MOTOR PROTEIN:
TO COOPERATE OR NOT TO COOPERATE ?

NEW INSIGHTS FROM AN OLD THEORY

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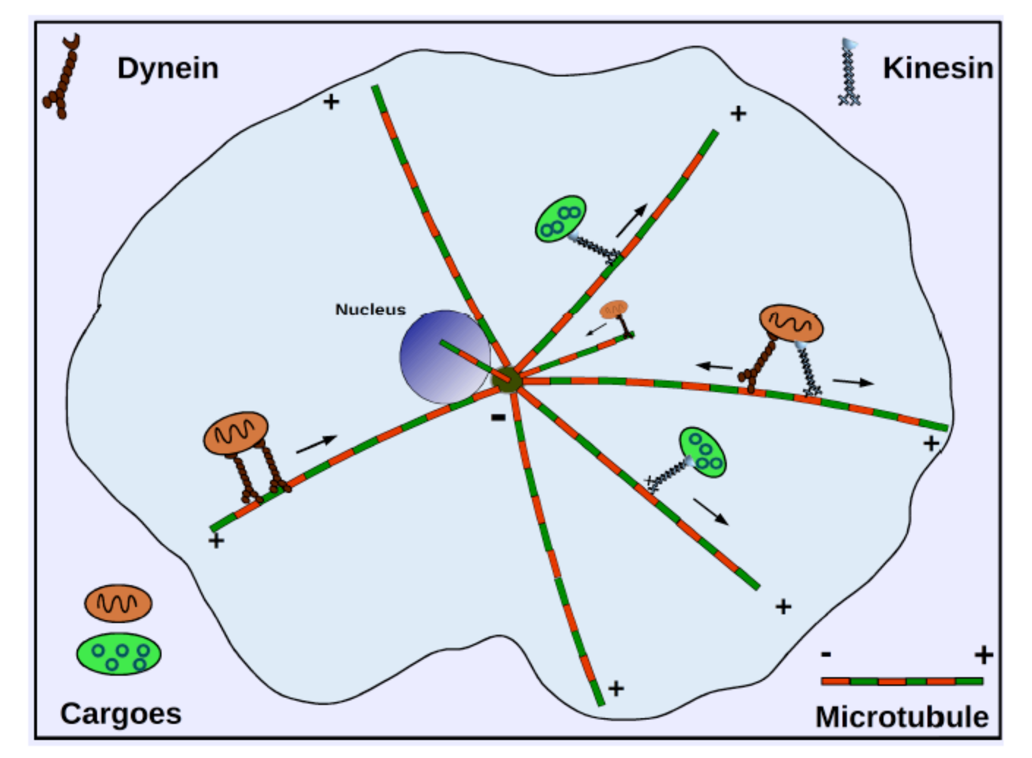
TRACKS AND TRANSPORT IN CELL

In eukaryotic cells, **microtubules** and **actin** filaments function as tracks for movement of motor proteins, which are sensitive to the structural polarity of the filament.

Kinesin and **dynein** are microtubule-associated motors, while myosin use actin filaments for motion.

Motors and associated filaments often work together in crucial cellular functions, eg., cell division.



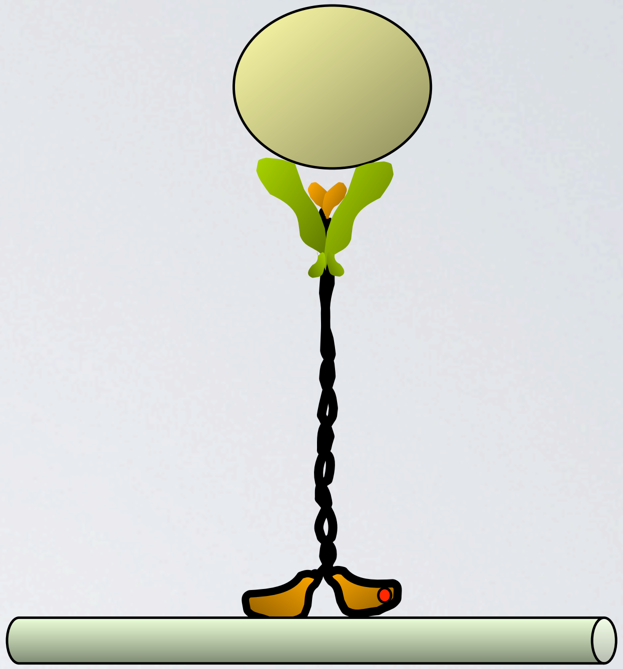
Desai and Mitchison, 1997

MOTOR: FORCE-VELOCITY RELATION

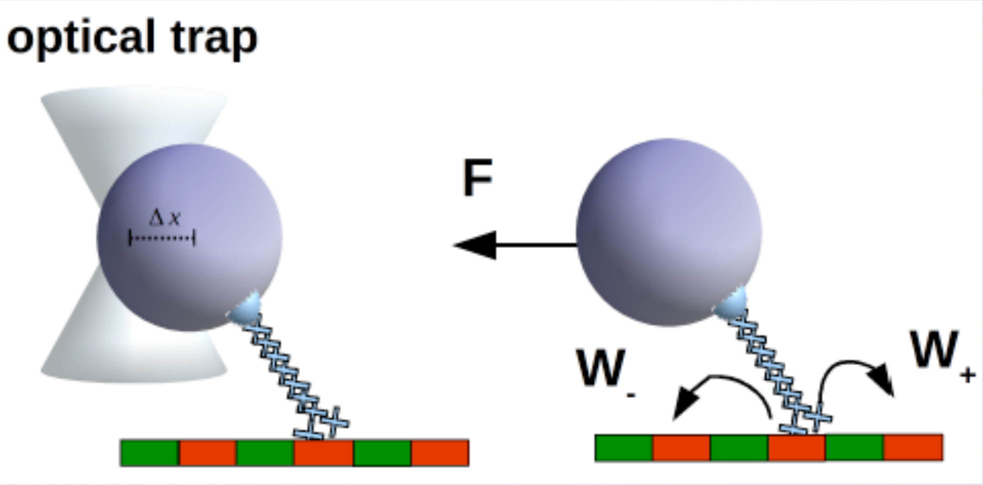
A motor protein is slowed down by applying force against its direction of motion.

$$v \simeq v_0(1 - F/F_s)$$

stall force



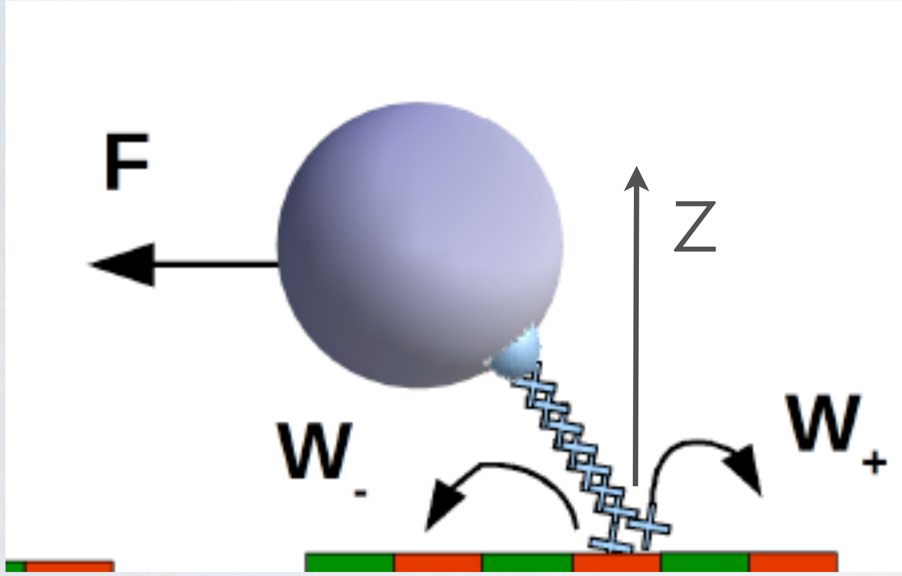
Stall force for a single motor protein ~ 1-10 pN.

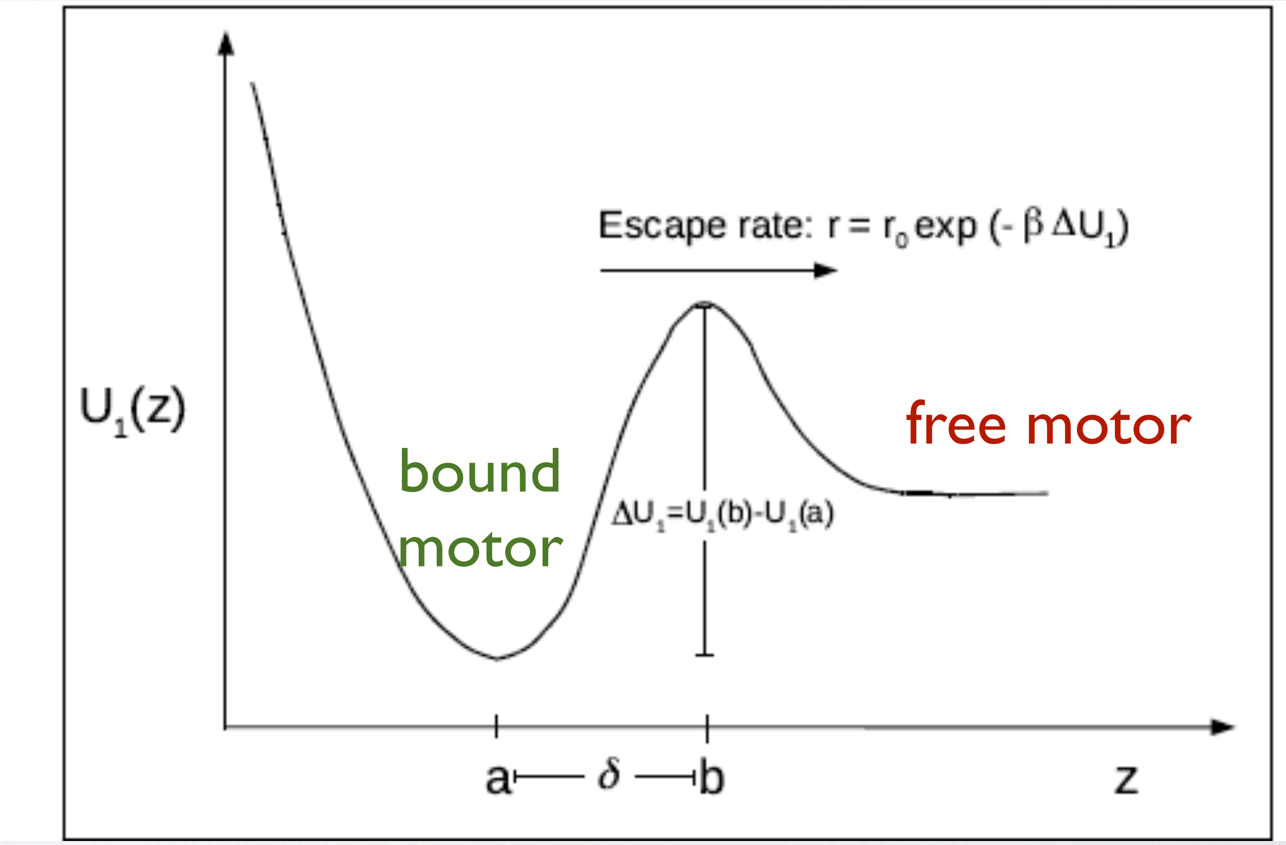


Some motors work in larger groups and generate more force.

DETACHMENT AGAINST APPLIED FORCE

The simple Kramers picture



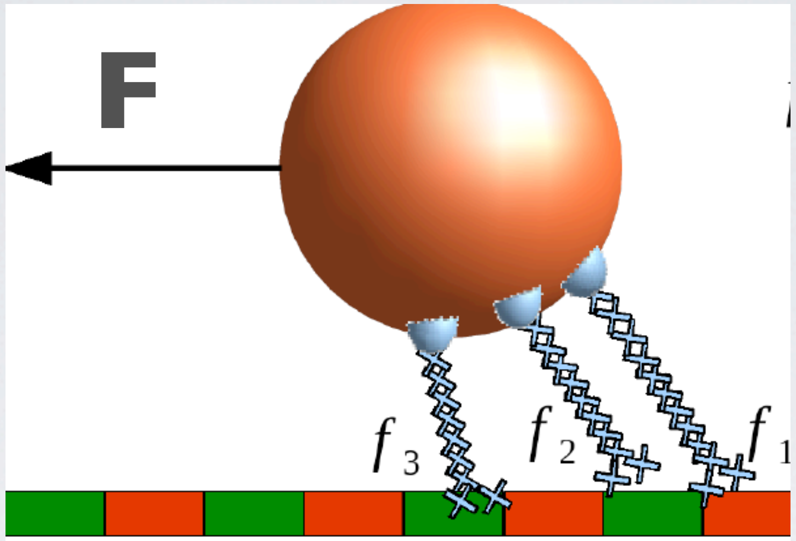


$$\epsilon(F) = \epsilon \exp(F/F_d)$$

detachment force $\boxed{F_d = \frac{k_B T}{\delta}}$

intrinsic unbinding rate

COLLECTIVE MOTION UNDER LOAD



A number of identical motors may be attached to the same cargo.

Load sharing

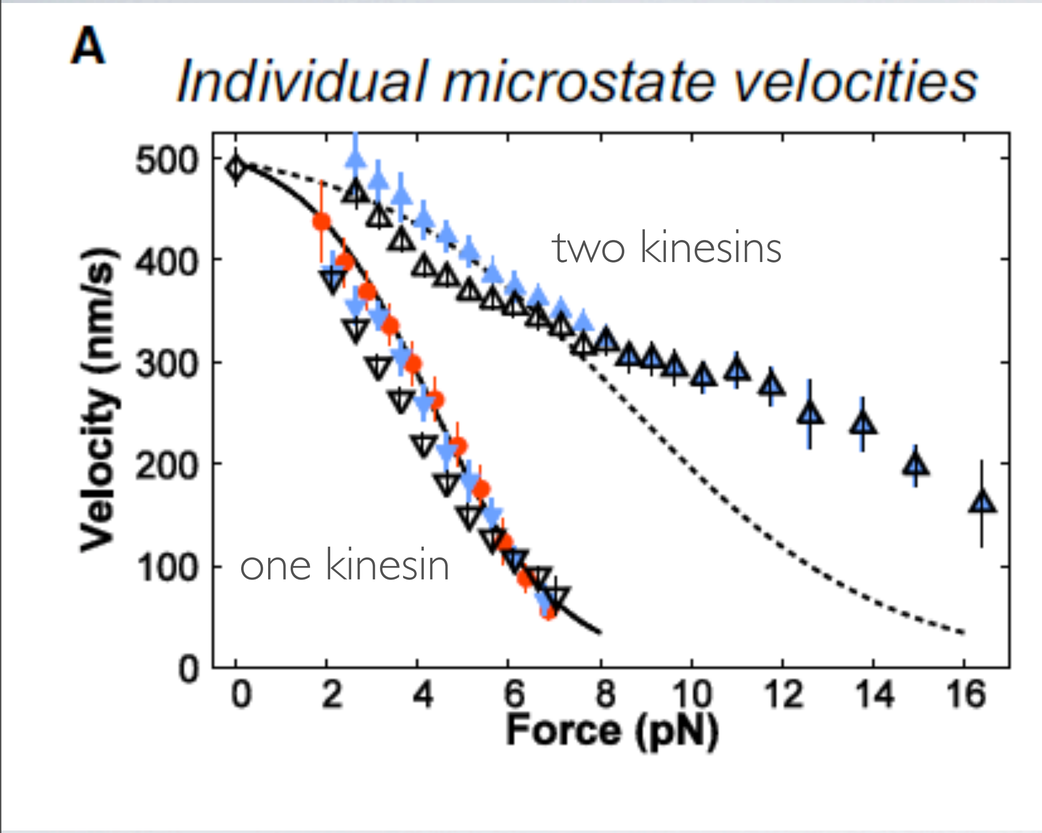
Equal $\boxed{f_1 = f_2 = f_3 = ..f_N \equiv f = F/N}$

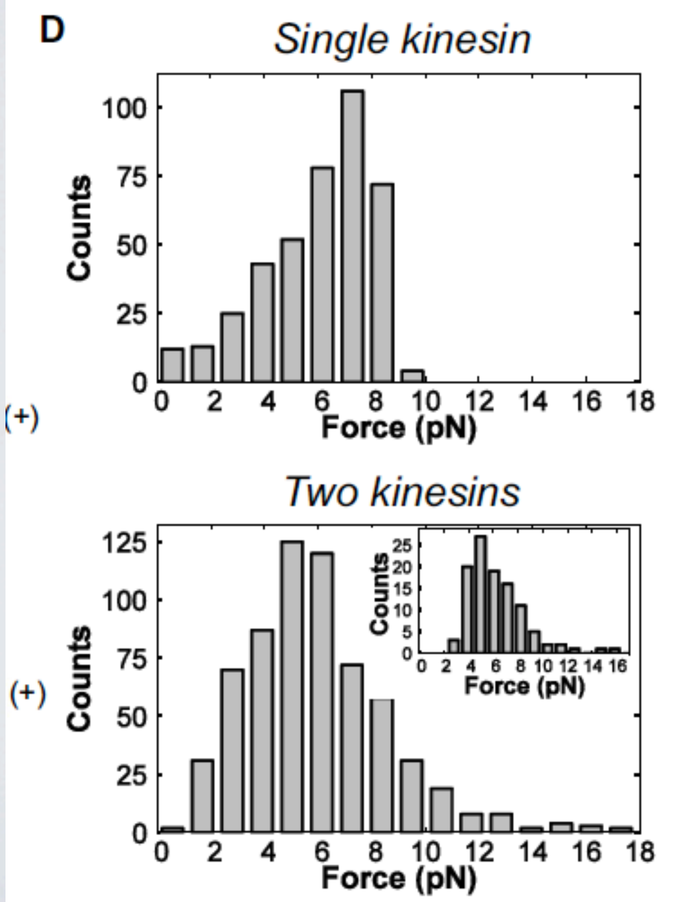
Klumpp and Lipowsky (2005)

Unequal/stochastic

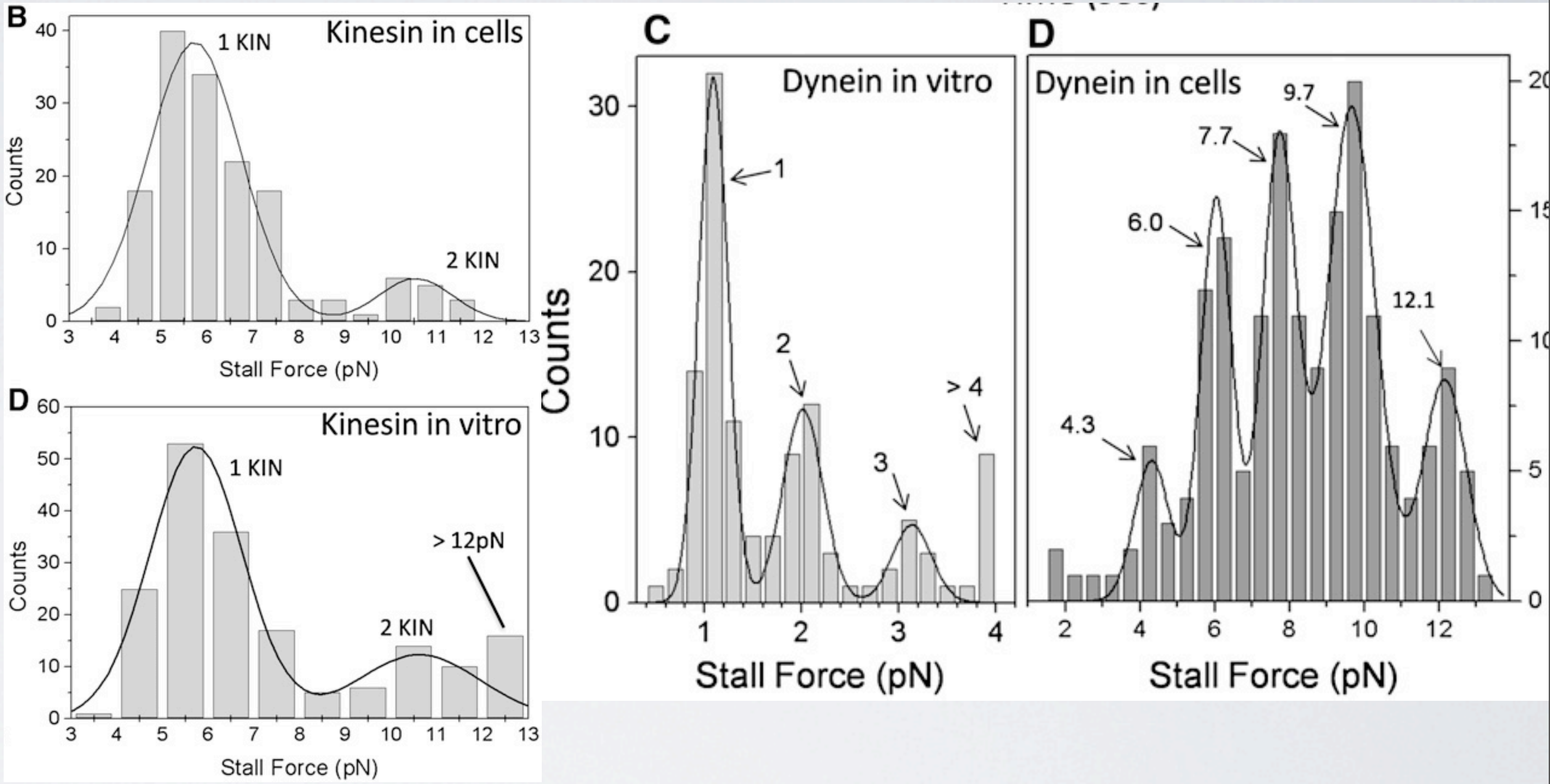
Kunwar and Mogilner (2010)

Force and velocity: one vs many motors





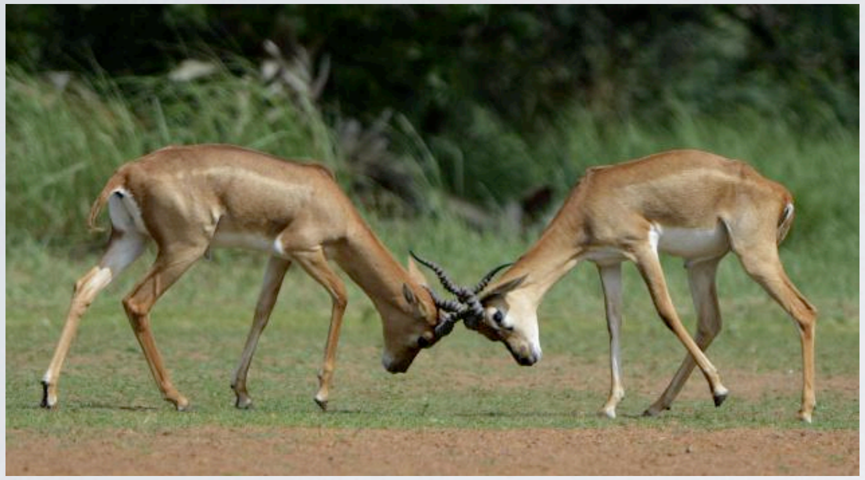
Jamison et al., Biophys. J (2010)

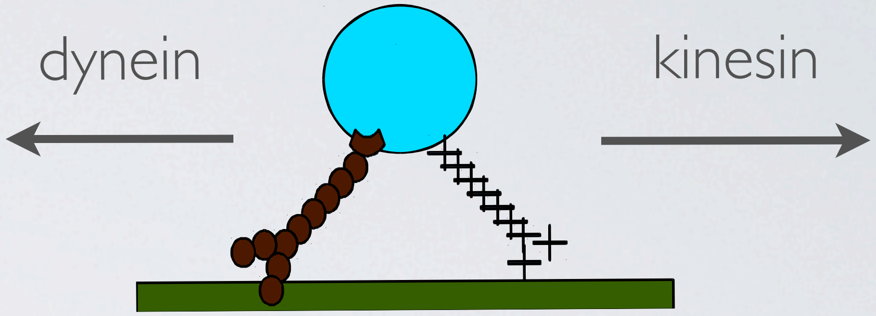


Rai et al., Cell (2013)

Total force generated by a multiple motor assembly is "additive".

TUG OF WAR BETWEEN MOTORS





Imagine a situation where N_+ plus end-directed motors and N_- minus end-directed motors pull a cargo simultaneously, such that

$$N_+ f_s^+ + N_- f_s^- = 0 \quad (f_s^+ > 0, f_s^- < 0)$$

f_s^+ = “stall force” of the plus directed motor

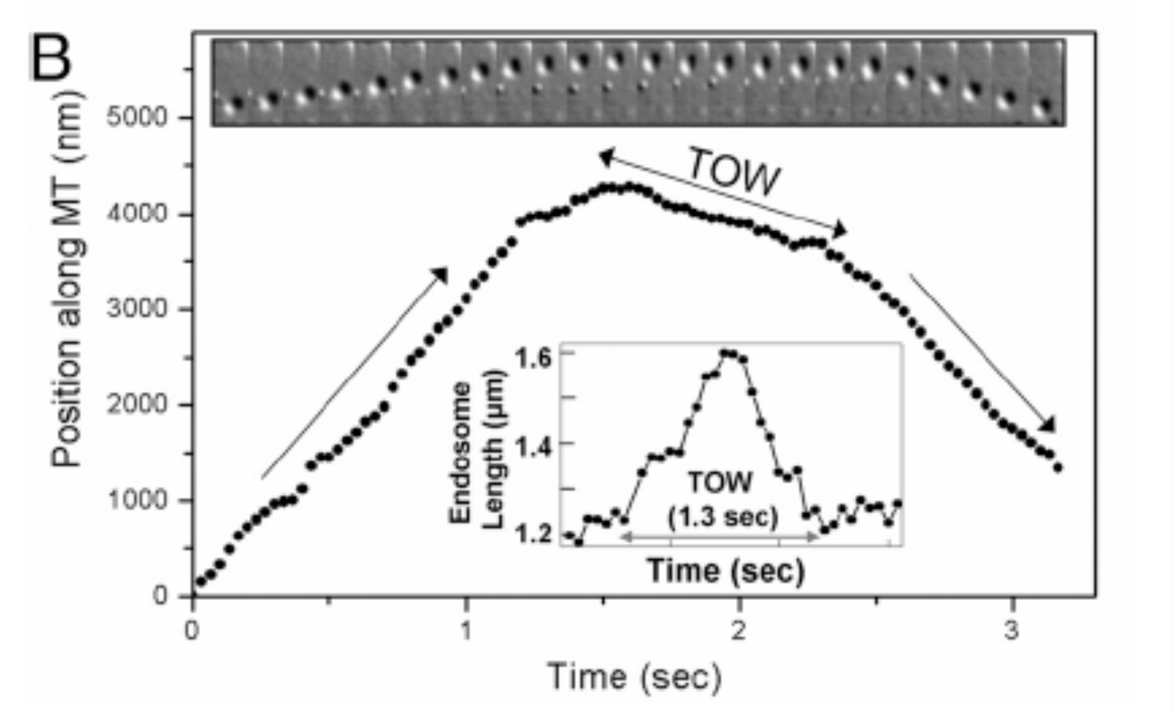
f_s^- = “stall force” of the minus directed motor

Since the net total force vanishes, the cargo reaches a state of “stall” , characterized by zero mean velocity.

TUG OF WAR LEADS TO BIDIRECTIONAL MOTION

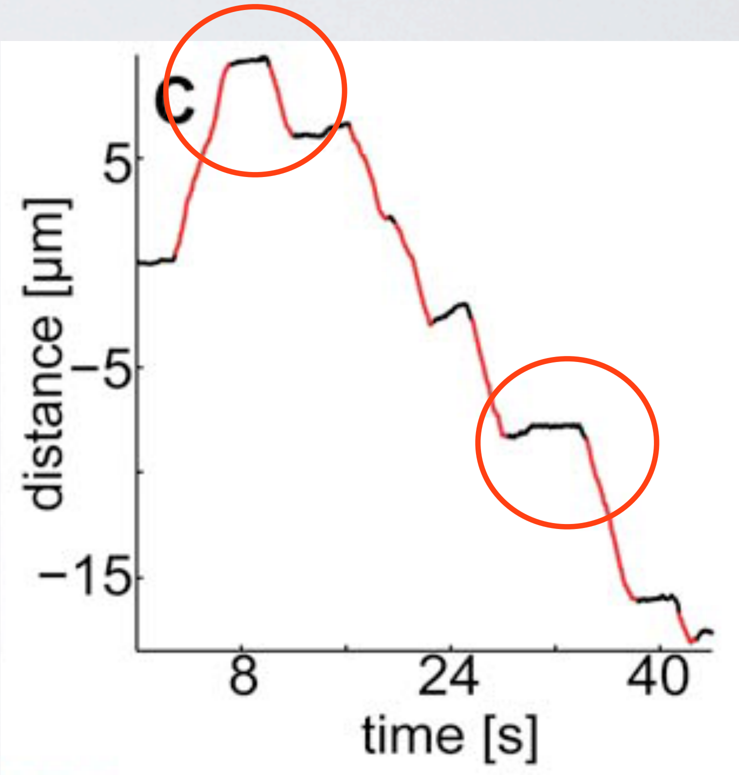
Many cellular organelles display bidirectional motion; in many cases, arising from tug of war between opposing motor teams.

Endosomes



Soppina. et al., PNAS (2010)

Human adenovirus

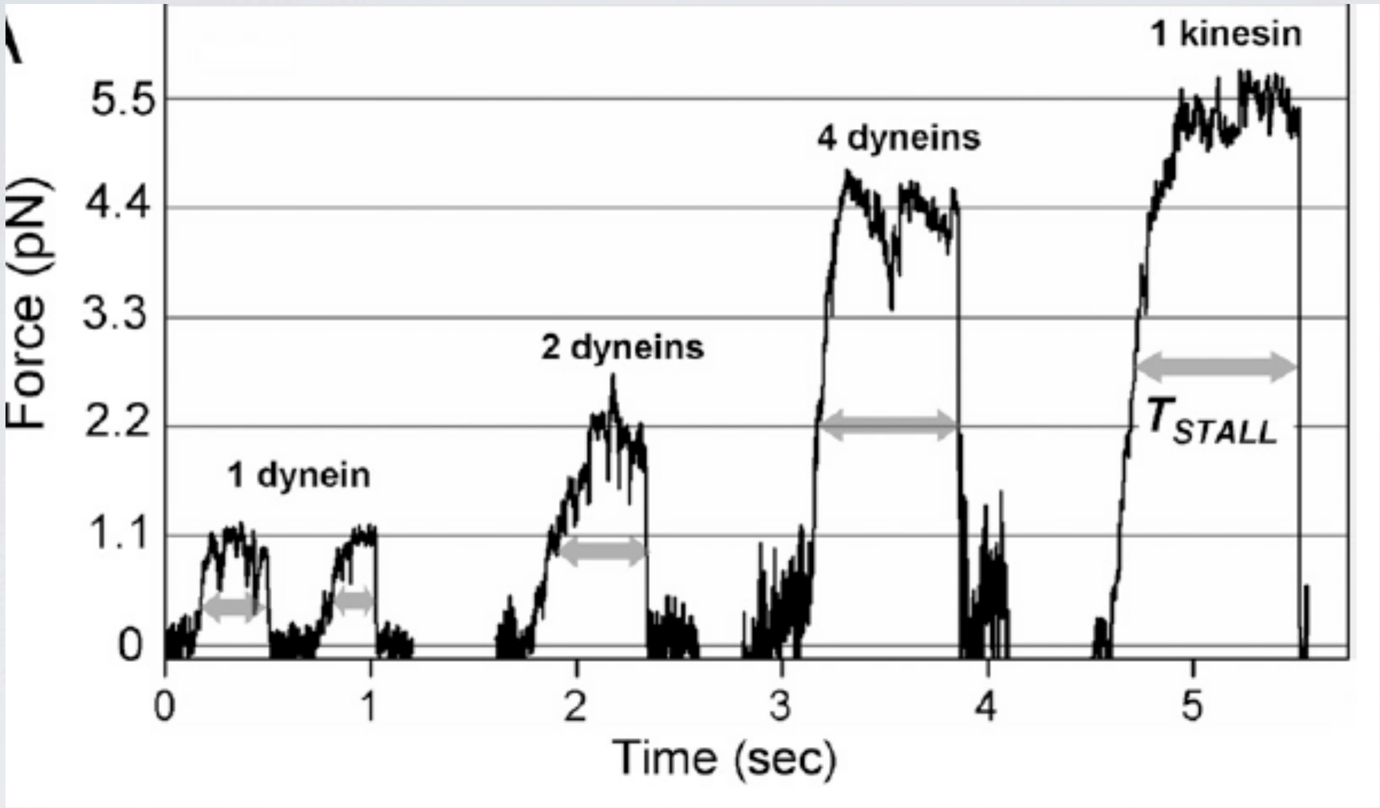


Gazzola et al., PLOS Comp. Biol. (2009)

Stall force (kinesin)= 5.5pN, stall force (dynein)= 1.1 pN.

5-6 dyneins are required to engage a kinesin in TOW.

SURVIVAL UNDER STALL

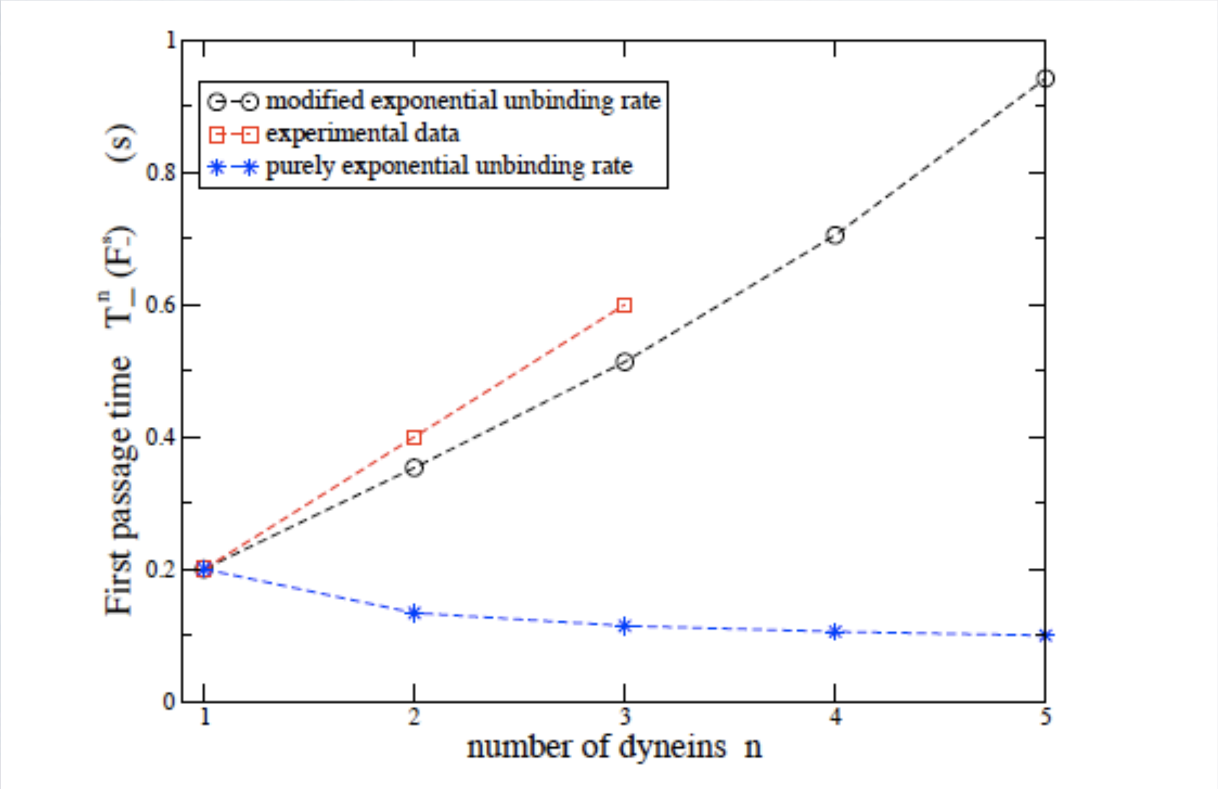


Soppina et al., PNAS (2010)

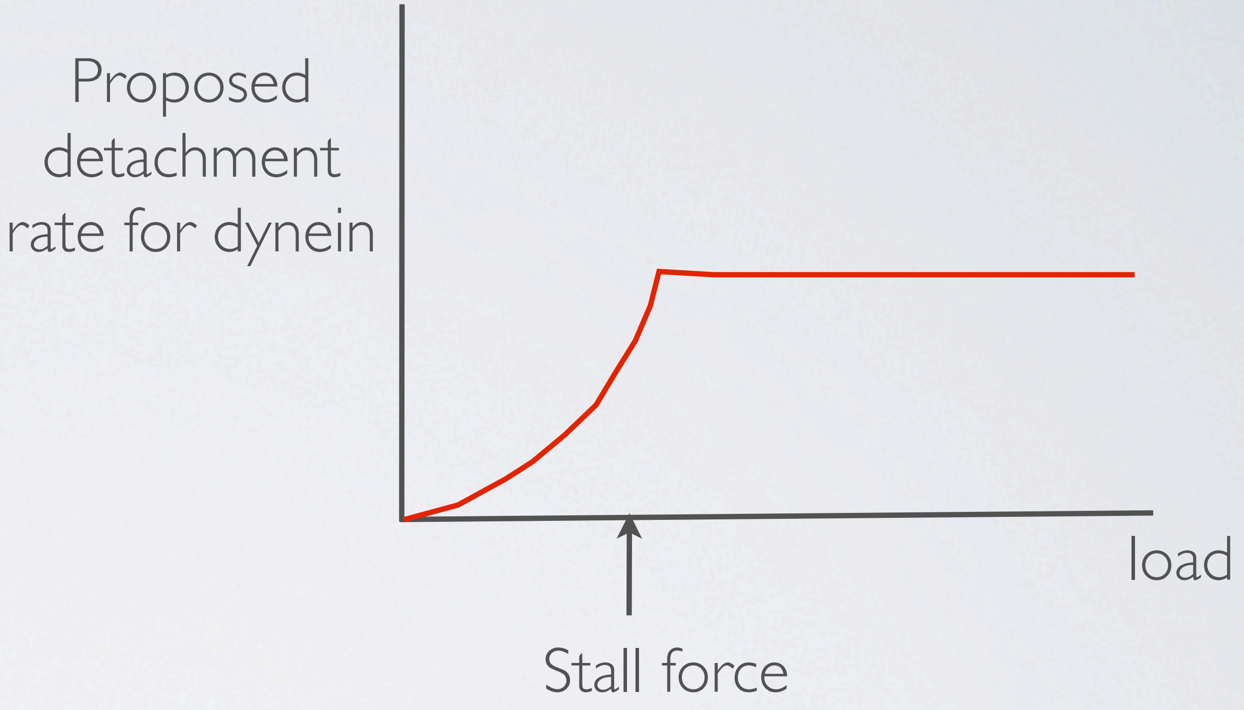
Dynein scales up the detachment time under stall load with number, nearly linearly.

A team of 5 dyneins equals a kinesin in ***force*** as well as ***tenacity***.

DYNEIN CATCH-BONDS UNDER HIGH LOAD



Deepak Bhat and M.G, Phys. Biol. (2012)


Proposed detachment rate for dynein

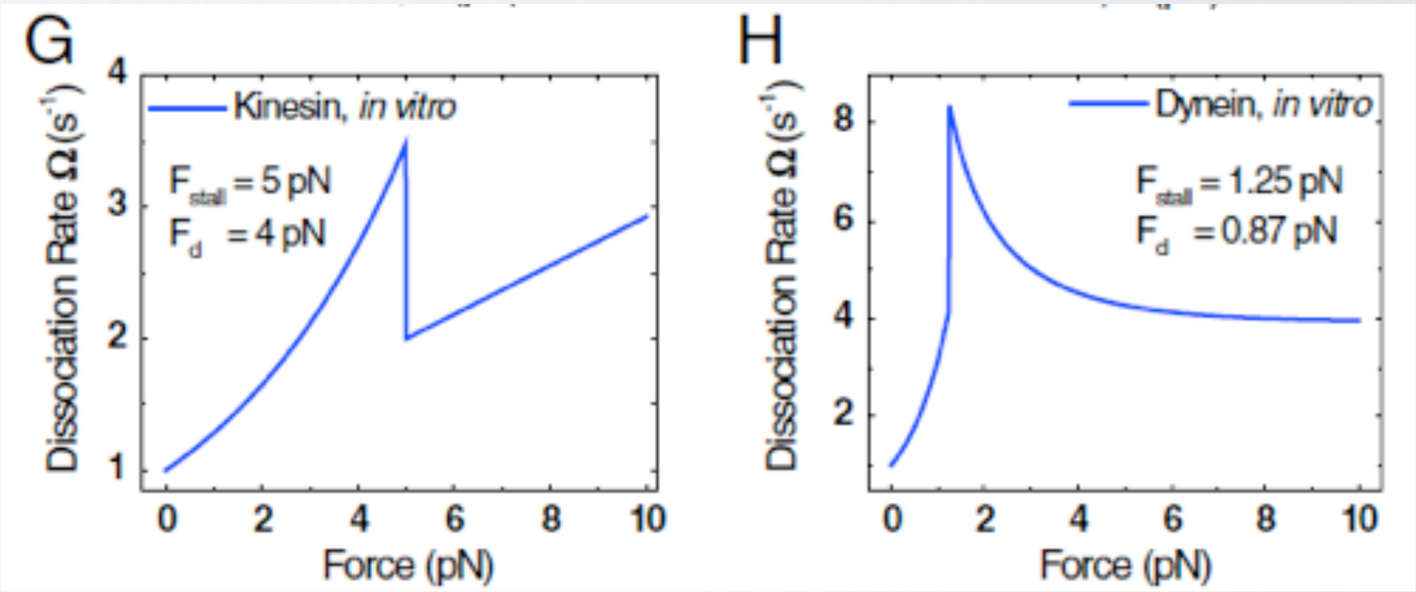
load

Stall force

Modified detachment-load curve for dynein

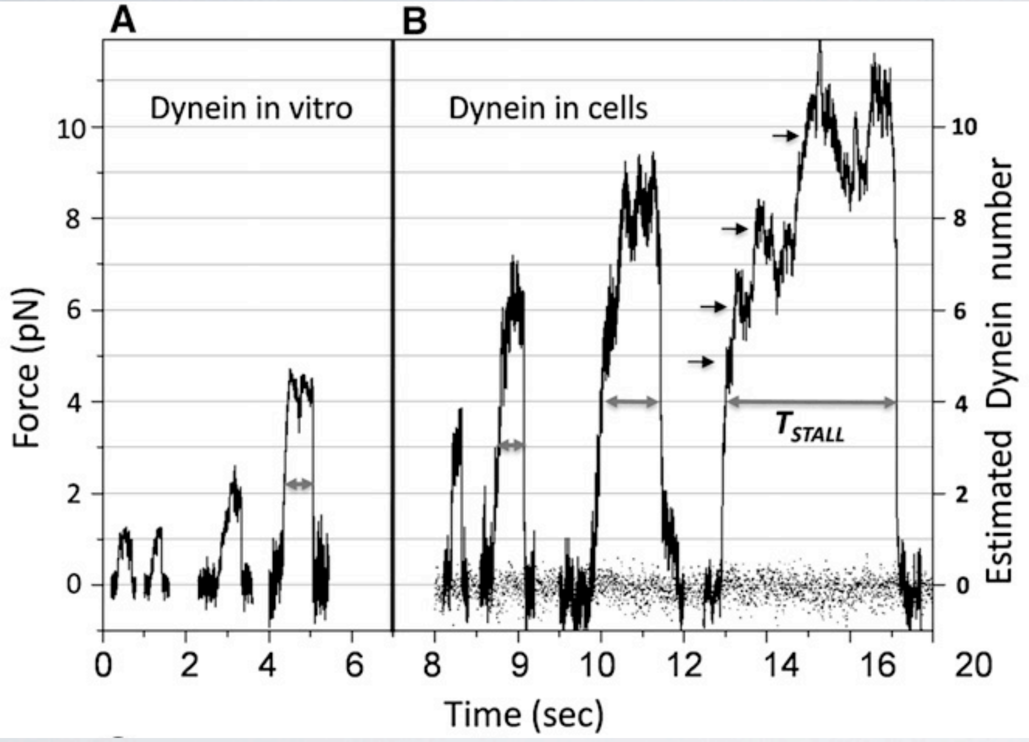
Dynein catch-bonds under high load-enables it to scale up survival time with number:

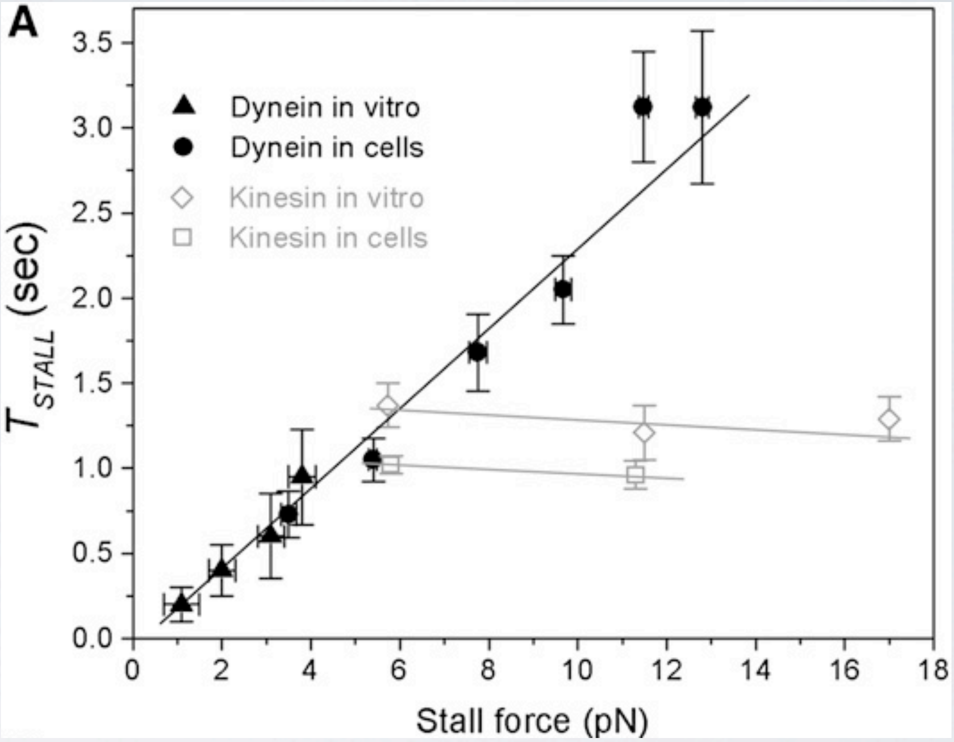
An effective team worker!

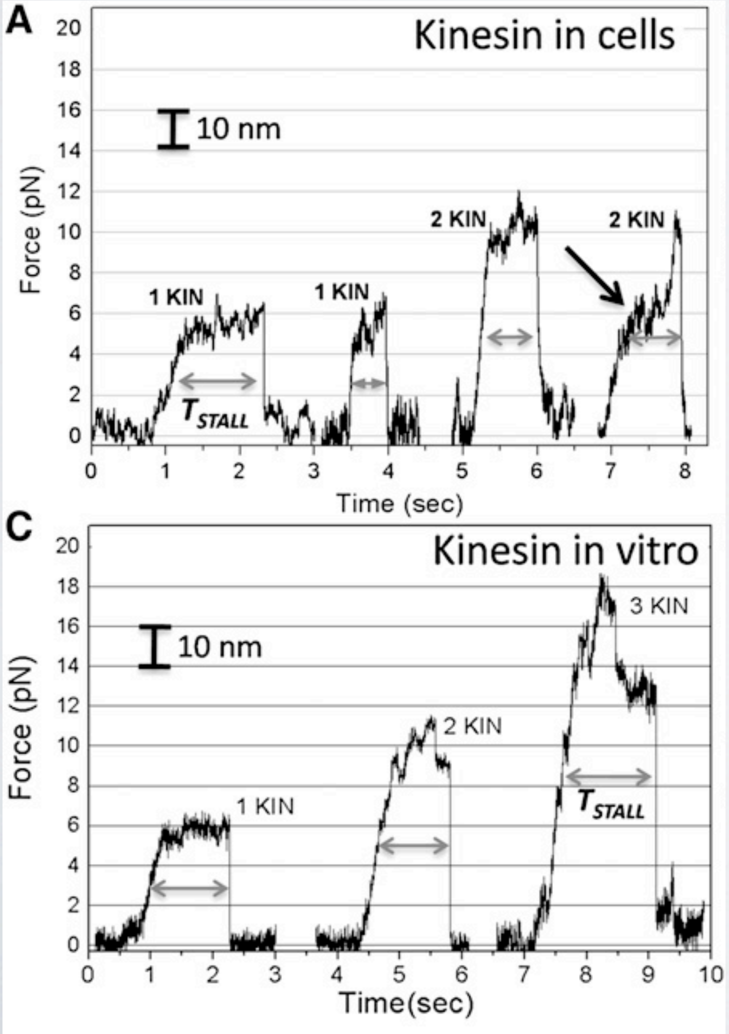


Kunwar et al., PNAS (2011)

Rai et al., Cell (2013)







Dynein increases survival time under stall with increasing N, but no tangible increase for kinesin.

A group of dyneins is more "effective" at exerting maximum force compared to a single dynein.

A group of kinesins is only as "effective" as a single kinesin.

Dynein is "cooperative" while kinesin is "non-cooperative".

(Rai et al., Cell (2013); Mallik et al., Trends in Cell Biol. (2013))

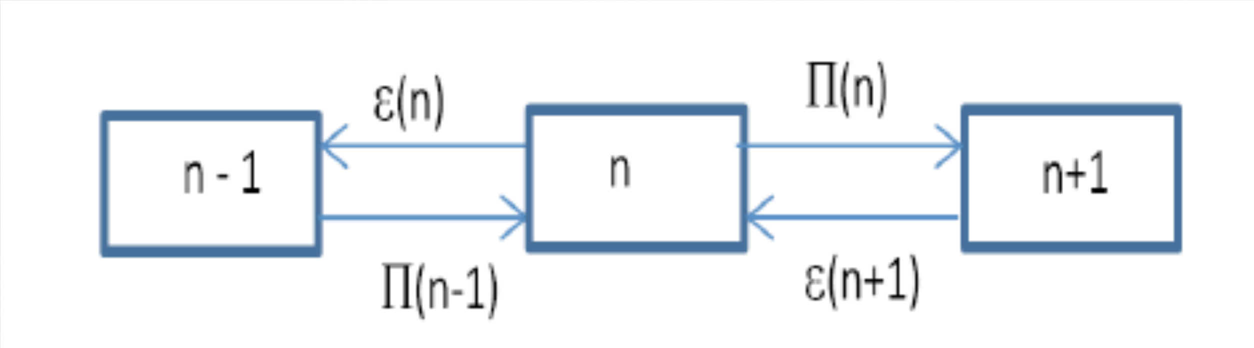
Why is dynein cooperative and kinesin non-cooperative ?

CARGO DETACHMENT AS A FIRST PASSAGE PROBLEM

System: a cargo coupled to a total of N identical motors

State characterized by number of filament-attached motors $n \leq N$

Fixed force ensemble: $F = N f_s$, shared equally between motors.



$$\epsilon(n) = n\epsilon\Psi(Nf_s/nf_d)$$

$$\pi(n) = \pi(N-n)$$ (reattachment rate)

$\epsilon = \epsilon_{\text{stall}}$ $\Psi(x) \sim 1$ (dynein)

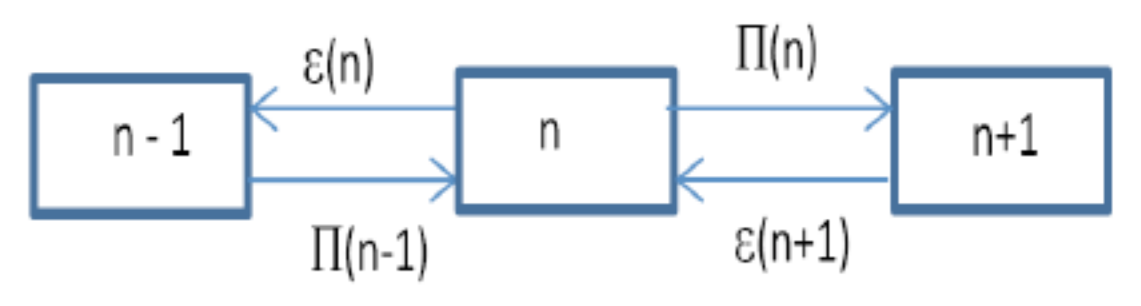
$\Psi(x) \simeq e^x$ (kinesin)

Time under stall ~ mean first passage time (MFPT) to reach n=0 from n=N

$$T_N = \sum_{n=1}^{N} \frac{1}{\epsilon_n} + \sum_{M=1}^{N-1} \sum_{m=1}^{N-M} \frac{1}{\epsilon_{M+m}} \prod_{n=0}^{M-1} \frac{\pi_{n+m}}{\epsilon_{n+m}}.$$

Klumpp and Lipowsky (KL)
PNAS (2005)

CONTINUUM THEORY



$P_n(t)$ = probability of finding n filament-engaged motors at time t

$$\dot{P}_n(t) = P_{n+1}\epsilon(n+1) + P_{n-1}\pi(n-1) - P_n[\pi(n) + \epsilon(n)]$$

Define $x = n/N$ and $\phi(x,t) = NP(Nx,t)$

Taylor-expand all quantities in powers of 1/N to find

$$\dot{\phi}(x,t) = -\frac{\partial}{\partial x}[\phi(x,t)v(x)] + \frac{\partial^2}{\partial x^2}[D(x)\phi(x,t)]$$

$$v(x) = \frac{1}{N}[\pi(Nx) - \epsilon(Nx)] = \pi[1 - x - \beta x \Psi(\alpha/x)]$$

$$D(x) = \frac{1}{2N^2}[\pi(Nx) + \epsilon(Nx)] = \frac{\pi}{2N}[1 - x + \beta x \Psi(\alpha/x)]$$

$$\beta = \frac{\epsilon}{\pi}$$

$$\alpha = \frac{f_s}{f_d}$$

$$\dot{\phi}(x,t) = -\frac{\partial}{\partial x}[\phi(x,t)v(x)] + \frac{\partial^2}{\partial x^2}[D(x)\phi(x,t)]$$

Motor detachment-reattachment dynamics

Smoluchowski equation for
probability distribution of position P(x,t)

$$\frac{\partial P}{\partial t} = \frac{\partial}{\partial x}\left[\frac{U'(x)}{\gamma}P\right] + \frac{k_B T}{\gamma}\frac{\partial^2 P}{\partial x^2}$$

Over-damped Brownian particle moving in a one-dimensional potential U(x)

Time under stall T_N= Mean first passage time taken by the particle to reach the absorbing boundary at x=0, starting from x=1.

Effective potential

$$U(x) \propto -\int^x v(y)dy = \epsilon\int^x y\,\Psi(\alpha/y)dy + \pi(x^2/2 - x) + \text{const}$$

$$k_B T \propto D(x) \longrightarrow T \propto N^{-1}$$

NOT the physical temperature!

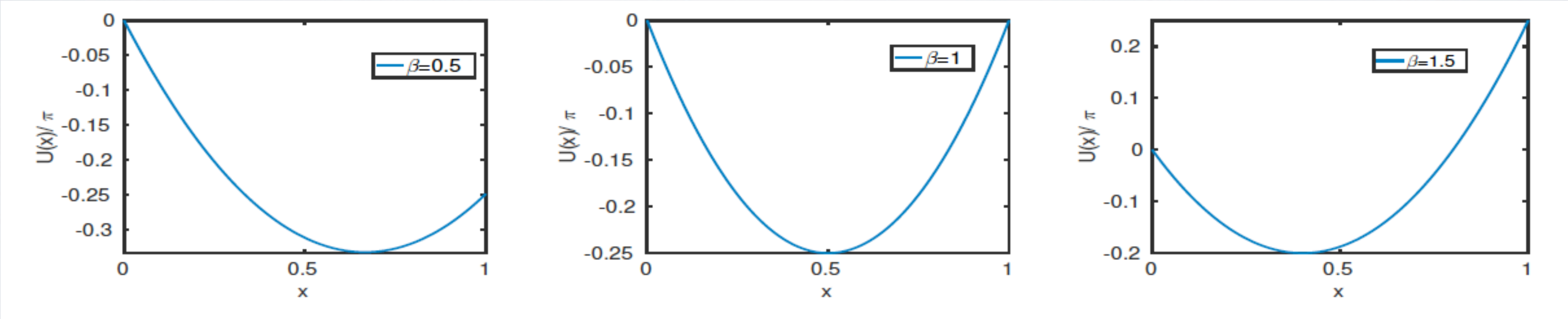
If U(x) has one or more minima, the "particle" gets trapped in them, which dominates the dynamics (necessary condition: v(x) vanishes at least once in [0:1]).

If U(x) has no minima or maxima, the "particle" may slide down, or forced to climb up from x=1 to x=0.

DYNEIN-LIKE MOTOR; $\Psi(x) \sim 1$

$$\frac{U(x)}{\pi} = (1+\beta)\frac{x^2}{2} - x$$

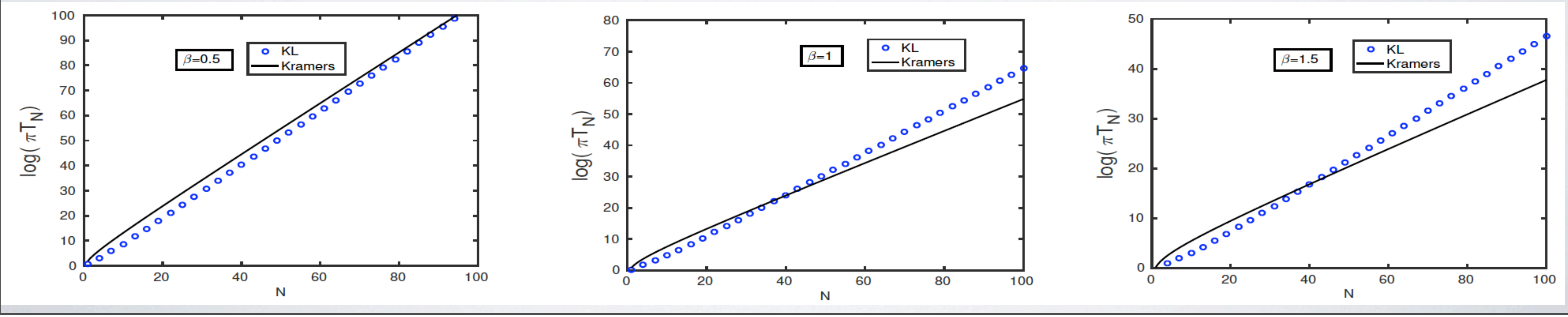
Single minimum at $x^* = \dfrac{1}{1+\beta}$



MFPT~ mean escape time from the potential well

$$\pi T_N \approx \frac{N e^{\frac{N}{2\beta}}}{1+\beta}\sqrt{\frac{2\pi^*}{\beta}}$$

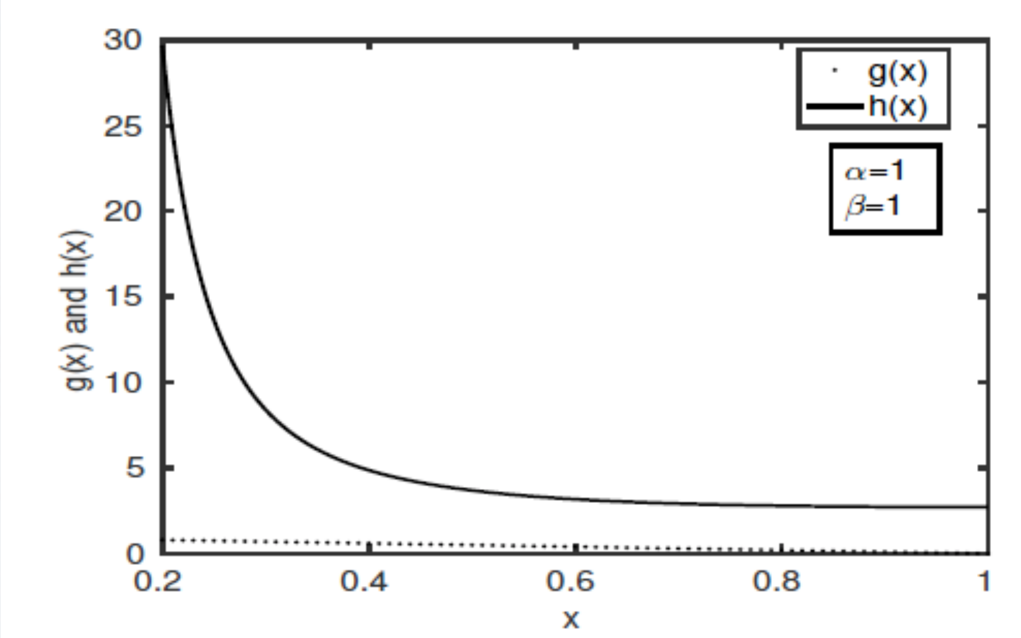
$$\pi^* = 3.1415...$$

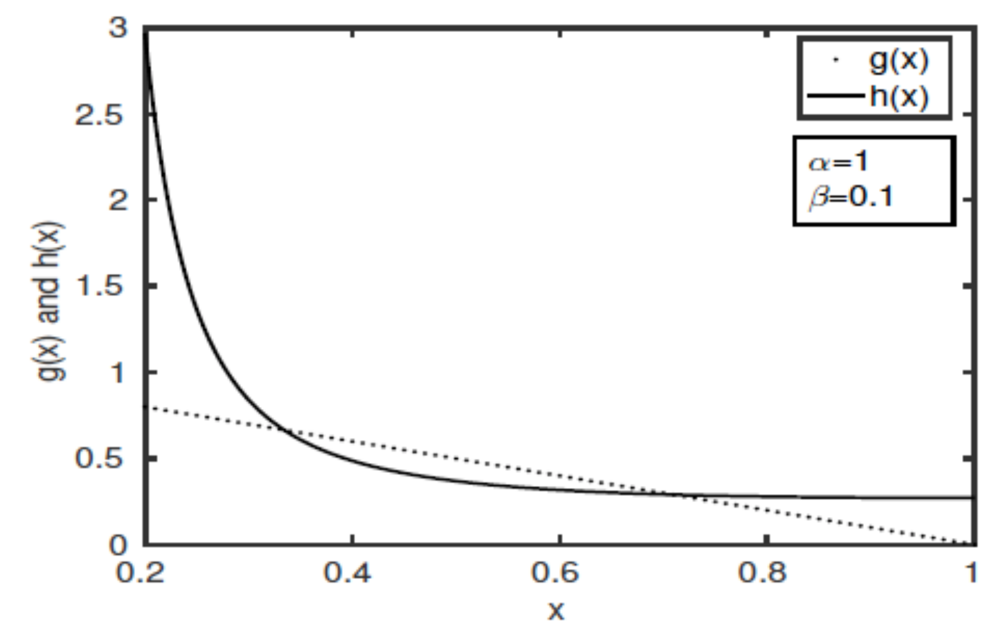


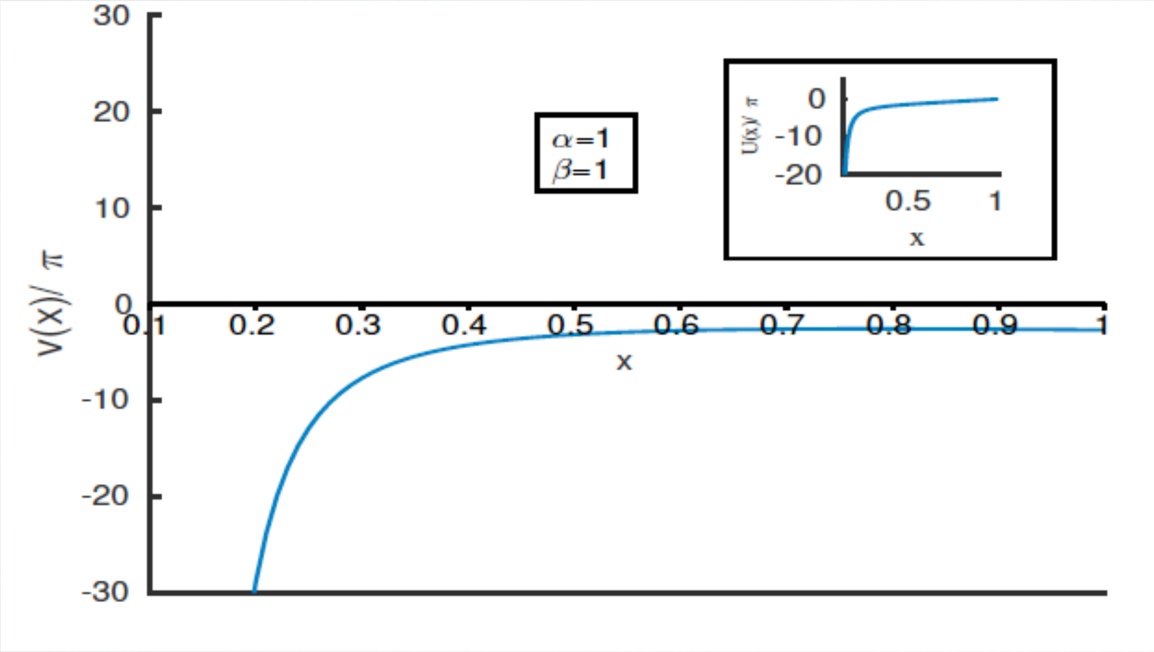
KINESIN-LIKE MOTOR; $\Psi(x) \simeq e^{x}$

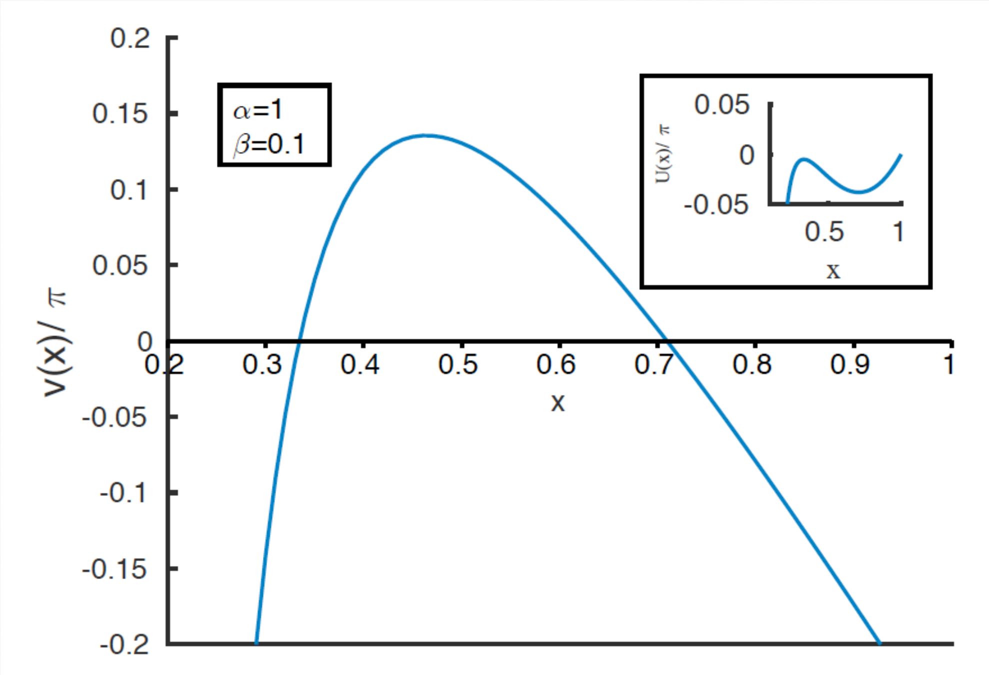
$$\frac{v(x)}{\pi} = g(x) - h(x) \qquad g(x) = 1 - x \qquad h(x) = \beta x e^{\frac{\alpha}{x}}$$

Decreasing β →



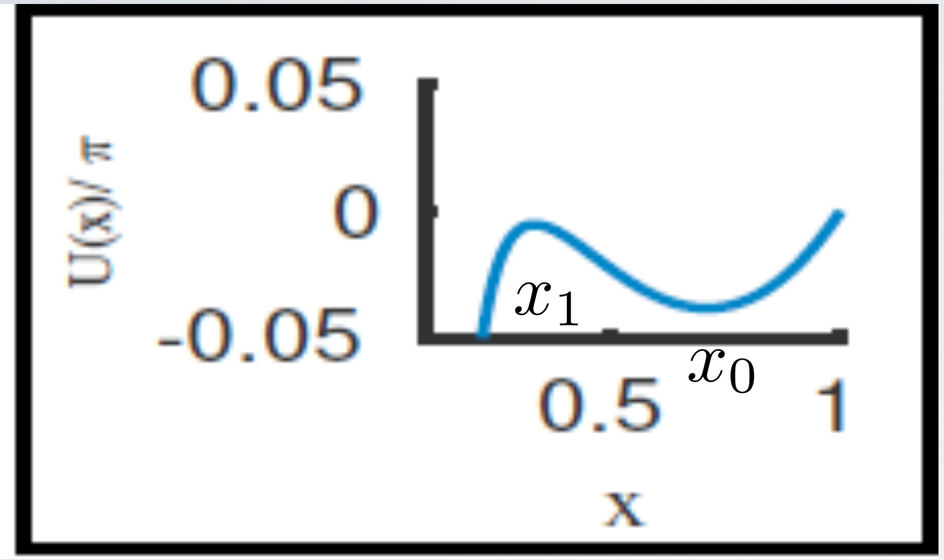






Both cooperative and non-cooperative behaviour is possible for a kinesin-like motor.

cooperative regime, D-dominated



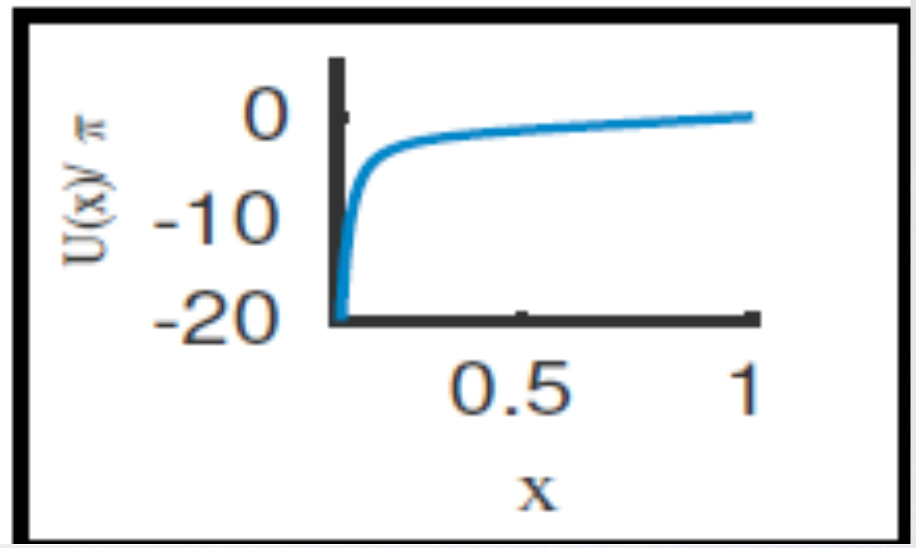
$$\boxed{T_N \simeq \frac{2\pi^*}{\omega_0\omega_1} e^{\frac{\Delta U}{D_0}}}$$

$$\omega_0 = U''(x_0)\ ,\ \omega_1 = -U''(x_1)$$

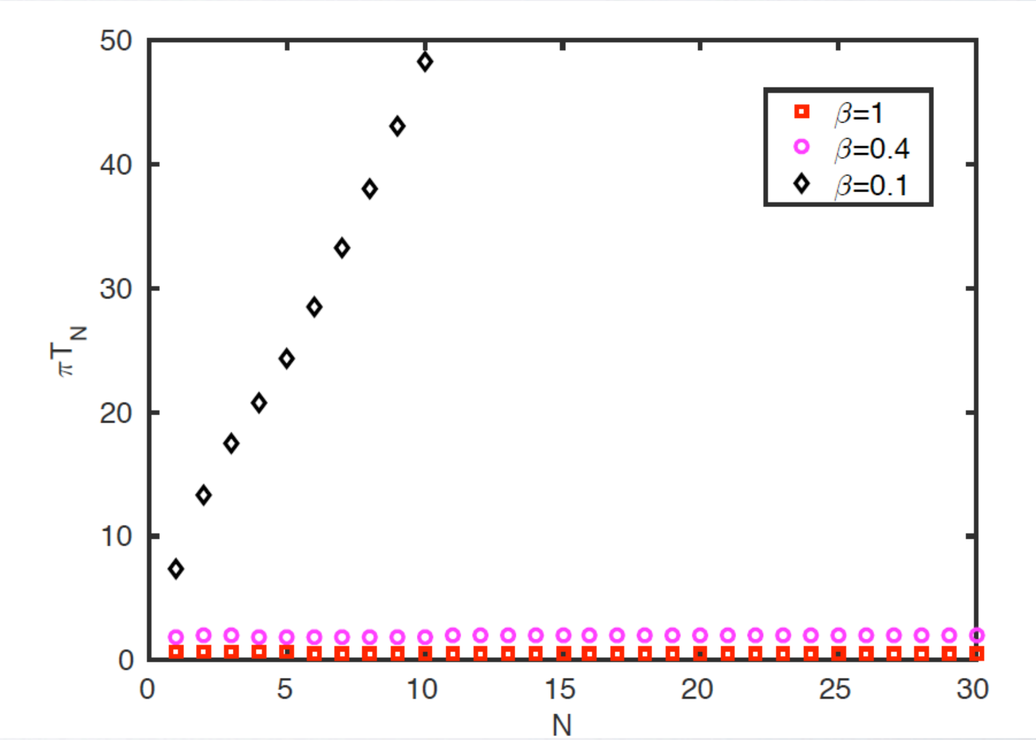
$$\Delta U = U(x_1) - U(x_0)$$

$$D_0 = D(x_0)$$

non-cooperative regime, v-dominated

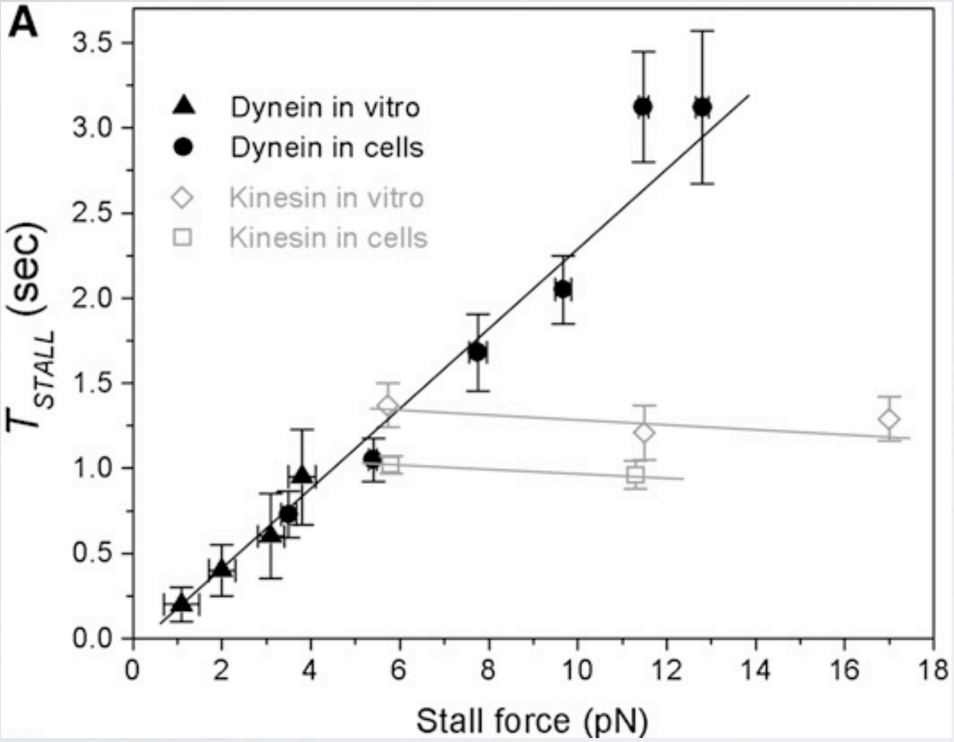


$$T_N \simeq -\int_0^1 \frac{dx}{v(x)} + O(1/N)$$

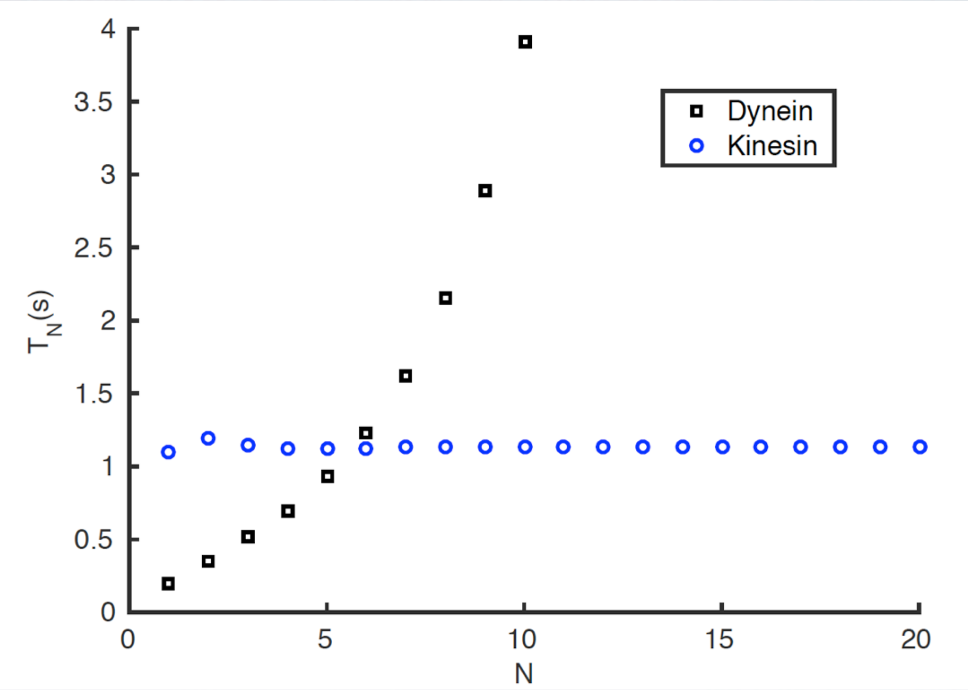


Cooperative dynein and non-cooperative kinesin

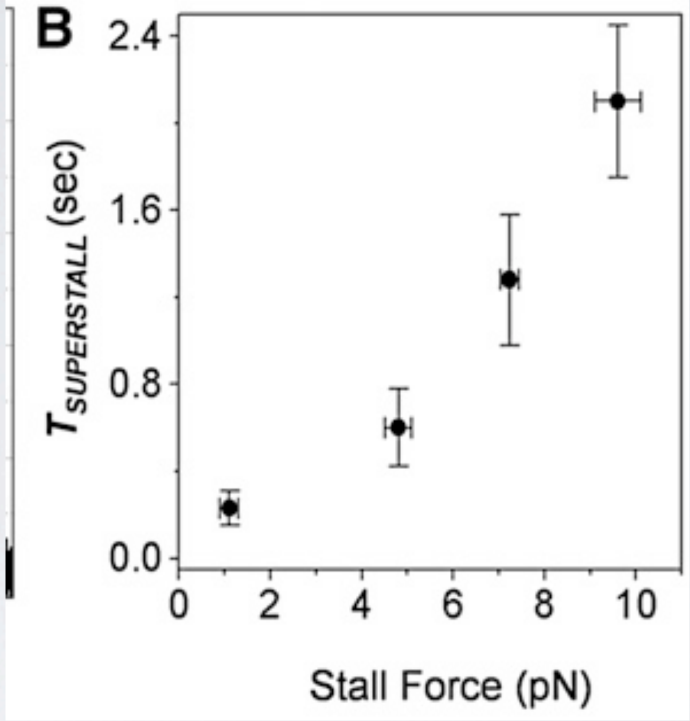
Rai et al., Cell (2013)



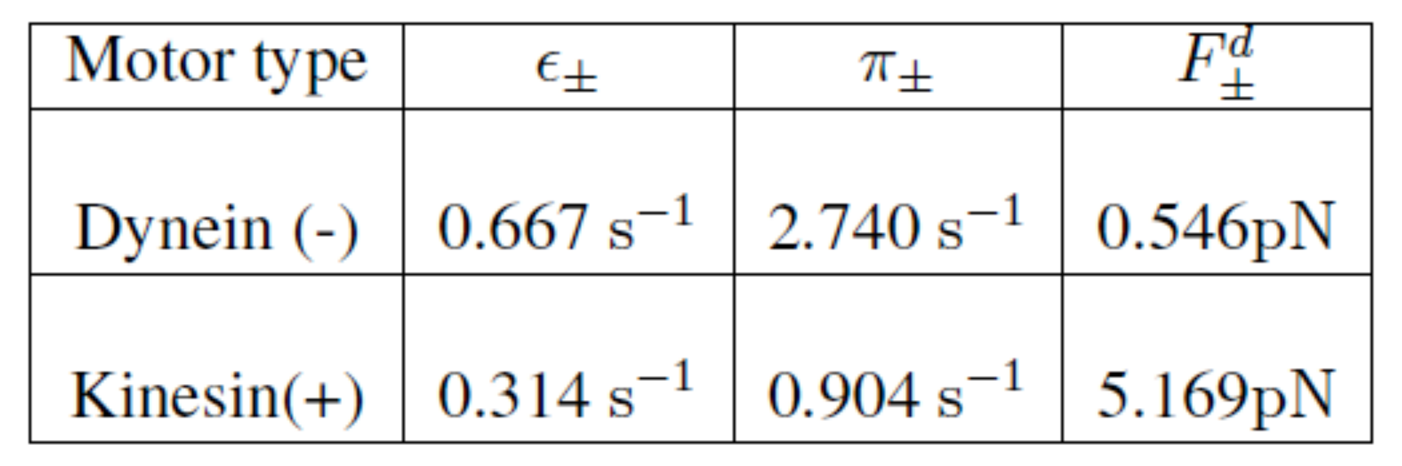
KL mean-field model



Rai et al., Cell (2013)



Fitting parameters extracted from in vitro experiments



Motor type	$\epsilon_\pm$	$\pi_\pm$	$F^d_\pm$
Dynein (-)	$0.667\ s^{-1}$	$2.740\ s^{-1}$	0.546pN
Kinesin(+)	$0.314\ s^{-1}$	$0.904\ s^{-1}$	5.169pN

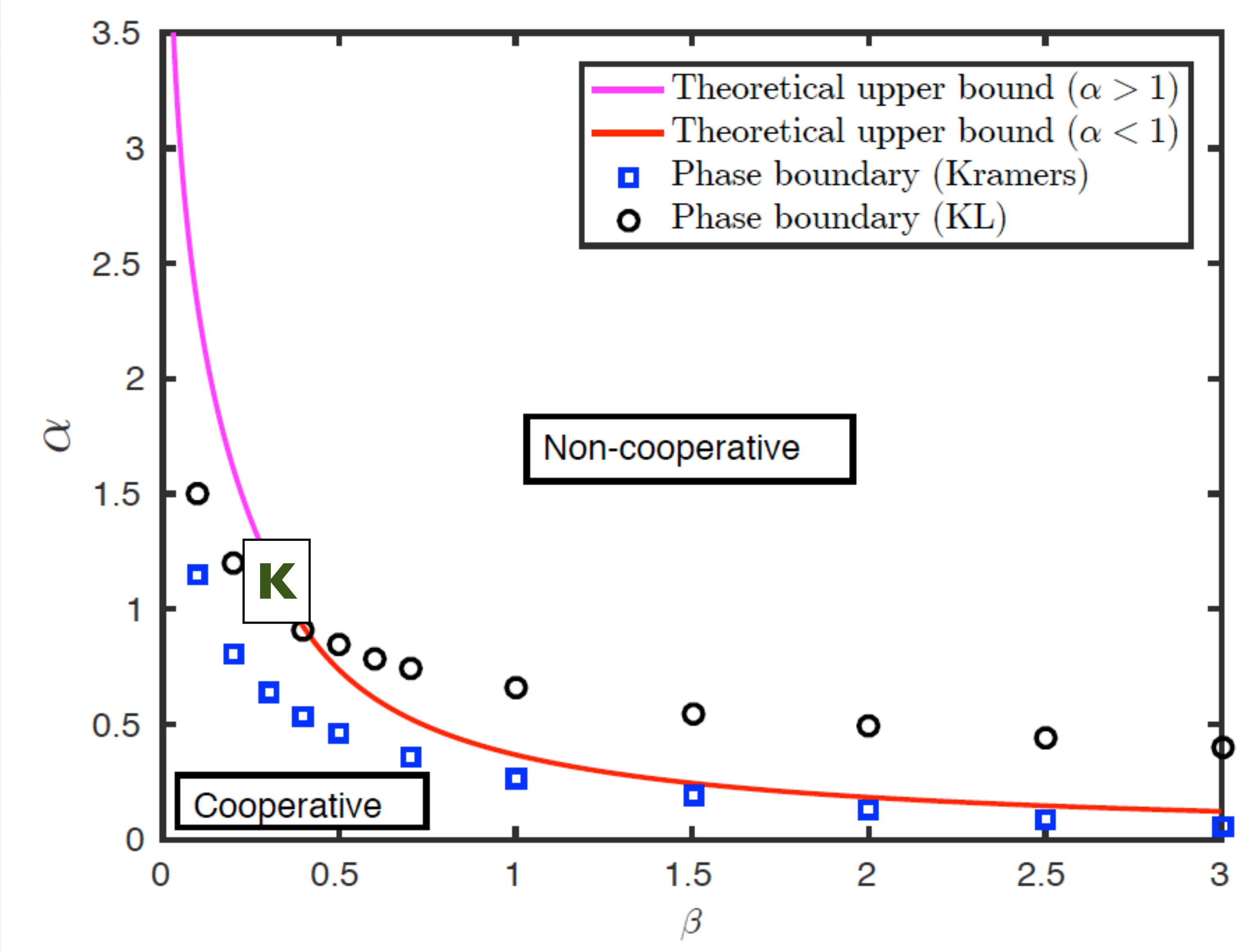
Stall force

dynein: 1.1pN

kinesin: 5.5 pN

Soppina et al. , PNAS (2009); Bhat and M.G, Phys. Biol. (2012)

THE “PHASE DIAGRAM” FOR MOTOR COOPERATIVITY



Theoretical upper bound

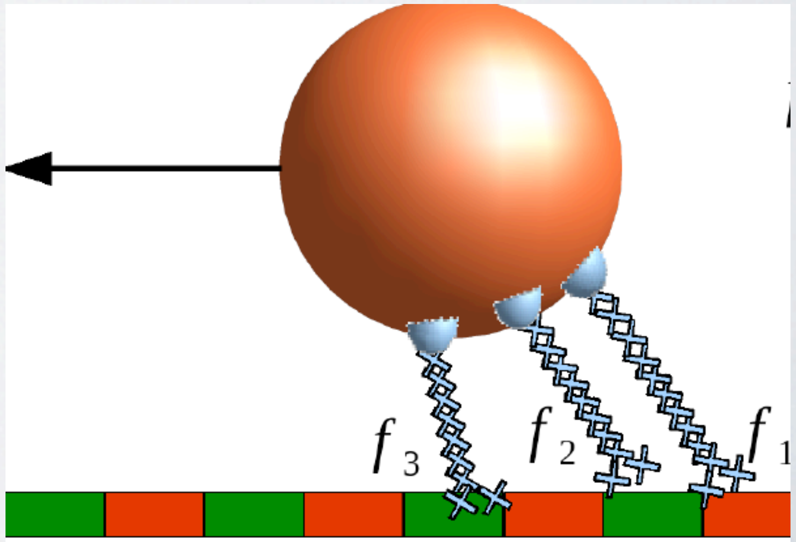
$$\alpha_{ub} = 1/\beta e \quad (\beta \leq e^{-1})$$

$$\alpha_{ub} = \ln(1/\beta) \quad (\beta \geq e^{-1})$$

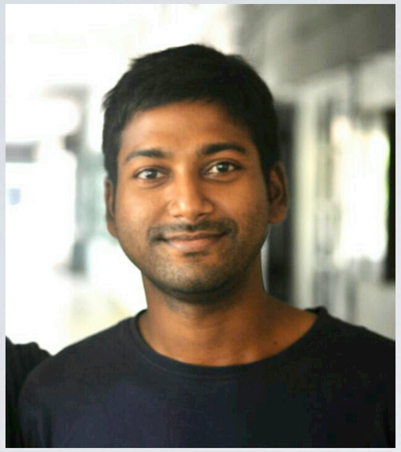
Planned extensions:

A. Including ATP dependence of various quantities

B. Elastically coupled motors and stochastic load-sharing



ACKNOWLEDGEMENTS



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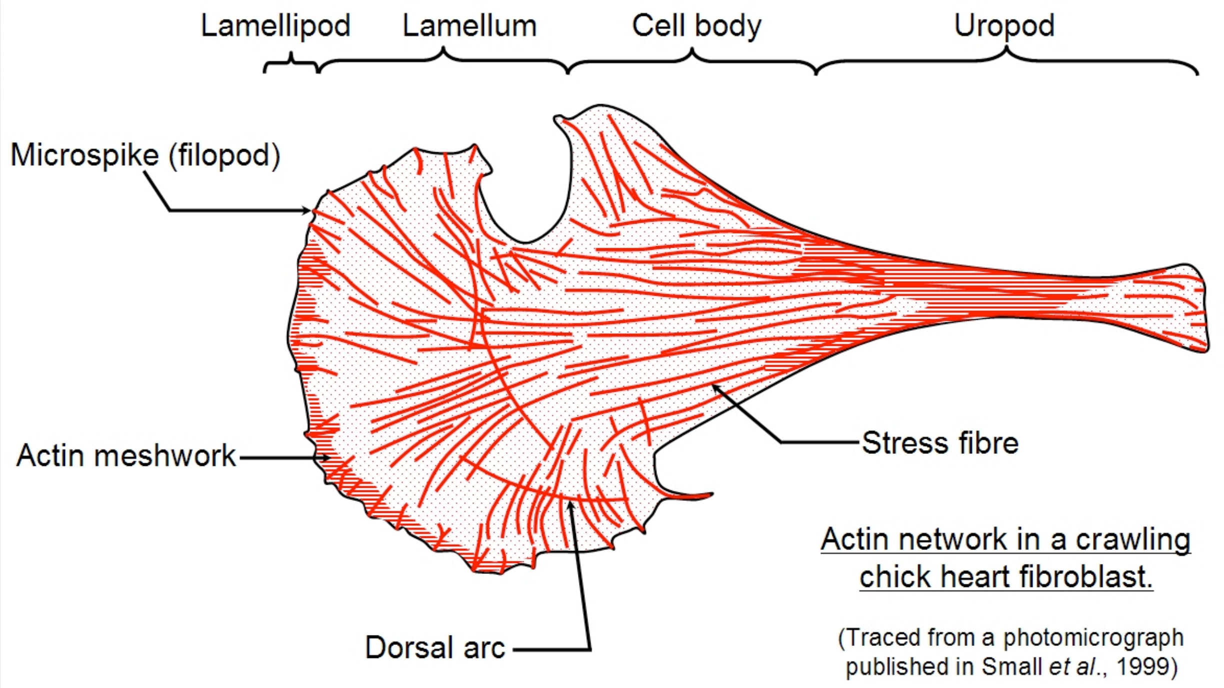
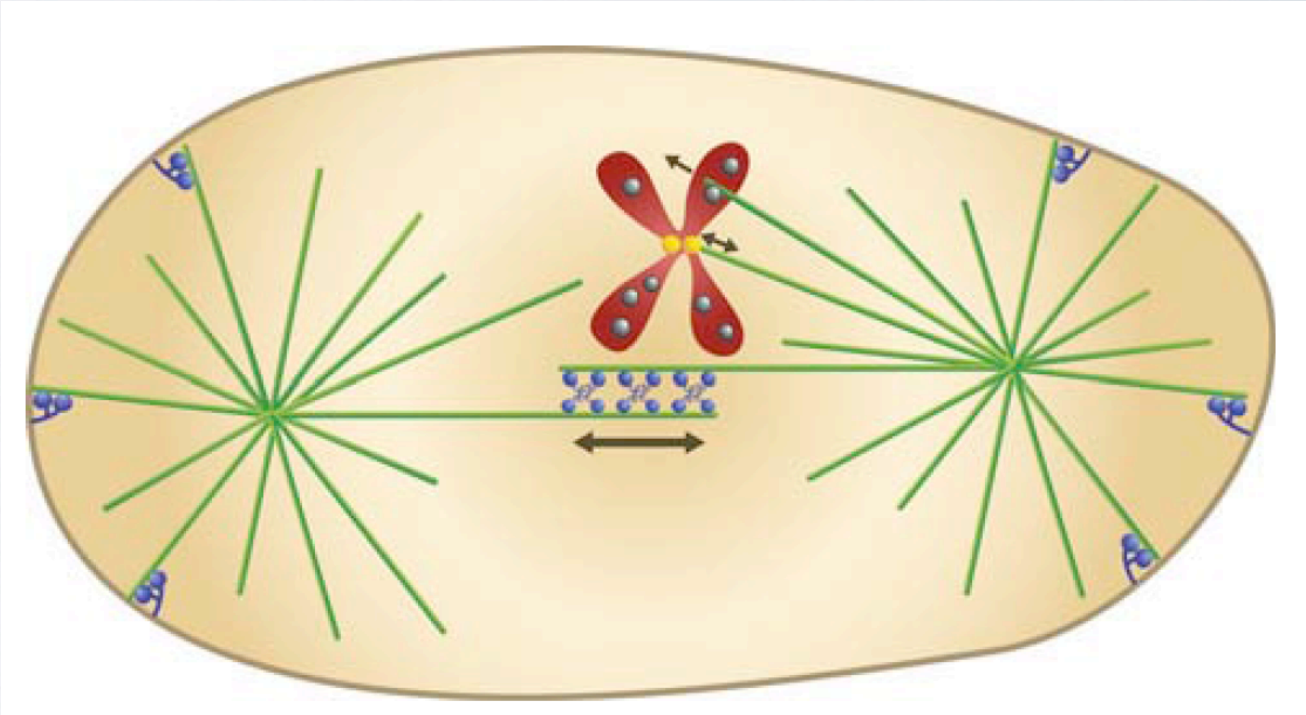
II. POLYMERIZATION FORCE OF A BUNDLE OF RIGID FILAMENTS

When growing filaments talk to each other



Actin network in a crawling chick heart fibroblast.

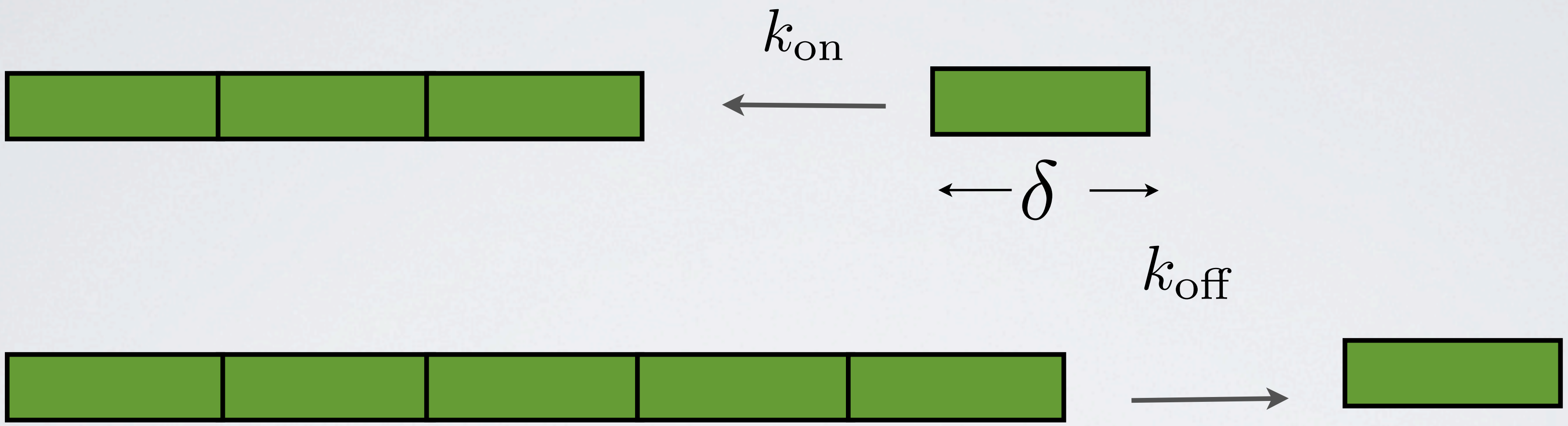
(Traced from a photomicrograph published in Small *et al*., 1999)



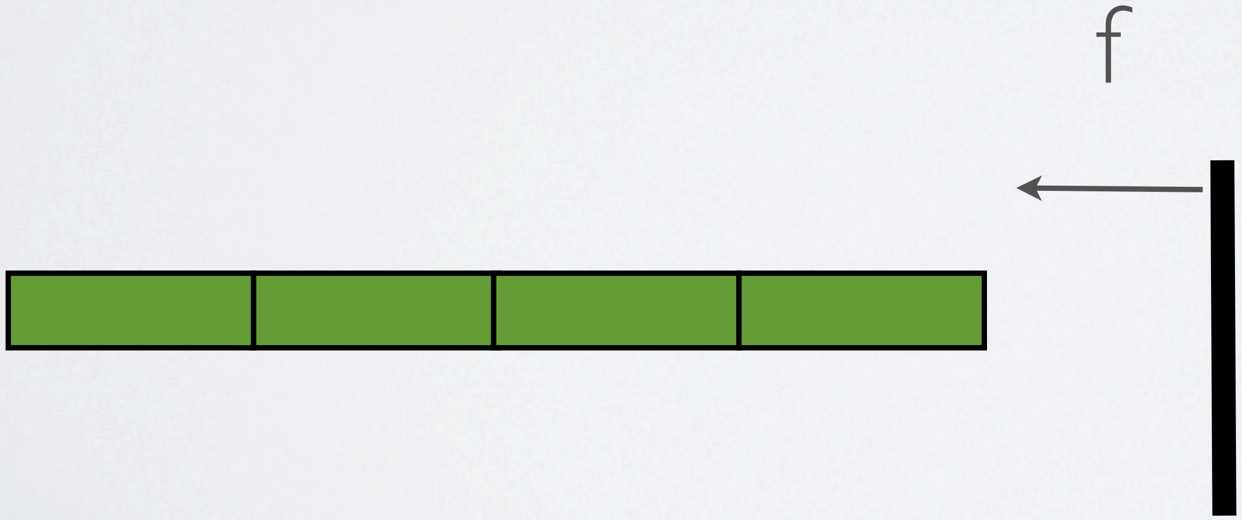
Small et al., Micros. Res. Tech.(1999)

I. Tolic-Norrelykke., Eur. Biophys. J (2008)

POLYMERIZATION FORCE



$$V = \delta(k_{\mathrm{on}} - k_{\mathrm{off}})$$



BROWNIAN RATCHET MODEL

When growth is obstructed by the barrier

$$k_{\mathrm{on}} \rightarrow k_{\mathrm{on}} e^{-\frac{f\delta}{k_B T}}$$

Force-velocity relation (single filament)

$$V_1(f) = \delta[k_{\mathrm{on}} e^{-\frac{f\delta}{k_B T}} - k_{\mathrm{off}}]$$

For N filaments, force per filament =f/N

$$V_N(f) = \delta[k_{\mathrm{on}} e^{-\frac{f\delta}{N k_B T}} - k_{\mathrm{off}}]$$

Stall force $$\boxed{f_s^{(N)} = \frac{N k_B T}{\delta} \ln(\frac{k_{\mathrm{on}}}{k_{\mathrm{off}}})}$$

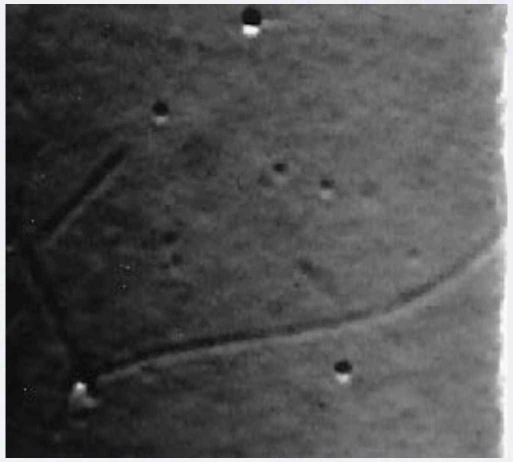
Peskin et al., Biophys. J (1993)

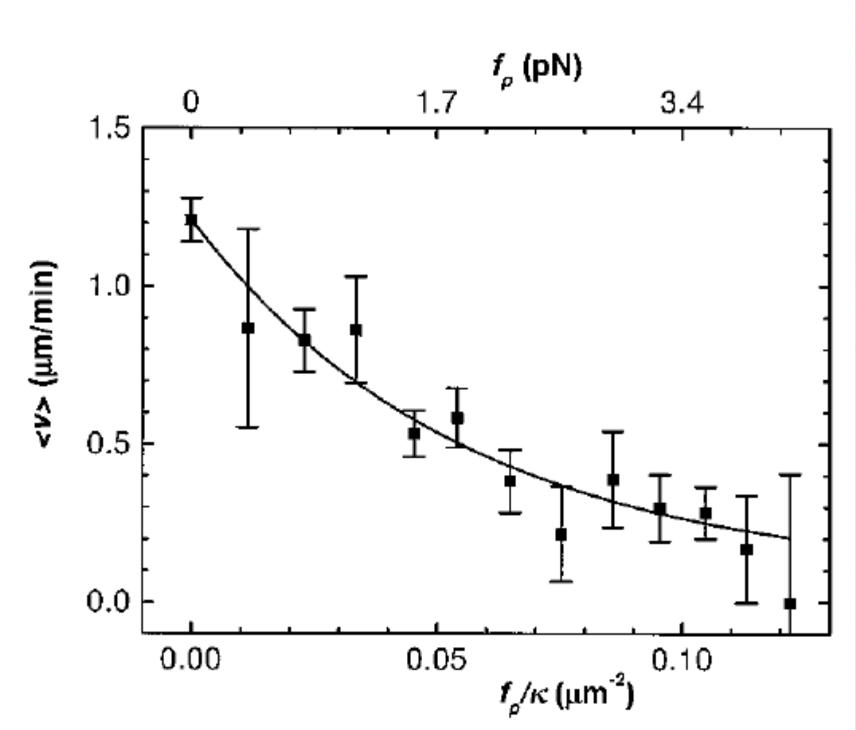
POLYMERIZATION FORCE OF A SINGLE MICROTUBULE

Measurement of the Force-Velocity Relation for Growing Microtubules
Marileen Dogterom* and Bernard Yurke
(*Science*, **278**, 856, 1997)

Forces generated by protein polymerization are important for various forms of cellular motility. Assembling microtubules, for instance, are believed to exert pushing forces on chromosomes during mitosis. **The force that a single microtubule can generate was measured by attaching microtubules to a substrate at one end and causing them to push against a microfabricated rigid barrier at the other end.** The subsequent buckling of the microtubules was analyzed to determine both the force on each microtubule end and the growth velocity. The growth velocity decreased from 1.2 micrometers per minute at zero force to 0.2 micrometer per minute at forces of 3 to 4 piconewtons. **The force-velocity relation fits well to a decaying exponential, in agreement with theoretical models, but the rate of decay is faster than predicted.**

- Microtubules were forced to grow towards a fixed wall.
- Some microtubules were forced to buckle after hitting the wall.
- From the shape of the buckled MT, the force exerted on the wall was determined.
- The rate of elongation was determined under various forces (measured as described above).

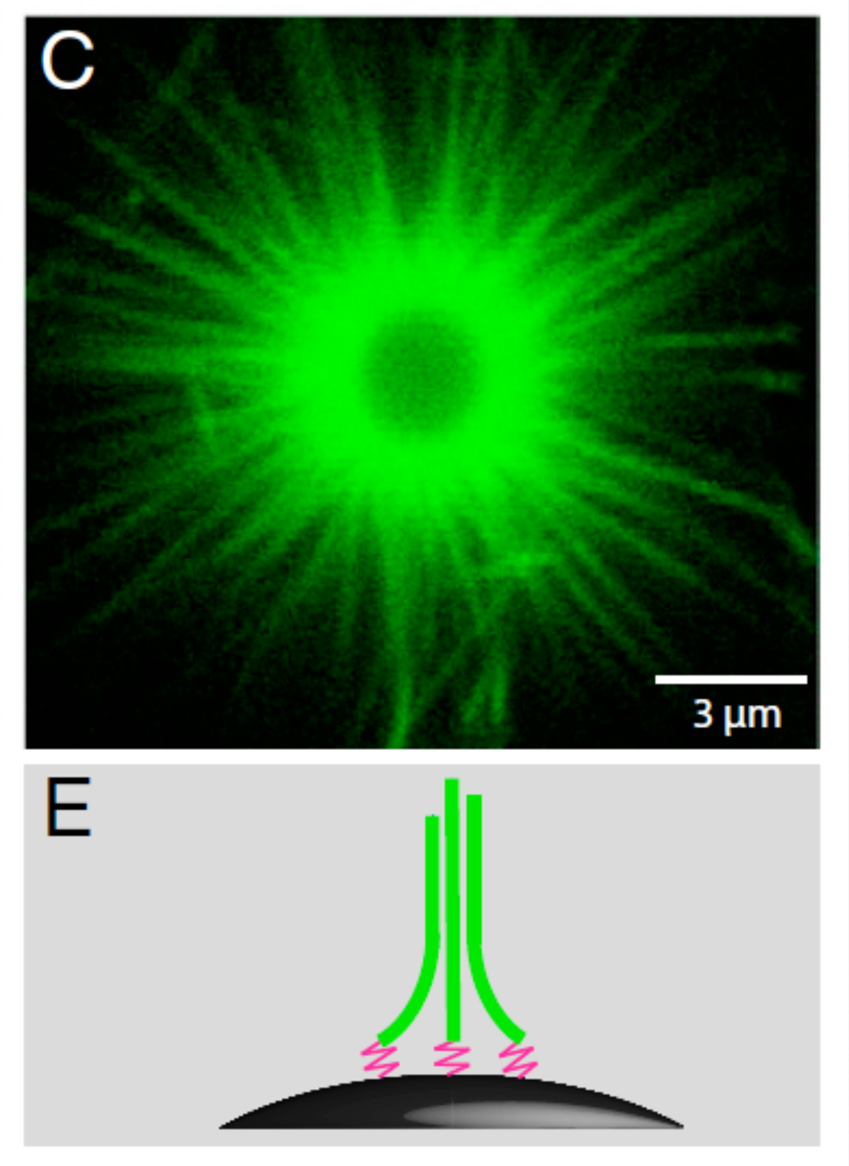




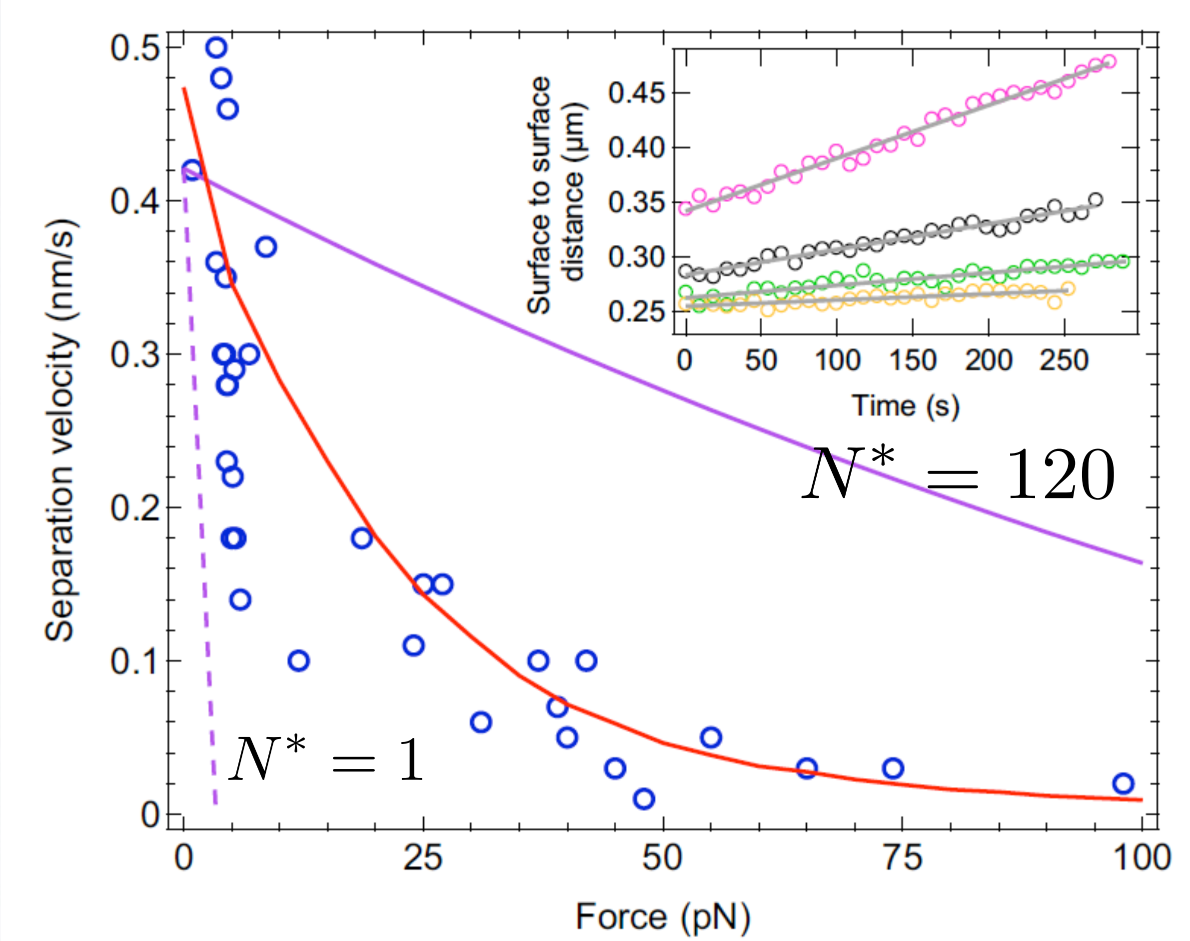
$$V(F) = Ae^{-\frac{F\delta}{N^* k_B T}} - B$$

Best fit: N*=3.6, not 13!

BUNDLE OF ACTIN FILAMENTS PUSHING AGAINST A LOAD



$$v \sim \delta[k_{\rm on} e^{-\frac{f\,\delta}{N^* k_B T}} - k_{\rm off}]$$

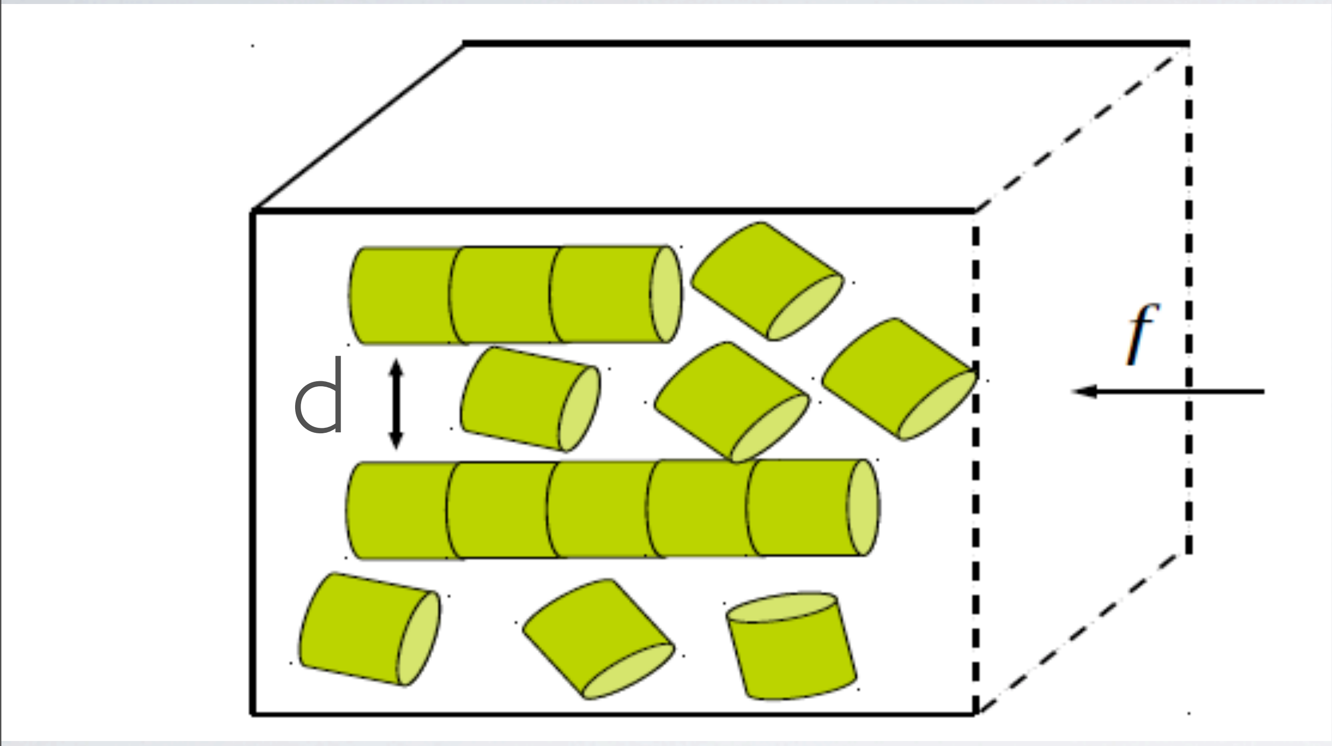


Demoulin et al., PNAS (2014)

Observed force-velocity curve lies between no load-sharing and perfect load-sharing.

GROWING POLYMERS PUSHING A FLAT WALL

BROWNIAN DYNAMICS SIMULATIONS

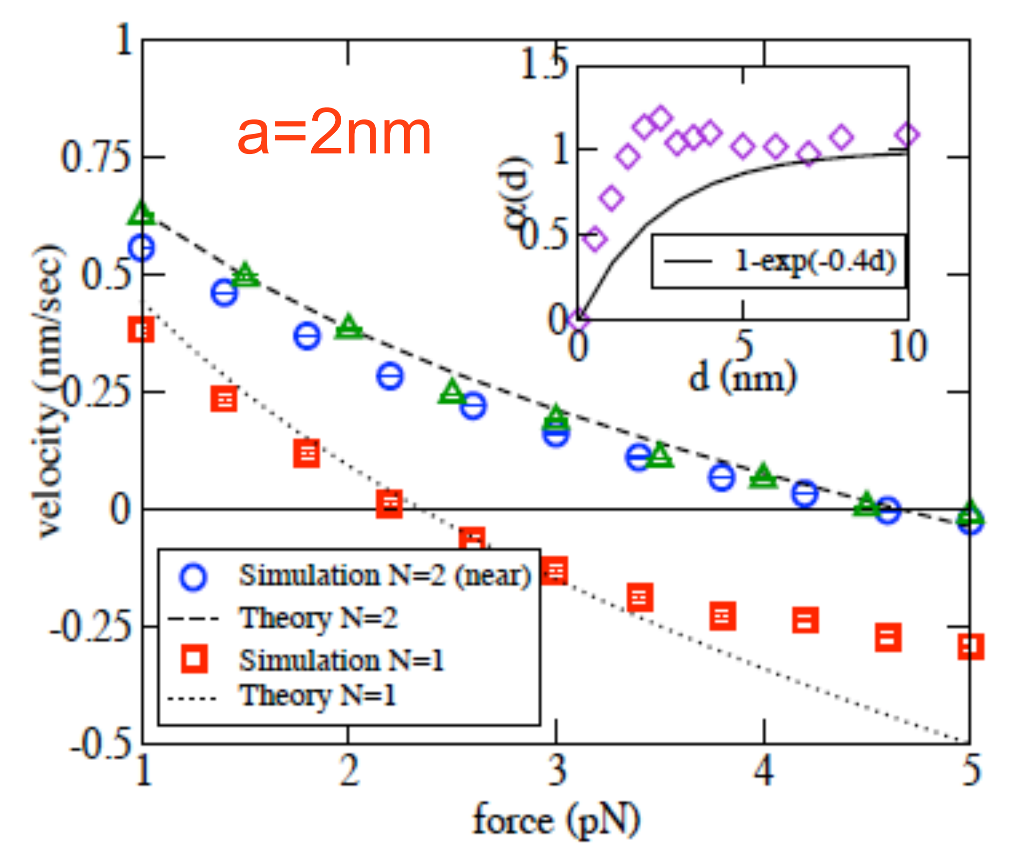


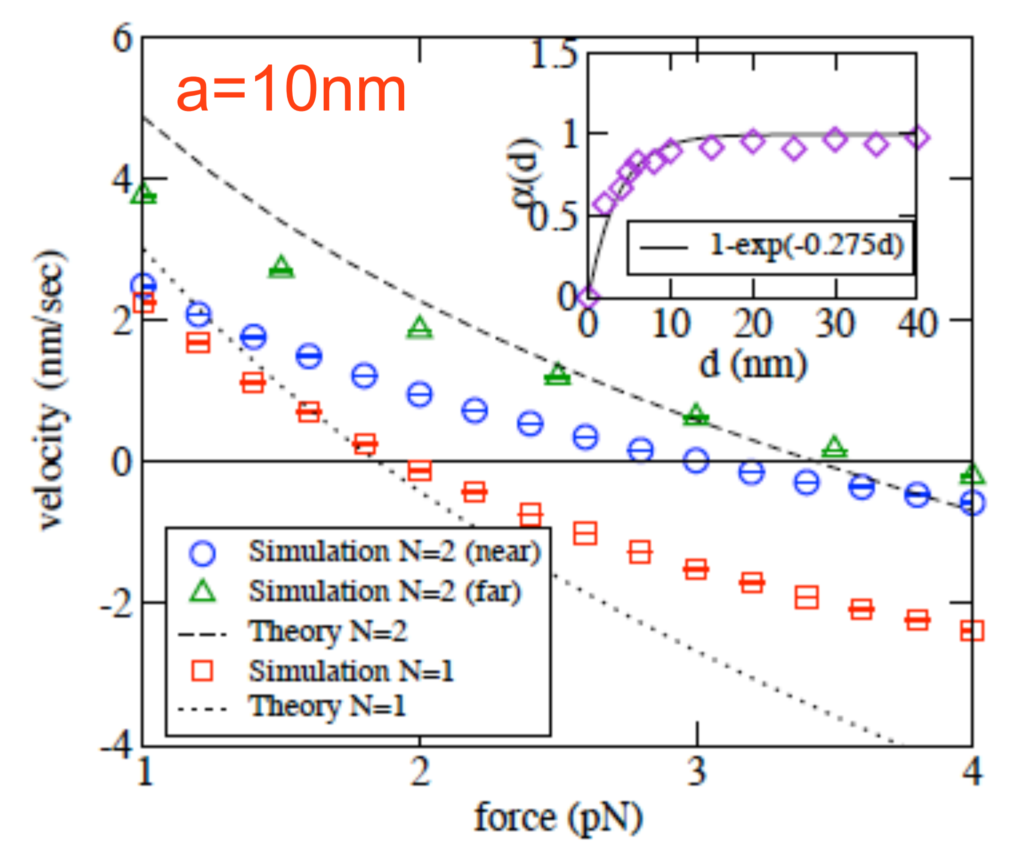
monomer length	δ	2 nm
radius	a	variable
diffusion coefficient(monomer)	D	$10^5\ \mathrm{nm}^2\mathrm{s}^{-1}$
diffusion coefficient(wall)	D_w	$10^3\ \mathrm{nm}^2\mathrm{s}^{-1}$
concentration	C_0	3.3 μM
monomer dissociation rate	k_{off}	variable

- Nucleate N linear polymers from one face of a rectangular box, equally spaced at the base (distance “d”).
- The opposite face is a moving wall, acted on by external force f, diffusion constant D.
- monomers are flat-faced solid discs, radius “a”, polymers grow by diffusion-limited adsorption.
- Reflecting boundary conditions imposed at the polymer-wall interface, when in contact.
- monomer concentration kept fixed during polymer growth.

V-F CURVE AND STALL FORCE

FLAT-FACED FILAMENTS, DIFFERENT RADII





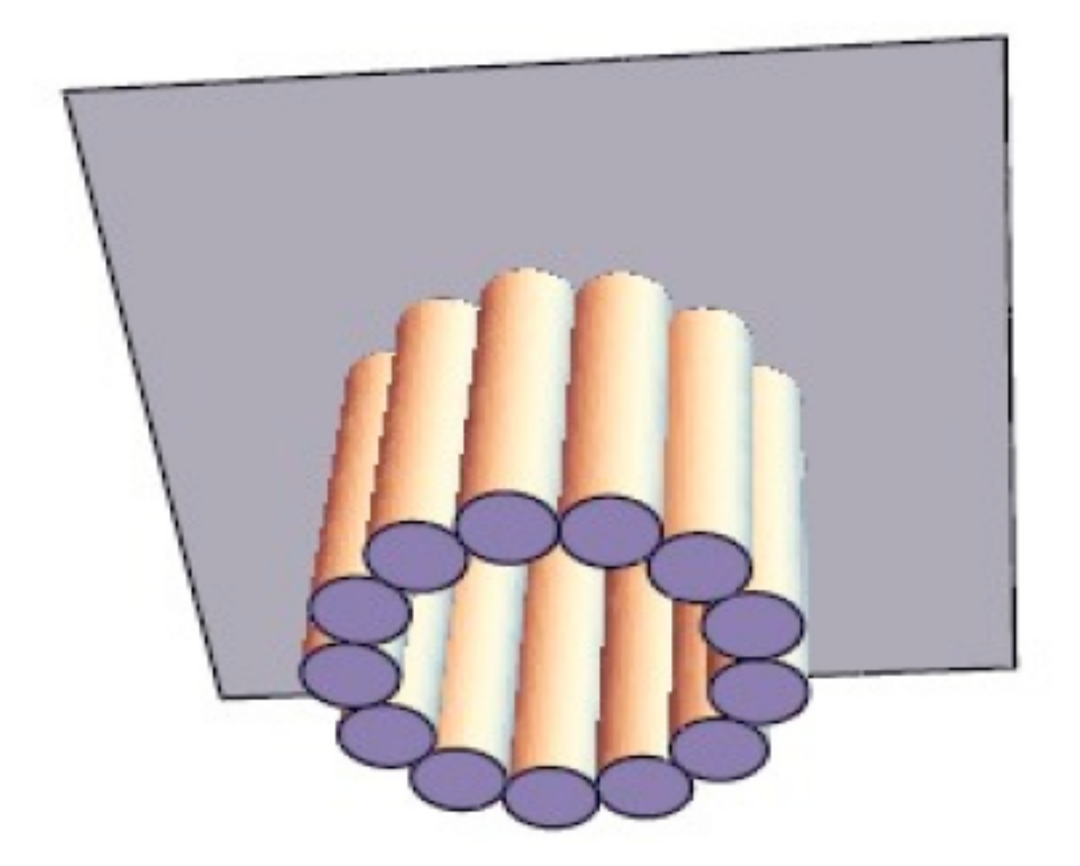
Filament	$f_s^{(1)}$(pN)	$f_s^{(2)}$(pN)	ν
FFD (a=2nm, near)	2.24 ± 0.05	4.53 ± 0.05	1.011 ± 0.0216
FFD (a=2nm, far)	2.24 ± 0.05	4.58 ± 0.05	1.022 ± 0.0435
FFD (a=10nm, near)	1.925 ± 0.05	3.05 ± 0.05	0.787 ± 0.026
FFD (a=10nm, far)	1.925 ± 0.05	3.725 ± 0.05	0.971 ± 0.012
FFD (a=20nm, near)	1.88 ± 0.25	2.76 ± 0.25	0.734 ± 0.075
FFD (a=20nm, far)	1.88 ± 0.25	3.55 ± 0.25	0.928 ± 0.059

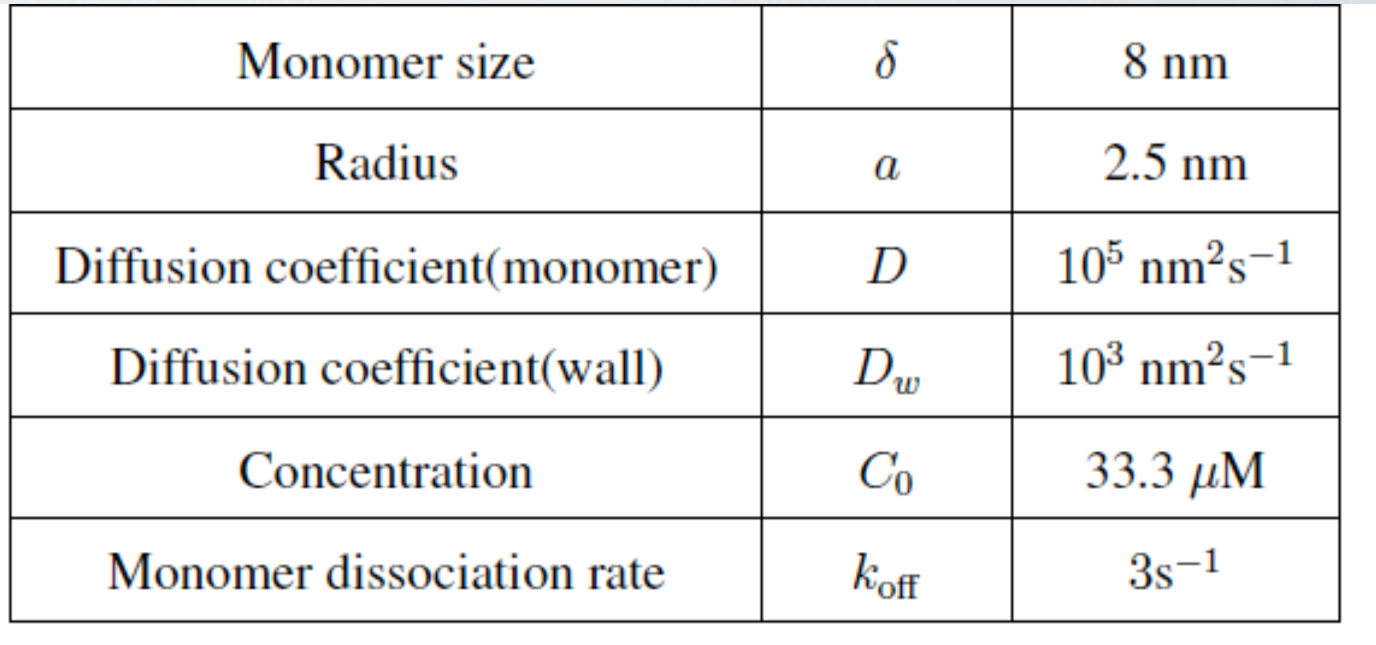
$$\boxed{\nu = f_s^{(2)}/2f_s^{(1)}}$$

$\nu < 1$ indicates sub-linear scaling of force with number.

Jemseena V and M.G, bioRxiv (2017)

MICROTUBULE-LIKE FILAMENTS



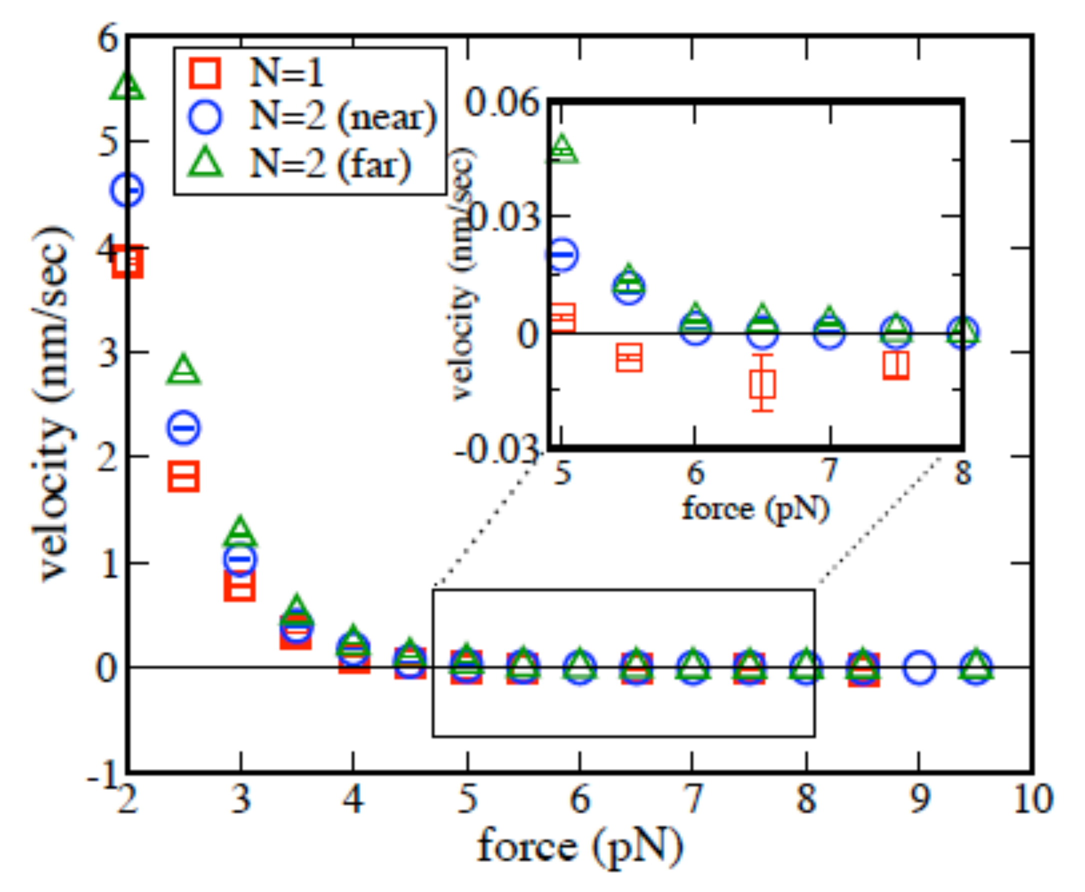


Monomer size	δ	8 nm
Radius	a	2.5 nm
Diffusion coefficient(monomer)	D	10^5 nm^{2}s^{-1}
Diffusion coefficient(wall)	D_w	10^3 nm^{2}s^{-1}
Concentration	C_0	33.3 μM
Monomer dissociation rate	k_{off}	3s^{-1}

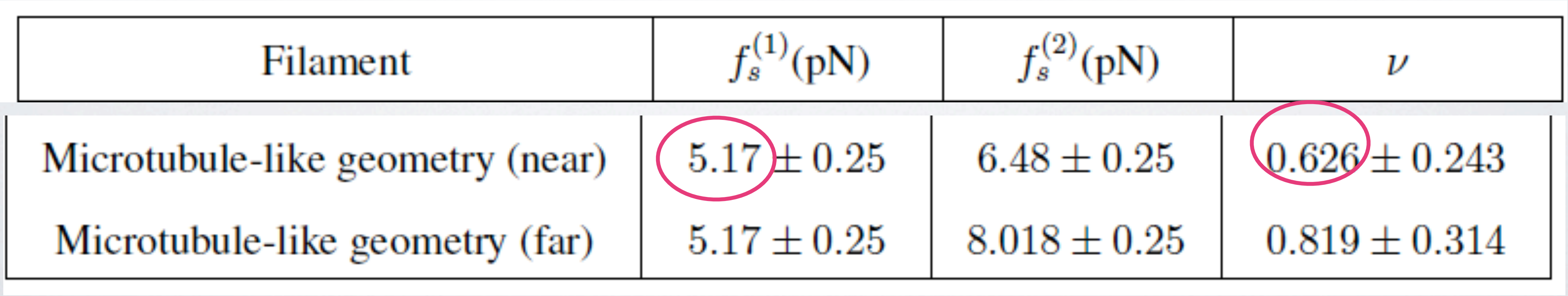
V-F CURVE AND STALL FORCE

SINGLE MICROTUBULE AND TWO MICROTUBULES

- For single microtubule, the stall force measured in our simulations is **5.17pN**.
- However, for 13 **independent** protofilaments, the combined stall force predicted by the Brownian ratchet model is **12.24pN**, for the present parameters.
- Discrepancy similar to experimental observations of Dogterom and Yurke (1997)
- Stall force for two filaments is, in general, **less than twice** that of a single filament.



Jemseena V and M.G, bioRxiv (2017)

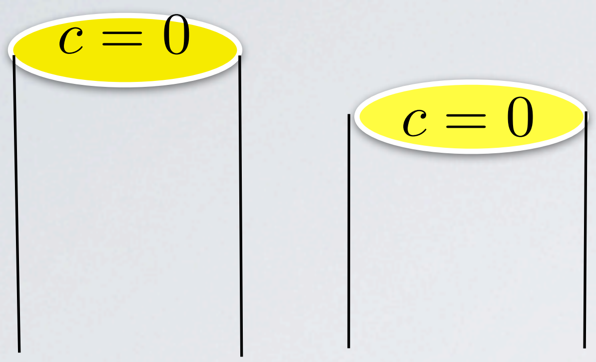


Filament	$f_s^{(1)}$(pN)	$f_s^{(2)}$(pN)	ν
Microtubule-like geometry (near)	5.17 ± 0.25	6.48 ± 0.25	0.626 ± 0.243
Microtubule-like geometry (far)	5.17 ± 0.25	8.018 ± 0.25	0.819 ± 0.314

AN ELECTROSTATIC ANALOGY

GROWING POLYMERS=CONDUCTORS AT FIXED POTENTIALS

$$\nabla^2 c = 0$$



$$c(\mathbf{r}) = c_0 \quad \text{as r} \to \infty$$

$$V = V_i \text{ on the conductors}$$

$$V(\mathbf{r}) = 0 \text{ as r} \to \infty$$

	Diffusion-limited growth	Electrostatics	
Monomer density	$D(c_0 - c(\mathbf{r}))$	$V(\mathbf{r})$	electric potential
Diffusive current density, normal	$J_n = D\frac{\partial c}{\partial n}$	$E_n = -\frac{\partial V}{\partial n}$	electric field, normal
monomer adsorption rate	$k_{\rm on}^{(i)} = \int J_n dS_i$	$4\pi Q_i = \int E_n dS_i$	electric charge

SELF AND MUTUAL CAPACITANCES

A general linear relation holds between charges and potentials in a system of conductors:

$$Q_i = \sum_j C_{ij} V_j \quad ; \quad V_i = \sum_j C^{-1}_{ij} Q_j$$

Simplifications: $V_i = Dc_0, \;\; k^{(i)}_{\rm on} = k_{\rm on}$ (for equally spaced filaments).

$$C_{ii} = \text{self} - \text{capacitance}$$

$$C_{ij} = \text{mutual capacitances}, \;\; i \neq j$$

$$\boxed{k_{\rm on} = \frac{4\pi D c_0}{\sum_j C^{-1}_{ij}}}$$

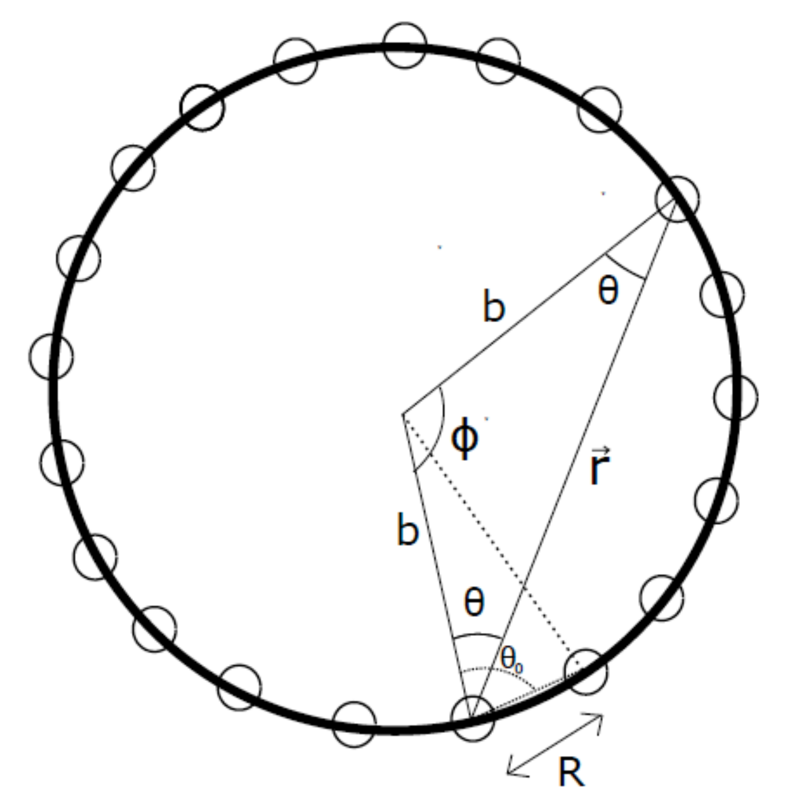
Disc-like conductors, radius a, mutual separation r_{ij}

$$C^{-1}_{ii} = \frac{\pi}{2a} \quad ; \quad C^{-1}_{ij} = \frac{1}{r_{ij}} + \ldots$$

("large distance/ monopole approximation")

L.D. Landau and E. M. Lifshitz, *Electrodynamics of continuous media*

DIFFUSIVE COUPLING “THEORY FOR N+1 ABSORBING DISCS IN A CIRCULAR RING

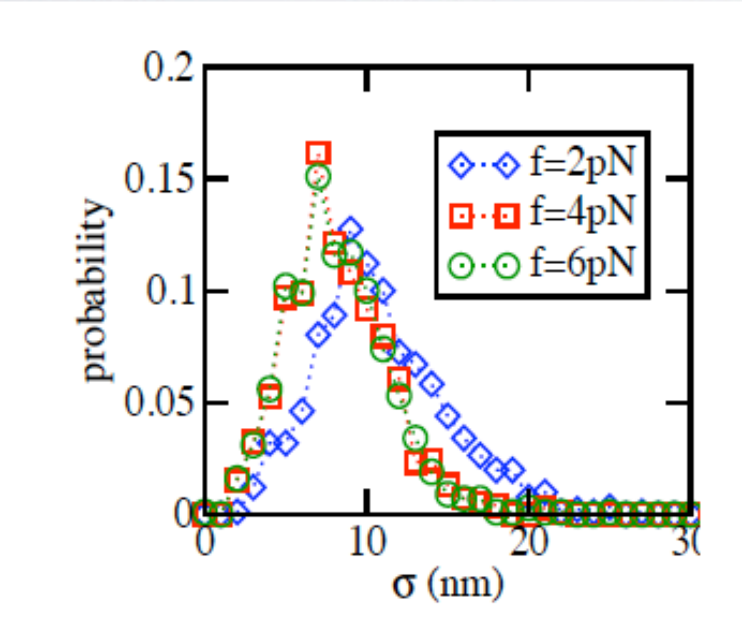


- Treat the array as a continuum distribution, replace the sum with integral:

$$\sum_{j\neq i} r_{ij}^{-1} = \frac{N}{2\pi}\int_0^{2\pi} \frac{d\phi}{r(\phi)}$$

$$r(\phi) = 2b\sin(\phi/2)$$

$$\sigma = \sqrt{\langle l_i^2\rangle - \langle l_i\rangle^2}$$



$$\boxed{k_{\mathrm{on}} = \frac{4Dac_0}{1+\frac{2a}{\pi R}\ln(2N/\pi)}}$$

Pranay Reddy, undergrad thesis, IIT Madras (2015)

Diffusive interaction leads to sub-additive polymerization force

$$f_s^{(N)} = N k_B T \ln\left(\frac{k_{\rm on}}{k_{\rm off}}\right) < N f_s^{(1)}$$

For a microtubule (N=12, R=2a,a=2nm), the theory predicts

$$f_s \simeq 4.48pN$$

in good agreement with simulations (~**5.17pN**)

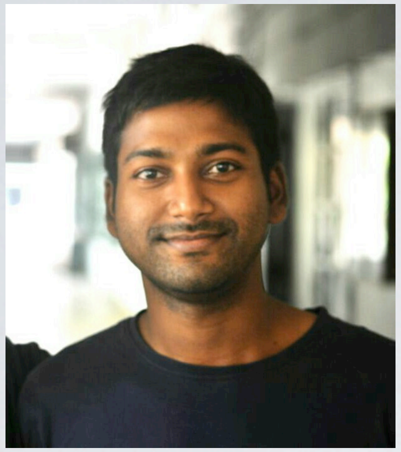
significantly different from **12.24pN** (non-interacting, additive scenario)

Planned extensions include

(a) catastrophe transition in microtubule bundles

(b) role of diffusive interaction in self-propelling colloids and eukaryotic chemotaxis

ACKNOWLEDGEMENTS



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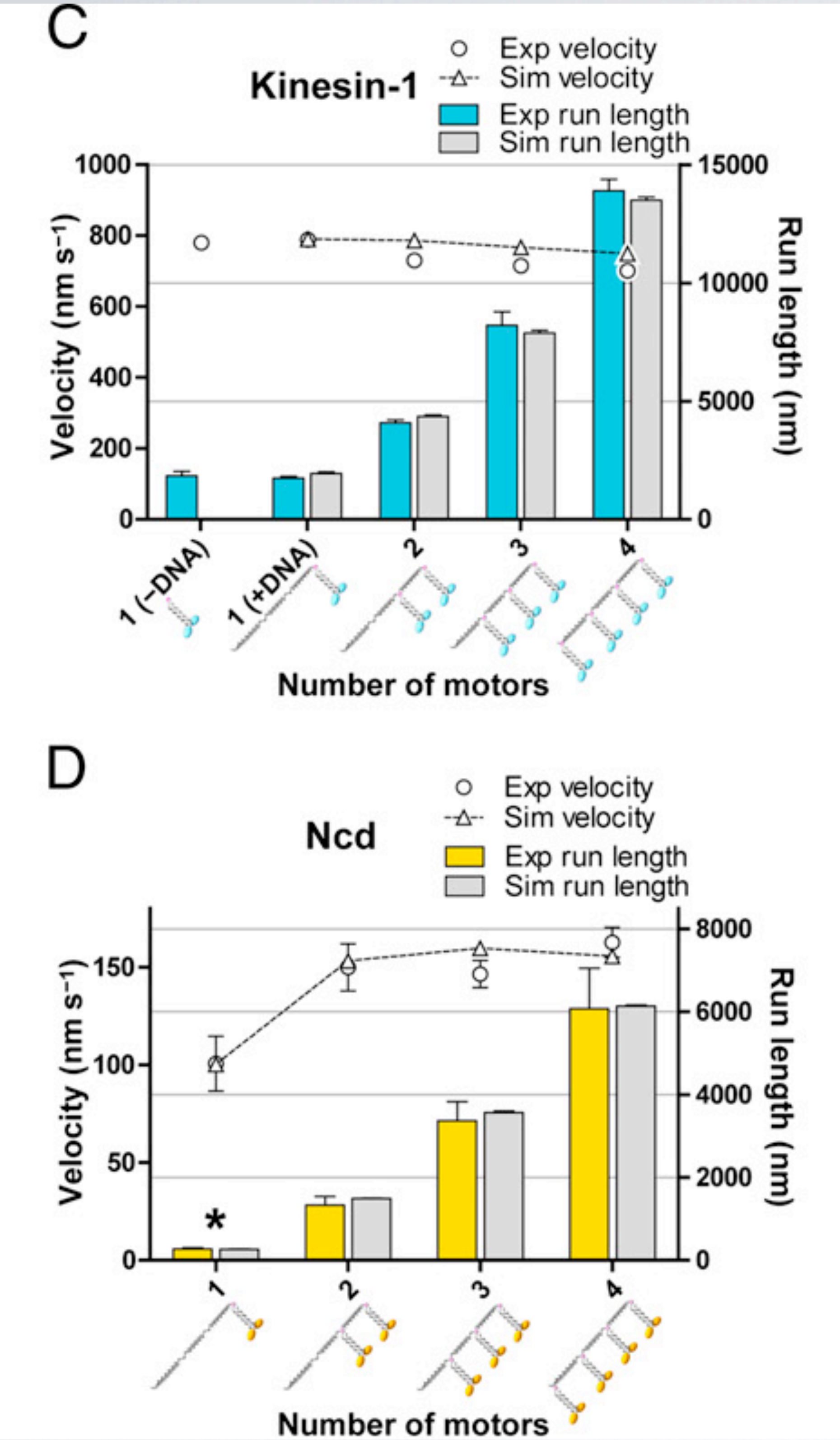
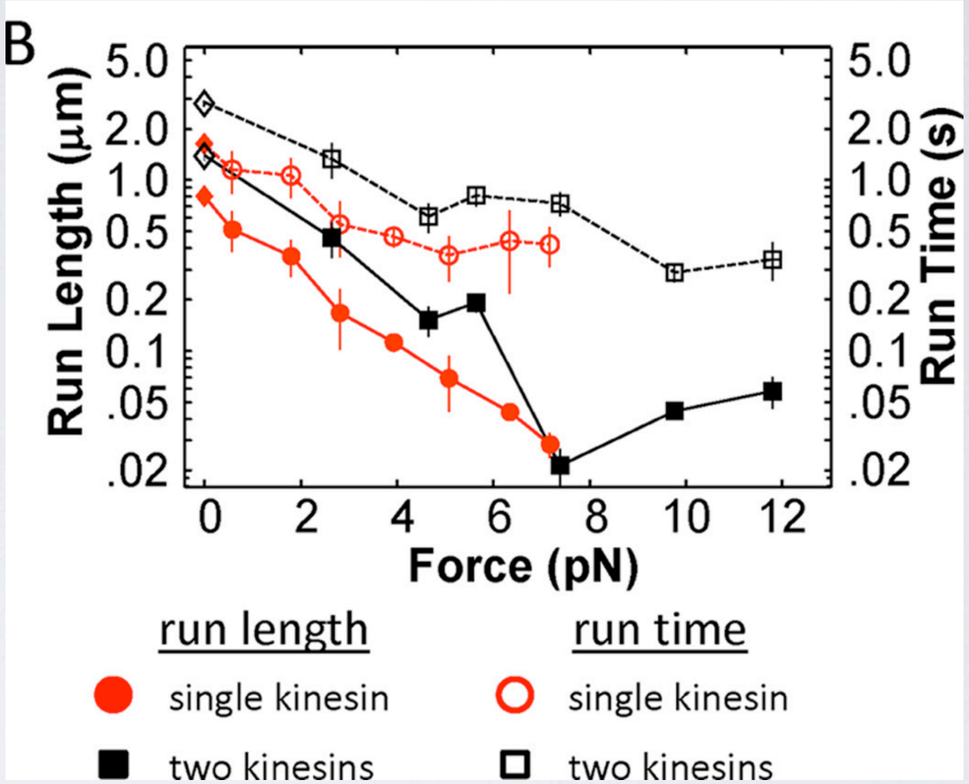
RUN LENGTH/TIME



Furuta et al., PNAS (2013)



Jamison, Driver, Diehl, J. Biol. Chem. (2012)

The fixed force ensemble

Cargo subjected to external force: F=Nf, but F is insensitive to detachment of motors.

Time of detachment, run length, velocity of motion

The fixed velocity ensemble

Force F is constantly adjusted to fix the cargo velocity V.

Time of detachment, force generated by motors

V=0 of special interest as motors exert maximum force