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Bringing the parts together: Steps towards an in-silico protocell

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Abstract: This article focuses on a system theoretic approach to synthetic biology, and in particular on the construction of a protocell model. The questions addressed here are: Which parts of functional modules are required to describe a protocell and which design methods are needed for self-replicating systems. We describe a model for an in-silico protocell that combines experimentally validated biological subsystems with theoretical studies.

Keywords: systems engineering, system analysis, synchronization, synthetic biology, bottom-up approach, artificial cell

1. INTRODUCTION

Synthetic Biology aims at creating new life forms with beneficial properties for chemical, pharmaceutical, or medical applications (Keasling, 2008; Trosset and Carbonell, 2015) but also plays a major role in the fundamental research. The top-down approach of Synthetic Biology, which equips existing organisms with additional capabilities, has recently proven to be feasible for solving technical problems in fuel production and drug production (Keasling, 2012). The complementary bottom-up approach, which tries to build life-like entities from molecular building blocks, is still in its infancy, but in the future may have the potential to provide simple, safe, and well predictable artificial organisms tailored to certain applications (Schwille, 2011).

Currently, the bottom-up approach is mainly driven by biophysical groups, who concentrate on mimicking certain cellular functions like membrane growth, cell division, or cell motility in experiments. Assembling these functions to an aggregate unit is the logical next step, but experimentally very challenging. The question is if one can construct artificial cell-like entities from certain functional devices in a similar way, as one can construct a chemical plant from process units. At this point, there is an obvious link to chemical engineering, systems engineering, and control engineering (Rollié et al., 2012). Engineering sciences are used to combine parts to complex systems with certain well-defined desired properties of the resulting aggregate system, and, in this sense, are mainly target-oriented. This could be a nice complement to the insight-driven approach of natural sciences that strives for understanding a certain part aspect in full detail.

This work uses a simple example of an artificial biological system to discuss possible engineering contributions to bottom-up Synthetic Biology. The idea is to define a certain desired functionality of the artificial system, to select building blocks that may fulfill the desired tasks and to assemble the models of the building blocks to a simple in-silico protocell. There are experimental results and mathematical models for the separate building blocks in literature, but the aggregate system has not been implemented in experiments yet. The aim of the theoretical study is to see if the selected building blocks are able to work in an ensemble, or if additional functionalities like control mechanisms are needed.

It should be noted that detailed whole-cell models built up from submodels exist in literature for real-life biological organisms, e.g. Karr et al. (2012). However, due to the complexity of biological organisms, these models tend to be very large and offer only a limited accessibility to theoretical analysis. The hope is that artificial biological systems are much simpler in their behavior, and that hence their system dynamics can be described and predicted more easily by smaller sets of mathematical equations.

2. STRUCTURED MODEL OF AN ARTIFICIAL CELL-LIKE ENTITY

The exemplary design task considered in the following is the construction of an entity that has the ability to grow, to determine the time point when its size has doubled compared to the original value, and finally to divide into two daughter cells. For this purpose, at least three different functional modules are needed. The first one is a container that forms the system boundary and grows, while its building blocks are generated inside the system. The second functional module is a length sensor that determines the position where the cell should divide. The third module is a divisome that performs the cell

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division by placing a contractile ring in the middle of the cell. In reality, at least one additional functional module would be needed for ATP regeneration as an energy supply. This is neglected here. For simplicity, it is assumed that the ATP level is always high enough to drive the required reactions. The models of the single modules presented in the following are largely taken from literature and adapted to our needs. The combination of the three parts to an artificial cell model is, to our knowledge, something new.

2.1 Expanding container

Mavelli et al. (2014) suggest a simple model for the growth of a vesicle or expanding container shown in Fig. 1.

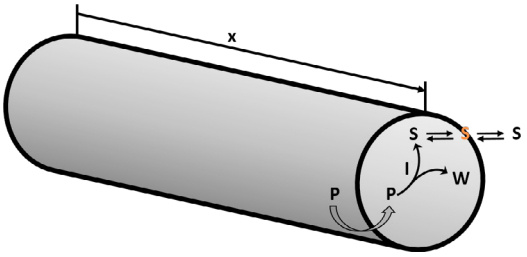

Fig. 1. Functional module-tube shaped membrane. P is a precursor, I is an enzyme, S is the surfactant and W is the waste (Mavelli et al., 2014). x denotes the length of the cylindric compartment.

A precursor P is metabolized by an enzyme I into a surfactant S and a waste W. The surfactant S is included along the entire membrane and increases its surface by a certain amount. While Mavelli et al. (2014) formulate a well-mixed model, we extend the approach to an one-dimensional spatially distributed system, assuming that our artificial cell is tube shaped with a variable length x, but a constant radius R. This assumption is mainly made to simplify the numerical computations, but is not unrealistic for some rod-shaped bacteria. A mass balances of the species P, S, and I lead to the balance equations (1)-(3):

$$\frac{\partial(R^2P)}{\partial t} + \frac{\partial(R^2P\dot{x})}{\partial x} = -\frac{\partial(R^2 j_P)}{\partial x} - R^2 r_S + 2R\wp(P_{ex} - P) \quad (1)$$

$$\frac{\partial(R^2S)}{\partial t} + \frac{\partial(R^2S\dot{x})}{\partial x} = -\frac{\partial(R^2 j_S)}{\partial x} + R^2 r_S - R^2 r_{up} \quad (2)$$

$$\frac{\partial(R^2I)}{\partial t} + \frac{\partial(R^2I\dot{x})}{\partial x} = -\frac{\partial(R^2 j_I)}{\partial x} \quad (3)$$

The concentration change of the precursor P depends on the precursor mass diffusion flow j_P, the surfactant formation rate r_S, and the membrane permeability of the precursor $\wp$. R is the radius of the cylinder and P_{ext} is the extracellular precursor concentration. The surfactant formation rate r_S is calculated as follows:

$$r_S = kIP, \quad (4)$$

where k is the rate constant for the surfactant formation. The term $\dot{x}$ in the equations (1)-(3) denotes the local length change due to growth of the cell; it has a diluting effect on the concentrations. Similarly, the surfactant concentration S in the bulk of the cell depends on diffusive transport j_S, the formation rate r_S, and an uptake rate r_{up}, which describes the transfer of surfactants to the membrane; r_{up} depends on an equilibrium surfactant concentration S_{eq} and is given as:

$$r_{up} = k_{up}(S - S_{eq}); \quad (5)$$

k_{up} is the rate constant for the surfactant uptake into the membrane. The concentration of the enzyme I changes locally due to diffusion, but in a growing cell also globally due to dilution caused by increasing cell volume. For unlimited growth, the dilution effect would have to be compensated by synthesizing I inside the cell or by providing additional I from outside. This is not done here. Instead, the initial value of I in the simulations is chosen sufficiently high to guarantee surfactant formation until the cell size has at least doubled.

It is assumed that the surfactant S built into the membrane contributes to area of the membrane with a specific surface α_s. Calculating the surface of the cylinder leads to the relation:

$$2\frac{\partial R}{\partial t} + 2\frac{\partial(R\dot{x})}{\partial x} = \frac{\alpha_S}{2} R^2 r_{up} \quad (6)$$

which is used to determine the local growth rate $\dot{x}$ for a given radius R.

2.2 Length sensor/positioner

The Min protein system that is an important mechanism in the cell division of *E.coli* is known to exhibit spatial concentration patterns on membrane surfaces. The pattern formation capability in combination with the property of $MinC$ to prevent proteins from attaching to the membrane is supposed to control the cell division of *E.Coli*: A contractile protein ring is placed in the middle of the cell membrane, where the time averaged concentration of Min proteins on the membrane has been shown to be lowest (Huang et al., 2003; Loose et al., 2008; Schweizer et al., 2012). Fig.2 shows a qualitative model of the interaction between the Min proteins and the membrane surface. $MinD$ proteins labeled by the energy rich ATP molecule are able to attach to the membrane. This attachment occurs primarily on membrane regions where already other $MinD$ proteins are attached. The membrane associated $MinD$ protein recruits the $MinE$ protein and a complex formation results. The $MinE$ protein in the complex causes the hydrolysis of the bound ATP molecule, the detachment of the $MinD : MinE$ complex from the membrane and the detachment of the $MinE$ protein from the $MinD$ protein. Finally, the $MinD$ protein is situated in the cytosol binding the less energy rich molecule ADP and the cycle can start again.

The intracellular concentration changes of the Min proteins are calculated by the balance equations (7)-(11) and the reaction kinetics characterized by the equations (12)-(15). The concentration changes of the intracellular Min proteins depend on the mass diffusion flows $j_{D_{ADP}}$, $j_{D_{ATP}}$, j_E and $j_{D_{ADP}}$ as well as the reaction kinetics r_1, r_2, r_3 and r_4 describing the formation of cytosolic $MinD_{ADP}$, membrane bound $MinD_{ATP} : E$, membrane bound $MinD_{ATP}$ and cytosolic $MinD_{ATP}$, respectively.