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Implementation of a concentration-controlled chemical clock

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Dear editor,

In recent decades, researchers have started seeking for substitutes for silicon based circuits to perform computation in other fields, including synthetic biology and molecular computing [1, 2].

Previous researches indicate that many circuit behaviors can be mapped to chemical reactions [3, 4]. Also, because chemical reactions satisfy mass action kinetics [5], the behaviors of chemical reactions are constrained by ordinary differential equations (ODEs) thus ensuring the predictability and programmability of chemical reaction networks (CRNs). Ref. [6] proved that researchers can utilize toehold mediated DNA displacement reactions [7] as substrates to implement arbitrary CRNs consisting of formal chemical reactions (reactions involving only formal species, i.e., symbols that do not correspond to any real world material) with less than or equal to two reactants in real world. With theoretical programmability and real-world implementability, CRNs become a powerful programming language in the implementation of different types of circuits. By designing CRNs, the functions of certain circuit components can be reliably implemented with chemical reactions.

As the operations of many digital circuit components such as D flip-flop largely depend on clock signals, it is essential to construct a chemical clock. Clocks that are capable of generating clock signals with arbitrary duty cycles can better aid molecular computing system design. In [8], a chemical clock named RGBY clock is proposed. However, it lacks the function of producing clock signals with duty cycles besides 1/2. Ref. [9] proposed a design method that can perfectly satisfy our requests. Nonetheless, it tunes duty cycles by redesigning the reaction system and is not convenient enough to implement in experiments.

In this study, a chemical clock with duty cycles that can be tuned by concentration is proposed. Our contributions are as follows.

(1) Proposing a novel architecture of a chemical clock in CRNs based on [8].

(2) Obtaining the expression of duty cycle.

(3) Mapping our clock implementation to DNA reactions and improving an existing mapping model proposed by [6].

In this study, let $[A](t)$ or $[A]$ denote concentration function of species A for simplicity, k or $k_1, k_2, \ldots$ denote rate constants.

CRN design. The chemical clock in this article can be considered as a combination of two two-phase RGBY clocks connected by absence indicators [8]. Within each two-phase clock, the phase signals are converted to each other periodically. Such a combination of the former model enables the adjustment of duty cycles.

For convenience and robustness, conforming to [8], only two rate constants are used in our system. k_{fast} denotes a relatively high rate constant while k_{slow} represents a relatively low one. k_{fast} and k_{slow} should satisfy $k_{\text{fast}} \gg k_{\text{slow}}$ to ensure the elimination of absence indicators and other fast reactions to finish in time. Also, this helps to increase the fault tolerance in rate constants.

Our CRN system consists of five types of species: (1) generator of absence indicators [8] ϕ, (2) phase signals X, Y, Z, W, (3) absence indicators x, y, z, w, (4) refinement species I_X, I_Y, I_Z, I_W, (5) output signals E_0, E_1. The reactions among such species are shown in Figure 1(a). The first four categories of formal reactions construct two two-phase clocks, one consisting of ϕ, phase signal species X, Z and their corresponding absence indicators and the other consisting of ϕ, phase signal species Y, W and their corresponding absence indicators. The fifth category of reactions acts as an interface between our clock and external applications and controls output clock signals E_0 and E_1.

The diagram presenting our design methodology is shown in Figure 1(b). From a qualitative perspective, for any reaction, larger densities of reactants give rise to a longer reaction time. Using this principle, different durations of time

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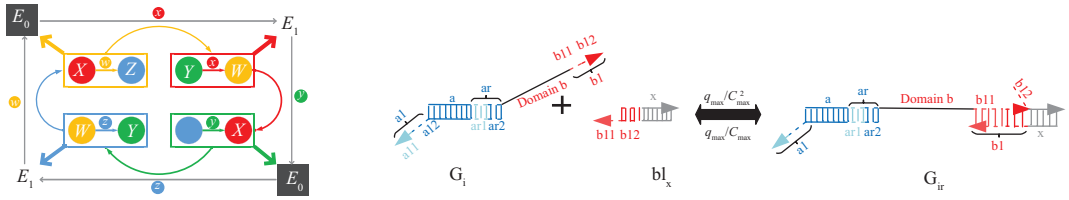
Category	Reaction
Generation of absence indicators	$\varphi \xrightarrow{k_{\text{slow}}} x,\ \varphi \xrightarrow{k_{\text{slow}}} y,\ \varphi \xrightarrow{k_{\text{slow}}} z,\ \varphi \xrightarrow{k_{\text{slow}}} w.$
Consumption of absence indicators	$X + x \xrightarrow{k_{\text{fast}}} X,\ Y + y \xrightarrow{k_{\text{fast}}} Y,\ Z + z \xrightarrow{k_{\text{fast}}} Z,\ W + w \xrightarrow{k_{\text{fast}}} W.$
Conversion of phase signals	$k_{\text{slow}}\ X + w \xrightarrow{k_{\text{slow}}} Z + w,\ Y + x \xrightarrow{k_{\text{slow}}} W + x,$ $Z + y \xrightarrow{k_{\text{slow}}} X + y,\ W + z \xrightarrow{k_{\text{slow}}} Y + z.$
Refinement reactions	$2Z \underset{k_{\text{fast}}}{\overset{k_{\text{slow}}}{\rightleftharpoons}} I_z,\ I_z + X \xrightarrow{k_{\text{fast}}} 3Z,\ 2W \underset{k_{\text{fast}}}{\overset{k_{\text{slow}}}{\rightleftharpoons}} I_w,\ I_w + Y \xrightarrow{k_{\text{fast}}} 3W,$ $2X \underset{k_{\text{fast}}}{\overset{k_{\text{slow}}}{\rightleftharpoons}} I_x,\ I_x + Z \xrightarrow{k_{\text{fast}}} X,\ 2Y \underset{k_{\text{fast}}}{\overset{k_{\text{slow}}}{\rightleftharpoons}} I_y,\ I_y + W \xrightarrow{k_{\text{fast}}} 3Y.$
Conversion of output signals	$E_0 + x \xrightarrow{k_{\text{fast}}} E_1 + x,\ E_1 + y \xrightarrow{k_{\text{fast}}} E_0 + y,$ $E_0 + z \xrightarrow{k_{\text{fast}}} E_1 + z,\ E_1 + w \xrightarrow{k_{\text{fast}}} E_0 + w.$

(a)



(b) (c)

Figure 1 (Color online) (a) Formal reactions used in our system; (b) the diagram presenting the relations of formal species in our CRNs; (c) the newly employed reaction in our DNA strand displacement reaction system.

can be created. By separating one period of an oscillation to several periods of time with different lengths, clock signals with different duty cycles can be attained. In our design, by employing two two-phase clocks, a period is partitioned to four parts which are four phase signal conversion stags (shown as inner cycles in Figure 1(b)): $X \to Z$, $Y \to W$, $Z \to X$, $W \to Y$. By controlling the conversion time via changing initial concentrations of phase signal species X, we can unequally partition the oscillation period thus creating different durations of time. We utilize species E_0 and E_1 to map the durations of each conversion reaction and generate clock signals as output.

Relation between initial concentration and duty cycle. We use $\langle E_0 \rangle$ and $\langle E_1 \rangle$ to denote the duration of over 90% maximum concentration of E_0 and E_1, respectively. The value of duty cycle can be expressed as $\langle E_0 \rangle / (\langle E_0 \rangle + \langle E_1 \rangle)$. Here, we define $D = \langle E_0 \rangle / \langle E_1 \rangle$ and the value of duty cycle equals to $D/(1+D)$. For simplicity, we only consider changing the concentration of X and maintaining the concentration of Y at 1. Instead of directly solving the ODEs depicting the system, we manage to give an approximated solution to the system by fitting data points. By randomly selecting values of initial concentrations of X in our model, data points of D are collected.

Consider a pair of fixed rate constant $k_{\text{slow}} = 100$ and $k_{\text{fast}} = 10^8$. We use linear model

$$D = a[X](0) + b \tag{1}$$

to fit our data points. The result of curve fitting is $a = 0.0746$, $b = 0.7962$. Once the expression is given, it is easy to implement a clock with an arbitrary given duty cycle by solving (1) to obtain the initial concentration of X. A corresponding analysis is provided in Appendix B.2.

DNA implementation. We use the model proposed by [6] to map our CRNs to DNA strand displacement reaction systems. In the model in [6], species from the formal reaction are represented by single stranded DNA. With the assistance of some DNA auxiliary species, a set of DNA strand displacement reactions can well approximate kinetic features of the original formal reaction. The highest rate constant of DNA reactions is $q_{\max}$, and such auxiliary species are assumed to maintain a relatively high concentration $C_{\max}$. A more detailed description of the model can be found in Appendix A.4. However, according to the analysis in our Appendix B.3, owing to the continuous consumption of auxiliary species in reaction systems involving oscillation, this assumption may not hold, and thus kinetic features may change.

Here we propose a refinement for the mapping model. For any auxiliary species A in an arbitrary reaction, a reversible reaction $A \underset{k_b}{\overset{k_f}{\rightleftharpoons}} A_i$ is employed in which A_i can be considered as the 'source' of A. We take a pair of rate constants as an example: $k_f = q_{\max}$ and $k_b = q_{\max}/C_{\max}$. We set the initial concentration of A_i as $C_{\max}^2$. Assume that in each reaction, the concentration of A being consumed is c. At the equilibration of $A \underset{k_b}{\overset{k_f}{\rightleftharpoons}} A_i$, we have $C_{\max}[A] = [A_i]$. Assume that in each reaction, this value is reduced by c thus after n cycles $[A] + [A_i] = C_{\max}^2 + C_{\max} - nc$ and $[A] = C_{\max} - nc/(C_{\max}+1)$. Compared to the concentration of A without refinement: $[A] = C_{\max} - nc$, the concentration consumed in each reaction is significantly reduced. By employing such refinement, our system is able to keep oscillating for a sufficiently long period of time. Also, we can see from the analysis that in fact, the actual rate constant is not important. We choose the values shown above just for simplicity. Any choice of k_f, k_b that has a large value of k_f/k_b will similarly slow the corruption of our system.

To implement such refinement in our DNA reaction system, some changes are made to our previous model. Firstly, every toehold in the previous substrate is separated into two toeholds. In reactions of our previous model, the two toeholds appear at the same time so they can be considered as a single toehold that behaves similarly to the previous toehold. Secondly, a new reaction is added to the previous substrate. In our new model, owing to the structures of auxiliary species, at least two toeholds in the upper strands of each auxiliary species are exposed. This attribute is used

in the design of a new strand displacement reaction to implement the formal refinement reaction $A \underset{k_b}{\overset{k_f}{\rightleftharpoons}} A_i$. The reaction is in Figure 1(c). Detailed results can be found in Appendix B.3.

Conclusion. In this study, the implementation of a molecular clock with a duty cycle that can be conveniently controlled by concentrations is proposed. Using parameter fitting, the relation between initial concentration and the duty cycle is given. By analyzing an existent substrate, we propose a refinement for the model and implement our CRNs using DNA reactions. According to Lemma 1 of Appendix B.2 and the method in Appendix B.4, clock periods can also be tuned.

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Supporting information Appendixes A and B. The supporting information is available online at info.scichina.com and link.springer.com. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

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