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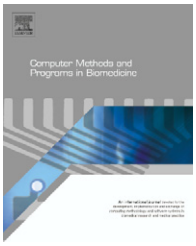

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HEMET: Mathematical model of biochemical pathways for simulation and prediction of HEpatocyte METabolism

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ABSTRACT

Many computer studies and models have been developed in order to simulate cell biochemical pathways. The difficulty of integrating all the biochemical reactions that occur in a cell in a single model is the main reason for the poor results in the prediction and simulation of cell behaviour under different chemical and physical stimuli. In this paper we have translated biochemical reactions into differential equations for the development of modular model of metabolism of a hepatocyte cultured in static and standard conditions (in a plastic multiwell placed in an incubator at 37 °C with 5% of CO_2). Using biochemical equations and energetic considerations a set of non-linear differential equations has been derived and implemented in Simulink®. This set of equations mimics some of the principal metabolic pathways of biomolecules present in the culture medium. The software platform developed is subdivided into separate modules, each one describing a different metabolic pathway; they constitute a library which can be used for developing new modules and models to project, predict and validate cell behaviour in vitro.

1. Introduction

In recent years the progress in high-throughput biotechnology [1–3] has enable a better understanding of cellular and molecular networks and systems on several scales. The attempt to understand the dynamics of living cells is, however, a difficult task, for example consequences of changes introduced in the cell and in its environment, able to alter cell activities such as cell growth rate and phenotype expression, are often unpredictable. Many attempts have been reported in the literature to simulate molecular processes [4], in particular through the kinetics of biochemical metabolic pathways. Several software packages for quantitative simulation of biochemical metabolic pathways, based on numerical integration of rate equations, have been developed, including GEPASI [5], KINSIM [6,7], MIST [8], METAMODEL [9], SCAMP [10] and E-CELL [11]. In predicting cell behaviour, it is important to model not only a single or a few interconnected pathways, but also the complete interactions between all the biochemical processes occurring in the cell. Mathematical modelling of biochemical networks has been attempted since the 1960s and, before genomic-scale pathway information became available, they mostly employed numerical integration of ordinary differential equations for obtaining reaction rates [12–16]. This kind of dynamic model allows the prediction of time dependent parameters such as metabolite concentration and reaction rate. To create accurate simulations, dynamic models require kinetic parameters and detailed rate-laws such as the MWC model [17] and those derived using the King–Altman method [18]. Another approach, such as metabolic flux analysis (MFA)

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using stoichiometric matrices, has been employed for large-scale analyses of metabolism [19,20]. Assuming a steady-state condition, MFA provides a flux distribution as the solution of the mass balance equation without the need for rate equations and kinetic parameters [20,21]. Since it is a "static" approach, the ability of MFA to predict the dynamic behaviour of metabolic pathways is limited. It provides a snapshot of a certain pathway in a single state, but it cannot predict the dynamic behaviour of metabolism [22]. Recently, this approach was extended to allow the prediction of dynamic behaviour, dynamic flux balance analysis (DFBA) [23]. It provides optimal time evolution based on pre-defined constraints, including kinetic rate equations. However, this extension was not intended to reduce the mass of information necessary for developing dynamic cell-scale simulation models. In addition, the DFBA study did not define the criteria for segmenting a whole metabolic pathway into parts defined by kinetic rate equations and by a stoichiometric model. Therefore this effort seems to lead to a generic modelling approach that does not allow evaluating the influence of a single-cell biochemical process on the others; it could be useful for having a generic transfer function of each cell type.

Recently a method for dynamic kinetic simulation of cell-wide metabolic pathways by applying the kinetics-based dynamic method to parts of a metabolic pathway and the MFA-based static method to the rest has been proposed [24].

However, all these models are focused on metabolic aspects, and is very difficult to interface them with those models that give an understanding of particular cell behaviours (neuron spikes [25]), or those that consider only one fixed cellular aspect (heat transmission) inside a tissue or organ [26], and for this reason arranging all aspects together in order to have a model of the whole cell or, indeed, of an organism is an unsolved challenge The difficulty of cross-talk between different models has led to the lack of a common language could allow the development of software platform.

The model reported in this paper goes in this direction because it has been developed starting from the biochemical reactions that underline the principal metabolic pathways in order to have a direct relationship between the "cell" system and the equations of the model that describe it. This approach could represent the basis for the development of a common language for cell modelling.

2. General outline of developed model

The model developed in this work and the implemented system have two principal goals:

- a modular structure, adaptable to each cell type and that could represent a milestone towards the development of new software platform for cell biology;
- a tool for design and validation of a cell experiment, in which the user can modify one or more parameters of the model and evaluate the effect of this variation on cell function in a simple and friendly way.

This type of software platform could be used to simulate in vitro experiments in a cell culture laboratory, optimize experimental protocols and, in the long run, reduce animal sacrifice.

The model schematizes the principal reactions that occur inside the cell using a formalism typical of systems theory. Each reaction is represented as a module. In the cell environment, the concept of feedback can be applied both to a global scale (the entire cell) and to local process (each metabolic or not reaction). In fact, a cell can be described as a feedback system that responds to an external stimulus, which modifies its status, through a series of reactions (metabolism), in order to restore its dynamic equilibrium (homeostasis) (Fig. 1). This is possible because all metabolic reactions are catalyzed by enzymes which allow the cell to have a fine control of energetic flow that comes from substrates. In our system the substrate is each biomolecule necessary for the development of a reaction. The principal ways that cells have to control the enzyme activity are two: the first is the auto-inhibition of a metabolic pathway due to a surplus of its products (local feedback), the other one is the energetic control that is regulated by the availability of energy or electron transporters (ATP, NADPH).

Cell metabolism is a function of ATP concentration, and its time dependent behaviour is related to cell energy, which activates or inhibits a metabolic pattern, shifting the equilibrium of the reaction or acting as a cofactor in enzymatic reactions, in which the kinetics are described by Michaelis–Menten model [27].

Because the typical cell concentration of metabolites is negligible [28] compared to K_M, the cell is sensitive to the concentration of substrates and the kinetics of reaction can be simplified:

$$v = \left|\frac{ds}{dt}\right| = \frac{v_{MAX} s}{K_M} \tag{1}$$

where v_{MAX} [M s^{-1}] is also a function of enzyme concentration and s [M] is the substrate concentration.

This approximation allows schematizing metabolic reactions with first order kinetics because their velocity increases with increase of reactants concentration, without arrive at saturation. Using this approximation, starting from a biochemical reaction:

$$A + B + \ldots + N \overset{\text{Enzyme}}{\longrightarrow} S \tag{a}$$

it is possible to obtain a differential equation:

$$\frac{ds}{dt} = A + B + \ldots + N - \alpha s \tag{2}$$

where A, B, …, N are the reactants, and s is the product of reaction. The production rate of s (and produced amount) is proportional to the substrate concentrations and decreases as the product increases, because a metabolic reaction is inhibited by plenty of its product. The parameter α depends on the rate of the consumption of s, which is one of the reactants of the following metabolic reaction.

Generally, the energetic control does not appear expressly in the stoichiomentrical equation of each reaction: for the purpose of this model we assume that ATP and NADPH have certain threshold concentrations (σ and θ) above which metabolic reactions stop or take place.