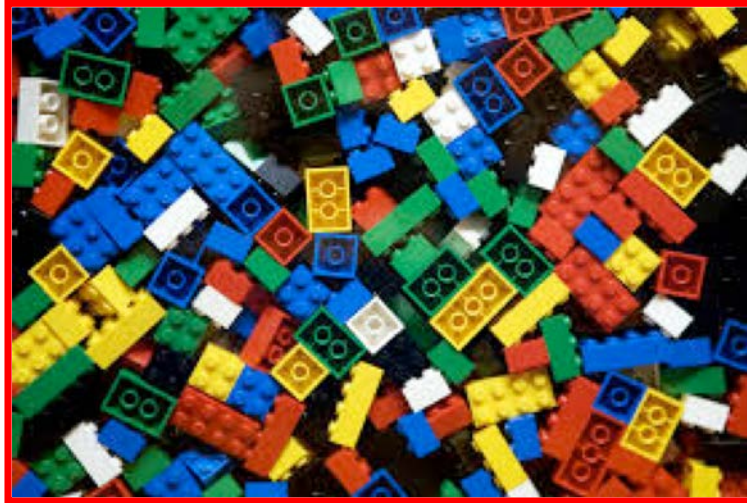


- **Biological question: organelle assembly**

● Biological question: organelle assembly

from components...



... to assembled structure



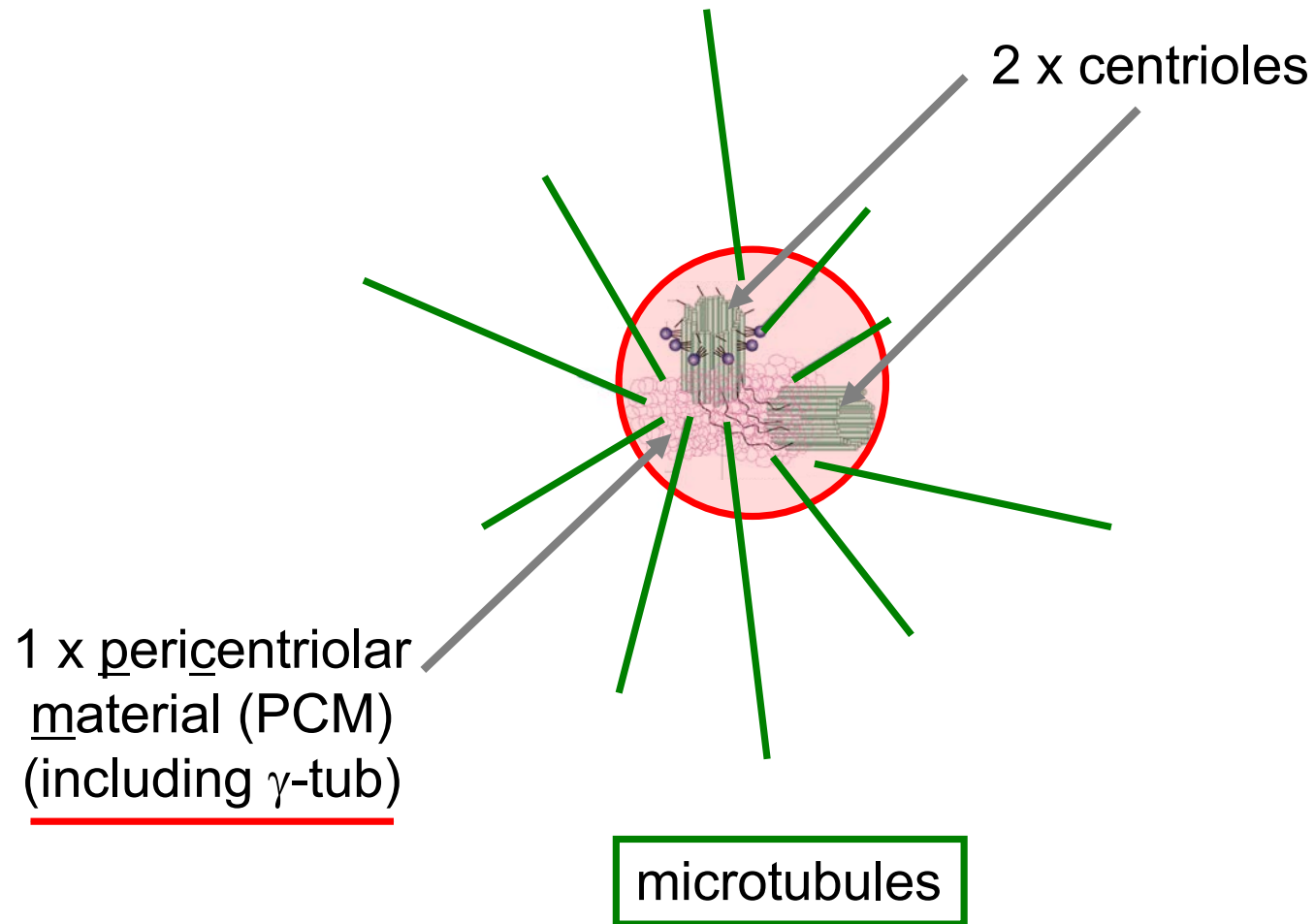
focus on centriole

- **The centrosome: an old friend**



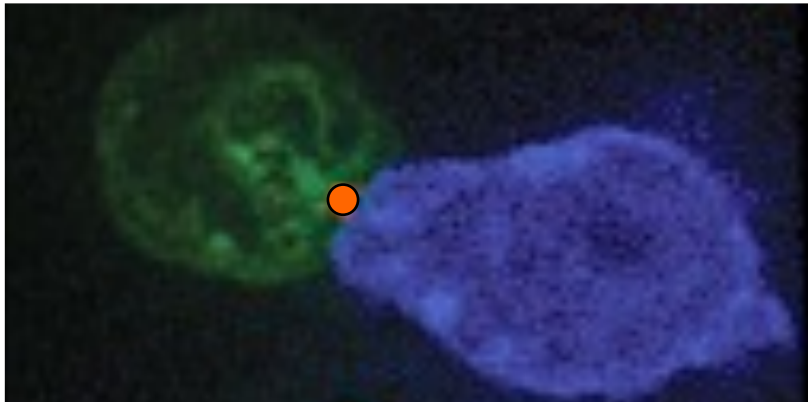
Parascaris equorum

● Centrosome: MTOC of most animal cells



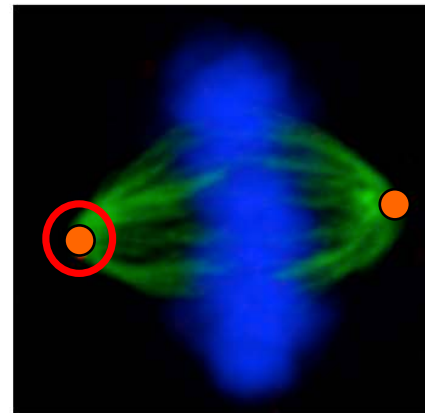
● Centrosome function (e.g.)

during interphase



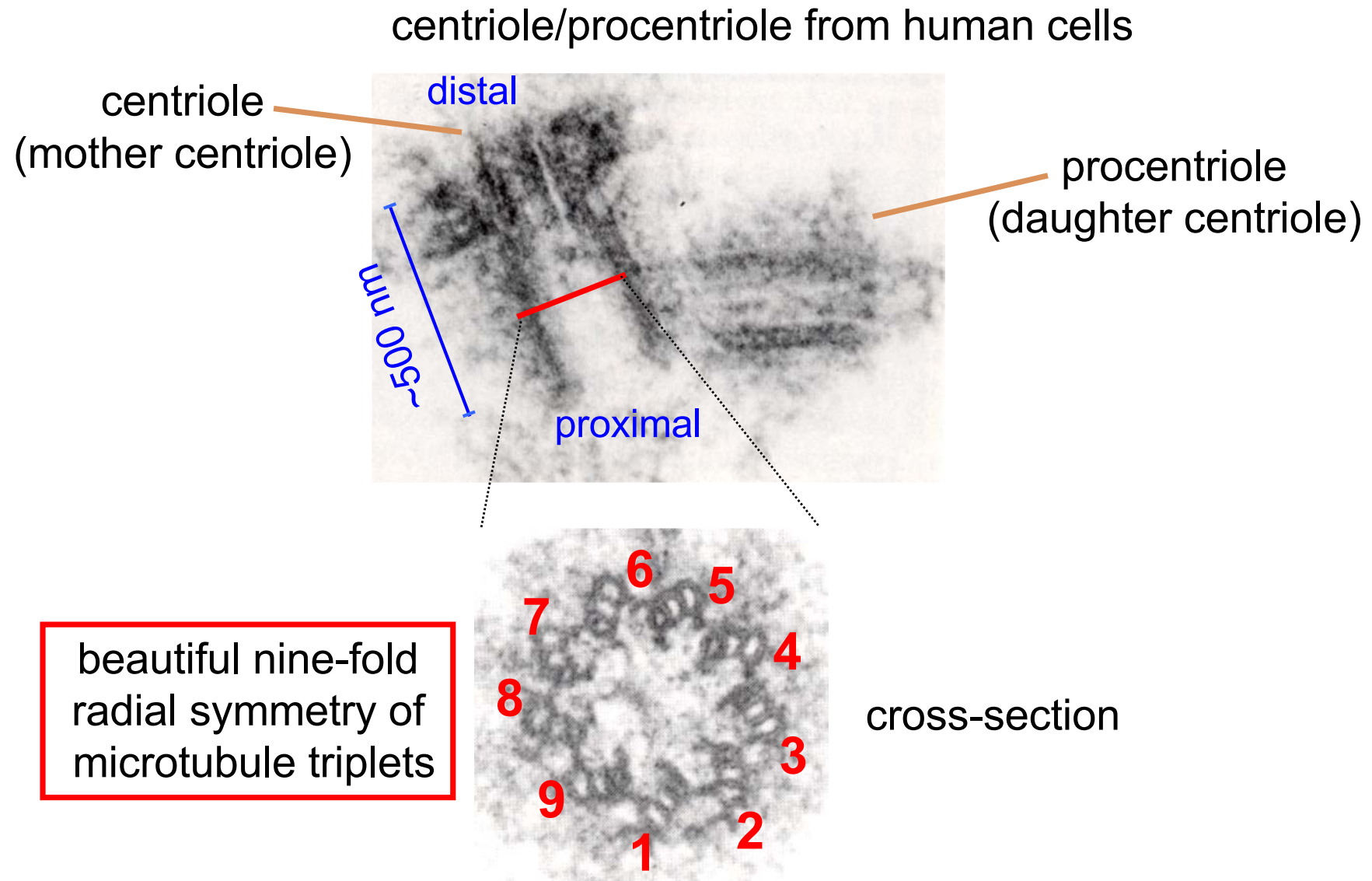
immunological synapse

during mitosis



spindle assembly

● Centriole architecture: EM

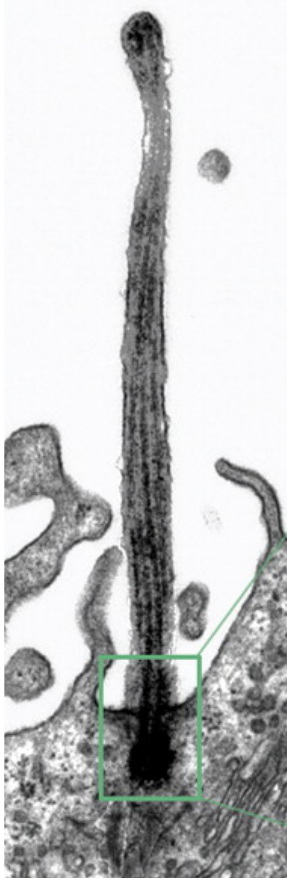


ancient organelle (present in LECA)

Paintrand et al., 1992

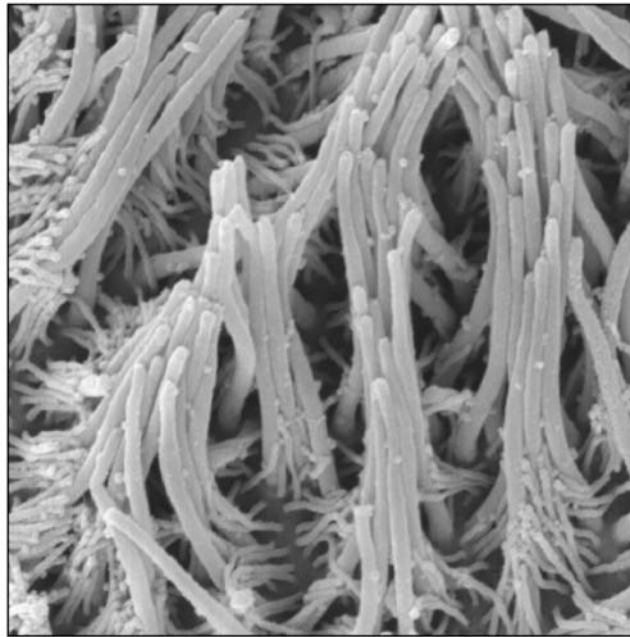
● Imparting the nine-fold symmetry

primary cilium

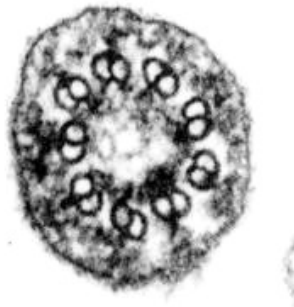
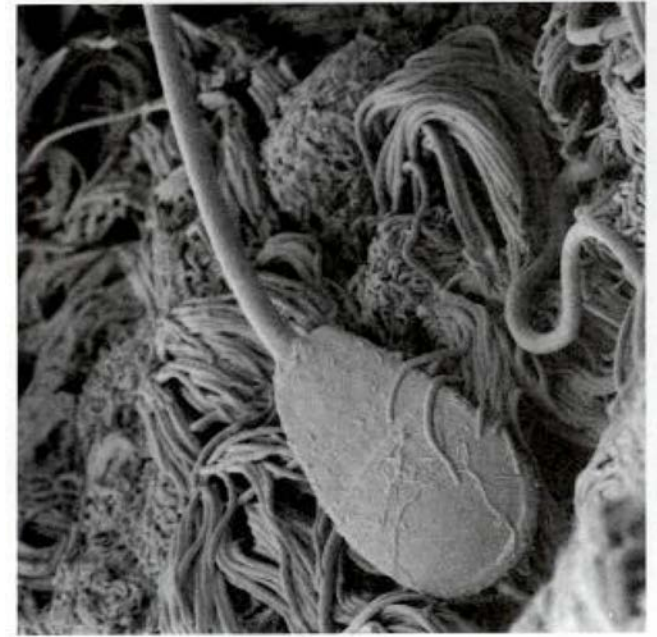


if defective/aberrant: ciliopathies

motile cilia



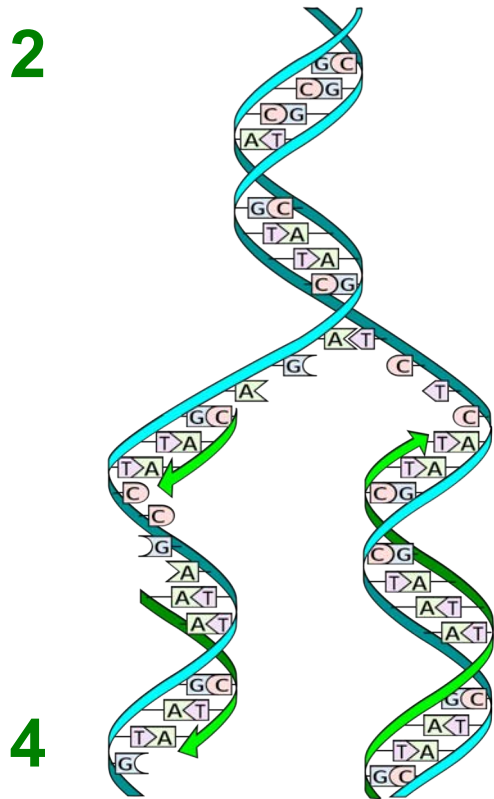
sperm flagellum



- **Canonical centriole duplication cycle**

DNA replication

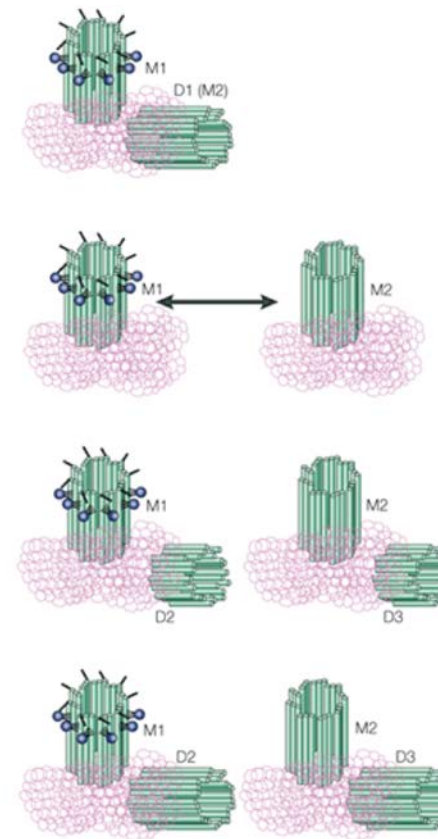
2



4

Centriole duplication

2



4

S phase

Modified from Doxsey, 2001

● Interesting exceptions to the $2 > 4$ rule

> Canonical cycle: $2 > 4$

> Multiciliated cells, e.g. human respiratory tract: $2 > \sim 200$

> *de novo* centriole assembly, e.g. mouse embryo: $0 > 2$

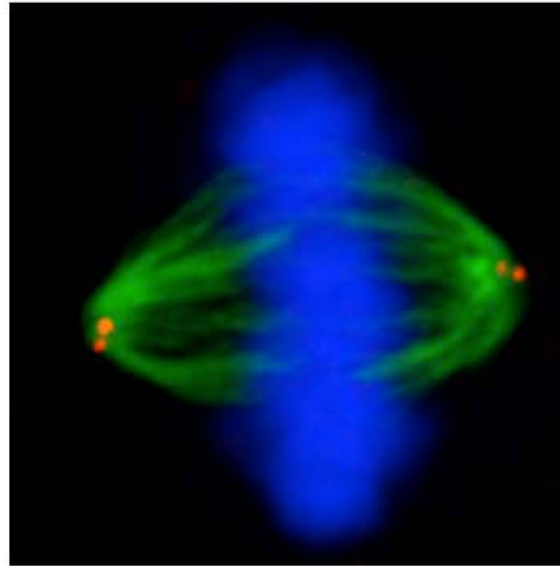
> Centriole elimination, e.g. metazoan oogenesis: $2 > 0$

> Many cancer cells: **more than 4**

> Plk4 overexpression: **sufficient to generate tumors**

● On the importance of centriole number

Control



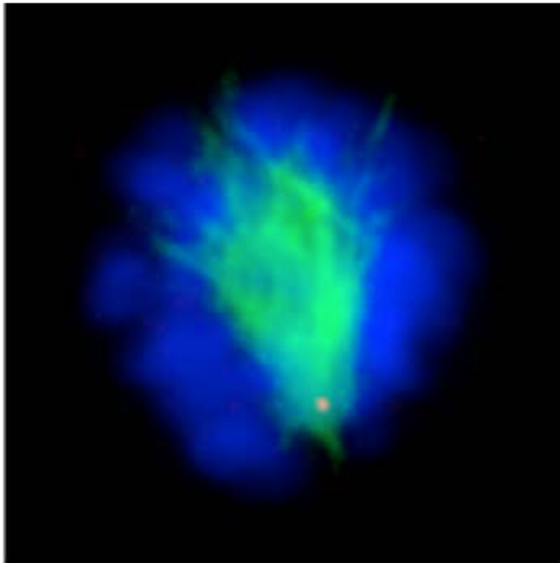
● DNA

● α -tubulin

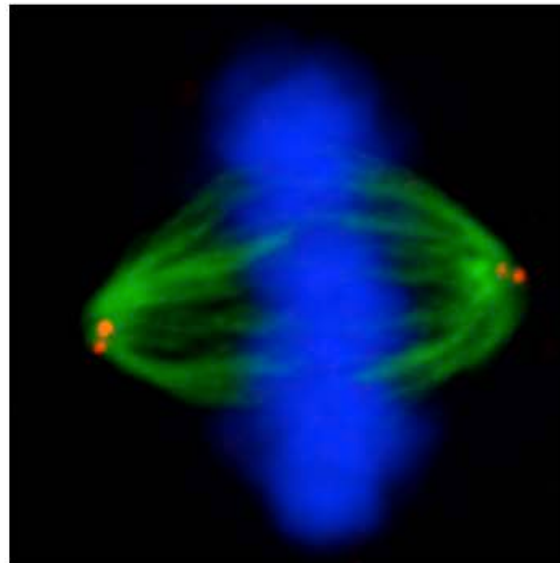
● Centrin (centriolar marker)

● On the importance of centriole number

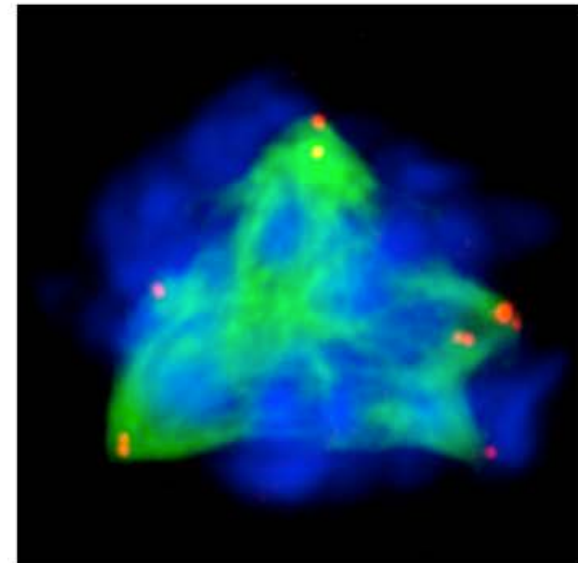
Underduplication



Control



Overduplication



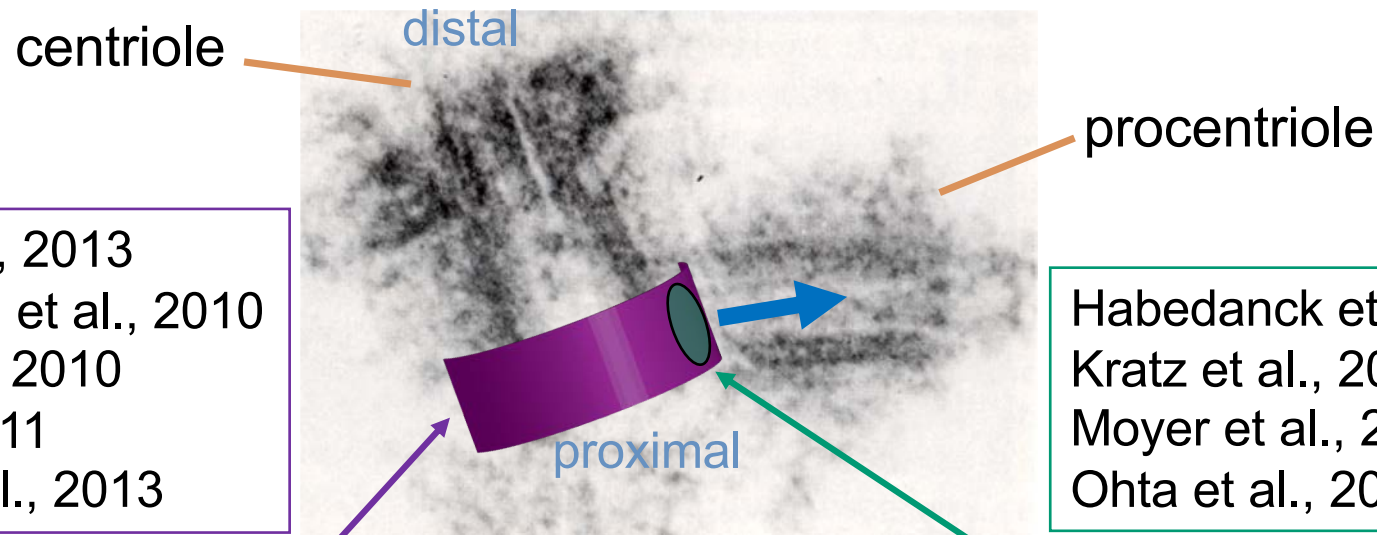
● DNA

● α -tubulin

● Centrin (centriolar marker)

● Cartwheel: seeded from a surface

centriole/procentriole (human cells)



Brown et al., 2013
Cizmecioglu et al., 2010
Hatch et al., 2010
Sir et al., 2011
Sonnen et al., 2013

Habedanck et al., 2005
Kratz et al., 2015
Moyer et al., 2015
Ohta et al., 2014

Cep152/Cep63/Cep57 torus

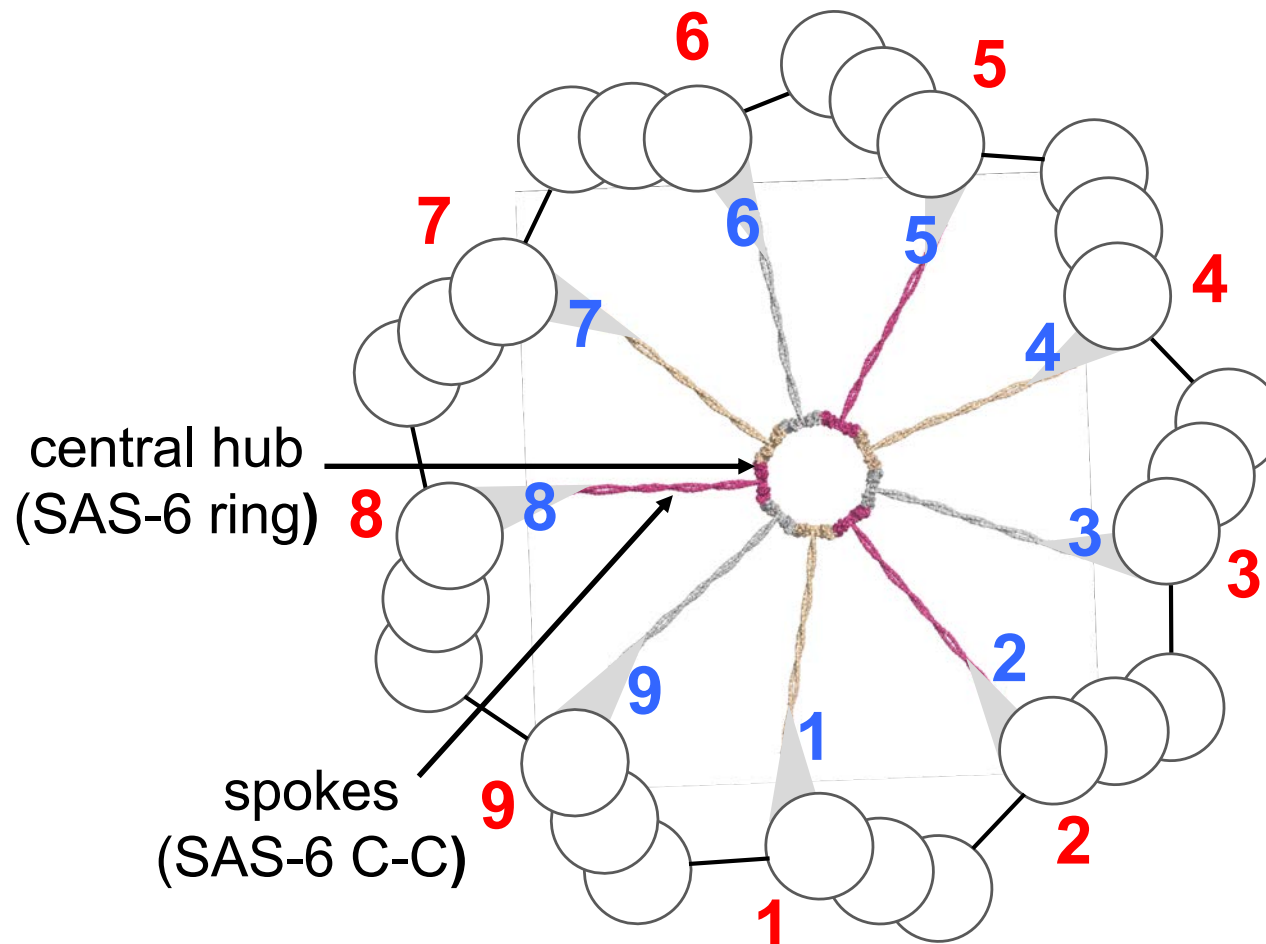
Plk4 + pSTIL focus

Paper

Paintrand et al., 1992

● Self-assembly as organizing principle

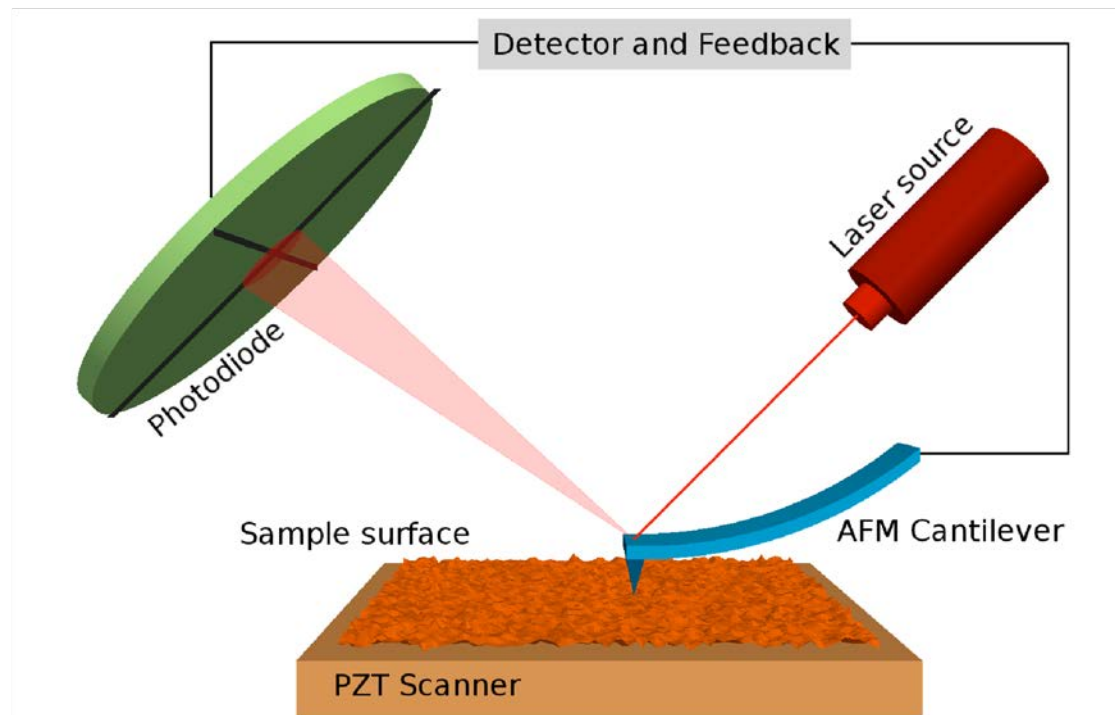
SAS-6 proteins as organizing principle at the root of the near-universal 9 fold symmetry of centrioles



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

● Atomic Force Microscope (AFM)

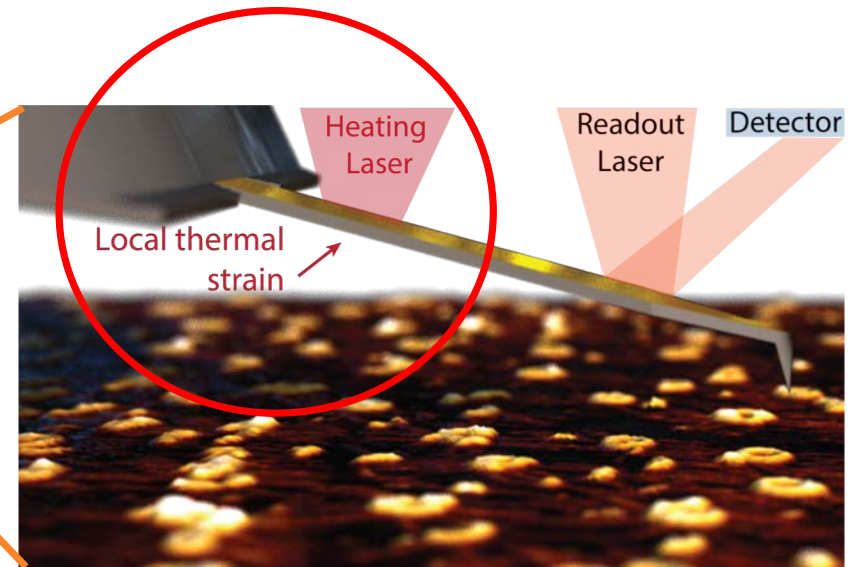
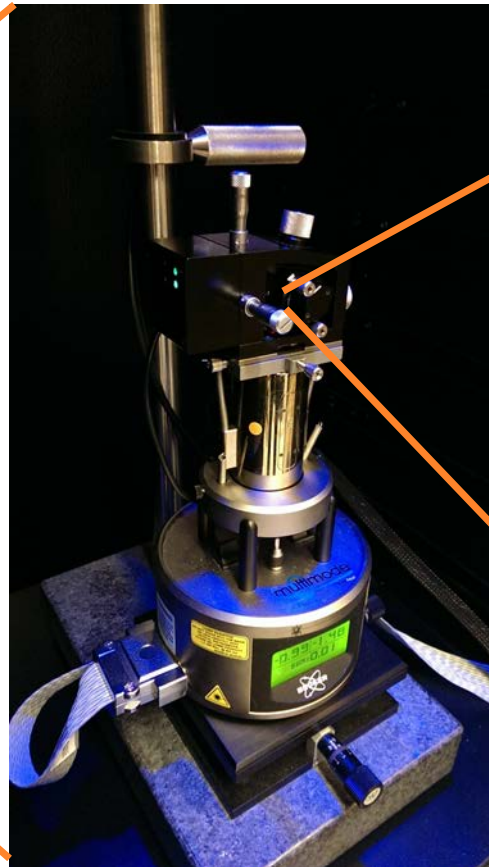
AFM to probe topology



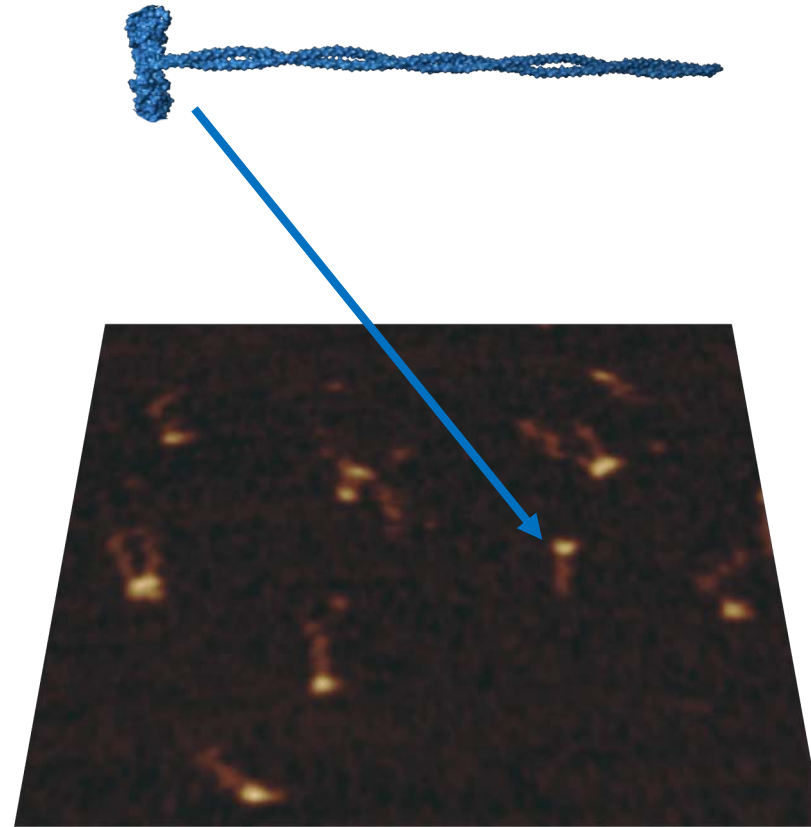
Conventional tapping
mode HS-AFM too
brutal for SAS-6
assembly dynamics!

● Photothermal off-resonance HS-AFM

Collaboration with the lab of Georg Fantner (EPFL)



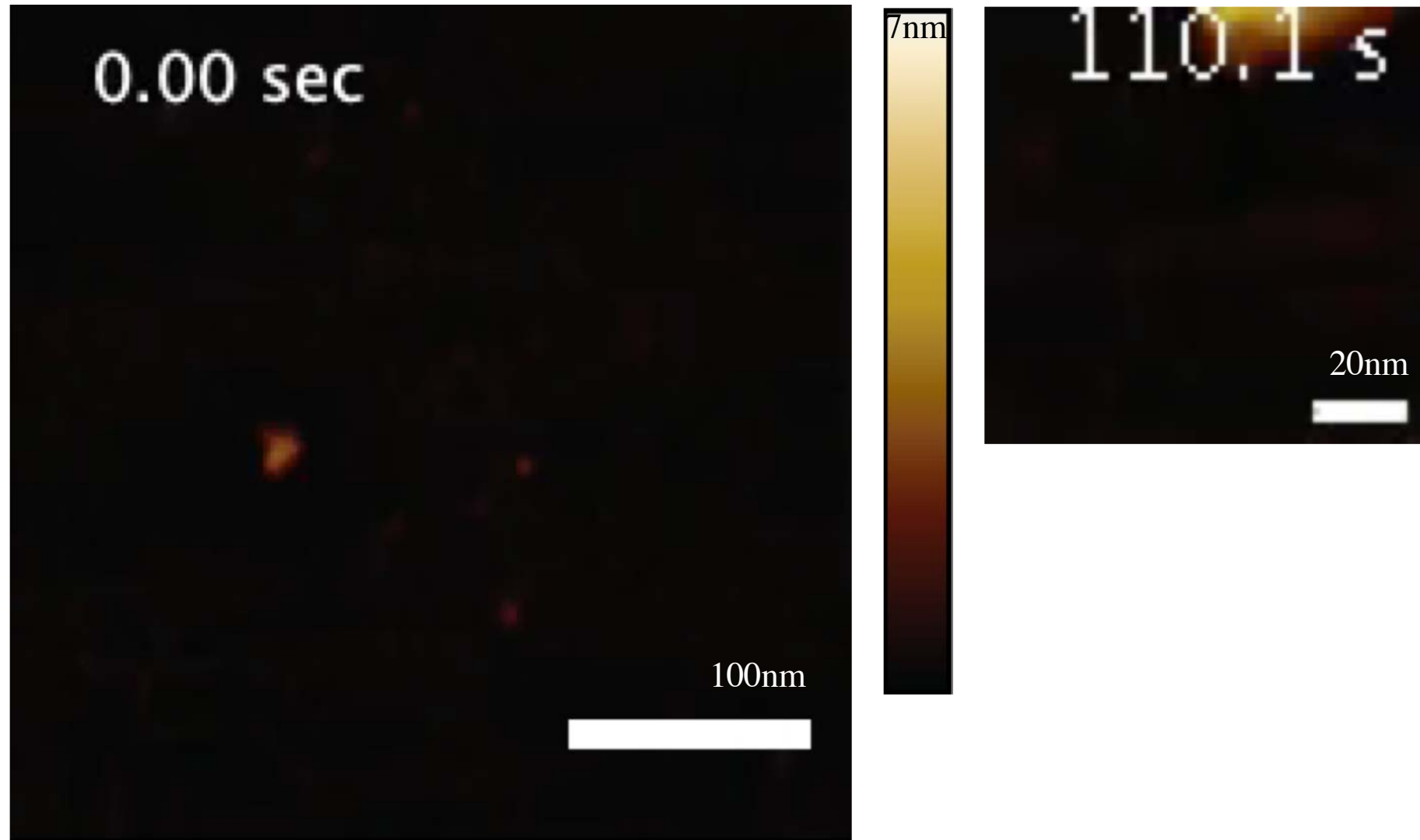
- **High speed AFM with CrSAS-6 (NL)**



CrSAS-6 (NL) on Mica

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

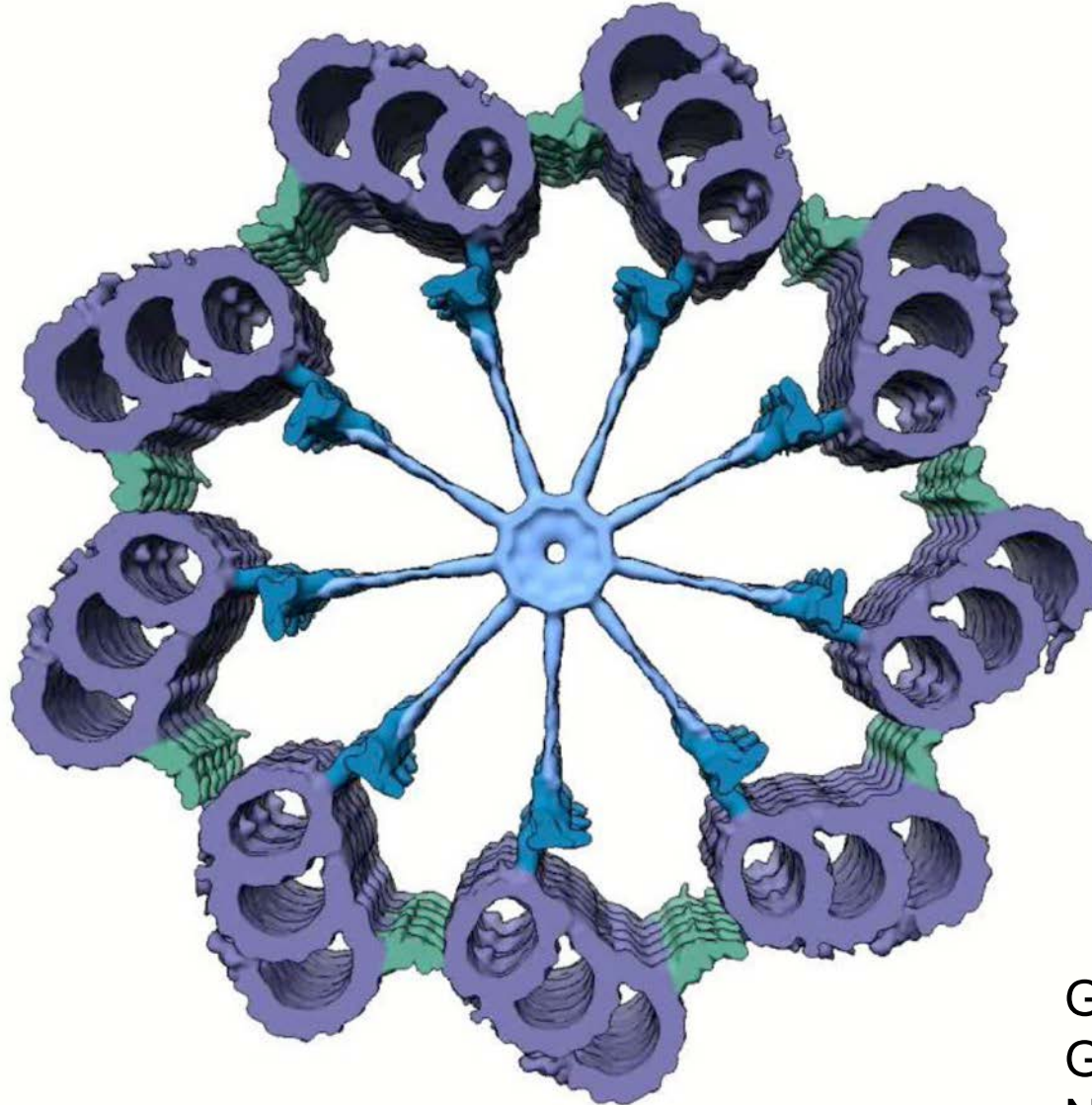
- **High speed AFM: scanning at ~ 0.4 Hz**
-



Nieveragelet 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

● Paper 3

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

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

● Questions?
