

# Designing Synthetic Biological Systems by Multiple Time Scale Networks

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## 1. Introduction

Rhythmic phenomena exist in living organisms at all levels with periods ranging from less than a second to years [1]. From both theoretical and experiment viewpoints, it is a great challenging problem in biological science to model, analyze and further predict the periodic behaviors of bio-systems. This paper aims to develop a new methodology to analyze and design biological oscillating networks with time delays, by using multiple time-scale networks (MTN) which are composed of a cyclic feedback network (CFN) and multiple positive feedback networks (PFN). We show that a MTN with certain conditions has no stable equilibria but stable periodic oscillations, depending on the total time delay of the CFN, although it has a complicated network structure including both positive and negative feedback loops. As an implementation example, a biologically plausible two-gene synthetic model with genes *lac* and *cl* is designed to demonstrate the theoretical result. The detail theoretical and numerical results are described in [1].

## 2. Multiple time-scale network (MTN)

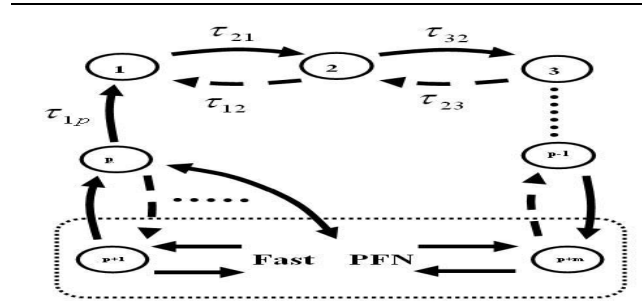
A basic MTN is a monotone dynamical system, i.e. its Jacobian matrix has elements of fixed signs, and consists of a fast PFN and a slow CFN. Assume that there are  $m$  fast variables  $y = (y_1, \dots, y_m)$  and  $p$  slow variables  $x = (x_1, \dots, x_p)$ , representing the concentrations of chemical components at time  $t$ , where  $p \geq 2$ . Then a MTN can be rewritten as

$$\dot{x}(t) = f(x_t, y_t), \quad (1)$$

$$\dot{y}(t) = \frac{1}{\epsilon} g(x_t, y_t), \quad (2)$$

where  $\epsilon$  is a small positive real parameter, and  $x_t$  and  $y_t$  are defined by  $x_t \equiv x(t + \theta)$  and  $y_t \equiv y(t + \theta)$ ,  $-r \leq \theta \leq 0$ . (1)-(2) are called a *singularly perturbed system* also known as a fast-slow system with slow  $x$  and fast  $y$ . Such multiple time-scale properties are found in many biochemical systems, in particular gene regulatory systems.

(2) is a PFN for the fixed  $x_t$ , i.e. there are only positive feedback loops except the self-feedback. (1) has a CFN



**Figure 1. An example of a basic MTN. Slow subnetwork is composed of  $p$  nodes. Fast subnetwork is comprised of  $m$  nodes from the  $(p+1)$ -th node to the  $(p+m)$ -th node, and is a PFN. All self-feedback loops are omitted.**

structure except two neighboring variables in  $x_t$  interacting with  $y_t$  or the PFN. Specifically, the structure of (1) has the following form.

$$\begin{aligned} \dot{x}_1(t) &= f_1(x_2(t - \tau_{12}), x_1(t), x_n(t - \tau_{1n})), \\ \dot{x}_i(t) &= f_i(x_{i+1}(t - \tau_{i,i+1}), x_i(t), x_{i-1}(t - \tau_{i,i-1})), \\ &\quad 2 \leq i \leq n-1, \\ \dot{x}_n(t) &= f_n(x_n(t), x_{n-1}(t - \tau_{n,n-1})), \end{aligned} \quad (3)$$

where  $\tau_{i+1,i} = \tau_{i,i+1} = 0$  if both  $\partial f_{i+1}/\partial x_i$  and  $\partial f_i/\partial x_{i+1}$  are non-zero for  $1 \leq i \leq n-1$ . Let the total delay be  $\tau = \sum_i \tau_{i,i+1}$ . Fig.1 is an example of MTN, which can be further extended to a more complex form [1]. Therefore, we have the following theoretical result.

**Theorem 1 (Poincaré-Bendixson Theorem)** *Let  $x(t)$  be a solution of (1) on the time interval  $[t^0, \infty)$ , and bounded. Let  $\omega(x)$  denote the omega-limit set of this solution. Then either*

1.  $\omega(x)$  is a single non-constant periodic orbit; or else
2. for each solution  $u(t)$  of (1) in  $\omega(x)$ , i.e., for solutions with  $u_t \in \omega(x)$  for all  $t$ , we have

$$\alpha(u) \cup \omega(u) \subseteq E, \quad (4)$$

where  $\alpha(u)$  and  $\omega(u)$  denote the alpha- and omega-limit sets, respectively, of this solution, and where  $E$  denotes the set of equilibria of (1).

This theorem does not provide sufficient conditions for periodic orbits, but indicates that the MTN of (1)-(2) has omega-limit sets composed of only periodic orbits and equilibria, which is a desirable property for modelling the oscillations of biological systems.

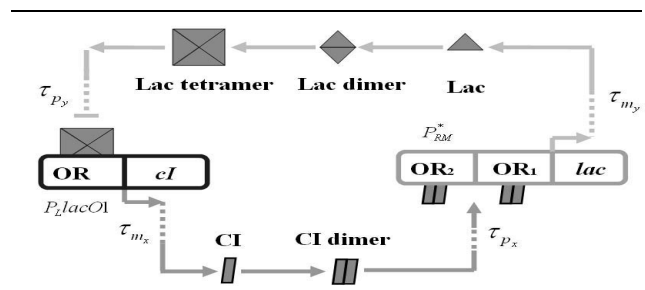
Next we give the following sufficient conditions for non-trivial periodic orbits.

**Theorem 2 (Convergence to periodic orbits).** *If the feedback for total one-direction interactions is sufficiently strong at any equilibrium, then there exists a total time delay  $\bar{\tau}$ , such that (1)-(2) converge to an orbitally and asymptotically stable periodic solution or a periodic self-oscillation when  $\tau > \bar{\tau}$ .*

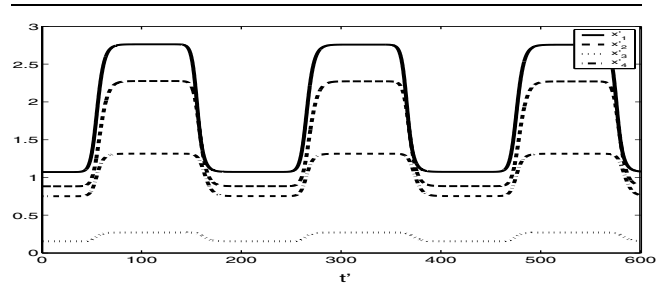
Therefore, we can ensure that the MTN with delays has no equilibrium but periodic oscillation in the domain, which can be used to design robust bio-oscillators in the synthetic gene regulatory networks.

### 3. Numerical implementation

As shown in Fig.2, the synthetic gene regulatory network is a simple two-gene model with genes  $cl$  and  $lac$  under the control of promoters  $P_{LlacO1}$  and  $P_{RM}^*$  respectively. All two genes are both well-characterized transcriptional regulators, which can be found in bacterium *E.coli* and  $\lambda$  phage. We assume that the designed gene network is implemented in a eukaryotic cell, e.g. in *yeast*, so as to examine the effect of time delays on the oscillation dynamics. mRNA of the gene  $cl$  translates the protein CI in cytoplasm, which in turn forms a homodimer and is transported or diffused into the nucleus to enhance the expression of the gene  $Lac$  by binding on the two operator sites of the promoter  $P_{RM}^*$ . On the other hand, mRNA of the gene  $lac$  translates the protein Lac, which forms a homodimer and further a tetramer in the cytoplasm. When moved to the nucleus, the tetramer is in the different form, which represses the expression of the gene  $cl$  by binding on the operator site of the promoter  $P_{LlacO1}$ . The promoters  $P_{LlacO1}$  has one binding site  $OR$  for the Lac tetramer, but the promoter  $P_{RM}^*$  has two binding sites  $OR_1$  and  $OR_2$  for the CI dimers with the affinity priority binding first on  $OR_1$  and second on  $OR_2$ . Note that  $P_{RM}^*$  is a mutated promoter from  $P_{RM}$ , which has no binding site for the tetramer Lac. The Schematic for the synthetic gene network is shown in Fig.2. Different from prokaryotes, there are significant time delays ( $\tau_{mx}$ ,  $\tau_{my}$ ,  $\tau_{px}$ ,  $\tau_{py}$ ) due to transportation or diffusion of mRNAs and transcriptional factors between the nucleus and cytoplasm, which may considerably affect the dynamics of the system.



**Figure 2. A synthetic gene network by  $cl$  and  $Lac$  genes.  $\tau$ 's represent time delays.**

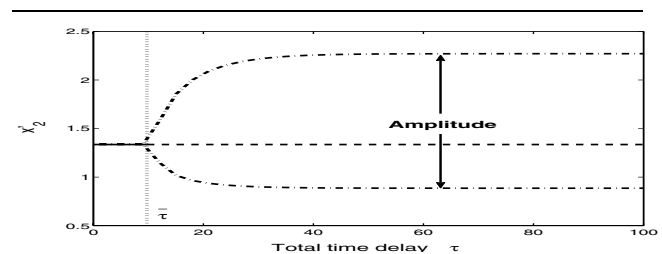


**Figure 3. Sustained oscillations of proteins.**

Fig.3 shows a case for the sustained oscillations and fig. 4 indicates the bifurcation diagram with the total delay as a parameter, which confirms our theoretical prediction.

### References

- [1] R. Wang, T. Zhou, Z. Jing, and L. Chen. Modelling periodic oscillation of biological systems with multiple time scale networks. *Systems Biology*, 1(1), 2004.



**Figure 4. A bifurcation diagram of a protein with total time delay  $\tau$  as a parameter. The solid line and dashed lines represent stable or unstable equilibrium, respectively.**