Organelle Size Control Systems

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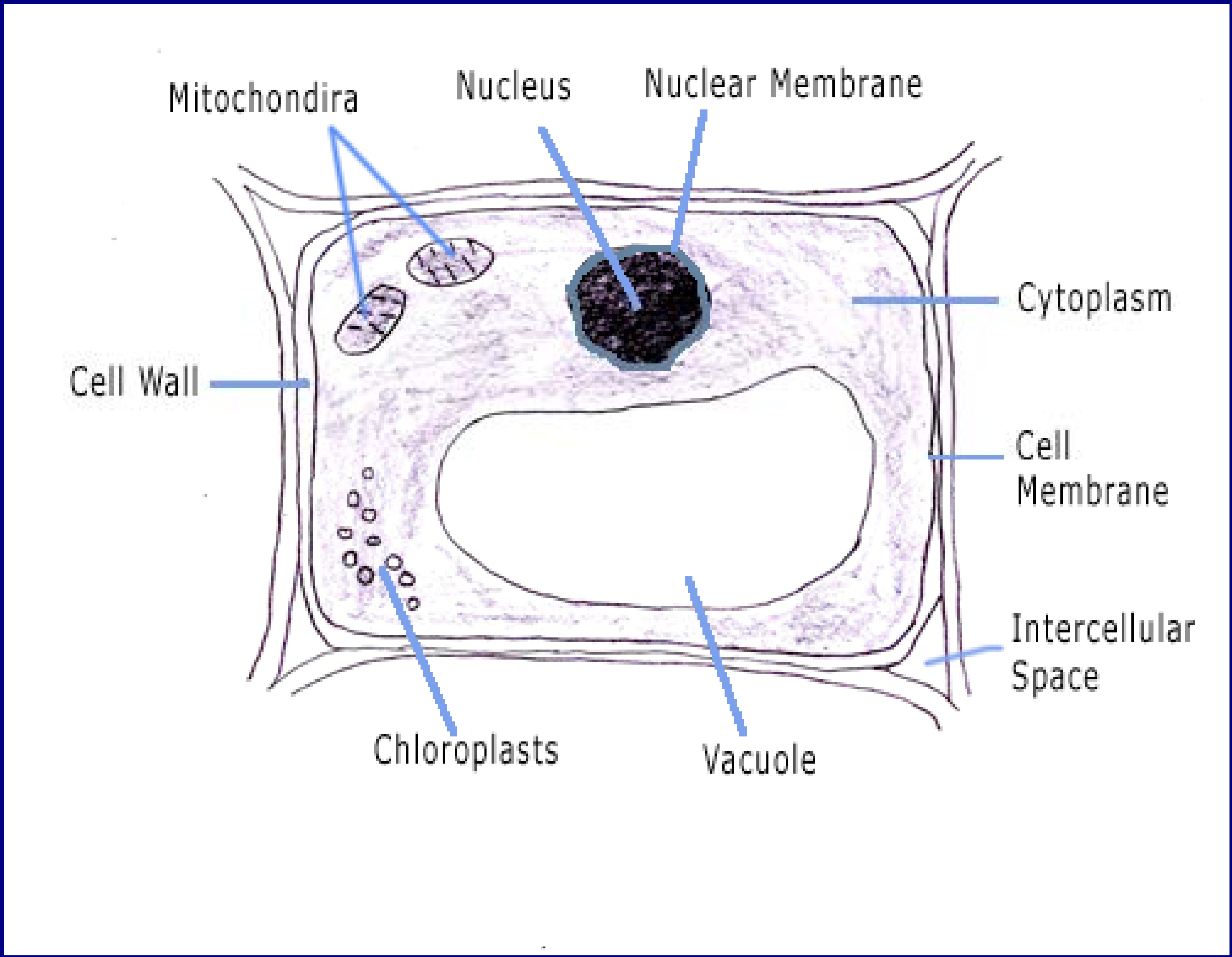
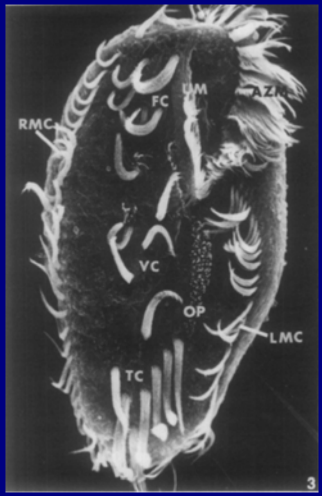
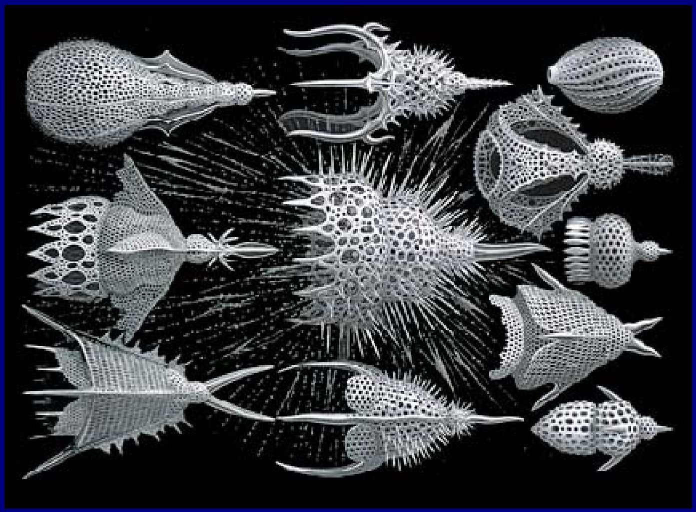
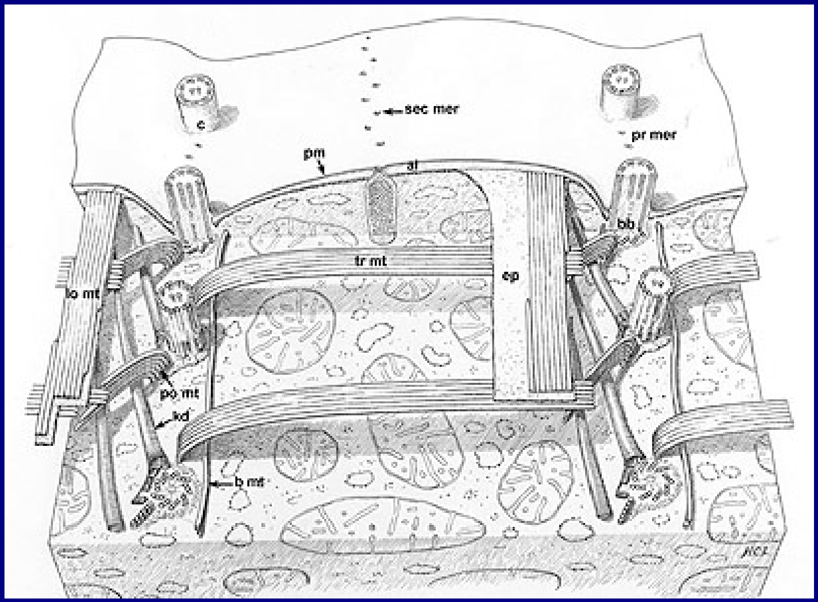
Cells aren't just bags of enzymes!



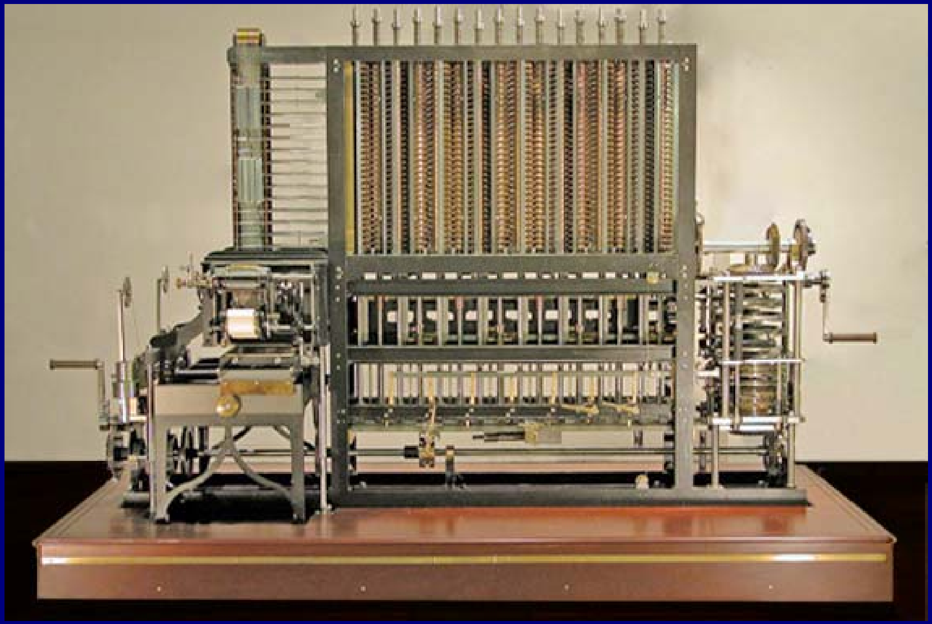
Cell are complex, precise machines



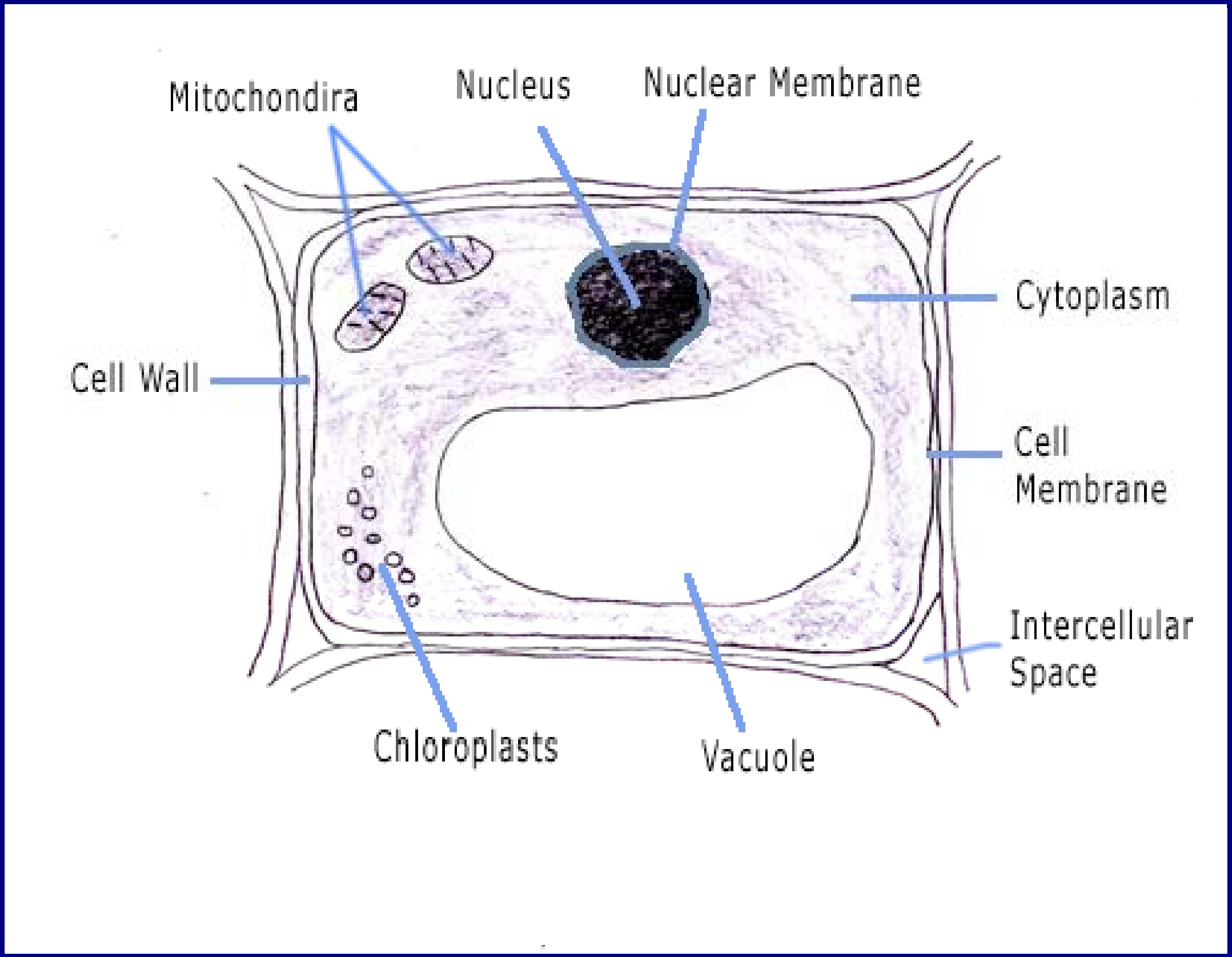




The cell as a self-organizing machine



Cell geometry at the level of organelles

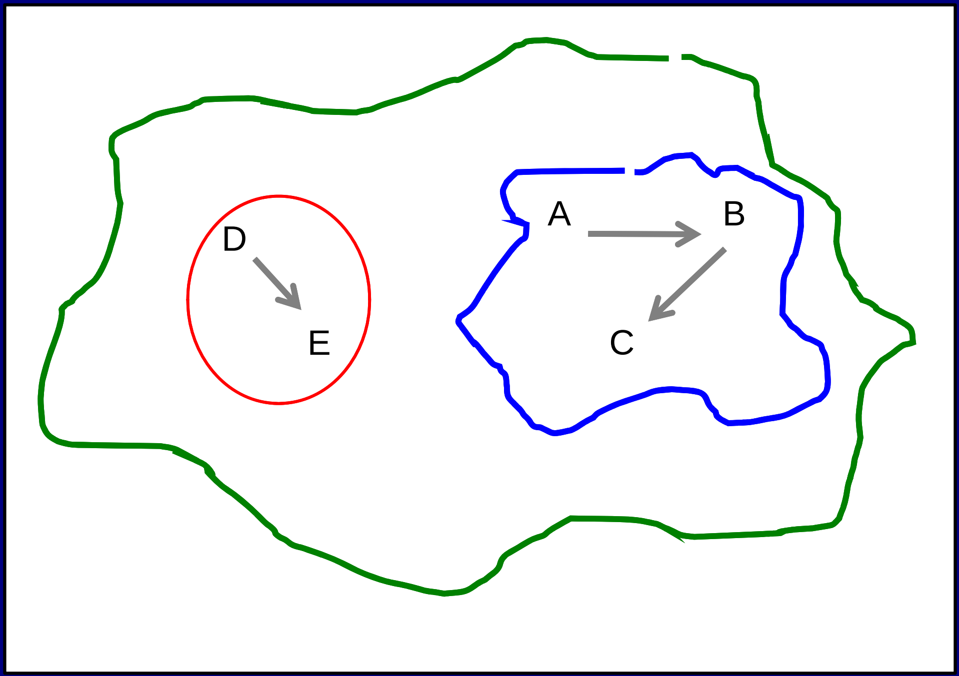


For each organelle:

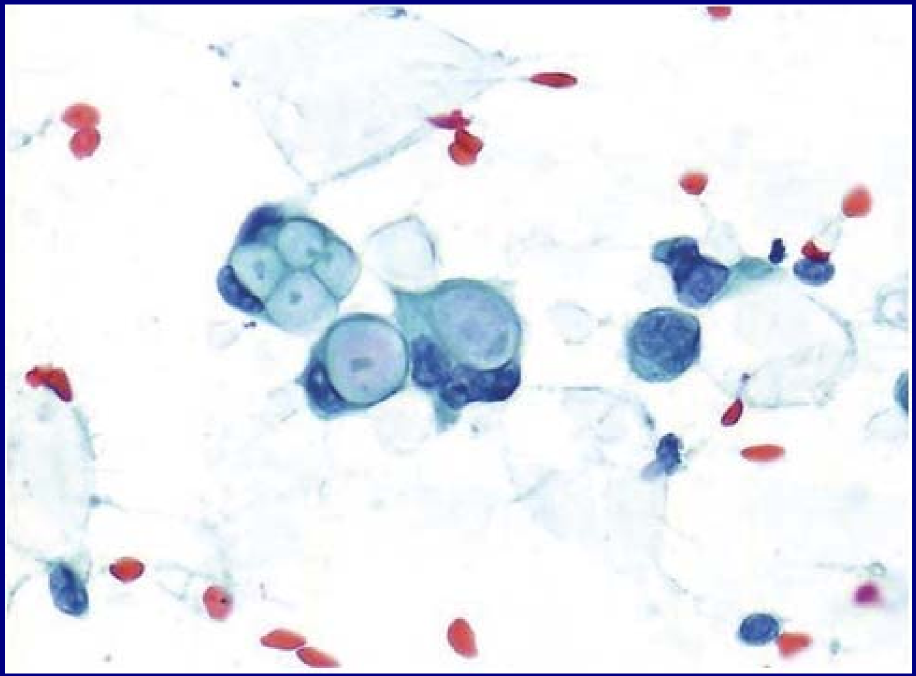
- size
- number
- position

Importance of Organelle Size

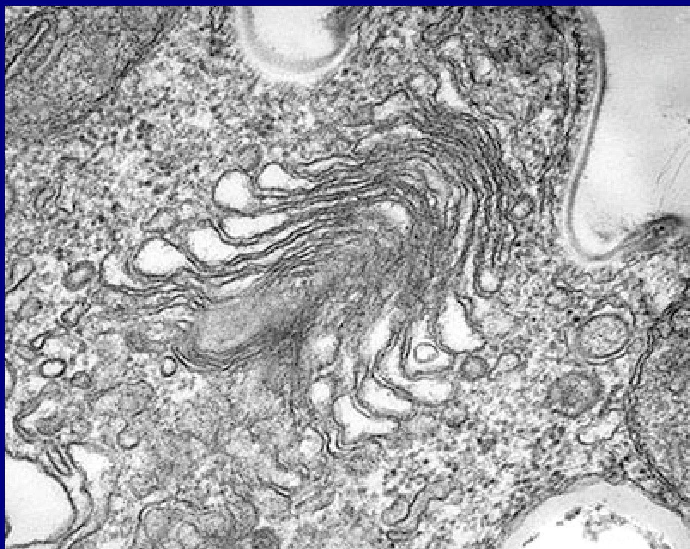
Physiological function,
Flux of intermediates



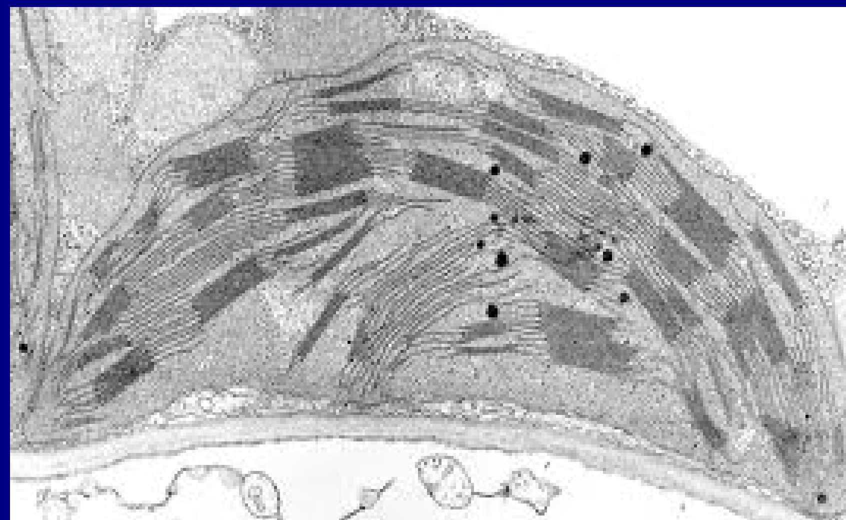
Disease



Why organelle size control is hard to study



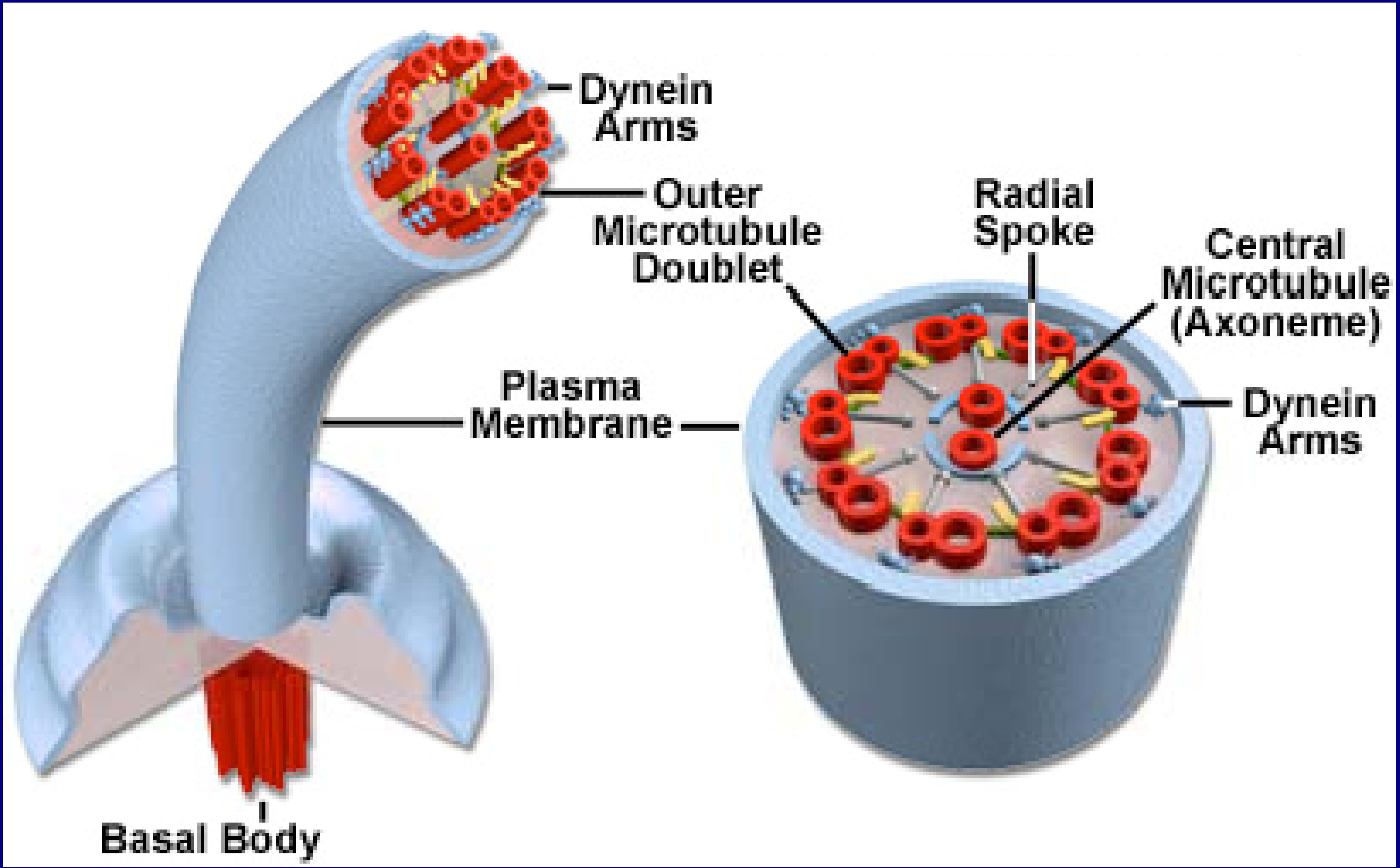
Golgi apparatus



chloroplast

We need a simpler system to study

Cilia/Flagella – a simpler organelle to study size control



Flagellar Length Control in Chlamydomonas

Flagella as model organelle

-Linear
-Easy to visualize and measure
-Number, shape, and position are constants
-same as cilia in animal cells
-size directly relates to fitness

Chlamydomonas as a model organism

-rapid growth
-yeast-like haploid genetics
-GFP, RNAi, microarrays
-genome sequence completed

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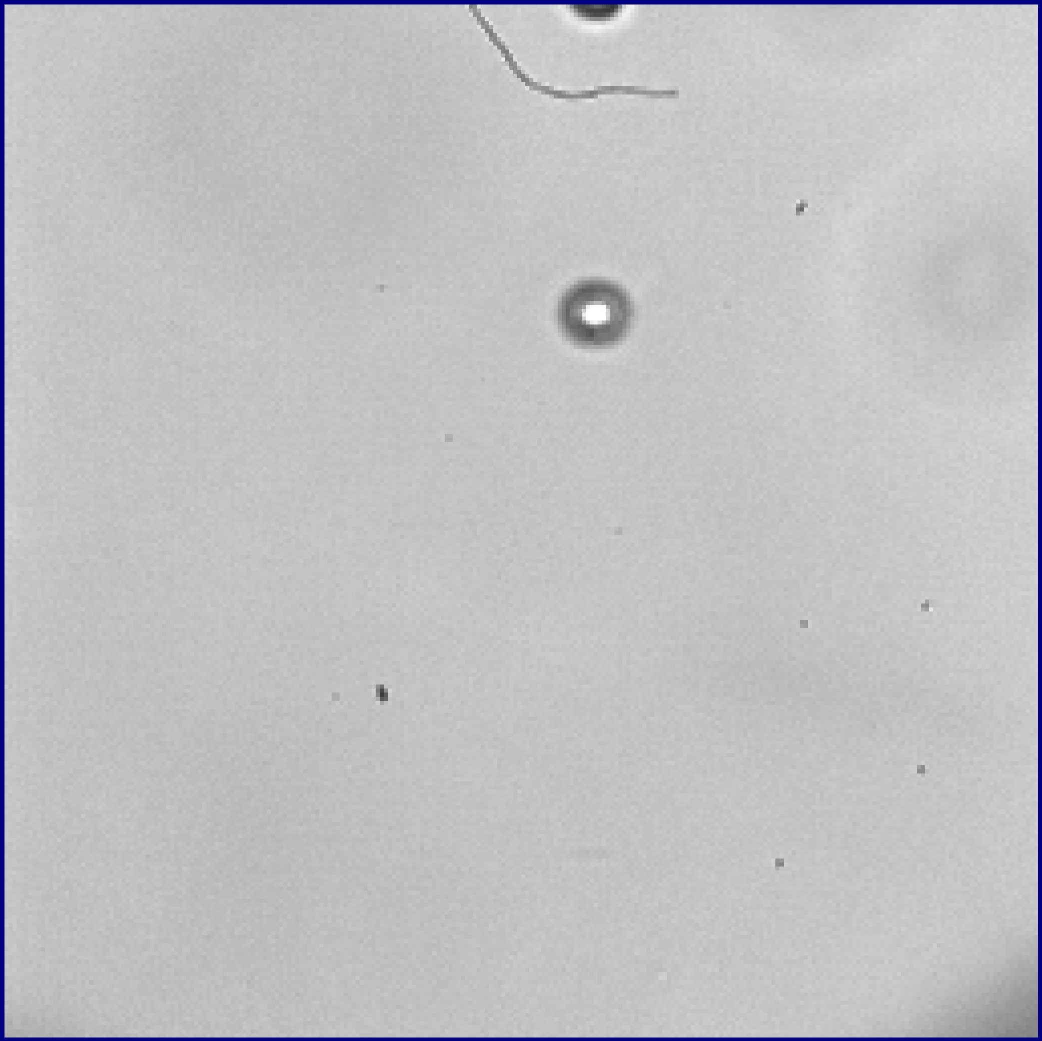
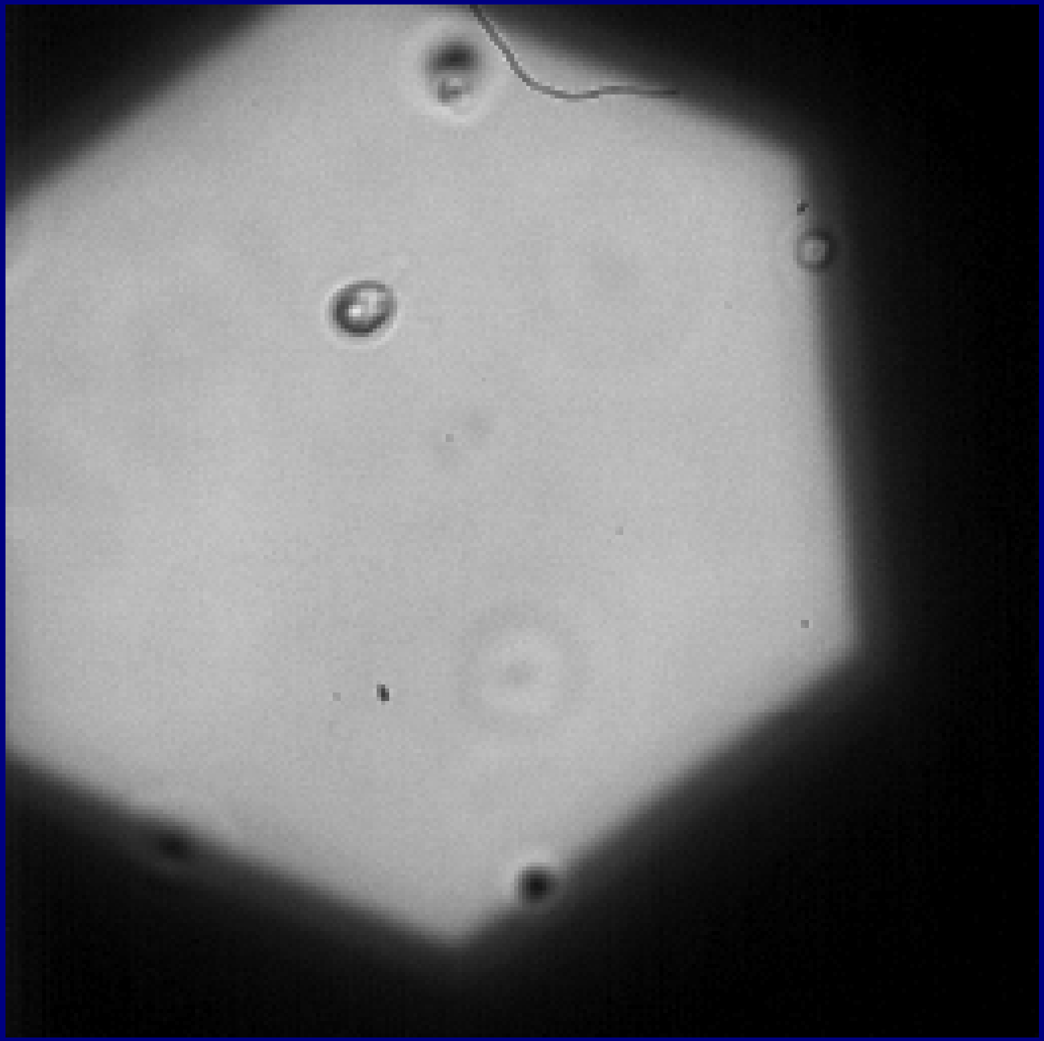
Fitness consequences of altered flagellar length

Wild-type

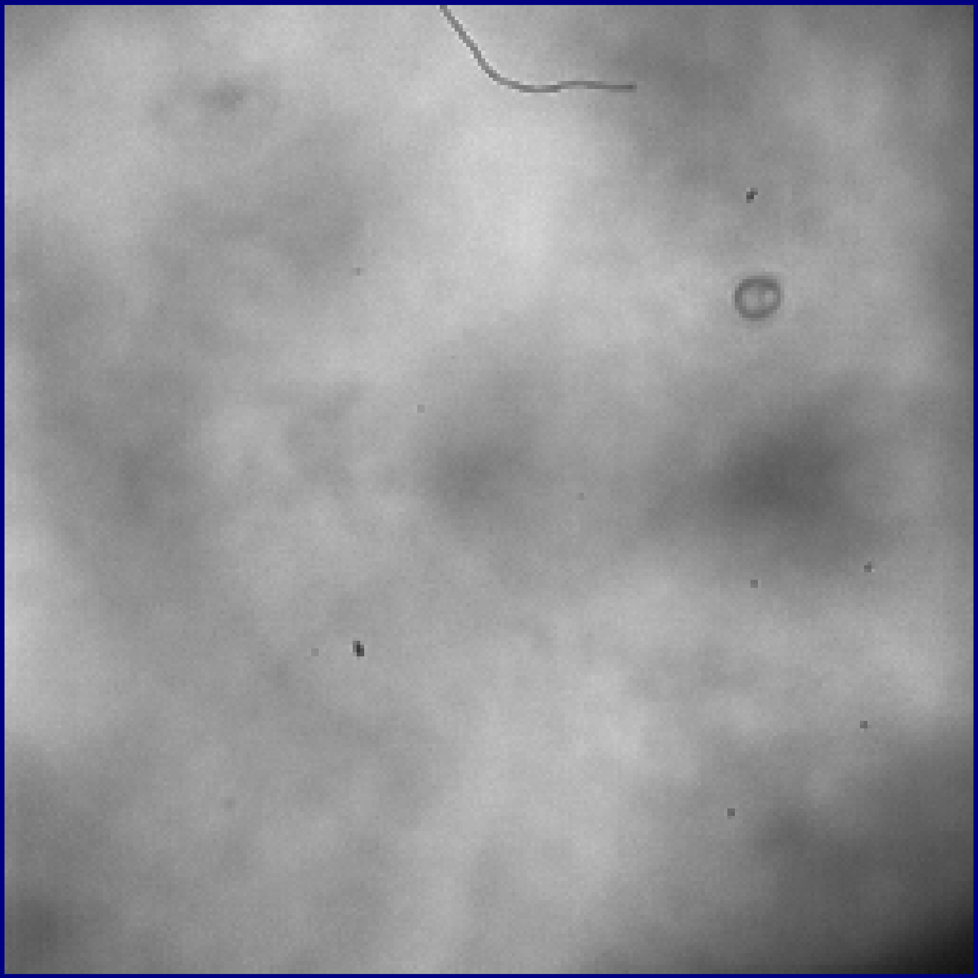


Long flagella



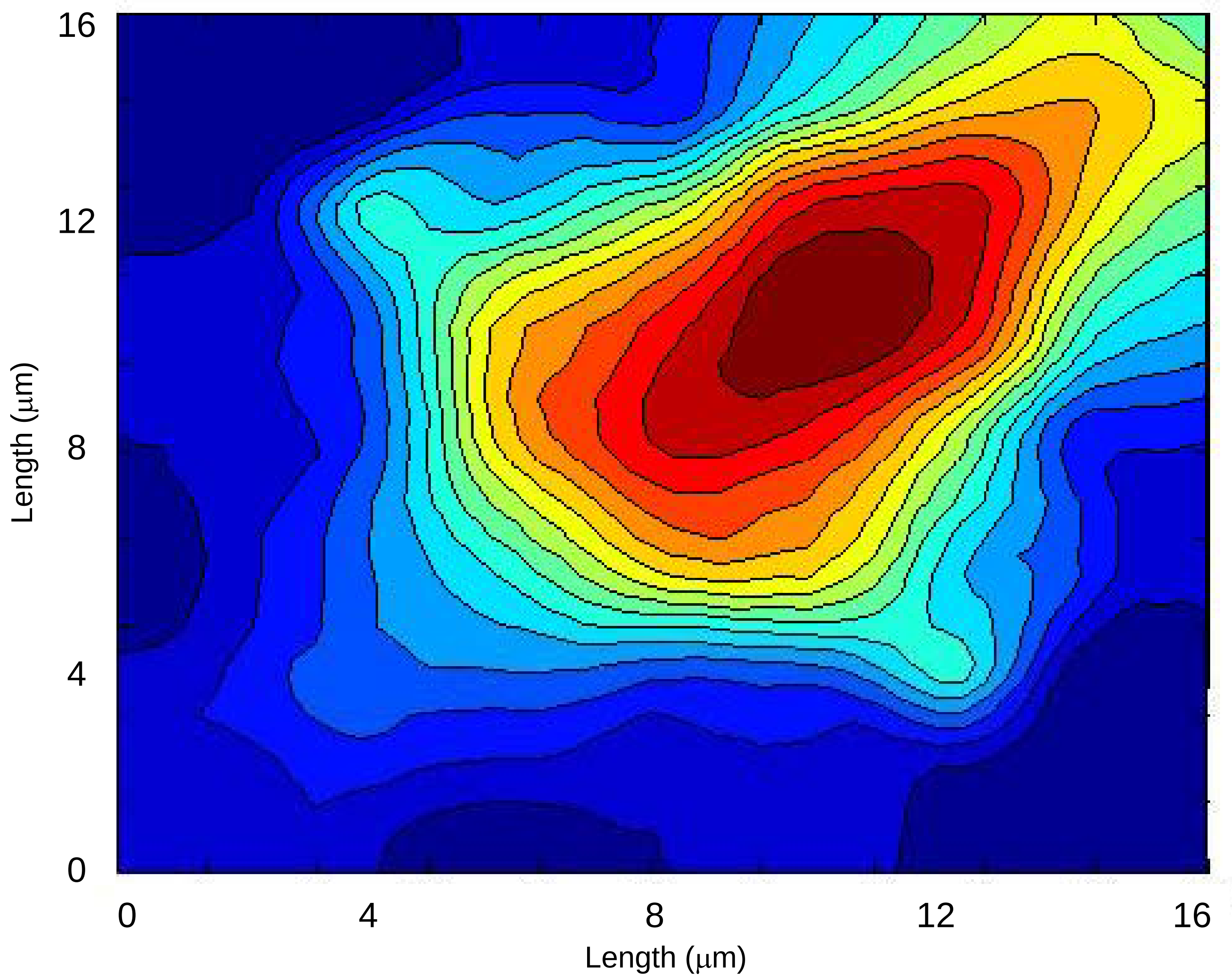
Unequal flagellar length

Short flagella

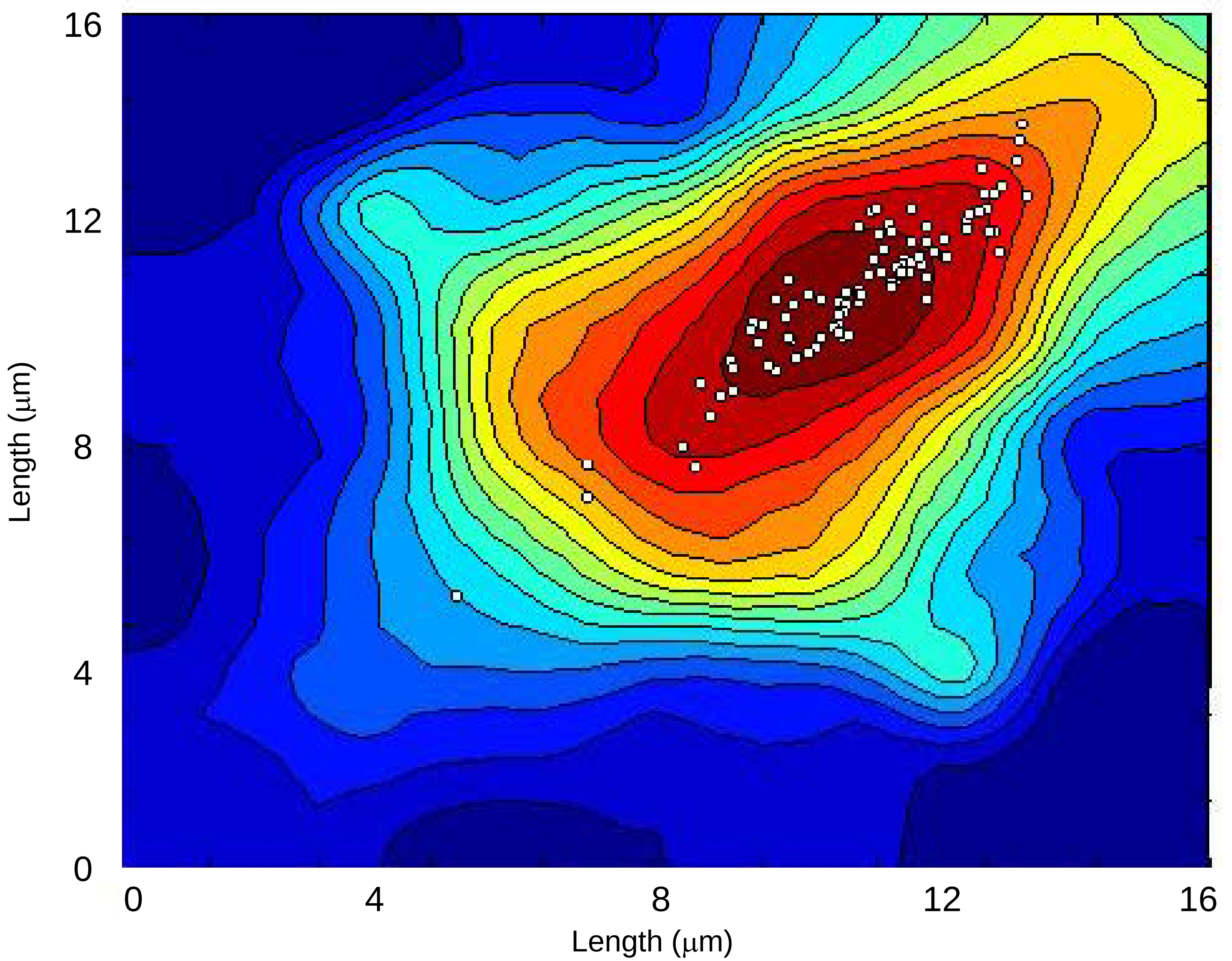


Important: not too long or too short
two flagella equal lengths

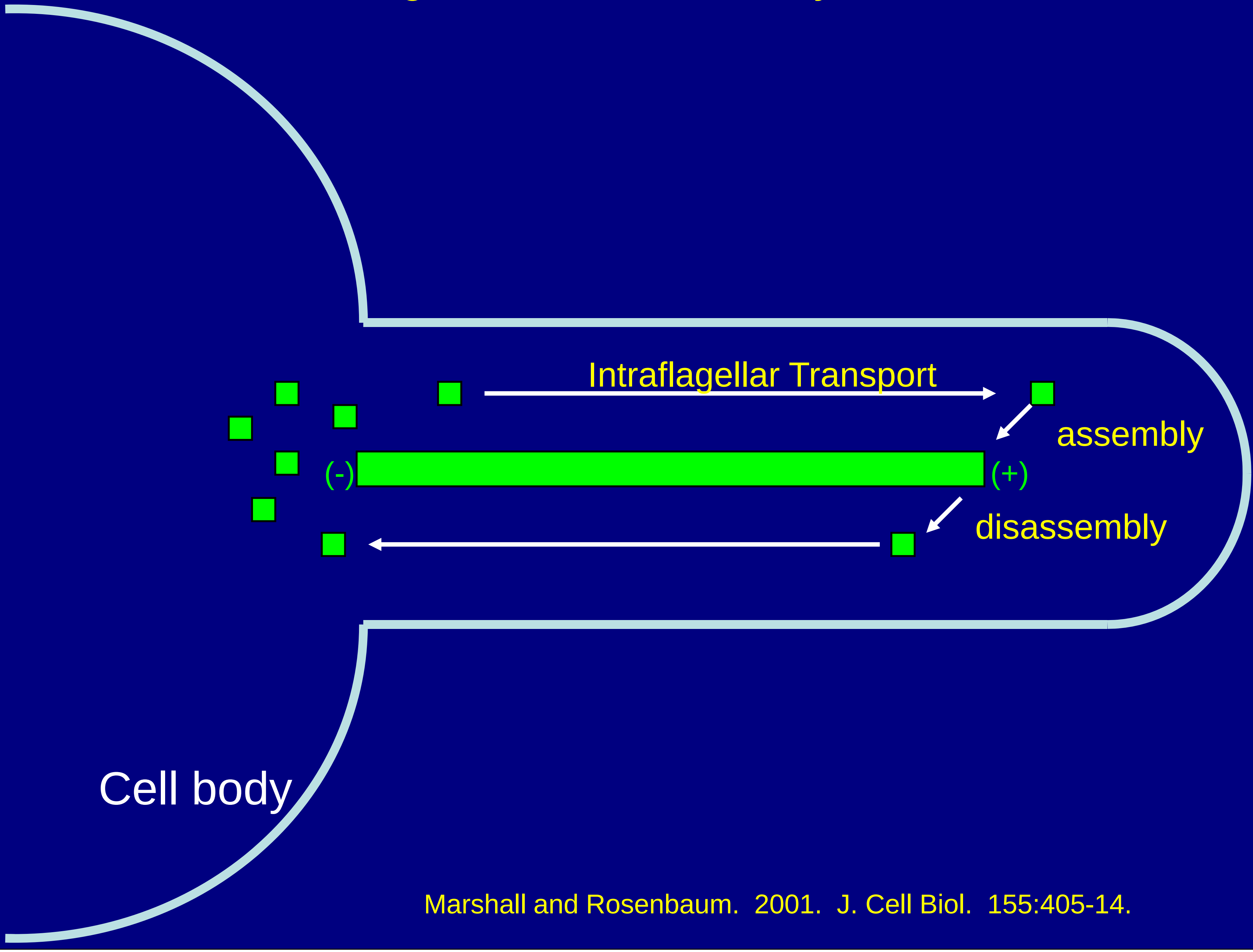
Swimming speed versus flagellar lengths



Wild-type flagellar lengths fall into optimal fitness range

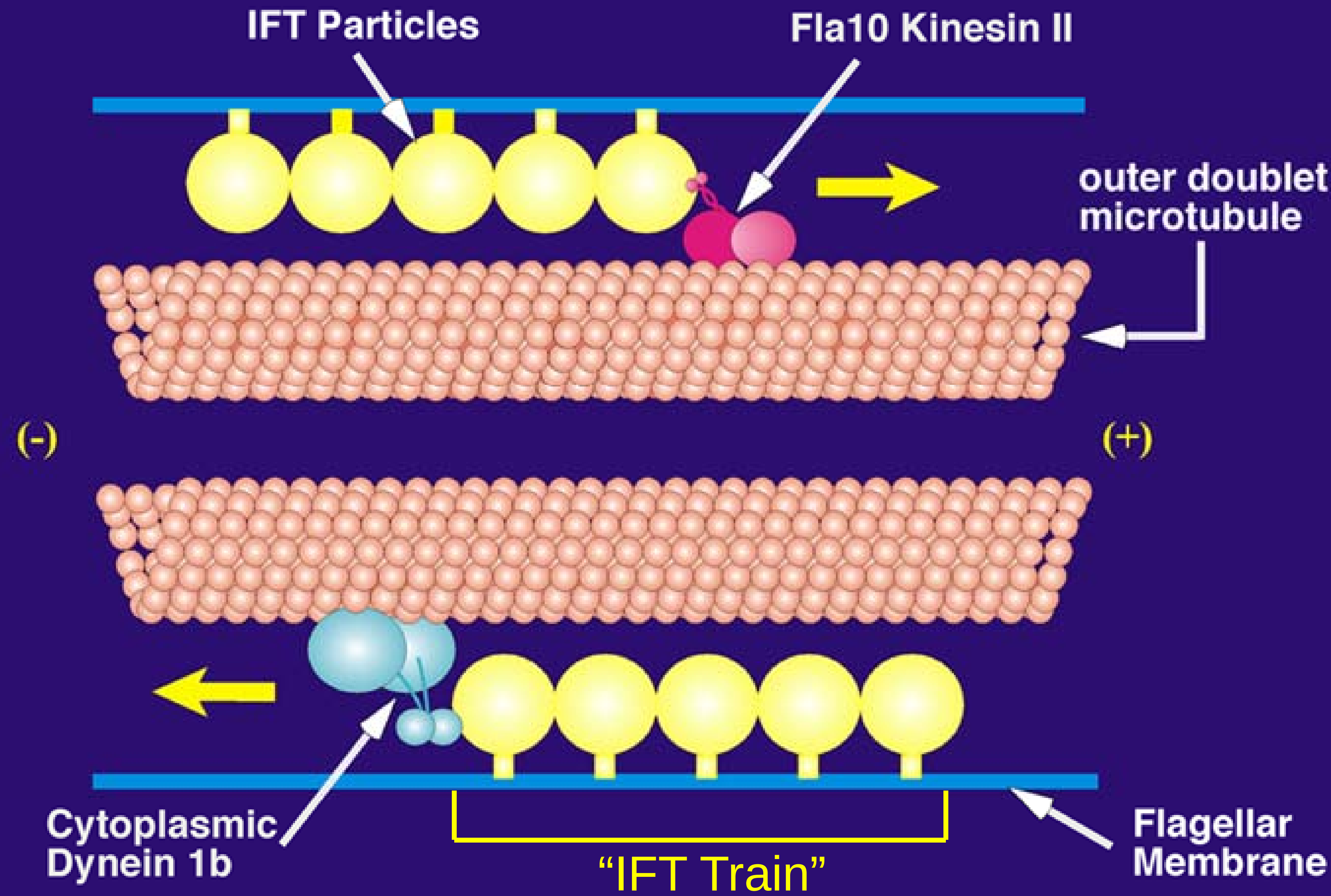


Flagellar Microtubule Dynamics

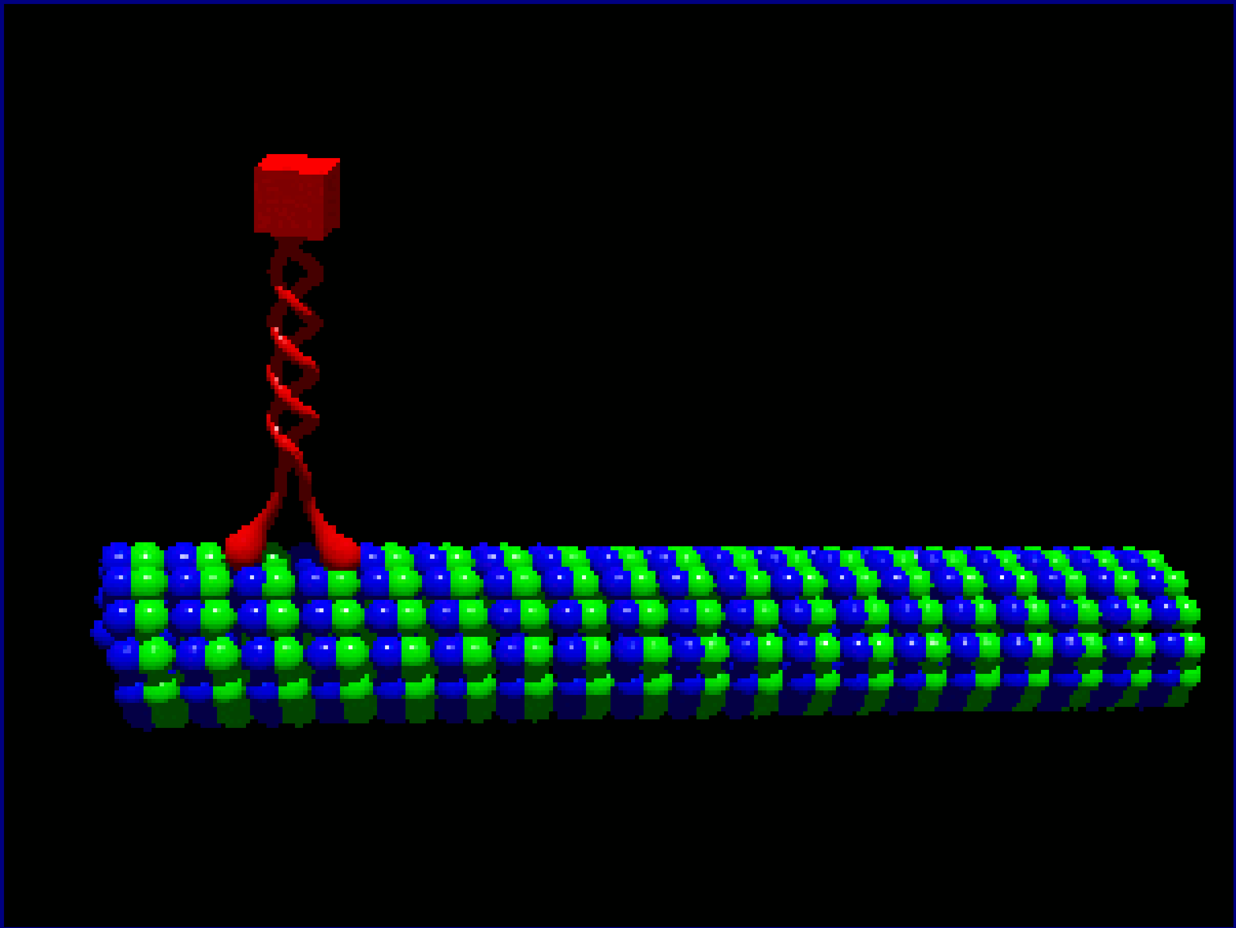


Marshall and Rosenbaum. 2001. J. Cell Biol. 155:405-14.

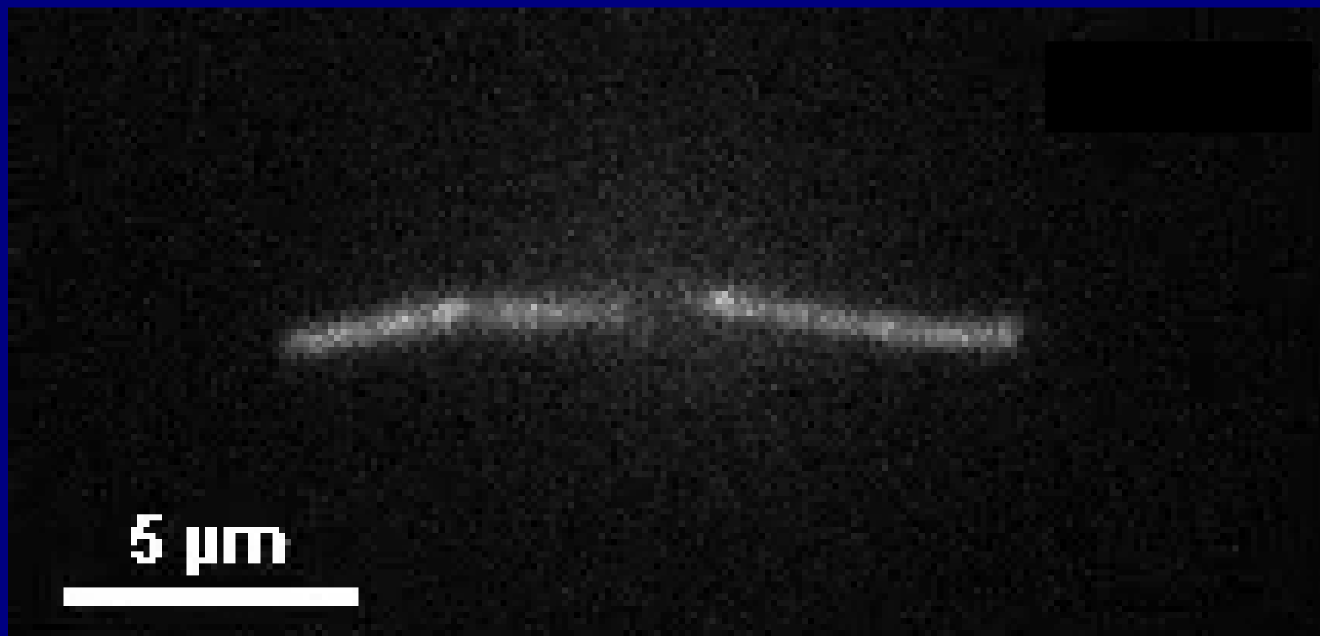
Intraflagellar Transport (IFT)



IFT movement is powered by a motor protein - kinesin



Imaging IFT by TIRF in Chlamydomonas



Goal of control system:

assembly = disassembly   iff   correct length

1. Disassembly length independent --> need to control assembly
2. Assembly rate-limited by IFT

How does transport by IFT depend on length?

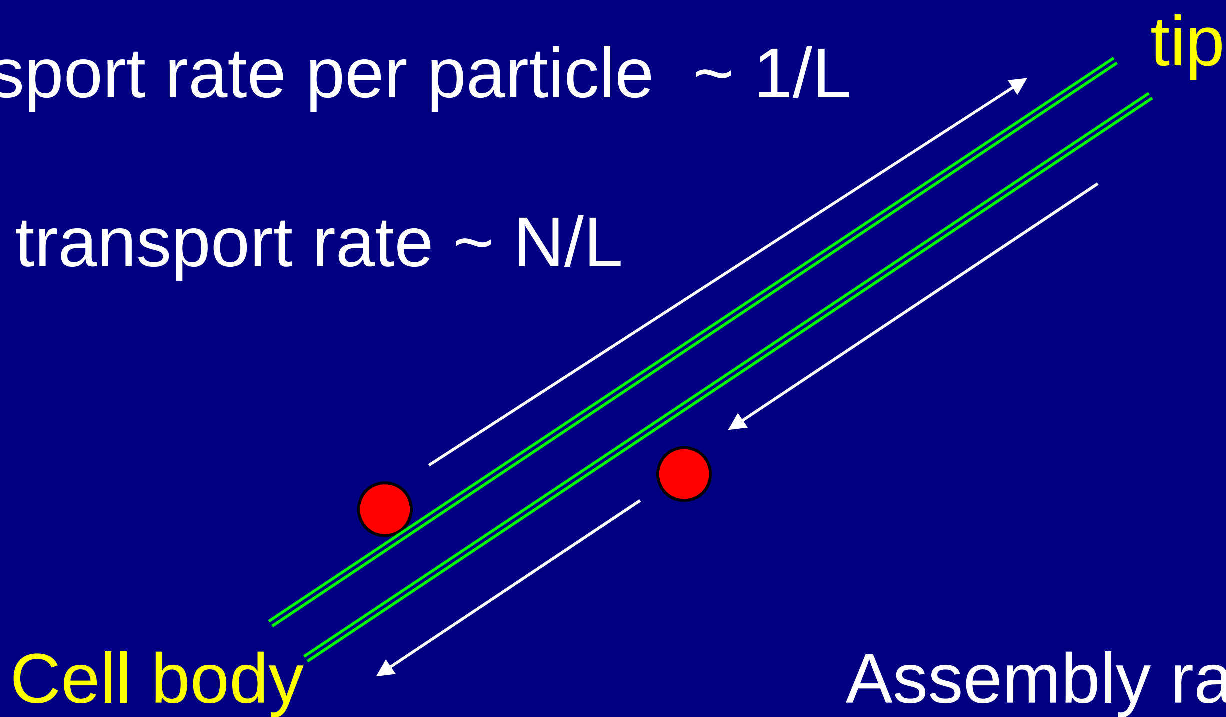
Marshall and Rosenbaum. 2001. JCB 155:405-1

Transport by IFT is inherently length-dependent

Round trip time ~ L

Transport rate per particle ~ 1/L

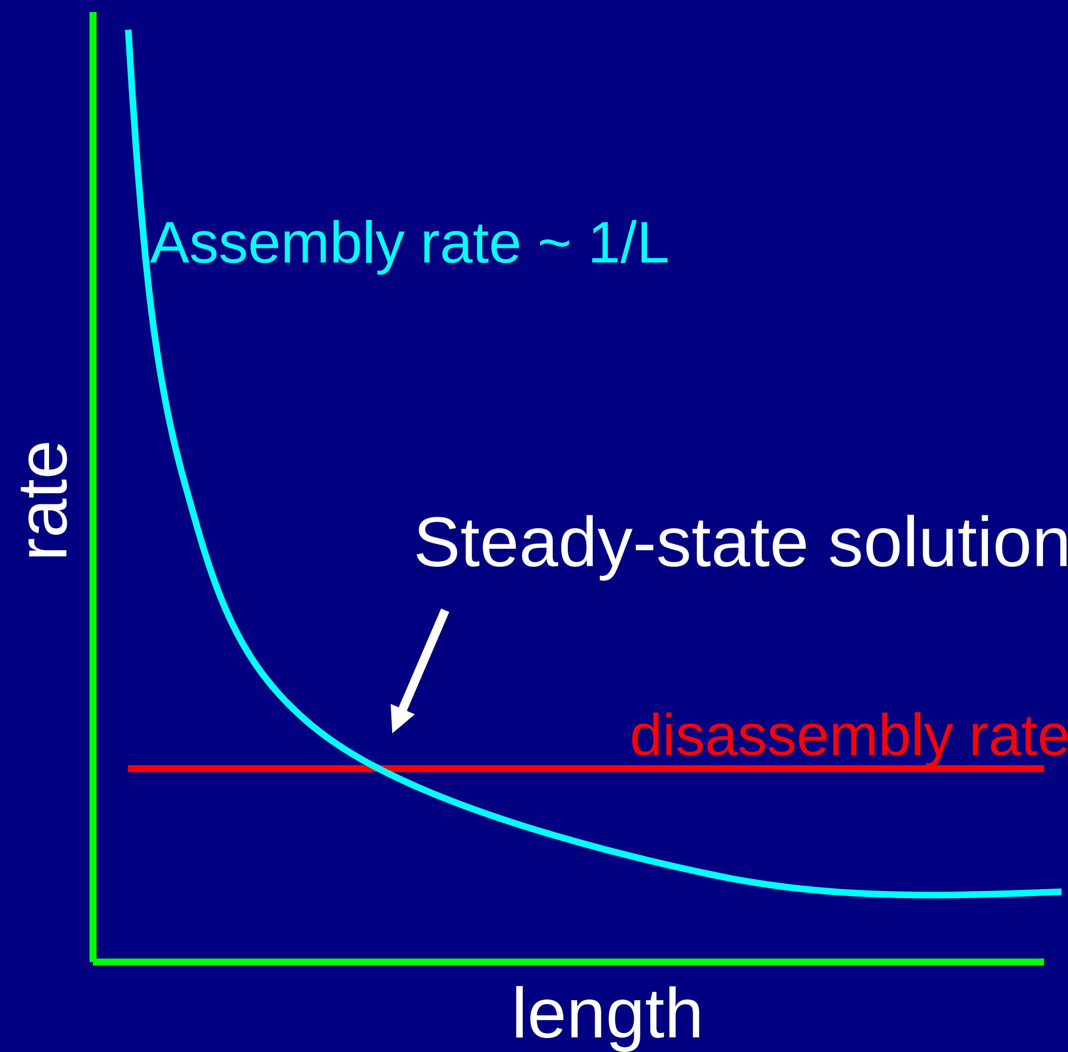
Total transport rate ~ N/L



Assembly rate ~ 1/L

Assuming N is independent of L

Balance-Point model for flagellar length control



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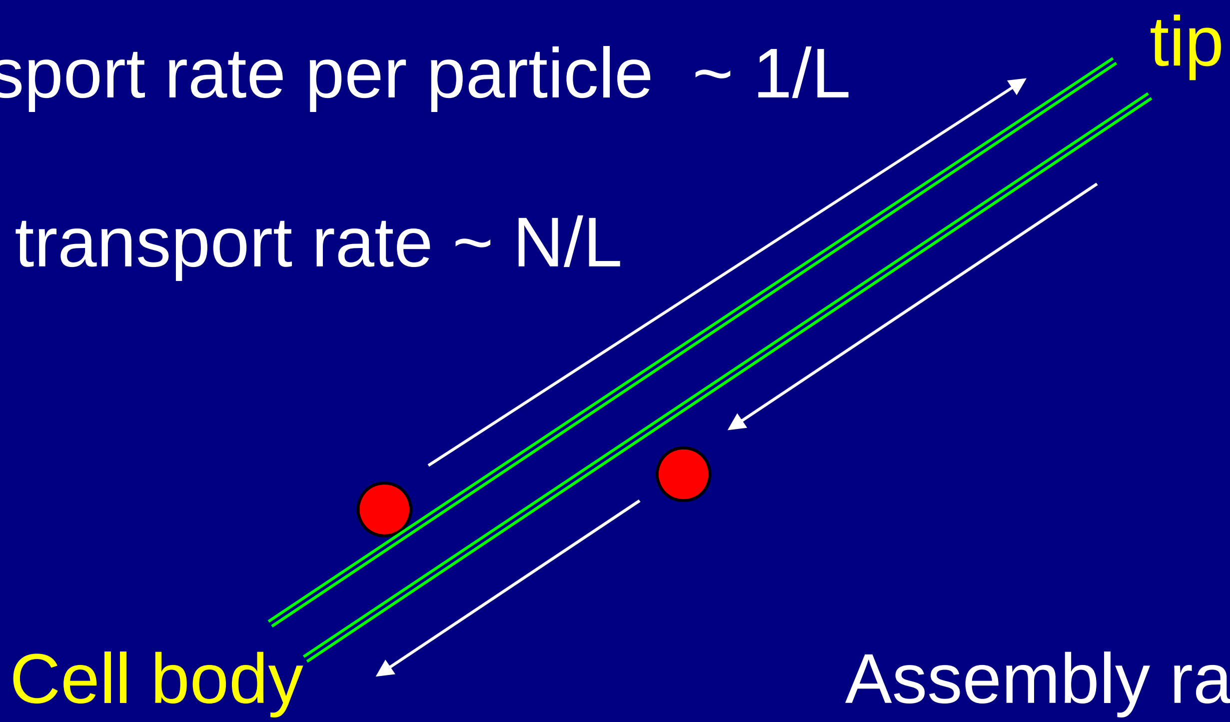
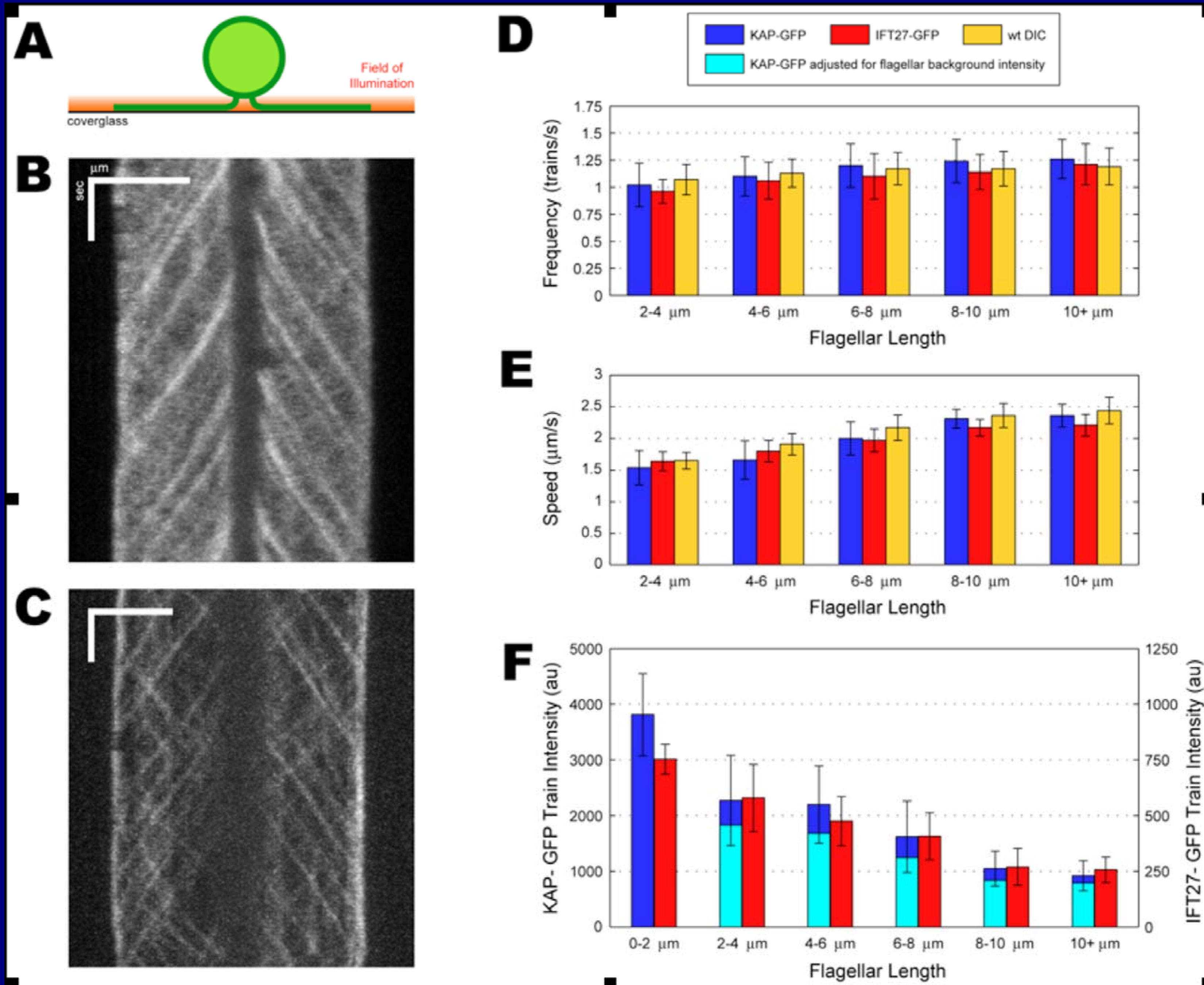


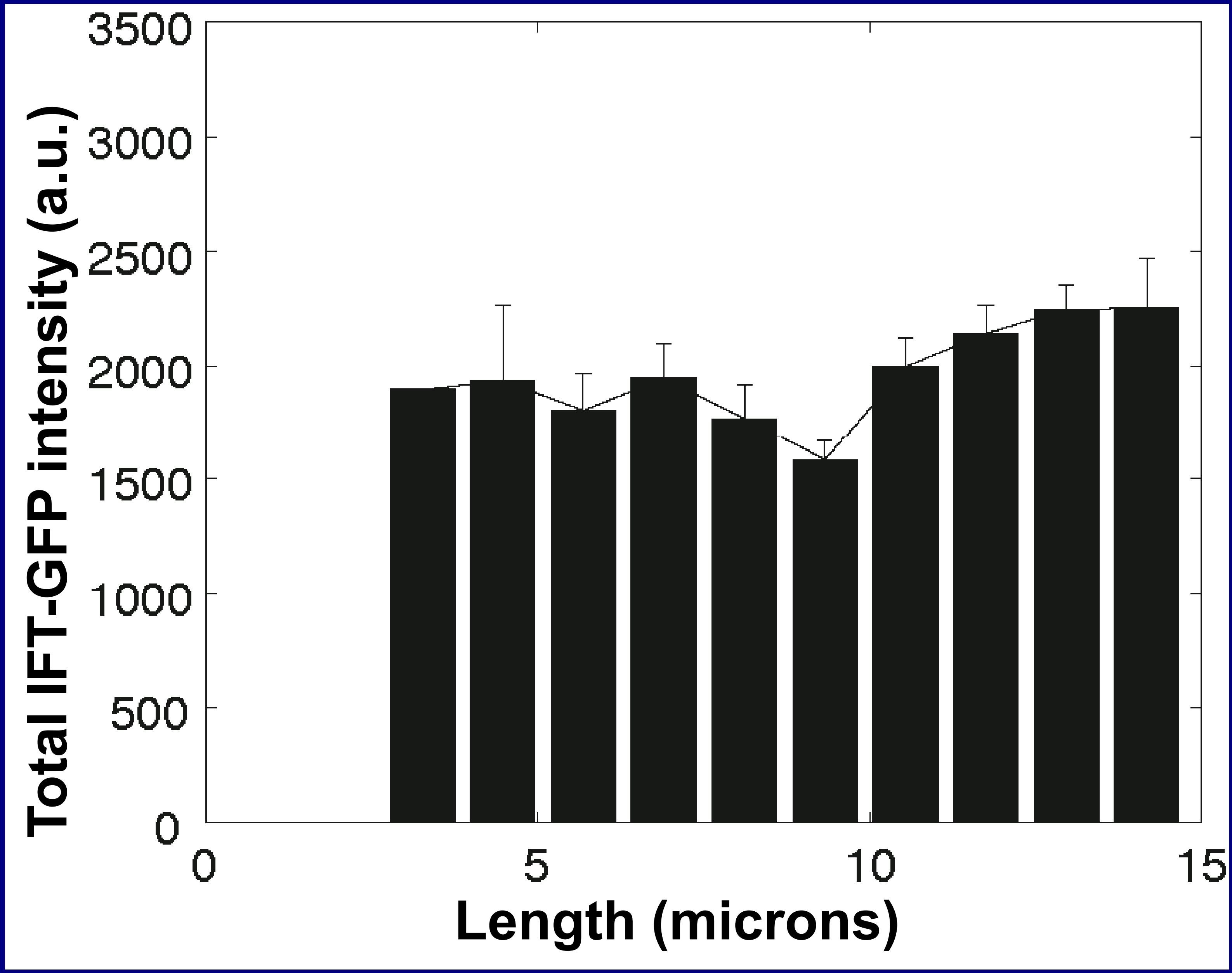
Assembly rate ~ 1/L

Assuming N is independent of L



Engel BD, Ludington WB, Marshall WF. 2009 JCB 187, 81-9

Total IFT content is approximately length-independent

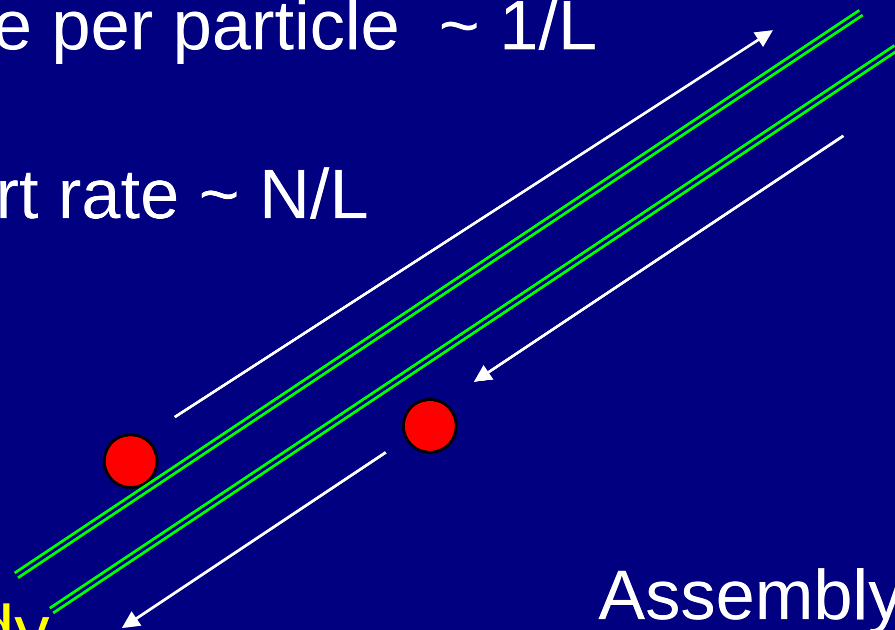


Length Regulation → Controlling IFT quantity

Round trip time ~ L

Transport rate per particle ~ 1/L

Total transport rate ~ N/L

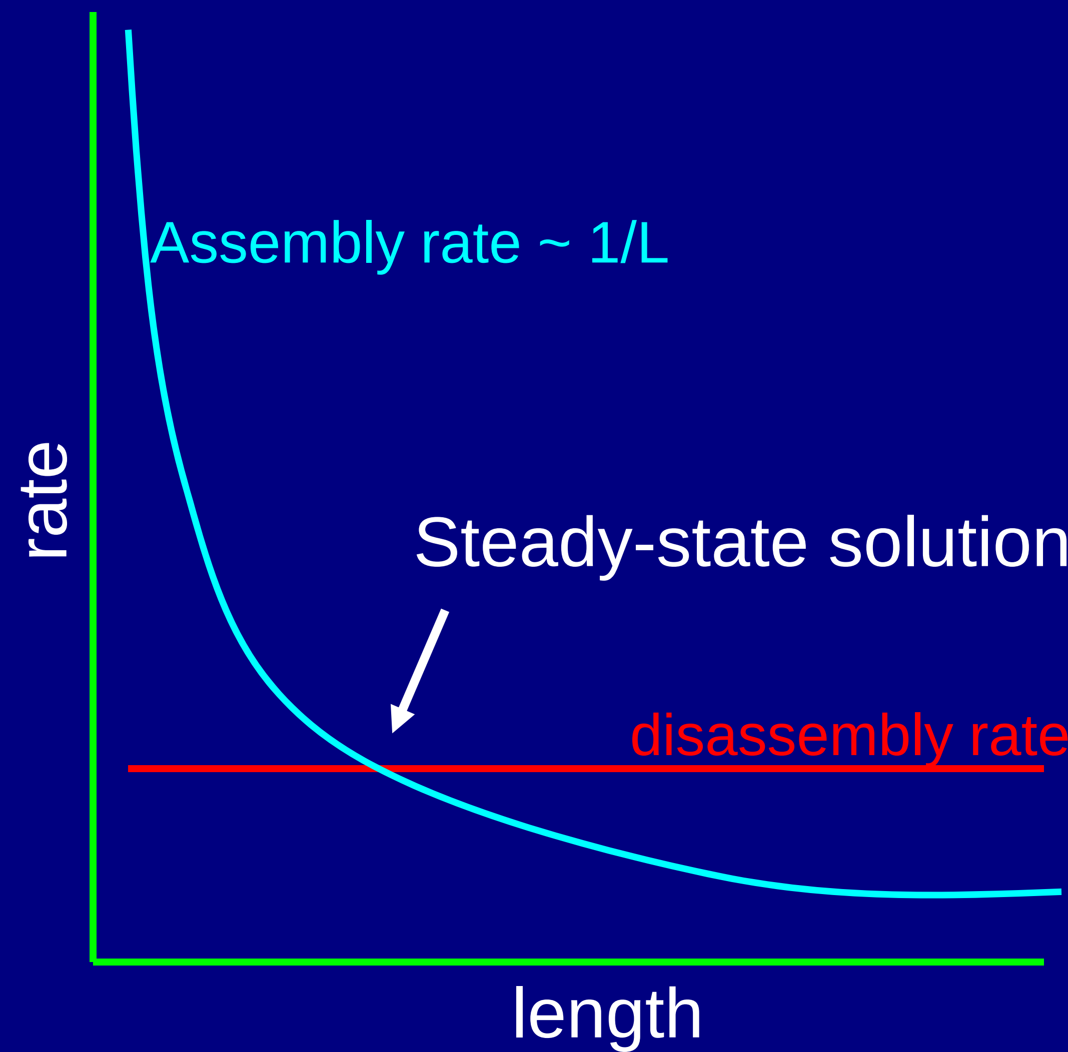


Assembly rate ~ 1/L

N is independent of L

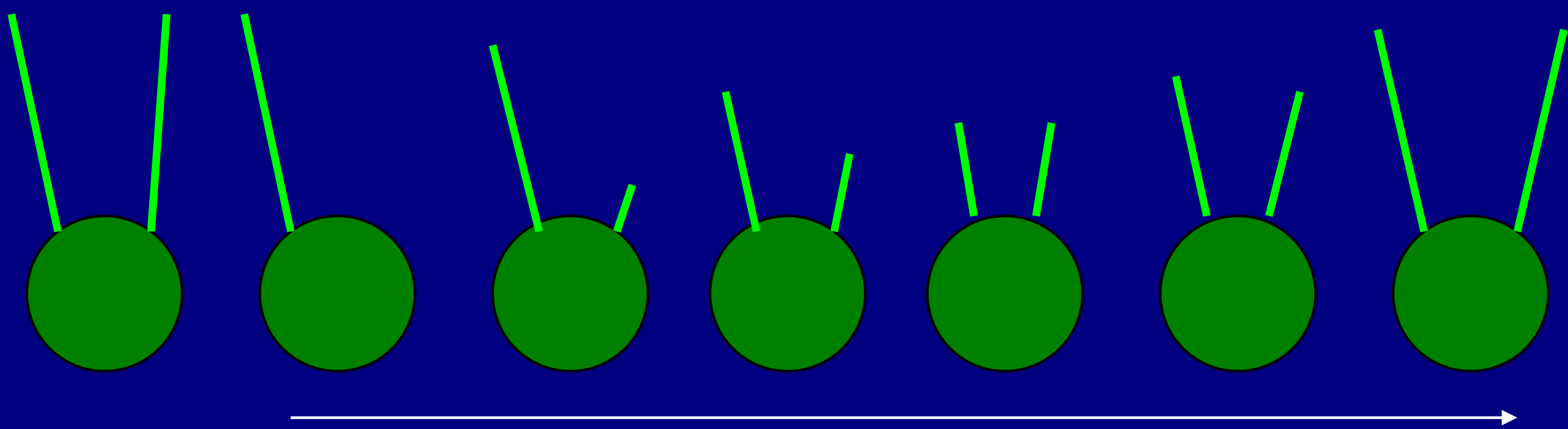
WHAT CONTROLS N?

Balance-Point model for flagellar length control



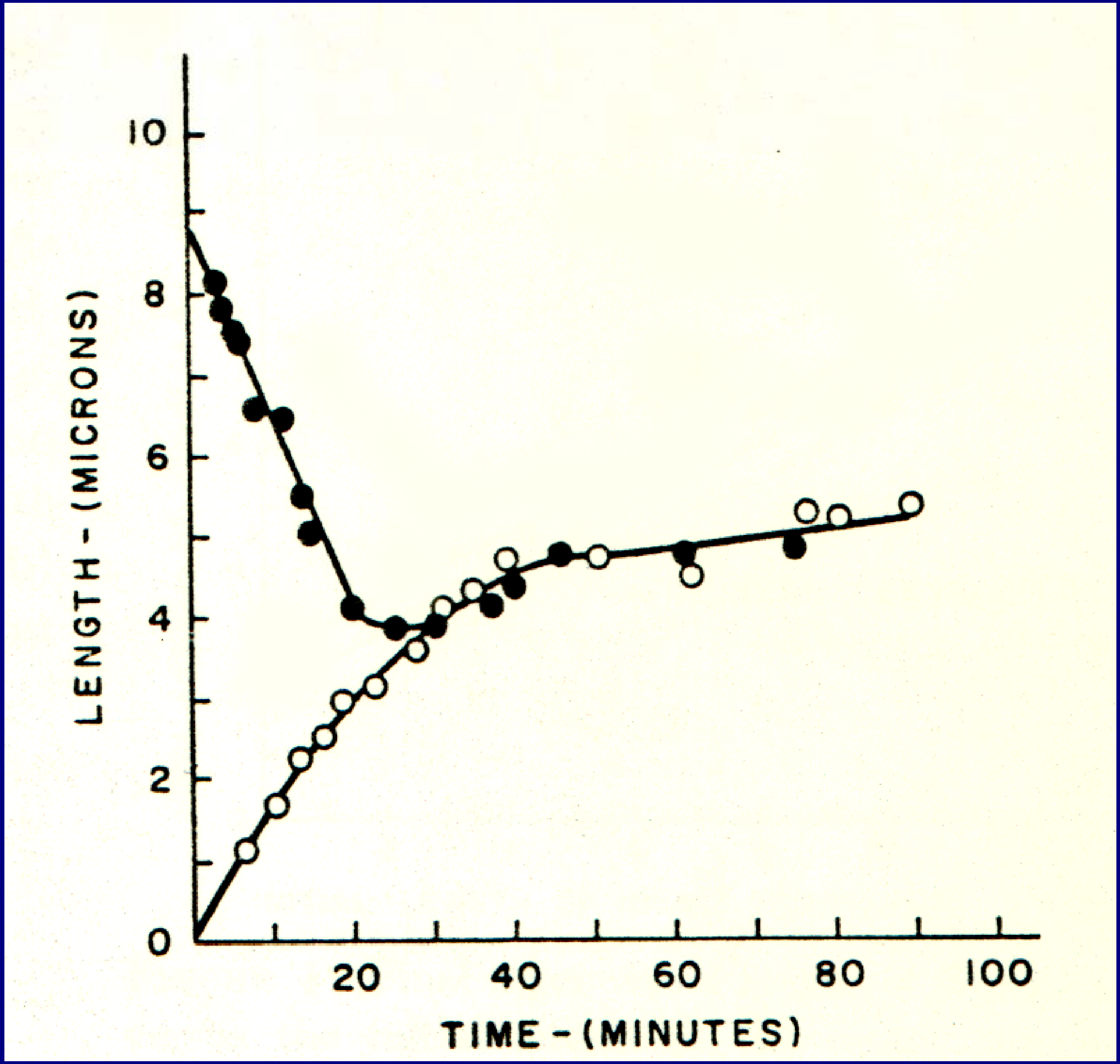
A challenge for the model:

Flagellar equalization after severing one flagellum

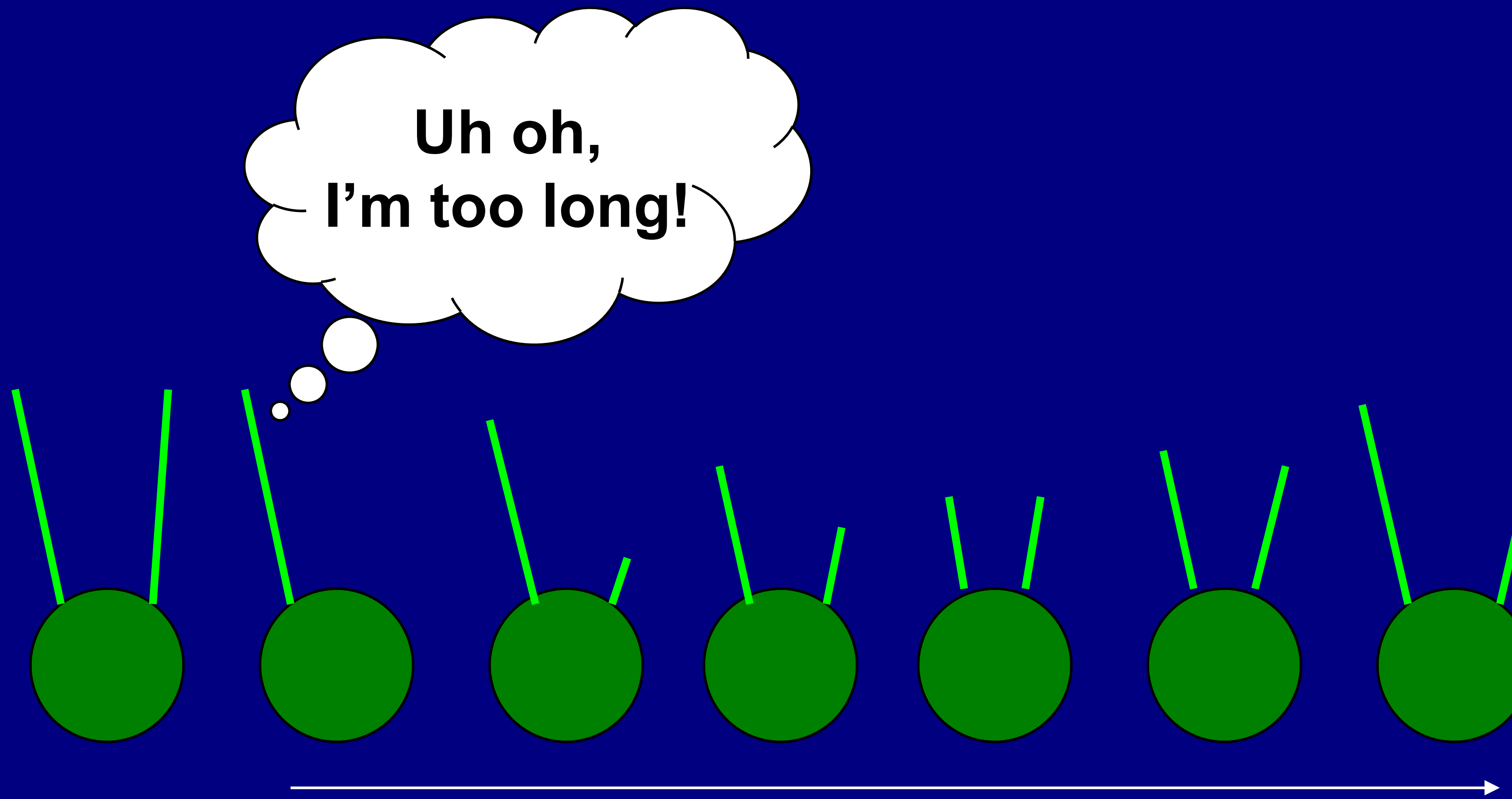


Flagellar length equalization following severing

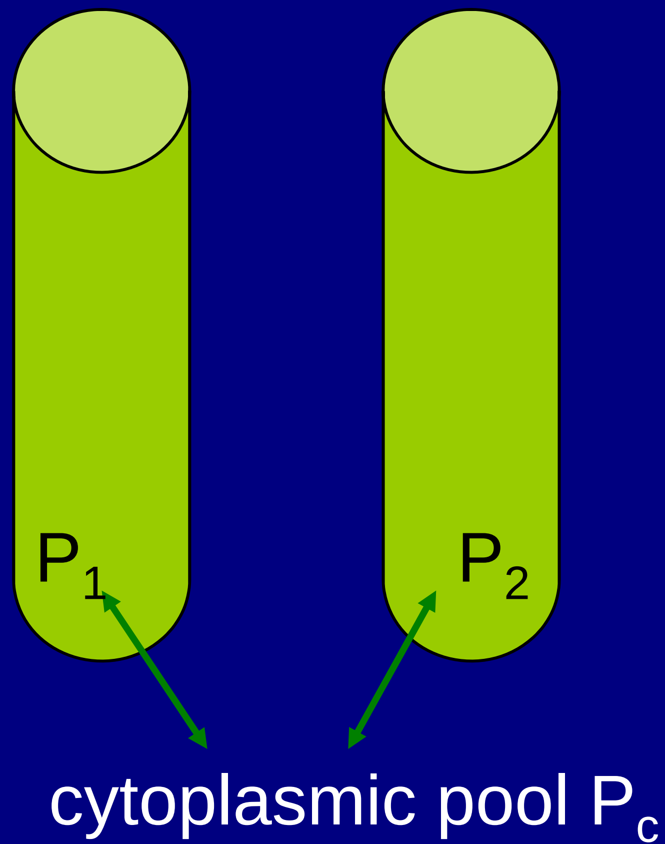
Coyne & Rosenbaum 1970



Traditional interpretation as evidence for length-sensor



Competition for cytoplasmic precursor pool



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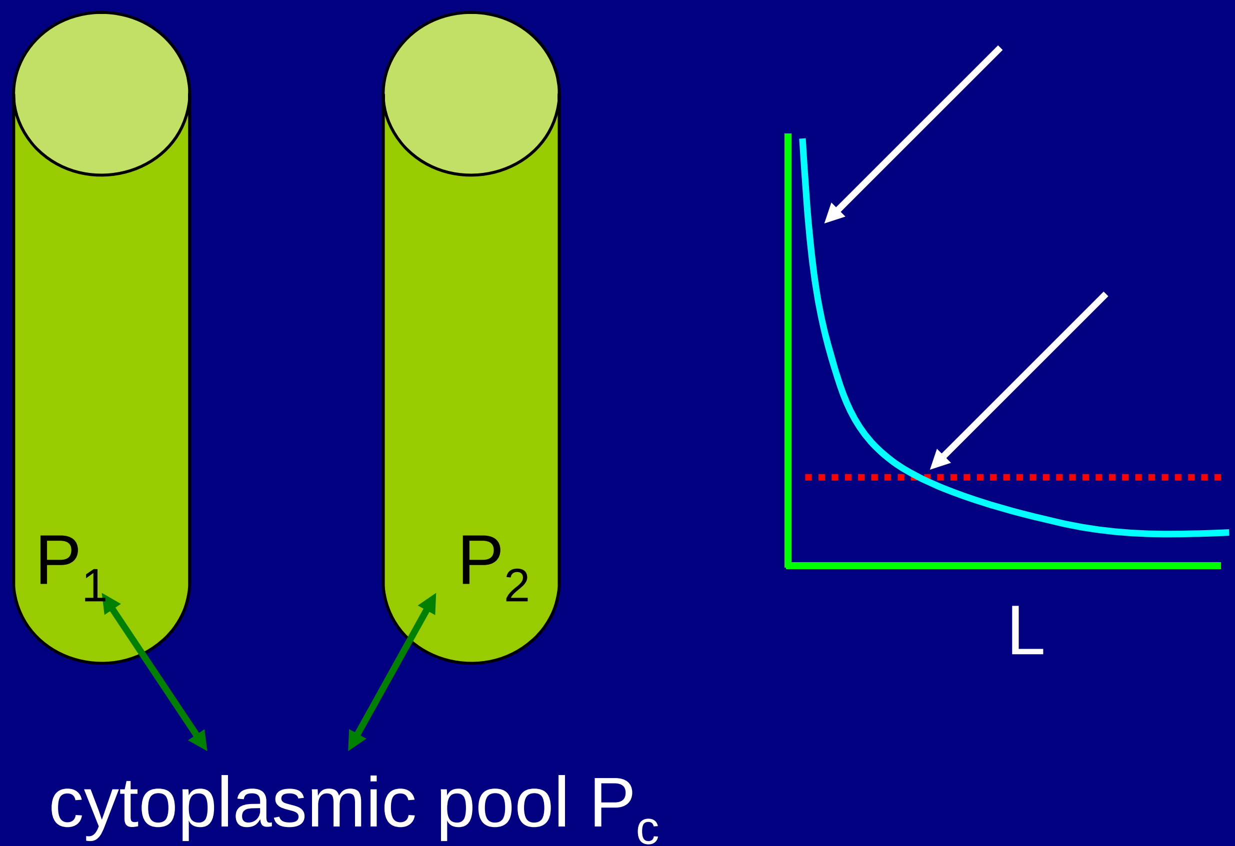
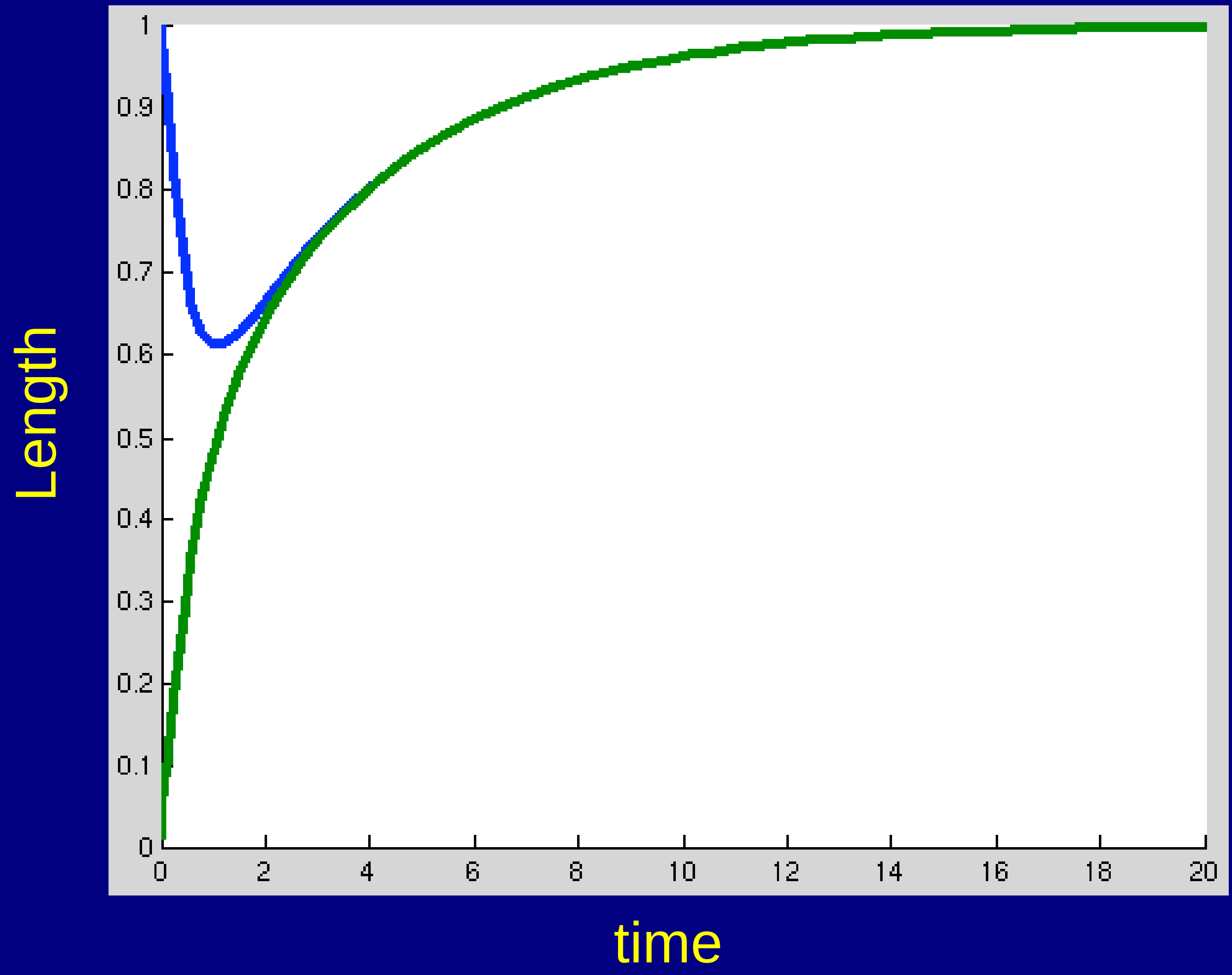
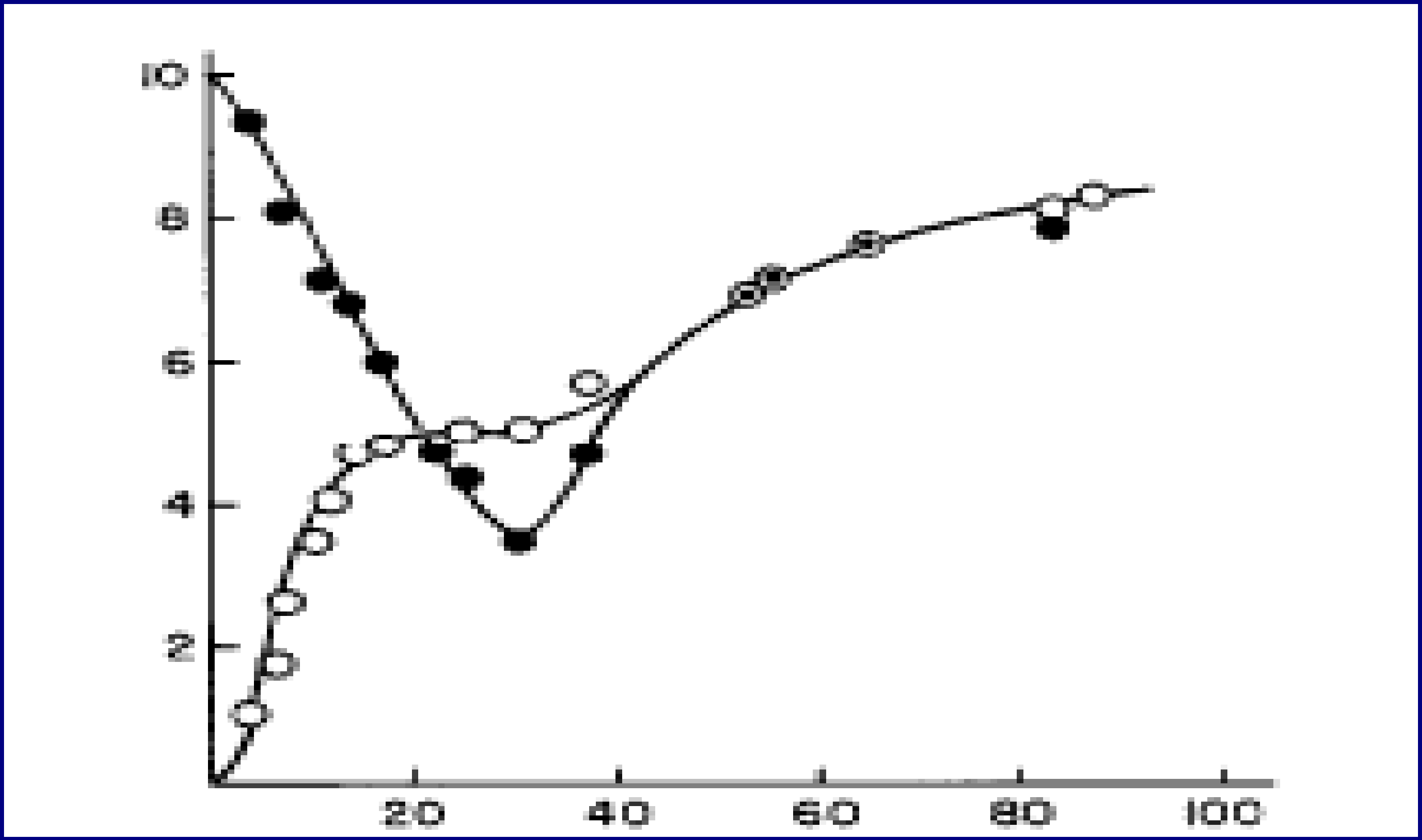


Balance-Point Model predicts Equalization of Lengths

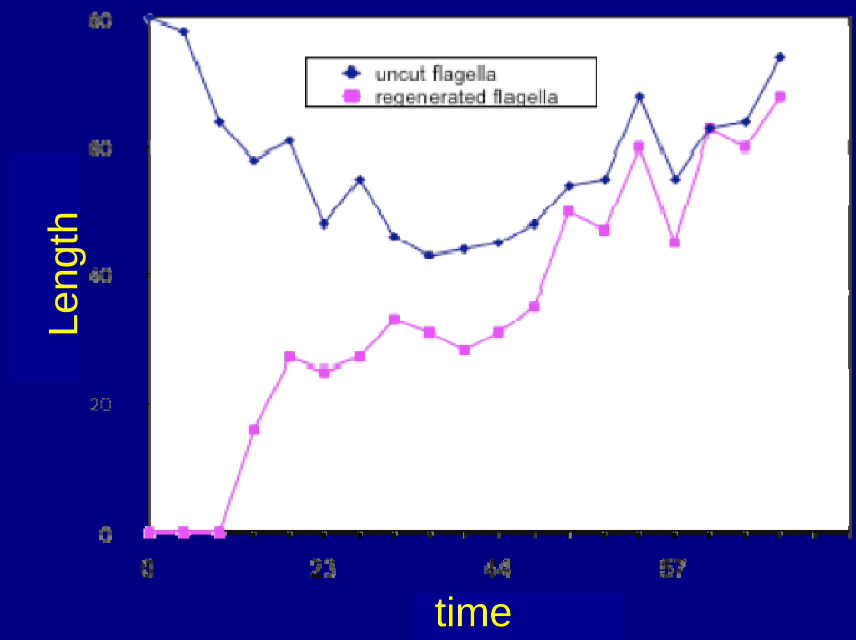


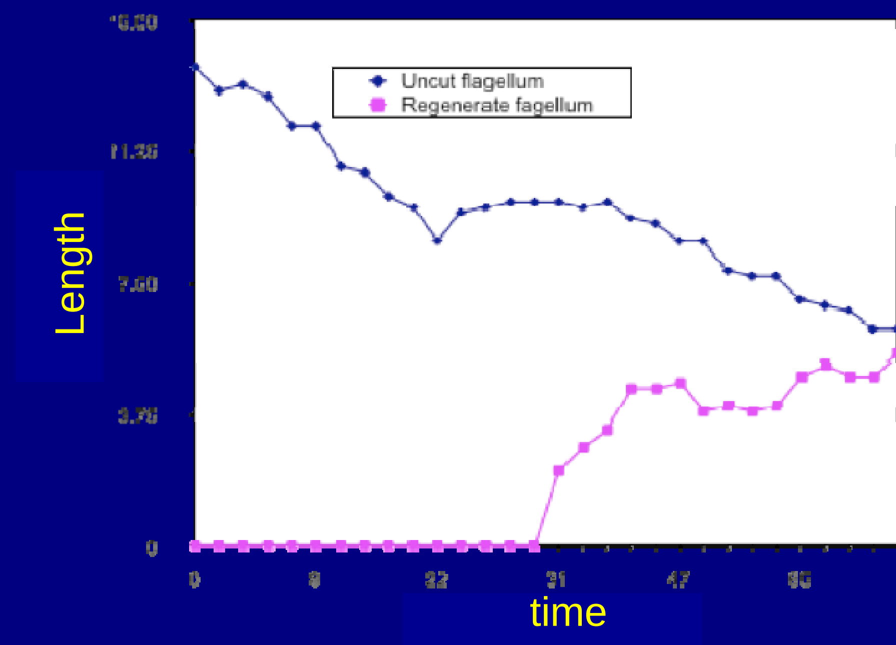
Overshoot – something the model does NOT predict

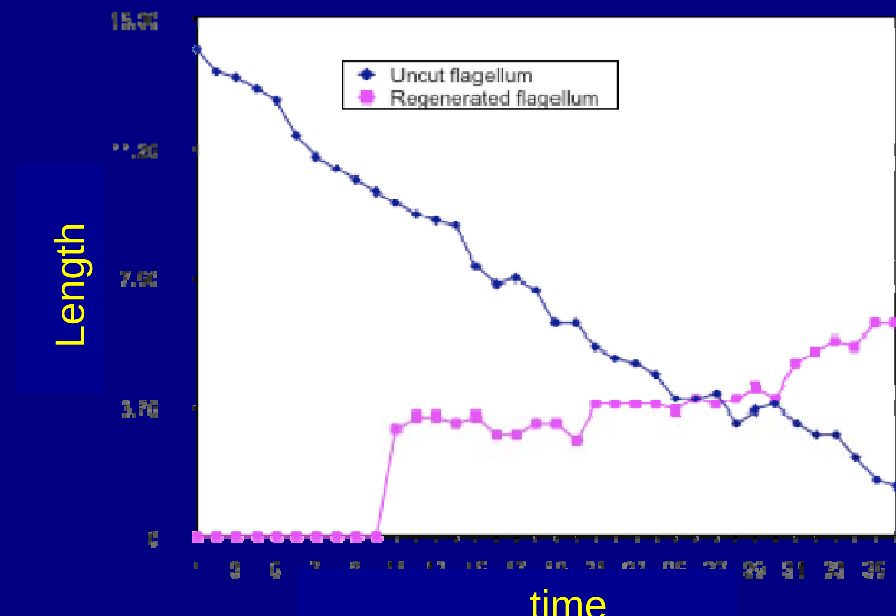


Requires equal-length flagella to have opposite behaviors

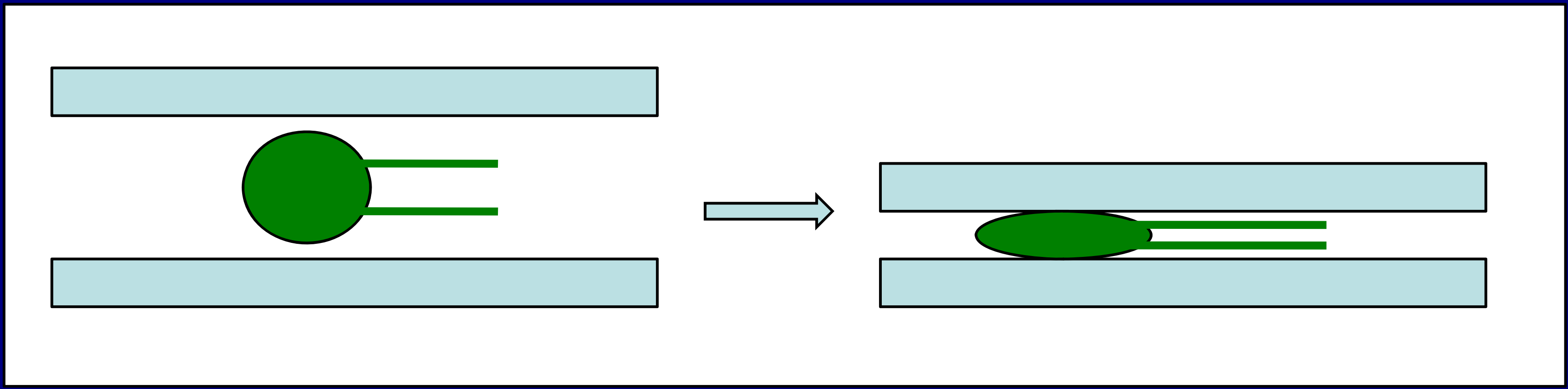
Checking for overshoot using laser scissors





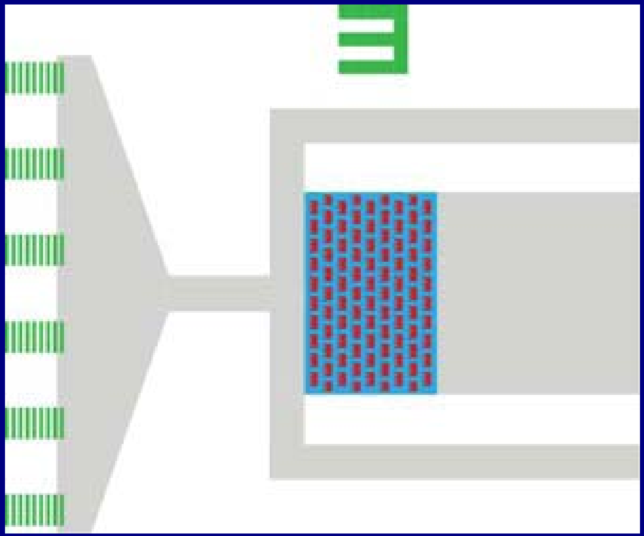


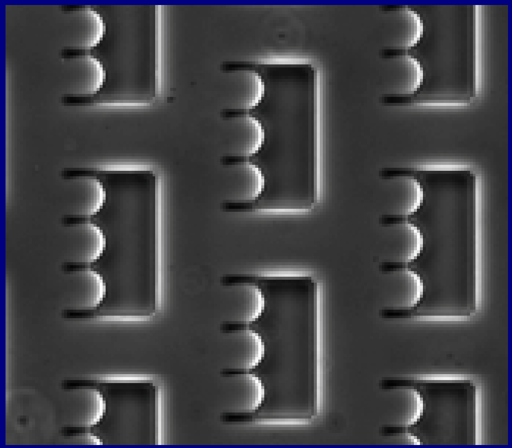
Standard method to immobilize cells

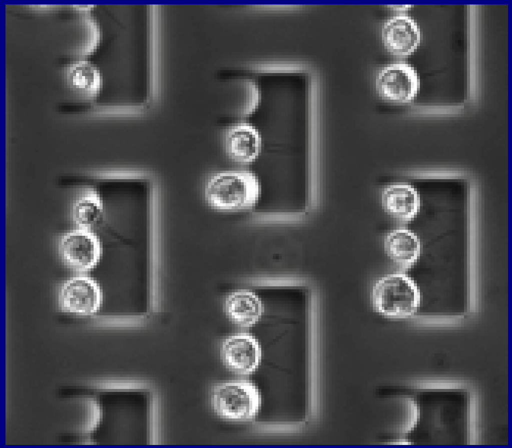


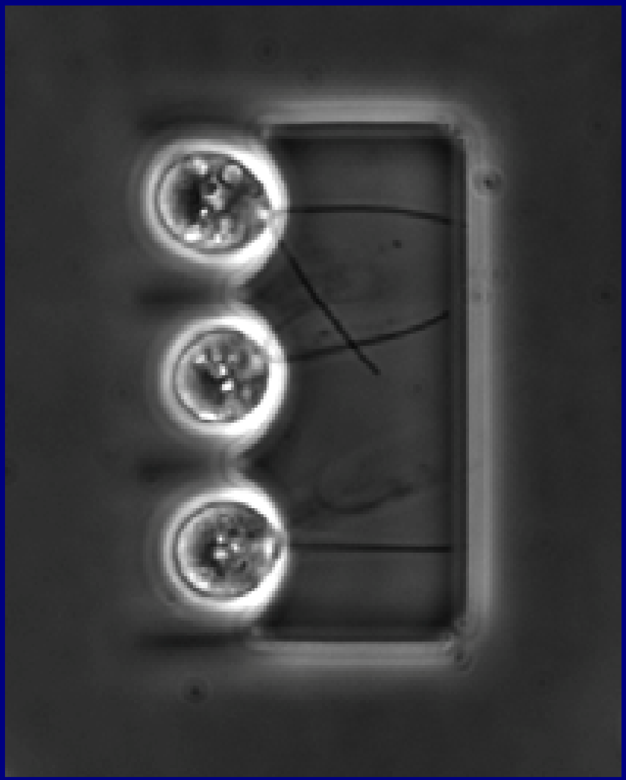
Cells do not divide
Become filled with clear vacuoles
Spontaneously drop their flagella

Custom microfluidic chamber for Chlamydomonas laser surgery

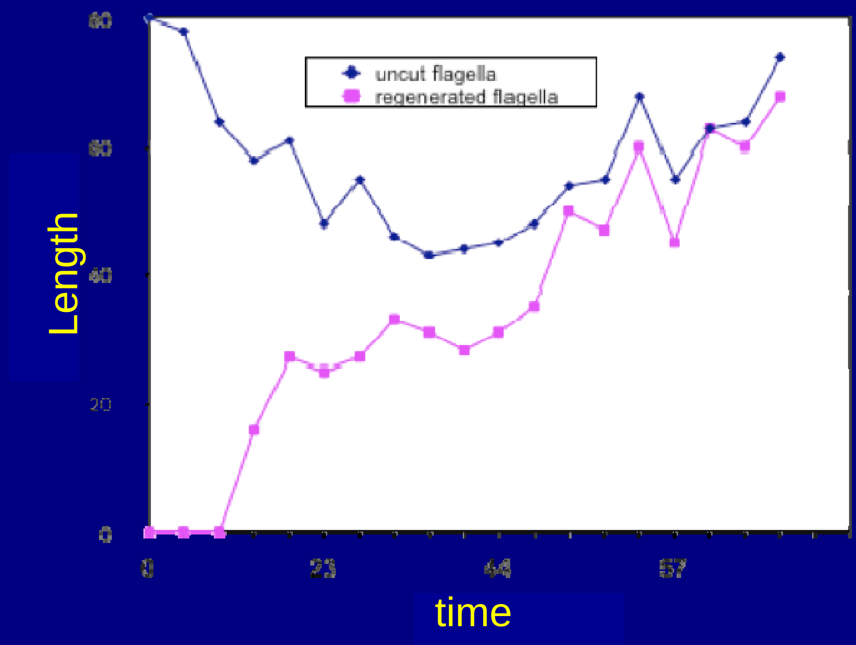


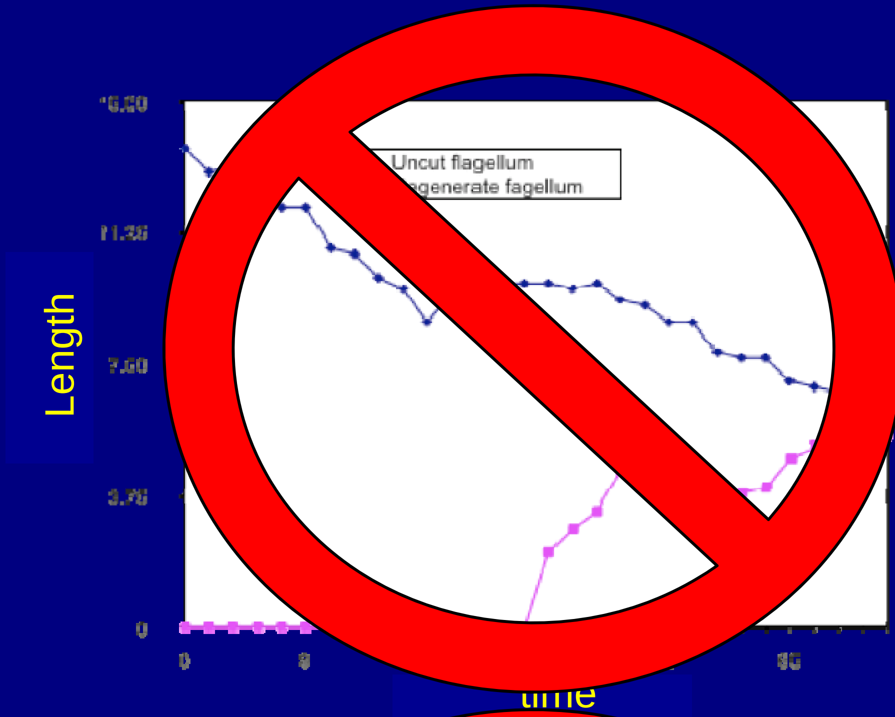


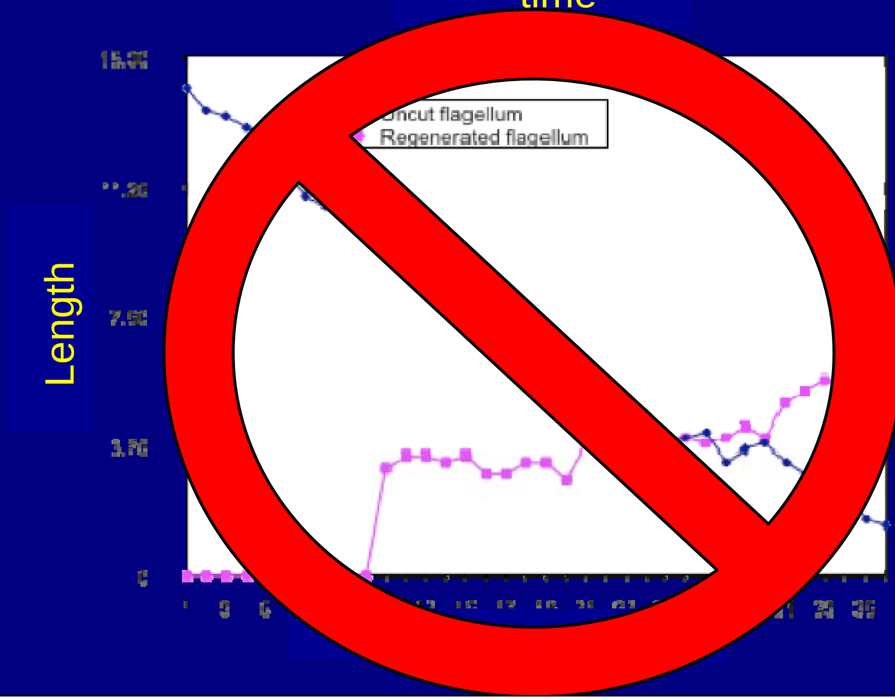




Overshoot vanishes in microfluidic chambers







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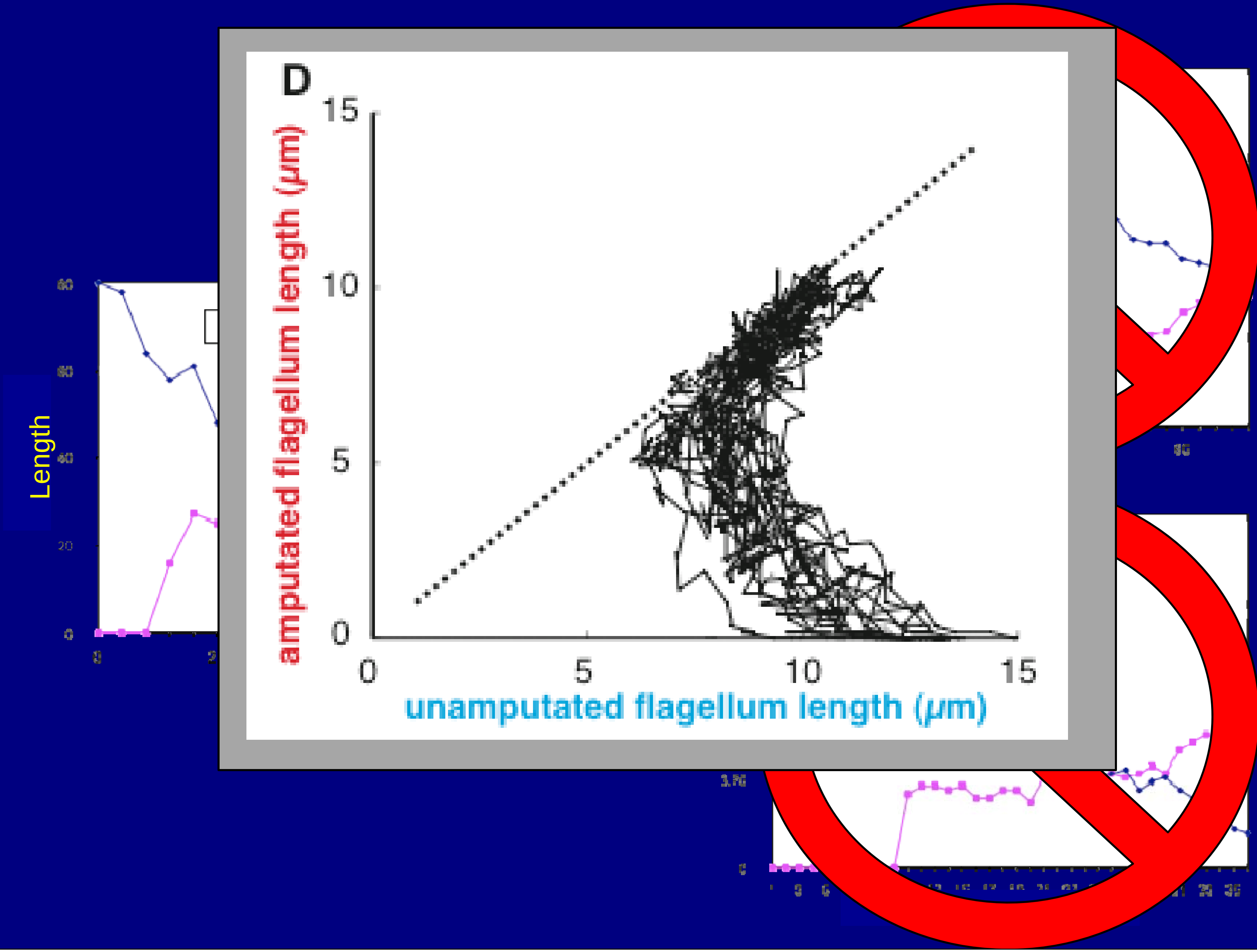
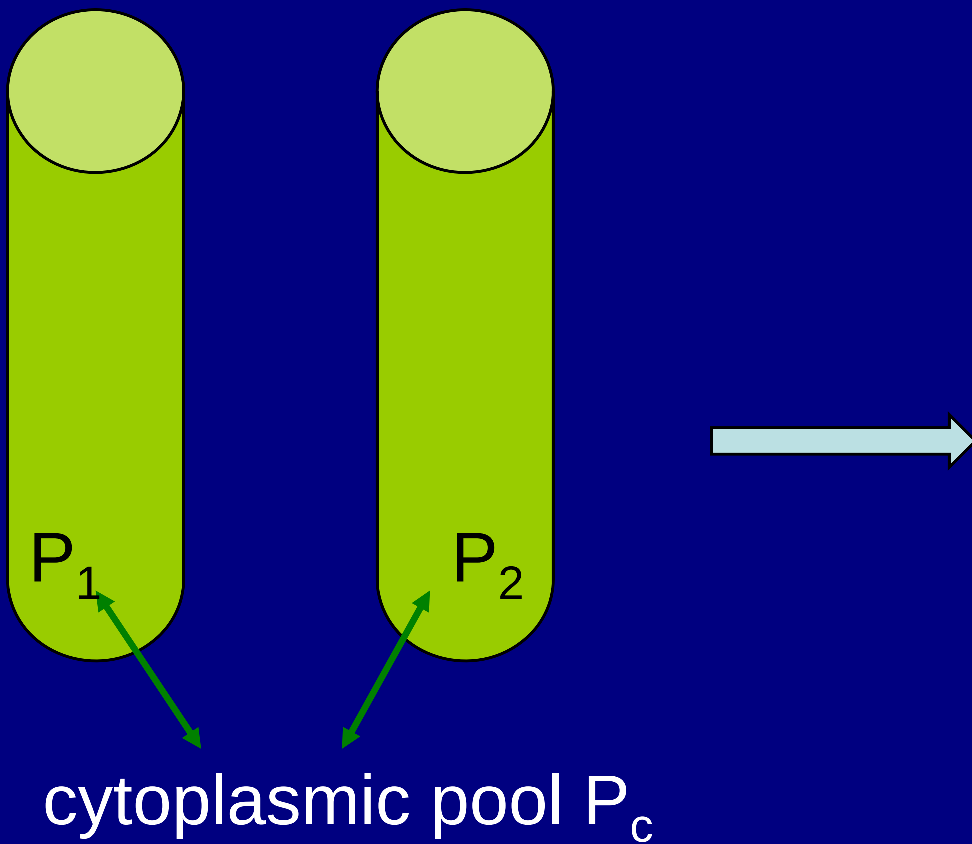
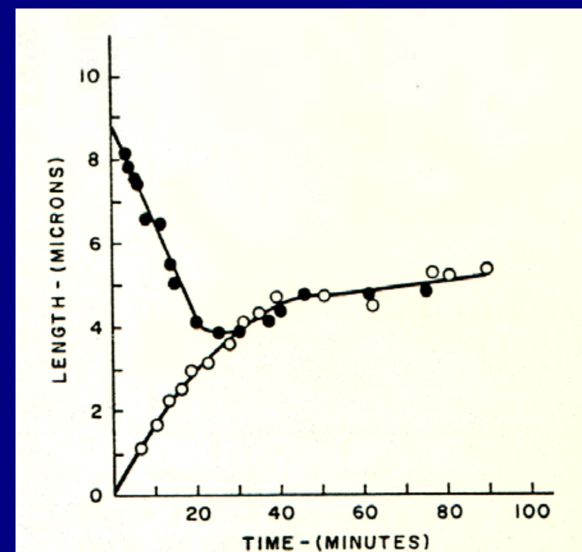
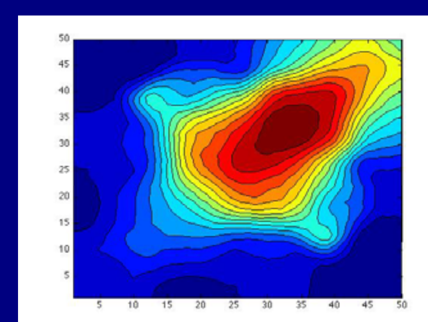


Precursor pool competition explains long-zero response



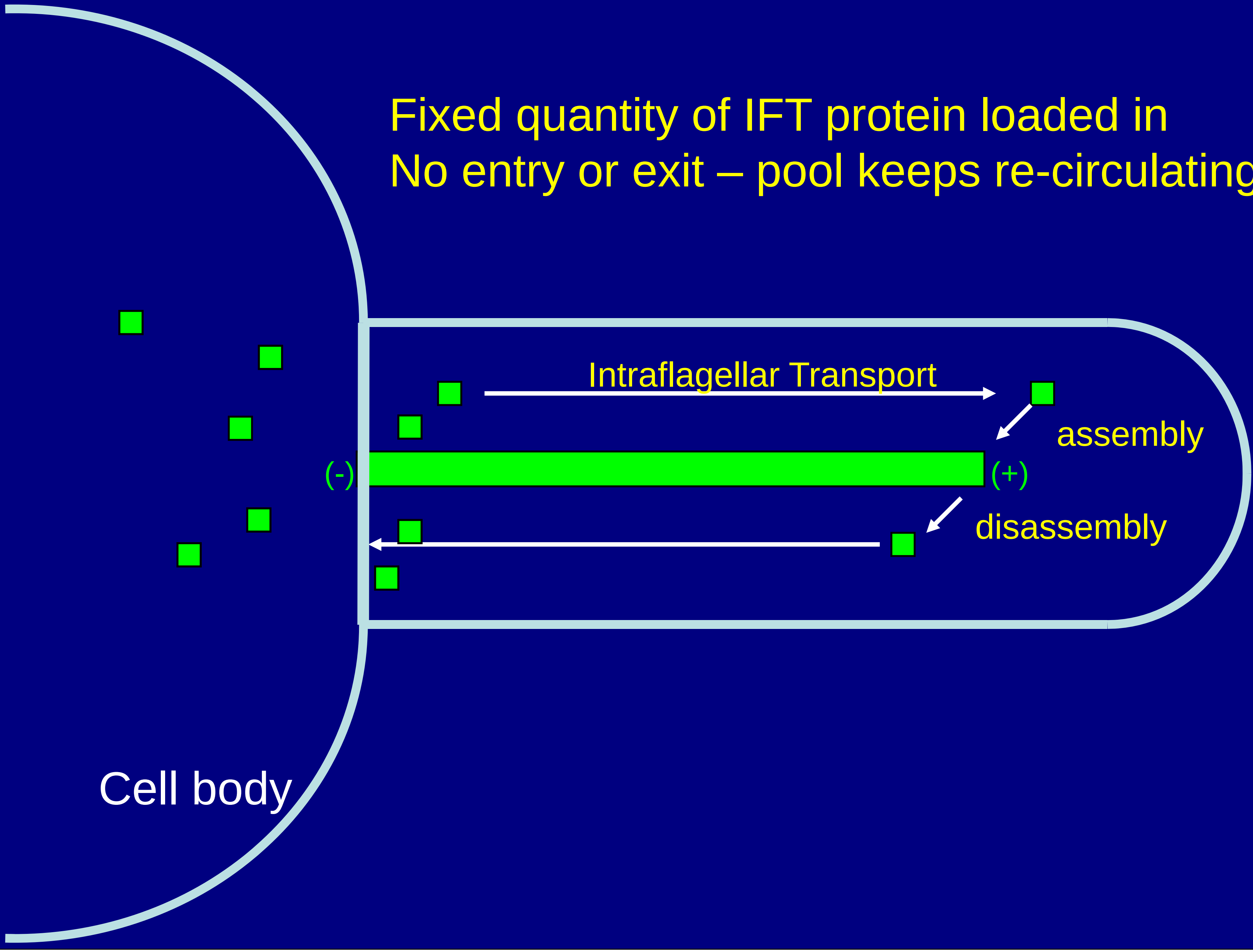


Dynamic length control → equal length flagella



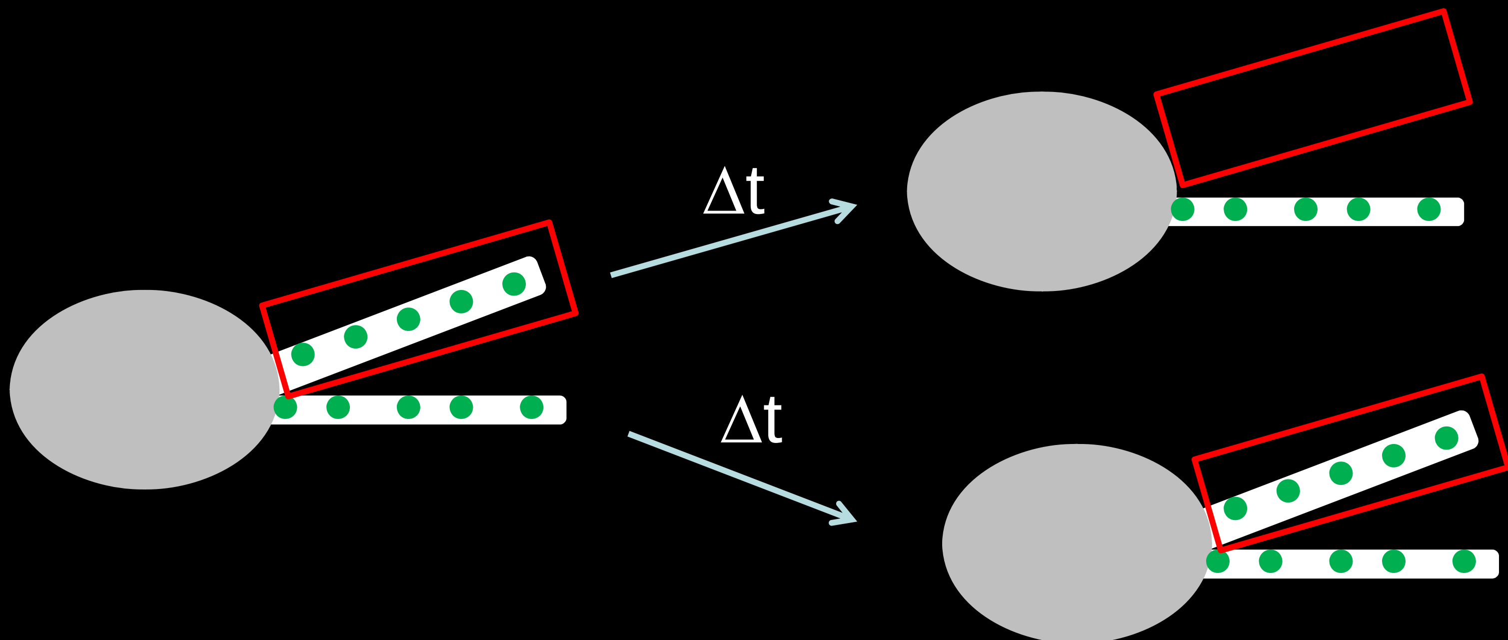
Initial Bolus Model

Fixed quantity of IFT protein loaded in
No entry or exit – pool keeps re-circulating

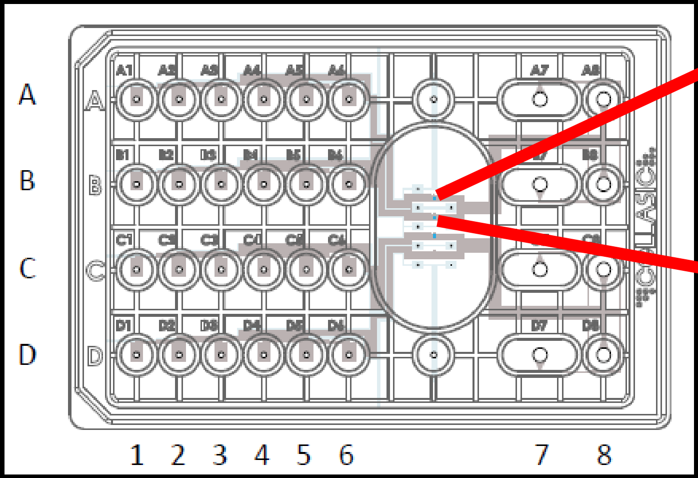


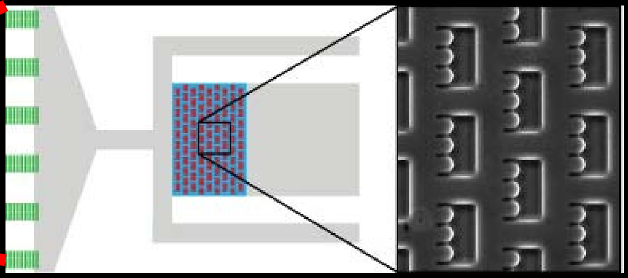
Testing Initial Bolus Model

Method: Fluorescence Recovery After Photobleaching (FRAP) of IFT proteins in one flagellum

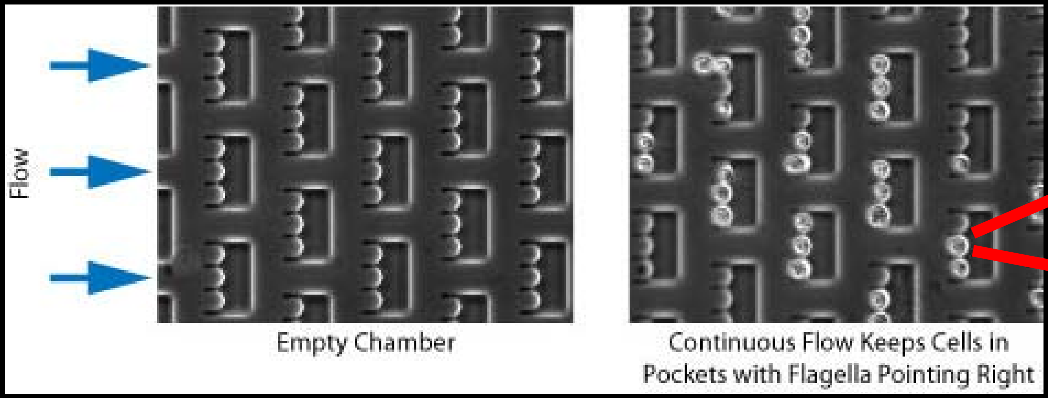


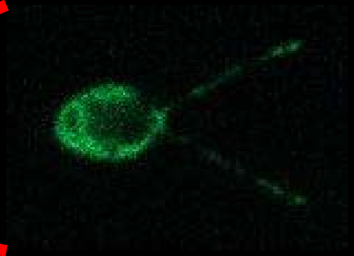
Microfluidic chamber for Chlamydomonas FRAP



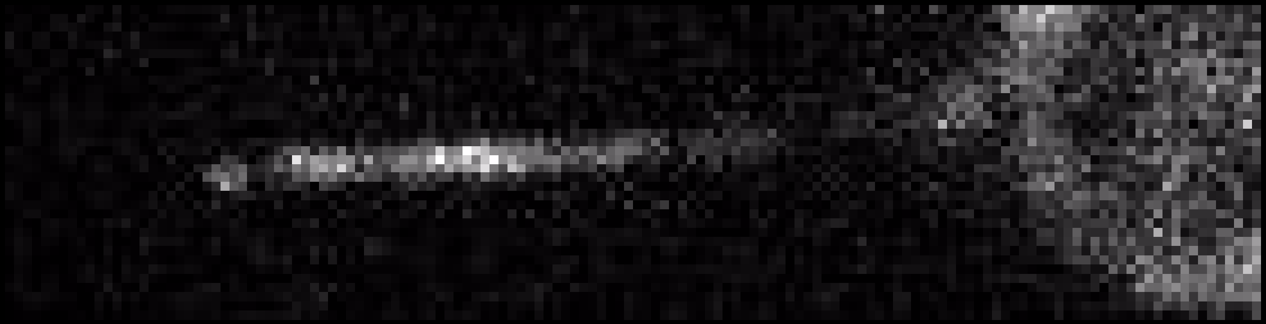


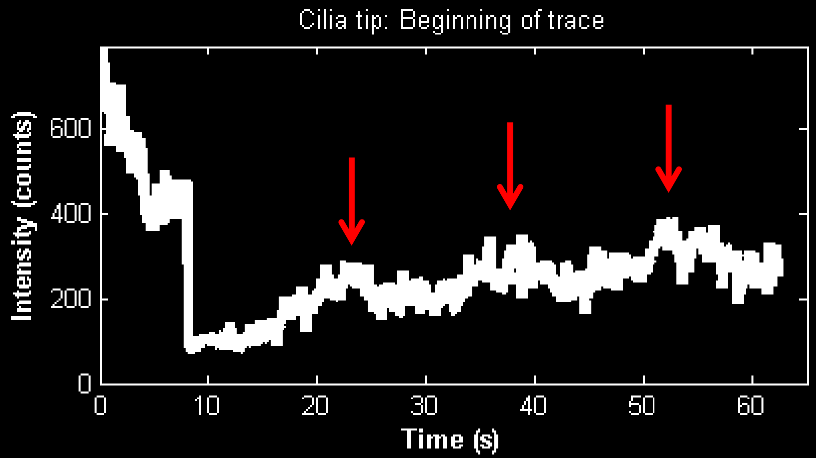
Will Ludington





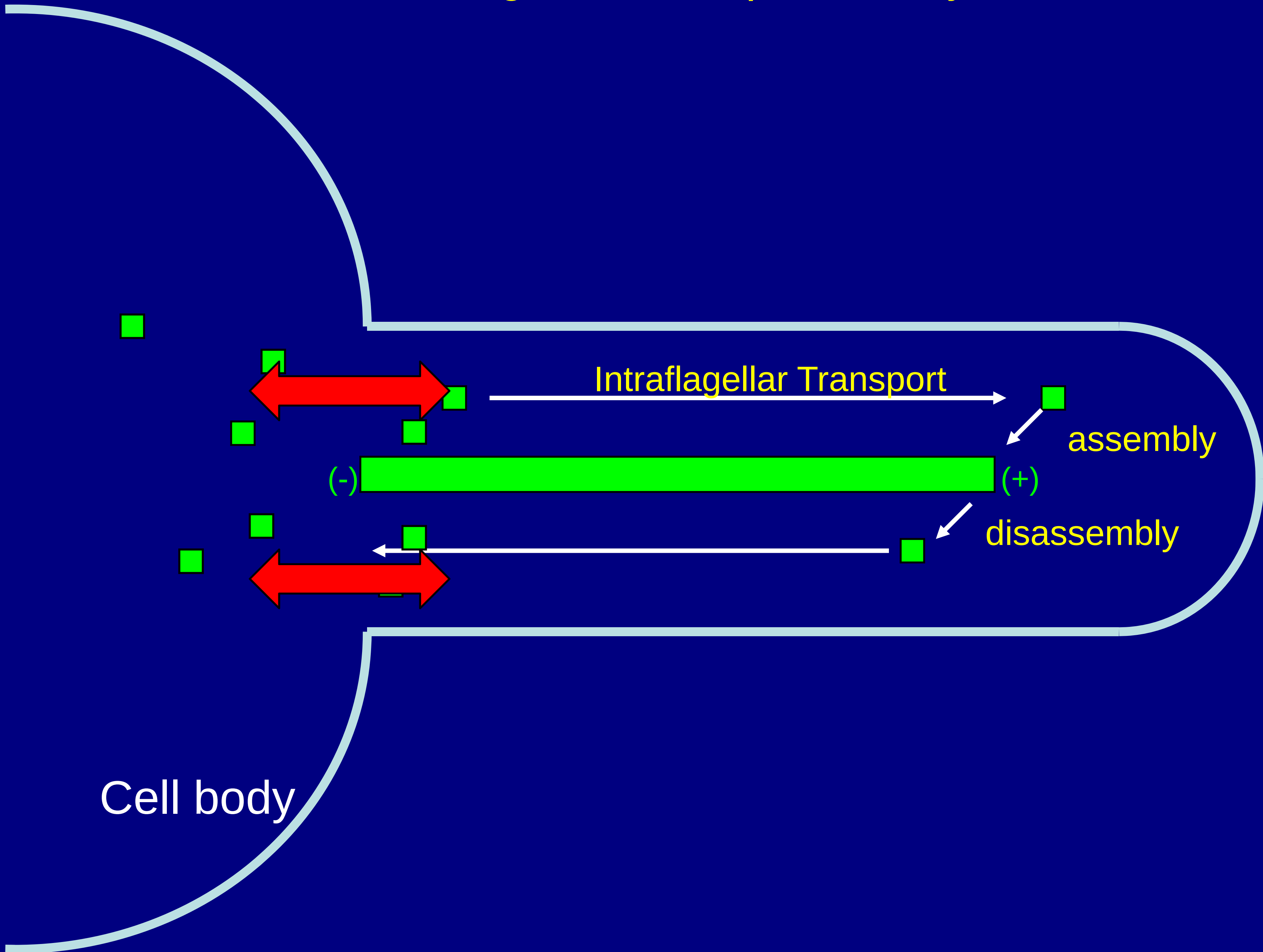
http://www.cellasic.com/ONIX_chlamy.html



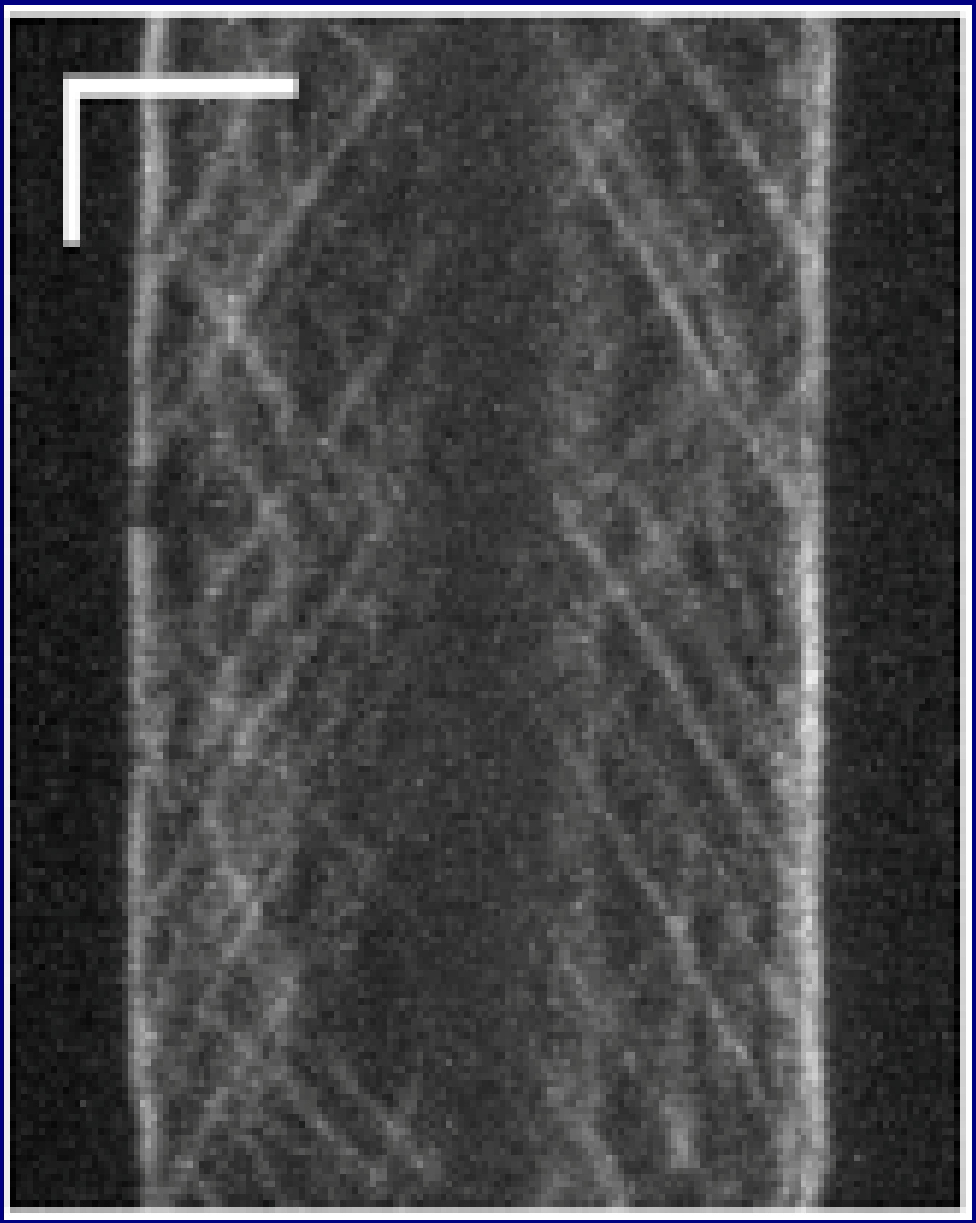


Hiro Ishikawa
Julia Gunzenhäuser
Rogelio Hernandez-Lopez
Alex Ritter

What regulates IFT particle Injection?



Quantifying IFT in living cells by TIRF imaging

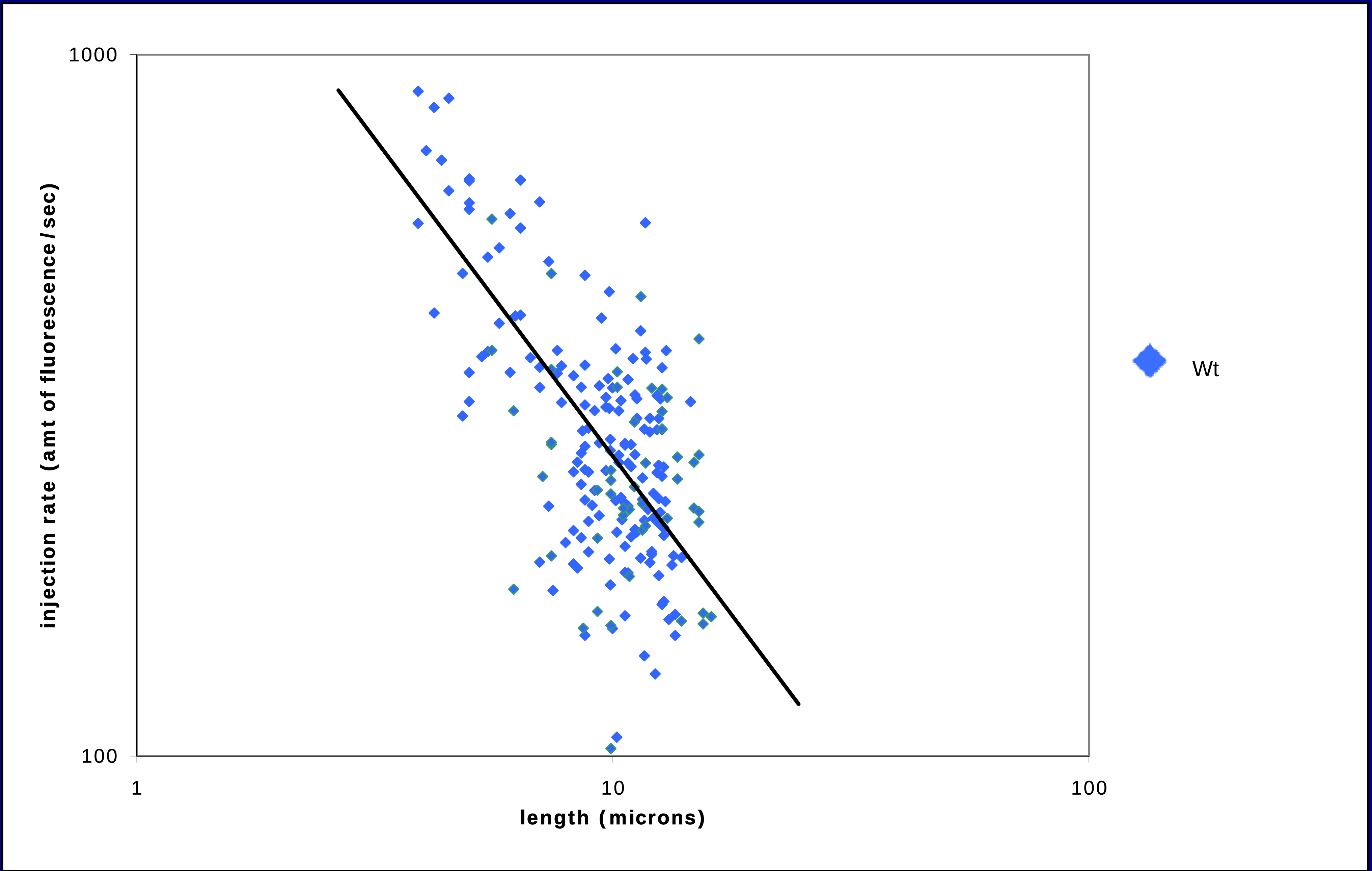


GFP tagged proteins:
KAP (Mary Porter)
IFT27 (Hongmin Qin)
IFT20 (Karl Lechtreck)

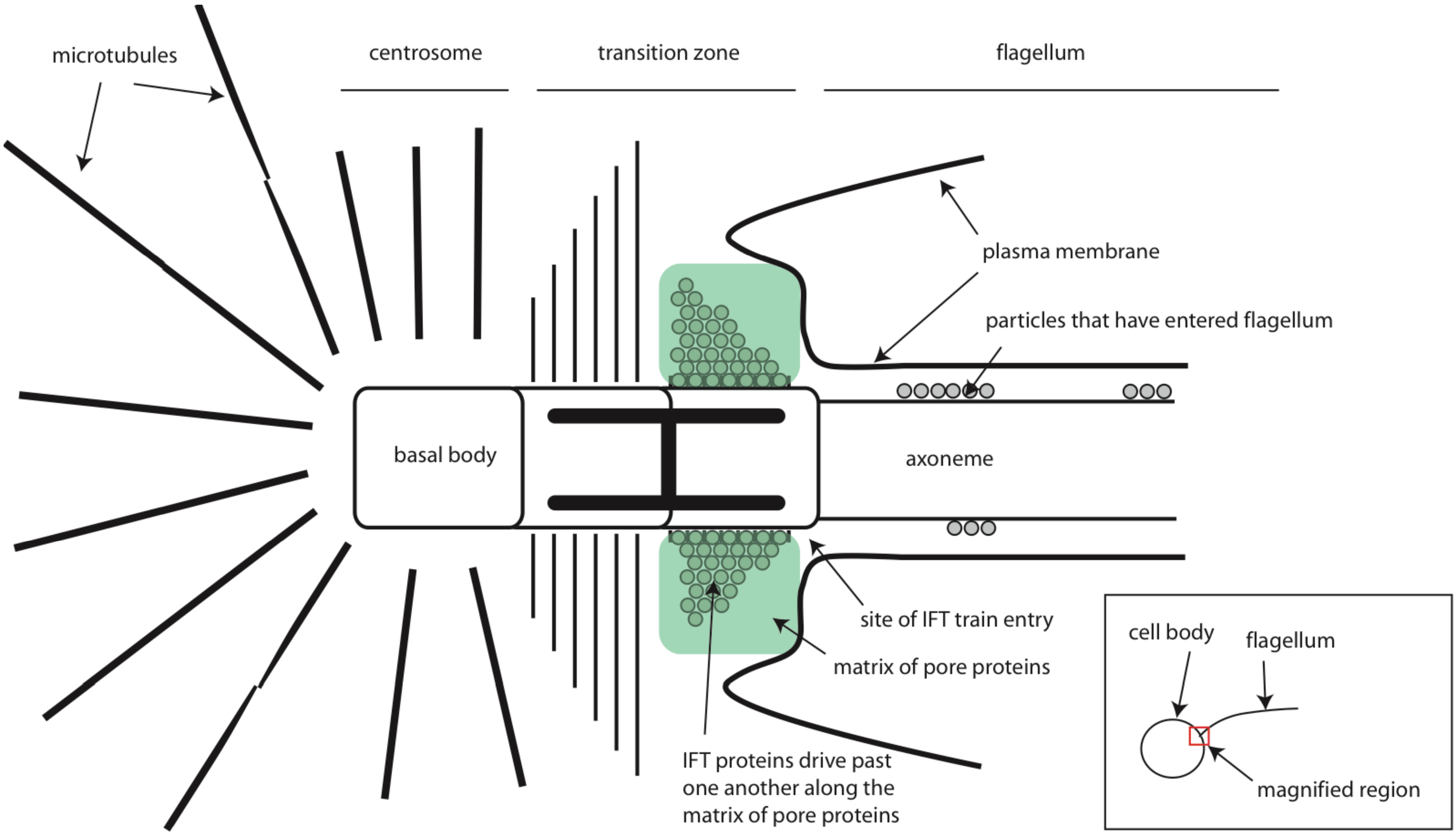
Analysis:
Frequency
Intensity
Speed

Injection Rate =
Frequency x Intensity

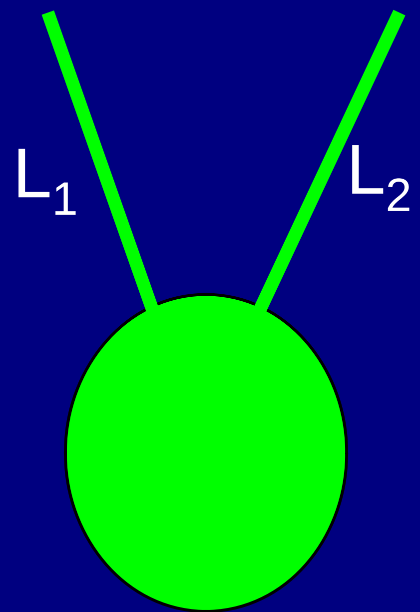
Injection rate is length-dependent



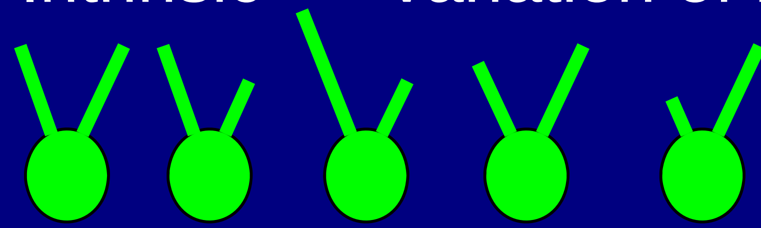
What regulates IFT injection into the flagellum?



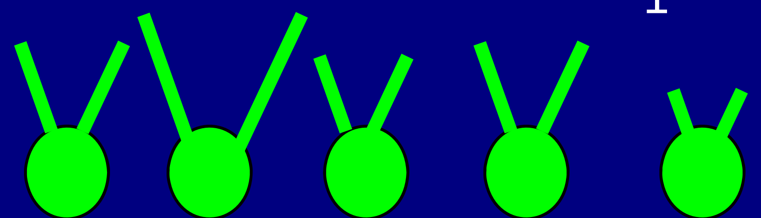
Probing length control system by measuring noise



Intrinsic --> variation of L_1 vs L_2

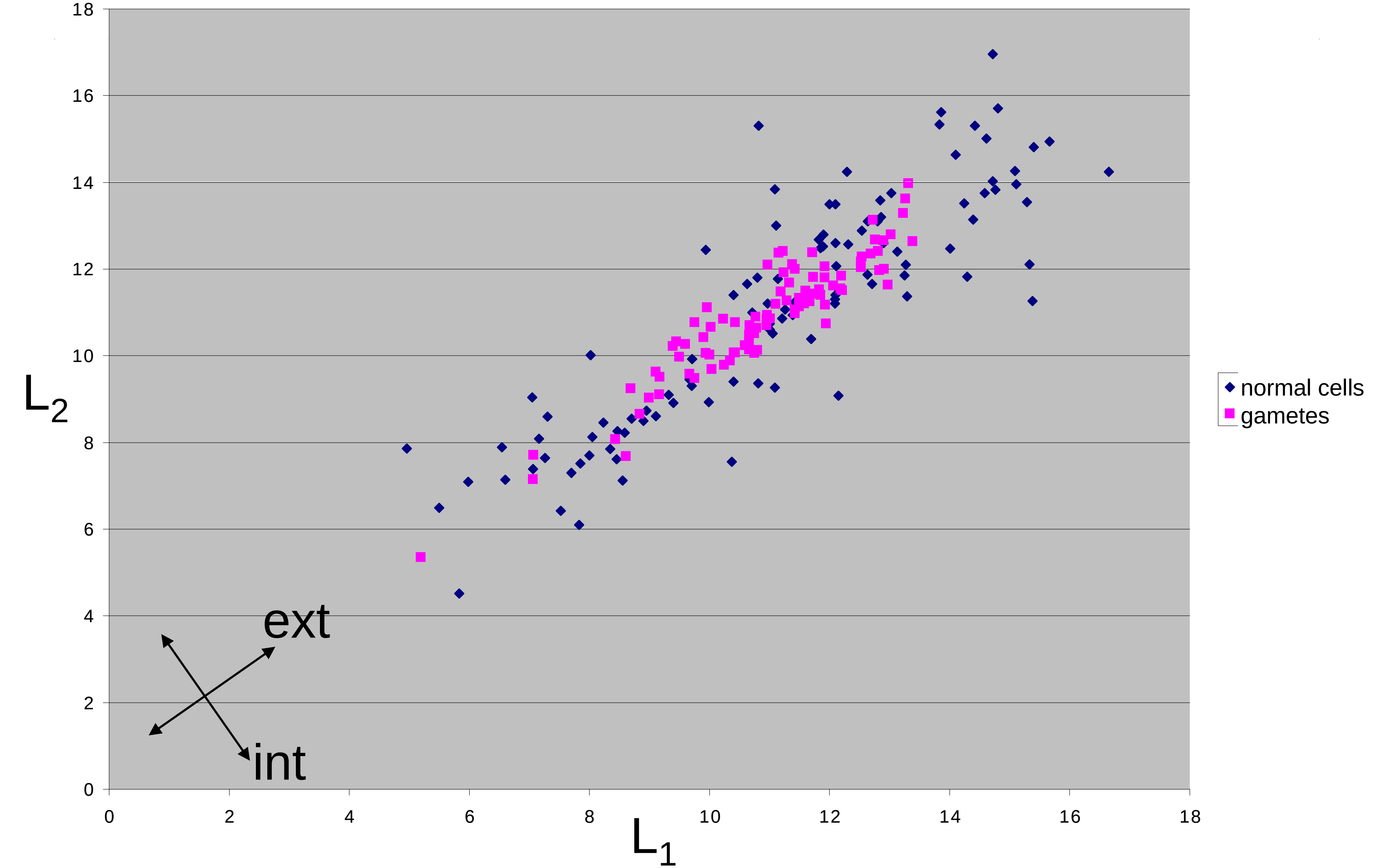


Extrinsic --> cell-cell variation of both L_1 and L_2

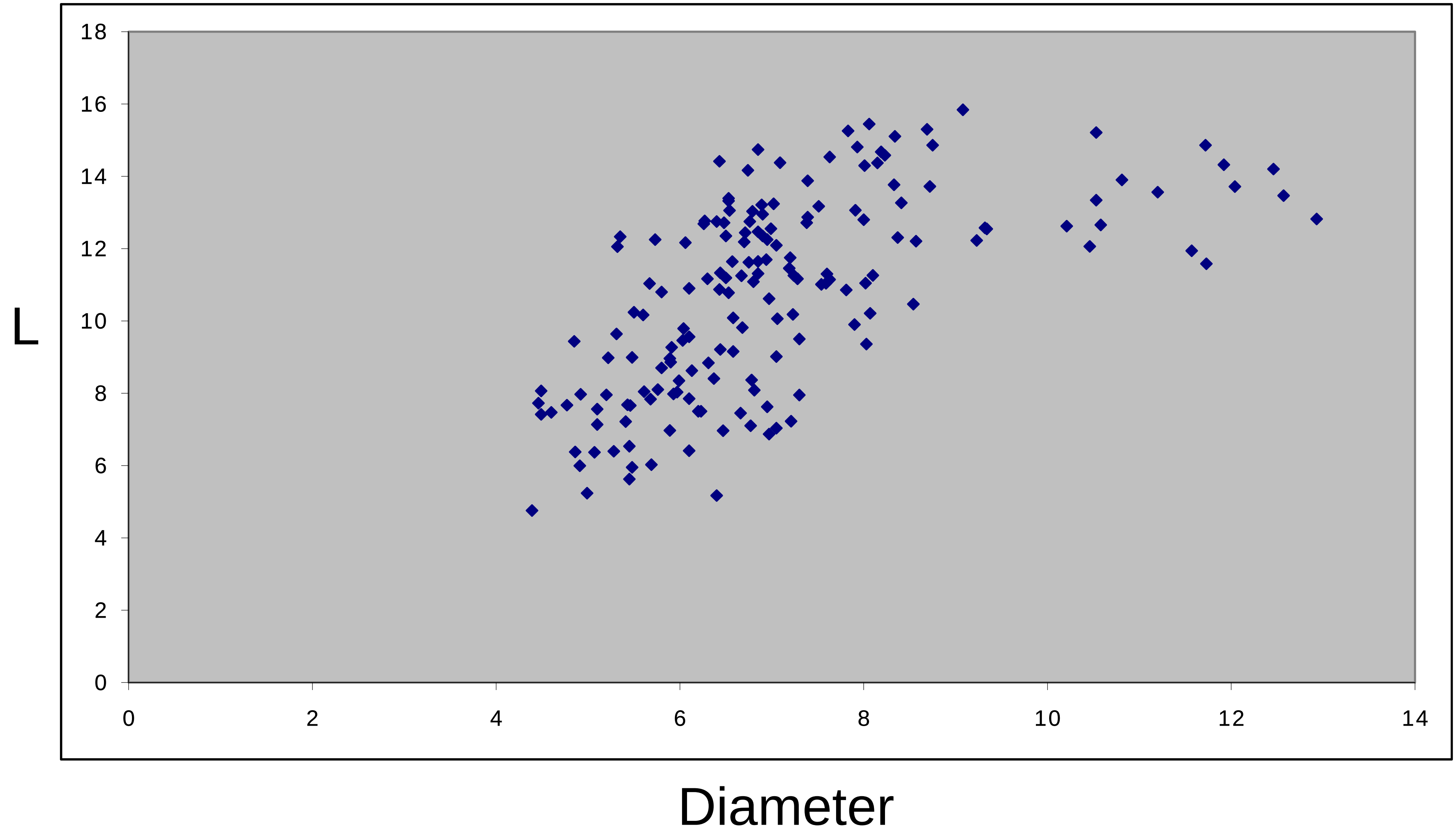


Analogous to “dual reporter” method
for noise in gene expression

Noise in wild type flagellar length

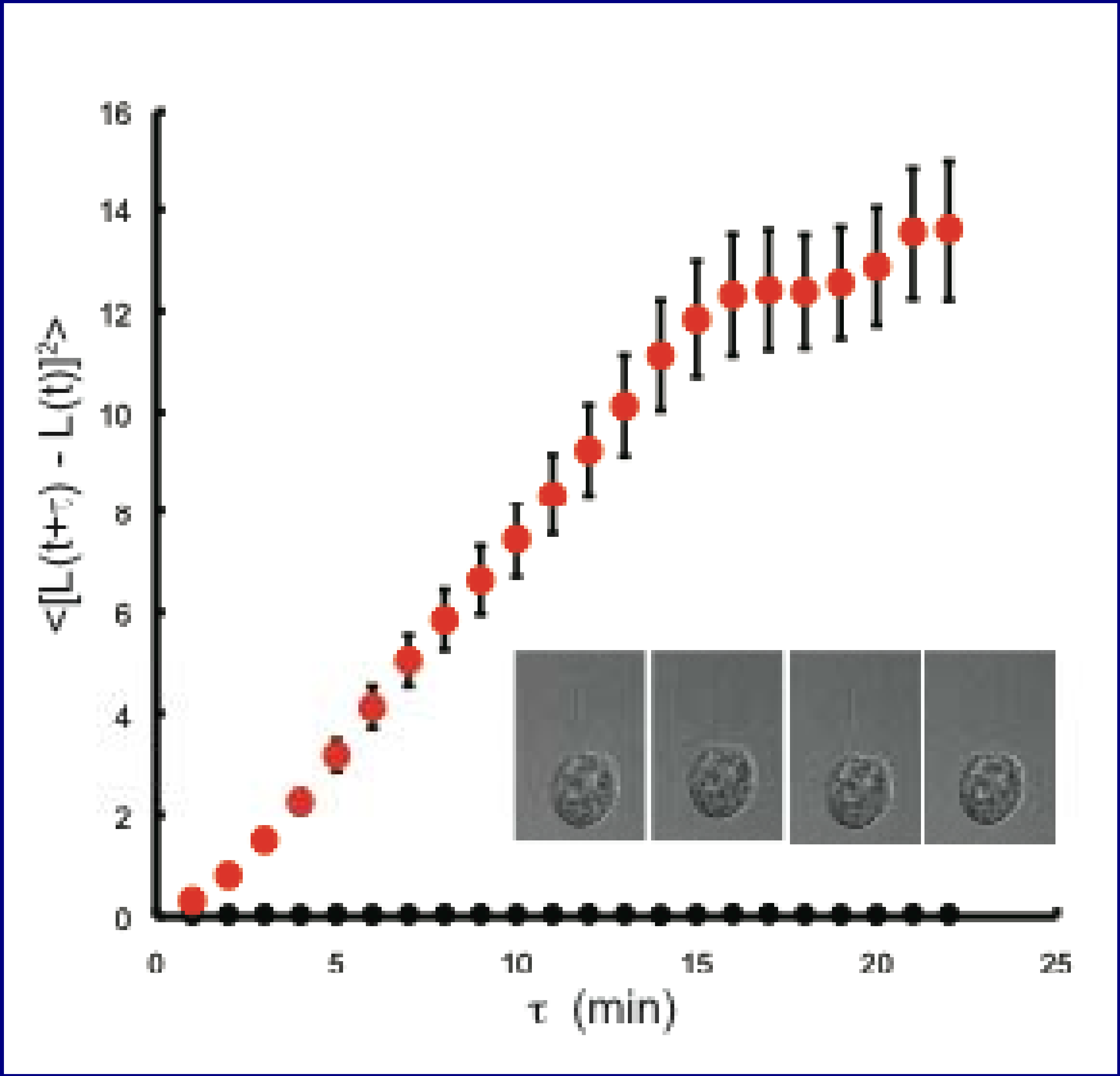


Length versus diameter



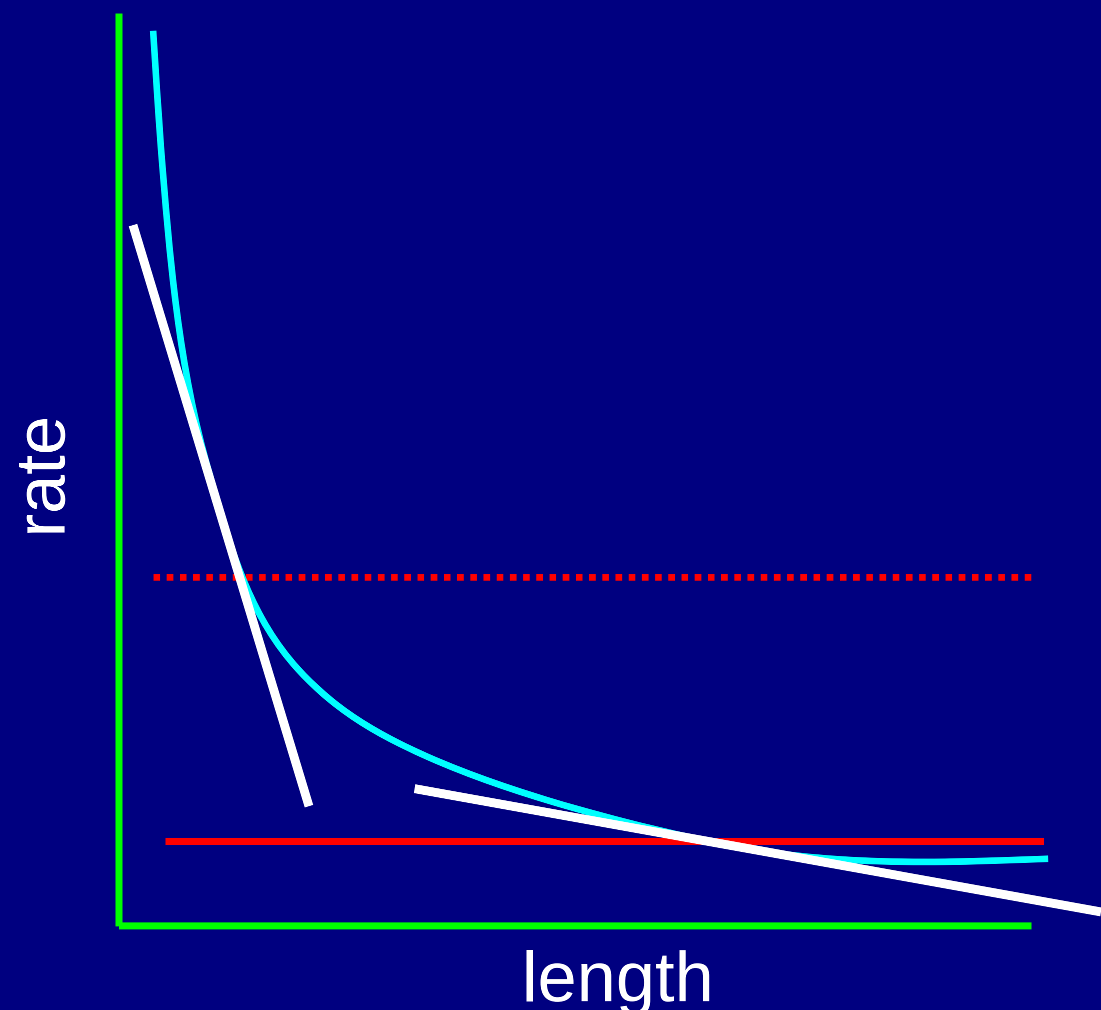
Some extrinsic noise is probably due to cell size variation

Fluctuations of flagellar length



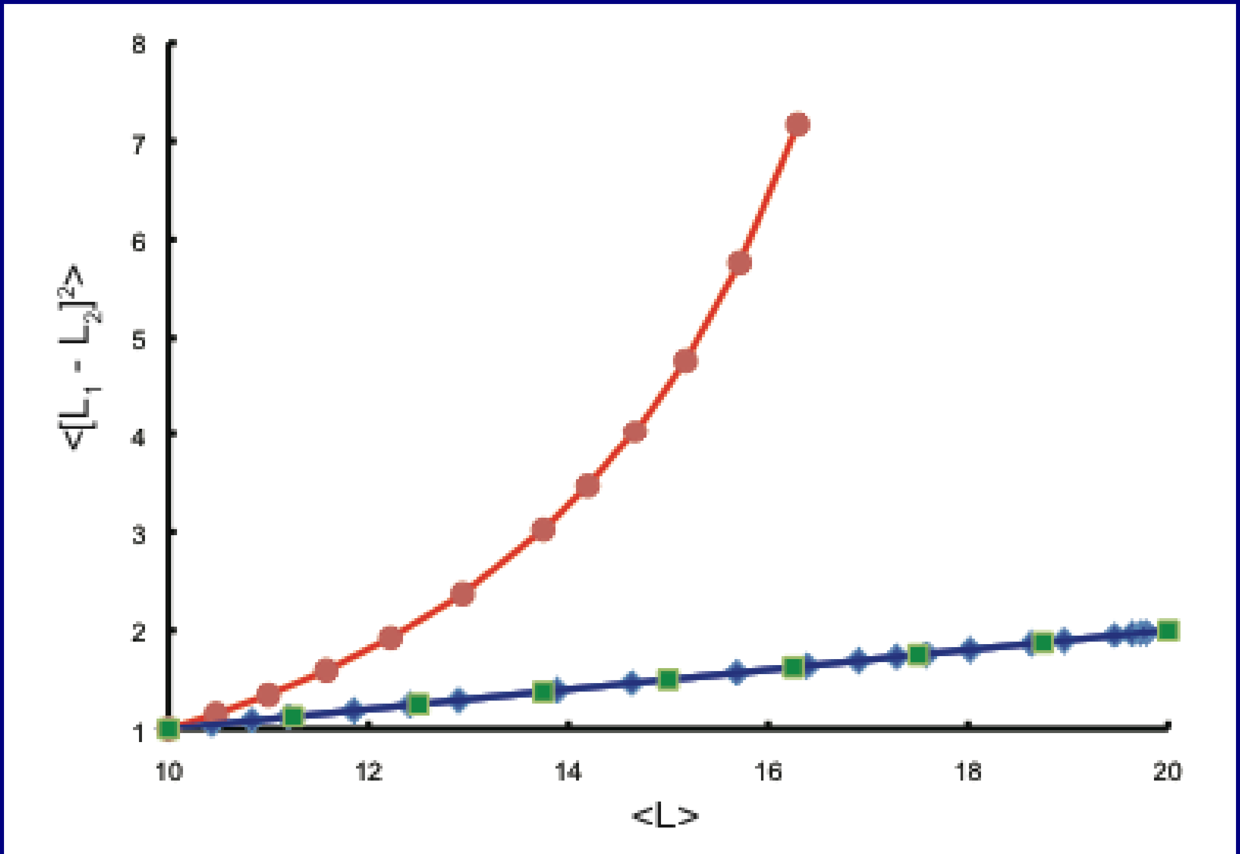
Linear Noise Model

Restoring “force” depends on length set-point



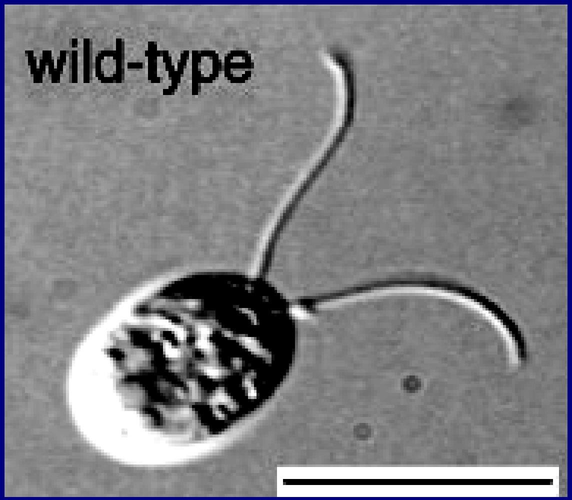
Fluctuations should be damped out more slowly in long-flagella mu

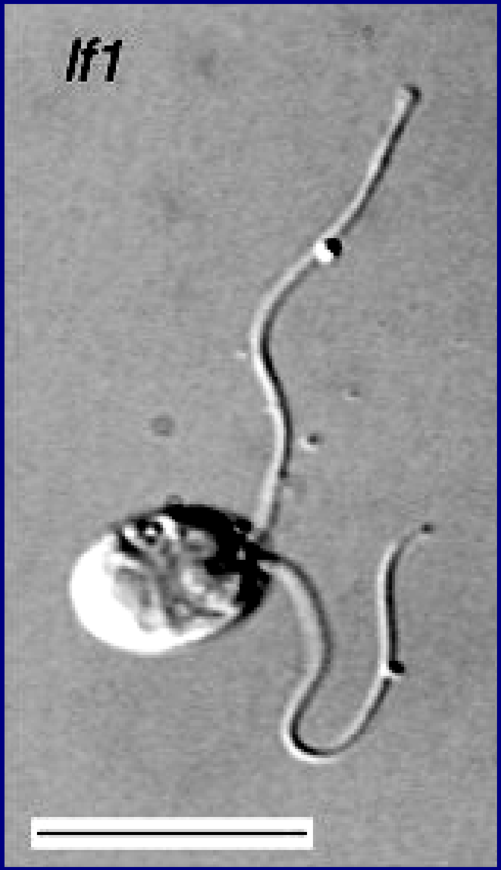
Prediction of noise model:
Any parameter change that increases length, increases noise



$$\frac{dL}{dt} = \frac{A(P - 2L)}{L} - D + \eta$$

Chlamydomonas lf mutants have increased length



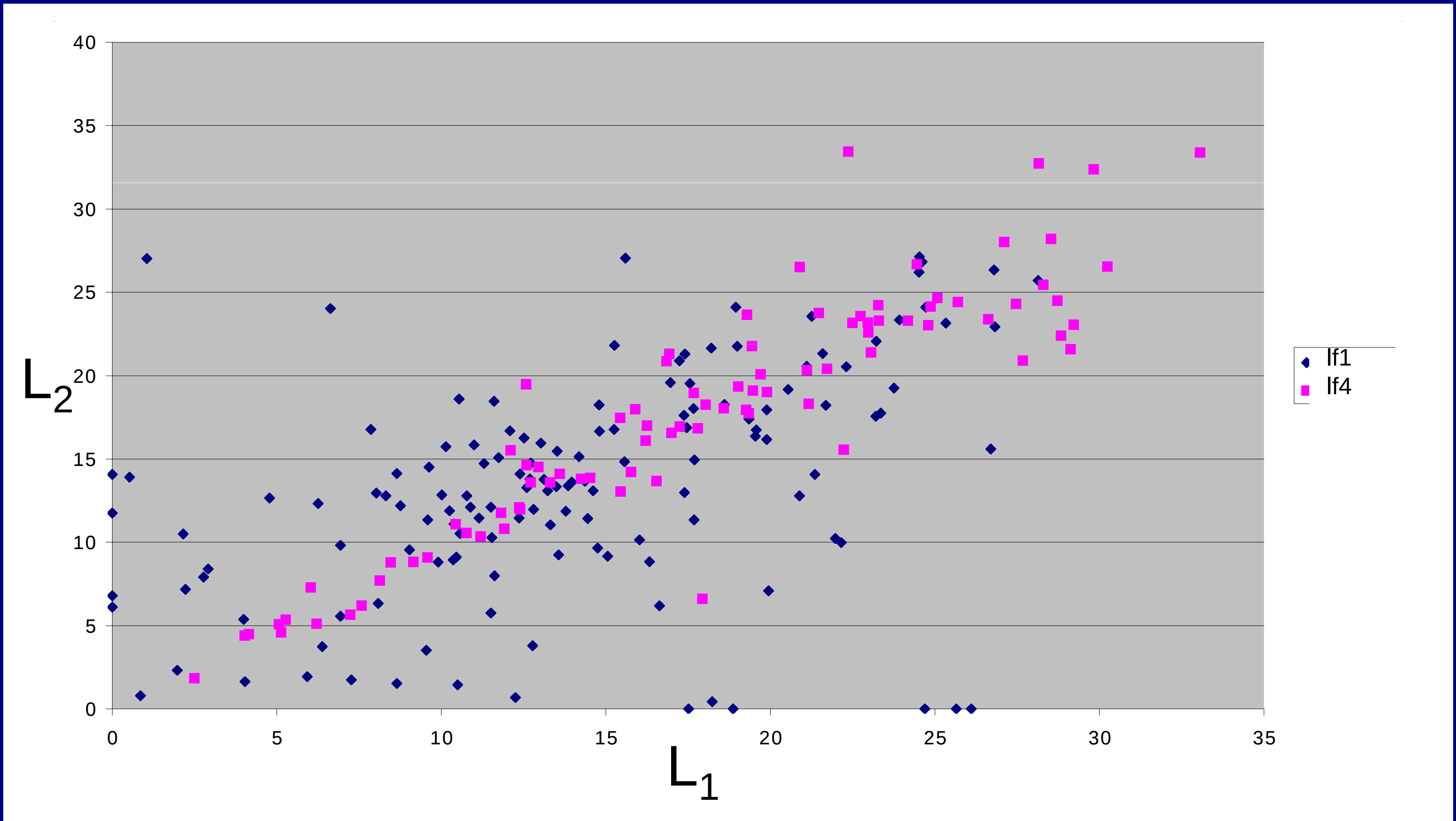


Lf1
Lf2
Lf3
Lf4

Li^+

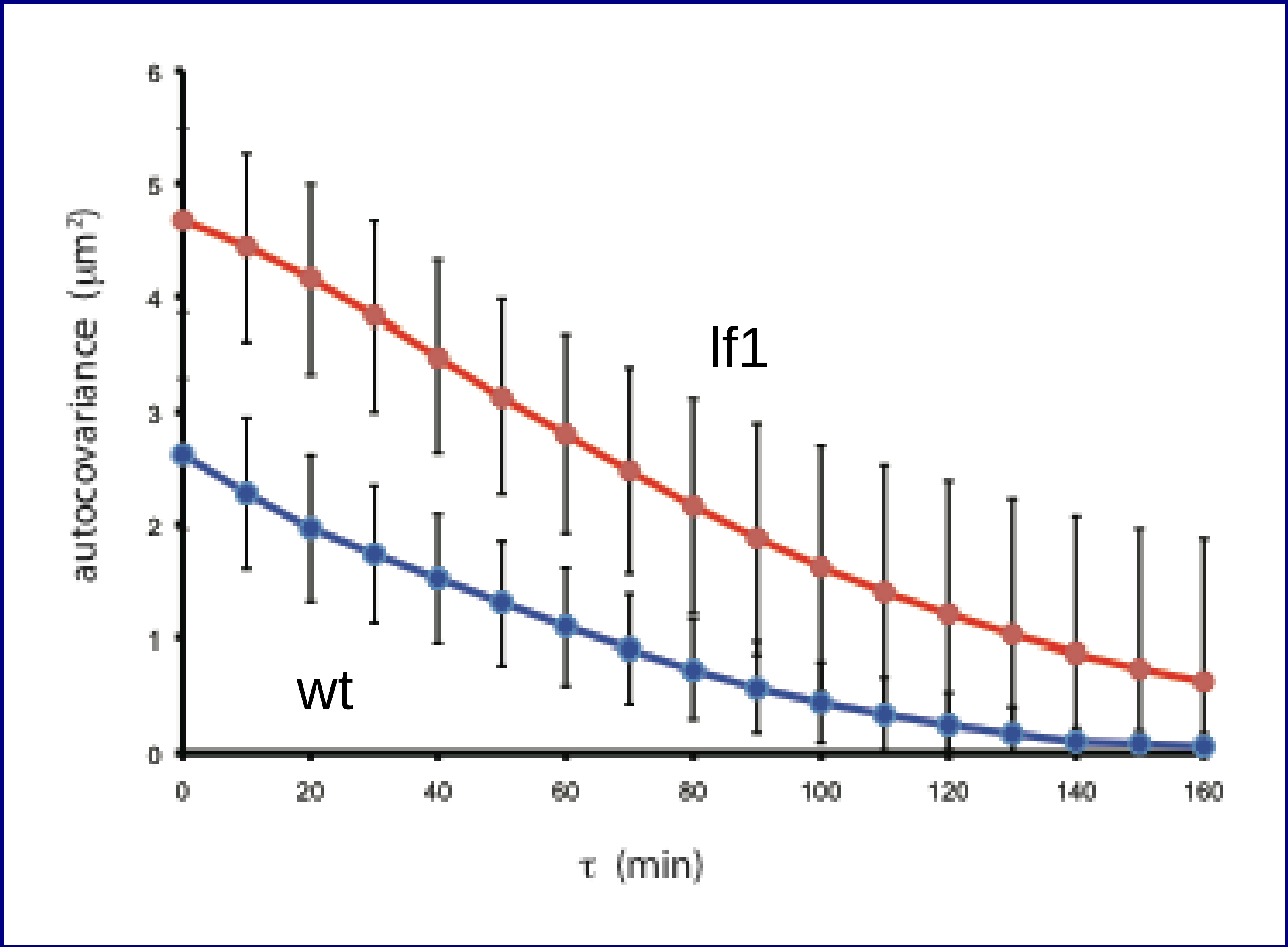
P. Lefebvre, UMN

Long-flagella mutants have increased noise

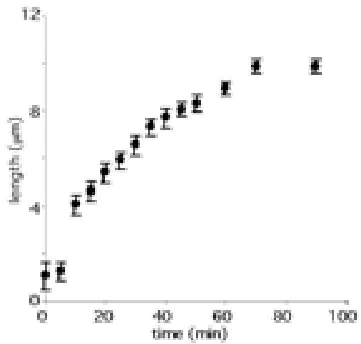


LF1 mutants - increased intrinsic noise
LF4 mutants - increased extrinsic noise

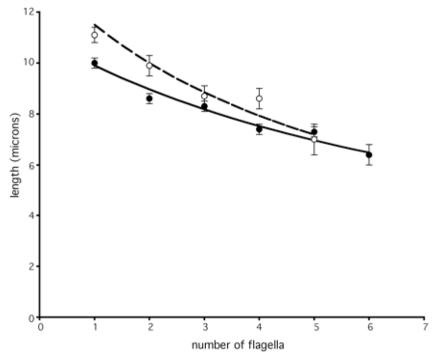
Slower damping of fluctuations in mutant with long flagella



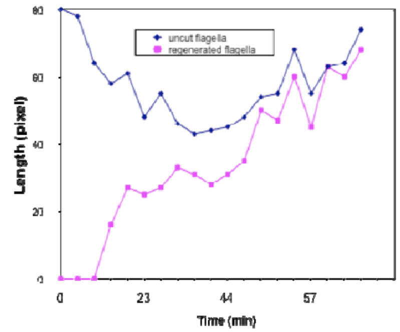
Future direction: experiment merging/parameter estimation



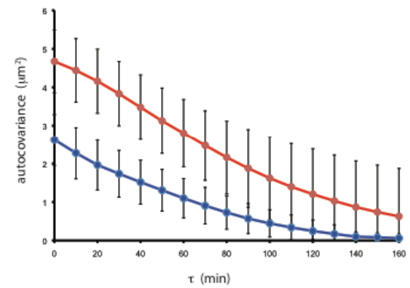
L vs t



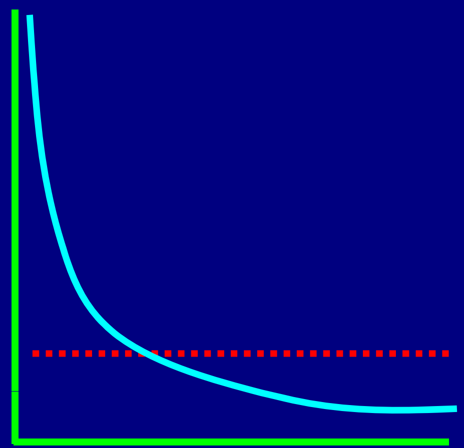
L vs N



long-zero



fluctuations



Directly measure parameters

Consistent parameter set ?

Hiroaki Ishikawa
Prachee Avasthi
Susanne Rafelski
Ben Engel
Will Ludington
Kimberly Wemmer

Mark Chan
Juliette Azimzadeh
Elisa Kannegaard
Mark Slabodnick

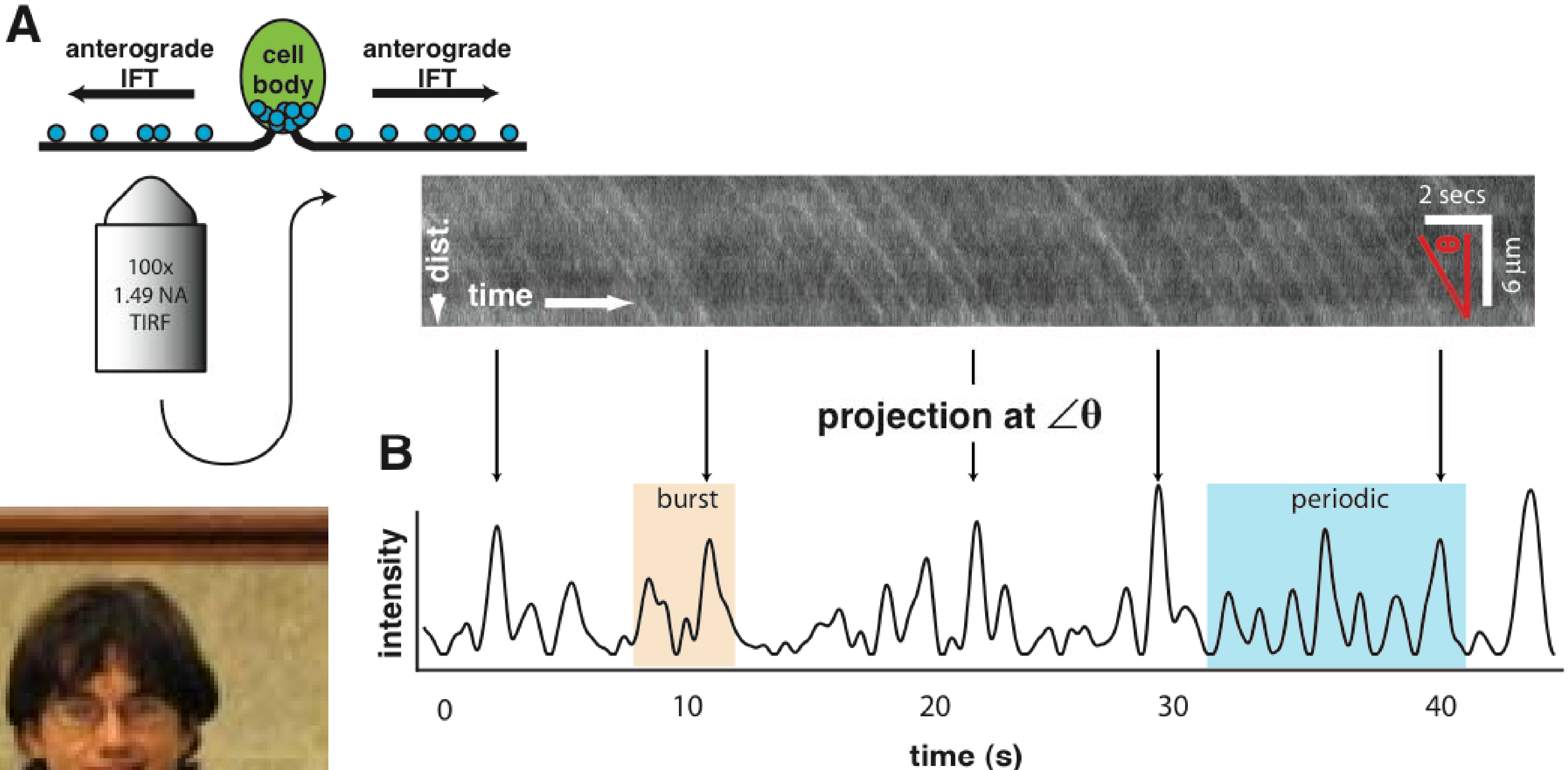
Amy Chang
Sarah Reiff

UCSF Center for Systems & Synthetic Biology

National Institute of General Medical Sciences

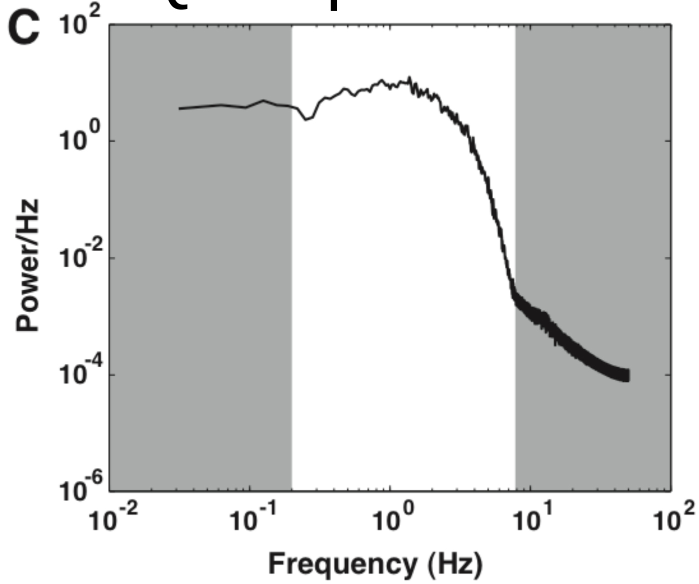
Additional Thanks Joel Rosenbaum, Mary Porter, Hongmin Qin, Karl Lechtreck, George Witman, Kurt Thorn, Linda Shi, Michael Berns (UCSD), David Kovar (U. Chicago)

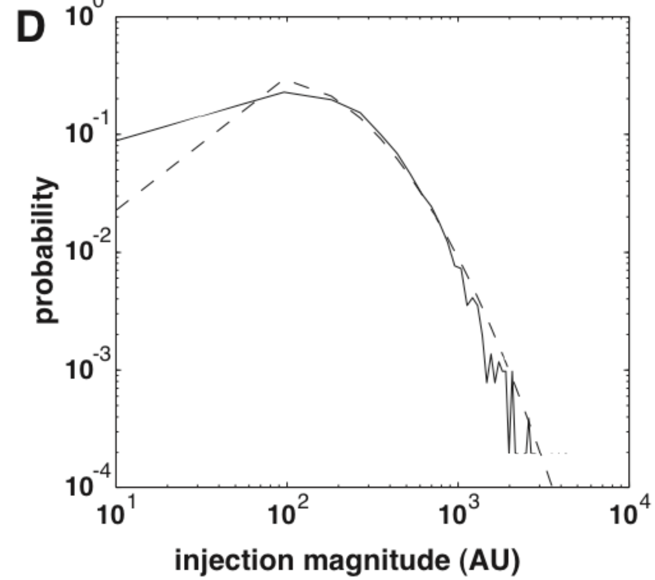
Time-series analysis of IFT injection



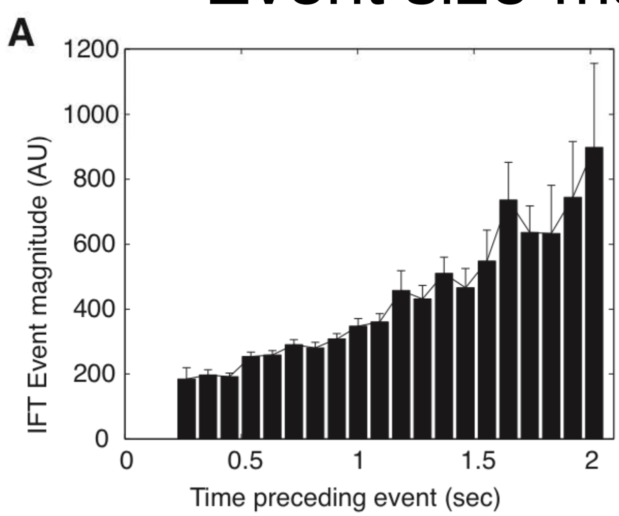
Will Ludington

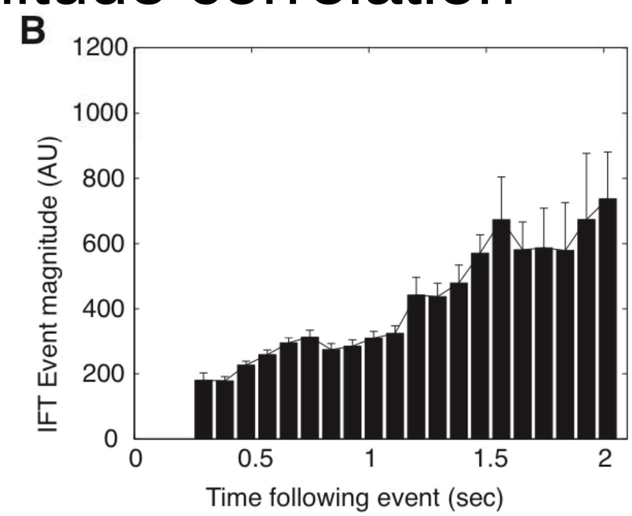
Quasiperiodic Fat-tail distribution





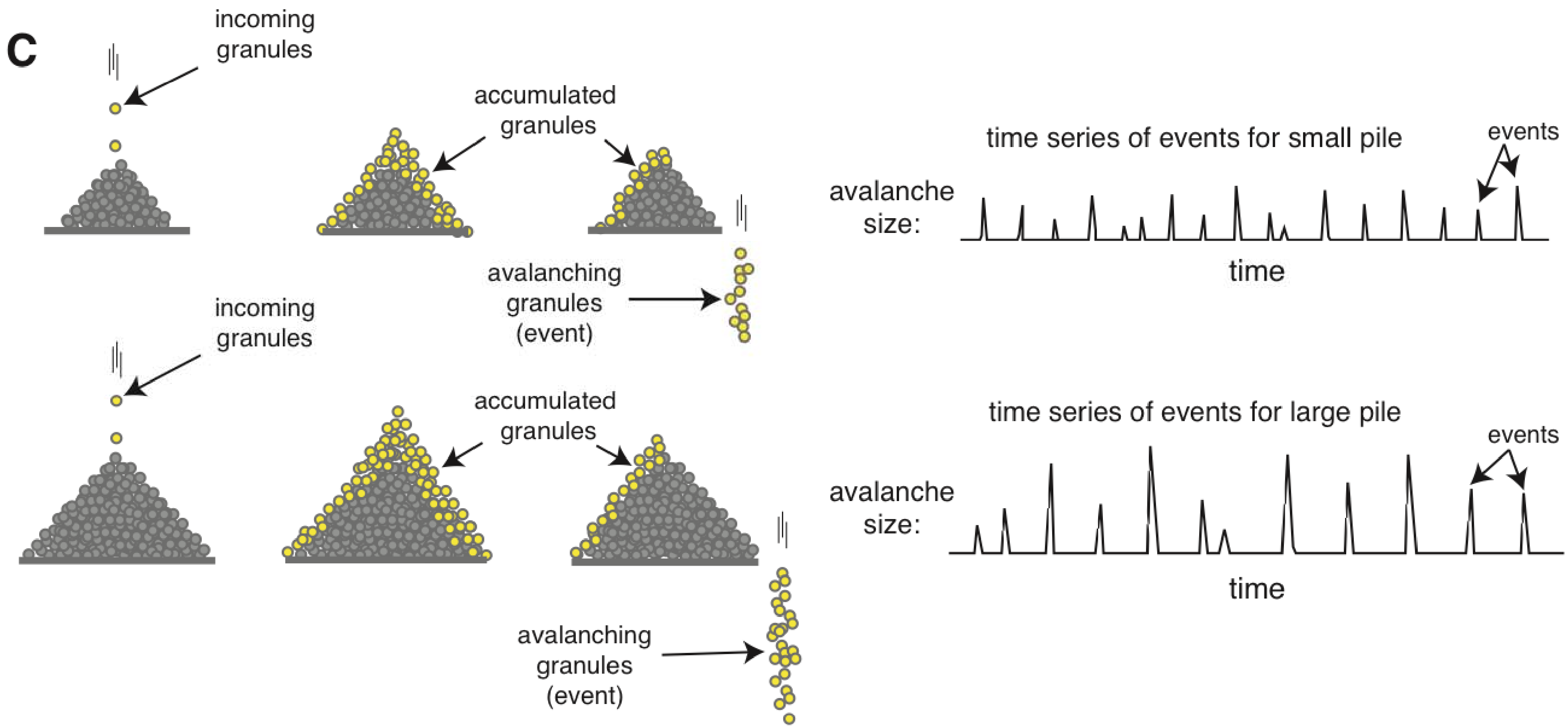
Event size-magnitude correlation





Also: bursting, long-memory process

IFT shares the properties of Avalanche-like Systems
Earthquakes, Sandpiles

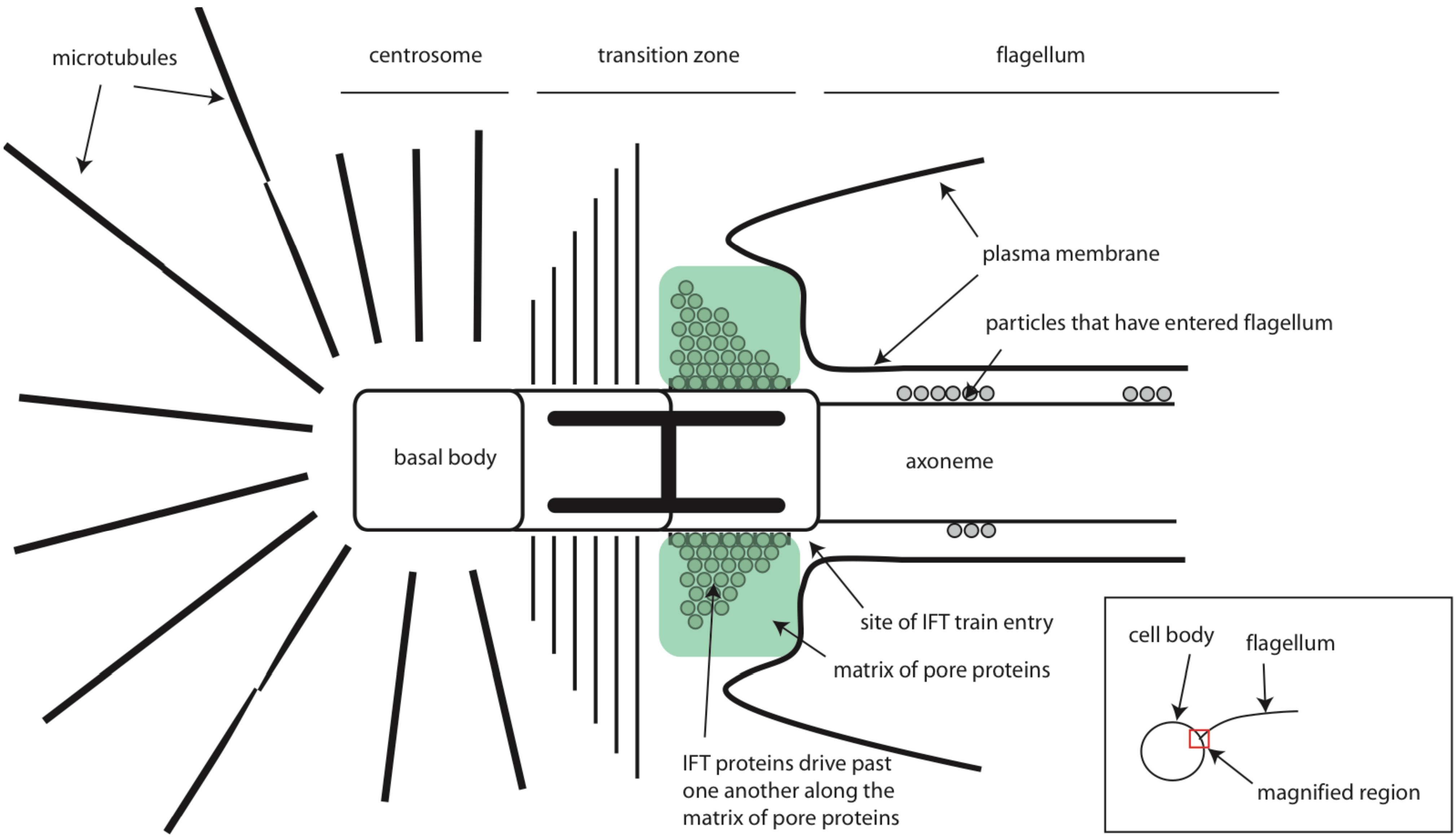


-Cellular automata-based avalanche model
yields observed IFT dynamics

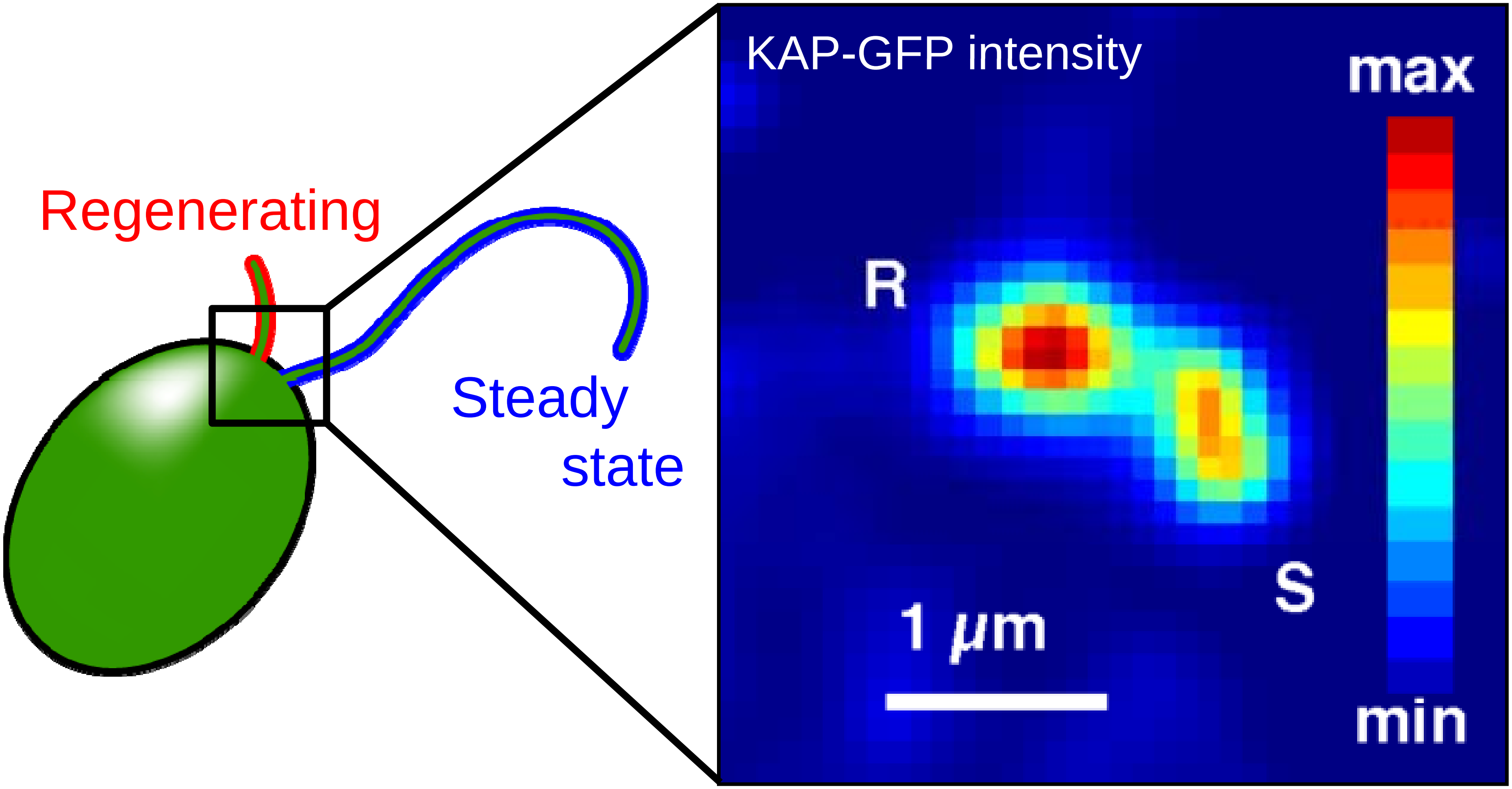
-Accounts for IFT train size & frequency changes

-Dynamics are regulated by <u>accumulation</u>

Could IFT injection be regulated by basal body recruitment?



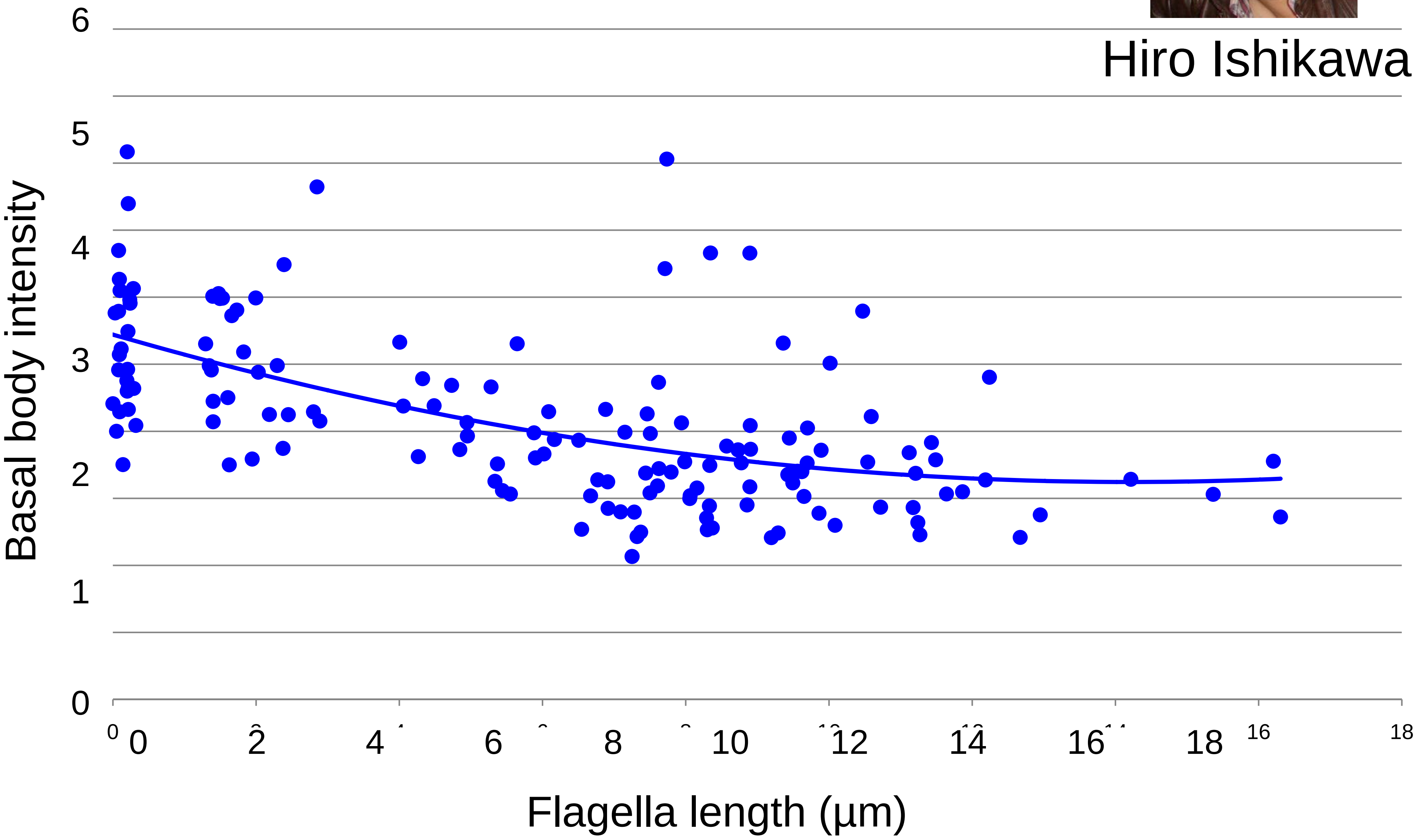
Short flagella recruit more IFT Protein than long flagella



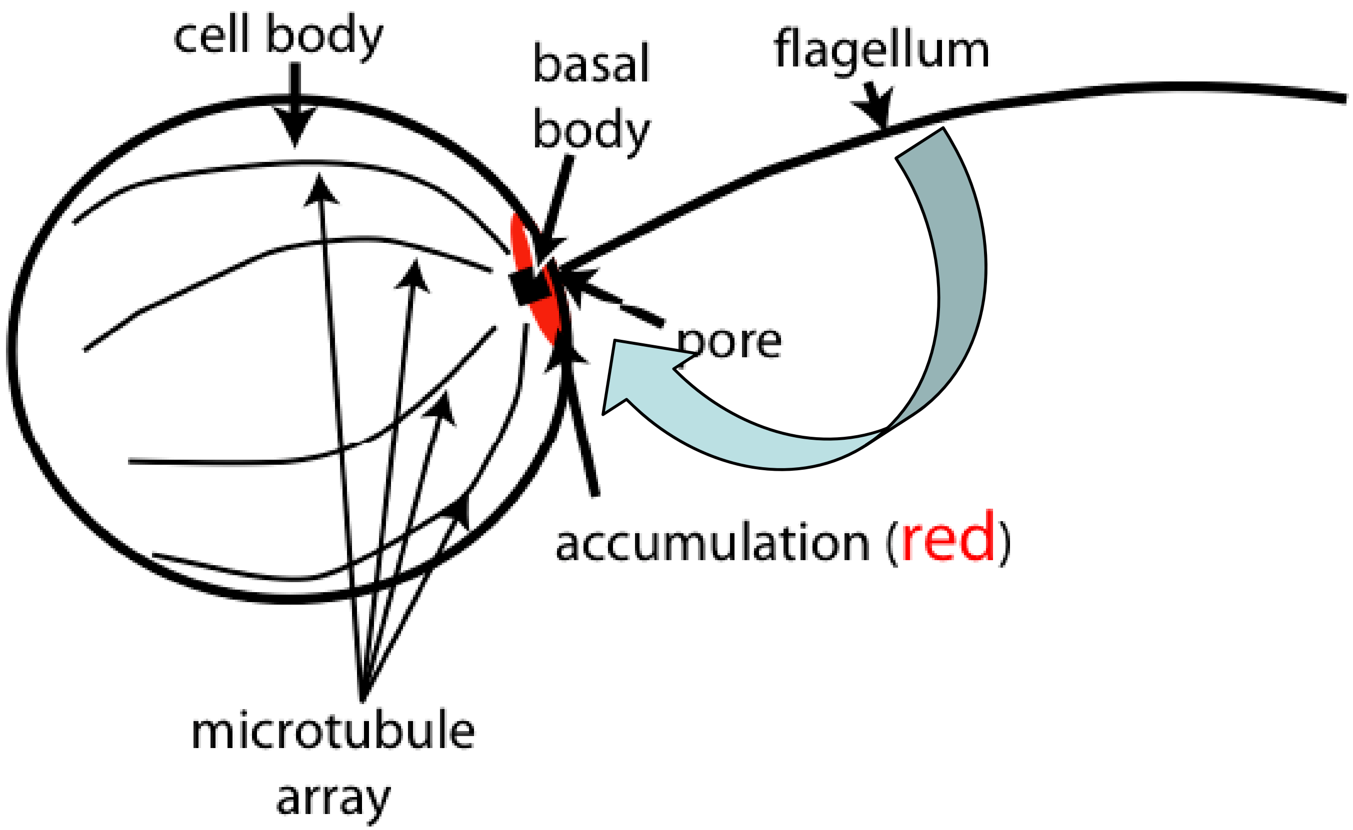
Will Ludington

Accumulation of IFT protein at basal body is a decreasing function of length

Hiro Ishikawa

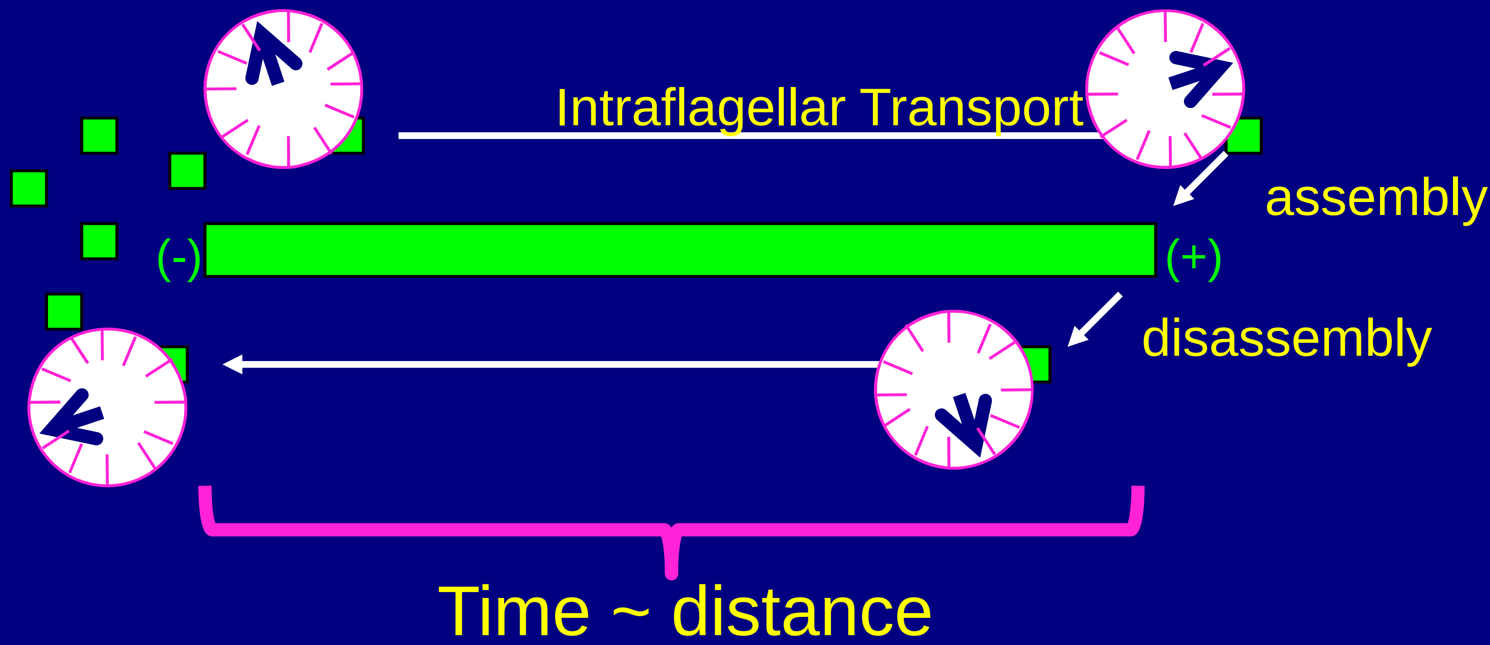


Flagellar length regulates accumulation at basal body



How does the cell know how long the flagellum is?

Approach #1: Use our imagination
Time-of-flight length sensor



Short: IFT particles return before hydrolysis
Long: Hydrolysis occurs en route

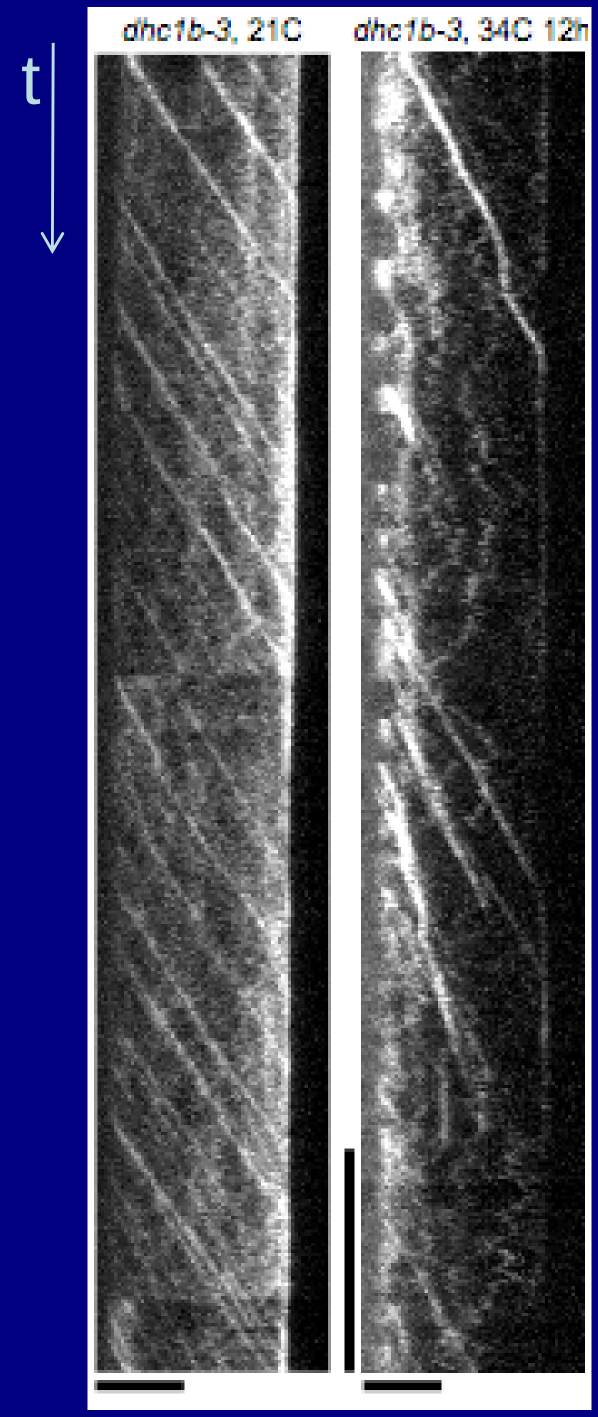
GTP/GDP ratio tells you about length

Prediction for time of flight:

slow retrograde IFT
should mimic long flagella
→ decrease injection
increase frequency
decrease magnitude

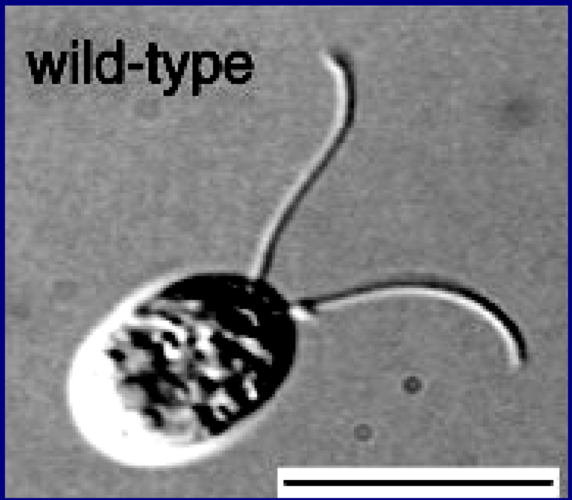
Result is the OPPOSITE ->
flagellum thinks it is too short

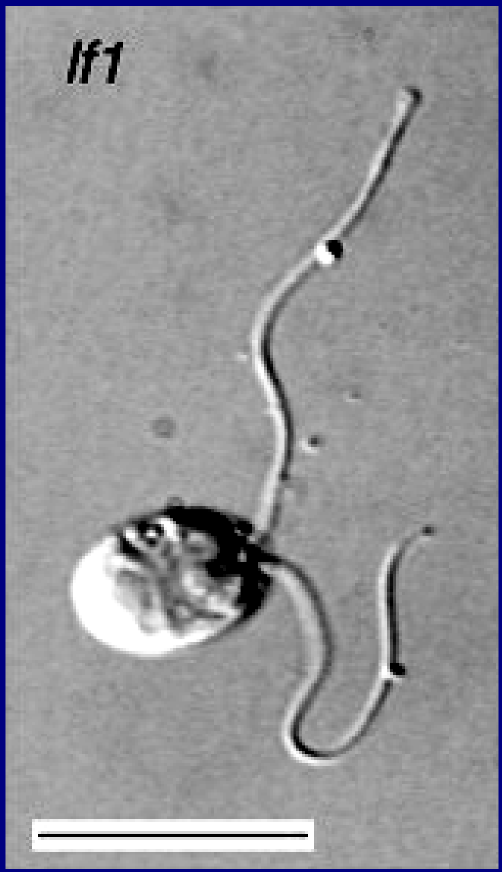
Ben Engel
(MPI Martinsried)



Approach #3: Genetics

Kim Wemmer

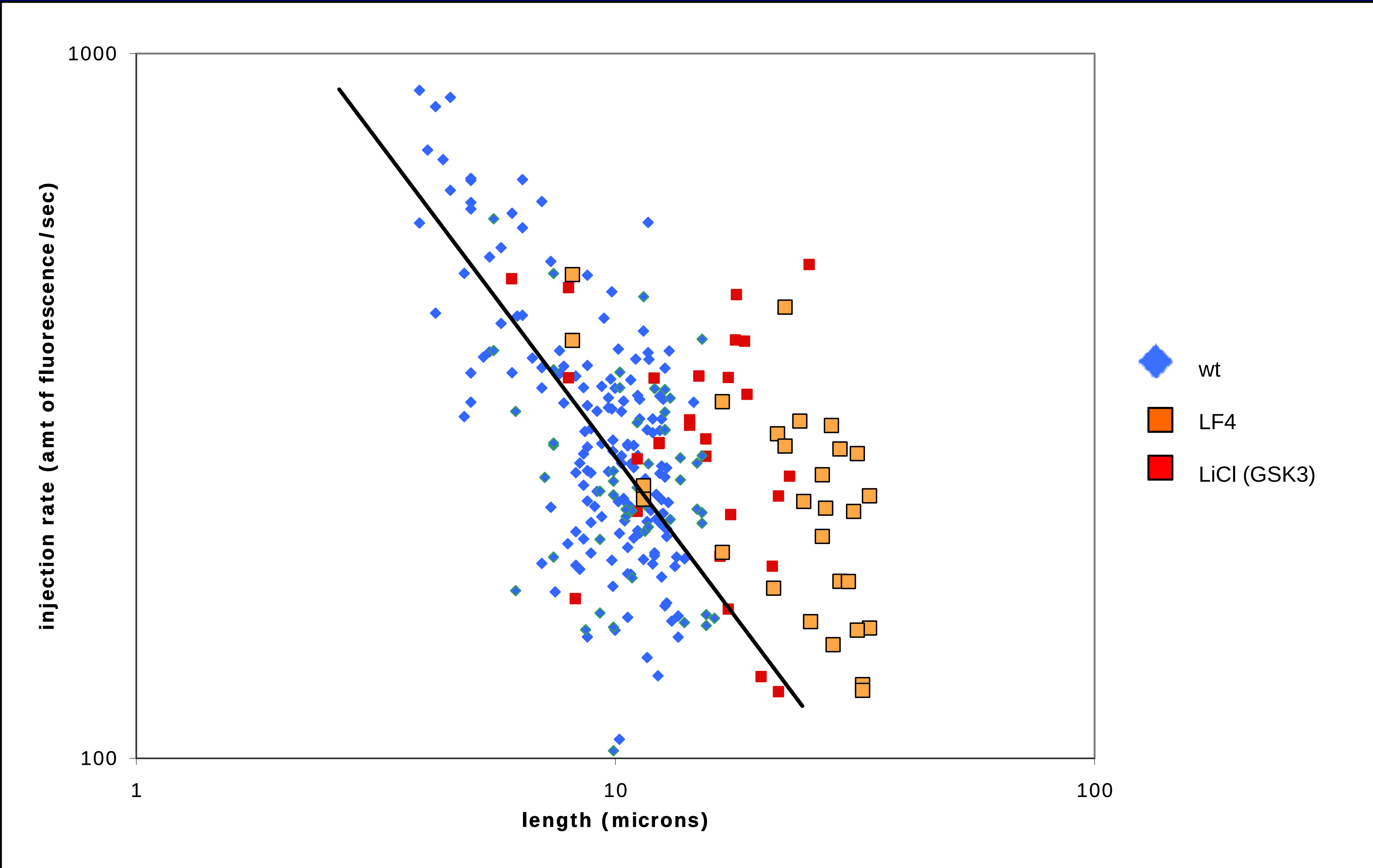




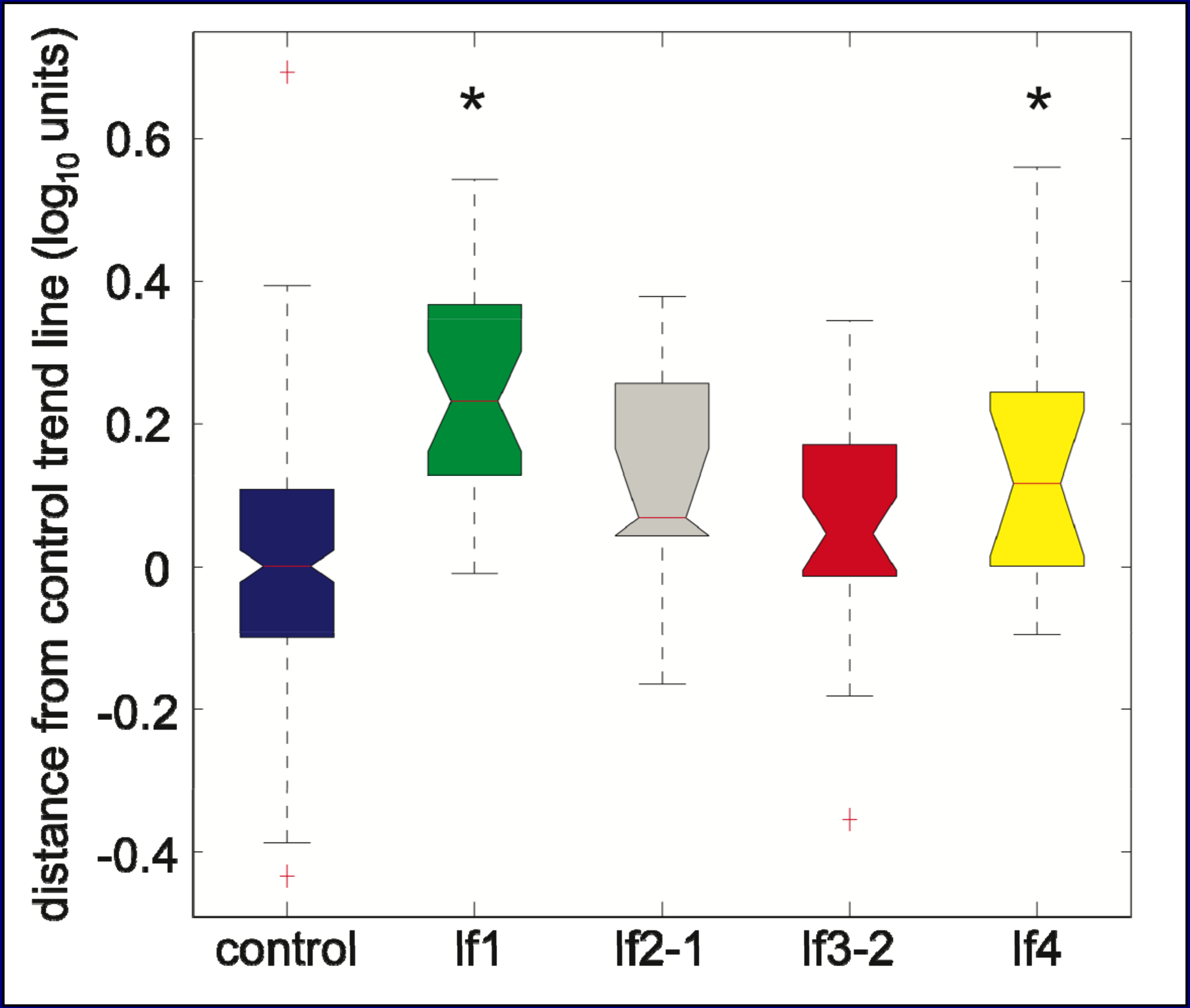
Lf1
Lf2
Lf3
Lf4

Li^+

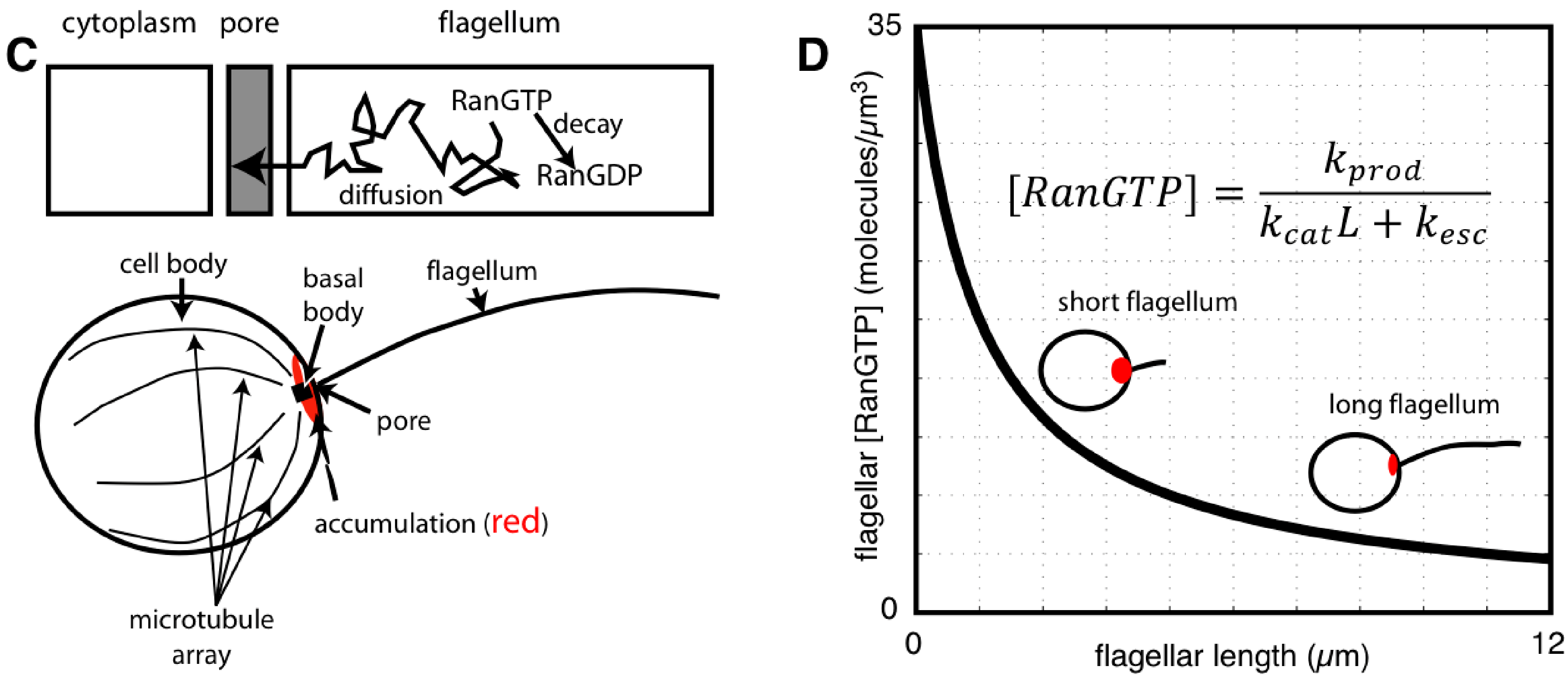
Injection rate changes in *lf4*
But remains length-dependent



lf mutants alter dependence of IFT injection versus length

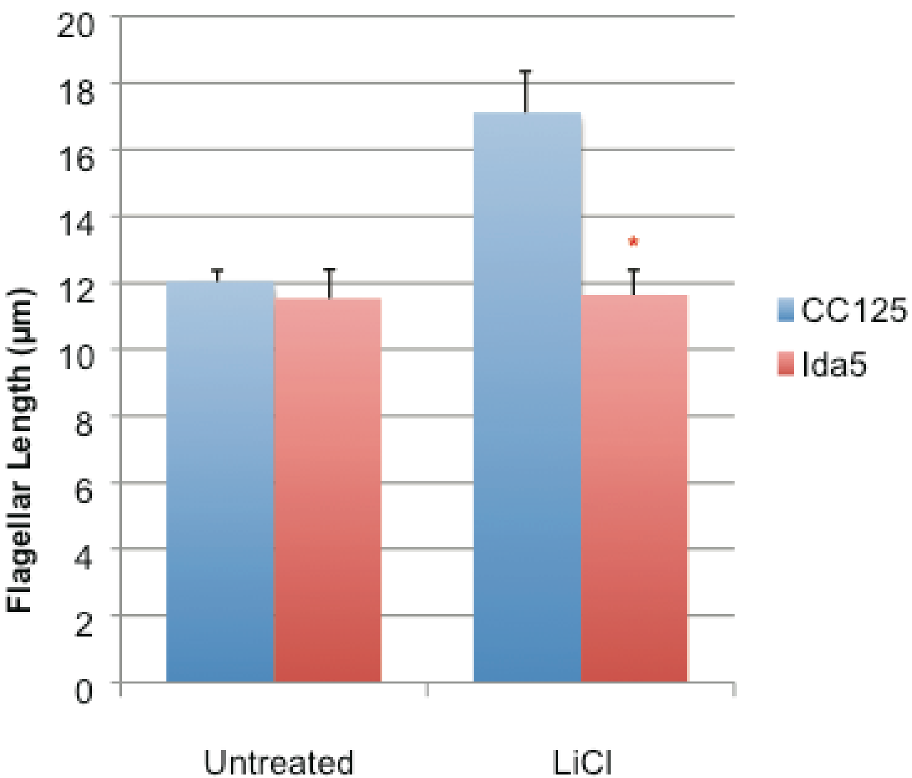


Approach #2:
Does known biochemistry of flagella suggest a plausible length sensor?



RanGTP could act as a flagellar volume sensor

Chemical/Genetic Epistasis:
Actin mutant suppresses effect of lithium on length



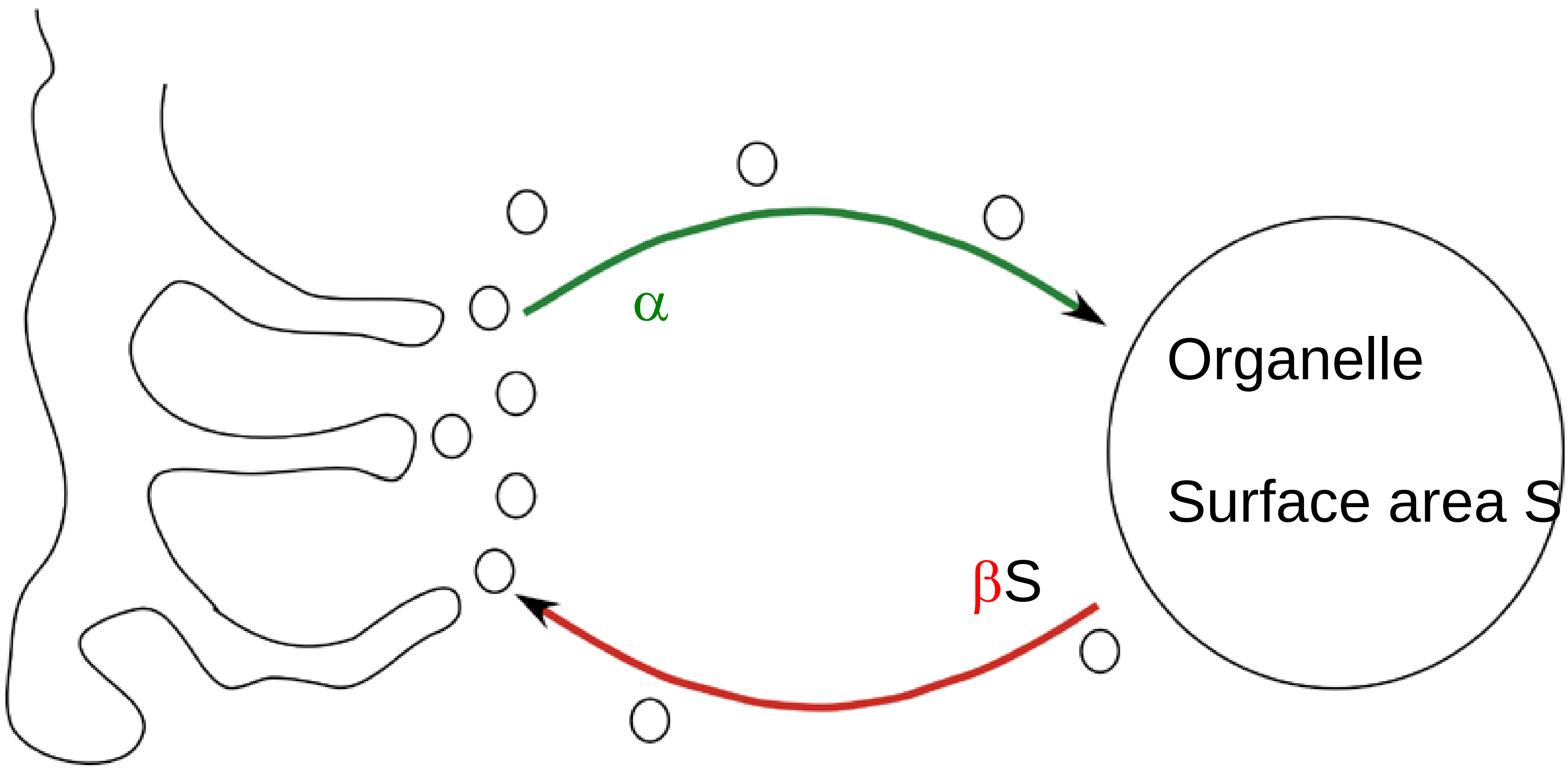
Lithium LF4

Actin

Length increase

Prachee Avasthi Crofts

What about other organelles?



Steady-state solution: $\alpha = \beta S$

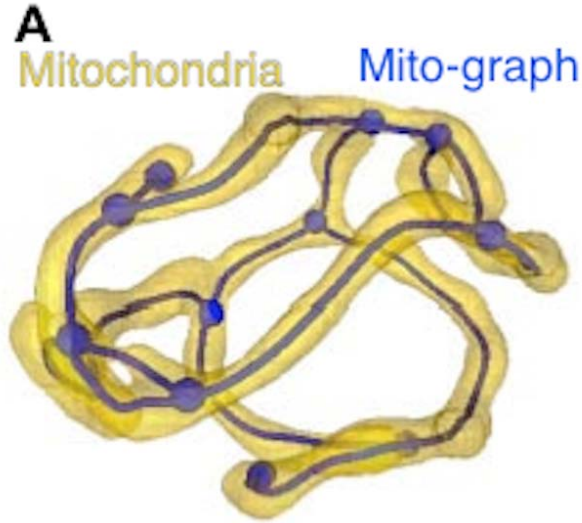
Steady-state surface area $S=\alpha/\beta$

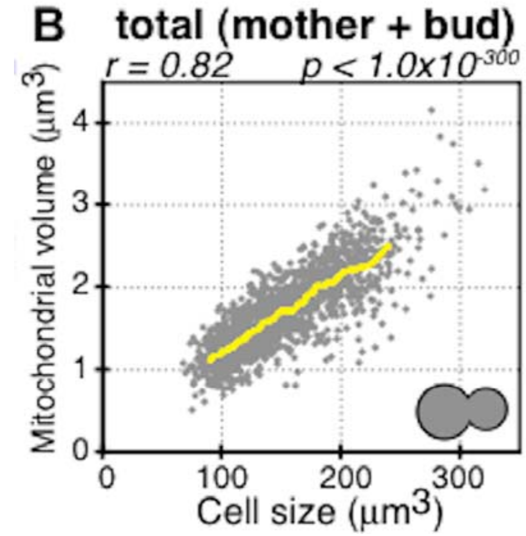
Marshall, WF. 2002. *Trends Cell Biol.* 12,414-9.

Organelle Size Scaling in Budding Yeast

Mitochondria

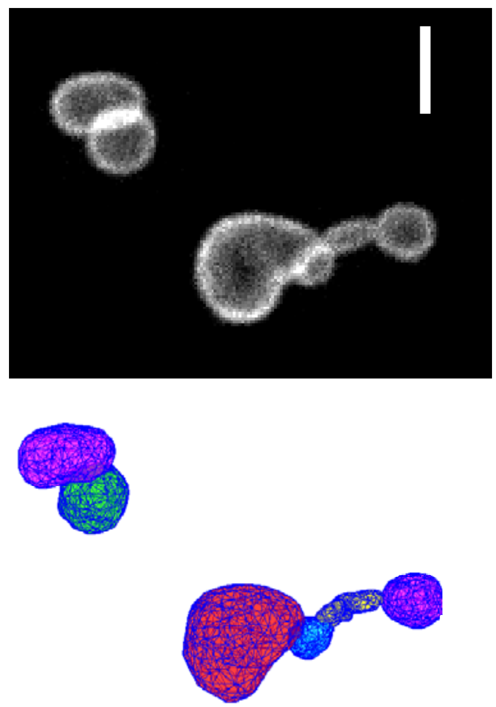
Susanne Rafelski

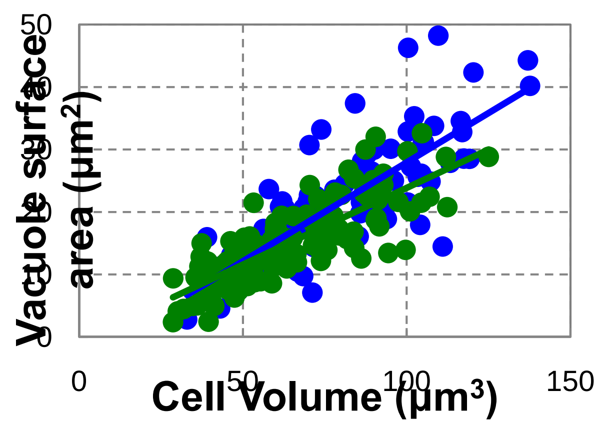




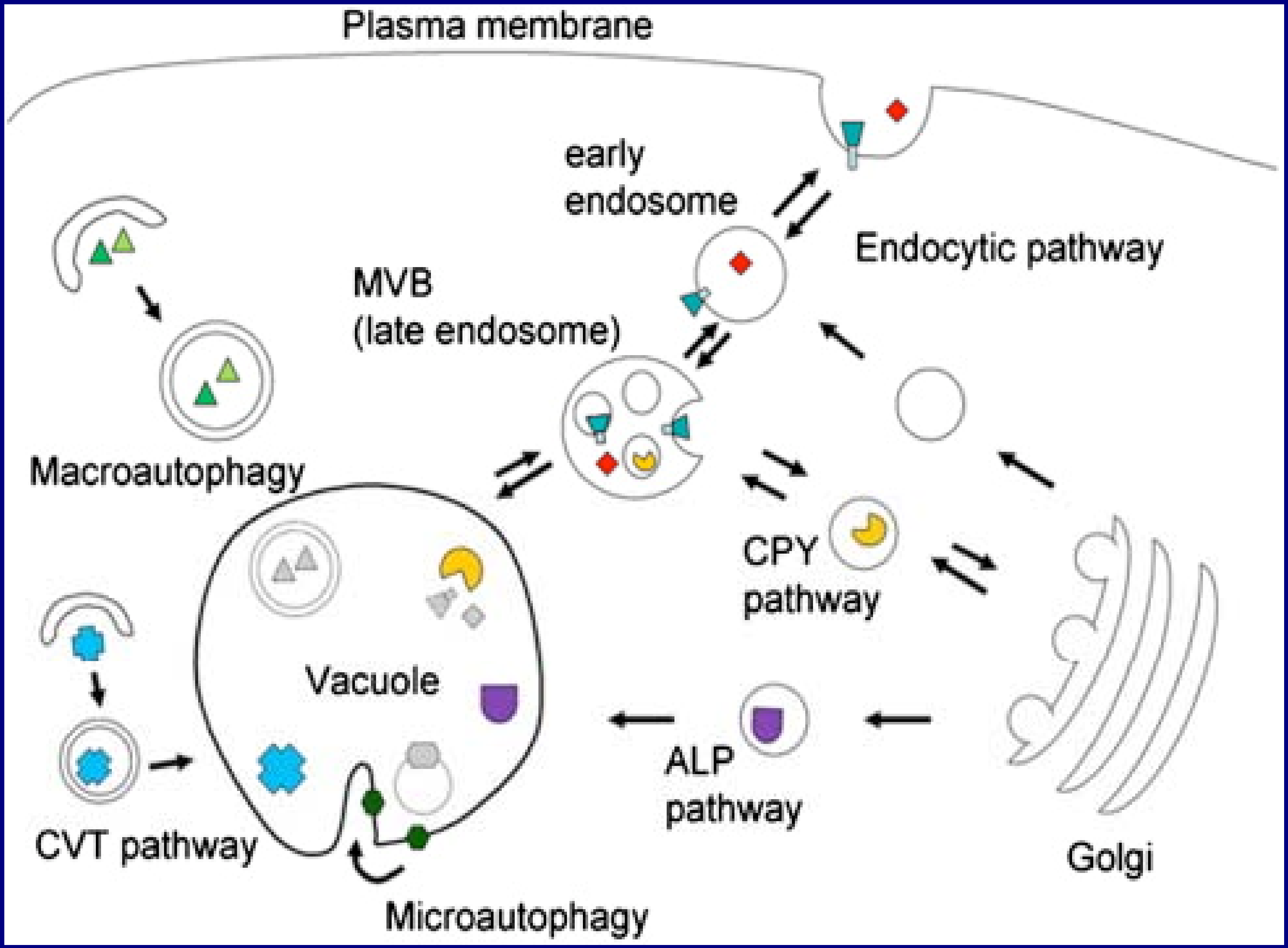
Vacuole

Mark Chan



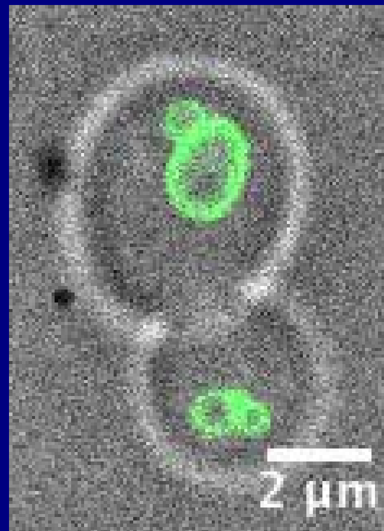


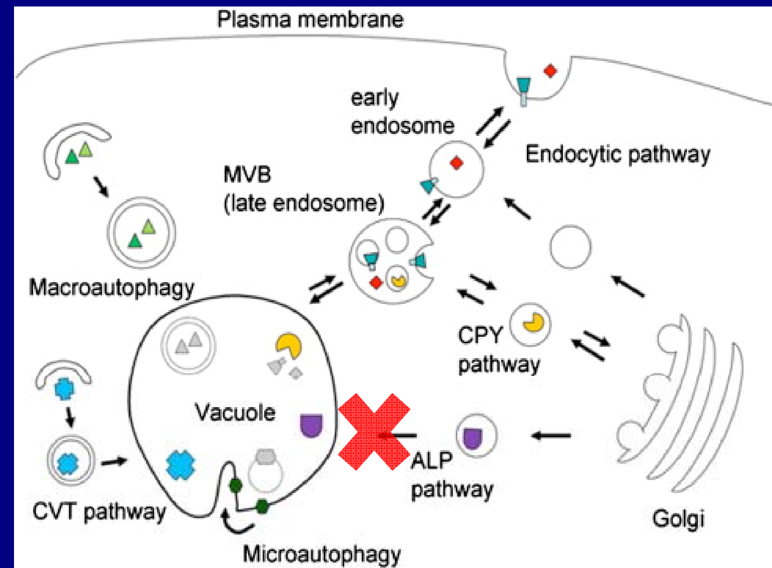
Prediction: reduced trafficking to vacuole → smaller size



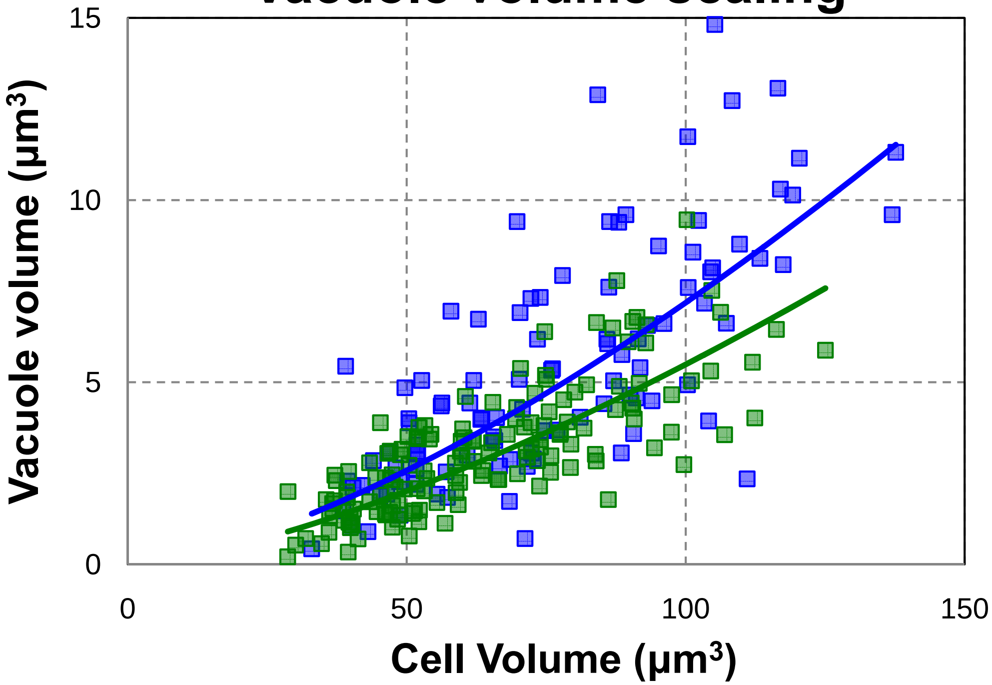
Tuning yeast vacuole size

apl5Δ
Deletion blocks ALP trafficking pathway

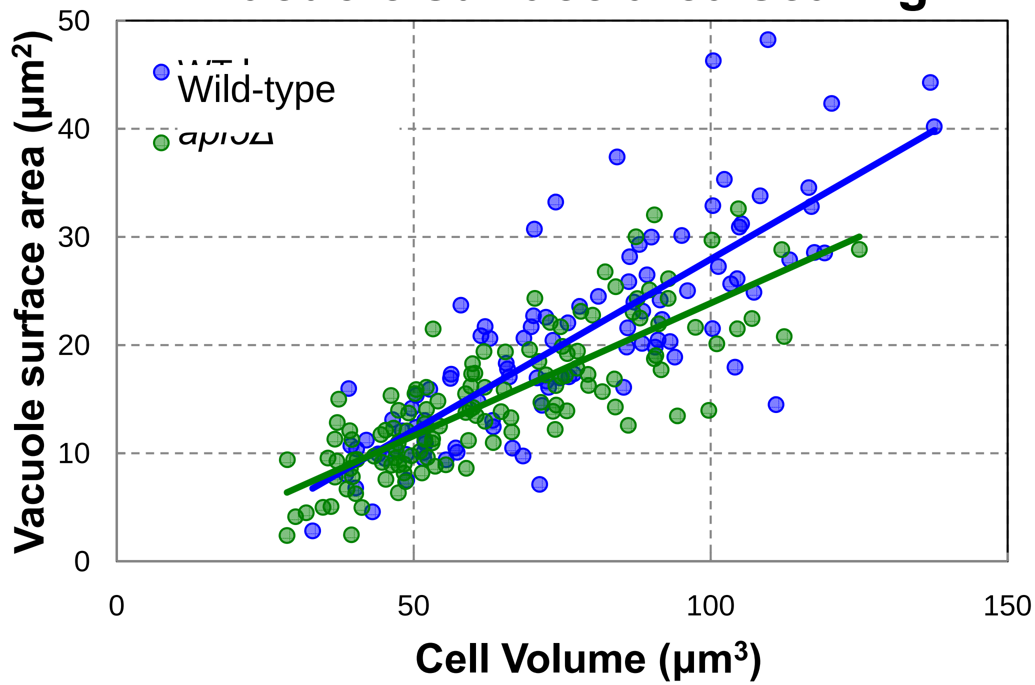




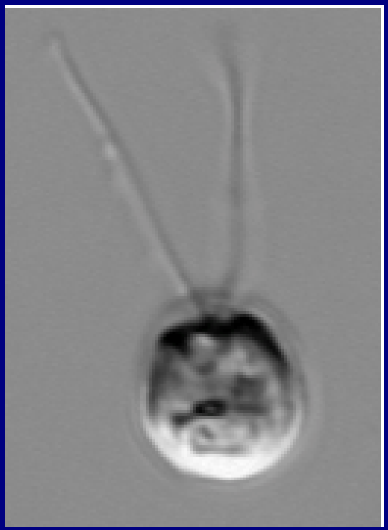
Vacuole volume scaling

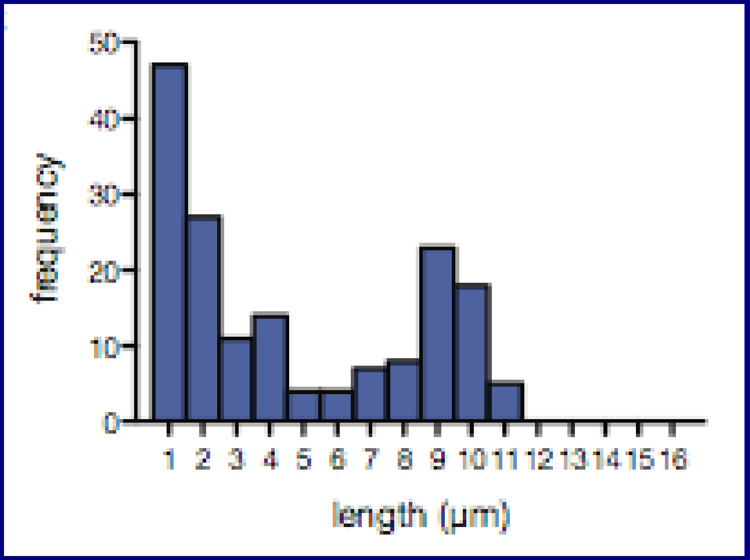


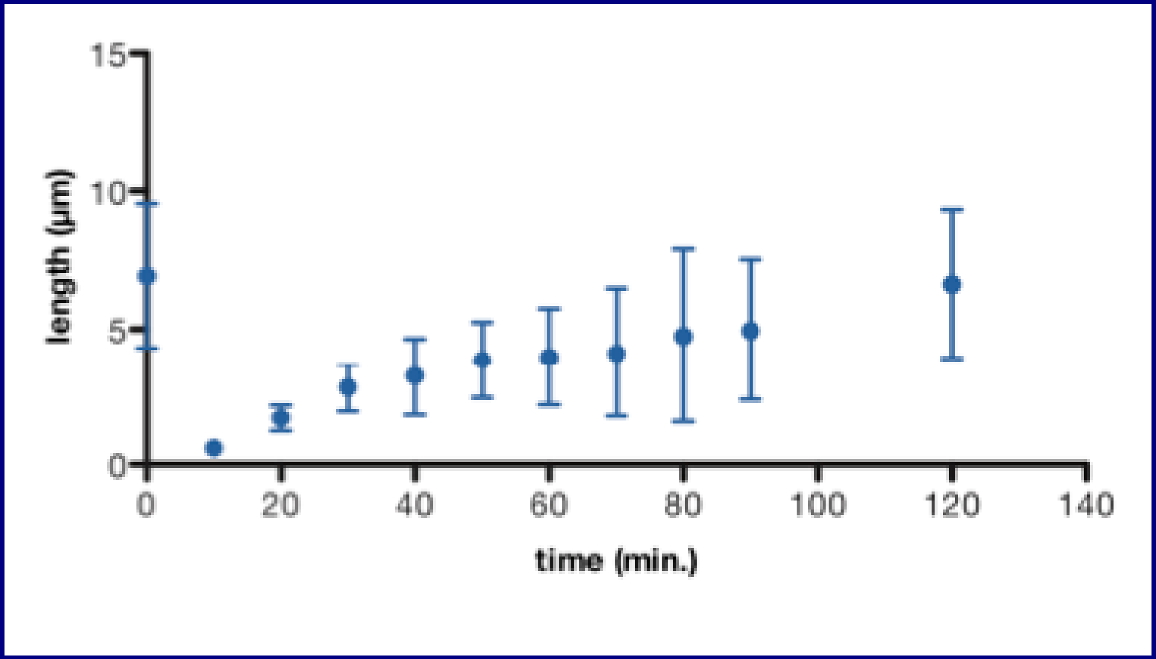
Vacuole surface area scaling



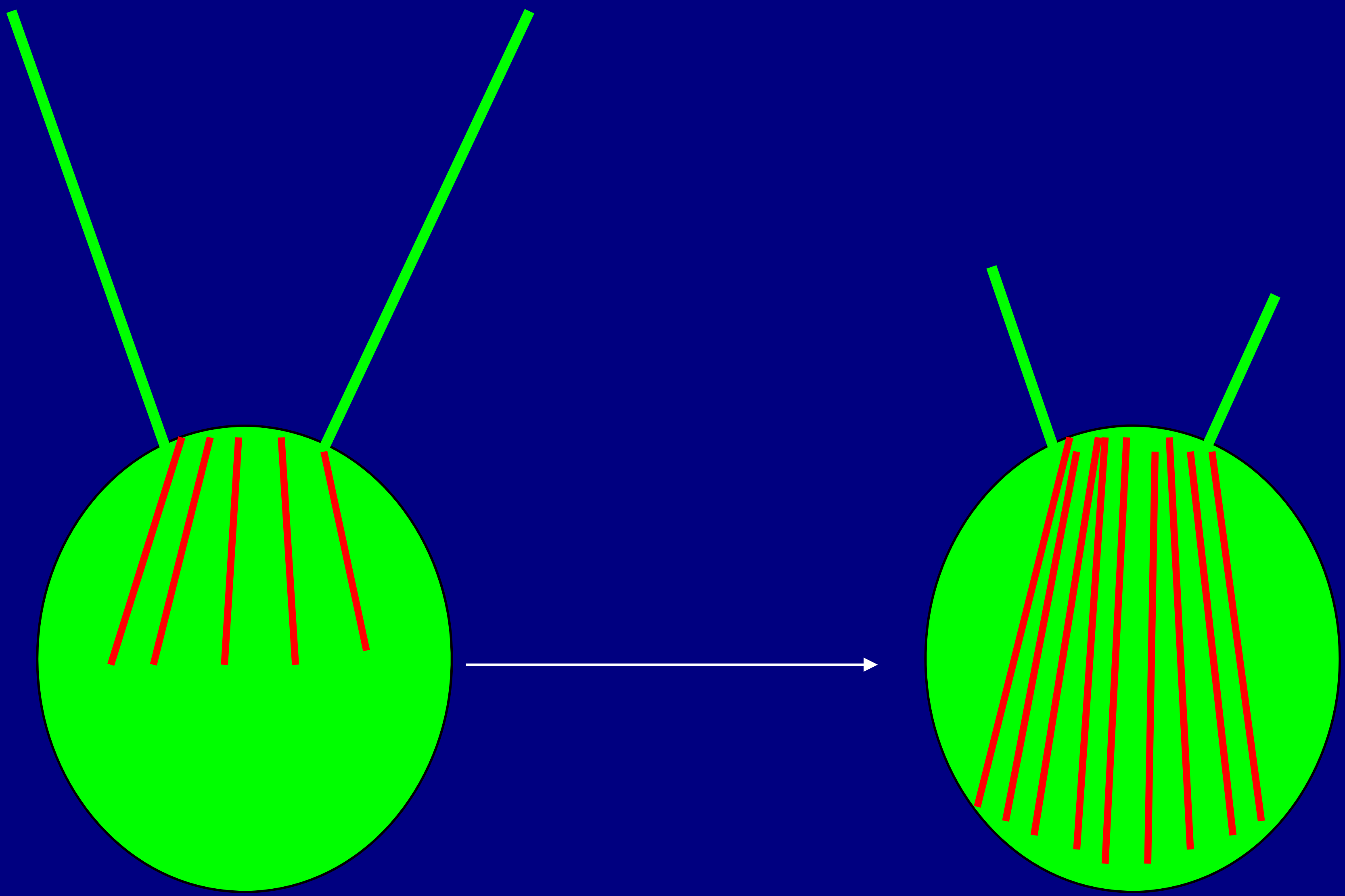
What about short flagella mutants?



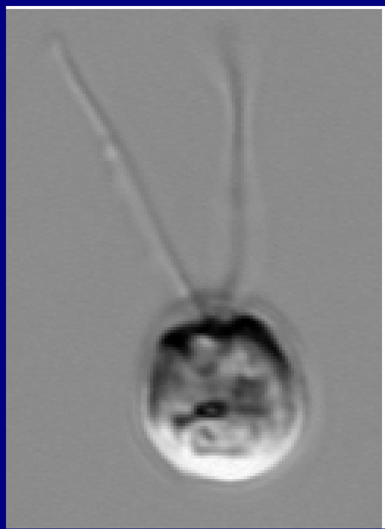


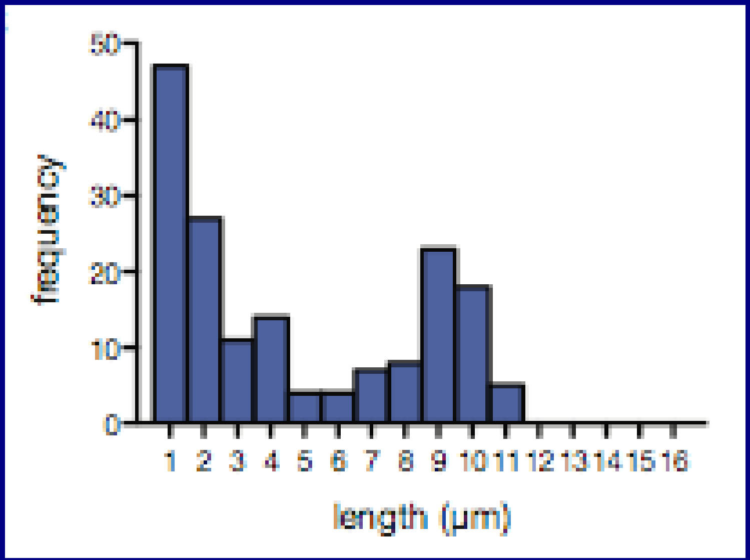


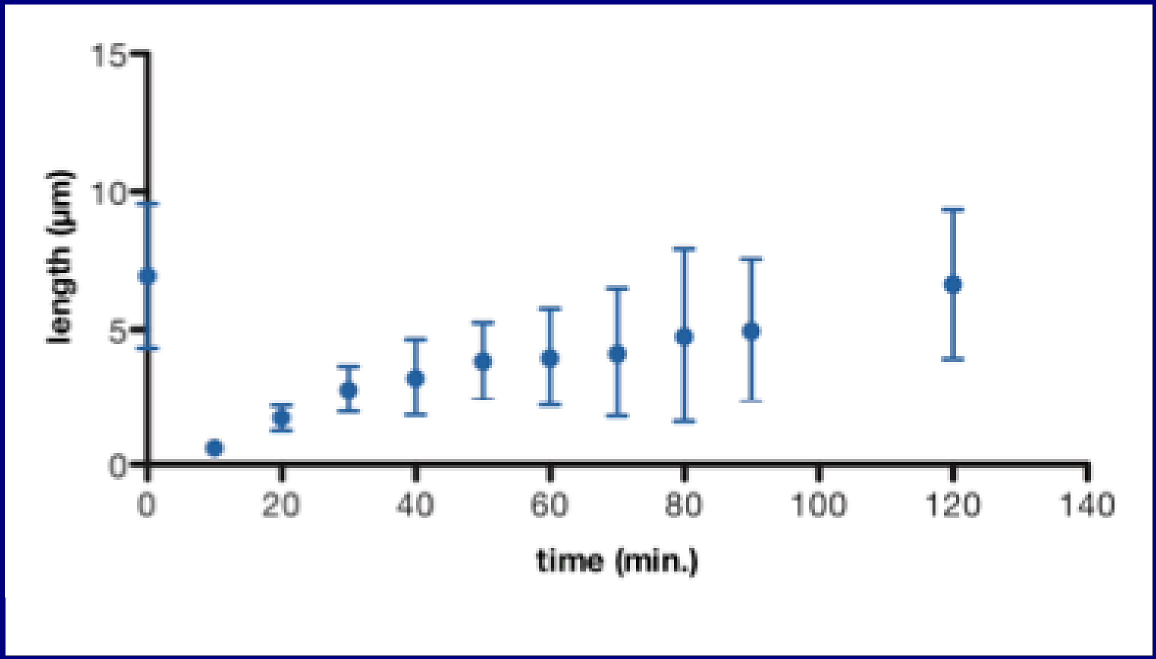
Conceptual model for katanin length phenotype: Competition for tubulin



What about short flagella mutants?

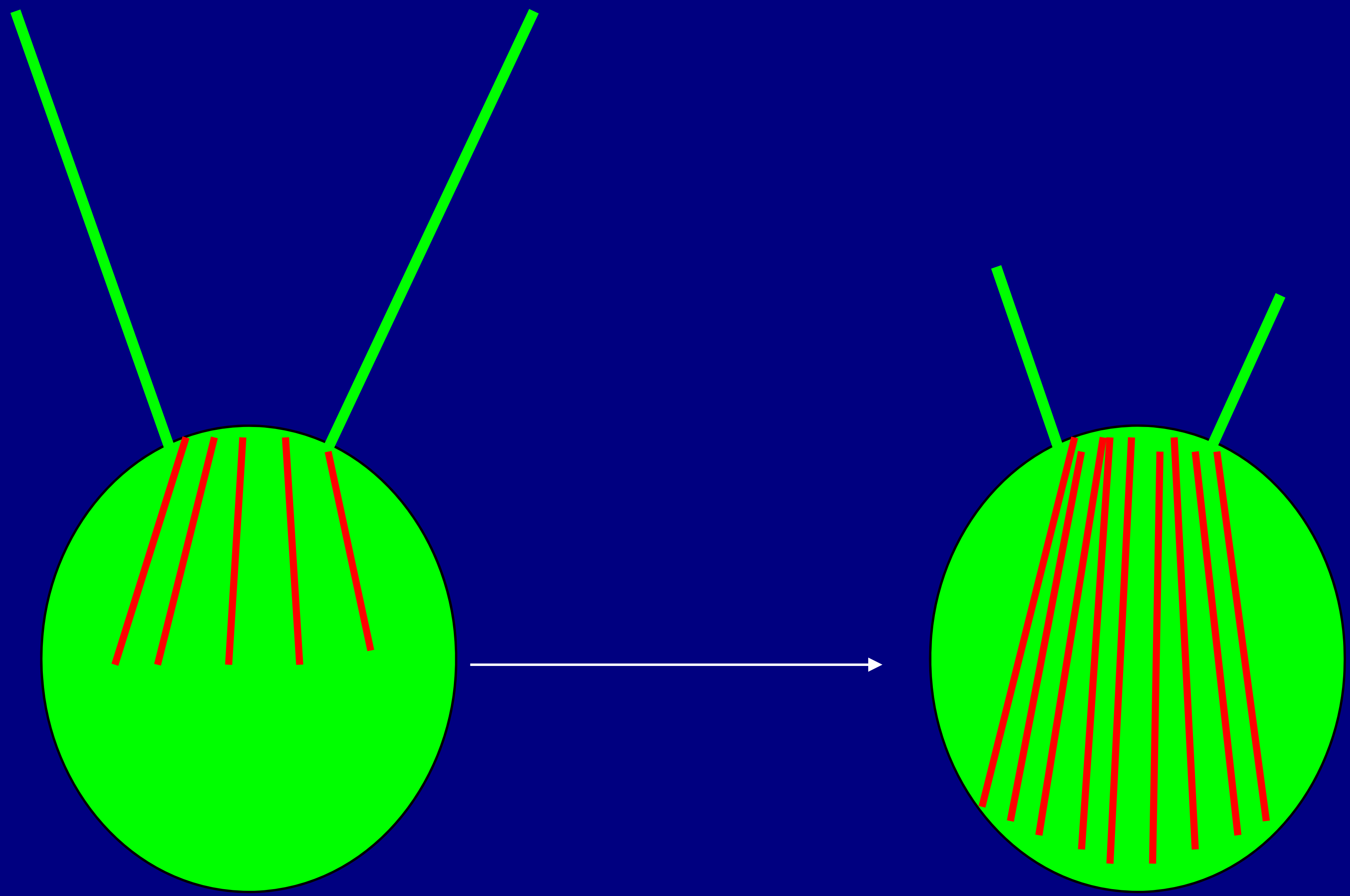






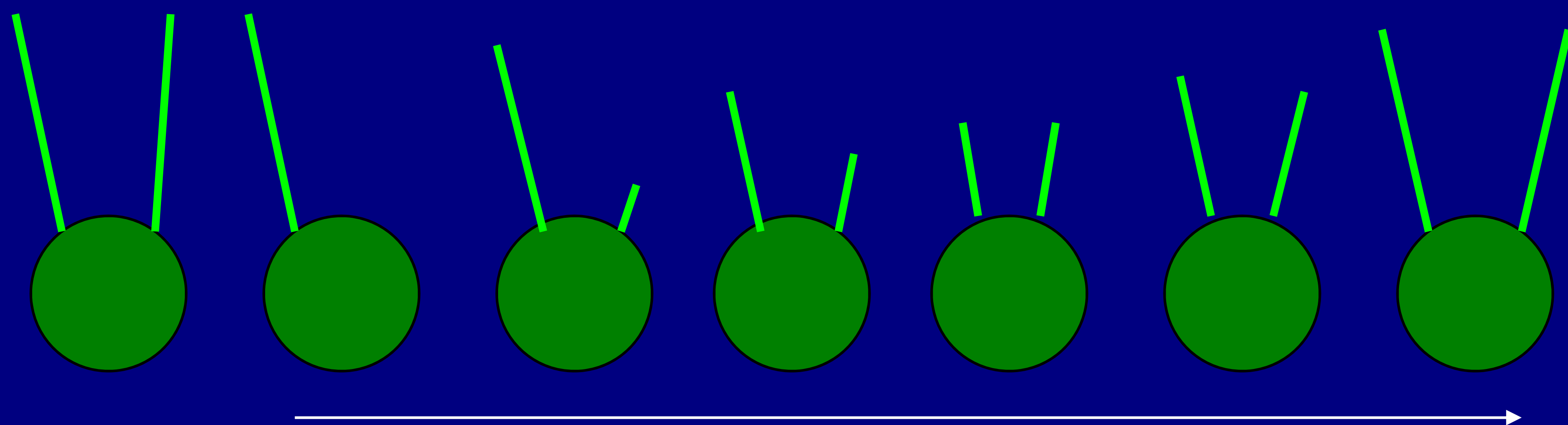
Elisa Kannegaard, Jessica Feldman

Conceptual model for katanin length phenotype: Competition for tubulin



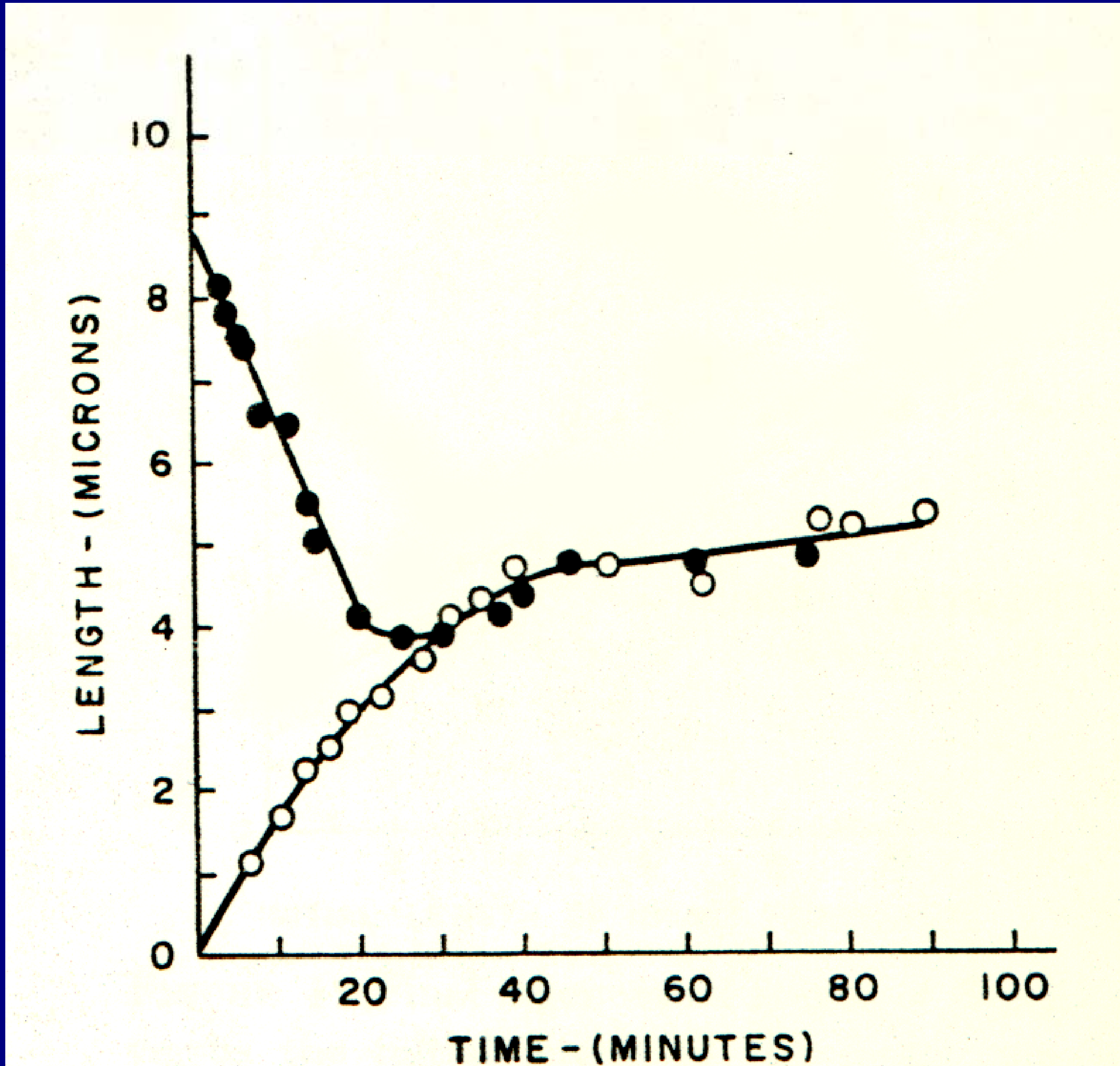
Example of what the model can explain:

Flagellar equalization after severing one flagellum

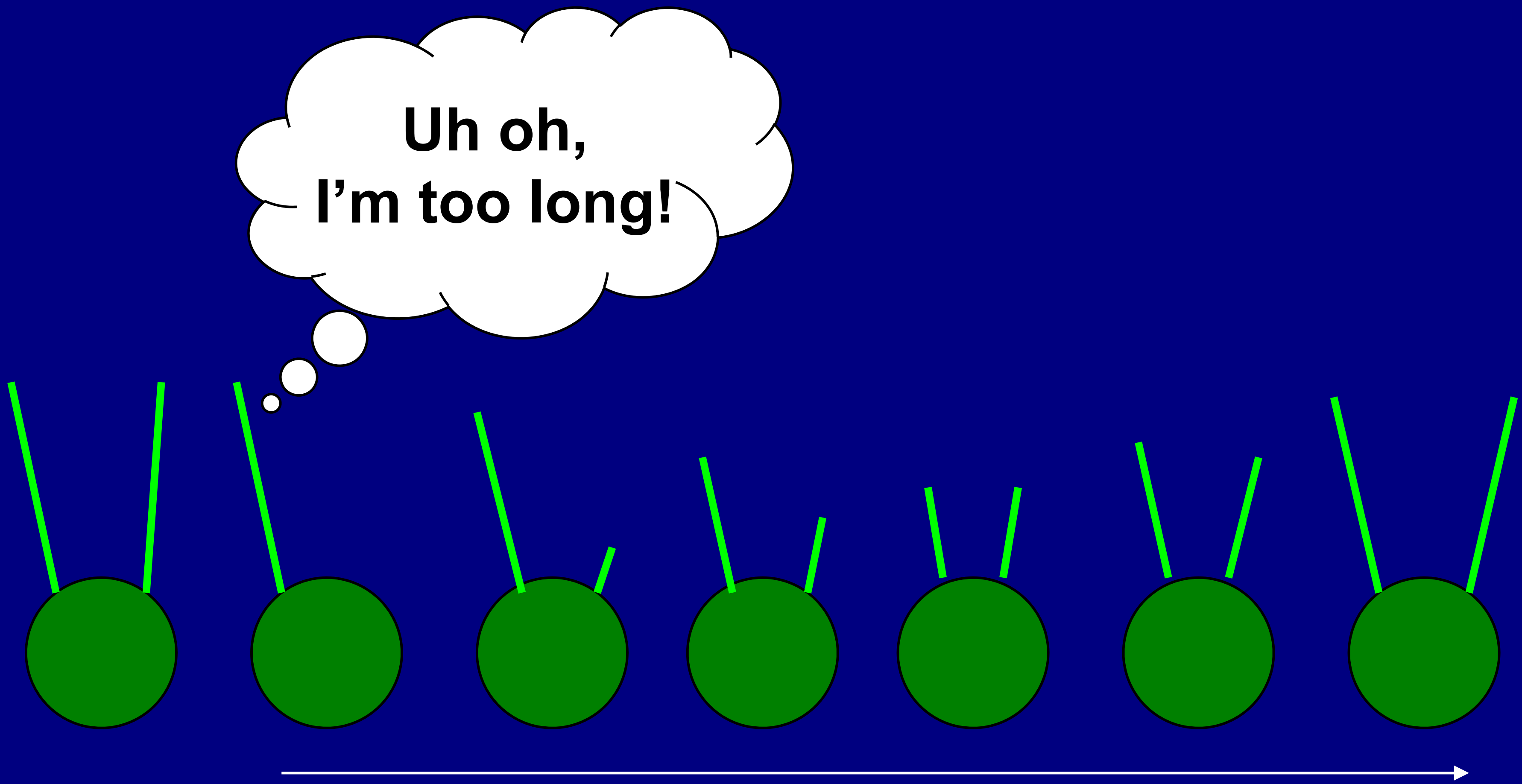


Flagellar length equalization following severing

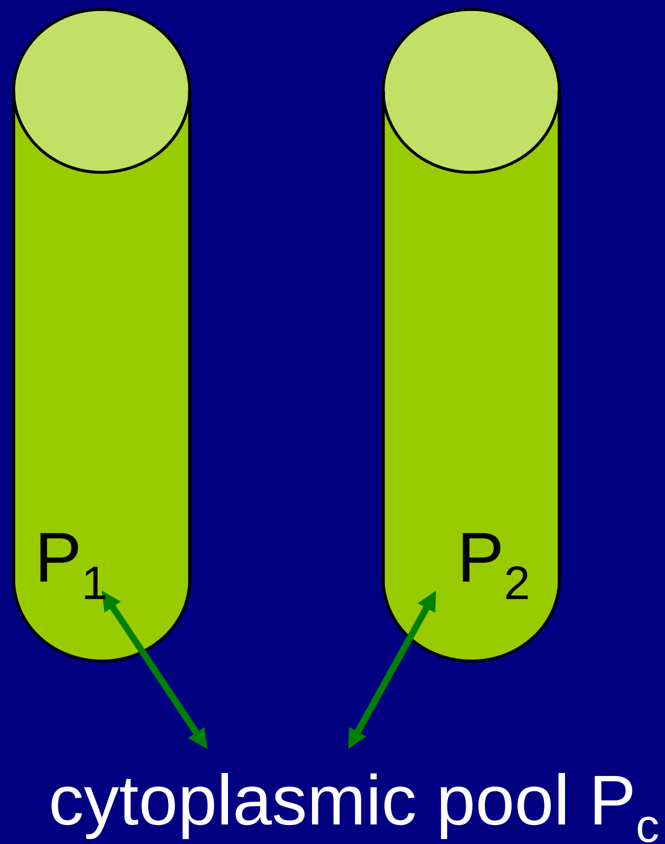
Coyne & Rosenbaum 1970



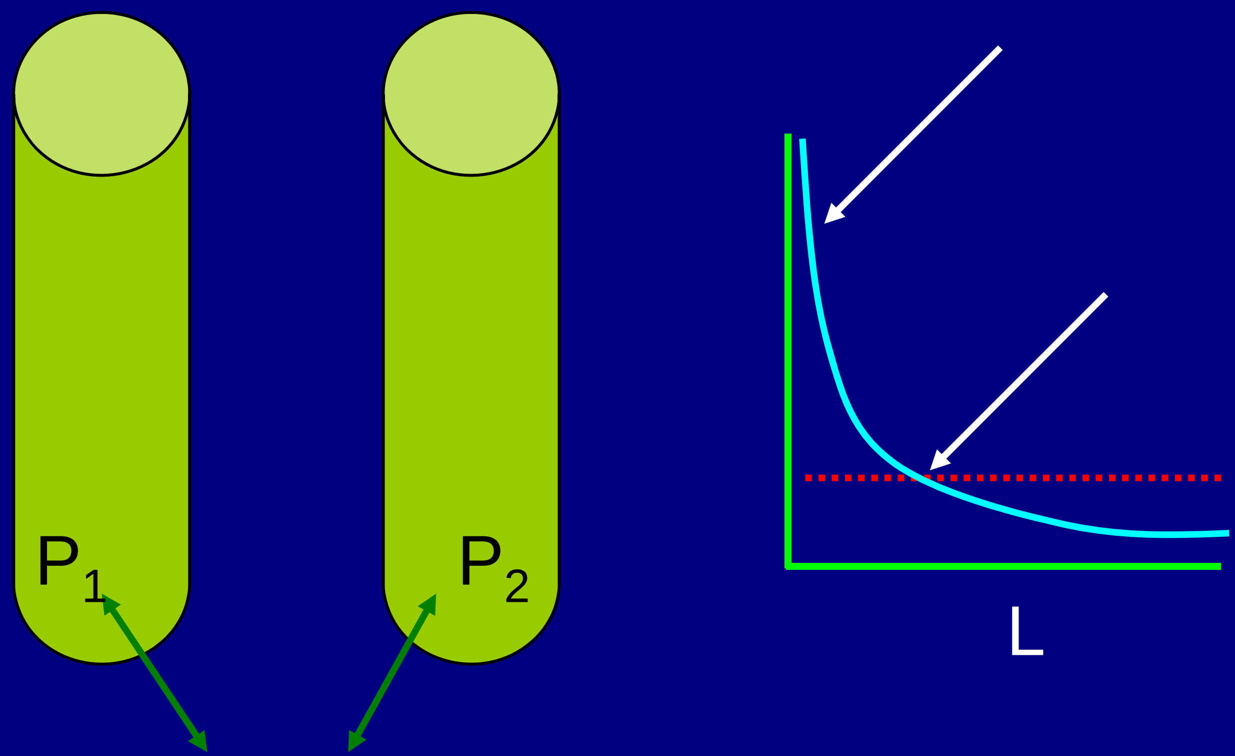
Traditional interpretation as evidence for length-sensor



Competition for cytoplasmic precursor pool

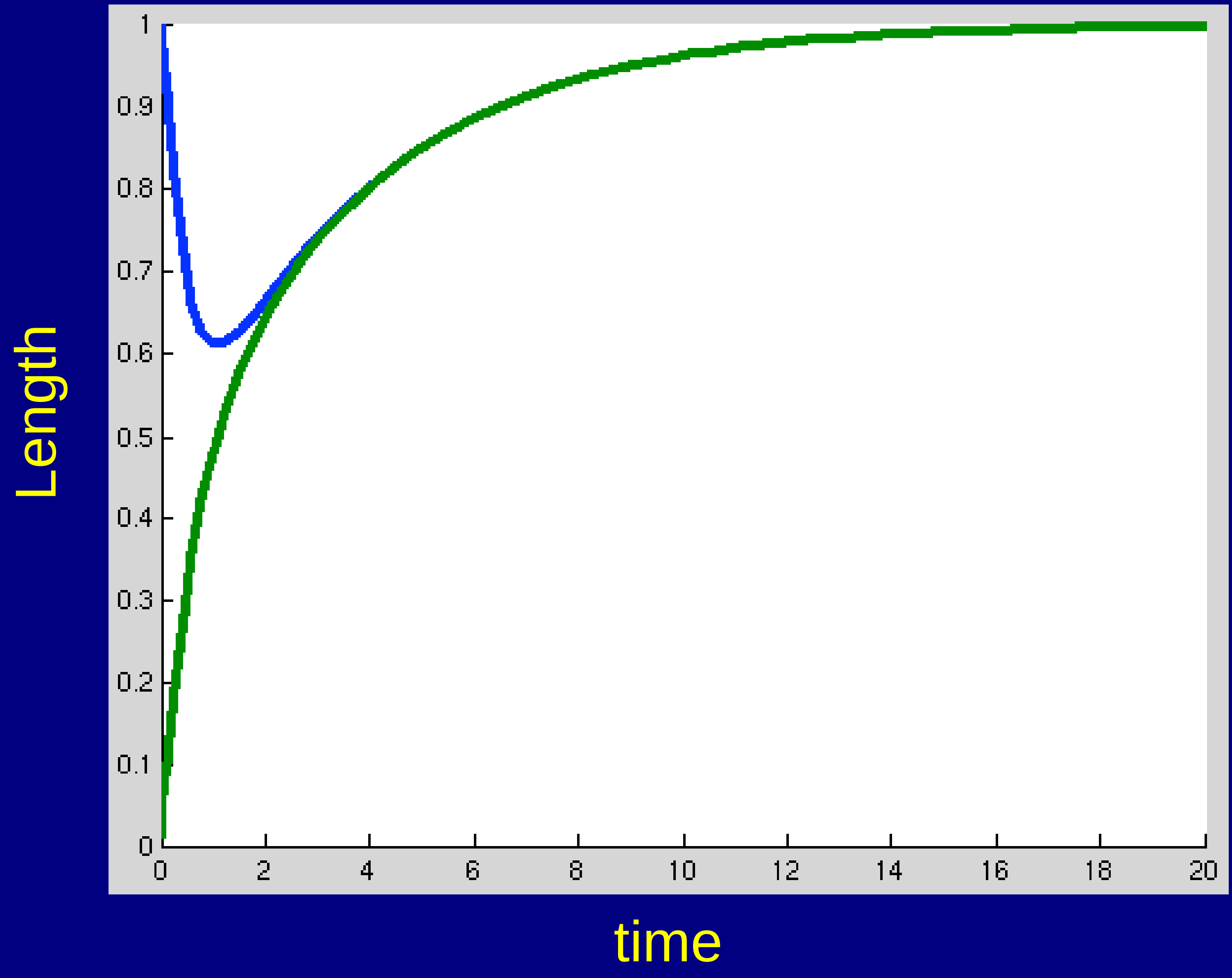


Competition for cytoplasmic precursor pool

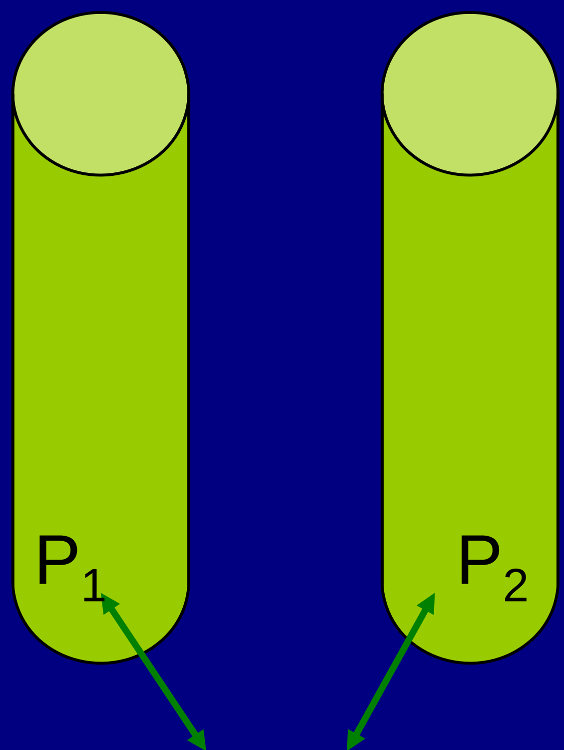


cytoplasmic pool P_c

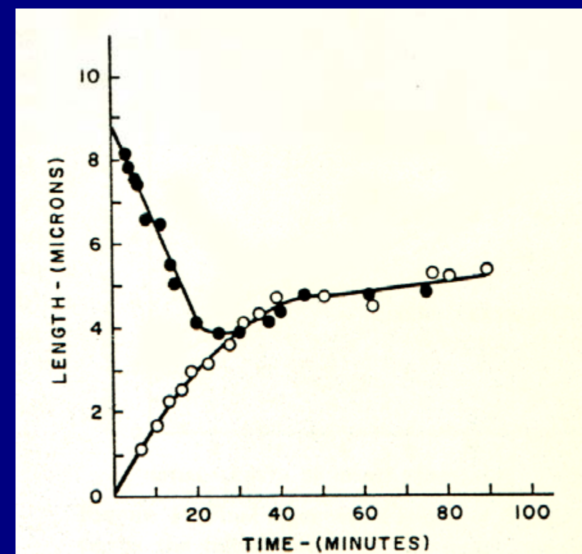
Balance-Point Model predicts Equalization of Lengths



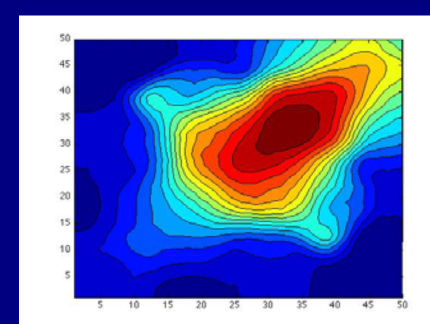
Precursor pool competition explains long-zero response



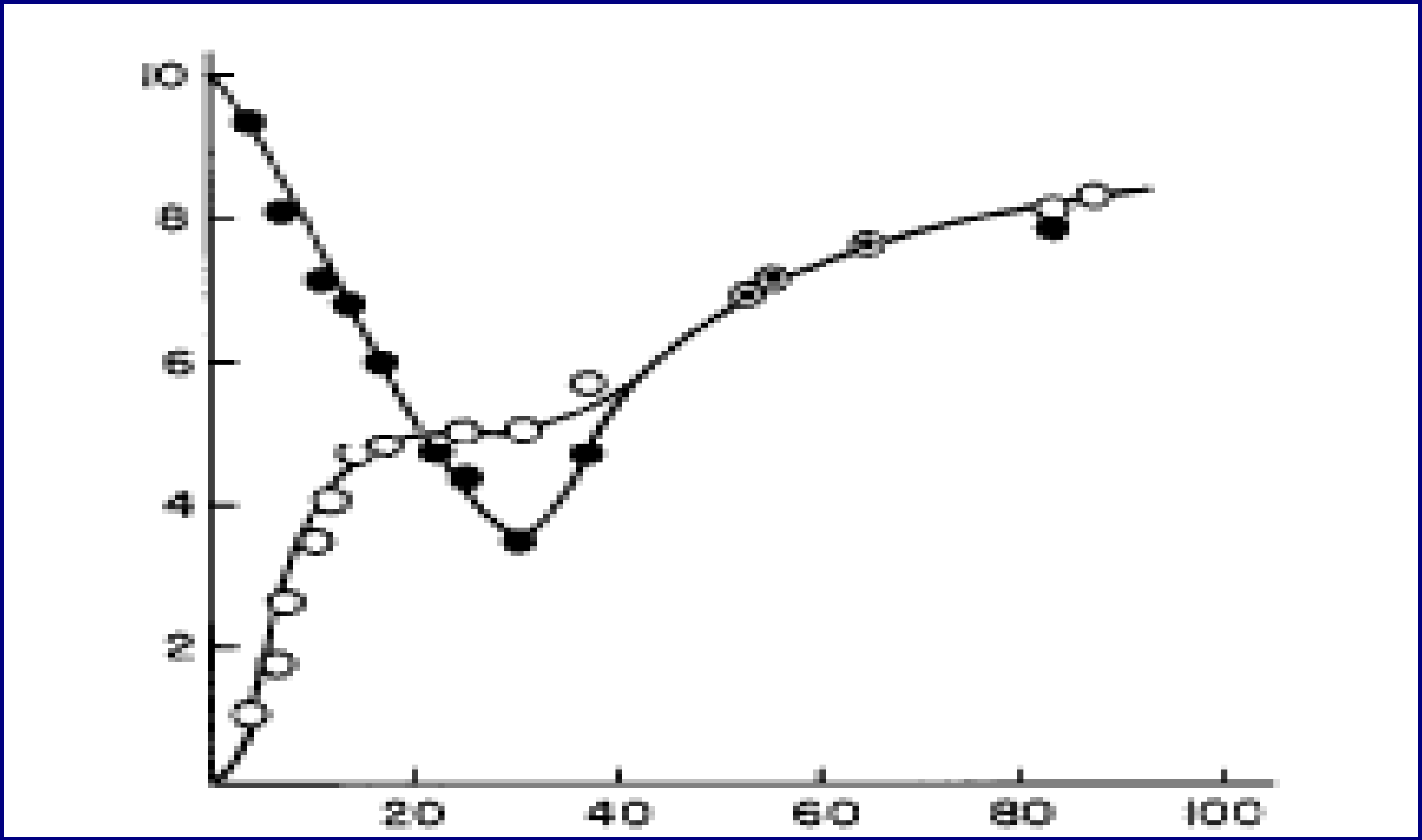
cytoplasmic pool P_c



Dynamic length control → equal length flagella

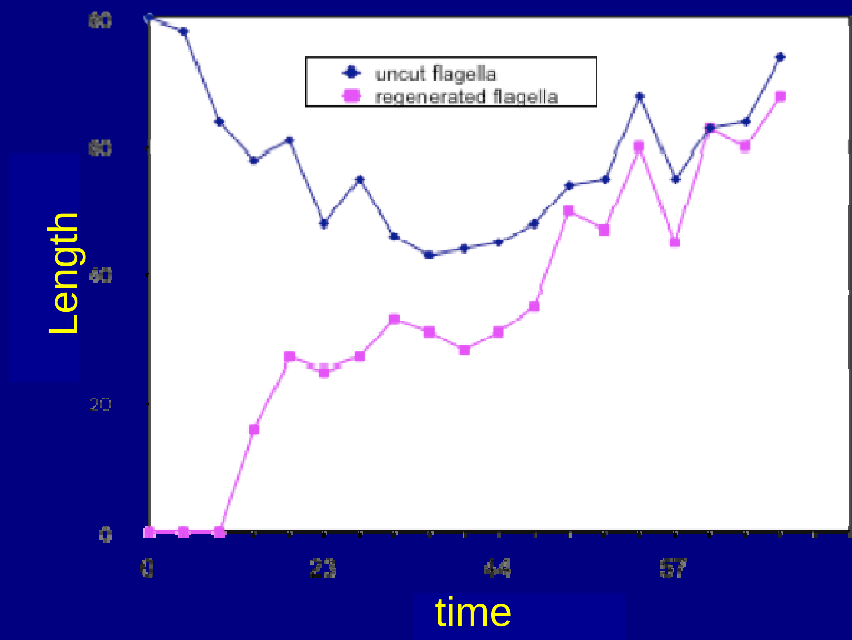


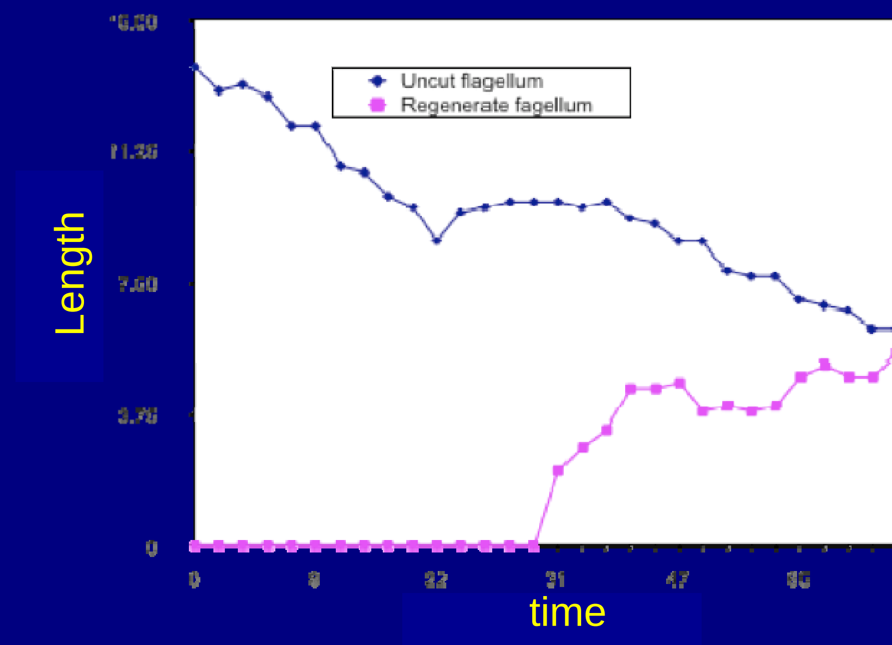
Overshoot – something the model does NOT predict

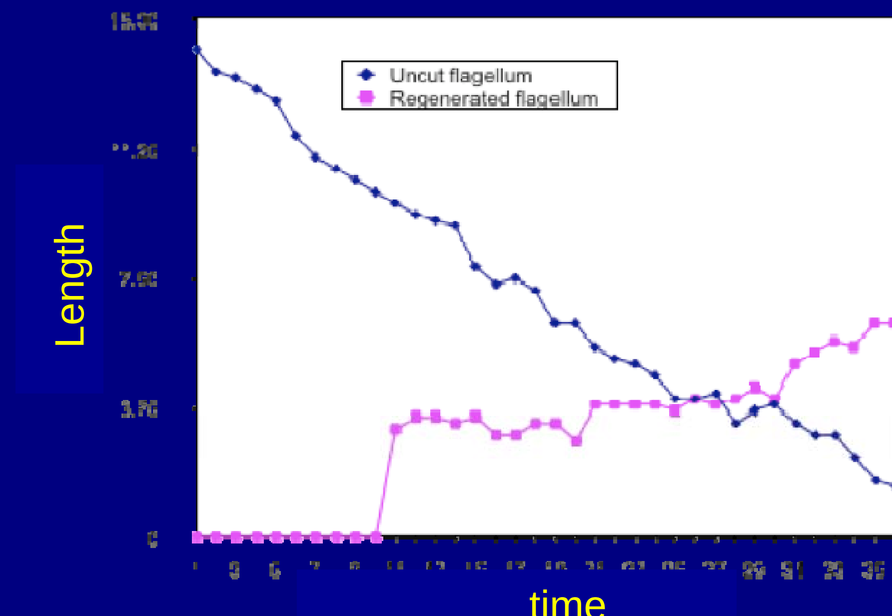


Requires equal-length flagella to have opposite behaviors

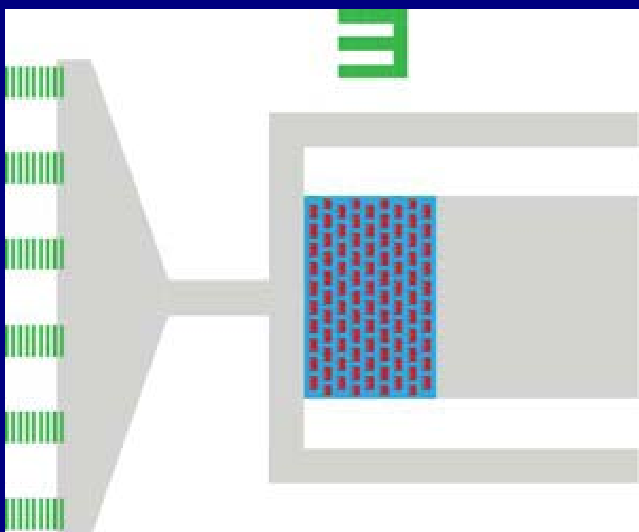
Checking for overshoot using laser scissors

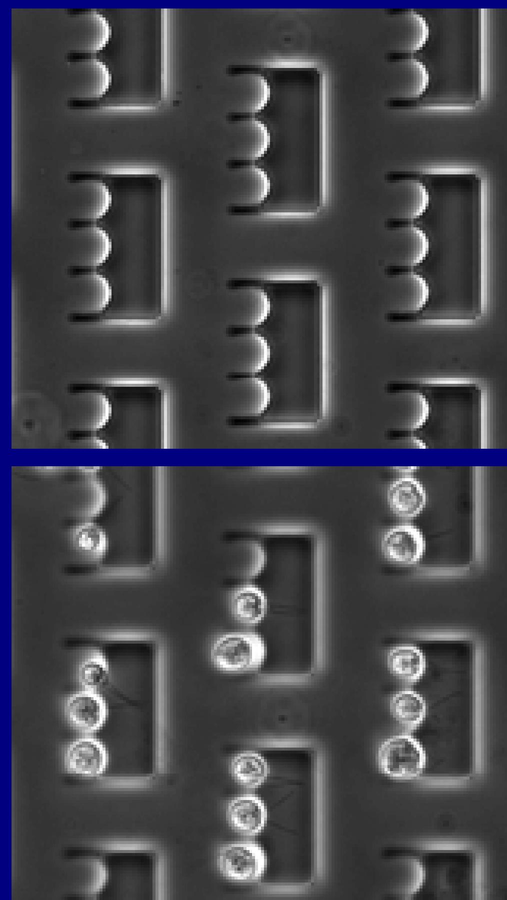


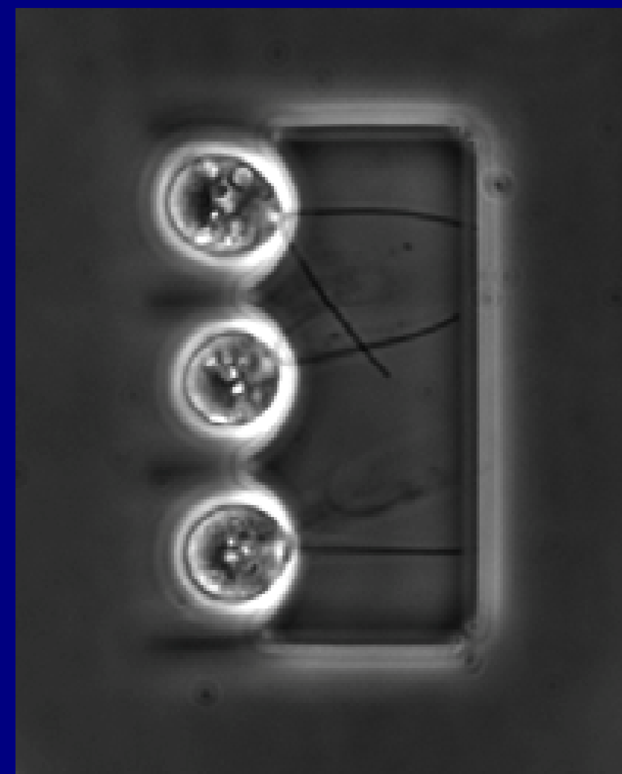




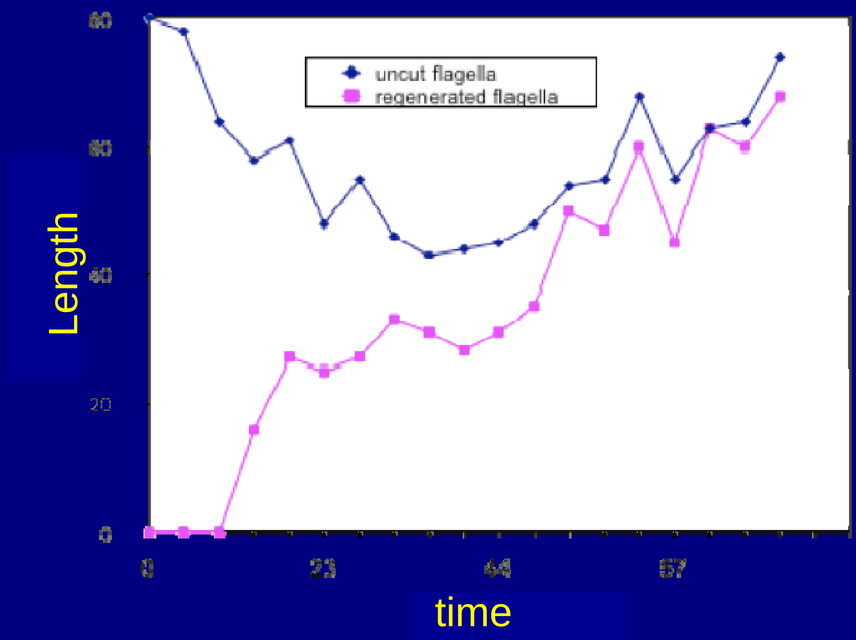
Custom microfluidic chamber for Chlamydomonas laser surgery

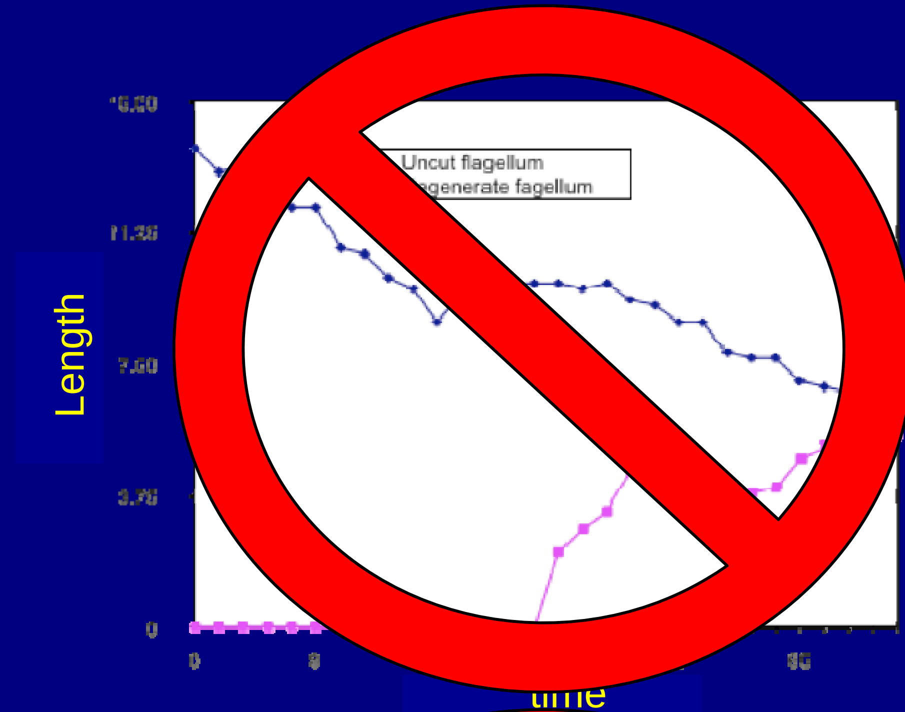


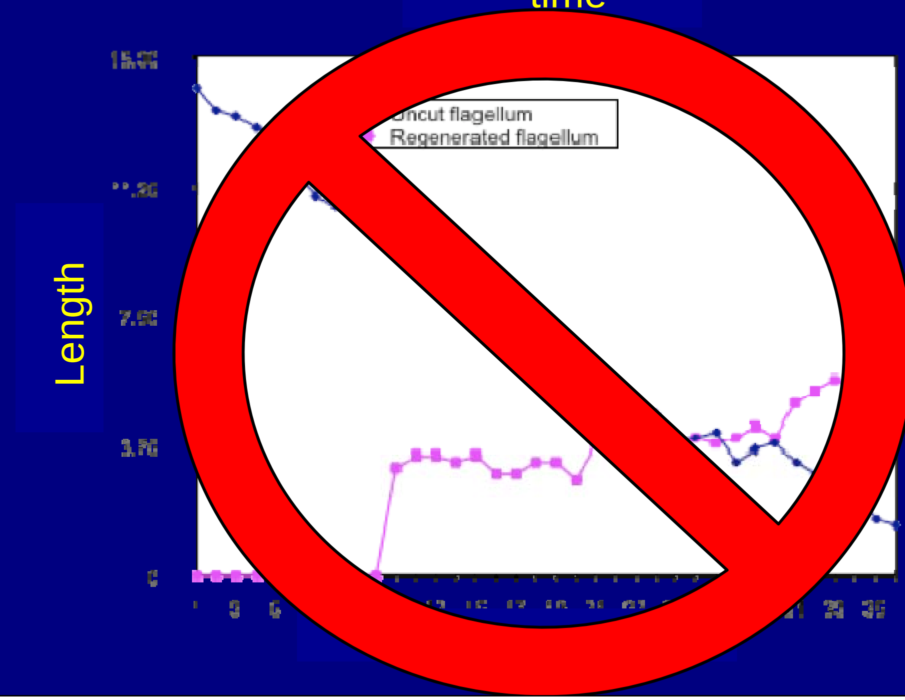




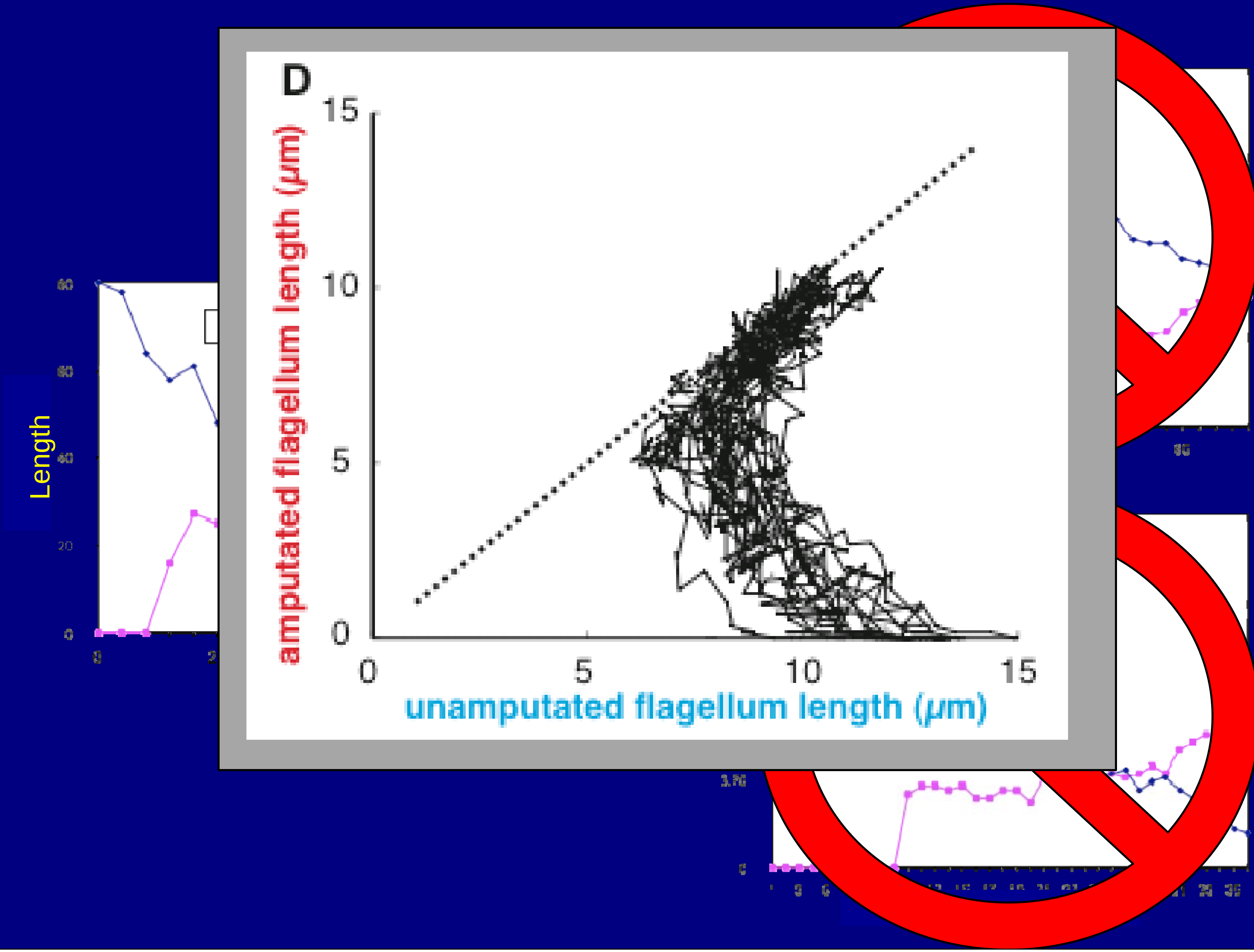
Overshoot vanishes in microfluidic chambers



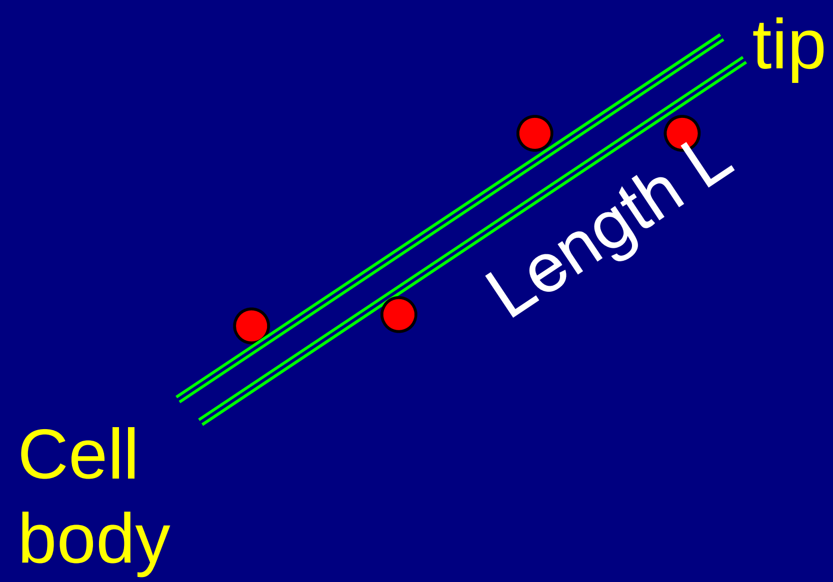




Overshoot vanishes in microfluidic chambers



IF number of IFT particles is length-independent:

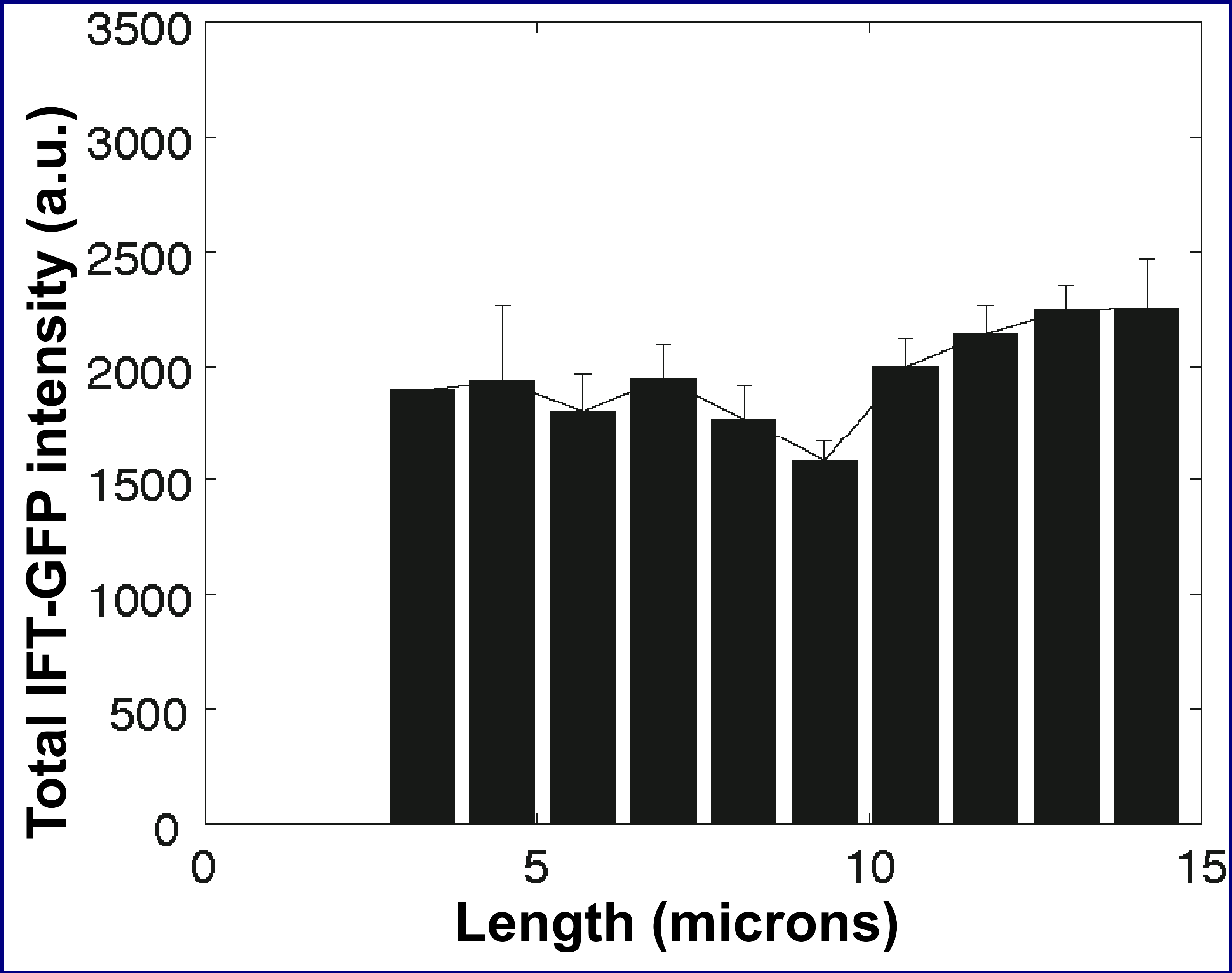


Round trip time ~ L PER PARTICLE

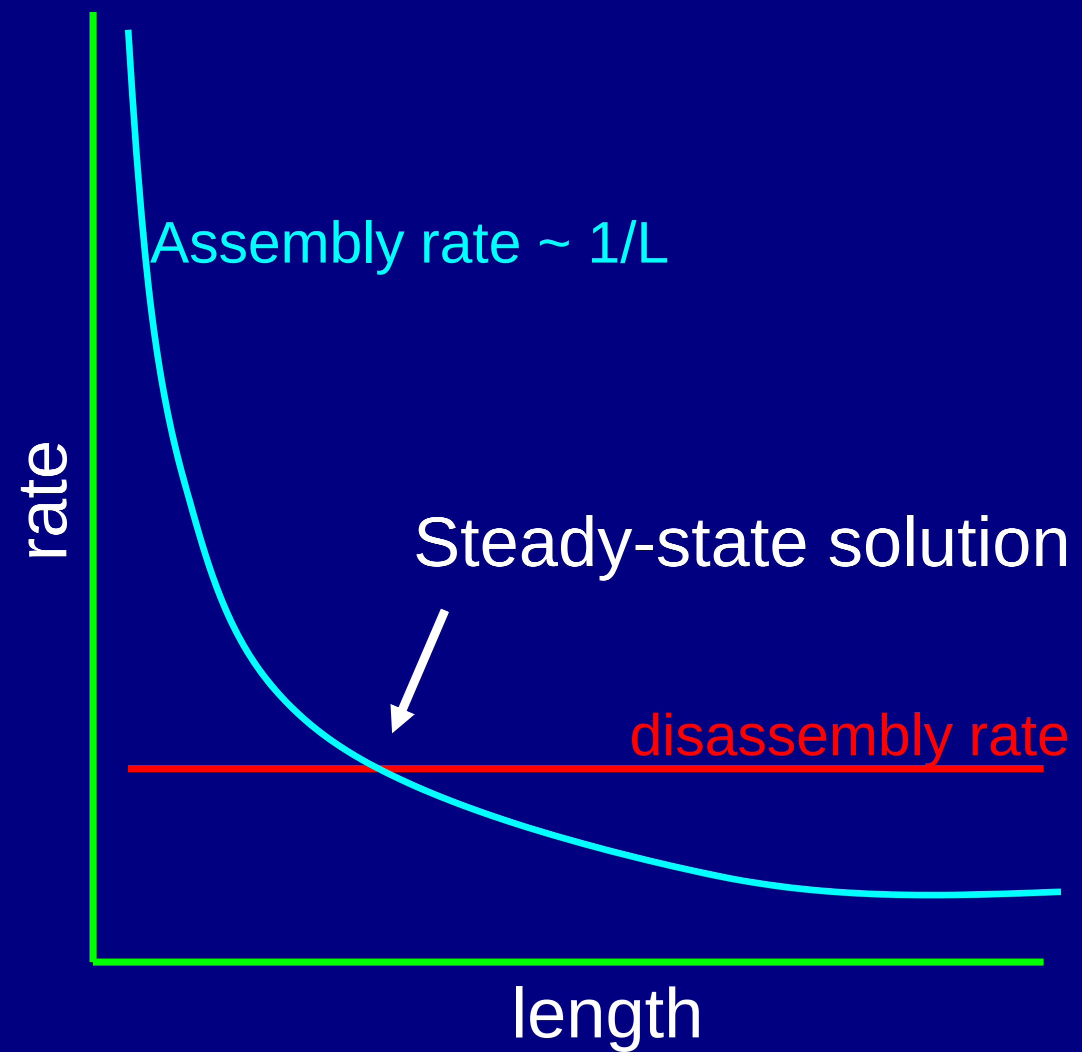
Transport rate ~ 1/L
iff N constant

Model breaks down completely if
N ~ L

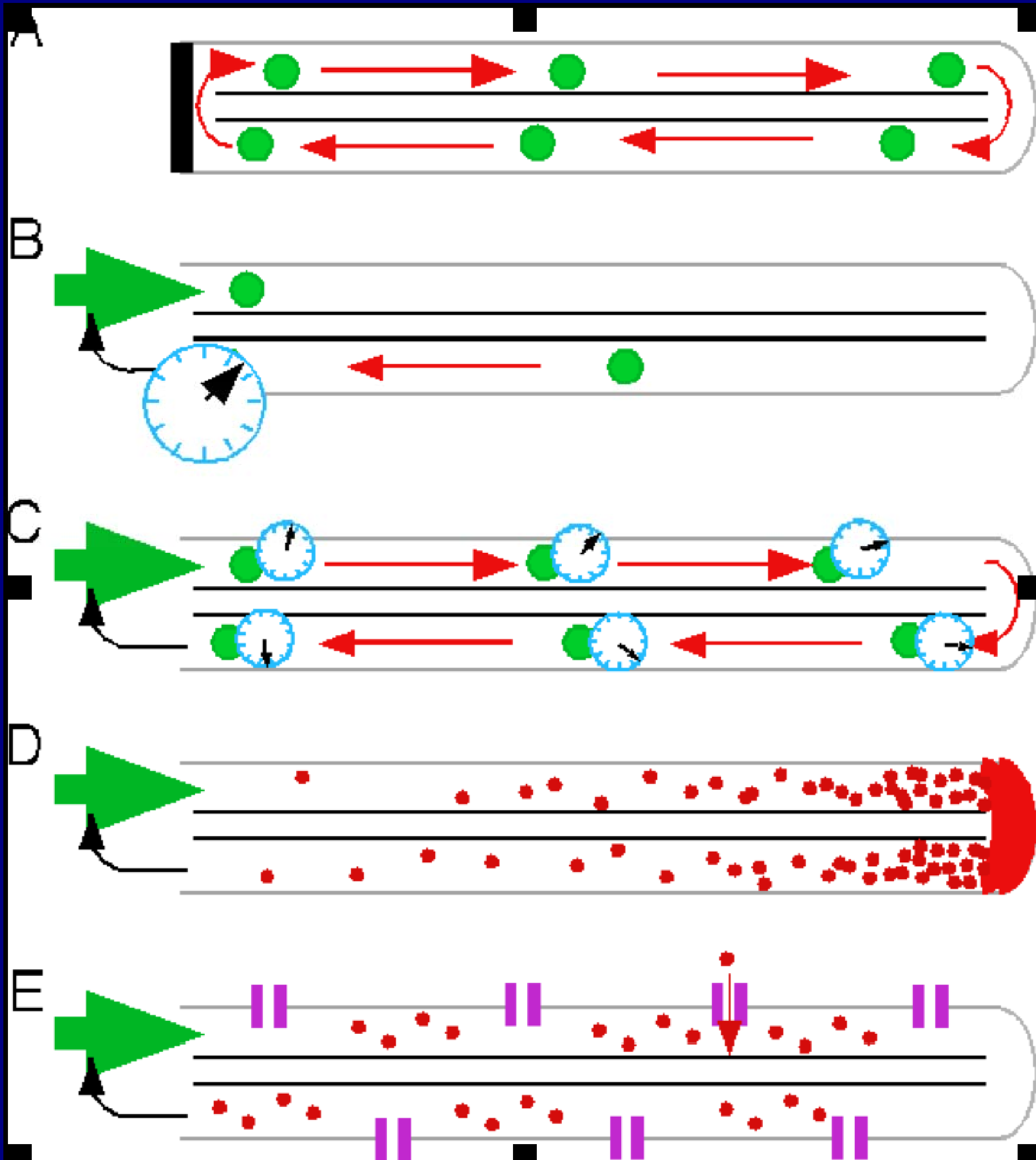
Total IFT content is approximately length-independent



Balance-Point model for flagellar length control



How does the flagellum know how long it is?



Initial Bolus

Wait-release

Time-of-flight

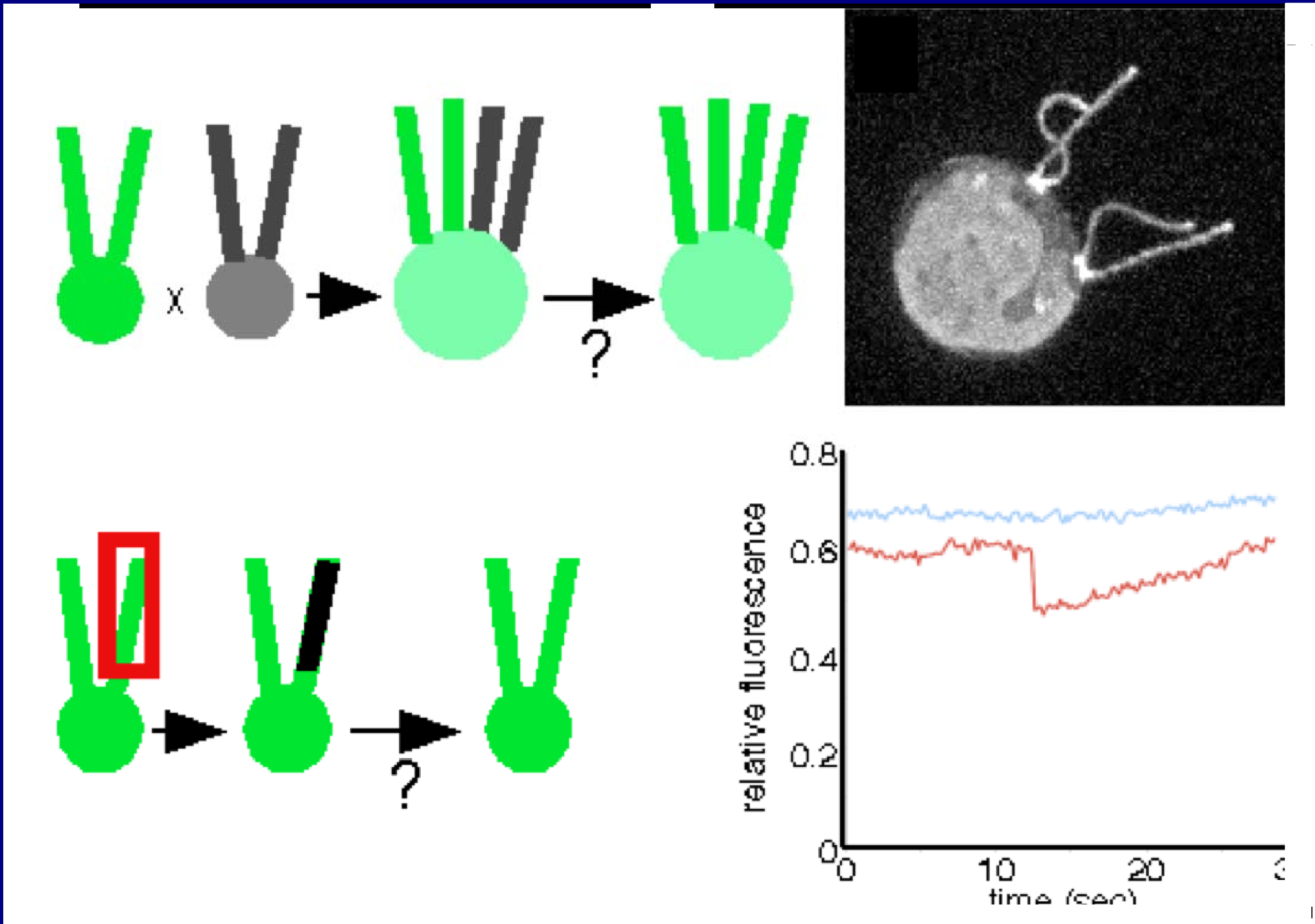
Tip diffusion

Levy 1974 J. Theor. Biol. 43, 133-49

Proportional current

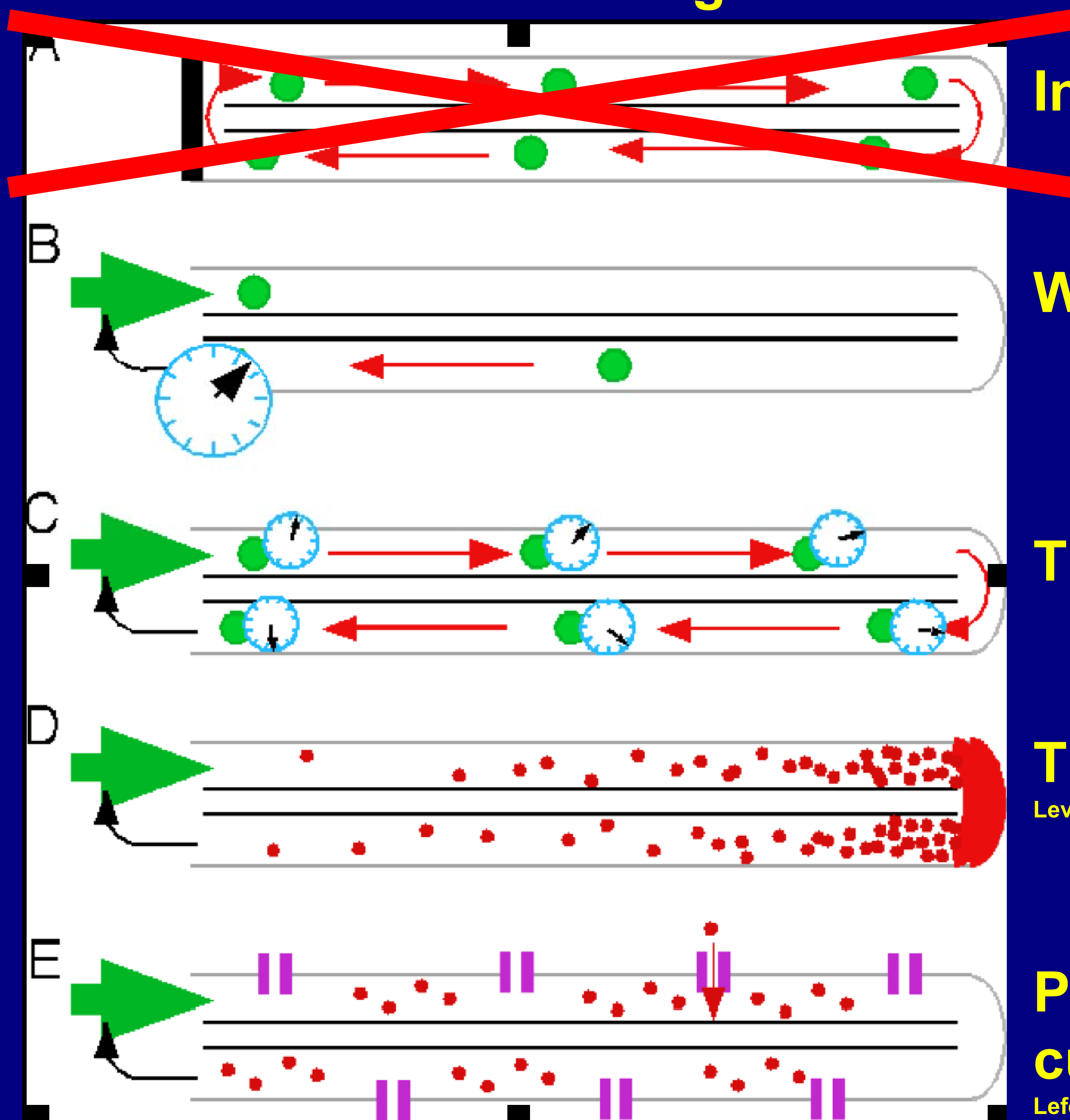
Lefebvre et al. 1978 JCB 78, 8-27
Beck & Uhl, 1994 JCB 125, 1119-25
Rosenbaum 2003 Curr. Biol. 13 R506-7

Rapid exchange of IFT proteins between flagella and cytoplasm



Not consistent with "initial bolus" mechanism

How does the flagellum know how long it is?



Initial Bolus

Wait-release

Time-of-flight

Tip diffusion

Levy 1974 J. Theor. Biol. 43, 133-49

Proportional current

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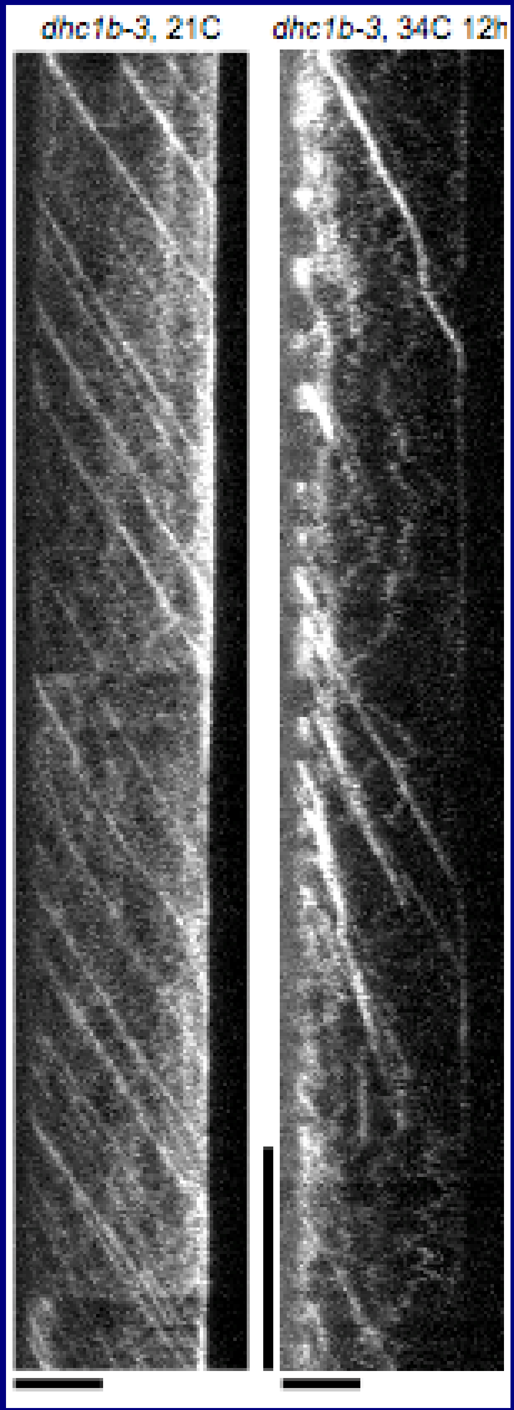
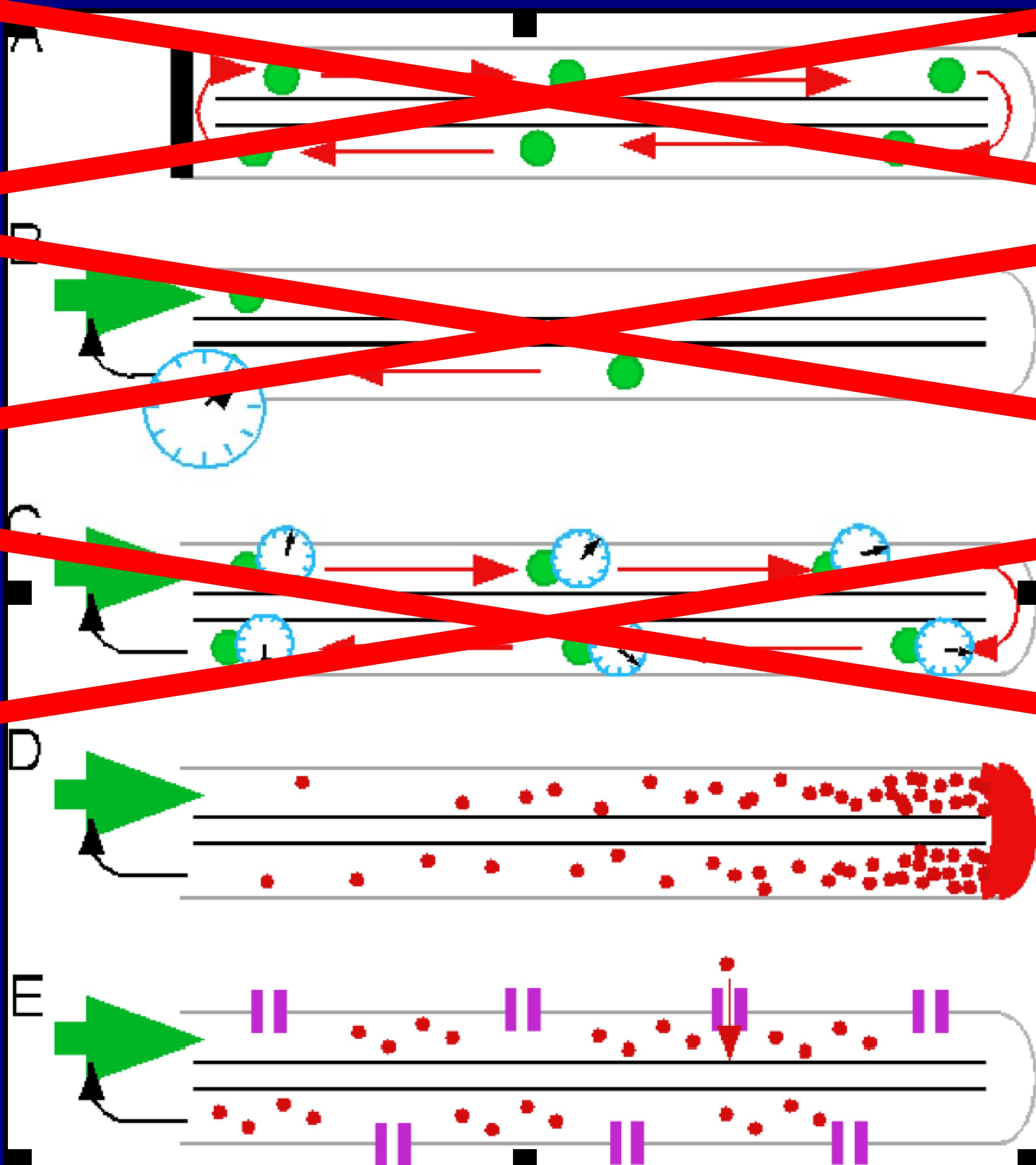
Prediction for time of flight:

slow retrograde IFT
should mimic long flagella

→ decrease injection
increase frequency
decrease magnitude



How does the flagellum know how long it is?



~~Initial Bolus~~

~~Wait-release~~

~~Time-of-flight~~

Tip diffusion

Levy 1974 J. Theor. Biol. 43, 133-49

Proportional current

Lefebvre et al. 1978 JCB 78, 8-27
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Possible implications of a RanGTP gradient