School of Computing Science,
University of Newcastle upon Tyne

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Oliver Shaw, Albert Koelmans, Jason Steggles,
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Applying Petri Nets to Systems Biology using XML Technologies

Oliver Shaw[1], Albert Koelmans[2], Jason Steggles[1], and Anil Wipat[1]

[1]School of Computing Science, University of Newcastle UK
[2]School of Electrical, Electronic and Computer Engineering, University of Newcastle UK
{o.j.shaw, albert.koelmans, l.j.steggles, anil.wipat}@ncl.ac.uk

Abstract Systems Biology is a rapidly developing discipline which utilises mathematical and computer science techniques to analyse and interpret biological models. Petri nets have been proposed as an effective formalism for Systems Biology but so far only a small number of simple manual Petri net models have been constructed and investigated. In this paper we present a scheme for automatically mapping biological systems into Petri net models using XML technology. We develop a simple prototype tool which translates Systems Biology Markup Language (SBML) to the Petri Net Markup Language (PNML). As an illustrative example we consider mapping a SBML description of the *Saccharomyces cerevisiae* glycolysis pathway into PNML. An analysis of the resulting PNML model then demonstrates that even basic invariant analysis can produce interesting results for biologists. Finally we discuss what features may be desirable to systems biologists in future modifications of the PNML standard.

1 Introduction

Advances in high-throughput molecular biology have resulted in an explosion in the data available to biologists. The ever increasing number of complete genome sequences, together with data arising from post-genomic studies makes the study of cellular systems at a global level a reality. *Systems Biology* [14] has arisen as a fast developing discipline that employs statistical, mathematical and computer science methods, together with wet, laboratory based research, to assemble, analyse and investigate models of biological systems at a holistic level. Research in this area is already producing valuable information about biological systems. With sciences current trend towards interdisciplinary collaboration it is becoming increasingly desirable to transfer models from one formalism to another and this is especially true of Systems Biology. To aid the transfer of this information a standard XML format for information interchange, SBML (Systems Biology Markup Language) [8] is being developed. Further techniques and tools are now required to exploit the expanding SBML data repositories available, such as the KEGG database [10].

Petri nets [21] are a well supported formal framework for modelling and analysing complex concurrent systems. Petri nets have a proven track record in modelling computing systems and a wide body of literature and tools are available on such methods, see [3,1,31]. Petri nets have recently been proposed as a potential tool for modelling, composing and analysing biological systems. To date a number of initial investigations

have appeared in the literature including the application of place transition nets (P/T nets) [22], stochastic nets [4], coloured nets [7] and hybrid nets [16]. However, these initial investigations have so far centered on the manual, expert translation of biologically systems in to the Petri net framework and are thus only feasible for the smallest examples. A method to automatically import biological information for Petri net analysis is now required to allow realistically large biological systems to be investigated.

In this paper we present a scheme for automatically mapping biological systems into Petri net models using XML technology. XML [19] is a platform independent information interchange format. The use of XML based languages as standards to transfer information and models between tools and disciplines is developing quickly. Languages such as Math-ML [29] and SBML [8] are in current use and are being continually developed. The Petri Net Markup Language (PNML) [30] has recently been formalised as a standard representation of Petri net models. The ability XML provides to transfer models between tools maximizes the analysis that can be carried out on a model, while extending its life beyond the time a particular tool becomes obsolete. We propose a direct mapping from SBML to PNML which serves as a starting point for the Petri net analysis of biological systems. Since both SBML and PNML are still developing our aim is to give an insight into what is currently possible and to consider what further features may be required to enhance the usefulness of PNML in such a translation role. We illustrate our approach using a SBML example of a simple biological pathway, the *Saccharomyces cerevisiae* glycolysis pathway [27] (Figure 2), whose SBML representation is available at [23]. This pathway metabolises glucose to produce the cellular energy sources ATP and NADH. We show how this SBML representation of the pathway can be mechanically translated into a corresponding PNML representation using a prototype Java based tool. We then analyse the resulting Petri net model and relate the results back to the initial biological system.

We propose and discuss several ideas to build on and facilitate the use of PNML for constructing and analysing models imported from SBML. We propose extensions to the mapping to allow the simulation of a model or view the network in a conceptually different way. We consider the benefit of allowing markup languages to be able to recognise specific tags from other markup languages. For example, a tool may primarily deal with Generalized Stochastic Petri Nets (GSPN) but may also be able to recognise Math-ML to allow for a different sampling policy for firing rates. Another important issue is tracking the evolution of a Petri net model of a biological system. A biologists needs to be able to discover the original data sources for the Petri net model and also be able to trace how additional information, such as firing rates in a Stochastic model, were derived. We discuss the problem of graph layout for automatically generated PNML models and the provisions needed in the PNML language for this. Finally we consider what support is needed to allow the modular development of PNML models.

The paper is structured as follows. In Section 2 we present background information on Systems Biology and its associated standard markup language SBML. We then briefly consider the applicability of Petri nets to biological modelling. In Section 3 we present a scheme for directly mapping biological systems expressed in SBML into PNML models. We illustrate this scheme by applying it to an example SBML biological pathway and then analysing the resulting Petri net model. Section 4 discusses

the insight gained during this research on PNML and proposes some further ideas for enhancing PNML to facilitate its use as an interdisciplinary research tool, such as in Systems Biology. Finally, we present our concluding remarks in Section 5.

Note for brevity, we assume in the sequel that the reader is familiar with the basic ideas of PNML (see [30] and [31] for an introduction).

2 Background

In this section we present an overview of Systems Biology and the Systems Biology Markup Language (SBML). We describe the components of SBML and how these make up a biological model. We then consider how Petri nets can be applied in Systems Biology and present a brief overview of what has been achieved in Systems Biology with Petri nets to date.

2.1 Systems Biology and SBML

Advancing techniques in the field of Molecular Biology have led to an explosion in the data available. This data now gives a global view of cellular and metabolic function. The field of Systems Biology [14] has arisen based on the use of this holistic data to strive to model and understand how subcellular and multicellular systems interact and operate. Systems Biology seeks to understand the functional roles of modules or systems, rather than focusing on their individual components.

Typically, Systems Biology relies on iterative cycles of computer based model construction, refinement and prediction, closely coupled to wet-lab experimental design, experimentation and data storage. Standards are required at both stages of the cycle to ensure that models are accurately constructed from experimental data. Thus, the standardisation of the software and modelling procedures is a separate, but very necessary issue, aside from the standardisation of experimental procedures and data. A number of world-wide projects are now in place to develop standard computational modelling software and platforms at the levels of the networks, the components, the model descriptions and for data storage and retrieval [23]. One goal of Systems Biology researchers is to provide tools and data standards that will facilitate the use of combinations of different modelling techniques and procedures. The Systems Biology Markup Language (SBML) is a standard format (currently implemented in XML) for the representation of models of biological networks that has arisen to try to meet this goal [8]. The SBML has had two official levels released since its conception and is still rapidly maturing. SBML representations of models developed for a variety of biological systems are now available from the SBML website [23] (for example ECell [28]) and SBML support from the Systems Biology modelling community is growing. A test suite of SBML models has been made available to test modelling techniques and to facilitate SBML related application developments. Modelling packages that support SBML are able to parse the SBML file and map it to their own internal format. Although SBML is aimed at machine readability it is also reasonably easy to follow by eye.

SBML has a simple set of building blocks to represent biological systems at its core. A number of key components are located at the top level of SBML forming the basis of

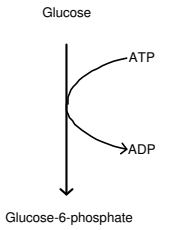
the language. Three components are aimed at defining the entities involved in a reaction and allow the structure of the model to be represented:

- *Species*: These are the substances (for example ions, metabolites or proteins) that take part in a reaction.
- *Reaction*: Describes the transformation process that change concentration of the species involved in a reaction. For example, the reaction could describe how reactants form products. Reactions can possess rate parameters that describe the rate of the transformation process.
- *Compartment*: A compartment specifies a finite container for species or substances. Examples could include the cell or cellular compartments such as the nucleus.

Further components allow the behavioural characteristics of the model to be captured:

- *Functional definitions* allow functions for a model to be defined.
- *Unit definitions* specify the units for the quantities used in the model.
- *Parameters* are quantities possessing a symbolic name that describe the variables that relate to individual reactions or act a global level.
- *Rules* are mathematical expressions that define how variables are calculated and constrained.
- *Events* describe the changes in sets of variables that may be triggered when a certain condition becomes satisfied.

An example of how a biological reaction may be captured using SBML is shown in Figure 1. The reaction in which Glucose is converted to Glucose-6-phosphate is shown




```
...
<listOfSpecies>
  <species name="Glucose" compartment="uVol"
      initialAmount="0.086999"/>
  <species name="Glucose-6-phosphate" compartment="uVol"
      initialAmount="1.39"/>
      ...
</listOfSpecies>
…
<listOfReactions>
 <reaction name="R1" reversible="false">
      <listOfReactants>
           <species Reference species="Glucose" stochemistry="1" />
           <species Reference species="ATP" stochemistry="1"/>
      </listOfReactantss>
      <listOfProducts>
           <species Reference species="Glucose-6-phosphate" stochemistry="1" />
           <species Reference species="ADP" stochemistry="1"/>
      </listOfProducts>
</reaction>
...
```

Figure 1. Conversion pathway of Glucose to Glucose-6phosphate and its SBML representation.

as a traditional biological diagram and represented in SBML (although some of the tags

have been omitted for clarity). In this simple reaction glucose is converted to glucose-6-phosphate, using ATP as an energy source. Hence the reactants are Glucose and ATP, the products are Glucose-6-phosphate and ADP. There are often enzymes associated with reactions in biological systems, these catalyse the reaction, speeding it up, and effectively allow the reaction to proceed. Currently SBML allows a `<modifier>` to be associated with a reaction, however there is no distinction between an enzyme or an inhibitor of a reaction. We hope that this will be resolved in future revisions allowing a more accurate, structural view of the system.

2.2 Petri Nets in Systems Biology

Biological molecular systems may be viewed as complex concurrent processes. Cellular mechanisms are complex and dynamic, multiple genes are transcribed, multiple types and copies of transcripts are translated to proteins, and those proteins partake in multiple signalling processes and metabolic reactions in a concurrent fashion. Petri net models have evolved to model complex concurrent computing systems in a discrete fashion and hence are inherently suitable for modelling biological networks in Systems Biology applications. For example, Figure 2 shows a complex system depicting the glycolysis

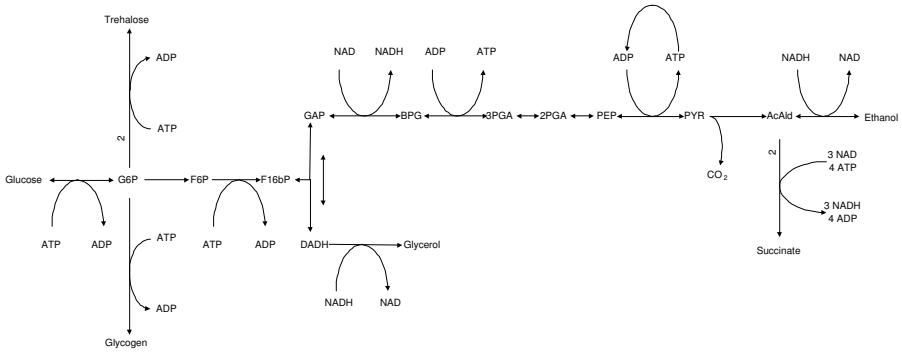


Figure 2. The *Saccharomyces cerevisiae* glycolysis pathway [27]

pathway in *Saccharomyces cerevisiae* [11] , with examples of concurrency and choice, demonstrating the similarity to traditional problems modeled as Petri nets. To date, there have been a limited number of initial reports into the use of Petri nets models in Systems Biology. P/T nets have been used to analyse a biological pathways in an erythrocyte cell [22]. Stochastic Petri nets have been used to model regulatory pathways in *E.coli* [25], blood coagulation [20] and COLE1 plasmid replication [4]. Coloured Petri nets have also been utilised as an avenue for investigation of biological pathways [7].

There are two aspects of biological networks that can be represented and analysed using Petri net based models:

- *Network structure.* Network structural properties can be analysed using methods already devised for the structural analysis of Petri nets. Some progress has been made in this area already, for example the use of Place and Transition invariants [22], although there are many interesting net properties such as boundedness, liveness and reachability for which a biological context still remains to be investigated.
- *Network behaviour.* Individual cellular processes can be viewed as discrete event systems in which sets of single molecules act as species, products and mediators in these reactions and processes. Hence, the concentrations of these entities can ultimately be expressed as integer units. The behaviour of biological networks over time has been modeled with stochastic [15] and hybrid Petri nets [16]. When simulating a network over time the rates of the reaction must be captured. Currently these rates are obtained from a combination of experimental results, expert knowledge and experimentally manipulating the network representation.

At present the structure and behavioural aspects of the Petri net models described in the literature have generally been encoded by hand, using expert knowledge. This is a time consuming and error prone process that may involve the duplication of models that have already been defined in a different modelling environment. The advent of SBML as a common model description language now makes it feasible to generate Petri net models from classical biological networks modelled by other means. A method to generate Petri net models from SBML would open up Petri net modelling techniques for application to many biological networks that have already been predefined and verified.

3 Mapping SBML to PNML

In this section we consider in detail the mapping between SBML and PNML models. We begin by describing how an SBML model can be directly mapped to a PNML description of a P/T net in which each token represents the presence of one molecule of a given species. We then consider alternatives ways of modelling biological systems with Petri nets and in particular, discuss how the basic PNML P/T net mapping can be extended to produce safe Petri net and stochastic Petri net models. We conclude this section by considering how existing Petri net tools can be used to analyse a PNML description of a biological system.

3.1 Mapping from SBML to a P/T Net

In this section we view biological pathways as a series of parallel discrete events, where one molecule of a substance is deemed equivalent to a single token [22]. Using this simple approach we show how an SBML model can be directly mapped to a PNML description of a P/T net. We illustrate the mapping we propose using the *Saccharomyces cerevisiae* glycolysis pathway introduced in Section 2.

Each SBML file will contain a list of species which take part in the biological reactions of the system in question. Each species is specified using a `<species>` field which contains a unique `<ID>` attribute which names the species, a `<compartment>` field identifying where the the given species is found, and an `<initialConcentration>` field.

Each species will be represented by a place in our Petri net model and mapped to a PNML <place> field. The species <ID> attribute then becomes the place <ID> attribute and <name> field. For example the following PNML would be used to represent the species glucose given in Figure 1.

```
<place ID="glucose">
<name>
<text>"glucose"</text>
</name>
<initialMarking>
<text>"0"</text>
</initialMarking>

</place>
```

One added complication is that a species may occur in more than one compartment in an SBML model. In this case it is clear why the <ID> attribute must be used as the identifier. The information about the compartment may be retained as part of the modular or graphical elements (see Section 4). The initial concentration field will be used to calculate the Petri nets initial marking and we discuss this in more detail below.

In an SBML file the <reaction> field is used to specify a single reaction that takes certain species as inputs, as specified by the <listOfReactions> field and produces certain species as products, specified by the <listOfProducts> field. Each reaction will be represented by a transition in the Petri net model using the PNML <transition> field and the unique <ID> attribute associated with each SBML reaction map to the <ID> attribute of the corresponding transition. We then need to define the arcs that will be used to connect the input/output places to the newly created PNML transition. We create an <arc> entry from each species place in the list of reactants to the new transition and from the transition to each species place in the list of products. Note that in SBML there is no concept similar to an arc so to satisfy the naming requirements of PNML each arc is given an <ID> *Ai* where *i* is an integer that relates to the order in which the arcs are created, (calculated as a global counter) and *A* represents arc. A simple example Figure 3 shows how the reaction shown in Figure 2 is mapped

```
- <reaction name=" vTreha " reversible=" false ">
- <listOfReactants>
<specieReference specie=" G6P " stoichiometry=" 2" />
<specieReference specie=" ATP " stoichiometry=" 1" />
</listOfReactants>
- <listOfProducts>
<specieReference specie=" Trh " stoichiometry=" 1" />
<specieReference specie=" ADP " stoichiometry=" 1" />
</listOfProducts>
…….
</reaction>
```

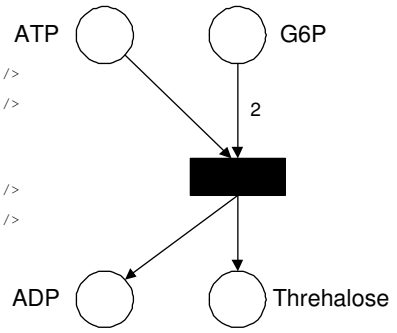


Figure 3. Creation of a simple transition from SBML.

to a transition in PNML. A reaction tag may have the <reversible> field set to true

to indicate that the reaction can also occur in the reverse direction. If this is the case then we create a second transition for the reaction which we name ID_R (where ID is the original reaction <ID>) such that the input/output places of the original transition are reversed. An example of such a Petri net for a reversible reaction and the associated SBML fragment is given in Figure 4.

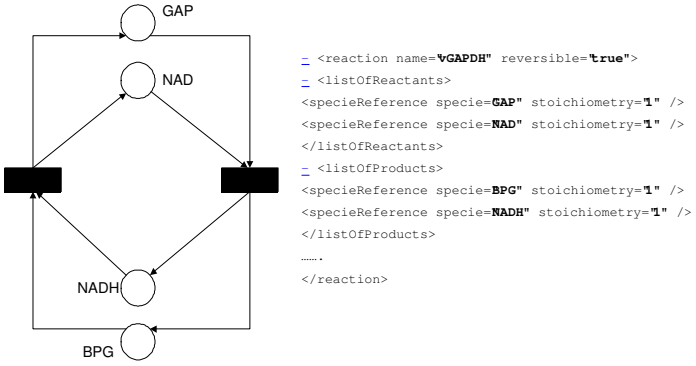


Figure 4. How a reversible reaction is mapped

Given the Petri net structure we now need to derive the initial marking for the PNML model. We set the <initialMarking> attribute in PNML using either the SBML species <initialAmount> or <initialConcentration> fields. The <initialAmount> field defaults to define the initial quantity of the species as a molar amount [8], if however a <substanceUnits> field is defined <initialAmount> is then defined in terms of that unit. If the value is in a molar form then the <initialMarking> can be obtained simply by multiplication by Avogadro's number. If the <substanceUnits> are a multiplication of a molar amount then a simple conversion can be carried out to obtain the molecular amount. In the unlikely event that the <substanceUnits> are not in a molar form then more user input is required. The <initialConcentration> field represents the concentration of a substance and can only be used if a volume is assigned to a compartment. The units used in the <initialConcentration> field take the form of <substanceUnits>/<spatialUnits>. (Note <initialConcentration> has only been included since the recent level 2 revision in SBML.) If the specified initial concentration is in a mole/litre amount then the molecules can be obtained by assuming one molecule is equal to $1n$M [25]. In the unlikely event that mole/litre concentrations are not used then more user input is required. The <initialConcentration> and <initialAmount> fields are mutually exclusive. If either of these fields is empty then it is implied that the values are unknown or are not needed for the analysis. If the values are missing from a SBML file then the <initialMarking> is set to 0.

The above mapping from SBML to a PNML model has been implemented as a prototype Java tool using JDOM [9]. JDOM was chosen due to its simplicity and platform independence, a JDOM/Java based tool will run on any system with a Java vir-

tual machine. This simple conversion tool is available on request from the authors. See www.bioinformatics.cs.ncl.ac.uk for more details.

3.2 Extending the Mapping to Other Net Types

Given the above view of an SBML system where tokens directly represent molecules and reactions are seen as discrete events we can always derive a P/T net model using the SBML to PNML mapping described. However, the proposed mapping is clearly not the only way a biological system can be modelled with Petri nets. For example instead of viewing the system as purely discrete it may be viewed as hybrid [16], with some places and transitions discrete and some continuous. This allows real values of concentrations to be used instead of discrete number of molecules. It is also possible to add time and quantitatively simulate the network. Stochastic effects have proven to be an important part of biological systems [17], and simulating a network in a stochastic manner would be beneficial. Another possible view is to consider biological pathways as Boolean networks where substances are denoted as present or absent depending on whether the current concentration meets a threshold requirement. Such a view leads naturally to a safe Petri net model in which structural analysis is simplified and inhibiting reactions can be easily modelled. In this section we briefly consider how our mapping could be extended to obtain safe Petri net and stochastic Petri net representations of biological systems.

In many biological systems researchers are interested in analysing the behavior of the system over time. Stochastic Petri nets [15] provide one means of modelling time information and have already been used to model biological systems (see for example [4,25]). Stochastic Petri nets are essentially P/T nets with a delay added to the transition. In Systems Biology rates are given for reactions and are held in the <KineticLaw> field (where available) in SBML files. The inverse of the rates can be mapped directly to the delay. There is a an issue as to how best to apply the rates to accurately capture the stochastic nature of biological systems (see section 4.2). An acceptable and usable mapping [25], assuming the existence of an appropriate PNML stochastic Petri net type, would proceed as follows. The <KineticLaw> field is read with its Math-ML fields describing the rate law. With in this there will constants which will have to be obtained from the <ListOfParameters> specified in the SBML file. From this information a single rate constant can be created. This would be added to the <delay> field of the appropriate transitions. There may, however be a case for storing the rate as a series of parameters, more like the original SBML file. This would be extremely beneficial if a series of experiments was to be run on a system, altering a single variable on each run. For example, if temperature was one of the variables that made up a rate law, the modelling of the effect of a change of temperature on the system would be greatly facilitated by having transition rates relative to a global temperature variable. This would allow the user to change one variable rather than possibly hundreds of individual rates.

Current attempts at biological modelling with Petri nets are based on relating the number of tokens on a place directly to the quantity of molecules present. This approach has produced some promising results but another possible view is to consider places to either be marked or not, with a token being put on a network place once a concentration of a molecule has reached some threshold concentration. The idea is to

only consider a place marked if a particular molecular species is present in a significant enough concentration to have an affect in the system. This representation would be particularly useful when investigating gene products and regulatory networks. For example if a protein is expressed in high enough quantities it may cause a group of genes to be expressed (a regulon). Here the exact concentration of the protein is not important, just that it is above a threshold level. Such a methodology is naturally suited to using safe Petri net models which benefit from many strong results that simplify net analysis [32]. Using this approach would also remove the need to spend time obtaining accurate molecular quantities for each place, which may not even be available for some SBML models. However it would be necessary to decide on the threshold levels and give initial markings as appropriate in the translation from SBML.

3.3 Analysis of a Biological Pathway

A SBML representation of the *Saccharomyces cerevisiae* glycolysis pathway (Figure 2) was converted to a PNML representation of a P/T net using a prototype tool based on our mapping. For full details of this conversion, including the complete SBML file and resulting PNML we refer the interested reader to [24]. We then considered analysing the PNML model using a number of Petri net tools. We began by using the *Petri net kernel* [12] to load the PNML. From the Petri net kernel an *Integrated Net Analyzer* (INA) [26] readable version of the system was obtained. This model was given a preliminary analysis in INA, looking for T-invariants and P-invariants. One P-invariants was discovered, one relating to the tight coupling between BPG and NAD. No other P-invariants were found. Fifteen T-invariants relating to the reversible reactions were found. Hence we see how Petri net properties can be directly related to biological phenomena.

Although we have only considered P and T invariants here there are many other interesting properties that may be useful to biologists. We now discuss how some of these Petri net properties can be related to biological systems.

- *Deadlock*. Deadlock would at the holistic level represent the death of the organism. Deadlock in smaller systems or sub models would represent a pathway no longer available to the organism, for example the lack of a resource would lead to deadlock in that pathway and an alternative pathway utilised.
- *Boundedness.* A particular molecule may be toxic, lethal to the organism at a certain level. Alternatively a molecule may induce further reactions at a certain threshold. Hence, boundedness analysis would be able to check if these conditions occur from an initial marking.
- *Traps*. A trap is a set of places which, once marked never loses all tokens. In biology this could for example represent a persistent protein or metabolite, i.e. one with no degradation pathway.
- *Siphons.* The presence of a siphon in a biological Petri net would indicate a store of proteins or metabolites that have no way of being replenished. For example these could be metabolites that are no longer available in a particular environment.
- *Reachability*. Reachability is potentially very interesting to biologists. By systematically deleting places from the net (the equivalent of a biological knock out experiment) the biologist may be able to find key genes that prevent certain states from occurring.

There are undoubtedly many other biological analogies in Petri net properties that could be exploited using analysis techniques such as model checking. Further research is needed to fully investigate this avenue in Systems Biology.

4 Proposals and Discussion

In this section we discuss the insights gained during our research on PNML and propose some ideas for enhancing PNML as an interdisciplinary research tool in new fields such as Systems Biology. We consider various aspects of PNML including timing issues, graphical layout, modularity concepts, and data management issues.

4.1 Data Management Facilities

The work discussed in this paper exposes what we believe is a shortcoming in the current version of PNML: the absence of data management facilities. Petri Net models often form part of a larger overall project, where it is necessary to trace its evolution through the use of version management and data management tools. We, for example, would wish to trace the source of the original SBML model from the PNML version produced, which would allow us to trust the data used in the model. The fact that PNML allows embedded tool specific information (e.g., stochastic code such as used by the Mobius tool [2]) makes it also imperative that extra information can be added to a PNML file to identify that information in detail.

We propose that every PNML file should have a compulsory (but possibly empty) header, to be used to deposit this kind of information (the importance of such features has previously been recognised in the design of interchange formats such as EDIF [5]). This could include (but should not necessarily be limited to) the following kind of data:

- the version number of the PNML language used in the file.
- the version number of the design contained in the file, as specified by the designer of the net or the tool that produced it. This could be part of a more elaborate version control string, maintained by the tool(s) used to control the overall project.
- the name of the tool that generated the net, with its version number.
- the data origin, which identifies where the net was created, possibly including a version number. This would be useful when the model is part of a project dispersed over many geographical locations.
- the designer(s) of the net.
- a time stamp, to identify when the net was written. The format of this could be a UTC (universal time coordinate).
- the name of the net.
- a set of annotation strings, to be displayed alongside the net.
- the names of files which contain libraries of already completed nets, to be included in the current net. The order in which the names are specified is relevant.

If the net contains tool specific code, it should be possible to provide similar data for each of those tools.

4.2 Time Issues

At present PNML only deals with straightforward P/T nets and no standards exist for other types of Petri nets such as stochastic Petri nets. Our work highlights how important it is to develop such Petri net type standards if PNML is to be accepted as an interdisciplinary tool. For example, stochastic Petri nets have already been shown to be useful in modelling biological systems [4,25] and it would have been natural to extend our mapping to this type of net. However, the lack of any PNML stochastic net type prevented us from undertaking this work in practice. Thus to facilitate the transfer of other formalisms to PNML it is essential that the full range of Petri net types is available. The inclusion of timing information is an important issue in its own right and it may well be desirable to augment PNML with a standard way of recording rate and delay information which is not tool specific.

An Achilles heel in biological modelling at present is a lack of reliable rate constants for all events in the system [18]. It is important that such practical limitation do not prevent a stochastic net being stored and analysed. It would therefore be advantageous for any time based PNML net to accept models where timing information is missing or unreliable for some transitions. It may useful to provide a means for biologists to record a "best guess" rate or a bound on the rates.

4.3 Graph Placement

The PNML standard specifications propose the inclusion of a number of features concerned with graphical information, layout and structure of Petri net models. This information is not necessary for the theoretical view of the model, but is mandatory if PNML is to be used in a large number of tools. Whilst these graphical features are valuable inclusions, features directly corresponding to these are missing from the SBML level 2 specification. This means that PNML models constructed automatically from biological models may not have any layout information available and so a layout procedure must be carried out prior to tool analysis.

It is feasible that a conversion process from SBML to PNML could involve the generation of layout data from information derived from the connectivity of components inherent in the SBML model, in combination with information from specific SBML components such as compartments. In addition specific data about SBML components derived from external sources such as the Gene Ontology [6], may also provide a means to add layout features to structured PNML during the mapping process. However, if PNML is to be used as a truly interdisciplinary interchange standard then it must also address this problem by providing appropriate tags and tools for the default layout of a Petri net. Work is currently underway at Newcastle to produce a tool which uses heuristics to automatically augment PNML models with appropriate layout information.

4.4 Modularity and Composition

Biological systems typically contain a number of recurring sub-pathways which are common to many different entities in that system. For example, each expressed gene

that encodes a protein involved in a particular pathway is subject to transcription (a separate process) and the mRNA that results is interpreted by the translational processes of the cell. Many components of the transcriptional and translational pathways are commonly applied to each gene. Variations in the application of different instances of these sub-pathways between different genes often can be mapped through to differences in the kinetic parameters that are applied to the steps in the pathway (e.g. promoter strength) [25]. The ability to have 'standard' or default sub-models such as these would facilitate the rapid construction of large networks. Biologists often view such sub-pathways at different levels of abstraction and then use the appropriate one depending on the detail needed for a particular analysis. This approach allows biologists to update the sub-pathways as they gain more knowledge and understanding of the processes involved. Although at present SBML does not support the above view of biological systems recent proposals for extending SBML have included the idea of a compositional approach (see [23]). To cope with the above compositional view we need to be able to build PNML models in a modular way allowing different PNML modules to represent the various recurring parts and views of a biological system. It will therefore be vital that a modular version of PNML, such as `modular PNML` [13], is available and supported to aid translation of biological models to PNML. Using a modular approach would also allow PNML models to be efficiently maintained and manipulated, and allow different abstract views of a biological system to be used. Providing modular concepts in PNML would also aid the composition of interconnected biological systems. For example, regulatory systems can be artificially viewed at the gene network level or at the protein network level, and composing such views is an important modelling problem.

5 Conclusions

In this paper we have considered using Petri net and XML techniques to model and analyse biological systems. We presented a scheme for automatically translating SBML models of biological systems into PNML and developed a prototype tool to implement this mapping. We illustrated our ideas using a SBML description of the *Saccharomyces cerevisiae* glycolysis pathway which was translated into PNML and then analysed using various Petri net tools.

This work has demonstrated how XML technologies can be used to open up new and productive links between different research communities which have their own data repositories and analysis tools. In particular, we have detailed how Petri net techniques and tools can be used to analyse biological systems and our work can be seen as providing further evidence of the usefulness of Petri nets as a modelling and analysis tool in Systems Biology.

XML technologies such as PNML and SBML are still developing and continued work is needed to refine these standards to enhance interdisciplinary model exchange. We have discussed the insights we have gained during our work and proposed a number of areas for consideration to enhance the use of PNML. These included a discussion of timing issues and the need to cope with partial or noisy information, as is often found in biological models. We also considered the problem of graphical layout for automatically generated PNML models and noted the need for additional tool support which we are

currently investigating and developing. Other important issues discussed included the need for modularity and encapsulation of traceability information for a PNML model.

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6 Appendix

Here the XML for both the input(SBML) and the output(PNML) of the tool are presented. The original PNML was obtained from the SBML website[23], and related to the work published in [27]. This was modified slightly by removing the kinetic rates for brevity.

6.1 Glycolysis SBML listing

```
<?xml version="1.0" encoding="UTF-8"?>
<!-- Created by Gepasi 3.30 on March 14, 2003, 13:42 -->
<sbml xmlns="http://www.sbml.org/sbml/level1" level="1" version="1">
<model name="Teusink">
<listOfCompartments>
<compartment name="uVol" volume="1"/>
</listOfCompartments>
<listOfSpecies>
<specie name="GLCi" compartment="uVol" initialAmount="0.08" boundaryCondition="false"/>
<specie name="G6P" compartment="uVol" initialAmount="1.39" boundaryCondition="false"/>
<specie name="F6P" compartment="uVol" initialAmount="0.28" boundaryCondition="false"/>
<specie name="F16P" compartment="uVol" initialAmount="0.1" boundaryCondition="false"/>
<specie name="TRIO" compartment="uVol" initialAmount="5.17" boundaryCondition="false"/>
<specie name="BPG" compartment="uVol" initialAmount="0" boundaryCondition="false"/>
<specie name="P3G" compartment="uVol" initialAmount="0.1" boundaryCondition="false"/>
<specie name="P2G" compartment="uVol" initialAmount="0.1" boundaryCondition="false"/>
<specie name="PEP" compartment="uVol" initialAmount="0.1" boundaryCondition="false"/>
<specie name="PYR" compartment="uVol" initialAmount="3.36" boundaryCondition="false"/>
<specie name="ACE" compartment="uVol" initialAmount="0.04" boundaryCondition="false"/>
<specie name="Pi" compartment="uVol" initialAmount="5" boundaryCondition="false"/>
<specie name="NAD" compartment="uVol" initialAmount="1.2" boundaryCondition="false"/>
<specie name="NADH" compartment="uVol" initialAmount="0.39" boundaryCondition="false"/>
<specie name="Glyc" compartment="uVol" initialAmount="0" boundaryCondition="true"/>
<specie name="Trh" compartment="uVol" initialAmount="0" boundaryCondition="true"/>
<specie name="CO2" compartment="uVol" initialAmount="1" boundaryCondition="true"/>
<specie name="SUCC" compartment="uVol" initialAmount="0" boundaryCondition="true"/>
<specie name="GLCo" compartment="uVol" initialAmount="50" boundaryCondition="true"/>
<specie name="ETOH" compartment="uVol" initialAmount="50" boundaryCondition="true"/>
<specie name="GLY" compartment="uVol" initialAmount="0.15" boundaryCondition="true"/>
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<listOfReactions>
 <reaction name="vGLK" reversible="true">
  <listOfReactants>
   <specieReference specie="GLCi" stoichiometry="1"/>
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```

```
 <listOfProducts>
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 </listOfProducts>
</reaction>
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  <specieReference specie="G6P" stoichiometry="1"/>
 </listOfReactants>
 <listOfProducts>
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 </listOfProducts>
</reaction>
<reaction name="vGLYCO" reversible="true">
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  <specieReference specie="G6P" stoichiometry="1"/>
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 </listOfReactants>
 <listOfProducts>
  <specieReference specie="Glyc" stoichiometry="1"/>
 </listOfProducts>
</reaction>
<reaction name="vTreha" reversible="true">
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  <specieReference specie="Trh" stoichiometry="1"/>
 </listOfProducts>
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 </listOfReactants>
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 </listOfProducts>
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```
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  <specieReference specie="P2G" stoichiometry="1"/>
 </listOfProducts>
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```
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 </listOfProducts>
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  <specieReference specie="NAD" stoichiometry="3"/>
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 <listOfProducts>
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```
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 </reaction>
</listOfReactions>
</model>
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6.2 Converted PNML listing

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```

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```

```
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```

```
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