Systems Mechano-Biology: Tension-inhibited Protein Turnover is sufficient to physically control Gene Circuits

Supporting Material

P. C. Dave P. Dingal[1] and Dennis E. Discher[1]

[1]Molecular and Cell Biophysics Laboratory, Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, Pennsylvania

Email: P. C. Dave P. Dingal - polimyr@seas.upenn.edu; Dennis E. Discher - discher@seas.upenn.edu

Contents

1 Mechanobiological gene circuit: systems of ordinary differential equations

1.1 Single-module gene circuits: reaction-order stability

A single module refers to a particular gene (S) and its corresponding protein (s) and is described by general rate equations that define what factors affects synthesis and turnover. To incorporate the stabilizing effect of tension (K_s) on protein we define degradation term δ as a Hill function as in Equation **(3)** in the main text:

$$\delta(s) = \frac{\delta_0 s^n}{K_s^n + s^n}$$

The set of ordinary differential equations (ODEs) for a single module is simply:

$$\begin{aligned}\frac{dS}{dt} &= \alpha(s) - \beta(S) \\ \frac{ds}{dt} &= \gamma(S) - \frac{\delta_0 s^n}{K_s^n + s^n}\end{aligned}$$

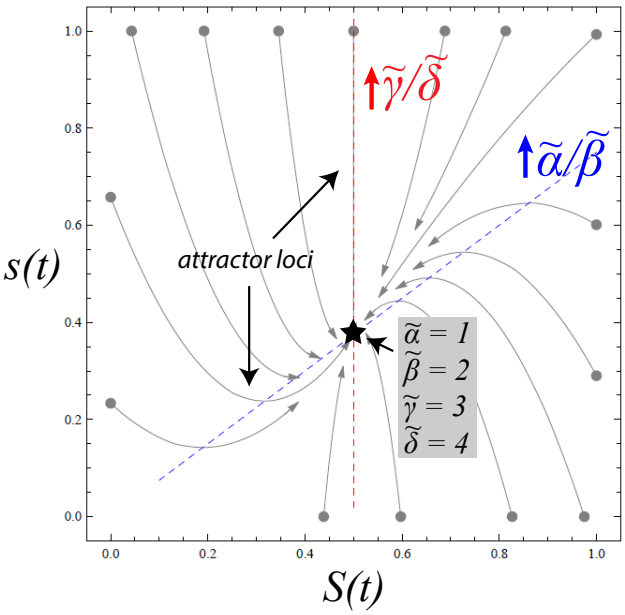
Ultimately, the value of K_s (and hence, E) dictates the steady state values, regardless of the initial condition. Here, we explored the functional forms of the various rates that dictate the stability and dynamics of the system. For the given Hill-functional form of δ above, we explored the various reaction-order forms of the other rates that converge to a steady state solution.

1.1.1 Rate-order forms by Schwanhausser *et al.*

Schwanhausser *et al.* [1] quantified proteomic and transcriptomic half-lives under the following rate equations:

$$\begin{aligned}\frac{dS}{dt} &= \tilde{\alpha} - \tilde{\beta} \cdot S \\ \frac{ds}{dt} &= \tilde{\gamma} \cdot S - \tilde{\delta} \cdot s\end{aligned}$$

where $\tilde{\alpha}, \tilde{\beta}, \tilde{\gamma}, \tilde{\delta}$ are rate constants. A phase plot of protein (s) and mRNA (S), while varying the ratio of synthesis/degradation rates for protein ($\tilde{\gamma}/\tilde{\delta}$) and mRNA ($\tilde{\alpha}/\tilde{\beta}$), shows that mRNA-relevant rates scale with mRNA and protein levels (blue line), but that the protein-relevant rates do not (red line). This was expected from the chosen forms of the rate equations above where



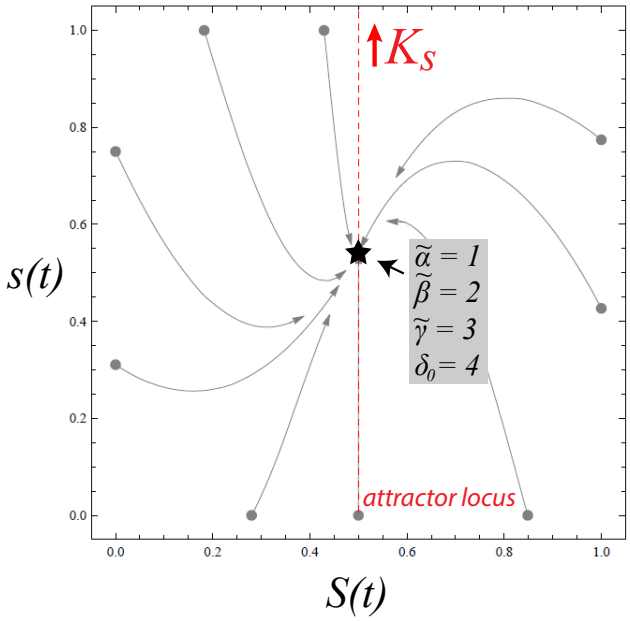
protein levels do not affect mRNA levels. Thus, in the search for the appropriate forms of the rate equations that capture the variations in structural protein expression with matrix elasticity, E, the ones described by Schwanhausser *et al.* are not sufficient.

1.1.2 Zeroth-order α, tension-dependent δ

Now if we consider changing degradation rate to be tension-dependent, keeping others similar to above:

$$\frac{dS}{dt} = \tilde{\alpha} - \tilde{\beta} \cdot S$$
$$\frac{ds}{dt} = \tilde{\gamma} \cdot S - \frac{\delta_0 s^n}{K_s^n + s^n}$$

In this case, we get the following phase plot where varying K_s, and hence protein levels, does not affect mRNA levels. This is again not physically relevant as we expect that both mRNA



and protein levels should respond to K_s (or E).

1.1.3 First-order rates, tension-dependent δ

First-order rates were chosen for the main text as they parsimoniously recapitulate experimental observations for the mechanobiological gene circuit of lamin A [2].

$$\frac{dS}{dt} = \tilde{\alpha} \cdot s - \tilde{\beta} \cdot S$$
$$\frac{ds}{dt} = \tilde{\gamma} \cdot S - \frac{\delta_0 s^n}{K_s^n + s^n}$$

With the lack of precise values for rate constants published in literature, we chose $O(1)$ values for the parameters. For the purposes of display in Figure 2 of the main text, we used the following values for each of the rate constants:

Parameter	Value
$\tilde{\alpha}$	1 s^{-1}
$\tilde{\beta}$	2 s^{-1}
$\tilde{\gamma}$	3 s^{-1}
δ_0	4 s^{-1}
n	2

For $n = 2$, constraints to rate-parameter values arise and can expressed in analytical form (see Section 2). Protein and mRNA levels were initialized in the range $\{0, 1\}$, as shown in the phase plot in Figure 2B.

1.1.4 Unstable steady states: higher-order rates

The following higher-order ($p \geq 2$) cases exhibit divergent steady states, and hence not biologically relevant:

- $\alpha = \tilde{\alpha} \cdot s^p$; $\beta = \tilde{\beta} \cdot S$; $\gamma = \tilde{\gamma} \cdot S$;
- $\alpha = \tilde{\alpha} \cdot s$; $\beta = \tilde{\beta} \cdot S$ $\gamma = \tilde{\gamma} \cdot S^p$;
- $\alpha = \tilde{\alpha} \cdot s^p$; $\beta = \tilde{\beta} \cdot S^p$ $\gamma = \tilde{\gamma} \cdot S^p$;

1.2 Two-Module Gene Circuit

Assuming first-order rates (except for protein degradation rates), the set of ODEs for coupled two-module gene circuit is:

$$\begin{aligned}
\frac{dL}{dt} &= \tilde{\alpha}_1 \cdot l - \tilde{\beta}_1 \cdot L \\
\frac{dl}{dt} &= \tilde{\gamma}_1 \cdot L - \delta_1 \cdot \frac{l^{n_l}}{K_l^{n_l} + l^{n_l}} \\
\frac{dM}{dt} &= \tilde{\alpha}_2 \cdot m \ \ + \tilde{\alpha}_3 \cdot l - \tilde{\beta}_2 \cdot M \\
\frac{dm}{dt} &= \tilde{\gamma}_2 \cdot M - \delta_2 \cdot \frac{m^{n_m}}{K_m^{n_m} + m^{n_m}}
\end{aligned}$$

where $K_s = f(s)$ or $K_s = f(tension)$ of some functional form (e.g. power-law). For lamin-myosin coupling, we chose $K_l = m^{x/n_l}$ and $K_m = E^{y/n_m}$.

The following values for rate constants were used in the simulations presented in Figures 3B, C of the main text:

Parameter	Value
$\{\tilde{\alpha_1}, \tilde{\alpha_2}\}$	$\{1.1\ s^{-1}, 1.1\ s^{-1}\}$
$\{\tilde{\beta}_1, \tilde{\beta}_2\}$	$\{5\ s^{-1}, 5\ s^{-1}\}$
$\{\tilde{\gamma_1}, \tilde{\gamma_2}\}$	$\{1.2\ s^{-1}, 1.2\ s^{-1}\}$
$\{\delta_1, \delta_2\}$	$\{5\ s^{-1}, 5\ s^{-1}\}$
$\{n_l, n_m\}$	$\{2, 2\}$
$\{x, y\}$	$\{0.44, 0.44\}$

with $E = 0.003 - 0.4$, to represent the order-of-magnitude range of elasticities in cell-on-gel experiments ($0.3 - 40$ kPa; ref. 2). The different molecular species were all initialized as $L(0) = l(0) = m(0) = M(0) = 0.005$.

1.3 Two-Module, Tissue-level Mechanobiological Gene Circuit

The set of ODEs for population-level coupling of two modules (e.g. collagen and myosin levels in a developing heart) are:

$$\begin{aligned}
\frac{dC}{dt} &= \tilde{\alpha}_1 \cdot \frac{c^{n_f-1}}{k_f^{n_f} + c^{n_f}} - \tilde{\beta}_1 \cdot C \\
\frac{dc}{dt} &= \tilde{\gamma}_1 \cdot C - \tilde{\delta}_1 \cdot \frac{c^{n_c}}{K_c^{n_c} + c^{n_c}} \\
\frac{dM}{dt} &= \tilde{\alpha}_2 \cdot m - \tilde{\beta}_2 \cdot M \\
\frac{dm}{dt} &= \tilde{\gamma}_2 \cdot M - \delta_2 \cdot \frac{m^{n_m}}{K_m^{n_m} + m^{n_m}}
\end{aligned}$$

where $K_c = m^{x/n_c}$ for some x, $K_m = c^{y/n_m}$, for some y.

The following values for rate constants were used in the simulations in Figure 5 (unless otherwise specified):

Parameter	Value
$\{\tilde{\alpha_1}, \tilde{\alpha_2}\}$	$\{5.2\ s^{-1}, 4.1\ s^{-1}\}$
$\{\tilde{\beta}_1, \tilde{\beta}_2\}$	$\{5\ s^{-1}, 3\ s^{-1}\}$
$\{\tilde{\gamma_1}, \tilde{\gamma_2}\}$	$\{3\ s^{-1}, 1.5\ s^{-1}\}$
$\{\delta_1, \delta_2\}$	$\{6.5\ s^{-1}, 7\ s^{-1}\}$
$\{n_f, n_c, n_m\}$	$\{2, 1.6, 3.7\}$
$\{x, y, k_f\}$	$\{0.51, 0.39, 0.89\}$

The different molecular species were initialized with the following values:

Species	Initial Value
C	0.00051
c	0.00051
M	0.011
m	0.011

2 Analytical Solution for Single-Module Gene Circuit

For the case that δ is tension-dependent and all other rates are first-order (Section 1.1.3), as in the main text, at steady state we have:

$$F(s,S) = \frac{dS}{dt} = 0 = \tilde{\alpha} \cdot s - \tilde{\beta} \cdot S \tag{2.0.1}$$

$$G(s,S) = \frac{ds}{dt} = 0 = \tilde{\gamma} \cdot S - \frac{\delta_0 s^n}{K_s^n + s^n} \tag{2.0.2}$$

2.1 Stability Analysis

First, we characterize the stability of fixed points (steady-state solutions) in the general case. The set of ODEs above include a nonlinear term, and so we perform linearization near a critical point (s^*, S^*) such that:

$$\begin{pmatrix} S \\ s \end{pmatrix}' = \begin{pmatrix} \frac{\partial F}{\partial S}(s^*,S^*) & \frac{\partial F}{\partial s}(s^*,S^*) \\ \frac{\partial G}{\partial S}(s^*,S^*) & \frac{\partial G}{\partial s}(s^*,S^*) \end{pmatrix} \cdot \begin{pmatrix} S - S^* \\ s - s^* \end{pmatrix}$$

The Jacobian matrix above simplifies to:

$$\begin{pmatrix} -\tilde{\beta} & \tilde{\alpha} \\ \tilde{\gamma} & \delta_0 \frac{nK_s^n (s^*)^{n-1}}{(K_s^n + (s^*)^n)^2} \end{pmatrix}$$

All solutions converge to the critical point when the eigenvalues (λ's) are all negative. The characteristic polynomial of the Jacobian matrix is:

$$\lambda^2 + \left[\tilde{\beta} + \delta_0 \frac{nK_s^n (s^*)^{n-1}}{(K_s^n + (s^*)^n)^2}\right] \cdot \lambda + \left[\tilde{\beta} \cdot \delta_0 \frac{nK_s^n (s^*)^{n-1}}{(K_s^n + (s^*)^n)^2} - \tilde{\gamma}\tilde{\alpha}\right] = 0 \tag{2.1.1}$$

The eigenvalues are:

$$\lambda_{1,2} = -\frac{1}{2}\left[\tilde{\beta} + \delta_0 \frac{nK_s^n (s^*)^{n-1}}{(K_s^n + (s^*)^n)^2}\right] \pm \frac{1}{2}\sqrt{\left[\tilde{\beta} + \delta_0 \frac{nK_s^n (s^*)^{n-1}}{(K_s^n + (s^*)^n)^2}\right]^2 - 4\left[\tilde{\beta} \cdot \delta_0 \frac{nK_s^n (s^*)^{n-1}}{(K_s^n + (s^*)^n)^2} - \tilde{\alpha}\tilde{\gamma}\right]} \tag{2.1.2}$$

In order to obtain a stable steady-state solution, the root term has to be less than the first term for the eigenvalues to be negative:

$$\left[\tilde{\beta} + \delta_0 \frac{nK_s^n (s^*)^{n-1}}{(K_s^n + (s^*)^n)^2}\right]^2 > \left[\tilde{\beta} + \delta_0 \frac{nK_s^n (s^*)^{n-1}}{(K_s^n + (s^*)^n)^2}\right]^2 - 4\left[\tilde{\beta} \cdot \delta_0 \frac{nK_s^n (s^*)^{n-1}}{(K_s^n + (s^*)^n)^2} - \tilde{\gamma}\tilde{\alpha}\right] \tag{2.1.3}$$

Simplifying, we get:

$$\frac{\tilde{\beta}\delta_0}{\tilde{\gamma}\tilde{\alpha}} > \frac{(K_s^n + (s^*)^n)^2}{nK_s^n (s^*)^{n-1}} \tag{2.1.4}$$

Thus, the parameter space of the rate constants must obey the above relationship with tension K_s and steady-state protein level s^*. One can immediately see that the null solution ($s^* = 0$) leads to non-negative eigenvalues, and hence an unstable steady state.

2.2 Steady-state solutions for $n = 2$ case

For the simplest case of $n = 2$ and substituting Eq. (2.0.2) to Eq. (2.0.1), we get (after removing null solution):

$$s^3 - \frac{\tilde{\beta}\delta_0}{\tilde{\gamma}\tilde{\alpha}} s^2 + \tilde{\gamma} K_s^2 s = 0 \tag{2.2.1}$$

$$s^2 - \frac{\tilde{\beta}\delta_0}{\tilde{\gamma}\tilde{\alpha}} s + \tilde{\gamma} K_s^2 = 0 \tag{2.2.2}$$

Equation (2.2.2) is simply a quadratic equation, which has two solutions:

$$s_{1,2} = \frac{1}{2}\left(\frac{\tilde{\beta}\delta_0}{\tilde{\gamma}\tilde{\alpha}}\right) \pm \frac{1}{2}\sqrt{\left(\frac{\tilde{\beta}\delta_0}{\tilde{\gamma}\tilde{\alpha}}\right)^2 - 4K_s^2} \tag{2.2.3}$$

Stability analysis shows that the larger solution does not satisfy Equation (2.1.4) and is an unstable node, such that we only have one biologically relevant, non-zero steady state:

$$\{s_{ss}, S_{ss}\} = \left\{\frac{1}{2}\left(\frac{\tilde{\beta}\delta_0}{\tilde{\gamma}\tilde{\alpha}}\right) - \frac{1}{2}\sqrt{\left(\frac{\tilde{\beta}\delta_0}{\tilde{\gamma}\tilde{\alpha}}\right)^2 - 4K_s^2}, \quad \frac{\tilde{\alpha}s_{ss}}{\tilde{\beta}}\right\} \tag{2.2.4}$$

where the rate constants must have values that obey the following:

$$\left(\frac{\tilde{\beta}\delta_0}{\tilde{\gamma}\tilde{\alpha}}\right)^2 - 4K_s^2 \geq 0 \tag{2.2.5}$$

or simply

$$\left(\frac{\tilde{\beta}\delta_0}{\tilde{\gamma}\tilde{\alpha}}\right) \geq 2K_s \tag{2.2.6}$$

It must be noted that Equation (2.2.6) can also be derived from Equation (2.1.4), when the smaller steady-state solution is used.

3 Numerical Solution for Two-Module Gene Circuit

For the coupled mechanobiological gene circuit (in Section 1.2), we employed a numerical solver (Mathematica; version 9, Wolfram Research) to conduct kinetic and steady-state analyses. The outputs for the code below are found in Figure 3B (kinetics plot with $E = 0.4$, and steady-state plot). To obtain Figure 3C plots, we changed the value of J (in code; equivalent to $\tilde{\alpha}_3$ in Section 1.2) from 0 to 1.8. To obtain plots for the case of $E = 0.003$, we changed the upper limit of the **For** loop to $\mathrm{i} < 0.0031$.

```
test = Partition[
 Flatten[Reap[
   For[i = .003, i < .4, i = i + .05, Clear[a, b, h, g, s, L, l, m, M, J, j, t, x, y, A1, A2, E1, q, r
       ];
     a = 1.10; b = 5; g = 1.20; h = 5; x = .440; y = .44; J = 0; j = 1.1; q = 5; r = 1.20; s = 5;
         A1 = 2.0; A2 = 2.0; E1 = i; Ko = 9.2;
     s = NDSolve[{l'[t] == a*L[t] - b*l[t],
      L'[t] == g*l[t] - h*L[t]^A1/(M[t]^x + L[t]^A1),
      m'[t] == J*L[t] + j*M[t] - q*m[t],
      M'[t] == r*m[t] - s*M[t]^A2/(E1^y + M[t]^A2),
      l[0] == .005, L[0] == .005, m[0] == .005, M[0] == .005}, {l, L, m, M}, {t, 0, 1000}];
     Plot[Evaluate[{l[t], L[t], m[t], M[t]} /. s], {t, 0, 1000}, PlotRange -> {{0, 15}, {0.00001,
         1.1}}, PlotStyle -> Thick] Sow[{L[1000] /. s, l[1000] /. s, M[1000] /. s, m[1000] /. s
         }]];][[2, 1]]], 4]; Lss = test[[All, 1]]; lss = test[[All, 2]]; Mss = test[[All, 3]]; mss
         = test[[All, 4]];
Plot[Evaluate[{200 L[t], 200 M[t]} /. s], {t, 0, 100},P lotRange -> {{0, 25}, {0., 10}}, PlotStyle ->
    Thick, PlotLegends -> {"Lamin protein", "Myosin protein"}]
ListLogLogPlot[{Partition[Riffle[200 Lss, 200 Mss], 2]}, PlotRange -> {{0.5, 20}, {0.5, 20}}]
```

4 Numerical Solution for Tissue-level Coupled Gene Circuit

The code listed below refers to Section 1.3 on modeling of the mechano-regulated dynamics of collagen production (by cardiac fibroblasts) and myosin expression and hence contraction levels (by cardiomyocytes), which prints out Figure 5C:

```
Clear[a, b, h, g, u, s, F, f, M, m, J, j, t, T, x, y, k0]
a = 5.2; b = 5; g = 3; h = 6.5; x = .51; j = 4.1; q = 3; r = 1.5; s = 7;
A1 = 1.6; A2 = 3.7; y = .39; z = 2; k0 = .89;
s = NDSolve[{F'[t] == a*f[t]^(z - 1)/(k0^z + f[t]^z) - b*F[t],
    f'[t] == g*F[t] - h*f[t]^A1/(m[t]^x + f[t]^A1),
    M'[t] == j*m[t] - q*M[t],
    m'[t] == r*M[t] - u*m[t]^A2/(f[t]^y + m[t]^A2),
    F[0] == .00051, f[0] == 0.00051, M[0] == 0.011, m[0] == 0.011},
    {F, f, M, m}, {t, 0, 1000}];
LogPlot[Evaluate[{100 F[t], 100 f[t], 100 M[t], 100 m[t]} /. s], {t, 0.5, 1000},
        PlotRange -> {{0, 15}, {0.1, 100.}}]
```

References

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[2] J. Swift, I. L. Ivanovska, A. Buxboim, T. Harada, P. C. D. P. Dingal, J. Pinter, J. D. Pajerowski, K. R. Spinler, J. W. Shin, M. Tewari, F. Rehfeldt, D. W. Speicher, and D. E. Discher. Nuclear lamin-a scales with tissue stiffness and enhances matrix-directed differentiation. *Science*, 341(6149):1240104, 2013.