

MPath2PN - Translating metabolic pathways into Petri nets

Paolo Baldan¹, Nicoletta Cocco², Francesco De Nes², Mercè Llabrés Segura³,
Andrea Marin², Marta Simeoni²

¹Università di Padova, Italy
baldan@math.unipd.it

²Università Ca' Foscari di Venezia, Italy
{cocco,marin,simeoni}@dais.unive.it, kekodenes@gmail.com

³Universitat de les Illes Balears, Spain
merce.llabres@uib.es

Abstract. We propose *MPath2PN*, a tool which automatically translates metabolic pathways, as described in the major biological databases, into corresponding Petri net representations. The aim is to allow for a systematic reuse, in the setting of metabolic pathways, of the variety of tools existing for Petri net analysis and simulation. The current prototype implementation of *MPath2PN* inputs the KEGG description of a metabolic pathway and produces two Petri nets, mainly differing for the treatment of ubiquitous substances. Such Petri nets are represented using PNML, a standard format for many Petri net tools. We are extending the tool by considering further formats for metabolic pathways in input and for Petri nets in output. *MPath2PN* is part of a more general project aimed at developing an integrated framework which should offer the possibility of automatically querying databases for metabolic pathways, producing corresponding Petri net models and performing analysis and simulation on them by means of various tools.

1 Introduction

Metabolic pathways are complex systems whose understanding is important in many fields, in particular in biology and medicine. Various techniques have been proposed to model and analyse metabolic pathways. Among these, Petri nets are a well-known formalism, used in computer science for modelling concurrent and distributed systems, which turns out to be particularly natural for representing metabolic pathways and with the advantage of the availability of many tools for visualisation, simulation and analysis. By using Petri nets it is possible to represent and analyse fundamental properties of metabolic pathways, like conservation relations on metabolites (corresponding to P-invariants), steady state flux distributions (corresponding to T-invariants), the rates of chemical reactions (corresponding to marking dependent rates in continuous transitions) or control mechanisms, such as positive or negative feedbacks.

When modelling a metabolic pathway as a Petri net one has to face several problems related to the multiplicity of data sources and formats. On the one

hand, the information on the pathway may be stored in different databases each using its own data format. On the other hand, once constructed, the Petri net model could be analysed with different Petri net tools, each one having its specific input format. Our proposal is aimed at alleviating this problem, automatising the recovery of metabolic data and their translation into corresponding Petri net models, which can be encoded using the input format of different tools available for Petri nets. This is part of a larger project - in progress - aimed at developing a framework able to automatically retrieve metabolic data from the web, produce corresponding Petri net representations and analyse them through the available tools. The framework should deal with the various databases for metabolic pathways and the different tools for Petri nets.

In this paper we present a prototype implementation of the automatic translation of the metabolic data into a Petri net model. The tool, *MPath2PN*, is written in Java and it is conceived to deal with different translations, that is different databases in input, such as KEGG and the BioModels Database, and different Petri net tools in output. At present it includes two specific translations from KEGG's data to PNML for PIPE2. The first translation is rather efficient since it considers a KGML file as the main source. The second translation is slower, since it gets most of the input data from the KEGG web service, but it provides a more detailed representation of the pathway which includes also ubiquitous substances.

The paper is organised as follows. In Section 2 we give a brief introduction to metabolic pathways and their main databases. In Section 3 we recall how to give a Petri net representation of a metabolic pathway. In Section 4 we describe the tool structure and the two translations from KEGG to PNML for PIPE2. Finally, in Section 5 we draw some conclusions.

2 Metabolic Pathways

An organism depends on its metabolism, the chemical system which generates the essential components for life and the energy necessary to synthesise and use them. Subsystems dealing with some specific function are called *metabolic pathways*. Biologists usually represent a metabolic pathway as a network of *chemical reactions*, catalysed by one or more *enzymes*, where some molecules (*reactants* or *substrate*) are transformed into others (*products*). Enzymes are not consumed in a reaction, even if they are necessary and used while the reaction takes place. The product of a reaction is the substrate of the next one.

To characterise a metabolic pathway, it is necessary to identify its components (namely the reactions, enzymes, reactants and products) and their relations. Such relations can be represented through a *stoichiometric matrix*. An element of the matrix, a stoichiometric coefficient n_{ij} , represents the degree to which the i -th chemical species participates in the j -th reaction. The kinetic of a pathway is determined by the *rate* associated with each reaction. It is represented by a rate equation, which depends on the concentrations of the reactants and on a reaction

rate coefficient (or rate constant) which includes all the other parameters (except for concentrations) affecting the rate.

A metabolic pathway contains many steps, one is usually irreversible, the other steps are usually reversible and in many cases the pathway can go in the opposite direction depending on the needs of the organism. *Glycolysis* is a good example of this behaviour: it is a fundamental pathway which converts glucose into pyruvate and releases energy. When glucose enters a cell, it is phosphorylated by ATP to glucose 6-phosphate in a first irreversible step, thus glucose will not leave the cell. When there is an excess of energy, the reverse process, the *gluconeogenesis*, converts pyruvate into glucose: glucose 6-phosphate is produced and stored as glycogen or starch. Most steps in gluconeogenesis are the reverse of those found in glycolysis, but the three reactions of glycolysis producing most energy are replaced with more kinetically favorable reactions. This system allows glycolysis and gluconeogenesis to inhibit each other.

Information on metabolic pathways are collected in many different databases. The **KEGG PATHWAY** database [9] contains the main known metabolic, regulatory and genetic pathways for different species. It integrates genomic, chemical and systemic functional information [38]. KEGG can be queried through a language based on XML [6], called **KGML** (KEGG Markup Language) [8], but also a web service for querying the system from users programs is available. Another important repository is the **BioModels Database** in the SBML.org site [17]. The models are coded in **SBML** (Systems Biology Markup Language), a language based on XML. Other free access databases are **MetaCyc** [11, 24], **Reactome** [15], **TRANSPATH**, which is part of **BIOBASE** [20] and **BioCarta** [1]. Relevant information can be found also in other databases, such as **BRENDA** [3, 25], **ENZYME** [5], **DIP** [4, 52], **MINT** [12, 27] and **BIND**.

3 Petri nets for modelling Metabolic Pathways

In some seminal papers Reddy et al. [50, 48, 49] and Hofestädt [36] propose Petri nets (PNs) for representing and analysing metabolic pathways. Since then a wide range of literature has grown on the topic (see, e. g., [26, 39, 21] for surveys on modelling metabolic pathways through PNs). PNs are a well-known formalism applied in computer science for modelling concurrent systems. They have an intuitive graphical representation which may help the understanding of the modelled system, a sound theory and many applications both in computer science and in real life systems (see [45, 51, 44, 28] for surveys on PNs and their properties). A PN model can be decomposed in order to master the overall complexity and it enables a large number of different analyses. Just to mention a few, one can determine conflicting evolutions, reachable states, cycles, states of equilibrium, bottlenecks or accumulation points. Additionally, once a qualitative PN model has been devised, quantitative information can be added incrementally. PNs seem to be particularly natural for representing metabolic pathways, as there are many similarities between concepts in biochemical networks and in PNs. They both consist of collections of reactions which consume and pro-

duce resources and their graphical representations are similar. This suggest to exploit the techniques developed for PNs also for metabolic pathways. In fact many tools are available for visualisation, analysis and simulation of PNs, a quite comprehensive list can be found at the Petri net World site [14].

Several generalisations of the basic PN formalism have been proposed to better modelling biological systems (such as PNs with test and inhibitor arcs [42, 43], Coloured PNs [35, 54], Timed PNs [29, 34, 46], Stochastic PNs [32, 41, 33], Continuous PNs [30, 23, 33, 39] and Hybrid PNs [42, 43]). Some extensions concern the qualitative aspects of the models and aim at increasing the expressive power or the modelling capabilities of the formalism. Other extensions introduce quantitative concepts, such as time and probability, thus allowing for the representation of temporal and stochastic aspects of biological systems, respectively. In this paper we will be concerned only with basic PNs, used for a qualitative modelling of metabolic pathways.

3.1 Petri net representation of a metabolic pathway

The qualitative representation of a metabolic pathway by means of a PN can be derived by exploiting the natural correspondence between PNs and biochemical networks. In fact, places in PNs are associated with molecular species, such as metabolites, proteins or enzymes; transitions in PNs correspond to chemical reactions; input places represent the substrate or reactants; output places represent reaction products. The incidence matrix of the PN is identical to the stoichiometric matrix of the system of chemical reactions. The number of tokens in each place of the PN indicates the amount of substance associated with that place. It may represent either the number of molecules expressed in moles or the level of concentration, suitably discretised by introducing a concept of concentration level [31].

Although the correspondence between metabolic pathways and PN elements is rather straightforward, some modelling choices have to be taken in the construction of a PN representation of a metabolic pathway. For example, enzymes and ubiquitous substances, such that H_2O , phosphate, ADP and ATP, might not be represented in the PN. Enzymes are taken and then released by the reactions and they are usually not represented in the PN model. This is an appropriate choice as long as their concentration do not change. Also ubiquitous substances, once assumed to be constant, can be omitted in the PN model. In this way the resulting model is greatly simplified, but, as an obvious drawback, processes involving such substances, such as the energy balance, are not modelled. In the PN models produced by the current prototype enzymes are not explicitly represented. Instead, as clarified later, the decision on whether to include information on the ubiquitous substances is left to the user.

Additionally, in a metabolic pathway one can distinguish between internal and external metabolites. The former are entirely produced and consumed in the network, while the latter represent sources or sinks, that is, connection points with other pathways producing or consuming them. External metabolites can be represented in the PN model in different ways, with different impacts on the

resulting net. In the translations currently performed by the prototype, external metabolites will simply result in places where connected transitions either all consume or all produce tokens. Their special status may be considered later in the simulation or analysis phase.

Another modelling problem arises from the fact that most of the reactions in a pathway are reversible. A reversible reaction is decomposed into two distinct reactions, a forward one and a backward one, leading to two corresponding transitions in the PN model. If the PN model does not represent the kinetic factors, the presence of the forward and backward transitions leads to a cyclic behaviour producing and destroying the same molecules, which might not be of biological interest. In the current implementation pairs of transitions corresponding to reversible reactions can be distinguished by their identifiers, so that the corresponding cyclic behaviours may be filtered out, if desired, in the analysis or simulation phase (e.g., an analysis based on T-invariants could ignore the trivial invariants consisting of pairs of transitions generated by a reversible reaction).

Once we have a qualitative model, quantitative data can be added to refine the representation of the behaviour of the pathway. In particular, extended PNs may have an associated transition rate which depends on the kinetic law of the corresponding reaction. This introduces further representation problems and choices, but in this paper we consider only qualitative modelling. A more detailed description of the representation of metabolic pathways with PNs can be found in [39, 21], where qualitative and quantitative modelling aspects are discussed and analysed.

4 The tool *MPath2PN*

The tool *MPath2PN* is intended to provide a way of automatically transforming a metabolic pathway, expressed in one of the various existing formalisms (e.g. KGML, SBML), into a corresponding PN, also expressed in one of the existing formalisms (e.g. PNML [13], a standard format used by many analysis tools for PNs, or the specific input formalism for PN tools, such as SNOOPY [18], INA [53] or TimeNET [19]).

We developed a prototype in Java with a structure which is modular enough to cope with many different translations (see Figure 1). We also implemented two specific translations which follow the modelling choices described in Section 3.1. Both of them derive the description of a metabolic pathway from the KEGG database and generate a corresponding PN. A basic source of information on the pathway is a file, in KGML format, which can be downloaded from KEGG. A file describing the corresponding PN model is produced, in PNML format for PIPE2 (Platform Independent Petri net Editor 2) [22], an open source platform independent tool for creating and analysing PNs. The two translations differ for the level of detail of the description of the pathway: the second translation considers also the presence of ubiquitous substances.

Since most of the descriptions of metabolic pathways and of PNs are based on XML formats, *MPath2PN* produces the translation by using XSLT (eXtensible

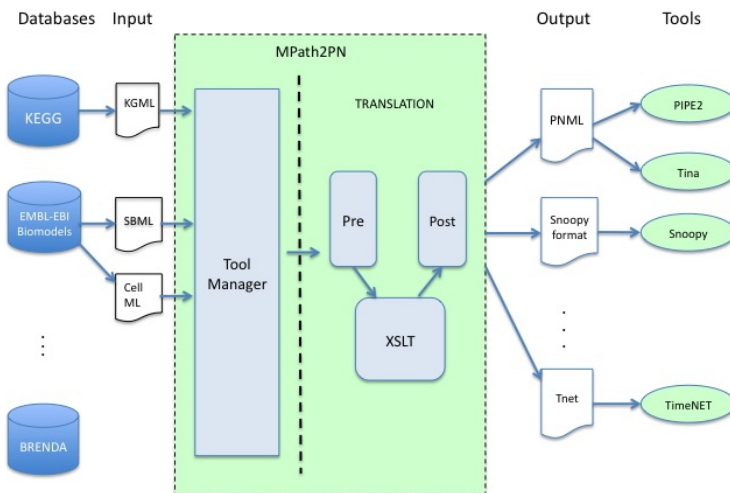


Fig. 1. Structure of *MPath2PN*

Stylesheet Language Transformation [7]) in the Saxon [16] open source version. Each translation requires the definition of an appropriate style sheet XSL which specifies the translation rules to be applied. Often there is the need to integrate various information in the translation, hence a translation is standardised into a three step process: pre-treatment, XSL translation and post-treatment. For the pre- and post-treatment, Java classes can be developed which modify respectively the input and the output files.

4.1 The first translation from KGML to PNML for PIPE2

The first translation implemented in *MPath2PN* consists of a plain transformation from a source KGML file describing the pathway downloaded from KEGG, to a target file describing the produced PN in PNML format for PIPE2.

Consider for example the KEGG pathway of the *Glycolysis / Gluconeogenesis* in *Homo sapiens* shown in Figure 2. We enclosed in a shaded box a small part of the pathway corresponding to a single reversible reaction, i.e., β -D-glucose 6-phosphate ketol isomerase (R03321). The KEGG page relative to such reaction is shown in Figure 3. The reaction is catalysed by the enzyme identified by the EC number 5.3.1.9 and it involves the compounds β -D-glucose 6-phosphate (C01172) and β -D-Fructose 6-phosphate (C05345). Note that KEGG uses its own identifiers for reactions and compounds. Let us take reaction R03321 as a running example for the translation from KGML to PNML.

The structure of the KGML format is shown in Figure 4. The root node represents the complete pathway, which is composed by nodes **entry**, **relation** and **reaction**, all with multiplicity $0, \dots, \infty$. A node **entry** represents a node in the KEGG pathway such as a compound, an enzyme or also a reference to another pathway. A node **relation** represents a relation between two proteins,

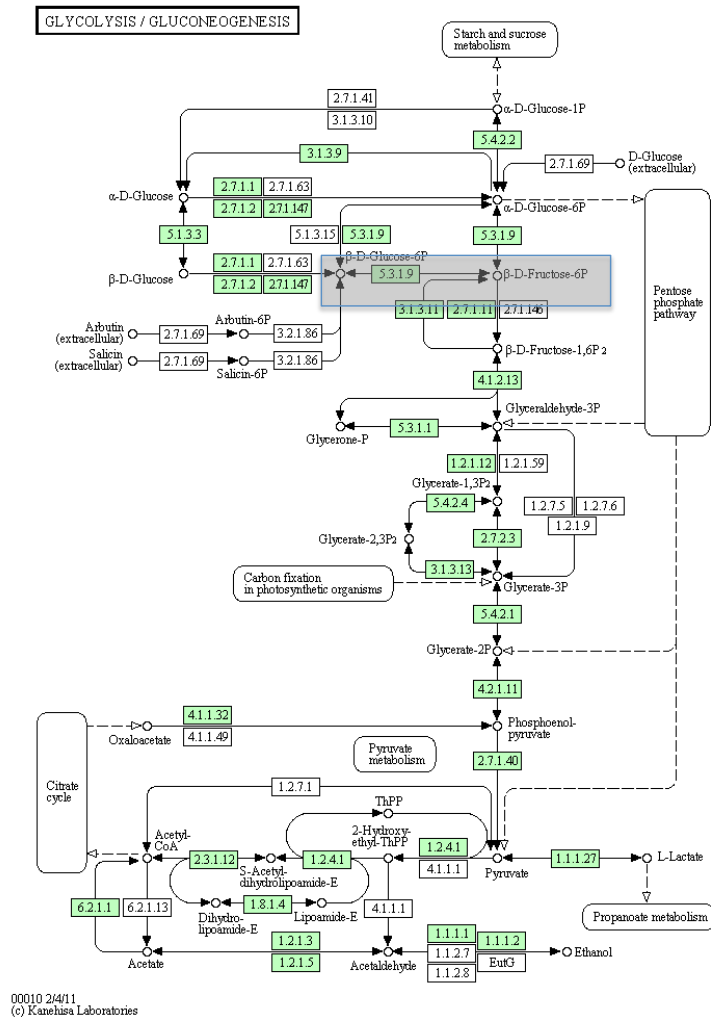


Fig. 2. KEGG pathway of the *Glycolysis / Gluconeogenesis* in *Homo sapiens*

or between a protein and a compound, or also a link to another map. A node **reaction** represents a pathway's reaction without dynamic information.

For instance, compound C01172 and reaction R03321 of our running example are represented in KGML as follows:

Entry	R03321	Reaction
Name	beta-D-Glucose 6-phosphate ketol-isomerase	
Definition	beta-D-Glucose 6-phosphate <=> beta-D-Fructose 6-phosphate	
Equation	C01172 <=> C05345	
RPair	RP02940	C01172_C05345 main
Enzyme	5.3.1.9	
Pathway	rn00010 Glycolysis / Gluconeogenesis rn01100 Metabolic pathways rn01110 Biosynthesis of secondary metabolites rn01120 Microbial metabolism in diverse environments	
Orthology	K01810 glucose-6-phosphate isomerase [EC:5.3.1.9] K06859 glucose-6-phosphate isomerase, archaeal [EC:5.3.1.9] K13810 transaldolase / glucose-6-phosphate isomerase [EC:2.2.1.2 5.3.1.9]	

Fig. 3. The KEGG page of reaction R03321

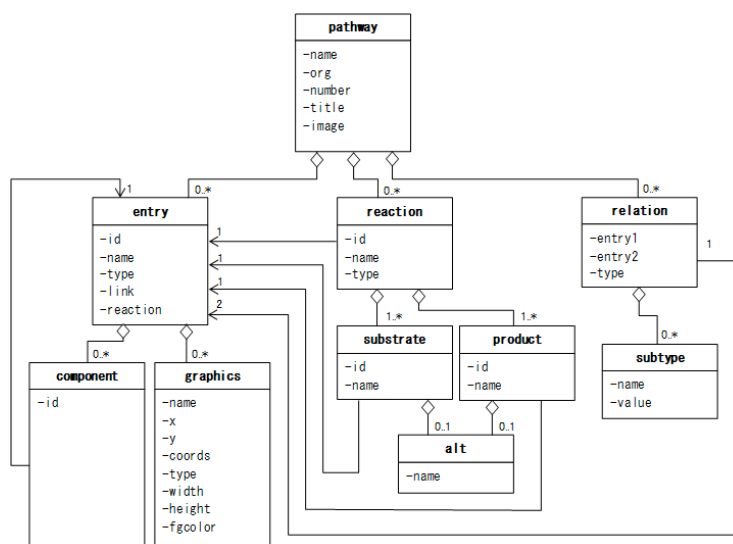


Fig. 4. Structure of a KGML file.

```

<entry id="90" name="cpd:C01172" type="compound"
  link="http://www.kegg.jp/dbget-bin/www_bget?C01172">
  <graphics name="C01172" x="332" y="301" type="circle" width="8"
    height="8" fgcolor="#000000" bgcolor="#FFFFFF"/>
</entry>
<reaction name="rn:R03321" type="reversible">
  <substrate name="cpd:C01172"/>
  <product name="cpd:C05345"/>
</reaction>

```


To build the PN representation of the pathway we use the nodes **entry** and **reaction**. Compounds correspond to places in the PN and reactions to transitions. The arcs are obtained by inspecting substrates and products in reactions.

The style sheet **net.xml** implements most of the translation. It uses other XSLs dealing with the various components: **labels.xml**, **places.xml**, **transitions.xml** and **arcs.xml**, as shown in Figure 5.

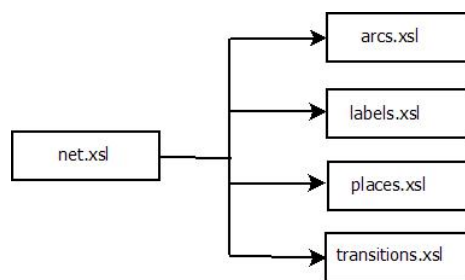


Fig. 5. The PNML style sheet structure

In **places.xml** the entries are checked to determine whether they have to be translated into places: only compounds are represented. The target code generated for compound C01172 of our running example is the following:

```
<place id="cpd:C01172" >
  <graphics><position x="332" y="301"/></graphics>
  <name><value>cpd:C01172</value></name>
</place>
```

Transitions are created by **transitions.xml** from the **reaction** nodes in the KGML format. As already mentioned, a non-reversible reaction produces a single transition, while a reversible reaction produces two transitions (a direct and an inverse one). The inverse transition is identified by the fact that its *id* obtained from the *id* of the direct transition by adding the string “**#rev**” as suffix. This allows to recognise cycles in the behaviour introduced by this encoding of reversible reactions.

The PNML code generated for reaction R03321 is the following:

```

<transition id="rn:R03321" >
  <name><value>rn:R03321</value></name>
  <rate><value>1.0</value></rate>
  <timed><value>>false</value></timed>
</transition>
<transition id="rn:R03321#rev" >
  <name><value>rn:R03321#rev</value></name>
  <rate><value>1.0</value></rate>
  <timed><value>>false</value></timed>
</transition>

```

The arcs are generated by templates in **arcs.xml**. They are inferred by the nodes **reaction** and their children **substrate** and **product** in the KGML format. For each pair (substrate, product) the following arcs are created,

substrate \rightarrow reaction, reaction \rightarrow product,

and, obviously, if the reaction is reversible, we will have also the inverse arcs:

inverse reaction \rightarrow substrate, product \rightarrow inverse reaction.

In our example the following arcs are generated in the target code:

```

<arc target="rn:R03321" source="cpd:C01172" id="cpd:C01172 to rn:R03321" >
  <inscription><value>1</value></inscription>
  <type value="normal" />
</arc>
<arc target="cpd:C05345" source="rn:R03321" id="rn:R03321 to cpd:C05345" >
  <inscription><value>1</value></inscription>
  <type value="normal" />
</arc>
<arc target="rn:R03321#rev" source="cpd:C05345" id="cpd:C05345 to rn:R03321#rev" >
  <inscription><value>1</value></inscription>
  <type value="normal" />
</arc>
<arc target="cpd:C01172" source="rn:R03321#rev" id="rn:R03321#rev to cpd:C01172" >
  <inscription><value>1</value></inscription>
  <type value="normal" />
</arc>

```

A KGML file representing a metabolic pathway does not provide any information on kinetic laws, initial concentrations of compounds and stoichiometric values. However, stoichiometric values, which are essential also for a qualitative modelling (they correspond to arc weights in the PN) can be retrieved through the KEGG web service. This is done in the post-treatment phase of the translation which, as a consequence of the multiple service invocations, is rather slow. In order to speed up this process, a caching of the information is introduced, so that each reaction is queried only once through the web service. Since KGML files do not provide information on ubiquitous substances, the resulting PN does not represent ubiquitous substances either.

5 Conclusions and future work

An obstacle to the use of PNs for modelling metabolic pathways seems to be, paradoxically, the amount of different sources of data on metabolic pathways and the number of simulation and analysis tools for PNs. This is due to the dishomogeneity both of databases formats for metabolic data and of input formats for PNs tools. To cope with this problem in the literature we find proposals for

- a standard format for metabolic data, such as SBML [17] or BioPAX [2], and a standard format for PN tools, such as PNML [13];
- unification or integration of different databases such as in [47] or [37], and translations between different data formats, such as in KEGGtranslator [10] or KGML2SBML and KGML2BioPAX [40].

In this paper we proposed a tool *MPath2PN*, for translating metabolic pathways into corresponding PN representations, coping with different input and output formats. The aim is to allow for a systematic reuse of the tools already developed for PNs also for the analysis and simulation of metabolic pathways. The input and output formats are generally based on XML. For this reason *MPath2PN* is based on XSLT and each translation can be defined by giving a corresponding style sheet XSL. Moreover *MPath2PN* allows for a pre-treatment and a post-treatment phase, implemented by Java classes, to permit the integration of different data sources on metabolic pathways.

We developed a prototype version of *MPath2PN* providing two rather standard translations. The first translation is from KGML to PNML for PIPE2 and it is rather efficient. The second translation is from KEGG to PNML for PIPE2 and it is slower, but it gives a more detailed representation of the pathway by considering also ubiquitous substances.

We are working on further translations to be included in *MPath2PN*:

- from KGML to the format of INA [53], a tool which allows for many different analysis of mainly qualitative Petri net models;
- from SBML to PNML;
- from SBML to the format of Snoopy [18], a tool which allows for analysis and simulation of stochastic/continuous PNs;
- from SBML to TimeNET/eDSPN format and from SBML to TimeNET/SCPN format. TimeNET is a tool that allows for analysis and simulation of extended deterministic and stochastic Petri nets (eDSPN) and stochastic coloured Petri nets (SCPN). Using this tool, it is possible to specify transition rates that may depend on the global state of the net. As a consequence, the translation of the dynamic information from the SBML specification into the TimeNET format can be done efficiently and without the need of further assumptions, in a purely syntactical way.

Further extensions of *MPath2PN* consist in providing different translations between the same input and output formats in order to implement different modelling choices, for example we could represent explicitly also enzymes or supply different ways of dealing with external metabolites.

When quantitative data are available, as in SBML, it is possible to obtain a quantitative PN model of a metabolic pathway. In this case further modelling decisions have to be taken in the translation, such as whether to consider all modifiers (such as inhibitors and cofactors) or not, whether and how to scale or discretise the amounts of substances, which kinetic model to choose, and, more generally, whether to give a continuous, a discrete or a stochastic representation.

Mpath2PN is freely available at:

<http://www.dsi.unive.it/~simeoni/MPath2PNtool.tgz>.

References

1. BioCarta: Charting Pathways of Life. <http://www.biocarta.com>.
2. BioPAX: Biological Pathway Exchange. <http://www.biopax.org/index.php>.
3. BRENDA: The Comprehensive Enzyme Information System. <http://www.brenda-enzymes.info>.
4. Database of Interacting Proteins. <http://dip.doe-mbi.ucla.edu>.
5. ENZYME: enzyme nomenclature database. <http://www.expasy.ch/enzyme>.
6. Extensible Markup Language. <http://www.w3.org/XML>.
7. Extensible Stylesheet Language Transformations. <http://www.w3.org/TR/xslt>.
8. Kegg Markup Language manual. <http://www.genome.ad.jp/kegg/docs/xml>.
9. KEGG pathway database. <http://www.genome.jp/kegg/pathway.html>.
10. KEGGtranslator. <http://www.ra.cs.uni-tuebingen.de/software/KEGGtranslator/>.
11. MetaCyc Encyclopedia of Metabolic Pathways. <http://metacyc.org>.
12. MINT: The Molecular INTeraction database. <http://mint.bio.uniroma2.it>.
13. Petri Net Markup Language. <http://www.pnml.org>.
14. Petri net tools. <http://www.informatik.uni-hamburg.de/TGI/PetriNets/tools>.
15. REACTOME. <http://www.reactome.org>.
16. SAXON: the XSLT and XQuery processor. <http://saxon.sourceforge.net/>.
17. SBML: Systems Biology Markup Language. <http://sbml.org>.
18. SNOOPY. <http://www-dssz.informatik.tu-cottbus.de/index.html?software/snoopy.html>.
19. TimeNET. <http://www.tu-ilmenau.de/fakia/TimeNet.timenet.0.html?&L=1>.
20. TRANSPATH: The Pathway Database. <http://www.biobase-international.com>.
21. P. Baldan, N. Cocco, A. Marin, and M. Simeoni. Petri nets for modelling metabolic pathways: a survey. *Natural Computing*, 9(4):955–989, 2010. ISSN: 1567-7818.
22. P. Bonet, C.M. Llado, R. Puijaner, and W.J. Knottenbelt. PIPE v2.5: A Petri net tool for performance modelling. In *Proc. 23rd Latin American Conference on Informatics (CLEI 2007), San Jose, Costa Rica*. ACM, 2007.
23. R. Breitling, D. Gilbert, M. Heiner, and R. Orton. A structured approach for the engineering of biochemical network models, illustrated for signalling pathways. *Briefings in Bioinformatics*, 9(5):404–421, 2008.
24. R. Caspi, H. Foerster, C.A. Fulcher, P. Kaipa, M. Krummenacker, M. Latendresse, S. Paley, S. Y. Rhee, A. G. Shearer, C. Tissier, T. C. Walk, P. Zhang, and P. D. Karp. The MetaCyc Database of metabolic pathways and enzymes and the BioCyc collection of Pathway/Genome Databases. *Nucleic Acids Research*, 36(Database issue: D623-D631), 2008.
25. A. Chang, Scheer M., Grote A., I. Schomburg, and D. Schomburg. BRENDA, AMENDA and FRENDA the enzyme information system: new content and tools in 2009. *Nucleic Acids Research*, 37(Database issue: D588-D592), 2009.

26. C. Chaouiya. Petri net modelling of biological networks. *Briefings in Bioinformatics*, 8(4):210–219, 2007.
27. A. Chatranyamontri, A. Ceol, L. Montecchi Palazzi, G. Nardelli, M. V. Schneider, L. Castagnoli, and G. Cesareni. MINT: the Molecular INTERaction database. *Nucleic Acids Research*, 35(Database issue: D572-D574), 2007.
28. J. Esparza and M. Nielsen. Decidability issues for Petri Nets - a survey. *Journal Inform. Process. Cybernet. EIK*, 30(3):143–160, 1994.
29. H. Genrich, R. Küeffner, and K. Voss. Executable Petri Net Models for the Analysis of Metabolic Pathways. *Proceedings of the Workshop on Practical Use of High-level Petri Nets*, pages 1–14, 2000.
30. D. Gilbert and M. Heiner. From Petri Nets to Differential Equations - An Integrative Approach for Biochemical Networks Analysis. In *Petri Nets and Other Models of Concurrency - ICATPN 2006*, volume 4024 of *LNCS*, pages 181–200. Springer, 2006.
31. D. Gilbert, M. Heiner, and S. Lehrack. A Unifying Frameworks for Modelling and Analysing Biochemical Pathways Using Petri Nets. *Proceedings of the Workshop on Computational Methods in Systems Biology (CMSB)*, pages 200–216, 2007.
32. P. J. Goss and J. Peccoud. Quantitative modeling of stochastic systems in molecular biology by using stochastic Petri nets. *Proc. Natl. Acad. Sci. USA*, 95(12):6750–6755, 1998.
33. M. Heiner, D. Gilbert, and R. Donaldson. Petri Nets for Systems and Synthetic Biology. In *Proc. of SFM'08*, volume 5016 of *LNCS*, pages 215–264. Springer, 2008.
34. M. Heiner, I. Koch, and S. Schuster. Using time-dependent Petri nets for the analysis of metabolic networks. In R. Hofstadt, K. Lautenbach, and M. Lange, editors, *Workshop Modellierung und Simulation Metabolischer Netzwerke*, Preprint No.10, pages 15–21. Faculty of Computer Science, Otto-von-Guericke University of Magdeburg, 2000.
35. M. Heiner, I. Koch, and K. Voss. Analysis and Simulation of Steady States in Metabolic Pathways with Petri nets. *Workshop and Tutorial on Practical Use of Coloured Petri Nets and the CPN Tools (CPN'01)*, pages 15–34, 2001.
36. R. Hofstädt. A Petri net application of metabolic processes. *Journal of System Analysis, Modelling and Simulation*, 16:113–122, 1994.
37. I. Kanaris, K. Moutselos, A. Chatziioannou, I. Maglogiannis, and F.N. Kolisis. Building in-silico pathway SBML models from heterogeneous sources. In *Bioinformatics and BioEngineering (BIBE2008)*, pages 1–6. IEEE, 2008.
38. M. Kanehisa, M. Araki, S. Goto, M. Hattori, M. Hirakawa, M. Itoh, T. Katayama, S. Kawashima, S. Okuda, T. Tokimatsu, and Y. Yamanishi. KEGG for linking genomes to life and the environment. *Nucleic Acids Research*, pages D480–D484, 2008.
39. I. Koch and M. Heiner. Petri nets. In B. H. Junker and F. Schreiber, editors, *Analysis of Biological Networks*, Book Series in Bioinformatics, pages 139–179. Wiley & Sons, 2008.
40. K.E. Lee, M.H. Jang, A. Rhie, C.T. Thong, S. Yang, and H.S. Park. Java DOM parsers to convert KGML into SBML and BioPAX common exchange formats. *Genomics & Informatics*, 8(2):94–96, 2010.
41. W. Marwan, A. Sujatha, and C. Starostzik. Reconstructing the regulatory network controlling commitment and sporulation in *Physarum polycephalum* based on hierarchical Petri net modelling and simulation. *Journal of Theoretical Biology*, 236:349–365, 2005.

42. H. Matsuno, Y. Tanaka, H. Aoshima, A. Doi, M. Matsui, and S. Miyano. Biopathway representation and simulation on hybrid functional Petri net. *In Silico Biology*, 3(0032), 2003.
43. S. Miyano and H. Matsuno. How to model and simulate biological pathways with Petri Nets - a new challenge for system biology. In *International Conference on Applications and Theory of Petri Nets, Bologna, Italy*, 2004.
44. T. Murata. Petri Nets: Properties, Analysis, and Applications. *Proceedings of IEEE*, 77(4):541–580, 1989.
45. J.L. Peterson. *Petri Net Theory and the Modelling of Systems*. Prentice-Hall, 1981.
46. L. Popova-Zeugmann, M. Heiner, and I. Koch. Timed Petri Nets for modelling and analysis of biochemical networks. *Fundamenta Informaticae*, 67:149–162, 2005.
47. Harsha K. Rajasimha. PathMeld: A methodology for the unification of metabolic pathway databases. Master’s thesis, Virginia Polytechnic Institute and State University, 2004.
48. V. N. Reddy. Modeling Biological Pathways: A Discrete Event Systems Approach. Master’s thesis, The University of Maryland, ISR-M.S. 1994-4, 1994.
49. V. N. Reddy, M.N. Liebman, and M.L. Mavrovouniotis. Qualitative Analysis of Biochemical Reaction Systems. *Comput. Biol. Med.*, 26(1):9–24, 1996.
50. V. N. Reddy, M. L. Mavrovouniotis, and M. N. Liebman. Petri net representations in metabolic pathways. In *ISMB93: First Int. Conf. on Intelligent Systems for Molecular Biology*, pages 328–336. AAAI press, 1993.
51. W. Reisig. *Petri Nets: An Introduction*. EACTS Monographs on Theoretical Computer Science. Springer Verlag, 1985.
52. L. Salwinski, C. S. Miller, A. J. Smith, F. K. Pettit, J. U. Bowie, and D. Eisenberg. The Database of Interacting Proteins: 2004 update. *Nucleic Acids Research*, 32(Database issue: D449-D451), 2004.
53. P.H. Starke and S. Roch. The Integrated Net Analyzer. *Humbolt University Berlin*, 1999. www.informatik.hu-berlin.de/~starke/ina.html.
54. K. Voss, M. Heiner, and I. Koch. Steady state analysis of metabolic pathways using Petri nets. *In Silico Biology*, 3(0031), 2003.