Standards, Platforms & Applications

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Summary

With the sequencing of the human genome, it has become apparent that Systems Biology, the understanding of cellular networks through dynamical analysis is becoming an important part of research for mainstream biologists. One of the indicative trends to emerge in recent years is the development of model interchange standards that permit biologists to easily exchange dynamical models between different software tools. In this chapter two chief model exchange standards, SBML and CellML are described. In addition, the development of extensible software frameworks, including SBW, BioSPICE and BioUML are discussed and the role they might play in stimulating the development of new tools and approaches. Finally, the range of possible computational applications is described, highlighting the rich set of tools that is emerging as systems biology becomes a mainstream science.

1 Introduction

Although Computational Systems Biology may seem to be a recent field of endeavor, its origins can be traced as far back as the 1920s and 30s (Wright 1929). During this period it was already believed by some that genes were responsible in some way for specifying enzymes. It was also around this time that glycolysis, the first metabolic pathway, was being elucidated and the beginnings of the idea that enzymes formed linked sequences called pathways. It is even more remarkable therefore that given the infancy of these concepts, Sewell Wright should attempt to give a physiological explanation for the occurrence of genetic dominance and recessivity (Wright 1934). Wright argued that the explanation for the origin of dominance lay with the properties of catalytic networks, and laid out an initial mathematical theory which described the properties of enzyme networks[1]. In the 1940's, as the first digital computers were being built, pioneering individuals such as Garfinkel, Higgins and Chance began investigating the possibility of modelling the subtle behavior of biochemical pathways. Even before the advent of the digital computer, the same group had been using using analog computers to model simple biochemical pathways for almost 15 years (Garfinkel et al. 1961; Higgins 1959; Chance 1943).

Since the work of the pioneers in the 1950's, there have been many small groups that have continued this line of inquiry and that together laid the foundation for many of the techniques and theory that we use today and take for granted, in contemporary systems biology. It should be noted that there is a large body of literature, particularly in the Journal of Theoretical Biology, dating back fifty years that many newcomers to the field will find useful to consider.

[1] This early work later became significant during the development of metabolic control analysis (Kacser and Burns 1981)

1.1 What is Systems Biology?

There are many conflicting opinions today on what exactly systems biology is. Historically the answer seems clear. The chief aim of systems biology is to understand how individual proteins, metabolites and genes contribute quantitatively to the phenotypic response. Lee Hood, president of the Institute of Systems Biology in Seattle, US, defines it similarly as "the identification of the elements in a system and the analysis of their interrelationships so as to explain the emergent properties of the system". Even so, some believe systems biology to be concerned with the collection of high-throughput data while others consider the elucidation of protein-protein networks and gene networks to be its hallmark. Certainly, both are vital prerequisites for understanding systems but neither alone can offer great *insight* into how networks operate dynamically.

Systems Biology is the natural progression of classical molecular biology from a descriptive to a quantitative science and is concerned with the dynamic response of biological networks.

1.2 Statement Of Problem

Building models is not an entirely new approach to biology. If one examines any text book on molecular biology or biochemistry, virtually every page has a diagram of a model. These models, which are often termed cartoon based models, represent the culmination of years of painstaking research; they serve as repositories of accepted doctrine and the starting point for the generation of new hypotheses. There are, however, limits to what can be done with these models, their predictive value tends to be poor, and the ability to reason using qualitative models is limited. In other sciences these limitations are avoided through the use of quantitative models, models which are described not just pictorially but also mathematically. Quantitative models by their nature have much better predictive value compared to qualitative models, but their real usefulness stems from the capacity to carry out precise reasoning with them.

2 Quantitative Approaches

There is a wide range of mathematical representations that one can use to build quantitative models, the choice of approach depending on the type of biological question, the accessibility of experimental data and the tractability of the mathematics. A short list of modelling representations is given in Figure 1. Probably the most successful and widely used kind of model are those based on differential equations (both ordinary and partial). These models assume a continuum of concentrations and rates. In reality of course, cellular systems are discrete at the molecular level, however, since the numbers of molecules is very large, the continuum approximation turns out to be very good. When the number of molecules drops to below a certain threshold the continuum model can break down and in these cases one must revert to stochastic simulation. The disadvantage of a stochastic simulation is that all the analytical methods available for continuous models no longer apply. One should therefore only use stochastic simulation if it is absolutely necessary and not in cases where an ODE based model adequately describes the data. This problem highlights the need to develop a new set of mathematical approaches in order to understand the dynamics of stochastic systems. There are other approaches, which include boolean, bayesian, formal logic and connectivity studies but these have yet to show any overwhelming advantage over continuum based models.

In this chapter I will be exclusively concerned with models based on differential equations and to a lesser extent stochastic equations.

Boolean: One of the simplest possible modelling techniques is to represent a network using Boolean logic (deJong 2002). This approach has been used to model gene networks.

Ordinary differential equations (ODEs): This is the commonest and arguably most useful representation. Although based on a continuum model, ODE models have proved to be excellent descriptions of many biological systems. Another advantage to using ODEs is the wide range of analytical and numerical methods that are available. The analytical methods in particular provide a means to gain a deeper insight into the workings of the model.

Deterministic hybrid: A deterministic hybrid model is one which combines a continuous model (e.g ODE model) with discrete events. These models are notoriously difficult to solve efficiently and require carefully crafted numerical solvers. The events can occur either in the state variables or parameters and can be time dependent or independent. A simple example involves the division of a cell into two daughter cells. This event can be treated as a discrete event which occurs when the volume of the cell reaches some preset value at which point the volume halves.

Differential-algebraic equations (DAEs): Sometimes a model requires constraints on the variables during the solution of the ODEs. Such a situation is often termed a DAE system. The simplest constraints are mass conservation constraints, however these are linear and can be handled efficiently and easily using simple assignment equations (see equation 2). DAE solvers need only be used when the constraints are nonlinear.

Partial differential equations (PDEs): Whereas simple ODEs model well stirred reactors, PDEs can be used model heterogenous spatial models.

Stochastic: At the molecular level concentrations are discrete, but as long as the concentrations levels are sufficiently high, the continuous model is perfectly adequate. When concentrations fall below approximately one hundred molecules in the volume considered (e.g. the cell or compartment) one has to consider using stochastic modelling. The great disadvantage in this approach is that one looses almost all the analytical methods that are available for continuous models, as a result stochastic models are much more difficult to interpret.

Figure 1: A non-exhaustive selection of mathematical techniques for mod-

2.0.1 Quantitative Models Based on Differential Equations

It is probably fair to say that most of the successful models to be found in the literature are based on ordinary differential equations. Many researchers will express these models using the following equation:

$$\frac{d\boldsymbol{S}}{dt} = \boldsymbol{N}\boldsymbol{v}(\boldsymbol{S}(\boldsymbol{p}), \boldsymbol{p}) \tag{1}$$

where $\boldsymbol{S}$ is the vector of molecular species concentrations, $\boldsymbol{N}$, the stoichiometry matrix; $\boldsymbol{v}$ the rate vector and $\boldsymbol{p}$ a vector of parameters which can influence the evolution of the system. Real cellular networks have an additional property that is particularly characteristic of biological networks, this is the presence of so-called moiety conserved cycles. Depending on the time-scale of a study, there will be molecular subgroups conserved during the evolution of a network, these are termed *conserved moieties* (Reich and Selkov 1981). The total amount of a particular moiety in a network is time invariant and is determined solely by the initial conditions imposed on the system[2].

In metabolism, conserved cycles act as common conveyers of energy (ATP) or reducing power (NAD); in signaling pathways they occur as protein phosphorylation states while in genetic networks, they occur as bound and unbound protein states to DNA. These conserved cycles will often have a profound effect on the network behavior and it is important that they be properly considered in computational models.

From the full set of molecular species in a model, it is customary to divide the set into two groups, the dependent ($\boldsymbol{S_d}$) and independent set ($\boldsymbol{S_i}$). This division is dependent entirely on the number and kind of conserved cycles in the network. If there aren't any conserved cycles in a model then the de-

[2]There are rare cases when a 'conservation' relationship arises out of a non-moiety cycle. This does not affect the mathematics but only the physical interpretation of the relationship. For example, A → B + C; B + C → D has the conservation, $B - C$ = constant,

pendent set is empty and the size of the independent set equals the number of molecular species in the model. For details on how to compute $\boldsymbol{S_d}$ and $\boldsymbol{S_i}$ the reader should consult (Sauro and Ingalls 2004) or refer to Box 1 in this chapter. In many cases it is vital that this separation into dependent and independent species be made. For simple time course simulations the separation is not so important, but for most other analyses it is critical and for stiff integration methods highly desirable. The reason is that many numerical methods, including the stiff integrators, employ a measure called the Jacobian matrix as part of the numerical calculation. If the separation is not carried out, the Jacobian becomes singular and thereby rendering most analyses (e.g. steady state location, bifurcation analysis, certain optimization methods and sensitivity methods etc.) numerically unstable if not impossible. Even when carrying out simple time course simulations, the separation is also useful because it enables the number of differential equations to be reduced in number and thereby improve computational efficiency.

Equation (1) is therefore better expressed as:

$$\begin{aligned} \boldsymbol{S_d} &= \boldsymbol{L_0 S_i} + \boldsymbol{T} \\ \frac{d\boldsymbol{S}_i}{dt} &= \boldsymbol{N_R v}(\boldsymbol{S_i}(p), \boldsymbol{S_d}, \boldsymbol{p}) \end{aligned} \tag{2}$$

In these equations, $\boldsymbol{S_i}$ is the vector of independent species, $\boldsymbol{S_d}$, the vector of dependent species, $\boldsymbol{L_0}$ the link matrix, $\boldsymbol{T}$ the total mass vector, $\boldsymbol{N_R}$ the reduced stoichiometry matrix, $\boldsymbol{v}$ the rate vector and $\boldsymbol{p}$ the vector of parameters. Equation (2) constitutes the most general expression of an ODE based temporal model (Hofmeyr 2001; Heinrich and Schuster 1996). The symbolism used in equation (2) is the standard notation used by many in the Systems Biology community.

Although mathematically, reaction based models are given by equations (1) and (2), many researchers are more familiar with expressing models in the form of a reaction scheme. For example, the following describes part of glycolysis:

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Glucose-6-P -> Fructose-6-Phosphate
Fructose-6-Phosphate + ATP -> Fructose-1-6-Bisphosphate + ADP
Fructose-1-6-Bisphosphate -> DHAP + GAP
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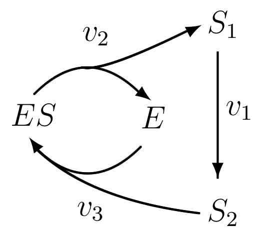
Figure 2: Part of Glycolysis described using a reaction scheme notation.

For brevity, the rates laws that accompany each reaction have been left out. Such notation is well understood by biologists. It is not straight forward however to convert this representation to the representation give by equation (2). However, many software tools will permit users to enter models as a list of reactions and then automatically generate the mathematical model (Sauro and Fell 1991; Sauro 2000; Sauro et al. 2003).

Box 1. Reaction Network Consider the simple reaction network shown on the left below:



$$\begin{array}{c c} & \begin{array}{ccc} v_1 & v_2 & v_3 \end{array} \\ \begin{array}{c} ES \\ S_1 \\ S_2 \\ E \end{array} & \begin{bmatrix} 0 & -1 & 1 \\ -1 & 1 & 0 \\ 1 & 0 & -1 \\ 0 & 1 & -1 \end{bmatrix} \end{array}$$

The **stoichiometry matrix** for this network is shown to the right. This network possesses two conserved cycles given by the constraints: $S_1 + S_2 + ES = T_1$ and $E + ES = T_2$. The set of independent species includes: $\{ES, S_1\}$ and the set of dependent species $\{E, S_2\}$.

The $\boldsymbol{L_0}$ matrix can be shown to be:

$$\boldsymbol{L_0} = \begin{bmatrix} -1 & -1 \\ -1 & 0 \end{bmatrix}$$

The complete set of equations for this model is therefore:

$$\begin{bmatrix} S_2 \\ E \end{bmatrix} = \begin{bmatrix} -1 & -1 \\ -1 & 0 \end{bmatrix} \begin{bmatrix} ES \\ S_1 \end{bmatrix} + \begin{bmatrix} T_1 \\ T_2 \end{bmatrix}$$

$$\begin{bmatrix} dES/dt \\ dS_1/dt \end{bmatrix} = \begin{bmatrix} 0 & -1 & 1 \\ -1 & 1 & 0 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix}$$

Note that even though there appears to be four variables in this system, there are in fact only two independent variables, $\{ES, S_1\}$, and hence only two differential equations and two linear constraints.

3 Standards

In recent years, particulary since the sequencing of the human genome, there has been an ever increasing list of wide ranging cellular models published in the literature. Each author has a particular notation that they use to publish the model. Some authors will publish the model as a reaction scheme, much like the notation given in Figure 2. Others will itemize the actual mathematical representation in the form of a list of differential equations. Some authors do not publish the model at all but provide the model as supplementary information. Until recently, there has been no way to publish models in a standard format. Without a standard format it has proved very difficult if not impossible in many cases to use published models without considerable effort.

As a result of this obvious shortcoming, a number of groups set out to gather community support to develop a standard that model developers would be happy to use. There was an early effort in 1998 by the BTK (BioThermoKinetics) group to standardize on a practical format for exchanging models between Gepasi (Mendes 1993) and SCAMP (Sauro and Fell 1991), both tools were widely used at the time. Around the same time, bioengineers at the University of Auckland began investigating the role that XML (Harold and Means 2001) could play in defining a standard for exchanging computational models in order to reduce errors that appeared frequently in published models. From the Auckland team emerged CellML (Lloyd et al. 2004). Members from the BTK group subsequently took their experience and contributed significantly to the other major model exchange standard, called SBML (Hucka et al. 2003). SBML was developed in 2000 at Caltech, Pasadena as a result of funding received from the Japanese ERATO program. Both CellML and SBML are today viewed as the main standards for exchanging cellular network models. There are however fundamental differences between the approaches that CellML and SBML take in the way models are represented.

3.1 CellML

CellML (Lloyd et al. 2004) represents cellular models using a mathematical description similar to equation (1). In addition, CellML represents entities using a component based approach where relationships between components are represented by connections. In many ways CellML represents a literal translation of the mathematical equations, except that the relationship between dependent and independent species is implied rather then explicit. The literal translation of the mathematics however goes much further, in fact the representation that CellML uses is very reminiscent of the way an engineer might wire up an analog computer to solve the equations (though without specifying the integrators). As a result CellML is very general and in principal could probably represent any system that has a mathematical description (and not just the kind indicated by equation (1)). CellML is also very precise in that every item in a model is defined explicitly. However, the generality and explicit nature of CellML also results in increased complexity especially for software developers. Another side effect of the increased complexity is that models that are represented using CellML tend to be quite large. On average, my own analysis of a sample from the CellML repository www.cellml.org/examples/repository/ indicates that each reaction in a model requires about 5Kbytes of storage.

Another key aspect of CellML is its provision for metadata support. The metadata can be used to provide a context for a model, such as the author name, when it was created and what additional documents are available for it's description. CellML uses standard XML based metadata containers such as RDF and within RDF the Dublin Core.

The CellML team has amassed a very large suite (hundreds) of models which provides many real examples of CellML syntax. This is an extremely useful resource for the community.

Owing to the complexity of CellML, one unfortunate side effect is that there are very few tools which can read and write CellML. As far as the author is aware there are only two third-party tools that can read and write CellML, these are VCell (Loew and Schaff 2001) and COR (Garny et al. 2003). The CellML team have recently (2004, http://cellml.sourceforge.net/) began to provide their own software tools to third-party developers. The delay in providing such tools to the community is probably one reason why CellML (given it's complexity) has not proved so popular relative to SBML which I will discuss next.

3.2 SBML

Whereas CellML attempts to be highly comprehensive, SBML was designed to meet the immediate needs of the modelling community and is therefore more focused on a particular problem set. One result of this is that the standard is much simpler and much less verbose. Like CellML, SBML is based on XML, however unlike CellML, it takes a different approach to representing cellular models. The way SBML represents models closely maps the way existing modelling packages represent models. Whereas CellML represents models as a mathematical wiring diagram, SBML represent models as a list of chemical transformations much like the example indicated in Figure 2. Since every process in a biological cells can ultimately be broken down into one or more chemical transformations this was the natural representation to use. However SBML does not have generalized elements such as components and connections, SBML employs specific elements to represent spatial compartments, molecular species and chemical transformations. In addition to these, SBML also has provision for rules which can be used to represent constraints, derived values and general math which for one reason or another cannot be transformed into a chemical scheme. Like CellML, the dependent and independent species are implied.

3.2.1 SBML Development Tools

Early on in the development of SBML, the original authors decided to provide software tools almost immediately for the community. Since XML at the time was not well understood by many software developers the provision of such assistance was crucial. In hindsight, this is probably one reason why SBML has become a popular standard. Initially the original authors provided a simple library for the Windows platform since the bulk of biology based users tend to be Windows users. Today this library is still used by a number of tools including Gepasi, Jarnac and JDesigner. With the growing popularity of SBML, the community has since developed a comprehensive cross platform tool (http://sbml.sourceforge.org) which is now the recommended SBML toolkit to use (libSBML). libSBML was developed in C/C++ for maximum portability.

3.2.2 Extensibility

It was realized early on by the authors of SBML that as systems biology developed there would be pressure from the community to make additional functionality available in SBML. To address this issue, SBML has a formal means for adding extensions in the form of the so-called annotations. There now exist a number of annotations that are used by software developers. Some of these address issues such as providing visualization information to allow software tools to render the model in some meaningful way (two examples of these will be given in a later section). Other extensions provide a means to store information necessary for flux balance analysis or to provide information for stochastic simulations. Ultimately some of the extensions will most likely be folded into the official SBML standard. This mechanism, a sort of Darwinian evolution, permits the most important and popular requests to be made part of SBML. It makes the process of SBML evolution more transparent and permits users to be more involved in the development of SBML.

3.2.3 Practical Considerations

While CellML is very general, SBML is more specific, as result, the storage requirement for SBML is much less. It takes on average roughly 1.5Kbytes to store a single chemical transformation in SBML Level 2 (compared to 5K for CellML). Interestingly it only takes roughly 50 to 100 bytes to store single transformations in raw binary format where there is minimal extraneous syntax. Some readers may feel that with today's cheap storage technologies, that discussions on storage requirements is unnecessary. Indeed for small models it is not an issue. However, in future very large models are likely to be developed. There is, for example a serious attempt (www.physiome.org) now underway to model in the long term entire organs and even whole organisms. The amount of information in these cases is huge and the question of efficient storage is not so trivial. Obviously XML is highly compressible and large models can be stored in this way. However, inefficient storage also increases the time taken to manipulate the models. Furthermore, in a modelling environment, model authors tend to generate hundreds of variants while developing the model. For a large model this clearly would generate huge amounts of XML based data. One of the things that has yet to be addressed by either standard is the how model variants can be efficiently stored.

3.2.4 Usage

Both SBML and CellML have been taken up by many software developers and implemented in their software. SBML in particular is being used in over 75 software projects. In addition, SBML is the official model interchange format for the BioSPICE project www.biospice.org, the SBW project www.sys-bio.org, the international *E. coli* alliance and the receptor tyrosine kinase consortium. Much of the SBML support is in stand-alone applications, however, a number of database vendors have also decided to provide export of SBML as an option, examples include reactome, stke and sigpath.

A related standard that has been proposed by (Yun et al. 2004) is for the storage of flux balance models. The proposed format is very similar to SBML but has the additional feature of storing the flux balance objective function.

3.3 Future Considerations

The development of standards for systems biology is still at a very early stage. I have not for example considered the problem of standardizing the formats for the experimental data that will be required for modelling. For example, there are no current standards for representing *quantitative* proteomic or metabolomic data, though efforts for defining a quantitative microarray format is maturing (www.mged.org).

More pressing from a modelling perspective is that there is currently no agreed way to merge smaller sub-models into larger models (composition). One of the few groups to have considered composition is Ginkel and Kremling (Ginkel et al. 2000). They have examined possible extensions to SBML to allow SBML to represent sub-models and models composed of sub-models. Additional issues include distinguishing different kinds of models, particularly ODE and stochastic models, currently there is no means to identify the kind of model an SBML file represents other than to use specific annotations. One unfortunate side-effect of using XML is the temptation to omit a detailed semantic specification. XML is often vaunted as a desirable technology because it is easily parsed, however, parsing and syntax checking is a very easy task to implement, the real difficulty comes when semantic checks are required and current XML technology offer no assistance in this task.

3.4 Other Standards

Apart from using XML to define an interchange format, there are two other mediums for representing models, these include, human readable text based formats and visual formats.

3.4.1 Visualization of Models

For many users, the ability to visualize models and to build models using visual tools is an important feature. There are currently a number of visualization formats that are in common use. One of the most comprehensive and freely available formats is the molecular interaction maps developed by Kohn (Kohn 1999) and more recently by Mirit Aladjem (Kohn et al. 2004). The Kohn format emerged from the need to represent complex signaling networks in a compact way. Unlike metabolic networks, signalling networks can be extremely complex with multiple protein states and interactions and therefore an alternative and more concise approach is desirable. At the time of writing there is no software for manipulating Kohn maps and no means to convert Kohn maps to SBML or any other standard. Hopefully this will change in the future.

An early computer based visual notation was proposed by Cook (Cook et al. 2001) who developed a notation called BioD. This notation has been implemented in a commercial software package called KineCyte (http://www.rainbio.com/Software.html).

Another proposal has been put forward by Kitano (Kitano 2003). This is a more traditional approach where different molecular entities (such as proteins, ions, transporters etc.) have particular pictorial representations. The software tool called cellDesigner (Funahashi et al. 2003) implements this proposed format.

One of the first visualization tools, JDesigner (Sauro et al. 2003) also implements a traditional way to depict networks (see Figure 3) using a pictorial representation to indicate substances and reactions. JDesigner also employs bezier curves to represent arcs in an attempt to make the diagrams similar to the notation found in many molecular biology text books. CellDesigner and JDesigner connect to the Systems Biology Workbench (SBW) for simulation support.

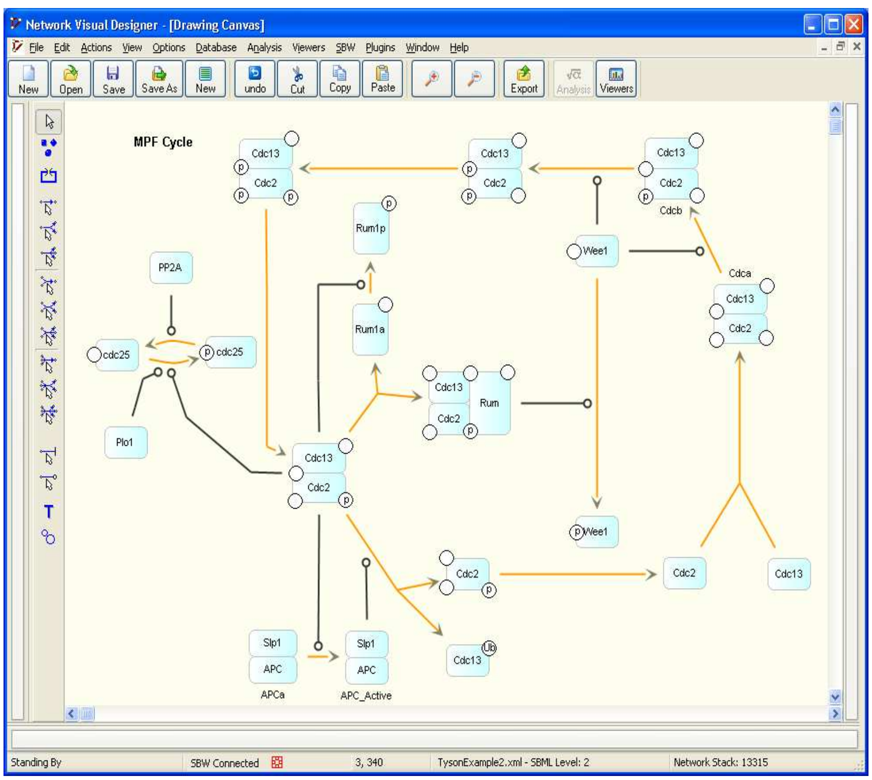


Figure 3: Example of JDesigner's visual format

Finally, there is a proposal from a commercial company called Gene Network Sciences which has devised a derivative of the Kohn notation called DCL. However this notation is proprietary and it's utility to the general scientific community is not certain at this time.

3.4.2 Human Readable Formats

In addition to visualization approaches and the use of XML to represent models, there has been a long tradition in the field to describe models using human readable text based formats. Indeed the very first simulator BIOSSIM, (Garfinkel 1968), allowed a user to describe a model using a list of reaction schemes. Variants of this have been employed by a number of simulators since, including, SCAMP (Sauro and Fell 1991), Jarnac (Sauro 2000), E-Cell (Tomita et al. 1999) and more recently Pysces (Olivier et al. 2005). Being able to represent models in a human readable format offers many advantages, including, conciseness, easily understood and manipulated using a simple editor, flexible, portable and above all extremely easy to include commenting and annotation.

3.5 Model Databases

At the time of writing there are, surprisingly, no model databases currently in existence. There are databases for almost every other kind of biological information except dynamic information. This is even more surprising since dynamic behavior is probably *the* key feature of biological systems.

There are a number of sites which include simple lists of models, for example the CellML Repository, but there are no searchable databases. Although such databases would be of great advantage to the community, the funding agencies have so far been reluctant to provide support. Instead a number of groups, including the original SBML group and the SBW group are instead developing model databases as part of other projects. In particular the Department of Energy through their GTL program are funding a small project to develop a database for microbial models. What features of a database might be useful? Probably one of the most useful features for such a database (apart from the obvious ability to query the database for particular models, organisms etc) would be the ability to deliver models in different computationally ready formats.

3.6 Other Related Standards

CellML and SBML are the primary formats used to store interchangeable dynamic models. Apart from the particular details on the model itself there is also the need to consider data that is used to build the models. Most models are built by laboriously searching the literature and carrying out additional experiments as necessary to fill in gaps in the data. This has proved to be an extremely effective method to building reliable models (Tyson et al. 2001; Tyson et al. 2002). However, Many inexperienced researches in Systems Biology feel that high-throughput data is the answer to the needs of the modelling community. Unfortunately much of the high-throughput data that is currently available is not appropriate. Much of the high-throughput data is very noisy and is probably more suitable for building qualitative models. More importantly, the bulk of high-throughput data is not generated with dynamic model building in mind and therefore is often not appropriate for this purpose. To date there has not been a single dynamic model that has been constructed as a result of high-throughput data. As systems biology and the construction of dynamic models becomes more important, it is very likely that the utility of high-throughput data will become much more significant. When this happens a proposed standard, called BioPAX (www.biopax.org) will most likely contribute.

BioPAX (Biological Pathway Exchange) is another proposed standard based on XML. BioPAX aims to integrate many of the incompatible pathway related databases (such as BioCYC, BIND, WIT, aMAZE, KEGG and others) so that data from any one of these databases can be easily interchanged. In future it should be possible to extract data from many of the pathway databases and integrate the data directly into SBML (or CellML) via BioPAX. The BioPAX group proposes to embed BioPAX elements onto SBML or cellML for unambiguous identification of substances (metabolites, enzymes) and reactions.

4 Platforms

Much of the current software development in the systems biology community concentrates on the development of stand-alone applications. Most of these tools are not easily extensible and many of them offer nearly identical functionality. One of the problems that currently plagues systems biology is the continual reinvention of the same kind of tool (called YADS - yet another differential equation solver). I believe it is not too unfair to suggest that in many cases our software capability today in systems biology is only marginally better than the first systems biology simulation package ever written (BIOSSIM) by David Garfinkel around 1960 (Garfinkel 1968). In many cases even the user interfaces are only marginally better. There are of course exceptions to this, VCell (Loew and Schaff 2001) in particular comes to mind as well as tools such as Gepasi (Mendes 1993) and Jarnac/JDesigner (Sauro et al. 2003). VCell is particularly suited to spatial modelling, Gepasi is well known for it's GUI user interface, the selection of optimization methods and it's ability to fit data to models, Jarnac was until very recently (See Pysces (Olivier et al. 2005)) the only script based programmable modelling tool which has a fairly complete set of tools for the analysis of time dependent ODEs and stochastic systems and finally

JDesigner because it was the first visual design model tool.

The reason for the repetitive nature of software in systems biology is that almost each and every group engaged in computational systems biology writes their own simulation package. Given the time constraints on the project, the software will only reach a level of maturity that is often equivalent to BIOSSIM. As a result, the provision of software does not appear to advance.

A number of groups have recognized this problem and instead of developing single isolated applications, they have chosen to develop a software infrastructure that permits and encourages extensibility and code reuse. The later is extremely important as it allows developers to build on existing code which in turn leads to new and interesting software tools. In this section I will describe three such environments, SBW, BioSPICE and BioUML. All three environments are open source.

4.1 SBW - Systems Biology Workbench

The SBW (Sauro et. al., 2004) is an extensible software framework that is both platform and language independent. Its primary purpose is to encourage code reuse among members of the systems biology community. Developers can run SBW on Linux, Windows or Mac OS and can develop software in a variety of different languages including C/C++, Java, Delphi, FORTRAN, Matlab, Perl, Python and any .NET language (e.g. Visual Basic or C#). The SBW was originally developed in parallel with SBML (Systems Biology Markup Language) as part of the Symbiotic Systems Project ERATO project at Caltech, Pasadena (Subsequent development was supported by DARPA through the BioSPICE program and development is now focused at the Keck Graduate Institute).

The central component of SBW is the broker, which is responsible for coordinating interactions among the different resources connected to it. These resources include simulation engines, model editors, SBML translators, databases, visualization tools and a variety of analysis packages. All modules in SBW connect via defined interfaces, which allows any one of the modules to be easily replaced if necessary. The key concept in SBW is that any new module may exploit resources provided by other modules; this dramatically improves productivity by allowing developers to build on existing tools rather than continuously reinvent.

In the past other similar architectures have been developed, most notably CORBA. When SBW was being developed, CORBA was seriously considered but a number of problems arose, first the learning curve for CORBA is very steep which means that it is out of reach for most developers except highly skilled individuals; the aim of SBW was to allow the average computational biologists to develop new SBW modules hence the programming model had to be simple. Finally, there were very few open source equivalents to the SBW broker and many of them were incompatible with each other.

An SBW module (the client) provides one or more interfaces or services. Each service provides one or more methods. Modules register the services they provide with the SBW Broker. The module optionally places each service it provides into a category. By convention, a category is a group of services from one or more modules that have a common set of methods.

One of the key advantages of SBW is it's language and OS neutrality. At a stroke this eliminates the irrational language and operating systems 'wars' that often plague software development. In addition to providing support for multiple languages there is also the facility to automatically generate web services from any SBW module (Frank Bergmann, personal communication).

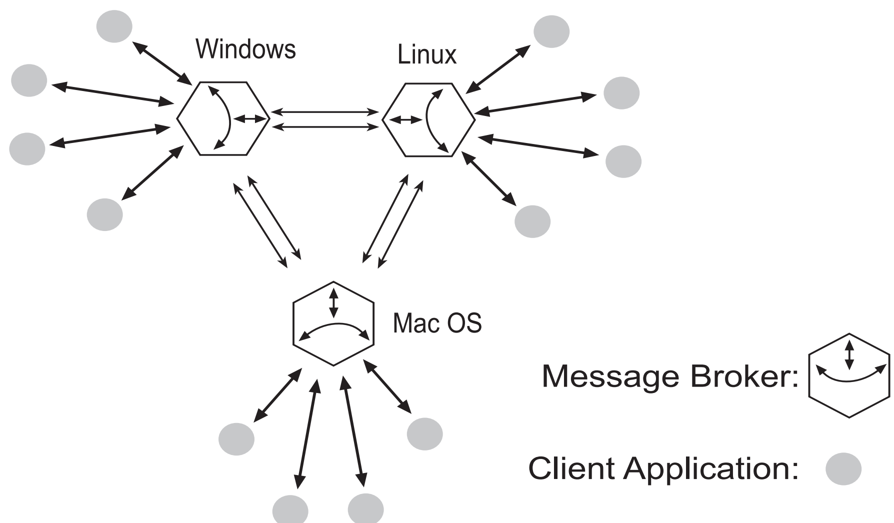


Figure 4: The Systems Biology Workbench (SBW) is a dynamic open-source distributed system. Client modules can attach and detach at run-time. Client modules can be written in a variety of languages, including, C/C++, Java, Delphi, FORTRAN, Python, Perl, Matlab, any .NET language. Data is exchanged between modules via binary messages which can include any combination of bytes, integers, floating point, complex numbers, strings, arrays and lists. Currently the available modules include, simulators, model editors, SBML manipulation, math library, frequency analyzer, bifurcation discover and analysis modules, structural analysis modules and others. Further details to be found at www.sys-bio.org

Messaging Protocols

At the heart of SBW is the messaging protocol used to exchange information between the different modules. For efficiency reasons, messages that are exchanged between modules are simple sequences of binary data. For each programming language there is a language binding library which takes care of much, if not all, of the housekeeping necessary to operate through SBW, including connection and transmission of data. In addition, issues such as little and big-endian byte ordering need not concern the developer as this is taken care of automatically by the binding libraries. Each binding also provides the necessary message packing and unpacking logic and exposes functionality in the form of an easy-to-use API.

Since SBW message passing is based on TCP/IP sockets it is straight forward to run SBW across the internet or more significantly across computational nodes on a supercomputer cluster.

4.2 BioSPICE

BioSPICE (www.biospice.org) is a DARPA funded effort to develop an open source framework and tool-set for modelling dynamic cellular network functions. The central component of BioSPICE is the dashboard which is used to construct work-flows between BioSPICE enabled applications. Both SBW and the dashboard encourage code reuse although in different ways. In the dashboard, code reuse is through the construction of work flows, in SBW code reuse is via programmatic interfaces and a plugable runtime architecture. The unit components in SBW tends to be more fine grained compared to BioSPICE modules. For example, SBW provides modules such as SBML support, frequency analysis, simulation methods, bifurcation analysis, which can be tied together at runtime to give the impression of a single application. The BioSPICE dashboard on the other hand allows the user to construct fixed work-flows prior to a run. The workflow configurations cannot be changed during runtime. In addition, where as SBW connects modules via interface specifications, the dashboard connects modules via data types. The BioSPICE dashboard is based on the Java netbeans application which makes it highly Java centric and interaction with applications written in other languages, though not impossible, non-trivial. It is possible to easily connect SBW modules to the dashboard (via the SBW Java interface) which greatly increases the flexibility of the dashboard combining the advantages of a work-flow approach to the free-flow approach of SBW.

4.3 BioUML

BioUML (www.biouml.org), developed by Fedor Kolpakov and his team, is a Java framework based around eclipse and targeted at the systems biology community. The authors state that the utility of BioUML covers access to databases with experimental data, tools for formalized description of biological systems structure and functioning, as well as tools for their visualization and simulations. BioUML is at an early stage of development but the central idea is of a plugable environment where plugins written in Java are used to extend the functionality of the framework. Much work remains to make the BioUML usable for the average biologists but the idea is interesting although the requirement to write all code in Java is limiting and some means to permit alternative language bindings would be useful. Recently the BioUML team developed a SBW interface, which permits access to plugins that are written in many different languages.

5 Applications

In recent years there has been a proliferation of software applications for the systems biology community (See Figure 5).

On the whole, many of these applications provide very similar functionality. The distinguishing feature among them is how easy they are to install and use. The more mature applications tend to be easier to install and have a much richer repertoire of functionality. Many of the applications are simple wrappers around standard ODE or Gillespie solvers and provide a simple means to load models and run time courses. Some of

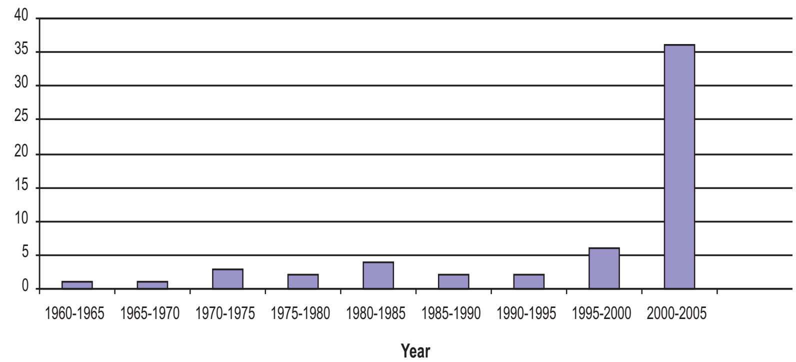


Figure 5: The Release of Software Tools for Computational Systems Biology over Time. Note the spike in the last five years

the applications fall by the wayside because the author has lost interest or funding has stopped. It is important therefore that what ever tool one uses, that the ability to export and import a recognized standard (or at least a documented format) such as SBML and/or CellML be available.

The original intention in this section was to list as many of the applications as possible together with their capabilities but given the large number now available it soon became clear that this task would be too great. Instead I refer the reader to the recent paper by Hucka et. al. (2004) where the authors describe almost forty applications. An even larger list can be found at the sbml.org web site.

There are some applications however that are worth mentioning specifically because they have some special characteristic. Table 1 lists a number of applications which are being actively maintained, have a reasonably large user base and offer facilities that are either unique or well done. I have not mentioned any stochastic simulators in Table 1 because many of these are still immature.

Application	Description
VCell	A very mature server based application that is specialized to build and simulate large scale spatial (PDE) models - Open Source, multiplatform (Loew and Schaff 2001).
Gepasi	This is a forms based application which has been maintained for many years by a dedicated author, the tool is particularly adapt at carrying out optimizations of ODE based models to data - Closed source, Windows, Linux (Mendes 1993).
WinSCAMP	A script based GUI application, which like Gepasi has a long tradition. Specialized for time course, steady state and metabolic control analysis of ODE based models. - Source available upon request, multiplatform (Sauro and Fell 1991; Sauro 1993)
Pysces	This is a very complete ODE based simulation environment built around the scripting language Python - Open Source, multiplatform (Olivier et al. 2005).
Jarnac/JDesigner	Jarnac is a script based application, JDesigner (See Figure 3) is a visual design tool which can use Jarnac via SBW to carry out simulations. The simulation capabilities of Jarnac are quite extensive, offering both ODE and stochastic simulation - Open Source, Windows, Linux (Sauro 2000; Sauro et al. 2003).

Table 1: Mature and Easily Accessible Tools for Modelling Cellular Networks.

There are also more general purpose tools available, both commercial and open-source which are worth considering. Probably the most well known commercial tool is Matlab (www.mathworks.com). Although Matlab is an excellent prototyping tool if suffers from poor performance when simulating systems larger than about thirty species if the model is not specified in the correct way. In fact a number of the open-source tools are orders of magnitude faster than Matlab. This stems from the fact that Matlab is a general purpose tool whereas the open-source tools are specialists and are therefore more heavily optimized for their specific application. The commercial tools require a high degree of programming skill because they do not have facilities for representing models in a way familiar to most biologists, instead users are required to derive the differential equations explicitly. Platforms, such as SBW make available translators from SBML to a variety of formats including Matlab, and in a number of cases, users employ tools such as JDesigner to maintain the model, but use a translator to generate Matlab (or any other supported format such as C or Java).

In addition to generic commercial modelling tools there are also now available a number of commercial tools specifically geared for modelling cellular networks. The most well known include Gene Networks Sciences, Berkeley Madonna and Teranode (These can easily be located on the web by using a reliable search engine).

5.1 Model Analysis

As a user, one of the most important aspects that I consider is the range of techniques that are available for analyzing the model. The purpose of building a model is not simply to generate a predictive tool, if it were we could probably get away with using empirical statistical techniques or machine learning approaches such a neural nets. An additional important role of model building is also to gain a deeper understanding into the properties of the model and to understand how the structure of the model leads it to behave the way it does. In order to answer these kinds of questions one needs techniques that can interrogate the model in a variety of different ways.

Table 2 lists some of the most important techniques that are available for analyzing models. Without these techniques, a model will often be as difficult to understand as the real system it attempts to model; the application of these techniques is therefore important.

All these techniques are extremely useful in gaining insight into how a model operates. The connectionist and structural analyses focus on the network properties of the model, that is they do not explicitly consider the dynamics of the model but on how the network connectivity sets the stage for generating the dynamics of the model. The last three techniques, CCA, frequency analysis and bifurcation analysis focus on the dynamical aspects of a model and are crucial to gaining a deep insight into the model (Bakker et al. 1997; Tyson et al. 2001).

5.2 Model Fitting and Validation

An important activity in systems biology modelling is the need to fit experimental data to models. There isn't sufficient space to cover to any great detail this topic but as time series data from microarray, proteomic and metabolomic data becomes more readily available the need to fit models to experimental data will become more acute. There are a number of issues related to this topic, one concerns the nature of the data that is generated by most of the current experimental techniques. In particular, most current techniques generate normalized data, that is absolute values are not given. This poses a number of problems to a fitting algorithm, since the underlying model is in terms of absolute quantities. A number of solutions are potentially available, however none are entirely satisfactory and ultimately the models generated by normalized data will most likely be only capable of reproducing trends in the data. Whether such models will have great predictive value is open to question and much research remains to be done in this area.

The other issue, is the intensive nature of the computations that are required to fit even a moderately sized model. One of the necessary requirements for fitting a model is estimating the confidence limits on the fitted parameters and the range of parameter space which describes the experimental data. This information is crucial to determine the validity of the model and can be used to design additional experiments to either refute the model or increase the precision of the model parameters. As a result of these requirements, computing a global optimization can take a considerable time. For example, in a recent study, Vijay Chickarmane (unpublished) estimated that the time required to fit a model of approximately three hundred parameters would be of the order of seven years on a normal desktop computer. Luckily, global optimization can be easily parallelized given a suitable optimizer (for example a genetic algorithm based optimizer) and the computation time can be reduced by hosting the problem on a cluster machine. Chickarmane estimates that using a one thousand node cluster, the optimization of a three hundred parameter model can be reduced to approximately two days of computation time. Such a computation can be easily setup using SBW. A single node on the cluster would act as the primary optimizer; this node in turn would farms out the time consuming simulation computations to the remaining nodes on the computer. For very large models, Grid computing (Abbas 2004) may be very appropriate for solving this kind of problem.

6 Future Prospects and Conclusion

The Systems Biology field has been developing rapidly in recent years but much remains to be done. One of the most useful developments must undoubtedly go to the development of standards such as SBML and CellML. Indeed the most recent of a long list of new systems biology journals, (Molecular Systems Biology) has stipulated that SBML is the preferred format for contributing models, hopefully other journals will follow. However, one aspect that still remains to be dealt with is to formalize the sematic rules for SBML. At the moment there is no guarantee that models written by different tools can be interchanged. If one focuses on the core specification in SBML that this is generally not an issue but it is vital that sematic validators be developed for SBML.

The other area that has received a lot of attention in recent years is the development of tools for systems biology. However, much of what is being developed is repetitious and little true advancement is being made. This is probably do to the large number of new comers to the field who are inexperienced and inevitably repeat what has gone before. A number of solutions exist to solve this problem, one is to develop extensible frameworks such as SBW, BioSPICE or BioUML, the other is to develop a suite of open-source libraries which can carry out specific functionality. An example of this is libSBML being developed by the SBML team. This library, written in C/C++ for maximum portability, enables other developers to concentrate on simulation capability rather than waste unnecessary effort developing their own SBML parser. In terms of other possible libraries, examples include, open-source Gillespie based stochastic solvers and ODE solvers. In both cases there is also the need to develop scalable and robust methods for computing the dependent and independent species. Further more, hybrid methods combining continuous and stochastic methods is a pressing need at the current time. Many biological systems interface noisy sensory apparatus (e.g. ligand binding to the surface of a cell membrane) to internal continuous analog networks (Sauro and Kholodenko 2004). In addition to the core solvers, we also need scalable analysis tools, particularly bifurcation analysis tools and sensitivity analysis tools. On the model validation front, much remains to be done, particularly the relationship between model validation and how this can direct future experimentation. This leads on to the development of new methods and algorithms for analyzing the complex networks in particular methods should be developed to modularize large networks since understanding an entire network is virtually impossible with out some recourse to a hierarchical modularization.

Finally, the role of high performance computing in systems biology is still very novel. In fact there appear to be very few applications to date of high performance computing to systems biology. One of the few useful applications is model fitting to data. When done correctly, this is an extremely computationally intensive calculation and is an ideal candidate for large cluster machines. In fact, one wonders whether this is the application for systems biology which could benefit from Grid computing.

7 Recommended Resources

Four web sources which are of interest to readers of this chapter include:

http://www.cellml.org This is the main CellML site. It has a very rich set of models expressed in CellML including specifications for the standard and pointers to software toolkits.

http://www.sbml.org This is the main SBML site. The site as ample documentation, examples illustrating how SBML is and should be used. In addition is has a rich set of software tools, in particular libSBML, which allows developers to easily add SBML support to their tools.

http://www.sys-bio.org This is the main SBW (Systems Biology Workbench) site. The latest versions for SBW, developer documentation, example models, screen shots, user guides can be obtained from this site. A link to the main sourceforge site is given where all the source code for SBW is made available.

http://www.biospice.org This is the main BioSPICE site. This site includes a description of BioSPICE and the large number of tools now available for the BioSPICE dashboard (including SBW itself).

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Approach	Description
Connectionist Theory	Connectivity studies are centered around the search for patterns in the way cellular networks are physically connected. (Barabasi and Oltvai 2004)
Structural Analysis	There are a wide range of useful techniques which focus on the properties of the networks that depend on the mass conservation properties of networks. These include, conservation analysis, flux balance and elementary mode analysis (Heinrich and Schuster 1996).
Cellular Control Analysis	CCA (also known as metabolic control analysis) is a powerful technique for analyzing the propagation of perturbations through a network. There exists a very large literature describing applications and theory (Fell 1997).
Frequency Analysis	Closely related to CCA is the analysis of how signals propagate through a network (Ingalls 2004; Rao et al. 2004).
Bifurcation Analysis	Bifurcation analysis is concerned with the study of how the qualitative behavior of steady state solutions change with changes in the model parameters (Tyson et al. 2001).

Table 2: Model Analysis Methods