INTERLOCKED MULTI-NODE POSITIVE AND NEGATIVE FEEDBACK LOOPS FACILITATE OSCILLATIONS

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Abstract. Positive and negative feedback loops in biological regulatory networks appear often in a multi-node manner since regulatory processes are in general multi-step. Although it is well known that interlocked positive and negative feedback loops (iPNFLs) can generate sustained oscillations, how the number of nodes in each loop affects the oscillations remains elusive. By analyzing a model of iPNFLs with multiple nodes, we find that the node number of the negative loop mainly plays a role of amplifying oscillation amplitudes whereas that of the positive loop mainly plays a role of reducing oscillatory regions, both depending on the (competitive or noncompetitive) way of interaction between the two loops. We also find that given an iPNFL network of the same structure, the noncompetitive model is more likely to produce large-amplitude oscillations than the competitive model. These results not only indicate that multi-node iPNFLs are an effective mechanism of promoting oscillations but also are helpful for the design of synthetic oscillators.

1. Introduction. Biochemical oscillations, as a kind of macroscopic phenomenon occurring in many biological regulatory systems, occur in many contexts such as signaling, metabolism and development, and control important aspects of cell physiology from signaling, motility and development to growth, division and death. The complexity of natural oscillatory systems makes it difficult to analyze their behaviors quantitatively. Uncovering functional properties of simple biological oscillators then becomes important for the understanding of various intracellular processes and the characterization of complex biological oscillators, and it is also helpful for the elucidation of design principles of biological oscillators [36, 16, 7]. In the spirit of synthetic biology, biological oscillators can be classified according to their topology [28, 18, 23, 19], e.g., the oscillators of negative feedback loops, and those of interlocked positive feedback loop (PFL) and negative feedback loop (NFL), which are denoted by iPNFL for brevity.

PFL and NFL, as two fundamental building blocks of many biological regulatory systems [13, 8, 3], provide basic functions requiring for cells to survive in complex

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environments. From the viewpoint of dynamics, however, positive and negative feedbacks can play different roles, e.g., negative feedback can induce oscillations (in fact it is indispensable for oscillations [30] whereas positive feedback is one mechanism to delay the negative-feedback signal [27]. A simple NFL has the potential to generate sustained oscillations, but this loop alone is often insufficient for reliable oscillations due to its weak robustness against stochastic fluctuations (or the noise) [2, 37]. On the other hand, iPNFL, which is also a fundamental building block of biological regulatory systems [36] and for which we can find its prototypes in many biological oscillators [1, 33, 42], can generate robust, tunable oscillations [36, 14, 32]. Interestingly, it has been shown that in contrast to NFL oscillators that in general have difficulty to adjust their frequencies without compromising their amplitudes, iPNFL oscillators can achieve widely tunable frequencies and near-constant amplitudes [37]. This tunability makes the latter design suitable for biological rhythms such as cell cycles and heartbeats that need to provide a constant output over a range of frequency. In addition, iPNFL oscillators seem to be more robust and easier to evolve [34]. In conclusion, oscillators of iPNFL can have more advantages over those of simple NFL from the viewpoint of robustness and tunability, and hence they are a main design subject in synthetic biology.

Multi-step processes are a remarkable characteristic of countless biological regulatory systems like gene regulatory networks. Feedback loops in these systems usually involve multiple intermediate processes of different functions such as transcription, translation, transport between the nucleus and cytoplasm, and posttranslational modifications [26, 35, 6, 40]. For a large-scale regulatory network, the feedback loops involving multi-step processes are not exceptional but would be very common. In previous studies, most multi-step processes were usually modeled by time delays [22, 11], and this is apparently a simplification. As a result, the practical effects of multi-step processes would not be well captured since each step may play a particular role. Although oscillators of interlocked simple PNFLs have extensively been studied [36, 30, 27, 37, 34, 11], those of interlocked multi-node PNFLs remain unexplored. In particular, it is not clear how the number of nodes in multi-node feedback loops impact oscillatory dynamics and what are the benefits of these loops.

In this paper, we systematically investigate a model of iPNFLs with multiple nodes, focusing on the effects of the numbers of nodes on oscillatory dynamics. By model analysis, we find that the number of the nodes on the NFL mainly influences oscillation amplitudes (precisely, more nodes lead to larger oscillation amplitude) whereas the number of the nodes on the PFL mainly influences oscillatory regions (specifically, more nodes result in the smaller oscillatory region in a competition mode). These results would be helpful for the design of robust, large-amplitude oscillators.

2. **Model description and simple analysis.**

2.1. **Model description.** Before describing our models to be considered, let us introduce definitions. Here, we use a directed graph to represent a network of gene regulation. In the regulatory network, if one gene promotes the expression of the other, we record the symbol of this pathway as positive. Similarly, if one gene represses the expression of another gene, the symbol of the pathway is marked negative. If the product of all the symbols on the loop is negative, the feedback loop is negative. Conversely, if the product is positive, the feedback loop is positive [30]. A feedback loop is homogeneous if the symbols of all the pathways on this loop are...
the same and heterogeneous otherwise (in other words, a heterogeneous feedback loop means that there are both positive and negative pathways on this loop). The length of a feedback loop is described as the number of the nodes on this loop.

Consider a biological network consisting of iPNFLs, which is schematically shown in Fig. 1(a). Assume that the NFL contains an odd number of nodes (denoted by $X_i$, where $1 \leq i \leq N$ with $N$ being an odd number) but the PFL may contain an arbitrary number of nodes (denoted by $X_j$, where $N \leq j \leq N + M$ with $M$ being an arbitrary non-negative integer). In principle, nodes in this network may represent genes, proteins, metabolites, or any other chemical species, but here we consider that they represent genes. In addition, we consider only homogeneous feedback loops. Thus, symbols $X_i$, where $1 \leq i \leq N$ represent activators whereas symbols $X_j$ with $N \leq j \leq N + M$ represent repressors. Symbol $X_N$ is assumed as the common node through which the two loops are coupled or interlocked to each other. We also consider the way of the interaction between two feedback loops, that is, the information carried by two transcription factors is integrated in a manner of combinatorial regulation at the region of the promoter for the common gene [25, 17], referring to Fig. 1(b, c). Thus, we have two kinds of gene regulatory models: noncompetitive and competitive. Here, the difference between the noncompetitive and competitive models is in the way of how the transcription factors $X_{N-1}$ and $X_{N+M}$ bind to the same binding site. In Fig. 1(b), transcription factors bind to the promoter binding sites independently, whereas in Fig. 1(c), transcription factors need to compete for the same binding site.

Let $x_k$ represent the concentration of $X_k$. According to Fig. 1, the deterministic differential equations governing dynamics of variables $x_k(1 \leq k \leq N + M)$ can be described as

$$\frac{dx_i}{dt} = g_i(x_i, x_{i-1}), i \in \{1, 2, \ldots, N + M\} \setminus \{N\}$$

(1a)

$$\frac{dx_N}{dt} = g_N(x_N, x_{N-1}, x_{N+M})$$

(1b)

where we stipulate that $x_0$ is the same as $x_N$. In Eq. 1a and Eq. 1b, $g_i(x_i, x_{i-1}) = c_i - \gamma_i x_i + \frac{\alpha}{1+(x_{i-1}/K_i)^n}$ if $X_i$ is a repressor whereas $g_i(x_i, x_{i-1}) = c_i - \gamma_i x_i +$
the system’s fixed point. For convenience, we denote by
\[ \alpha \frac{(x_i-1/K_1)^{h_i}}{1+(x_i-1/K_1)^{h_i}} \]
if \( x_i \) is an activator, and
\[ g_N(x_N, x_{N-1}, x_{N+M}) = c_N - \gamma_N x_N + \]
\[ \frac{\alpha_N}{1+(x_{N-1}/K_N)^{h_N}} x_N \frac{\alpha_{N+M}(x_{N+M}/K_{N+M})^{h_{N+M}}}{1+(x_{N+M}/K_{N+M})^{h_{N+M}}} \]
for the noncompetitive binding whereas
\[ g_N(x_N, x_{N-1}, x_{N+M}) = c_N - \gamma_N x_N + \]
\[ \frac{\alpha_N a_{N+M}(x_{N+M}/K_{N+M})^{h_{N+M}}}{1+(x_{N+M}/K_{N+M})^{h_{N+M}}} \]
for the competitive binding. \( c_k \) (\( 1 \leq k \leq N + M \)) are basal production rates, \( \gamma_k \) are degradation rates, \( h_k (1 \leq k \leq N + M) \) are Hill coefficients, \( K_k (1 \leq k \leq N + M) \) are dissociation constants, and \( \alpha_k (1 \leq k \leq N + M) \) are the largest production of node that is activated or repressed by another node.

For simplicity but without loss of generality, we consider only the case of homogeneous feedback loop. To reduce the number of system parameters but to keep the qualitative behavior of the system unchanged, we assume \( g_i = c_n - \gamma_n x_i + \frac{\alpha_n(x_i-1/K_1)^{h_i}}{1+(x_i-1/K_1)^{h_i}} \) for \( 1 \leq i \leq N \) (here \( N \) is an odd number), \( g_{N+j} = c_p + \gamma_p x_{N+j} + \frac{(x_{N+j-1}/K_2)^{h_p}}{1+(x_{N+j-1}/K_2)^{h_p}} \) for \( 1 \leq j \leq M \) (here \( M \) is a positive integer) and
\[ g_N = c_n + \gamma_n x_N + \frac{\alpha_n(x_N+1/K_1)^{h_n}}{1+(x_N+1/K_1)^{h_n}} \]
for the noncompetitive binding whereas \( g_N = c_n + \gamma_n x_N + \frac{\alpha_n a_{N+M}(x_{N+M}/K_{N+M})^{h_{N+M}}}{1+(x_{N+M}/K_{N+M})^{h_{N+M}}} \)
for the competitive binding.

2.2. Stability analysis of the fixed point. The purpose of this subsection is to derive conditions on Hopf bifurcation by performing the linear stability analysis of the system’s fixed point. For convenience, we denote by \( \mathbf{x}^* = (x_1^*, \ldots, x_{N+M}^*)^T \) the steady state of system (1), that is, \( \mathbf{x}^* \) is a solution of the algebraic equation group: \( g_k = 0, 1 \leq k \leq N + M \). Note that the Jacobian matrix of system (1) at fixed point \( \mathbf{x}^* \), denoted by \( \mathbf{J} \), takes the form
\[
\begin{bmatrix}
\frac{\partial g_1}{\partial x_1} & 0 & 0 & \frac{\partial g_1}{\partial x_N} & 0 & 0 & 0 \\
\frac{\partial g_2}{\partial x_1} & 0 & 0 & 0 & 0 & 0 & 0 \\
\vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \vdots \\
0 & 0 & \frac{\partial g_N}{\partial x_N} & 0 & 0 & \frac{\partial g_N}{\partial x_{N+1}} & 0 \\
0 & 0 & 0 & \frac{\partial g_{N+1}}{\partial x_N} & \frac{\partial g_{N+1}}{\partial x_{N+1}} & 0 & 0 \\
0 & 0 & \vdots & \vdots & \vdots & \ddots & \vdots \\
0 & 0 & 0 & 0 & 0 & \frac{\partial g_{N+M}}{\partial x_{N+M}} & \frac{\partial g_{N+M}}{\partial x_{N+M+1}}
\end{bmatrix}
\]
(2)

where the elements in the empty place are all zero. We can show that the characteristic equation of \( \mathbf{J} \) takes the following form
\[ \prod_{k=1}^{N+M} (\lambda + \gamma_k) - F_1 \prod_{i=1}^{N-1} (\lambda + \gamma_i) - F_2 \prod_{j=1}^{M} (\lambda + \gamma_j) = 0 \]
(3)

where we denote \( \gamma_k = -\frac{\partial g_k}{\partial x_k} \big|_{x=x^*} \) with \( 1 \leq k \leq N + M \), \( F_1 = \frac{\partial g_N}{\partial x_N + M} \prod_{j=1}^{M} \left. \frac{\partial g_{N+j}}{\partial x_{N+j-1}} \right|_{x=x^*} \), and \( F_2 = \frac{\partial g_N}{\partial x_{N+1}} \prod_{i=1}^{N-1} \left. \frac{\partial g_{i+1}}{\partial x_i} \right|_{x=x^*} \). According to the properties of positive and negative feedback loops, we can verify \( F_1 > 0 \) and \( F_2 < 0 \).

Without loss of generality, we assume that for the NFL, all basal production rates \( (c_k) \), all degradation rates \( (\gamma_k) \), Hill coefficients \( (h_k) \), and all strengths \( (\alpha_k) \),
are the same, respectively. We denote by $c_n$, $\gamma_n$, $h_n$ and $\alpha_n$ these common parameters, respectively. The similar assumption is made for the PFL, and the common parameters are denoted by $c_p$, $\gamma_p$, $h_p$ and $\alpha_p$ respectively. With these setting and by noting that $N$ is an odd number due to the property of the NFL, Eq. (3) can be rewritten as the following form
\[
(\lambda + \gamma_n)^N(\lambda + \gamma_p)^M - F_1(\lambda + \gamma_n)^{N-1} - F_2(\lambda + \gamma_p)^M = 0 \tag{4}
\]
In principle, this algebraic equation can be solved numerically to obtain characteristic values, however the sign of the real parts of these values cannot be determined analytically.

In order to obtain an explicit result of the characteristic values, we consider the special case of $\gamma_n = \gamma_p = \gamma$. If the number of nodes on the NFL is less than that on the PFL, i.e., if $N \leq M + 1$, then we have
\[
f(\lambda) \equiv (\lambda + \gamma)^{M+1} - F_2(\lambda + \gamma)^{M+1-N} - F_1 = 0 \tag{5}
\]
In this case, if $N = M$ the node number of the NFL is one more than that of the PFL, then Eq. (5) becomes
\[
(\lambda + \gamma)^{M+1} - F_2(\lambda + \gamma) = F_1 \tag{5a}
\]
We can verify that this equation has at least a root with positive real part, implying that the fixed point is unstable. More generally, we can verify that Eq. (5) has a root with positive real part, implying that the fixed point is unstable.

If the number of the NFL is more than that of the PFL, i.e., if $N \geq M + 1$, then we have
\[
(\lambda + \gamma)^N - F_1(\lambda + \gamma)^{N-M} - F_2 = 0 \tag{6}
\]
In this case, if $N = M + 1$ (the node number of the NFL is the same as that of the PFL), then Eq. (6) is reduced to
\[
(\lambda + \gamma)^N = F_1 + F_2 \tag{6a}
\]
from which we can obtain the explicit expressions of $N$ characteristic values (the results are omitted here).

Next, we focus on the special case of $N = M + 1$. Note that $F_1$ is always positive whereas $F_2$ is always negative. We will distinguish three cases: (1) $F \equiv F_1 + F_2 > 0$, i.e., the positive feedback dominates. In this case, if $[F(x^*)]^{1/N} > \gamma$, then the real parts of the characteristic values are all positive, implying that the fixed point $x^*$ is unstable; if $[F(x^*)]^{1/N} \leq \gamma$, then the eigenvalues are zero, and the stability of the fixed point $x^*$ cannot be determined and needs further analysis. (2) $F = 0$, i.e., the positive and negative feedbacks keep balance. In this case, the real parts of the characteristic values are all negative, implying that the fixed point $x^*$ is stable. (3) $F < 0$, i.e., the negative feedback dominates. In this case, the characteristic values are all complex and all the real parts are negative. Moreover, the characteristic values appear in a complex conjugate manner since the characteristic polynomial does not have a positive real root.

In conclusion, the system undergoes Hopf bifurcation at the critical point determined by $[F(x^*)]^{1/N} \cos(\pi/N) = \gamma$, and has a limit cycle as the system parameters satisfy $[F(x^*)]^{1/N} \cos(\pi/N) > \gamma$. More precisely, the fixed point $x^*$ is stable as long as $[F(x^*)]^{1/N} \cos(\pi/N) < \gamma$, and unstable if $[F(x^*)]^{1/N} \cos(\pi/N) > \gamma$. In the
latter case, by the Hopf theorem we know that there exists a periodic orbit and the period is approximately given by
\[ T = \frac{2\pi}{\left| \left( F(x^*) \right)^{1/N} \sin(\pi/N) \right|} \]
near the critical point determined by \[ \left| \left( F(x^*) \right)^{1/N} \cos(\pi/N) \right| = \gamma. \]

In order to clearly illustrate the above theoretical predictions, we perform numerical analysis results displayed in Fig. 2. We take the non-competition model as an example and distinguish three cases according to the numbers of nodes on positive and negative feedback loops. This figure shows that regardless of the relationship between \( N \) and \( M \), i.e., whatever \( N < M + 1 \), \( N = M + 1 \) or \( N > M + 1 \), the stability of the system’s fixed point changes accordingly with changing the value of degradation rate \( \gamma \) more precisely, the real part of the characteristic value changes from positive to negative values).

**Figure 2.** Influence of parameter \( \gamma \) on the real and imaginary parts of the root of the characteristic equation \( f(\lambda) = 0 \); non-competition model. (a) \( N = 3, M = 8 \) (corresponding to the case of \( N < M + 1 \)); (b) \( N = 3, M = 2 \) (corresponding to the case of \( N = M + 1 \)); (c) \( N = 5, M = 2 \) (corresponding to the case of \( N > M + 1 \)); (d) Bifurcation diagram of \( x_3 \) versus \( \gamma \) for \( N = 3, M = 2 \). Here green solid and red dashed lines represent stable and unstable steady states respectively, and the symbol HB represents the Hopf bifurcation point. Other parameter values are set as \( \alpha_n = \alpha_p = 3, c_n = c_p = 0.1, h_n = 3, h_p = 1, \alpha_n = \alpha_p = \alpha \), and \( K_1 = K_2 = 1 \).

Specifically, the real part of the eigenvalue, i.e., \( Re(\lambda) > 0 \), is a monotonically decreasing function of the degradation rate \( \gamma \) (referring to the green lines in Fig. 2a-c) whereas the imaginary part of the eigenvalue, i.e., \( Im(\lambda) > 0 \), is a monotonically
increasing function of the parameter $\gamma$ (referring to insets of Fig. 2a-c), whatever the relationship between the node numbers of the two feedback loops. Moreover, the real part of the eigenvalue changes from positive to negative, crossing the line of $Re(\lambda) = 0$ (referring to the red dashed lines). This implies that Hopf bifurcation occurs when the degradation rate $\gamma$ crosses a critical value. This result is in consistent with the one predicted by the above theory. Although this bifurcation induces limit-cycle oscillations, the range of the limit cycle is different between the cases of $N \leq M + 1$ and $N > M + 1$. If the other parameters are fixed, the range of parameter $\gamma$ that guarantees the existence of the limit cycle for the $N \leq M + 1$ case is the interval of , which is apparently narrower than that for the $N > M + 1$ case where the range of the limit cycle is . The reason for this difference is that when $N > M + 1$ (implying that the NFL has more nodes than the PFL), negative feedback plays a dominant role in the system of coupled feedback loops, and hence the range of parameter $\gamma$ that makes the system generate oscillations in this case is wider than that in the other two cases of $N < M + 1$ and $N = M + 1$ (since the negative feedback is a major driver of sustained oscillations). This result implies that oscillations are more likely to occur in the iPNFLs dominated by negative feedback, and this is consistent with the previous results that NFL promotes oscillations [36, 28, 27].

Three insets of Fig. 2a-c show the influence of the change of $\gamma$ on the imaginary part of the eigenvalue, $Im(\lambda)$. We observe that with the increase of parameter $\gamma$, the imaginary part $Im(\lambda)$ tends to become larger, indicating that parameter $\gamma$ may amplify the oscillation period of the underlying system. In addition, Fig. 2d is a bifurcation diagram and shows how the bifurcation dynamics of transcription factor $x_3$ changes after $\gamma$ crosses the bifurcation point indicated by HB.

3. Main results. Through model analysis, we find that the numbers of nodes on positive and negative feedback loops can have significantly different influences on oscillations, depending on the interactive way of the two loops [9, 10]. We also find that the effect of the node number of the NFL is equal to that of a time delay to some extent.

3.1. Oscillatory regions. According to previous studies [37, 20], we know that iPNFLs allow for tunable and robust oscillations with widely tunable frequency and nearly constant amplitude. Here, we first consider whether the competition model or the non-competition model can indeed generate sustained oscillations as predicted by theory, for some values of system parameters. Fig. 3a and 3b show two numerical examples of oscillations, where the only difference is in the interacting way between the two feedback loops, and the system parameter values are set the same in both cases of combinatorial regulation. We observe that oscillations (including oscillation period and amplitude) shown in the two panels are nearly the same. The reason may be that negative feedback is dominating in both cases.

We further explore the role of positive and negative feedback loop node numbers in generating oscillations in the two different cases of combinatorial regulation. From Fig. 3c and 3d, we observe that if the number of nodes on the PFL is fixed, then the system may jump from the non-oscillating region to the oscillating region as the NFL node number increases. However, if the number of the NFL nodes is fixed, then increasing the PFL node number can make the system have opposite transition (i.e., from oscillations to non-oscillations). From Fig. 3c and 3d, we observe that if the number of nodes on the NFL is larger than that of nodes on the PFL, that is, if the negative feedback dominates in the coupled loops, then the underlying
Figure 3. The occurrence of oscillations in the system of interlocked multi-node positive and negative loops. (a, c) correspond to the noncompetitive model whereas (b, d) to the competitive model. (a, b) show time series of component $x_5$, where the inset is a phase trajectory in the $(x_4, x_5)$ plane. (c, d) show both a stable region for the fixed point (corresponding to nonoscillation indicated in the diagram) and an oscillatory region (corresponding to oscillation indicated in the diagram) in the $(N, M)$ plane, where the green dashed line is the border of the two regions. Parameter values are set as $\alpha_n = \alpha_p = 3, c_n = c_p = 0.1, h_n = 3, h_p = 1, \alpha_n = \alpha_p = 1$, and $K_1 = 0.5$, and $K_2 = 4$.

It is worth mentioning that if the numbers of nodes on the NFL and the PFL are all large, e.g., $N = 11$ and $M = 10$ used in Fig. 3c and 3d, the competition mechanism is more likely to generate oscillations than the non-competition mechanism (comparing the boundaries between oscillating and non-oscillating regions in the two cases of combinatorial regulation). A possible interpretation of this phenomenon is that if the numbers of nodes on feedback loops are large enough and the positive and negative feedback strength is comparable, then the former mechanism may more efficiently transfer information than the latter mechanism.

3.2. Effect of the node number of negative feedback loop on oscillations.

It is well known that oscillations of large amplitude can enhance robustness [11, 21], regulation of downstream clock-controlled genes [43], and accuracy for telling time
In the previous subsection, we have studied the influences of the numbers of PFL and NFL nodes on the oscillatory behavior of the underlying system, but how the node numbers affect amplitude and period remains unclear. Here, we focus on investigating this issue. For this, we fix the node number of the PFL at $M = 2$, but distinguish two ways of combinatorial regulation: competition and non-competition, referring to Fig. 1b and 1c.

In our model, parameters $K_1$ and $K_2$ are assumed as dimensionless dissociation constants for repressors and activators, respectively. However, promoter occupancy of the competitive binding sites may change over time. Previous studies revealed that in the case of competitive binding, there is an optimal ratio of the dissociation constant of an activator over that of a repressor, such that the oscillation amplitude of a key clock gene is optimally enlarged \[25, 38\] (note: in our case, when $K_1/K_2$ is larger than the optimal ratio, the activator of the PFL always occupies the binding sites, implying that the occupancy of the NFL repressor to the binding sites is rejected). Our numerical results shown in Fig. 4a and 4b verify this qualitative result. Specifically, there is an optimal ratio between the two dissociation constants such that the oscillation amplitude is largest, regardless of the number of the NFL nodes or the way of combinatorial regulation on the cis-regulatory module of the common gene. The similar result holds for the oscillation period, referring to Fig. 4c and 4d. Note that in order to obtain these results, we change the value of $K_1$ while keeping the value of $K_2$ fixed at $K_2 = 0.5$.

Figure 4 shows numerical results for four different values of (note: for the homogeneous feedback loop, takes only odd numbers), where the number of the nodes on the PFL, $M$, is fixed at $M = 2$. From Fig. 4a and 4b, we observe that the oscillation amplitude increases with the increase of $N$, regardless of regulatory way (competitive or non-competitive). This increase is very apparent for the small node number of the NFL, e.g., the largest oscillation amplitude at $N = 3$ is below 1 but the one at $N = 5$ is approximately equal to 6 for the non-competitive model and is beyond 6 for the competitive model. However, the change in the oscillation amplitude will become slower and slower if $N$ is further increased, e.g., the largest oscillation amplitudes at $N = 7$ and $N = 11$ are approximately equal. On the other hand, we observe from Fig. 4c and 4d that the oscillation period also increases with the increase of $N$, regardless of regulatory way, but the increase degree is different from that in the case of oscillation amplitude. The increase in period is always very apparent if the number of nodes in the NFL is added by 1, e.g., the largest oscillation period changes from approximately 150 to approximately 230 as $N$ changes from 7 to 11.

For both noncompetitive and competitive models, the amplitude and period of oscillations depend on the ratio of the dissociation constants of the repressor and the activator, $K_1/K_2$. In general, oscillations occur only when this ratio is within a small range. More precisely, the range is up to the interval of 0 and 4 for the non-competitive model but it is not beyond the interval of 0 and 2 for the competitive model, implying that the combinatorial regulation of noncompetitive type is more likely to induce oscillations than that of competitive type. As mentioned above, there is an optimal ratio, $K_1/K_2$, the amplitude and period of oscillations all reach the maximum.

### 3.3. Effect of the node number of positive feedback loop on oscillations.

Different from the investigation done in the above subsection, here we focus on investigating the influence of the number of nodes on the PFL ($M$) on the amplitude...
Figure 4. The effect of the node number of the negative feedback loop on the amplitude and period of oscillations, where $M = 2$. (a, c) correspond to the noncompetitive model, (b, d) correspond to the competitive model. (a, b) for oscillation amplitude, (c, d) for oscillation period. Parameter values are set as $\alpha_n = \alpha_p = 3, c_n = c_p = 0.1, h_n = 3, h_p = 1, \alpha_n = \alpha_p = 1$, and $K_2 = 0.5$.

and period of oscillations. For this, we keep the number of nodes on the NFL ($N$) fixed. We obtain qualitative results, which are significantly different from those obtained in the previous subsection (comparing Fig. 5 and Fig. 4).

Figure 5 shows results for four different values of $M$ (note: for the homogeneous feedback loop, $M$ may take arbitrary positive integer), where the number of the nodes on the NFL, $N$, is fixed at $N = 5$. The increase in the number of nodes on the PFL has no significant effect on the amplitude and period of oscillations, but there are some differences in effect between the non-competition model and the competition model. In fact, from Fig. 5a and 5c that correspond to the competition model, we observe that for the same rate $K_1/K_2$, the period and amplitude of oscillations are almost kept invariant for different values of the number of the nodes on the PFL. However, from Fig. 5b and 5d that correspond to the non-competitive model, we observe that the change tendency is different. For example, if $K_1/K_2$ is greater than the optimal value, then the oscillation amplitude and period are all greater in the case of $M = 1$ than those in the other three cases (i.e., $M = 4, 6, 10$). If the number of the PFL nodes, $M$, increases from 1 to 4, both the amplitude and the period of the oscillations decrease slightly. However, if $M$ increases from 4 to 6 or 10, neither the amplitude nor the period of the oscillations increase or decrease.

Recall that in Fig. 4, the range of $K_1/K_2$ that can increase the amplitude and period of oscillations becomes significantly larger as the number of NFL nodes ($N$)
increases. In contrast, we observe from Fig. 5 that increasing the number of PFL nodes \( (M) \) cannot significantly change the range of \( K_1/K_2 \) that guarantees the existence of oscillations. In addition, we observe that the range of \( K_1/K_2 \) for the amplitude and period of oscillations in the non-competitive model is approximately twice as large as those of the competitive model. These results indicate that the parameter space for the existence of oscillations is larger in the non-competitive model than in the competitive model. In other words, the non-competitive mechanism is more likely to produce oscillations than the competitive mechanism, although the optimal value of \( K_1/K_2 \) is almost the same in both cases.

It is worth pointing out that the remarkable differences in results shown in Fig. 4 and Fig. 5 can help us understand and even can in turn be used to infer the internal mechanisms of biological oscillators.

3.4. The global effects of the node numbers on NFL and PFL. In the previous subsections, we assumed \( \gamma_n = \gamma_p = 1 \) and showed that the noncompetitive model is more likely to produce oscillations of large amplitude and long period. In this subsection, we try to reveal the global effects of the NFL and PFL node numbers on oscillations by letting parameter pair \((\gamma, K)\) change, where we assume \( \gamma_n = \gamma_p = \gamma \) and \( K_1 = K_2 = K \). For clarity, we distinguish three cases of the relationship between \( N \) and \( M \): \( N > M + 1 \), \( N = M + 1 \) and \( N < M + 1 \). Numerical results are shown in Fig. 6 and Fig. 7.
First, we observe from Fig. 6 that for the non-competition model, the increase in the number of PFL nodes $M$ has no distinct effect on the amplitude of oscillations. However, for the competitive model, the difference between the oscillation amplitudes corresponding to $M = 1$ and $M = 2, 6, 8$ is very significant [comparing Fig. 6(e) with Fig. 6(f-h)]. A possible reason for this difference is that the competitive model is more sensitive to the influences of the number of PFL nodes, $M$ and parameter $\gamma$. Note that when the number of PFL nodes, $M$ is large (i.e., $N < M + 1$), the positive feedback dominates in iPNFLs. In this case, the competitive mechanism would cause the biological signal to spend more time in the transmission process, thus weakening the role of negative feedback and making the oscillation behavior less likely to occur.

Comparing two arrays of panels in Fig. 6, regardless of the relationship between the numbers of NFL nodes and PFL nodes, the oscillation amplitude is always larger in the non-competitive model than that in the competitive model. However, we observe from this figure that for both models, both a smaller $\gamma$ (close to 0.3) and a larger $K$ (close to 1) can result in a larger oscillation amplitude.

We also analyze the joint effects of the numbers of PFL and NFL nodes on the period of oscillations, with numerical results shown in Fig. 7. Similar to the case of the oscillation amplitude analyzed above, we observe from this figure that for the non-competitive model, $M$ increasing the number of PFL nodes, has no significant effect on the oscillation period. For the competitive model, however, there is a remarkable difference between the oscillation periods corresponding to $M = 1$ and $M = 2, 6, 8$.

It is worth mentioning that when the system arrives at the maximum period of oscillations, the values of parameters $\gamma$ and $K$ are always negatively correlated.
Therefore, for the non-competitive model, if we want to amplify the oscillation period, then there is a trade-off relationship between parameters $\gamma$ and $K$. For the competitive model with multiple PFL nodes (i.e., if $M \geq 2$), the maximum oscillation period is obtained at a smaller $\gamma$ and at a larger $K$ (close to 1). In addition, we observe from Fig. 7 that the oscillation period is always greater in the non-competitive model than in the competitive model.

![Figure 7](image_url)

**Figure 7.** Three dimensional pseudo-diagram in the $(\gamma, K)$ plane, where the color bar represents oscillation period, where parameter $N$ is fixed at $N = 3$. (a, b, c, d) correspond to non-competitive binding with $M = 1, 2, 6, 8$ from left to right (corresponding to the cases of $N > M + 1$, $N = M + 1$ and $N < M + 1$, respectively); (e, f, g, h) correspond to competitive binding with $M = 1, 2, 6, 8$ from left to right (corresponding to the cases of $N > M + 1$, $N = M + 1$ and $N < M + 1$, respectively). Other parameter values are set as $\alpha_n = \alpha_p = 3, c_n = c_p = 0.1, h_n = 3, h_p = 1$.

Taken the above analysis together, we can conclude that no matter how the number of nodes changes, the non-competitive model performs better than the competitive model in enlarging the amplitude and period of oscillations. In other words, the noncompetitive mechanism is more likely to facilitate sustained oscillations than the competitive mechanism.

3.5. **The influence of node number and time delays on the oscillating region.** Traditionally, time delays were often used to model multi-step processes in biological information transmission systems [28, 11, 39, 41, 15]. Such a simplification is only for convenience of establishing mathematical models in the case that molecular details cannot be specified. Here we want to answer the issue: to what extent, the effect of time delay is equal to that of a certain multi-step process. For clarity, we use the non-competition model as an example to briefly discuss the impact of the number of NFL nodes and the time delay on the oscillatory region, respectively. The time-delayed model to be studied is described as follows

$$\frac{dx_1}{dt} = c_n - \gamma_n x_1 + \frac{\alpha_n \alpha_p}{1 + (x_1(t - \tau)/K_1)^{h_n}} \frac{(x_3/K_2)^{h_p}}{1 + (x_3/K_2)^{h_p}}$$
\[
\frac{dx_2}{dt} = c_p - \gamma_p x_2 + \frac{\alpha_p (x_1/K_2)^{h_p}}{1 + (x_1/K_2)^{h_p}}
\]

\[
\frac{dx_3}{dt} = c_p - \gamma_p x_3 + \frac{\alpha_p (x_2/K_2)^{h_p}}{1 + (x_2/K_2)^{h_p}}
\]

In this model, intermediate steps in the NFL are replaced with a one-step process with the delay of \(\tau\). The number of PFL nodes is fixed at 2. The non-competitive models dynamics of \(N = 3\) can be described by Eq. (1a and 1b). Numerical results are presented in Fig. 8.

**Figure 8.** Comparison between influences of NFL node number and time delay on oscillating region, where the number of PFL nodes, \(M\) is fixed at \(M = 2\). (a) displays the dependence of the maximum oscillation amplitude on the ratio of \(K_1/K_2\); (b) shows the oscillation region of in the \((K_1, K_2)\) plane; (c) shows the oscillation region of \(\tau = 1.04\) also in the \((K_1, K_2)\) plane. Other parameter values are set as \(\alpha_n = \alpha_p = 3, c_n = c_p = 0.1, h_n = 3, h_p = 1\).

For comparison, we utilize the maximum oscillation amplitude as a measure of the effect of the number of NFL nodes and time delay. By numerical calculations, we found that \(N = 3\) and \(\tau = 1.04\) each can make the system reach the same maximum oscillation amplitude of 0.8, as shown in Fig. 8a. In this case, we say that the effect of the three nodes on negative feedback is equal to that of time delay \(\tau = 1.04\). However, as can be seen from Fig. 8b and 8c, the oscillation regions are significantly different between the cases of \(N = 3\) and \(\tau = 1.04\). The apparent difference between these two oscillation regions indicates that the number of NFL nodes and the time delay can have different effects on the robustness of the oscillation. Therefore, simply modeling a multi-step process using a time delay would be inappropriate and must be carefully considered.

4. Conclusion and discussion. Biochemical oscillations are a very interesting phenomenon [28, 37, 29]. Unraveling which factors and how they affect oscillations would be fundamentally important for the understanding of biological oscillators. In recent years, many efforts have been made to explore the performance advantages of iPNFLs from different aspects [36, 37, 31, 4], e.g., Tsai et al. reported that iPNFLs allow for tunable and robust oscillations with widely tunable frequency and nearly constant amplitude [37]. Here, we have analyzed the dynamics nature of iPNFLs from a distinct perspective. Based on the regulation of competitive sites in a gene promoter region, we have introduced two toy models to investigate the influences of the feedback loop node numbers on oscillations. We found that the numbers of nodes in the NFL and PFL play a different role in adjusting the amplitude and period
of oscillations. Specifically, increasing the number of NFL nodes can significantly amplify the oscillation amplitude and period, but increasing the number of PFL nodes has no significant influence in the non-competition model but can reduce the oscillation region in the competitive model.

Our models only considered homogeneous feedback loops, but heterogeneous feedback loops exist extensively in biological regulatory systems. The models considered combinatorial regulation, which however in general takes place in transcriptional regulatory networks, e.g., in the mammalian circadian clock, the activator ROR and the repressor REV-ERB form interlocked feedback loops, and compete for the same binding sites in the key gene Bmal1 promoter [38]. In addition, the system of iP NFLs may exhibit other complex behaviors (e.g., the coexistence of diverse behaviors), but the mechanism behind it remains elusive. Our models did not consider the response of iP NFLs to stochastic fluctuations [12], nor different time scales of positive and negative feedback loops [5]. Apart from node numbers of feedback loops, combinatorial regulation, and time delays, there exist many other factors that can influence oscillatory behaviors of cell or gene regulatory systems.

Finally, understanding and dissecting the design principle of genetic oscillators is one central topic in synthetics and systems biology. In summary, in this paper we have demonstrated that the number of NFL nodes in the system of iP NFLs can enlarge the amplitude and period of oscillations, and interlocked multi-node positive and negative feedback loops are an effective mechanism to promote oscillations. These qualitative results can provide useful guidance for future studies in the design of synthetic oscillatory networks and may offer additional understanding of natural oscillatory systems as well.

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