Building a polymer: microtubule dynamics

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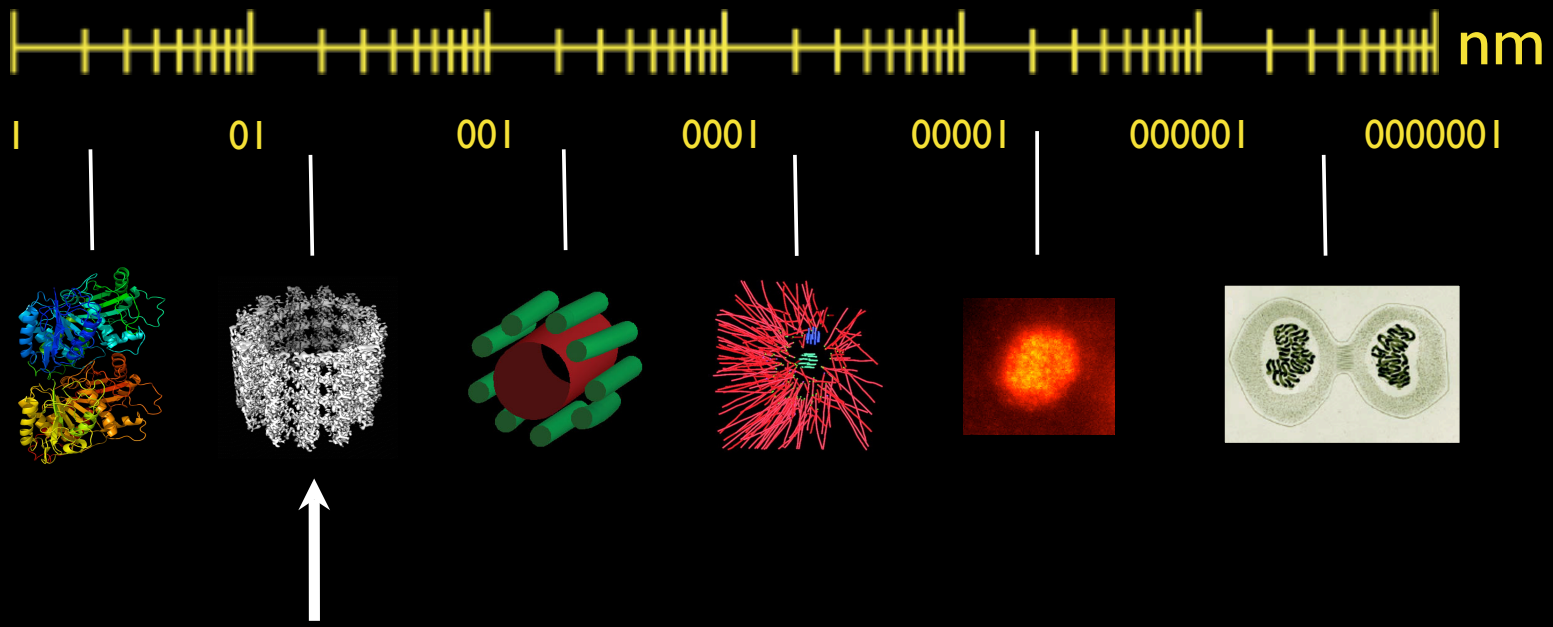
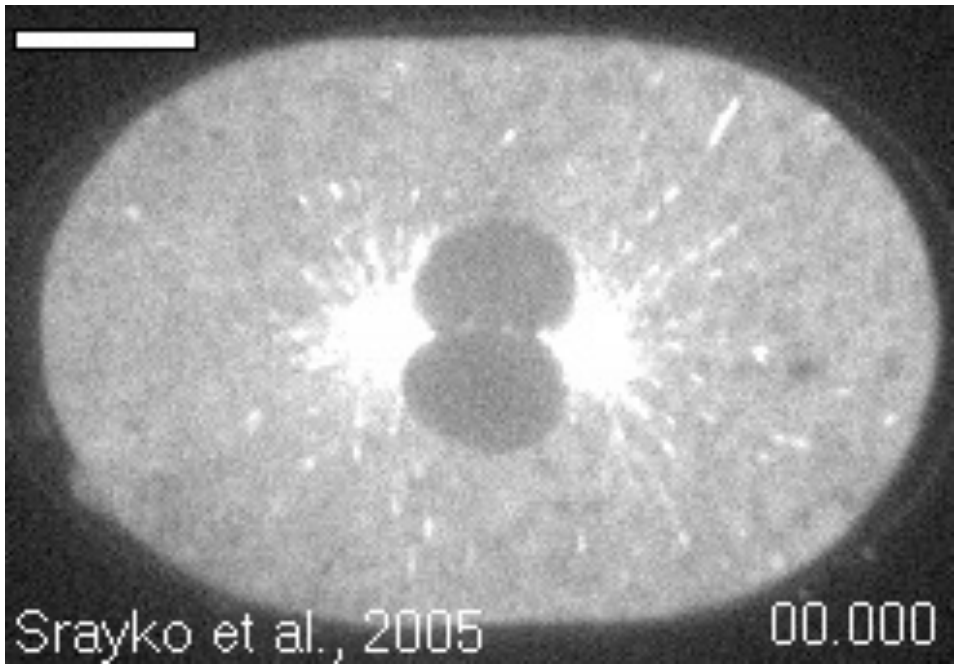
Scale in analysis of biological systems



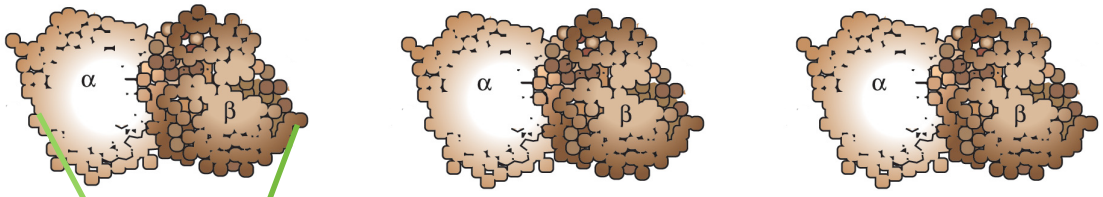
Microtubules growing from centrosomes



EB1-GFP marks the ends of microtubules

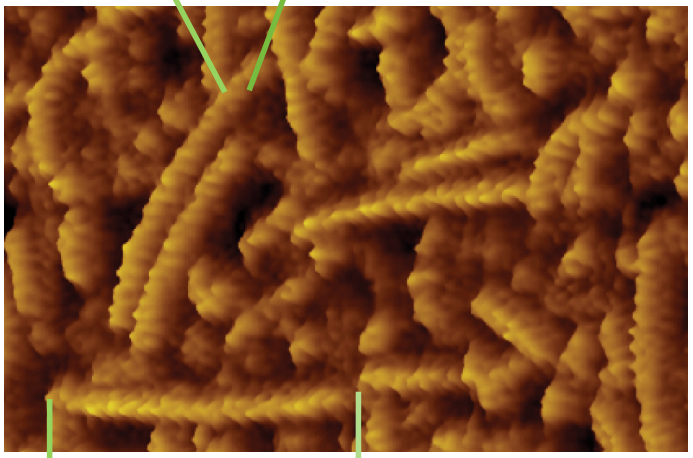
Subunits
(crystallography)

8 nm



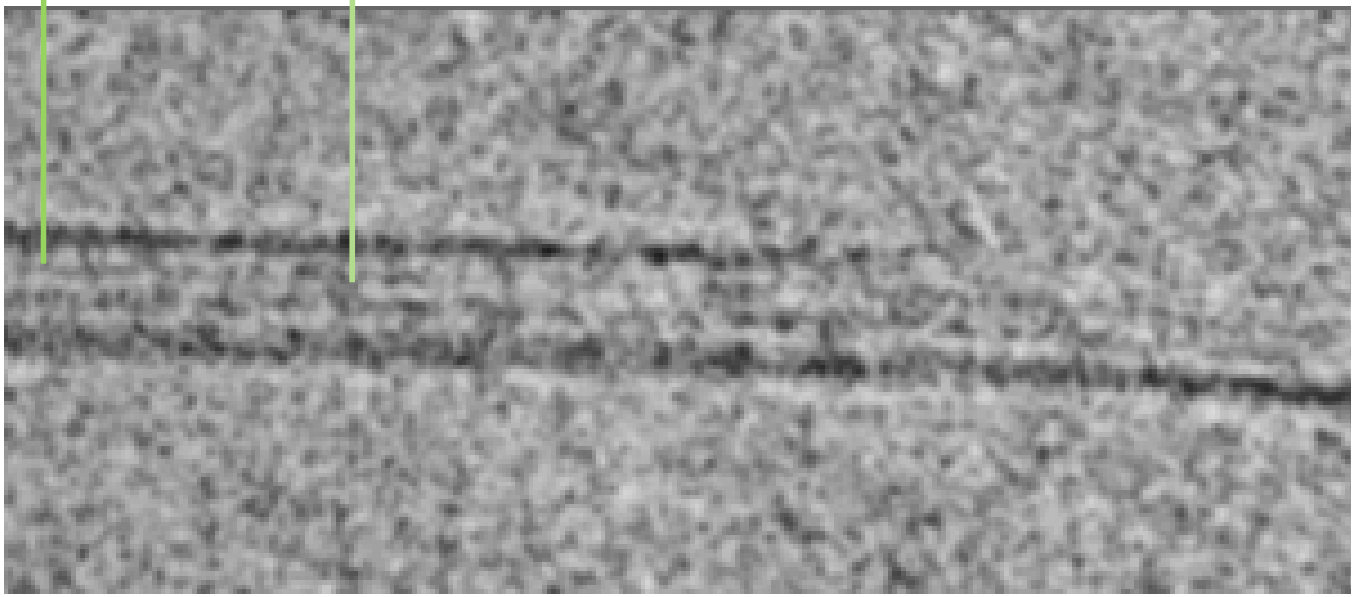
Protofilaments
(atomic Force)

30 nm

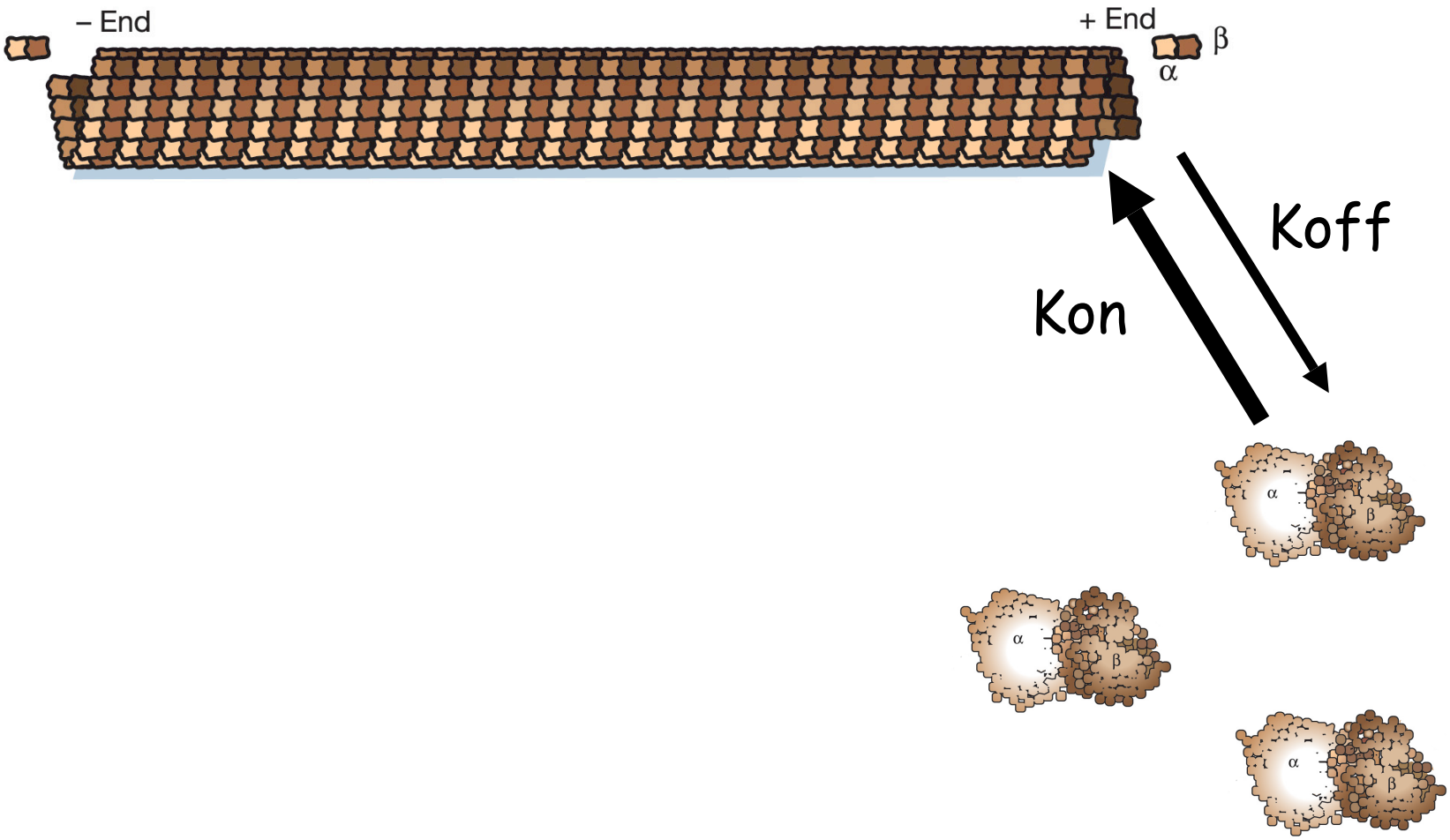


Microtubules
(vitreous ice)

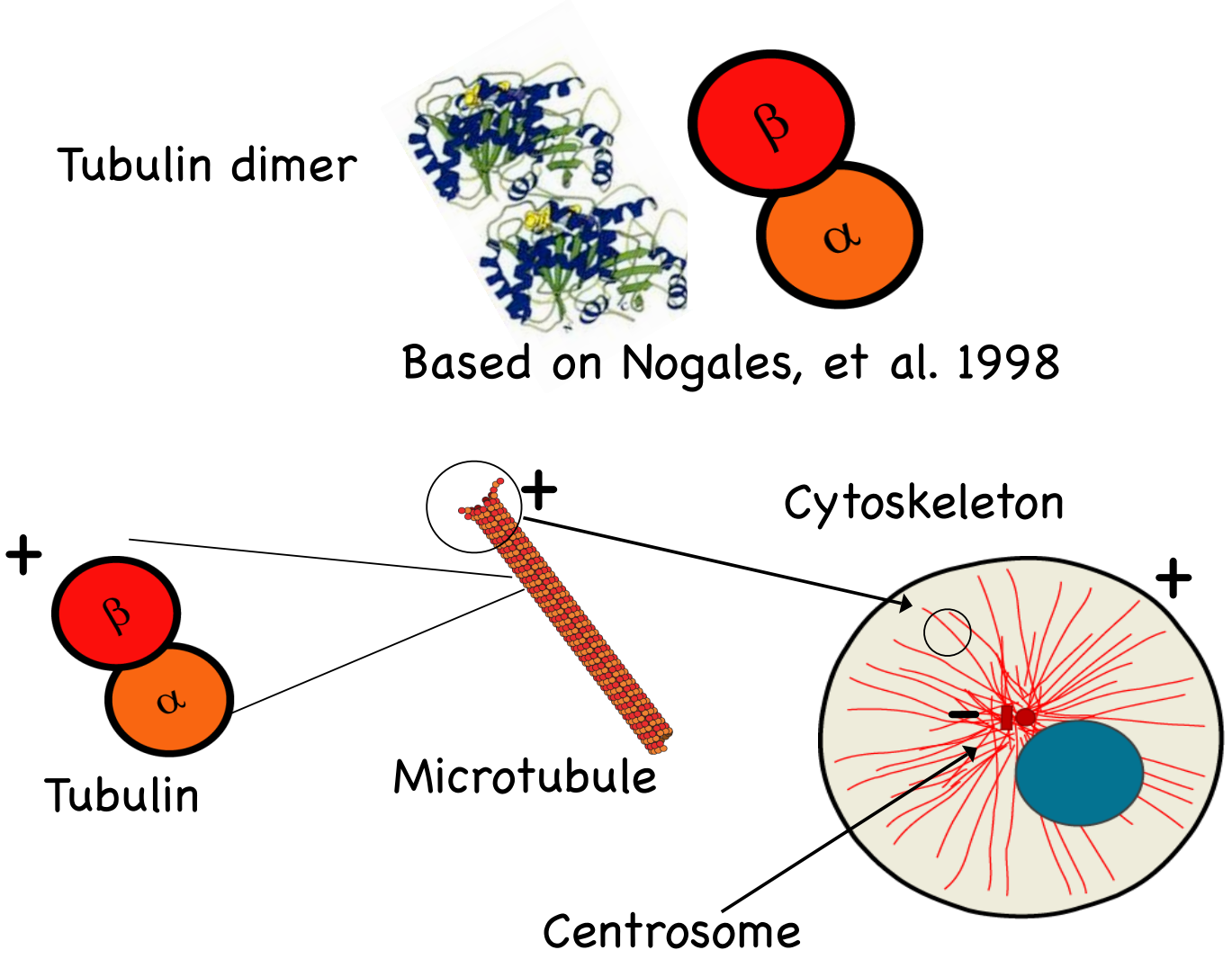
60 nm



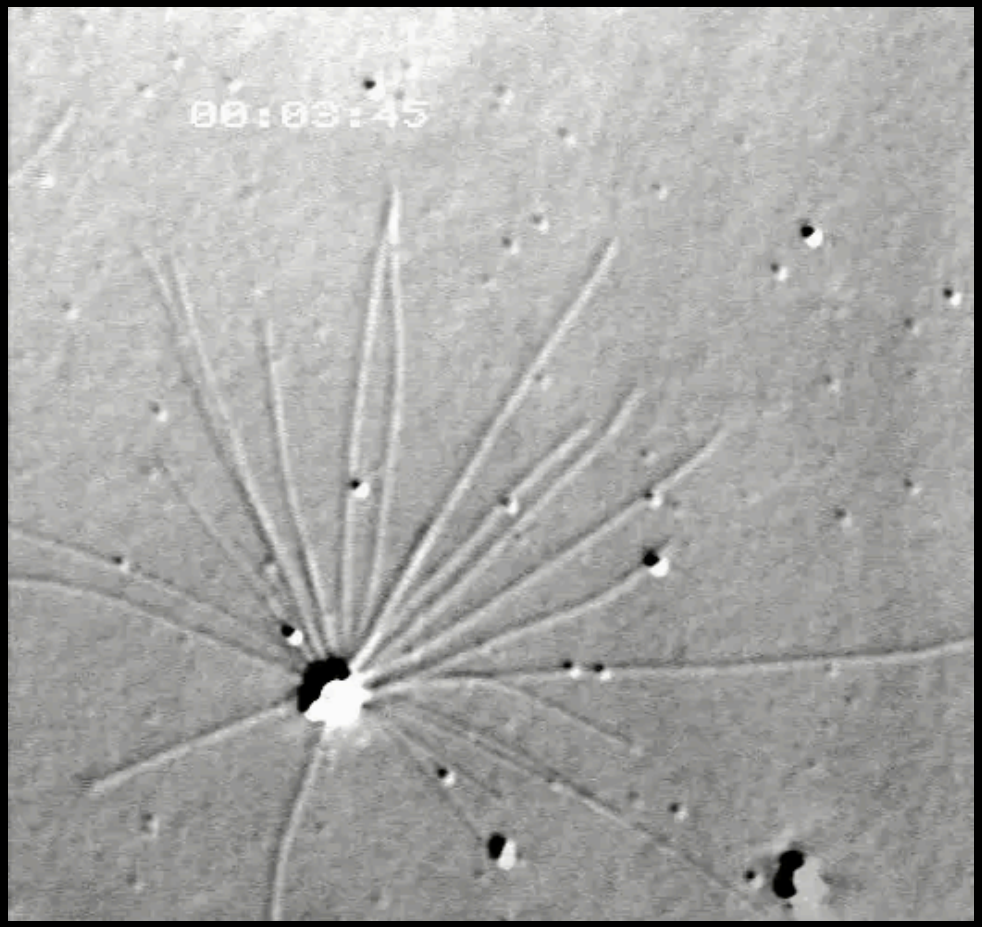
Microtubules grow from their ends.



Microtubules have polarity

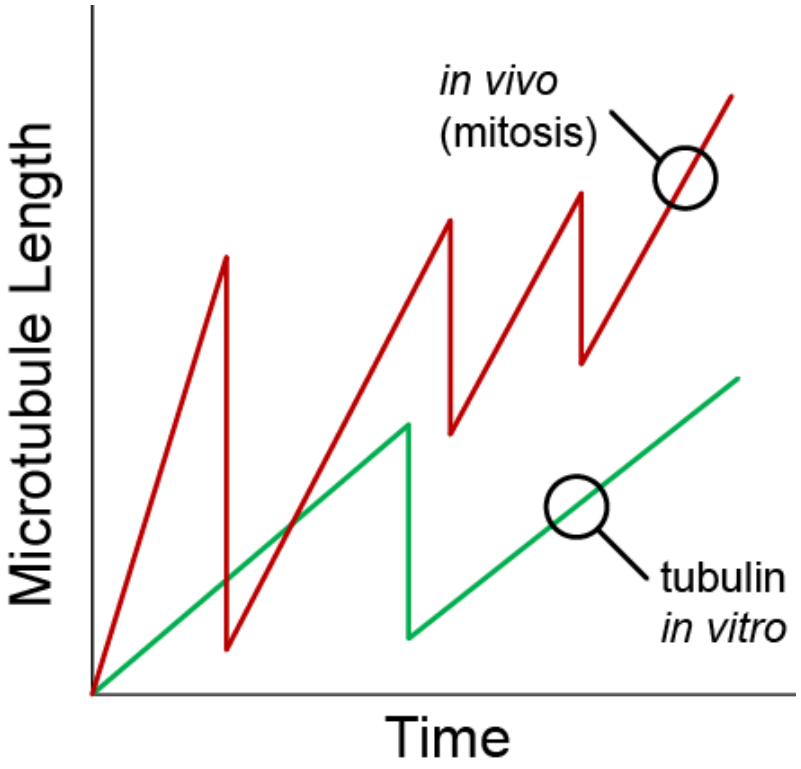


Microtubule growing from isolated tubulin

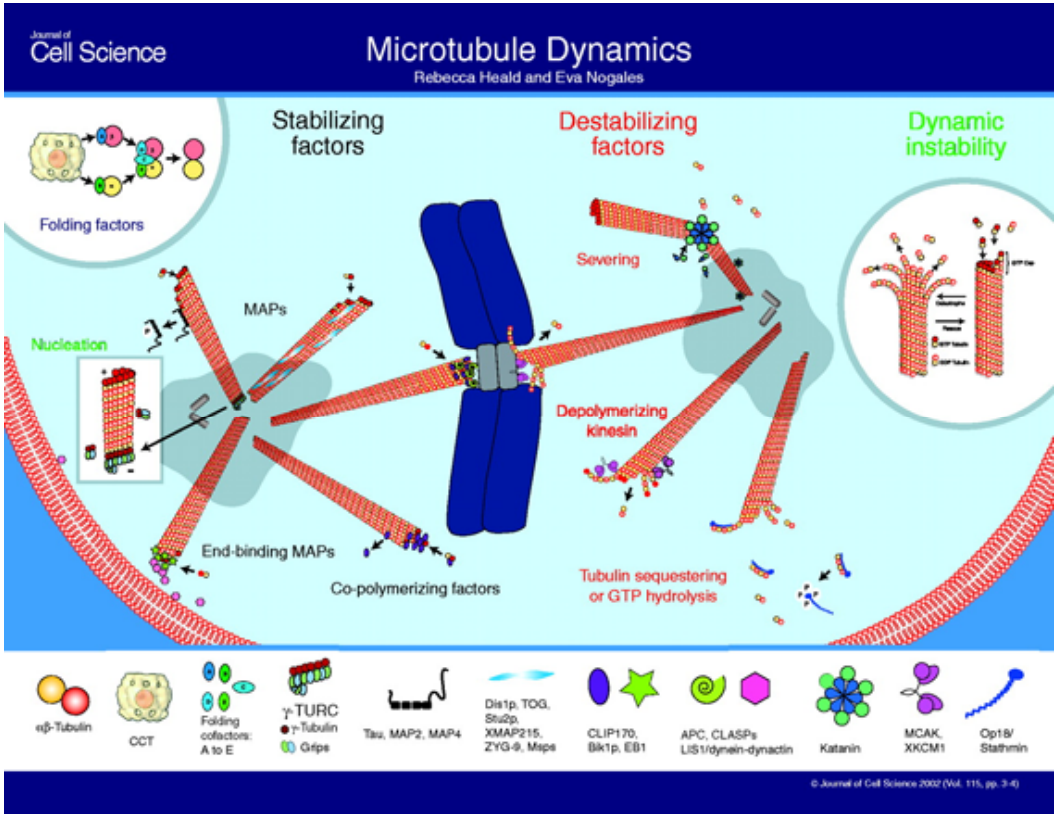


(Nomarski Microscopy)

K.Kinoshita

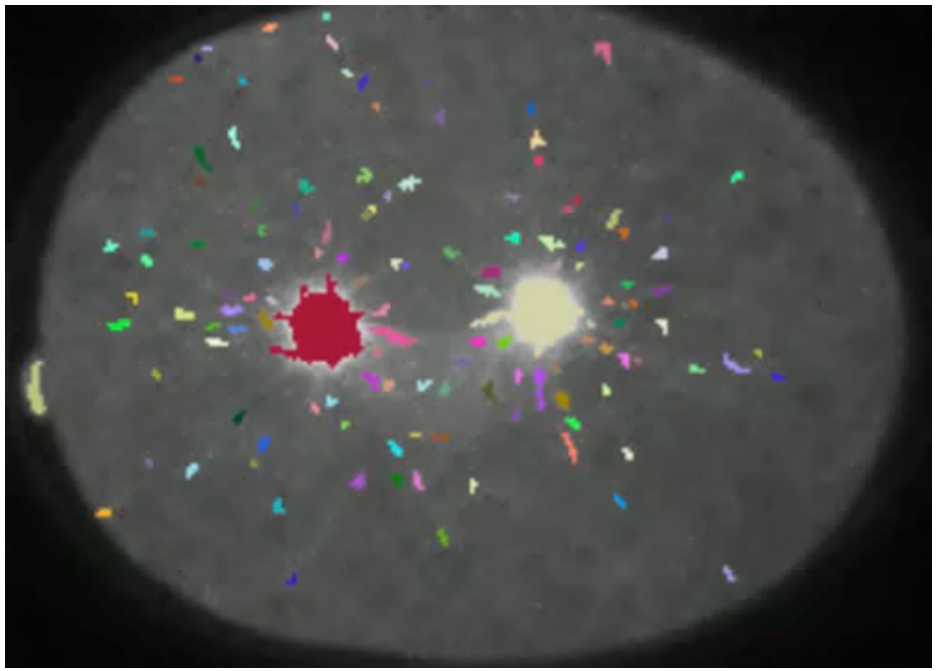

Microtubule Length
in vivo
(mitosis)
tubulin
in vitro
Time

How complicated is the control of microtubules in cells?



Heald, R. et al. J Cell Sci 2002;115:3-4

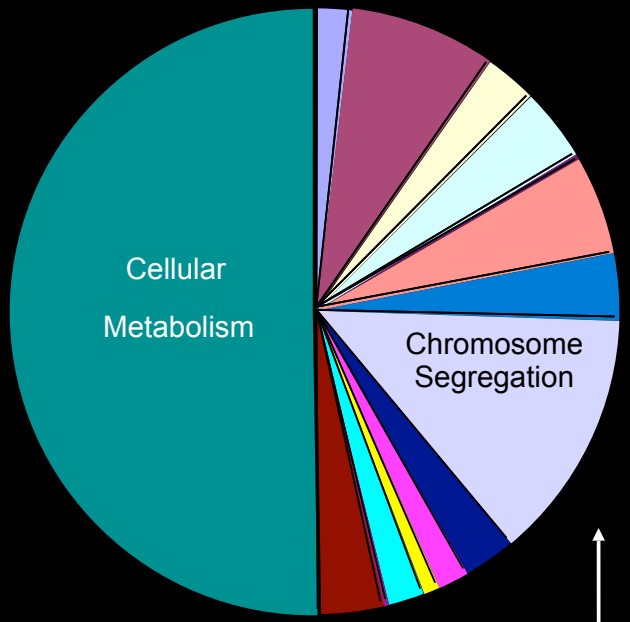
Genome-wide RNAi screen for genes required for microtubule growth



M.Srayko

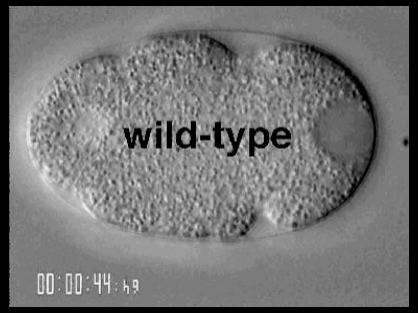
Secondary screens refine phenotype.

800 genes



19,000 genes

DIC screen



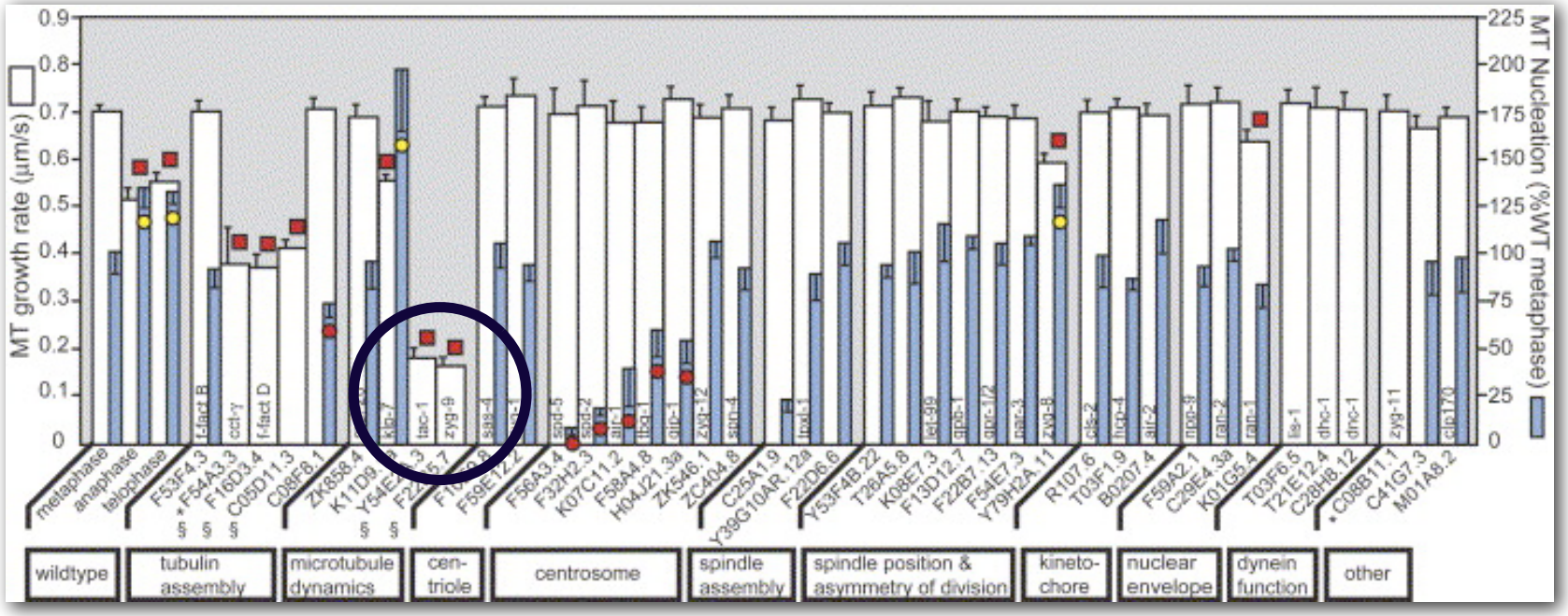
Fluorescent screen

220 genes

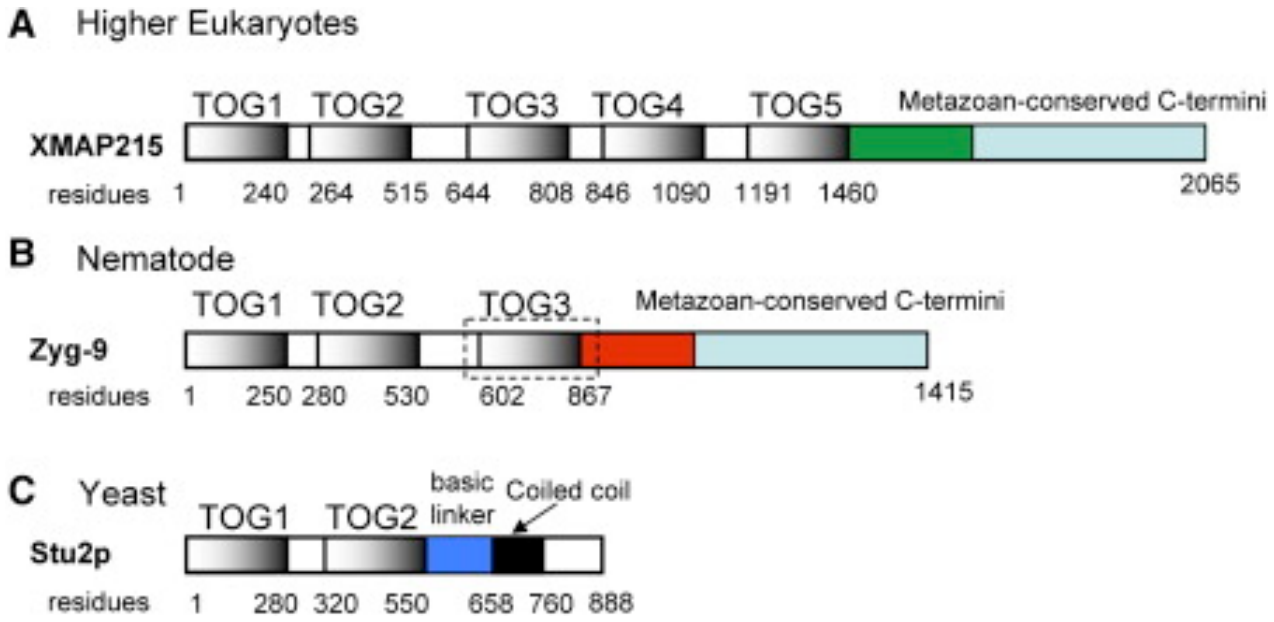
10 genes
required
for microtubule
growth

Bioinformatics

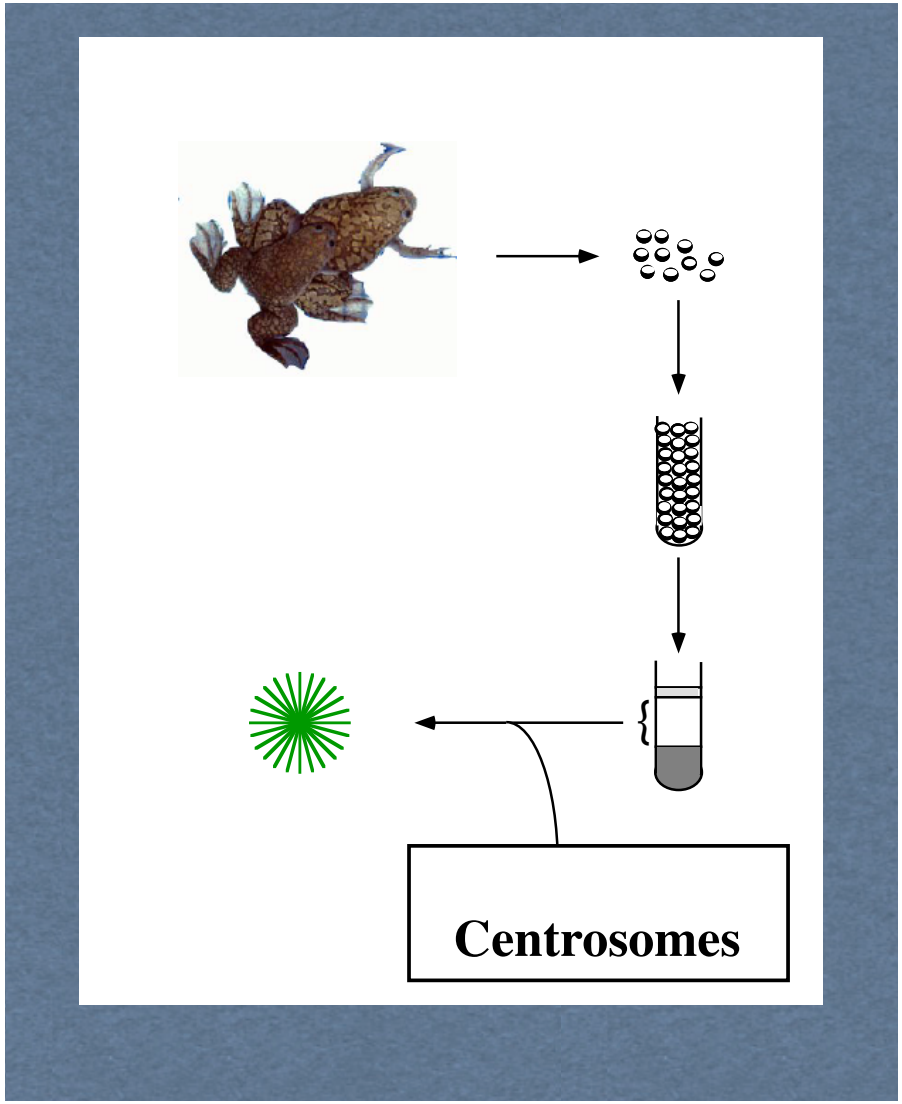
Microtubule growth in C.elegans requires few genes



A family of microtubule binding proteins



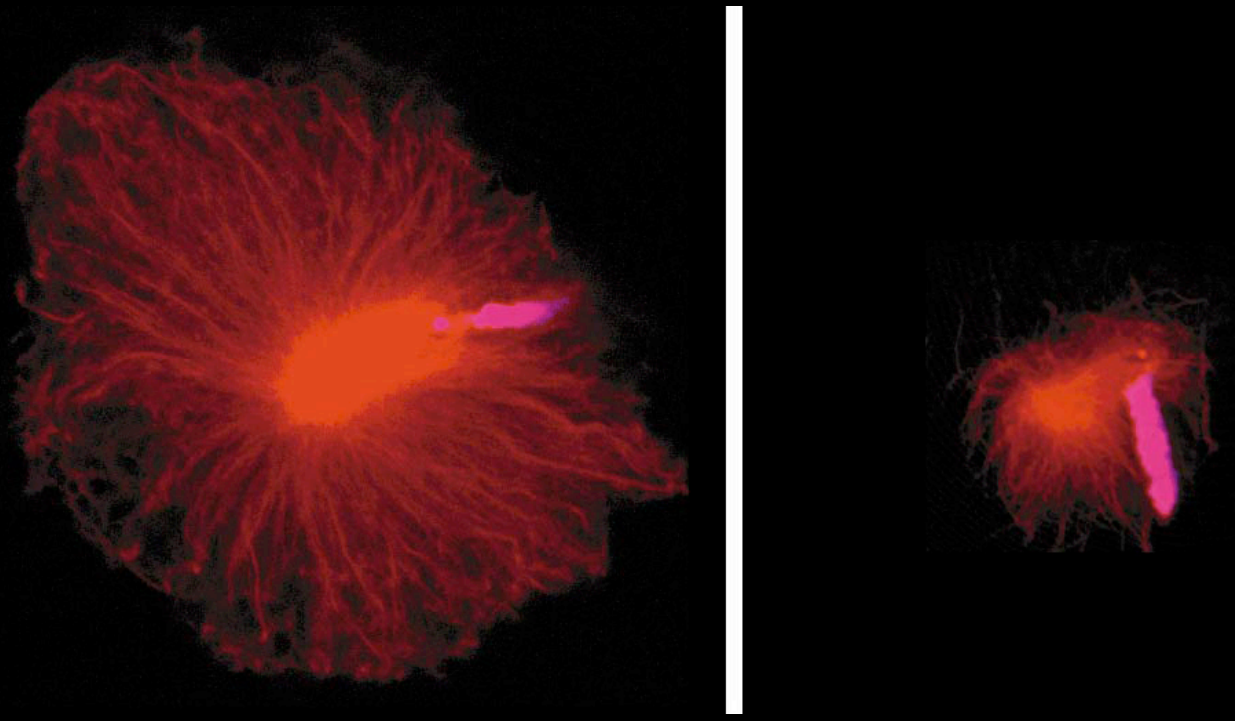
Studying microtubule growth in Xenopus extracts



XMAP is required for microtubule growth in Xenopus embryonic extracts

With XMAP

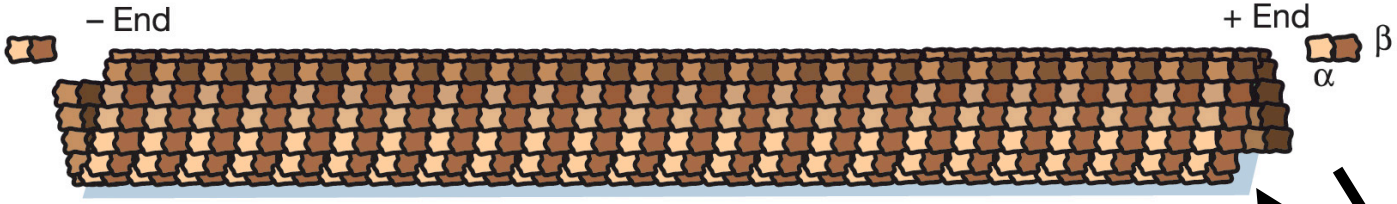
Without XMAP



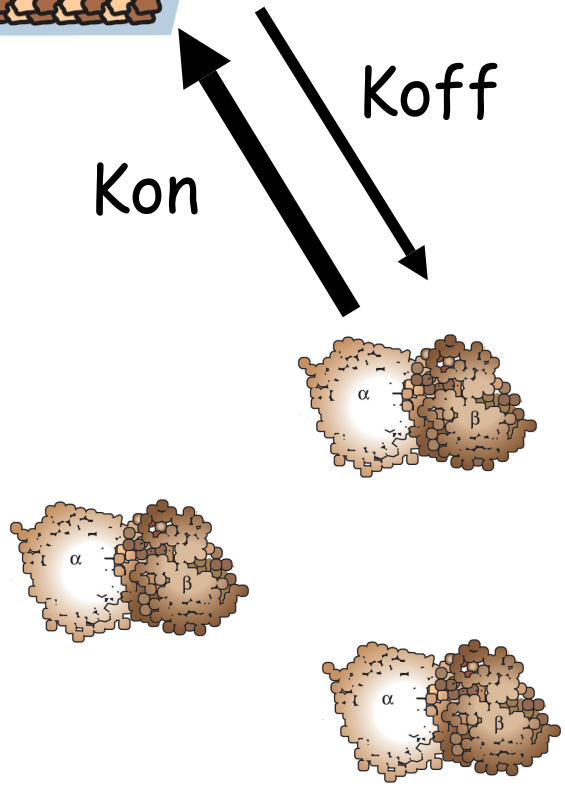
Gard and Kirschner 1987
Tournebize... Hyman, 2000

R. Tournebize

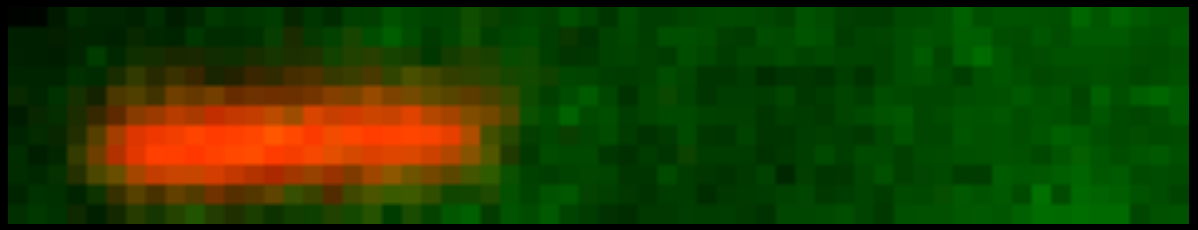
How does XMAP increase the growth rate of a microtubule plus end?



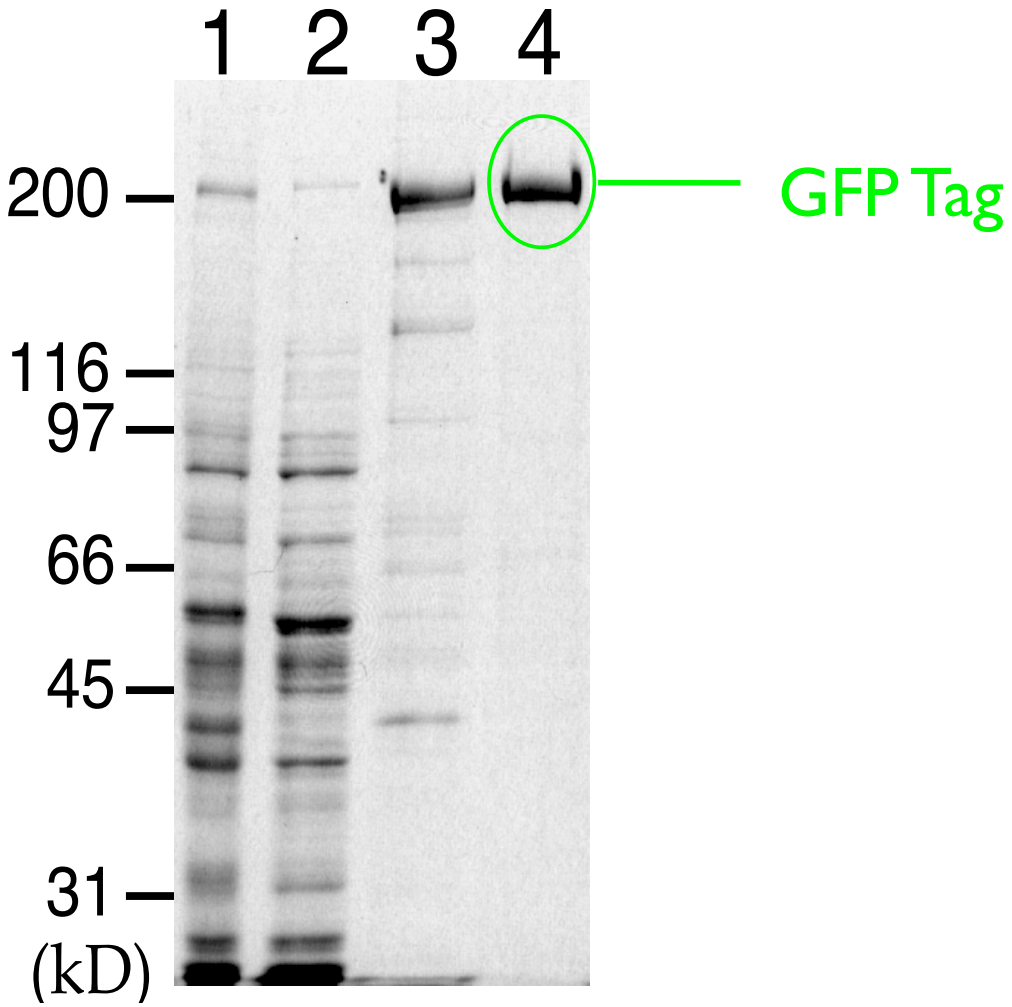
Joe Howard



Monitoring growth of microtubule plus ends

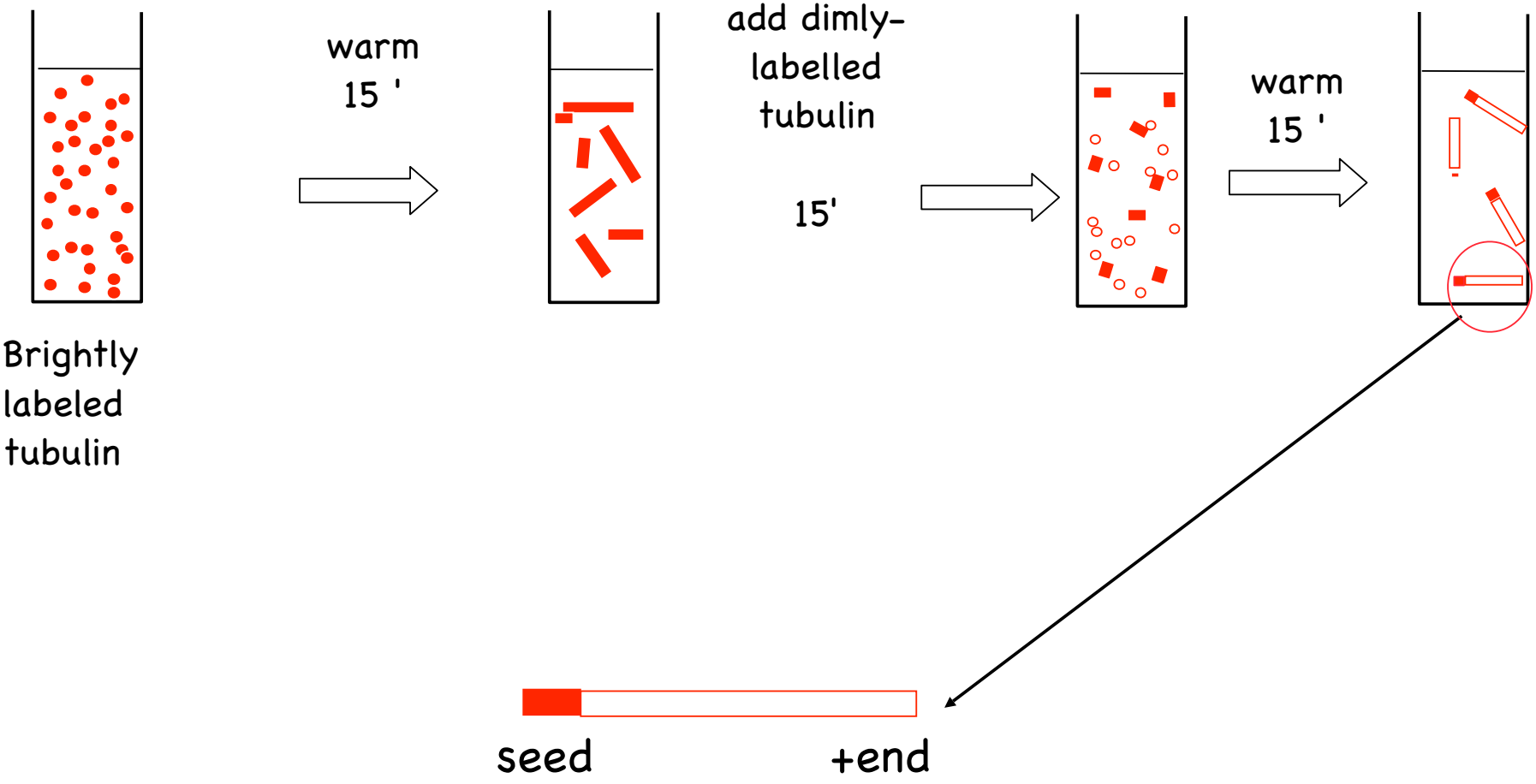


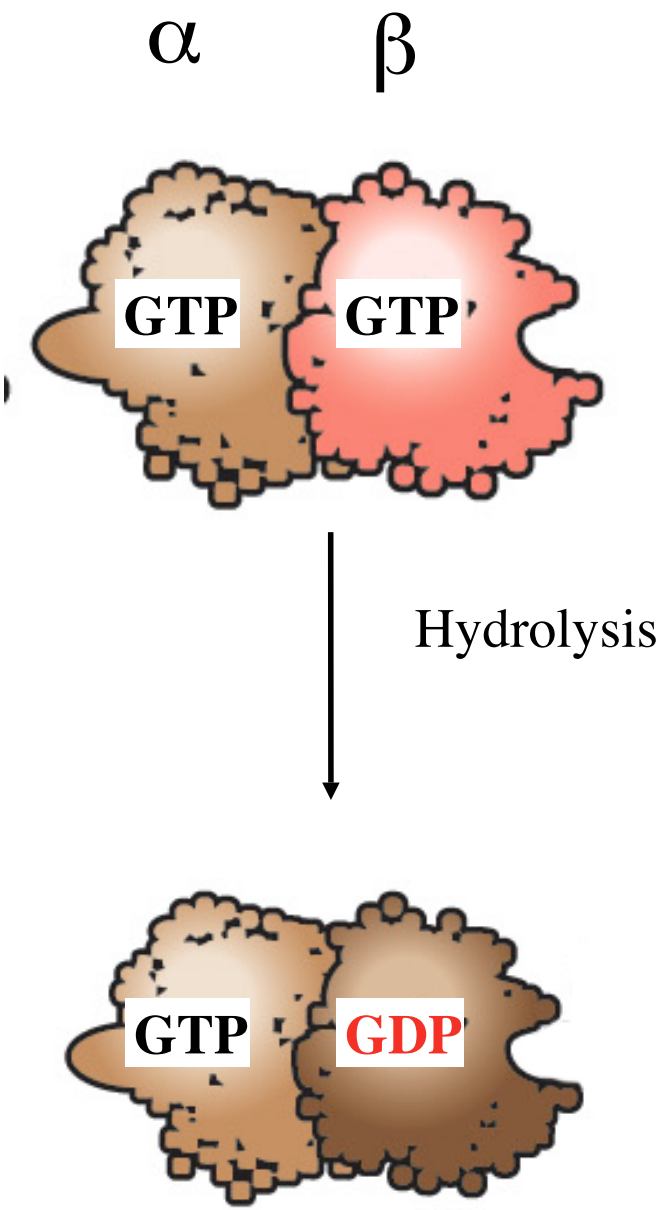
Production of XMAP215 in Baculovirus

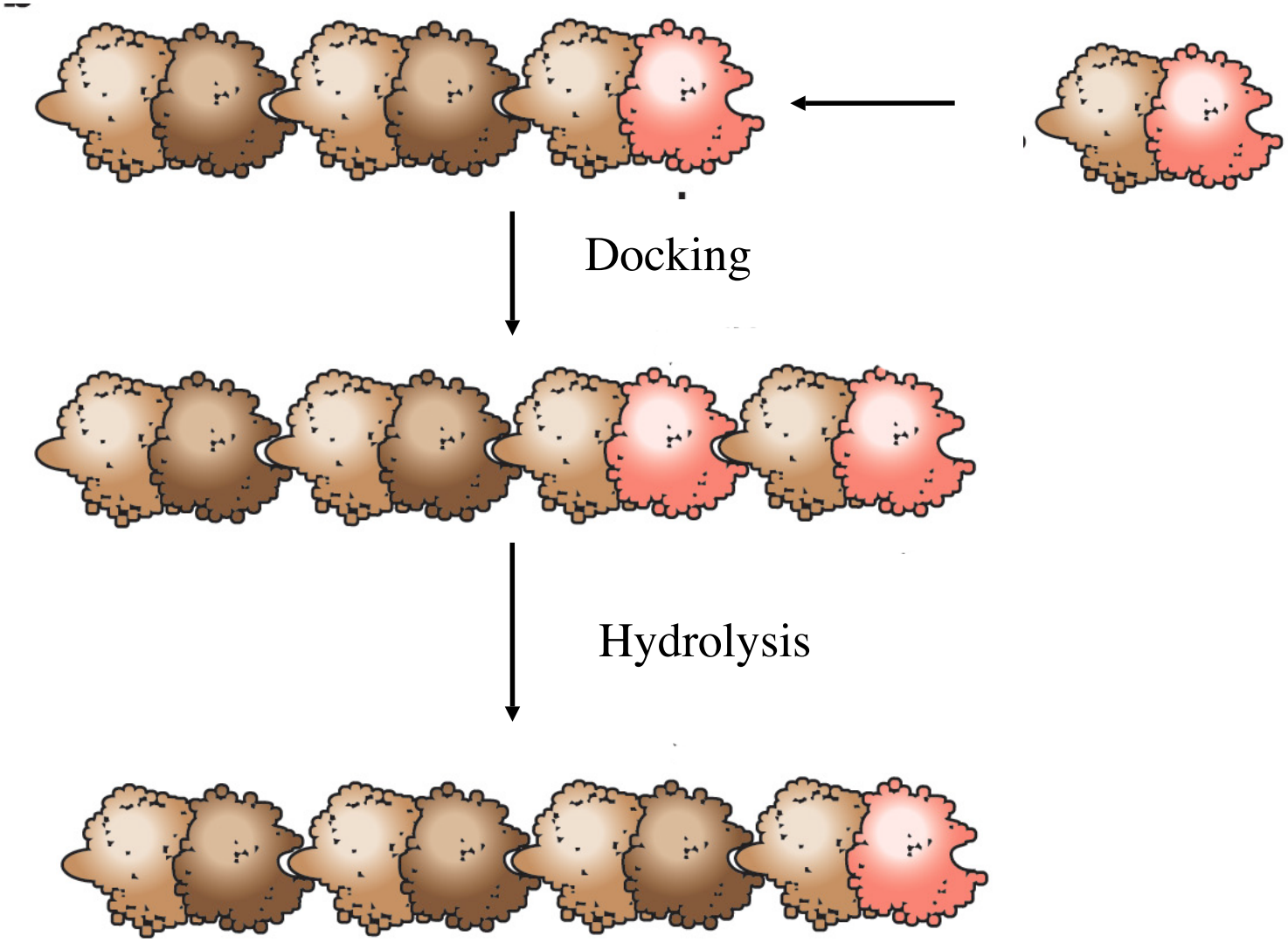


D.Drechsel

Polarity-marked microtubules (in vitro)

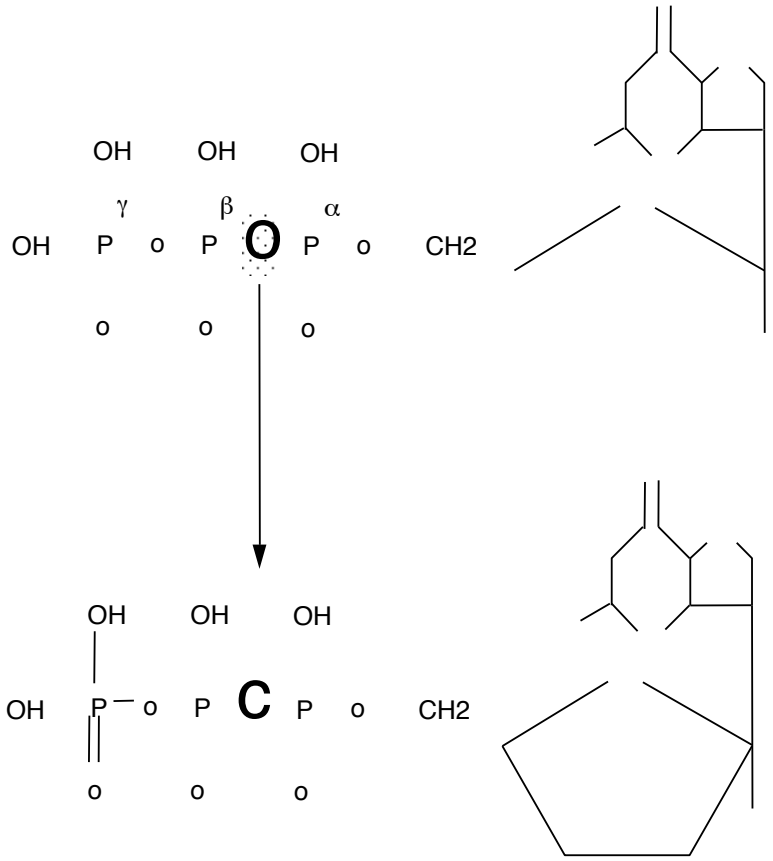



α
β
GTP
GTP
Hydrolysis
GTP
GDP

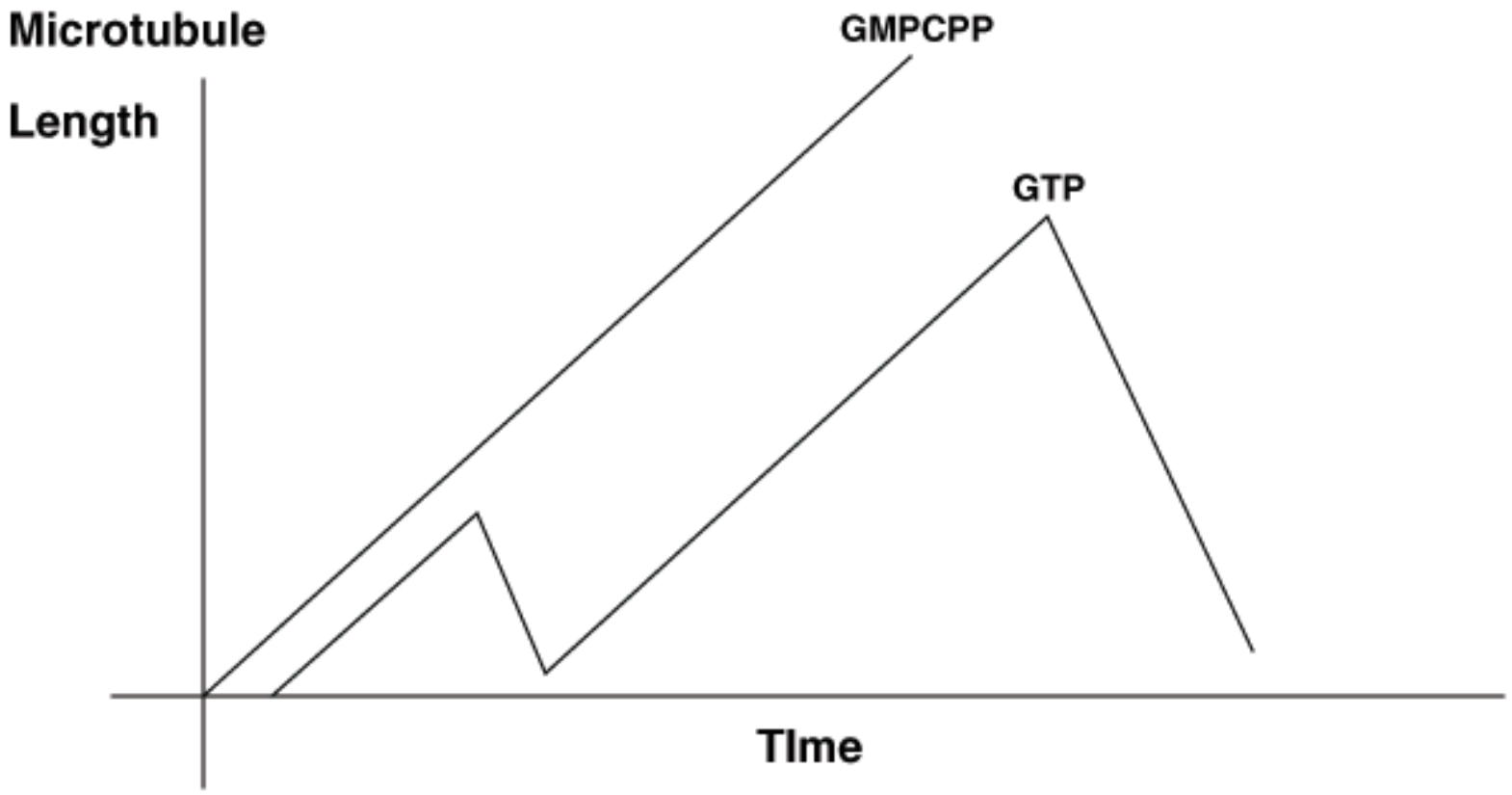

Docking
Hydrolysis

Guanalyl $\alpha-\beta$ -methylene diphoshphonate :non-hydrolysable analogue of GTP

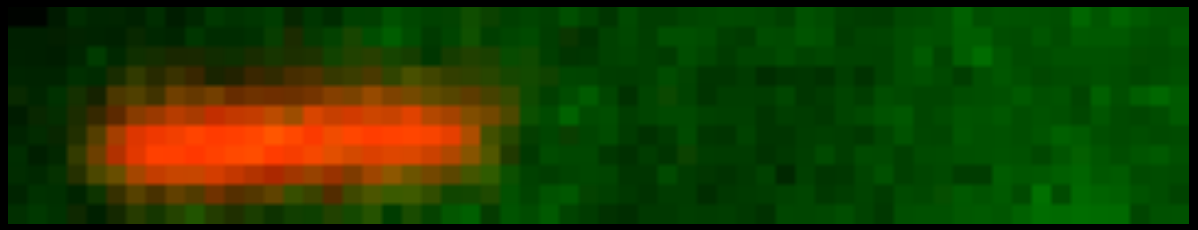
(GMPCPP)



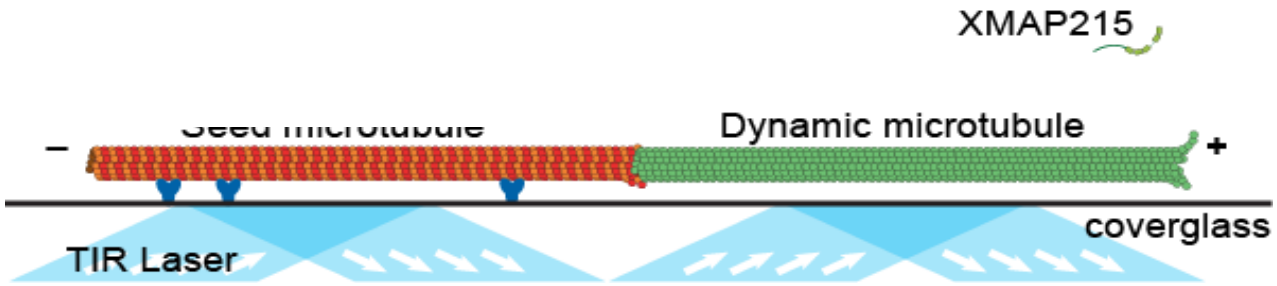
GMPCPP Microtubules do not exhibit dynamic instability

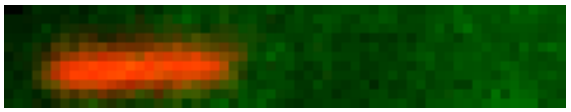


Monitoring growth of microtubule plus ends

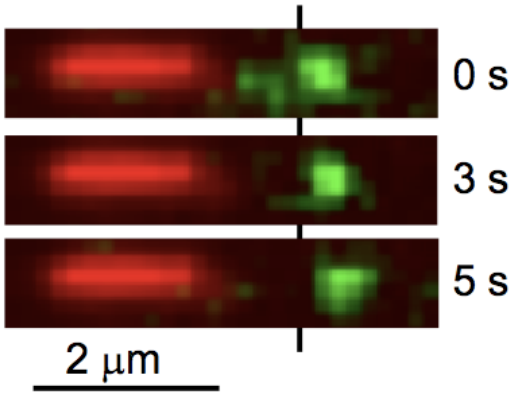


Microtubule growth in vitro can be visualized by Total Internal Reflection Microscopy



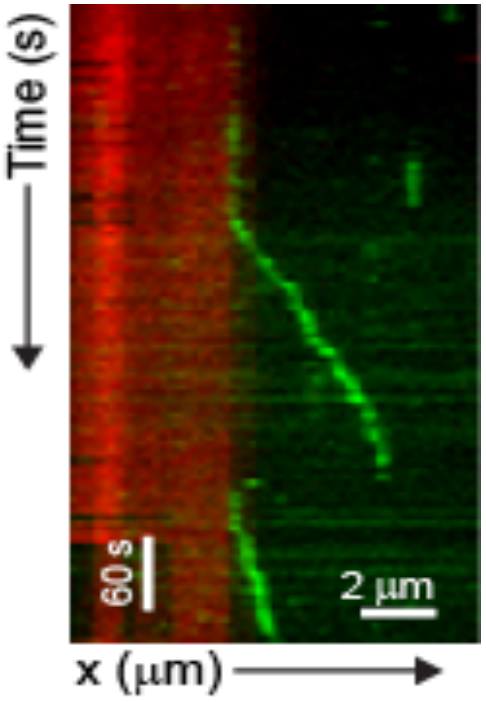


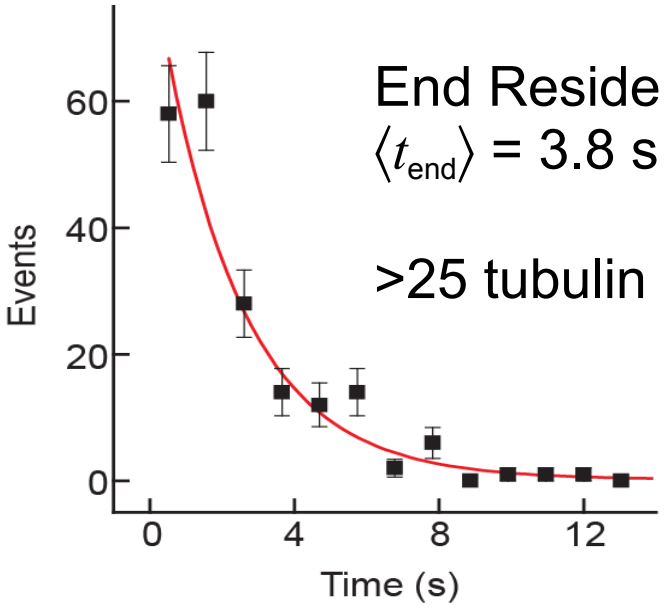
XMAP215 is processive (it surfs at the ends)



Residency time of XMAP215 at microtubule ends

(by looking at single molecules of GFP-XMAP).





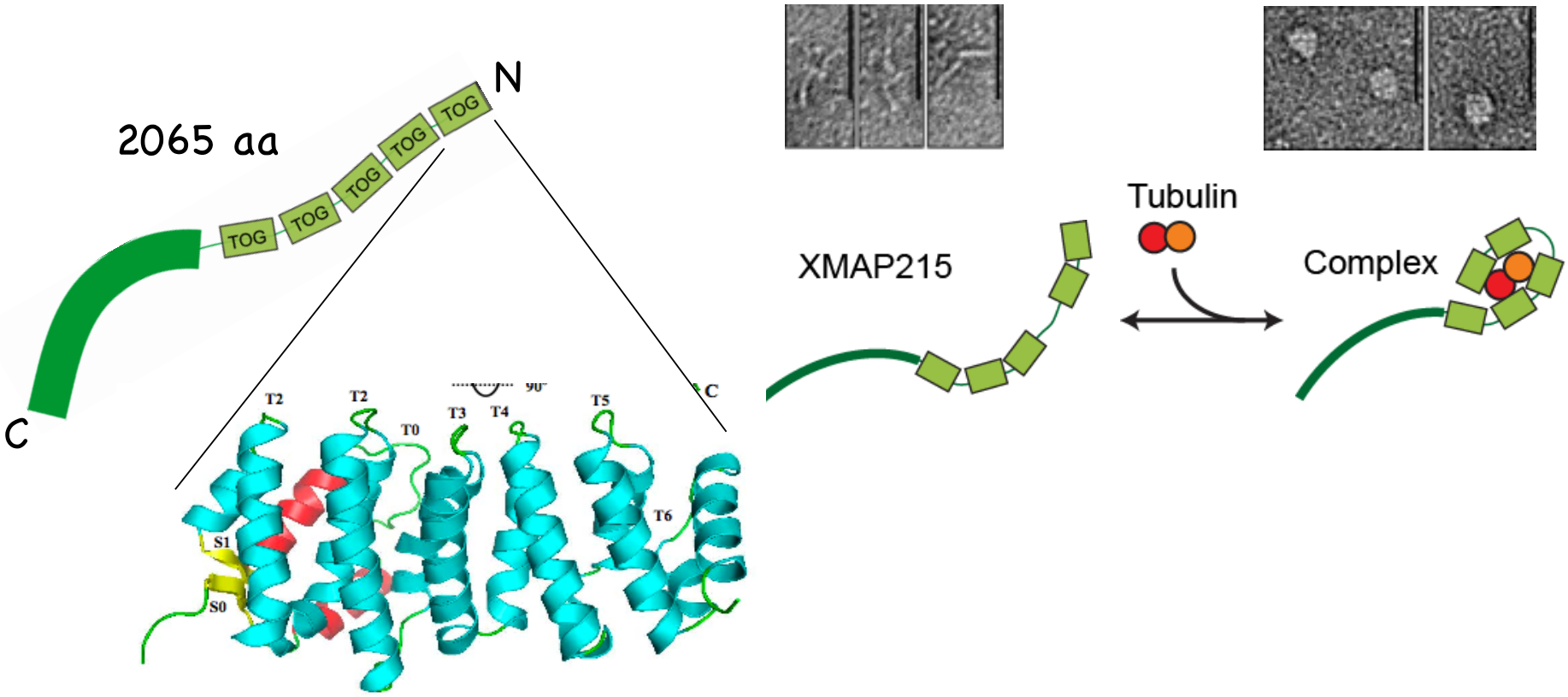
End Residence

$\langle t_{end} \rangle$ = 3.8 s

>25 tubulin dimers

- From the 4 s residency time, we can calculate that XMAP arrives at 8 molecules per second.
- Microtubules grow at 80 dimers per second
- During the 4 s residency time of XMAP, about 350 tubulin dimers arrive at the end.

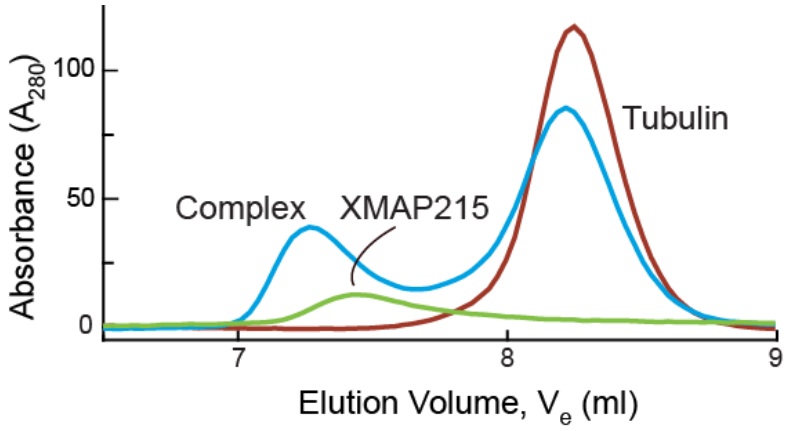
TOG domains bind tubulin



Al Bassam, Hyman, Harrison 2007

XMAP215 binds one tubulin dimer

Size exclusion chromatography



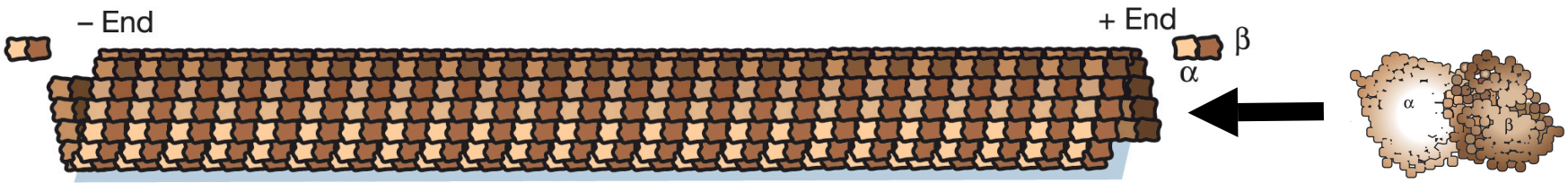
Analytic Ultracentrifugation:

XMAP215 = 221 ± 10 kDa

Complex = 320 ± 30 kDa

Jawdat Al-Bassam, Tim Noetzel; Steve Harrison

How could XMAP215 enhance plus-end growth rate?



The association constant we measured is:

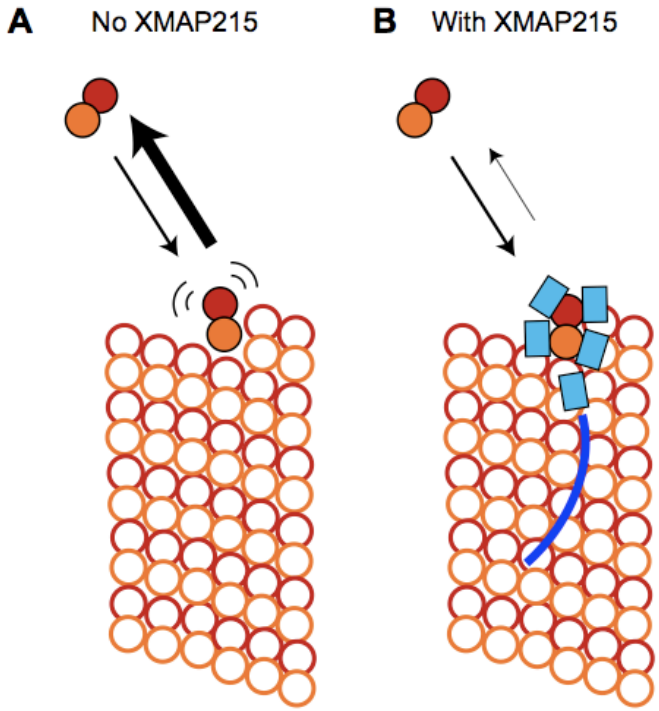
0.3 per protofilament

The association constant we measured is:

1.5 per protofilament

Association constant: Theoretical limit 5 ,

XMAP215 could act as a classic catalyst

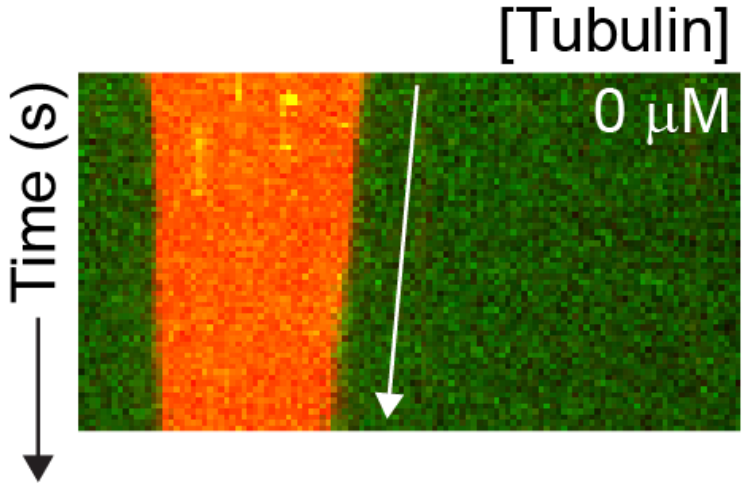


If XMAP215 stabilizes an intermediate

Then,

-
-

XMAP215 depolymerises microtubules, in the absence of tubulin

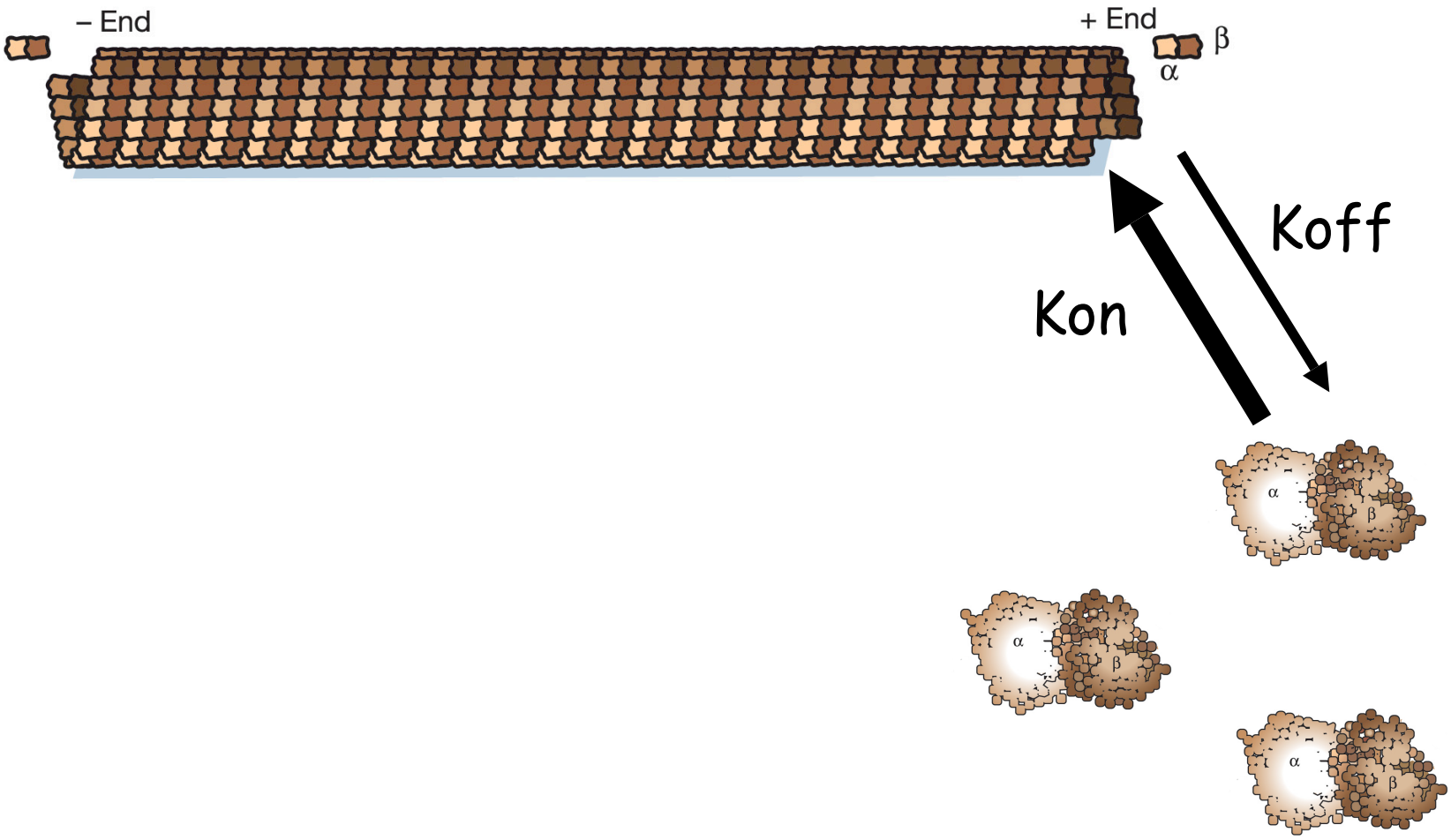


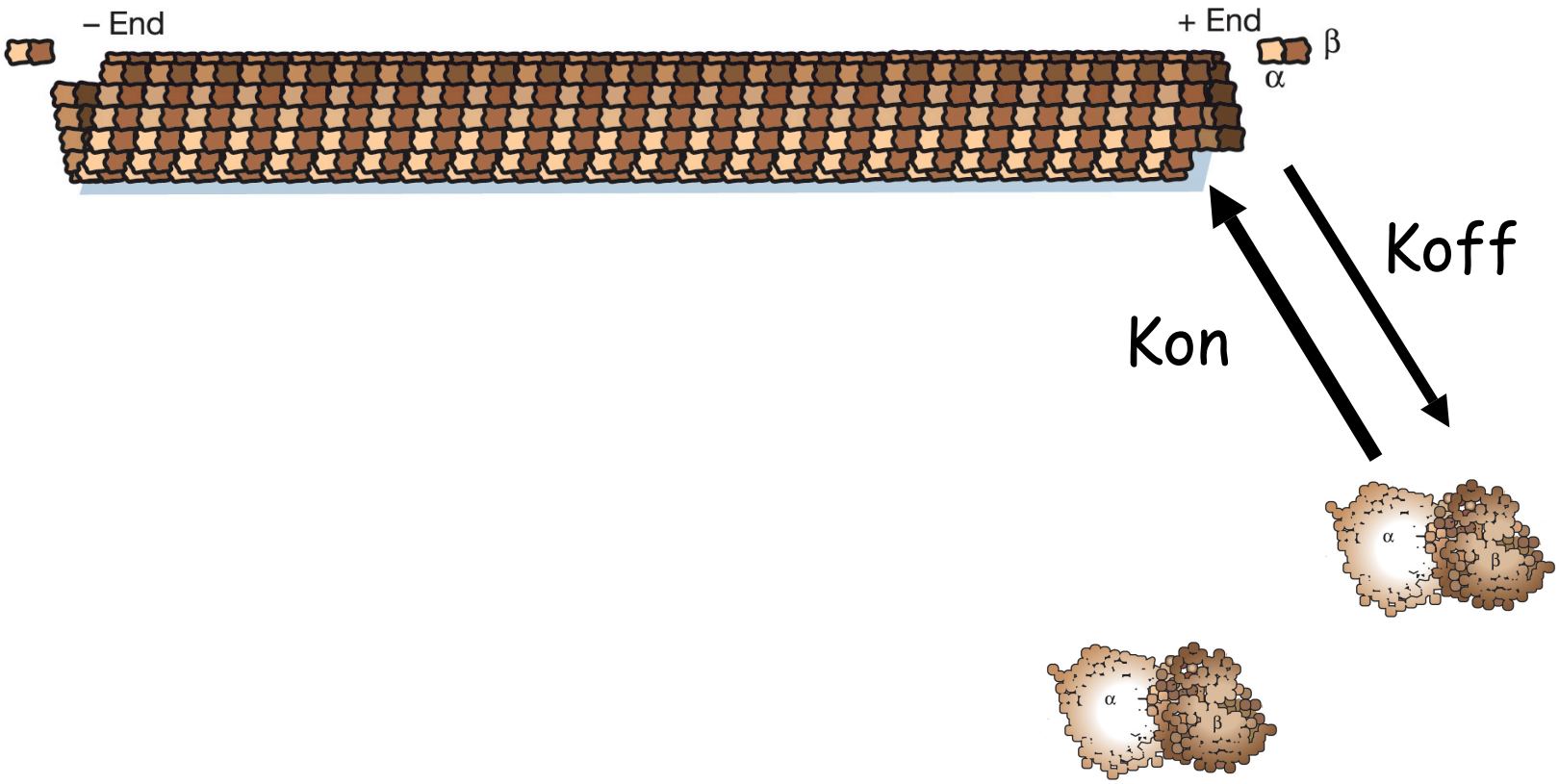
If XMAP215 stabilizes an intermediate

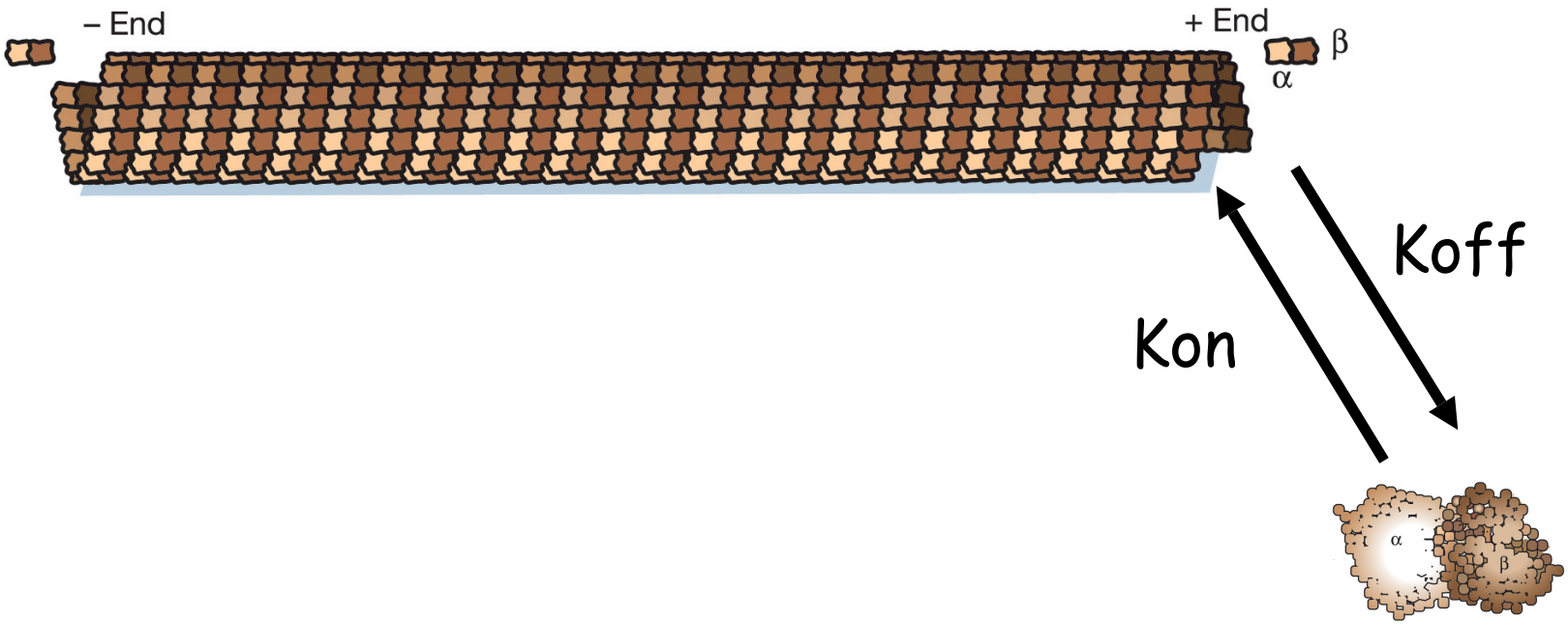
Then,

-
-

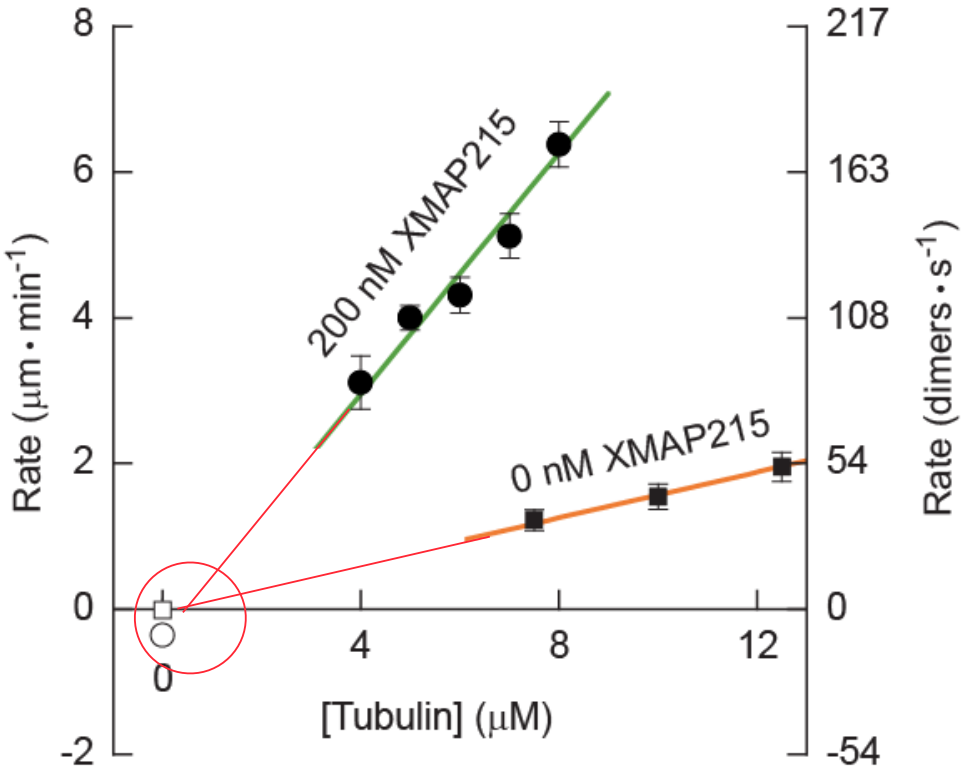
The critical concentration for microtubule growth



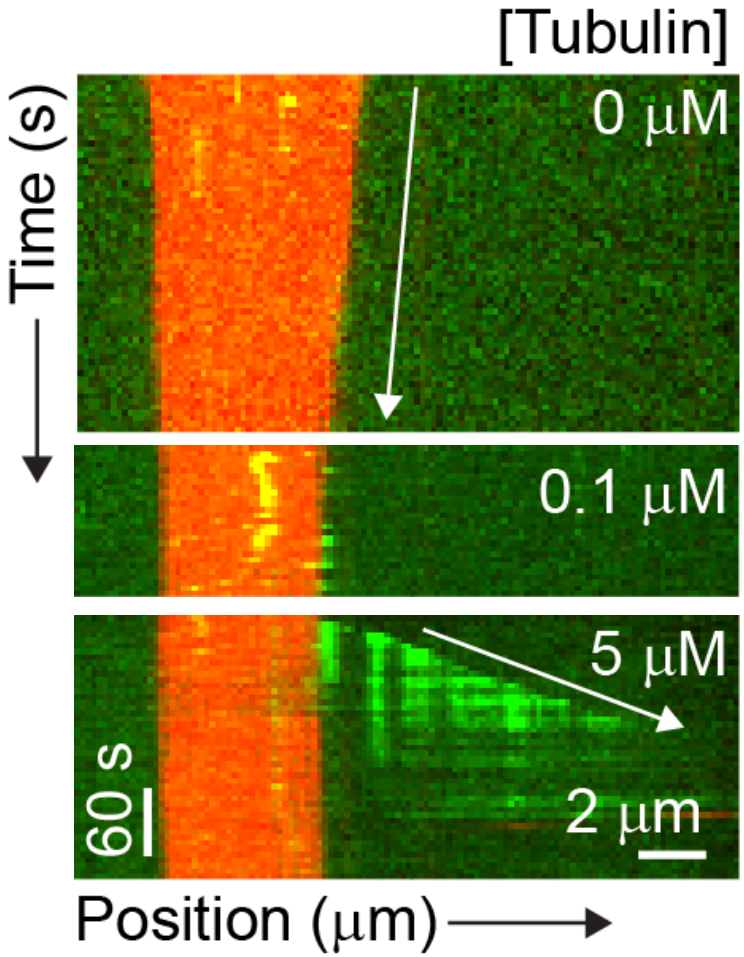

– End
+ End
β
α
Koff
Kon
α
β
α
β


– End
+ End
β
α
Koff
Kon
α
β

Critical concentration for growth does not change



XMAP215 acts as a polymerase



XMAP215 acts as a polymerase