Non-normality Can Facilitate Pulsing in Biomolecular Feedforward Loops

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Abstract
Non-normality can underlie pulse dynamics in engineering contexts. However, its role in pulses generated in biological contexts is generally unclear. Here, we address this issue using tools of linear algebra and control systems theory on simple computational models of biomolecular circuits. We find that non-normality is present in standard models of feedforward loops. We used a generalized framework and pseudospectrum analysis to identify non-normality in larger biomolecular circuit models, finding that it correlates well with pulsing dynamics. Finally, we find that the interplay of non-normality and zero dynamics of the system is important in determining the pulse characteristics in response to an input. These results should be useful in analysis, to screen for pulsed dynamics in biomolecular circuits, and in design, by highlighting tunable system properties.

1 Introduction
A transient growth of a system output followed by a decay, or a pulse, is observed in diverse contexts in engineering and science. An example is the transient change in the output speed of a motor from its desired value when the input load increases before its controller resets the speed back to the desired value [1]. For step changes in the input, this is typically achieved by designing the overall input-output response dynamics to have a zero at the origin [1]. Another example is the transient growth thought to be associated with the transition from laminar flow to turbulence [2]. The core mechanism for this is non-normality, whereby dominant modes in
the inherent system dynamics are oriented in such a manner that specific directions of perturbations or initial states are transiently amplified (Fig 1a).

Recent advances in high resolution temporal imaging have highlighted the functional importance of pulse behaviour in biomolecular systems as well as their underlying dynamical mechanisms \[3\]. One example of pulse behaviour is in the transient differentiation process of competence in the bacteria \textit{Bacillus subtilis} \[4\]. It has been shown that the underlying network has both positive and negative transcriptional feedback which can combine to generate excitability, or a parameter regime where small amplitude random fluctuations can generate large amplitude pulses in the output. Similar mechanisms are believed to underlie neuron dynamics \[5\]. Another biomolecular circuit that can generate pulses is an incoherent feedforward loop \[6\], an overrepresented motif in genetic networks \[7\]. In a simple realization (Fig. 1b), a step input $u$ activates the expression of two proteins $x$ and $y$. The values of both proteins start to increase. $x$ is also a transcriptional repressor of $y$ and, due to the repressing action of $x$ on $y$, the values of protein $y$ decrease. This generates a pulse in $y$ in response to a step input. Such feedforward loops occur in diverse contexts in biology \[8\] and, recently, there have been synthetic demonstrations of such designs \[10\]. Finally, such pulsed behaviour is also seen in bacterial chemotaxis, as an indirect consequence of perfect adaptation \[11\]. In such systems, the output exhibits a transient deviation from a fixed value on the application of a stimulus. This transient deviation often takes the form of a pulse. Underlying mechanism for this is the presence of integral feedback in the underlying circuit \[8\], a feature which is similar to the design of system zero from the input to the output mentioned above. These examples present a subset of pulsed behaviour in biomolecular systems whose functional roles and mechanisms are starting to be understood.

There are at least three interesting dynamical mechanisms that can generate pulsed behaviour. One is excitability, a nonlinear dynamical phenomenon which requires small amplitude random input. Second is based on integral feedback and involves placing a system zero in how a step change in input affects the output. This can also function in linear systems. Third is non-normality, where the inherent system dynamics are such that certain initial conditions can generate transient growth in output before it settles to the equilibrium point. Whether or not non-normality can underlie pulsing in biomolecular circuits is generally unclear.

Here we ask if pulsing in feedforward loops is related to non-normality. Standard models of feedforward loops are analyzed to address this. It is observed that non-normal growth is key factor for pulsing in the standard incoherent feedforward loop. We develop and apply a mathematical framework based on matrix norms and pseudospectrum calculation to identify
non-normality in larger, more complicated models, finding that existence of non-normality correlates well with pulsing. Finally, we investigate the contribution of system zeros, that can facilitate pulsing in response to step inputs, finding that it has an additive effect to non-normality-based pulsing. This interplay between non-normality property and system zeros, which determine response to external inputs, can be used to shape the pulse dynamics in biomolecular circuits.

Figure 1: a) The role of non-normality for system output when there is a fold change in the input. The dotted arrow represents the eigenvector of the system, which shapes the system response [5]. The normal system gives a monotonic response where as for non-normal dynamics a pulse may be observed. b) A standard two node feedforward loop and illustrative sketch of system responses for a step input.

2 RESULTS

2.1 Non-Normality in Standard Incoherent Feedforward Loop

We begin by investigating the role of non-normality in a standard model of an incoherent feedforward loop (Fig. 1b [7]).

2.1.1 Model of Incoherent Feedforward Loop

In this model, a transcription factor $u$ acts as the input to the system, activating the expression of proteins $x$ and $y$. Further, the protein $x$ is a transcriptional repressor of $y$. With a step change in input $u$, the expression of proteins $x$ and $y$ increase. As levels of $x$ increase, they repress the production of protein $y$, and its levels decline. Therefore, a step change in input $u$ generates a pulse in the output $y$. 
A simple mathematical model of this is

$$\begin{align*}
\frac{dx}{dt} &= \beta_x u - \alpha_x x, \\
\frac{dy}{dt} &= \beta_y u \frac{K_{xy}}{x} - \alpha_y y,
\end{align*}$$

(1)

where $x$ and $y$ represents the concentration of the proteins, $u$ is the input, $\alpha_x$ is the degradation rate for protein $x$, $\alpha_y$ is the degradation rate for protein $y$, $\beta_x$ is the production rate of protein $x$, $\beta_y$ is the production rate of protein $y$, and $K_{xy}$ is the dissociation constant for the binding of $x$ to the promotor of $y$. Similar models has been used in the literature to study fold-change detection, scale invariance and perfect adaptation in feedforward loop [9, 12–14]. These models help to understand and quantitate the dynamics of this system.

A step increase in $u$ (0.1 $\rightarrow$ 0.2) results in a pulse response of the system output $y$ (Fig. 2a). For the simulation, we have set the nominal value of parameters to be unity. As expected, the output $y$ transiently changes from its steady-state value before returning to the pre-step value.

The linearized dynamics of (1) around the operating point $(x_e, y_e, u_e)$, where $x_e$, $y_e$, $u_e$ are the steady state value of $x$, $y$ and $u$ before the step change) are,

$$\delta \dot{z} = A \delta z + B \delta u,$$

(2)

where

$$\delta z = \begin{bmatrix} \delta x \\ \delta y \end{bmatrix}, \quad A \triangleq \begin{bmatrix} -\alpha_x & 0 \\ -\frac{\beta_y K_{xy} \alpha_x^2}{\beta_x u_e} & -\alpha_y \end{bmatrix}, \quad B \triangleq \begin{bmatrix} \beta_x \\ \beta_y \alpha_x \end{bmatrix}.$$

Similar to the nonlinear model, a step increase in $\delta u$ produces a pulse in the output $\delta y$ (Fig. 2a). As this is a linear system, the natural solution (no input, $\delta u = 0$) of the state can be written as a linear combination of eigenvectors,

$$\delta z(t) = c_1 v_1 e^{-\alpha_x t} + c_2 v_2 e^{-\alpha_y t},$$

(3)

where $c_1$, $c_2$ are the scalar values, dependent on the initial conditions and, $v_1$ and $v_2$ are the eigen vectors of the system matrix $A$. For the feedforward loop (2), the eigenvectors are $v_1 = [0 \ 1]^T$ and $v_2 = \left[ \frac{\alpha_y - \alpha_x}{H} \ 1 \right]^T$, where $H = -\frac{\beta_y \alpha_x^2}{\beta_x u_e}$. The angle between the eigen vectors depends upon system parameters.

2.1.2 Eigenvector Analysis

The time evolution of output for a step change in input can be visualized with the help of eigenvectors. A step change in input changes the equilibrium point of the system. The path from the past equilibrium point to the new equilibrium point is determined by the direction of the eigenvectors.
In Eqn. (3), $\delta z$ is the resultant of two vectors, $c_1 v_1 e^{-\alpha_x t}$ and $c_2 v_2 e^{-\alpha_y t}$. Here, the magnitude of the individual vectors decreases, but the orientation of the vectors is such that the resultant of the two vectors increases for a short time before decreasing. This can be analytically expressed as follows. For $X = |c_1 v_1 e^{-\alpha_x t}|$ and $Y = |c_2 v_2 e^{-\alpha_y t}|$ the resultant $R = |\delta z(t)|$ of the vectors can be written from the parallelogram law,

$$R^2 = X^2 + Y^2 + 2XY \cos \theta,$$

where $\theta$ is the angle between the eigenvectors $v_1, v_2$ [15]. We note that when $\theta = 0^\circ$, the magnitude of $R$ is greater than when $\theta = 90^\circ$. The resultant $R$ exhibits a transient growth prior to settling to a steady value. The condition for transient growth is that the rate of change of $R$ when the step change is applied is positive,

$$\left.\frac{dR}{dt}\right|_{t=0} > 0. \quad (4)$$

For the feedforward loop model considered above, the resultant increases for a short time if,

$$\theta < \sin^{-1} \left( \frac{1 - \bar{\alpha}}{1 + \bar{\alpha}} \right), \quad (5)$$

where $\bar{\alpha} = \frac{\alpha_x}{\alpha_y}$ is the ratio of the eigenvalues. If the eigenvalues are very close to each other such that $\bar{\alpha} \sim 1$ then the eigenvectors have to be almost colinear to have a transient growth. The condition can be used to generate pulse in a system or shape it. As the angle between the eigenvectors depend upon the parameters, which can be manipulated so as to satisfy Eqn. (5) for a pulsing output.

For the purpose of analysis, different degradation rates $\alpha_x$ and $\alpha_y$ are considered in the above model and correlated with their effect on the pulse height. The changes in these parameters changes the angle between the eigenvectors. The corresponding output responses in response to an identical step change in $\delta u$ are shown in Fig. 2b (inset). When the eigenvectors are colinear, or approximately so, the largest growth is observed in the output pulse. As the angle between eigenvectors increases, the pulse height starts to decrease. We conclude that non-normality is inherent in the standard model of a feedforward loop circuit.

### 2.2 Non-Normality in Larger Scale Networks

Next, we analyze the presence of non-normal dynamics in larger biomolecular circuits. We start by developing and adapting mathematical methods that provide a generalized framework for this.
Figure 2: Analysis of an incoherent feedforward loop. (a) A step change input of two fold results a pulse output for the linearized and nonlinear model of incoherent feedforward loop (inset). (b) The change in pulse shape with change in angle between eigenvectors. The pulse height decreases as the angle between vector increases on change of parameters. (c) The pseudospectrum of the incoherent feedforward loop showing the non-normality. The right hand bar shows the perturbation value in the order of ten in log scale. The color in the spectrum is related to the perturbation value in the bar.

2.2.1 Transient Growth in General Context

The pulsing behaviour need not be restricted to second order systems, such as the model considered in the previous section. For a general $n^{th}$ order system, sufficient conditions to obtain pulsing behaviour (Eqn. (5)) can be generalized. Consider a general $n^{th}$ order system,

$$\delta \dot{z} = A \delta z,$$

where $A$ is a $n \times n$ square matrix and output $\delta z$ is an $n \times 1$ vector. The autonomous solution of the system states can be written as,

$$\delta z(t) = e^{A(t-t_0)} \delta z(t_0) = \Phi(t, t_0) \delta z(t_0).$$
The growth of solutions is tightly upper bounded by $r$ [16],

$$r = \max_{\delta z(t_0)} \frac{\|\delta z(t)\|}{\|\delta z(t_0)\|}.$$  

The sufficient condition for transient growth is $\frac{dr}{dt}|_{t=t_0} > 0$. The maximum value of this rate can be explicitly calculated for systems where the matrix $A$ is stable, having all eigenvalues in the left-half of the complex plane. For such systems, the maximum value is

$$W(A) = \sigma_{\text{max}}(A + A^T),$$  

where $\sigma_{\text{max}}(X)$ denotes the maximum eigenvalue of the matrix $X$. The proof of this is in the Supplementary Material. A positive value of $W(A)$ ensures a pulsed output (Fig. 2). This condition is a sufficient condition for pulsing in higher order systems and may be viewed as a generalization to Eqn. (5). For the incoherent feedforward loop model considered previously, $W(A) = 8$, consistent with the pulsed output observed.

### 2.2.2 Pseudospectrum Analysis

For second order systems, non-normal dynamics that generate pulsed behaviour can be quantified using eigenvectors. As the order of the system increases, using eigenvectors to quantify pulses becomes increasingly complicated. To quantify pulse characteristics in higher order systems, we present the notion of pseudospectrum, a tool that has been used to study non-normal dynamics [17]. We briefly restate standard definitions for this.

**Definition-1:** [17] Let $A \in \mathbb{C}^{N \times N}$ and $\epsilon > 0$ be arbitrary scalar. The pseudospectrum $\sigma_{\epsilon}(A)$ of $A$ is the set $z \in \mathbb{C}$ such that

$$\|(zI - A)^{-1}\| \geq \epsilon^{-1},$$

where $\|(zI - A)^{-1}\|$ is called as the resolvent of $A$ at $z$.

A simpler notion of pseudospectrum $\sigma_{\epsilon}(A)$ is the set of $z$ such that

$$z \in \sigma(A + E),$$

where $E \in \mathbb{C}^{N \times N}$ and $\|E\| < \epsilon$. In other words, a system is normal if the magnitude of perturbation in eigenvalues are less than the magnitude of perturbation in system matrix $A$.

**Definition-2:** [17] The $\epsilon$-pseudospectral abscissa is the supremum of the real parts of $z \in \sigma_{\epsilon}(A)$,

$$\gamma_{\epsilon} = \sup_{z \in \sigma_{\epsilon}(A)} \text{Re}(z)$$
Theorem 1. \[18\] For an autonomous system $\dot{x} = Ax(t)$, where $x \in \mathbb{R}^n$, $A \in \mathbb{R}^{n \times n}$, and $\sigma(A) < 0$ state solution depends on transition matrix $\Phi(t, t_0) = e^{A(t-t_0)}$ and the lower bound of the maximum transient growth can be bounded as

$$\sup_{t \geq 0} \|\Phi(t)\| \geq \frac{\gamma_\epsilon(A)}{\epsilon},$$

and the upper bound of the transient growth can be quantify as,

$$\sup_{t \geq 0} \|\Phi(t)\| \leq \frac{L_\epsilon(A)}{2\pi \epsilon} e^{\gamma_\epsilon t},$$

where $L_\epsilon$ is the contour of the pseudospectrum.

At $t = t_0$, $z(t) = z(t_0)$ and $\|e^{A(t-t_0)}\| = \Phi(t, t_0) = 1$ and for the transient growth $\|\Phi(t)\| > 1$ for short period of time as $t > t_0$. The bounds of transition matrix guarantee transient growth in a pulsed output. For completeness, a simple proof is reproduced in the Supplementary Material.

We performed this pseudospectrum analysis on the feedforward model considered previously (Fig. 1c). We find that small perturbations can generate large changes in the eigenvalues, showing that transient growth leading to pulsed output is possible.

Figure 3: The characterization of biomolecular circuit pulse with quantitative measure from Theorem-1

2.2.3 Non-Normality in 3-Node Networks

To test the role of non-normality in generating pulse dynamics in higher-order systems, we used the above-mentioned methods on a set of randomly generated three-node circuit topologies \[19\]. Some of the circuit topologies were shown to generate a pulsed response. Here, we show that the circuits that generate a pulsed response exactly correlate with inherent
non-normal dynamics. We illustrate this using an example circuit (Fig. 4a). The mathematical model of this circuit (from [19]) is,
\[
\begin{align*}
\dot{x}_1 &= k_1 u - k_2 x_2, \\
\dot{x}_2 &= k_3 x_1 - k_4 x_2, \\
\dot{x}_3 &= k_5 x_1 - k_6 x_2 x_3.
\end{align*}
\] (9)

where, \( u \) is the input, \( x_i (i = 1, 2, 3) \) are the circuit nodes and \( k_j (j = 1, 2, \ldots, 6) \) are the reaction rates. For this model, the parameters were set to \( k_i = 1 \) for \( i \neq 4 \), \( k_4 = 2 \). A step change in \( u \) produces a pulse in \( x_3 \) (Fig. 4a).

To analyze the role of non-normality in these dynamics, we first linearized the model around the pre-step equilibrium \( (x_{10} = 2, x_{20} = 1, x_{30} = 2) \),
\[
\dot{x} = Ax.
\]

The linearized model exhibit a pulsing similar to nonlinear model. The system matrix \( A \) has three eigenvalues \( \lambda_1 = -0.1, \lambda_{2,3} = -1 \). The corresponding eigenvectors are \( v_1 = [0 \ 0 \ 1]^T \), \( v_{2,3} = [0.55 \ 0.55 \ 0.62]^T \). As there are three eigenvectors, a visual illustration of non-normality is harder relative to the simpler two-dimensional model analyzed in the previous section. Therefore, we computed the abscissa \( W(A) \) and the pseudospectrum.

For this circuit, \( W(A) = 1.313 \), which is positive. This implies that there is initial transient growth consistent with the observed pulsed output. Then, we computed the pseudospectrum (Fig. 4b). We find that a perturbation of \( \epsilon = 0.1 \) gives \( \sigma_\epsilon > 0.1 \), implying the presence of non-normality. For the entire set of topologies considered in [19], we find that pulsed behaviour exactly correlates with inherent non-normal dynamics (please see Supplementary Material).

2.2.4 Non-Normality in eukaryotic chemotaxis signaling pathway

Next, we analyzed the role of non-normality in the pulse behaviour of a eukaryotic signaling pathway [20]. This system adapts to changes in chemoafferent cyclic adenomonophosphate (cAMP). A mathematical model of this system has been developed previously [19]. It models two different affinity receptors \( R_1 \) and \( R_2 \) that bind cAMP. This complex activates two proteins Ras-GEF and Ras-GAP that incoherently act on activated Ras-GTP. Activated Ras is measured using a cytosolic reporter \( RBD-GFP \).
Figure 4: Non-normality in larger biomolecular circuits. (a) A step change 0.1 to 0.2 in $u$ results a pulse output $x_3$ in the three node pulsing circuit (inset). (b) Pseudospectrum of the three node linearized model. The right hand bar shows the perturbation value in the order of ten in log scale. The color in the spectrum is related to the perturbation value in the bar. (c) Output $Ras^{GTP}$ when $cAMP$ changes from 1 to 2 in adaptive Ras-cAMP signaling network(inset). (d) Pseudospectrum of 6-node system showing non-normality in system dynamics. The right hand bar shows the perturbation value in the order of ten in log scale. The color in the spectrum is related to the perturbation value in the bar.
The model equations are,
\begin{align*}
\dot{x}_1 &= k_1(u + r_1)(\bar{x}_1 - x_1) - k_2x_1, \\
\dot{x}_2 &= k_3(u + r_2)(\bar{x}_2 - x_2) - k_4x_2, \\
\dot{x}_3 &= k_5(x_1 + x_2) - k_6x_3, \\
\dot{x}_4 &= k_7(x_1 + x_2) - k_8x_4, \\
\dot{x}_5 &= k_9x_3(\bar{x}_5 - x_5) - k_{10}x_4x_5, \\
\dot{x}_6 &= k_{11}(\bar{x}_6 - x_6) - k_{12}x_6x_5,
\end{align*}

where \( x_1 = R_1, \ x_2 = R_2, \ x_3 = GEF, \ x_4 = GAP, \ x_5 = Ras^{GTP}, \ x_6 = RBD^{cyt} \) are the states of the system. The values of the system parameters are considered as in \[19\].

A step change in cAMP results in a pulse of activated Ras (Fig. 4c). To check if non-normality has a role in these dynamics, we computed the numerical radius \( W \) and pseudospectrum of the linearized system obtained by linearization around the pre-step equilibrium point. We find that the numerical radius \( W = 491.3 > 0 \) indicates the presence of the pulse. The computed pseudospectrum (Fig 4d) also indicates that the system admits non-normality.

These results show that non-normality can underlie pulses in larger more realistic models of biomolecular circuits and highlights the utility of the numerical radius and pseudospectrum as tools that can be used to screen for pulsed behaviour.

### 2.3 Interplay with zero dynamics

Finally, we investigate the interplay between pulse dynamics based on non-normality and system zeroes, another dynamical mechanism that can generate pulses. System zeroes can be defined as input excitations that give zero steady-state output and have been shown to be mathematically equivalent to adaptation in a biological system \[21\].

We studied the role of non-normality and zero in the pulse amplitude numerically to understand the interplay. A system represented as in (2) inherits the non-normality, a system property, with \( A \) and zero dynamics, an input-output property, is characterized with \( A \) as well as \( B \). For the study, we varied the nonnormality by changing the nondiagonal term while the zero is on fixed location. An increasing magnitude of nondiagonal term implies increase in non-normality \[22\]. The perturbation in zero due to \( A \) is compensated with a change in \( B \) such that the zero remains unchanged while varying only non-normality. The location of zero is varied by changing the parameter of \( B \) which do not have any effect on non-normality of the system. It is important to note that the change of zero in also changes the steady-state value of output. For, the computation initial conditions are
selected as the final value so that the step change gives a perfect pulse. We find that adding non-normality to the system enhances the pulse height. The combined non-normality and zero dynamics can give a higher value of a pulse (Fig. 5a). This indicates that the combination of non-normality and system zeros can act synergistically in increasing pulse magnitudes.

To understand the relation between system input and non-normal dynamics further, we considered the effect of a step change in the input on the equilibrium point of a system. As the input level changes, there is a change in the equilibrium point of the system. The previous equilibrium point acts as the initial condition of this trajectory. Supposing that the new equilibrium point has non-normal dynamics with perturbations in a specific region of phase space generating a transient pulse. Then the trajectory in response to a step change in input will be a pulse if the initial equilibrium point is in this region. This is illustrated in Fig. 5b.

Figure 5: The role of zero in pulsing. (a) The system exhibit pulse much more stronger when non-normality is embedded along with zero-dynamics. (b) The non-normal growth for selective initial conditions region in phase plane.

3 Discussion

Pulses are functionally important dynamical behaviour observed in biomolecular systems, such as in feedforward loops. Here, we investigated the dy-
namic mechanisms underlying such pulses and presented three main results. First, we find that non-normality can play a role in generating these pulses, similar to its role in fluid mechanics. Second, we demonstrated how pseudospectrum analysis can be used to screen for such behaviour. Third, we find that the zero dynamics of the system can also play a role in generating the pulses, especially in response to an external input. The interplay between non-normality and zero dynamics provides a design framework for such systems.

It is interesting to highlight that the pulsing behaviour can be achieved and understood using essentially linear system tools. Here, we have seen this from the point of view of non-normal dynamics as well as system zeros. Non-normality is a property of inherent system dynamics that can enable pulse behaviour. A system zero enables pulse behaviour in the output depending on the input form. Together, they can act in combination to generate pulses. As linear system methods are relatively simpler to understand and scale up, this can be advantageous from a design perspective as well.

An important direction for future work is to extend this analysis to that of the overall nonlinear system. Tools such as Lyapunov exponents may help in quantifying the extent of the deviation of the transient pulse behaviour away from the fixed equilibrium point.

Pulse dynamics are prevalent in multiple contexts in engineering and science. Here, we have highlighted the role of non-normal dynamics in the pulsing mechanism prevalent in biomolecular feedforward loops. This is a linear dynamical mechanism and so, as investigated here, can be directly used for screening for pulse behaviour in large scale models. For the same reason, this can help in the design of biomolecular circuits with pulse dynamics.

d provide a more systematic understanding of this phenomenon. Also, it can be helpful to design biomolecular circuit to have pulse characteristics. As the pulse can be generated with linear circuits, the synthesis and controlling the system can be more flexible.

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