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Modeling Hormone-induced Calcium Oscillations in Liver Cell with Membrane Computing

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Abstract. The capability of membrane computing to deal with distributed and parallel computing models, allows it to characterize the structure and processes of biological systems. With this advantage, membrane computing provides an alternative modelling approach to conventional methods such as ordinary differential equations, primarily in preserving the discrete and nondeterministic behavior of biological reactions. This paper investigates the implementation of the framework for modelling and verification based on membrane computing with a biological process of hormone-induced calcium oscillations in liver cell. The biological requirements and properties of this process are formalized in membrane computing. The model of membrane computing is verified with the simulation strategy of Gillespie algorithm and the model checking approach of the Probabilistic Symbolic Model Checker. The results provided by the simulation and model checking approaches demonstrate that the fundamental properties of the biological process have been preserved by membrane computing model. The results have emphasized that membrane computing provides a better approach in accommodating the structure and processes of hormone-induced calcium oscillations compared to the approach of the ordinary differential equations. However other biological aspects such as the selection of parameters based on the stochastic behavior of biological processes have to be tackled to strengthen membrane computing competence in modelling biological processes.

Key-words: membrane computing; hormone induced calcium oscillations; Gillespie algorithm; PRISM.

1. Introduction

Modelling can provide valuable insights into the working and general principles of organization of biological systems, and it also can suggest novel experiments for testing hypotheses, based on the modelling experience [1]. The modelling of biological reactions by using deterministic rate laws has been in practice for many years. This model focus on measuring the concentrations of objects with the simple relation between reaction rates and objects concentrations in a continuous and deterministic way [2]. This approach does not consider the structure and microscopic characteristics of the biological processes by concentrating on the macroscopic behavior of the system. Although such approach as in ordinary differential equations (ODE) allows the processes to be described in detail, a number of implicit assumptions underlying ODE are no longer applicable on the molecular level [3]. As such, the adequacy of ODE has been questioned for describing microscopic reactions and processes. With this limitation in conventional mathematical models, a number of alternative approaches have been explored for modelling biological processes, among them are amorphous computing [4], brane calculus [5], petri net [6] and process algebra [7].

Membrane computing [8] [9] is another alternative to solve the limitations in conventional mathematical models such as ODE by taking into considerations its essential features that are of interest to biological applications. Some of those features are distribution capability, discreet nature, algorithmically represented, scalability, transparency, massive parallelism, non-determinism and communication [10]. Membrane computing is a formal specification that inspired from the structure and functionality of the living cell, as models and simulators of cellular phenomena, and hence producing theoretical paradigm defined in a language that is close to molecular biology. In recent periods, research in modelling and validating biological systems with membrane computing have been carried out to reinforce membrane computing as a tool to analyse biological systems [11]. Important advances in computability, which provides the feasibility to handle huge volumes of data, which is already available in biology, support a new period of mathematization of biology possible by means of computer science model, such as membrane computing.

This paper describes in-silico analysis of hormone-induced calcium oscillations in liver cell (HCOLC) based on the framework for modeling and verifying biological system with membrane computing as outlined in Muniyandi [11]. The framework is categorized into four steps as illustrated in Figure 1.

The first step is to extract the required elements and properties from the biological system that essential for modelling the system in membrane computing. This is followed by the building of membrane computing model of the biological system, in which the membrane computing formalism [8] is used accordingly to represent the elements and attributes extracted from the system. The third step is to verify the correctness of the membrane computing model by simulating it with membrane

computing simulation strategy. The results of the simulations are compared to the results in vivo or in vitro experiments. Finally, the membrane computing model is formally verified to ascertain that it has sustained the fundamental behavior or property of the biological system. In this step, the model checking approach is utilized to verify membrane computing model based on the properties of biological system.

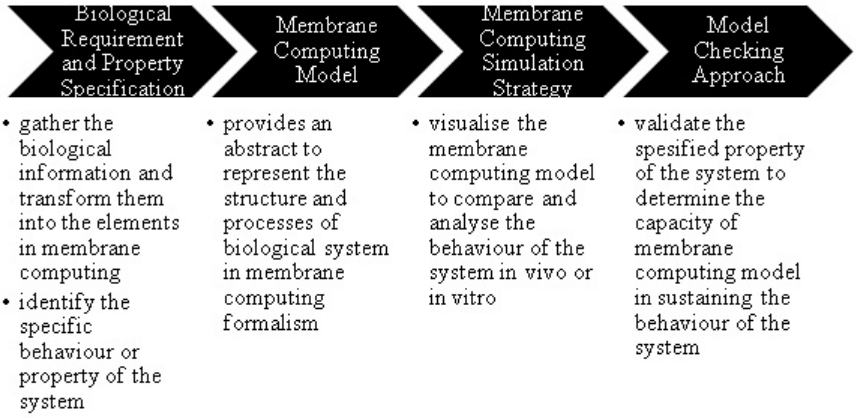

Fig. 1. Framework of modelling and verifying a biological system.

2. Biological Requirements and Property Specifications

In the main tissue of the liver, liver cell makes up the three quarter of the livers cytoplasmic mass. The process in liver cell with receptor mediated inducement produces $InsP_3$ (inositol 1,4,5-trisphosphate) which inspires the escalation of calcium concentration in the compartment of cytosol. This process is called hormone-induced calcium oscillations in liver cell (HCOLC) [12], which is used to regulate the frequency encoding of hormone signals in cytosol.

The main components of the system are: (i) Endoplasmic reticulum (ER) as calcium storage compartment; (ii) An $InsP_3$ and calmodulin to manage calcium release channel at the interface of ER and the cytosol. (Calmodulin plays a role in mediating the active regulatory function of calcium in cytosol and coordinating the calcium release from the ER); (iii) Two ATP-driven pumps responsible for restoring calcium in cytosol to the resting level by compensating the calcium fluxes through the calcium release channel and calcium leaks.

The calcium concentration in cytosol is examined to define the temporal pattern of calcium. With persistent hormone stimulus, the temporal pattern relates to sustained oscillations of calcium in the form of repetitive spikes. The system has two important behaviors:

- The system is not stable when it is under-stimulated. In this case, the system obtains sustained oscillations and after each spike of the oscillation, the calcium

concentration in cytosol returns to the base value. This implies when the system reaches high number of spikes, there are high hormone channelings. The numbers of spikes increase with the increase of hormone level.

- The system is stable when it is over-stimulated. In this case, the system achieves a nonoscillatory state after an initial spike regardless of the level of the hormone.

The HCOLC is monitored by adapting the process within a three compartments system which involves the extracellular, cytosol and endoplasmic reticulum (ER). The respective processes in extracellular, ER and cytosol are: (i) to flux in or out the calcium to or from the cytosol; (ii) to moves calcium to and from cytosol; and (iii) to determine the concentration of calcium. The system behaviors can be extracted into five processes that are pairwise interactions among the compartments for facilitation based on the characterization of HCOLC system by Somogyi and Stucki [12]. The five processes are: (i) calcium fluxing from extracellular into the cytosol; (ii) calcium translocation from cytosol to ER; (iii) calcium translocation from ER to cytosol; (iv) calcium pumped out of cytosol into extracellular; and (v) hormone channeling from ER to cytosol.

Based on the behaviours of HCOLC system, the main properties are categorised into two:

- Property (1): The reaction of hormone channeling will be actively begun during the formation of spikes with the high concentration of calcium in the cytosol.
- Property (2): Hormone channeling is stabilized to achieve a steady nonoscillatory state with low concentration of calcium in the cytosol after a small initial spike.

3. Membrane Computing Model

The task of creating a membrane computing model is done by converting the ODE model described by Somogy and Stucki [12] into a discrete system by using rewriting rules [13]. The ODE model of the system is as follow:

$$\frac{dx}{dt} = k'y - k''x - \alpha f[y]x, \tag{1}$$

$$\frac{dy}{dt} = k''x - k'y + \alpha f[y]x + \gamma - \beta y, \tag{2}$$

with $f[y] = \dfrac{y^n}{(a^n + y^n)}$.

$f[y]$ is a function that relates to the mediation of calmodulin to determine the feedback of calcium concentration in cytosol. x and y are calcium concentration in ER and cytosol. a, k', k'', α, γ and β are parameters used for assigning the weight to prioritize the execution of the processes. a is to determine whether the system

under-stimulated or overstimulated, and α is to specify the low and high level of the hormone. n is an integer and t is the time unit. In the ODE model, values of parameters are as follows: $k' = 2$, $k'' = 0.01$, $\gamma = 1$, $\beta = 1$, $n = 4$. The initial concentration for both x and y are 1. Arbitrary values for parameters a and α are chosen to conclude whether the system was stable or unstable.

Before the system is modeled with membrane computing it should be converted into discrete form. By taking into consideration the two differential equations, (1) and (2), the following rewriting rules for each of the processes involved in the system, are formulated. The details of this conversion is provided by Muniyandi and Zin [15].

The membrane system of $HCOLC$ is represented as:

$$HCOLC = (V, \mu, \omega_E, \omega_C, \omega_{ER}, R_E, R_C, R_{ER}).$$

This system consists of three compartments: extracellular (E), cytosol(C) and endoplasmic reticulum (ER). There is no direct linkage between E and ER, and communications between them are done through C. Therefore the membrane structure of this biological system is represented as:

$$\mu = [\quad [\quad [\quad]_{ER}\quad]_C\quad]_E.$$

The object in this system is calcium. To differentiate the concentration of calcium in each compartment, it is denoted with the label of the compartment.

$$V = \{Ca_E, Ca_C, Ca_{ER}\}.$$

The initial multisets are:

$$\begin{aligned} \omega_E &= \{Ca_E\}, \\ \omega_C &= \{Ca_C\}, \\ \omega_{ER} &= \{Ca_{ER}\}. \end{aligned}$$

The evolution rule has the form:

$$R_i j : u[v]_i \xrightarrow{k_i} u'[v']_i$$

where u, v, u', v' are multisets and the rule j occurs in compartment i. k_i is a real number representing the kinetic constant i. i is the label of the compartment. i and j are integers.

There are five rules in HCOLC system:

Rule 1: Calcium flux from extracellular into the cytosol:

$$R_C 1 : Ca_E[\ ]_C \xrightarrow{k_1} [Ca_C]_C, \quad k_1 = \gamma$$

Rule 2: Calcium translocation from cytosol to ER:

$$R_{ER}1 : Ca_C[\ ]_{ER} \xrightarrow{k_2} [Ca_{ER}]_{ER}, \quad k_2 = k'$$

Rule 3: Calcium translocation from ER to cytosol:

$$R_{ER}2 : [Ca_{ER}]_{ER} \xrightarrow{k_3} Ca_C[\ ]_{ER}, \quad k_3 = k''$$

Rule 4: Calcium pumped out of cytosol into extracellular:

$$R_C2 : [Ca_C]_C \xrightarrow{k_4} Ca_E[\ ]_C, \quad k_4 = \beta$$

Rule 5: Hormone channeling:

$$R_{ER}3 : [Ca_{ER}]_{ER} \xrightarrow{k_5} Ca_C[\ ]_{ER}, \quad k_5 = \alpha * f[Ca_C]$$

4. Simulating the Membrane Computing Model

Simulation is a technique of solving problems by the observation of the performance, over the time, of a dynamic model of the system. Computer simulations have become a useful part of mathematical modelling of many natural or biological systems.

The membrane computing simulations strategies used are based on discrete evolution procedure of objects either with deterministic or stochastic selections of the rules. The metabolic algorithm [14] uses discrete and deterministic evolution strategy and Gillespie algorithm [17] uses discrete and stochastic evolution strategy for simulating membrane computing. Stochastic simulation is to describe the time evolution of a system in a way that exhibits some degree of randomness in its dynamical behaviour [16]. Multi-compartment Gillespie Simulator [18] based on Gillespie algorithm is used to simulate the membrane computing model [22].

There are four experiments that have been carried out. The purpose of these experiments is to analyze how different values of a and α affect the behavior of calcium in cytosol of the HCOLC system to determine the stability of the system. Parameters used in the experiments are similar to parameters and initial multisets that were used in ODE model. The value of a and α are determined through black box testing [23] in which the inputs are selected based on the expected output of the system. The selected values of a and α for the four different experiments are:

Experiment A: $a = 20$ and $\alpha = 5$;
Experiment B: $a = 20$ and $\alpha = 200$;
Experiment C: $a = 0.2$ and $\alpha = 5$;
Experiment D: $a = 0.2$ and $\alpha = 200$.

The result of the experiments is shown in Figure 2. These results are compared with the results generated by ODE approach discussed by Somogyi and Stucki [12]. Experiments A and B show that the processes in the system occur with the understimulated environment when the value of a is high. If the hormone level of α is low as shown in experiment A then there are less spikes compared with when the

hormone level of α is high as in experiment B. Meanwhile, the process in the system is over-stimulated when the value of a is low but the low or high hormone level of α do not affect the behaviours of the system as shown in experiments C and D.

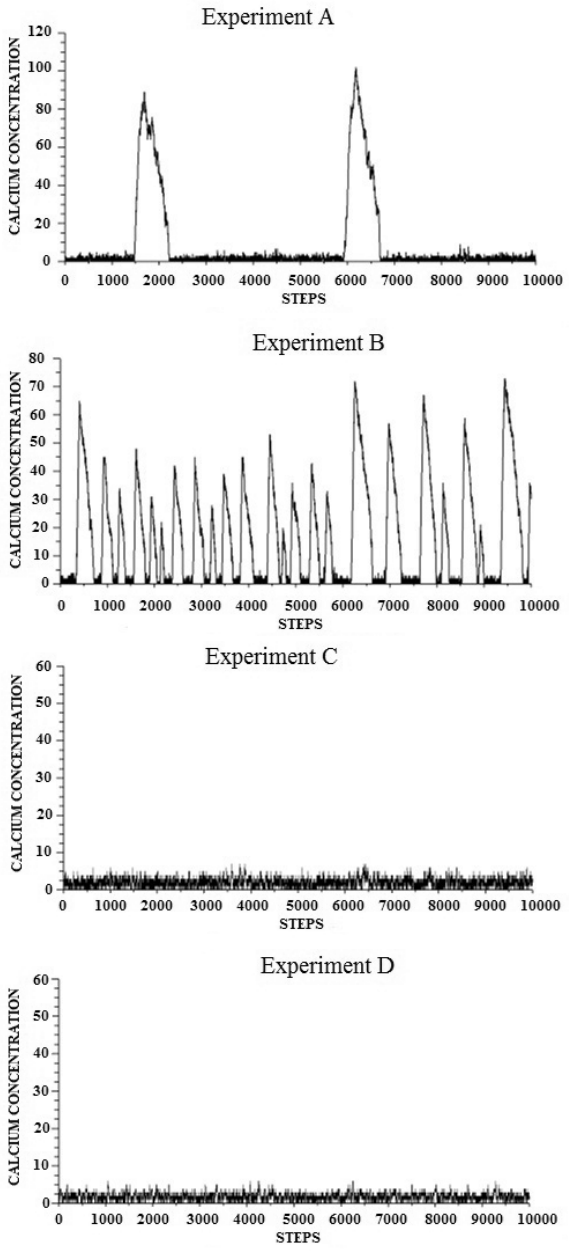

Fig. 2. Experiments with the value of a and α.

Although, the simulation results of membrane computing model establishes almost similar results as generated by the ODE model [12], the non-deterministic and parallel executions of processes in different compartments of HCOLC system are obvious in membrane computing model. This is in accordance with the behaviors of processes in biological systems.

However the simulation of membrane computing model could not differentiate the small changes between the experiments C and D. The similar experiments with the ODE model showed that after an initial spike, the concentration of calcium adopts steady state with approximately small frequency of oscillations and it will be more pronounced with the high hormone as in the experiment D. The initial spike in the simulation of membrane computing model is not clear though there is steady state of small frequency of oscillations. This is mainly because similar value of parameters that utilized in the continuous model of ODE is also employed in the discrete and stochastic model of membrane computing. Consequently the small changes between the two experiments could not be distinguished by membrane computing model.

5. Model Checking the Membrane Computing Model

Simulation results could not deduce that the properties of the biological systems have been safeguarded. In order to verify these properties, other approaches need to be explored. Ipate and Gheorghe [19], have surveyed three verification approaches utilized so far for applications of membrane computing. The approaches are based on grammar, finite state machine and model checking. The grammar based methods encountered difficulties when dealing with hierarchical compartments, parallel behavior, communication mechanism and multisets of objects [19]. The approach based on finite state machine verifies the transition coverage, aims to produce a test set in a way every single transition of the model is covered [19]. The model checking tools such as the Simple Promela Interpreter (SPIN) and the reimplementation and extension of symbolic model checking (NuSMV) have been used to cover the main features of membrane computing, including parallelism and communication [19]. However, the finite state machine methods and, the model checking approaches of SPIN and NuSMV do not emphasize on the verification of stochastic aspects embodied in the membrane computing model of biological systems.

In stochastic modeling, Continuous Time Markov Chain (CTMC) is one such method that use Continuous Stochastic Logic (CSL), a stochastic variant of Computational Tree Logic (CTL) which allows to state properties over states as well as over paths. Probabilistic Symbolic Model Checker (PRISM) [20] supports a probabilistic model based on CTMC and systems specifications through CSL for stochastic systems. Therefore PRISM model checker can be used as the model checker tool for membrane computing model [20] [24].

The membrane computing model of HCOLC system is translated into the PRISM formalism by using a technique proposed by Romero-Campero et al. [21]. The analysis of the properties of the system is done by using the concept of rewards. Rewards are used to reason the behavior of the model in a certain fashion by measuring the

probability as well as to identify a wide range of quantitative measures relating to modeling behavior.

There are five processes in the system:

R1: Calcium fluxing from extracellular into the cytosol.
R2: Calcium translocation from cytosol to ER.
R3: Calcium translocation from ER to cytosol.
R4: Calcium pumped out of cytosol into extracellular.
R5: Hormone channeling from ER to cytosol.

To verify these processes, rewards $Flux\_In$, $Translocation\_Cytosol$, $Translocation\_ER$, $Flux\_Out$ and $HormoneChanneling$ are defined for each of the experiments A, B, C and D. When the process is activated, the reward determines the concentration of calcium in cytosol. For instance, for the rewards $Flux\_In$, the calcium (Y) concentration is updated when the process R1 is activated during the simulation.

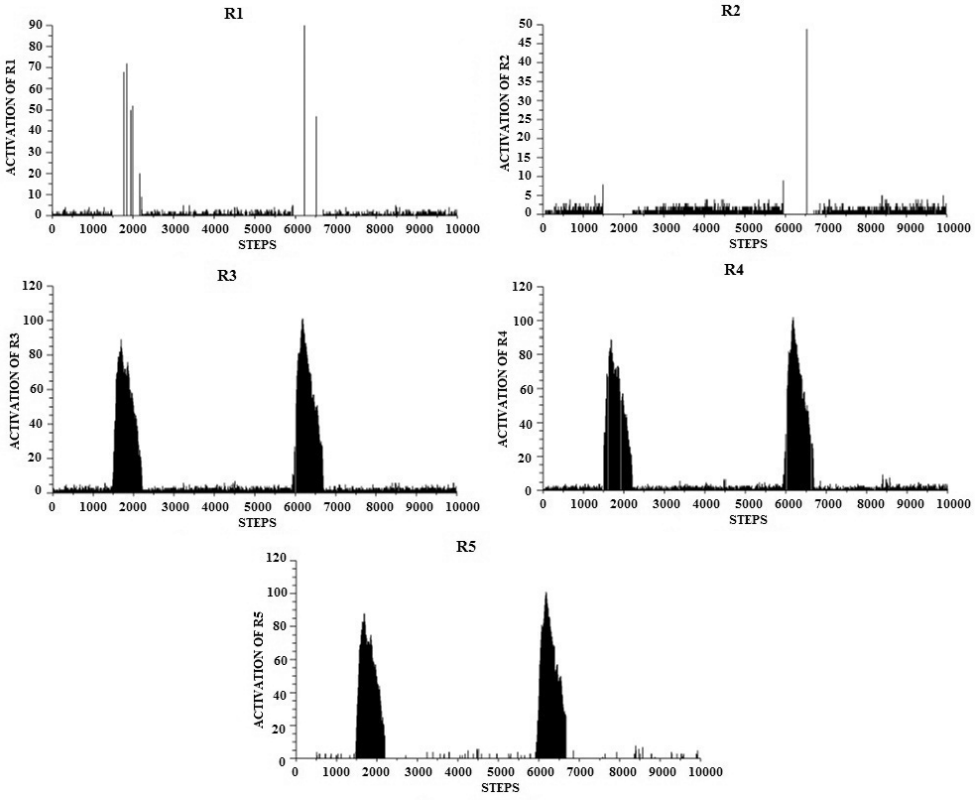

Fig. 3. Activation of the processes in the Experiment (A).

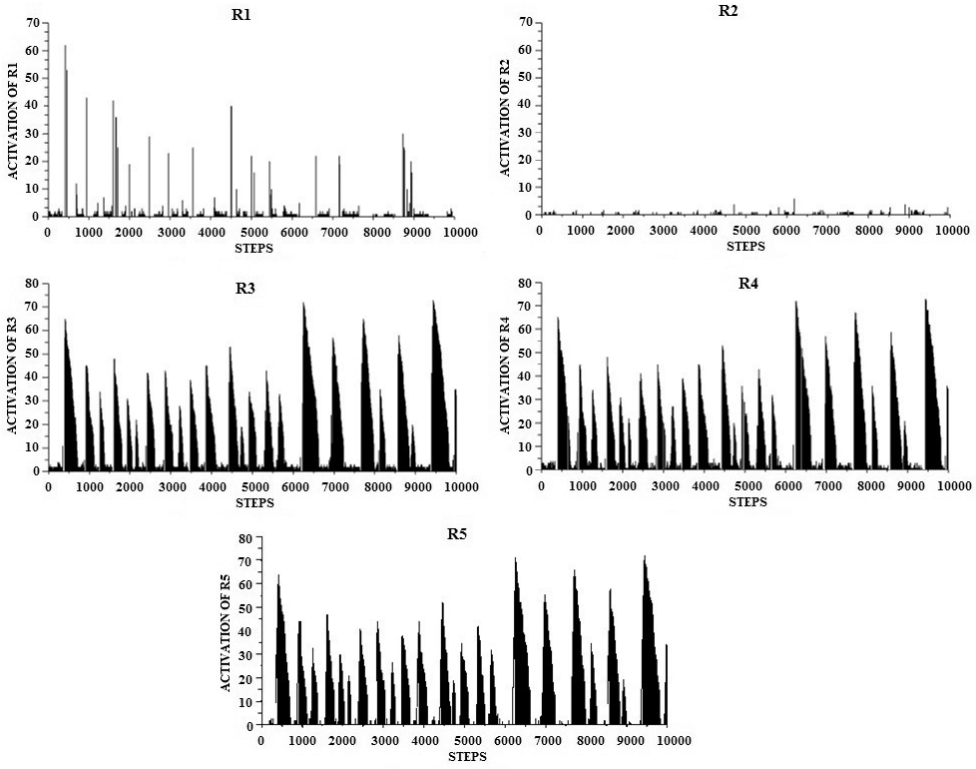

Fig. 4. Activation of the processes in the Experiment (B).

The rewards are described in PRISM as follow:

$rewards$ "$Flux\_In$"
$[R1]$ $true : Y$
$endrewards$

$rewards$ "$Translocation\_Cytosol$"
$[R2]$ $true : Y$
$endrewards$

$rewards$ "$Translocation\_ER$"
$[R3]$ $true : Y$
$endrewards$

$rewards$ "$Flux\_Out$"
$[R4]$ $true : Y$
$endrewards$

$rewards$ "$HormoneChanelling$"

$[R5]\ true : Y$
endrewards

Property (1) is analyzed with experiments A and B. The results of the experiments are shown in Figure 3 and Figure 4. The results show that the hormone channeling is high when the spikes are formed as shown by R5 in both figures. This process is stabilized by regular occurrence of the flux-out process (R4) and translocation process from cytosol to ER (R3). Meanwhile the flux-in process (R1) and translocation process from ER to cysotol (R2) are suppressed. More regular spikes are occurring when the hormone level is high with increasing hormone channeling (R5) as shown by Figure 3 with experiment B.

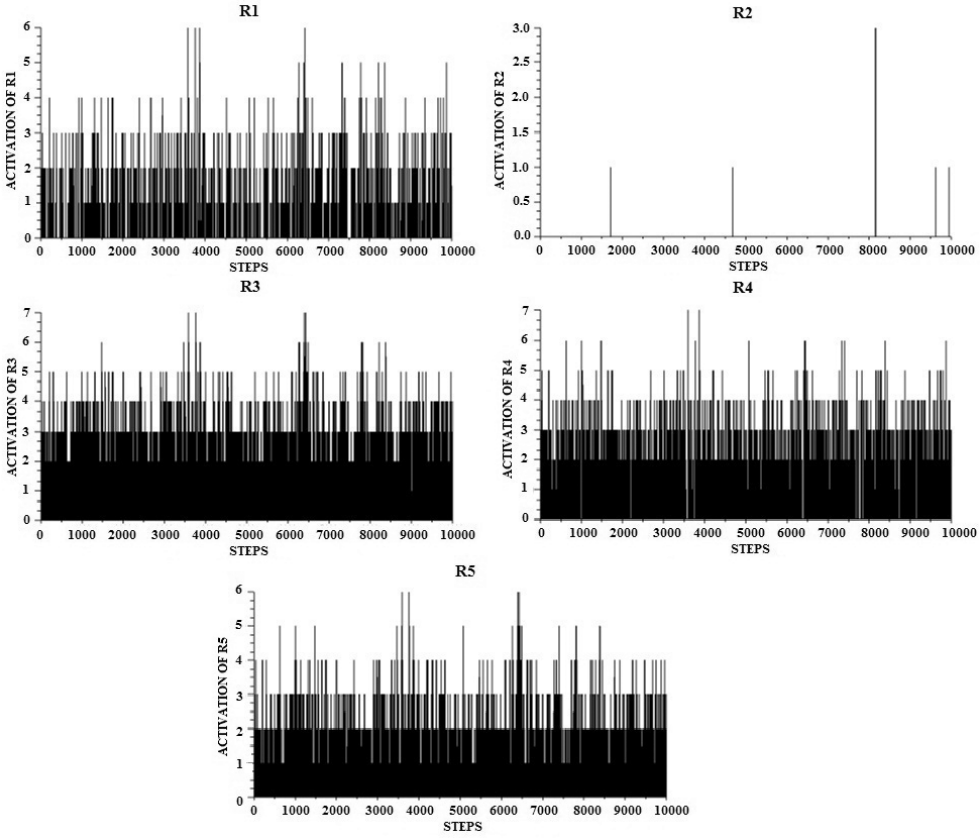

Fig. 5. Activation of the processes in the Experiment (C) and (D).

Property (2) is analyzed with experiments C and D. The result of the experiments is shown in Figure 5. From the figure, it is clear that both experiments generate about the same results. It shows that the hormone channeling (R5) is moderate and the spikes are not formed at the start of the simulation but in the ODE model there was a spike at initial stage of the simulation. This process is stabilized by regular

occurrence of the flux-out process (R4) and translocation process from cytosol to ER (R3). Meanwhile the flux-in process (R1) and translocation process from ER to cysotol (R2) are suppressed.

6. Discussion and Conclusions

The results of the verification of the membrane computing model of HCOLC system demonstrates that properties of the biological system have been captured by the model. On top of that, membrane computing model provides alternative ways to symbolize the structure and the processes in the system compared to ODE model. Processes and the movement of objects involved between compartments in this hierarchical arrangements of HCOLC system can be distinguished by the membrane computing model.

The membrane computing model can also be used to generate the behavior of the overall system by accommodating different processes within and between the compartments. The results demonstrate the capability of membrane computing model to capture the structure and the processes involved in the multi-compartments biological system as well as the ability to preserve the behavior and the properties of the processes. Hence, membrane computing provides a formal way to represent multi-compartment biological system without discounting biological characteristics.

Nonetheless the capability of membrane computing needs to be fortified by enhancing membrane computing features to model various behaviors of biological system. The formation of parameters primarily for simulating stochastic system of membrane computing is among the features that need to be addressed as demonstrated in the experiments C and D. Appropriate parameters to characterize the stochastic behavior of the rules to regulate the execution of specific process at certain time steps have to be correctly determined based on the behavior of the system. As stated by Phair [25], a lot of information contained in dynamic biological data and successful dynamic or kinetic models are heavily depend on knowing the underlying biological mechanisms. He further mentioned that much of the value of kinetic modeling lies in the ability of dynamic experiments to reveal useful information about the complex biological systems.

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References

[1] Meng T. C., Somani S., Dhar P., *Modeling and Simulation of Biological Systems with Stochasticity*, Silico Biology, 2004, Vol. **4**(3), pp. 293–309.

[2] Rubinov S. I., *Introduction to Mathematical Biology*, Courier Dover Publications: USA, 2003.

[3] Jong H. D., *Modeling and Simulation of Genetic Regulatory Systems: A Literature Review*, Journal of Computational Biology, 2002, Vol. **9**(1), pp. 67–103.

[4] Abelson H., Allen D., Coore D., Hanson C., Homsy G., Knight T. K., Nagpal R., Rauch E., Sussman G. J., Weiss R., *Amorphous Computing*, Communications of the ACM, 2000, Vol. **43**(5), pp. 74–82.

[5] Cardelli L., *Brane Calculi*, Communications of the ACM, Lecture Notes in Computer Science, 2005, Vol. **3082**, pp. 257–278.

[6] Peleg M., Rubin D., Altman R. B., *Using Petri Net Tools to Study Properties and Dynamics of Biological Systems*, J. Am. Med. Inform. Assoc., 2005, Vol. **12**(2), pp. 181–199.

[7] Baeten J. C. M., Basten T., Reniers M. A., *Process Algebra: Equational Theories of Communicating Processes*, Cambridge University Press: UK, 2009.

[8] Păun G., *Computing with Membranes*, Journal of Computer and System Science, 1998, Vol. **61**, pp. 108–143.

[9] Păun G., Rozenberg G., Salomaa A., *The Oxford Handbook of Membrane Computing*, Oxford University Press: USA, 2010.

[10] Păun G., Ciobanu G., Perez-Jimenez M. J., *Application of Membrane Computing*, Springer-Verlag (Natural Computing Series): Berlin, 2006.

[11] Muniyandi R., *A Framework for Modeling and Verifying Membrane Computing of Biological Systems*, PHD Thesis, Universiti Kebangsaan Malaysia, Malaysia, 2011.

[12] Somogyi R., Stucki J. W., *Hormone-induced Calcium Oscillations in Liver Cells Can Be Explained by a Simple One Pool Model*, The Journal of Biological Chemistry, 1991, Vol. **26**(17), pp. 11068–11077.

[13] Bezem M., Klop J. W., Vrijer R. D., *Term rewriting systems*, Cambridge University Press: London, 2003.

[14] Manca V., *The metabolic algorithm for P systems: Principles and applications*, Theoretical Computer Science, 2008, Vol. **404**(1–2), pp. 142–155.

[15] Muniyandi R., Zin A. M., *Comparing Membrane Computing with Ordinary Differential Equation in Modeling a Biological Process in Liver Cell*, The Sixth International Conference on Bio-Inspired Computing: Theories and Applications (BIC-TA 2011), 2011.

[16] Gillespie D. T., *Stochastic Simulation of Chemical Kinetics*, Annual Review of Physical Chemistry, 2007, Vol. **58**, pp. 35–55.

[17] Gillespie D. T., *Approximate Accelerated Stochastic Simulation of Chemically Reacting Systems*, Journal of Chemical Physics, 2001, Vol. **115**(4), pp. 1716–1733.

[18] Romero-Campero F., Gheorghe M., Auld J., *Multicompartment Gillespie simulator in C*: `http://www.dcs.shef.ac.uk/ marian/PSimulatorWeb/PSystemMF.htm`, The Sheffield University: UK, 2004.

[19] Ipate F., Gheorghe M., *Testing based on P systems - an overview*, CMC10 Proceedings of the 11th international conference on Membrane computing, 2010.

[20] Kwiatkowska M., Norman G., Parker D., *Using probabilistic model checking in systems biology*, Association for Computing Machinery, 2008, Vol. **35**(4), pp. 14–21.

[21] Romero-Campero F. J., Gheorghe M., Bianco L., Pescini D., Perez-Jimenez M. J., Ceterchi R., *Towards Probabilistic Model Checking on P Systems Using PRISM*, Lecture Notes Computer Science, 2006, Vol. **4361**, pp. 477–495.

[22] Muniyandi R., Zin A. M., *Experimenting the simulation strategy of membrane computing with Gillespie algorithm by using two biological case studies*, Journal of Computer Science, 2010, Vol. **6**, pp. 525–535.

[23] Beizer B., *Black-Box Testing: Techniques for Functional Testing of Software and Systems*, Wiley: London, 1995, pp. 13–15.

[24] Muniyandi R., Zin A. M., Shukor Z., *Model checking the biological model of membrane computing with probabilistic symbolic model checker by using two biological systems*, Journal of Computer Science, 2010, Vol. **6**, pp. 669–678.

[25] Phair R. D., *Integrative BioInformatics: Practical Kinetik Modeling of Biological Systems*, `http://www.bioinformaticsservices.com/bis/resources`, The John Hopkins University: US, 1995.