

Compositionality, stochasticity, and cooperativity in dynamic models of gene regulation

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We present an approach for constructing dynamic models for the simulation of gene regulatory networks from simple computational elements. Each element is called a “gene gate” and defines an input/output relationship corresponding to the binding and production of transcription factors. The proposed reaction kinetics of the gene gates can be mapped onto stochastic processes and the standard ordinary differential equation (ODE) description. While the ODE approach requires fixing the system’s topology before its correct implementation, expressing them in stochastic π -calculus leads to a fully compositional scheme: network elements become autonomous and only the input/output relationships fix their wiring. The modularity of our approach allows to pass easily from a basic first-level description to refined models which capture more details of the biological system. As an illustrative application we present the stochastic repressilator, an artificial cellular clock, which oscillates readily without any cooperative effects. [DOI: 10.2976/1.2804749]

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Providing efficient ways to model the dynamics of gene regulatory networks is an important challenge in systems biology. Many different methods have been proposed in the past for such dynamical networks. One prominent approach is based on discrete logical methods, going back to the pioneering work by Kauffman on synchronous Boolean networks (Kauffman, 1969) and Thomas on asynchronous Boolean networks (Thomas, 1973, 1991); reviews of the current state of such approaches are in De Jong, 2002, Smolen *et al.*, 2002. A different, independent approach is based on rate equations, hence on the continuous dynamics of nonlinear ordinary differential equations (ODEs) (Goldbeter, 1996). Finally, there are various variants of stochastic methods, based either on the master equation approach (Van Kampen, 1992) or on the equivalent Gillespie algorithm (Gillespie, 1977).

The basic underlying problem for the quantitative description of the dynamics of gene regulatory networks is the enormous diversity of the “actors” involved, i.e., the biomolecules

which determine the network structure and dynamics. Both from an analytic and a computational point of view, one therefore needs to simplify in order to make simulations of such networks feasible: representing all actors by individual computational elements is simply unfeasible. But this is not the only problem. Two obvious other challenges are: (i) to have flexible modeling schemes, and (ii) schemes which do not grow too fast with the increase of the number of reactions included.

In this paper we propose an approach to models of gene regulatory network dynamics which is both flexible and has such advantages in terms of system size. It combines two features:

First, our modeling approach to gene regulatory networks is based on an abstraction of the genome as a set of input-output elements, the *gene gates* (Blossey *et al.*, 2006). The properties of each gate are defined by a set of abstract kinetic reactions. (In the simplest - Boolean - setting, a gate would be either on or off.) Based on these modules, regulatory circuits

can be constructed by formulating input-output relationships between the gates. An advantage of this modeling approach is that it allows to start with a very simple construction of the gates to represent the overall topology of the network. We show how more biological detail can be added to the model while leaving the underlying topology of the network unaltered. The approach therefore permits to build computational models with variable degrees of detail which is highly desirable given the incomplete knowledge of most biological systems.

Second, the full advantage of our compositional approach can be seen by formulating the networks in terms of processes defined in a process calculus, the π -calculus, which originates in the field of programming languages in theoretical computer science (Milner, 1999), and has been proposed for applications to systems biology only recently (Priami *et al.*, 2001; Regev and Shapiro, 2002; Regev, 2003). Not only do the compositional features of this calculus allow to express each gate as an autonomous network element, they also significantly reduce the system size (Cardelli, 2007). For $2n$ elements, the size of the input/output interface equals $2n$, while the number of kinetic reactions can be in the worst case n^2 . An introduction to the stochastic π -calculus and its use in simulations is presented in the Supplementary Material (see also Phillips and Cardelli, 2007).

The process calculus directly allows for a stochastic formulation of the dynamics, which is clearly more realistic for networks of molecules with small copy numbers than the deterministic dynamics. This feature can indeed be critical for the description of the network dynamics.

We illustrate this by our application of the approach to the repressilator, a three-gate inhibitory network which is an artificial cellular clock realized experimentally (Elowitz and Leibler, 2000). While the repressilator readily oscillates within a stochastic dynamics without cooperative mechanisms in the interaction between genes and transcription factors, such cooperativity is required to bring about oscillations in a deterministic (nonlinear) gate dynamics as we show by mapping the gene gate kinetics onto deterministic ODEs.

Finally, we demonstrate the refinement of the basic description to include more details of the biological system for the repressilator by an inclusion of the transcription, translation and repressor binding processes. Protein complexation is found to regularize the oscillations.

MODELING GENE REGULATORY NETWORKS BY GENE GATES

Definition of a gene gate

To be specific, in this work we want to consider genetic interactions in genomes similar to those of prokaryotes (bacteria). In such organisms, the basic regulatory mechanism follows the classical dogma of molecular biology, according to which DNA “makes” RNA which in turns “makes” protein (Alberts *et al.*, 2002).

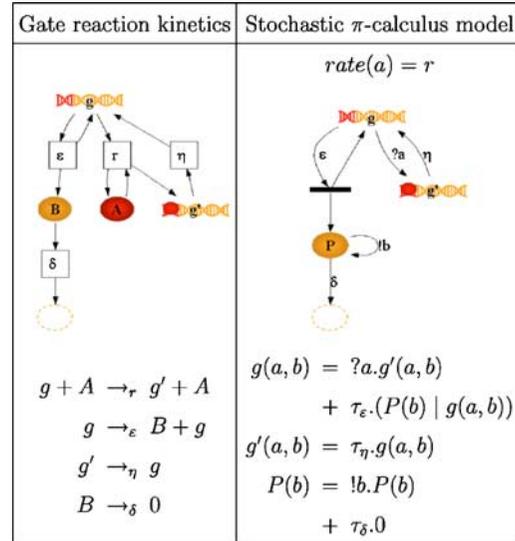


Figure 1. Gate reaction kinetics and stochastic π -calculus model of a gene gate. The kinetics denote stochastic reactions, where all the rates are of dimension s^{-1} .

The modeling scheme we propose for the gene regulatory circuits of such organisms is based on the idea that the action of each gene is uniquely identifiable by its regulatory input (activation/inhibition) and its regulatory output. In a first modeling step this therefore amounts to neglecting all intermediate steps, which are the formation of the gene-transcription factor complex, the recruitment of the polymerase for basal transcription, the transcription process of the gene, the translation process of the mRNA. Only the blocking of the gene by transcription factor recruitment (since in the present paper we only discuss repressed genes) and the production of a corresponding transcription factor from the gene is retained.

We represent the whole gene network as a composition of “gene gates.” A gene gate comprises not only all of the processes listed above but in addition also the degradation machinery of the proteins. In a gene gate, transcription and translation are lumped together in one parameter set, and protein degradation will be controlled by a separate parameter.

The physical basis for this initial modeling employing a reduction of variables is based on the common distinction between slow and fast variables. The selection of these variables is indeed important, as has been discussed in detail, e.g., in Bundschuh *et al.*, 2003. The advantage of our compositional/modular approach is that we can add all neglected intermediate layers of regulation in an easy fashion without affecting the basic topology of the network. In this way, the faster processes that were neglected in the beginning can be added in principle without any further approximations, as we demonstrate here.

We thus arrive at the representation of a gene regulator element as shown schematically in Fig. 1 (top left). The

double helix represents an active gene g , while the double helix with a blocked promoter region represents a blocked gene g' . The red and orange ovals represent different types of proteins, while the dotted ovals represent degraded proteins. The shapes are labeled with a gene name (g, g') or a protein name (A, B).

The graphical representation has a precise correspondence with the gate reaction kinetics shown at the bottom of the figure, which summarizes the possible reactions between the gate and the proteins. These reactions are the blocking of the gene by protein A , the production of protein B by the unblocked gene, the unblocking of g' , and the decay of the protein, all with their corresponding rates.

Gene gates in stochastic π -calculus

The stochastic π -calculus is essentially a modular language for describing the dynamics of a biological system, from which a set of reaction equations can subsequently be derived. The stochastic π -calculus differs from reaction equations in two fundamental ways. First, instead of modeling the individual reactions of a system, we model its components. This allows a system to be described in a modular fashion, so that each component can be modified independently. Second, instead of explicitly saying which component can interact with which other component, we describe the different sites on which a component can interact. This adds a layer of abstraction to the model, where two components can interact if they have complementary sites.

Figure 1 (left and right) compares the reaction equation model and a stochastic π -calculus model of the gene gate. As for the reaction kinetics, the graphical representations at the top of the figure are equivalent to the textual representations at the bottom. Each shape in the graphs represents a protein or gene in a particular state.

For the stochastic π -calculus model on the right, each labeled edge represents a reaction, which can be either unary or binary. Unary reactions are labeled with a reaction name, where each name is associated with a corresponding rate. For example, a protein can degrade by doing a reaction δ , and a blocked gene can unblock by doing a reaction η . A gene can also produce a new protein in parallel with itself by doing a reaction ε , where a horizontal bar represents parallel composition. Binary reactions are labeled with a reaction site preceded by a send (?) or receive (!), where each site is associated with a corresponding rate. For example, a gene can become blocked by receiving on site a , and a protein can react by sending on site b . Two entities can interact by sending and receiving on the same site, where the rate of the reaction is equal to the rate of the site. As a result, a protein that sends on site a can interact with a gene that receives on a , causing the gene to block.

Each shape in the model is parameterized by its interaction sites. The genes g, g' are parameterized by sites a and b , while the protein P is parameterized by site b . Thus $g(a, b)$

denotes a gene that receives on a and that produces proteins which send on b . The parameters allow networks of arbitrary complexity to be constructed from a single model of a gene gate. For instance, an autoinhibitory gate can be defined as $g(a, a)$, i.e., a gene that receives on a and that produces proteins which send on a . A bistable network can be defined as $g(a, b) | g(b, a)$ and a repressilator network can be defined as $g(a, b) | g(b, c) | g(c, a)$.

If we compare the two models in Fig. 1 we observe that the reaction equation model contains two proteins A, B , but does not fully describe the behavior of either. In particular, there is no information on how protein A is produced or degraded, or on how protein B interacts. In contrast, the stochastic π -calculus model describes the complete behavior of the protein P that is produced by the gene. Furthermore, the model does not need to explicitly mention protein A , since it only considers the site on which the gene can interact. This ability to describe the components of a system in a modular way is one of the main advantages of the stochastic π -calculus. Not only does this allow for more maintainable models, but it can also help to significantly reduce the model size. Consider the gene network described in Fig. 2, consisting of N proteins P_1, \dots, P_N , each of which can block M genes g_1, \dots, g_M . For the reaction equation model we need to explicitly state which protein can block which gene, resulting in a model of size $N \times M$. In contrast, for the stochastic π -calculus model we only need to state that each protein can send on site a and that each gene can receive on a , resulting in a significantly smaller model of size $N + M$.

APPLICATION: THE REPRESSILATOR IN STOCHASTIC π -CALCULUS

Parameter variation of a basic repressilator

In the first instance we explore the parameter space of a simple repressilator network, constructed using the gene gate described in Fig. 1. Our compositional approach to modeling allows the network to be defined in a straightforward manner as $g(a, b) | g(b, c) | g(c, a)$. Note that the initial population of proteins is empty: they are produced constitutively and stochastically by the gates. We assume that the sites a, b, c are associated with the same reaction rate r , resulting in a model with four parameters $r, \varepsilon, \eta, \delta$. Furthermore, since the dynamics of the network depends only on the relative rates of these parameters, we can arbitrarily fix the value of one parameter in order to study the effects of the other three. Here we fix the constitutive rate of protein production ε at a nominal value of 0.1, and vary the rates of protein degradation δ , gene unblocking η and gene repression r . The results of the parameter variation are shown in Fig. 3.

Figure 3(i) shows the simulation results for $\eta=0.00001$, $\delta=0.001$ and $r=1.0$. We observe alternate cycles of protein production, where each cycle is characterized by a dominant protein. The cycles alternate in a specific sequence of proteins $P(c)$, $P(b)$, $P(a)$ and the population of the

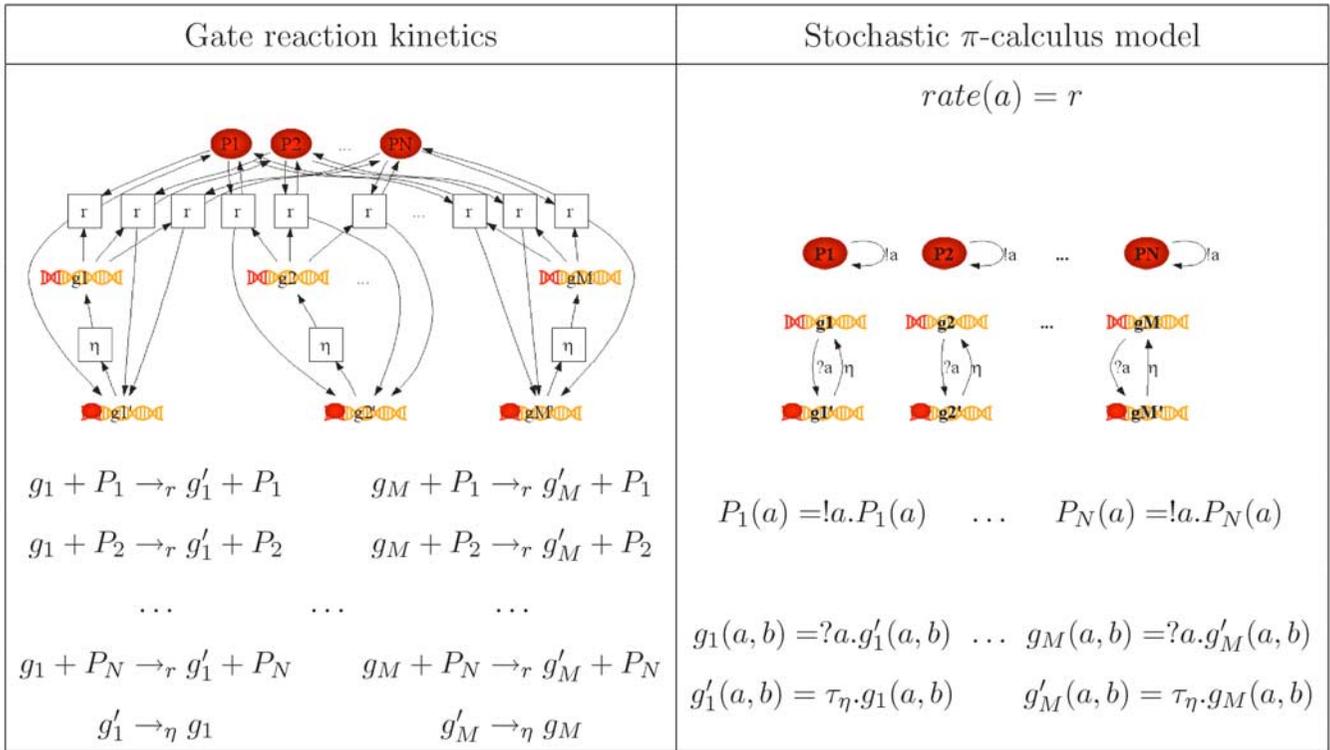


Figure 2. Reducing the combinatorial explosion in stochastic π -calculus.

dominant protein stabilizes at about 100 in each cycle. The dominant population fluctuates significantly due to stochastic noise in the system, and the duration of the cycles also varies considerably. We can improve on all these aspects of the repressilator model by adjusting its parameters appropriately.

First, we observe that the dominant protein population stabilizes at an equilibrium between production and degradation, given by ε/δ . We can limit the relative size of the fluctuations by decreasing the degradation rate to $\delta=0.0001$, resulting in a dominant population of about 1000, as shown in Fig. 3(ii).

Next, we observe that when one protein is dominant the other two proteins are absent and their corresponding genes are blocked, where one of the blocked genes is actively repressed. If the repressed gene unblocks then it is immediately blocked again by the dominant protein. If the unrepressed gene unblocks then it can start to produce proteins, which will repress the dominant gene and will themselves become dominant. The duration of protein cycles is highly irregular, since it depends on the rate of unblocking of the unrepressed gene, which is characterized by an exponential distribution. Furthermore, both blocked genes are in a stochastic race to unblock, and the duration of protein cycles will also depend on how far apart they unblock from one another, which is highly variable. We can reduce this variability by increasing the rate of gene unblocking to $\eta=0.0001$. As this rate is in-

creased, the effect of degradation plays a role in improving the regularity of oscillations: if a gene unblocks, it is immediately blocked again by any repressors that have not yet degraded. As a result, a gene can only start producing proteins when all residual repressors are degraded. Since the decay curve of each protein is fairly regular, we observe an increased regularity in the oscillations. In this setting, a gene can repeatedly block and unblock many times while waiting for the residual repressors to degrade. Unfortunately, this also increases the likelihood of a leaky production of proteins, which results in a stuttering of the oscillations, as observed in Fig. 3(iii).

We can compensate for this by increasing the rate of gene repression to $r=10.0$. In this setting, even if there is one protein remaining, it will still have a high probability of blocking the corresponding gene. This significantly reduces the probability of a leaky production of proteins, thereby reducing the stuttering effects, as shown in Fig. 3(iv).

We summarize the results of our parameter analysis for the repressilator network:

- The rate of protein degradation δ should be low enough so that the population of the dominant protein is large relative to its fluctuations.
- The rate of gene unblocking η should be higher than the rate of protein degradation, to enable protein cycles of regular duration.

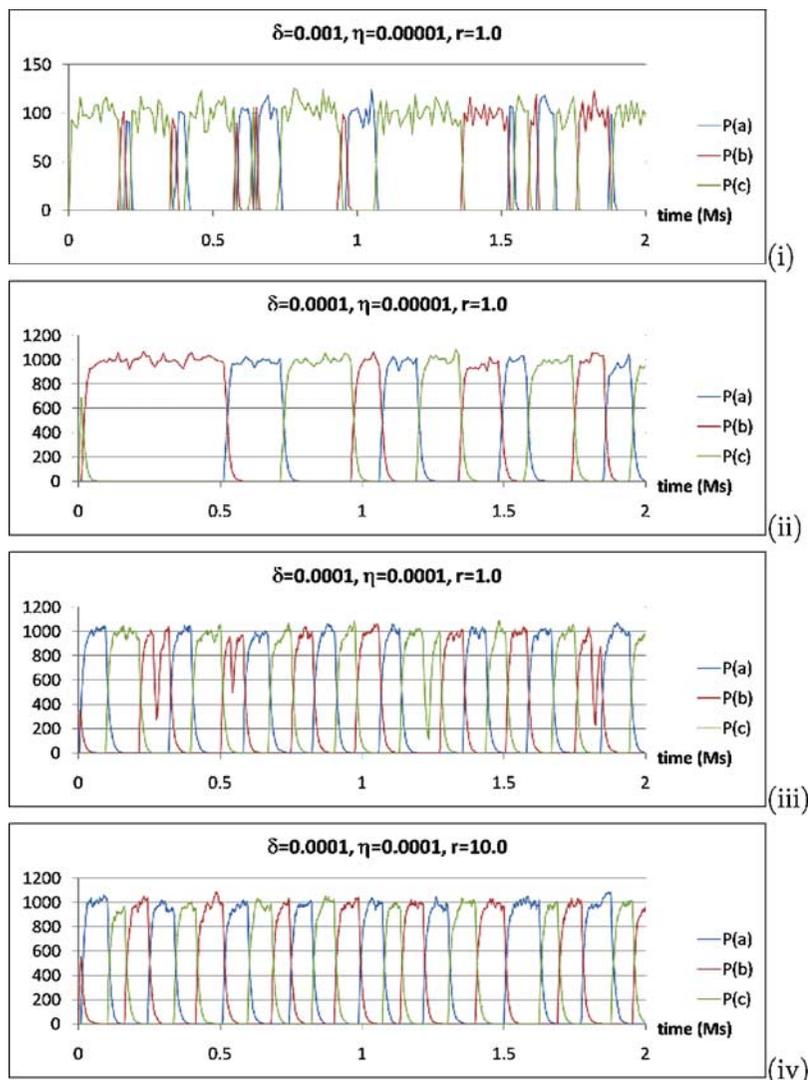


Figure 3. Parameter variation for a simple repressator network, based on the gene gate model of Fig. 1. The plots show the populations of proteins $P(a)$, $P(b)$, $P(c)$ over time (Ms).

- The rate of gene repression r should be high enough that a single protein will cause the gene to block before transcription can occur, to prevent the leaky production of proteins. The rate should also take into account the number of times that a gene can attempt to produce before the last repressor has degraded, which is determined by η/δ .

Using these basic principles we can design effective repressator networks with a wide range of parameters. In particular, successful designs should include all models that satisfy the constraints $\delta < \varepsilon/1000$, $\eta > \delta$ and $r > 100 \cdot \varepsilon \cdot \eta/\delta$. Additional details are provided in the online supplementary material.

We also note that the behavior of the stochastic repressator significantly differs from its deterministic counterpart. Later, we provide the derivation of the ODE system that follows from the kinetic reaction scheme. While the stochastic

repressator oscillates readily without cooperativity, it can be shown that this is not the case for the deterministic dynamics.

Transcription, translation, and repressor binding

The repressator network in the previous section was constructed using a highly simplistic model of a gene gate. In this section we examine various refinements to our gene gate model, and test whether the results of our parameter analysis are still applicable. Note that the high-level definition of the repressator network remains unchanged as $g(a,b)|g(b,c)|g(c,a)$. We simply refine our model of a gene gate to include more biological details.

Figure 4 presents a model of a gene gate which considers gene transcription and RNA translation. The simulation results with $\delta = \eta = 0.0001$, $r = 10$ and $\varepsilon_2 = \delta_2 = 0.01$ are almost identical to those of Fig. 3(iv), suggesting that our parameter analysis is still applicable. Here we fix $\varepsilon/\delta_2 = 10$ so that there is a continuous supply of a few RNA molecules to enable

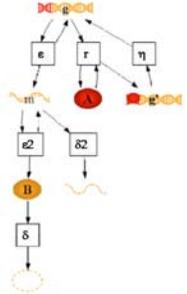
Gate reaction kinetics	Stochastic π -calculus model
 $ \begin{aligned} g + A &\rightarrow_r g' + A \\ g &\rightarrow_\epsilon m + g \\ g' &\rightarrow_\eta g \\ m &\rightarrow_{\epsilon 2} B + m \\ m &\rightarrow_{\delta 2} 0 \\ B &\rightarrow_\delta 0 \end{aligned} $	$ \begin{aligned} \text{rate}(a) &= r \\ g(a, b) &= ?a.g'(a, b) \\ &\quad + \tau_\epsilon.(m(b) g(a, b)) \\ g'(a, b) &= \tau_\eta.g(a, b) \\ m(b) &= \tau_{\epsilon 2}.(P(b) m(b)) \\ &\quad + \tau_{\delta 2}.0 \\ P(b) &= !b.P(b) \\ &\quad + \tau_\delta.0 \end{aligned} $

Figure 4. Gate reaction kinetics and stochastic π -calculus model of a gene gate with gene transcription and RNA translation.

steady translation, and we fix $\epsilon 2 / \delta = 100$ so that the dominant protein population stabilizes at about 1000.

Figure 5(i) presents a model of a gene gate in which a repressor must remain bound in order to block the gene. In this situation the simulation results do not produce alternating protein cycles when the rate of repressor binding is high, as shown in Fig. 6(i). This is because, when a repressor unbinds from a gene it has a high probability of re-binding, which gives rise to a situation in which all three genes are blocked. However, we do get oscillations when the rate of repressor binding is very low ($r=0.00001$) as shown in Fig. 6(ii), though the cycles are irregular. The low repression rate ensures that a single repressor has a low probability of switching off a gene. This allows the gene to produce proteins when the repressor finally does unbind, in order to start the next cycle. This also means that a large number of repressors is required in order for a gene to be switched off. We observe that a gene is typically switched off after about 100 repressors are produced. In this model it is also important for the DNA-TF complex to be long lived ($\eta=0.00001$) so that the repressor remains tightly bound for a sufficient length of time, comparable to the duration of a protein cycle. Unfortunately, low η also means that the oscillations do not occur at regular intervals, since the duration of protein cycles is determined by η as opposed to the smooth repressor degradation curve. If we increase η to 0.0001 we no longer obtain distinct oscillations, since the repressor can unbind too soon, after which the gene has a much higher probability of

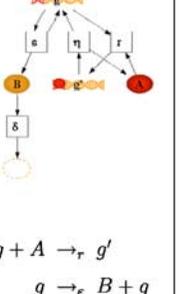
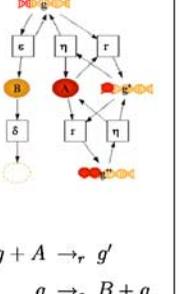
	Gate reaction kinetics	Stochastic π -calculus model
(i)	 $ \begin{aligned} g + A &\rightarrow_r g' \\ g &\rightarrow_\epsilon B + g \\ g' &\rightarrow_\eta g + A \\ B &\rightarrow_\delta 0 \end{aligned} $	$ \begin{aligned} \text{rate}(u) &= \eta \\ g(a, b) &= ?a(u).g'(a, b, u) \\ &\quad + \tau_\epsilon.(P(b) g(a, b)) \\ g'(a, b, u) &= !u.g(a, b) \\ P(b) &= (\nu u) !b(u).P'(b, u) \\ &\quad + \tau_\delta.0 \\ P'(b, u) &= ?u.P(b) \end{aligned} $
(ii)	 $ \begin{aligned} g + A &\rightarrow_r g' \\ g &\rightarrow_\epsilon B + g \\ g' &\rightarrow_\eta g + A \\ g' + A &\rightarrow_r g'' \\ g'' &\rightarrow_\eta g' + A \\ B &\rightarrow_\delta 0 \end{aligned} $	$ \begin{aligned} \text{rate}(u) &= \eta \\ g(a, b) &= ?a(u).g'(a, b, u) \\ &\quad + \tau_\epsilon.(P(b) g(a, b)) \\ g'(a, b, u) &= !u.g(a, b) \\ &\quad + ?a(u').g''(a, b, u, u') \\ g'(a, b, u, u') &= !u'.g(a, b, u) \\ P(b) &= (\nu u) !b(u).P'(b, u) \\ &\quad + \tau_\delta.0 \\ P'(b, u) &= ?u.P(b) \end{aligned} $

Figure 5. Gate reaction kinetics and stochastic π -calculus models of gene gates with repressor binding (i) and with two repressor binding sites (ii). In the stochastic π -calculus models the protein P is characterized by a local binding site u , written (νu) . In this setting, $!b(u)$ can be understood as “send on site b and bind to site u ,” and $?b(u)$ can be understood as “receive on site b and bind to site u .”

producing a protein than becoming blocked again. This causes the protein cycles to interfere with each other, as shown in Fig. 6(iii).

Interestingly, we can solve this problem by allowing proteins to degrade when still bound to a gene. We model this by replacing the definition of P' with $P'(b, u) = ?u.P(b) + ?u$ in Fig. 5(i), which is equivalent to adding a reaction $g' \rightarrow_{\eta g}$. This produces the desired oscillations, shown in Fig. 6(iv). At first glance the degradation of bound repressors may seem counterintuitive, but it can also be viewed as an abstraction of a more general requirement, which is that a repressor can somehow dissociate from a DNA binding site in an inactive form, such that it has very low probability of re-binding. One

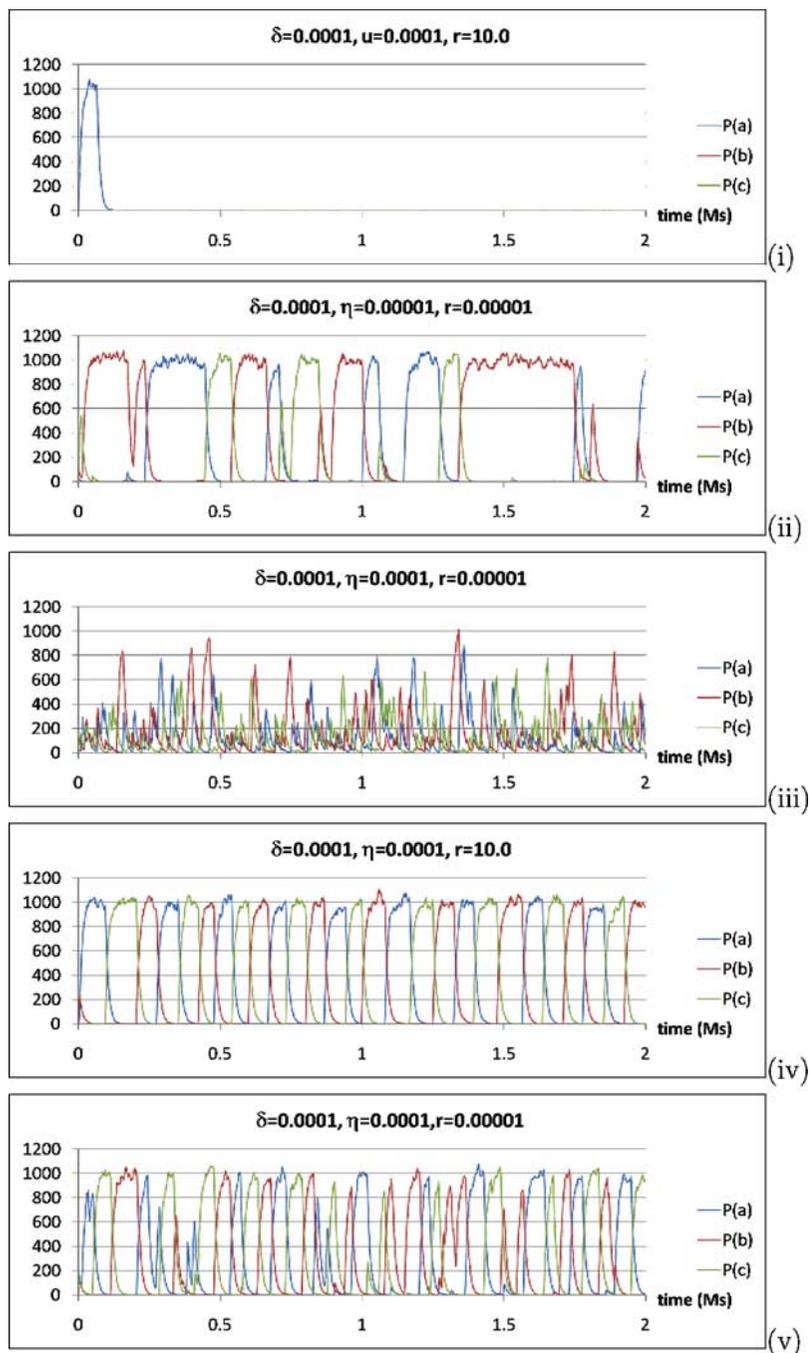


Figure 6. Simulation results for gene gates repressor binding (i)–(iii), for degradation of bound repressors (iv) and for gene gates with two repressor binding sites (v). The plots show the populations of proteins $P(a)$, $P(b)$, $P(c)$ over time (Ms).

way of achieving this is to allow two repressors to bind to the DNA, as shown in Fig. 5(ii). For large repressor populations, when a repressor unbinds it is more likely to bind again than for the second repressor to unbind. Conversely, for small repressor populations when a repressor unbinds it is much less likely to bind again. In this way, the population of repressors can be used to control the likelihood of gene activation, giving rise to more regular cycles. Corresponding simulation results are shown in Fig. 6(v). Although the protein cycles are still noisy, they are nevertheless of reasonably similar duration.

We summarize the results for our more detailed repressilator models:

- The presence of gene transcription and RNA translation does not significantly perturb the dynamics of the repressilator network, provided there is a continuous supply of a few RNA molecules.
- If we assume that a protein must remain bound in order to repress a gene then we can still obtain the desired repressilator dynamics, provided the bound proteins can also degrade. The degradation of bound

	Gate reaction kinetics	Stochastic π -calculus model
(i)	$ \begin{aligned} g + A2 &\xrightarrow{r} g' + A2 \\ g &\xrightarrow{\epsilon} B + g \\ g' &\xrightarrow{\eta} g \\ B &\xrightarrow{\delta} 0 \\ B + B &\xrightarrow{r_2} B2 \\ B2 &\xrightarrow{\delta} 0 \end{aligned} $	$ \begin{aligned} \text{rate}(a) = r, \text{rate}(b2) = r2/2 \\ g(a, b, b2) &= ?a.g'(a, b, b2) \\ &\quad + \tau_{\epsilon}.(P(b, b2) g(a, b, b2)) \\ g'(a, b, b2) &= \tau_{\eta}.g(a, b, b2) \\ P(b, b2) &= ?b2.P2(b) + !b2.0 \\ &\quad + \tau_{\delta}.0 \\ P2(b) &= !b.P2(b) \\ &\quad + \tau_{\delta}.0 \end{aligned} $
(ii)	$ \begin{aligned} g + A4 &\xrightarrow{r} g' + A4 \\ g &\xrightarrow{\epsilon} B + g \\ g' &\xrightarrow{\eta} g \\ B &\xrightarrow{\delta} 0 \\ B + B &\xrightarrow{r_2} B2 \\ B2 &\xrightarrow{\delta} 0 \\ B2 + B2 &\xrightarrow{r_4} B4 \\ B4 &\xrightarrow{\delta} 0 \end{aligned} $	$ \begin{aligned} \text{rate}(b4) = r4/2 \\ g(a, b, b2, b4) &= ?a.g'(a, b, b2, b4) \\ &\quad + \tau_{\epsilon}.(P(b, b2, b4) g(a, b, b2, b4)) \\ g'(a, b, b2, b4) &= \tau_{\eta}.g(a, b, b2, b4) \\ P(b, b2, b4) &= ?b2.P2(b, b4) + !b2.0 \\ &\quad + \tau_{\delta}.0 \\ P2(b, b4) &= ?b4.P4(b) + !b4.0 \\ &\quad + \tau_{\delta}.0 \\ P4(b) &= !b.P4(b) \\ &\quad + \tau_{\delta}.0 \end{aligned} $

Figure 7. Models of gene gates with dimerization (i) and tetramerization (ii) of repressors.

repressors is not essential for oscillations, but it does produce a significant improvement in their regularity.

Cooperativity by repressor dimerization and tetramerization

As a final modification of the stochastic repressilator we discuss the effect of cooperativity in transcription factor binding. For this we address the cases of dimerization and tetramerization. The gate reaction kinetics and the stochastic π -calculus models for these two cases are depicted in Fig. 7.

In the first model the gene produces a protein that can form a dimer by sending or receiving on site b2, and the resulting dimer can send on site b. In the second model, the dimer can form a tetramer by sending or receiving on site b4, and the resulting tetramer can send on site b. This way of

modeling dimerization is also compatible with biological reality, since a protein must be able to interact both on a site and on its complement in order to dimerize.

Figure 8 shows the effect of cooperativity on the repressilator network. The results on the left correspond to the repressilator with no cooperativity, while the results on the right correspond to the repressilator with tetramerization. The program code for the simulations is given in supplementary online material. Figure 8(i) shows the populations of the three proteins $P(a)$, $P(b)$, $P(c)$ over time. We observe that the populations fluctuate significantly less in the presence of cooperativity. We can quantify this by measuring the variability of the dominant protein populations over time. In order to obtain a clean separation of protein cycles, we only consider the dominant population of a given protein when the remaining two proteins are off. The principle of the approach is illustrated in Fig. 8(ii). We assume that a protein is on when its population is above a certain threshold, and off when its population is below this threshold, and we fix the threshold at roughly 10% of the observed steady state of protein levels, i.e., at about 100. We use this definition to extract the dominant protein populations from the simulation results in row (i) by application of a simple filter, in order to obtain the plots in row (ii). The gaps in the plots correspond to situations where multiple proteins are *on* simultaneously, which we deliberately ignore. This is a convenient metric for comparing the variability of dominant protein populations, since it filters out situations where multiple proteins have competing populations. We quantify the difference between the two models by measuring the mean and standard deviation of the dominant protein populations over a time period of 10^7 time units. In absence of cooperativity we observe a mean of 880 and a standard deviation of 196, whereas with tetramerization we observe a mean of 935 and a standard deviation of 98. For clarity, only the first 10^6 units are shown in Fig. 8(i),

(ii), and (iii). For a more coarse-grained comparison over the same time period, in the absence of cooperativity we observe that the dominant protein population falls below a threshold of 800 roughly 23% of the time, whereas with tetramerization it falls below this threshold only 3% of the time. In this setting, cooperativity acts to improve the regularity of oscillations by reducing the fluctuations in protein levels. In the presence of cooperativity, the leaky transcription of a gene is less likely to perturb the oscillations, since at least two proteins must be produced in order to have an effect in the case of dimerization, and at least four proteins are required in the case of tetramerization. Thus, cooperativity can be seen not as an essential requirement for oscillations, but as a means of improving the stability of oscillations over a wider range of parameters.

We can compare the regularity of oscillations by measuring the duration of protein cycles. Here we assume that a protein cycle starts when the protein is switched on, and ends when the next cycle starts. Figure 8(iii) shows histograms of

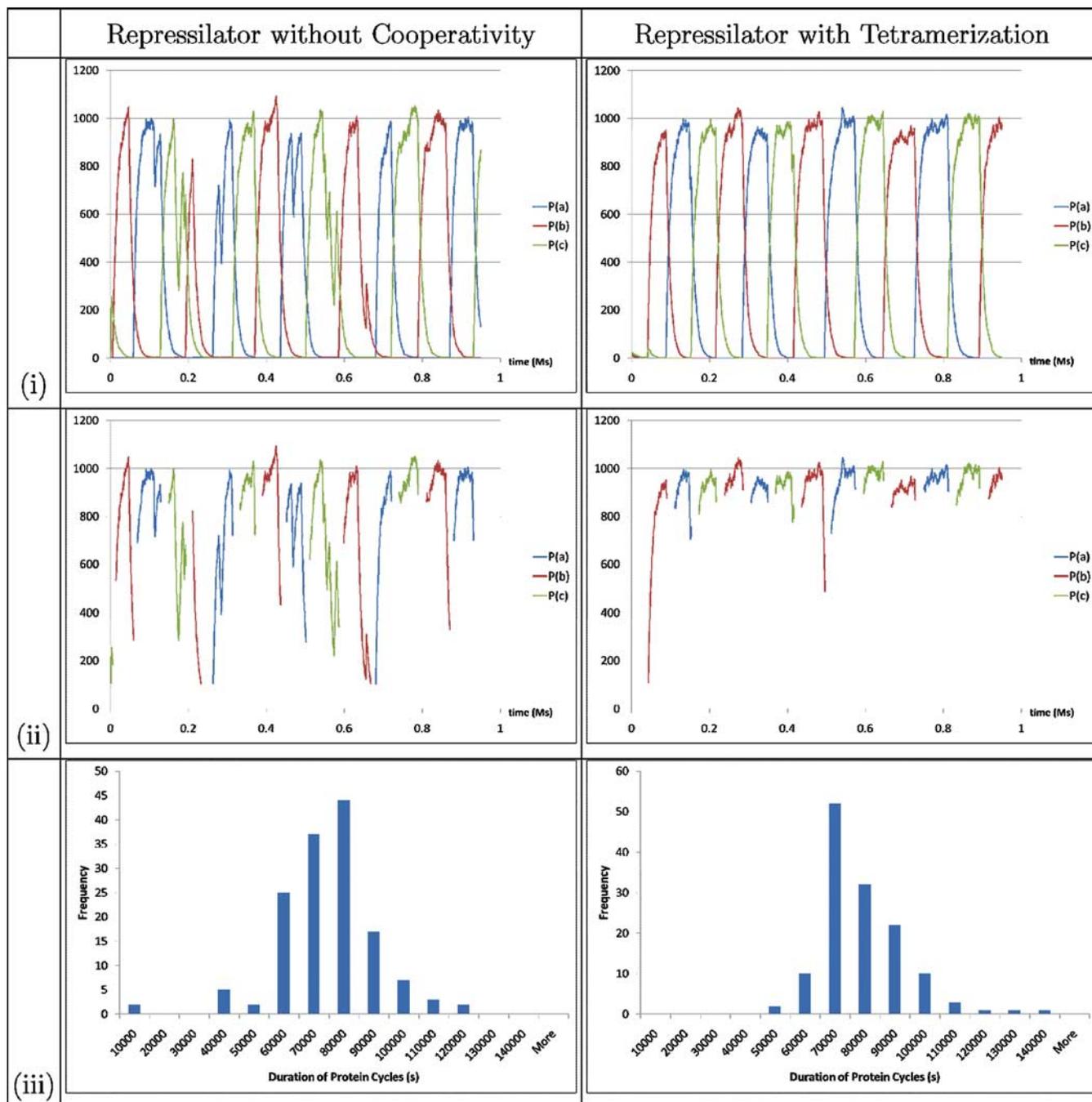


Figure 8. Observed effects of cooperativity on the Repressilator Network. The plots in row (i) and (ii) show the populations of proteins $P(a)$, $P(b)$, $P(c)$ over time (Ms). The plots in row (iii) show histograms of the duration of protein cycles for all three proteins. The results without cooperativity were obtained for $\varepsilon=0.1$, $\delta=0.0001$, $\eta=0.001$ and $r=1.0$, while the results with tetramerization were obtained for $\varepsilon=0.4$, $r=0.0001$ and $r4=0.0001$, with δ , η and r unchanged.

the duration of protein cycles for the three proteins. For both models there are approximately 140 protein cycles, and we observe a moderate improvement in cycle regularity in the presence of cooperativity. Without cooperativity we observe a mean duration of 69 000 and a standard deviation of 16 000, and with tetramerization we observe a mean duration of 75 000 and a standard deviation of 14 000.

Note that in presence of cooperativity the rate of gene repression r can be significantly lower than in absence of cooperativity, while still observing regular protein cycles. Not only does this improve the robustness of the network by allowing for a broader range of parameters, it could also be important in situations where the rate of repression is limited by cellular constraints. For example, if we assume that the

rate of protein-gene interaction is determined by random diffusion, it may be physically impossible for this rate to be above a certain threshold. Cooperativity could be one way for a cell to overcome this limitation.

RATE EQUATIONS OF THE GENE GATES

For completeness we establish how the gate reaction kinetics can be expressed in terms of rate equations (ODEs) by making use of the mass action law.

We demonstrate this by applying the scheme to the simplest circuit that can be built from the inhibitory gate, the autoinhibitory loop (Fall *et al.*, 2002), where the output B acts upon its own gate, hence B and A have to be identified; we first ignore the formation of protein complexes.

With the identification $A=B$ in the gate reaction kinetics in Fig. 1, the autoinhibitory loop is given by



In order to have a well-defined continuous setting we consider a cellular environment with a protein concentration $[A]_c$ (mol/L). We choose a population of autoinhibitory loops with a concentration $[N]_c$ (mol/L) and normalize according to

$$[A] \equiv \frac{[A]_c}{[N]_c}, \quad [g] \equiv \frac{[g]_c}{[N]_c}, \quad [g'] \equiv \frac{[g']_c}{[N]_c} \quad (5)$$

so that $[A]$, $[g]$, $[g']$ are concentration ratios, hence dimensionless quantities. For the gate states we have the conservation condition $[g] + [g'] = 1$. Casting the reaction kinetics into ordinary differential equations we have

$$\dot{[A]} = \varepsilon[g] - \delta[A], \quad (6)$$

$$\dot{[g]} = -r[g][A] + \eta[g'], \quad (7)$$

$$\dot{[g']} = r[g][A] - \eta[g']. \quad (8)$$

Note that due to our choice of dimensionless variables the kinetic parameters carry the same dimensions as the rates in the reaction scheme (s^{-1}) and we can therefore leave the same symbols. Using the conservation condition we can eliminate the equation for $[g']$ and end up with only one equation for the unblocked gate state $[g]$, i.e.,

$$\dot{[g]} = \eta(1 - (1 + \nu[A])[g]), \quad (9)$$

where $\nu \equiv r/\eta$. The inhibitory loop is therefore described by two ODEs, one each for $[A]$ and $[g]$.

We can relate the gene gate description to the common continuum description of the dynamics of gene networks. This can be achieved by making some additional simplifications which are of approximate nature. First, we observe that in the limit $\eta \rightarrow 0$, when $\nu \equiv r/\eta$ is kept finite, $[g]$ can be made very small without affecting the equation for $[A]$ since it does not depend on η . Therefore, this limit allows to separate the time scales of the dynamics of $[A]$ and $[g]$. For $\dot{[g]} \approx 0$, $[g]$ varies according to

$$[g](t) \approx \frac{1}{1 + \nu[A](t)}. \quad (10)$$

Inserting this equation into Eq. (6) one obtains an equation for $[A]$ which is given by

$$\dot{[A]} = \frac{\varepsilon}{1 + \nu[A]} - \delta[A]. \quad (11)$$

This is the standard Hill-type equation for an inhibitory loop (Cherry and Adler, 2000; Fall *et al.*, 2002) in the case of a noncooperative inhibition.

We can easily check the quality of this approximation. From Eq. (10) we obtain

$$\dot{[g]} = -\frac{\nu\dot{[A]}}{(1 + \nu[A])^2} = -\frac{\nu}{(1 + \nu[A])^2} \left(\frac{\varepsilon}{1 + \nu[A]} - \delta[A] \right) \quad (12)$$

which shows that $\dot{[g]}=0$ is strictly fulfilled only at the stationary points of the dynamics of $[A]$. Due to the $[A]$ dependence of the denominator in the equation the time variation of $[g]$ becomes indeed small if $[A]$ is large; but for small values of $[A]$, and away from the stationary points, the approximation becomes increasingly poor. For the autoinhibitory loop it can indeed be seen from the numerical solutions of the equations that for large initial values of $[A]$, and near the stationary state for $t \rightarrow \infty$, the exact and approximate solutions coincide, but for the intermediate range of concentrations, both do differ quantitatively (not shown).

For the fixed points of the full system we find from $\dot{[A]} = \dot{[g]} = 0$ the conditions

$$\varepsilon g_0 = \delta A_0, \quad g_0 = \frac{1}{1 + \nu A_0} \quad (13)$$

which lead to a unique equilibrium solution. Perturbations around the fixed-point value (A_0, g_0) in the form $A = A_0 + \delta A$ obey

$$\begin{pmatrix} \dot{\delta[A]} \\ \dot{\delta[g]} \end{pmatrix} = \begin{pmatrix} -\delta & \varepsilon \\ -\frac{\eta\nu}{1 + \nu A_0} & -\eta(1 + \nu A_0) \end{pmatrix} \cdot \begin{pmatrix} \delta[A] \\ \delta[g] \end{pmatrix}. \quad (14)$$

Denoting the matrix in this equation by \mathbf{J} , one has $\text{Det } \mathbf{J} > 0$ and $\text{Tr } \mathbf{J} < 0$ independent of the parameter values, hence the equilibrium state is indeed stable (Fall *et al.*, 2002).

We now allow for dimerization of the transcription factor A . In the gate reaction kinetics we have



and the dimers degrade according to



We now assume that the dimers activate the gene according to (see Fig. 6, top)



The rate equation for the dimers thus reads as

$$[\dot{A}_2] = d[A]^2 - \delta[A_2]. \quad (18)$$

Upon assuming that the dimerization reaction is in equilibrium, $[\dot{A}_2]=0$, we can relate the concentrations of dimers, $[A_2]$, to $[A]^2$, and define an equilibrium constant K_D . This leads to a modification of Eq. (9)

$$[\dot{g}] = \eta(1 - (1 + \nu_D[A]^2)[g]), \quad (19)$$

with $\nu_D \equiv \nu K_D$. The denominator in Eq. (11) is then replaced by

$$\frac{\varepsilon}{1 + \nu[A]} \rightarrow \frac{\varepsilon}{1 + \nu_D[A]^2}, \quad (20)$$

where the exponent $h=2$ is the Hill-exponent corresponding to dimerization. The case of tetramerization can be treated analogously.

We finish this section by writing down the ODE equations of the repressilator. It consists of a three-gene circuit in which each gene represses the transcription of one of the other genes in a circular manner, e.g., $g_1 \dashv g_2 \dashv g_3 \dashv g_1$.

For the deterministic version of the repressilator the gene gate equations read as follows. Denoting the corresponding transcription factors of the repressilator genes by $[A_i]$, with $i=1, 2, 3$, the rate equations of the repressilator are given by the six ODEs

$$[\dot{A}_i] = \varepsilon[g_i] - \delta[A_i] \quad (21)$$

$$[\dot{g}_i] = \eta(1 - (1 + \nu_h[A_{i-1}]^h)[g_i]) \quad (22)$$

with periodic conditions on the indices ($[g_4] \equiv [g_1]$).

In Eqs. (21) and (22), the gene-transcription factor interaction is assumed to be cooperative with a general Hill exponent h whose value is left unspecified here; dimerization corresponds to the value $h=2$ and tetramerization to $h=4$. For a deterministic version of the repressilator, called ‘‘RepLeaky’’ by the authors, it has recently been shown that a sufficient criterion on the Hill exponent is $h > 4/3$ in order to bring about sustained oscillations (Müller *et al.*, 2006). Although the RepLeaky repressilator is formulated in terms of a protein-mRNA model, and hence its nonlinear dynamic equations thus differ from those of our gate-based version, it

turns out that the result by Müller *et al.* also applies to our case. This follows from a comparison of the stability analysis of both models, which shows that the equations governing the linear stability of the fixed points can be mapped onto each other. Hence, according to the sufficient criterion for oscillations developed by Müller *et al.*, the noncooperative repressilator which we found to oscillate readily in its stochastic case does not oscillate in its deterministic version since $h=1$.

Finally, it is instructive to compare the ODE description of the repressilator based on the gene gates with other ODE description of gene regulatory circuits, see, e.g., Widder *et al.* (2007). Here, the modeling of simple gene circuits starts with considerably more biological detail than our gene gate description, which is minimalistic. However, keeping all the details is often difficult if not impossible, and sometimes not even needed. We believe it is therefore more reasonable to start with a basic model and do refinements at a later stage. At the lowest level of detail, the basic model could ultimately be simplified to the level of stochastic boolean networks, by ignoring the protein species and modeling the interaction between gene gates directly. This is clearly difficult in modeling schemes that are not sufficiently modular, one clear advantage of the stochastic π -calculus, as presented in this work.

CONCLUSION: CONTRIBUTIONS AND RELATION TO OTHER WORK

To conclude, we have presented an approach to model gene regulatory networks which is fully compositional and stochastic due to the use of a process calculus description of gene gates. It is made possible by exploiting the compositional features of the stochastic π -calculus, which greatly facilitates the exploration of model design through simulation.

Our approach demonstrates that for a better understanding of the effect of regulatory mechanisms, a coarse model can indeed be a useful starting point; we have shown how a stepwise modification of such a model can provide novel insights into their role. For our system at hand, the repressilator, we could establish that stochasticity alone is sufficient to bring about oscillatory behavior in the three-gene network, and that, contrary to the deterministic case, cooperative mechanisms are not needed. The latter are not without effect, as we could see: cooperativity of binding regularizes the oscillations. Furthermore, we have shown how additional mechanisms in transcription, translation and repressor binding influence the oscillatory behavior of the network.

We like to note that the noncooperative case is also not entirely academic: while in protein binding cooperativity is mostly present in in-vivo cellular genetic networks, it now becomes technically feasible to build artificial transcriptional oscillators based on DNA and RNA which lack protein binding cooperativity in the transcriptional initiation process (Simmel F, private communication, 2007; Kim *et al.*, 2006).

The properties of such artificial and in-vitro networks can thus be expected to yield novel information about the functional constraints of gene regulation systems, in particular when combined with mathematical modeling.

We have also established the relationship between the stochastic and the deterministic (ODE) description of the gene gates. While both descriptions are valid representations of the underlying gate reaction kinetics, the example of the repressilator clearly shows that both descriptions do not yield equivalent system behavior.

Stochastic effects in networks have previously been studied mostly in the context of their role in perturbing an underlying deterministic dynamics. Also there surprising effects were observed, like the occurrence of oscillatory behavior at a finite distance from a Hopf bifurcation, or even oscillations via a different type of bifurcation (Freidlin, 2001; Lee Deville *et al.*, 2006). We stress that in our context stochastic effects do not act merely as perturbations of an underlying deterministic dynamics, but bring about the dynamic behavior in the first place.

It remains a challenge to find the correct abstraction level for the representation of the biologically relevant features of a regulatory network in terms of computable elements. In this respect our compositional approach is of advantage, since it permits to modify the properties of the individual components by fine tuning without affecting the overall network topology. Supporting information is available in an EPAPS document.

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