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Synthesising periodic triggering signals with genetic oscillators

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Abstract: The potential of the clock lies in its role of triggering logic reaction for sequential biological circuits. This research introduces an idea of designing a genetic clock generator by Fourier series based on the genetic oscillators. The authors generalise the design idea using a combination of fundamental sinusoidal signals. Since biochemical reaction of the biological system is extremely slow, however, transition between minimal and maximal levels is instantaneous for an ideal clock signal; it is thus not directly realisable in biological systems. That means it would be hard to directly synthesize a square wave generator for use as a genetic clock. They apply Fourier series to represent a square wave as a finite summation of sinusoidal waves generated by some genetic oscillators with different harmonic oscillating frequencies, in which the amplitude alternates at a constant frequency between the fixed minimal and maximal levels with the same duration of time.

1 Introduction

In recent years, design and construction of biological circuit has been regarded as a significant tendency in synthetic biology. It is commonly treated as an interdisciplinary application in molecular biology and engineering.

Synthesis of biological logic circuits is one of the research interests in systems biology. Many basic biological logic behaviours have been developed since the appearance of the repressilator in 2000 by Elowitz and Leibler [1], after that there were optimisation-based oscillator design [2, 3] and synthesis of fundamental biological gates including NOT, AND, OR, NAND, NOR and XOR [4, 5]. In particular, some specific bio-combinational logic circuits and sequential logic circuits have also been unveiled [6–8]. However, previous works for synthesising biological oscillators only focus on the sine wave generation, which cannot be used to trigger bio-sequential logic circuits. That needs a square wave as the clock input.

Synchronisation of logic circuits with a special signal, that is, clock signal, has many benefits. Among others, main benefits are

- The ability to memorise states is gained with the introduction of synchronisation with clock signals.
- Behaviour of synchronous circuits can be predicted precisely if there is a stable clock signal. Therefore performance analysis is easier to be made.
- Design of biological circuits with higher complexity in their (time-related) operational functions would be possible, such as molecular computers.

In this study, we attempt to bring our previous design of a gene oscillator [3] to synthesize the square wave generator. The aim is to create a biological triggering signal, such as a clock signal, for use in the bio-sequential logic circuits. We apply Fourier series [9] to decompose the periodic square signal as a finite summation of sinusoidal waves generated by the genetic oscillators with different harmonic oscillating frequencies. A Fourier series decomposes periodic signals into the sum of a set of simple oscillating functions (usually lower oscillating frequencies). The advantage is that one only needs to develop a fundamental genetic oscillator with fundamental frequency and combines a limited number of its harmonics to approximate a clock. The harmonic functions can be directly generated from the fundamental one without the need to redesign the higher harmonic waves. The second advantage is that it avoids directly designing a square wave generator which needs a genetic network with extremely fast response to create a sharp change from 'logic low' to 'logic high'. The genetic clock is composed of three genetic oscillators in our study. The combination of these genetic oscillators is made possible by protein binding [10]. In silico experiments confirm applicability of the approach by showing its application in triggering a *J–K* flip-flop (FF) as a biological 2 bit asynchronous counter.

2 Design of genetic oscillators using real structured genetic algorithm (RSGA)

Design of a genetic oscillator was previously treated as a common tracking problem [2, 3]. In [3], the RSGA was used to simultaneously determine the optimal structure and parameters for synthetic genetic oscillators. The operational process of RSGA includes structured genetic mapping, reproduction, crossover and mutation. A chromosome consists of control genes and parameter genes; both of them

are real numbers in the chromosome structure. Repeating an evolutionary process is able to generate the fittest chromosome after several generations of evolution. We specify an objective function to search for the optimal decay rates of proteins, the transcription rates of mRNA and the number of genes for oscillation.

2.1 Genetic oscillator

Elowitz and Leibler used three transcriptional repressor systems (*tetR*, *lacI* and *λcI*) to construct a biological oscillating network [1]. A fundamental transcription repressor is described by two coupled first-order differential equations

$$\begin{aligned}\frac{\mathrm{d}m_i}{\mathrm{d}t} &= -\gamma_{m_i} m_i + \alpha_i b_i\left(p_j^n\right) + \alpha_0 + w_{i1}(t),\\ \frac{\mathrm{d}p_i}{\mathrm{d}t} &= \beta m_i - \gamma_{p_i} p_i + w_{i_2}(t)\end{aligned} \tag{1}$$

where

$$b_i\left(p_j^n\right) = \frac{1}{1+p_j^n}$$

and (i, j)°(lacII, λcI), (tetR, lacI), or (λcI, tetR), $m_i \in \mathbb{R}_+$ is concentration of mRNA, p_i, $p_j \in \mathbb{R}_+$ are concentrations of proteins for three genes, $\alpha_i \in \mathbb{R}$ denotes transcription rate of mRNA, α_0 is leakiness of the promoter, $\beta \in \mathbb{R}$ is ratio of the protein decay rate to the mRNA decay rate, $\gamma_{p_i} \in \mathbb{R}_+$ and $\gamma_{m_i} \in \mathbb{R}_+$ are decay rates of proteins and mRNA, respectively and $n \in \mathbb{R}$ is a Hill coefficient, w_{ij}, $j = 1, 2$ reflect the effect of environmental noises.

The three-genes oscillation model was extended to an N coupled genetic oscillation model in [11–14], we represent a class of stochastic models for the N-stage gene oscillator with intrinsic fluctuations and extrinsic noises [12] as follows

$$\dot{X} = f(X) + \sum_{i=1}^{4} g_i(X) n_i + w \tag{2}$$

where (see equation at the bottom of the page)

and $X = [m_1\ p_1\ m_2\ p_2\ \ldots\ m_N\ p_N]^{\mathrm{T}}$ is state vector, $f(X)$ is non-linear interactions of gene oscillation, $g_i(X)n_i$ are intrinsic parameter fluctuations for the ith random intrinsic noise n_i, δ is a random constant, $\boldsymbol{w} = [w_1\ w_2\ \ldots\ w_{2N-1}\ w_{2N}]^{\mathrm{T}}$ is extrinsic disturbance vector. The intrinsic noise in gene expression mostly stems from promoter fluctuations and low copy mRNA fluctuations.

It has been known that stable behaviour is exhibited when oscillators are constructed with an odd number of repressor genes [15]. Protein concentration of that kind of oscillators attracts globally to a stable limit cycle. However, oscillators with even number of genes tend to be a quasi-stable periodic cycle. It will diverge after a long period of time. When the number of genes is even, the number of repressive loops should also be even. We adjust the following non-linear term to make sure an odd number of the repressive loops

$$b_i\left(p_j^n\right) = \frac{1}{1+p_j^n}, \quad n \triangleq \begin{cases} \geq 0, & \text{for repression} \\ < 0, & \text{for activation} \end{cases} \tag{3}$$

This would ensure that the biological systems are sufficiently robust and attracted to a stable limit cycle [14, 15] for oscillation.

2.2 Fitness function

Given a sinusoidal signal denoted as

$$r_i(t) = A_i \sin(\omega_i t + \phi_i), \quad i = 1, 2, \ldots, N \tag{4}$$

$$f(X) = \begin{bmatrix} -\gamma_{m_1} m_1 + b_1\left(p_N^n\right) \\ \beta_1 m_1 - \gamma_{p_1} p_1 \\ -\gamma_{m_2} m_2 + b_2\left(p_1^n\right) \\ \beta_2 m_2 - \gamma_{p_2} p_2 \\ -\gamma_{m_3} m_3 + b_3\left(p_2^n\right) \\ \beta_3 m_3 - \gamma_{p_3} p_3 \\ \vdots \\ -\gamma_{m_N} m_N + b_N\left(p_{N-1}^n\right) \\ \beta_N m_N - \gamma_{p_N} p_N \end{bmatrix}, \quad g_1(X)n_1 = \begin{bmatrix} \delta\gamma_{m_1} \\ 0 \\ \delta\gamma_{m_2} \\ 0 \\ \delta\gamma_{m_3} \\ 0 \\ \vdots \\ \delta\gamma_{m_N} \\ 0 \end{bmatrix}$$

$$g_2(X)n_2 = \begin{bmatrix} \delta b_1\left(p_N^n\right) \\ 0 \\ \delta b_2\left(p_1^n\right) \\ 0 \\ \delta b_3\left(p_2^n\right) \\ 0 \\ \vdots \\ \delta b_N\left(p_{N-1}^n\right) \\ 0 \end{bmatrix}, \quad g_3(X)n_3 = \begin{bmatrix} 0 \\ \delta\beta_1 \\ 0 \\ \delta\beta_2 \\ 0 \\ \delta\beta_3 \\ \vdots \\ 0 \\ \delta\beta_N \end{bmatrix}, \quad g_4(X)n_4 = \begin{bmatrix} 0 \\ \delta\gamma_{p_1} \\ 0 \\ \delta\gamma_{p_2} \\ 0 \\ \delta\gamma_{p_3} \\ \vdots \\ 0 \\ \delta\gamma_{p_N} \end{bmatrix}$$

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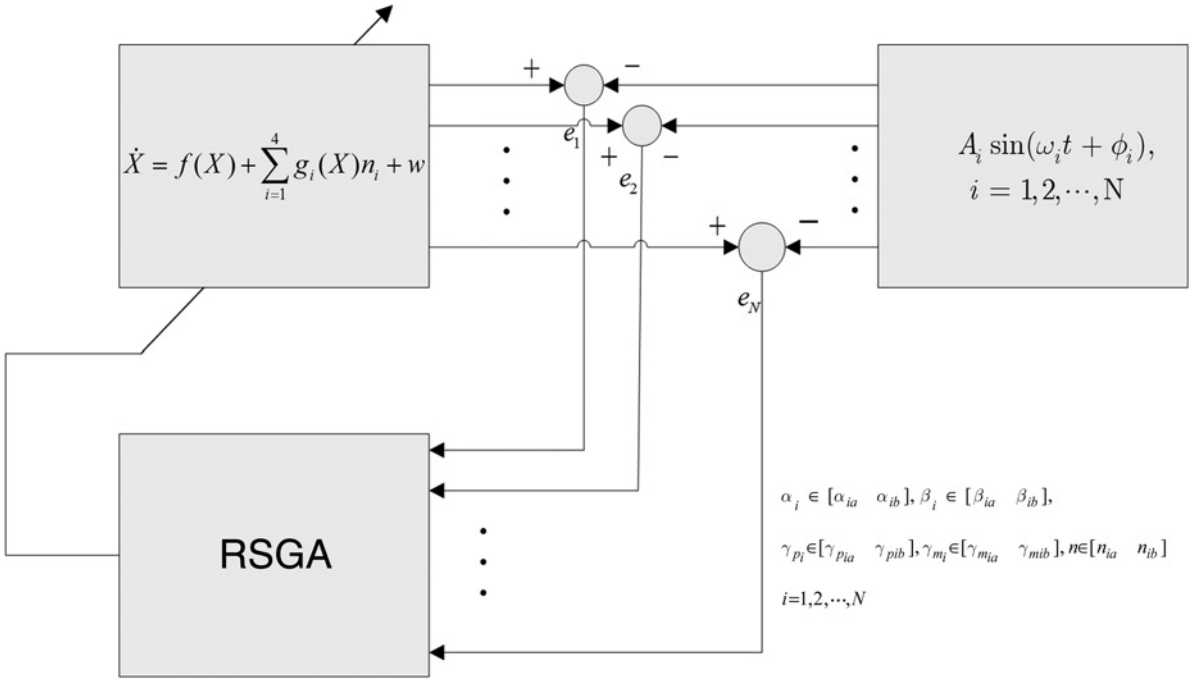

Fig. 1 *RSGA-based synthetic genetic oscillator design*

where A_i, ω_i and ϕ_i are amplitude, frequency and phase, respectively; a genetic oscillator was designed in [3] to track this sinusoidal wave to facilitate oscillation behaviour with the desired amplitude, phase and frequency.

For the current purpose, the normalised performance index J_p and the structure index J_s are defined, respectively, as

$$J_p = \sqrt{\frac{\sum_{i=1}^{N} \int_0^T e_i^2 \, dt}{T \sum_{i=1}^{N} A_i^2}}, \quad J_s = \frac{N}{n_{max}} \tag{5}$$

where

$$e_i = p_i - A_i \sin(\omega_i t + \phi_i)$$

and J_p represents the objective function of tracking error e_i, J_s represents the objective function specifying the order and n_{max} is the allowable maximal order.

The objective function for optimisation consisting of two parts related, respectively, to parameter selection and structure determination is defined by (see (6))

where $\rho \in [0, 1]$ is the weighting factor, J_p is the normalised performance index and J_s is the normalised structure index. J_{tot} can be explained as the objective value of the tracking error and J_s represents the objective function for the number of genes to generate oscillation. The smaller J_p is, the higher tracking accuracy will be. As for J_s, a small J_s implies a simpler structure of the oscillator. We simply set the fitness function as follows

$$F\left(\alpha_i, \ \gamma_{p_i}, \ N, \ n\right) = 1/J_{tot}\left(\alpha_i, \ \gamma_{p_i}, \ N, \ n\right) \tag{7}$$

Appropriately selecting α_i, γ_{p_i} and N to maximise $F(\alpha_i, \gamma_{p_i}, N, n)$ is equivalent to minimising $J(\alpha_i, \gamma_{p_i}, N, n)$. The approach can be illustrated as in Fig. 1 [3].

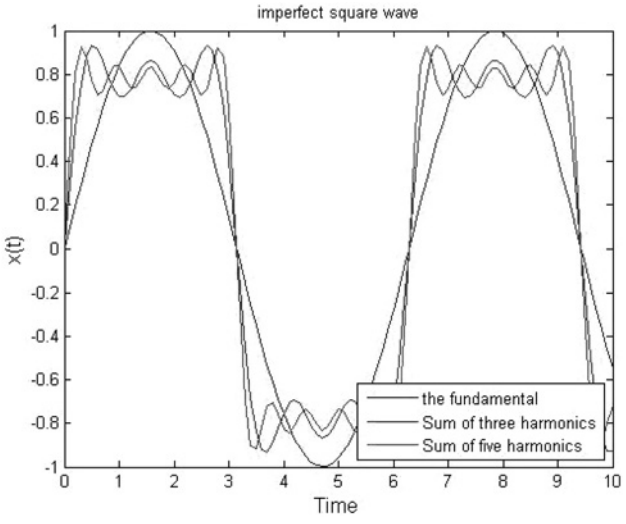

Fig. 2 *Periodic square waves synthesised by Fourier series*

$$J_{tot}\left(\alpha_i, \ \gamma_{p_i}, \ N, \ n\right) = \min_{\substack{\alpha_i \in [\alpha_{ia} \quad \alpha_i], \quad \gamma_{p_i} \in [\gamma_{p_{ia}} \quad \gamma_{p_{ib}}], \\ n \in [n_{ia} \quad n_{ib}], \quad i = 1, 2, \ldots, N}} \left[\rho J_p + (1 - \rho) J_s\right] \tag{6}$$

2.3 Brief introduction to RSGA

2.3.1 Structured genetic mapping: The chromosomes $Y = \langle c, p \rangle$ of each individual in RSGA is an order set consisting of the set of control genes c and the set of parameter genes $p = \langle p_c, p_u \rangle$ with p_c and p_u representing the control dependent and control independent parameter genes, respectively. All genes are expressed in real numbers within ($R_{\min}$, $R_{\max}$). The parameter genes are regulated by the control gene.

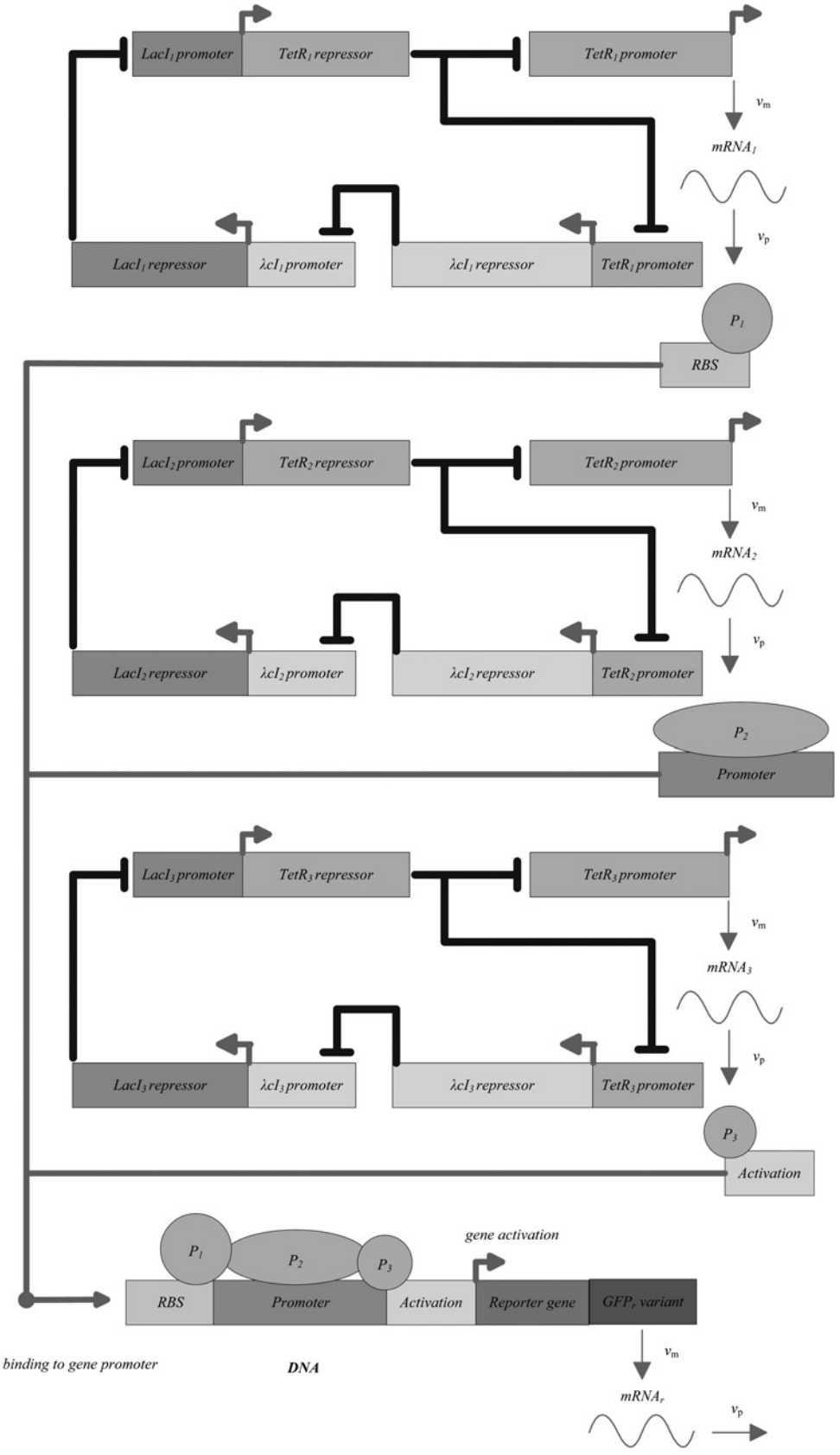

Fig. 3 *Combination of three genetic oscillators*

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For the current problem, the order set of parameter genes p contains the key parameters for transcription of the biogenetic oscillator which are to be determined including transcription rate and sensitivity of mRNA and decay rates of protein and mRNA etc.

The structured genetic mapping from Y to $\tilde{Y}$ is defined as

$$\tilde{Y} = \langle c,\ \tilde{p} \rangle \tag{8}$$

where $\tilde{Y}$ represents an ordered set consisting of c and $\tilde{p}$

$$\tilde{p} = \langle \tilde{p}_c,\ p_u \rangle \tag{9}$$

The operator $\otimes$ denoting the genetic switch is defined by

$$\tilde{p}_c = [c_i] \otimes \left[p_{ij} \right]_{n_j} \equiv \begin{cases} p_{ij}, & \text{if} \quad B_{\max} \le c_i \\ p_{ij}t, & \text{if} \quad B_{\min} \le c_i \le B_{\max} \\ \phi, & \text{if} \quad c_i \le B_{\min} \end{cases} \tag{10}$$

where $j = 1,\ \ldots,\ n_j$, and $t = (c_i - B_{\min}/B_{\max} - B_{\min})$ with ϕ denoting an empty element. Variations of $B_{\max}$ and $B_{\min}$ are generation dependent and is defined by

$$\begin{cases} B_{\max} = B_{\min} = B_{\text{mid}}, & \text{if} \quad g_i = g_{\text{init}} \\ B_{\max} = R_{\text{mid}} + 0.5\Delta B, \quad B_{\min} = R_{\text{mid}} - 0.5\Delta B, & \\ \quad \text{if} \quad 0 < g_i < g_{\text{fin}} & \\ B_{\max} = R_{\max}, \quad B_{\min} = R_{\min}, & \text{if} \quad g_i = g_{\text{fin}} \end{cases} \tag{11}$$

with $R_{\text{mid}} = 0.5(R_{\max} + R_{\min})$, $R_{\max}$ and $R_{\min}$ are maximum and minimum boundaries, g_i denotes current generation, g_{init} and g_{fin} are initial and final generations and $\Delta B = kg_i$ with k being a positive constant. The current boundaries $B_{\max}$ and $B_{\min}$ would be shifted when g_i increases such that the parameter searching range extends. Through the boundary sizing technique, the RSGA is able to provide a switch function in the early stage of evolution, emphasising structural optimisation. Although the boundary increases

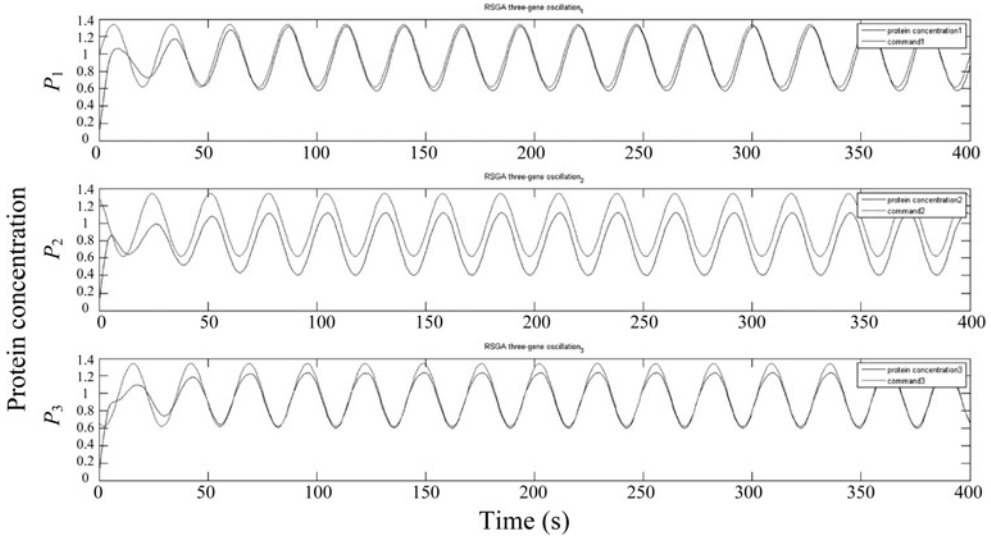

Fig. 4 *Oscillation of the genetic oscillator obtained by RSGA*

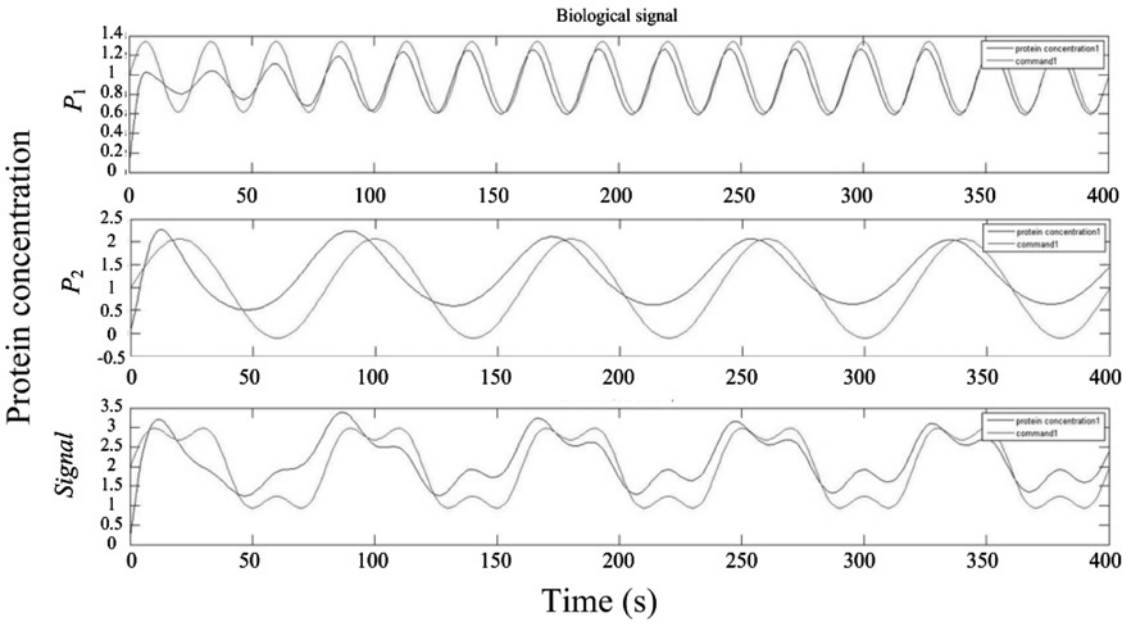

Fig. 5 *Biological signal synthesised by two sinusoidal waves*

gradually with the generation number, it becomes a linear scaling function. When the evolution process gradually reaching the end of the generation, ($B_{\min}$, $B_{\max}$) replaces ($R_{\min}$, $R_{\max}$) and the working algorithm gradually becomes the parameter optimisation.

2.3.2 Reproduction: In the reproduction process, the chromosomes are selected for mating, which depends on their relative fitness values, that is, roulette wheel selection. The chromosome selection probability is, as usual genetic algorithms, given by

$$P_{\mathrm{r}} = \frac{f_i}{\sum_{j=1}^{m} f_j} \tag{12}$$

where f_i is fitness value of the ith member and m is population size. The chromosomes of higher probability associate with a relative high-fitness value among the population of all chromosomes.

2.3.3 Crossover: The crossover mechanism utilises extrapolation or interpolation to generate new individuals. Initially, it operates in extrapolation. When the parameters of the offspring exceed $R_{\max}$ and $R_{\min}$, it changes to interpolation. The operation is determined by

$$\begin{cases} \tilde{x}_{di} = x_{di} - \lambda\left(x_{di} - x_{mi}\right) \\ \tilde{x}_{mi} = x_{mi} + \lambda\left(x_{di} - x_{mi}\right) \end{cases}, \text{ if } x_{di} > R_{\max} \text{ or } x_{mi} < R_{\min} \tag{13}$$

$$\begin{cases} \tilde{x}_{di} = x_{di} + \lambda\left(x_{di} - x_{mi}\right) \\ \tilde{x}_{mi} = x_{mi} - \lambda\left(x_{di} - x_{mi}\right) \end{cases}, \text{ if } R_{\min} \le x_{mi} \le x_{di} \le R_{\max} \tag{14}$$

where $\lambda = \lambda_0[1 - (g_i/g_{\mathrm{fin}})]$, x_{di} and x_{mi} refer to parent individuals, $\tilde{x}_{di}$ and $\tilde{x}_{mi}$ are offsprings of x_{di} and x_{mi}, respectively and $\lambda_0 \in [0, 1]$ is a uniformly distributed random number. Both of the control and parameter genes are real numbers and share the same operation of mutation.

2.3.4 Mutation: The mutation operator applies randomly chosen individuals to gain fine tuning in chromosomes. The non-uniform mutation method to change genes in a chromosome is adopted

$$\tilde{x}_{ij} = \begin{cases} x_{ij} + \Delta\left(g_i,\ x_{ij\max} - x_{ij}\right), & \text{if} \quad h = 0 \\ x_{ij} - \Delta\left(g_i,\ x_{ij} - x_{ij\min}\right), & \text{if} \quad h = 1 \end{cases} \tag{15}$$

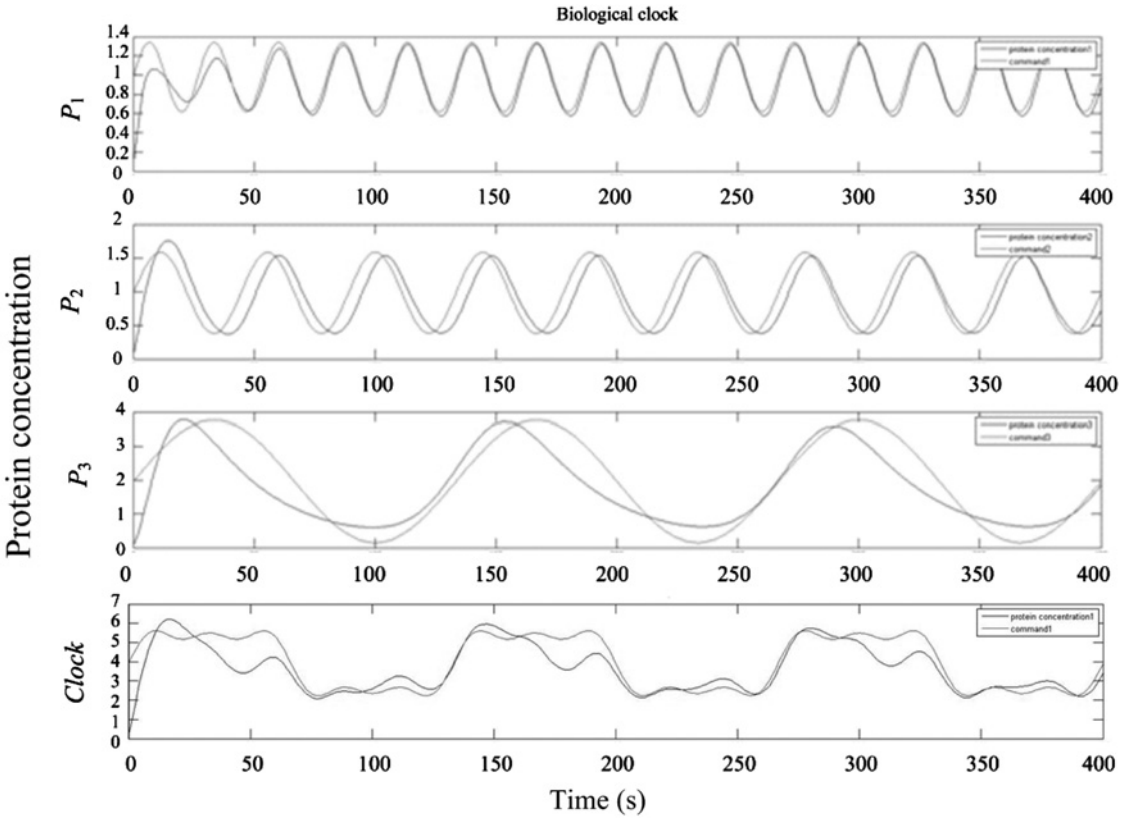

Fig. 6 *Biological clock synthesised by three sinusoidal waves*

Table 1 Comparison of slope of the fundamental sine waves

	Fundamental wave	Third harmonic wave	Fifth harmonic wave	Synthetic wave
slope at the mid-point of the positive edge	0.0951	0.0855	0.0760	0.2597

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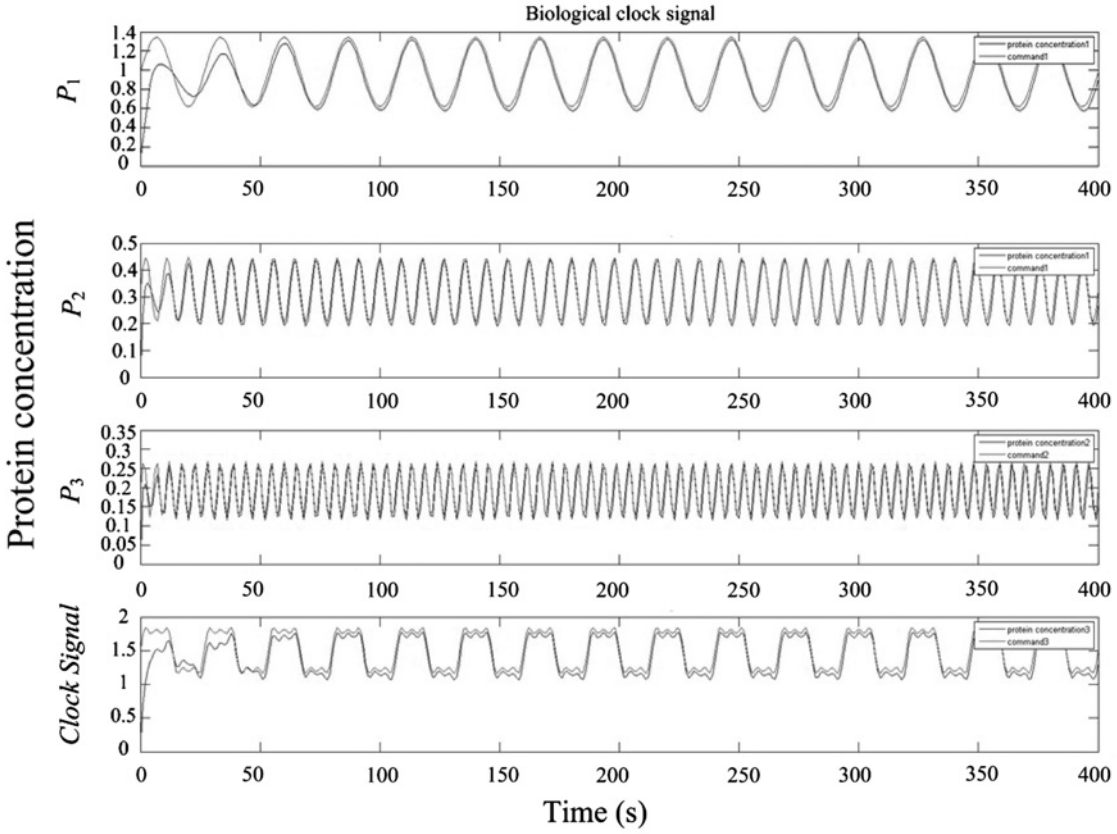

Fig. 7 *Biological clock signal synthesised by combining three sinusoidal waves; those were originated from the fundamental sine wave (the top one) generated by a genetic oscillator*

where

$$\Delta(g_i, y) = y\lambda_0\left(1 - \frac{g_i}{g_{\text{fin}}}\right), \quad x_{ij} \notin \{\phi\}$$

is jth element of the ith individuals in the current generation, the value $h \in \{0, 1\}$ depends on the random production, $x_{ij\max}$ ($x_{ij\min}$) is maximal (minimal) jth element of the ith individual in the current generation and y is a scaling factor.

One can pick good genes by evaluating the fitness values and generating elite chromosomes to the next generation as in the usual evolutionary process by the following procedures: structural genetic mapping, reproduction, crossover, mutation, elitism and then go back to the structural genetic mapping and move on provided that the terminal condition is not reached. One is referred to [3] for more details.

3 Synthesising an approximate square wave

In mathematics, any periodic function can be constituted by an infinite series of the sum of fundamental periodical functions, known as Fourier series. Since the square wave considered here is well behaved, a Fourier series is appropriate to decompose the square wave into a combination of several fundamental sine waves where each fundamental sine wave is the output of a fundamental genetic oscillator.

3.1 Square wave using Fourier series

The try here is to produce a periodic triggering signal for sequential biological circuits. In those circuits, a series of square wave is used as a clock signal to trigger a synchronous circuit. Using Fourier series expansion by a finite number of fundamental sine waves with cycle frequency ω_i over time t, one can approximate the square wave as

$$f(t) = \sum_{n=1}^{M} \frac{\tilde{A}_i \sin\left((2n-1)\omega_i t\right)}{2n-1} \tag{16}$$

where $\tilde{A}_i$ is the Fourier coefficient and M is the sum of the total number of harmonics. Certainly, the more the number of harmonics, the waveform is more close to an ideal square wave. The square wave synthesised by a finite Fourier series in different harmonic number can be illustrated as in Fig. 2. Obviously, the wave's edge determining the triggering effect exhibits a significant improvement in the sum of more harmonics. Increasing the number of fundamental sine wave contributes to reducing the rise time and fall-off time on the transient behaviour. This makes the synthetic signal more close to a square wave. However, instantaneous changes are unlikely realistic for biological signals. In general, the biochemical reactions change slowly with time, the reaction may be up to several hours. Therefore the requirement for a sharp change of status between logics '0' and '1' is not as strict as that in

Table 2 Comparison of slope of synthetic square waves

	Synthetic wave of Fig. 7	Synthetic wave of Fig. 6
slope	0.2853	0.2597

digital circuits. Thus, one might use a limited number of harmonic series to resemble a square wave.

The following inequality specifies the requirement for synthesising a clock with the triggering slope at $t = t_0 + (m-1)T$, $m = 1, 2, \ldots$, with T being period of the periodical signal, for a positive-edge-triggered circuit

$$\lim_{t \to t_0 + (m-1)T} \frac{\partial}{\partial t} \left\| \sum_{n=1}^{N} \frac{\tilde{A}_i \sin\left((2n-1)\omega_i t\right)}{2n-1} \right\| \geq \varepsilon, \quad m = 1, 2, \ldots \tag{17}$$

where ε is a positive constant specifying the positive-edge-triggered level. Since this is a periodical signal, one only needs to consider the slop at $t = t_0$. Based on (17), the value of N can be determined which conforms to the limit.

Consider, for example, the requirement of the triggering slope is $\varepsilon = 10$ at $t = t_0$. One first calculates the slope of the fundamental that the genetic oscillator generated at $t = t_0$. A genetic oscillator generates the fundamental sine wave $\sin(\pi t)$ where $A_i = 1$ and $\omega_i = \pi$. One finds the slope to be 3.1416. This reveals that the requisite number N of the

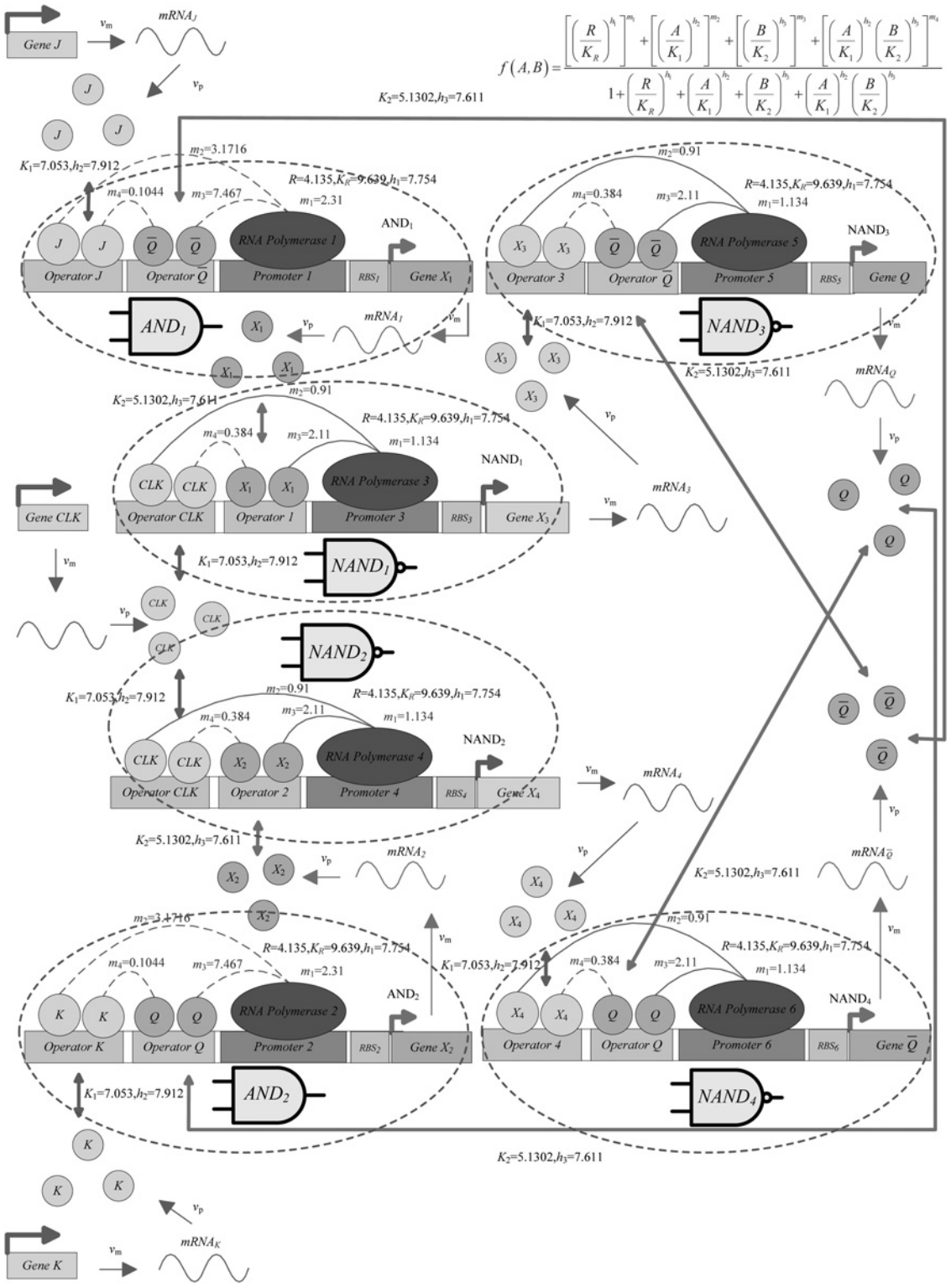

Fig. 8 *Schematic diagram of cis-regulatory implementation of the J–K FF*

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genetic oscillator is $N \geq 3.1831$ at $t = t_0$. The approximate square wave synthesised by four harmonic terms, that is, $N = 4$, should satisfy the requirement.

3.2 Combination of fundamental genetic oscillators

It has been revealed in [3] that the frequency and wavelength can be regulated in the genetic oscillator. Therefore the key of using a Fourier series for realisation of a genetic clock is how to combine a variety of fundamental sine waves with different frequency and amplitude. We refer to the method of protein binding [10] and confirm applicability of the idea.

In Fig. 3, we consider three separate genetic oscillators. They generate the particular protein concentrations (P_1, P_2 and P_3). The working mechanism depends on yeast two-hybrid system in 2002 by Liebler [10]. Assuming three different genetic oscillators, they oscillate and produce proteins in the case of synchronisation. The oscillation frequency is specified individually. Those three proteins are produced by TetR promoters binding to three different cis-regulatory sequences without considering the affinity of each protein. The particular signals are placed in the specific location of ribosomal binding site, activation domain and promoter, respectively. Using the method of fusion which binds to three different cis-regulatory sequences without regard to fusion fail.

It should be noted that the approach herein avoid directly using a genetic network with extremely fast response to realise the square wave generator. The later may not be practically realisable because limited response speeds of real genetic networks.

3.3 Practical concerns

The previous design only considers the biochemical process which is totally under control. That means that the clock signal should be faster than the process response, that is, the processes should not change significantly within one clock cycle.

When the oscillators are multiples of the fundamental they will all be in sync, but when they are separate, there might be phase differences between them during implementation. This might distort the clock signal and corrupt the desired slope. The concern remains to be observed.

Practically, it might also become difficult for placing larger numbers of regulatory molecules at the promoter. This causes a limit on the maximum number of harmonics one can add, and consequently on the slope of the square wave. This raises one of the concerns while realising the synthesised square wave generator.

4 Numerical experiments

Referring to (2), consider the cyclic gene regulatory network designed by applying the RSGA which consists of 1–5 genes to track the sinusoidal wave $A_i \sin(\omega_i t + \phi_i)$ where $A_i = 0.362$, $\omega_i = 0.075\pi$, $\phi_i = (i2\pi/N)$, i represents the number of genes worked at the same time. The genetic oscillator tracking $A_i \sin(\omega_i t + \phi_i)$ is illustrated in Fig. 4 where $\beta_i = 0.3465$ and $\gamma_{m_i} = 0.167$. The parameters $N = 3$, $n = 4.3936$, $\alpha_1 = 1.2716$, $\alpha_2 = \alpha_3 = 1.5278$, $\gamma_{p_1} = 0.3745$ and $\gamma_{p_2} = \gamma_{p_3} = 0.5623$ were searched and determined. This serves as the fundamental source for synthesising the clock generator.

4.1 Synthetic biological clock

4.1.1 Synthetic biological signal: In the preliminary experiment, we have completed the synthesis of a square wave by the combination of two sinusoidal harmonic waves $A_i \sin \omega_i t$ and $3A_i \sin(1/3\omega_i t)$, see Fig. 5. For the synthesis of three sinusoidal harmonic waves $A_1 \sin \omega_1 t$, $5A_i \sin[(1/5)\omega_i t]$ and $(5/3)A_i \sin[(3/5)\omega_i t]$, see Fig. 6.

We adopt a fundamental sine wave generator which possesses lower frequency. The slow reaction is to match the nature of gene networks in biochemical applications. In Table 1, the slopes of various fundamental waves at the mid-point of the positive edge in one cycle are listed. It is seen that the synthetic wave exhibits a larger triggering slope than that of the individual waves.

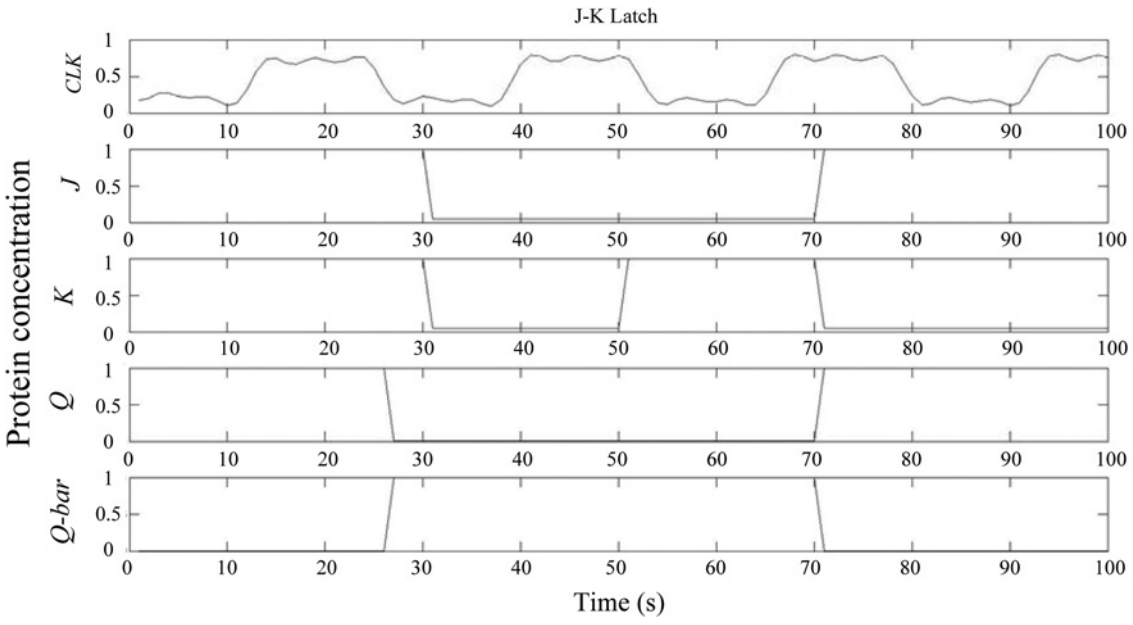

Fig. 9 *Output response of the biological J–K FF when $Q_0 = 1$*

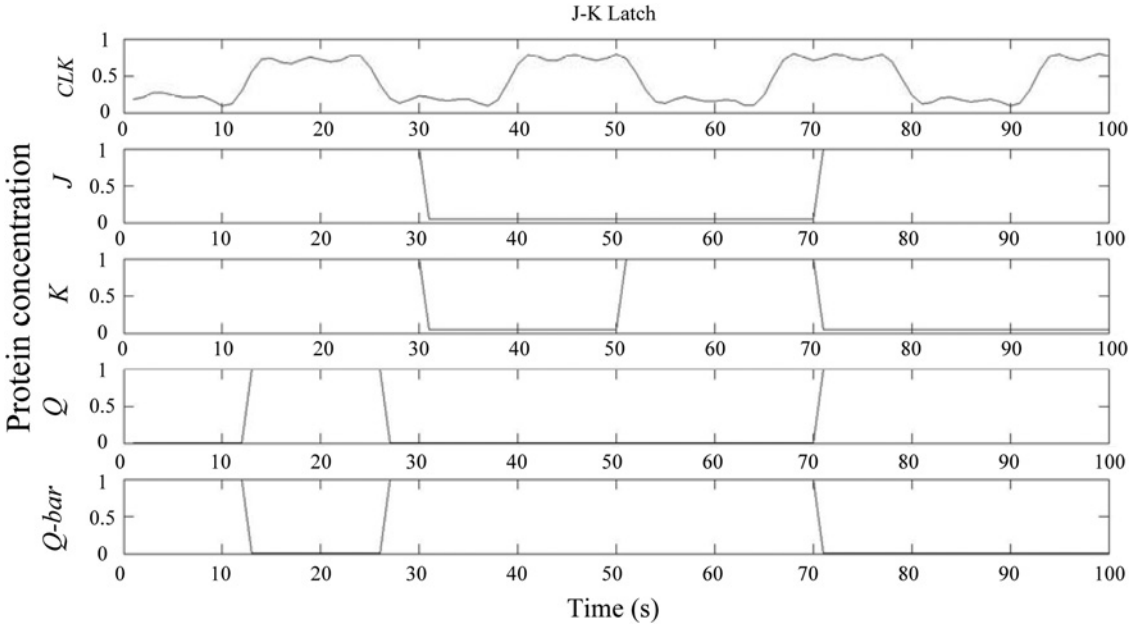

Fig. 10 *Output response of the biological J–K FF when $Q_0 = 0$*

Inequality (17) determines whether the triggering effect of the synthetic signal complies with the requirement for triggering a sequential logic circuit. We take the fundamental sine wave $0.362 \sin(0.075\pi t)$ in the original approximate square wave model and calculate the slope of the fundamental wave at $t_0 = 200$ s which gives 0.0853 and the value of the slopes for three harmonic terms added together is 0.2597.

4.1.2 Biological clock: For comparison, we directly deal with a single genetic oscillator, rather than separately produce three different signals then add together. We use a fundamental oscillating signal which is similar to a sine wave to synthesize other harmonics. In Fig. 7, we use the original signal $A_i \sin(\omega_i t)$ to duplicate the third harmonic wave $(1/3)A_i \sin(3\omega_i t)$ and the fifth harmonic wave $(1/5)A_i \sin(5\omega_i t)$ and combine the three together to synthesize a square-wave signal. The last subfigure shows the result. From Table 2, it is seen that the maximal slope of the synthesised wave is larger than that generated by the previous method after the transient stage. The slope of the synthesised wave is 0.2853 at $t_0 = 200$ s. The result shown in Fig. 7 reveals that the method can achieve better triggering effect. In actual biological systems, the same species or biological signals combined with each other is relatively easier, this idea is thus quite consistent to the natural biological behaviour.

Next, we apply the previous design to trigger a *J–K* FF which is composed of several biological logic gates [6]. These biological logical gates contain two AND gates and four NAND gates as illustrated in Fig. 8. The synthesised square wave is used as a biological clock while adding high or low protein concentration as *J* and *K* inputs.

4.2 Sequential logic circuits

4.2.1 J–K FF: The result verifies our proposed approach when matching the logic responses presented in Figs. 9 and 10 to the truth table of *J–K* FF of Table 3. Although the clock signal is slightly distorted, it did not bring much impact on the desired output since the response of biogenetic networks is dominated by the slow dynamics rather than the fast digital response. The edge-triggered effect of this clock signal is evident. From Fig. 10, one can observe that *Q* starts the reaction when the time is about the moment that the clock signal changes. This means that the amount of status change, at the moment the clock signal

Table 3 Truth table of *J–K* FF

J	*K*	CLK	Q	$\bar{Q}$	Comments
0	0	↑	Q_0	$\bar{Q}_0$	no change
0	1	↑	0	1	latch RESET
1	0	↑	1	0	latch SET
1	1	↑	$\bar{Q}_0$	Q_0	toggle

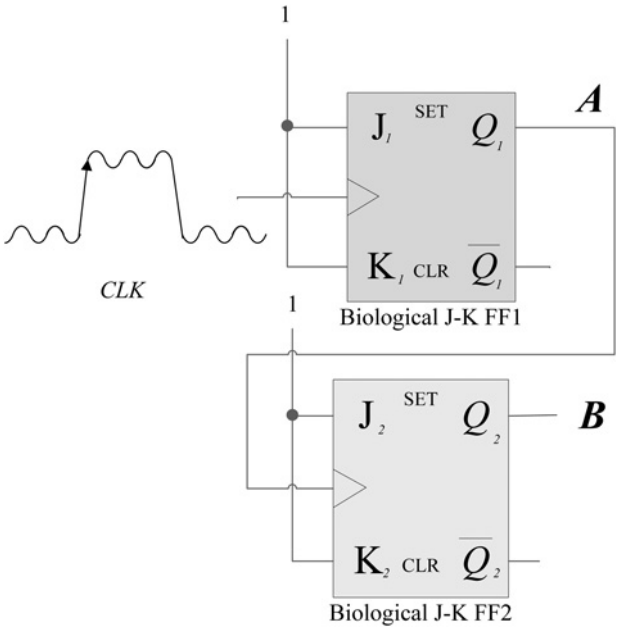

Fig. 11 *Biological 2 bit asynchronous counter*

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triggering, is enough to simulate the exact system response. Although comparing it to the ideal signal, there is a tiny amount of time delay; this would be acceptable in the real biological systems because biochemical reactions are relatively slow in this kind of biological systems.

4.2.2 Frequency divider: As a practical extension, we use a biological *J–K* FF and a synthetic biological clock to realise a frequency divider. In digital logic and computing, a counter constituted by an FF can be used to record changes of logic states. Functionally, a counter with a clock pulse excitation can be categorised into different kinds: synchronous counter and asynchronous counter, binary counter and decade counter, up counter, down counter and ring counter. Here, we realise a binary asynchronous counter and drive the counter with a positive-edge trigger. Displayed in Fig. 11 is a clock signal (CLK) used as the biological triggering signal with all inputs K and inputs J set to logic high (i.e. the normalised high-level protein concentration). We use a positive-edge trigger to drive the FF1, and its output Q_1 is as the *J–K* FF2's clock input. Functionally, the output Q_2's frequency is 1/4 to the CLK's frequency.

When setting input J and input K to logic high, one can observe from Fig. 12 that terminal A reflects exact logic reaction of the *J–K* FF1. It can be observed that the output change exactly occurs at the positive edge of CLK. This causes its period two times the CLK frequency. This displays exact response of a *J–K* FF as in digital circuits. Next, we consider cascaded connection of two biological *J–K* FFs as illustrated in Fig. 11 where terminal A is connected to the clock input of the *J–K* FF2. Setting all J and K to logic high forms a 2 bit asynchronous counter. By

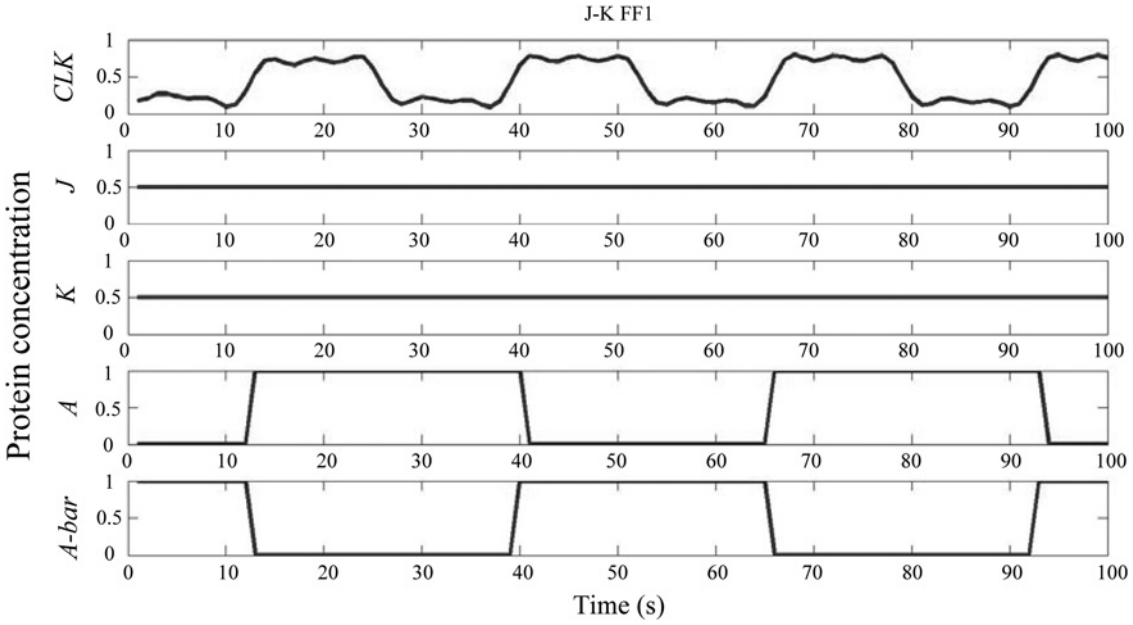

Fig. 12 *Output response of the biological J–K FF1 when $J_1 = 1$ and $K_1 = 1$*

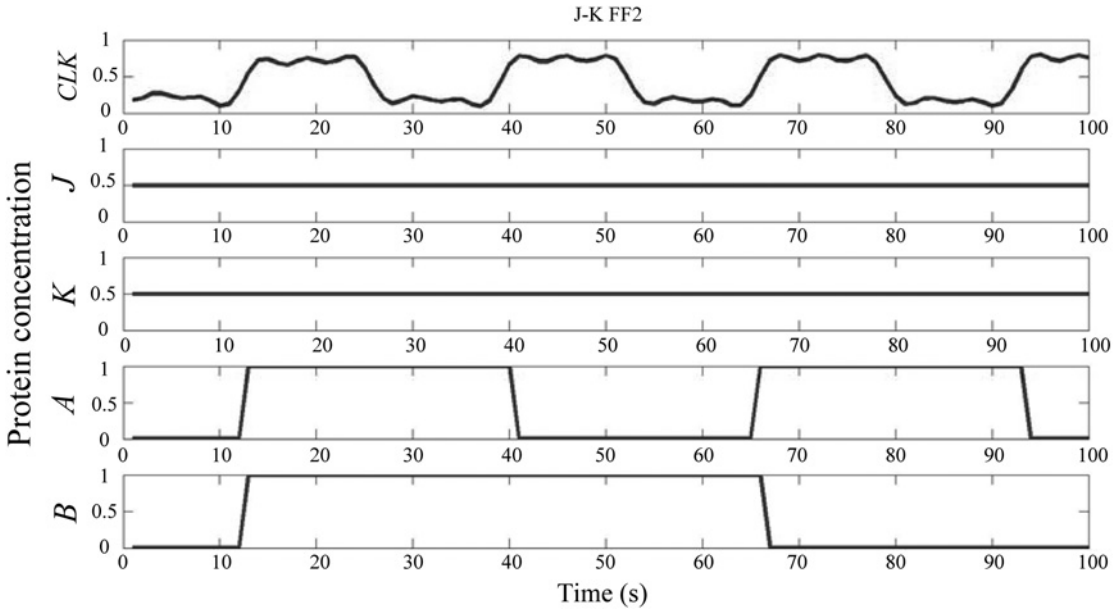

Fig. 13 *Output response of the biological J–K FF2 when $J_2 = 1$ and $K_2 = 1$*

adding the synthetic clock signal to the system generates the response shown in Fig. 13. The change of the logic status within a period of 50 sampling cycles at terminal A is twice to that at the terminal *B*. The response is consistent to the logic reaction of a 2 bit asynchronous counter in electronics. This confirms correctness of our design.

5 Conclusions

This research presents a new attempt to construct a genetic clock which can be used to activate biological sequential logic circuits such as FFs, counter and memory etc. We apply Fourier series to approximate a clock pulse series as a finite summation of sinusoidal waves generated by some fundamental genetic oscillators with different oscillating frequency and amplitude. The method is simple and easy to implement. In silico experiments confirm applicability of the approach by showing its application in triggering a *J–K* FF. Application to form a frequency divider is also presented.

6 Acknowledgment

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