Modeling of intercellular transport for emerging applications in synthetic biology

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Abstract

Synthetic biology is a way to create new biological functions that do not exist in nature (e.g. targeted drugs, diagnostic microsystems for healthcare applications, green fuels in the field of the environment ...) to meet specific needs. Nowadays, artificial bio-functions become more and more complex. Nevertheless, one of the main bottlenecks is the integration of a large number of artificial genes in the host cell. A promising way to get around consists in dispatching the function in multiple host cells and make them work together in a kind of micro-ecosystem. *In silico* design of such systems requires predictive models of intercellular transport of molecules. This issue has been tackled through two projects carried out by master students from different background (biotechnologies for some of them, microelectronics and computer science for the others). An overview of intercellular transport modeling is given in the first part of this paper. Then, models are illustrated in two examples developed during student projects.

1 Emergence of multi-cellular systems in synthetic biology

Synthetic biology can be defined as the application of engineering principles to the fundamental components of biology. In particular, the design of artificial gene regulatory network is one of the most investigated way to design new biological functions. Although very promising, this technology suffers from two main drawbacks that may limit the complexity of the artificial function. First, the number of artificial genes that can be added to the genome of a given microorganism is generally quite small (some units). Second, artificial genes designed for the application should be orthogonal with each other and with the genome of the host cell. A way to overcome these drawbacks is to split the main function and to implement each sub-function into different host cells [1]. By this way some components (regulating proteins, promoters …) may be used several times inside different host cells. However, sub-functions are not completely independent, that is to say that signal transfer mechanisms between cells have also to be designed [2].

Examples of systems composed with several reprogrammed bacteria achieving a complex function have already been developed (digital gates [3], prey-predators ecosystems [4], programmable pattern generator [5]). For such systems, the proof of concept is carried out through *in sillico* simulations but most of the time, models are simplified and the intercellular transport of species is described roughly. Assumptions used are valid for small systems but may lead to inaccuracy for complex ones. This paper deals with the design-oriented modeling of such mechanisms. The first section is an overview of the intercellular transport mechanisms and the associated models. Then, two examples are given.

2 State-of-the-art-in intercellular transport modeling

Exchange of chemical species between cells involves many steps and these steps are spatially spaced: the molecule have to move inside the sender organism to the plasma membrane, cross this membrane, difuse through the intercellular medium, cross again the plasma membrane of the receptor and move inside the receptor cell to the place where it may have an activity. All

these displacements could be modeled thanks to four different mechanisms: a simple random diffusion, a passive transport, an active transport and exo/endocytosis. Let us first have an overview of these mechanisms and the associated models.

The **random diffusion** corresponds to the displacement of chemical species inside cells or through the extracellular medium. Except for very specific application, the intracellular displacement is ignored (concentration of the species near the membrane is equal to the mean concentration inside the cell). Conversely, extracellular displacement needs to be taken into account. There are several ways to model 2-D or 3-D species displacement [6]. To save simulation time, compartmental model is often implemented, at least during the upstream stages of the design process. The equation which describes the diffusion of molecules from a point A to a point B in an unbounded plane is:

$$\frac{d[X_B]}{dt} = \frac{\gamma}{d_{AB}} \cdot ([X_B] - [X_A]) - D \cdot [X_A]$$

where $[X_A]$ and $[X_B]$ are the concentration of X respectively at the point A and B, d_{AB} is the distance between A and B, γ is the diffusion constant (in $um \cdot s^{-1}$) and D is a decay constant modeling the probability that the species is degraded before reaching B.

The **passive transport** could be compared to simple diffusion through the plasma membrane. As for other diffusion problems in physics, the transport rate is directly proportional to the gradient of the concentration between both sides of the membrane. It does not consume energy. The differential equation that governs this passive transport is:

$$\frac{d[Xout]}{dt} = -\frac{d[Xin]}{dt} = \alpha \cdot S \cdot ([Xout] - [Xin])$$

where $[Xout]$ and $[Xin]$ are the concentration inside and outside the cell, α is a surface permeability coefficient (in $s^{-1} \cdot \mu m^{-2}$) which depends on physic-chemical parameters of the membrane and S is the membrane surface.

The **active transport** of molecules through the membrane requires two elements: energy (ATP in biology) and a specific transporter integrated in the plasma membrane. This transporter fixes ATP, which creates a species

flow that occurs with respect to or against their concentration gradient. Every time an ATP binds on a transporter, it is hydrolyzed and the transporter is recycled. Thus, to simplify the model, the transporter is never consumed by the transport mechanism. Most of the time, the transport occurs only when the concentration of the species to transport is above a given threshold Xth. The model is the following:

$$\frac{d[Xout]}{dt} = -\frac{d[Xin]}{dt} = \begin{cases} 0 & if\ [X_{in}] < Xth \\ V_{max} \cdot [Y] \cdot \frac{[X_{in}]}{[X_{in}]+k_P} - \frac{Xth}{Xth+k_P} & else \end{cases}$$

where V_{max} is the maximum transport rate (in s^{-1}), $[Y]$ is the concentration of the transporter and k_P is a dissociation constant. In order to avoid discontinuities, this equation may be replaced by a standard smoothing function as following:

$$\frac{d[Xout]}{dt} = \frac{1}{2}\left(f(x) + \sqrt{\varepsilon^2 + f^2(x)}\right)$$

where $f(X)$ is the transport rate for $[X_{in}] > Xth$ and ε corresponds to the transport rate for $X = Xth$. It should be noticed that active transport may occur in both direction. Model is the same and the sign of the constant V_{max} corresponds to the transport direction.

The **exocytosis** is the third kind of transportation of molecules. This way of displacement requires energy and the formation of vesicle that will fuse to the membrane. Exocytosis is very important in the brain for the displacement of the neurotransmitter through the synapse. The modeling of the exocytosis is very tricky because of the large number of involved factors [7] and is not discussed in this paper.

3 Example #1: Modeling of a transport mechanism with a competition between passive and active transport

In order to illustrate the purpose, we model a simple system which consists in a source of N-Acyl Homoserine Lactone (AHL), a hormone widely used in synthetic biology because of its natural quorum sensing and

communication roles in bacteria [8], and a receiver cell. The system has been described and simulated with The Virtual Cell (Fig. 1).

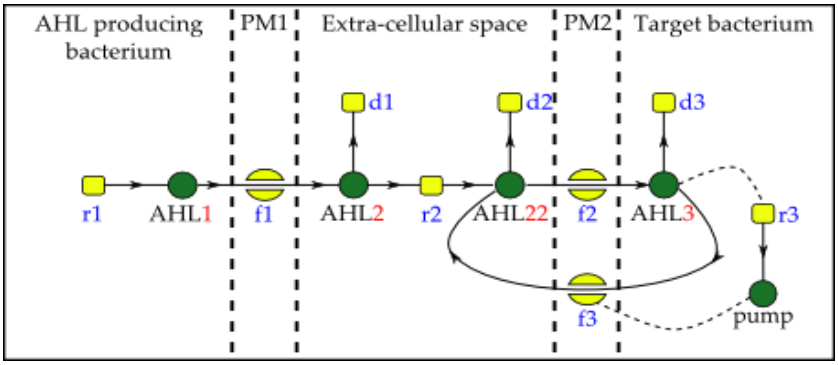


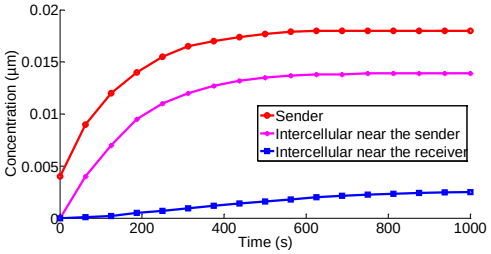
Figure 1 — The Virtual Cell cartoon which corresponds to the Example #1. AHLs are produced inside the first bacteria and freely diffuse through its membrane and the extracellular medium to the second bacteria. Two transports are in competition at this point: a passive transport through which AHLs may go inside the receiver and an active transport (efflux pump) that send back AHLs in the extracellular medium. The active transport is controlled by the concentration of pump which is itself synthesized by a gene activated by the AHL. AHL1, AHL2, AHL22 and AHL3 are respectively the concentration of AHLs inside the sender, in the extracellular medium near to the sender, in the extracellular medium near to the receiver and inside the receiver.

In more details, the model consists in 7 rate equations: (i) the constitutive synthesis of AHL in the production bacteria, (ii) the passive transport through the membrane of the production bacteria, (iii) the random diffusion of AHL between the two bacteria (including decays), (iv) the passive diffusion through the sender membrane, (v) the decay of AHL inside the sender, (vi) the synthesis of the pump which is activated by AHL and (vii) the active transport through the sender membrane which is controlled by the concentration of pump. Equations used correspond to the ones described in previous section.

The simulation parameters are the following. AHL production rate is set to 0.05 $nM \cdot min^{-1}$ and its decay rate to $1.8 \cdot 10^{-3}$ min^{-1}. The surface permeability coefficient of the sender membrane is 0.5 $min^{-1} \cdot \mu m^{-2}$ and the surface of the *E.coli* is estimated to about 4.83 μm^2. The diffusion in the extracellular medium is set to 5 $\mu m \cdot min^{-1}$ and the distance between the sender and the receiver to 10 mm. For the active transport, the maximal transport rate is

4000 nM·min^{-1} and the dissociation constant is 0.25 min^{-1}. The complete Virtual Cell model can be found in the public BioModels library (talide: assb_blanck_talide).

Simulation results are given in Fig. 2. The concentration of AHL in the sender, the receiver and the intercellular medium is monitored. As expected, a small decay is observed between AHL1 and AHL2 due to the degradation and the dilution that occurs in both intra and extra-cellular compartment. The shape of the AHL22 concentration curve really suggests a delay between the time when AHLs go out of the cell and the moment when they are near the target bacteria. The difference of steady state between AHL2 and AHL22 corresponds to the random diffusion in extracellular medium. Finally, the level of AHL3 in the target cell depends on the efficiency of the efflux pump (V_{max}) as well as the threshold concentration (X_{th}) beyond which the pump is not active.



(a)

V_{max} **uM·min^{-1}**	X_{th} **uM**	*AHL3* **uM**
0.4	0	0.1
4	0	0.01
40	0	0.001
4	0.001	0.001
4	0.1	0.005

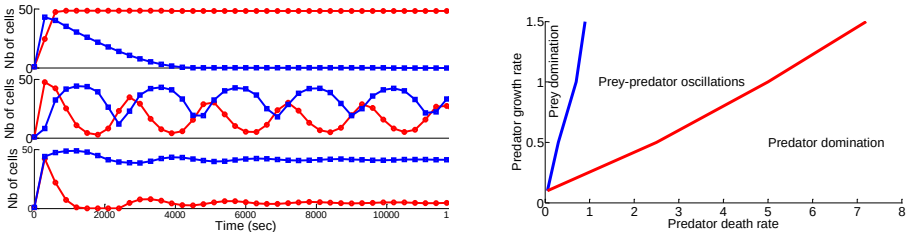
(b)

Fig. 2 ─ Simulation results: transient evolution of AHL concentration as a function of the position (on the left) and concentration of AHL at the steady state in the receiver as a function of the maximum efflux pump rate and the threshold value.

4 Example #2: Improvement of the model of a prey-predator ecosystem with intercellular transport considerations

The second example concerns the prey-predator ecosystem described in [4]. It is composed of two reprogrammed *E.coli* strains. The *prey* system consists in constitutive expression of a suicide gene which can be repressed by an antidote activated by the AHL (3O6HSL) synthesized by the *predator*. The *predator* has a non-constitutive suicide gene that requires another AHL (3OC12HSL), synthesized by the *prey*, to be expressed. This double

feedback loop leads to equilibrium between the number of *predators* and *preys* that strongly depends on the death and grow rate of both bacteria. Three states may be reached: domination of prey, domination of predators or oscillation. In [4], a rough model of the system is established in order to predict these states through static and transient simulations. The aim of this work is to improve this model in order to take into consideration a passive transport of AHL between both cells. The complete mode which consists in 21 differential equations and 58 parameters is implemented in VHDL-AMS, a hardware description language mostly used in microelectronics domain for the description and the simulation of complex heterogeneous systems. The possibility to efficiently describe biological systems through this language has recently been demonstrated [9]. Simulation results, given in Fig. 3, show that the behavior of the system is in accordance with experimental results and simulations obtained with the rough model and described in [4]. Nevertheless, quantitative results are not exactly the same.



(a) (b)

Fig. 3 — Simulation results: on the left, transient evolution of the number of prey (red dots) and predators (blue squares) as a function of the growth rate ratio vs death rate of predators. The ratio is equal to 0.7 for the top curve, 4 for the second and 7 for the third. On the right, representation of the border between the three states as a function of the couple (growth rate of predators, death rate of predators).

5 Conclusions and outlook

This work presented in this paper gives an overview of the way that intercellular transport of chemical species can be modeled. While the discussed models are very basic they are sufficient to take into account the main effect that may occur. The main difficulty encountered in model development is the choice of consistent values for parameters involved in the transport equations. Indeed, most of them are empirical and do not

necessarily correspond to measurable biological signal. They were therefore estimated from values from the literature and / or extracted from experimental results and / or set to an arbitrary value.

The VHDL-AMS implementation performed on the second model is very interesting. Up to now, we demonstrated that the gene regulatory network inside a cell can be modeled by equivalent electronics circuits and, as a consequence, widely analyzed with electronics simulators [9]. According to the equations, transport mechanisms between cells might also been represented by electronic equivalents circuits. As a consequence, this work paves the way to the extension of our modeling formalism to multi-cellular systems.

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