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MODEL-BASED DESIGN OF SYNTHETIC NETWORKS

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Model-Based Design of Synthetic Networks

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Abstract. While significant progress had been made in synthetic biology over the last decade, researchers still lack a reliable tool for computer-aided design of gene regulatory networks (GRN), one that can reveal the full range of nonlinear dynamic behaviors in a single run. Here, we propose a network design cycle that utilizes both the qualitative simulation of GRNs modeled by a class of ODE equations and the intrinsic stochasticity of regulation to ultimately design a network that exhibits a specified desired behavior with the highest probability. Finally, to show the power and ease of our method, we perform a case study with a real-life benchmark gene network to get a synthetic oscillator.

Keywords: synthetic biology, qualitative dynamics, gene regulatory networks, stochastic parameters, network design

1 Introduction

In synthetic biology, *in silico* approaches are a must in the design of a gene regulatory network (GRN) before costly *in vitro* experiments. Thus, synthetic biology needs a reliable tool that is able to reveal the full range of network dynamics. But, the traditional mathematical modeling cycle still heavily depends on trial-and-error via numerical investigations that, in most cases, are both nontrivial and time-consuming.

To address this problem, we implemented a tool for automated qualitative analysis and simulation of GRNs modeled by a class of ODE equations that adopts steep sigmoidal response functions to capture the intrinsic nonlinearity and temporal multi-scale of GRN dynamics. The resulting tool calculates the dynamics of these models on the basis of sound rules established by [2]. Under specific biologically reasonable assumptions and sufficient conditions, our tool provides sound and complete predictions [3], *i.e.* in a *single run*, it provides all and only possible dynamics of the GRN network at study, where each predicted trajectory is characterized by ranges of parameter values as well as by its qualitative dynamical property, namely stable, cyclic, or spiraling dynamics. The proposed simulator also takes into account the inherent stochasticity of regulation, and calculates the probability of occurrence of each trajectory derived from the deterministic model by assigning a measure of uncertainty to the parameter values [1] and by propagating such uncertain information along the transitions of each trajectory. Then, we can develop robust control strategies aimed at leading a cell system towards or away from a desired or undesired state with the highest probability of success.

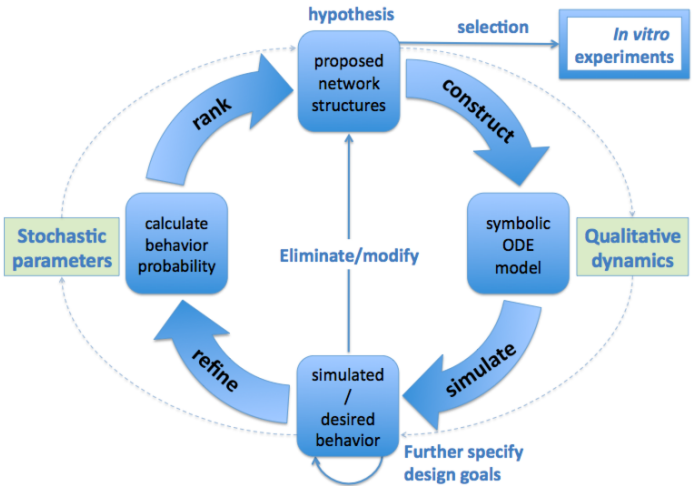

Fig. 1. Design cycle of a synthetic network to display a desired dynamic behavior. Qualitative simulations allow the prediction of all qualitatively distinct network behaviors, which can be used to verify if the design goals are satisfied by the hypothesized network. Then, the introduction of stochasticity on parameter values allows the network to achieve the desired behavior with the highest probability. The top ranking design can be passed onto *in vitro* experimentation for the next stages of implementation.

Network design is made easier by our simulator as the responses to perturbations (*e.g.* addition/deletion of genes, changes in connectivity, external stimulation, *etc.*) will become apparent as they can be quickly and rigorously calculated. Likewise, changes in the initial conditions are conveniently explored and their consequences on system dynamics will be more obvious. Thus, model-based network design becomes a development cycle that consists of the following phases:

1. **Hypothesized networks.** Plausible GRN network structures are conceptualized from preexisting functional modules/smaller circuits of larger networks or created *ex novo* with the goal of exhibiting a desired dynamic behavior.
2. **Model construction.** Formalization of a symbolic ODE model based on the regulatory interactions of one selected gene network.
3. **Qualitative simulation**. Prediction of all the potential qualitative behaviors from specific set of initial conditions and parameter constraints.
4. **Hypothesis testing**. Comparison of simulated results to the desired behavior. A model that is unable to reproduce the desired behavior is eliminated and the design cycle restarts anew. Otherwise, the simulated results contain rich information, such as all transient states before the final behavior and the parameter conditions to reach any state, that can be used to revise the original design goal. For example, a designer may specify requirements about the transient states before arriving at the desired behavior; and consequently,

the designer is able to define an idealized temporal profile of gene network activity.

5. **Stochastic parameters.** Distribution functions representing the stochastic values of model parameters are refined to calculate and maximize the probability of the desired behaviors and minimize undesired ones.
6. **Network selection.** The proposed networks can be ranked by scoring the probability of success alongside the designer's preference. The most plausible model can then be selected for interpretation by synthetic biology and adapted for *in vitro* experimentation.

Figure 1 shows the phases of the model-based design cycle. To demonstrate the power and ease of our method in the design of a synthetic network, as a benchmark we take a real-life example from synthetic biology, the so-called IRMA network that has been extensively studied in both computer modeling and experiments [4, 5]. Its dynamical properties and parameter specifications are well-known, giving us the flexibility to operate in various knowledge scenarios: unknown, well-defined, or a mixture. Thus, using the five-gene synthetic network constructed in yeast *Saccharomyces cerevisiae*, we are able to accurately predict the dynamics of the IRMA network in various modification scenarios, which concur with the findings of [5], and detect, within a set of candidate network models, the one that reproduces the desired behavior with the highest probability.

2 IRMA: a Synthetic Network Case-Study

The main aim of this section is to demonstrate how to use the model-based design cycle to obtain a specific desired behavior in a well-known, reasonably-sized gene regulatory network. Let our design goal be the introduction of oscillatory dynamics into the IRMA network. Similarly to [5], we start from a network structure that under certain conditions has stable steady-state dynamics. Let us refer to this network as the original structure. By following the carefully-designed phases of the design cycle, we construct, simulate, and modify the five-gene network until oscillations are found. In addition to the original structure, we specifically explore two hypothesized networks: the first has the same structure with a different parameter space, while the second has a re-engineered network connection. Though the re-engineered networks are adapted from [5], our results are much improved compared to the various numerical studies previously employed by the authors, which were not only computationally taxing but also limited, as each run only provides a snapshot of the dynamics in a single instance of parameter values. Then, these numerical methods are time-consuming and exhaust valuable resources. Whereas, in this preliminary study, we show the ease of predicting the complete picture of network behaviors over a specified range of parameters *in a single simulation.* This implies a dramatic reduction in the computational times.

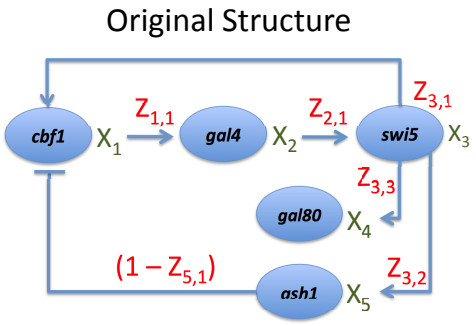

Fig. 2. Original Structure. The IRMA network structure in galactose growing conditions. As in (1), x_1=[Cbf1], x_2=[Gal4], x_3=[Swi5], x_4=[Gal80], and x_5=[Ash1]. Gene activation and inhibition are marked by arrow and bar, respectively. The response functions (red) that affect the change in the x_i variable (green) were labeled for convenience.

2.1 Selection of Plausible Network Structures

Let us consider the first four phases in the model-based design cycle (*i.e.* network hypothesis, model construction, qualitative simulation, and hypothesis testing) that are necessary to design a GRN that reproduces the desired behavior. To start, we consider the original IRMA network.

1. Hypothesized network. Since its realization, the IRMA model has often been used as a benchmark in synthetic biology [4]. For our case study, we start with the IRMA network structure under galactose growing conditions, diagramed in Fig. 2. The network topology consists of two transcriptional feedback loops: one positive and one negative. This network structure is known to have stable steady-state dynamics; however, we take it as a basis for successive modifications on this model structure to obtain oscillatory dynamics.

2. Model construction. To formulate the IRMA model into the mathematical framework given in [2] that considers a class of ODE models where regulation is threshold-dependent, let x_i represent the concentrations of protein for the following genes: *cbf1*, *gal4*, *swi5*, *gal80*, and *ash1* respectively for $i = 1, 2, 3, 4, 5$. In literature and in the framework, gene activation is best approximated by a continuous yet very steep sigmoid function that switches from 0 to 1 when the gene product reaches a critical threshold concentration at $x_i = \theta_{ij}$. Usually, it is mathematically expressed by the Hill function:

$$Z_{i,j}(x_i, q, \theta_{ij}) = \frac{x_i^{1/q}}{x_i^{1/q} + \theta_{ij}^{1/q}},$$

where $0 < q \ll 1$ is the measure of steepness and non-negative $j = 0, 1, \ldots, m_i$ represents the threshold index for each gene action. Instead, gene inhibition is modeled by $(1 - Z_{i,j})$. So, by taking into account all the network connections for each gene in the original IRMA (Fig. 2), we can write the model with the

following system of ODE equations:

$$\begin{aligned}
\dot{x_1} &= k_{11}Z_{3,1} + k_{12}Z_{3,1}(1 - Z_{5,1}) - \gamma_1 x_1\,, \\
\dot{x_2} &= k_{21} + k_{22}Z_{1,1} - \gamma_2 x_2\,, \\
\dot{x_3} &= k_{31} + k_{32}Z_{2,1} - \gamma_3 x_3\,, \\
\dot{x_4} &= k_{41} + k_{42}Z_{3,3} - \gamma_4 x_4\,, \\
\dot{x_5} &= k_{51} + k_{52}Z_{3,2} - \gamma_5 x_5\,,
\end{aligned} \tag{1}$$

where k_{il} is the transcription rate for the l^{th} synthesis term and γ_i is the degradation rate. By our assumptions, $\theta_{i0} = 0$ for any i and the thresholds must be ordered, *i.e.* $\theta_{ij} < \theta_{ik}$ for $j < k$. Here, we have ordered the three threshold concentrations of x_3 in accordance with [6], and all other x_i variables only have one threshold. For the symbolic parameter relationships between k_{il}, γ_i, and θ_{ij}, we have constructed the initial parameter inequalities according to the nominal values given in [5]. Inherently, we assume positive biological rates $k_{il} > 0$, $\gamma_i > 0$ and threshold values $\theta_{ij} > 0$. We define the set of inequalities as $I_0 = \{k_{il} > 0$; $\gamma_i > 0$; $\theta_{ij} > 0$; $k_{11} > \gamma_1\theta_{11}$; $k_{12} > \gamma_1\theta_{11}$; $k_{21} < \gamma_2\theta_{21}$; $k_{22} > \gamma_2\theta_{21}$; $k_{51} < \gamma_5\theta_{51}$; $k_{52} > \gamma_5\theta_{51}\}$.

3. Qualitative simulation. The automated simulator [3], based on the mathematical results given in [2], soundly predicts the set of all possible system behaviors exhibited by a GRN model for the given set of initial parameters and starting domain, which are symbolically expressed by ranges of parameter inequalities and x_i ranges with respect to their thresholds. The system phase space is partitioned by the threshold hyperplanes into domains characterized by the gene regulatory dynamics that are either linear or nonlinear. In a *regular* domain, all genes are far from threshold values, making the Hill function take value of either 0 or 1, and then the system dynamics are linear. Otherwise, one or more genes has a nonlinear response and the domain is *switching*. For algorithmic purposes, each qualitatively distinct domain is unequivocally labeled D_j. By using the sound rules established in [2] to qualitatively analyze the regulatory dynamics locally in each domain, we can compose a solution trajectory that traverses the system phase space through a sequence of local transitions from one domain to another [3]. Then, each solution trajectory is characterized by a unique sequence of traversed domains. Since each local transition from a domain D_i to the successive D_j is associated with a set of parameter inequalities $I_{i,j}$, which must be satisfied for the transition to occur, the solution trajectory is also characterized by the joint set of inequalities constructed from all the parameter constraints in the initial set and all local transitions that occurred in the trajectory. Let the output from a single simulation be a *behavior tree* such that all possible solution trajectories starting from same initial domain are organized into a tree data rooted in the initial domain.

Starting in a regular domain where x_1 and x_2 are above threshold (D_9), we simulate the model given by (1) with the initial inequalities: $I_0 \cup \{k_{31} + k_{32} < \gamma_3\theta_{31}\}$ to obtain Fig. 3. Let us refer to this model as M_0 to distinguish from subsequent modifications. As expected, the behavior tree shows that all solution trajectories end in stable points as indicated by the double hexagon.

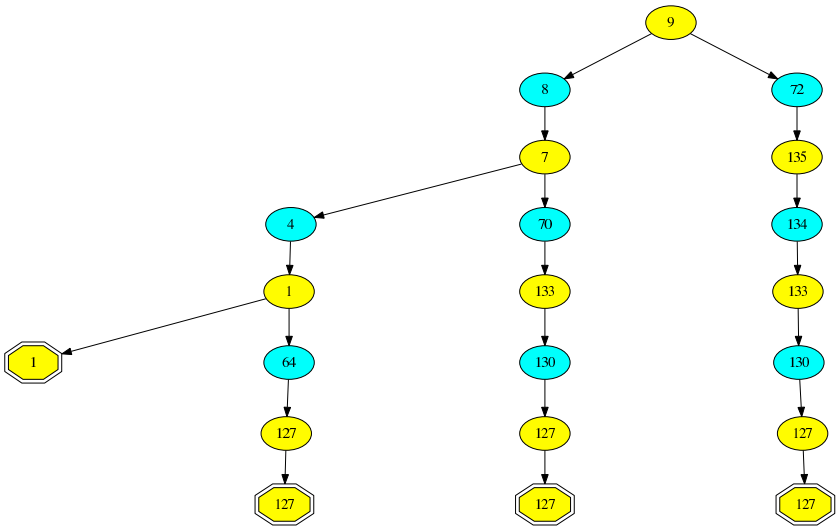

Fig. 3. Solution tree of the qualitative simulation of the original IRMA network (M_0) described in (1) starting at D_9 with the given initial parameters. Yellow and blue color represents regular and switching domains, respectively. Hexagon indicates that solution trajectory has stopped at a stable point inside the numbered domain.

4. Hypothesis testing. The simulation results confirm the stable steady-state dynamics in the original IRMA model as all solution trajectories end in a stable point in the regular domain where all genes are below threshold (D_1) or where only one x_4 is above threshold (D_{127}). We remark, in general, that the different sequences of transition states before the final behavior may provide useful insight to the synthetic biologist later in the design process, but as our design goal only focuses on obtaining oscillatory dynamics in the final behavior, we do not make use of this information in this case study. As the original model does not display our desired oscillatory behavior, in our model-based design cycle, this network must be eliminated or modified. Now, we will return to Phase 1 for evaluation and re-engineering.

2.2 Changing the Parameter Space: from M_0 to M_1

1. Hypothesized networks. To keep the original network structure and obtain oscillatory behavior, we observe that it is necessary to find a balance between the two feedback loops in the original model to obtain oscillatory dynamics. The *swi5* gene product (x_3) seems to be the key player as the first and second thresholds participate in the positive and negative feedback of *cbf1* expression (x_1), respectively. However, the original model is imbalanced and has a weak positive feedback loop because of the initial constraint on x_3, *i.e.* $k_{31} + k_{32} < \gamma_3\theta_{31}$, which restricts the ability of x_3 to become active in the GNR dynamics. Then, we should modify M_0 by using a different set of the initial inequalities, which in effect, puts the network into a different the parameter space.

2. Model construction. In the re-engineered model M_1, we change the initial inequalities to let the synthesis rates of x_3 be between the first and second thresholds, *i.e.* $\gamma_3\theta_{31} < k_{31} + k_{32} < \gamma_3\theta_{32}$, such that each k_{3l} still remains relatively small, *i.e.* $k_{3l} < \gamma_3\theta_{31}$ for $l = 1, 2$. Then, the initial inequalities for the M_1 network is: $I_0 \cup I_{M_1}$, where $I_{M_1} = \{\gamma_3\theta_{31} < k_{31} + k_{32} < \gamma_3\theta_{32};\ k_{31} < \gamma_3\theta_{31};\ k_{32} < \gamma_3\theta_{31}\}$.
3. Qualitative simulation. The simulation of M_1, starting from the same initial domain D_9, results in a larger behavior tree with 49 trajectories, seven of which are cycles. This tree is too large to be displayed here, but we noticed that the behavior tree is actually made of 7 sub-trees that have similar dynamics as each sub-tree has 7 trajectories: one of them is cycling and the rest are stable. We have displayed one of the subtrees in Fig. 4 by simulating the model in an initial domain where only x_3 is above threshold (D_3). The cyclic trajectory starts from D_3, traverses a series of domains, and then returns to D_3. Since the trajectory returns to a regular domain, it will encounter the same set of linear ODE equations that will direct the motion to the same subsequence sequence of domains. All of the inequality constraints that allow the motion of the cyclic trajectory will have been satisfied the first time the trajectory passes through that sequence of domains; therefore, this trajectory is able to repeatedly travel this sequence of domains in a cycle. In the cycle's path, there are six different places in the behavior tree where the trajectory could have branched to become a stable trajectory. Then, the inequalities at the branching points are critical to the final behavior. These inequalities are displayed in Table 1. The left and right column lists of all the inequalities required to branch off into either a stable or cyclic trajectory, respectively. We remark that these results provide valuable information on how and when to restrict the parameters to obtain the desired behavior.
4. Hypothesis testing. Here, the results are more promising as the re-engineered network actually displays our desired behavior where the original model did not. We proceed by saving this network for Phase 5 where we will use stochastic parameters to rank each candidate model by the probability to obtain the desired behavior. For the meantime, we return back to Phase 1 to see if we can improve the behavior tree; more specifically, we have the goal of getting a smaller behavior tree with more instances of cycles.

2.3 Re-engineering the Network Structure: from M_1 to M_2

1: Hypothesized networks. We hypothesize that the strength and timing of the positive feedback loop on *swi5* (x_3) can be increased by removing the connection from *swi5* (x_3) to *cbf1* (x_1) and by directly amplifying *swi5* (x_3) expression with a positive auto-regulatory loop. Let us construct this auto-regulation as drawn in Fig. 5 and refer to the network as M_2.
2. Model construction. For M_2, the modifications are only related to the change equations for x_1 and x_3. The variable x_1 is no longer activated by x_3; it has a small basal expression rate k_{11} and is still inhibited by x_5. Then, let $Z_{3,1}$ be the auto-regulation that activates itself (x_3) with a maximal transcription

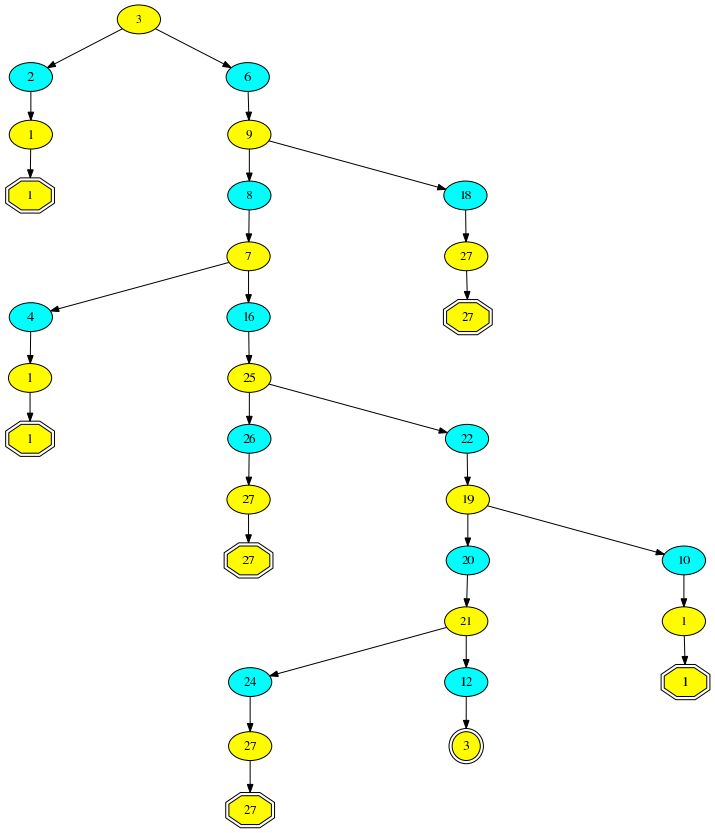

Fig. 4. Simulated sub-tree of modified network (M_1) with given initial conditions. Colors and shapes are as in Fig. 3. Double circle marks cyclic trajectory that returns to the starting domain in D_3.

Table 1. Branch point inequalities $I_{i,j}$ listed by individual local transitions $D_i \to D_j$.

stable		cycle	
$I_{3,2}$	$0 < \gamma_1\theta_{11}$	$I_{3,6}$	$k_{21} + k_{22} > \gamma_2\theta_{21}$
$I_{9,18}$	$k_{31} + k_{32} > \gamma_3\theta_{31}$	$I_{9,8}$	$0 < \gamma_1\theta_{11}$
$I_{7,4}$	$k_{21} < \gamma_2\theta_{21}$	$I_{7,16}$	$k_{31} + k_{32} > \gamma_3\theta_{31}$
$I_{25,26}$	$k_{11} + k_{12} > \gamma_1\theta_{11}$	$I_{25,22}$	$k_{21} < \gamma_2\theta_{21}$
$I_{19,10}$	$k_{31} < \gamma_3\theta_{31}$	$I_{19,20}$	$k_{11} + k_{12} > \gamma_1\theta_{11}$
$I_{21,24}$	$k_{21} + k_{22} > \gamma_2\theta_{21}$	$I_{21,12}$	$k_{31} < \gamma_3\theta_{31}$

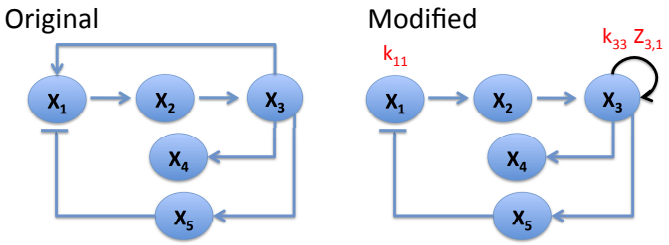

Fig. 5. Modified structure. Comparison of the original IRMA network structure (M_0) compared to the modified network (M_2) that includes a positive auto-regulatory feedback loop on x_3. The changes made to the original network are highlighted.

rate of k_{33}. So, the ODE equations are the same as (1) with the exception to x_1 and x_3, which shall be written as below:

$$\begin{aligned} \dot{x_1} &= k_{11} + k_{12}(1 - Z_{5,1}) - \gamma_1 x_1 \,, \\ \dot{x_3} &= k_{31} + k_{32} Z_{2,1} + k_{33} Z_{3,1} - \gamma_3 x_3 \,. \end{aligned} \tag{2}$$

Parameter inequalities must be added to state the relationship in the modified parameters of x_1 and x_3. The initial inequalities to be appended are $I_{M_2} = \{k_{11} < \gamma_1\theta_{11};\ k_{33} < \gamma_3\theta_{31};\ k_{31} + k_{32} < \gamma_3\theta_{33};\ \gamma_3\theta_{31} < k_{31} + k_{33} < \gamma_3\theta_{33};\ \gamma_3\theta_{31} < k_{32} + k_{33} < \gamma_3\theta_{33}\}$. Thus, the entire set of initial inequalities is: $I_0 \backslash \{k_{11} > \gamma_1\theta_{11}\} \cup I_{M_1} \backslash \{k_{31} + k_{32} < \gamma_3\theta_{32}\} \cup I_{M_2}$. For easy comparison, we have summarized the initial inequalities for the original model M_0, the previously reengineered network M_1 and the current network M_2 in the table below.

Model M_0	Model M_1	Model M_2
I_0	I_0	$I_0 \backslash \{k_{11} > \gamma_1\theta_{11}\}$
$k_{31} + k_{32} < \gamma_3\theta_{31}$	$k_{31} < \gamma_3\theta_{31}$	$k_{31} < \gamma_3\theta_{31}$
	$k_{32} < \gamma_3\theta_{31}$	$k_{32} < \gamma_3\theta_{31}$
	$k_{31} + k_{32} > \gamma_3\theta_{31}$	$k_{31} + k_{32} > \gamma_3\theta_{31}$
	$k_{31} + k_{32} < \gamma_3\theta_{32}$	$k_{31} + k_{32} < \gamma_3\theta_{33}$
		$k_{11} < \gamma_1\theta_{11}$
		$k_{33} < \gamma_3\theta_{31}$
		$k_{31} + k_{33} > \gamma_3\theta_{31}$
		$k_{31} + k_{33} < \gamma_3\theta_{33}$
		$k_{32} + k_{33} > \gamma_3\theta_{31}$
		$k_{32} + k_{33} < \gamma_3\theta_{33}$

3. Qualitative simulation. Starting in the same initial domain (D_9), the behavior tree shows nine possible behaviors with four cyclic trajectories, shown in Fig. 6. All stable trajectories either end in D_{27} (described above) or where only x_4 and x_3 are above threshold, *i.e.* $\theta_{31} < x_3 < \theta_{32}$, in D_{163}. All cycles start and end in in the same domain D_{27}. As before, the inequalities at the branching points into stable or cyclic trajectory are displayed in Table 2.

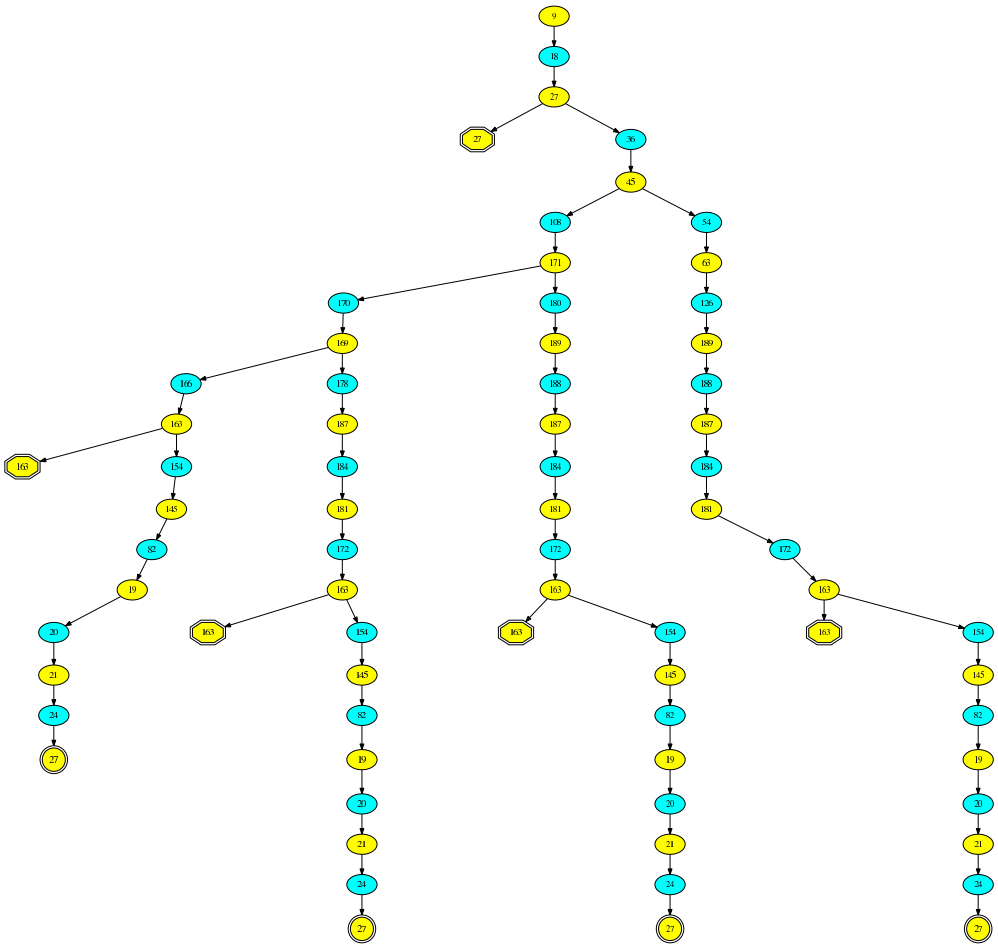

Fig. 6. Solution tree of network M_2 with initial conditions in D_9. All notation is the same as Fig. 4.

Table 2. Inequalities $I_{i,j}$ for local transition $D_i \to D_j$ at each branching point.

stable		cycle	
$I_{27,27}$	$\gamma_1\theta_{11} < k_{11} + k_{12} < \gamma_1\theta_{12}$	$I_{27,36}$	$k_{31} + k_{32} + k_{33} > \gamma_3\theta_{32}$
	$\gamma_2\theta_{21} < k_{21} + k_{22} < \gamma_2\theta_{22}$	$I_{45,54}$	$k_{31} + k_{32} + k_{33} > \gamma_3\theta_{33}$
	$\gamma_3\theta_{31} < k_{31} + k_{32} + k_{33} < \gamma_3\theta_{32}$	$I_{45,108}$	$k_{51} + k_{52} > \gamma_5\theta_{51}$
	$0 < k_{41} < \gamma_4\theta_{41}, 0 < k_{51} < \gamma_5\theta_{51}$	$I_{171,170}$	$k_{11} < \gamma_1\theta_{11}$
$I_{163,163}$	$0 < k_{11} < \gamma_1\theta_{11}, 0 < k_{21} < \gamma_2\theta_{21}$	$I_{171,180}$	$k_{31} + k_{32} + k_{33} > \gamma_3\theta_{33}$
	$\gamma_3\theta_{32} < k_{31} + k_{33} < \gamma_3\theta_{33}$	$I_{169,166}$	$k_{22} < \gamma_2\theta_{21}$
	$0 < k_{41} < \gamma_4\theta_{41}$	$I_{169,178}$	$k_{31} + k_{32} + k_{33} > \gamma_3\theta_{33}$
	$\gamma_5\theta_{51} < k_{51} + k_{52} < \gamma_5\theta_{52}$	$I_{163,154}$	$k_{31} + k_{33} < \gamma_3\theta_{32}$

4. Hypothesis testing. By comparing the results of the two re-engineered models, we argue that the behavior tree of M_2 is intuitively more favorable to produce cycles than M_1 as there are 4 cycles over 9 trajectories compared to the 7 cycles over 49 trajectories. In M_2, the inequality requirements for a stable point are quite strict as there are restrictions on parameters that appear in all five state equations, as seen in Table 2. Then, the parameter space to get a stable trajectory is better defined, and then it will be more feasible to find parameter values that lead to oscillatory dynamics. We keep both models M_1 and M_2 for the next phase of development in the model-based design cycle. The current model M_2 seems more favorable to produce oscillatory behaviors with a higher probability, but for confirmation, stochastic methods should be applied to both trees.

2.4 Refinement of Plausible Network Parameters

5. Stochastic parameters. At this stage in the design cycle, we have looked at three GRN networks and models. The original IRMA network M_0 did not produce oscillations, so we discard it. Over the first four phases of the model-based design cycle, we have developed two hypothesized networks M_1 and M_2 that were both able to exhibit oscillatory dynamics. In this phase, we assign distribution functions to each parameter and, by using the method given in [1], we calculate the probability of each solution trajectory. The simulated behavior trees and the inequalities for each local transition are useful information to propagate the uncertainty down the behavior tree to obtain the probability of the stable trajectories versus cycles. Knowledge of parameter values is good as the IRMA model has been well-studied. Then by using well-defined distribution functions, the probability of occurrence is more accurate. However, in the case of a wide range of parameter values, we are still able to maximize the probability of the oscillatory behavior and minimize the occurrence of stable trajectories.
6. Network selection. The combination of the qualitative and stochastic aspects of the computational tool provide powerful insights to the study of GRNs because: (i) the qualitative simulation allows the ability to predict all possible system behaviors, and (ii) the stochastic parameters aids in the prediction of likeness of occurrence of a specific system behavior and incorporates the inherent stochasticity of regulation. Taken all-together, this becomes a process of development, ranking, and eventually selection of a network for implementation by the synthetic biologists. The top-ranking model, with the highest probability of getting the desired dynamics, might be difficult to implement in an experimental laboratory. Then, the final selected network will be a balance between the most plausible networks from this design cycle and experimental difficulties in *in vitro* and/or *in vivo* implementation.

3 Discussion

The time-consuming development of gene regulatory networks in synthetic biology is much improved with the use of mathematical modeling. However, the

available tools lack the ability to soundly analyze the hypothesized GRN and show a panoramic view of the possible dynamic behaviors in a qualitative context. The main goal of this article was to propose a new design cycle *in silico* that has the dual ability to apply a sound and complete qualitative tool [3] along with stochasticity [1] to obtain a desired behavior from a particular GRN model with the highest probability.

To provide a proof of principle, we apply the model-based design cycle to a real-life, well-studied gene network in the synthetic biology literature. Here, we successfully delve into the five-gene IRMA network to achieve oscillatory dynamics by modifying the parameter space and/or network structure. While these are preliminary findings, they show the ease of modifying a GRN design and the power of predicting all possible dynamical behaviors. Computational resources and valuable time is reduced dramatically by the symbolic approach when compared to the various numerical attempts of [5]. Also, the parameter search is quickly and easily modified with specifications in the initial inequalities and does not require the extensive computational approaches previously taken by [6].

The proposed simulator serves as a general-purpose automated-simulator; therefore, the implementation into different network structures only requires the GRN system to be formulated into a certain mathematical framework. This feature makes it an ideal candidate for network design and development, especially in the light of clear design goals such as achieving a desired behavior or specific requirements on transitional states before the desired outcome. Thus, the carefully designed phases of the model-based design cycle make it a promising tool for the engineering and re-engineering of future synthetic networks.

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