

Supplementary Information

Contribution of myosin II activity to cell spreading dynamics and mechanics

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I. EFFECTS OF τ_p AND τ_q ON THE DYNAMICS OF CELL AREA AND FORCE

Solving the more general case where the dynamics of $Q(t)$ are explicitly taken into consideration we find the following third-order linear differential equation for $P(t)$, from which a solution for $R(t)$, $f(t)$ and $Q(t)$ can also be obtained by direct substitution:

$$A\tau_p\tau_q\frac{\partial^3 P}{\partial t^3} + (A\tau_q + B\tau_p)\frac{\partial^2 P}{\partial t^2} + C\frac{\partial P}{\partial t} + DP + E = 0, \quad (\text{S1})$$

where,

$$\begin{aligned} A &= k_m\left(\xi_c + \frac{R_0}{h}\xi_s^{\max}\right) + k_l\xi_c \\ B &= k_m(1 + \beta)\frac{R_0}{h}\xi_s^{\max} + (k_m + \tilde{k}_l)\xi_c + 2\kappa_c\tau_q(k_m + k_l) \\ C &= k_m(1 + \beta)\frac{R_0}{h}\xi_s^{\max} + (k_m + \tilde{k}_l)\xi_c + 2\kappa_c\tau_p(k_m + \tilde{k}_l) + 2\kappa_c\tau_q(1 + \alpha)(k_m + k_l) \\ D &= 2(k_m + \tilde{k}_l)\tilde{\kappa}_c \\ E &= 2\alpha(1 + \beta)\kappa_c k_m \xi_s^{\max} v_{pol}/h \end{aligned} \quad (\text{S2})$$

and,

$$\tilde{k}_l \equiv (1 + \beta)k_l; \quad \tilde{\kappa}_c \equiv (1 + \alpha)\kappa_c \quad (\text{S3})$$

This equation can be solved analytically as a sum of three exponents with rather complicated expressions of the model parameters. We thus demonstrate here the essential consequences that may arise when the dynamics of $Q(t)$ are taken explicitly. Because the actively generated stress, $Q(t)$, operates in a direction that facilitates spreading it generally acts in opposition to myosin contractility in the lamellar bulk and thereby suppresses the dynamical effects that $P(t)$ has on spreading. As seen in Fig. S1c including $Q(t)$ mainly has the effect of smoothing out the local maxima resulting from $P(t)$ dynamics since these forces resist the late decrease in cell area. The

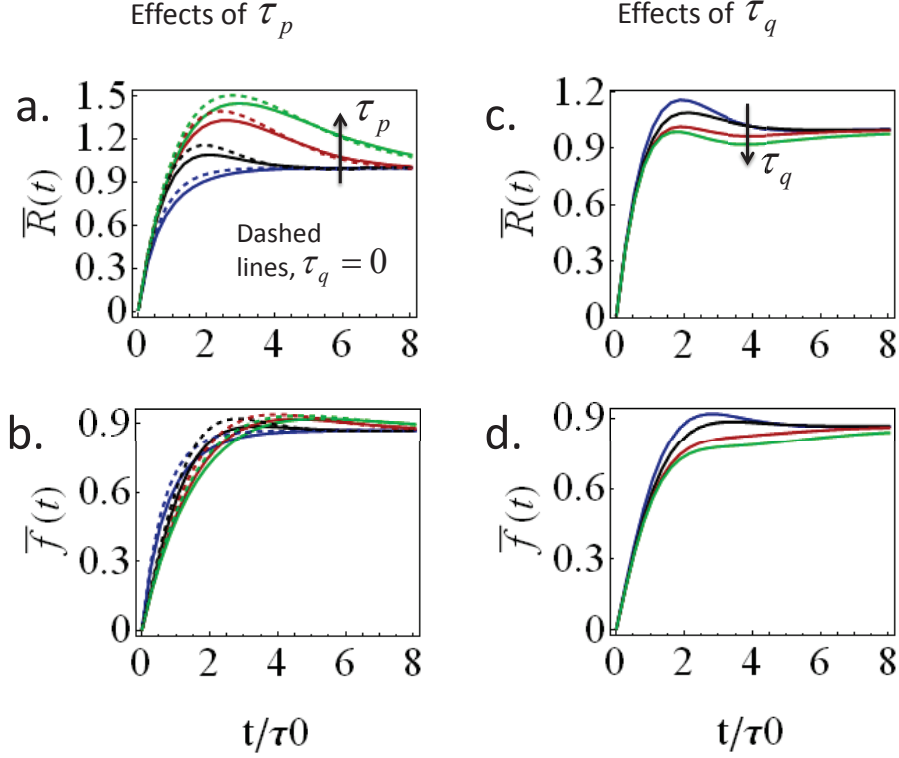


FIG. S1. Effects of τ_p and τ_q on the dynamics of cell area and force during spreading. Left panel curves are plotted for $\tau_p/\tau_0 = 0, 1, 2, 3, 5$, from bottom to top respectively, solid lines are for $\tau_q/\tau_0 = 1$, dashed lines are for $\tau_q = 0$. Right panel curves are for $\tau_q/\tau_0 = 0, 1, 2, 3, 5$, from top to bottom respectively, and $\tau_p/\tau_0 = 1$. Other factors are $k_m/k_l = 1$, $\alpha = \beta = 1$, $\tau_c = \xi_c/(2\kappa_c) = 0.16\tau_0$. Scaled quantities $\bar{R}(t)$ and $\bar{f}(t)$ are as defined in main text figures. The irregular shape of the $R(t)$ curves is seen to mainly depend on the dynamics of $P(t)$; because the cell is free to contract, the force $f(t)$ only weakly depends on the dynamics of $P(t)$. Slow myosin contractility in the lamellar bulk results in a late decrease in cell area. Dashed lines in panel a. and b. show that taking $\tau_q = 0$ provides a good approximation to the full solution. $Q(t)$ dynamics has little effect on the shape of the $R(t)$ and $f(t)$; slower $Q(t)$ dynamics generally tends to smoothen the curves since these forces act in opposition to the active contractility, $P(t)$, of myosin motors in the lamellar bulk. An interesting consequence of very slow $Q(t)$ dynamics is a late increase in cell area and force as seen by the red and green curves in panels c and d.

green curve in panel c shows that a sufficiently slow $Q(t)$ dynamics ($\tau_q \sim 5\tau_0$) may give rise to a late phase of slow increase in cell area. Compared to the bold consequences of $P(t)$ on the shape of the $R(t)$ curves, $Q(t)$ has a much less observant effect. Panel a shows that reducing the value of τ_q from $1\tau_0$ to zero has only a minor effect on the shape of the spreading curves. This justifies the

simplifying approximation we adopt in the manuscript in setting $\tau_q = 0$.

II. CONTRIBUTION OF MYOSIN FORCE TO TOTAL TRACTION DURING SPREADING

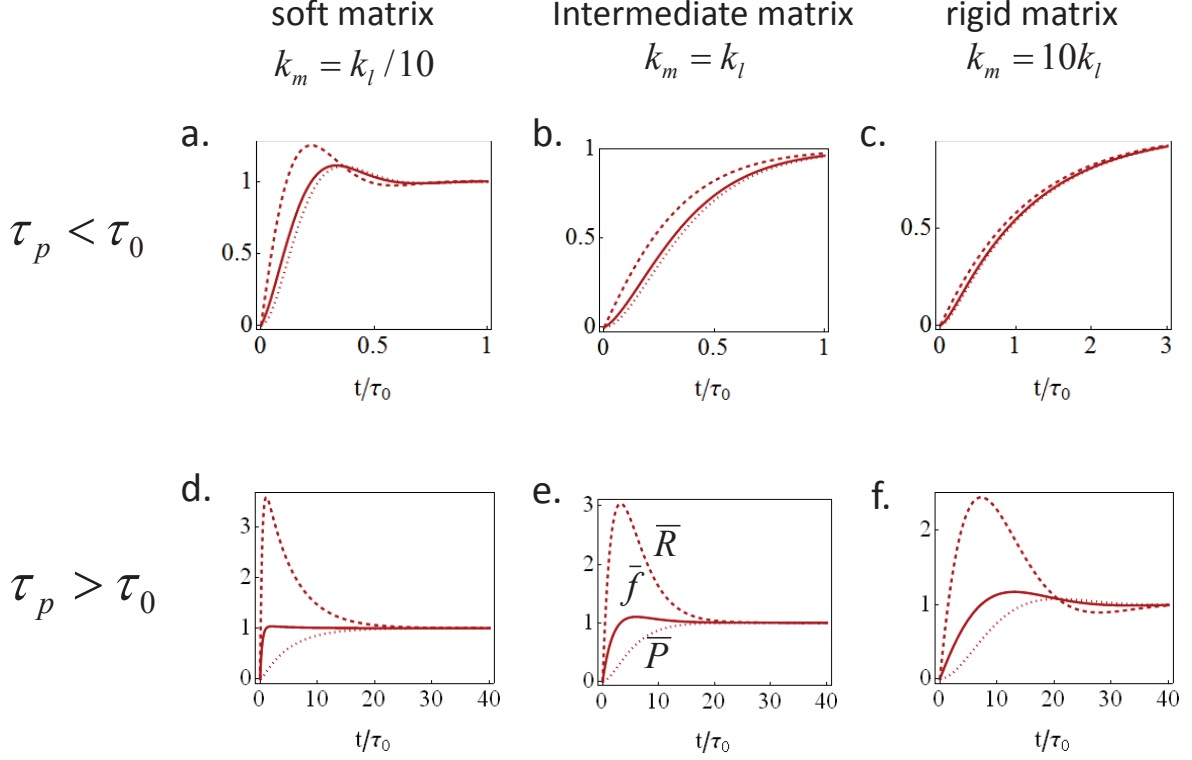


FIG. S2. Evolution of (normalized) cell radius, force and myosin dipolar stress, for three cases of matrix to cell rigidity ratio and for the two points a. and b. in panel 4a. The plots complement the analysis shown in Fig. 4b and 4c. We use $\alpha = \beta = 3$ and $\tau_p/\tau_0 = 0.11$ $\tau_p/\tau_0 = 20.9$, for the points a. and b., respectively. τ_0 is defined as the value of τ for $\beta = 0$ and $k_m = k_l$. The scaled quantities are defined as follows: $\bar{R}(t) = [R(t) - R_0]/[R_{ss} - R_0]$, $\bar{f}(t) = [f(t) - f_0]/[f_{ss} - f_0]$, $\bar{P}(t) = P(t)/P_{ss}$, where R_{ss} , f_{ss} and P_{ss} are the steady-state values of cell radius, force and actomyosin contractility, and R_0 , f_0 are the respective initial values of cell radius and force; $P(t)$ is assumed to polarize from an initial value $P_0 = 0$.

The total elastic force exerted onto the matrix during spreading includes two distinct contributions that are experimentally indistinguishable. Those are the active myosin force and the passive viscoelastic force resulting from the stretching of the cytoskeleton network and the membrane during spreading. Our theory allows us to examine these two contributions during spreading. Fig. S2 shows the variations of the normalized quantities, $\bar{P}(t) = \frac{P(t) - P_0}{P_{ss} - P_0} = \frac{P(t)}{P_{ss}}$, $\bar{f}(t) = \frac{f(t) - f_0}{f_{ss} - f_0}$ and

$\bar{R}(t) = \frac{R(t) - R_0}{R_{ss} - R_0}$, during spreading for the cases shown in Fig. 4b and 4c. Examining panel f., for example, allows us to physically explain how an oscillatory behavior may arise in case of a rigid substrate when $\tau_p > \tau_0$. Because τ_p is relatively long, the cell may rapidly extend in size with relatively weak resistance - resulting in a first peak in cell radius. Once myosin polarization takes an effect it causes a slow contraction of cell radius - giving rise to a first minimum in $R(t)$. This elastic contraction is accompanied by relaxation of cellular stress and, via the coupling expressed in Eqs. 5 – 7, leads to subsequent reduction in myosin force. This allows the cell to accelerate to a second (smooth) peak in $R(t)$; these oscillatory dynamics are damped due to various frictional interactions involved hence a steady-state is eventually reached. Similar reasoning explains other spreading phenomena predicted by our theory.

III. A MICROSCOPIC STOCHASTIC MODEL OF CELL SUBSTRATE INTERACTION

The details of the microscopic stochastic model used in the derivation of Eq. 2 have been discussed elsewhere [1, 2] and we repeat them briefly here for completeness. Upon forming a connection between the actin network and ligands in the ECM, molecular elements in both the lamella and the ECM are elastically deformed by the retrograde flow of actin. We hypothesize that the total density of integrin linkers in the membrane, N , is fixed, and that integrins may either be engaged in an actin-ECM connection, or be disconnected. We thus assume a two-state model in which N_b/N is the fraction of integrins that are engaged in an elastic coupling between the actin network and the ECM, and $N_{ub}/N = 1 - N_b/N$ is the remaining fraction of unbound integrins. In the following only these two states are considered while the reorganization dynamics of integrins in the membrane to form focal complexes with a distribution of cluster sizes are ignored for simplicity. Each connection behaves as a molecular clutch [3] that couples the motion of the actin network to an elastic deformation of the ECM. Once bound and pulled backward by the tensile forces in the cytoskeleton, a local elastic deformation is created in both the ECM and the lamellar cytoskeleton. We denote by $s = s_l + s_m$ the total local displacement of one actin-ECM connection, where s_l and s_m are, respectively, the separate displacements of the lamella and matrix springs. We define these displacements to be positive when stretched in the direction of the retrograde flow, v_F . The density of engaged adhesion sites, N_b , and the mean displacement, $\langle s \rangle$, are in general functions of the sliding speed, v_F , the density of ligands on the surface, N_L , and the binding/unbinding kinetic rates of ligands to the cytoskeleton. To predict these dependencies we exploit a simple

stochastic model for the binding kinetics at the cell-matrix interface, inspired by Walcott et al. [2]. We denote by $n(s, t)$ the number of engaged actin-ECM adhesions present in a unit area of the the adhesion layer with displacement between s and $s + ds$ at time t . The rate of change in $n(s, t)$ is given by :

$$\frac{\partial n(s, t)}{\partial t} = -v_F \frac{\partial n(s, t)}{\partial s} + k_b g(s) N_{ub}(t) N_L - k_{ub}(s) n(s, t) \quad (\text{S4})$$

with the normalization condition given by $\int_{-\infty}^{\infty} n(s, t) ds = N_b(t)$ and $N_{ub}(t) = N - N_b(t)$. Here, $k_b N_L$ and $k_{ub}(s)$ are the binding/unbinding pseudo-first-order rate constants. The binding rate constant, $k_b N_L$, is assumed to be strain-independent and to linearly depend on the density of ligands on the surface, N_L . The function $g(s)$ accounts for the probability that a connection will form with non-vanishing displacement, s . Because the time scale at which the number of connected integrins reaches a steady-state is likely to be much shorter than the time scale of minutes with which we are concerned, we seek for steady-state solutions of Eqs. S4 and consequently all relevant symbols hereafter refer to the steady-state solution. A particular (Green's function) solution is obtained by assuming that $g(s) = \delta(s)$ is a delta function; this is applicable to the case that connections always form with zero displacement initially. One finds the following solution for $n(s)$ in the steady state:

$$n(s) = \frac{N \exp\left[\frac{-1}{v_F} \int_0^s k_{ub}(s') ds'\right] \theta(s)}{\frac{v_F}{k_b} + \int_0^{\infty} \exp\left[\frac{-1}{v_F} \int_0^s k_{ub}(s') ds'\right] ds} \quad (\text{S5})$$

where $\theta(s)$ is the Heaviside step function. This solution can be used to find a general solution for any choice of $g(s)$ by linear superposition (e.g., see [4]). Once $n(s)$ has been determined, the mean displacement, $\langle s \rangle = \langle s_l + s_m \rangle$, and density of bound integrin connections, N_b , can be calculated via:

$$\langle s \rangle = \frac{1}{N_b} \int_0^{\infty} s n(s) ds \quad \text{and} \quad N_b = \int_0^{\infty} n(s) ds \quad (\text{S6})$$

Eqs. S5, S6 can be used to study different forms of the displacement-dependence of the rate constants, and one can distinguish between catch-bond and slip-bond dynamics, see [1] for a discussion of these cases. A significant simplification is obtained with the assumption that $g(s)$ is symmetric and that the binding/unbinding rate constants are displacement independent, i.e., that $k_b N_L = 1/\tau_{ub}$ and $k_{ub} = 1/\tau_b$ are constants, where τ_{ub} and τ_b are the corresponding mean life time of the unbound and bound states. In this case, Eqs. S5, S6 directly lead to the results stated in Eq. 2:

$$N_b = \frac{\tau_b}{\tau_b + \tau_{ub}} N \quad \text{and} \quad \langle s_l + s_m \rangle = \tau_b v_F \quad (\text{S7})$$

The validity of the assumption that τ_{ub} and τ_b are fixed during spreading has been examined with experiments on endothelial cells by measuring the temporal dependence of the (mean) radial cell tractions on cell spreading speed [1]. The analysis suggested that cell-substrate adhesions behave as catch-bonds, namely that $k_{ub}(s)$ is, on average, a decreasing function of s in the course of spreading. Finally, Eq. S7 can also be derived directly from Eq. S4 by multiplying both sides of the equation by s and integrating over the whole range of s from minus-infinity to infinity.

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