# Supplementary Table 1: Model Assumptions

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Rationale for assumption</th>
<th>Level of confidence in assumption</th>
<th>How critical is the assumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>There is a constant number of branching events per second per cell.</td>
<td>It produces prediction of the scaling property of the graded actin filament density that many models fail to produce.</td>
<td>Moderate</td>
<td>Moderately critical; other models could predict graded density.</td>
</tr>
<tr>
<td>The density of pushing filaments at the rear corners of the lamellipodium is zero.</td>
<td>It is likely that this density is small; assuming it zero simplifies the model and produces excellent parameter-less fit.</td>
<td>Moderate</td>
<td>Moderately critical; assuming a small, non-zero density produces reasonable fits as well.</td>
</tr>
<tr>
<td>Membrane tension is spatially constant.</td>
<td>Known for in vitro membrane physics; see estimates in this paper.</td>
<td>High</td>
<td>Highly critical.</td>
</tr>
<tr>
<td>Cell shape can be approximated by a slightly bent rectangle.</td>
<td>Shape analysis.</td>
<td>High</td>
<td>Not very critical; it makes the model algebra much easier.</td>
</tr>
<tr>
<td>Membrane resistance is distributed equally locally among the growing filaments.</td>
<td>Theoretical arguments previously published.</td>
<td>High</td>
<td>Highly critical.</td>
</tr>
<tr>
<td>Protrusion is force-limited; the force–velocity relation is concave down.</td>
<td>Indirectly indicated by our data; previously published measurements.</td>
<td>High</td>
<td>Highly critical.</td>
</tr>
<tr>
<td>Filaments grow on average in a direction locally normal to the boundary.</td>
<td>Previously published work.</td>
<td>Moderate</td>
<td>Moderately critical; other mechanisms would complicate the model.</td>
</tr>
<tr>
<td>Growing filaments are stalled or buckled at the cell sides.</td>
<td>Speculation</td>
<td>Moderate</td>
<td>Highly critical; this is the central assumption of our force-balance model.</td>
</tr>
<tr>
<td>Myosin-powered contraction produces a significant centripetal actin network flow only at the very rear of the cell.</td>
<td>Measurements of actin network flow.</td>
<td>High</td>
<td>Not very critical; otherwise, relatively small corrections to the model required.</td>
</tr>
</tbody>
</table>
Supplementary discussion

1. Mathematical model of cell shape

Sub-model of actin filament distribution along the leading edge

Following our previous work\(^8\), we observe that the actin network is organized in a dendritic array with actin filaments oriented at approximately \(\pm 35^\circ\) relative to the direction of protrusion, and model the densities of right- and left-oriented growing barbed ends along the leading edge with functions \(b^* (l,t)\). We assume that Arp2/3-mediated filament branching takes place at the same rate for each leading-edge filament. This per-filament rate is equal to the total number of filaments nucleated over the whole leading edge per second divided by the total number of the uncapped leading-edge filaments. The molecular pathway determining this rate is unknown; a plausible mechanism could be based on rapidly diffusive molecules, the total number of which is conserved, controlling the total number of branching events per cell. Assuming that the branching takes place only along the leading edge, each filament has equal probability to become a “mother” filament. Then, as the total number of growing filament ends increases, the branching rate per filament decreases inversely. A filament at \(+35^\circ\) branches off filaments oriented at \(-35^\circ\), and vice versa. As filaments are skewed with respect to the direction of protrusion, their barbed ends slide laterally along the leading edge as they grow. Finally, the filaments get capped and lag behind the leading edge. These assumptions lead to the following equations for the densities of growing barbed ends along the leading edge:

\[
\frac{\partial b^*}{\partial t} = V \frac{\partial b^*}{\partial l} + \frac{\beta b^*}{B} - \frac{\gamma b^*}{\text{capping}} + \frac{1}{\text{total number of filaments at the leading edge}} \int_{-L/2}^{L/2} [b^* (l,t) + b^- (l,t)] \, dl
\] (1)

Here \(V\) is the lateral flow rate (which is close to cell speed), \(l\) is the arc length along the leading edge, \(\gamma\) represents the constant rate of capping and \(\beta\) is the total number of nascent filaments branching out per cell per second. The actual lateral flow rate is graded along the leading edge (rather than constant as assumed here) due to the curvature of the leading edge, but this does not affect the results, as the respective term is but a small perturbation\(^8\).

We choose the boundary conditions at the rear corners of the leading edge, \(l = \pm L/2\) (where \(L\) is the total length of the leading edge) as follows:

\[
b^* (-L/2,t) = 0, b^- (L/2,t) = 0
\] (2)

The biological meaning of these conditions is that at the rear sides of the leading edge, where large adhesions are located\(^9\), the “age” of any right (left)-oriented filaments at \(-L/2\) (\(+L/2\)) is zero, since they immediately glide to the right (left) away from the side. These boundary conditions are the simplest possible ones, but others are also possible (discussed previously\(^8\)). Note that the solutions of (1-2) derived below have the following feature: \(b^* (\pm L/2,t) + b^- (\pm L/2,t) = 0\), so the total barbed end density at the rear corners
of the leading edge is close to zero according to this model. We chose these boundary conditions because they fit the data well. These conditions would occur naturally if branching ceased at the large adhesions located at the rear corners of the leading edge, where vigorous actin network flow and remodeling take place. Regardless of the exact mechanism, we assume that there are specific local conditions responsible for maintaining a constant, very low density of uncapped barbed ends at the sides. All the modeling results remain valid if the boundary conditions at the sides are changed from zero to a low but non-zero density (at least 4-5 times lower than that at the center).

To non-dimensionalize equations (1-2), we choose the cell’s leading edge length, $L$, as the length scale; the characteristic time of capping, $\tau = 1/\gamma$, as the time scale; and the total number of nascent filaments branched out per unit length of the leading edge over the characteristic time scale, $\hat{b} = \beta \gamma / L$, as the filament density scale. This allows us to rescale the equations introducing the non-dimensionalized time, distance and densities:

$$t = \tau \tau' = 1/\gamma, \quad l = L L = L, \quad b = \hat{b} \gamma / \gamma, \quad b = \hat{b} \gamma / \gamma,$$

dimensional variables into equations (1-2) leads to the non-dimensional system:

$$\frac{\partial b}{\partial t} = \mp \lambda \frac{\partial b}{\partial s} + b^+ - b^- B = \int_{-1/2}^{1/2} \left[ b^+ + b^- \right] ds, b^+ (-1/2, t) = 0, b^- (1/2, t) = 0. \quad (3)$$

Here $\lambda = V L / (\gamma L)$. The capping rate, $\gamma$, is of the order of $1/$sec$^{10}$, the lateral flow rate $V \sim 0.1 \mu m/$sec, and the leading edge length $L \sim 30 \mu m$. Thus, in the biologically relevant regime, barbed ends are capped within seconds, long before they move laterally across the leading edge: $\lambda \sim 0.01 \ll 1$. We are interested in the steady state actin distribution, so equations (3) becomes,

$$\mp \lambda \frac{db}{ds} + b^+ - b^- = 0. \quad (4)$$

Equations (4) can be solved with the standard substitution,

$$b^+ = c_+ \cdot \exp (\kappa s), b^- = c_- \cdot \exp (\kappa s),$$

which turns the differential equations (4) into an algebraic system:

$$\begin{bmatrix}
(1 + \lambda \kappa) & -1/B \\
-1/B & (1 - \lambda \kappa)
\end{bmatrix}
\begin{bmatrix}
c_+ \\
c_-
\end{bmatrix} = \begin{bmatrix} 0 \\
0 \end{bmatrix}.$$  

This system has a non-trivial solution if

$$\det \begin{bmatrix}
(1 + \lambda \kappa) & -1/B \\
-1/B & (1 - \lambda \kappa)
\end{bmatrix} = 0,$$

leading to the equation $1/B^2 = 1 - \lambda^2 \kappa^2$ (below, we demonstrate that $|\kappa| \sim 1$, and so $B = 1$ and $b^+ = b^-$, so the local densities of left- and right-oriented filaments are almost equal). This equation with the boundary condition (3) allows two solutions, both with $\kappa$ an imaginary number: $\kappa = \pm i \kappa_0, \kappa_0 = \frac{1}{\lambda} \sqrt{1/B^2 - 1}$. Then,
\[
\begin{bmatrix}
 b^+ \\
 b^-
\end{bmatrix} = C_1 \begin{bmatrix} 1 \\
 1
\end{bmatrix} \exp(\kappa_0 s) + C_2 \begin{bmatrix} 1 \\
 1
\end{bmatrix} \exp(-\kappa_0 s).\]

Due to the symmetry of the problem, \(C_1 = C_2 = C\), and \(b^+ = b^- = C \cos(\kappa_0 s)\) (the \(\sin(\kappa_0 s)\) term disappears due to the symmetry). A system of three equations then determines three unknowns, \(B, C, \kappa_0\):

\[
B = \frac{4C}{\kappa_0} \sin\left(\frac{\kappa_0}{2}\right), \quad B^2 = \frac{1}{1 + \lambda^2 \kappa_0^2}, \quad C \cos\left(\frac{\kappa_0}{2}\right) = 0
\]

From (5), we find \(\kappa_0 = \pi, B = 1, C = \pi/4\). The approximate analytical solution for the stationary non-dimensional actin filament density along the leading edge, \(b^+ + b^- = (\pi/2) \times \cos(\pi s)\), is in excellent agreement with the numerical solution, as well as with the data (Fig. 3b; see also Lacayo et al.). Specifically, the actin filament density distribution exhibits the predicted scaling – the distribution is a function of the ratio of \(l/L\) – so when the distance is scaled by cell length, and the density by its maximal value, the distributions in all cells are similar.

The predicted dimensional actin filament distribution has the form:

\[
D(l) = b^+(l) + b^-(l) = \frac{\pi}{2} \frac{\beta}{L^\gamma} \cos\left(\frac{\pi l}{L}\right)
\]

This distribution has a concave down profile with a maximum \(-\beta/\gamma L\) at the center of the edge and a minimum \(-\beta/\gamma L\) at the rear side corners. For the following, we approximate this distribution with an inverted parabolic function (Fig. 3c):

\[
D(l) = \frac{\beta}{L^\gamma} \left(1 - \left(\frac{l}{L/2}\right)^2\right)
\]

This approximation to the trigonometric function is very close and makes all subsequent algebra much simpler. We tested the results from all formulae derived from this approximation against those from the trigonometric equations and found that the results are not affected by this approximation.

With the model’s boundary conditions, more nascent filaments branch out closer to the center of the cell. This, in turn, increases the net branching rate at the center, because more nascent filaments branch off the higher number of the existing filaments at the center. The existing growing barbed ends start to effectively compete for resources (because the total number of branching events per second is conserved), and if the actin filament density at the cell sides is kept low, the center ‘wins’. This positive feedback is the reason for the characteristic inverted parabolic profile of the actin filament distribution.

It is worth mentioning that our measurements show inverted parabolic actin filament distributions along the central part of the leading edge, as predicted. However, the filamentous actin density at the sides does not decrease significantly from the front corners to the rear corners, so we cannot directly test the validity of the boundary conditions (the parameter-free fit of the prediction to the data (see below) is an indirect test). Our explanation of why the measured actin filament density at the sides does not
decrease significantly hinges on the fact that with actin labeling experiments we cannot distinguish between growing, pushing filaments (the density of which is expected to decrease), and capped/stalled/buckled filaments. According to the model, actin filaments are either stalled or buckled at the front side corners of the lamellipodium. These stalled/buckled filaments contribute to the measured filamentous actin density along the lamellipodial sides, but do not contribute to protrusion. As the cell moves forward, more stalled/buckled filaments accumulate towards the rear of the lamellipodial sides, so the fraction of the filamentous actin density that is due to those filaments has to grow from front to rear along the sides. Thus, the fact that the measured filament density along the sides is more or less constant likely indicates that the density of growing, pushing filaments indeed decreases from front to rear at the sides.

Finally, note that according to the model, the actin filament density along the leading edge is proportional to the density of the uncapped growing barbed ends there. The density of uncapped growing barbed ends at the leading edge is approximately determined by the following balance: \( \frac{db}{dt} = \left( \frac{\beta}{B} \right) - \gamma b \). On the other hand, the density of capped barbed ends increases due to capping and decreases due to the fact that capped ends cease to grow and thus lag behind the cell front (which continues to protrude with rate \( V \)). Thus, within a narrow zone of width \( u \) at the leading edge, the density, \( c \), of the capped barbed ends is determined by the following balance: \( \frac{dc}{dt} = \gamma b - \left( \frac{V}{u} \right) c \), where the rate of the capped ends leaving the edge is equal to the width of the zone divided by the protrusion rate. At steady state, \( b = \left( \frac{\beta}{\gamma B} \right) \), and \( c = \left( \frac{\gamma u}{V} \right) b \). The total number of the leading edge filaments is thus, \( (c+b) = \left[ 1 + \frac{\gamma u}{V} \right] b \). The natural scale for the parameter \( u \) is the average actin filament length, which is of the order of \( V/\gamma \).

Thus, the factor \( \left[ 1 + \frac{\gamma u}{V} \right] \) is a dimensionless parameter of order unity, which is independent of any cell-dependent variables, so the total density of actin filaments at the leading edge, both capped and uncapped, is proportional to the capped filaments’ density there. The actin filament number density, in turn, is proportional to the total density of filamentous actin at the leading edge.

### Cell geometry

As illustrated by our shape analysis (Fig. 1b), keratocyte shapes can be largely described by two parameters – shape modes 1 and 2, mostly corresponding to cell area and aspect ratio, respectively. Thus, keratocyte shapes can be approximated reasonably well by a simple geometric figure, such as a rectangle (inset, Fig. 3d).

Let us assume therefore that the lamellipodium is a rectangle characterized by its area \( (A) \) and aspect ratio \((S)\), or alternatively by its length \((x)\) and width \((y)\), so that \(xy = A\), \(x/y = S\). Note, that the total leading edge length is, \( L = x + 2y \). The following are obvious geometric relations:
\[ x = \sqrt{A/S} , \quad y = \sqrt{A/S} , \quad L = \sqrt{A/S} + 2\sqrt{A/S} \]  

(8)

For the observed range of aspect ratios, \(1 < S < 4\), the total leading edge length varies only \(~ 10\%\) as the aspect ratio varies 4-fold, and the approximate expression for this length is \(L \approx 3\sqrt{A}\), which is supported by the data.

The validity of the rectangular approximation is supported by the observation that across the population of cells, the measured perimeter \(P_m\) is nearly equal to the perimeter calculated from the rectangle approximation using each cell’s measured area and aspect ratio, \(P_c = 2(x + y) = 2\left(\sqrt{AS} + \sqrt{A/S}\right)\) (Fig. S7). It is worth noting that keratocyte shapes could also be approximated by other relatively simple geometric figures quantified by two parameters, such as a half-ellipse, rather than a rectangle. In those cases, a model similar to the one presented here could be built and similar predictions would be generated, though the algebra would become much more involved. Note however, that in most cases, the shapes qualitatively appear more rectangular than half-elliptical (Fig. 1a), so such approximation apart from being less tractable would also lead to larger errors.

Let us also note that approximating the actual cell shape by a rectangular one introduces small errors that are hard to control. For example, both the front and the sides of cells are convex, and we, in fact, estimate the leading edge curvature below. Rough geometric estimates (not shown) indicate that this could lead to systematic underestimation of the leading edge length by \(~ 10-20\%\). Also, there are small ambiguities in the locations of the front and rear corners in real cells, and therefore in the appropriate boundary conditions. The accumulated errors jeopardize neither the orders of magnitude of the theoretical estimates, nor the predicted correlations between variables; however, together with experimental errors and stochastic effects in cell behavior, this limits our ability to make exact fits and calculations at this point.

**Force balance determines cell shape**

A growing actin network can be characterized by the so-called force–velocity relation\(^{11}\). When no force resists filament growth, protrusion occurs at the free polymerization rate \(V_0\). The free polymerization rate is equal to \(V_0 = k_{on} \delta G - k_{off} \delta\), where \(k_{on} = 10/\left(\mu M \cdot \text{sec}\right)\) and \(k_{off} = 1/(\text{sec})\) are, respectively, the monomer assembly and dissociation rates at the barbed ends of actin filaments\(^{10}\), \(\delta = 3\, \text{nm}\) is the length increment of a filament upon an assembly event, and \(G\) is the actin monomer concentration at the leading edge. Note that \(k_{off} \delta \sim 1\,\text{nm/s} \ll V_0\), so we can approximate the free polymerization rate as, \(V_0 \approx k_{on} \delta G\). As the load force, \(f\), applied to a filament’s barbed end increases, the protrusion rate, \(V\), decreases until at the stall force, \(f_{\text{stall}}\), it ceases. Thus, \(V(f = 0) = V_0, V(f = f_{\text{stall}}) = 0\). In this force–velocity relation the force is per filament. The stall force per filament, \(f_{\text{stall}} \sim 2 - 3\,pN\), has been measured, albeit
indirectly\textsuperscript{12,13}. In fact, longer filaments can buckle\textsuperscript{14} rather than stall; the resistance could bend a filament so that it starts to grow in parallel with the boundary, which is equivalent to stalling protrusion. There are experimental indications that this buckling mechanism is plausible in motile cells\textsuperscript{15}; estimates show that with the observed lengths and elastic properties of actin filaments, the buckling force would be of the same order of magnitude as the stall force\textsuperscript{16}. Whether filaments in motile keratocytes are buckled or stalled at the sides is an unresolved question that will also depend on the local mesh organization.

In the lamellipodium, the membrane resistance force is distributed among neighboring filaments almost equally\textsuperscript{2}, so the force per filament can be approximated by $f = T / D$. Here $T$ is the membrane tension (force per unit length of the cell boundary), which, as described below, is spatially constant (but can, in principle, fluctuate in time). As $D$, the number of pushing barbed ends per unit length of the boundary, is graded in space, the force per filament also varies along the leading edge with a minimum at the center and increasing toward the sides. In subsequent derivations, we use the following notations: the actin filament density at the center of the leading edge is denoted $D_c$, and from (7), $D_c = \beta / (L \gamma)$. At the sides of the front edge defined by $l = \pm x / 2$, the density is

$$D_s = \frac{\beta}{L \gamma} \left(1 - \left(\frac{x}{L}\right)^2\right).$$

Finally, we denote the ratio $D_c / D_s$ as $D_{cs}$.

It is very convenient to introduce the lumped model parameter:

$$z = \frac{T \gamma}{f_{\text{stall}} \beta},$$

which is the ratio of membrane tension to the total force needed to stall the network of growing actin filaments at the central lamellipodium. Recall that $D_c = \beta / (\gamma L)$, so

$$\frac{T}{f_{\text{stall}} D_c} = \frac{T \gamma L}{f_{\text{stall}} \beta} = z L.$$

We assume that the sides of the leading edge are defined by where filaments are mechanically stalled by the membrane tension: $V_s = 0, T / (f_{\text{stall}} D_s) = 1$. Then,

$$T = f_{\text{stall}} \frac{\beta}{L \gamma} \left(1 - \left(\frac{x}{L}\right)^2\right),$$

or,

$$z = \frac{1}{L} \left(1 - \left(\frac{x}{L}\right)^2\right).$$

From (8), $\frac{x}{L} = \frac{S}{S + 2}, 1 - \left(\frac{x}{L}\right)^2 = \frac{4(S + 1)}{(S + 2)^2}$, and $\frac{1}{L} = \frac{\sqrt{S}}{\sqrt{A(S + 2)}}$ so,

$$z = \frac{4\sqrt{S}(S + 1)}{\sqrt{A(S + 2)}},$$

and (see also Fig. 3d),
Force–velocity relation determines leading edge radius

The shape of the lamellipodium in real cells is obviously rounded (Fig. 1a). Thus to further characterize lamellipodial shape, in addition to length and width, we introduce the approximate leading edge radius, R. The rounded shape of the leading edge of motile keratocytes is maintained through a graded distribution of protrusion along the edge. As explained in the main text, we hypothesize that membrane tension is the crucial limiting factor for filament elongation and protrusion, and explain the curvature of the leading edge as follows. At the maximal actin filament density in the middle of the leading edge, the load imposed by membrane tension is carried by many filaments and thus the force per filament is small, so filament growth rate is high. Toward the sides, the filament density decreases and the load per filament gradually increases, so the growth rate decreases. This smoothly decreasing growth rate toward the sides of the lamellipodium then leads to a curved leading edge. While the exact form of the force–velocity relation was not required above in determining the approximate cell shape (i.e. the cell aspect ratio in the rectangular shape approximation), it is required for the calculation of the effective curvature of the leading edge described below as well as for determining the relationship between cell speed and cell morphology discussed in the next section.

The force–velocity relation for individual actin filaments has not been measured directly (theory suggest that it is concave up, or more complex: concave down at small forces and concave up at greater loads). Moreover, the single filament force–velocity relation does not directly determine the relation for a network of actin filaments; while the stall force for a network of filaments is approximately equal to the stall force per filament times the number of filaments, the network force–velocity relation elsewhere is not simply obtained from the individual filament relation assuming the force is divided by the filament number. One possible reason for this is the existence of a mechanochemical feedback between filament number and force. In the most extreme form of this feedback, the filament number is proportional to force, so that velocity is simply independent of force until the whole network crushes at the stall force. This extreme case would lead to a step-function-like force–velocity relation (and in our case, a rectangular shaped cell as the protrusion rate along the front of the leading edge would be uniform). Other reasons for a more complicated network force–velocity relation include properties of force-sensitive adhesions and elastic recoil of the actin network (discussed elsewhere).

In a very general form, the force–velocity relation can be expressed by the formula:

\[ V = V_0 \left( 1 - \left( \frac{f}{f_{\text{stall}}} \right)^w \right). \]  

(14)
(There are many other elementary functions that could be used to express this relation, e.g. \[ (1 + \exp[w(f - f_{\text{stall}})])^{-1} \]; the results do not depend on the particular choice of functions, but the calculations are simplest when power functions of the force are used.) By varying the parameter \( w \), all ever-observed force–velocity relations can be approximated, except for a few cases where non-monotonic\textsuperscript{18}, or crossover\textsuperscript{19} relations were predicted or observed. In the latter cases, the mechanism involved is too complex or not well understood, so at present such cases are ignored.

The parameter \( w \) determines how robust protrusion is. If \( w = 1 \), the protrusion rate decreases linearly with increasing resistive force; at half the stall force, the protrusion rate goes at half the free polymerization rate. If \( w < 1 \), the force–velocity relation is concave up, so the protrusion rate decreases rapidly with small increases in the resistive force; at half the stall force, the protrusion rate is less than half the free polymerization rate. Such a concave up force–velocity relation was measured, for example, for \textit{in vitro} actin networks assembled from purified proteins\textsuperscript{20}. These results, however, do not agree with a more directly relevant measurement performed on the lamellipodial network in fish keratocytes which showed a concave down force–velocity relation\textsuperscript{5} or with measurements on \textit{in vitro} actin networks assembled in cytoplasmic extracts\textsuperscript{21} which are discussed below. Finally, if \( w > 1 \), the force–velocity relation is concave down, so the protrusion rate decreases slowly with small increasing resistive force; at half the stall force, the protrusion rate is greater than half the free polymerization rate. In this case protrusion is robust: the protrusion rate is nearly constant at loads weak relative to the stall force, while at greater loads the speed decreases drastically. Mathematically, as \( w \) increases, the force–velocity relation becomes closer to a step function. We show the shapes of the force–velocity relations given by (14) for \( w = 1, 2, 4, 8 \) (Fig. S6b).

Direct measurements in motile keratocytes\textsuperscript{5} and in \textit{in vitro} actin networks\textsuperscript{21} both resulted in a concave down force–velocity relation that we approximated mathematically, based on the published data, by a relation of the form (14) with \( w \approx 4 \) (fits not shown). However, the value of the parameter \( w \) is by no means established for the force–velocity relation characterizing a lamellipodium that is protruding steadily. It is notoriously difficult to measure the force–velocity relation and interpret the results of those measurements. Moreover, both cited experimental studies did not measure the force–velocity curve directly, but rather derived it from dynamic data: the measurements were performed in a transient regime in which growing actin networks were slowed down rapidly, over seconds, by increasing the force imposed by an AFM cantilever. There is no guarantee that actin networks in such experiments have enough time to adjust to changing conditions (the characteristic time of network re-modeling is in the second to tens of seconds range\textsuperscript{11}), and indeed one of the studies found hysteresis behavior\textsuperscript{21}. It is therefore plausible that in a steadily protruding network, the actin architecture adapts to achieve a more robust force–velocity relation that is less sensitive to the resistive force, so the actual force–velocity relation would be characterized by a more step-like function with \( w > 4 \).

Assuming that filaments grow in a direction locally normal to the boundary, and taking into account the persistence of keratocyte shape through time\textsuperscript{6,22}, we can relate the
angle between the normal to the cell edge and the direction of crawling with the local filament growth rate (Fig. S6a):

\[ \cos \theta (l) = \frac{V(l)}{V(0)} \]  \hspace{1cm} (15)

where \( \theta (l) \) is the orientation of the normal to the leading edge at position \( l \), and \( V(l) \) is the normal protrusion rate there; note that \( V(0) = V_{cell} \). As the protrusion rate decreases toward the sides, the angle \( \theta \) increases leading to the curved leading edge. From trigonometry, for small \( l \) and \( \theta \), \( \cos \theta = 1 - \theta^2 / 2 \), and \( l = R \sin \theta = R \theta \), where \( R \) is the approximate lamellipodial radius in the middle of the leading edge. So, \( R = l / \theta \). Taking the force–velocity relation in (14) we have,

\[ V(l) = V_0 \left( 1 - \left( \frac{T}{f_{stall} D(l)} \right)^w \right). \]  \hspace{1cm} (16)

Using formulae (7, 16) and simple calculus, we find the graded lateral change of the locally normal protrusion rate: \( V(l) = \frac{dV}{dD} \left( \frac{D^c}{L/2} \right)^2 \). Substituting this expression into (15) and using the approximation \( \cos \theta = 1 - \theta^2 / 2 \), we obtain the formula,

\[ 1 - \theta^2 / 2 = 1 - \frac{dV}{dD} \left( \frac{D^c}{L/2} \right)^2, \]  \hspace{1cm} (17)

from which we can express the angle \( \theta \) in terms of the coordinate \( l \) as \( \theta \approx 2 \sqrt{\frac{2dV/dD \cdot l}{V_{cell}/D_c \cdot L}} \). Thus, \( R = \frac{l}{\theta} \approx \frac{L}{2} \sqrt{\frac{V_{cell}/D_c}{2dV/dD}} \). Using (10, 16), we have:

\[ \frac{V_{cell}}{D_c} = \frac{V_0}{(1/2L)} \left[ \left( \left( \frac{zL}{w} \right)^w \right) - 1 \right], \]  \hspace{1cm} (18)

finally giving,

\[ R \approx L \frac{\sqrt{(zL)^w - 1}}{2\sqrt{2w}}. \]  \hspace{1cm} (19)

Using equations (8, 13), \( R \) can now be expressed as a function of cell area and aspect ratio:

\[ R \approx \frac{\sqrt{AS + 2\sqrt{AS}}}{2\sqrt{2w}} \left[ \frac{4(S+1)}{(S+2)^2} \right]^w - 1. \]  \hspace{1cm} (20)

We used (18) to calculate the expected leading edge radius of cells from the measurements of \( A \) and \( S \) using different force–velocity relations characterized by \( w = 1, 2, 4, 8 \), and plotted the predicted versus the measured radii in Fig. S6c. For all these values of the parameter \( w \), the theory gives the right order of magnitude for the radius, and correctly predicts its correlations with the area and aspect ratio of the cell. Quantitatively, \( w = 8 \) gives a very good fit between experiment and theory.

Note also that this model explains a curious observation: high-aspect-ratio cells have relatively broad (high radius) lamellipodia, which implies uniform protrusion.
velocities across the leading edge, yet these cells also have high actin filament density at
the cell midline and steep actin density gradients toward the sides. Conversely, low-aspect-ratio cells have round lamellipodia, which imply steeply graded velocity
differences, but have relatively flat, uniformly low filamentous actin density profiles.
This apparent paradox can be resolved by noting that at high actin densities (low force
per filament) the force–velocity relation is saturated, so steep actin-density fall-off does
not translate to great changes in velocity along the leading edge; the opposite is true at
low actin densities, allowing for relatively flat density profiles to yield highly graded
velocities.

**Force–velocity relation determines cell speed**

Cell speed is approximately equal to the protrusion rate at the center of the leading edge
since the retrograde actin network flow at the center of the leading edge is very low
\(^7,22,25\). In order to calculate cell speed, again knowledge of the specific dependence of the
protrusion rate on the membrane tension-generated resistive load is needed. Taking, as
above, the force–velocity relation in (14) and again assuming that the cell speed, \(V_{\text{cell}}\),
is equal to the maximum protrusion rate (found at the middle of the lamellipodium, \(l=0\)), we
obtain,

\[
V_{\text{cell}} = V_0 \left(1 - \left(\frac{T}{f_{\text{stall}}D_c}\right)^w\right) = V_0 \left(1 - (zL)^w\right) = V_0 \left(1 - \left(\frac{4(S+1)}{(S+2)^2}\right)^w\right)
\]  

(19)

In Fig. S6d, we compare the fits obtained using equation (19) for cell speed as a function
of aspect ratio to the data for \(w = 1,2,4,8\). It is clear that \(w = 1\), though qualitatively
predicting the correct trend, is quantitatively poor; \(w = 4\) is better, and again \(w = 8\) gives a
very good fit. Thus, two experimentally independent observations regarding (a) the
relation between cell speed and aspect ratio, and (b) the geometrical relation between the
lamellipodial radius and cell area and aspect ratio (previous section), are both well fit by
our model when we take \(V = V_0 \left(1 - \left(\frac{f}{f_{\text{stall}}}\right)^8\right)\) as the force–velocity relation. Explicitly
for cell speed this implies:

\[
V_{\text{cell}} = Gk_{\text{on}} \delta \left(1 - (zL)^8\right),
\]

(20)

where we have neglected the contribution of the monomer off-rate.

**Membrane physics relevant to the cell shape model**

Experimental and theoretical estimates of physical parameters characterizing the plasma
membrane of the cell can be gleaned from the literature\(^26-27\). Most of the measurements
reported were made for pure lipid bilayers; however, the corresponding parameters in
biological membranes, when measured, are typically only a few fold different than those
in pure lipid bilayers\(^28\), which will not change the order-of-magnitude estimates presented
here.
First, we argue that the membrane tension is spatially constant; it is the same at the front, side and rear of the cell. This assumption is crucial for our model. The membrane tension can, in principle, change in time, but synchronously at all locations around the cell. The theoretical argument is very straightforward: if there is a difference in membrane tension between the rear and front in the cell, then the rate of membrane flow would be \( v \sim \frac{\sigma x^2}{\eta y} \), where \( y \) is the lamellipodial length, \( x \) is the lamellipodial width, and \( \eta \) is the membrane viscosity. Characteristic viscosity is \( \eta \sim 0.01 \text{pN} \cdot \text{s} / \mu \text{m} \)\(^{29,30} \); characteristic tension amplitude is \( \sigma \sim 100 \text{pN} / \mu \text{m} \)\(^{4} \); other characteristic values are \( y \sim 10 \mu \text{m} \), \( x \sim 30 \mu \text{m} \). So, the membrane would flow with an enormous rate of \( v \sim 10^6 \mu \text{m} / \text{s} \). Thus, even negligibly small differences in membrane tension \( \sim 1 \text{pN} / \mu \text{m} \) would be diminished to zero by lipid flow in just \( \sim 10 \mu \text{m} / \left(10^4 \mu \text{m} / \text{s} \right) \sim 0.001 \text{s} \). Hence on the scale of seconds and minutes relevant to cell motility, the membrane tension is constant in space. Note that experimentally it is established that there is no lasting membrane flow in keratocytes\(^31 \).

Second, simple estimates demonstrate that membrane tension is not due to membrane elasticity, but arises primarily from the resistance of the membrane to actin pushing forces at the front and sides of the cell. At the rear of the cell, this tension is maintained by the actin network’s resistance. Let us introduce the effective line tension at the membrane edge, \( \Gamma \). The physical meaning of this line tension is the thermodynamic work required to elongate the edge by a unit length. There is a ‘horizontal’ edge curvature contribution to the line tension; the corresponding free energy per unit area of membrane is equal to \( \frac{\kappa}{R^2} \), where \( \kappa \sim 20k_B T \) is the membrane bending modulus\(^{26,32} \), and \( R \) is the effective vertical radius of lamellipodial curvature; plus energy of tension \( e \) concentrated at the edge. To find \( \Gamma \), let us add length \( \delta \) to the edge. If \( h \) is the lamellipodial height, we add a membrane area \( a = h \delta \). The effective radius of “vertical” curvature of the edge is simply \( \sim h \). So, the added energy is \( E \sim \left( \frac{\kappa}{h^2} + e \right) \times h \delta \sim \left( \frac{\kappa}{h^2} + e \right) \times h \delta \sim \left( \frac{\kappa}{h} + eh \right) \times \delta \).

The effective line tension is \( \Gamma = E / \delta \sim \left( \frac{\kappa}{h} + eh \right) \). In this expression, \( h \sim 0.1 \mu \text{m} \) and \( \kappa \sim 20 k_B T \). If \( e \sim 100 \text{pN} / \mu \text{m} \) (see below), then \( eh \sim 10 \text{pN} \), while \( \frac{\kappa}{h} \sim 1 \text{pN} \), so the bending part of the energy can be neglected, and \( \Gamma \sim eh \). Now, the actin filament pressure pushing the membrane at the leading edge generated by filaments, \( T \), is balanced by the membrane tension, \( e \). According to Laplace’s Law, if a pressure \( (T - e) \) is restrained by a curved membrane boundary under tension, then \( \frac{\Gamma}{R} = T - e \), or \( e \left( 1 + \frac{h}{R} \right) = T \). Because \( \frac{h}{R} \sim 0.01 \), \( e = T \), so the membrane tension is simply equal to the pressure from the growing actin filaments at the edge.
The magnitude of this pressure can be estimated theoretically; the force per filament is in the pN range\(^1\), and there are \(\sim\)100 filaments per micron at the leading edge\(^3\), so \(T \sim 100 \, \text{pN/\mu m}\). Tension of similar magnitude was estimated from experiments in which a membrane tether was pulled from the surface of a motile fibroblast\(^4\). Note, that if this value is substituted into the definition of the lumped parameter \(z\) in (10), then agreement with the data is achieved if there are a few thousands of pushing filaments per cell, which agrees with previous experimental estimates\(^3\).

This tension is too weak to stretch the membrane significantly: to change membrane area by a few percent, a tension of \(\sim 10^5 \, \text{pN/\mu m}\) is needed\(^2\). Note also, that the membrane tension is not only constant across the membrane of an individual cell; measurements in a population of fibroblasts suggest that there is little variation in tension across a population of cells\(^4\). There is also independent evidence that protrusion rate in fibroblasts is limited by membrane tension\(^4\).

The simplest interpretation of our observation that cell area fluctuates only \(\sim\)1\% on the scale of seconds to an hour is that the membrane is pulled taut around the lamellipodium and cell body, and there is very slow exchange of membrane between the exterior cell membrane and its intracellular stores. Indeed, in motile fibroblasts, the endo/exocytosis rate is \(\sim 1 \, \text{\mu m}^2/\text{s}\)\(^{28}\), so about an hour would be needed to replace the plasma membrane; thus it is possible that on the seconds-to-minutes time scale a motile keratocyte keeps cell area constant mechanically. Furthermore, it is known that keratocyte fragments, whose movement is essentially indistinguishable from whole keratocytes, have minimal intracellular membrane stores\(^3\). Note that we actually measure the projected cell area, rather than the actual cell area. However, since the projected area changes little and the cell is at steady state, all respective areas and volumes, actual and projected, are steady.

The mechanism by which animal cells control their total plasma membrane area is still not well understood\(^2\), and it is possible that cell area is dynamically maintained constant\(^3\). It is worth keeping in mind that a reservoir of plasma membrane sometimes serves to buffer against fluctuations in the plasma membrane tension for the whole cell, so the membrane tension itself may be maintained constant as well\(^3\), justifying its use as a model parameter. Finally, note that in some cells, a major component of the membrane tension is the energy of transient attachments between the membrane and the actin cytoskeleton\(^2\). This energy has to be included into the formulae for the energy \(E\) used above to calculate the membrane tension. Our model corresponds to the assumption that such attachments in motile keratocytes do not contribute significantly; future research will have to test this assumption.
2. Model relation to measurements and correlations not addressed explicitly in the main text

Scaling of model parameters

The lumped parameter \( z \) is defined in (10) as \( z = \frac{T\gamma}{f_{\text{stall}}B} \). Understanding how these cellular parameters, and \( z \) overall, scale with large-scale morphological properties like cell area, is of distinct biological interest, as this may provide insight into the biochemical and biophysical mechanisms that determine the value of these parameters. Our model provides a prediction regarding the scaling of \( z \) with cell geometry:

\[
z(A, S) = \frac{4\sqrt{S} (S + 1)}{\sqrt{A} (S + 2)^3},
\]

as well as a prediction in terms of the geometrical parameters and the actin distribution ratio: \( z(L, D_{cs}) = \frac{1}{L \cdot D_{cs}} \). The predicted dependence of \( z \) on cell geometry is illustrated in Fig. S5a and b, in which \( z(A, S) \) is evaluated at all of the \((A, S)\) positions in our dataset and plotted against either \( A \) or \( S \). This demonstrates the general predicted scaling of \( z \) with these parameters over physiologically-relevant ranges, and also shows that \( z \) is relatively constant in individual cells through time. The concordance of these two different predictions of the model (\( z(A, S) \) and \( z(L, D_{cs}) \)) is shown in Fig. S5c. Note that this concordance is driven entirely by the relation between \( D_{cs} \) and \( S \) shown in Fig. 3: here, we examine the correlation between two different estimates of a cellular parameter, \( z \), based on different observables, while Fig. 3 shows the relation between two different observables based on their hypothesized molecular underpinnings.

At present, we cannot simultaneously measure \( T, \gamma, f_{\text{stall}}, \) and \( \beta \) (and hence \( z \)), so we cannot directly verify the predicted relations. However, we can clarify the model’s prediction for specific scaling relations, which are in principle measurable. We expect that \( \gamma \) and \( f_{\text{stall}} \) will not depend on cell size, whereas \( \beta \) and \( T \) may well vary with cell area. What, then, is the model’s prediction of how \( T/\beta \) ought to scale with cell area? The expression \( z = \frac{4\sqrt{S} (S + 1)}{\sqrt{A} (S + 2)^3} \) obviously implies that \( z \), and thus \( T/\beta \), vary with cell area. If cell aspect ratio and area were uncorrelated across the population, then we would expect \( z \propto A^{-1/2} \); on the other hand, if aspect ratio correlated with area, then \( z \) would scale differently as a function of area. Detailed analysis of the population data set reveals that cell area and aspect ratio are not independent. Cells with larger area tend to be more canoe-like, illustrated by the fact that the principal axis of shape variation (shape mode 1; Fig. 1b) describes a continuum of shape phenotypes in which area and aspect ratio are coupled. This is further demonstrated by the statistically significant correlation that we observe between cell area and aspect ratio (Fig. 2b; Fig. S2). However, is this dependence enough to substantially modify the predicted scaling of \( z \propto A^{-1/2} ? \) We examined our live
cell data set to find the best-fit exponent $\nu$ for the relation $z(A,S) = \tilde{z} A^\nu$, where $\tilde{z}$ is a constant. The best-fit exponent was $\nu = -0.54$ (95% bootstrap confidence interval for $\nu$ is [-0.55, -0.53]; Fig. S5d), indicating that the dependence of $S$ on $A$ influences the scaling of $z$ with $A$ to a detectable but nevertheless minor degree.

Given that $\nu \sim -\frac{1}{2}$, we have essentially $\tilde{z} \propto \frac{T}{\beta} $ $\propto A^{-1/2}$. We observed that the total amount of filamentous actin in cells increases with cell area, indicating that the parameter $\beta$ that determines the number of actin filaments must increase with cell area as well. It may scale as $\beta \propto \sqrt{A}$, which would mean that the total branching rate is proportional to the cell perimeter, or to the leading edge length, and could suggest perhaps that there is a molecular complex at the cell edge that regulates actin network assembly by activating Arp2/3\textsuperscript{37}. If this were true, the membrane tension, $T$, would vary little with cell size. Another possibility is that $T \propto \sqrt{A}$. In that case, $\beta$ would increase essentially linearly with cell area; this scaling would also be a plausible biologically and suggest that filament branching is triggered by a membrane associated signal (rather than a leading-edge associated complex). Further research will be needed to address this distinction\textsuperscript{38}, and to verify the overall prediction of the model that $\frac{T}{\beta} \propto A^{-1/2}$.

Front roughness and angular speed

Angular speed anti-correlates strongly with cell aspect ratio (and, to a lesser extent, with all observables correlating with aspect ratio; Fig. S2). The model does not address the angular speed directly; qualitatively, cell turning is due to stochastic imbalances between different parts of the cell and spatial-temporal instabilities of the protrusion at the leading edge. Previously, we demonstrated that in cells with low aspect ratio (and lower levels of filamentous actin), the leading edge is much more unstable, causing more frequent and significant turns\textsuperscript{8}. These instabilities were explained by a model similar to the model we use here. This connection between an unstable leading edge and less persistent directional motion is further reflected in the observed correlation between the front roughness measure (not directly addressed in the model, but which we believe to be indicative of the global order or disorder of the leading edge) and angular speed (Fig. S2).

Similarly, front roughness strongly anti-correlates with cell speed (Fig. S2). This could be explained by stochastic variations of the protrusion along the ‘rough’ leading edge, which are not synchronized as needed for rapid locomotion. The correlation of front roughness with actin monomer concentration could be explained if high actin monomer concentrations drive localized intermittent rapid growth of actin filaments. This probably contributes to greater observed variations of the cell speed for the lower aspect ratio cells.
Filamentous actin concentration

The average concentration of actin filaments in cells correlates with the actin density ratio, $D_{cs}$, and cell aspect ratio, $S$. This qualitatively agrees with the model, because when the total branching rate is elevated or the capping rate is lowered, or both, then the assembly of actin filaments is enhanced. At the same time, according to the model, $S$ and $D_{cs}$ increase.

Front-to-rear slope in actin filament density

In smooth, high-aspect-ratio (i.e. “coherent”) canoe-shaped cells, the actin filament density decreases from the leading edge toward the rear (Fig. 8b,c), indicating that filaments’ appearance and growth are enhanced in a narrow band near the leading edge and disassemble uniformly throughout the lamellipodium. This observation is supported by kabiramide C staining of live cells. This front-to-rear decrease in actin filament density occurs along the entire leading edge. Indeed, the ratio of actin filament density near the leading edge to that farther rearward correlates with cell aspect ratio (Fig. 8d): it is largest in canoe-shaped cells with high aspect ratio; it becomes lower in cells with lower aspect ratios, and can even become less than one in the very decoherent, lowest aspect ratio cells. Though the model does not address these observations directly, they deserve some discussion. We explain these results as follows: when the overall branching rate is lower, cells for some reason are unable to focus most of the growing filaments at the cell edge, and new filaments grow to a greater extent throughout the lamellipodium (supported by kabiramide C staining, which is more uniform in cells with low aspect ratio). Thus, in low aspect ratio cells, additional assembly of actin filaments occurs throughout the lamellipodium and partially compensates for the disassembly of filaments created at the leading edge.

Note that in cells with low aspect ratios, the actin filament density at the leading edge is lower, but this density hardly decreases toward the rear, while in canoe-shaped cells, the filament density at the front is high, and decreases toward the rear. Thus, at the rear, the actin filament density probably varies much less than at the front. This supports our argument that membrane tension (which depends on the force needed to crush and push the actin network at the rear) varies little from cell to cell.

Some other correlations

Correlations that we did not comment on explicitly can be simply explained indirectly. For example, $R$ correlates with $V_{cell}$ and $D_{cs}$ because all of these observables strongly correlate with $S$. 

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3. Model relation to data from individual cells through time

The data for individual cells’ speed and shape as a function of time allowed us to characterize individual cell variability through time and compare it to the behavior of cell populations, most notably demonstrating the constancy of cell area within a given cell (Fig. 2a). We found that individual variability did not appreciably increase with time in the ranges observed (up to a few hours); thus individual cells do not appear to be ergodically exploring the phase space of the full population. In addition, these data provide further clues regarding the correlations between observables and the nature of the variability in the population data. First, we observe a strong correlation between the actin filament densities at the center and sides of the leading edge (Fig. S4a), for cells on the highly-ordered “coherent” side of the phenotypic spectrum. This indirectly supports one of the main modeling assumptions about the global regulation of filament branching in cells; if the total number of branching events per cell is regulated (as we assume in the model) than the actin density at different positions along the leading edge is expected to be correlated as observed, whereas if the branching was regulated locally such correlation would not be expected.

Second, the time-lapse data demonstrate that the actin filament density at the center of the leading edge correlates with aspect ratio, while the filament density at the side of the leading edge does not (data not shown). Roughly, this means, according to the model, that the membrane tension does not fluctuate in time much, while the parameter $\beta$ (or perhaps $\gamma$), and the amplitude of the actin profile with it, do. The actin filament density at the sides of the leading edge stays more or less constant to balance membrane tension, while the length of the front of the leading edge (and as area is constant, the cell’s aspect ratio with it) fluctuates together with $D_c$ and cell speed. This is reflected in the larger temporal variation observed in $D_c$ relative to the variation in $D_s$, within the same cell as a function of time: 

$$\frac{std(D_c(t))}{mean(D_c(t))} = 0.22;$$

$$\frac{std(D_s(t))}{mean(D_s(t))} = 0.18;$$

the analysis was done for data points acquired at 10 second intervals over 200 seconds for 11 cells. Thus, significant fluctuations in either $\beta$, $\gamma$, or both, augmented by some variation in $T$, appear to explain the large variation in cell geometry and speed, both through time for individual cells, and across a population of cells.

4. Model limitations in explaining the data

An obvious limitation of the simple model presented here is that, for reasons of mathematical simplicity and more transparent comparison of model predictions with data, the complex cell shape is approximated simply by a bent rectangle. More sophisticated models based on differential equations lead to a more detailed description of the leading edge and sides of the cell, but quantitative comparison of the results of these models with experiments is not easy, and the biological conclusions are harder to decipher.
Moreover, the exact shape of the cell rear is beyond the current model framework. Implicitly, the model assumes that the rear edge is a circular arch of the same average radius as that of the leading edge, which is in fact not far from the truth. Additional simplifications of our model are the fact that we treat the lamellipodium as two-dimensional and that we ignore the effects of the cell body. However, these approximations are justifiable given the observations that the lamellipodium is flat and extremely thin\textsuperscript{33,34} and that lamellipodial fragments of keratocytes (without a cell body) move in a manner essentially indistinguishable from whole cells\textsuperscript{40,41}.

Another problem in relating the model to experimental data is that the measured profile of filamentous actin density along the leading edge includes not only filaments growing and pushing the edge, but also presumably some filaments that are capped, stalled and/or buckled and thus not contributing to protrusion. The fraction of the latter is likely to be higher at the lamellipodial sides complicating quantitative interpretation of the actin density ratio. This problem is reflected in the deviation of the theoretical prediction from the experimentally measured relation $D_{cs}(S)$ for small values of $D_{cs}$ ($S\sim1$). The reason for this discrepancy is that the theory predicts only values $D_{cs} > 1$, and in low aspect ratio cells, where the actin filament distribution along the leading edge is relatively flat, there is probably a large fraction of capped, buckled and stalled filaments at the sides so the error in interpreting $D_{cs}$ becomes larger.

The model prediction also becomes poor for very large values of $D_{cs}$ ($S>1.6$), albeit for a different reason. Cells characterized by high values of $D_{cs}$ are canoe-like, with an aspect ratio much greater than 1, which means that their widths, $x$, are large, while their lengths, $y$, are very small. It is plausible then that the actin network does not have enough time to disassemble during its transit from cell front to rear, causing an increase in membrane tension. Mathematically, this would lead to a non-linear correction for cell shapes characterized by high values of $D_{cs}$. Explicitly including such non-linearity into the model at this point would only complicate matters without adding biological insight.

Finally, there are many processes that are each of secondary importance to the overall cell shape for keratocytes, yet together they introduce additional “noise” in cell behavior and errors in the theory-experiment relation. Among those are corrections to the sides’ shape due to the myosin-powered centripetal actin network flow, retraction fibers at the cell rear, and potential contribution of the adhesions to membrane tension and protrusion.

Note also that the situation in other cell types is likely more complicated. While other well-characterized motile cells such as fibroblasts and neutrophils rely on the same molecular components and mechanisms involved in keratocyte motility\textsuperscript{9,42,43}, keratocyte shapes are considerably simpler and far more stereotyped. In particular, other cell types typically have more variable shapes, both across the population and in individual cells through time. Unlike keratocytes, more complex cells are likely to exhibit significant hysteresis (history-dependence) in shape – for example, the shape of a fibroblast at any given time is likely to be a reflection of that cell’s immediate past history of protrusions.
and retractions, and is not likely to reconstitute in the same configuration after actin network collapse as do keratocytes (Fig. 2c-e).

5. Molecular perturbation experiments and their theoretical interpretation

To further investigate the role of various molecular processes in determining cell shape, we targeted specific components of the cytoskeleton in live cells with pharmacological agents. The agents employed included several actin drugs: cytochalasin D, which caps actin filaments and affects nucleotide hydrolysis on monomers; latrunculin, which sequesters actin monomers; and jasplakinolide, which stabilizes actin filaments and slows depolymerization. We also employed two compounds that target myosin: blebbistatin, which is a specific myosin II inhibitor; and calyculin A, which enhances myosin II activity by inhibiting a myosin light chain phosphatase. All these treatments elicited statistically significant morphological changes, but their extent was rather small, as illustrated by the similarity of the mean shape of each perturbed population to that of the untreated cells. In particular, the natural shape variation in the population was substantially larger than the shifts induced by any of these perturbations. Furthermore, the phase space of cell shapes under these perturbations was nearly identical to that spanned by the population of unperturbed cells. Specifically, the first two shape modes were essentially unchanged by these molecular perturbations, and in all treatments but jasplakinolide, modes three and four remained intact (data not shown). Note that concentrations of these drugs higher than those we employed typically lead to “catastrophic” phenotypes with no movement and no lamellipodium. However, as long as keratocytes are moving and possess a lamellipodium, the spectrum of cell shapes remains largely unchanged.

Two parameters in our simple model describe an individual cell, and are thus of use in relating the effects of these perturbations to their molecular mechanisms: area, $A$, and the lumped parameter $z = T \gamma / (f_{\text{stall}} \beta)$, where $T$ is the membrane tension, $\gamma$ is the capping rate, $f_{\text{stall}}$ is the per-filament stall force, and $\beta$ is the branching rate per cell per second. The outcome of the model with regards to cell speed also depends on the value for the cellular actin monomer concentration (see eq. (20) above), so we use $G$ as an additional parameter (we do not have experimental data on how the perturbations change the actin monomer concentration). The distribution of cell areas of perturbed cells does not change much compared to untreated cells. Therefore, we attempt to explain the observed changes in cell geometry and speed quantitatively by changes in the parameters $z$ and $G$. Some perturbations show a change in $z$ that is relatively straightforward to explain; however other perturbations may either affect more than one of the parameters that go into the lumped parameter $z$ (and since the relative magnitude of these changes is hard to anticipate, $z$ can go either up or down), or require the invocation of compensatory mechanisms for a satisfactory explanation.
We focus here on the effect these agents have on (i) aspect ratio, (ii) lamellipodial radius, (iii) cell speed. Other observables, such as cell perimeter, actin ratio, roughness, etc. are logically connected to these main ones. The shape of the cell rear and its reaction to perturbations are not discussed here and will be addressed in future work. Note that we will discuss the perturbation-induced changes qualitatively, as there is no quantitative information on the molecular effects of these perturbations. In order to consider these three observables, the following formulae derived above are used:

\[ z = \frac{T}{f_{\text{stall}}} \gamma, S = 10(1 - (zL)), R \sim \sqrt{(zL)^{w} - 1}A, V \sim G\left(1 - (zL)^{w}\right) \]  

(21)

The formula for \( S \) is a linear approximation to equation (13); also, recall that \( L \approx 3\sqrt{A} \), and so \( L \) does not change significantly upon perturbations.

**Cytochalasin** The measured changes can be explained neatly by the model: effectively, cytochalasin increases the capping rate, \( \gamma \) [44], so, according to (21), \( z \) goes up, and \( S, R, V \) all decrease, as observed.

**Blebbistatin** These effects are also very easy to understand: according to our model, myosin-powered contraction assists actin filament disassembly at the rear of the cell. Blebbistatin inhibits myosin contraction [47] and without this assistance, tension \( T \) is likely to increase as the membrane encounters a greater resistance of a more intact actin network at the rear. Thus, \( z \) goes up, and \( S, R, V \) all go down, as observed.

**Calyculin** The effects of this treatment can be explained as follows. Calyculin brings the tension \( T \) down, likely by increasing the centripetal actin flow [48]. However, it may also drive actin filament assembly down by mechanically sliding and/or straining actin filaments and inhibiting branching, so if \( \beta \) decreases to a greater extent than \( T \), then \( z \) increases, and \( S, R \) decrease, as observed. At the same time, calyculin may indirectly enhance actin network disassembly by making myosin break actin filaments more vigorously. This could significantly increase the actin monomer concentration, and this increase could overcome the decrease of the \( z \)-related factor in the formula for cell speed, leading to increased speed as observed. Also, higher actin monomer concentration could elevate the branching rate, if Arp2/3-mediated branching depends on the rate of monomer addition.

Note also, that the perturbations of myosin contraction (blebbistatin and calyculin) are the only ones significantly affecting the angular speed of cells (Fig. S1). While the model does not address this directly, we hypothesize that this is related to the subtle balance of retractions of the right and left rear corners of the cell being dependent on myosin-generated centripetal actin flow.

**Latrunculin** Latrunculin sequesters actin monomers [45], and since Arp2/3-mediated branching depends on the rate of actin monomer addition [49], it could thereby decrease the branching rate, \( \beta \). This would increase parameter \( z \) and decrease \( S \) and \( R \), as observed. The observed decrease in cell speed is easy to explain because the actin monomer concentration likely decreases due to monomer sequestration.
**Jasplakinolide** The effects of this drug are not easy to explain, but the following mechanism is plausible. Jasplakinolide stabilizes actin filaments, so it probably enhances branching and increases parameter $\beta$, despite inverse effect of the lower actin monomer concentration. $T$ is also likely to increase, but maybe not to the same extent, so as a result parameter $\varepsilon$ decreases slightly, so $S$ increases. It is easy to explain the decrease in cell speed: actin filaments are stabilized, so the actin monomer concentration is reduced resulting in lower speed (despite the slight increase of the $\varepsilon$-related factor).

6. **Ruling out other potential models**

Our quantitative model for the fronts/sides of the cell is based on the hypothesis that protrusion is force-limited, and that the actin filament density along the leading edge is graded. Thus, the filaments at the sides are stalled because they are fewer in number and collectively unable to protrude against the membrane tension, while the numerous filaments at the front together push the leading edge effectively. The model for the rear edge of the cell, which is much less explicit or quantitative at present, is based on the idea that the membrane tension pushes forward debris of the actin network that is likely largely disassembled at the rear. Here, we discuss possible alternative hypotheses and their relations to the data. These alternatives include:

a) Global angular actin network architecture  
b) Graded actin monomer concentration  
c) ‘Central organizer’ of the cell shape, i.e., MTOC  
d) Myosin-powered graded centripetal flow  
e) Pre-set leading edge length  
f) Depolymerization ‘clock’

Hypotheses (a-d) are illustrated schematically in Fig. S3. A role for microtubules is ruled out by the lack of a shape phenotype under nocodazole treatment (data not shown) and the observation that microtubules are not required for keratocyte motility. Likewise, a hypothetical “morphogen field” or other central organizing principle (hypothesis (c)) fail to explain the observed correlations among different morphological characteristics (Fig. 2b). The discussion below details how the extensive dataset acquired in this work allows us to test and refute the other alternative hypothesis mentioned above.

It is important to note here that while our model explains the main trends in the data, we do not rule out some additional contributions from other mechanisms including those listed above. The arguments given below explain why the above mechanisms cannot by themselves be the main factor determining cell shape, and show how such mechanisms would lead to predictions that are inconsistent with the data. However, more subtle contributions, particularly for the more extreme shapes, may well occur. As an example, it is possible that the depolymerization clock mechanism sets a lower limit on cell length ($y$) and may affect the shape of cells if their width approaches this limit.
Global angular actin network architecture. It is possible, in principle, that actin filaments are oriented on average in the direction of protrusion globally everywhere in the cell, rather than locally normal to the cell edge (Fig. S3a). According to this idea and the branching geometry, only roughly half of the filaments would be pushing at the cell sides relative to the front, so that the sides could be stalled while the front protrudes forward. Graded actin filament density would be irrelevant, and this hypothesis would explain the geometry of the cells with relatively flat actin filament density profiles along their leading edges.

Arguments against this hypothesis: A flat actin density profile would result in a very flat leading edge, with an enormous lamellipodial radius. This hypothesis would certainly not predict the observed correlation of radius with area and aspect ratio, which requires graded actin filament density profile. Also, it fails to predict the observed relation between the actin density ratio and aspect ratio.

Graded actin monomer concentration. If most actin filament depolymerization took place in the cell body, then actin monomer diffusion from the cell body across the lamellipodium and ‘consumption’ along the leading edge would lead to roughly radial downward gradient of actin monomer density with its center at the cell body. In this scenario, the lamellipodial sides, to which the distance from the cell body is longest, would have lower actin monomer concentration (Fig. S3b). At the lamellipodial front, which is closest to the cell body, the monomer concentration would be higher. In canoe-shaped cells, if protrusion was limited by actin monomer availability, protrusion at the sides would be slowed down due to lower monomer concentration there, and the resulting graded protrusion could explain cell shape.

Arguments against this hypothesis: First, there is experimental evidence that depolymerization is distributed laterally more or less uniformly in the cell24. It is possible that myosin could move filamentous actin toward the cell center and thus concentrate disassembly there; however, myosin inhibition, which therefore forces depolymerization to occur along the whole rear edge of the cell, produces no dramatic effect on cell shapes (Fig. 1c). Second, our data indicate that actin monomer concentration anti-correlates with aspect ratio (in fixed cells), which in turn correlates with cell speed (in live cells), arguing against actin monomer-limited protrusion. Third, this model could not explain the shape of low aspect ratio cells where the lamellipodial sides are as close to the cell body as the lamellipodial front. Finally, this model would predict anti-correlation of cell speed and cell area, which is not observed. We can similarly rule out various “morphogen field” hypotheses, which propose that reaction-diffusion of some regulatory molecule results in a concentration gradient of that molecule, establishing spatial cues that determine cell shape50.

Myosin graded inward flow: Myosin-powered centripetal flow of the lamellipodial actin network is graded24,25, so that the flow in the laboratory frame of reference is slow at the lamellipodial front and faster at the sides and rear. According to this hypothesis, even if actin filament growth rate was uniform around the cell boundary, this growth could be
balanced by the inward actin network flow at the sides of the lamellipodium and overcome at the rear, while only slightly perturbed at the front (Fig. S3d).

Arguments against this hypothesis: First, actin network flow maps obtained in our lab⁷ show almost no flow at the lamellipodial sides near the front of the cell. More importantly, this hypothesis would predict that blebbistatin (calyculin) inhibiting (strengthening) myosin would cause significant changes in cell geometry and speed, which are not observed. More subtly, some of our observations, such as the correlations between aspect ratio and cell speed, and between lamellipodial radius and area, are hard to explain quantitatively in the framework of this hypothesis. We emphasize though that none of these observations rule out the possibility for myosin-powered graded inward flow to play an important role in shape determination of other motile cell types.

Regulated leading edge length: One very hypothetical possibility is that a linear supramolecular structure, the length of which is determined by some pathway(s) that are not part of the self-organizing mechanisms that we discuss in this paper⁵¹, defines the length of the leading edge, x. Then, given a constant cell area, the aspect ratio would be uniquely defined.

Arguments against this hypothesis: It would be hard to explain why aspect ratio correlates with cell area, why speed correlates with aspect ratio, and why lamellipodial radius correlates with cell area; many more correlations would require very elaborate assumptions to explain them.

Depolymerization clock: This hypothesis states that the distance between the front and the rear of the lamellipodium, y, is determined by the time τ needed for disassembly of the actin network: \( y = V_{cell} \tau \).

Arguments against this hypothesis: It would predict a correlation of cell speed with the front-to-rear distance, which is not observed. Also, this hypothesis would predict a much stronger correlation between aspect ratio and area than observed (y would be more or less constant for all cells, so x would increase significantly as A grows). However, the depolymerization clock could be a crucial mechanism determining the rear edge shape in the sub-population of the cells characterized by small y, as discussed above.

Supplementary methods

Cell culture, fixation and microscopy.
To obtain additional information about the distributions of both actin monomers and actin filaments and their correlations with cell morphology, the population-based analysis described in the main text for live cells (Figures 1,2) was repeated for a population of fixed keratocytes co-stained for actin monomers and filamentous actin. All treatments prior to fixation were identical to the live cell experiments described in the main text. Briefly, keratocytes were cultured from the scales of the Central American cichlid Hypsophrys nicaraguensis as described previously⁸. Keratocyte sheets from one day old
cultures were disaggregated by incubating in 85% PBS and 2.5 mM EGTA, pH 7.4, for 5 min, followed by incubation in normal media for an additional ~1.5–2 hours. Cells were fixed with 4% Formaldehyde as described elsewhere\(^2\) followed by co-staining of filamentous actin with Rhodamine-phalloidin (Cytoskeleton Inc.) and actin monomers with FITC-DNAseI (Molecular Probes). We attempted to keep the concentration and duration of staining constant, but still we observed considerable variation of staining intensities between different coverslips. To compare relative amounts of actin between cells on different coverslips we employed a coverslip based normalization scheme described below. In addition, comparison of the fixed cell data set with the live cell one, revealed that the fixation process introduced moderate, yet statistically significant, changes in cell shape (Fig. S1).

Images of fixed cells were collected with a microscope (Axioplan 2; CarlZeiss MicroImaging, Inc.) using a 63× NA 1.4 oil plan-Apochromat objective (Carl Zeiss MicroImaging, Inc.). Images were collected with a cooled back-thinned CCD camera (MicroMax 512BFT; Princeton Instruments) with a 2× optovar attached using MetaMorph software version 6 (Molecular Devices). For each coverslip, ~10–35 polarized cells were randomly chosen for imaging.

Filamentous and monomeric actin concentrations in fixed cells were measured by phalloidin and DNAseI staining intensities, respectively. The total integrated intensity within the cell outline was divided by the cell area to provide a crude measure of concentration. These values varied greatly between coverslips because of differential staining; thus the values were re-centered to set the median of each coverslip to 1. (Values were not re-scaled: though the distributions of values for each coverslip had different centers, the spreads were comparable and thus did not warrant correction.)

**Algorithm 1: Alignment of two polygons with weighted landmark**

It is simple to find the rotation and translation that optimally align two collections of points via a closed-form procedure that minimizes the sum of squared distances between corresponding points known as Procrustes analysis\(^5\). The complexity here is that the exact point correspondences between cell shapes represented as polygons are not known: for a 200-point polygon, there are 200 possible different point orderings for a given winding direction (clockwise or counterclockwise). A brute-force method to align two 200-point polygons would simply be to apply the Procrustes method 200 times, and take the alignment with the smallest squared error as optimal. We obtain a significant speedup over this approach by trying only eight possible orderings for each direction and “hill-climbing” to a local optimum from each of those starting points. This procedure is not guaranteed to find the global optimum, but we have found it very reliable on the shapes used in this work. To further help the alignment of keratocyte shapes, we have found it helpful to define an approximate “cell body position,” determined either manually or from the bright spot of fluorescence produced by the cell body, as a landmark to be aligned in the Procrustes procedure. (This landmark is of course not re-ordered with other points, as a general correspondence in the cell body positions is assumed across all cells.) The Procrustes method allows for different points to be differently weighted, and we have
found that assigning a weight of 0.3 to the point at the center of the cell body, and a weight of 0.7 shared among polygon points allows for good alignment. Note that while in some cases it might be reasonable to allow for reflections of shapes if that provides a smaller squared error term, it is not reasonable to do so for keratocytes, as these cells have distinct top and bottom surfaces, which reflecting would implicitly disregard.

Algorithm 2: Mutual alignment of a population of shapes
This method has been previously described$^{54}$; briefly, an expectation-maximization procedure is used to simultaneously estimate the mean cell shape in the population and align all cells to that mean. As an initialization step, the polygons are roughly aligned along their long axes and ordered winding counterclockwise from the rearmost point. Then, the mean of the (unaligned) population is calculated, and each cell is aligned to that mean via Algorithm 1. The mean is re-computed from the newly aligned cells, which are then aligned to the new mean, until no cells change their position (above a certain low threshold). At this point, the cells are considered aligned.

Supplementary references


**Figure S1** – Molecular perturbations shift cell populations in distinct but subtle manners.  
(a) Shown are the mean ± standard deviation of each of the measured properties for cell populations under different molecular perturbations. Significant changes in means (p<0.05; determined from a bootstrap confidence interval on the difference in means) are colored and shown in bold face. At the right of the table a dendrogram shows the relationship between the different perturbations as determined by average-linkage hierarchical clustering based on the Mahalanobis distance between the mean measurement vector for each perturbation.  
(b) Molecular perturbations have only a modest effect on cell shape. The top row shows the mean shapes of populations of cells treated with different agents at least 10 min before imaging; the mean shape of the unperturbed population is superimposed (dashed line). In the lower rows, the first and second principal modes of shape variation in these populations are illustrated by superimposition of the mean shape and shapes at one and two standard deviations away from that mean along each mode.
**Figure S2** – Correlations between measurements reveal a phenotypic continuum.
Pairwise correlations between measurements gathered from live and fixed keratocyte populations are presented. Scatter-plots of the various measures (in pairwise combinations) are shown; the plot’s row corresponds to the measurement plotted on the x-axis, and the column indicates the measurement on the y-axis. Measurements are named on the diagonal, and the range of values plotted is shown. Measurements of the fixed population are shown in the upper-right triangle, and the live population in the lower-left triangle. Each data point in the scatter-plots represents an individual cell. Plots are colored by the Spearman rank-order correlation score, which is comparable to Pearson’s r statistic, but is more robust to outliers, with negative correlations in shades of blue and positive correlations in shades of red. Numeric correlation values are shown for data that have values significantly different from zero (p < 0.05; determined from a bootstrap confidence interval on the Spearman score). A small number of outliers were excluded, as follows. Area: 1 (live); front radius: 7 (live), 1 (fixed); front roughness: 21 (live), 7 (fixed); actin ratio: 5 (fixed); angular speed: 8 (live); actin filament concentration: 2 (fixed); actin monomer concentration: 1 (fixed).
**Figure S3** – Schematic illustrations of alternative models for possible mechanisms of cell shape determination in keratocytes.

(a) The actin network is organized so that filaments are oriented on average in the direction of protrusion (rather than locally normal to the cell edge) everywhere in the cell. Only roughly half of the filaments would be pushing at the cell sides relative to the front, so that the sides could be stalled while the front is protruding. (b) A gradient of actin monomer concentration centered at cell body leads to graded protrusion rate due to differences in local actin monomer concentration; monomer concentration at the sides is lower (since they are further away from the cell body) leading to slower protrusion there and limiting the ability of the cell sides to extend further. (c) Microtubules (with the microtubule-organizing center (MTOC) localized at the cell body) play a central role in shape determination. (d) Myosin-powered graded actin network centripetal flow balances uniform network growth to yield the observed graded extension; at the front myosin-driven flow is small and protrusion is dominated by polymerization, at the sides myosin-driven flow cancels polymerization to yield no net protrusion, and finally at the rear myosin-driven flow dominates leading to retraction of the cell rear.
Figure S4 – Data from individual cells through time.

Data from a “coherent” cell (see Supplementary Movie 2) and a “decoherent” cell (see Supplementary Movie 3) stained with kagohimide C and imaged over 10 minutes at 10-second intervals. (a) $D_c$ and $D_s$ are depicted as a function of time for both cells. “Coefficients of variance” (standard deviation over the first 200 seconds, divided by average staining intensity) are shown for $D_c$ and $D_s$ traces. (b) $D_{cs}$ in individual cells is shown to correlate with aspect ratio $S$, as predicted by the model and illustrated by the population data in Fig. 3. Each point represents a measurement of the cells shown in panel (a) at a specific time; solid lines are linear fits to the data points. The dashed line shows the predicted relation between $D_{cs}$ and $S$. 
Figure S5 – Scaling of the model parameter $z$ with cell area and aspect ratio. (a and b) According to the model, the cell-specific parameter $z$ can be calculated as a function of cell area $A$ and aspect ratio $S$. Here, $z(A, S)$ is plotted versus $A$ (a) and $S$ (b). Each blue circle depicts data from a single cell in the population data set. The colored lines represent data from measurements of eleven individual cells taken at 10s interval over a total of 10 minutes (each color depicts a different cell). Together, (a) and (b) demonstrate that the $z$-values for the population of cells lie relatively homogeneously within a small range of possible $z$-values, and that for a given cell over time, $z$ remains largely constant. (c) $z$-values calculated as a function of cell area and aspect ratio (as in the previous panels) are plotted against $z$-values as calculated from area, aspect ratio, and the observed actin center-to-sides ratio $D_{cs}$. For reference, $z=z$ is plotted in red. (d) log($z$) is plotted as a function log($A$) for the population data. The solid line depicts the best linear fit to the log-transformed data, suggesting that the model parameter $z$ scales with cell area as $z \propto A^{-0.54}$, which is consistent with the observation that cell area is slightly correlated with aspect ratio in the population (see Fig. S2 as well). For comparison, the dashed line represents the best fit to the data with the exponent of $A$ fixed at $-0.5$. 

Supplementary Information
Figure S6 – Force–velocity relation links graded actin distribution along the leading edge to cell speed and lamellipodial radius.

(a) Schematic illustration of the graded extension model; cell protrusion is fastest at the cell midline and decreases toward the sides. Cell speed is equal to the maximal protrusion rate at the midline: $V_{\text{cell}} = V(0)$. The lamellipodial radius can be calculated using the graded actin distribution, the force–velocity relation and geometrical formulae from the radial extension model. (b) Possible force–velocity relation for a protruding actin network. The polymerization rate, $V$, is plotted as a function of the force per filament, $f$, for $w = 1, 2, 4, 8$. (c) The measured radii of curvature are compared to those calculated using the different force–velocity relations shown in (b). (d) Cell speed is depicted as a function of aspect ratio. Dashed lines indicate the model predictions for this relationship using the different force–velocity equations shown in (b). Dots represent measured values from a population of live cells, with the Gaussian-weighted moving average ($\sigma=0.25$) ± one standard deviation shown by the solid line and shaded region (as in Fig. 4b).
Figure S7 – The rectangle assumption approximates cell perimeter well. The calculated cell perimeter based on the measured cell area, $A$, and aspect ratio, $S$, using the rectangle approximation is plotted as a function of the measured perimeter. The close fit indicates that the rectangle assumption gives a good approximation of cell shape.
Figure S8 – Front-to-rear slope in actin filament density correlates with cell aspect ratio. Phase-contrast (a) and fluorescence (b) images of a fixed cell stained with TRITC-phalloidin. (c) The profile of the (background-subtracted) fluorescence intensity of that cell is shown as a function of distance from the leading edge. The profile was averaged along the width of the region highlighted in (a). The measured profile was fit in the indicated region (solid line; fitting region is bounded per-cell by the maximum intensity within the first μm of depth and the minimum intensity beyond 3.5 μm) and the front-to-rear ratio (the ratio of the height of the fit line at 1 and 4 μm from the leading edge) was used as a measure of the slope. (d) The front-to-rear ratio is plotted against the cell aspect ratio. Each data point corresponds to an individual cell.