Deterministic and Stochastic Aspects of Actin Filament Dynamics

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Overview

• A bit of background on cell motility
• Deterministic analysis of actin dynamics in solution
  (i) Analytical results on existence, uniqueness, qualitative properties, and convergence to equilibrium in solution
  (ii) Computational results on the population dynamics on fast and slow time scales
  (iii) Effects of sequestration
• Stochastic analysis of filament dynamics accounting for nucleotide profiles
  (i) Polymerization, treadmilling and fluctuations of individual filaments
  (ii) The evolution of length distributions under various scenarios of capping, fragmentation, etc
  (iii) Clarifying the role of twinfilin
Examples of individual and collective cell movement
Actin dynamics at the leading edge

Actin dynamics at the leading edge – cont.
Details of the actin control network
Basic questions about actin dynamics ...

1. How do cells maintain a pool of unpolymerized actin subunits?
2. How are signals directed to Arp2/3 complex?
3. How do cells create actin filaments with free barbed ends?
4. How do new filaments elongate?
5. How do growing filaments push the membrane forward?
6. What limits the growth of filaments?
7. How are filaments marked for depolymerization?
8. How do filaments depolymerize?
9. How do stable filaments survive in cytoplasm?
10. How are subunits recycled to the ATP-actin profilin pool?

How do these processes balance to control the length distribution and the dynamic response?

Previous work


Single filament dynamics

Polymerization Rate

Points:
- **Pointed end**
- **Barbed end**

Equations:
- \[ c - c + c_s \]
- **Barbed end**
- **Pointed end**

Parameters:
- \( K = 0.6 \)
- \( K = 0.5 \)
- \( K = 0.12 \)
- \( K = 0.5 \)

Diagram:
- ATP
- Monomer
- 0
Let $M_i$ denote a filament of length $i$ and let $C_i$ be the corresponding concentration. Then we consider the sequence

$$
\begin{align*}
\mathcal{M}_1 & \xrightarrow{k_1^+} \mathcal{M}_2 \xrightarrow{k_2^+} \mathcal{M}_3 \xrightarrow{k_3^+} \cdots \mathcal{M}_n \xrightarrow{k_n^+} \mathcal{M}_{n+1} \\
\mathcal{M}_1 & \xleftarrow{k_1^-} \mathcal{M}_2 \xleftarrow{k_2^-} \mathcal{M}_3 \xleftarrow{k_3^-} \cdots \mathcal{M}_n \xleftarrow{k_n^-} \mathcal{M}_{n+1}
\end{align*}
$$

<table>
<thead>
<tr>
<th>Nucletation</th>
<th>Propagation</th>
</tr>
</thead>
</table>

$k^+ = 10$ for all $n$  
$k_1^- = 10^6$  
$k_2^- = 10^3$  
$k^- = 1$  
$n \geq 3$

The governing equations ....

Define the flux from a filament of \( n-1 \) into a filament of length \( n \).

\[
j_n \equiv k_{n-1}^+ C_1 C_{n-1} - k_{n-1}^- C_n
\]

\[
\frac{dC_1}{dt} = -2(k_1^+ C_1^2 - k_1^- C_2) - \sum_{n=2}^{N}(k_n^+ C_1 C_n - k_n^- C_{n+1}) = -2j_2 - \sum_{n=3}^{N} j_n
\]

\[
\vdots
\]

\[
\frac{dC_n}{dt} = (k_{n-1}^+ C_1 C_{n-1} - k_{n-1}^- C_n) - (k_n^+ C_1 C_n - k_n^- C_{n+1}) = j_n - j_{n+1}
\]

\[
\frac{dC_N}{dt} = (k_{N-1}^+ C_1 C_{N-1} - k_{N-1}^- C_N) = j_N
\]

Conservation condition:

\[
\sum_{n=1}^{N} nC_n = C_0
\]

This implies that solutions exist globally in time for any finite \( N \).
The steady state ....

Define $K_n \equiv k_n^+/k_n^-$; then the equilibrium relations $j_i = 0$ lead to

$$C_n = K_{n-1} C_1 C_{n-1} = K_{n-1} K_{n-2} C_1^2 C_{n-2} = \cdots = \left( \prod_{i=1}^{n-1} K_i \right) C_1^m \equiv \Lambda_n C_1^m$$

and the conservation condition becomes

$$\sum_{n=1}^N nC_n = \sum_{n=1}^N n\Lambda_n C_1^m = C_0$$

The left-hand side is monotone increasing and vanishes at zero, and therefore the steady-state is unique. One can also prove that

- For any fixed $N > 3$ and $K = K_j$ for $j \geq 3$, there exists a critical concentration $C_0^*$ such that the profile is monotone increasing for $C_0 > C_0^*$ and monotone decreasing for $C_0 < C_0^*$. The critical $C_0^*$ gives $C_1 = K^{-1}$.

- For any fixed $C_0 > 0$ there exists an $N > 3$ such that the profile is monotone decreasing.

- The Gibb’s free energy is a Lyapunov function.
Steady-state profiles for various $C_0$

Are the monotone increasing profiles ever physically relevant?
Why does the distribution ‘stall’ with a unimodal profile when the asymptotic distribution is monotone increasing?
Phase 1 (Nucleation): flux from monomer pool to dimers and trimers and initiation of filaments. Trimers rapidly equilibrate with monomers.

Phase 2 (Polymerization): Elongation of filaments due to polymerization. This persists until the monomer pool reaches a QSS.

Phase 3: Redistribution of monomers amongst filaments with few new filaments added. This is a long, slow ‘diffusive’ process.

The monomer concentration will equilibrate when the net flux to the dimer/trimer pool balances that from the long filament pool to the monomers.
The convective or hyperbolic phase

For $n \geq 4$ we can write

$$\frac{dC_n}{d\tau} + \left( k^+ C_1 - k^- \right) (C_n - C_{n-1}) = k^- (C_{n-1} - 2C_n + C_{n+1})$$

Convective speed

(a) Number of filaments against filament length in monomers

(b) Speed of peak against time in seconds
Understanding the shape of the intermediate profile

\[
\frac{dC}{dt} = \begin{bmatrix} K_1 & K_2 \\ K_3 & K_4 \end{bmatrix} C + \Gamma
\]

\[
C = (C_2, C_3, \cdots,)^T \quad \Gamma = (k_1^+ C_1^2, 0, \cdots 0)^T
\]

\[
K = \begin{bmatrix}
-(k^+ C_1 + k_1^-) & k_2^- & 0 & 0 & \cdots \\
 k^+ C_1 & -(k^+ C_1 + k_2^-) & k^- & 0 & \cdots \\
0 & k^+ C_1 & -(k^+ C_1 + k^-) & k^- & \cdots \\
\vdots & k^+ C_1 & -(k^+ C_1 + k^-) & k^- & \cdots \\
\vdots & \vdots & \vdots & \vdots & \vdots
\end{bmatrix}
\]
Asymptotic analysis of the spectral problem

- The matrix $K$ has two large negative eigenvalues given approximately by $-k_1^-$ and $-k_2^-$. 
- The remaining eigenvalues are those of $K_4$ to lowest order. $K_4$ is a perturbation of a tridiagonal matrix (in the $(N,N)$ entry) of the form

\[
K_4 = (k^+ C_1)J - (k^+ C_1 + k^-)I + k^- J^T
\]

where $J$ is the lower diagonal shift. 
- The eigenvalues of $K_4$ are

\[
\lambda_p = -(k^+ C_1 + k^-) + 2\sqrt{k^- k^+ C_1} \cos \theta_p \quad \theta_p \equiv \frac{p\pi}{N + 1}
\]

- For $k^+ = 10$, $k^- = 1$, $C_{1,\text{crit}} = 0.1$, and therefore

\[
\lambda_N \to 0 \quad \text{as} \quad N \to \infty
\]

This shows that the quasi-attractor is \textit{not} an artifact of the assumption that the maximum filament length is finite.
Decomposition of the quasi-stationary profiles

<table>
<thead>
<tr>
<th>#</th>
<th>Eigenvalue</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>$-6.1778 \times 10^{-7}$</td>
</tr>
<tr>
<td>2</td>
<td>$-5.5600 \times 10^{-6}$</td>
</tr>
<tr>
<td>3</td>
<td>$-1.5444 \times 10^{-5}$</td>
</tr>
<tr>
<td>4</td>
<td>$-3.0271 \times 10^{-5}$</td>
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<tr>
<td>5</td>
<td>$-5.0040 \times 10^{-5}$</td>
</tr>
<tr>
<td>6</td>
<td>$-7.4750 \times 10^{-5}$</td>
</tr>
<tr>
<td>8</td>
<td>$-1.0440 \times 10^{-4}$</td>
</tr>
<tr>
<td>9</td>
<td>$-1.3900 \times 10^{-4}$</td>
</tr>
<tr>
<td>10</td>
<td>$-1.7853 \times 10^{-4}$</td>
</tr>
</tbody>
</table>
Polymerization with fragmentation and annealing

\[
\frac{dC_k}{dt} = \frac{1}{2} \sum_{l=1}^{k-1} A_{l,k-l} C_{k-l} C_l - \left( \sum_{l=1}^{N-k} A_{k,l} C_l \right) C_k
\]

Annealing of fil\(_{k-l}\) with fil\(_l\)

\[
+ \sum_{l= k+1}^{N} B_{l,k} C_l - \left( \sum_{l=1}^{k-1} B_{k,l} \right) C_k
\]

Breakage of fil\(_l\) into fil\(_k\) and fil\(_{l-k}\)

Breakage of fil\(_k\) into fil\(_l\) and fil\(_{k-l}\)
The effects of fragmentation

Fragmentation rate:

\[ 1 \cdot 10^{-8} \text{s}^{-1} \]

\[ 1 \cdot 10^{-6} \text{s}^{-1} \]
Evolution with and without fragmentation
Nucleotide profiles matter!
Objectives of a stochastic model/simulation

- What is the average nucleotide profile of an actin filament?
- How do different biochemical factors such as ADF/cofilin alter the average nucleotide profile and conversely, how does the nucleotide profile affect the action of these factors?
- What is the dynamic response of the distribution of length and nucleotide profiles to an “external” signal that produces an increased amount of globular actin?
- In what regimes are both the continuous and the stochastic models valid, and when must one use a stochastic model?
The stochastic model for filament evolution

• Assume that the system is well-mixed
• Each filament is characterized by its length and nucleotide sequence, and the state of the system is characterized by the numbers of filaments of various lengths and nucleotide sequences.
• The filament population is represented abstractly as follows:
  ♦ Define an alphabet $\mathcal{A} = \{1, 2, 3\}$ for monomer states, where 3 stands for ATP, 2 for ADP-Pi and 1 for ADP. The internal state of a filament, i.e. its nucleotide profile, is represented by a string over the alphabet $\mathcal{A}$.
  ♦ we let $\mathcal{A}^*$ be the set of all strings (“words”) over $\mathcal{A}$. More precisely,

$$\mathcal{A}^* = \bigcup_{n} \mathcal{A}^n$$

• The state of a well-mixed solution of actin filaments is encoded by the random variable $X : \mathcal{A}^* \rightarrow \mathbb{N}$
The master equation

\[ \frac{dc}{dt} = \nu \mathbf{E} \hat{\mathcal{R}}(c), \]

For mass action kinetics

\[ \hat{\mathcal{R}}_\ell(c) = k_\ell \prod_{i=1}^{n} (c_i)^{\nu_{ij}} \]
Let \( n = (n_1, n_2, \ldots, n_s) \) be the number of molecules of different species, \( V \) the volume, and \( N_A \) Avagadro’s number.

\[
\frac{dn}{dt} = \nu \mathcal{E} \tilde{R}(n)
\]

For MAK

\[
\tilde{R}_\ell(n) = N_A V k_\ell \prod_{i=1}^{s} \left( \frac{n_i}{N_A V} \right)^{\nu_{ij}} = \frac{k_\ell}{(N_A V) \sum_i \nu_{ij} - 1} \prod_{i=1}^{s} (n_i)^{\nu_{i\ell}} = \hat{k}_\ell \prod_{i=1}^{s} (n_i)^{\nu_{ij}}.
\]

\[
\frac{d}{dt} P(n, t) = \sum_\ell \mathcal{R}_\ell(n - \nu \mathcal{E}(\ell)) \cdot P(n - \nu \mathcal{E}(\ell), t) - \sum_\ell \mathcal{R}_\ell(n) \cdot P(n, t)
\]

\[
\mathcal{R}_\ell = c_\ell h_{j(\ell)}(n)
\]

and

\[
c_\ell = \frac{k_\ell}{(N_A V) \sum_i \nu_{ij(\ell)} - 1} = \hat{k}_\ell
\]
The stochastic simulation algorithm

Gillespie’s direct method

(1) Initialization (set the initial numbers of molecules, and set $t = 0$).

(2) Calculate the reaction rate functions $R_i (i = 1, \ldots, r)$.

(3) Generate two random numbers $r_1$ and $r_2$ from a uniform distribution on $(0, 1)$.

(4) Calculate $\tau$ as follows:

$$R_0(n) = \sum R_j(n), \quad \tau = \frac{1}{R_0(n)} \ln \frac{1}{r_1}$$

(5) Determine the smallest integer $n_0$ that satisfies

$$\sum_{i=1}^{n_0} R_i(n) > r_2 R_0(n)$$

(6) Update the states of the species to account for changes due to reaction $n_0$ and set $t = t + \tau$.

(7) Go to 2.
Modifications of the algorithm

• A major issue in stochastic simulation of polymerization reactions is that one has to account for the fact that new ‘species’ are created continuously.

• We define equivalence classes of species for specific kinetic steps, rather than treating each filament individually. As we’ll see this leads to a dramatic reduction in the computational time.

• We don’t update the reaction rates at every step; knowing which reactions occur we only update the appropriate steps.

Chemical steps included in the equation

• Polymerization at barbed and pointed ends
• Hydrolysis
• Fragmentation
• Capping and Sequestration
• Annealing of filaments
# Kinetic parameters

<table>
<thead>
<tr>
<th></th>
<th>$k_D^+$</th>
<th>$k_{DP}^+$</th>
<th>$k_T^+$</th>
<th>$k_D^-$</th>
<th>$k_{DP}^-$</th>
<th>$k_T^-$</th>
<th>$k_F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbed end</td>
<td>3.8</td>
<td>7.4</td>
<td>7.4</td>
<td>1.5</td>
<td>0.9</td>
<td>0.9</td>
<td>—</td>
</tr>
<tr>
<td>Pointed end</td>
<td>0.16</td>
<td>0.56</td>
<td>0.56</td>
<td>0.26</td>
<td>0.19</td>
<td>0.19</td>
<td>—</td>
</tr>
<tr>
<td>Sequestration by twinfilin</td>
<td>180.0</td>
<td>—</td>
<td>—</td>
<td>1.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Capping by twinfilin</td>
<td>10.0</td>
<td>1.0</td>
<td>10.0</td>
<td>1.0</td>
<td>10.0</td>
<td>10.0</td>
<td>—</td>
</tr>
<tr>
<td>Sequestration by thymosin</td>
<td>1.0</td>
<td>—</td>
<td>1.0</td>
<td>100.0</td>
<td>—</td>
<td>0.9</td>
<td>—</td>
</tr>
<tr>
<td>Capping by gelsolin</td>
<td>20</td>
<td>—</td>
<td>—</td>
<td>0.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gelsolin F-actin binding</td>
<td>20</td>
<td>—</td>
<td>—</td>
<td>0.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fragmentation by gelsolin</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.25</td>
</tr>
<tr>
<td>Profilin/monomer binding</td>
<td>—</td>
<td>—</td>
<td>50</td>
<td>—</td>
<td>—</td>
<td>5</td>
<td>—</td>
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<tr>
<td>Profilin/filament binding</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2250</td>
<td>2250</td>
<td>2250</td>
<td>—</td>
</tr>
</tbody>
</table>

- D: ADP, DP: ADP-P$_i$, T: ATP, F: fragmentation
- Second-order rates in (µM·sec)$^{-1}$, first-order rates in sec$^{-1}$. 
How pseudo-reactions affect the computational time

- Graph showing the relationship between the initial number of filaments in the system and CPU time in seconds for both non-optimized and optimized versions.

- The graph demonstrates a significant increase in CPU time as the initial number of filaments increases, with the optimized version showing a more level curve compared to the non-optimized version.
Evolution of the nucleotide profile for a single filament
Evolution of the length distribution with fragmentation
The long-time evolution
Long-time single filament evolution
The role of twinfilin in recent experiments

- Gelsolin is thought to cap barbed ends with high affinity
- Thymosin $\beta_4$ sequesters G-ATP-actin
- Twinfilin caps barbed end
- Helfer, et al. suggest that the biphasic behavior in the presence of twinfilin is due to sequestration of G-ADP-actin

The kinetic interactions

\[ \text{G–ADP–actin} \xrightarrow{k_{D \rightarrow T}} \text{G–ATP–actin} \]

\[ k_D^- + k_{D-Pi}^- \]

\[ k_{sqD}^- \]

\[ k_{sqD}^+ \]

\[ k_{cP}^- \]

\[ k_{cP}^+ \]

\[ \text{Filaments} \]

\[ \text{TWN–G–ADP} \]

\[ \text{TWN–Filaments} \]

\[ \text{Free twinfilin in solution} \]
Sequestration by twinfilin

(a) No twinfilin present

(b) 0.8 μM of twinfilin

(c) 1.7 μM of twinfilin

(d) 5.4 μM of twinfilin
An interesting consequence of this ..
Something that is hard to detect experimentally

(b) Mean filament length and fluorescence

- Mean length in # of monomers
- Scaled fluorescence (a.u.)

Number of monomers vs Time in seconds

- 0 to 1.5 x 10^4 seconds
Capping and sequestration by twinfilin

(a) No twinfilin present

(b) 0.8 μM of twinfilin

(c) 1.7 μM of twinfilin

(d) 5.4 μM of twinfilin
Evolution in time of the capped filament population

- 0.8 µM of twinfilin
- 1.7 µM of twinfilin
- 5.4 µM of twinfilin

Percentage of capped filaments
Sequestration only...
Capping only

(a) No twinfilin present
(b) 0.8 μM of twinfilin
(c) 1.7 μM of twinfilin
(d) 5.4 μM of twinfilin
The effect of other control proteins – profilin
Summary

- Computational models (deterministic or stochastic) allow for controlled experimentation that is difficult to do in the lab.
- A model of the actin filament dynamics predicts a broad range of time scales in the approach to the steady state.
- The early evolution shows a convective behavior in the length distribution, but after equilibration of the monomer pool there is a very slow diffusive rearrangement of the length distribution.
- The results show that experimental controversies as to the nature of the steady-state profile may arise in part because experiments are not run long enough.
- These results also emphasize the need for control molecules that fragment filaments, cap pointed ends, and create new branches in order to control actin formation on a time scale relevant to cell movement.
- The stochastic simulations validate the conclusion reached by Helfer et al, that a major role for twinfilin is sequestration of G-ADP-actin.
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