- Molecular Biology of the Cell Alberts et al.
- Cell Movements D. Bray
- Mechanics of motor proteins and the cytoskeleton J. Howard
- Physics of biomolecules and Cells Les Houches H. Flyvbjerg, F. Jülicher, P. Ormos, F. David

Outline

I. Force Production by polymerization
   - single filament
   - Listeria motion

II Molecular motors
   - single motor
   - motors acting collectively

III Active polar gels
   - Hydrodynamic theory
   - Application to cell motility

I Actin and Microtubule

1. Structure of Actin and microtubule

a. Actin protein = globular protein mass = 45 kDa
size = 5.5 nm = d

Actin monomer contain a ATP pocket ATP can hydrolyze to give ADP
Mg^{2+} ion

- Actin filament 2 protofilaments parallel and oriented : shifted by 1/2 actin monomer
  Right handed helix 72 nm pitch 24 monomer / turn
  Each added monomer increase the size by 2.77 nm
  Diameter 6 nm

- F Actin and G Actin

- Protein and drugs interacting with actin

- Actin filaments are polar : the monomer is polar + ▷▷▷▷ -

  \+ ——————— -
  Barbed end pointed end

- Actin persistence length 17 μm

b. Microtubule

- Tubulin dimer : association between α and β monomers

Both monomers are polar and have a GTP pocket α/β dimer is very stable and the trapped GTP does not hydrolyze Dimer size = period = 8 nm mass 50 kDa

- 13 parallel protofilaments shifted by 0.92 nm after 13 protofilament shift by 13 × 0.92 = 12 nm = 3 monomers Perfectly periodic structure if we ignore the difference between α and β tubulin. One can see the structure as 3 rotating helices

- In nature there are microtubule between 8 and 19 microfilaments, but to accomodate the structure filaments must rotate into a helix. Pitch 4,5 μm for 12 filaments
  - 5.8 μm for 14 filaments
  - 3.3 μm for 15 filament

- Proteins and drugs interacting with microtubule MAP
  Taxol

- Microtubule persistance length 5 mm

Microtubule in a cell : mitotic spindle
axonemes → cilia + flagellae
used for internal transport.

2. Polymerization and treadmilling

- Actin treadmilling

$\underset{n}{\text{OOOOOOOO}}$ $\frac{dn}{dt} = k_{on} C - k_{off}$ C being the monomer concentration. $K_c = \frac{k_{off}}{k_{on}}$ is the equilibrium concentration critical concentration constant

ad (+ -)

$k_{on}^{+} = 11,6\ s^{-1}\ \mu M^{-1}$ $\quad K_c = 0,12\ \mu M$ in vitro

$k_{off}^{+} = 1,4\ s^{-1}$

In the cytoplasm $c = 30\ \mu M \gg K_c$ tendancy to polymerization

But the rapid ATP hydrolysis after polymerization

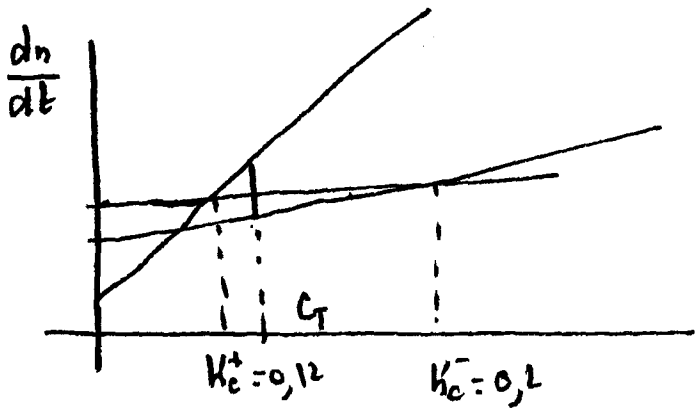
DDDDTTTTT

The ATP for polymerization because the ATP concentration is much higher

The ADP for depolymerization at - end K_c is different at both extremities

At - end k_{on}^{-} (ATP) $= 1,3\ \mu M^{-1} s^{-1}$

k_{off}^{-} (ADP) $= 0,24\ s^{-1}$ $\quad K_c^{-} = 0,2\ \mu M$



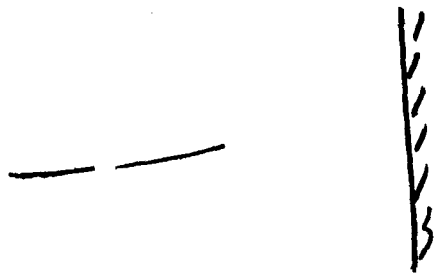
Dynamic equilibrium if polymerization at + end compensates depolymerization at

Treadmilling is observed in vivo but at a much higher concentration because of protein interacting with actin.

Rq : Actin polymerization velocity $v = k_{on} \cdot c \cdot \delta = 11,6 \times 30 \times 2,7 \times 10^{-3} = 1\ \mu$

Janson, Dogterom PRL 92 248101 (2004)

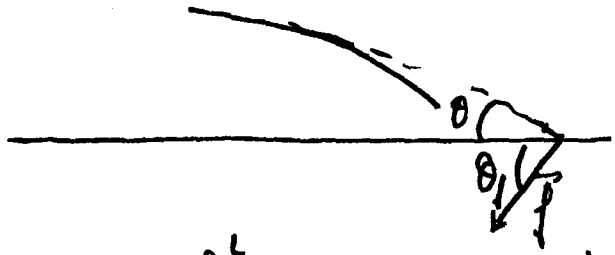
Dogterom Yurke Science 278 856 (1997)



- Microtubule seed → polymerization

  Before the wall free polymerization

  Against the wall → buckling → force velocity relation



Energie $F = \int_0^L ds \left[\frac{1}{2} k_B T l_p \left(\frac{d\theta}{ds} \right)^2 \right] + \int \vec{f} \cdot d\vec{r}$

$\frac{d\vec{r}}{ds}$ is the tangent vector $\vec{t}$ $F = \int_0^L ds \frac{k_B T l_p}{2} \left[\left(\frac{d\theta}{ds} \right)^2 + 2 q^2 (\cos\theta - \theta_1) \right]$

where $q^2 = \frac{f}{k_B T l_p}$

Minimization gives $\frac{d^2\theta}{ds^2} + q^2 \sin(\theta - \theta_1) = 0$

or $\frac{1}{2} \left(\frac{d\theta}{ds} \right)^2 = q^2 \cos(\theta - \theta_1) + \text{cst}$

Integration gives $s = \int^{\theta(s)} d\theta$ ———

$f_s = f(\cos(\theta_b \cdot \theta_f))$. One then measures the force velocity relation $f_s(v_T)$

- Microtubule kinetics $v_T = k_{on}(f_s)\, c - k_{off}(f_s)$

- Kramers rate theory $k_{on}(f_s) = k_{on}(0) \exp - f_s \delta_{on}/kT$

$k_{off}(f_s) = k_{off}(0) \exp - f_s \delta_{off}/kT$

The best fits are obtained with $\delta_{off} = 0$. Only one adjustable parameter δ_{on} (of the order of nanometers)

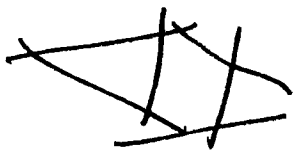
The polymerization at velocity v_T has thus created a force $f_s = \frac{kT}{\delta_{off}} \text{Log} \frac{k_{on}(0)}{k_{on}(f}$

- Stall force $f_s = \frac{kT}{\delta_{on}} \text{Log} \frac{k_{on}(0)}{\omega_+ + k_{off}}$

III Listeria Propulsion

1. Actin gel

In the presence of certain proteins Actin forms a crosslinked gel: Arp 2/3



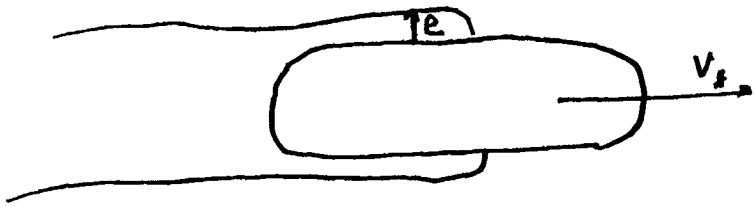
This gel has a modulus of order 10^4 Pa = E

2. Listeria

Images - Comet growth from the surface of the bacteria

- Soap like motion due to elastic deformation

1. Rest Phase of Bromobacteria and cells



- Growth from the surface : rubber band model (1) require stretching

$$\sigma_{\theta\theta} = E\frac{e}{R} \qquad \sigma_{rr} = \frac{2\gamma}{R} = l\int_0^e \sigma_{\theta\theta}\, dz \sim E\left(\frac{e}{R}\right)^2$$

Thin shell approximation

- Elastic energy $E_{el} \sim \frac{1}{2}\frac{\sigma_{\theta\theta}^2}{E} \cdot LeR \sim E\frac{e^3}{R}L$

Elastic force $F_{el} = E\frac{e^3}{R}$

- Viscous force
  - Hydrodynamic friction negligible
  - Attachment - detachment of proteins at the surface of the bacteria

$F_f \sim \Xi RLv$ $\quad W = p\frac{1}{2}\langle a^2\rangle \omega_{eff}\, p$

$\langle a^2\rangle \sim \frac{v}{\omega_{eff}}\, p = \frac{\omega_{off}}{\omega_{off}+\omega}$

Total force a bacteria $\quad F = E\frac{e^3}{R} - \Xi RLv$

Steady state motion

$V = v_p \qquad V = N_0 L \qquad F = E(N_p)^3 L^3 = \Xi RLv$

Biochemistry problem

4 - Stress distribution : soft bacteria

- At the back $v_p(0) = v$ advancing velocity
- On the side $v_p(0) \sim \frac{e}{L} v \ll v$

The polymerization velocity depends on local stress $v_p = v_p^0 \exp \frac{f\delta}{kT}$ ← force a filament

$f = \sigma a^2$

$$v_p = v_p^0 \exp \frac{\sigma a^2 \delta}{kT}$$

At the back $\sigma > 0$ the gel pulls the bacteria backwards

at the front $\sigma > 0$ the gel pushes the bacteria

Direct visualization : soft bacteria ⇒ oil drop $v = 0,15$ μm/min

Elastic stress balanced by local Laplace pressure

Equation of motion

radial $\frac{2\gamma_0}{R} = \gamma H - \delta P - \sigma_{nn}$ $H = \frac{\cos\theta}{h} + \frac{d\cos\theta}{dh}$

$$v \sin\theta = v_p = v_p^0 \exp \frac{\sigma_{nn}}{\sigma_0}$$

Fit $v_p^0 = 1,4$ nm/s

$\sigma_0 \sim 1$ kPa

Molecular motors

I Structure and function of molecular motors

1. Function of motor proteins

- Muscle contraction due to motion of myosin II fibers walking a actin (Huxley)
- Glia and flagella are usually powered by dynein motors that force sliding of microtubules against one another
- Cellular transport 

kinesin motor → ballistic motion faster than random diffusion

- Mitosis 
- Internal ear: ~~stereocilia~~ bundled ciliated cells → transport channel opening

- All these motors consume energy in the form of ATP hydrolized to ADP.
- They all walk a polar filament: a motor always walk in the same direction { + end motors / - end motors

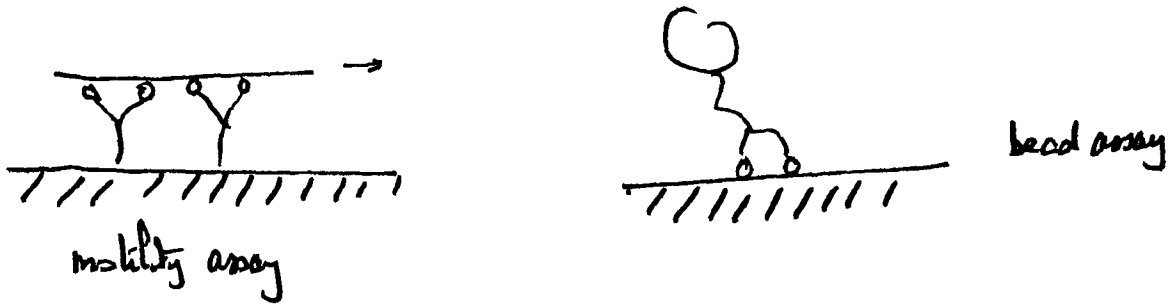
2. Structure of motor proteins

- Motors walking on actin: Myosins → myosin II Velo → + end
  myosin V + step 36 nm
  myosin VI move toward - end
- Motors walking on microtubule kinesin Velo step of 8 nm → + end

$F_0 F_1$ ATPase

- Motors walking on DNA RNA polymerase : beautiful recent work S. Block.

3. Bead assay - Motility assays



4 - Processivity

A molecular motor is processive if it remains bound to the filament during several steps. Kinesin a microtubule are processive. This allows transport by individual kinesin (and bead test)

Myosin II are non processive. One needs several myosins attached by their tail to move a filament.

The duty ratio is defined as $R = \frac{t_{on}}{t_{on} + t_{off}}$ t_{on} = attached time, t_{off} = detached time

Kinesin are processive $R > 0,5$ to have only one attached head (out of 2)

For myosin $R \approx 0,01 - 0,02$ $N = \frac{1}{R}$ myosins are required acting together in a muscle $N \approx 50$

5. Thermodynamics of molecular motors

Coupled driving reaction $\Delta\mu = \Delta\mu_{ATP} - \Delta\mu_{ADP} - \Delta\mu_{Pi}$

Mechanical f

2 fluxes v, r Onsager relations

$$v = \lambda_{11} f + \lambda_{12} \Delta\mu \qquad \text{Dissipated energy } W = r\Delta\mu + fv$$
$$r = \lambda_{12} f + \lambda_{22} \Delta\mu$$

$W > 0 \quad \lambda_{11} > 0 \quad \lambda_{22} > 0 \quad \lambda_{11}\lambda_{22} - \lambda_{12}^2 > 0$ Crossed terms are equal $\lambda_{12} = \lambda_{21}$

The protein is a motor if $r\Delta\mu > 0$ (it consumes energy) and $fv < 0$ it produces mechanical work.

The yield is $\eta = \dfrac{-fv}{r\Delta\mu}$ The motor is isothermal, nothing to do with Carnot cycle. $0 < \eta < 1$

The stall force is the force that stops the motor $v = 0$ $f = -\dfrac{\lambda_{12}}{\lambda_{11}}\Delta\mu$

it is of the order of a few picoNewton 4 - 5 pN for kinesin.

II Modeling molecular motors

1 - Multistate systems and transitions between states

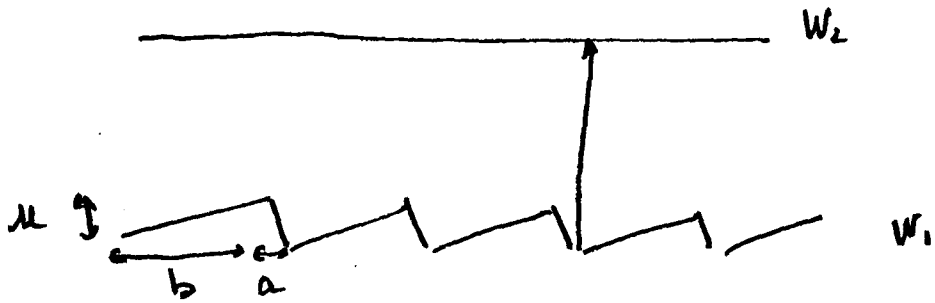
Identify several states in the system

Write Chemical kinetics relations between these states → rate

2. Two state model

a - Qualitative description.

The motor has two states a weakly bound state and a strongly bound state. In the weak state, the motor sees the period of the filament and sees a potential W_1 and W_2



ATP consumption induces transition between the states. It is important that the potential W_1 is asymmetric : the system is not a motor if it is symmetric

By Many chemists try to build artificial motors on this idea : Stoddard Sauvage Mallik ...

ω_1 transition rate from $1 \to 2$ for simplicity ω_1 very large and localized at minimum

ω_2 desexcitation from $2 \to 1$ uniform

After excitation at the minimum, the motor diffuse over a length $\sqrt{\frac{2D}{\omega_2}}$

If $\frac{2D}{\omega_2} < a^2$ the motor goes back to its original position.

If $\frac{2D}{\omega_2} \sim a^2$ probability $p \sim \frac{1}{2}$ to come back to its original position

$p = \frac{1}{2}$ to move to the left

If $2D \sim a^2$ ..... the critical value is $2D \sim a^2$

$\Sigma N_p = \frac{M}{b} \qquad t_1 = \frac{\Sigma b^2}{M}$

The velocity is $V = \frac{a+b}{t_1 + 1/\omega_1} p = p \frac{a+b}{1/\omega_1 + \frac{\Sigma b^2}{M}}$ p being the probability that the motor moves to the left

An external force f biases the potential and $p \neq 0$ if $f \geq \frac{kT}{a}$

opposes the motion in the sliding down $v_p = \frac{1}{\Sigma}\left(\frac{M}{b} - f\right)$

The smallest of $\frac{kT}{a}$ and $\frac{M}{b}$ is the stall force

Rq - Backwards steps are allowed with a probability $\sim \exp - \frac{b^2}{4 D \omega_2^{-1}} \sim \exp - \frac{b^2}{a^2}$ very small if the potential is really asymetric $b \gg a$. They are sometimes observed with kinesin (a motor) see Coppellotal. - Noise (diffusion is needed for a motor)

b. Fokker Planck Equations

P_1 is the probability to be in state 1

P_2 2

The probability currents are $J_1 = -D\left\{\frac{\partial P_1}{\partial x} + \frac{P_1}{kT}\frac{\partial W_1}{\partial x} - P_1 \frac{f}{kT}\right\}$

$J_2 = -D\left\{\frac{\partial P_2}{\partial x} + \frac{P_2}{kT}\frac{\partial W_2}{\partial x} - P_2 \frac{f}{kT}\right\}$

The Fokker Planck equation for the conservation of [illegible] are

$\frac{\partial}{\partial t} + \frac{\partial}{\partial x} = - \omega_1(x) + \omega_2(x)$

Probability is conserved

$$\frac{\partial P_1}{\partial t} + \frac{\partial J_1}{\partial x} = \omega_1 P_1 - \omega_2 P_2$$

The average velocity over a period is $v = \left[\int_0^l (P_1 + P_2)\, dx\right]^{-1} \int_0^l (J_1 + J_2)\, dx$

Rq Thermal equilibrium : $P_1(x) = \rho_1 e^{-W_1/kT}$ + Detailed balance $\omega_1 P_1 = \omega_2$

$P_2(x) = \rho_2 e^{-W_2/kT}$

$\omega_1 = \omega(x) \exp \frac{W_1}{kT}$ $\omega_2 = \omega(x) \exp W_2/kT$

$J_1 = J_2 = 0$ $v = 0$ no motion.

Long time behavior : At long times $P_i(x) = \frac{1}{\sqrt{4\pi D_{eff} t}} \exp - \frac{(x - vt)^2}{4 D_{eff} t}$ $g_i \frac{x}{e}$

g_i being a periodic function of x. If we normalize $\int_0^e (g_1 + g_2) dx = 1$

$$\Sigma v = \int_0^l \left\{ g_1 \left(-\frac{\partial W_1}{\partial x}\right) + g_2 \left(-\frac{\partial W_2}{\partial x}\right) \right\} dx$$ average force balance

The effective diffusion constant D_{eff} (including thermal and stochastic noise) can also be calculated

c. General remarks

- Motor must be out of equilibrium
- The potential must be asymmetric otherwise g_i symmetric $\frac{\partial W}{\partial x}$ asymmetric

Other potential

{ no noise needed
{ good for 2 heads linear

III Collective behavior of processive motors : Pulling tubes from vesicle

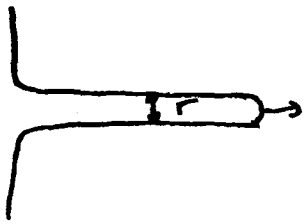
1- Membrane and Vesicle Physics :

as a paper sheet

Helfrich Hamiltonian $\mathcal{H} = \int ds \left\{ \frac{1}{2} \kappa H^2 + \sigma \right\}$

ignore gaussian curvature κ = bending modulus = energy to ~ bokT

H = total curvature σ = vesicle tension very low ~ 10^{-5} N/m



tube of length L radius R pulled by a force f

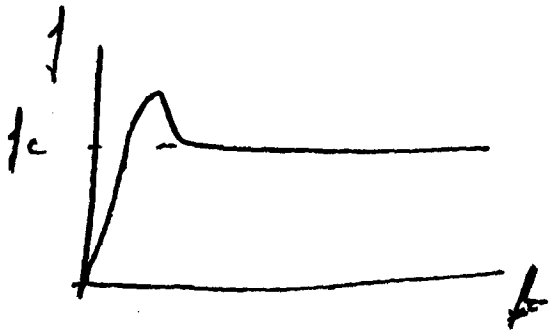
$$F = \frac{1}{2} \frac{\kappa}{R^2} 2\pi R L + \sigma 2\pi R L - f L$$

radius $\frac{\partial F}{\partial r} = 0$ $\quad 2\pi\sigma = \frac{\kappa}{r^2}\pi$ $\quad r = \sqrt{\frac{\kappa}{2\sigma}}$

$$F = L \left\{ f_c - f \right\} \qquad f_c = 2\pi \sqrt{\frac{\kappa}{2\sigma}} \cdot 2\sigma = 2\pi \sqrt{2\kappa\sigma} = 2\pi \frac{\kappa}{r}$$

Si $f < f_c$ ~~pas de formation de~~ no tube formation

Tube de taille finie $f < f_c$



I. Derenyi et al.

In order to pull a tube motors must exert a force f_c depending only on the tension σ (controlled by osmotic pressure) and the bending energy κ known

$r \sim 40 - 100$ nm

$f_c \simeq 10$ pN

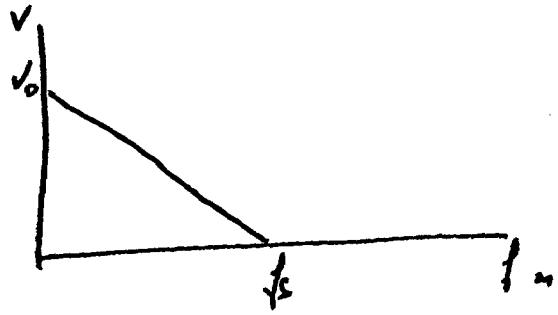
2. Membrane tube pulled by molecular motors

- Experiment

- Idea cluster of motors at the end of the tube with n_b motors exert a force f_c and pulls the tube. Each motor exerts a force $f_m = \frac{f_c}{n_b}$

- Motor constitutive equation

$$v = v_0 \left(1 - \frac{f_m}{f_s}\right)$$



The tube advances at velocity $v < v_0$ velocity of free motor $v_0 = 0.6$ μm/s

Free motor advance at velocity v_0 and pile up at the tip to make clusters

ρ_b density of bound motor k_u = unbinding rate of bound motor = 0,42 s⁻

ρ_u density of unbound motor k_b binding rate of unbound motor

ρ_{ad} = density a vesicle at equilibrium $k_u \rho_b = k_b \rho_u$

$2\pi r \rho_{ad} = \rho_u + \rho_b$ Flux conservation

Close to the vesicle $\rho_u = \frac{k_u}{k_u + k_b} 2\pi r \rho_{ad}$ $\rho_b^\circ = \frac{k_b}{k_u + k_b} 2\pi r \rho_{ad}$

Motor conservation equation in the tube reference frame

$\frac{\partial \rho_b}{\partial t} + \frac{\partial}{\partial z} j_b = - k_u^\circ \rho_b + k_b \rho_u$ $j_b = \rho_b (v_0 - v)$ ballistic

$\frac{\partial \rho_u}{\partial t} + \frac{\partial}{\partial z} j_u = k_u \rho_b - k_b \rho_u$ $j_u = -D \frac{\partial \rho_u}{\partial z}$

Rq No force exerted on motor $k_u = k_u^\circ$

Equation at the tip $\frac{\partial n_b}{\partial t} = j_b - k_u(n_b) n_b$

$j_u + k_u(n_b) n_b = 0$

For the motor at the tip they exert a force $f_m = \frac{f_c}{n_b}$ and k_u varies with f_m $k_u = k_u^\circ \exp + \frac{f_m a}{kT}$ (Kramers rate theory)

b. Threshold

For short tubes $\rho_u \simeq \rho_u^\circ$ is constant $1. \simeq D \cdot (V_0 - V) \sim \rho_u^\circ N_0$

$k_b^- n_u n_b$ $\quad k_b^0 \sim n_u n_b \exp \frac{1}{n_b k_L}$

Motor constitutive equation $N = N_b \left(1 - \frac{f_c}{n_b f_s}\right) \quad V - V_0 = - \frac{N_b f_c}{n_b f_s}$

The velocity is given by $\rho_b^* = \frac{k_u^0 f_s}{N_0 f_c} n_b^2 \exp \frac{f_c a}{n_b k T}$

Bifurcation $\rho_b^0 < \rho_b^c$ no cluster at the tip $\Rightarrow$ no tube

$$\rho_b^c \simeq \frac{k_u^0 f_c a^2 f_s}{(kT)^2 N_0}$$

Critical value of ρ_s for tube formation $\rho_s^c \sim \frac{k_u^0 + k_b}{k_b} k_u^0 \left(\frac{a}{kT}\right)^2 f_s \underbrace{\left(\frac{f_c}{r}\right)}_{\sim \sigma}$

for $\sigma = 2\,10^{-4}$ $\quad \rho_s^c \simeq 200$ motors / μm^2

$\rho_s \sim \sigma$ roughly satisfied

c. Motor distribution along the tube

$x = 0$ at the tip $\quad \rho_b = \rho_b^* + A(t)\, e^{qx}$

At long times $q = \frac{N_0 f_c k_B}{D f_s n_b k_u^0} \quad n_b \sim t^{1/4}$

d - Limitations of the theory

. Mean field low density theory ⟹ simulations

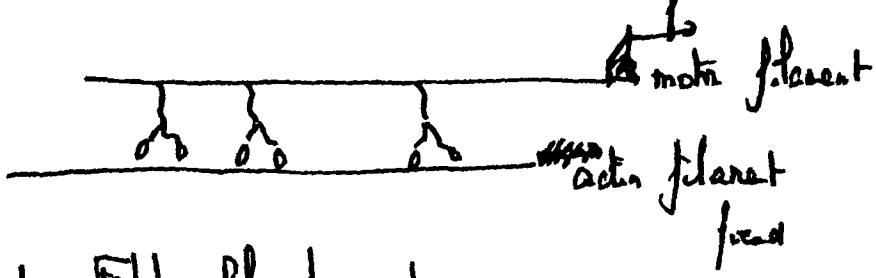
- Motor behavior at the tip are all motors independent

Simulations V. Zeldovich

More detailed study Y. K'afri, D. Campos, JF discrete model

. Oscillations of the tube (σ increases with tube length)

IV Collective behavior of non processive motors Aydan, Lübber Prost (Rev Mod Phys 1997)



see muscle structure

1 - Fokker Planck equation

We will ignore thermal fluctuations. When a motor is in a weakly bound state, it is rapidly attached to the filament and it is carried by the motion of other motors.

We will also assume that the potential in the strongly bound state is

We will assume that there are N motors randomly distributed the density $\rho_1 + \rho_2$ is constant we normalize it to 1 over a period $l = a + b$

$$\rho_1 + \rho_2 = \frac{1}{l}$$

$$\frac{\partial \rho_1}{\partial t} + \frac{\partial J_1}{\partial x} = -\omega_1 \rho_1 + \omega_2 \rho_2 \qquad \omega_2 = \omega t$$

$$\omega_1 = \Omega\, \theta(x) \qquad \theta(0) = 1$$

θ is a localized function

$$\frac{\partial \rho_2}{\partial t} + \frac{\partial J_2}{\partial x} = \omega_1 \rho_1 - \omega_2 \rho_2$$

In a stationnary state the velocity is constant $J_i = \rho_i v$

The force exerted by the filament potential on the motor is

$$f_m = N \int -\frac{\partial W_1}{\partial x}\, \rho_1\, dx \qquad (W_1 = \omega t)$$

The force balance on the thick motor filament reads $f + f_m = 5v$

2. Dynamic phase transition

One can calculate ρ_1 and f in powers of v

$$\rho_1(x) = \sum_n \rho_n(x)\, v^n$$

$$f_m(x) = \sum_n f_m^{(n)}\, v^n$$

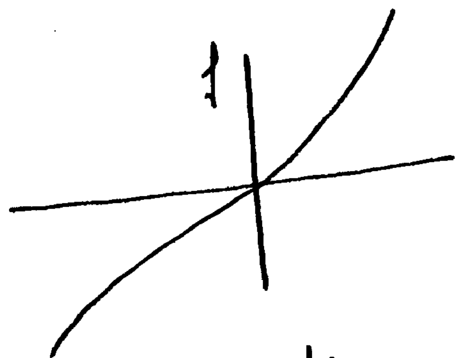
Plugging into the [illegible]

$$f = \Gamma v + u v^3 \qquad u > 0$$

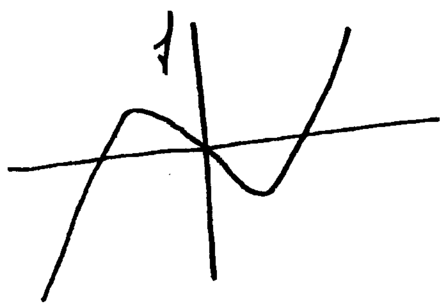
Γ is positive if $\Omega < \Omega_c$
negative if $\Omega > \Omega_c$

Ω_c given by $\dfrac{Nu}{2a^2\omega_2} = \dfrac{\Omega(\Omega+2\omega_2)}{(\Omega+2\omega_2)^2} = \zeta$

$\Omega < \Omega_c$



$\Omega < \Omega_c$



Spontaneous symmetry breaking and dynamic phase transition at $\Omega = \Omega_c$

- Hysteresis
- Oscillations

Add random electric fields: D. Rivelin et al