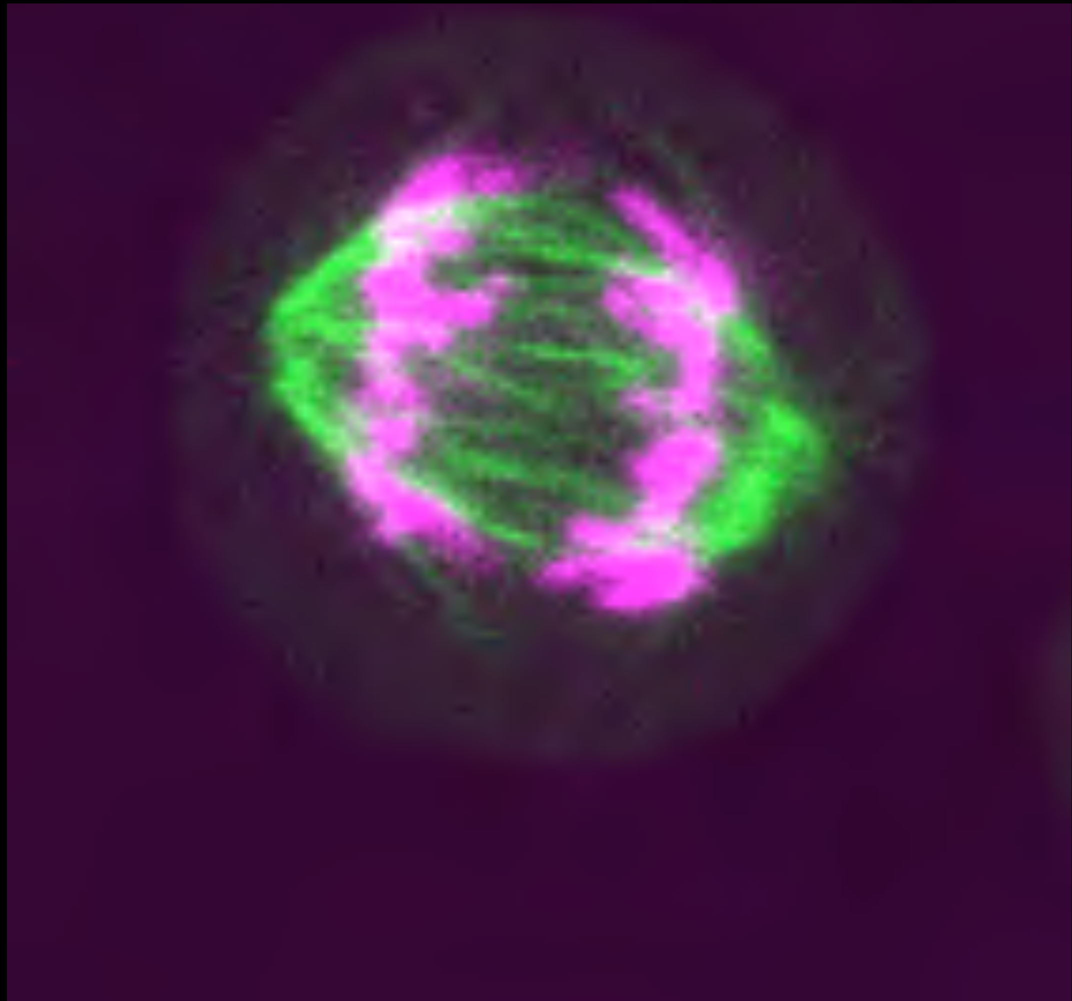
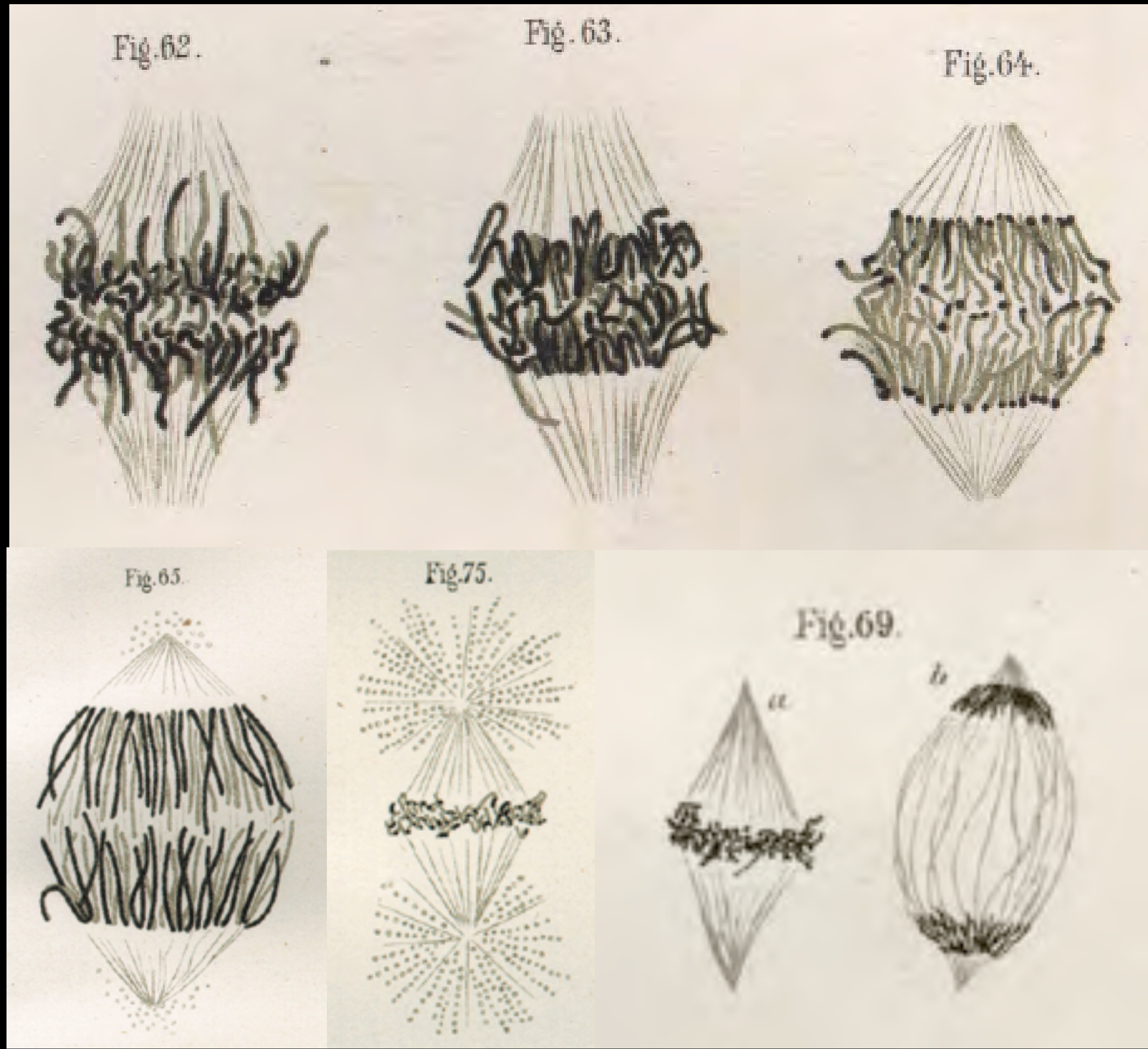
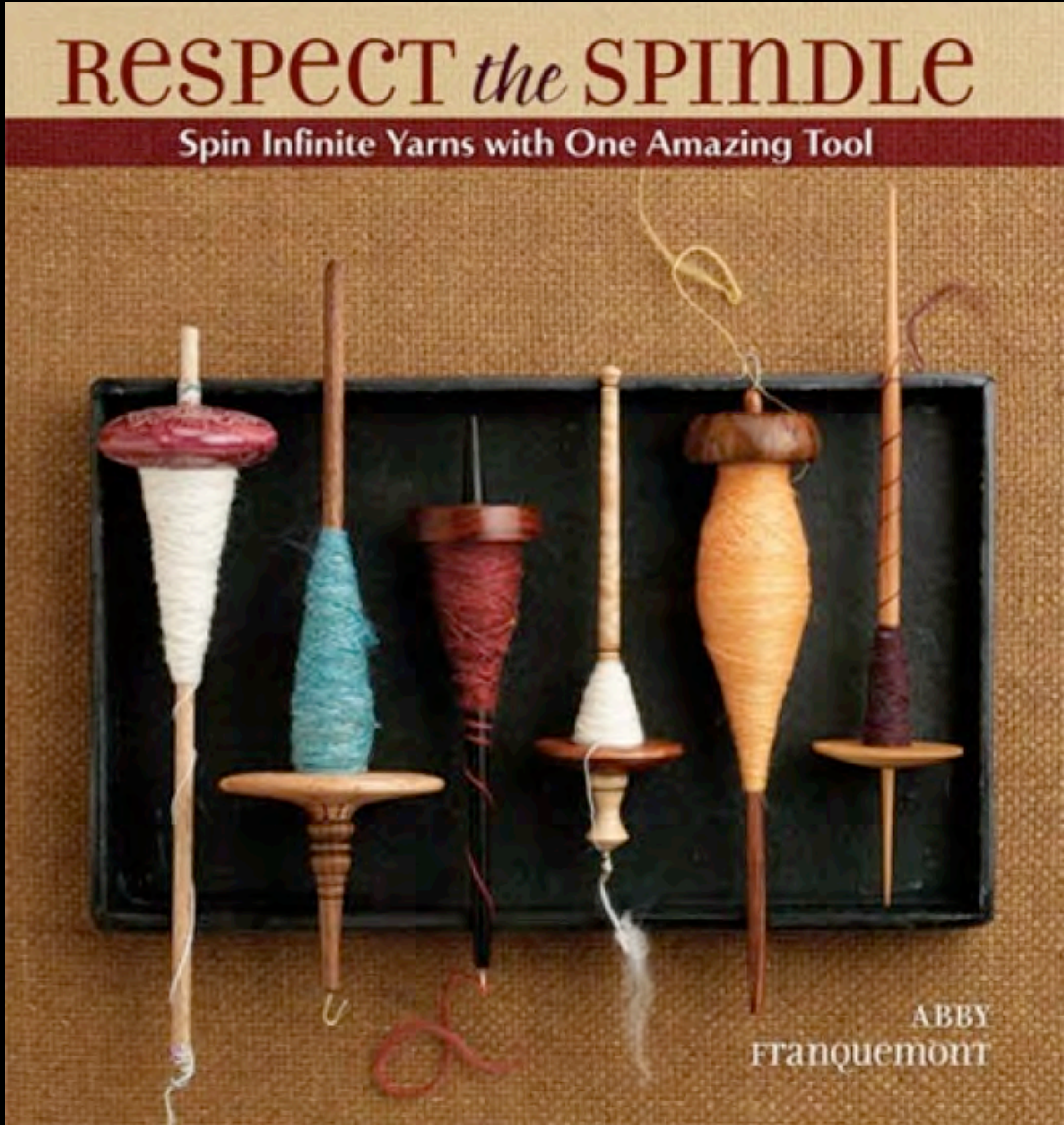
Forces in the mitotic spindle



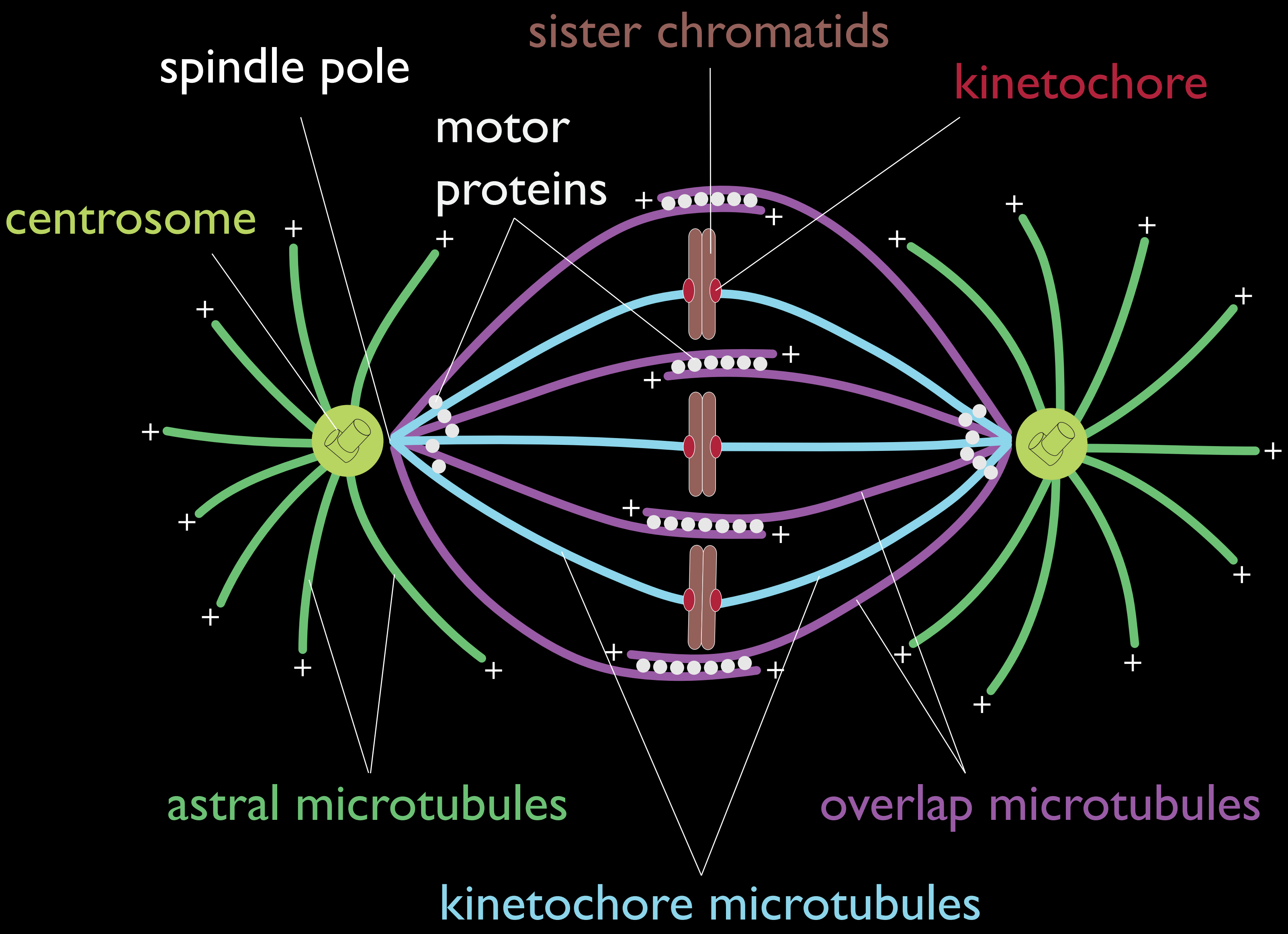
Iva Tolić
Ruđer Bošković Institute, Zagreb



W. Flemming. Zellsubstanz, Kern und Zelltheilung
(F. C. W. Vogel, Leipzig, 1882)

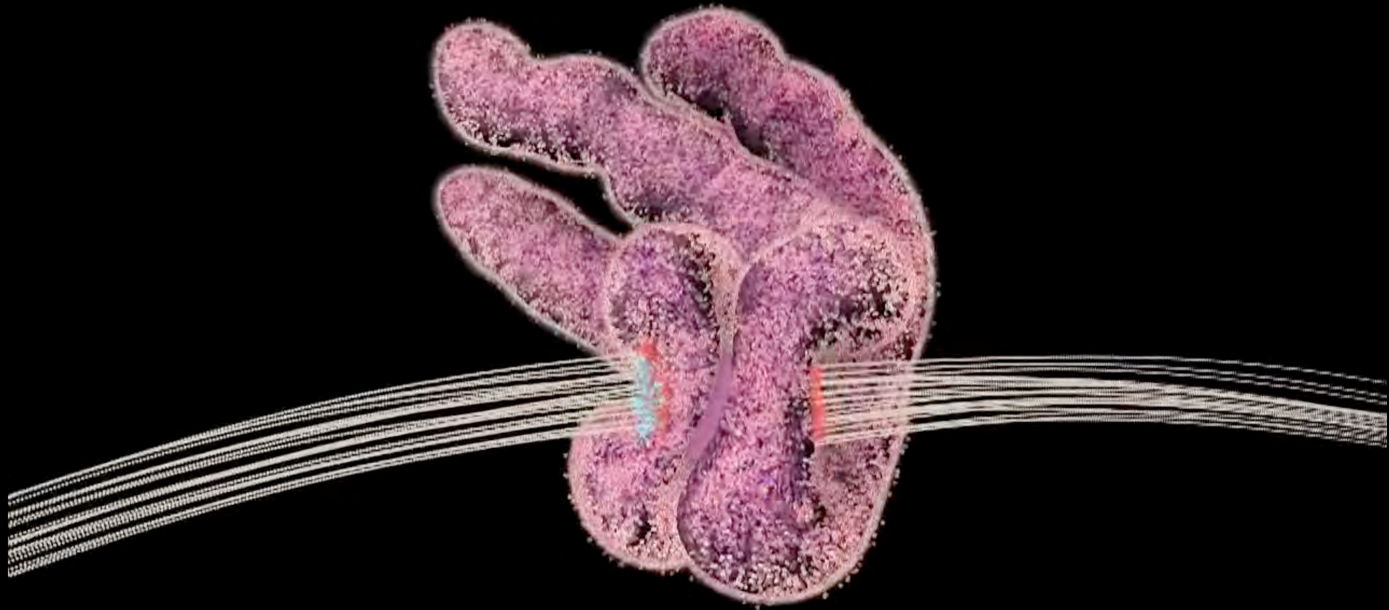

RESPECT the SPINDLE
Spin Infinite Yarns with One Amazing Tool
ABBY
FRANQUEMONT

The mitotic spindle



Alberts *et al.*, Molecular Biology of the Cell

Kinetochores

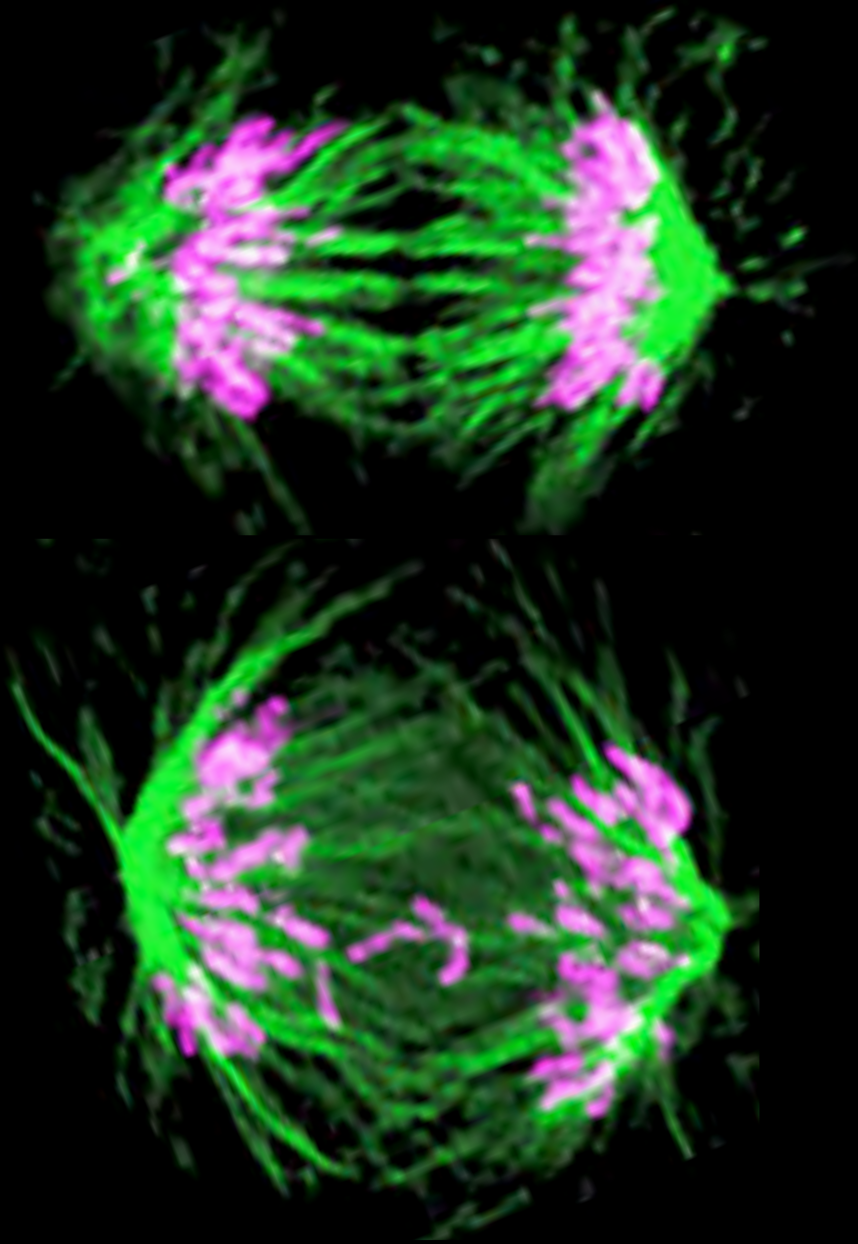


Drew Berry

Functions of the kinetochore

- attachment of chromosomes to spindle MTs
- activation of the spindle checkpoint (tension sensing)
- force generation for chromosome movement

Accuracy



The principal task of the spindle is to segregate the chromosomes without errors.

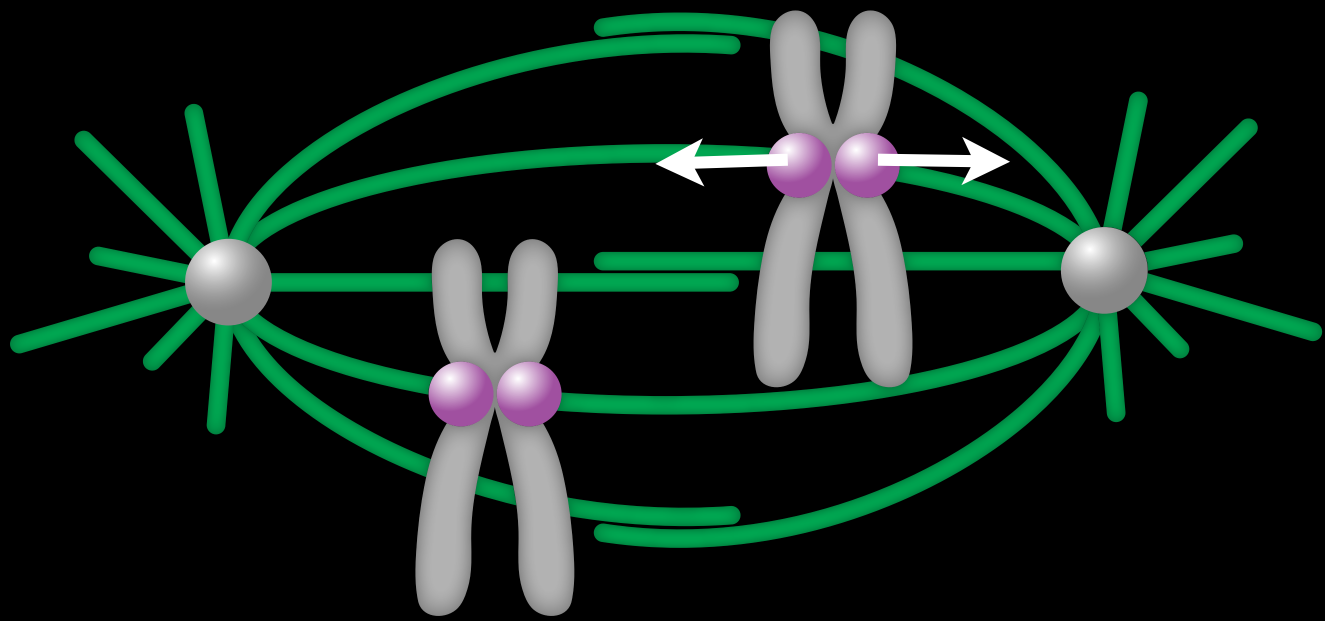
Chromosome loss:
1 in 10,000 cell divisions in yeast
(Hartwell & Smith, 1985).

Aneuploidy:
genetic disorders, cancer

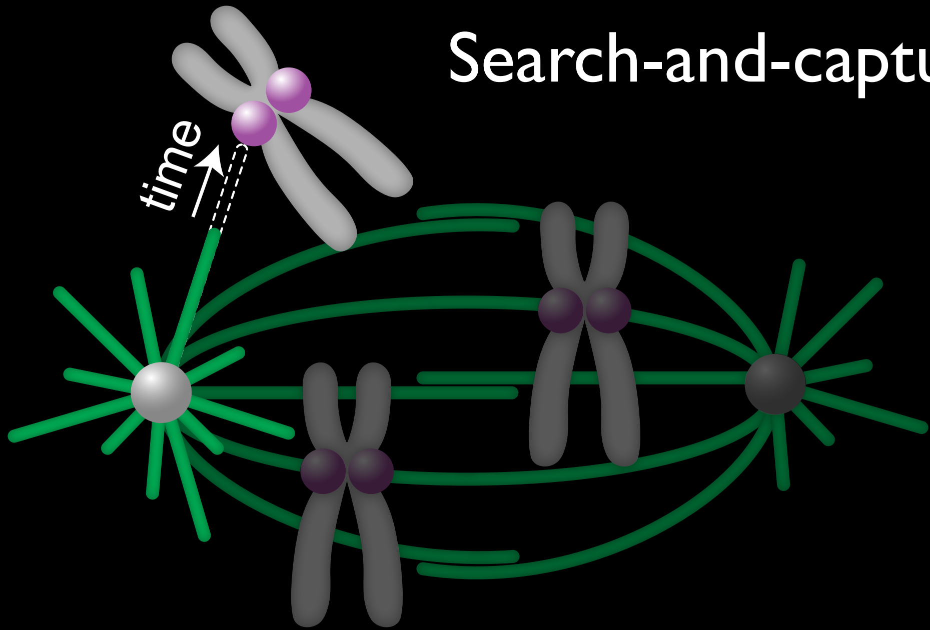
The challenge

1. How does the spindle self-assemble?

2. What forces act on chromosomes?



How does the spindle self-assemble?



Search-and-capture

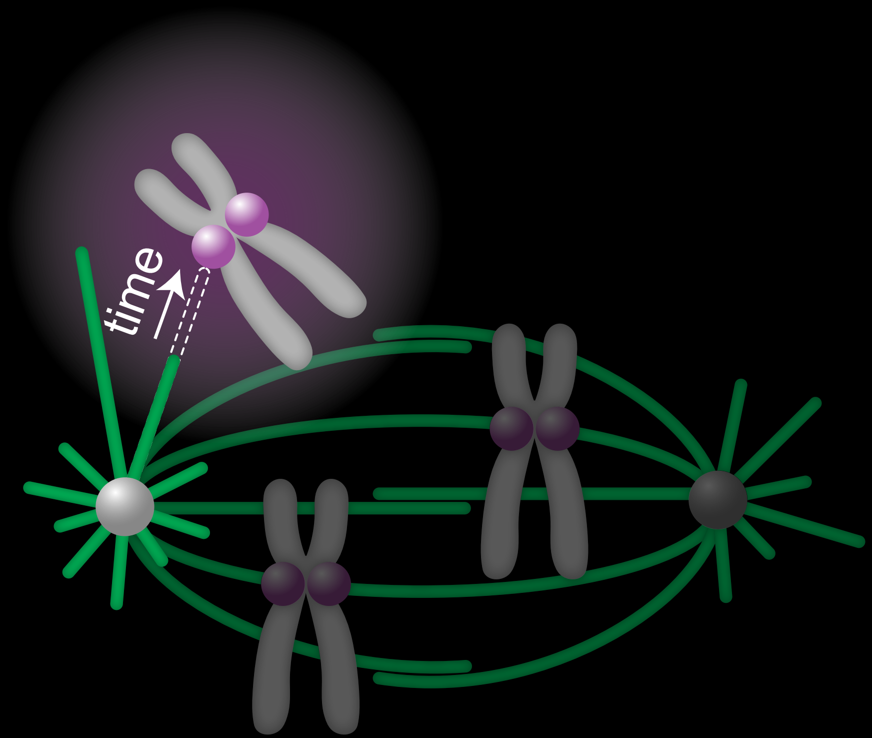
Kirschner and Mitchison, 1986

The time required for microtubules to capture all kinetochores exceeds the duration of prometaphase

⇨ other mechanisms.

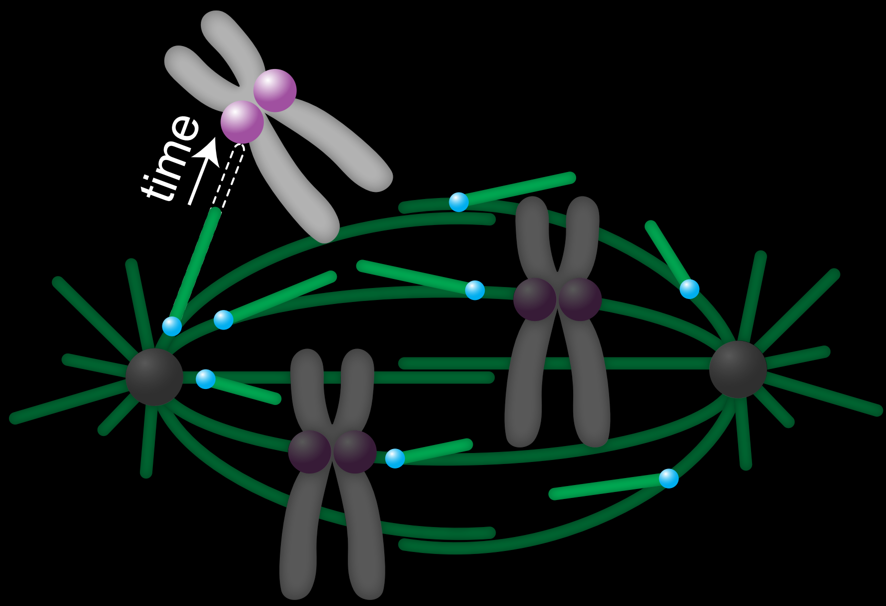
Mechanisms that accelerate kinetochore capture

Bias in MT dynamics towards the chromosomes



Carazo-Salas et al. 2001
Wilde et al. 2001

Nucleation of MTs at spindle MTs

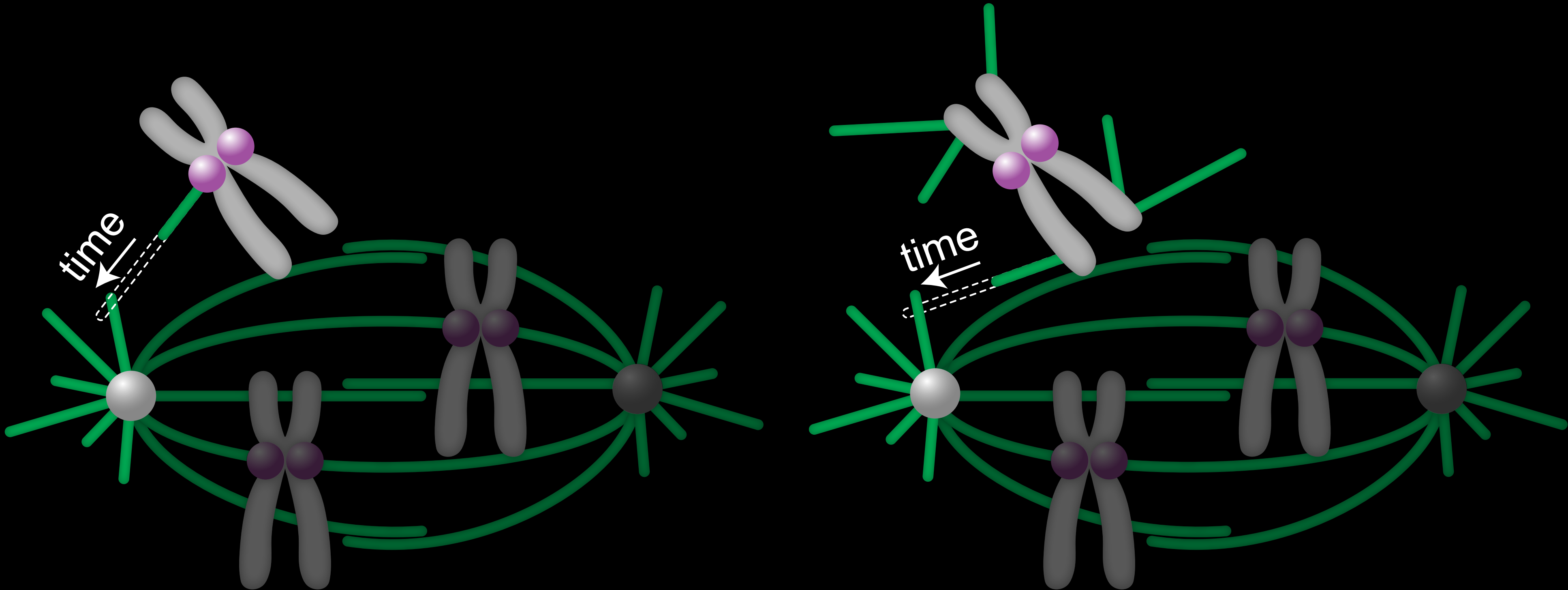


Goshima et al. 2008

Mechanisms that accelerate kinetochore capture

Nucleation of MTs at the kinetochore

Nucleation of MTs at the chromosome

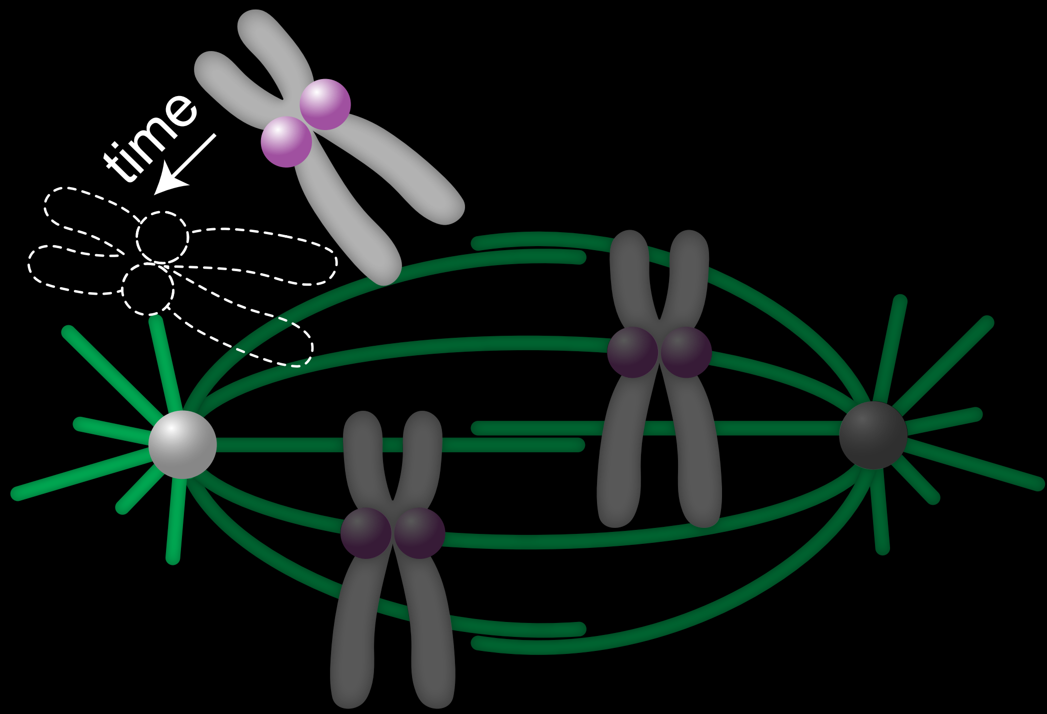


Witt et al. 1980
De Brabander et al. 1981

Karsenti et al. 1984
Carazo-Salas et al. 1999

Mechanisms that accelerate kinetochore capture

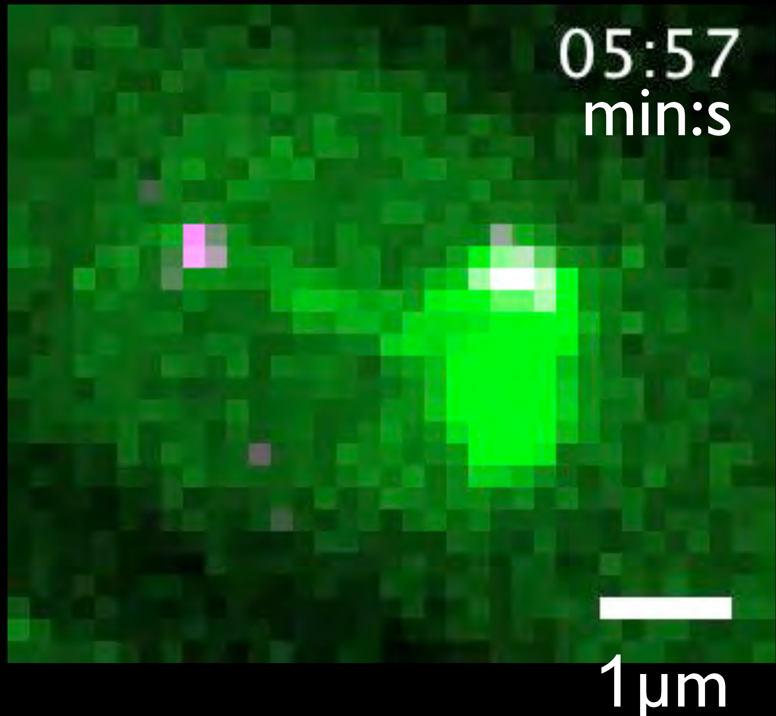
Chromosome movements



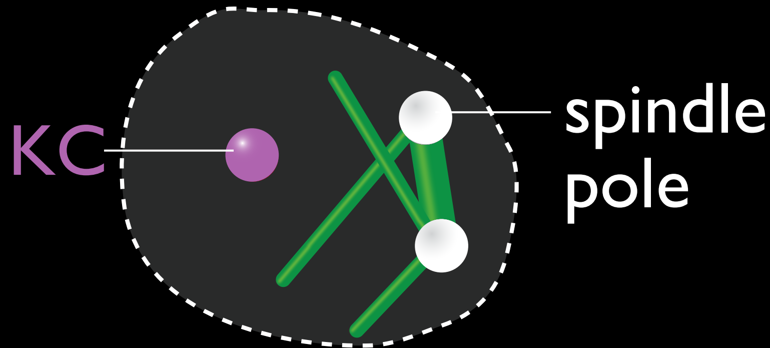
Paul et al. 2009

Observation of individual capture events

Fission yeast
S. pombe

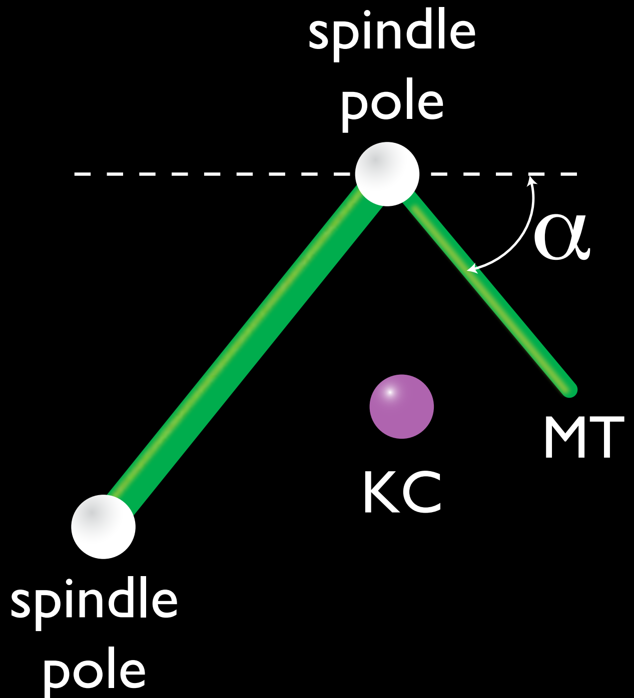


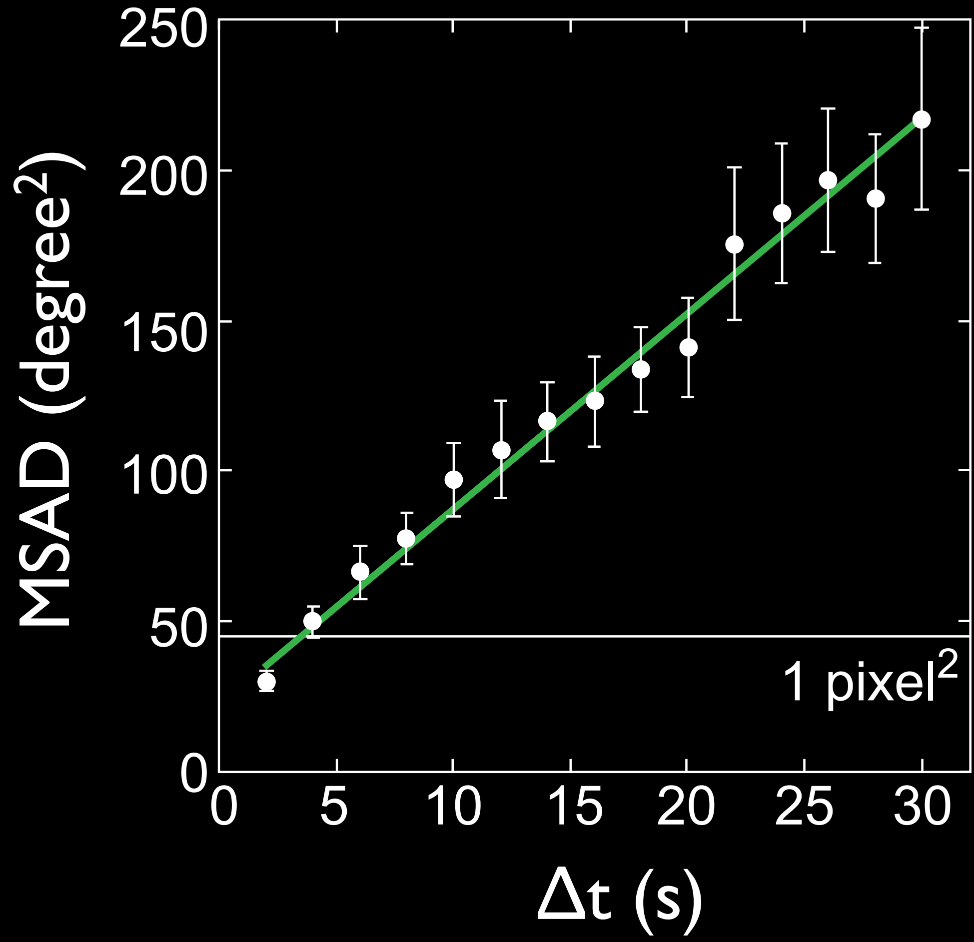
GFP-tubulin
ndc80-tdTomato



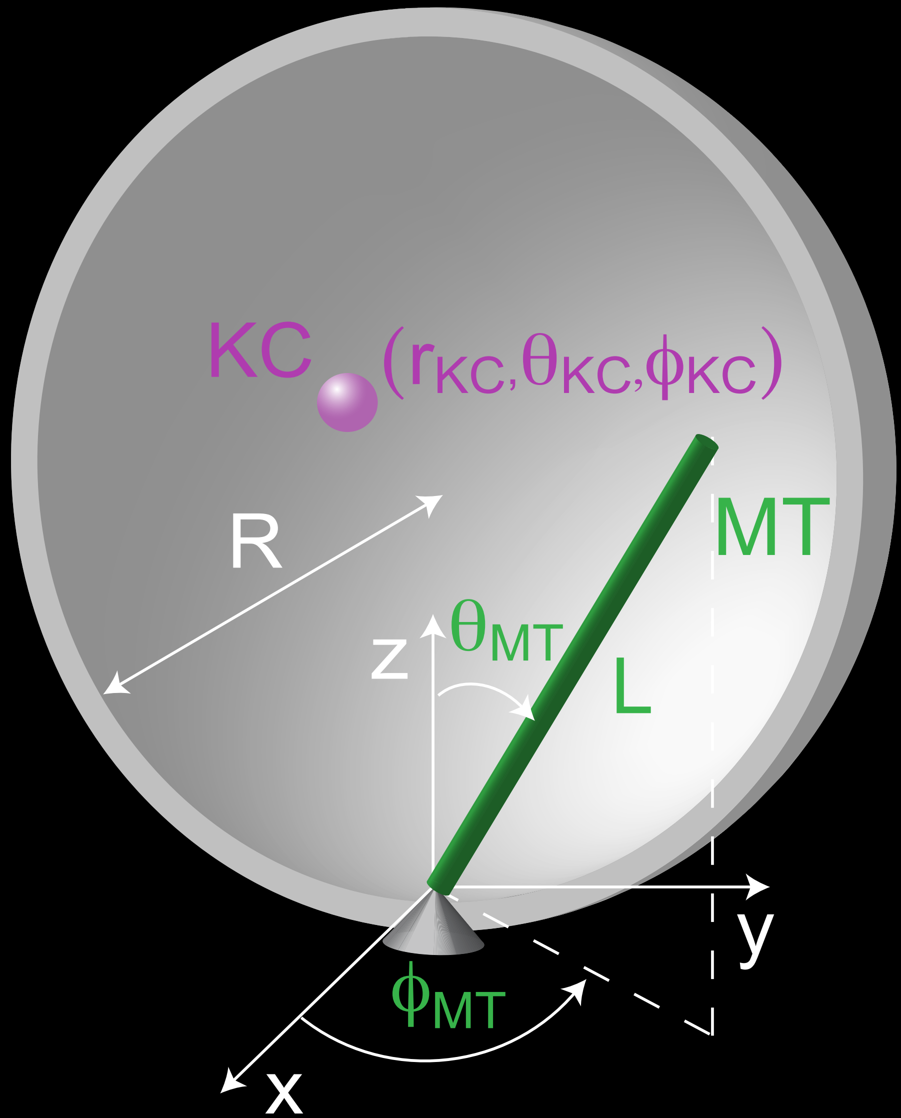
Iana Kalinina

Pivoting of microtubules is random





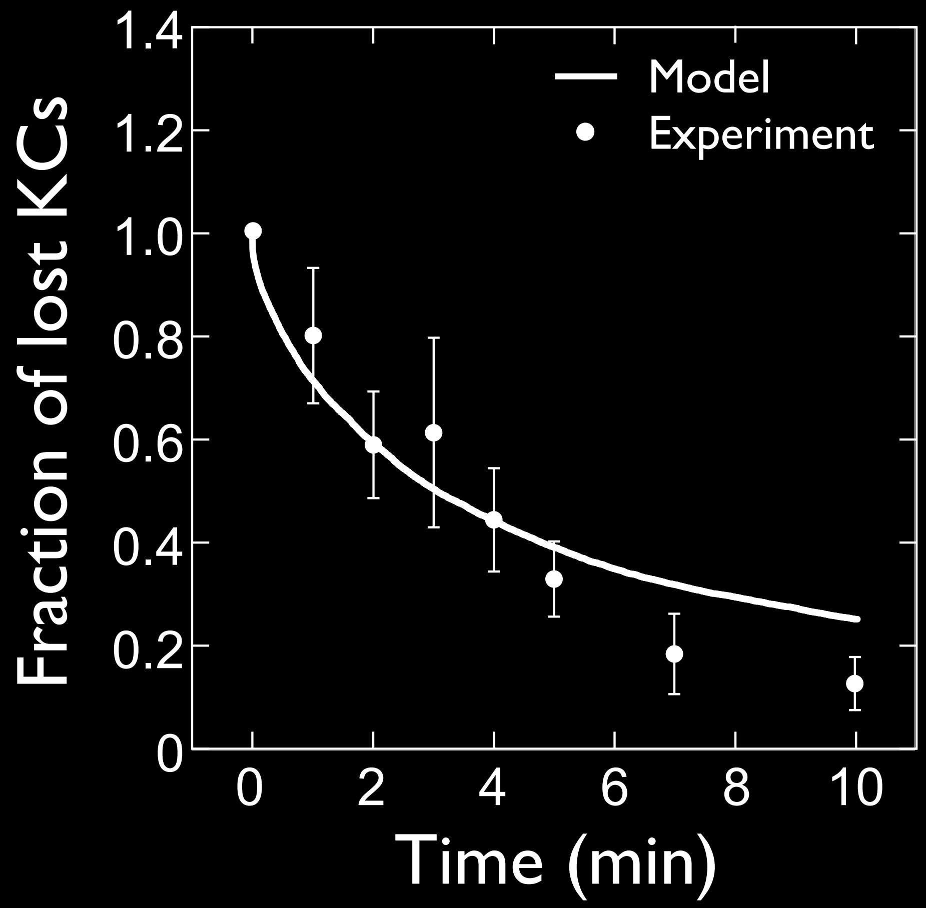
Theoretical model



- Microtubule pivots around the spindle pole.
- Kinetochore diffuses.

Amitabha Nandi, Benjamin Lindner, Nenad Pavin

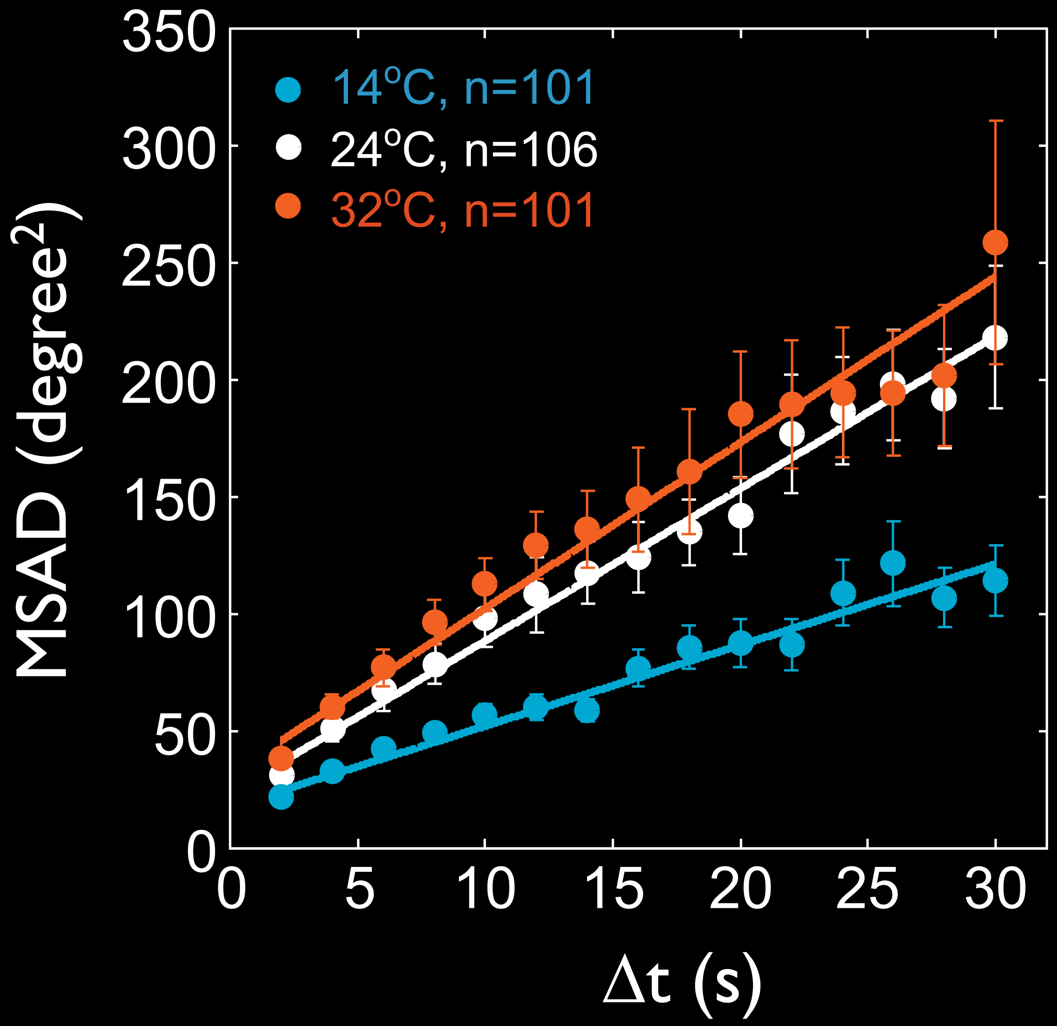
Test of the model



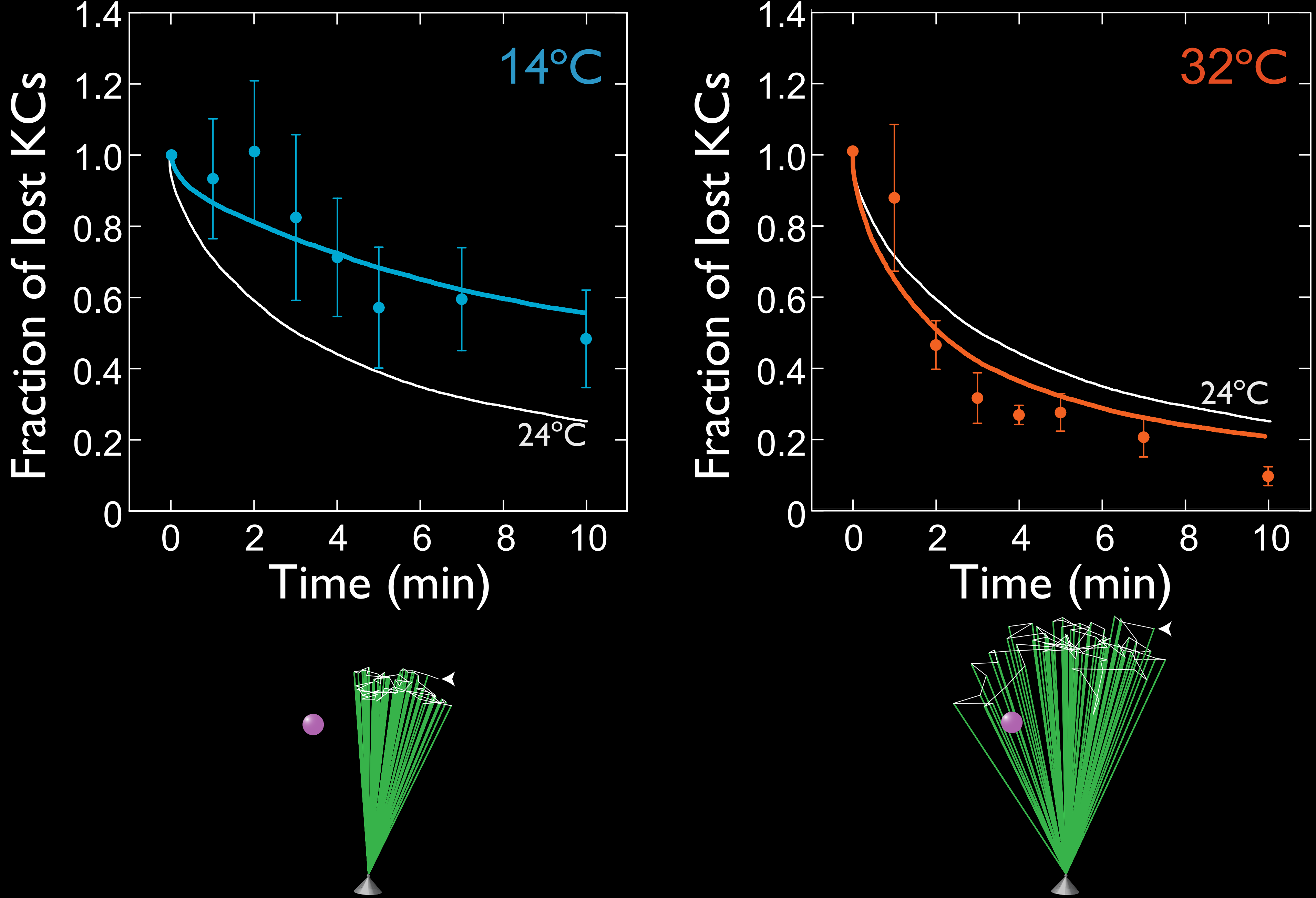
Pivoting model – **3** minutes

"Search-and-capture" model – **100** minutes

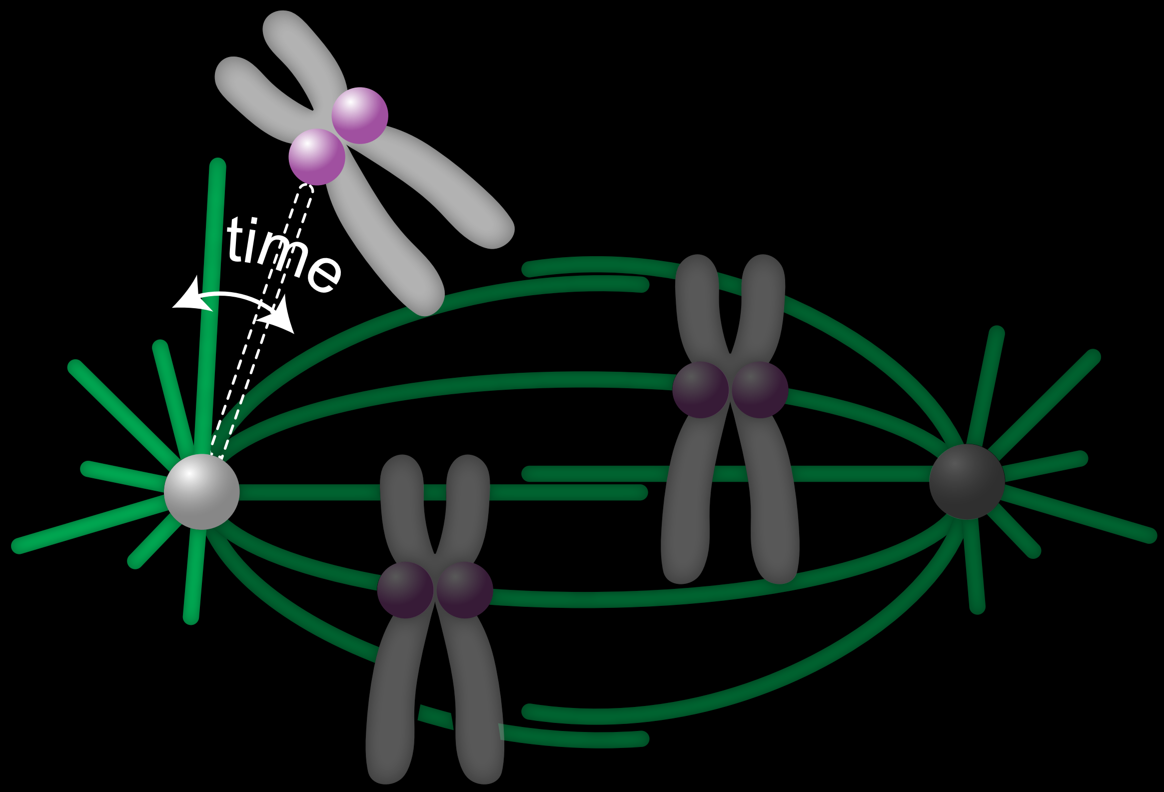
Pivoting of microtubules changes with temperature



Tests of the model



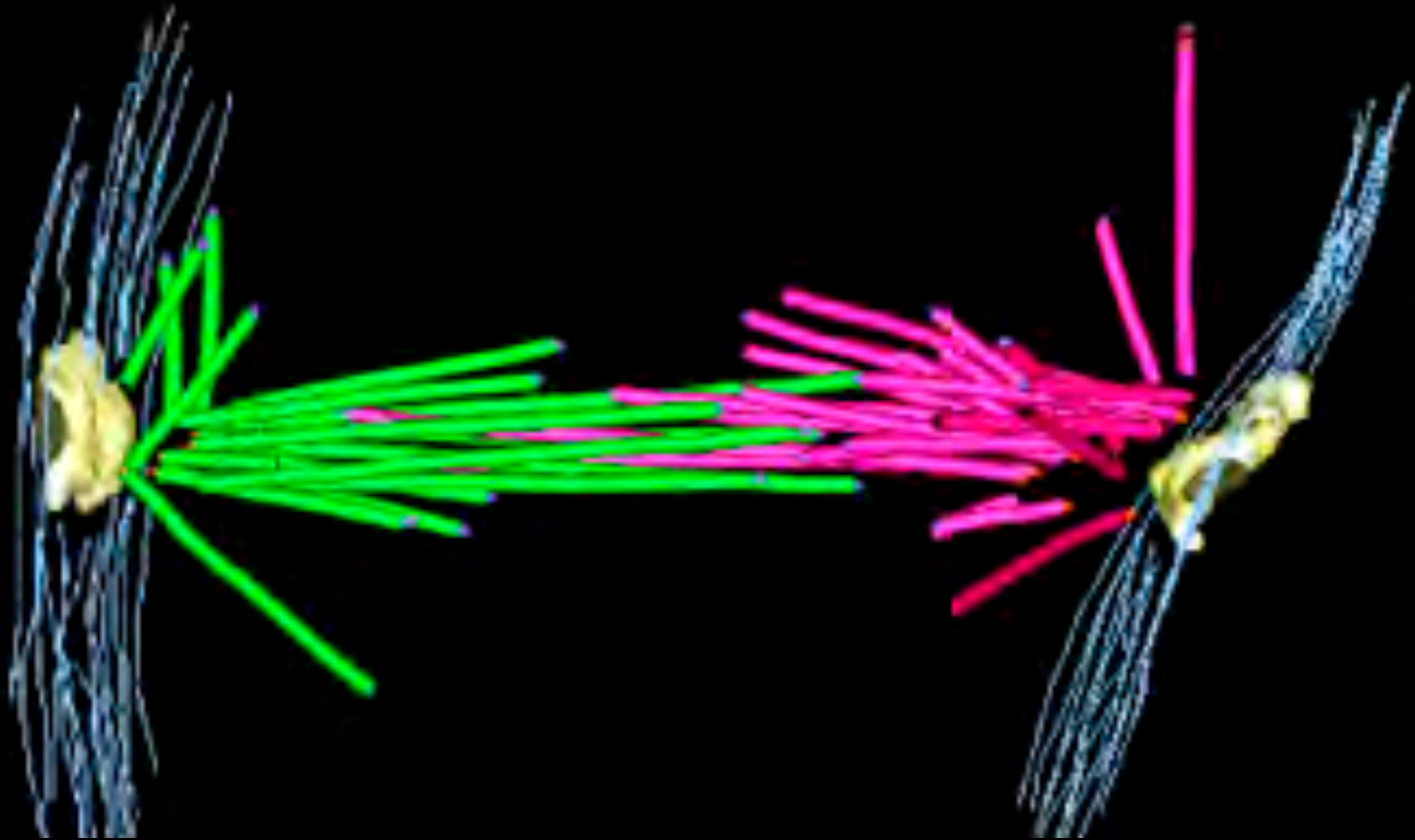
MT pivoting around the spindle pole accelerates capture



Kalinina, Nandi, ..., Lindner, Pavin, Tolić-Nørrelykke, *Nat Cell Biol* 2013

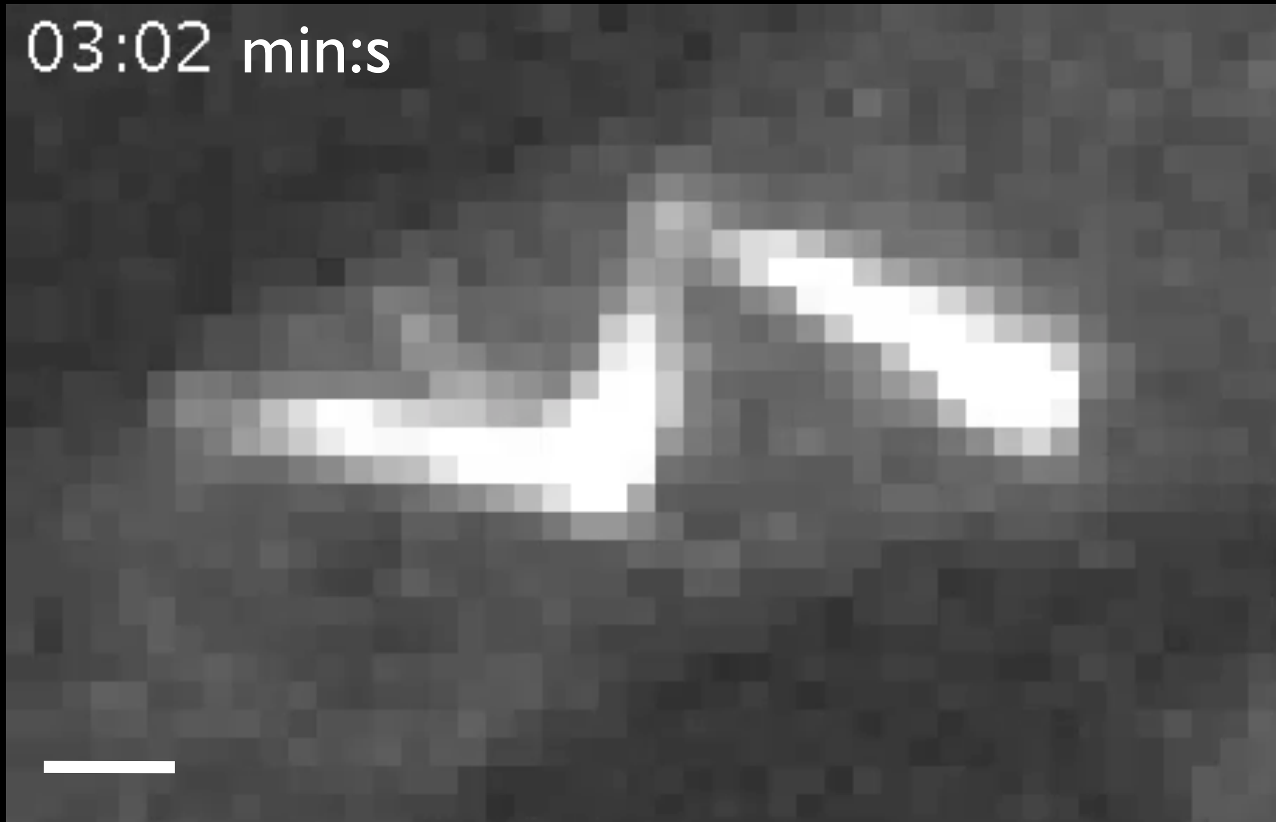
Pavin & Tolić-Nørrelykke, *Syst Synth Biol* 2014

The spindle consists of parallel and antiparallel MT bundles



Grishchuk & McIntosh, 2006

MT pivoting accelerates spindle assembly



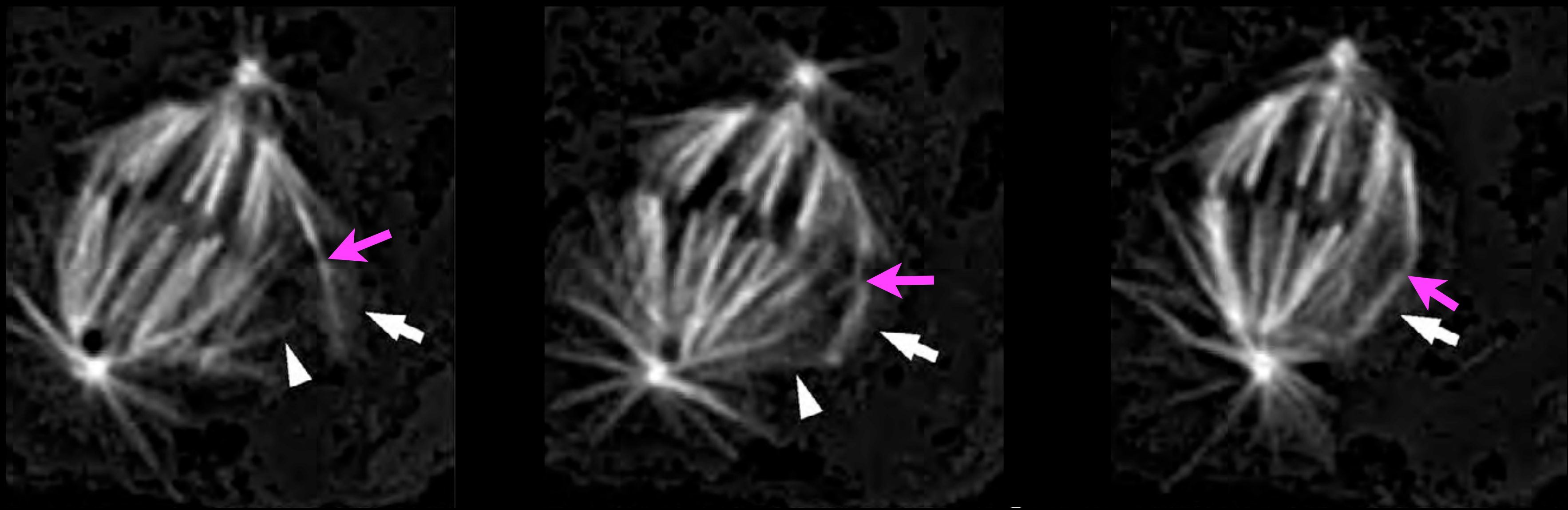
1µm

GFP-tubulin

Lora Winters

MTs growing from kinetochores pivot

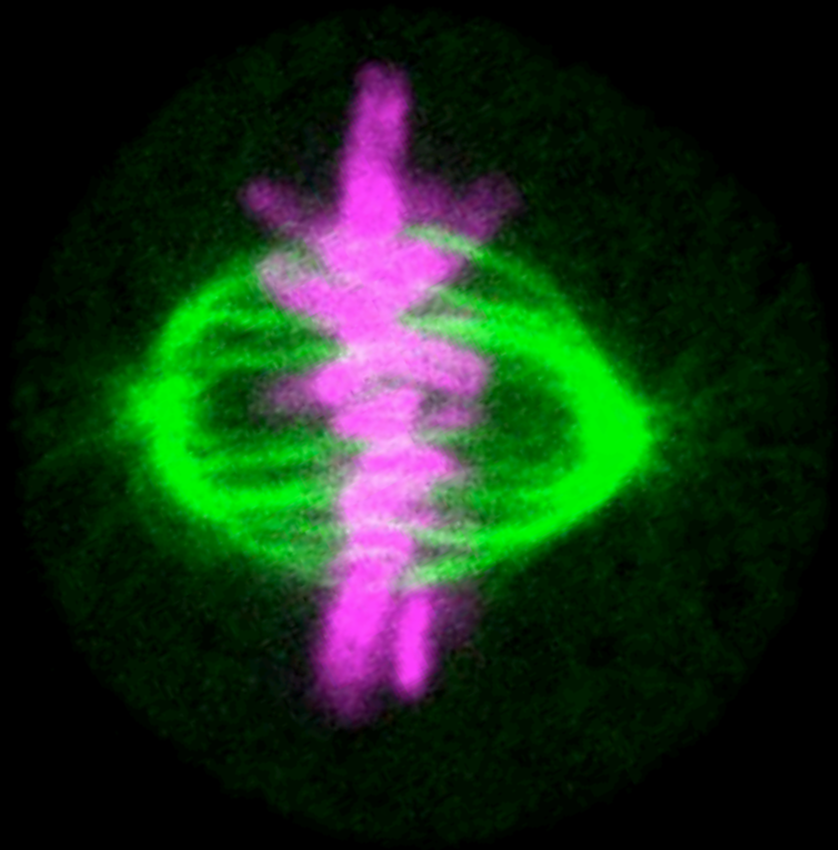
Pivoting motion of kinetochore-bound MTs helps spindle assembly in higher cells.



Drosophila S2 cells

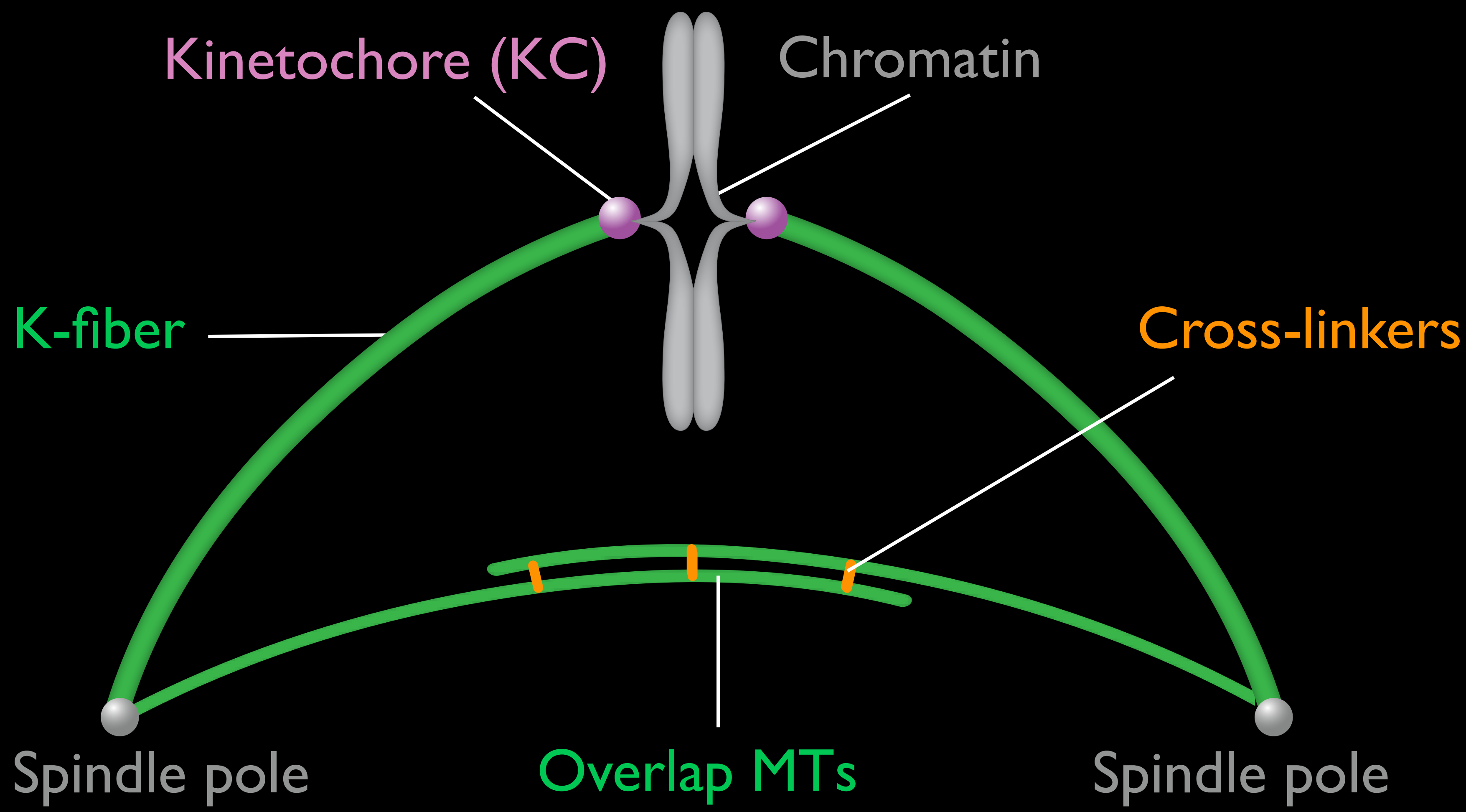
Maiato et al. 2004

What forces act on chromosomes?



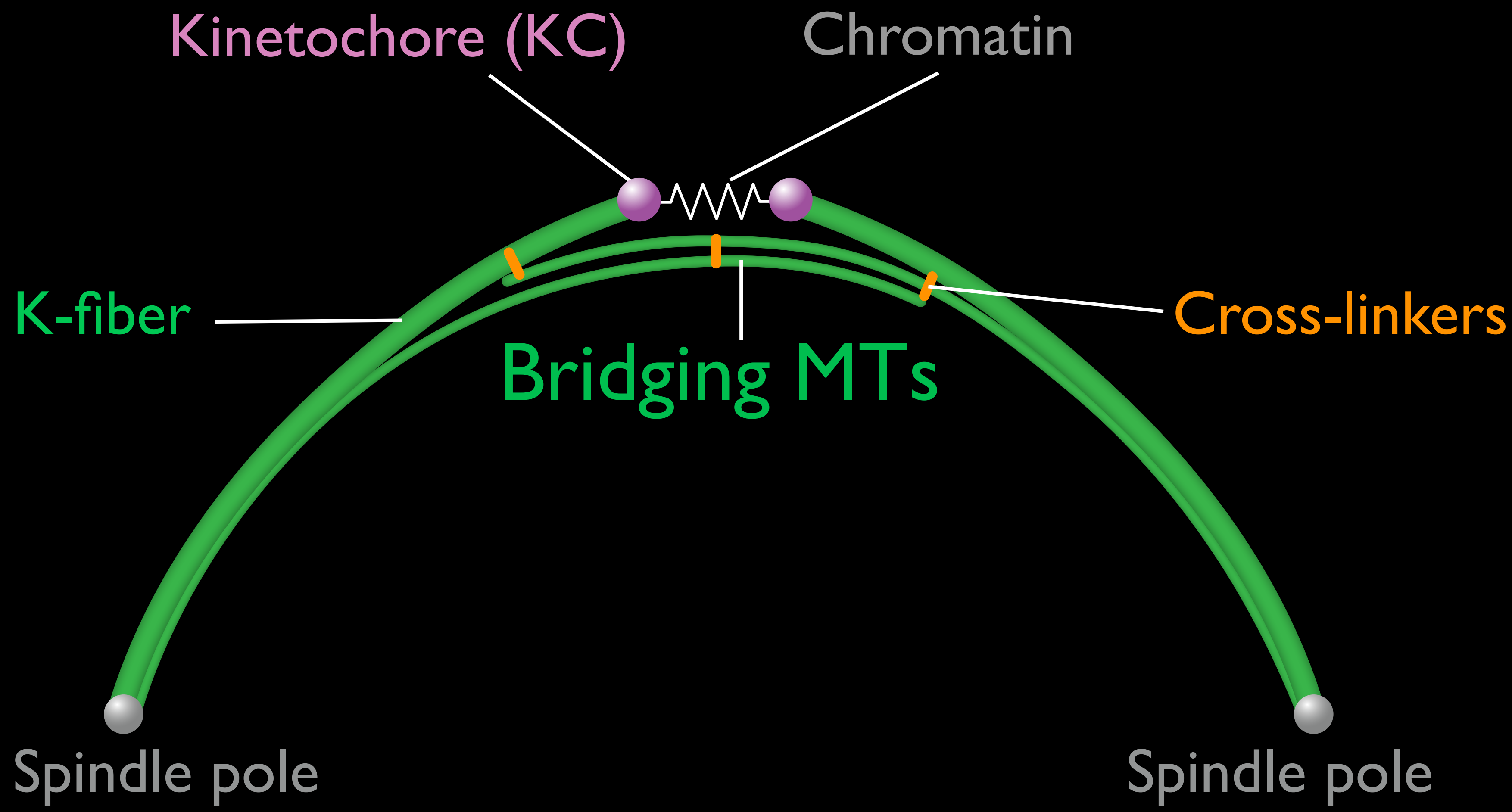
Force generation is a key task of the spindle.

Current paradigm



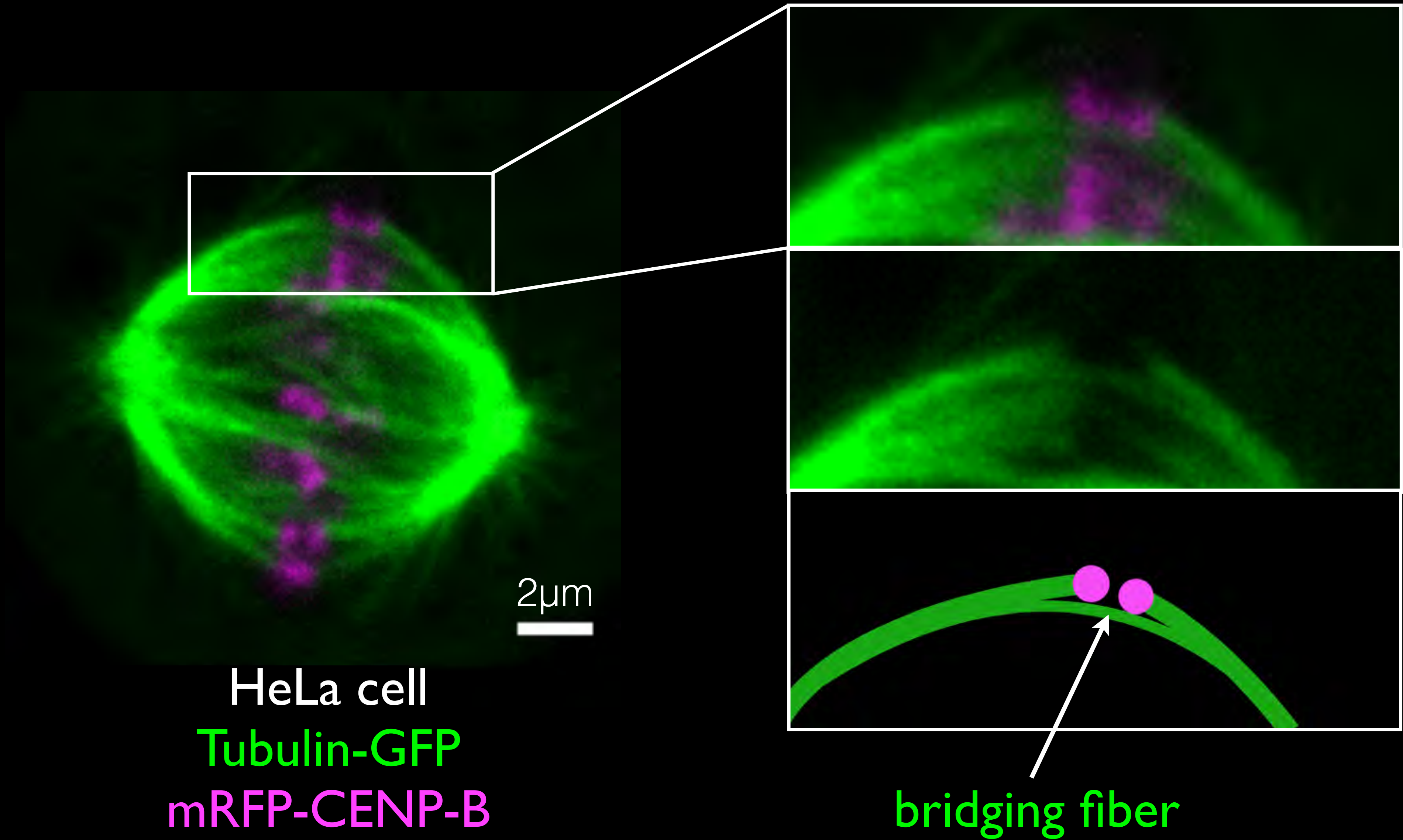
Forces on kinetochores are generated only by k-fibers.

Our hypothesis

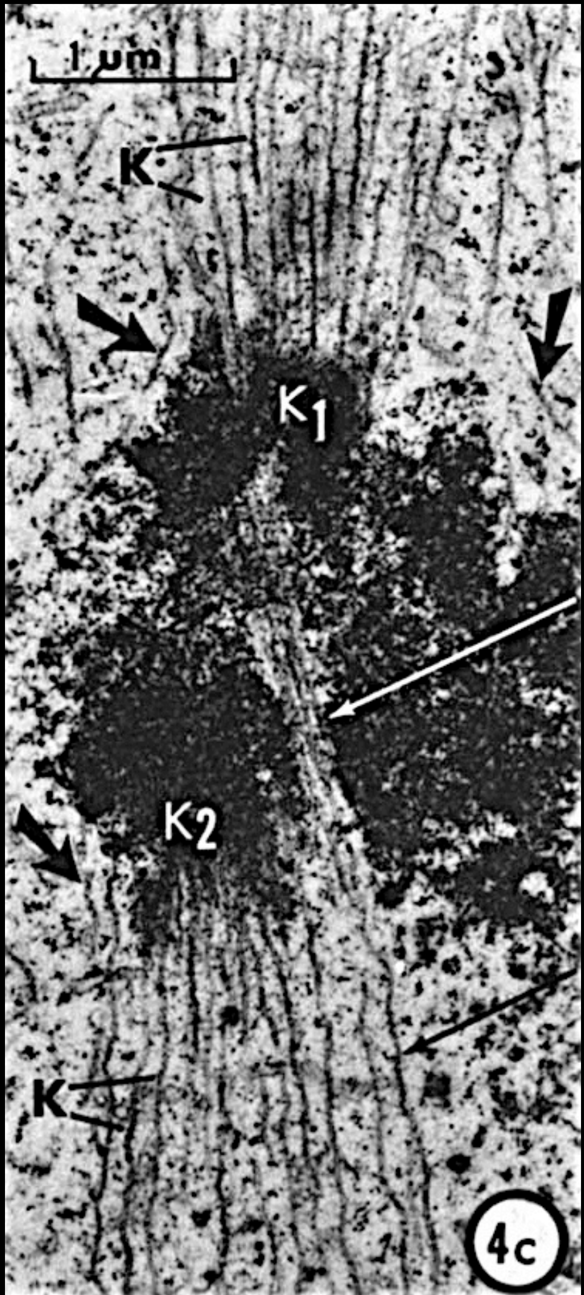


Sister k-fibers are connected by MTs
in addition to the chromatin spring.

Non-kinetochore MTs bridge sister k-fibers



Non-kMTs have been seen near kinetochores in EM

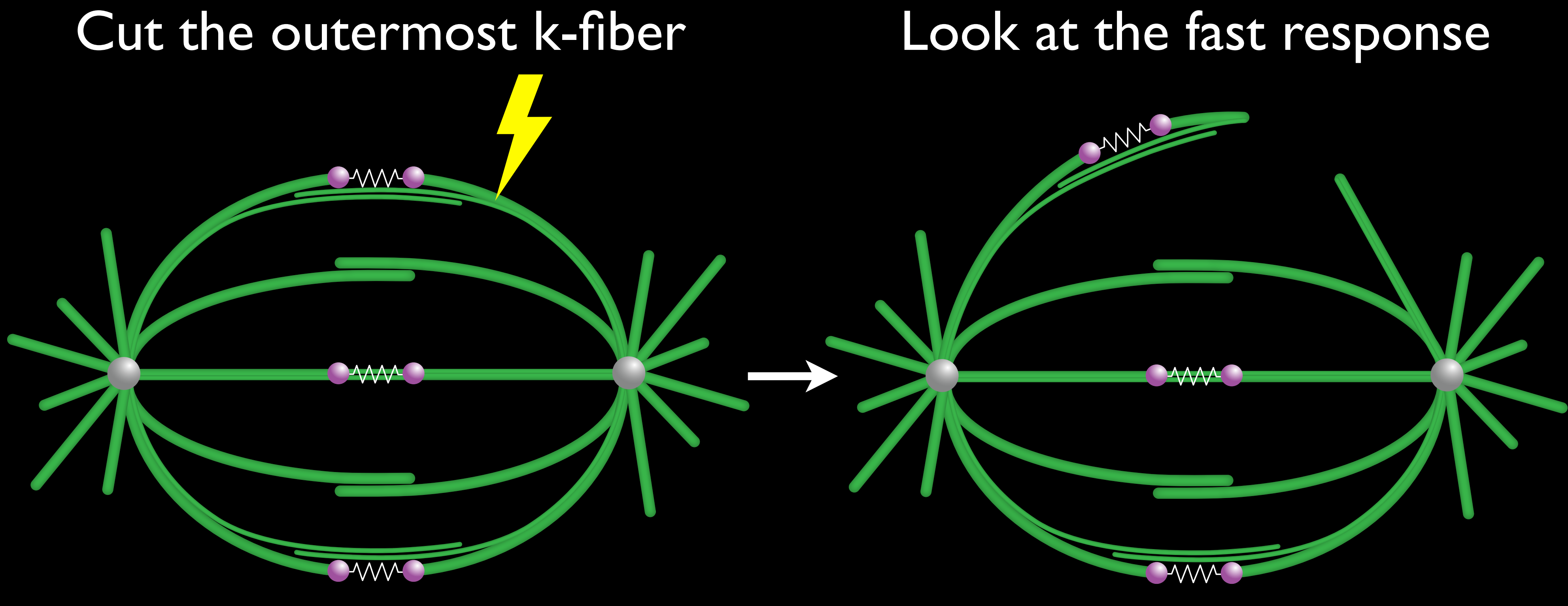


African blood lily

Jensen et al., *J Cell Biol* 1982 (lily)
McDonald *et al.*, *J Cell Biol* 1992 (PtK1)
Ohi *et al.*, *Dev Cell* 2003 (Xenopus)

Function of these MTs?

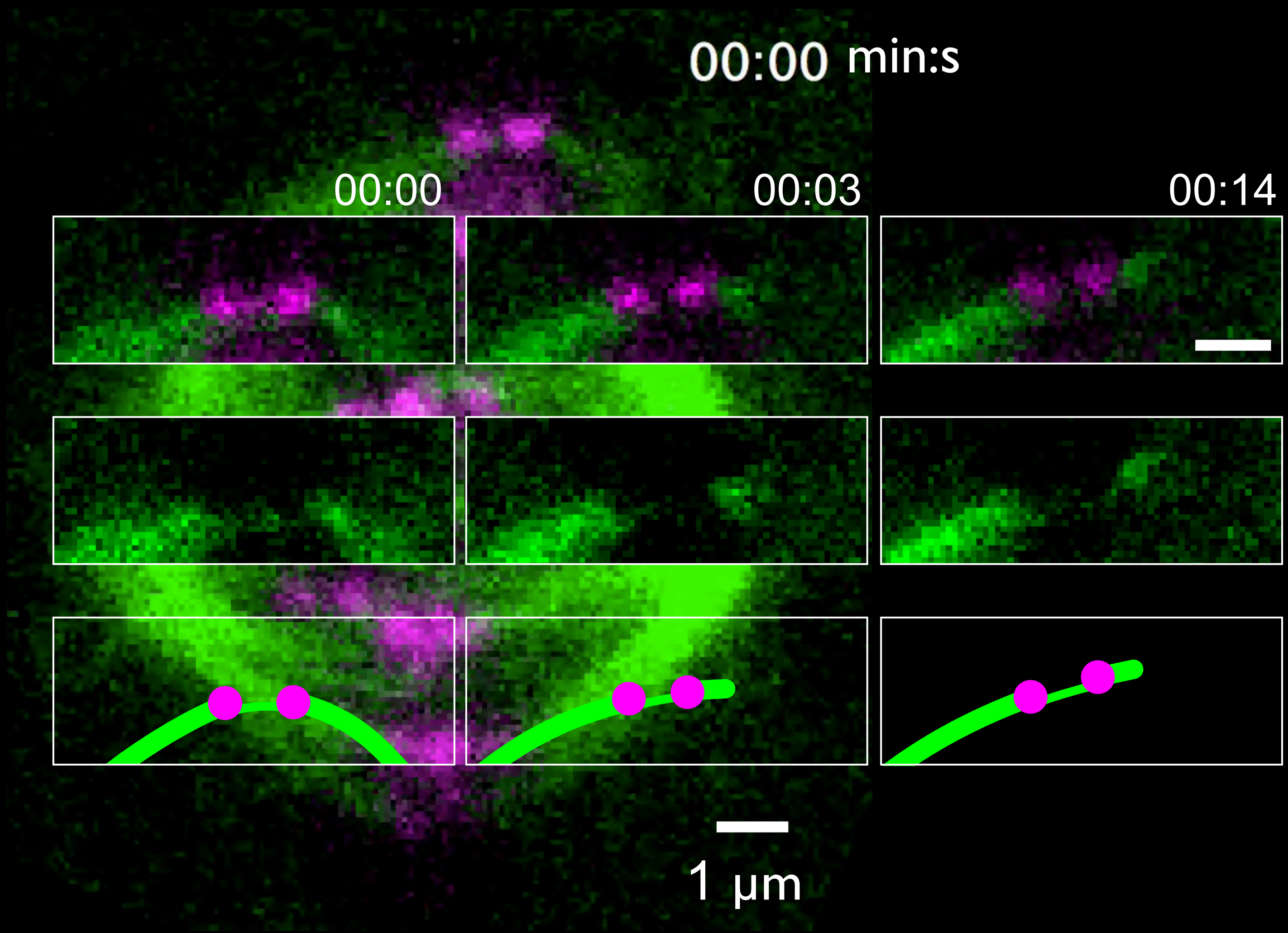
Laser-cutting assay for the study of bridging MTs



Similar laser-cutting of k-fibers:

Maiato *et al.*, *J Cell Biol* 2004
Dick and Gerlich, *Nat Cell Biol* 2013
Sheykhani et al., *Cytoskeleton* 2013
Sikirzhytski *et al.*, *J Cell Biol* 2014
Elting *et al.*, *J Cell Biol* 2014

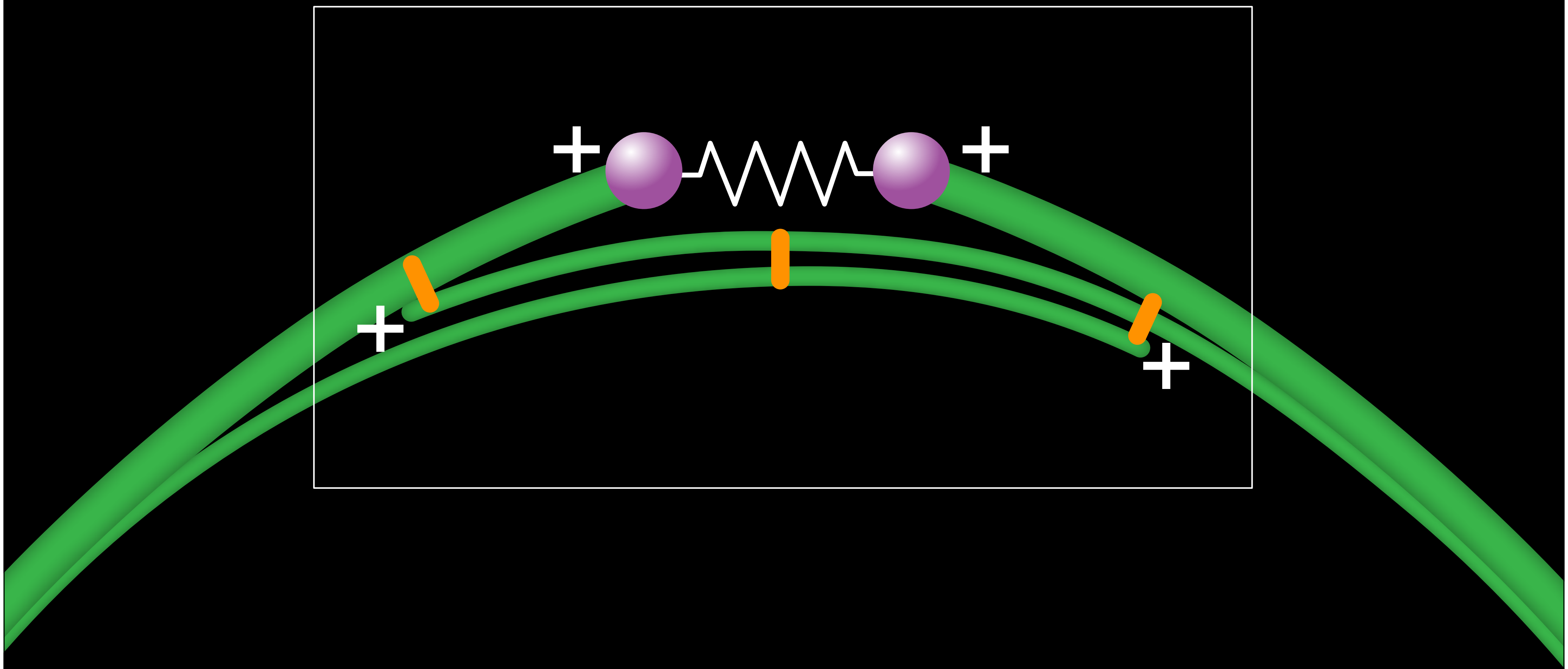
Bridging MTs are connected with k-fibers in HeLa cells



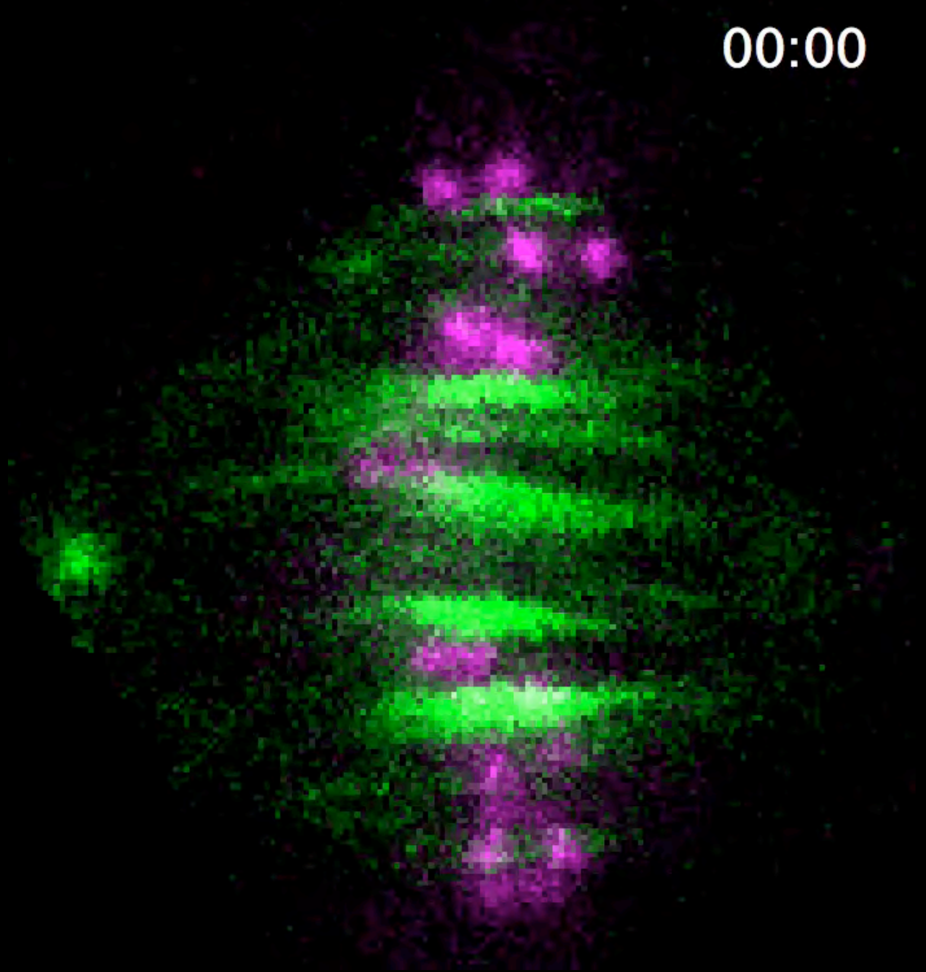
Tubulin-GFP
mRFP-CENP-B

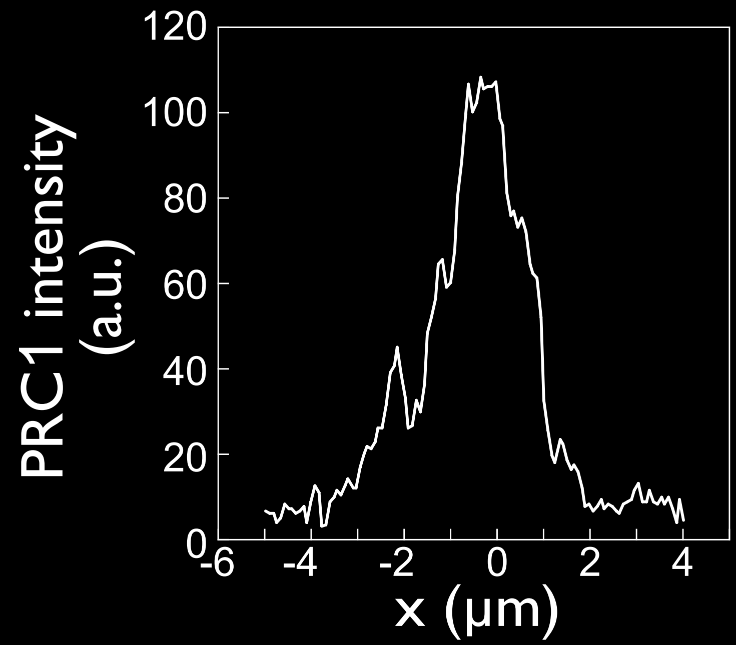
Janko Kajtez, Anastasia Solomatina

Do bridging fibers contain antiparallel overlaps?



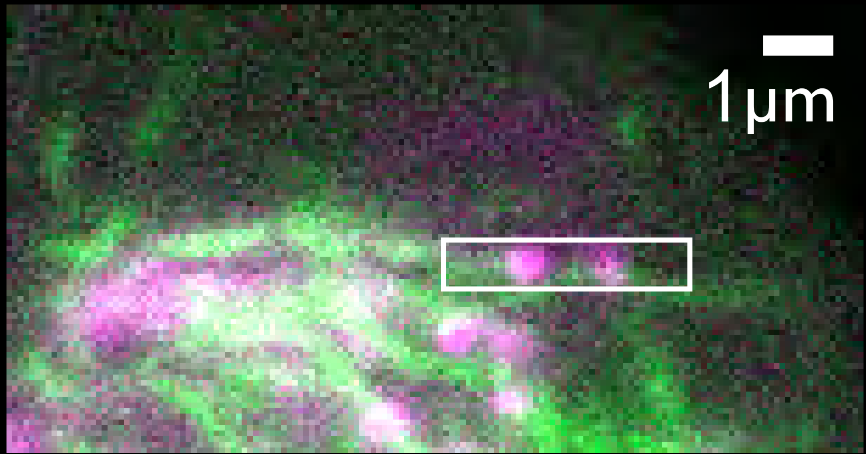
PRC1 is found in the bridging fiber



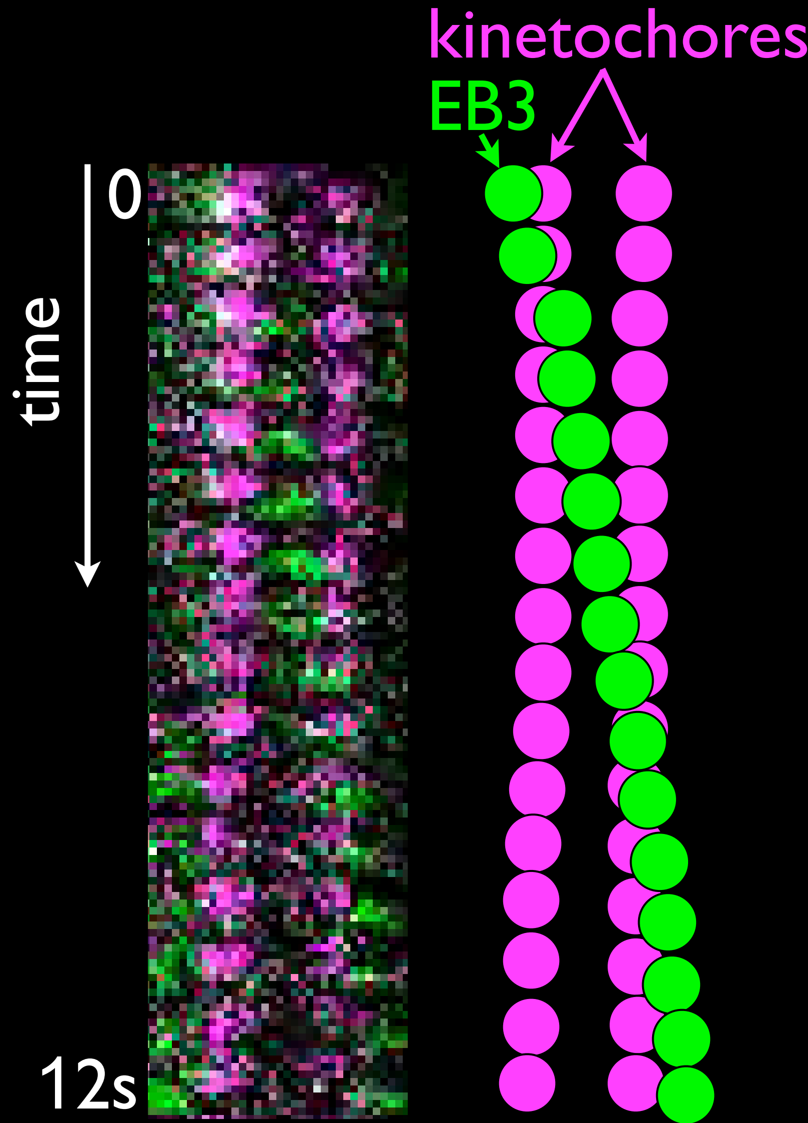


PRC1-GFP
(overlap regions of MTs)
mRFP-CENP-B

Bridging fiber contains dynamic MTs



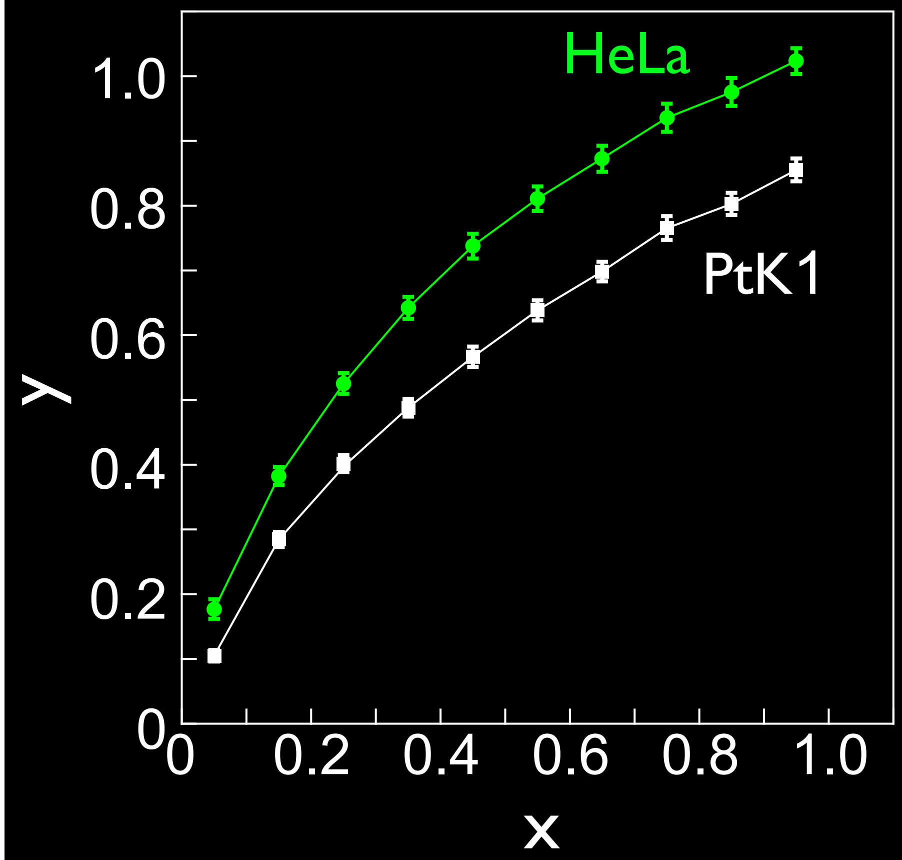
2xGFP-EB3
mCherry-CENP-A

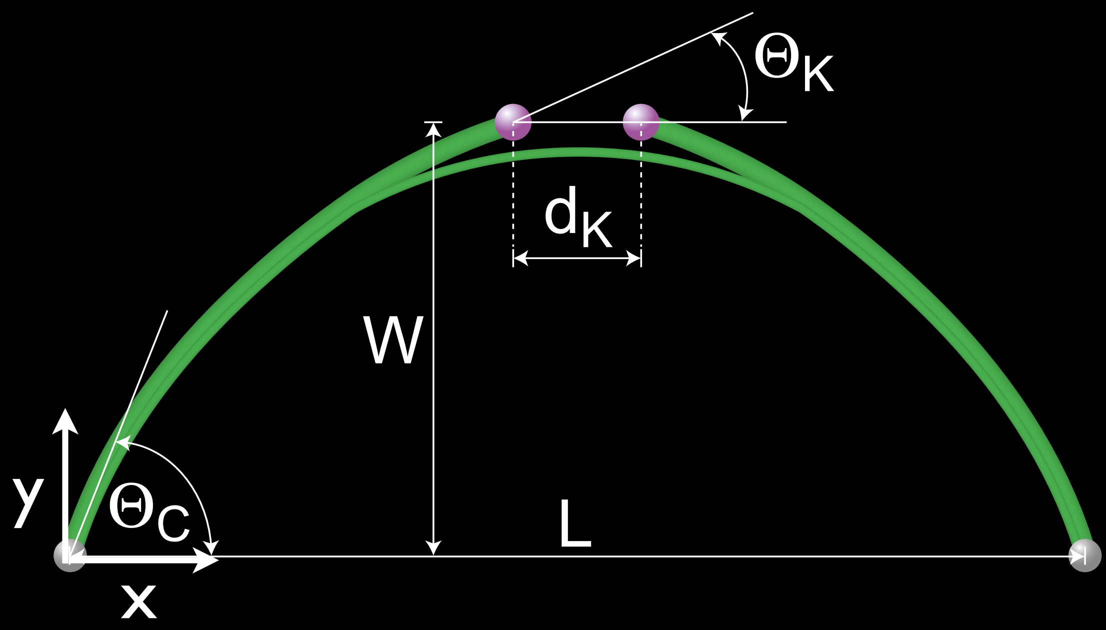


Bruno Polak

1.9±0.4 EB3 spots/min

Shape of the k-fiber

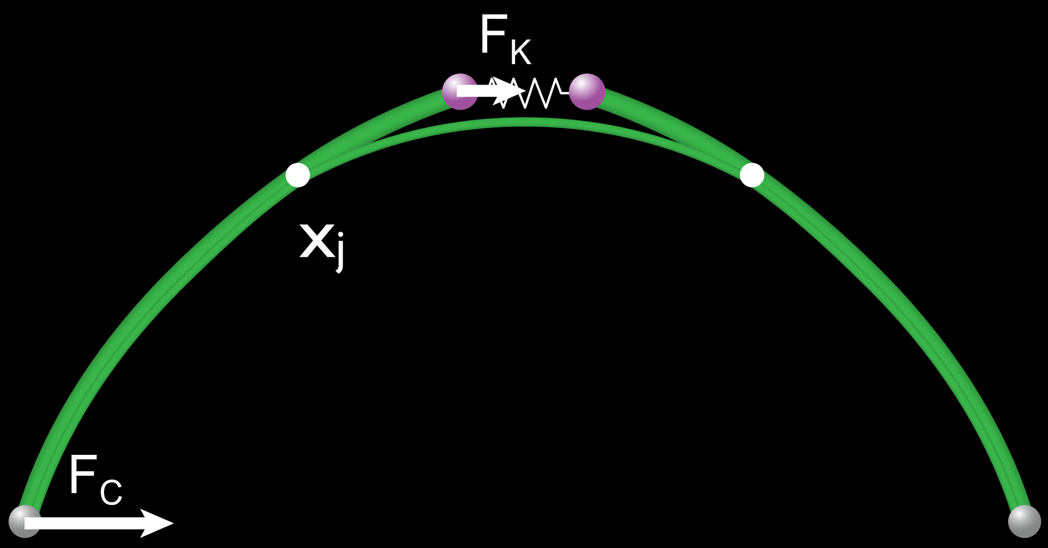




Θ_C did not change after the cut.

Θ_K increased after the cut.

Theory



Maja Novak
Nenad Pavin

In:

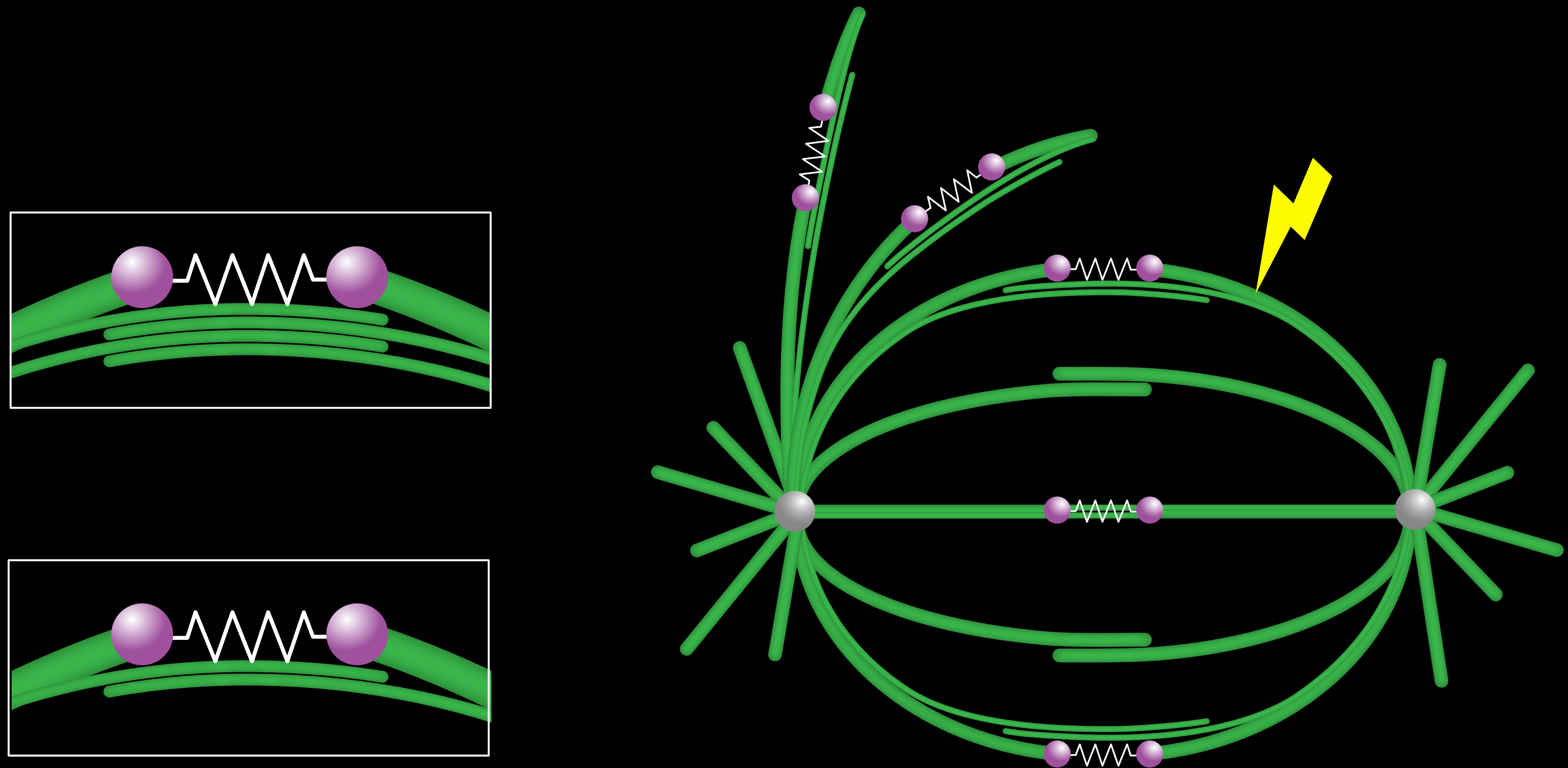
- Measured geometry of the spindle:
  - spindle length and width
  - angles at the spindle pole and at the kinetochore
- Bending rigidity of the k-fiber and of the bridging fiber
  - bending rigidity of a single MT
  - number of MTs in the k-fiber and in the bridging fiber

Out:

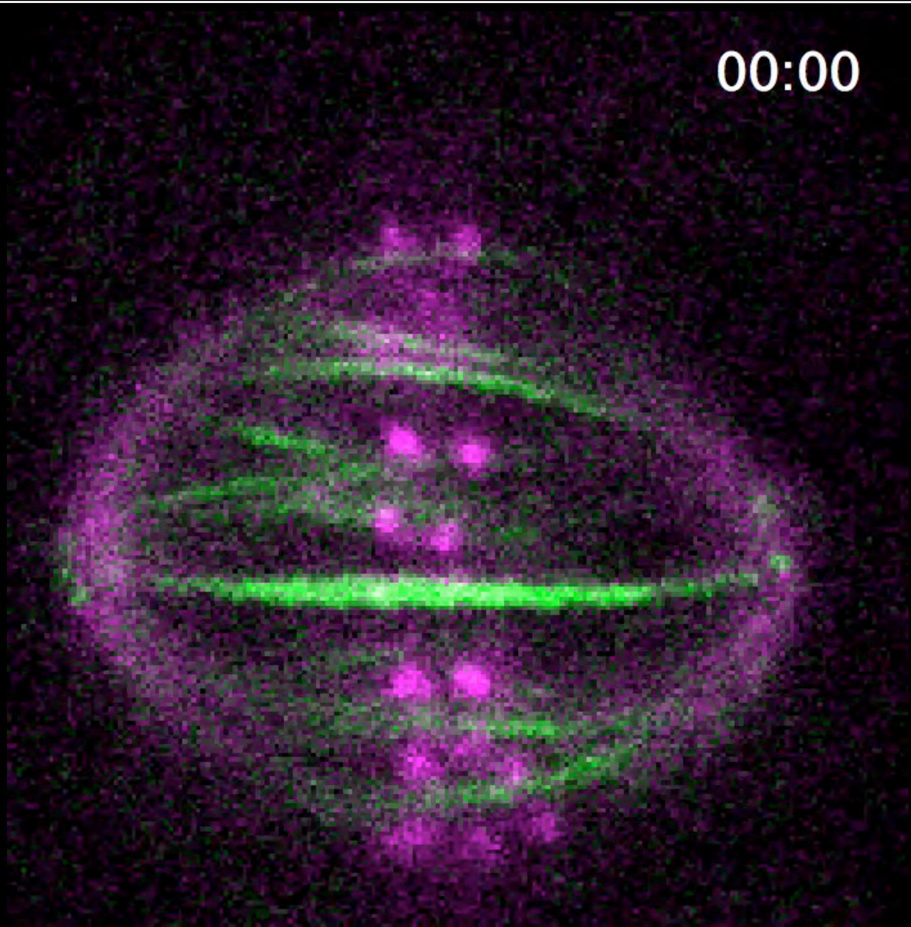
- position of the junction, x_j ~1 μm away from the KC
- force at the spindle pole, F_C ~50 pN
- force at the kinetochore, F_K ~300 pN

How important is the bridging fiber for the force balance?

Bridging fiber with more MTs ⇨ larger force at the pole
⇨ larger force in the bridging fiber
⇨ faster straightening after the force is released



Cells with thicker bridging fibers

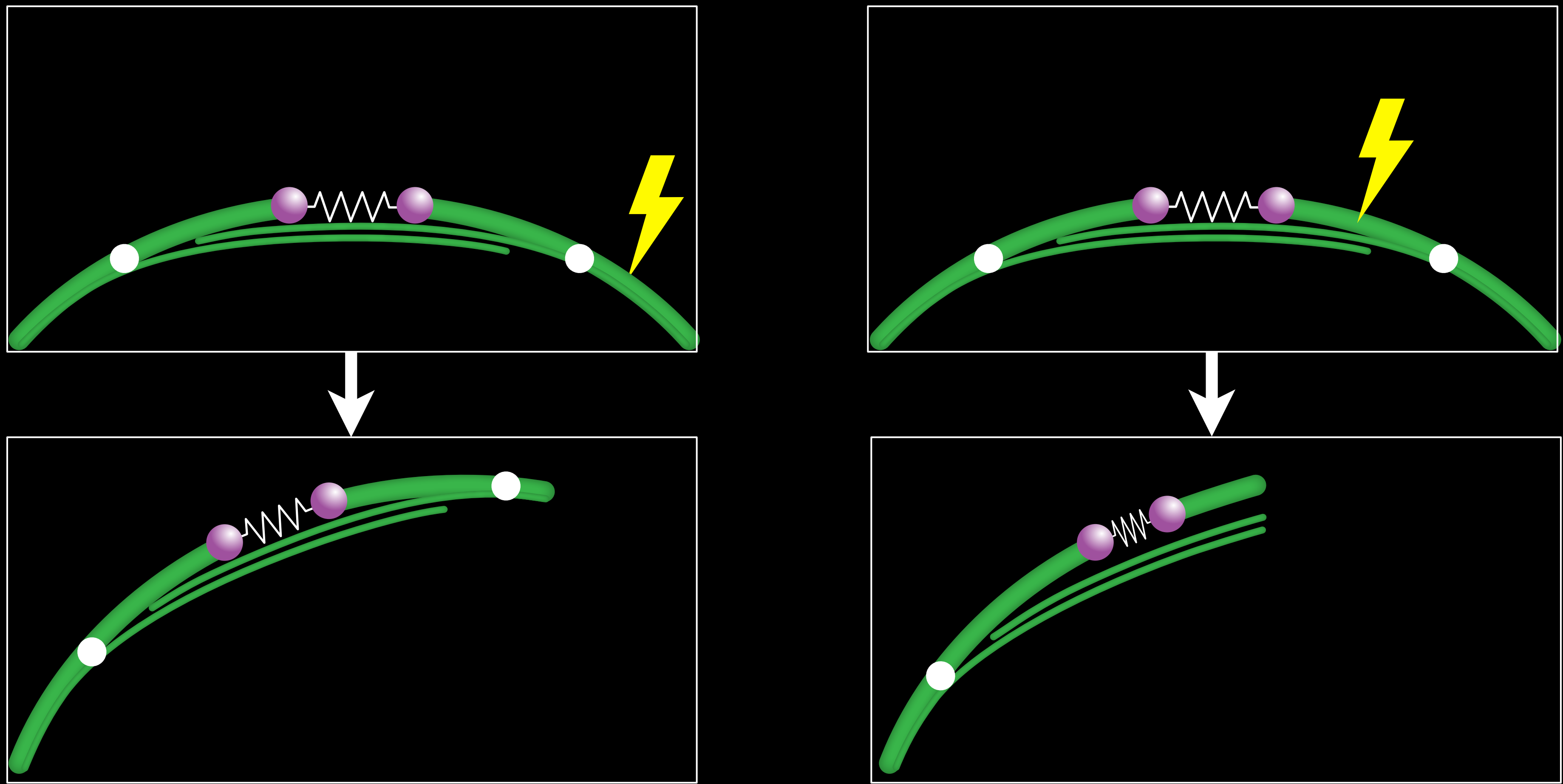


PRC1-GFP
Tubulin-mCherry
mRFP-CENP-B

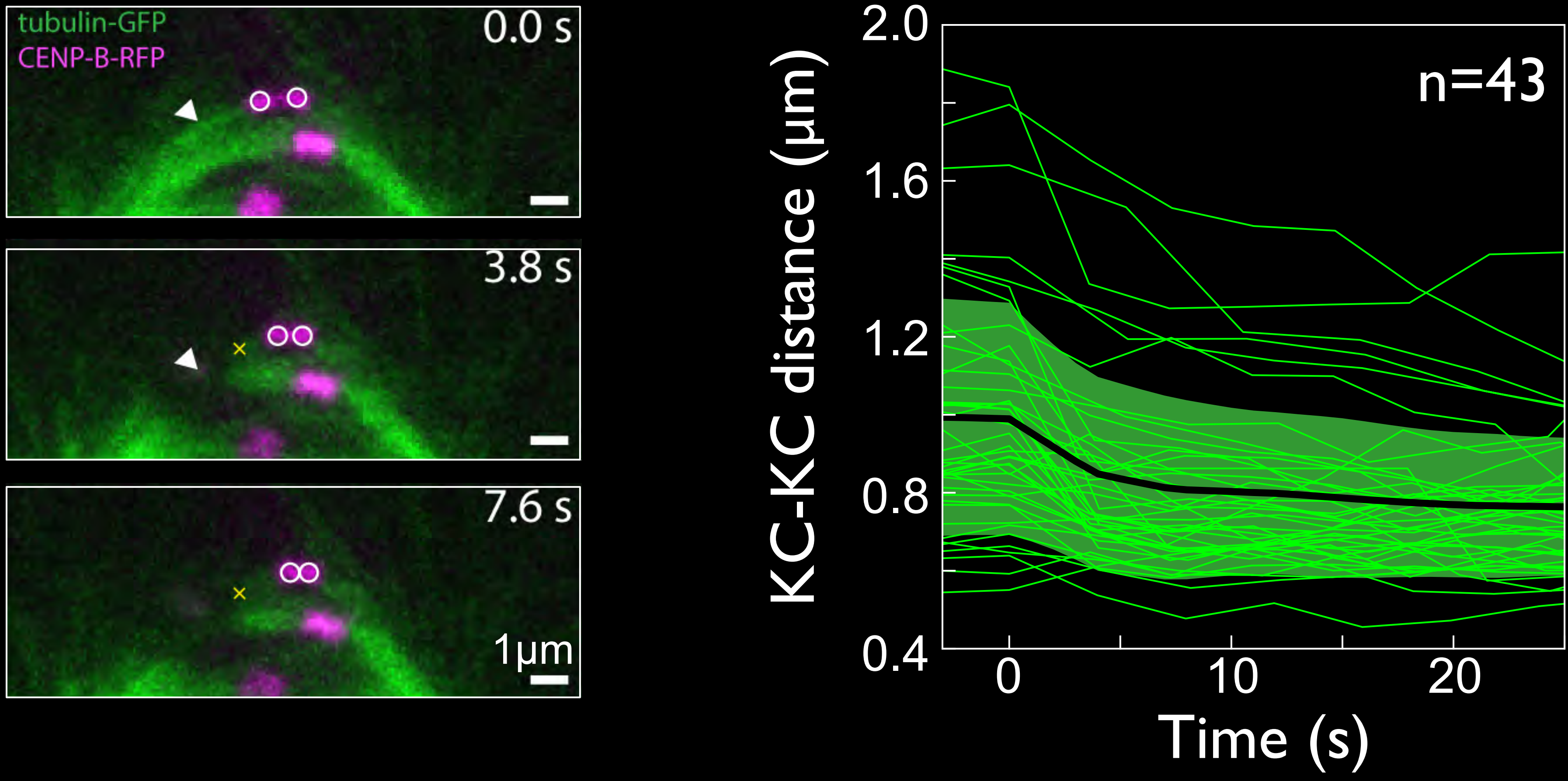
n = 23 ± 5 MTs

What happens with the tension on KCs after cutting?

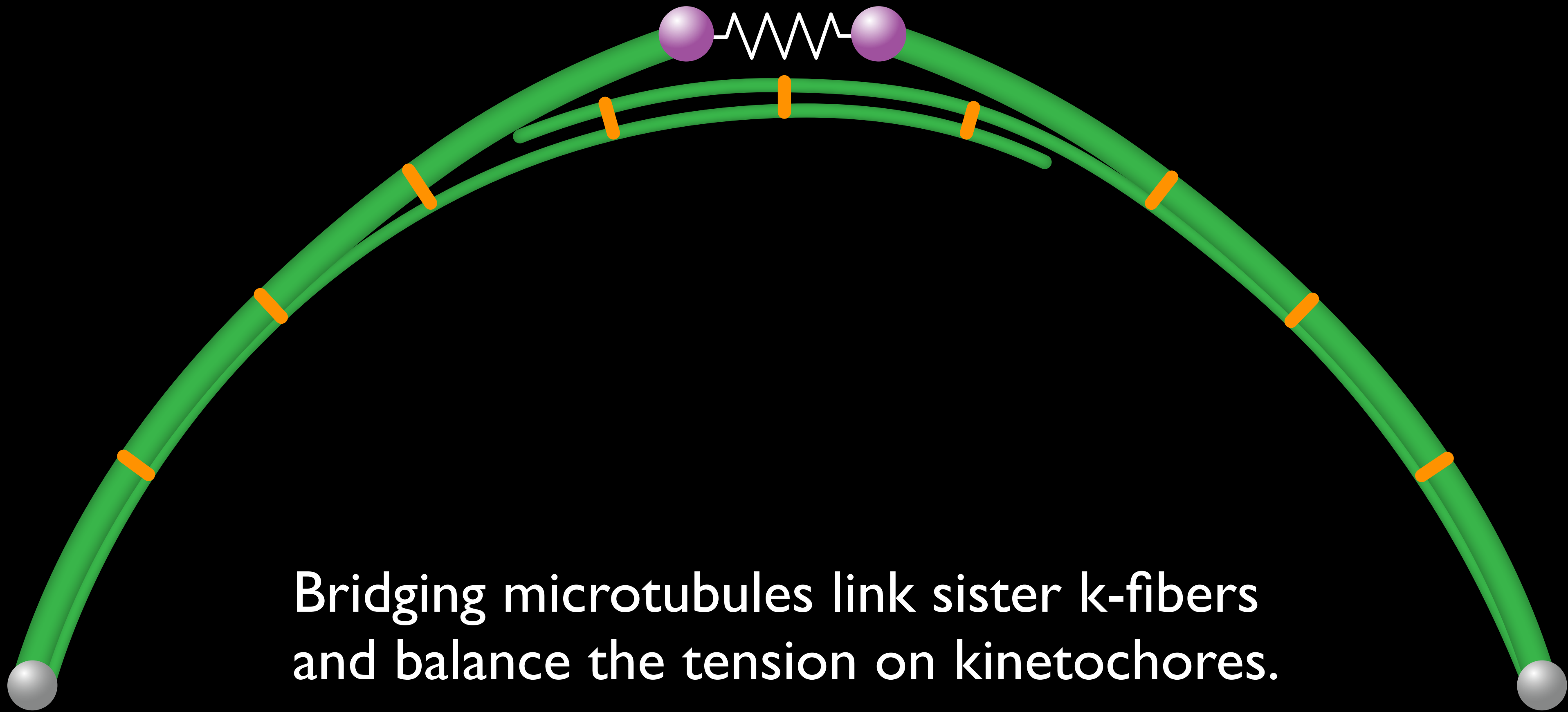
Prediction: Cutting closer to the KC should result in a greater release of tension at KCs.



The distance between KCs decreases after cutting



Conclusion



Bridging microtubules link sister k-fibers and balance the tension on kinetochores.

Acknowledgments

MPI-CBG, Dresden

Iana Kalinina, Lora Winters, Janko Kajtez, Anastasia Solomatina

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Petrina Delivani, Alexander Krull,
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Julie Welburn (Edinburgh)
Emanuele Roscioli, Daniela Cimini (Virginia Tech)
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Linda Wordeman (Washington)
Graphics: Ivana Šarić (Zagreb)

University of Zagreb

Maja Novak

Matko Glunčić

Nenad Pavin