Intracellular Transport & Collective Force Generation by Motor Proteins

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I. Background

II. Single Motors

III. Collections of Motors

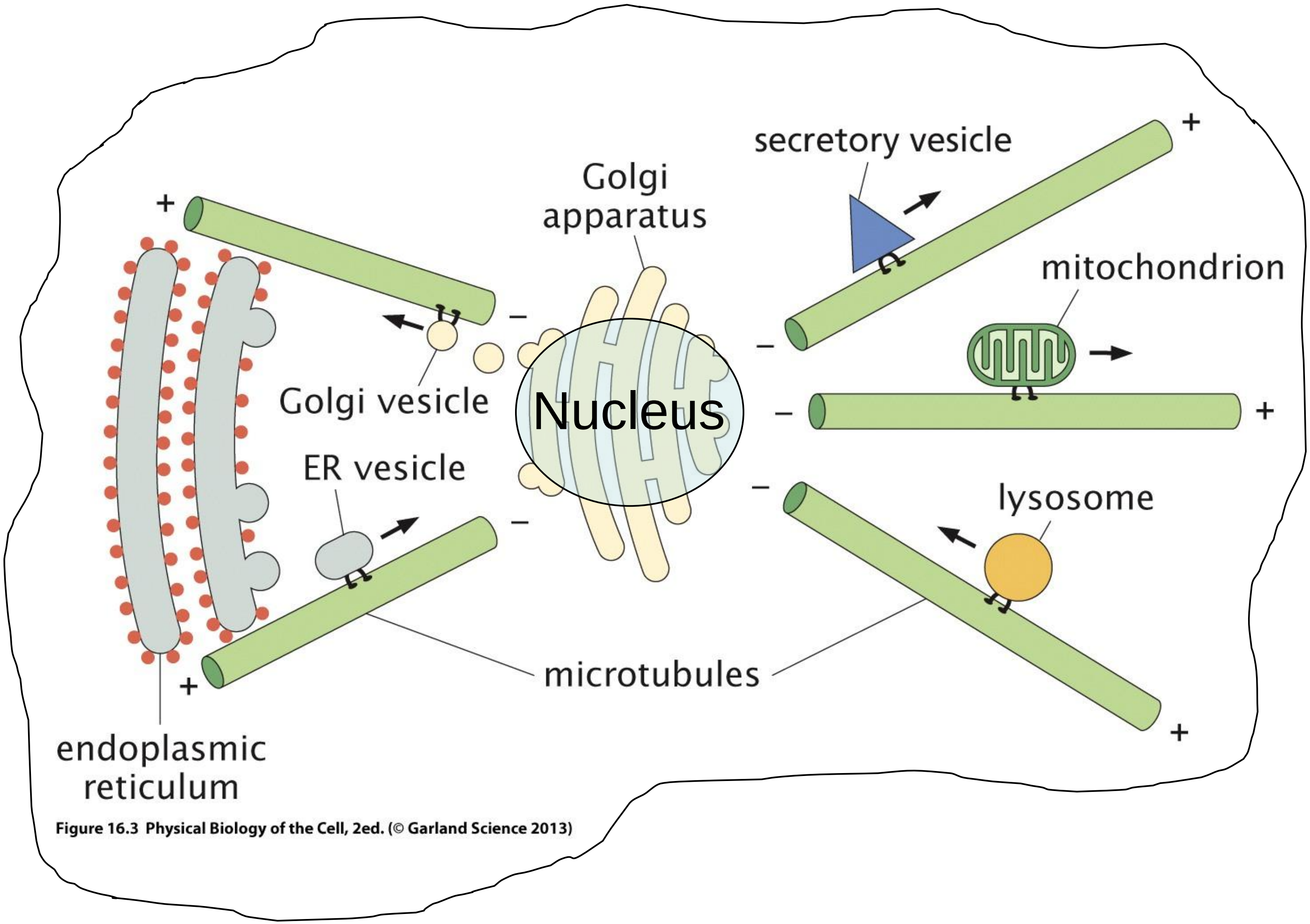
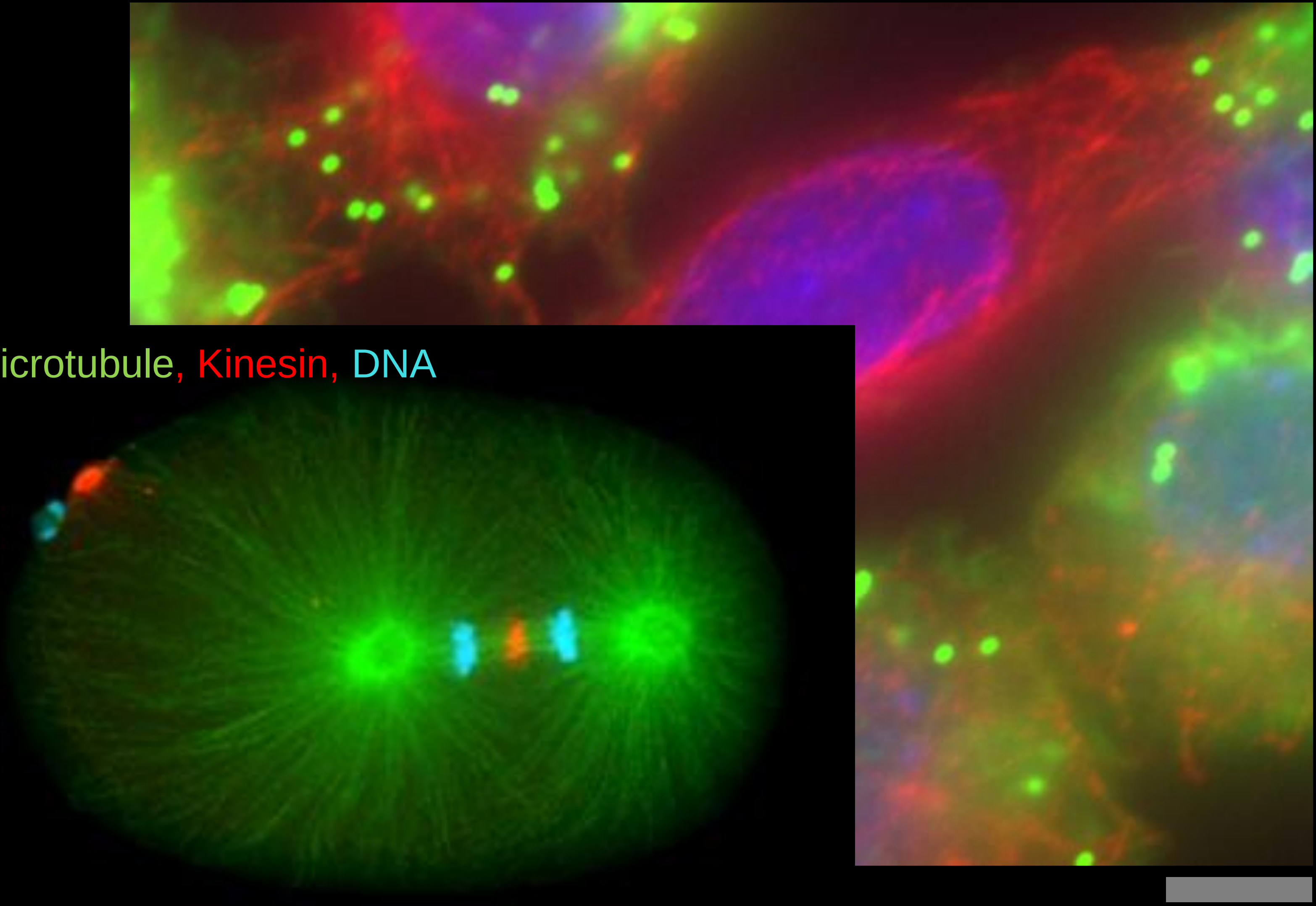


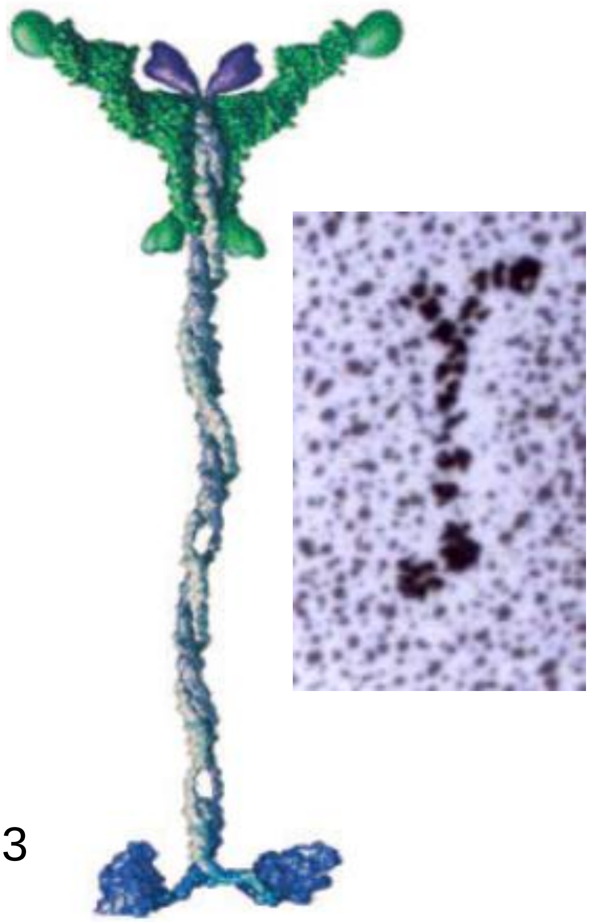
Figure 16.3 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Pigment granules, Chromosomes, Bacteria, Virus, …….



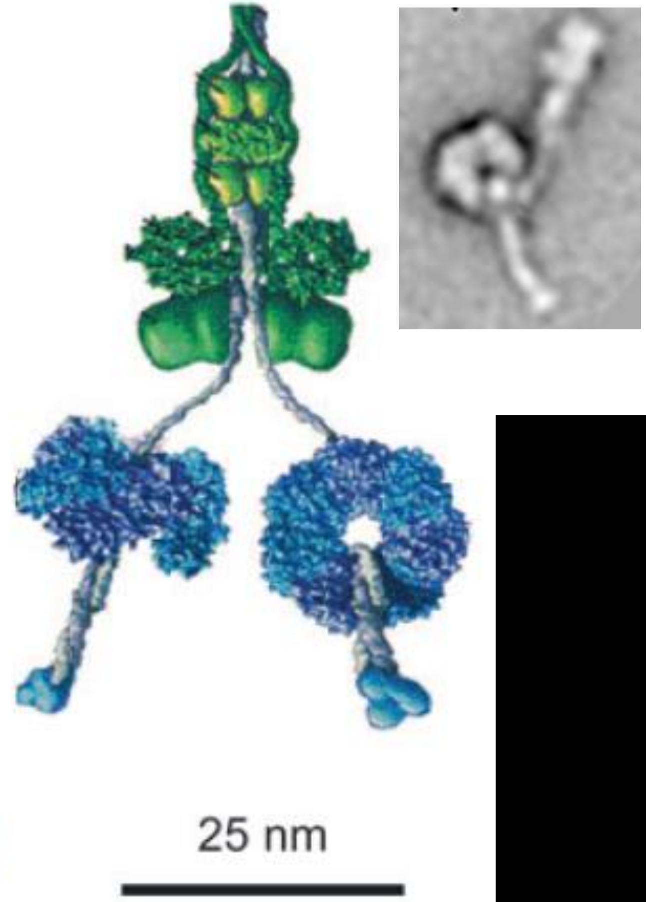
C. Elegans mitotic division, Canman Lab, Columbia

Kinesin



Vale 2003

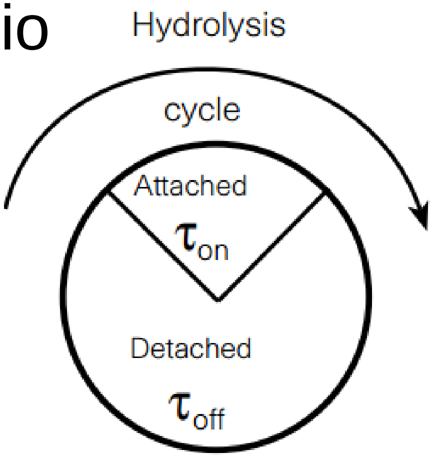
Dynein



Inner Life of a Cell

Typical numbers

Duty Ratio



Dimension of cells → 10 Microns

Size of a Motor → 50-100 nm

Cargoes carried by Motors → 50nm – Few microns

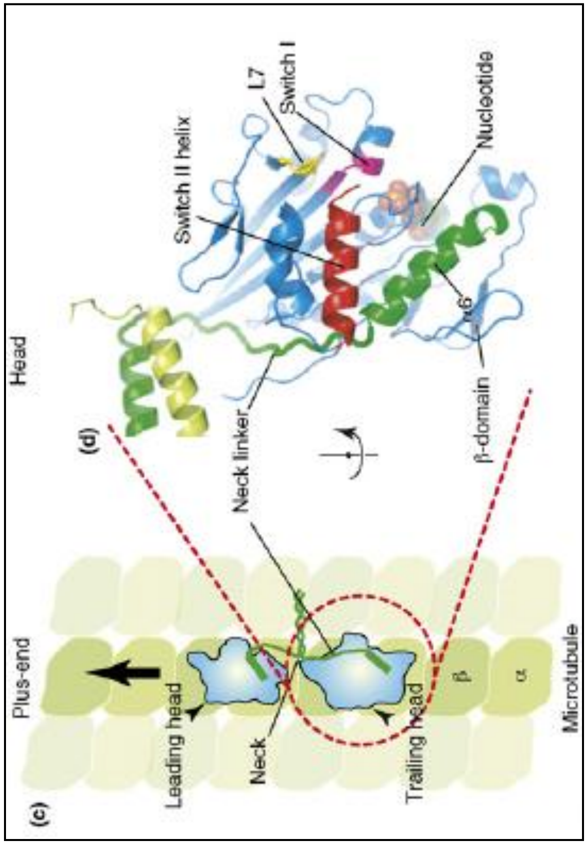
Velocity of motion → 1-2 microns/sec

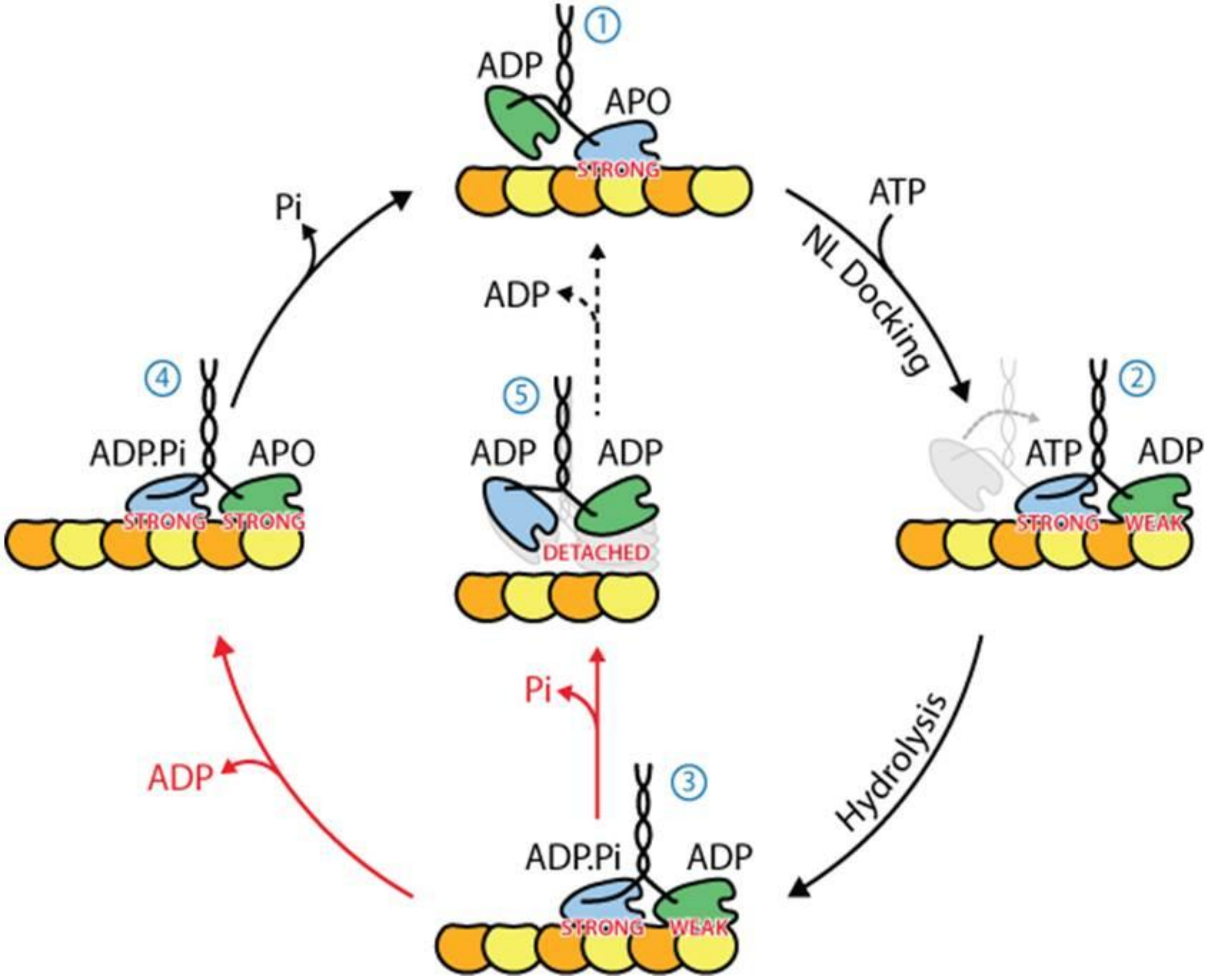
100 cycles completed in 1 second

Energy available from 1 ATP = 25 K_bT = 100 pN-nm

Work done per cycle ~ 50 pN-nm

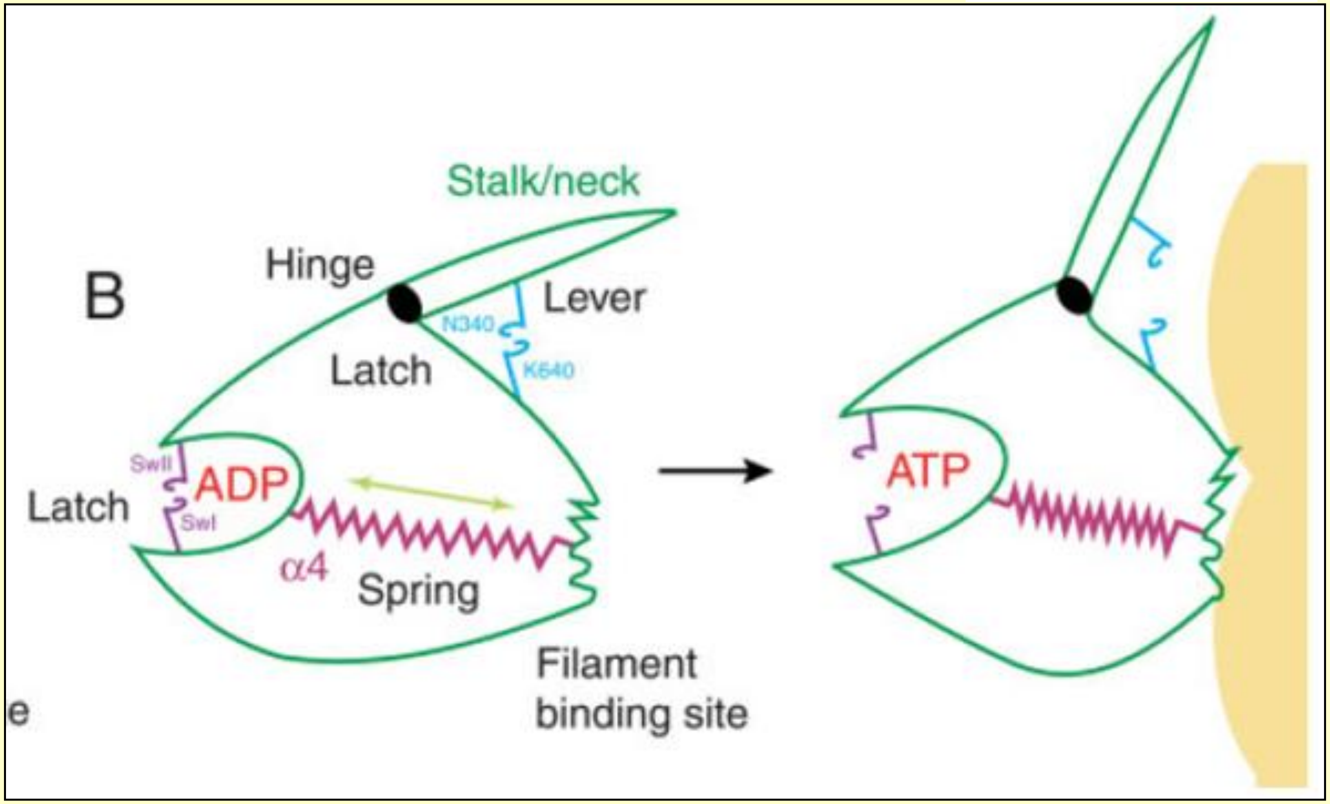
Diffusion constant for 50nm object ~ 1 micron^2/sec

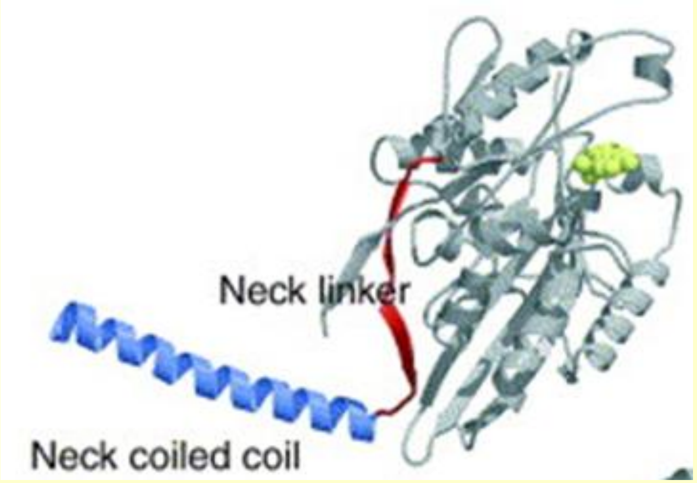


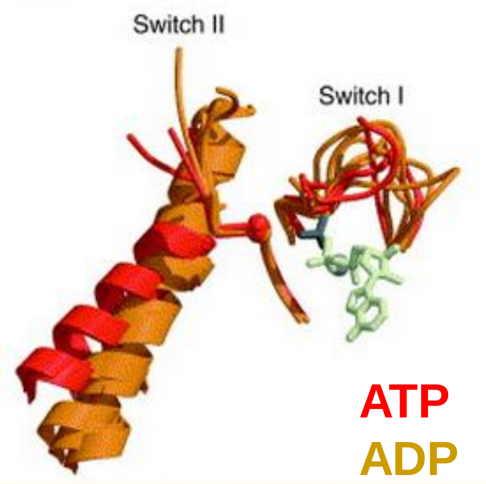


Weakly bound	ADP
Strongly bound	ATP, ADP-Pi andNucleotide free (Apo)

Sharyn A. Endow BioEssays 25.12

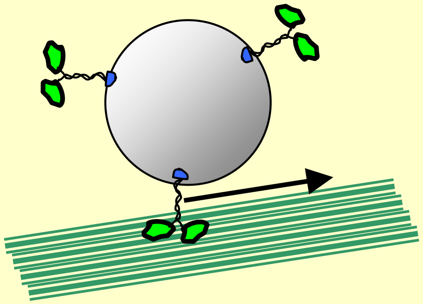


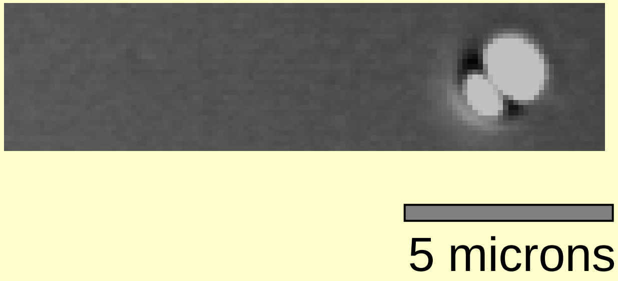


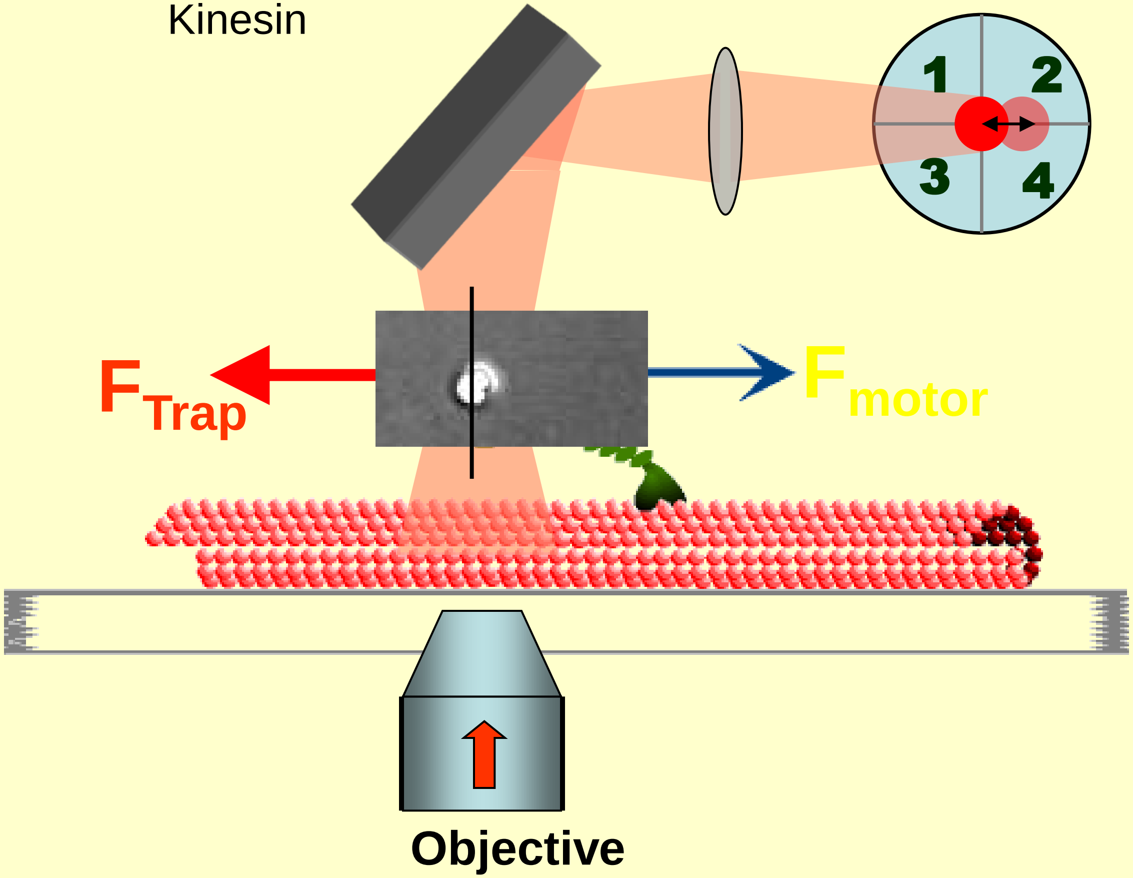


Sablin and Fletterick, Current Opinion in Structural Biology 2001

I. Studying Single Motors …







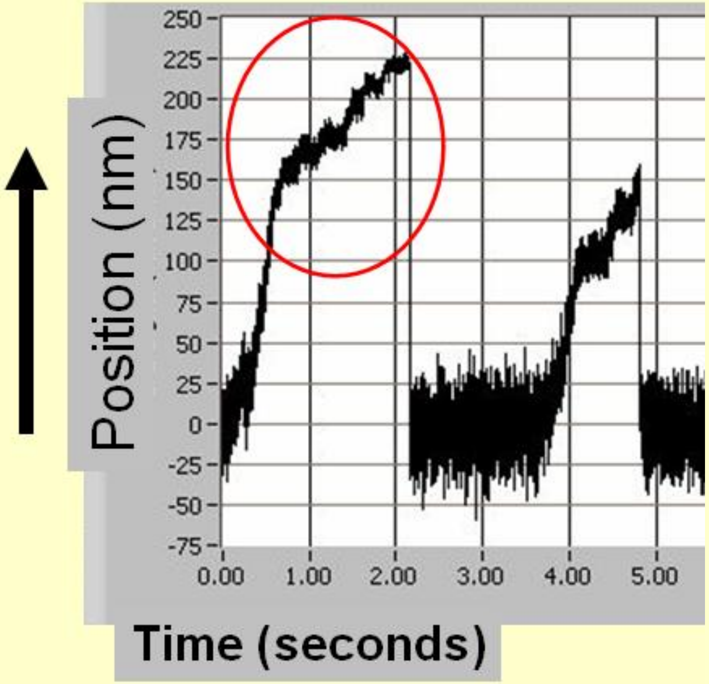
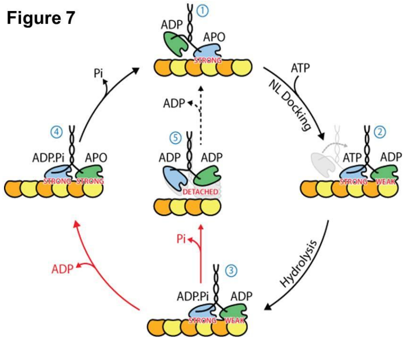
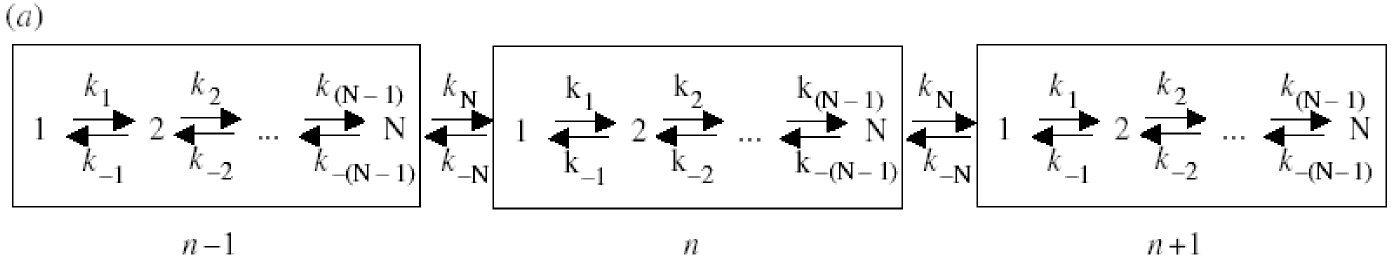


Figure 7





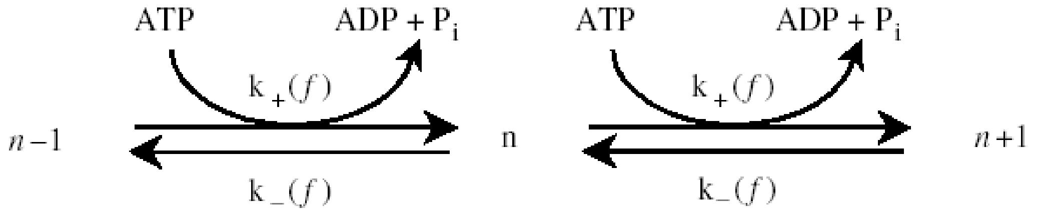


Figure 2. Kinetic schemes for a tightly coupled motor that hydrolyses one molecule of ATP in moving forwards from site n to $n+1$ while pulling against a constant load f. (a) A general N-state motor. (b) An idealized one-state motor.

Thomas et al,
Proc Roy Soc 2001

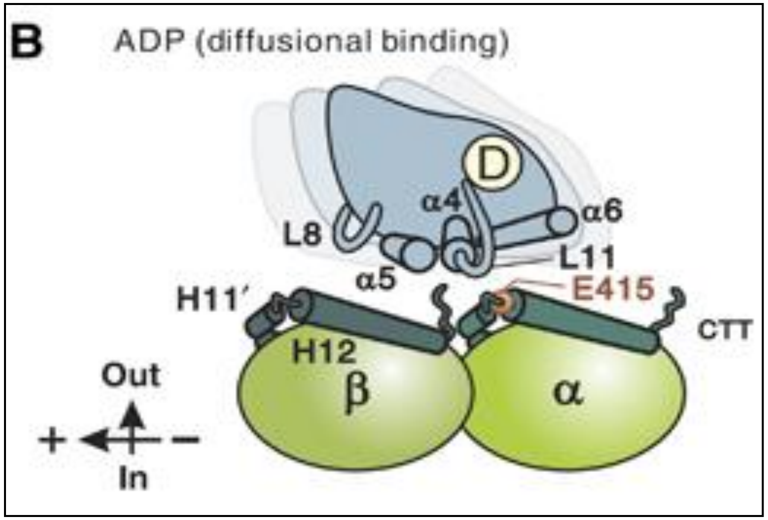
$$Keq = [k_1(f)k_2(f)k_3(f),\ldots,k_N(f)]/[k_{-1}(f)k_{-2}(f)k_{-3}(f),\ldots,k_{-N}(f)] = A\exp(-\Delta G/kT). \qquad (3.5)$$

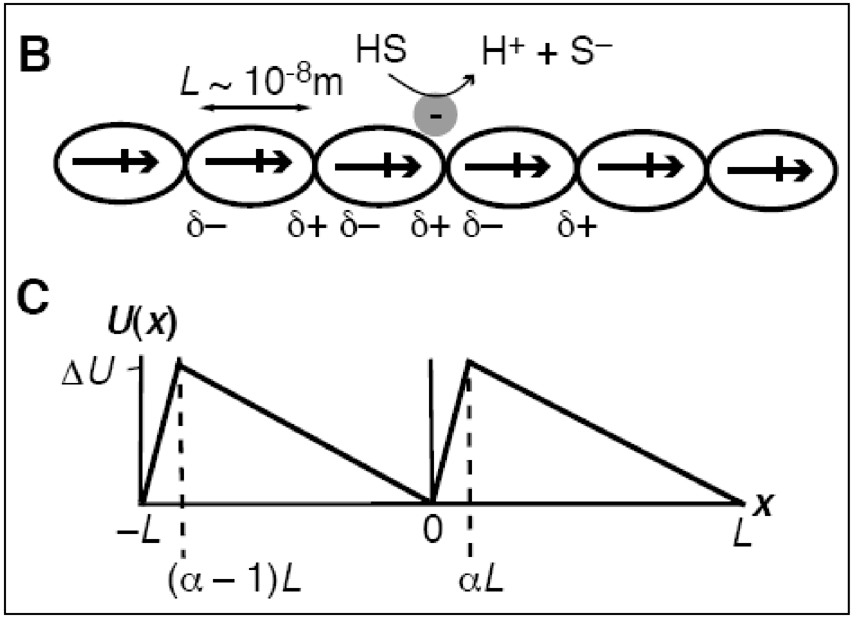
Equation of motion (overdamped)

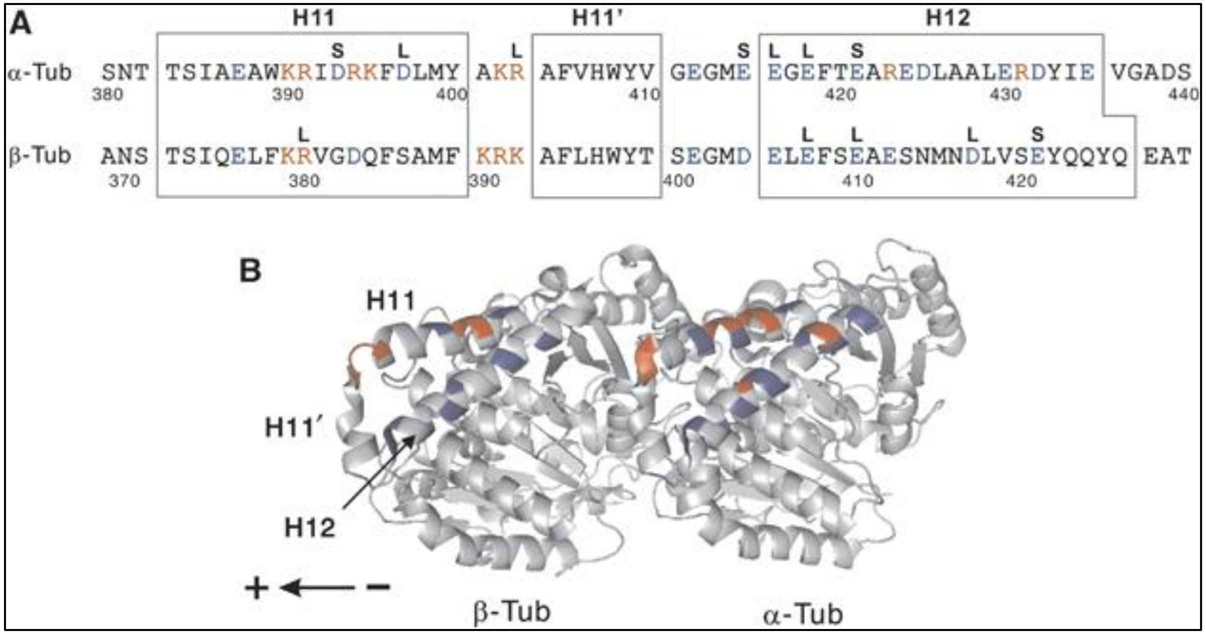
$$0 = -\gamma\frac{dx}{dt} - \frac{dV_\mu(x)}{dx} + F_{ext} + \xi(t),$$

Astumian, 1997

Uchimura et al, 2010







(B) Dipoles within units of a protein polymer
(C) Anisotropic sawtooth potential

Continuum Ratchets

Ajdari, A. and Prost, J. (1992)

Flashing Ratchet..

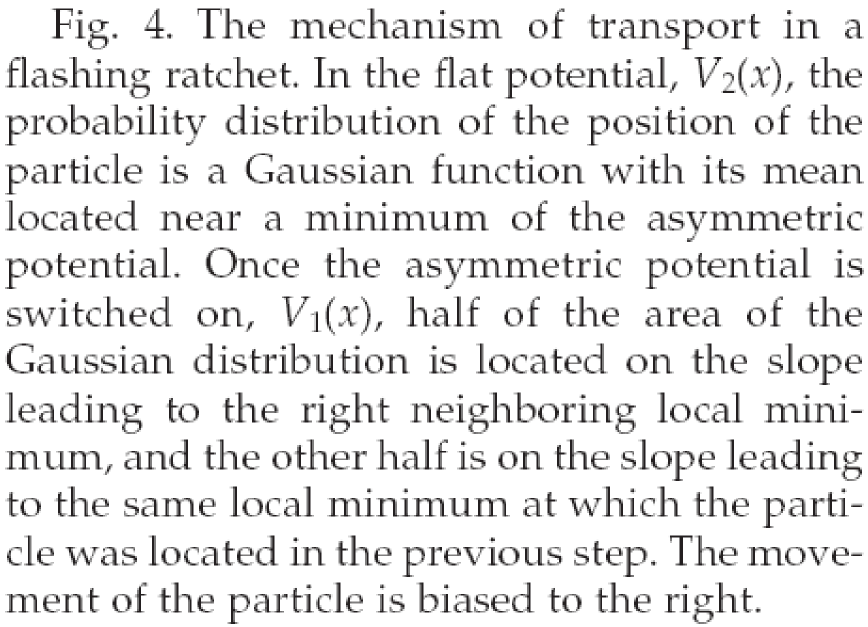
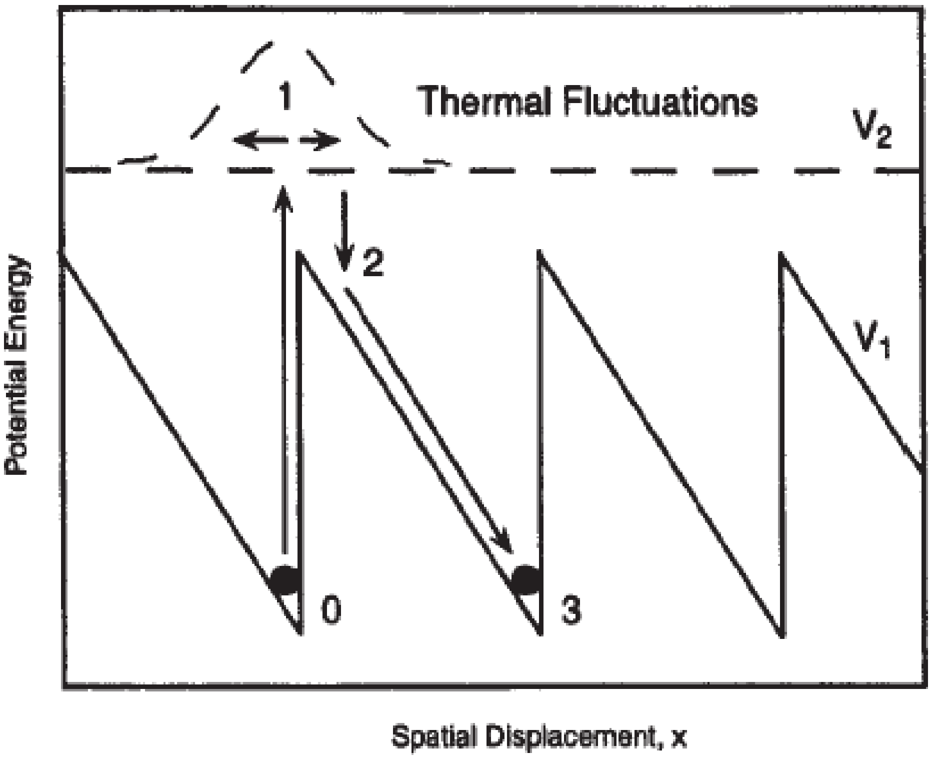


Fig. 4. The mechanism of transport in a flashing ratchet. In the flat potential, $V_2(x)$, the probability distribution of the position of the particle is a Gaussian function with its mean located near a minimum of the asymmetric potential. Once the asymmetric potential is switched on, $V_1(x)$, half of the area of the Gaussian distribution is located on the slope leading to the right neighboring local minimum, and the other half is on the slope leading to the same local minimum at which the particle was located in the previous step. The movement of the particle is biased to the right.



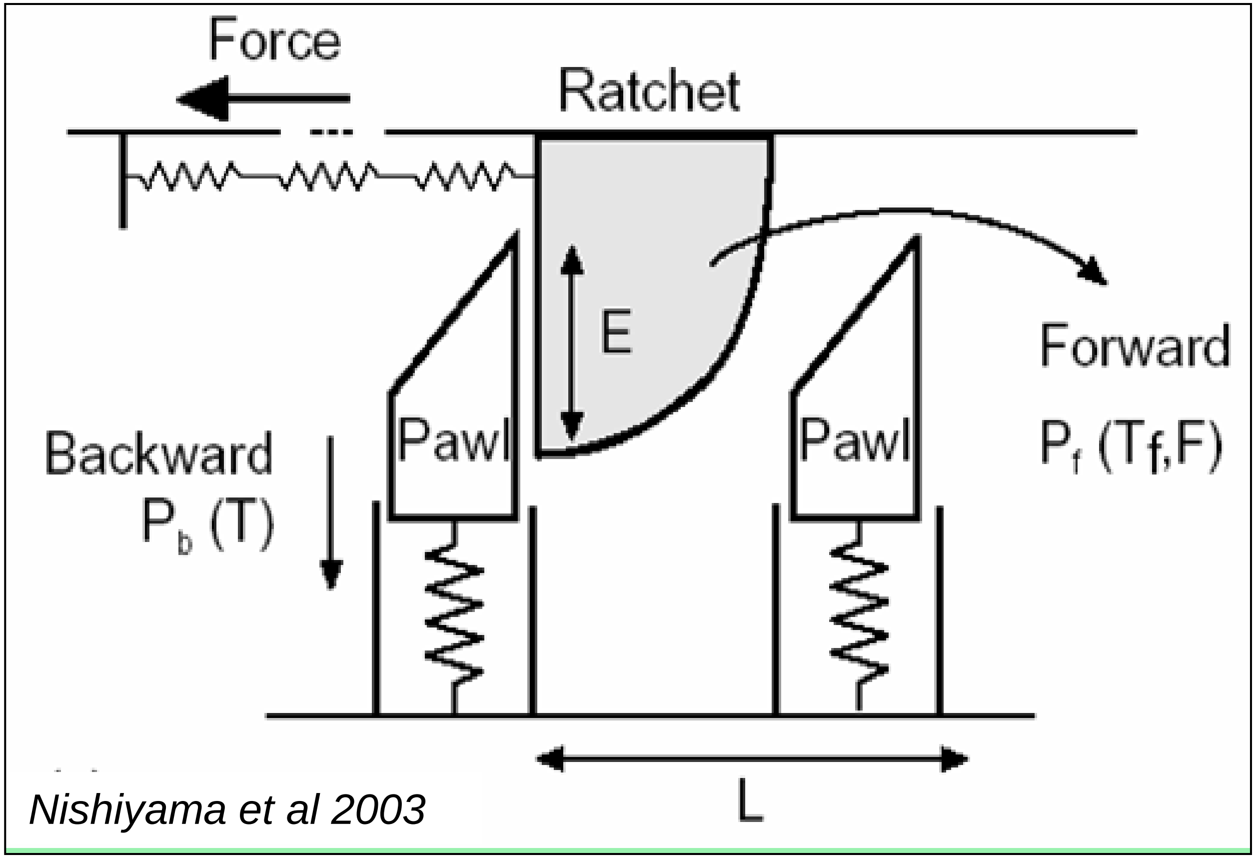
Chowdhury, 2008

Chemical state evolves following the Discrete master equation

$$\frac{\partial P_{\mu}(x,t)}{\partial t} = \sum_{\mu'} P_{\mu'}(x,t) W_{\mu' \to \mu}(x) - \sum_{\mu'} P_{\mu}(x,t) W_{\mu \to \mu'}(x)$$

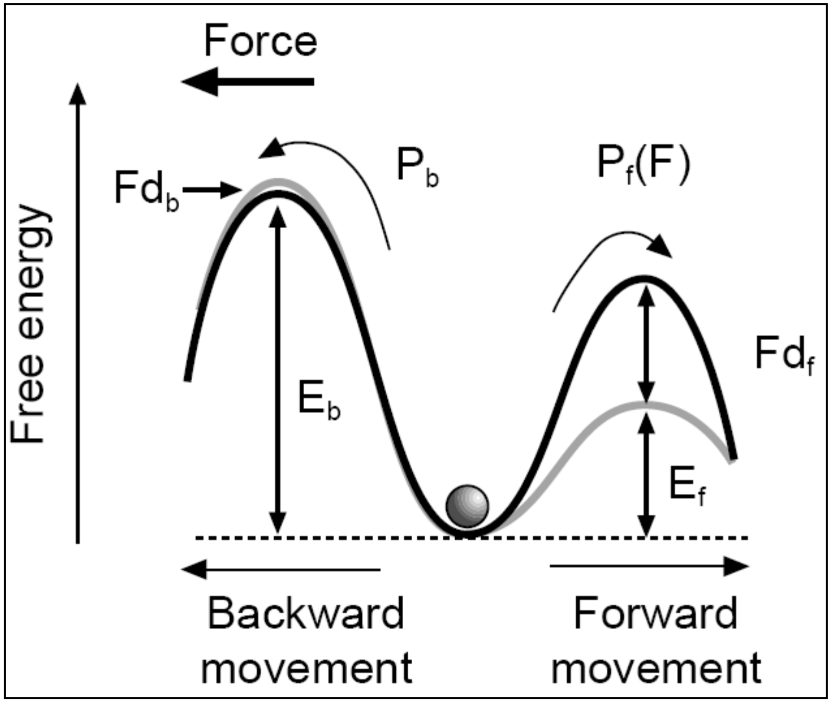
Fokker Planck equation for motion

$$\frac{\partial P_\mu(x,t)}{\partial t} = \underbrace{\frac{1}{\eta}\frac{\partial}{\partial x}\left[\left\{V'_\mu(x) - F\right\} P_\mu(x,t)\right] + \left(\frac{k_B T}{\eta}\right)\frac{\partial^2 P_\mu(x,t)}{\partial x^2}}_{\text{Hydrodynamics}} \underbrace{+\sum_{\mu'} P_{\mu'}(x,t) W_{\mu'\to\mu}(x) - \sum_{\mu'} P_\mu(x,t) W_{\mu\to\mu'}(x).}_{\text{Discrete Chemical transitions (Markov)}}$$



Nishiyama et al 2003

Nishiyama et al, 2003

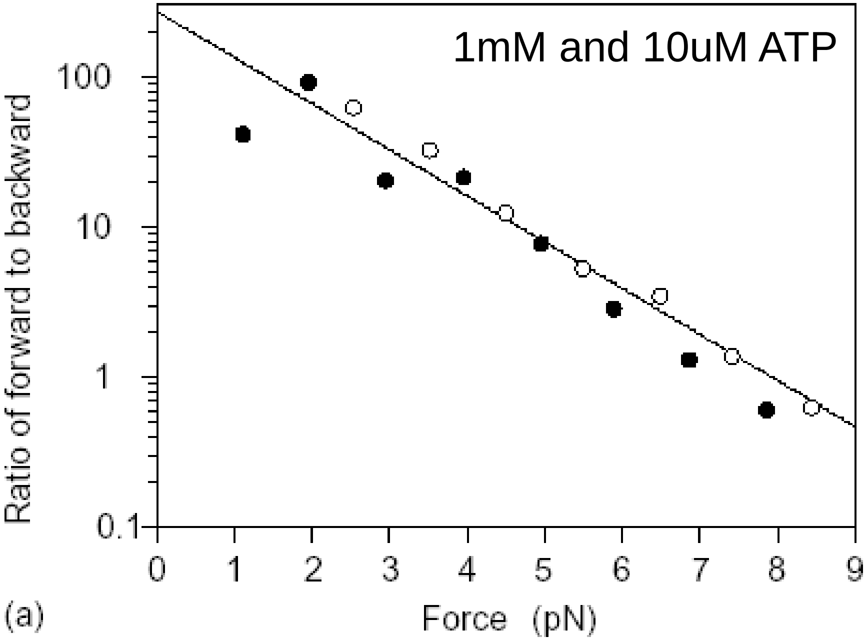


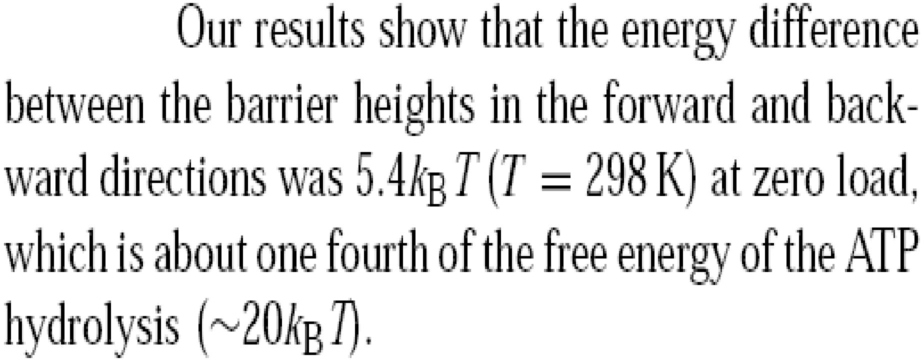
$$\frac{k_{3\mathrm{f}}}{k_{3\mathrm{b}}} = \exp\left[\frac{E_\mathrm{b} - E_\mathrm{f}}{k_\mathrm{B}T}\right]\exp\left(-\frac{Fd}{k_\mathrm{B}T}\right)$$

$$d\ (=d_\mathrm{f} - d_\mathrm{b})$$

Number of movements in the forward direction relative to those in the backward direction

→ Can be obtained from exp data (a)




Our results show that the energy difference between the barrier heights in the forward and backward directions was $5.4k_\mathrm{B}T$ ($T = 298$ K) at zero load, which is about one fourth of the free energy of the ATP hydrolysis ($\sim 20k_\mathrm{B}T$).

II Collections of Motors -- Forces in the Cell

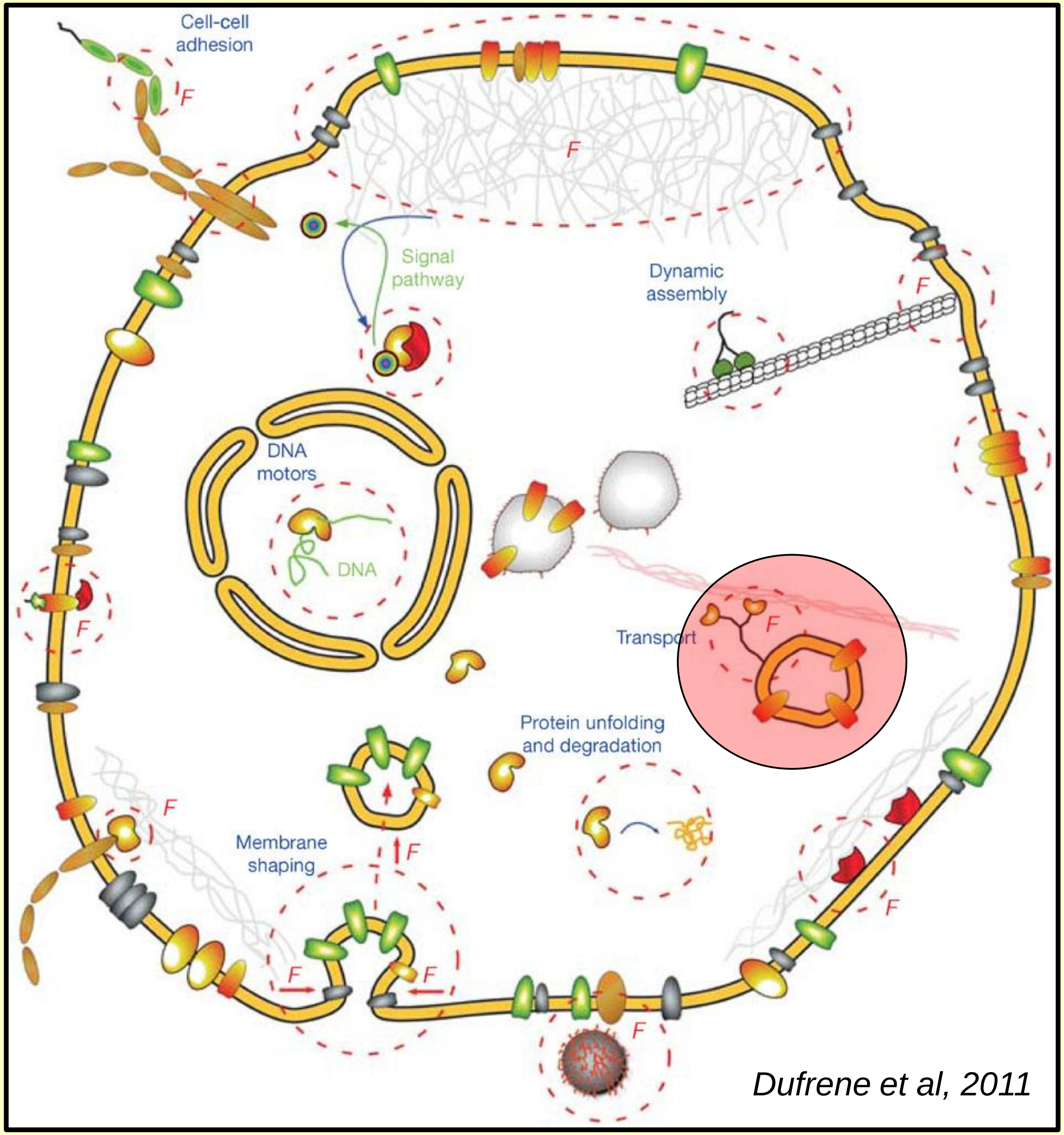
Molecular Forces
1-10 pN

Cellular Forces
10 -1000 pN

Heterogeneous, Difficult to measure

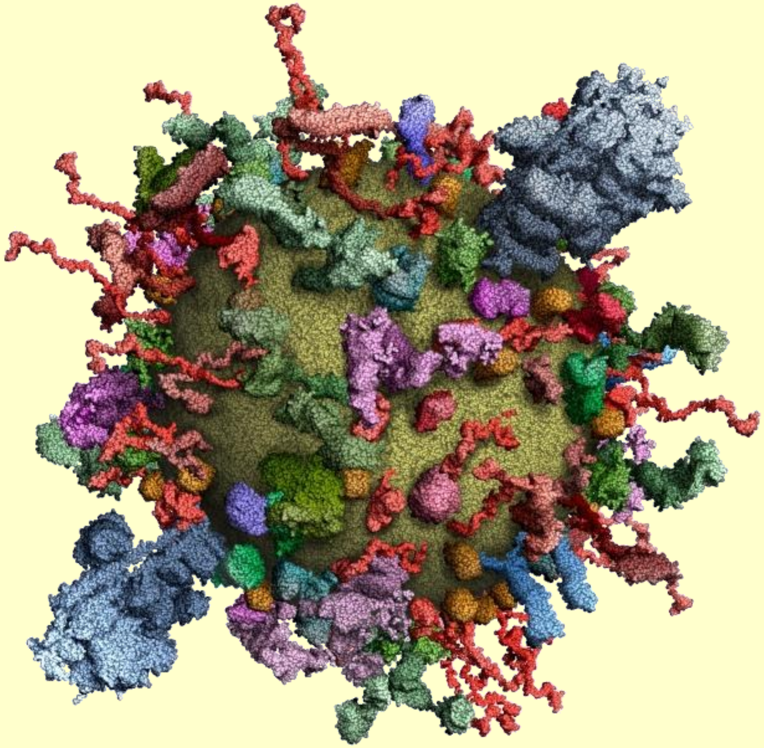
Contribution of individual molecules?

Reduced complexity:
Look at Single cargoes in the cell



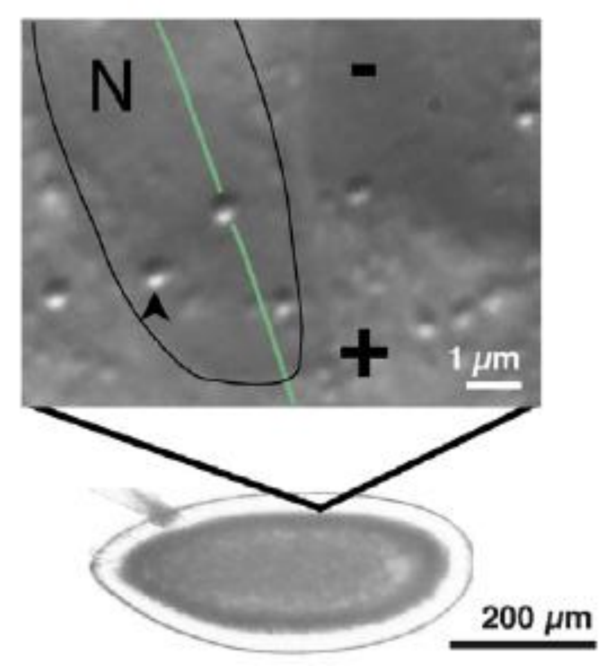
Dufrene et al, 2011

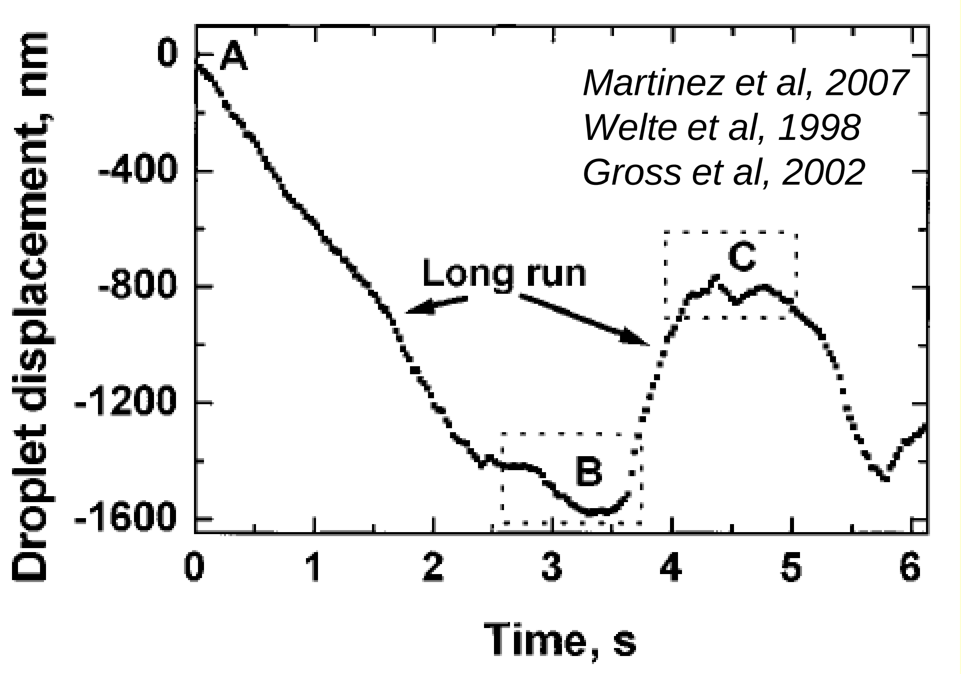
Reality …



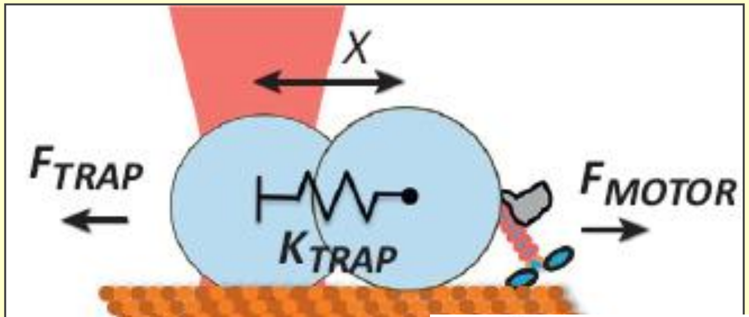
www.biotechnologie.de

Motion of Lipid droplets in *Drosophila* embryos



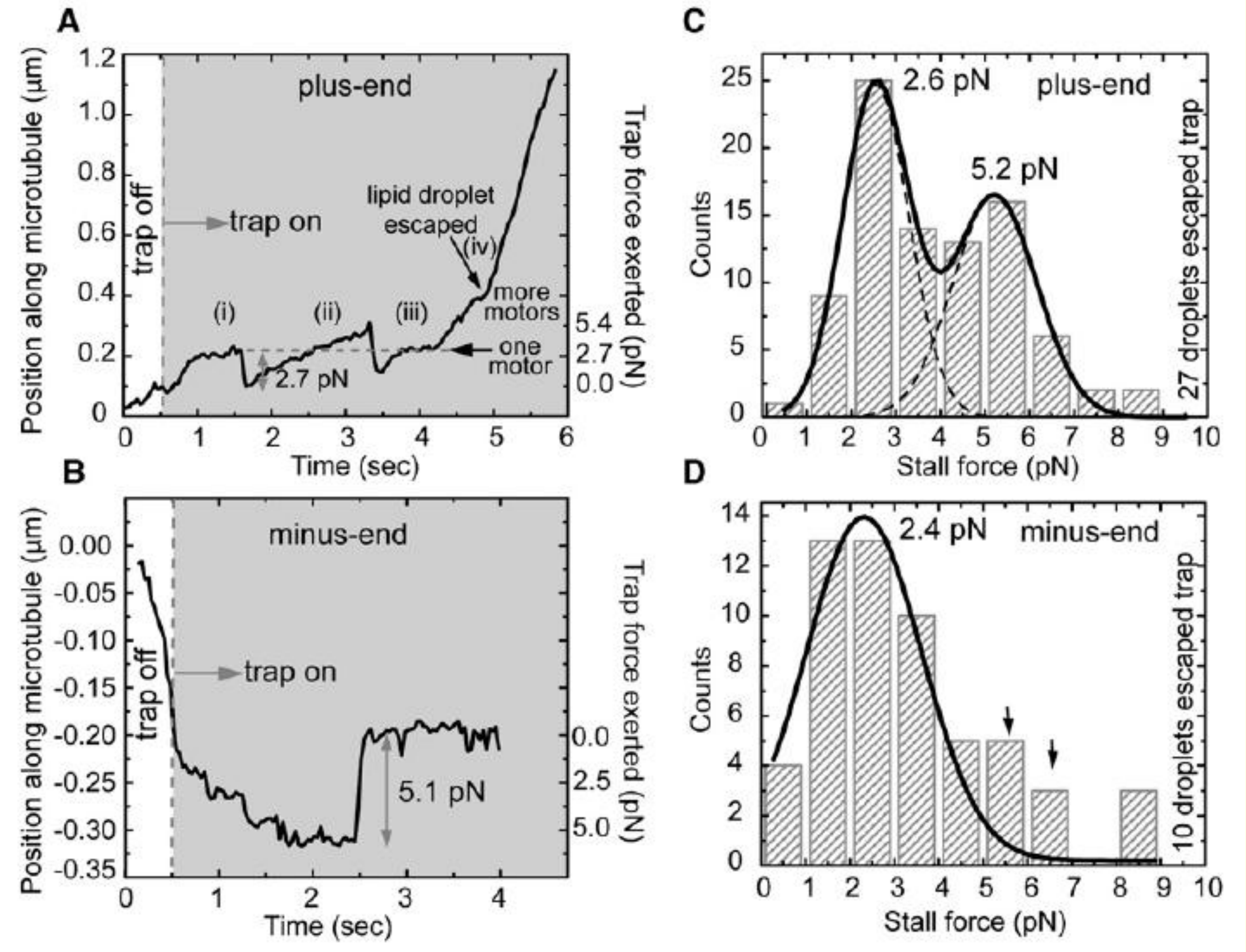


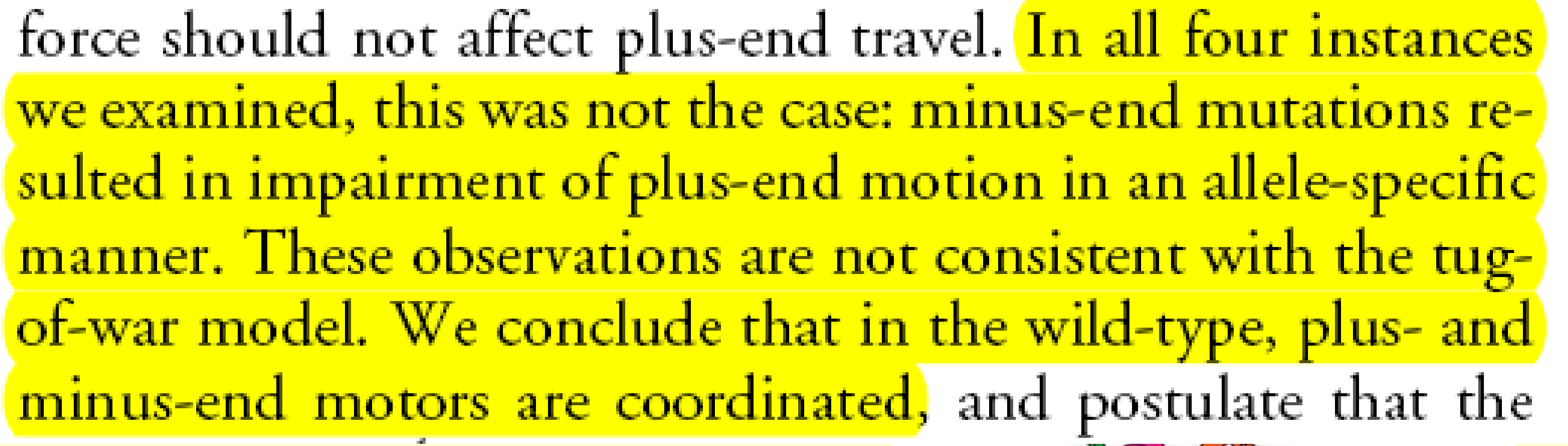
Martinez et al, 2007
Welte et al, 1998
Gross et al, 2002

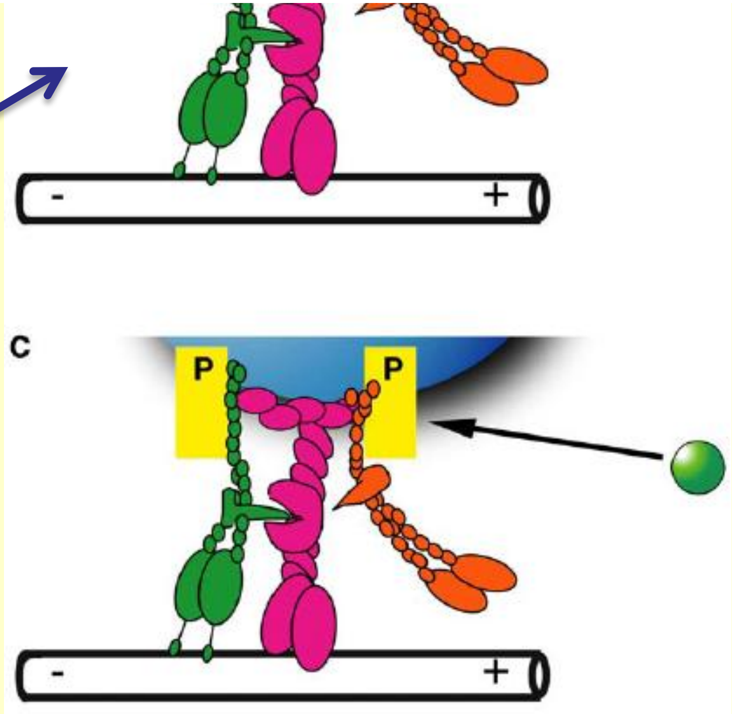


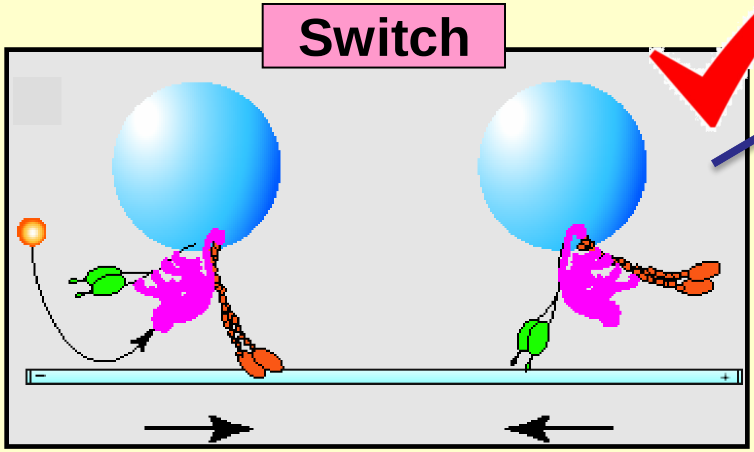
Force measurements in embryos

Shubeita et al, 2008




force should not affect plus-end travel. In all four instances we examined, this was not the case: minus-end mutations resulted in impairment of plus-end motion in an allele-specific manner. These observations are not consistent with the tug-of-war model. We conclude that in the wild-type, plus- and minus-end motors are coordinated, and postulate that the



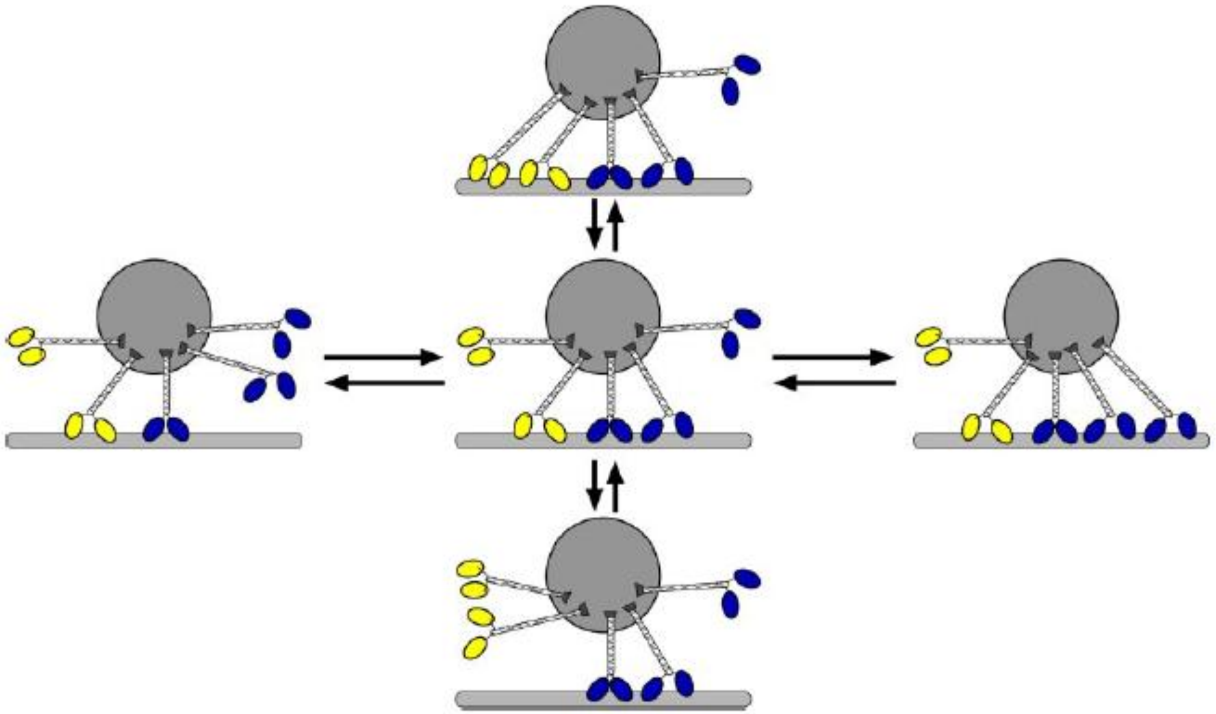


Welte et al, 2004, 2005

Tug-of-war as a cooperative mechanism for bidirectional cargo transport by molecular motors

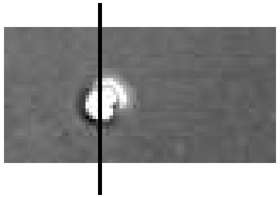
Melanie J. I. Müller*, Stefan Klumpp†, and Reinhard Lipowsky*‡

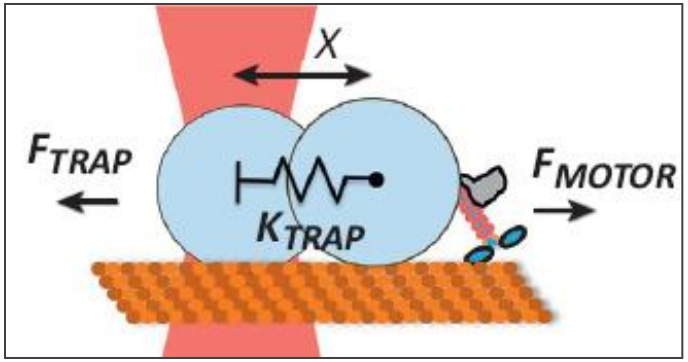
PNAS | March 25, 2008

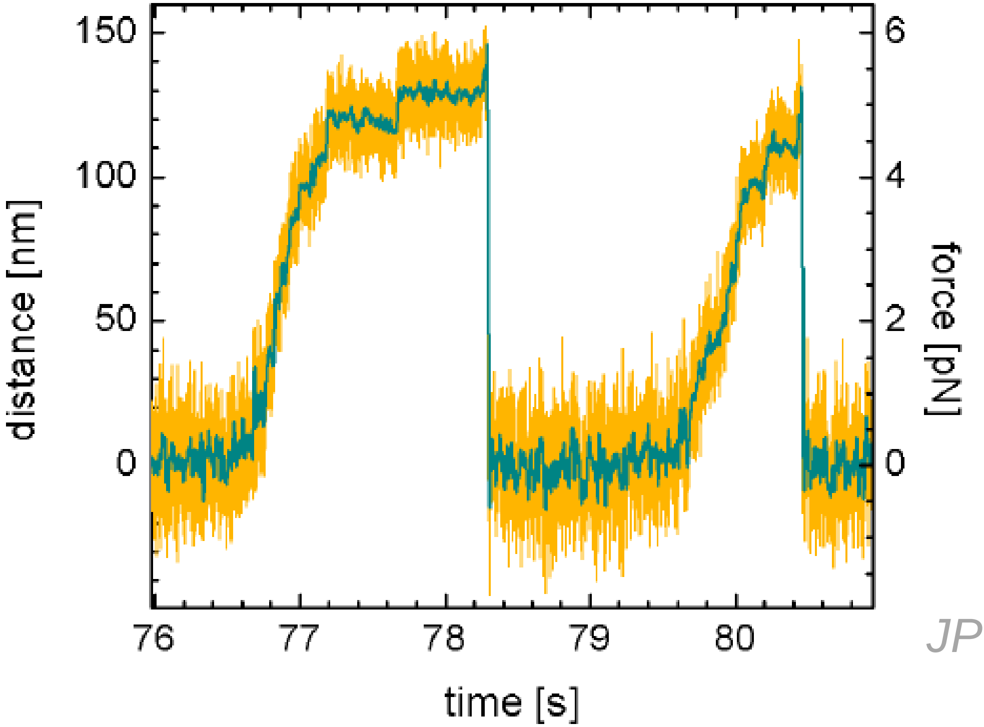


- Stochastic transitions between two species of motor yields Bidirectional motion
- Tuning of single-motor parameters
- No need to invoke a third “coordination complex”

To model the motion







JPK

Velocity
$$v(F) = \begin{cases} v_F\left(1 - F/F_s\right) & \text{for} \quad F \leq F_s \\ v_B\left(1 - F/F_s\right) & \text{for} \quad F \geq F_s \end{cases}$$

Unbinding rate
$$\varepsilon(F) = \varepsilon_0 \exp[F/F_d],$$

Binding rate
$$\pi(F) = \pi_0$$

Muller et al, 2008

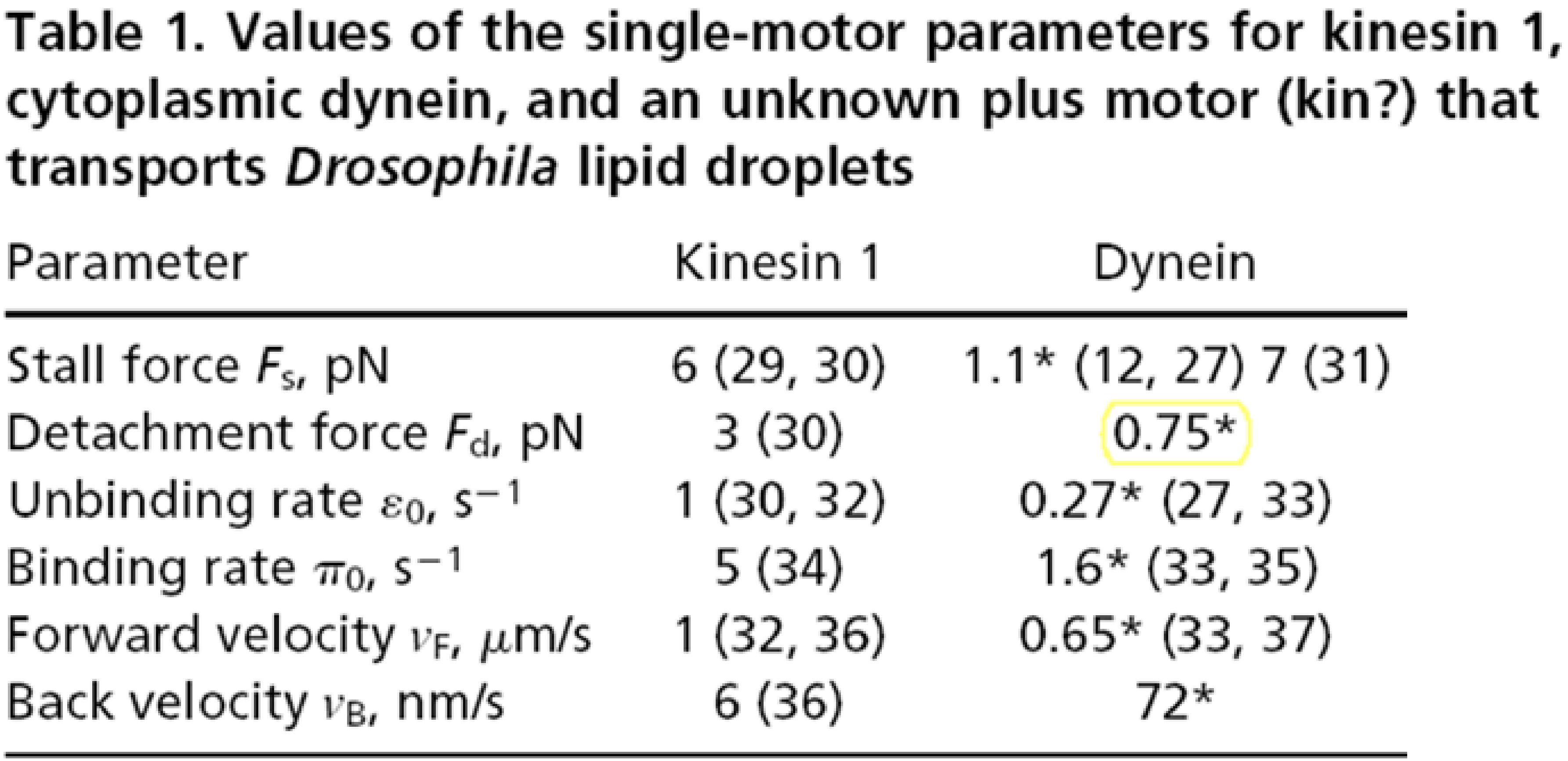
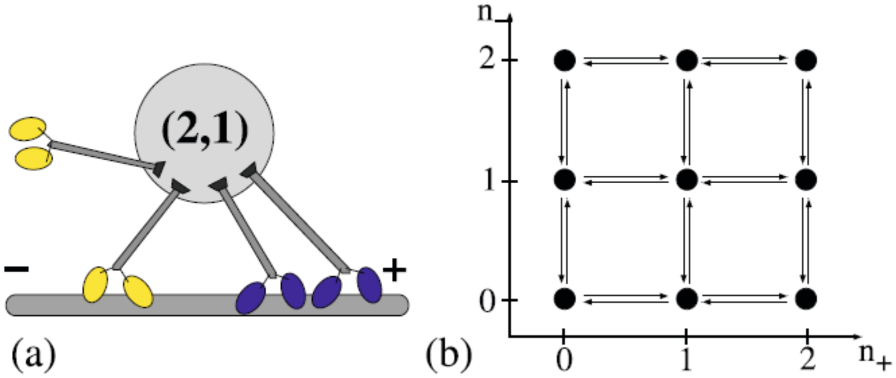


Table 1. Values of the single-motor parameters for kinesin 1, cytoplasmic dynein, and an unknown plus motor (kin?) that transports *Drosophila* lipid droplets

Parameter	Kinesin 1	Dynein
Stall force F_s, pN	6 (29, 30)	1.1* (12, 27) 7 (31)
Detachment force F_d, pN	3 (30)	0.75*
Unbinding rate ε_0, s^{-1}	1 (30, 32)	0.27* (27, 33)
Binding rate π_0, s^{-1}	5 (34)	1.6* (33, 35)
Forward velocity v_F, μm/s	1 (32, 36)	0.65* (33, 37)
Back velocity v_B, nm/s	6 (36)	72*

Unbinding rate ε

Binding rate π



Steady state solution to …

$$\frac{\partial}{\partial t} p(n_+, n_-, t)$$

$$= p(n_+ + 1, n_-, t)\, \varepsilon_+(n_+ + 1, n_-) + p(n_+, n_- + 1, t)\, \varepsilon_-(n_+, n_- + 1)$$

$$+ p(n_+ - 1, n_-, t)\, \pi_+(n_+ - 1, n_-) + p(n_+, n_- - 1, t)\, \pi_-(n_+, n_- - 1)$$

$$- p(n_+, n_-, t)[\pi_+(n_+, n_-) + \pi_-(n_+, n_-) + \varepsilon_+(n_+, n_-) + \varepsilon_-(n_+, n_-)].$$

Force Balance $\quad n_+ F_+ = -n_- F_- \equiv F_c(n_+, n_-),$

Mean field $\quad F_+ = F_c / n_+$

$$\Rightarrow \quad \varepsilon_+(n_+, n_-) = n_+\, \varepsilon_{0+} \exp[F_c(n_+, n_-)/(n_+ F_{d+})].$$

Velocity Balance $\quad v_c(n_+, n_-) = v_+(F_+) = -v_-(-F_-).$

Muller et al, 2008

Muller et al, 2008

The force and velocity balances as given by (6) and (9) lead to the cargo force

$$F_c(n_+, n_-) = \lambda(n_+, n_-)\, n_+ F_{s+} + [1 - \lambda(n_+, n_-)]\, n_- F_{s-}, \tag{10}$$

with

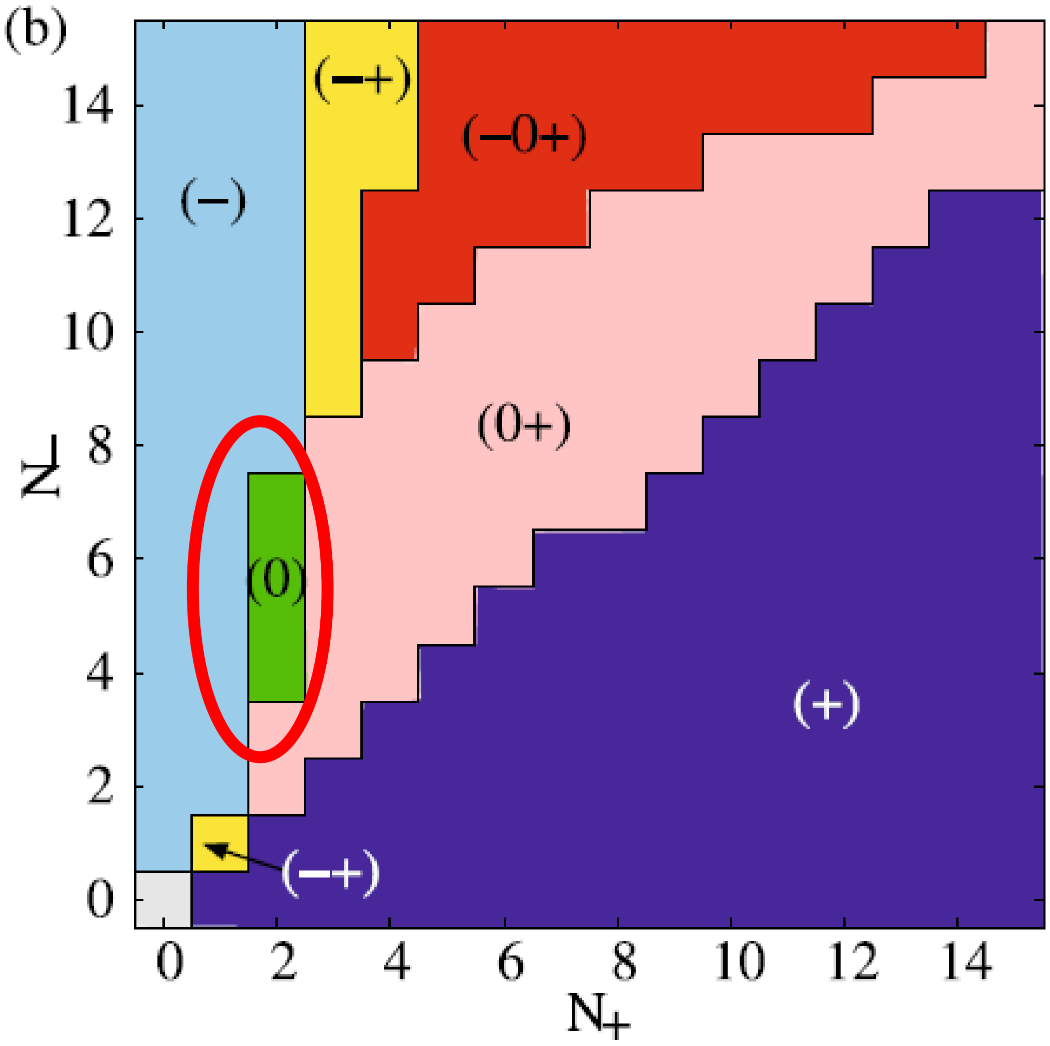
$$\lambda(n_+, n_-) = 1/[1 + (n_+ F_{s+} v_{0-})/(n_- F_{s-} v_{0+})], \tag{11}$$

and to the cargo velocity

$$v_c(n_+, n_-) = \frac{n_+ F_{s+} - n_- F_{s-}}{n_+ F_{s+}/v_{0+} + n_- F_{s-}/v_{0-}}. \tag{12}$$

We solve the Master equation (1) for the steady state by determining the eigenvector of the associated transition matrix with eigenvalue zero. In addition, we simulate individual cargo trajectories by using the Gillespie algorithm [10] for the binding/unbinding dynamics as given by (7) and (8) and let the cargo move with velocity v_c in the intervals between (un-)binding events.

Phase diagram of Bidirectional motion



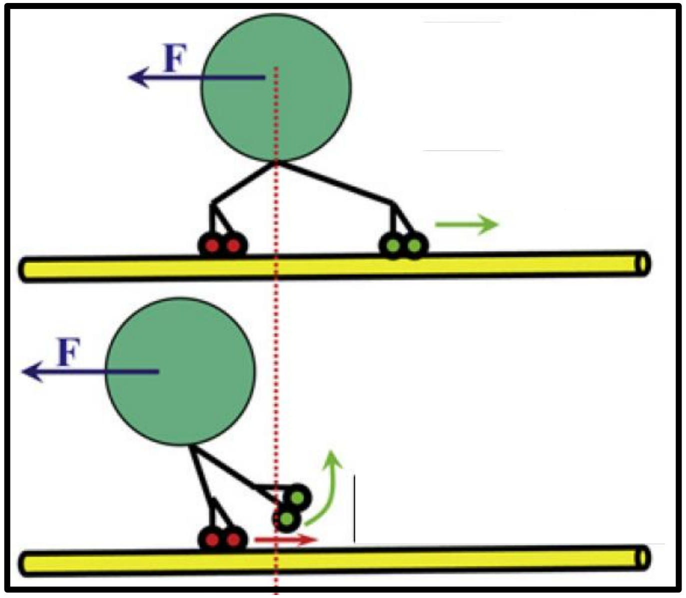
Muller et al, 2008

Criticisms …

Equal Load Sharing

Linear Force Velocity

Detachment under load



Tug-of-war between dissimilar teams of microtubule motors regulates transport and fission of endosomes

Virupakshi Soppina, Arpan Kumar Rai, Avin Jayesh Ramaiya, Pradeep Barak, and Roop Mallik[1]

Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Colaba, Mumbai 400005, India

Edited by J. Richard McIntosh, University of Colorado, Boulder, CO, and approved September 14, 2009 (received for review June 12, 2009)

PNAS, 2009

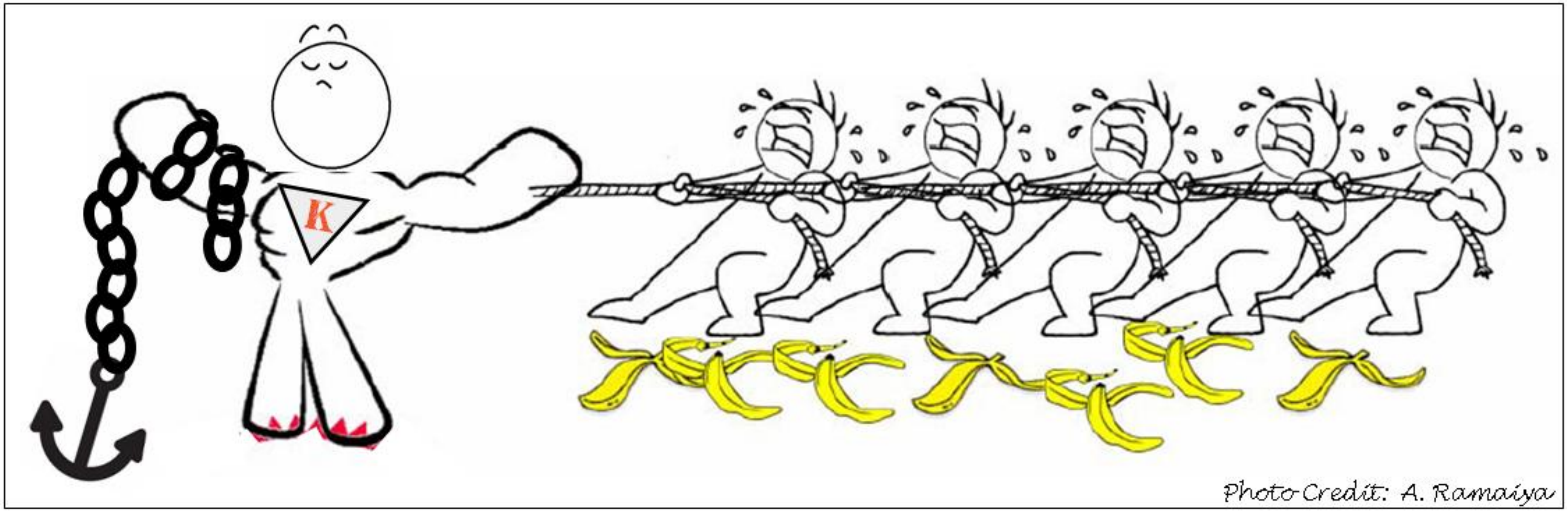
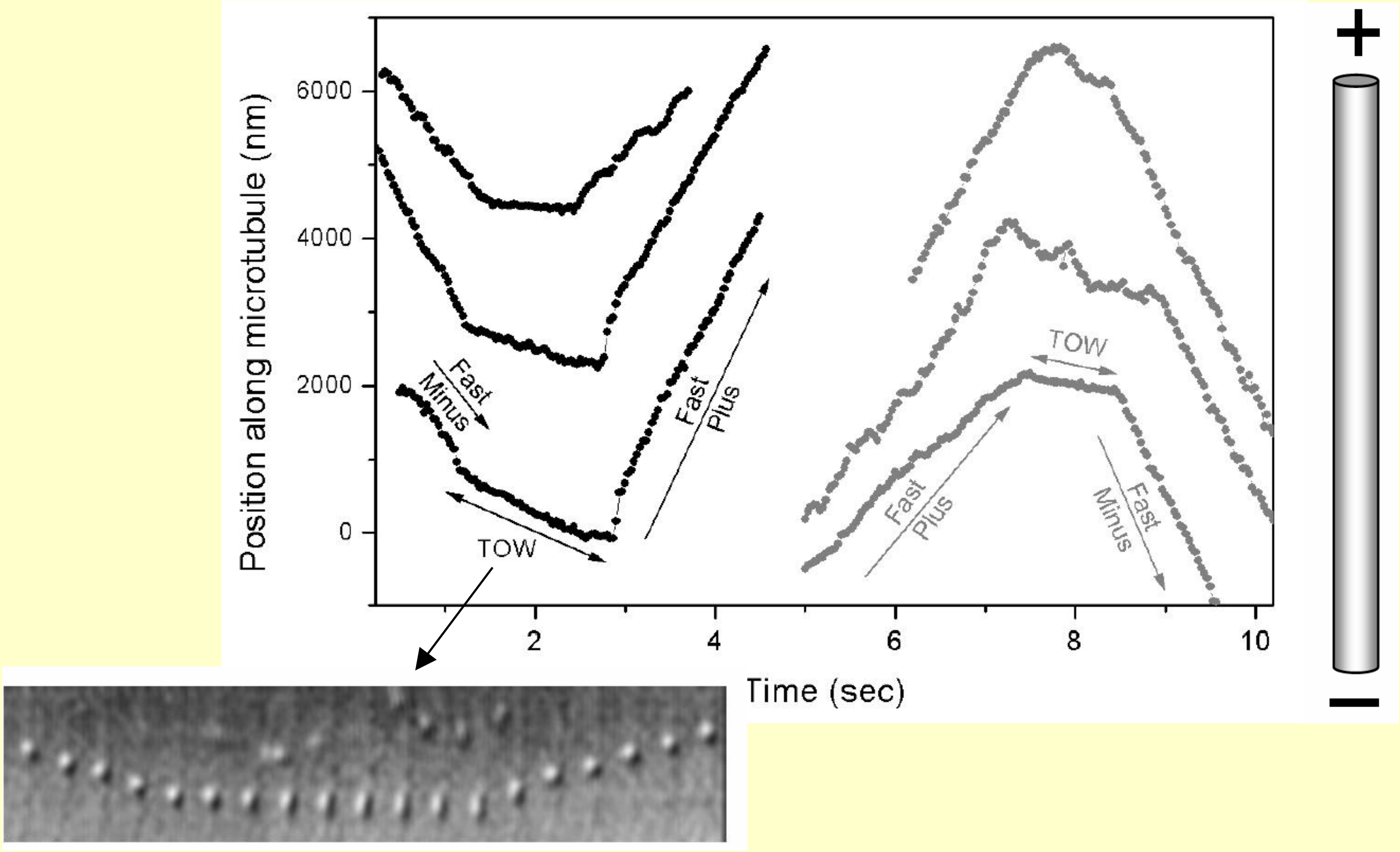


Photo Credit: A. Ramaiya

5.5 pN

1.1 x 5 = 5.5 pN

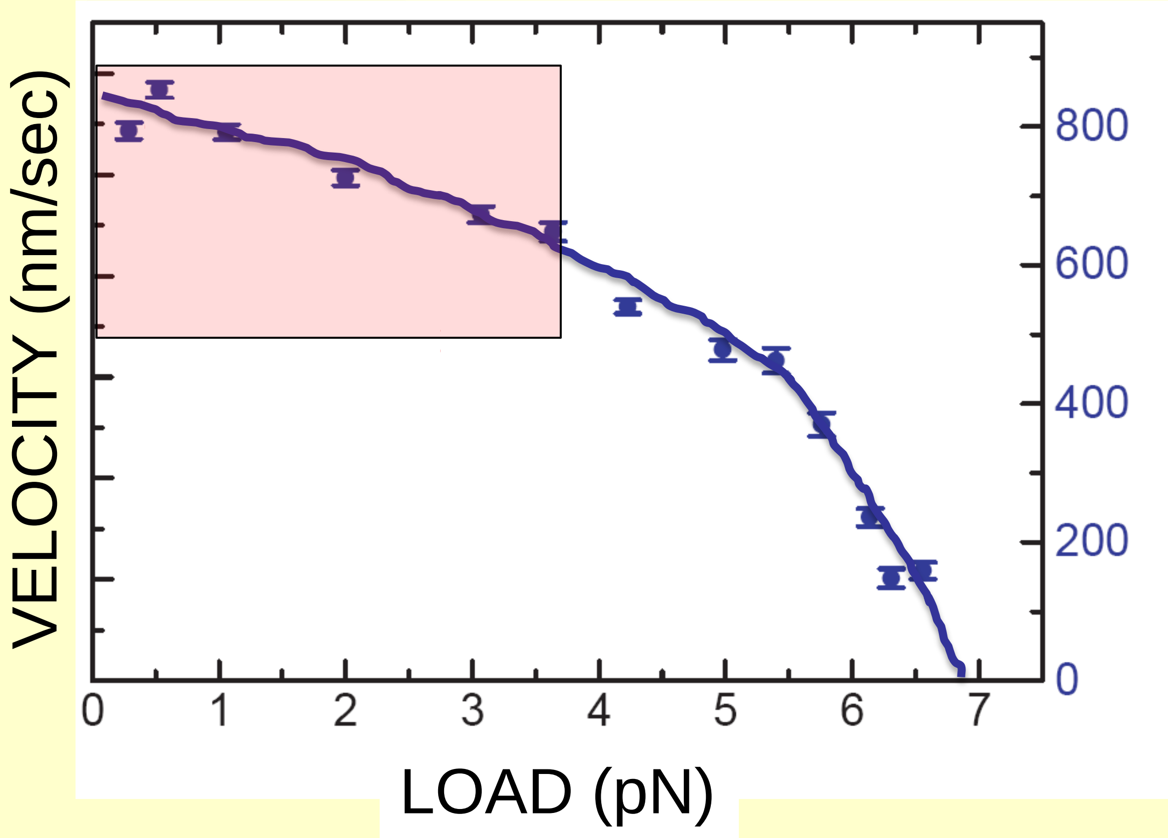
Motion of Endosomes



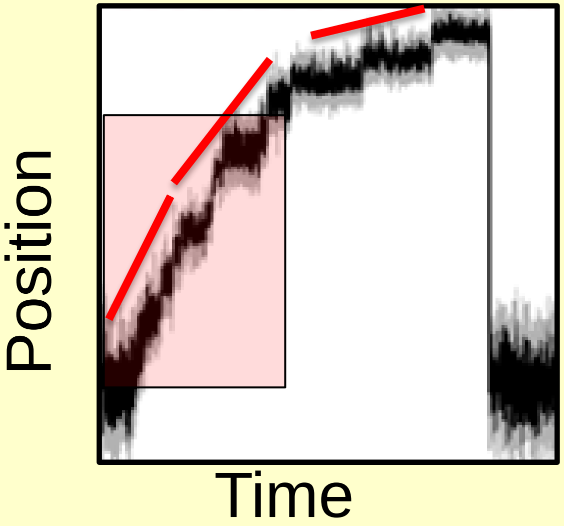
5-8 Dyneins in Tug Of War against 1-2 Kinesins
… In good agreement with Lipowsky (??)

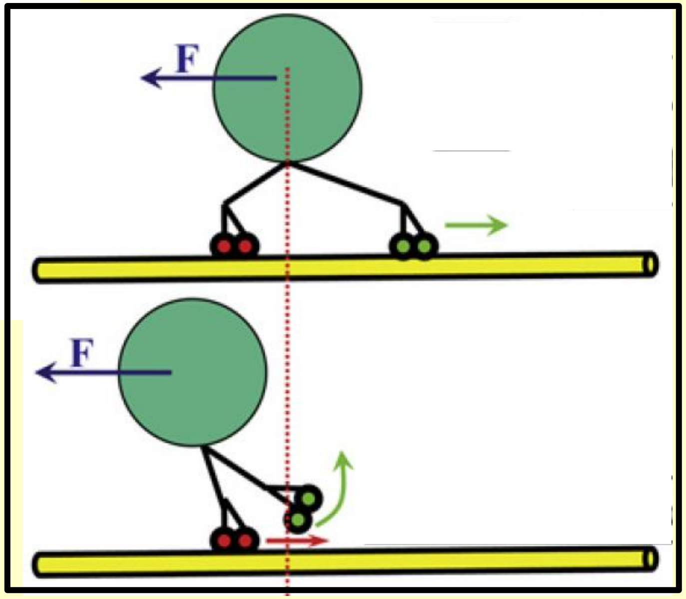
Kinesin's response to Load …

F-V curve



Visscher et al, Nature 1999

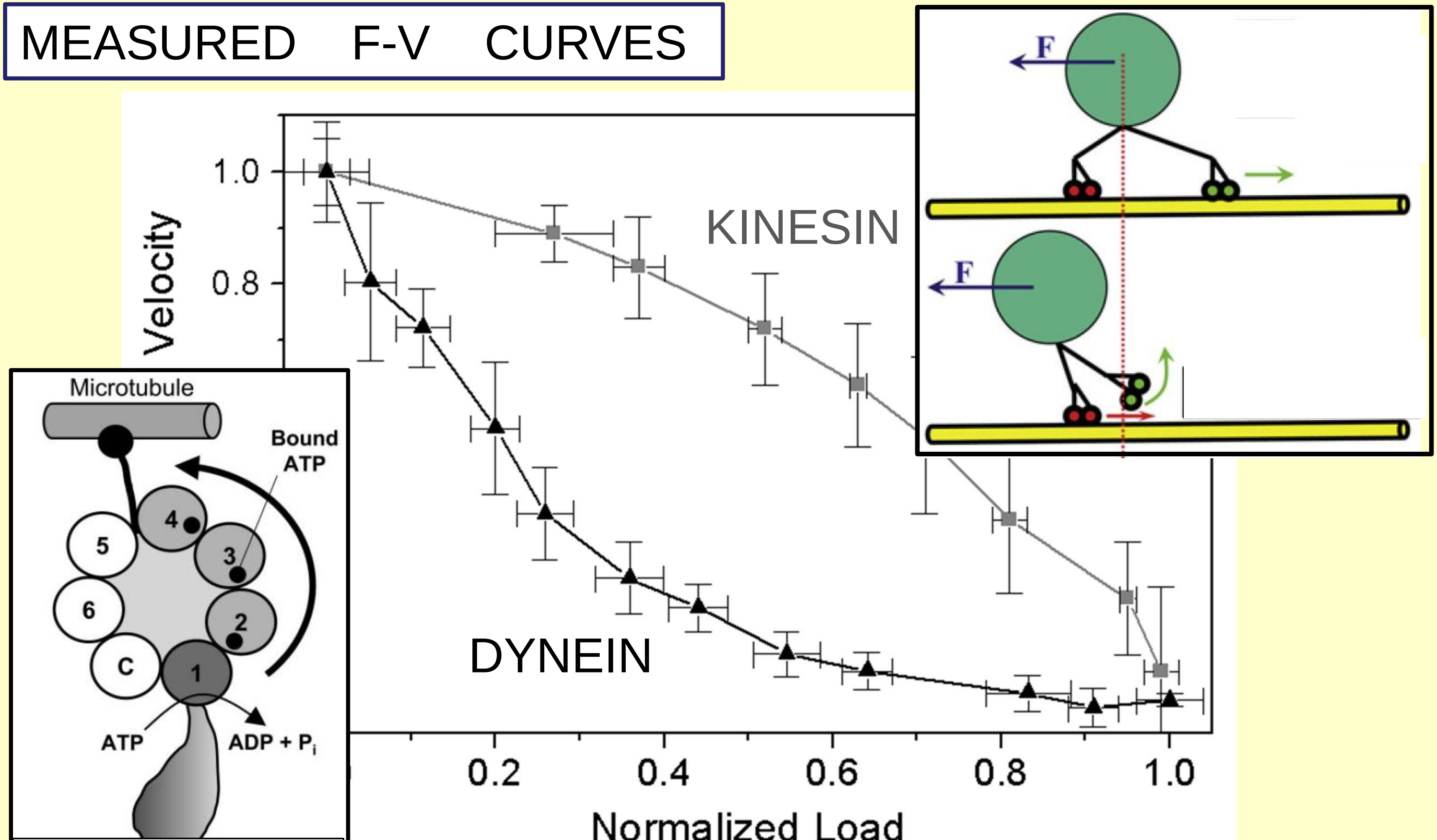




Jamison et al 2010
Driver et al 2011
Kunwar et al 2008

Mechanism for Dynein’s improved collective Function ?

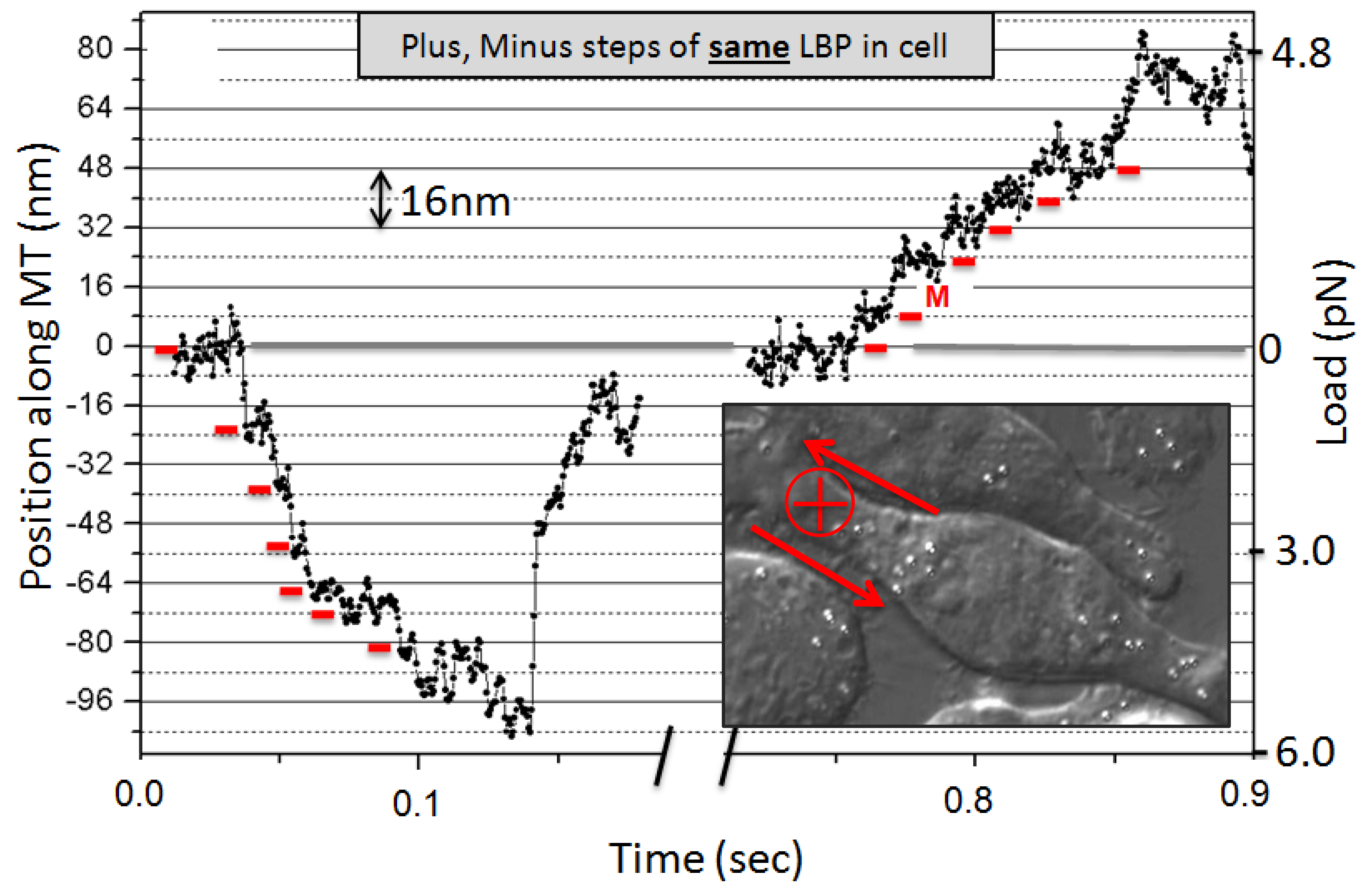
MEASURED F-V CURVES



Dynein as a Gear
Mallik et al,
Nature 2004

Crucial input from MC
--- Singh et al , PNAS 2005

LOAD-DEPENDENT STEP SIZE INSIDE CELLS



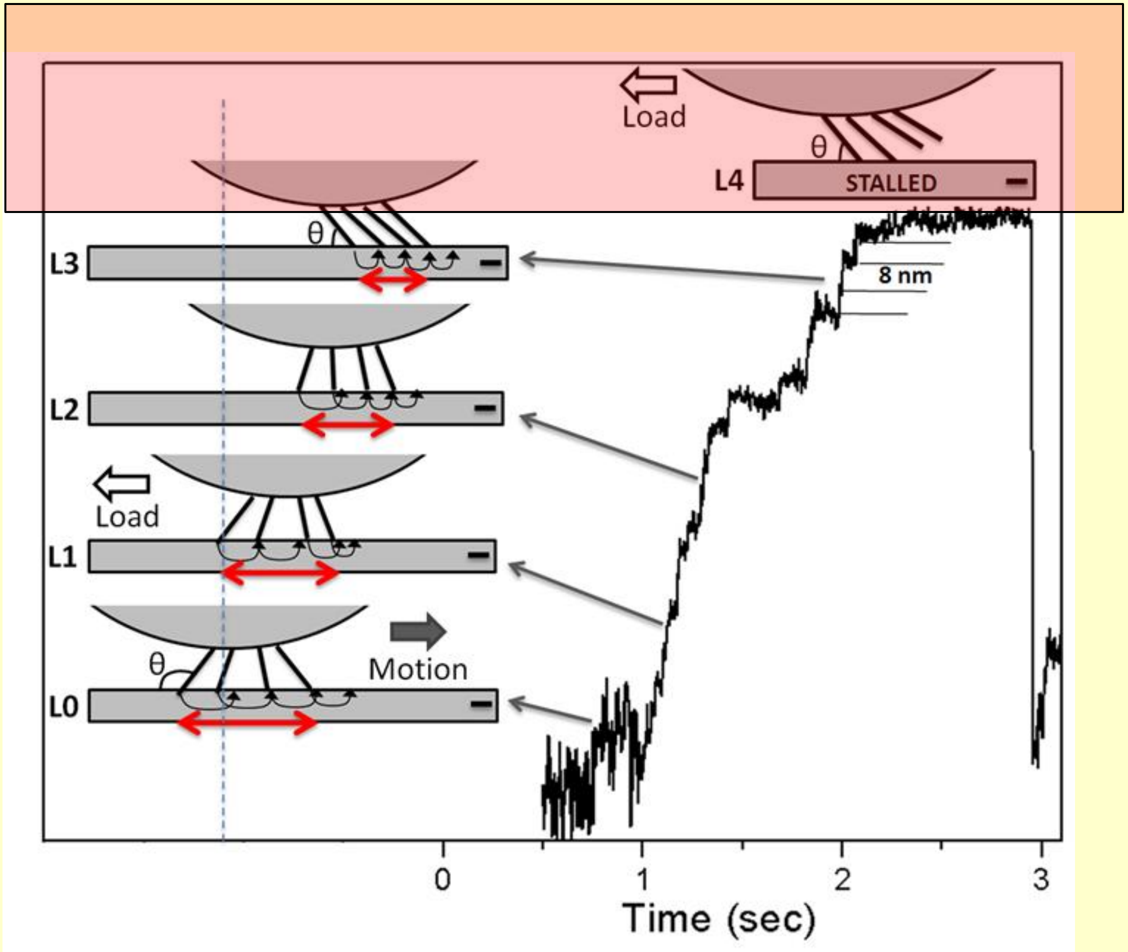
Problem at high load (Bhat et al, 2012)

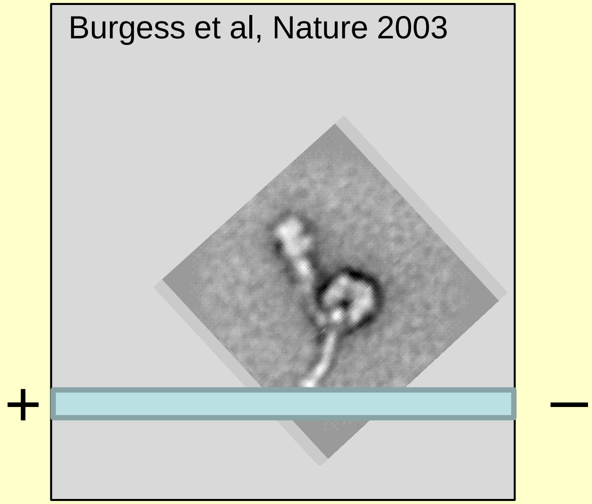
$$\epsilon(F) = \epsilon_0 \exp\left(|F|/F_d\right)$$

$$\varepsilon_+(n_+, n_-) = n_+ \varepsilon_{0+} \exp[F_c(n_+, n_-)/(n_+ F_{d+})].$$

F_s

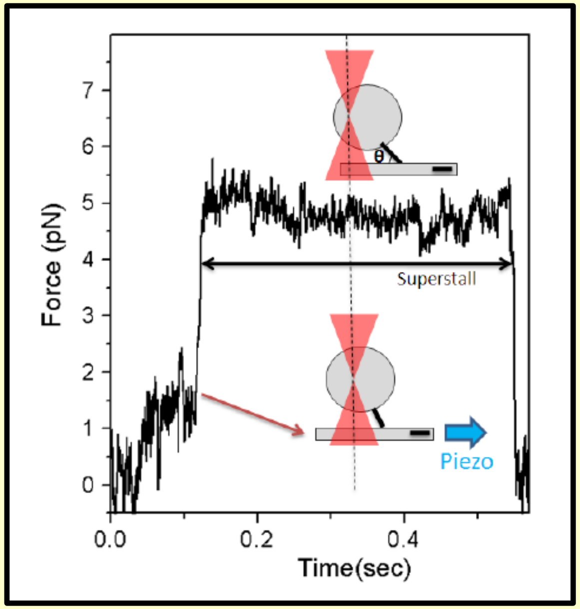
$$\epsilon(F) = \epsilon_0 \exp\left(|F|/F_d\right)$$

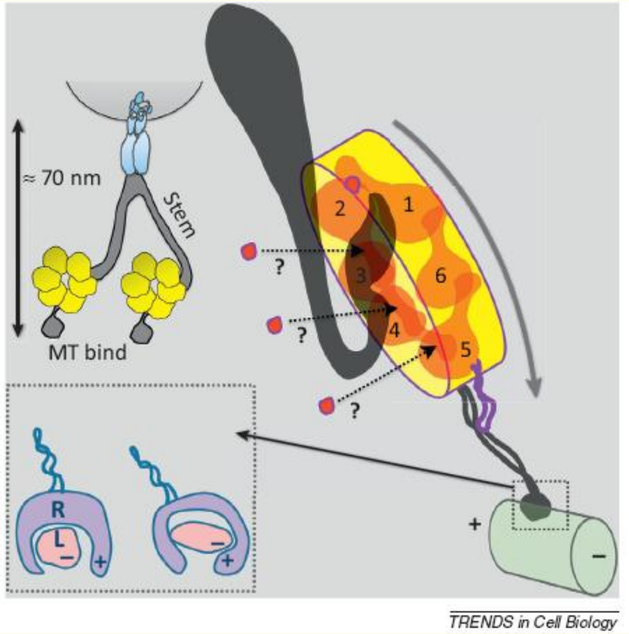




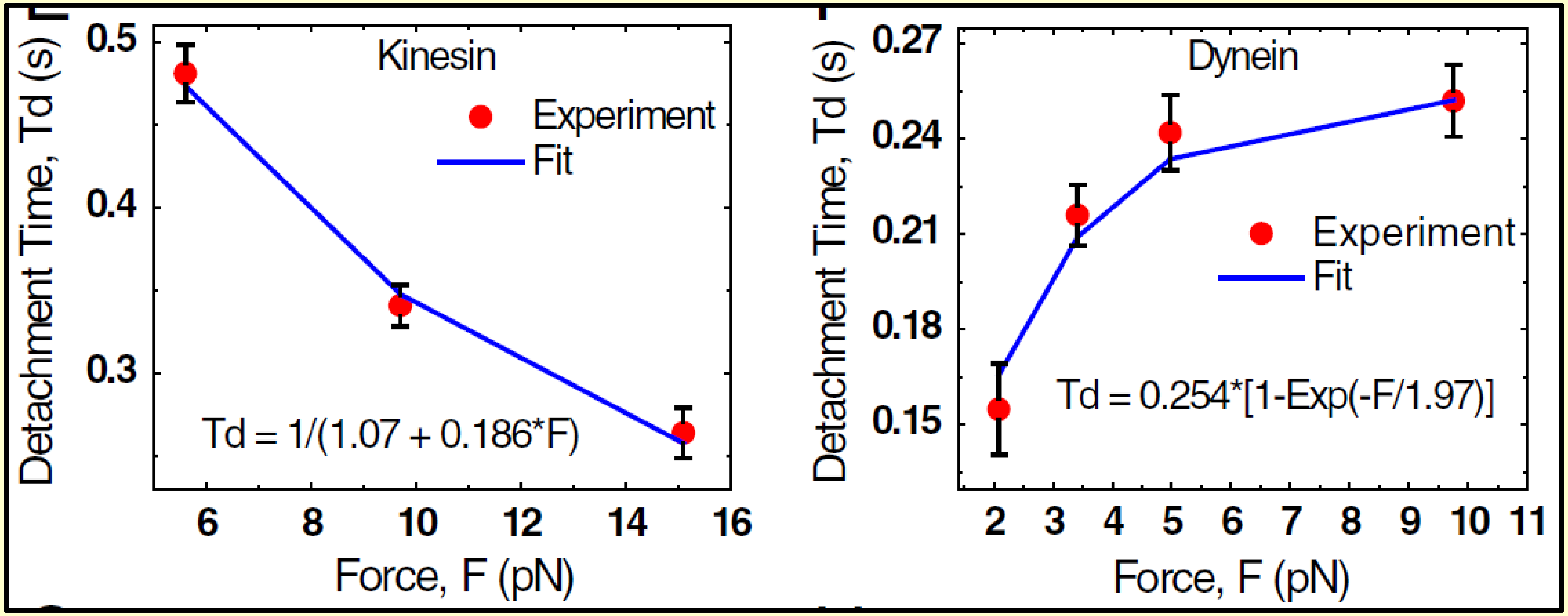
Burgess et al, Nature 2003

Catch bond In Dynein





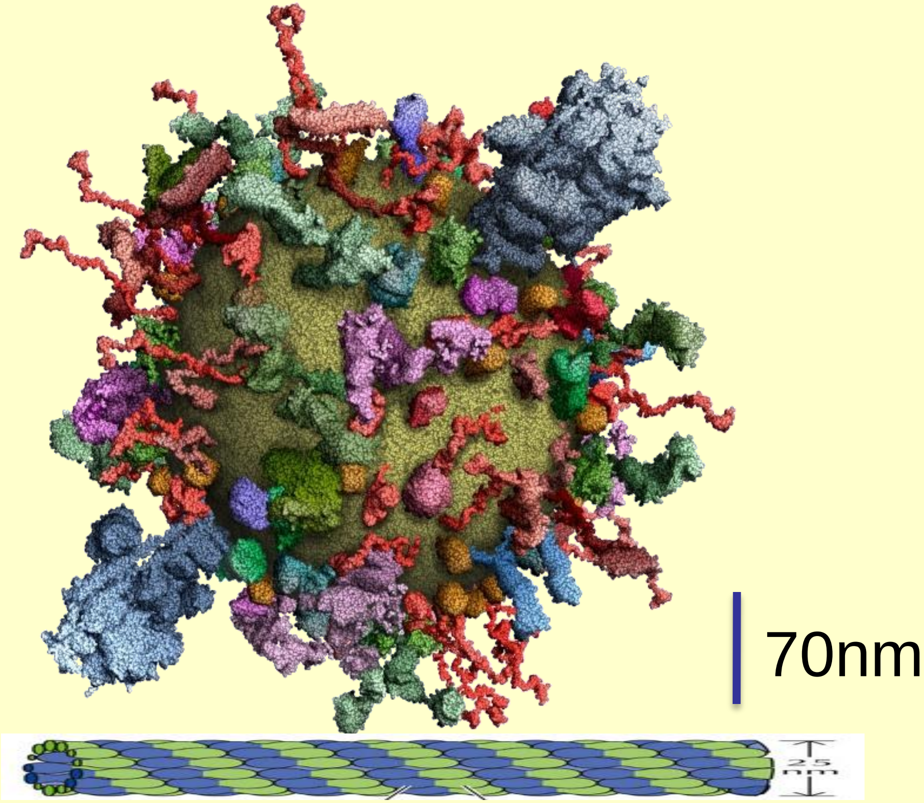
Kunwar et al, PNAS 2011



Rai *et al*, 2013

- Roles are reversed between Single and Collective behaviour
- Dyneins appears molecularly adapted to generate large collective forces → Gear, Catch-bond
- Possible to tune forces, and therefore processes with Dynein. Less so with Kinesin.
- 8 -12 Dyneins can generate force simultaneously.

What next ? … Geometry



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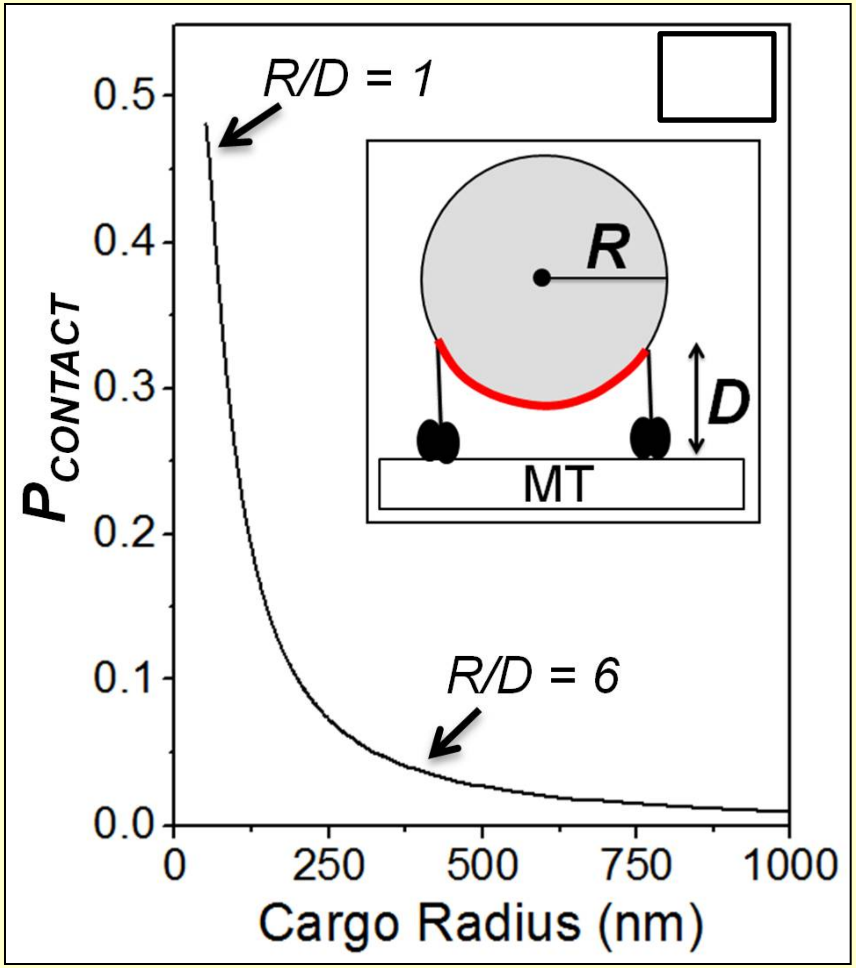
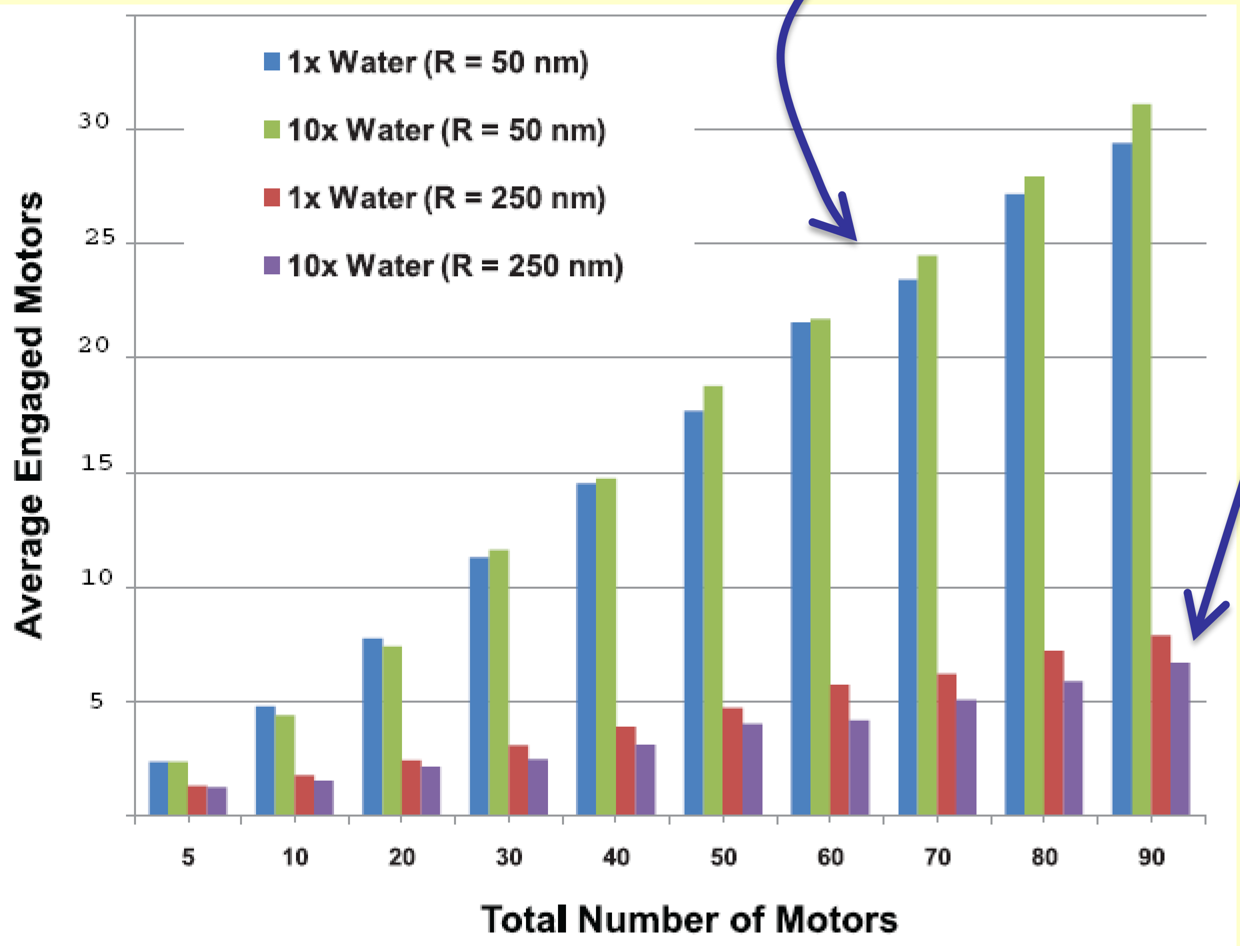
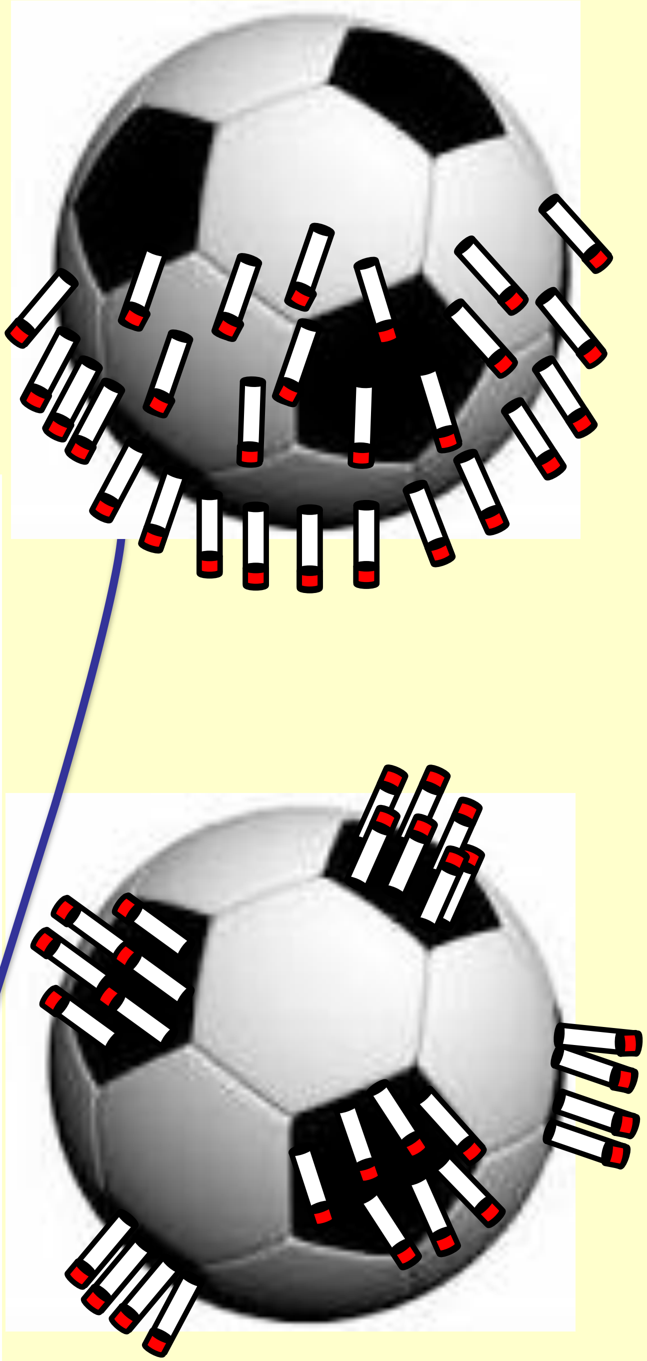


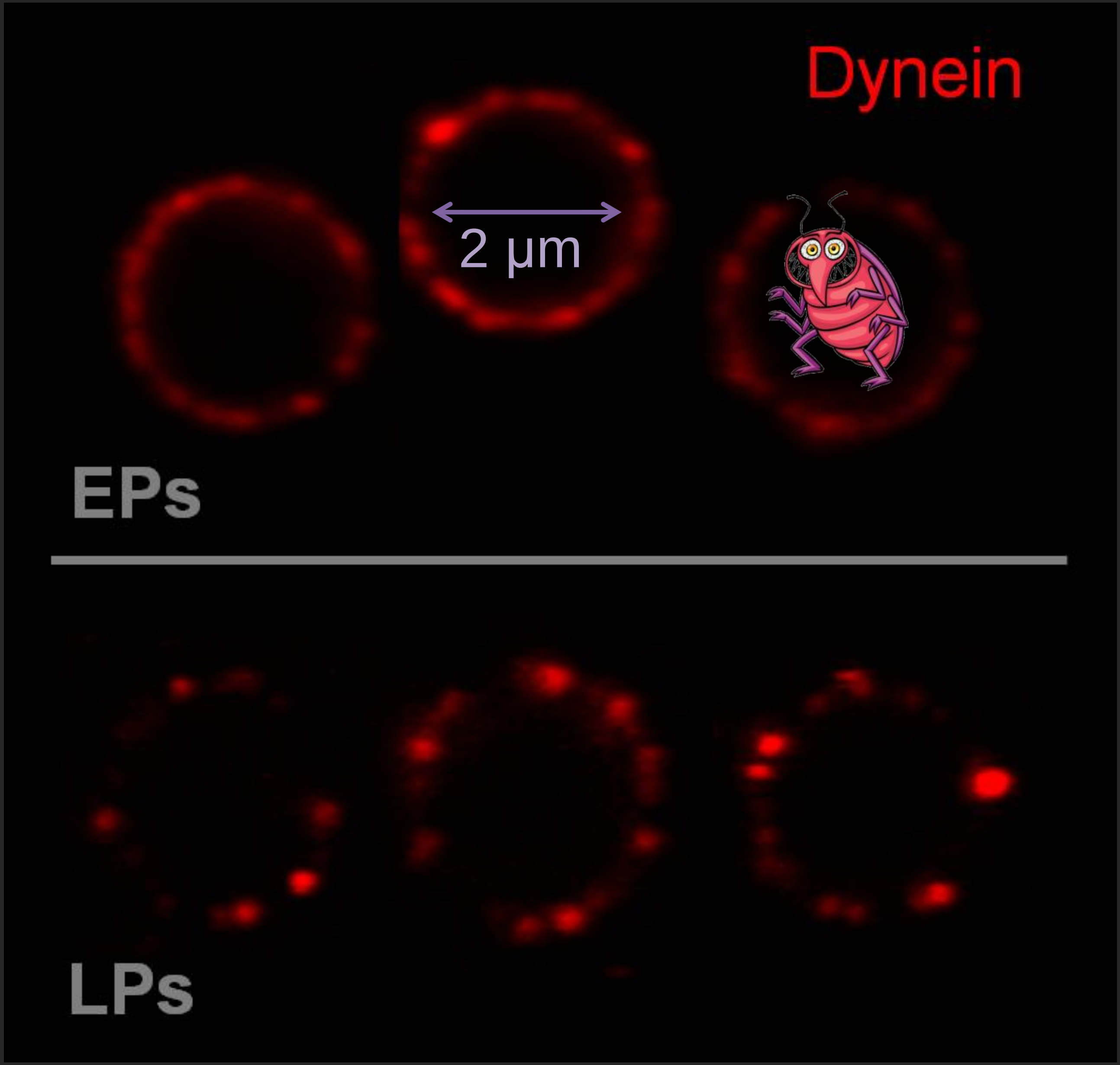
D_{CARGO} / L_{MOTOR}

Erickson et al, PLoS Comp Biol 2011





Lipid induced “Memory” in Bidirectional Transport ??



Ashim

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Reagents/Discussion/Criticism

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