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JWS ONLINE CELLULAR SYSTEMS MODELLING AND THE SILICON CELL

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ABSTRACT

Rapid developments in bioinformatics over the last decade, coupled with a dramatic increase in the amount of available, quantitative data has necessitated the need for good analysis tools to quantitatively understand the functioning of biological systems. Detailed kinetic models offer one such tool and while such models have been developed since the 1960s little attention has been paid to the presentation and conservation of such models. Here we focus on the JWS Online project (http://jjj.biochem.sun.ac.za) and its role in 1) offering a web based tool for analysis of kinetic models, 2) acting as a repository for published kinetic models and 3) facilitating the reviewing of new models. In addition we advocate the use of a specific type of kinetic models, the so-called "Silicon Cell" models (http://www.siliconcell.net). By elaborating on the process of constructing one such model, based on yeast glycolysis, we illustrate the approach of "modular modelling" and "model combining." This approach is presented as a preferred method to model biological systems, as opposed to the building of single large models.

INTRODUCTION

Models are tools that can be used to address many different questions. From the multitude of different kinds of models we focus here on detailed kinetic models using ordinary differential equations (ODEs) to describe biological systems. Our aim in using such models is to get a quantitative understanding of the functioning of the living cell.

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The ultimate and very ambitious goal is to build a computer replica of a living cell. Our approach in doing so is a modular one. Build detailed kinetic models - modules - that can be studied, at the enzyme level, as isolated units of the complete system. The approach is a bottom up approach; the model is constructed on the basis of characterization of the individual components and their interactions. Such models are validated in independent experiments and posted in a model database. Interacting modules can be grouped together and a larger model can be constructed and revalidated. In this way the model will be gradually extended and upon each module addition a new validation can be done, eliminating the overwhelming parameter optimization that would be necessary if a large model were constructed in one step.

The level at which these detailed models are build is at the enzyme level, the enzymes being the catalytic units in the cell, the synthesis of which is regulated as a part of the cellular process. In addition many drugs are effective at the level of the enzyme activity and if our models are to be important in drug development strategies we need a good representation at the enzyme activity level. Of course here the modeller can use the huge body of knowledge that is available from the field of enzymology. At the core of the models lie the kinetic rate equations, which have been studied for over a century by enzymologists. However enzymologists and system biologists have a different topic of study, where for the former the enzyme is the system, the latter is interested in a set of enzymes working together in a cellular environment. This has important implications for the conditions under which the enzymes are characterized.

Of course an ambitious project such as attempting to build a silicon cell cannot be achieved in a single research group, but will be dependent on the collaboration between a large number of groups active in experimental as well as modelling and theoretical fields. Of crucial importance in such collaborations will be the standardization of experimental conditions for enzyme kinetic measurements, decision on model organism(s) and growth condition(s) that will be modelled.

Global initiatives such as the newly formed *Systems Biology for Yeast* might play an important role in coordinating such projects while organizations such as the Beilstein Institut could play an important role in catalysing the standardization procedures.

In these proceedings we will treat several of the aspects that are, in our view, important in the process of building the silicon cell and show how the JWS Online Cellular System modelling project can be used to achieve them.

We will start by introducing the JWS project and explain its function as an easy to use, web-based modelling tool, a model repository, and its role in model curating. We will finish by illustrating the bottom-up modelling approach as advocated in the Silicon Cell project using our detailed kinetic model of yeast glycolysis as an example.

MOTIVATION OF THE JWS PROJECT

ODE based models of biological systems have been used for over 40 years and many models have been published. Analysis of these models is usually heavily based on computer simulations due to the non-linear character of the ODE's, and whereas such analyses were limited by computer strength in the early years, these limitations have been largely overcome and it is now possible to simulate large sets of ODE's on todays desktop computers. Not only have there been rapid advances in the development of computer hardware but the development of good numerical algorithms for solving differential equations have made it easier to build and analyse kinetic models. In addition, several specialist software packages for simulations as well as general mathematical programmes now incorporate these algorithms, allowing the user to work in a high level environment. Still a certain amount of knowledge is necessary to build and analyse kinetic models and for someone that is not initiated in this theory and who would like to quickly check an existing model for its ability to describe a set of experimental data there is no off the shelf tool to do so. First, a new user would have to figure out what software tools are available and make a decision on which one to use. Second, the user would have to acquire the software (this is often easy as most of the packages are free of charge and can be downloaded from the internet), and install the software on his/her computer. Thirdly, the user would have to learn how to use the software and with many of the packages having a multitude of options this is not necessarily simple. After all this, there is still no model to run and this might be the biggest hurdle to overcome. The user is interested in running an existing model but where can such models be obtained? Of course the user may contact the author and in such a way receive the model in digital form but this might be in the dedicated format of whatever software package the model builder has used and this is not necessarily compatible with the easy to use packet the user has selected. Often model descriptions only exist in literature, an electronic description of the model is no longer available and the user will have to code the model from the manuscript, a non-trivial task.

To overcome these problems we have started the JWS Online Cellular Systems modelling project, a repository of kinetic models that can be run over the internet using a standard web browser [1].

JWS AS A REPOSITORY OF MODELS

The JWS project was started as an effort to collect existing kinetic models of biological systems and present/preserve them in an easy to use format. It quickly became apparent that many of the models described in the literature run the risk of being lost. The description of models in manuscripts is often very poor and electronic versions of the models have been lost or formats have become outdated. No official repository of kinetic models existed and no standard way of presenting kinetic models in the literature has been agreed upon.

Two initiatives to standardize a model description of biological systems, using XML based exchange formats, should be mentioned: CellML (http://www.cellml.org) and SBML (http://www.sbml.org). Both these initiatives also have a list of kinetic models that can be downloaded in either CellML or SBML format. The number of programs that can load these file formats and that can be used to run these models is still limited (especially for CellML) but the list of programs supporting SBML is growing rapidly.

The focus of the JWS project is not so much on an exchange format but on building a repository of kinetic models. These models can be directly run on the web site (see below), thus no exchange format is necessary. However, in order to allow users to run the listed models on a stand-alone computer we are making a number of different formats available. Currently a download feature for models in SBML format is available for a number of models, illustrating the collaboration between the SBML group at CalTech and the JWS project in exchanging models. Future downloads will be made available as Mathematica notebooks Copasi, and PySCeS formats.

Currently the database holds 19 models in the Silicon Cell category, shown in Fig. 1. In addition to the Silicon Cell category two other types of models are stored, 1) Core models, minimal models that illustrate a specific hypothesis or idea and are not necessarily based on realistic kinetics, and 2) Demonstration models, simple models mostly used for teaching or demonstration purposes.

We try to include as many models as possible and invite users to submit to us models that they would like to include in the database. Please contact us via e-mail (jls@sun.ac.za) for submission formats.

The Silicon Cell: detailed metabolic models			
Detailed glycolytic model in *Lactococcus lactis* - model	Hoefnagel *et al.* - 2002	more	
Glycolysis in *Trypanosoma brucei* - model	Bakker *et al.* - 2001	more	sbml
A Computational Model for Glycogenolysis in Skeletal Muscle - model	Lambeth *et al.* - 2002	more	sbml
Pyruvate branches in *Lactococcus Lactis* - model	Hoefnagel *et al.* - 2002	more	sbml
Glycolysis in *Saccharomyces cerevisiae* - model	Teusink *et al.* - 2000	more	sbml
Sucrose accumulation in sugarcane - model	Rohwer *et al.* - 2001	more	
Bacterial phosphotransferase system - model	Rohwer *et al.* - 2001	more	
Threonine synthesis pathway in E. coli - model	Chassagnole *et al.* - 2001	more	
Kinetics of Histone Gene Expression - model	Koster *et al.* - 1988	more	
Glycolysis in *Saccharomyces cerevisiae*, 6 variables - model	Galazzo *et al.* - 1990	more	
Full scale model of glycolysis in *Saccharomyces cerevisiae* - model	Hynne *et al.* - 2001	more	
Quantification of Short Term Signaling by the Epidermal GFR - model	Kholodenko *et al.* - 1999	more	
Red Blood Cell Model - model	Mulquiney *et al.*		
Mechanism of protection of peroxidase activity by oscillatory dynamics - model	Olsen *et al.* - 2003	more	
Dynamic model of *Escherichia coli* tryptophan operon - model	Bhartiya *et al.* - 2003	more	
MCA of Glycerol Synthesis in *Saccharomyces cerevisiae* - model	Cronwright *et al.* - 2003	more	
Mathematical modelling of the urea cycle - model	Maher *et al.* - 2003		
A kinetic model of the branch-point between the methionine ... - model	Curien *et al.* - 2003	more	
Modelling Photosynthesis and its control - model	Poolman *et al.* - 2000	more	

Figure 1. Screen capture of the current (December 2003) models in the Silicon Cell category of the JWS repository of kinetic models. Each of the models can be interrogated by clicking on its specific model link. Literature references to the specific models are listed under 'more links'. Download options in SBML format is limited to only four models at present via the SBML link.

JWS AS AN EASY TO USE WEB BASED SIMULATOR

JWS allows users to run and interrogate kinetic models via any browser that is capable of running Java2 applets. Most modern browsers support the SUN Microsystems J2RE plug-in and modern versions have this plug-in pre-installed. The system has been tested using the Microsoft windows operating system 98 and higher using Internet Explorer 5+ and Netscape 6+, using Mac OS X and the SAFARI browser, and MOZILLA under Linux. Using any of these browsers the user can type in the URL for the JWS site (http://jjj.biochem.sun.ac.za) and after selecting the database link on the home page the user can interrogate any of the models listed in the database by clicking on the model link (see Fig. 1). Upon selection of a model a graphical interface is downloaded to the user as an applet, in which the user can change parameter values and select the type of simulation requested.

An example of such an applet is given in Fig. 2.

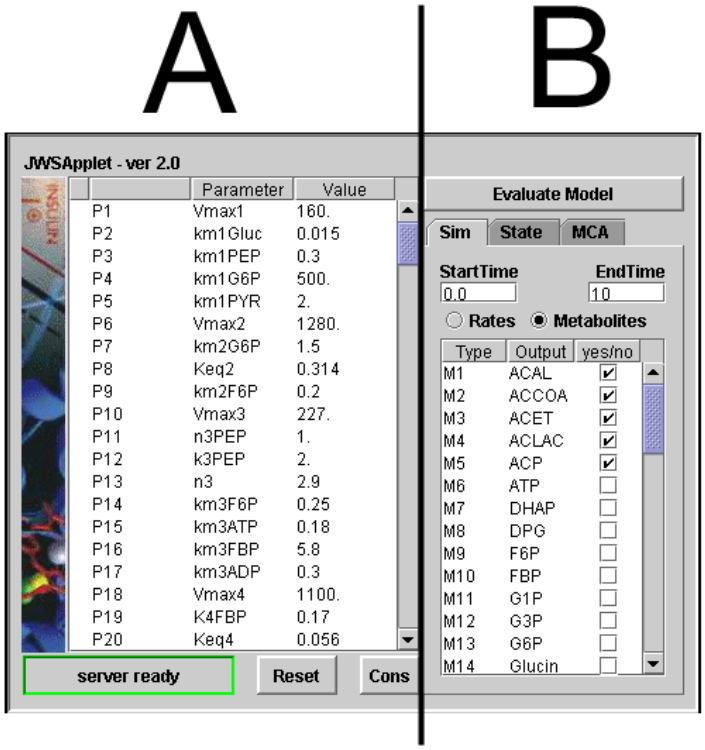

Figure 2. Screen capture of an example model applet. The applets consist of two panels: **A)** a scrollable table listing all parameter values and initial conditions, **B)** a control panel on which the user can select to either do a time simulation (Sim), a steady-state (State) analysis or a control analysis (MCA) by clicking on either of the three tabs at the top of the panel. The model simulation is started by pressing the "evaluate model" button.

In the left hand panel, (Fig. 2A) model parameters and initial conditions can be changed by the user in the scrollable table. The right hand panel (Fig. 2B) is used to select between the different simulations, i.e. time simulations (Sim), steady-state analysis (State) or metabolic control analysis (MCA), by clicking on the respective tabs. In Fig. 3 the respective panels are shown as if each of the three options were selected. In Fig. 3A the user has selected the Sim option, (default) and in this option the user can set the begin and end time of the simulation, and whether metabolites or rates should be plotted in the resulting graph. Figure 3B lists the options available for steady-state analysis. These options include: Steady State, N, K, or L matrix, Jacobian and Eigenvalues.

After the relevant options have been selected and the evaluate button pressed, a table with the steady-state metabolite concentrations, fluxes or the requested structural matrix will be displayed.

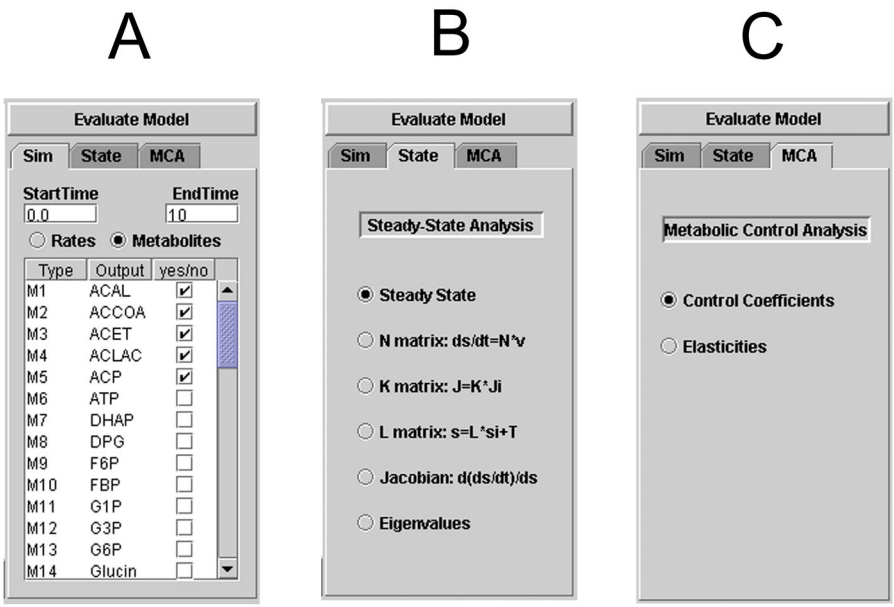

Figure 3. A), B), C), the control panel of the JWS applet showing the three types of simulation and their options.

In addition to the applet, the user also downloads a scheme of the model system. In this reaction scheme each of the catalytic steps is indicated and by moving the mouse cursor over the enzyme steps, the rate equation used in the model is shown in a panel below the applet.

Upon pressing the evaluate model button the user sends a request to the JWS server to analyse the model according to the selected options. After the analysis is complete a GIF file containing the result is send back to the user and displayed in a separate window (Fig. 4).

The best way to try the JWS simulation engine is of course to simply point your browser to the URL of any of the mirror sites and try it out yourself. Currently there are three sites for accessing the JWS models: the main site at the University of Stellenbosch, South Africa (http://jjj.biochemistry.sun.ac.za), and two mirror sites, one at the Vrije Universiteit in Amsterdam (http://www.jjj.bio.vu.nl) and at the Virginia Bioinformatics Institute, U.S.A. (http://jjj.vbi.vt.edu). Try the site and give us feedback either via e-mail (jls@sun.ac.za) or via the model forums.

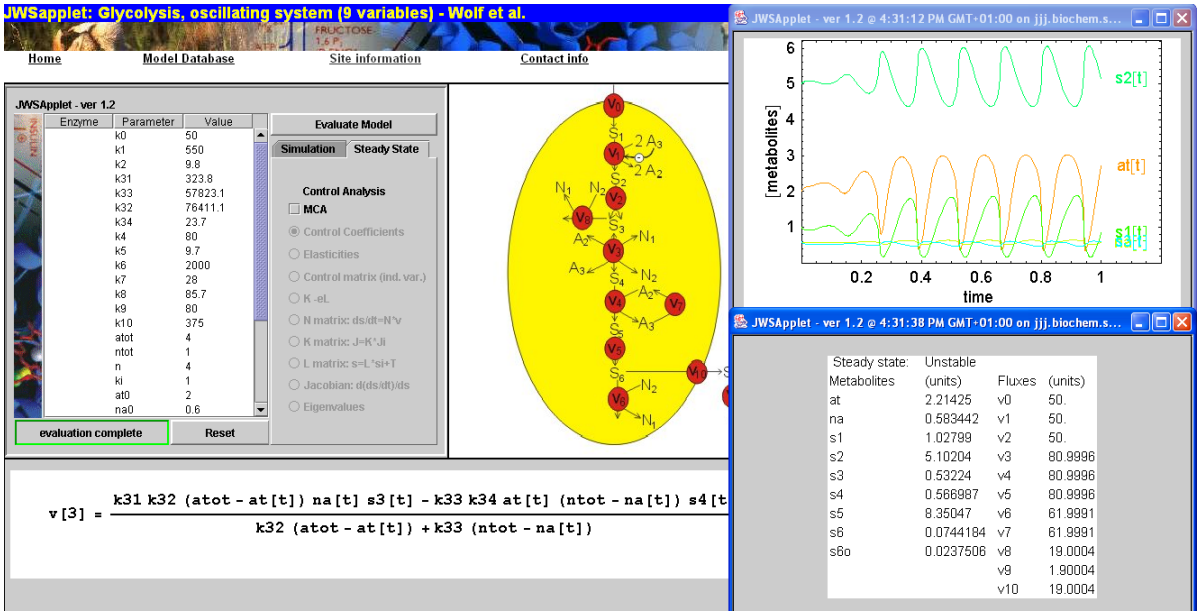

Figure 4. A typical screen capture with applet, scheme, a rate equation and some result windows.

JWS AS A CURATION TOOL FOR MODELS

Initially models to be included in the JWS repository were coded from the literature and a surprisingly high percentage of model descriptions were not complete, vague or lead to results different from those published. This indicated a weakness in the reviewing process of manuscripts containing kinetic models. Whereas it is assumed that a manuscript containing a model description is also checked on the correctness of the model, in practice such a check is usually not done. This is not surprising since it takes a lot of time to code a model from the literature and to rerun the simulations performed by the authors might not be a trivial task.

To improve the quality of model descriptions in manuscripts, and to assist in the reviewing process of manuscripts containing models, we recently started collaborating with the journals *Microbiology* and *European Journal of Biochemistry* to upload models onto a secure part of the JWS site. If a manuscript containing a suitable kinetic model is submitted to these journals the authors are requested to submit a model description in electronic form to JWS Online. Subsequently, the model will be made accessible to the authors and reviewers of the manuscript. After acceptance of the manuscript the model will be transferred to the public part of the database.

This collaboration with scientific journals assures a steady supply of new models to the database. In addition it makes models described in the literature more accessible, a direct link in the manuscript is given to the model applet, thus a reader of the manuscript has direct access to the model via the Internet.

THE SILICON CELL PROJECT

We initiated the Silicon Cell project at the Vrije Universiteit in Amsterdam to advocate the use of specific kinds of kinetic models (http://www.siliconcell.net). Such models, coined Silicon Cell models are intended to be replicas of (parts of) the cellular metabolism. What distinguishes this approach from other modelling strategies is that the parameters must be experimentally determined. The ultimate aim is to be able to make a quantitative description of the cellular metabolism using a kinetic model. Such models would be very powerful tools, for instance, in medical and metabolic engineering studies.

The Silicon Cell models are collected in the JWS database and separate models can easily be linked to bigger models (see below).

BUILDING A SILICON CELL TYPE MODEL OF YEAST GLYCOLYSIS

As an example model to illustrate the Silicon Cell approach we will use the detailed kinetic model on yeast glycolysis published by our group [2]. Although several kinetic models have been published on yeast glycolysis, we were interested in a specific question, can we describe yeast glycolysis on the basis of its enzyme kinetics as measured in isolated form *in vitro*?
This is an important question, rooted in a reductionist approach, can we describe the whole system on the basis of the characteristics of the isolated components? If this question can be answered positively this would help tremendously in building kinetic models of the living cell since we can then use the whole field of biochemistry in an integrated approach for studying systems biology.

The first step in the modular approach of building a Silicon Cell is to delineate the module. We were interested in studying yeast glycolysis under conditions where this pathway was isolated, as much as possible, from the rest of metabolism. Thus, in our first attempt we chose the linear pathway from glucose to ethanol as our system. We worked under anaerobic conditions so no carbon was converted via the citric acid cycle and since we worked under non-growing conditions we ignored anabolic routes.

Thus, we focused on the enzymes in the Embden-Meyerhof-Parnas pathway with as additional enzymes glucose transport, pyruvate decarboxylase and alcohol dehydrogenase.

As stated above, we should be careful in choosing the conditions under which to study the isolated components. In a systems biology approach it is important to study the systems components under the conditions under which they are active in the cell. This could be a very difficult task, it would be virtually impossible to mimic the cellular environment precisely and if the enzyme characteristics are crucially dependent on these conditions then erroneous results might be obtained.

We deliberately chose yeast glycolysis as a system because it has been studied extensively. Thus, many of the enzymes had been characterized in terms of kinetic mechanisms, and parameter values have been determined. As a first assumption we used the kinetic mechanism as has been published but noticed that for many of the enzymes the assay conditions were different from those prevailing in the cytosol. Thus we re-measured many of the enzyme kinetic parameters using the same assay buffer for all the enzymes [2].

After the enzyme kinetic information was thus collected, a kinetic model was build and analysed. This initial kinetic model did not lead to a steady state. When subsequently compared to experimental *in vivo* data (i.e. substrate consumption and product formation rates by a yeast culture) a number of branches were shown to be active in the system. This result shows the importance of delineating the system correctly; clearly we had not incorporated all the reactions that were active in the system. When branches to glycogen, trehalose, succinate and glycerol were added to the model (with simplified kinetics as only limited information on the kinetics of these reactions was available), a steady state was obtained.

Validation is an important aspect of the modelling process and should be done independent of the model construction, i.e. one cannot fit kinetic parameters on the same data set that will be used for the validation. In our silicon cell approach there is an even stronger restriction; kinetic parameters must be determined on enzymes and cannot be determined on system data sets. This does not mean that only *in vitro* measurements on purified enzymes can be used, with NMR techniques one can also obtain kinetic information on enzyme activities *in vivo* as a function of its substrate and product concentrations.

Validation of the yeast glycolytic model revealed that the model quite accurately described the pathway fluxes, i.e. within 10% of the experimentally determined values, but that some of the intermediate concentrations were not accurately described i.e. some were more than a factor 5 off. Important as a next step of validation is to go back to the model and check whether the model can describe the experimental data precisely given a certain error range of the experimentally determined parameter values. The model could, within a 5% error margin for the kinetic parameter values, give a precise description of the experimental data set. This result shows that the deviation observed between the model prediction and the experimental *in vivo* data set does not mean that the model is essentially wrong. A next important step would be to validate the model for different steady-state conditions. Validation should be an iterative process between model and experiment. Thus, after model construction it is tested against an experiment and differences are analysed, preferably going back to the isolated step. If a difference can be linked to a specific enzyme in the model but cannot be resolved within the measurement error of the kinetic parameters of that enzyme then this indicates that the rate equation used is not correct. Possibly a regulatory link is not included in the rate equation or the assay conditions under which the enzyme was measured were too different from the *in vivo* conditions. The problem can be addressed from both the modellers and the experimentalists side. The modeller can check what needs to be changed to the existing kinetic parameters to make the rate equation fit the experimental data and check experimentally whether such a value is realistic. The experimentalist can further characterize the enzyme *in vitro*. Also *in vivo* data, for instance other steady-state conditions might help to pinpoint errors in the rate equation.

In addition to validations using steady-state experimental data a more stringent validation test can be made on the dynamic behaviour of the model. Experimentally yeast has been observed to show limit cycle oscillations in the glycolytic pathway. Our model shows a stable steady-state solution and at first sight this might appear to be in disagreement with the dynamic experimental data. However here one should realize that the limit cycle oscillations in yeast glycolysis are only observed under specific experimental conditions. These include harvesting the yeast cells in a specific phase of the growth curve (several hours after glucose run-out) and after addition of cyanide and working at relatively high cell densities [3, 4]. It is known that yeast has a rapid change in expression of glucose transporters after the run out of glucose and our model could be made to oscillate after changing the activity of the glucose transport and the ATP hydrolysis reactions.

Thus, qualitatively the model is in agreement with the experimental dynamic behaviour in that it can show limit cycle oscillations. However, the description is not very precise; the frequency of the oscillations is 0.5 min^{-1} while the experimental frequency is 1.5 min^{-1} and the amplitude of the oscillations of the metabolites is too low.

To be able to observe the limit cycles experimentally the oscillations in different cells must be synchronized. Without synchronization small phase differences in the yeast cells would level out the oscillations of the population. Our hypothesis that synchronization works through acetaldehyde, a volatile compound that diffuses rapidly through the membrane and can thus act as a communicating molecule [5]. Cyanide complexes with acetaldehyde and oscillations are only observed in a narrow range of cyanide concentrations. The detailed kinetic model also showed synchronisation via acetaldehyde if the concentration of yeast cells chosen was high enough.

BUILDING THE SILICON YEAST CELL

One of the biggest challenges of systems biology is to construct detailed models of complete cells. The large number of parameters and limited information on interactions especially between macromolecules makes the construction of such models a daunting task. We would propose to tackle the problem by dividing the cell up in a large number of modules for each of which a model is build that is validated on its own. These models are collected in the JWS Online database and can be linked to other existing models on parts of yeast metabolism. When models are grouped together another round of validations needs to be made after which subsequent models can be added.

To illustrate the approach we have linked three independently build models of parts of yeast metabolism. We have taken the yeast glycolytic model as described by Teusink et al. [2] as the core and have replaced the simple description for glycerol formation by a detailed description as given by Cronwright et al. [6]. We have also added another branch of glycolysis, the Methylglyoxal pathway as described by Martins et al. [7]. Importantly, the overall description of the steady-state metabolite levels was significantly improved in the total model as compared to the glycolysis model on its own.

Of course such a project would need the support of a large number of experimental and modelling groups to work together. At the ICSB 2003 the international workshop for yeast was started and such a workgroup could possibly coordinate such a project.

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