Steady-state solution of probabilistic gene regulatory networks

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We introduce a probability model for gene regulatory networks, based on a system of Chapman-Kolmogorov equations that represent the dynamics of the concentration levels of each agent in the network. This unifying approach includes the representation of excitatory and inhibitory interactions between agents, second-order interactions which allow any two agents to jointly act on other agents, and Boolean dependencies between agents. The probability model represents the concentration or quantity of each agent, and we obtain the equilibrium solution for the joint probability distribution of each of the concentrations. The result is an exact solution in "product form," where the joint equilibrium probability distribution of the concentration for each gene is the product of the marginal distribution for each of the concentrations. The analysis we present yields the probability distribution of the concentration or quantity of all of the agents in a network that includes both logical dependencies and excitatory-inhibitory relationships between agents.

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I. GENE REGULATORY NETWORKS

Kaufman [1] pioneered models of gene regulatory networks [2] that have been extended [3,4] in order to include logical dependencies between agents [5–8], as well as stochastic dynamics [9–11].

In this paper we develop a unifying approach to model the noisy behavior of regulatory networks that includes (i) excitatory and (ii) inhibitory interactions between agents, and (iii) second-order interactions which allow any two agents to jointly act on other agents. We also show that Boolean dependencies between agents can be modeled with our approach by using second-order interactions.

The model studied in this paper represents the *concentration levels* or quantity of each agent in the network. All transitions in the model are probabilistic. Time is represented via random transition times whose average value depends on which agents are involved in each transition. The work in [12,13] is a precursor of the approach that we develop here. The present paper extends our prior work on G networks [14,15], so as to compute the probability of activation of the agents in the presence of complex interactions.

The model in this paper differs from probabilistic Boolean networks [10] in that we propose an integer valued concentration level for each agent i, denoted by $K_i(t)$, and we study the stochastic dynamical behavior of the vector $K(t)$ whose elements are the $K_i(t)$, with the probability distribution $P(k,t)=P[K(t)=k]$. On the other hand, we define a mapping of the variables $K_i(t)$ into binary variables $B_i(t)$ such that $B_i(t)=1$ if $K_i(t)>0$, and $B_i(t)=0$ if $K_i(t)=0$, and also compute the steady-state probabilities of the $B_i(t)$ from the corresponding distribution for the $P(k,t)$. However, we do not deal directly with the dynamics of the vector $B(t)$ whose elements are the $B_i(t)$. In our model $K(t)$ is a Markov chain in continuous time, but $B(t)$ is *not* a Markov chain.

In [16] a deterministic population model is considered; it uses nonlinear ordinary differential equations [Eq. (1) in [16]] to represent the concentration or quantity of different genes. The approach in [16] is similar to the use of the general mass equations (GMA) of chemistry, and variability due to biology and measurement noise is represented in [16] by modifying the parameter values in the data sets. Our approach uses a probabilistic model similar to the chemical master equations (CME) of chemistry, and noise is intrinsically part of the model. In chemistry, the GMA are a "macroscopic" deterministic approximation of the "microscopic" probabilistic representation provided by the CME. The work of Ribeiro *et al.* [11] also considers the latter approach, and in [11] the system is analyzed using Monte Carlo simulations, while our work pursues an analytic approach. Also, [11] describes the logical dependencies between agents via rate equations, while here we present both the probabilistic CME and derive explicit Boolean dependencies between these equations.

Since practical measurements with microarrays will deal with large populations of cells each of whose individual instantaneous behavior may not be synchronized, in [17] the effect of the variation in the number of cells which have a given gene expression at a given measurement instant is studied; signal processing techniques are used to derive the correct gene expression for cyclic gene expression from a large number of cells with specific reference to a single gene. This paper presents a model based on a single cell and multiple agents, and includes the time-dependent probabilistic dynamics as presented in the differential equation (5).

II. REGULATORY NETWORKS AND G NETWORKS

We will first begin by presenting the model that we propose, which is based on G networks [14,18,15,19], which are probabilistic dynamical models with an unbounded discrete state space, operating in continuous time. The model is composed of the following:

(i) *Agents*, which are the primary objects of interest; they represent genes or other active biochemical or living objects whose levels of activity we wish to represent.

(ii) *Gates*, which represent the interactions between

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agents; gates are either *binary* in nature (i.e., they describe the effect of agent i on agent j), or they are *ternary* and describe the joint effect of two agents on a third agent, or they are multivalued, representing the impact of a set of agents on a given agent. By chaining sequences of agents with ternary gates, we obtain joint effects of multiple agents on a single agent.

We will now set up the *probability model* for regulatory networks, and discuss its analytical solution. The probability model is defined via the following quantities defined for any agents $i,j,l \in \{1,\ldots,N\}$:

(i) The $K_i(t) \geq 0$ are integer-valued *random variables* which represent the *concentration or quantity* of the agents i at time $t \geq 0$. In the following equations, we assume that any agent i will be able to interact with other agents as long as $K_i(t)$ is positive, while it cannot interact with other agents when $K_i(t)=0$.

(ii) Each $\Lambda_i \geq 0$ is a real number representing the rate at which agent i is being replenished from some external source. Furthermore $\Lambda_i \Delta t + o(\Delta t)$ is the probability that in a time interval $[t,t+\Delta t[$ the variable $K_i(t)$ increases by +1 due to the replenishment of agent i from an external source. Notice that $1/\Lambda_i$ is the average time between increases in $K_i(t)$ that are caused by external replenishment of the agent.

(iii) Similarly, if $K_i(t)>0$ then the agent may be depleted and $\lambda_i \Delta t + o(\Delta t)$ is the probability that in a time inerval $[t,t+\Delta t[$ the level of $K_i(t)$ will drop by 1, so that $\lambda_i \geq 0$ is the rate at which agent i is being depleted, provided that agent i is present in some positive concentration.

(iv) The $r_i \geq 0$ are real numbers representing the activity rates of each agent i, provided again that the agent is present in some nonzero amount. Then $1/r_i$ is the average time between successive interactions of agent i with other agents, and $r_i \Delta t + o(\Delta t)$ is the probability that in a time interval $[t,t+\Delta t[$, agent i interacts with another agent. If agent i does interact with another agent, then the following events occur:

(a) With probability $P^+(i,j)$, it interacts with agent j in a facilitating (excitatory) mode; when this happens, $K_i(t)$ is depleted by 1 and $K_j(t)$ is increased by 1.

(b) With probability $P^-(i,j)$, it interacts with agent j in an inhibitory mode; when this happens, $K_i(t)$ is depleted by 1 and $K_j(t)$ is also depleted by 1.

(c) With probability $Q(i,j,l)$ agent i joins with agent j to act upon agent l in excitatory mode, as a result of which both $K_i(t)$ and $K_j(t)$ are reduced by 1, while $K_l(t)$ is increased by 1.

Finally for any i,

$$d_i + \sum_{j=1}^{n} \left(P^+(i,j) + P^-(i,j) + \sum_{l=1}^{n} Q(i,j,l) \right) = 1, \tag{1}$$

where d_i is the probability that agent i does not interact with other agents and its content is depleted due to some natural effect. If $d_i=1$, then the agent does not act on other agents at all, for instance, if it is the end product of a series of other interactions. In order to use a more compact notation, we replace the rates r_i and the probabilities by "weights" in the following manner:

$$w^+(i,j) = r_i P^+(i,j), \tag{2}$$

$$w^-(i,j) = r_i P^-(i,j), \tag{3}$$

$$w(i,j,l) = r_i Q(i,j,l). \tag{4}$$

Note that from the above assumptions $\Lambda_i, \lambda_i, r_i$ are the parameters of exponential distributions, and Λ_i, λ_i are the arrival rates of independent Poisson processes which, respectively, increase or decrease the level of the variables $K_i(t)$.

The dynamics of the G network can now be represented by a system of Chapman-Kolmogorov (CK) differential and difference equations that govern the *random process* $K(t) = [K_1(t),\ldots,K_n(t)]$, $t \geq 0$. This process represents the number of units, or the concentration, of the n different types of agents.

Denote by $k=[k_1,\ldots,n]$ an n-vector of non-negative integers, and let $P(k,t)=P[K(t)=k]$ be the probability that $K(t)$ takes that particular value k. In order to write the CK equations, define e_i to be the n vector all of whose elements are zero *except* for the ith element whose value is +1. The dynamic behavior of the G network is then given by

$$\begin{aligned}
\frac{dP(k,t)}{dt} = \sum_{i=1}^{n} \Bigg(& P(k+e_i,t)(\lambda_i + r_i d_i) + \Lambda_i P(k-e_i,t) 1(k_i > 0) \\
& - P(k,t)[\Lambda_i + (\lambda_i + r_i) 1(k_i > 0)] \\
& + \sum_{j=1}^{n} \Bigg\{ [P(k+e_i-e_j,t) 1(k_j > 0)] w^+(i,j) \\
& + [P(k+e_i+e_j,t) + P(k+e_i,t) 1(k_j = 0)] w^-(i,j) \\
& + \sum_{l=1}^{n} P(k+e_i+e_j-e_l,t) 1(k_l > 0) \\
& \times [w(i,j,l) + w(j,i,l)] \Bigg\} \Bigg),
\end{aligned} \tag{5}$$

where all of the terms $P(y,t)$ on the right-hand or left-hand side of the equation are zero if any of the elements of the vector y are negative.

A. Exact solution

The model we have presented is a special case of the "G network with triggered customer movement," which we have introduced previously in the context of queueing theory [15]. Consider now the manner in which the system behaves in the long run, represented by its equilibrium probability distribution $P(k)=\lim_{t\to\infty} P(k,t)$, and introduce the term

$$q_i = \min\left[1, \frac{\Lambda_i + \sum_{j=1}^{n} q_j w^+(j,i) + \sum_{j,l=1,l\neq j}^{n} q_j q_l w(j,l,i)}{r_i + \lambda_i + \sum_{j=1}^{n} q_j w^-(j,i) + \sum_{j,l=1,l\neq j}^{n} q_l w(l,i,j)}\right] \tag{6}$$

for $i=1,\ldots,n$, which represents the probability that agent i is activated in steady state.

Theorem 1. For any subset $I\subset\{1,\ldots,n\}$ such that $q_m<1$ for each $m\in I$, and $I=\{m_1,\ldots,m_{|I|}\}$,

$$P(K_m = k_m) = q_m^{k_m}(1-q_m), \tag{7}$$

$$P[(K_{m_1},\ldots,K_{m_{|I|}}) = (k_{m_1},\ldots,k_{m_{|I|}})] = \prod_{i=1}^{|I|} q_{m_i}^{k_{m_i}}(1-q_{m_i}). \tag{8}$$

The proof of this theorem, stated in a slightly different manner, can be found in [15].

Note. Consider the following behavior of a cell with respect to a given single gene: On for time $T1$, off for time $T2$, on for time T_1, off for time T_2, and so on indefinitely. This is one possible example of a deterministic steady-state behavior. If an observer measures this behavior at a random instant $t\gg T_1,T_2$, the observer will fall upon either the on or the off state and the probability that it will observe the on state is $P(\mathrm{ON})=\frac{T1}{T1+T2}$. A similar statement holds if T_1,T_2 are random variables; writing $E[\ ]$ as the expectation, we would have $P(\mathrm{ON})=\frac{E[T_1]}{E[T_1]+E[T_2]}$. In the context of the probability model in the paper, $P(k)=\lim_{t\infty} P(k,t)$ provides the corresponding quantity. $P(k)$ does not say that the state is always the same; it simply says that $P(k)$ is the probability that the state we observe in steady state (i.e., for large t) has the value k.

Notice that (6) is a system of nonlinear equations; thus we need to determine the conditions under which these equations have a solution, and also to determine whether they have a *unique* solution. Fortunately this was also proved in [15].

Theorem 2. The solution of (5) as provided by (6), (8), and (9) exists and is unique.

B. An example

In this section we develop a simple example to illustrate the use of the approach we have introduced. In this example, three types of agents interact. The agents or entities (C, V, A) interact via facilitation-excitation, inhibition, and joint facilitation of an agent by two others.

Agent C in isolation. In the system we consider, we would like to observe whether the agent C is activated. When it exists in isolation, with a replenishment rate Λ_c and a depletion rate r_c, using (6) we have

$$P(K_c>0) = \rho_c = \frac{\Lambda_c}{r_c}. \tag{9}$$

If $\Lambda_c<r_c$, while if $\Lambda_c\geqslant r_c$ then $P(K_c>0)=1$ and agent C is constantly activated; in particular, if $r_c=0$ there is no natural depletion of agent C.

The effect of agent V. If agent V is introduced into the system at some rate Λ_v, it has the effect of combining with C to have a joint excitatory effect on itself (positive self-feedback) represented by $w(v,c,v)$. Thus V depletes itself and depletes C, but it also reactivates itself in the process, so that it is both depleting C and maintaining its own level of activation. We suppose that agent V is not subject to some other natural form of removal from the medium, except through its effect on agent C. Thus $r_v=w(v,c,v)$. As a result when V is present we now have

$$q_c = \frac{\Lambda_c}{r_c + w(v,c,v)q_v} = \frac{\Lambda_c}{r_c+\Lambda_v}, \tag{10}$$

$$q_v = \frac{\Lambda_v + q_v q_c w(v,c,v)}{w(v,c,v)}, \tag{11}$$

so that

$$\frac{\Lambda_c}{r_c+\Lambda_v} \leqslant q_c \leqslant \frac{\Lambda_c}{r_c + w(v,c,v)} < \frac{\Lambda_c}{r_c}. \tag{12}$$

In particular, when $r_c=0$, we see that the introduction of agent V results in having

$$P(K_c>0) = \frac{\Lambda_c}{\Lambda_c+\Lambda_v} < 1, \tag{13}$$

instead of $P(K_c>0)=1$. In fact, if $\Lambda_v>\Lambda_c$, then $P(K_c>0)<0.5$ which may be unacceptably low. As a result, we now take the following step.

Introducing agent A. Now in order to limit the effect of V we introduce an agent A which has an inhibitory effect on V so that, still assuming that $r_c=0$, we have

$$q_a = \frac{\Lambda_a}{w^-(a,v)}, \tag{14}$$

$$q_v = \frac{\Lambda_v + q_v q_c w(v,c,v)}{w(v,c,v) + q_a w^-(a,v)}, \tag{15}$$

$$q_c = \frac{\Lambda_c}{q_v w(v,c,v)}. \tag{16}$$

Conclusion. From the above equations, if agent A is introduced in sufficient concentration or at sufficient rate so that

$$\Lambda_a > \frac{w(v,c,v)\Lambda_v}{\Lambda_c} \tag{17}$$

then $P(K_c>0)=1$ and agent C remains constantly activated despite the presence of agent V.

C. A second example

As a second example, consider the following toy regulatory network [20] composed of four agents, call them $\{M_0,\ldots,M_3\}$ connected cyclically so that the ith agent inhibits agents $(i+1)\bmod 4$ and agent $(i+2)\bmod 4$, and there are no other dependencies. Assume that agents have just two states (on and off).

The timing in this simple model may be either deterministic, where each agent changes state in exactly unit time, or random (e.g., exponentially distributed) of average value 1 for all agents, or each agent can have a different timing behavior. Thus the resulting behavior of this synchronous or asynchronous system can be quite different depending on what is assumed about the time between state transitions of the agents. Another important assumption about such a network concerns the state the agents will enter when they are quiescent, i.e., when they are left to themselves. Clearly, if the agents left to themselves all enter the 0 (off) state, then the model has little interest since all agents will remain in that state, assuming that they start there. On the other hand, if we assume that they spontaneously enter the 1 or "on" state when they are not acted upon by another agent, then more interesting behaviors can result. Also, the meaning of these interconnections can be interpreted in at least two different ways, for any $i=1,\ldots,3$,

Interpretation (1). $M_i=\neg M_{(i-1)\bmod 4}\wedge M_{(i-2)\bmod 4}$.

Interpretation (2). Both agents $M_{(i-1)\bmod 4}$ and $M_{(i-2)\bmod 4}$ inhibit the activation of agent M_i.

Assume now that all agents start in the same initial state, that all state transition times are exponentially distributed with average value 1, and that when they are quiescent (i.e., free of inputs from other agents) they all reset themselves to the value 1 ("on"). For both interpretations the probabilistic state of all agents will be identical, and their stationary distribution $q=\lim_{t\to\infty}P[A_i(t)=1]$ is given by the following:

Interpretation (1). Using (35) we write $q=(1-q)(1-q)$ so that $q=0.382$.

Interpretation (2). Using (7) we have $q=\frac{1}{1+2q}$ so that $q=0.5$.

Under interpretation (2), the agents will all spend *on the average* half of the time being "on" and the other half being "off." All 16 states, represented by the vector of four binary variables, will be equally likely with probability 1/16 in steady state. With interpretation (1) they spend more time in the "off" state than in the "on" state; in fact in this case the state (0,0,0,0) is 6.854 times more likely to occur than the state (1,1,1,1). Thus, the manner in which the interactions between agents are precisely defined has significant impact on the analysis that our modeling approach can offer.

III. DEPENDENCY OF AN AGENT'S STATE ON MORE THAN TWO OTHER AGENTS

We have seen that an agent can act on another agent via facilitation-excitation or inhibition, or two agents can jointly act on a third via inhibition. These relationships result in the expression (6) where we have the following:

(i) In the *numerator* on the right-hand side of the expression for q_i we have the excitation terms $q_jw^+(j,i)$ and $q_jq_lw(j,l,i)$.

(ii) In the *denominator* on the right-hand side, we have the inhibition terms $q_jw^-(j,i)$ and the terms $q_lw(l,i,j)$.

Thus in (6), although the state of an agent depends on that of many others, we do not have expressions on the right-hand side of the form $q_{l_1}q_{l_2}\cdots q_{l_m}$, $m\geqslant 3$ which would be necessary if we would like to have dependencies such as "the state of agent i depends on the joint probabilities of agents $l_1,l_2,\ldots,l_m$."

Therefore, we construct an extended G network that responds to this need:

(i) Consider a G network Γ which contains the agents $\{1,\ldots,n\}$, and the additional agents $\Theta(a)=\{a_1,\ldots,a_\alpha\}$, $\Theta(b)\{b_1,\ldots,b_\beta\}$.

(ii) We construct another G network $\hat{\Gamma}$ that contains all the agents of Γ, and in addition contains the "dummy agents" specified below.

(iii) Introduce two sets of "dummy agents" $A_1,A_2,\ldots,A_\alpha$ and $D_1,\ldots,D_\beta$ that are used as intermediaries between some of the agents of Γ.

(iv) a_1 excites A_1, (a_2,A_1) jointly excite A_2 and so on. Finally $(a_\alpha,A_{\alpha-1})$ excite A_α, and A_α acts on agent l in an *excitatory* manner. The related parameters are $w^+(a_1,A_1)$, $w(a_2,A_1,A_2)=1$, until $w(a_\alpha,A_{\alpha-1},A_\alpha)=1$, and finally $w^+(A_\alpha,l)=1$.

(v) We set $\Lambda_{A_s}=\lambda_{A_s}=0$, $r_{A_s}=1$ for $1\leqslant s\leqslant\alpha$.

(vi) We also introduce dummy agents $D_1,\ldots,D_\beta$ so that (b_1) acts upon D_1 in an excitatory manner with $w^+(b_1,D_1)=1$, (b_2) acts upon D_2 similarly, and so on, and b_β acts upon D_β in an excitatory manner with D_β with $w^+(b_\beta,D_\beta)=1$.

(vii) Then each D_β acts upon agent l in an *inhibitory* manner with $w^-(D_\alpha,l)=1$.

(viii) We set $\Lambda_{D_s}=\lambda_{D_s}=0$, $r_{D_s}=1$ for $1\leqslant s\leqslant\beta$.

Using (7), we immediately obtain

$$q_{A_1}=q_{a_1}w^+(a_1,A_1),$$

$$q_{A_s}=q_{a_s}q_{A_{s-1}},\quad s=2,\ldots,s=\alpha,$$

$$q_{D_1}=q_{b_1}w^+(b_1,D_1),$$

$$q_{D_s}=q_{b_s}q_{D_{s-1}},\quad s=2,\ldots,s=\beta, \tag{18}$$

so that we have

$$q_l=\min\left(1,\frac{\Lambda_l+\sum_{j=1}^{n}q_jw^+(j,l)+w^+(a_1,A_1)\prod_{s=1}^{\alpha}q_{a_s}}{r_l+\lambda_l+\sum_{j=1}^{n}q_jw^-(j,l)+w^-(b_1,D_1)\prod_{s=1}^{\beta}q_{b_s}}\right). \tag{19}$$

IV. BOOLEAN DEPENDENCIES BETWEEN AGENTS

For each G network we can also derive a "Boolean B network" via a homomorphic mapping H as follows.

Let Z^+ be the set of non-negative integers, and let $k \in \{Z^+\}^N$ be an N vector of non-negative integers. The mapping H is defined by

$$H:\{Z^+\}^N \to \{0,1\}^N, \tag{20}$$

such that

$$H(k) = [h(k_1), \ldots, h(k_N)],$$

where

$$h(k_i) = 1 \quad \text{if } k_i > 0,\ h(k_i) = 0 \quad \text{if } k_i = 0. \tag{21}$$

We define the Boolean B network as the system obtained from a given G network simply by applying the mapping H to the G-network state vector $K(t)$,

$$B(t) = [B_1(t), \ldots, B_N(t)], \tag{22}$$

where $B_i(t)=h[K_i(t)]$, $B(t)=H[K(t)]$.

Similarly we can define the probability distribution for the random binary N vector $P[B(t)=v]$ for the Boolean N vector $v=(v_1,\ldots,v_N)$ which represents a specific value taken by $B(t)$.

As a direct consequence of Theorem 1 we have the steady-state probability distribution for the B network.

Theorem 3. The steady-state probability distribution of $B(t)$ is given by

$$P[B = v] = \lim_{t\to\infty} P[B(t) = v] = \prod_{i=1}^{N} q_i^{v_i}(1 - q_i)^{(1-v_i)}, \tag{23}$$

where the q_i are given by (6), and obviously the ith term in (23) is q_i when $v_i=1$ and is $(1-q_i)$ when $v_i=0$.

Thus the B-network state is simply computed from the G-network state. Its use will become apparent in Sec. IV, we study logical dependencies between the agents in a regulatory network.

Note also from Theorem 3 that the marginal probability distributions of the B_l also have a simple form,

$$P[B_i = 1] = q_i, \quad p[B_i = 0] = (1 - q_i). \tag{24}$$

We can now use (19) to compute the state of an agent as a function of the product of the activation probabilities of other agents, using just the dummy agents A_s, and we have the following direct consequence of (19).

Theorem 4. Consider the B network $\hat{\mathbf{B}}$ which results from the G network $\hat{\Gamma}$, and from (24) let $q_l=P[B_l=1]$ be the steady-state probability that agent l is activated in the network $\hat{\mathbf{B}}$. Then using (19), if $w^+(b_1,D_1)=0$, $w^+(j,l)$ for all $j \neq A_\alpha$, $w^-(j,l)=0$ for any other agent j, with $w^+(A_\alpha,l)=1$, and $\Lambda_l=\lambda_l=r_l=0$, we have

$$q_l = \prod_{s=1}^{\alpha} q_{a_s}, \tag{25}$$

which may be written as

$$q_l = P[B_l = 1] = \prod_{s=1}^{\alpha} q_{a_s} = \prod_{s=1}^{\alpha} P[B_{a_s} = 1],$$

$$= P[\mathop{\wedge}_{s=1}^{\alpha} B_{a_s} = 1]. \tag{26}$$

The *conjunctive (CNF) and disjunctive (DNF) normal forms* are standard representations for Boolean functions. Each of them is universal in the sense that it allows the representation of any Boolean function. Consider a set of binary literals $B_j \in [0,1]$ which represent the activated (1) or inactivated (0) state of an agent, $j \in [1,\ldots,n]$, and consider a term $T_i=X_{i1}\vee\cdots\vee X_{in}$ where X_{ij} is either B_j or it is $\neg B_j$.

The Boolean function $F:[0,1]^n \to [0,1]$ is in DNF if it is written as

$$F = \mathop{\wedge}_{i=1}^{m} T_i, \tag{27}$$

while it is in CNF when it is written as

$$F = \mathop{\vee}_{i=1}^{m} \tau_i, \tag{28}$$

where the $\tau_i=X_{i1}\wedge\cdots\wedge X_{in}$ with X_{ij} being either B_j or $\neg B_j$, and they too are disjoint. Clearly we can transform an expression in CNF into DNF and vice versa using the well-known Boolean identity,

$$\neg[X \vee Y] = \neg X \vee \neg Y.$$

In the following we will shall show how the expression (19) can be used to derive the state probability for a logical expression in CNF. Before doing so, we will introduce the *complement* of an agent, and also show how the joint effect of two agents on a third one that is available in the numerator of (6) can be exploited to obtain a product term of the probabilities associated with *several* agents in the right-hand side of (6).

A. Constructing an agent's complement

In order to obtain the probability of a term in the expression on the right-hand side of (27), within the G-network model we also need a "complement" a_c for any agent a so that $B_{a_c}=\neg B_a$ so that $\lim_{t\to\infty} P[B_{ca}=1]=\lim_{t\to\infty} P[B_a=0]$ and

$$q_{a_c} = P[B_{a_c} = 1] = 1 - q_a. \tag{29}$$

Let $\Theta \subseteq \{1,\ldots,n\}$ be a subset of the set of agents in Γ. We construct a new G network Γ_Θ which contains all the agents in Γ, plus agents $\Theta_c=\{a_c : a \in \Theta\}$ which are the complements of the agents Θ. To construct the additional agents, we must do two things:

(i) Relate the parameters and interconnections of an agent's complement to the original agent in Γ so that (29) is satisfied, and obtain sufficient conditions concerning the agents in the original network so that this can be accomplished.

(ii) Show that the values of the stationary probabilities of the original agents in Γ have not been modified in the new network.

We will assume that the following conditions are satisfied for the agents $a \in \Theta$: these will be the sufficient conditions for the existence of the G network Γ_Θ.

(I) $r_a + \lambda_a \geq \Lambda_a$.

(II) For any agents $j, l \neq a$ in the original G network Γ we must have $w(j,a,l)=0$. This implies that agent a cannot be involved in second-order effects *initiated* by other agents in the network, although it is possible that $w(a,j,l)>0$.

(III) Finally, we require that $w^-(j,a) \geq w^+(j,a)$.

Note that as a result of these three conditions we obtain

$$1 \geq \frac{\Lambda_a + \sum_{j=1}^{n} w^+(j,a)}{r_a + \lambda_a + \sum_{j=1}^{n} q_j w^-(j,a)}.$$

From (6) we have

$$q_a = \frac{\Lambda_a + \sum_{j=1}^{n} q_j w^+(j,a)}{r_a + \lambda_a + \sum_{j=1}^{n} q_j w^-(j,i)} \tag{30}$$

and the steady-state probability that agent a's complement is activated given by

$$1 \geq q_{a_c} = \frac{r_a + \lambda_a - \Lambda_a + \sum_{j=1}^{n} q_j [w^-(j,i) - w^+(j,a)]}{r_a + \lambda_a + \sum_{j=1}^{n} q_j w^-(j,i)}, \tag{31}$$

so that for each $a \in \Theta$ we have

(i) $\Lambda_{a_c} = r_a + \lambda_a - \Lambda_a$.

(ii) $r_{a_c} + \lambda_{a_c} = r_a + \lambda_a$ which can be obtained by setting $r_{a_c} = r_a$, $\lambda_{a_c} = \lambda_a$.

(iii) $w^-(j,a_c) = w^-(j,a)$.

(iv) $w^+(j,a_c) = w^-(j,a) - w^+(j,a)$.

(v) Finally we set $w(j,a_c,l)=0$ for any $j,l \in \{1,\ldots,n\} \cup \Theta \cup \Theta_c$.

Thus all the parameters of the complement agent a_c are now known since they are obtained directly from the parameters of a, or they are set to zero.

Of course from (30) we can also obtain the original value $q_a = 1 - q_{a_c}$.

B. Expressions in conjunctive normal form

Consider the Boolean function $F:[0,1]^n \to [0,1]$ in CNF, written as the conjunction of disjoint terms

$$F = \bigwedge_{u=1}^{m} \tau_u, \tag{32}$$

where we have the following:

(i) A term is written as $\tau_u = X_{u1} \wedge \cdots \wedge X_{u\alpha}$ with X_{us} being either B_{a_s} or $B_{a_{cs}} = \neg B_{a_s}$,

(ii) $m = 2^\alpha$ since all possible terms are being summed, and obviously

(iii) the terms are disjoint, i.e., $\tau_u \wedge \tau_v = \phi$ for $u \neq v$.

Notice that if the B_{a_s} and $B_{a_{cs}}$ are independent Boolean variables, which is the case when $P[B_{as}=1] = \lim_{t\to\infty} P[B_{a_s}(t)=1]$, then

$$P[F=1] = \sum_{u=1}^{m} \prod_{s=1}^{\alpha} P[X_{us}=1], \tag{33}$$

and we will use the developments in Secs. IV A and III to prove the following result.

Theorem 5. For any expression in CNF (32), there exists a G network with a set of agents A, which contains the agent F, the agents $\{a_1,\ldots,a_\alpha\}$ and their complements $\{a_{c1},\ldots,a_{c\alpha}\}$, as well as dummy agents $\{A_{us}: 1 \leq u \leq 2^\alpha, 1 \leq s \leq \alpha\}$, such that for $q_F = \lim_{t\to\infty} P[B_F(t)=1]$ is given by (33).

Before we detail the proof let us indicate that this result states that, given a specified Boolean dependency between agents of a regulatory network, one can use the G-network model to represent these Boolean dependencies. Since the regulatory network itself is *probabilistic*, these Boolean dependencies will be reflected in equations about the *probabilities of the state of the agents*, i.e., these probabilities will be are consistent with the Boolean dependencies that have been given.

Proof of Theorem 5. From Sec. IV A we know that

$$q_{a_s} = \frac{\Lambda_{a_s} + \sum_{jA} w^+(j,a_s)}{r_{a_s} + \lambda_{a_s} + w^-(j,a_s)},$$

$$q_{a_{cs}} = \frac{r_{a_s} + \lambda_{a_s} - \Lambda_{a_s} + \sum_{jA} q_j [w^-(j,a_s) - w^+(j,a_s)]}{r_{a_s} + \lambda_{a_s} + \sum_{jA} w^-(j,a_s)}.$$

Using Sec. III, we consider the dummy agents $\{A_{us}: 1 \leq u \leq 2^\alpha, 1 \leq s \leq \alpha\}$, with

(i) $w^+(j,F)$ for all $j \neq A_\alpha$, $w^-(j,F)=0$ for any other agent $j \neq F$, $w^+(A_\alpha,F)=1$, and $\Lambda_F = \lambda_F = r_F = 0$,

(ii) $w^+(a_1,A_{u1})=1$ and $w^+(a_{c1},A_{u1})=0$ if $X_{u1}=B_{a_1}$, otherwise if $X_{u1}=\neg B_{a_1}$ then $w^+(a_1,A_{u1})=0$ and $w^+(a_{c1},A_{u1})=1$, for all $1 \leq u \leq 2^\alpha$,

(iii) $w^+(a_s,A_{u,s-1},A_{us})=1$ and $w^+(a_{cs},A_{u,s-1},A_{u,s})=0$ if $X_{us}=B_{a_s}$, otherwise if $X_{us}=\neg B_{a_s}$ then $w^+(a_s,A_{u,s-1},A_{u,s})=0$ and $w^+(a_{cs},A_{u,s})=1$, for $2 \leq s \leq \alpha$ and all $1 \leq u \leq 2^\alpha$.

(iv) $w^+(A_\alpha,F)=1$,

(v) $r_{a_s} = r_{a_{cs}} = 2^{\alpha-1}$ because, from (33) there are exactly $2^{\alpha-1}$ terms with either B_s or $\neg B_s$ in the sth position,

(vi) We select $\Lambda_{a_s} \geq 0$ in a manner which satisfies any constraint we may have on the value of q_{a_s} when the agent does not receive signals from other agents (agent "at rest"). For instance, if $\lambda_{a_s}=0$, if we take $\Lambda_{a_s} = r_{a_s} = 2^{\alpha-1}$, for the agent at rest we will have $q_{a_s}=1$, while if we take Λ_{a_s}

$=2^{\alpha-2}$ we would have $q_{a_s}=0.5$ as the agent's activation probability at rest.

(vii) $w^+(a_s,j)=w^-(a_s,j)=0$ and $w^+(a_{cs},j)=w^-(a_{cs},j)=0$ if j is not a dummy agent. With these values we will have

$$q_F=\sum_{u=1}^{m}\prod_{s\in\Upsilon_u}^{\alpha} q_{a_s}\prod_{s\in\Phi_u}[1-q_{a_s}], \tag{34}$$

where $\Upsilon_u=\{s:X_{us}=B_{a_s}\}$ and $\Phi_u=\{s:X_{us}=\neg B_{a_s}\}$, completing the proof. QED

Note that the expression for the state probability of an agent whose state depends on the state of others' according to a Boolean function in DNF can also be similarly constructed.

V. SUMMARY

The model we have presented computes the probability distribution of the concentration or quantity of all of the agents in a network, in a general framework that includes both logical dependencies and excitatory-inhibitory relationships between agents.

Our analysis proves that the equilibrium *joint probability distribution of the activation level* of the N agents in a regulatory network is given by the formula

$$p(k_1,\ldots,k_N)=\prod_{i=1}^{N}(1-q_i)q_i^{k_i}, \tag{35}$$

where k_i is the value taken by the concentration or quantity of agent i, and the q_i are governed by equations of the form

$$q_i=\sum_{u=1}^{m}\prod_{s\in\Upsilon_u}^{\alpha} q_{a_s}\prod_{s\in\Phi_u}[1-q_{a_s}] + \frac{\Lambda_i+\sum_{j=1}^{N} q_j[w^-(j,i)-w^+(j,a)]}{r_i+\lambda_i+\sum_{j=1}^{N} q_j\left[w^-(j,i)+\sum_{l=1}^{N} w(j,i,l)\right]}, \tag{36}$$

where the first term comes from (34), so that both the logical dependencies (in the first term) and the excitatory-inhibitory dependencies (in the second term) are captured in the solution.

We can consider that a regulatory network acts as the control system of a "biochemical nanofactory," with the rate of production of certain compounds being determined by the probability that certain sets of agents are activated. Then our analysis would enable the computation of the rate of production of these compounds over a period of time. Thus our approach does not replace a discrete event or Monte Carlo simulation of a regulatory network based on the full semantics of agent interactions such as [11], but does offer a means to evaluate and predict the behavior of the network over a period of time which is one or two orders of magnitude longer than the time associated with the individual biochemical reactions that are involved. The q_i and *Theorem 1* can provide simple computations, as illustrated in the examples of Secs. II B and II C, while the more detailed estimates provided by Monte Carlo simulations need to be evaluated in terms of statistical confidence intervals, which often require a large number of independent simulation runs.

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[1] S. A. Kauffman, J. Theor. Biol. **22**, 434 (1969).
[2] M. Ptashne and A. Cann, *Genes and Signals* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 2002).
[3] R. Thomas, J. Theor. Biol. **42**, 565 (1973).
[4] R. Thomas, A. M. Gathoye, and L. Lambert, Eur. J. Biochem. **71**, 211 (1976).
[5] M. Kaufman, F. Andris, and O. Leo, Proc. Natl. Acad. Sci. U.S.A. **96**, 3894 (1999).
[6] G. Bernot, J. Guespin-Michel, J.-P. Comet, P. Amar, A. Zemirline, F. Delaplace, P. Ballet, and A. Richard, "Modeling, observability and experiment, a case study," *Proceedings of the Dieppe School on Modeling and Simulation of Biological Processes in the Context of Genomics* (Frontier Group, New York, 2003), pp. 49–55.
[7] G. Bernot, J.-P. Comet, A. Richard, and J. Guespin, J. Theor. Biol. **229**, 339 (2004).
[8] J.-P. Comet, thesis, Université d'Evry-Val-d'Essonne, 2006.
[9] A. Arkin, J. Ross, and H. H. McAdams, Genetics **149**, 1633 (1998).
[10] I. Shmulevich, E. R. Dougherty, S. Kim, and W. Zhang, Bioinformatics **18**, 261 (2002).
[11] A. Ribeiro, R. Zhu, and S. A. Kauffman, J. Comput. Biol. **13**, 1630 (2006).
[12] A. Arazia, E. Ben-Jacob, and U. Yechiali, Physica A **332**, 585 (2004).
[13] E. Gelenbe, "Keeping viruses under control," in *ISCIS05—Computer and Information Sciences, 20th International Symposium, Istanbul, Turkey*, edited by P. Yolum, Lect. Notes Comput. Sci. Vol. 3733, (Springer-Verlag, Berlin, New York, 2005), pp. 304–311.
[14] E. Gelenbe, J. Appl. Probab. **28**, 656 (1991).
[15] E. Gelenbe, J. Appl. Probab. **30**, 742 (1993).

[16] P. Mendes, W. Sha, and K. Ye, Bioinformatics **19**, 122 (2003).
[17] H. Lähdesmäki, H. Huttunen, T. Aho, M.-L. Linne, J. Niemi, J. Kesseli, R. Pearson, and O. Yli-Harja, Signal Process. **83**, 835 (2003).
[18] E. Gelenbe, Neural Comput. **5**, 154 (1993).
[19] E. Gelenbe, Probab. Eng. Mech. **7**, 335 (1993).
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