

- **Biological question: organelle assembly**
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from components...



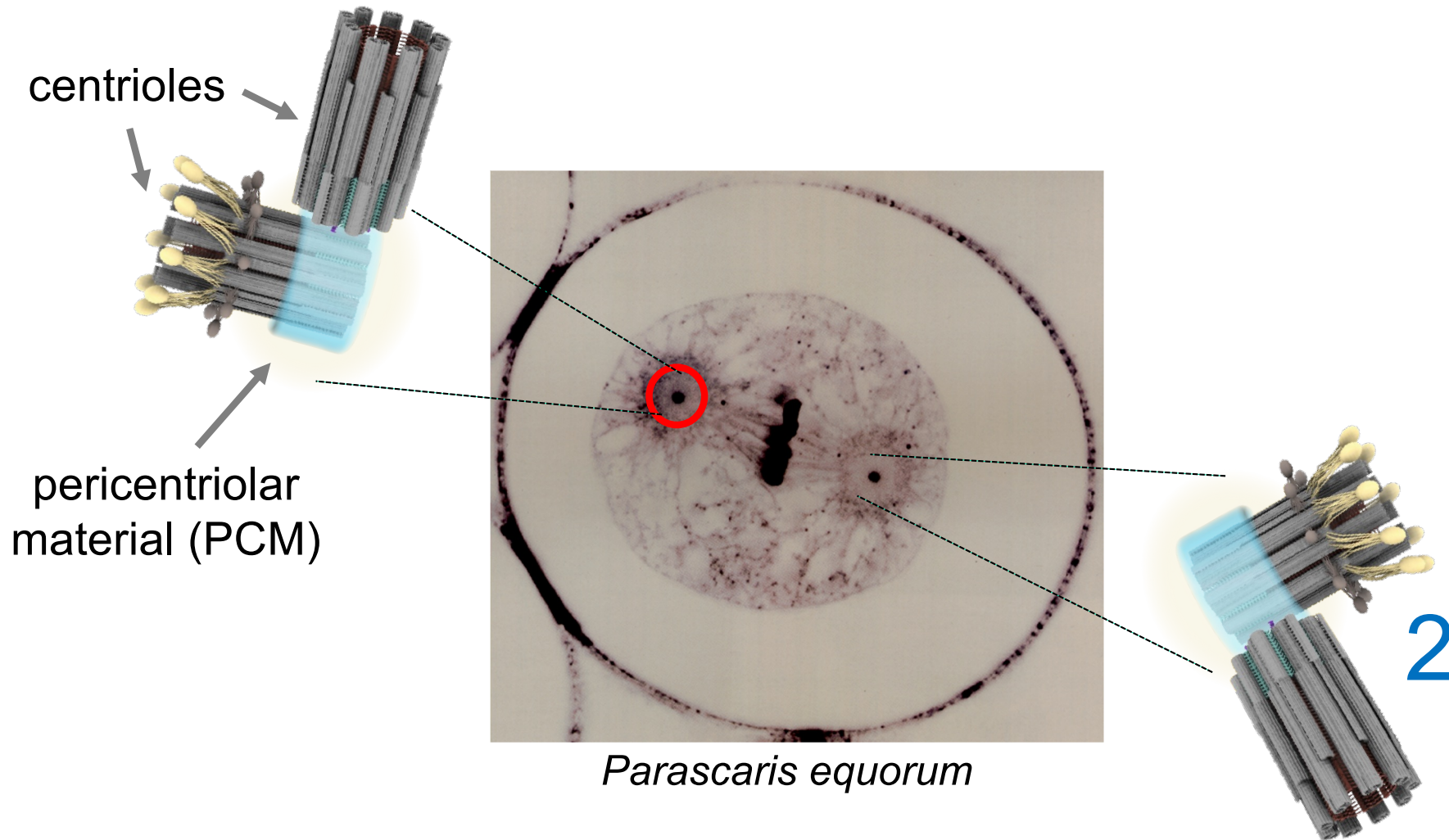
... to assembled structure



focus on centriole

- **Project topic –hence longer introduction**
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● The centrosome: an old friend

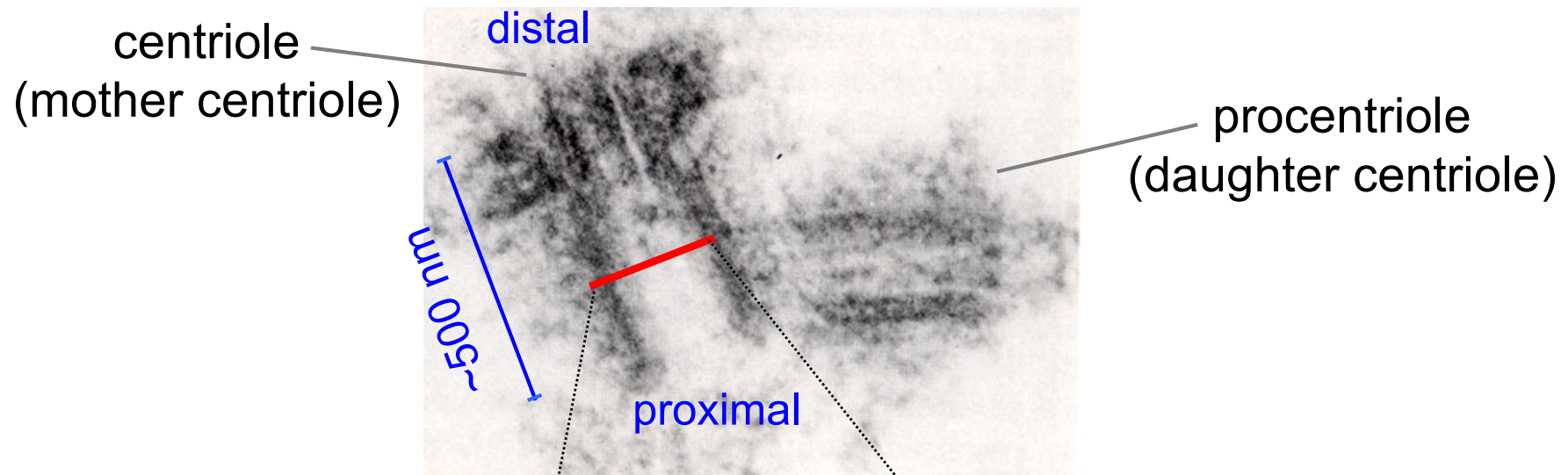


Parascaris equorum

Boveri, 1887
van Beneden and Neyt, 1887

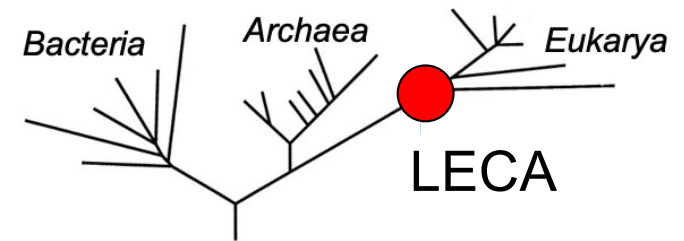
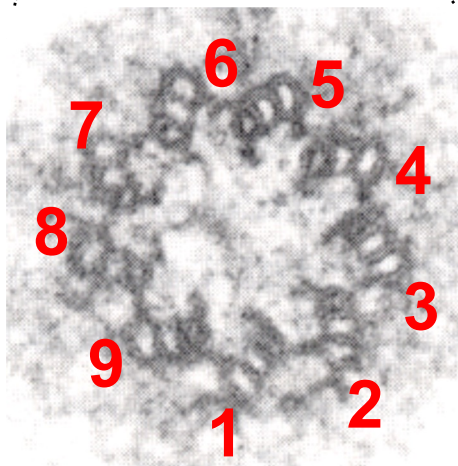
● Centriole architecture: EM

centriole/procentriole from human cells



cross-section

beautiful nine-fold radial symmetry of microtubule triplets



~ 2 billion years!

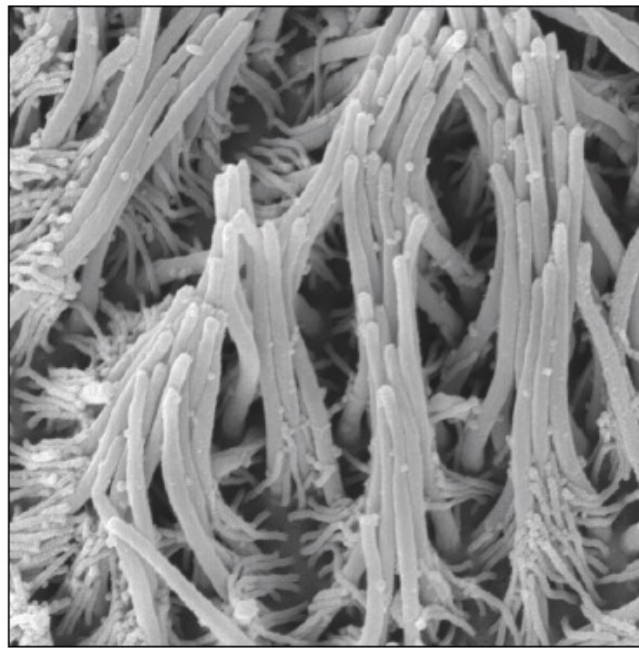
● Imparting nine-fold symmetry

primary cilium

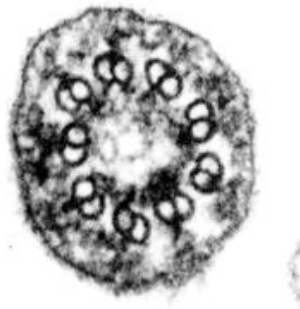
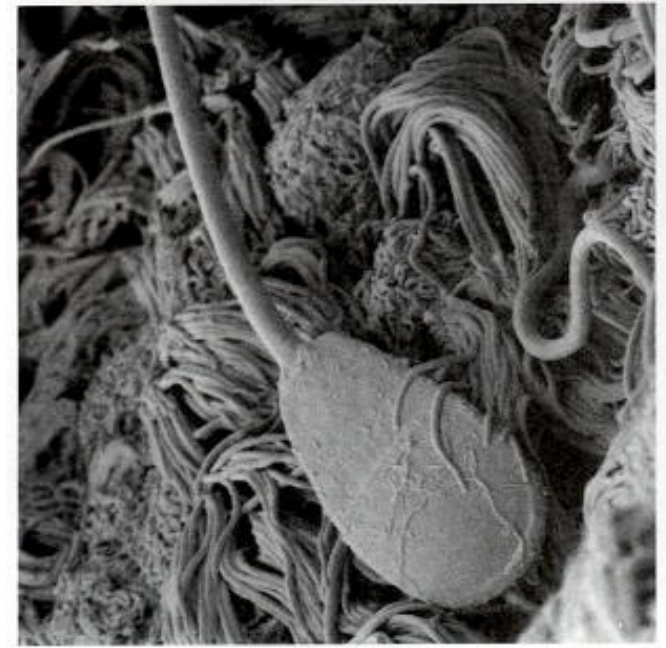


if defective/aberrant: ciliopathies

motile cilia



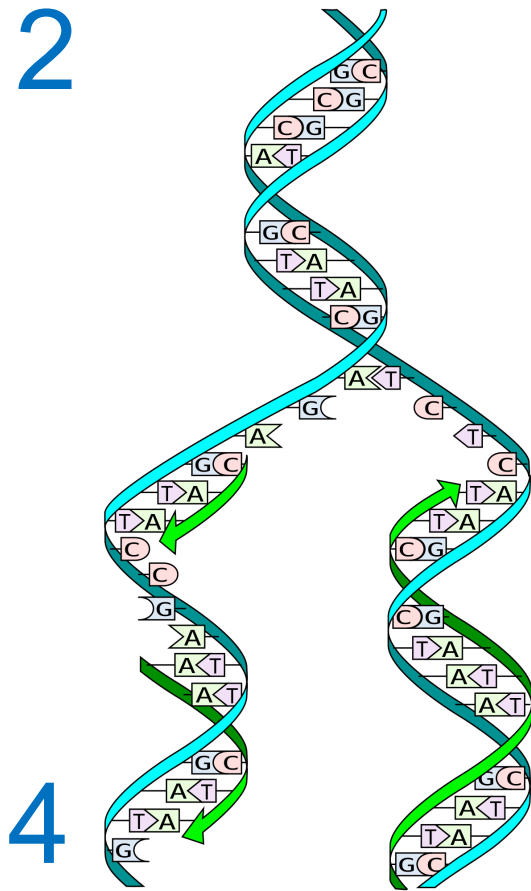
sperm flagellum



- **Centriole number control**

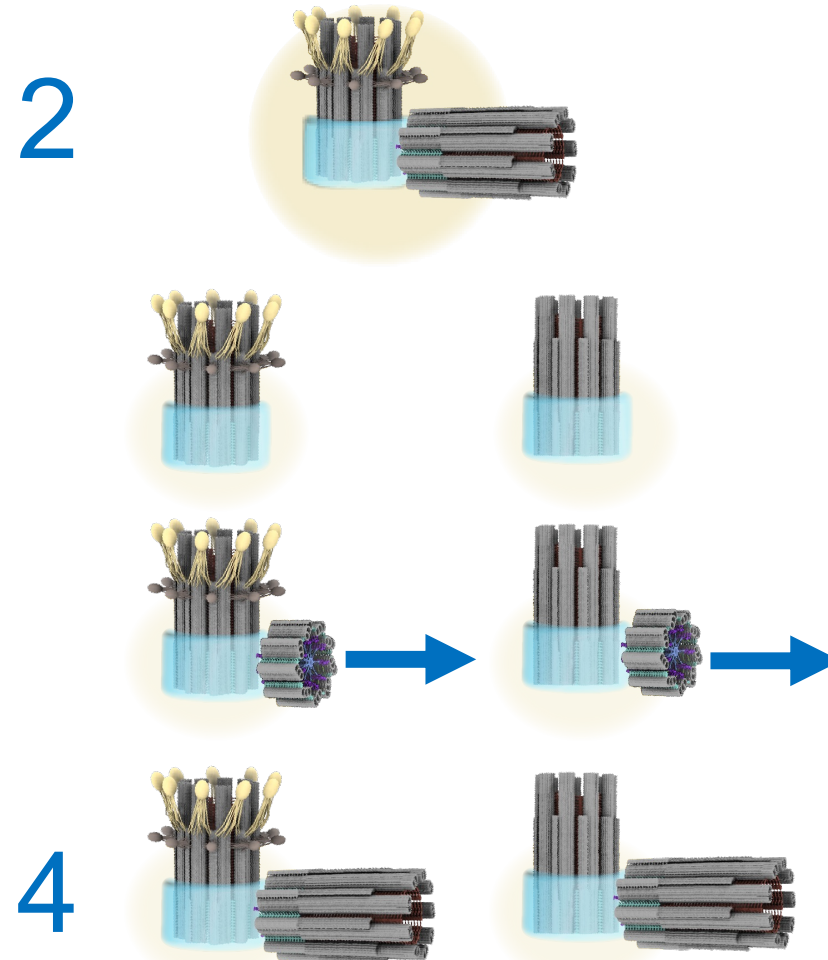
- **Duplication: once per cell cycle**

DNA replication



S phase

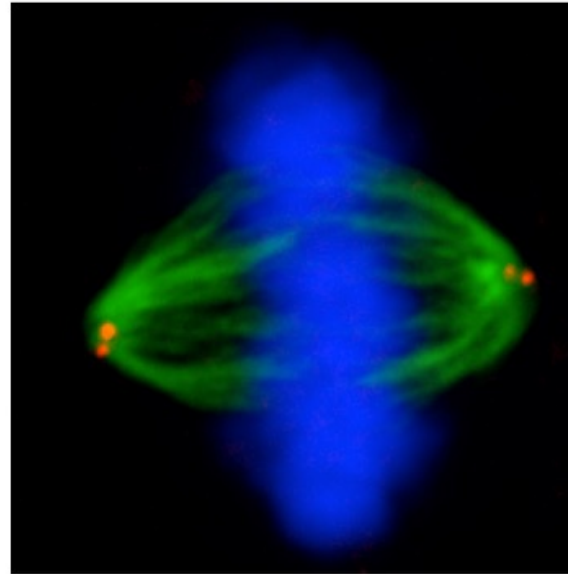
Centriole duplication



● On the importance of centriole number

4

Control



● DNA

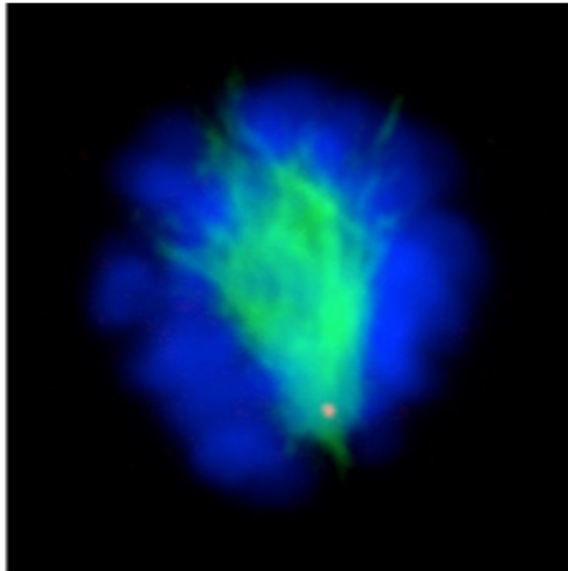
● α -tubulin

● Centrin (centriolar marker)

● On the importance of centriole number

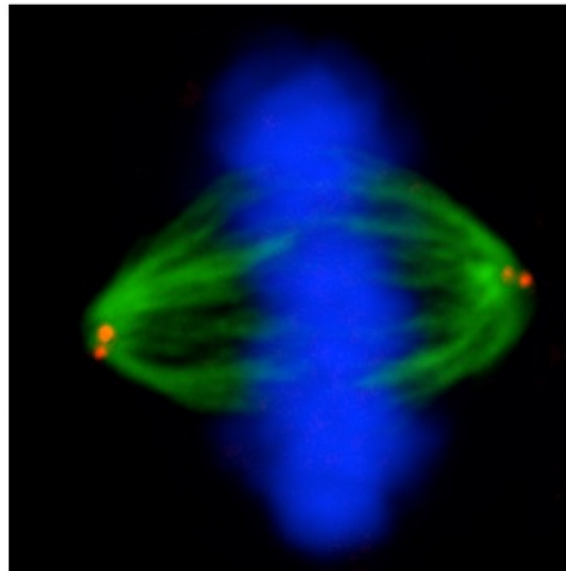
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Underduplication



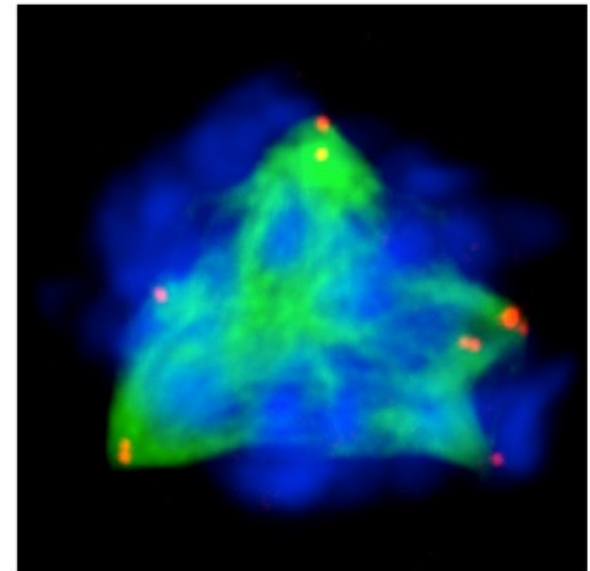
4

Control



>4

Overduplication



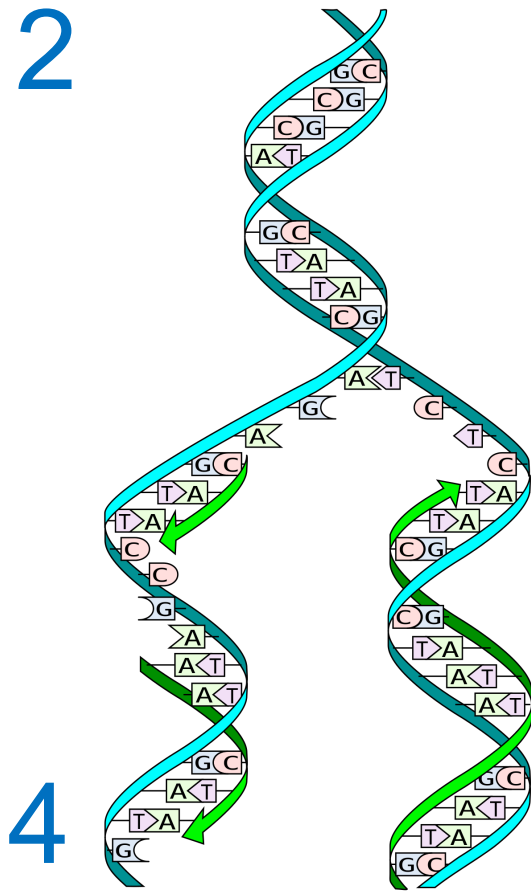
● DNA

● α -tubulin

● Centrin (centriolar marker)

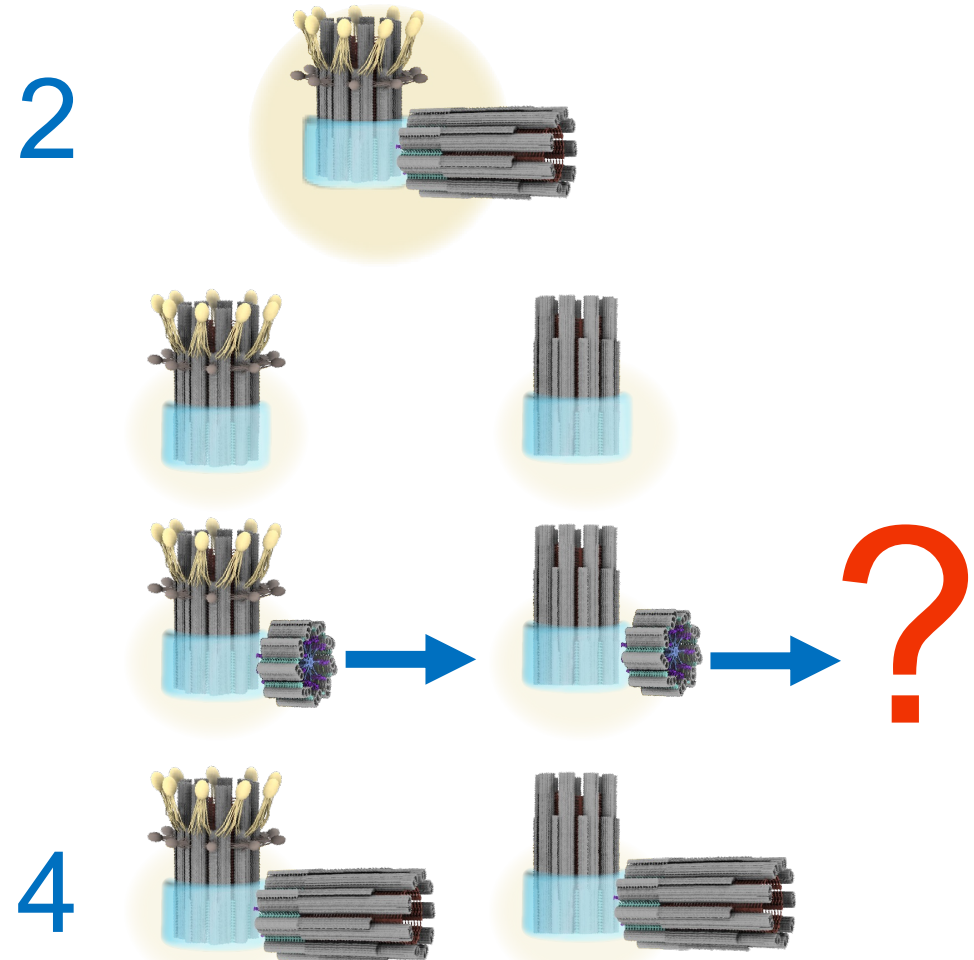
- **Duplication: once per cell cycle**

DNA replication

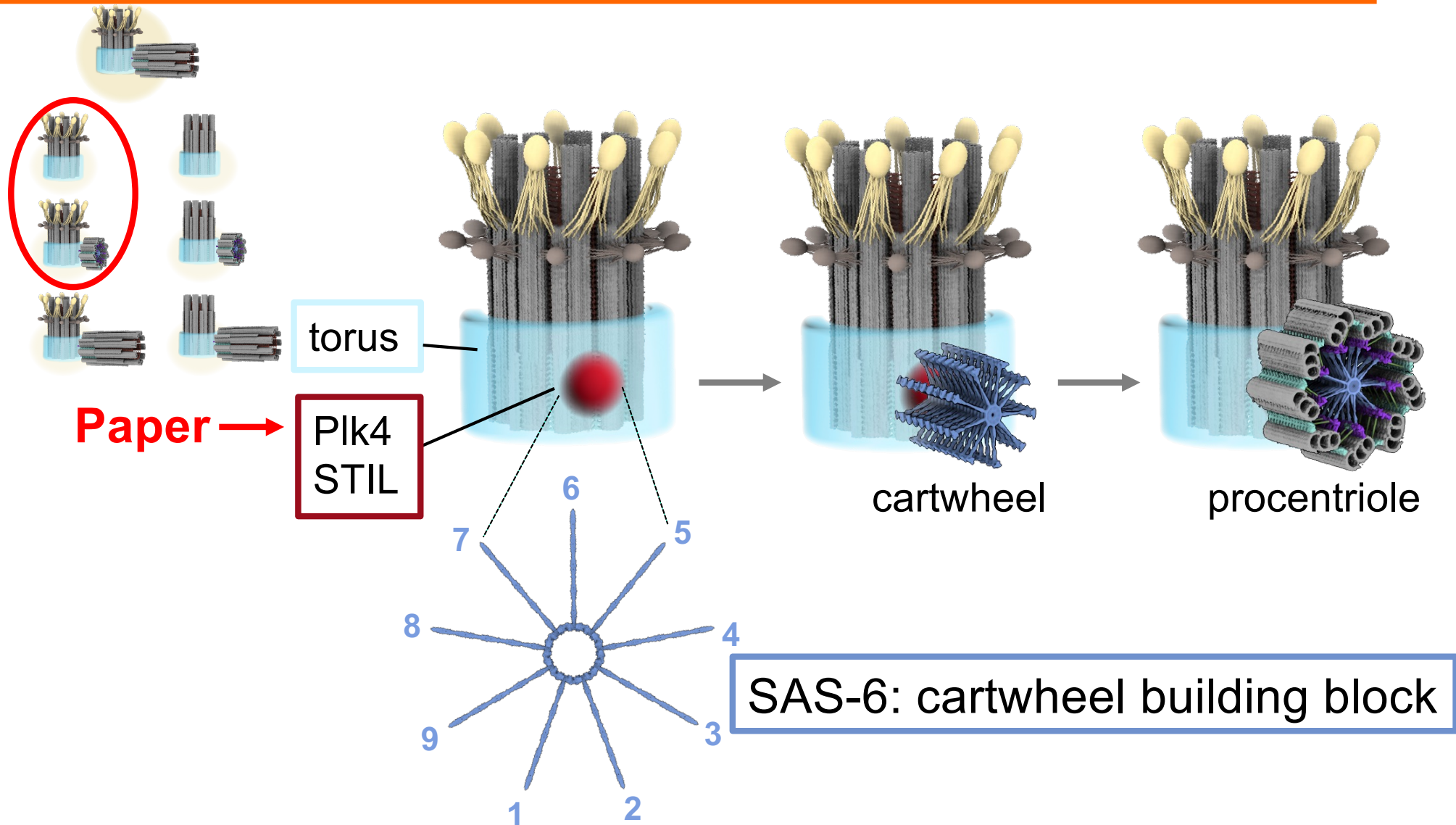


S phase

Centriole duplication



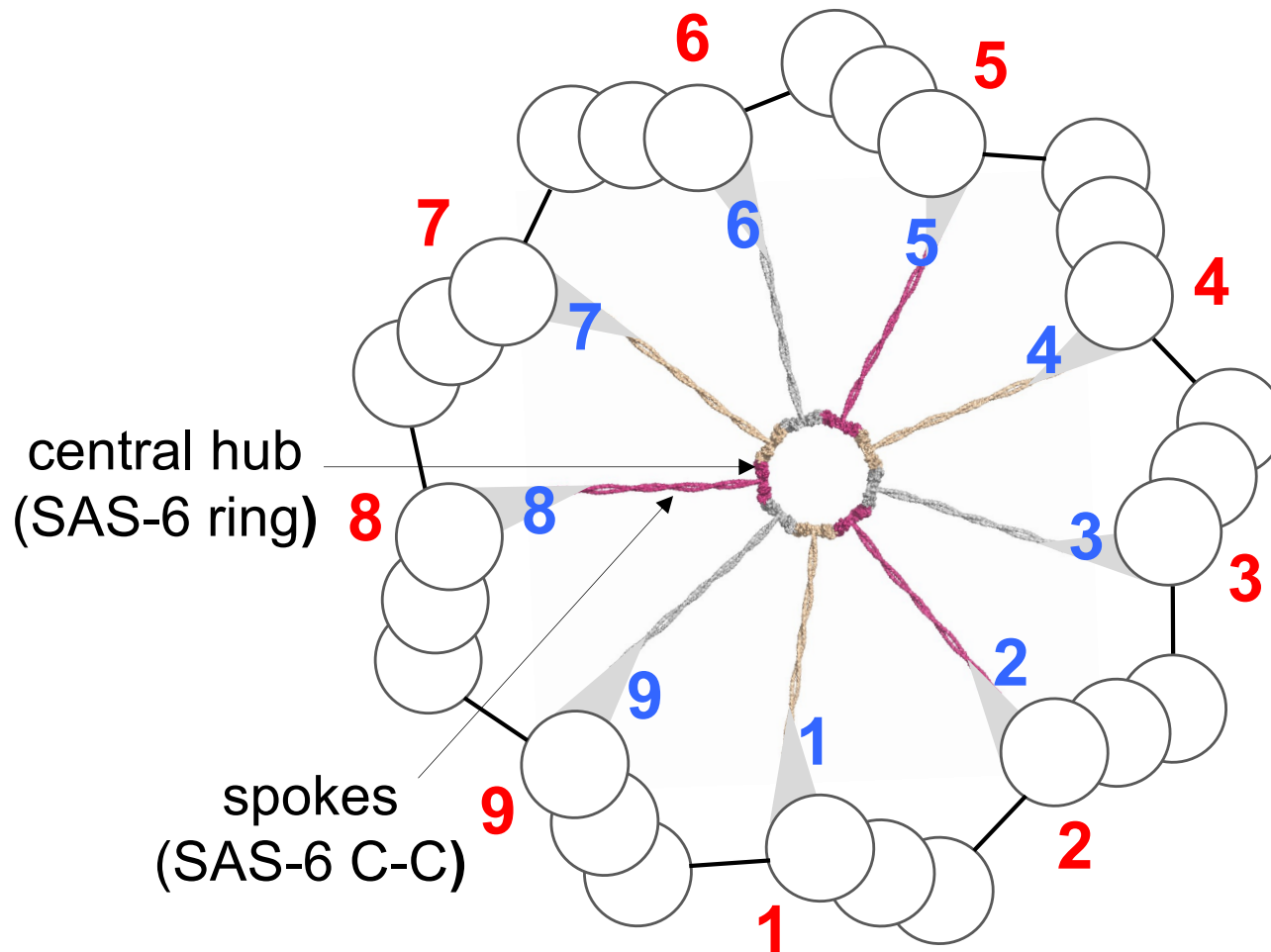
● Onset of mentored centriole duplication



Collaboration with Michel Steinmetz (PSI, Switzerland)

● Self-assembly as organizing principle

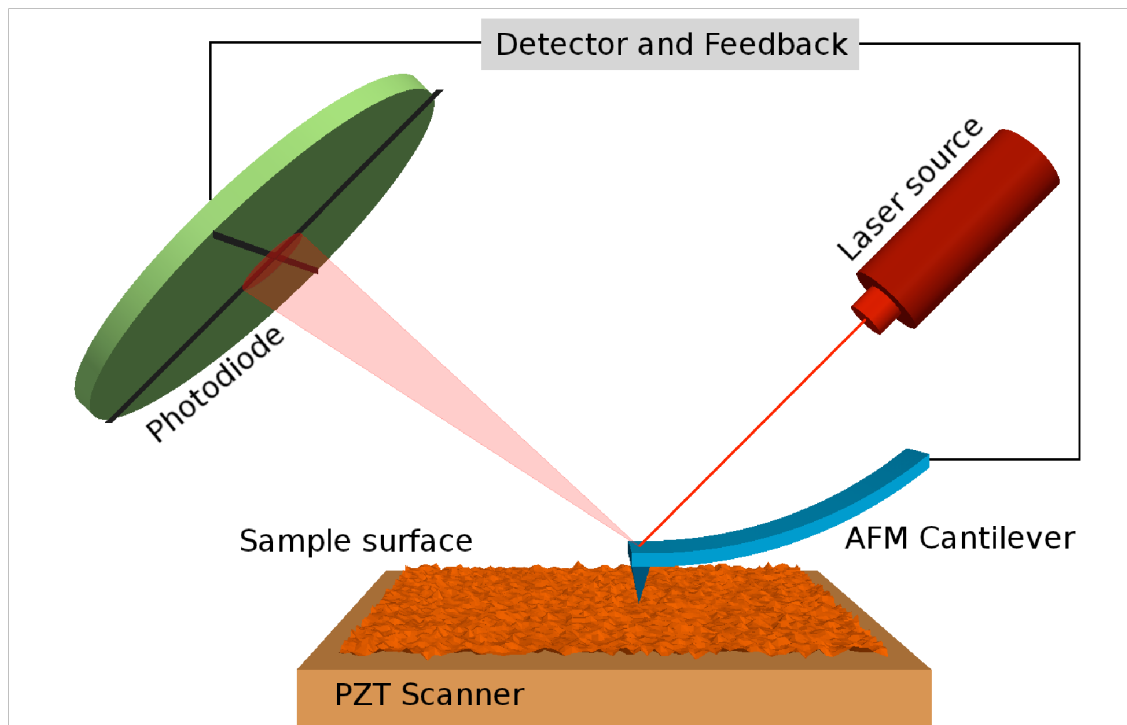
SAS-6 proteins as organizing principle at the root of the near-universal 9 fold symmetry of centrioles



Kitagawa et al., 2011
van Breugel et al., 2011

● Atomic Force Microscope (AFM)

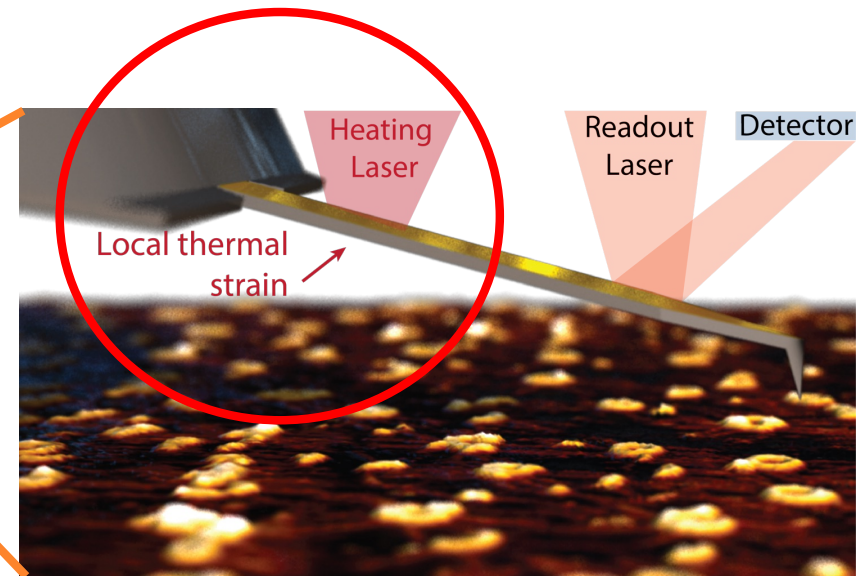
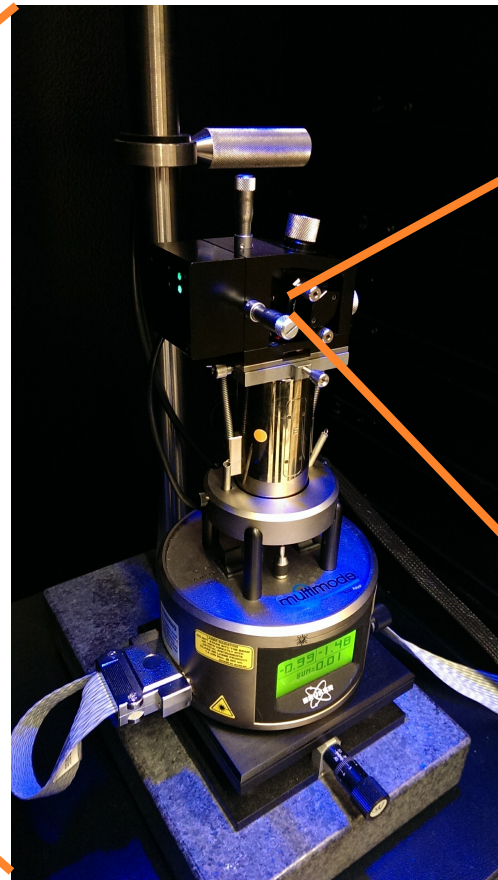
AFM to probe topology



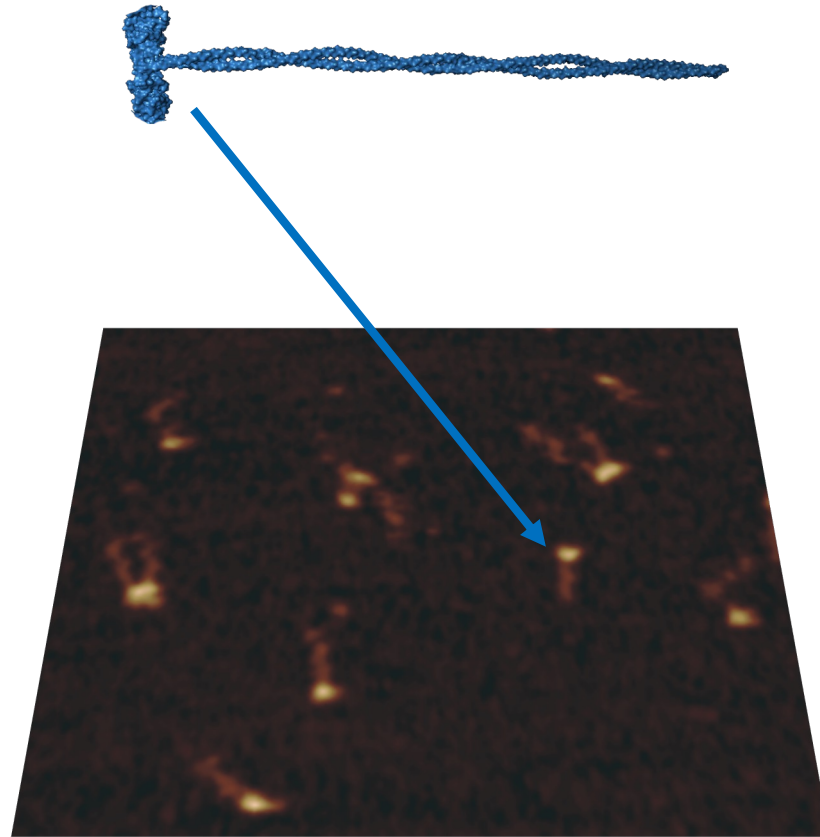
Conventional tapping mode HS-AFM too brutal for SAS-6 assembly dynamics!

● Photothermal off-resonance HS-AFM

Collaboration with the lab of Georg Fantner (EPFL)



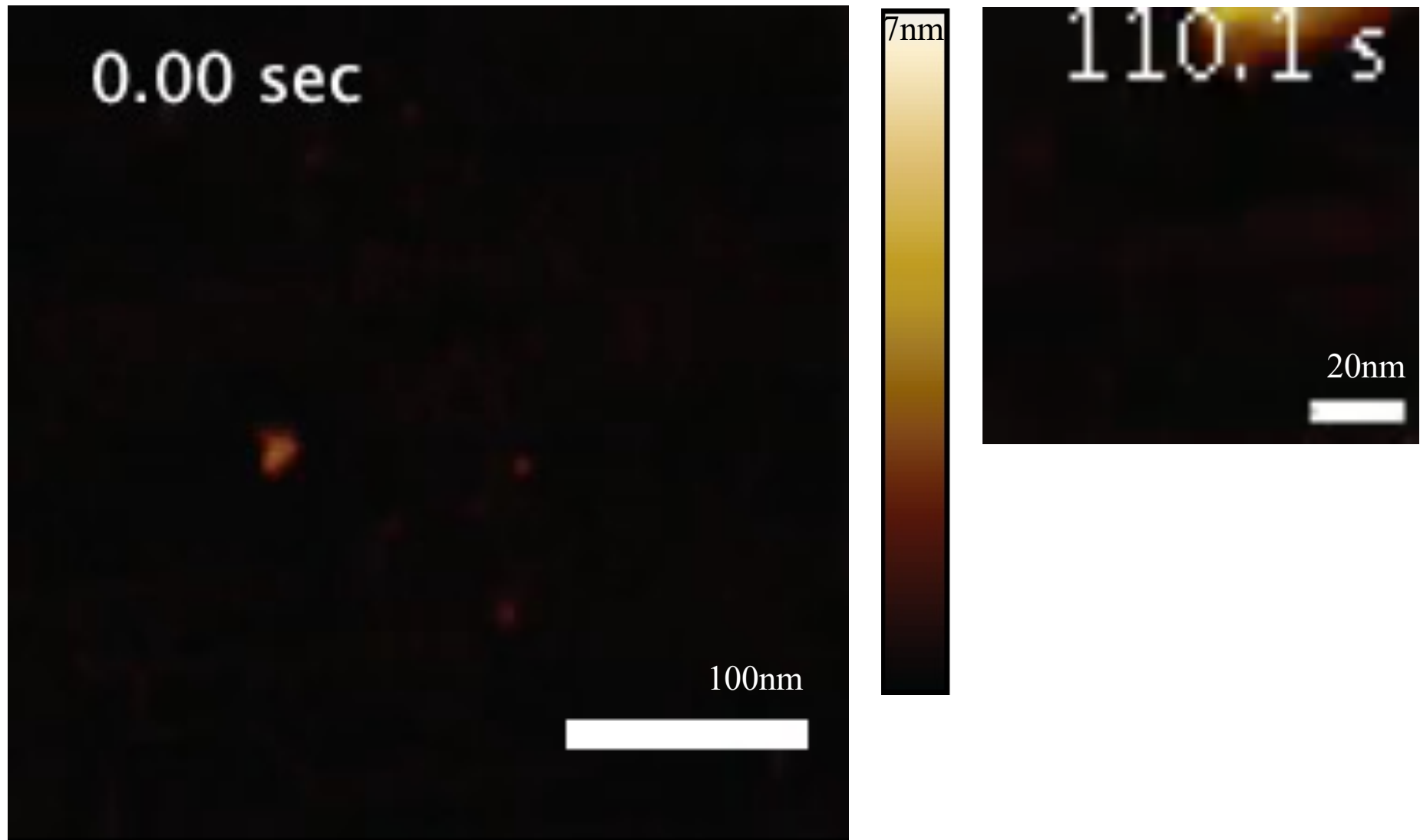
- **High speed AFM with CrSAS-6 (NL)**



CrSAS-6 (NL) on Mica

$\frac{1}{4}$ of scanned area (total: 800 nm²)

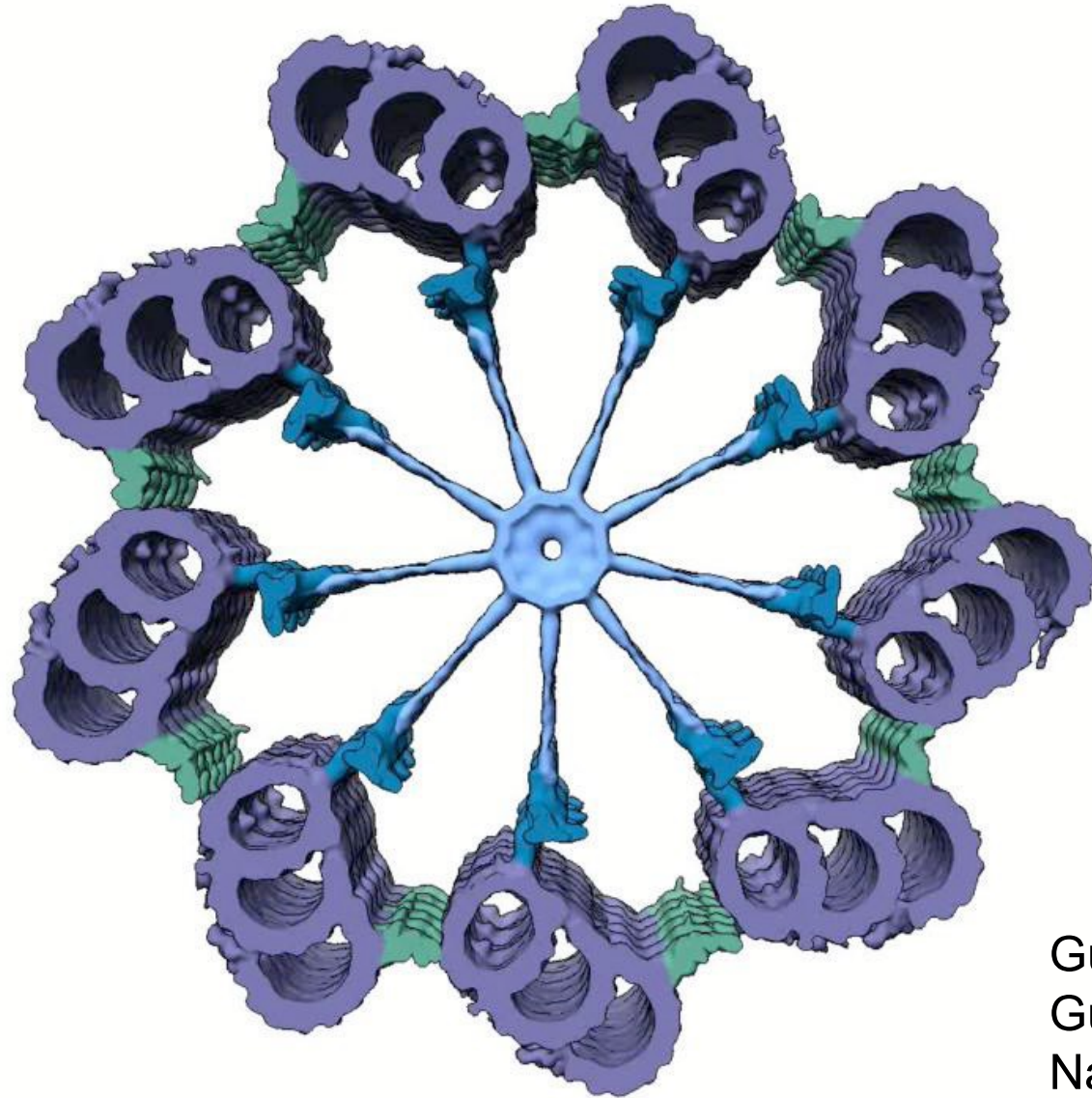
- **High speed AFM: scanning at ~ 0.4 Hz**
-



Nievergelet et al., 2019
Banterle et al., 2021

● Centriole architecture: the movie

Trichonympha spp.



Guichard et al., 2012
Guichard et al., 2013
Nazarov et al., 2020
Klena et al., 2020

● Interesting exceptions to the 2 > 4 rule

- > Mentored cycle: **2 > 4**
- > Multiciliated cells, e.g. human respiratory tract: **2 > ~200**
- > *de novo* centriole assembly, e.g. mouse embryo: **0 > 2**
- > Centriole elimination, e.g. metazoan oogenesis: **2 > 0**
- > Many cancer cells: **more than 4**
- > Plk4 overexpression: **sufficient to generate tumors**

● Paper 3

Reversible centriole depletion with an inhibitor of Polo-like kinase 4.

Wong et al., *Science* 348: 1155 (2015)

● Questions?

