Software tools for structure analysis of biochemical networks

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SOFTWARE TOOLS FOR STRUCTURE ANALYSIS OF BIOCHEMICAL NETWORKS

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Abstract

In the cell, tissue, organ and organism under different conditions operate metabolic, gene regulation and signaling networks that determine biochemical reactions, biochemical or biophysical process.
Biochemical networks can present the relationships between genes and genes products, between proteins and etc.. Exploring of these networks helps better understand some cellular process or properties of biological system. Therefore, there is a growing need for software tools that allow simulation and modeling.
There are many network visualization, simulation and analysis tools, which play key role in systems biology. Each of these tools is devised for some specific need, for example, representation of cellular state, dynamic of cellular process, time-dependent behavior, etc..
We provide researchers and coterie information on existing tools with goals: 1) to describe briefly existing tools for data visualization, simulation or analysis of different network types; 2) to make easy selection of tool corresponding to research problem and requirements. In this manuscript we focus on tools which include topological analysis of network structure.
Within this manuscript we examine 20 existing tools. 18 tools of them are freely available for academic use and 12 provide source code. During this work we did not found a tool which provides imitation/modeling of evolution. In accordance with main comparison parameter – structure analysis - we prefer VisANT, Cytoscape with BiNoM and NetworkAnalyzer plugins, Biological Networks and CelNetAnalyser.

Types of biochemical networks

From the viewpoint of network architecture, main ingredients are molecules, interactions, pathways and networks. A cellular system can be viewed to be formed conceptually from individual molecules, to pairwaise interactions, to local structures (including network motifs, molecules, pathways, subnetworks) and eventually to global biomolecular networks (Zhang, 2009). Biomolecular networks allow visualizing and describing of intracellular molecular interactions of cellular system by using available metabolic and gene regulation experimental data (Zhang, 2009), as well as representation of many biological processes such as metabolism, gene regulation, signal transduction (Zhenjun Hu et al, 2005). In terms of interactions, each type of biomolecular network is assembled by the following different pairwise interactions: *transcription regulatory network*: TF-DNA interactions; *gene regulatory networks*: gene-gene interactions (genetic interactions); *protein interaction network*: protein-protein interactions; *metabolic networks*: enzyme-substrate interactions; *signaling networks*: molecule-molecule interactions (Zhang, 2009).
Component data such as genomic and proteomic data provide a specific molecular content of a cellular system. Also dependent on omic data 4 network types can be defined (Zhang, 2009):

- Transcriptiomic data - Transcription regulatory networks
- Proteomic data – Protein Interaction network
- Metabolic data – Metabolic network
- Integrated data - Signaling network

The goals of systems biology are to understand the mechanisms of how biochemical networks generate particular cellular functions in response to environmental stresses or genetic changes. To design biological systems at the molecular interaction level, it is essential to identify a biochemical network map, to build a dynamic model of the system, and to perform system analysis (Nishio et al, 2008).

Modeling and types of models

The main goal of modeling is to supplement researchers understanding of biological system properties (local, global) or behavior corresponding to different perturbations. We briefly characterize here two types of computational models: kinetic (dynamic) and structural models, as well goals of these modeling.

Kinetic models

Cellular systems are commonly modeled by nonlinear dynamical systems such as ordinary differential equations or stochastic processes such as the chemical master equation, based on mass action low and enzyme reaction kinetics.

The term „kinetic model" is used in dual sense. In the biological sense, it is a network of interactions between biological entities. In the mathematical sense, "kinetic" refers to a system of mechanistic ordinary differential equations that determine the temporal state of the corresponding system of biochemical reaction. There are two types of kinetic models that are devised to present system dynamics: a classical chemical kinetic model and stochastic chemical kinetic model. A **classical chemical kinetics (CCK) model** composed of *n* chemical species and *m* chemical reaction channels. CCK model utilizes ordinary differential equations to present system dynamics (Myers, 2010).
Dynamic simulations of a cellular system can provide a more thorough quantitative understanding of cellular system principles, mechanism and function (Zhang, 2009). Simulation results allows researchers to test their understanding, to explore „what-if" scenarios (Alberghina and Westerhoff, 2005), make predictions about the system for situations that have not yet been studied and make the design of new synthetic biological systems (Myers, 2010).

Structural (topological) models

An important class of methods in Systems Biology deals with structural or topological (parameter-free) analysis of cellular networks (Klamt et al, 2007). This is a type of model that is used for graphical representation and topological analysis of biological networks.
In structural or topological (parameter-free) models networks are represented in graph form [Klamt et al, 2007, Zhenjun Hu et al, 2005, Zhenjun Hu et al, 2007, Klamt et al, 2006, Zinovyev et al, 2008) (directed, undirected (Klamt et al, 2007) and mixed (Zhenjun Hu et al, 2005)), that consist of nodes and edges. Nodes dependent of number of connections and type of graph can be hubs (highly connected nodes) and metanodes which contains all its descendents and genes annotated directly under it (Zhenjun Hu et al, 2009). Nodes represent genes, gene products, proteins, chemical compounds or small molecules and links (edges) represent various types of interactions or associations between pair of nodes, e.g. metabolic events, protein/protein-nucleotide interactions, regulatory relationships or signaling pathways (Suderman and Hallett, 2007, Zhenjun Hu et al, 2007). Connections can be directed or undirected; they can have physical meaning, denote general associations; they can represent shared characteristics between components (Zhenjun Hu et al, 2005).
Choice of network representation is often dictated by the research problem. Directed networks are suitable when the interactions between two components have a well-defined direction, for example, the direction of metabolic flow from substrates to products, or the information flow from transcription factors to the genes that they regulate. Undirected networks, such as protein interaction networks, represent mutual relationships: if protein A binds to protein B, then protein B binds to protein A. This type of representation also often applies to predictions made by high-throughput proteomic or genomic analysis, or indirect links based on shared genes or protein components between pathways and complexes (Zhenjun Hu et al, 2005).
To represent and analyze networks developers may use different formalisms, for example, CellNetAnalyser use interaction graphs and (logical) interaction hypergraphs (Klamt et al, 2006), but VisAnt use metagraph to (Zhenjun Hu et al, 2009).
Structural analysis of cellular interaction networks contributes to a deeper understanding of network-wide interdependencies, causal relationships, and basic functional capabilities. Structural analysis, towards a functional analysis of the structure is not based on quantitative and dynamic properties and can thus only provide qualitative answers (Klamt et al, 2006). However, some insights into the dynamic properties can nevertheless often be obtained, because fundamental properties of the dynamic behavior are often governed by the network structure (Klamt et al, 2006).
The functions of biomolecular networks are closely related to their topologies and facilitated by characteristic topological patterns. Components of cellular networks including genes, proteins, and other molecules usually act in collaboration to carry out specific biological processes and biochemical activities, by forming relatively isolated functional units called modules. From the topological perspective, a model can be understood as a subnetwork that is densely connected within itself but sparsely connected with the rest of the network. In cellular networks, a module refers to a group of physically or functionally connected biomolecules that work together to achieve some desired cellular function (Luonan Chen et al, 2009).
Structural models characterize and provide information of the connectivity (topology) of the interactions involved in a biological process. Identifying topological features in networks is an important part of understanding the relationship between structure and function of network motifs, e.g. feedback, feed-forward loops (Zhenjun Hu et al, 2005). In biomolecular networks are motifs such as feedback loops and single input motifs, autoregulation loops in transcriptional regulatory networks, and short cycles in protein interaction networks (Luonan Chen et al, 2009). A network motif is a subunit of a complex network that appears much more frequently in the given network than expected by chance alone. Such subnetworks are considered to be basic building blocks of many real complex networks.

Software tools, that represent biochemical network structure

There are many network visualization, simulation and analysis tools, which play key role in systems biology and each meets some specific need. Within this manuscript we consider 20 existing tools (see Table 1). Most available tools are suited to biological network visualization and use definitions, methods and algorithms (Suderman and Hallett, 2007) from graph and control theory. Some of these tools analyze network structure with help of a number of graph structural analysis methods. We discuss here the main differences and similarities of these tools.

Software tools developed for Systems Biology depending on functionality and field of use can be divided in three main groups:

(1) tools for modeling, simulation and/or analyses metabolic networks (biochemical reaction or process networks) allowing definition of a system like a collection of connected chemical reactions and the boundary of the system where the set of reactions intersects with its environment,
(2) tools for modeling and analyses genetic regulatory networks,
(3) tools for visualization and analyses of structure topology.

The list of groups can be extended with other groups, for example, tools for network drawing for presentations, publications, tools for modeling Boolean or logical networks etc.

During software tool estimation, we establish that 8 of examined tools provide modeling of metabolic networks, 4 tools are suitable for modeling gene regulatory networks, 6 tools allow visualization and analysis of network structure (see Figure 1).

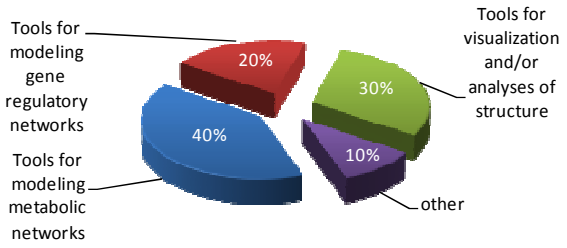

Fig.1. Tools classification and percentage distribution by functionality and field of usage

Tools that contain model simulation are designed for modeling and analysis of dynamic system.

Dependent on usage permissions and license type, software tools can divide in several groups:

1) License for free academic use in accordance with license terms
2) Free software license
3) Open source license
4) Commercial license

Within this manuscript we establish that 18 of examined 20 tools are freely available for academic use. In all 8 tools are commercial product and 5 of them are freely available for academic use. 12 tools provide source code (see Figure 2).

Depending on built-in function are software tools that support SBML format (allows to import/export models or only export in SBML format) and conversely do not have this function (see Figure 3, 4).

SBML is System Biology Markup Language standard for representing models of biochemical and gene-regulatory networks (Funahashi et al, 2008, Hucka et al, 2008). It is a machine-readable model definition language based upon XML. SBML can encode models consisting of biochemical entities linked by reactions to form biochemical networks (Wanner et al., 2005).

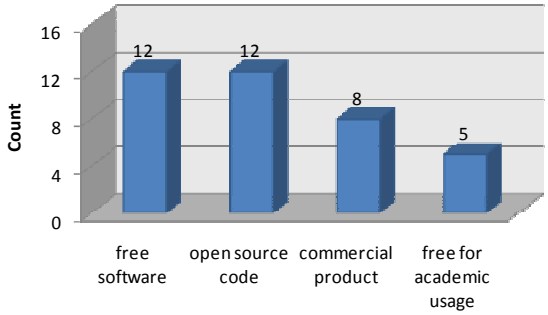

Fig.2. Tools rating by type of license and use permisions

SBML is developed in levels and represent substantial changes to the composition and structure of the language. Level 2 represents an evolution of the language. Minor versions of SBML are termed versions and constitute changes within the Level to correct, adjust and refine language features (Hucka et al, 2008). SBML 1 Level has 2 versions and 2 Level now has 4 versions. One must note that SBML Levels are not linked. If user wants to open the model (saved in SBML Level 1 Version 2 format) on tool that supports SBML Level 2 Version 4, it will not succeed. SBML developers have not provided inheritance between SBML Levels and Versions.
By supporting SBML as a format for reading and writing models, different software tools (including programs for building and editing models, simulation programs, databases, and other systems) can directly communicate and store the same computable representation of those models, removing opportunities for translation errors and assuring a common starting point for analyses and simulations (Hucka et al, 2008). Summary on SBML supporting software can be found at www.sbml.org.

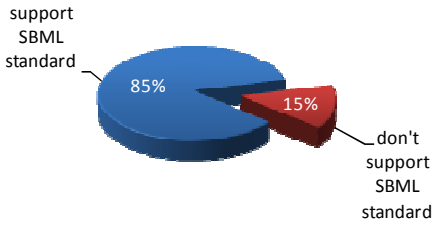

Fig.3. Tools rating by supporting of SBML standart

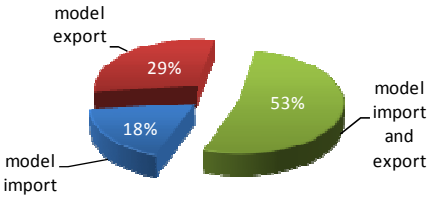

Fig.4. Supported functions of SBML standart

Depending on built-in function are software tools that support SBGN format (allows to import/export or only export models in SBGN format) and conversely do not have this option.
SBGN (Systems Biology Graphical Notation) is a visual language developed by a community of biochemists, modelers and computer scientists that consists of three complementary languages: process diagram, entity relationship diagram and activity flow diagram. Together they enable scientists to represent networks of biochemical interactions in a standard, unambiguous way. A process diagram represents all the molecular processes and interactions taking place between biochemical entities, and their results. The SBGN notation for entity relationships puts the emphasis on the influences that entities have upon each other's transformations rather than the transformations themselves. SBGN activity flow diagram permit modulatory arcs to directly link different activities, rather than entities and processes or relationships as described previously. SBGN can foster efficient and accurate representation, visualization, storage, exchange and reuse of information on all kinds of biological knowledge, from gene regulation, to metabolism, to cellular signaling (Le Novère et al, 2009).

Table 1.

Summary of software tools that was examined, their main goal and functions

Group	Software tool	Main goal	Functions
Tools for modeling metabolic networks or chemical reaction networks	CellDesigner	Tool for kinetic modeling of biochemical networks	CellDesigner includes building, visualizing, and kinetic modeling of biochemical networks (chemical reactions and processes); browse and modify existing SBML models with references to existing databases, simulate and view the dynamics.
	JDesigner	Tool for visual simulation biochemical reaction networks	Tool includes kinetic modeling of biochemical networks, permit users to graphically specify the model, derive the set of differential equations automatically and generate a solution. It allows selecting the appropriate kinetic laws from a wide selection of inbuilt rate laws or defining new user defined rate laws. The graph shows how changes for ex. concentration all nodes in time.
	Pathway analyser		Tool for analysis of metabolic pathways, particularly by flux based analyses and simulations on SBML Models. [http://pathwayanalyser.sourceforge.net]
	PathwayLab	Tool for visualization, documentation and in silico analysis of biochemical pathways	PathwayLab allows to create and store new reaction objects with user specified kinetics, including efficient transient analysis (simulation) and Metabolic Control Analysis (MCA), dynamic biochemical simulation, robust steady-state analysis. This tool allows to create and layout pathways drawings. [http://www.innetics.com/]
	Cobra	COnstraint-Based Reconstruction and Analysis Toolbox for Matlab	Cobra includes implementations of many of the commonly used forms of constraint-based analysis such as FBA, gene deletions, flux variability analysis, sampling, and batch simulations together with tools to read in and manipulate constraint-based models [*].
	Pathway Tools	Development of organism-specific databases (also called model-organism databases) that integrate many bioinformatics datatypes, from genomes to pathways.	Tool includes scientific visualization, web publishing, and dissemination of those organism-specific databases, visual analysis of omics datasets. Computational inferences including prediction of metabolic pathways, prediction of metabolic pathway hole fillers, and prediction of operons, that can be used for genome analysis. Analysis of biological networks: 1) Interactively tracing metabolites through the metabolic network; 2) Finding dead-end metabolites in metabolic networks; 3) identifying choke points (potential drug targets) in metabolic networks. [http://bioinformatics.ai.sri.com/ptools/ptools-overview.html]
	GEPASI	Gepasi is a software package for modeling biochemical systems	It simulates the kinetics of systems of biochemical reactions and provides a number of tools to fit models to data, optimize any function of the model, perform metabolic control analysis and linear stability analysis. Gepasi's *scan* utility provides a way for advanced exploration of a model's behaviour in multi-dimensional parameter space, is capable of doing data fitting (parameter estimation) with experimental data, of finding maxima or minima of any model variables with any number of adjustable model parameters. The results of simulations can be plotted in 2D and 3D directly from the program
	COPASI (Complex Pathway Simulator)	Software application for simulation and analysis of biochemical networks	Copasi supports chemical reaction network; arbitrary kinetic functions; ODEs for compartments, species, and global quantities, assignments for compartments, species, and global quantities; initial assignments for compartments, species, and global quantities. It includes: stochastic and deterministic time course simulation; steady state analysis (including stability); metabolic control analysis/sensitivity analysis; elementary mode analysis; mass conservation analysis; time scale separation analysis; calculation of Lyapunov exponents; parameter scans; optimization of arbitrary objective functions; parameter estimation using data from time course and/or steady state experiments simultaneously.
Tools for modeling gene regulatory networks	BioTapestry	Tool for simulating genetic regulatory networks	BioTapestry includes building, visualizing, and simulating genetic regulatory networks; creation dynamic submodels; dynamic presentation (graph) of the network behavior in time.
	GenMapp	Tool for visualization gene expression data on maps representing biological pathways and groupings of genes.	GennMap focuses on annotated pathways, using simple graphics and labels to provide biological context to molecular objects Tool allows visualizing an expression data along pathways (called mapps), creating new mapps, identifying global biological associations within an expression dataset. MAPPFinder Integrated with GenMAPP is to perform a global analysis of gene expression or genomic data in the context of hundreds of pathway MAPPs and thousands of Gene Ontology Terms.

Deleted:

Group	Software tool	Main goal	Functions
	NetBuilder	Tool for modeling and simulation environment of genetic regulatory networks	NetBuilder allows users to create a picture of the (known) components and interactions in the system, and enter quantitative information, such as know or estimated quantities and rates. creating and manipulating the mathematical representations they need to predict the behaviour of their systems. NetBuilder' has simulators to carry out stochastic or deterministic numerical integration on the basis of a given initial state and the expressions for the flux that are constructed (by hidden functions in NBMathModel) on the basis of the model parameters.
	Genetic Network Analyzer	Tool for the modeling and simulation of genetic regulatory networks	GNA consists of a simulator of qualitative models of genetic regulatory networks in the form of piecewise-linear differential equations. Instead of exact numerical values for the parameters, which are often not available for networks of biological interest, the user of GNA specifies inequality constraints. This information is sufficient to generate a state transition graph that describes the qualitative dynamics of the network.
Tools for visualisation and analyses of network structure	Cytoscape	Tool for visually exploring biological networks	Tool for visualizing molecular interaction networks and integrating these interactions with gene expression profiles and other state data which includes complex network analysis and visualization. Cytoscape have a powerful Visual Styles (3D).
	Visant	An integrative software platform for the visualization, mining, analysis and modeling of the biological networks	Tool for creating multi-scale networks, representing many types of biological data, such as biomolecular interactions, cellular pathways and functional modules; provides: a visual interface for combining and annotating network data, supporting function and annotation data for different genomes from the Gene Ontology and KEGG databases and the statistical and analytical tools needed for extracting topological properties of the user-defined networks. Tool introduces extensive functionalities to visualize and integrate the gene ontology with biological networks using metagraph technology.
	Edinburgh Pathway Editor	Tool designed for annotation, visualization and presentation of wide variety of biological networks, including metabolic, genetic and signal transduction pathways.	It allows visual representation to field standards (SBGN), storage and retrieval of annotation such as kinetic and other numerical data in relational databases (local and remote for enterprise development) and links graphical objects to external databases and web resources to show all available information on demand.
	Biological Networks	Application for visualization and analysis of biological pathways	Biological Networks is a graph-based system for creating a combined database of biological pathways, gene regulatory networks and protein interaction maps. After importing expression data, users can apply sorting, normalization and clustering algorithms on the data and then create various tables, heat maps and network views of the data.
	BiNoM (Biological Network Manager)	BiNoM is a Cytoscape plugin, developed to facilitate the manipulation of biological networks	BiNoM include structural analysis of the networks (strongly connected components, path and cycle analysis, network clustering, etc.) and support of generating network modular view.
	NetworkAnalyser	This tools is a Cytoscape plugin, developed to analyze biological and other networks	Networkanalyser computes and displays a large number of simple and complex topological network parameters for directed and undirected networks loaded into Cytoscape using efficient graph algorithms, for example, the number of nodes, edges, and connected components, the network diameter, radius, density, centralization, heterogeneity, and clustering coefficient, the characteristic path length, and the distributions of node degrees, neighborhood connectivities, average clustering coefficients, and shortest path lengths.
	CellNetAnalyzer	CNA is a package for MATLAB and provides a environment for structural and functional analysis of biochemical networks	CNA facilitates the analysis of metabolic (stoichiometric) as well as signaling and regulatory networks solely on their network topology, i.e. independent of kinetic mechanisms and parameters. CNA includes Metabolic flux analysis, Analysis of basic topological / structural properties, Metabolic pathway analysis and for Signal flow (signaling, regulatory) networks: Analysis of interaction graphs, Analysis of logical (Boolean) interaction networks.
	Pathway Builder	Tool for drawing signal transduction pathways for presentation, publications,	Pathway Builder contains pathway templates and illustration items. It allows you to make web pages from the pathways images of the Pathway Builder tool. [http://avaxhome.ws/software/software_type/scientific/Medical/pathway_builder.html]

Group	Software tool	Main goal	Functions
		posters, etc.	
	SBMLToolBox	SBMLToolbox provides functions for reading, writing, and validating SBML models.	SBMLToolbox provides functions for reading, writing, and validating SBML models; viewing model structures in a simple GUI; converting models into a symbolic form suitable for use with MATLAB's Symbolic Math Toolbox; and simulate models using MATLAB's ordinary differential equation solver.

Next Table 2 summarizes properties and facilities of examined software tools.

Table 2.

Summary of examined software tools properties and facilities

Nr.	Software tool	Licence (free software, open source, free use, comercial)	Base	OS	Model creation	Model simulation	Model creation or simulation using differential	Model analysis	Topological analysis	Support of SBML standart (Import/Eksport)	Support of SBGN standart	Data (Gene data, Pathways) or model import form public available DataBases
1	Cytoscape [37,5, 36]	(LGPL license) open source	Java	L, W, M	**Y**				**Y**	I (sif, gml, XDMML, xls) /E		KEGG, Pathway Commons, IntAct, BioMart, NCBI Entrez Gene,PICR
2	Visant [37, 39, 40]	Free and open source code	Java	L, W, M	**Y**	**Y**		**Y**	**Y**			GenBank, KEGG, SwissProt
3	BioTapestry [25]	Free and open source code	Java	L, W, M	**Y**	**Y**	**Y**			E		
4	CellDesigner [12-15]	free use license, open source code	-	L, W, M	**Y**	**Y**	**Y**			I/E	**Y**	KEGG, SGD, DBGET, IHOP, Genom Network Platform, PUBMED, Entrez Gene
5	JDesigner [17]	LGPL license Free and open source code	Win32 appl.	W	**Y**	**Y**	**Y**	**Y**		I/E		
6	Edinburgh Pathway Editor [27]	non-commercial License	Java	L, W, M	**Y**		**Y**			E	**Y**	
,7	GenMapp [4,6-8,9,16]	Open source	-		**Y**	**Y**						Swiss-Prot, Entrez Gene, PubMed, Unigene, UniProt, Ensembl
8	NetBuilder [31]	free and open source code	C++ 6.0	L, W, M						E	**Y**	
9	Pathway analyser	free and open source code	C++	L		**Y**	**Y**	**Y**		I		
10	Biological Networks [37]	Free for academic use, commercial	Java		**Y**			**Y**	**Y**	I		Pathway import: KEGG, BIND, GO
11	PathwayLab	commercial		W	**Y**	**Y**	**Y**	**Y**		E	**Y**	
12	Pathway Tools	free for academic use, commercial						**Y**		E		
13	Pathway Builder	free and open source		L, W, M	**Y**					I		
14	BiNoM [43,44]	free and open source code		L, W, M	**Y**				**Y**	I/E (BioPAX, Cell-Designer)	**Y**	
15	CellNet-Analyser [10]	free for academic use, commercial		L, W, M				**Y**	**Y**	I/E		
16	Cobra [11]	Free and open source code		L, W, M				**Y**		I/E		
17	Network-	free open-source	Java	L,					**Y**			

Nr.	Software tool	Licence (free software, open source, free use, comercial)	Base	OS	Model creation	Model simulation	Model creation or simulation using differential	Model analysis	Topological analysis	Support of SBML standart (Import/Eksport)	Support of SBGN standart	Data (Gene data, Pathways) or model import form public available DataBases
	Analyser [1]			W, M								
18	GEPASI [28]	free		W	**Y**	**Y**	**Y**	**Y**		I/E (L1)		
19	Genetic Network Analyzer [10]	Free for academic use, commercial	Java	L, W, M	**Y**	**Y**	**Y**	**Y**		I/E		
20	COPASI [18]	Open source code, free for academic use, commercial	C++, Java	L, W, M	**Y**	**Y**	**Y**	**Y**		I (L1V1-2,L2V1-3) /E (L1V2, L2V1-3)		

* OS is Operating System that can be Linux (L), Windows (W) and MacOS (M).

There are many public available databases like KEGG (Kanehisa et al, 2006), GO (Raychaudhuri et al, 2002), GenBank (Benson et al, 2008), etc., that store and share experimental information. There are software tools (see Table 2.) that allow data import from these databases and data connection with set model.

Software tools of structure analysis

Here we describe three software tools that analyze network structure. During survey of these tools we have found 22 structure topological parameters and features which are described here to.

VisAnt

VisANT is a web-based software framework (Java application) for visualizing and analyzing many types of networks of biological interactions and associations, as well as an especially useful tool for integrating information from a wide variety of sources. VisAnt is freely available. VisANT provides (Zhenjun Hu et al, 2005):

(1) a visual interface for combining and annotating network data.
(2) supporting annotation data for different genomes from the Gene Ontology. GO terms can be easily dropped into the network to group genes annotated under the term, thereby integrating the hierarchical ontology with the network (Zhenjun Hu et al, 2009). GO is gene ontology, wich provides hierarchically organized information about gene products, their activity, biological functions and cellular location (Zhenjun Hu et al, 2009).
(3) visualization, mining, analysis and modeling of the biological networks, which extend the application of GO (Zhenjun Hu et al, 2009).
(4) supporting exploratory pathway analysis using metagraphs (type of graph), which includes multi-scale visualization of multiple pathways, editing and annotating pathways using a KEGG compatible visual notation and visualization of expression data in the context of pathways (Zhenjun Hu et al, 2007).
(5) the statistical and analytical tools needed for extracting topological properties of the user-defined networks.

VisAnt provide functions to annotate genes at any customized level of abstraction (Zhenjun Hu et al, 2009).

VisAnt is capable of easily exploring pathways at different scales: a pathway overview enables user to observe the topology of large sets of pathways, while the detailed internal structure of any particular pathways or set of pathways is easily revealed by mouse-clicking (Zhenjun Hu et al, 2007).

VisANT explicitly allows creation of mixed networks involving different types (Zhenjun Hu et al, 2005), i.e. networks containing both directed and undirected links (Suderman and Hallett, 2007). Networks can also be analyzed for topological characteristics to identify larger global properties, such as degree distribution, path length, shortest path and clustering coefficient calculations (Suderman and Hallett, 2007, Zhenjun Hu et al, 2007), connections finding between a given set of genes/proteins.

CellNetAnalyser

CellNetAnalyser is a package for MATLAB and provides a comprehensive and user-friendly environment for structural and functional analysis of metabolic (stoichiometric) as well as signaling and regulatory networks [CellNetAnalyser Manual]. CAN allows to analyze mass-flow (i.e. stoichiometric or metabolic) networks, signal-flow (signaling and regulatory) networks and stoichiometric networks.
The particular strengths of CellNetAnalyzer are methods for functional network analysis, i.e. for characterising functional states, for detecting functional dependencies, for identifying intervention strategies, or for giving qualitative predictions on the effects of perturbations (Klamt et al, 2007).
CNA provides a powerfull battery of methods for metabolic and stoichiomic network analysis:

(1) Metabolic flux analysis
   - classification of flux scenarios (determinacy and redundancy) and rates (balanceability and calculability)
   - calculation of flux distributions (for all types of flux scenarios)
   - consistency checks in redundant systems
   - flux optimization subject to an arbitrary linear objective function (flux balance analysis, FBA)
   - sensitivity analysis of calculated rates
   - feasibility check of a given scenario

(2) Analysis of basic topological / structural properties
   - graphical display of the stoichiometric matrix
   - computation of graph-theoretical path lengths in an directed and undirected graph representation of the reaction network; determination of network diameter
   - detection of (elementary) conservation relations, enzyme subsets, isozymes, blocked and parallel reactions

CAN provides a algorithms for a functional analysis of signal-flow networks - Analysis of interaction graphs

- basic topological properties
- large-scale computation of all positive and negative signaling paths connecting inputs with outputs or of all signaling paths between a given pair of nodes; statistical analysis of these paths
- large-scale computation of all positive and negative feedback loops; statistical analysis of these routes
- computation of minimal cut sets (removing reactions or species) for a given set of paths or/and loops
- computation of distance (shortest paths) matrices; separately for positive and negative paths
- large-scale dependency analysis (which species has (positive/negative) influence on which species; identification of activators and inhibitors of a given species enabling predictions on perturbation experiments)connectivity histogram.

CellNetAnalyzer support calculations of global topological properties of the network. It is useful to detect errors in the network structure after the network has been composed (Klamt et al, 2007, Klamt et al, 2006).
CNA is programmed with MATLAB under LINUX as operating system and it is a commercial product.

Cytoscape with BiNoM AND NETWORKANALYSER

Cytoscape is a complex network visualization and analysis tool supporting a core set of features including standarts and customizable network display styles, ability to import a large variety of interaction files, and zoomable network views (Suderman and Hallett, 2007). Cytoscape is suitable for analyzing molecular interaction networks and biological pathways and integrating these networks with annotations, gene expression profiles and other state data. Cytoscape specializes in the representation of interaction networks. Automatic layout algorithms help to organize massive amounts of interaction data relating to a set of molecules (in orange). Cytoscape include filter of the network to select subsets of nodes and/or interactions based on the current data, allows to find active subnetworks/pathway modules and find clusters (highly interconnected regions) (Cline et al, 2007).
BiNoM (BIological NetwOrk Manager) is a Cytoscape plugin, developed to facilitate the manipulation of biological networks represented in standard systems biology formats (SBML, SBGN, BioPAX) and to carry out studies on the network structure. Binom allows the analysis of networks created with CellDesigner software and their conversion in BioPAX format. BiNoM proposes to generate three standard interfaces, during BioPAX import operations: reaction network, pathway structure and protein-protein interaction (Zinovyev and Calzone).
BiNoM provides the user with a complete interface for the analysis of biological networks in Cytoscape environment, for example, structural analysis of the networks (strongly connected components, path and cycle

analysis, network clustering, etc.). BiNoM provide simplification and analysis of the network representation are achieved by use of the build-in library of graph analysis tools, including:

- analysis of connected and strongly connected components,
- path analysis (finding shortest, suboptimal, all paths),
- modular decomposition of the network using node semantics, cycle analysis, subnetwork clustering, decomposition of network into modules and clipboard operations (Zinovyev et al, 2008).

Cytoscape is Java application whose source code is released under the Lesser General License (Suderman and Hallett, 2007). This system has plugins (Additional features) that can construct any developers.

NetworkAnalyzer is a software tool for the analysis of biological and other networks. This is the versatile Cytoscape plug-in that computes a comprehensive list of simple and complex topology parameters (single values and distributions) for directed and undirected networks using efficient graph algorithms. Simple parameters are the number of nodes, edges, self-loops, and connected components, the average number of neighbors, the network diameter, radius, density, centralization, hetero-
geneity, and clustering coefficient, the number of shortest paths, and the characteristic path length. Complex parameters are distributions of node degrees, neighborhood connectivities, average clustering coefficients, topological coefficients, shortest path lengths, and shared neighbors of two nodes. Network-Analyzer displays the distributions as histograms or scatters plots and allow export them as chart images in the formats JPG/PNG/SVG or as tables in plain text files (Assenov et al., 2008).

Biological Networks

BiologicalNetworks is a software platform for biological pathways analysis, querying and visualization of gene regulation and protein interaction networks, metabolic and signaling pathways, and is an information management framework over PathSys. This software integrates over 20 curated and publicly contributed data sources, biological experimental and PubMed data for the 8 representative genomes (*S. cerevisiae*, *D.melanogaster*, etc.). BiologicalNetworks is supported with curated pathways from a number of public databases like KEGG. BiologicalNetworks software is available at http://biologicalnetworks.net/index.php and it is a free Integrated Research Environment for biologists.

In BiologicalNetworks is developed a preliminary standard for exchanging files that have visual markup and annotation of network layouts. Users of BiologicalNetworks can input several basic data types, including data in standardized network and interaction data exchange formats, such as PSI-MI, BioPAX and SBML (Baitaluk et al., 2006).

Biological networks include following main functions: Data integration, Data representation, Data analysis, Microarray data analysis (Baitaluk et al., 2006).

Once a network dataset has been imported or loaded into BiologicalNetworks, the genes or proteins within it can be queried for other known and predicted interactions from the PathSys's database. To enable data analysis, the following tools are available (Baitaluk et al., 2006):

1) Import and export user data
2) Create, save, edit user pathways
3) Optimize the view by filtering, pathway expansion, and protein classification.
4) Perform graphic drawing and layout optimization
5) Search: find and display a list of objects based on a name or a keyword.
6) Expand: searches the database and displays objects functionally linked to a selected node or a set of nodes. Thus, by alternating expand and filtering options, users can browse through the database building their favorite pathways.
7) Build pathways: finds a set of links between two or more nodes by searching for the shortest path in the total network of all links in the database. This tool assists in finding regulatory paths between all selected objects.
8) Find common targets/regulators: searches for common targets or regulators for the group of molecules. This tool as well as Build Pathway can find functional links between proteins in the lists imported from other programs (e.g. gene expression clusters).
9) Find intersection with curated pathways: searches a group of nodes for other known and predicted interactions from the PathSys's repository of curated pathways.
10) BiologicalNetworks provides an advanced querying facility for retrieving the data of user's interest by querying Nodes and Properties types. User friendly querying interface allows user to make query with any logical combination of conditions both on Node and Property trees.

Networks can also be analyzed for graph topological properties, such as degree distributions, path lengths, shortest paths or clustering coefficients (Baitaluk et al., 2006).

Topological parameters and features

Topological analysis of biomolecular networks can provide quantitative insights into biological networks. Given the network G={V,E}, where V is the node set and E is the edge set, the topological indices for characterizing a network include average degree K, clustering coefficient C, average path length L, and network diameter D. The topological distribution include degree distribution P(k), cluster coefficient distribution C(k), shortest path distribution SP(k). The most elementary characteristic of a node is its degree or connectivity k. Topological analysis of cellular networks helps to understand the biological roles and functions of the network. The topological measures (see Table 3) can capture the cellular features of cellular networks and provide broad insight into cellular evolution, molecular function, network stability, and dynamic responses (Luonan Chen et al, 2009).

Table 3:
Summary of computed structure parameters by software tools Visant, Cytoscape with BiNoM, CellNetAnalyzer and Biological Networks

Nr	Parameters and features	Visant	Cytoscape with BINOM and NetworkAnalyser	CellNet-Analyser	Biological Networks
1.	Node degree	**Y**			
1.1.	In-degree	**Y**			
1.2.	Out-degree	**Y**			
2.	Degree distribution	**Y**	**Y**		**Y**
2.1.	In-Degree distribution	**Y**			
2.2.	Out-Degree distribution	**Y**			
3.	Degree distribution (average value)	**Y**			
4	Shortest Path	**Y**	**Y** (directed or undirected)	**Y**	**Y**
4.1.	Shortest path between two given components	**Y**	**Y**	**Y**	**Y**
4.2.	Shortest path between many given components	**Y**			
4.3.	Shortest path between all components	**Y**		**Y**	**Y**
4.4.	Optimal and suboptimal shortest paths		**Y**		
4.5.	All the paths leading from one component		**Y**		
4.6.	All non-intersecting paths		**Y**		
5	Shortest Path length		**Y**	**Y**	**Y**
6	Longest path length			**Y**	
7	Average path length			**Y**	
8	Network diameter		**Y**	**Y**	
9	Path finding		**Y**	**Y**	**Y**
9.1.	Path between two given components	**Y**			
9.2.	Path find between a given set of nodes	**Y**		**Y**	**Y**
11	Positive (Negative) Feedback loop	**Y**		**Y**	
12	Feedforward loop (cycle, circuits)	**Y**	**Y**	**Y**	**Y**
13	Self-Loop	**Y**			
14	Randomize Undirected network	**Y**			
15	Statistical Randomization of undirected network	**Y**			
16	Randomize Directed networks	**Y**			
17	Clustering coefficient				**Y**
18	Average clustering coefficient		**Y**		
19	Clustering-Coefficient distribution	**Y**		**Y**	
20	Average-Clustering- Coefficient distribution	**Y**			
21	Cluster finding		**Y**		**Y**
22	Number of components		**Y**	**Y**	
23	Number of edges		**Y**		
24	Number of self-loops		**Y**		

Nr	Parameters and features	Visant	Cytoscape with BINOM and NetworkAnalyser	CellNet-Analyser	Biological Networks
25	Number of connected components		**Y**		
26	Number of shortest paths		**Y**		
27	The average number of neighbors		**Y**		
28	Radius		**Y**		
29	Density		**Y**		
30	Centralization		**Y**		
31	Heterogeneity		**Y**		
32	Neighborhood connectivities		**Y**		
33	Shared neighbors of two nodes		**Y**		
34	Topological coefficients		**Y**		
35	Modular view of the network		**Y**		
36	Clustering and Decomposition				
36.1.	Cycle clustering and cycle decomposition		**Y**		
36.2.	Material component clustering and material decomposition		**Y**		

Almost all of software tools use their own file format (see Table 4.). However, part of them support data exchange formats (se Table 5) that are generally accepted in Systems biology branch, e.g. SBML, SBGN. Implementation of these standards makes easy representation of networks in form that is understandable both for human, and machine, and facilitate communication between different software tools and data exchange, as well as stimulate experience exchange between researchers. Next table represent summary of file formats that supports Cytoscape, VisAnt and CellNetAnalyser.

We should note that BiNoM supports conversion between standards (CellDesigner->BioPAX, BioPAX->SBML) (Zinovyev et al, 2008). But, CellNetAnalyser supports SBML models with help of SBMLToolBox module. Development of VisAnt is also underway for support of the SBML (Zhenjun Hu et al, 2005).

Table 4:

Summary of existing file format supported by Visant, Cytoscape, CellNetAnalyzer and BiologicalNetworks

File format	Tool	Explanation
BioPAX	VisAnt Cytoscape BiologicalNetworks	Biological Pathway eXchange standard format for pathway information supported by multiple pathway databases.
Edge List	ViSant	Default text format for non-XML-based data.
Expression	VisAnt	Expression matrix file, the first line must start with #!Expression. Optional parameter addNewNode to determine whether to abandon the nodes that are not in the current network, e.g. addNewNode=false.
GML	VisAnt Cytoscape BiologicalNetworks	Graph Markup Language, a common graph file format supported by several network software.
ID-Mapping	VisAnt	This file format is designed to allow user to add various database IDs in VisAnt, as well as alias and functional descriptions, to the nodes in a network, must start with #!ID Mapping.
KGML	VisAnt	The KEGG Markup Language (KGML) is a data exchange format of the KEGG graph objects, especially the KEGG pathway maps that are manually drawn and updated. The KGML files for KEGG metabolic pathways specify how enzymes (boxes) are linked by a relation and how compounds (circles) are linked by a reaction (Zhenjun Hu et al, 2007).
M	CellNetAnalyzer	MatLab M-file (file-programm, file-function) format.
Macro/Batch	VisAnt	The file format to store a list of commands for VisANT to carry out.
MAT	CellNetAnalyzer	MatLab file format.
PSI-MI	VisAnt BiologicalNetworks	XML standard format for molecular interactions supported by molecular interaction databases. It is a data exchange format for protein-protein interactions.

File format	Tool	Explanation
SBML	Cytoscape CellNetAnalyzer BiologicalNetworks	System Biology Markup Language standard for representing models of biochemical and gene-regulatory networks.
SIF	Cytoscape BiologicalNetworks	The simple interaction format is convenient for building a graph from a list of interactions. It also makes it easy to combine different interaction sets into a larger network, or add new interactions to an existing data set.
VisML	VisAnt	Default XML format, containing all the network information. Network stored as VisML format can be safely replayed as it was stored.
XGMML	Cytoscape	XGMML is the XML evolution of GML and is based on the GML definition. In addition to network data, XGMML contains node/edge/network attributes.
XML	BiologicalNetworks	XML is (Extensible Markup Language) a set of rules for encoding documents electronically.
XLS	Cytoscape	The tables in these files can have network data and edge attributes. Users can specify columns containg source nodes, target nodes, interaction types, and edge attributes during file import.

Table 5:

Summary of standardized network and interaction data exchange formats supported by public databases, software tools and directly by Cytoscape, VisAnt, CellNetAnalyser and BiologicalNetworks

File format	KGML	GML	PSI-MI	BioPAX	SBML	SBGN	XGMML
Cytoscape with BiNoM		Y	Y (L1, L2.5)	Y (L1,L2)	Y	Y	Y
VisAnt	Y	Y	Y	Y			
CellNetAnalyser					Y (L2)		
Biological Networks		Y	Y	Y	Y (L1, L2)		

This and other programs that work with graphs use mainly their own file format. Therefore, exchanging graphs between different programs is almost impossible. Simple tasks like exchange of data, externally reproducible results or a common benchmark suite are much harder than necessary (Available at: http://www.infosun.fim.uni-passau.de/Graphlet/GML/, 20.12.2009). For that reason we suggest to use the data exchange formats like GML or SBML. In this way (By this means) user can directly communicate between different software tools and store the same computable representation of model.

We don't switch BioPAX data exchange format, while it is a standardized format for exchanging pathway information and integrate knowledge from multiple pathway databases.

Conclusions

Within this manuscript we examine 20 existing tools. 18 tools of them are freely available for academic use, among them 5 are commercial product for non academic use. 12 tools provide source code.

8 tools provide modeling of metabolic networks, 4 tools are suitable for modeling gene regulatory networks, 6 tools allow visualization and analysis of network structure.

Structural model characterize and provide information of the connectivity (topology) of the interactions involved in a biological process. Identifying topological features in networks is an important part of understanding the relationship between network structure and functions of their subunits such as motifs.

Analyzing software tools and scientific literature we found 47 structure topological features, 27 of them are structure parameters that can be used to characterize biomolecular network but there is no software tool that could calculate them all. It is essential to be indicated Cytoscape with BINOM and NetworkAnalyser plugins can calculate large number of above-mentioned topological parameters and features.

Almost all of software tools use their own file format. However, part of them support data exchange formats that are generally accepted in Systems biology branch, e.g. SBML, SBGN. Implementation of these standards makes easy representation of networks in form that is understandable both for human, and machine, and facilitate communication between different software tools and data exchange, as well as stimulate experience exchange between researchers.

Working with graphs we suggest using the data exchange formats like GML or SBML. In this way user can directly communicate between different software tools and store the same computable representation of model. 17 of 20 software tools support SBML standard. 9 tools of them allow both model import and export, 5 tools provide only model export function and 3 tools support model import.

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