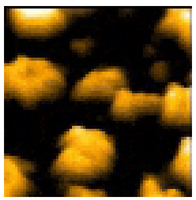
ESC7: Single molecule approaches

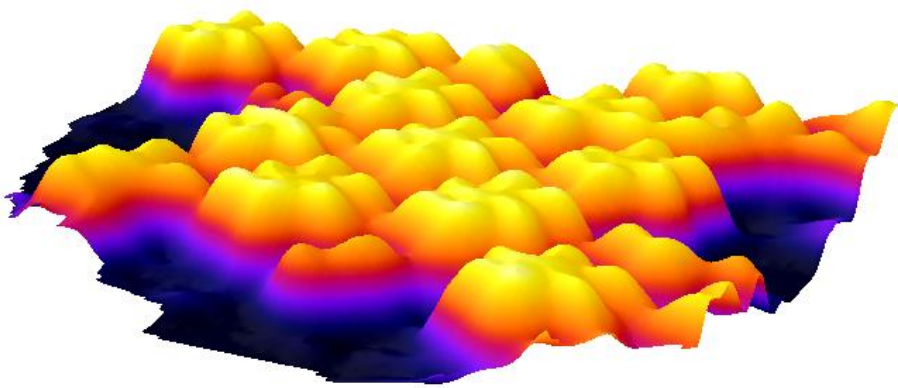
Atomic Force Microscopy and Optical Tweezers – Applications

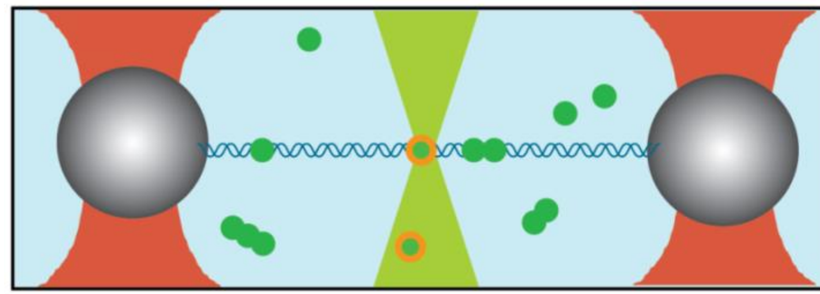
Wouter Roos

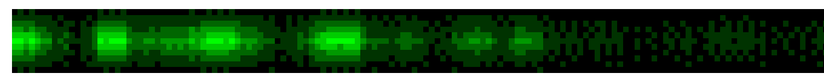
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

Atomic Force Microscopy and Optical Tweezers







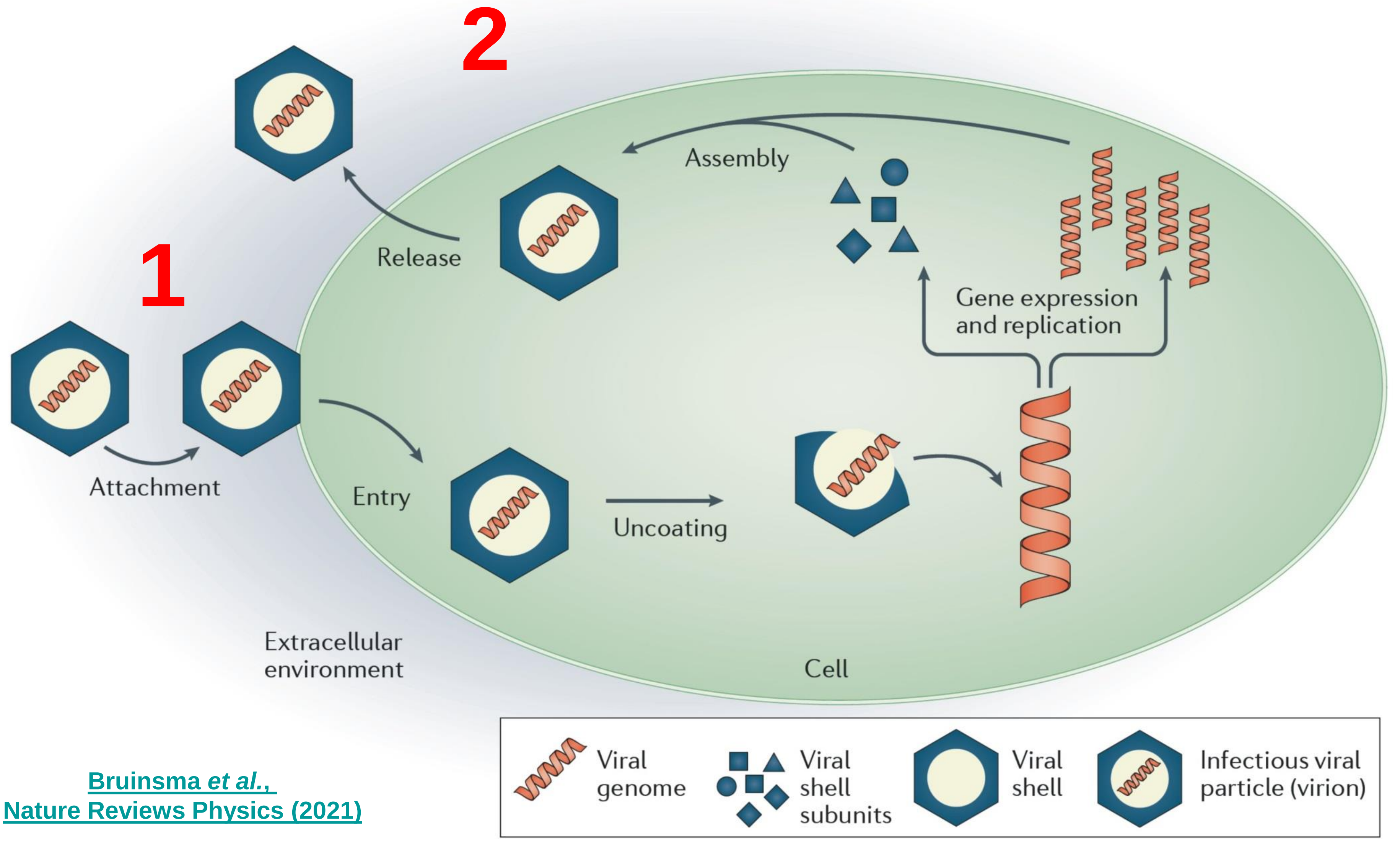


Wouter Roos

rijksuniversiteit groningen

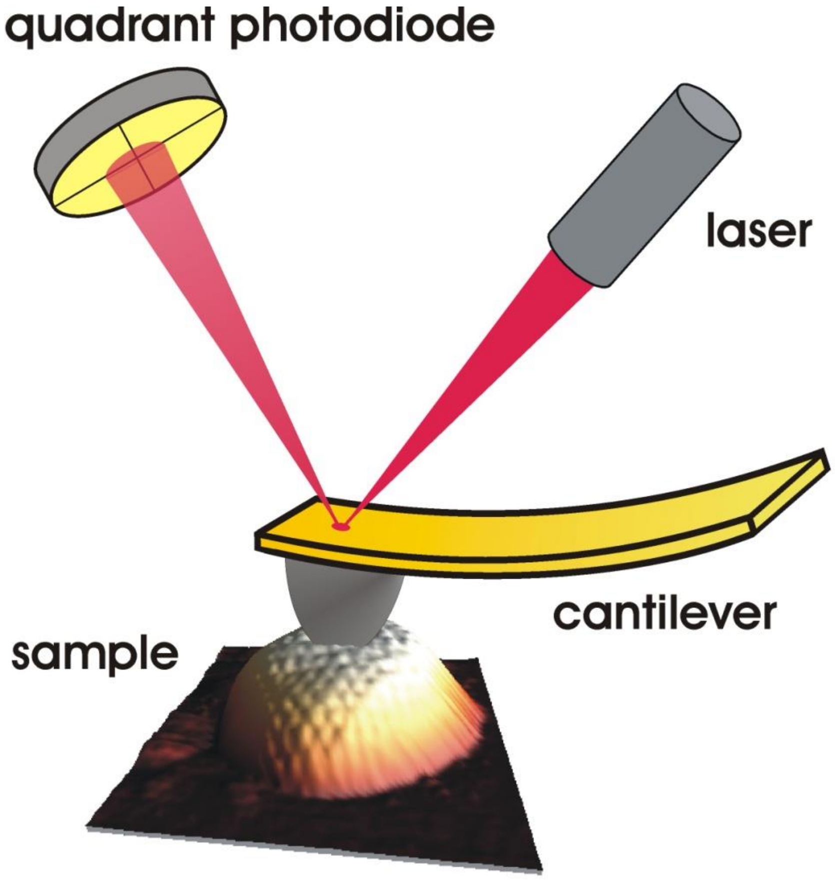
zernike institute for advanced materials

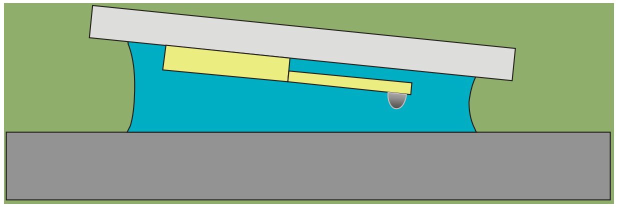
Viral life cycle



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

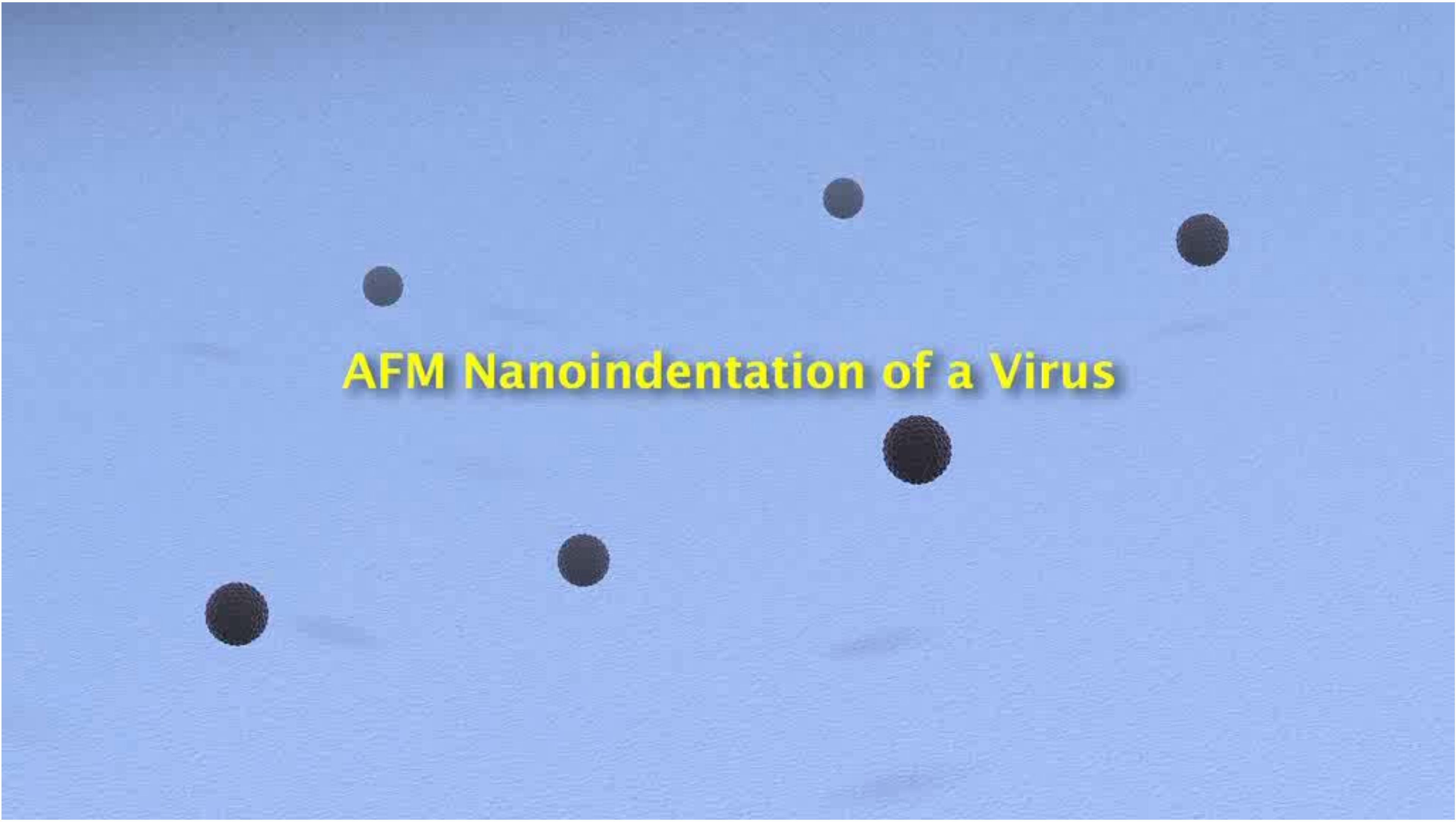
Atomic Force Microscopy





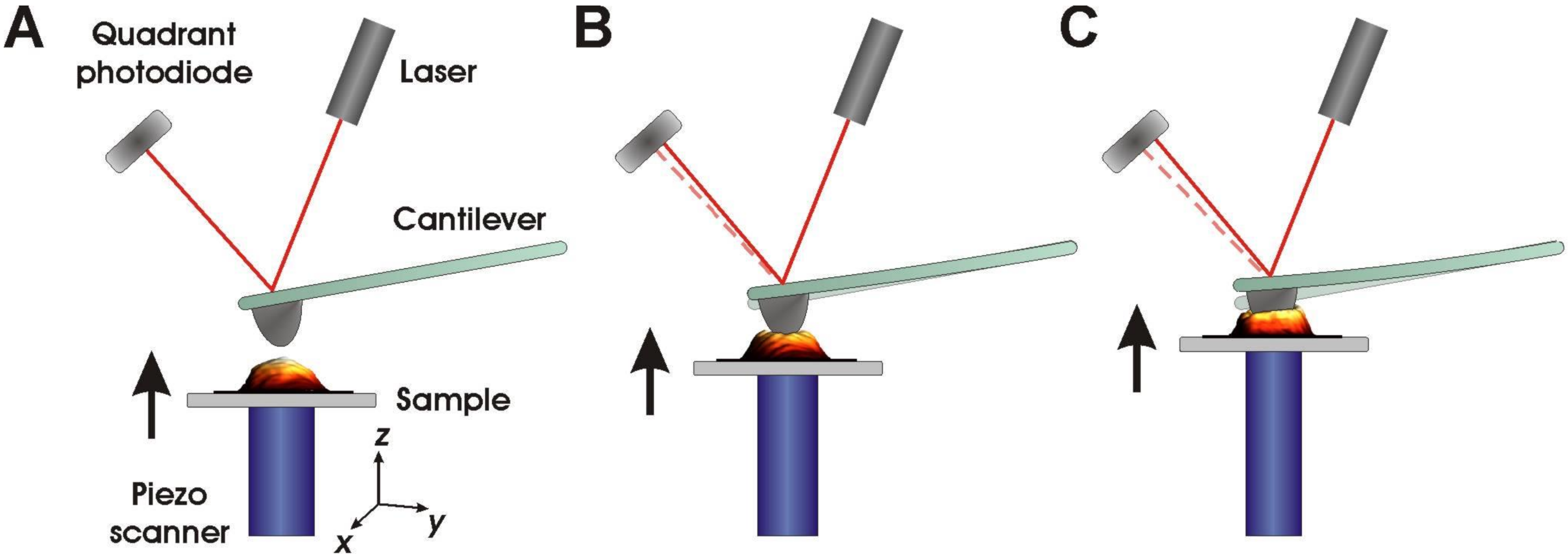
AFM:

- nm resolution
- force measurements
- dynamic measurements
- measurements in liquid

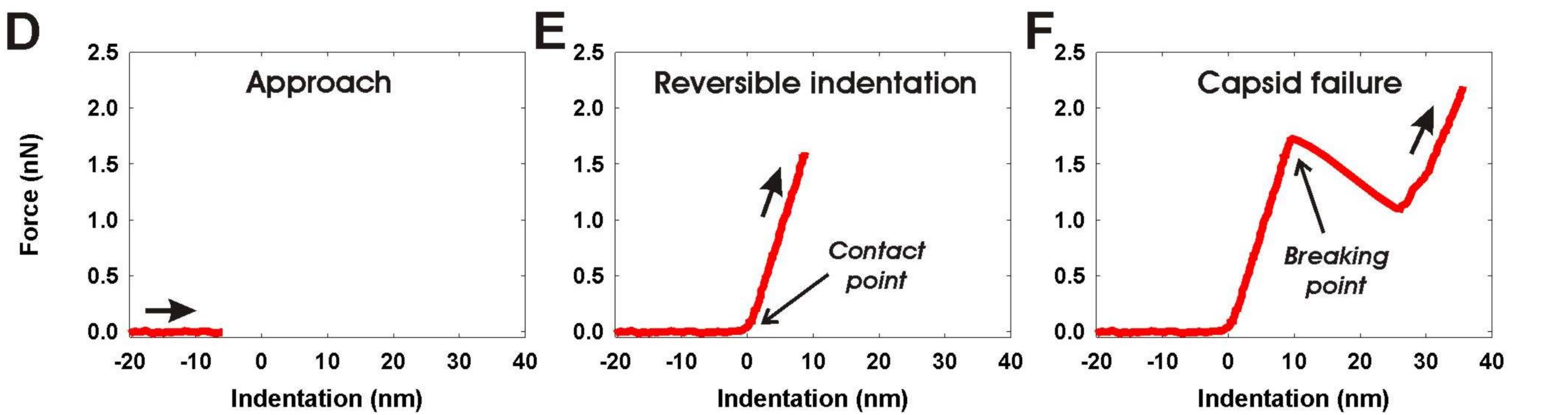


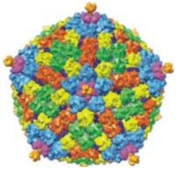
https://www.rug.nl/research/zernike/molecular-biophysics/roos-group/

Atomic force microscopy-based mechanobiology



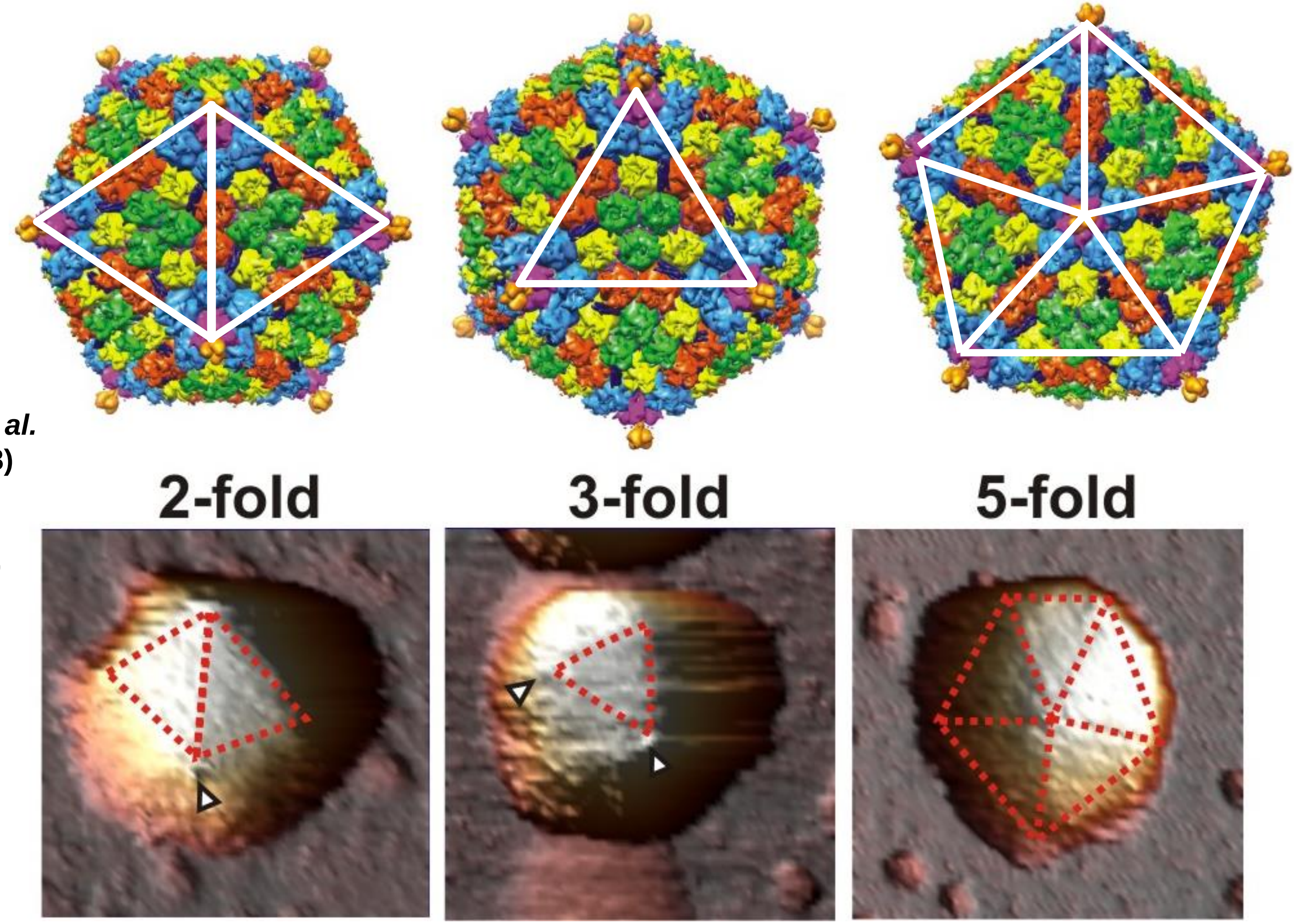
Roos *et al.*, Nature Physics 2010
Krieg *et al.*, Nature Reviews Physics 2019





Linking virus mechanics to infectivity

Imaging Adenovirus along its principle symmetry axes

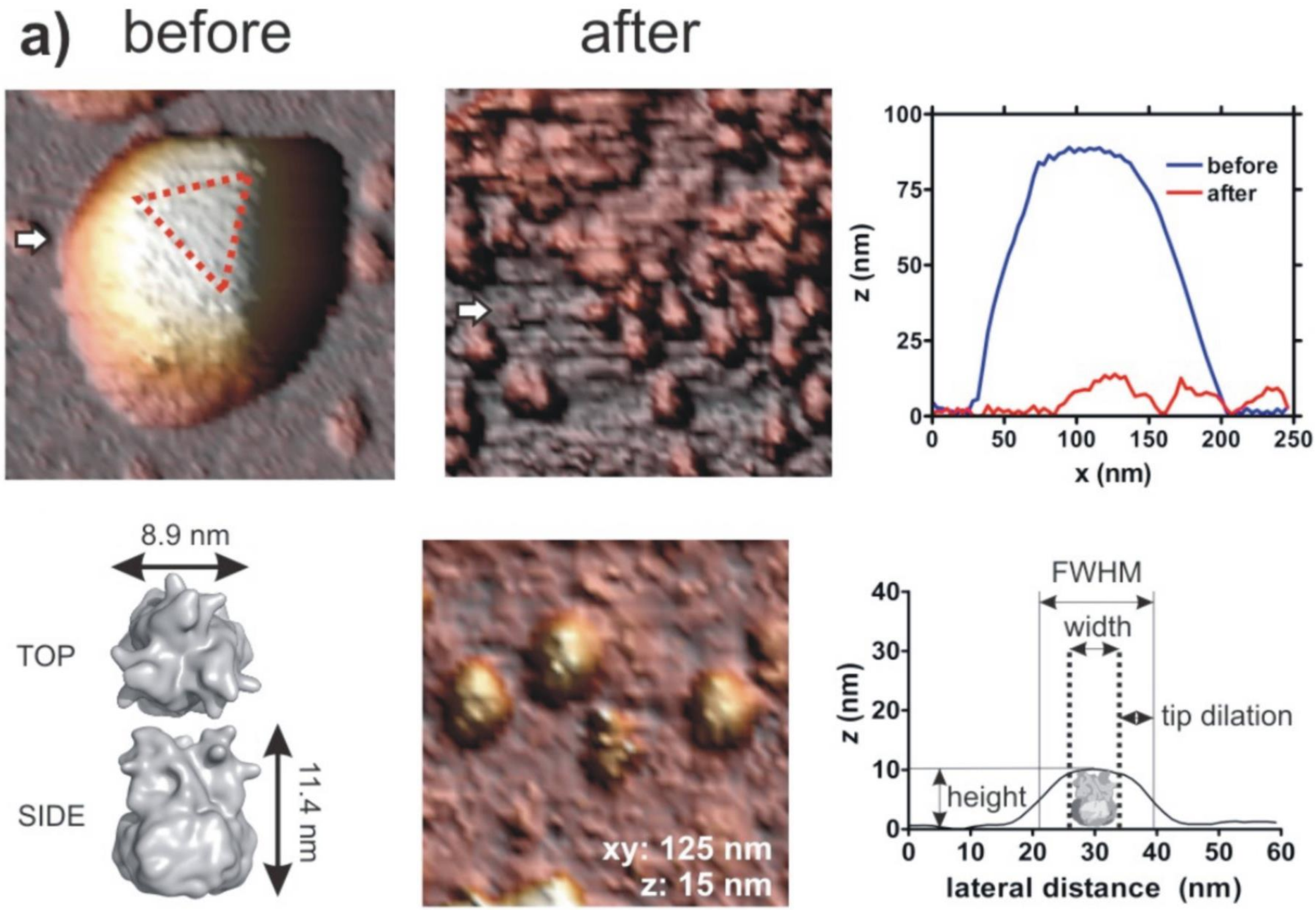


Snijder *et al.*
J Virol (2013)

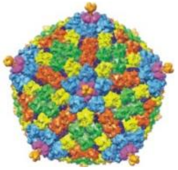
Van Rosmalen *et al.*
J Biol Phys (2018)

Denning *et al.*
Nanoscale (2019)

Mechanical probing of Adenovirus



240
hexons
in
1 AdV

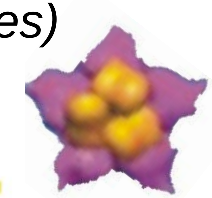


Host factors, mechanics & infectivity

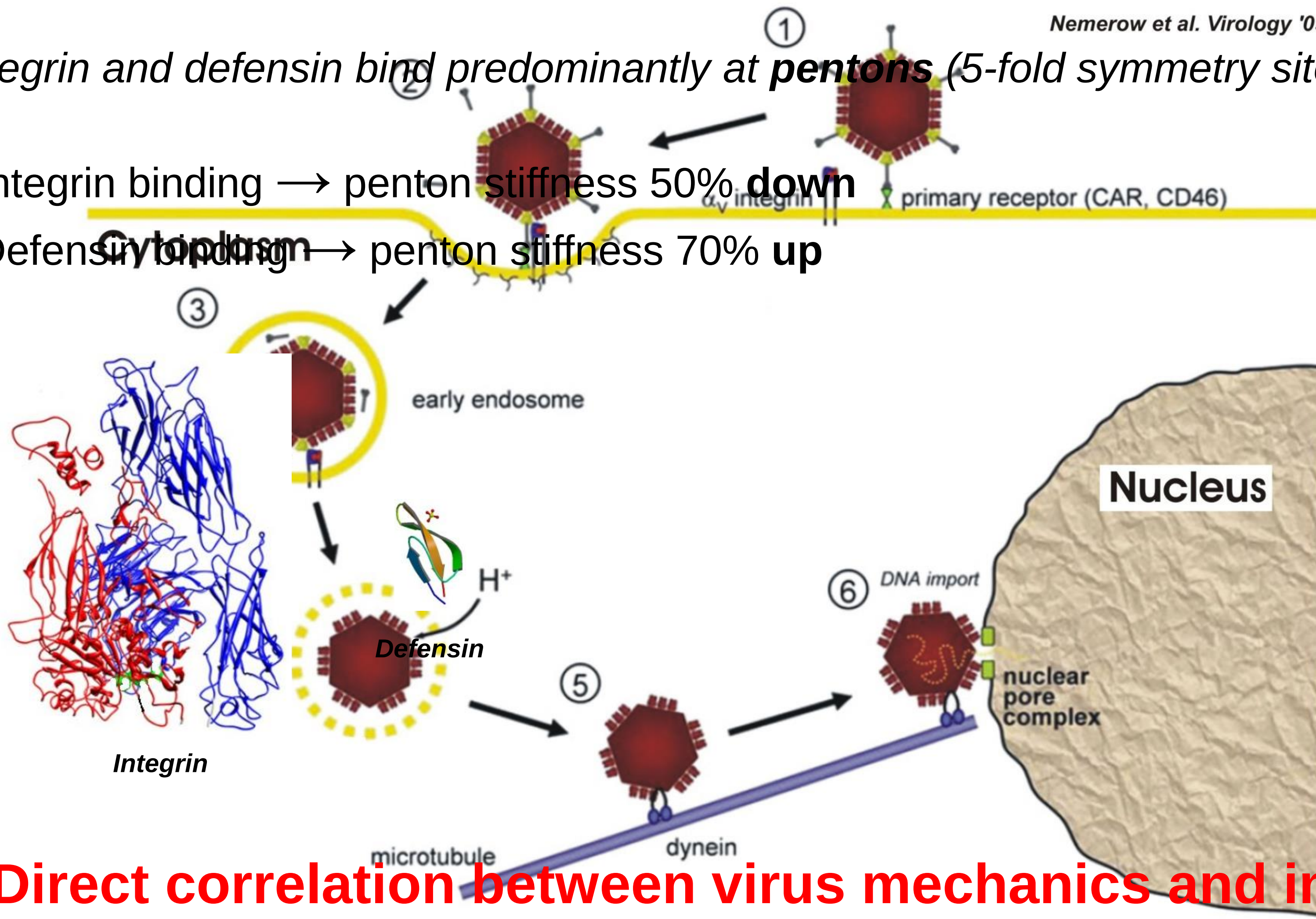
- Integrin αvβ5 is cell surface receptor for adenovirus: promotes DNA uncoating
- Human alpha defensin (HD5, antimicrobial protein): blocks adenovirus infection

Nemerow et al. Virology '09

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

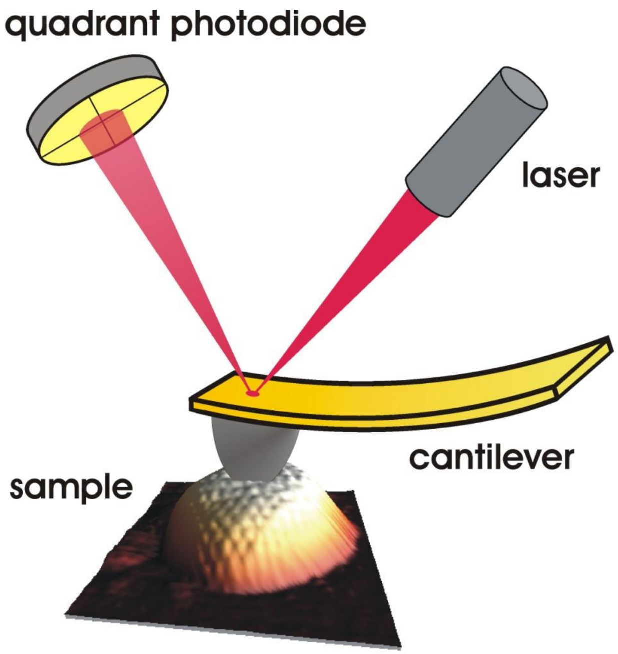


- Integrin binding ⟶ penton stiffness 50% **down**
- Defensin binding ⟶ penton stiffness 70% **up**



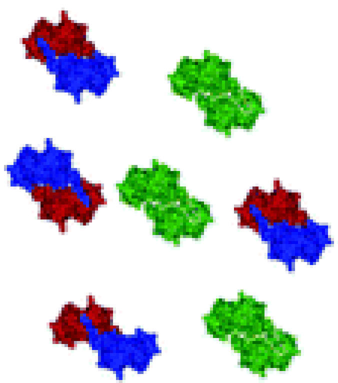
Direct correlation between virus mechanics and infectivity

Viral structure and assembly



Viral structure:
Increasingly better view on mechanical & atomic structure

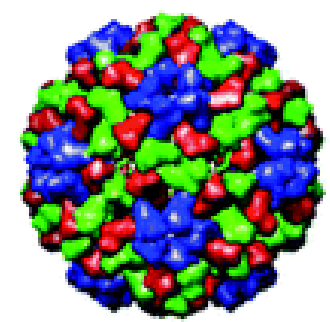
Nat Chem (2013), J Virol (2017), Nanoscale (2019), Viruses (2023)



Protein Dimers

? ? ? ? ? ?

Viral assembly:
Fascinating but still poorly understood self-organisation process

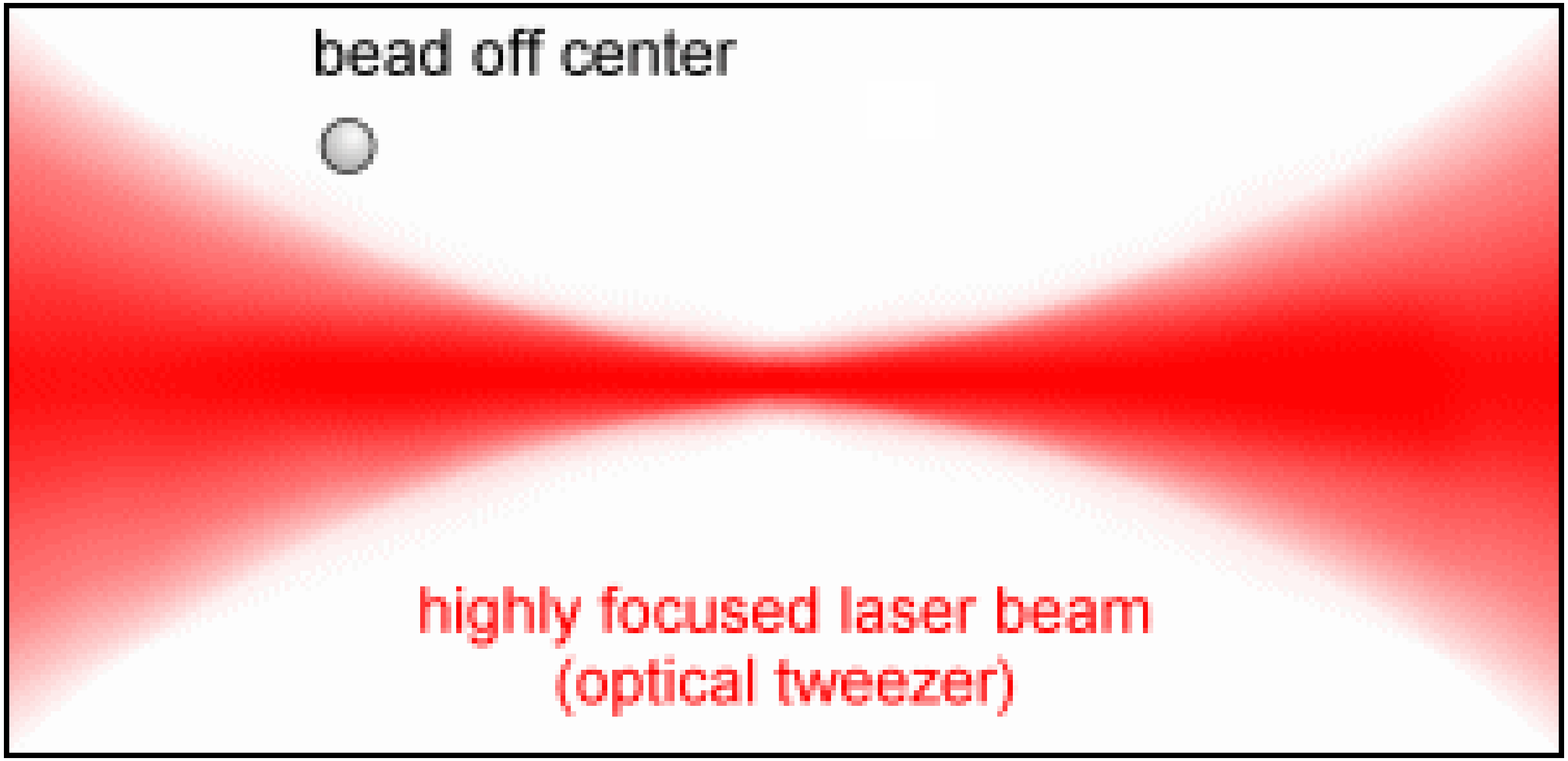


Virus Capsid

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

Optical Tweezers

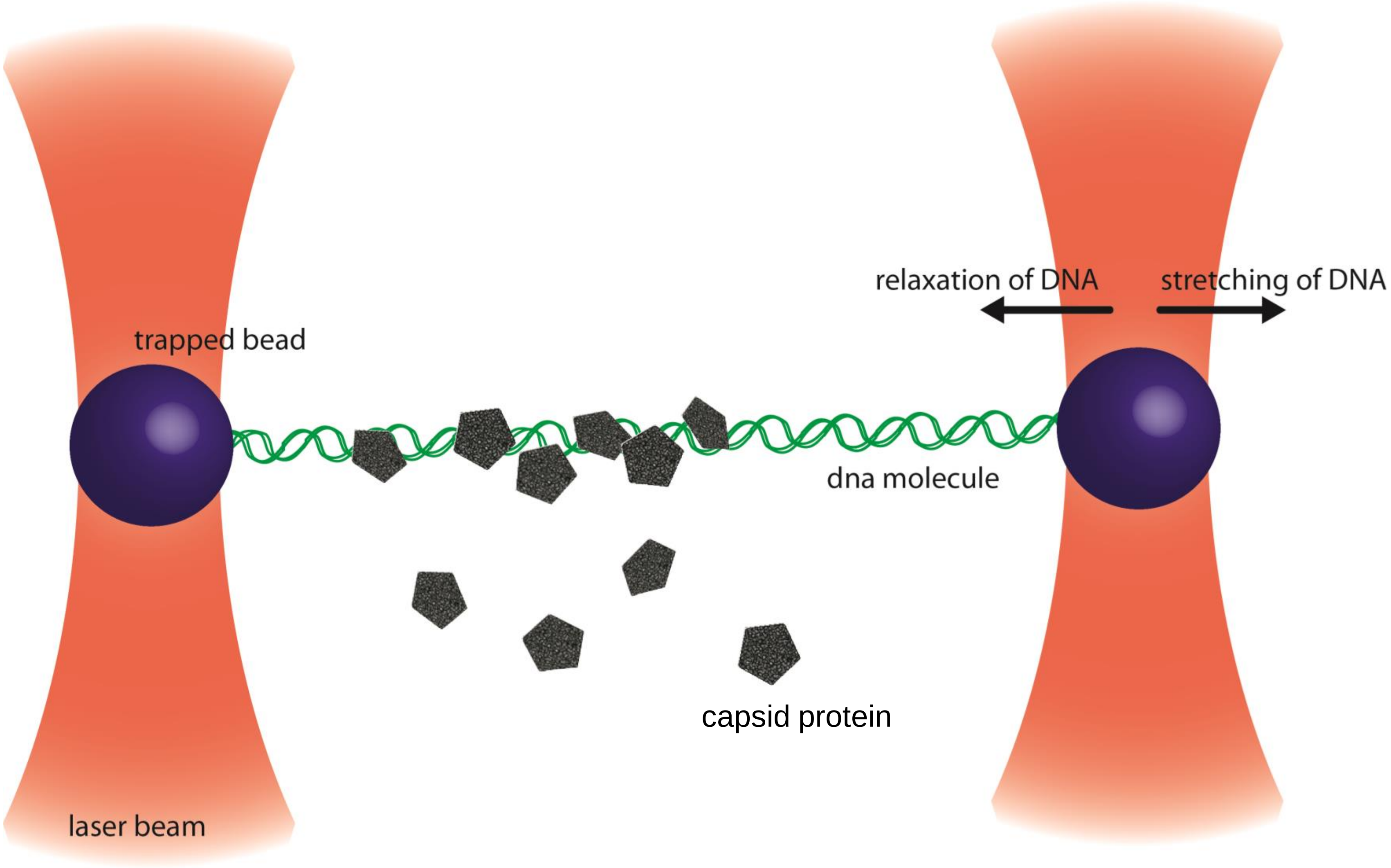
micron-sized particles can be stably trapped in the focus of a laser beam



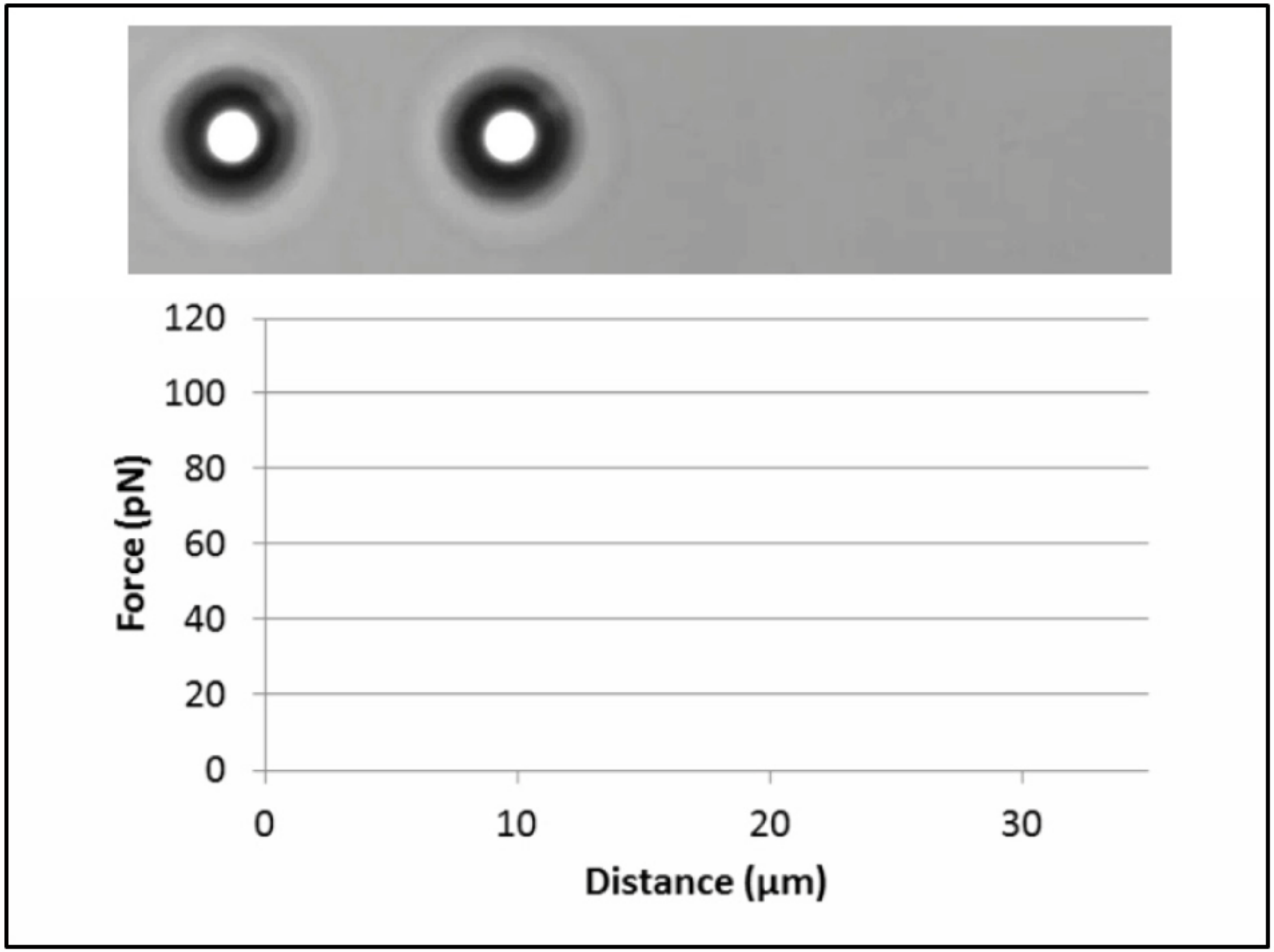
https://home.uni-leipzig.de/pwm/web/?section=introduction&page=opticaltraps

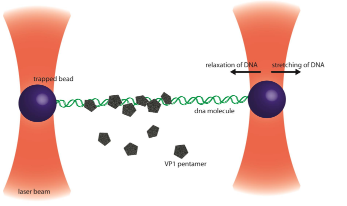
1986 Arthur Ashkin (Bell labs) ***Nobel Prize Physics 2018***

Following virus assembly by optical tweezers



Following assembly by optical tweezers

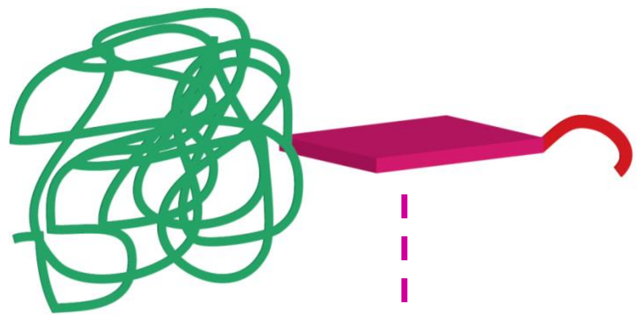




Synthetic Virus-Like-Particles

Artificial
virus-like particles are
controlled systems

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



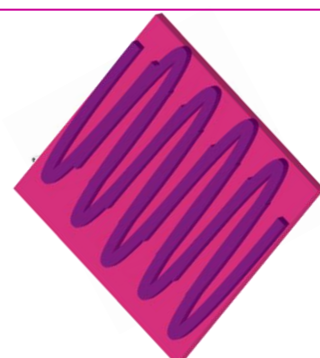
Biosynthetic coat protein: polypeptide

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

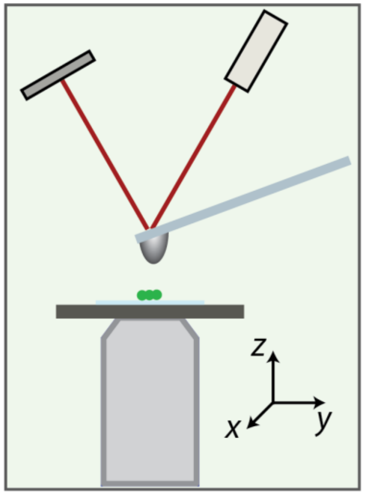
Folds and stacks in solution into stiff filamentous structures.
Precisely tunes cooperativity:
Silk-like sequence S_{10} = $(GAGAGAGQ)_{10}$

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

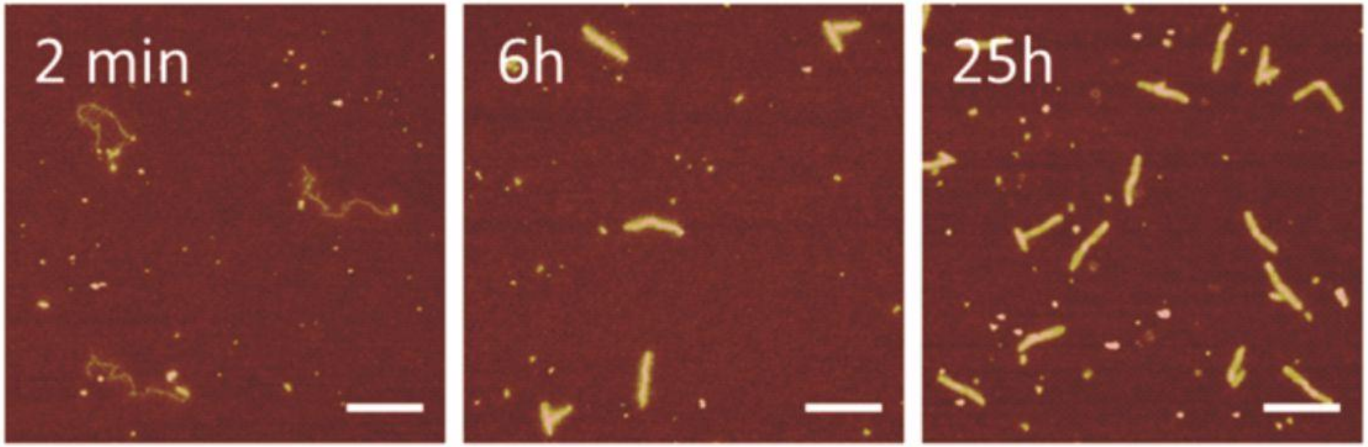


De Vries Lab:
A. H. Garcia et al., *Nature Nanotechnology* (2014)
B. Zhao et al., *Soft Matter* (2016)

Particle visualisation by AFM



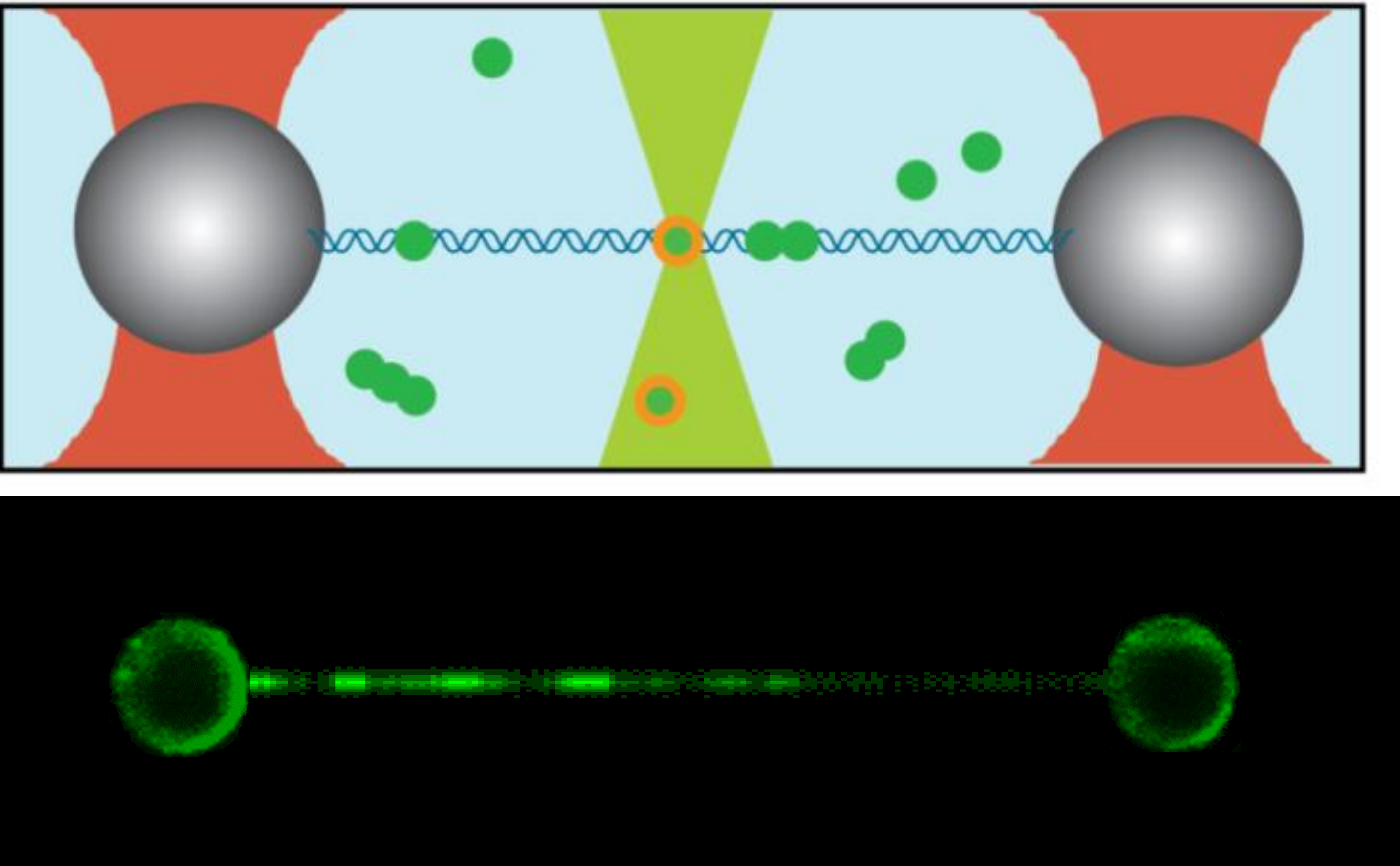
DNA 2.5 kbp ~ 1 μm



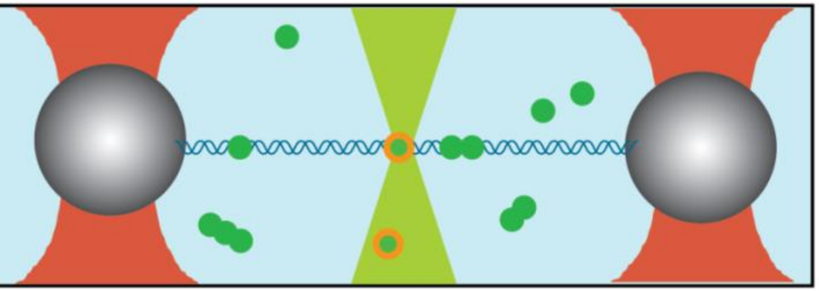
350 nm

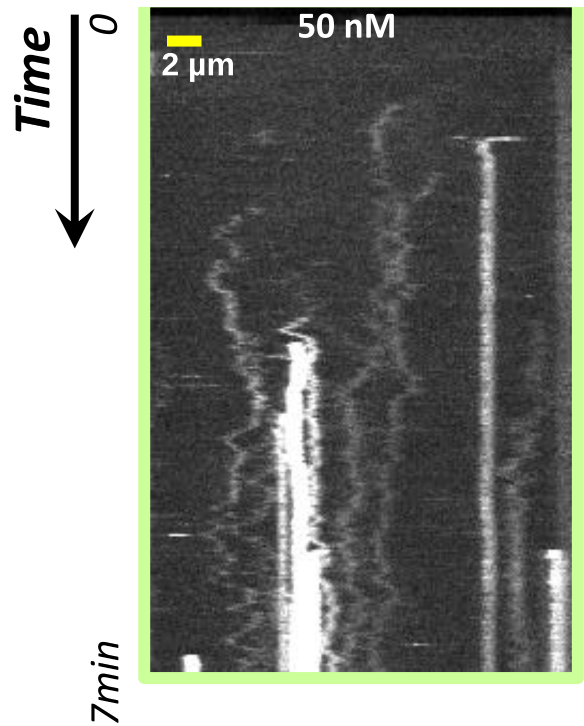
- Stiff rod-like appearance of the VLPs (300nm)
- DNA compacted 1/3 its original length
- Cooperative self-assembly.

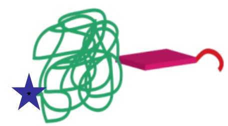
Protein attachment, in real-time

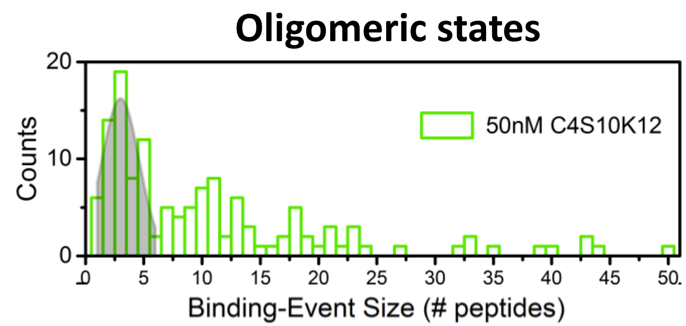


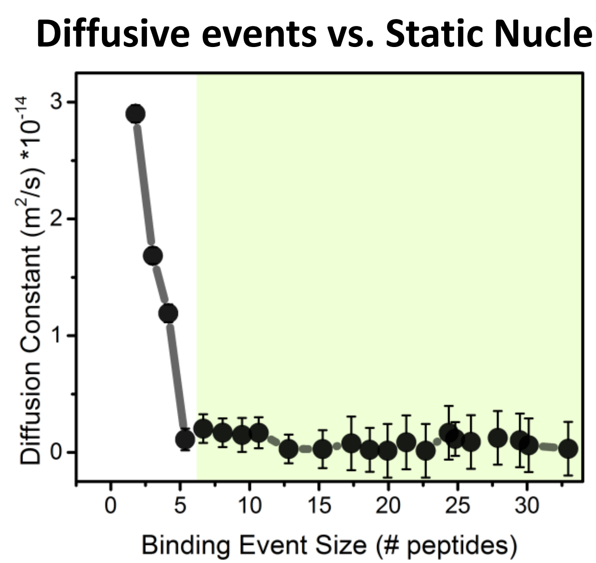
Protein attachment, in real-time



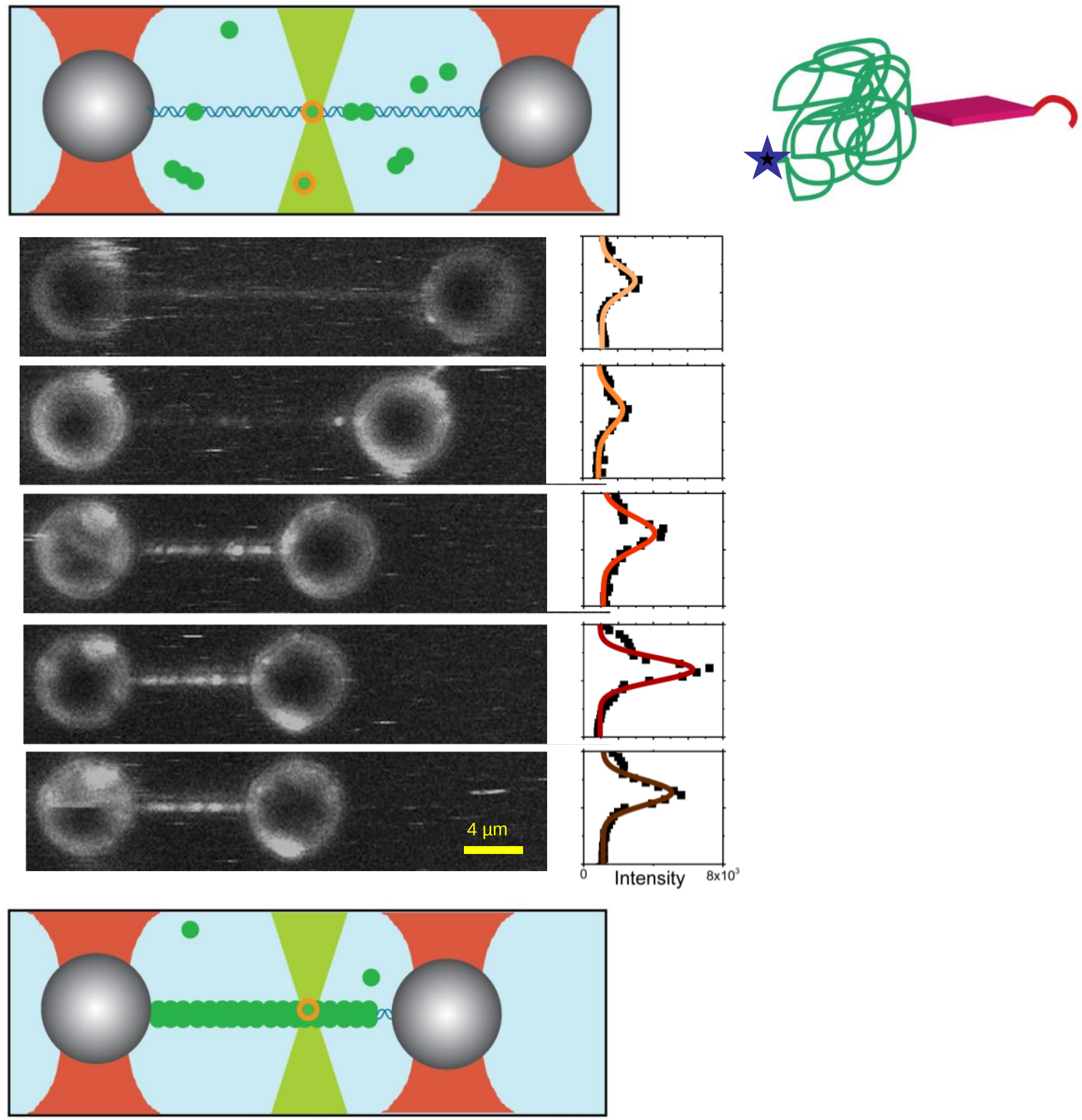






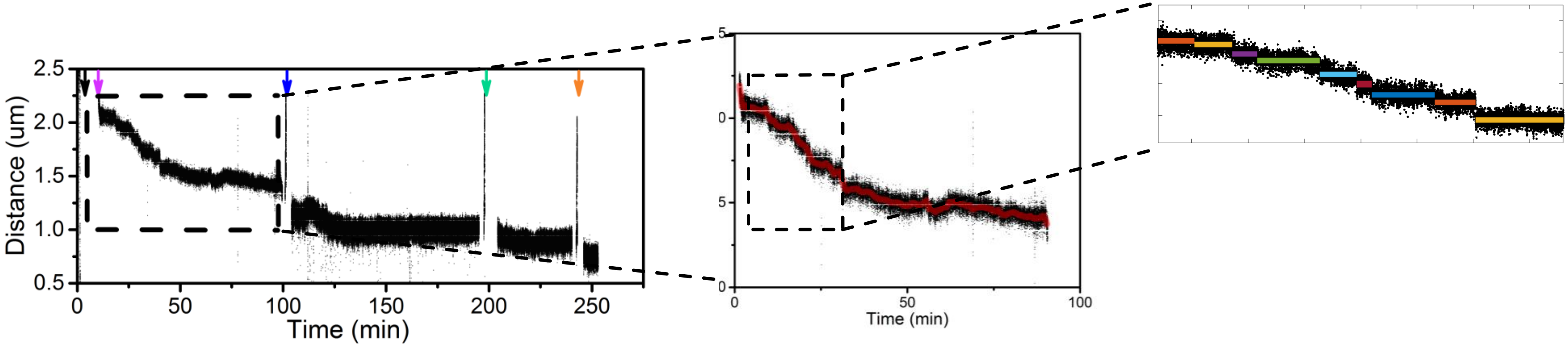


DNA compaction by rod shaped VLP

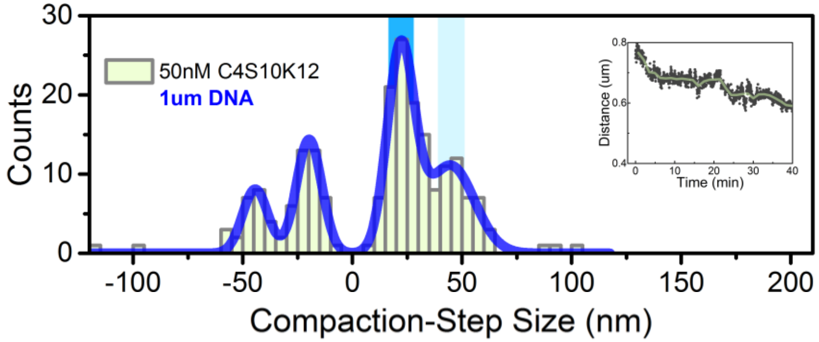


[Marchetti *et al.*
Nano Letters (2019)]

DNA compaction in real-time

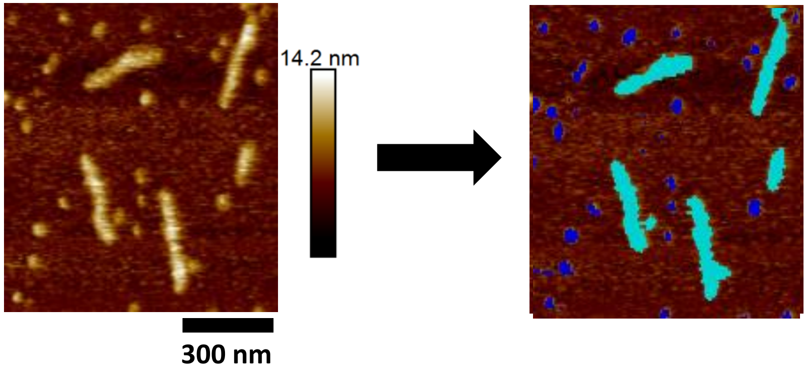


Regular compaction steps for assembly model



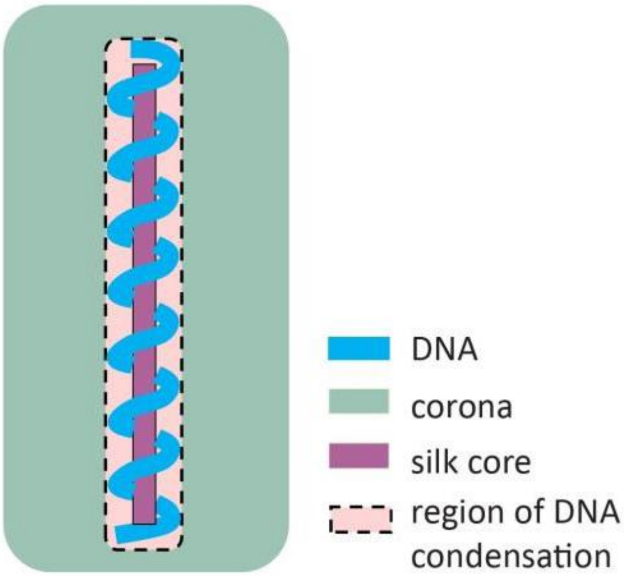
Average Compaction-Step:

~ 30 nm

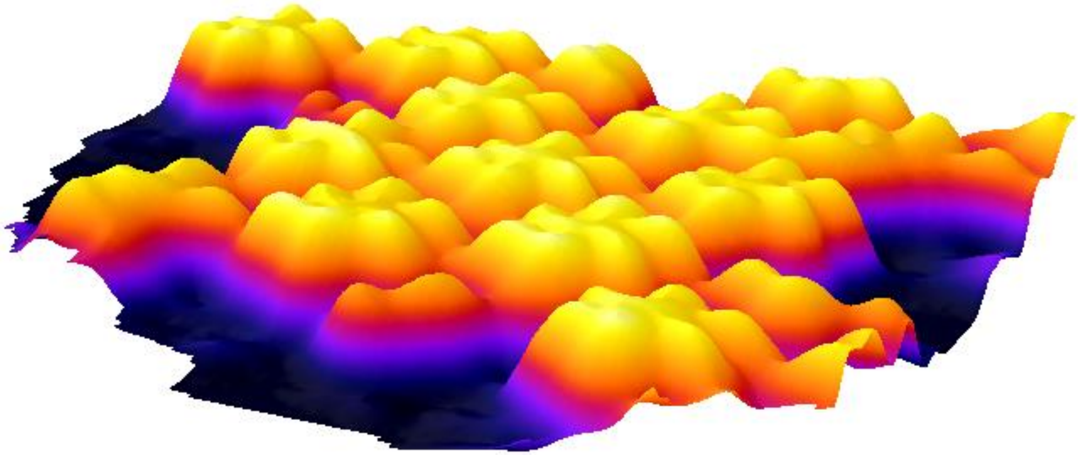


Mean particle HEIGHT:

9.1 ±0.5 nm



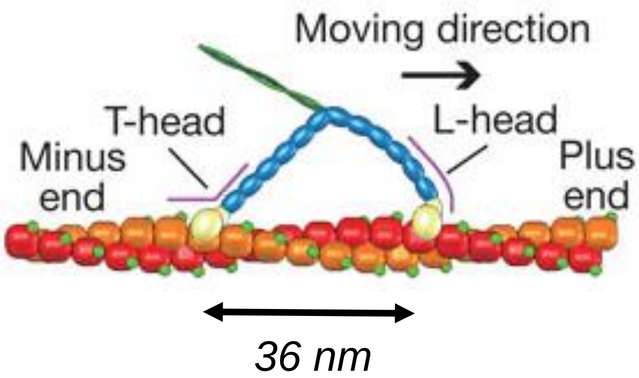
High Speed AFM studies of dynamics



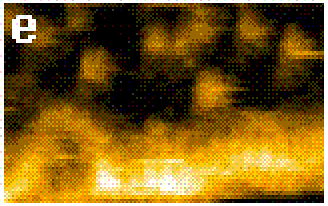
<u>Are we able to follow assembly in real-time?</u>

High speed AFM

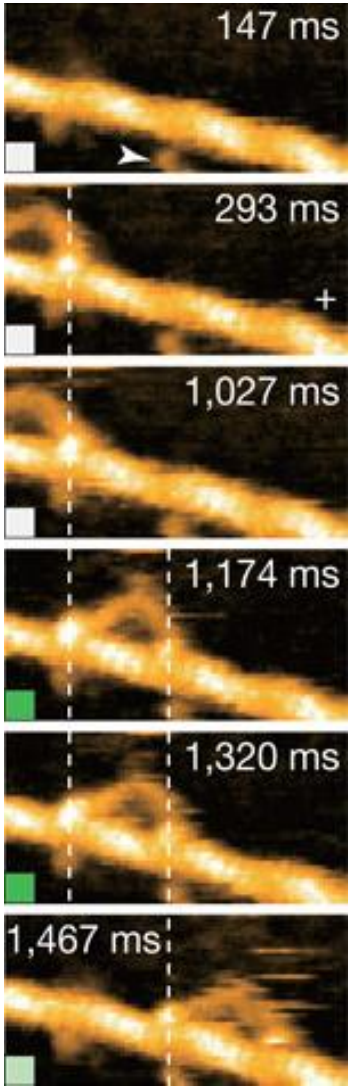
Toshio Ando
Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University



Myosin molecular motor on actin filaments



7 frames s^{-1}



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

Kodera,…,Ando, Nature (2010)

High Speed AFM studies of 2D HIV assembly

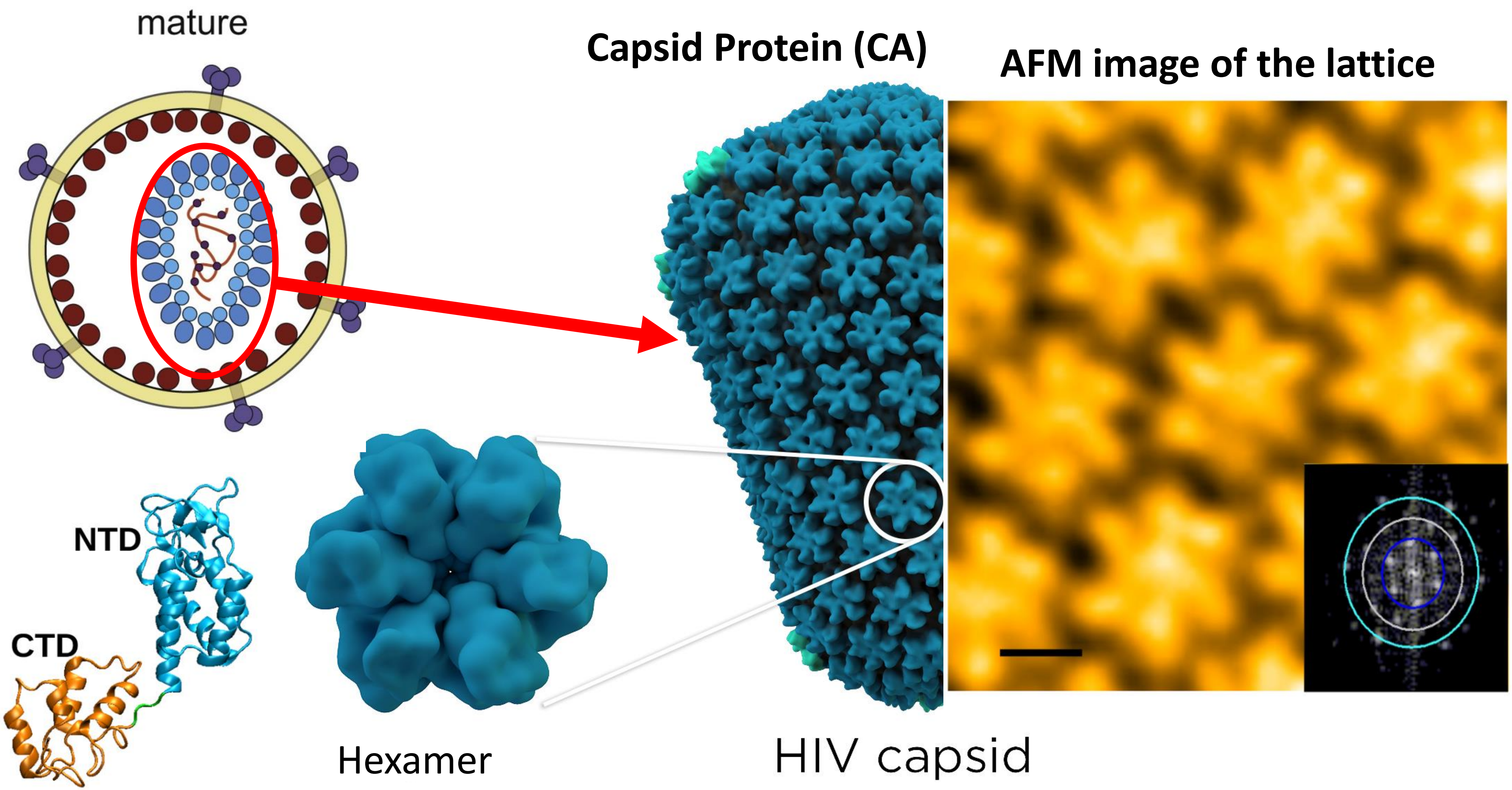
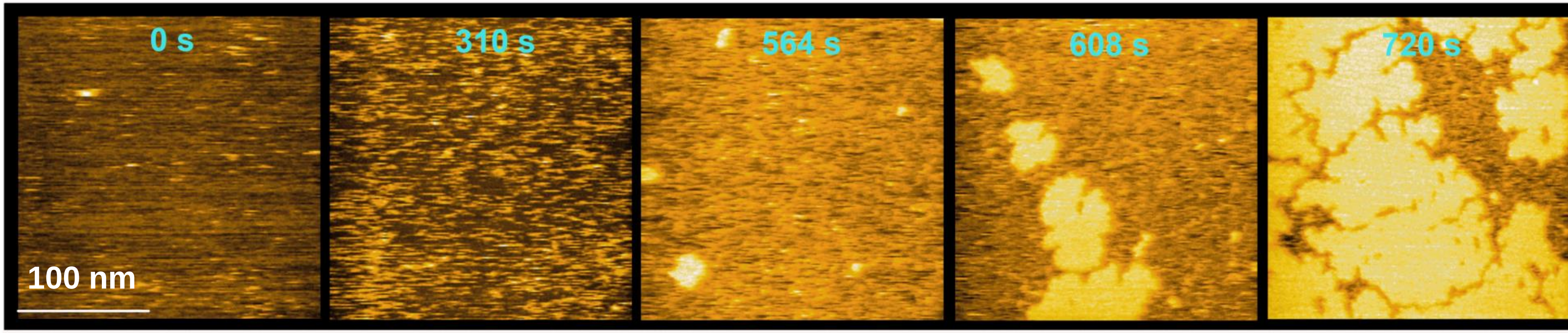
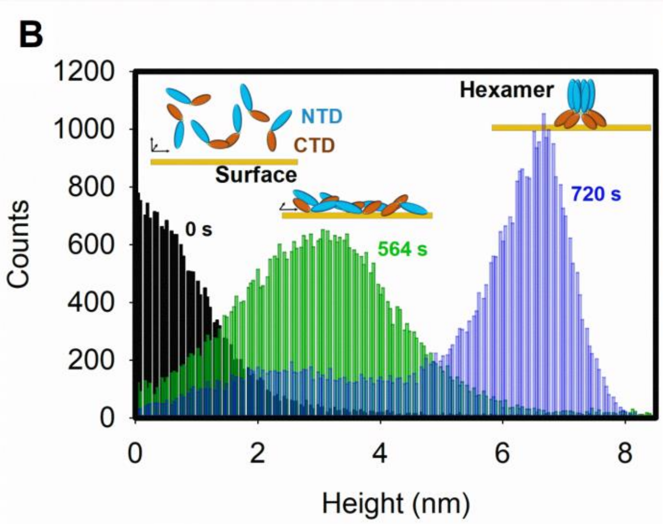
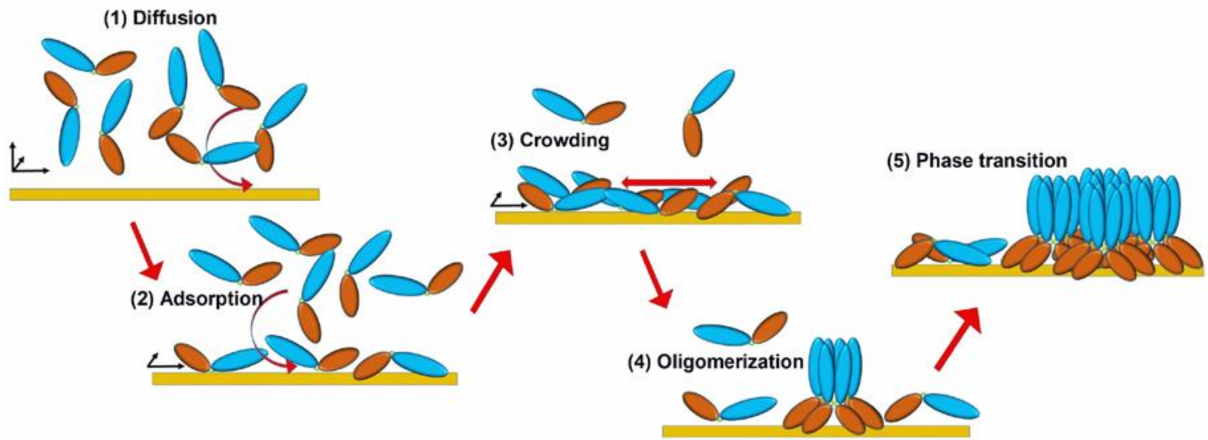


Figure credits: Monroe et al., ***Structure***, 2010 and Thomas Splettstoesser

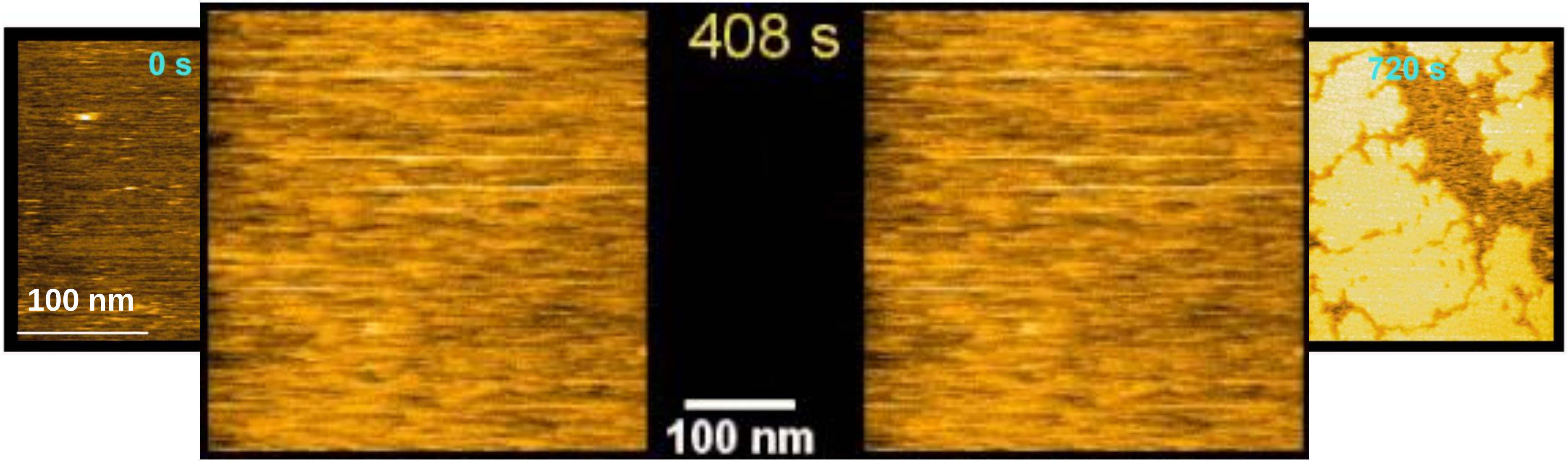
High Speed AFM studies of 2D HIV assembly

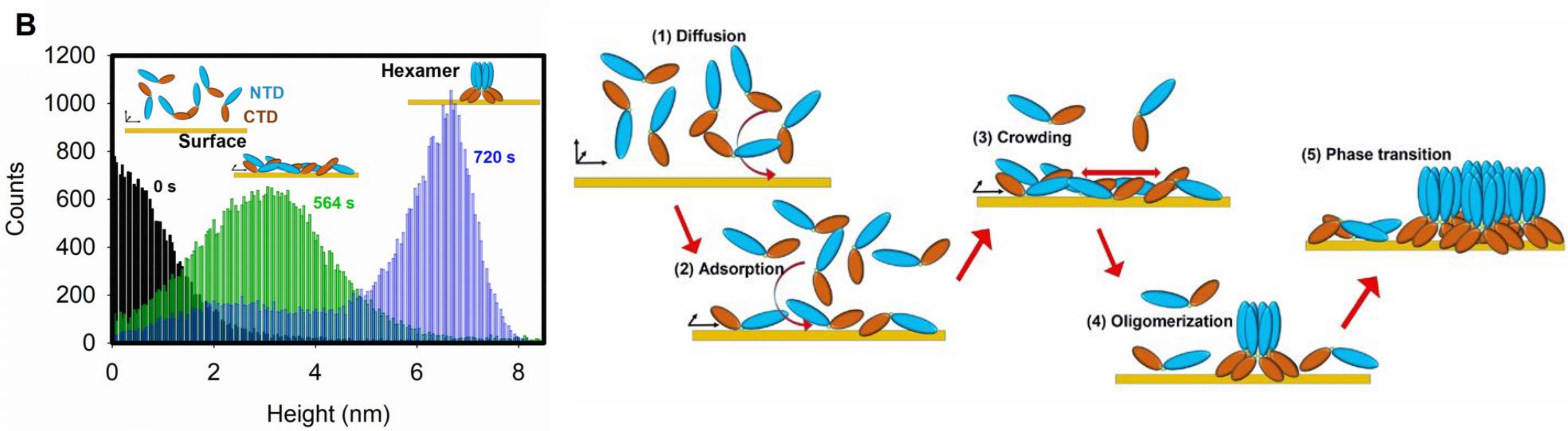






High Speed AFM studies of 2D HIV assembly





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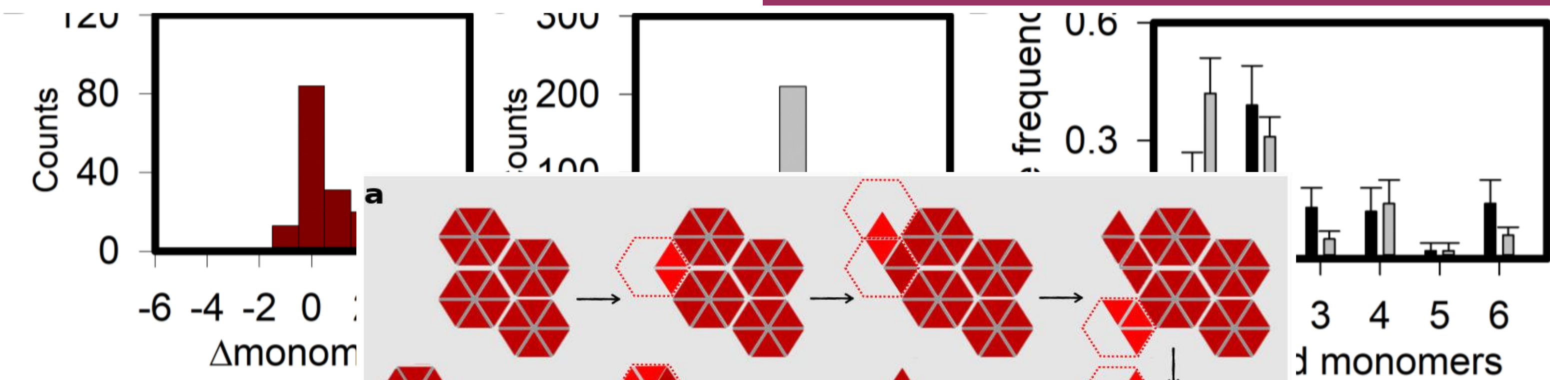
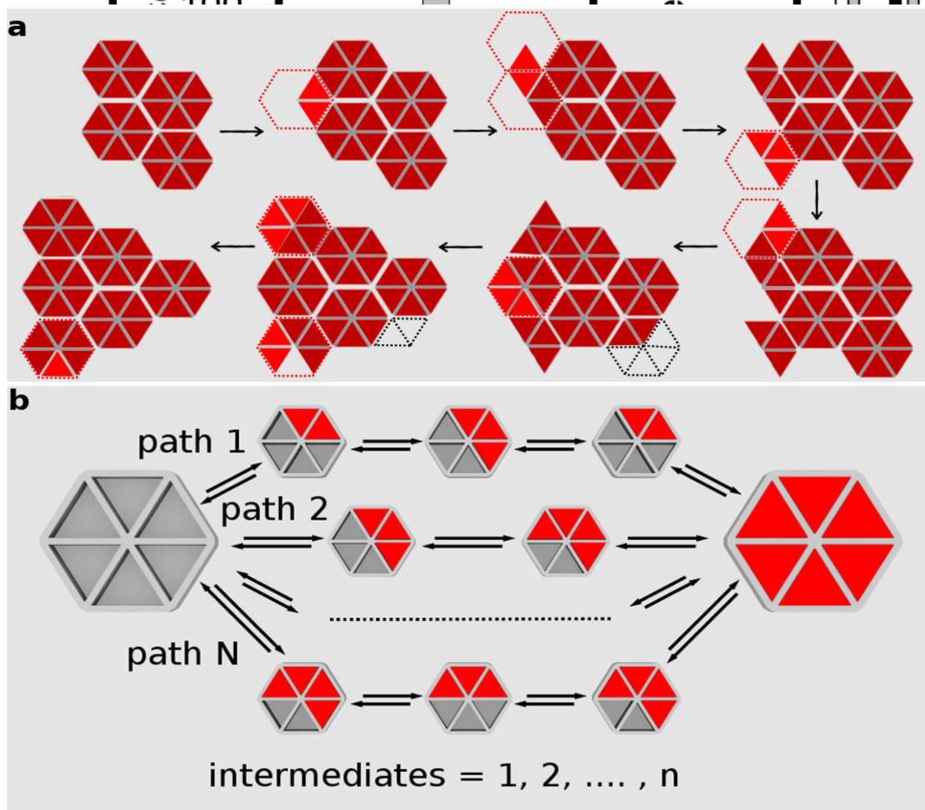
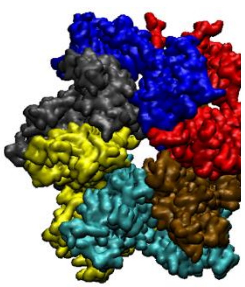
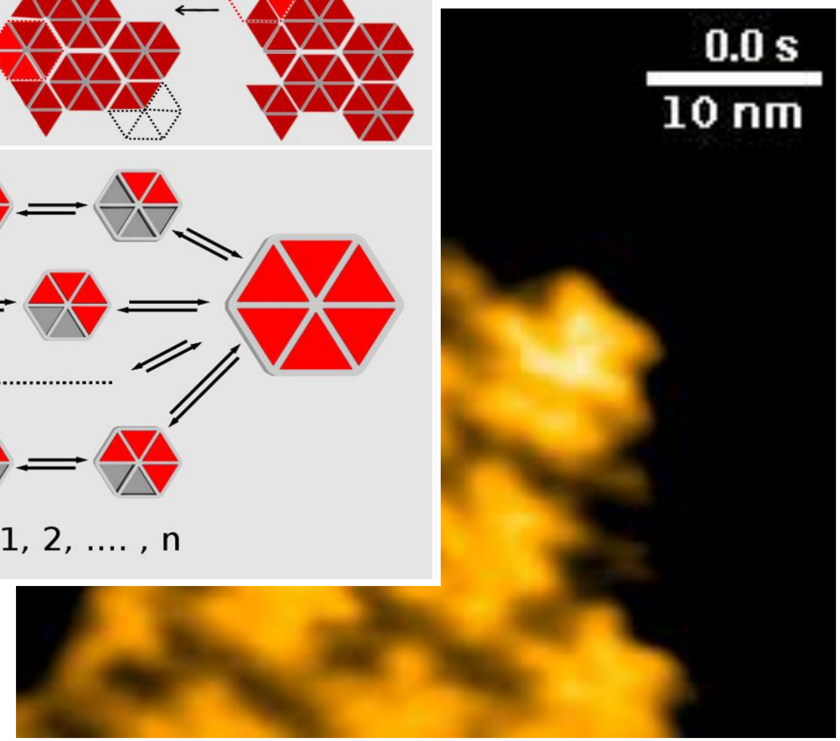


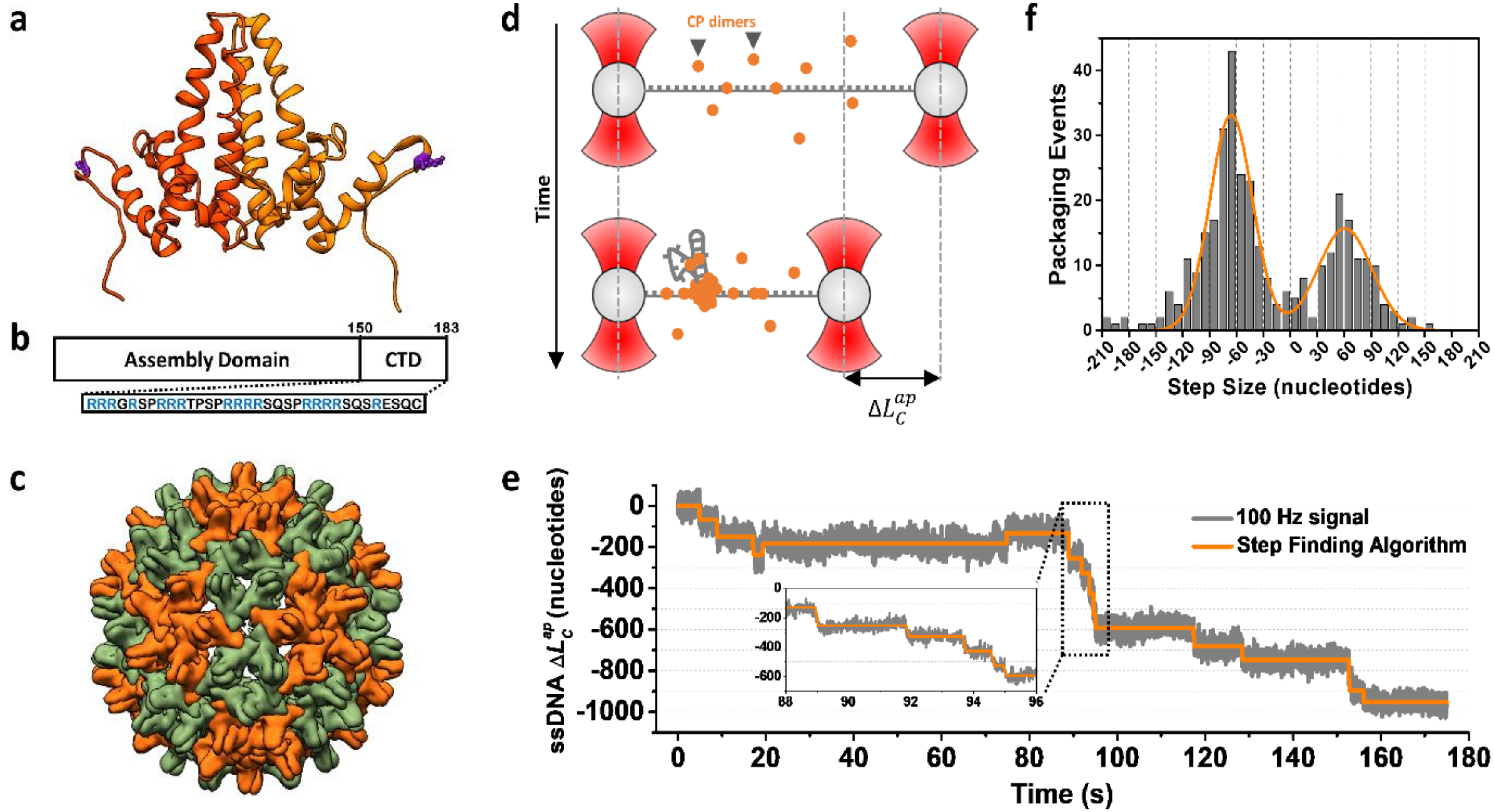


[Valbuena *et al.* ACS Nano (2020)]

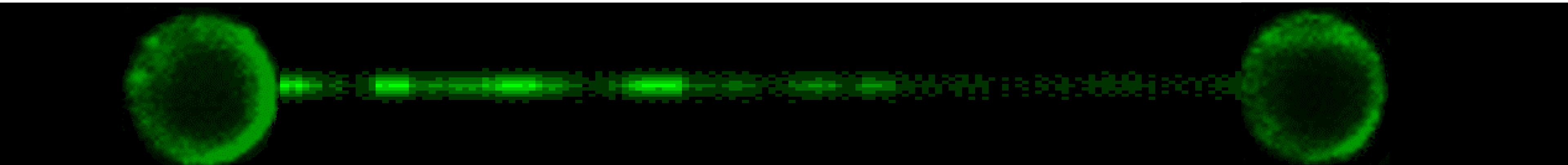




Hepatitis B Virus assembly

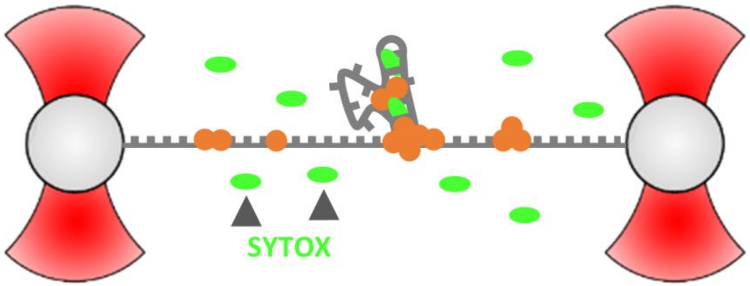


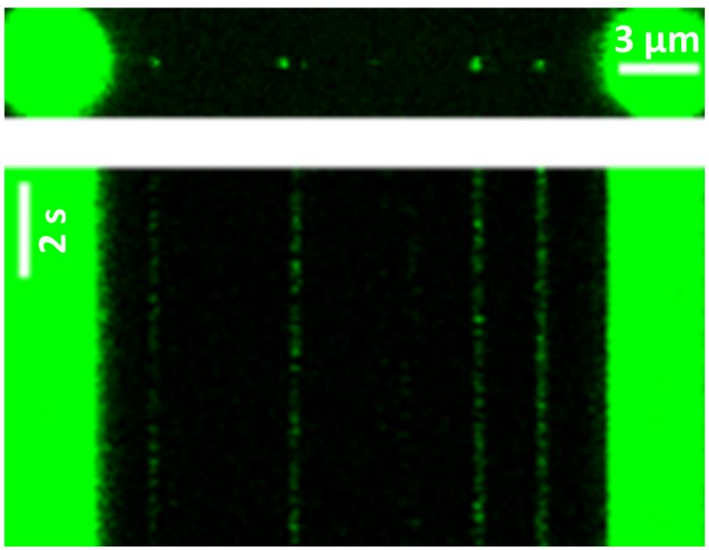
[Buzon *et al.* Science Advances 2021]

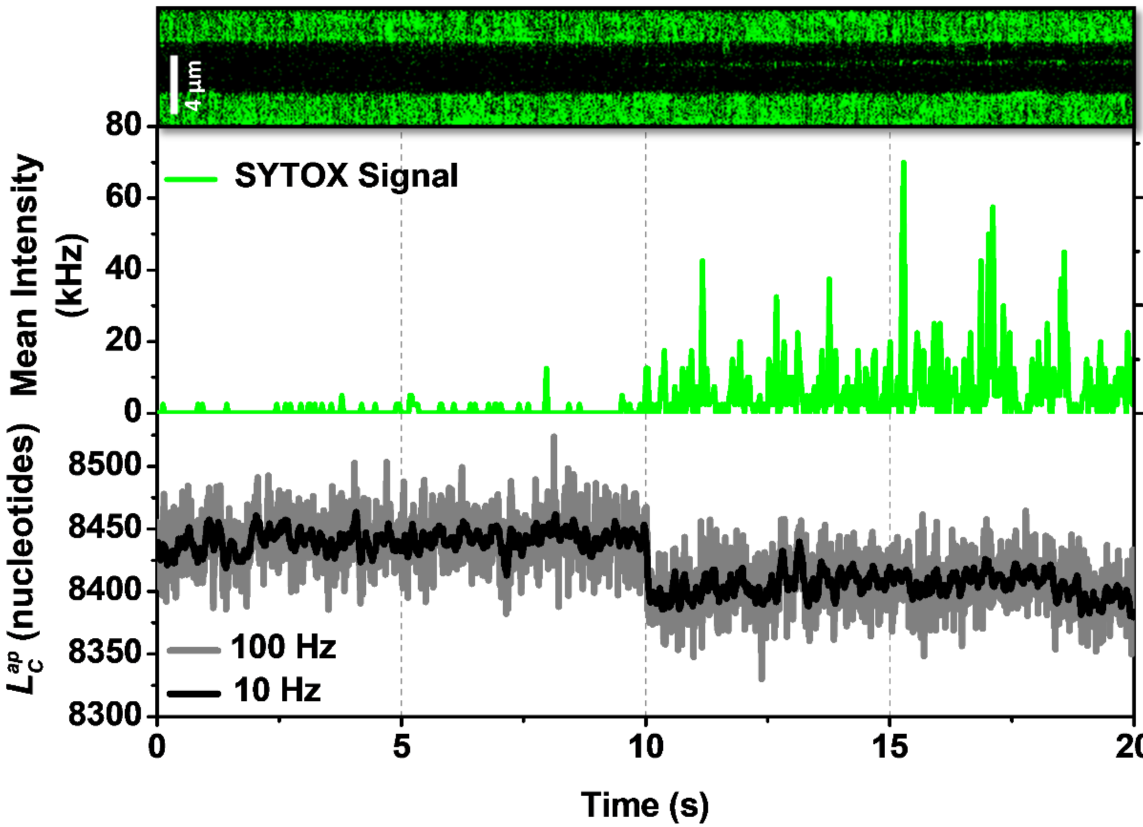


Hepatitis B Virus assembly

Cp has the capability to chaperone the formation of dsDNA







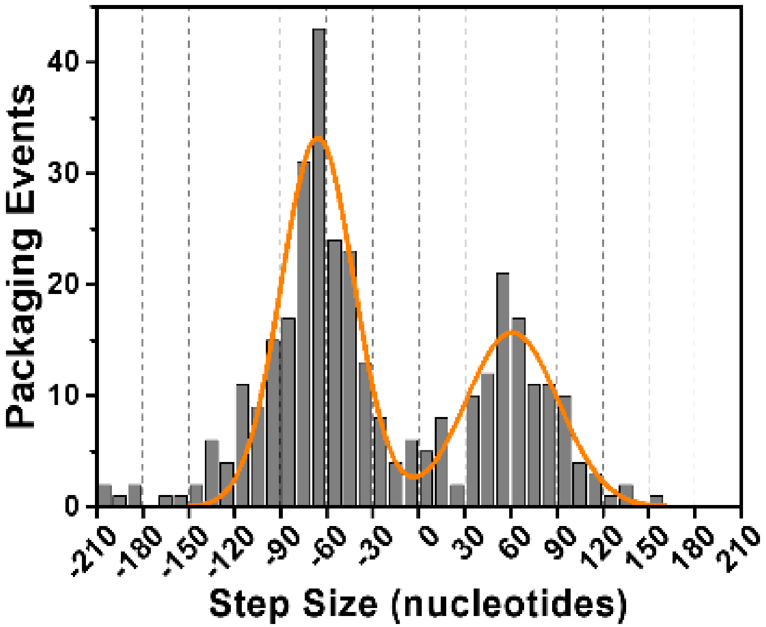
Hepatitis B Virus assembly

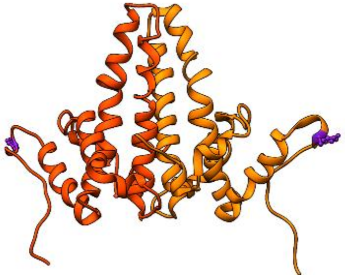
Cp condenses nucleic acids, even under tension (F = 11 pN)

Assembly footprint: ~70 nt

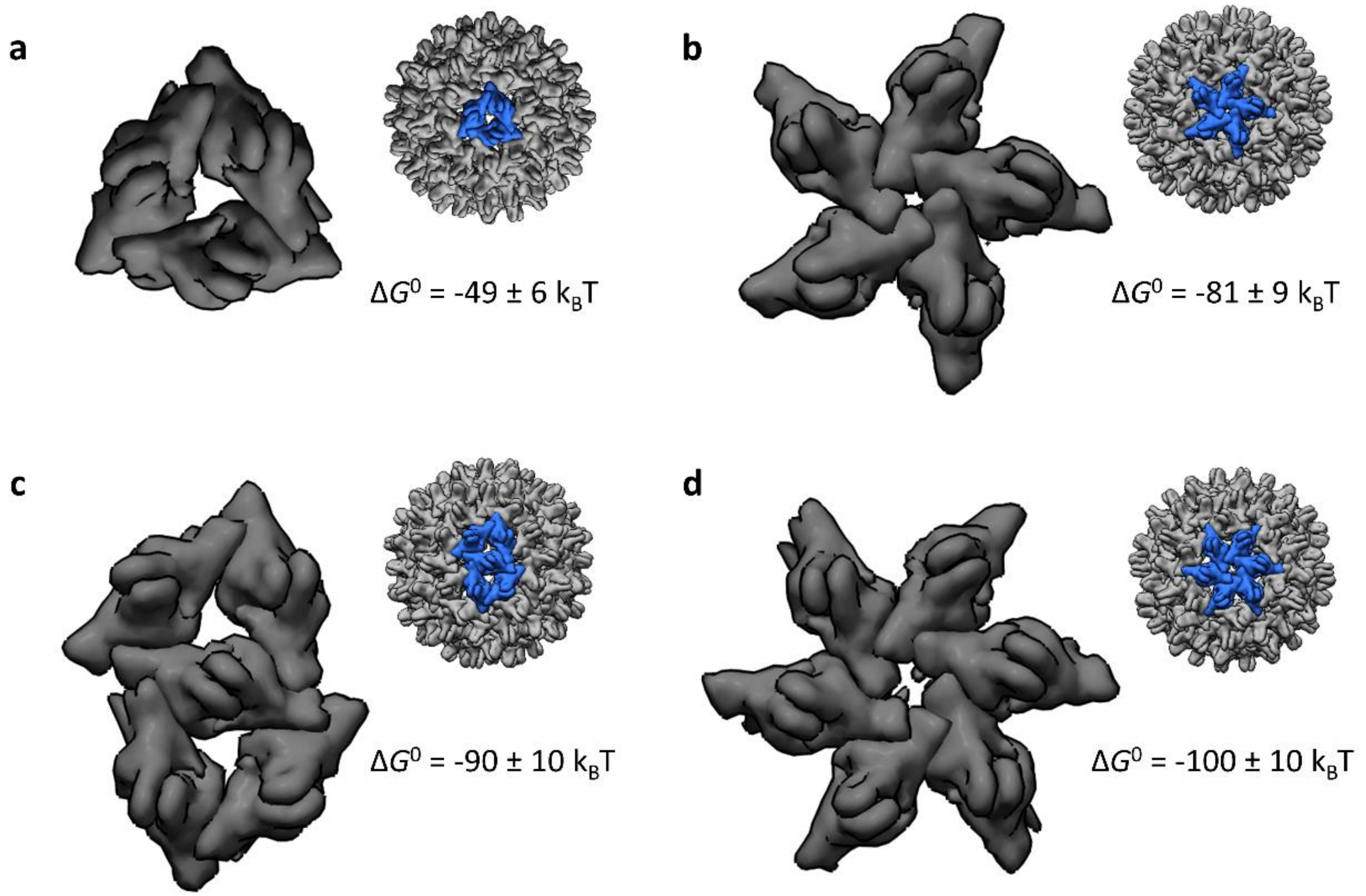
Work per condensation step:
F* *step size* = ~ 100 k_BT

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

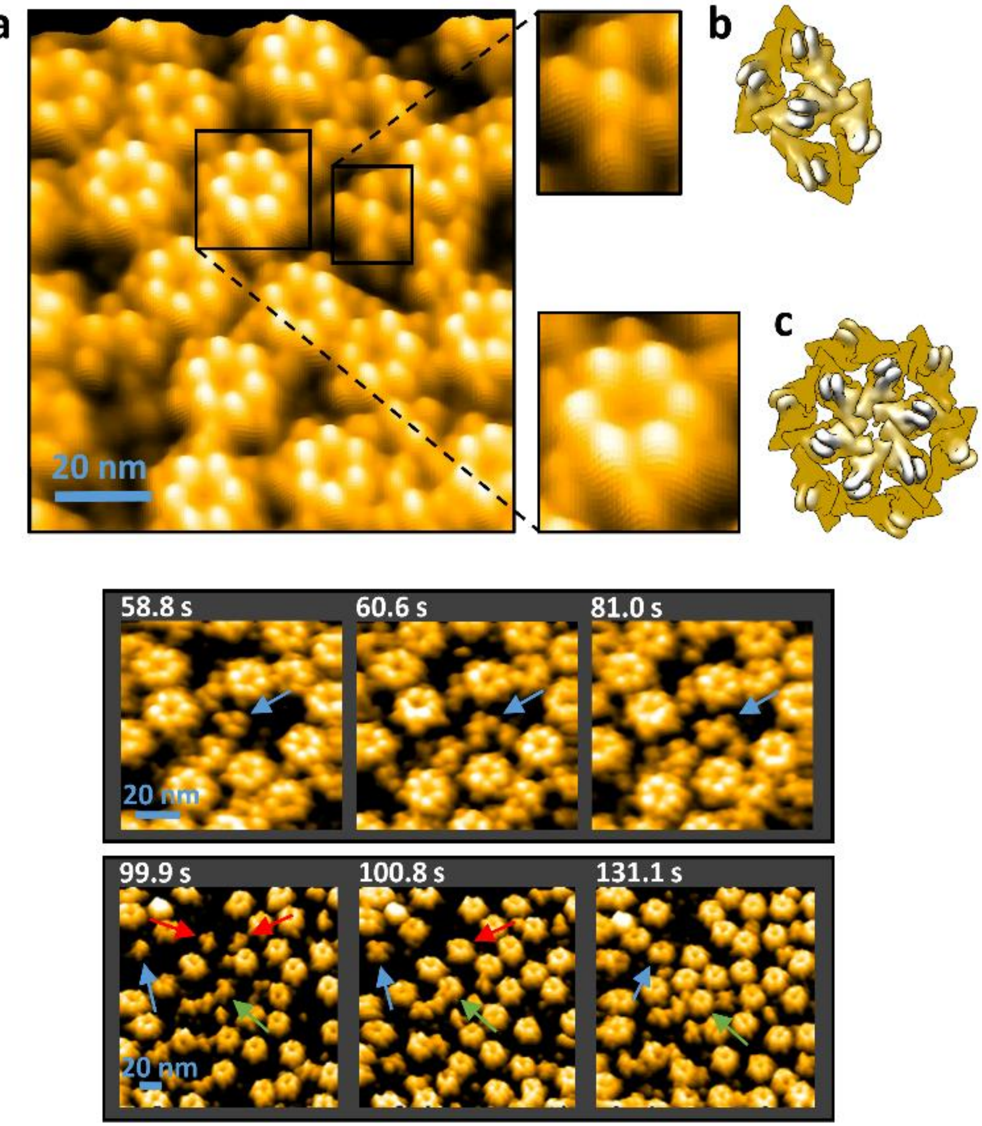


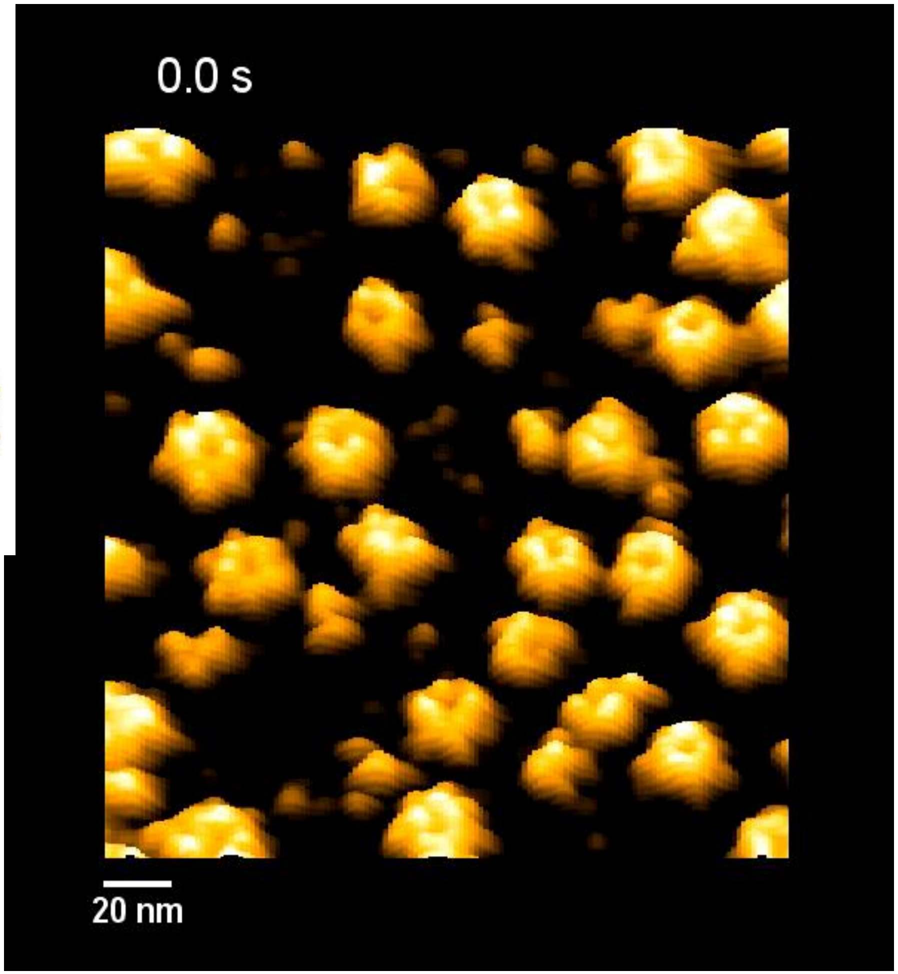


Hepatitis B Virus assembly

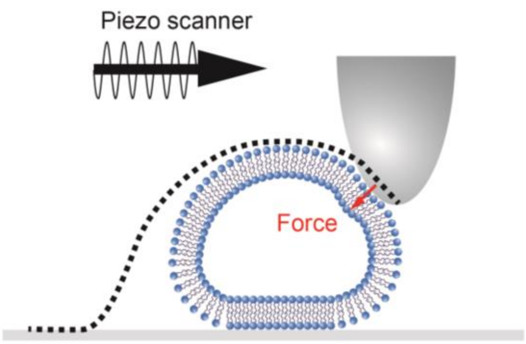


Hepatitis B Virus assembly





For those not (only) interested in viruses

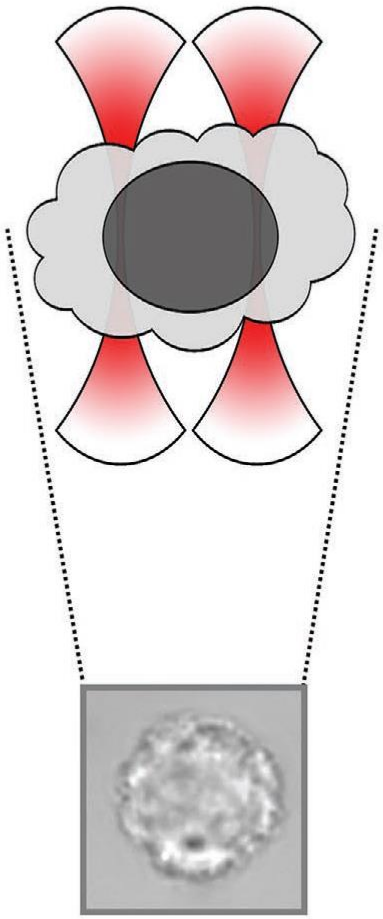


<u>Extracellular vesicles / lipoprotein particles</u>

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

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

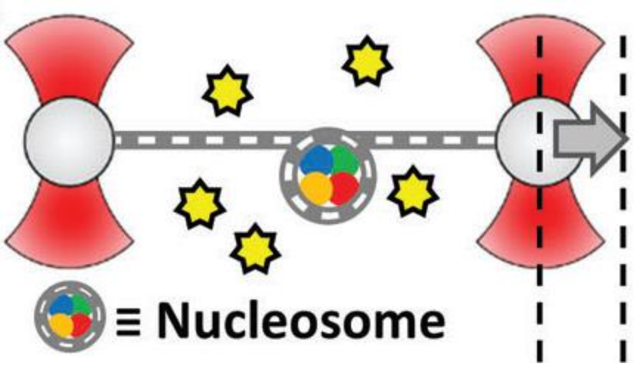
AFM nanoindentation (mechanical probing)



<u>Single-Cell Activation Kinetics</u>

Vasse *et al.*
Small Methods (2021)

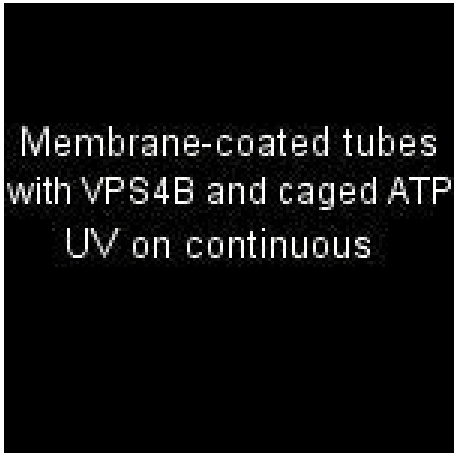
Optical Tweezers studies of cell and histone dynamics



<u>Histone-chaperone based nucleosome remodelling</u>

Buzón *et al.* Adv. Scie. (2023)

For those not (only) interested in viruses

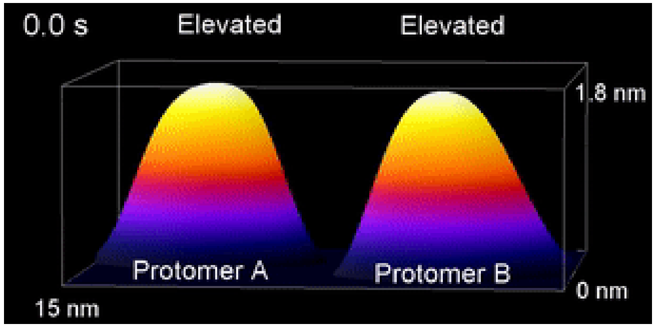


ESCRT:

Maity *et al.* Science Advances (2019)

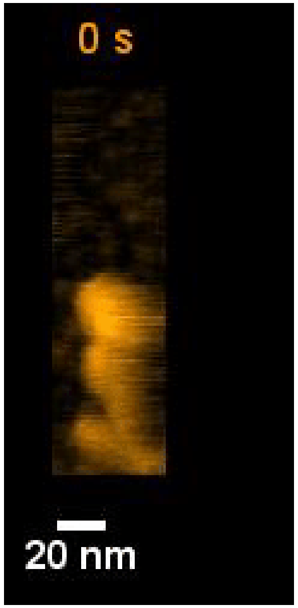
Bertin *et al.* Nat. Comm. (2020)

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



Bacterial transporters:

Maity *et al.* PNAS (2022)



Assembly of synthetic systems:

Maity *et al.* JACS (2020)

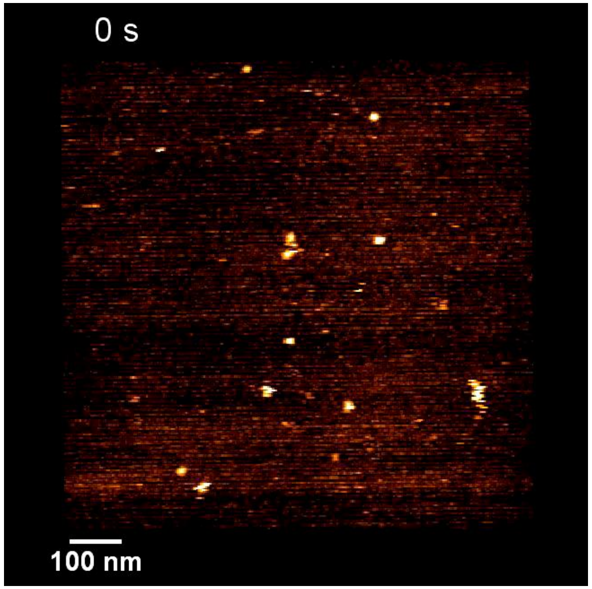
Liu *et al.* Nat. Chem. (2023)

Assembly of antibiotics:

Shukla *et al.* Nature (2022)

Melcrova *et al.* Nat. Comm. (2023)

Shukla *et al.* Cell (2023)



High Speed AFM studies of dynamics

Acknowledgments

Collaborators

S. Otto, S. Marrink. D. Slotboom (***RUG***)
G. Wuite, F. MacKintosh (***VU A'dam***)
R. Schiffelers, A. Heck,
M. Weingarth (***U Utrecht***)
M. Mateu (***CSIC***), G. Nemerow (***Scripps***)
A. Zlotnick (***IU Bloomington***)
N. Kodera, K. Ngo, T. Ando (***Kanazawa***)
C. Uetrecht (***CSSB***), R. de Vries (***WUR***)
A. Garcia (***UNAM***), P. Bassereau (***Curie***)
W. Weissenhorn (***IBS***)
M. Schelhaas (***U Münster***)

MOSBRI Molecular-Scale Biophysics Research Infrastructure

Roos Lab

G. v/d Borg, S. Maity, Y. Feng, A. Melcrova, C. Richards, Y. Gong, M. Middelkamp,
C. v. Ewijk, S. Sasidharan, S. de Weerd, L. Presutti, Y. Knelissen

ViruScan

R. Sorkin, D. Denning, M. Baclayon, J. Snijder, R. Lira
D. Vorselen, M. v Rosmalen, M. Marchetti, P. Buzón, M. Piontek

O-ChemS Out-of-equilibrium Chemical Systems

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