Stochasticity in signal transduction pathways

Vidal Rodriguez, J.

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Summary

The research presented in this thesis is focused on the study of various stochastic and spatial effects concerning signalling systems in prokaryotic cells. Signalling systems are widespread mechanisms that enable cells to sense and respond to stimuli. In some cases, such as the two-component type, this response may result in the activation or inhibition of gene expression of a response. Due to the inherent spatial and stochastic nature of signalling systems, we have made use of explicit simulations to gain insight into their complex functioning phenomena and to stir the development of mathematical models. In Chapter 1 we have given a general overview of the role of computer simulations in biochemistry and the various flavours of methodologies available. We also cite relevant applications and results. Finally we introduce for the non-biologist the basics of two-component signalling systems in bacteria.

Generally, complex biochemical systems have been studied by means of explicit simulations. In Chapter 2 we have developed the Gillespie Multiparticle method (GMP), which is suited for the study of complex systems with spatial and stochastic components. GMP follows the principles of the Reaction-Diffusion Master Equation, which is the theoretical framework, with the particular feature of being an approximate solver. The GMP method separates the reaction and the diffusion processes that are executed alternatively at predetermined intervals. This makes it a hybrid stochastic and deterministic method. By using such approach we simplify the diffusion process by following a Cellular Automata approach. This allows for enhancements in the diffusion of several (or many) particles in a single diffusion step, rather than the one-by-one approach used in exact solvers. We have shown in Chapter 3 that the fluctuation levels produced by our approximate solver (GMP) give, on average, comparable results to those of an exact solver (MesoRD). Only the noise, or variation, of the fluctuations is slightly reduced due to the introduction of the semi-deterministic diffusion mechanism. We expected that the performance of the GMP method would be better than that of the exact. However, for the cases that we studied, in the low number of molecules regime, we experienced no significant gains. We have, however, detailed the cost and the situations in which the methods would be more efficient. One final word is that the methods that use a regular lattice and use point-like particles suffer from discretization errors that affect the reaction mechanism used (Gillespie) and consequently the noise in steady state.

The second half of this thesis focuses on the study of two-component sig-

nalling systems and the gene expression system and mechanism responsible for the synthesis of its sensory and signal transport molecules. Due to the numerous different actual two-component signalling systems we have taken an abstract and general conceptualization of the problem. Thus, it is not the study of a particular system, which would require detailed knowledge of the kinetic and diffusion related parameters. Rather, an abstract generalization with which we shed light into the basic properties that drive these systems.

Chapter 4 deals with the implications of spatial distribution and localization of sensors and the inherent stochastic diffusion and stochastic processes of the transporter molecules in two-component signalling systems. We concluded that despite the diffusion-limited character of the reactions (reaction upon collision) the system effectively works as a memory less system, which was corroborated by simulations. We also have realized that under the constraint of using a fixed number of molecules in the signalling system there is an optimal ratio, and thus and optimal response time, between sensors (HK) and response regulator (TF) which lays around 2:3 and favouring higher number of response regulators in the case of not instantaneous reactions. This is because of the higher cost of finding a single target, once the response regulator has been activated on the membrane by the rest of sensory molecules in the system. Thanks to the symmetry of the problem and some simplifications it has been possible to derive afterwards a mathematical model.

The analysis of the optimal response time raised the question of whether the organization of the genes involved in the expression of the sensory and response regulator molecules has been under evolutionary pressures. These would have evolved to minimize, for example, resource utilisation of additional regulatory mechanisms. In chapter 5 we made a statistical analysis of the length and ordering of genes suggest that there is a slight bias towards a favourable ordering of the genes, in which the first gene is more likely to be transcribed. However, the dynamic analysis draws a more complex picture in which translation, translational couple and multiple ribosome binding sites contribute to the regulation of the final protein number count. The dynamic seem to agree with the picture that few proteins are synthesized in each burst of transcription. Thus although the order of the genes may play a role, the last word resides in secondary structure of the mRNA that seems to have the potential for a larger impact in the final expressions levels by strengthening translational coupling.