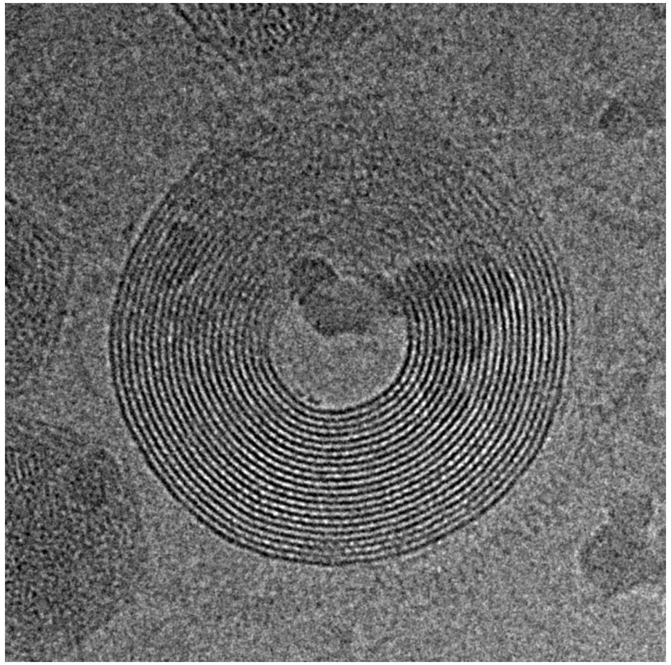


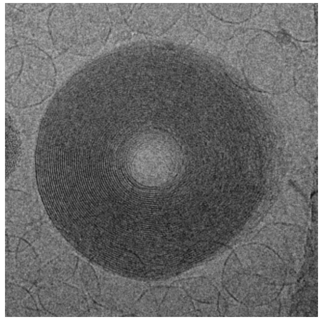
Morphogenesis and ultrastructure of condensed DNA toroids

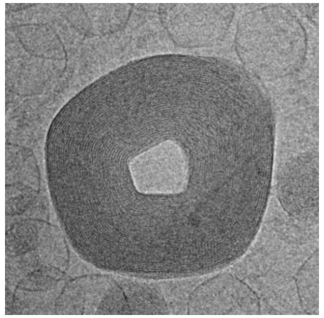
analysed by cryo-TEM

Kahina VERTCHIK[1,2]

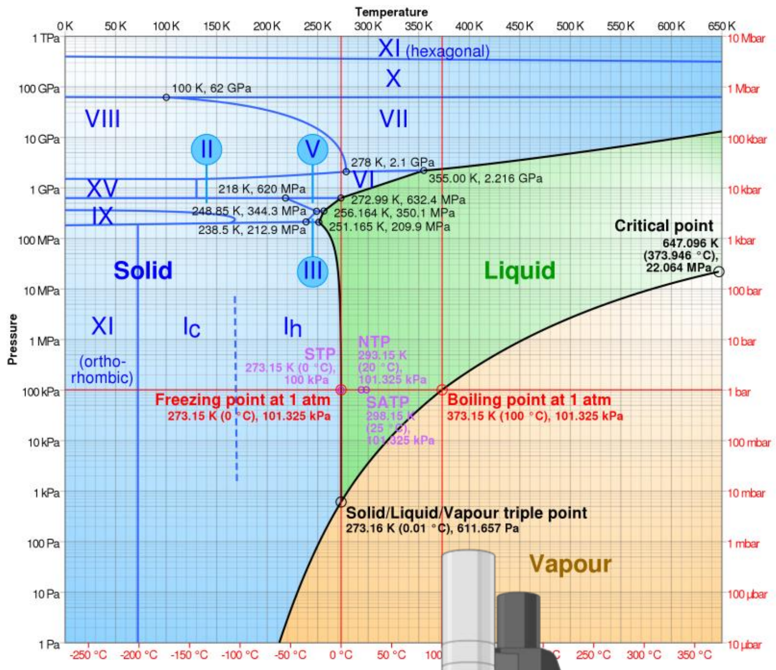
[1] JEOL Europe (SAS), 78290 Croissy sur Seine, France

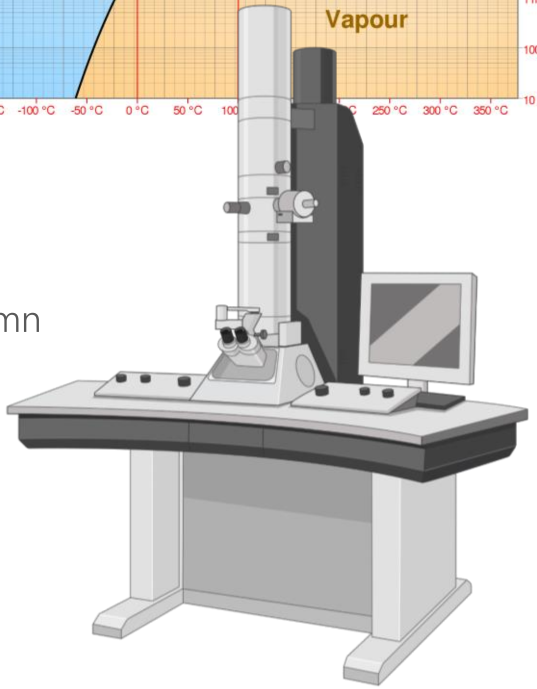
[2] Université Paris-Saclay, CNRS, Laboratoire de Physique des Solides, 91405, Orsay, France





What is cryo electron microscopy ?





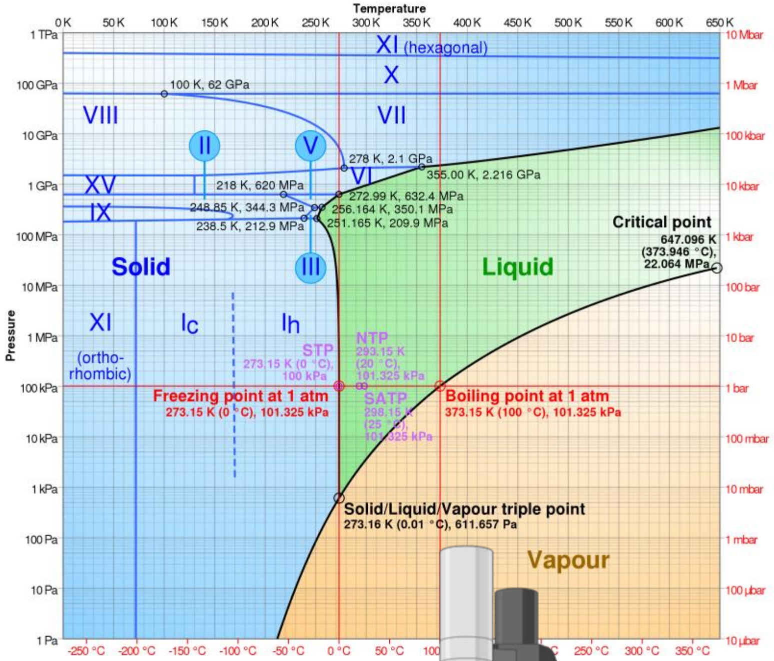
P≈ $10^{-10}\ Bar$
inside the column

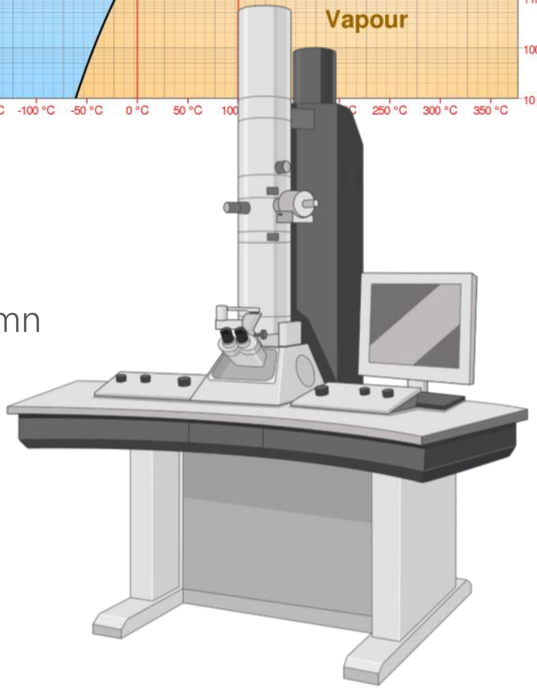
Observing biological samples with nm resolution ?
proteins, viruses, DNA …

- composed of H2O, C, and low atomic nb atoms

- Systems in solution = do not resist to high vacuum

What is cryo electron microscopy ?





P$\approx 10^{-10}\,Bar$
inside the column

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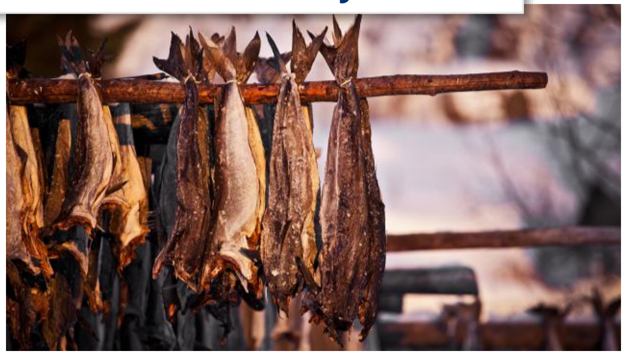
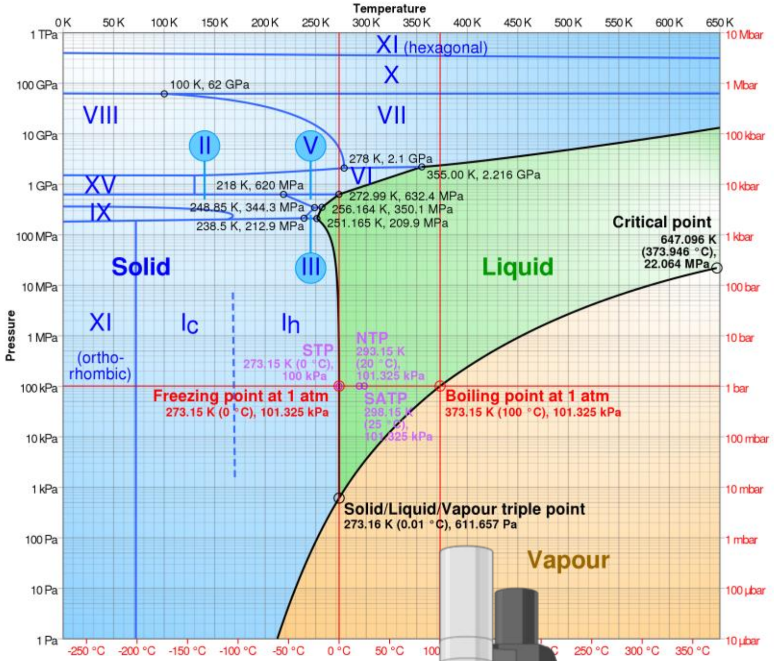
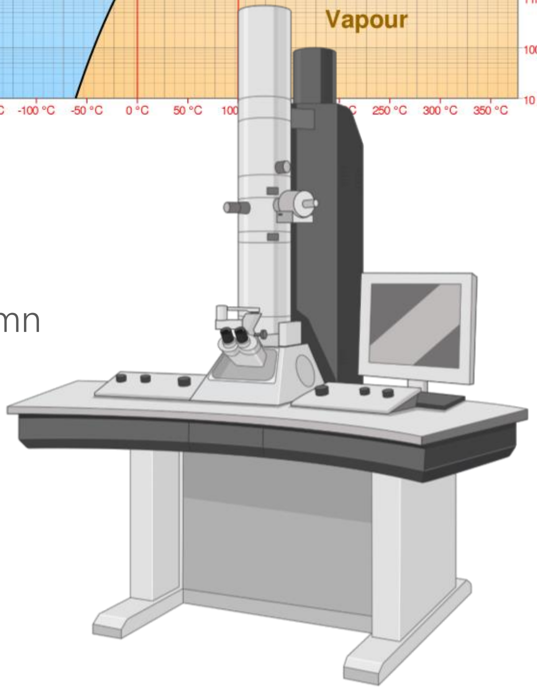
Usual method = **dehydration**



Far from the native state of the biological sample …

What is cryo electron microscopy ?





$P \approx 10^{-10}$ *Bar*
inside the column

Observing biological samples ?

proteins, viruses, DNA …

- composed of H2O, C, and low atomic nb atoms

- Systems in solution = do not resist to high vacuum

Usual method = **dehydration**

Far from the native state of the biological sample …

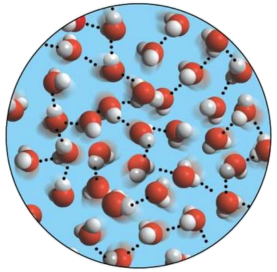
Freezing

Keep water in a solid state

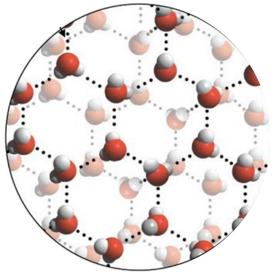
Native environment is preserved

What is cryo electron microscopy ?

Classical freezing
Not enough to preserve the sample



Liquid state

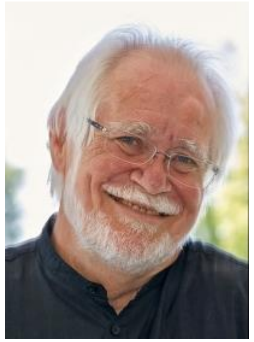


Solid state
Nucleation of ice crystal

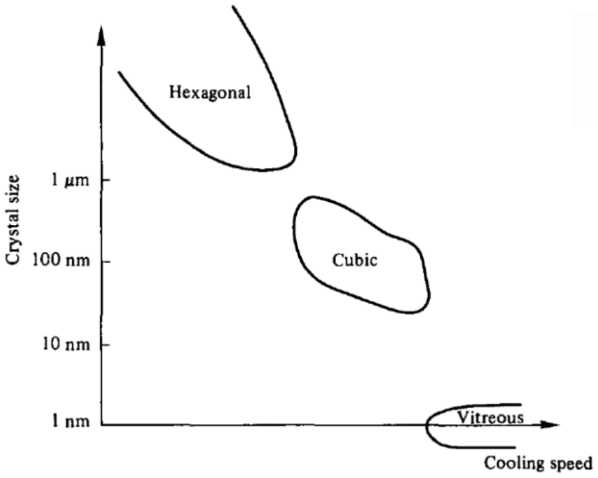
Deformation of the structure

Vitrification
To avoid crystallization

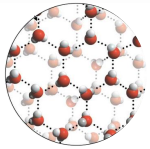
Cooling fast enough to avoid crystallization > 100000 °/sec with cryogenic fluids

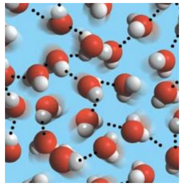


J. Dubochet
EMBL, Univ. Lausanne



crystalline

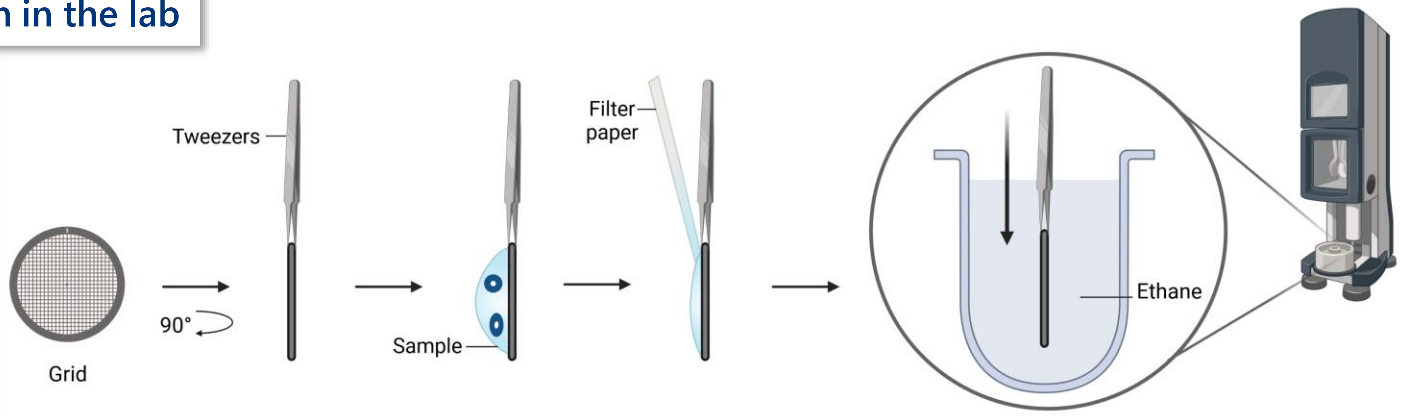




amorphous

Relationship between cooling speed and crystal ice size

Vitrification in the lab



What is cryo electron microscopy ?

Cryo-TEM = **Electron microscopy** + **Vitrification technics** to observe hydrated samples in their native environment

DNA toroids - introduction

DNA strand = semi-flexible
polymer negatively charged

DNA toroids - introduction

Coil

DNA strand in solution

Worm-like random coil conformation

DNA toroids - introduction

Coil

Phase transition

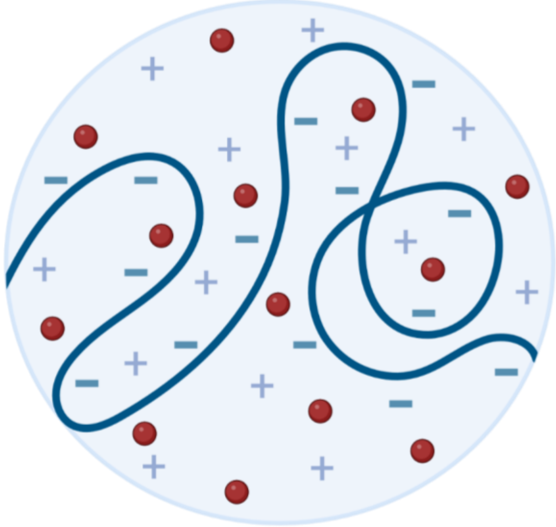
Globule

Adding
multivalent
cations

spermine (4+)

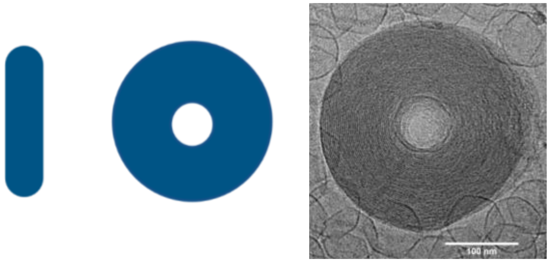
DNA strand in solution

Worm-like random coil conformation



Spontaneous
CONDENSATION
Self-assembly

DNA toroid

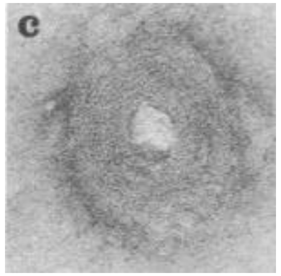


DNA rod

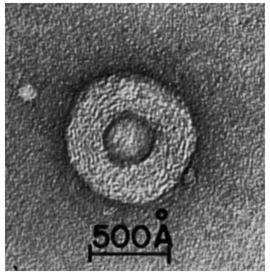
DNA toroids - introduction

A model system for physics and biology

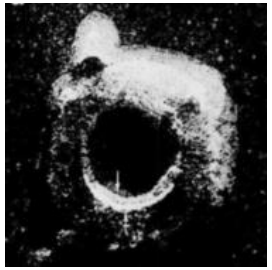
Typical of semi-flexible polymer
Different condensing agents, different molecules



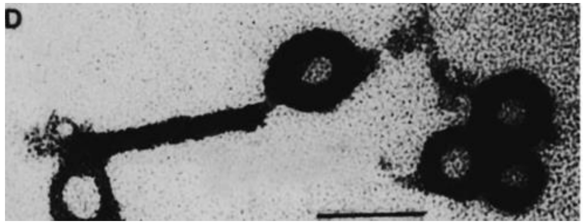
Laemmli, 1975
Polylysine+ DNA



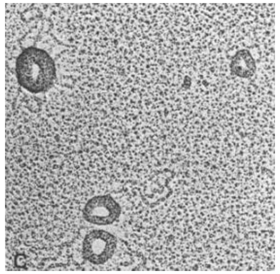
Gosule et al, 1976
Spermidine+ DNA

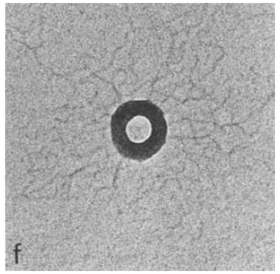


Marx et Ruben, 1986
Spermidine+ DNA



Arscott et al, 1990
Hexamine Cobalt+ DNA





Garcia-Ramirez et al, 1994

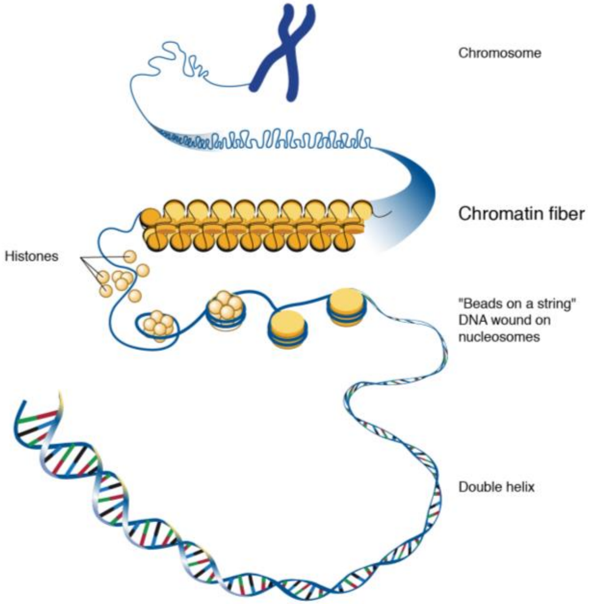
Φ0 + DNA

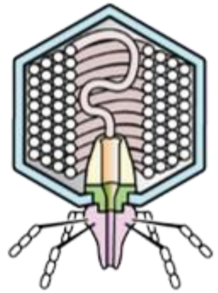
H5+ DNA

Clupeine+ DNA

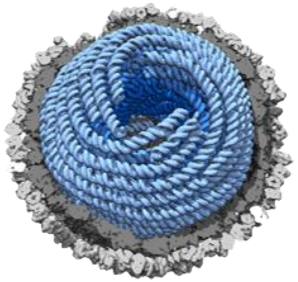
A phenomenon vastly studied experimentally but no complete model exists yet …

Understanding condensed states of DNA
in biological systems

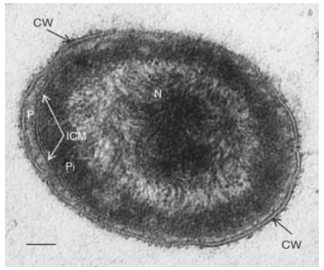




Bacteriophage T3/T7
Serwer et al, 2019



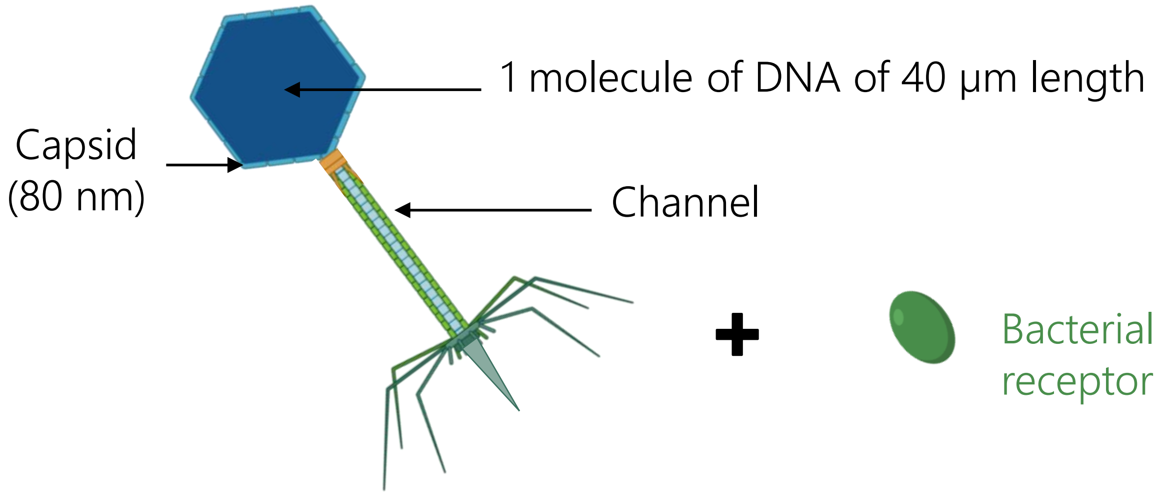
Double stranded ARN virus
Huiskonen et al, 2019



Bacterial nucleoid
Hobbot & Kellenberger, 1985

Preparation of DNA toroids

✓ DNA reservoir : **T5 bacteriophage**



✓ Condensing agent :

Preparation of DNA toroids

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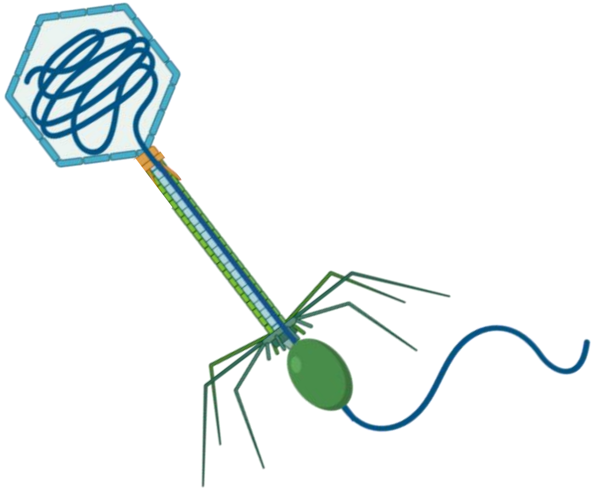

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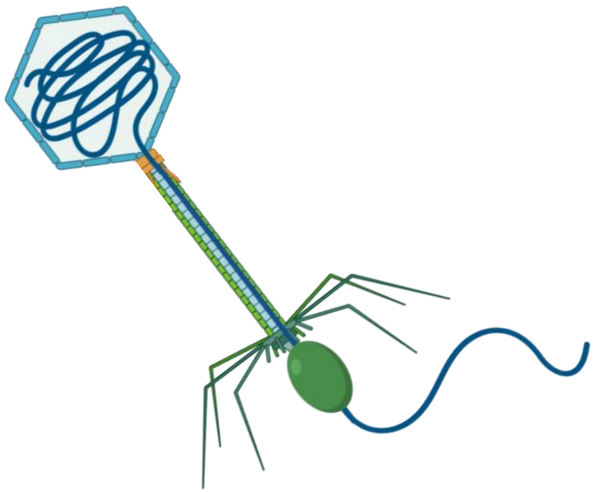
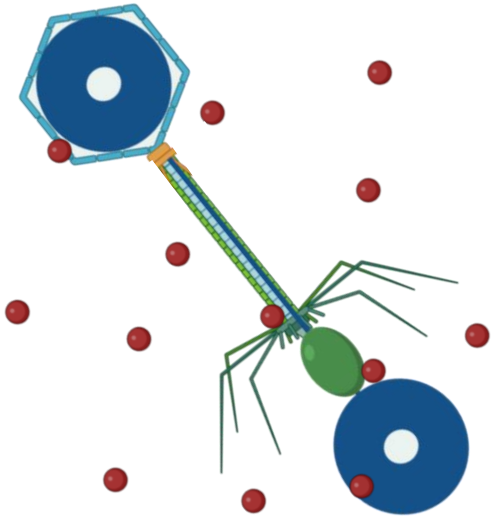


✓ Condensing agent : **Spermine 4+**



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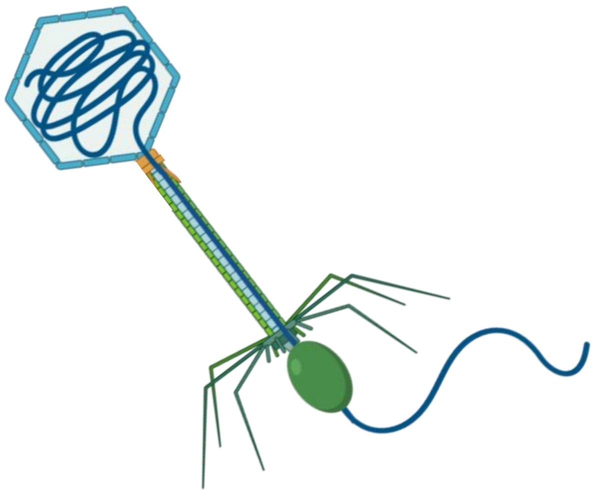

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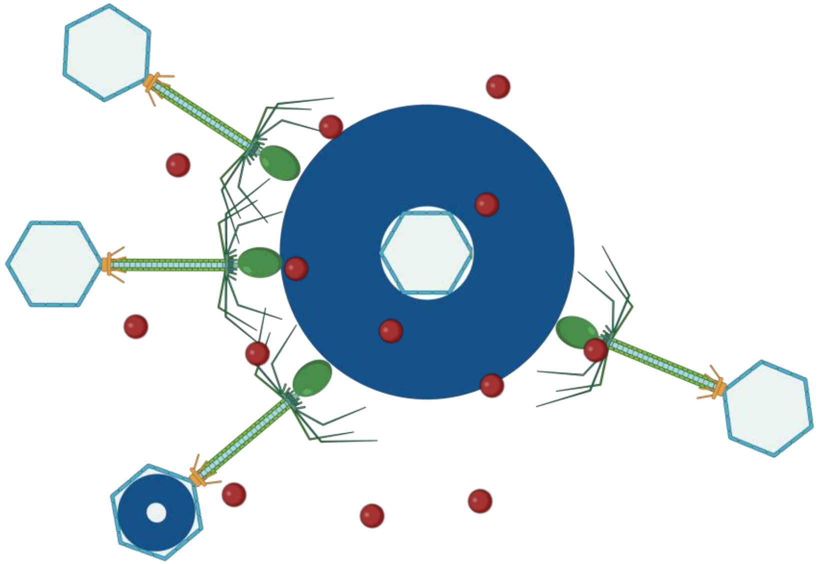

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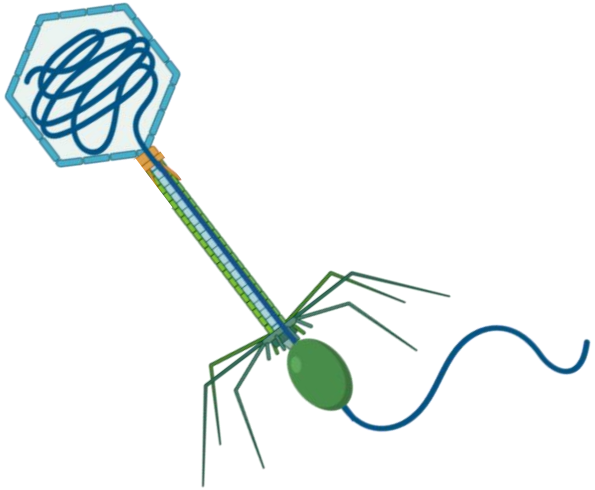

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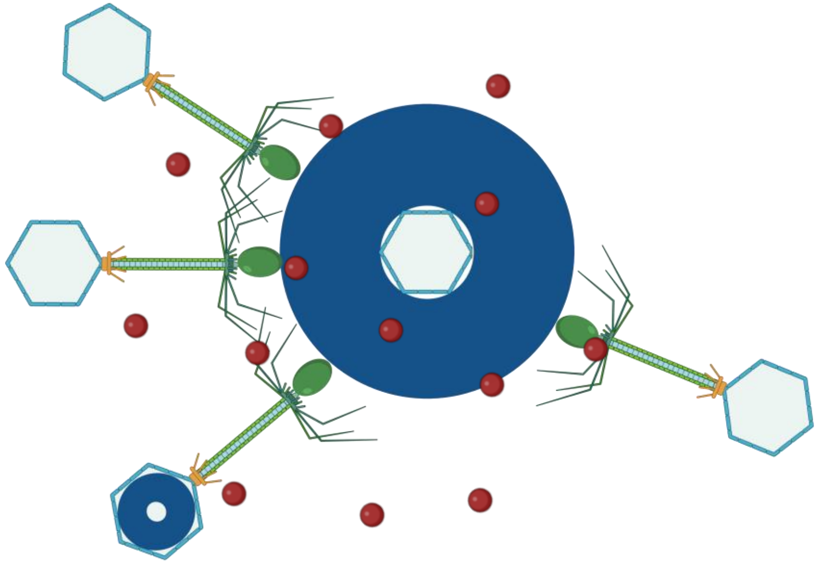
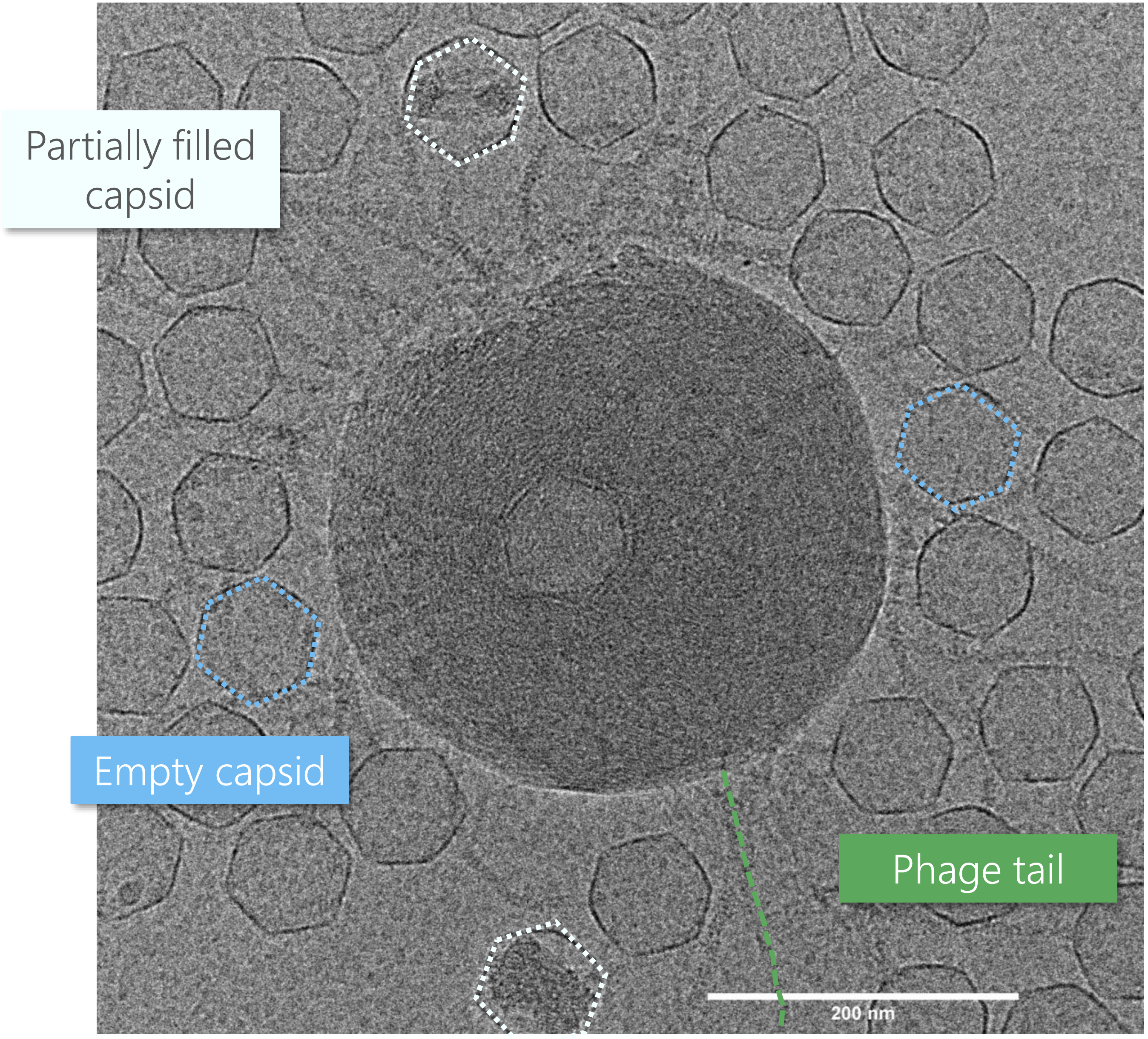
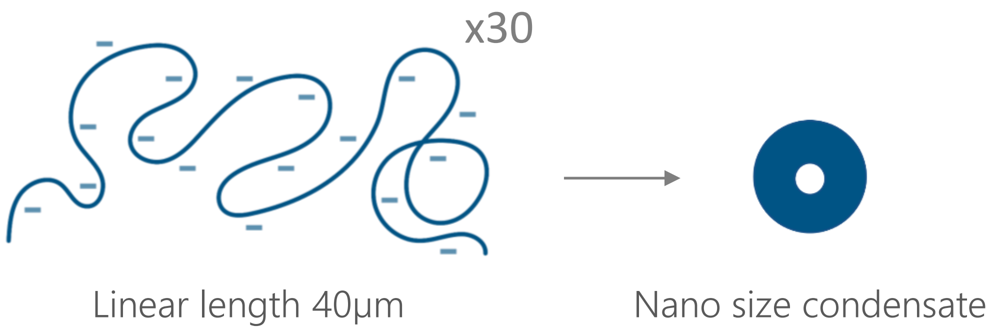


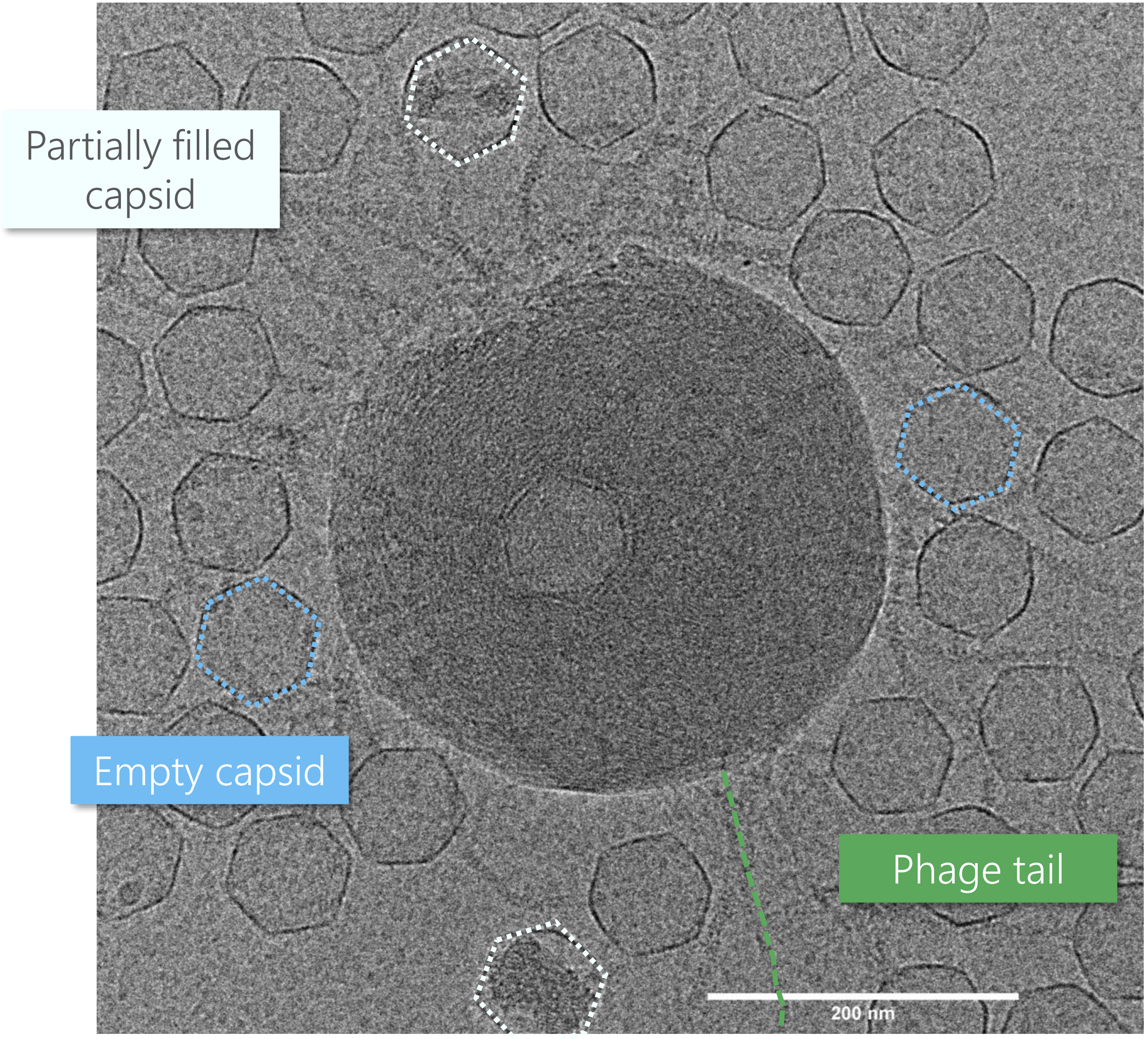


Preparation of DNA toroids

Typical dimensions of our final objects

- External diameter ~ 250 nm
- Internal diameter ~ 90 nm
- Thickness ~ 200 nm
- 1 toroid ~ **30 DNA molecules**





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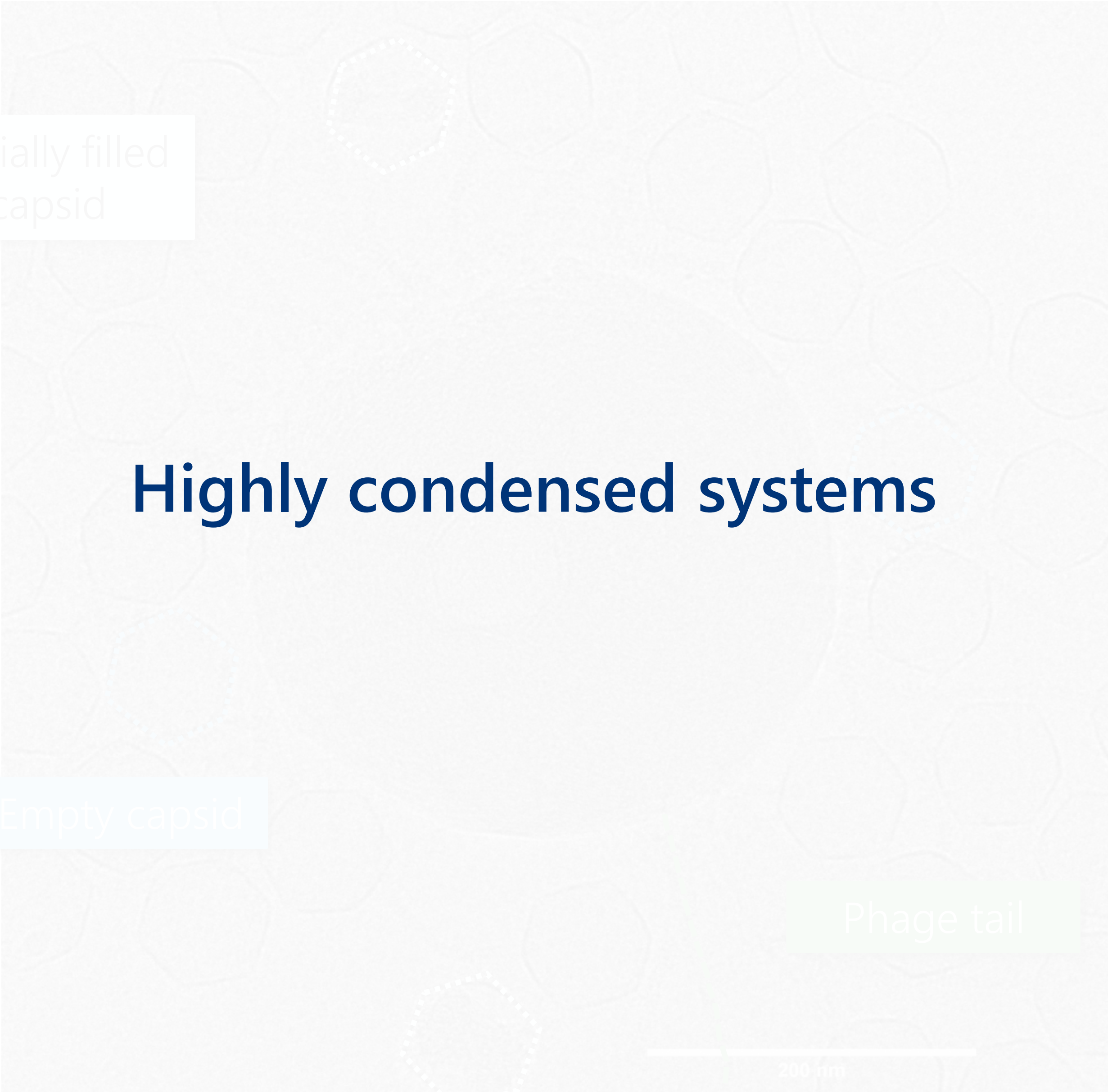
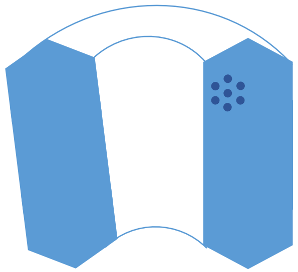
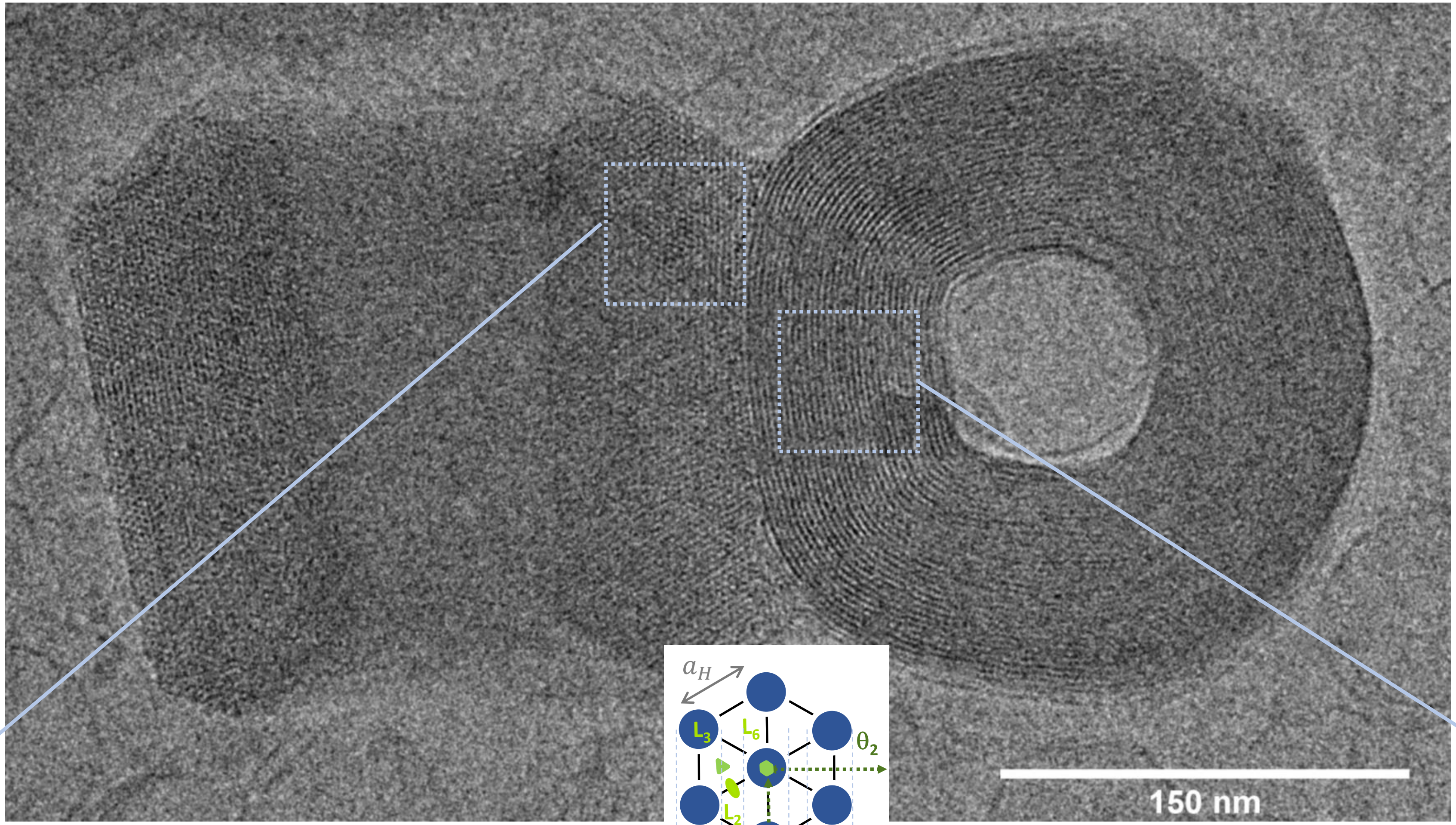
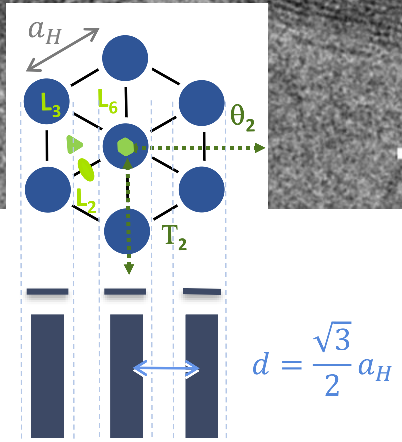


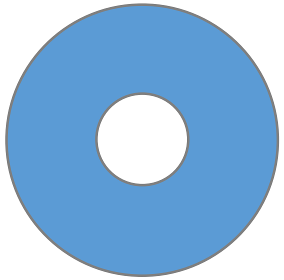
DNA toroids = Highly condensed systems

Microscopic structure : Local hexagonal order

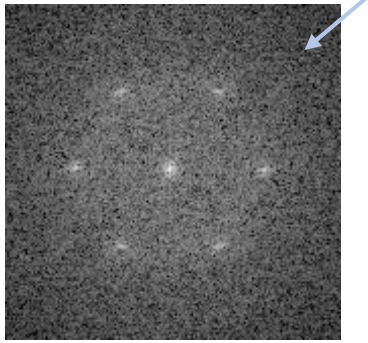




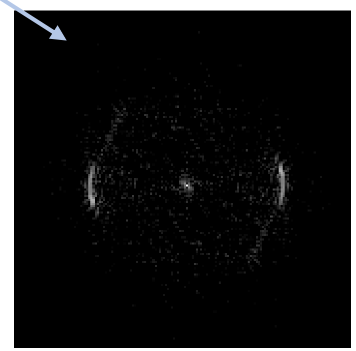




Hexagonal lattice (d)



Lattice spacing d



Microscopic structure : Local hexagonal order

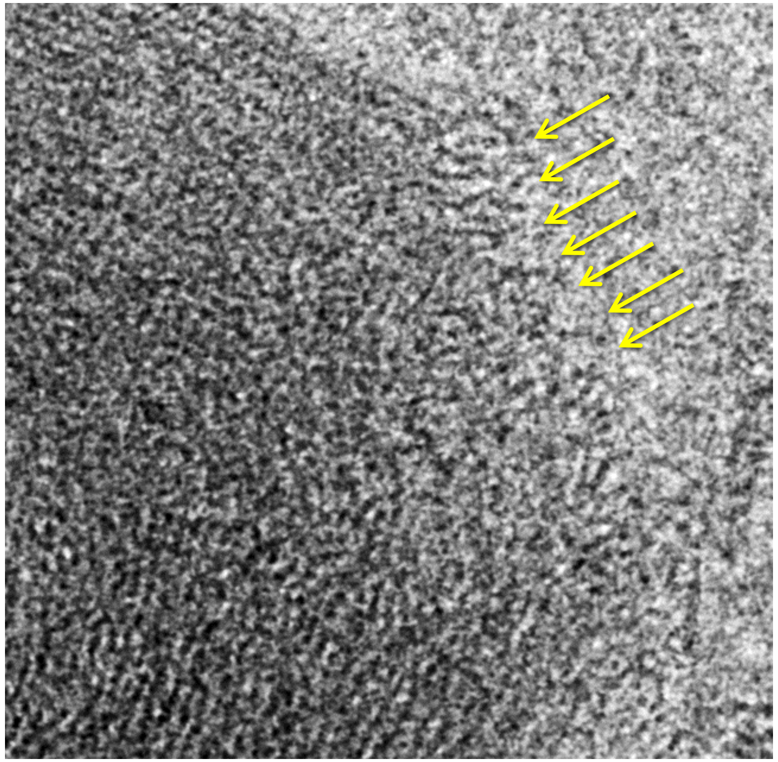
DNA toroids = Ordered systems

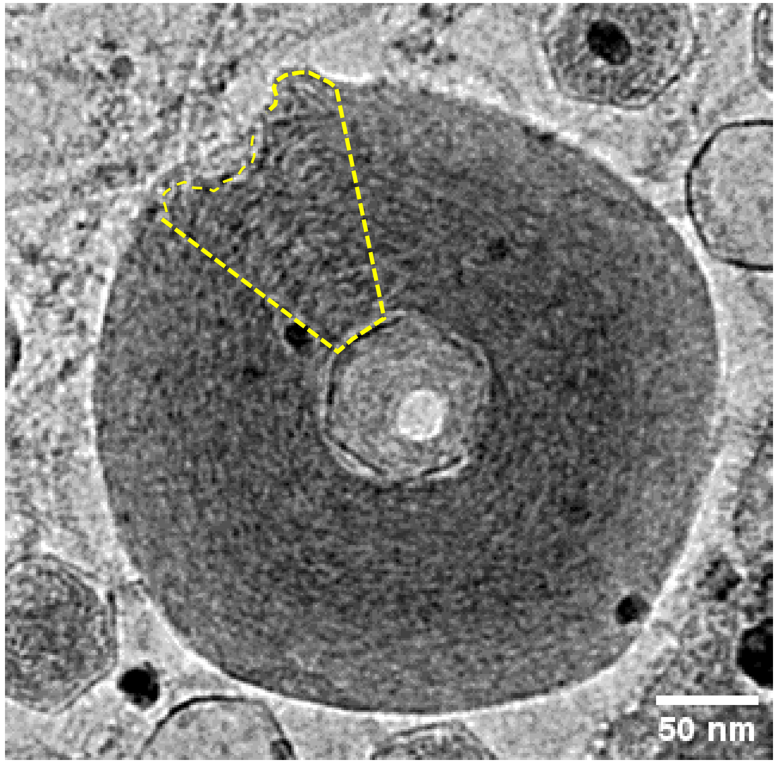
Made of ~30 DNA strands
that spontaneously organized in an hexagonal lattice

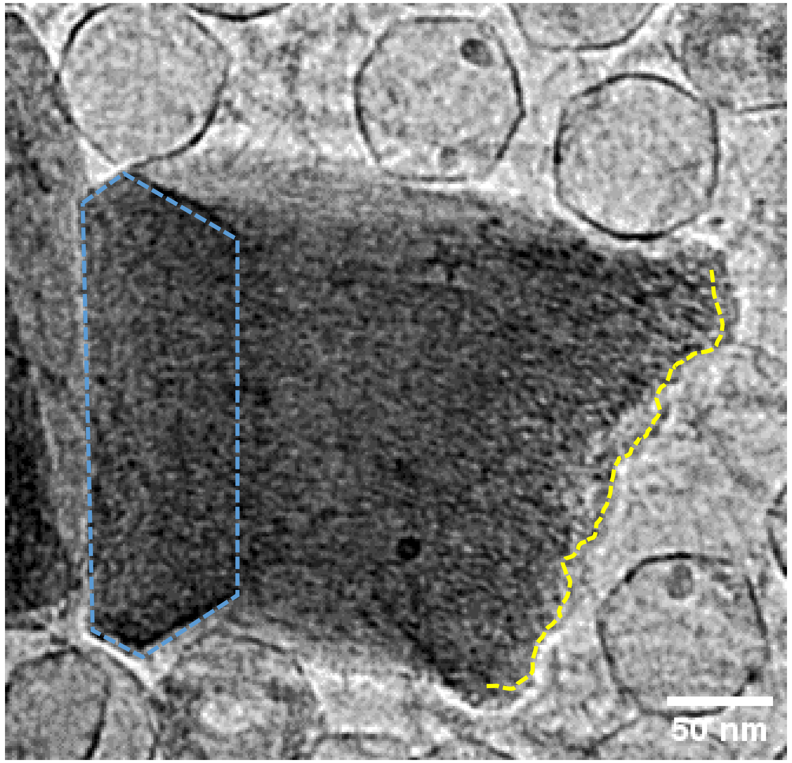
Hexagonal lattice (*d*)

Lattice spacing *d*

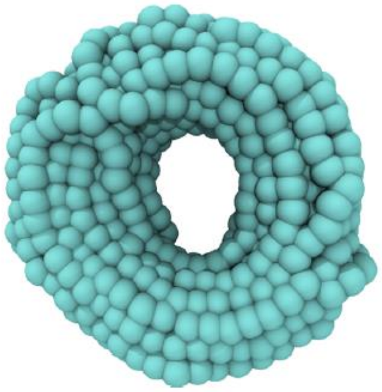
Existence of a disordered radial region

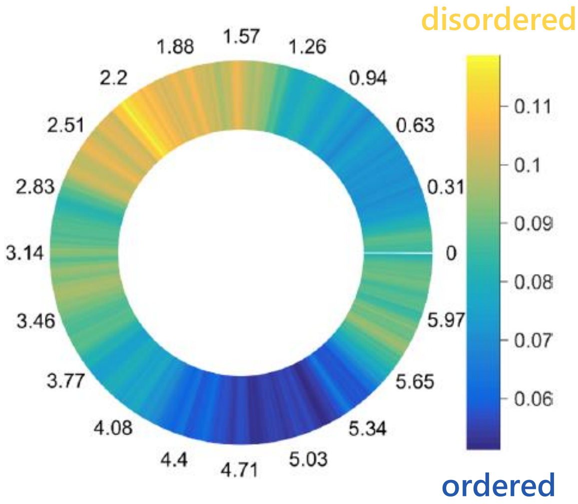






Segregation order / disorder



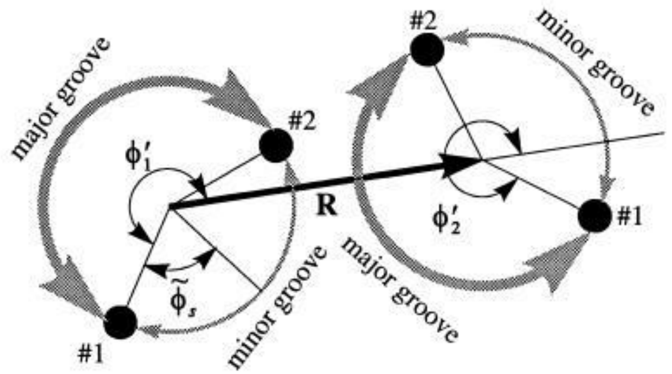


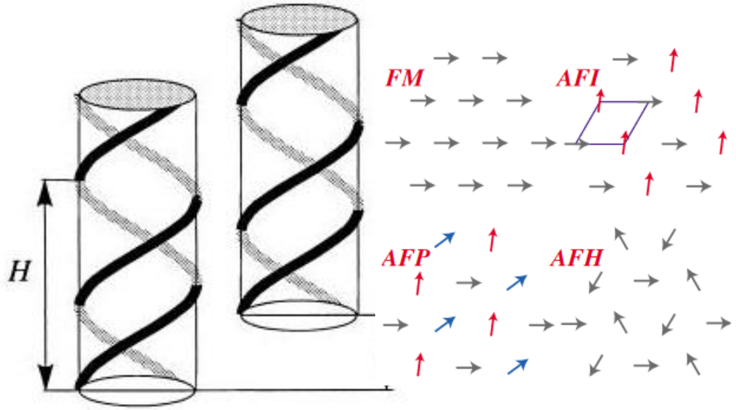
Simulations

Dey et al, J. Phys. Chem B, 2017

Correlations between DNA helices

Kornyshev – Leikin theory
« electrostatic zipper »



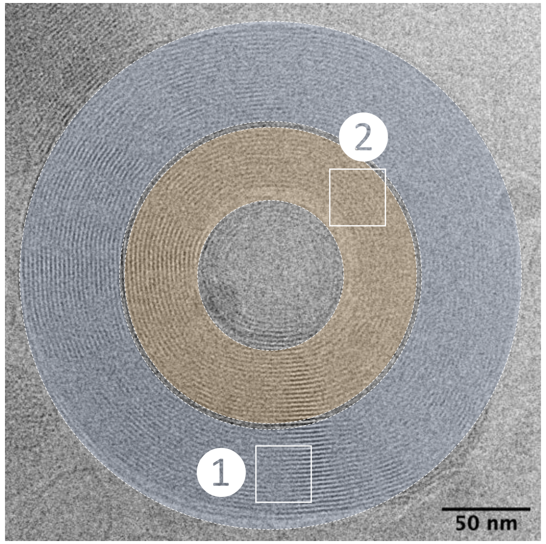


Kornyshev & Leikin, 1998; Harreis et al, 2001;
Wynveen et al, 2005

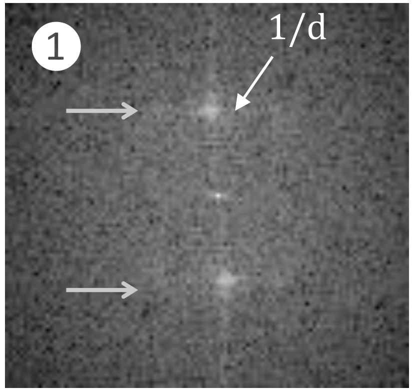
Radial curvature variation

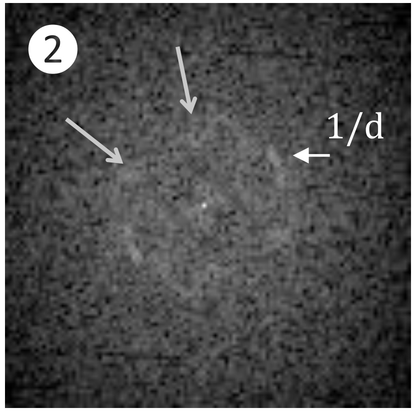
Barberi et al, 2021

Outer region
Low curvature

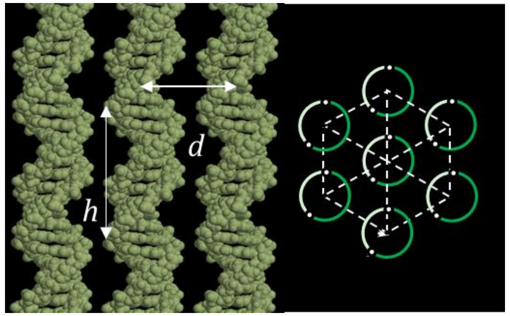


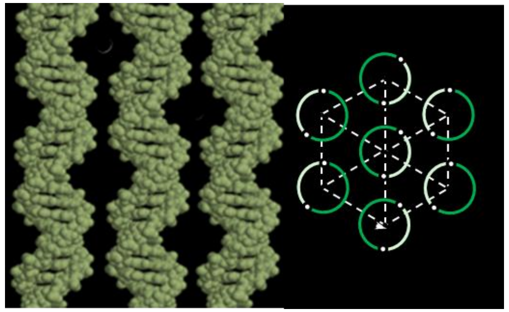
Inner region
High curvature





ferromagnetic order





antiferromagnetic order

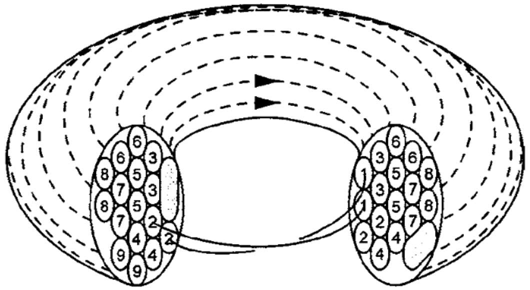
Observation

Kornyshev et al, 2005; Leforestier & Livolant, 2009; Barberi et al, 2021

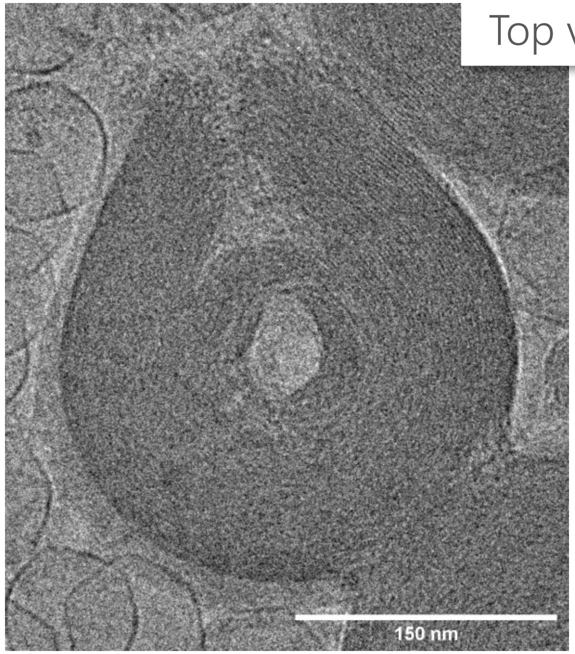
Molecular pathway and toroid morphogenesis

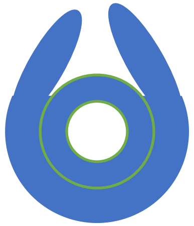
Coil spooling model

Marx & Ruben, 1984, 1985;
Böttcher et al, 1998;
Hud & Downing, 2001

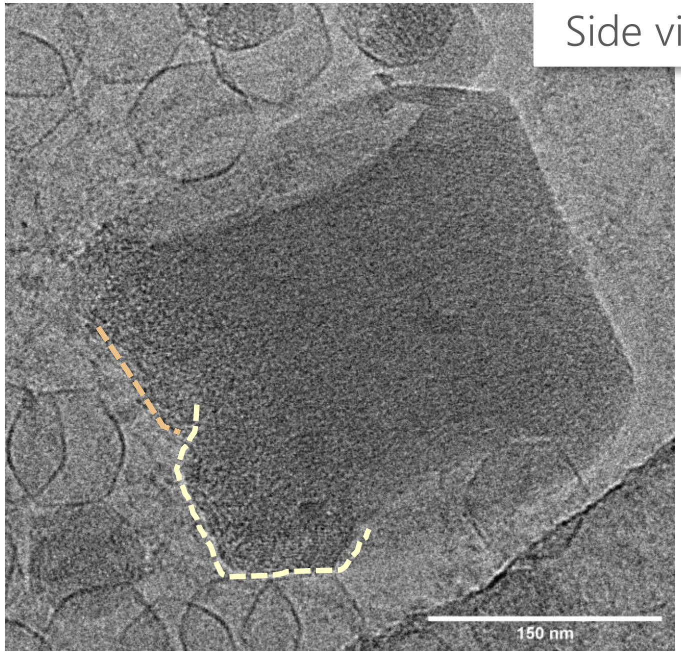


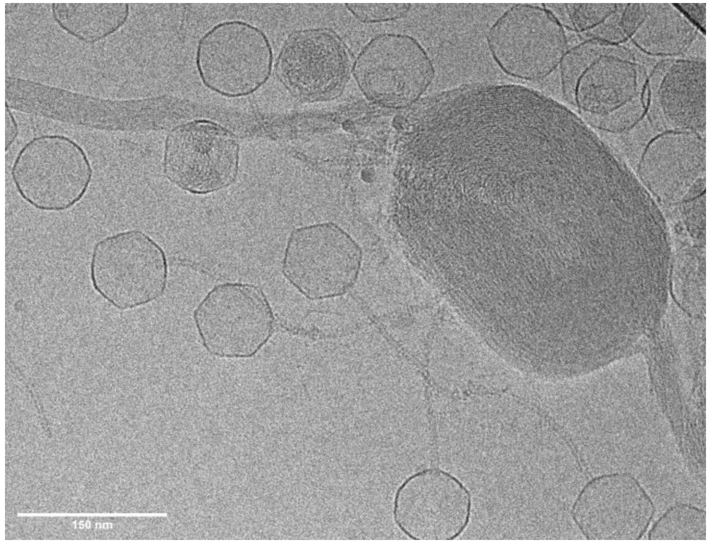
Top view





Side view





Molecular pathway and toroid morphogenesis

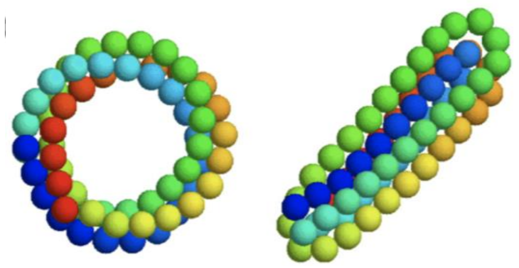
Competition toroid / rod
As ground state of DNA condensation

Sakaue & Yoshikawa, 2002
Hoang … Podgornik & Maritan, 2015
Dey & Redding, 2017
Sun, … & Nordenskiöld 2019

Loop nucleation

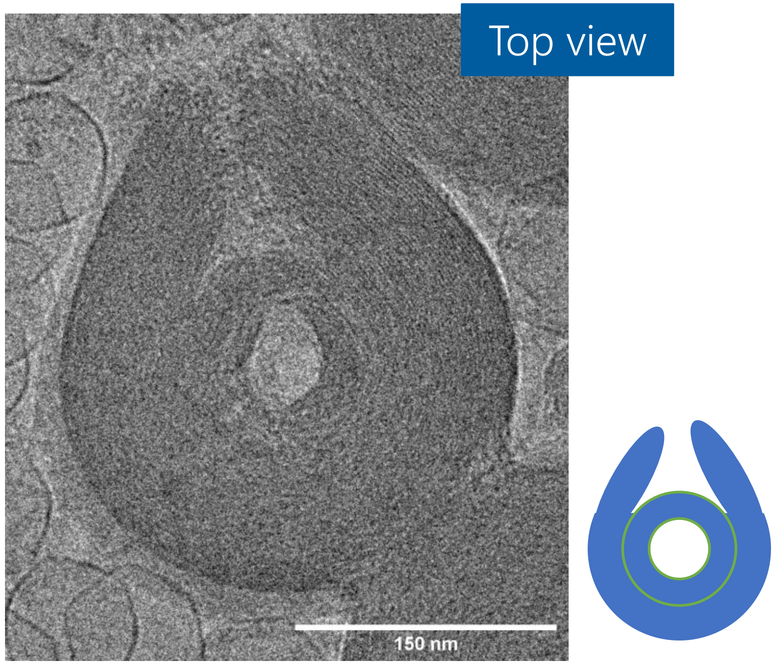
Toroid

Rod (hairpin)

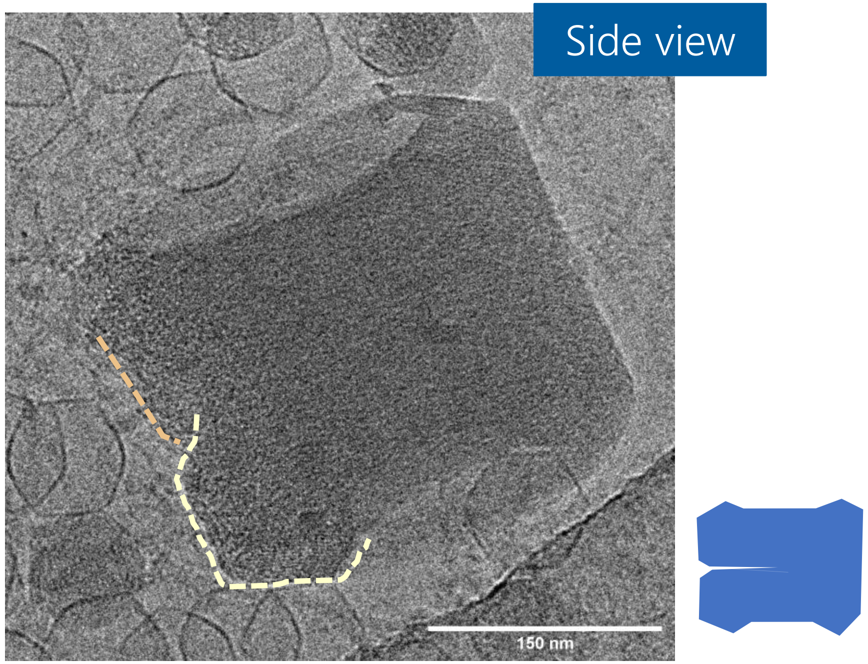


Hoang … Podgornik & Maritan, 2015

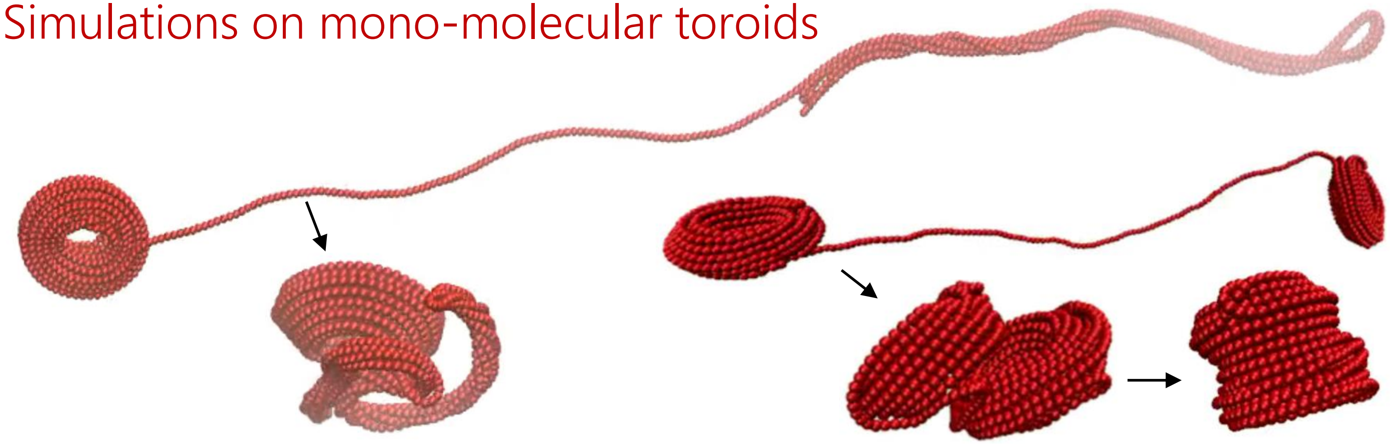
Top view



Side view



Simulations on mono-molecular toroids



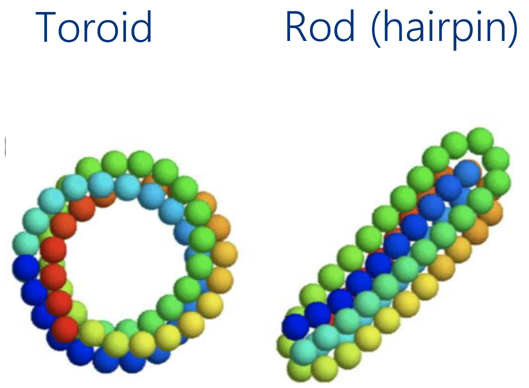
Sun, … & Nordenskiöld, NAR 2019

Molecular pathway and toroid morphogenesis

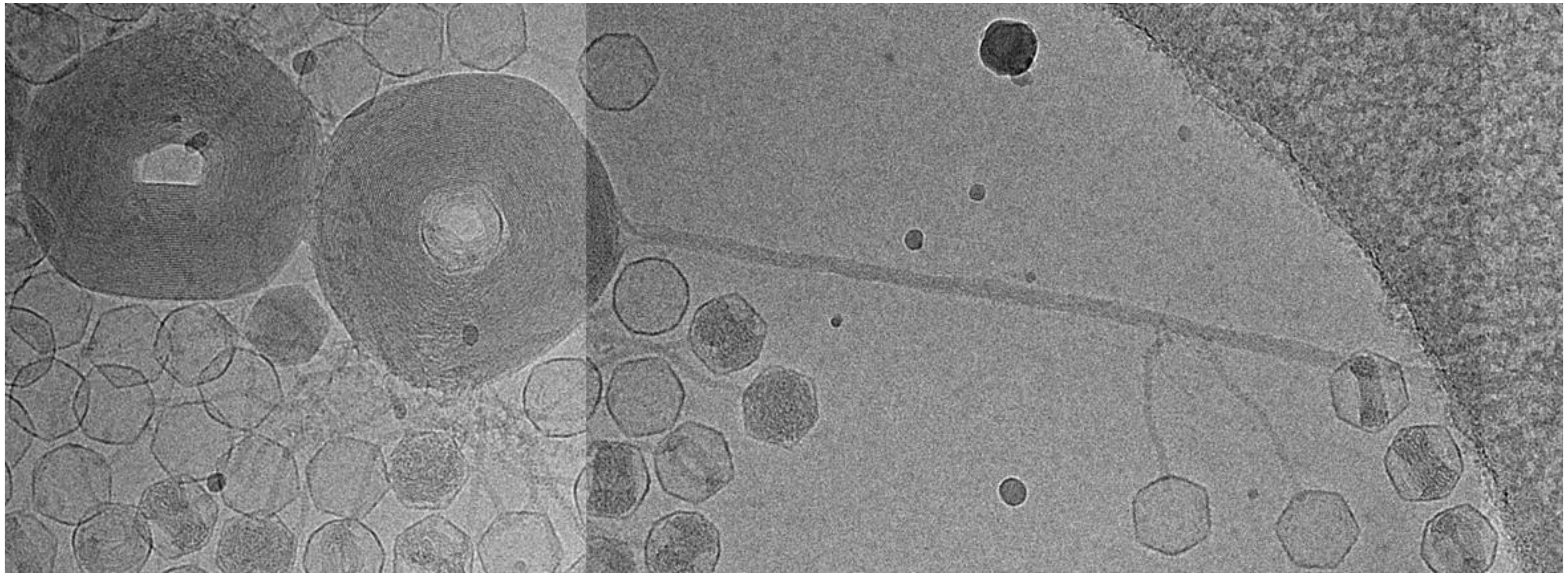
Competition toroid / rod
As ground state of DNA condensation

Sakaue & Yoshikawa, 2002
Hoang ... Podgornik & Maritan, 2015
Dey & Redding, 2017
Sun, ... & Nordenskiöld 2019

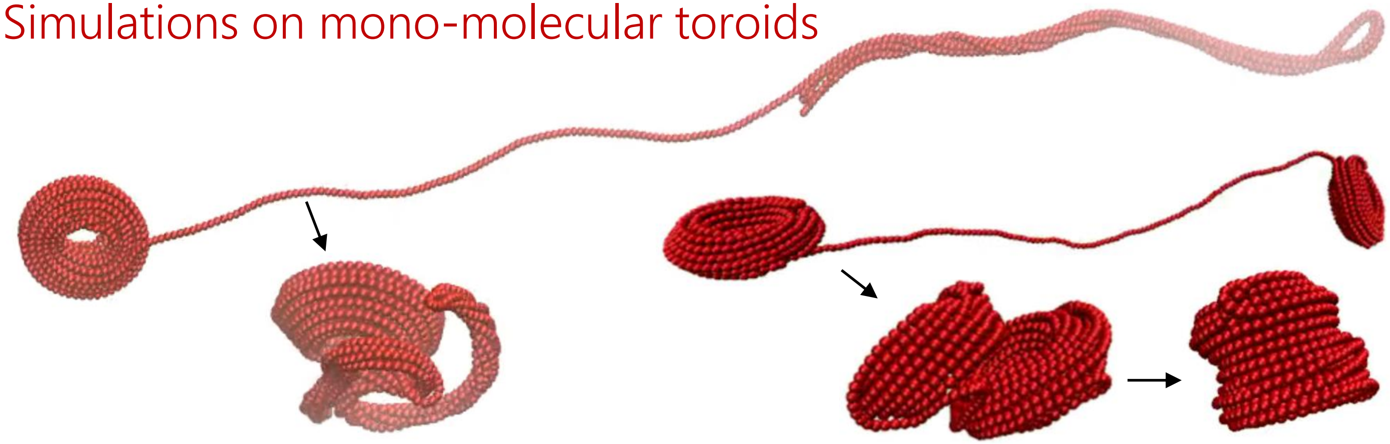
Loop nucleation



Hoang ... Podgornik & Maritan, 2015



Simulations on mono-molecular toroids

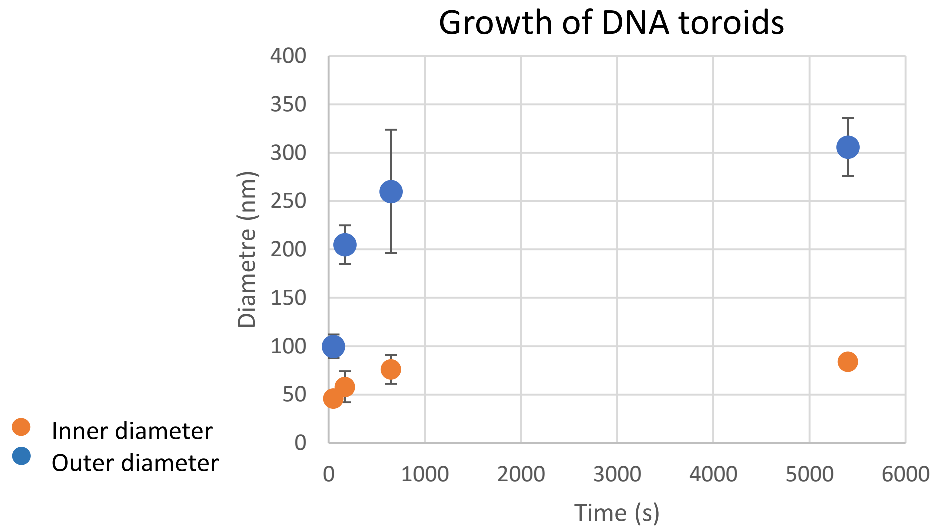


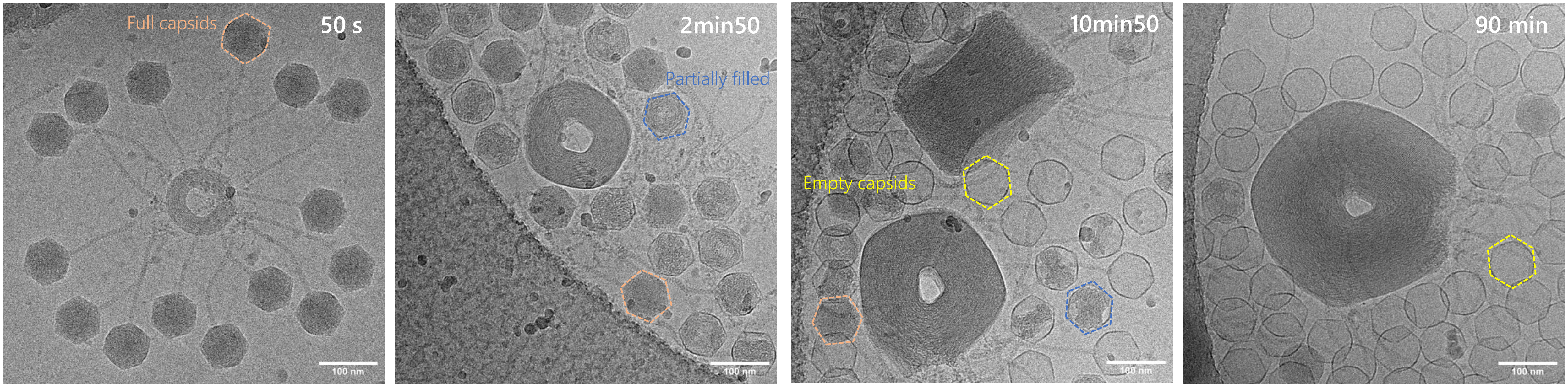
Sun, ... & Nordenskiöld, NAR 2019

Time resolved experiments

Initial motivations

- How fast does the torus form?
- Can we image the first moments of the formation with our technical constraints? Can we see rods ?
- can we observe the nucleation of the first loops?

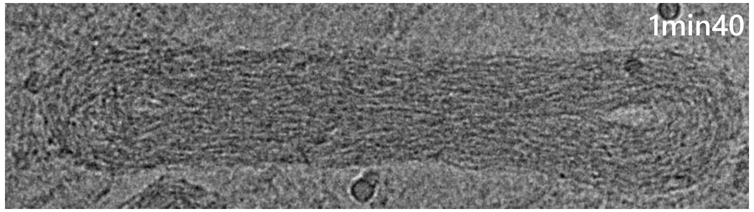


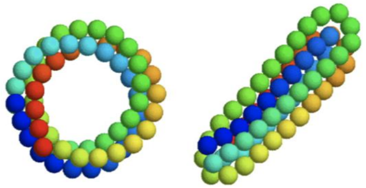


Time resolved experiments

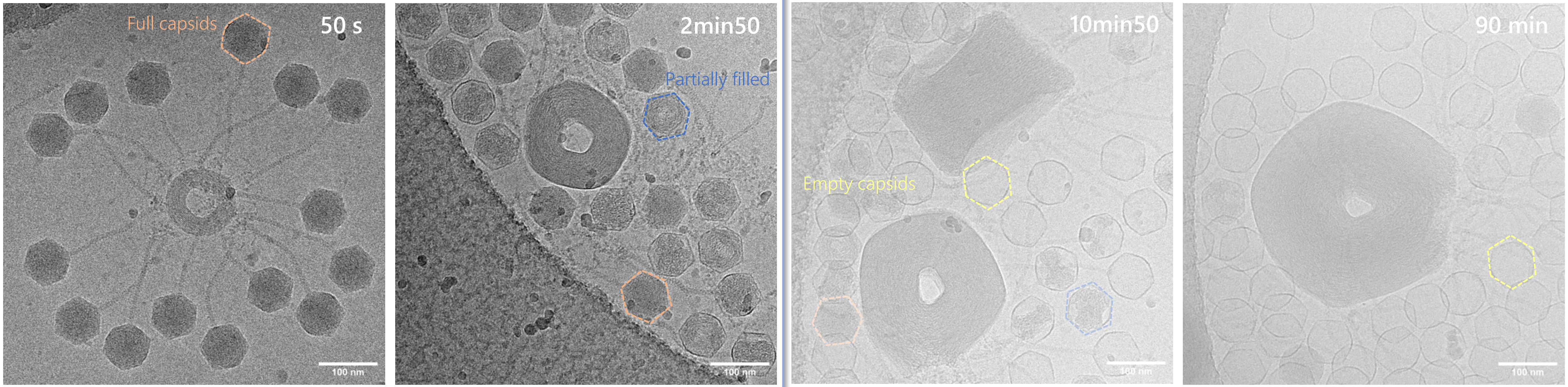
Work in progress

- Observation of rods at short time scale

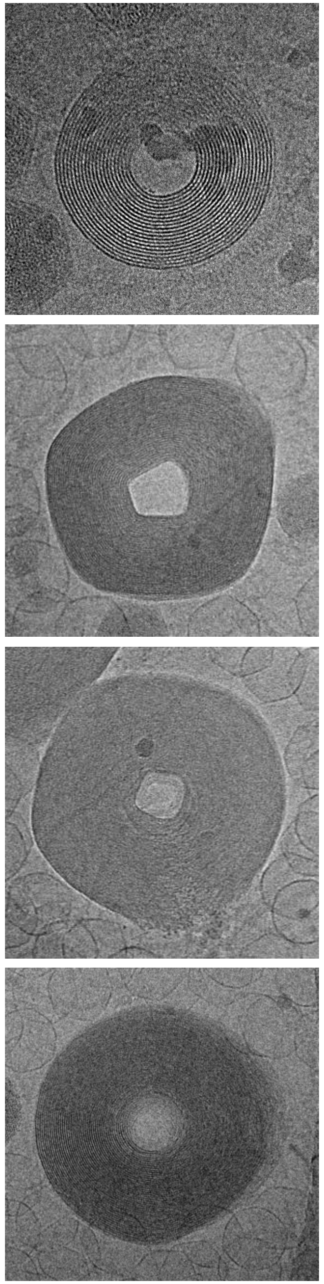




Hoang ... Podgornik & Maritan, 2015



Conclusion and perspectives



- DNA toroids = Highly **condensed** object (linear lenght of 1 strand 40 µm (x30) -> 250 nm diameter toroid)
- Segregation **order & disorder** = optimization of helical **correlations**
- Morphogenesis = **No simple winding** of the molecule into a coil <-> Competition between nucleation and growth of toroidal / rod-shaped loops (hairpins)

Next steps :

Towards a comprehensive model of the DNA toroid ?

- Understanding **limiting factors** of toroid growth -> phage concentration (DNA reservoir)

  Monomolecular toroids *vs* giant toroids
- Understanding **complex interplay** between helical pitch and helical correlations
- **Tomography** study to model our objects

THANK YOU FOR YOUR ATTENTION !

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