Resources, standards and tools for systems biology

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Abstract

Modelling and simulation techniques are valuable tools for the understanding of complex biological systems. The design of a computer model necessarily has many diverse inputs, such as information on the model topology, reaction kinetics and experimental data, derived either from the literature, databases or direct experimental investigation. In this review, we describe different data resources, standards and modelling and simulation tools that are relevant to integrative systems biology.

Keywords: Bioinformatics; systems biology; pathway databases; systems biology standards; modelling and simulation; modelling tools

INTRODUCTION

For a long time, research in molecular biology has been focused on the analysis of relevant components of the cellular network (proteins, metabolites) in isolation. By this approach, thousands of genes have successfully been characterized and functionally annotated. But biological systems are complex and their characteristics are the result of a highly interwoven interaction network developing through time and space. Fundamental characteristics of living systems, such as the assimilation of nutrients, growth and reproduction, the perception of (environmental) signals and its processing can be narrowed down ultimately to the single unit that all living things are composed of: the cell. Thus, the understanding of the characteristics of cellular systems is essential, but this requires an approach that takes into account both interactions at the molecular level as well as physiological functions that are characteristics of the whole organism. In particular, in the light of understanding multigenic and complex diseases that cannot be pinned down to a single gene or component, systems approaches become increasingly important.

Systems biology explanations of physiology and disease should be multi-level; from molecular pathways and regulatory networks, through cells and organs, ultimately to the level of the whole organism. With the use of computer models for such processes, insilico predictions can be generated on the state of the disease or the effect of the individual therapy [1]. Models are partial representations and their aim is to explain which features of a system are necessary and sufficient to understand it [2]. The performance of a model is mainly defined by its predictive power. What is predicted depends on the task and of course is general.

Systems biology is going to revolutionize our knowledge of disease mechanisms and the interpretation of data from high-throughput technologies. Biological systems can be studied by (i) investigating the components of cellular networks and their interactions, (ii) applying experimental high-throughput and whole-genome techniques and (iii) integrating computational methods with experimental efforts [3]. This approach requires an integration of experimental and computational methods [4] and, thus,
an iterative, integrated process of data mining and data gathering (e.g. from scientific literature, databases and experiments), data integration, computational modelling and analysis and finally validation of specific observations that were not explicit beforehand (Figure 1).

Using data mining steps, one agglomerates sufficient details for the generation of model prototypes of the biological system under investigation. Finally, using analysis methods, the mathematical model is refined, cross-validated with regard to internal and external features (for example using parameter estimation and validation methods) and being used for the formulation of new hypotheses that in turn are subject to further experimental investigation.

Systems biology methodology and approaches have evolved rapidly over the past few years, driven by emerging new high-throughput technologies. The most important boost was given by the large sequencing projects such as the human genome project that resulted in the full sequence of the human and other genomes [5, 6]. This knowledge builds the theoretical basis to compute gene regulatory motifs, to determine the exon–intron structure of genes and to derive the coding sequence of potentially all genes through many organisms. From the exact sequences, probes for whole genome DNA arrays have been constructed that allow monitoring of the transcriptome of a given cell- or tissue-type. Proteomics technologies have been used to identify translation status on a large scale (2D-gels, mass spectrometry). Protein–protein interaction data involving thousands of components have been measured to determine information on the proteome [7]. Multiple databases exist, a variety of experimental techniques have produced gene and proteome expression data from various tissues and samples and important disease-relevant pathways have been investigated. Information on promoter regions and transcription factors is available for many genes as well as sequence information. This information—although extremely helpful—cannot be utilized efficiently, because of the lack of integrative analysis tools.

To validate such data in the system-wide hierarchical context ranging from DNA to RNA to protein to interaction networks and further on to cells, one needs to correlate and integrate such information. Thus, an important part of systems biology is data integration.

In this work, we review key genomic and computational resources available for systems biology approaches. The first part of our review is devoted to databases and public repositories that store functional high-throughput data, for example, gene expression data, sequences, disease information, annotation of protein function and biological pathways. Creating a fundamental knowledgebase is the first essential step in the development of computer models for cellular processes and these data repositories can be used in order to derive the ‘parts list’ of the biological process under study. By combining these data and by adding functional relationships they deliver an initial topology (or reaction network) that is the basis for dynamic modelling.

In the second part of this review, we describe some important standards for exchanging relevant data. Experimental investigation in functional genomics and proteomics, e.g. of disease processes, typically involves several steps of experimental testing using heterogeneous data techniques. The integration of those data requires common schema for data storage, data representation and data transfer. For particular experimental techniques (e.g. in transcriptome and proteome research), such schema has already been established. On a more complex level, schema has also been defined for biological models and pathways such as SBML [8, 9], CellML [10] and BioPAX. Most of these repositories use an XML-based language style.

In the third part of the review, we introduce state-of-the-art tools for dynamic computational modelling. For dynamical modelling, common approaches are based on systems of ordinary differential equations

Figure 1: Modelling biological systems.
(ODEs) that describe biochemical reaction networks. Other possible deterministic approaches are e.g. neural networks, cellular automatons, Boolean or Petri nets [11, 12]. Computer tools allow the analysis of the dynamic behaviour of the reaction networks with the given model parameters. It is a very important feature of such systems to allow the estimation of these parameters from experimental data and the analysis of the behaviour of the system with respect to changes of these parameters. We give an overview on the features of several tools and highlight results from the PyBioS system developed in our laboratory.

DATA RESOURCES FOR SYSTEMS BIOLOGY
The development of mathematical models of cellular systems requires a lot of information on different aspects of the system. Data typically arises from several levels of cellular information quantified by different functional genomics technologies such as DNA, RNA or protein sequence data, gene expression data from array experiments, abundance data of proteins and metabolites from diverse experimental techniques (e.g. mass spectrometry, 2D-gels, blots), information on protein–protein interactions or protein modifications or kinetics of enzyme activities or binding affinities, among others. The most important resource for such information is the scientific literature and human expertise curated in public databases. In particular, for the development of mathematical models standardized resources that provide their data in a computational amenable and reusable manner are a preferable resource. Table 1 gives a brief list of some important databases. A large compilation of relevant database resources is given in [13]. Moreover, *Nucleic Acids Research* offers a yearly database issue in January, providing a broad overview of diverse databases.

### Primary data resources
The National Center for Biotechnology Information (NCBI) [14] and the European Bioinformatics Institute (EMBL–EBI) [15] provide several databases that are widely used in biological research offering information about nucleotide sequences, proteins, genes, molecular structures and gene expression. Among the nucleotide sequence databases, the Genetic Sequence database (GenBank), the Reference Sequence Database (RefSeq) and UniGene can be found at the NCBI. Related databases at the EMBL–EBI are the EMBL Nucleotide Database or the Ensembl automatic genome annotation database. The Ensembl project is developing and maintaining a system for the management and presentation of genomic sequences and annotation for eukaryotic genomes [16–19]. Similarly to nucleotide sequence

<table>
<thead>
<tr>
<th>Data resource</th>
<th>URL</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ontology</strong></td>
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<td></td>
</tr>
<tr>
<td>GO</td>
<td><a href="http://www.geneontology.org/">http://www.geneontology.org/</a></td>
<td>[71]</td>
</tr>
<tr>
<td><strong>Pathway databases</strong></td>
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</tr>
<tr>
<td>KEGG</td>
<td><a href="http://www.genome.jp/kegg/">http://www.genome.jp/kegg/</a></td>
<td>[31]</td>
</tr>
<tr>
<td>Reactome</td>
<td><a href="http://www.reactome.org/">http://www.reactome.org/</a></td>
<td>[32]</td>
</tr>
<tr>
<td>BioCyc (including EcoCyc, MetaCyc, HumanCyc)</td>
<td><a href="http://www.biocyc.org/">http://www.biocyc.org/</a></td>
<td>[33]</td>
</tr>
<tr>
<td>Pathway Interaction Database (PID)</td>
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</tr>
<tr>
<td>BioCarta</td>
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<td></td>
</tr>
<tr>
<td>Spike</td>
<td><a href="http://www.wcs.tau.ac.il/~spike/">http://www.wcs.tau.ac.il/~spike/</a></td>
<td></td>
</tr>
<tr>
<td>IntAct</td>
<td><a href="http://www.ebi.ac.uk/intact/">http://www.ebi.ac.uk/intact/</a></td>
<td>[40]</td>
</tr>
<tr>
<td>Database of interacting proteins (DIP)</td>
<td><a href="http://dip.doe-mbi.ucla.edu/">http://dip.doe-mbi.ucla.edu/</a></td>
<td>[41]</td>
</tr>
<tr>
<td><strong>Kinetics databases</strong></td>
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<tr>
<td>BRENDAX</td>
<td><a href="http://www.brenda.uni-koeln.de/">http://www.brenda.uni-koeln.de/</a></td>
<td>[46]</td>
</tr>
<tr>
<td>SABIO-RK</td>
<td><a href="http://sabio.villa-bosch.de/SABIORX/">http://sabio.villa-bosch.de/SABIORX/</a></td>
<td>[47]</td>
</tr>
<tr>
<td><strong>Expression data resources</strong></td>
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<td></td>
</tr>
<tr>
<td>ArrayExpress</td>
<td><a href="http://www.ebi.ac.uk/arrayexpress/index.html">http://www.ebi.ac.uk/arrayexpress/index.html</a></td>
<td>[29]</td>
</tr>
<tr>
<td><strong>Disease specific databases</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>Systems biology model repositories</strong></td>
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<td></td>
</tr>
<tr>
<td>BioModels</td>
<td><a href="http://www.biomodels.org/">http://www.biomodels.org/</a></td>
<td>[48]</td>
</tr>
<tr>
<td>JWS</td>
<td><a href="http://jjj.biochem.sun.ac.za/">http://jjj.biochem.sun.ac.za/</a></td>
<td>[49]</td>
</tr>
</tbody>
</table>
databases, Swiss-Prot, TrEMBL [20] and UniProt [21], provide information on protein sequences and annotations. Moreover, there are databases for protein families, domains and functional groups such as InterPro [22, 23] or those with a focus on protein structures like Protein Data Bank (PDB) [24]. Since a few years, also non-translated RNAs and microRNAs revealed to be highly important in the control of cellular systems and gave rise to the implementation of related databases, like RNAdb [25] or miRBase [26], with the objective of gathering current information. Microarray data provide a valuable resource in the interpretation of the transcriptome levels of genes. Large repositories store these data from multiple studies such as the Gene Expression Omnibus (GEO) [27, 28] at NCBI and the ArrayExpress [29] at EMBL-EBI. These databases provide free distribution and shared access to comprehensive gene expression datasets. Data include single and multiple channel microarray-based experiments measuring the abundance of mRNA, genomic DNA and protein molecules. Data from non-array-based high-throughput functional genomics and proteomics technologies are also archived, including SAGE and mass spectrometry peptide profiling.

**Pathway and interaction databases**

Pathway databases are particularly interesting for modelling approaches, since they offer a straightforward way of building network topologies by the annotated reaction systems [30]. These databases provide integrated representations of functional knowledge of the different components of a biological system and constitute a basis for the topology of mathematical models. The databases Kyoto Encyclopedia of Genes and Genomes (KEGG) [31], Reactome [32] and BioCyc [33] contain metabolic reactions and several signal transduction pathways. KEGG is a reference knowledgebase offering information about genes and proteins, biochemical compounds, reactions and pathways. It provides 317 reference pathways that are linked to genes and reactions of 38 eukaryotes and many microorganisms. It can be accessed via the web, FTP and web services. Reactome [32] is managed as a collaboration of the Cold Spring Harbor Laboratory, the EBI and the Gene Ontology Consortium. It uses a very precise specification (ontology) of components and interactions that comprises details on stoichiometry, localization, references to external databases, etc. This covers also processes like complex formation events or translocations of molecules. A further pathway database with a similar scope is BioCyc [33] that covers pathway data on *Escherichia coli* (EcoCyc), and predicted metabolic pathways of other microorganisms (MetaCyc) and human (HumanCyc). Databases with a specific focus on signalling events are BioCarta [34], Spike [35], Transpath [36], STKE [37], NetPath [38] and the Pathway Interaction Database (PID) [39]. An inherent aspect of the pathway concept is protein–protein interaction subject of the databases IntAct [40] or database of interacting proteins (DIP) [41]. Gene regulation processes and gene regulatory networks are not yet covered in as much detail as metabolic processes or signalling. However, there are databases that store information on transcription factor binding sites such as RegulonDB [42], TRED [43] and Transfac [44]. The lack of uniform data models and data access methods of the existing almost 224 interactions and pathway databases make data integration very difficult [30, 45]. Table 2 illustrates the overlap of several of these pathway resources in human.

<table>
<thead>
<tr>
<th>Pathway databases</th>
<th>Reactome</th>
<th>KEGG</th>
<th>HumanCyc</th>
<th>PID</th>
<th>Biocarta</th>
<th>IntAct</th>
<th>Dip</th>
<th>Spike</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactome</td>
<td>12,042</td>
<td>209</td>
<td>93</td>
<td>8</td>
<td>62</td>
<td>78</td>
<td>15</td>
<td>55</td>
</tr>
<tr>
<td>KEGG</td>
<td>209</td>
<td>1498</td>
<td>199</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td>HumanCyc</td>
<td>93</td>
<td>199</td>
<td>1077</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PID</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>1064</td>
<td>114</td>
<td>42</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Biocarta</td>
<td>62</td>
<td>0</td>
<td>1</td>
<td>2160</td>
<td>114</td>
<td>1152</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IntAct</td>
<td>78</td>
<td>0</td>
<td>1</td>
<td>5690</td>
<td>114</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dip</td>
<td>15</td>
<td>0</td>
<td>2</td>
<td>25</td>
<td>114</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spike</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>125</td>
<td>976</td>
<td>114</td>
<td>112</td>
<td>181</td>
</tr>
</tbody>
</table>

**Table 2**: Numbers of overlapping reactions/interactions from different pathway databases that can be mapped to each other in respect of identical substrates and products.
Besides topological information about cellular reaction networks, kinetic data, such as kinetic laws and kinetic constants, are of particular interest for the generation of mathematical models. Two databases that are concerned with such data are BRENDA [46] and SABIO-RK [47].

Mathematical models of a biochemical reaction system have been made available to the scientific community in form of publications often depicting a diagram of the reaction system or a list of the reaction equations, along with a mathematical description (e.g. as a differential equation system), and lists of kinetic parameters and concentrations of specific states. Recently, model databases have been installed such as the BioModels database [48] or JWS [49]. Both are public, centralized databases of curated, published, quantitative kinetic models of biochemical and cellular systems. For instance, the BioModels database currently provides 87 curated and 40 non-curated models.

**Theme-specific databases**

Whereas most of the above-mentioned databases are fairly general, there exist multiple databases with a specific focus. For instance, there are databases that are focused on a certain species, for example MGD for mouse [50], Flybase for *Drosophila melanogaster* [51], wormbase for *Caenorhabditis elegans* [52] or SGD for yeast [53], or they contain information on specific diseases, such as cancer (e.g. COSMIC [54]) and diabetes (e.g. T1DBase [55]), or they contain information on a specific subject such as chemical compounds found in biological systems (ChEBI [56], the Human metabolome database [57], PRIDE [58], LipidMaps [59], the Human serum metabolome project [60]).

**Mining literature for systems biology**

Finally, the integration of literature information is highly important. Literature is accessed in a derived form such as the concepts represented by the Medical Subject Headings (MeSH) and Gene Ontologies (GO). A further approach that is recently applied for building systems biology resources is text mining [61]. Text mining can either be used for pre-selection of appropriate literature or the automatic extraction of data from literature. In particular, systems biology can benefit significantly from the extraction of data on molecular interactions of cellular components and related information about the kinetics of the interactions [62]. However, text mining of scientific literature is still in its early phase and the precision of its results, as given by false-positive and false-negative rates, has to be improved. For further review on literature mining see [63–65].

**STANDARDS USED IN SYSTEMS BIOLOGY**

An important part of systems biology is data integration. Although data integration itself cannot explain the dynamical behaviour of biological systems, it is useful for increasing the information content of the individual experimental observation, enhancing the quality of the data and identifying relevant components in the model. On the basic level of complexity data integration consists of the integration of heterogeneous data resources and databases with the aim of parsing data from these databases, to query for information and to make it usable for modelling. Technically, database integration requires the definition of data-exchange protocols and languages and the development of parsers that interconnect the databases to a data layer that is able to display the heterogeneous data sources in a unified way.

A standard for representation, storage and exchange of data is a convention about the information items necessary to describe the experiment and the encoding of this information (e.g. expression data of microarray experiments or information about the relations between components and interactions of a pathway). The standard has to enable an unambiguous transfer and interpretation of the data and information. Developing a standard involves four steps: an informal design of a conceptual model, a formalization, the development of a data exchange format and the implementation of supporting tools [66].

**Conceptual design**

The first step, the conceptual model design, gives an informal description of the related domain and specifies its delimitation. The description should address the minimal number of most informative parameters but should still provide a common ground for all related applications [66]. For instance, for the microarray domain a conceptualization is provided by Minimum Information about a Microarray Experiment (MIAME) [67] and Minimum
Information about a Proteomics Experiment (MIAPE) [68] which gives guidelines for the standardized collection, integration, storage and dissemination of proteomics data. Like specifications for experimental data also concepts for the description of mathematical models such as Minimum Information Requested in the Annotation of Biochemical Models (MIRIAM) [69] have been elaborated.

Data representation formalisms and languages
The description of a given domain can be represented in any format, but the use of common representation formalisms and languages makes it easier to compare and interpret data from similar domains and it facilitates the integration, computational processing and comprehensive interpretation of that data. Controlled vocabularies are a prerequisite for a consistent data description. They contain sets of words or phrases representing particular entities, processes or abstract concepts [70]. Within a particular controlled vocabulary, individual terms are usually associated with a unique identifier, an unambiguous definition and occasionally also synonyms to prevent misinterpretations.

Furthermore, ontologies are used for conceptualization of a knowledge domain. An Ontology defines terms and relations along with a vocabulary of a topic area and thus, provides a common terminology over a certain domain. Relations are, for example, ‘is-a’ relations that describe a generalization, forming a term hierarchy. An example is the GO that builds the basis for a generalized functional annotation of genes and their products. The naming of genes and gene products is not necessarily systematic and genes having identical functions are given different names in different organisms or the verbal description of location and function might be different. To address this problem the GO was initiated as a collaborative effort [71]. GO terms have a parent–child relationship. GO defines three top-level categories, ‘molecular function’, ‘biological process’ and ‘cellular component’ and organizes all keywords in a hierarchical graph-like structure. The terms defined in GO form a directed acyclic graph. The power of the GO project lies in the fact that many applications have been developed that use GO terms to validate other data for functional information.

Data exchange formats
During the last years, the eXtensible Markup Language (XML) [72] has been proofed to be a flexible tool for the definition of standard formats not only for applications in different fields of information technology, but also for the management of data from diverse experimental platforms. One example designed for data from microarray experiments is MAGE-ML [73]. Others are, for instance, those dealing with pathway data and mathematical models. Table 3 gives an overview of some XML-based data exchange formats used in systems biology. SBML [8, 9], CellML [10] and BioPAX [74] have the potential to become de facto standards for their respective application area.

BioPAX is defined by the BioPAX working group and is designed for handling information on pathways and topologies of biochemical reaction networks. The Systems Biology Markup Language (SBML) is a format for ‘describing models common to research in many areas of computational biology, including cell-signalling pathways, metabolic

**Table 3: Examples of XML-based standards used in systems biology**

<table>
<thead>
<tr>
<th>Modelling system</th>
<th>Description</th>
<th>Application area</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BioPAX</td>
<td>Biological Pathways Exchange</td>
<td>Description and exchange of biological pathway data</td>
<td>[74]</td>
</tr>
<tr>
<td>SBML</td>
<td>Systems Biology Markup Language</td>
<td>Describing and exchange of mathematical models</td>
<td>[8, 9]</td>
</tr>
<tr>
<td>SBGN</td>
<td>Systems Biology Graphical Notation</td>
<td>Visual notation for computational models of biological systems</td>
<td>[96]</td>
</tr>
<tr>
<td>CellML</td>
<td>Cell Markup Language</td>
<td>Describing and exchange of mathematical models</td>
<td>[10]</td>
</tr>
<tr>
<td>PSI-MI</td>
<td>Proteomics Standards Initiative Molecular Interaction</td>
<td>Description and exchange of protein-protein interactions</td>
<td>[97]</td>
</tr>
</tbody>
</table>
pathways, gene regulation and others’ [8, 9]. Major releases of the SBML standard are called levels, where level 2 is the most recent. SBML defines list of species (entities of the model), compartments, parameters and reactions, among others. SBML is widely used—it is supported by over 110 software systems.

A comparison of SBML and BioPAX comes to the conclusion that, while the main structures of these formats are similar, SBML is tuned towards simulation models of molecular pathways. BioPAX turns out to be the most general and expressive format [75], even if it is lacking definitions for the representation of dynamic data such as kinetic laws and parameters.

It is argued that the syntactic and document-centric XML cannot achieve the level of interoperability required by the highly dynamic and integrated bioinformatics applications. Therefore, semantic web technology like resource-description framework (RDF) and the web ontology language (OWL) have been proposed as alternatives to current XML technology [76].

Using standards brings several advantages, e.g. an ontology along with a defined vocabulary is used that promotes an accurate description of the data and it provides a software-independent common representation of the data. One of the most important general problems in building standards for biology is that our understanding of living systems is not static but rather constantly developing what necessitates a regular update of these standards [66].

**MODELLING TOOLS**

**Annotation tools**
The first step in setting up a model is by summarizing in the computer, all reactions, interactions and processes that are relevant to the model, either as a list of reactions or as a diagram that is describing those processes and depicts the network structure. There are several software tools that can be used for this purpose, for example JDesigner [77] or the graphical and user-friendly interface of the CellDesigner software [78]. Another software that has a sophisticated graphical user interface and supports BioPAX for model exchange is Cytoscape [79]. A more advanced but comprehensive tool for annotation is the Reactome Curator Tool that can take advantage of the already existing data provided by a local copy of the Reactome MySQL database. Reaction systems designed by this tool have to be converted into appropriate interchange formats, like SBML or BioPAX.

**Modelling tools**

Once the model topology is designed, a mathematical model can be created. If this is, for example, a kinetic model, further data on the kinetic laws and kinetic parameters has to be identified or appropriate assumptions have to be made. For this purpose, diverse software tools have been developed. One can use commercial tools like Mathematica or Matlab that are well elaborated and offer broad spectra of functionalities. One disadvantage of using these programs is that the differential equation system of the mathematical model has to be formulated explicitly by the user. Overviews of current software platforms and projects that face up to this as well as an overview about computational requirements for this purpose is given in [3, 80, 81]. Common systems among others—for this purpose are Gepasi [82–84], COPASI [85], E-Cell [86, 87], ProMoT/Diva [88], Virtual Cell [89–91] or the Systems Biology Workbench (SBW) and its add-ons [92]. Table 4 summarizes some modelling and visualization tools for systems biology. A comprehensive list of modelling and simulation tools is also given in [93] that reports the results of an online survey of systems biology standards. This report identified CellDesigner [78] as the most popular stand-alone application in respect to its graphical functionalities.

**Table 4: Selected modelling and simulation tools for systems biology**

<table>
<thead>
<tr>
<th>Modelling system</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>General Modelling Tools</td>
<td></td>
</tr>
<tr>
<td>GEP ASI [82–84]</td>
<td></td>
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<tr>
<td>CellDesigner [78]</td>
<td></td>
</tr>
<tr>
<td>E-Cell [86, 87]</td>
<td></td>
</tr>
<tr>
<td>ProMoT/Diva [88]</td>
<td></td>
</tr>
<tr>
<td>Virtual Cell [89–91]</td>
<td></td>
</tr>
<tr>
<td>Systems Biology Workbench (SBW) and its add-ons [92]</td>
<td></td>
</tr>
<tr>
<td>COPASI [85]</td>
<td></td>
</tr>
<tr>
<td>PyBioS [3, 95]</td>
<td></td>
</tr>
<tr>
<td>Model Visualization Tools</td>
<td></td>
</tr>
<tr>
<td>BioTapestry [98]</td>
<td></td>
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<tr>
<td>Cytoscape [99]</td>
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<td>VisANT [100]</td>
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</table>
Gepasi and COPASI come up with user-friendly interfaces for the simulation and analysis of biochemical systems. They support the definition of compartments. Common kinetic types as well as user-defined kinetic types are available. They provide time-course simulation and steady-state calculation and the ability to explore the behaviour of the model over a wide range of parameter values using a parameter scan that runs one simulation for each parameter combination. Gepasi and COPASI can characterize steady states using metabolic control analysis (MCA) and linear stability analysis and they are capable of doing parameter estimation with experimental data and optimization.

E-Cell is based on the modelling theory of the object-oriented Substance–Reactor Model. Models are constructed with three object classes, substance, reactor and system. Substances represent state-variables, reactors describe operations on state variables and systems represent logical or physical compartments. Time-course calculation is done by the use of a simulation engine. Numerical integration is supported by first-order Euler or fourth-order Runge–Kutta method.

ProMoT/Diva consists of the modelling tool ProMoT and the simulation environment Diva. The workbench deals with modular models and can handle Differential Algebraic Equation (DAE) systems. Modelling is supported with a graphical user interface and a modelling language. The modelling tool provides the possibility to use existing modelling entities out of knowledge bases.

The Virtual Cell is a web-based client-server architecture with central databases of user models. It provides a formal framework for modelling biochemical, electrophysiological and transport phenomena while considering the sub-cellular localization of the molecules that take part in them [91].

The SBW provides a server that acts as a broker between different modelling and simulation tools (clients) via a common interface. These clients (add-ons) cover graphical tools for model population, deterministic and stochastic simulators and analysis tools like the integration of MetaTool [94]. Closely related to the SBW is the development of SBML that is used for communication by SBW.

A modelling and simulation platform for systems biology that is developed in our laboratory is PyBioS [3, 95]. PyBioS has a web-based user interface and provides functionalities for the model development, simulation and analysis (Figure 2). Compared to other systems biology modelling and simulation tools PyBioS provides interfaces to external pathway data resources that can be searched and directly be used for the generation of the model topology in mind. The system can handle large ODEs with thousands of reactions. Furthermore, PyBioS provides an interface for the upload of experimental data that can directly be used as initial values for model components such as mRNA, protein, metabolite or enzyme abundances. Appropriate kinetics can be chosen from a repository of pre-defined kinetic laws. From this information—the model topology and the reaction kinetics along with the respective parameters—PyBioS automatically creates a mathematical model that can be used subsequently for simulation and analysis. Besides plotting graphs of time-course data (concentrations or fluxes versus time), PyBioS also provides a visualization of the interaction network graph comprising coloured nodes according to simulation results. The latter is very helpful for the interpretation of simulation results with respect to the network structure. For the creation of a first model prototype, PyBioS has an interface to external pathway databases, such as Reactome, that can be used for the automatic generation of a model based on the respective network topology. Via the web-interface one can search for specific reactions or pathways and by this enrich the model automatically with reactions from pathway databases (Figure 3).

**SUMMARY AND FUTURE NEEDS**
The new functional genomics techniques will elevate our knowledge on biological networks to a great extent. Systems biology will play a key role in future research in the interpretation of such data. The information that we can gain about a biological system (for example a disease process) appears in practice as an experimental observation, and research is restricted to the granularity and the precision of the experimental techniques in use. It is very likely that the range of experimental granularity will increase in the next years utilizing heterogeneous techniques that target a biological question of interest at different points so that data integration...
remains a major challenge of future biomedical research.

In the case of complex disease conditions, it is clear that such integrated approaches are required in order to link clinical, genetic, behavioural and environmental data with diverse types of molecular phenotype information and to identify correlative associations. Such correlations, if found, are the key to identifying biomarkers and processes that are either causative or indicative of the disease.

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