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Supplemental information

Deconstructing the role of myosin contractility in force fluctuations within focal adhesions

Debsuvra Ghosh, Subhadip Ghosh, and Abhishek Chaudhuri

Supplementary Material to Deconstructing the role of myosin contractility in force fluctuations within focal adhesions

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Appendix A: Dimensionless Equations

Following the physical parameter values used in our model described in the Table 1, we proceed to turn our dynamical equations dimensionless as prescribed in the main text. The characteristic scales for length, time, velocity and force are calculated as $l_0 = 1.76392$ nm, $\omega_d^{-1} = 0.00285714$ s, $v_0 = 617.373$ nm/s and $f = 2.33854$ pN.

$$
\begin{aligned}
\frac{dn_m}{d\tau} &= \tilde{\omega}(N_m - n_m) - n_m \exp\left(\frac{\tilde{\kappa}_m \tilde{y}}{\tilde{f}_d}\right) \\
\frac{d\tilde{x}_c}{d\tau} &= -n_c \tilde{\kappa}_c \tilde{x}_c - n_m \tilde{\kappa}_m \tilde{y} \\
\frac{d\tilde{y}}{d\tau} &= \tilde{v}_u \left(1 - \frac{\tilde{\kappa}_m \tilde{y}}{\tilde{f}_s}\right) + \frac{d\tilde{x}_c}{d\tau} \\
\frac{dn_c}{d\tau} &= \tilde{k}_{\text{on}}(N_c - n_c) - \tilde{k}_{\text{off}} n_c \exp\left(\frac{-\tilde{\kappa}_c \tilde{x}_c}{\tilde{F}_b}\right)
\end{aligned}
\tag{S.1}
$$

Appendix B: Jacobian

The Jacobian matrix ($\mathcal{J}$) is computed to obtain the linearisation about the fixed points of the system which are calculated in the main text. The number of dynamical variables and concerning differential equations is 4, therefore the Jacobian matrix is of the order 4×4 and contains 16 elements as shown in Eq. (S.2).

$$
\frac{d}{d\tau}\begin{pmatrix} \tilde{x}_c \\ \tilde{y} \\ n_m \\ n_c \end{pmatrix} = \mathcal{J} \begin{pmatrix} \tilde{x}_c \\ \tilde{y} \\ n_m \\ n_c \end{pmatrix} = \begin{bmatrix} J_{11} & J_{12} & J_{13} & J_{14} \\ J_{21} & J_{22} & J_{23} & J_{24} \\ J_{31} & J_{32} & J_{33} & J_{34} \\ J_{41} & J_{42} & J_{43} & J_{44} \end{bmatrix} \begin{pmatrix} \tilde{x}_c \\ \tilde{y} \\ n_m \\ n_c \end{pmatrix}
\tag{S.2}
$$

The elements of the Jacobian matrix, J_{ij}, are explicitly calculated and the full matrix is depicted below,

$$\mathcal{J}=\begin{bmatrix} -\tilde{k}_{\mathrm{on}}-\tilde{k}_{\mathrm{off}}\exp\left(\frac{n_m^0\tilde{f}_s}{n_c^0\tilde{F}_b}\right) & \tilde{k}_{\mathrm{off}}\frac{n_c^0\tilde{\kappa}_c}{\tilde{F}_b}\exp\left(\frac{n_m^0\tilde{f}_s}{n_c^0\tilde{F}_b}\right) & 0 & 0 \\ \frac{\tilde{f}_s n_m^0}{n_c^0} & -n_c^0\tilde{\kappa}_c & -n_m^0\tilde{\kappa}_m & -\tilde{f}_s \\ \frac{\tilde{f}_s n_m^0}{n_c^0} & -n_c^0\tilde{\kappa}_c & -\tilde{v}_u\frac{\tilde{\kappa}_m}{\tilde{f}_s}-n_m^0\tilde{\kappa}_m & -\tilde{f}_s \\ 0 & 0 & -n_m^0\frac{\tilde{\kappa}_m}{\tilde{f}_d}\exp\left(\frac{\tilde{f}_s}{\tilde{f}_d}\right) & -\tilde{\omega}-\exp\left(\frac{\tilde{f}_s}{\tilde{f}_d}\right) \end{bmatrix} \tag{S.3}$$

The characteristic polynomial of the Jacobian has the form mentioned in Eq. 7,

$$P(\lambda)=\lambda^4+\mathcal{A}\lambda^3+\mathcal{B}\lambda^2+\mathcal{C}\lambda+\mathcal{D}=0 \tag{S.4}$$

which is a fourth order polynomial equation, where $\mathcal{A}$ is trace of matrix $\mathcal{J}$ or –Tr[$\mathcal{J}$], and $\mathcal{D}$ is determinant or Det[$\mathcal{J}$]. The coefficients are explicitly calculated as follows,

$$\mathcal{A} = \tilde{k}_{\mathrm{on}}+\tilde{k}_{\mathrm{off}}\exp\left(\frac{n_m^0\tilde{f}_s}{n_c^0\tilde{F}_b}\right)+n_c^0\tilde{\kappa}_c+\tilde{v}_u\frac{\tilde{\kappa}_m}{\tilde{f}_s}+n_m^0\tilde{\kappa}_m+\tilde{\omega}+\exp\left(\frac{\tilde{f}_s}{\tilde{f}_d}\right) \tag{S.5}$$

$$\begin{aligned}\mathcal{B} &= \frac{1}{\tilde{F}_b\tilde{f}_d\tilde{f}_s}\Bigg[\exp\left\{\tilde{f}_s\left(\frac{1}{\tilde{f}_d}+\frac{n_m^0}{\tilde{F}_b n_c^0}\right)\right\}\tilde{F}_b\tilde{f}_d\tilde{f}_s\tilde{k}_{\mathrm{off}}+\exp\left(\frac{\tilde{f}_s}{\tilde{f}_d}\right)\tilde{F}_b\left\{\tilde{f}_d\tilde{f}_s(\tilde{k}_{\mathrm{on}}+n_c^0\tilde{\kappa}_c)+\tilde{f}_d\tilde{v}_u\tilde{\kappa}_m\right.\\ &+ \left.(\tilde{f}_d-\tilde{f}_s)\tilde{f}_s n_m^0\tilde{\kappa}_m\right\}+\exp\left(\frac{\tilde{f}_s n_m^0}{\tilde{F}_b n_c^0}\right)\tilde{f}_d\tilde{k}_{\mathrm{off}}\left\{\tilde{F}_b\tilde{v}_u\tilde{\kappa}_m-\tilde{f}_s^2 n_m^0\tilde{\kappa}_c+\tilde{F}_b\tilde{f}_s\left(n_c^0\tilde{\kappa}_c+n_m^0\tilde{\kappa}_m+\tilde{\omega}\right)\right\}\\ &+ \tilde{F}_b\tilde{f}_d\left\{\tilde{f}_s\tilde{\omega}(n_c^0\tilde{\kappa}_c+n_m^0\tilde{\kappa}_m)+\tilde{v}_u\tilde{\kappa}_m(\tilde{k}_{\mathrm{on}}+n_c^0\tilde{\kappa}_c+\tilde{\omega})+\tilde{f}_s\tilde{k}_{\mathrm{on}}(n_c^0\tilde{\kappa}_c+n_m^0\tilde{\kappa}_m+\tilde{\omega})\right\}\Bigg]\end{aligned} \tag{S.6}$$

$$\begin{aligned}\mathcal{C} &= \frac{1}{\tilde{F}_b\tilde{f}_d\tilde{f}_s}\Bigg[\exp\left\{\tilde{f}_s\left(\frac{1}{\tilde{f}_d}+\frac{n_m^0}{\tilde{F}_b n_c^0}\right)\right\}\tilde{k}_{\mathrm{off}}\left[\tilde{F}_b\tilde{\kappa}_m\left\{(\tilde{f}_d-\tilde{f}_s)\tilde{f}_s n_m^0+\tilde{f}_d\tilde{v}_u\right\}-\tilde{f}_d\tilde{f}_s\tilde{\kappa}_c(\tilde{f}_s n_m^0-\tilde{F}_b n_c^0)\right]\\ &+ \exp\left(\frac{\tilde{f}_s}{\tilde{f}_d}\right)\tilde{F}_b\left\{\tilde{f}_d\tilde{v}_u\tilde{\kappa}_m(\tilde{k}_{\mathrm{on}}+n_c^0\tilde{\kappa}_c)+\tilde{f}_d\tilde{f}_s\tilde{k}_{\mathrm{on}}(n_c^0\tilde{\kappa}_c+n_m^0\tilde{\kappa}_m)-\tilde{f}_s^2\tilde{k}_{\mathrm{on}}n_m^0\tilde{\kappa}_m\right\}\\ &+ \tilde{F}_b\tilde{f}_d\left\{\tilde{k}_{\mathrm{on}}n_c^0\tilde{v}_u\tilde{\kappa}_c\tilde{\kappa}_m+\tilde{v}_u\tilde{\kappa}_m\tilde{\omega}(\tilde{k}_{\mathrm{on}}+n_c^0\tilde{\kappa}_c)+\tilde{\omega}\tilde{f}_s\tilde{k}_{\mathrm{on}}(n_c^0\tilde{\kappa}_c+n_m^0\tilde{\kappa}_m)\right\}\\ &+ \exp\left(\frac{\tilde{f}_s n_m^0}{\tilde{F}_b n_c^0}\right)\tilde{f}_d\tilde{k}_{\mathrm{off}}\left\{\tilde{F}_b\tilde{\kappa}_m\tilde{\omega}\left(\tilde{v}_u+\tilde{f}_s n_m^0\right)+\tilde{\kappa}_c(\tilde{F}_b n_c^0-\tilde{f}_s n_m^0)(\tilde{v}_u\tilde{\kappa}_m+\tilde{f}_s\tilde{\omega})\right\}\Bigg]\end{aligned} \tag{S.7}$$

$$\mathcal{D} = \tilde{v}_u\frac{\tilde{\kappa}_c\tilde{\kappa}_m}{\tilde{f}_s\tilde{F}_b}\left\{\tilde{\omega}+\exp\left(\frac{\tilde{f}_s}{\tilde{f}_d}\right)\right\}\left[n_c^0\tilde{k}_{\mathrm{on}}\tilde{F}_b+\tilde{k}_{\mathrm{off}}\exp\left(\frac{n_m^0\tilde{f}_s}{n_c^0\tilde{F}_b}\right)\left(\tilde{F}_b n_c^0-\tilde{f}_s n_m^0\right)\right] \tag{S.8}$$

Nature and properties of the eigenvalues are dependent on the sign of the coefficients $\mathcal{A}$, $\mathcal{B}$, $\mathcal{C}$ and $\mathcal{D}$. We explore the algebra of polynomial equations to ascertain the features of the roots that they possess, which in turn provides us with the dynamical phases without explicitly solving the differential equations governing the system. In the following section, we shall detail a method to systematically determine the characteristics of algebraic roots of a real valued polynomial equation.

Appendix C: Newton's Rules for computing types and signs of roots

Newton formulated a set of rules that furnishes a lower bound for the cardinality of imaginary roots of a polynomial, in addition to the upper bound of positive roots, by taking into account the permanences and variations in an order of signs as procured from the polynomial.

Given a polynomial $P(x)$,

$$P(x) = {}^nC_0 a_n x^n + {}^nC_1 a_{n-1} x^{n-1} + {}^nC_1 a_{n-1} x^{n-1} + \cdots + {}^nC_{n-1} a_1 x + {}^nC_0 a_0 \tag{S.9}$$

Simple elements are denoted as a_n, a_{n-1}, a_{n-2}, ..., a_1, a_0. **Quadratic elements** are denoted as Q_r, where Q_r is defined as follows,

$$\text{For} \quad P(x) = \sum_{i=0}^{n} p_{n-i} x^{n-i}, \quad Q_r = \frac{p_r^2}{({}^nC_r)^2} - \frac{p_{r+1}}{{}^nC_{r+1}} \frac{p_{r-1}}{{}^nC_{r-1}} \tag{S.10}$$

$$= \frac{1}{({}^nC_r)^2}\left[p_r^2 - \frac{{}^nC_r}{{}^nC_{r+1}} \frac{{}^nC_r}{{}^nC_{r-1}} (p_{r+1})(p_{r-1})\right]$$

$$= \frac{1}{({}^nC_r)^2}\left[p_r^2 - \frac{\frac{n!}{r!(n-r)!}}{\frac{n!}{(r+1)!(n-r-1)!}} \frac{\frac{n!}{r!(n-r)!}}{\frac{n!}{(r-1)!(n-r+1)!}} (p_{r+1})(p_{r-1})\right]$$

$$\text{Finally,} \quad Q_r = \frac{1}{({}^nC_r)^2}\left[p_r^2 - \frac{r+1}{n-r} \frac{n-r+1}{r} (p_{r+1})(p_{r-1})\right] \tag{S.11}$$

Theorem 0.1 (Newton's Incomplete Rule) *Supposing that the quadratic elements for a polynomial $P(x)$ are all non-zero, the number of variations of signs in the sequence $Q_n, Q_{n-1}, \ldots, Q_0$ provides a lower bound for the number of imaginary roots of $P(x)$.*

To obtain Newton's complete rule, one has to look at the sequences of both simple and quadratic elements,

$$\begin{array}{cccccc} a_n & a_{n-1} & a_{n-2} & \cdots & a_1 & a_0 \\ Q_n & Q_{n-1} & Q_{n-2} & \cdots & Q_1 & Q_0 \end{array}$$

By concentrating on associated pairs i.e.,

$$\begin{array}{cccc} \cdots & a_{r+1} & a_r & \cdots \\ \cdots & Q_{r+1} & Q_r & \cdots \end{array}$$

We are to look for possibilities of sign changes in the aforementioned pair by denoting them by their permanence i.e. no changes in sign and variance i.e. changes in sign in the following manner: a lowercase **v** denotes variance in sign of upper element of the pairs, an upper-case **V** denotes variance in the sign of lower element of the pairs, a lower-case **p** denotes permanence of sign of upper element of the pairs and an upper-case **P** denotes permanence of sign of lower element of the pairs. By instating this schema, we obtain four possible ways the signs can change in a pair — vV, vP, pV and pP.

Theorem 0.2 (Newton's Complete Rule) *Supposing a non zero simple and quadratic elements of $P(x)$, then the total number of double permanences, written as $\sum pP$ is an upper bound of number of negative roots and total number of variance-permanences, written as $\sum vP$ is the upper bound of positive roots.*

Corollary 1 *The total number of real roots are the sum of double permanences and variance-permanences.*

Lower Limit	Coefficient	Upper Limit
8.35772	$\mathcal{A}$	10.2638
-2.53967	$\mathcal{B}$	13.2611
-0.0142965	$\mathcal{C}$	4.15717
0	$\mathcal{D}$	0.0129296

Table S1: The limits on the values of coefficients $\mathcal{A},\mathcal{B},\mathcal{C}$ & $\mathcal{D}$

Therefore total number of real roots is equal to total number of permanences in quadratic elements i.e. $\sum P$. This is an upper bound of the real roots. Thus $n-\sum P = \sum V$ is the lower bound of number of complex roots. We may now proceed with using these rules to obtain the bounds on types of roots for a quartic polynomial with real coefficients that appears as a characteristic polynomial for our system.

A quartic polynomial $P_4(x)$ has the following form,

$$\begin{aligned} P_4(x) &= {}^4C_0 a_4 x^4 + {}^4C_1 a_3 x^3 + {}^4C_2 a_2 x^2 + {}^4C_3 a_1 x + {}^4C_4 a_0 \\ &= a_4 x^4 + 4a_3 x^3 + 6a_2 x^2 + 4a_1 x + a_0 \end{aligned} \tag{S.12}$$

Comparing it with the quartic polynomial of the form $x^4+\mathcal{A}x^3+\mathcal{B}x^2+\mathcal{C}x+\mathcal{D}$, as used in the main text, the simple elements are calculated to be $a_4 = 1, a_3 = \mathcal{A}/4, a_2 = \mathcal{B}/6, a_1 = \mathcal{C}/4$ and finally $a_0 = \mathcal{D}$. Similarly, the quadratic elements are $Q_4 = 1, Q_3 = \mathcal{A}^2/16-\mathcal{B}/6, Q_2 = \mathcal{B}^2/36-\mathcal{A}\mathcal{C}/16, Q_1 = \mathcal{C}^2/16-\mathcal{B}\mathcal{D}/6$ and $Q_0 = \mathcal{D}^2$.

It is possible to numerically show that any quartic polynomial will have at most 14 different combinations of roots. Our system has two constraints on the characteristic polynomial due to the fact that two of the coefficients, $\mathcal{A}$ and $\mathcal{D}$ are entirely positive inside the relevant parametric space, thus leaving only 4 possible combinations of signs for $\mathcal{B}$ and $\mathcal{C}$, as we shall observe. The coefficients of the characteristic polynomial have the following limits in the parametric space,

We proceed with finding the bounds on cardinality of different types of roots for our system by calculating the simple and quadratic elements as described earlier with different combinations of coefficients under the bounds laid down in Table S1.

CASE I Both $\mathcal{B}$ and $\mathcal{C}$ are positive

The simple elements do not have a change in sign which prohibits roots with positive $\mathbb{R}$ part. ΣvP being zero throughout confirms this.

a	+	+	+	+	+	ΣpP is 4, either 2 (–) $\mathbb{R}$ roots & 2 $\mathbb{C}$ roots with (–) $\mathbb{R}$ part,
Q	+	+	+	+	+	or 4 (–) $\mathbb{R}$ roots

a	+	+	+	+	+	ΣpP is 2, 2 (–) $\mathbb{R}$ roots & 2 $\mathbb{C}$ roots with (–) $\mathbb{R}$ part
Q	+	+	+	–	+	

a	+	+	+	+	+	ΣpP is 2, 2 (–) $\mathbb{R}$ roots & 2 $\mathbb{C}$ roots with (–) $\mathbb{R}$ part
Q	+	+	–	+	+	

a	+	+	+	+	+	$\Sigma p P$ is 2, 2 (–) $\mathbb{R}$ roots & 2 $\mathbb{C}$ roots with (–) $\mathbb{R}$ part
Q	+	+	−	−	+	

CASE II $\mathcal{B}$ is positive but $\mathcal{C}$ is negative

a	+	+	+	−	+	$\Sigma p P = \Sigma \upsilon P = 2$, maximum 2 (+) and 2 (–) $\mathbb{R}$ roots
Q	+	+	+	+	+	

a	+	+	+	−	+	$\Sigma p P$ is 2, and $\Sigma \upsilon P$ is 0, i.e. maximum 2 (–) $\mathbb{R}$ roots but no (+) $\mathbb{R}$ roots
Q	+	+	+	−	+	

CASE III $\mathcal{B}$ is negative but $\mathcal{C}$ is positive

a	+	+	−	+	+	$\Sigma p P = \Sigma \upsilon P = 2$, maximum 2 (+) and 2 (–) $\mathbb{R}$ roots
Q	+	+	+	+	+	

a	+	+	−	+	+	$\Sigma p P$ is 2, and $\Sigma \upsilon P$ is 0, i.e. maximum 2 (–) $\mathbb{R}$ roots but no (+) $\mathbb{R}$ roots
Q	+	+	−	+	+	

CASE IV Both $\mathcal{B}$ and $\mathcal{C}$ are negative

a	+	+	−	−	+	$\Sigma p P = \Sigma \upsilon P = 2$, maximum 2 (+) and 2 (–) $\mathbb{R}$ roots
Q	+	+	+	+	+	

We proceed to collate various possible combinations of roots, as predicted by Newton's rules of signs, in Table S2.

From Table S2, we can conclude, with λ_js, where $j = 1, \ldots, 4$, denoting four eigenvalues, miscellany of positive and negative $\mathcal{B}$, and $\mathcal{C}$ lead to the following combination of eigenvalues: (i) $\lambda_{1,2,3,4}$ all are real negative, (ii) $\lambda_{1,2}$ real negative and $\lambda_{3,4}$ real positive, (iii) $\lambda_{1,2}$ real negative and $\lambda_{3,4} = -\alpha \pm i\beta$, and (iv) $\lambda_{1,2}$ real negative and $\lambda_{3,4} = \alpha \pm i\beta$. α and β are real positive numbers. Case (i) corresponds to linearly stable (s) phase where a perturbation decays exponentially with time and the system returns to its fixed point. Case (ii) is characterized by exponentially growing perturbations in time and called unstable (u) phases. Instability in our system is established when all the clutches are detached from the actin filament and it is is freely pulled by the molecular motors. Stable spiral (ss) or oscillation decaying with time is the characteristic property of case (iii), which reaches stable (s) phase at long time scale. Growing oscillation in time is a hallmark of unstable spiral (us) that originates from the presence of positive real part of the complex eigenvalues as indicated in (iv). Going beyond the ambit of linear stability and numerically solving the coupled non-linear equations, presents the unstable spiral phase as a precursor of stable oscillation in the system, as shown in Fig. 3.

Signs of Coefficients		Types of Roots			
$\mathcal{B}$	$\mathcal{C}$	λ_1	λ_2	λ_3	λ_4
$+$	$+$	$-\mathbb{R}$ $-\mathbb{R}$	$-\mathbb{R}$ $-\mathbb{R}$	$-\mathbb{R}$ $-\Re(\mathbb{C})$	$-\mathbb{R}$ $-\Re(\mathbb{C})$
$+$	$-$	$-\mathbb{R}$ $-\mathbb{R}$	$-\mathbb{R}$ $-\mathbb{R}$	$+\mathbb{R}$ $+\Re(\mathbb{C})$	$+\mathbb{R}$ $+\Re(\mathbb{C})$
$-$	$+$	$-\mathbb{R}$ $-\mathbb{R}$	$-\mathbb{R}$ $-\mathbb{R}$	$+\mathbb{R}$ $+\Re(\mathbb{C})$	$+\mathbb{R}$ $+\Re(\mathbb{C})$
$-$	$-$	$-\mathbb{R}$ $-\mathbb{R}$	$-\mathbb{R}$ $-\mathbb{R}$	$+\mathbb{R}$ $+\Re(\mathbb{C})$	$+\mathbb{R}$ $+\Re(\mathbb{C})$

Table S2: Possible roots (λ_i) resulting from various combination of signs of $\mathcal{B}$ and $\mathcal{C}$. $\mathbb{R}$ denotes real roots and $\Re(\mathbb{C})$ refers to real parts of complex roots.

Appendix D: Coefficients for the case where the clutches are always bound to the filament

The coefficients $\mathcal{A}', \mathcal{B}'$ and $\mathcal{C}'$ are given as follows:

$$\mathcal{A}' = \tilde{\omega} + \exp\left(\frac{\tilde{f}_s}{\tilde{f}_d}\right) + \frac{1}{\epsilon^2}\left(N_c \tilde{\kappa}_c + n_m^0 \tilde{\kappa}_m\right) + \frac{\tilde{v}_u \tilde{\kappa}_m}{\tilde{f}_s} \tag{S.13}$$

$$\mathcal{B}' = \frac{\tilde{v}_u \tilde{\kappa}_m N_c \tilde{\kappa}_c}{\tilde{f}_s \epsilon^2} + \left[\tilde{\omega} + \exp\left(\frac{\tilde{f}_s}{\tilde{f}_d}\right)\right]\left[\frac{\tilde{v}_u \tilde{\kappa}_m}{\tilde{f}_s} + \frac{N_c \tilde{\kappa}_c}{\epsilon^2} + \frac{n_m^0 \tilde{\kappa}_m}{\epsilon^2}\right] - \frac{\tilde{f}_s n_m^0 \tilde{\kappa}_m}{\epsilon^2 \tilde{f}_d} \exp\left(\frac{\tilde{f}_s}{\tilde{f}_d}\right) \tag{S.14}$$

$$\mathcal{C}' = \frac{\tilde{v}_u \tilde{\kappa}_m N_c \tilde{\kappa}_c}{\tilde{f}_s \epsilon^2}\left[\tilde{\omega} + \exp\left(\frac{\tilde{f}_s}{\tilde{f}_d}\right)\right] \tag{S.15}$$