Motor Powered Alignment of Cytoskeletal Filaments

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The Cell Cytoskeleton

Mechanical Modes
1. - Twisting
2. - Bending
3. - Breakage
4. - Alignment

Functions
1. - Architecture
2. - Transport
3. - Cell \ Organelle Dynamics
Actin Organization in Cells

[Actin] ~ 10 to 500µM (~1mM in muscle)

[Myosin] ~ 1 to 50µM (~0.2mM in muscle)
In Vitro Motility Assay

Ingredients
1. Skeletal muscle meromyosin
2. Rhodamine-phalloidin F-actin.
3. 2 mM ATP
4. Nitrocellulose coated glass coverslips
5. Non-fluorescent F-actin

Alignment of Cytoskeletal Polymers - Theory

Kierfeld, Kraikivsky and Lipowsky. 2008. Biophys. Revs. Letts. 4:363-
Shearing - Control of Filament Length

Mean actin filament length (µm)

Number of pipetting cycles

Relative Frequency

Filament length (µm)
“Normal” *In vitro* Motility Assay (~ 2 pMolar F-actin)
In vitro Motility with 20µM unlabelled F-actin (~10^7 x “Normal” amount)
A Quantitative Measure of Alignment

(A) A qualitative measure of alignment.

(B) A quantitative measure of alignment.

(C) KS value

KS = d₁ + d₂

(D) N_{obs}
Alignment Strongest when Filament Length > 1\(\mu\)m
Formation of Aligned Domains - an Active Process

1. Alignment takes about 5-10 minutes to establish.

2. Rapid solution flow causes immediate alignment of filaments in bulk phase (in direction of flow).

**A**

**B**

KS

Time (min)

0 10 20 30 40 50 60

0.1 0.2 0.3 0.4 0.5 0.6
Pattern Formation

See also Schaller, V et al. 2010. Nature. 467, 73.
Microdomain Dynamics

(A) Relative Frequency

KS: 0.096

(B) Relative Frequency

KS: 0.187

Angle θ
Myosin Filaments do not disrupt Oriented Actin Domains

Fields of oriented actin also appear when synthetic filaments formed by native myosin are used.

![Myosin E.M.](image1)
![Actin Fluorescence](image2)

- Relative Frequency
  - KS: 0.24975
  - KS: 0.33644
Persistence of motion is slightly greater at higher filament “crowding”

- +0.5mg/ml non-fluorescent actin
- No added non-fluorescent actin
Filament Collisions

A. Filaments bend due to motor forces. Segmentation of a collision complex into individual filaments is a challenging computer vision problem.

B. At low densities filaments transiently align by “kinking” upon collision.
Observations of Single Collisions

High Motor Density

*Entrainment*

*Bending*

Low Motor Density

*Crossover*

*Weak alignment*
Alignment Probability depends on Motor Density

Incident filament

Target filament

Alignment Probability depends on Motor Density

Low Motor Density

High Motor Density

Emergent Angle (degrees)

Incident Angle (degrees)

N_{obs}

Angle
Does Surface Filament Sliding produce Convection Currents?

Surface

2 µm Defocus

10 µm

20x normal speed

CONCLUSIONS

1. Quantitative measure of population orientation.
2. Microdomain formation increases with filament crowding.
3. Microdomain formation is an active process.
4. Optimal filament length ca. $1 \mu m$.
5. Actin microdomains reorganize on micrometer – second time scales.

PROSPECTS

Extension of theory and measurement in 2D and towards 3D

a- Finite crossover probability.
b- Relaxation of rigid rod approximation
c- Non-processive motors
d- Fluctuations in motor surface density
e- Weak forces