

# Modeling of Hysteresis in a Mammalian Gene Regulatory Network

J. Hu, K.R. Qin, C. Xiang\*, and T.H. Lee

*Department of Electrical and Computer Engineering, National University of Singapore  
Graduate School for Integrative Sciences and Engineering, National University of Singapore  
Email: {hujuan, eleqk, elexc, eleleeth}@nus.edu.sg*

Hysteresis, observed in cell cycle or gene regulatory network experimentally, has a pivotal impact on biological systems, in the sense of enabling cells to adopt multiple internal expression states in response to a single external input signal. In a synthetic hysteretic mammalian transcription network, the transactivator (TA) cotranscribed by TA's cognate promoter is repressed by the transrepressor, whose activity is modulated by the macrolide antibiotic erythromycin (EM). The SEAP (human placental secreted alkaline phosphatase) is expressed cocistronically with TA. The interconnection of SEAP concentrations versus EM concentrations is demonstrated to be hysteresis in the experiment. In this paper, the modified Bouc-Wen hysteresis model is developed to describe the hysteresis in the mammalian gene network. Simulation result is presented to verify the capability and accuracy of the mathematical model to describe the hysteresis phenomenon in the mammalian gene regulatory network. Comparative study has shown better performance with this model than previous one in the literature.

## 1. INTRODUCTION

Hysteresis is a ubiquitous phenomenon that occurs in diverse disciplines ranging from electronics to economics, from mechanics to life science.<sup>1</sup> Roughly speaking, hysteresis refers to the phenomenon that the response of a system takes on different values for an increasing input than for a decreasing one. In biology, hysteresis provides a mechanism that enhances the robustness of cell functions against random perturbations.<sup>2-4</sup> Hysteresis exists in Cell cycle of *Xenopus* egg extracts as the amount of cyclin needed to induce entry into mitosis is bigger than the amount of cyclin needed to hold the extract in mitosis, which results in a bistable system with a ratchet to prevent slipping back from mitosis to interphase.<sup>2</sup> The hysteretic response of CDK activity to total cyclin is examined to be a much more robust mechanism for generating cell cycle dynamics than non-hysteretic mechanisms.<sup>3</sup>

Hysteresis also occurs in the synthetic gene regulatory networks.<sup>4, 5</sup> In Kramer and Fussengger's experiment, the existence of hysteresis is verified in a mammalian gene regulatory network.<sup>5</sup> In this pioneering work, hysteresis loop of the experiment data is presented, characterizing by requiring bigger signal to switch from OFF-to-ON than from ON-to-OFF, and a mathematical model is constructed to model

the mammalian synthetic hysteretic gene regulatory network by characterizing every component of the network. Simulation study by fitting the model to the experiment data shows that the model has captured the important characteristic of the hysteretic gene regulatory network, but the shape of the simulated loop doesn't match the experiment data well. Therefore, a more accurate mathematical model for the mammalian hysteretic gene regulatory network is needed. The Bouc-Wen model is very popular in mechanical engineering due to the ease of its numerical implementation and its ability to represent a wide range of hysteresis loop shapes.<sup>7, 9</sup> But this original Bouc-Wen model can only generate stable clockwise hysteresis loop while hysteresis is often counter clockwise in biological systems, either the cell cycle<sup>2, 3</sup> or the gene regulatory network.<sup>5</sup> Therefore, in this paper the Bouc-Wen model is modified to describe hysteresis in the mammalian gene regulatory network studied in the literature<sup>5</sup>. Simulation study shows that this model is more consistent with the experiment data compared with the previous model in the literature<sup>5</sup>.

In this paper, we first present the gene regulatory network with hysteresis and the development of the modified Bouc-Wen hysteresis model in Section 2 and 3. Simulation results and comparison study are given in Section 4. The final section offers discussion

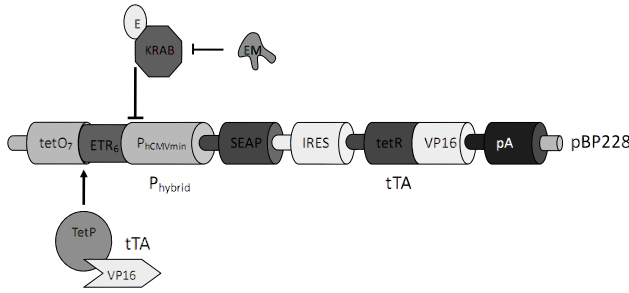
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\*Corresponding author.

and conclusion.

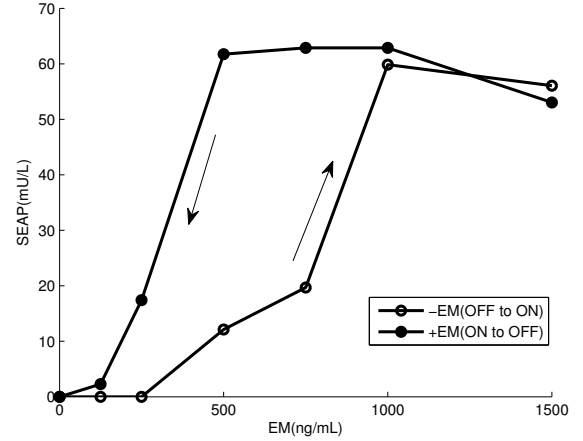
## 2. The gene regulatory network with hysteresis

In Kramer and Fussengger's experiment<sup>5</sup>, the tetracycline dependent transactivator (TA) induces a hybrid promoter (tetO7-ETR8-PhCMVmin) driving its own as well as SEAP (human placental secreted alkaline phosphatase) expression by means of a positive feedback loop. The macrolide dependent transrepressor represses tetO7- ETR8-PhCMVmin-driven transcription in an erythromycin(EM)-responsive manner. The molecular configuration is shown in Fig.1<sup>5</sup>, capitalizing the interconnection of tetracycline- and macrolide- responsive transgene control modalities.



**Fig. 1.** Hysteretic synthetic mammalian gene regulatory network

The clinically licensed antibiotic EM modulates the activity of The transactivator TA through abolishing the transrepressor capacity, which also binds to the hybrid promoter with an inhibition effect. SEAP is expressed cocistronically with TA. Therefore, Varying  $[EM]$  concentrations result in different active transrepressor concentrations and can be used to regulate  $[SEAP]$ . The square brackets  $[.]$  denote concentration. Experiment result in Fig.2 has shown that the existence of the hysteresis in the dynamic behavior of  $[SEAP]$  versus  $[EM]$ , which is the EM-SEAP kinetics. The gene regulatory network shows hysteretic expression behavior characterized by higher  $[EM]$  required for OFF-to-ON than ON-to-OFF expression changes. Correspondingly, the hysteresis loop in Fig.2 is counter clockwise.<sup>5</sup>

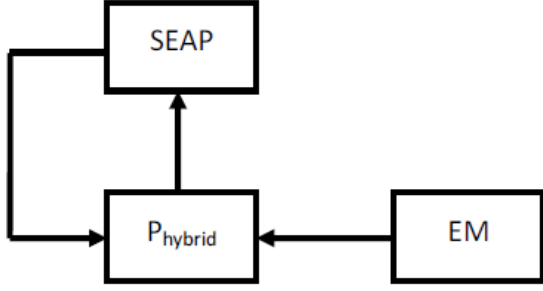


**Fig. 2.** Hysteresis in the gene regulatory network in Cell B

The Chinese hamster ovary (CHO) cells CHO-HYST were cultured in the experiment and two different clones CHO-HYST42 (Cell A) and CHO-HYST44(Cell B) are chosen. In the following sections, we are going to use the experiment data from Cell A to develop our model and use that from Cell B to test the model and also do comparison study with other model.

## 3. Mathematical model development

The fundamental point in mathematical modeling of biological system is that the model should be built upon the understanding of the inside biological mechanism. In the mammalian hysteretic gene regulatory network, EM inhabits the action of the transrepressor and transrepressor itself binds to the hybrid promoter (tetO7-ETR8-PhCMVmin, in Fig.1) by repression effect. As a whole effect, it equals that EM activates the hybrid promoter which then drives the expression of SEAP and TA. The kinetics of coupling between EM and SEAP is posited as a two-step process shown in Fig.3. Correspondingly, the mathematical model is developed in two steps. First, a Hill function is used to describe the interaction between EM and the chimeric promoter. Second, the hill function is incorporated into the modified Bouc-Wen model which is used to model the hysteresis in the gene regulatory network.



**Fig. 3.** configuration of the mammalian gene regulatory network

Hill function is commonly used in biochemistry and its mathematical form is as follows,

$$x_1 = \frac{ax_0^n}{b^n + x_0^n} \quad (1)$$

where  $x_0$  denotes the  $[EM]$  in the experiment.  $x_1$  is a variable representing the conformational changes in the hybrid promotor.  $a, b$  are constant parameters and  $n$  is the Hill coefficient describing cooperativity and its value is system-dependent.  $n > 1$  means positively cooperative reaction between  $x_0$  and  $x_1$ ,  $n < 1$  means negatively cooperative reaction while  $n = 1$  means noncooperative reaction. Therefore, it can be predicted that the parameter  $n$  should be bigger than 1 in this gene regulatory network as the interconnection of EM and hybrid promotor is positively cooperative.

In second step, the modified Bouc-Wen model is used to describe the Counter clockwise hysteresis loop observed in EM-SEAP kinetics in the experiment. The model is expressed by the following differential equation.

$$\dot{w} = \rho(\dot{x} - \sigma|x|w + \delta x|\dot{w}| - \gamma w|\dot{w}|) \quad (2)$$

where

$$x = -1 + 2 \frac{x_1 - c_0}{c_m - c_0} \quad (3)$$

$$c_0 = \frac{ax_{0min}^n}{b^n + x_{0min}^n} \quad (4)$$

$$c_m = \frac{ax_{0max}^n}{b^n + x_{0max}^n} \quad (5)$$

$$w = -1 + 2 \frac{x_2 - x_{2min}}{x_{2max} - x_{2min}} \quad (6)$$

In the above equations,  $x_1$  from Hill function is incorporated into the modified Bouc-Wen model as  $x$  is the normalized variable of  $x_1$ .  $w$  is the normalized variable of  $x_2$ , which denotes the  $[SEAP]$ .  $x_{0min}, x_{0max}$  are the minimum and maximum values of the  $x_0$  or  $[EM]$ , and  $x_{2min}, x_{2max}$  are the minimum and maximum values of  $x_2$  or  $[SEAP]$ , respectively.  $\rho, \sigma, \delta, \gamma$  are the model parameters to be determined. Compared to the original normalized Bouc-Wen model in the literature<sup>7-9</sup>, the third term on the right side is modified, and an additional term, i.e, the fourth term is added. The reason for this modification is that the hysteresis loop generated by Bouc-Wen model is clockwise while the hysteresis loop observed in the experiment is clearly counter clockwise. It has been shown by extensive simulation studies that the modified Bouc-Wen model can describe both the clockwise and counter clockwise stable hysteresis loops.

In the right hand side of the modified Bouc-Wen model,  $\rho(\dot{x} + \delta|\dot{w}|x)$  can be explained as the activation part between the  $[EM]$  and  $[SEAP]$  while  $-\rho(\sigma\dot{x}w + \gamma|\dot{w}|w)$  can be considered as the self degradation part of  $[SEAP]$ . Here these two parts are nonlinear activation and nonlinear degradation.<sup>6</sup>

### 3.1. Parameter Identification

Parameter identification from experiment data is still the bottleneck of model development and incorporating techniques from systems engineering is an effective strategy to deal with the problem. As widely used in system engineering and also in systems biology, the basic idea of parameter identification strategy is using optimization algorithm to find the optimal parameter values which minimize E, the difference between the model-predicted  $[SEAP]$  and the experiment value defined by

$$E = \sum_{m=1}^M ([SEAP]_{pred}(m) - [SEAP](m))^2 \quad (7)$$

where  $M$  is the total number of experiment samples,  $[SEAP](m)$  denotes the observed value of  $m$ th experiment sample point,  $[SEAP]_{pred}(m)$  denotes the corresponding  $m$ th predicted value.

modified Bouc-Wen model in Eq.2 is complex and nonlinear. Therefore, it is challenging to solve the above optimization problem as all gradient-based algorithms can not be directly used. In this gene regulatory network, experimentally measured values of  $[EM]$  and  $[SEAP]$  are provided to validate the hysteretic EM-SEAP kinetics, which is essentially the phase portrait between  $[EM]$  and  $[SEAP]$ . The variation of  $[SEAP]$  versus the variation of  $[EM]$  is concerned rather than the reaction rate of  $[EM]$  and the reaction rate of  $[SEAP]$ , expressed by  $\dot{x}$  and  $\dot{w}$  in the model, respectively. Therefore, the term  $\frac{dw}{dx}$ , expressing the variation of  $[SEAP]$  versus the variation of  $[EM]$  is derived by rewriting the modified Bouc-Wen model in Eq.2 as follows,

$$\dot{w} - \rho\delta x|\dot{w}| + \rho\gamma w|\dot{w}| = \rho(\dot{x} - \sigma|\dot{x}|w) \quad (8)$$

Where

$$|\dot{w}| = \dot{w}sgn(\dot{w}), |\dot{x}| = \dot{x}sgn(\dot{x}) \quad (9)$$

Substituting Eq.9 into Eq.8, we get

$$\dot{w}(1 - \rho\delta xsgn(\dot{w}) + \rho\gamma wsgn(\dot{w})) = \dot{x}\rho(1 - \sigmasgn(\dot{x})w) \quad (10)$$

Dividing both sides by  $\dot{x}$ , we have

$$\frac{dw}{dx} = \frac{\rho(1 - \sigmasgn(\dot{x})w)}{(1 - \rho\delta xsgn(\dot{w}) + \rho\gamma wsgn(\dot{w}))} \quad (11)$$

By analyzing the interconnection between EM and SEAP,  $[SEAP]$  increases as  $[EM]$  increases and decreases as  $[EM]$  decreases. Therefore the sign of  $\dot{w}$  is the same as the sign of the  $\dot{x}$ . Hence, in the process of OFF-to-ON,  $[EM]$  increases and the signs of  $\dot{x}$  and  $\dot{w}$  are positive. In this case, Eq.11 can be simplified as follows,

$$\frac{dw}{dx} = \frac{\rho(1 - \sigma w)}{(1 - \rho\delta x + \rho\gamma w)} \quad (12)$$

while in the process of ON-to-OFF,  $[EM]$  decreases and the signs of  $\dot{x}$  and  $\dot{w}$  are negative. In this case, Eq.11 can be simplified as follows,

$$\frac{dw}{dx} = \frac{\rho(1 + \sigma w)}{(1 + \rho\delta x - \rho\gamma w)} \quad (13)$$

Now for any given set of parameters, the model-predicted value of  $[SEAP]$  can be calculated from

Eq.12 and Eq.13 numerically and the simplex algorithm<sup>10</sup> can be used to solve the optimization problem in Eq.7 iteratively.

In the process of parameter identification, normalization is very simple but critical technique especially with large set of parameters whose ranges of values vary sharply. Otherwise, the computation algorithm will be very sensitive with the initial values. Therefore, before optimization, test on parameter set has to be done to approximate the ranges of different parameters using random initial values. If the ranges of different parameters are observed to have a big difference after several tests, normalization is needed to make all the parameters have similar scales. In the modified Bouc-Wen model, a set of seven unknown parameters,  $a, b, n, \rho, \sigma, \delta, \gamma$  is to be estimated. Parameter normalization is applied before optimization. Matlab optimization toolbox *fmincon* is applied to implement the above parameter identification strategy. All the estimated parameter values are listed in Table 1.

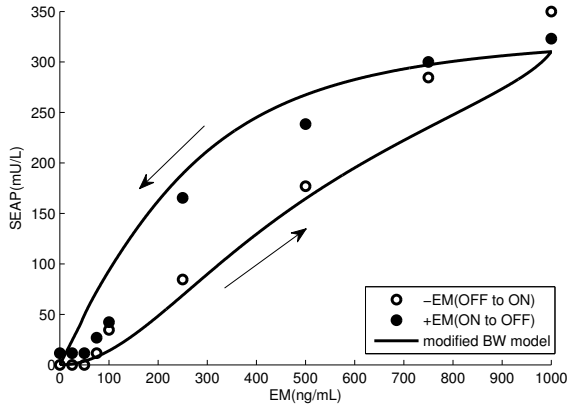
#### 4. Simulation Results

Fig.4 has shown both the hysteresis loop from the modified Bouc-Wen model and the experimentally measured hysteretic EM-SEAP kinetics in the gene regulatory network in Cell A. The arrows that goes up and goes down denote the simulated OFF-to-ON and ON-to-OFF, respectively. This has shown that the hysteresis loop from the model is counter clockwise which agrees with the experiment result, as higher  $[EM]$  is required for OFF-to-ON than ON-to-OFF expression changes. The hysteresis loop from the simulation study fits the experiment data well. Therefore, the model has captured the cooperativity relationship correctly between  $[EM]$  and  $[SEAP]$ . This verifies the capability and accuracy of the mathematical model to emulate natural gene expression behavior with hysteresis. In Fig.5, the experiment data, the simulation results from two different mathematical models describing the hysteresis in the gene regulatory network in Cell B are presented. The two models are the previous model in the literature<sup>5</sup> and the modified Bouc-Wen model, respectively. The hysteresis loops from the two models demonstrate that both of them have captured the most important feature of the hysteretic gene regulatory net-

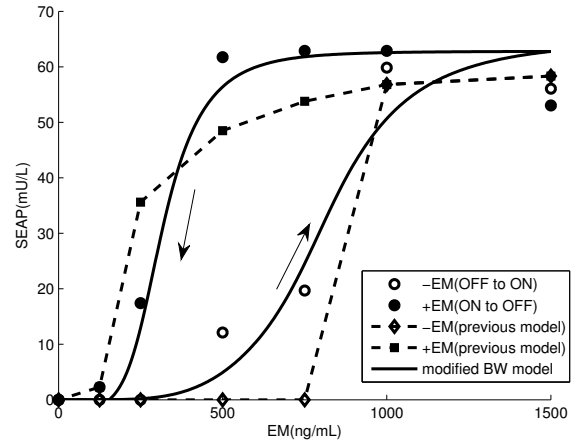
**Table 1.** Model parameter values for validation of EM-SEAP hysteretic kinetics in gene network.

Parameter	Description	Unit	Value for Cell A	Value for Cell B
$x_{0min}$	Minimum value of $[EM]$	ng/mL	0	0
$x_{0max}$	Maximum value of $[EM]$	ng/mL	1000	1500
$x_{2min}$	Minimum value of $[SEAP]$	mU/L	0	0
$x_{2max}$	Maximum value of $[SEAP]$	mU/L	350	70
a	Constant in Hill function		375.46	236.12
b	Constant in Hill function	ng/mL	376.4	498.8
n	Hill coefficient		2	5
$\rho$	Parameter in modified Bouc-Wen model		0.36	0.39
$\sigma$	Parameter in modified Bouc-Wen model		1.85	0.01
$\delta$	Parameter in modified Bouc-Wen model		3.18	2.52
$\gamma$	Parameter in modified Bouc-Wen model		0.52	0.01

work, that is, higher  $[EM]$  is required for OFF-to-ON than ON-to-OFF expression changes. This can also be considered as the validation of the modified Bouc-Wen model by using another gene regulatory network. Compared with the result from the previous model, the hysteresis loop from the modified Bouc-Wen model agrees with the experiment data much better than the previous model. Therefore, the modified Bouc-Wen model is more accurate to describe the bistable response profile in the gene regulatory network in Cell B.



**Fig. 4.** Model validation test on EM-SEAP kinetics in Cell A



**Fig. 5.** Comparison with previous model on EM-SEAP kinetics in Cell B

## 5. Discussion

The mammalian gene regulatory network consisting of a transgene and transactivator (TA) cotranscribed by TA's cognate promoter, repressed by constitutive expression of a macrolide-dependent transcriptional silencer, whose activity is modulated by the macrolide antibiotic erythromycin (EM), shows hysteresis in the interconnection of EM-SEAP. Inspired by the similarity in the shape of the experiment loop and the one from mathematical hysteresis model, a new hysteresis model is developed to describe the counter clockwise hysteresis in the mammalian gene regulatory network. The new hysteresis model is extended from the well-known Bouc-Wen model for hysteresis in mechanical engineering. Because the Bouc-Wen model can only generate stable clockwise hysteresis loop, it can not be directly used to de-

scribe the stable counter clockwise loop observed in the experiment.

Simulation results from this model (solid line in Fig.4 and Fig.5) exhibit adequate agreement with the experimental results in Cell A and Cell B. This verifies that the modified Bouc-Wen model is valid and accurate for modeling of the hysteresis in the mammalian gene regulatory network. Comparison study shows that this model fits the experiment data significantly better than the previous one (the dashed line in Fig.5). The hysteresis loop from the modified Bouc-Wen model is more accurate to capture the interconnection of tetracycline- and macrolide- responsive transgene control modalities in the mammalian gene regulatory network.

Considering the positively cooperativity between the EM and the hybrid promotor as a whole in the system level, a Hill function, widely used in biochemistry is incorporated into the modified Bouc-Wen model. The parameter Hill coefficient  $n$  is predicted to be bigger than 1 as the interconnection between EM and SEAP is positively cooperative and simulation result agrees with this prediction. According to table 1, the value of the Hill coefficient  $n$  in these two different gene regulatory networks is different as  $n$  is 2 in Cell A and  $n$  is 5 in Cell B, respectively. This addresses the fact that the EM-SEAP kinetics are different as Cell A shows a graded response profile while Cell B shows a bistable response profile<sup>5</sup>, which in turns shows the decisive effect of the value of Hill coefficient on the shape of the hysteresis loop from the modified Bouc-Wen model.

In conclusion, a mathematical description of the hysteresis phenomenon in a synthetic mammalian gene regulatory network is presented in this paper. The modified Bouc-Wen model is developed to describe the hysteretic EM-SEAP kinetics with adequate understanding of the biological mechanisms. Simulation result has shown the hysteresis loop from the model agrees with the experiment results in both Cell A and Cell B. This demonstrates the ability and accuracy of the mathematical model to emulate natural gene expression behavior with hystere-

sis. Comparison study has shown that this model fits the experiment data better than previous one in the literature<sup>5</sup>. Therefore, this modified Bouc-Wen model is valid and accurate to describe the hysteresis phenomenon in the mammalian gene regulatory network. In the future work, sensitivity analysis on the modified Bouc-Wen model will be applied to investigate the robustness of the results relative to small variations of all the parameters. Furthermore, more hysteresis examples in biological network will be studied to investigate whether the modified Bouc-Wen model can be generalizable to other cases of hysteresis beyond this mammalian gene regulatory network.

## ACKNOWLEDGMENTS

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