Flexing Protein muscles: How to Pull with a "Burning Rope"

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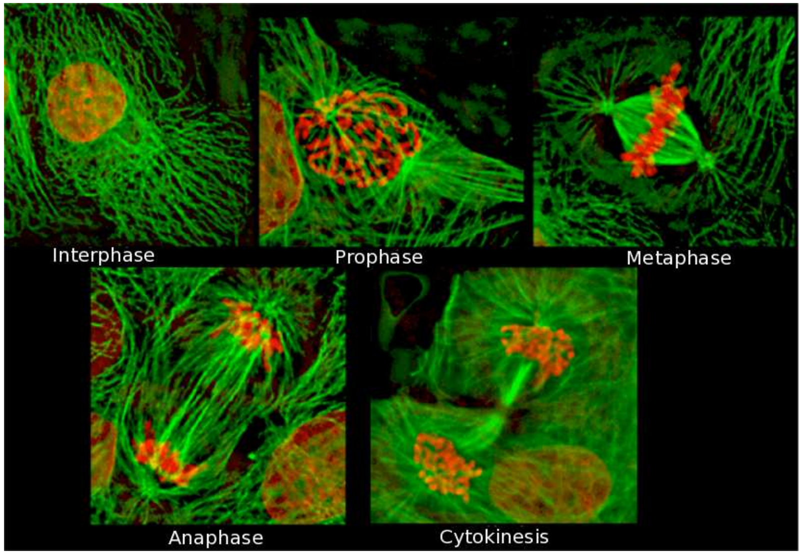
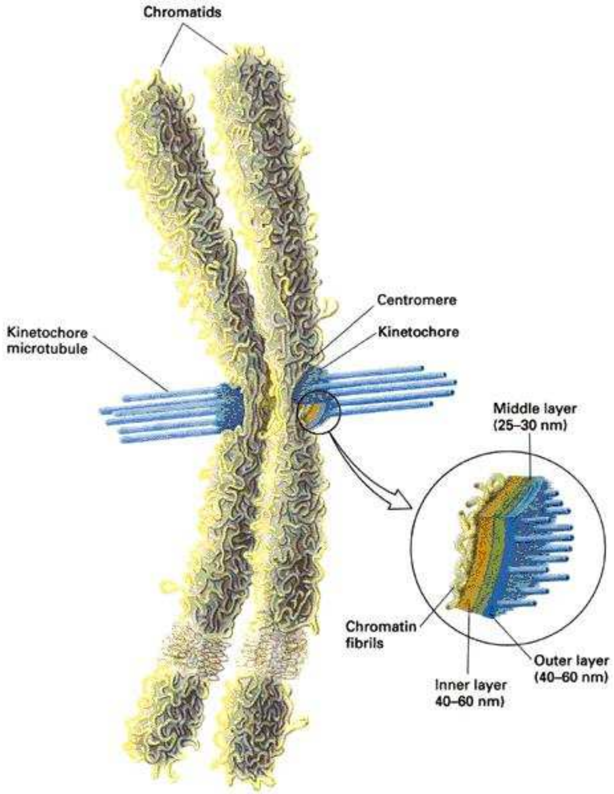
Eukaryotic Chromosomal Segregation



- Before cells divide their chromosomes are duplicated and then moved to the center (midline) of the dividing cell.
- Once they are all centered, they are separated into two equal sets, enabling cell division.
- Question: How are chromosomes moved?

Chromosome/microtubule interactions

Chromosomes are connected to microtubules (MT) at the KINETOCHORES where kinetochore structural complexes (such as Ndc80) bind the MT lattice.

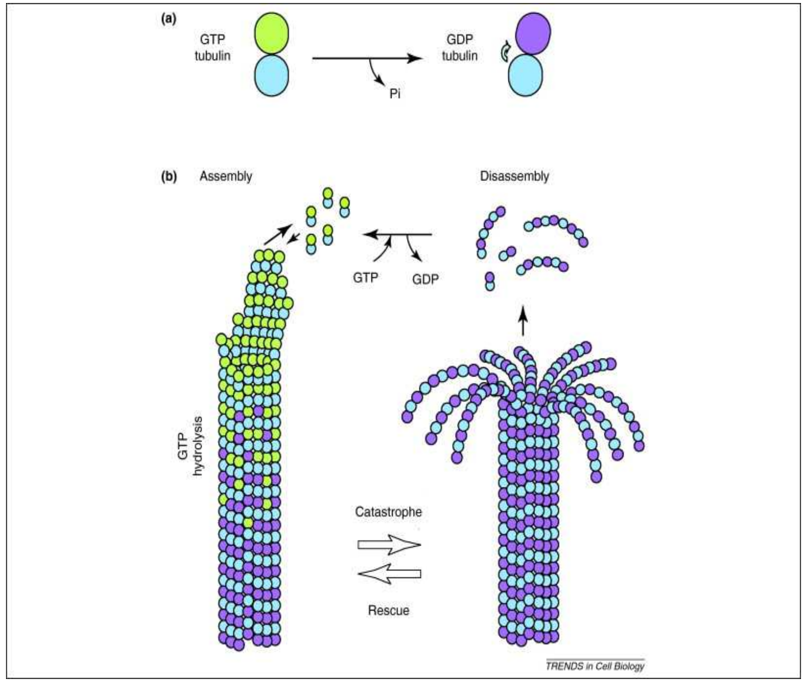


Chromosome/microtubule interactions

Microtubules change their length by polymerizing and depolymerizing at their tip.

DYNAMIC INSTABILITY describes the process by which microtubules switch between polymerizing and depolymerizing states.

Chromosome velocities are related to the MT polymerization/depolymerization rates.

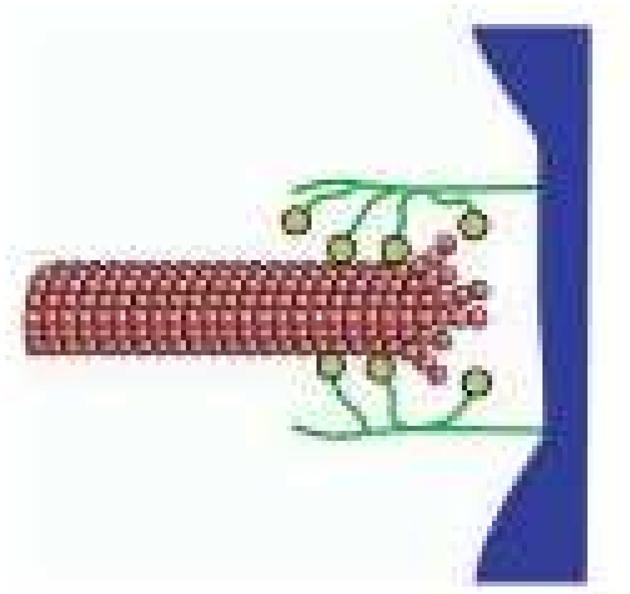


Question: How can depolymerization/polymerization affect chromosomal movement?

Ndc80 Complex

Question: How can depolymerizing microtubules pull the chromosomes?
Remark: It is known to not be due to walking molecular motors (i.e., dynein).

Proposed Answer:
Biased diffusion of Ndc80 proteins along the MT.



Before now there has been no mechanistically satisfactory model of how biased diffusion actually works.

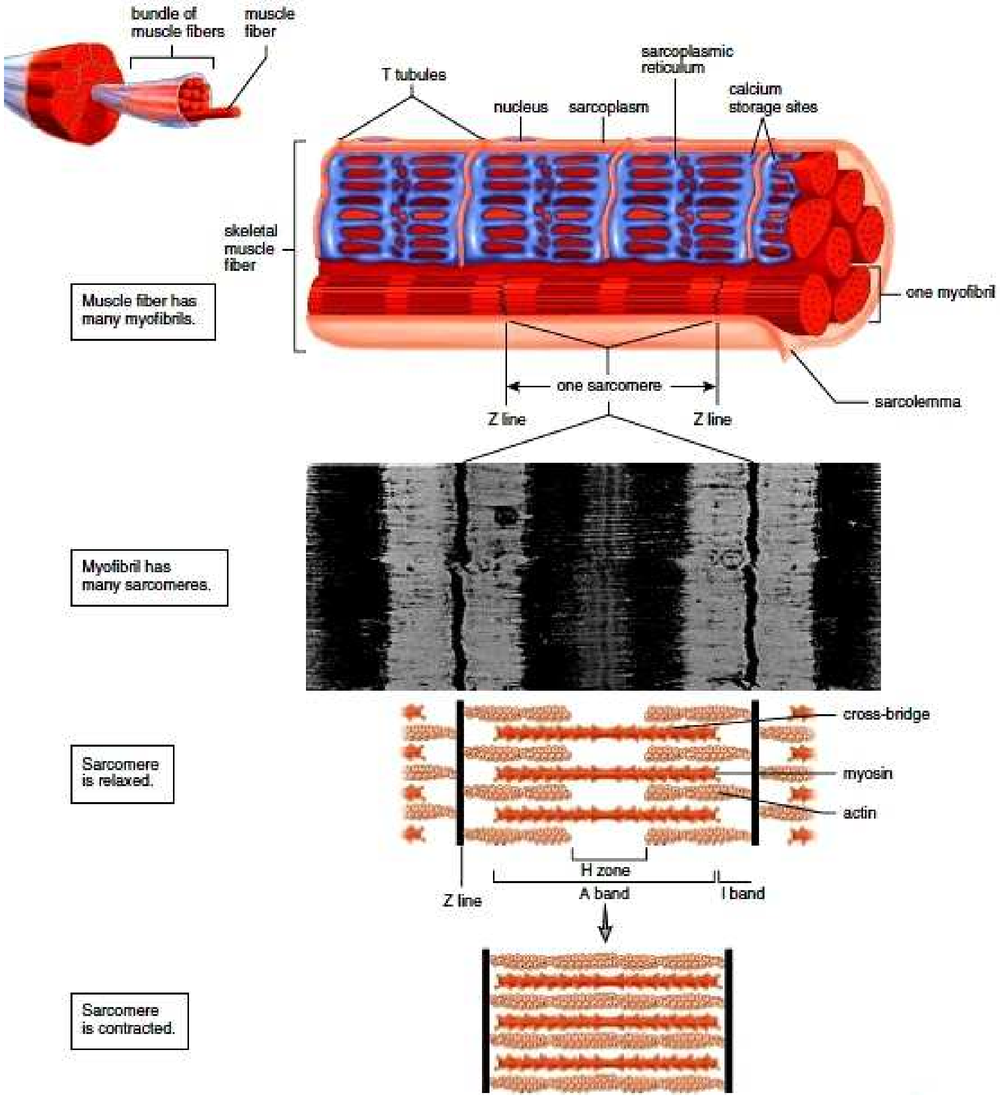
Outline of this Talk

The main question: how do kinetochores work, i.e., how to pull with a depolymerizing microtubule?

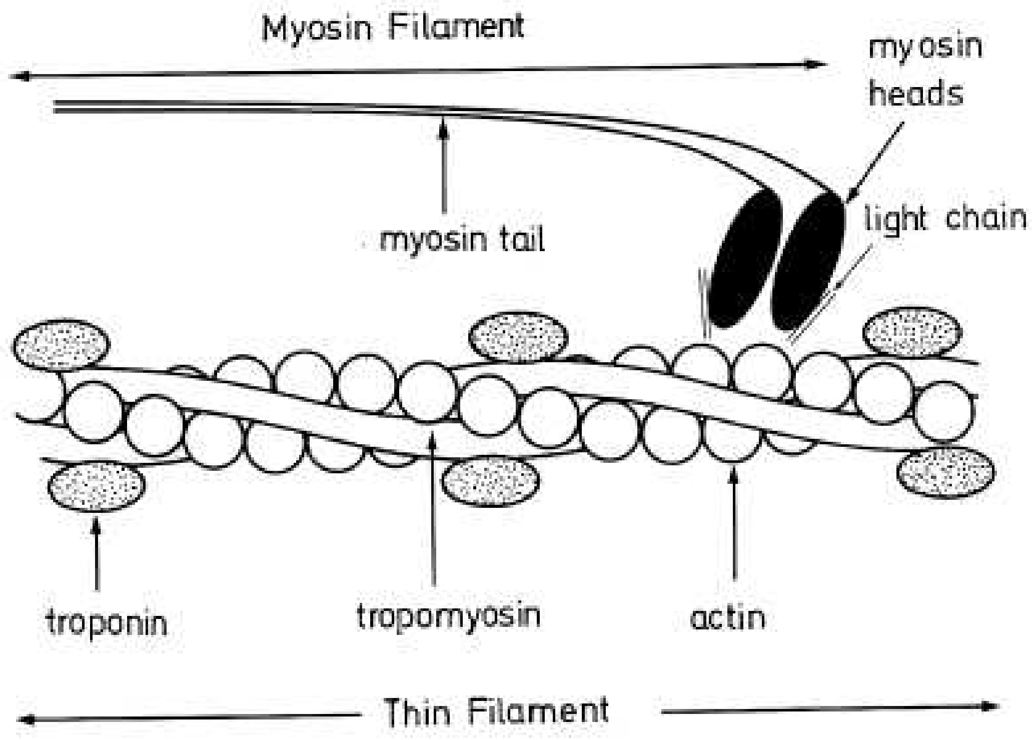
Issues to discuss:

- Models for load-velocity curves
  - Huxley model of actin-myosin crossbridges
  - Binding and unbinding of flexible proteins
    - Bond breaking - Bell's law
    - Bond formation - OU process
  - Sliding platelet in a flow
  - Kinetochore motion

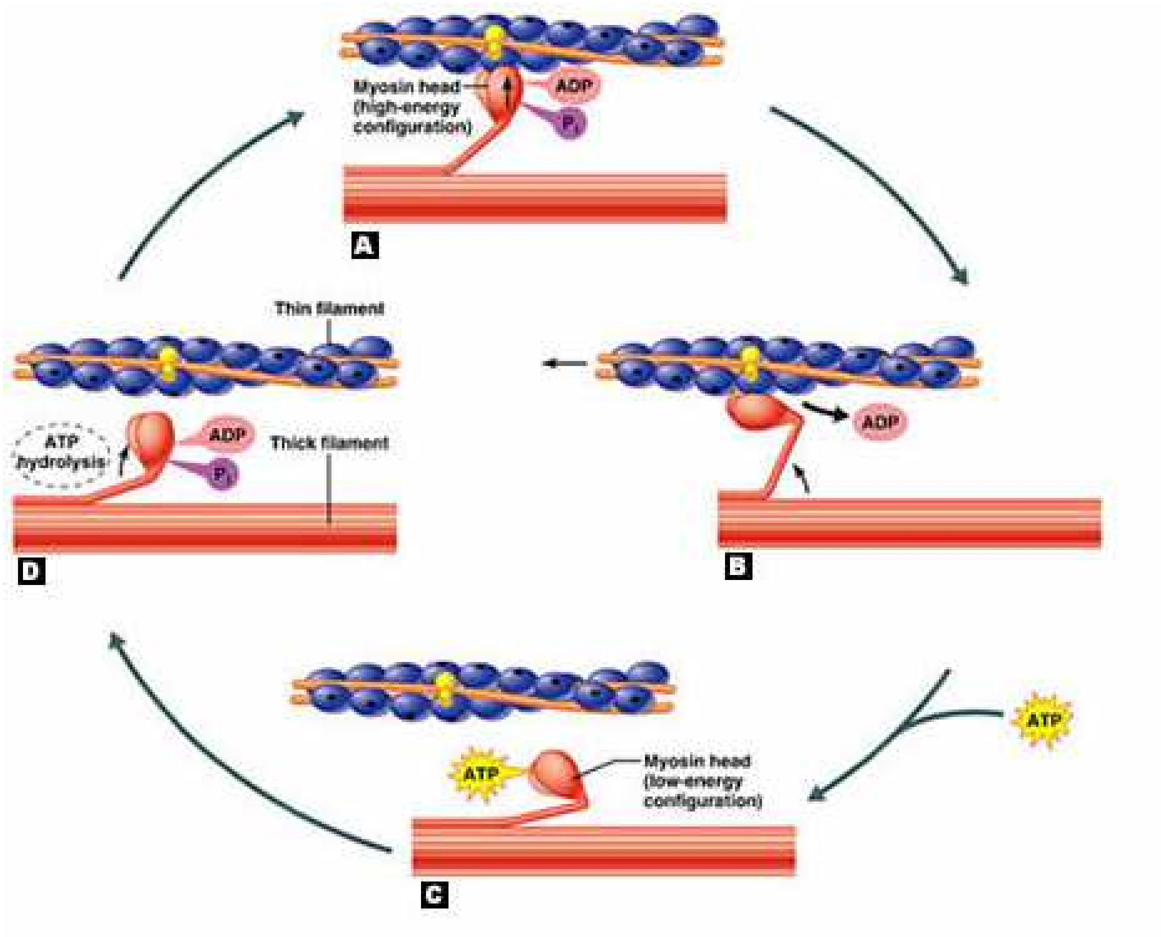
Warmup Exercise: Skeletal Muscle



Crossbridges



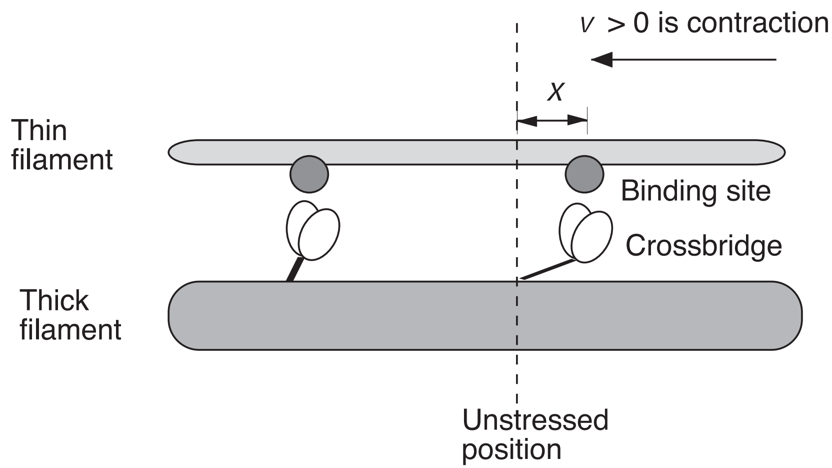
The Power Stroke



Kinesin Animation

The Huxley Model

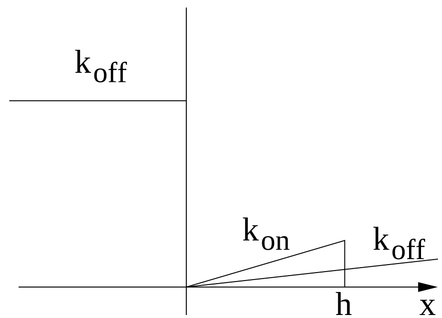
Suppose a crossbridge can be bound (B) or unbound (U)



$$U \underset{k_{off}(x)}{\overset{k_{on}(x)}{\rightleftarrows}} B,$$

Let $n(x,t)$ be the probability density of bound crossbridges with extension x,

$$\frac{\partial n}{\partial t} = v\frac{\partial n}{\partial x} + k_{on}(x)\left(1 - \int_{-\infty}^{\infty} n dx\right) - k_{off}(x)n.$$



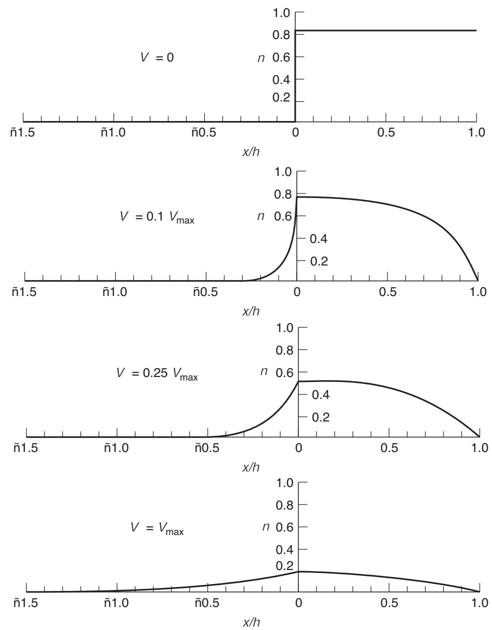
The force generated is $F = \rho \int_{-\infty}^{\infty} kx\, n(x,t)dx$.

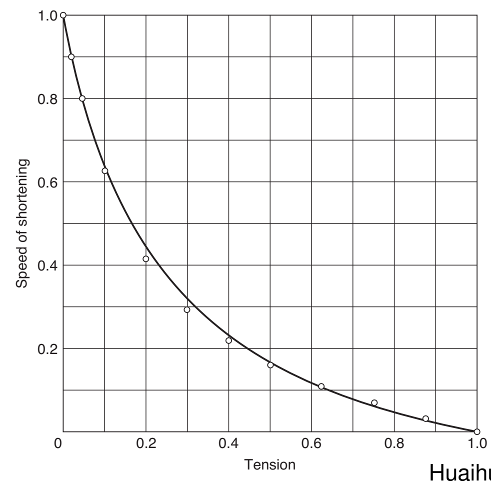
Load-Velocity Curve

To find the load-velocity curve

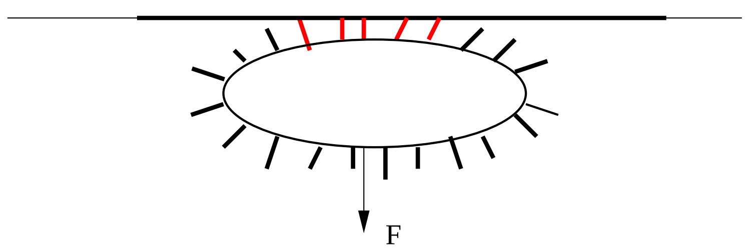
- Pick a velocity v,
- Calculate steady state $n(x; v)$
- Calculate the force F,
- Plot v vs. F.

Remark: Although not biophysically accurate, this is a "typical" load-velocity curve for molecular motors.



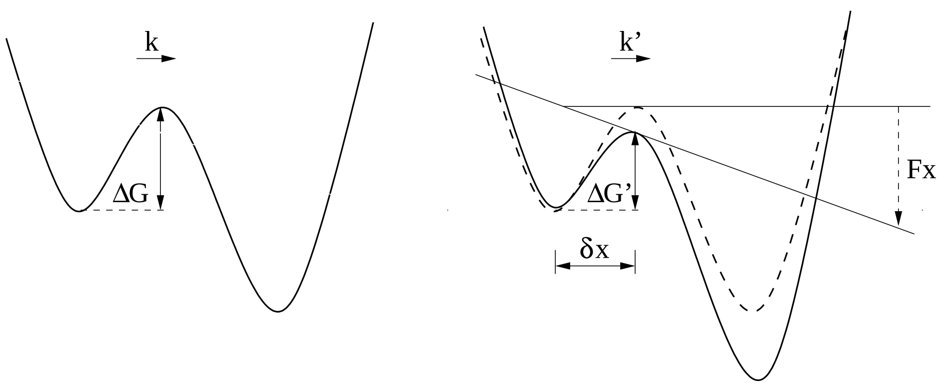


Load Dependent Unbinding



Bonds break in a load dependent fashion

$$U \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} B, \qquad k_{off} = \beta \exp(\frac{F}{F_0}) \text{ (Bell's Law)}.$$



Consequences of Bell's Law

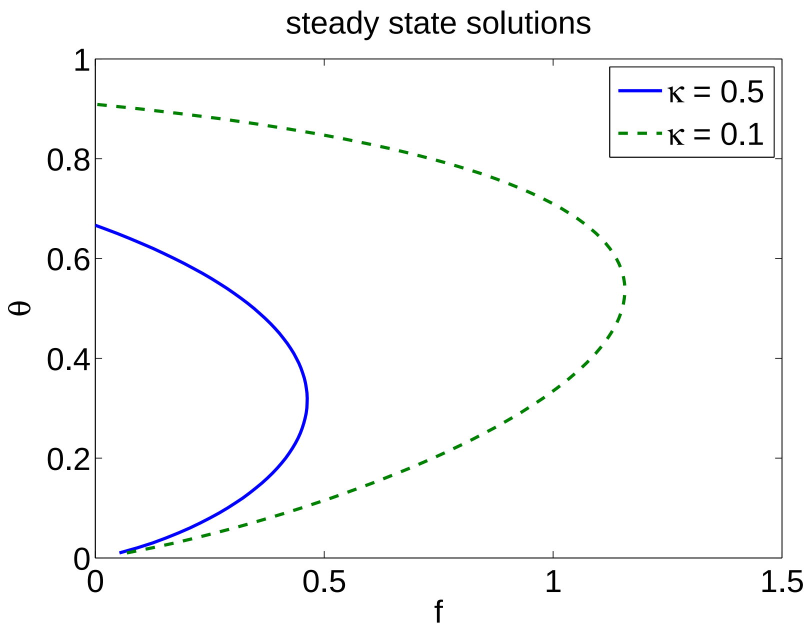
Suppose the load is spread uniformly among all bonds

$$S_n \underset{\beta_n}{\overset{\alpha_n}{\rightleftarrows}} S_{n+1}, \qquad \alpha_n = (N - n)k_{on}, \qquad \beta_n = n\beta \exp\left(\frac{F}{nF_0}\right)$$

leads to the deterministic equation

$$\frac{d\theta}{dt} = (1-\theta) - \kappa\theta\exp\left(\frac{f}{\theta}\right)$$

with $\theta = \frac{n}{N}$, $\kappa = \frac{\beta}{k_{on}}$, $f = \frac{F}{NF_0}$
exhibits critical behavior.

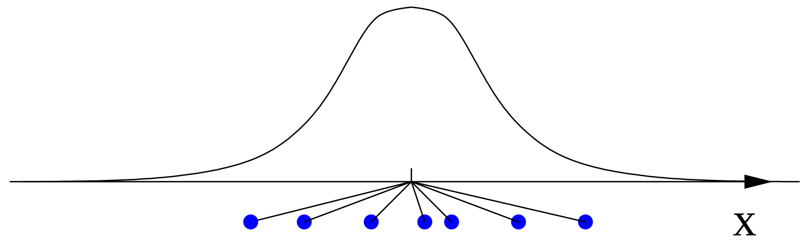


Remark: This is an interesting stochastic exit time problem.

Position Dependent Binding

Unbound flexible proteins undergo an Ornstein-Uhlenbeck process (diffusion with linear restoring force)

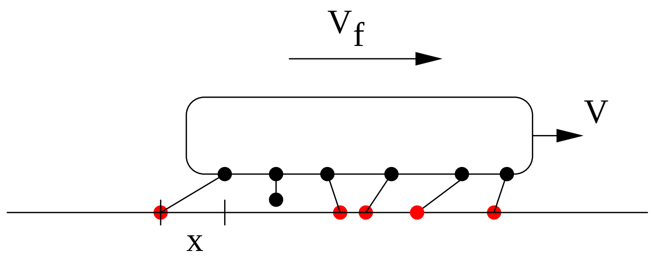
$$\xi dx = -kx dt + dW$$



with spring constant k, have Gaussian equilibrium distribution, so that the binding rate is also Gaussian

$$k_{on} = \kappa \exp\left(\frac{-kx^2}{2k_BT}\right).$$

Sliding in a Flow



Let $n(x,t)$ be the density of bound binders with extension x,

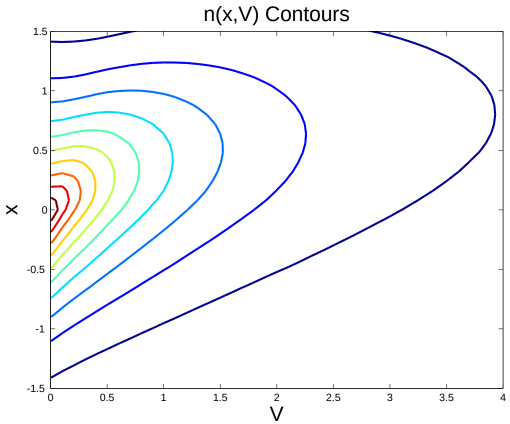
$$\frac{\partial n}{\partial t} = -V\frac{\partial n}{\partial x} + \alpha(x)\Big(N_T - \int_{-\infty}^{\infty} n(x,t)dx\Big) - \beta(x)n,$$

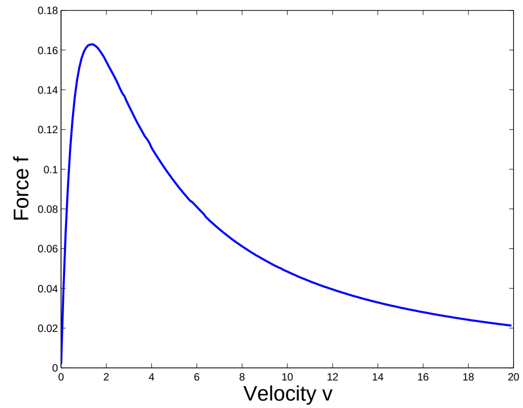
$$\alpha(x) = \kappa\exp\left(\frac{-kx^2}{2k_BT}\right), \qquad \beta(x) = k_{off}\exp\left(\frac{k|x|}{F_0}\right),$$

The force generated is

$$F = k\int_{-\infty}^{\infty} xn(x,t)dx.$$

And the Answer is...

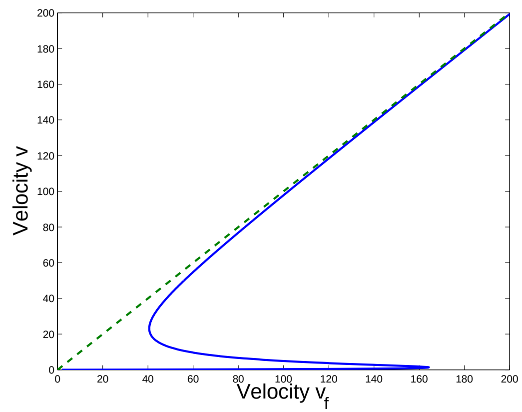




with the motion determined by the force balance equation

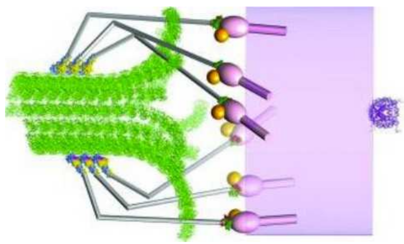
$$\xi(V_f - V) - F = 0.$$

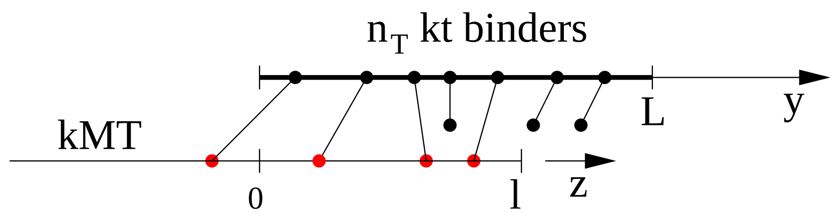
- biphasic Force vs. Velocity curve,
- Velocity vs. V_f has hysteretic behavior (explaining the static-kinetic friction transition).



Kinetochore Motion

How can depolymerizing microtubules pull?





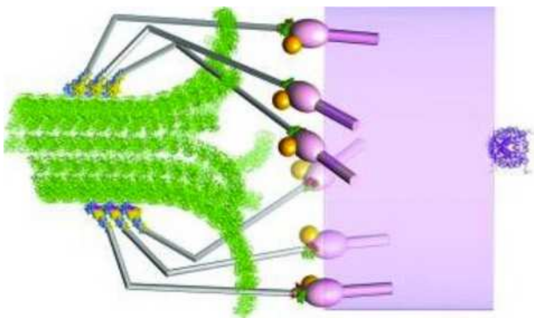
Let $n(z, y, t)$ be the density of bound kinetochore binders with rest position y and binding position z,

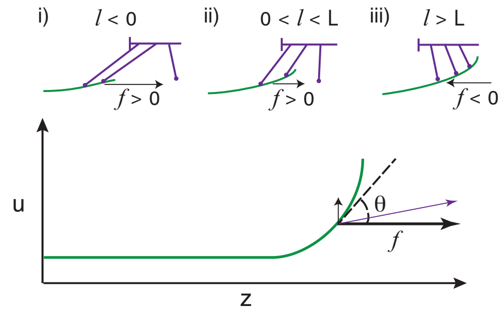
$$\frac{\partial n}{\partial t} = -\frac{\partial}{\partial z}(vn) + k_{on}(z,y)\Big(\frac{n_T}{L} - \int_{-\infty}^{l} n dz\Big) - k_{off}(z,y)n,$$

$$k_{on} = \kappa \exp\left(-\frac{k(y-z)^2}{2k_BT}\right) H(l-z), \qquad k_{off} = \kappa_{off} \exp\left(\frac{k|z-y|}{F_0}\right)$$

Protofilament Shape

What is the shape of the depolymerizing protofilament?





Total energy for a protofilament is

$$E = \int_{-\infty}^{l} \left(\frac{\alpha}{2}(\dot{\theta} - \phi)^2 + \frac{k_{lat}}{2} u^2 + zf \right) ds,$$

$$\frac{du}{ds} = \sin\theta, \qquad \frac{dz}{ds} = \cos\theta.$$

To find the equilibrium shape, set $\delta E = 0$.

Shape Equations

Shape Equations (linearized):

$$\frac{d^2 w}{dz^2} = k_{lat}\theta, \qquad \alpha \frac{d^2\theta}{dz^2} = -w + \rho\theta, \qquad \frac{d\rho}{dz} = f,$$

$$f(z) = \frac{k}{n_p}\int_0^L (y-z)n(z,y)dy$$

with boundary conditions

$$\dot{\theta} = \phi, \qquad w = 0, \qquad \rho = 0, \text{ at } z = l$$

$$\theta = 0, \qquad u = 0, \text{ at } z = -\infty$$

Remark: With $f = 0$ these reduce to the classical beam equation $\frac{d^4\theta}{dz^4} - \frac{k_{lat}}{\alpha}\theta = 0$.

Imagine the Possibilities
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Depolymerization Rate

Curled or loaded filaments break more easily:

$$k_{break}(z) = \beta \exp(\kappa_b \dot{\theta} - \frac{\rho}{\rho_0})$$

because of curvature and load - (Bell's law).
Consequently, the depolymerization velocity is

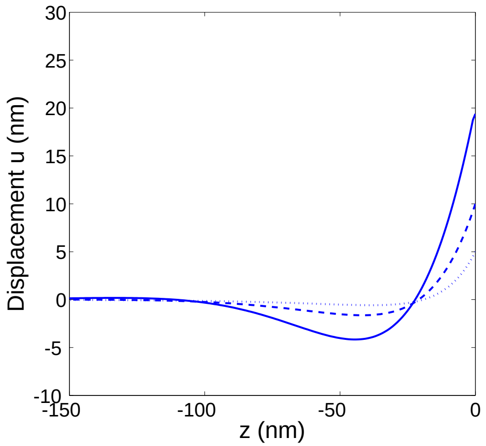
$$v_d = \int_{-\infty}^{l} k_{break}(z) dz,$$

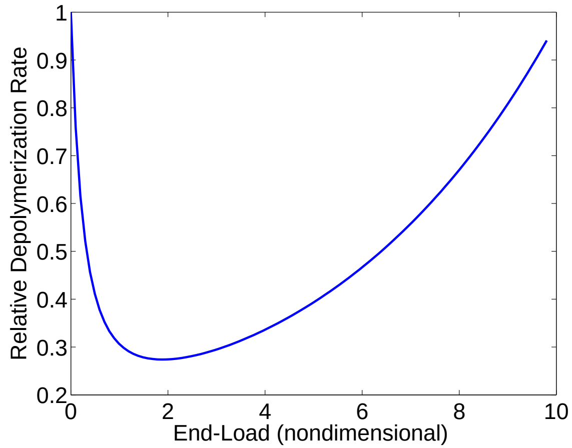
and total force generated by the bonds is

$$F = k \int_0^L \int_{-\infty}^{l} (y - z) n(z, y, t) dz dy.$$

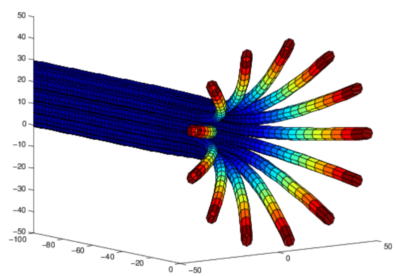
Depolymerization Rate

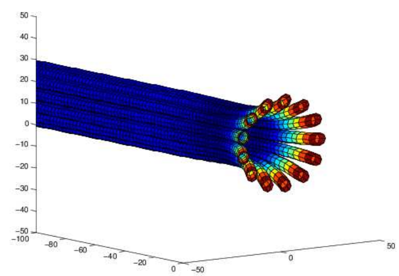
For an end-loaded protofilament:





Typical of "catch-bonds"

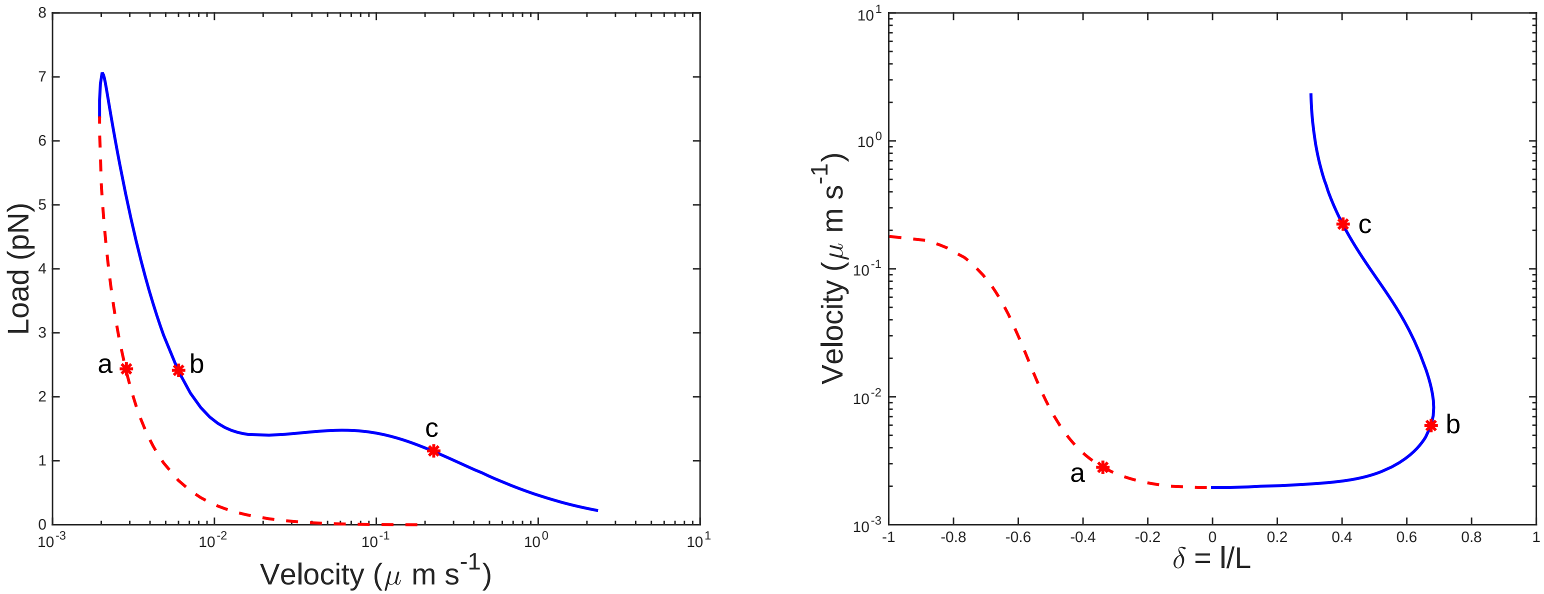




Finding the solution

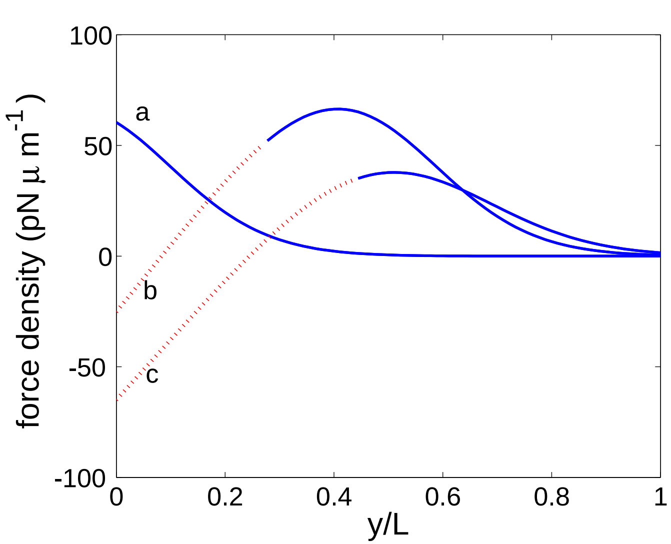
- Pick a value of v and l;
- Find $n(z,y;v)$: $0 = -\frac{\partial}{\partial z}(vn) + k_{on}(z,y)\left(\frac{n_T}{L} - \int_{-\infty}^{l} n dz\right) - k_{off}(z,y)n$
- Calculate $f(z)$: $f(z) = \frac{k}{n_p}\int_0^L (y-z)n(z,y)dy$
- Find protofilament shape,
- Find v_d: $v_d = \int_{-\infty}^{l} k_{break}(z)dz$
- Adjust l until $v_d = v$ (a fixed point problem)
- Calculate the total force: $F = k\int_0^L \int_{-\infty}^{l} (y-z)n(z,y,t)dzdy.$
- Make lots of plots.

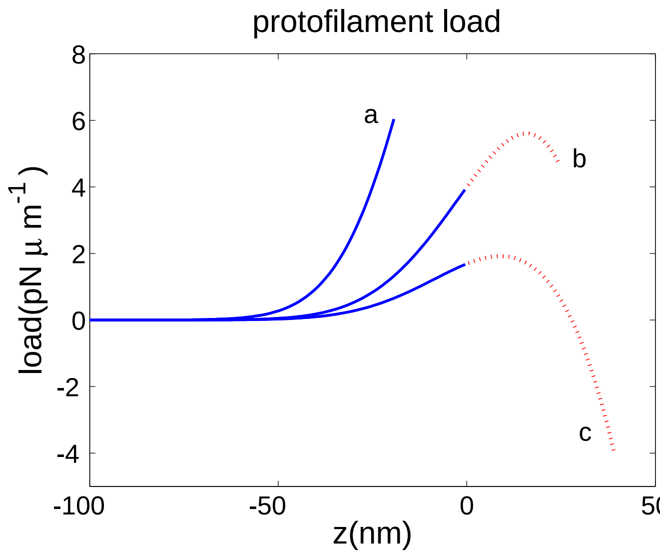
And the Answer is . . .

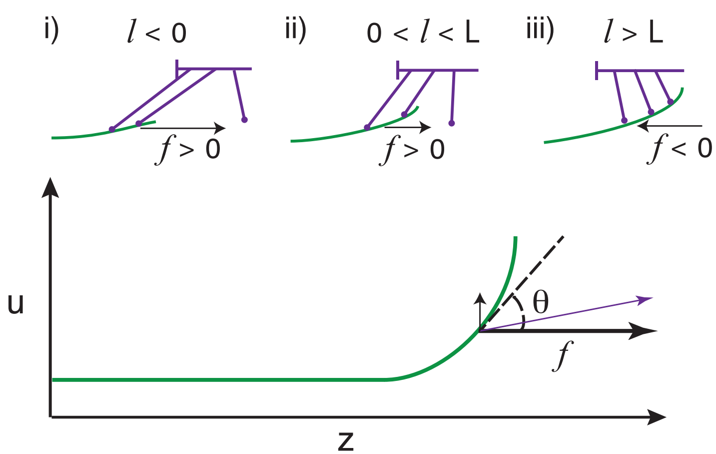


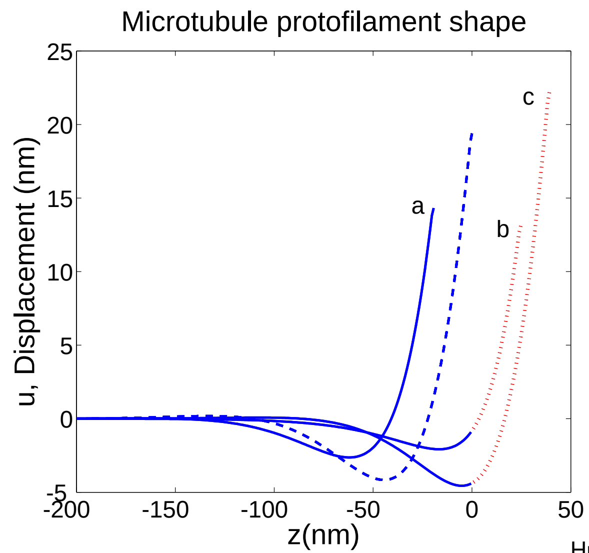
Remark: There is no stall force. There is "disconnect" at high loads.

How Does that Work?



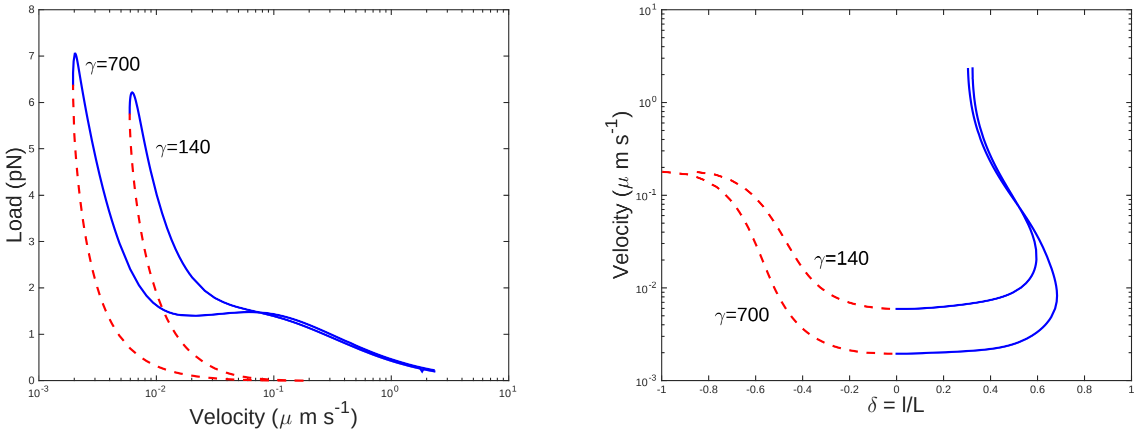




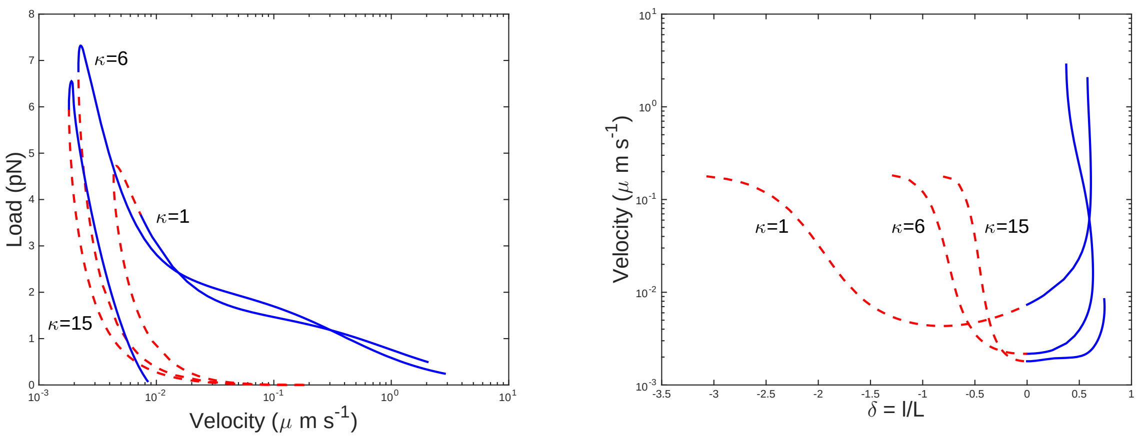


Effects of Parameters?

$\gamma = \frac{L^2 k}{\alpha}$ (protein stiffness/protofilament bending stiffness):

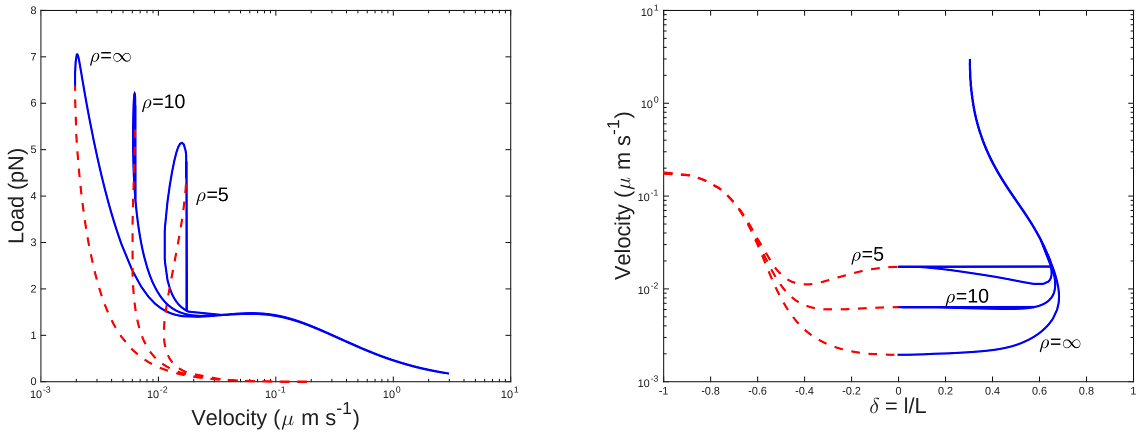


k (Ndc80 spring constant): There is an "optimal" k.



Effects of Parameters?

ρ_0 (Bell's law coefficient for protofilaments):



Remark: Notice the "flat" load-velocity profile.

Conclusion

- Protein flexibility plays an important role in their binding and unbinding, etc.,
- and may lead to critical behaviors (i.e., thresholds, bifurcations, etc.).
- Biased diffusion of Ndc80 proteins enables depolymerizing microtubules to pull chromosomes,
- answering the question of how to pull with a "burning rope."

Thanks!

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- Blerta Shtylla, Pomona College
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National Science Foundation
WHERE DISCOVERIES BEGIN