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Fully Stochastic Simulation of Calcium Microdomains

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Ionized Calcium (Ca^{2+}) is a versatile second messenger, orchestrating a great variety of vital cellular functions such as cell motility, regulation of gene transcription, neurotransmitter release and cytoskeleton dynamics. Its low intracellular resting concentration is maintained and tightly regulated by ion channels, ion pumps and Ca^{2+} buffers. The inositol 1,4,5-trisphosphate receptor (IP_3R) is an essential Ca^{2+} release channel that orchestrates the liberation of Ca^{2+} from the endoplasmic reticulum in many different cell types. Its biphasic non-linear regulation by Ca^{2+} and its structural organization in channel cluster are the basis of complex intracellular Ca^{2+} signal pattern such as waves and oscillations. The complexity and universal importance of Ca^{2+} signals motivated the use of mathematical models to gain a deeper understanding of Ca^{2+} dynamics. Classical deterministic simulation strategies are based on the assumption that stochastic fluctuations in molecular populations are negligible and are thus adequately represented by molecular concentrations. This is only true for reaction diffusion system with large molecular populations. In case of Ca^{2+} signaling, this assumption does not hold. Due to the low resting [Ca^{2+}] of approximately 0.1 μM (≈ 60 Ca^{2+} ions/fl) and the quantization of Ca^{2+} release sites in aggregated channel cluster microdomains, stochastic fluctuations do not average out. The results are apparently random occurrences of Ca^{2+} puffs, elementary Ca^{2+} release events (ECRE), arising from the synchronized opening of channel clusters. To understand the stochasticity of ECRE, it becomes necessary to take the stochastic nature of underlying chemical reaction diffusion system into account. The most fundamental formulation of exact stochastic reaction kinetics is the chemical master equation (CME). It describes the evolution of a system as a discrete time Markov process, based on reaction propensities that are closely related to macroscopic reaction rate constants. In contrast to stochastic differential equations where noise terms are usually arbitrary chosen, this approach reveals stochastic fluctuations with exact statistical properties. For complex systems, the CME becomes quickly analytically intractable and numerical simulations are the only option to gain information about the dynamic behavior of the underlying system. Furthermore, due to the hierarchical spatial organization of Ca^{2+} signals ranging from the nm scale of single channels to the μm scale of global Ca^{2+} waves, the numerical solution of the CME becomes computational infeasible. One solution for these problems are hybrid algorithms that treat functionally important non-linear components (such as the IP_3R) stochastically while passive bulk reactions and diffusion are treated deterministically. Even though more exact, this approach still neglects intrinsic Ca^{2+} signal noise with its system specific statistical properties, arising from Ca^{2+} buffer association/dissociation reactions and diffusion. The potential relevance of noise on non-linear system has been described before, hence the question arises how fluctuations in the molecular population of Ca^{2+} influence the non-linear gating of IP_3Rs. In this thesis, a fully stochastic simulation software for chemical reaction diffusion systems has been developed, to study Ca^{2+} microdomains including a single IP_3R in its naturally 'noisy' molecular environment. In consecutive steps, the simulation software has been developed, validated and applied to Ca^{2+} systems: (a) As a first step, the influence of mobile and stationary Ca^{2+} buffers on the gating dynamics of a conducting IP_3R was investigated. The used model was thereby able to reproduce experimental findings of buffer dynamic dependent variations of mean channel open/close times, open probability and peak Ca^{2+} amplitudes. (b) Thereupon, potential noise reducing effects of Ca^{2+} buffers in signaling pathways was examined. It was shown that Calmodulin acts as a low-pass noise filter in downstream Ca^{2+} signaling pathways. (c) Furthermore a detailed simulation of an isolated non-

conducting IP_3R, exposed to Ca^{2+} fluctuations with varying temporal characteristics arising from diffusion and Ca^{2+} buffer dynamics was carried out. Detailed analysis of the channel gating dynamics revealed the influence of Ca^{2+} signal noise on the non-linear gating dynamics of the IP_3R. In summary all these findings confirmed the necessity of fully stochastic simulation strategies and revealed functional relevant aspects of Ca^{2+} signal noise.