

# Boolean Network Approach to Negative Feedback Loops of the p53 Pathways: Synchronized Dynamics and Stochastic Limit Cycles

Hao Ge\*      Min Qian †

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## Abstract

Deterministic and stochastic Boolean network models are build for the dynamics of negative feedback loops of the p53 pathways. It is shown that the main function of the negative feedback in the p53 pathways is to keep p53 at a low steady state level, and each sequence of protein states in the negative feedback loops, is globally attracted to a closed cycle of the p53 dynamics after being perturbed by outside signal (e.g. DNA damage). Our theoretical and numerical studies show that both the biological stationary state and the biological oscillation after being perturbed are stable for a wide range of noise level. Applying the mathematical circulation theory of Markov chains, we investigate their stochastic synchronized dynamics and by comparing the network dynamics of the stochastic model with its corresponding deterministic network counterpart, a dominant circulation in the stochastic model is the natural generalization of the deterministic limit cycle in the deterministic system. Moreover, the period of the main peak in the power spectrum, which is in common use to characterize the synchronized dynamics, perfectly corresponds to the number of states in the main cycle with dominant circulation. Such a large separation in the magnitude of the circulations, between a dominant, main cycle and the rest, gives rise to the stochastic synchronization phenomenon.

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\*Centre for Computational System Biology and Mathematical Department, Fudan University, Shanghai 200433, P.R.China; email: edmund\_ge@tom.com

†School of Mathematical Sciences, Peking University, Beijing 100871, P.R.China

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## I Introduction

Thousands of papers have been reported in the field of p53 protein during the last three decades since 1979, including both the experimental and theoretical analysis. p53, the tumor suppressor, transcriptionally activates Mdm2, which in turn targets p53 for degradation (Piette et al. 1997, Prives et al. 1998, Vogelstein et al. 2000, Momand et al. 2000, Michael et al. 2003), keeping p53 at a low steady state level. The low steady state of p53 is essential for normal cell proliferation because p53 induces either cell cycle arrest or programmed cell death. The concentration of p53 increases in response to stress signals, such as DNA damage, inducing a transition to oscillations of p53 level. Following stress signal, p53 also activates transcription of several hundred genes, which are involved in the program of cell cycle arrest, apoptosis, cellular senescence and DNA repair. Therefore, it is important to note that many additional proteins interact with p53 and Mdm2, so that a number of positive and negative autoregulatory feedback loops acting upon the p53 response are embedded inside a network with a few additional interactions (Harris et al. 2005).

Several mathematical models have been proposed to explain the damped oscillations of p53, either in cell population or in a single-cell, most of which are deterministic model of ordinary differential equations (Mihalas et al. 2000, Lev Bar-Or et al. 2000, Monk et al. 2003, Ma et al. 2003, Ciliberto et al. 2005). On the other hand, the stochastic nature of p53 dynamics has also been observed and caused more and more interest nowadays (Ma et al. 2003).

However, in many cellular biochemical modelling, a detailed model, whether the deterministic one based on mass-action law, Michaelis-Menten kinetics and Hill function, or the stochastic molecular number-based CME(**chemical master equation**), is not warranted because of a lack of enough quantitative experimental data. Alternatively, one can develop discrete state network model, i.e., deterministic and stochastic Boolean networks, of a complex biological system using the available information on the activation and repression from one signaling molecules to another, e.g., the kind of signaling wiring diagram as the Fig. 1 in (Li et al. 2004), where

Li et. al. have developed a discrete Boolean model by applying the approach of Hopfield for neural networks for yeast cell-cycle regulatory network with 11 nodes. The main results of (Li et al. 2004) are that the network is both dynamically and structurally stable. The biological steady state, known as the G1 phase of a cell cycle, is a global attractor of the dynamics; the biological pathway, i.e., the returning to G1 phase after perturbation, is a globally attracting dynamic trajectory. The deterministic Boolean model has been further extended to incorporate stochastic dynamics (Zhang et al.2006). They found that both the biological steady state and the biological pathway are well preserved under a wide range of noise level. Furthermore, recently we investigated the synchronized dynamics and nonequilibrium steady states in the stochastic yeast cell-cycle network (Ge et al. 2007), applying the mathematical circulation theory of Markov chains(Jiang et al. 2004).

In the present paper, deterministic and stochastic Boolean network models are build for the dynamics of negative feedback loops of the p53 pathways. It is shown that the main function of the negative feedback in the p53 pathways is to keep p53 at a low steady state level, and each sequence of protein states in the negative feedback loops, is globally attracted to a closed cycle of the p53 dynamics after being perturbed by outside signal (e.g. DNA damage). Our theoretical and numerical studies show that both the biological stationary state and the biological oscillation after being perturbed are stable for a wide range of noise level. Applying the mathematical circulation theory of Markov chains, we investigate their stochastic synchronized dynamics and by comparing the network dynamics of the stochastic model with its corresponding deterministic network counterpart, a dominant circulation in the stochastic model is seen to be the natural generalization of the deterministic limit cycle in the deterministic system.

It is shown that a large separation in the magnitude of the circulation, between a dominant main cycle and the rest, gives rise to the stochastic synchronization phenomenon and the stochastic global attractive behavior, and moreover the power spectrum of the trajectory has a main peak, whose period converges just to the number of states in the dominant cycle. Furthermore, the net circulation of the dominant cycle increases monotonically with the noise-strength parameter  $\beta$ , approaching its deterministic limit; while the circulation of all the other cycles approaches zero very fast when  $\beta$  is quite large. Together, these observations provide a clear picture of the nature of the synchronization and stochastic limit cycles in a stochastic network in

terms of the probabilistic circulation of NESS (nonequilibrium steady states) (Jiang et al. 2004).

For the completeness of the work, in supporting information, we give a theoretical sketch of some relevant results on biological networks, including a classification of the deterministic and stochastic Boolean networks and their correspondence. Also a short introduction of the mathematical theory of stochastic circulation for Markov chains is introduced and applied to deterministic and stochastic networks. It is shown that the stochastic Boolean network is reversible if and only if the matrix  $T$  in the model is symmetric, and the net NESS circulation is strictly positive as long as the probability of the directed cycle is larger than that of its reversal.

## II Boolean network approach

Since the influential work of J.J. Hopfield in 1980s' (Hopfield 1982, Hopfield 1984), the deterministic Boolean (Hopfield) network has been applied to various fields of sciences. Amit (Amit 1989) has introduced a temperature-like parameter  $\beta$  that characterizes the noise in the network and constructed a probabilistic Boolean network called Boltzmann machine. The deterministic and stochastic Boolean networks have found wide range of applications in biological networks.

In our model, the states of the nodes (proteins, DNAs or RNAs) in the network at the  $n$ -th step are represented by variables  $X_n = (X_n^1, X_n^2, \dots, X_n^N)$  respectively, where  $N$  is the number of nodes in the network. Each node  $i$  has only two values,  $X_n^i = 1$  and  $X_n^i = -1$ , representing the active state and the reset state of this node respectively.

### Deterministic Boolean network model:

The deterministic model consider in the present paper is a deformation of model A1 in supporting information. Let us suppose  $N$  is a fixed integer,  $S = \{1, 2, \dots, N\}$ . We take the state space as  $\{-1, 1\}^S$ . Denote the state of the  $n$ -th step as  $X_n = (X_n^1, X_n^2, \dots, X_n^N)$ , then the dynamic is as follows:

If  $X_n \neq (-1, -1, \dots, -1)$ , then

$$X_{n+1}^i = \begin{cases} \text{sign}(H_i), & \text{if } \sum_{j=1}^N T_{ij} X_n^j \neq U_i; \\ X_n^i, & \text{if } \sum_{j=1}^N T_{ij} X_n^j = U_i; \end{cases} \quad (1)$$

where the function  $\text{sign}(x) = \begin{cases} 1 & x > 0; \\ 0 & x = 0; \\ -1 & x < 0, \end{cases}$   $H_i = \sum_{j=1}^N T_{ij}X_n^j - U_i$  is the input to the  $i$ -th node and  $U_i$  ( $1 \leq i \leq N$ ), given *a priori*, are called the threshold of the  $i$ -th unit.

And if  $X_n = (-1, -1, \dots, -1)$ , then  $X_{n+1} = X_n$  when the parameter  $\gamma = 0$  representing that the system is under normal environment, and  $X_{n+1} = (1, -1, -1, \dots, -1)$  when the parameter  $\gamma = 1$  representing there are some perturbation(signal) of the system (e.g. DNA damage) emerge.

### Stochastic Boolean Network model:

The stochastic model consider in the present paper is a deformation of model  $C$  in supporting information, which is similar to the model in (Ge et al. 2007). Consider a Markov chain  $\{X_n = (X_n^1, X_n^2, \dots, X_n^N), n = 0, 1, 2, \dots\}$  on the state space  $\{-1, 1\}^S$ , with transition probability given as follows:

$$P(X_{n+1}|X_n) = \prod_{i=1}^N P_i(X_{n+1}^i|X_n), \quad (2)$$

where

$$P_i(X_{n+1}^i|X_n) = \frac{\exp(\beta X_{n+1}^i H_i)}{\exp(\beta H_i) + \exp(-\beta H_i)},$$

in which  $H_i = \sum_{j=1}^N T_{ij}X_n^j - U_i$  is the input to the  $i$ -th node, if  $\sum_{j=1}^N T_{ij}X_n^j \neq U_i$  and  $X_n \neq (-1, -1, \dots, -1)$  or  $i \geq 2$ ,

$$P_i(X_{n+1}^i|X_n) = \begin{cases} \frac{1}{1+e^{-\alpha}}, & X_{n+1}^i = X_n^i; \\ \frac{e^{-\alpha}}{1+e^{-\alpha}}, & X_{n+1}^i = 1 - X_n^i, \end{cases}$$

if  $\sum_{j=1}^N T_{ij}X_n^j = U_i$  and  $X_n \neq (-1, -1, \dots, -1)$  or  $i \geq 2$ , and

$$P_1(X_{n+1}^1|X_n) = \begin{cases} 1 - \gamma, & X_{n+1}^1 = X_n^1, \\ \gamma, & X_{n+1}^1 = 1 - X_n^1, \end{cases}$$

if  $X_n = (-1, -1, \dots, -1)$ , where the parameter  $0 \leq \gamma \leq 1$  still represents the stochastic perturbations of the system from extracellular environment.

In the above equation,  $\alpha(> 0)$ ,  $\beta(> 0)$ ,  $T_{ij}$  and  $U_i$  ( $1 \leq i, j \leq N$ ) are parameters of the model. The positive temperature-like parameter  $\beta$  represents noise in the system from the perspective of statistical physics (Amit 1989, Zhang et al. 2006). Noticeably, the actual noises within a cell might not be constant everywhere, but here

we use a system-wide noise measure for simplicity. To characterize the stochasticity when the input  $H_i$  to a node is zero, we have to introduce another parameter  $\alpha$ . This parameter controls the likelihood for a protein to maintain its state when there is no input to it.

The previous parameters  $\beta$  and  $\alpha$  represent the intracellular noise due to thermodynamic fluctuations, and on the other hand, we need another parameter  $\gamma$  to characterize the extracellular signal strength with appropriate stochasticity. Since the signal is not purely disordered, we can not express the probability  $P_1(X_{n+1}^i|X_n)$  when  $X_n = (-1, -1, \dots, -1)$  as the exponential form analogous to the Boltzmann distribution, hence we directly regard  $\gamma$  as the probability transiting from  $(-1, -1, \dots, -1)$  to  $(1, -1, \dots, -1)$ .

In our models below,  $T_{ij} = 1$  for a arrow from protein  $j$  to protein  $i$ , and  $T_{ij} = -1$  for a horizontal bar instead of arrowhead from protein  $j$  to protein  $i$ (Fig. 1, 2). And it is indispensable to point out that self-connections haven't been taken into consideration in our models for simplicity.

Similar to Proposition II.6 in supporting information, with the same initial distribution, when  $\gamma \rightarrow 0$ , and  $\alpha, \beta \rightarrow \infty$ , then the stochastic Boolean network model converges to the corresponding deterministic Boolean network model with parameter  $\gamma = 0$ ; and when  $\gamma \rightarrow 1$ , and  $\alpha, \beta \rightarrow \infty$ , then the stochastic Boolean network model converges to the corresponding deterministic Boolean network model with parameter  $\gamma = 1$ .

### III Steady states and synchronized dynamics of the p53 pathways

From biochemical perspective, the microscopic variables for a cellular regulatory network are the concentrations, or numbers, of various mRNAs, regulatory proteins, and cofactors. If all the biochemistry were known, then the dynamics of such a network would be represented by a chemical master equation (McQuarrie 1967, Gillespie 1977). Unfortunately, much of the required information is not available, nor such a “fully-detailed” model will always be useful. Phenomenologically the concentrations of key players of a biochemical regulatory network can often be reduced to two or three states, such as resting state, activated state, inactivated state,

etc. (Fall et al. 2002). The interactions between these states are usually determined from experimental data.

The present study will build simple deterministic and stochastic Boolean network models for several negative feedback loops of the p53 pathways, and more important, provides a sound mathematical explanation of the synchronized dynamics and stochastic limit cycles in the stochastic Boolean network model after being perturbed by stress signals, in terms of the theory of nonequilibrium circulations (Jiang et al. 2004). This makes the description of pathway more definite and penetrating.

### III.1 The core regulation

Negative feedback loops, composed of one transcription arm and one protein-interaction arm, are a common network motif across organisms. p53, the tumor suppressor, transcriptionally activates Mdm2, which in turn targets p53 for degradation. Although it is believed that the existence of negative feedback loop in biological systems can always give rise to oscillations, yet it is not sufficient for the appearing of a limit cycle in the deterministic ODE model from the mathematical point of view.

A bit out of expectation is, if we use the deterministic Boolean network even to the simplest case(Fig.1), the existence of negative feedback already gives rise to a limit cycle corresponding to oscillations in a biological system, when there exists the outside signal (i.e.  $\gamma = 1$ ).

Fig 1.

**Model:** From Section II(For Fig.1).

The first node  $X_n^1$ : p53; and the second node  $X_n^2$ : Mdm2.

$N = 2$  is the number of nodes in the model. For simplicity, we set thresholds as  $U_1 = U_2 = 0$ . And the interacting matrix

$$T = \begin{bmatrix} 0 & -1 \\ 1 & 0 \end{bmatrix}$$

The deterministic model with  $\gamma = 0$  has a global attractor  $(-1, -1)$ , which corresponds to the fact that the main function of the negative feedback between p53 and Mdm2 is to keep p53 at a low steady state level in normal cells.

On the other hand when  $\gamma = 1$ , the deterministic model has a unique limit cycle consist of 4 state, which is described by Table 1. It corresponds to the fact that the stress signal (e.g. DNA damage) will activate the protein p53(i.e. time-2 point in

Table 1) and induce a transition to oscillations of p53 level after being perturbed from the outside environment.

Time	p53	Mdm2
1	1	-1
2	1	1
3	-1	1
4	-1	-1

Table 1: Cycle evolution of the protein states in the negative feedback loop of p53 and Mdm2 after being perturbed.

Moreover, as long as the stress signal strength  $\gamma$  is sufficiently low ( $0 < \gamma \ll 1$ ) or sufficiently high ( $0 < 1 - \gamma \ll 1$ ), the stochastic Boolean network model would preserve the dynamics of the corresponding deterministic model at a certain low level of noise. As it illustrates the same phenomenon as the Cyclin G/Mdm-2 loop given below, so we won't show the data here.

Similar model can also be built and analyzed exactly by the same reasoning, to other ubiquitin ligases that promote p53 ubiquitination and subsequent proteasomal degradation (Fig. 10 in Harris et al. 2005). We only use the simplest example as a start, and pass directly to a really delicate interesting case.

### III.2 Cyclin G/Mdm2 loop

Fig 2.

**Model:** From Section II.

The first node  $X_n^1$ : p53; The second node  $X_n^2$ : cyclin G; The third node  $X_n^3$ : PP2A cyclin G; The last node  $X_n^4$ : Mdm-2.

$N=4$  is the number of nodes in the model. For simplicity, we set thresholds as  $U_1 = U_2 = U_3 = U_4 = 0$ . And from Fig.2, the interacting matrix

$$T = \begin{bmatrix} 0 & 0 & 0 & -1 \\ 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \end{bmatrix}.$$

The deterministic model with  $\gamma = 0$  has a global attractor  $(-1, -1, -1, -1)$ , which corresponds to the fact that p53 is kept at a low steady state level in normal

cells.

On the other hand, the deterministic model when  $\gamma = 1$  has a unique limit cycle consist of 8 state, which is described by Table 2. It corresponds to the fact that the stress signal (e.g. DNA damage) will activate the protein p53(i.e. time-2 point in Table 2) and induce a transition to oscillations of p53 level after being perturbed from the outside environment, which roughly corresponds to the p53 pathway or biological trajectory described in (Harris et al. 2005) “One of the most active of the p53-responsive genes is the cyclin G gene. It is rapidly transcribed to high levels after p53 activation in a wide variety of cell types.”(i.e. Time-3 point in Table 2) “The cyclin G protein makes a complex with the PP2A phosphatase, which removes a phosphate residue from Mdm-2, which is added to the Mdm-2 protein by a cdk kinase” (i.e. Time-4,5 points in Table 2). Our language in the present paper is more definite and penetrating than the quotations.

Time	p53	Cyclin G	PP2A	Cyclin G	Mdm-2
1	(-1	-1		-1	-1)
2	(1	-1		-1	-1)
3	(1	1		-1	-1)
4	(1	1		1	-1)
5	(1	1		1	1)
6	(-1	1		1	1)
7	(-1	-1		1	1)
8	(-1	-1		-1	1)

Table 2: Cycle evolution of the protein states in the Cyclin G/Mdm-2 loop after being perturbed.

### Numerical Simulation of the stochastic Boolean network model

The numerical results about the cyclic motion of this stochastic model are quite similar to the stochastic Boolean network model of the cell-cycle, which we have recently discussed (Ge et al. 2007). The main conclusion is that, given the structure of the negative feedback present in Fig. 2, the stochastic model still exhibits well pronounced oscillations after being seriously perturbed, which is excellently characterized by the circulation theory introduced in the supporting information.

Numerical computations for the current model(Fig.2), are carried out with the

famous Gillespie's method (Gillespie 1977) of the stochastic Boolean network model using MATLAB, and the results are given in the following figures. The network with 4 binary nodes has a total of  $2^4 = 16$  number of states. Here, we can present the dynamics of the network in terms of the integer states  $0, 1, 2, \dots, 2^4 - 1 = 15$  on a line. This 1-d system is reversible if and only if the 4-d system is reversible.

Fig. 3 are the basic behavior of a random trajectory. The upper panel shows that there arises the phenomenon of local rapid synchronization like that observed in (Hopfield 1995) during a very short time period after the value of  $\gamma$  transits from 0.01 to 0.99 at time  $n = 50$ , when  $\beta$  and  $\alpha$  are sufficiently large. The lower panel is a random trajectory over a longer time. Little deviation is shown from the deterministic trajectory in Table 2 after being perturbed at time  $n = 50$ , which implies that the stochastic model still leads to well pronounced oscillations when the perturbation from the environment is sufficiently high.

Fig 3.

Fig. 4 shows the stationary distribution of the state  $(-1, -1, -1, -1)$  in the stochastic model which increasingly approaches 1 when  $\beta$  tends to infinity. This excellently corresponds well to the dynamics of the deterministic model when  $\gamma = 0$ . At large  $\beta$  (low temperature or small noise level), the low level state  $(-1, -1, -1, -1)$  is the most probable state of the system. So analogous to the concept of the deterministic model, this state  $(-1, -1, -1, -1)$  can be regarded as the global attractor of the stochastic model. Moreover, one observes a phase-transition like behavior of the stationary distribution of the state  $(-1, -1, -1, -1)$  while varying the noise level  $\beta$  (similar behavior has been seen in (Qu et al. 2002)).

Fig 4.

Then we turn to investigate the synchronized dynamics when the signal parameter  $\gamma$  is sufficiently high (i.e.  $0 < 1 - \gamma \ll 1$ ). The keys to understand synchronization behavior in stochastic Boolean network models are (i) establishing a correspondence between a stochastic dynamics and its deterministic counterpart; and (ii) identifying the cyclic motion in the stochastic models.

As there is a growing awareness and interest in studying the effects of noise in biological networks, it becomes more and more important to quantitatively characterize the synchronized dynamics mathematically in stochastic models, because the concepts of limit cycle and fixed phase difference no longer holds in this case. Instead, physicists and biologists always have to characterize synchronized dynamics by the distinct peak of some spectrum or just only by observing the stochastic trajectories, which however may cause ambiguities in the conclusion. Therefore,

a logical generalization of limit cycle to the stochastic model is well worth to be developed.

In case of the stochastic models of biological networks, there does exist a rather complete mathematical theory for the cyclic motion of the corresponding Markov chains, which has been developed for more than twenty years (Jiang et al. 2004, Kalpazidou 1995). One of the most important concepts in this mathematical theory of NESS is the circulation, which corresponds to the cycle kinetics in open chemical systems (Hill 1989, Qian 2005). The details of mathematical theory is supplied in the supporting information.

To further characterize the synchronized dynamics, we give Fig. 5 which shows the Fourier power spectrum of the stochastic trajectory with different values of  $\beta$  respectively. Using MATLAB, the discrete Fourier transform for time series  $\{x_1, x_2, \dots, x_n\}$  is defined as

$$y_m = \left| \sum_{k=1}^n x_k e^{-i(2\pi/n)(m-1)(k-1)} \right|, \quad \left( \frac{m-1}{n} 2\pi, 1 \leq m \leq n \right). \quad (3)$$

Therefore, by the Herglotz theorem (Qian et al. 1997 p. 331), the power spectrum of discrete trajectory has a symmetry  $y_m = y_{n+2-m}$ . For different sets of parameters, we found all the calculations give the same outstanding main peak in the Fig. 5. It is important to mention here that by ergodicity, different trajectories give the same power spectrum for any  $\beta$  that is sufficiently large.

Fig 5.

The single dominant peak in Fig. 5 implies there exists a global synchronization and a globally attractive phenomenon. Note that by representating our maps, one-to-one, from the  $N$  binary nodes to the integers  $0 - 15$ , the synchronized behavior is preserved. It is possible that the map will cause some distortion in the power spectrum.

To further illustrate the synchronized behavior, Fig. 6 shows the power spectra of all the 4 individual nodes in the network. While subtle details are different, all exhibit the dominant peak, similar to that of the overall dynamics. This demonstrates further that synchronized dynamics is presented in the network.

Fig 6.

Fig. 7 plots the magnitude and the period of the dominant peak of the power spectrum in Fig. 5 respectively, as functions of the noise strength, i.e., the parameter  $\beta$ . It shows, as we have predicted, that the period converges to 8 which corresponds perfectly to the number of states in the main cycle of Table 2 when  $\beta$  is large. We also put error bars on the upper panel of Fig. 7 with various values of  $\beta$ .

Fig 7.

Finally, Fig. 8 shows how the net circulation of the dominant, main cycle varies with  $\beta$ , applying the determinant presentation of circulations according to Theorem II.4 in the supporting information. Note that circulation is just the time-averaged number of appearance of certain cycle along the stochastic trajectory of our model, and net circulation is just the difference between the circulations along the positive direction and negative direction of a specific cycle. It is clearly seen that the net circulation of the main cycle increases monotonically to  $\frac{1}{8}$  that is just the reciprocal of the number of states in the main cycle, which implies the appearing of more and more distinct synchronization and global attractive behavior with increasing  $\beta$ . The direction of the net circulation does not change. It is also found that the circulation of negative direction along the main cycle is always quite low compared to the circulation of positive direction.

Fig 8.

The net circulations of all the cycles are very small when  $\beta$  and  $\alpha$  are near zero, since the system is close to equilibrium(reversible) state when  $\alpha = \beta = 0$  according to lemma II.9 in supporting information.

For large  $\beta$ , the net circulations of all the cycles except the main cycle are also very small by numerical simulation using the determinant expression in Theorem II.4 of supporting information. All the circulations of non-main cycles actually decrease with increasing  $\beta$  when  $\beta$  is large. Examples are shown in Fig. 9. This large separation in the magnitudes of the weights gives rise to the stochastic synchronization, and this stochastic limit cycle can be defined as a “**stable**” one, whose attractive domain is global.

Fig 9.

As in (Zhang et al. 2006, Ge et al. 2007), we also notice that there exists an inflection point in the curve in Fig. 8. This implies a cooperative transition of the net circulation of the main cycle while varying the noise level  $\beta$ , which equivalently means some sort of “phase transition” around  $\beta = 2$  from chaotic fluctuations(period=infinity) at small  $\beta$  to periodic fluctuations (period=8) at large  $\beta$ . Certainly, the implication of this observation remains to be further elucidated.

## IV Discussion

### From detailed models to simplified Boolean network approach

Deterministic nonlinear mathematical models, based on the Law of Mass Action, have been traditionally used for biochemical reaction networks. Furthermore, noises

are unavoidable in small biochemical reaction systems such as those inside a single cell. Stochastic models with chemical master equations (CME) (McQuarrie 1967, Van Kampen 1981) should be developed, which has already provided important insights and quantitative characterizations in some cases of the biochemical system (Fox et al. 1994, Fox 1997, Zhou et al. 2005). Ref. (Wilkinson 2006) is a good introduction to the stochastic modelling in biology.

In many cellular biochemical modelling, it is impossible to build a detailed, molecular number-based CME model due to a lack of quantitative experimental data. Thus, one often seeks a simplified network model based on simple binary states of the signaling molecules. This leads to the Boolean, Markov network model of the p53 pathways we studied in the present paper.

### **Modelling the oscillatory dynamics of p53 pathways after being perturbed**

The realization that p53 is a common denominator in human cancer has stimulated an avalanche of research since 1989. The p53 gene can integrate numerous signals that control cell life and death, and damped oscillations for p53 and Mdm2 has been observed and modeled (Lev Bar-Or et al. 2000, Ma et al. 2003). The Boolean network models built in the present paper, which omit some parameters to represent the degradation of the p53 protein in cells, only try to explain the mechanism of the oscillatory behavior of the p53 dynamics after being perturbed by stress signals rather than exhibiting the damped oscillations. The signal parameter  $\gamma$  plays a very important role in the model similar to the cell cycle model in (Ge et al. 2007), which can induce the level of p53 from a low steady state to oscillated behaviors.

On the other hand, ideally, one should try to combine these Boolean network models of negative feedback loops together to construct a clear and integrated picture of the p53 pathways, maybe especially including several positive feedback loops (Harris et al. 2005). But unfortunately we haven't developed a reasonable way to do so.

### **Synchronization and circulation in stochastic Boolean network**

Synchronization is an important characteristics of many biological networks (Strogatz 2003, Winfree 2000) whose dynamics has been modelled traditionally by deterministic, coupled nonlinear ordinary differential equations in terms of regulatory mechanisms and kinetic parameters (Murray 2002, Fall et al. 2002). Two

important classes of biological networks which have attracted wide attentions in recent years are neural networks (Scott 2002) and cellular biochemical networks (Goldbeter 1996).

As we know, the occurrence of a deterministic limit cycle in an ODE model is the hallmark of a synchronization phenomenon, while such a definite concept no longer holds in a stochastic system. It is observed that in our present model of p53 pathways, the trajectory concentrates around a main cycle, which we call **stochastic limit cycle** and is very similar to in many aspects the limit cycle of a deterministic model. Furthermore, the present work shows that the circulation in an irreversible Markov chain (Jiang et al. 2004) is a reasonable generalization of the concept of deterministic limit cycles, and provides a sound mathematical explanation of the synchronization in the stochastic Boolean network model.

### **Stability and robustness of the p53 genetic networks**

Biological functions in living cells are controlled by protein interaction and genetic networks, and have to be robust to function in complex (and noisy) environments. More robustness would also mean being more evolvable, and thus more likely to survive.

More precisely, these molecular networks should be dynamically stable against various fluctuations which are inevitable in the living world. Therefore, the stability and robustness of Boolean network model can not be determined if the noise hasn't been introduced into the model, since it is not reasonable to simply apply the Euclidean topology in mathematics to such a real biological discrete model not to say the magnitudes of the different attractive basins of fixed states and limit cycles as in the deterministic models. For instance, the recent analysis of stochastic cell-cycle Boolean networks (Ge et al. 2007, Zhang et al. 2006) just claimed the stability and robustness of the previous deterministic model (Li et al. 2004), in which Li, et.al. announced but didn't really investigate the robustness of this model against perturbation. Hence, the numerical and theoretical studies in the present paper in some sense excellently exhibit the stability and robustness of the p53 genetic networks.

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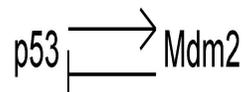


Figure 1: Negative feedback loop of p53 and Mdm2. Arrows denote stimulatory interactions, whereas horizontal bars instead of arrowheads indicate inhibitory influences.

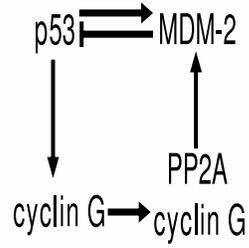


Figure 2: Cyclin G/Mdm2 loop, redrawn from (Fig. 8 in Harris et al. 2005).

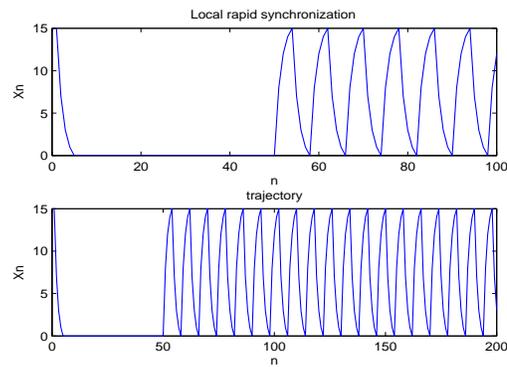


Figure 3: Stochastic trajectory and synchronization of the Cyclin G/Mdm-2 loop. Simulations are carried out with the parameters  $\alpha = 5$ ,  $\beta = 5$  and the values of  $\gamma$  transits from 0.001 to 0.999 at time  $n = 50$ .

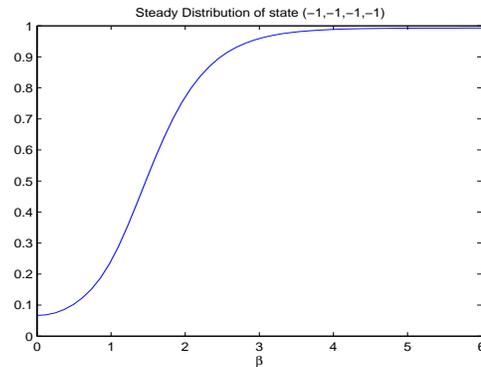


Figure 4: Stationary distribution of the state  $(-1, -1, -1, -1)$  in the stochastic model. Simulations are carried out with the parameters  $\alpha = 5$  and  $\gamma = 0.001$ .

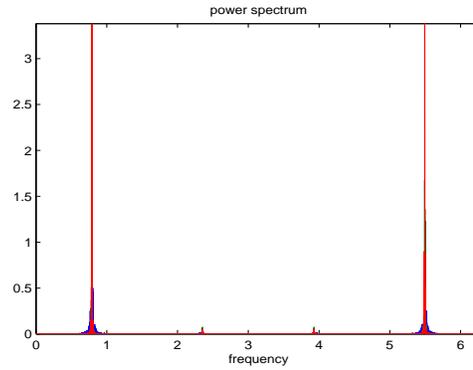


Figure 5: Power spectrum of the overall trajectory in the Cyclin G/Mdm-2 loop with the parameter  $\alpha = 5$  and  $\gamma = 0.999$  fixed, and with different  $\beta$ : blue:  $\beta=2.4$ ; green:  $\beta=4.8$ ; red:  $\beta = 6$ . The discrete Fourier transform causes an alias; hence the spectrum is symmetric with respect to  $\pi$  on the  $[0, 2\pi]$  interval.

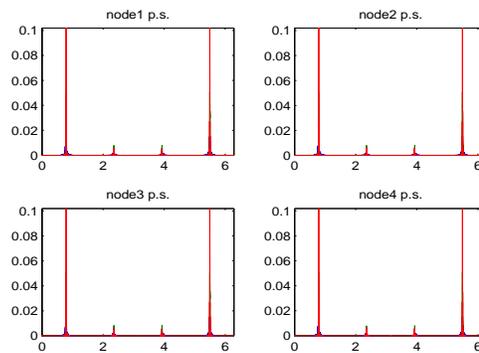


Figure 6: Power spectra of individual nodes in the Cyclin G/Mdm-2 loop show a synchronization among all the nodes with the parameter  $\alpha = 5$  and  $\gamma = 0.999$  fixed, and different  $\beta$ : blue:  $\beta=2.4$ ; green:  $\beta=4.8$ ; red:  $\beta=6$ .

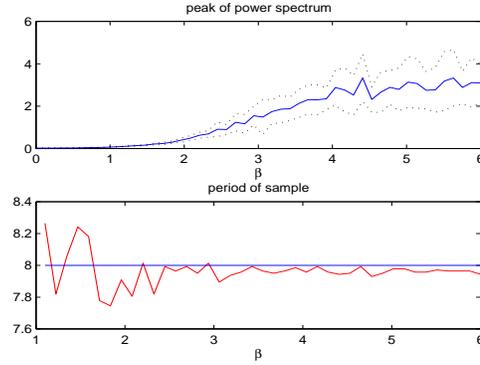


Figure 7: Magnitude and period of the dominant power-spectral peak as functions of  $\beta$  in the Cyclin G/Mdm-2 loop, with  $\alpha = 5$  and  $\gamma = 0.999$ . In the upper panel, the magnitude of the dominant power-spectral peak is averaged over 20 simulations. The solid curve is the mean, and the dotted curves are the mean  $\pm$  standard deviation. The period approaches to 6 (the horizontal line) with increasing  $\beta$ .

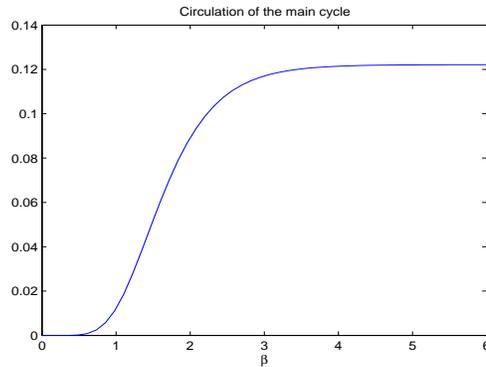


Figure 8: Net circulation of the main cycle in the Cyclin G/Mdm-2 loop as a function of the noise strength,  $\beta$ , with the parameter  $\alpha = 5$  and  $\gamma = 0.999$  fixed.

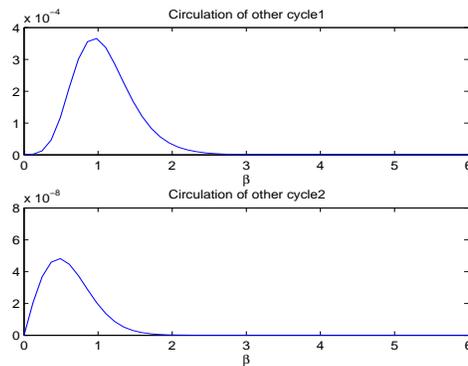


Figure 9: Net circulation of several other cycles in the Cyclin G/Mdm-2 loop as a function of the noise strength,  $\beta$ , with the parameter  $\alpha = 5$  and  $\gamma = 0.999$  fixed.

# Supporting Information

Hao Ge\*      Min Qian †

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## I Theoretical Sketch of Some Relevant Results on Deterministic and Stochastic Boolean Networks

This section is mainly restated from (Ge et al. 2007).

We first give a brief account of the deterministic Hopfield networks and its stochastic incarnation, the stochastic Boolean networks (sometimes called Boltzmann machines). They can be mainly categorized into several classes as follows (Amit 1989).

Let us suppose  $N$  is a fixed integer,  $S = \{1, 2, \dots, N\}$ . We take the state space as  $\{-1, 1\}^S$ .

### Model A: deterministic

*A1 (discrete time, synchronous, McCulloch-Pitts):* Denote the state of the  $n$ -th

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\*School of Mathematical Sciences, Peking University, Beijing 100871, P.R.C.; email: edmund\_ge@tom.com

†School of Mathematical Sciences, Peking University, Beijing 100871, P.R.C.

step as  $X_n = (X_n^1, X_n^2, \dots, X_n^N)$ , then the dynamic is

$$X_{n+1}^i = \text{sign}^1 \left( \sum_{j=1}^N T_{ij} X_n^j - U_i \right), \text{ if } \sum_{j=1}^N T_{ij} X_n^j \neq U_i; \quad (1)$$

and if  $\sum_{j=1}^N T_{ij} X_n^j = U_i$ , then  $X_{n+1}^i$  randomly choose 1 or  $-1$  with probability  $\frac{1}{2}$  respectively.  $U_i$  ( $1 \leq i \leq N$ ), given *a priori*, are called the threshold of the  $i$ th unit.

*A2 (continuous time, synchronous)*: Every state has an exponentially distributed stochastic waiting-time, with mean waiting-time  $\lambda^{-1}$ , then chooses the next state by the same rule of model A1.

*A3 (discrete time, asynchronous, Hopfield)*: The neurons are updated one by one, in some prescribed sequence, or in a random order. If the previous state  $\sigma$  satisfies  $\sum_{j=1}^N T_{ij} \sigma_j > U_i$ , then  $\sigma_i$  changes to be 1, otherwise changes to be  $-1$ .

*A4 (continuous time, asynchronous)*: Every state has an exponentially distributed stochastic waiting-time, with rate constant  $\lambda$ , then chooses the next state by the same rule of model A3.

**Remark I.1** *Note that by deterministic, we mean the transition from one state to the next is deterministic. But the systems with continuous-time will still behave stochastically due to the Poisson nature in the transition time.*

## Model B: stochastic Boolean networks

*B1 (discrete time, synchronous)*: Consider the Markov chain

$$\{X_n = (X_n^1, X_n^2, \dots, X_n^N), n = 0, 1, 2, \dots\} \quad (2)$$

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$${}^1\text{The function } \text{sign}(x) = \begin{cases} 1 & x > 0; \\ 0 & x = 0; \\ -1 & x < 0. \end{cases}$$

on state space  $\{-1, 1\}^S$ , with transition probability given as follows: for each pair of states  $\sigma, \eta \in \{-1, 1\}^S$ , the probability transiting from  $\sigma$  to  $\eta$

$$p_{\sigma\eta} = \prod_{i=1}^N \frac{\exp(\beta\eta_i(\sum_{j=1}^N T_{ij}\sigma_j - U_i))}{\exp(\beta(\sum_{j=1}^N T_{ij}\sigma_j - U_i)) + \exp(-\beta(\sum_{j=1}^N T_{ij}\sigma_j - U_i))}, \quad (3)$$

where  $\beta(> 0)$ ,  $T_{ij}$  and  $U_i$  ( $1 \leq i, j \leq N$ ) are parameters of the model.

*B2 (continuous time, synchronous):* Consider the continuous-time Markov chain  $\{\xi_t : t \geq 0\}$  on state space  $\{-1, 1\}^S$ . Every state waits an exponential time with meantime  $\lambda^{-1}$  until choosing the next state by the rule of model *B1*. So the transition density matrix is

$$q_{\sigma\eta} = \lambda p_{\sigma\eta}, \quad \forall \sigma, \eta \in \{-1, 1\}^S. \quad (4)$$

*B3 (discrete time, asynchronous):* Denote  $\sigma^i$  to be the new state which changes the sign of the  $i$ th coordinate of  $\sigma$ . Consider the Markov chain  $\{X_n = (X_n^1, X_n^2, \dots, X_n^N), n = 0, 1, 2, \dots\}$  on state space  $\{-1, 1\}^S$ . The neurons are updated one by one, in some prescribed sequence, or in a random order. Then choose the next state according to the probability:

$$p_{\sigma\sigma^i} = \frac{\exp(\beta\sigma^i(\sum_{j=1}^N T_{ij}\sigma_j - U_i))}{\exp(\beta(\sum_{j=1}^N T_{ij}\sigma_j - U_i)) + \exp(-\beta(\sum_{j=1}^N T_{ij}\sigma_j - U_i))}, \quad \sigma \in \{-1, 1\}^S,$$

where  $\beta > 0$ ; and  $p_{\sigma\eta} = 0$ , if  $\eta \neq \sigma^i$  for each  $i$ .

*B4 (continuous time, asynchronous):* Every state waits an exponential time with meantime  $\lambda^{-1}$  until choosing the next state by the rule of model *B3*. So the transition density matrix is

$$q_{\sigma\sigma^i} = \lambda p_{\sigma\sigma^i}, \quad \forall \sigma \in \{-1, 1\}^S. \quad (5)$$

The third class given below is a variant of the model *B1*. It is included here since it is the model used for the probabilistic Boolean network of cell-cycle regulation in

(Ge et al. 2007).

### Model C: deformation

Fix  $\alpha > 0$ . Consider a new Markov chain  $\{X_n = (X_n^1, X_n^2, \dots, X_n^N), n = 0, 1, 2, \dots\}$  on the state space  $\{-1, 1\}^S$  taking the model B1 as defined initially, with transition probability given as follows:

$$P(X_{n+1}|X_n) = \prod_{i=1}^N P_i(X_{n+1}^i|X_n), \quad (6)$$

where we define

$$P_i(X_{n+1}^i|X_n) = \begin{cases} \frac{\exp(\beta X_{n+1}^i (\sum_{j=1}^N T_{ij} X_n^j - U_i))}{\exp(\beta (\sum_{j=1}^N T_{ij} X_n^j - U_i)) + \exp(-\beta (\sum_{j=1}^N T_{ij} X_n^j - U_i))} & \text{if } \sum_{j=1}^N T_{ij} X_n^j \neq U_i \\ \frac{1}{1+e^{-\alpha}} & \text{if } \sum_{j=1}^N T_{ij} X_n^j = U_i \text{ and } X_{n+1}^i = X_n^i \\ \frac{e^{-\alpha}}{1+e^{-\alpha}} & \text{if } \sum_{j=1}^N T_{ij} X_n^j = U_i \text{ and } X_{n+1}^i = 1 - X_n^i. \end{cases}$$

**Remark I.2** *The model C differs from B1 when  $\sum_{j=1}^N T_{ij} X_n^j - U_i = 0$ , and the latter is also a special case of the former when  $\alpha = 0$ .*

## II Mathematical Circulation Theory of Network

### Nonequilibrium Steady States

This section is also mainly restated from (Ge et al. 2007), ignoring those detailed proofs.

There are many different approaches to the theory of nonequilibrium statistical mechanics in the past (Nicolis et al. 1977, Keizer 1987), mathematical theories of which have emerged in the last two decades, and Jiang et.al. have summarized their results of this theory in a recent monograph (Jiang et al. 2004). The most

important concepts in the theory are (i) reversibility of a stationary process that corresponds to thermodynamic equilibrium, and (ii) the circulation in a stationary process which corresponds to NESS. A key result of the theory is the circulation decomposition of NESS.

## II.1 Circulation theory of nonequilibrium steady states

Hill (Hill 1989) constructed a theoretical framework for discussions of vivid metabolic systems, such as active transport, muscle contractions, etc. The basic method of his framework is diagram calculation for the cycle flux on the metabolic cycles of those systems (Hill 1989). He successively found that the result from diagram calculation agrees with the data of the numbers of completing different cycles given by random test (Monte Carlo test), but did not yet prove that the former is just the circulation rate in the sense of trajectory of a corresponding Markov chain. In Chapter 1 and 2 of (Jiang et al. 2004), Markov chains with discrete time and continuous time parameter are used as models of Hill's theory on circulation in biochemical systems. The circulation rate is defined in the sense of trajectories and the expressions of circulation rate are calculated which coincide with Hill's result obtained from diagrams. Hence the authors verify that Hill's cycle flux is equivalent to the circulation rate defined in the sense of trajectories.

Below we only state the circulation theory of discrete-time Markov chains, and refer to Chapter 2 in (Jiang et al. 2004) for the quite similar circulation theory of continuous-time Markov chains.

First of all, we state the main results, rewritten in terms of the cycle representation of stationary homogeneous Markov chains (Kalpazidou 1995, Theorem 1.3.1),

which is analogous to the Kirchoff's current law and circuit theory in the networks of master equation systems (Schnakenberg 1976).

**Theorem II.1** *Given a finite oriented graph  $G = (V, E)$  and the weight on every edge  $\{w_e > 0 : e \in E\}$ . If the weight satisfies the balance equation(input=output)*

$$\sum_{e^+=i} w_e = \sum_{e^-=i} w_e, \quad \forall i \in V, \quad (7)$$

*then there exist a positive function defined on the oriented cycles  $\{w_c : c = (e_1, e_2, \dots, e_k), k \in \mathbb{Z}^+\}$ , such that*

$$w_e = \sum_{e \in c} w_c, \quad \forall e \in E. \quad (8)$$

For a finite stationary Markov chain  $X$  with finite state space  $S = \{1, 2, \dots, N\}$ , transition matrix  $P = \{p_{ij} : i, j \in S\}$  and invariant distribution  $\bar{\pi} = \{\pi_1, \pi_2, \dots, \pi_N\}$ , one can take its state space to be the group of vertexes, and  $E = \{e : e^- = i, e^+ = j, p_{ij} > 0\}$  with weight  $\{w_e = \pi_i p_{ij}\}$ . Then notice that (7) is satisfied, since  $\sum_j p_{ij} = 1$  for each  $i$  and

$$\sum_j \pi_i p_{ij} = \pi_i = \sum_j \pi_j p_{ji}, \quad \forall i \in V,$$

we can conclude that

**Corollary II.2** *(cycle decomposition) For an arbitrary finite stationary Markov chain, there exists a positive function defined on the group of oriented circuits  $\{w_c : c = (i_1, i_2, \dots, i_k), k \in \mathbb{Z}^+\}$  such that*

$$\pi_i p_{ij} = \sum_c w_c J_c(i, j), \quad \forall i, j \in V, \quad (9)$$

*where  $J_c(i, j)$  is defined to be 1 if the cycle  $c$  includes the path  $i \rightarrow j$ , otherwise 0.*

**Definition II.3**  $w_c$  is called the **circulation** along cycle  $c$ .

For any  $i, j \in S, i \neq j$ ,

$$\pi_i p_{ij} - \pi_j p_{ji} = \sum_c (w_c - w_{c_-}) J_c(i, j), \quad (10)$$

where  $c_-$  denotes the reversed cycle of  $c$ . Equation (10) is called the **circulation decomposition** of the stationary Markov chain  $X$ , and the difference between the circulations of positive direction and negative direction along the cycle  $c$  (i.e.  $w_c - w_{c_-}$ ) is called the **net circulation** of cycle  $c$ , which actually represents the true flux of this cycle.

It can be proved that generally the circulation decomposition is not unique, i.e. it is possible to find another set of cycles  $\mathcal{C}$  and weights on these cycles  $\{w_c | c \in \mathcal{C}\}$  which also fit (10).

However, the most reasonable choice of circulation definition is the one defined in the sense of trajectories from the probabilistic point of view. Along almost every sample path, the Markov chain generates an infinite sequence of cycles, and if we discard every cycle when it is completed and at the meantime record it down, then we can count the number of times that a specific cycle  $c$  is formed by time  $t$ , which we denote by  $w_{c,t}(\omega)$ .

The following theorem is recapitulated from Theorem 1.3.3 in (Jiang et al. 2004).

**Theorem II.4** Let  $\mathcal{C}_n(\omega)$ ,  $n = 0, 1, 2, \dots$ , be the class of all cycles occurring until  $n$  along the sample path  $\{X_l(\omega)\}$ . Then the sequence  $(\mathcal{C}_n(\omega), w_{c,n}(\omega)/n)$  of sample weighted cycles associated with the chain  $X$  converges almost surely to a class

$(\mathcal{C}_\infty, w_c)$ , that is,

$$\mathcal{C}_\infty = \lim_{n \rightarrow +\infty} \mathcal{C}_n(\omega), \quad \text{a.e.} \quad (11)$$

$$w_c = \lim_{n \rightarrow +\infty} \frac{w_{c,n}(\omega)}{n}, \quad \text{a.e.} \quad (12)$$

Furthermore, for any directed cycle  $c = (i_1, i_2, \dots, i_s) \in \mathcal{C}_\infty$ , the weight  $w_c$  is given by

$$w_c = p_{i_1 i_2} p_{i_2 i_3} \cdots p_{i_{s-1} i_s} p_{i_s i_1} \frac{D(\{i_1, i_2, \dots, i_s\}^c)}{\sum_{j \in S} D(\{j\}^c)}. \quad (13)$$

where  $D = \{d_{ij}\} = I - P = \{\delta_{ij} - p_{ij}\}$  and  $D(H)$  denotes the determinant of  $D$  with rows and columns indexed in the index set  $H$ . The function  $\delta_{ij} = \begin{cases} 0, & i \neq j; \\ 1, & i = j, \end{cases}$  is the well known Kronecker delta function.

It is important to emphasize that the circulations defined in the above theorem also satisfy the circulation decomposition relation (10).

The above theorem not only rigorously confirms the Hill's theory, but also gives a prior substitute method of the widely used diagrammatic method. The complexity of directed diagrams and cycles increases rapidly with the number of states in the model, while the determinant interpretation is much more systematic and easy to be applied using the mathematics softwares. But it will still cost excessive time to compute these determinants if there are hundreds of states in the model.

Luckily, a Monte Carlo method using the so-called **derived chain method** (Section 1.2 in Jiang et al. 2004) to compute the cycle fluxes has already been developed according to the above theorem, the main idea of which is just simply to discard every cycle when it is completed and at the meantime record it down so as to count the number of times that a specific cycle  $c$  is formed by time  $t$  (i.e.  $w_{c,t}(\omega)$ ).

Then when the time  $t$  is long enough, one gets the approximated circulation of the cycle  $c$  (i.e.  $w_c \approx \frac{w_{c,t}(\omega)}{t}$ ).

Recently, the Boolean yeast cell-cycle network model discussed in (Ge et al. 2007) has 2048 states and it is found that the Monte Carlo method is much more efficient than the method of determinant interpretation, because the latter is even impossible to be applied to such a large model upon a normal computer.

The relationship between circulation and NESS is as follows, which is recapitulated from Theorem 1.4.8 in (Jiang et al. 2004).

**Theorem II.5** *Suppose that  $X$  is an irreducible and positive-recurrent stationary Markov chain with the countable state space  $S$ , the transition matrix  $P = (p_{ij})_{i,j \in S}$  and the invariant probability distribution  $\Pi = (\pi_i)_{i \in S}$ , and let  $\{w_c : c \in \mathcal{C}_\infty\}$  be the circulation distribution of  $X$ , then the following statements are equivalent:*

- (i) *The Markov chain  $X$  is reversible.*
- (ii) *The Markov chain  $X$  is in detailed balance, that is,*

$$\pi_i p_{ij} = \pi_j p_{ji}, \forall i, j \in S.$$

- (iii) *The transition probability of  $X$  satisfies the **Kolmogorov cyclic condition**:*

$$p_{i_1 i_2} p_{i_2 i_3} \cdots p_{i_{s-1} i_s} p_{i_s i_1} = p_{i_1 i_s} p_{i_s i_{s-1}} \cdots p_{i_3 i_2} p_{i_2 i_1},$$

*for any directed cycle  $c = (i_1, \cdots, i_s)$ .*

- (iv) *The components of the circulation distribution of  $X$  satisfy the symmetry condition:*

$$w_c = w_{c^-}, \forall c \in \mathcal{C}_\infty.$$

Consequently, when the system is in a nonequilibrium steady state, there exists at least one cycle, containing at least three states, round which the circulation rates

of one direction and its opposite direction are asymmetric (unequal), so as to cause a net circulation of the cycle. In theoretic analysis, if there is a large separation in the magnitude of the circulation, between few dominant, main cycles and the rest, it gives rise to the stochastic synchronization phenomenon and helps to distinguish the most important main biological pathways, which can be observed in experiments.

## II.2 Applied to deterministic and stochastic Boolean networks

The keys to understand synchronization behavior in stochastic models are (i) establishing a correspondence between a stochastic dynamics and its deterministic counterpart; and (ii) identifying the cyclic motion in the stochastic models.

In the framework of the stochastic theory, deterministic models are simply the limits of stochastic processes with vanishing noise. This is best illustrated in the following proposition.

**Proposition II.6** *With the same initial distribution, when  $\beta \rightarrow \infty$ , the model  $B_k$  converges to the model  $A_k$  in distribution, for  $k = 1, 2, 3, 4$ .*

We shall further discuss the necessary condition for stochastic Boolean networks to have cyclic motion. In the theory of neural networks, one of Hopfield's key results (Hopfield 1982,1984) is that for symmetric matrix  $T_{ij}$ , the network has an energy function. In the theory of Markov processes, having a potential function (Kolmogorov cyclic condition in Theorem II.5) is a sufficient and necessary condition for a reversible process. A connection is established in the following theorem.

**Theorem II.7** For  $k = 1, 2, 3, 4$ , the Markov chain  $\{X_n\}$  in the model  $B_k$  is reversible if and only if  $T_{ij} = T_{ji}, \forall i, j = 1, 2, \dots, N$ , i.e. the matrix  $T$  is symmetric.

From the above two conclusions, it is obvious that

**Corollary II.8** When  $T_{ij} = T_{ji}, \forall i, j = 1, 2, \dots, N$ , there doesn't exist any limit cycle consist of more than two states in model  $A_k, k = 1, 2, 3, 4$ .

**Lemma II.9** For  $k = 1, 2, 3, 4$ , the Markov chain  $\{X_n\}$  in the model  $B_k$  is reversible when  $\beta$  is zero, and the Markov chain  $\{X_n\}$  in the model  $C$  is reversible when  $\beta$  and  $\alpha$  are both zero.

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