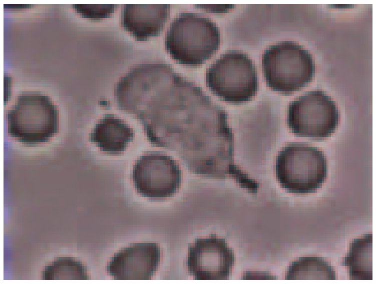
Mechanical Properties of Filament/Motor Mixtures



David Rodgers `50s

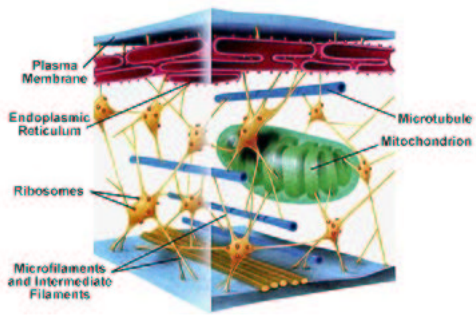
Tanniemola B Liverpool
Blackett Laboratory, Imperial College, London

Plan

1) Cell Movement and Mechanics
   a) The Cell Cytoskeleton
   b) Filaments and Molecular Motors
2) Semiflexible Polymers
3) Rheology of Semiflexible polymers
4) Motile Polymers
   a) Active cross-links
   b) Active forces
5) Conclusions and outlook

Inside the Cell

- The Cell Cytoskeleton



Prentice Hall

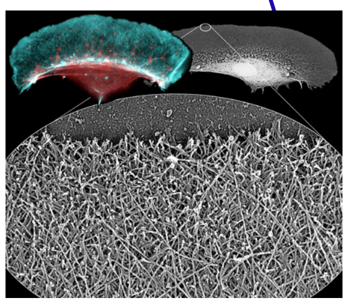
Cell Movement

- Actin cytoskeleton - retrograde flow

QuickTime™ and a
None decompressor
are needed to see this picture.

Cell Movement

- Cell Fragment (Keratocyte)

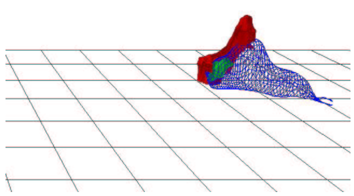


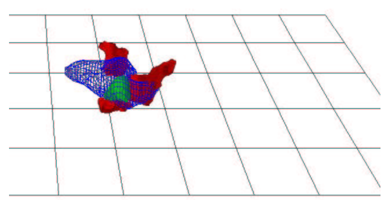
QuickTime™ and a
None decompressor
are needed to see this picture.

Svitkina et al, ‘99

Cell Movement

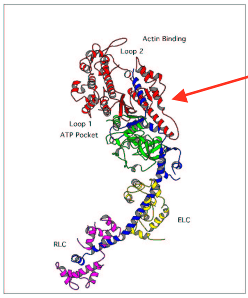
Dictostylium with/without Myosin 1B



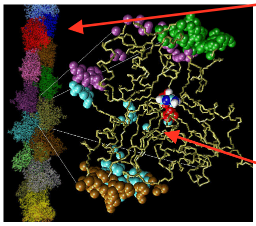


Titus Lab (U Minnesota)

The Players



- Myosin



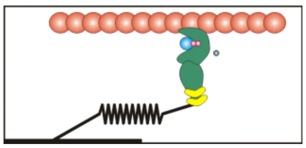
- F-actin (polar)
- G-actin

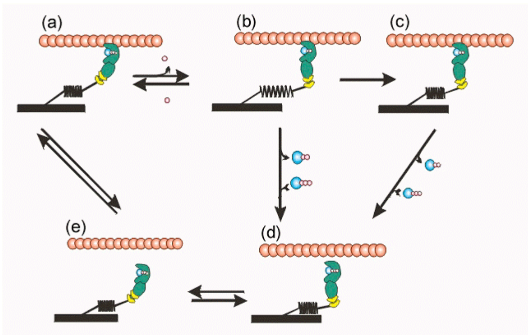
Acto-myosin molecular motor

- F-actin and Myosin use Adenosine TriPhosphate (ATP) to turn chemical energy into mechanical work

Muscle !

Rigor





Far from equilibrium

In vitro experiments

- Mechanical experiments on purified cytoskeletal filamentous proteins (F-actin) - early `90s
  Janmey, Sackmann, Schmidt ….
- Actin Filaments + different types of Myosin + ATP- last couple of years
  Sackmann, Käs, Amblard ….
- THEORY?

Linear Rheology

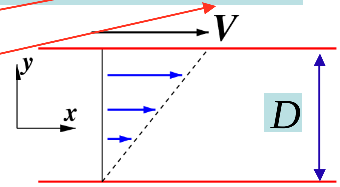
- Apply small shear strain and measure shear stress

Strain rate

$$\sigma_{xy}(t) = \int_{-\infty}^{t} dt' \, G(t-t') \dot{\gamma}_{xy}(t')$$

- Shear Modulus

$$\dot{\gamma}_{xy} = V / D$$



- Frequency dependent modulus

$$G^{*}(\omega) = i\omega \int dt \, G(t) \, e^{i\omega t} = G'(\omega) + iG''(\omega)$$

Storage modulus (elastic)

Loss modulus (viscous)

Questions/Goals

- What happens to the viscoelasticity of an F-actin solution due to the presence of Myosin II/ S1 Myosin and ATP?
- Develop a microscopic theory which can explain the viscoelastic response of F-actin solutions.
- Compare in vitro mixture of Actin/Myosin/ATP with just Actin
- (Lots of unjustified approximations and assumptions)

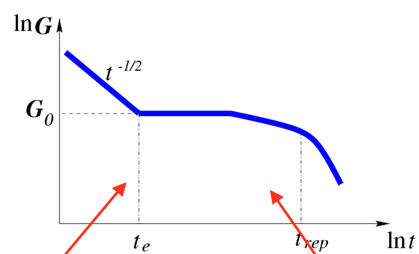
Rheology of Polymers

- Entangled flexible polymer solutions are viscoelastic

Entanglement time t_e

Plateau modulus G_0

Reptation time t_{rep}



'Theory': Free chains move by 'reptation'

Tube model: deGennes; Doi/Edwards

Semiflexible Polymers

- Wormlike Chain (WLC) Bending Energy

$$H_{WLC}[\boldsymbol{R}(s)] = \frac{A}{2}\int ds\left(\frac{\partial^2 \boldsymbol{R}}{\partial s^2}\right)^2$$

- Inextensible

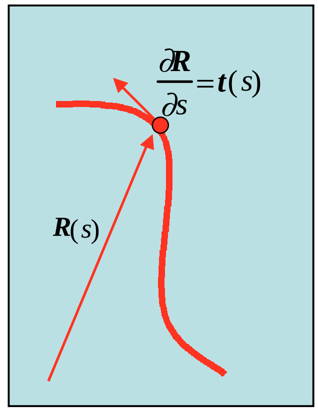
$$\left(\frac{\partial \boldsymbol{R}}{\partial s}\right)^2 = 1$$

- Tangent correlation function

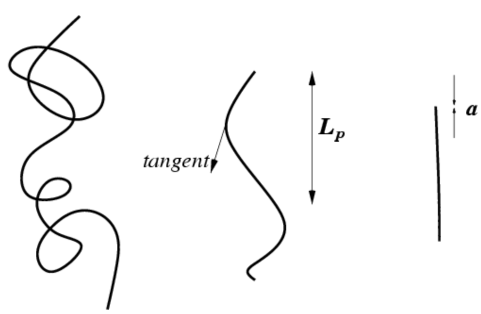
$$\langle \boldsymbol{t}(s)\bullet\boldsymbol{t}(0)\rangle = \exp(-s/L_p)$$

- Persistence length

$$L_p = \frac{A}{k_B T}$$



Persistence Length



Coil	Semiflexible	Rod
$L >> L_p$	$L \approx L_p$	$L << L_p$

Semiflexible Polymer Dynamics

Viscous Friction, Shear, Bending Elasticity, 'Tension', Thermal, Active

$$\underline{\underline{\zeta}}\cdot\frac{\partial}{\partial t}\boldsymbol{R}(s,t) - \underline{\underline{\zeta}}\cdot\dot{\gamma}\boldsymbol{R} = -A\frac{\partial^4 \boldsymbol{R}}{\partial s^4} + \frac{\partial}{\partial s}\left(\Lambda(s,t)\frac{\partial \boldsymbol{R}}{\partial s}\right) + \boldsymbol{f}(s,t) + \boldsymbol{f}_{act}(s,t)$$

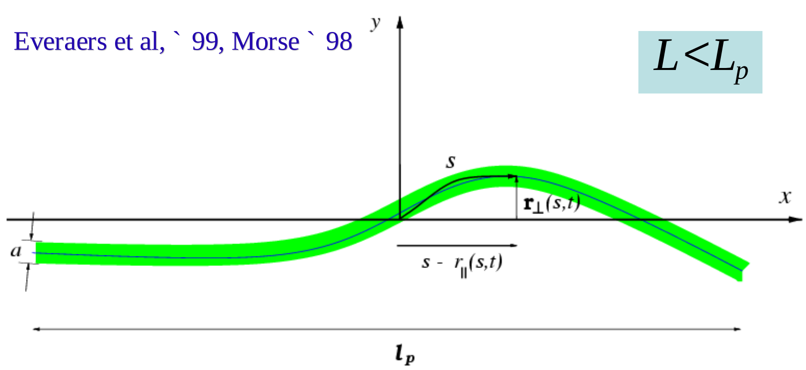
$$\left(\frac{\partial}{\partial s}\boldsymbol{R}(s,t)\right)^2 = 1 \quad \Rightarrow \quad \Lambda(s,t)$$

- Dynamics with constraints

Anisotropic Dynamics

Everaers et al, ` 99, Morse ` 98

$L < L_p$



- Transverse motion (Bending) $\boldsymbol{r}_\perp(s,t)$
- Longitudinal motion (Inextensibility) $r_\parallel(s,t)$

Anisotropic Dynamics

Everaers et al, `99, Morse `98

$\ell_\perp(t) \approx \left(t L_p k_B T/\zeta\right)^{1/4}$ Transverse relaxation length

$\ell_\parallel(t) \approx \left(t L_p^5 k_B T/\zeta\right)^{1/8}$ Longitudinal relaxation length (friction)

$$\partial_t \mathbf{r}_\perp(s,t) = \frac{1}{\zeta_\perp}\left[-A\,\partial_s^4 \mathbf{r}_\perp + \partial_s\left(\Lambda(s,t)\,\partial_s \mathbf{r}_\perp\right)\right] + \mathbf{f}_\perp(s,t)$$

$$\partial_t r_\parallel(s,t) = \frac{1}{\zeta_\parallel}\left[-A\,\partial_s^4 r_\parallel - \partial_s \Lambda\right] + f_\parallel(s,t)$$

$$\partial_s r_\parallel = \frac{1}{2}\left|\partial_s \mathbf{r}_\perp\right|^2 + \ldots \qquad \zeta_\perp = 2\zeta_\parallel = 4\pi\eta/\mathrm{Log}(\ell/a)$$

TBL,Maggs , *Macrom.* **34**, 6064, 2001

Self consistent dynamics

- Transverse fluctuations

$$\mathbf{r}_\perp(q,\omega) = \int ds\,dt\,e^{i\omega t + iqs}\,\mathbf{r}_\perp(s,t) \approx \frac{\mathbf{f}_\perp(q,\omega) + \int_{k\Omega} qk\,\Lambda(q-k,\omega-\Omega)\,\mathbf{r}_\perp(k,\Omega)}{i\omega + \alpha q^4}$$

- Inextensibility

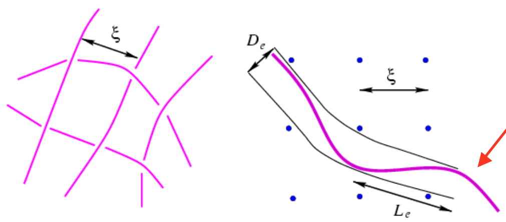
$$iq\left(r_\parallel(q,\omega) - r_\parallel^{eq}(q,\omega)\right) = K(q,\omega)\Lambda(q,\omega)$$

$$K(q,\omega) = 2^{-3/4} k_B T A^{-5/4}\left(i\omega\zeta_\perp\right)^{-3/4} f(q^4/\omega)$$

- Longitudinal fluctuations

$$r_\parallel(q,\omega) - r_\parallel^{eq}(q,\omega) = \frac{\mathbf{f}_\parallel(q,\omega)}{i\omega + K(q,\omega)q^2}$$

Tightly Entangled Polymers



Tube model

Monomer conc.

Polymer diameter

- Mesh-size $\xi = 1/\sqrt{\rho_a a} << L_p$
- Entanglement length $L_e \approx L_p (\xi / L_p)^{4/5}$
- Tube diameter $D_e \approx L_p (\xi / L_p)^{6/5}$
- Monodisperse L

Semenov/Odijk

Stress Tensor

- Kramers-Kirkwood formula for stress in fluctuating 'beads'

$$\sigma_{ij}(t) = -\frac{\rho}{L}\int_0^L ds \langle F_i(s,t)\, R_j(s,t)\rangle \approx \sum_{n=1}^{4} \sigma_{ij}^n$$

$$\sigma_{ij}^1(t) = u_i u_j \frac{\rho}{\ell}\int_0^\ell ds \langle \Lambda(s,t)\rangle$$ Tension stress

$$\sigma_{ij}^2(t) = A\frac{\rho}{\ell}\int_0^\ell ds \langle r_{\perp i}(s,t)\partial_s^4 r_{\perp j}(s,t)\rangle$$ Curvature stress

$$\sigma_{ij}^3(t) = u_i u_j \frac{\rho}{\ell}\int_0^\ell ds \langle \partial_s r_{\parallel}(s,t)\Lambda(s,t)\rangle$$ Longitudinal stress

$$\sigma_{ij}^4(t) = k_B T \frac{\rho}{L}\left\langle u_i u_j - \frac{1}{3}\delta_{ij}\right\rangle$$ Orientational stress

Shear modulus

- Linear response

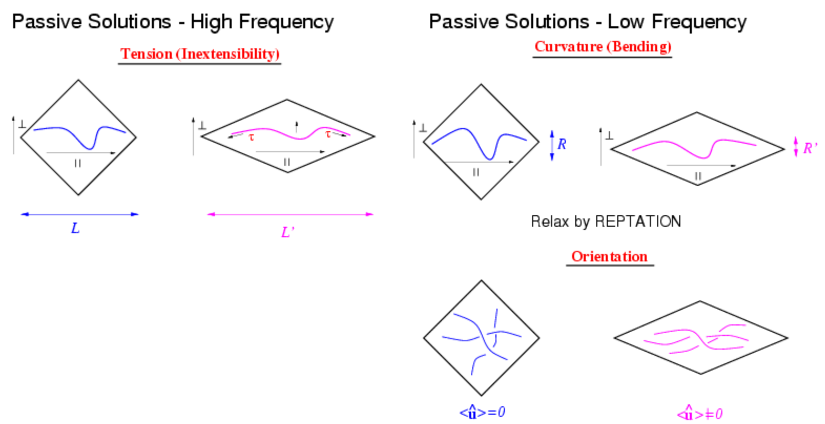
$$G^1(t) \approx \frac{\xi^{-2} k_B T L_p^2}{(t k_B T L_p / \zeta_\perp)^{3/4}}$$ Tension

$$G^2(t) \approx \frac{\xi^{-2} k_B T}{(t k_B T L_p / \zeta_\perp)^{1/4}}$$ Curvature

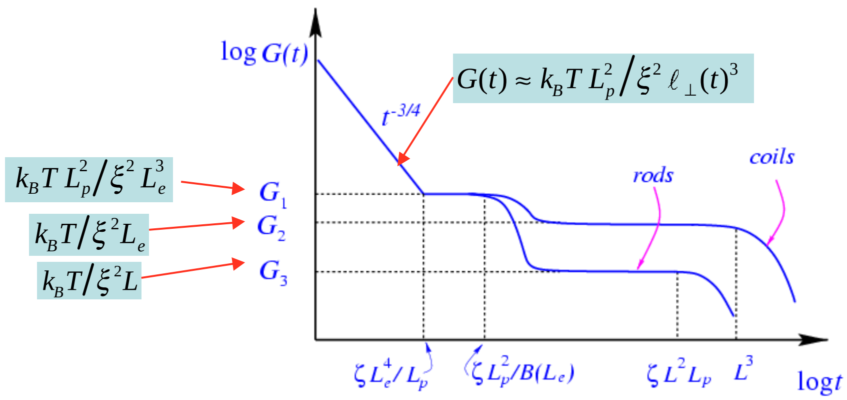
$$G^4(t) = k_B T \frac{3\rho}{5L} \exp(-6 t k_B T / \zeta_u) \; ; L < L_p$$ Orientation

$$G^3(t) \approx \frac{\xi^{-2} k_B T}{(t k_B T (2L_p)^5 / \zeta_\perp)^{1/8}}$$ Longitudinal

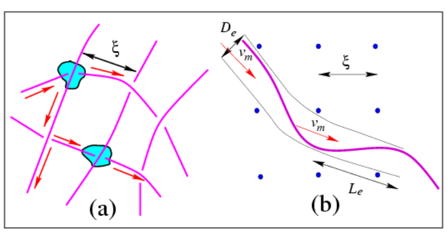
Contributions to Stress



Shear modulus



Motile Solution-Active X-links



ACTIVE temporary cross-links

LARGE longitudinal fluctuations

$k_B T \;\Rightarrow\; k_B (T + T_{act})$

DIRECTED motion in 'tube'

polar filament!

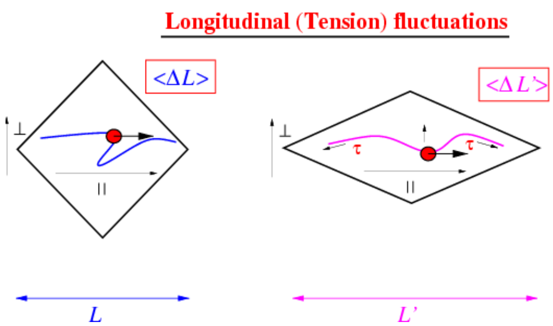
v_m

QuickTime™ and a Cinepak decompressor are needed to see this picture.

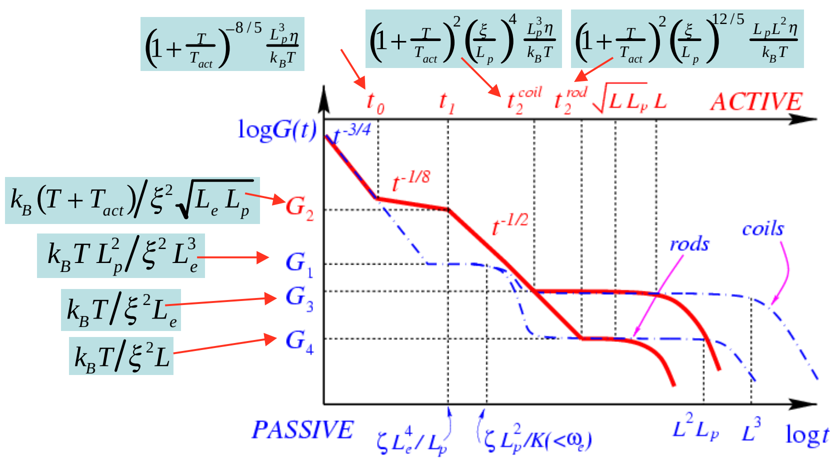
J. Käs' Lab

TBL,Maggs & Ajdari, PRL **86**, 4171, 2001

New Contributions to Stress



Shear modulus



Numbers

- Distance between aggregates $\ell_m \approx 1/\rho_m \xi^2$
- Active temperature

$$k_B T_{act} \approx f_0^2 t_s / \zeta \ell_m$$

- Tube velocity

$$v_m \approx f_0 / \zeta \ell_m$$

Motor concentration

Motor cycle time

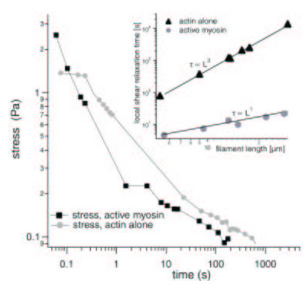
Typical Motor force

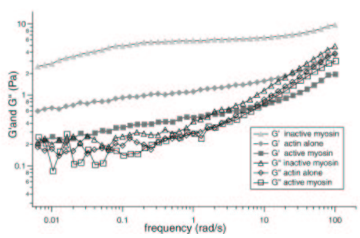
F-actin $\rho_a = 100\mu g/ml$, Myosin $\rho_m = 0.1\mu M$

$$T_{act}/T \approx 100 \quad \Rightarrow \quad t_{rep} \ (10^4 s \Rightarrow 1s)$$

Experiments on MII/Actin/ATP

- Longest relaxation time



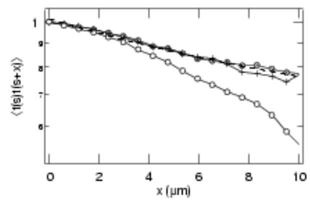


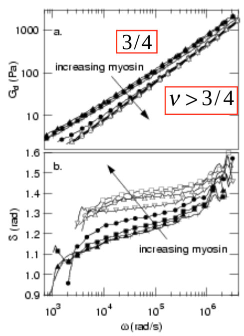
- Plateau modulus

Josef Käs' Lab

Experiments on S1/Actin/ATP

- Tangent correlations
  - not WLC!

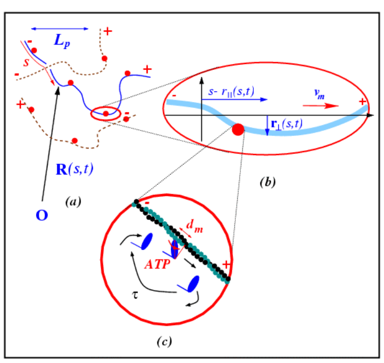




- High freq. shear modulus

Le Goff et al

Motile Solution-Active Forces



Myosin S1 fragments do not form clusters

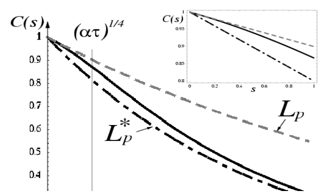
ACTIVE forces due to myosin interacting with single filaments

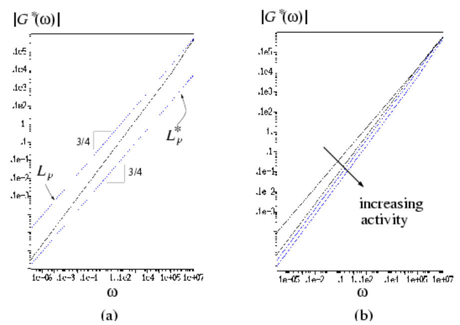
Time-scale of activity

τ

TBL (preprint) , 2001

Polar active filaments





Lengthscale dependent rigidity

Crossover scale

$$\ell_C \approx \left(\tau\, L_p\, k_B T / \zeta\right)^{1/4}$$

Conclusions

- F-actin solutions have a rich viscoelastic response over a large range of moduli and time-scales
- The rheological properties are changed dramatically by myosin activity
  - Non-equilibrium motile solution is harder on short timescales and softer on longer timescales than the equivalent passive solution
  - Non-equilibrium active filaments have different static and dynamic fluctuations which affects high frequency response

Acknowledgements

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The Royal Society