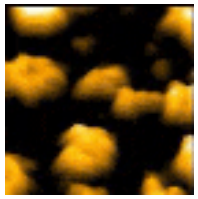
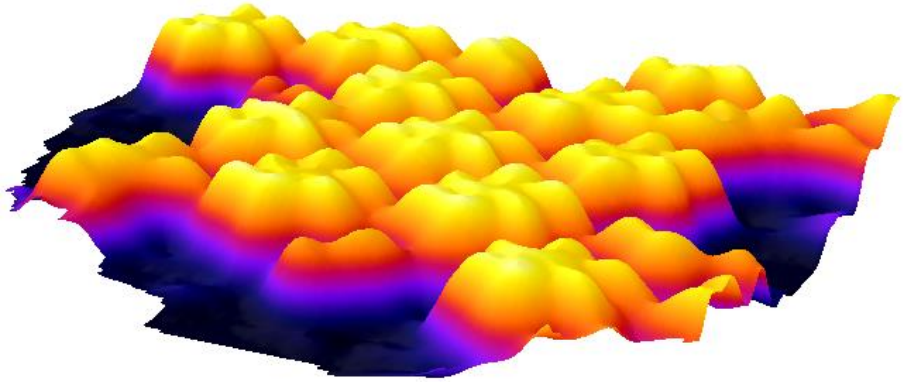
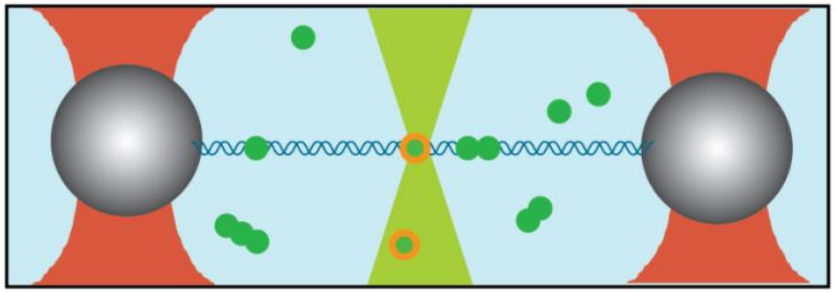
Single-molecule approaches to study viral self-assembly in real-time





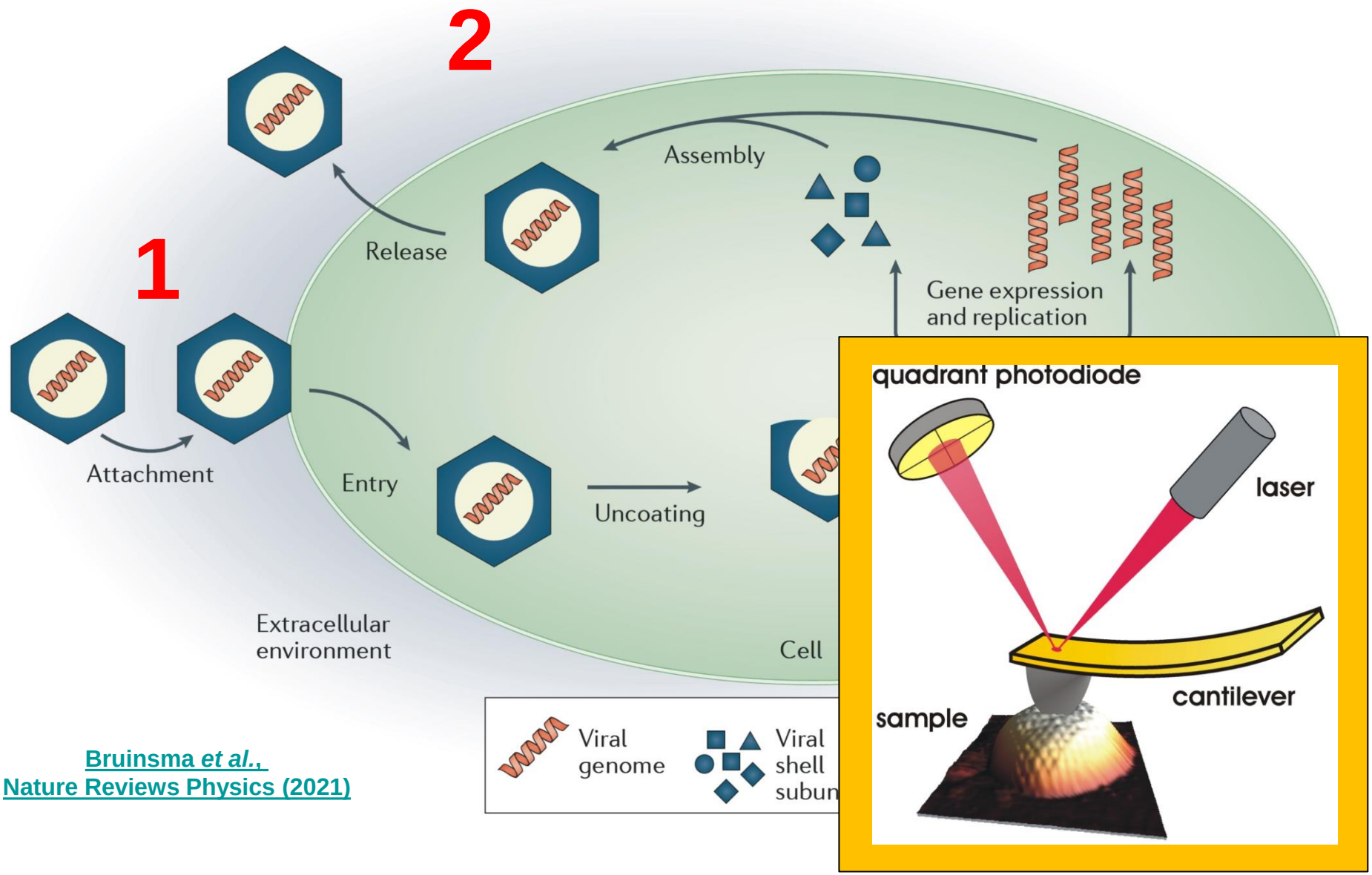


Wouter Roos

rijksuniversiteit groningen

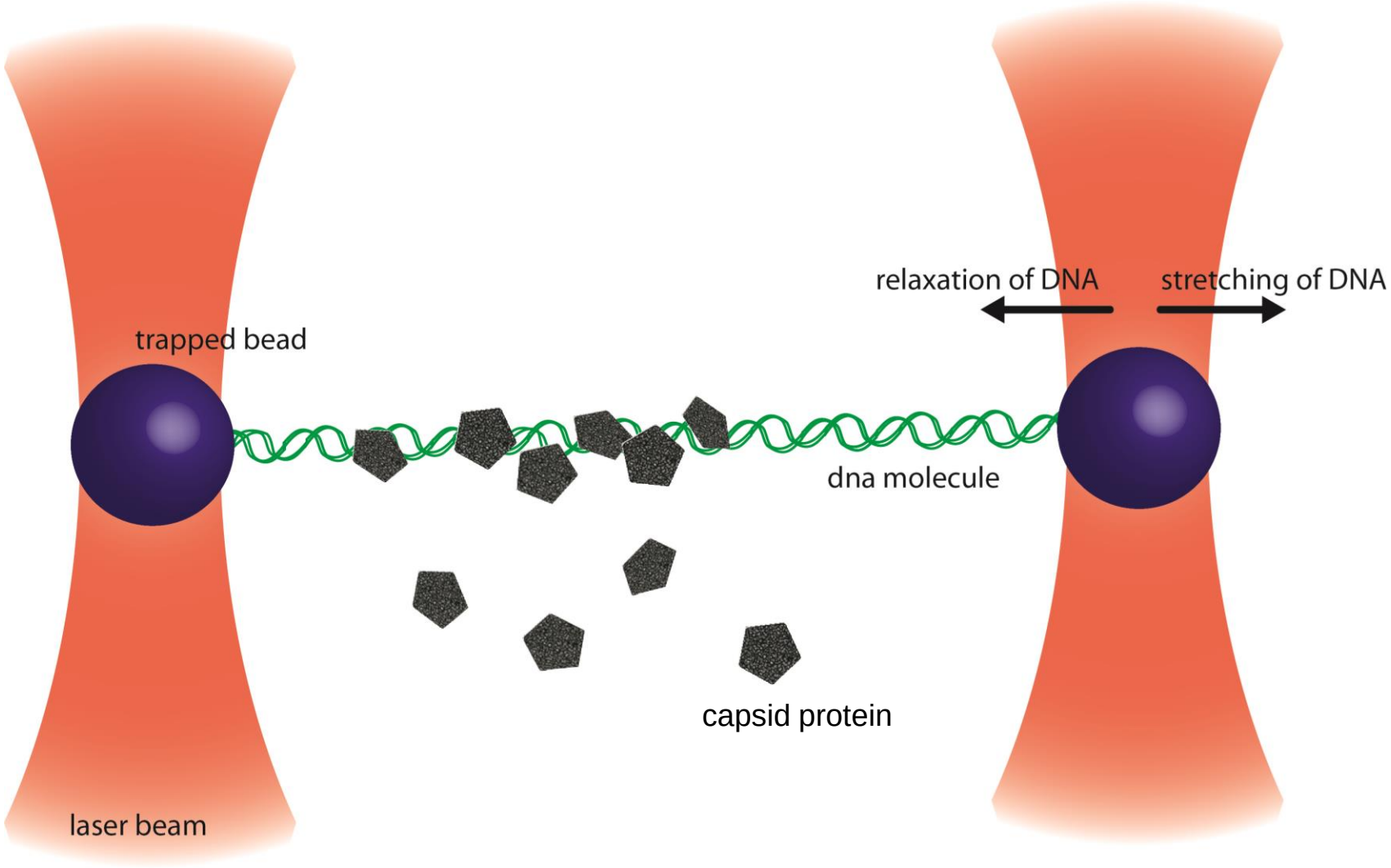
zernike institute for advanced materials

Viral life cycle



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

Following virus assembly by optical tweezers

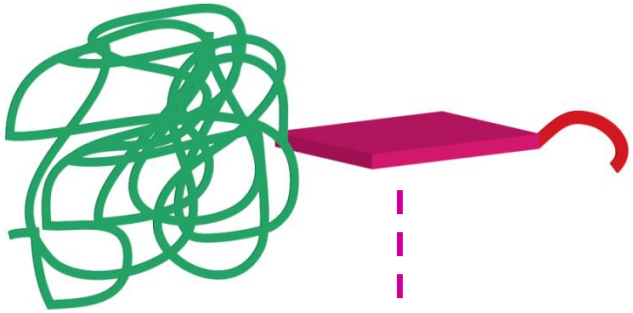


Synthetic Virus-Like-Particles

Artificial
virus-like particles are controlled systems

→

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



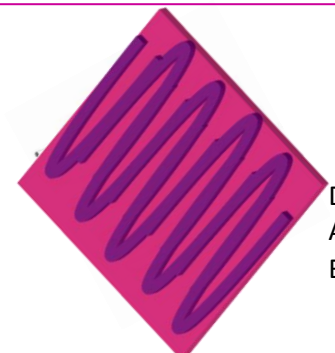
Biosynthetic coat protein: polypeptide

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

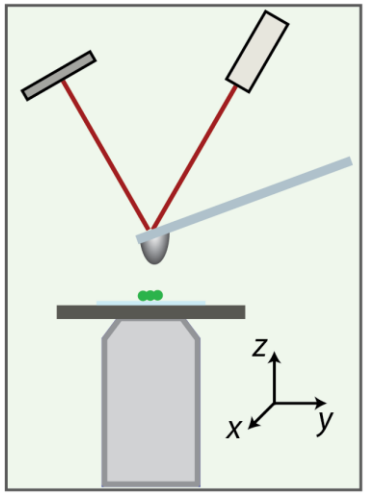
Folds and stacks in solution into stiff filamentous structures.
Precisely tunes cooperativity:
Silk-like sequence S_{10} = $(GAGAGAGQ)_{10}$

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

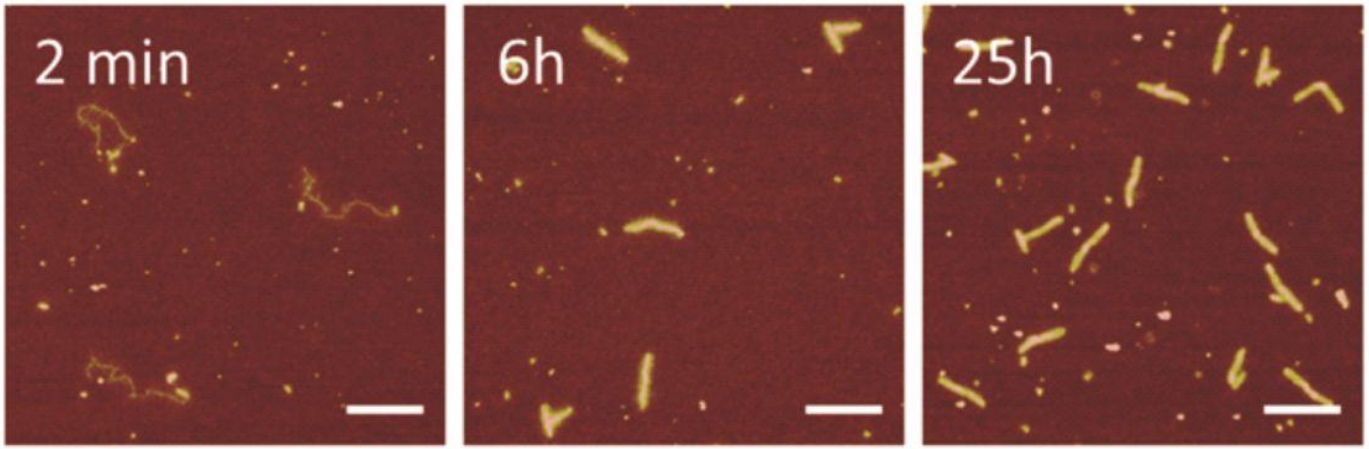


De Vries Lab:
A. H. Garcia et al., *Nature Nanotechnology* (2014)
B. Zhao et al., *Soft Matter* (2016)

Particle visualisation by AFM



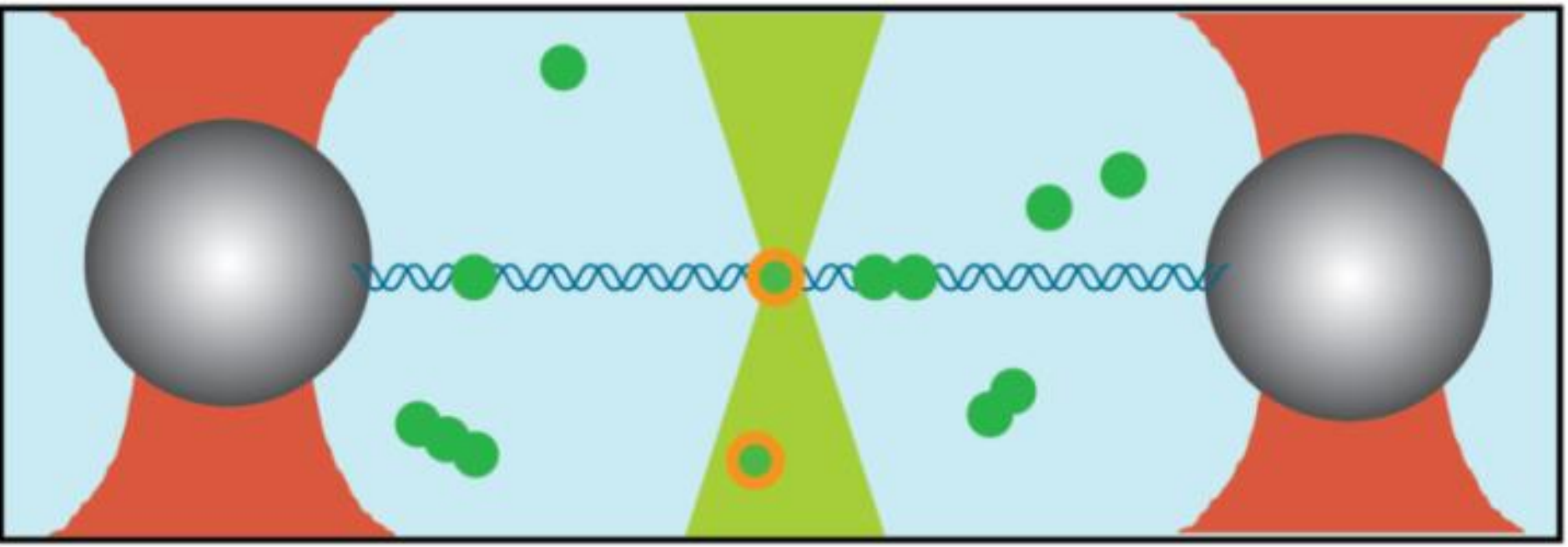
DNA 2.5 kbp ~ 1 µm

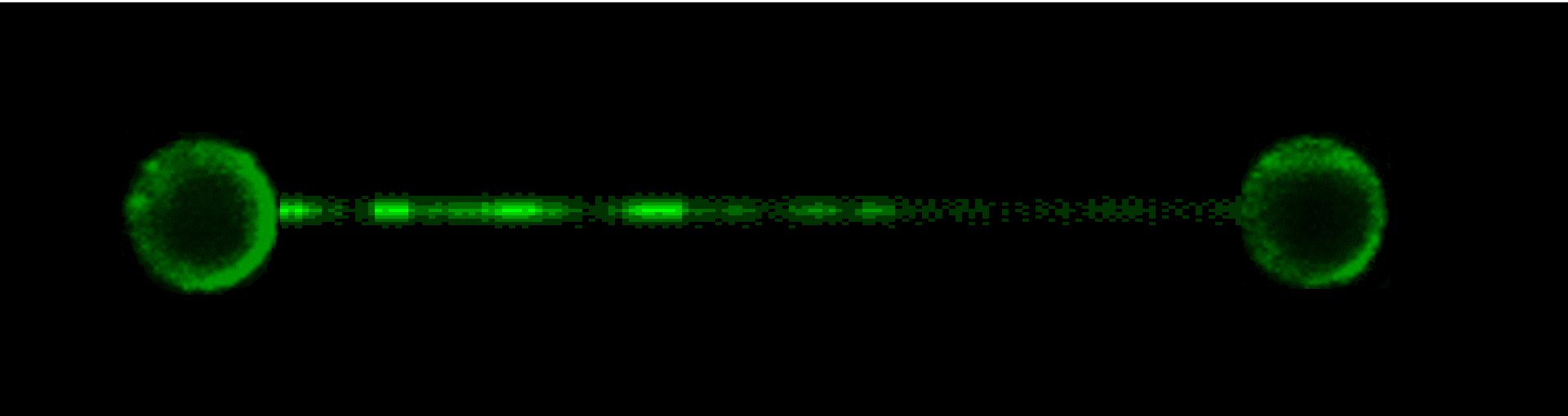


350 nm

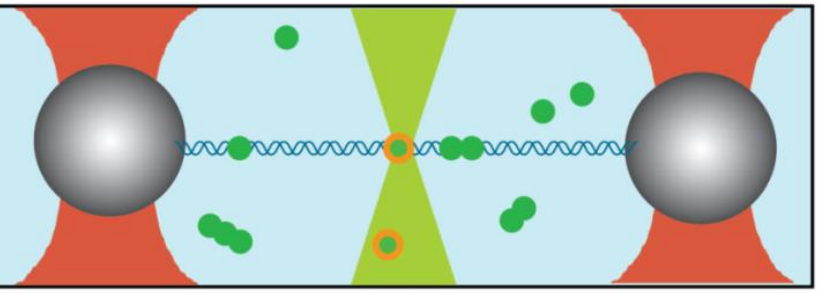
- Stiff rod-like appearance of the VLPs (300nm)
- DNA compacted 1/3 its original length
- Cooperative self-assembly.

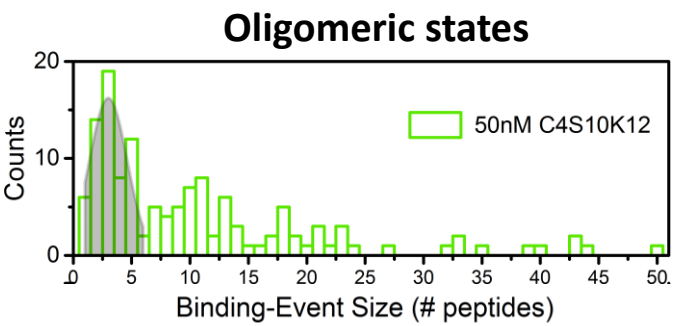
Protein attachment, in real-time

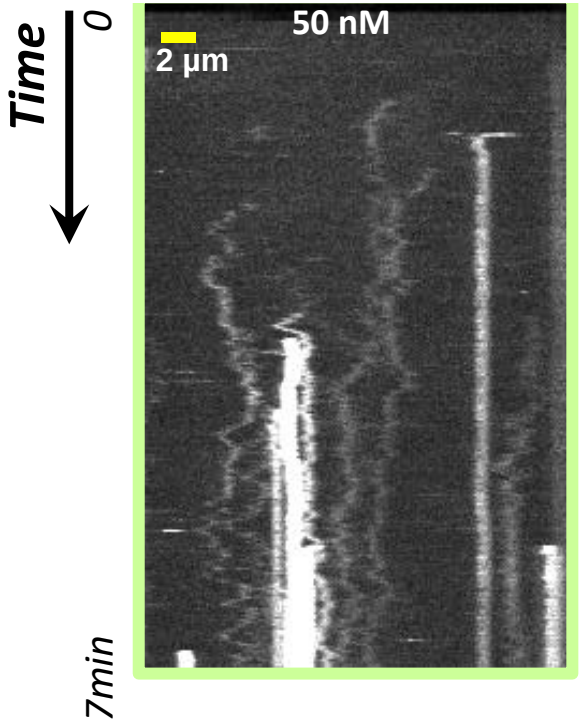


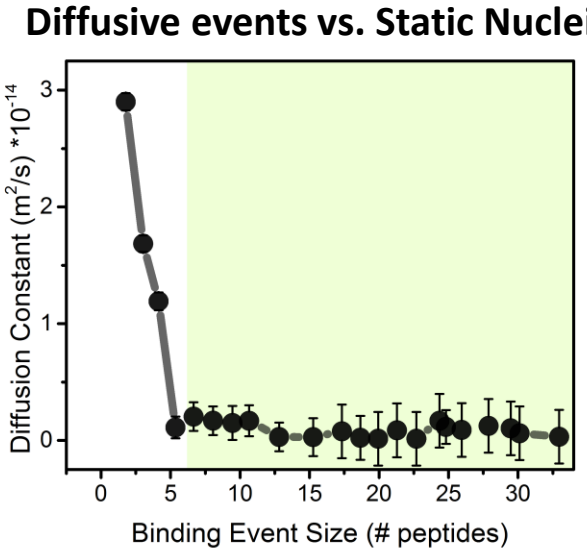


Protein attachment, in real-time

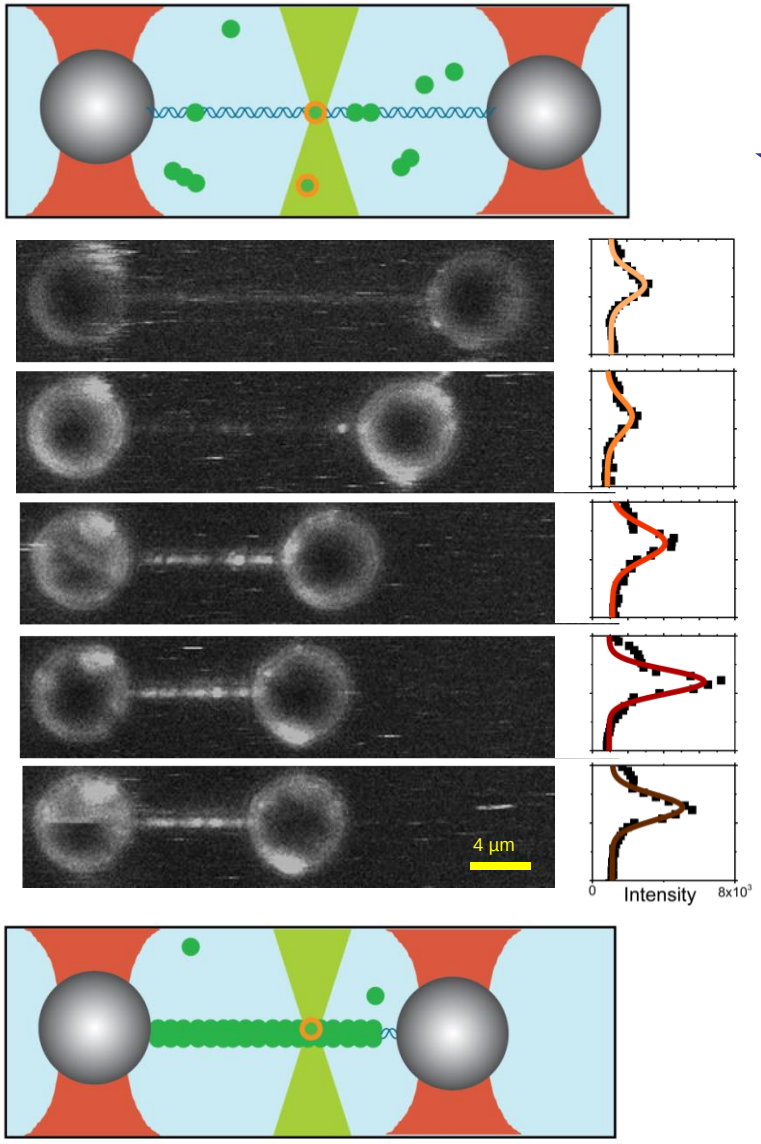


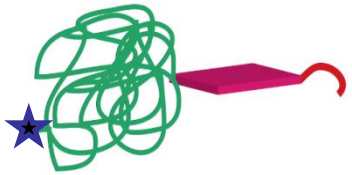






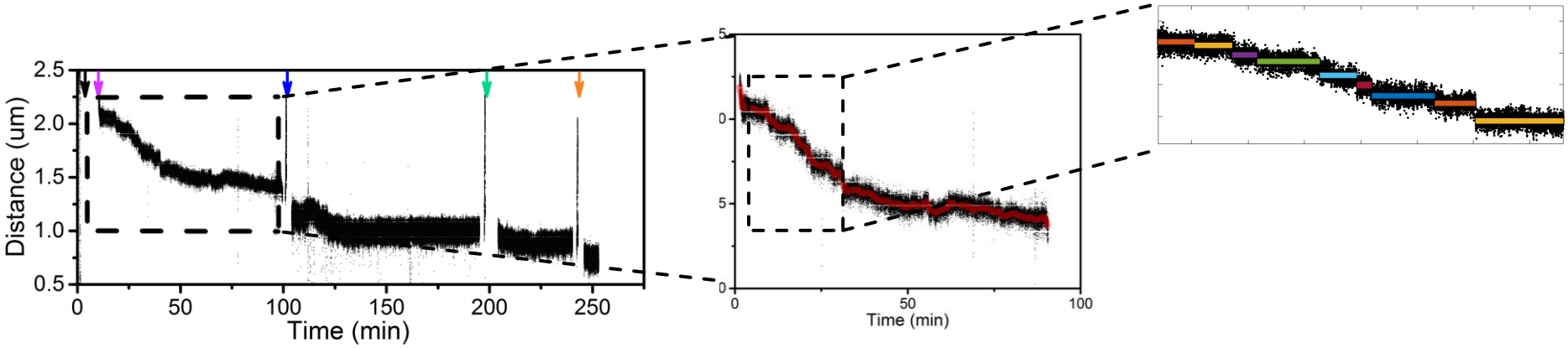
DNA compaction by rod shaped VLP



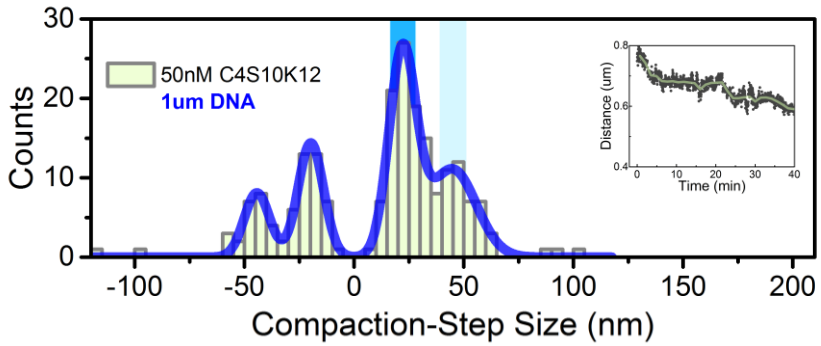


Marchetti *et al.*
Nano Letters (2019)

DNA compaction in real-time

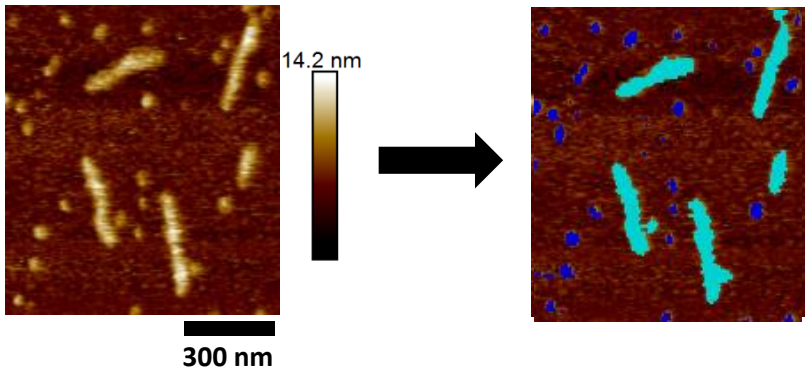


Regular compaction steps for assembly model



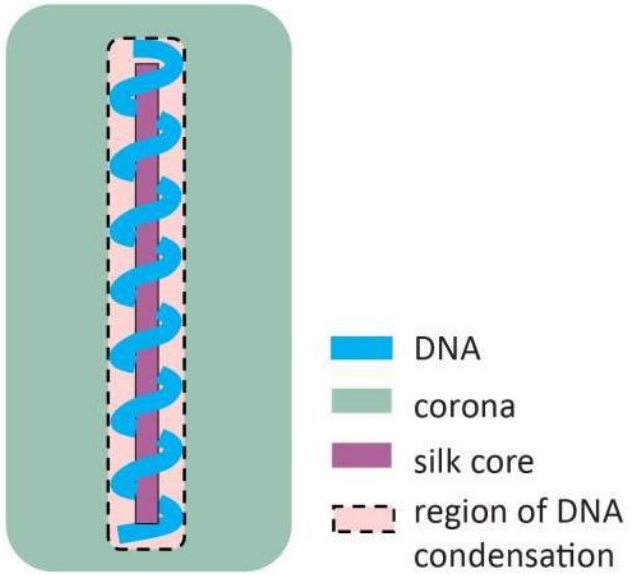
Average Compaction-Step:

~ 30 nm

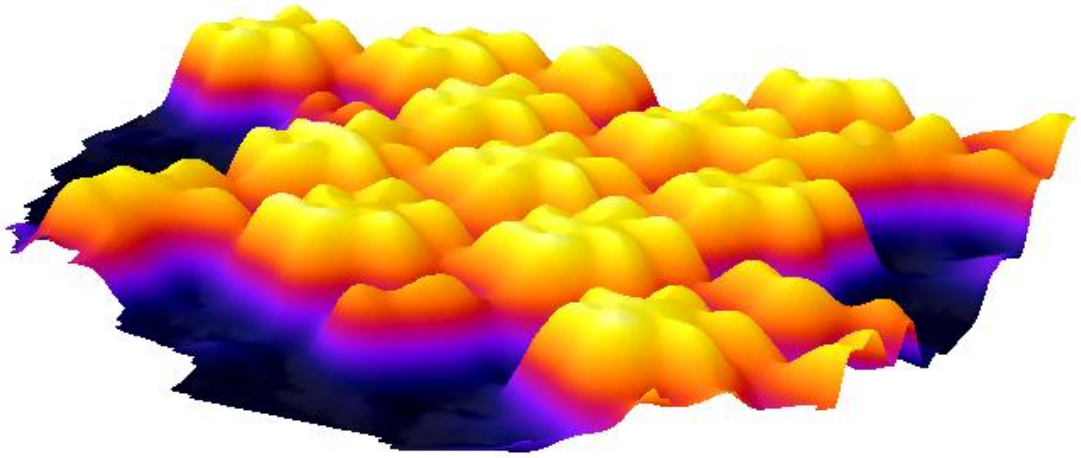


Mean particle HEIGHT:

9.1 ±0.5 nm



High Speed AFM studies of dynamics



Are we able to follow assembly in real-time?

High Speed AFM studies of 2D HIV assembly

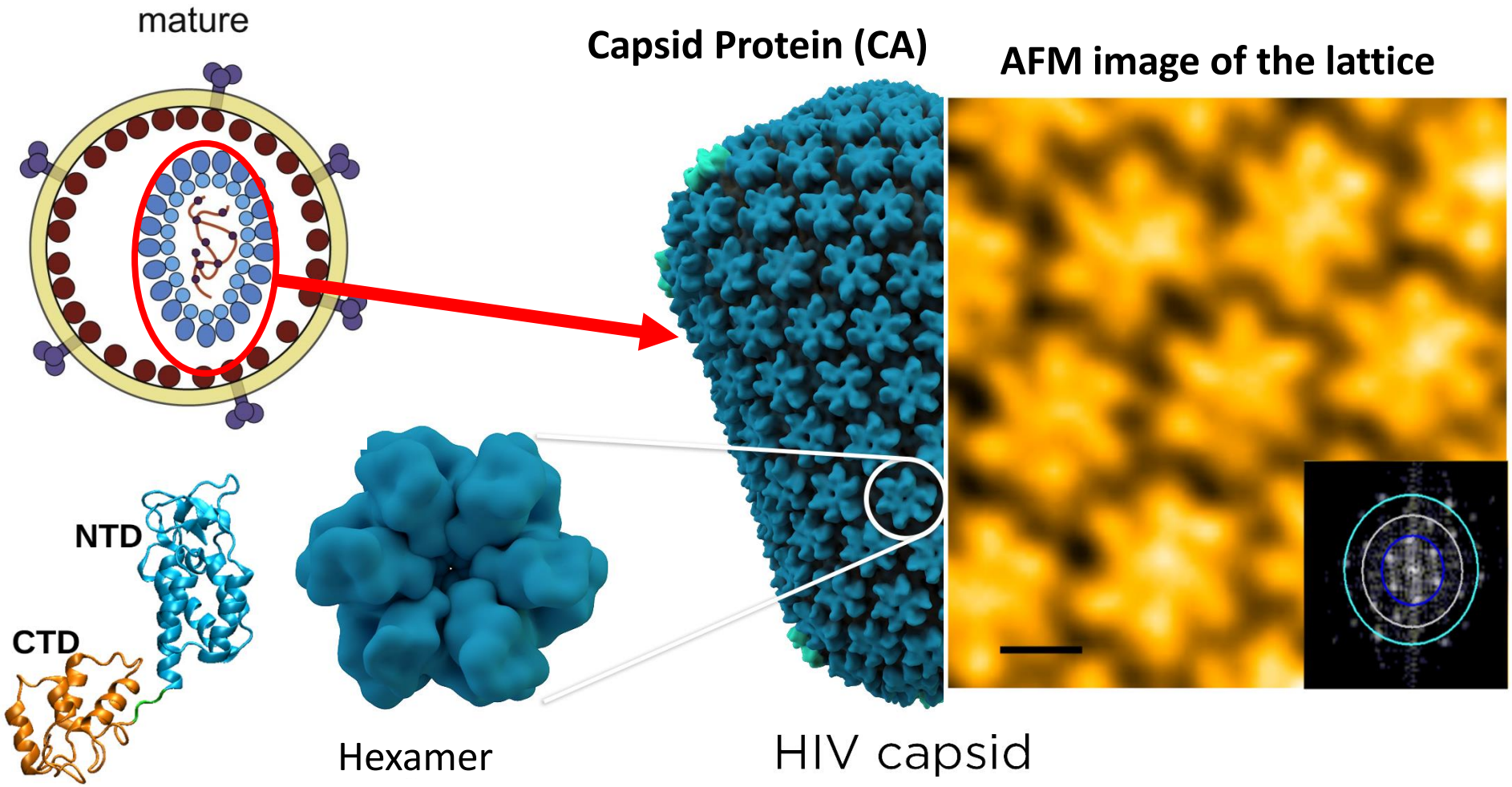
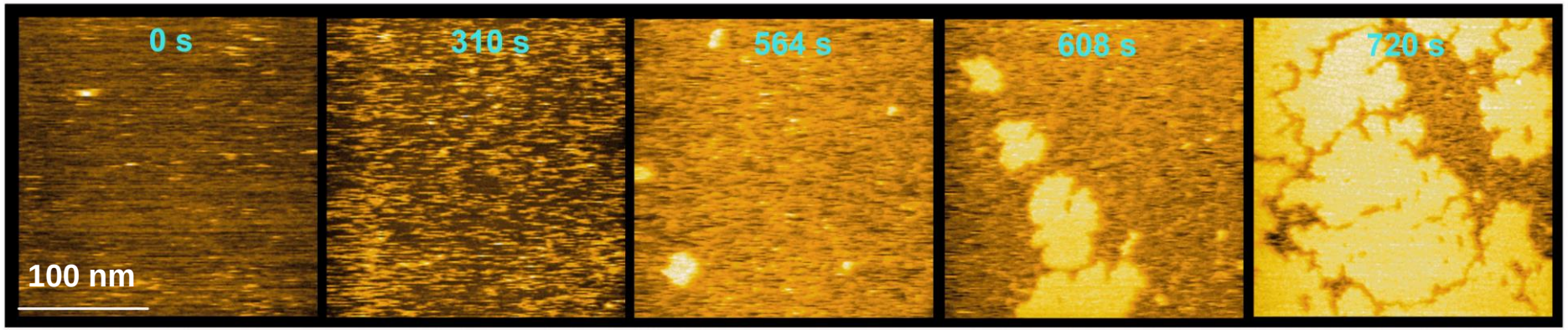
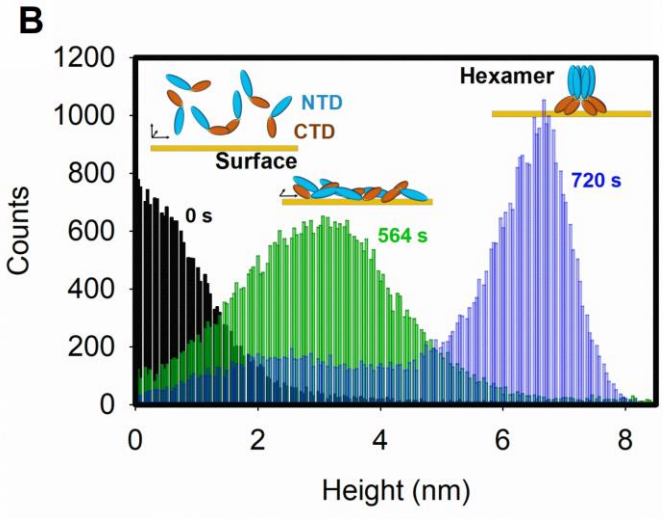
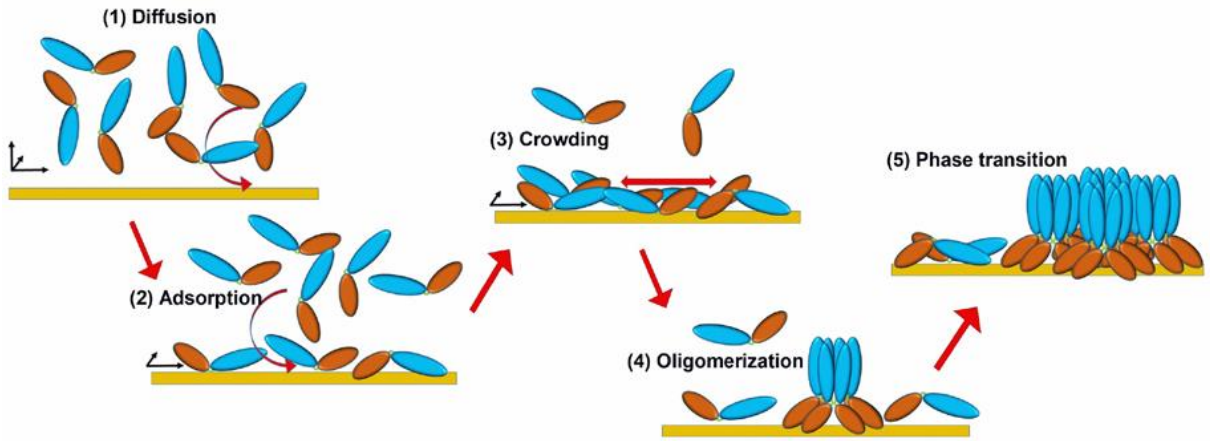


Figure credits: Monroe et al., ***Structure***, 2010 and Thomas Splettstoesser

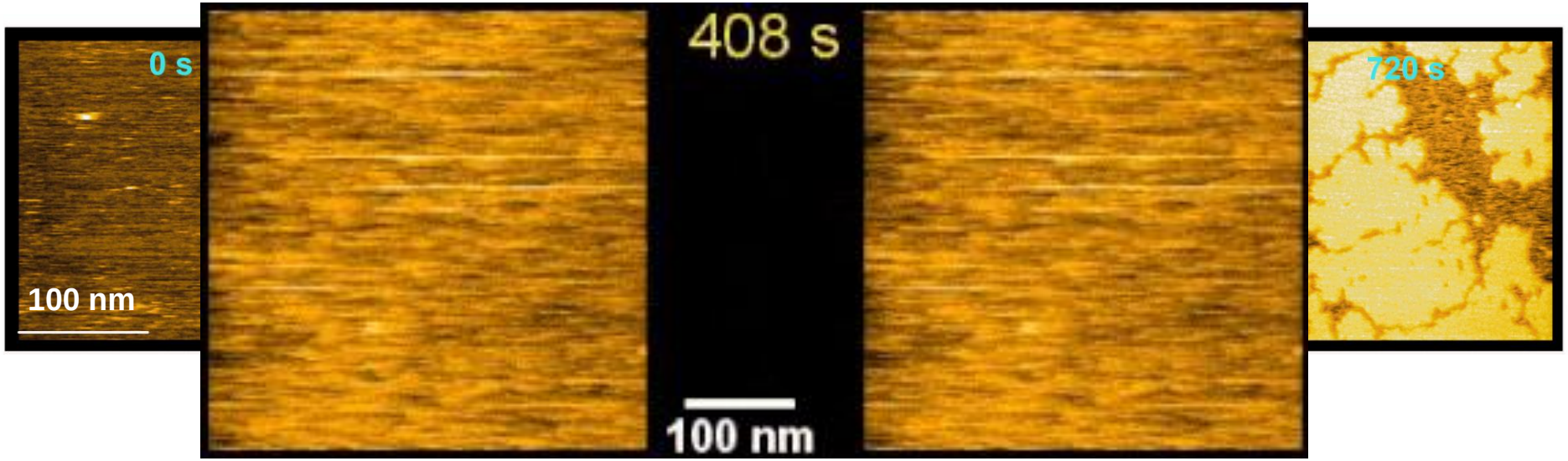
High Speed AFM studies of 2D HIV assembly

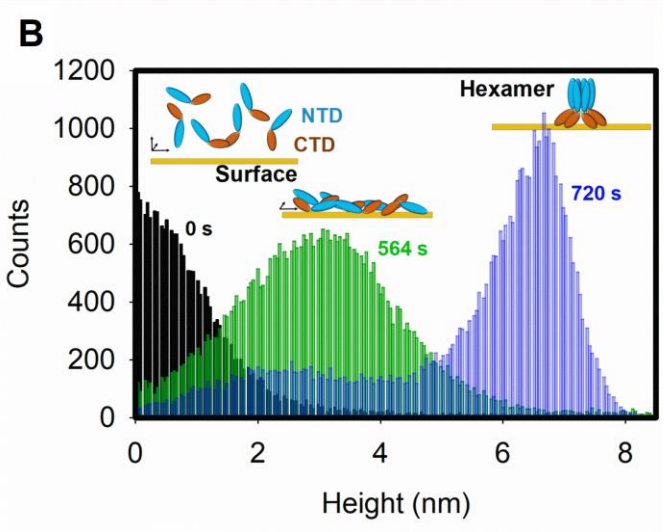


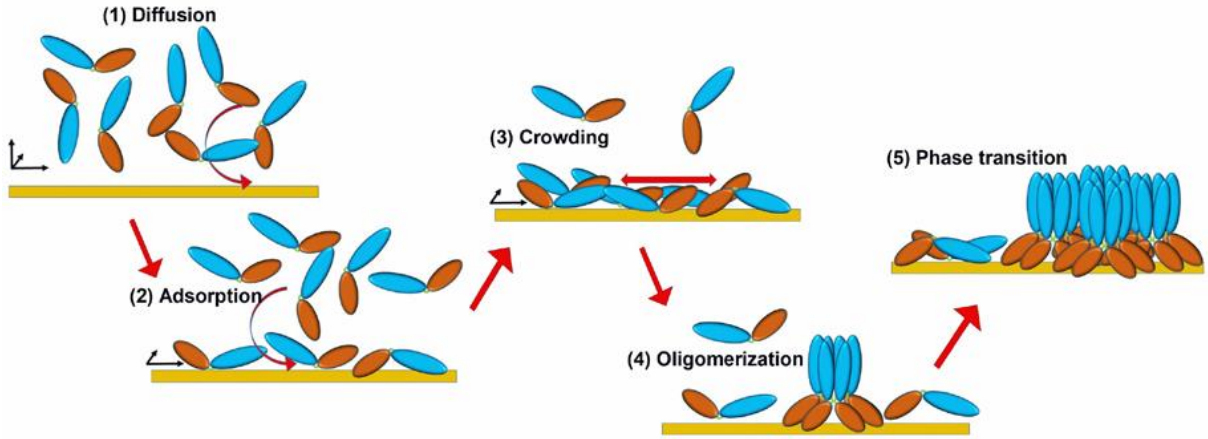




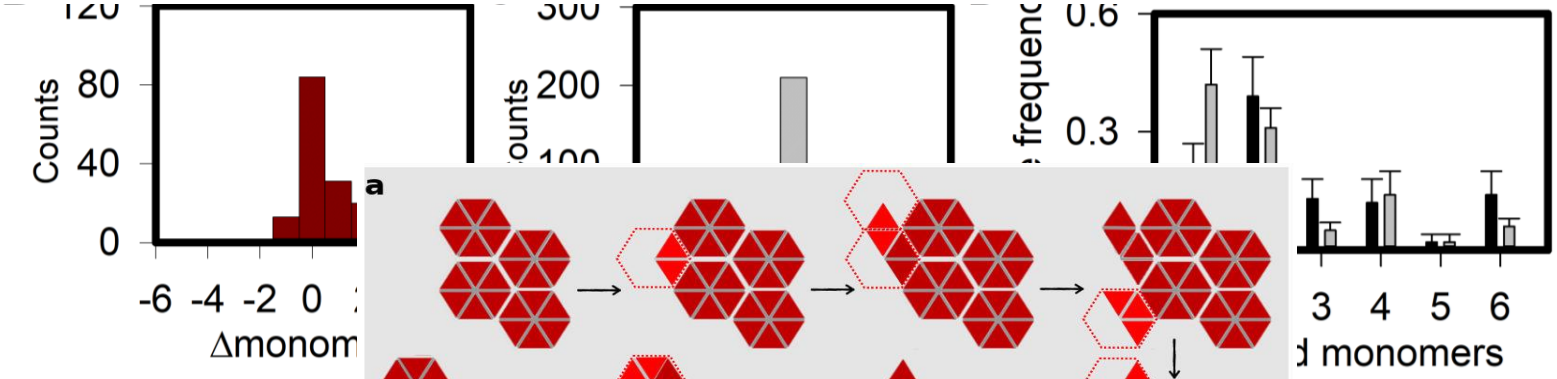
High Speed AFM studies of 2D HIV assembly

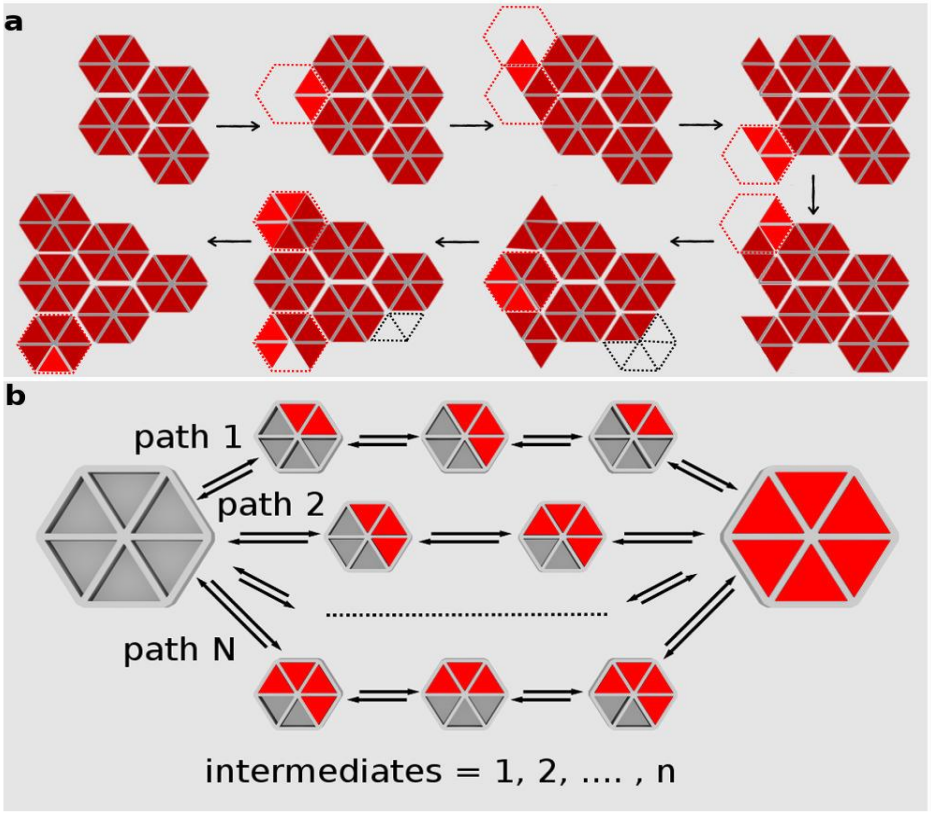




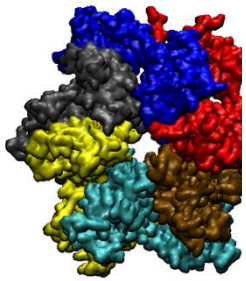


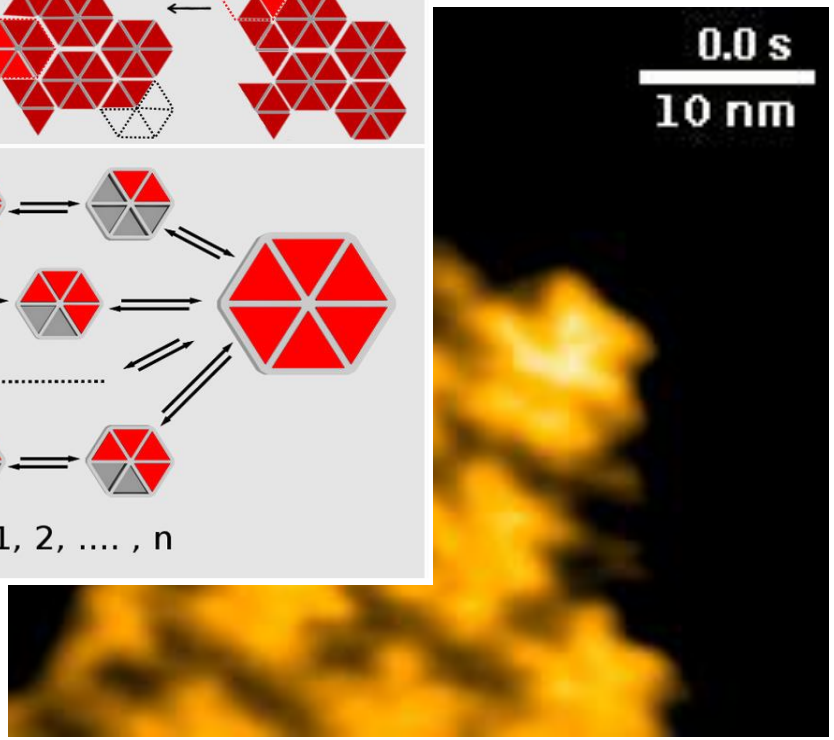
High Speed AFM studies of 2D HIV assembly



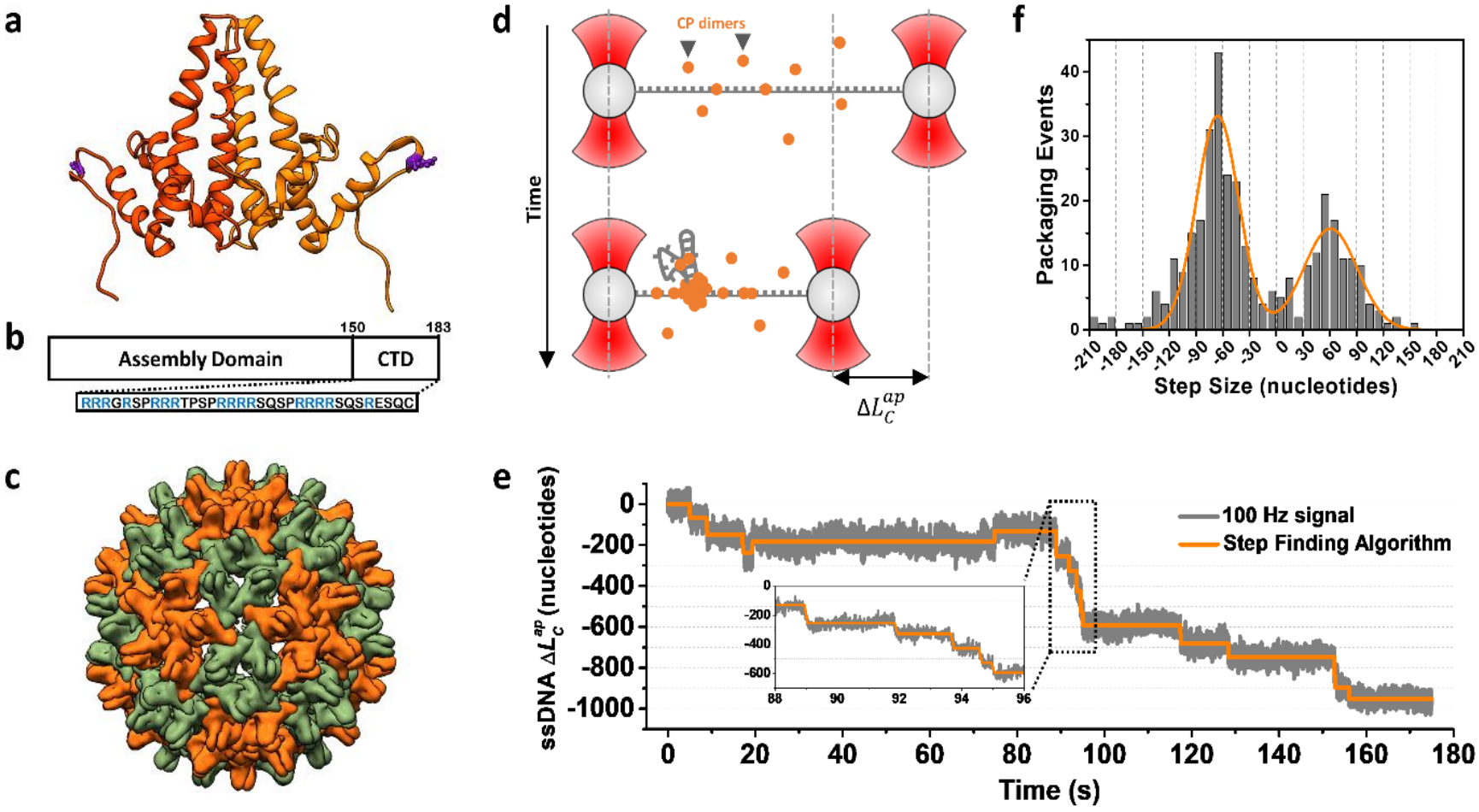


Valbuena *et al.*
ACS Nano (2020)

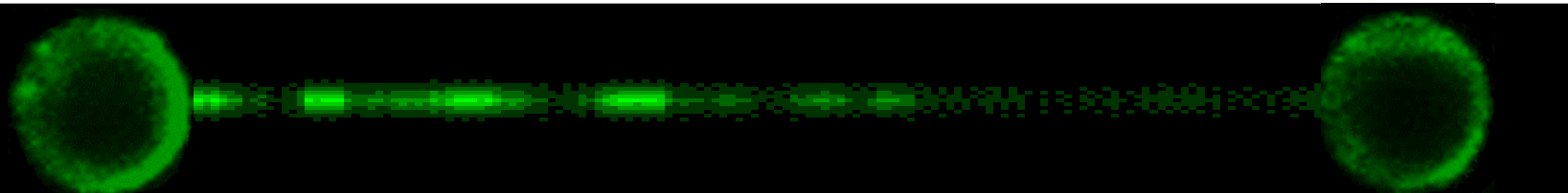




Hepatitis B Virus assembly



Buzon *et al.* Science Advances 2021



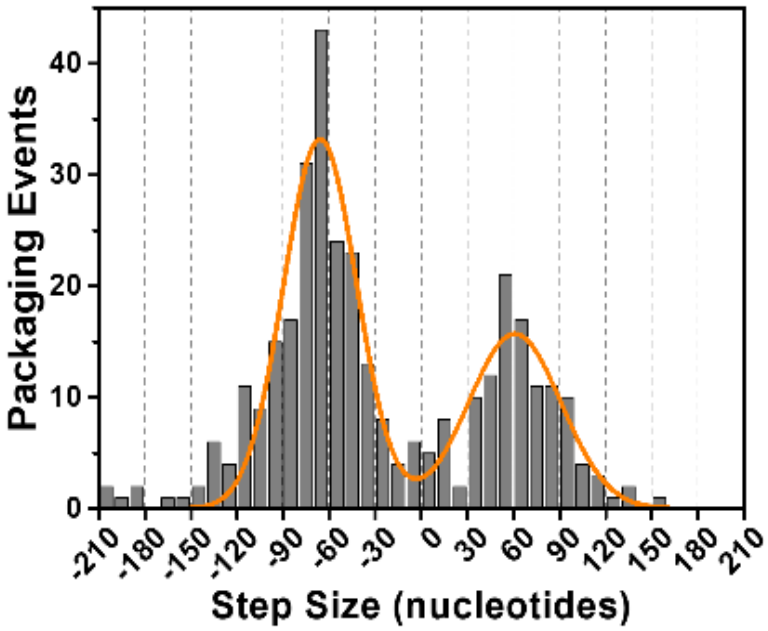
Hepatitis B Virus assembly

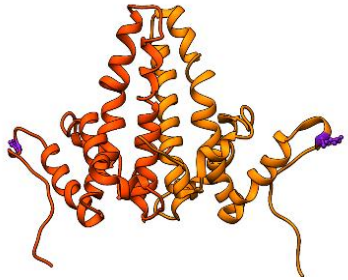
Cp condenses nucleic acids, even under tension (*F* = 11 pN)

Assembly footprint: ~70 nt

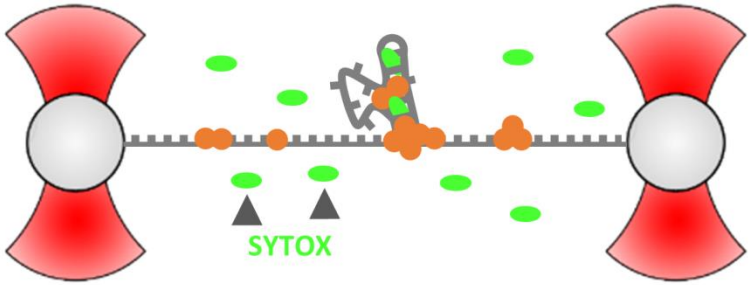
Work per condensation step:
F step size* = ~ 100 k_BT

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

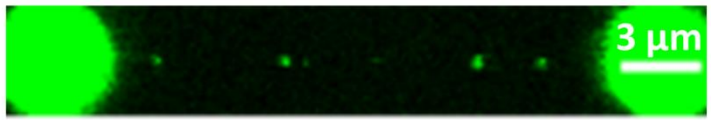


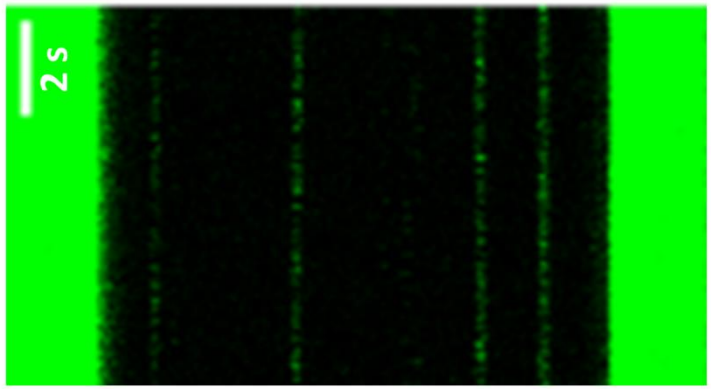


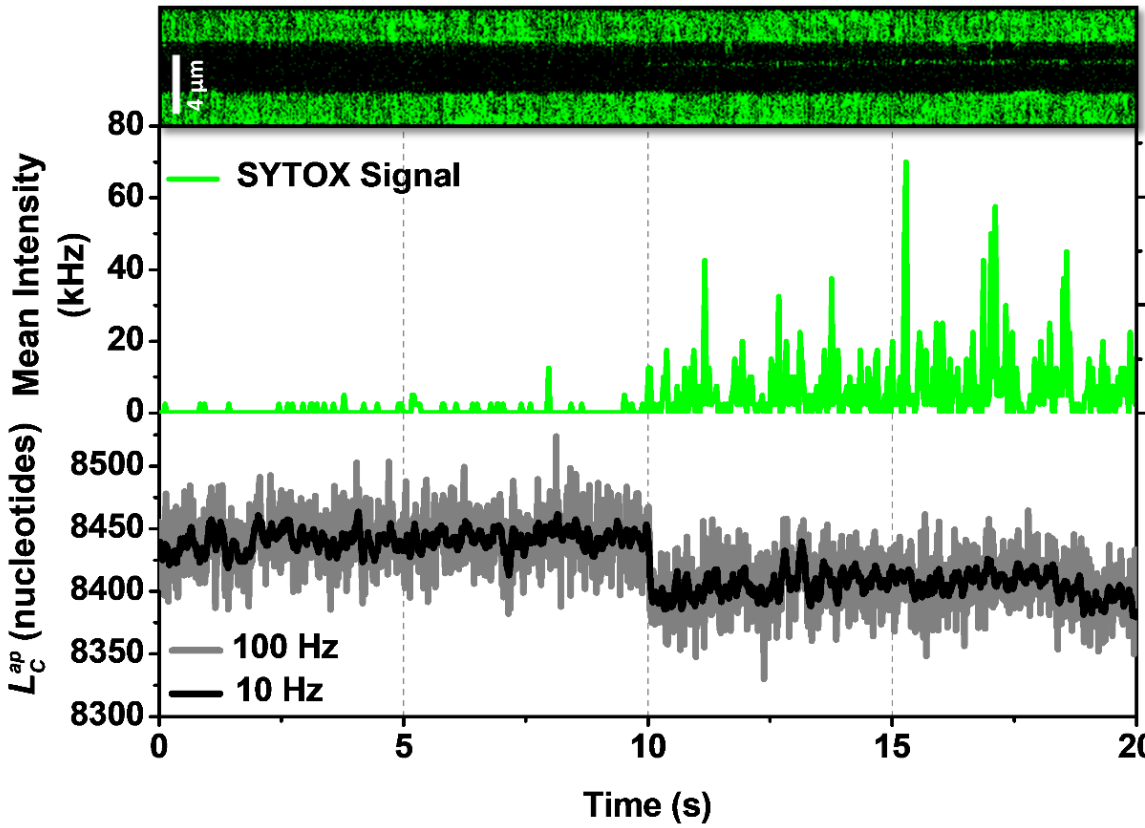
Hepatitis B Virus assembly



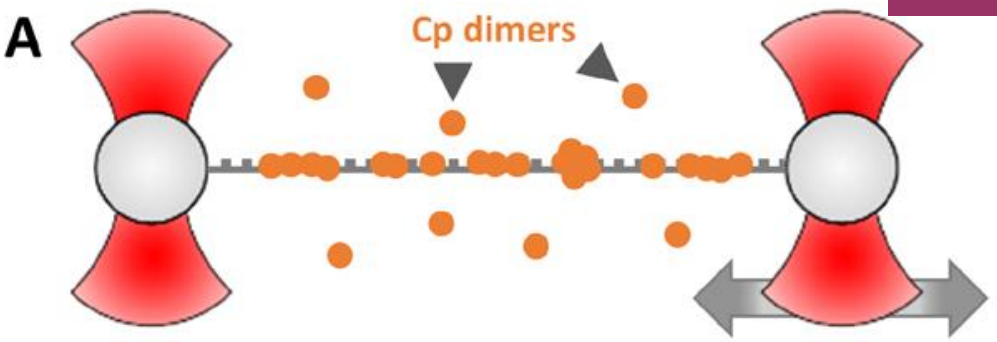
Cp has the capability to chaperone the formation of ds genome patches

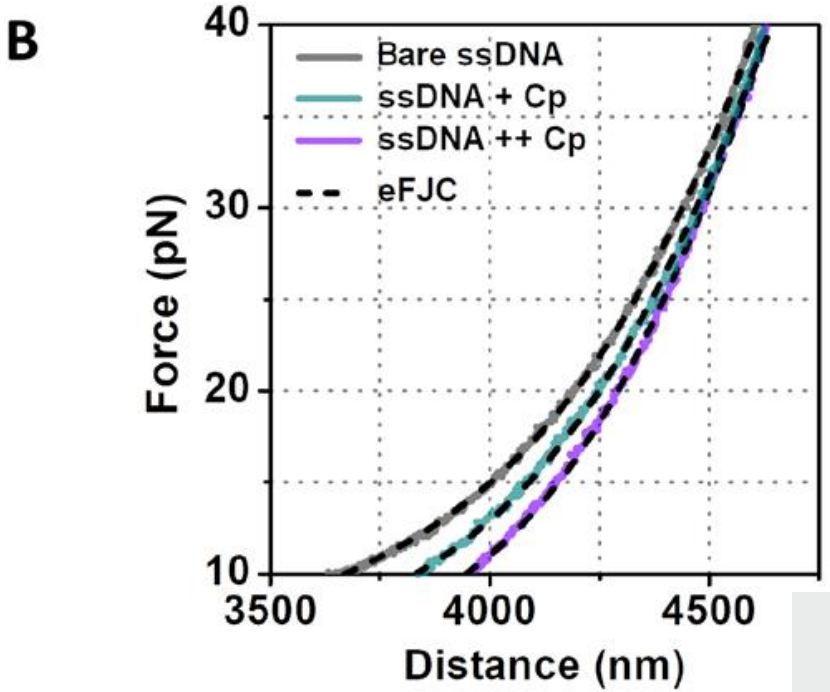






Hepatitis B Virus assembly



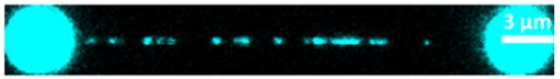


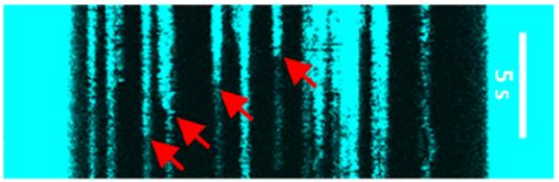
Force-extension curves for varying Cp concentrations

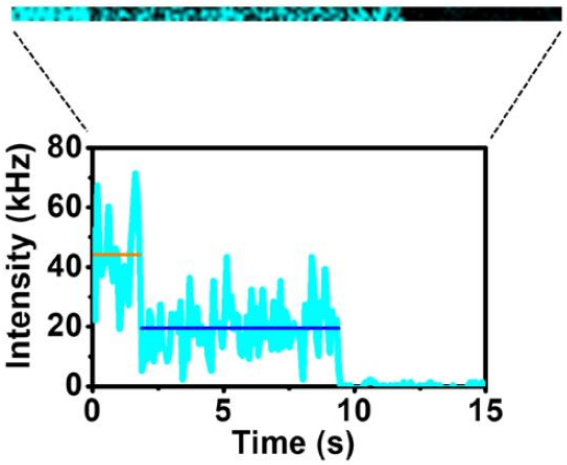
extensible Freely Jointed Chain fitting yields L_p as function of [Cp]

$\Delta G^0_{\text{prot–DNA}}$ (k_BT)	$\Delta G^0_{\text{prot–prot}}$ (k_BT)	ΔG^0_{bind} (k_BT)
-10 ± 2	-7 ± 1	-16 ± 2

Hepatitis B Virus assembly



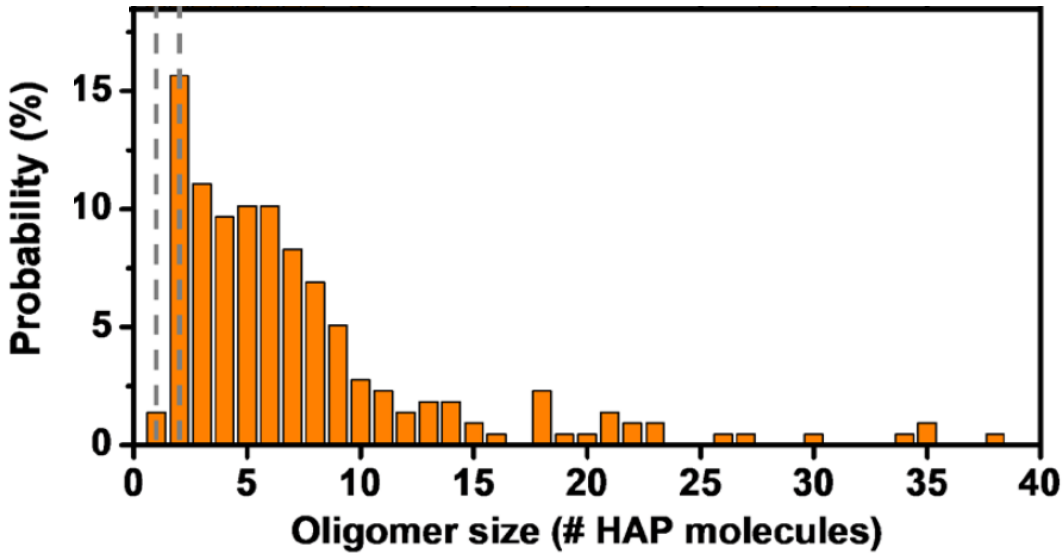




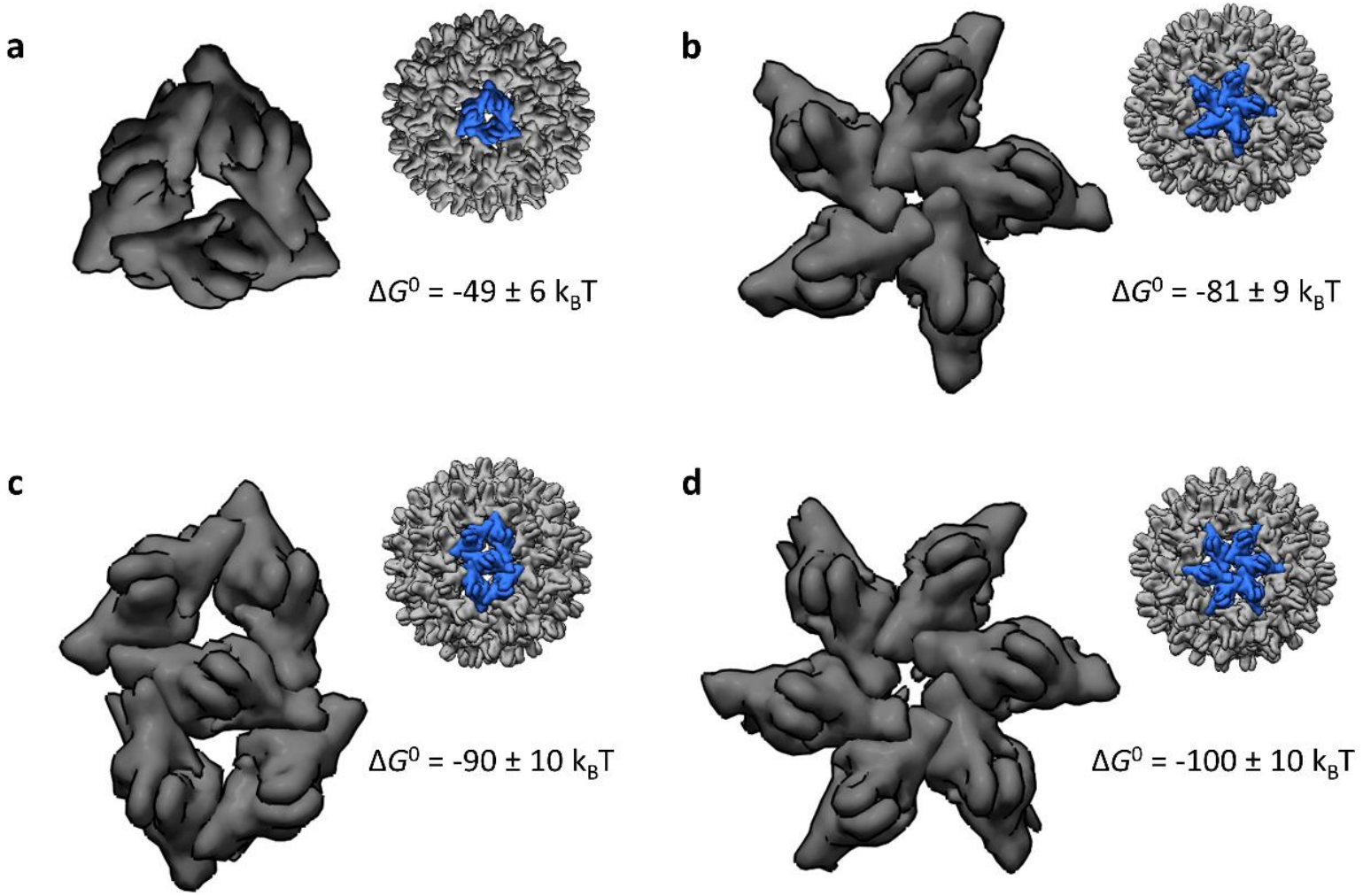
Assembly nucleus?

HAP only binds dimer-dimer interfaces

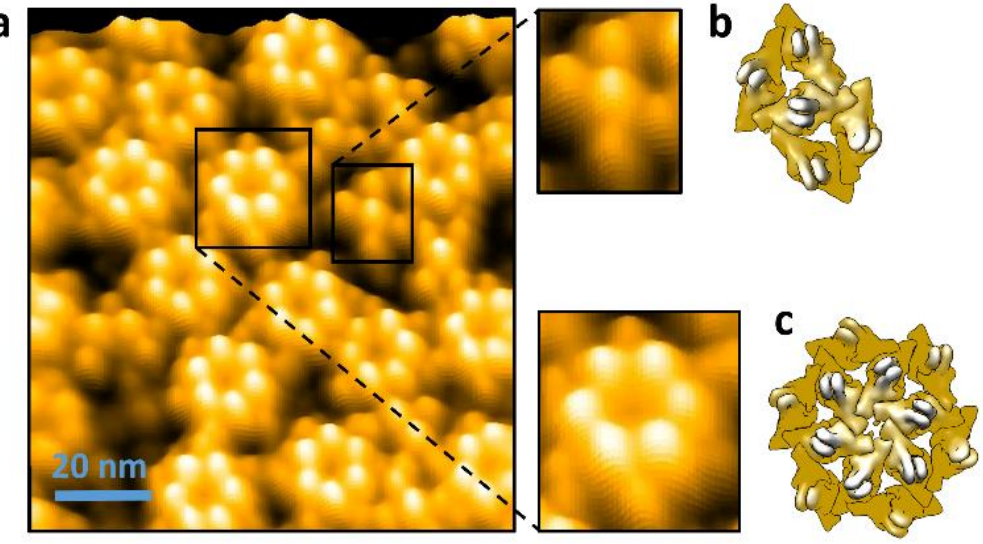
Discrete bleaching steps yields # HAP

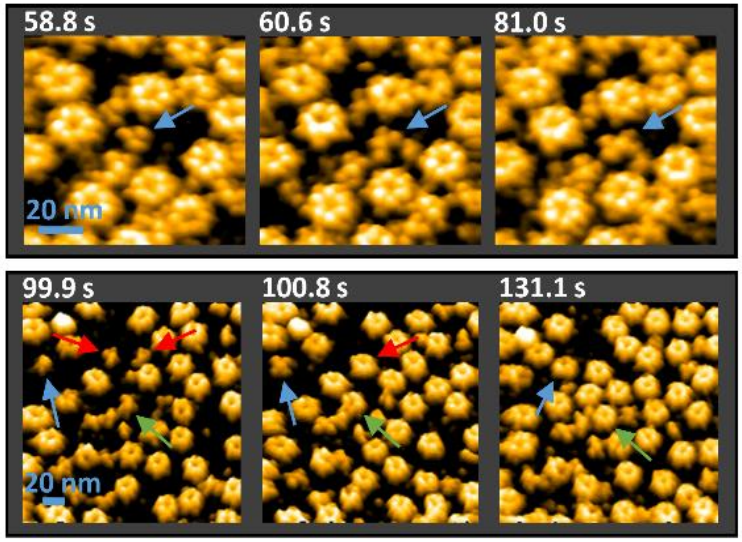


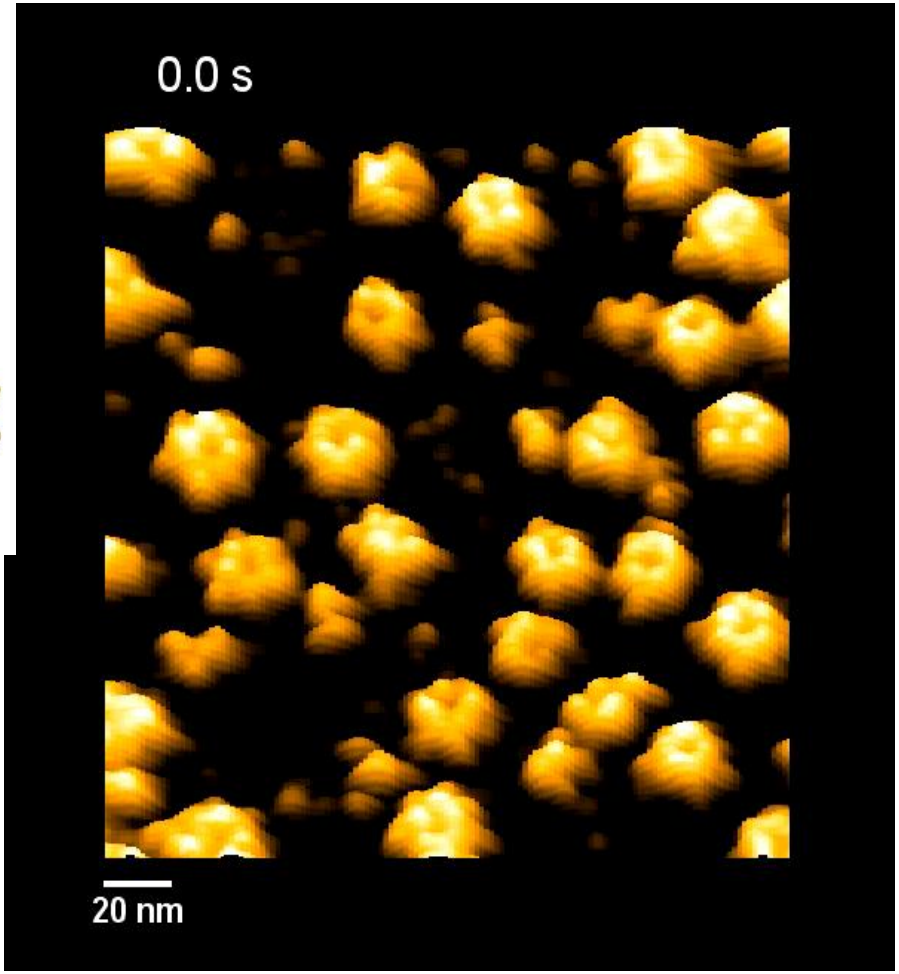
Hepatitis B Virus assembly



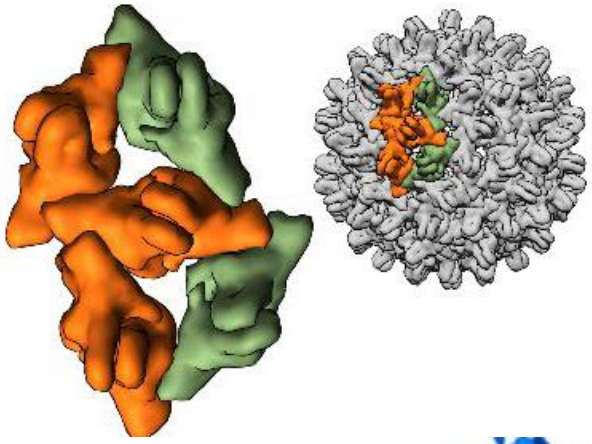
Hepatitis B Virus assembly

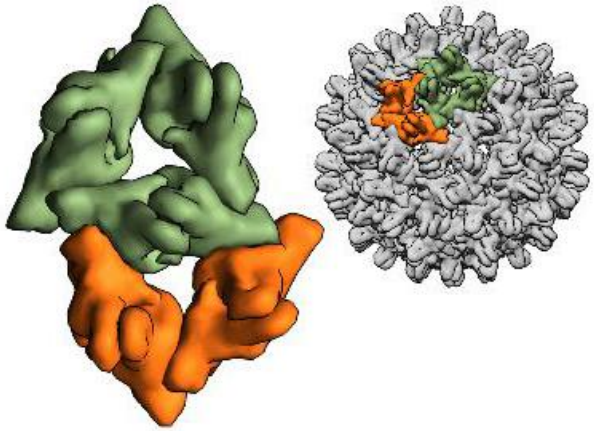






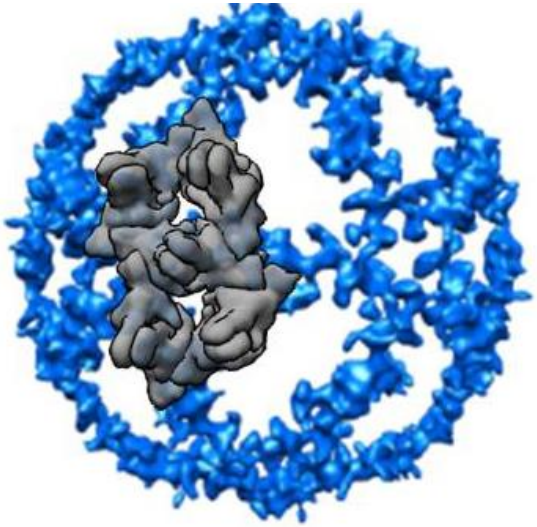
Hepatitis B Virus assembly

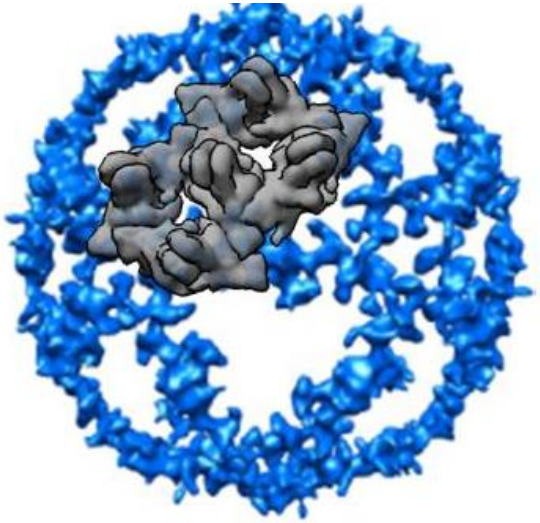




A-B dimer

C-D dimer

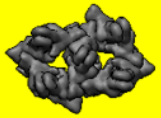




bound to three or two double-stranded regions

HBV assembly pathway proceeds by the formation of diamond pentamers and dodecamers, instead of through the fivefold symmetric pentamer

Hepatitis B Virus assembly

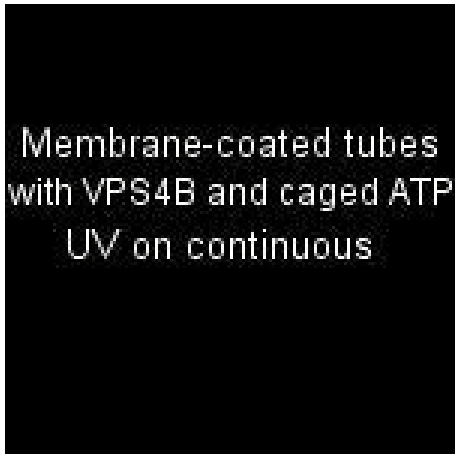
Index	Structure	Single contacts	Total contacts
1.1		0	0
2.1		1	1
3.1		2	2
3.2		2	2
3.3		2	4
4.1		1	4
4.2		3	3
4.3		3	3
5.1		0	6
5.2		4	5

Diamond pentamer represents local minimum of energy as the smallest configuration of dimers that has more interfacial contacts than subunits.

Assembly proceeds by reversible reactions, where subunits forming part of contact-rich structures are likely to persist.

Virus self-assembly proceeds through contact-rich energy minima

For those not only interested in viruses

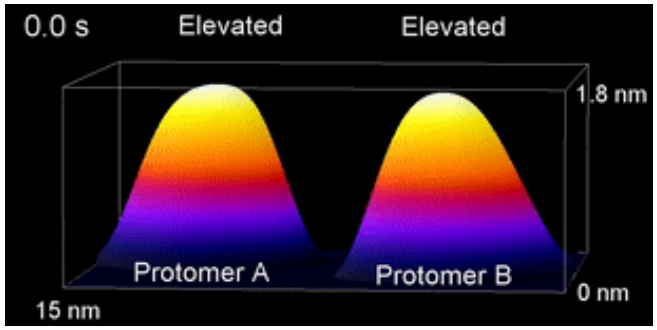


ESCRT:

Maity *et al.* Science Advances (2019)

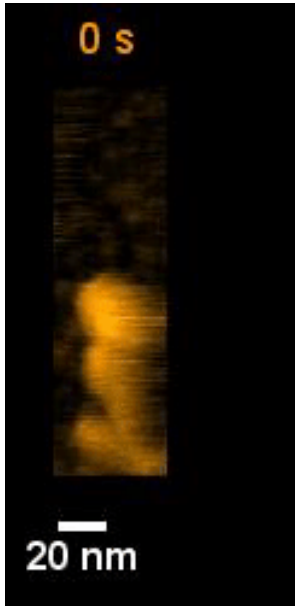
Bertin *et al.* Nat. Comm. (2020)

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



Bacterial transporters:

Maity *et al.* PNAS (2022)



High Speed AFM studies of dynamics

Assembly of synthetic systems:

Maity *et al.* JACS (2020)

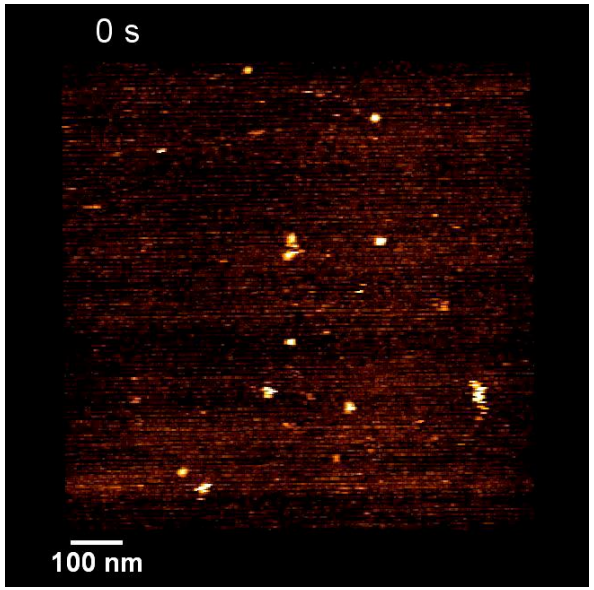
Liu *et al.* Nat. Chem. (2023)

Assembly of antibiotics:

Shukla *et al.* Nature (2022)

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

Shukla *et al.* Cell (2023)



Antimicrobial peptides in action

January 20, 2022

Articles

THE LANCET

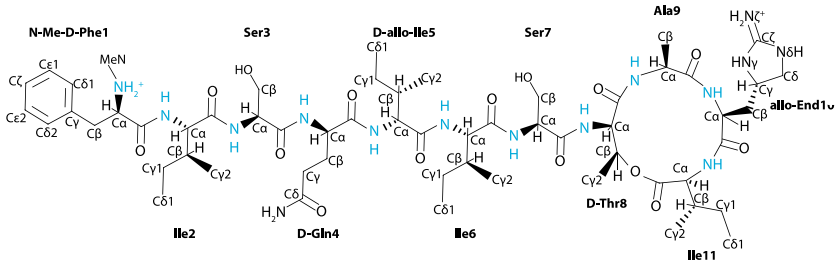
Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis

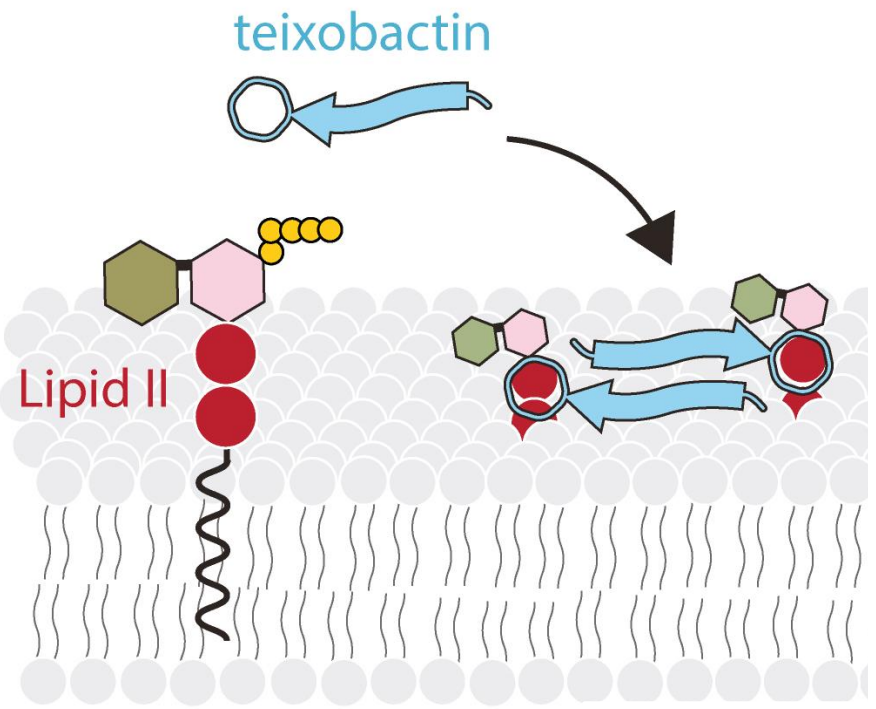
Antimicrobial Resistance Collaborators*

- ***First comprehensive analysis of global impact of antimicrobial resistance (AMR) estimates resistance itself caused 1.27 million deaths in 2019, and that antimicrobial-resistant infections played a role in 4.95 million de***

Molecular Mechanism of Antimicrobials…

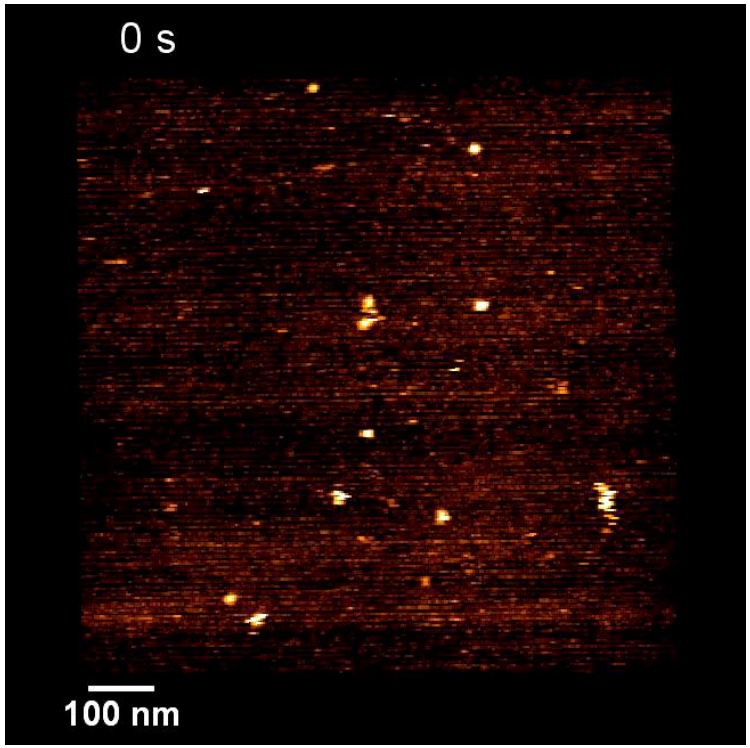
Antimicrobial peptides in action: Teixobactin

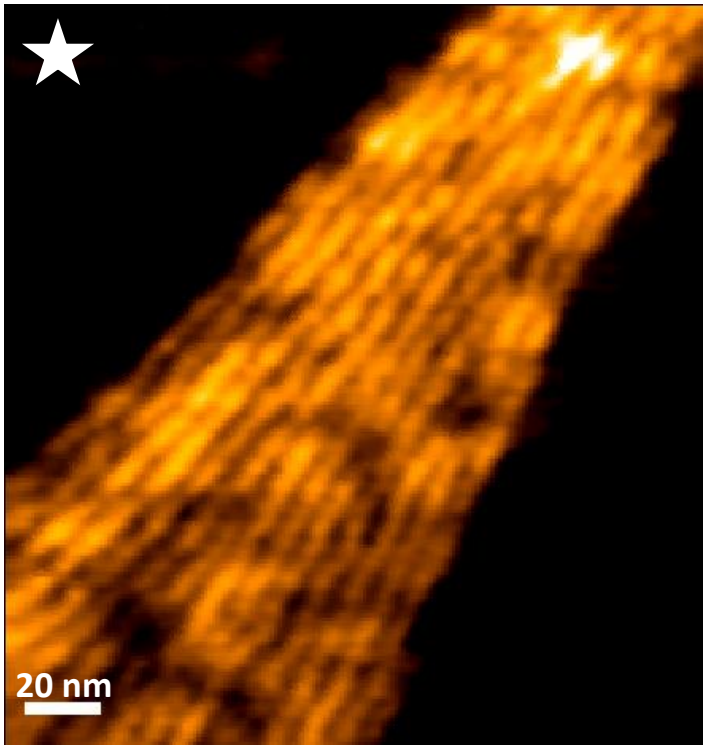




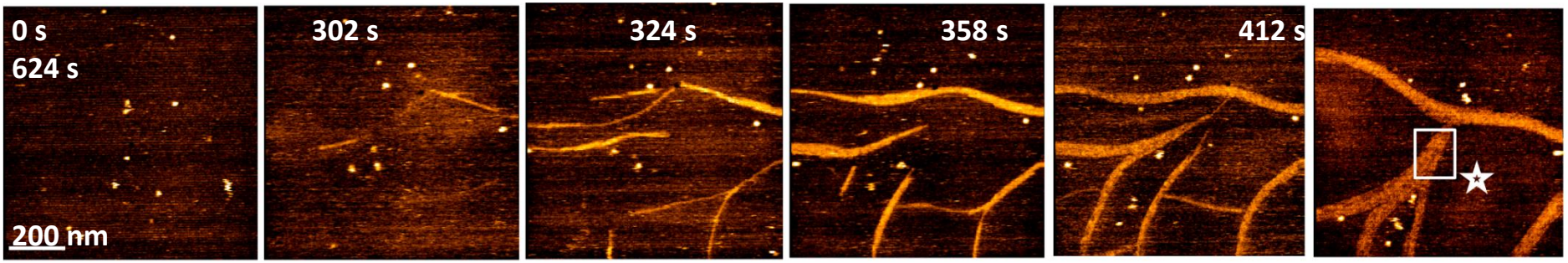
- **Active against methicillin-resistant *Staphylococcus aureus* and *Mycobacterium tuberculosis*.**
- **Teixobactin targets Lipid II (precursors for peptidoglycan synthesis).**

Antimicrobial peptides in action: Teixobactin



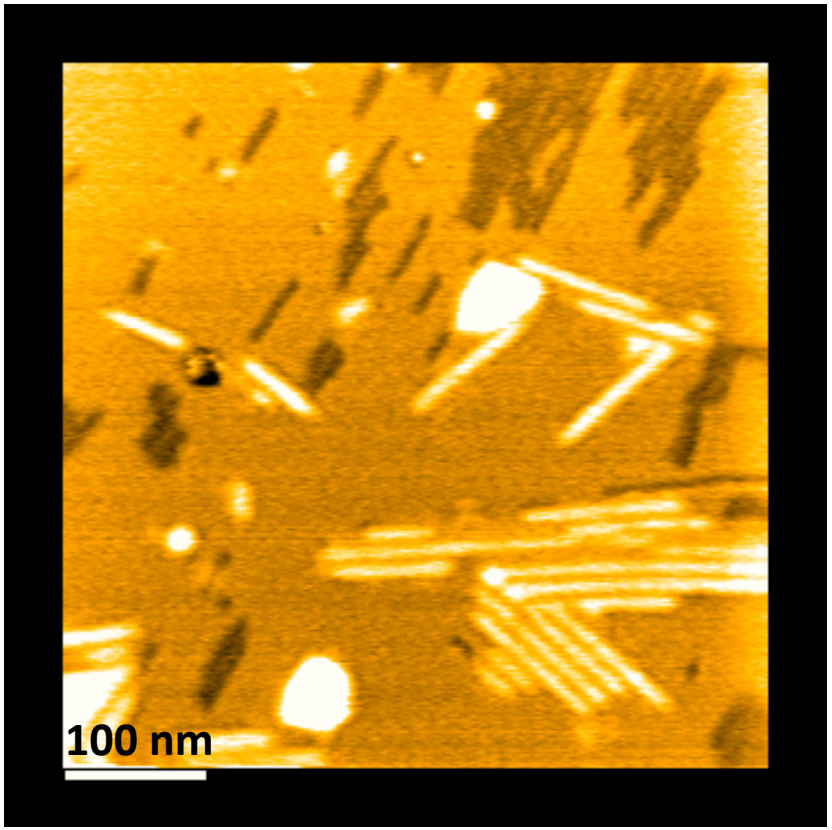


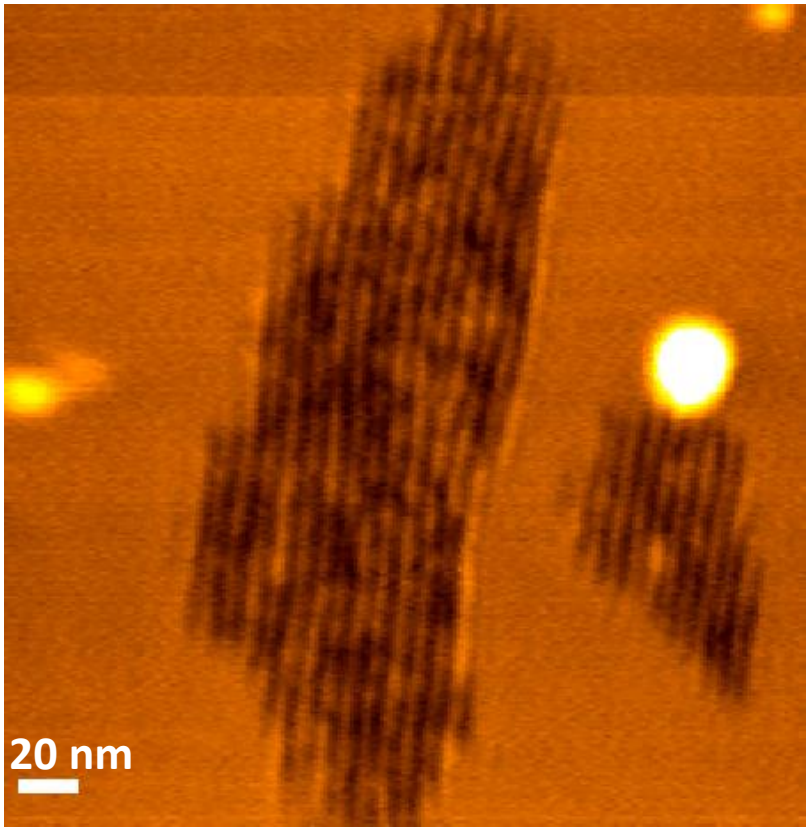
Shukla *et al.* Nature (2022)



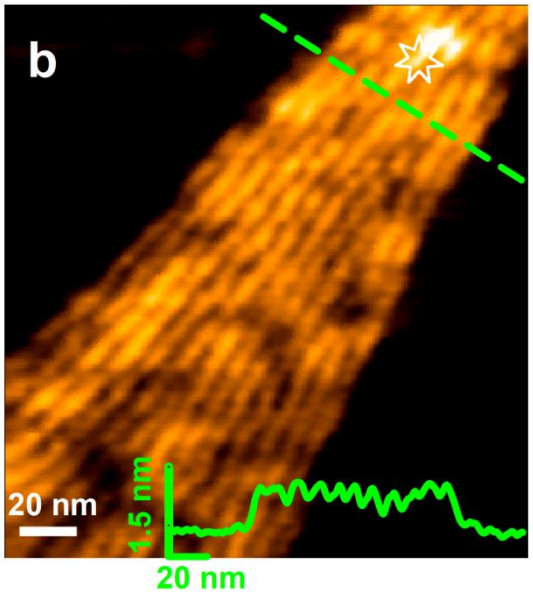
Antimicrobial peptides in action: Teixobactin

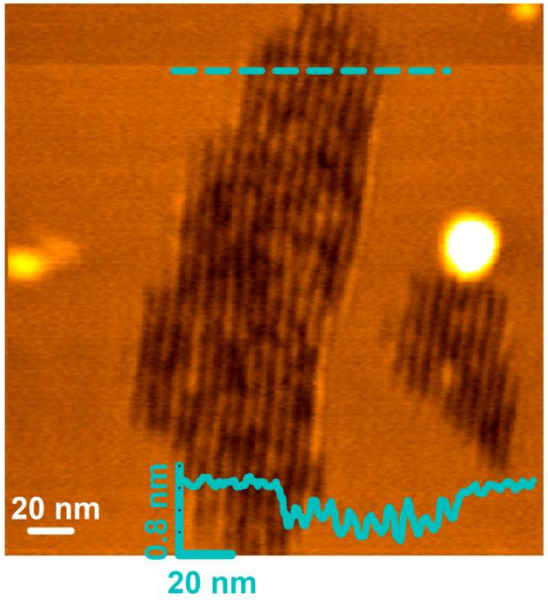
After 30 minutes….

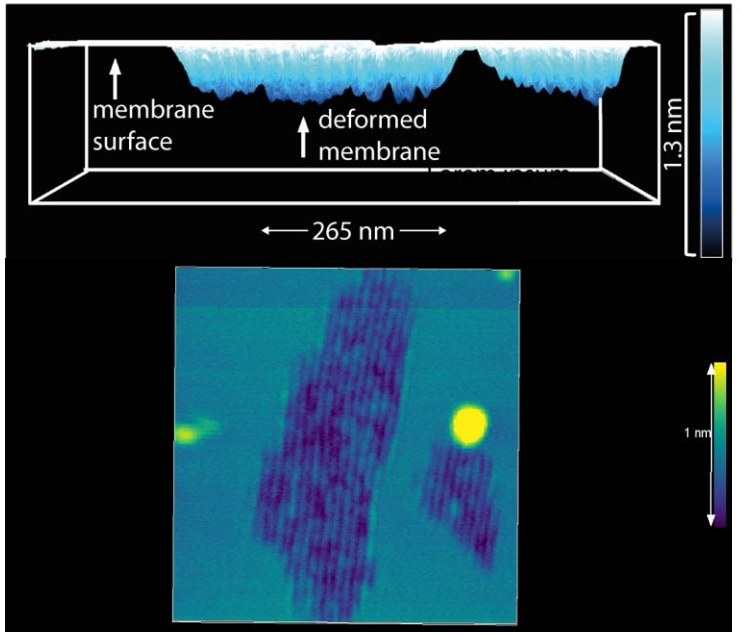


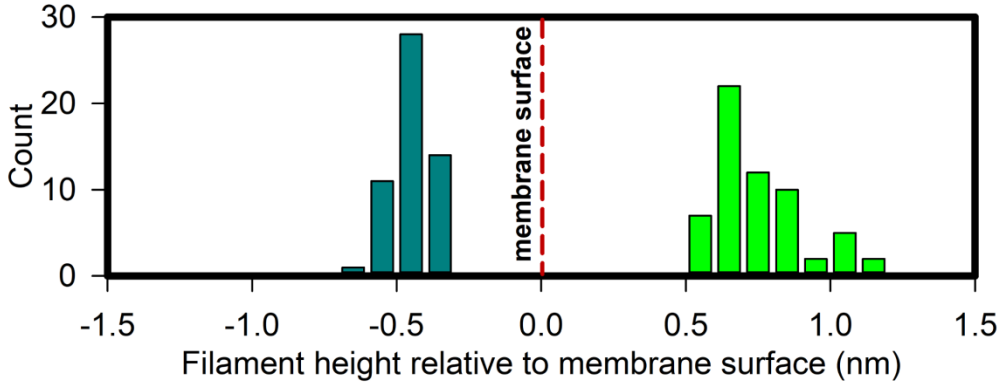


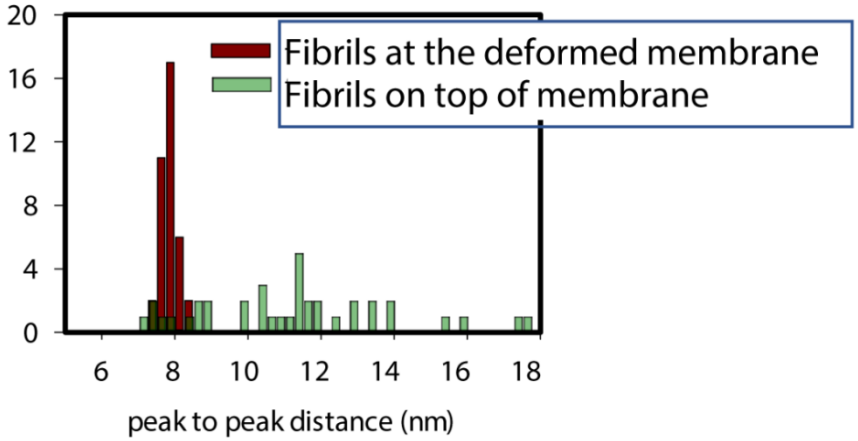
Antimicrobial peptides in action: Teixobactin



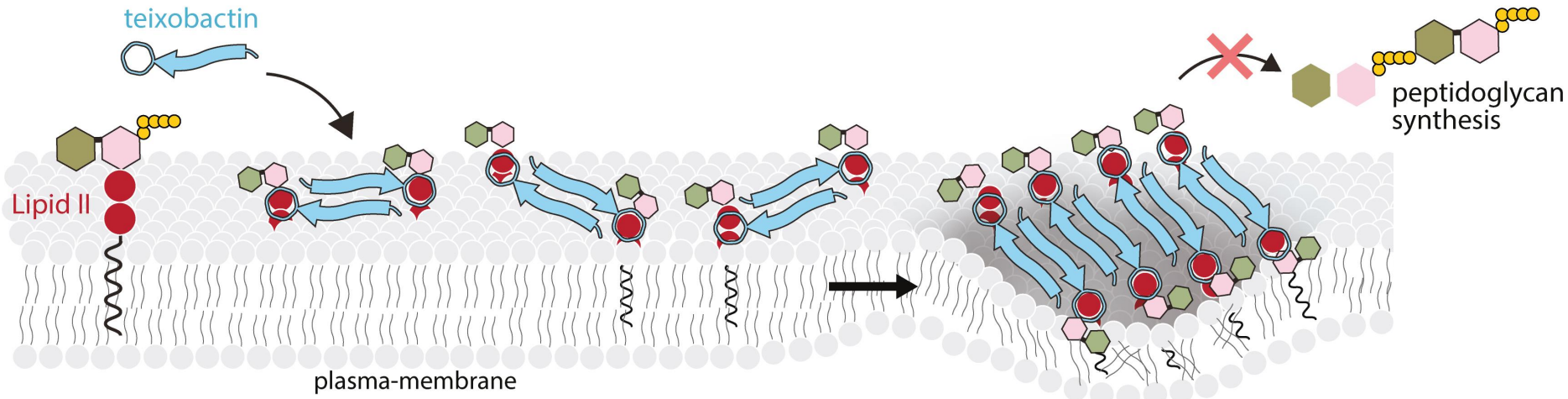


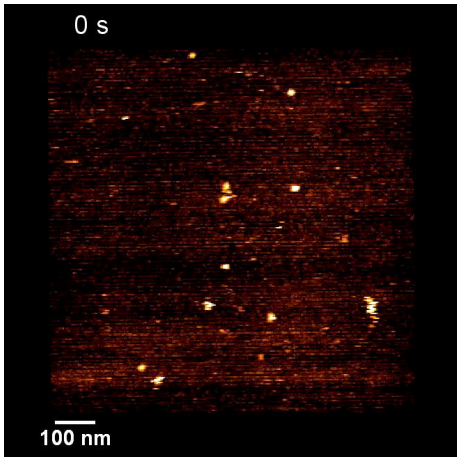


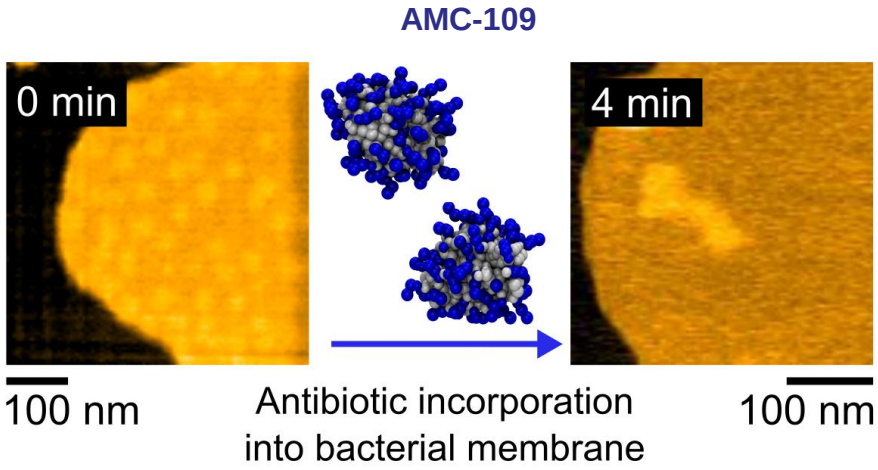




Antimicrobial peptides in action







Teixobactin: Shukla *et al.* Nature (2022)
Clovibactin: Shukla *et al.* Cell (2023)

Melcrova *et al.* Nature Communications (2023)

Acknowledgments

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O-ChemS
Out-of-equilibrium Chemical Systems

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