Software

MetaReg: a platform for modeling, analysis and visualization of biological systems using large-scale experimental data
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Abstract

MetaReg http://acgt.cs.tau.ac.il/metareg/application.html is a computational tool that models cellular networks and integrates experimental results with such models. MetaReg represents established knowledge about a biological system, available today mostly in informal form in the literature, as probabilistic network models with underlying combinatorial regulatory logic. MetaReg enables contrasting predictions with measurements, model improvements and studying what-if scenarios. By summarizing prior knowledge and providing visual and computational aids, it helps the expert explore and understand her system better.

Rationale

Given the recent accumulation of high throughput biological data, the task of integrating and analyzing large-scale data-sets is a major challenge. A variety of computational modeling approaches have been developed for the analysis of such data-sets, such as clustering [1,2] and topological interaction network models [3,4]. While these approaches give a broad, low resolution picture of cellular processes, many biologists are interested in a specific subsystem, and wish to use the results from experiments in order to refine the current knowledge on the system. This analysis of data in the context of the available knowledge is often performed in an informal manner: The researcher sketches a diagram of a relevant subsystem according to the current knowledge. This diagram summarizes and organizes the available knowledge, and assists the expert in analyzing the predicted state of the system in various possible experiments. The predictions are then compared to experimental measurements, and if a discrepancy is found, additional experiments are performed, and the diagram is iteratively refined.

In the case of complex biological systems and massive amounts of data, manual construction of the model, state predictions, comparison with data and systematic model refinements are impractical, and automatic computational methodologies must be employed [5,6]. To address the need for such an analysis workflow, we developed MetaReg, an integrative tool for analysis of steady-state, high-throughput data in the context of specific biological systems. The theoretical foundations of the MetaReg methodology and algorithms are outlined below in the 'MetaReg’s algorithmic layer' section (for a complete description, see [7]). While making some gross simplifying assumptions about the behavior of real biological systems, the model was demonstrated to be highly effective on several systems [7-9]. MetaReg enables easy conversion of the current qualitative knowledge on a particular
subsystem into a mathematical model, including logical relations among the biological components. The system is represented by a probabilistic graphical model called a Bayesian network [10], which allows distinguishing between regulatory relations that are known at a high level of certainty and those that are more speculative. Given the model, MetaReg predicts the level of each variable under any given genetic perturbation or environmental stimuli. Moreover, MetaReg allows incorporation of high throughput data, and graphical comparison between model predictions and measurements. The most advanced MetaReg capability is suggesting model refinements by systematically seeking changes that increase the fit between model predictions and experimental measurements.

The MetaReg application

MetaReg core functionality

Figure 1 illustrates the key features of the MetaReg application and its workflow. The basic workflow begins with model construction and its initial analysis through simulations. Once a current-knowledge model is established, it can be used to predict component values under any experimental treatment (for example, genetic perturbation, growth environment). Next, we compare these predictions to the values observed in the actual experiments under the same treatments, and highlight the discrepancies between them graphically. MetaReg can also automatically refine the model in order to reduce such discrepancies. Screenshots of the main windows from the application are shown in Figure 2. A comprehensive manual of the application is available online [11].

Model construction

The first step in utilizing MetaReg is the construction of the biological system model on the 'model canvas' (Figure 2a). A MetaReg model consists of a set of biological variables and their regulatory logics. The 'variables' represent different biological entities (mRNA, protein, metabolite, and so on). Each variable may attain several discrete 'states' (three states by default), representing, for example, the transcript level of an mRNA, or the activity level of an enzyme. The state of a variable \( v \) is influenced by the states of the variables that are connected to \( v \) by incoming edges. These variables are called the 'regulators' of \( v \). Most importantly, every variable is assigned a discrete 'logic', which defines its state given the states of its regulators. For example, if variable A has two activators B and
C, its logic might be Max(B, C). We assume all the logics represent steady-state regulatory relations, and thus the model represents the steady-state behavior of the biological system. Every logic is associated with a probability that indicates the certainty in the prior biological knowledge. For example, if a logic is known with high certainty, it will be assigned with a high probability (for example, 90%), and alternative logics will have low probabilities.

The application offers several tools to help in model construction. Variables can be selected from and automatically linked to known databases, such as SGD [12] and NCBI Gene [13] (Figure 2b). Each variable can be attributed with links to relevant journal publications from PubMed, enabling further model curation. The application provides several gadgets for logic definition, including scripting, a tabular editor and a logic wizard (Figure 2c) for hierarchical construction of complex logics. The type of each regulation, activation (→), repression (∴) or other (❍) is automatically deduced based on the logic of the regulatee (the regulated variable). The model canvas is fully interactive, including capabilities for manual or automatic variable positioning and highlighting of different sets of variables, such as all the metabolites or all the cycles in the model.

Model simulation
In order to view the behavior of the model in response to different experimental treatments, simulations can be per-
formed. Given a particular experimental treatment, the possible system states are computed as described in [8]. A 'system state' is an assignment of states to all the variables in the model. The user can dynamically design an experimental treatment scenario and visually analyze the system state on the model canvas. If the model contains cycles, several system states might be feasible, and the user can navigate among them.

**Data integration**

The application can integrate 'observations' (measurements) from multiple studies. The measured biological components are automatically matched to the model variables. For example, gene expression data are automatically matched to the corresponding mRNA variables, and protein measurements are matched to the corresponding protein variables. As part of the data import, the user must specify the 'experimental treatment' used in each experiment, including the environmental stimulations and genetic perturbations performed in each particular experiment. For example, if the experiment was performed in surplus of nitrogen and on a yeast strain where Leu3 is knocked out, the experimental treatment is 'Leu3 = 0; Nitrogen = 2', where Leu3 and Nitrogen are model variables. Once the data are imported, it is possible to visualize all measured variables under each of the experiments in a single data matrix (Figure 2d; see below), or to view the measurements of a specific experiment projected on the model canvas (Figure 2a).

**Comparing predictions with observations**

In order to evaluate the model, the 'predicted' levels of each variable are compared to its 'observed' levels under each experiment. MetaReg provides a prediction engine that infers probabilistically the expected level of each variable in each experiment, given the network model and the experimental treatment (see [7]). MetaReg supports two visualization tools to compare these predictions with the observations, both designed to highlight cases of discrepancies, which are often the starting point of further research. First, the observed and the predicted values for a single experiment can be projected side by side on the model canvas (Figure 2a). The second visualization tool provides a comprehensive view of the discrepancies across all the experiments, in which each cell contains color-coded representation of the observed and the predicted values, along with a representation of the discrepancy between them (Figure 2d). This view allows simple detection of discrepancy 'hot-spots' in which the model fails to explain the data.

**Model refinement**

Our methodology enables refinement of the model to obtain better fit between model predictions and observations. The input of the refinement process is the target variable and a set of regulators. MetaReg searches among all possible regulatory logics and outputs the most significant one. The suggested logic can be further edited by the user (Figure 2e). This way the user can test hypotheses about variable regulation.

**Case study: leucine biosynthesis in Saccharomyces cerevisiae**

**Modeling and simulations**

We present a model for leucine biosynthesis and related signaling pathways in *Saccharomyces cerevisiae*. Building on literature reports, we constructed a detailed model of known regulatory relations in this system. The model contains 47 variables (nodes) and 67 regulations (arcs). The model is available from our web site [14].

Leucine is an essential branched-chain amino acid generated from pyruvate via α-ketoisovalerate (α-IPM) and β-IPM in a linear pathway in which nine catalyzing enzymes are involved (Ilv2, Ilv3, Ilv5, Leu9, Leu4, Leu1, Leu2, Bat1, Bat2). The regulation of leucine production is controlled by several known mechanisms [15].

Several leucine biosynthetic enzymes are subject to transcription regulation via the general regulatory pathway of amino acid biosynthesis. Starvation for any amino acid induces the translation of Gcn4 via Gcn2. Gcn4 is a transcriptional activator of enzymes that catalyze several amino acid biosynthesis pathways, including the leucine biosynthetic pathway.

The control of several catalyzing enzymes is regulated by the transcriptional activator Leu3. The activity of Leu3 is regulated by α-IPM, an intermediate of the pathway acting as a co-inducer. When α-IPM is present, Leu3 acts as activator; when α-IPM is absent, Leu3 acts as repressor [15]. Hence, α-IPM serves as a sensor of leucine production.

The enzymatic activity of Leu4 is subject to two major controls by metabolites. The first is feedback (end product) inhibition by leucine. At high levels of leucine, Leu4 activity is inhibited, and causes a reduction in the production of the pathway. The second control is inactivation by coenzyme A, a product of the reaction catalyzed by Leu4 and a central energy metabolite in the mitochondria. This control serves as a link between the metabolic process and the energy metabolism context.

In Figure 3, we present a diagram of our model. It includes the leucine biosynthetic pathway, the catalyzing enzymes and their transcriptional control. The state of internal leucine depends on the leucine transport into the cell and on the yield of the leucine biosynthetic pathway. The transport is facilitated via amino acid permeases (Bap2, Bap3, Gap1, Tat1) that are regulated by Gcn4, Leu3, and the TOR signaling pathways. The model includes four environmental stimulators: 'NH₃' (ammonium), 'rapamycin', 'leucine', and 'amino acids', which indicates availability of all amino acids except leucine that are needed to represent the environmental con-
conditions enforced on the system. The model graph contains many cycles. For example, the general nitrogen control regulation (for example, Gcn4 $\rightarrow$ biosynthetic enzymes $\rightarrow$ leucine biosynthesis pathway $\rightarrow$ internal amino acids $\rightarrow$ Gcn2 $\rightarrow$ Gcn4), the leucine-specific transcriptional regulation via Leu3 (Leu3 $\rightarrow$ biosynthetic enzymes $\rightarrow$ leucine biosynthesis pathway $\rightarrow$ $\alpha$-IPM $\rightarrow$ Leu3), and autoregulation of Leu3 transcription factor (TF) on LEU3 gene transcription (LEU3 $\leftrightarrow$ Leu3). The variables that are part of cycles in the model are highlighted in Figure 3.

We used three states for each mRNA variable: state ‘0’ represents reduced transcription level compared to the wild type, state ‘1’ represents the wild-type transcription level when cells are grown on YPD medium, and state ‘2’ represents increased transcription level. Similarly, each protein has three states reflecting its activity level (high = ‘2’, medium = ‘1’, low = ‘0’). The modeling of Leu3 is a special case, since we had to represent its dual role as activator and repressor. We used state ‘0’ for its repressive mode, state ‘1’ represents no effect (for example, in the leu3 mutant), and state ‘2’ indicates the Leu3 activator mode. For example, a simulation of the system behavior in leucine starvation is shown in Figure 3.

Data preparation
We integrated expression profiles from four datasets that contain treatments pertinent to our model: seven profiles in rapamycin treatment after 15, 30, 60 and 120 minutes of incubation and in amino acid deprivation after 1, 1.5 and 2 hours of incubation [16]; six profiles in histidine starvation and various Gcn4 perturbations [17]; six profiles of chemostat growth in nitrogen limiting conditions with and without Leu3 perturbation [18]; and six profiles in nitrogen depletion after 8, 12 and 24 hours of treatment and in amino acid and adenine starvation after 1, 2 and 4 hours of treatment [19]. A complete description of the profiles, the experimental treatments under which they were obtained and the data pre-processing, is available in Additional data file 1.

Evaluation of the model in accordance with data
We applied the prediction engine of MetaReg to the collection of experimental treatments described above. The matches
and mismatches between the predictions and the observations are displayed by the discrepancy matrix in Figure 4b. While there is a good match for the majority of the components and conditions, the matrix reveals several major discrepancies between the model and the microarray experiments.

The leucine biosynthetic genes *LEU1*, *LEU2*, *LEU4* and *BAP2* show an unexpected decrease in expression in the *leu3* mutant strain (Figure 4b, columns 16-18). The reduction was surprising since Leu3 is known to act as a repressor in these experiments.

In *gcn4* mutant strains, we observed an increase in the mRNA levels of the leucine biosynthetic genes *BAT1*, *ILV2*, *ILV3* and *ILV5* following 3AT treatment (histidine starvation; Figure 4a, columns 11-12). In our model the effect of general amino acid control on these genes is mediated solely by Gcn4.
Gcn4 is absent in these experiments, our model does not predict such an increase, and a discrepancy appears (Figure 4b, column 12).

For LEU3, we observed an increase in expression in two gcn4 mutant strains and in nitrogen limitation experiments (Figure 4b, row 11, columns 11-15). According to the literature, LEU3 mRNA is upregulated by either Gcn4 or Leu3 TFs. As no amino acid shortage occurs in these experiments, neither Gcn4 nor Leu3 are expected to be active, hence the model predicts a low level of LEU3 mRNA, in contradiction to the observed increase.

Following a rapamycin treatment, we observed a consistent decrease in the levels of four biosynthetic genes, BAT1, ILV3, ILV5 and LEU1. The effect of rapamycin on the biosynthetic genes is known to be mediated by the TOR pathway through Gcn4 [20]. It is thus expected that under rapamycin treatment, Gcn4 will be active, while Leu3 will not be active. Consequently, the levels of the leucine biosynthesis genes (LEU1, ILV3, ILV5, BAT1) regulated by Gcn4 should be alleviated. Surprisingly, we witness a down-regulation of these genes.

For LEU9, BAT2, BAP3 and TAT1, we could not find any report on their regulation in the literature, and thus their predicted level is constant. Hence, the discrepancies merely reflect the lack of knowledge about them.

Leucine model refinement

In order to improve the fit of the model’s predictions to the observed data, we used MetaReg’s refinement algorithm. We focus here on two representative examples of model refinement. In these examples we suggest improved logics for the way in which Leu3 and Gcn4 jointly regulate LEU9, BAT2 and LEU2.

LEU9 and BAT2 have similar expression patterns (Figure 4a), but we could not find any report on their regulation in the literature. MetaReg suggests that LEU9 is regulated solely by Leu3 with no definite regulatory role for Gcn4 (Figure 5a, LEU9 table, rows 1 and 3). A similar logic is obtained for BAT2. Note that for Leu3, MetaReg’s refinement matches its known repressive role: when Leu3 acts as a repressor (Leu3 = 0), we observed medium/low transcription of LEU9, even though the level of the activator Gcn4 is high (Figure 5a, LEU9 table).

LEU2 expression is known to be affected only by Leu3 [15]. Indeed, the suggested logic (Figure 5) shows that the state of Gcn4 does not influence Leu2. As expected, when Leu3
should act as activator (Leu3 = ‘2’) there is high transcription (LEU2 = ‘2’). However, we do not detect the expected repressive effect of Leu3 on its targets. When Leu3 should act as repressor (Leu3 = ‘0’), we observe medium LEU2 transcription (LEU2 = ‘1’) instead of the expected low transcription.

Figure 5b,c illustrates the refinement process. During refinement, MetaReg tests the predicted activity levels of the TFs (Gcn4 and Leu3) against the observed level of the mRNA in each experiment (Figure 5b), and computes the best logic between the regulators’ predicted level and the observations. Consequently, the discrepancies observed for LEU2 and LEU9 in our initial model (before refinement) are drastically reduced after refinement (Figure 5c).

In the case of LEU1, BAT1, LEU4, ILV2, ILV3 and ILV5, the results were similar to LEU2 (not shown). For BAP2, BAP3, TAT1 and LEU3, MetaReg did not succeed in deriving a high confidence logical relation, due to inconsistent effects that could not be explained by the model. For example, for TAT1, only down-regulation is observed in the data (Figure 4a, last row). For BAP3, we observe an inconsistency between two sets of nitrogen depletion experiments in different studies (Figure 4a, columns 13-15 versus 19-21). This probably indicates that each of those genes is regulated by additional elements that are not included in the model.

**MetaReg’s algorithmic layer**

In this section, we briefly outline the algorithmic layer behind the MetaReg application. A full description can be found in [7].

**Modeling prior knowledge**

Our model consists of variables X1...Xn, represented by nodes, and regulations among them, represented by arcs. The set of variables that together regulate variable Xi are called its ‘regulatory unit’, denoted Pa_i. This is the set of nodes that have arcs directed into Xi. Each variable can be in one of several discrete ‘states’, and its state in any condition is assumed to be determined by its ‘logic’, that is, a discrete function of its regulators’ states in that condition. Note that this assumption implies that the relevant conditions are in steady state. In order to model our confidence in the prior knowledge, the logic of a variable Xi is formulated probabilistically as our level of certainty that the variable attains a certain state given the state of its regulatory unit. The uncertainty is modeled by the conditional probability \( \theta(X_i \mid Pa_i) \). This approach allows us to distinguish between regulatory logics that are known at a high level of certainty and those that are more speculative.

The experimental treatment is modeled by fixing the states of each variable that correspond to the environment, and by changing the regulation function priors to reflect the perturbations (for example, when a gene is knocked out, its level is set to zero under that condition, irrespective of the levels of its regulators).

**Data integration**

In practice, biological measurements are continuous, and one does not know in advance how to translate them into discrete states. To overcome this, each logical variable Xi is associated with an observed real-valued variable Yi, and the conditional distribution \( \psi(Y_i \mid X_i) \) specifies the probability of the variable Yi to attain a certain observed real value given its state. Hence, \( \psi(Y_i \mid X_i) \) translates the actual measurements into the discrete model without applying any a priori discretization to the data. In MetaReg, each \( \psi \) is modeled as a mixture of Gaussians.

**The complete computational model**

Our probabilistic model defines a ‘Bayesian score’, which evaluates the fit of the model predictions to the data, measured as the log likelihood of the data given the model:

\[
\log Pr(X,Y \mid Model) = \log \left( \frac{1}{Z} \prod_i \theta_i(X_i \mid Pa_i) \cdot \psi(Y_i \mid X_i) \right)
\]

where Z is a normalization constant. The conditional probabilities \( \theta \) are known from our prior knowledge of the biological system, and \( \psi \) are determined by maximizing a likelihood score using an Expectation-Maximization procedure. This model corresponds to a Bayesian network in the case of acyclic dependencies, or to a factor graph in the more general case where the model contains feedback loops.

**Computing model predictions**

The ‘predicted level’ is the expected value of a variable Xi given the model and the experimental procedure applied. This is obtained by first computing the posterior states distribution of Xi using a standard probabilistic inference method called Loopy Belief Propagation [21]. This way we obtain a probabilistic average of all its possible system modes. Then, the (continuous) predicted level of Xi is its expectation given \( \theta \) and its states distribution. The comparison of predicted and observed levels (both on the model canvas and in a discrepancy matrix) displays both levels as real values.

**Logic refinement**

Given a target gene and its candidate regulatory unit, the refinement process searches in the space of discrete regulatory logics in order to achieve a logic with a locally maximum Bayesian score, while fixing the logics of all other variables. Due to an exponential number of possible logics, we apply a greedy heuristic. In the case of ties the algorithm chooses randomly among the equally scored improvements. The \( \psi \) parameters depend strongly on the particular model logics, and thus we re-optimize them using an expectation-maximization (EM)-like procedure during each step of the logical refinement procedure. Note that the refinement process uti-
Table 1

Available tools related to MetaReg

| Tool type                     | Tools                                       | Description                                                                 | Relation to MetaReg                                                                 |
|------------------------------|---------------------------------------------|----------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Network or model visualization tools | Cytoscape [28]                             | Tools for constructing visualizations of interaction and regulatory networks. These networks can then be integrated with high-throughput data | These tools offer powerful visualization aids and other analysis aids, but they do not address regulatory logics and do not offer model evaluation or refinement mechanisms |
|                              | Visant [29]                                |                                                                            |                                                                                     |
|                              | CellDesigner [30]                           |                                                                            |                                                                                     |
|                              | Reviewed in [31]                           |                                                                            |                                                                                     |
| Kinetic and continuous modeling tools | Gepasi [32]                                | Tools allowing detailed dynamical modeling with kinetic parameters and differential equations | These tools can perform detailed model analysis by accurate dynamical simulations, but they cannot discover new mechanisms and rely on detailed mechanistic understanding of the system1 |
|                              | BioNetS [33]                               |                                                                            |                                                                                     |
|                              | Dynetica [34]                              |                                                                            |                                                                                     |
|                              | PyBioS [35]                                |                                                                            |                                                                                     |
|                              | Reviewed in [36-38]                        |                                                                            |                                                                                     |
| Logical modeling tools       | BIOCHAM [39]                               | Tools for modeling regulatory systems using various formalisms, for example, Boolean, discrete, fuzzy logic and so on | Allow model evaluation through simulations, but are not designed for model evaluation and refinement in accordance with high throughput data |
|                              | Bionet [40]                                |                                                                            |                                                                                     |
|                              | CellNetAnalyzer [41]                       |                                                                            |                                                                                     |
|                              | GINsim [42]                                |                                                                            |                                                                                     |

1 In several cases the kinetic and continuous modeling tools have parameter optimization capabilities, but only for the given differential equations included in the model. Hence, these tools lack MetaReg's ability to discover unknown mechanism of reaction and changes in model topology. Furthermore, kinetic and continuous modeling approaches require either detailed mechanistic understanding or known model parameters (e.g., reaction constants), which are commonly unknown, even in well studied systems (e.g., the Leucine biosynthesis pathway studied here).

Discussion

MetaReg provides a framework for the modeling and analysis of a biological network vis-à-vis high throughput data. A major practical need of molecular biologists today is to generate hypotheses based on network modeling and to iteratively refine the network. MetaReg is designed exactly for this purpose - it allows mathematical modeling of a biological system, interpretation of high throughput data in the context of the prior model, and computational refinement of the model based on the high throughput data. Several other tools with related capabilities, emphasizing visualization or simulations, are being developed (Table 1). The MetaReg platform is unique in its modeling and refinement capabilities, which fit the needs and workflow of biological investigations. It allows streamlined cycles of probabilistic modeling, laboratory experimentation and systematic refinement.

MetaReg is implemented efficiently, computing predictions and logic refinements within a few seconds for 100 nodes, and within an hour for 6,000 nodes (using a network with no more than three regulators per variable, 90% certainty level in all logics, and 100 gene expression profiles). However, the model has practical size limitations: the prediction algorithm run-time increases exponentially with the average number of regulators per variable. Also, for large models with over 300 variables, the automatic layout of the model topology may take several minutes.

MetaReg formalizes the biological system using discrete component states, assuming that the system is in steady state. Clearly these crucial assumptions are a simplification of the biological reality. By making such assumptions, we tried to strike a practical balance between our wish to enable a faithful description of the biological system and the scarcity of accurate knowledge at very high resolution. Indeed, biological processes are inherently temporal, but when the sampling rate (the number and time resolution of experiments) is low relative to the rate of the regulatory mechanisms, we believe that our results here as well as in [7-9] show that the steady state assumption is reasonable.

The accuracy of the prediction and refinement processes may be sensitive to the model size and the certainty in the logics. We have shown previously that the algorithms are highly robust to certainty level on small networks [7]. Indeed, the results shown in the leucine example were obtained using a uniform certainty level of 0.99 for all variables, but we obtained very similar results when using certainty levels of 0.95 and 0.9 (not shown). However, the robustness of our methods to model size and to certainty levels requires further systematic exploration.

A major prerequisite to using MetaReg is formalizing high quality prior knowledge on the pathway of interest. Several efforts to generate databases of curated knowledge on signaling pathway are currently under way (for example, BioModels [22], Reactome [23] and SPIKE [24]). Thanks to such efforts, it will soon be relatively easy to apply the MetaReg methodology in studying many additional biological systems.

Availability and requirements

Project name: MetaReg (home page at [25]).

Operating system(s): Windows.

Programming language: Java for the envelope and C++ for the algorithms.
Other requirements: Java 1.5 or higher.

License: free for non-commercial users.

Any restrictions to use by non-academics: License needed.

Abbreviations
EM, expectation maximization; IPM, isopropylmalate; TF, transcription factor.

Authors’ contributions
IU developed the tool, performed the analysis and co-wrote the paper. IG-V conceived the study, developed MetaReg, performed the analysis and co-wrote the paper. RS conceived and supervised the study and co-wrote the paper.

Additional data files
The following additional data are available with the online version of this paper. Additional data file 1 provides a complete description of the profiles, the experimental treatments under which they were obtained and the data preprocessing.

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