Integrating Bioinformatics and Computational Biology: Perspectives and Possibilities for *In Silico* Network Reconstruction in Molecular Systems Biology

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Abstract: There is a flood of molecular data about many aspects of cellular functioning. This data ranges from sequence and structural data to gene and protein regulation data, including time dependent changes in the concentration. Integration of the different datasets through computational methods is required to extract biological information that is relevant from a systems biology perspective.

In this paper we discuss how different computational tools and methods can be made to work together integrating different types of data, mining these data for biological information, and assisting in pathway reconstruction and biological hypotheses generation. We review the recent body of literature where such integrative approaches are used and discuss automation of data integration and model building to generate testable biological hypotheses. We analyze issues regarding the design of such automated tools and discuss what limitations and pitfalls can be foreseen for the automation and what solutions can computer science and biologists provide to overcome them.

INTRODUCTION

Molecular systems biology is a broad discipline in which computational methods play a central role. Most researchers will agree that molecular systems biology ultimately aims at understanding how molecular systems function when they are assembled. Thus, pathway and circuit reconstruction and mathematical modeling of the corresponding networks, are central issues in this research area. Because of its inherent diversity, different "origins" are acknowledged for the field [1, 2].

From an historical point of view, the use of mathematical models and computer simulations in molecular biology can be traced at least to the early fifties. Its use appears to have been introduced by Britton Chance, Benno Hess, Joe Higgins, David Garfinkel and their colleagues [3-7]. They used modeling to achieve a more systemic understanding of the processes they were studying experimentally. Such processes ranged from the kinetics and mechanism of catalase action to glycolytic oscillations [3-7]. In the sixties and seventies a few other research groups entered the field, and applied engineering control theory to the study of biological systems [8-16]. Also in the sixties, researchers started taking advantage of the computer capabilities for data organization and analysis. The work of Fitsch [17-19], and its further development by Needleman and Wunsch in 1970 [20] and by Smith and Waterman in their seminal 1981 paper [21], would set the stage for the bioinformatics revolution that took place during the last decade in the twentieth century.

This revolution was in all likelihood prompted by an accumulation of gene sequences and protein sequences and structures. The accumulated data required the development of advanced computational tools for their analysis and organization, leading to the bioinformatics burst. As a consequence of the working capacity of the post-genomic high throughput (HTP) techniques and of the capabilities of computer tools, the old reductionism paradigm shifted towards an integrative view of the molecular biology problems. Albeit not new, this integrative view shifts attention from bioinformatics into (molecular) systems biology, which is sensed as a new frontier in biology. This shift was predicted by Bertalanffy and others as early as in 1940 [22]. The current recognition of this new paradigm has been fueled, among others, by Horishi Kitano and John Doyle [22-28].

The size and scope of the accumulated HTP data sets have made it impossible for any one person to analyze and integrate them all, even if one is only interested in a specific molecular biology process on a given organism. However, such integration is fundamental to reconstruct the molecular networks involved in cellular processes and to obtain a systemic perspective of how those networks function. Thus, researchers need tools that assist with processing, filtering, organizing, and appropriately displaying the complex information that is available. Furthermore, automation of analysis and integration is also fundamental for an effective use of that information.

The increasing availability of computational power and storage capabilities, described for example by Moore's law [29], facilitates the creation of CPU-demanding algorithms. These algorithms can be used to build software applications that can manipulate the different available datasets, integrate the information they contain, and display the end result of the analysis in a user friendly format. Web based applications play an important role in making all these software tools available to most scientists. Efficient information mining and integration, and an appropriate display of the results facilitates *in silico* reconstruction of the molecular networks

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that regulate and execute relevant molecular biology processes.

In silico pathway and circuit reconstruction is an important area within systems biology, and it has consequences for molecular biology, genetics, biotechnology and others. The reconstruction ultimately generates hypotheses regarding the connectivity and dynamic behavior of pathways and circuits that must be validated against experimental predictions data. Different types of *in silico* reconstruction problems exist:

(1) Identifying the pathways, genes and processes/functions that are encoded in the entire genome of an organism. This provides information regarding possible *qualitative* systemic responses of the cell. As a first approach to this reconstruction problem, one may map the annotated genes onto pre-existing charts of metabolism, gene circuits and signal transduction. This action may reveal limitations in adaptive responses of that organism. For example, if no amino acid biosynthetic pathways are found, then this organism is not likely to survive in a medium without and external amino acid source.

(2) Reconstructing the detailed reaction network that exists within specific pathways or circuits (see for example [53-59]). An important aspect in this type of research is the reconstruction of novel pathways and the identification of new components in classical pathways. This is a relevant problem because, either in well characterized organisms or with the sequencing of metagenomes [60], many genes of unknown function exist and several previously unknown pathways are being discovered. This type of reconstruction may allow for more precise predictions regarding how specific parts of the cellular response are regulated and executed, facilitating the creation of mutated organisms with biotechnological interest.

(3) Reconstructing regulatory networks in gene expression and signal transduction. For example, identifying regulatory motifs in DNA will provide indication of what transcription factors may regulate the expression of different proteins and genes that are involved in specific processes (e. g. [61-63]). At the metabolic and signal transduction level, reconstruction of the detailed regulatory networks for the enzymes in a pathway is a requirement for accurate and quantitative prediction of the dynamic cellular behavior in response to environmental challenges.

In this paper we focus on and review the use of HTP datasets for the *in silico* reconstruction of metabolic pathways, signal transduction pathways, and gene circuits.We frame the reconstruction in the context of computational molecular systems biology, an emerging field that merges bioinformatics, and computational biology. Within this context, we shall discuss the data sets and computational approaches that can facilitate that reconstruction. Then, we review the literature for work that uses an integrative computational approach for this reconstruction and discuss automating the integration process. Finally, we discuss the various problems that hinder automation of integration and analysis of the data sets. We discuss in more details some of the technical aspects of the research reviewed in the main paper and provide further references to other sources of information in a supplementary appendix.

WHAT IS OUT THERE? AUTOMATED DATA ANALYSIS IN THE AGE OF OMICS

Pathway reconstruction requires integration of knowledge at many different levels. Before discussing this integration, we briefly characterize each of the various types of datasets that are available for *in silico* reconstruction of molecular biology pathways. Also, we shall discuss the different methods and tools that are more commonly used a) to mine these datasets, and b) to extract network information and facilitate pathway reconstruction.

Bibliomic Data

Bibliographic data has been accumulating for more than a century. Databases such as MEDLINE [64] or the Web of Science Citation Index [65] collect and organize data from this published literature. Through automated keyword analysis, search engines can identify relevant documents in these databases. These documents can be mined for information on genes, pathways, and networks of interest. Most of these documents concern specific, detailed studies about small sets of genes, proteins, RNAs or metabolites within specific organisms. In many cases, this data provides a detailed functional analysis of many individual genes, using methods that are more accurate than those used to obtain HTP data.

Manual and electronic literature analysis has been used for metabolic reconstruction since researchers started creating mathematical models for molecular biology processes [4]. Currently, literature searches are limited at automatically generating a possible network structure for a given molecular process, although they can easily identify papers containing information that is relevant for the reconstruction of that network. In fact, most mathematical models of molecular pathways and circuits are based upon information that was manually retrieved from literature (for example [66] or [67]).

The recent development of tools such as iHOP [68-71] or biobibliometrics [71] allows researchers to automatically reconstruct networks of genes and proteins from automated literature analysis [68-71]. The underlying assumption of these methods is that identifying gene and protein names that co-occur in the same document(s) generates a network of genes/proteins that are functionally related among them. These network reconstruction methods have been used as a starting point to reconstruct pathways such as the Iron Sulfur Cluster (ISC) biogenesis in *Saccharomyces cerevisiae* [55] or to identify genes involved in some types of cancer [72]. However, such a network should be viewed as a low level reconstruction of the molecular pathways that are involved in the processes for which those specific genes are important. Further analysis is advisable before one claims that the automatically generated network is a complete conceptual model of the processes of interest.

The largest fraction of the textual scientific information contained in a paper is unavailable if one mines Medline

exclusively, because the full text of the papers is not included in this database. The PubMed, SCOPUS, PLOS, and BioMedCentral initiatives are crucial in making full text of scientific papers publicly available [73-76]. The ongoing efforts from scientific journal to make their contents fully available on line also contribute to this effort. There is strong awareness that molecular systems biology research will greatly benefit if information mining from text can be automated to a higher degree than it currently is [61, 69, 77].

Sequence Data, Functional Data, and Structural Data

Databases of annotated gene and protein sequences facilitate the subsequent functional annotation of new genomes, through the use of homology comparisons. Gene or protein sequences from different organisms that have very high similarity (homology) are likely to have the same function. Sequence data can also be mined to predict a) regulatory regions and open reading frames of genes, b) RNA genes, and c) targets for regulation by these RNA genes (see supplementary appendix). The accumulated functional knowledge about genes and proteins facilitates the creation of charts for metabolism, signal transduction and gene circuit for the different organisms with fully sequenced and annotated genomes (see for example [78-81]). In such charts, the individual function of a protein is superimposed onto the particular steps where that protein is active. An example where genome annotation has been used to reconstruct the full complement of metabolic pathways for *Lactobacillus plantarum* can be found in [45, 49]. Sequence based annotation is not possible when a new gene is not homologous to any gene of known function. However, if structural information can be gathered for the protein coded by that gene, structural homology comparisons may also facilitate attributing general or specific functions to individual genes, for example using classifications such as SCOP or CATH [82-92]. Knowing the structure of a protein (or RNA gene) can elucidate the mechanism by which these molecules perform their function. Furthermore, having structural templates allows the prediction of structures for other homologous proteins.

Even when homology is not useful for functional annotation, sequence information can still be used to infer some functional information. For example, one can use phylogenetic conservation to investigate possible functions of the genes. The logic behind phylogenetic conservation analysis is as follows. If a set of homologous genes with unknown function is present (absent) with other genes of known function in the same set of genomes, then it is possible that evolution acted simultaneously on that set of genes because somehow they share a function. This may with other genes of known function allow the researcher to predict that some genes are involved in the same processes, although their individual function may remain uncertain. Such an approach has been combined with other lines of evidence, to identify the proteins Yfh1 [93] and Grx5 [58] as being involved in ISC biogenesis. Another example is the application of phylogenetic analysis to the reconstruction of the Coenzyme A biosynthesis pathway in different archaeal genomes [94] and for the reconstruction of parasite nucleotide biosynthesis [95]. A similar method for inferring function is that of finding gene fusion events. Such events imply that two genes share common function [96, 97] and maybe even common regulation, as appears to be the case for example in the biosynthesis of aromatic amino acids in low GC Gram-positive bacteria [98].

Sequence based reconstruction of gene circuits can be more complex than sequence based reconstruction of metabolic and signal transduction pathways. The reason for this is that regulatory sequences in DNA are shorter than coding sequences. This creates a lower signal to noise ratio in their detection, when compared to identifying a full gene. Identification of gene circuits units within a genome can be done for example by searching for regulatory motifs or sequence patterns upstream of gene promoters. Confidence in the accuracy of the regulatory units predicted by this method is increased by finding that such motifs are phylogenetically conserved in different organisms [99-108]. This type of analysis has been used for example to identify novel targets of the Eyeless transcription factor in *Drosophila* [109], to identify transcription factors that regulate human gene expression [110], to identify novel gene circuits for amino acid transport and glucose in *S. cerevisiae* [111, 112] or to predict operons in *Pyrococcus furiosus* [113]. An intense research effort to understand RNA gene circuits is also under way [114-121]. Only when this new layer of regulation is fully understood and integrated into the reconstruction efforts, can one have full reconstruction of gene circuits. Progress in this area is fast and different groups are already reconstructing RNA gene circuits in bacteria and other organisms by integrating the distinct types of information discussed in this section of the review [122-127].

Gene Expression Data

Different types of mathematical analysis allow researchers to infer functional genetic modules and circuits from the analysis of high and low throughput gene expression data [128-136]. Statistical theory and information theory is extensively used to infer regulatory network structures from gene expression data [137-147]. For example, if a gene/protein of unknown function is differentially regulated during some cellular response, then one might infer that this gene is involved in that response. The set of genes responsive to human interferon beta [148, 149] or that of genes involved in the development of rat central nervous system [150] have been reconstituted from microarray data. However, one must always keep in mind that post-translational regulation and fine tuning of enzyme activities may work to modify the importance of changes in gene expression. This may complicate the interpretation of gene expression profiles.

Available gene expression data is often static, in the sense that it is measured at a specific time during the response and no other previous or subsequent measurements are available. Static gene expression data, in general, do not provide significant information about the specific function of a gene in the response. Exceptions might be for example situations where only one protein is missing in a circuit or pathway that is well known. In such cases, if only one gene of unknown function is identified in the expression data, its function is likely to be the one that is missing. Alternatively, it may be possible to infer sequential action of transcription factors in the gene circuit, by using time series data from microarray experiments [151-153]. For example, the network of gene activation in macrophage response [154], the p53

network in human leukemia cell lines [155] and the regulation of galactose biosynthesis [156] have been studied using this type of analysis.

Proteomics Data

Proteomics experiments in which measurements of protein levels and activity are made can also assist in network reconstruction. Having such data is important and complementary to the gene expression data. In fact, proteomic data is required to ultimately refine the network structure of circuits and pathways and assess their functionality *in vivo*. This is so because there are cases in which the levels of the protein that is coded by a gene can change in response to some stimulus, even though expression of that gene is unchanged and no interaction with other proteins has been previously reported. In addition, proteomics analysis may reveal changes in the activity of proteins in responses to challenges that were previously unknown to affect their activity. This kind of information is still lacking for most organisms and cell types.

Proteomics studies are in their infancy, when compared to gene expression studies. Nevertheless, they have been used for pathway reconstruction in various cases. HTP proteomic experiments led to the discovery of networks of direct physical interaction between proteins in different organisms [157-172]. Finding which proteins interact physically with those of unknown function sheds light upon the processes in which the later proteins may be involved in, thus facilitating the reconstruction of their role in the cell. However, one should keep in mind that these experiments may detect false positive interactions and fail to detect real interactions, due to the experiments being made under inappropriate physiological conditions. In addition to detection of protein interactions, proteomic approaches have been used to a) identify the kinases that are necessary for cell cycle progression in *Drosophila* [173], b) reconstruct human phosphorylation networks [174], c) reconstruct the network of growth factor signaling in cancer cells [175], d) study the pheromone response in yeast [176], and e) study signal transduction in plants [177, 178].

It is still unfeasible to use large scale proteomic assays to measure changes in the activity of the full protein complement of an organism. However, the development of protein chips that identify binding of protein to DNA [179] and allow measuring of enzyme activity for whole pathways [180, 181] suggests that such a goal may be attainable in the future.

Metabolomic Data

Metabolomics data can also, in principle, be used to reveal information about pathway and circuit connectivity [182, 183], to estimate parameter values, and to validate and refine models of specific cellular processes [184, 185]. These very important points would justify a wide program of metabolomic experiments. Without this kind of measurements, validation of mathematical models for specific metabolic processes may be difficult. Furthermore, one can infer which reactions and which steps causally precede others in a metabolic or signal transduction network, by measuring the changes in the concentration of metabolites or signaling molecules over time. Such an analysis has been performed to predict the sequence of glycolytic reactions [186] and to further refine the regulatory network of glycolysis in *Lactococcus lactis* [184].

USING MOLECULAR DATA TO PREDICT SYSTEMIC BEHAVIOR

In the previous section we presented a selection of the data that are available for *in silico* inference and reconstruction of network and pathway topology. A given network topology should be able to explain the experimental behavior of a system, if that topology underlies the process that regulates the biological response being measured. However it is often impossible to use common sense for judging how well a given network explains some dynamic response, because the dynamic behavior of biological systems is non-linear. To overcome such a limitation, topological schemas can be used to create mathematical models whose dynamical behavior can be rigorously analyzed and compared to what is experimentally observed. This validation process is fundamental in testing the correctness of *in silico* pathway reconstruction.

Building Mathematical Models

Mathematical models can be created for networks of different scales. Some researchers are interested in modeling the entire metabolic network found in a genome (e.g. [31-52]. Such models have been analyzed to predict both, growth characteristics, and essentiality of genes in different organisms using flux balance analysis (FBA) [35, 38, 39, 46]. Although in mathematical terms FBA models are linear, the qualitative predictions of phenotype have so far held for between 60% and 80% of the genes [39, 46, 187]. These models inappropriately represent dynamic regulatory effects, which could account for some of the erroneous predictions (see [188] for details).

Smaller scale models that accurately account for dynamic regulation are important for testing more detailed hypotheses about the functioning of specific processes. For example, the regulatory structure of the Iron-Sulfur Cluster biogenesis pathway in *S. cerevisiae* has been validated by comparing the dynamic behavior of mathematical models for alternative regulatory topologies to experimental results [55]. Similarly, models of cell cycle have been validated by comparing its dynamic behavior to experimental results [189-191], and models of the pentose phosphate pathway have been validated by predicting the best targets for treatment of metabolic diseases [192-195].

Statistical methods can be used how well alternative networks can reproduce experimental behavior, if sufficient information is available. For example, one can use optimization algorithms to determine which network more accurately fits the known quantitative behavior of a system [196-198]. Qualitative statistical methods should be used for this evaluation, if the available experimental data is not quantitative. For example tree decision algorithms have been used to analyze the qualitative behavior of signal transduction modeling [199, 200].

Different types of mathematical modeling can be used to study the same biological problem. The choice of a particular type depends on the level of complexity and details one wishes to consider, on the available data, on personal prefer-

ence, etc. Differential equations, either ordinary (for well stirred systems) or partial (for systems with spatial differentiation), are used to define mathematical models that can quantitatively simulate the dynamics of a molecular system [4, 201]. This type of mathematical approach is still the most prevalent choice as a modeling tool, although stochastic approaches are becoming popular. Alternative approaches are further discussed in the supplementary appendix.

Bottlenecks in Model Building

One limitation to the creation of mathematical models is that, for many processes, the parameter values are unknown. Even when parameter values are available, locating them and evaluating if the experimental conditions under which they have been measured are appropriate for our modeling purposes is not easy. Furthermore, automatically identifying documents from the literature in which such information is available is still a task that is hard to automate. Another limitation in creating a mathematical model is when the knowledge about the actual mechanism that underlies many biological processes is lacking. This makes it impossible to derive classical Michaelis-Menten like expressions for the kinetics of those processes. Mathematical representations based on approximate formalisms, such as power-law models, help side-step this lack of information (see supplementary appendix and [202]). Creating a mathematical model is a very work intensive task that is prone to human error. As the dimension of a model increases, automated model building becomes important in decreasing modeling errors. General automated model building is possible only by using structured and systematic formalisms.

Most of these formalisms are mathematical approximations that simplify the exact kinetic expressions representing the dynamical behavior of individual molecular biology processes and reduce the dimension of the system of equations to be solved (for example [203]). Using structured formalisms that are based in approximation theory also sidesteps the lack of knowledge regarding mechanism while building a mathematical model [204]. These approximate representations are accurate over a varying range of values about their operating point [205] and they can be invaluable as canonical formalisms that facilitate automated generation, analysis, and exchange of mathematical models [204]. For a review of these ideas see [202].

INTEGRATION OF BIOINFORMATICS AND COMPUTATIONAL BIOLOGY FOR AUTOMATED NETWORK EXTRACTION AND MODEL BUILDING

Automatic model building that is based on the topological representation of a network requires integration of information at different levels. Fig. **1** outlines in a flow chart how one can integrate the bioinformatics data mining process to the model building process in order to complete the *in silico* reconstruction of networks. In general, the integration needs to be highly parallelized, allowing for flexible exclusion of one or more types of datasets and for an appropriate interaction with the user.

Outline of the Integration

A first layer of information (sequence data, structural data, literature data, gene expression data, proteomics data and metabolomics data) available in HTP data sets and published material can be integrated to derive a second layer of processed data. This second data layer contains information about a) gene/protein function and interactions, b) pathway and gene circuit topology, and c) parameter values. The second layer of data also receives direct experimental inputs at different levels. For example, experimentally determined parameter values can be directly integrated into this layer. The data from the second layer can in turn be integrated to generate alternative topological schemas that describe how the pathways and circuits of interest may function. Statistical algorithms, such as decision tree methods or Bayesian analysis, can be used at different stages of the reconstruction to rank the relevance of the information and give users an objective means of deciding what is the most appropriate information to include in the reconstruction.

The topological schemas can be used either to refine preexisting mathematical models or to generate new ones. The dynamical behavior of alternative models can then be compared to the experimental behavior of the system. By using statistical methods to analyze these comparisons, one may objectively rank which schema are more likely to correctly describe the topology that underlies the observed behavior. (Fig. **2**) details how the analysis and integration of the different datasets can be accomplished and used for pathway and circuit reconstruction.

The Role of Text-Mining

Fig. **2A** suggests how literature analysis (***bibliomics***) can be used to generate conceptual models for pathway and circuit reconstruction. Web crawling robots, using Artificial Intelligence (AI) algorithms, can perform automated keyword and contextual analysis of information contained either in literature databases or in live web documents. They can also identify lists of genes that appear to be relevant for the process of interest and generate a tentative topological network by suggesting functions for those genes.

Automated and contextual text analysis may be extremely important to locate information about parameter values and enzyme kinetics and in automatically integrating such information into models. However, human intervention is still the most accurate way for mining this type of information from the literature, which suggests that the need for human curation should always be kept in mind during the different stages of the reconstruction process.

If appropriate standards of model and parameter reporting are defined, automated text mining of parameter values from the literature may be possible. One must be cautious regarding these standards. Many may be tempted to use parameter values and models that are automatically found, without considering the appropriateness of the models for the goals one has and the compatibility between different parameter determinations.

The Role of Mining "omics"

Fig. **2B** suggests how functional information, sequence information and gene expression information (***genomics***) can be used within a global strategy for pathway reconstruction. By processing sequence information one can automatically identify genes and proteins that participate in a given process

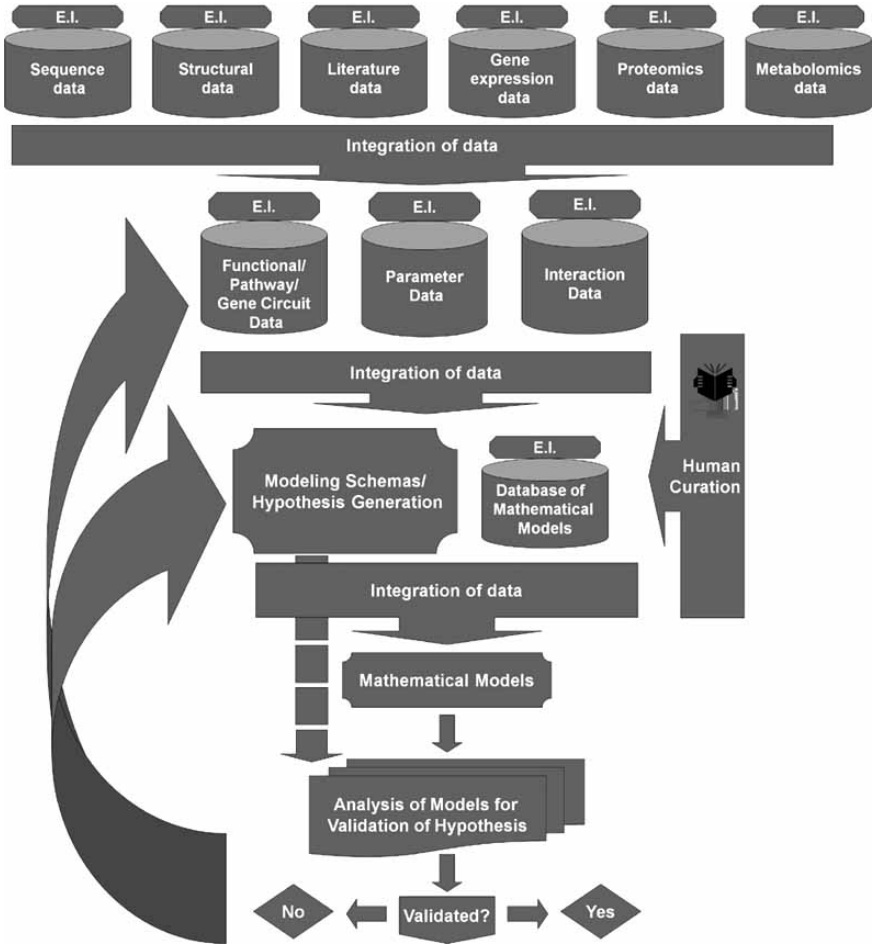


Fig. (1). Integrating the different datasets for pathway reconstruction: An overview. E.I. stands for direct experimental input to a given class of data. Different types of primary data can be used and integrated to infer functional information. Functional information can also be experimentally generated. All the relevant information can be integrated, thus generating conceptual schemas that can be used to generate and test hypotheses regarding the behavior of the molecular system of interest.See text for details.

and generate functional information for those molecules. In parallel, gene expression data can, in principle, also be used to derive some form of causal relationship between changes in the expression of the different genes. Besides identifying/confirming genes involved in the processes of interest, the information processing shown in Fig. **2B** can also be used to generate alternative architectures for the topology of gene circuits and pathways.

Fig. **2C** suggests that ***proteomics*** and ***interaction data*** can be used to derive a network of protein and gene interactions. In addition to identifying or confirming previously identified proteins, the interaction data can provide additional hypotheses regarding the previously unknown connectivity in a pathway.

Proteomics and metabolomics data can also be used to infer the sequence in which different proteins act in a pathway and how the activity of the different steps is regulated. "Omics" data may also contain essential information for identifying appropriate parameters for the mathematical models [2], as long as structured formalisms are used to write the model equations and sufficiently dense time series are available in the data. For more information on this subject see the supplementary appendix and references therein.

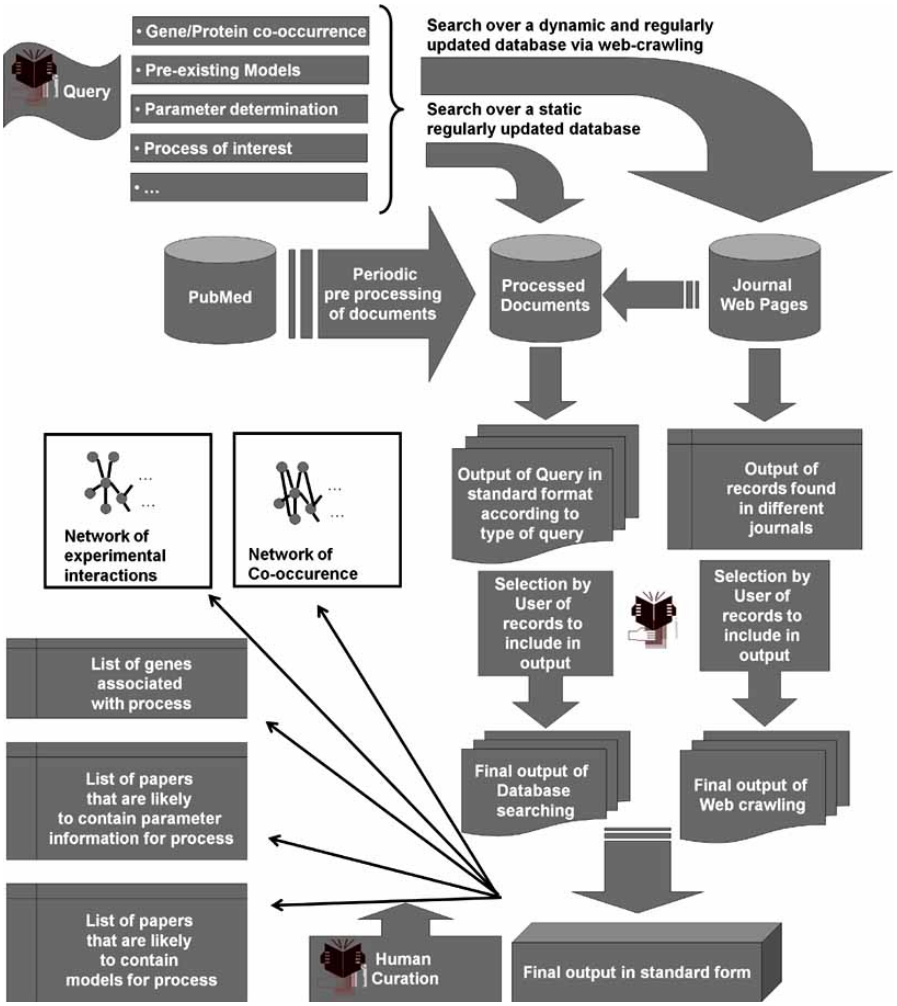
From Bioinformatics to Computational Systems Biology Through Model Building

Fig. **2D** details the final stage of the integration between bioinformatics and computational systems biology. The user can attribute alternative roles in the network to genes even if they have not been automatically assigned to specific reaction steps or regulatory interactions. Once a final set of alternative schemas for the pathways and circuits of interest are created, the users must decide upon the level of detail that is necessary for their research. Automated search of databases with pre-existing models can find previously build mathematical models of the reconstructed network, if they exist. Once the models are created, they can then be analyzed, validated, and refined. The process of model validation, described in previous sections, can stimulate new research, assisting in the design of experiments that clarify unsolved questions and allowing for rational testing of hypotheses about the systemic behavior of the network.

Examples of Bioinformatics and Computational Biology Integration in Molecular Biology

It is consensual that the use and integration of different datasets, combined with mathematical modeling is important

A


Query
• Gene/Protein co-occurrence
• Pre-existing Models
• Parameter determination
• Process of interest
• ...
Search over a dynamic and regularly updated database via web-crawling
Search over a static regularly updated database
PubMed
Periodic pre processing of documents
Processed Documents
Journal Web Pages
Network of experimental interactions
Network of Co-occurence
Output of Query in standard format according to type of query
Output of records found in different journals
Selection by User of records to include in output
Selection by User of records to include in output
List of genes associated with process
List of papers that are likely to contain parameter information for process
List of papers that are likely to contain models for process
Final output of Database searching
Final output of Web crawling
Human Curation
Final output in standard form

B

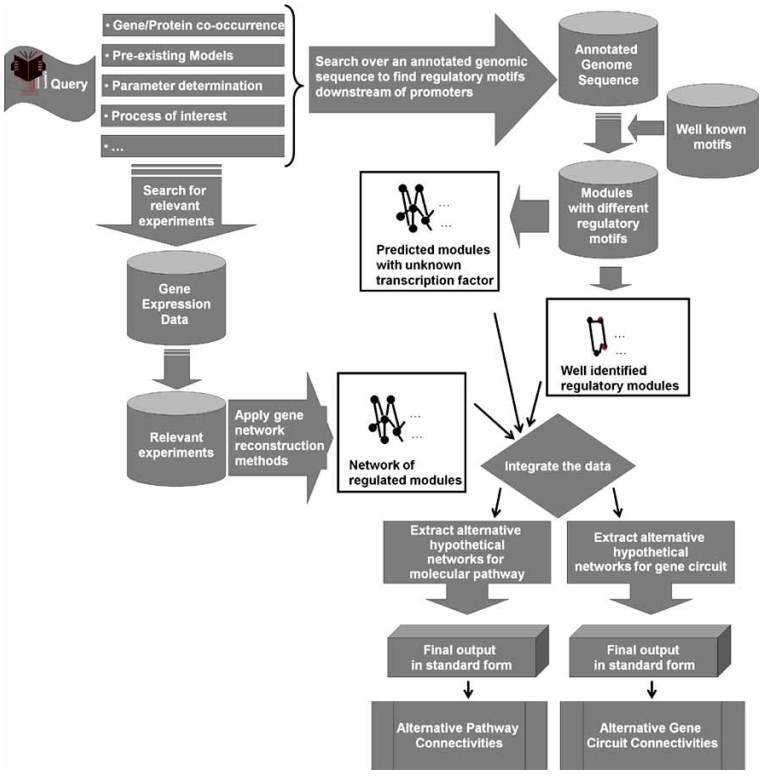
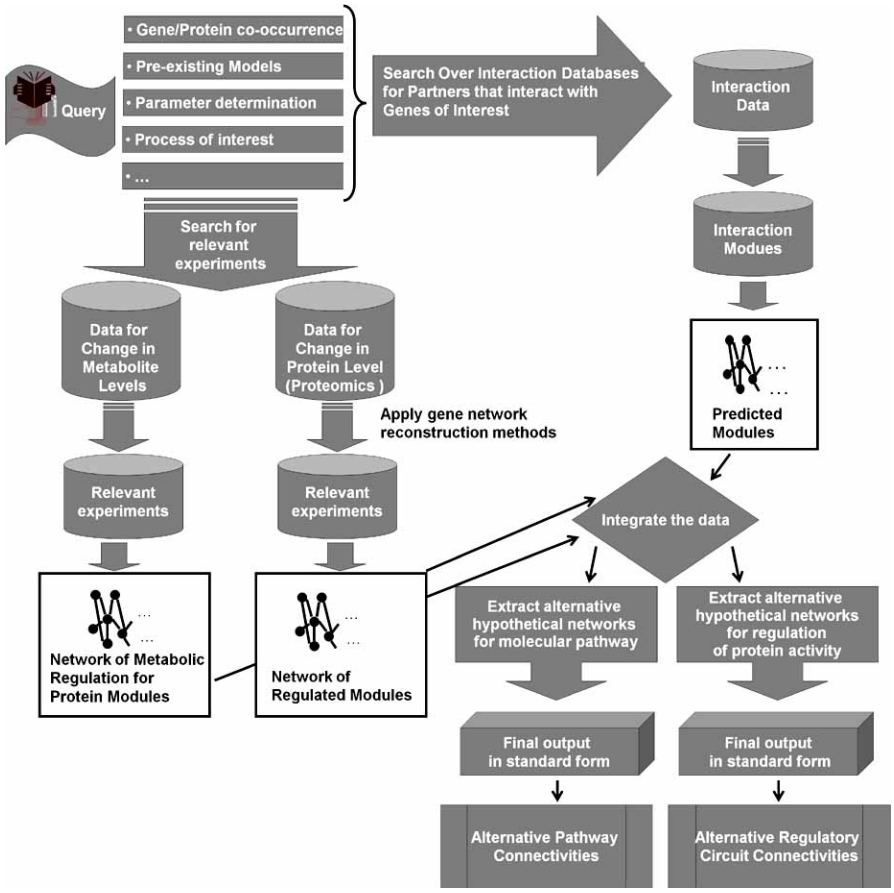

Query
• Gene/Protein co-occurrence
• Pre-existing Models
• Parameter determination
• Process of interest
• ...
Search over an annotated genomic sequence to find regulatory motifs downstream of promoters
Annotated Genome Sequence
Well known motifs
Search for relevant experiments
Predicted modules with unknown transcription factor
Modules with different regulatory motifs
Gene Expression Data
Well identified regulatory modules
Relevant experiments
Apply gene network reconstruction methods
Network of regulated modules
Integrate the data
Extract alternative hypothetical networks for molecular pathway
Extract alternative hypothetical networks for gene circuit
Final output in standard form
Final output in standard form
Alternative Pathway Connectivities
Alternative Gene Circuit Connectivities

Fig. (2). Contd…

C



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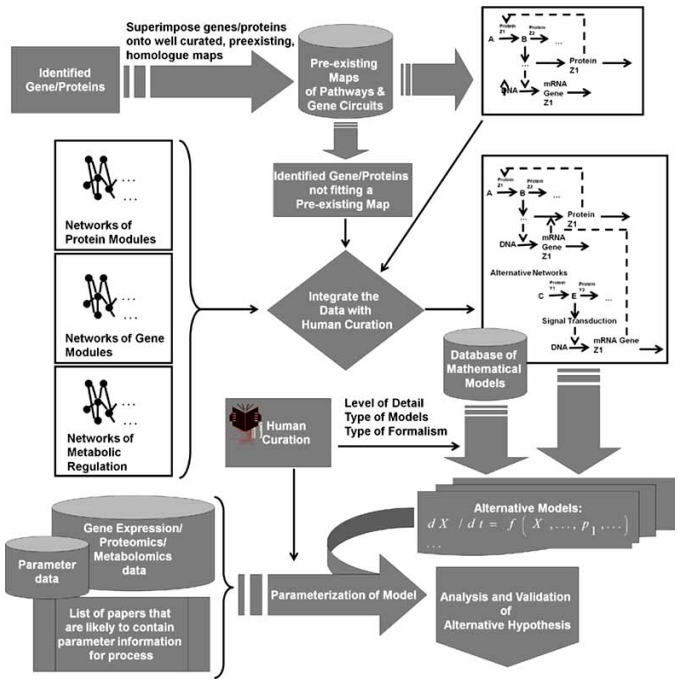

Fig. (2). Detailed schemes for the data mining of the different datasets for pathway and circuit reconstruction. **A –** Mining the literature. Automated analysis of abstracts and full text of papers can assist in reconstructing functional molecular networks of the cell. **B –** Mining gene expression data and genome sequence. Combining the analysis of gene expression data with that of sequence data, functional networks of gene regulation can be generated. **C –** Mining Proteomics and Metabolomics datasets. Proteomics and metabolomics information can be combined to derive protein modules with causal interactions. **D –** Integrating the data to generate hypotheses and validate models. Integrating the data from **A**, **B**, and **C** one can generate mathematical models that can be used to generate and test hypotheses regarding the behavior of molecular systems. See text for a more detailed discussion

in facilitating and improving the accuracy of *in silico* reconstruction of molecular pathways in cells [37, 41, 53-55, 61, 206-208]. For example, Alves and Sorribas have combined the use of literature analysis, phylogenetic profiling, analysis of sequences, and protein interaction prediction to reconstruct the regulatory network of mitochondrial ISC biogenesis in yeast [53-55, 58]. Bas Teusink's group has been developing a method, AUTOGRAPH, that combines sequence homology analysis with the existence of well curated metabolic maps to reconstruct the complete metabolic networks of new genomes. They have applied their method to the reconstruction of *L. lactis* metabolism [41]. Su *et al.* [209, 210] reconstruct both the pathway of phosphate assimilation and the gene circuits that regulate the expression of that pathway in *Synechococcus*, by combining genomic information with information about interactions between different genes and proteins. A combination of literature analysis and microarray data analysis has also been used to derive a regulatory network for *E. coli* and to test the consistency of microarray data based predictions [211]. There is an overlap of approximately 80% between the networks derived by the two methods. A reconstruction analysis of the regulatory network for the galactose biosynthesis pathway in yeast successfully predicts the genes with stronger regulatory influence on the pathway. The analysis has also been made by combining microarray data and protein interaction data [156]. A combination of time series analysis of gene expression and *in silico* prediction of transcription factor biding sites has been used to define regulatory modules in the inflammatory response of the macrophage, suggesting novel roles for the transcription factors ATF3 and NRF2 [154]. MAPK signaling pathways in the human blood fluke have been reconstructed by integrating phylogenetic conservation analysis and experimental gene expression measurements [212]. An analysis of the human phosphoproteome by combining consensus substrate motifs with context modeling was used for improved prediction of cellular kinase-substrate relationships [174, 213, 214].

Currently, the process of combining different datasets to generate testable biological hypotheses lacks a well defined structure and can only be partially automated. Nevertheless, there is constant progress along the road towards such automation, and for example, both Su *et al.* [209, 210] and Alves and Sorribas [55] propose and apply structured integrative approach for network reconstruction in molecular biology. An area where integration of bioinformatics and computational biology may be a powerful tool is that of synthetic biology. We will not discuss this subject any further in this paper and refer to the literature for reviews on the subject [215-221].

CHALLENGES TO AUTOMATION IN COMPUTATIONAL SYSTEMS BIOLOGY

Automatically integrating the information from the different datasets for pathway and circuit reconstruction in molecular systems biology is not trivial. The design of a global solution for this integration requires careful consideration of the goals, challenges, and limitations of the available a) data, b) data mining methods, and c) mathematical models.

At a first glance, one might think that the amount of data being generated by HTP methods is the most difficult challenge for the integration process. However, enough computational power is available to deal with this problem and data accumulation may not be a major issue. In our perspective, the major challenges are likely to be a) the definition of the necessary information content for a given type of data, b) the development of universal standards for the reporting and deposition of that data into databases, and c) the integration of information mined from different types of data into a coherent whole. It is crucial that the relevant information that is needed to address a given biological problem is well organized and easily accessible. Developing and applying standards in data reporting plays a central role in facilitating the automation of integrative approaches, because the existent of regularities in data structure is fundamental for the development of efficient computational methods.

Minimal Information Content and Data Reporting Standards

Achieving consistency in data organization, classification and storage into databases is crucial for achieving automated data integration. An important step in achieving such consistency is deciding what information to store in databases and how to report and organize that information.

It is not easy to define the minimal information required to describe the validity and scope of the deposited data, because the relevant information will change depending on the problem one wants to address when using the deposited such data. In some cases the data will have less than the minimal information required to correctly assess the conditions in which the experiment was done and the caveats that apply to the results. In other cases there will exist an excess of information that may be confusing and difficult to organize for one's purpose. An additional problem in comparing similar data from different origins is the following. When different groups replicate experiments using similar techniques, most of the times there are differences either in the exact conditions of the experiment or in the method for data acquisition and treatment. This may lead to contradictory results for what is apparently the same experiment.

If one wants to potentiate automated data mining and integration, a uniform language and ontology that can be used to report all experiments and data of a given type is needed. This ontology and language should contain sufficient information to decide if two data sets replicate the same experiment or not. Whatever standard ontologies evolve for the different datasets, such classification schemes must a) be self consistent, b) be applicable to any organism, c) have the fullest possible coverage of biological/experimental functions, d) adapt to new knowledge and information, and e) be easily integrated with ontologies for other types of datasets.

Several ontologies have been developed to describe the different datasets described in the previous sections (for example [222-233]). No set of ontologies is yet universally accepted. However, the Open Biomedical Ontologies (OBO), and the Gene Ontology (GO) are becoming *de facto* standards in the area [227]. Creating an ontology or a report language is not an easy task and the lack of an appropriate initial structure may compromise future utility. For example,

the GO has an uneven amount of information given for different classes of terms at the same depth of classification [234], which may become a problem in future. An area where information content standards are essential if one wants to facilitate automated model building is that of reporting parameter values for kinetic and thermodynamic experiments (see above section 3 and supplementary appendix). Standard report languages that, to some extent integrate relevant ontology information, have been proposed for HTP experiments [235], mathematical models [24, 236], gene that is expression data [237], mass spectrometry data [http://psi-dev.sourceforge.net/ms/xml/mzdata/mzdata.html], protein interaction data [160], and metabolomics data [225]. It is unclear if any of these languages obeys appropriate criteria for minimum information content and they are still far from being universally applied. However, computer-based algorithms and methods will only be able to automatically cross-correlate all available information in an efficient and error-free way when reporting of HTP experiments and data has converged to some standard.

Imagine that such standards exist and that one wants to automatically build the topology of the complete network of molecular interactions for an organism. An appropriate classification of the different data would, in principle: a) organize all enzymes into metabolic pathways; b) organize all signal transduction proteins, into signal transduction pathways; and c) organize all transcription factors (TF) and genes with binding sites for those TF, into gene circuits (Fig. **3**). Whenever a new genome is sequenced and annotated, its genes can be inserted into the maps, displaying which pathways and circuits are present in that specific organism. As differences in regulation between organisms are found or new pathways and circuits are discovered and reported, the classifications and maps themselves can be updated.

Informational Models for Integrated Data Analysis

There are different organizational models that can be used to store and integrate molecular biology data. One model is that of a central warehouse that stores database information (e.g. [238-243]). These databases are then linked to a server that provides different types of analytical tools. These tools allow users to mine and, up to a point, integrate information from the databases. Often, these central warehouses have a few mirrors distributed over the world wide web. Another type of integration service that is often found on the internet is the Metaserver. These servers, which usually analyze one type of data (e.g. [244-266]), submit queries to many different central warehouses and then process and integrate all the outputs.

Another organizational model for information storage and analysis is that of distributed sources of information and analytical services. Such an approach underlies GRID technology. At this moment there are at least three GRID-based approaches to the development of integrative platforms: BioMOBY [267-271], myGRID [272] or caBIO [273]. They rely on different levels of analysis and aim at integrating services and databases provided by different providers on the web. Such distributed architectures take advantage of the decentralized nature of the information and crawl over the internet space, identifying and accessing different types of data. The data are then pipeline into appropriate services, also decentralized and identified over the internet or in a central repository of services, to perform the analysis required by the user. However, there is still a long way to go before full automated integration of all available types of biological data that are available on the web is achieved.

Currently, the semantic web or web 3.0 is under development [274]. This new technology is expected to integrate information using machine learning methods. The effect of such technology in facilitating automated analysis and model building in molecular biology is potentially large [274-281].

Constraints to Automated Reconstruction of Biological Circuits

It is a truism that no one can be an expert in everything, which makes collaborative work fundamental in an integrative endeavor such as pathway reconstruction in molecular systems biology. However, it is also true that sometimes the demand for collaborative work placed upon researchers by both funding agencies and lack of time to familiarize themselves with different areas, rather than enabling research synergisms, is a hindrance to the progress of the research. Development of a structured integrative approach for network reconstruction and hypotheses evaluation would be an important contribution towards increasing the synergism among groups interested in different parts of the same biological problem.

Independently of the informational model(s) for data reporting, storage and integration, the user interface must be designed taking into account the target audience(s) for the different services. That audience will potentially be as diverse as the entire molecular biology community itself is. Hence, an integrative software service must take into account users with different expertise and different goals. Therefore, a flexible pipeline should exist for data processing and analysis. This pipeline should allow for different types of reconstruction questions to be asked within the integrative architecture. Some users will be satisfied by clicking in buttons and obtaining the final result, while other will want to manipulate the parameters and algorithms behind the interface for their own purposes and for refining standard analysis. There is a need to provide tools that are sufficiently constrained to reduce scientifically unsound use and sufficiently flexible to allow expert users to refine methods and data inputs. It is possible that the development of different tools for the different audiences is the most adequate way to address this problem.

Furthermore, one must be prepared to allow the users to perform different levels of reconstruction and to introduce new qualitative and quantitative information based on their own expertise. While some will be interested in the static reconstruction of pathways and circuits, others will be interested in the dynamic aspects of that reconstruction. Additionally, while some will be interested in a broad picture of what an organism can do, others will want to pursue a more detailed analysis of parts of the cellular response of that organism and only be interested in AI specific pathways. Thus, if one wishes to develop a methodology and implement it in a set of tools that can be useful to a wide audience and used in a scientifically sound way, one has to take several, sometimes contradictory, constraints into account. Human cura-

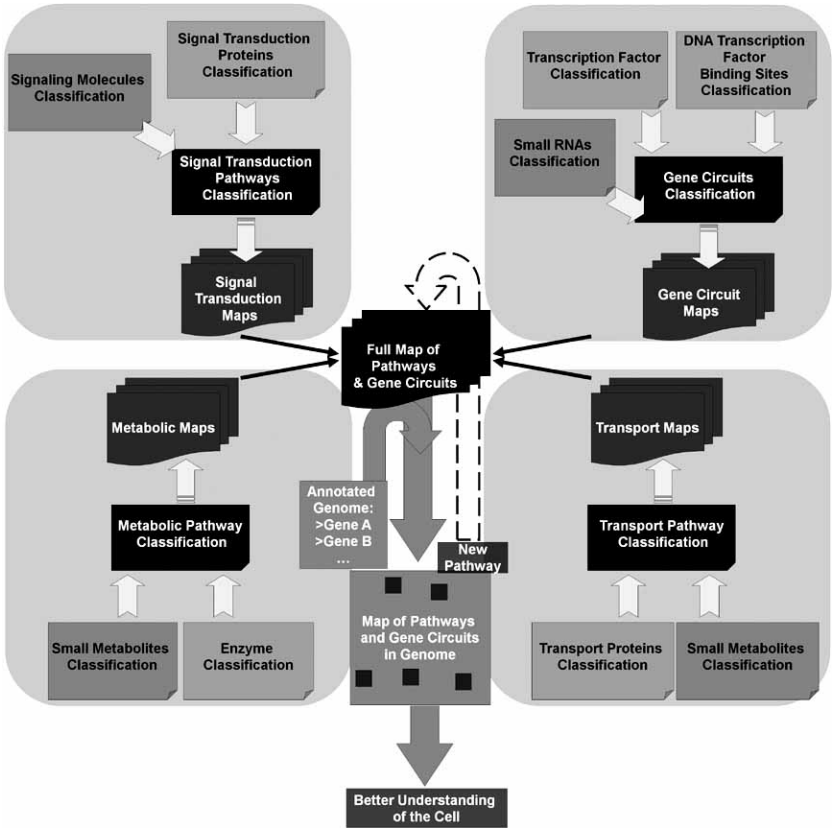

Fig. (3). Creating and integrating maps for pathways and gene circuits and applying them to the reconstruction of pathways in a genome will enable a better understanding of cellular physiology and behavior. Pre-existing gene circuits, metabolic pathways, transport pathways and signal transduction pathways can be used as a template for the assembly of the cellular pathways in newly annotated genomes. New pathways that are found in those genomes can be added to the set of pre-existing pathways. See text for a more detailed discussion

tion is and will continue to be an important part in the process, at even when AI development is able to generate tools that, to some degree, can replace expert intervention.

From a technical point of view, it is unlikely that a single research group will be able to come with an all-encompassing software platform that will allow for such an enormous diversity of reconstructions and goals. Thus, one of the requirements for such platforms is that they are open to external contributions. However, this openness must be somewhat more controlled than what is one finds in most of the open source projects that exist nowadays. Contributing programmers would have to adhere to some programming, organizational, and nomenclature standards, in a structure similar to that of wikipedia. The reason for such constraints is that in many cases there exists both duplication of efforts and inconsistent nomenclature even in complementary packages. An example for both these problems are many packages for the analysis of HTP data for developed the R platform.

Finally, an integrative platform should include some form of machine learning algorithms to analyze older datasets. It is unlikely that many resources are dedicated to reformatting data from previous decades, making that data compatible with whatever standards percolate for each type of dataset. Trainable machine learning algorithms are currently the best hope of mining the older data efficiently. This is an important challenge for the AI community.

SUMMARY AND CONCLUSIONS

In this work we analyze the *in silico* reconstruction of cellular pathways within the context of molecular systems biology. We start with a discussion of the data sets that are available for this reconstruction in the post genomic era. We describe how systematic integration of the different types of information can be used to create mathematical models that generate testable biological hypotheses about the functioning of the reconstructed system. We then review examples of how such data have been integrated to reconstruct pathways and obtain a systemic understanding of how those pathways work. Finally, we provide a general discussion of how different bioinformatics and computational biology tools can be integrated for automated model generation. We are aware that a full automation of this process may not be possible. However, if we are ever to approach such a goal, at least two issues must be addressed:

a) Better and uniform standards of reporting for the different datasets.

b) Better machine learning methods/artificial intelligence algorithms to facilitate automation of the analysis.

Any improvement in these areas will translate into a more efficient and more accurate network reconstruction and facilitate the systemic understanding of molecular pathways and circuits.

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SUPPLEMENTARY APPENDIX: AVAILABLE DATASETS, ANALYTICAL METHODS AND TOOLS

Here we provide a more in depth analysis of the different datasets that are available for metabolic reconstruction and of the tools that can be used to mine those data sets for information.

S.1 Literature Data

Available Datasets

Because thousands of scientific papers are published monthly within many areas of molecular biology, it is not feasible for anyone to read them all. A digest of the information contained in the literature is available on-line, in literature databases that contain the abstract, title and keyword information such as ***MEDLINE*** [1], ***PUBMED*** [2], ***Web of Science*** [3, 4] or others [5]. The full text of many papers and some books is also available in databases available in journal home pages. The information in these databases is organized according to strict criteria that facilitate finding papers reporting on many different subjects. The various databases are associated to diverse search engines that have different sensitivities and goals [6-11]. Automated analysis of a natural text within a given literature database to find specific pieces of information (gene names, gene function, parameter values, interaction information between individual molecular components, etc.) is a rapidly evolving field [12]. Truly flexible, multipurpose, and automated network mining from the full text of live documents brings enormous added value from the literature datasets to molecular systems biology research [13-16]. Values for kinetic and thermodynamic parameters for many different reactions and processes are also buried in the literature. Mining for this data has been mostly manual, and parameter information stored in databases is not in general sufficient to evaluate the usability of such data (e.g. ***BRENDA*** and enzyme parameters [17-19]).

Analytical Methods and Tools for Text Mining

Natural language processing and mining is developing rapidly [3, 6, 20-39]. Currently, several alternative models are being used to develop tools that mine the literature. One of the models is ontology-based and relies on creating classifications that reduce ambiguity in the text to be mined (e.g. [3, 22, 25]). Another model is based upon the existence of thesaurus and dictionaries of synonyms [26, 40]. A third model, based on machine learning and using a combination of ontologies and thesaurus, allows the search algorithm to be trained while mining text information (e. g. [20, 21]). This should improve the performance of the methods in a near future. As a general goal, it is increasingly important to make the methods for automated text mining available to the biological researchers in applications that facilitate the derivation of biologically relevant information. Some text mining tools that fulfill those requirements are already available (e. g. [20, 36, 40-48]). For example, they can be used to extract network information [41, 42, 44, 47] or protein localization [48] from raw text and literature analysis. It would also be invaluable to develop specific software applications that allow researchers to accurately identify parameter value reports. Ideally, an automated mining of parameter information would allow for the identification of organism, reaction, units and conditions of parameter determination.

It is likely that the major hindrance to a precise and complete mining of the literature information by automated text mining tools will be the decades of accumulated literature using non-standardized nomenclature and classifications (see main text). Possible remediation for these problems are a) creating better dictionaries of synonyms, and b) using artificial intelligence and machine learning methods to increase the size of the dictionaries and allow the algorithms to make educated guesses regarding correspondence of terms. Part of this upgrading of the dictionaries could be done according to a combination of machine learning and a "wiki-like" model. That is, as users executed their jobs they could provide information and feedback to the methods regarding new synonyms and correct/incorrect guesses made by the software.

S.2 Sequence Data

Available Datasets

With the increasing number of genome sequencing projects that are completed or underway, increasing amounts of sequence data are available. Sequence datasets are too numerous to mention. However, a few central repositories contain most of the sequence information, often duplicated among the repositories. The ***NCBI*** web pages [36-42] and the ***KEGG*** web pages [43-47] contain annotation information for most organisms with fully sequenced genomes. ***Expasy*** [48, 49] also has this information with a focus on the proteins. The ***TIGR*** institute [50, 51] web pages and the ***DOE Joint Genome Institute*** [52] web pages also contain much of this information. Then, individual genome project web pages contain a wealth of functional details regarding their genome of interest and its genes. This is the case for example of the ***SGD*** resource [53, 54] for the yeast ***Saccharomyces cerevisiae***, of WormBase for *C. elegans* [49] or ***FlyBase*** for *Drosophila* [50, 51]. There are also databases available for RNA genes and gene targets (e. g. [52-55]) as well as for regulatory sequences in the genome of different species (e. g. [56]).

Analytical Methods and Tools for Functional Annotation of Sequences

We will not discuss the computational tools and methods used for genome sequencing and annotation. Those interested can find detailed explanations of the historical evolution of genome sequencing methods in the paper by Ankeny [57] and the references therein. For more recent reviews on the subject see [58-61].

Once the genome sequence is completed and assembled, one faces the task of finding the open reading frames (ORFs). Searching for homology to known genes is one of the methods for genome annotation. The *de facto* standard tool for aligning homologous sequences is the ***BLAST*** alignment program [62]. When no sufficient homology can be found, other methods and tools exist to predict ORFs. An overview of such methods can be found in [63]. By and large, the gene finding methods split into signal finding methods or content finding methods. Signal finding methods look for elements that signal the beginning and the end of genes, such as the ATG codon, or sequences that are recog-

nized as promoter sequences. However, many other signals that can be used for this purpose exist in genomes. These signals include for example transcription factor binding sites or ribosome binding sites in prokaryotes. Content finding methods are based upon finding differences in general statistical properties between coding and non-coding sequences of the genome. For example, in a genome the frequency of dinucleotides is different in coding and non-coding sequences, as is the GC content of the sequence. These differences can be used to separate the two types of sequences. Different gene finding tools use alternative combinations of methods to find signals and content, thus improving their probability of finding true ORFs [64-70]. The available tools for gene finding include ***GENEMARK*** [64], ***SNAP*** [68], ***Ensembl*** [71], ***SPG2*** or ***TWINSCAN*** (e.g. [67]), among many others.

Metagenomic sequencing efforts, where the DNA sequence of an environment is sequenced and must be assembled, are now emerging [72-76]. Finding genes in these sequences is not easy because often one does not know to which genome a sequence belongs to. Ideally, the sequences should be assembled, whenever possible, into its individual genomes which can be analyzed to find genes. However, if such assembly is not possible, specialized programs must be used to predict genes in large fractions of orphan DNA [77, 78]. ***Metagene*** [69] is an example of a tool developed specifically for prokaryotic gene finding in metagenomic samples.

Several centralized servers such as ***Expasy*** [79], ***KEGG*** [80-83] or ***NCBI*** [84] provide tools that allow researchers to infer functional information from sequence data. The information made available for any given sequence ranges from the actual molecular function of an individual gene/protein to the prediction of post translational modifications in a protein [77] or the prediction of its location within the cell [85-88]. Also, several on-line services provide maps of metabolic pathways, gene circuits, and signal transduction pathways [89-100]. By superimposing the different genes identified within a genome onto those maps, one gains knowledge about qualitative aspects of cellular behavior. For example, if there are no genes coding for a given amino acid pathway within a genome one can expect that the organism will require that amino acid as a part of its diet. Some tools and servers that allow for analysis of phylogenetic conservation and congruence studies between genes in sets of fully sequenced genomes are also available [101-108]. There are also tools available for the analysis of RNA genes and for the analysis of regulation by RNA [109-115].

S.3 Structural Data: Mining for Function

Available Datasets

As is the case for sequence data, the amount of structural data for the molecular components of cells is also increasing steadily. The whole proteome structural genomics projects [116, 117] that are currently under way contribute to this increase [118]. There is a central repository of protein and nucleic acid structures at the protein databank (***PDB***) [119]. The structures deposited at the ***PDB*** include complexes formed between proteins and even provide information regarding the stoichiometry of those complexes. Many of the structures contained in the ***PDB*** can also be found elsewhere, for example at the ***NCBI*** [84].

Analytical Methods and Tools

Computational methods and tools are necessary from the ground up in the analysis of structural data. The most common ways to obtain protein structure are:

a) Submitting purified protein crystals to high energy radiation bombardment (e. g. X-Ray or neutrons) and capturing snapshots of the radiation diffracted by the protein crystals. These snapshots are then reconstituted into a 3D structure using appropriate software [120-125].

b) Using NMR technology to take spectral snapshots of the protein in solution [126-130], that are then reconstituted into a 3D structure using appropriate software.

Technical details regarding structure determination are well beyond the scope of this review. For practical reasons, our main focus will be on structure visualization, analysis and prediction. Once the proteins structures are available they are reported and stored using a specific data format [131-133] that includes, among other things, the 3D coordinates of the different atoms in the protein, the native organism, and the oligomerization state of the biologically relevant form of the protein. An analysis of these structures requires tools for their visualisation. One of the simplest tools available is ***RasMol*** [134]. This free tool allows the researcher to visualize and do minimal manipulation of the structure files. Another free tool is ***DEEPVIEW*** [135]. This tool is more sophisticated and, for example, it allows for point mutations to be introduced into the structure and energy minimization to be performed. It also allows researcher to do their own structural modelling of their protein of interest if the structure has not been determined yet. There are alternative methods for structural modelling. The most successful can be roughly grouped as follows:

a) **Fold prediction.** In some cases, when not enough information is available to obtain a 3D structure prediction, there are methods that allow the prediction of the secondary structure of a protein [136, 137]. Currently, many of the methods used for fold prediction are used as intermediate steps for obtaining a 3D structure prediction of proteins for which there are no known homologues in the PDB.

b) **Homology modelling.** The researcher has a sequence of interest and finds homologues of that sequence that have had their structure determined. By doing a sequence alignment, the researcher can then superimpose the sequence onto the known structure, followed by energy optimization. Many servers do homology modelling. ***SWISSMODEL*** [135] and ***3DJIGSAW*** [138] are a few of these. To find a list of other such servers one can consult the special issues of the journal Proteins that report the ***CASP*** (critical assessment of structural prediction) results [139-145].

c) **Threading.** In the absence of homologues with known structure, one can thread the sequence over a known structure of a protein that one thinks is likely to have structural similarity, followed by energy minimization.

d) ***Ab initio* modelling.** By using short stretches of homology over different protein structures one can also create models for the structure of a protein that has no homologues with a known structure. ***Robetta*** is an example of a server that allows researchers to do *ab initio* modelling from sequence [146]. To find a list of other such servers see the special issues of the journal Proteins that report the ***CASP*** results [139-145]. An alternative *ab initio* method is starting with the linear alpha helical structure of a sequence and by doing molecular dynamics simulations study how the protein folds. However, there is not enough computational power available to use this approach as a standard procedure.

The use of *in silico* protein docking can also reveal details about how different biological molecules physically interact. Interested readers can use different programs and servers to perform such docking studies [147-164].

S.4 Gene Expression Data

Available Data

Large datasets measuring changes in gene expression for entire genomes, under different conditions are accessible for many different organisms. These data can be used to infer which genes and proteins are important for a specific cellular response, thus giving researchers information about the role of genes with unknown function. Additionally one can infer information regarding the dynamics of the function, which is something harder, or even impossible, to do from sequence or structural data. Gene expression data is deposited in different databases. The Gene Expression Omnibus ***(GEO),*** at the ***NCBI*** offers the raw data for many of the micro array experiments published in the literature [165]. Other general databases exist, both for microarray data and for other types of gene expression experiments (e. g. [166-171]). There are also databases for gene expression for specific human diseases (e.g. [172, 173]) in specific organisms, such as mouse [174], *Arabidopsis* [175] and others.

Analytical Methods and Tools

With the advent of fully sequenced genomes, there came the invention of DNA chips and microarrays [176, 177]. In microarray experiments, probes that hybridize mRNA for a large fraction of the individual genes in a genome are imprinted onto a slab of material. Cells are grown and subject to some stressful condition, which may include mutation. Then cells are collected and their mRNAs are purified, amplified and hybridized with the probes in the microarray. By comparison with a control condition, these experiments reveal how much the expression of individual genes changed in response to the stress. For a more detailed description of microarray experiments see for example [178].

Other methods also allow measuring changes in gene expression at the whole genome level. Such methods include for example SAGE [179, 180] or CAGE [181] experiments, where mRNAs are directly amplified and identified in solution without the need of an imprinted microarray. A variation of the classical DNA microarray is the tiling microarray. The probes in these arrays are designed to cover entire regions of the genome, rather than individual genes [182]. These microarrays have shown that there are large islands of expression, for example in the human genome, that are either outside the genes or constitute only fragments of those genes [182, 183].

As is the case with the determination of protein structure, computational methods are needed to process the data in microarray experiments that measure changes in gene expression. The determination of change fold for gene expression in microarray experiments is usually done using differential measurements of fluorescence or radioactivity. Image capture, processing and noise filtering are areas where appropriate algorithms must be used in order to ensure acceptable data acquisition and normalization [178, 184, 185].

Once the data are collected and organized in its final form, then a different set of computational methods is required in order to determine whether a change in gene expression is actually significant or whether it can be attributed to noise [186-189]. There are no general methods that are universally considered as more appropriate to analyze the significance of a given change fold. This is an area of intensive research. Microarray data have problems of reproducibility and robustness, which underlines the importance of experimental design, replicate experiments and appropriate statistical methods as a means to identify significant changes in gene expression.

There are several tools to analyze microarray gene expression data and cluster genes according to their changes in expression. For example, the tools provided in ***GEO*** [165, 190] can be used to comparing different gene expression profiles. ***TM4*** [191] and ***BASE*** [192] are examples of software platforms that allow for the analysis and comparison of gene expression profiles. ***Bioconductor*** [193] is a popular open source software platform that uses a set of functions and procedures written in R to analyze microarray data.

S.5 Proteomics Data

Available Data

What information from proteomics experiments should be stored in databases and how it should be organized is still not consensual [194-200]. In consequence there is still no central repository of proteomics data. ***PRIDE*** [199, 201] and the ***Peptide Atlas*** [202] projects are probably the resources that contain more data from proteomics experiments. The global proteomics machine [203] provides software and internet services that mine data from these and other proteomics data sources. On a smaller scale, the ***NetworKIN*** database stores proteomics information about the phosphorylation state of human phosphoproteins [204].

There is, to our knowledge, no good and general HTP method to characterize protein activity on a large scale (however, see the main text for a promising new method.) The development of such methods would allow for the large scale determination of enzyme parameters, binding, and

thermodynamics constants and would likely create a revolution in mathematical modeling. Nevertheless, and independently of the limitations, the application of proteomics methods to the study of the dynamics of protein post translational modification can be a step forward in obtaining this type of information.

Analytical Methods and Tools

The progresses in 2D gel electrophoresis and in Mass Spectrometry (MS) and NMR technology have permitted the analysis of whole cell samples in order to identify the protein complement of the cell [205-225]. In general, proteins are separated via electrophoresis. The different proteins are then identified using MS techniques or NMR techniques. Unfortunately, datasets from these studies are not as abundant nor publicly available as those from microarray experiments. Furthermore, a central repository for all the proteomics experimental data is still lacking. Nevertheless, the available proteomics data already provide information regarding community proteomics [226], subcellular localization of proteins [227-229], dynamics of protein turnover [207, 208, 230, 231], post-translational modifications of proteins [217, 232-240], markers of specific responses, and many other aspects of protein function.

Most of the software effort in proteomics is still dedicated to creating tools for identifying proteins from the MS or NMR spectra. These tools work, for example, by using Fourier analysis for spectral identification. The spectra are compared to know protein spectra, thus allowing for the identification of proteins in sample. The data sources mentioned in the previous section provide tools that allow researchers to identify the proteins that are present or absent in each sample.

S.6 Metabolomics Data

Available Data

There is, to the knowledge of the authors, no general central public repository of metabolomics data. A few databases already exist, but their content is fragmentary, with respect to the amount of reported metabolomics data [241-244]. Furthermore, the problem of developing a standard in data reporting is a difficult one that has only now began to be addressed [245-249]. Links to some of the current repositories of metabolomics data available on the web can be found at http://www.bmrb.wisc.edu/metabolomics/external_metab_links.html. As time progresses and standardization settles into the field, it is likely that central repositories of metabolomics data will be developed. Such data will be invaluable for example to derive parameter values and perform mathematical model validation [250].

Analytical Methods and Tools

Cells use proteins to take up nutrients and make other small molecular species. There may be more than 200 000 metabolites in the plant kingdom alone [200]. Unlike proteomics or genomics, in metabolomics experiments hundreds of types of molecules with different chemical properties must be detected. Thus, detection techniques must be sensitive, robust and versatile. Technological advances in NMR spectroscopy [251, 252] and MS have facilitated the use of these two types of techniques to measure the changes in the concentration of small metabolites over time [245, 250, 253-269]. NMR metabolomics methods are inherently quantitative but have a low sensitive and require larger amounts of a metabolite for identification than MS methods. MS is more sensitive but cannot provide absolute quantification. A more detailed analysis of these and other experimental techniques used in metabolomics experiments can be found in the literature [268, 270, 271].

Computational methods are required at every step to process and analyze metabolomics data [263]. A single spectrum in a metabolomics experiment may contain many different signals. This requires powerful computational and statistical methods as well as accurate spectral maps for the many different types of metabolites. Statistical and computational methods are needed to deconvolute the metabolomics spectra. Pattern recognition methods are required when comparing different metabolomics experiments of the same system under different conditions. Methods for pattern recognition are reviewed for example in [248, 249, 272]. The comparisons between different metabolomics profiles help identifying metabolites whose levels are significantly modified, thus pointing at direct changes in regulation of metabolism between the two conditions. Algorithms such as those developed by Vance *et al.* [273] can be applied in determining the causal sequence of events in metabolomics experiments. Parameter fitting methods and algorithms can use the data to obtain parameter values for mathematical models [274-278].

S.7 Interaction Data

Available Data

Interaction data can be divided into three categories:

Genetic and functional interactions. By genetic interactions data we mean data that provide information regarding essentiality of genes (e.g. [279]) or complementation between different genes [280]. For example, in *S. cerevisiae*, a multi-copy vector containing the ISA2 gene rescues the Δgrx5 mutant phenotype [281]. By functional interaction data we mean data that provide information about which processes and functions are genes and proteins involved in. Genetic and functional interaction data provides very useful information regarding the function of the different genes. It may be invaluable in providing information regarding the pathways and responses in which genes of unknown function are involved. ***Prophecy*** is a database that collects this data for *S. cerevisiae* [282]. However, there is, to our knowledge no other resource that systematically collects this information for other organisms. Another type of functional interaction data is that of genes and proteins that are known to belong to specific metabolic pathways, signal transduction pathways and gene circuits. By homology analysis many proteins of any genome can be placed into the different pathways and circuits, thus providing functional information.

Physical interactions. By physical interactions data we mean data that provides information regarding actual physical interactions between different proteins or between proteins and nucleic acid. There are many different types of experiments that provide data regarding these interactions. Automated and large scale Two Hybrid Screens (THS) systematically take the proteins of a genome and scan for physical interactions between each pair of proteins [283-285]. If

an interaction exists, a chemical product that can be detected is produced or photons are emitted [285, 286]. A systematic application of the THS technology has been reported for yeast, worm, fly, human, *H. pylori* and other organisms (see [286-296]). Different reporter methods will provide different sensitivity and robustness in THS [291]. The THS assays also have problems with false positive and false negative interactions. This is so because the interaction between proteins is often measured in conditions that may not be the native conditions under which the two proteins meet in the cell. An alternative method for HTP assays of protein interactions is by using classical biochemical co-purification methods followed by MS identification of the purified proteins [287, 297]. So far there is a 10-20% overlap between dataset of protein interactions that are determined independently [298]. It is conceivable that in the near future protein chips, where the individual proteins of the genome are secured to some support material, will be devised and used to fish out interacting partners from whole cell protein extracts. There are already affinity chips that are used for purification of proteins [211]. Additional sources for protein-protein interaction information are *in silico* predictions. By using sequence information and evolutionary analysis there are methods that predict, for example, co-evolution of residues in different proteins or gene fusion events in different genomes. Predictions of co-evolution or detection of gene fusion events are interpreted as indicating that there is a physical interaction between two proteins (see [299-302] for a review). On-line resources where information from HTP interaction experiments is deposited include the ***BIND*** database [303] the ***PRIME*** database [304], the ***MIPS*** database [305], the ***DIP*** database [306] and the ***MINT*** database [307]. All these databases also contain information regarding small scale interaction experiments, mined from the literature. Some databases focus on specific organisms, as is the case for example of the *E. coli* database ***EchoBase*** [308].

Another type of physical interaction between cellular components that has implications in cell function is the one occurring between proteins and nucleic acids. Information regarding which regulatory motifs are bound by the different transcription factors (TF) in a genome is important because it allows the identification of which genes are regulated by each transcription factor. The development of ChIP-chip technology [309, 310] enables HTP research about which genes are regulated by which TFs in a genome. In ChIP-chip experiments, cells are grown under the conditions of interest and then they are lysed. Then one of a series of methods is used to crosslink DNA and protein, followed by fragmentation of the genomic DNA. The DNA fragments are then co-purified with the transcription factors of interest, amplified, and hybridized with a genomic DNA microarray. This hybridization reveals where in the genome the TF binds, allowing the inference of gene circuits controlled by each TF. As is the case for protein-protein interactions, there is no large scale central repository for this type of information. There are however smaller databases that contain information regarding the different aspects of protein-DNA interaction. The ***NDB***, ***PSIBASE*** and ***AANT*** databases contain information obtained from DNA protein complexes deposited at the PDB [311-313]. ***NPInter*** also has information about interactions between protein and non coding RNA in *E. coli, S. cerevisiae, C. elegans, D. melanogaster, M. musculus,* and *H. sapiens* [314]. The ***BDTNP*** database at Berkeley (https://bdtnp.lbl.gov/Chipper-/index.jsp) is a secured access resource for the analysis of fly related DNA-protein interactions.

Analytical Methods and Tools

The computational methods used for mining interaction data range from those used to find co-occurrence of genes in the literature, to those used to create protein alignments and to calculate co-evolution of specific residues, and to those used for *in silico* protein docking. Many algorithms use graph theory to derive networks of functional interactions from the different types of data. From a mathematical modeler's perspective, the representation of networks obtained from mining interaction data is in general far from perfect. Most representations of these networks show a set of nodes (the genes/proteins) connected by edges whenever there is some form of interaction between them (e.g. [315]). This kind of representation is ambiguous and prevents direct utilization of the networks for the generation of mathematical models. Some tools (e.g. pathway tools [96, 100]) represent networks with diagrams that can be easily and unambiguously parsed as models. However, this is mostly for enzyme network data. Building something similar for systematic reconstruction of gene circuits and signal transduction pathways is difficult until a standardized classification is accepted for signal transduction proteins and transcription factors.

S.8 Kinetic Data

Kinetic data are fundamental for the development of mathematical models in molecular biology. Having such data in abundance and devising good classification systems for the different functions of cellular species facilitates automated model creation. This job has so far been easier for enzymes than for other types of molecules. Many years ago, the enzyme commission (EC) developed a functional classification scheme for the activity of enzymes that, although not perfect, is very useful when annotating proteins with enzyme functions [316]. Databases such as ***BRENDA*** [19, 317-319] include information about the function of different types of enzymes, and in some cases also about kinetic parameters and thermodynamic energy measurements. Kinetic information can also be found for example in ***KDBI*** [320]. ***EzCatDB*** [321, 322] and ***MaCie*** [323, 324] include information regarding catalytic mechanisms. ***ProTherm*** provides thermodynamics data for mutant proteins [325]. However, the kinetic information is not classified and organized in such a way that researchers can directly take the parameter values and use them to build models. Anyone who is interested in doing so must consult the original sources where the parameter values are reported and make sure that units are consistent and that the experimental conditions during the determination are appropriate for the purposes of the model.

It would be important to have similar functional classifications for other types of proteins, such as receptors, structural proteins, transcription factors and so on. This would facilitate automated building of schema that could then be used to build mathematical models. However, proposed standard for such classifications are still far from perfect or universal. Some classifications based upon the DNA binding

motifs or the structural binding domain of transcription factors have been proposed (see e.g. [326, 327]). Classifications similar to that proposed by the EC for enzymes have been proposed for signal transduction molecules [328] and transport proteins [329]. However, their use is far from widespread and it is unclear if they will be adopted as a standard.

It would also be extremely useful to have standard functional classifications for:

a) **Lipid components**. The chemical structure of these lipids may constitute a good basis for such a classification.

b) **Nucleic acid molecules**, including the different types of small RNAs that are currently being shown to play important roles in regulating and catalyzing functional aspects of the cell metabolism. Although a general classification has been proposed for RNA [330], it focuses on structure, rather than function.

c) **Small metabolites**. Again, the chemical structure of these small metabolites may constitute a good basis for a functional classification. The *Ligand* database [331] is a good repository of small metabolic molecules. Other methods, based on chemical and structural information allow for the development of different types of metabolite classifications (e. g. [332]).

Having a classification for the different proteins, genes and other components of the cell has allowed the creation of many resources with more systemic functional information such as lists of pathways, gene circuits and signal transduction pathways (e.g. ***Metacyc*** [95], ***KEGG*** [80, 82] or ***EMP*** [333]). Such resources provide annotated information regarding the function of the different cellular components.

S.9 Mathematical Models

Available Data

Mathematical models of specific molecular processes in different organisms are an increasingly important source of information for the understanding of the systemic behavior in molecular biology. Such models have been published since the 1950s and they are mostly scattered in the literature. Datasets that collect and organize these models have been recently started, but relatively few models are included [334-341]. Model annotation is much more challenging than annotation of genes and proteins for several reasons. For any given system, one can create models using different formalisms and at different levels of detail. This depends, among other things, upon the questions one wants to ask of the model. Models of similar processes on different organisms may involve different enzymes, different number of steps and branch points, etc. Finally, the existence of specific regulatory signals that may vary from one condition to another, from one protein isoform to another, or from one organism to the next, introduces further complexity into the modeling process.

Despite all these difficulties, it is clear that a standard model reporting language is useful to reutilize models or part of models that have been previously defined and curated. Building models is a challenging task and any tools that facilitate the model building process are useful. Currently there is an effort to make newly published models comply with minimal information criteria for publication [338]. There is also a drive to facilitate the interchange of model files between different programs and researchers by asking that models be deposited in central databases, using a standardized modeling language such as ***SBML*** or ***CellML*** [342-345]. A large investment is also being made in the development of tools for model set up and analysis [346-352]. The model database efforts are currently in their infancy and the databases are still small. However, if one is willing to search the literature, one will find many models that are useful in understanding the systemic behavior of molecular biology processes.

Analytical Methods and Tools

There are different formalisms for writing models and different classes of models. The choice of a given mathematical representation depends on the goals of the modeling exercise, the complexity of the target system, and the available data (either in terms of parameters or in terms of time course data, metabolite levels, enzyme activities, etc.). Furthermore, the same problem can be studied at different levels of complexity. For example, one can find models that range from the modeling of a single enzyme reaction to those that attempt to model the whole cell, going through intermediate scale models that consider small sets of cellular pathways and circuits.

Among the smaller scale models that consider only a very limited number of molecules and reactions we find many different types of modeling. Mainly, quantum modeling is used for investigating the active state of an enzyme reaction [353], molecular dynamics is used to study protein folding [354], stochastic modeling can be used for modeling networks of reactions with a small number of molecules [346]. For large scale models of many pathways, the quantum modeling, molecular dynamics modeling, Monte Carlo modeling and stochastic modeling become unfeasible due to lack of computer power. Sets of ordinary and partial differential equations are common in exploring the dynamic behavior of complex pathways [275, 355, 356]. Qualitative types of modeling such as discrete binary mode modeling [357-359] or flux balance analysis [360-363] are also used to study large networks.

Integration of models from different origins and using different mathematical forms is a very challenging problem. Before discussing possible solutions to this problem, it is worth it briefly detail some of the specific requirements of the most common formalisms. A simple description of a process is appealing because one require less information to build a model from such a description. However, the utility of the resulting models may be limited if our goal is to understand dynamic behavior and design principles. More complex descriptions of a process have better changes of producing models that reproduce a wide range of behavior for the system one is studying. However, the complexity of such models combined with lack of information regarding the process may prevent the creation of more detailed models.

A similar argument can be made for mathematical representations. Simple linear representations will facilitate the analysis of models but will prevent the model from capturing non linear behavior that is typical of biological systems. Approximation theory provides many alternative formalisms that combine simplicity with non linearity [364]. We now briefly discuss different types of models used in molecular biology.

Finite State Models

Finite state models analyze the dynamic behavior of networks in which the nodes are allowed only a finite number of states (e.g. on-off). Such a mathematical description is very simple, thus allowing the analysis of complex and large networks. The basic information required to built such models include: (i) the stoichiometric matrix of the reactions taking place in the model, which can be derived from the conceptual scheme of the target system, (ii) the relevant regulatory signals, so that we can appropriately describe the on-off states of the system's elements, and (iii) a set of functions, usually sigmoid, that are used to decide the on-off state of the different elements (e.g. [365]). Finite state models are appealing because they need a minimum of information as input and provide clues about the dynamic properties of a system [358]. However, in general, these models will not be useful if detailed quantitative predictions are needed.

Models Based on Stoichiometry

Flux-balance analysis (FBA) and graph theoretical approaches study network topology and relate changes in that topology to qualitative changes in the dynamics of the system. These models often study the entire metabolic network of an organism [360, 366-381]. As in the case of the finite state models FBA requires a minimum of information, besides the stoichiometric matrix of the model. From this matrix and using graph theoretical methods and optimization techniques, FBA can, for example evaluate the effect of deleting a gene on the flux distribution, thus identifying the expected phenotypes for mutants in the different enzymes. A disadvantage of these approaches is that they fail to account for dynamic regulation. FBA models are a good choice for genome-wide descriptions and can be used as a base for more detailed modeling of specific pathways.

Stochastic Modeling

Stochastic models consider the details of the system and either a) use a Master equation to study the time distribution of the metabolite concentration [382-385], or b) consider every molecule in the system and follow their time evolution using chemical kinetics and algorithms that are able to statistically predict each elementary reaction event in the system. *Gillespie like algorithms* require that all reactions are mass action in order for the methods to work [346-349]. There are now newer stochastic methods that are being developed and loosen this constraint [386]. These models are computationally very heavy. Whenever dealing with processes that involve large pools of molecules, deterministic approaches are good approximations to the more detailed stochastic approach.

Continuous Modeling

The use of differential equations, either ordinary (for well stirred systems) or partial (for systems with spatial differentiation) to define mathematical models that can be used to quantitatively simulate the dynamics of a molecular system was the first [387, 388] and is still the more prevalent choice as a computational modeling tool. There are mathematical rational expressions that simplify complex mechanisms and are considered to represent more or less accurately the dynamics of many different types of individual molecular biology processes. For example, for a simple one substrate enzyme, the Michaelis-Menten rate expression can represent the dynamics of the reaction in well stirred systems [389]. Many other rational expressions have been derived for more complex enzyme atic mechanisms. However, we lack the knowledge of how many processes happen, which prevents the use of these pre-defined kinetic expressions in modeling. Even if the mechanism is known, in most cases the parameter values have not been measured and thus the mathematical representation using rational expression cannot be parameterized. Furthermore, when parameter values are available, the experimental conditions under which they have been measured may invalidate their use.Additional complications occur when one is interested in modeling spatially non-homogenous systems. In such a case PDEs must be used and many of the simplifying assumptions for obtaining rational rate expressions such as the Michaelis Menten kinetics often break down. Thus, depending on the level of mechanistic, spatial and mathematical detail one wants to consider, a given conceptual scheme can generate many different models.

One way to side-step the lack of knowledge regarding mechanism and, in some cases, parameter values is by using approximation theory. Mathematical theory allows us to approximate functions of known and unknown form with structured, canonical, representations that are precise at the operating point of the approximation and accurate over a varying range of values about that operating point. The most widespread and successful approaches use Taylor series to approximate the kinetic functions. This strategy was used by Savageau in the late sixties to generate a non linear representation of molecular biology systems, known as the *Power-law* formalism [390-392]. The Power-law representation can be derived from the conceptual model and some systemic properties can be analyzed without the need of a detailed kinetic characterization [393-406]. Furthermore, we can easily modify a given model by adding terms to any of the equations, which can be an advantage for sharing models. Alternative representations to the Power-law formalism that share some of its advantages are the *Lin-log* [265, 407, 408] and *(log)linear* [409] formalisms. Both formalisms require the same information as required for the Power-law formalism, although the final representation differs [364, 410]. Recently, a *Saturable and Cooperative (SC)* formalism has been derived by using Taylor series approximation after a power-inverse transformation [364, 410]. In a different strategy, the use of a special rate-law called *Convenience kinetics* has been proposed as a way of obtaining a general representation [411]. Because power-law models can be easily and automatically obtained from other representations, the use of this

(and other) formalism(s where such usage is possible) may facilitate HTP modelling building and analysis.

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