Modeling, Simulation and Analysis of Metabolic Networks.

Allapalli Bharadwaja
University of Windsor

Abstract

The simulation and analysis of bio molecular networks especially metabolic pathways has been an area in which there is always a need for improvement. With that motivation researchers are trying to provide efficient and considerably fast techniques to model and analyze various kinds of biological networks in the field of Systems biology.
To understand how a system of the basic unit of biology, a cell behaves in dynamically changing surroundings is an issue for biologists.
This brings into picture the emergence of mathematical modeling as an invaluable tool to understand the behaviour of a cell. Mathematical models have proven invaluable in several fields of science and engineering.

This survey reviews research on proposed methodologies that have been implemented for modeling and analysis of complex metabolic networks.

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1. INTRODUCTION

The survey reviews research on various modeling methods implemented for analysis and simulation of metabolic networks, "a complex network of biochemical reactions describing flows of" metabolites. [Baldan et al 2010]

- In computational biology any biological system can be represented as a pathway with numerous communicating nodes arranged in a certain well known order. This paved the way for mathematical models such as graph theories, ordinary differential equations, petri nets etc., to play a vital role in simulation of pathways in Systems Biology.
- There are three major issues faced during modeling i). data requirement ii). scalability of the network size i.e., how well the modeling works when size of the network increases and iii). computational effort.

Thus, there is a need of efficient techniques in modeling of metabolic pathways and computational biologists have been making a notable progress in this area and in the further sections of this article the contribution of their work has been discussed.

The sources used for this survey are Google Scholar, Silico Biology, ACM and IEEE web resources.

This survey is based on 12 journal papers, 8 conference papers, 1 Master's thesis, 1 PHD thesis, 1 workshop paper and a reference book.

1.1 Structure of the survey

The survey is categorized into seven sections:
Section one presents a brief introduction to the topic. A review on the petri modeling of metabolic networks both qualitative and quantitative modeling methods is presented in section two of the paper. Section three focuses on various other modeling techniques for simulation of metabolic networks namely machine learning techniques, dynamic simulators, modeling based on fuzzy concepts and Agent based modeling approach. The fourth section summarizes the methods discussed followed by conclusion. Acknowledgements in fifth section are followed by a complete list of works referred for the survey in the sixth section.

One interesting observation can be made through a comprehensive review on this issue is that the majority of computational biologists have a penchant for Petri nets computational tool used to model a complex biological systems such as metabolic pathway / network.

1.2 Challenges faced during modeling in Systems biology

The aim of Systems biology is twofold: the first is to predict how a biological system behaves in different environments and second is to study how a particular biological system is connected (interrelated connections inside the system). Even the simplest of metabolic network is a complex system involves a number of enzymatic reactions. "This level of complexity stretches the ability of experienced and dedicated modellers to build, analyze,

simulate, verify, and test their models by hand. For example, when a model fails to account for all the observations in the experimental data set, the modellers must determine where the problem lies:

1. With the parameter set?
2. With the model itself? Maybe the molecular wiring diagram is incorrect. Maybe some crucial molecular interactions have been left out of the model.
3. With the experimental data? Maybe the unfitted experimental observations are mistaken in some way." [Sauro et al 2006].

2. PETRI NET MODELING AND ANALYSIS OF METABOIC NETWORKS

In the fields of Real life systems and Computer science, Petri nets are widely used computational tools in modelling complex parallel systems. Their strong theoretical background, ability to modularise and breakdown the complexity of biological systems and innate graphical representation ease the task of understanding a modelled pathway leading to a number of practical applications in the above mentioned areas of study.
"Additionally, once a qualitative Petri Net model has been devised, quantitative information can be added incrementally." [Baldan et al 2010].

2.1. Qualitative Modeling and Analysis

Qualitative modeling wisely ignores the kinetics of the reactions involved in a metabolic network and focuses more on stochastic modeling techniques based on the factors such as liveness, deadlocks, s-invariants and t-invariants etc.

The aim of the works discussed below is to provide an efficient method for modeling and analysing metabolic pathways so that biologists can note clinical observations related to genetic diseases and understands the behaviour of a cell / biological unit so as to address a biological phenomenon more efficiently and easily.

2.1.1. *Petri net representations in metabolic pathways.*

The work of Reddy et al [1993, 1994] was an important breakthrough in the field of metabolic engineering. The authors describe a way of representation for metabolic pathway modeling as discrete event systems. This method delineates the dynamics of an individual reaction and emphasizes on discrete systems approach.
In this work Petri nets are described with a detailed explanation from the authors point of view and as a tool Petri nets is used for computer implementable representation of fructose metabolism (in liver) pathway.

Reddy et al [1993, 1994] state that the analysis which they conducted proves that most metabolic pathways are not literally reversible, due to thermodynamic irreversibility of many reactions. But an alternate set of reactions can reproduce the precursor metabolites. And further they also claim that in the PN model of Fructose metabolism they developed, the path to Glycolysis is possible

since there is at least one possible firing sequence from Fructose to GAP (viz. Fructose, F1P, Glyceraldehyde, GAP, and finally Glycolysis. (Shown in the figure below)

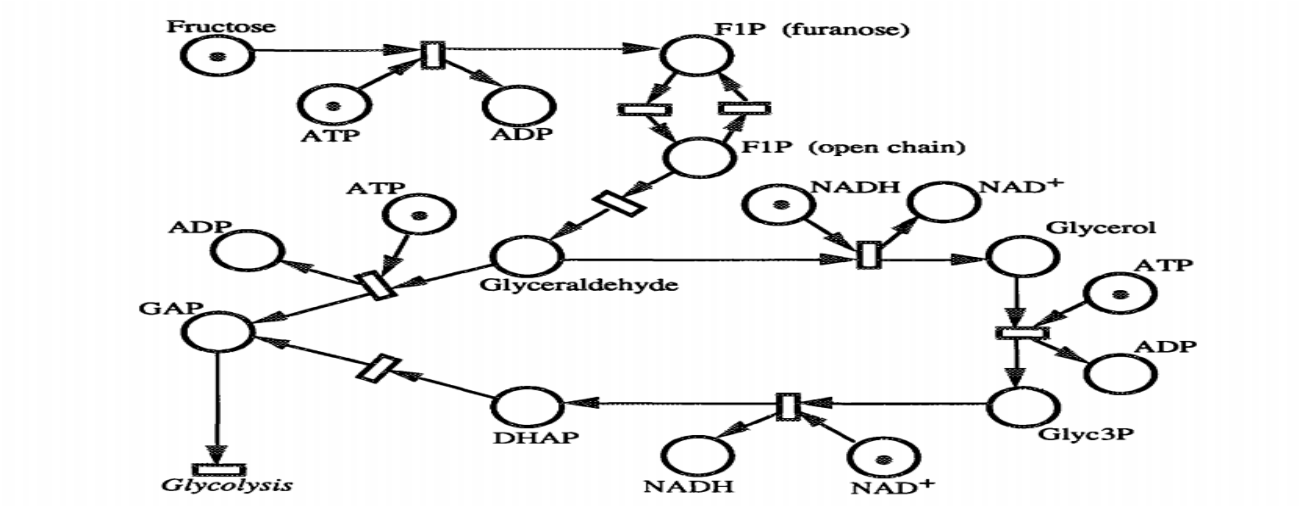

Fig. Representation of Fructose-Glycolysis metabolism using Petri Nets
[Reddy et al 1993, page 333].

Thus, Reddy et al [1993, 1994] successfully modeled metabolic networks using Petri net theory and claim that their approach, "representation of metabolic pathways by Petri nets is a promising modeling approach and should enable many types of qualitative analysis of pathways." The authors conclude by stating that "the representation of metabolic pathways as Petri nets is of value because the properties of Petri nets can be applied to the pathway, leading to relevant observations".

2.1.2. *Simulation and analysis of metabolic networks by time-dependent petri nets.*

The works published by Reddy et al [1993, 1996] were very important and Koch et al [1999] using the foundation laid by Reddy et al [1993, 1996] made a notable contribution in the field of metabolic pathway modeling and analysis.

The new model created by Koch et al [1999] is an extended version of the model proposed by Reddy, et al [1996].
The model proposed by Reddy et al [1993] uses discrete event methodology for representing and analysing bio chemical networks (metabolic networks) which are modeled with Petri Nets. The new idea, the authors describe here implements the same methodology by also considering the factors which are present in a biological system: a).Reversible reactions and b).Time dependencies, thus including quantitative issues.

Koch et al [1999] modeled, analysed and simulated combined Glycotic and Penta phosphate pathway using time –dependent petri nets. Modeling is done using Petri net Editor (PED), Analysis using Integrated Net Analyzer and simulation using PEDVisor.
Thus, the authors claim to have found out that there exists a vector describing a special type of marking in the modeled petri net (graph), which always results in a constant by scalar multiplication with any reachable state of the net.
The authors state that the analysis which they conducted shows that the net does not have any deadlocks and dynamic conflicts and thus the net being live (absence of deadlocks).

The authors state that Time-dependent petri nets exhibit more better and efficient way for modeling and verification in the process of analysis and simulation of metabolic networks with or without agitations.

2.1.3 *Qualitative modelling of regulated metabolic pathways*

The research work of Simao et al [2005] in computational biology on modeling and analysing metabolic pathways address the following problems.

1. To analyze and understand biological process through simulation using computational methods. (Because this is beyond the capability of humans).
2. To provide with an efficient method for modeling, analysing and simulating metabolic pathways so that biologists can understand the behaviour of a cell / biological unit so as to address a biological phenomenon more efficiently and easily.

Simao et al [2005] referred to the works of Reddy et al [1996], Zevedei-Oancea et al [2003], Goss et al [1998], Santillán et al [2001], Xiu et al [2002], Bhartiya, et al [2003] and identified certain limitations. They are as follows

a) for the idea presented in Goss et al [1998], in the case of the two regulatory processes considered, a PN modelling is not so direct because regulators are not consumed and the stoichiometry not as evident.

b) Simao et al [2005] state that Santillán et al [2001], Xiu, et al. [2002] and Bhartiya et al [2003] "progressively include more regulatory processes, the quantitative model becomes increasingly complex, impeding proper analytical approaches. Dynamical insights are then obtained solely on the basis of numerical analyses, which are *per se* incomplete."

The new model proposed by Simao et al [2005] is a generic approach covering metabolic reactions and regulatory processes in a single PN-based qualitative modelling. In summary, the procedure consists of three processes:

(1) The metabolic pathway converting chorismate into trypto-phan
(2) The transcriptional inhibition of trpEDCBA operon by the dimmeric holorepressor, which results from the combination of the product of the repressor gene *trpR* with the amino acid Trp
(3) The inhibition of the enzyme involved in the first biosynthetic step, TrpE, by the final product, the amino acid Trp.

The authors state that qualitative modelling of the biosynthesis of tryptophan (Trp) in *Escherichia coli*, taking into account two types of regulatory feedbacks: the direct inhibition of the first enzyme of the pathway by the final product of the pathway and the transcriptional inhibition of the Trp operon by the Trp–repressor complex. On the basis of this integrated PN model, the researchers further indicate how available dynamical analysis tools can be applied to obtain significant insights in the behaviour of the system.
The authors claim to have achieved that the resulting integrated PN model is readily amenable to classical PN analysis techniques, leading to the delineation of the asymptotic behaviour for different income fluxes of external Trp. For low Trp influx, they obtain a cycle representing the homeostatic regulation of both Trp and TrpE. For moderate Trp influx, they obtain a dead marking with both TrpE and the repressor inactive. Finally, for high Trp influx, they obtain a dead marking characterized by the absence of all Trp catalytic enzymes and the presence of active repressor (holorepressor).

Simao et al [2005] about their approach state that "this new model is still too simple to represent all the qualitative subtleties" of the biological system and consider several extensions to take into account the additional regulatory feedbacks occurring in E.coli.
In conclusion, they state that the method have provided a first step towards the integration of regulatory and metabolic processes into a coherent qualitative modelling framework, in terms of standard PNs and with the help of existing techniques "extensions of this formalism to refine qualitative models and generate more quantitative results" [Simao et al 2005], e.g. using hybrid or stochastic PNs extensions.

Year	Author	Title	Previous woks referred	Major contribution
1993	Reddy et al	Petri net representations in metabolic pathways	None	First paper to make a strong proposition of petri net methodology for modeling metabolic networks as a discrete event system
1999	Koch et al	Simulation and analysis of metabolic networks by time-dependent petri nets.	Reddy et al [1993, 1996]	The new model created by the authors of this paper is an extended version of the model proposed by Reddy et al [1996] by also considering Reversible reaction and Time dependencies, thus integrating qualitative information with quantitative analysis.
2005	Simao, E et al	Qualitative modeling of regulated metabolic pathways	Reddy [1996] et al, Goss et al [1998], Santillán et al [2001], Xiu et al [2002], Bhartiya et al [2003], Zevedei-Oancea et al [2003]	In terms of standard PNs, the method provided a first step towards the integration of regulatory and metabolic processes into a coherent qualitative modeling framework

2.2 Qualitative modeling and analysis of metabolic networks

Quantitative modeling and analysis considers the mathematical and statistical data involved in a biological system. For example the reaction rates etc., i.e., kinetics of reactions taking place in metabolism.

2.2.1. *Petri net based modelling and simulation of metabolic networks in the cell*.

The problem Chen and Freier [2002] tried to solve is to improve the current standards in the study and understanding of cell behaviour as physiological, biochemical and genetic systems through modeling and simulation techniques of bio molecular (metabolic) networks in a cell.

Authors pointed out some of the shortcomings in previous research works as follows:

1. Lack of interoperability among different biological networks.
2. Freier, Hofestädt, Matthias Lange et al., [2002] the databases like Federated Database systems (ISYS and Discovery Link), multi database systems (TAMBIS) and data ware houses (N C B I/Entrez and SRS) attain only well-known pathways, lack of automation in constructing dynamic and graphical metabolic pathways and also they do not contain comprehensive information about the metabolic pathways such as physical and chemical properties of metabolic pathways.

The new idea, presented in this paper is as follows:

1. A system called IIUDB (Individually Integrated User Data base) is developed to integrate the access to several biological data bases and to offer a possibility to model and simulate gene regulated metabolic networks based on the Petri net modelling idea.
   The aim of IIUDB is to support the integration of multiple heterogeneous biological data bases and also to enhance the execution of applications that extend beyond individual databases.

2. A new idea of developing a common exchange language (between different data bases) a standard BioPNML (Biology Petri Net Markup Language), for modeling metabolic networks. [Chen and Freier 2002] claim that this eases the process of converting original XML source file to another XML format having different definitions and ontologies.

A petri net model of urea cycle was simulated (by VON++) for analysis.The dynamic behaviour of the model system was studied for example metabolic fluxes, and the regulation of urea cycle enzyme activities etc.

Thus based on the experiment conducted

1. The authors claim that the analysis which they conducted proves that IIU Data base architecture, by integrating access to various biological data bases, helps to develop user designed-models of a whole cell that include gene regulation and metabolic pathways and
2. Hybrid petri nets are user friendly graphical interfaces helps in simulation and visualization of biological networks.

Therefore, Chen and Freier [2002] conclude by stating that their new architecture IIUD has better access to various biological data than any known data base such as TAMBIS or Discovery Link.

2.2.2. *The biology petri net markup language.*

The research work of Chen et al [2002] aims to provide with an efficient method for modeling and analysing metabolic pathways so that biologists can note clinical observations related to genetic diseases and understand the behaviour of a cell / biological unit so as to address a biological phenomenon more efficiently and easily.

The methodology proposed by Chen et al [2002] is based on the works of Reddy et al [1993] [1996], Koch et al [1999].
Chen et al [2002] pointed out notable drawbacks in the previous research works and mention that they identified a common limitation in proposed methodologies of Valk, [1978], Hofestaedt, [1998], Goss [1999], Koch [1999], Chen [2000], Kueffner [2000], Matsuno . [2000], Genrich [2001] and Heiner [2001], by stating that "they lack unity in their concepts, notations, and terminologies making it tedious for naive researchers to understand the potential applications of Petri nets due to the various interpretations presented by different authors. And also states that no Petri net tools exist which satisfies completely all requirements in simulating a biological network."
The authors state that in the model proposed by Reddy et al [1996] "the reactions and other biological processes were modeled as discrete events and it was not possible to simulate kinetic effects." And the PN XML standards executed in the work of Matthias Jüngel et al [2000] is incompatible for different design destinations.

Therefore, a new methodology Biology Petri Net Markup Language (BioPNML) is proposed, which the Chen et al [2002] believe, is the initial step in formulation of a standard interchange platform for Bioinformatics and Petri nets. Main aim of this approach is to bridge Petri net models to other metabolic pathway simulators.
Using BioPNML the researchers presented XML syntax of a biochemical reaction involving the formation of L-ornithine from L-arginine in the presence of arginase (enzyme). Kinetics related to the reaction was also considered and a basic model was presented.

An enzymatic (arginase acting as a catalyst) metabolic pathway (biochemical) reaction formation of L-ornithine from L-arginine modeled using petri nets was successfully represented using a markup language (strongly based on XML) could connect petri nets to XML based simulators was also able to integrate different biological databases.

Based on the above work the authors claim that their proposed markup language "BioPNML makes it easier for users and developers of biological software to map data in different formats. Easier mapping enables developers of biological software who are using open standards, such as XML, to adopt changes in biological data formats faster." [Chen et al 2002]. And about their contribution the authors also claim that "It serves as a starting point for the development of a standard interchange format for Petri nets and other molecular biological modeling and simulation tools."

2.2.3. *Time petri nets for modelling and analysis of metabolic networks.*

The problem addressed by Popova-Zeugmann et al [2005] is to provide a better way for computational modeling and simulation of biochemical networks for analysis. The medical society benefits in effectively tackling a biological malfunction at a cell / genetic level.

The authors Popova-Zeugmann et al [2005] referred to the works of Reddy et al [1993] and Chen and Hofestadt [2003]. And according to the authors, no paper is known to discuss and present an approach how to derive the quantitative model in a systematic manner from the qualitative one.

In this paper the authors used time Petri nets to develop a discretely treatable quantitative model for biochemical networks. Their approach starts with the qualitative model and the well-established structural analysis method to compute the minimal transition invariants. After converting the qualitative Petri net into a quantitative one, they give a structural technique to prove the time dependent reliability of a given transition sequence, and by this means of a transition invariant. The crucial point of the whole approach is the total avoidance of any state space construction. Therefore, it may be applied also to infinite systems, i.e. unbounded Petri nets.

Using time Petri nets authors developed a discretely treatable quantitative model for a special metabolic network of the central carbon metabolism in the potato tuber.

The authors state that by the analysis they conducted, the following results were obtained

1. The entries in the vector sum of all minimal T-invariants vary between 6 and 236.
2. The least common multiple of all entries in the vector sum exceeds the standard computer accuracy. Thus, it is required to skip the last normalization step and the firing times have to be approximated by rational numbers and
3. Using the parametric description approach, as summarized in section 4 of the paper, it can be shown that all minimal T-invariants are still realisable in the steady state of the derived time Petri net model.

The authors claim that their approach starts with the qualitative model and the well-established structural analysis method to compute the minimal transition invariants. After converting the qualitative Petri net into a quantitative one, they have used a structural technique to prove the time-dependent reliability of a given transition sequence, and by this means of a transition invariant.
Moreover, they also claim that they were able to calculate the shortest and longest time length of transition sequences using linear programming.
And state that the crucial point of the whole approach is the total avoidance of any state space construction. Therefore, it may be applied also to infinite systems, i.e. unbounded Petri nets.

Year	Author	Title	Previous works referred	Major contribution
2002	Chen and Freier	Petri net based modeling and simulation of metabolic networks in the cell.	None	It successfully integrated various biological data bases, by proposing common Bio XML standards. Thus enabling researchers to obtain metabolic data at a single platform thus improving portability of data files.
2002	Chen et al	The biology petri net markup language.	Reddy [1993, 1996] and Koch et al [1999]	First paper to propose and implement BioPNML, formulation of a standard interchange platform for Bioinformatics and Petri nets. Successfully bridge Petri net models to other metabolic pathway simulators.
2005	Popova-Zeugmann, L et al	Time petri nets for modeling and analysis of biochemical networks.	Reddy [1993], Chen and Hofestadt [2003]	This paper is a solid reference on how to make use of qualitative analysis to model metabolic networks quantitatively. Thus it provides a basis in modeling using unbounded petri nets

3. OTHER APPROACHES IN MODELING AND ANALYSIS OF METABOLIC NETWORKS

This section reviews research on various other methodologies used to model and analyse metabolic networks. As metabolism is a complex biological process involving numerous biochemical reactions, computational biologists, in order to provide with an efficient technique to model and analyze metabolic pathways name different methods which include agent based modeling systems, modeling of metabolic pathway using fuzzy concepts, machine learning techniques etc., and were also abled to design dynamic simulators.

3.1 *Modeling and analysis of metabolic networks using Fuzzy concepts*

This section reviews “the use of fuzzy logic concepts in modelling biochemical reactions involved in metabolism. In fact, each enzymatic reaction is modelled by means of a "sigmoidal transfer function" relating input and output substrate concentrations.

The slant of this function is adjusted using fuzzy concepts. This adjustment is conducted depending on the type of enzymatic reaction." [Solaiman and Picart 1996].

The main aim behind the work of Solaiman and Picart [1996] is to provide with an efficient method for modeling and analysing metabolic pathways so that biologists can note clinical observations related to genetic diseases and understand the behaviour of a cell / biological unit so as to address a biological phenomenon more efficiently and easily.

Solaiman and Picart [1996] referred to the previous works of Reder [1988] and Solaiman et al [1994] and state that the approach of Reder [1988] is a Numerical modeling (NM) approach in which numerical measurements for enzymatic parameter reactions are inaccurate and physician reports do not provide with quantitative results.

The new approach described by the Solaiman and Picart [1996] in this paper is a new enzymatic reactions modelling approach. This model is based on the use of a physical reasoning method describing the enzymatic reaction (ER) functioning. Parameters updating is conducted using fuzzy concept; by considering the qualitative alphabet, already used, as a set of linguistic variables modelled by their membership function.

According to their analysis, the authors state that "an enzymatic reaction (ER) permitting to transform an input substrate Sin to output substrate Sout is governed by a set of differential equations. And that the input and the output substrate concentrations (SC) are related together through a reaction advancement degree transfer function."
In the proposed model, the substrate concentrations are described using the qualitative alphabet
Therefore, a SC "x" is considered through a membership vector: p = (p AL, p N, p AH) instead of a single value.

In the proposed model, all the analytic components that may compose the function f(t) are reduced to a single sigmoidal transfer function in which the slant is adapted in a dynamic way in order to take into account the dynamic behaviour of different SCs.
In order to describe the proposed method of slant adaptation, the following configurations are discussed:

1. Basic Enzymatic Reaction (ER) (no activating or inhibiting substrates)
2. ER with activating substrates
3. ER with inhibiting substrates and
4. ER with activating and inhibiting substrates.

Solaiman and Picart [1996] claim to have achieved the following results.
The analysis which they conducted proves that going by the Qualitative modeling approach proposed by Solaiman et al [1994], the results obtained helped in reproducing the known and existing metabolic reaction networks in a fast and simple way.

The authors state that their new model seems promising in order to permit quantitative results to process data concerning adverse drug reactions and the first advantage of this model is that it takes into account the continuous substrate production through an important parameter, generally

ignored in Qualitative Modeling, the time parameter. They also claim that in their proposed model the common use of the NM (sigmoidal transfer function) and QM (linguistic variables controlling the sigmoidal slant values) has permitted to resolve the following difficulties:
a) Major / normal production pathways through varying slant values,
b) Modelling all ER using a unified approach.

3.2 *Modeling using Dynamic Simulators*

The problem addressed by Kurata et al [2005] is to understand how all of the cellular molecules work in concert as a living system and predict the dynamics at molecular-interaction level. This is done using computers because the whole process is beyond the capability of human.

The authors refer to the papers by Mendes [1993], Kauffman [1993], Bhattacharjya et al [1996], Goryanin et al [1999], Bhalla et al [1999], Chen et al [2000], Hasty et al [2001], Schoeberlet et al [2002] and Hatakeyama et al [2003].

Kurata et al [2005] state that:

- The simulator described in Mendes [1993] does not support the automatic conversion of biochemical network maps into dynamic models.

- The model proposed in Kauffman [1993], Bhattacharjya et al [1996] may not truly represent biological reality.

- The simulator described in Goryanin et al. [1999] does not support the automatic conversion of biochemical network maps into dynamic models and

- also mention that the concrete model(s) presented in Bhalla et al [1999], Chen et al [2000], Hasty et al [2001], Schoeberl et al [2002], and Hatakeyama et al [2003] require decades of research to obtain the exact kinetic parameters of detailed molecular mechanisms in vivo, resulting in restricting the application of the model(s).

The new simulator proposed by the researchers convert the maps into dynamic models, the technology of three layers and two stages has been originally developed with the elaborately designed rules in an XML representation, which divides a biochemical network into three layers, i.e., gene, protein, and metabolic layers, and partitions the conversion process into two stages. The simulation consists of forward and reverse engineering; forward engineering converts gene regulatory and metabolic networks into dynamic models, and reverse engineering explores the kinetic parameters of the model to fit to experimental data. Once a biochemical map is provided, CADLIVE automatically builds a dynamic model, thereby facilitating simulation and analysis.

The authors use a CADLIVE Simulator to build a mathematical model for the nitrogen assimilation system, and the kinetic parameters were estimated by genetic algorithms. They analyzed the *Escherichia coli* nitrogen-assimilation system that consists of multiple and

complicated negative and positive feedback loops, where the activity and synthesis of glutamine synthetase is regulated to adapt to the great changes in the ammonia concentration.

In their experiment, the authors simulated and analyzed the dynamics of the mutant lacking GlnK and a wild type in order to predict the mechanism for "runaway expression" of the *Ntr* genes.

Based on the conducted experiment the authors claim that their simulator CADLIVE predicted that the *glnK* gene is responsible for hysteresis or reversibility of nitrogen-related (*Ntr*) gene expression with respect to the ammonia concentration, supporting the experimental observation of the runaway expression of the *Ntr* genes, the authors state that the analysis which they conducted proves that the generated model was able to satisfy the behaviors of a wild-type and knockout mutants or the biological rationalities in terms of transient responses and robustness.

As a concluding remark Kurata et al [2005] state that
a). their model captures the intrinsic features of transient response and the robustness to the changes in the ammonia concentration.
b). Simulated results demonstrate that the dynamic model is able to predict not only the qualitative behaviors of the wild type, but also those of several mutants".
c). One of the most important breakthroughs for CADLIVE is the proposition of efficient and practical rules to integrate all possible molecular interactions into mathematical models, thereby simulating the dynamic features underlying their molecular architectures.
d). The proposed CADLIVE Simulator has been demonstrated to handle gene regulatory and metabolic networks at an intermediate level without going all the way down to exact biochemical reactions. [Kurata et al 2005].

3.3 *Modeling and analysis of metabolic networks using an Agent based modeling approach*

The problem Hossain [2008] tried to solve is to improve the current standards in the study and understanding of cell behaviour as physiological, biochemical and genetic systems through modeling and simulation techniques of bio molecular (metabolic) networks in a cell.

Hossain [2008] analyzed the works of Varma and Palsson [1994], Heinrich and Schuster [1996], Schilling et al [1999], Papin et al [2004] and Price et al [2004] and state that "all the approaches discussed above mainly capture the structural aspects of the network without considering the kinetic properties of the enzymatic reactions involved in the network."

The thesis by Hossain [2008] presents an application of an Agent based modeling and simulation of cell metabolism. "A new agent-based model has been proposed with the ability to simulate and analyze the metabolic network of prokaryotic cell. The dynamic model of carbon metabolism described by [Chassagnole et al 2002] is used here to validate the proposed agent based dynamic simulation of cellular metabolism. The dynamic responses of intracellular metabolites to a pulse of glucose were experimentally measured for E.coli K-12 strain W3110 culture." [Hossain 2008]

Hossain [2008] claims that "during the dynamic simulation of E.coli, the injection pulse experimental technique was easily designed as an additional agent and effectively implemented into the main structure" and also claims to have "developed a framework which can efficiently identify and fill gaps in linear and branched networks using the central metabolism of E.coli as a model system."

Table 4-6: Comparison between Agent-based simulation and MATLAB Simulation

Metabolite	Glu_{ext}	fdp	g1p	G6p	pep	pyr	f6p	gap	6pg
Agent-Based Simulation									
% error	1.04	19.69	17.93	6.99	5.28	15.07	19.44	15.0	5.50
Simulation Time	50 sec simulation need 6792 sec of computational time								
MATLAB Simulation using ode45 solver									
% error	1.70	67.72	15.71	6.78	16.73	18.82	19.41	15.30	6.18
Simulation Time	50 sec simulation need 211 sec of computational time								

Table 4-6. Chapter 4. Hossain [2008, page 90]

The author claims that according to the table above, his methodology can produce efficient simulation results.

Hossain [2008] based on his approach claim that it is the "first agent based model of metabolic networks, a new and potential tool for modeling the complex organization in the cell and can be extended to model eukaryotes with multiple compartments."
The author also claims about his approach has the natural helps one to exploit all the advantages related to an object-oriented paradigm and "the simulation results prove the effectiveness of the proposed modeling and simulation approach as it successfully captured the dynamics of the system and reproduced the same results as reported in literature."
Finally, the author claims that the "key benefit of this agent-based model is that it enables one to identify inconsistencies in metabolic network through qualitative simulation." [Hossain 2008]

3.4 *Machine learning techniques in modeling and analysis of metabolic networks*

The research work of Biba et al [2011] aims

1. To improvise the methodologies used to model, analyse and simulate complex biological systems (metabolic networks) for the purpose of understanding these systems thoroughly so as to uncover the functionalities and behaviour of biological systems expressed through complex interactions among its building blocks.
2. Developing and applying hybrid practices (by combining symbolic and stochastic models) to model biological systems

The shortcomings of previous works referred in their paper were mentioned by Biba et al [2011]. They are:

1. The authors state that in the works of Le Novre and Shimizu. [2001], Klamt and Stelling [2003], "The tools used lack Machine Learning abilities to infer dynamics from observations. Only simulating the model is not sufficient for a thorough understanding of metabolic networks. Most of their nature and behavior remains hidden or unobserved".
2. The authors also mention that the work of Koyuturk et al [2004] "does not deal with automatic reconstruction or simulation of the model".
3. The authors also state that the methods proposed in: i). R. Hoffmann, M. et al [2005] "usually produce is just the structure of the network which gives only a static view of the real stochastic biological environment." and
4. ii). You et al [2006] "do not deal with automatic reconstruction or simulation of the model."

In their work Biba et al [2011] introduce a hybrid method that combines both symbolic (structure of the model) and statistical machine learning (unpredictable nature of biological systems) approaches for modeling, analysing and simulation of biological systems (metabolic networks).

The experiment conducted by the authors involved in modeling in PRISM of the Bisphenol A Degradation pathway of Dechloromonas aromatica.
a). Performing simulations of the network by changing the parameters of the model and performing stochastic runs.
b). Performing stochastic simulations to see which is the most probable path in the network by computing the probability of the related predicate through the Viterbi algorithm.

1. The authors claim that their experiments show the feasibility of discovering significant active paths from metabolomics data in the form of traces of sequences of reactions, by running and simulating the reconstructed model.
2. They also claim that by simulating the model, we can imply what is the effect of changing parameters of the basic distribution, on the output of the model. Therefore, this gives further insight into the structure of the model and how it can be optimized and its output by changing the parameters of the model and
3. from empirical observations, they noted that all the stochastic execution of the model performed by the two predicates 'hindsight' and 'probf' (discussed in the paper) reflected real biological paths that are supposed to have produced the sequences.

Therefore based on their research Biba et al [2011]

1. State that the strength of the proposed approach stands in the description language that allows the modeling relations and in the ability to model stochastic dynamics in a robust manner. (Simulating the model helps to understand the behavior of the observed part of the model, while machine learning helps to uncover the unobserved part).
2. Authors in this paper also state that graphical EM algorithm used in PRISM, is a version of algorithms employed for learning in the presence of missing data and believe that this will ease the process in tackling with incomplete real datasets [Biba et al 2011].

Year	Author	Title	Previous works referred	Major contribution
1996	Solaiman, B and Picart, D	Biochemical metabolic modeling using fuzzy concepts.	Reder [1988] and Solaiman et al [1994]	Paper presents an improvised methodology (based on the previous works), enzymatic modeling approach using the concept of Fuzzy logic
2005	Kurata, H et al	Cadlive dynamic simulator: direct link of biochemical networks to dynamic models.	Mendes [1993], Kauffman [1993], Bhattacharjya et al [1996], Goryanin et al. [1999], Bhalla et al [1999], Chen et al [2000], Hasty et al [2001], Schoeberl, et al [2002] and Hatakeyama et al [2003]	One of the best computational tool enabling dynamic simulation from a biological map and will be a core system to combine large scale dynamic biological systems. The simulations being very close to real biochemical systems at finer levels of interaction.
2008	Hossain, M.	Multi-agent based modeling and simulation of metabolic networks.	None	In the presence of contemporaries, agent based modeling is implemented for simulation of metabolic pathways that was able to provide a clear analysis of metabolism
2011	Biba. M et al	Using machine learning techniques for modeling and simulation of metabolic networks	None	Unpredictable nature of biological systems was easily analyzed and simulated by integrating structural machine learning techniques with statistical data, providing a new break -through in the field of systems biology

4. CONCLUSION

The survey reviewed 12 journal papers, 8 conference papers, a master's, thesis PHD thesis, a workshop paper and a reference book. This survey has been classified into various sections based on different techniques proposed and used in the process of modeling metabolic networks to analyze and understand metabolism from a biological perspective.

Simulation and modeling of different pathways in Computational biology is to understand biological networks as a dynamic molecular system and learn how various molecular reactions work within a cell.

The first part of the survey reviewed research on modeling of metabolic networks using petri nets. Petri net theory and the process of metabolism have interesting similarities in their concepts and fundamentals. From this observation researchers have argued that petri nets as a modeling tool is widely used methodology in simulation of complex biological pathways like signal pathways, gene regulatory networks and metabolic networks. When dealing with biological databases require transparent flow of data. When data is readily available petri net representation is easy leading to qualitative analysis. The issue which boggles computational biologists is that the data inferred from different biological databases is incomplete and inconsistent, therefore quantitative modelling of metabolic pathways is not as simple as expected. And it becomes difficult in Petri net representation of this inconsistent data with the help of suitable tools. Researchers have considered that by not considering certain factors petri nets for modeling metabolic pathways is simple, easy to visualize and it allows user to make required changes using computers thus a more preferred modeling technique.

In the later sections of the survey we discussed and review various other works that propose equally effective methodologies for metabolic network modeling and analysis. In addition to Petri net modeling researchers have used other notable approaches in reconstruction of metabolic networks such as Agent based modelling, machine learning methods etc., and were able to derive important observations leading to significant results. Another effective method of integrating logical modeling and Petri net theory is influential as the presence of certain feedback circuits helped the biologists to analyze metabolism at deeper level of precision.

Year	Authors	Title	Paper's referred to	Major Contribution
1988	Reder	Metabolic control theory.	None	It proposed an efficient way (at that period of time), mathematical modeling of metabolic networks based on structural characterizations
1993	Kauffman	The origin of order, self-organization and selection in Evolution.	None	A comprehensive book explaining evolutionary biology, which extends the basic concepts of Darwinian evolution. First of its kind.
1993	Mendes	A software package for modeling the	None	Microsoft windows software coded in C for modeling biochemical reaction networks. The input can be user

		Dynamics, steady state and control of biochemical and other systems.		defined or 35 predefined rate equations thus, a notable breakthrough in the field of Computational biology. A direct computational tool for modeling biochemical pathways.
1993	Reddy et al	Petri net representations in metabolic pathways.	None	First paper to make a strong proposition of petri net methodology for modeling metabolic networks as a discrete event system
1996	Solaiman and Picart	Biochemical metabolic modeling using fuzzy concepts.	Reder [1988] and Solaiman et al [1994]	Paper presents an improvised methodology (based on the previous works), enzymatic modeling approach using the concept of Fuzzy logic
1999	Koch et al	Simulation and analysis of metabolic networks by time-dependent petri nets.	Reddy et al [1993, 1996]	The new model created by the authors of this paper is an extended version of the model proposed by Reddy et al 1996 by also considering Reversible reaction and Time dependencies, thus integrating qualitative information with quantitative analysis.
2002	Chen et al	Petri net based modeling and simulation of metabolic networks in the cell.	None	It successfully integrated various biological data bases, by proposing common Bio XML standards. Thus enabling researchers to obtain metabolic data at a single platform thus improving portability of data files.
2002	Chen and Freier	The biology petri net markup language.	Reddy [1993], [1996] and Koch et al [1999]	First paper to propose and implement BioPNML, formulation of a standard interchange platform for Bioinformatics and Petri nets. Successfully bridge Petri net models to other metabolic network simulators.
2005	Kurata et al	Cadlive dynamic simulator: direct link of biochemical networks to dynamic models.	Mendes [1993], Kauffman [1993], Bhattacharjya et al [1996], Goryanin et al. [1999], Bhalla et al [1999], Chen et al [2000], Hasty et al [2001], Schoeberl, et al [2002] and Hatakeyama et al	One of the best computational tool enabling dynamic simulation from a biological map and will be a core system to combine large scale dynamic biological systems. The simulations being very close to real biochemical systems at finer levels of interaction.

			[2003]	
2005	Popova-Zeugmann, L et al	Time petri nets for modeling and analysis of biochemical networks.	Reddy [1993], Chen and Hofestadt [2003]	This paper is a solid reference on how to make use of qualitative analysis to model metabolic networks quantitatively. Thus it provides a basis in modeling using unbounded petri nets.
2005	Simao et al	Qualitative modeling of regulated metabolic pathways.	Reddy [1996] et al, Goss et al [1998], Santillán et al [2001], Xiu et al [2002], Bhartiya et al [2003], Zevedei-Oancea et al [2003]	In terms of standard PNs, the method provided a first step towards the integration of regulatory and metabolic processes into a coherent qualitative modeling framework.
2008	Hossain	Multi-agent based modeling and simulation of metabolic networks.	None	In the presence of contemporaries, agent based modeling is implemented for simulation of metabolic pathways that was able to provide a clear analysis of metabolism.
2011	Biba et al	Using machine learning techniques for modeling and simulation of metabolic networks	None	Unpredictable nature of biological systems was easily analyzed and simulated by integrating structural machine learning techniques with statistical data, providing a new break -through in the field of systems biology.

And going by the survey it can be said that (it depends on the reader) for modeling a complex biological network systems, petri nets (as a computational tool) is one of the better available and preferred solutions to solve the problem of modeling or simulation in the field of Systems Biology.

Future works based on the research works reviewed in this survey are as follows.
Reddy et al [1993] state that "The further possibilities Petri net modeling of metabolic networks would also encompass the identification and regulation of alternate routes of biotransformation, e.g. to overcome metabolic blocks due to defective enzymes, or to obtain greater yields in bioprocesses".
Solaiman et al [1996] state that "a promising approach using fuzzy concepts is proposed in modelling enzymatic reactions. In fact, the common use of the NM (sigmoidal transfer function) and QM (linguistic variables controlling the sigmoidal slant values) has permitted to resolve the innate difficulties:"
Chen et al [2002] state that "BioPNML serves as a starting point for the development of a standard interchange format for Petri nets and other molecular biological modeling and simulation tools."

Kurata et al [2005] state that "In the future, CADLIVE will be a core system to integrate various biochemical reactions into large-scale dynamic systems, and may construct virtual cells that produce all possible features of real biological systems at the molecular interaction levels."

Hossain [2008] state that the current agent based model can be improvised to directly suit System Biology Markup Language and there is a need for creating a new parser module "that can access online databases and convert them to a compatible framework for the agent."

In the future, availability of specific standardised formats and readily available quantitative data will increase the readability and reliability of biological databases. And thus improve the feasibility of semiautomatic translation of metabolic pathways to Petri nets. [Baldan et al 2010].

Biba et al [2011] state that by considering stoichiometric constraints to PRISM will generate a better simulation of the metabolic network and will enable to reconstruct large scale metabolic network systems.

5. ACKNOWLEDGEMENTS

Author is grateful to Dr. Richard Frost and Dr. Alioune Ngom department of Computer Science, University of Windsor for their valuable comments and suggestions.

Personally thank friends and family who constantly provided motivation.

6. REFERENCES

Bhattacharjya, A. and Liang, S. 1996. Power laws in some random Boolean networks. *Phys. Rev. Lett.* 77, 1644.

Bhalla, U. and Lyengar, R. 1999. Emergent properties of networks of biological signaling pathway. *Science,* 283,381-387.

Bhartiya,S. et al. 2003. Dynamic model of Escherichia coli tryptophan operon shows an optimal structural design. *Eur. J. Biochem*., 270, 2644-2651.

Baldan, P., Cocco, N., Marin, A., and Simeoni, M. 2010. Petri nets for modelling metabolic pathways: a survey. *Natural Computing,* 9, 4, 955-989.

Biba, M., Xhafa, F., Esposito, F., and Ferilli, S. 2011. Using machine learning techniques for modeling and simulation of metabolic networks. *International Conference on Complex, Intelligent and Software Intensive Systems,* 85-92.

Chen, K.C., Csikasz-Nagy, A., Gyorffy, B., Val, B. Novak, J., and Tyson, J.J. 2000. Kinetic analysis of a molecular model of the budding yeast cell cycle. *Mol. Biol. Cell,* 1, 369-391.

Christensen, B. and Nielsen, J. 2000. Metabolic network analysis. *Bio Analysis and Biosensors for Bioprocess Monitoring,* 209-231.

Chen, M. and Freier, A.2002. Petri net based modelling and simulation of metabolic networks in the cell. *In Bioinformatics Research and Education Workshop*, 1-5.

Chen, M., Freier, A., Kohler, J., and Ruegg, A.2002. The biology petri net markup language. *Lecture Notes in Informatics: Proceedings of Promise2002, Desel J. et al*, 150-161.

Chen, M. and Hofestadt, R.2003. Quantitative petri net model of gene regulated metabolic networks in the cell. *In Silico Biology* 3, 3, 347-365.

Chen, L., Wang, R., and Zhang, X.2009.Biomolecular networks: methods and applications in systems biology.10. *John Wiley & Sons Inc.*, 281-300.

Goss, P.J.E and Peccoud, J. 1998. Quantitative modeling of stochastic systems in molecular biology by using stochastic petri nets. *Proc. Natl. Acad. Sci. USA.*, 95,6750-6755.

Goryanin, I., Hodgman, T.C and Selkov, E.1999. Mathematical simulation and analysis of cellular metabolism and regulation. *Bioinformatics* 15, 749 –758.

Goel, G., Chou, I., Voit, E., et al. 2008. System estimation from metabolic time-series data. *Bioinformatics*, 24, 21, 2505.

Hasty, J., McMillen, D., Isaacs, F., and Collins, J. 2001. Computational studies of gene regulatory networks: *In numero molecular biology. Nat. Rev. Genet*.2: 268 –279.

Hatakeyama, M., Kimura, S., Naka, T., Kawasaki, T., Yumoto, N., Ichikawa, M., Kim, J.H., Saito, K., Saeki, M., Shirouzu, M., et al. 2003. A computational model on the modulation of mitogen-activated protein kinase (MAPK) and Akt pathways in heregulin-induced ErbB signalling. *Biochem. J.* 373,451 –463.

Hossain, M. I. 2008. Multi-agent based modeling and simulation of metabolic networks. *A Thesis submitted for the degree of Master of Engineering, Department of Chemical and Bimolecular Engineering, National University of Singapore*, 1-102.

Kauffman, S.A. 1993. The origin of order, self-organization and selection in evolution. *Oxford University Press.*

Koch, I., Schuster, S., and Heiner, M.1999. Simulation and analysis of metabolic networks by time-dependent petri nets. *InComputer Science and Biology-Proceedings of the German Conference on Bioinformatics, ISBN*. 1-3.

Kurata, H., Masaki, K., Sumida, Y., and Iwasaki, R. 2005. Cadlive dynamic simulator: direct link of biochemical networks to dynamic models. *Genome research*, 15, 4, 590-600.

Lee, J., Gianchandani, E., and Papin, J.2006. Flux balance analysis in the era of metabolomics. *Briefings in Bioinformatics*, 7, 2, 140-150.

Llaneras, F. and Pico, J.2008. Stoichiometric modelling of cell metabolism. *Journal of bio-science and bioengineering*, 105, 1-11.

Mendes, P. 1993. GEPASI: A software package for modeling the dynamics, steady state and control of biochemical and other systems. *Comput. Appl. Biosci*. 9, 563–571.

Popova-Zeugmann, L., Heiner, M., and Koch, I.2005. Time petri nets for modelling and analysis of biochemical networks. *Fundamenta Informaticae* 67, 1-3, 149-162.

Qeli, E. 2007. Information visualization techniques for metabolic engineering. *Ph.D. thesis, University atsbibliothek Marburg*.

Reder, C. 1988. Metabolic control theory, *Journal of Theoretical Biology*, 135, 175-201.

Reddy, V., Mavrovouniotis, M., Liebman, M., et al. 1993. Petri net representations in metabolic pathways. *InProc Int Conf Intell Syst Mol Biol. Vol. 1*. 96038982. 328-336.

Reddy, VN et al. 1996. Qualitative analysis of biochemical reaction systems. Computu. *Biol. Med.*, 26, 9-24.

Raman, K. and Chandra, N. 2009. Flux balance analysis of biological systems: applications and challenges. *Briefings in bioinformatics* 10, 4, 435-449.

Solaiman and Picart, D. 1994. Neural networks modelling of biochemical reactions, *IEEE International Conference on Neural Networks, ICNN94, June 26-Jul 2, Orlando, USA.* 3348-3351.

Solaiman, B. and Picart, D. 1996. Biochemical metabolic modeling using fuzzy concepts. *In Engineering in Medicine and Biology Society, Bridging Disciplines for Biomedicine. Proceedings of the 18th Annual International Conference of the IEEE. Vol. 5. IEEE*, 1895-1896.

Santillan, M. and Mackey, MC. 2001. Dynamic regulation of the tryptophan operon: a modeling study and comparison with experimental data, *Proc. Natl. Sci. USA*, 98, 1364-1369.

Schoeberl, B., Eichler-Jonsson, C., Gilles, E.D., and Muller, G. 2002. Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors. *Nat. Biotechnol*. 20, 370 –375.

Simao, E., Remy, E., Thieffry, D., and Chaouiya, C. 2005. Qualitative modelling of regulated metabolic pathways: application to the tryptophan biosynthesis in e. coli. *Bioinformatics 21, Suppl* 2, 190-196.

Sauro, H., Harel, D., Uhrmacher, A., Hucka, M., Kwiatkowska, M., Shaffer, C., Mendes, P,Stromback, L., and Tyson, J. 2006. Challenges for modeling and simulation methods in systems biology. *In Proceedings of the Winter Simulation Conference. WSC. IEEE*, 1720-1730.

Tenazinha, N. and Vinga, S. 2011. A survey on methods for modeling and analyzing integrated biological networks. *IEEE/ACM Transactions on Computational Biology and Bioinformatics (TCBB)* 8, 4, 943-958.

Xiu, ZL et al. 2002. Nonlinear dynamics of regulation of bacterial trp operon: model analysis of integrated effects of repression, feedback inhibition and attenuation. *Biotechnol, Prog,* 18,686-693.

Zevedei-Oancea, I., Schuster, S., et al. 2003. Topological analysis of metabolic networks based on petri net theory. *In silico biology* 3, 3, 323-345.