Nonlinear Dynamics Effects in Synthesis and Degradation of Proteins

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Abstract

The cellular function is regulated by synthesis and degradation of proteins. Due to the complexity of underlying processes, their complete structure and functionality remains still mainly unexplained. However, recent progress of computer facilities has allowed to perform in silico experiments to deal with complex molecular biology systems. Here we report the results of two such numerical simulations. First, we have numerically analyzed different dynamical regimes in a system of genetic oscillators with intracell coupling. Second, we have performed simulations of a protein degradation by a proteasome, the complex molecular machine, intended to destroy malfunctioning proteins. These results have several applications. The construction of synthetic genetic network or control of the protein degradation represent, on one side, a basic step towards understanding of logical cellular control, whereby biological processes could be monitored or manipulated at the protein level. On the other hand, from the construction of simple switches or oscillators, one can envision the design of devices capable of performing elaborate functions in living cells [1, 10].

Introduction

Protein synthesis in the course of gene expression and protein degradation by proteasomes are key processes in the cellular metabolism. In this simulation project we have studied selected nonlinear effects of gene expression and protein degradation in which the complexity and stochasticity of the system seriously influence the system behavior. In particular, we have considered two related tasks: i) influence of stochasticity, quorum-sensing, cell growth and mutations on the behavior of synthetic genetic oscillators, i.e on its synchronization and clusterization, and ii) degradation of different proteins in the kinetic model of the proteasome. The solution of these task is important for design of synthetic genetic networks, simulation of new drugs and understanding of cellular metabolism. Moreover, this investigation will contribute into the design of software for creating a virtual immune system.

Control of synchronization in inhibitory coupled synthetic genetic oscillators

We have considered a model of hysteresis-based relaxation genetic oscillators coupled via quorum-sensing mechanism, recently proposed in [8]. The oscillator is constructed by combining two engineered gene networks, the toggle switch [3] and an intercell communication system, based on the dynamics of the autoinducer (AI). Due to the presence of multiple time scales in the system, the synthetic genetic oscillator can produce relaxation oscillations.

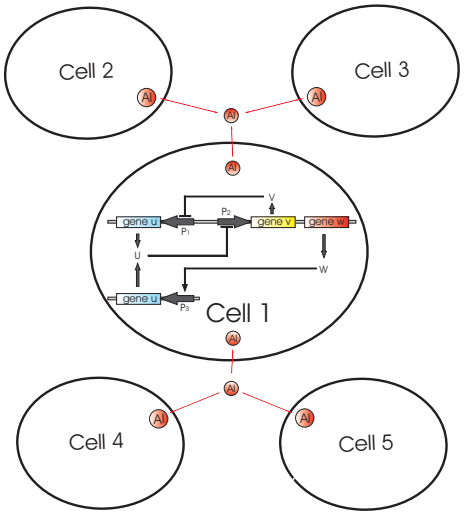

Fig.1. A scheme of coupled genetic relaxators. The genetic network inside each cell is coupled with another cell by a diffusion of small autoinducer molecules AI, which influence the gene expression.

We have shown [6] that the appearance of multistability, multirhythmicity and clusterization is immanent for this type of synthetic genetic networks. Performing extensive simulations we have distinguished between two different types of clusters: steady state clusters (inhomogeneous steady state, or the "oscillation death" regime) and oscillatory clusters (in-phase, anti-phase and asymmetric oscillations, as well as organization in multiple cluster regimes). For each separate cluster formation, we have also demonstrated how the dependence on initial conditions can lead to different distributions of the oscillators between the clusters. Despite the multitude of rhythms and regimes produced, their control and manipulation is also very important. We have therefore shown [7] how the dynamics of the system can be manipulates using the quorum sensing mechanism, the biologically occurring noise and the size of the system. The main effects are switching between synchronous and asynchronous oscillations, suppression of the oscillations, as well as optimal behavior of the system for intermediate noise intensities or exact size of the system.

The effects obtained can be considered as a significant advantage for a multitude of applications. For example, it has been reported that multistability is a main mechanism for memory storage and temporal pattern recognition in artificial and natural neural networks [2]. Moreover, the effect of multistability is also used to create an electrically addressable passive device of organic molecules [4] for registration, storage and processing of information. Therefore, it is logical to assume that the ability of the genetic circuits to display multistability opens the possibility for construction of a "new era" computational devices, based on genetic and DNA computing, which can be used, e.g., for programming of the drug therapy or cancer treatment. In addition, it is very important to note that the presence of different periods for different

oscillator distributions in every regime reported here, opens the possibility for a resonant behavior of the system on multitude frequencies. This result can be important, e.g., for the construction of genetic networks driven by a periodic signal [5] coupled with cell cycle regulation. It also means that different synchronization regions can be obtained for different external frequencies, an effect which can have impact in cancer chronotherapy or cell cycle regulation. We emphasize the generality of these results, although derived for this particular model of genetic network, since no special properties of the given system were used to obtain the appearance of effects presented. The results obtained have been submitted in two papers [7, 6].

The kinetic modeling of the protein degradation by the proteasome

Proteasomes are multicatalytic cellular protease complexes that degrade intracellular proteins into smaller peptides. They are present in all eukaryotic cells, archaea, and certain bacteria. In experiments about 500000 proteasomes have been found in the nucleus and the cytoplasm of one eukaryotic cell. Proteasomes are absolutely essential for the homeostasis because the removal of proteasome genes in eukaryotes is lethal. Many roles in the cell's metabolism are played by proteasomes: they destroy abnormal and misfolded proteins tagged with Ubiquitin and are an essential component of the ATP-Ubiquitin-dependent pathway for protein degradation. Proteasomes play an important role in the immune system by generating antigenic peptides of 8-12 residues to be presented by the MHC class I molecules and hence are the main supplier of peptides for its recognition by killer T-cells. As a part of the Ubiquitin system, proteasomes are involved in the regulation of the cell cycle and the cell stress response. Recently the proteasome inhibition has been suggested as a new successful target for cancer treatment.

In our previous work we have suggested two models of the proteasome function. The first transport model describes the the protein translocation and show that the differences in the length dependent velocity rates can be of crucial importance for the proteasome output [13, 11, 12]. The second model describes the kinetics of the proteasome degradation and predicts the protein concentration dynamics. To model the proteasome kinetics we have taken as a template the model from [9]. This model describes the concentration of a certain length peptides inside and outside the proteasome. The parameters include the influx and exit rates which can depend on the length. The model has Michaelis-Menten kinetics and takes into account the cleavage inside the proteasome. In order to develop a new model it was decided to add a position specific cleavage pattern. This pattern for the initial substrate can be taken from the experiment or from cleavage prediction algorithms, making in this way the virtual model of the proteasome to predict the substrate and certain length fragment dynamics. Preliminary simulations have shown that one can achieve good matching with the experiment. This model is now under the detailed investigation and have been compared by extensive numerical simulations with experimental data. Using the developed model of the protea-

some kinetics we have performed extensive simulations to model degradation of different proteins, i.e. substrates.

In particular, we have performed the following numerical experiments:

- Computation of proteasomal length distribution for long peptides. As known, this length distribution, i.e. the probability to find a fragment with certain length is nonmonotonous and has a peak at 8-12 aminoacids, corresponding to the typical length of epitopes, produced for the immune system.
- Computation of short protein degradation by the proteasome. From the experiments the following effect has been detected: longer peptides can be degraded faster. The same effect has been detected in the model with nonmonotonous cleavage function.
- Computation of the dynamics of fragment production in time. We have compared the mass spectroscopy results with simulation results for several substrates and all fragments and achieved a good correspondence.

These results will be published in a separated paper .

Methodologies applied and resources used

Each genetic relaxator has been described by a system of three first order stochastic differential equations (SDE). To study a population of many coupled relaxators, simultaneous simulation of many SDE have been used. Sometimes, to check the population size effects and related with that reduction of stochasticity the simulation of up to 300,000 differential equations with noise was necessary. For this research the usage of high-performance computing with functional parallelization was of absolute importance. I have used facilities and help of personnel of CESCA-CEPBA computer centre in Barcelona (Kadesh computer). Such massive and extensive simulations have been impossible without support of the HPC-EUROPA project.

For the simulation of proteasomal degradation two separate differential equations have been needed for each possible fragment produced. It means that, e.g., for the substrate of 300 amino acids length the simultaneous solution of $90,000$ equations was needed. The application of supercomputers have helped a lot to put these simulations in a reasonable time frame.

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References

[1] D. Bray. Protein molecules as computational elements in living cells. *Nature*, 376:307, 1995.

[2] J. Foss, A. Longtin, B. Mensour, and J. Milton. Multistability and delayed reccurent loops. *Phys. Rev. Lett.*, 76:708–711, 1996.

[3] T. S. Gardner, C. R. Cantor, and J. J. Collins. Construction of a genetic toggle switch in escheeichia coli. *Nature*, 403:339–342, 2000.

[4] H. Gudesen, P. Nordal, and G. Leistad. Electrically addressable passive device, method for electrical addressing of the same and uses of the device and the method. *United States Patent 6055180*, 1999.

[5] J. Hasty, J. J. Collins, F. Isaacs, M. Dolnik, and D. McMillen. Designer gene networks:towards fundamental cellular control. *Chaos*, 11:207–220, 2001.

[6] A. Koseska, E. Volkov, A. Zaikin, and J. Kurths. Inherent multistability in arrays of autoinducer coupled genetic oscillators. *Phys. Rev. E* 75: 031917, 2007.

[7] A. Koseska, A. Zaikin, J. J. García-Ojalvo, and J. Kurths. Stochastic suppression of gene expression oscillations under intercell coupling. *Phys. Rev. E*, 75: 031917, 2007.

[8] A. Kuznetsov, M. Kern, and N. Kopell. Synchrony in a population of hysteresis-based genetic oscillators. *SIAM J. Applied Math.*, 65:392–425, 2005.

[9] F. Luciani, C. Kesmir, M. Mishto, M. Or-Guil, and R. J. de Boer. A mathematical model of protein degradation by the proteasome. *Biophysical Journal*, 88:2422–2432, 2005.

[10] R. Weiss and T. Knight. Engineered communications for microbial robotics. *DNA6: 6th International Meeting on DNA Based Computers (Leiden, the Netherlands)*, 2000.

[11] A. Zaikin and J. Kurths. Optimal length transportation hypothesis to model proteasome product size distribution. *Journal of Biological Physics*, 32:231–243, 2006.

[12] A. Zaikin, A.K. Mitra, D.S. Goldobin, and J. Kurths. Influence of transport rates on the protein degradation by proteasomes. *Biophysical Reviews and Letters*, 1:375–386, 2006.

[13] A. Zaikin and T. Pöschel. Peptide-size-dependent active transport in the proteasome. *Europhysics Letters*, 69:725–731, 2005.