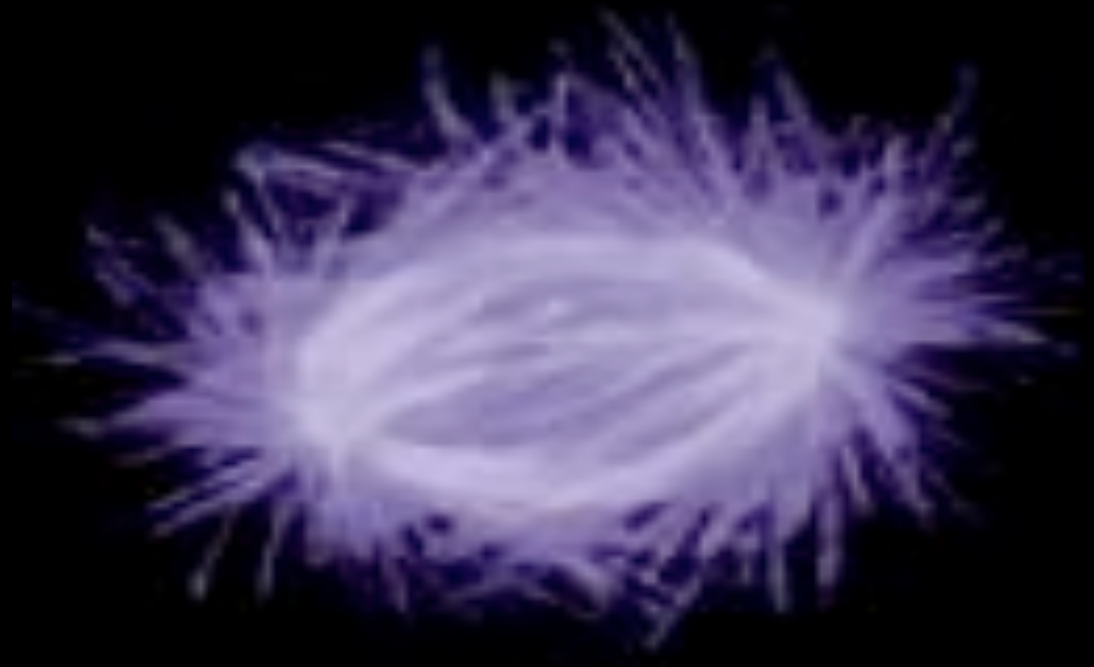
Departments of Mathematics & Neurobiology, Physiology and Behavior
University of California at Davis

Reverse engineering of molecular machines: mitotic spindle

Alex Mogilner

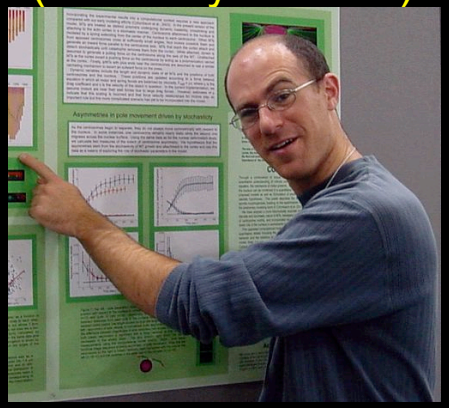


Kyoto, October 2007

People who did it:

Roy Wollman

Eric Cytrynbaum
(currently at UBC)



Collaborators:

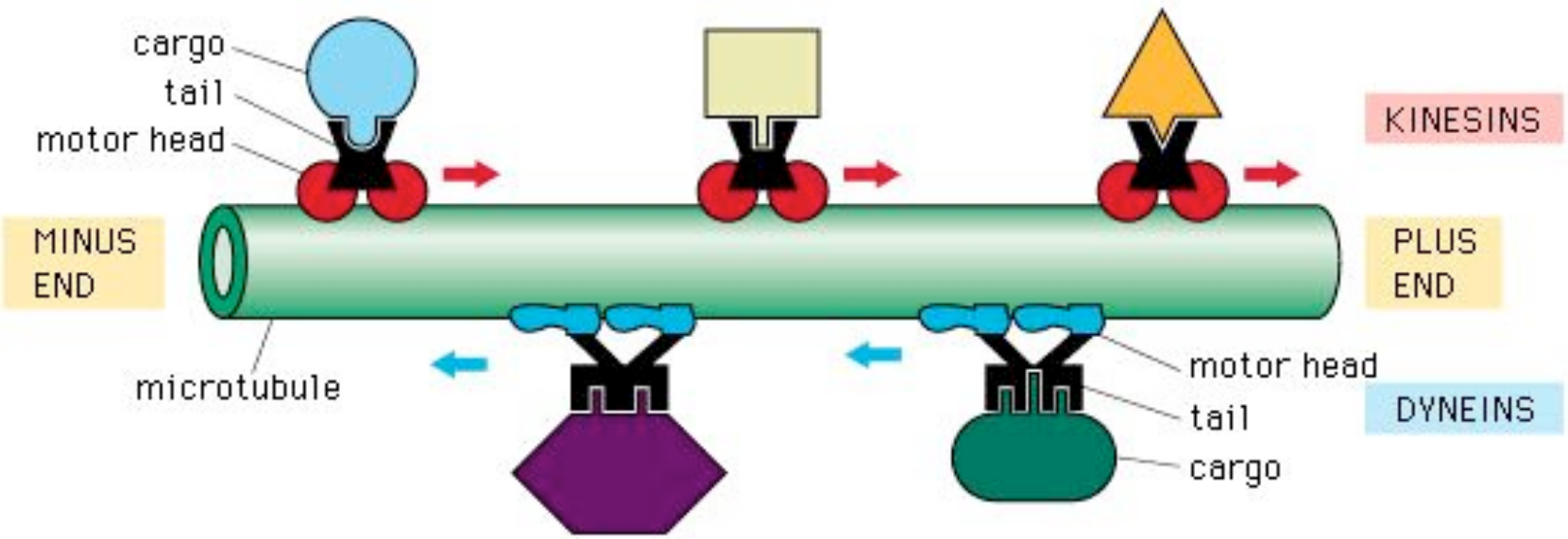
Josh Jones and Tobias Meyer (Stanford)

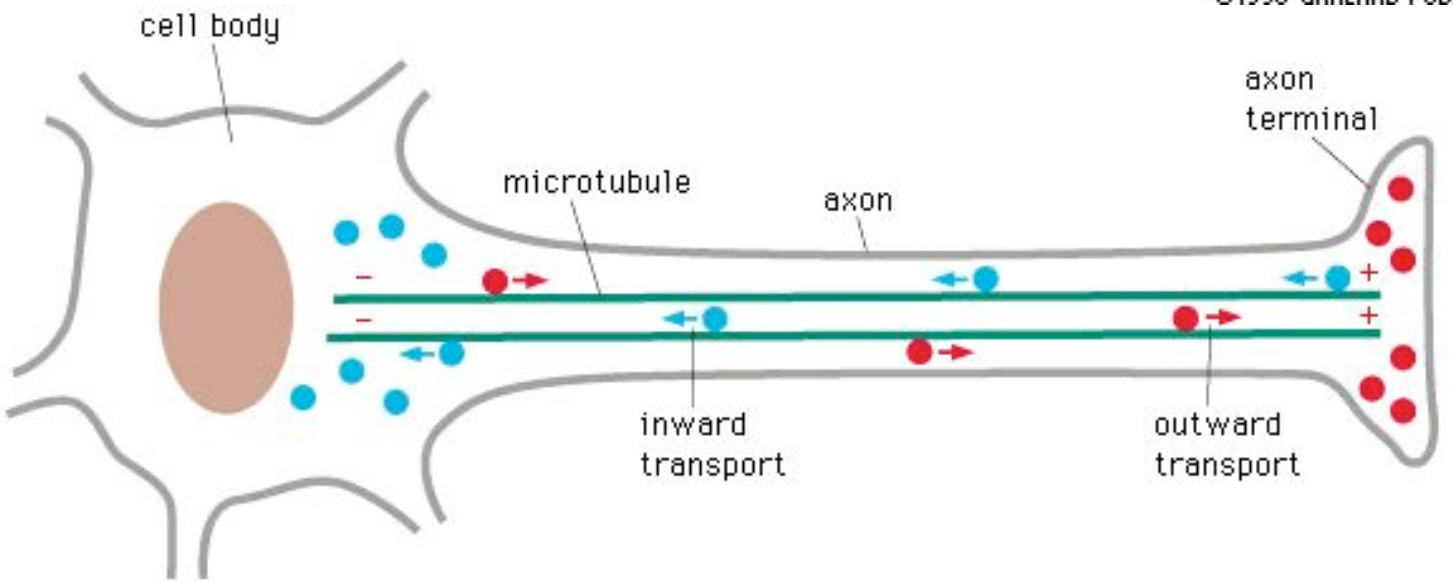
Jon Scholey and Gul Civelekoglu (Davis)

Supported by NSF, NIH

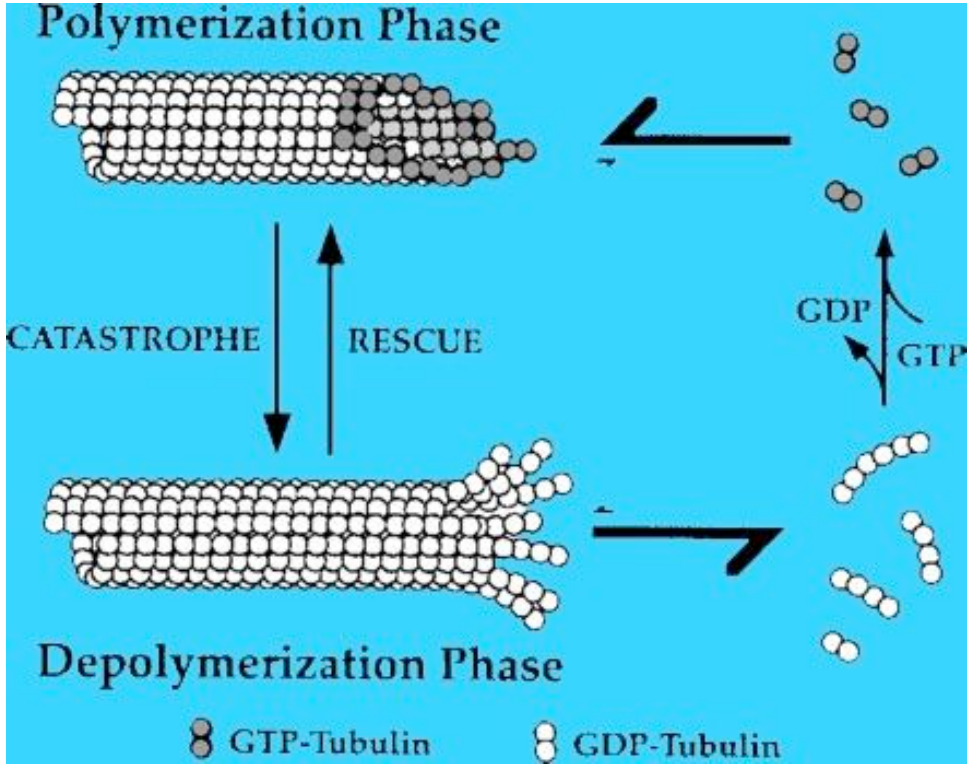
Relevant papers and about our lab at:

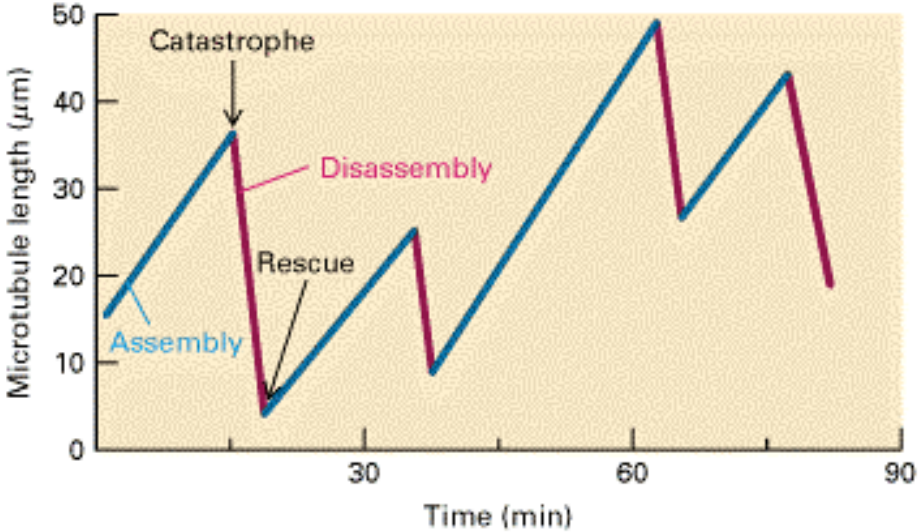
www.math.ucdavis.edu/~mogilner

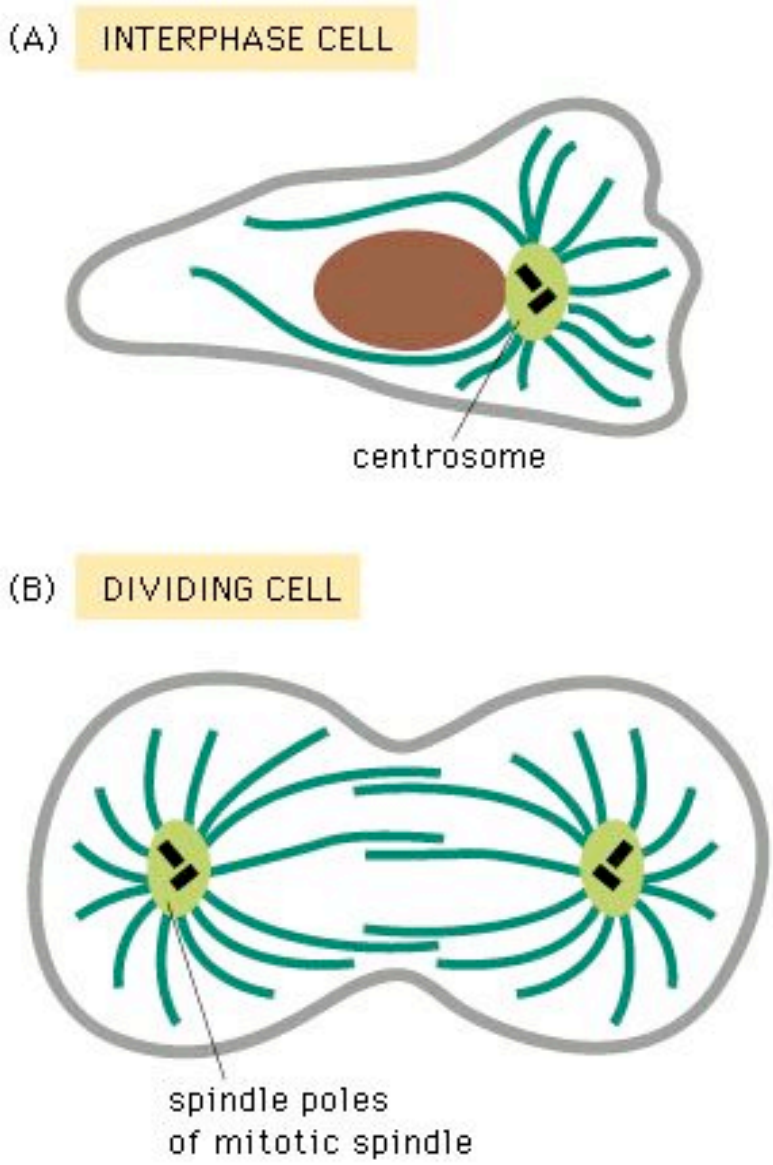




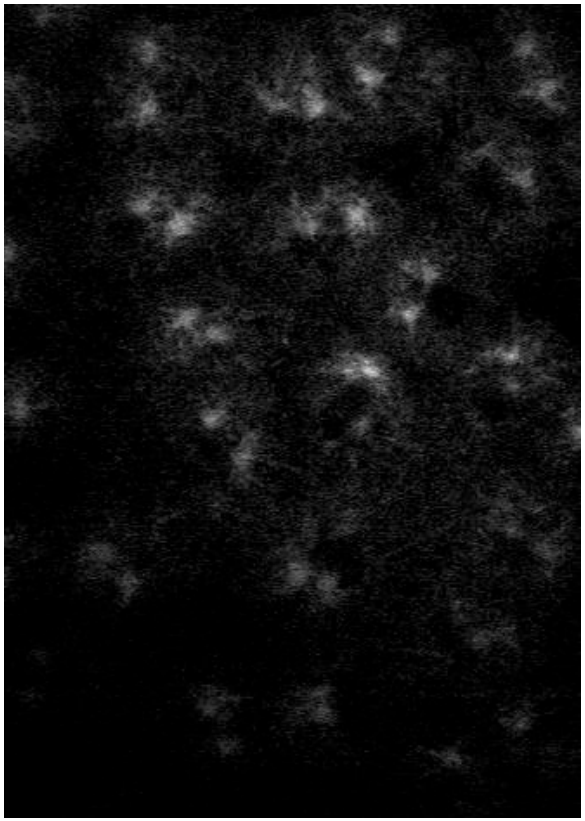
Dynamic instability

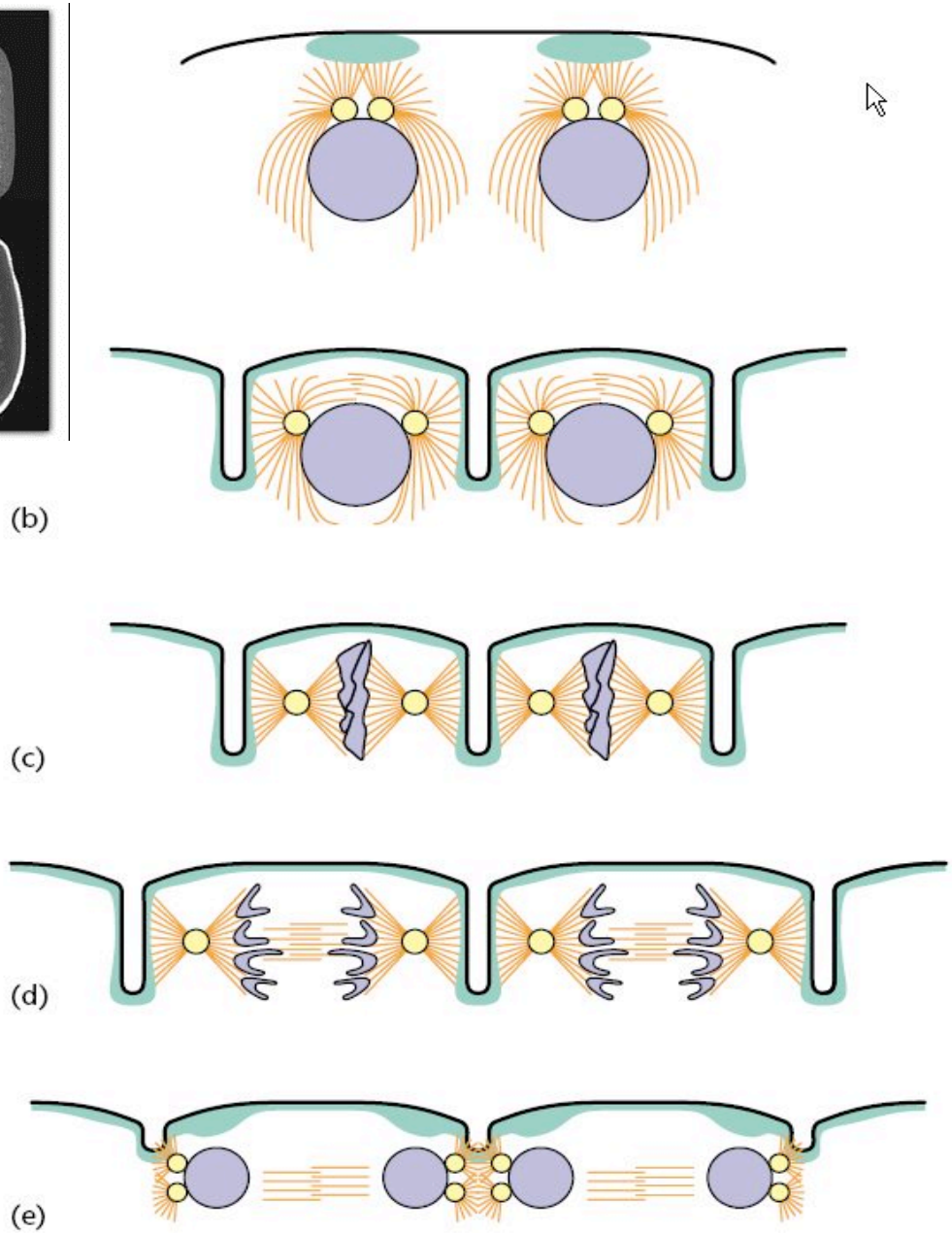




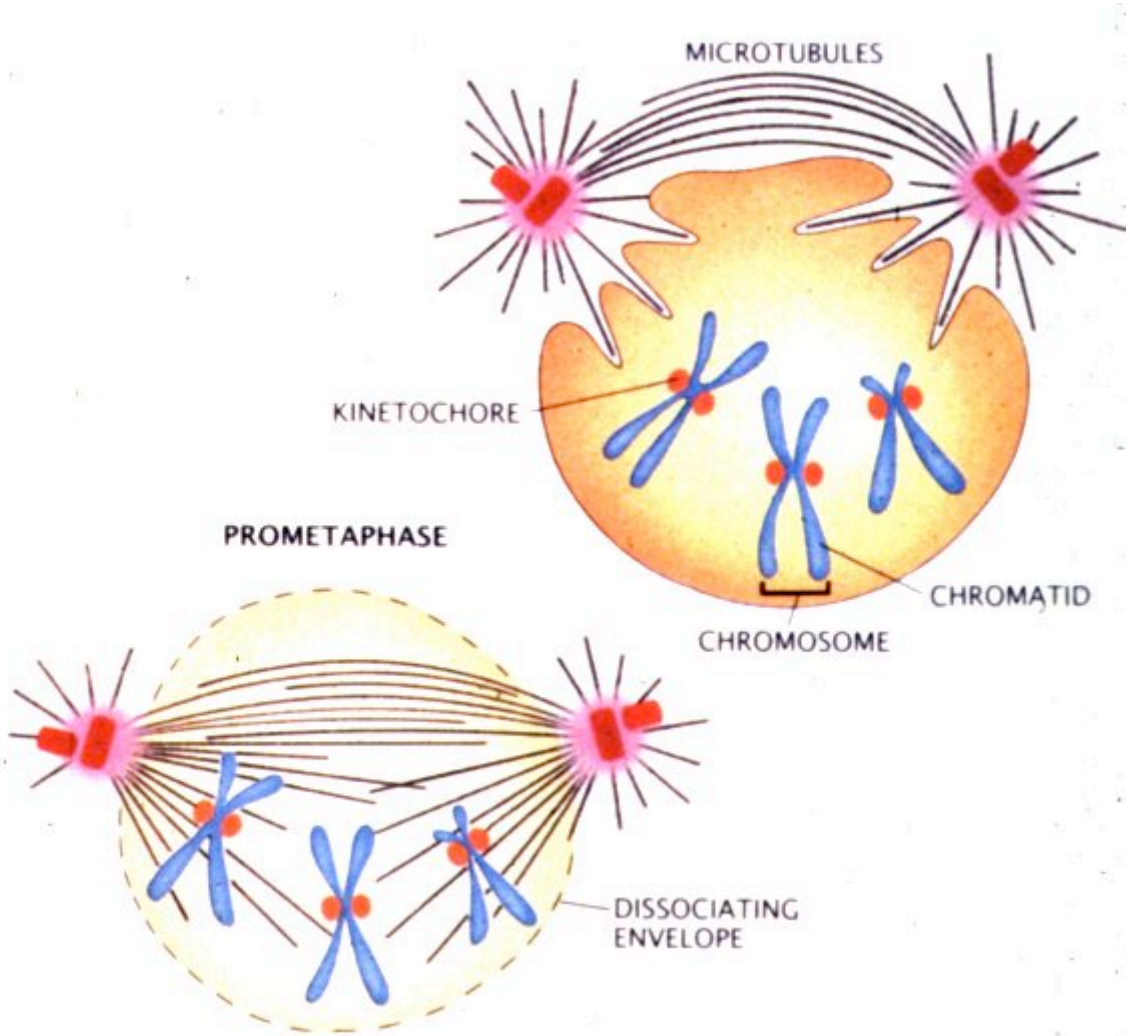


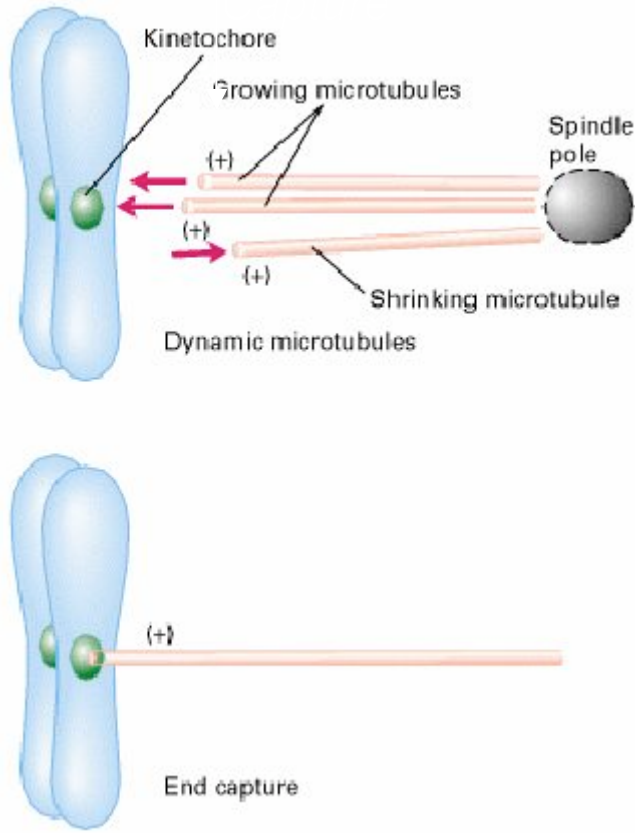
Mitosis (segregation of chromosomes before cell division) in Drosophila Embryo





Search and Capture





Holy & Leibler, 1994:

p – probability of a successful search
t_s – average time of a successful search
t_u – average time of an unsuccessful search
τ - average search time
q – prob. to grow in the right direction, ~ 1/3
p^* - probability to grow to length d

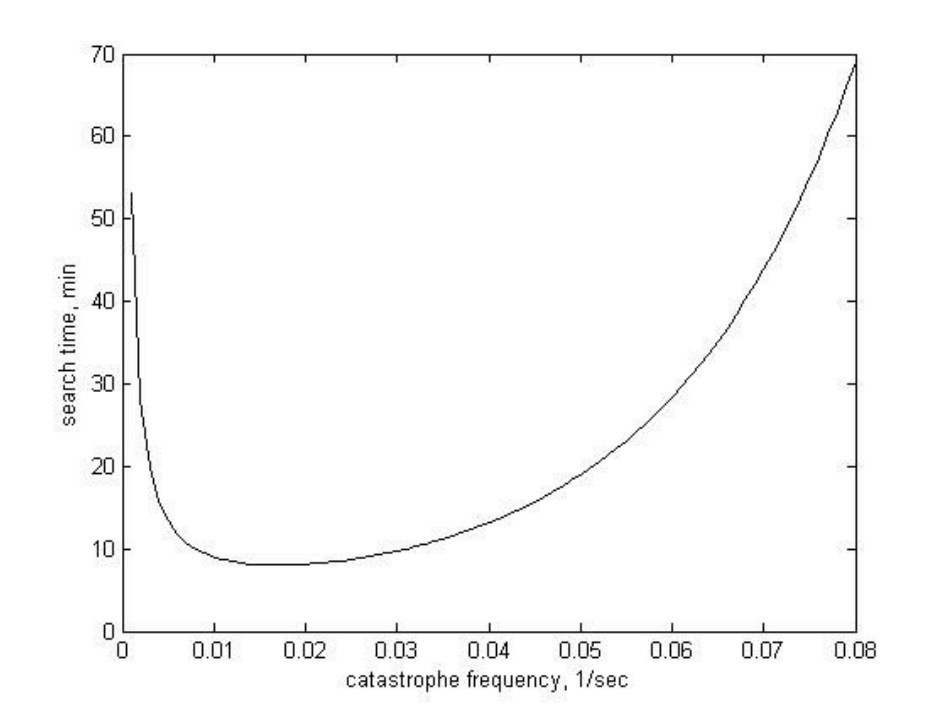
$$\tau = pt_s + (1-p)p(t_s + t_u) +$$

$$(1-p)^2 p(t_s + 2t_u) + \ldots = t_s + \frac{1-p}{p} t_u$$

$$p << 1, \quad \tau \approx \frac{t_u}{p}, \quad p = qp^*, \quad p^* \sim e^{-d/l}$$

$$l = \frac{V_g}{f_{cat}}, \quad t_u \approx \frac{l}{V_g}, \quad \tau \approx \frac{le^{d/l}}{qV_g},$$

$$\min(\tau) = \frac{de}{qV_g} \sim 10 \text{ min} \quad \text{at} \quad l = d$$



For Newt lung cell, distance between spindle pole and chromosome, d ~ 10 μm. Rescue frequency is very small, and average microtubule length, l ~ 10 μm. Growth rate, V_g ~ 10 μm/min. Time in prometaphase, τ ~ 10 min.

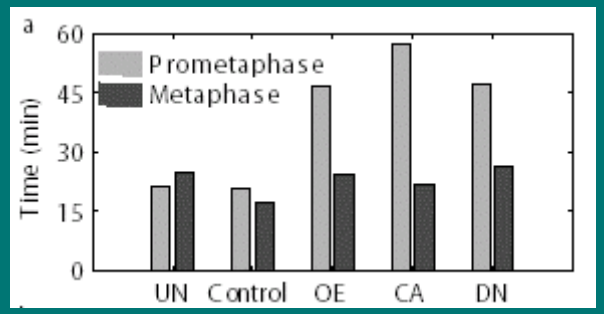
Optimal unbiased '*Search and Capture*' is not fast enough:

Multiple chromosomes - greater time to capture:

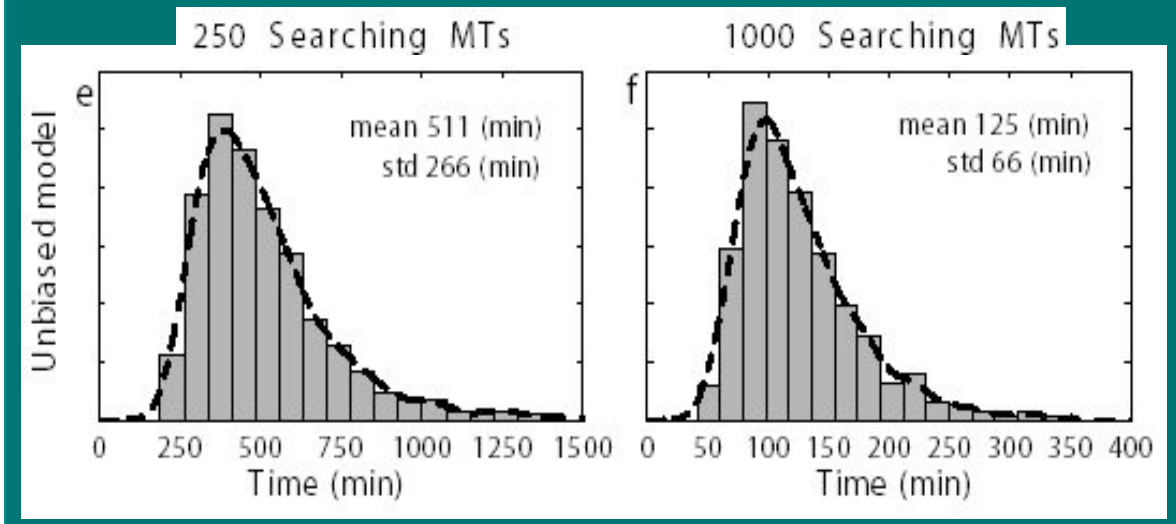
1) Capture of the last chromosome corresponds to the longest search; time ~ logarithm of the number of chromosomes.
2) Geometric effect: a few-fold increase.

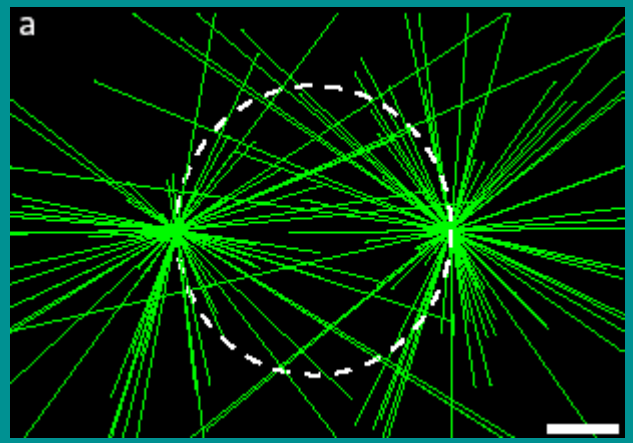
Experiment with Hela cells:

prophase – prometaphase – metaphase

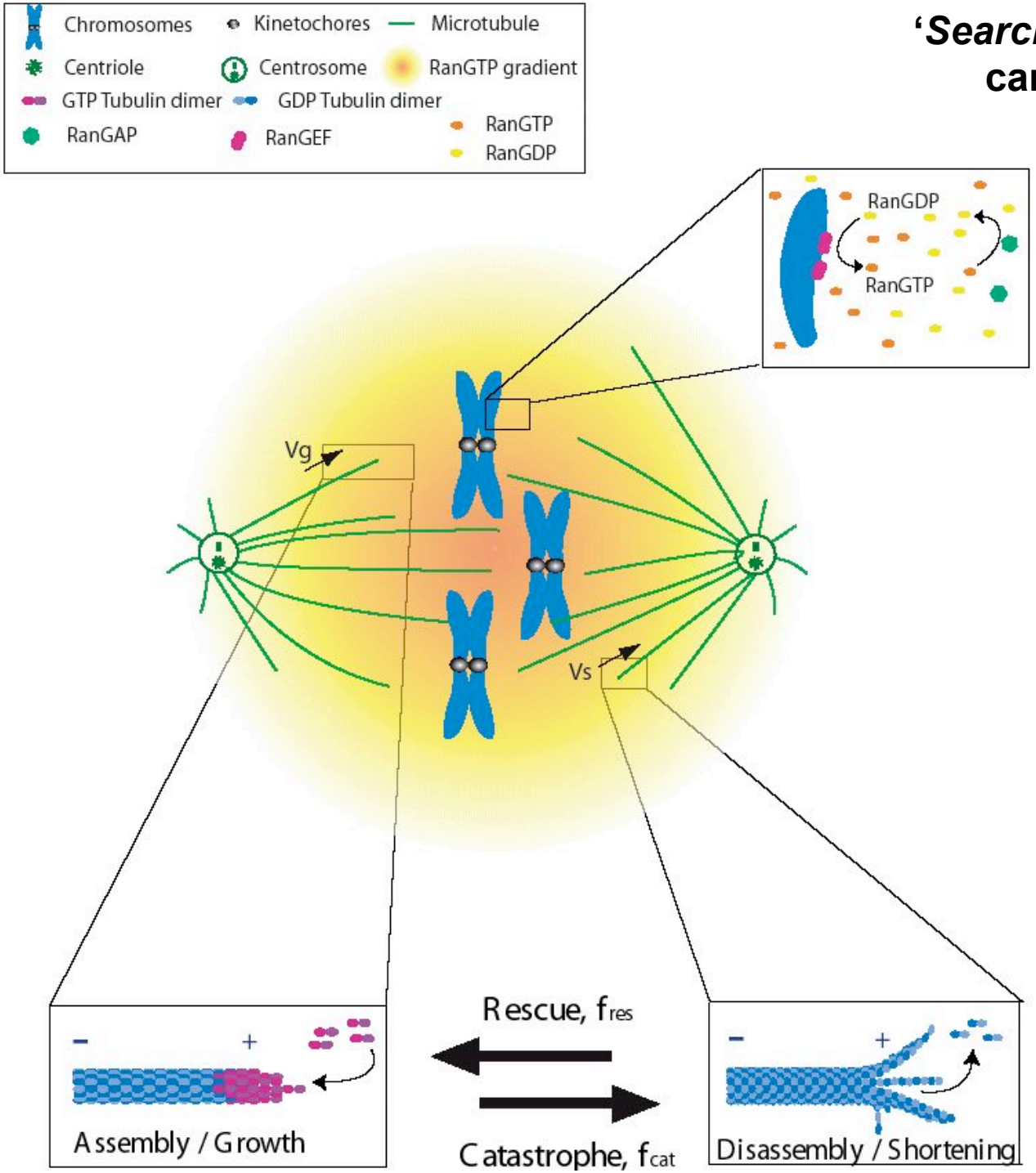


Numerical experiment:

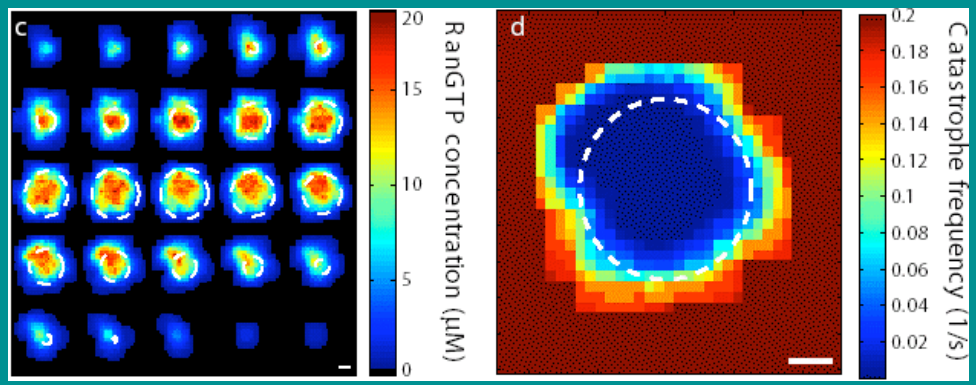


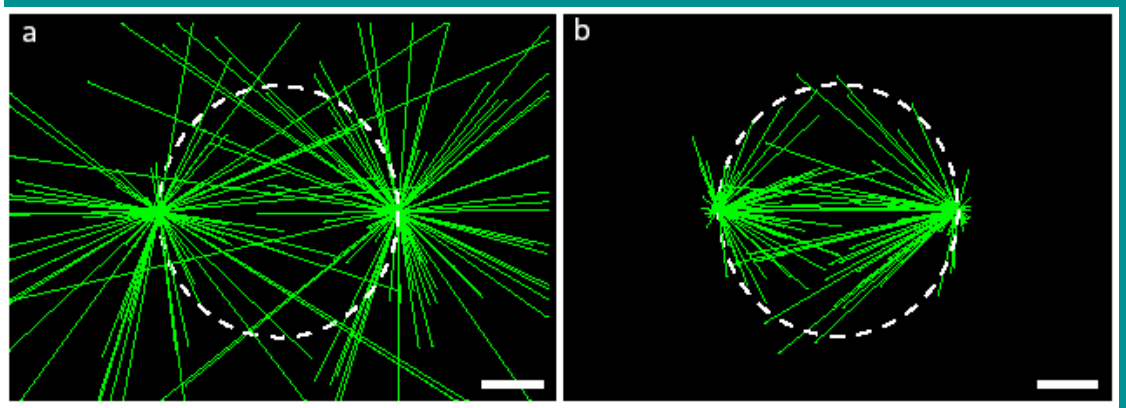


‘*Search and Capture*’ can be biased



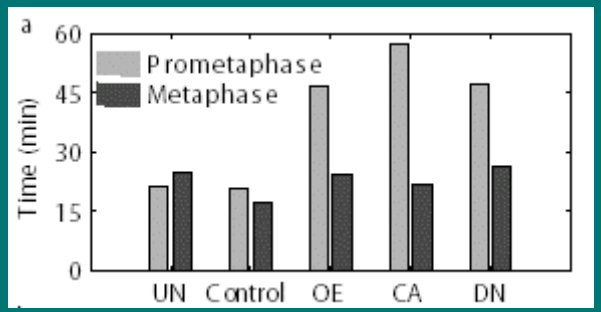
Optimal biased '*Search and Capture*' is fast enough:



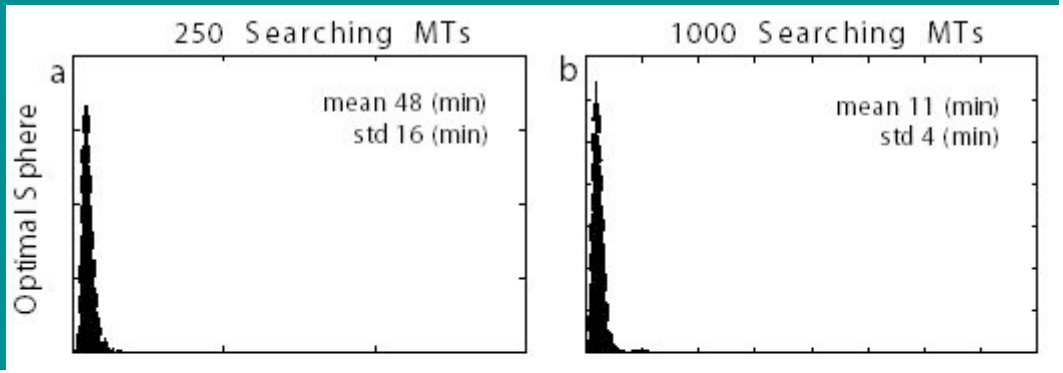


Experiment with Hela cells:

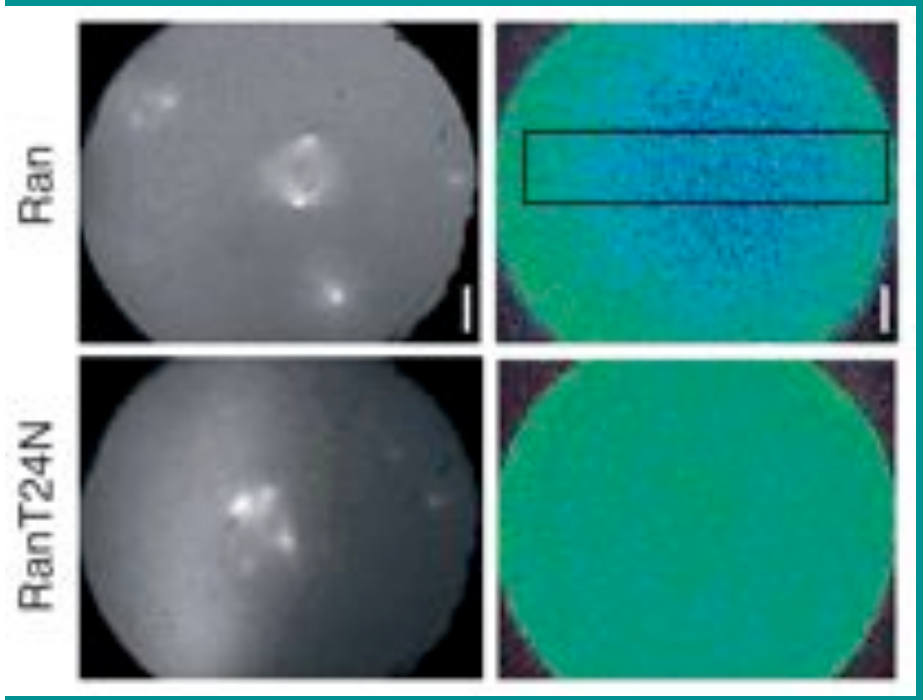
prophase – prometaphase – metaphase



Numerical experiment:

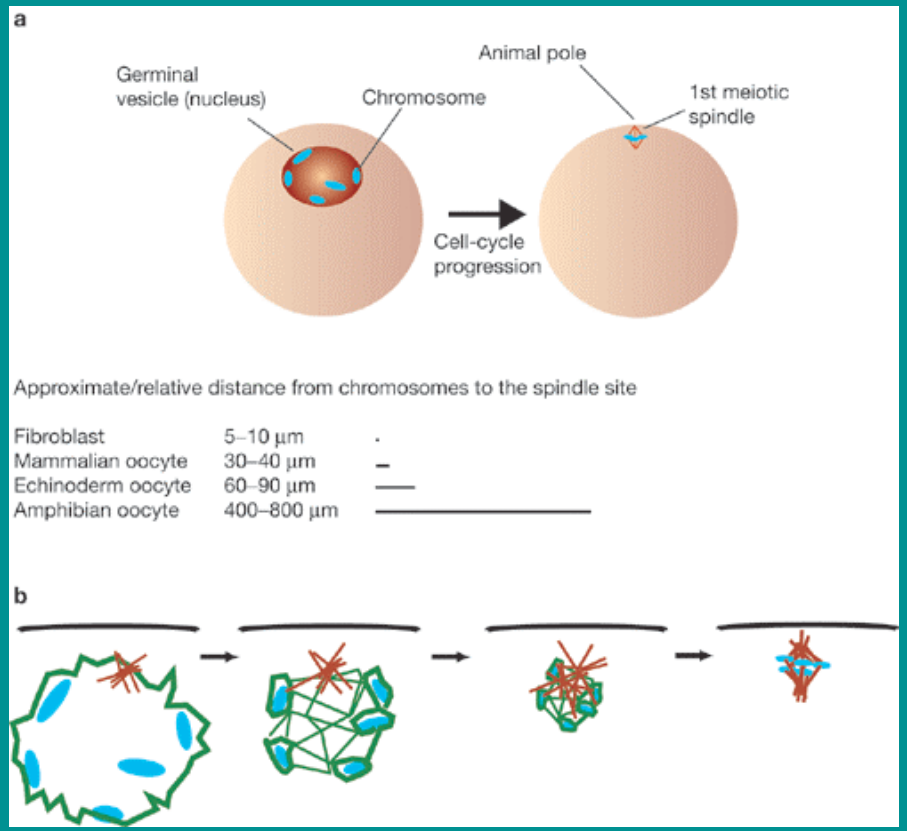


The model inspired two recent studies:

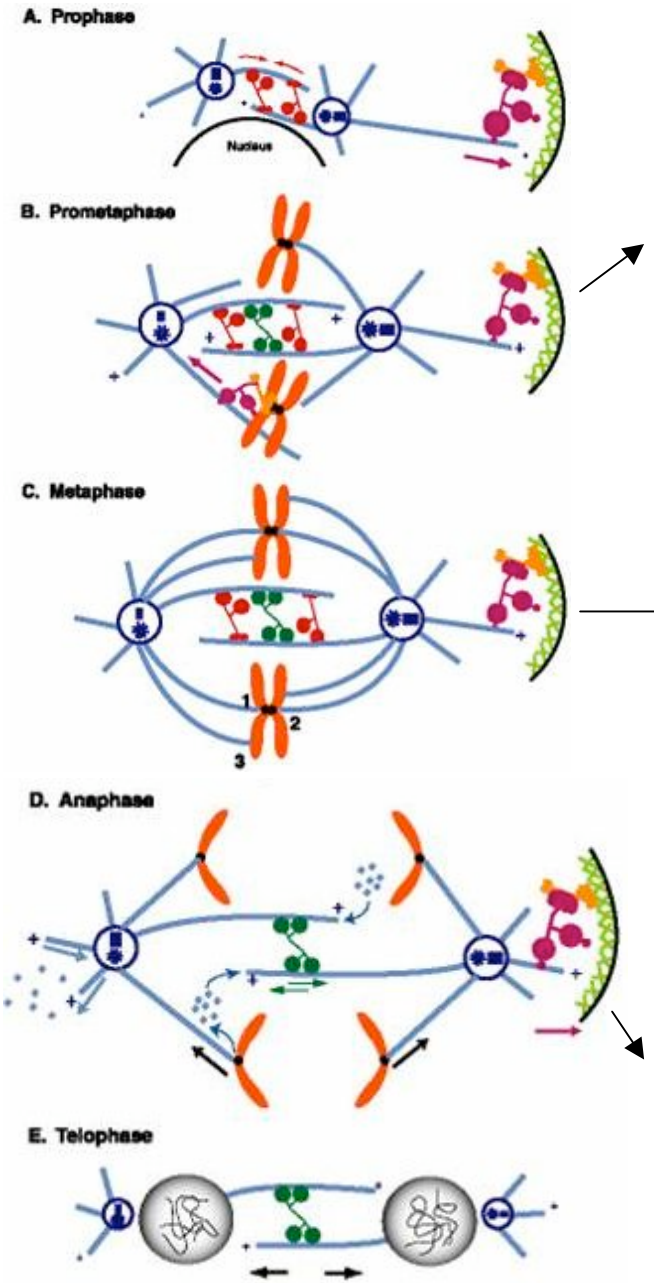


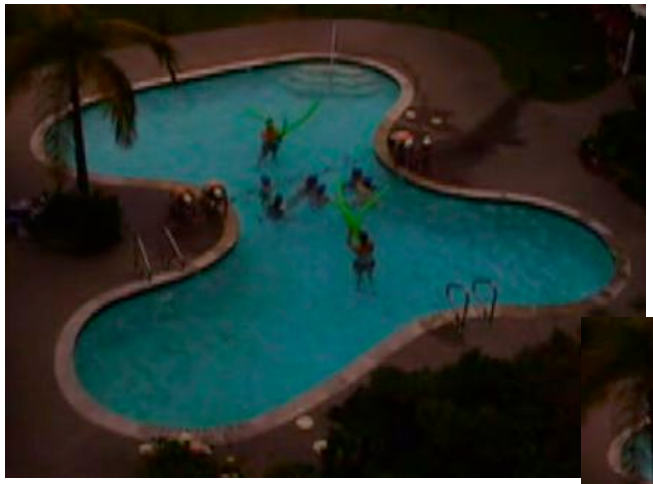
Caudron et al, 2005:
Ran gradients exist and bias microtubule asters

Lenart et al, 2005:
at large centrosome-chromosome distances, “Search&Capture” is not efficient, and actin-myosin “fishnet” mechanism works first

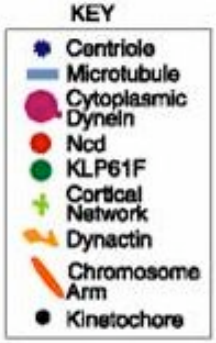


Multiple parts of the spindle are involved in stochastic, yet robust and predictable 'dance'

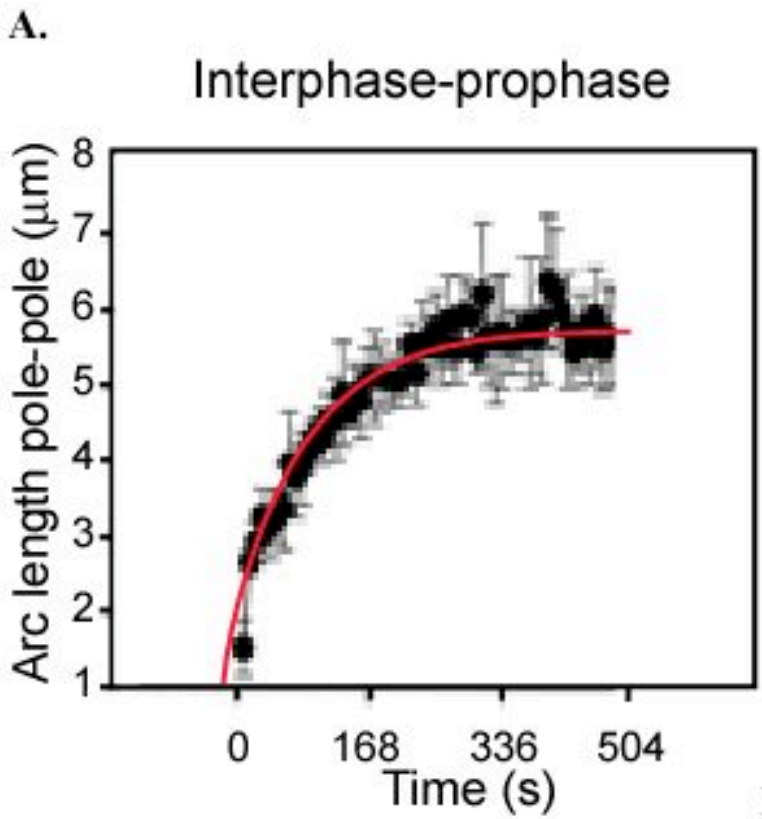


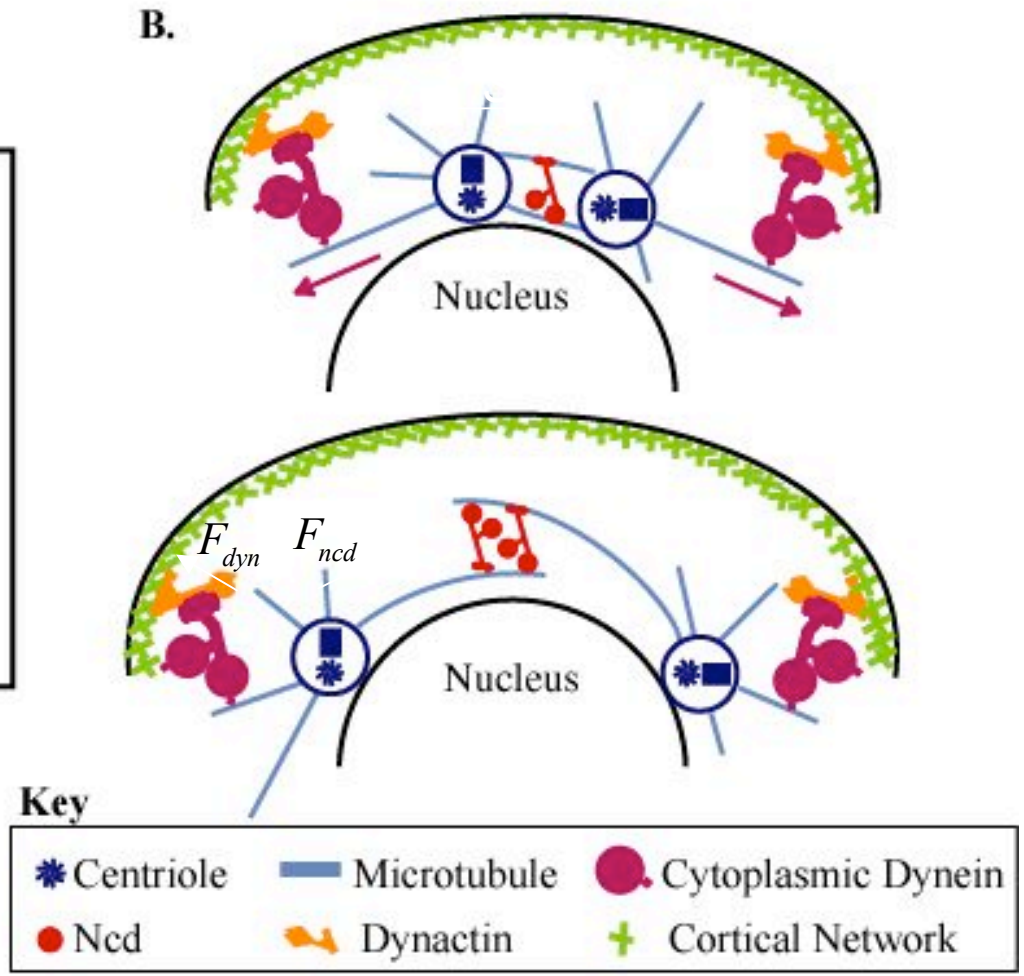


Force-balance model;
Motors switch on and off at times that are tightly regulated



Balance of dynein (outward) and ncd (inward) forces explains pole separation and transient steady state in interphase - prophase

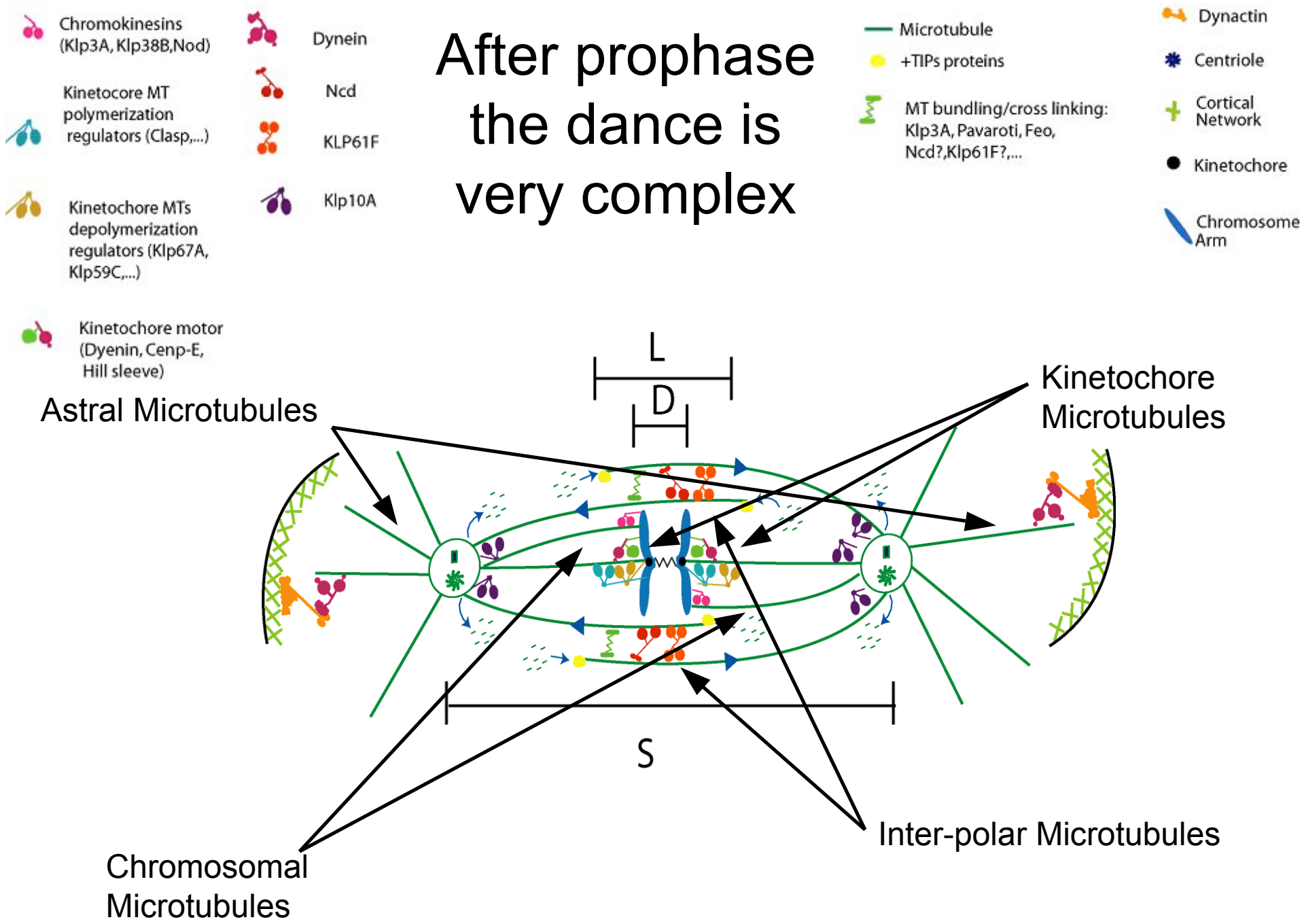




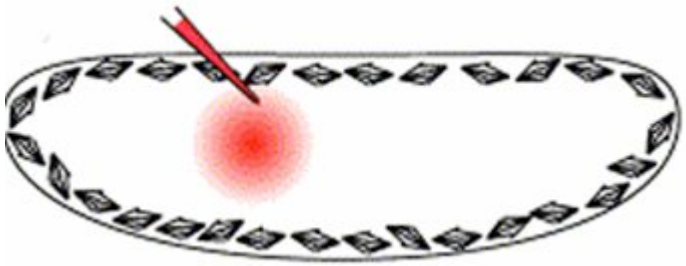
$$\frac{dS}{dt} = \frac{2}{\mu}\left(F_{dyn} - F_{ncd} \times S\right)$$

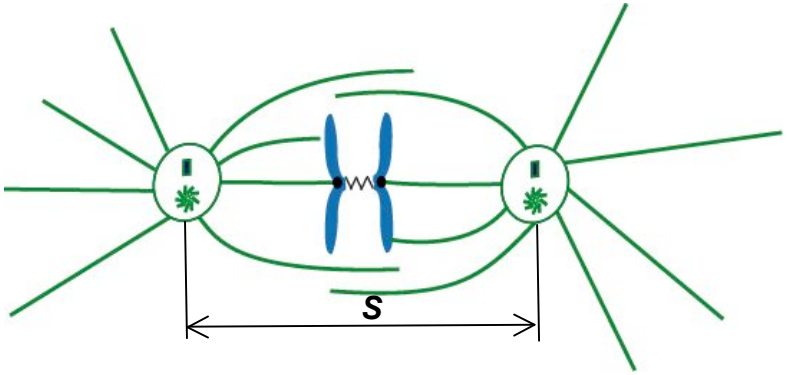
Cytrynbaum et al., 2003, 2005

After prophase the dance is very complex

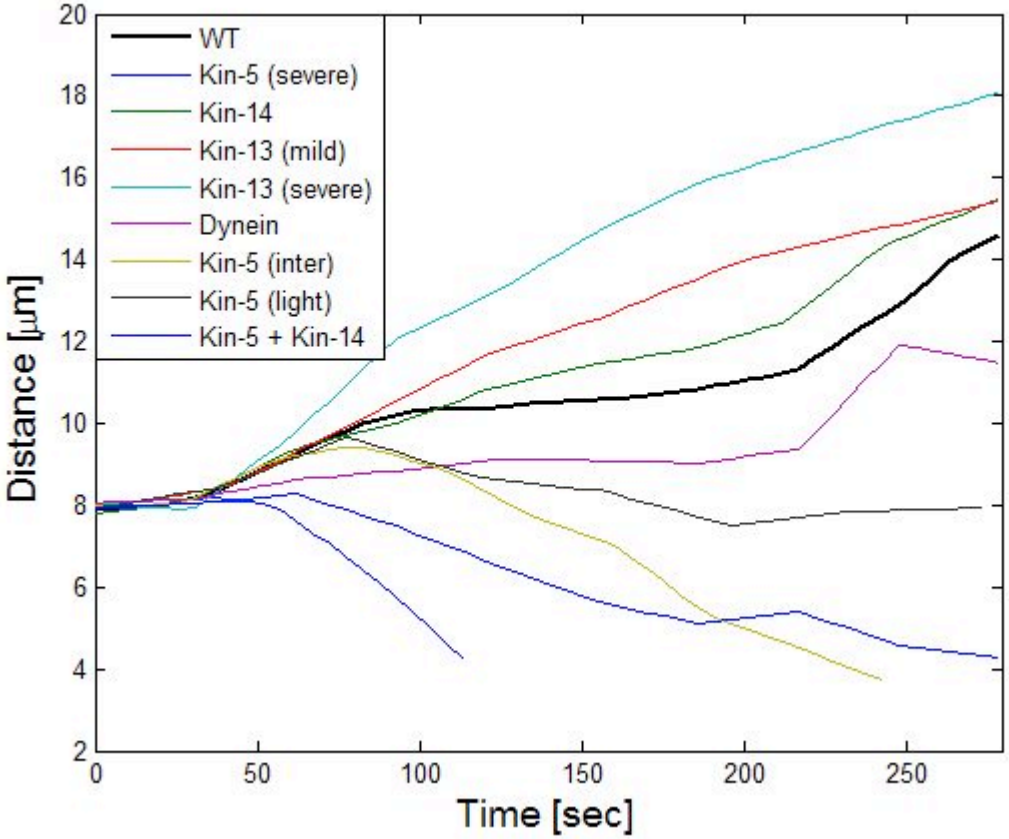


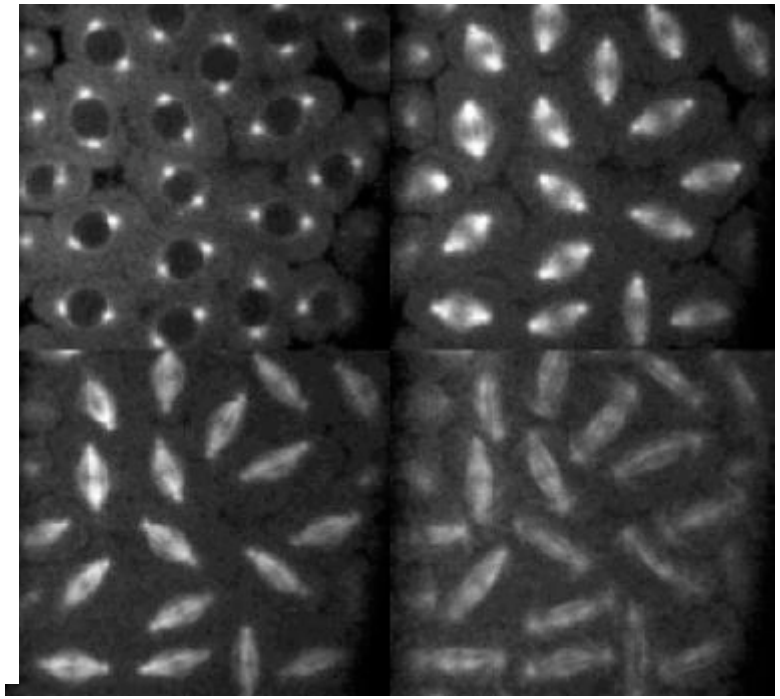
Quantitative measure of mitotic progression





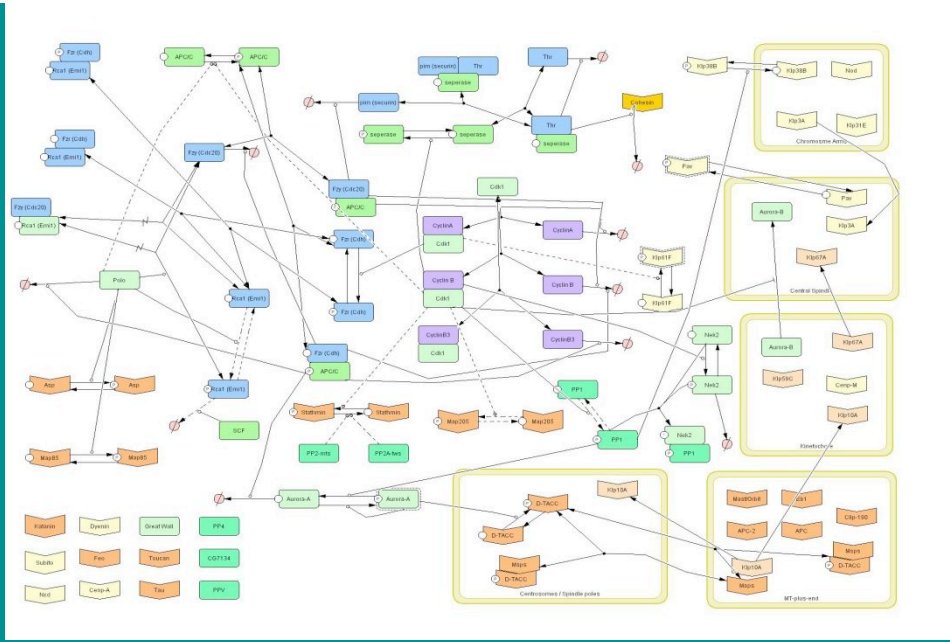
Pole – Pole distance *s* over time

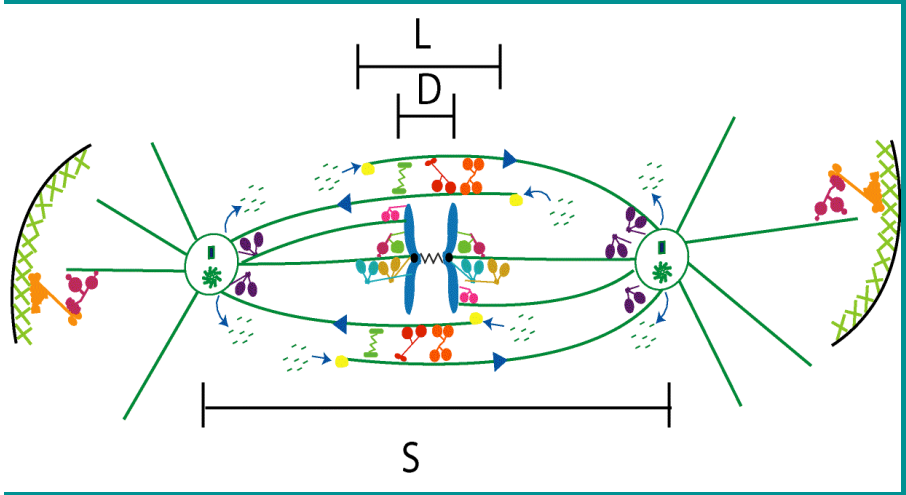




Biochemical regulation

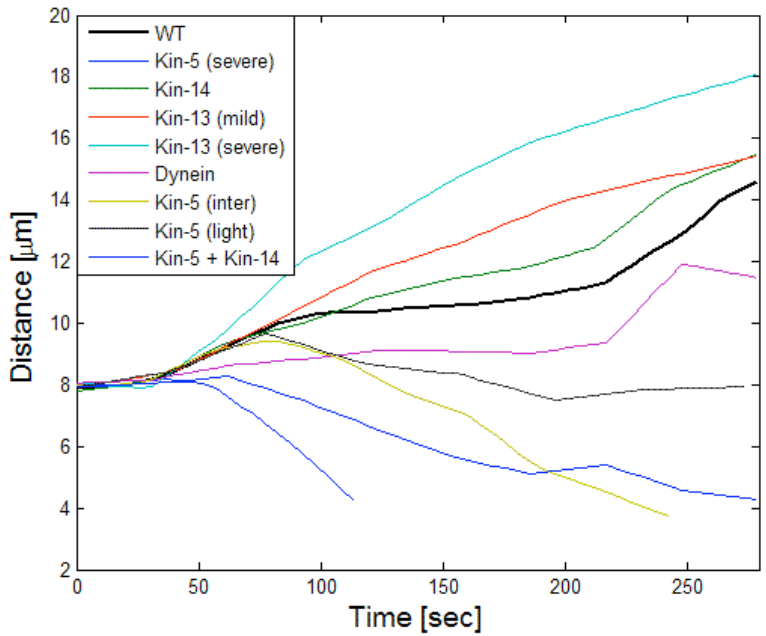
Switches





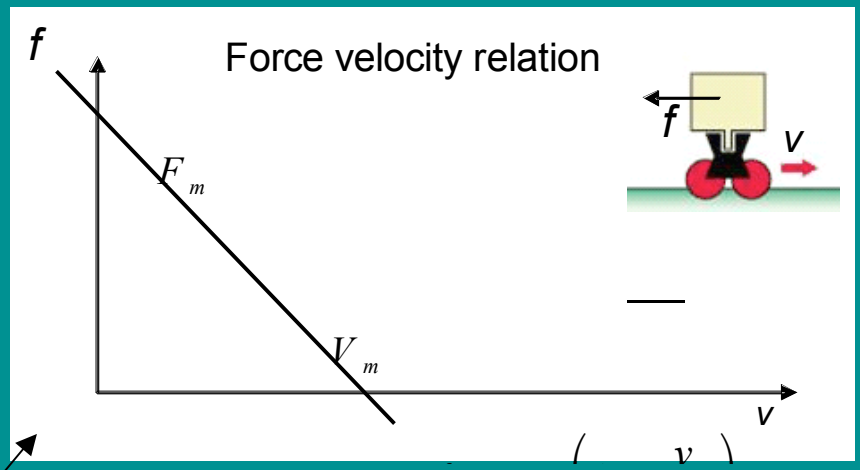
Mechanics

Phenotype

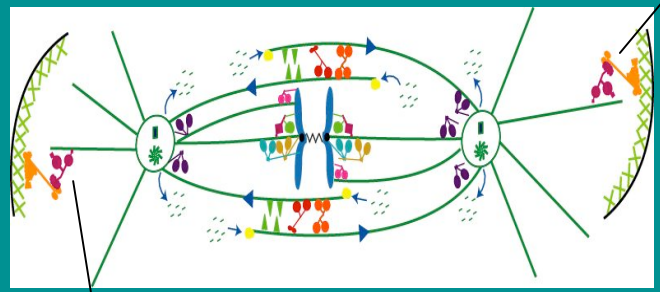


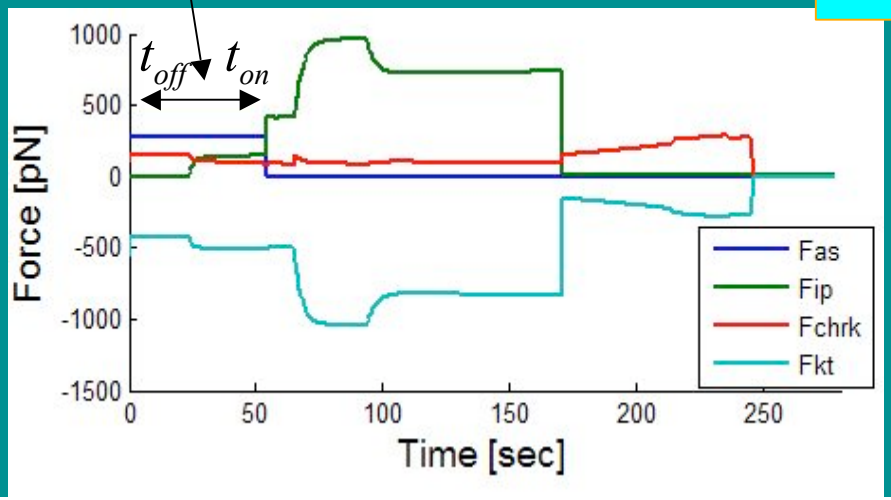
Defining the puzzle: Find activation sequence and mechanical characteristics of motors that explains the data

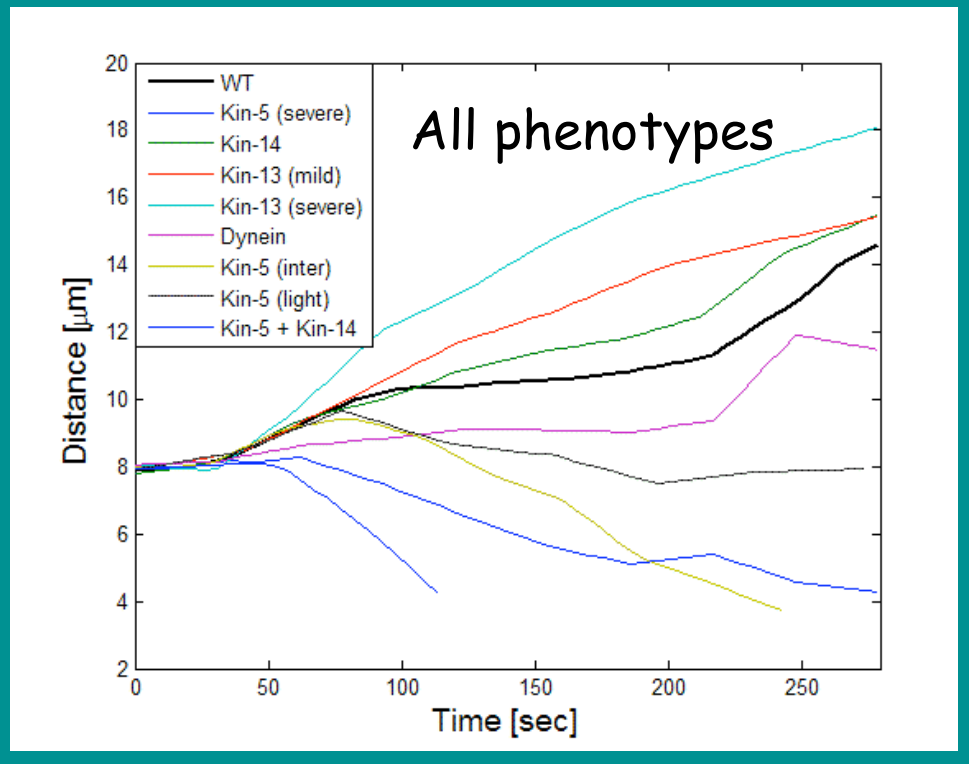
$$\left[t_{off}, t_{on}\right], F_m, V_m \; - ?$$



Motor activation profiles



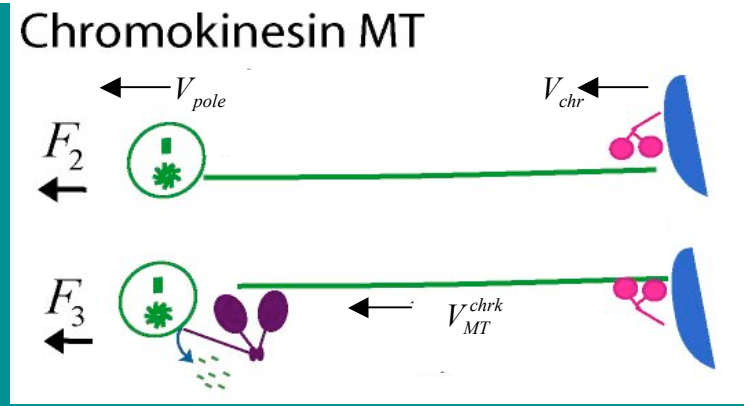




Building the model (example of chrMTs)

$$-F_{chrk,mx}\left(1-\frac{V_{MT}^{chrk}-V_{chr}}{V_{chrk,mx}}\right)=F_{dep,mx}\left(1-\frac{V_{MT}^{chrk}-V_{pole}}{V_{dep,mx}}\right)$$

$$V_{MT}^{chrk}=\frac{\left(F_{dep,mx}\left(1+\frac{V_{pole}}{V_{dep,mx}}\right)+F_{chrk,mx}\left(1+\frac{V_{chr}}{V_{chrk,mx}}\right)\right)V_{dep,mx}V_{chrk,mx}}{V_{dep,mx}F_{chrk,mx}+V_{chrk,mx}F_{dep,mx}}$$



$$F_2=F_{chrk,mx}\left(1-\frac{V_{pole}-V_{chr}}{V_{chrk,mx}}\right)$$

$$F_3=-F_{dep,mx}\left(1-\frac{V_{MT}^{chrk}-V_{pole}}{V_{dep,mx}}\right)$$

3. Forces on microtubule population

$$F_{chrk}=P_{chrk}\frac{N_{chrk}A_{chr}}{(S-D)^2}\left(\left(1-P_{dep}\right)F_2+P_{dep}F_3\right)$$

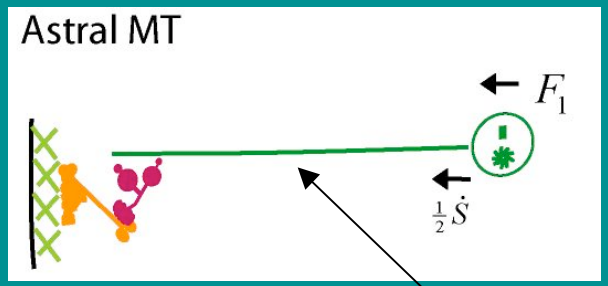
Regulatory switch

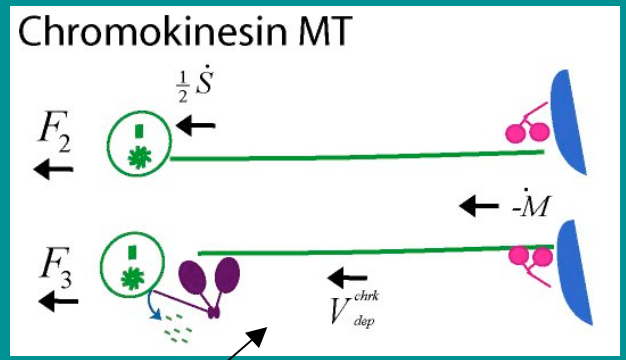
Number of microtubules

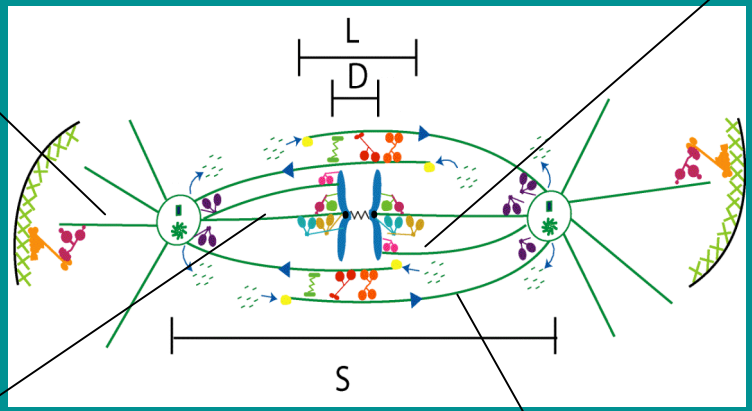
Single Microtubule equations

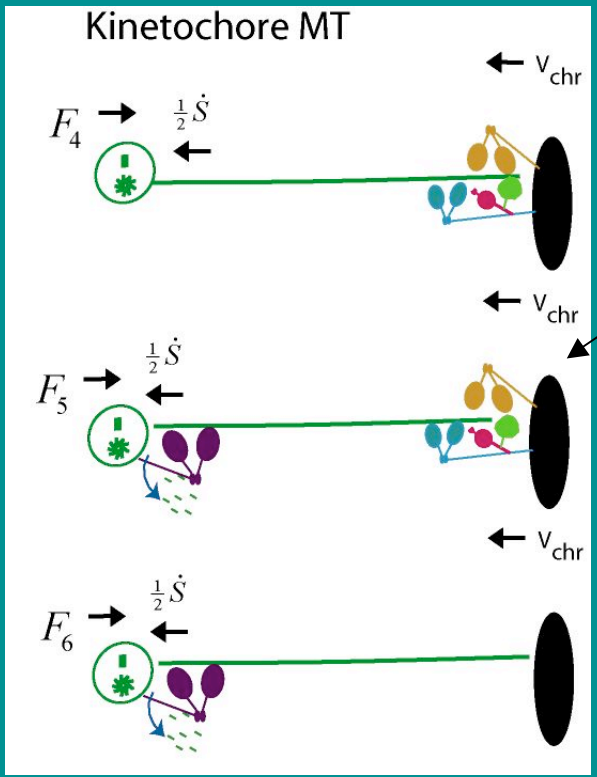
$$P_{chrk}=\begin{cases}0 & inactive\\ 1 & active\end{cases}$$

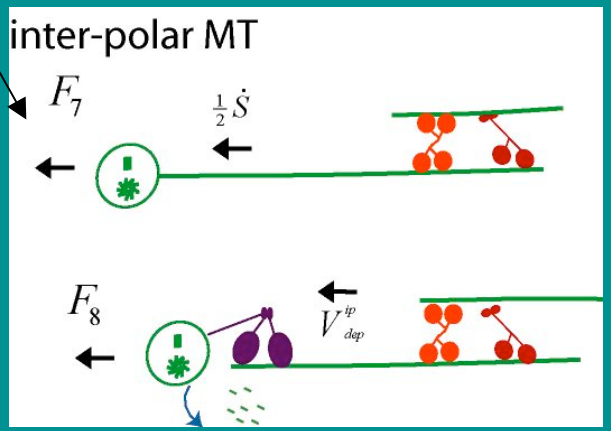
4 types of microtubule populations











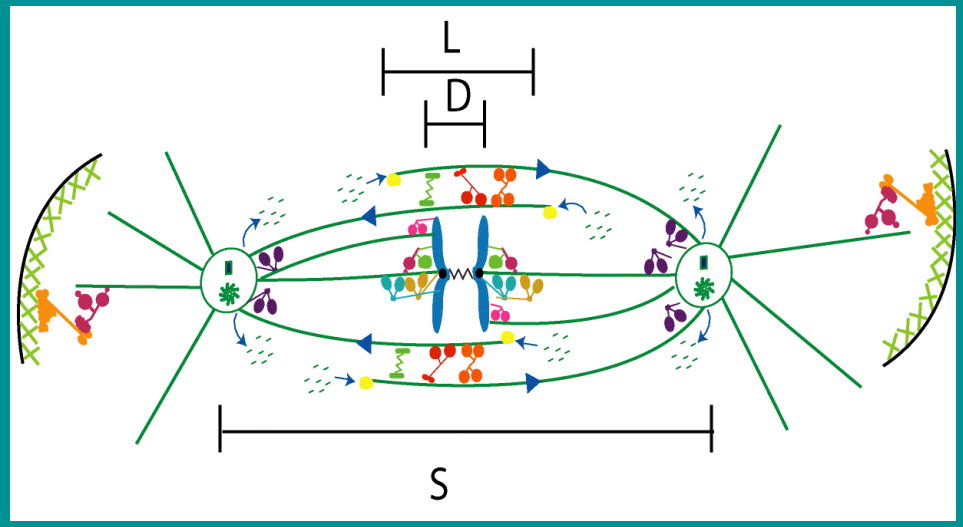
Forces on the spindle and dynamic equations

$$\frac{dS}{dt} = \frac{2\left(F_{ip} + F_{chrk} + F_{aster} - F_{kt}\right)}{\mu_{pole}}$$

$$\frac{dD}{dt} = \frac{2\left(F_{kt} - F_{chrk} - F_{cohesion}\right)}{\mu_{chr}}$$

$$\frac{dL}{dt} = 2V_{ploy}^{ip} - 2\left(\left(1 - P_{dep}\right)V_{pole} + P_{dep}V_{MT}^{ip}\right)$$

$$V_{pole} = \frac{1}{2}\frac{dS}{dt} \qquad V_{chr} = \frac{1}{2}\frac{dD}{dt}$$



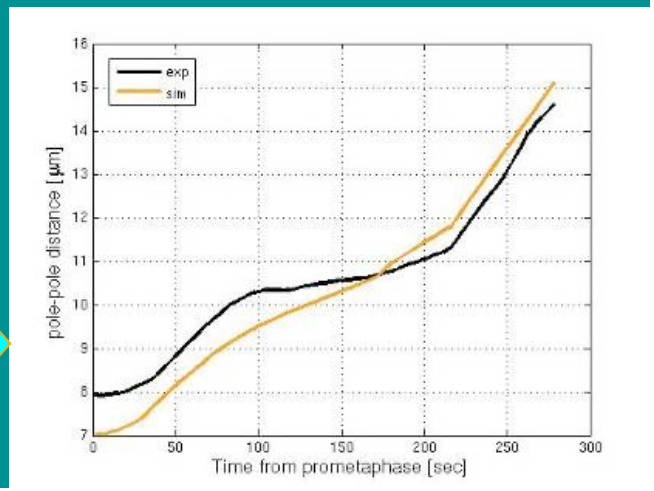
Numerical solution of the model equations (stiff ODE solver)

Solve for single microtubule ($v \rightarrow f$)

Integrate for microtubule population ($f \rightarrow F$)

Integrate for entire spindle ($F \rightarrow v$)

Score



Impossibility of systematic scanning of the parameter space (~ 50 parameters)
So: genetic algorithm based optimization

1. Start with random population of solutions

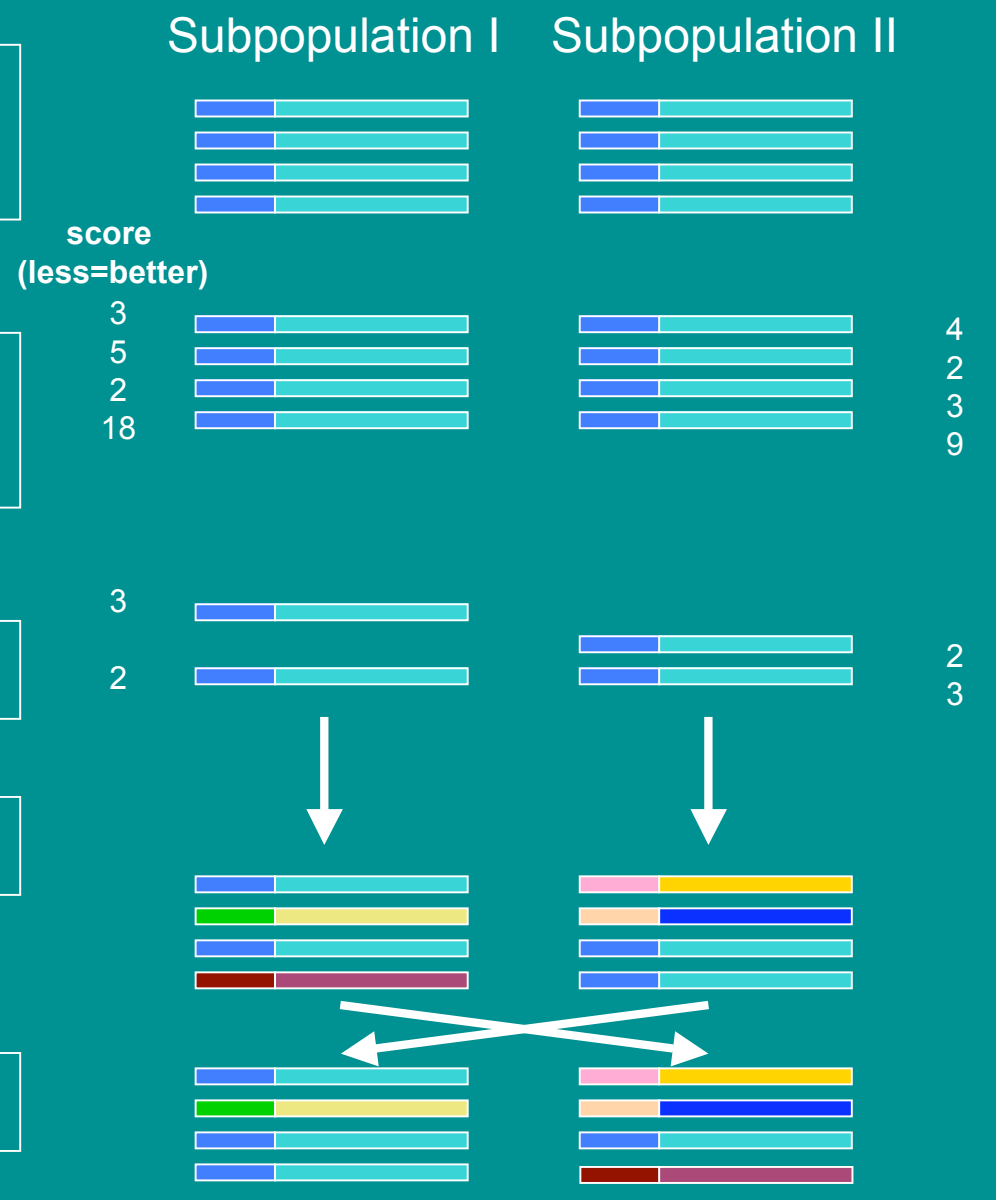
2. Evaluate goodness of solutions.

3. ‘Survival of the fittest’

4. Mutation

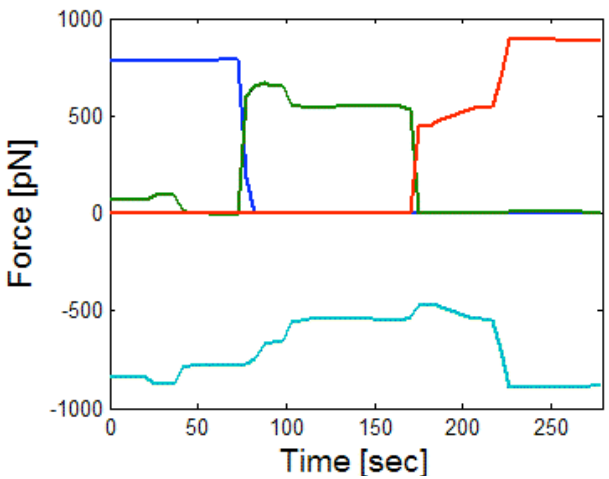
5. Recombination

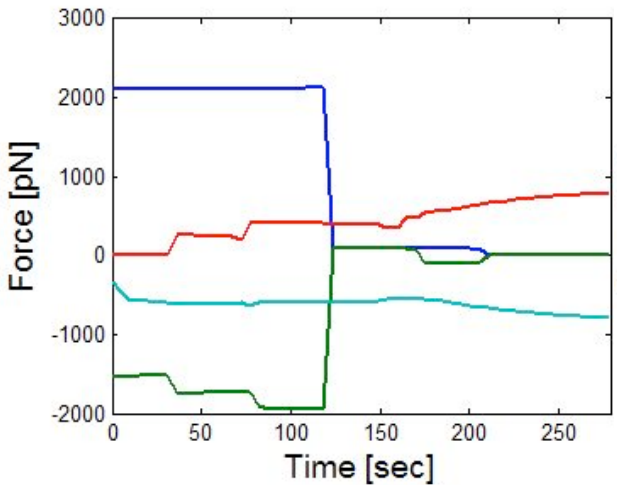
x 200

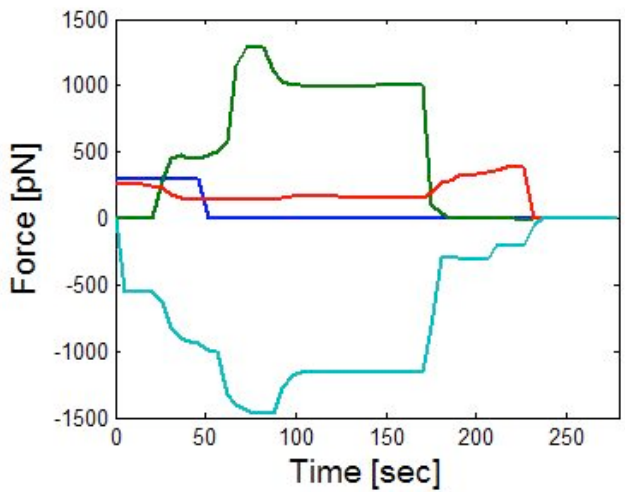


Examples of good models

$$\frac{dS}{dt} = \frac{2\left(F_{ip} + F_{chrk} + F_{aster} + F_{kt}\right)}{\mu_{pole}}$$

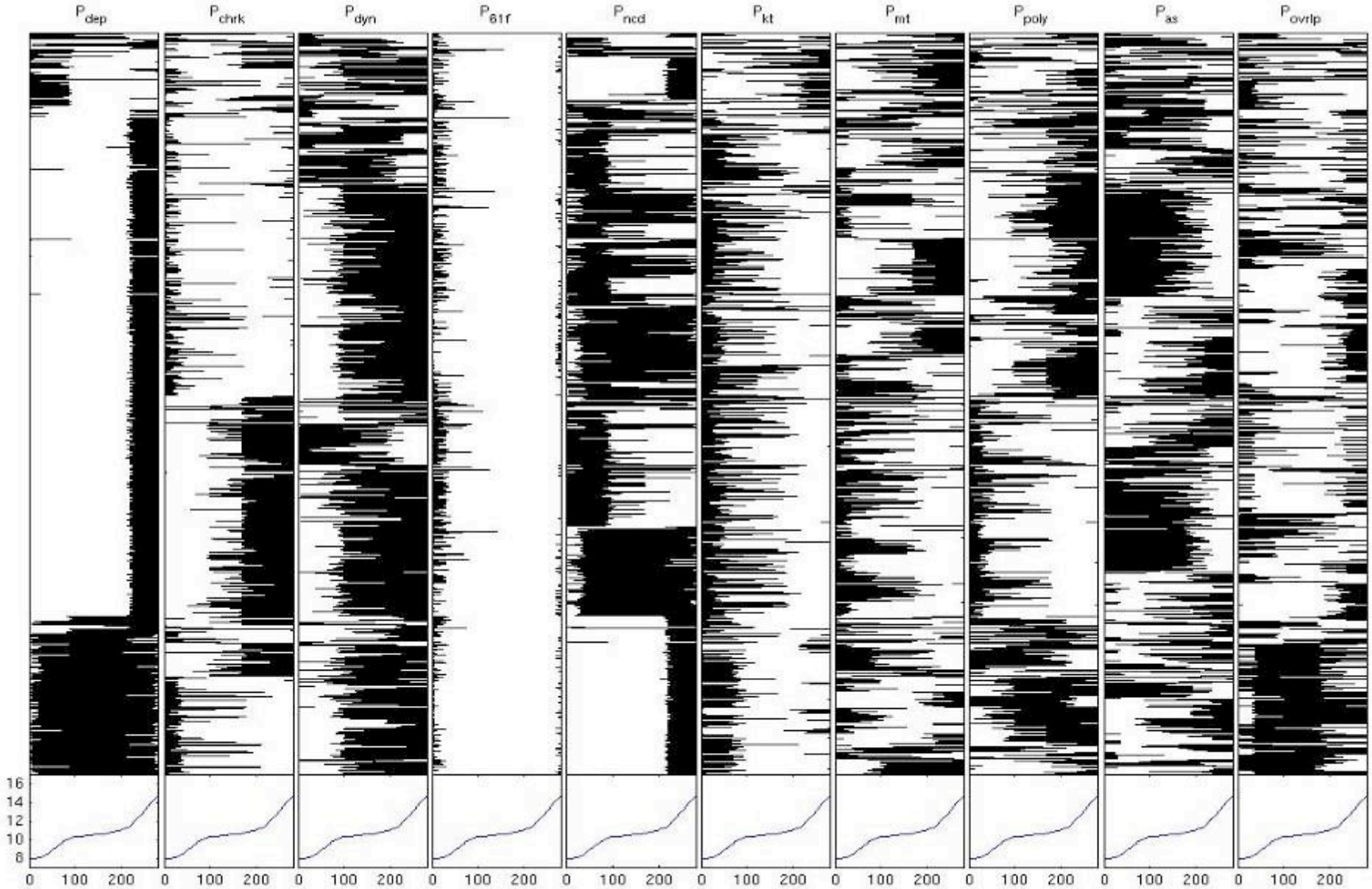




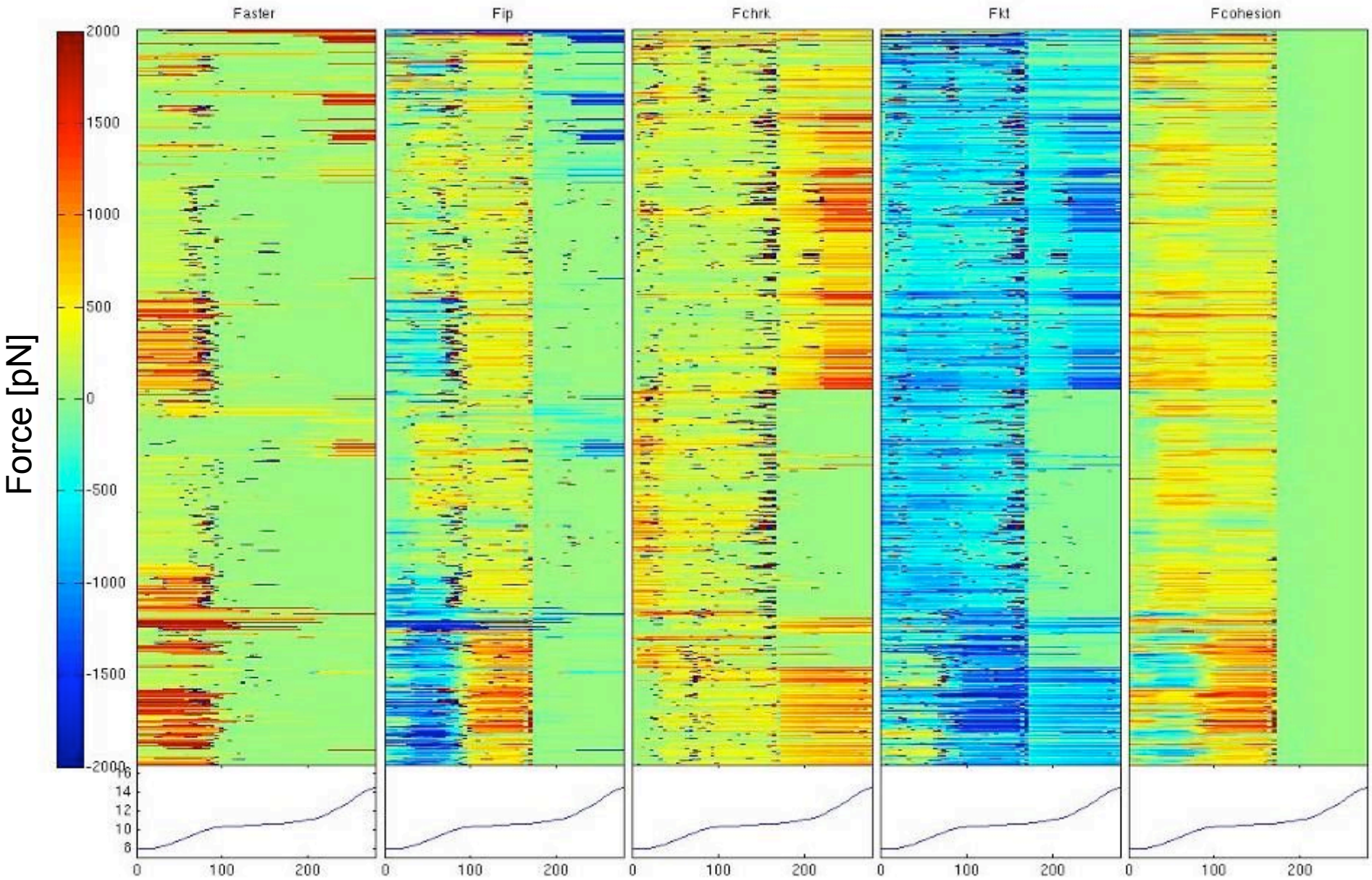


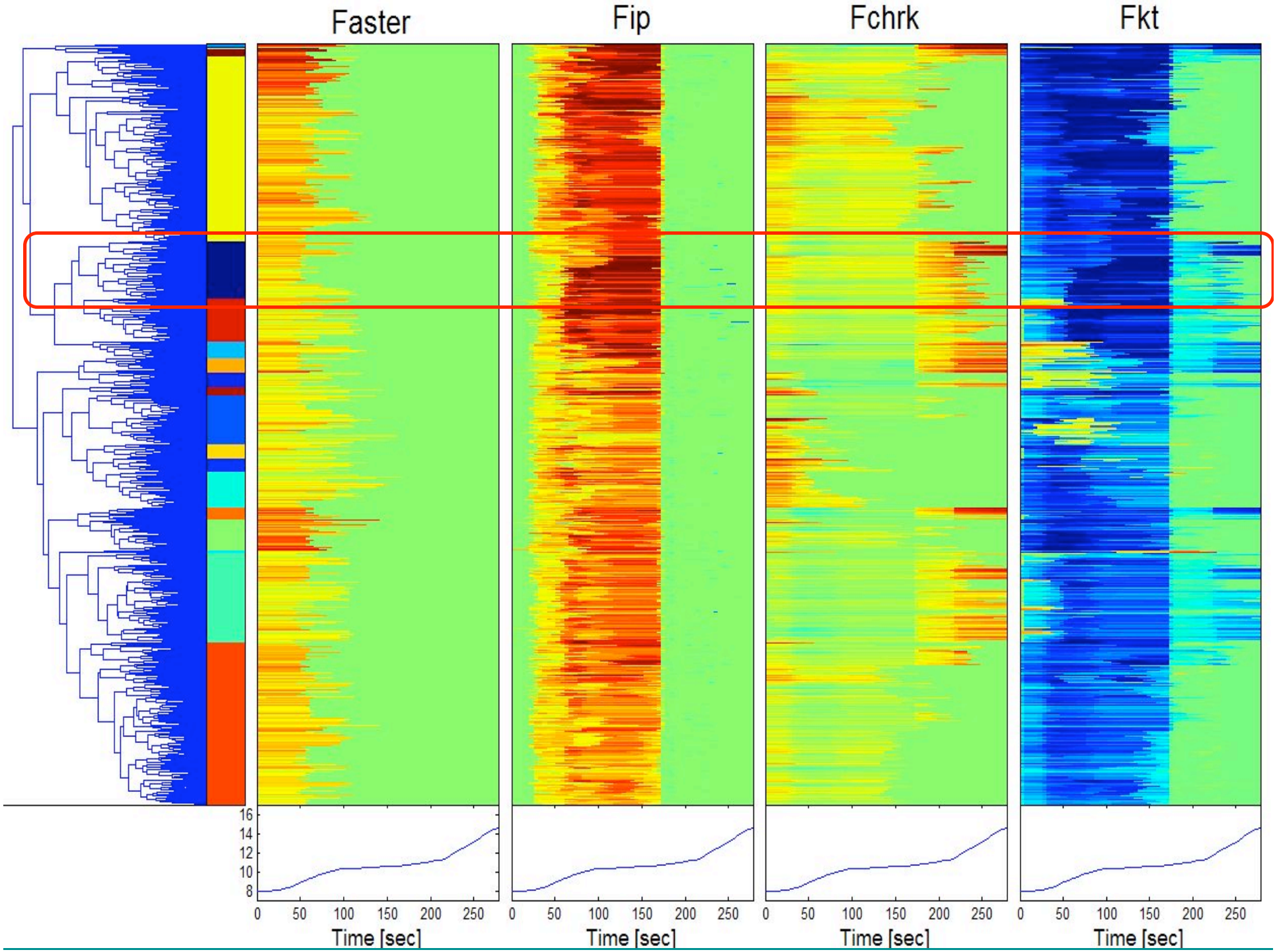
We have 10,000+ more examples…

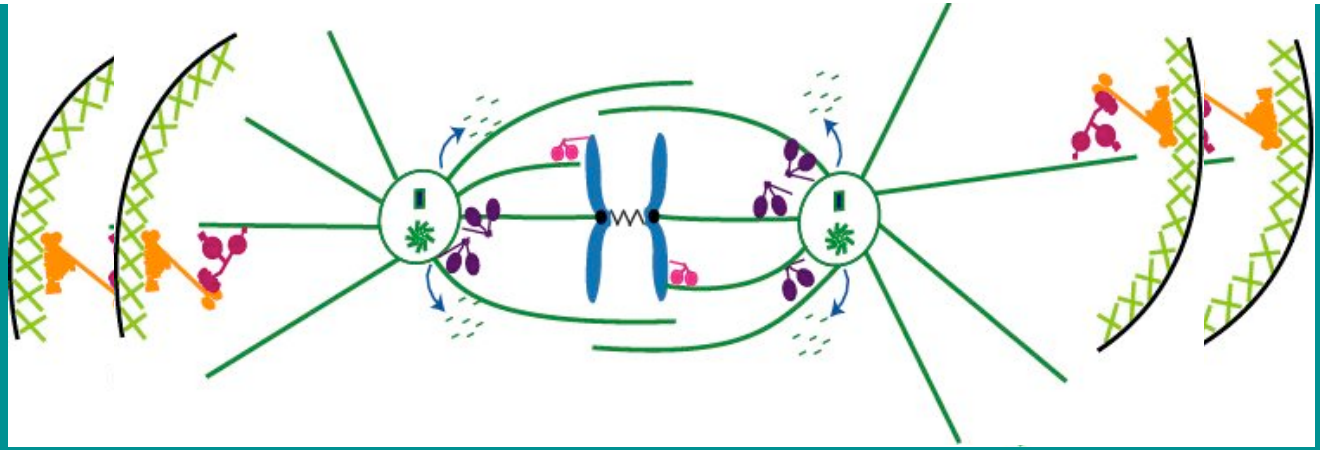
Bewildering variety of the ‘perfect’ models: switches



Bewildering variety of the ‘perfect’ models: forces

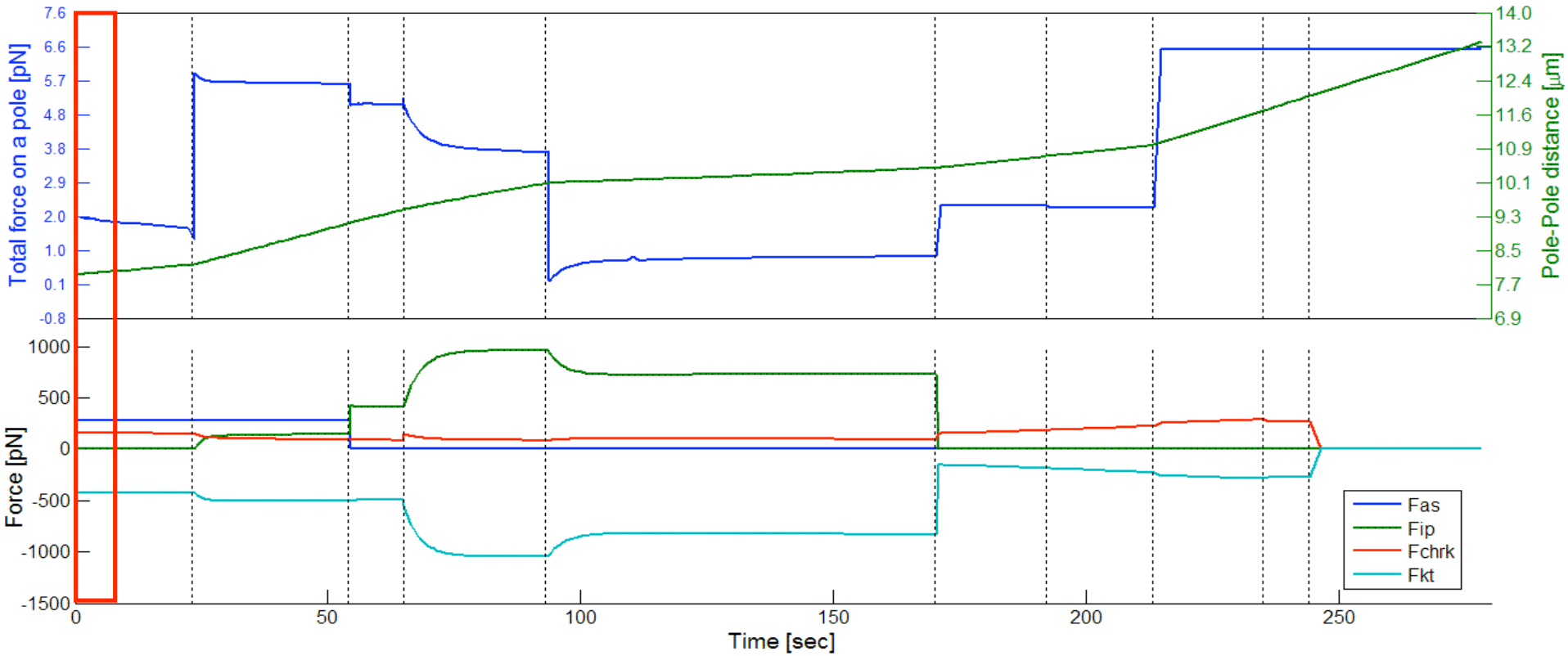


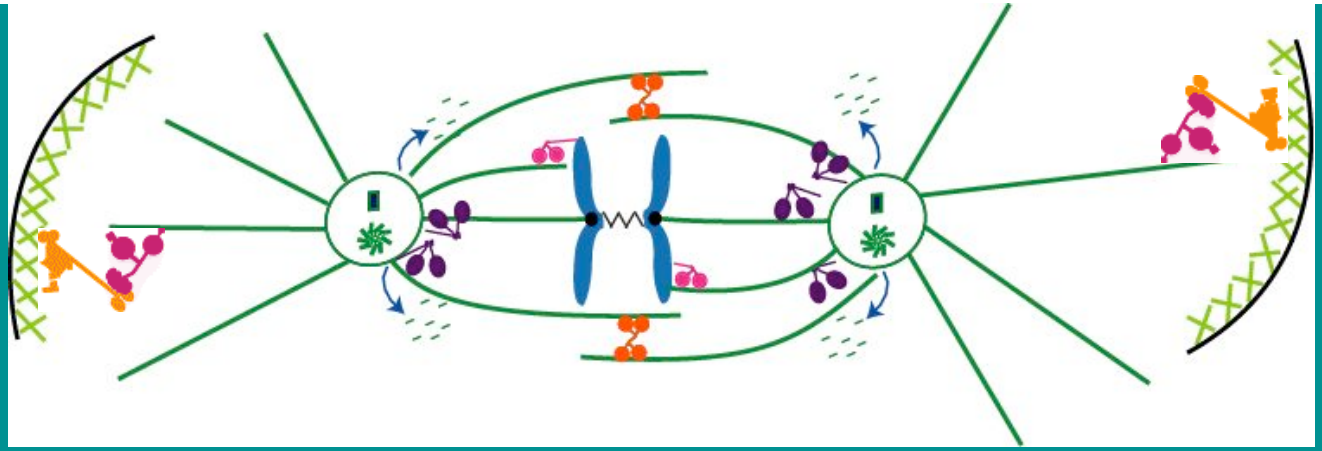

Faster
Fip
Fchrk
Fkt
16
14
12
10
8
0
50
100
150
200
250
Time [sec]



Outwards: Dynein, Chr-Arms
Kinesin-5

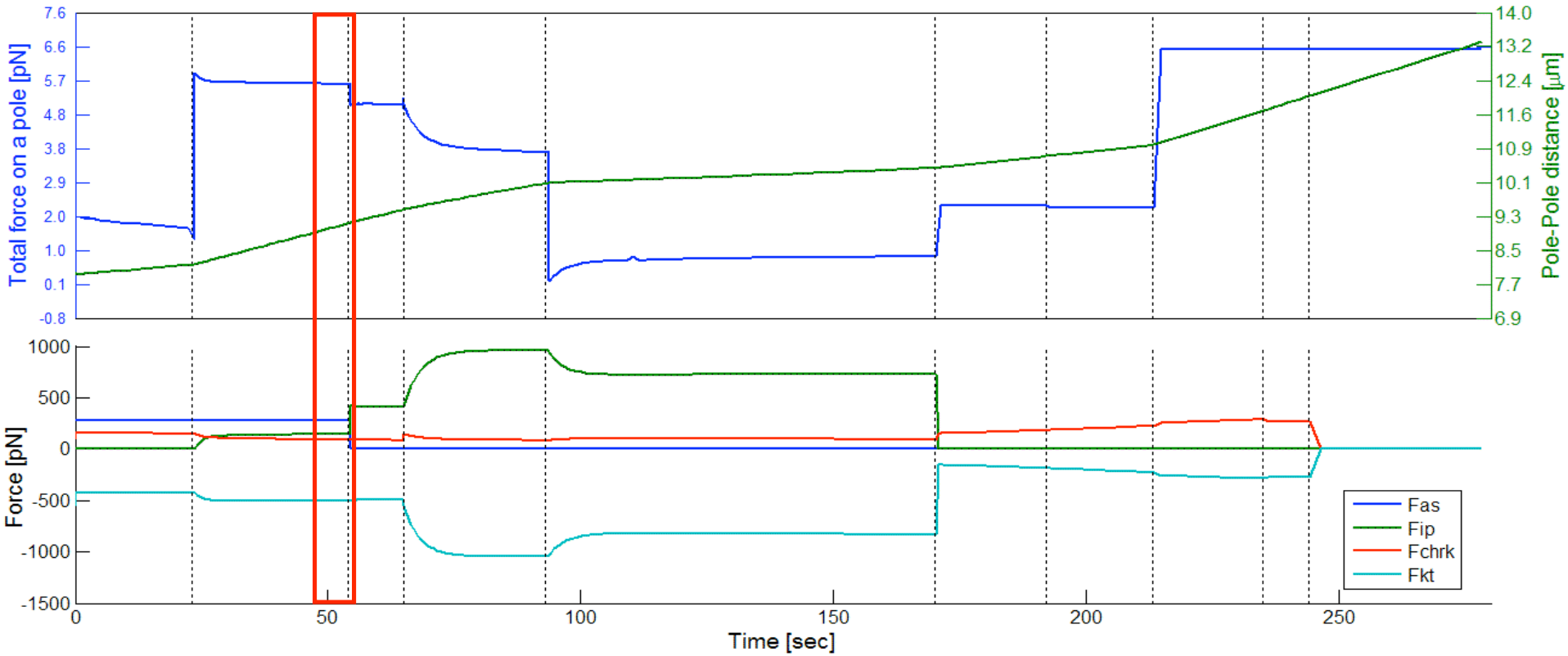
Inwards: Kinesin-13

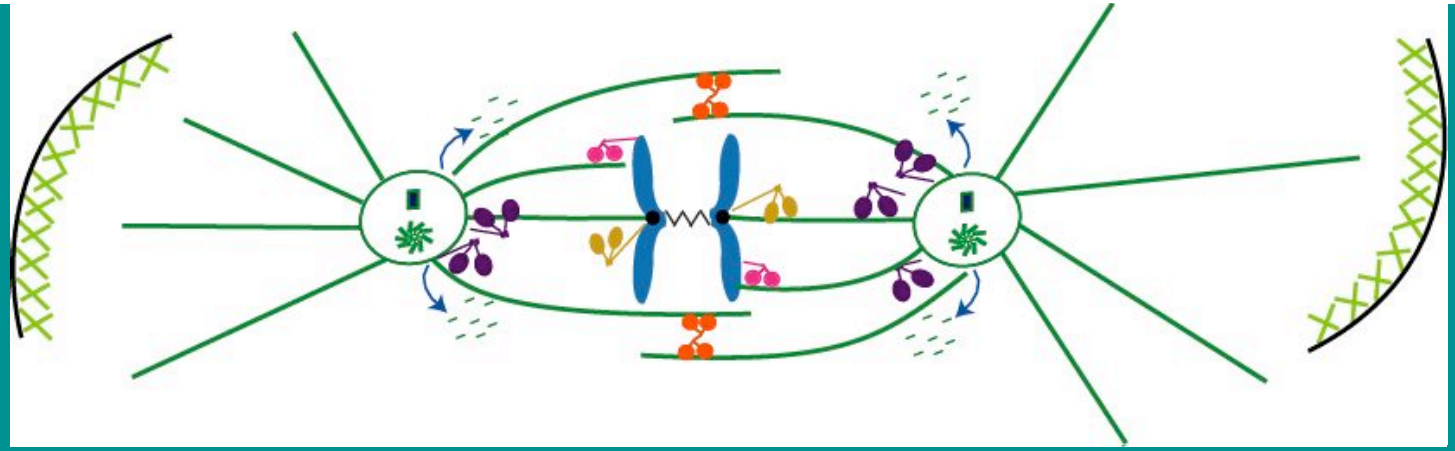




Outwards: Dynein, Chr, Arms Kinesin-5

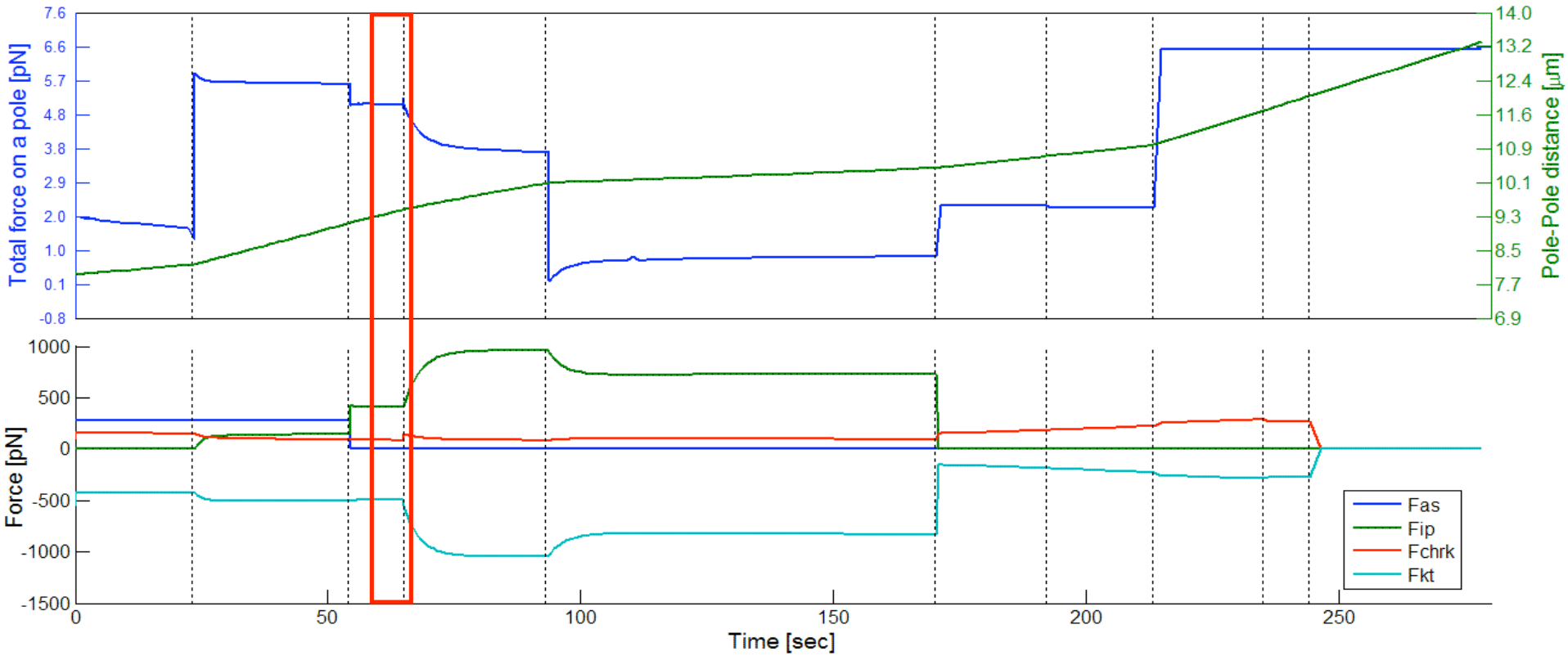
Inwards: Kinesin-13

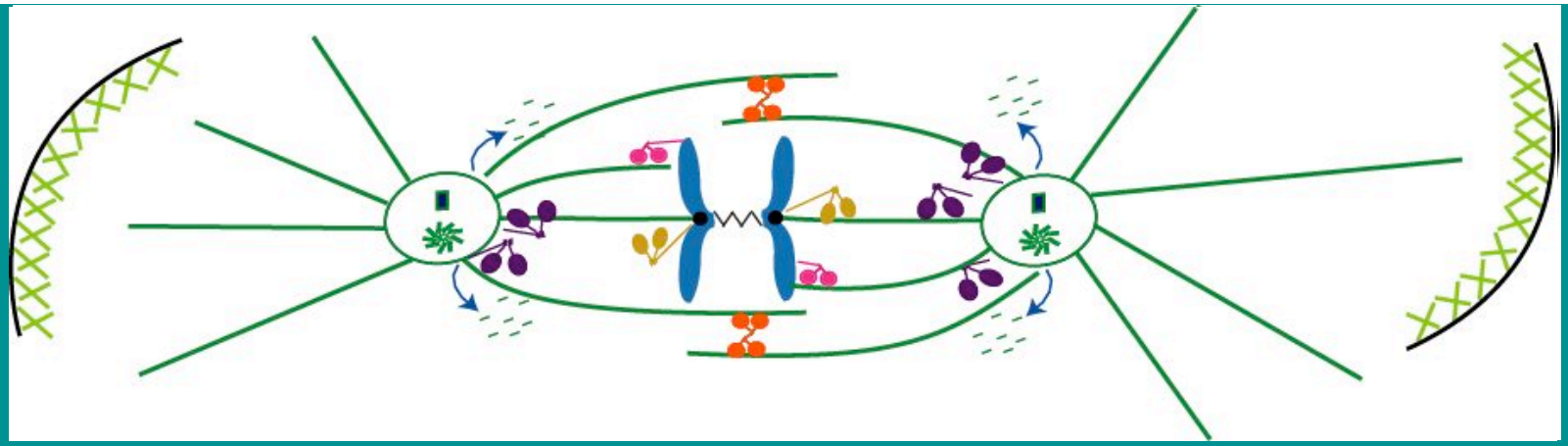




Outwards: Chr-Arms
Kinesin-5

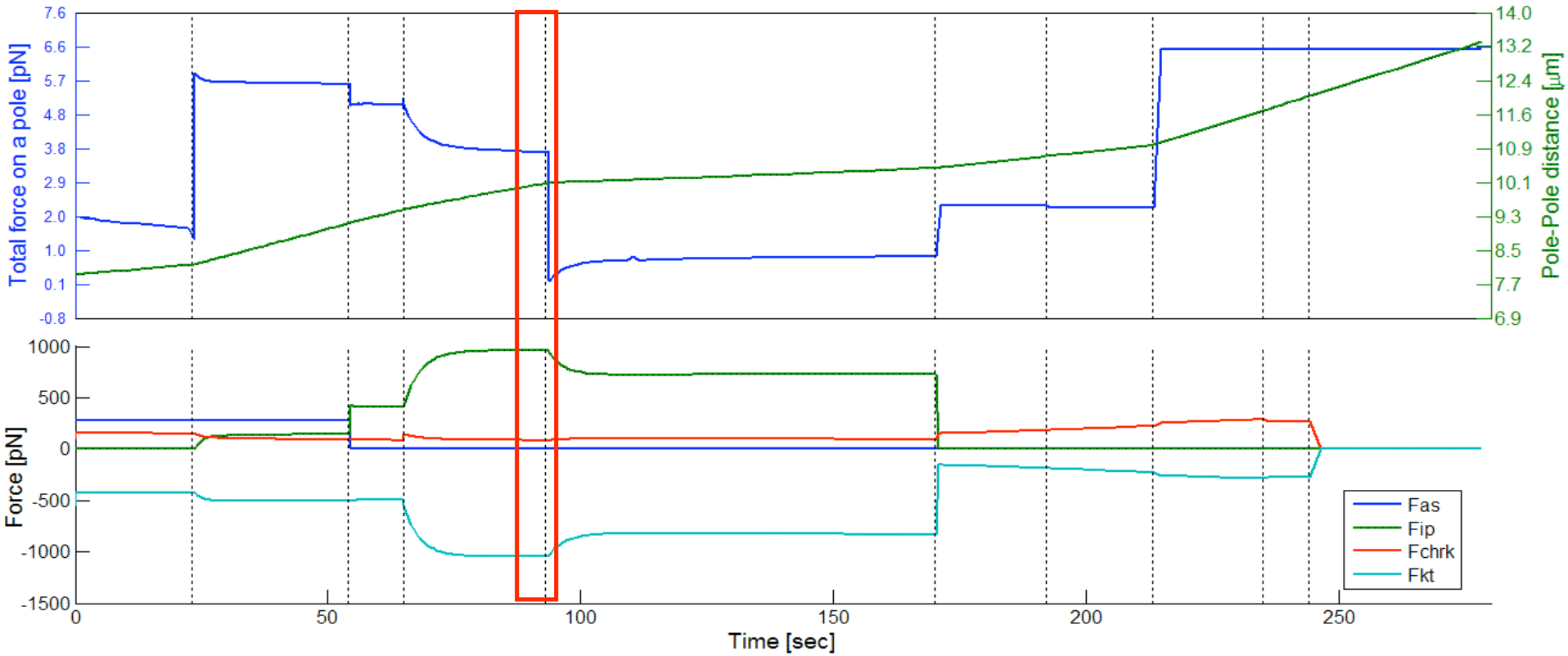
Inwards: Kinesin-13, KT-dep

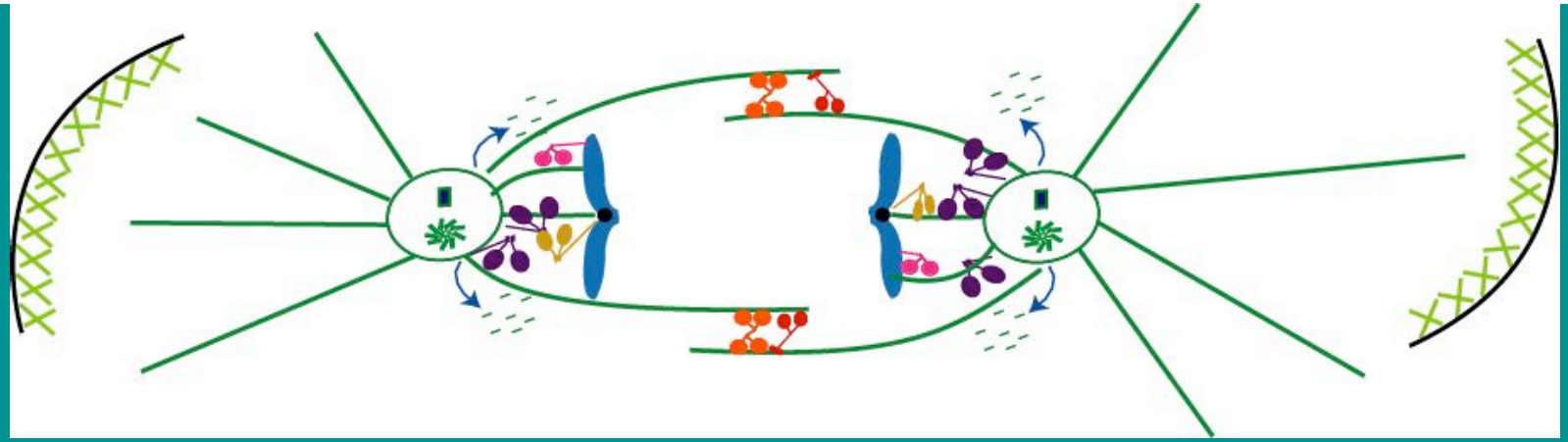




Outwards: Chr-Arms
Kinesin-5

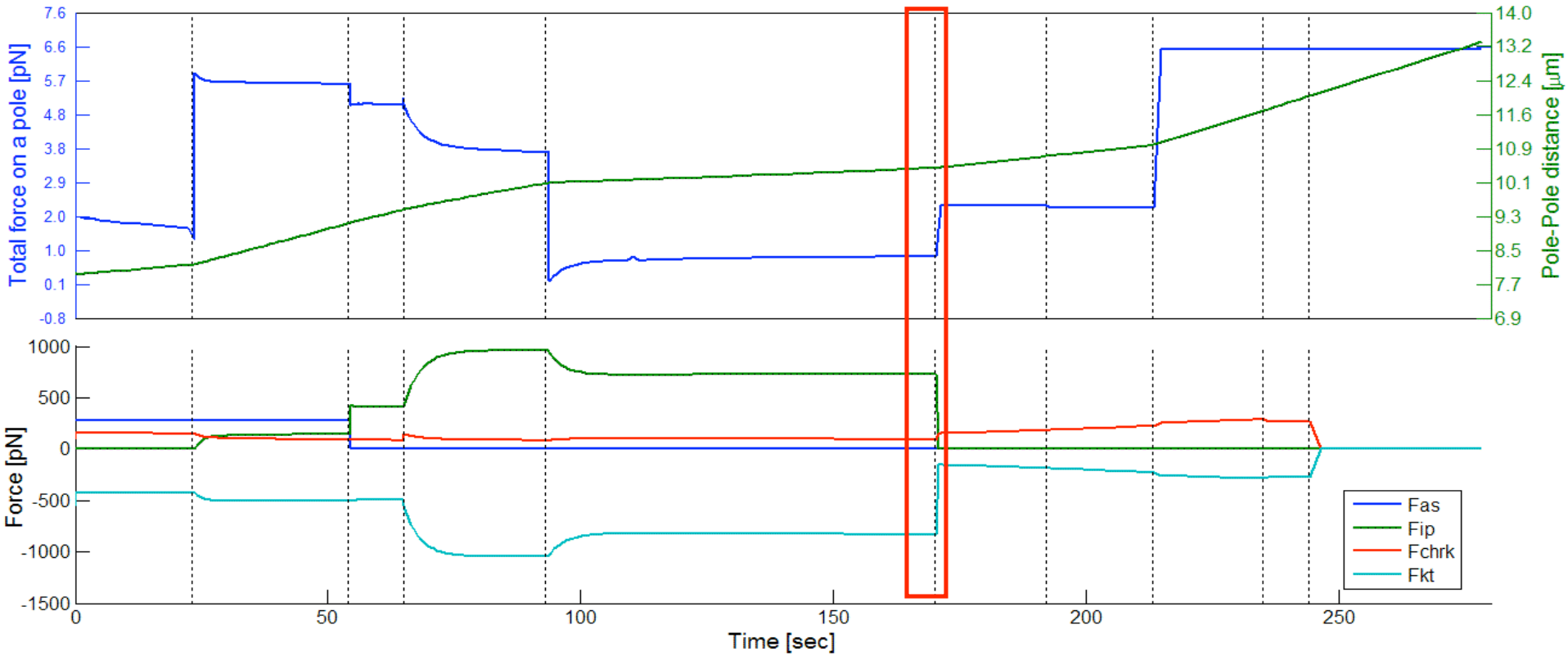
Inwards: Kinesin-13, KT-dep
Kinesin-14

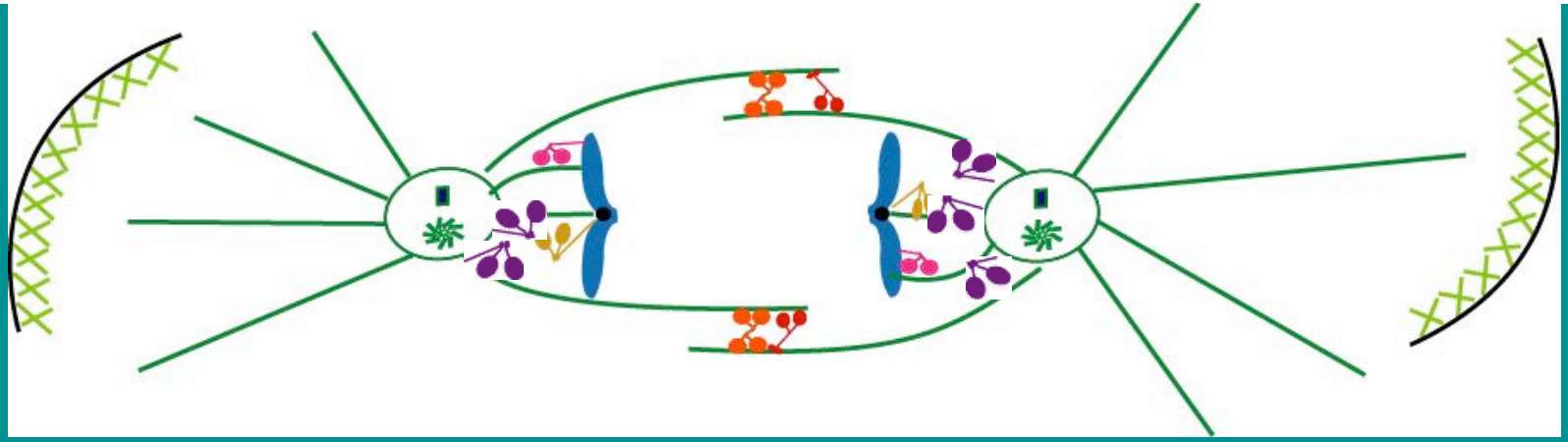




Outwards: Chr-Arms, Kinesin-5

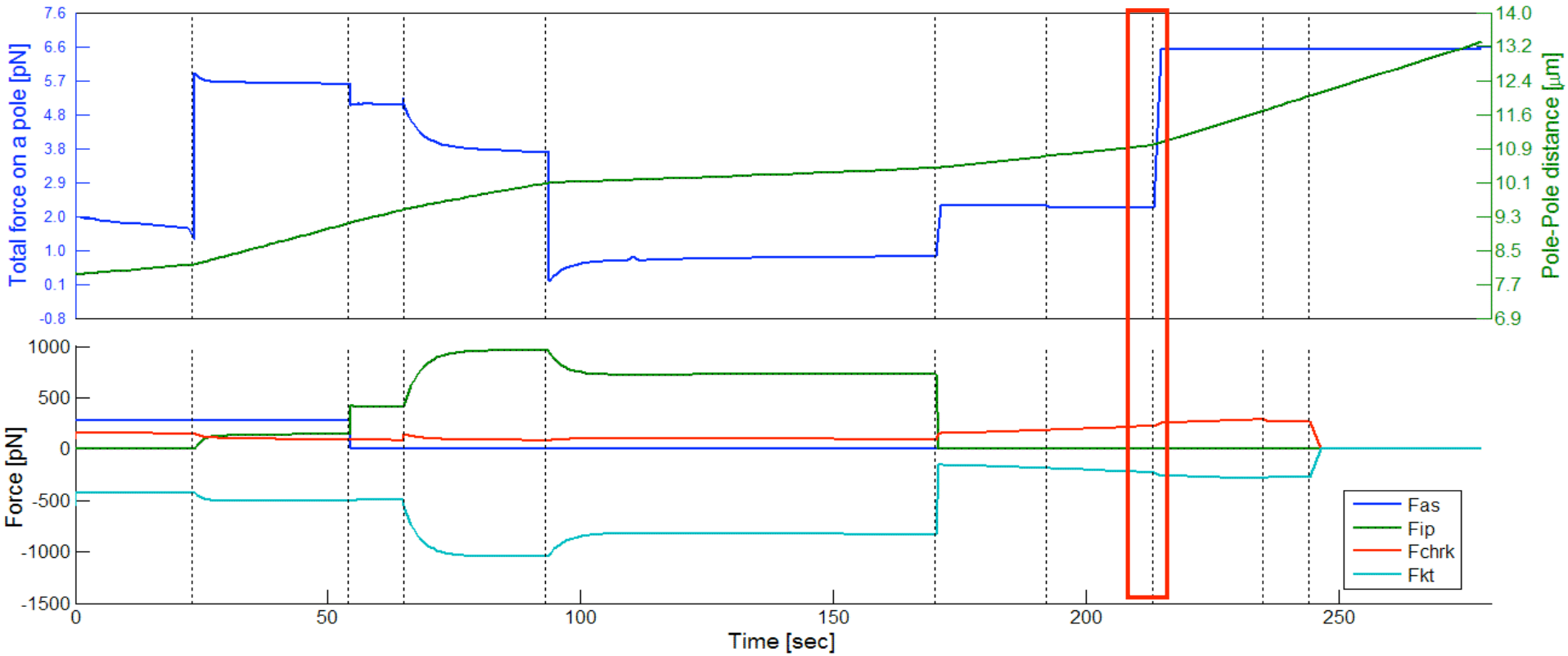
Inwards: Kinesin-13, KT-dep, Kinesin-14

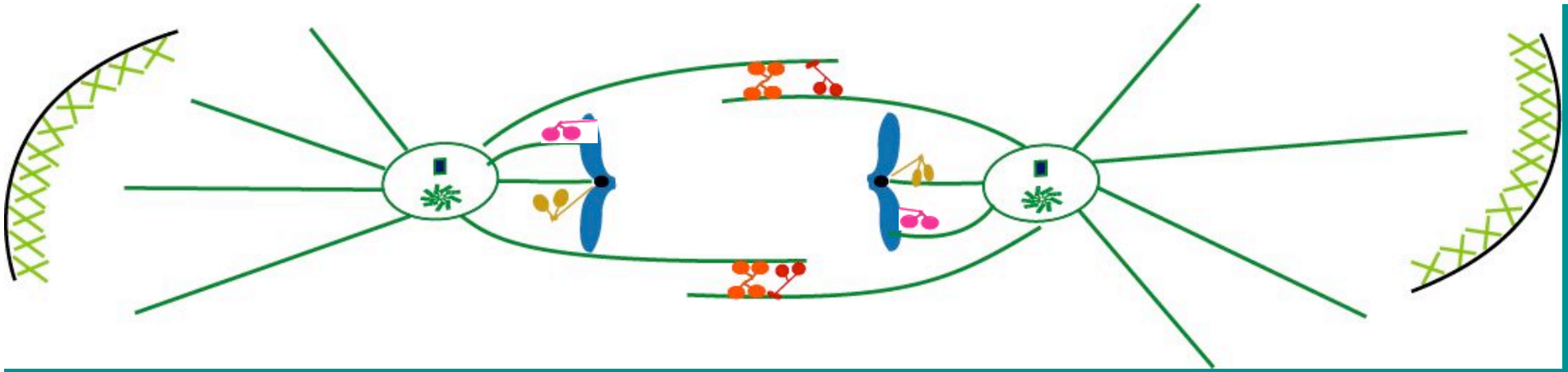




Outwards: Chr-Arms Kinesin-5

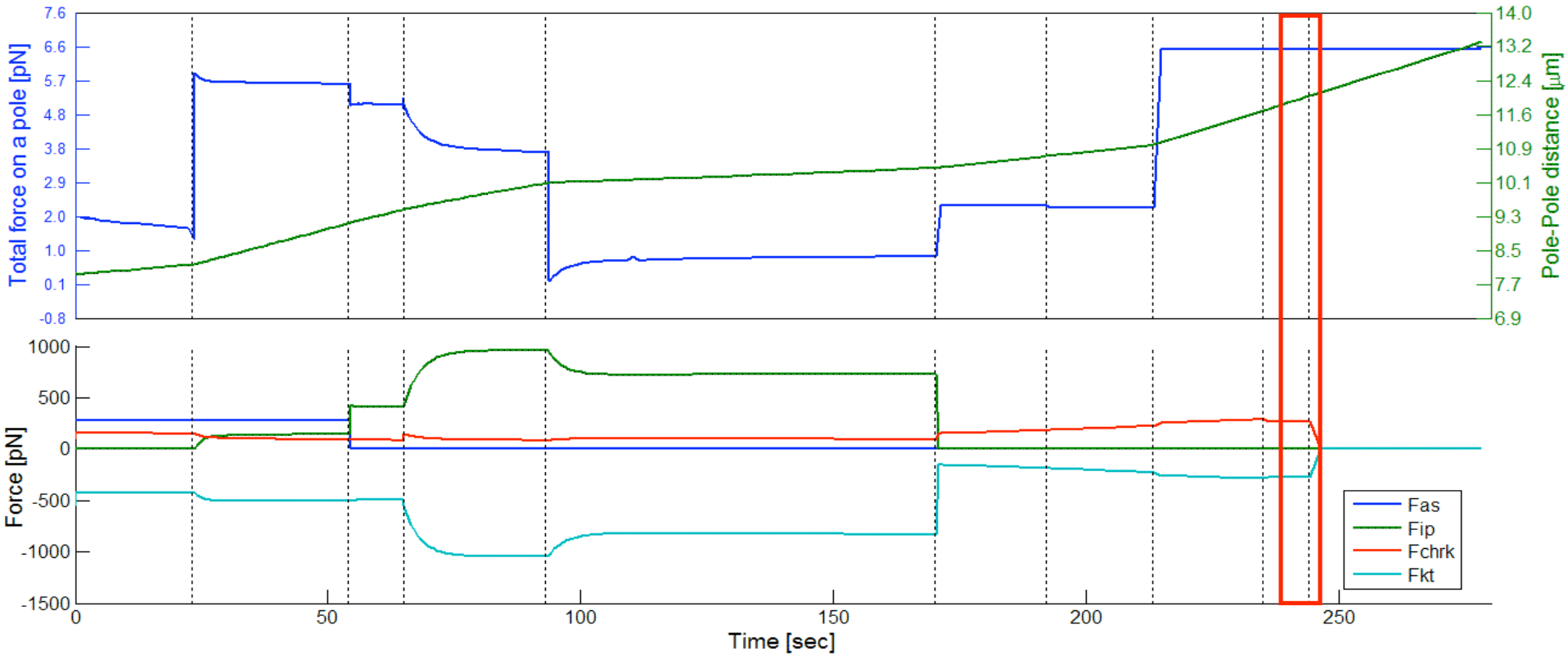
Inwards: Kinesin-13, KT-dep Kinesin-14



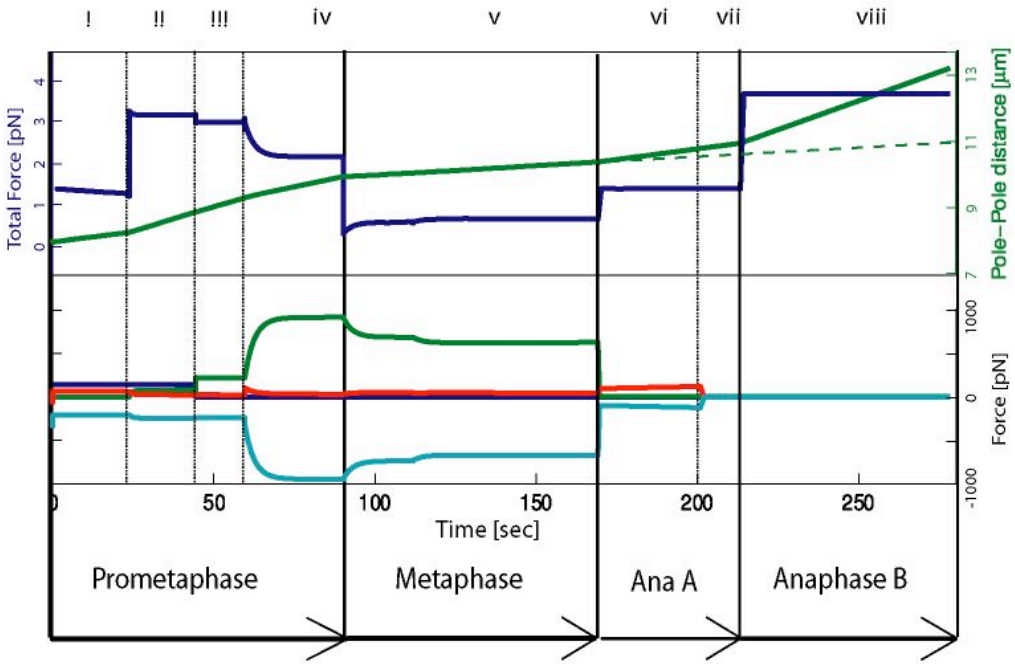


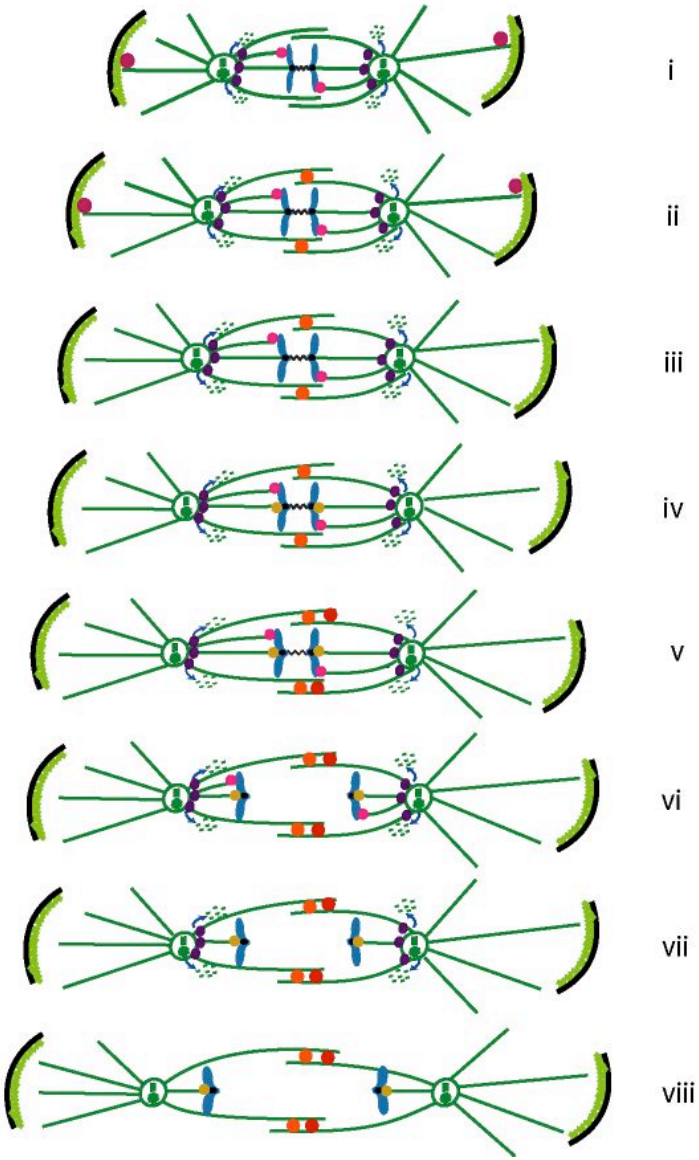
Outwards: Chr-Arms, Kinesin-5

Inwards: KT-dep, Kinesin-14



Final Model

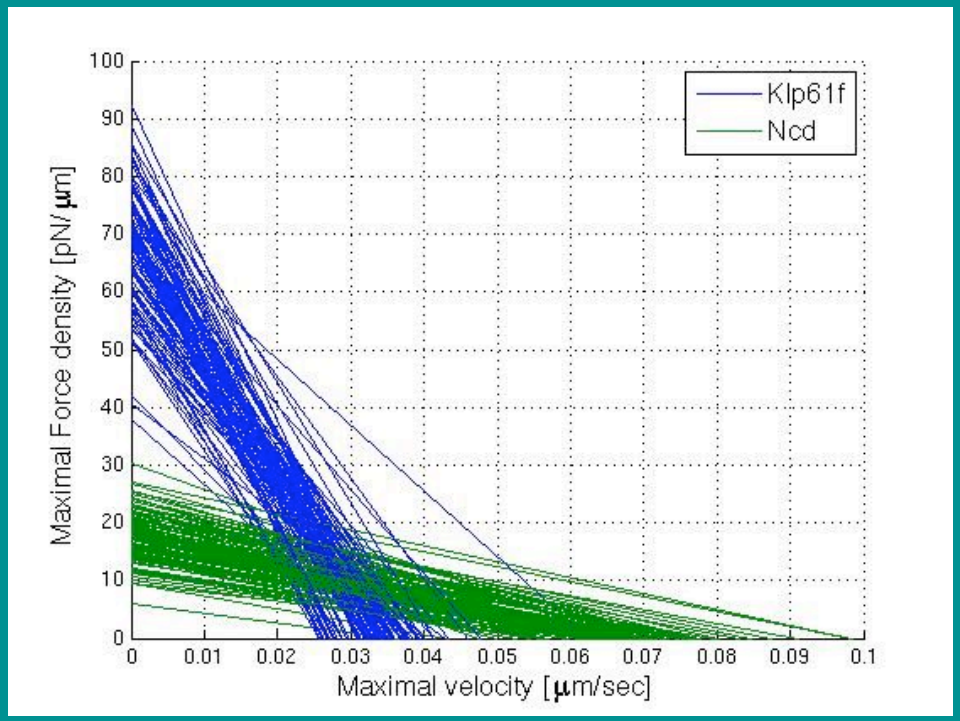




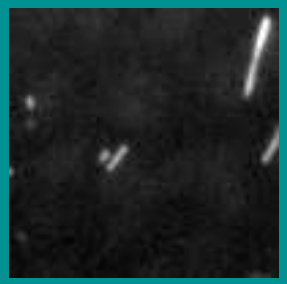
Key
- Kinesin-5
- Kinesin-14
- Chrk-5
- Dynein
- Kt-depoly
- Kinesin-13

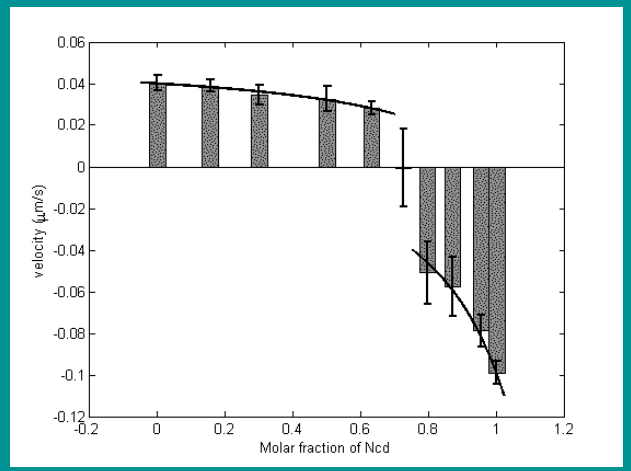
Model prediction:
estimates of the mechanical parameters

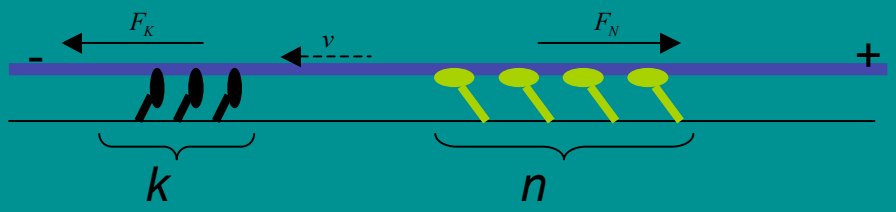
Kinetic / mechanic properties of participating motors



In agreement with
in vitro biochemical study:







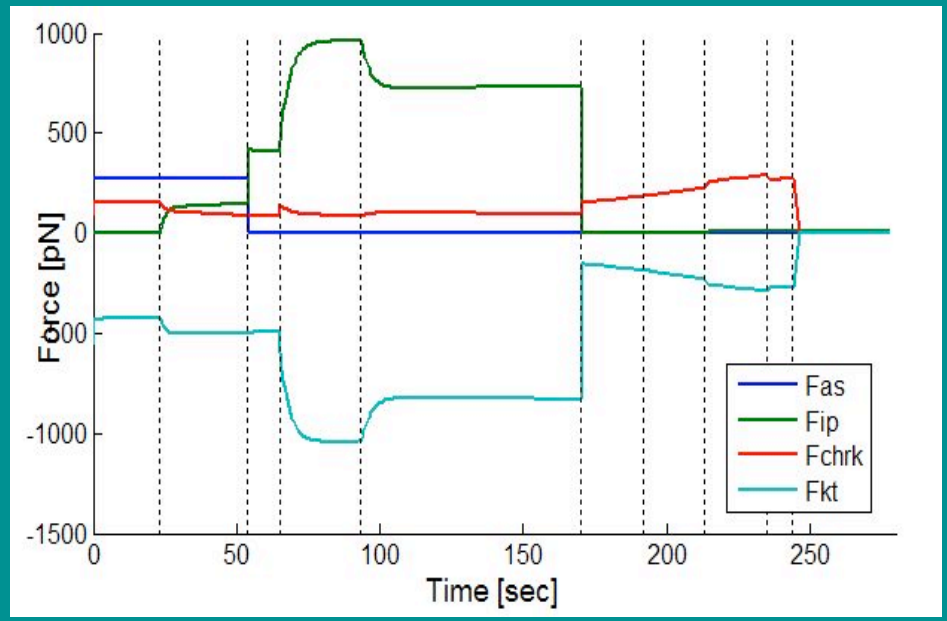
Design principle: Balance of large forces

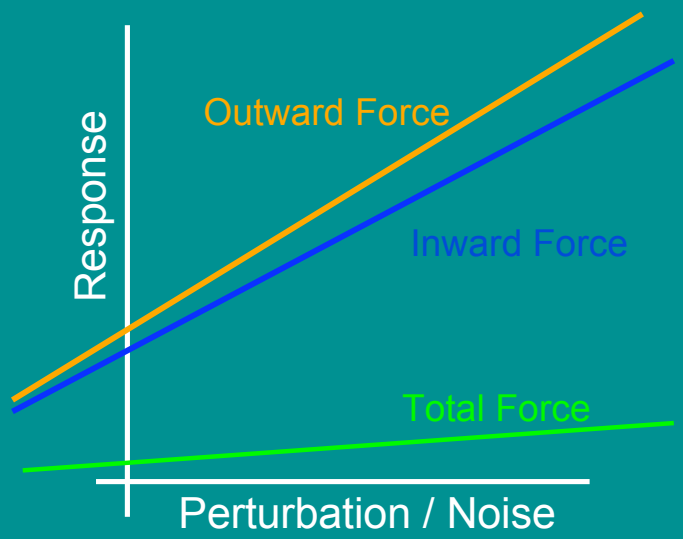
$$\mu_{pole}\frac{dS}{dt} = 2\sum_{Pole} F$$

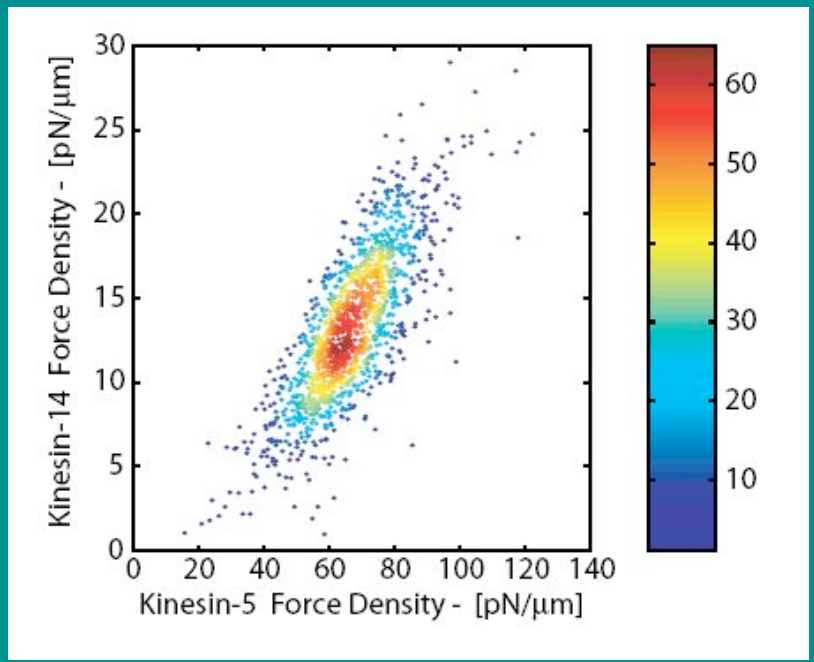
$$\mu_{chr}\frac{dD}{dt} = 2\sum_{Chr} F$$

Typical drag ~ 100 [pN sec/μm]

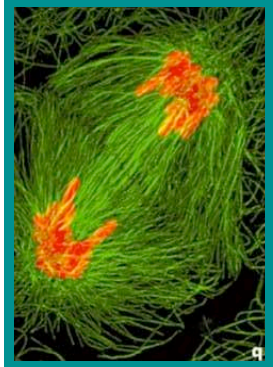
$$\frac{dS}{dt} \sim 0.03\left[\mu m/\sec\right] \Rightarrow \sum_{Pole} F \sim 1-10\left[pN\right]$$



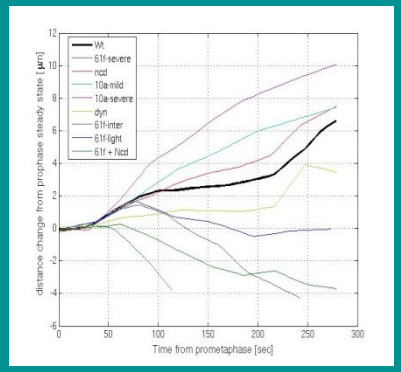




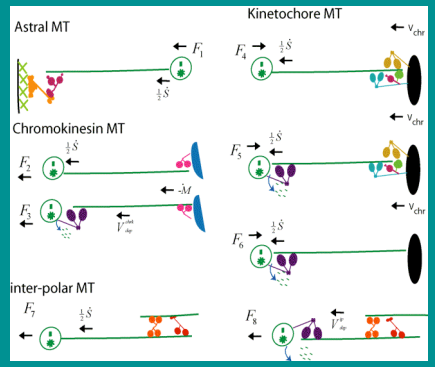
General paradigm for system level analysis of mechanical (and other) systems



Biological problem



Global quantitative measurements

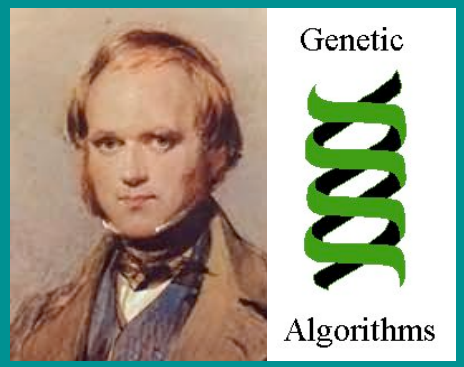


Detailed mechanistic description of **multiple possibilities**

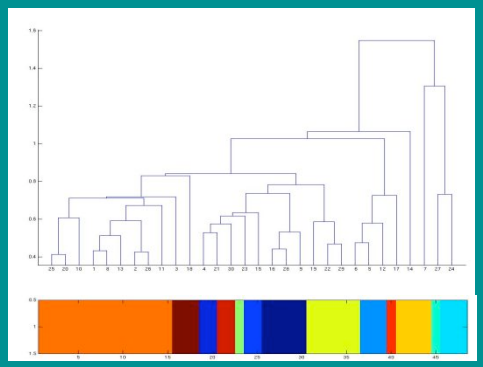
$$\frac{dS}{dt} = \frac{2\left(F_{ip} + F_{chrk} + F_{aster} - F_{kt}\right)}{\mu_{pole}}$$

$$\frac{dL}{dt} = 2V_{ploy}^{ip} - 2\left(\left(1 - P_{dep}\right)V_{pole} + P_{dep}V_{MT}^{ip}\right)$$

Mathematical description



Repeated Stochastic optimization



Clustering / Unsupervised learning

Back to the biology

General paradigm for system level analysis of mechanical (and other) systems

Biological problem

Global quantitative measurements

Detailed mechanistic description of **multiple possibilities**

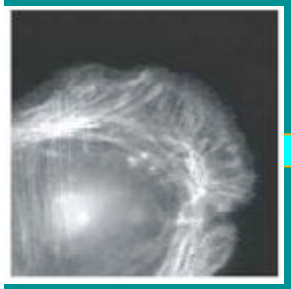
Mathematical description

Repeated Stochastic optimization

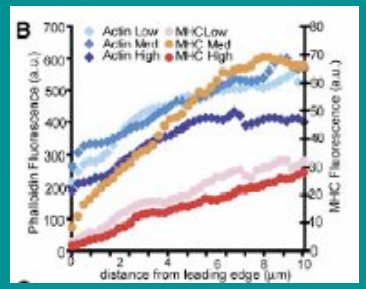
Clustering / Unsupervised learning

Back to the biology

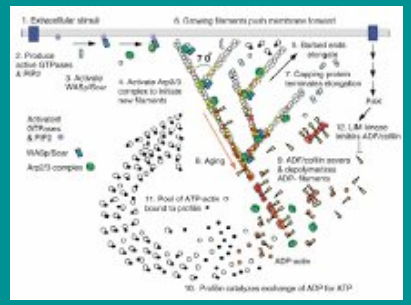
General paradigm for system level analysis of mechanical (and other) systems



Biological problem



Global quantitative measurements



Detailed mechanistic description of **multiple possibilities**

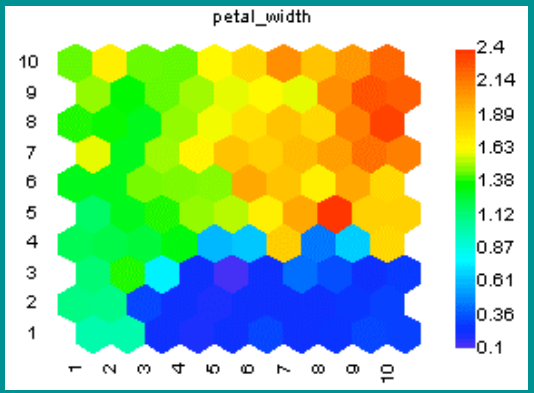
$$\frac{\partial a}{\partial t} = \tilde{D}\frac{\partial^2 a}{\partial x^2} - \frac{\partial}{\partial x}\left[\left(\frac{dL}{dt}\right) a\right]$$

$$a(0)=1, \quad \frac{\partial a}{\partial x}\Big|_{x=L(t)} = -a(L)$$

$$\frac{dL}{dt} = (e^{-N_0/N} a(L) - \cos\theta_c).$$

Mathematical description

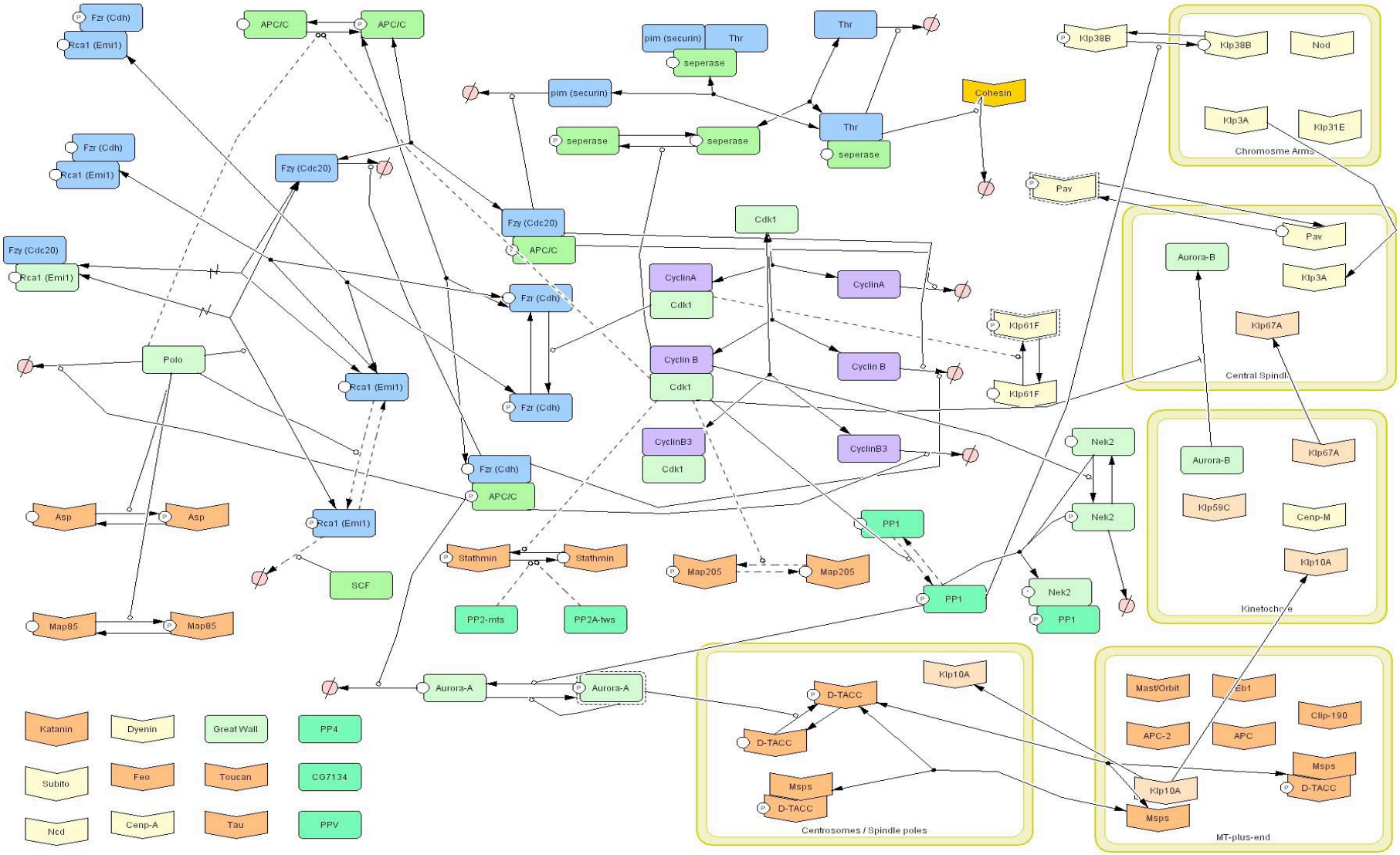
Repeated Stochastic optimization



Clustering / Unsupervised learning

Back to the biology

Future challenge: add biochemical regulation



General principles of complex systems' design:

Robustness, Redundancy

Open system, consuming lots of energy

Multi-objective optimization: speed and accuracy

Inter-connectedness, impermanence