

Compositionality, stochasticity and cooperativity in dynamic models of gene regulation

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We present an approach for constructing dynamic models of gene regulatory networks from simple computational elements. Each element is termed a gene gate and defines an input/output-relationship corresponding to the binding and production of transcription factors. The proposed gene gate kinetics is mapped onto standard rate equations and stochastic processes. 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. 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 . As an application we present the stochastic repressilator, which we show oscillates readily without any cooperative effects. Modifications due to the inclusion of cooperative mechanisms are discussed.

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Providing efficient ways to model the dynamics of gene regulatory networks is an important challenge. Many different methods have been proposed, ranging from discrete, logical approaches [1] to rate equations (ode's) [2], and master equations [3, 4]. An underlying problem for the quantitative description of the dynamics of gene regulation is the enormous diversity of the 'actors' involved, i.e., the biomolecules which determine the network structure and dynamics. Both from an analytic and computational point of view, one therefore needs to simplify: 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 modelling schemes, and ii) schemes which do not grow too fast with the increase of the number of reactions included.

We propose a *compositional* approach to models of gene regulatory network dynamics which is both flexible and has such advantages in terms of system size. It is based on an abstraction of the genome as a set of input-output elements, the *gene gates*. The properties of each gate are defined by a set of abstract kinetic reactions. Based on these modules, simple circuits can be constructed by formulating input-output relationships between the gates. We show that the reaction kinetics of the gates maps to the standard description by ode's in the deterministic case. However, the full advantage of our approach can only be seen by formulating the network in terms of processes defined in a process calculus, the π -calculus, originating in the field of programming languages in theoretical computer science [5]. Not only do the compositional features of this calculus allow to express each gate as an autonomous network element, but they also significantly reduce the system size: if there are $2n$ species

in the system, it is described by n^2 reactions and a stoichiometric matrix of size $2n^3$ [6]. The *process calculus* keeps the system size description manageable: as in programming notations, the system description is a compact representation of the combinatorial state space.

Furthermore, the process calculus directly leads to a stochastic formulation of the dynamics, which is clearly more realistic for networks of molecules with small copy numbers. This feature can indeed be critical for a correct description of the network dynamics, as we illustrate by our application of the approach to the repressilator, a three-gate inhibitory network [7].

To be specific, in this work we want to consider interactions between genes in genomes equivalent 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 [8]. Since in prokaryotes the role of RNA is fairly passive, it seems feasible to coarse-grain over its degrees of freedom. One is then left with a network in which only DNA and proteins occur. As a further simplification, we consider only single and not multiple inputs and outputs, but this restriction can easily be dropped.

We abstract the whole gene network as one composed of "gene gates". A gene gate comprises not only the reading process of the gene - transcription - by RNA polymerase, but also the translation of the mRNA transcript into protein, and finally also the degradation machinery of the proteins. Transcription and translation will be lumped together in one parameter set, and protein degradation will be controlled by a separate parameter set. We thus arrive at the representation of a gene reg-

FIG. 1: i) A gene gate g which receives a transcription factor A as input and gives off a transcription factor B as its output. ii) Three elementary gene circuits constructed from the basic gene gate shown in i). From top to bottom: autoinhibitory circuit; bistable switch; repressilator (for explanation, see text). For simplicity, the gate names and in- and outputs are suppressed.

ulatory base element as shown in Figure 1 i). Here, a protein A can repress the action of gene gate g . If not repressed, the gene gate produces a protein B which can, in turn, act upon either the same gene gate (if $B = A$) or, within a regulatory circuit, on other gene gates. In this abstraction, the proteins can also be interpreted to act like messages that are being exchanged between the gene gates via their inputs and outputs.

The action of a gene gate can be described in a formal gate reaction kinetics. The first reaction is the action of A on g ,



where A binds on g with rate r and g is turned into the state g' . The factor A is released unaltered.

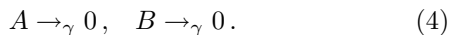
The gene gate g can undergo two kinds of reactions. We assume that it constitutively produces a protein B at a basal rate ε ; the corresponding reaction reads as



Further, since the transcription factor on input represses the gate, we have the reaction



i.e., the gate state g' simply relaxes back to g . A low rate of relaxation will leave the gate in the state g' and hence effectively repress transcription factor production. Finally, we list the degradation reactions for the proteins,



Eqs.(1) - (4) constitute the basis of our gate-reaction scheme.

We now apply this scheme to the simplest circuit that can be built from the inhibitory gate, the autoinhibitory loop [9], where the output B acts upon its own gate, hence B and A have to be identified. We first show that, on a deterministic level, our description reduces to the standard ode-based approach. For this, we cast the gate kinetics into rate equations. The autoinhibitory loop is then described by the system of ordinary differential equations

$$\dot{A} = \varepsilon g - \gamma A, \quad (5)$$

$$\dot{g} = -rgA + \eta g' \quad (6)$$

$$\dot{g}' = rgA - \eta g'. \quad (7)$$

Since $g + g' = 1$ is a constant (there is only one gene), we can eliminate the equation for g' and then end up with one equation for the gate, i.e.,

$$\dot{g} = \eta - (\eta + rA)g. \quad (8)$$

The inhibitory loop is then described by two ode's, one each for A and g . If the relaxation rate η is high and $\nu \equiv r/\eta = \text{finite}$, $\dot{g} \approx 0$, and we can insert eq.(8) into the equation for A which yields

$$\dot{A} = \frac{\varepsilon}{1 + \nu A} - \gamma A, \quad (9)$$

i.e., a standard Hill-type equation for an inhibitory loop [9].

This approach allows us to describe any given network with single input/output relations in terms of a collection of ode's; also gates with positive activation can be formulated in the same way [10]. The network construction is however not compositional: for any given network, we have to predefine its topology first, via the kinetic reactions, and then translate them into ode's. This problem arises already for the simple autoinhibitory gate. Suppose we had started with the reaction equations eqs.(1) - (4), had written down the ode's, and only *then* identified A with B : the network topology would have been fixed afterwards. In this case, we would then have been left with two identical expressions for the degradation of the protein A , one stemming from the original equation for A , the other from the equation for B . This problem is clearly easy to fix for such a trivial case, but becomes very difficult to tackle systematically for large networks.

It is hence desirable to define each network element as an autonomous object with its proper actions on input and output, and then to be able to build the networks only by fixing the wiring. To achieve this for our gene gates, we invoke an approach originating in theoretical computer science. This so-called *process calculus* approach was pioneered for computational biology by A. Regev and E. Shapiro [11, 12]. They proposed that the interaction of two biomolecules, e.g. the complexation of two proteins, can be understood as a communication in which messages are exchanged. In the simple protein example the messages correspond to the binding actions of the two interacting protein domains: these have to convey the message to each other that they are "ready to bind", or, "ready to be bound", respectively. The exchange of these messages abstracts the physical interactions [5].

The process calculus approach has a distinct advantage over other schemes exactly due to its compositionality properties. In our example of the autoinhibitory switch we define the gate as a *process* in π -calculus by $neg(A, B)$ where A, B are *channels* on which input and output messages are sent [13]. Transcription is thus described by a process rule in which outputs are defined by their dependence on input. Going back to the kinetic

FIG. 2: Output generated by the stochastic bistable switch without cooperativity. Time-series of the fluctuating output of the protein numbers. The parameters in the simulation are $r = 1.0$, $\varepsilon = 0.1$, $\eta = 1 \times 10^{-3}$, $\gamma = 1 \times 10^{-2}$.

reactions, eqs.(1) and (2), we see that we have to define two rules, one of basal transcription with a rate ε , which does not depend on input, and inhibition of transcription with a rate η , which depends on the input.

Further, and importantly, the degradation mechanism is bound to the channel on which the messages pass. Therefore, the process describing the autoinhibitory gate $neg(A, A)$ automatically calls only the channel A , while a gate $neg(A, B)$ would call both channels A and B . Each neg -gate acts as a fully autonomous computational module which can be composed at will without predefining the systems' topology.

E.g., a bistable switch circuit can easily be represented by parallel composition (indicated by the symbol $|$) of inhibitory gate processes $neg(x, y)$ in the form of

$$neg(A, B)|neg(B, A) \quad (10)$$

A further advantage of the process description are its size requirements. For a system of $2n$ elements, writing down the reaction equations leads in general to a set of n^2 equations (the reaction matrix has size $2n^3$ in the worst case, but it can be sparse). Since in the process calculus approach one describes the “compatible complementary interaction surfaces” of each species and leaves the matching table implicit, this amounts to computing an input/output interface of $2n$, hence that of the number of elements to begin with [14].

Finally, in the process calculus approach, compositionality and its advantages of system size scaling are accompanied by its stochastic features. The interaction dynamics of the gates can be computed by selecting input and output calls via the Gillespie algorithm [10, 15]. The dynamics produced by the gates then admits to calculate the stochastic traces of the system evolution. This is illustrated in Figure 2, which shows the switching dynamics of the bistable switch, as given by eq.(10). Such behaviour was found previously from a solution of a master equation for the system [16]. The stochastic π -calculus result obtained via the Gillespie algorithm is fully equivalent. Inspection of the master equation as given in [16] shows immediately, however, that it is not compositional in our sense. In fact, writing down master equations for more complex systems becomes rapidly a daunting task. This is not so with the gene gates in π -calculus. The repressilator, e.g., is easily spelled out as

$$neg(A, B)|neg(B, C)|neg(C, A) \quad (11)$$

where the placement of input and output channels inside each gate again fully specifies the wiring topology of the

FIG. 3: Stochastic repressilator output generated by variation of the gene relaxation rate over one order of magnitude. The parameters in the stochastic simulation are $r = 1$, $\varepsilon = 0.1$, $\gamma = 1 \times 10^{-4}$, $\eta = 1 \times 10^{-5}$ (top), $\eta = 1 \times 10^{-4}$ (middle), and $\eta = 1 \times 10^{-3}$ (bottom).

circuit. The same holds true for networks of arbitrary wiring complexity, and the approach then becomes even more advantageous.

The usefulness of having a stochastic rather than a deterministic description had been advocated before for the bistable switch, which readily switches without the need for any kind of nonlinearity in the inhibition, or for explicit time-delays between inhibition and degradation [9, 16]. In order to achieve this in a deterministic set-up, the nonlinearity in eq.(9) must be modified according to the replacement

$$\frac{\varepsilon}{1 + \nu A} \rightarrow \frac{\varepsilon}{1 + \nu A^h} \quad (12)$$

with a Hill-exponent $h > 1$ in the denominator. A Hill exponent $h > 1$ is indicative of “positive cooperativity”: the exponent ensures that the inhibition mechanism is sufficiently steep upon concentration changes. Microscopically, cooperativity is produced by protein complexation, e.g. by the formation of transcription factor dimers in the cell, which then interact with the DNA. In the case of the bistable switch, the presence of cooperativity is crucial for the deterministic system: otherwise it would not even have two stable states [17].

The effect of stochasticity on the network dynamics is even more dramatic for the inhibitory three-gate circuit, the repressilator. In the deterministic setting, a Hill exponent of at least $h = 4/3$ is needed for sustained oscillations [18]. By contrast, the stochastic repressilator readily produces oscillations without any cooperative mechanism. Figure 3 displays three time-series generated by the repressilator kinetics; what is changed from the top to the bottom figure is the relaxation rate of the gene gates, by two orders of magnitude (see Figure caption). One full oscillation cycle consists in the alternation of the three different proteins, whereby always one protein dominates over the other ones. The protein populations stabilise at an equilibrium between transcription and degradation. For low relaxation rates we observe an irregular oscillation, since the relaxation of the genes then is highly variable. As this rate is increased, the effect of degradation plays a role in improving the regularity of oscillations: if a gene unblocks, it is immediately blocked again by any inhibitory proteins that have not yet degraded. As a result, a gene can only start transcribing when all residual inhibitory proteins are degraded. Since the decay curve of each protein is fairly regular, we observe an increased regularity in the oscillations. For even higher relaxation rates, a gene can repeatedly block and unblock many times while waiting for the residual proteins to degrade. This increases the likelihood of a leaky

FIG. 4: The effect of cooperativity on the stochastic repressilator. Values indicated where different from Figure 3. Top: cooperativity by dimerization; $\varepsilon = 0.1$, $\eta = 1 \times 10^{-3}$, $\gamma_2 = 1 \times 10^{-4}$ (degradation of the dimer), $hom2 = 1 \times 10^{-4}$ (dimerization). Bottom: cooperativity by tetramerization; same rates as for dimerization, with $\gamma_4 = 1 \times 10^{-4}$ (degradation of the tetramer), $hom4 = 1 \times 10^{-4}$ (tetramerization).

transcription of proteins, which results in a stuttering effect in the oscillations, as observed in Figure 3 (bottom).

Further, we have studied the effect of cooperativity on the stochastic repressilator, see Figure 4. In the top graph, cooperativity is introduced by requiring the proteins produced from one gate to dimerize first before being able to bind to the subsequent gate. In the bottom graph, these dimers must also bind to form tetramers before being able to bind the gate. In this setting, cooperativity acts to improve the regularity of oscillations by reducing the stuttering effects observed in Figure 3 (bottom). 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.

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. Our application to the repressilator poses interesting questions on the role of cooperativity in the dynamics of stochastic networks. So far, stochastic effects in networks have been studied in the context of their role in perturbing an underlying deterministic dynamics. Also there surprising effects were observed, like the occurrence of oscillatory behaviour at a finite distance from a Hopf bifurcation, or even oscillations via a different type of bifurcation [19, 20]. In our context stochastic effects do not act merely as perturbations of an underlying deterministic dynamics. It remains a challenge for the modeler and the experimenter alike to find the correct abstraction level for the representation

of a real regulatory network in terms of computable elements.

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