Quasi-cellular systems: stochastic simulation analysis at nanoscale range

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Motivations

The artificial creation of the simplest forms of life (minimal cells) is a challenging aspect in modern synthetic biology. Quasi/cellular systems able to produce proteins directly from DNA can be created by encapsulating a cell-free transcription/translation system (PURESYSTEM) in microemulsion droplets and liposomes (10^(-5) - 10^(-7) m of diameter). It is possible to detect the overall protein production inside these compartments using DNA encoding for GFP and monitoring the fluorescence emission over time. The entrapment of solutes in microemulsion droplets and liposomes is a complex process that creates a population of compartments with different internal compositions of molecular species, which affects the final protein production. A complete understanding of the distribution of solutes inside the different compartments and on its effect on the course of internal reactions are two relevant and still open issues in the field. Stochastic simulation is a valuable tool in the study of biochemical reaction at nanoscale range; QDC (Quick Direct-Method Controlled), a stochastic simulation software which uses the well-known Gillespie's SSA algorithm, was used; a suitable model reproducing the PURESYSTEM reactions network was hence created, with the aim to describe how the different composition of species affects the overall translation process, thus trying to infer the internal composition of each microcompartment from its observable fluorescent signal emission.

Methods

In order to understand how the protein production is affected by the reactants concentration, we first generate experimental data by combining different amounts of DNA, enzymes, translation factors and consumable. Consequently, a set of fluorescence vs. time curves were generated. Next, the pre-existing translation model was improved to describe in detail a coupled transcription/translation system with simultaneous elongation events on the same molecule. The dynamical coupling between the transcription and translation systems was assessed using logical formulations allowed in QDC's syntax, thus creating sequentially dependent processes in the concurrent-only environment of Gillespie's algorithm. Stochastic simulations were performed in order to globally fit, by sigmoid curves (R^2 > 0.98), the entire experimental dataset for protein production. The comparison of the parameters estimates for the different inputs showed how protein production is strongly affected by enzymes concentration.

Results

Different kinetic parameters were considered, such as the final plateau of production and the initial time required to release the first complete proteins from elongating ribosomes. Further analysis demonstrates how the transcription process plays a fundamental role, determining the rate of GTP depletion from the system, hence preventing ulterior elongation of peptide chains.To the best of our knowledge, the present work is the first one describing in detail the stochastic behavior of the PURESYSTEM™. The developed model allows us to scale the system down to compartment diameters where anomalous entrapment phenomena are presumed. Thanks to our results, an experimental approach is now possible, aimed at recording the GFP production kinetics in very small microemulsion droplets or liposomes, and inferring, by using the simulation as a hypotheses test benchmark, the internal solutes distribution, and shed light on the still unknown forces driving the entrapment phenomenon.