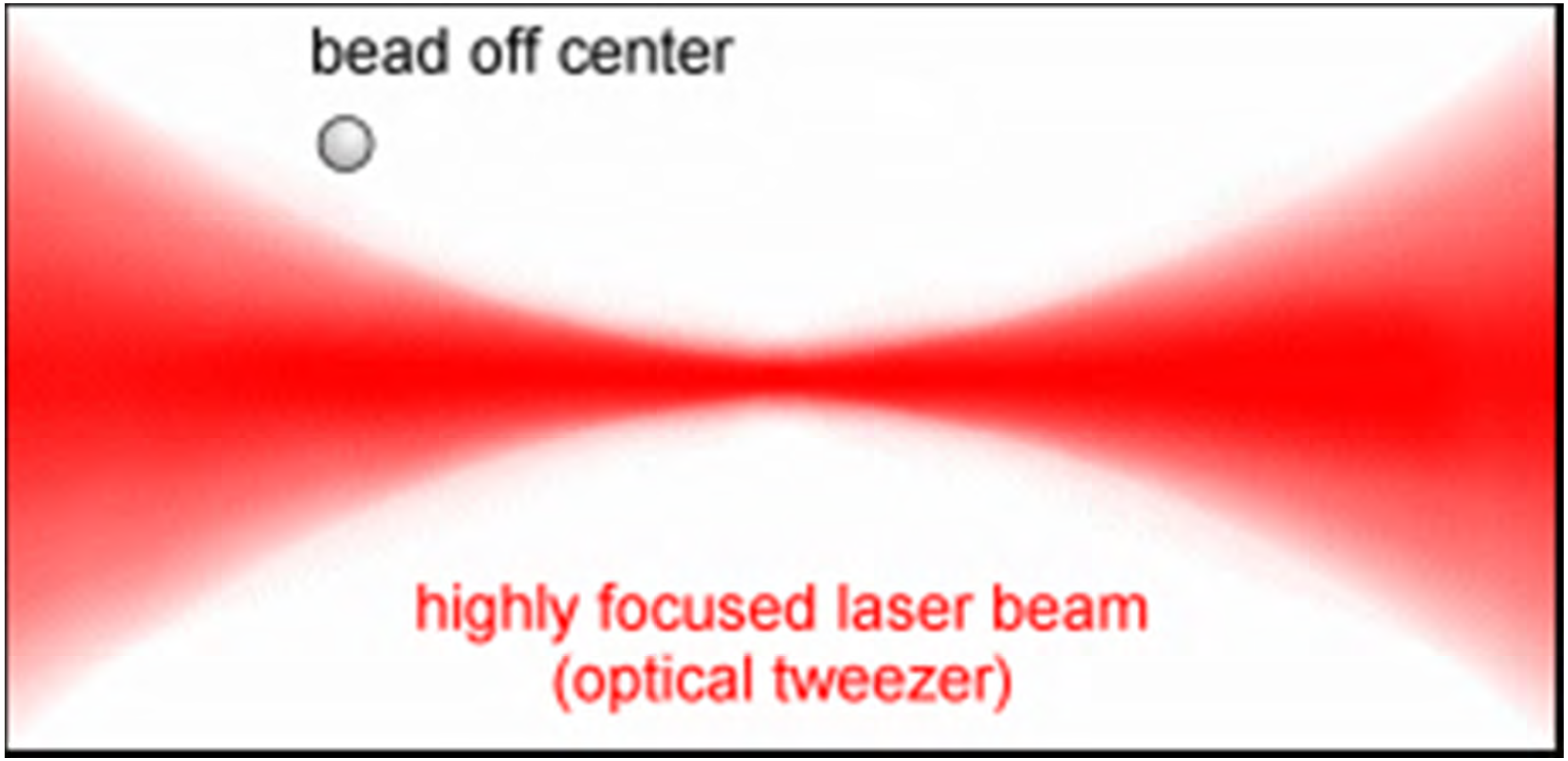


Viral assembly:
Fascinating but poorly understood self-organisation process

Image: Comellas *et al.* Soft Matter (2011)

Optical Tweezers

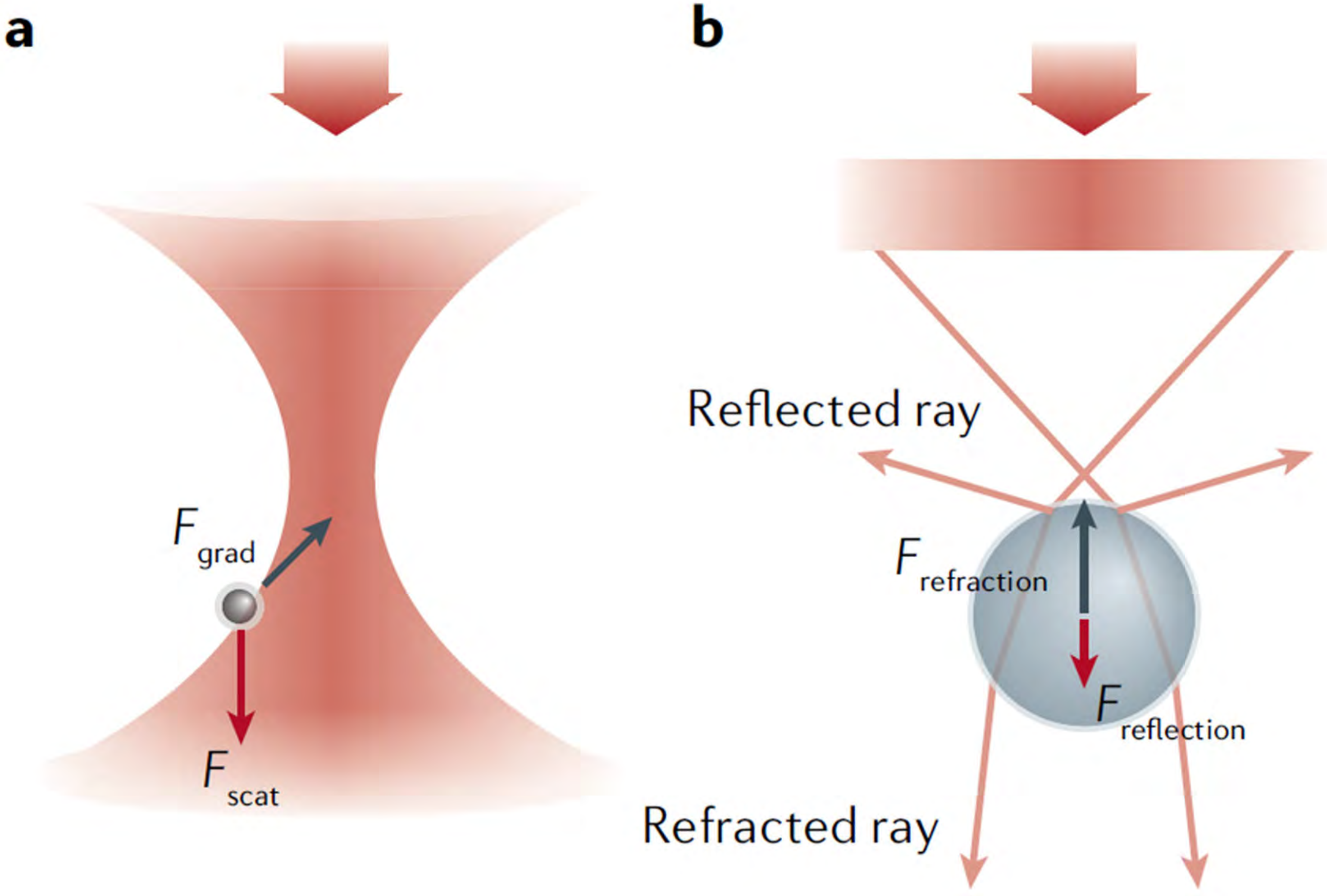
micron-sized particles can be stably trapped
in the focus of a laser beam



https://home.uni-leipzig.de/pwm/web/?section=introduction&page=opticaltraps

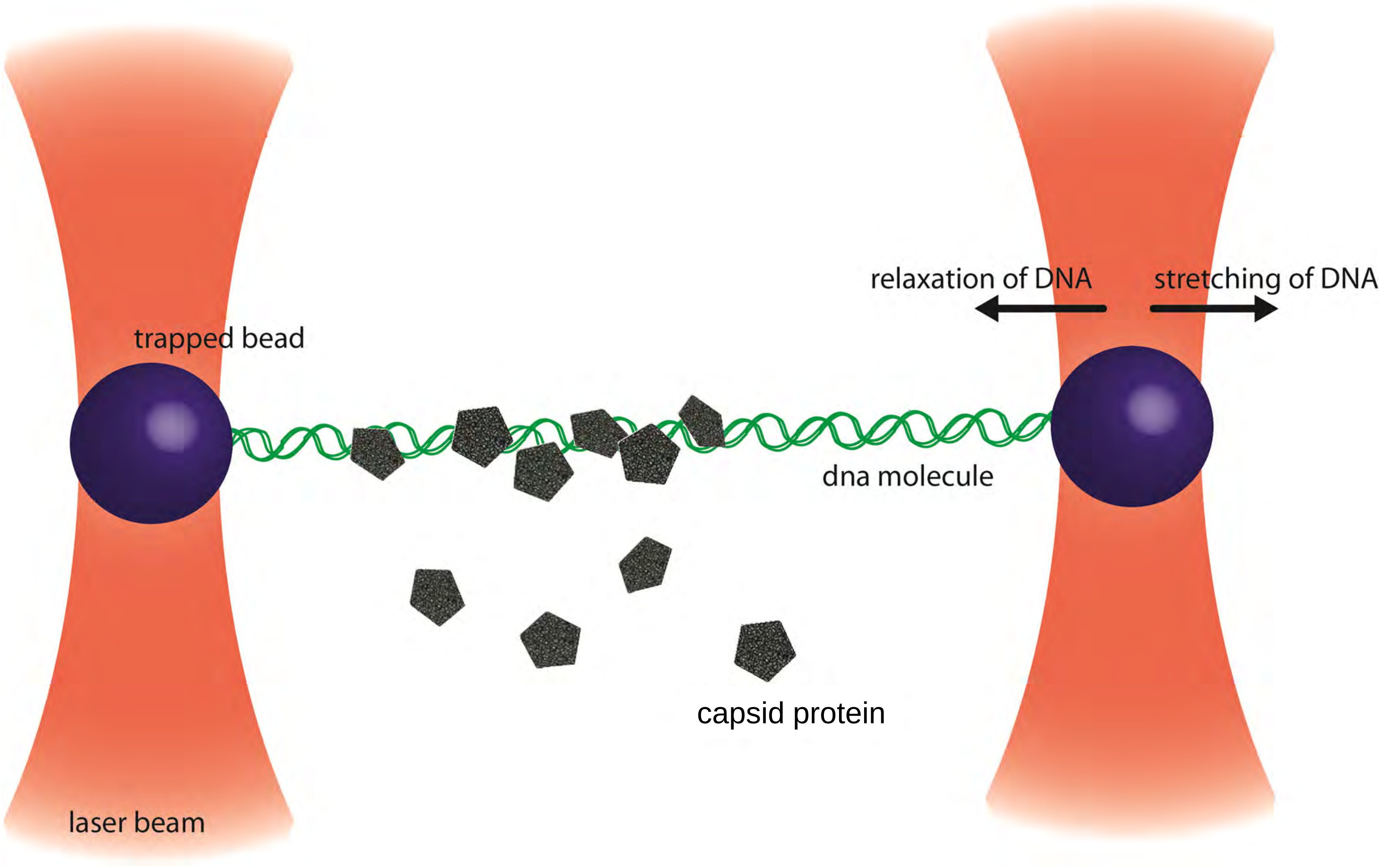
1986 Arthur Ashkin (Bell labs) ***Nobel Prize Physics 2018***

Optical Tweezers

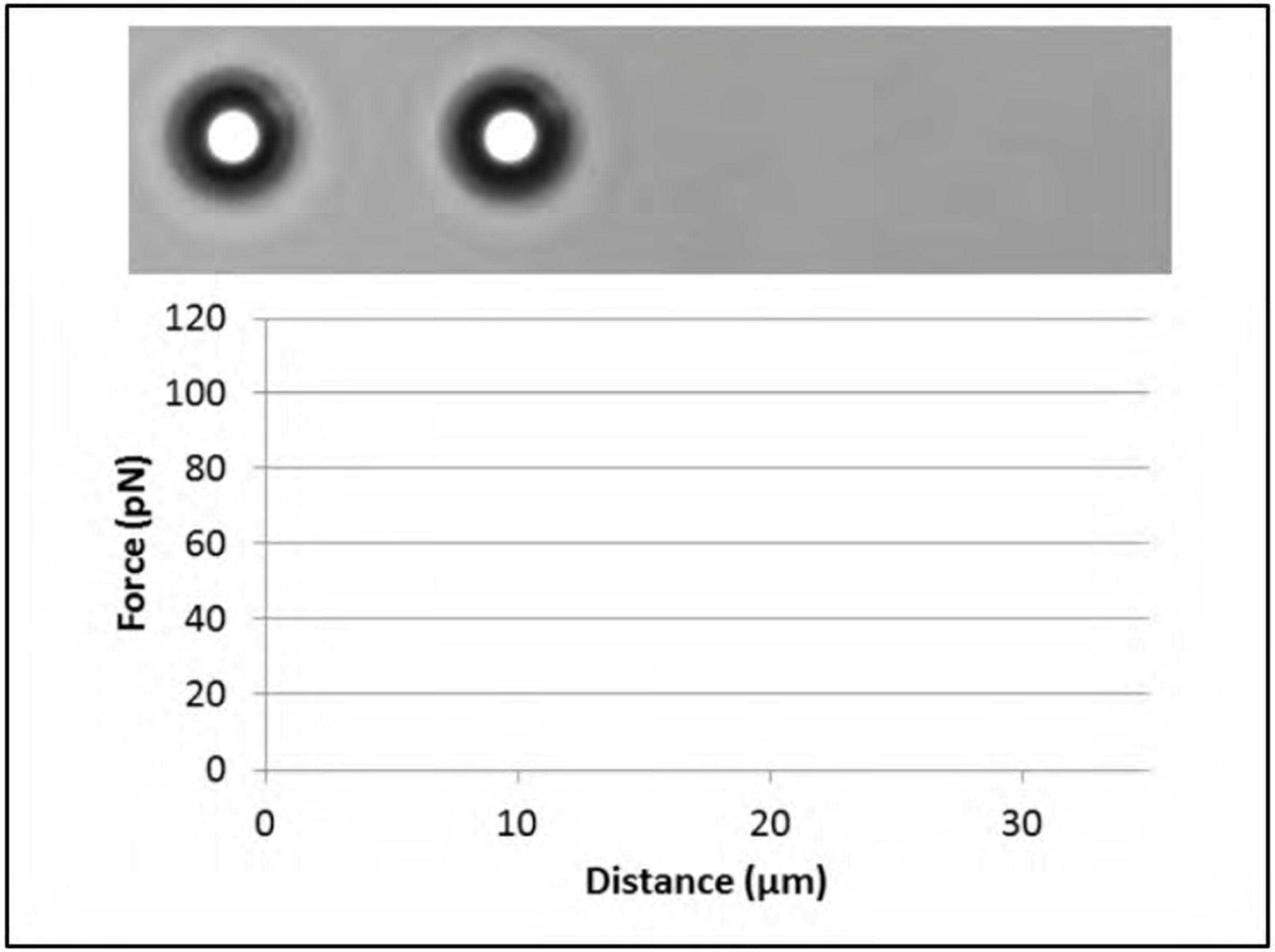


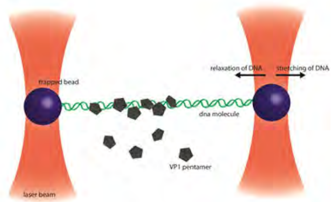
Review by Bustamante and co-workers:
Nature Reviews Methods Primer (2021) 1:25

Following virus assembly by optical tweezers



Following assembly by optical tweezers



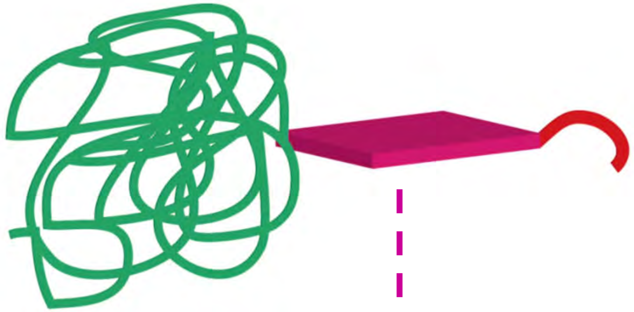


Synthetic Virus-Like-Particles

Artificial
virus-like particles are controlled systems

→

Our goal:
unravel the assembly process of a **rod-shaped artificial VLP**



Biosynthetic coat protein: polypeptide

44.8 kDa

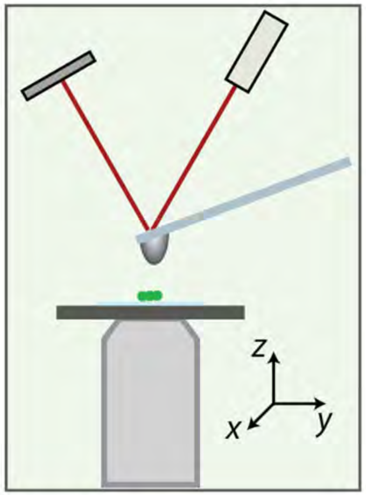
Prevent aggregation of the assembled VLPs.
High fraction of prolines and hydrophilic (mostly uncharged) amino acids:
~400-amino-acid- hydrophilic random-coil C

Folds and stacks in solution into stiff filamentous structures.
Precisely tunes cooperativity:
Silk-*like* sequence S_{10} = $(GAGAGAGQ)_{10}$

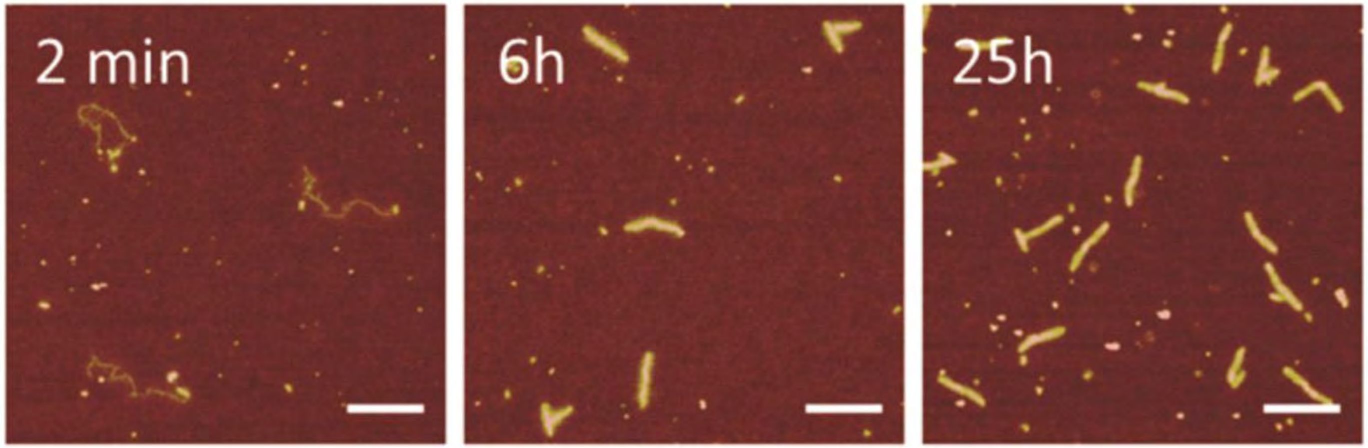
Binds DNA non-sequence-specifically through electrostatic interactions:
Oligolysine block B

De Vries Lab:
A. H. Garcia et al., *Nature Nanotechnology* (2014)
B. Zhao et al., *Soft Matter* (2016)

Particle visualisation by AFM



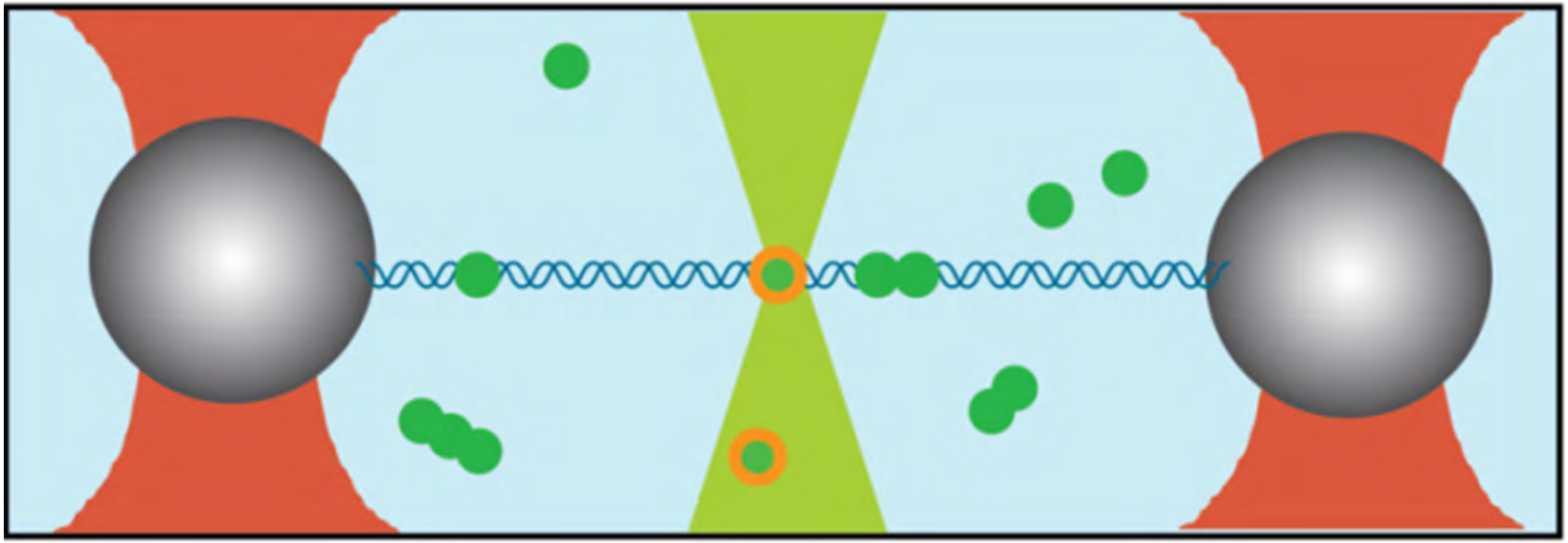
DNA 2.5 kbp ~ 1 µm

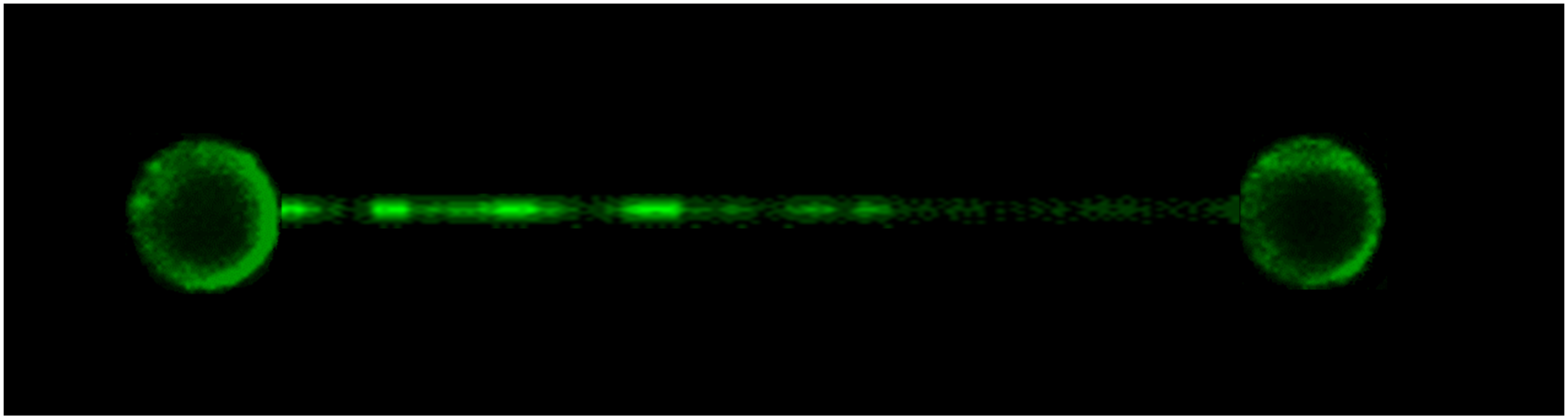


350 nm

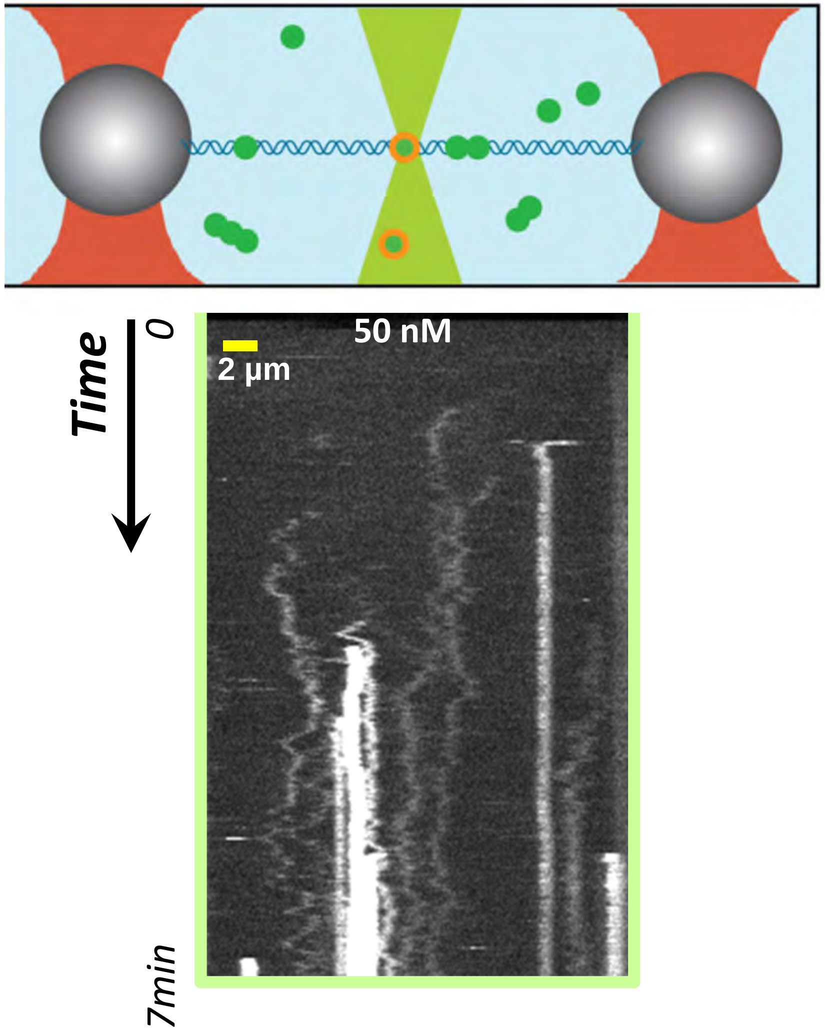
- Stiff rod-like appearance of the VLPs (300nm)
- DNA compacted 1/3 its original length
- Cooperative self-assembly.

Protein attachment, in real-time

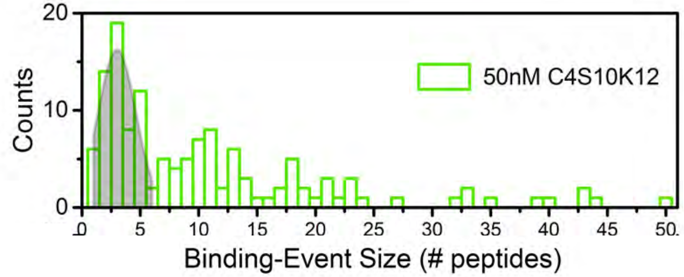




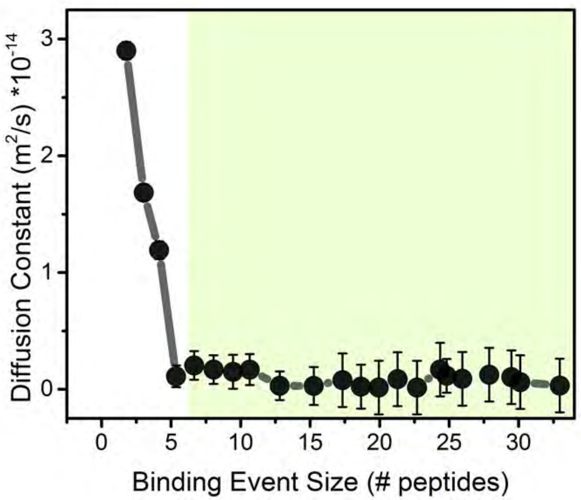
Protein attachment, in real-time



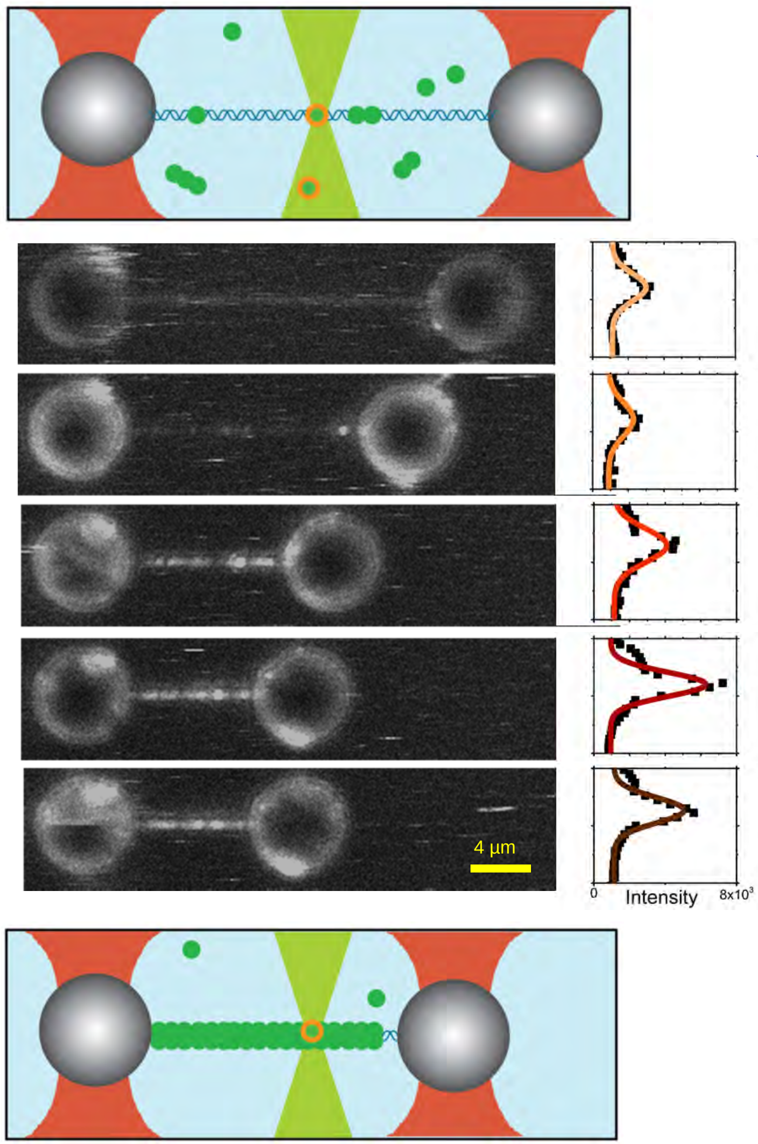
Oligomeric states



Diffusive events vs. Static Nuclei

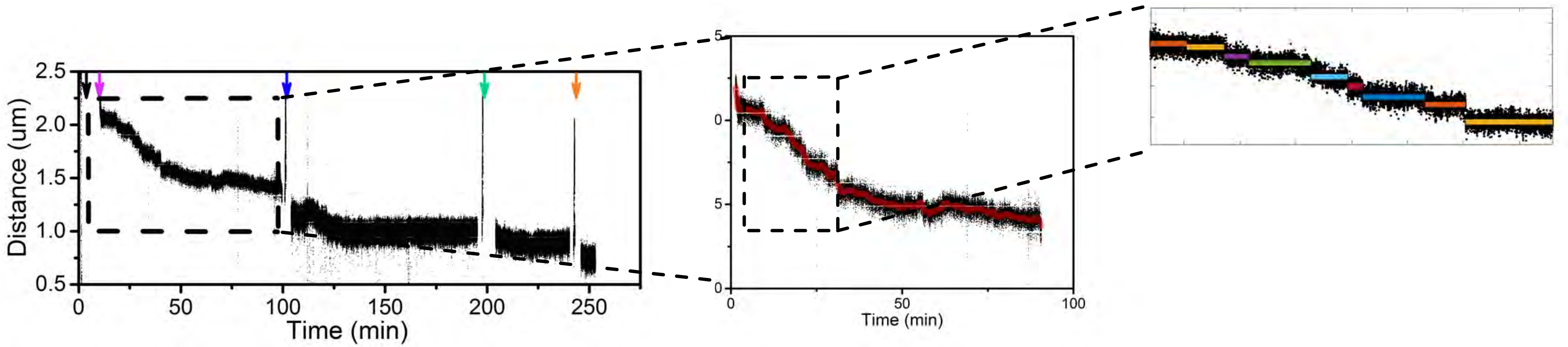


DNA compaction by rod shaped VLP

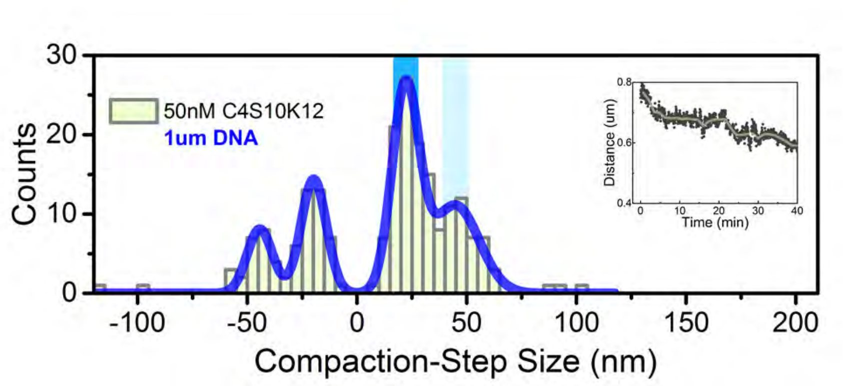


Marchetti *et al.*
Nano Letters (2019)

DNA compaction in real-time

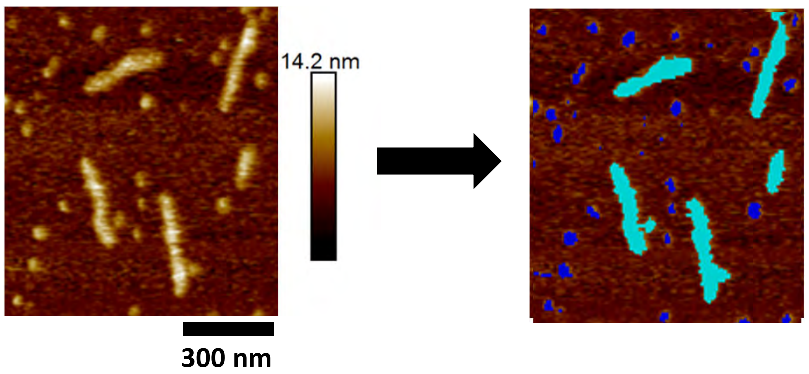


Regular compaction steps for assembly model



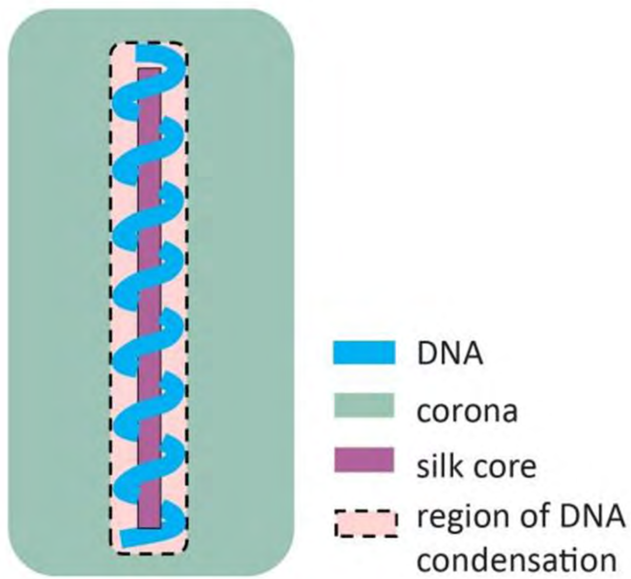
Average Compaction-Step:

~ 30 nm

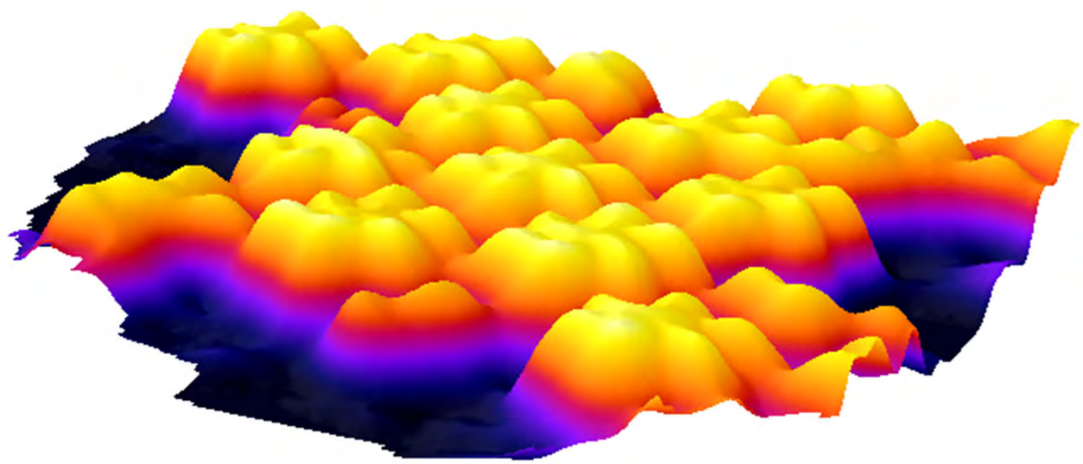


Mean particle HEIGHT:

9.1 ±0.5 nm



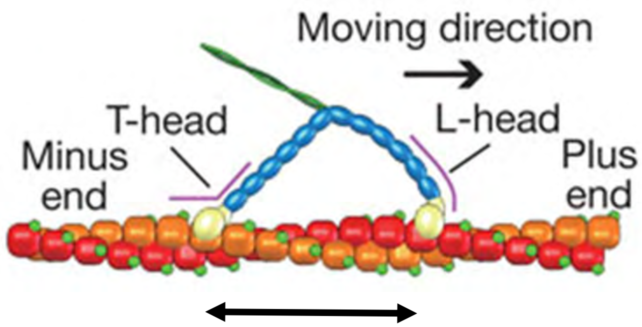
High Speed AFM studies of dynamics



<u>Are we able to follow assembly in real-time?</u>

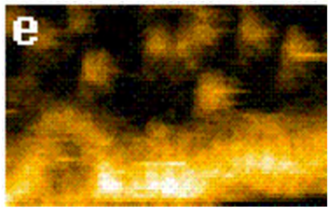
High speed AFM

Toshio Ando
Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University

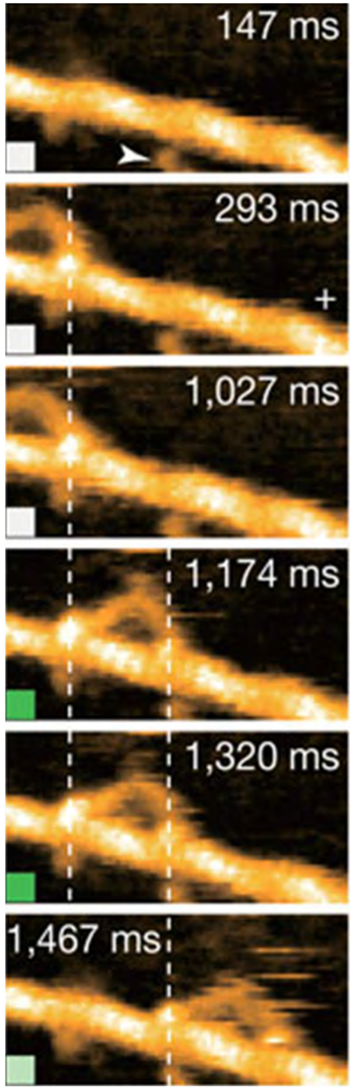


36 nm

Myosin molecular motor on actin filaments



7 frames s^{-1}



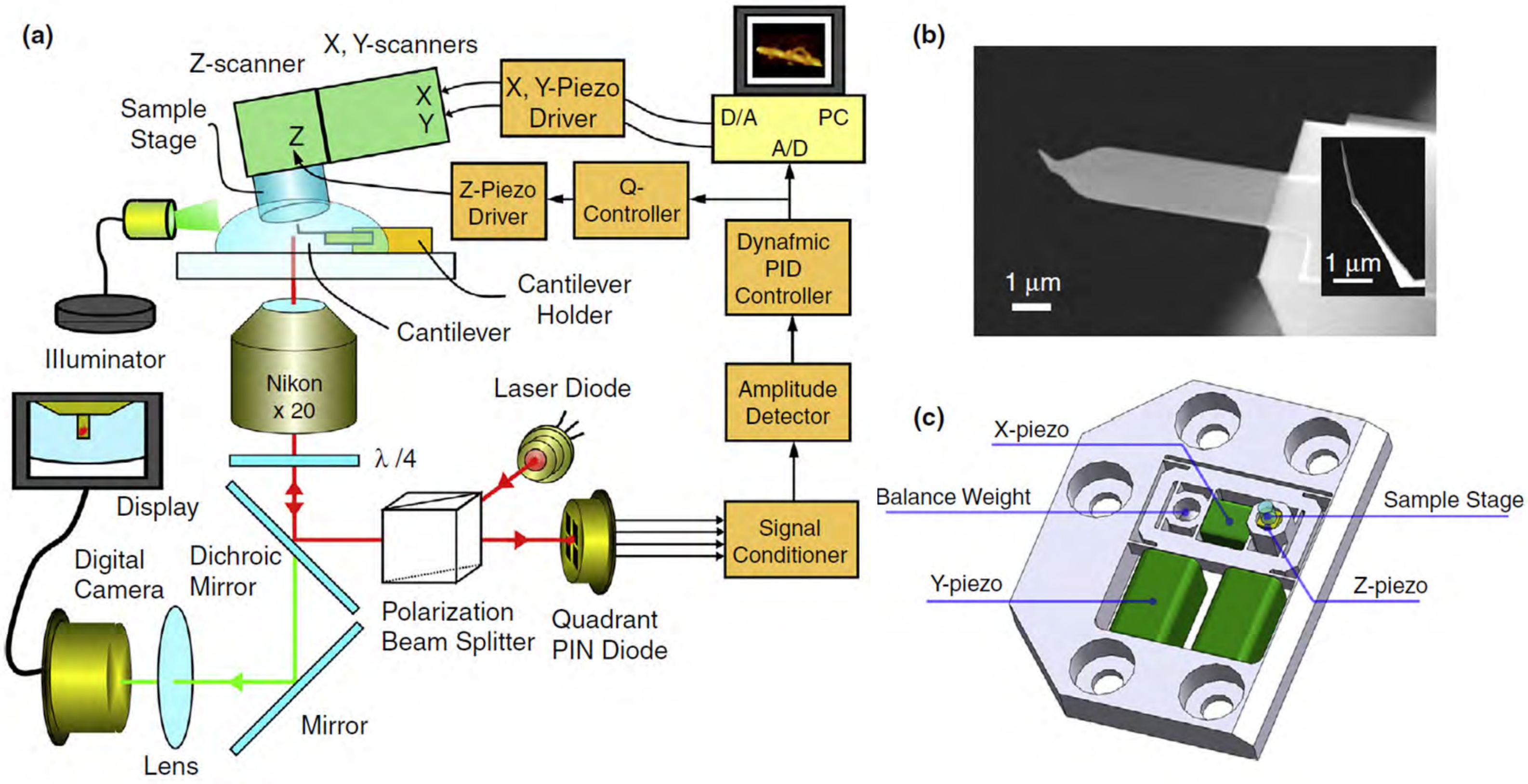
Kodera,…,Ando, Nature (2010)

Ando,...,Kodera, Annu. Rev. Biophys. (2013)
Ando,…,Scheuring, Chem. Rev. (2014)
Ando, Biophys Rev. (2018)

High speed AFM

(1) Ultrasmall cantilevers (low cantilever mass allows high resonance frequencies)
(2) Fast actuators (dummy piezo, balance weight)
(3) Fast amplitude detectors
(4) Adaptive/dynamic feedback (against ‘parachuting’)

Casuso,…,Rico, Phil. Trans. R. Soc. A (2020)



Ando, Curr. Opin. Struct. Biol. (2014)

High Speed AFM studies of 2D HIV assembly

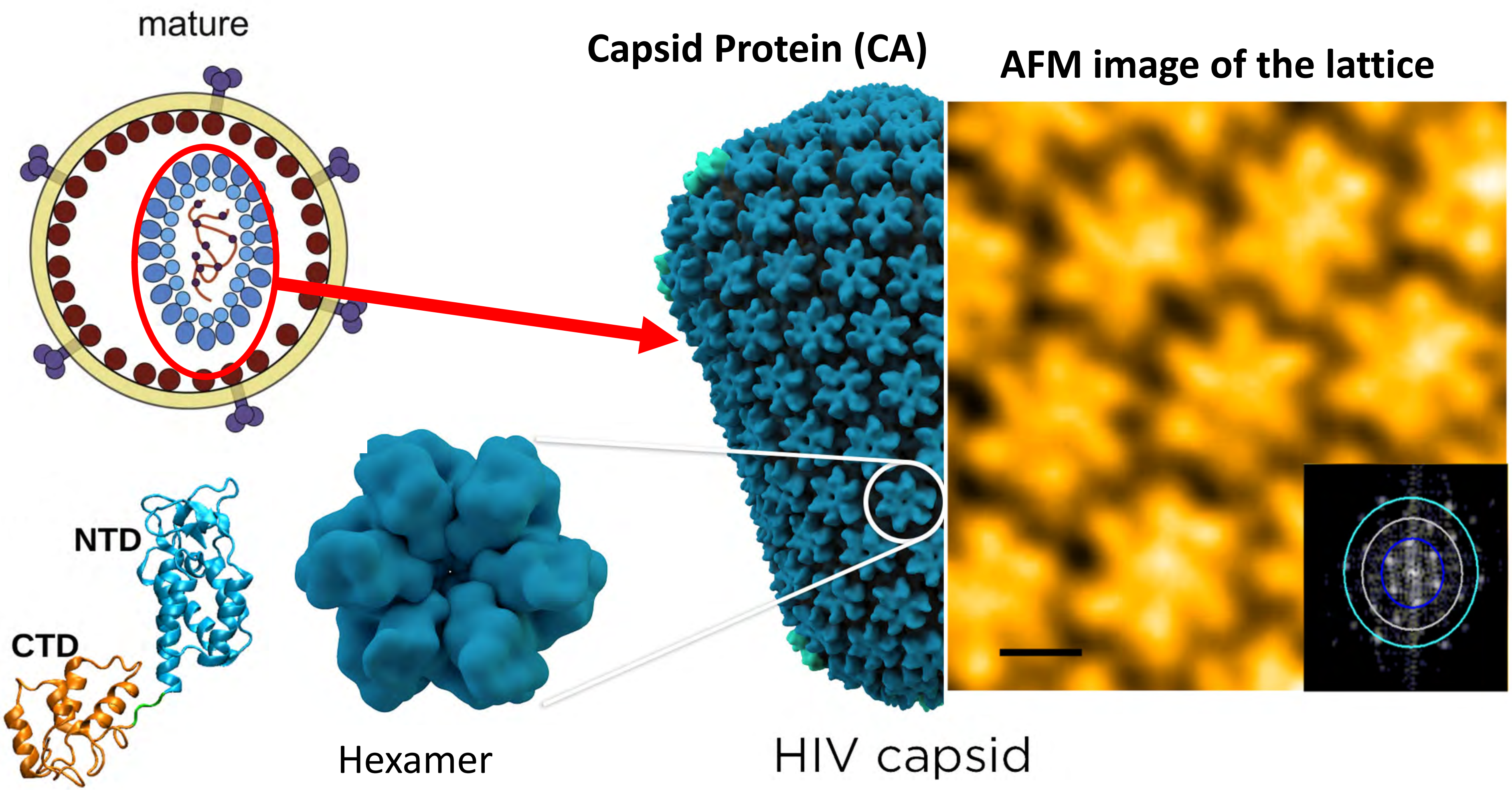
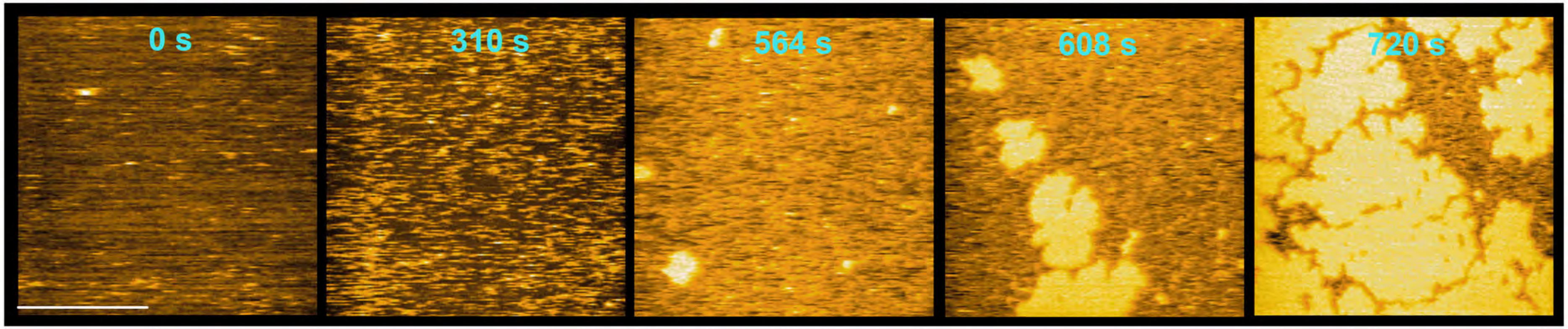
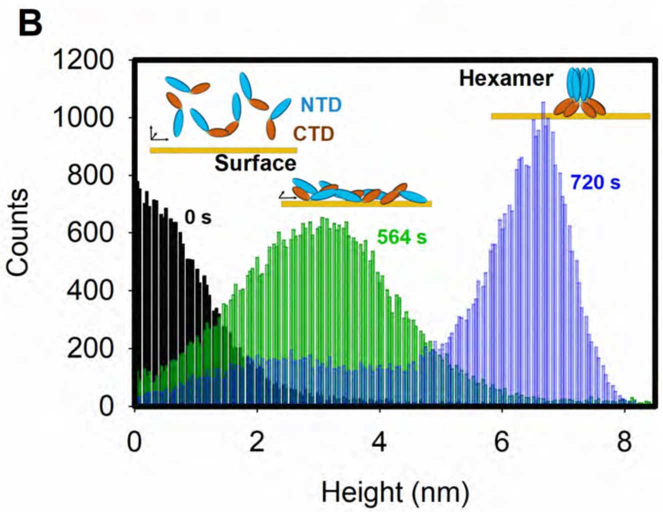
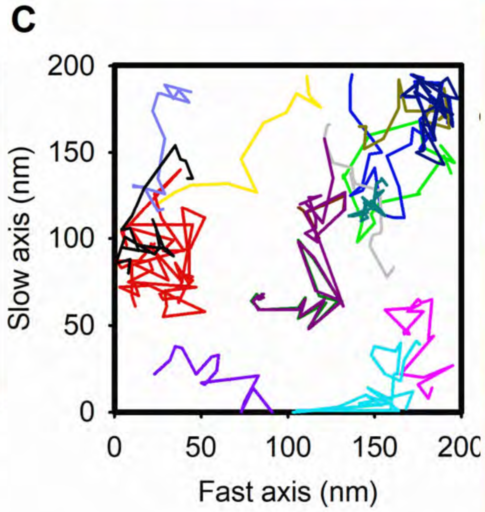


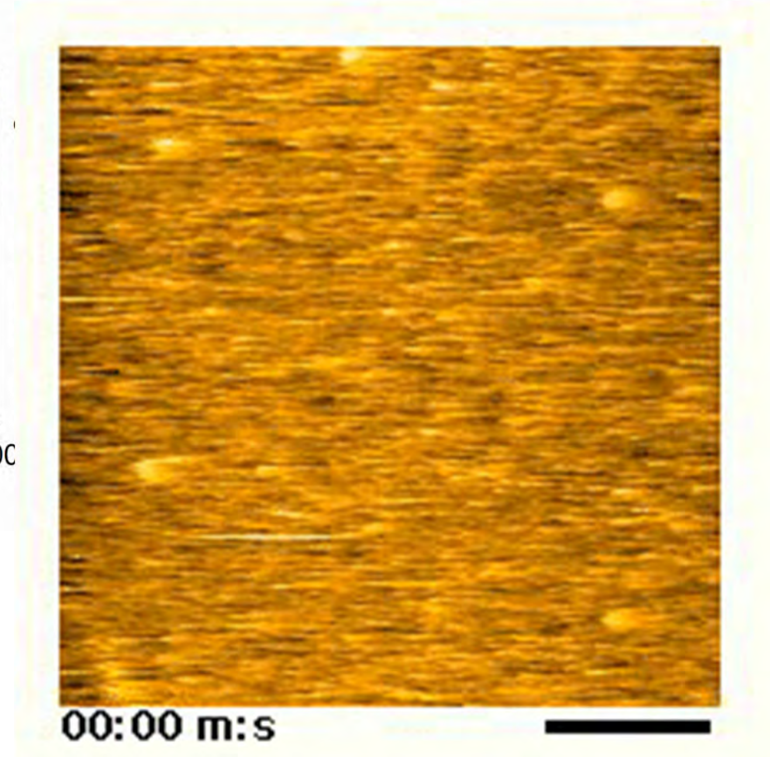
Figure credits: Monroe et al., ***Structure***, 2010 and Thomas Splettstoesser

High Speed AFM studies of 2D HIV assembly

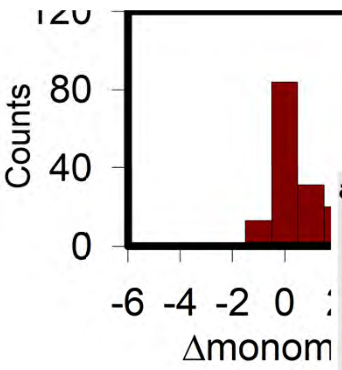




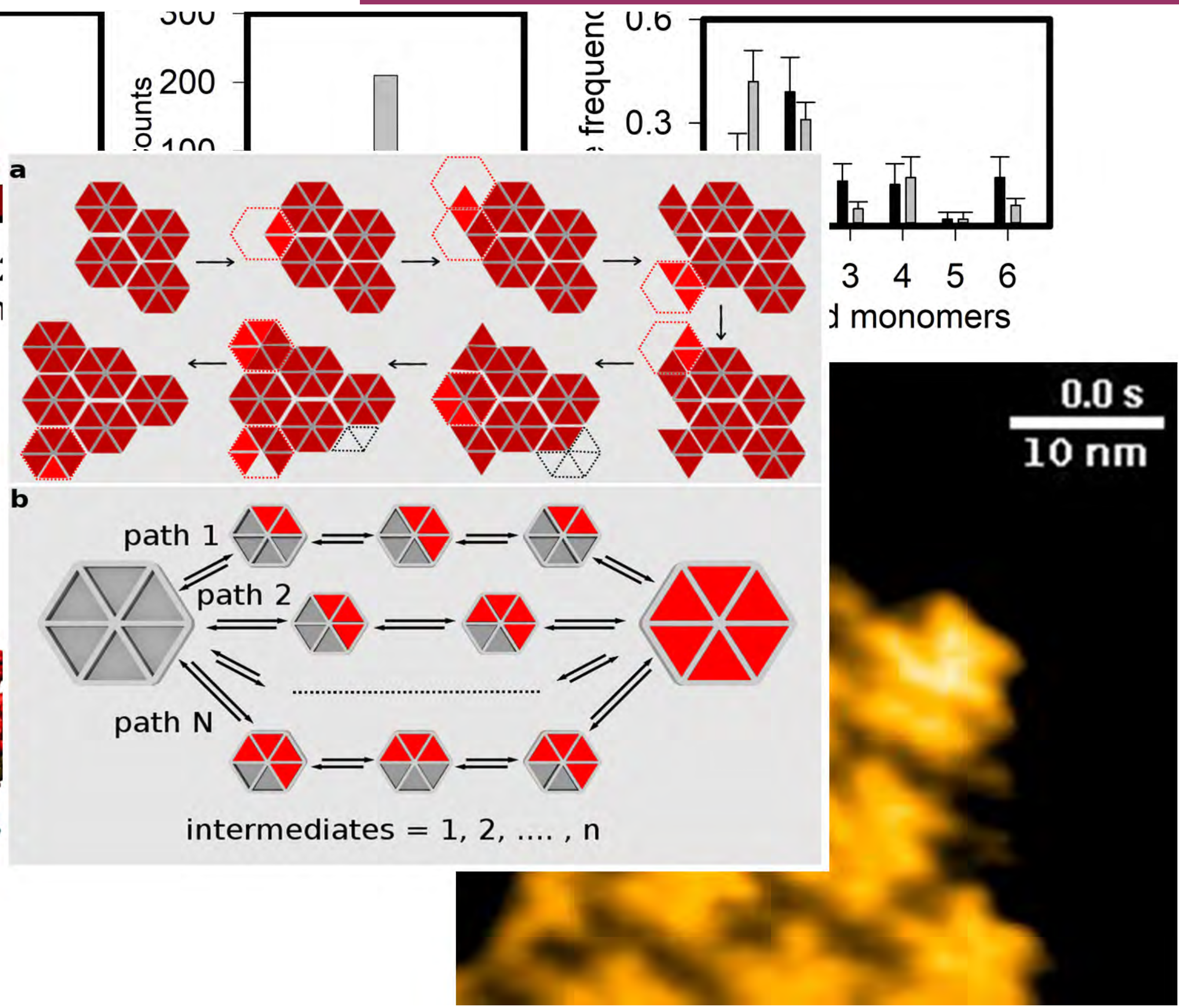




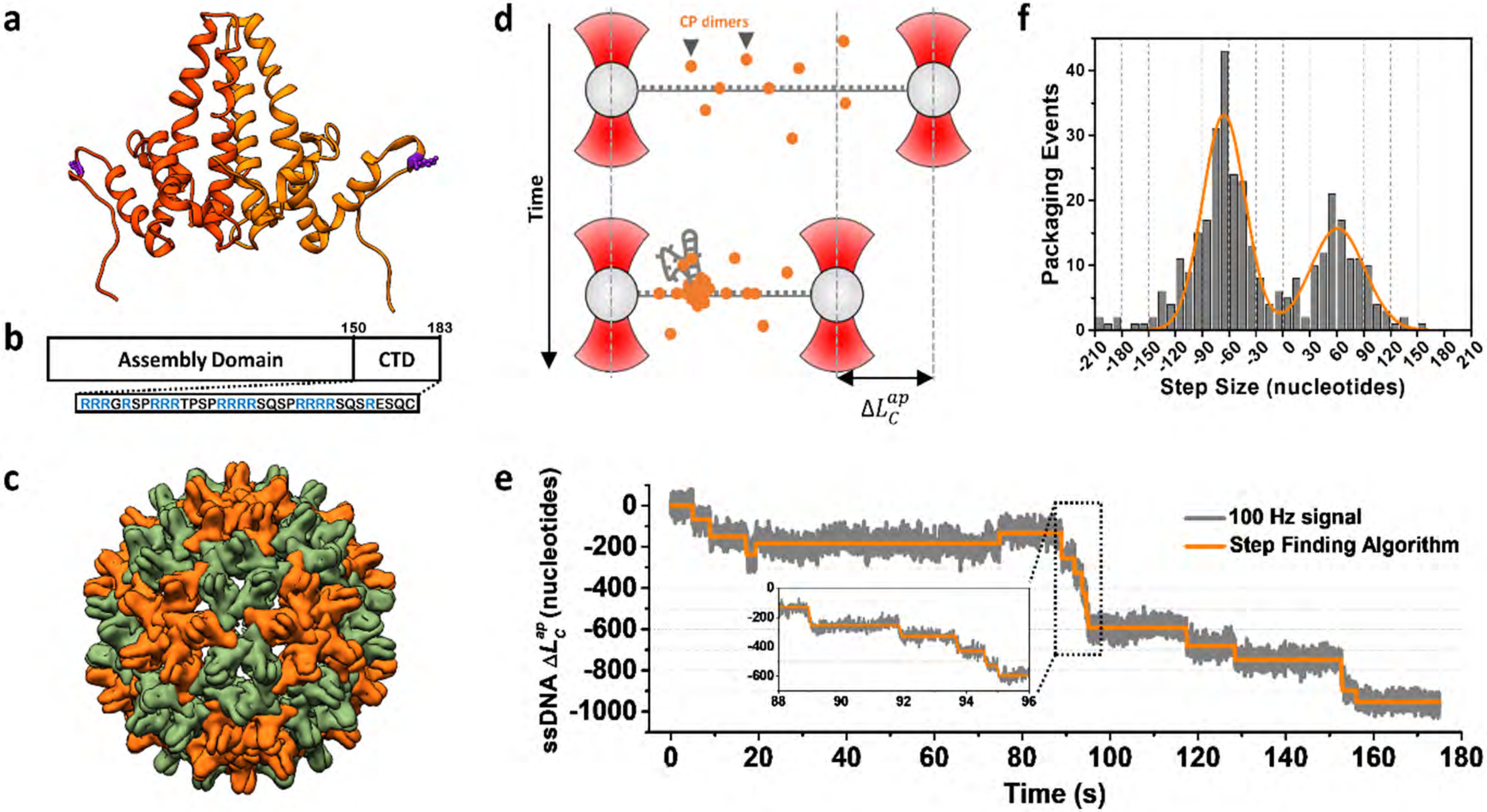
High Speed AFM studies of 2D HIV assembly



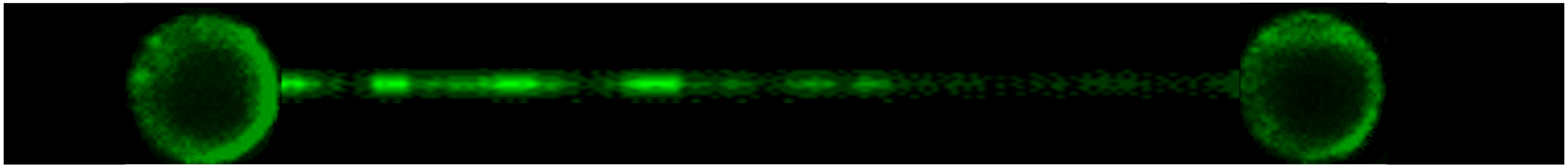
Valbuena *et al.*
ACS Nano (2020)



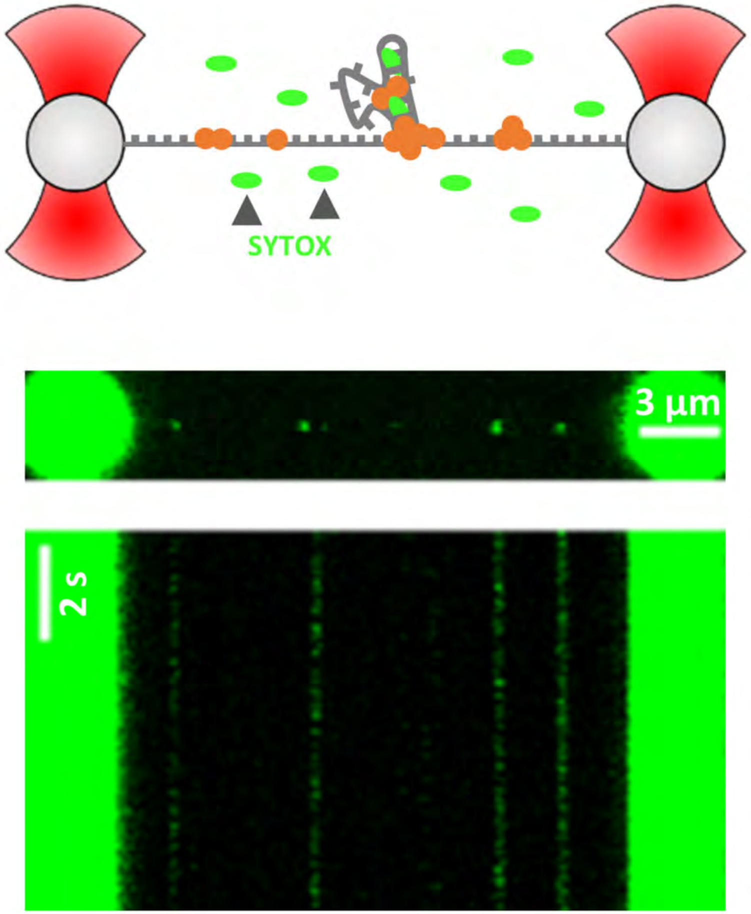
Hepatitis B Virus assembly



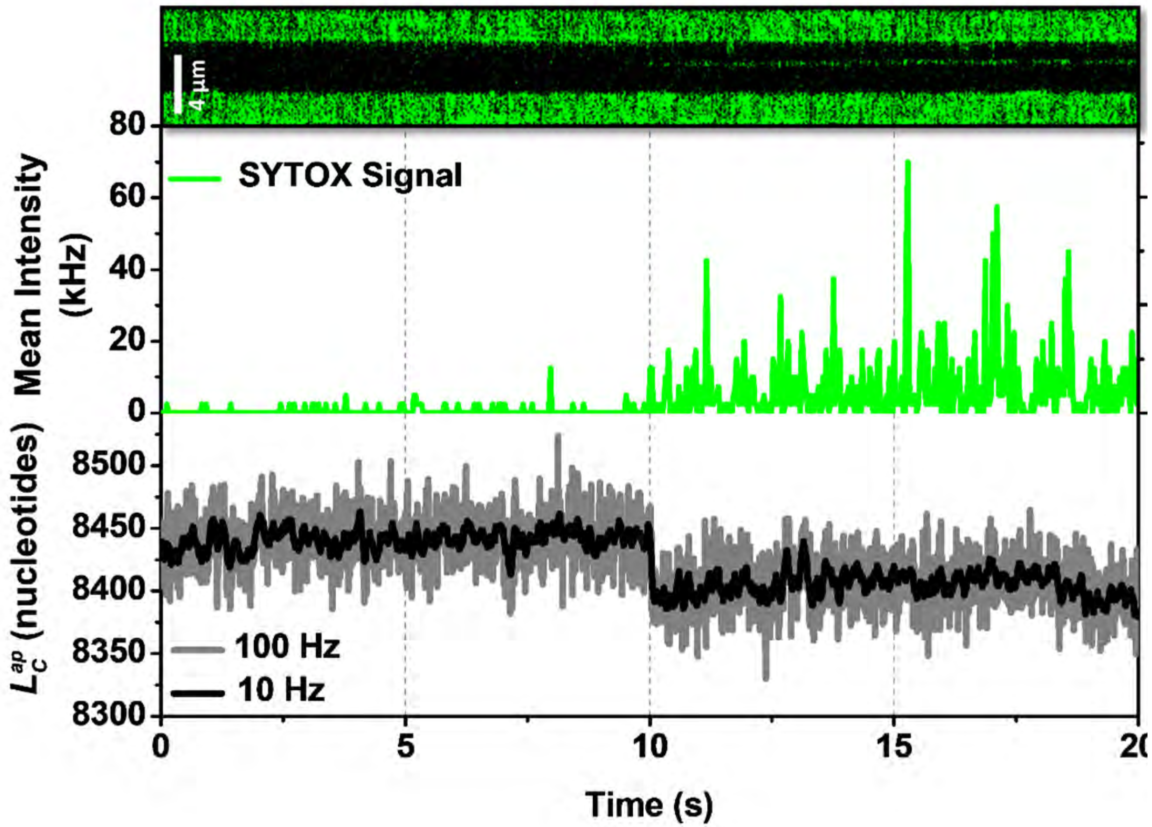
Buzon *et al.* Science Advances 2021



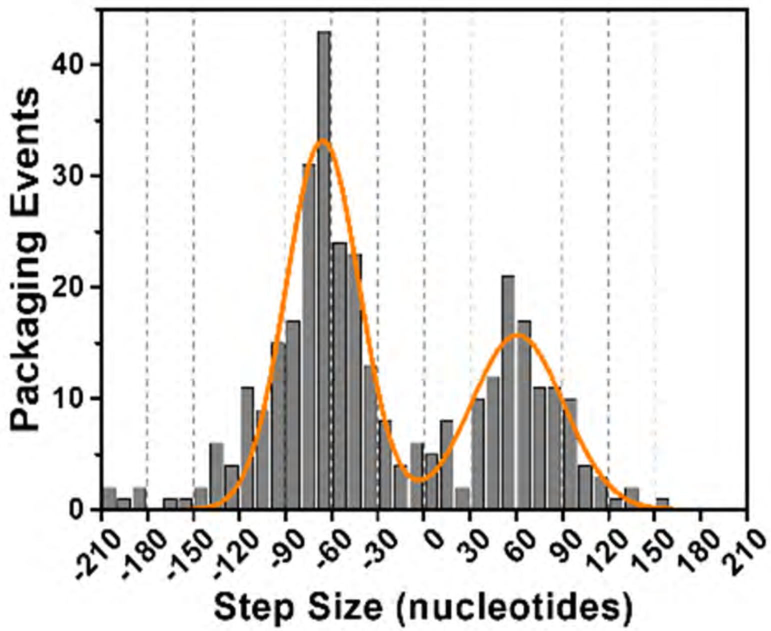
Hepatitis B Virus assembly



Cp has the capability to chaperone the formation of dsDNA



Hepatitis B Virus assembly



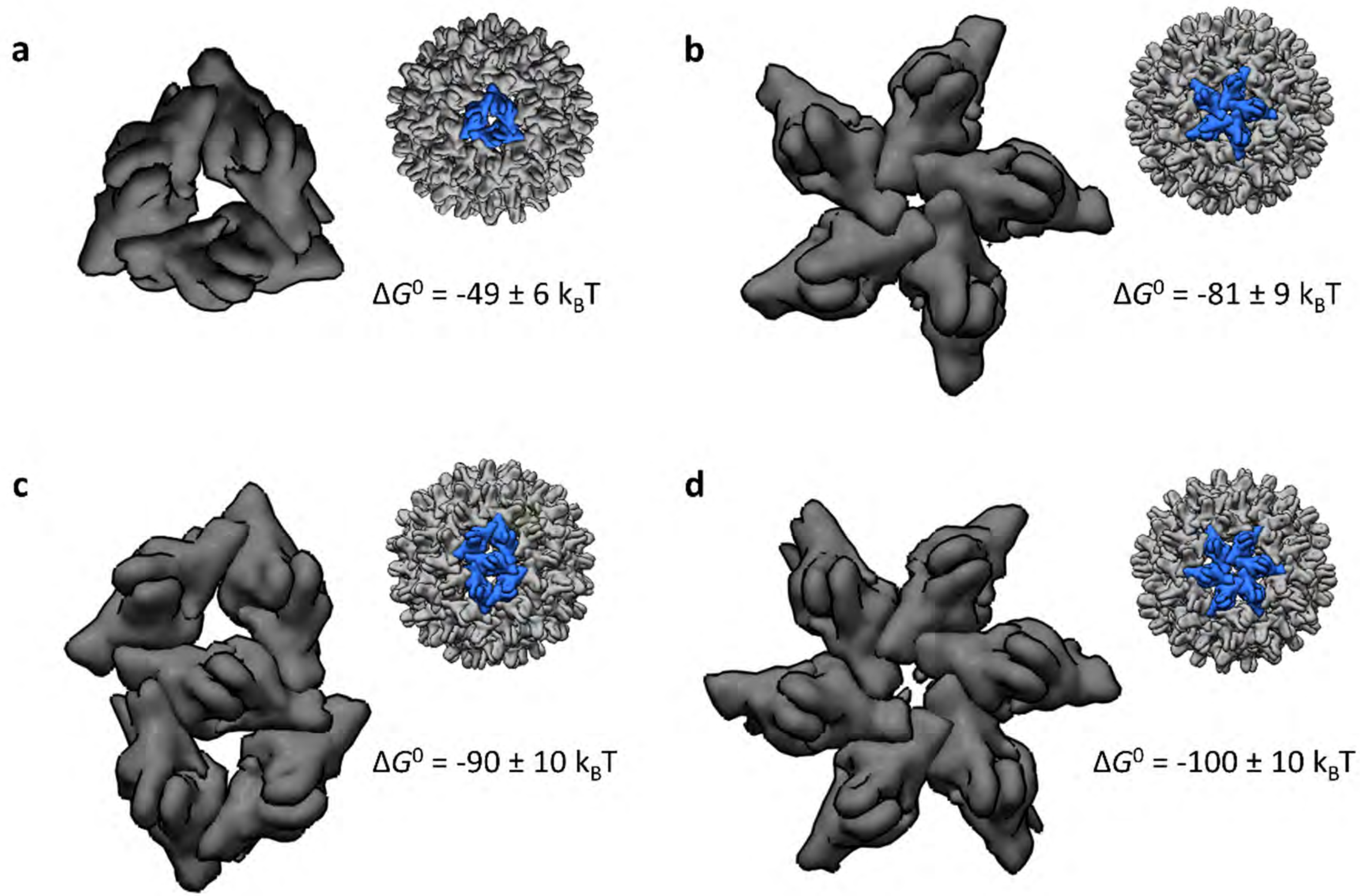
Cp condenses nucleic acids, even under tension (F = 11 pN)

Assembly footprint: ~70 nt

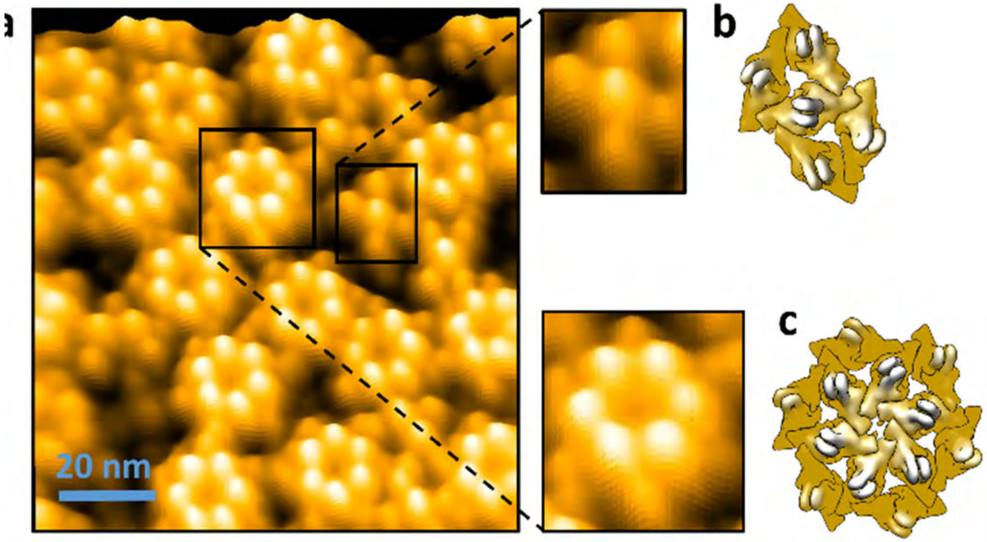
Work per condensation step:
F* *step size* = ~ 100 k_BT

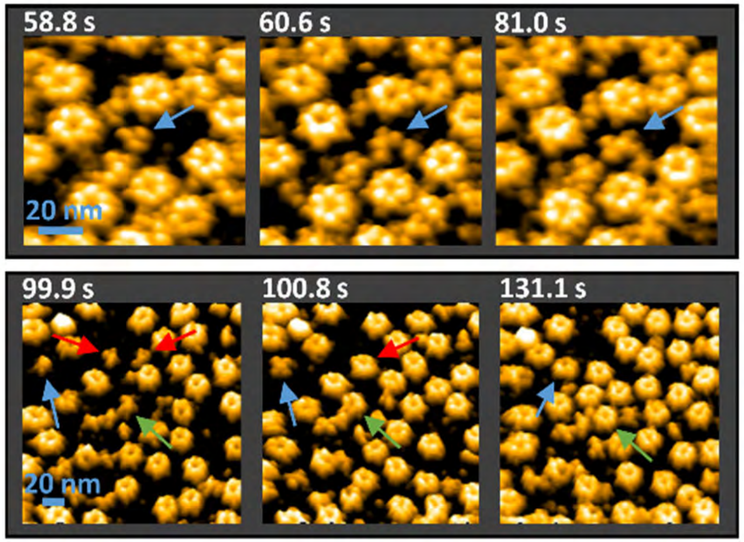
This corresponds to ~1.4 k_BT/nt (*compare to ~5 k_BT/bp for ATP driven packaging motor of Φ29*)

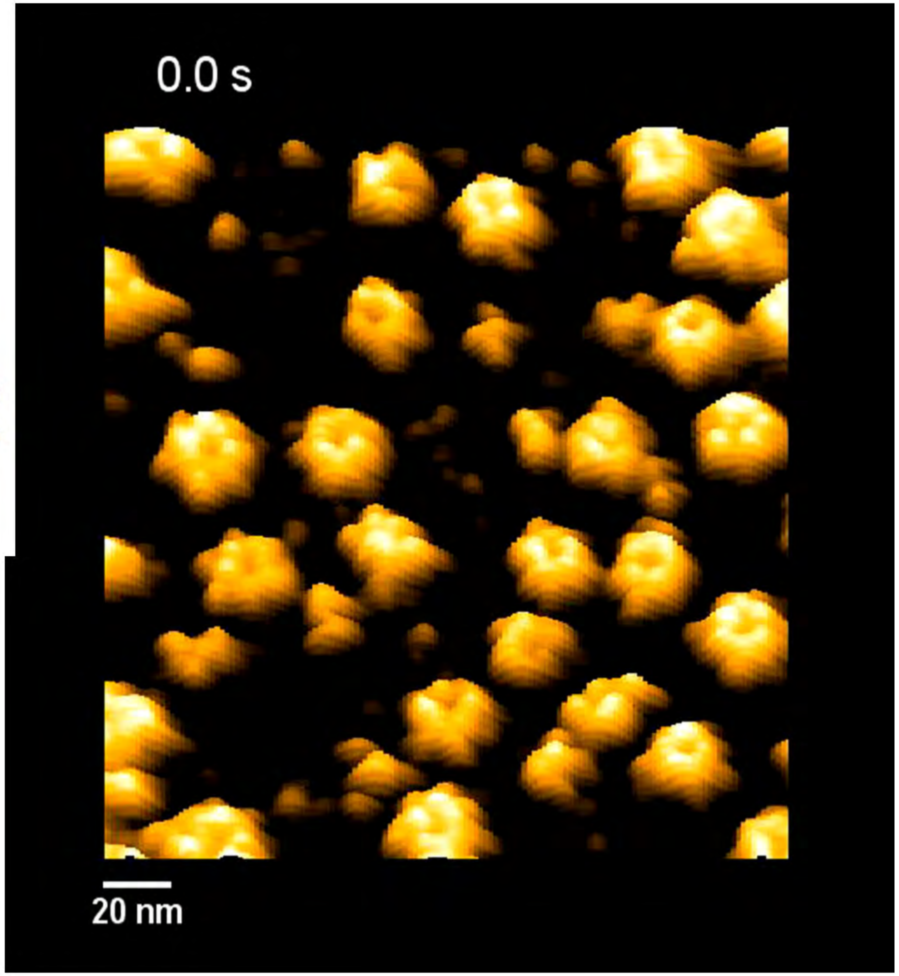
Hepatitis B Virus assembly



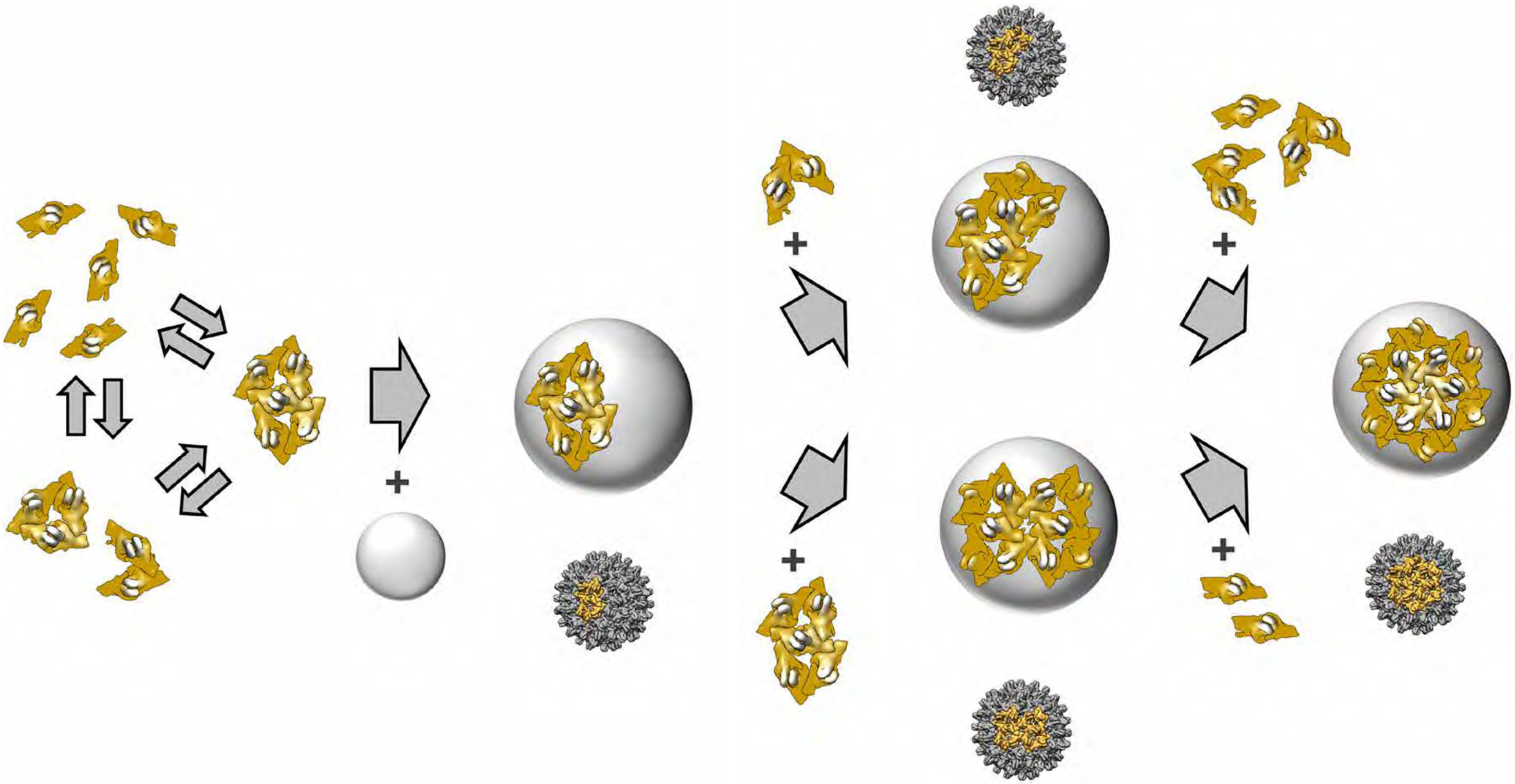
Hepatitis B Virus assembly



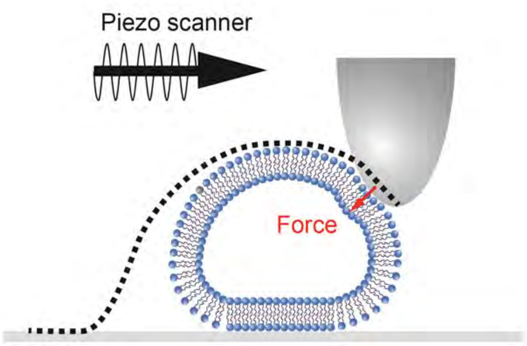




Hepatitis B Virus assembly



For those not (only) interested in viruses



Extracellular vesicles / lipoprotein particles

Vorselen *et al.*
Nat. Comm. (2018)

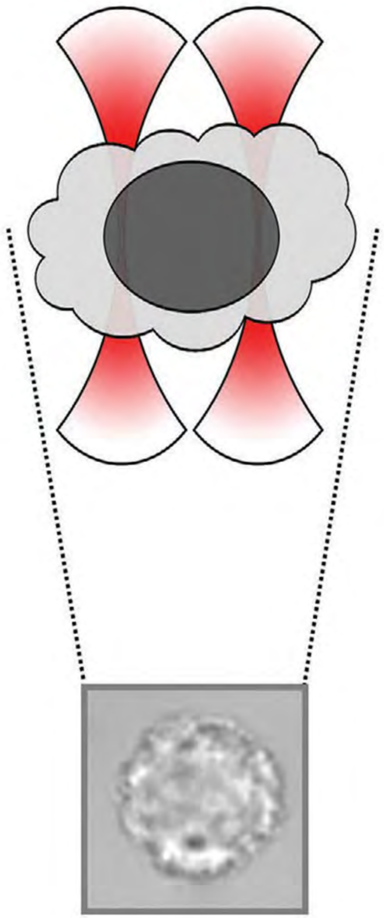
Piontek *et al.*
J Extracell. Biol. (2022)

AFM nanoindentation (mechanical probing)

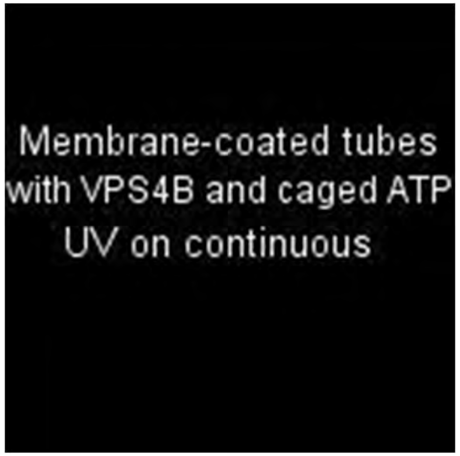
Single-Cell Activation Kinetics

Vasse *et al.*
Small Methods (2021)

Optical Tweezers studies of cell dynamics



For those not (only) interested in viruses



ESCRT:

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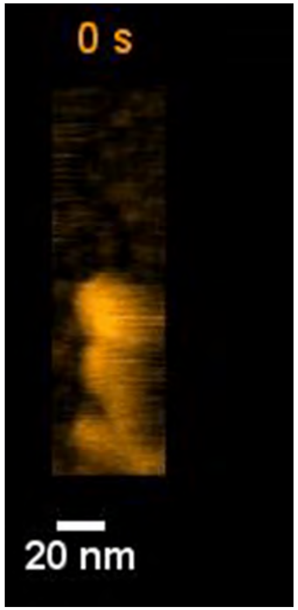
Azad *et al.* Nat. Struct. Mol. Biol. (2023)



Assembly of synthetic systems:

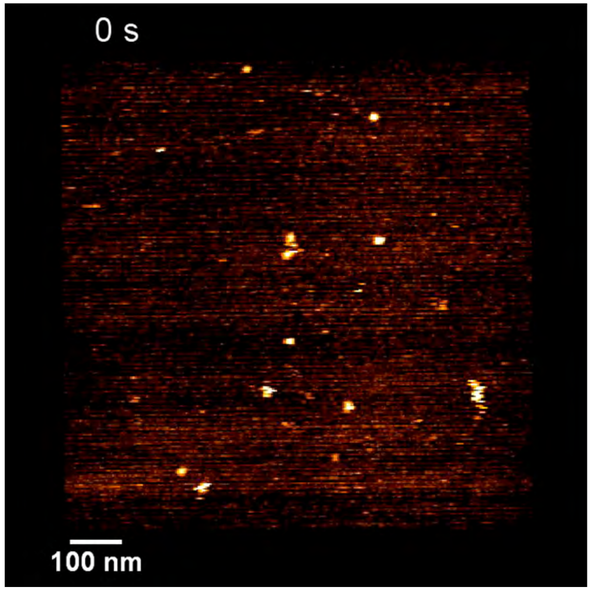
Maity *et al.* JACS (2020)

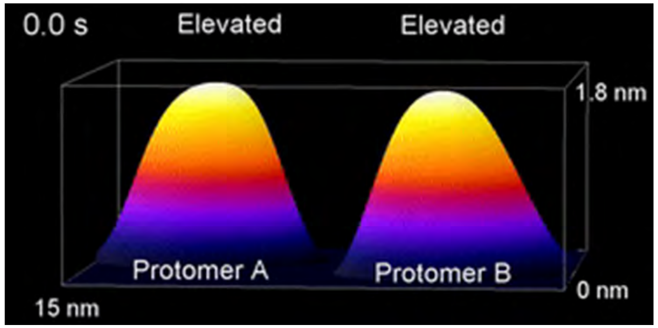
Liu *et al.* Nat. Chem (2023)

Assembly of antibiotics:

Shukla *et al.* Nature (2022)

Melcrova *et al.* bioRxiv (2023)





Bacterial transporters:

Maity *et al.* PNAS (2022)

High Speed AFM studies of dynamics

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MOSBRI Molecular-Scale Biophysics Research Infrastructure

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ViruScan

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O-ChemS Out-of-equilibrium Chemical Systems

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