Membrane Waves Driven by Actin and Myosin

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We present a model which couples the membrane with the protrusive forces of actin polymerization and contractile forces of molecular motors, such as myosin. The actin polymerization at the membrane is activated by freely diffusing membrane proteins that have a spontaneous curvature. Molecular motors are recruited to the polymerizing actin filaments, from a constant reservoir, and produce a contractile force. All the forces and variables are treated in the linear limit. Our results show that for convex membrane proteins the myosin activity gives rise to robust transverse membrane waves, similar to those observed on different cells.

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The phenomenon of membrane waves and ruffles in motile cells has attracted a lot of recent attention [1–5]. These waves are membrane protrusions that form on the surface of cells, usually at their periphery (leading edge of moving cells), and propagate along the cell membrane. This puzzling behavior involves a high activity of actin polymerization and many different proteins [6] and is influenced by myosin contraction and adhesion to the external substrate. Despite the apparent complexity of this system [7], we wish to explore here a minimal physical model that gives rise to some of the observed behavior. We have recently presented a model that couples the membrane elasticity and protrusive forces of actin polymerization through the introduction of freely diffusing membrane proteins (MP) that activate the actin polymerization [8]. This model gives rise to several observed patterns, depending on the spontaneous curvature of the MP. In particular, we found that for convex MP, thermal fluctuations become unstable, which we identify with the phenomenon of MP aggregation (forming a “tip complex” [9]) resulting in fingerlike protrusions such as filopodia. In the present work we extend our model to include the effect of the contractile forces due to molecular motors such as myosin. These motors are found to be active in the lamellipodia [10] and membrane ruffles [1,2]. Within our model, these contractile forces give rise to robust traveling waves on the membrane, which we compare to experimental observations.

In our model actin polymerization is activated at the membrane by the MP [Fig. 1(a)], so that the filaments are mostly directed towards the membrane [7]. We therefore restrict and simplify our model by assuming that the actin-induced protrusion forces are normal to the membrane. The myosin motors attach to the actin meshwork and produce a contractile normal force at the membrane by pulling on the other ends of the actin filaments [Fig. 1(a)], as was shown in [10]. In a branched actin gel each myosin motor can affect a relatively wide region of membrane, while when the filaments are bundled and span the entire lamellipodia, the myosin motors affect a highly localized part of the membrane [11]. Because of branching, the membrane at position $r$ is linked by actin filaments to motors that are not directly below it [Fig. 1(a)]. The resultant force is therefore the sum of those contributions, with some appropriate weighing function. Each myosin motor is at an average distance $L$ from the membrane, attached to a network of branched filaments with branch spacing $d$. By counting the number of parallel paths that connect the motor (at point $r'$) with different locations ($r$) on the membrane, we can estimate the spatial force distribution: $M(r - r') = \exp(-|r - r'|^2/2\xi^2)/\sqrt{2\pi}\xi^2$, where $\xi^2 = d^2/(L/d - 1)$. In the limit of highly branched network (small $d/L$) each myosin affects a membrane patch of radius $\sim \sqrt{Ld}$, while in the limit of no branching ($d/L \rightarrow 1$) we get a localized force. Additionally, since the motor pulls both the filaments and the membrane, the branching increases the overall friction, which grows with the branching as:

![FIG. 1](color). (a) Schematic description of our model (see text for details). (b) Strong contraction (large downward black arrow) leads to MP aggregation on the two sides (small arrows on the membrane), followed by the motors (dashed red arrows), leading to wave propagation.
Γ = \exp(-\xi^2/d^2). In the limit of dense branching (d → 0) we get a highly diminished contractile force. Note that the above description breaks in the limit where d approaches the diameter of the filaments. In this limit the excluded volume of the branches makes the force distribution uniform. We will work with a two-dimensional density \( m(r) \) that represents the projection of these attached myosin motors onto the plane of the membrane [Fig. 1(a)]. The full three-dimensional structure of the actin network and the forces it produces in the whole cell [12] are not captured by our simple two-dimensional model. It may, however, be a good starting point to describe the cytoskeleton-induced dynamics in close proximity to the membrane.

The equation of motion for the membrane height deflection \( h \) is

\[
\frac{\partial h}{\partial t} = \mathcal{O}(r-r') \left[ -\frac{\delta F}{\delta h(r')} - \int \mathcal{M}(r'-r'')A^* m(r'')dr'' + \text{An}(r') \right] d^2r' \\
+ \int \mathcal{O}(r-r') \left[ -\kappa \nabla^4 h(r') + \sigma \nabla^2 h(r') + \frac{k\hat{H}}{n_0} \nabla^2 n \right. \\
- \Gamma \int \mathcal{M}(r'-r'')A^* m(r'')d^2r'' + \text{An}(r') \right] d^2r',
\]

where \( F \) is the free energy of the membrane with MP [13], \( A, A^* \) are the protrusion and contraction proportionality constants, respectively, \( \hat{H} \) is the spontaneous curvature of the MP, \( \mathcal{O}(r-r') \) the Oseen tensor describing the viscous drag of the fluid surrounding the membrane, and \( \kappa, \sigma \) the bending modulus and the surface tension of the membrane,

\[
\begin{pmatrix}
\hat{h} \\
\hat{\eta} \\
\hat{n}
\end{pmatrix} = \begin{pmatrix}
\hat{\mathcal{O}}(-\kappa q^4 - \sigma q^2) & -\hat{\mathcal{O}} \mathcal{M}_q A^* & \hat{\mathcal{O}}(A - \kappa \hat{H} q^2/n_0) \\
0 & -k_{\text{off}} & k_{\text{on}} \\
0 & -D q^2 & 0
\end{pmatrix} \begin{pmatrix}
h \\
m \\
n
\end{pmatrix},
\]

where \( \mathcal{M}_q \) is the Fourier transform of the myosin force kernel, including the factor \( \Gamma \). Solving the homogeneous coupled equations, we find solutions of the form: \( (h, n, m)(q, t) \sim \exp[\omega(q)t] \), where \( \omega(q) = \omega(|q|) + \text{i} \omega'(|q|) \). The dispersion \( \omega(q) \) can be solved analytically but the resulting expressions are rather complex so we give them here in graph form (Fig. 2).

We now concentrate on convex MP, i.e., \( \hat{H} < 0 \). Without the contraction of motors these MP give rise to an unstable mode \( \omega'(q) > 0 \) (Fig. 2) [8]. This instability corresponds to actin bundles and protrusions that are static laterally, i.e., \( \omega''(q) = 0 \). An experimental realization of the interplay between actin and myosin appears in [10]. In this experiment the application of myosin-inhibiting drug produced a regular array of straight, static, and equally spaced actin bundles in the growth cone of a neuron, in agreement with our model [8] (Fig. 2). We use the observed average spacing of \( L_{\text{bun}} \sim 0.5 \mu\text{m} \), to fit the parameters of our model such that \( q_m \sim 2\pi/L_{\text{bun}} \) where \( \omega'(q) \) is maximal, which we list in Table I.

respectively. In the present Letter we shall work with the approximate form of the Oseen tensor, which is valid for restricted fluid flows in the actin-rich lamellipodia. In Fourier space we get: \( \hat{\mathcal{O}} = d/4\pi \), where \( d \) is the length scale of the fluid confinement inside the actin network.

For the local density of MP \( n \) we have a conservation equation

\[
\frac{\partial n}{\partial t} = D \nabla^2 n - \Lambda \kappa \hat{H} \nabla^2 n + \nabla f_n,
\]

where \( D \) is the in-membrane diffusion coefficient, \( \Lambda \) is the mobility of the MP, and \( f_n \) correspond to the conserved thermal noise.

The density of attached myosin motors depends on the adsorption rate and the local actin concentration, \( k_{\text{on}} n \), and on the detachment rate \( k_{\text{off}} \). We assume the existence of an infinite reservoir of free myosin in the cytoplasm with a fixed density. When the actin gel is thick and the motors are distributed far from the membrane [10], we can neglect the direct dragging of the motors by the in-plane motion of the MP. In this case the dynamics of the myosin is simply given by

\[
\frac{\partial m}{\partial t} = -k_{\text{off}} m + k_{\text{on}} n + f_m,
\]

where \( f_m \) is a thermal noise term in the myosin on-off rate. At steady state we have: \( n_0 = n_0 k_{\text{on}}/k_{\text{off}} \), where \( n_0 \) is the average density of MP. In order to have analytic results we will work in the linear limit of small fluctuations from the average values of \( n_0, m_0 \) and flat membrane \( h_0 = 0 \).

Fourier transforming Eqs. (1)–(3) without the thermal noise terms, we can write them in matrix form

When the motors activity is added in our model, i.e., \( A^* k_{\text{on}} \neq 0 \) (4), we find that these protrusions become oscillatory \( \omega''(q) \neq 0 \) (Fig. 2). In the limit of \( A^* k_{\text{on}} \rightarrow 0 \) (weak coupling) we first get \( \omega''(q) \neq 0 \) around the wave vector \( q_w \), where \( \omega'(q_w) = -k_{\text{off}} \) (Fig. 2). This corresponds to a resonance between the frequency of the membrane-actin mode and the myosin internal mode. As \( A^* k_{\text{on}} \) increases, the increased myosin-induced contraction reduces the actin-induced instability and may cause it to vanish (Fig. 2). At the same time the range of wave vectors for which we have an oscillatory solution increases, as does the magnitude of \( \omega''(q) \), but we are still in the highly damped regime; \( |\omega''(q)| \leq |\omega(q)| \).

When the myosin activity increases further and becomes dominant (\( A n_0 - A^* m_0 < 0 \)), the oscillatory mode becomes unstable (Fig. 2), due to the following effect [Fig. 1(b)]: a strong local contraction and a dip in the membrane (large negative curvature) will cause the appearance of large positive curvature at its sides (shoulders). This will drive the MP to aggregate there, so that an
unstable mode appears. Nevertheless, the myosin follows the aggregation of the actin to the shoulders, and we get a propagating mode. A unique feature of our model is robust membrane waves, which are weakly damped (or even unstable). The results shown in Fig. 2 are in the limit of \( M_\eta \rightarrow 1 \), which corresponds to local myosin activity (for simplicity). We also plot the dispersion with the full expression (dashed lines), and as expected the branching smears the myosin forces and therefore suppresses the instability at high \( q \), while the overall results are qualitatively unchanged. Indeed, the effect of myosin is observed in laterally moving bundles [10].

In the unstable region we get an almost linear dispersion relation \( \omega''(q) \approx q \) (Fig. 2, inset of Fig. 3), which corresponds to a constant group velocity: \( \nu = \partial \omega'(q) / \partial q \). Using some approximation of our model we get (using a local myosin kernel)

\[
\omega'' \approx \sqrt{-\frac{DA^*\Lambda kHn}{D} q} \Rightarrow \nu = \sqrt{-\frac{DA^*\Lambda kHn}{D}}. \tag{5}
\]

In Fig. 2 we plot the calculated dispersion of Eq. (5) which compares well with the result of the full model in the linear regime.

Experiments [3,4,6,10] have shown traveling membrane protrusions in many cell types along the leading edge of the lamellipodia, with a velocity in the range of [3,4] \( \nu \sim 100-200 \) nm/sec, similar to what we calculate (Fig. 2, inset of Figs. 3). Furthermore, it was found that the velocity of these waves is independent of the wavelength (Fig. 5 of [4]) and the actin polymerization activity [3]. Both of these properties follow from our calculations [Eq. (5)].

Recent experiments [4] have measured the correlation function for the normal membrane velocity \( \nu_n = \dot{h} \), which we compare to our calculations [Eqs. (1)–(3)] in Fig. 3, using the parameters given in Table I [14]. We find good agreement with the typical measurements [4]. The periodicity in \( x \) space is given by \( q_m \), while the periodicity in \( t \) space is given by \( \omega'' \sim \nu q_m \). The temporal decay is determined by the values of \( \omega'(q) \) around \( q_m \), while the spatial decay is determined by the width of the peak of the function \( \omega'(q) \) at \( q_m \) (inset of Fig. 3). We show in Fig. 3 that an increase of the surface tension will induce a faster temporal decay and increase the oscillations time and length, while the wave velocity is unaffected.

Membrane ruffles [1,2,5], which are linear protrusions that travel away from the leading edge, have velocities similar to those we calculated above. The initiation of these ruffles was observed to be concurrent with the event of membrane-substrate adhesion [1], but this connection is still not understood. We show here the results of a one-dimensional numerical simulation of our model (without thermal noise), where the membrane is perturbed by a Gaussian dip at time \( t = 0 \) (Fig. 4), to mimic the effect of the adhesion event. A traveling wave of membrane...
undulation followed closely behind by a wave of actin and myosin (in this order) is formed and propagates rearwards. These calculated features have been recently observed in great detail [1,2]. In the inset we show the waves at longer times, and therefore demonstrate that they propagate while maintaining their overall shape.

Our prediction that the wave velocity depends on the myosin activity [Eq. (5)] is in excellent agreement with the observations of EGF-activated cells (increased myosin activity) [15], while in cells treated with a drug that inhibits myosin activity, these ruffles either disappear [1] or their velocity diminishes [2].

To conclude, we presented a minimal model coupling the membrane curvature, actin protrusion, and myosin contraction forces, which gives rise to robust membrane waves, as observed on the surface of living cells. The real cell is much more complicated than described here, and therefore other physical and biochemical processes [2,16], in different cell types, may be the dominant mechanisms for the observed membrane oscillations. Our simple model allows us to make the following testable predictions: (i) the wave velocity is predicted to depend on the myosin activity according to Eq. (5) and (ii) the velocity-velocity correlation function should depend on the membrane surface tension, as shown in Fig. 3.

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**TABLE I.** List of the parameters used in the calculations; when two numbers are given the first corresponds to Fig. 2 and the second to the inset of Fig. 3; otherwise, the same value is used for both. The value of A’ corresponds to the inset of Fig. 3. The values of \( n_0, A \) and A’ are chosen to give the measured range of actin flow velocities \( \sim 0.01 \mu \text{m/sec} \) [10].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H ) [( \mu \text{m}^{-1} )]</td>
<td>-200, -50</td>
<td>0, 9</td>
</tr>
<tr>
<td>( D ) [( \mu \text{m}^2/\text{sec} )]</td>
<td>0.402, 1</td>
<td>0.25, 2.5</td>
</tr>
<tr>
<td>( \eta ) [g/( \mu \text{m sec} )]</td>
<td>100, ( \eta_{\text{water}} = 10^{-4} )</td>
<td>2.4 ( \times 10^{-7} ), 4.5 ( \times 10^{-8} )</td>
</tr>
<tr>
<td>( \Lambda ) [sec/g]</td>
<td>10^4</td>
<td>4.5 ( \times 10^{-8} )</td>
</tr>
<tr>
<td>( d ) [( \mu \text{m} )]</td>
<td>0.1</td>
<td>10^3, 300</td>
</tr>
<tr>
<td>( n_0 ) [( \mu \text{m}^{-2} )]</td>
<td>10^3</td>
<td>0.1, 0.0153</td>
</tr>
</tbody>
</table>

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**FIG. 4** (color). One-dimensional simulation using our linearized model (without thermal noise), showing the formation of moving waves due to sudden membrane perturbation. We plot here the membrane height \( h(x) \), myosin \( m(x) \), and actin \( n(x) \) density fluctuations as a function of position and time; \( t = 0 \) sec (light blue), \( t = 1 \) sec (blue), \( t = 2 \) sec (green), and \( t = 3 \) sec (red). Note that for the height profile at \( t = 0 \) use the right-hand scale. In the inset we show the waves at longer times; \( t = 60 \) sec (blue), \( t = 90 \) sec (green), and \( t = 120 \) sec (red).