Sparse essential interactions in model networks of gene regulation

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Gene regulatory networks typically have low in-degrees, whereby any given gene is regulated by few of the genes in the network. What mechanisms might be responsible for these low in-degrees? Starting with an accepted framework of the binding of transcription factors to DNA, we consider a simple model of gene regulatory dynamics. In this model, we show that the constraint of having a given function leads to the emergence of minimum connectivities compatible with function. We exhibit mathematically this behavior within a limit of our model and show that it also arises in the full model. As a consequence, functionality in these gene networks is parsimonious, i.e., is concentrated on a sparse number of interactions as measured for instance by their essentiality. Our model thus provides a simple mechanism for the emergence of sparse regulatory networks, and leads to very heterogeneous effects of mutations.

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I. INTRODUCTION

During the last decade, genomic studies have revealed that complex organisms typically do not have many more genes than less complex ones. Because of this, the paradigm for thinking about biological complexity has shifted from the number of genes to the way they may work together: higher complexities might be associated with a greater proportion of regulatory genes. In particular, there are strong indications in eukaryotes and prokaryotes that for increasing genome size the number of regulatory genes grows faster than linearly in the total number of genes [1, 2]. Hence it is appropriate to consider biological complexity in the framework of interaction networks. This shift from components to the associated interactions has received increasing attention in many scientific communities, with applications ranging from network biology to sociology. The relevance of this conceptual framework for biology has been repeatedly emphasized (see, for example the review [3]) and has benefited from inputs from other fields and from statistical mechanics in particular [4]. We will therefore freely use the network terminology, referring to nodes, their degrees, distinguishing between in and out degrees etc.

From studies that strive to unravel gene regulatory networks (GRN), several qualitative properties transpire: (i) a given gene is generally influenced by a “small” number of other genes (low in-degree of the network of interactions when compared to the largest possible degree); (ii) some genes are very pleiotropic (the out-degree of some nodes of the network can be high); (iii) GRN seem to be robust to change (e.g. to environmental fluctuations or to mutations), a feature that is also found at many other levels of biological organisation [5, 6, 7, 8]. A simple way to build robustness into a network is to have rather dense connections, effectively incorporating redundancy in a local or global way. Furthermore, the number of networks having m interactions grows very quickly with m. Thus when modeling GRN, the network realizations that perform a given regulatory function are dominantly of very high degree. However this is not the case experimentally, at least with respect to the in-degree, and so models so far have had to build in limitations to the accessible connectivities [9, 10, 11]. In this work we show that such shortcomings of models can be overcome by taking into account the known mechanisms underlying genetic interactions: gene regulation is mediated via the molecular recognition of DNA motifs by transcription factors, and this leads to biophysical constraints on interaction strengths. Within this relatively realistic framework, we shall see that networks are in fact driven to be parsimonious (the essential interactions are sparse) for the in-degree while the out-degree is unconstrained.

We begin by explaining the mechanisms incorporated into the model, in particular the determinants of the interactions. We follow standard practice [12, 13, 14] when modeling interactions between DNA binding sites and transcription factors: the affinity is taken to depend on the molecular recognition of DNA motifs by transcription factors, and this leads to biophysical constraints on interaction strengths. Within this relatively realistic framework, we shall see that networks are in fact driven to be parsimonious (the essential interactions are sparse) for the in-degree while the out-degree is unconstrained.

Before proceeding, it is perhaps useful to point out briefly the main similarities and differences between the approach adopted in this work and in previous literature. Our model belongs to the class known as “threshold
models”, used widely in describing neural networks [15] and more recently in GRN modeling [16]. Within such a framework, one represents the GRN by its matrix of connections, and mutations correspond to random modifications of this matrix. In our approach, mutations are also random of course, but we mimic the underlying microscopic effects of a mutation and this forces us to work with weighted interactions; this more realistic way of treating mutations has rather striking consequences as we shall see. Note that we focus on generic aspects of the problem, without attempting to reproduce specific experimental data.

After setting the general framework, we present some of the mathematical and numerical tools we use to analyze the model. Results are first given for the full model, derived using computational methods. Then we focus on a limiting case of the model for which a mathematical analysis can be pushed rather far. We demonstrate there that the constraint of having a given function makes the networks be marginally “viable” and that the corresponding connectivity is in a sense minimal, i.e., networks are as sparse as they can be subject to maintaining their function. This same principle applies to the full model and remarkably, the simple limit proves to be an excellent approximation. Along with the spontaneous appearance of sparseness, we find that the network interactions are quite robust to change [8; 17]: only those few binding sites that are “effectively” used are fragile, mutations of the other (little used) binding sites have almost no effect. Thus robustness to mutational changes is very high for most binding sites while the “essential” interactions have much lower robustness; robustness is heterogeneously distributed in the network. Implications of this sparseness are developed in the discussion; in particular, a consequence is that redundant interactions are rapidly eliminated under evolution if no new function arises which might change the selection pressure.

II. FRAMEWORK: MODEL OF INTERACTING GENES

Gene expression dynamics and viability

Our framework is an abstract GRN model belonging to a family of models that has been used many times by different authors [16; 18; 19; 20; 21]. We consider $N$ genes whose products can have regulatory influences on the same set of genes (retroaction) and possibly also have some “down-stream” consequences. However the consequences of these last effects can be ignored for our purposes since they lead to no feedback on the $N$ “core” genes. Call $S_j(t)$ the expression level of gene $j$ at time $t$, in practice thought of as the concentration of the transcription factor [22] it produces. The dynamics of the $S_j(t)$ takes place on biochemical time scales (typically minutes). To model that, we keep the spirit of earlier work [16; 19; 20; 21], taking the genes to be either on ($S_j = 1$) or off ($S_j = 0$). Furthermore, these expression levels are updated synchronously at discrete time steps: to go from time step $k$ to $k + 1$, we take

$$S_i^{(k+1)} = H \left( \sum_{j=1}^{N} W_{ij} S_j^{(k)} - h \right).$$

(1)

Here, $h$ is a threshold and $H$ is the Heaviside function, $H(x) = 0$ for $x \leq 0$ and $H(x) = 1$ for $x > 0$, while $W_{ij}$ denotes the strength of the interaction that gene $j$ has on gene $i$. A priori, the $W_{ij}$ can be arbitrary and we have no built-in restriction on the network’s connectivity.

The “integrate and fire” functional form of Eq. (1) is inspired by that arising in perceptrons [15]. The main family of models that have been used for modeling gene expression dynamics involve random boolean functions of the inputs [9; 23]. Since that framework does not provide a central role for weighted interactions, we have not considered here the use of this second family of models.

Eq. (1) defines a deterministic discrete dynamical system. After possible transient behavior, at large $k$ the set of expression levels $\{S_i^{(k)}\}_{i=1,...,N}$ will either go to a (time-independent) steady state or will go into a cycle (periodic behavior). Which case arises may depend on the initial state of the expression levels. Following the motivation [16] coming from early embryo development, we consider given the initial expression levels, $\{S_i^{(\text{ini})}\}_{i=1,...,N}$. Furthermore, a network will “perform the desired function” if and only if, starting with the initial expression pattern, it will lead to the desired steady-state gene expression levels; these must also be given a priori and correspond to the “target” $\{S_i^{(\text{target})}\}_{i=1,...,N}$. (More complicated choices, such as limit cycles, would also be possible.) Hereafter we say that a network is “viable” if it satisfies this functional property. Note that in our model, all genes are on an equal footing; it is then easy to see that the model’s properties depend not on the details of the initial and target patterns, but only on the number of indices $i$ where $S_i^{(\text{ini})} = S_i^{(\text{target})} = 0$ and 1. We shall show results when these numbers are set to their average values if each pattern is taken at random, but the results are not sensitive to this choice.

Microscopic modeling of the interactions

So far the framework is rather abstract, the interactions $W_{ij}$ are arbitrary. However much is known about how interactions are mediated in reality, and so it is appropriate to include this knowledge to obtain more realistic models. To begin, the product of gene $j$ is a transcription factor, hereafter denoted $TF_j$, i.e., a protein which modulates the rate at which other genes are transcribed. This modulation arises from the binding of $TF_j$ to the regulatory region of other genes (cf. Fig. 1). In the absence of any bound transcription factors in its regulatory
region, gene $i$’s level of transcription will be low (considered here as off, $S_i = 0$). We allow all of the $N$ types of transcription factors to access all the regulatory regions, but binding depends on the affinity between the TF and the DNA content of these regions. To keep the model simple, we consider that each gene’s regulatory region consists of $N$ putative binding sites, one for each of the $N$ types of TFs as illustrated in Fig. 1. If gene $j$ is “on”, it will produce a certain number $n$ of TF molecules of type $j$; if it is off, it produces no TFs. We shall consider different values of $n$ in our study, using the biologically relevant range $100 \leq n \leq 10^4$. (The lower value comes from the multiplicity of transcripts in E. coli [24] and the expected numbers of protein copies produced thereof, while the upper value comes from direct measurements of numbers of transcription factor molecules [25].)

To model the affinity between TFs and binding sites, we follow standard practice and represent each TF and binding site by a character string using a 4 letter alphabet. The binding free-energy is then simply proportional to the mismatch between the two chains [12, 13, 14, 26, 27]. This leads to the inverse Boltzmann factor $\hat{n}_{ij} \approx C e^{d_{ij}}$, for which Gerland et al. [14] have shown that the constant $C$ is close to 1 and thus will be dropped hereafter. In this formula, $d_{ij}$ is the Hamming distance (number of mismatches) between $TF_j$ and the $j$th binding site of gene $i$; furthermore, $\varepsilon$ is the penalty for each mismatch (contribution to binding energy in units of $k_b T$). Experimentally, $\varepsilon$ is inferred to have a value between one and three if we think of each base pair of the DNA as being represented by one character [28, 29, 30].

The number $L$ of characters used to represent a TF or its binding site is set using the typical number of base pairs in experimentally studied binding sites, $10 \leq L \leq 15$.

When $S_j = 1$, there are $n$ TF molecules of type $j$ that can bind to the $j$th site of gene $i$’s regulatory region; given that this site can be occupied only by one TF at a time, it is common practice to take this occupation probability to be [14]:

$$p_{ij} = \frac{1}{1 + \hat{n}_{ij}/n}. \quad (2)$$

For our purposes, we simplify this relation by working in the regime of low competition as follows. First we define our $W_{ij}$ to be proportional to the Boltzmann factor:

$$W_{ij} = e^{-\varepsilon d_{ij}} \quad i, j = 1, \ldots, N. \quad (3)$$

When $n$ times $\sum_{j=1}^{N} W_{ij} S_j$ is large enough, following Eq. [2], there is a high probability that at least one of the binding sites in gene $i$’s regulatory region will be occupied [14]. We thus set the threshold in Eq. [1] at a value $h$ which is inversely proportional to the number $n$ of TF molecules, $h = 1/n$. This parameter $h$ plays a central role in the model so we shall investigate how its value influences the behavior of the network.

![FIG. 1: Schematic representation of the regulatory region of gene $i$: there are $N$ binding sites, each labeled by an index $j (1 \leq j \leq N)$. Represented is the interaction $W_{ij}$ mediated by the binding of TF $j$ to the $j$th site of that region. The binding affinities depend on the mismatch between the string of length $L$ representing the TF and that representing the DNA of the corresponding binding site.](image)

### III. METHODS FOR MODEL ANALYSIS

**Uniform sampling of viable genotypes**

One can think of a GRN’s genes and DNA binding sites as specifying its “genotype”; equivalently, the genotype can be thought of as being given by the list of weights $W_{ij}$, corresponding to a weighted oriented graph. Because TFs are typically pleiotropic, they are generally thought to evolve slowly, while DNA regulatory regions typically have a high level of polymorphism and may evolve more quickly. Thus in all our study we shall consider that the genes (and thus the TF they code) are fixed whereas the strings of characters representing the DNA binding sites are unconstrained. This defines the scope of the genotype space of our model.

Now within this genotype space lies a small subset of viable genotypes since they are specified by character strings. However, only a tiny part of this space corresponds to viable genotypes, the kind we are concerned with. To sample this much smaller space, we rely on Monte Carlo Markov Chains (MCMC). Within such a procedure, we start by producing (if necessary by design) a viable GRN; then we perform a random walk in the viable subspace of genotypes: at each step we propose a small change of the characters in one of the binding sites; if the new genotype to phenotype is generally many to one. It is relatively straightforward to sample uniformly all genotypes since they are specified by character strings. However, only a tiny part of this space corresponds to viable genotypes, the kind we are concerned with. To sample this much smaller space, we rely on Monte Carlo Markov Chains (MCMC). Within such a procedure, we start by producing (if necessary by design) a viable GRN; then we perform a random walk in the viable subspace of genotypes: at each step we propose a small change of the characters in one of the binding sites; if the new genotype is viable, we accept it, otherwise we reject it and stay with the current genotype. From this procedure, we sample uniformly the space of viable genotypes, which allows us to generate many random viable networks; from these unbiased samplings we examine the statistical properties imposed by viability. Properties include network sparseness, robustness to mutations and essentiality of interactions. Furthermore, the results will depend on the
“specificity” \cite{13, 24, 31} of the interactions between genes (through the alphabet size and the length of the character strings used in our matching process): this aspect plays an important role in understanding how such networks can both function and evolve, so we will consider how our results depend on it.

Model implementation

The DNA sequences of one of our networks of \(N\) genes can be represented via \(N^2\) strings of \(L\) characters in a four letter alphabet; indeed, a binding site for a given TF is represented by \(L\) characters, there are \(N\) binding sites per gene and a total of \(N\) genes. In our framework, each TF is considered as given while the space of all considered networks arises from letting the DNA sequences be variable. Thus, instead of having an explicit representation of the strings associated with TFs and binding sites, it is enough to track a binary string of length \(L\) for each binding site, where for each entry the bit 0 (respectively 1) stands for a mismatch (respectively a match) with the corresponding TF. The genotype then reduces to \(N^2\) such binary strings. It is important to remember that there are 3 underlying possible characters for each bit at 0 and only one if the bit is 1. When using this representation for our MCMC, the transition rate from a 0 to a 1 bit must be three times smaller than the transition rate from a 1 to a 0 bit.

To ensure that our MCMC is ergodic on the time scales accessible to our computational resources, we use a swap operator whereby we exchange the content of two randomly chosen neighbor binding sites. We call “step operation” the following: (1) propose successively \(L\) random point mutations; (2) propose a single swap. A “sweep” is then the application of \(N^2\) random step operations. The autocorrelation time of this MCMC was estimated to be less than the time of 1 sweep.

Concerning the choices for \(S^{(\text{ini})}_{i=1,...,N}\) and \(S^{(\text{target})}_{i=1,...,N}\): if drawn at random, the fraction of terms set to 1 would be approximately equal to that set to 0 when \(N\) is large. To reduce finite size effects, we force the equality at our levels of \(N\) which are multiples of 4. Because of the permutation symmetry of the model, one can always permute the indices so that \(S^{(\text{ini})}_{i} = 1\) for \(i \leq N/2\) and 0 otherwise; furthermore we also impose without loss of generality \(S^{(\text{target})}_{i} = 1\) for \(N/4 < i \leq 3N/4\) and 0 otherwise. Notice that \(\sum_i S^{(\text{ini})}_{i} = \sum_i S^{(\text{target})}_{i} = N/2\) and \(\sum_i S^{(\text{ini})}_{i} S^{(\text{target})}_{i} = N/4\).

Finally, we need to start the MCMC with a viable GRN. To generate an initial genotype, we first set

\[
W_{ij} = S^{(\text{target})}_{i} S^{(\text{target})}_{j} \quad i,j = 1,\ldots,N
\]

and then construct the bit strings of the binding sites by taking values that approximate this equation. In practice this initial setting nearly always leads to a viable genotype; if not, other approximations are tried. From this procedure an initial viable genotype is constructed and then the MCMC can begin.

IV. Results

Some qualitative properties

The total number of genotypes is \(4^{LN^2}\); since realistic values of \(L\) are at least 10, this number is astronomical even for rather modest \(N\). However only a tiny fraction of these genotypes are viable. Naively, since we want the gene expression pattern in the steady state to be given by \(S^{(\text{target})}\), and since there are \(2^N\) possible patterns, one may expect only a fraction of order \(2^{-N}\) of the genotypes to be viable. In fact, the fraction is even smaller, especially as \(h\) grows. Thus if one seeks to generate random viable GRN by producing random genotypes (strings of characters for the binding sites), most attempts will be unsuccessful and it will be near impossible to sample the space of viable GRNs when \(N\) is 3 or more. This is why we relied on Monte Carlo Markov Chains to perform the sampling of viable networks. (Such an approach is computationally efficient if the Markov Chain has a short auto-correlation time, which is the case here as mentioned in the methods section.) Although it is difficult to derive properties of viable genotypes, general genotypes are relatively easy to understand because there is no viability constraint. The statistical properties of the interaction strengths \(W_{ij}\) follow from the distribution of the mismatch between the character strings for a binding site and a TF. Each of the \(L\) characters of a binding site gives a mismatch with probability \(3/4\); the total mismatch \(d\) thus follows the binomial distribution of mean \(3L/4\):

\[
p(d) = \binom{L}{d} (1/4)^{L-d} (3/4)^d
\]

Sparse essential interactions

In contrast to general genotypes, viable genotypes are subject to the constraint of reaching (after some transients) the steady state expression given by \(\{S^{(\text{target})}_{i}\}_{i=1,...,N}\) when initialized in the state \(\{S^{(\text{ini})}_{i}\}_{i=1,...,N}\). How do the set of interactions \(W_{ij}\) differ when comparing viable and general networks? We first address this question computationally by considering statistical properties of random genotypes, subject to being viable or not.

The main control parameter is the threshold value \(h\), which must be compared to the typical value of \(\sum_j W_{ij} S_j\) in the absence of the viability constraint; denote this value by \(\omega\). Representing the averages over all genotypes by \(\langle \rangle\), we have \(\omega = N \langle W_{ij} \rangle / 2\). For this formula we have used the fact that because of the symmetry of the model
in the absence of the viability constraint, the \( \langle W_{ij} \rangle \) are independent of \( i \) and \( j \); we have also used \( \langle S_i \rangle = 1/2 \) for random initial and target expression states. Now when \( h \) is significantly larger than \( \omega \), nearly all random genotypes will have their expression levels go to 0 at large times and thus will not be viable. If a genotype is viable, it must be that \( \sum_i W_{ij} S_i^{\text{target}} \) is anomalously large for all \( i \) such that \( S_i^{\text{target}} = 1 \). When this happens for such a row \( i \), one may have one entry \( j^* \) for which \( W_{ij^*} \) is very large, or one may spread the “burden” among several interactions \( W_{ij_1}, W_{ij_2}, \ldots W_{ij_h} \) where each weight is a bit larger than average. In the space of all viable genotypes, does one more often resort to the first or second strategy? To find out, we begin by considering the distribution of the mismatch between TFs and their binding sites in viable networks. We denote the mismatch by \( d \); it is perhaps useful to regard the quantity \( L - d_{ij} \) as a measure of the affinity between a TF and the corresponding DNA binding site in this model. In Fig. 2 we show the distribution of \( d \) when there are \( N = 20 \) genes, for increasing values of the threshold parameter \( h \). At the low values of \( h \), \( d \) has a binomial distribution with a peak near \( d = 3L/4 \) as expected. However one observes at small \( d \) significant deviations. In fact, as \( h \) increases, the viability constraint become marked and the distribution becomes bimodal: a peak appears at low mismatch values. Note that this peak shifts as \( h \) increases, indicating that there are nearly perfect matches that appear in that regime.

To distinguish the two scenarios, namely whether the burden in each row \( i \) is concentrated on one vs. on multiple \( j \) indices, it is natural to consider the inverse participation ratio (IPR), a commonly used measure of how many terms effectively participate in a weighted list. The straightforward use of this approach for the matrix \( \{W_{ij}\} \) is the IPR \( I = \sum_{i,j} W_{ij}^2 / (\sum_{i,j} W_{ij})^2 \). If many elements effectively participate, the value will be \( O(1/N^2) \), while if only a few contribute per line the value will be not much less than \( 1/N \). However this index is a poor indicator of sparseness for several reasons. First, in the absence of the viability constraint and when \( \varepsilon \) is of order unity, the value is found to be of \( O(1) \) ! The reason can be traced to the distribution of the \( W_{ij} \) when the mismatches are binomially distributed: because the weights \( W_{ij} \) are exponentially in the mismatches, \( I \) is often dominated by one or two interactions. Second, we are actually more interested in what happens for each row \( i \) such that \( S_i^{\text{target}} = 1 \); the other rows don’t need good matches and just add noise to \( I \). Thus it is appropriate to focus on the IPR restricted to one such line at a time. We therefore define

\[ A_{ij} = S_i^{\text{target}} S_j^{\text{target}} (d_{ij} - 3L/4)^2 \tag{6} \]

and the associated IPR for the \( i \)-th line

\[ I_i = \frac{\sum_j A_{ij}^2}{(\sum_j A_{ij})^2}. \tag{7} \]

Then we average these \( I_i \)’s over \( i \)’s for which \( S_i^{\text{target}} = 1 \). This is the IPR quantity plotted in Fig. 3. Except at very low values of \( h \), only one or two weights per row are significantly larger than the others in that same row. Note that the “staircase” structure of the plot is a consequence of the discrete nature of the mismatch value.

It is possible to further explore the statistical properties of viable genotypes by considering not the weights themselves but their function. One can ask whether “essential” interactions are sparse (few essential interactions per row), \( i.e., \) in a given viable genotype, how many of its interactions have the property that viability is lost when the interaction is removed (\( W_{ij} \) is set to 0). We find that as soon as \( h \) is not too small, there is almost always just one essential interaction per gene as shown in Fig. 4 for \( N = 20 \) and \( L = 12 \). The same result holds for other relevant values of \( N \) and \( L \), suggesting that within our models, the drive towards sparse interactions arises

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FIG. 2: Distribution of the Hamming distance between a TF and the receiving DNA site for \( N = 20, L = 12, \varepsilon = 1.5 \) and for various values of the threshold parameter: (circle) \( h = 0.3 \), (square) \( h = 0.1 \), (diamond) \( h = 0.02 \), (triangle up) \( h = 0.005 \), (triangle down) \( h = 0.001 \). The lines are to guide the eye.

FIG. 3: The mean of the inverse participation ratio per line (cf. Eq. 7) versus \( h \) for \( N = 20, L = 12, \varepsilon = 1.5 \). The line is to guide the eye.
in regimes of biological relevance. We also considered a stronger measure of essentiality: we asked that the viability be lost when the interaction’s mismatch is increased by one. Remarkably, the rule “one essential interaction per gene” generally held here too. Thus mutations in these interactions are typically deleterious, while mutations in the vast majority of the other interactions have no consequence on viability. This shows that mutational robustness is very heterogeneously distributed among the interactions in the network.

Mathematical analysis in a simple limit

As already mentioned, under the dynamical process Eq. [11], when the expression levels reach the target, the dynamics must be at a fixed point if the genotype is viable. Then the different lines of the matrix \( \{W_{ij}\} \) have to satisfy the “fixed point” constraint and in each line it is sufficient to consider those elements which are multiplied by 1. It is therefore worth considering a toy model where transient effects in the dynamics are neglected. In such a framework, we consider only the fixed point conditions that now can be thought of as coming from the particular choice \( S_i^{(\text{target})} = S_i^{(\text{target})} \) for each \( i \). For each such index \( i \) (or equivalently line of the matrix \( \{W_{ij}\} \)), the toy model leads to the partition function

\[
Z_{\text{toy}}^{(K)}(h) = \sum_{d_1,\ldots,d_K} p(d_1,d_2,\ldots,d_K) H\left(\sum_{j=1}^{K} e^{-d_j\varepsilon} - h\right) \tag{8}
\]

where \( K = N/2 \) and \( H \) is the Heaviside function. We have also assumed that \( i \) is such that \( S_i^{(\text{target})} = 1 \). For the other lines (for which \( S_i^{(\text{target})} = 0 \), if \( h \) is not too small the fixed point condition will nearly always be satisfied and so can be ignored. Because in this toy model the different lines are independent, we can focus on one line at a time, in line with what arises when analyzing fixed points in neural network systems [12].

In this reduced problem, the state space is a \( K \)-dimensional hypercube \( \mathcal{C} \) with edge length \( L \), \( \mathcal{C} : (d_1, d_2, \ldots, d_K), \) \( d_j = 0, 1, \ldots, L \) being the \( j \)th mismatch. The a priori mismatch probability in fact factorizes:

\[
p(d_1, d_2, \ldots, d_K) = \prod_{i=1}^{K} p(d_i) \tag{9}
\]

with \( p(d) \) given by Eq. [3]. From this we see that \( \left[Z_{\text{toy}}^{(K)}(h)\right]^{K} \) gives the fraction of random genotypes that are viable. Notice, that for \( L \) large and for \( d \) small enough

\[
p(d) \approx \frac{L^d}{4L} \ll 1, \quad d \ll L. \tag{10}
\]

We wish to understand the effect of the Heaviside constraint on the probability distribution of the mismatches and compare with what happens in the full model. We first define \( d_h = d_h(\varepsilon, h) \) by the equation \( e^{-\varepsilon d_h} = h \) and assume that \( h \) is such that Eq. [10] holds for \( d < d_h \). Now remove from the state space \( \mathcal{C} \) the sub-hypercube \( \mathcal{C}' : d_i \geq d_h \) for all \( i = 1, \ldots, K \). In this reduced state space we keep the same probabilities up to a normalization factor:

\[
\tilde{p}(d_1, d_2, \ldots, d_K) = p(d_1) \ldots p(d_K) \left( \prod_{j} \left( 1 - H(d_h - d_j) \right) \right) / A. \tag{11}
\]

The factor in brackets is 1 within the reduced state space and vanishes outside of it, so it serves simply to filter out elements in \( \mathcal{C}' \). Also,

\[
A = 1 - \sum_{d_1,\ldots,d_L} \prod_{j=1}^{K} p(d_j) H(d_j - d_h) = 1 - \left( 1 - \sum_{m} p(m) H(d_h - m) \right)^K \tag{12}
\]

Eqs. [11][12] are exact. To proceed further we take advantage of Eq. [10]: in the r.h.s. of Eqs. [11][12] we expand when possible the products and drop all quantities where \( p(d) \)'s with \( d < d_h \) appear at higher order than first, e.g., \( \ldots p(d_i) p(d_j) \ldots \) with \( d_{i,j} < d_h \). This yields

\[
\tilde{p}(d_1, d_2, \ldots, d_K) \approx p(d_1) \ldots p(d_K) \sum_{j=1}^{K} H(d_h - d_j) / K \sum_{m<d_h} p(m) \tag{13}
\]

The marginal distribution of a mismatch, say \( d_1 \), is obtained by summing over all \( d_i \) with \( i > 1 \). (Note that the marginals distributions do not depend on the value of the index.) Changing notation \( d_1 \rightarrow d \) one easily obtains

\[
\tilde{p}(d) \approx \frac{K-1}{K} p(d) + \frac{1}{K} \sum_{m<d_h} p(m) \tag{14}
\]
Eq. [14] has a very simple interpretation: in the reduced state space, the shape of the probability distribution of any mismatch is essentially that without the viability constraint, but with an additional peak at small values of the mismatch. There is thus one “leading” mismatch taking care of most of the constraint, while the other mismatches behave approximately as if they were unconstrained. Such a behavior is exactly what we saw happened in the full model. In brief, the effect of the viability constraint condenses on one of the entries of the row considered, the other entries behave as if there were no constraint. Furthermore, one has sparseness of the essential interactions and a high IPR. This situation, where the IPR goes from low to high values as a parameter (here) is increased, is reminiscent of many “condensation” phase transitions. In a biological context, such a transition has been observed in another genetic system, but based on epistatic interactions [32] and otherwise unrelated to our framework. It has also been seen in statistical physics problems [33].

In Eq. [14], the neglected terms are order $Kp(d)$ smaller than those kept. Hence to justify dropping those terms we must have

$$KL^d/4^L \sim KL^{[\ln(1/h)/\epsilon]/4^L} \ll 1.$$  \hspace{1cm} (15)

The constraint imposed so far on the state space (exclusion of $C'$) leads to simple formulæ but it is stronger than the one imposed by the toy partition function, namely

$$\sum_{j=1}^{K} e^{-\epsilon d_j} > h.$$  \hspace{1cm} (16)

If we replace in this relation the inequality by an equality and assume that the $d_j$'s are continuous variables, we obtain the definition of a hypersurface, call it $S$, which is included in $C'$. From this we see that the constraint Eq. [10] removes not the whole hypercube $C'$ but only the points lying beyond $S$. The perturbative relation Eq. [14] is nevertheless an excellent approximation provided the probabilities associated with those points of $C'$ which remain in the state space are very small. This is usually the case for values of parameters we consider and that are of biological interest. In fact, the quality of the approximation could have been guessed given the form of the data shown in Fig 2.

V. DISCUSSION AND CONCLUSIONS

We considered a fairly general model of a gene regulatory network (GRN) in which function is identified with reaching given target gene expression levels. By simulation and mathematical analysis, we investigated the properties of networks in this model under the constraint that they be “viable” (i.e., have the desired function). We find that for a certain range of values of the model’s parameters, the viability constraint leads to sparse GRN; we have quantified this through both inverse participation ratios of the interactions and through the sparsity of “essential” interactions. Interestingly, the effects of the viability constraint condense onto just a few of the possible binding sites, the others being non-functional. As a result, nearly all mutations of the binding sites have no effect on the viability and so such sites have a very high mutational robustness. However, for those few sites which bear the burden of the constraint, the majority of mutations are deleterious so their mutational robustness is low. Thus in our GRN, the mutational robustness is extremely heterogeneous from site to site. In addition, any “redundant” interaction is expected to become lost under evolutionary dynamics since mutations will remove it and condense the burden of viability onto a smaller number of interactions.

Although our modeling of the regulation of gene expression is relatively idealized (cf. the simple dynamical process Eq. [1]), other features of the model presented in this paper are fairly realistic; in particular we have insisted on including interactions through the biophysical mechanism of molecular recognition and affinity. It is therefore interesting that the sparseness of GRNs comes out very naturally in this framework. This is well illustrated by our numerics and by the analytic calculation in the previous section. It should be clear that this sparseness is a result of the combined effect of several causes: the viability constraint, the low probability of a small mismatch between TF and the binding site of DNA, the size $L$ of this segment, the not-too-small spacing (in units of $k_B T$) between the energy levels that determine the strength of TF-DNA interactions, and finally the value of the threshold $h$ itself in the expression dynamics of Eq. [1]. A necessary condition for our GRNs to be sparse is that this threshold $h$ be significantly larger than the total strength contributed by random gene interactions in the absence of the viability constraint. For a four letter code, assuming, as we do, that the binding energy is additive in mismatches and that every mismatch costs
the same, one gets the condition
\[ h \gg \frac{N}{2}(0.25 + 0.75e^{-\varepsilon})^L \]  
(17)

Note that within a two letter code, the condition forces one to larger values of \( L \) (close to 20) and thus beyond what is realistic biologically.

Since the model parameters correspond to measurable quantities, it is appropriate to compare to biological values. According to Eq. [2] the probability that a TF occupies a DNA site is controlled by \( n \), the number of these molecules. Thus, this probability is greater than 1/2 when the corresponding Boltzmann factor is larger than 1/n. Hence, 1/n is an estimate of our threshold \( h \); the reasonable range is roughly 1/10000 < \( h < 1/100 \). Interpreting \( \gg \) as “larger by one order of magnitude”, i.e., by a factor 10, one gets an allowed region in parameter space as illustrated in Fig. [5].

The predicted domain of relevance is above the corresponding curves (taken at \( N = 20 \) and illustrative values of \( h \)). We see that \( \varepsilon \) and \( L \) should not be too small. Moreover, it is gratifying that the experimental range of these parameters (indicated by a rectangle) is near the border and, most of it, within this region. The model would remain meaningful if \( L \) and \( \varepsilon \) were even larger. However, in the analogue of Fig. [2] the point at \( d = 0 \) would dominate strongly over the few neighboring \( d \) points, and the system would be robust but not evolvable [26, 27]. Presumably, both to reach functional GRN and to allow these to be evolvable, it is desirable not to be too dominated by essential interactions, so the probability of having two co-dominant mismatches should not be completely negligible. Such effects go beyond our model as we work within a “strong selection” limit: a GRN is viable or not, no graduation is allowed; when a continuous fitness replaces viability, evolvability should be strongly enhanced.

It is worth emphasizing that as the number \( N \) of genes grows, it is necessary to increase slowly either \( L \), \( \varepsilon \) or \( h \). \( \varepsilon \) is constrained by biophysical processes and thus not evolvable, and \( L \) seems the best candidate for the system to adapt to increasing \( N \) [31]. Note nevertheless that the effects of growing \( N \) are mild and that in practice regulation is modular, so effectively biological GRN have only modest values of \( N \). Finally, as already mentioned in the introduction, our model belongs to the class of threshold models that have a much wider applicability than GRN. Therefore, the emergence of essential interactions following the mechanisms outlined in this paper is expected in all cases where the typical magnitude of the “local field” \( \sum_i W_{ij} S_j \) is small compared to the threshold.

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