Supplementary Material: Experimental Evidence for Constraints in Amplitude-Timescale Co-variation of a Biomolecular Pulse Generating Circuit Design

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S1 Area under the pulse

In the main text, the numerical computation approach is presented for amplitude-timescale co-variations, which may be better understood with the following analysis. To study the linearized model of the system \((G(s))\), which follows the same characteristics of pulse as of nonlinear model, to study the constraint analytically. The area under the pulse, which roughly depends on the product of height and timescale can give more insight of such behavior. The area under the curve \((A)\) is,

\[
A = \int_{0}^{\infty} y(t) dt = \lim_{s \to 0} \int_{-\infty}^{\infty} y(t) e^{-st} dt,
\]

\[
= \lim_{s \to 0} Y(s) = \lim_{s \to 0} G(s) U(s),
\]

\[
= \lim_{s \to 0} \frac{\alpha_y \gamma_x}{\alpha_x \gamma_y},
\]

\[
= \frac{\alpha_y}{\alpha_x \gamma_y}.
\]

Note that the area under the curve \(A\) is independent of \(\gamma_x\). This is why an in increase \(\gamma_x\) increases the pulse height but results in a decrease in width so that the area remains unchanged. Similarly an increase in \(\gamma_y\) decreases the area through decrease the height as well as width, this is evident from simulations. In the same way, an increase in \(\alpha_x\) results in a decrease in area, resulting from a decrease in height. The increase of area due to increase in \(\alpha_y\) comes from increase of pulse height, which we noted from the simulations.

In summary, we found that the analysis supports the amplitude and timescale co-variations obtained from numerical simulations.

S2 Co-variations of amplitude and pulse width

In the main text, we addressed the amplitude and timescale co-variations, where we have used pulse height as a metric for amplitude and rise time as a metric for timescale. Another metric that can be used to characterize the timescale is pulse width, which is defined as timespan between half of the pulse height in rising edge to the half of the pulse height in falling edge (Fig. S1A inset).

Computational Analysis

For individual parameter variations, we found that amplitude and pulse width can mutually increase as degradation rate of the output protein \((\gamma_y)\) increases, and that amplitude can decrease with an increase in pulse width as degradation rate of intermediate protein \((\gamma_x)\) increases. Further, we found that only the pulse amplitude, not the pulse width, change when the production factors \((\alpha_x, \alpha_y)\) change. These are summarized in Fig. S1A.

For random individual parameter perturbations, change in pulse width is the \(\Delta t_w = t_{w1} - t_{w2}\), where \(t_{w1}\) is the pulse width when the parameter set is \(\theta_1\) and \(t_{w2}\) is the pulse width when the parameter set is \(\theta_2\), is computed along with change in pulse height \(\Delta p_h\) (as defined in Main Text). For degradation rate parameter of output protein \((\gamma_y)\), the points lie in the first quadrant implying that for an increase in pulse height the pulse width also increases. For the degradation rate parameter of intermediate protein \((\gamma_x)\),

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the points lie in the second quadrant implying that an increase in pulse height results in a decrease in pulse width. For the production rate of the proteins ($\alpha_x$, $\alpha_y$), the points lie on the y-axis showing that the amplitude can increase or decrease without altering the pulse width. Therefore, the trends noted earlier persist across these parameter sets as well. For simultaneous random parameter set perturbations, parametric density plot on the amplitude and pulse width space shows a larger density of relatively lower amplitude and faster pulse width (Fig. S1 C).

Figure S1: Trends for amplitude-timescale co-variations as pulse height and pulse width with parameter space exploration. A) Solid lines are the amplitude and pulse width co-variations as the parameters are individually varied for the nominal parameter parameters. Inset represents a typical pulse trajectory and pulse height as amplitude metric and pulse width as timescale metric. B) Symbols represent the change in pulse height and change in pulse width as different randomly sampled points in parameter space as individually parameters are perturbed. C) Colorbar represents the density of parameters at a particular point in the amplitude-pulse width space as the multiple parameters are simultaneously perturbed. The encircled point shows the nominal parameter set.

Experimental Evidence

We computed pulse width for the pulses reported in the main text (Fig. 3B). We find that as arabinose levels increase pulse height increases and pulse width increases (Fig. S2B). Further, we find that as aTc levels increase, the pulse height increases and pulse width increases. These results shows that for these experimental conditions, there is a constraint that as the pulse height increases, the pulse width increases, making a higher amplitude pulse also wider (Fig. S2B).

Systematic Model Analysis

Similar to main text, we analyzed the systematic models to correlate amplitude and pulse width qualitatively (as done for amplitude and rise time). We noted that for the standard model, the amplitude changes but the pulse width remains fixed for change in aTc and amplitude increases and pulse width decreases for increase in arabinose (Fig. S3A). For modified models, amplitude and pulse width increases for increase in aTc and amplitude increases and pulse width decreases for increase in arabinose (Fig. S3B-C).

We note that for computations as well as experiment, the results of the pulse height and pulse width co-variations are qualitatively same as that of pulse height and rise time co-variation presented in the main text.
Figure S2: A) Pulse height is $p_h$ and pulse width ($t_w$) is time span from $p_{mid} = p_h/2$ (where $p_h$ is the pulse height) on rising edge to falling edge of the pulse output. B) Experimental evidence for pulse height and pulse width co-variations.

Figure S3: Amplitude-rise time co-variation for change of the inducers in computational models A) Solid lines represent the amplitude and pulse width co-variations in the standard model for inducers (indicated with arrow for aTc and arabinose as ara). B) Solid lines represent the amplitude and pulse width co-variations in the modified model of aTc-TetR dynamics for inducers. C) Solid lines represent the amplitude and pulse width co-variations in the modified model of aTc-TetR and arabinose-AraC dynamics for inducers. The change in dynamics from Fig A to C as same as the main text Fig 4 A to C.

S4 Change in inducer level is equivalent to change in dissociation constant

To numerically transform the change in inducer level into a parameter in the system, we need to find the equivalence relations. The feedforward loop based on AraC, TetR and deGFPssrA (in Fig. S3A inset) is equivalent to the $u = [AraC : arabinose]$, $x = [TetR]$ and $y = deGFPssrA$ of Fig 1A (inset).
For araC-arabinose dynamics

Assuming that araC level is at steady-state equals to \([araC]_T\). The binding-unbinding reactions of AraC and arabinose are,

\[ AraC + \text{arabinose} \xrightarrow{k_-} k_+ u. \]  

(2)

At equilibrium,

\[ k_- u = k_+ [\text{arabinose}][\text{AraC}], \]

\[ \implies k_- u = k_+ [\text{arabinose}][[\text{AraC}]_T - u], \]

\[ \implies u = \frac{[\text{AraC}]_T [\text{arabinose}]}{[\text{arabinose}] + k_- / k_+} = \frac{[\text{AraC}]_T [\text{arabinose}]}{[\text{arabinose}] + k_u}. \]

The activation Hill term with respect to \(u\) is,

\[ \frac{u}{u + K_u} = \frac{[\text{AraC}]_T [\text{arabinose}]}{[\text{arabinose}] + k_u + K_{u0}}, \]

\[ = \frac{[\text{AraC}]_T}{[\text{arabinose}] + k_u + K_{u0}} = \frac{[\text{AraC}]_T}{[\text{arabinose}] + K_u}. \]

Here, \(K_{u0}\), for arabinose = 0, changes to \(K_u = K_{u0} c + k_u\) for arabinose = \(c\), \(c > 0\). In summary, the change in arabinose in the experiment is equivalent to change the dissociation constant \(K_u\) of the model.

For TetR-aTc dynamics

The TetR and aTc binding and unbinding reactions are,

\[ x + aTc \xrightarrow{k_{2-}} x : aTc. \]  

(3)

At equilibrium,

\[ k_{2-} [aTc] = k_{2-} [x : aTc], \]

\[ \implies k_{2-} [aTc] = k_{2-} (x - x_T), \]

\[ \implies x = \frac{k_{2-} / k_{2+}}{[aTc] + k_{2-} / k_{2+}} = \frac{k_x}{[aTc] + k_x}. \]

The repression Hill term with respect to \(x\) is,

\[ \frac{K_{x0}}{x + K_{x0}} = \frac{K_{x0}}{x_T [aTc] + k_x + K_{x0}}, \]

\[ = \frac{K_{x0} [aTc] + k_x}{x_T + K_{x0} [aTc] + k_x} = \frac{K_x}{x_T + K_x}. \]
It can be noted, $K_{x0}$, for $aTc = 0$, changes to $K_x = K_{u0} \frac{d + k_x}{k_x}$ for $[aTc] = d$, $d > 0$.

In summary, the change in $aTc$ in the experiment is equivalent to change the dissociation constant $K_x$ of the model.

**S4 Co-variations in standard model for pair wise perturbation**

In the main text Fig. 2A-B, we perturbed one parameter at a time and in Fig 2C we perturbed all the parameters all together. To smoothen the transition we perturbed the parameters pairwise. Specifically, instead of perturbing all parameters in the standard model, we perturbed two parameters at a time (Fig. S4). We note that for production factors the amplitude changes without changing timescale. However, for the degradation rate parameters, there is a change in the timescale as well as in the amplitude of the pulse.

**S5 Co-variations in different model variants of incoherent feedforward loop**

**S5.1 Input dynamics**

In the main text, we considered models of second order, which is a standard way to model the feedforward loop [1–3]. If we consider the input (u) dynamics, the second order model is becomes a third order model [4]. Here, the goal is to investigate whether the co-variations persist we consider such a model with input dynamics. This model is,
Figure S5: Amplitude-rise time co-variation for change of the inducers in a third order model.

\[
\frac{du}{dt} = \alpha_u - \gamma_u u,
\]
\[
\frac{dx}{dt} = \alpha_x \frac{u/K_u}{1 + u/K_u} - \gamma_x x,
\]
\[
\frac{dy}{dt} = \alpha_y \frac{u/K_u}{1 + u/K_u} \frac{1}{1 + x/K_x} - \gamma_y y,
\]

where \( \alpha_u, \alpha_x, \) and \( \alpha_y \) are the production factors in the model for \( u, x \) and \( y \), respectively. Here, \( \gamma_u, \gamma_x \) and \( \gamma_y \) are degradation rates of proteins \( u, x \), and \( y \) respectively. For simulations the parameter values considered are in Table 1. The changes in the inducers are modelled with change in the dissociation constants as done in the main text.

The amplitude-rise time co-variation for these parameters remains the same as second order model: amplitude and rise-time are directly proportional for \( aTc \) variations, and inversely proportional for arabinose variations (Fig. S5).

### S5.2 mRNA dynamics

In the model discussed in the main text (Eqn. 6), we assumed the mRNA dynamics to be faster than protein dynamics and considered the protein dynamics only. Here, we incorporate transcription (DNA to mRNA) as well as translation (mRNA to protein) for a more detailed model,

\[
\dot{x}_m = \alpha_x \frac{u}{u + K_u} - \gamma_x x_m,
\]
\[
\dot{x}_p = \beta_x x_m - \delta_x x_p,
\]
\[
\dot{y}_m = \alpha_y \frac{u}{u + K_u} \frac{K_x}{K_x + x} - \gamma_y y_m,
\]
\[
\dot{y}_p = \beta_y y_m - \delta_y y_p,
\]

where \( x_m, x_p \) are the concentration of mRNA and protein for X gene and, \( y_m \) and \( y_p \) are the concentration of mRNA and protein for Y gene respectively. The parameter \( \alpha_x \) is
Figure S6: Amplitude and timescale co-variations for inducer perturbations. Blue line indicates aTc variation, black line indicates arabinose variations.

| Parameter | Value | Unit   |
|-----------|-------|--------|
| $\alpha_u$ | 10   | nM/hr  |
| $\alpha_x$ | 10   | nM/hr  |
| $\alpha_y$ | 10   | nM/hr  |
| $\gamma_u$ | 10   | 1/hr   |
| $\gamma_x$ | 1    | 1/hr   |
| $\gamma_y$ | 10   | 1/hr   |
| $\beta_x$  | 10   | nM/hr  |
| $\beta_y$  | 10   | nM/hr  |
| $\delta_x$ | 1    | 1/hr   |
| $\delta_y$ | 10   | 1/hr   |
| $n$       | 3    | -      |

Table 1: Parameter values considered for simulation

the mRNA production rate of X, $\alpha_y$ is the mRNA production rate of Y, $\gamma_x$ is the mRNA degradation rate of X, $\gamma_y$ is the mRNA degradation rate of Y, $\beta_x$ is the protein production rate of X, $\beta_y$ is the protein production rate of Y, $\delta_x$ is the protein degradation rate of X, $\delta_y$ is the protein degradation rate of Y, $K_x$ is the dissociation constant for X and $K_u$ is the dissociation constant for u. Parameter values considered for the simulation are in Table 1.

We note that the rise time as well as the pulse height can mutually increase for an increase in aTc and the pulse height increases and the rise time decreases for an increase in arabinose in Fig. S6. These co-variations are largely similar to noted trends in the main text (Fig. 4C).

S5.3 Co-operativity

Transcription regulation may have co-operative dynamics [4]. This process can be modelled by modifying the Hill function with a power factor ‘n’ \( \{ K/(x+K) \rightarrow K^n/(K^n+x^n) \} \), where ‘n’ is the degree of co-operativity,
Figure S7: Amplitude and timescale co-variations for inducer perturbations. Blue line indicates aTc variation, black line indicates arabinose variations.

\[
\dot{x}_m = \alpha_x \frac{u}{u + K_u} - \gamma_x x_m, \\
\dot{x}_p = \beta_x x_m - \delta_x x_p, \\
\dot{y}_m = \alpha_y \frac{u}{u + K_u} \frac{K_y}{K_x + x^n} - \gamma_y y_m, \\
\dot{y}_p = \beta_y y_m - \delta_y y_p,
\]

(5)

where \(x_m\), \(x_p\) are the concentration of mRNA and protein for X gene and, \(y_m\) and \(y_p\) are the concentration of mRNA and protein for Y gene respectively. The parameter \(\alpha_x\) is the mRNA production rate of X, \(\alpha_y\) is the mRNA production rate of Y, \(\gamma_x\) is the mRNA degradation rate of X, \(\gamma_y\) is the mRNA degradation rate of Y, \(\beta_x\) is the protein production rate of X, \(\beta_y\) is the protein production rate of Y, \(\delta_x\) is the protein degradation rate of X, \(\delta_y\) is the protein degradation rate of Y, \(K_x\) is the dissociation constant for X and \(K_u\) is the dissociation constant for u. Parameter values considered for the simulation are in Table 1.

We note that the rise time as well as the pulse height can mutually increase for an increase in aTc and the pulse height increases and rise time decreases for an increase in arabinose in Fig. S7. These co-variations are largely similar to noted trends in the main text (Fig. 4C).

**S5.4 Maturation of Protein**

GFP undergoes maturation and this was not captured in previously described models. The model after including the maturation process is
Figure S8: Amplitude and timescale co-variations for inducer perturbations. Blue line indicates aTc variation, black line indicates arabinose variations.

\[
\dot{x}_m = \alpha_x \frac{u}{u + K_u} - \gamma_x x_m \\
\dot{x}_p = \beta_x x_m - \delta_x x_p \\
\dot{y}_m = \alpha_y \frac{u}{u + K_u} \frac{K^n_x}{K^n_x + x^n} - \gamma_y y_m \\
\dot{y}_p = \beta_y y_m - \delta_y y_p \\
\dot{y}_f = \theta_y y_p - \delta_y y_f
\] (6)

where \(x_m, x_p\) are the concentration of mRNA and protein for X protein and, \(y_m, y_p,\) and \(y_f\) are the concentration of mRNA and unmatured protein (dark GFP), matured protein for Y (functional GFP) respectively. The parameter \(\alpha_x\) is mRNA production rate of X, \(\alpha_y\) is mRNA production rate of Y, \(\gamma_x\) is mRNA degradation rate of X, \(\gamma_y\) is mRNA degradation rate of Y, \(\beta_x\) is protein production rate of X, \(\beta_y\) is protein production rate of Y, \(\delta_x\) is the protein degradation rate of X, \(\delta_y\) is the protein degradation rate of Y, \(\theta_y\) is the rate of maturation, \(K_x\) is the dissociation constant for X and \(K_u\) is the dissociation constant for u Parameter values considered for the simulation is in Table 1.

We note that the rise time as well as the pulse height can mutually increase for an increase in aTc and the pulse height increases and rise time decreases for an increase in arabinose in Fig. 8. These co-variations are largely similar to noted trends in the main text (Fig. 4C).

**S5.5 Resource Competition**

In the main text, we noted that the mismatch in the experiment and computational model predictions which could be due to resource limitations in the cell. This was not considered in the model in the main text (Eqn. 6). In the computational model, the growth of cells is assumed to be constant irrespective of gene expression. However, it has been noted that as gene expression increases it consumes more resources, such as ribosomes, which results in slower growth \([5–7]\). Including this in the model gives,
\[
\begin{align*}
\dot{x}_m &= \alpha_x \frac{u}{u + K_u} - \gamma_x x_m, \\
\dot{x}_p &= \beta_x x_m - \delta_x(x_m, y_m)x_p, \\
\dot{y}_m &= \alpha_y \frac{u}{u + K_u} \frac{K_x}{K_x + x} - \gamma_y y_m, \\
\dot{y}_p &= \beta_y y_m - \delta_y(x_m, y_m)y_p,
\end{align*}
\]

(7)

where \(x_m, x_p\) are the concentration of mRNA and protein for X protein and, \(y_m\) and \(y_p\) are the concentration of mRNA and protein for Y protein respectively. The parameter \(\alpha_x\) is the mRNA production rate of X, \(\alpha_y\) is the mRNA production rate of Y, \(\gamma_x\) is the mRNA degradation rate of X, \(\gamma_y\) is the mRNA degradation rate of Y, \(\beta_x\) is the protein production rate of X, \(\beta_y\) is the protein production rate of Y, \(\delta_x(x_m, y_m)\) is the effective protein degradation rate of X, \(\delta_y(x_m, y_m)\) is the effective protein degradation rate of Y, \(K_x\) is the dissociation constant for X and \(K_u\) is the dissociation constant for u. Parameter values considered for the simulation is in Table 1. As the concentration of mRNA increases, it sequesters ribosome that results in fewer resources for the cell to grow. In the above model, growth of the cell is modelled as [7],

\[
\delta_{x,y} = \frac{K}{x_m + y_m + K},
\]

(8)

where \(\delta\) represents the ‘burden-free’ growth of the cell and parameter \(K\) is a resource sharing coefficient. This modified model partially captures the resource competition between the host cell and the biomolecular circuit.

Figure S9: Amplitude and timescale co-variations for inducer perturbations. Blue line indicates aTc variation, black line indicates arabinose variations.

Co-variations of this model can match the experimental measurements (Fig. S9). In this computational model, the rise time and pulse height increase together for a change in arabinose as well as in aTc.
Optical Density (OD) as growth phases with indicated inducer stages

A) aTc induced in LB media  
B) aTc induced in M9 media  
C) arabinose induced in LB media  
D) arabinose induced in M9 media

Figure S10: Optical Density (OD) as growth phases with indicated inducer stages A) aTc induced in LB media B) aTc induced in M9 media C) arabinose induced in LB media D) arabinose induced in M9 media
Plasmid and IFFL mechanism

Figure S11: Plasmid map and illustration of inducer action. A) Plasmid map B) Working mechanism of inducers

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