



Biochemical relaxation oscillator designed to control molecular computation[☆]

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ABSTRACT

Embedding efficient calculation instructions into biochemical systems has always been a research focus in synthetic biology. Amongst this, the main challenge is how to overcome the contradiction between the parallel occurrence of biochemical reactions and the ordinal implementation of calculation instructions. In response to this challenge, the current work presents a new 4-dimensional biochemical oscillator model based on the relaxation oscillation to generate a pair of symmetric clock signals for dual-module regulation tasks through chemical reaction networks. The pair of symmetric clock signals separately act as a catalyst to participate the corresponding reaction modular of performing calculation, and control their on/off switch if the concentration of the clock signal is nonzero. The detailed discussions are subsequently made on the important effect of the oscillator parameters on controlling the period and the occurrence order of clock signals. Further, the oscillator is applied to control the iteration calculation, along with which a termination strategy is proposed to jump out of the loop.

1. Introduction

A main objective of synthetic biology is to design programmable biochemical controllers that can operate in molecular contexts incompatible with traditional electronics [1,2]. The execution of operations in the biochemical environment needs to follow specific lines of reasoning logics, and the chemical reaction network theory [3] is precisely the medium that helps embedding classical deep algorithms into organisms. Many related works have been published in recent years. Moorman et al. proposed a biomolecular perceptron network in order to recognize patterns or classify cells *in vivo* [4]. The work of Vasic et al. transformed the feedforward ReLU neural network into chemical reaction networks (CRNs for short), and performed their model on standard machine learning training sets [5]. There are also some attempts to build CRNs with learning capabilities [6,7]. However, few have implemented the whole neural network computations into complete biochemical systems. The main reason is that the algorithm based on computer instruction performs operations in a sequential manner, whereas chemical reactions may proceed in parallel. This contradiction calls for an appropriate regulation strategy that isolates two reaction

modules from co-occurring and controls their occurrence order [2]. For example, Blount et al. built cell-like compartments and added electrical clock signals artificially in order to solve this problem [6], which increased the difficulty of biochemical implementation. A more natural idea is to design biochemical oscillators that can automatically generate chemical species acting as periodical clock signals, taking advantage of the oscillatory changes of their concentrations between positive and zero values to turn corresponding reaction module on or off.

Oscillation phenomena are often encountered in chemical and biological systems and the design of oscillators has been extensively studied in most aspects of industrial applications [8–10]. The ideas of designing biological components and circuits with oscillatory characteristics are generally divided into two categories. One is based on the real biological oscillation mechanism, utilizing the existing biological networks (such as genetic regulatory networks and phosphorylation networks) to synthesize the desired oscillatory structures, which usually serves for the simulation and explanation of specific phenomena observed in biochemical experiments [11,12]. The other category originates directly from the chemical reaction network theory [3], designing abstract CRN structures that can lead to specific

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dynamical behavior and then utilizing DNA strand displacement technology to transform the abstract CRNs into real biochemical reactions. Specifically, Arredondo and Lakin [13] utilized a 20-dimensional CRN oscillator extended from an ant colony model [14] to coordinate and arrange the execution sequence of all parts of their chemical neural networks. We also follow the logic of the latter because it is more befitting to our design of the desired oscillation configurations, with mature theoretical guarantees [15–17] and practical successful applications in chaotic oscillatory system [18] and biological chaotic circuits [19] provided by the DNA strand displacement technology. There are two main drawbacks in these designs. The first one is that the oscillation mechanisms are usually ambiguous; and the second drawback is the set of initial concentrations of these oscillators is usually too strict to be achieved accurately in real biochemical environments. In view of this, we summarize the requirements for species as clock signals and design a universal 4-dimensional biochemical relaxation oscillator, which generates the expected clock signals to make two reaction modules execute alternately. The main contributions of the paper include:

- (1) The design of a 4-dimensional biochemical relaxation oscillator to generate coordinated clock signals, which can switch on/off dual-module reaction blocks of performing computations by acting as catalysts participating reactions;
- (2) The quantification of the effect of the oscillator parameters and initial values on controlling the period and occurrence order of clock signals, which reveals that our biochemical oscillator has a much clearer mechanism about oscillation and a more practical choice of initial concentrations as compared to existing ones;
- (3) The building of a specific truncated subtraction module to generate the species that can control the termination of the computation;

The rest of this paper is organized as follows. Preliminaries and problem statements are given in Section 2. Section 3 introduces the structure of our 4-dimensional biochemical relaxation oscillator with detailed dynamical analysis on the oscillation mechanism. In Section 4 we discuss how to control the period and occurrence order of the oscillation. Then we illustrate the loop control based on a dual-module regulation example and present a strategy of loop termination for it in Section 5. The last two sections are concerned to conclusion of the whole paper and the appendix.

2. Preliminaries and problem statement

In this section we introduce the basic knowledge on CRNs from Feinberg [3], and further formulate the problem by a motivating example of how to construct a biochemical oscillator to control the occurrence order of two reaction modules.

2.1. CRN

Consider a network with n species $S_1, \dots, S_n$, and m chemical reactions with the i th reaction $\mathcal{R}_i$ written as



where $a_{ij}, a'_{ij} \in \mathbb{Z}_{\geq 0}$ represent the complexes of reactant and product, respectively, and the j th ($j = 1, \dots, n$) entry of a_{ij} represents the stoichiometric coefficient of species S_j in complex a_{ij} . Some basic concepts about CRNs may be defined based on the above information [3].

Definition 2.1 (Chemical Reaction Network). For the finite sets of species, complexes and reactions, denoted by $S = \{S_1, \dots, S_n\}$, $C = \cup_{i=1}^m \{a_{ij}, a'_{ij}\}$, and $\mathcal{R} = \{a_1 \rightarrow a'_1, \dots, a_m \rightarrow a'_m\}$, respectively, the triple $(S, C, \mathcal{R})$ is called a CRN if the following conditions hold

- there is no reaction $a_{ij} \rightarrow a'_{ij} \in \mathcal{R}$ ($i = 1, \dots, m$) such that $a_{ij} = a'_{ij}$;

- $\forall a_{ij} \in C$, there exists $a'_{ij} \in C$ supporting either $a_{ij} \rightarrow a'_{ij} \in \mathcal{R}$ or $a'_{ij} \rightarrow a_{ij} \in \mathcal{R}$.

By denoting the concentration of species S_i by $s_i \in \mathbb{R}_{\geq 0}$, the dynamics describing the change of all species concentrations can be written as

$$\frac{ds}{dt} = \Xi \cdot r(s), \quad (2)$$

where $s \in \mathbb{R}_{\geq 0}^n$ is the n -dimensional concentration vector, $\Xi \in \mathbb{Z}^{n \times m}$ is called the stoichiometric matrix with every column defined by $\Xi_{ij} = a'_{ij} - a_{ij}$, and $r(s)$ is the m -dimensional vector-valued function evaluating the reaction rate. The most common model to specify the reaction rate is the *mass-action kinetics* [3] by

$$r(s) = \left(k_1 \prod_{i=1}^n s_i^{a_{1i}}, \dots, k_m \prod_{i=1}^n s_i^{a_{mi}} \right)^\top \quad (3)$$

with $k_j > 0$ to represent the reaction rate constant of reaction $\mathcal{R}_j$. The CRN equipped with *mass-action kinetics* is called mass-action system, which is essentially a group of polynomial ordinary differential equations (ODEs) and able to implement some specific operations [20]. We place the further discussion of the *mass-action kinetics* in Appendix, together with a CRN example implementing an addition calculation, and directly enter the problem statement part.

2.2. Problem statement

When implementing calculation with chemical reactions, the core difficulty is to deal with the contradiction between the sequential execution of calculation instructions and intrinsically parallel occurrence of chemical reactions. This is especially true for those compound algorithms such as loop calculation, in which the calculation procedures usually have a specific priority. We take the frequently-used loop iteration calculation $s_1 = s_1 + 1$ appearing in many machine learning algorithms as an example to formulate the problem under study.

Example 2.2. Given two reaction modules ($\mathcal{M}$)



with all the reaction rate constants to be 1 (In the context, when the reaction rate constant is 1, we just omit it), and the ODEs are

$$\begin{aligned} \mathcal{M}_1 : \frac{ds_2}{dt} &= s_1 + s_3 - s_2, & \mathcal{M}_2 : \frac{ds_1}{dt} &= s_2 - s_1, \\ \frac{ds_1}{dt} &= \frac{ds_3}{dt} = 0; & \frac{ds_2}{dt} &= 0. \end{aligned}$$

Their solutions are

$$\mathcal{M}_1 : s_2(t) = s_1(0) + s_3(0) - (s_1(0) + s_3(0) - s_2(0))e^{-t}, \quad (4a)$$

$$\mathcal{M}_2 : s_1(t) = s_2(0) - (s_2(0) - s_1(0))e^{-t}. \quad (4b)$$

$\mathcal{M}_1$ is actually the network given in the Appendix, called addition module, and $\mathcal{M}_2$ finishes the load task, called load module [2]. When these two modules work independently, $\mathcal{M}_1$ can perform the calculation of $s_2^* = s_1(0) + 1$ by setting $s_3(0) = 1$ while $\mathcal{M}_2$ realizes $s_1^* = s_2(0)$. Note that if $s_3(0)$ is given by other non-negative real value, then other iteration computation may be reached. Moreover, the expressions of solutions (4) imply that both of them converge to equilibrium exponentially. Therefore, alternation and loop of $\mathcal{M}_1$ and $\mathcal{M}_2$ could perform the desired loop iteration calculation $s_1 = s_1 + 1$. However, if we directly put these two reaction modules together, there is strong coupling on the dynamics of S_1 and S_2 , and their concentrations would increase to infinity in the absence of regulation, which fails to execute the calculation instruction.

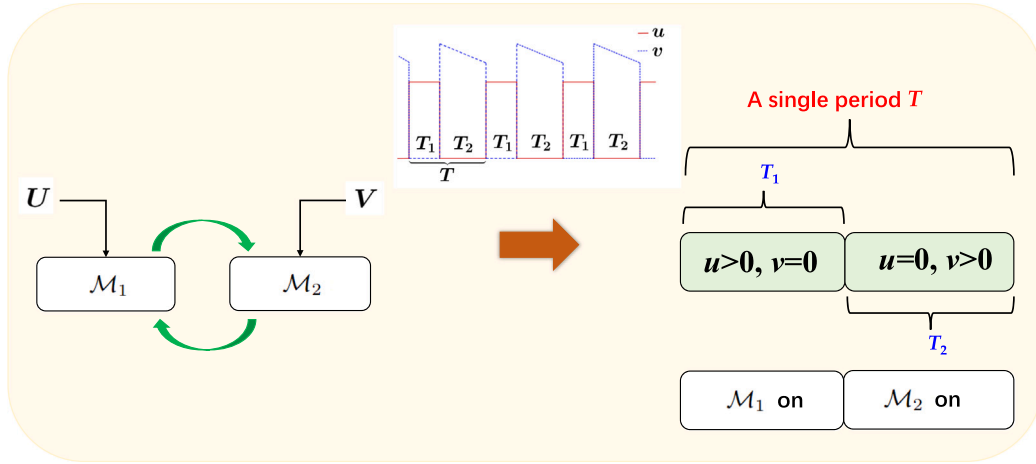
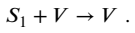
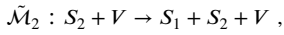
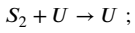
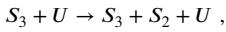
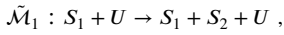


Fig. 1. A schematic diagram of a pair of standard clock signals U and V for module regulation.

The above example suggests that we need to find a new tool to control strictly and alternatively the “turn on” and “turn off” of occurrence of the two modules $\mathcal{M}_1$ and $\mathcal{M}_2$. A possible solution is to introduce a chemical oscillator that produces periodical signals for order regulation. For this purpose, we modify $\mathcal{M}_1$ and $\mathcal{M}_2$ as follows.

Example 2.3. The modified reaction modules are



Here we introduce two species U and V that are involved in reactions as catalysts. The ODEs of dynamics of the whole network ($\tilde{\mathcal{M}}_1$ plus $\tilde{\mathcal{M}}_2$) are expressed as

$$\frac{ds_1}{dt} = (s_2 - s_1)v , \quad (5a)$$

$$\frac{ds_2}{dt} = (s_1 + s_3 - s_2)u , \quad (5b)$$

$$\frac{ds_3}{dt} = 0 . \quad (5c)$$

From the above equations, it can be concluded that whether species U (resp., V) has a non-zero concentration, it will “turn on” or “turn off” $\tilde{\mathcal{M}}_1$ (resp., $\tilde{\mathcal{M}}_2$). Hence, as long as species U and V are designed to be a pair of clock signals whose concentrations oscillate with the same period, they will be able to generate “loop” so as to control the execution sequence of $\tilde{\mathcal{M}}_1$ and $\tilde{\mathcal{M}}_2$, and finally to realize the loop iteration calculation $s_1 = s_1 + 1$. Fig. 1 gives a schematic diagram of a pair of standard clock signals to alternatively and periodically “turn on” and “turn off” two reaction modules.

Based on Example 2.3 and the usual requirements for clock signals [21], we conclude ours for the current work, called symmetrical clock signals.

Definition 2.4 (Symmetrical Clock Signals). A pair of oscillatory species U and V are called symmetrical clock signals if their concentrations u and v satisfy

1. u and v have the same oscillation period T which can be divided into two separate parts as $T = T_1 + T_2$. During the duration of T_1 (resp., T_2), there is $u > 0$ while $v = 0$ (resp., $v > 0$ while $u = 0$).
2. There exist abrupt transitions between adjacent phases of u and v and their values in every phase are complementary, that is to

say, $\exists u_{middle} > 0$ (resp., $\exists v_{middle} > 0$), for any sufficiently small $\Delta t > 0$, $u(t) > u_{middle} > 0$ (resp., $v(t) = 0$) whenever $k * T + \Delta t < t < k * T + T_1 - \Delta t$ and $u(t) = 0$ (resp., $v(t) > v_{middle} > 0$) whenever $k * T + T_1 + \Delta t < t < (k + 1) * T - \Delta t$ with $k \in \mathbb{Z}_{\geq 0}$.

Remark 2.5. When $u(t) > u_{middle} > 0$, species U turns on the $\mathcal{M}_1$; when $u(t) = 0$, then the species U turns off the $\mathcal{M}_1$. The different situations of $u(t)$ divided by u_{middle} refer to adjacent different phases, and we expect $u(t)$ and $v(t)$ to switch between adjacent phases abruptly, with the time cost characterized by the sufficiently small Δ .

The requirement of abrupt transitions between adjacent phases motivates us to consider relaxation oscillation [22,23] as the basic dynamical structure. Thus the following task is on the construction of biochemical relaxation oscillator towards controlling molecular computation.

3. Biochemical relaxation oscillator

In this section, we introduce the mechanism of relaxation oscillation and develop a 4-dimensional biochemical relaxation oscillator for the current task.

3.1. Mechanism of 2-dimensional relaxation oscillator

Relaxation oscillation is a type of common oscillation in biochemical systems [24], whose general form, as an example of 2-dimensional case, is

$$\begin{aligned} \epsilon \frac{dx}{dt} &= f(x, y) , \\ \frac{dy}{dt} &= g(x, y) , \quad x \in \mathbb{R}, y \in \mathbb{R}, \end{aligned} \quad (6)$$

where f, g are C^∞ -functions, $\epsilon > 0$ is sufficiently small and the critical manifold $\mathcal{C}_0$ is defined as $\mathcal{C}_0 \triangleq \{(x, y) : f(x, y) = 0\}$. The above system is a standard singular perturbation system.

There have been a great deal of studies [24–26] on the dynamics of (6), where we are rather concerned with those related to oscillation. When the critical manifold $\mathcal{C}_0$ is “S-shaped” (See Fig. 2 as an example), that is to say, the function $y = \varphi(x)$ induced by $\mathcal{C}_0$ has precisely two critical points, a non-degenerate minimum x_m and the other non-degenerate maximum x_M satisfying $x_M > x_m$ which together divide the $\mathcal{C}_0$ into three segments: the left and right segment allow stable equilibrium points on them, while when the equilibrium point is on the middle segment, it is usually unstable [24]. The two critical points

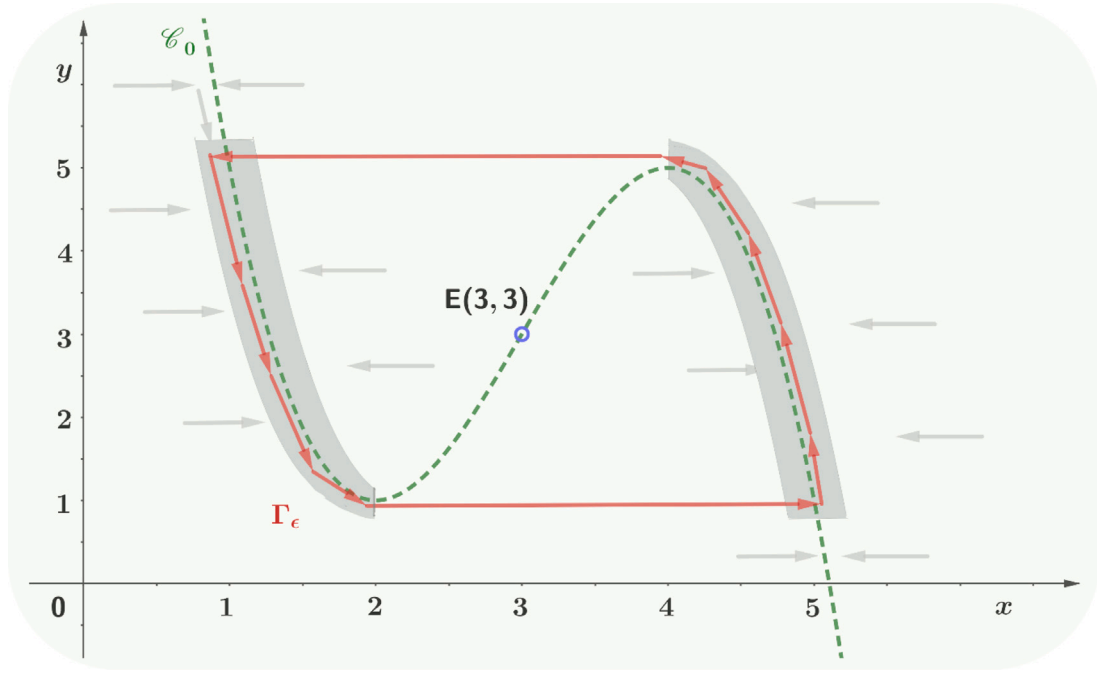


Fig. 2. Phase plane portrait of the relaxation oscillation model (8).

together with $x_l : \varphi(x_l) = y_M = \varphi(x_M)$, $x_r : \varphi(x_r) = y_m = \varphi(x_m)$ define a singular trajectory Γ_0 by

$$\Gamma_0 = \{(x, \varphi(x)) : x_l < x \leq x_m\} \cup \{(x, y_m) : x_m < x \leq x_r\} \cup \{(x, \varphi(x)) : x_M \leq x < x_r\} \cup \{(x, y_M) : x_l \leq x < x_M\}. \quad (7)$$

For this class of systems, Krupa and Szmolyan [24] gave a detailed geometric analysis of the existence and property of relaxation oscillations. Since the relaxation oscillation orbit usually revolves around an unstable equilibrium point, the stability of equilibrium points directly determines the existence of relaxation oscillation. In high-dimensional systems whose critical manifold also presents as ‘S-shaped’ (as shown in Fig. 2, it means that the slow variable has two fold points on the critical manifold), equilibrium points lying on the left or right part are stable (or saddle), then the relaxation oscillation is forbidden. Only if a unique equilibrium point moves to the middle segment of the critical manifold $\mathcal{C}_0$, it destabilizes and initiates oscillation. Namely, a relaxation oscillation orbit Γ_ϵ exists and lies in the $\mathcal{O}(\epsilon)$ -neighborhood of Γ_0 . Here we ignore the details about this condition and recommend readers to refer to Theorem 2.1 in that paper for more information.

Remark 3.1. The dynamics of (6) serving as a biochemical relaxation oscillator should satisfy the following three conditions:

1. it allows for the existence of a relaxation oscillation orbit in the first quadrant;
2. its detailed expression should match a mass-action system;
3. the signals generated should be symmetrical according to Definition 2.4.

The van der Pol equation [27] is a typical case of structure (6) with S-shaped manifold, which acts as a basis for the oscillator design. We provide a coordinate-transformed version with given coefficients, written as

$$\epsilon \frac{dx}{dt} = -x^3 + 9x^2 - 24x + 21 - y, \quad (8a)$$

$$\frac{dy}{dt} = x - 3, \quad x, y \in \mathbb{R}_{>0}, \quad (8b)$$

with a sufficiently small $\epsilon > 0$. Clearly, $(x^*, y^*) = (3, 3)$ is the unique equilibrium lying on the middle segment of the critical manifold $\mathcal{C}_0$:

$\{(x, y) : y = \varphi(x)\}$ with $\varphi(x) = -x^3 + 9x^2 - 24x + 21$, and the singular trajectory is

$$\Gamma_0 = \{(x, \varphi(x)) : 1 < x \leq 2\} \cup \{(x, 1) : 2 < x \leq 5\} \cup \{(x, \varphi(x)) : 4 \leq x < 5\} \cup \{(x, 5) : 1 \leq x < 4\}. \quad (9)$$

The phase plane portrait is exactly presented in Fig. 2, where the green dotted broken curve represents the critical manifold $\mathcal{C}_0$, the shadows refer to $\mathcal{O}(\epsilon)$ -neighborhood of the corresponding $\mathcal{C}_0$ parts, the rail enclosed by red broken lines approximates position of the relaxation oscillation orbit Γ_ϵ , the gray arrows describe direction of the flows and $E(3, 3)$ is the unique unstable equilibrium. The corresponding $x_l = 1, x_m = 2, x_M = 4, x_r = 5, y_m = 1$ and $y_M = 5$. As it can be seen, the flows starting from points in the first quadrant, except the unstable equilibrium, will converge to Γ_ϵ through the horizontal motions. Note that the model of (8) does not match the expressions of the kinetic Eqs. (2) and (3) for a certain mass-action system, since the terms “ $-y$ ” and “ -3 ” cannot reflect the consumption of species X and Y , respectively. To fix this, and also to avoid destroying the inherent dynamical property of (8), a naive idea is to multiply the first equation by x and the second equation by y , which gives a modified version as follows.

The modified van der Pol model is governed by

$$\epsilon \frac{dx}{dt} = (-x^3 + 9x^2 - 24x + 21 - y)x, \quad (10a)$$

$$\frac{dy}{dt} = (x - 3)y, \quad x, y \in \mathbb{R}_{>0}, \quad (10b)$$

with a sufficiently small $\epsilon > 0$. Compared with (8), the current model adds the y -axis into the critical manifold and creates saddle equilibrium points on the axes, which do not affect the dynamical behavior of flows starting from points in the first quadrant. Fig. 3 displays a diagram of oscillatory x and y by taking $(x_0, y_0) = (5, 5)$ and $\epsilon = 0.001$. Obviously, the corresponding species X and Y cannot directly play the role of a pair of symmetrical clock signals as Definition 2.4 requires. However the oscillatory species X satisfies the requirement that x has abrupt transitions between adjacent phases and we will further calculate the corresponding Δt .

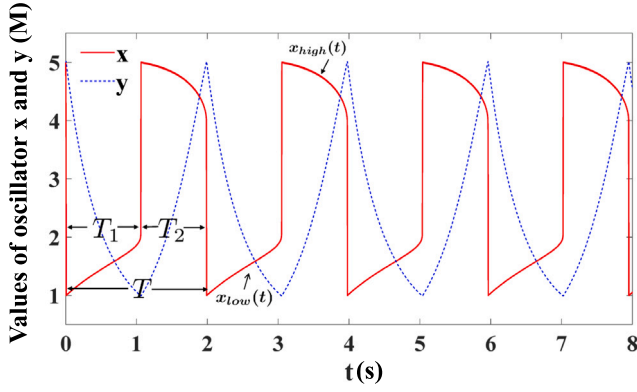


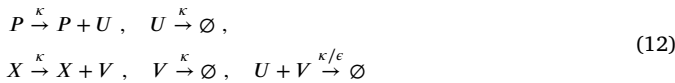
Fig. 3. Diagram of oscillators x and y of the model (10) starting from (5,5) when $\epsilon = 0.001$. Here, M denotes mol/L.

3.2. Development of 4-dimensional biochemical relaxation oscillator

Although the modified van der Pol model (10) fails to directly generate a signal whose concentration could stay close to 0 when it is low, there exits abrupt transitions between adjacent phases in the value of x as Definition 2.4 requires. Thus we treat the species X as a driving signal and introduce a “subtraction operation” to pull down its low value to 0, i.e., considering the known truncated subtraction module



This module may implement the operation $u^* = p(0) - x(0)$ when $p(0) > x(0)$ or $u^* = 0$ when $p(0) \leq x(0)$. Note that here we limit the initial concentrations of U and V are bounded so that the system is bounded. Therefore, as long as the initial concentration of species P is less than that of species X , the equilibrium u^* will be “pulled down” to 0, i.e., U has the potential to be one of the symmetrical clock signals. Based on this understanding, we set up the species X to follow the dynamics of (6) which acts as a relaxation oscillator shown in (10), and further couple it with the following modified module



with a sufficiently small $\epsilon > 0$. Apparently, the modifications include: (i) a new outflow reaction $V \xrightarrow{\kappa} \emptyset$ is added; (ii) the reaction rate constant of $U + V \xrightarrow{\kappa/\epsilon} \emptyset$ is set to be rather large compared to others; (iii) the overall reaction rate constants are uniformly controlled by a sufficiently large κ . We will give reasons of making these modifications during the subsequent analysis.

By combining the dynamics for the driving signal X , i.e., (6), and that of the mass-action system (12), we get

$$\epsilon_1 \frac{dx}{dt} = \eta_1 f(x, y), \quad (13a)$$

$$\frac{dy}{dt} = \eta_1 g(x, y), \quad (13b)$$

$$\epsilon_1 \epsilon_2 \frac{du}{dt} = \eta_1 (\epsilon_1 (p - u) - uv), \quad (13c)$$

$$\epsilon_1 \epsilon_2 \frac{dv}{dt} = \eta_1 (\epsilon_1 (x - v) - uv), \quad (13d)$$

with sufficiently small $\epsilon_1, \epsilon_2 > 0$ and $\eta_1, p > 0$. We reshape the dynamics (6) by multiplying η_1 with the main purpose of conveniently adjusting the oscillation period of species concentrations as we will state in Section 4. The ODEs (13a) and (13b) will degenerate to (6) if η_1 is contained in $f(x, y)$ and $g(x, y)$. Here $f(x, y)$ and $g(x, y)$ are assumed to guarantee the existence of relaxation oscillation [24], and moreover,

the relaxation oscillation orbit Γ_ϵ lies strictly in the first quadrant along with a unique unstable equilibrium; p is a constant representing the initial concentration of catalyst P ; and η_1/ϵ_2 is sufficiently larger than η_1 , which ensures that U and V response quickly enough to the driving oscillator X so that they can oscillate synchronously with X .

Remark 3.2. In the ODEs of (13c) and (13d), the very large reaction rate constant for $U + V \rightarrow \emptyset$ is set as $\eta_1/(\epsilon_1 \epsilon_2)$, which directly “borrows” the small parameter ϵ_1 from the perturbed system (13a) and (13b). This is just for the purpose of the unified notation.

For this 4-dimensional oscillator model describing the evolution of species set $S = \{X, Y, U, V\}$, there are two time-scale parameters ϵ_1 and ϵ_2 that motivate us to analyze the dynamics using the geometric singular perturbation theory [28,29]. Let $\alpha = (u, v) \in \mathbb{R}_{\geq 0}^2$, $\beta = (x, y) \in \mathbb{R}_{> 0}^2$, $F(\alpha, \beta) = (\eta_1(p - u - uv/\epsilon_1), \eta_1(x - v - uv/\epsilon_1))$ and $G(\alpha, \beta) = (\eta_1 f(x, y)/\epsilon_1, \eta_1 g(x, y))$, then we define the corresponding slow-fast systems (labeled by σ_{sl} and σ_{fa} , respectively) as follows

$$\sigma_{sl} \triangleq \left\{ (\alpha, \beta) \left| \epsilon_2 \frac{d\alpha}{dt} = F(\alpha, \beta), \frac{d\beta}{dt} = G(\alpha, \beta) \text{ as } \epsilon_2 \rightarrow 0 \right. \right\}, \quad (14a)$$

$$\sigma_{fa} \triangleq \left\{ (\alpha, \beta) \left| \frac{d\alpha}{d\tau} = F(\alpha, \beta), \frac{d\beta}{d\tau} = \epsilon_2 G(\alpha, \beta) \text{ as } \epsilon_2 \rightarrow 0 \right. \right\}, \quad (14b)$$

which are equivalent essentially with $\tau = t/\epsilon_2$. The system σ_{sl} is a direct transformation of (13) and σ_{fa} is the corresponding fast system. We can also define their reduced version by letting $\epsilon_2 \rightarrow 0$ as follows

$$\sigma_{rsl} \triangleq \left\{ (\alpha, \beta) \left| 0 = F(\alpha, \beta), \frac{d\beta}{dt} = G(\alpha, \beta) \right. \right\}, \quad (15a)$$

$$\sigma_{rfa} \triangleq \left\{ (\alpha, \beta) \left| \frac{d\alpha}{d\tau} = F(\alpha, \beta), \frac{d\beta}{d\tau} = 0 \right. \right\}. \quad (15b)$$

The flows generated from σ_{rsl} and σ_{rfa} are named slow flows and fast flows, respectively. They are utilized to approximate the flows of σ_{sl} and σ_{fa} under the condition of sufficiently small ϵ_2 . The critical manifold $\mathcal{C}_0$, induced by σ_{sl} according to $\mathcal{C}_0 = \{(\alpha, \beta) : F(\alpha, \beta) = 0\}$, gives

$$\mathcal{C}_0 : \begin{cases} \epsilon_1(p - u) - uv = 0, \\ \epsilon_1(x - v) - uv = 0. \end{cases} \quad (16)$$

Definition 3.3 (Normally Hyperbolic Manifold). [29] The manifold $\mathcal{C}_0$ is said to be normally hyperbolic if the linearization of the reduced fast system (15b) at each point of $\mathcal{C}_0$ has exactly r eigenvalues on the imaginary axis, with r referring to the dimension of the variable β .

Lemma 3.4. The critical manifold $\mathcal{C}_0$ given in (16) is normally hyperbolic.

The proofs of this lemma and other theoretical results are all put in Appendix B.

We then provide the celebrated Fenichel Slow Manifold Theorem as following lemma for the description of the slow manifold of $\mathcal{C}_0$:

Lemma 3.5 (Fenichel Slow Manifold Theorem [28]). Assume that D_0 is a compact normally hyperbolic manifold of the slow system σ_{sl} with boundary, for any sufficiently small $\epsilon_2 > 0$, there exists a manifold D_{ϵ_2} lying within the $\mathcal{O}(\epsilon_2)$ -neighborhood of D_0 which satisfies:

1. D_{ϵ_2} is differential homeomorphic to D_0 ;
2. D_{ϵ_2} is locally invariant under the flows of σ_{sl} , i.e., no flow of σ_{sl} can leave D_{ϵ_2} without also leaving its neighborhood;
3. D_{ϵ_2} is C^r for $0 < r < \infty$.

The manifold D_{ϵ_2} is called the slow manifold corresponding to the manifold D_0 .

Remark 3.6. According to Lemma 3.5, there exists a slow manifold in the $\mathcal{O}(\epsilon_2)$ -neighborhood of $\mathcal{C}_0$, denoted by $\mathcal{C}_{\epsilon_2}$, such that any flow of σ_{sl} will remain in motion on the manifold once it enters the neighborhood of $\mathcal{C}_{\epsilon_2}$. $\mathcal{C}_{\epsilon_2}$ can therefore be treated as a perturbation of $\mathcal{C}_0$.

We can depict the evolution of trajectory of slow-fast system (14) more concretely through the following theorem, which also implies that the oscillatory signals U and V can respond to the driving signal X quickly enough due to the introduction of the sufficiently small ϵ_2 .

Theorem 3.7. For a sufficiently small $\epsilon_2 > 0$, any trajectory of the slow system σ_{sl} originating from the region $\{(\alpha, \beta) : \alpha \in \mathbb{R}_{\geq 0}^2, \beta \in \mathbb{R}_{> 0}^2\}$ will merge into the slow manifold $\mathcal{C}_{\epsilon_2}$ along the fast flow within $0 < t < \epsilon_2 |\ln \epsilon_2|$, and moreover, will not leave the manifold.

Theorem 3.7 means that the long-term dynamical behavior of the slow system σ_{sl} is fully decided by $\mathcal{C}_{\epsilon_2}$, and the instantaneous behavior occurs within a time interval $\epsilon_2 |\ln \epsilon_2|$ which actually corresponds to the Δt in Definition 2.4. We will not dwell on the error introduced by this Δt and the cumulative consequences here because we are more interested in the long-term behavior of the system and its effect on the task of module regulation. Since $\mathcal{C}_{\epsilon_2}$ lies in the $\mathcal{O}(\epsilon_2)$ -neighborhood of $\mathcal{C}_0$, we pay attention on the structure of $\mathcal{C}_0$. The following theorem gives a more clear description of $\mathcal{C}_0$. Note that “S-shaped” critical manifold of Σ_{xy} allows the similar “ x_{middle} ” to strictly distinguish the different segment of x denoted as $x_{high}(t)$ and $x_{low}(t)$ with $\sup x_{low}(t) < x_{middle} < \inf x_{high}(t)$. We then use this concept to give the value of p .

Theorem 3.8. For the critical manifold $\mathcal{C}_0$ shown in (16) with any sufficiently small $\epsilon_1 > 0$ and a relaxation oscillation occurring in the subsystem Σ_{xy} in system (13), if the initial concentration of catalyst P is set as $p = (\sup x_{low} + \inf x_{high})/2$, then the concentrations of the oscillatory signals U and V can be described as functions of the concentration of X and the parameter ϵ_1 as

$$\begin{cases} u(x, \epsilon_1) = p - x + u_h(x, \epsilon_1), \\ v(x, \epsilon_1) = v_l(x, \epsilon_1), \end{cases} \quad (17)$$

when $x < x_{middle}$, and

$$\begin{cases} u(x, \epsilon_1) = u_l(x, \epsilon_1), \\ v(x, \epsilon_1) = x - p + v_h(x, \epsilon_1), \end{cases} \quad (18)$$

when $x > x_{middle}$. The expression of u and v satisfies that $|u_h(x, \epsilon_1)|, |u_l(x, \epsilon_1)|, |v_h(x, \epsilon_1)|, |v_l(x, \epsilon_1)| < \mathcal{O}(\epsilon_1)$.

Remark 3.9. Note that (17) and (18) are the description of the critical manifold $\mathcal{C}_0$ in (16), while the long-term behavior of (13) is based on the description of the slow manifold $\mathcal{C}_{\epsilon_2}$ which is a perturbation of $\mathcal{C}_0$ under the sufficient small ϵ_2 . We provide the asymptotic solution of (13c) and (13d) on $\mathcal{C}_{\epsilon_2}$ as follows based on the result of Theorem 3.8 and the Taylor expansion at $\epsilon_2 = 0$

$$\begin{cases} u(x, \epsilon_1, \epsilon_2) = p - x + u_h(x, \epsilon_1) + \epsilon_2 f(x, y) + \mathcal{O}(\epsilon_2^2), \\ v(x, \epsilon_1, \epsilon_2) = v_l(x, \epsilon_1) + \mathcal{O}(\epsilon_2), \end{cases} \quad (19)$$

when $x < x_{middle}$, and

$$\begin{cases} u(x, \epsilon_1, \epsilon_2) = u_l(x, \epsilon_1) + \mathcal{O}(\epsilon_2), \\ v(x, \epsilon_1, \epsilon_2) = x - p + v_h(x, \epsilon_1) + \epsilon_2 f(x, y) + \mathcal{O}(\epsilon_2^2), \end{cases} \quad (20)$$

when $x > x_{middle}$, and $f(x, y)$ is from (13a). The whole formulas above describe the dynamics of subsystem Σ_{uv} on the slow manifold.

Now, we can announce that the constructed chemical relaxation oscillator (13) is qualified to produce symmetrical clock signals as required.

Theorem 3.10. For a 4-dimensional system (13) describing the concentrations evolution of species set $S = \{X, Y, U, V\}$ with sufficiently small $\epsilon_1, \epsilon_2 > 0$, assume that the initial concentration of catalyst P is set as $p = (\sup x_{low} + \inf x_{high})/2$, and the initial concentration $(x(0), y(0), u(0), v(0)) \in \mathbb{R}_{> 0}^4$ is not the unique unstable equilibrium of the subsystem Σ_{xy} (governed only by (13a) and (13b)). Then the species U and V can act as a pair of symmetrical clock signals as requested by Definition 2.4.

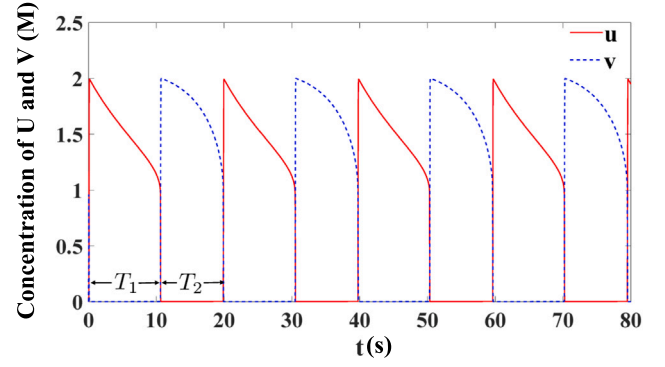


Fig. 4. The symmetrical clock signals U and V suggested in Example 3.11.

At the end of this section, we provide an instance of system (13) to examine the results of our clock signal design.

Example 3.11. We use the modified van der Pol model as a specific 2-dimensional relaxation oscillator to produce the driving signal X . Through replacing (13a) and (13b) by (10) in (13), we obtain the 4-dimensional biochemical oscillator model to be

$$\epsilon_1 \frac{dx}{dt} = \eta_1(-x^3 + 9x^2 - 24x + 21 - y)x, \quad (21a)$$

$$\frac{dy}{dt} = \eta_1(x - 3)y, \quad (21b)$$

$$\epsilon_1 \epsilon_2 \frac{du}{dt} = \eta_1(\epsilon_1(p - u) - uv), \quad (21c)$$

$$\epsilon_1 \epsilon_2 \frac{dv}{dt} = \eta_1(\epsilon_1(x - v) - uv). \quad (21d)$$

We name this 4-dimensional model (21) as the standard biochemical relaxation oscillator in the context. With $\epsilon_1 = \epsilon_2 = 0.001, \eta_1 = 0.1, p = 3$ and initial point $(x(0), y(0), u(0), v(0)) = (5, 5, 0, 0)$, we show the simulation result of the oscillatory components u and v in Fig. 4. Obviously, the corresponding species U and V satisfy the requirements in Definition 2.4. We will further analyze the dynamical properties and discuss the CRN implementation of the standard biochemical relaxation oscillator in subsequent chapters.

4. Period and occurrence order control of symmetrical clock signals

In this section, based on the standard symmetrical clock signals, we discuss how to control the period and occurrence order of the oscillatory species via adjusting oscillator parameters and initial values, respectively.

In the standard biochemical relaxation oscillator of (21), species X is the driving signal for symmetrical clock signals U and V , so we are only concerned with the effect of parameters encountered in (21a) and (21b) on them. However, to ensure a S-shaped critical manifold constructed and the unique equilibrium assigned on the repelling part as Fig. 2 shows, we keep the current structure of the modified van der Pol model and redefine the subsystem Σ_{xy} of (21a) and (21b) as

$$\epsilon_1 \frac{dx}{dt} = \eta_1(-x^3 + 9x^2 - 24x + 21 - y)x, \quad (22a)$$

$$\frac{dy}{dt} = \eta_1(x - \ell)y, \quad (22b)$$

where $\ell \in \mathbb{R}_{> 0}$ replaces the original “3”, and induces an indefinite equilibrium $x^* = \ell$ for species X . Based on the work of Krupa and Szmolyan (2001), it can be inferred that there exists a relaxation oscillation orbit along with the instability of the equilibrium in the first quadrant when $2 < \ell < 4$. Note that ℓ is still in need of avoiding being too close to 2 or 4 in order to exclude canard explosion, and ℓ just

affects the position of equilibrium of system (22) along the repelling part of the critical manifold without affecting its instability, so the related singular trajectory is exactly the same with Γ_0 given in (9) under this interval.

Based on (22), we provide the following theorem to calculate the value of T_1 and T_2 .

Theorem 4.1. For system (22), a whole period $T = T_1 + T_2$ with the following description:

$$T_1 = \int_1^2 \frac{(\varphi'(x) - \frac{d\psi_1}{dx}(x, \epsilon_1))dx}{\eta_1(x - \lambda)(\varphi(x) - \psi_1(x, \epsilon_1))}, \quad (23)$$

$$T_2 = \int_5^4 \frac{(\varphi'(x) + \frac{d\psi_2}{dx}(x, \epsilon_1))dx}{\eta_1(x - \lambda)(f(x) + \psi_2(x, \epsilon_1))}, \quad (24)$$

where $\varphi(x) = -x^3 + 9x^2 - 24x + 21$, ψ_1 and ψ_2 are differentiable function defined separately on $(1, 2) \times (0, \epsilon_0)$ and $(4, 5) \times (0, \epsilon_0)$, and $\exists \xi(\epsilon_1) = O(\epsilon_1^{2/3})$, such that

$$\forall x \in (1, 2), |\psi_1(x, \epsilon_1)| < \xi(\epsilon_1), \quad (25)$$

$$\forall x \in (4, 5), |\psi_2(x, \epsilon_1)| < \xi(\epsilon_1), \quad (26)$$

ϵ_0 is sufficiently small.

Formula of T_1 and T_2 as in Eqs. (23) and (24) are not convenient to estimate the period of x in practice due to the lack of an explicit expression of $\psi_i(x, \epsilon_1)$, $i = 1, 2$. Note that $|\psi_i(x, \epsilon_1)| < \xi(\epsilon_1) = O(\epsilon_1^{2/3})$, $i = 1, 2$, for sufficiently small ϵ_1 , we give more simplified estimate of T_1 and T_2 as

$$T_1 \approx \int_1^2 \frac{\varphi'(x)dx}{\eta_1(x - \ell)\varphi(x)}, T_2 \approx \int_5^4 \frac{\varphi'(x)dx}{\eta_1(x - \ell)\varphi(x)}. \quad (27)$$

We omit the difference between the actual oscillation orbit Γ_{ϵ_1} and the critical manifold Γ_0 because the error can be expressed as $\mathcal{O}(\epsilon_1)$, and just use (27) as a period estimation of u and v under a sufficiently small ϵ_2 . By substitute $\ell = 3$ and $\eta_1 = 0.1$ into (27), we obtain period estimation of U and V in Example 3.11 as $T_1 \approx 10.470$, $T_2 \approx 9.193$, which is consistent with the results in Fig. 4.

The estimation formulas (27) reveal that both ℓ and η_1 can adjust T_1 and T_2 , while regulation through the latter one seems more direct because both T_1 and T_2 are approximately inversely proportional to η_1 . T_1 and T_2 actually correspond to the maximum time provided for the controlled reaction modules, such as $\tilde{\mathcal{M}}_1$ and $\tilde{\mathcal{M}}_2$ in Example 2.3. Logically, both T_1 and T_2 need to be larger than the reaction time for the corresponding reaction module. The request is easily achievable, since reaction time is usually quite short. Of course, the time required for the reactions to reach the equilibrium is often different under different actual regulation tasks. To perform appropriate computation, η_1 can help to increase or shorten the execution time of reaction modules quantitatively. Similarly, subject to the number and rate of reactions in practical applications, there is often a difference in execution time between the two modules to be regulated, the parameter ℓ can coordinate the execution time allocated to the two modules within a certain period of time through affecting the ratio of T_1 and T_2 . We show the relationship between ℓ and T_1/T_2 in Fig. 5. The ratio of T_1 over T_2 decreases as ℓ increases based on the simulation result, and actually $0.256 < T_1/T_2 < 6.987$ within $2 < \ell < 4$. We also estimate the value of ℓ as 3.127 such that $T_1 \approx T_2$, which allocates the execution time between the two modules equally and may contribute to multi-module regulations in our future work.

We also consider the effect of the initial values of species appearing in subsystem Σ_{xy} of the standard biochemical relaxation oscillator (21), i.e., the effect of $(x(0), y(0))$ on oscillatory behaviors of u and v . We look at the middle part of the critical manifold $\mathcal{C}_0$, which takes $\{(x, \varphi(x)) : 2 < x < 4\}$ with $\varphi(x) = -x^3 + 9x^2 - 24x + 21$, and is identified by $\varphi_{re}(x)$ for distinguishing from $\varphi(x)$. Through rewriting it as $\{(\varphi_{re}^{-1}(y), y) : 1 < y < 5\}$, where $\varphi_{re}^{-1}(y)$ means the inverse function

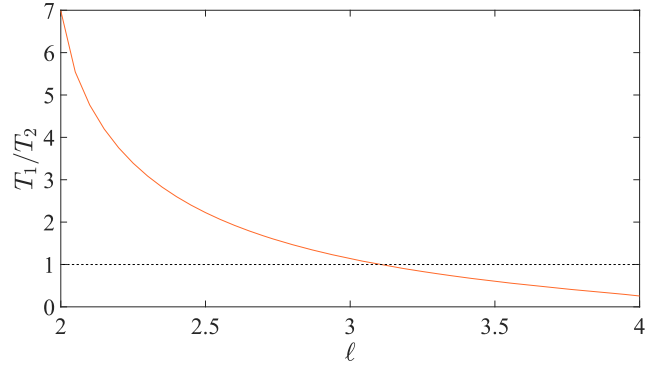


Fig. 5. Diagram of T_1/T_2 related to ℓ within $2 < \ell < 4$.

of $\varphi(x)$, we can divide the first quadrant of the phase plane of Σ_{xy} into two regions

$$\begin{aligned} \mathcal{A}_1 : & \{(x, y) : y \geq 5\} \cup \{(x, y) : x < \varphi_{re}^{-1}(y), 1 < y < 5\} \\ & \cup \{(\varphi_{re}^{-1}(y), y) : 3 < y < 5\}, \\ \mathcal{A}_2 : & \{(x, y) : y \leq 1\} \cup \{(x, y) : x > \varphi_{re}^{-1}(y), 1 < y < 5\} \\ & \cup \{(\varphi_{re}^{-1}(y), y) : 1 < y < 3\}, \end{aligned}$$

where only the unstable equilibrium $(x^*, y^*) = (3, 3)$ is excluded. As illustrated in Fig. 6, the region $\mathcal{A}_1$ and $\mathcal{A}_2$ actually distinguish the case where U or V turns on the module to be regulated first, determining the computation order of reaction modules $\tilde{\mathcal{M}}_1$ and $\tilde{\mathcal{M}}_2$.

Arredondo and Lakin [13] required the initial concentrations of some species to differ by an order of 10^6 from others in their model, specifically, some species at 10^{-6} while others are at 1. Although such a large concentration difference may not inherently cause issues in all biochemical contexts, maintaining precise control over concentrations as low as 10^{-6} in a biological setting is experimentally challenging. However, for our oscillator, a key advantage lies in its clear mechanism between the choice of initial conditions and the emergence of oscillatory behavior. Moreover, our model also supports sustained oscillations across a wide range of initial values. All of these suggest that the current oscillator looks more encouraging and competitive.

Naturally, except for the oscillation period and emergence sequence, the proposed relaxation oscillator can modulate other parameters, such as the amplitudes of the periodical signals. In the current requirement for each oscillating signal, their low amplitudes should be zero while their high amplitudes can be any nonzero values. Theoretically, they can be modulated to be any one precisely, but we need not discuss this point due to the current design requirement. In our another paper [30], we discuss the change of $T = T_1 + T_2$ will affect the amplitudes of oscillating signals, and also use the following system

$$\begin{aligned} \epsilon_1 \frac{dx}{dt} &= \eta_1(-x^3 + ax^2 - bx + c - y)x, \\ \frac{dy}{dt} &= \eta_1(x - \ell)y. \end{aligned}$$

to modulate the oscillator, where a, b, c are parameters. More details may be found there.

5. Loop control, feasibility and termination of molecular computations

In this section we apply the standard biochemical relaxation oscillator to the example of the iteration calculation, along with the discussion of feasibility, and finally provide a strategy for automatic loop terminations.

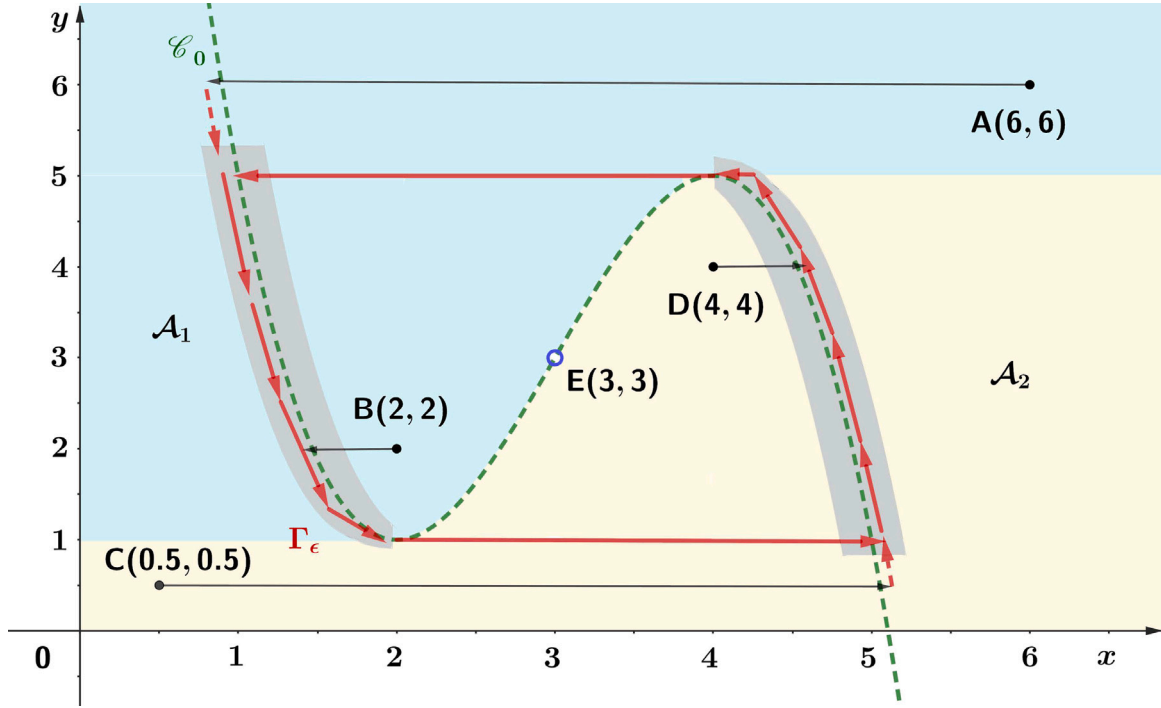


Fig. 6. Trajectory evolution diagram of subsystem Σ_{xy} of (21) starting from four different initial points $A(6,6)$, $B(2,2) \in \mathcal{A}_1$ and $C(0.5,0.5)$, $D(4,4) \in \mathcal{A}_2$, where the green, red and black lines express the same information as given in Fig. 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

5.1. Loop control of molecular computations

We recall the motivating example of Section 2 in which two reaction modules $\mathcal{M}_1$ and $\mathcal{M}_2$ are designed with the target of making the loop iteration calculation $s_1 = s_1 + 1$. Here their calculation control is just taken as an application scenario for the standard biochemical relaxation oscillator. As stated in Section 2, these two modules need to be modified as the form of $\tilde{\mathcal{M}}_1$ and $\tilde{\mathcal{M}}_2$, in order to avoid coupling between s_1 and s_2 . Then we can apply the standard biochemical relaxation oscillator to control their calculations through combining all related dynamical equations, i.e., combining (21) with (5), which gives

$$\begin{aligned} \epsilon_1 \frac{dx}{dt} &= \eta_1(-x^3 + 9x^2 - 24x + 21 - y)x, \\ \frac{dy}{dt} &= \eta_1(x - 3)y, \\ \epsilon_1 \epsilon_2 \frac{du}{dt} &= \eta_1(\epsilon_1(p - u) - uv), \\ \epsilon_1 \epsilon_2 \frac{dv}{dt} &= \eta_1(\epsilon_1(x - v) - uv), \\ \frac{ds_1}{dt} &= (s_2 - s_1)v, \\ \frac{ds_2}{dt} &= (s_1 + s_3 - s_2)u, \\ \frac{ds_3}{dt} &= 0. \end{aligned} \quad (28)$$

The structure consists of the following three steps: (1) the first 2 equations produce the periodical oscillator x ; (2) x drives the next two equations to generate the concentration variables of the symmetrical clock signal U and V ; (3) U and V participate in the $\tilde{\mathcal{M}}_1$ and $\tilde{\mathcal{M}}_2$ to realize the iteration calculation $s_1^* = s_1^* + s_3^*$.

As an illustration, Fig. 7 presents the time evolution of s_1 and s_2 in the system (28), starting from $(x(0), y(0), u(0), v(0), s_1(0), s_2(0), s_3(0)) = (5, 5, 0, 0, 0, 0, 1)$ with the original choice of model parameters $\epsilon_1 = \epsilon_2 = 0.001, \eta_1 = 0.1, p = 3$. Note that the curve of s_1 is a staircase-like function, and s_1 periodically increases by 1. This indicates that the standard biochemical relaxation oscillator of (21) is quite valid to

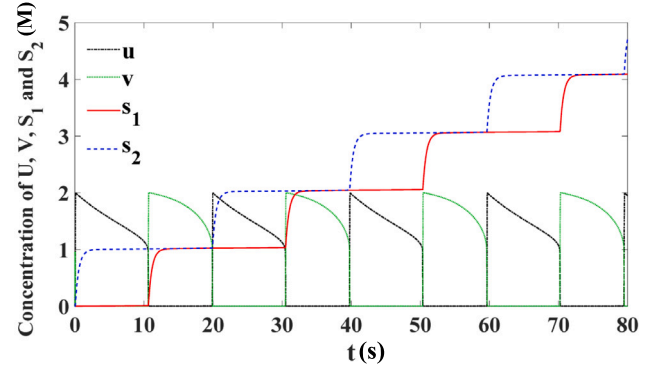


Fig. 7. Time evolution of species S_1 and S_2 of the system (28) in response to symmetrical clock signals U and V .

periodically control the execution of computation modules $\tilde{\mathcal{M}}_1$ and $\tilde{\mathcal{M}}_2$. A further look at Fig. 7 reveals that the calculation time for these two modules, i.e., time allocated for the curve of s_1 or s_2 to “climb” a stair, is very short compared to the oscillation period of u and v . The main reason is that both $\tilde{\mathcal{M}}_1$ and $\tilde{\mathcal{M}}_2$ perform the calculation instruction at exponential speeds, whose time consumption (estimated by 2.014) is much lower than $T_1 + T_2 \approx 19.663$. There is still a large room to reduce the oscillation period of the driving signal X in the standard biochemical relaxation oscillator for the current purpose.

5.2. Feasibility of molecular computations

Although there are reaction networks corresponding to the dynamical Eqs. (21c), (21d) and (5), i.e., network (12) with $\kappa = \eta_1/\epsilon_2$ and $\tilde{\mathcal{M}}_1$ plus $\tilde{\mathcal{M}}_2$ given in Example 2.3, respectively, the ODEs of (28) are not enough to be representative of calculation made by chemical molecules since there is no reaction network corresponding to the equations of

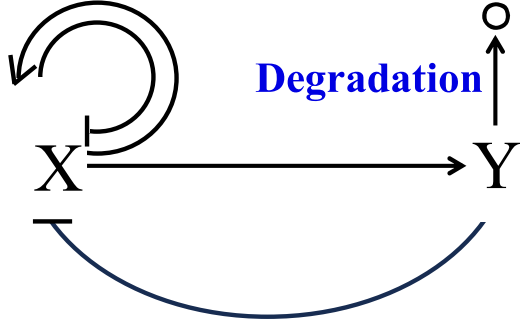
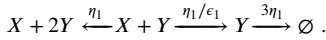
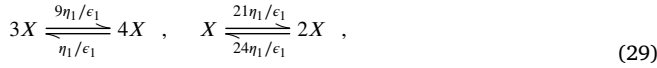


Fig. 8. Diagram of motif structure corresponding to the CRN of (29), with the interactions expressed as activation ($\rightarrow$) and inhibition ($\dashv$).

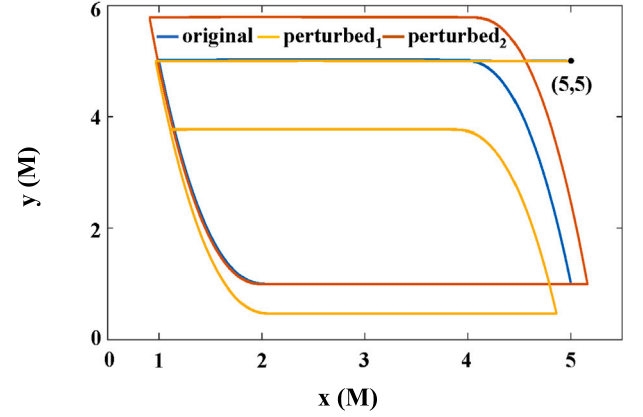
(21a) and (21b). It is necessary to find a mass-action system that has the dynamics of (21a) and (21b), which refers to CRN realization of the kinetic equations. Note that there should be many CRN realizations for the same kinetic equations, and a great deal of algorithms have been developed to reach this objective. Since how to realize a CRN from a group of kinetic equations is not the main topic of this work, we only provide a naive realization by directly transforming each monomial in (21a) and (21b) into an abstract reaction where the species coefficients on the left and right sides are completely determined by the order of the monomial, and both the parameter η_1 and ϵ_1 appear as the rate constants. The result is as follows



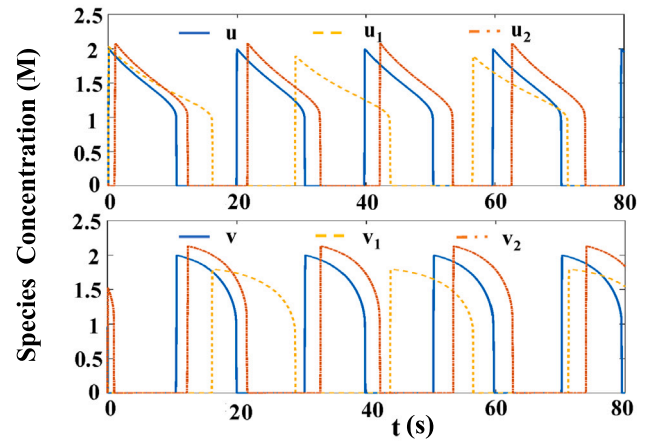
Physical explanation. Note that the above reaction network consists of two reversible reactions and a chain reaction, where the chain reaction is widely mentioned in the book of Feinberg (2019) (e.g. network (4.14) and (6.1)), and the collisions of microscopic particles dominate the reaction direction with different rates. X and Y in this CRN (29) can be viewed to form a simple negative feedback, as species X activates the generation of Y while species Y inhibits X , which is a classical motif for biochemical oscillations [31] shown in Fig. 8.

Heterogeneity of rate parameters. Another point that should be pointed out is that the rate constants are assigned so accurately using very small ϵ_1 , which does not seem biochemically realistic. The heterogeneity of rate parameters should be discussed. Indeed, parameter η_1 and ϵ_1 along with the coefficients in the S-shaped function are not necessary to fit perfectly the assigned values. Their real values only ensure the system to generate oscillation and have a unique equilibrium which lies on the repelling part of the critical manifold and stays away from the two non-hyperbolic points. We perturb the reaction rates in network (12) and (29) with noise controlled by 1%, and simulate the oscillation orbit in the phase plane of x and y , along with perturbed values of u and v in Fig. 9. The perturbation causes a shift in the oscillation orbit and affects both the period of u and v and their behavior during the first period with unchanged initial point (5, 5, 0, 0), while not destroying the oscillation structure and the generation of the clock signals. This means that our oscillator exhibits a certain degree of robustness in rate constant selection. This inherent flexibility avoid the stringent initial condition requirements required in the study [13], as well as extremely precise parameter values to sustain oscillatory behavior.

Note that the above exhibited robustness does not mean our intention to achieve oscillation through tuning rate constants in our method. Actually, the tune on rate constants would require molecular engineering, e.g., modulating the toehold length in a strand displacement



(a)



(b)

Fig. 9. Perturbation of the reaction rates in network (12) and (29) with 1% noise. (a) Phase portrait of x and y with the original oscillation orbit in the blue line and two perturbed cases colored in red and orange; (b) the corresponding values of u and v under perturbations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

implementation. Zhang & Winfree [32] showed a maximum 6-order of magnitude speedup possible via lengthening reaction toeholds, which places a hard limit on the rates that could be accessed via this strategy.

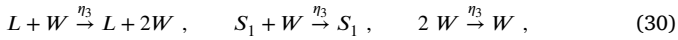
Biomolecules realization. We now discuss the feasibility of inserting the abstract network of (29) into a real biochemical environment. Similar to the strategy of Salehi et al. [17], we take the DNA strand displacement reactions as a practical medium for real realization. Actually, Soloveichik et al. claimed that DNA strand displacement reactions can emulate the kinetics of any CRN and provided a software tool mapping arbitrary CRN to DNA reactions [15]. Besides, the realization of DNA reactions fully retains the reaction rates of the original abstract reactions, that is, the restrictions on the rate constants of original CRN can be well satisfied through the DNA strand displacement. According to the method shown in the work of Soloveichik et al. [15], the abstract chemical reaction with m reactants and nonzero products can be emulated by $m + 1$ DNA strand displacement reactions. This means that unimolecular and bimolecular reactions can be mapped to two and three DNA strand displacement reactions, respectively. The abstract CRNs in (29) can be all translated into specific DNA reactions by similar methods, so the implementation in the biochemical environment is expected.

Notably, although there is a guarantee (at least in theory) of using the DNA strand displacement technique to transform the abstract reactions involved in the oscillation model into real reactions, this does not mean the current system is going to work in the real world. The main reason is that biological implementation will face multiple challenges, such as noise/uncertainties, unintended interactions, resource competition, etc., in the biological environment. More biochemical experiments (assisted by biologists) should be made for the purpose of practical applications in the future. In other words, the current work is not intended as an immediate blueprint for experimental realization but rather as a necessary theoretical exploration step.

5.3. Loop termination of molecular computations

We have completed the task of realizing iteration computation $s_1 = s_1 + 1$ through the standard biochemical relaxation oscillator of (21). This operation will never stop. However, there is usually a restriction of upper bound on s_1^* , e.g., when it is used to model the “iteration times” in machine learning algorithm, an instruction of iteration termination should be necessary. For this reason, we introduce a new counter species W to utilize the concentration w to measure the difference between a given loop times l and the concentration of S_1 , finally controlling the occurrence and termination of the computation modules.

We construct the following specific truncated subtraction module to generate the species W



whose ODEs are expressed as

$$\frac{dw}{dt} = \eta_3(l - s_1 - w)w, \quad (31a)$$

$$\frac{ds_1}{dt} = \frac{dl}{dt} = 0. \quad (31b)$$

Remark 5.1. The analytical solution of the ODEs (31) is

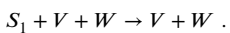
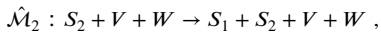
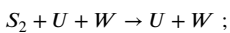
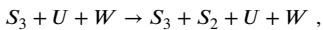
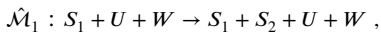
$$w = \frac{(l - s_1)w(0)}{w(0) + (l - s_1 - w(0))e^{-\eta_3(l - s_1)t}} \quad (32)$$

with $l \neq s_1$. In the case of $l > s_1$, w converges exponentially to $l - s_1$; in the case of $l = s_1$, w degenerates to the linear form of

$$w = \frac{w(0)}{1 + \eta_3 w(0)t}; \quad (33)$$

and in the case of $l < s_1$, the equilibrium of w is 0. The reaction rate constant η_3 plays the role of regulating the convergence speed of w .

To turn off the computation modules $\hat{\mathcal{M}}_1$ and $\hat{\mathcal{M}}_2$ after looping the given times l , the species W should behave as a catalyst and its concentration vanishes as the reactions (30) proceed. We show the two new computation modules $\hat{\mathcal{M}}_1$ and $\hat{\mathcal{M}}_2$ as



This time the dynamics become

$$\frac{ds_1}{dt} = (s_2 - s_1)vw, \quad \frac{ds_2}{dt} = (s_1 + s_3 - s_2)uw, \quad \frac{ds_3}{dt} = 0. \quad (34)$$

We couple all ODEs of (21), (30) and (34), and get the whole ODEs to be

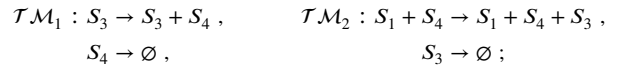
$$\begin{aligned} \epsilon_1 \frac{dx}{dt} &= \eta_1(-x^3 + 9x^2 - 24x + 21 - y)x, \\ \frac{dy}{dt} &= \eta_1(x - 3)y, \\ \epsilon_1 \epsilon_2 \frac{du}{dt} &= \eta_1(\epsilon_1(p - u) - uv), \\ \epsilon_1 \epsilon_2 \frac{dv}{dt} &= \eta_1(\epsilon_1(x - v) - uv), \\ \frac{ds_1}{dt} &= (s_2 - s_1)vw, \\ \frac{ds_2}{dt} &= (s_1 + s_3 - s_2)uw, \\ \frac{dw}{dt} &= \eta_3(l - s_1 - w)w, \\ \frac{ds_3}{dt} &= \frac{dl}{dt} = 0, \end{aligned} \quad (35)$$

which has the function of automatically performing loop iteration calculation and timely terminating it when the loop goes beyond a desired times. Fig. 10 shows the loop termination results by species W , where Fig. 10(a) (resp., Fig. 10(b)) simulates the case of $\eta_3 = 1$ (resp., $\eta_3 = 50$), $w(0) = l(0) = 4$, with the same choice of other parameters and initial values as given for Fig. 7. When $\eta_3 = 1$, neither s_1 nor s_2 stabilizes at 4, and both go a little bit beyond. This means there is a lag when terminating $\hat{\mathcal{M}}_1$ and $\hat{\mathcal{M}}_2$. The reason is that when s_1 increases to be close to $l = 4$, w converges towards 0 at a nearly linear speed as (33) shows, leading to a delay of shutting down the two modules with the counter species W . However, when a larger $\eta_3 = 50$ is selected to accelerate the convergence of w , shown in Fig. 10(b), the lag phenomenon is weakened and s_1 stabilizes exactly at 4. In practice a relatively large η_3 is thus desired to be selected, corresponding to relatively fast reaction rates of the truncated subtraction module (30).

Remark 5.2. Our biochemical relaxation oscillator design and loop termination strategy achieve the iteration computation $s_1 = s_1 + 1$ well through biochemical molecular reactions in the form of (29) plus (30) plus $\hat{\mathcal{M}}_1$ and $\hat{\mathcal{M}}_2$. The whole modular system can work as a counter CRN to control the alternation calculations of any other two modules, labeled by $\mathcal{T}\mathcal{M}_1$ and $\mathcal{T}\mathcal{M}_2$. As long as the symmetrical clock signals U and V generated by the standard biochemical relaxation oscillator are fed into both $\mathcal{M}_1$, $\mathcal{M}_2$ and $\mathcal{T}\mathcal{M}_1$, $\mathcal{T}\mathcal{M}_2$ in parallel as catalysts, and the counter species W is used to monitor the alternation times of their computations, the target calculation can be implemented. We exhibit the corresponding flowchart in Fig. 11. This application is quite promising, and may push the development of achieving all kinds of molecular computations.

We finally provide an realization example of factorial calculation based on the counter CRN, with target modules $\mathcal{T}\mathcal{M}_1$ and $\mathcal{T}\mathcal{M}_2$ as follows, which may contribute to the approximation of the Euler's number e [2].

Example 5.3. Given these two modules to be regulated



alternation and loop of these two modules could complete the factorial calculation like $s_3 = n!$ as long as the concentration of counter species S_1 terminates at n . Take the strategy described in Fig. 11 and couple the following dynamics with (35):

$$\frac{ds_3}{dt} = (s_1 * s_4 - s_3)vw, \quad \frac{ds_4}{dt} = (s_3 - s_4)uw, \quad (36)$$

simulation result of s_3 is shown in Fig. 12, along with the concentration of S_1 and W . Our module regulation on $\mathcal{T}\mathcal{M}_1$ and $\mathcal{T}\mathcal{M}_2$ approximately realizes the calculation $s_3 = 4!$ after 4 automatic loops. Note that a lag

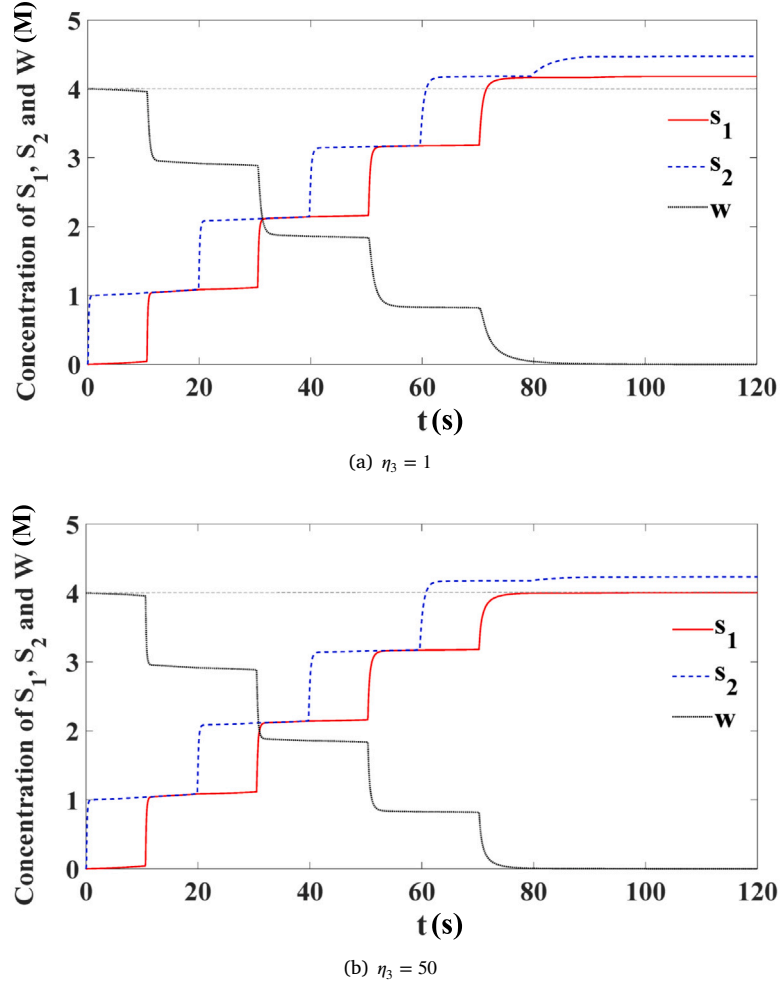


Fig. 10. Loop termination of iteration computation $s_1 = s_1 + 1$ by counter species W in system (35).

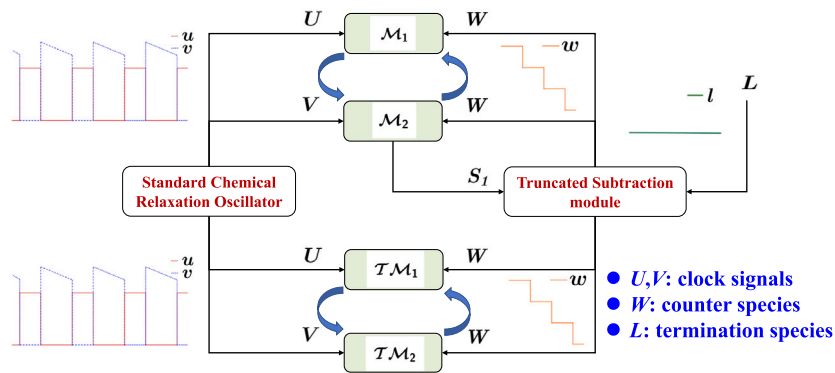


Fig. 11. A schematic diagram of applying chemical oscillation-based iteration computation modules $\mathcal{M}_1$ plus $\mathcal{M}_2$ to control the alternative calculation and termination of two target modules $\mathcal{T}\mathcal{M}_1$ and $\mathcal{T}\mathcal{M}_2$.

exists in the segment when s_3 climbs to 24, the reason is that we set $\mathcal{M}_2$ and $\mathcal{T}\mathcal{M}_2$ in the same period to execute, corresponding to $v > 0$, so the concentration changes of S_1 and S_3 are in parallel, we actually get the result $s_3 = 4!$ after s_1 reaches 4. Although lags like this are common in our dual-module regulation, they do not hamper our regulation goals.

6. Conclusions

Our 4-dimensional oscillator model solves the task of dual-module regulation well, but is a little weak when faced with regulation tasks of three or more modules. Also, our strategy fails to achieve accurate

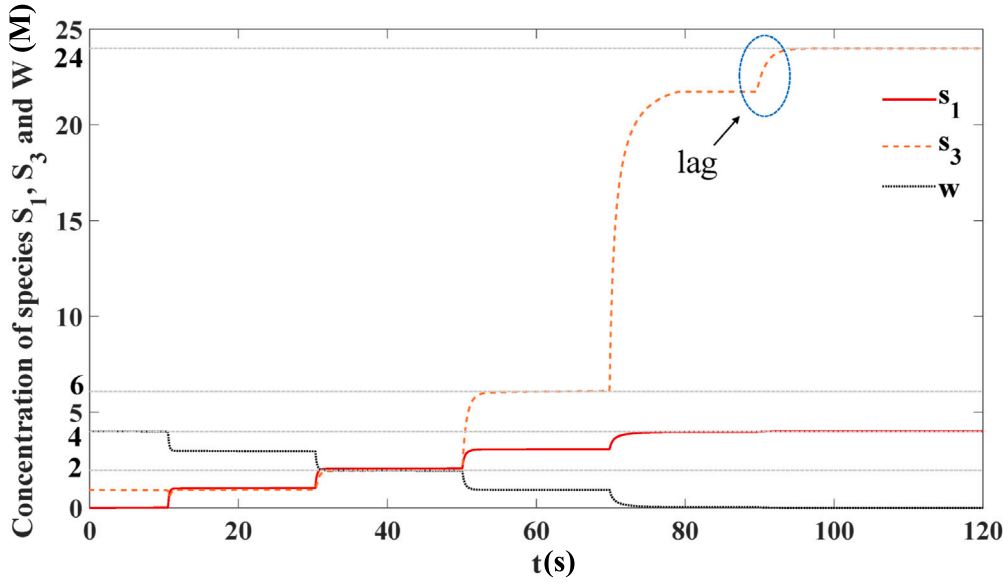


Fig. 12. Realization of factorial calculation $s_3 = n!$ with $n = l = 4$, $\eta_3 = 50$ and other parameters remaining the same.

loop termination because the update of the counter species W is always coupled with the counter CRN, which is inevitable in the regulation case of two modules, but may be handled under multi-module regulation. It will be the focus of our future work to find suitable oscillation structure and design corresponding biochemical oscillator model for multi-module regulation, and a more accurate, more flexible strategy in practical use for loop termination may be developed based on this.

CRedit authorship contribution statement

Xiaopeng Shi: Writing – original draft, Investigation, Formal analysis. **Chuanhou Gao:** Writing – review & editing, Supervision. **Denis Dochain:** Writing – review & editing. **Yuzhen Fan:** Writing – review & editing, Visualization, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Chuanhou Gao reports financial support was provided by National Nature Science Foundation of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. An example of CRN with mass-action kinetics for addition calculation

In this appendix, we give an example of CRN to exhibit the mass-action kinetics, and also use it to implement the addition calculation.

It has been proven that the mass-action chemical kinetics is Turing universal [20]. This means that any functional computation can be firstly embedded into solutions of a group of polynomial ODEs [33], and then realized into mass-action chemical systems whose dynamics correspond to the ODEs. In practice, this process is implemented by mapping the input of calculation into the initial concentrations of some species of the network and the output into the limiting concentrations of other species, usually based on the equilibrium. Then the evolution of species concentration over time can be used to characterize the specific functional computation. We present an example of “addition calculation” to give readers more clear illustration which is inspired by Vasic et al. [2].

Example A.1. A CRN follows



where the last reaction refers to an outflow reaction. The stoichiometric matrix is $\Xi = \begin{pmatrix} 0 & 0 & 0 \\ 1 & 1 & -1 \\ 0 & 0 & 0 \end{pmatrix}$ and the reaction rate function is $r(s) = (s_1, s_3, s_2)^T$. We write the ODEs of the dynamics as

$$\frac{ds_2}{dt} = s_1 + s_3 - s_2, \quad \frac{ds_1}{dt} = \frac{ds_3}{dt} = 0 \quad (\text{A.2})$$

and claim that this network can serve for implementing addition calculation, like $a + b = c$, $a, b, c \in \mathbb{R}_{\geq 0}$. Clearly, when all reactions reach equilibrium, the equilibrium point vector s^* satisfies $s_1^* = s_1(0)$, $s_2^* = s_1^* + s_3^*$ and $s_3^* = s_3(0)$. Therefore, by letting $s_1(0) = a$, $s_3(0) = b$, and $s_2^* = c$, we realize the addition calculation by this network.

Appendix B. Proofs of theoretical results

Proof of Lemma 3.4. The eigenvalues of the linearization at any point of $\mathcal{C}_0$, other than the double eigenvalues at 0, correspond to the eigenvalues of $\frac{\partial F}{\partial a}(\alpha, \beta)$, that is $\lambda_1 = -\eta_1$ and $\lambda_2 = -\eta_1 - \eta_1(u+v)/\epsilon_1$. Both λ_1 and λ_2 are negative, so the linearization of the reduced fast system (15b) at each point of $\mathcal{C}_0$ has exactly 2 eigenvalues on the imaginary axis, which completes the proof. ■

Proof of Theorem 3.7. The fast system σ_{fa} describes the instantaneous behavior corresponding to the slow system σ_{sl} with $\tau = t/\epsilon_2$, and the time can be caught as $t < \epsilon_2 |\ln \epsilon_2|$ with the sufficiently small ϵ_2 [28]. As we have shown in Lemma 3.4, the eigenvalues of the linearization of the critical manifold $\mathcal{C}_0$ are negative, so the fast flows converge towards $\mathcal{C}_0$ in σ_{fa} which constitutes a rough characterization of the instantaneous behavior of the slow system σ_{sl} , and similarly, the slow flows constrained on the critical manifold $\mathcal{C}_0$ describe the long-term behavior. Note that $\mathcal{C}_0$ is perturbed to the slow manifold $\mathcal{C}_{\epsilon_2}$ by ϵ_2 , the trajectory originating from the region will converge actually towards $\mathcal{C}_{\epsilon_2}$ along the fast flows within $t < \epsilon_2 |\ln \epsilon_2|$, and the trajectory will be constrained on the $\mathcal{C}_{\epsilon_2}$ forever once entering its neighborhood. ■

Proof of Theorem 3.8. From (16), it is easily to get

$$v^2 + (p - x + \epsilon_1)v - \epsilon_1 x = 0, \quad (\text{B.1a})$$

$$u^2 - (p - x - \epsilon_1)u - \epsilon_1 p = 0. \quad (\text{B.1b})$$

For the relaxation oscillator x , we can also set a positive constant δ as $\delta = (\inf x_{\text{high}} - \sup x_{\text{low}})/4$, then we have $p - x > \delta$ when $x < x_{\text{middle}}$ and further $p - x \pm \epsilon_1 > 0$ so long as $\epsilon_1 < \delta$. Under the constraint of $u, v \geq 0$, the above equations may be solved as

$$u(x, \epsilon_1) = \frac{(p - x - \epsilon_1) + \sqrt{(p - x - \epsilon_1)^2 + 4\epsilon_1 p}}{2}, \quad (\text{B.2a})$$

$$v(x, \epsilon_1) = \frac{-(p - x + \epsilon_1) + \sqrt{(p - x + \epsilon_1)^2 + 4\epsilon_1 p}}{2}. \quad (\text{B.2b})$$

The solutions can be further analyzed as

$$\begin{aligned} |u_h(x, \epsilon_1)| &= |u(x, \epsilon_1) - (p - x)| \\ &= \left(\frac{2p}{\sqrt{(p - x - \epsilon_1)^2 + 4\epsilon_1 p} + (p - x - \epsilon_1)} - 1 \right) \epsilon_1 \\ &< \left(\frac{p}{p - x - \epsilon_1} - 1 \right) \epsilon_1 < \left(\frac{\sup x_{\text{low}}}{\delta} + 1 \right) \epsilon_1 = \mathcal{O}(\epsilon_1), \end{aligned}$$

and

$$\begin{aligned} |v_l(x, \epsilon_1)| &= |v(x, \epsilon_1) - 0| \\ &= \frac{2x}{\sqrt{(p - x + \epsilon_1)^2 + 4\epsilon_1 p} + (p - x + \epsilon_1)} \epsilon_1 \\ &< \frac{x}{p - x + \epsilon_1} \epsilon_1 < \left(\frac{\sup x_{\text{low}}}{2\delta} \right) \epsilon_1 = \mathcal{O}(\epsilon_1), \end{aligned}$$

which leads to (17). For the case of $x > x_{\text{middle}}$, the analysis can be performed based on the same logic, which completes the proof. ■

Proof of Theorem 3.10. From Theorem 3.7, for the system considered any trajectory originating from the region $\{(x, y, u, v) : x, y > 0, u, v \geq 0\}$ will merge into the $\mathcal{O}(\epsilon_2)$ -neighborhood of critical manifold $\mathcal{C}_0$ within $t < \epsilon_2 |\ln \epsilon_2|$. This actually corresponds to the transitions between adjacent phases of u and v as they are oscillating synchronously with the driving oscillator x , as long as we choose $\Delta t = \epsilon_2 |\ln \epsilon_2|$. Remark 3.9 provides the description of $\mathcal{C}_2$, so the low value of u and v can stay close to 0 if we treat $\mathcal{O}(\epsilon_1) + \mathcal{O}(\epsilon_2)$ as the sufficient small ϵ_0 . One can set $u_{\text{middle}} = v_{\text{middle}} = (\inf x_{\text{high}} - \sup x_{\text{low}})/2$, then the oscillation diagram of u and v in each period can be divided into the corresponding segment $u > 0$ (resp. $v > 0$) and $u = 0$ (resp. $v = 0$). Therefore, the two items in Definition 2.4 can be satisfied as long as $(x(0), y(0))$ is not the unstable equilibrium point, and the relaxation oscillatory signal X will drive U and V to oscillate synchronously, acting as the desired symmetrical clock signals. ■

Proof of Theorem 4.1. We first confirm the formula for T_1 . Consider $\Gamma_1 = \{(x, \varphi(x)) : 1 < x < 2\}$ and label the part of relaxation oscillation orbit that lies in the $\mathcal{O}(\epsilon)$ -neighborhood of the left part of the critical manifold as Γ_{1,ϵ_1} , then Γ_{1,ϵ_1} can be viewed as perturbation of Γ_1 according to Fenichel Slow Manifold Theory. We describe Γ_{1,ϵ_1} as

$$\Gamma_{1,\epsilon_1} : y = \chi_l(x, \epsilon_1), 1 < x < 2, \quad (\text{B.3})$$

and for sufficiently small $\epsilon_0, \forall \epsilon_1 \in (0, \epsilon_0)$,

$$\exists \psi_1 : (x, \epsilon_1) \rightarrow \varphi(x) - \chi_l(x, \epsilon_1). \quad (\text{B.4})$$

It is obvious that $\psi_1 > 0$ and is differentiable w.r.t x , and $|\psi_1(x, \epsilon_1)| < \xi(\epsilon_1) = \mathcal{O}(\epsilon_1^{2/3}), \forall x \in (1, 2)$. Then we can substitute $y = \varphi(x) - \psi_1$ into $\frac{dy}{dt}$ in system (22) and get:

$$\begin{aligned} \frac{dy}{dx} &= \varphi'(x) - \frac{d\psi_1(x, \epsilon_1)}{dx}, \\ \frac{dy}{dt} &= \eta_1(x - \lambda)(\varphi(x) - \psi_1(x, \epsilon_1)). \end{aligned} \quad (\text{B.5})$$

The time it takes to travel along Γ_{1,ϵ_1} is given by

$$\begin{aligned} T_1 &= \int_{\Gamma_{1,\epsilon_1}} dt = \int_1^2 \frac{1}{\frac{dx}{dt}} dx = \int_1^2 \frac{\frac{dy}{dx}}{\frac{dy}{dt}} dx \\ &= \int_1^2 \frac{(\varphi'(x) - \frac{d\psi_1}{dx}(x, \epsilon_1)) dx}{\eta_1(x - \lambda)(\varphi(x) - \psi_1(x, \epsilon_1))}. \end{aligned} \quad (\text{B.6})$$

We can take a similar strategy to prove the formula of T_2 . Then the whole period of x can be approximated as $T_1 + T_2$. ■

Data availability

No data was used for the research described in the article.

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